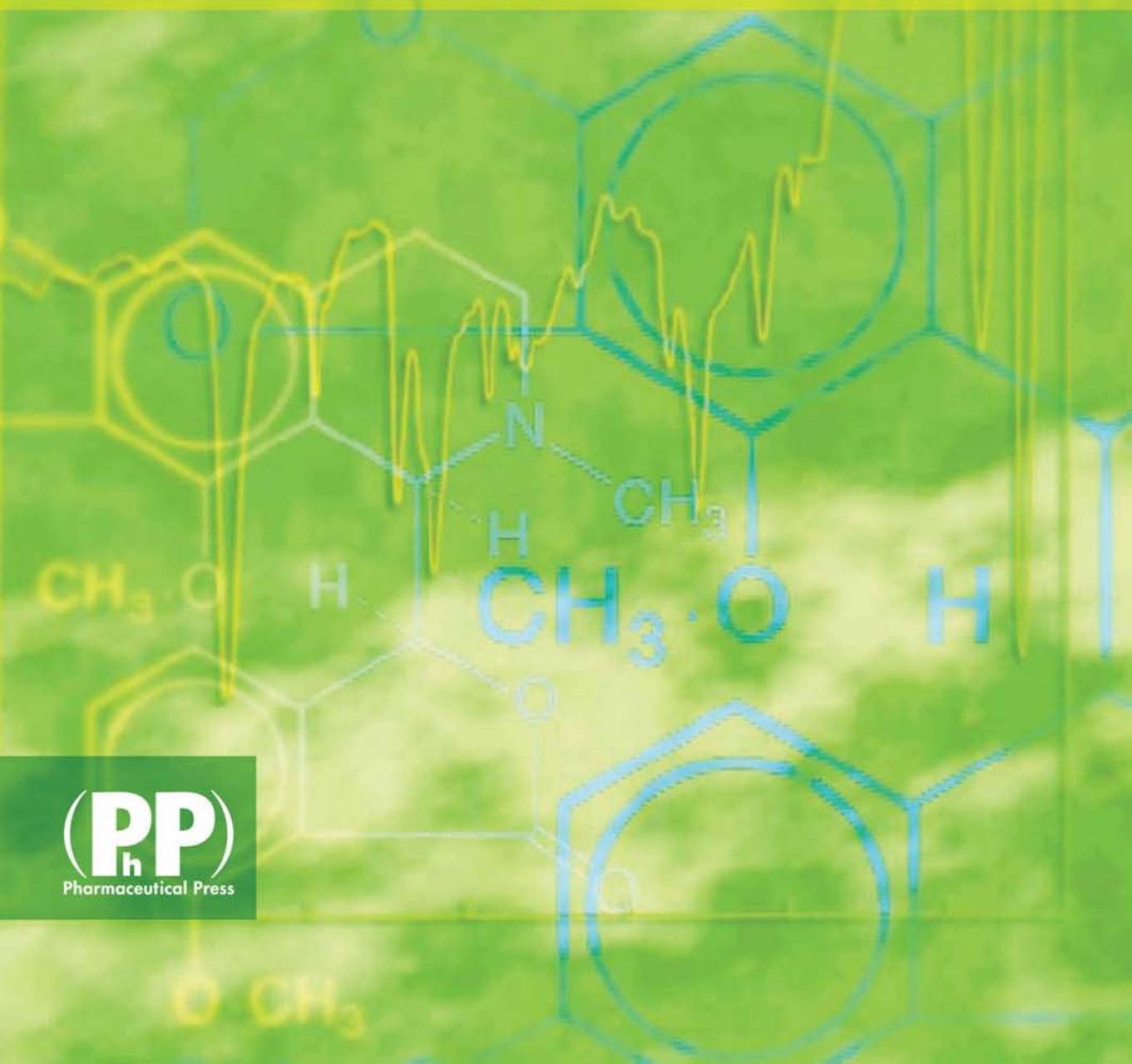
Clarke's Analysis of Drugs and Poisons

Fourth edition

Edited by Anthony C Moffat, M David Osselton and Brian Widdop



Pharmaceutical Press

Clarke's Analysis of Drugs and Poisons

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in pharmaceuticals, body fluids and postmortem material

FOURTH EDITION

Consulting Editors
Anthony C Moffat
M David Osselton
Brian Widdop

Executive Development Editor
Jo Watts

London • Chicago
Pharmaceutical Press

Published by Pharmaceutical Press

1 Lambeth High Street, London SE1 7JN, UK
1559 St Paul Avenue, Gurnee, IL 60031, USA

First edition, edited by EGC Clarke, published 1969 (Vol. 1) and 1975 (Vol. 2)
Second edition (in one volume) published 1986
Third edition published 2004
Fourth edition published 2011

Typeset by Thomson Digital, Noida, India
Printed in Italy by LEGO S.p.A.

ISBN 978 0 85369 711 4

A catalogue record for this book is available from the British Library.

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Contributors

Dr Federica Bortolotti
Department of Public Health and Community Medicine, Section of Forensic Medicine, University of Verona, Verona, Italy

Dr Robin A Braithwaite
Regional Toxicology Laboratory, City Hospital NHS Trust, Birmingham, UK

Dr David E Bugay
SSCI Inc., West Lafayette, USA

Dr Jan Cordonnier
Dorpsstaat 106, B-8340 Sysele-Damme, Belgium

Professor David A Cowan
Drug Control Centre, King's College London, UK

Dr Alastair G Davidson
Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK

Dr Sheila Dawling
Vanderbilt University Medical Centre, Diagnostic Labs – TVC, Nashville, Tennessee, USA

Professor Frederik A de Wolff
Toxicology Laboratory, Leiden University Medical Center, Leiden, The Netherlands

Dr Jacob F de Wolff
University College London Hospitals NHS Foundation Trust, London, UK

Dr Olaf H Drummer
Victorian Inst. of Forensic Medicine, Southbank, Victoria, Australia

Dr Ameriga Fanigliulo
Department of Public Health and Community Medicine, Section of Forensic Medicine, University of Verona, Verona, Italy

Dr Robert J Flanagan
Medical Toxicology Unit, Guy's and St Thomas' Hospital Trust, London, UK

Dr Jesus M Gonzalez
Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania, USA

Dr Paul Graham
Walker Graham Pharma Consulting Ltd, Ashington, Northumberland, UK

Mr Mike Hallworth
Department of Clinical Biochemistry, Royal Shrewsbury Hospital, Shrewsbury, UK

Dr Roger D Jee
The School of Pharmacy, University of London, UK

Dr Graham Jones
Office of Chief Medical Examiner, Edmonton, Canada

Dr A Wayne Jones
National Board of Forensic Medicine, Department of Forensic Chemistry, Linkoping, Sweden

Dr Maria Kała
Department of Forensic Toxicology, Institute of Forensic Research, Cracow, Poland

Dr Steven Karch
PO Box 5139, Berkeley, CA 94705, USA

Dr Phil Kemp
Analytical Research Laboratories, Oklahoma City, USA

Dr Sarah Kerrigan
SHSU Regional Crime Laboratory, The Woodlands, Texas, USA

Dr Pascal Kintz
Laboratoire Chemtox, Illkirch-Graffenstaden, France

Dr Thomas Kupiec
Analytical Research Laboratories, Oklahoma City, USA

Dr Marc LeBeau
Chemistry Unit, FBI Laboratory, Quantico, Virginia, USA

Professor John C Lindon
Division of Biomedical Sciences, Imperial College of Science, Technology and Medicine, London, UK

Dr Barry K Logan
NMS Labs, Willow Grove, Pennsylvania, USA

Dr Pamela A Martoglio Smith
SSCI Inc., West Lafayette, USA

Professor Hans H Maurer
Department of Experimental and Clinical Toxicology, University of Saarland, Homburg/Saar, Germany

Dr Sean D McDermott
Drugs Intelligence Unit, Forensic Science Laboratory, Dublin, Ireland

Professor Anthony C Moffat
Formerly, Royal Pharmaceutical Society of Great Britain and, The School of Pharmacy, University of London, London, UK

Professor Jeremy K Nicholson
Department of Surgery and Cancer, Imperial College of Science, Technology and Medicine, London, UK

Dr R Sam Niedbala
Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania, USA

Professor M David Osselton
University of Bournemouth, Dorset, UK

Dr Jennifer Pascali
Department of Public Health and Community Medicine, Section of Forensic Medicine, University of Verona, Verona, Italy

Dr Michael A Peat
Quest Diagnostics, Houston, Texas, USA

Dr Frank T Peters
Institut für Rechtsmedizin, Universitätsklinikum Jena, Jena, Germany

Professor Colin F Poole
Department of Chemistry, Wayne State University, Detroit, Michigan, USA

Dr John Ramsey
Division of Cardiological Sciences, St George's Hospital Medical School, London, UK

Dr David Reith
Dunedin School of Medicine, University of Otago, New Zealand

Dr Dave Rudd
GlaxoSmithKline Manufacturing, Ware, Hertfordshire, UK

Dr Johan Schaep
Chemiphar n.v., Brugge, Belgium

Dr Vina Spiehler
422 Tustin Avenue, Newport Beach, CA 92663, USA

Dr Thomas Stimpfl
Department of Forensic Medicine, Wien, Austria

Professor Franco Tagliaro
Department of Medicine and Public Health, University of Verona, University Hospital, Verona, Italy

Dr Andrew Taylor
Royal Surrey County Hospital, Guildford, Surrey, UK

Dr Phil Teale
Medication and Doping Control, HFL Sport Science Quotient Bioresearch, UK

Dr Fiona C Thorley
SSCI, A Division of Aptuit, Abingdon, Oxfordshire, UK

Professor Donald RA Uges
Laboratory for Clinical and Forensic Toxicology and Drug Analysis, University Hospital Groningen and University Centre of Pharmacy, Groningen, The Netherlands

Dr Alain Verstraete
Klinische Biologie, Universitair Ziekenhuis, Ghent, Belgium

Dr David Watson
Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK

Dr Jo Watts
Pharmaceutical Press, Royal Pharmaceutical Society of Great Britain, London, UK

Dr Brian Widdop
Formerly, Medical Toxicology Unit, Guy's and St Thomas' Hospital Trust, London, UK

Dr Steve HY Wong
Department of Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

About the Editors

Professor Anthony C Moffat
BPharm, PhD, DSc, CChem, FRSC, FRPharmS, FFIP, FFSSoc

Professor Anthony C Moffat is Emeritus Professor of Pharmaceutical Analysis at The School of Pharmacy, University of London, where he was previously Head of the Centre for Pharmaceutical Analysis. He was also Chief Scientist at the Royal Pharmaceutical Society. He has over 350 publications as well as the co-authorship of eight books.

Previously he worked for the Forensic Science Service for 23 years as Research Co-ordinator (Birmingham Laboratory), Resources Manager (Huntingdon Laboratory), Head of Quality Management (HQ, London), Assistant Director (Huntingdon Laboratory), and Head of the Drugs and Toxicology Division at the Home Office Central Research Establishment, Aldermaston. He has also been a Superintendent Pharmacist in a community pharmacy, Assistant Professor of Biochemistry, Baylor College of Medicine, Houston, Texas, and Chief Pharmacist, St Leonard's Hospital, London.

An active member of many professional and learned societies, his fellowships include the Royal Pharmaceutical Society, Royal Society of Chemistry, Forensic Science Society, International Pharmaceutical Federation and the American Association of Pharmaceutical Scientists as well as the membership of the International Association of Forensic Toxicologists.

Professor M David Osselton
BSc, PhD, CSci, CChem, FRSC, MEWI

Professor M David Osselton started his forensic toxicology career in 1974 when he went to work with Dr Alan Curry at the Home Office Central Research Establishment, Aldermaston. He gained casework experience as Senior Toxicologist working at the Home Office Forensic Science laboratories in Nottingham and Huntingdon before returning to Aldermaston in 1984 to succeed Dr Anthony Moffat as Head of Research in Alcohol, Drugs and Toxicology. In 1991, he was appointed Head of Toxicology for the Forensic Science Service. In 2007, Professor Osselton went to Bournemouth University as Head of the Centre for Forensic Sciences. He has wide experience in toxicology casework and has been involved in numerous high profile cases working for the defence and prosecution both in the UK and overseas. He is internationally known for his research interests in toxicology and lectures widely at conferences as a plenary and keynote speaker. Between 2003 and 2009 he was Lead Assessor (Toxicology) for the UK Council for the Registration of Forensic Practitioners (CRFP) and was Visiting Professor to the Department of Forensic Science and Drug Monitoring at Kings College, University of London (2004–2007). He is a Fellow/Member of a number of professional and learned bodies including the Royal Society of Chemistry, Royal Society of Medicine, Expert Witness Institute, International Association of Forensic Toxicologists (TIAFT), Society of Forensic Toxicologists (SOFT), LTG (formally the London Toxicology Group), UK Workplace Drug Testing Forum and is chair of the United Kingdom and Ireland Association of Forensic Toxicologists.

Dr Brian Widdop
BSc, PhD, SRCS, CChem, FRCS, FRCPath

Dr Brian Widdop was Director of the Medical Toxicology Unit Laboratory at Guy's Hospital, London, from 1970 until 2002. Dr Widdop has been a Speciality Assessor for the Council for the Registration of Forensic Practitioners, a member of the WHO IPCS Working Group on Analytical Toxicology, Chief Advisor to the South East Asia Regional Office of the WHO on analytical toxicology and was a Director of the Board of the UK Horse-racing Forensic Laboratory from 1991 to 2002. From 1997 to 2001, he was joint co-ordinator of the European proficiency Testing Scheme for Drugs of Abuse. Dr Widdop has published over 80 papers on various aspects of clinical and analytical toxicology and has spoken at many international meetings. He is also a member of the editorial board of the Journal of Analytical Toxicology.

Dr Widdop belongs to several international scientific societies and was a founder member of the London Toxicology Group. He has been a member of The International Association of Forensic Toxicologists for 42 years and was the recipient of the Alan Curry Award in 2002.

Dr Jo Watts
BSc, PhD

Dr Jo Watts attained her degree in pharmacology and toxicology followed by a PhD in neuropharmacology, both at The School of Pharmacy, University of London. She is a member of TIAFT and the LTG.

Foreword

As one of the past presidents of the International Association of Forensic Toxicologists (TIAFT) it is an honour as well as a pleasure for me to write a foreword for the fourth edition of this prestigious publication. Indeed, when a publication is prepared by an impressive number of leading toxicologists working in world-famous institutions, as editors or as former or new authors – all outstanding specialists in their respective fields of activity – we as toxicologists can only expect to have another great database in our hands with which to do research or our daily work.

In addition to the monographs revised from the previous editions, dealing with physicochemical and pharmacotoxicological properties of drugs and poisons, and the indexes of analytical data, review chapters have been included on various basic subjects of toxicology, such as hospital and forensic toxicology; immunoassays; analysis of alternative matrices; doping; driving under the influence of drugs and alcohol; therapeutic drug monitoring; workplace testing; quality assurance; pharmacokinetics; pesticides; volatile organic substances; natural toxins; different separation technologies; and spectrometric methods. New chapters by renowned experts have been added that deal with method development and validation; sampling, storage and stability; extraction; more recently developed analytical technologies such as liquid chromatography coupled to mass spectrometry; atomic absorption spectrometry, or inductively coupled mass spectrometry for the determination of inorganic poisons; pharmacogenomics; measuring and reporting uncertainty; drug facilitated sexual assaults; and paediatric forensic toxicology. These topics enable our younger and less young colleagues alike to familiarise themselves with these developments or to improve their knowledge.

Especially at a time when shortcuts are made for budgetary reasons in healthcare and forensic systems, we need to document our performances of reliable analytical results followed by correct interpretations of these results to proof our usefulness to decision makers. Therefore, this fourth edition is published at the perfect moment.

I remember very well at the beginning of my career in Luxembourg the moment I held the first edition from 1969, which was recommended to me by a French colleague. It was always a bible for me and is still an important part of my personal library. Several similar publications have been produced in the past, but they have never had the same impact on toxicology as *Clarke*. My professional work was also influenced by the renowned British scientists who I met during a TIAFT conference in Ghent back in the early '70s. What a lot has changed since those days!

It is an important advance that a chapter on interpretation has been added, as this is the major difference between analytical toxicology and analytical chemistry. Toxicological analysis is not analytical toxicology. From my work in forensic toxicology, I know that in court we are questioned more about interpretation of our results than about the performance of the analytical methods. Our customers should be aware that we are not 'only making measurements'.

Since toxicology is a multidisciplinary science, toxicologists need not only to have comprehensive knowledge of analytical methodology, but also to be proficient in the basics of medicine, physiology, clinical chemistry, biochemistry, pharmacodynamics and pharmacokinetics in order to provide the best possible contributions to clinicians and to forensic authorities. Toxicology is a difficult and a complex issue requiring collective information exchange among toxicology specialists from all related fields. Even the publishing of routine cases that may not be routine for other colleagues should be encouraged. In spite of the fantastic efforts in recent years to establish correlations between toxicant concentrations in body tissues and pharmacodynamic action, behaviour impairment, interindividual variability, pharmacogenetics, postmortem changes or concurrent existing pathologies, there is still a lot of research required to improve our knowledge. So, I can only recommend to my colleagues: let us do it!

Even now that a staggering amount of information is available via the internet, *Clarke* remains a reference for old and young toxicologists. It is an easily accessible tool which can be consulted either by reading the book or by browsing and searching the online version, to give us useful structured, and peer-reviewed information written by well-known experts.

I wish *Clarke* the best success that it deserves, to equal that of the previous three editions of this publication.

Robert Wennig, PhD
Past President of TIAFT

Preface

Clarke's Analysis of Drugs and Poisons aims to be the world's leading text on the analysis of drugs and poisons. Not only does it contain chapters on the methodology and techniques of modern analytical toxicology, but the monographs include analytical data on therapeutic drugs, drugs of abuse, drugs misused in sport as well as pesticides, metals and other poisons. This fourth edition builds on the previous editions with significant updating and improvements in scope and electronic form.

Clarke, as it is affectionately known, has gained a world-wide reputation as a reliable source of toxicological information. Its presence on the benches of many different types of pharmaceutical and toxicological laboratories is a testament to its usefulness.

Since the third edition was published in 2004, there have been about 120 new chemical entities brought onto the market. Some of these come from completely new chemical or pharmacological groups, but most are 'me too' drugs. In addition, there has been a growth of drugs misused in sport and those subject to abuse; eg legal cannabinoids and Mephedrone, and their derivatives. Nearly 400 monographs have been added to the fourth edition, with priority based on the importance of the substances covered in one of the following areas: drugs of abuse, forensic toxicology, hospital emergency toxicology, doping in sport, drugs subject to therapeutic drug monitoring and environmental toxicology. It has been impossible to include all the new drugs and poisons available, but work is continuing to add further data in the future.

The information in *Clarke* has been designed to provide methods and data to enable analysts to detect, identify, quantify and profile drugs and poisons in a wide variety of situations. In addition, information on how to interpret the analytical data is included, since this is often the most difficult part.

The book has been designed for use not only in hospital and toxicology laboratories, but also in numerous other analytical establishments. This includes quality control laboratories, and clinical laboratories engaged in drug investigations for purposes such as therapeutic drug monitoring or research into pharmacokinetics and patterns of drug metabolism. In addition, there is much information that will be of use in environmental toxicology, particularly the analysis of toxic metals and pesticides.

The needs of students studying analytical and forensic toxicology have not been forgotten and the chapters form an excellent basis for study. The spin-off book *Clarke's Analytical Forensic Toxicology* from the third edition of *Clarke* is a testament to meeting the requirements of the university teaching sector.

The book is in two volumes to make it easier to use. Volume 1 contains chapters comprising methodology and analytical techniques, and the subject index to both volumes; Volume 2 contains the analytical and toxicological data, indexes to the analytical data, a list of reagents and a repeat of the subject index to both volumes. Those who regularly use *Clarke* will be pleased to see that the original style and form of presentation of the information has been retained from the previous edition. This tried and tested format is clear, making it easy to find relevant information.

Clarke is now an established publication on MedicinesComplete, which provides online access to some of the world's leading drug and healthcare references. This includes such reference sources as *Martindale: The Complete Drug Reference*, *British National Formulary*, *The Merck Index* and *Stockley's Drug Interactions*. The online version of *Clarke* has the advantage that text searches can be performed thus aiding the reader to access relevant information more rapidly, either in *Clarke* alone or across multiple reference sources. Another advantage of the online version is that it can be updated online far more frequently and easily than the conventional book form.

Volume 1

Part 1: methodology and analytical chapters

This part now contains 44 chapters describing methodology and analytical techniques, which is an increase of 13 chapters from the previous edition. Three of the previous chapters have each been split into two because of the increased complexity of the topics covered. Thus there are now chapters on Driving Under the Influence of Alcohol as well as Driving Under the Influence of Drugs; Drugs in Human Sport as well as Drugs in Animal Sport; and Quality Control and Assessment in the Pharmaceutical Industry as well as Quality Control and Assessment in the Toxicology Laboratory. This latter chapter recognises the increase role of accreditation in the forensic toxicology laboratory and gives guidance on how to achieve this.

A new chapter on methodology in Drug-facilitated Sexual Assault has been included to recognise the rise in this type of crime and the need for good forensic toxicological analyses.

In terms of the use of particular analytical techniques, Method Development and Validation is a new chapter to assist those who need to develop their own methods and demonstrate that they are fit for purpose. Also included in this area are two new chapters on Sampling, Storage and Stability, as well as Extraction, since many toxicologists have asked for information on these topics. The increased use of liquid chromatography–mass spectrometry to replace gas chromatography–mass spectrometry in the analysis of organic compounds has been covered by a new chapter on this topic. Similarly, a new chapter on Atomic Absorption Spectroscopy, Inductively Coupled Plasma–Mass Spectrometry has been added to recognise the increased use of this combination of techniques in inorganic analysis.

Four new chapters have been included to assist the toxicologist to interpret analytical data and report the results in a meaningful and clear manner. The chapter on Measuring and Reporting Uncertainty is a clear exposition that all measurements are subject to error and gives guidance on how to measure and report the uncertainty. A chapter on Paediatric Forensic Toxicology recognises that children are not just small adults and need to be treated as a separate population. Similarly, the chapter on Pharmacogenomics clearly shows how we as individuals differ in our genetic makeup and how that might affect our response to drugs. Often one of the most difficult tasks a toxicologist has is to do is to interpret the results of the analyses; a new chapter on Interpretation of Results, together with the updated chapter on Pharmacokinetics, aims to assist toxicologists in this area. This backs up the information on interpretation given in each of the methodology chapters.

All the other chapters have been revised to bring them fully up to date.

The structure of the spectroscopic and chromatographic chapters has been retained from the previous edition to ensure that all the relevant information is given in an easy-to-read form. The chapter on emerging techniques has been completely rewritten to acknowledge the regulatory aspects of introducing new techniques and what new instrumentation might be available in the future.

The chromatographic and capillary electrophoresis systems have been extensively expanded and revised to include general screening systems as well as specialised systems for particular classes of drugs and poisons. The general systems for use have all been proven as robust

and reproducible over the years, and give excellent results for use in systematic toxicological analysis.

Subject index

The subject index covering both volumes can be found at the end of Volume 1.

Volume 2

Part 2: analytical and toxicological data

This part contains monographs for 2111 drugs and poisons, which is an increase of around 370 from the last edition. Not only have totally new monographs been introduced, but monographs from previous editions that were excluded from the third edition have been reinstated because the drugs concerned are still used in some parts of the world. The new additions have been chosen for drugs and poisons that are new and widely used prescription drugs, novel drugs of abuse or common poisons not previously included. For example, there are now 15 new monographs on metal salts. All the other monographs have been updated from the third edition. The use of the Recommended International Non-proprietary Name (rINN) for the drug name has been continued as this is now the international standard method of nomenclature.

The orientation of the chemical structures has been normalised so that the structures of similar compounds may be compared more easily. In addition, new chemical and analytical data have been added to aid the toxicologist and pharmaceutical analyst. This includes information on stability of drugs in solution and biological fluids at different temperatures, 1-chlorobutane extraction data, and infrared spectra of drug salts.

Analytical data for compounds on colour tests, thin-layer chromatography, gas chromatography and high performance liquid chromatography are given from which to choose systems that will separate and identify drugs, poisons and their metabolites. This is followed by full ultraviolet, infrared and mass spectral data together with listings of the major peaks to assist further in identifying compounds.

A major change has been made to the Quantification section of each monograph: it has been rearranged to give details of the analysis of each biological fluid or tissue separately instead of being ordered by technique. This makes finding an analytical method to use for a particular tissue very much easier. Additional data such as a method's limit of detection and limit of quantification have been added when available. This has meant a considerable increase in the size of each monograph and the list of the references at the end of the monograph, but it has improved the usability of the information. All the monographs have been brought up to date by the inclusion of new references and the deletion of old ones whenever possible. The references cited give further information on published methods for separating, identifying and quantifying drugs, poisons and their metabolites. Review articles are given whenever relevant to act as a further source of concise information.

The section entitled Disposition in the Body gives data on therapeutic concentration, toxicity, bioavailability, half-life, volume of distribution, clearance, distribution in blood, plasma : saliva ratio, protein binding and dose to enable analytical data to be interpreted in the context of a given case. In addition, abstracts from published clinical studies and case histories are included.

Part three: indexes to analytical data

This part contains indexes of analytical, chemical and therapeutic data, arranged in a similar order to how they appear in the monographs: CAS numbers, molecular formulae, therapeutic classes, molecular weights, melting points, colour tests, thin-layer chromatographic data, gas chromatographic data, high performance liquid chromatographic data, ultraviolet absorption maxima, infrared peaks, mass spectral data of drugs, and mass spectral data of pesticides. A list of reagents and proprietary test materials mentioned in the analytical procedures in Parts One and Two is also provided, as is a list of pharmacological terms.

Subject index

The subject index covering both volumes is repeated at the end of Volume 2.

Preparation of this edition

We are grateful to the editorial and production staff at Pharmaceutical Press who have helped in this project: Emma Burrows, Helen Carter, Tamsin Cousins, Amy Cruse, Simon Dunton, Marian Fenton, Rebecca Garner, Austin Gibbons, David Granger, Jo Humm, Jean Macpherson, Julie McGlashan, Louise McIndoe, Ithar Malik, Jason Norman, Karl Parsons, The Prescribers at The School of Pharmacy (London), Lucy White and John Wilson.

There were also the freelance staff who wrote and updated the monographs, to whom we owe thanks: Irene Chiwele, Mildred Davies, Laurent Y Galichet, Poppy McLaughlin, and Eva Reichardt. A team of copy-editors, proofreaders and indexers also contributed to the production of this publication.

Without the enthusiasm and dedication of these people this work would not have been published.

The Editorial Board members have also assisted in many ways: they authored, refereed manuscripts and monographs, and provided analytical data from their own laboratories. They and the authors have done a great job in providing up-to-date information in an easily accessible and readable manner.

A C Moffat
M D Osselton
B Widdop
J Watts
January 2011

General Notices

Health and Safety

This work is intended to be used by appropriately qualified and experienced scientists. Processes and tests described should be performed in suitable premises by personnel with adequate training and equipment. Care should be taken to ensure the safe handling of all chemical or biological materials, and particular attention should be given to the possible occurrence of allergy, infection, fire, explosion or poisoning (including inhalation of toxic vapours). Cautionary notes have been included in a number of monograph entries, but the possibility of danger should always be kept in mind when handling biological samples, and medicinal or other chemical substances.

Classification

At the head of each monograph, an indication is given of the classification of the compound according to its therapeutic or commercial use, its pharmacological action and/or its chemical group. The substance may, of course, have other uses or actions in addition to that stated.

Nomenclature

Monograph Titles

The main titles of the monographs are the Recommended International Non-Proprietary Names (rINNs), this includes both drugs and pesticides. For drugs of abuse, the most common chemical names or abbreviations have been used. It is worth noting that for rINNs and chemical nomenclature, it is now general policy to use 'f' for 'ph' (e.g. in sulpha), 't' for 'th' and 'i' for 'y'. For this reason, entries in alphabetical lists and indexes should be sought in alternative spellings if the expected spellings are not found.

The main title of a monograph is generally that of the free acid or base as this is the form in which the compound will usually be isolated in an analysis; details of the commonly available salts are included in subsidiary paragraphs within the monograph.

The following abbreviated names for radicals and groups are used in the titles.

Recommended name	*Chemical name*
acetonide	(isopropylidenedioxy)
aceturate	*N*-acetylglycinate
amsonate	4,4′-diaminostilbene-2,2′-disulfonate
besilate	benzenesulfonate
camsilate	camphorsulfonate
caproate	hexanoate
cipionate	cyclopentanepropionate
closilate	*p*-chlorbenzenesulfonate
edetate	ethylenediaminetetraacetate
edisilate	1,2-ethanedisulfonate
eglumine	*N*-ethylglucamine
embonate	4,4′-methylenebis (3-hydroxy-2-naphthoate) (=pamoate)
enantate	heptanoate
erbumine	*tert*-butylamine
esilate	ethane sulfonate
gluceptate	glucoheptonate
hibenzate	*o*-(4-hydroxybenzoyl)benzoate
isetionate	2-hydroxyethanesulfonate
lauril	*n*-dodecyl
laurilsulfate	*n*-dodecylsulfate
meglumine	*N*-methylglucamine
mesilate	methanesulfonate
metilsulfate	methylsulfate
mofetil	2-morpholinoethyl
napadisilate	1,5-naphthalenedisulfonate
napsilate	2-naphthalenesulfonate
octil	octyl
pivalate	trimethylacetate
steaglate	steroyl-glycolate
tebutate	*tert*-butylacetate
teoclate	8-chlorotheophyllinate
tosilate	*p*-toluenesulfonate
xinafoate	1-hydroxy-2-naphthoate

IUPAC Names

The nomenclature generally follows the definitive rules issued by IUPAC, 1993.

Proprietary Names and Synonyms

A selection of proprietary names have been included in the monographs. These can generally be applied to the UK, USA, Japan and a selection of African, Asian and European countries. Comprehensive lists of proprietary names worldwide, can be found in *Martindale: The Complete Drug Reference*, 37th edn, London, Pharmaceutical Press, 2011. Only single-substance preparations have been included except in the case of certain major classes of drugs for which the names of some compound preparations have been added. Some proprietary names that are not in current use have been retained. Names under the heading 'Synonyms' include alternative names, common titles, abbreviations and drug trial numbers.

CAS Registry Numbers

Chemical Abstract Service (CAS) registry numbers are provided, where available, in the monographs to assist readers to refer to other information databases.

Molecular Weights

Molecular weights have been calculated using the table of Atomic Weights as revised in 2001 by the Commission on Atomic Weights, IUPAC General Assembly, and based on the ^{12}C scale. Molecular weights have been corrected to one decimal place and are listed in ascending order in the index of Molecular Weights.

Physical Characteristics

Dissociation Constants

Numerous methods can be used for the determination of dissociation constants, and there are often differences in the various values reported in the scientific literature. The pK_a values given in the monographs have been taken from published data and should be regarded only as approximate. The temperature at which the determination was made is given where known.

Information on the theory, measurement and evaluation of dissociation constants is given in *The Pharmaceutical Codex*, 12th edn, London, Pharmaceutical Press, 1994.

Melting Points

The melting points recorded in the individual monographs are listed in ascending order in the index of Melting Points.

Partition Coefficients

Values for log *P* are given in a number of monographs. Where the pH of the aqueous phase is stated, the values given are apparent coefficients at that pH (not ion-corrected). Where no pH is stated for the aqueous phase, it can be assumed that log *P* is for the neutral form of the substance even though it is potentially ionisable.

The values given are approximates only but they serve to indicate the characteristics of the substance when it is submitted to an extraction process.

For a comprehensive collection of partition coefficients for drugs see C. Hansch *et al.*, *Exploring QSAR: Hydrophobic, Electronic and Steric Constants,* Washington, American Chemical Society, 1995. Information on the theory of partition coefficients can also be found in J. Sangster, *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*, New York, John Wiley, 1997.

Solubility

The solubilities given in the monographs, unless otherwise stated, apply at ordinary room temperature. They have been obtained from various sources and should not be regarded as precise because of variations depending on the method and condition of determination. In general, approximate values are given when a substance is soluble in less than1000 parts of solvent. Where no figure is given, the usual solubility terms have been adopted:

Very soluble	1 part in less than 1
Freely soluble	1 part in 1–10
Soluble	1 part in 10–30
Sparingly soluble	1 part in 30–100
Slightly soluble	1 part in 100–1000
Very slightly soluble	1 part in 1000–10000
Practically insoluble or insoluble	1 part in more than 10000

In the solubility statements, the word 'water' refers to purified water, the word 'ether' refers to diethyl ether and the word 'ethanol', without qualification, refers to ethanol (95%).

Temperature

Temperatures are expressed throughout the text in degrees Celsius (centigrade).

Analytical Data

All analytical data in the monographs apply to the form of the substance described in the main title of the monograph, unless otherwise specified.

In all lists or indexes of chromatographic data, a dash indicates that the value is not known, not that the substance does not elute.

Extraction

It has not been possible to give direct information on the best method for extracting individual substances from various biological samples. However, useful information can be gained from the data on solubility, dissociation constant and partition coefficient. The best solvent can be chosen by reference to solubility, the pH for extraction is indicated by the pK_a value, and the partition coefficient gives a quantitative measure of the phase volume ratios needed for a successful extraction from 1-chlorobutane.

Colour Tests

Where colour tests are given in the monographs, these names refer to the tests described in the Colour Tests chapter, where complete tables of colours are provided. Reference should be made to this chapter for an explanation of the system used for describing the colours. The reagents used for the colour tests are also listed within the list of reagents and additional colour reaction data for approximately 250 compounds is also presented. Colour tests applicable to biological fluids are described under the Hospital Toxicology chapter.

Thin-layer Chromatography

The thin-layer chromatographic systems referred to in the monographs are described in the TLC chapter on together with lists of data for drugs in important chemical and pharmacological classifications. General screening systems (systems TA to TF and systems TL, TAD, TAE, TAF, TAJ, TAK and TAL), which include over 1500 drugs and metabolites, are provided (see Chapter 39 for system details and references). In order to clarify the presentation of values, the data are expressed in terms of $R_f \times 100$ (hR_f). Complete lists of data, in ascending order, are given in the index of Thin-layer Chromatographic Data.

Gas Chromatography

The gas chromatographic systems referred to in the monographs are described in the GC chapter, together with lists of retention data for drugs in important chemical and pharmacological classifications. A general screening system (system GA), which includes over 1500 drugs and metabolites, is provided. An alternative screening system (system GB) is also included (see Chapter 40 for system details and references).

For most of the systems, the data are given in terms of Retention Index. Retention times or relative retention times are used in a few systems. Complete lists of retention data, in ascending order, are given in the indexes of Gas Chromatographic Data.

High Performance Liquid Chromatography

The HPLC systems referred to in the monographs are described in the HPLC chapter, together with lists of retention data for drugs in important chemical and pharmacological classifications. Six general screening systems (systems HA, HX, HY, HZ, HAA and HBK) covering between400 and 1600 drugs are provided (please note that values for system HBK have not been included within monographs and can only be found in the index) (see Chapter 41 for system details and references). The data are given in terms of Retention Index, retention time, relative retention time and column capacity ratio *k* (see Chapter 41). Complete lists of retention data, in ascending order, are given in the indexes of High Performance Liquid Chromatographic Data.

Ultraviolet Absorption

The wavelengths of principal and subsidiary peaks are recorded in each monograph for acid, alkaline and neutral solution, where available. These are generally listed from 230 nm.

Values in neutral solution are given for compounds for which values in acid or alkaline solution are not available or when the values in neutral solution differ significantly from those in acid or alkaline solution.

In many monographs, the ultraviolet spectrum is reproduced. In these spectra, the following notation is used:

———————	acid solution
.....	alkaline solution
- - - - - - - - - - - - - - -	neutral solution

Where more than one curve is shown, they do not necessarily relate to the same concentration and, consequently, points where the curves cross cannot be taken as true isosbestic points. The wavelengths of peaks in a few of the spectra may differ very slightly from those stated in the text. Where there is doubt, the values given in the text should be used. In monographs where the spectrum is reproduced, the A_1^1 value for each peak is stated, if available. The A values apply to the form of the substance described in the main title of the monograph, unless otherwise stated.

The A_1^1 values are divided into 3 categories in order to provide an indication of reliability:

- The letter 'a' after a figure indicates that the value is a mean value based on several reported figures, all of which lie within a range of ±10% of the mean.
- The letter 'b' after a figure indicates that the value is a single reported value of unknown reliability.
- The letter 'c' after a figure indicates that the value is a mean value based on several reported figures, some of which lie outside ±10% of the mean.

The phrase 'no significant absorption' indicates that no peaks are found at the concentrations normally used.

The A_1^1 values quoted in the monographs may be useful in identification, and may help in determining the strength of a solution which is required to obtain a curve within the instrumental range of absorption. They may also be useful to give an approximate indication of the amount of drug in a solution. However, because of instrumental differences and the possible effect of solvent and pH, A_1^1 values are subject to considerable variation and the values quoted should not be used when an accurate assay is required. In this case, a reference specimen should be examined at the same time as the sample.

The wavelengths of main peaks are listed for acid, alkaline and neutral solution from 230 nm in the index of Ultraviolet Absorption Data.

Infrared Absorption

The wavenumbers of the 6 major peaks in the range 2000–650 cm^{-1} (5–15 μm), in descending order or amplitude, are recorded in the monographs. In many cases, the infrared spectrum is also reproduced. When selecting the 6 principal peaks, those which are in the region where Nujol absorbs (1490–1320 cm^{-1}, 6.7–7.6 μm) have been omitted. Corrections for calibration errors have been applied where these are known.

The 6 principal peaks, in ascending order of the main peak, are listed in the index of Infrared Peaks.

Mass Spectrum

The *m/z* values of the 8 most abundant ions, in descending order of intensity, are included in many monographs. Where dashes occur in the listing, this indicates that less than eight ions have been observed.

The 8 principal ions, in ascending order of the main peak, are listed in the index of Mass Spectral Data of Drugs. A separate index for pesticides can also be found. The full mass spectra for the majority of the listed compounds are displayed within the monographs.

Quantification

The methods referred to in the references quoted under the heading 'Quantification' in the monographs are not intended to be recommended methods. These references are intended to be used as a guide to the literature on the particular subject.

Reagents

Reagents required for specific tests or methods are generally described fully in the appropriate place in the text. However, certain common reagents that are used throughout the book are described in the list of Reagents and Proprietary Test Materials. Reagent solutions are made in purified water unless otherwise specified. When ethanol, without qualification, is stated to be used, this refers to ethanol (95%).

Unless otherwise stated, solutions of solids in liquids are expressed as percentage w/v, and solutions of liquids in liquids as percentage v/v. When acids of various strengths are specified, e.g. 50% sulfuric acid, this implies the appropriate dilution by volume of the strong acid in water.

Disposition in the Body

Many of the monographs contain a section with the heading 'Disposition in the Body'. The information in these statements has been obtained from a detailed survey of published papers and other reference sources. Certain monographs have a single reference at the end of the statement, and this indicates that all the disposition information has been obtained from that source. Wherever possible, information is included on absorption, distribution, metabolism, excretion, therapeutic concentration, toxicity and pharmacokinetic parameters.

Entry to the literature is provided by the inclusion of abstracts of published papers on clinical studies or case histories. These abstracts include details of drug concentrations in plasma or other body fluids or tissues; in these data a dash means that the particular value was not determined, and ND or 0 means that the substance was not detected. Concentrations in body fluids or tissues are expressed in mg/L or μg/g.

In some monographs, the information is incomplete, the amount of detail being dependent upon that available in the literature searched. It should not be assumed that the statements presented reflect the only significant factors in the disposition of the drug concerned.

Therapeutic Concentration

This is the concentration range usually observed after therapeutic doses, as reported in clinical studies and other research projects. It should not be interpreted as the concentration range required for optimum therapeutic effects.

Toxicity

This statement may include drug concentrations in blood or other body fluids or tissues, which have been reported to be associated with toxic or lethal effects. Because of inter-subject variations or other variable factors, the reported toxic or lethal concentrations may occasionally lie close to or within the therapeutic range.

In some monographs, the toxic or lethal blood concentrations are stated in the form 60–89–150 mg/L. These figures have been obtained from a survey of a number of reported cases and represent the maximum concentrations found in 10, 50 and 90% of the subjects, respectively.

Maximum permitted concentrations in air (8–h exposure limit) are those recommended by the Health and Safety Executive in *Occupational Exposure Limits 2002*, Guidance Note EH40/2002 Supplement, London, HMSO, 2003.

Volume of Distribution

This relates to plasma concentrations after IV administration, unless otherwise stated. Values are based in a body-weight of 70 kg.

Clearance

This usually refers to the total plasma clearance (or total whole blood clearance) after IV administration. In some instances, the total clearance after an oral dose has been included if the drug is known to be well absorbed and is not subject to significant first-pass metabolism.

Numerous factors and inter-subject variations may affect the absorption, distribution, metabolism and excretion of drugs. These include age, sex and disease states such as renal impairment. In addition, results of analyses may be subject to unavoidable analytical inaccuracies. Consequently, there may be considerable variations in the observed drug concentrations and in values for pharmacokinetic parameters in individual cases. Hence, the values given in the monographs should be used only as a guide and should not be taken as absolute values.

Dose

The dose recorded under this heading in the monographs indicates the usual daily dose (oral unless otherwise stated) that may be administered for therapeutic purposes. It is intended solely as a guide in deciding whether the amount taken by an individual falls within the normal dosage range and should not be taken as a recommendation for treatment.

More detailed information on doses in different conditions and age groups may be found in *Martindale: The Complete Drug Reference*, 37th edn, London, Pharmaceutical Press, 2011; the *British National Formulary*, latest edition; or in the manufacturers' data sheets for the products.

Comments

This edition of *Clarke* could not have been completed without the comments on the second and the first editions, and the contribution of analytical data from many scientists involved in the analysis of drugs. In order to assist in the preparation of the next edition, the reader is invited to send any constructive comments and relevant new data concerning the analysis of drugs in biological materials to the Editor, Clarke's Analysis of Drugs and Poisons, Royal Pharmaceutical Society of Great Britain, 1 Lambeth High Street, London SE1 7JN, UK. In this way, future editions will be improved to the benefit of all of those who use it.

Deletions

The following substances which were included in Volumes 1 and 2* of the 1st and 2nd editions are now included in this edition:

Acetyldihydrocodeine
Adrenalone
Allantoin
Allylprodine
Alphameprodine
Alphamethadol
Aminometradine
Aminopentamide
Amisometradine
Amolanone
Amopyroquine
*Amotriphene
Amprotropine
Amydricaine
Amylocaine
Apoatropine
Azacosterol
Azamethonium Bromide
Benzalkonium Bromide
Benzamine
Benzathine Penicillin
Benzethidine
Betameprodine
Betaprodine
*Brocresine
Butallylonal
Butethamine
Butoxamine
Cetoxime
Chlorisondamine Chloride
Citronella Oil
Clamoxyquin
Clonitazene
*Cloponone
Codeine *N*-Oxide
Cyclamic Acid
Cyprenorphine
Demecolcine
Demeton-O
Desomorphine
Diampromide
Dibutoline Sulphate
*Diethylaminoethyl Diphenylpropionate
Dimenoxadole
*Dimethocaine
Dimethylthiambutene
*Dimophebumine
Dioxaphetyl Butyrate
Dioxathion
*Dioxyamidopyrine
*Diphenazoline
Dithiazanine Iodide
Embramine
Erythrityl Tetranitrate
*Ethylisobutrazine
Ethylmethylthiambutene
*Ethylpiperidyl Benzilate
Etonitazene
Etoxeridine
Etymide
Fenimide
*Fenmetramide
Furethidine
Hydromorphinol
Hydroxypethidine
*Imidocarb
*Iminodimethylphenylthiazolidine
*Iopydol
*Iopydone
Isobutyl Aminobenzoate
*Isometamidium
Isomethadone
Laudexium Methylsulphate
Leucinocaine
Levomethorphan
Levomoramide
Levophenacylmorphan
Lucanthone
Metabutethamine
Metabutoxycaine
Metazocine
*Methadone Intermediate
Methaphenilene
Methoxypromazine
Methylaminoheptane
Methyldesorphine
Methyldihydromorphine
Methylhexaneamine
Methyridine
Metofoline
Metopon
*Moramide Intermediate
Morpheridine
Morphine *N*-Oxide
Mustine
Myrophine
Naepaine
*Naftazone
Narcobarbital
Nicocodine
Nicomorphine
*Nifuroxime
Noracymethadol
Norbutrine
*Nordefrin
*Norgestrel
Norlevorphanol
*Octacaine
Octaverine
Orthocaine
Pamaquin
*Panidazole
*Paromomycin
Pentaquin
*Pethidine Intermediate A
Phenadoxone
Phenamidine
Phenampromide
*Phenatine
Phenisonone
Phenomorphan
Phenoxypropazine
Phenylpropylmethylamine
*Phthivazid
*Picloxydine
Pipamazine
Piperoxan
Pipethanate
Plasmocide
*Proadifen
Probarbital
Proheptazine
Properidine
Pulegium Oil
*Pyrrocaine
Quinapyramine Chloride
Racemethorphan
Racemoramide
*Resorantel
*Rifamide
*Rolicypram
Stilbamidine
Sulphasomizole
Sulphonal
*Taurolin
Teclothiazide
*Terodiline
*Tetracosactrin
*Tetraethylammonium Bromide
*Thozalinone
Thurfyl Nicotinate
*Tiletamine
Tolonium Chloride
Tolycaine
Triclobisonium Chloridc
Tropacocaine
Tropine
Tymazoline
Viomycin
Xenysalate

Abbreviations

A_1^1	Specific absorbance (abbreviation of $A_{1cm}^{1\%}$)
AAFS	American Academy of Forensic Sciences
AAS	Anabolic/androgenic steroids; atomic absorption spectrometry
4-ABA	4-Aminobenzoyl-β-alanine
ABFT	American Board of Forensic Toxicology
ABP	2-(2-Amino-5-bromobenzoyl)pyridine
ABV	Alcohol percentage by volume
AC	Acetylated
2-ACB	2-Amino-5-nitrobenzophenone
2-ACDP	2-Amino-5-chlorodiphenylamine
ACDP	2-Amino-2′-chloro-5-nitrobenzophenone; 2-Amino-5,2′-dichlorobenzophenone
ACE	Angiotensin-converting enzyme
ACFP	2-Amino-5-chloro-2′-fluorobenzophenone
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACNB	2-Amino-2′-chloro-5-nitrobenzophenone
ACPO	Association of Chief Police Officers
AD	Alzheimer's disease
ADC	Analogue-to-digital converter
ADCB	2-Amino-5,2′-dichlorobenzophenone
ADH	Alcohol dehydrogenase
ADHD	Attention deficit hyperactivity disorder
ADI	Acceptable daily intake
AED	Atomic emission detector
AEME	Anhydroecgonine methylester
AES	Atomic emission spectrometry
AFID	Alkali flame ionisation detection
AFM	Atomic force microscopy/microscope
AFMAB	5-Amino-2′-fluoro-2-methylaminobenzophenone
AFNB	2-Amino-2′-fluoro-5-nitrobenzophenone
AFS	Atomic fluorescence spectrometry
agg.	aggregate (in botanical names), including two or more species which resemble each other closely
AGP	α_1-Acid glycoprotein
AIDS	acquired immunodeficiency syndrome
ALDH	Aldehyde dehydrogenase
ALL	Acute lymphoblastic leukemia
ALS	Amyloid lateral sclerosis
ALT	Alanine transaminase (alanine aminotransferase)
6-AM	6-Acetylmorphine
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	AMP-activated protein kinase
AMT	α-Methyltryptamine
amu	Atomic mass units
ANB	2-Amino-5-nitrobenzophenone
AO	Aldehyde oxidase
AOAC	Association of Analytical Chemists
AORC	Association of Official Racing Chemists
APB	3-Amino-1-phenylbutane
APC	7-Ethyl-10-[4[*N*-(5aminopentanoic acid)-1-piperidino]-carbonyloxycamphothecin
APCI	Atmospheric Pressure Chemical Ionisation
APDC	Ammonium pyrrolidine dithiocarbamate
APEI	Atmospheric pressure electrospray ionisation
API	Atmospheric pressure ionisation; active pharmaceutical ingredients
APL	Acute promyelocytic leukaemia
APT	Attached proton test
AR	Analytical reagent
Art	artefact
5-ASA	5-Aminosalicyclic acid
ASL	Average signal level
ASP	Amnestic Shellfish Poisoning
AsPEX	Allele-specific primer extension
AST	Aspartate transminase (aspartate aminotransferase)
ASTM	American Society for Testing and Materials
ASV	Anodic stripping voltametry
ATD	Automated thermal desorption
ATR	Attenuated total reflectance
AUC	Area under the curve
AUFS	Absorbance units full scale
AV	Atrioventricular
BAC	Blood alcohol concentration
BBA	Butyl boronic acid
BBR	Blood-to-breath ratio
BC	Background correction
BCRP	Breast cancer resistance protein
1,4-BD	1,4-Butanediol
BDB	3,4-Benzodioxazol butanamine
BDMPEA	4-Bromo-2,5-dimethoxyphenethylamine
BE	Benzoylecgonine
BEN	Balkan endemic neuropathy
BGE	Background electrolyte
bid	Twice daily
BMAA	β-*N*-Methylamino-L-alanine
BMC	4-Bromomethyl-7-methoxycoumarin
BMI	Body mass index
BNCT	Boron neutron capture therapy
BOAA	β-I-Oxalylamino-L-alanine
BP	Blood pressure; Bristish Pharmacopoeia; butyrylated; benzophenone
Bp	Boiling point
B.P.	British Pharmacopoeia
BPH	Benign prostatic hyperplasia
BrAC	Breath alcohol concentration
BRP	Biological reference preparation
BSA	Bovine serum albumin; body surface area
BSH	Mercaptoundecahydrododecaborate
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
BuChE	Butyrylcholinesterase
BUN	Blood urea nitrogen
BZP	*N*-Benzylpiperazine
CA	Carbonic anyhdrase
CAM	Base-modified PEG
CAP	College of American Pathologists
CAS	Chemical Abstracts Service
2-CB	4-Bromo-2,5-dimethoxyphenethylamine
CBD	Cannabidiol
CBN	Cannabinol
CBQCA	3-(4-Carboxy-benzoyl)-2-quinoline carboxaldehyde

CCD	Charge-coupled device
CD	Circular dichroism
2C-D	2,5-Dimethoxy-4-methyl-β-phenethylamine
CDT	Carbohydrate-deficient transferrin
CE	Capilary electrophoresis
CEC	Capillary electrophoresis; collision energy
2C-E	2,5-Dimethoxy-4-ethyl-β-phenethylamine
CEDIA	Cloned enzyme donor immunoassay
CFP	Ciguatera fish poisoning
CFTB	5-Chloro-2′-fluoro-2-(2,2,2-trifluroethylamino)-benzophenone
CG	Chorionic gonadotrophin
CGE	Capillary gel electrophoresis
cGMP	Cyclic GMP; current good (pharmaceutical) manufacturing practice
CHE	Cholinesterase
ChE	Cholinesterase
CHF	Congestive heart failure
-CHNO	Descarbamoyl artefact
CI	Chemical ionisation
2C-I	2,5-Dimethoxy-4-iodo-β-phenethylamine
CIA	Chemiluminescent immunoassay, capillary ion analysis
CID	Collision induced dissociation
CIEF	Capillary isoelectric focusing
CIn	Colour index
CIRMS	Combustion isotope ratio MS
CITP	Capillary isotachophoresis
CL	Clearance
Cl	Clearance
CL_{CR}	Creatinine clearance
C_{max}	Mean maximum plasma concentration
CMC	Critical micelle concentration
CNS	Central nervous system
$-CO_2$	Artefact formed by decarboxylation
COHb	Carboxyhaemoglobin
COMT	Catechol-*O*-methyltransferase
COPD	Chronic obstructive pulmonary disease
COSY	Correlation spectroscopy
COX	Cyclooxygenase
CPMACB	2-Cyclopropylmethylamino-5-chlorobenzophenone
CRA	Controlled Substances Act
CRS	Chemical reference substance
CSEI	Cation selective exhaustive injection
CSF	Cerebrospinal fluid
CSP	Chiral stationary phase
CT	Computed tomography
2C-T-2	2,5-Dimethoxy-4-ethylthio-β-phenethylamine
2C-T-7	2,5-Dimethoxy-4-propylthio β-phenethylamine
CTAB	Cetyl trimethyl ammonium bromide
CTFEAB	5-Chloro-2-(2,2,2-trifluoro)-ethylaminobenzophenone
CTX	Ciguatoxin
CV	Coefficient of variation
CVAA	2-Chlorovinylarsenous acid
CVAO	2-Chlorovinyl arsenous oxide
CVVHDF	Continuous veno-venous haemodiafiltration
CYP	Cytochrome P450
CZE	Capillary zone electrophoresis
2,4-D	2,4-Dichlorophenoxyacetic acid
DA	Dialkylated
DAB	2,5-Diaminobenzophenone
DACB	2,5-Diamino-2′-chlorobenzophenone
DAD	Diode array detection/detector
DAFB	2,5-Diamino-2′-fluorobenzophenone
DART	Direct analysis in real time
DBD	3,4-Benzodioxazol butanamine
DBQ	2,6-Dibromoquinone-4-chlorimide
DBZ	Dibenzosuberamine
DC	Direct current
DCCA	(3(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid
DCMAB	2′,5-Dichloro-2-(methylamino) benzophenone
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDS	Drug detection system
DEA	Drug Enforcement Agency
DEACFB	2-Diethylaminoethylamino-5-chloro-2′-fluorobenzophenone
DECP	Drug Evaluation and Classification Program
DEG	Diethylene glycol
dEPO	Darbepoietin
DEPT	Distortionless enhancement by polarisation transfer
DESI	Desorption electrospray ionisation
DFA	Drug-facilitated assault
DFSA	Drug-facilitated sexual assault
DHEA	Dehydroepiandrosterone
DHHS	Department of Health and Human Services
dH_2O	Distilled water
DHPLC	Denaturing HPLC
DIPT	Diisopropyltryptamine
DLLME	Dispersive liquid–liquid microextraction
DLS	Dynamic light scattering
DMA	2,5-Dimethoxyamfetamine
p-DMAB	*p*-Dimethylaminobenzaldehyde
DME	Dimethyl ether
DMES	Dimethylethylsilyl
DMF	Dimethylformamide
DMS	Differential mobility spectrometry
DMSA	Dimercaptosuccinic acid
DMSO	Dimethylsulfoxide
DNOC	Dinitro-*o*-cresol
DNS-Cl	Dansyl chloride
DOB	4-Bromo-2,5-dimethoxyamfetamine
DOD	(US) Department of Defense
DOM	2,5-Dimethoxy-4-methylamfetamine
DON	Deoxynivalenol
DOT	(US) Department of Transport
DPA	Diphenylamine
DPASV	Differential pulse anodic stripping voltametry
DPI	Dry powder inhalation/inhaler
DPV	Differential pulse voltametry
DQ	Design qualification
DRESS	Drug rash with eosinophilia and systemic symptoms
DRIFT	Diffuse reflectance IR Fourier transform spectroscopy
DSHEA	Dietary Supplement and Health Education Act
DSP	Diarrhetic shellfish poisoning
DTAB	Dodecyl trimethyl ammonium bromide
DUI	Driving under the influence
DUIA	Driving under the influence of alcohol
DUID	Driving under the influence of drugs
DVT	Deep vein thrombosis
DWI	Driving while intoxicated/impaired
EA	Enzyme acceptor
EAAS	Electrothermal AAS
EC	Electrochemical
ECD	Electron capture detection
ECG	Electrocardiogram
ECM	Enteric coated microcapsules
ECT	Electrical capacitance tomography
ED	Erectile dysfunction; enzyme donor
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EDT	1,2-Ethanedithiol

EDTA	Ethylene diamine tetra-acetate
EDXRF	Energy-dispersive XRF
EEG	Electroencephalogram
EI	Electron Impact
EIA	Enzyme immunoassay
ELCD	Electrolytic conductivity detection
ELF	Epithelial lining fluid
ELISA	Enzyme-linked immunosorbent assay
ELS	Evaporative light-scattering
EMC	Erythromycylamine
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EME	Ecgonine methyl ester
EMEA	European Agency for the Evaluation of Medicinal Products
EMIT	Enzyme-multiplied immunoassay technique
EMPA	Ethyl methylphosphonic acid
ENFSI	European Network of Forensic Science Institutes
EOF	Electroosmotic flow
EPBRP	European Pharmacopoeia biological reference preparations
EPI	Enhanced product ion
EPO	Erythropoietin
ESA	Electrostatic analyser
EQA	External quality assurance/assessment
ESI	Electrospray ionisation
ET	Ethylated
ETAAS	Electrothermal atomic absorption spectrometry
EtG	Ethyl glucuronide
EtS	Ethyl sulfate
ETV	Electrothermal vaporisation
EU	European Union
eV	Electron volts
EWDTS	European Workplace Drug Testing Society
FAAS	Flame atomic absorption spectrometry
FAB	Fast atom bombardment
FAEE	Fatty acid ethyl esters
FAIMS	Field asymmetric waveform ion mobility spectrometry
FAME	Fatty acid methyl ester
FASS	Field-amplification sample stacking
FDA	Food and Drug Administration
FEI	Federation Equestre Internationale
FFAP	Acid-modified PEG
FFT	Fast Fourier transform
fg	Femtograms
FIA	Flow injection analysis; fluorescent immunoassay
FID	Flame ionisation detection; free-induction decay (NMR)
FISH	Fluorescence in-situ hybridisation
Fp	Freezing point
FPBA	4-Fluoro-3-phenoxybenzoic acid
FPD	Flame photometric detector
FPIA	Fluorescence polarisation immunoassay
FPLC	Fast protein liquid chromatography
FPN	Ferric(III) chloride-perchloric acid-nitric acid
ft	Foot (feet)
FSH	Follicle stimulating hormone
FT	Fourier transform
FTD	Flame thermionic detection
FTIR	Fourier transform infrared
FTIRD	Fourier transform infrared detector
GABA	γ-Aminobutyric acid
GBL	γ-Butyrolactone
GC	Gas chromatography
GC-HRMS	High resolution mass spectrometry
GC-MS(-MS)	Tandem GC-MS
GCS	Glasgow Coma Scale
G-CSF	Granulocyte colony-stimulating factor
GFAAS	Graphite furnace atomic absorption spectrometry; electrothermal atomic absorption spectrometry
GFR	Glomerular filtration rate
GH	Growth hormone
GHB	γ-Hydroxybutyric acid
GI	Gastrointestinal
GLC	Gas-liquid chromatography
GLP	Good laboratory practice
GMND	Guamanian motor neuron disease
GMP	Good manufacturing practice
G6PDH	Glucose-6-phosphate dehydrogenase
GPS	Genomic prescribing system
GRM	Gastric release microcapsules
GSR	Gunshot residue
GTX	Gonyautoxins
h	Hour(s)
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
hCG	Human chorionic gonadotrophin
HCL	Hollow cathode lamp
-HCl	Artefact formed by the elimination of hydrochloric acid
-HCN	Artefact formed by the elimination of hydrogen cyanide
HCV	Hepatitis C virus
HD	3β-Hydrosteroid dehydrogenase
HDO	Mustard sulfoxide
HDO_2	Mustard sulfone
HEACFB	2-Hydroxyethylamino-5-chloro-2′-fluorobenzophenone
HEPES	*N*-(2-Hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid
HERG	Human ether-a-go-go-related gene
HFB	Heptaflurobutyrate
HFBA	Heptaflurobutyric anhydride
HGN	Horizontal gaze nystagmus
HHD	2-Chloro-2-hydroxyethyl sulfoxide
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMBC	Heteronuclear multiple bond correlation
HMMC	4-Hydroxy-3-methoxymethcathinone
HMQC	Heteronuclear multiple quantum coherence
HMT	Hexamethylenetetramine
$-H_2O$	Artefact formed by dehydration of an alcohol or by rearrangement of an amino oxo compound
HOM	Humic organic matter
HPLC	High performance liquid chromatography
HR	Heart rate
HR-MS	High resolution mass spectrometry
HS	Headspace
HS-GC	Headspace gas chromatography
HSQC	Heteronuclear single quantum coherence
-HY	Acid-hydrolysed/acid hydrolysis
I	Spin quantum number
IA	Immunoassay
IBS	Irritable bowel syndrome
ICADTS	International Council on Alcohol, Drugs and Traffic Safety
ICH	International Conference on Harmonisation
ICP	Inductively coupled plasma
ICR	Ion cyclotron resonance
ICRAV	International Conference of Racing Analysts and Veterinarians
ID	Isotope dilution
i.d.	Internal diameter
IDA	Information dependent acquisition
IDLH	Immediately dangerous to life
IEC	Ion exchange chromatography

IFHA	International Federation of Horseracing Authorities
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor-1
ILAC	International Laboratory Accreditation Co-operation
IM	Intramuscular
IMPA	Isopropylmethylphosphonic acid
IMS	Ion mobility spectrometry
INAA	Instrumental neutron activation analysis
INR	International normalised ratio
IOC	International Olympic Committee
IP	Identification points/intraperitoneal
IQC	Internal quality control
IR	Infrared
IRMA	Immunoradiometric assay
IRMS	Isotope ratio mass spectrometry
IS	Internal standard
ISE	Ion selective electrode
IT	Ion trap
IU	International unit
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
J	Indirect spin coupling
JRES	*J* resolved experiment
k	Column capacity ratio
K-EDTA	Potassium ethylenediamine tetraacetic acid
KIMS	Kinetic interaction of microparticles in solution
LA	Laser ablation
LAAM	Levomethadyl acetate
LAL	Limulus amoebocyte lysate test
λ	Wavelength
LAMPA	Lysergic acid *N*-(methylpropyl) amide
LC	Liquid chromatography
LC-MS(-MS)	Tandem LC-MS
LCTF	Liquid crystal tuneable filter
LD_{50}	Lethal dose to 50% of a population
LFA	Lateral flow assay
LH	Luteinising hormone
LIF	Laser or light induced fluorescence
LLE	Liquid–liquid extraction
LLOQ	Lower limit of quantification
ln	Logarithm to the base e (natural logarithm)
LOCI	Luminescent oxygen channeling immunoassay
LOD	Limit of detection
log	Logarithm to the base 10
LOQ	Limit of quantification
LPG	Liquified petroleum gas
LSD	Lysergic acid diethylamide; lysergide
LTFS	Low temperature fluorescence spectroscopy
M	Molar (moles per L)
M (COOH-)	Carboxy metabolite
M (nor-)	*N*-Desmethyl metabolite
M (OH-)	Hydroxy metabolite
M (ring)	Ring compound as metabolite
MACB	2-Methylamino-5-chlorobenzophenone
MACDP	2-Methylamino-5-chlorodiphenylamine
MALDI	Matrix assisted laser desorption and ionisation
6-MAM	6-Monoacetyl morphine
MANFB	2-Methylamino-5-nitro-2′-fluorobenzophenone
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MAS	Magic-angle-spinning
mAU	Milli-absorbance units
MBA	Methyl boronic acid
MBDB	*N*-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine
2,3-MBDB	Methyl-2,3-benzodioxazol butanamine
MBTFA	*N*-Methylbis(trifluoroacetamide)
MCF	(1*R*,2*S*,5*R*)-(–)-Menthylchloroformate
MCPA	Methylchlorophenoxy acetic acid
MCPA-CoA	Methylenecyclopropylacetyl-coenzyme-A
MCPP	2-(2-Methyl-4-chlorophenoxy)propionic acid
*m*CPP	1-(-3-Chlorophenyl)piperazine
MDA	Methylenedioxyamfetamine
MDE	Methylenedioxyethamfetamine
MDEA	Methylenedioxyethylamfetamine
MDI	Metered-dose inhalers
MDMA	3,4-Methylenedioxymetamfetamine
MDP2P	1-(3,4-Methylenedioxyphenyl)-2-propanone
MDPPP	3,4-Methylenedioxy-α-pyrrolidinopropiophenone
Me	Methyl
MECC (or MEKC)	Micellar electrokinetic capillary chromatography
MECK	Micellar electrokinetic chromatography
MEKC (or MECC)	Micellar electrokinetic capillary chromatography
MEL	Maximum exposure limit
mEq	Milliequivalent(s)
µg	Microgram(s)
µm	Micrometer(s)
MFD	Mass fragmentographic detection
MGF	Mechano growth factor
µM	Micrometre(s)
MHRA	Medicines and Healthcare Products Regulatory Agency
MIBK	Methyl isobutyl ketone
MID	Multiple ion detector
Min	Minute
MLR	Multiwavelength linear regression
MLS	Multi-angle light scattering
MMA	Multi-angle light scattering; 2-methoxy-metamfetamine
MMDA	3,4-Methylenedioxy-5-methoxyamfetamine
MMDBB	2,3-Dimethylbenzodioxazolbutanamine
6-MNA	6-Methoxy-2-naphthyl acetic acid
MND	Motor neurone disease, mono-*N*-dealkyldisopyramide
mol	Mole
8-MOP	8-Methoxypsoralen
MOPPP	4-Methoxy-α-pyrrolidinopropiophenone
MO/TMS	Methoxime/trimethylsilyl
Mp	Melting point
MPA	Methylphosphonic acid; *N*,*N*-dimethyl-*p*-phenylenediamine hydrochloride
MPHP	4′ Methyl-α-pyrrolidinohexanophenone
MPPP	4-Methyl-α-pyrrolidinopropiophenone
MQL	Minimal quantifiable limit
M_r	Relative molecular mass
MR	Metabolic ratio
MRI	Magnetic resonance imaging
MRL	Maximum residue limits
MRM	Multiple reaction monitoring
MRO	Medical Review Officer
MRPL	Minimum required performance level
MRS	Magnetic resonance spectroscopy
MS	Mass spectrometry
MSC	Multiplicative scatter correction
MSTFA	*N*-Methyltrimethylsilyltrifluoroacetamide
MTA	4-Methylthioamfetamine
MTSS	Merck tox screening system
m/z	Mass to charge ratio
NA	Numerical aperture
NAA	Neutron activation analysis
NACE	Non-aqueous capillary electrophoresis
NAD	Nicotinamide–adenine dinucleotide
NAPA	Acecainide; *N*-acetylprocainamide
NAPQI	*N*-Acetyl-*p*-benzoquinoneimine

NAT2	*N*-Acetyltransferase 2
NBD-F	4-Fluoro-7-nitro-2,1,3-benzoxadiazole
NBP	4-(4-Nitrobenzyl)pyridine
NCE	New chemical entity
NCI	Negative chemical ionisation
NC-SPE	Non-conditioned SPE
ND	Nordiazepam
NDPX	Norpropoxyphene
ng	Nanogram(s)
$-NH_3$	Artefact formed by elimination of ammonia
NHTSA	(US) National Highway Traffic Safety Administration
NIAPCI	Negative ion atmospheric pressure chemical ionisation
NICI	Negative ion chemical ionisation
NIDA	National Institute for Drug Abuse
NIR	Near-infrared imaging
NIST	National Institute of Standards and Technology
NLCP	National Laboratory Certification Program
nm	Nanometer(s)
NMDA	*N*-Methyl-D-aspartate
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOE	Nuclear Overhauser enhancement
NOESY	Nuclear Overhauser enhancement spectroscopy
NPC	Normal phase chromatography
NPD	Nitrogen phosphorus detection
NRC	Nuclear Regulatory Commission
NRG-1	Naphthylpyrovalerone (naphyrone)
NSAI	Non-steroidal anti-inflammatory
NSAID	Nonsteroidal antiinflammatory drug
NSD	Nitrogen specific detector
NSP	Neurotoxic shellfish poisoning
OAB	Overactive bladder
$OATPT_2$	Organic anion transporting polypeptide 2
OC	Oesophageal cancer
ODS	Octadecylsilane
OECD	Organization for Economic Development and Cooperation
OES	Occupational exposure standard
OF	Oral fluid
OOS	Out-of-specification
OTA	Ochratoxin-A
P	Apparent partition coefficient
PAD	Peripheral arterial disease
PAGE	Polyacrylamide gel electrophoresis
PBMS	Particle beam MS
PC	Precipitation chromatography; principle component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCC	Pyridinium chlorochromate
PCEEA	*N*-(1-Phenylcyclohexyl)-2-ethoxyethenamine
PCEPA	*N*-(1-Phenylcyclohexyl)-3-ethoxypropanamine
PCMEA	N-(1-Phenylcyclohexyl)-2-methoxyethenamine
PCP	Phencyclidine
PCPR	*N*-(1-Phenylcyclohexyl)-propanamine
PCR	Polymerase chain reaction; principal component regression
PD	Pulsed discharge
PDA	Photodiode array
PDHID	Pulsed discharge helium ionisation detector
PDT	1,3-Propanedithiol
PEEK	Polyether etherketone
PEG	Polyethylene glycol
PEL	Permissible exposure limit
PFB	Pentafluorobenzoyl
PFK	Perfluorokerosene
PFP	Pentafluoropropionate; puffer fish poisoning
-PFP	pentafluoropropionylated
PFPA	Pentafluropropionic anhydride
PFTBA	Perfluorotributylamine
pg	Picogram(s)
pGp	*p*-Glycoprotein
PGRN	Pharmacogenetics Research Network
PGx	Pharmacogenomics
PH	Permethylated hydroxypropyl
PHA	4-Hydroxyamfetamine
PhAsO	Phenylarsine oxide
Ph. Eur.	European Pharmacopoeia
Ph. Int	International Pharmacopoeia
P*I*	PH of the isoelectric point of a protein
PIAPCI	Positive ion atmospheric pressure chemical ionisation
PICI	Positive ion chemical ionisation
PID	Photoionisation detection
PIFAB	Positive ion fast atom bombardment
PIS	Product ion spectrum
PJ	Personalised justice
pK_a	Negative logarithm of the dissociation constant
PLA	Phospholipase A
PLOT	Porous layer open tubular
PLS	Partial least-squares
PLSR	Partial least-squares regression
PM	Permethylated; personalised medicine
PMA	4-Methoxyamfetamine
PMEA	4-Methoxyethylamfetamine
PMMA	4-Methoxy-methamfetamine
PMN	Polymorphonuclear leukocytes
PMPA	Pinacoylmethylphosphonic acid
PN	Propionylated
PO	*per os* (oral)
p.o.	Per oral
P-III-P	Procollagen type III
PPARδ	Peroxisome proliferator activated receptor δ
ppb	Part(s) per billion
PPC	4-Phenyl-4 piperidinocyclohexanol
PPD	*p*-Phenylenediamine
ppm	Part(s) per million
PPP	α-Pyrrolidinopropiophenone
PQ	Performance qualification
PRP	Polyribosylribitol phosphate
PSI	Pre-column separating inlet
PSP	Paralytic shellfish poisoning
PSX	Polysiloxane
PtE	Phosphatidylethanol
PTFE	Polytetrafluoroethylene
PTV	Temperature-programmed sample inlet, programmable temperature vaporising
PVP	Poly(vinylpyrrolidone)
QA	Quality assurance
QC	Quality control
qPCR	Quantitative PCR
QQQ	Triple quadrupoles
QTOF	Quadrupole TOF
RCI	Racing Commissioners International
r.d.	Relative density
rDNA	Recombinant DNA
rf	Radio frequency
RFLP	Restriction fragment length polymorphism
rhEPO	Recombinant human erythropoietin
rhGH	Recombinant human growth hormone
RI	Retention index
RIA	Radioimmunoassay
RMTC	Racing Medication and Testing Consortium
RNA	Ribonucleic acid
RPC	Reversed-phase chromatography
RRT	Relative retention time

RSD	Relative standard deviation
RSS	Root sum square
RT	Retention time
s	second(s)
SAMHSA	Substance Abuse and Mental Health Services Administration
SARM	Selective androgen receptor modulator
SBW	Spectral band width
SC	Subcutaneous
SCF	Supercritical fluid
SCFC	Supercritical fluid chromatography
SCOT	Support-coated open tubular
SDS	Sodium dodecyl sulfate; standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	Standard error of calibration; size exclusion chromatography
SEP	Standard error of prediction
SERM	Selective estrogen receptor modulator
SFE	Supercritical fluid extraction
SFST	Standardised field sobriety test
SHGB	Sex hormone binding globulin
SI	Système international d'unités
SID	Surface ionisation detection
SIM	Selected ion monitoring
SIMCA	Soft independent modelling of class analogies
SIR	Selected ion recording
SMAP	2-Sulfamoylacetylphenol
SNAP-25	Synaptosome-associated protein of 25,000 daltons
SNARE	Acronym derived from "soluble NSF attachment receptor"
SNP	Single nucleotide polymorphism
SNPA	*N*-Succinimidyl-*p*-nitrophenylacetate
SNR	Signal-to-noise ratio
SNV	Standard normal variate
SOFT	Society of Forensic Toxicologists
$-SO_2NH$	Artefact formed by elimination of the sulfonamide group
SOP	Standard operating procedure
SORS	Spatially offset Raman spectroscopy
sp.	Species (plural spp.)
sp.gr.	Specific gravity
SPE	Solid-phase extraction
SPME	Solid phase microextraction
SPR	Surface plasmon resonance
SRM	Selected reaction monitoring; standard reference materials
SSI	Sonic spray ionisation
SSNMR	Solid-state NMR
SSRI	Selective serotonin reuptake inhibitor
STA	Systematic toxicological analysis
STIP	Systematic toxicological identification procedure
STOCSY	Statistical TOCSY
STP	2,5-Dimethoxy-4-methylamfetamine; short tandem repeat
STR	Short tandem repeat
STX	Saxitoxin
SVT	Supraventricular tachycardia
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
$t_{1/2}$	Half-life
T_1	Spin-lattice or longitudinal relaxation time
T_2	Spin–spin or transverse relaxation time
TBA	Tetrabutyl ammonium hydrogen sulfate
TBAF	Tetrabutyl ammonium fluoride
TBAH	Tetrabutylammonium hydroxide
TBDM	Tert-butyldimethylsilyl
TBPE	Tetrabromophenolphthalein ethyl ester
TBSA	Total body surface area
TBW	Total body water
TCA	Tricyclic antidepressant
TCD	Thermal conductivity detector
TCM	Traditional Chinese medicine
TCP	3,5,6-Trichloro-2-pyridinol
TCRC	Time-coupled time-resolved chromatography
TDGO	Thiodiglycol sulfoxide
$TDGO_2$	Thiodyglycol sulfone
TDI	Tolerable daily intake
TDM	Therapeutic drug monitoring
TdP	torsades des pointes
TEA	Triethylamine
Tf	Transferrin
-TFA	Trifluoroacetylated
TFAA	Trifluroacetic anhydride
TFMPP	1-(3-Trifluoromethylphenyl)piperazine
TFPI	Tissue factor pathway inhibitor
TGS	Triglycine sulfate
THA	Tetrahexylammonium hydrogensulfate
THC	Tetrahydrocannabinol
THCA	11-Carboxytetrahydrocannabinol
THC-COOH	Tetrahydrocannabinol-11-oic-acid
THEED	Tetrahydroxyethylene diamine
THF	Tetrahydrofuran
TIAFT	The International Association of Forensic Toxicologists
TIC	Total ion current
TID	Thermionic detection
TIS	Turbo ion spray
TLC	Thin-layer chromatography
2,3,5-TMA	2,3,5-Trimethoxyamfetamine
3,4,5-TMA	3,4,5-Trimethoxyamfetamine
TMAH	Tetramethylammonium hydroxide
t_{max}	Time to maximum plasma concentration
TMCS	Trimethylchlorosilane
TMMA	2,3,5-Trimethoxymethamfetamine
TMS	Trimethylsilyl
TMSI	Iodotrimethylsilane
TMSTFA	Trimethylsilyltrifluoroacetyl
TNF	Tumour necrosis factor
TOC	Total organic carbon
TOCSY	Total correlation spectroscopy
TOF	Time of flight
TPAH	Tetrapentylammonium hydroxide
TPI	Terahertz pulsed imaging
TPMT	Thiopurine methyltransferase
TRXRF	Total reflection XRF
TSD	Thermionic specific detection
TSP	Trimethylsilyl [2,2,3,3-$^2H_4$]-proprionic acid sodium salt
TTX	Tetrodotoxin
TVAC	Total viable aerobic count
UAC	Urine alcohol concentration
UDP	Uridine diphosphate
UGT	UDP-glucuronosyltransferase
UHPLC	Ultra-high pressure LC
UK	United Kingdom
ULOQ	Upper limit of quantification
UN	United Nations
UPLC	Ultra performance liquid chromatography
USA	United States of America
USP	United States Pharmacopeia
UV	Ultraviolet
V	Volt(s)
VAMP	Vesicle associated membrane protein
var.	Variety
V_D	Volume of distribution
Vet.	Veterinary
VGDS	Voluntary genomics data submission

Vol	Volume(s)	Wt	Weight
VSA	Volatile substance abuse	w/v	Weight in volume
v/v	Volume in volume	w/w	Weight in weight
WADA	World Anti-Doping Agency	XRD	X-ray diffraction
WCOT	Wall-coated open tubular	XRF	X-ray fluorescence
WDXRF	Wavelength dispersive XRF	XRPD	X-ray powder diffraction
WHO	World Health Organization		

PART ONE

Chapters

Methodology and analytical techniques

CHAPTER

1 Hospital Toxicology

DRA Uges

Hospital toxicology is concerned with individuals admitted to the hospital with suspected poisoning and its prime aim is to assist in the treatment of the patient. The range of substances that may be encountered is huge and ideally the hospital laboratory will have the capability to identify and, if required, quantify pharmaceutical agents, illicit drugs, gases, solvents, pesticides, toxic metals and a host of other industrial and environmental poisons in biological fluids. In practice, few laboratories can offer such a comprehensive menu and resources are concentrated on those compounds most often involved in poisoning and for which toxicological investigations are particularly useful to the clinical services. In developed countries, hospital clinical chemistry laboratories are geared to provide these basic services and rely on support from central specialised toxicology laboratories for the rarer cases. Fortunately, in the vast majority of cases the diagnosis can be made on circumstantial and clinical evidence; there is no need for urgent analyses and these can be carried out as a routine exercise. However, when the patient's condition is severe and the diagnosis is not clear, toxicological tests may be crucial and the analytical results must be furnished quickly (usually within 1–2 h of the patient's arrival) if they are to have any bearing on diagnosis and treatment. Ideally, the toxic substance can be both identified and quantified within this time frame. When this is not possible, a qualitative result still has considerable value if the symptoms are consistent with the identified toxin and should be communicated to the clinician without delay.

These time constraints entail an inevitable compromise between speed and analytical accuracy and precision. Consequently, the quantitative methods used may fall short of the standards required, for example, for pharmacokinetic investigations. However, they must be of sufficient quality to allow an appropriate clinical decision to be made (Peters, Maurer 2002). In this area, close liaison between the laboratory personnel and the clinician who manages the patient is essential and can save hours of fruitless effort. An attempt must be made to obtain as much information about the patient as possible. This should include not only the clinical picture, but also any previous medical history of poisoning, details of drugs or other substances to which the patient may have had access and, in cases of accidental poisoning, substances to which the patient may have been exposed. This sort of dialogue between the clinician and an experienced analytical toxicologist can often yield clues as to what the cause of toxicity might be and therefore suggest which tests should be performed as a priority. Close communication must continue if the initial tests prove negative, so that the search can be widened, or if the clinician requires advice on the interpretation of positive results.

Laboratories that provide analytical toxicology analyses to assist with cases of acute and chronic poisoning often offer additional services in the area of drug abuse. An increasing number of central laboratories started with providing blood spot services. Dried whole spots on printed paper are sent to these laboratories for analysis, e.g. tacrolimus, anticonvulsants, antibiotics (tuberculosis, cystic fibrosis). This can range from diagnostic tests to uncover the covert misuse of laxatives and diuretics through to routine screening of urine samples from patients assigned to treatment and rehabilitation programmes. For the latter, the requirement is to establish the drug-taking patterns of new patients and to monitor their subsequent compliance with the prescribed treatment regime. Details of techniques suitable for these services are given in separate sections.

Causes of hospital admissions for poisoning

Social and economic stresses or mental disorders often result in suicide attempts, particularly through drug overdose, one of the most common causes of emergency hospital admissions. Homicidal poisoning is relatively rare, but surviving victims of this practice are often investigated initially in the hospital environment. Individuals who have been administered substances without their knowledge to facilitate robbery or sexual abuse may also be admitted to hospital. Although in the latter scenario the victims tend to contact the medical services several days after the incident, if teenagers or young adults arrive in hospital semiconscious or disorientated, the administration of so-called date-rape drugs such as alcohol, gamma-hydroxybutyric acid (GHB), flunitrazepam or ketamine must always be considered. Poisoning in children is mainly accidental, but deliberate poisoning by parents, guardians or siblings does occur. Accidental poisoning usually takes place in the domestic environment, with young children and the elderly particularly at risk. Children may gain access to pharmaceutical products, cleaning agents (bleach, disinfectants), pesticides, alcoholic drinks and cosmetics. The confused elderly may misjudge their intake of medications or be poisoned by inappropriate handling of toxic household products. Both are susceptible to acute or chronic poisoning with carbon monoxide emitted by faulty domestic heating appliances. The workplace is another environment in which accidental poisoning occurs and the analytical results from the hospital laboratory can be important not only in medical diagnosis but also in any subsequent legal investigations that involve insurance claims.

Iatrogenic intoxications occur through inappropriate medical or paramedical treatment. Neonates require intravenous dosing and the need to work out doses per kilogram of body mass or per square metre (m^2) of body area introduces the risk that the total amount and volume of medicine to be administered may be miscalculated. Other causes of iatrogenic poisoning include drug interactions, use of the wrong route of administration and failure to take note of impaired liver or renal function, which reduces the patient's ability to eliminate the drug. A common example is the accumulation of digoxin in elderly patients with reduced renal function.

Qualitative screening or quantitative analysis?

Laboratories adopt different approaches to hospital toxicology. To a large extent, the range of equipment available and the skills and knowledge of the staff govern the policy adopted. Where resources are scarce, only a limited screen for common drugs and poisons may be carried out, with the main effort directed towards quantitative analyses for toxins indicated by circumstantial evidence and the patient's clinical signs. Specialised toxicology laboratories may pursue a systematic and comprehensive toxicological screen in every case, on the grounds that the clinical and circumstantial indicators are seldom reliable, and then proceed to quantify any substances detected. While the latter approach is more likely to yield useful information, it is expensive and time-consuming. As stated above, close liaison with the clinicians to obtain a comprehensive case history and a full clinical picture can often help to focus the resources on the qualitative and quantitative tests that are most relevant. The guidelines given in Table 1.1 are useful in this context.

Table 1.1 Guidelines to help focus resources on the most relevant qualitative and quantitative tests

Indications for qualitative screening	*Indications for quantitative analyses*
To distinguish between apparent intoxication and poisoning	When the type and duration of treatment depends on the concentration (e.g. antidotes for paracetamol and thallium)
When information about the patient is lacking (no medical history)	When the prognosis is gauged by the plasma concentration (e.g. paraquat)
When the clinical picture is ambiguous (e.g. seizures)	To distinguish between therapeutic and toxic ingestion of drugs
Where the clinical picture may be caused by a pharmacological group of drugs rather than one particular substance (e.g. laxatives, diuretics)	Mixed intoxications (e.g. methanol and ethanol)
Cases of mixed intoxication (drugs of abuse, alcohol)	Toxicological monitoring (e.g. aluminium, Munchausen's syndrome)
Poisoning with no immediately evident clinical picture (e.g. paracetamol)	Toxicokinetic calculations
Where no reliable or selective quantitative method is available (e.g. herbal preparations)	Research (e.g. efficacy of treatment), education, prevention, etc.
For forensic reasons	
At the special request of the clinician	
For purposes of statistics, research, education, prevention, etc.	

In larger clinical laboratories, the use of various liquid chromatography–mass spectrometry (LC-MS) techniques for therapeutic drug monitoring and toxicological assays has increased considerably and the introduction of liquid chromatography linked to triple quadrupole mass spectrometry (LC-MS(-MS)) has brought about an enormous increase in reliability and sensitivity both in this application (Boermans *et al.* 2006) and in forensic toxicology (Roman *et al.* 2008). Although LC-MS(-MS) is not a comprehensive screening method, if sufficient information on the likely cause of poisoning in a drugs overdose case is available, it is possible to obtain both qualitative and quantitative data for a selection of up to 15 drugs and their metabolites within 40 minutes.

Applications

Confirmation of diagnosis

Most patients who reach hospital in time respond well to measures designed to support the vital processes of respiratory and cardiovascular function and, as mentioned above, toxicological investigations are of only historical value. However, it is still useful to have objective evidence of self-poisoning as this usually instigates psychiatric treatment and follow-up.

Differential diagnosis of coma

When circumstantial evidence is lacking, a diagnosis of poisoning may be difficult to sustain simply on the basis of clinical examination, since coma induced by drugs is not readily differentiated from that caused by disease processes. Apparent poisonings can be caused by hypoglycaemic coma, a cerebrovascular accident, exhaustion (after seizures), brain damage, meningitis, withdrawal symptoms, idiosyncratic reactions (e.g. to theophylline and caffeine), allergic reactions (shock), viral infections or unexpected symptoms of a disease (e.g. Lyme disease). In these situations, toxicological analyses serve either to confirm poisoning as the cause of coma or to rule it out in favour of an organic disorder that requires alternative medical and pathological investigations.

Diagnosis of brain death

A patient with brain death may be a potential donor of organs. In such cases, the patient should have a deep coma of known origin with no indication of a central infection and normal metabolic parameters. When the primary cause of coma is drug overdose, it is important to ensure that the drug has been eliminated prior to confirming the diagnosis of brain death. This also applies to drugs that may have been given in therapy. For example, thiopental is often given in the treatment of brain oedema and during neurosurgery. The half-lives of thiopental and its metabolite, pentobarbital, increase if cardiac function is diminished or the patient is hypothermic, and therefore plasma concentrations of both compounds must always be measured. Midazolam and diazepam are also administered frequently in treating cases of brain damage, and the continued presence of active concentrations of these drugs and their metabolites should also be excluded using specific and sensitive procedures, such as high performance liquid chromatography (HPLC) and LC-MS. Even if benzodiazepines or their metabolites cannot be detected, there remains the possibility that some may still be present; for instance, active concentrations of hydroxymidazolam glucuronide may be present, since the half-life may be increased considerably with end-stage organ failure. This may suggest a provocation test with the specific benzodiazepine antagonist flumazenil. Similarly, the presence of active levels of anticonvulsants (phenobarbital, carbamazepine, phenytoin and valproate), which are also given in the treatment of brain damage, must be excluded. Again, the use of sensitive and specific chromatographic methods is essential.

Influence on active therapy

Although supportive therapy remains the cornerstone of the management of acute poisoning, specific antidotes are available for metals (chelation agents), anticholinesterase inhibitors (atropine), methanol and ethylene glycol (ethyl alcohol, formepizole, 4-methylpyrazole), paracetamol/acetaminophen (*N*-acetylcysteine), digoxin (antibody fragments), calcium blockers (calcium salt), cumarines (phytonadione) and opioids (naloxone). Given a clear diagnosis, a clinician usually administers the antidote without waiting for laboratory confirmation, but subsequent analyses may help to decide whether to continue with the therapy. For example, both parenteral and oral therapy with desferrioxamine in cases of iron poisoning is indicated if patients deteriorate and the serum iron concentration is extremely high. Measurements of cholinesterase activity in serum or red cells are useful in a situation of high-dose infusions of atropine into patients exposed to organophosphate insecticides or thiocarbamates. Measures designed to reduce the absorption of poisons from the gut, such as the use of emetics, purgatives, gastric lavage and irrigation, are now considered to be of limited value and unwarranted in most cases of poisoning. The efficacy of whole-bowel irrigation is also questionable, although some advocate its use to remove sustained-release or enteric-coated preparations of, for example, iron salts and other potentially lethal poisons that have passed into the small bowel, and in the decontamination of body packers. A single oral dose of activated charcoal has largely replaced other means of reducing absorption, although it is generally useful only when given within 1 h of ingestion and fails to absorb inorganic ions, alcohols, strong acids or alkalis, or organic solvents.

Techniques to increase the rate of elimination of poisons, such as diuresis, adjusting the urinary pH, haemodialysis and peritoneal dialysis, venous–venous haemofiltration and charcoal haemoperfusion, are now rarely used. Forced diuresis is now frowned upon; it is probably beneficial only in cases of poisoning with thallium and, when coupled with alkalisation of urine, chlorophenoxy herbicides. Alkalisation of urine effectively increases the elimination of salicylates, phenobarbital and chlorophenoxy herbicides. Acidification of the urine has little merit in increasing the elimination of weakly basic substances, such as amfetamines and phencyclidine. New insights provide the indication of high-dose Intralipid after a severe overdose of a wide variety of drugs, e.g. lidocaine, antidepressants (see www.lipidrescue.org). Haemofiltration also has a role in this context. 'Gut dialysis', or the use of multiple oral doses of activated charcoal, is thought to operate by creating a drug

concentration gradient across the gut wall that leads to movement of the drug from the blood in the superficial vessels of the gut mucosa into the lumen. So far, its efficacy has been demonstrated for carbamazepine, dapsone, phenobarbital, quinine and theophylline, and there is evidence for its application in poisoning with calcium antagonists. Most of these procedures carry inherent risks to the patient and, as pointed out already, their applications are limited to only a handful of poisons. Toxicological analyses to identify and quantify the poison should be used to ensure that they are used appropriately and at the same time to prevent overtreatment of patients who would recover without such interventions.

Medicolegal aspects of hospital toxicology

The primary role of the hospital toxicologist is to assist clinicians in the treatment of poisoned patients, irrespective of any other aspects that surround the case. However, some cases may have a criminal element. These can range from iatrogenic poisoning, in which a patient or relative sues a health authority and its staff for neglect, through to the malicious administration of drugs or poisons by a third party. The latter category includes victims of drug-facilitated sexual assault who have been administered drugs such as flunitrazepam or GHB to induce confusion and amnesia, and non-accidental poisoning in children. Mothers are the most frequent perpetrators of child poisoning and do so to attract sympathy and attention as a consequence of the child's illness (Munchausen's syndrome by proxy). When these situations arise, the hospital toxicologist is obliged to take special precautions to conserve all residual samples (human matrices as well as medicines) and documentation that may feature subsequently as part of a forensic investigation (see Chapter 9).

Clinical manifestations and biomedical tests

Specific acute clinical manifestations and vital signs of the patient that can be important in suggesting the cause of poisoning are set out in Table 1.2.

Biochemical tests that gauge the physiological status of the patient are more important in terms of the immediate management of the condition and some of the abnormalities found can also be diagnostic of the type of agent involved (see Table 1.6). These, together with the clinical manifestations and history, provide the basis for the order in which the toxicological tests are carried out.

Other indicative features

Some poisons have characteristic odours that may be discerned on the patient's body or on clothes, or in breath and samples of vomit, as listed in Table 1.3. Colours of the skin and of urine samples can also be useful indicators (Tables 1.4 and 1.5). However, these clues should be interpreted with caution and are not a substitute for proper clinical and toxicological evaluation.

The results of biomedical tests are usually available before any toxicological tests have been completed; Table 1.6 highlights their potential diagnostic value.

Assays required on an emergency basis

Table 1.7 lists the toxicological assays (mainly in serum, plasma or blood) that should be performed as soon as possible after admission and highlights those that should preferably be provided by all acute hospital laboratories. Emergency requests for the analysis of rarer poisons may be referred to a specialised centre. Such lists vary according to the pattern of poisoning prevalent in different countries or regions, and Table 1.7 is therefore presented only as a guideline. Notes that indicate the relevance of the assays are also included.

Table 1.2 Disturbance of clinical features and indications of possible causes

Clinical feature	*Disturbances and poisons indicated*
General appearance	Restlessness or agitation (amfetamines, cocaine, lysergide (LSD), opiate withdrawal), apathy, drowsiness, coma (hypnotics, organic solvents, lithium)
Neurological disturbances	Electroencephalogram (EEG) (central depressants), motor functions (alcohol, benzodiazepines, GHB), speech (alcohol, drugs of abuse), movement disorders (hallucinogens, amfetamines, butyrophenones, carbamazepine, lithium, cocaine, ethylene glycol), reflexes, seizures (most centrally active substances in overdose or withdrawal), ataxia
Vital signs	
Mental status	Psychosis (illicit drugs), disorientation, stupor
Blood pressure	Hypotension (phenothiazines, beta-blockers, nifedipine, nitroprusside and other vasodilators)
	Hypertension (corticosteroids, cocaine, phenylpropanolamines, anticholinergics)
Heart	Pulse, electrocardiogram (ECG) elevation of QT-time (tricyclic antidepressants, orphenadrine, calcium blockers, class III antiarrhythmics, fluoroquinolones, macrolide antibiotics, antipsychotics, antimycotics, lithium and many drug–drug interactions)
	Irregularities, torsades de pointes (phenothiazines, procainamide, amiodarone, lidocaine), heart block (calcium blockers, beta-blockers, digoxin, cocaine, tricyclic antidepressants)
Temperature	Hyperthermia (LSD, cocaine, methylenedioxymetamfetamine (MDMA), selective serotonin reuptake inhibitors (SSRIs), dinitro-*o*-cresol (DNOC))
	Hypothermia (alcohol, benzodiazepines)
Respiration	Depressed (opiates, barbiturates, benzodiazepines)
	Hypoventilation (salicylates)
Muscles	Spasm and cramp (strychnine, crimidine, botulism)
Skin	Dry (parasympatholytics, tricyclic antidepressants)
	Perspiration (parasympathomimetics, cocaine)
	Gooseflesh (strychnine, LSD, opiate withdrawal)
	Needle marks (parenteral injections: drugs of abuse, insulin),
	Colour (red, carboxyhaemoglobin; blue, cyanosis, e.g. with ergotamine; yellow, DNOC)
	Blisters (paraquat, barbiturates)
Eyes	Pinpoint (opiates, cholinesterase inhibitors, quetiapine)
	Dilated pupils (atropine, amfetamines, cocaine)
	Reddish (cannabis)
	Reflex, movements, lacrimation, nystagmus (phenytoin, alcohol)
Nose	Nasal septum complications (cocaine)
Chest	Radiography (bronchoconstriction, metals, aspiration)
Abdomen	Diarrhoea (laxatives, organophosphates)
	Obstruction (opiates, sympatholytics such as atropine)
	Radiography (lead, thallium, condoms packed with illicit drugs)
Smell	Sweat, mouth, clothes, vomit (see Table 1.3)

Quality management

It is essential that the whole laboratory process be controlled strictly and subjected to regular internal and external assessments. All administrative and analytical activities should be described in detailed standard operating procedures (SOPs), which should be reviewed and, if necessary, updated at regular intervals. The laboratory should have in place a system of internal quality controls and also participate in external proficiency-testing schemes. Particular attention should be given to the storage of raw analytical data, results and residual samples, and no unauthorised person should have access to patient information.

Table 1.3 Odours associated with poisoned patients

Odour	*Potential agents or situation*
Acetone/nail polish remover	Acetone, propan-2-ol, metabolic acidosis
(Aeroplane) Glue	Toluene, aromatic hydrocarbon sniffing
Alcohol	Ethanol (not with vodka), cleaners
Ammonia	Ammonia, uraemia
Bitter almonds, silver polish	Cyanide
Bleach, chlorine	Hypochlorite, chlorine
Disinfectant	Creosote, phenol, tar
Formaldehyde	Formaldehyde, methanol
Foul	Bromides, lithium
Hemp, burnt rope	Cannabis
Garlic	Arsenic, dimethyl sulfoxide (DMSO), malathion, parathion, yellow phosphorus, selenium, zinc phosphide
Mothballs	Camphor, naphthalene, *p*-dichlorobenzene
Smoke	Nicotine, carbon monoxide
Organic solvents	Diethyl ether, chloroform, dichloromethane
Peanuts	Rodenticide
Pears	Chloral hydrate, paraldehyde
Plants with special odours	For example *Taxus*, *Convallaria*
Rotten eggs	Disulfiram, hydrogen sulfide, hepatic failure, mercaptans (additive to natural gas), acetylcysteine
Shoe polish	Nitrobenzene
Turpentine	Turpentine, wax, solvent of parathion, polish

Table 1.4 Typical colours of the skin with poisoning

Colour of skin	*Poison or situation*
Blue, cyanosis	Hypoxia, methaemoglobinaemia, sulfhaemoglobin
Blue, pigment	Dye (amitriptyline or chloral hydrate tablets), paint
Yellow (jaundice)	Liver damage (alcohol, borate, nitrites, scombroid fish, rifampicin, mushrooms, metals, paracetamol, phosphorus, solvents)
Yellow	DNOC
Reddish	Carbon monoxide
Black, necrosis	Sodium or potassium hydroxide, sulfuric acid, burning, intra-arterial injection

Table 1.5 Urine colours associated with various poisons

Colour of urine	*Poison or drug*
Red/pink	Ampicillin, aniline, blackberries, desferrioxamine, ibuprofen, lead, mercury, phenytoin, quinine, rifampicin
Orange	Warfarin, rifampicin, paprika
Brown/rust	Chloroquine, nitrofurantoin

Table 1.6 Biochemical and haematological abnormalities in poisoning

Abnormality	*Indication*
Acid–base disturbances	
Metabolic acidosis	Ethylene glycol, salicylate, methanol, cyanide, iron, amfetamines, MDMA
Metabolic alkalosis	Chronic use of diuretics or laxatives
Respiratory acidosis	Opiates
Respiratory alkalosis	Salicylates, amfetamines, theophylline
Increased anion gap	Ethylene glycol
Increased osmolar gap	Alcohols, glycols, valproate
Electrolyte disturbances	
Hypocalcaemia	Ethylene glycol, oxalates, phosphates, diuretics, laxatives
Hyperkalaemia	Digoxin, potassium salts
Hypokalaemia	Theophyllline, insulin, oral antidiabetic drugs, diuretics, chloroquine
Hypernatraemia	Sodium chloride, sodium bicarbonate
Hyponatraemia	MDMA, diuretics
Glucose	
Hypoglycaemia	Insulin, oral antidiabetic drugs, ethanol (children), paracetamol (with liver failure)
Liver enzymes	
Raised transaminases	Paracetamol, amfetamines, MDMA, iron, *Amanita phalloides*, strychnine
Haematological	
Anaemia, raised zinc protoporphyrin, basophilic stippling	Lead
Carboxyhaemoglobin	Carbon monoxide
Methaemoglobinaemia	Chlorates, nitrites
Raised prothrombin time	Paracetamol, coumarin anticoagulants

Where possible, the laboratory should seek accreditation by an external authority (see Chapter 22).

Request forms

A specially designed request form for toxicological analyses is a useful way not only to obtain essential demographic information on the patients and the analyses required but also to gather details of symptoms, drugs prescribed, biochemical abnormalities and previous medical history. This supplements the oral information provided by the clinician. On completion of the analyses, a copy of the form with the results and interpretation entered can be returned to the clinician. An example of a request form is shown in Fig. 1.1.

Collection and choice of samples

Blood, serum or plasma

Blood is usually easy to obtain and the analytical results can be related to the patient's condition and also be used in pharmacokinetic or toxicokinetic calculations. A 10 mL sample of anticoagulated blood (sodium edetate) and 10 mL of clotted blood should be collected from adults on admission (proportionately smaller volumes from young children). Most quantitative assays are carried out on the plasma, but anticoagulated whole blood is essential if the poison is associated mainly with the red cells (e.g. carbon monoxide, cyanide, lead, mercury). Serum from coagulated blood can also be used, although the levels are almost always the same as those in plasma. Serum has the advantage that there is no potential interference from any additive. The disadvantage is that clotting takes time and occurs only at room temperature, which creates problems with the analysis of unstable analytes that require samples to be cooled immediately by immersion in ice. It is advisable in addition to collect a 2 mL blood sample into a fluoride/oxalate tube if ethanol ingestion is suspected. However, since most of the fluoride tubes used in hospitals do not contain enough sodium fluoride to completely inhibit microbial production of alcohol (the minimum fluoride concentration required in blood is 1.5% w/v), these samples are not acceptable for forensic purposes. There are conflicting reports of the dangers of contamination of samples collected after the use of disinfectant swabs containing ethanol or 2-propanol and then analysed for ethanol content. Volunteer studies (Malingre *et al.* 2005) have suggested that this is not a

Table 1.7 Emergency toxicological assays

Assay(s)	*Intervention*	*Comments*
Anticholinesterase inhibitors[a]	Atropine (since 2008 the use of pralidoxime or obidoxime is contraindicated)	Measure serum (or preferably red cell) cholinesterase activity
Antiepileptics (carbamazepine, phenytoin)	Multiple-dose activated charcoal	—
Benzodiazepines	Flumazenil antidote only in severe cases	Consider presence of active metabolites; withdrawal seizures
Beta-blockers	Glucagon, isoprenaline	—
Calcium antagonists	Calcium salt infusions, Intralipid	Verapamil: severe prognosis
		Nifedipine: acidosis
Carboxyhaemoglobin[a]	Hyperbaric oxygen	No value after administration of oxygen
Chloroquine	High doses of diazepam	Monitor serum K^+
Cocaine	Diazepam, haloperidol	—
Digoxin[a]	Potassium salts, Fab antidote	Monitor serum K^+, measure serum digoxin prior to giving Fab fragments
Ecstasy group (methylenedioxyamfetamine (MDA), MDMA)	Single-dose activated charcoal, diazepam, dantrolene	Check for metabolic acidosis and hyponatraemia, hyperthermia
Ethanol[a]	Haemodialysis, vitamin B	Monitor blood glucose in children
Iron[a]	Desferrioxamine, IV + PO	Measure unbound iron; colorimetric assays for serum iron unreliable in presence of desferrioxamine
Isoniazid	Pyridoxine	—
Lithium[a]	Haemodialysis, vitamin B	Measure serum level 6 h after ingestion
Methaemoglobin[a]	Methylene blue	Methaemoglobinaemia caused by nitrites, chlorates, dapsone, aniline
Methanol, ethylene glycol plus other alcohols	Methylpyrazole or ethanol and haemodialysis	Monitor serum ethanol levels to ensure optimum antidote administration
Methotrexate	Folinate, venous–venous haemofiltration	Measure plasma methotrexate level 4–6 h after ingestion
Opiates	Naloxone	—
Osmolality	—	Increased by alcohol, glycols, severe valproate overdose
Paracetamol[a]	*N*-Acetylcysteine, methionine	Measure serum level at least 4 h after ingestion; prothrombin time and international normalised ratio (INR) are useful prognostic indicators
Paraquat (qualitative urine test)[a]	Activated charcoal	Urine test diagnostic; plasma levels useful in predicting outcome
Salicylate[a]	HCO_3^- infusion, haemodialysis	Repeat serum salicylate assays may be needed because of continued absorption of the drug
Strychnine	Diazepam	—
Thallium	Prussian (Berlin) blue orally	Treatment continued until urine thallium levels <0.5 mg/24 h
Theophylline[a]	Multiple-dose activated charcoal	Measure serum theophylline in asymptomatic patients 4 h after ingestion
Tricyclic antidepressants	Multiple-dose activated charcoal, Intralipid, HCO_3^-	QT time

[a]To be provided by all acute hospital laboratories.

significant problem, whereas Higuchi *et al.* (2005) reported a definite risk during collection from hospital patients, particularly when the swab was used to wipe the syringe needle after sampling. On balance it is advisable to avoid the use of these swabs in the hospital setting where careless sampling techniques are most likely to occur. The vigorous discharge of blood through a syringe needle can cause haemolysis and invalidate a serum iron or potassium assay.

Urine

Urine usually contains higher concentrations of drugs, poisons and their metabolites than blood and is therefore ideal for qualitative screening. Most drugs remain detectable for much longer periods in urine than in blood. For example, GHB will have almost entirely disappeared from blood by the time a patient reaches hospital but can often still be found in a urine sample. Some substances are detectable only as a metabolite in urine (e.g. oxaxepam as oxazepam glucuronide; nicotine as cotinine). However, the detection of a substance in urine does not necessarily mean that this is the cause of the poisoning since it may have been taken days before the event and may not be related to the acute situation. In emergency cases, particularly when the patient is unconscious, the delay in obtaining a urine sample may be unacceptable and many clinicians are now reluctant to use catheterisation routinely on unconscious patients. Where a sample can be obtained, a volume of <25 mL is sufficient for most purposes.

Stomach contents

This sample includes vomit, gastric aspirate or stomach washout. Stomach washout is no longer a routine treatment procedure, but when it is carried out it is important to obtain the first sample of washout rather than a later sample, which will be diluted considerably. If the sample is obtained soon after the overdose, it may be possible to recognise the presence of undegraded tablets and capsules, or the characteristic odour of certain compounds. Stomach contents can be substituted for urine in toxicological screening, and are useful for identifying poisons derived from plants and fungi, and for other poisons that are difficult to detect in blood or urine. However, as with urine, quantitative

Request form for toxicological analysis

Name of Your Hospital and laboratory
Address
Telephone and fax numbers
E-mail address for information and requests
Your home page for background information

• Unique **sample number**.....
Name of responsible toxicologist, chemist,
and / or pharmacist in your laboratory
telephone and bleep numbers

Information about the applicant / answer to:
Name clinician:.....
Telephone + bleep:.....
Ward / clinic / hospital.....
Address of the hospital.....
.....
Code of the clinic / hospital.....

Information about the samples
Serum / whole blood / urine / stomach/
other:.....
number of sample tubes: .....
date and time of sampling.....
.....
Received in lab: date:..... / time:.....

Information about patient
Surname:.....
Forename(s)..... .....
Birth date: M/F
Hospital reg. number: .....
Address:.....
.....
General practitioner: name + address
.....
Insurance: firm / class / number .....
.....

Medical Information
Weight Height Coma Creatinine
.kg; .... cm .....(I/IV) ..... µM

motivation for analytical request
O right dose / medication
O coma / poisoning?
O uncommon reaction
O misuse / abuse
O research
O other reasons:.....

Background data
Known prescribed drugs with dose.....
Other packaging found: .....
Abnormal or significant vital signs .....
Abnormal biochemistry .....

Requested analysis
O Specific screening for: .....
.....
O Quantitative analysis of: ethanol / paracetamol/ .....
.....

Results of analysis
In the sample was found (mg/L):
.....
.....
.....
.....
.....
.....

Reference ranges (mg/L):
therapeutic toxic

Analyst:
.....
Date analysis
.....
Toxicologist
.....
Pass on by
.....
day/time:

Remarks, conclusions
.....
.....
.....

Financial information:

Without *special request the samples will be kept at* –20 °C *for* 14 *days after analysis and then destroyed.*

Figure 1.1 Request form for toxicological analysis.

analyses serve no purpose, for example, in reflecting the amount of poison absorbed.

Saliva, sweat and/or oral fluids

There is growing interest in the use of saliva (see Chapter 18) or sweat (Barnes *et al.* 2008) as an alternative non-invasive test sample (see Chapter 18) and in its potential uses in hospital toxicology. Other human matrices (e.g. oral fluid, sweat) as well as other matrices including dried whole blood spot (for transportation), hair (patient non-compliance), plasters (drugs of abuse), liquor (antibiotics, MTX), pus (antibiotics) and tissue are sent to TDM and toxicology laboratories.

Toxicological screening

Toxicological screening schemes can be divided into limited, specific or extensive ('general unknown') screening. The choice of scheme is clearly strongly dependent on the range of technical equipment available, the expertise of the laboratory staff and the information provided about the patient. All analytical techniques have their own advantages and disadvantages and in this chapter several different methods and approaches are described.

Fast limited screening

Immunoassays

Commercially available immunoassays (IAs), such as fluorescence polarisation immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA; see Chapter 31), give quick qualitative results and, in some cases, a semi-quantitative result in plasma for a variety of substances or groups of compounds. Their limitations in terms of specificity and sensitivity must always be considered when interpreting results. Hospital laboratories that provide therapeutic drug monitoring (see Chapter 2) and screening services for drugs of abuse are ideally placed to invoke these assays as part of a toxicological investigation. However, in recent years, larger hospital laboratories have tended to move to LC-MS(-MS) for therapeutic drug monitoring, especially for immunosuppressant drugs (e.g. ciclosporin, everolimus, sirolimus, mycophenolate) on the grounds of better specificity, more flexibility and lower costs.

Alcohol dehydrogenase test for ethanol

This quantitative test is based on the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase (ADH) in the presence of nicotinamide adenine dinucleotide (NAD) and is applicable to serum and plasma. Several commercial ADH kits are available and the test can be performed on routine clinical chemistry analysers. Propan-2-ol and other higher alcohols can also reduce NAD to give positive readings. Methanol and acetone do not react and therefore a gas chromatographic method for alcohols is much preferred. Ethylglucuronide, as a marker for strong or recent ethanol use, is easily measured by LC-MS(-MS) in urine or plasma.

Direct (colour tests) in serum or urine samples

Blood glucose Hypoglycaemia is a feature of overdose with insulin, hypoglycaemic agents and ethanol. It can also occur in the early stages of liver damage after severe poisoning with paracetamol.

Ketones in urine Dip a 'Labstix' strip briefly into the urine and read after 10–15 s. A positive result for ketones may indicate intoxication by acetone or isopropyl alcohol. This test may also be positive in starvation or in diabetic ketosis.

Carbon monoxide in whole blood Dilute a sample of the blood 1 in 20 with 0.01 mol/L ammonia and compare the colour with a sample of normal blood treated similarly. A pinkish tint suggests the presence of carboxyhaemoglobin (COHb). Modern clinical gas analysers automatically measure the COHb and therefore this colour test is rarely required.

Salicylates Trinder's reagent is used (40 mg of mercuric chloride in 850 mL of water; add 120 mL of 1 mol/L hydrochloric acid and 40 g of hydrated ferric nitrate and dilute to 1000 mL with water).

Trinder's test in serum Add 4.5 mL of Trinder's reagent to 0.5 mL of serum, shake well and centrifuge. A violet colour in the supernatant liquid indicates the presence of salicylate or salicylamide.

Trinder's test in urine Add 5 drops of Trinder's reagent to 1 mL of urine (pH 5–6). A violet colour indicates the presence of salicylate or salicylamide. A positive result is obtained after therapeutic doses of acetylsalicylic acid (aspirin) or aminosalicylic acid. The red colour is formed with a phenolic OH group.

Volatile reducing substances (including alcohols and aldehydes): dichromate test in serum or urine

- Add 1 mL of urine or serum to a test-tube.
- Apply one drop of 2.5% (w/v) potassium dichromate in 50% (v/v) sulfuric acid to a strip of glass-fibre filter paper.
- Insert this paper in the neck of the test-tube.
- Lightly cork the tube, and place it in a boiling water-bath for 2 min.

A colour change to green indicates a positive result. Ethanol gives a positive reaction if present above 400 mg/L, in which case the blood-ethanol concentration should be measured.

Confirmation of methanol

- Add one drop of 2.5% (w/v) potassium dichromate in 50% (v/v) sulfuric acid to 1 mL of urine and allow to stand at room temperature for 5 min.
- Add one drop of ethanol and a few milligrams of chromotropic acid.
- Gently add about 0.5 mL of concentrated sulfuric acid down the side of the tube so that it forms a layer in the bottom.

A violet colour at the interface indicates methanol. Note that the metabolite formaldehyde also gives a positive reaction in this test.

Organophosphorus compounds: cholinesterase inhibitors in serum

- To each of three tubes add 2 mL of 0.02% (w/v) dithiobisnitrobenzoic acid in 0.1 mol/L sodium dihydrogenphosphate buffer, pH 7.4 solution and 1.0 mL of 0.5% (w/v) aqueous acetylthiocholine iodide solution.
- To the first tube add 20 µL of control plasma and stand next to the second 20 µL of sample plasma.
- To the third tube add 20 µL of 20% (w/v) aqueous pralidoxime or obidoxime chloride solution (which reverses the inhibitor activity) and 20 µL of test plasma.
- Vortex the contents of all three tubes and allow them to stand at room temperature for 2 min.

If a cholinesterase inhibitor is present, the yellow colour in the control tube will be deeper than that in the sample tube. Further confirmation is provided if the depth of colour in the pralidoxime tube is similar to that in the control tube.

Trichloro-compounds: Fujiwara test in serum or urine

- To 1 mL of urine add 1 mL of 20% (w/v) sodium hydroxide solution and 1 mL of pyridine.
- Heat in a boiling water-bath for 2 min.
- A blank urine sample and an authentic solution of trichloroacetic acid should be tested at the same time, both blank and control solutions being treated in a similar fashion to the sample.

The development of a red colour in the pyridine layer indicates ingestion of a trichloro-compound.

Metabolites of carbon tetrachloride may also give a positive result with this test, but carbon tetrachloride is metabolised only partially to trichloromethyl compounds and the test may fail to detect this agent. If carbon tetrachloride poisoning is suspected, evidence of hepatotoxicity should be sought by carrying out the appropriate serum-enzyme assays.

Paraquat and diquat – dithionite test in urine

- To 1 mL of urine add 1 mL of a freshly prepared 0.1% (w/v) solution of sodium dithionite in 1 mol/L sodium hydroxide.

A blue colour indicates the presence of paraquat.

A green colour is given by diquat, but paraquat may also be present.

A strong blue colour obtained with a urine sample taken more than 4 h after ingestion suggests a poor prognosis.

Confirmation of the severity of the poisoning is obtained by measuring the paraquat concentration in plasma.

Paracetamol: cresol–ammonia test in urine

- To 0.5 mL of urine add 0.5 mL of 36% (v/v) hydrochloric acid.
- Heat the mixture for 10 min at 100°C.
- Mix two drops of the mixture with 10 mL of water and add 1 mL of 1% (w/v) *o*-cresol in water, and 4 mL of 2 mol/L ammonium hydroxide.

A blue colour appears if paracetamol is present. The test is very sensitive and can detect therapeutic concentrations. The presence of the parent drug and its conjugated metabolites can be detected for several days after

overdose. If a positive result is obtained, a plasma-paracetamol determination should be carried out immediately.

Cyanide in blood

Dräger test-tube method

- Dispense 5 mL of heparinised blood into a special 25 mL test-tube (about 10 cm long) fitted with a rubber stopper with two holes.
- Insert into one hole a newly opened Dräger test-tube for cyanide.
- Add 5 mL of 10% (v/v) sulfuric acid and close the tube immediately.
- Close the open hole with a finger while sucking the cyanide air from the mixture through the Dräger test-tube with a Dräger pump for 3 min.

An orange–red colour indicates a positive reaction. This can be made semi-quantitative by treating a standard solution of potassium cyanide (0.625 mg/L is equivalent to 0.5 mg/L CN) in the same way (toxic concentrations >0.5 mg/L).

Use of the Cyantesmo kit for rapid detection of cyanide in blood has been described by Rella *et al.* (2005).

Thallium in urine

- Dissolve 1.6 g of sodium hydroxide, 1.2 g of potassium sodium tartrate and 1.36 g of potassium cyanide in 10 mL of water.
- Freshly prepare a 250 mg/L solution of dithizone in chloroform (reagent should be green).
- Add 1 mL of the cyanide reagent to 5 mL of urine in a glass-stoppered test-tube and vortex for 10 s.
- Add 2 mL of the dithizone solution, vortex for 1 min and centrifuge (5 min).

Thallium produces a pink–red layer in the chloroform. A blank urine sample and a standard solution of thallium (1 mg/L) should be treated in the same way for comparison.

Toxicological screening by thin-layer chromatography

Thin-layer chromatography (TLC) is still sometimes applied to urine samples. Unfortunately, TLC methods for newer drugs are rarely developed anymore.

Many TLC systems have been developed for use in hospital toxicology. These include the commercial Toxilab system, which provides standards for the substances and metabolites most commonly encountered in intoxicated patients. The most generally used mobile phase is chloroform–methanol (9 : 1 v/v), although some countries now require the less toxic dichloromethane instead of chloroform. In hospital toxicology it is advisable to use at least two separate mobile phase systems to obtain a more definitive result. Silica-gel plates of 20 × 20 cm with or without fluorescent indicator are the most popular, although smaller sizes can also be used.

Solvent extraction procedure for acidic, neutral and basic drugs

- Pipette 10 mL of the sample (urine or stomach contents) in each of two 20 mL screw-capped glass bottles or stoppered test-tubes.
- To one bottle (A) add 1 to 2 mL of 1 mol/L sulfuric acid.
- To the other bottle (B) add of 1 to 2 mL of 1 mol/L sodium hydroxide solution (check the pH with an indicator paper).
- Add 10 mL of dichloromethane to each bottle or tube. Shake gently for about 5 min, and centrifuge for 5–10 min.
- Remove the top aqueous layer using a Pasteur pipette connected to a water-operated vacuum pump.

Back-extraction procedure for stomach contents extracts

- Add 3 mL of 0.5 mol/L sodium hydroxide solution to the acid dichloromethane extract (A), and 3 mL of 0.25 mol/L sulfuric acid to the alkaline dichloromethane extract (B).
- Shake the bottles again, centrifuge and discard the organic solvent layers.
- Make bottle A acidic by the addition of 0.5 mL of 3 mol/L sulfuric acid, and make bottle B alkaline by the addition of 0.5 mL of 6 mol/L sodium hydroxide solution.
- Add 10 mL of dichloromethane to each bottle, shake, centrifuge and remove the aqueous layers as above.

Drying and concentration

- Remove residual moisture from the two extracts (from either urine or stomach contents) by filtering through phase-separating paper, and collect the filtrates in 10 mL conical test-tubes.
- Add a little tartaric acid to the basic extract to prevent the loss of volatile bases, and evaporate to dryness under a stream of air or nitrogen.
- Dissolve each residue in 0.1 mL of methanol.

Chromatography of acidic and neutral drugs

Location reagents

- Mercuric chloride–diphenylcarbazone: (a) dissolve 0.1 g of diphenylcarbazone in 50 mL of ethanol; (b) dissolve 1 g of mercuric chloride in 50 mL of ethanol; prepare both solutions daily. Mix solutions (a) and (b) just before spraying.
- Mercurous nitrate: a saturated aqueous solution of mercurous nitrate.
- Dragendorff: (a) mix 2 g of bismuth subnitrate, 25 mL of acetic acid and 100 mL of water; (b) dissolve 40 g of potassium iodide in 100 mL of water. Mix together 10 mL of (a), 10 mL of (b), 20 mL of acetic acid and 100 mL of water. Prepare the mixture every 2 days.
- Furfuraldehyde: (a) dilute 2 mL of redistilled furfuraldehyde to 100 mL with acetone; (b) dilute 4 mL of sulfuric acid to 100 mL with acetone; prepare immediately before use. Spray with (a) first, followed by (b).

Reference solutions Prepare solutions (1 mg/mL) in methanol of authentic samples of drugs, as indicated in Table 1.8.

Method

- Divide a TLC plate (silica-gel G, 250 μm) into eight equal columns by scoring lines with a spatula.
- Apply 10 μL aliquots of the reference solutions and 25 μL aliquots of the sample extract to the columns on the plate in the sequence shown in Table 1.8.
- Evaporation of the spots can be hastened by the use of a cold-air blower.
- Develop the plate for a distance of about 10 cm from the origin in a tank that contains 100 mL of a 4 : 1 mixture of chloroform–acetone (system TD). Alternatively, system TE or system TF may be used (Table 1.9).
- After development, remove the plate from the tank and dry under a stream of cold air.

Visualisation

- Examine the plate under ultraviolet (UV) light.
- Cover columns 3 to 8 (Table 1.8) with a glass plate and spray columns 1 and 2 with mercuric chloride–diphenylcarbazone reagent. White spots on a violet background indicate the presence of barbiturates and related compounds.
- Cover columns 1 and 2 and 5 to 8 with glass plates, and spray columns 3 and 4 with mercurous nitrate spray. Black spots are given by barbiturates and related compounds.
- Cover columns 1 to 4 and 7 and 8 with glass plates, and spray columns 5 and 6 with Dragendorff spray. An orange spot is given by methaqualone.

Table 1.8 Sequence of application of acidic extract to TLC plate

Column number	*Solution*
1	Amobarbital and phenobarbital
2	Sample extract
3	Phenobarbital and phenytoin
4	Sample extract
5	Methaqualone
6	Sample extract
7	Meprobamate
8	Sample extract

Table 1.9 TLC data for some acidic and neutral drugs

Compound	R_f *value in systems*			*Mercuric chloride–diphenylcarbazone reagent*	*Mercurous nitrate spray*
	TD	*TE*	*TF*		
Primidone	08	39	26	Positive	Positive
Meprobamate[a]	09	60	34	—	—
Paracetamol	15	45	34	—	—
Phenytoin	33	36	53	Positive	Positive
Salicylamide	38	46	55	—	—
Barbital	41	31	61	Positive	Positive
Phenobarbital	47	28	65	Positive	Positive
Cyclobarbital	50	35	64	Positive	Positive
Butobarbital	50	38	65	Positive	Positive
Heptabarbital	50	30	65	Positive	Positive
Amylobarbital	52	36	65	Positive	Positive
Pentobarbital	55	45	66	Positive	Positive
Quinalbarbital	55	44	68	Positive	Positive
Glutethimide[b]	63	78	62	Positive	Positive
Methaqualone[c]	63	—	—	—	—
Phenylbutazone	78	66	68	Positive	—

[a]Violet with furfuraldehyde reagent.
[b]Weak reaction with Dragendorff spray.
[c]Positive reaction with Dragendorff spray.

- Cover columns 1 to 6, and spray columns 7 and 8 with furfuraldehyde reagent. A violet spot is given by meprobamate.
- The chromatographic system distinguishes between certain types of barbiturates (Table 1.9), which is sufficient for most clinical situations. If doubt exists, or if it is crucial to know which barbiturate is present, the sample should be examined by HPLC or GC. Certain antibiotics give white spots with mercurous nitrate spray, but do not react with mercuric chloride–diphenylcarbazone reagent (Table 1.9).

Chromatography of basic drugs

Solvent extraction from urine

- Mix together 5 mL of urine, 0.5 mL of 4 mol/L sodium hydroxide solution and 2.5 mL of dichloromethane.
- Centrifuge and remove the aqueous phase.
- Pass through a filter paper containing 1 g of anhydrous sodium sulfate and evaporate the filtrate to dryness.
- Dissolve the residue in 50 µL of dichloromethane or methanol.

Location reagents/spray reagents

- Acidified iodoplatinate: dissolve 0.25 g of platinic chloride and 5 g of potassium iodide in sufficient water to produce 100 mL and add 5 mL of concentrated hydrochloric acid.
- Mandelin's reagent: dissolve 0.5 g of ammonium vanadate in 1.5 mL of water and dilute to 100 mL with concentrated sulfuric acid. Filter the solution through glass wool.
- Sulfuric acid (9 mol/L).

Reference solutions Prepare solutions in methanol (1 mg/mL) of authentic samples of drugs as listed in Table 1.10.

Method

- Divide a TLC plate (silica-gel G, 250 µm) into eight equal columns by scoring lines with a spatula.
- Apply 10 µL aliquots of the authentic solutions and 25 µL aliquots of the sample extract to the columns on the plate in the sequence shown in Table 1.10.
- Evaporation of the spots can be hastened by the use of a cold-air blower.
- Develop the plate for a distance of about 10 cm from the origin in a tank that contains a 100 : 1.5 mixture of methanol–strong acetic acid (system TA). After development, remove the plate from the tank and dry under a stream of cold air until the plate no longer smells of ammonia. (Avoid using hot air to dry the plate, as this may volatilise certain drugs.)
- Alternatively, system TB or system TC may be used.

Visualisation

- Examine the plate under UV light ($\lambda = 254$ and 350 nm).
- Cover columns 3 to 8 with a glass plate and spray columns 1 and 2 with acidified iodoplatinate solution. Most basic drugs give violet or blue colours.
- Cover columns 1 and 2 and 5 to 8 with glass plates, and spray columns 3 and 4 lightly with Mandelin's reagent. Various colours are given by many basic drugs (see Chapter 30).
- Cover columns 1 to 4 and 7 and 8, and spray columns 5 and 6 lightly with 9 mol/L sulfuric acid. Most phenothiazines are extensively metabolised, and urine extracts yield a number of spots on the chromatogram with colours ranging from pink to blue.
- If a pure solution of a suspected drug has been applied to column 7, spray this and column 8 with a reagent with which it is known to react. Alternatively, if the R_f values and spray reagent reactions derived from columns 1 to 6 suggest the presence of a drug for which a further detection reagent exists, use this reagent on column 8 to obtain additional evidence.

Details of R_f values and spot colours are given in Table 1.11. Acidified iodoplatinate solution reacts with many basic drugs to give violet or blue

Table 1.10 Sequence of application of basic extract to TLC plate

Column number	*Solution*
1	Codeine
2	Sample extract
3	Amitriptyline and nortriptyline
4	Sample extract
5	Chlorpromazine
6	Sample extract
7[a]	Suspected drug
8	Sample extract

[a]Column 7 is reserved for an authentic solution of any basic drug that may be suspected on clinical or circumstantial evidence.

Table 1.11 TLC data for some common basic drugs

Compound	R_f *values in systems*			*Acidified iodoplatinate*	*Mandelin's reagent*		*Metabolites in system TA*
	TA	*TB*	*TC*		*Visible*	*Ultraviolet (350 nm)*	
Maprotiline	15	17	05	—	—	—	
Protriptyline	19	17	07	Violet	Pink	Green	
Desipramine	26	20	11	Violet	Blue	—	
Dihydrocodeine	26	08	13	Blue	White	—	One at R_f 16; drug and metabolite have elongated spots
Codeine	33	06	18	Blue	—	—	
Nortriptyline	34	27	16	Violet	Violet	Yellow (violet centre)	
Morphine	37	00	09	Blue	—	—	
Promazine	44	41	30	Green	—	—	Many, which give pink or blue spots with 9 mol/L sulfuric acid
Chlorphenamine	45	33	18	Violet	—	—	One, below the parent drug
Imipramine	48	49	23	Violet	Blue	Quenches	Desipramine; a second metabolite sometimes occurs between imipramine and desipramine
Methadone	48	61	20	Pink (grey rim)	—	—	Methadone degradation product at R_f 15
Procyclidine	48	63	31	Violet	—	—	
Thioridazine	48	43	30	Brown (blue rim)	Blue (violet rim)	Quenches	Pair of blue spots, with pink spots above and below with Mandelin's reagent
Chlorpromazine	49	49	35	Violet (blue rim)	Pink	Yellow (weak)	Many, which give pink or blue spots with 9 mol/L sulfuric acid
Promethazine	50	37	35	Violet (blue rim)	Pink	—	One, below the parent drug
Quinine	50	02	11	Violet	—	Blue (strong)	One immediately below and one immediately above the parent drug; both strongly fluoresce
Amitriptyline	51	55	32	Violet	Violet	Yellow (violet centre)	Nortriptyline; a second metabolite sometimes occurs between amitriptyline and nortriptyline
Clomipramine	51	54	34	Violet	Blue	Quenches	One or two, both below the parent drug
Dosulepin	51	50	42	Red (blue rim)	White	Blue (weak)	One or two, both below the parent drug
Doxepin	51	52	37	Violet	Grey	Blue (orange rim)	One or two, both below the parent drug
Pethidine	52	37	34	Violet	—	—	One, below the parent drug
Dibenzepin	54	20	35	Violet	Blue	Quenches	Two, both just below the parent drug
Nicotine	54	39	35	Brown	—	—	Metabolites coalesce to give spot at R_f 60
Opipramol	54	06	22	Blue	Yellow	Green	
Diphenhydramine	55	45	33	Violet	—	—	One or two, both just below the parent drug
Orphenadrine	55	48	33	Violet	Yellow	Blue	One, below the parent drug and with the same reactions to Mandelin's reagent
Chlorprothixene	56	51	51	Violet	Pink	Orange	
Cyclizine	57	49	41	Violet (blue rim)	—	—	One, below the parent drug
Mianserin	58	39	58	Blue	Violet	Quenches	Two, below the parent drug
Butriptyline	59	61	48	Pink	Grey	Green	One or two, both below the parent drug
Trimipramine	59	62	54	Violet	Blue	Quenches	One or two, both below the parent drug
Carbamazepine	60	04	56	—	Yellow (blue rim)	Green (strong)	
Pentazocine	61	15	12	Violet	Grey	White	
Dextropropoxyphene	68	59	55	Violet	Grey	—	Several; one at R_f 40 which gives a blue streak with acidified iodoplatinate solution; the parent drug is rarely seen in urine extracts
Lidocaine	70	35	73	Blue	—	—	Metabolites of lidocaine are not extractable from urine
Buclizine	75	61	83	Red	—	—	

colours. False-positive reactions can occur with endogenous urine components; urine extracts from heavy smokers contain nicotine, the metabolites of which coalesce to give a brown spot. Mandelin's reagent reacts with fewer compounds, but gives more distinct colours and some spots exhibit characteristic fluorescence under UV light. The presence of drug metabolites in urine extracts can result in a characteristic pattern of spots on the chromatogram.

Mobile phase A mixture of ethyl acetate–cyclohexane–methanol–25% (v/v) aqueous ammonia (70 : 15 : 10 : 5) (for basic neuroleptic drugs).

Chromatography

- Apply two 5 µL portions of the extract to a plastic silica-gel plate F 254 nm (10 cm × 20 cm) and the same amount of a standard mixture of drugs.
- Allow the solvent front to travel 8 cm, remove from the tank and dry with a hot-air blower.
- Remove any residual traces of ammonia by heating the plate at 100°C for 2–5 min.

Visualisation procedure

- Examine the plate under UV light (254 nm; see Table 1.12).
- Cut the plate into two sections and spray one half with acidified iodoplatinate reagent and the other with Mandelin's reagent and examine the plates in daylight (Table 1.12).
- Heat the Mandelin-sprayed section at 100°C for 5 min and re-examine (Table 1.12).

If after TLC, the same extract is used for GC analysis, an acid hydrolysis step (10 mL urine refluxed with 3 mL 36% hydrochloric acid for 15 min; neutralised) has to be carried out before extraction.

Gas–liquid chromatography screening for alcohols and other volatile substances

In normal practice it is advisable to measure the more volatile alcohols (methanol, ethanol, acetone and isopropyl alcohol) separately from the higher alcohols, trichloroethanol and the metabolites of GHB, but for screening it is possible to detect all at two different temperature steps.

Standard solutions in water

- Ethanol: 0.5, 1.0, 2.0, 3.0 and 5.0 g/L.
- Acetaldehyde, acetone, methanol and isopropyl alcohol: 0.1, 0.3, 0.5, 1.0 and 2.0 g/L.
- Ethylene glycol, propylene glycol and trichloroethanol: 0.05, 0.1, 0.2, 0.5 and 1.0 g/L.

Internal standard solution in water

- 0.3 g/L propan-1-ol or 0.1 g/L propylene glycol.

Apparatus

- Gas chromatograph with flame ionisation detector (FID) on 275°C.
- Column DBWAX (60 m × 0.53 mm i.d., 1.0 µm; Megabore J&W Scientific, 125-7062; Fisons).
- Injector temperature 250°C, split mode; He, 4.3 mL/min. He 3.0 mL/min; headspace injector at 60°C.
- Temperature programme: 100°C for 5 min to 175°C for 15 min.

Table 1.12 Thin-layer chromatography of some neuroleptic drugs

Drug	*hR_f*		*Acidified iodoplatinate*		*Mandelin's spray*	
	8 min	*254 nm*	*Daylight*	*Daylight*	*5 min, 100°C, daylight*	*5 min, 100°C, 350 nm*
Alimemazine	79	Positive	Positive	Red	Red	Red–purple
Amitriptyline	69	Purple	Brown	Grey	Blue–grey	Yellow–red
Chlorprothixene	73	Purple	Brown	—	—	—
Citalopram	55	Deep blue	Brown	—	White	Pink fluorescence
Clomipramine	70	Deep blue	Brown	Blue	Blue	Green
Clozapine	55	Deep blue	Brown	Red–brown	Grey	Rose
Desipramine	37	Purple	Brown	Blue	Brown–blue	Yellow
Nordoxepin	39	Purple	Brown	—	—	—
Doxepin	64	Purple	Brown	Yellow–grey	Brown	Brown–red
Fluphenazine	50	Purple	Brown	Orange	Red	Red–purple
Fluoxetine	42	Deep blue	Yellow–brown	White	White	Yellow
Fluvoxamine	44	Deep blue	Yellow–brown	—	White	Purple
Imipramine	65	Purple	Brown	Blue	Brown–blue	Yellow
Levomepromazine	73	Purple	Brown	Purple–brown	Blue	Red–purple
Maprotiline	35	Positive	Brown	Not visible (grey)	Beige–brown	Red–brown
Nefazadon	63	Deep blue	Yellow	Pink–red	Grey–pink	Pink
Norfluoxetine	40	Deep blue	Red–brown	White	White	Yellow
Nortriptyline	42	Purple	Brown	Grey	Brown–blue	Yellow fluorescence
Olanzapine	53	Deep blue	Brown	Grey	Grey	Blue
Paroxetine	35	Deep blue	Brown	Blue	Green	Blue
Perazine	46	Purple	Blue	Orange–grey	Red	Red–purple
Pericyazine	45	Blue	Brown	Brown	Brown	Positive
Perphenazine	36	Purple	Blue	Pink	Red	Red–purple
Promazine	60	Purple	Blue	Orange	Red	Red–purple
Protriptyline	35	Blue	Brown	Positive	Positive	Blue
Thioridazine	65	Purple	Brown	Blue	Blue	Positive
Trifluoperazine	52	Purple	Brown	Brown	Brown	Positive
Trimipramine	78	Purple	Brown	Blue	Blue	Yellow fluorescence
Venlafaxine	69	Deep blue	Yellow	Grey–pink	Grey–pink	Pink

Assay
- Pipette 0.1 mL of whole blood (serum or urine), or aqueous standard into a tube of about 1.5 mL with a stopper.
- Add 0.5 mL of internal standard solution.
- Close the tube and vortex.
- Inject 0.2 μL onto the gas chromatograph.
- Calculate the retention times and prepare a calibration curve of concentration versus peak height ratios of the standards and the internal standard.

The elution order of the compounds is: acetaldehyde (about 3.3 min), acetone, methanol, propan-2-ol, ethanol (about 4.1 min), propan-1-ol (internal standard, about 5.0 min), trichloroethanol, propylene glycol and ethylene glycol. Acetonitrile may have the same retention time as ethanol.

Gas chromatographic screening for drugs

Gas–liquid chromatography (GLC) with capillary columns and a nitrogen–phosphorus detector (NPD), or with an electron capture detector (ECD) in series, is a powerful screening system that is sensitive enough to detect many of the compounds of interest in small samples of serum, plasma or whole blood, as well as in urine specimens.

Much greater selectivity and specificity are obtained by coupling the gas chromatograph to a mass spectrometer (see Chapter 37).

Solvent extraction of serum, plasma or whole blood for basic drugs
- To 1 mL of plasma add 0.1 mL of the internal standard (3.0 mg promazine hydrochloride per litre of water), 0.2 mL of 1 mol/L sodium hydroxide and 10 mL of a mixture of hexane–isoamyl alcohol (99 : 1 v/v).
- Vortex or shake mechanically for 5 min.
- Centrifuge at 2000*g* for 10 min.
- Place the tubes into a freezing bath at −40°C until the water phase is frozen, or remove the intermediate and water phases by suction.
- Transfer the organic layer into a clear test-tube and evaporate to dryness under a stream of nitrogen in a water-bath at about 60°C.
- Redissolve the extract in 100 μL of methanol.
- Inject 3 μL onto the GC column (splitless with a delay of 30 s at 250°C).

Chromatography
- Column: Cp-SiL 5 CB (10 m × 0.32 mm i.d., 0.12 μm) or Cp-SiL 19 CB (10 m × 0.32 mm i.d., 0.19 μm).
- Temperature programme: 130°C to 230°C at 100°/min for 30 s.
- Gas flow rates: bypass He 30 mL/min, septum purge He 5 mL/min, overall He 150 mL/min and column flow He 1 mL/min.
- Detector: NPD (300°C).

HPLC screening using the systematic toxicological identification procedure

The systematic toxicological identification procedure (STIP) system (system HZ) is based on a rapid and simple extraction method followed by isocratic reversed-phase HPLC with diode-array detection (DAD). A library of retention times and UV spectra is available for about 400 common drugs. A disadvantage of the system is that a large number of drugs elute between 1 and 3 min and this problem is exacerbated with substances devoid of a characteristic UV spectrum (e.g. maximum <210 nm). In such cases a second chromatographic analysis may be required. The technique is also less sensitive than GC screening methods.

Extraction procedure This is applied to serum or plasma to prepare extracts of both acidic and basic drugs:
- Add 1 mL acetonitrile to 1.0 mL of sample (serum or plasma) and vortex for 2 s.
- Add 6 mL of dichloromethane and vortex for a further 2 s.
- Add 100 μL of 2 mol/L hydrochloric acid or 2 mol/L of sodium hydroxide.
- Mix gently for 2 min and centrifuge.
- Remove the water and protein layer and transfer the organic layer into a new tube.
- Evaporate the organic layer to dryness at 40°C under a steam of nitrogen.
- Dissolve the residue in 100 μL of the mobile phase.
- Inject an exact volume of 40 μL onto the HPLC column (the acidic extract and the basic extract are analysed consecutively).

STIP column Lichrospher RP–18e; (125 mm × 4.0 mm i.d., 5 μm; Merck, Darmstadt, no. 21568).

Mobile phase
- Mix 530 mL of ultrapure water, 146 μL of triethylamine and about 750 μL of phosphoric acid (85%). (Note that the number of free silol groups differs with each column batch. The column supplier should therefore provide the exact volume of phosphoric acid per column for neutralising the free silol groups.)
- Add 10% (w/v) potassium hydroxide to pH 3.3.
- Add 470 mL acetonitrile (pH ± 4.0), de-gas (e.g. by sonication); flow rate 0.6 mL/min.

The retention times in the library and on the chromatogram have to be the same (window <10%), otherwise the retention times have to be corrected by changing the phosphate concentration or the flow rate of the mobile phase.

Detection The diode array spectra are matched with the reference spectra present in the database. The retention times and UV maxima of drugs eluted with the STIP mobile phase are given in the Index of High Performance Liquid Chromatographic Data in volume 2.

Screening and quantification using LC-MS(-MS)

The following LC-MS(-MS) method can qualitatively and quantitatively determine over 100 drugs and metabolites in serum or plasma with the use of one precipitation/dilution step. In comparison with the STIP procedure it provides faster analyses with lower limits of quantification, less analytical interference and more selectivity, and is sensitive enough to detect and quantify drugs with low molecular weights (100–500). Moreover, assays are generally linear over larger ranges than with HPLC-DAD methods. The method described uses the mass of the mother ion and just one transition (daughter) ion and is suitable for both clinical toxicology and therapeutic drug monitoring applications. In laboratories with a high throughput, the reduction in reagent usage, simplified and more rapid sample preparation, and shorter analysis times and subsequent lower operational costs compensate for the initial high outlay on the equipment.

As LC-MS has fewer disadvantages than HPLC-DAD and as LC-MS (-MS) is superior to all other chromatographic systems, we describe just LC-MS(-MS).

Apparatus Fisher Scientific LC Triple Quad Mass detector system consisting of a Surveyor MS pump, a Surveyor plus Autosampler and a TSQ Quantum Mass Selective Detector with instrument control and data analysis for the mass spectrometer and related instruments provided by Xcalibur software, all obtained from Thermo Fisher Scientific (Waltham, MA, USA).

Other LC-MS(-MS) systems can be used with approximately the same degree of sensitivity.

Mass detector settings
- ESI, positive ion mode
- Spray voltage 3500 V
- Sheath gas (high-purity N_2) pressure 35 arbitrary units (AU)
- Auxiliary gas (high-purity N_2) pressure 5 AU
- Collision gas (Ar) pressure 1.5 mtorr
- Capillary temperature 350°C.

Sample analyses are performed in the selected-reaction monitoring mode (SRM) with a dwell time of 0.1 s per mass unit and the scan width set at *m/z* 0.5.

Column Silica-based polar end-capped C_{18}; (50 mm × 2.1 mm i.d., 5 μm; HyPurity Aquastar, Interscience, Breda, The Netherlands).

Mobile phase Acetonitrile–water–buffer (5 g ammonium acetate, 35 mL 100% acetic acid, 2 mL trifluoroacetic acid made up to 1000 mL with water (pH 3.4–3.60)).

Table 1.13 Chromatographic gradient

Time (min)	*TFA buffer (%)*	*Water (%)*	*ACN (%)*
0.00	5.0	95	0.0
2.00	5.0	0.0	95
3.00	5.0	0.0	95
3.10	5.0	95	0.0
3.60	5.0	95	0.0

Chromatographic separation is performed by means of a gradient with a flow rate of 0.3 mL/min and an analysis time of 3.6 min (Table 1.13).

Assay

- To 100 µL of serum or edetate plasma in an HPLC vial, add 750 µL of protein precipitation reagent (acetonitrile–methanol (840 : 160)) containing a suitable internal control substance (e.g. 0.04 mg/L cyanoimipramine or a deuterated substance).
- Vortex for 1 min and store at −20°C for at least 10 min to stimulate protein precipitation.
- Vortex for a further 1 min; centrifuge at 11 000*g* for 5 min
- Using the auto-injector, inject 5 µL of the clear supernatant onto the HPLC column every 3.6 min.

Notes: (1) A mixture of methanol and water (50 : 50) is used as the wash solvent for the injector. (2) Owing to the high sensitivity of the LC-MS(-MS) system it may be necessary to reduce the volume of sample extracted to 10 µL for some substances.

Calibrators and quality control samples Stock solutions of drugs or metabolites are prepared and spiked into blank serum or edetate plasma samples to yield calibration and quality control standards at appropriate concentrations which are carried through the analytical procedure.

Ionisation suppression TFA is known to cause signal suppression during ionisation (Annesley 2003), but it provides superior peak shapes in comparison to other volatile acids. When TFA is used at low concentrations, the positive effect on the peak shape overrules the negative effect of the signal suppression. Another source of ion suppression is the matrix effect and any method developed must always be tested for this eventuality. Possible ways to resolve ion suppression are: switching to atmospheric pressure chemical ionisation (APCI), changing ionisation polarity, improving the sample preparation procedure and/or changing the chromatographic conditions (Jessome, Volmer 2006; Leverence et al. 2007).

Interpretation of chromatograms It is strongly advised that the retention times and peak shapes of the chromatograms are always visually checked by an experienced analyst. The peak height of the internal control should be approximately the same (within ±10%) during the whole analytical run to assure that no preparation errors have occurred and the LC-MS(-MS) system is working correctly.

As stated previously, the method is designed to be operated throughout the day in high-volume laboratories dealing primarily with therapeutic drug monitoring assays. Urgent clinical toxicology assays can be given priority at any time by preparing the patient sample, a calibration standard and a control standard, and the results are available to the clinician within 45 minutes.

Drugs and active metabolites analysed by the described LC-MS(-MS) method are indicated in Table 1.14.

Table 1.14 Drugs and active metabolites analysed by the described LC-MS(-MS) method[(a) (b)]

Substance (or metabolite)	*Sample volume (µL)*	*Parent mass (amu)*	*Product mass (amu)*	*CE (eV)*	*LOQ (µg/L)*
Alprazolam	100	309.1	281.1	28	1
Amantadine	100	152.0	135.1	19	50
Amphotericin B	100	924.5	743.3	23	100
Amitriptyline	100	278.2	233.1	18	5
Amlodipine	100	409.2	238.0	11	5
Amprenavir	10	506.3	245.1	17	200
Aripiprazol	100	448.1	285.0	28	10
Atazanavir	10	705.4	168.0	41	160
Baclofen	100	214.0	151.0	19	2
Biperiden	100	312.2	98.1	21	2
Bromazepam	100	316.0	182.0	31	50
Bromperidol	100	420.0	165.0	27	0.5
Bupivacaine	10	289.2	140.1	20	50
Chlordiazepoxide	100	299.9	227.0	27	50
Desmethyl-chlordiazepoxide	100	286.0	233.1	36	50
Chlorprothixen	100	316.0	271.0	21	5
Citalopram	100	325.1	262.0	20	5
Desmethylcitalopram	100	311.2	262.1	18	10
Clarithromycin	10	748.5	590.2	18	100
Hydroxyclarithromycin	10	764.4	606.2	20	100
Clindamycin	10	425.2	126.1	26	0.6
Clobazam	100	301.0	259.0	21	10

table continued

Table 1.14 continued

Substance (or metabolite)	*Sample volume (μL)*	*Parent mass (amu)*	*Product mass (amu)*	*CE (eV)*	*LOQ (μg/L)*
N-Desmethyl-clobazam	100	287.0	245.0	21	10
Clomipramine	100	315.2	227.0	47	5
N-Desmethyl-clomipramine	100	301.2	227.0	37	10
Clonazepam	100	316.0	270.1	23	10
(Zu)Clopenthixol	100	401.1	356.2	21	1
Clozapine	100	327.2	269.9	23	15
Desmethyl-clozapine	100	313.2	269.9	23	15
Colchicine	100	400.2	358.1	23	—
Cortisone	100	361.1	121.1	51	50
Darunavir	100	548.3	392.1	14	300
Demoxepam	100	286.9	207.1	34	20
Desipramine	100	267.2	72.2	14	5
Dexamethasone	100	393.1	355.1	14	50
Diazepam	100	285.1	193.1	35	20
Diltiazem	100	415.2	178.0	22	10
Diphenhydramine	100	256.2	167.0	15	5
Etravirine	100	435.1	303.9	37	200
Flecainide	10	415.0	398.0	24	10
Flucloxacillin	100	454.1	160.0	16	200
Fluconazole	10	307.2	219.9	20	500
Fluoxetine	100	309.9	148.2	10	10
Desmethyl-fluoxetine	100	296.0	134.2	11	10
Flunitrazepam	100	314.0	268.1	26	10
Flupethixol	100	435.0	264.9	35	1
Flurazepam as *N*-desalkyl-flurazepam	100	288.9	140.0	32	10
Fluvoxamine	100	318.9	200.1	21	5
Haloperidol	100	378.1	165.0	22	1
Hydrocortisone	100	404.1	363.2	11	50
Imipramine	100	281.2	86.2	16	5
Indinavir	10	614.3	421.1	32	300
Itraconazole	100	705.2	392.0	36	100
Hydroxy-itraconazole	100	721.2	408.0	42	100
Lamotrigine	10	256.2	210.9	28	500
Levomepromazine	100	329.2	100.1	21	5
Lopinavir	10	629.5	155.0	39	350
Lorazepam	100	320.9	274.9	26	20
Lormetazepam	100	335.1	289.0	27	2
Maprotiline	100	278.2	250.1	18	10
Desmethyl-maprotiline	100	264.2	169.1	18	5
Metoclopramide	100	300.1	227.0	18	10

Table 1.14 continued

Substance (or metabolite)	*Sample volume (μL)*	*Parent mass (amu)*	*Product mass (amu)*	*CE (eV)*	*LOQ (μg/L)*
Metoprolol	100	268.1	159.1	21	5
Mianserin	100	265.1	208.1	22	5
Desmethyl-mianserin	100	251.0	208.1	18	10
Midazolam	100	325.8	291.0	24	10
1-Hydroxy-midazolam	100	342.0	168.0	34	10
4-Hydroxy-midazolam	100	342.0	297.0	28	—
Mirtazepine	100	266.2	195.0	30	5
Desmethyl-mirtazepine	100	252.1	195.0	22	5
Moclobemide	10	268.9	139.0	33	—
Mycophenolic acid	100	321.0	207.0	24	200
Nelfinavir	10	568.2	330.0	32	150
M8-Nelfinavir	10	584.4	330.0	32	40
Nevirapine	10	267.0	226.1	26	200
Nitrazepam	100	282.1	236.1	24	10
Nordiazepam	100	270.9	140.0	29	20
Nortriptyline	100	264.2	233.1	15	5
Olanzapine	100	313.2	256.1	24	5
Oxazepam	100	287.0	241.0	23	40
Paroxetine	100	330.2	192.1	21	10
Pericyazine	100	365.9	142.1	24	3
Perphenazine	100	404.1	171.1	23	0.75
Phenprocoumon	100	280.9	203.0	16	100
Pipamperone	100	376.2	291.0	16	5
Pimozide	100	461.9	328.2	28	4
Posaconazole	100	701.3	614.2	33	100
Prazepam	100	325.0	271.0	24	30
Prednisolone	100	361.1	147.1	21	20
Methyl-prednisolone	100	375.1	161.1	21	50
Prednisone	100	359.1	237.1	23	40
Promethazine	100	285.1	198.0	30	—
Propafenone	10	342.2	116.1	22	50
Hydroxy-propafenone	10	358.1	116.1	24	25
Propranolol	100	260.2	155.0	25	—
Quetiapine	100	384.1	253.0	23	5
Rifampicin	10	823.3	791.3	17	200
Desacetyl-rifampicin	10	781.4	749.2	20	200
Risperidone	100	411.2	191.1	32	2
9-Hydroxy-risperidone	100	427.2	207.0	28	2
Ritonavir	10	721.2	296.0	19	200
Ropivacaine	100	275.0	126.2	22	50

table continued

Table 1.14 continued

Substance (or metabolite)	*Sample volume (μL)*	*Parent mass (amu)*	*Product mass (amu)*	*CE (eV)*	*LOQ (μg/L)*
Saquinavir	10	671.3	570.2	30	40
Scopolamine	100	303.9	138.1	21	8
Sertraline	100	305.9	158.9	32	5
Desmethyl-sertraline	100	292.0	158.9	27	510
Strychnine	100	335.2	184.0	39	—
Temazepam	100	301.1	255.0	24	20
Tipranavir	10	603.2	411.0	22	200
Tramadol	100	264.2	264.2	10	25
Tipranavir	10	603.2	411.0	22	200
Trazodone	10	372.0	176.0	24	50
Trimipramine	100	295.2	100.1	16	—
Desmethyl-trimipramine	100	281.2	86.1	16	—
Venlafaxine	100	278.2	121.1	31	20
o-Desmethyl-venlafaxine	100	264.2	107.1	40	20
Verapamil	100	455.5	165.1	28	20
Desmethyl-verapamil	100	441.2	165.0	24	20
Voriconazole	100	350.0	281.1	17	100
Zolpidem	100	308.1	235.1	34	10

[a]MS settings: CE = collision energy (eV); LOQ = lower limit of quantification.
[b]For the therapeutic and toxic ranges see www.bioanalysis.umcg.nl.

Tests for specific compounds and groups of compounds

Alcohols, acetone, acetaldehyde and glycols

Ethanol is frequently taken at the same time as other drugs and can intensify the action of depressant drugs. A blood-ethanol determination helps to distinguish this from normal alcoholic intoxication; it is also useful in the clinical assessment of unconscious patients admitted with head injuries and smelling of drink, especially since younger and younger children are binge drinking. Children are particularly at risk from hypoglycaemia following the ingestion of alcohol. Methanol is available in a variety of commercial products (antifreeze preparations, windscreen washer additives, duplicating fluids). Acetone is sometimes consumed by alcoholics as a substitute for ethanol; children may take nail cleaner fluid; diabetics may be comatose from high endogenous acetone levels. Acetone is also a metabolite of isopropyl alcohol. It can be useful to measure acetaldehyde as a toxic metabolite of ethanol, since some patients are unable to metabolise this compound for genetic reasons or because of an interaction with disulfiram, metronidazole, tolbutamide, watercress and other substances. Acetaldehyde is also a major metabolite of paraldehyde. Ethylene glycol is a principal component of automotive antifreeze products. Poisoning by either methanol or ethylene glycol is often associated with severe metabolic acidosis and electrolyte imbalance; therapy with ethanol infusions or other antidotes must be instituted without delay.

Enzymatic assays based on ADH and breath analysers are applicable only to ethanol; a qualitative and quantitative GLC method is required for the other alcohols.

Gas chromatography of alcohols

- Column: 0.3% Carbowax 20M on 80–100 mesh Carbopak C (2 m × 2 mm i.d.) glass
- Column temperature: 120°C
- Carrier gas flow rate (N_2): 30 mL/min
- Detection: FID.

Reference solutions Prepare an aqueous solution containing 0.2 mg/mL each of ethanol, methanol, propan-2-ol and propan-1-ol and inject 1 μL onto the column. The retention times for these compounds are listed in Table 1.15.

Identification assay

- Add 50 μL of whole blood or urine to 0.5 mL of distilled water in a stoppered test-tube.
- Vortex for 10 s.
- Inject 1 μL onto the column.
- Identify any peaks that appear by reference to the standard chromatogram.

Quantification

Ethanol standard solutions Dilute 8 g of ethanol in 100 mL of distilled water to give a stock solution of 80 g/L. Dispense 100, 250, 500, 750 and 1000 μL volumes into 20 mL graduated flasks using an accurate microsyringe and fill to the mark with distilled water. This gives standard ethanol solutions containing 0.4, 1, 2, 3 and 4 g/L.

Methanol standard solutions Dilute 8 g of methanol in 100 mL of distilled water to give a stock solution of 80 g/L. Dispense 50, 150, 250, 500 and 1000 μL volumes into 20 mL graduated flasks using an accurate microsyringe and fill to the mark with distilled water. This gives standard methanol solutions of 0.2, 0.6, 1, 2 and 4 g/L.

Table 1.15 Retention times of alcohols relative to propan-1-ol

Alcohol	*Relative retention time*
Water	0.30
Methanol	0.36
Ethanol	0.50
Propan-2-ol	0.79
Propan-1-ol	1.00

Store the standards at 4°C and prepare fresh from the stock solutions at least once a week.

Internal standard Add 100 µL of propan-1-ol to 500 mL of distilled water to give a solution containing 0.16 g/L.

Assay

- Add 50 µL of sample and 50 µL aliquots of standards to 0.5 mL of internal standard solution.
- Mix for a few seconds.
- Inject 1 µL onto the column.
- Construct a calibration graph of concentration versus the peak-height ratios of the ethanol or methanol standards to the internal standard and read off the ethanol or methanol concentration in the sample.

Accuracy is improved by injecting duplicates and calculating mean peak-height ratios.

Ethylene glycol and diethylene glycol in serum by HPLC

- Apparatus: HPLC with UV detection ($\lambda = 238$ nm)
- Column: RP 8 select B column (4 mm × 150 mm i.d.)
- Mobile phase: methanol–water (72 : 28 v/v); flow rate 1.5 mL/min.

Assay

- Add 0.1 mL internal standard solution (1 mg/mL propane-1-3-diol in water) to 0.1 mL of sample and mix.
- Add 0.2 mL of 5 mol/L sodium hydroxide and 5 µL benzoyl chloride.
- Close the test-tube and mix for exactly 10 min.
- Wait for exactly 10 min.
- Add 0.4 mL pentane; vortex for 5 min and centrifuge for 3 min at 3000*g*.
- Inject 20 µL of the pentane layer onto the HPLC.

The retention time of the diethylene glycol derivative is about 3 min, and of the ethylene glycol derivative about 4 min.

Alcohols in serum by osmolality If no specific assay for alcohols is available, the osmolal gap should be measured:

$$(\text{measured mOsmol/kg in patient's serum}) - (\text{calculated osmolality}/0.93) = \text{osmol gap}$$

- In practice, osmol gap = measured mOsmol/kg − 290.
- Milligrams of alcohol per litre of serum = osmol gap × relative molecular mass.
- Each measured osmol gap unit = F g/L alcohol in serum; $F = 0.026$ for methanol; 0.043 for ethanol; 0.05 for ethylene glycol; 0.055 for acetone; and 0.059 for propan-2-ol.

Screening for abuse of solvents

The term 'glue-sniffing' comes from the abuse of adhesives, which often contain solvents such as toluene, ethyl acetate, acetone or ethyl methyl ketone. These, and similar compounds, also occur in a diverse range of other commercial products that may be abused, such as shoe-cleaners, nail varnish, dry-cleaning fluids, bottled fuel gases (butane and propane), aerosol propellants and fire extinguishers (bromochlorodifluoromethane). The identification, quantification and interpretation of solvents abused are described in detail in Chapter 11.

Antidepressants and antipsychotics

Antidepressants and antipsychotics comprise a diverse group of compounds that includes the tricyclic antidepressants, antipsychotic agents and lithium. Other substances, mainly the newer drugs, include the selective serotonin reuptake inhibitors (SSRIs), monoamine oxide inhibitors (MAOIs) and atypical antipsychotics such as clozapine and olanzapine. Tricyclic antidepressants remain an important cause of suicide, and serious poisoning can lead to cardiac disturbances (increasing QRS-complex), respiratory depression, metabolic acidosis, convulsions and coma. These drugs are gradually being replaced by the less toxic SSRIs such as citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. However, these drugs can induce aggressive and suicidal behaviour. These drugs are also used as drugs of abuse.

Analysis of antidepressants and antipsychotics by GLC To detect the misuse of these drugs, especially the more recent ones and depot preparations, GLC methods have the advantage of producing a lower limit of quantification. Alternative systems are described in Chapter 40.

Extraction procedure

- To 1.0 mL of blood, plasma or urine in a 15 mL Pyrex centrifuge tube, add 1.5 mL of ammonium chloride buffer (pH 9.5) and 5 mL of the extraction solvent (chloroform–propan-2-ol–*n*-heptane (10 : 14 : 26 v/v/v)).
- Vortex for 1 min; centrifuge at 2000*g* for 5 min.
- Remove the aqueous layer (by suction or freezing).
- Transfer the organic layer to a clean brown glass test-tube and evaporate to dryness under a stream of nitrogen in a water-bath at about 60°C.
- Redissolve the extract in 100 µL of methanol.
- Inject 3 µL onto the GC column.

Chromatography

- Column: Cp-SiL 5 CB (10 m × 0.32 mm i.d, 0.12 µm) or Cp-SiL 19 CB (10 m × 0.32 mm i.d, 0.19 µm).
- Temperature programme: 130°C to 230°C at 100°/min for 30 s.
- Gas flow rates: bypass He 30 mL/min, septum purge He 5 mL/min, overall He 150 mL/min, and column flow He 1 mL/min.
- Detector: NPD (300°C).

The retention indices for various substances are given in Table 1.16.

Table 1.16 Retention indices for antidepressant and antipsychotic drugs and their active metabolites

Name	*Retention index, CP-SiL 5 CB*
Alimemazine	2313
Amitriptyline	2201
Biperiden	2292
Bromperidol	3066
Butriptyline	2201
Chlorpromazine	2500
Chlorprothixene	2523
cis-Flupentixol	3087
Clomipramine	2433
Clozapine	2859
Cyclizine	1943
Desipramine	2251
Desmethylclomipramine	2453
Desmethylmaprotiline	2304
Desmethylmianserin	2214
Desmethylpromethazine	2225
Dextropropoxyphene	2219
Dibenzepin	2456
Diphenhydramine	1867
Dosulepin	2392
Doxepin	2243
Fenfluramine	1252
Fluphenazine	3081
Fluoxetine	1872
Fluvoxamine	1898
Haloperidol	2942
Hydroxyzine	2897
Imipramine	2238
Levomepromazine	2547
Levomepromazine-*S*-oxide	2666
Maprotiline	2350

table continued

Table 1.16 continued

Name	*Retention index, CP-SiL 5 CB*
Meclozine	3054
Melitracen	2306
Mesoridazine	3362
Mianserin	2166
Nomifensine	2068
Nordosulepin	2404
Nordoxepin	2253
Norfluoxetine	1841
Nororphenadrine	1909
Nortriptyline	2216
Orphenadrine	1913
Perazine	2803
Perphenazine	3348
Pericyazine	3256
Pipamperone	3014
Pipotiazine	3888
Prochlorperazine	2939
Promazine	2323
Promethazine	2281
Promethazine sulfoxide	2633
Protriptyline	2260
Sulforidazine	3384
Thioridazine	3120
Thioridazine ring-*S*-oxide	3493
Trazodone	3293
Trifluoperazine	2663
Trifluoperazine-S-oxide	2954
Trifluopromazine	2249
Trimipramine	2251
Zuclopenthixol	3320

Table 1.17 Exceptions to UV maxima at 254 nm

Drug	*UV maxima (nm)*
Butriptyline	265
Clopenthixol	240
cis-Flupentixol	240
Fluoxetine	240
Fluvoxamine	245
Haloperidol	245
Maprotiline	265
Nomifensine	293
Norfluoxetine	240
Paroxetine	293
Pipamperon	244
Pipotiazine	267
Protriptyline	244

Table 1.18 Fluorescence detector in series with UV detector

Drug	*Excitation (nm)*	*Emission (nm)*
Fluoxetine	280	310
Maprotiline	280	310
Orphenadrine	265	310
Protriptyline	280	310
Paroxetine	290	340

Analysis of antidepressants by HPLC

Extraction procedure

- To 1.0 mL of blood, plasma or urine in a 15 mL centrifuge tube, add 1.5 mL of ammonium chloride buffer (pH 9.5) and 5 mL of the extraction solvent (chloroform–propan-2-ol–*n*-heptane (10 : 14 : 26 v/v/v)).
- Vortex for 1 min; centrifuge at 2000*g* for 5 min.
- Remove the aqueous layer (by suction or freezing).
- Transfer the organic layer into a clean brown glass test-tube and evaporate under vacuum or stream of nitrogen to dryness.
- Redissolve the residue in 0.1 mL of the mobile phase.
- Inject 60 µL of the serum extract.

Chromatography

- HPLC apparatus with an isocratic pump.
- Detector: UV (0.01 absorption units full scale, AUFS) and for some drugs a fluorescence detector in series. UV maxima mostly at 254 nm, except those shown in Table 1.17. Optionally, a fluorescence detector in series with UV detector can be used (Table 1.18).
- Column: Microspher Si (100 mm × 4.6 mm, i.d.) (Chrompack, Middelburg, The Netherlands, no. 28400) or Lichrosorb 60 Si5 m (150 mm × 3 mm i.d.) (Merck, Darmstadt, Germany).
- Mobile phase: methanol–dichloromethane–buffer (30% acetic acid–diethylamide (20 : 1 v/v) pH 3.2; 10 : 90 : 1.5); flow rate 1.0 mL/min.
- The flow rate of the mobile phase is 1.0 mL/min, except for hydroxyzine and mianserin when it is 0.8 mL/min, and for chlorphenamine, fluoxetine, fluvoxamine, maprotiline, oxomemazine, pipamperone, protriptyline, tiapride and their main metabolites when it is 1.3 mL/min.
- The limit of quantification (LOQ) for most drugs is 5–10 µg/L of serum or less and 25 µg/L or less for the metabolites. Haloperidol's LOQ is 3 µg/L. The coefficient of variation (CV) in the therapeutic range is mostly 1–5%. The newer SSRIs (fluoxetine, fluvoxamine, paroxetine and sertraline) can also be determined easily and reliably by this method. Antipsychotics not measurable by this method are trazodone, droperidol, penfluridol, fluspirilene and biperiden.

Benzodiazepines, zolpidem and zopiclone

Benzodiazepine tranquillisers are prescribed widely and therefore occur more frequently than any other type of drug in overdose cases. The effects of these drugs in overdose are usually mild, although they may have a synergistic effect when taken with alcohol or other drugs. The anticonvulsive benzodiazepine clonazepam (Rivotril) is also used to detoxify patients with very severe (other) benzodiazepine dependence. Although these drugs do not seem to cause lethal intoxications, reports of deaths from benzodiazepines have been published, most of which refer to elderly people or cases of combined overdose of flunitrazepam and opiates. Over 30 benzodiazepines are available; some of these are both the parent compound and a metabolite of other benzodiazepines. The intrinsic activity varies enormously from one to the other. For example, alprazolam has a therapeutic effect at a serum concentration of 1 µg/L, whereas oxazepam becomes effective only at 1000 µg/L. This phenomenon makes comprehensive screening for the group very difficult. Several of the metabolites (including some glucuronides) are also active. In patients with renal failure the metabolite midazolam glucuronide can still be active even if the parent compound and its hydroxy-metabolite are no longer measurable (see Diagnosis of brain death). Several immunoassays are available to screen for the benzodiazepines in urine. However, in most of these the antibodies do not react with the glucuronides and prior enzyme hydrolysis of the urine is therefore required. The hypnotics zolpidem and zopiclone have similar dynamic and toxic activity to the benzodiazepines. Although these are not benzodiazepines, they exhibit cross-reactivity with some benzodiazepine immunoassays.

Analysis All benzodiazepines and their unconjugated metabolites (except the parent drug potassium clorazepate) are extractable from body fluids into an organic solvent and can be quantified in serum or plasma by normal-phase HPLC with UV detection. GC with ECD can also be used (see Chapter 40). All analytes of benzodiazepines, whatever the matrix (blood, urine) or analytical method (immunoassay or chromatography), require a hydrolysis step (see also Analytical methods below). Smith *et al.* (2006) described as LC-MS(-MS) system for the screening of 30 benzodiazepines.

Benzodiazepines by reversed-phase HPLC

Reagents

- Borax buffer (pH 9.0): 43 mmol/L, 1.621 g disodium tetraborate plus 15 mL of 0.1 mol/L HCl and sufficient water to make 100 mL.
- Buffer (pH 5.9): 1.19 g disodium hydrogenphosphate·$2H_2O$ plus 8.17 g potassium dihydrogenphosphate plus sufficient water to make 1 L. Add 1 mol/L sodium hydroxide or 1 mol/L phosphoric acid to pH 5.9.
- Aqueous methanol: 150 mL of methanol plus 350 mL of water.

Extraction procedure

- Pipette 0.5 mL serum plus 0.2 mL pH 9 buffer into a centrifuge tube.
- Add 0.1 mL internal standard A or 0.2 mL of B and 5.0 mL dichloromethane.
- Vortex for 1 min; centrifuge at 2000*g* for 5 min.
- Remove the aqueous and interface layers.
- Transfer the organic layer to a clean test-tube and evaporate under vacuum or a stream of nitrogen to dryness.
- Redissolve the residue in 0.1 mL mobile phase.
- Inject 50 µL onto the HPLC column.

Table 1.19 gives the retention times.

Internal standard Two internal standards can be used: A, 2.0 mg nitrazepam/L in aqueous methanol; or B, 4.0 mg nordazepam/L in aqueous methanol.

Mobile phase Tetrahydrofuran (THF)–methanol (40 : 60), buffer pH 5.9; flow rate 1.0 mL/min.

Table 1.19 Retention times and LOQ data for benzodiazepines

Benzodiazepine	*Retention time (min)*	*LOQ (µg/L)*	
		254 nm (A)	*310 nm (B)*
Bromazepam	7.4	50	—
Camazepam	26.6	30	—
Chlordiazepoxide	14.4	10	—
Clobazam	10.6	20	—
Clonazepam	9.3	—	10
Demoxepam	7.2	10	—
Desalkylflurazepam	12.5	5	25
Desmethylchlordiazepoxide	11.2	10	—
Desmethylclobazam	8.5	15	—
Desmethylflunitrazepam	8.0	—	3
Diazepam	20.8	10	—
Flunitrazepam	10.0	—	10
Ketazolam	23.0	20	—
Lorazepam	10.8	20	—
Lormetazepam	13.6	2	—
Midazolam	30.0	25	—
Nitrazepam	9.0	—	10
Nordazepam	17.0	10	—
Oxazepam	11.0	5	—
Temazepam	13.2	10	—

Apparatus HPLC with UV detection ($\lambda = 254$ nm (0.02 AUFS) and 310 nm (0.005 AUFS) for clonazepam, nitrazepam and flunitrazepam and their metabolites).

Column Nucleosil $5C_{18}$ (250 × 4.6 mm i.d., 5 µm).

Cholinesterase inhibitors (organophosphate and carbamate pesticides)

There are no simple direct chemical tests for these compounds. The toxic effects are usually associated with depression of the cholinesterase activity of the body, and measurement of the plasma or serum cholinesterase can be used as an indication of organophosphorus or carbamate poisoning. Plasma or serum cholinesterase (pseudocholinesterase) is inhibited by a number of compounds and can also be decreased in the presence of liver impairment. Erythrocyte cholinesterase (true cholinesterase) reflects more accurately the cholinesterase status of the central nervous system. However, pseudocholinesterase activity responds more quickly to an inhibitor and returns to normal more rapidly than erythrocyte cholinesterase activity. Thus, measurement of pseudocholinesterase activity is quite adequate for diagnosing acute exposure to organophosphorus or thiocarbamate compounds, but cases of illness that may be caused by chronic exposure to these compounds should also be investigated by determining the erythrocyte cholinesterase activity. A colorimetric method for this purpose is described in Chapter 16.

Paraquat and diquat

Paraquat (1,1-dimethyl-4,4-bipyridylium chloride) is the most important bipyridyl herbicide. Although deaths are reported from accidental paraquat exposure by inhalation and transdermal absorption, accidental or deliberate intake is nearly always oral ingestion. Diquat is less toxic than paraquat. Granular preparations usually contain 2.5% w/w of paraquat and 2.5% w/w of diquat; liquid preparations may contain 20% w/v of paraquat alone. A qualitative test for paraquat in urine is described earlier in the section 'Paraquat and diquat – dithionite test in urine'. Measurement of the plasma paraquat concentration is a useful prognostic test and Scherrmann *et al.* (1987) published a nomogram of the interrelationship of time after ingestion, plasma concentration and probable outcome. The main use of an assay is to prevent overtreatment of patients who either are not at risk or have no chance of survival. Paraquat can be measured in plasma by immunoassay, although the methods are not widely available. HPLC methods have also been described (see Chapter 16). An alternative colorimetric method is described below.

Quantification of paraquat in plasma by colorimetry This method is based on that of Jarvie and Stewart (1979).

Standard solutions Prepare standard solutions of paraquat in plasma containing 0, 0.1, 0.2, 0.5, 1.0 and 2.0 mg of paraquat per litre; store frozen until required.

Extraction solvent Mix 50 mL of water-saturated isobutyl methyl ketone and 50 mL of water-saturated isobutyl alcohol and then add 0.5 g of sodium dodecylsulfate.

Method

- Add 2 mL of water and 10 mL of the extraction solvent to 2 mL of plasma (or standard solution) in a 15 mL glass-stoppered centrifuge tube.
- Mix gently for 5 min on a roller mixer.
- Centrifuge for 5 min at 1500*g*.
- Carefully transfer 8 mL of the solvent phase into a second tube that contains 0.8 mL of 2.5 mol/L sodium chloride and stopper the tube.
- Vortex for 5 min and centrifuge for 2 min at 1500*g*.
- Carefully remove the solvent phase.
- Transfer 0.7 mL of the aqueous phase into a 4 mL glass tube.
- Add 100 µL of a freshly prepared 3% (w/v) solution of sodium dithionite in 0.3 mol/L sodium hydroxide.
- Shake the mixture briefly and transfer to a semi-micro-quartz cuvette.
- Scan the solution immediately between 384 and 460 nm against a blank that contains 0.7 mL of 2.5 mol/L sodium chloride and 100 µL of the sodium dithionite solution. The absorbance caused by paraquat is obtained by subtracting the reading at 460 nm from that at

397 nm. A more specific assay can be obtained by using the second derivation of the UV maximum.
- The LOQ in serum is 50 μg/L.
- If diquat is also present, it will be extracted and gives rise to additional small peaks between 430 and 460 nm. Corrections can be made for this, but the increase in paraquat concentration is small (10–15%) and is usually insignificant in clinical situations.

Chlorophenoxyacetic acid herbicides

Poisoning with chlorophenoxyacetic acids, such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and methylchlorophenoxyacetic acid (MCPA), causes metabolic acidosis, myoglobinuria, rhabdomyolysis, elevated liver function tests, hypophosphataemia, miosis and tachycardia (Roberts *et al.* 2005). Plasma levels above 100 mg/L are associated with toxic symptoms.

These compounds can be measured spectrophotometrically at a maximum of about 284 nm, or after acid extraction and methylation by gas chromatography with FID or MS detection (see Chapter 16).

Chlorophenoxyacid herbicides in serum or urine by GLC

- To 1 mL of serum or urine add 0.1 mL of internal standard (2 mg/mL of MCPA or 2,4-D in methanol), 1 mL of 2 mol/L hydrochloric acid, and 5 mL of diethyl ether.
- Vortex for 1 min, centrifuge for 5 min at about 2000*g*.
- Transfer the ether layer to a conical test-tube.
- Add 0.1 mL of methylation reagent (0.2 mol/L trimethyl anilinium hydroxide in a 1 : 1 (v/v) mixture of methanol (freshly prepared)).
- Vortex for 30 s and centrifuge for 5 min at 1500*g*.
- Inject 1 μL of the lower layer onto the GC column.

Chromatography

- Column: 2% OV17 or Gaschrom Q.
- Oven temperature: 205°C.
- Injector temperature: 310°C.
- Detector (FID) temperature, 300°C.

The retention indices are 1605 (2,4-D), 1740 (2,4,5-T) and 1580 (MCPA).

Analgesics: paracetamol, salicylates and other non-steroidal anti-inflammatory drugs

Acute overdose with most of the analgesics rarely causes severe toxicity with the exceptions of paracetamol (acetaminophen) and salicylates.

Paracetamol Paracetamol (acetaminophen) is widely available as an over-the-counter medicine and is frequently taken in overdose. Paracetamol is metabolised by the liver to *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which is normally inactivated by liver glutathione. After paracetamol overdose, the glutathione stores become depleted to leave toxic amounts of NAPQI to bind to proteins and cause centilobular necrosis. Drugs that induce hepatic CYP450 enzymes (e.g. phenobarbital) and chronic high ethanol abuse may enhance paracetamol toxicity. Intravenous infusion of *N*-acetylcysteine to replenish the glutathione stores is an effective treatment, especially when given during the early stages of poisoning. During the first 12 h after ingestion of a severe overdose, no clinical features other than vomiting may occur. After 12 h, hepatic necrosis causes continued vomiting, which may also induce abdominal pain after 24 h. Signs of jaundice become apparent after 36–72 h and the patient may develop hepatic encephalopathy and hepatic failure. Serum or plasma paracetamol measurements play a crucial role in the early diagnosis; management protocols and nomograms that relate these to time after ingestion and the likelihood of developing liver damage have been published (Smilkstein *et al.* 1991). The sample analysed should ideally not be taken until 4 h after ingestion, as prior to this the processes of absorption and distribution are incomplete. However, in practice this is not always feasible since the exact time of ingestion may not be known. Measuring a second paracetamol level about 4 h after the first can be useful, especially in cases of staggered overdose, and can give a better indication of prognosis. A half-life of about 4 h indicates a healthy liver and one of about 12 h predicts a severe necrosis.

Reliable commercial kits are available for paracetamol measurements in serum or plasma, designed for use on routine clinical analysers and based on either immunoassays (Edinboro *et al.* 1991) or enzymatic reactions (Morris *et al.* 1990). Numerous GC and HPLC methods have also been published.

Salicylates Salicylic acid is most often derived from acetylsalicylic acid (aspirin) and severe overdose results in respiratory alkalosis and metabolic acidosis. Children below the age of 4 years are particularly susceptible to salicylate poisoning. Continued absorption of aspirin is common after the initial admission to hospital. Sustained-release salicylate preparations may form concretions in the stomach that result in prolonged absorption as they gradually disintegrate. Application of salicylate-containing ointments to abnormal skin can also lead to significant toxicity, as can the use of teething gels in infants. Chronic salicylate poisoning can occur in rheumatic patients who take large doses of aspirin, and salicylism should be considered in any elderly patient with unexpected delirium or dementia. Ingestion of methyl-salicylate is rare, but it is potentially more dangerous because of rapid absorption. Treatment of severe salicylate poisoning involves sodium bicarbonate infusions, multiple doses of oral-activated charcoal and, in severe cases, haemodialysis. Toxicity is associated with plasma salicylate concentrations of 300 mg/L or greater. Adults with plasma salicylate concentrations less than 450 mg/L and children with plasma salicylate concentrations less than 350 mg/L do not require specific treatment. The slow and continuous absorption of the drug may necessitate repeat plasma salicylate determinations.

Colorimetric assay for salicylates

Standard solutions Prepare aqueous solutions that contain 0, 200, 500 and 800 mg/L of salicylic acid. These should be stored frozen when not in use.

Assay

- Add 5 mL of Trinder's reagent to 1 mL of the serum sample in a 15 mL centrifuge tube.
- Vortex for 30 s and centrifuge for 5 min at 1500*g*.
- Measure the colour intensity of the supernatant liquid at 540 nm.
- Determine the concentration in the sample by comparison with the standard solutions.

Other NSAIDs Other NSAIDs include the arylacetic acids (e.g. diclofenac), arylpropionic acids (e.g. ibuprofen, ketoprofen, naproxen), heterocyclic acetic acids (e.g. indometacin, ketorolac, sulindac), pyrazolones (phenylbutazone), oxicams (e.g. piroxicam) and mefenamic acid. Most patients who take an overdose of these drugs are asymptomatic, but the chronic use of mixtures of analgesic drugs has been linked to renal damage, including papillary necrosis and chronic interstitial nephritis. These compounds are extractable at acidic pHs and most can be determined by the STIP method or other suitable HPLC methods.

Antiepileptics (carbamazepine, oxcarbazepine, phenytoin, phenobarbital, primidone, valproate, ethosuximide, clonazepam, clobazam)

Antiepileptic drugs are commonly prescribed in combination in epilepsy treatment. Symptoms of acute overdose simulate those of barbiturate poisoning. Laboratories that offer a routine therapeutic drug monitoring service for these drugs have little difficulty in adapting their normal procedures to the occasional overdose case (see Chapter 2). Immunoassays for some of the antiepileptic drugs lack linearity and exhibit different cross-reactivities in the toxic range, and HPLC or GC (see Chapter 40) assays are preferred alternatives in toxicological investigations. 10-Hydroxycarbazepine, the active metabolite of oxcarbazepine, can be determined in plasma by the Pinkerton method (detection at 240 nm), as described under caffeine.

Normal-phase HPLC determination of phenobarbital, phenytoin, primidone, carbamazepine, clobazam, diazepam, ethosuximide, clonazepam and nitrazepam

This method uses the same column, reagents and apparatus as the normal-phase HPLC method described for antidepressants above.

Valproic acid determination by GC

Apparatus Gas chromatograph provided with a capillary column, but also an all-glass column (1.8 m × 3 mm i.d., with 5% (w/w) FFAP on Gas-Chrom Q 60/80 mesh). Temperature 170°C. Carrier gas N_2, 30 mL/min.

Internal standard 150 mg cyclohexane carboxylic acid per litre of dichloromethane.

Standards 0, 25, 50, 100 and 200 μg valproic acid per mL of human or calf serum.

Assay
- Pipette 0.2 mL of serum into a nipple tube.
- Add 20 μL 6 mol/L sulfuric acid and 200 μL internal standard solution.
- Vortex for 30 s and centrifuge for 10 min at 1500*g*.
- Inject 2 μL into the gas chromatograph.
- LOQ is 2 mg/L.

This method is also suitable for measuring gamma-hydroxybutyric acid in serum. An alternative method for GHB and its metabolites using micellar kinetic chromatography has been reported by Dahlen and Vriesman (2002).

Reagents
- Buffer: 93.6 g sodium dihydrogenphosphate per L of water (pH 3.3).
- Dichloromethane 50% water saturated: dichloromethane washed with ultrapure water, dried over a molecular sieve (0.4-nm Perl form plus 2 mm; Merck) and mixed with equal parts of water-saturated dichloromethane.
- THF (HPLC grade).

Mobile phase THF–methanol–dichloromethane (50% saturated; 6 : 0.2 : 93.8 v/v/v); degassed; flow rate 1.0 mL/min.

Apparatus HPLC with UV detection ($\lambda = 254$ nm).

Column 150 mm × 3 mm i.d., with regular 5 μm silica gel.

Standards
- In dichloromethane make a stock solution with 2.5 mg diazepam, 2.5 mg desmethyldiazepam, 50 mg carbamazepine, 100 mg phenytoin (as acid), 200 mg phenobarbital (as acid), 400 mg ethosuximide, 0.5 mg nitrazepam and 0.5 mg clonazepam per litre.
- Store in ampoules at −20°C.
- Pipette 0, 50, 100, 200 and 400 μL into separate tubes and add 1.0 mL of human or calf serum; mix.
- Prepare a calibration curve.

Internal standard Use 2 mg hexobarbital/L of dichloromethane.

Assay
- Into a small test-tube or injection vial, pipette 0.25 mL of serum, 0.25 mL of buffer and 2.5 mL of internal standard.
- Vortex for 1 min and centrifuge at 2000*g* for 5 min.
- Inject approximately 50 μL of the lower layer (dichloromethane) into the HPLC column (the needle of the automatic injector is arranged to pass through the serum–water layer into the dichloromethane layer or the water layer can be removed by a vacuum pump).
- If clonazepam or nitrazepam has to be measured, evaporate the dichloromethane layer to dryness under vacuum or a stream of nitrogen at 40°C.
- Redissolve the residue in 50 μL of mobile phase and inject 30 μL into the HPLC column.

Retention times for antiepileptic drugs are given in Table 1.20.

Valproic acid is normally measured by immunoassay (see Chapter 31) or by GC.

Table 1.20 Retention times for antiepileptic drugs

Anticonvulsant	*Retention time (min); strongly dependent on pH of mobile phase*
Trimethadione	1.0
Methylphenobarbital	1.1
Hexobarbital	1.2
Phenobarbital	1.25
Barbital	1.3
Ethosuximide	1.35
Diazepam	1.4
Methylphenytoin	1.4
Heptobarbital	1.4
Phenytoin	1.6
Sulthiame	1.8
Clonazepam	2.0
Nitrazepam	2.15
Nordiazepam	2.6
Carbamazepine	3.0
Carbamazepine-epoxide	(5.6, often undetectable)
7-Aminoclonazepam	8.8
Primidone	No detection

Carbon monoxide

Carbon monoxide is one of the most frequent causes of fatal poisoning. Common sources of carbon monoxide are vehicle exhaust fumes, smoke from fires, and improperly maintained and ventilated heating systems. More rarely, exposure to dichloromethane vapours from paint strippers, degreasing agents and aerosol propellants can lead to carbon monoxide poisoning because the solvent can be metabolised by mixed-function oxidases to carbon dioxide and carbon monoxide. The affinity of carbon monoxide for haemoglobin is 200–300 times that of oxygen and therefore most of the toxic effects result from diminished oxygen delivery to the tissues. Symptoms progress from headache, nausea, gastrointestinal upset, hyperventilation, hypertension and drowsiness to coma. Chronic poisoning as a result of continuous exposure to small amounts of carbon monoxide leads to non-specific symptoms such as headaches, dizziness, fatigue and general malaise, and is often undiagnosed. Elevated carboxyhaemoglobin (COHb) concentrations confirm a diagnosis of carbon monoxide poisoning. When a patient is removed from the contaminated atmosphere, the COHb disappears rapidly, particularly if oxygen is administered. A qualitative test for COHb is described under 'Carbon monoxide in whole blood' in the section 'Direct (colour tests) in serum or urine samples'.

Hospital clinical chemistry laboratories are usually equipped with automated differential spectrophotometers (CO-oximeters) that simultaneously measure the absorption of a blood haemolysate at four or more wavelengths to determine total haemoglobin, the percentage saturation of oxyhaemoglobin and COHb, as well as methaemoglobin and sulfhaemoglobin (Widdop 2002).

If such an apparatus is not available, the spectrophotometric method of Rodkey *et al.* (1979) can be used.

Quantification of carboxyhaemoglobin in blood by spectrophotometry

Principle When a reducing agent (sodium dithionite) is added to the blood, both the oxygenated form and the methaemoglobin are converted quantitatively to the reduced form, which has the visible spectrum B shown in Fig. 1.2. Carbon monoxide has a much greater affinity for haemoglobin than oxygen, and the COHb is not reduced by sodium dithionite. Thus, even when treated with sodium dithionite, COHb retains its normal twin-peaked spectrum, marked A in Fig. 1.2. The maximal difference between the spectra of A and B is at 540 nm, while at 579 nm the spectra have the same absorbance (isosbestic point). The percentage saturation of carbon monoxide in a blood sample (A) can be calculated from measurements of the absorbance of the carbon monoxide-free sample (B) and the untreated sample (C), after reduction of each with sodium dithionite.

Standards Gas bottles of pure carbon monoxide can be obtained. Alternatively, commercial reference standards of haemolysed blood in sealed glass ampoules are available (IL, Warrington, UK).

Assay
- Dilute 0.2 mL of the heparinised whole-blood sample with 25 mL of a 1 mL/L solution of aqueous ammonium hydroxide.
- Mix well.

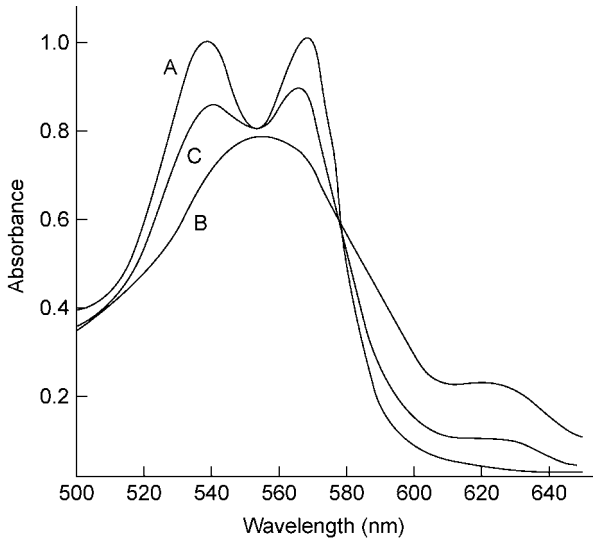

Figure 1.2 Ultraviolet spectra of (A) carboxyhaemoglobin, (B) reduced haemoglobin and (C) a blood sample from a patient poisoned with carbon monoxide.

- Transfer 5-mL aliquots of this mixture into three tubes, labelled A, B and C.
- Saturate A with carbon monoxide by bubbling the gas very slowly through the mixture for 5–10 min (take care to avoid frothing) to give a 100% COHb standard.
- Saturate solution B with pure oxygen by bubbling the gas slowly through the mixture for at least 10 min to displace all the bound CO (again take care to avoid frothing) to give a 0% COHb standard.
- Add to all three tubes a small amount of sodium dithionite (approximately 150 mg) and 10 mL of ammonium hydroxide solution (the sodium dithionite must be freshly obtained or have been stored in a sealed container in a dessicator to prevent inactivation by contact with moist air).
- Vortex for a few seconds.
- Record the absorbances of A, B and C against the ammonium hydroxide solution between 500 and 650 nm and measure the absorbances at 540 nm and at 579 nm. (Wash out the sample cell thoroughly between the recordings and wash the cell with a little of the solution, the absorbance of which is about to be recorded.)
- Calculate the ratios of the absorbances at 540 and 579 nm for each of the solutions A, B and C.
- Calculate the percentage COHb saturation as follows:

$$\%\text{Saturation} = \frac{\text{ratio for C} - \text{ratio for B}}{\text{ratio for A} - \text{ratio for B}} \times 100$$

- Interpretation: normal, <5%; smokers, <10%; headaches, 10–20%; toxic, 20–30%; potentially fatal, >45%.

Metals

The detection of poisoning with toxic metals is an important feature of hospital toxicology; in modern laboratories the favoured techniques are atomic absorption spectrophotometry (AAS) and inductively coupled plasma-MS (ICP-MS). These techniques and their applications are described in Chapters 17 and 43.

Theophylline and caffeine

Theophylline is prescribed to asthmatic children and adults, but serious toxicity can be caused by both therapeutic excess and overdose. Clinical features include severe hypotension, cardiac arrhythmias and convulsions. Biochemical disturbances include hyperinsulinaemia, hyperkalaemia, glycosuria and metabolic acidosis. Many theophylline preparations are of the slow-release type, so that the onset of toxic symptoms may be delayed for up to 12 h following overdose. Treatment consists of gastric lavage for patients who reach hospital within 1 h of the overdose and multiple oral doses of activated charcoal, which is thought to be as efficient as charcoal haemoperfusion as an elimination procedure. The plasma theophylline concentration is an important diagnostic test and should be measured urgently. In asymptotic patients levels should be measured 4 h or more after ingestion.

Caffeine is prescribed for neonatal apnoea. It is also an ingredient of many proprietary stimulant preparations and an important adulterant in drugs of abuse, such as the so-called 'smart drugs'. Some patients have an idiosyncrasy for theophylline or caffeine, developing tachycardia at low serum concentrations. Although the lethal dose is large (about 10 g), severe caffeine intoxication with tachyarrhythmias followed by cardiovascular collapse has caused deaths in children. Caffeine also potentiates the effects of sympathomimetic drugs, which contribute to adverse cardiac disorders.

Commercial immunoassay kits are available to determine theophylline and caffeine in serum or plasma. These drugs can be determined by the STIP chromatography system.

Measurement of xanthines in serum by normal-phase HPLC

Apparatus HPLC with UV detection ($\lambda = 273$ nm).

Column Normal-phase ODS-silica (5 μm).

Mobile phase Methanol–THF–water–acetic acid (100 : 30 : 900 : 1); add sufficient sodium hydroxide solution to give pH 5.6 (pH is critical).

Assay

- To 50 μL of serum (plasma) add 100 μL of internal standard solution (5 mg 8-chlorotheophylline per L of 1 mol/L sodium acetate) and 2 mL of a mixture of chloroform (or dichloromethane) and isopropyl alcohol (1 : 9 v/v).
- Vortex, centrifuge at 1500*g* for 5 min.
- Cool the tube to −40°C to freeze the water layer.
- Transfer the organic layer into a second test-tube.
- Evaporate to dryness under a stream of nitrogen at 50°C or under vacuum.
- Re-dissolve the residue in 20 μL of mobile phase solution.
- Inject 10 μL into the HPLC.

Other xanthines and their metabolites also elute. Retention times relative to theophylline are 0.49 for 3-methylxanthine, 0.63 for theobromine, 0.88 for paraxanthine, 1.36 for caffeine and 1.46 for the internal standard 8-chlorotheophylline.

Measurement of caffeine in serum by reversed-phase HPLC with direct injection

Apparatus HPLC with a 10 μL loop injector and DAD ($\lambda = 273$ nm; 0.1 AUFS).

Column

- Pinkerton ISRP GFF II (250 × 4.6 mm i.d., code 731472 W; LC Service, Emmen, The Netherlands).
- Guard column: ISRP GFF II (10 × 4.6 mm i.d., code 731474; LC Service, Emmen, The Netherlands). (Flush the guard column backwards once a week with pure acetonitrile.)

Mobile phase 0.1 mol/L potassium dihydrogenphosphate–acetonitrile (80 : 20); add sufficient 4 mg/L potassium hydroxide to pH 6.8; flow rate 1.0 mL/min (for 10-hydroxycarbazepine use 1.2 mL/min).

Assay

- Centrifuge 0.5 mL of serum in a microcentrifuge at maximal speed or filter through a 0.45 μm filter.
- Inject enough serum (approximately 30 μL) to flush and fill the 10 μL loop.
- Rinse the injector with water prior to each injection.
- Calculate the concentration from the peak height ratios.
- If the concentration is too high, dilute the sample with blank serum.

The analytical range of the method is 1–40 mg/L for caffeine in serum.

Cardioactive drugs

There are several classes of cardiac drugs. The cardiac glycoside digoxin is the oldest still in use and therapeutic overdose is far more common

than deliberate overdose. Serious digoxin overdose has a mortality rate of up to 20% and may be combated by the administration of oral activated charcoal, magnesium sulfate and ovine fragment antidigoxin antibodies. Digoxin is usually measured in serum by immunoassay (see Chapter 31), but the presence of Fab fragments interferes with the assay, as does that of other cardiac glycosides such as digitoxin. When considering the use of Fab-fragment therapy, a serum digoxin immunoassay carried out prior to administration can be used to calculate the total body burden and the amount of antidote required. Other cardioactive drugs can be measured in serum or plasma by HPLC (Chapter 41), by GC (Chapter 40) or by the LC-MS(-MS) method described above.

Measurement of common antiarrhythmic drugs in serum or plasma by HPLC

Apparatus HPLC column with UV detection ($\lambda = 205$ nm). For flecainide, fluorescence detection can be used ($\lambda_{ex} = 205$ nm, $\lambda_{em} = 365$ nm).

Column Nucleosil $5C_{18}$ (150 × 4.6 mm i.d., 5 μm).

Mobile phase Acetonitrile–buffer (4 g/L KH_2PO_4 adjusted to pH 4.45 with phosphoric acid, 72 : 28), flow rate 1.5 mL/min. For flecainide assay use 68 : 32.

Assay

- To 0.5 mL serum add 0.2 mL borax buffer (pH 9.0), 0.1 mL internal standard solution (e.g. 5 mg/L of chlorodiisopyramide) and 5 mL dichloromethane.
- Vortex for 2 min and centrifuge for 5 min at 1500*g*.
- Place the tube in a freezing bath at −40°C for sufficient time to freeze the water layer.
- Transfer the organic layer into a clean test-tube.
- Evaporate to dryness under a stream of nitrogen, or under vacuum.
- Add 0.2 mL of mobile phase and re-dissolve.
- Inject 50 μL onto the HPLC column.

Retention times for antiarrhythmic drugs are given in Table 1.21.

Measurement of the calcium antagonists verapamil and diltiazem in serum or plasma by HPLC

Apparatus HPLC column with UV detection ($\lambda = 206$ nm, 0.02 AUFS).

Column Nucleosil $5C_{18}$ (150 × 4.6 mm i.d., 5 μm).

Mobile phase Acetonitrile–buffer (13.6 g/L KH_2PO_4 adjusted to pH 2.5 with phosphoric acid, 70 : 30), flow rate 1.5 mL/min.

Assay

- To 1 mL serum add 0.1 mL internal standard (1 mg/L of the other drug) and 0.3 mL buffer pH 2.5 and mix.
- Add 5 mL diethyl ether; vortex for 1 min.
- Centrifuge for 5 min at 2000*g*.
- Place the test-tube in a freezing bath at −40°C until the water layer is frozen.

Table 1.21 Retention time and LOQ data for antiarrhythmic drugs

Antiarrhythmic	*Retention time (min)*	*LOQ (mg/L serum)*
Tocainide	3.0	0.1
Nordisopyramide	4.0	0.1
Lidocaine	4.5	0.1
Mexiletine	4.8	0.1
Disopyramide	7.5	0.25
Quinidine	9.0	0.25
Bupivacaine	12	0.1
Internal standard	13.5	
Flecainide	20 [(a)]	0.05
Dipyridamole	23 [(a)]	0.1

[(a)]For these drugs a higher concentration of acetonitrile in the mobile phase is recommended.

- Transfer the organic layer to a conical test-tube.
- Add 0.1 mL 0.5 mol/L sulfuric acid.
- Vortex for 1 min; centrifuge for 5 min at 1500*g*.
- Inject 50 μL of the water layer onto the HPLC column.

Retention times are verapamil 2.7 min, norverapamil 3.8 min and diltiazem 5.8 min.

Quantitative fluorescence method for quinine and quinidine in serum Quinine and quinidine are stereoisomers. Quinine is still widely used as an antimalarial as well as in elderly patients to treat night cramps. Quinidine is used as an antiarrhythmic agent. The isomers have similar toxic properties and severe overdose causes cardiac arrhythmias and visual disturbances that may result in permanent blindness. Multiple-dose oral activated charcoal is thought to increase the elimination of these drugs. Chloroquine is often used for self-euthanasia and, in contrast with quinine, it is mainly present in the erythrocytes. Therefore, whole blood analysis by LC-STIP or LC-MS (-MS) is required.

Standard Prepare solutions in human or calf serum that contain 5, 10, 20 and 40 mg/L.

Assay

- To 0.2 mL of serum (plasma) add 40 μL of 2 mol/L tris(hydroxymethyl)methylamine and 4 mL of dichloromethane–isopropyl alcohol (3 : 1).
- Vortex for 30 s and centrifuge at 2000*g* for 5 min.
- Carefully transfer the solvent layer to a second test-tube.
- Add 4 mL of 0.05 mol/L sulfuric acid.
- Vortex for a few seconds and centrifuge at 2000*g* for 5 min.
- Transfer the aqueous layer to a fluorimeter cell and measure the fluorescence ($\lambda_{ex} = 350$ nm, $\lambda_{em} = 450$ nm).

Drugs of abuse

Drugs of abuse may be taken deliberately or accidentally in overdose, or administered to others by a third party. Laboratory personnel should be aware of potential legal implications that might arise subsequently from any cases that involve drug abuse, and make sure that full documentation is collected and retained. Hospital toxicologists include drugs of abuse screening as part of their portfolio of tests provided to aid diagnosis and treatment, and for this purpose urine is the sample of choice. Quantitative assays in serum or plasma are rarely needed urgently and are usually reserved for cases with medicolegal implications. Routine analysis of drugs of abuse in urine also forms part of drug-dependence treatment programmes in which laboratory tests are used to assess the drug-taking pattern of new patients and subsequently to monitor their compliance with treatment. Apart from classic drugs of abuse such as heroin, cocaine and amfetamines, misuse of common medicines (codeine, oxycodone, benzodiazepines, etc.) is also widespread. In addition, the Internet is an increasing source of information on the purported recreational pleasures induced by a variety of substances, many of which provide little more than a placebo effect. Other forms of drug misuse include the covert use of laxative or diuretic drugs; early detection of this practice can avoid a futile search for a medical cause of the symptoms (see Detection of diuretics and laxatives). Tests for the abuse of volatile substances are described in Chapter 14.

Analytical methods

Fast immunoassay screening tests are described above and more information can be found in Chapters 3, 18 and 31. For routine drug-dependence screening programmes, in which large batches of urine samples are analysed daily, the analytical protocol usually comprises rapid automated immunoassay screening using a clinical chemistry analyser followed by the re-examination of positive samples using a more selective chromatographic technique.

Chromatographic analysis of drugs of abuse

Deglucuronidation Several of these drugs are excreted extensively as glucuronides in the urine. It is therefore recommended that acid or

enzymatic hydrolysis of the urine is carried out prior to extraction. Enzymatic hydrolysis is a gentler procedure that avoids the destruction of drugs that are acid labile.

Acid hydrolysis

- To 10 mL of urine in a screw-capped bottle add 2 mL of 36% (v/v) hydrochloric acid.
- Screw the cap on to the bottle and allow it to stand in a boiling water-bath for 15 min.
- Cool the bottle, and transfer the contents to a clean beaker.
- Add solid sodium bicarbonate to adjust the pH to 8.5.
- When frothing has subsided, transfer the solution back into the screw-capped bottle.

Enzyme hydrolysis

- Dissolve 5000 Fishman units of mixed glucuronidase–sulfatase (from *Helix pomatia)* in 1 mL of acetate buffer (pH 5).
- Adjust 10 mL of the urine to pH 5.0 (4.9–5.1) with 0.1 mol/L hydrochloric acid.
- Add 1 mL of the enzyme solution and incubate at 37°C overnight.

Thin-layer chromatography procedure for drugs of abuse in urine
Table 1.22 gives R_f values and colour reactions for drugs of abuse.

Standard solutions Dissolve 100 mg of each drug in 10 mL of methanol containing 1 drop of acetic acid:

- Standard I: methadone, heroin, codeine and morphine.
- Standard II: methaqualone, pethidine and ephedrine.
- Standard III: dextropropoxyphene, cocaine, benzoylecgonine and amfetamine.
- Standard IV: dextromoramide, pentazocine and methylamfetamine.
- Standard V: the urine extract of a drug-free heavy smoker.
- Standard VI: MDMA and methylenedioxyethylamfetamine (MDEA).

TLC plates Plastic or glass plates coated with silica gel (10 × 20 cm 60 GF 254, 250 μm), with fluorescence indicator.

Assay

- Hydrolyse 20 mL of the urine (see above).
- Add 50 mg of ammonium chloride and adjust the pH to 9.2–9.4 with 25% (v/v) aqueous ammonia (use a pH-meter).
- Transfer this mixture on to an Extralute column (Merck); wait for 5 min.
- Add two consecutive 20 mL volumes of the extraction solvent (dichloromethane–propan-2-ol (1 : 1 v/v)). (For safety reasons, dichloromethane can be substituted for chloroform.)
- Collect both extracts into a conical test-tube.
- Add 1 drop of acetic acid (to avoid volatilising amfetamines) and dry under air or nitrogen in a water-bath at 50°C or under vacuum.
- Re-dissolve the residue in 50 μL of methanol.
- Apply, on a line marked 1.5 cm from the bottom of the plate, 5 μL of the standard solutions and the urine extracts, about 15 mm from each other in the following order: standard I, II, patient extracts, III, IV, V.

Plate A, general drugs (mobile phase: ECMA)

- The mobile phase is ethyl acetate–cyclohexane–methanol–25% (v/v) aqueous ammonium hydroxide (70 : 15 : 10 : 5; mix shortly before use). Use 100 mL for one or two plates.
- Development time exactly 8.0 min (about 8 cm).
- Dry the plate in air, or use a handheld hot-air blower.
- The plate has to be free of ammonia; place it at 100°C for 2–5 min before spraying. Examine the plate at 254 and 366 nm.
- Place above hydrochloric acid vapour for 3 min.
- Spray with acidified iodoplatinate solution.
- Spray again with sodium nitrite solution (approximately 5 g of sodium nitrite in 20 mL of water; freshly prepared).

Plate B, opiates (mobile phase: EMA)

- The mobile phase is ethyl acetate–methanol–25% (v/v) aqueous ammonium hydroxide (85 : 10 : 5).
- Develop the plate for 30 min.
- Air-dry for 15–20 min or use an air blower.
- Elute the plate again for 15 cm in ethyl acetate.
- Air dry and then heat for 2 min at 100°C.
- Apply the detection sequence described for plate A.

Plate C, amfetamines, ephedrine (mobile phase: BMA)

- The mobile phase is *n*-butyl alcohol–methanol–acetic acid (70 : 20 : 10).
- Develop the plate over a distance of 10 cm.
- Air-dry briefly and then use a hot-air blower until visually dry.
- Heat for 15 min at 100°C.
- Cool to ambient temperature.
- Observe under UV light at 254 nm and then spray with 1% (w/v) ninhydrin in ethanol.

Table 1.22 R_f values and colour reactions for drugs of abuse

	R_f *in mobile phases*			*Acidic iodoplatinate*	*Ninhydrin*		*Copper spray*
	CMA	*EMA*	*BMA*		*366 nm*	*100°C; 366 nm*	
Standard I							
Methadone	81	67	—	Orange-brown	—	—	—
Heroin (powder only)	50	44	7	Violet-brown	—	Blue	Orange
Codeine	30	27	20	Violet-grey	—	—	—
Morphine	17	15	7	Blue	—	Blue	Orange
Standard II							
Methaqualone	87	78	77	Brown	—	—	—
Pethidine	60	52	28	Brown	(Pink)	Pink-red	Orange
Ephedrine	22	22	10	(Grey)	(Violet)	Red-violet	Brown-red
Standard III							
Dextropropoxyphene	91	75	15	Brown	—	—	—
Cocaine	84	70	38	Red-brown	—	—	—
Amfetamine	39	36	34	Blue-violet	Violet	Red-brown	Orange-violet
Standard IV							
Dextromoramide	89	74	30	Brown	—	—	—
Pentazocine	75	62	58	Red-brown	—	Red	Orange-red
Metamfetamine	36	33	20	Violet	—	Violet	Red
Nicotine	60	50	1	Blue-violet	—	(Blue-violet)	(Orange-violet)

- Expose the plate to UV light at 366 nm for 5 min (amfetamine violet).
- Spray again with 1% (w/v) ninhydrin reagent and leave the plate in an oven at 80–100°C for about 20 min.
- Expose to UV light again at 366 nm for 5 min.
- Spray with copper nitrate reagent (1 mL of saturated aqueous solution of copper nitrate, 0.1 mL of 4 mol/L nitric acid and 100 mL of methanol) to improve the differentiation between amfetamine and ephedrine.

Notes on specific compounds and groups

- Many amfetamine derivatives are in use and it is preferable to detect these by HPLC or GLC. In the mobile phase BMA, spraying with ninhydrin after development at 366 nm and 20 min at 80°C to 100°C causes the following amfetamine derivatives to turn violet:
  - MDMA, $R_f = 0.19$
  - MDA, $R_f = 0.32$
  - 4-Methoxyamfetamine (4-MA), $R_f = 0.31$.
- After spraying with methanolic copper nitrate spray, amfetamine, metamfetamine and most amfetamine derivatives turn red. Only MDEA, $R_f = 0.27$, remains colourless with ninhydrin as well as with the copper nitrate spray.
- Cocaine gives a bright blue colour with acidified iodoplatinate solution. In practice, cocaine is rarely detected in urine samples by this technique because the drug is rapidly metabolised to benzoylecgonine, a polar substance that extracts poorly into organic solvents.
- It is essential to distinguish between codeine (a common constituent of cough medicines) and morphine. A small amount of codeine is thought to be *O*-demethylated to morphine in humans. A high intake of codeine can give rise to the detection of the *N*-demethylated metabolite, norcodeine.
- Dextropropoxyphene is metabolised extensively in the liver, but the parent drug is not detected in urine samples. The appearance of the metabolites is quite characteristic.
- Heroin (diacetylmorphine) is rapidly deacetylated to morphine in the body and is not excreted in urine. The detection of heroin is based, therefore, on the identification of morphine in urine (see below). Heroin detected in an extract may indicate an attempt by the patient to deceive the analyst by the direct addition of the drug to the urine sample.
- Methadone is *N*-demethylated in the body to give a product that undergoes spontaneous cyclisation to yield two metabolites in the urine. Methadone and the metabolites are usually found together, but occasionally only the metabolites are detected. Detection of a large amount of methadone in a urine sample in the absence of metabolites suggests direct addition by the patient to deceive the analyst.
- Morphine and codeine appear as blue spots. The TLC system described above is useful after an immunoassay for opiates as it can differentiate between morphine (as a metabolite of heroin) and codeine. Less than 10% of morphine is excreted as unchanged morphine and therefore the sensitivity of the test is increased by hydrolysing the conjugated metabolites prior to extraction.

Screening for drugs of abuse by chromatography with mass selective detection (GC-MS)

GC-MS is the method of choice for confirming the results of immunoassay tests, and the procedure described by Maurer (2011a, 2011b) is ideal for this purpose (see also Chapters 26 and 40). GC-MS analysis of drugs is also applied to hair testing (Paterson *et al.* 2001).

Liquid chromatography with MS is used increasingly in clinical and forensic toxicology. An important advantage of LC-MS over GC-MS is that no derivatisation is needed (Marquet 2002). With the introduction of LC-MS(-MS) into clinical laboratories, this technique may eventually supersede GC-MS in this area.

Screening for drugs of abuse by gradient-elution HPLC with diode array, fluorescence or electrochemical detection

Apparatus Gradient HPLC set-up, DAD ($\lambda = 215$ nm; 0.100 AUFS) and optionally an electrochemical detector (0.85 V/Ox; ATT = 128) and a fluorescence detector ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 340$ nm).

Table 1.23 HPLC gradient mobile phase at 1 mL/min

Time (min)	*% A*	*% B*
0	100	0
30	30	70
35	30	70
40	20	80
45	100	0
60	100	0

Column Lichrospher RP-18e (125 × 4.0 mm, 5 µm).

Mobile phases

- Mobile phase A is 0.025 mol/L triethylammonium phosphate buffer in water (pH 3.0).
- Mobile phase B is acetonitrile (HPLC grade).

Activation and cleaning of the column with 50% A plus 50% B Use HPLC gradient mobile phase at 1 mL/min, as in Table 1.23.

Solid-phase extraction column A 10 mL Certify solid-phase extraction (SPE) column (Varian), conditioned with 2 mL of methanol and 2 mL of buffer pH 6.0.

Assay

- To 1.0 mL serum or urine add 3.0 mL of 0.1 mol/L phosphate buffer pH 6 and 50 µL of the internal standard (25 µg/L of nalorphine in water).
- Vortex for a few seconds and centrifuge if not clear.
- Transfer the diluted mixture to the SPE column without vacuum.
- Wash the column without vacuum with 1.0 mL buffer pH 6.0.
- Wash with 1 mL of phosphate buffer pH 6–methanol (80 : 20), then with 1.0 mL of 1 mol/L acetic acid.
- Dry the SPE column for 2 min under maximal vacuum.
- Dry the wall of the SPE column.
- Wash the column under light vacuum with 1.0 mL hexane.
- Dry under maximal vacuum for 5 min.
- Centrifuge at 1500*g* for 5 min.
- Elute the column without vacuum with 1.2 mL of a mixture of hexane–ethyl acetate (80 : 20 v/v). This is fraction 1 (acid components).
- Elute the SPE column without vacuum with 2.4 mL (dichloromethane–propan-2-ol–25% ammonium hydroxide (80 : 20 : 2)) into a second test-tube. This is fraction 2 (basic extract).
- Dry both extracts under a stream of nitrogen.
- To fraction 1 add 100 µL of 33% (v/v) acetonitrile in water and vortex for 30 s.
- To fraction 2 add 100 µL of 0.025 mol/L of triethylammonium phosphate buffer pH 3; vortex for 30 s.
- Inject 60 µL of both extracts on to the HPLC column and start the gradient elution.

The LOQ of each substance is about 50 µg/L in urine with UV detection (Table 1.24), and much lower with fluorescence or electrochemical detection.

Detection of diuretics in urine by thin-layer chromatography

Reagents

- Ammonium amidosulfonate (5% w/v in water).
- Mandelin's reagent (50 mg ammonium monovanadate in 10 mL of 96% sulfuric acid; warm until a clear solution is obtained and use as spray reagent while still warm).
- Naphthylethylenediamine dihydrochloride (5% (w/v) in 100 mL of acetone–water, 3 : 1 (v/v)).
- Sodium 1,2-naphthaquinone-4-sulfonate solution (200 mg sodium 1,2-naphthaquinone-4-sulfonate in 15 mL water–ethanol, 1 : 1 (v/v)).
- Sodium hydroxide (0.1 mol/L and 6 mol/L).
- Sodium nitrite (1% w/v in water).
- Van Urk's reagent (100 mg 4-dimethylaminobenzaldehyde in 10 mL ethanol; add 1 mL 36% HCl; use freshly prepared).
- Sulfuric acid 9 mol/L (96% sulfuric acid–water, 1 : 1 (v/v)).

Table 1.24 HPLC retention time and detection data for drugs of abuse

Illicit substance or metabolite	*Retention time (min)*	*UV maximum (nm)*	*Electrochemical detection (0.85 V)*	*Fluorescence (λ_{ex} = 290 nm; λ_{em} = 340 nm)*
Morphine-3-glucuronide	1.81	212, 240, 283	−	−
Normorphine	3.85	211, 240, 283	+	+
Morphine	4.79	212, 242, 285	+	+
Oxymorphone	5.52	205, 227, 277	+	−
Morphine-6-glucuronide	6.55	211, 282	+	+
Dihydromorphine	6.86	205, 228	+	−
Nalorphine	7.66	211, 242, 285	−	+
Amfetamine	7.79	207	−	−
Hydromorphine	7.86	205	+	−
Metamfetamine	9.16	208	−	−
Dihydrocodeine	9.68	210	+	+
Codeine	9.89	212, 242, 285	+	+
MDA	10.64	200, 235, 284	+	+
Oxycodone	10.85	206, 230	+	−
Morphine acetate	10.88	210, 240, 285	−	−
4-Methoxyamfetamine	11.05	225, 275	−	+
Hydrocodone	11.53	260, 231	+	−
MDMA	11.66	200, 235, 284	+	+
6-Monoacetylmorphine	11.96	210, 282	+	+
MDEA	11.98	200, 235, 284	+	+
Ethylmorphine	12.18	209, 240, 284	+	+
Ethylamfetamine	12.77	208	−	−
Ethyltryptamine	13.70	220, 280	+	+
Benzoylecgonine	13.99	200, 234	−	±
6-Nicotylmorphine	14.50	211, 264	+	−
Norcocaine	14.69	200, 234	−	−
Acetylcodeine	14.79	211, 240, 285	−	+
Cocaine	15.41	200, 234	−	+
Methylenedioxyethylamfetamine	15.80	206, 278	−	+
Papaverine	16.55	201, 252	+	−
Noscapine	16.81	214, 311	+	−
Pentazocine	17.00	225, 280	−	+
LSD	17.03	240, 310	+	−
Nicomorphine	17.07	270, 222, 265	+	−
Coca ethylene	17.88	200, 234	+	−
Normethadone	18.34	200	−	+
Methadone	20.30	200	−	−
EDDP[a]	21.07	209	−	−
THC-COOH[b]	30.57	210	−	−
Cannabidiol	34.40	210, 232, 275	±	−
Cannabinol	35.63	222, 285	+	−

[a]2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
[b]11-Carboxytetrahydrocannabinol.

Reference mixture Use mixtures of 0.2% (w/v) in methanol of hydrochlorothiazide, sulfafurazole, phenacetin and prazepam.

Standards Prepare 0.1% (w/v) in methanol of the following standard solutions:

- I: amiloride, clopamide, quinethazone, and triamterene.
- II: chlorothiazide, etacrynic acid, indapamide and spironolactone.
- III: acetazolamide, chlorotalidone, furosemide and polythiazide.
- IV: bumetanide, cyclopenthiazide, hydrochlorothiazide, mefruside and canrenoate.

Reference mixture (R_f values) Hydrochlorothiazide $R_f = 0.11$, sulfafurazole $R_f = 0.33$, phenacetin $R_f = 0.52$ and prazepam $R_f = 0.72$ on TLC glass plates with silica-gel F254 indicator.

Method

- Consider first a deglucuronidation step (see Deglucuronidation).
- Solution A: mix 25 mL of urine with 1 mL of 36% (v/v) hydrochloric acid.
- Solution B: mix 25 mL of urine with 1 mL 6 mol/L sodium hydroxide.
- Transfer solutions A and B on to separate Extrelut-20 SPE columns.

- Wait for 5–10 min.
- Elute both columns twice with 20 mL of ethyl acetate.
- Transfer both extracts into a conical tube.
- Dry under vacuum.
- Re-dissolve the residue in 0.1 mL of methanol.

Chromatography Add standards, extracts and reference solution to a silica-gel F254 indicator on four TLC plates (20 × 20 cm) in the sequence:

- I, 5 µL
- II, 5 µL
- Urine, 2 µL
- Urine, 5 µL
- Urine, 10 µL
- III, 5 µL
- IV, 5 µL
- Reference, 2 µL.

Elution (for all four TLC plates)

- 20 min in chloroform–methanol (9 : 1 v/v).
- Dry with hot-air blower.

Detection A

- Observe under a UV-lamp at 254 and 366 nm; calculate the R_F values.

Detection B

- Plate I: spray with warm Mandelin's reagent.
- Plate II: spray with naphthylethylenediamine solution, then spray with 36% (v/v) hydrochloric acid.
- Heat at 100°C for 10 min.
- Spray with 9 mol/L sulfuric acid, and dry with hot-air blower.
- Spray with 1% sodium nitrite solution, and dry with a hot-air blower.
- Spray with 5% ammonium aminosulfonate, and dry a with hot-air blower.
- Spray with 0.5% naphthylethylenediamine, and dry with a hot-air blower.
- Plate III: spray with naphthoquinone sulfonate solution.
- Spray lightly with 0.1 mol/L sodium hydroxide, and dry with hot-air blower.
- Spray with sodium 1,2-naphthaquinone-4-sulfonate solution.
- Dry with a hot-air blower for 10 min until the standards become a clear orange.
- Plate IV: spray with Van Urk's reagent, and dry for 5 min at 100°C.

Observe each plate after each spraying procedure (Table 1.25 gives the data for diuretic drugs). Interference may arise from the presence of benzodiazepines, which are hydrolysed to aminobenzophenones that also undergo diazotisation. Sulfonamides are also detected by this procedure and give yellow colours with the Van Urk's reagent after heating.

Detection of laxatives in urine by thin-layer chromatography

Reference solutions

1. Dissolve 2 mg of phenolphthalein in 1 mL of ethanol and dilute to 10 mL with dichloromethane.
2. Dissolve 2 mg of danthron in 10 mL of dichloromethane.
3. Dissolve 2 mg of rhein in 10 mL of dichloromethane. (A method for preparing rhein from sennoside A or B has been described by Lemli (1965).)
4. Bisacodyl and oxyphenisatin metabolites:
   - — Dissolve 2 mg of each substance in 2 mL of ethanol.
   - — Add 20 µL of 6 mol/L sodium hydroxide, heat for 30 min at 70°C.
   - — Cool, neutralise with 20 µL of 6 mol/L hydrochloric acid.
   - — Add 8 mL of dichloromethane and 2 mg of the parent substance to each corresponding solution.

The precipitate of sodium chloride that forms on neutralisation does not interfere with the chromatography.

Method Enzymatic hydrolysis of the glucuronide metabolites is essential before extraction.

Assay

- Adjust 20 mL of urine to pH 5 with 0.1 mol/L hydrochloric acid.
- Add 2 mL of acetate buffer (pH 5), and 10 000 units of mixed glucuronidase/sulfatase (from *Helix pomatia*).
- Heat in a water-bath at 60°C for 2 h.
- Pour the urine on to an unbuffered Extrelut column (see TLC diuretics above) and leave for 2–3 min to allow the sample to soak into the column.
- Place a 50 mL test-tube under the column outlet and pour 20 mL of a mixture of dichloromethane–isopropyl alcohol (9 : 1 v/v) on to the column.
- When the elution is complete, insert into the top of the column a hollow rubber stopper connected by a rubber tube to a pressure bulb.
- Apply gentle pressure to discharge residual solvent.
- Place the tube that contains the eluate in a beaker of hot water.
- Evaporate the extract to dryness under a stream of nitrogen.
- Dissolve the residue in 100 µL of dichloromethane just prior to examination by the following TLC systems.

System A

- High-performance silica gel (10 × 20 cm) with fluorescent indicator and concentration zone.
- Mobile phase: *m*-xylene–isobutyl methyl ketone–methanol (10 : 10 : 1).

System B

- As used for system A.
- Mobile phase: hexane–toluene–acetic acid (3 : 1 : 1).

Table 1.25 Thin-layer chromatographic data for diuretic drugs

Substance	R_f[a]	*TLC results*				
		Before spraying[b]			After spraying[b]	
		254 nm	366 nm	Mandelin's	254 nm	366 nm
Plate I						
Amiloride	50	+	+	+Blue		
Acetazolamide	18					
Chlorothiazide	11					
Hydrochlorothiazide	11	White	+Violet			
Quinethazone	15	+	White	+Blue		
Triamterene	8 T	+	+	+Blue	+Blue	
Chlortalidone	23	+Yellow				
Clopamide	39					
Furosemide	7 T	+	Dark brown			
Bumetanide	6 T	+	Red-brown			
Polythiazide	32	+	White	+Violet	+Violet	

table continued

Table 1.25 continued

Substance	R_f[a]	*TLC results*						
Cyclopenthiazide	27	Grey						
Spironolactone	75	Red–brown	+White	Yellow				
Etacrynic acid	11	White						
Indapamide	46	Violet						
Canrenone	73	Orange	+Yellow	+Yellow				
Mefruside	55							
							After spraying	
		36% HCl	**9 mol/L H_2SO_4**	**Nitrite**	**Sulfonate**	**Naphthyl**	**254 nm**	**366 nm**
Plate II								
Amiloride	50							
Acetazolamide	18							
Chlorothiazide	11							
Hydrochlorothiazide	11	Pink						
Quinethazone	15	Pink						
Triamterene	8 T	Blue	Blue					
Chlortalidone	23							
Clopamide	39							
Furosemide	7 T	Brown–grey	Brown–grey	Brown–grey Brown–grey	Violet			
Bumetanide	6 T	Pink						
Polythiazide	32	Pink						
Cyclopenthiazide	27	Pink						
Spironolactone	75	Light brown–green	Light brown[c]	Yellow–brown	Orange–brown	Yellow	Yellow–green	
Etacrynic acid	11							
Indapamide	46	Pink	Violet	Violet	Brown	Brown		
Canrenone	73	Yellow	Yellow	Yellow[c]	Yellow–brown	Orange	Yellow	Yellow–green
Mefruside	55							
		Naphthaquinone	**254 nm**	**366 nm**	**Van Urk's**			
Plate III					**Plate IV**			
Amiloride	50			+	Yellow			
Acetazolamide	18	Orange						
Chlorothiazide	11	Orange						
Hydrochlorothiazide	11	Orange			Yellow			
Quinethazone	15	Orange						
Triamterene	8 T		+	+	Yellow			
Chlortalidone	23	Orange						
Clopamide	39	Orange						
Furosemide	7 T	Red-orange			Yellow			
Bumetanide	6 T	Orange			Yellow			
Polythiazide	32	Orange			Yellow			
Cyclopenthiazide	27	Orange			Yellow			
Spironolactone	75	Grey						
Etacrynic acid	11							
Indapamide	46	Orange			Pink			
Canrenone	73							
Mefruside	55	Red–violet						

[a] T = tailing.
[b] + = fluorescence.
[c] At 366 nm strong fluorescence (yellow–green).

Assay

- Score two plates vertically to divide them into two (10 × 10 cm) zones.
- Score a horizontal line parallel to the concentration zone at a distance of 5 cm.
- To one plate apply 5 µL aliquots of the reference solutions of bisacodyl, danthron, oxyphenisatin and phenolphthalein to each zone, together with a 3 µL and a 10 µL aliquot of the urine extract, also to each zone.
- Develop the plate using system A.
- To the second plate apply 5 µL aliquots of the reference solutions of danthron, phenolphthalein and rhein to each zone, together with a 3 µL and a 10 µL aliquot of the urine extract. Develop this plate using system B.
- Remove the plates and dry under a hot-air blower.

Detection

- Danthron is visible as a yellow spot on the system A plate, or as an orange spot on the system B plate. Examine the plates under UV light at 254 and 366 nm. All the substances absorb at 254 nm; danthron and rhein give an orange fluorescence at 366 nm.
- Spray the left-hand side of each plate with 6 mol/L sodium hydroxide. Bisacodyl and its hydrolysis products give violet spots; oxyphenisatin appears as three faint pink spots; phenolphthalein and rhein give a violet and a red spot, respectively.
- Spray the left-hand side of the system A plate with a freshly prepared 1% (w/v) solution of potassium ferricyanide.
- Heat both plates to 100°C and spray the right-hand side immediately with Mandelin's reagent (see TLC of diuretics above).

The R_f values and colour reactions of these compounds and their hydrolysis products are given in Table 1.26.

Detection of diuretics and laxatives in urine by HPLC

Apparatus

- Gradient HPLC with DAD ($\lambda = 250$ nm; 0.08 AUFS; ABS mode: 2) and optionally an electrochemical detector; 0.85 V/Ox, ATT = 128 and a fluorescence detector, sensitivity low; $\lambda_{ex} = 290$ nm and $\lambda_{em} = 340$ nm; range −25 to + 250 mV.
- Pre-column: Lichrospher 60, RP-select B, 5 µm (Merck no. 1.50963).
- Column: Lichrospher 60, RP-select B (125 × 4.0 mm, 5 µm, Merck no. 1.50981).
- Mobile phase A: 0.025 mol/L triethylammonium phosphate buffer (pH 3.0).
- Mobile phase B: acetonitrile; flow rate 1.0 mL/min.

The HPLC gradient programme is given in Table 1.23.

Reagents

- Acetonitrile
- Dichloromethane
- Disodium hydrogenphosphate
- Ethyl acetate
- Potassium hydrogenphosphate
- Sodium hydroxide
- 1 mol/L triethylammonium phosphate
- 37% hydrochloric acid
- 0.1 mol/L phosphate buffer pH 6.0 (13.6 g potassium dihydrogenphosphate in water to 1 L, add sufficient 1 mol/L potassium hydroxide to pH 6.0)
- 2 mol/L sodium hydroxide solution (80 g sodium hydroxide per litre of water)
- Phosphate-buffered saline (PBS) pH 7.2 (1.41 g disodium hydrogenphosphate dihydrate and 0.27 g potassium dihydrogenphosphate plus 8.75 g sodium chloride in sufficient water to 1 L, pH 7.2)
- 0.025 mol/L triethylammonium phosphate buffer pH 3.0
- 2 mol/L hydrochloric acid
- Beta-glucuronidase/arylsulfatase.

Method

- Pipette into each of two test-tubes 1.0 mL standard solution I and into two other test-tubes 1.0 mL of standard solution II (see Detection of diuretics in urine by thin-layer chromatography for preparation of standard solutions).
- Add to each tube 1.0 mL of phosphate buffer pH 6.0.
- Pipette into each of two other test-tubes 1.0 mL of the patient's urine.
- Add 1.0 mL phosphate buffer pH 6.0 and beta-glucuronidase–arylsulfatase (75 : 1).
- Incubate at 37°C for 14 h.
- Add to one series of test-tubes (with standard solutions I and II and patient's urine) 100 µL of 2 mol/L hydrochloric acid.
- Add to the other series 100 µL of 2 mol/L sodium hydroxide.
- Check the pH of both with pH test-paper; one tube has to be acidic and one basic. (Note: chrysophanic acid and phenolphthalein colour the solution reddish.)
- Add to both tubes 5 mL ethyl acetate.
- Shake for 20 min in a rotary mixer and allow to stand for 5 min; centrifuge for 5 min at 1500*g*.
- Transfer both organic layers into two clean tubes (this is easier if the tubes are first put into a freezing bath at −40°C for a short time).
- Add to both tubes 5 mL of PBS buffer pH 7.2.
- Mix for 5 min in a rotary mixer, stand for 5 min; centrifuge for 5 min at 1500*g*.
- Transfer both organic phases into two clean test-tubes.
- Dry under stream of nitrogen at 40°C; add to the extract 150 µL 20% (v/v) acetonitrile in triethylammonium phosphate buffer (pH 3).
- Vortex for 1 min and centrifuge for 5 min at 1500*g*.
- Inject 20 µL onto the HPLC column.

Always run a blank gradient with 65 µL triethylammonium phosphate buffer before the patient's serum or urine extracts. Table 1.27 gives HPLC retention time and detection data for diuretics and laxatives.

GC systems for the detection of diuretics and laxatives are described in Chapter 40.

Interpretation and advice

An experienced hospital toxicologist is expected not only to provide valid analytical data but also to assist the clinician in relating the findings to a particular case of poisoning. This may be quite straightforward

Table 1.26 Thin-layer chromatographic data for laxatives and their hydrolysis products

Substance	*System A R_f relative to phenolphthalein*	*System B R_f relative to rhein*	*Visible*	*Colour after spray with Mandelin's*	*Colour with NaOH*
Bisacodyl	0.98	—	—	Violet	Violet
Hydrolysed bisacodyl	0.71	—	—	Violet	Violet
Danthron	1.38	1.38	Orange	Red	brown
Oxyphenisatin	0.98	—	—	Pink[(a)]	Pink
Hydrolysed spot 1	0.80	—	—	Pink[(a)]	Pink
Hydrolysed spot 2	0.63	—	—	Pink[(a)]	Pink
Phenolphthalein	1.00	0.08	—	Violet	Brown
Rhein	—	1.00	Yellow	Red	Green–yellow

[(a)]Violet after spraying with potassium ferricyanide.

Table 1.27 HPLC retention time and detection data for diuretics and laxatives[a]

Substance	*Retention time (min)*	*B=basic; A=acid*	*D=diuretic, L=laxative*	*Detector response*	
				Electrochemical	*Fluorimetric*
Mix I					
Acetazolamide	7.9	B/A	D	−	±
Hydrochlorothiazide	9.4	B/A	D	±	±
Clopamide	14.9	B/A	D	±	−
Indapamide	18.9	B/A	D	++	−
Phenolphthalein	20.4	B/A	L	±	−
Polythiazide	21.7	B/A	D	−	±
Bisacodyl	22.3	A	L	−	−
Canrenoate (metabolised canrenone)	24.3	B/A	D	−	−
Danthron	25.2	B/A	L	−	−
Chrysophanic acid[b]	27.0	B/A	L	−	−
Mix II					
Amiloride	7.4		D	−	±
Chlorothiazide	8.8	B/A	D	−	±
Triamterene	10.6	B	D	−	±
Quinetazone	11.4	B/A	D	+	−
Chlortalidone	14.2	B/A	D	−	±
Furosemide	18.6	A	D	±	±
Mefruside	19.7	B/A	D	−	−
Cyclopenthiazide	21.4	B/A	D	−	−
Etacrynic acid	22.3	A	D	−	−
Spironolactone (metabolised canrenone)	24.2	A	D	−	−
Bisoxatin	24.9	A	L	−	−

[a]Standard mix (5 mg of each substance in water–methanol = 1 : 1 (keep at −80°C).
[b]Chrysophanic acid is the major metabolite in urine of several anthraquinone stimulant laxatives, such as senna, aloes, danthron, rhubarb (*Rhei radix*) and buckthorn (*Rhamnus*).

when the presence of a high concentration of a drug or poison is consistent with the patient's symptoms and the circumstantial evidence. In other cases, factors such as the patient's age, sex, health and previous exposure must be taken into account. For example, addicted patients may have developed a tolerance to extremely high concentrations of opiates, benzodiazepines and ethanol, and exhibit relatively mild toxicity. An elderly invalid with respiratory problems is far more susceptible to an overdose of a central depressant drug than a healthy young adult, and so may have life-threatening symptoms with only moderate plasma concentrations. The route of administration (inhalation, oral ingestion, intravenous injection, etc.) can have a very significant effect on the subsequent toxicity, which must also be taken into account when interpreting plasma concentrations (see Chapter 24). Mixed overdoses of drugs and alcohol are common, and synergistic reactions can confuse the clinical picture. The hospital toxicologist must therefore develop a good background knowledge of drug interactions. However, there are situations in which the analytical results fail to offer an adequate explanation. This can result from mistakes in sample collection; for example, blood samples taken from an arm being used to infuse a therapeutic agent may have very high concentrations of that agent because of contamination. The ingestion of large amounts of sustained-release preparations can result in disproportionately low blood concentrations. Systems for collecting and referring samples to the laboratory can occasionally break down and the samples received and analysed (with negative results) may be from the wrong patient. Negative results on the correct samples must also be interpreted with caution. The patient may not be poisoned after all and the clinical effects may be caused by an organic disorder. Alternatively, the toxic agent responsible is not detected, which may instigate a wider analytical search or application of a more sensitive assay. A list of therapeutic and toxic concentration ranges for drugs is given in Table 1.28. The hospital toxicologist may also be asked to apply toxicokinetic principles (see Chapter 24) to the quantitative data to answer questions raised by the clinician; examples in which this is relevant are described below.

How much (A) of the poison is still in the body at a serum concentration C?

$$A = C \times V_d(\text{L/kg body mass}) \times \text{body mass(kg)}$$

where V_d is the volume of distribution.

How long will it take for a measured serum concentration (C_o) to decrease to below the toxic concentration (C_{tox})?

- $C_{tox} = C_o \times e^{-k_{el}t}$
- k_{el} is the elimination constant, $0.693/t_{1/2}$.
- If the elimination is not saturated, the kinetic parameter k_{el} of the patient can be calculated as follows, using two serum concentrations measured during the elimination phase:
  - Elimination constant $k = (\ln C_1 - \ln C_2)/(t_2 - t_1)$
  - $t_{1/2} = (\ln 2)/k = 0.69/k$
  - Clearance $Cl = kV_d$, where V_d is volume of distribution.

What is the efficiency of an extracorporeal elimination treatment?
Severe cases of intoxication sometimes require extracorporeal elimination treatments, such as haemodialysis or haemoperfusion. For the clinician it is important to have an estimate of how many hours the dialysis or haemoperfusion has to be continued and when the next blood

Table 1.28 Therapeutic and toxic concentrations

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Acamprosate	440.5	Pl	0.03–0.075	
Acebutolol	336.4	S	0.5–1.25	L 15–20
Diacetalol	308.4	S	0.65–4.45	L 90–150
Acecarbromal	279.1	Pl	10–20	25–30
Acenocoumarol	353.3	S	T 0.03–0.09	T 0.1–0.15
			P 0.1–0.5	
Acetaldehyde	44.1	B	0–30	100–125
Acetazolamide	222.2	S	In general 10–20	25–30
			Glaucoma 4–5	
Acetohexamide	324.4	Pl	20–60	500
Acetone	58.1	B	5–20 endogenous in diabetes	After acetone drinking/ sniffing 200–400
				Diabetic ketoacidosis 325–450
				L 550
Acetonitrile	41.1	B		L 0.75
Cyanide	26.0	B	0.001–0.012(–0.15)	
Acetylsalicylic acid	180.2	Pl *very unstable*		
Salicylic acid	138.1	S	Rheumatism 200–300 (child 150)	400–500 (child 300) L 500–900
			Anticoagulant 50–125 Prostaglandin synthetase inhibitor 50–150	
Aciclovir	225.2	S	T 0.5–1.5	
			P 5–15	
Acitretin	326.4	Pl	(0.05) 0.1–0.7	
Ajmaline	326.4	S	0–2.1	L 5.5[d]
Albendazole	265.3			
Albendazole sulfoxide		S	0.5–1.5	
Alcuronium chloride	827.9	Pl	0.3–3	
Aldrin	364.9	S	0–0.0015	0.0035
Alfentanil	416.5	S	0.03–0.6	
Alfuzosine	389.5	Pl	0.003–0.06	
Alimemazine	298.4	S	0.05–0.4	0.5
				L 1–3.2
Allobarbital	208.2	S	2–5	10
				L 20–30
Allopurinol	136.1	S	P 1–5	
Oxypurinol	152.1	S	5–15	20
Alphaprodine	261.4	S	0.87–1.0	
Alprazolam	308.8	S	0.02–0.04	0.075
Alprenolol	249.4	Pl	0.01–0.2	T 0.1
				P 1
				L 40
Hydroxyalprenolol	265.4	Pl	0.04–0.06	sum T 0.25–0.3
			sum 0.1–0.2	
Aluminium	27.0	S	0–0.02 (0.1)	0.05–0.15
				L 4.4[d]
Amantadine	151.3	S	0.3–0.6 (1)	1
				L 21[d]
Amikacin	585.6	S	T 1–4 (10)	T 10
			P 15–25 (30)	P 30

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Aminobenzoic acid	137.1	S	300–600	600
Aminophenazone	231.3	S	10–20	
Aminoglutethimide	232.3	S	0.5–25	
4-Aminopyridine	94.1	S	0.025–0.075	0.15–0.2
Amiodarone	645.3	S	1–2.5	3
			T 0.5–2	
Desethylamiodarone	617.3	S	sum (1–5)	sum 5–8
Amisulpride	369.5	Pl	0.1–0.4	10[d]
Amitriptyline	277.4	S	0.05–0.2	
Nortriptyline	263.4	S	0.007–0.2	sum 0.5
			sum 0.1–0.25	L 1.5–2
Amlodipine	408.9	Pl	0.006–0.018	0.088
				L 0.1–0.2
Ammonia	17.0	Pl	0.5–1.7	
Amobarbital	226.3	Pl	2–12	>9 L 13–96
Amodiaquine	355.9	S	0.3–0.7	
Amoxapine	313.8	S	0.01–0.02	
8-Hydroxyamoxapine	329.8	S	sum 0.2–0.6	sum 3
				L sum 5
Amoxicillin	365.4	S	T 0.5–1	
			P 5–15	
Amfetamine	135.2	S	(0.02) 0.05–0.15	0.2
				L 0.5–1[d]
				(for addicts 1–10)
Amphotericin B	924.1	S	T 0.025–1	T (3–) 5–10
			P 1.5–3.5	
Ampicillin	349.4	S	T 0.02–1	
			P 2–20	
Amprenavir	505.6	Pl S	P (2 h) 4-6; (4–6 h) 1.5–4.5	
			T (10–12 h) 0.4–1	
Amrinone	187.2	Pl	1–2 (4)	
Amsacrine	393.5	Pl *add 1 drop lactic acid then 48 h at −20° add*	Continuous infusion 0.1–0.5	
			T 0.03	
			P 0.15–5.5	
Anileridine	352.5	S	<0.5	L 0.9[d]
Aniline	93.1	S		L 6
Antimony	121.8	B	0.01	0.2
Apomorphine	267.3	S	0.002–0.02	
Aprindine	322.5	S	0.7–2	2
Aprobarbital	210.2	Pl	10–40	40 L 50
Aripiprazole	448.4	S Pl	0.1–0.45	
Dehydro-aripiprazole		S Pl	0.15–0.55	
Arsenic	74.9	B	0.002–0.07	0.05–0.25 (1)
				L 9–15
Arsenic	74.9	U	0–0.1	Chronic 0.2–1
				Acute 1
				As(III) more toxic than As(V)
Ascorbic acid	176.1	Pl	5–12	
Astemizole and desmethylastemizole	458.6	Pl	0.003–0.005	0.14[d]
Atazanavir	704.9	S Pl	P (1–2 h) 3.5–6; (4–8 h) 2–4	
			T (10–24 h) >0.15	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Atenolol	266.3	S	0.1–0.6 (1)	2
				L 27[d]
Atovaquone	366.8	Pl	(10) 15–30 (50)	
Atracurium	929.2	S	0.1–1 (5)	
Atropine	289.4	S	0.002–0.025	0.03–0
				L 0.2
Azapropazone	336.4	Pl	30–90	
Azathioprine	277.3	S Pl	P 0.05–0.3	
6-Mercaptopurine	152.2	S	0.04–0.3	1–2
Azelastine	383	Pl	0.002–0.003 (0.01)	
Azithromycin	749.0	Pl	0.04–1	
			Tissue >2 µg/g	
Aztreonam	435.4	S	T 1–10	
			P 50–250	
Baclofen	213.7	S	0.08–0.6	1.1–3.5
				L 6–9.6
Barbital	184.2	Pl	5–30	20
				L 90
Barbiturates				
Intermediate acting		S	1–5	10–30
				L >30
Long acting		S	10–40	40–60
				L >80
Short acting		S	1–5	7–10
				L >10–15
Barium	137.3	Pl	0.001	
Bendroflumethiazide	421.4	Pl	0.05–0.1	
Benoxaprofen	301.7	Pl	Peak <50	
Benperidol	381.4	Pl	0.002–0.01	
Benzbromaron	424.1	S	2–10	
Benzene	78.1	B	0.0002 (smokers 0.0006)	L 0.95
Benzphetamine	239.4	S	0.025–0.5	0.5
				L 14[d]
Benztropine	307.4	S	0.01–0.2	0.05
				L 0.7
Benzyl alcohol	108.1	B	18	
Bepridil	366.6	Pl	0.6–2.5	
Beryllium	9.0	B U	0.0009	0.7–1.7 (urine)
Betaxolol	307.4	S	0.005–0.05	L 36[d]
Bethanidine	177.2	S	0.02–0.5	
Bevantolol	345.4	Pl	0.2–2	
Bezafibrate	361.8	S	3–15	
Bicalutamide	430.4	Pl	1.5–17.5 (25) as *R*-enantiomer	
Biperiden	311.5	S	0.005–0.1	L 0.25[d]
Bismuth	209.0	B	0–0.05	0.1
Bisoprolol	325.5	S	0.01–0.06	
Bopindolol	380.5	Pl	0.001–0.015 after hydrolysis	
Borate	58.8	Pl	0–7	20
				L 200
Boron	10.8	S	0.8–6	20–50
				L 50–150

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Brallobarbital	287.1	S	4–8	8–10
				L 50–150
Bretylium	414.4	S	0.08–2.4	
Brodifacoum	523.4	S		0.02
				L 0.03–0.17
Bromadiolon	527.4	S		0.02
Bromazepam	316.2	S	0.08–0.17	03–0.4
				L (1)–2
Bromide	79.9	S	Normal 3–30	Bromism >200
			Therapeutic 75–100 (300)	500–1000 (1500)
				L 2000
Bromisoval	223.1	S	10–20	30–40
Bromoxynil	276.9	Pl		20
Bromperidol	420.3	S	0.001–0.02	
Brompheniramine	319.2	S	0.005–0.015	0.2[d]
Brotizolam	393.7	S	0.001–0.02	
Buflomedil	307.3	S	0.2–0.5	15–25
				L 25
Bunitrolol	248.3	S	0.001–0.015	
Bupivacaine	288.4	S	Epidural anaesthesia (e.a.) 0.25–0.75	4–5 as cardiac drug 1.5 e.a.
			P 1–4	
Buprenorphine	467.6	S	0.0001–0.001	0.02–0.003
			P 0.001–0.005	L (1.1[d]) 4–13
			As substitute therapy 0.001–0.008	
Bupropion	239.7	S	0.025–0.1	0.17
				L 0.45
Buspirone	385.5	S	0.0009–0.005 (0.01)	
Butabarbital (secbutobarbital)	212.2	S	5–15	10
				L 30
Butalbital	224.3	Pl	1–10	10–15
				L 15–30
Butanone	72.1	Pl	0–10	500
Butaperazine	409.6	Pl	0.02–0.7	
Butobarbital	212.3	Pl	2–15	(14) 32–98
				L (11) 30–75
Butorphanol	327.5	S	0.0003–0.002	
Butriptyline	293.5	S	0.07–0.15	0.4–0.5
Cadmium	112.4	B	0–0.0065	0.015–0.05
Cadmium	112.4	U	0–0.005/g creatinine	
Caffeine	194.2	S	T 8–20 (drink 2–5)	30–50
				L >80–100
Camazepam	371.8	S	0.1–0.6	2
Camphor	152.2	Pl		0.3–0.4 1 (1.7)
Candesartan	440.5	Pl	0.08–0.18	
Captopril	217.3	S	0.05–0.5 (1)	5–6
				L 60
Carazolol	298.4	Pl	0.015	
Carbamazepine	236.3	S	4–12	15
Carbamazepine epoxide		S	0.5–6	15
Carbaryl	201.2	Pl		5
				L 6

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Carbenoxolone	570.8	Pl	~5–30	50
Carbimazole	186.2			
Methimazole	114.2	S	T 0.5–3.4	
Carbinoxamine	290.8	Pl	~0.02–0.04	
Carbocromen	361.4	Pl	0.8–2.4	
Carbon monoxide HbCO	28.0	B	1–5% (smoker 8–10%)	25–35% L 50–60%
Carbon tetrachloride	153.8	B	0.07	20–50 L 100–200
Carboplatin (free fraction)	371.3	Pl		
Platinum	195.1	S	P 10–25	T 0.1–0.2
Carbromal	237.1	S	2–10	15–20 L 40
Bromide	79.9	S	5–30	300
Carisoprodol	260.3	S	2.5–10	
Carteolol	292.4	Pl	0.01–0.1	
Carvedilol	406.5	Pl	~0.02–0.15 (–0.3)	
Cefaclor	385.8	Pl *20°C unstable*	Oral 13–35 IV to 900	
Cefalexin	365.4	Pl *20°C unstable*	P 10–65 T 1–5	
Cefaloridine	415.5	Pl *20°C unstable*	T 0.5–1 P 20–80	
Cefalothin	396.4	Pl *20°C unstable*	P 15–30	
Cefamandole	462.5	Pl *20°C unstable*	T 1–5 P 10–40–150	
Cefazolin	454.5	Pl *20°C unstable*	P 50–150	
Cefotaxime	477.5	Pl *20°C unstable*	T 0.5–2 P 10–50 IV to 225	
Cefoxitin	427.4	Pl *20°C unstable*	P 25–220	
Cefsulodin	554	Pl *20°C unstable*	20–100	
Ceftazidime		Pl *20°C unstable*	T20–40 P50–200	
Ceftizoxime	383.4	Pl *20°C unstable*	P 40–160	
Ceftriaxone	598.5	Pl *20°C unstable*	15–75	
Cefuroxime	424	Pl *20°C unstable*	T 0.5–1 P 10–60 IV to 180	
Celiprolol	379.5	Pl	0.05–1	
Cephradine	349.4	Pl *20°C unstable*	T 0.5–1 P 20–50	
Cerivastatin	459.6	Pl	0.002–0.04	
Cetirizine	388.9	Pl	~0.02–0.4	
Chloral hydrate	165.4			
Trichloroethanol	149.4	S	(1.5) 5–15	40 L 60
Chlorambucil	304.2	Pl	0.15–0.3 (–1.0)	
Chloramphenicol	323.1	S	5–15 T 5–10 P 10–20 (25)	25 T 10

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Chlordane	409.8	Pl	0.001	0.0025
				L 1–7
Chlordecone	490.6	Pl		0.5
Chlordiazepoxide	299.8	S	0–4.4	3–10
				L 20
Demoxepam	286.7	S	0.5–0.74	1
				L 2.7
Chlormezanone	273.7	S	2.5–10	20
				L 53[d]
Chlorobutanol	177.5	Pl		100[d]
				L 64
Chloroform	119.4	B	20–50	70–260
				L 390
Chlorophacinone	374.8	Pl		0.1
Chlorophenoxyacetic acid	221.0	S		100
Chloroprocaine	270.8	S	2–4	
Chloroquine	319.9	S	0.02–0.2	0.6–1
				L 3
Chlorothiazide	295.7	S	~6	
Chlorpheniramine	274.8	S	0.001–0.017	0.02–0.03 1.1[d]
Chlorphentermine	183.7	S	0.32	
Chlorpromazine	318.9	S	0.03–0.3	0.5–2
				L 2
Chlorpropamide	276.7	Pl	30–250	200–750
Chlorprothixene	315.9	S	0.02–0.2	0.4–0.7
				L 0.8
Chlorpyrifos	350.6	Pl		0.2
Chlortalidone	338.8	S	Blood: 5–10	(Plasma) ~2
			Plasma: 0.14–1.4	
Chlortetracycline	478.9	S	1–5 (–10) (plasma)	30
Cholinesterase, pseudo-		S	2000–7000 U/L	1000 U/L
Chromium	52.0	Pl	0.00035	32[d]
Cibenzoline		Pl	0.2–0.4 (–0.9)	(0.5–) 1
Ciclosporin	1203	*EDTA blood*	T 0.1–0.4	T 0.35–0.4
			After 2 h 0.75–1.5 depending on transplanted organ or disease	
			Immunoassay ~10–20% higher due to metabolites	
Cidofovir	279.2	Pl	10–50	
Cilazapril	435.5	Pl	0.003–0.09	
Cimetidine	252.3	Pl	0.5–1.5	T 1.3
				L 110[d]
Ciprofloxacin	331.4	S	T 0.05–0.5	12[d]
			P 1–5	
Cisapride	466.0	Pl	0.04–0.08	
Cisplatin (free fraction)	300.1	Pl	P 1–5	T 0.1
Platinum		S	P 10–25	T 0.1–0.2
Citalopram	324.4	Pl	0.02–0.2	L 0.5
Clarithromycin	748.0	Pl	~0.2–2	
Clemastine	343.9	Pl	~0.001–0.002	20
Clenbuterol	277.2	Pl	0.0003–0.0006	0.003[d]
Clindamycin	425.0	Pl	T 0.05–2 P	
			1.5–9.5	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Clobazam	300.7	S	0.1–0.4	
N-Desmethylclobazam	286.7	S	2–4	
Clofibrate	242.7	S	50–250	
Clomethiazole	161.7	Pl	0.1–5	13–26
Clomipramine	314.9	S	(0.02) 0.09–0.25	
N-Desmethylclomipramine	300.8	S	sum 0.15–0.55	sum 0.4
				L sum 1–2
Clonazepam	315.7	Pl *may be reduced by microorganisms*	0.02–0.07	0.1
Clonidine	230.1	S	0.0002–0.002	0.025–0.06
				L 0.23[d]
Clopenthixol (Zu)	401.0	S	T 0.002–0.010 (0.015)	0.05–0.1
Clorazepic acid (clorazepate)	314.7	S	only detectable after IV administration	
Nordazepam	270.7	S	sum 0.02–0.8	sum 1.5–2
Clotiazepam	318.8	Pl	0.1–0.7	
Cloxacillin	435.9	S	5–30	P 85
Clozapine	326.8	S	Adult 0.35–0.6 (0.8)	(Seizures >0.5) 0.8–1.3
			Child 0.2–0.4	L 3
Desmethylclozapine	312.8	S	0.1–0.6	0.7
Cobalt	58.9	B	0.0001–0.0022	
Cocaine	303.4	S	0.05–0.3	0.25–5
				L 1–20
Codeine	299.4	S	T 0.01–0.05	0.3–1
			P 0.05–0.250	L 1.6
Morphine	285.4	S		0.15
Colchicine	399.4	S	0.0003–0.0024	0.005
			P 0.003	L 0.024[d]
Colistin	~1170	S	1–5 -(10)	
			Cystic fibrosis 10–350	
			[d]10^6 units	
Copper	63.6	Pl	0.6–1.5	2
				L 5
Cresol	108.1	Pl		~50
				L 120
Cromoglicate sodium	512.3	Pl	~0.01	
Cyanide	26.0	B	0.001–0.006	0.5
			Smoker 0.005–0.012 (–0.15)	L (1) 4–5
Cyclizine	266.4	S	0.1–0.25 (0.03–0.3)	0.75–1
				L 15
Norcyclizine	252.5	S	0.004–0.025	
Cyclobarbital	236.3	S	2–10	10–15
				L 20
Cyclobenzaprine	275.4	S	~0.003–0.036	0.4
Cyclophosphamide	279.1	Pl	10–25	
Cyclopropane	42.1	Pl	80–180	
Cyproheptadine	287.4	Pl	~0.05	
Cytarabine (Ara C)	243.2	Pl *add 1 drop 1 mol/L tetrahydrouridine for stabilisation*	0.05–0.5	
Danazol	337.5	Pl	~0.2	

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Dantrolene	314.3	S	0.4–1.5	
			T 0.3–1.4	
			P 1–3	
Dapsone	248.3	S	0.5–5	10–20
				L 18[d]
Deptropine	333.5	S		0.015
Desferrioxamine	406.8	S	3–15	
Ferrioxamine	1082.5	S	0.5–3	
Dexamethasone	392.5	Pl	~0.05–0.265	
Dexfenfluramine	231.2	S	0.03–0.06	0.15–0.25
Dextromethorphan	271.4	S	0.01–0.04	0.1
				L 3
Dextromoramide	392.5	S	0.075–0.15	0.2
				L 0.9
Dextropropoxyphene	339.5	Pl	0.05–0.75	1
				L 2
Nordextropropoxyphen		Pl	sum 0.75–3	sum 3
Diazepam	284.7	Pl	Anxiolytic 0.125–0.25	1.5
			Antiepileptic 0.25–0.5	L 5
			Eclampsia, tetanus, strychnine poisoning 1–1.5	
Nordazepam	270.7	S	Approximately the same as diazepam 0.2–1.8	
Diazinon	304.3	S	0.05–0.1 (0.5)	
Diazoxide	230.7	S	10–50	50–100
Dibenzepin	295.4	S	T 0.025–0.15	
			P 0.1–0.5	
Desmethyldibenzepin		S	sum 0.2–0.4	sum 3
				L 18
Dichlorodiphenyltrichloroethane	354.5	S	0–0.013	
Dichloromethane	84.9	S		L 280
2,4-Dichlorophenoxyacetic acid	221.0	S		~100
				L 200
Dichlorvos	221.0	B		L 29[d]
Diclofenac	296.2	S	T 0.05–0.5	50–60[d]
			P 0.1–2.2	
Dicoumarol	336.3	S	8–30 (50)	50–70
Dicycloverine	309.5	Pl	0–0.1	~0.2
				L 0.5
Didanosine	236.2	Pl	0.5–2.9	
Dieldrin	380.9	S	0–0.0015	0.15–0.3
Diethylcarbamazine	199.3	Pl	0–0.2	
Diethylpropion	205.3	Pl	0.007–0.2	2
				L 5.4[d]
Difenacoum	444.0	Pl		0.5
Diflunisal	250.2	S	(9) 40–100 (200)	300–500
				L 600
Digitoxin	764.9	S	0.01–0.03	0.03
				L 0.04–0.1
Digoxin	780.9	S	T 0.0005–0.001 (was 0.002)	T (0.0014) 0.0025–0.007
				L (0.0015) 0.01–0.03 depending on potassium level

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Dihydrocodeine	301.4	S	0.03–0.25	0.5–1
				L 2
Dihydroergotamine	583.7	Pl	0.001–0.01	
Diltiazem	414.5	S	0.05–0.4	0.8
				L 2–6
Dimethadione	129.1	S	500–1000	1000
Dimethyltryptamine	188.3	S	0.001–0.1	
Dimetindene	292.4	S	P 0.01–0.05	
Dinitro-*o*-cresol (DNOC)	198.1	S	1–5	30–60
				L75–100
Diphenhydramine	225.4	Pl	0.1–1	1
				L 5
Diphenoxylate	452.6	S	~0.01	
Dipipanone	349.5	Pl	~0.05	0.2
Dipyridamole	504.6	S	1–2	4
			T 0.1–1	
Diquat	184.2	S U		0.1–0.4
Disopyramide	339.5	Pl	2–7	8
Nordisopyramide	297.5	S	20% active stronger anticholinergic	sum 8–10
Disulfiram	296.5	S	0.05–0.4	0.5–5
				L 8
Diethyldithiocarbamate	171.3	S	0.3–1.4	
Divinyl oxide	70.1	B		L 700
Dixyrazine	427.6	B	~0.3	L 5.5[d], 9.4[d]
Domperidone	425.9	S	0.005–0.025 (0.04)	
Donepezil	379.5	Pl	0.03–0.075	[d]Active metabolite 6-*O*-desmethyldonepezil
Dosulepin (dothiepin)	295.4	S	0.02–0.15 (0.4)	0.8
				L (1) 5–19
Desmethyldosulepin		S	0.1–0.2	0.75
Dosulepin *S*-oxide		S	0.04–0.4	0.65–2.2
Doxacurium chloride	1106.1	Pl	0.01–0.3	
Doxapram	378.5	S	(1.5) 2–5.2	9 (doxapram and keto-doxapram
Doxazosin	451.5	S	0.01–0.05	
Doxepin	279.4	Pl	sum 0.05–0.35	0.1
				L 1–18
Nordoxepin	265.4	S	sum 0.2–0.35	0.5–1
				L 2–4
Doxorubicin	543.5	S	0.006–0.02	
Doxycycline	444.5	S	(1-) 5–10	30
Doxylamine	270.4	S	0.05–0.2	1–2
				L 5
Dronabinol (Δ^9-tetrahydrocannabinol, THC)	314.5	Pl	0.01–0.2	
Droperidol	379.4	Pl	~0.05	
Dyphylline	254.3	S	6.5–14 (–20)	40
Edrophonium	165.2	S	0.15–0.2	~0.15
Efavirenz	315.7	Pl	1.0–4.0	4–6
Emetine	480.6	Pl	~-0.1	0.5
Enalapril	376.5			
Desethylenalapril		S	0.01–0.05 (0.1)	

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Encainide	352.2	S		
3-Methoxy-*O*-desmethylencainide (MODE)	368.5	S	0.06–0.28	
O–Desmethylencainide (ODE)	338.5	S	0.1–0.3	0.3
Endrin	380.9	S	0–0.003	0.01–0.03
Enoximone	248.3	Pl	≥0.2	
Entacapone	305.3	Pl	0.4–1.0 (–7.0)	
Ephedrine	165.2	S	0.02–0.2	1
				L 5[d]
Epirubicin	543.5	S	0.01–0.05	
Eprosartan	520.6	S	0.01–0.04	
Erythromycin	733.9	S	0.5–6	12–15
			T 0.5–1	
			P 4–12	
Esmolol	295.4	Pl	0.15–2	
Estazolam	294.8	S	0.055–0.2	
Etacrynic acid	303.1	S	0.05–0.1	
Ethambutol	204.3	S	0.5–6.5	6–10
Ethanol	46.1	B	0–25	1000–4500 L (2250) 4000–6000
Ethchlorvynol	144.6	S	0.5–8	20 L 50
Ethinamate	167.2	S	5–10	50–100
				L 100 (200[d])
Ethosuximide	141.2	S	40–100	(100) 150–200
				L 250
Ethyl ether	74.1	S	500–1500	L (1400) –1900
Ethylene glycol	62.1	S		200–500
				L 2000
Etidocaine	276.4	S	0.5–1.5	1.6–2
Etilefrine	181.2	S	P 5–15	
Etodolac	287.4	S	20–50	
Etomidate	244.3	Pl	0.1–0.5 (–1)	
Etoposide	588.6	S	T 2–6	
			P 8–14	
Everolimus	958.2	B	T 0.002–0.006 (0.01)	0.006–0.009
			At 2 h 0.003–0.015	
			Immunoassay ~30–35% higher due to metabolites	
Famotidine	337.4	S	0.02–0.06 (0.2)	0.42[d]
Felbamate	238.2	Pl	30–60	70–120
Felodipine	384.3	S	0.001–0.008 (0.012)	0.01–0.015
Fenbufen	254.3	S	~-60	
Fenfluramine	231.3	Pl	0.05–0.15	0.5–0.7
				L 6
Fenitrothion	277.3			1
Fenofibrate	360.8	Pl	5–30	
Fenoldopam	305.8	Pl	0.003–0.06	
Fenoprofen	242.3	S	15–65	
Fenoterol	303.4	Pl	(0.001–) 0.01–0.04	
Fentanyl	336.5	S	0.001–0.002	0.002–0.02
Fexofenadine	501.7	Pl	0.3–0.6	
Finasteride	372.6	Pl	0.008–0.01	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Flecainide	414.4	S	T 0.45–0.9	1.5–3
			P 0.75–1.25	L 2.6[d],13[d]
Flucloxacillin	453.9	Pl	3–30	
Fluconazole	306.3	S	5–15 (40)	50–75
Flucytosine	129.1	S	T 25–50	100
			P 50–100	
Flumazenil	303.3	S	0.01–0.05	0.5
			P 0.2–0.3	
Flunarizine	404.5	S	0.025–0.2	0.3
Flunitrazepam	313.3	S *may be reduced by microorganisms*	0.005–0.015	0.05
Fluoride	19	S U *not in glass container*	After 24 h stop medication (equilibrium) 0.08–0.15	T 0.5–2 L 3
5–Fluorouracil	130.1	S	0.05–0.3	0.4–0.6 1 neurotoxic
Fluoxetine	309.3	S	0.1–0.45	1.5–2
Norfluoxetine	295.3	S	sum 0.15–0.5 (0.9)	0.4
				L 0.9–5.0
Flupentixol	434.5	S	0.001–0.015	
Fluphenazine	437.5	S	(0.0002–) 0.001–0.017	0.05–0.1
Flupirtine	304.3	Pl	0.5–1.5	~3–4
Flurazepam	387.9	S	0.0005–0.03	0.15–0.2
			Sedation 0.007	L 0.5–17
N–Desalkylflurazepam	288.9	S	0.04–0.15	sum 0.2–0.5
Flurbiprofen	244.3		5–15	
Fluvoxamine	318.3	S	0.05–0.25	0.65
Furosemide	330.8	S	2–5 (10)	25–30
Gabapentin	171.2	Pl	2–20	25
Galantamine	287.4	Pl	0.03–0.14	
Gallopamil	484.6	Pl	0.02–0.1	L 8[d]
Gamma-hydroxybutyric acid (GHB) see Hydroxybutyrate				
Ganciclovir	255.2	S	0.5–5	T 3–5
			T 0.2–1	P 20
			P 5–12.5	
Gemcitabine	263.2	Pl	3–6	
Gemfibrozil	250.3	Pl	~-25	
Gentamicin	449–477	S	P 4–15	T 2
			T 0.05–2	
Glibenclamide	494.0	S	0.03–0.35	0.6
Glipizide	445.5	Pl	0.1–1.0 (2.5)	2
Glutethimide	217.3	S	2–12	12–20
				L 30
Gold	197.0	S	3–8	10–15
Granisetron	312.4	Pl	0.009–0.017	
Griseofulvin	352.8	Pl	0.3–1.3 (2.5)	
Guaifenesin	198.2	B	~0.3–1.4	
Guanethidine	198.3	S	0.01	
Haloperidol	375.9	S	0.005–0.015 (0.04)	0.05–0.1
				L 0.5
Halothane	197.4	B	22–84	L 200
Heptabarb(ital)	250.3	Pl	1–4	8–15
				L 20

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Heptaminol	145.2	Pl	~0.2–1(–1.5)	
Heptobarbital	218.2	S	50–100	125–150
Hexachlorophene	406.9	S	0.003–0.65 (1)	L 35
n–Hexane	86.2	Pl	0.01	
Hexapropymate	181.2	S	2–5	10–20
Hexobarbital	236.3	S	1–5	8 (10–20)
				L 50
Hydralazine	160.2	S	(0.05) 0.2–0.9	
Hydrochlorothiazide	297.7	S	0.07–0.45	
Hydrocodone	299.4	S	0.002–0.024 (0.05)	0.1
				L 0.1 (0.2)
Hydrogen sulfide	34.1	S		L 0.92
Hydromorphone	285.3	S	0.008–0.032	L >0.1
4–Hydroxybutyrate (GHB)	104.1	Pl	~50–120	80 (abuse)
				L 250–280 (abuse)
Hydroxychloroquine	335.9	S	T 0.1–0.4	0.5–0.8
			P 0.5–2.0	L 4
Hydroxyzine	374.9	S	P 0.05–0.09	0.1
				L 39[d]
Ibuprofen	206.3	S	15–30 (5–50)	100
Idebenone	338.4	S	0.05–0.2	
			P 0.65–0.85	
Imipenem	317.4	S	T 0.5–5	
			P 20–75	
Imipramine	280.4	S	0.045–0.15	
Desipramine	266.4	S	0.075–0.25 sum 0.15–0.3	sum 0.5
				L sum (0.8–) 4.5–13
Indinavir	613.8	Pl S	P (1–3 h) 7–12; (4–7 h) 3–7	10
			T (10–12 h)	
			Pl neg 0.1–0.5; resistant >0.75	
Indometacin	357.8	S	0.5–3	4–6
Indoramin	347.5	Pl	~0.025–0.1	
Iproniazid	179.2	Pl	~5	
Iron	35.8	S P *non-haemolytic*	0.5–2	6 child 2–8
				L 17
Iron	35.8	B	380–625	
Isoniazid	137.1	S	T 0.2–1	20
			P 3–10	L (30–) 100
Isopropanol	60.1	B		200–400
				L 1000
Acetone	58.1	B	5–20	200–400
				L 550
Isosorbide dinitrate	236.1	S	0.003–0.018	
isosorbide mononitrate	191.1	S	0.2–0.5	
Isotretinoin	300.4	Pl	T 0.4–1.8; oral 0.1–0.5	
Isoxicam	335.3	S	5–25	
Isradipine	371.4	Pl	0.0005–0.002 (–0.01)	
Itraconazole	705.6	S	T >0.25	
Hydroxyitraconazole		S	sum 1–4	sum 6
Kanamycin	484.5	S	T 1–4	T 5–10
			P 15–25	P 25–30

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[(a)]	*Reference concentration (mg/L)*	
			Therapeutic[(b)]	*Toxic*[(c)]
Ketamine	237.7	S	0.5–6.5	7 (abuse)
Ketanserin	395.4	S	0.015–0.2	
			P 0.08–1	
Ketazolam	368.8	S	0.001–0.02	
Nordazepam	270.7	S	0.2–0.6	1–2
Ketobemidone	247.3	Pl	0.025–0.030	
Ketoconazole	531.4	S	T 0.3–0.5	
			P 3–10 (20)	
Ketoprofen	254.3	S	1–5	
			P 5–15 (–20)	
Ketorolac	255.3	S	0.22–0.35	5 (plasma)
Ketotifen	309.4	Pl	0.001–0.004	0.02
				L 1.2[(d)]
Labetalol	328.4	S	0.025–0.2	0.5–1
Lacidipine	455.5	Pl	0.003–0.006	
Lamotrigine	256.1	S	2–15	15
				L 50[(d)]
Lead	207.2	B *Heparinised*	<0.3	Acute 0.45–0.6
			Child <0.2	Chronic 0.4
				L 3
Leflunomide	270.2	Pl	As metabolite A77172618-63[α]	0.02 teratogeric
Levetiracetam	170.2	S	6–20–(40)	400[(d)]
Levocabastine	420.5	Pl	Topical, nasal or ocular administration 0.001–0.01	
Levodopa	197.2	Pl	0.3–1.6	L 650[(d)]
Levomepromazine (methotrimeprazine)	328.5	S	0.02–0.15	0.5
Levomethadone	345.9	S	0.04–0.3	1
				L 0.2
Levorphanol	257.4	Pl	0.007–0.02	0.1
				L 0.8[(d)] (2.7[(d)])
Levothyroxine	776.9	Pl	0.045–0.14	
Lidocaine	234.3	S	(1–) 1.5–5	6–10
				L 10–25
Monoethylglycinexylidide (MEGX)	206.3	S	0.07–0.175	
Lisinopril	405.5	S	(0.005–) 0.02–0.07	0.5
Lithium	6.9	S	4–10 mg/L	T 1.5 (2) mmol/L (15 mg/L)
			T 0.5–1.2 mmol/L	L >35 mg/L
Lofepramine	419.0	Pl	0.003–0.03	
Loperamide	477.0	Pl	P 0.001–0.003	
Lopinavir	628.8	Pl S	P (3–5 h) 7–14	
			T (8–12 h) 5–7	
Loprazolam	464.9	S	0.03–0.01	
Loratadine	382.9	S	0.015–0.027	
Descarboethoxyloratadine	311.0	S	0.007–0.028	
Lorazepam	321.2	S	0.02–0.25	0.3–0.6
Lorcainide	370.9	Pl	(0.04–) 0.1–0.4 (–0.9)	
Lormetazepam	335.2	S	0.001–0.02	
Loxapine	327.8	S	0.01–0.03 (–0.1)	1
				L 7.7
Lysergide (LSD)	323.4	S	0.0005–0.005	0.001
				L 0.002

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Magnesium	24.3	Pl	17–25	T 85–120
				L 200–350
Malathion	330.4	Pl		0.5 L
				1.7[d]
Manganese	54.9	Pl	Plasma 0.0005–0.0015	
			Blood 0.004–0.012	
Maprotiline	277.4	S	0.075–0.25 (0.1–0.6)	0.3–0.8
				L 1–5
Desmethylmaprotiline	263.4	S	sum 0.1–0.4	sum 0.75–1
Mebendazole	295.3	Pl	≥0.1	0.5
Medazepam	270.8	S	0.01–0.15	0.6 (–1)
			P 0.1–0.5	
Nordazepam (= desmethyldiazepam)	270.7	S	0.2–0.6	1–2
Mefenamic acid	241.3	S	0.3–20	25
Mefloquine	379.3	Pl	0.4–2 (1.5–5.5[β])	1.5–2 carboxylic acid metabolite
Melitracen	327.9	Pl	0.01–0.1	
Meloxicam	351.4	Pl	0.4–2	
			P 1–4	
Melperone	299.8		0.05–1	L 17.1[d]
Melphalan	305.2	S	0.2–1.5	
MEGX (liver test)	206.3	S	T 0.070–0.175	0.05
Mephenesin	182.2	S	3–10	
Mephenytoin	218.3	S		
Desmethylmephenytoin	204.3	S	sum of mephenytoin + metabolite 15–40	sum of mephenytoin + metabolite 50
Mepindolol	262.4	Pl	0.007–0.07	
Mepivacaine	246.4	S	2–5.5	6–10
				L 50
Meprobamate	218.3	S	10–30	30–50
				L 110
Meptazinol	233.4	Pl	0.025–0.25	
6-Mercaptopurine	152.2	Pl *add 0.1 mol/L dithiothreitol for stabilisation*	0.03–0.08	1–2
Mercury (organic)	200.6	B U	0–0.01	0.1–0.3
				L 0.4–22
Mercury (inorganic)	200.6	B	0–0.08	0.2
				L>0.6
Mesalazine (mesalamine)	153.1	Pl	0.1–0.18	
Mesoridazine	386.6	S	0.1–1.1	3–5
				L 3[d],4[d]16[d]
Mesuximide	203.2	S	0.04–0.08	
N-Desmethylmesuximide (normesuximide)	189.2	S	10–30 (40)	40–50
Metaclazepam	393.7	Pl	0.05–0.2 (0.4)	
Metamfetamine (D-methylamfetamine; Ice)	149.2	Pl	0.01–0.05	0.2–1 (5) erratic driving
				0.05–2.5 (blood)
				L (2) 10–40
Metformin	129.2	S	1.0–4.0	45–70
Methadone	309.4	S	0.05–0.5 (1)	Naive users 0.2
			Analgesic 0.1–0.3	Users >0.75
			Addicts 0.2–0.75	L (acute) 0.2–1

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Methanol	32.0	B	Endogenous ~–1.5	200
				L >900
Methapyrilene	261.4	Pl	~0.1	>30 (4)
				L 2–380[d]
Methaqualone	250.3	Pl	0.4–5.0	>2
				L >8
Methazolamide	236.3	S	40	
Methimazole (thiamazole)	114.2	Pl	0.5–2 (–3)	
Methocarbamol	241.2	S	25–40 (–50)	250
Methohexital	262.3	Pl	(0.5–) 1–6	
Methotrexate	454.4	S	Active > 0.005	T >4.5 (24 h after dose); >0.45 (48 h after dose)
Methoxsalen (8-methoxypsoralene)	216.2	S	0.1–0.2	1
			T 0.025–0.1	
			P 0.1–0.4	
Methoxyflurane	165.0	B	30–200	
2-Methyl-4-chlorophenoxyacetic acid (MCPA)	200.6	Pl	~100 (500)	
			L ~180	
2-Methylchlorophenoxypropionic acid (MCPP)	214.6			~100 (500)
				L 669[d]
Methyldopa	211.2	S	1–5	7–10 9[d]
Methylenedioxyamfetamine (MDA, love drug))	179.2	S	0.4	1 (1.5)
				L 2
Methylenedioxyethylamfetamine (MDEA, Eve)	207.3	Pl	0.2	L 1
3,4-Methylenedioxymethylamfetamine (MDMA, XTC)	193.2	S	0.1–0.35	0.35–0.5
Methylenedioxyamfetamine	179.2	S		L 0.4–0.8
Methylfentanyl	350.5	S		L 0.002–0.011
Methylphenidate	233.3	S	0.005–0.06	(0.5) 0.8
				L 2.3
Methylthioamphetamine (4-MTA, *p*-MTA)	181.3	Pl		1 2[d],4.2[d]
Methyprylon	183.2	Pl	10–20	12–75 (–128)
				L 50 (–100)
Metiamide		S	0.01–0.06	
Metildigoxin	795.0	Pl	0.0005–0.0008 (–0.003)	0.0025–0.003
				L 0.005
Metipranolol as desacetylmetipranolol	267.3	Pl	0.02–0.08[γ]	
Metoclopramide	299.8	S	0.04–0.15	0.1–0.2
				L 4.4[d]
Metoprolol	267.4	S	0.1–0.6	(0.65[d]) 1
			T 0.02–0.34	L (4.7[d]) 12–18
Metronidazole	171.2	S	(3–)10–30	150 (200[d])
Mexiletine	179.3	S	0.5–2	2–4
				L 35[d]
Mianserin	264.4	S	0.015–0.07 (0.14–)	0.5–5
Desmethylmianserin	250.3	S	sum 0.04–0.125	sum 0.3–0.5 L
Miconazole	416.1	Pl	~1 (2–9)	
Midazolam	325.8	S	0.08–0.25	1–1.5 (glucuronide)
			(postoperative awake 0.1–0.04)	-metabolites also active

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Mifepristone	429.6	Pl	1–2	
Milrinone	211.2	S	0.15–0.25	0.3
Minoxidil	209.3	Pl	0.04–0.25 (oral)	1.4[d],3.1[d] L 2.7[d]
			<0.03 (topical)	
Mirtazepine	265.4	S	0.02–0.1 (–0.3)	
Desmethylmirtazepine	251.4	S	sum 0.05–0.3	sum 1
Mizolastine	432.5	Pl	~0.2–0.8	
Moclobemide	268.7	S	P 1.5–4	5–8
			T 0.4–1	L 16
Modafinil	276.4	Pl	~2–3 (9)	
Molindone	276.4	Pl	~0.5	
Molsidomine	242.2	Pl	0.001–0.02 (–0.2)	
Molybdenum	95.9	Pl	0.005	
Montelukast	586.2	Pl	0.05–0.3	
Moricicine	427.5	Pl	~0.12–1.27	
Morphine	285.4	S	0.01–0.12	0.15–0.5 L 0.05–4 strongly depending on route, how long abused (glucuronide) -metabolites also active
			Neonates under artificial breathing 0.08–0.12	
Moxonidine	241.7	Pl	0.001–0.002 (–0.004)	
Muromonab-CD3 (OKT 3)	50 000 + 25 000	Pl	~0.7–1.3	
Nabumetone	228.3	Pl	20–70	
6-Methoxy-2-naphthylacetic acid	216.3	Pl	~10–37 (–100)	
Nadolol	309.4	Pl	0.02–0.25	L 1.3[d]
Naftidrofuryl (Nafronyl)	383.5	Pl	<0.5	
Nalbuphine	357.4	Pl	0.03–0.6 (0.2)	
Nalidixic acid	232.2	S	10–30	40–50
Nalidixic acid	232.2	U	50–200	
Nalmefene	339.4	Pl	IV 0.010–0.2	
			Oral 0.01–0.2	
Naloxone	327.4	S	0.01–0.03	
Naltrexone	341.4	Pl	0.001–0.005	
6β-Naltrexol	343.4	Pl	0.05–0.15	
Naproxen	230.3	S	25–70 (90)	200–400
				414[d]
Naratriptan	335.4	Pl	(0.008–) 0.01–0.05	
Nebivolol	405.4	Pl	0.001 (fast metabolisers)	0.48[d]
			0.003 (slow metabolisers) <0.02	
Nedocromil	371.3	Pl	(0.003) <0.025	
Nefazodone	470.0	Pl	~0.01–0.3 (–1)	5.5[d]
Nefopam	253.3	Pl	0.01–0.1 (–0.2)	4[d];12[d]
Nelfinavir	567.8	Pl	P (2–4 h) 3–5; (4–7 h) 2–3	
			T (8–12 h) 0.75–1.5	
Neostigmine	223.3	Pl	0.001–0.01	
Netilmicin	475.6	S	P 7–15 (–18)	T 4
			T 0.5–2 (3)	
Nevirapine	266.3	Pl S	P (1–2 h) 4–8	
			T 3–5	
Nicardipine	479.5	S	0.035–0.135	
Nickel	58.7	S	0.0015–0.005	
Nickel	58.7	U	0.0005–0.006	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Nicotine	162.2	S	sum T 0.001–0.275 Transdermal 0.004–0.03 Heavy smoker T 0.003–005 P 0.03–0.35	
Cotinine	176.2	S	sum P 0.025–0.35 Light smoker T 0.175–0.275 P (end of day) 0.25 –0.34	sum 0.3–1 L 5
Nicotinic acid	123.1	Pl	3–19	
Nifedipine	346.3	S	0.02–0.1(–0.15)	0.15–0.2 L 5.4[d]
Niflumic acid	282.2	Pl	2–35	
Nilvadipine	385.4	Pl	<0.01	
Nimesulide	308.3	Pl	10–75	
Nimodipine	418.4	S	0.01–0.05	
Nimustine	272.7	S	0.0002–0.0005	
Nisoldipine	388.4	Pl	0.0003–0.001 (–0.01)	
Nitrazepam	281.3	S *may be reduced by microorganisms*	0.03–0.12	0.2–0.5
Nitrendipine	360.4	Pl	0.01–0.05	
Nitrofurantoin	238.2	S	0.5–2 (–3)	3–4 (child 2)
Nitrofurantoin	238.2	U	10–400	
Nitroglycerin	227.1	Pl	~0.015	
Nitroprusside	218.0	S		
Thiocyanate	57.1	S	(5) 6–30	50–100 L 200
Nizatidine	331.5	S	0.15–1	
Nomifensine (unbound)	238.3	S	T 0.02–0.06 P 0.2–0.6	0.8–1; total 8–10
Nordazepam (desmethyldiazepam)	270.7	S	0.2–0.8 (1.8)	1.5–2
Norfloxacin	319.3	S	0.5–5	
Nortriptyline	263.4	S	0.075–0.025 (0,4)	>0.25 L 1–3
10-Hydroxynortriptyline	279.4	S	0.04–0.4	
Obidoxime	359.2	S	1–10	
Ofloxacin	361.4	S	1–4 T 0.05–5 P 1–7	30– (40[d])
Olanzapine	312.4	S	0.02–0.08 (0.1)	0.2 L 1[d]
Ondansetron	293.4	S	0.03–0.3	
Opipramol	363.5	S	0.05–0.2 (0.5)	0.5–2 (3) L 7–10
Orphenadrine	269.4	Pl	0.05–0.2 (0.6)	0.5–1
Tophenacine	255.4	S	sum 0.05–0.2	sum 0.5–2 L4–8
Oxatomide	426.6	Pl	0.02–0.1	
Oxazepam	286.7	S	(0.15) 0.5–2	2 L 3–5
Oxcarbazepine	252.3	S		
10-Hydroxycarbazepine	254.3	S	12–35	45
Oxprenolol	265.4	S	0.05–0.3 (–1.0)	2–3 L 10

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Oxycodone	315.4	S	(0.005–) 0.02–0.05	0.2
				L 0.6
Oxytetracycline	460.5	S	5–10	30
Paclitaxel	853.9	Pl	P 2–12	
			T 0.5–1	
Pamidronate	235.1	Pl	~0.5–1	
Pancuronium	652.8	S	0.1–0.6	0.4
				L 1.6
Pantoprazole	383.4	Pl	~4.6	
Papaverine	339.4	S	0.2–0.6 (2)	
Paracetamol (acetaminophen)	151.2	S	10–20 (2.5–25)	T 75–100
				P 100–150
				L 160
Paraldehyde	132.2	B	sedative 30–100	200–400
			hypnotic 100–300	L >500 (less with alcohol ingestion)
Acetaldehyde	44.1	S B	0–30	100–125
Paramethadione	157.2	S	1.1–5	
Paraoxon	275.2	Pl		>0.005
Paraquat	186.3	S U		0.05
				L 2 (4 h); 0.1 (24 h)
Parathion	291.3	Pl		0.01–0.05 0.05–0.08
Paraoxon	275.2			
Paroxetine	329.4	S	(0.01) 0.07–0.15 (0.25)	0.3
Pefloxacin	333.4	S	T 0.1–6	25
			P 5–10	
Pemoline	176.2	Pl	1–7	
Penbutolol	291.4	S	(0.01–) 0.3–0.7 (1.0)	
Penciclovir	253.3	S	T 0.1–0.3	
			P 1.75–2 (oral); 10–20 (IV)	
Penfluridol	524.0	S	0.004–0.025	
Penicillin (benzyl)	334.4	S	1–10	
D-Penicillamine	149.2	Pl	~1.7–5.6 (–11)	
Pentachlorophenol	266.3	S	0–0.1 (–0.2 dietary)	30
				L >45
Pentamidine	340.4	Pl	~0.3–0.5	
Pentazocine	285.4	S	0.01–0.2 (0.5)	1–2
				L >1 (3)
Pentobarbital	226.3	Pl	1–10 (25–40)	(5–) 8–10
				L (8–) 15–25
Pentoxifylline (oxpentifylline)	278.3	Pl	~0.5–2	
Perazine	339.5	S	0.02–0.35	0.5
Desmethylperazine	325.5	S	0.04–0.55	
Pericyazine (periciazine)	365.5	S	0.005–0.03	0.1
Perindopril	368.5	Pl	(0.05–) 0.08–0.15	
Perphenazine	404.0	S	0.0004–0.03	0.05
Pethidine (meperidine)	247.3	S	0.1–0.8	(1–) 2
				L >5
Norpethidine	233.3	S	0.3	0.5
Phenacetin	179.2	S	5–20	50
Phenazone (antipyrine)	188.2	S	5–25	50–100

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Phencyclidine (PCP)	243.4	S		0.007–0.24
				L (0.3–) 1–5
Phendimetrazine	191.3	S	0.02–0.24 (–0.3)	
Phenelzine	136.2	S	0.001–0.002 (–0.2)	0.5
				L >1.5
Phenformin	205.3	Pl	0.03–0.1 (–0.3)	0.6
				L 3
Pheniramine	240.3	Pl	0.01–0.27	L 1.9[d]
Phenmetrazine	177.2	S	0.02–0.25	0.5 L 4
Phenobarbital	232.2	S	2–30 (–40)	30–40
				L 45–120
Phenol	94.1	S		50
				L 90
Phenprocoumon	280.3	S	1–3	5
Phensuximide	189.2	S	4–10	80
			P 10–20	
Phentermine	149.2	S	0.03–0.1	L 7.6[d]
Phenylbutazone	308.4	S	50–100	120–200
				L 400–500
Phenylephrine	167.2	S	0.03–0.1 (–0.3)	
Phenylpropanolamine (norephedrine)	151.2	S	0.05–0.5	2
				L 48
Phenytoin	252.3	S	8–20; baby 6–14	25; baby 15
				L 70
Free fraction		S	0.2–2	2–2.5
Physostigmine	275.3	S	<0.001–0.005	
Pimozide	461.6	S	0.001–0.02	
Pinazepam	308.8	Pl	0.01–0.05	
Pindolol	248.3	S	0.02–0.08 (–0.15)	0.7
Pipamperone	375.5	S	0.1–0.4	0.5–0.6
Piperacillin	517.6	S	T 1–5	
			P 20–70	
Piperazine	86.1	S	0.02–0.1	0.5
Pipotiazine	457.7	S	0.001–0.06	0.1
Piracetam	142.2	S	20–75	
Pirenzepine	351.4	S	0.03–0.45	
Pirmenol	338.5	S	1–3	
Piroxicam	331.3	S	5–10 (–20)	14[d]
Pizotifen (pizotyline)	295.4	Pl	0.007–0.009 (–0.01)	
Platinum	195.1	S	0.5–5	30
			P 10–30	T 10
Polythiazide	439.9	S	0.002–0.007	
Prajmalium	369.6	Pl	0.06–0.5	
Pramipexole	211.3	Pl	~0.0002–0.007	
Pranlukast	481.5	Pl	0.2–1.2	
Prazepam	324.8	S	0.01–0.04	
Nordazepam (desmethyldiazepam)	270.7	S	0.2–0.8	1–2
Prazosin	383.4	S	0.001–0.075	0.9
Prednisolone	360.4	Pl	0.5–1	
Prilocaine	220.3	S	0.5–2 (–5)	5
				L ~20

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Primaquine	259.4	Pl	~0.1–0.	
Carboxyprimaquine	274.4	Pl	1–2	
Primidone	218.3	Pl	T 5–12	10 (15–20)
				L 50
Phenobarbital	232.2	S	20–40	60–80
Probenecid	285.4	S	Inhibition penicillin excretion, 40–60; uricosuric 100–200	
Procainamide	235.3	S	4–10	10–15
				L >20
N–Acetylprocainamide (acecainide)	277.4	S	2–12	sum 40
			sum T 5–30	
Procaine	236.3	S	2.5–10	15–20
				L 20
Prochlorperazine	373.9	S	0.01–0.05	0.2–0.3
				L 5
Procyclidine	287.4	S	0.08–0.63	1–2
				L 7.8[d]
Proguanil	253.7	Pl	0.04–0.15	
Cycloguanil	251.7	Pl	0.02–0.06	
Promazine	284.4	S	0.01–0.4	(1–) 2–3
				L >5
Promethazine	284.4	S	(0.05) 0.1–0.4	1
				L 2.4[d]
Propafenone	341.5	S	0.4–1.1 (1.6)	1.1–3 L 7.7[d]
			β-Blocker >0.8	
Norpropafenone	415.5	S		sum 2–3
Propallylonal (Ibomal)	289.1	S	0.3–10	>10
Propantheline	368.5	Pl	~0.02 (–0.04)	
Propofol	178.3	B	Narcosis 2–4 (8)	Abuse L 0.22
			Induction –8	
Propoxyphen (dextro)	339.5	S	0.1–0.75	1
Norpropoxyphen	325.5	S	0.1–0.15	2
Propranolol	259.3	S	T 0.05–0.15	1–2
			P 0.1–0.3	L 4–10
Propylene glycol	76.1	S	0.05–0.5	1000
Propylhexedrine	155.3	S	0.01	0.5
				L 2–3
Propyphenazone	230.3	S	3–12	
Prothipendyl	285.4	S	0.05–0.2	0.5 (–1)
Protionamide	180.3	S	T 0.5	
			P 3–8	
Protriptyline	263.4	S	0.07–0.17 (0.38)	0.5
				L 1
Pseudoephedrine	165.2	S	0.5–0.8	L 19
Pyrazinamide	123.1	S	30–75	
Pyridostigmine	181.3	S	0.05–0.1 (–0.2)	
Pyridoxine	169.2	Pl	0.003–0.018	
Pyrimethamine	248.7	Pl	0.05–0.75	
			P 0.25–4	
Pyrithyldione	167.2	Pl	1–10	
Quazepam	386.8	S	0.01–0.15	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Quetiapine	383.5	S	(0.025) 0.75–0.5 (0.9)	1.8[d]
				L 7
Quinidine	360.5	S	(1) 2–6	(6–) 10 (–15)
				L 30
Quinine	378.5	S	1–7 (–9.5)	10
Rabeprazole	359.4	Pl	0.25–1	
Ramipril	416.5	Pl	~0.001–0.01 as metabolite ramiprilat	
Ranitidine	314.4	S	(0.05) 0.15–0.5	
Recainam	263.4	Pl	1.3–6	
Remoxipride	371.3	Pl	0.7–8	L 41–150
Retinol		Pl	0.2–0.8	
Ricin	30 000 + 33 000	S	0.0005	
Rifabutin	847.0	S	T 0.05	
			P 0.15	
Rifampicin	823.0	S	sum with metabolite	
Desacetylrifampicin	781.0	S	sum P 5–20	sum P 20
			sum T 0.5–1	sum L 55[d]
Riluzole	234.2	Pl	0.05–0.5 (–1.5)	
Risperidone	410.5	S	0.003–0.03	0.08
9-Hydroxyrisperidone	426.5	S	sum 0.02–0.06	0.08
Ritonavir	721.0	Pl	P (2–6 h) 5–8	
			T (8–12 h) 2–4 (12)	
			As booster 0.3–2	
Rizatriptan	269.4	Pl	0.005–0.1	
Ropinirole	260.4	Pl	0.0004–0.006	
Ropivacaine	274.4	Pl	<4 (mean maximum tolerated)	(1–2) >4
			0.6 (free arterial plasma)	
Roxatidine	384.9	Pl	0.1–0.8	
Roxithromycin	837.0	Pl	4–12	
Salbutamol	239.3	S	0.004–0.018	0.03
				L 0.16
Salicylamide	137.1	Pl	5–40 (50)	
Salicylic acid	138.1	S	Rheumatism 200–300 (child 150)	400–500 (child 300)
			anticoagulant 50–125	L 500–900
			prostaglandin synthetase inhibitor 50–150	
Saquinavir	721.0	Pl	P (2–5 h) 0.8–1.6; medium (5–10 h) 0.3–0.8	
			T (10–12 h) 0.1–0.4	
Scopolamine (hyoscine)	303.4	S	0.0001–0.0003 (–0.001)	
Secbutabarbital (butabarbital)	212.2	S	5–15	10
				L 30
Secobarbital	238.3	S	2–10	>8
				L (4–) 10–50
Selenium	79.0	Pl	0.045–0.13	0.4
Sertraline	306.2	S	0.05–0.25 (–0.5)	0.29[d];1.6[d]
Sildenafil	474.6	Pl	0.025–0.25 (0.5)	Depending on cardiac function
Silver	107.9	B	0–0.005	
			with silver sulfadiazine ointment for burns 0.06–0.6	

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Sirolimus	914.2	Pl	0.004–0.015	0.020
			single therapy 0.012–0.020	
Sotalol	272.4	S	0.5–3 (5)	5–10
				L 40[d]
Sparteine	234.4	Pl	0.5–1	
Spironolactone	416.6	S	0.1–0.5	
Canrenone	340.5	S	0.05–0.25 (–0.5)	
Stiripentol	234.3	Pl	4–20	20
Streptomycin	581.6	S	T 1–5	T 5
			P 15–40	P 40–50
Strontium	87.6	S	0.03	
Strychnine	334.4	S	0.075–0.1	
			L 0.2–2	
Sufentanil	386.6	S	0.0005–0.005	L 0.001–0.007[d]
			P 0.01–0.02	
Sulfaguanidine	214.2	S	30–50	
Sulfamethoxazole	253.3	S	30–60 (100–200)	200–400
Sulfanilamide	172.2	S	100–150	
Sulfasalazine	398.4	Pl	5–30 (–70)	
Sulfinpyrazone	404.5	Pl	6–17 (–21)	
Sulfonamides		S U	T 35–75	200
			P 80–150	
Sulindac	356.4	S	0.5–5	
Sulindac sulfide	340.4	S	sum (including sulfone) 1–5	
Sulpiride	341.4	S	0.04–0.6	L 3.8[d]
			P 0.15–0.75	
Sultiam (sulthiame)	290.4	S	0.5–12.5	12–15
				L 20–25
Sumatriptan	295.4	Pl	0.018–0.06	
Suramin	1407.2	Pl	150–250	300
Tacrine	198.3	Pl	0.007–0.03	0.02
Tacrolimus	804.0	B	T 0.003–0.01	T 0.003–0.01
Talinolol	363.5	Pl	0.04–0.15	L 5[d]
Talipexole	282.2	Pl	Continuous infusion 0.003–0.01	
Tamoxifen	371.5	S	0.05–0.5	
Teicoplanin	189.4	S	10–40	200
Temazepam	300.7	S	0.3–0.9	1 L 8.2[d]
			T 0.02–0.15	
Tenoxicam	337.4	Pl	5–10	
Terazosin	387.4	Pl	0.02–0.08 (–0.1)	
Terbinafine	291.4	S	T 0.01–0.03	
			P 0.5–3	
Norterbinafine	277.4	S	P 0.4–0.8	
Terbutaline	225.3	S	0.001–0.006 (–0.01)	L 0.04
Terfenadine	471.7	Pl	0.0015–0.0045	0.06
				L 0.4[d]
Tetrachloroethylene	165.8	S		L 4–5
Tetracycline	444.4	S	5–10	30
			T 1–5	
Tetrahydrocannabinol	314.5	Pl (unstable)	P 0.05–0.125	
			0.001–0.01	0.004–0.0015
Tetrazepam	288.8	Pl	0.05–0.6 (–1)	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Thalidomide	258.2	Pl	0.5–1.5 (–8)	
Thallium	204.4	B	<0.005	0.1–0.5
				L 0.5
Thallium	204.4	U		0.1
Theobromine	180.2	S	10–15	20
Theophylline	180.2	S	8–20; baby 5–10	25–30; baby 15
				L 50–250
Thiamphenicol	356.2	S	3–10 (peak 5–15)	>20
			P 5–15	
Thiazinamium	299.5	S	0.05–0.15	0.3
Thiocyanate	58.0	S	Non-smokers 1–4	35–50
			Smokers 3–12	L 200
			As metabolite of nitroprusside 6–30	
Thiopental	242.3	S	1–5 (flat EEG: 25–40)	10 (40–50)
				L 10–100
Pentobarbital	226.3	S	5–10	10–15
Thioproperazine	466.6	S	~0.001–0.02	0.1
Thioridazine	370.6	S	0.2–1	2 (5)
				L 3–10
Mesoridazine	386.6	S	0.3 (0.2–1.6)	
Sulforidazine	402.6	S	<0.6; sum 0.75–1.5	sum 3
Tiagabine	375.6	Pl	0.04–0.1 (0.2)	0.4–0.5
Tiapride	328.4	Pl	P1–2	
Tiaprofenic acid	260.3	S	15–35 (–40)	
Ticlopidine	263.8	Pl	<1–2	
Tilidine (tilidate)	273.4	Pl	0.05–0.12	1.7[d]
Nortilidine (nortilidate)	259.4	Pl	0.2	4.4[d]
Tiludronate (tiludronic acid)	318.6	Pl	0.2–1.5	
Timolol	316.4	S	T 0.005–0.05	
			P 0.02–0.1	
Tin	118.7	S	0.03–0.14	
Tinidazole	247.3	S	<60	
Tiopronin	163.2	Pl	2–5	
Tiotixene	443.6	S	T 0.001–0.02	0.1
			P 0.01–0.1	
Tipranavir	602.2		P (2–4 h) 45–90	
			Medium (4–8 h) 30–75	
			T 12–40	
Tizanidine	253.7	Pl	~0.015	
Tobramycin	467.5	S	T 0.1–1 (2)	T 2 (chronic treatment)
			P 7–12 (15)	
Tocainide	192.3	S	4–12	(13–15) 25
				74[d]
Tofenacin	255.4	S	P 0.025–0.1	0.5–1
Tolbutamide	270.3	S	45–100	400–500
				L 640[d]
Tolmetin	257.3	S	10–80	
Toluene	92.1	B		L 10
Topiramate	339.4	Pl	(2) 4–10 (20)	20–25

table continued

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Topotecan	421.5	Pl	sum ~0.001–0.01	
Tramadol	263.4	B	0.01–0.25	0.8
Tranexamic acid (tranexamate)	157.2	Pl	10–50	
Tranylcypromine	133.2	S	0.05–0.3	0.3–0.5
Trapidil	205.3	S	(4–) 6–10	
Trazodone	371.9	S	0.5–2.5	4
			T 0.3–1.5	L 12–15
			P 1.5–2.5	
Triamterene	253.2	S	T 0.01–0.1	
			P 0.05–0.2	
Triazolam	343.2	S	0.002–0.02	0.04
2,2,2-Tribromoethanol	282.8	B		50
				L 90
Trichlorfon (metrifonate)	257.4	Pl	1.5–4	
2,2,2-Trichloroethanol	149.4	S	5–15	40–70
				L 60–100
2,4,5–Trichlorophenoxyacetic acid	255.5	S		~100
				L 200
Trifluoperazine	407.5	S	(0.001–) 005–0.05	0.1–0.2
Triflupromazine	352.4	S	0.03–0.1	0.3–0.5
Trihexyphenidyl	301.5	S	0.05–0.2	0.5
Trimethadione	143.1	S	20–40	
Dimethadione	129.1	S	500–1000	1000
Trimethobenzamide	388.5	S	1–2	
Trimethoprim	290.3	S U	1.5–2.5 (5–10)	15–20
Trimipramine	294.4	S	0.07–0.3	0.5
				L 8.7[d]
Triprolidine	278.4	S	0.004–0.045	
Tropisetron	284.4	S	T 0.02–0.03	
			P 0.05–0.1	
Tubocurarine	646.2	S	0.7–3 (–6)	
Tungsten	183.8	Pl	0.035	
Urapidil	387.5	S	~0.1–0.2	
Valaciclovir	360.8			
Aciclovir	225.2	S	T 0.2–1.5	
			P 0.5–1.5	
Valnoctamide	143.2	S	5–25	40
Valproic acid	144.2	Pl	50–100 (150)	150–200
				L 720[d]
Vanadium	50.9	Pl	0.05	
Vancomycin	1449.3	S	T 8–15	P (30) 50
			P 20–40; continuous infusion 13–25	
Vecuronium	557.8	Pl	~0.2–0.37 (–0.5)	
Venlafaxine	277.4	S	sum 0.2–0.75	
O–Desmethylvenlafaxine	263.4	S	sum 0.25–0.75	sum 1–1.5
				L 6.6[d]
Verapamil	454.6	S	0.05–0.35	0.9
				L 2.5–4
Norverapamil (desmethylverapamil)	440.6	S	sum 0.15–0.6	sum 1
Vigabatrin	129.2	S	T (1) 5–25	

Table 1.28 continued

Compound	*Relative molecular mass*	*Material*[a]	*Reference concentration (mg/L)*	
			Therapeutic[b]	*Toxic*[c]
Viloxazine	237.3	Pl	P 5–10	
Vinblastine	811.0	Pl	P 0.25–0.4	
Vincristine	824.9	Pl	P 0.3–0.4	
Vinylbital	224.3	S	1–4	5
				L 8
Viquidil	360.9	Pl	0.15–0.25	
Voriconazole	349.3	S	0.5–4	6
Warfarin	308.3	S	1–3 (7)	10–12
			T 0.3–3	L 100
			P 5–10	
Xamoterol	339.4	Pl	0.02–0.15	
			P 0.1–0.27	
Xylene	106.2	B		L 3–40
Yohimbine	354.4	Pl	0.05–0.3	
Zafirlukast	575.5	Pl	0.005–0.03	
			P 0.1–0.7	
Zalcitabine	211.1	Pl	P 0.02	
Zidovudine	267.2	S	T 0.1–0.3	0.5–3
			P 1–1.5	
Zinc	65.4	Pl	0.6–1.6	2
				L 42[d]
Zipeprol	384.5	Pl	0.1–0.7	L 5.8[d]
Ziprasidone	412.9	Pl	(0.02) 0.05–0.12	
Zolmitriptan	287.4	Pl	(0.003–) 0.007–0.01	
Zolpidem	307.4	S	0.08–0.3	0.5
				L 2–4
Zoniramide	212.2	Pl	10–40	50–70
Zopiclone	388.8	S	0.01–0.05	0.15
			P 0.04–0.07	L 0.6[d]
Zotepine	331.9	Pl	0.001–0.12	0.15–0.2
Zuclopenthixol	401.0	Pl	0.005–0.07	0.15–0.3

[a]B, whole blood (heparinised or edetate); S, serum; U, urine; Pl, plasma.
[b]Reference concentration (mg/L) during steady state; T, trough level just before drug administration; P, peak level 1–2 h after drug administration.
[c]Minimum level or range for which concentration-dependent side-effects or toxic effects have been noted; blank, no values because toxic concentrations are not available; T, trough level just before drug administration; P, peak level 1–2 h after drug administration; L, lethal.
[d]Case report.

sample should be withdrawn. The efficiency of haemodialysis (or haemoperfusion) can be determined as follows:

- Measure the blood flow rate (mL/min) through the artificial kidney.
- As the drug levels are measured in plasma and the drug is cleared from the plasma, the blood flow rate has to be converted into the plasma flow rate [blood flow rate × (1 − haematocrit)].
- Measure the drug plasma levels in samples taken before (C_{bef}) and after (C_{aft}) the artificial kidney.
- $[(C_{bef} - C_{aft})/C_{bef}] \times$ blood flow $\times (1 -$ haematocrit$) =$ clearance (mL/min).
- This extracorporeal clearance has to be added to the physiological clearance of the poisoned patient: $(Cl_{own} + Cl_{extra} = Cl_{total})$.
- The half-life time during extracorporal clearance is $(\ln 2)/k = 0.69V_d/Cl_{total}$.

As an alternative to these formulae, the toxicologist can use a commercially available toxicokinetic or pharmacokinetic software program. The pharmacokinetics software package MW/Pharm (Mediware, University Groningen, The Netherlands) is very suitable and flexible for both therapeutic drug monitoring (TDM) and toxicological calculations. AutoKinetic by SW Tönnes, Frankfurt, Germany, is a less comprehensive program that uses Excel. A method for calculating the dialysis time needed to alleviate cases of alcohol poisoning has been described by Hirsch *et al.* (2001)

Reporting results

Reports (verbal or written) should be submitted to the clinician by an authorised toxicologist who is fully responsible for the results and the advice provided. If the methods used were not validated, this should be indicated to the clinician so that he or she can judge the possible margin of error.

Sources of information

The practice of hospital toxicology requires knowledge and experience of pharmacotherapy, bioanalyses, good laboratory practice, pharmacokinetics, toxicokinetics, pharmacodynamics, basic toxicology,

clinical toxicology, forensic toxicology, chemistry, and indications and contraindications of the different treatments. Numerous sources of information are available and those listed in Further reading are among the most useful. It is advisable to consult several sources before giving advice.

Books that deal specifically with poisoning by industrial chemicals, household products and natural toxins are also useful sources of reference.

Therapeutic and toxic concentrations

Table 1.28 lists the therapeutic and toxic serum concentration ranges for a large number of drugs. Therapeutic serum levels are the steady-state concentrations that need to be reached for the drug to exert a significant clinical benefit without unacceptable side-effects. Where concentrations are shown in brackets this refers to extreme, but still acceptable, values. Toxic serum levels are concentrations above which unacceptable, concentration-dependent, toxic effects may appear. The toxic levels are expressed as a range, which means that the toxic effects may start somewhere in this range, depending on the patient and his or her clinical history. It should be taken into account that these values are never static and may change with advancing knowledge or with other (therapeutic) uses of the drug. Toxic and, where applicable, normal ranges are also given for substances that have no therapeutic use. It is emphasised that these data are intended merely as guidelines and that there is wide individual susceptibility towards the effects of drugs and poisons. In other words, the physician should treat the patient according to his or her clinical signs and not the analytical results. The University Medical Center of Groningen (The Netherlands) provides a list with all their determinations in human matrices, including clinical chemistry, TDM and kinetic parameters. See http://www.bioanalysis.umcg.nl/.

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CHAPTER

2 Therapeutic Drug Monitoring

M Hallworth

Introduction

Therapeutic drug monitoring (TDM) may be defined as the use of drug or metabolite monitoring in body fluids as an aid to the management of therapy (the term therapeutic drug *management* is now also employed as an alternative description). Since antiquity, physicians have adjusted the dose of drugs according to the characteristics of the individual being treated and the response obtained, and this practice is easiest when the response is readily measurable, either clinically (e.g. in the case of antihypertensive drugs, analgesics or hypnotics) or with an appropriate laboratory marker (e.g. in the case of anticoagulants, hypoglycaemic agents or lipid-lowering drugs). Dose adjustment is much more difficult (but no less necessary) when drug response cannot be rapidly assessed clinically (e.g. in the prophylaxis of seizures or mania), or when toxic effects cannot be detected until severe or irreversible (e.g. nephrotoxicity or ototoxicity). Provided that certain basic conditions are satisfied and appropriate analytical methods are available, the plasma concentration of a drug or metabolite may serve as an effective and clinically useful surrogate marker of response in these cases. However, it must be stressed that TDM is not simply the provision of an analytical result but a process that begins with a clinical question and continues by devising a sampling strategy to answer that question, determining one or more drug concentrations using a suitable method and interpreting the results appropriately.

TDM has been practised routinely in clinical laboratories since the mid-1970s, but the scientific foundations of the subject date back to the 1940s, when Marshall first tested the concept that the activity of a drug is dependent on its plasma concentration. In 1960, Buchthal showed a relationship between seizure control and plasma phenytoin concentration in patients being treated for epilepsy, and Baastrup and Schou described the plasma concentration–pharmacological effect relationship for lithium in 1967. This work coincided with the rise of clinical pharmacology during the 1960s and the demonstration of the fundamental concepts of pharmacokinetics and pharmacodynamics, which underpin the interpretation of drug concentration measurements.

Fundamental concepts

Different patients need different doses of drug to produce optimum pharmacological effect because individuals vary widely in the way that they absorb and dispose of drugs, and in the way that they respond to drugs. The steps between prescribing a drug to a patient and obtaining the desired response are summarised in Fig. 2.1, which also indicates the distinction between pharmacokinetics and pharmacodynamics. *Pharmacokinetics* describes the way in which a patient's system handles drugs, and encompasses uptake of drugs into the body, distribution throughout body compartments, and the metabolism and elimination of drugs (and their metabolites) from the body. These processes are described in more detail in Chapter 24.

Pharmacodynamics is concerned with the interaction of pharmacologically active substances with target sites (receptors), and the biochemical and physiological consequences of these actions. For example, the effects of a given tissue concentration of digoxin on cardiac muscle are modified by the local potassium concentration, which affects the concentration–response relationship and means that plasma drug concentrations are not the sole determinant of response. In simple terms, pharmacokinetics may be said to be the study of what patients do to drugs, and pharmacodynamics of what drugs do to patients.

If plasma drug concentrations are to be a useful surrogate marker of response, two premises must be satisfied. The first is that the drug concentration in plasma accurately reflects the concentration at the site of action (the receptor), which may be located in the plasma compartment itself or may be deep in target tissue. This assumption is true for many drugs, but is far from universal – for example, the blood–brain barrier may mean that plasma concentrations of drugs that act on the brain are unrepresentative of concentrations at the site of action, and adequate concentrations of an antibiotic in the blood may not guarantee effective concentrations at the centre of a poorly perfused abscess. There may also be significant time differences between peak concentrations of a drug in plasma and maximum penetration to the receptor, which complicates the interpretation of plasma concentration measurements. Further, the total (free plus protein-bound) concentration of drug in plasma may not reflect the pharmacologically active free-drug concentration, especially for those drugs that are strongly bound by plasma proteins (see below).

The second premise is that drug concentration at the receptor should provide an accurate indication of pharmacological response. This may not be true if other drugs interact with the receptor, if receptor numbers are reduced (e.g. in the phenomenon of tolerance when patients have been on a drug for some time), or if the coupling of receptors to signal transduction pathways is modified.

Criteria for assessing the clinical value of drug monitoring

The essential criteria for TDM to be clinically useful for a particular drug may therefore be developed and summarised as:

- Poor correlation between the dose given and the plasma concentration obtained in different patients (wide inter-individual pharmacokinetic variability). Clearly, if the dose given is an effective predictor of plasma concentration in all patients, then measuring an individual's plasma concentration is superfluous. Adherence testing (determining whether patients actually take their drugs) may be a useful adjunct to TDM programmes (see below), but is unlikely to justify such a programme in the absence of other indications for monitoring.
- Good correlation between plasma concentration and pharmacological effect in different patients (low inter-individual pharmacodynamic variability). If plasma concentration measurements do not give accurate information about response, they are at best useless and at worst misleading. So both the premises stated above must be satisfied (plasma drug concentration predicts receptor concentration and receptor drug concentration predicts response). Active metabolites are generally undesirable as they contribute to the effect but make a variable (or zero) contribution to the measured concentration, depending on the assay system; for example, a metabolite may have 10% of the biological activity of the parent drug but show 100% cross-reactivity in an immunoassay. This criterion also normally requires that the action of the drug at the receptor site be essentially reversible. If this is not the case, and the drug binds irreversibly to the receptor, the pharmacodynamic half-life (or duration of effect) may be markedly longer than the pharmacokinetic half-life (or length of time that the drug can be detected in the circulation). For example, the anticonvulsant vigabatrin acts as a suicide inhibitor for the enzyme γ-aminobutyric acid transaminase and irreversibly inactivates the enzyme. In this situation, the drug may not develop its

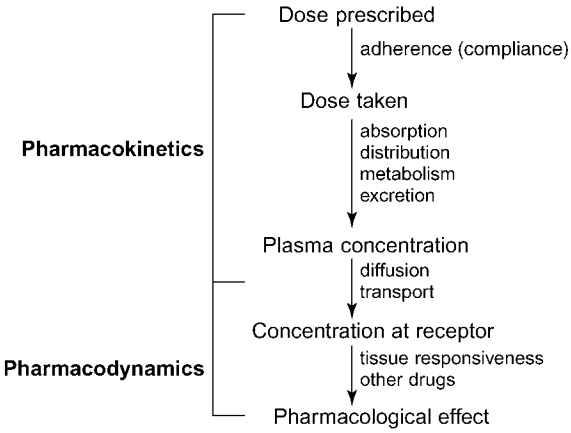

Figure 2.1 Processes involved in drug action.

maximal clinical effect until days or weeks after dosing has been started, and it may still be exerting an effect when no drug can be detected in the plasma. It is difficult to argue that TDM has a useful contribution to make in these circumstances. Exceptions to this general rule are certain anticancer agents where, even though the action of the drug is irreversible, an index of the body's total exposure to the drug can be used to predict subsequent response.

- TDM is clinically relevant only for drugs that show significant toxic or undesirable effects at plasma concentrations only slightly above those required for useful effects. These drugs are described as having a low therapeutic index, defined as the mean toxic dose divided by the mean effective dose. If there is a wide margin between effective concentrations and undesirable effects, as is the case for penicillin, effective therapy may be achieved by giving the drug to all patients in large excess, with no need for individualisation of therapy. In contrast, the aminoglycoside antibiotics have relatively narrow margins between effective concentrations and those that produce unacceptable toxicity, and concentration monitoring has an essential role in ensuring maximal effect with minimal toxicity.
- Similarly, TDM is redundant for drugs where there is a better clinical marker of both the desired effect and any associated adverse effects, for example blood pressure, plasma glucose concentration, prothrombin time or plasma creatinine concentration. Plasma drug concentrations have little to offer in this situation, except in the elucidation of rare cases where a high dose of drug fails to produce the desired effect, when TDM may help differentiation between poor adherence (non-compliance), poor drug absorption, receptor dysfunction or the use of the wrong drug for the situation.

Application of these criteria results in a relatively short list of drugs for which concentration monitoring has a proved clinical role (those marked with +++ in Table 2.1). Measurement of other drugs may contribute in certain cases or in particular clinical circumstances, but cannot be said to be generally useful in dosage optimisation (marked as ++ in Table 2.1). Concentration monitoring has not been shown to be applicable to the drugs marked as ± in Table 2.1.

Indications for drug monitoring

The main reasons for measuring drugs in plasma may be summarised as:

- To ensure that sufficient drug is reaching the drug receptor to produce the desired response (which may be delayed in onset, e.g. for antidepressant drugs)
- To ensure that drug (or metabolite) concentrations are not so high as to produce signs or symptoms of toxicity
- To guide dosage adjustment in clinical situations where pharmacokinetic parameters are changing rapidly (e.g. in neonates or in patients with changing hepatic or renal function)
- As an aid to defining the pharmacokinetic and pharmacodynamic parameters of new drugs.

A fifth indication, the assessment of adherence (compliance), remains a matter of some controversy. Clearly, if the assessment of adherence is accepted as a valid indication for concentration monitoring, it could be necessary to provide an analytical service for virtually every drug in the pharmacopoeia, at enormous cost. Furthermore, although gross non-adherence to a regime is associated with very low or undetectable concentrations of drug in blood, variable adherence can be difficult or impossible to detect, and the variability between patients that makes dosage individualisation necessary also implies that surprisingly low plasma concentrations for a given dose of drug are not necessarily due to poor adherence. Adherence can be assessed in other ways, for example by tablet counting, by supervision of medication or by the use of carefully designed questions that are non-judgmental ('How often do you forget to take your tablets?'). Assessment of adherence is thus not a primary indication for measuring plasma drug concentrations, although, if an analytical service is provided for other reasons, concentration monitoring may have a role for patients with poor symptom control who deny poor adherence despite careful questioning and reinforcement.

Even if concentration measurements of a drug have been shown to be of value, this does not mean that they are required in all situations in all patients who receive the drug. Indeed, indiscriminate use of TDM services has done much to erode the cost-effectiveness of the process and has frequently harmed rather than helped patient care. As with any laboratory test, a clear clinical question should be formulated before recourse is made to concentration measurements. The question helps to decide what measurements should be made and how the results can be interpreted. Examples of suitable questions might be 'My patient is not responding to therapy – could this be because of inadequate plasma concentration or is a different drug required?' or 'Could this patient's symptoms be explained by drug toxicity?'. When requesting physicians lose sight of this fundamental principle, and do tests as 'routine', answers without questions are obtained. The dangers of this are well illustrated by the all too frequent example of a patient on anticonvulsant therapy for epilepsy who has a 'routine' blood test done in primary care, with the result found to be significantly above the target or 'therapeutic' range for the drug. The laboratory may then telephone the result as a matter of urgency and cause an inexperienced physician to react with an inappropriate dose reduction, which precipitates seizures in a patient who was perfectly well controlled on the original dose.

Analytical requirements

Sample

Blood, urine, saliva and hair may be considered as possible samples for TDM analyses. By its very nature, hair is a retrospective medium which can give an indication of drug concentrations in the weeks or months preceding sampling. This is a valuable property in the field of detection of drugs of abuse (see Chapter 19), but it has little relevance to individualisation of therapy. Applications of hair analysis to TDM are therefore limited at present, although there have been studies on long-term monitoring of antipsychotic drugs, such as haloperidol, in psychiatric in-patients. Similarly, the variation of drug concentrations in urine brought about by factors such as the state of hydration and urinary pH means that there are no applications in TDM in which urine is preferred to plasma as a sample matrix.

Plasma (or serum) is normally the preferred sample for TDM analyses, but has the disadvantage that it requires an invasive procedure for collection (venepuncture). Some drugs (e.g. many immunosuppressants) are concentrated in the red cells and so whole blood (with an appropriate anticoagulant – e.g. ethylenediamine tetraacetic acid (EDTA)) is more suitable than plasma. When plasma samples are used, care must be taken with anticoagulants and the use of gel separation barriers, both of which can cause interference with some drugs or assay systems. However, in the absence of such effects there are no clinically significant differences between serum and plasma and either may be used.

Table 2.1 Main drugs subject to therapeutic monitoring

Drug	*Notes*	*Suggested assay method (* = commercial kits available)*	*Plasma concentrations associated with optimum response*	*Clinical value of TDM*
Amikacin	Monitoring essential in ensuring efficacy with minimum toxicity.	1. Immunoassay* 2. GLC (Isoherranen, Soback 1999; Mayhew, Gorbach 1978) 3. HPLC (Isoherranen, Soback 1999; Soltes 1999)	Vary with dose schedule.	+++
Amiodarone	Effects may last months after drug stopped (elimination half-life 50 days). Active metabolite (desethylamiodarone, DEA).	1. HPLC (Lesne, Pellegrin 1987) 2. LC-MS (Kollroser, Schober 2002) 3. Capillary electrophoresis (CE) (Zhang *et al.* 1996)	0.5–2.5 mg/L (0.7–3.7 μmol/L) (amiodarone + DEA)	++
Amitriptyline	Large genetic differences in rate of metabolism. Some evidence for concentration–effect relationship. Metabolised to nortriptyline.	1. GLC (Ulrich *et al.* 1996) 2. HPLC (el Yazigi, Raines 1993; Kabra *et al.* 1981) 3. LC-MS (Kirchherr, Kühn-Velten 2006; Sauvage *et al.* 2006)	100–250 μg/L (amitriptyline + nortriptyline)	++
Antiretroviral drugs (protease inhibitors [-avir suffix]) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) – efavirenz, nevirapine	Large inter-individual variability in drug concentrations. Appropriate TDM may have a role in maximising efficacy and reducing toxicity.	1. HPLC (Titier *et al.* 2002) 2. LC-MS (D'Avolio *et al.* 2007; Ghoshal, Soldin 2003)	Suggested minimum target trough concentrations: amprenavir 400 μg/L, indinavir 100 μg/L, lopinavir 1000 μg/L, nelfinavir 800 μg/L, ritonavir 2100 μg/L, saquinavir 100 μg/L, efavirenz 1000 μg/L, nevirapine 3400 μg/L	++
Amprenavir	See Antiretroviral drugs			
Caffeine	More predictable concentration–effect relationship than for theophylline. Low toxicity.	1. Immunoassay* 2. HPLC (Dobrocky *et al.* 1994; Meatherall, Ford 1988; Schreiber-Deturmeny, Bruguerolle 1996) 3. LC-MS (Zhang *et al.* 2008)	5–20 mg/L (25–100 μmol/L) (in neonatal apnoea)	++
Carbamazepine	Active metabolite present (carbamazepine 10,11-epoxide). The drug induces its own metabolism, so levels fall after a few weeks on the same dose.	1. Immunoassay* 2. HPLC (Kouno *et al.* 1993; Matar *et al.* 1999; Meatherall, Ford 1988; Pienimaki *et al.* 1995; Romanyshyn *et al.* 1994) 3. GLC (Burke, Thenot 1985; Chen, Khayam-Bashi 1991; Kumps 1982) 4. GC-MS (Speed *et al.* 2000) 5. LC-MS (Subramanian *et al.* 2008) 6. Electrokinetic chromatography (Pucci, Raggi 2005)	4–12 mg/L (17–51 μmol/L)	+++
Chloramphenicol	Clearance reduced by hepatic or renal dysfunction. Potentially fatal concentration-dependent toxic reaction in neonates with immature hepatic metabolism.	1. HPLC (Davidson, Fitzpatrick 1986; Meatherall, Ford 1988)	10–25 mg/L (31–77 μmol/L)	++
Chlorpromazine	Extensively metabolised, some metabolites have pharmacological activity. TDM rarely used.	1. HPLC (Mercolini *et al.* 2007) 2. LC-MS(-MS) (Kirchherr, Kühn-Velten 2006)	30–500 μg/L (94–1600 nmol/L)	±
Ciclosporin	Complex pharmacokinetics and distribution. Monitoring useful in differentiating underdosage and toxicity	1. Immunoassay* (Schutz *et al.* 1998) 2. HPLC (Holt, Johnston 1995) 3. LC-MS (Keevil *et al.* 2002; Poquette *et al.* 2005)	Vary with type of transplant and time post-transplantation. Whole-blood concentrations (EDTA anticoagulant) are determined.	+++
Clobazam	Value of monitoring limited by development of tolerance to drug. Active metabolite (*N*-desmethylclobazam) present in higher concentrations than parent drug.	1. HPLC (Kunicki 2001) 2. GLC (LeGatt, McIntosh 1993)	—	±
Clonazepam	Value of monitoring limited by development of tolerance to drug.	1. HPLC (de Carvalho, Lanchote 1991; Le Guellec *et al.* 1998) 2. GLC (de Carvalho, Lanchote 1991) 3. GC-MS (Song *et al.* 1996) 4. LC-MS (Marin *et al.* 2008)	15–60 μg/L (50–190 nmol/L)	±

table continued

Table 2.1 continued

Drug	*Notes*	*Suggested assay method (* = commercial kits available)*	*Plasma concentrations associated with optimum response*	*Clinical value of TDM*
Clozapine	Wide inter-patient pharmacokinetic variability; problems with toxicity. Dose–concentration relationship for therapeutic effect and some side-effects. Full effect may take weeks to develop.	1. HPLC (Weigmann *et al.* 1997; Wilhelm, Kemper 1990) 2. LC-MS (Kirchherr, Kühn-Velten 2006; Niederlander *et al.* 2006)	Target clozapine concentration: 350 µg/L (1100 µmol/L) Clozapine : norclozapine ratio at steady state is approximately 1.32	++
Darunavir	See Antiretroviral drugs			
Desipramine	Curvilinear concentration–response relationship. Concentrations above and below range are associated with lower efficacy.	1. HPLC (Chen *et al.* 1997; el Yazigi, Raines 1993; Kabra *et al.* 1981; Yoo *et al.* 1995) 2. GC-MS (Pommier *et al.* 1997) 3. LC-MS (Kirchherr, Kühn-Velten 2006)	100–160 µg/L (380–600 nmol/L)	++
Diazepam	No defined therapeutic range – tolerance develops rapidly.	1. GLC (Karnes *et al.* 1988) 2. GC-MS (Kudo *et al.* 1988) 3. HPLC (Azzam *et al.* 1998) 4. LC-MS (Marin *et al.* 2008)	-	±
Digitoxin	See Digoxin	1. Immunoassay* 2. LC-MS (Tracqui *et al.* 1997)	12–25 µg/L (15–33 nmol/L)	++
Digoxin	Elimination prolonged in renal failure. Significant pharmacodynamic variability.	1. Immunoassay* (Wahyono *et al.* 1991) 2. HPLC (Tzou *et al.* 1995) 3. LC-MS (Tracqui *et al.* 1997)	0.8–2.0 µg/L (1.0–2.6 nmol/L) In heart failure: 0.5–1.0 µg/L (0.6–1.3 nmol/L)	++
Disopyramide	Concentration-dependent protein binding complicates concentration–effect relationship.	1. Immunoassay* 2. GLC (Kapil *et al.* 1984; Vasiliades *et al.* 1979a) 3. HPLC (Mayer *et al.* 1991; Vasiliades *et al.* 1979a)	2.0–5.0 mg/L (5.9–14.7 µmol/L)	++
Doxepin	Large genetic differences in rate of metabolism. Some evidence for concentration–effect relationship. Metabolised to desmethyldoxepin (active).	1. GLC (Vasiliades *et al.* 1979b) 2. HPLC (Kabra *et al.* 1981) 3. LC-MS (Badenhorst *et al.* 2000; Kirchherr, Kühn-Velten 2006; Sauvage *et al.* 2006)	50–250 µg/L (as doxepin + metabolite) (180–900 nmol/L)	++
Efavirenz	See Antiretroviral drugs			
Ethosuximide	Poor relationship between dose and plasma concentration, but effect usually quantifiable by EEG monitoring.	1. HPLC (Matar *et al.* 1999; Meatherall, Ford 1988) 2. GLC (Grgurinovich, Miners 1980; Kumps 1982; Riva *et al.* 1982) 3. GC-MS (Speed *et al.* 2000)	40–100 mg/L (280–710 µmol/L)	++
Everolimus	Analogue of sirolimus.	1. Immunoassay* 2. LC-MS (Korecka *et al.* 2006)	3–8 µg/L (3.1–8.3 nmol/L)	++
Felbamate	Risk of aplastic anaemia and hepatic failure. Interactions with other anticonvulsants.	1. HPLC (Tang 2008) 2. Electrokinetic chromatography (Pucci, Raggi 2005) 3. LC-MS (Thompson *et al.* 1999)	20–60 mg/L (85–250 µmol/L)	±
Flecainide	Elimination prolonged in renal failure. Significant pharmacokinetic variability (CYP2D6 polymorphism).	1. Immunoassay* 2. HPLC (Wilson *et al.* 1998) 3. LC-MS (Breindahl 2000) 4. GLC (Stas *et al.* 1989)	0.2–1.0 mg/L (0.4–2.1 µmol/L)	++
Flucytosine	Wide pharmacokinetic variability. Serum concentrations relate well to efficacy and toxicity. Monitoring may reduce risk of toxicity.	1. HPLC (Bury *et al.* 1979)	2 h (peak) concentration >25 and <100 mg/L	++
Fluoxetine	Long half-life. Active metabolite norfluoxetine.	1. LC-MS (Kirchherr, Kühn-Velten 2006; Sauvage *et al.* 2006)	150–800 µg/L (fluoxetine + norfluoxetine)	±
Gabapentin	Good relationship between plasma concentration and dose, but no relationship between plasma and CSF concentrations. Simple pharmacokinetics, short half-life.	1. HPLC (Ratnaraj, Patsalos 1998) 2. LC-MS (Ifa *et al.* 2001) 3. GC-MS (Borrey *et al.* 2005)	2–20 mg/L (12–120 µmol.L) suggested	±

Table 2.1 continued

Drug	*Notes*	*Suggested assay method (* = commercial kits available)*	*Plasma concentrations associated with optimum response*	*Clinical value of TDM*
Gentamicin	Monitoring essential in ensuring maximum effect with minimum toxicity. Once-daily dosing requires different target ranges.	1. Immunoassay* 2. HPLC (Adams *et al.* 1998; Delaney *et al.* 1982; Isoherranen, Soback 1999; Soltes 1999; Yusuf *et al.* 1999) 3. GLC (Isoherranen, Soback 1999; Mayhew, Gorbach 1978) 4. GC-MS (Preu *et al.* 1998)	Vary with dose schedule.	+++
Haloperidol	Large genetic differences in rate of metabolism (CYP2D6 substrate). Monitoring is useful in assessing phenotype and adjusting therapy.	1. HPLC (Angelo, Petersen 2001; Wilhelm, Kemper 1990) 2. LC-MS (Hoja *et al.* 1997) 3. GLC (Tyndale, Inaba 1990)	5–15 μg/L (13–40 nmol/L)	++
Imipramine	Large genetic differences in rate of metabolism. Plasma concentrations correlate with clinical improvement.	1. GC-MS (Pommier *et al.* 1997) 2. HPLC (Chen *et al.* 1997; el Yazigi, Raines 1993; Kabra *et al.* 1981; Yoo *et al.* 1995) 3. LC-MS (Kirchherr, Kühn-Velten 2006; Sauvage *et al.* 2006)	150–300 μg/L (imipramine + desipramine)	++
Itraconazole	Variable absorption. Monitoring is advisable when the drug is used for prolonged periods.	1. HPLC (Uno *et al.* 2006) 2. LC-MS (Vogeser *et al.* 2003)	Trough concentration >0.5–1.0 mg/L	++
Indinavir	See Antiretroviral drugs			
Kanamycin	See Amikacin	1. Immunoassay* 2. GLC (Isoherranen, Soback 1999) 3. HPLC (Adams *et al.* 1997; Isoherranen, Soback 1999; Kubo *et al.* 1985; Soltes 1999) 4. GC-MS (Preu *et al.* 1998)	Vary with dose schedule	+++
Lamotrigine	Wide range of concentrations associated with seizure control. Complex interactions with other anticonvulsants.	1. HPLC (Croci *et al.* 2001; Greiner-Sosanko *et al.* 2007; Matar *et al.* 1999) 2. LC-MS (Subramanian *et al.* 2008) 3. Electrokinetic chromatography (Pucci, Raggi 2005)	1–15 mg/L (?) (4–60 μmol/L)	++
Levetiracetam	Good safety profile, no evidence to justify monitoring.	1. HPLC (Contin *et al.* 2008) 2. GC (Greiner-Sosanko *et al.* 2007) 3. LC-MS (Guo *et al.* 2007) 4. Electrokinetic chromatography (Pucci, Raggi 2005)	6–20 mg/L (35–118 μmol/L) suggested	±
Lidocaine	Good concentration–effect relationship. Active metabolite complicates interpretation.	1. GLC (Willis *et al.* 1984) 2. HPLC (Barat *et al.* 1996; Halbert, Baldwin 1984) 3. LC-MS (Abdel-Rehim *et al.* 2000)	2–6 mg/L (9–26 μmol/L)	++
Lithium	Variable absorption characteristics. Sodium balance affects excretion.	1. Flame emission spectroscopy/AAS (Popov *et al.* 1986) 2. Ion-selective electrodes (Bertholf *et al.* 1988; Christian 1996) 3. Colorimetry (Christian 1996; Gorham *et al.* 1994)	0.5–0.8 mmol/L (prophylaxis); up to 1.2 mmol/L in acute mania	+++
Lopinavir	See Antiretroviral drugs			
Methadone	Monitoring may be valuable for drug addicts on methadone substitution. Administered as racemate.	1. Immunoassay* 2. HPLC (Pham-Huy *et al.* 1997) 3. LC-MS (Moody *et al.* 2008) 4. GC-MS (Bermejo *et al.* 2000) 5. CE (Kelly *et al.* 2007)	150–50 μg/L (430–720 nmol/L) (total plasma methadone – *R*+*S* isomers)	++
Methotrexate	Concentration monitoring necessary to assess need for 'rescue' therapy (folinic acid) on high-dose regimes.	1. Immunoassay* (Aherne *et al.* 1978) 2. HPLC (Emara *et al.* 1998)	<5–10 μmol/L at 24 h post-dose; <0.5–1.0 μmol/L at 48 h; <0.1 μmol/L at 72 h	+++
Mycophenolate mofetil	Pre-dose concentrations vary widely and are poor reflections of total exposure to the drug. Measures of area under the curve preferred.	1. Immunoassay* 2. HPLC (Hosotsubo *et al.* 2001; Shipkova *et al.* 1998; Svensson *et al.* 1999) 3. LC-MS (Brandhorst *et al.* 2006; Premaud *et al.* 2006)	3.0–9.0 μmol/L (1.0–3.0 mg/L) (as mycophenolic acid; depends on transplanted organ) AUC in range 30–60 μg h/L recommended	++
Nelfinavir	See Antiretroviral drugs			

table continued

Table 2.1 continued

Drug	*Notes*	*Suggested assay method (* = commercial kits available)*	*Plasma concentrations associated with optimum response*	*Clinical value of TDM*
Netilimicin	See Gentamicin	1. Immunoassay* 2. HPLC (Isoherranen, Soback 1999; Soltes 1999) 3. GLC (Isoherranen, Soback 1999; Mayhew, Gorbach 1978) 4. GC-MS (Preu *et al.* 1998) 5. CE (Calcara *et al.* 2005)	Vary with dose schedule.	+++
Nevirapine	See Antiretroviral drugs			
Nortriptyline	Large genetic differences in rate of metabolism. There is an optimum therapeutic window; plasma concentrations above or below this are associated with worsening mood.	1. GLC (Ulrich *et al.* 1996) 2. HPLC (el Yazigi, Raines 1993; Kabra *et al.* 1981) 3. LC-MS (Kirchherr, Kühn-Velten 2006; Sauvage *et al.* 2006)	50–150 µg/L (190–570 nmol/L)	++
Oxcarbazepine	Prodrug for 10-hydroxycarbamazepine. No evidence to justify routine monitoring.	1. HPLC (Greiner-Sosanko *et al.* 2007; Levert *et al.* 2002; Matar *et al.* 1999; Pienimaki *et al.* 1995) 2. GLC (von Unruh, Parr 1985) 3. GC-MS (von Unruh, Paar 1986) 4. LC-MS (Paglia *et al.* 2007; Subramanian *et al.* 2008)	13–28 µg/L (50–110 nmol/L) suggested	±
Phenobarbital	Now rarely used. The value of monitoring is limited by development of tolerance to the drug.	1. Immunoassay* 2. HPLC (Kouno *et al.* 1993; Matar *et al.* 1999; Meatherall, Ford 1988; Romanyshyn *et al.* 1994) 3. LC-MS (Subramanian *et al.* 2008) 4. GLC (Burke, Thenot 1985; Kumps 1982) 5. GC-MS (Speed *et al.* 2000)	10–40 mg/L (40–170 µmol/L) (tolerance means these ranges need to be interpreted flexibly) 15–20 mg/L (65–85 µmol/L) in prophylaxis of febrile convulsions in children	++
Phenytoin	Saturable metabolism means that the concentration–dose relationship is unpredictable. Monitoring vital for safe use of drug.	1. Immunoassay* 2. HPLC (Kouno *et al.* 1993; Matar *et al.* 1999; Meatherall, Ford 1988; Romanyshyn *et al.* 1994) 3. LC-MS (Subramanian *et al.* 2008; Zhang *et al.* 2008) 4. GLC (Burke, Thenot 1985; Kumps 1982) 5. GC-MS (Speed *et al.* 2000) 6. Electrokinetic chromatography (Pucci, Raggi 2005)	5–20 mg/L (20–80 µmol/L)	+++
Primidone	Metabolised to phenobarbital. No evidence that monitoring primidone concentration improves management.	1. HPLC (Matar *et al.* 1999; Meatherall, Ford 1988; Romanyshyn *et al.* 1994) 2. GLC (Burke, Thenot 1985; Kumps 1982) 3. GC-MS (Speed *et al.* 2000)	See Phenobarbital	±
Procainamide/*N*-Acetylprocainamide	Active metabolite (*N*-acetyl-procainamide, NAPA) complicates the concentration–effect relationship.	1. Immunoassay* 2. HPLC (Lessard *et al.* 1998) 3. GLC (Kessler *et al.* 1982)	4–10 mg/L (17–42 µmol/L) Procainamide + NAPA should not exceed 30 mg/L	++
Propranolol	No evidence that monitoring improves management.	1. HPLC (Braza *et al.* 2000; Sood *et al.* 1988) 2. LC-MS (Umezawa *et al.* 2008) 3. GC-MS (Quaglio *et al.* 1993)	—	±
Quinidine	Active metabolite (3-hydroxyquinidine) complicates interpretation.	1. Immunoassay* 2. HPLC (Carignan *et al.* 1995; Meineke *et al.* 1995) 3. GLC (Kessler *et al.* 1982) 4. GC-MS (Quaglio *et al.* 1990)	2–6 mg/L (6.2–18.5 µmol/L)	++
Risperidone	Active metabolite 9-hydroxyrisperidone. No clear role for monitoring.	1. HPLC (Balant-Gorgia *et al.* 1999) 2. LC-MS (Flarakos *et al.* 2004; Kirchherr, Kühn-Velten 2006)	20–60 µg/L (including metabolite)	±
Ritonavir	See Antiretroviral drugs			
Salicylate	Role for monitoring in avoiding toxicity on chronic anti-inflammatory therapy	1. Colorimetry (Stewart, Watson 1987; Trinder 1954) 2. HPLC (Dawson *et al.* 1988; Stewart, Watson 1987) 3. LC-MS (Bae *et al.* 2008)	150–300 mg/L (110–220 µmol/L)	±

Table 2.1 continued

Drug	*Notes*	*Suggested assay method (* = commercial kits available)*	*Plasma concentrations associated with optimum response*	*Clinical value of TDM*
Saquinovir	See Antiretroviral drugs			
Sirolimus	Monitoring is a regulatory requirement. Pre-dose concentrations are a good index of exposure	1. Immunoassay* 2. HPLC (Shaw *et al.* 2000) 3. LC-MS (Poquette *et al.* 2005)	4–12 µg/L (chromatographic assay) when given with ciclosporin, increasing to 12–20 µg/L when ciclosporin discontinued. Whole blood samples determined.	+++
Tacrolimus	Monitoring of value in ensuring maximum effect with minimal toxicity. Pre-dose concentrations reflect total exposure.	1. Immunoassay* 2. LC-MS (Poquette *et al.* 2005; Taylor *et al.* 1997)	Typically 15 µg/L following kidney transplantation, reducing to 5–10 µg/L in stable patients. Whole blood samples used.	++
Teicoplanin	See Vancomycin	1. Immunoassay* 2. HPLC (Mochizuki *et al.* 2007)	20–60 mg/L (trough) for *Staphylococcus aureus*, 10–60 mg/L for other infections.	++
Theophylline	Highly variable hepatic metabolism. Monitoring helps with dosage individualisation.	1. Immunoassay* 2. HPLC (Kizu *et al.* 1999; Meatherall, Ford 1988; Schreiber-Deturmeny, Bruguerolle 1996) 3. LC-MS (Zhang *et al.* 2008)	10–20 mg/L (55–110 µmol/L)	+++
Tiagabine	No evidence to justify routine monitoring at present.	1. GC-MS (Chollet *et al.* 1999) 2. HPLC (Gustavson, Chu 1992)	20–40 µg/L (50–97 µmol/L) suggested	±
Tipranavir	See Antiretroviral drugs			
Tobramycin	See Gentamicin	1. Immunoassay* 2. GLC (Isoherranen, Soback 1999; Mayhew, Gorbach 1978) 3. HPLC (Isoherranen, Soback 1999; Soltes 1999)	Vary with dose schedule.	+++
Topiramate	No evidence to justify routine monitoring at present.	1. Immunoassay* 2. GLC (Holland *et al.* 1988; Wolf *et al.* 2000) 3. LC-MS (Christensen *et al.* 2002; Subramanian *et al.* 2008)	5–25 mg/L (15–60 µmol/L) suggested	±
Valproic acid	Complex pharmacokinetic profile. Poor relationship between plasma concentration and effect (in epilepsy). The role of monitoring in bipolar affective disorder is unproven.	1. Immunoassay* 2. GLC (Grgurinovich, Miners 1980; Kumps *et al.* 1981; Kumps 1982; Riva *et al.* 1982) 3. GC-MS (Speed *et al.* 2000) 4. LC-MS (Cheng *et al.* 2007) 5. Electrokinetic chromatography (Pucci, Raggi 2005)	Concentrations above 50 mg/L (350 µmol/L) are more likely to be associated with efficacy.	±
Vancomycin	Monitoring is useful in ensuring maximum effect with minimum toxicity	1. Immunoassay* 2. HPLC (Favetta *et al.* 2001; Li *et al.* 1995; Pfaller *et al.* 1984)	Peak (1 h) 20–40 mg/L (14–28 µmol/L) Trough 5–10 mg/L (3–7 µmol/L)	++
Vigabatrin	Plasma concentrations show linear relationship to dose. Irreversible mode of action.	1. HPLC (Franco *et al.* 2007; Ratnaraj, Patsalos 1998; Tsanaclis *et al.* 1991) 2. GC-MS (Borrey *et al.* 2005)	Not relevant.	±
Voriconazole	High pharmacokinetic variability. Relationship between concentration and efficacy is unclear.	1. HPLC (Pennick *et al.* 2003) 2. LC-MS (Egle *et al.* 2005)	2–6 mg/L (?)	±
Zonisamide	Little evidence to justify routine monitoring at present.	1. HPLC (Furuno *et al.* 1994; Greiner-Sosanko *et al.* 2007; Shimoyama *et al.* 1999) 2. LC-MS (Subramanian *et al.* 2008) 3. CE (Makino *et al.* 1997)	10–20 mg/L (47–94 µmol/L)	±

+++ Monitoring has proven clinical value.
++ Monitoring may have value in isolated cases or specific circumstances.
± Monitoring has not been shown to be applicable.

Plasma also contains a considerable amount of protein and many drugs of interest in the TDM field (e.g. phenytoin) show significant protein binding. This means that the total (free plus protein-bound) concentration of the drug in plasma varies with protein concentration, even though the free (pharmacologically active) concentration remains constant. This variation of measured drug concentration with plasma protein concentration complicates the interpretation of plasma drug levels, and has led to strategies to measure only the circulating free (unbound) drug. This can be achieved either *in vitro* by determining the concentration of drug in a plasma ultrafiltrate (obtained by centrifugation of plasma through an appropriate filter or by equilibrium dialysis across a semipermeable membrane) or by sampling an *in vivo* ultrafiltrate. Saliva is sometimes used as the latter, although care must be taken to ensure that the saliva : plasma concentration ratio is constant and unaffected by salivary pH or salivary flow rate. This is not always the case, but where these conditions are satisfied (for drugs that are not ionisable or are un-ionised within the salivary pH range, e.g. theophylline, carbamazepine and phenytoin), saliva can provide an effective, non-invasive sample matrix for determination of the pharmacologically active component of drug in plasma. Saliva sampling can be particularly useful in children or in adults with needle phobia, although there are still problems with collection and potential contamination. Drug concentrations in saliva are normally lower than in plasma or serum and the matrix itself provides some analytical challenges, as mucoproteins present in saliva make samples difficult to handle or pipette accurately, and centrifugation is time-consuming and not completely effective in removing debris. For these reasons, salivary monitoring still has not found wide application, though it undoubtedly has a role in some circumstances (see Baumann (2007) and Chapter 18).

Timing of measurements

The primary requirement for an appropriate specimen if TDM is to be used to assess the adequacy of response is that the specimen be taken when the drug concentration in the body is at steady state, that is when the effects of any recent dose changes have been allowed to stabilise. The time to reach steady state is determined by the elimination half-life of the drug and there is a fixed relationship between the number of half-lives that have elapsed since the drug was commenced and the progress towards steady-state concentration (see Chapter 24). The plasma concentration after 3.3 half-lives have elapsed is 90% of the predicted steady-state concentration and 94% of steady-state concentration is reached after four half-lives. For drugs with a long plasma half-life (e.g. digoxin, 20 to 50 h; phenytoin, 22 h) a week or two (or even longer) may be required before steady state is reached, especially if renal function is poor as in the case of digoxin. It is usual to allow at least four half-lives to elapse before monitoring the effect of any dose change. Obviously, if toxicity is suspected after a dose change it would be unwise to wait for steady state before checking the plasma concentration. Computer programs with the ability to predict steady-state concentrations from measurements made before steady state is attained may also be helpful.

The other issue relating to appropriate sample timing is the question of when the sample should be taken in relation to the last dose of the drug. As drugs are normally administered at fixed time intervals, there is inevitably variation of plasma concentration between one dose and the next. For most purposes, the most reproducible parameter for long-term monitoring is the steady-state trough concentration – a measurement immediately prior to the next oral dose. The importance of precise sample timing depends somewhat on the half-life of the drug: drugs with long half-lives (e.g. phenytoin) show little variation in concentration across the dosage interval and accurate specimen timing is less important than for drugs with short half-lives such as theophylline (half-life 7 h). For these drugs, toxic symptoms may correlate better with peak plasma concentrations than with trough concentrations and, if toxicity is suspected, a sample timed at 1–6 h post-dose (depending on the release characteristics of the preparation and the speed of absorption) may be more appropriate. Some antibiotics require high peak concentrations and low trough concentrations for optimal effects (e.g. aminoglycosides, for which this is now achieved by once-daily dosing regimes). In the case of lithium, a strong case has been made for standardising the sampling time at exactly 12 h post-dose. The area under the concentration–time curve (AUC) may also be a better predictor of response than either the peak or the trough concentration (e.g. in the case of ciclosporin), but the determination of AUC requires more complex sampling regimes. Sampling at exactly 2 h post-dose has been shown to predict AUC for ciclosporin more closely than trough sampling but it presents practical difficulties for routine monitoring.

Some drugs exhibit a distribution phase immediately after the dose has been given and for the duration of this phase the plasma concentration will be unrepresentative of the pharmacologically relevant receptor concentration. This is particularly the case for digoxin, where clinically misleading concentrations may be obtained in the first 4 or 5 h following a dose (whether oral or intravenous) while the drug equilibrates between plasma and tissue compartments.

Case example

A 70-year-old woman was seen by her general practitioner at a 09:30 appointment. She described a one-week history of feeling tired and nauseated, and was on long-term digoxin therapy. A blood sample taken for various biochemical tests including digoxin was received in the laboratory at 11:30. The digoxin concentration was 3.1 μg/L (3.9 nmol/L) (target range 0.8–2.0 μg/L; 1.0–2.6 nmol/L). A telephone call to the surgery established that the patient had taken her digoxin tablets that morning and thus the 09:30 sample was approximately 2 h post-dose. A repeat sample taken the following day, 8 h after the morning dose, showed a digoxin concentration of 2.0 μg/L (2.6 nmol/L). A modest reduction in digoxin dose was advised, but the specimen at 09:30 gave a misleading view of the severity of the situation.

Measurement techniques

A suitable analytical technique is obviously the bedrock of drug analysis, and studies to establish a correlation between plasma drug concentration and response cannot begin until a measurement technique has been developed and validated. The final stage of establishing the clinical value of an assay is prospective randomised controlled trials comparing patients who have been monitored with a control group who have been managed without the aid of concentration monitoring. Unfortunately, there are virtually no drugs for which unequivocal evidence of the clinical benefit of monitoring has been obtained by large-scale trials. Lithium and phenytoin provide the best examples.

Much early pharmacokinetic knowledge was obtained from studies using colorimetry, spectrophotometry and spectrofluorimetry, but these methods have now been virtually abandoned for quantitative clinical work because of their lack of specificity. The exception is for lithium, which is still frequently measured by colorimetric methods or flame atomic emission spectroscopy, although ion-selective electrodes are slowly displacing spectrometric techniques. Many antibiotics were once measured by bioassay, but this has poor specificity for combinations of drugs and is too slow, imprecise and labour intensive for present-day applications. Thin-layer chromatography (Chapter 39) is also now rarely used for quantitative measurement of therapeutic concentrations.

The techniques that are most widely used for routine clinical measurement of drug concentrations are immunoassays (Chapter 31), gas chromatography (GC) (Chapter 40) and high performance liquid chromatography (HPLC) (Chapter 41), either alone or, increasingly, coupled with mass spectrometry (Chapter 37) as the hyphenated methods GC-MS or LC-MS (Chapter 38). Selection of the most appropriate method for a given drug or clinical situation is not easy, and the choice depends on the availability of staff, expertise and equipment, the nature of the service to be provided, and the range of drugs to be assayed. The widely varying chemical nature of the substances to be assayed for TDM purposes means that it will rarely be possible to offer a comprehensive service based on a single analytical principle. Compromises will usually have to be made between using the best method for each individual analyte and using techniques that allow quantification of a wide range of substances.

Table 2.2 Comparison between chromatography and immunoassay for therapeutic monitoring

	Chromatography	*Immunoassay*
Applicability	Wide	Limited
Specificity (metabolites, etc.)	Yes	Sometimes
Speed of analysis	Slow–medium	Slow–fast
Capital cost (equipment0	High	High–low
Reagent costs (consumables)	Low	Medium–high
Skill required	Medium–high	Low-medium
Suitability for urgent/ point-of-care testing	Poor	Good

The basic requirements of any method are that it should be specific for the substance being assayed (without interference from structurally related compounds or endogenous plasma components), capable of precise quantification, and sufficiently sensitive to detect concentrations normally found in therapy in a sample small enough for clinical work (certainly less than 1 mL plasma and ideally 10–100 μL). Immunoassays and chromatographic techniques each have their own strengths and weaknesses, which are summarised in Table 2.2.

Chromatographic methods

Chromatographic methods are flexible and adaptable to a wide range of compounds. In most cases methods for new compounds can be devised relatively quickly compared with immunoassays, for which development times can be significant, particularly if a new antiserum must be raised. Chromatographic techniques frequently allow quantification of a range of related compounds in a single run, which has advantages when a number of drugs are prescribed together (e.g. anticonvulsants) or when separate quantification of a drug and its active metabolites is required. The combination of flexibility, specificity and sensitivity makes chromatographic techniques the method of choice for many toxicological applications. However, for TDM purposes they have a number of disadvantages. In comparison with immunoassays they are slower and more labour-intensive, frequently demanding a significant level of technical expertise. Sample throughput is usually lower than for automated immunoassays, although partially or fully automated chromatographic systems can improve this. Sample volume is often higher for chromatographic procedures, which is a particular disadvantage for paediatric applications. Sample preparation is also more laborious, since extraction or formation of a chemical derivative may be required before the chromatographic step. Total analytical time from a 'cold' start is also significantly longer, which makes chromatographic techniques less applicable for urgent measurements outside normal laboratory hours, or for point-of-care applications.

Both GC and HPLC still have a role, but the previously dominant position of GC in clinical work has steadily been eroded and HPLC or LC-MS are now the chromatographic methods of choice for most TDM applications (see Table 2.1). LC-MS and LC tandem-mass spectrometry (LC-MS[-MS]) provide highly specific and accurate methods and are rapidly becoming routine procedures (Chapter 1). The cost of instruments has fallen dramatically in recent years, but remains considerable and the skilled labour required to develop and operate these systems has important revenue consequences. LC-MS and LC-MS(-MS) offer great advantages in terms of speed, flexibility, sensitivity, reproducibility, reliability and low sample volume requirements. Moreover, they are invaluable in measuring difficult compounds such as immunosuppressants and for establishing reference procedures. A more comprehensive treatment of chromatographic and MS techniques is given in Chapters 37, 38, 40 and 41.

Immunoassays

Immunoassays, as conventional separation radioimmunoassays, have been applied to the determination of therapeutic drugs since the late 1960s, and these techniques still have a role when very high sensitivity is required, though safety and ease of use are significant disadvantages. However, the advent of homogeneous (i.e. not requiring a phase separation step) non-isotopic immunoassays in the mid-1970s proved to be the foundation for widespread adoption of commercial immunoassay kits into clinical laboratories. A bewildering variety of techniques is now available and more details will be found in Chapter 31. These kits have obvious advantages. They are generally technically simple, requiring little operator skill, and are amenable to automation on equipment commonly found in routine clinical laboratories. Sample throughputs can thus be very high and analysis times very short. Their main disadvantage is lack of specificity, either because of interference from endogenous plasma components (haemoglobin, bilirubin, etc.) that can affect the efficiency of the detection system, or because of cross-reactivity of the primary antibody with metabolites or structurally related compounds. Drugs with a large number of metabolites with similar structures (e.g. benzodiazepines, ciclosporin) pose particular problems in this respect. The development of sophisticated homogeneous non-isotopic systems is now virtually exclusive to large diagnostic companies, which limits the practical applicability of immunoassays to compounds for which there is a commercial market for an assay system. Cross-reactivity, specificity and sensitivity of available immunoassays may vary, even with the same technique on different platforms.

Free-drug concentrations

Free-drug concentrations, as indicated above, may be determined by measuring drug concentrations in a plasma ultrafiltrate obtained by centrifugation of plasma through an ultrafiltration membrane with a molecular weight cut-off of approximately 30 000 daltons, by equilibrium dialysis or by ultracentrifugation. The three separation methods usually give similar results, although systematic differences have been reported and it is advisable to compare the results of two or more techniques when validating a new method. Ultrafiltration and centrifugation are non-equilibrium techniques and are often preferred in the clinical setting as they can be completed more rapidly than the other methods. Where drug binding to protein is temperature dependent, careful control of temperature during the separation step is essential. Monitoring of free-drug concentration undoubtedly provides better clinical information than total concentration monitoring, but the increased methodological complexity and time required have limited its widespread application.

Chirality

The question of stereoselective analyses for therapeutic drugs has attracted increasing attention in recent years, Many pharmacologically active compounds contain a carbon atom linked to four different substituents and thus have the potential to exist in two different stereoisomeric forms. This property is called *chirality,* and the pairs of mirror-image isomers are called *enantiomers*. Frequently, only one enantiomer possesses a particular pharmacological action and the other may be inactive, or active in a different way. For example, the *d*-isomer of propoxyphene is a narcotic analgesic, while the *l*-isomer has no narcotic properties and is used as a cough suppressant. Enantiomers may also show marked differences in pharmacokinetic properties, such as clearance and volume of distribution (Chapter 24), and stereoselective analytical methods capable of resolving individual isomers are required if meaningful TDM information is to be obtained. These methods rely on HPLC. Immunoassays are of little use in this situation because it is difficult or impossible to produce antibodies that have enough specificity towards different enantiomers to enable them to be measured separately.

No chiral drugs are currently monitored routinely for TDM purposes, although the separate determination of the *d*- and *l*- isomers of amfetamine in urine samples from drug users prescribed dexamfetamine has been used to distinguish those compliant with the therapy from those also using 'street' amfetamine, which is a racemate.

Quality assurance

Rigorous quality assurance is essential in TDM analyses, as clinical decisions depend on the analytical results. Both internal quality control

procedures and external quality assessment schemes are necessary, and further details are given in Chapters 21 and 22. External assessment schemes are available for the majority of drugs routinely monitored and often have an important role in highlighting inappropriate methods or those that perform poorly. The potential for interference by other drugs or metabolites in TDM assays means that particular care needs to be taken in preparing specimens for external quality assurance schemes to ensure that they are as clinically realistic as possible. 'Spiking' parent drug into a plasma base material is unlikely to produce an effective material for method comparison.

Interpretation of results

In the 40 years or so that TDM has been practised in routine clinical laboratories, it has been demonstrated repeatedly that making drug concentration measurements available to clinicians does not in itself result in improved clinical care. Improved outcome depends on the application of the result to a specific clinical situation with appropriate expertise. This is facilitated by a multidisciplinary approach in which pharmacists, laboratory staff and clinicians work together to share expertise and promote best use of the service. If the laboratory is to provide an effective service, clinicians must be prepared to provide basic information, such as the reason for a particular request, the dose regime, the time of the last dose and the presence of any drugs that might cause pharmacological or analytical interference.

In particular, it is important to understand that the widely quoted (and just as often misused) 'therapeutic ranges' for drugs represent a guide to the approximate concentrations that produce a therapeutic response in the majority of patients rather than a set of inflexible limits between which patients must be forced. 'Target ranges' has been suggested as a better term, which at least carries the connotation that the ranges are something to aim at rather than implying that all concentrations within the range are therapeutic (and all outside are not). Many patients need plasma drug concentrations above (sometimes substantially above) the upper limit of the therapeutic range for effective therapy, and such concentrations must not provoke a knee-jerk dosage reduction. Specialist clinicians usually appreciate this fact, but non-specialists frequently do not and the laboratory or pharmacist has an important educational role here. Conversely, plasma drug concentrations below the lower limit of the therapeutic range may produce perfectly satisfactory responses in some patients, and arbitrary dose increases to get concentrations into the 'therapeutic range' will merely increase the likelihood of toxicity without added benefit. 'Interpretation' of plasma drug concentrations by comparing them with an arbitrary range and commenting 'sub-therapeutic' or 'toxic' does far more harm than good.

Optimum drug concentrations for a particular patient are highly individual and depend on many pharmacodynamic factors as well as the severity of the underlying disease process. This does not undermine the relevance of TDM, but it does require a degree of interpretative expertise and an understanding of the reason behind a particular request and how the result obtained relates to the clinical question. Whether this expertise resides with the clinician, the pharmacist or the laboratory scientist is less important than the fact that it exists somewhere and can be accessed when needed, although, in the nature of the service, laboratory staff are likely to be best placed to monitor interpretation across a range of clinical situations. The cardinal principle, oft repeated but still forgotten, is to treat the patient, not the drug level.

Case example

A 52-year-old woman with complex partial epilepsy treated with phenytoin 200 mg/day was still experiencing frequent seizures (1–2 per week). Her plasma phenytoin concentration over this period was in the range 8.8 to 10.1 mg/L (35–40 μmol/L) (target range 10–20 mg/L; 40–80 μmol/L). The dose was (rather rashly) increased to 300 mg/day, which saturated the patient's capacity to metabolise phenytoin and provoked a marked rise in plasma concentration to 30.3 mg/L (120 μmol/L). The patient was fit-free but severely ataxic, and the dose was reduced to 250 mg/L. On this dose, plasma concentrations were within the target range at approximately 14 mg/L (55 μmol/L), but fits recurred, albeit at decreased frequency, indicating that the broad population target range of 10–20 mg/L (40–80 μmol/L) was not optimal for this particular individual. A dose increase to 275 mg/day produced plasma concentrations a month apart of 19.7 and 17.7 mg/L (76 and 70 μmol/L), and the patient was untroubled by toxicity and remained fit-free for the next 2 years. Clearly, optimum phenytoin concentrations in this patient are confined to the upper reaches of the population 'target range'.

Factors affecting interpretation

As implied above, many factors may affect interpretation of plasma drug concentrations and it is impossible to go into specific detail in the confines of this chapter. Each of the following may have a bearing on the significance of a particular drug concentration at a particular time:

- Pharmacokinetic factors such as the age of the patient, the time since the last dose, administration of a loading dose and whether steady state has been achieved.
- Pharmacodynamic factors such as receptor density, presence of interfering drugs or drug metabolites, and concentration of endogenous substances such as potassium.
- Clinical factors such as the severity of the primary condition and the presence of other diseases.

Pharmacodynamic monitoring, biomarkers and pharmacogenetics

This chapter began by defining TDM as the use of drug or metabolite measurements in body fluids as an aid to monitoring therapy. In recent years, other methods of controlling drug therapy have been introduced and, though they do not fit the strict definition of TDM, they merit discussion as they are becoming increasingly important. Pharmacodynamic monitoring is the study of the biological effect of a drug at its target site, and has been applied in the areas of immunosuppressive therapy and cancer chemotherapy. For example, the biological effect of the immunosuppressant ciclosporin (see above) can be assessed by measuring the extent of inhibition of calcineurin phosphatase, or the interleukin-2 concentration of peripheral blood lymphocytes. The main disadvantage of pharmacodynamic monitoring is the fact that the assays involved are often significantly more complex and time-consuming than the measurement of a single molecular species by chromatography and immunoassay.

Any biochemical measurement that can be used to determine efficacy, extent of toxicity or individual pharmacodynamics for a therapeutic agent is termed a therapeutic *biomarker*. Biomarker monitoring can provide an integrated measure of all biologically active species (parent drug and metabolites) so that therapeutic ranges can be defined more closely. In addition, biomarkers are often free from the matrix and drug disposition problems that bedevil TDM in some areas, notably immunosuppressants.

Pharmacogenetic studies (studies of hereditary influences, including ethnicity, on pharmacological responses) have clear and wide-ranging clinical relevance. The enzymes that are responsible for the metabolism of drugs and other compounds exhibit wide inter-individual variation in their protein expression or catalytic activity, resulting in different drug metabolism phenotypes between individuals. This variation may arise from transient effects on the enzyme, such as inhibition or induction by other drugs, or may be at the gene level and result from specific mutations or deletions. *Pharmacogenetic polymorphism* is defined as the existence in a population of two or more alleles (at the same locus) that result in more than one phenotype with respect to the effect of a drug. The term 'pharmacogenomics' was coined to describe the range of genetic influences on drug metabolism, and its application to the practice of tailoring drugs and dosages to individual genotypes to enhance safety and/or efficacy ('personalised medicine') undoubtedly represents a massive growth area for 21st-century medicine (Chapter 25).

Determination of an individual's ability to metabolise a specific drug, by administering either a test dose of the drug or a compound

metabolised by the same enzyme system (phenotyping) or by specific genetic analysis (genotyping) can inform and improve the clinician's ability to adjust drug dosing according to the specific requirements of the individual patient. For example, a number of enzymes of the cytochrome P450 superfamily show genetic polymorphisms that account for differences in clinical response. The CYP2D6 isoform has more than 75 allelic variants, and metabolises a range of drugs widely used in medicine, including many antiarrhythmics and antidepressants. Debrisoquine is also a substrate for this isoform, and debrisoquine hydroxylase activity determined by the rate of metabolism of a test dose of debrisoquine has been widely used for the determination of CYP2D6 phenotype and the differentiation of poor metaboliser (PM), extensive metaboliser (EM) and ultra-extensive metaboliser (UEM) phenotypes. Debrisoquine is no longer available and dextromethorphan has replaced it as a probe drug for clinical use. Alternatively, genetic analysis can be used to define the CYP2D6 phenotype and identify the alleles associated with the PM phenotype (of which the most common are CYP2D6*3, *4, *5, *6 and *7). Once determined, the phenotype or genotype can be used to guide dosing for any of the drugs metabolised by the CYP2D6 isoform. CYP2D6 is absent in 15–30% of Asian people.

The clinical applications of pharmacogenomics are extensive. Examples might include anticoagulation (polymorphism of the CYP2C9 isoform), oncology (thiopurine methyltransferase isoforms and the serum Her2/neu receptor), psychiatry (CYP2D6 isoforms), epilepsy and pain control.

The combination of classic TDM, pharmacodynamic biomarkers and pharmacogenetics will undoubtedly accelerate the development of new drugs and facilitate their clinical use. It will also have a major role in delivering therapeutic efficiency and improved patient outcome with less need for plasma concentration monitoring. However, integrating the information available from all three strands is a complex challenge that will require sophisticated decision support software and effective strategies for presenting the information in an accessible format to those responsible for patient care. Pre-treatment pharmacogenetic profiling will allow identification of individuals who are likely to be particularly susceptible to or resistant to a proposed treatment strategy, allowing better choice of starting dose or the use of a different drug. Pharmacodynamic factors such as age, disease and other drugs mean that pharmacogenetics can never tell the whole story, and biomarkers of effect and drug or metabolite concentration measurements will be needed to complete the picture and deliver truly personalised therapeutics.

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CHAPTER

3 Workplace Drug Testing

A Verstraete and M Peat

Introduction

Workplace drug testing began in the USA during the 1980s as a result of accidents in the railroad industry, and the political environment of the 'war on drugs' and the 'crack' epidemic. It is now an accepted practice, with between 30 and 40 million such tests being carried out in the USA annually. In Europe, Australia and other industrialised countries, workplace drug testing has gained increasing acceptance over the past decade. For example, in the UK, workplace testing was established about 15 years ago and in 2004 it had an estimated annual turnover of £12 million. European laboratories have followed the American lead in setting up careful protocols for sample collection, analysis and medical review of results. However, there are differences both in criteria for the minimum concentration of a drug or metabolite in a urine specimen that constitutes a positive result, and in the selection of target drugs. Moreover, in the UK in particular, the ethos is that testing should be part of a package of measures that includes formulation of company policy, education of the workforce as regards the dangers of drug misuse, and treatment and/or rehabilitation programmes. Specific legislation has been enacted in Finland, Norway and Ireland to govern the principles and practices of workplace testing. The European Guidelines drawn up by representatives of the leading European laboratories are designed to establish best practice and at the same time take account of the individual requirements of national custom and legislation. The Guidelines relate to collection of samples, laboratory analyses and interpretation of the results.

Evolution of workplace testing in the USA

The major milestones in the evolution of workplace testing in the USA were:

- A major railroad accident in which there were several fatalities. The National Transportation Safety Board determined that use of cannabis (marijuana) by one of the engineers was a causal factor in the accident.
- The introduction of 'crack' cocaine and the deaths of high-profile sports and entertainment figures from cocaine use.
- The declaration of a 'war on drugs' and a change of the US Government's policy to focus on reducing the supply of drugs.

In 1986 President Reagan issued an Executive Order (Federal Register 1986) that required those federal employees in safety- and security-conscious positions to be drug tested. This Order also led to the publication by the Department of Health and Human Services (DHHS) of the *Mandatory Guidelines for Drug Testing of Federal Employees* (Federal Register 1988a), which became known as the National Institute for Drug Abuse (NIDA) Guidelines (or today as the Substance Abuse and Mental Health Services Administration (SAMHSA) Guidelines). These were modified in 1994 (Federal Register 1994) to change the cut-offs for cannabis detection and in 1998 (Federal Register 1998) to change the cut-offs for opiate detection. SAMHSA currently has responsibility for the *Guidelines*.

This confluence of events set the stage for the introduction of drug-testing programmes to the non-federal workplace. Their introduction focused on the expected improvement in safety and public health, and on the economic savings to be expected from decreased absenteeism, staff turnover rate and reduced health care costs. A limited number of major corporations were already performing pre-employment testing and claimed improvements in these, although they generally had not published their data. As the decade progressed, a number of studies (see Peat (1995) for earlier references and American Management Association (2001) for a more recent study) were published, the majority of which showed that some benefits were to be expected.

A major impetus to more widespread workplace drug testing was the publication by the Department of Transport (DOT; Federal Register 1988b) in 1988 of rules for drug testing of workers in the airline, pipeline, railroad and trucking industries. Those involved in mass transit were included originally, but were removed from the rules after a court challenge. Eventually, they were included under the 1991 Omnibus Transportation Employee Testing Act (US US Senate Public Law 1991). The DOT rules, known as *CFR Part 40* (Federal Register 1988b), originally covered between two and three million workers. After the rules were amended to cover alcohol testing in 1994 and to include mass transit workers, the number increased to approximately eight million. *CFR Part 40* was revised in 2000 (Federal Register 2000); the revision did not extend the scope of drug testing, but has had a significant impact on how it is practised.

In the USA, the DHHS *Guidelines* and *CFR Part 40* are the two major sets of rules that govern the practice of drug testing in the Federal workplace and in that regulated by the DOT. Similar rules for testing are performed under the auspices of the Nuclear Regulatory Commission (NRC) and the Department of Defense (DOD).

As stated previously, although there is far less regulation of workplace drug testing in other countries, the standards for acceptable practice are similar to those of the USA and cover specimen collection, laboratory analysis and medical review of results.

The question that underlies these drug-testing programmes is 'Have they been effective in reducing drug use in the workplace and/or in the general population?' For the USA, there are certainly data (Quest Diagnostics 2008) that suggest a reduction in the number of positives throughout the 1990s and that this reduction correlates with a reduction in the admitted use of illicit drugs (SAMHSA 2000). In all probability, numerous factors are responsible for the reduction in drug use, including targeted education programmes and supply reduction programmes, in addition to workplace drug testing.

Prevalence of drug use in the workforce and in the workplace

In the USA, of the 16.4 million illicit drug users aged 18 years or older in 2004, 12.3 million (75.2%) were employed either full or part time (SAMHSA 2005). In a survey in 1991–1993 the highest rates of current and past-year illicit drug use were reported by construction workers, food preparation workers, waiters and waitresses. The lowest rates of current illicit drug use were found among police officers and detectives, administrative support workers, teachers and childcare workers (SAMHSA 1996). A recent survey in the USA showed (Frone 2006) that illicit drug use in the workforce involved an estimated 14.1% of employed adults (17.7 million workers): 11.3% used cannabis, 1.0% used cocaine and 4.9% used psychotherapeutic drugs (sedatives, tranquillisers, stimulants and analgesics) for non-medical purposes. Illicit drug use in the workplace involved an estimated 3.1% of employed adults (3.9 million workers): 1.6% used cannabis, 0.1% used cocaine and 1.8% used psychotherapeutic drugs. In terms of being at work under

the influence of an illicit drug, 1.7% were impaired from cannabis, 0.2% from cocaine and 1.4% from psychotherapeutic drugs.

Illicit drug use in the workforce and in the workplace is not distributed uniformly in the employed population. At-risk, though circumscribed, segments of the US workforce (men aged 18–30 years in building and grounds maintenance occupations) were identified with prevalence rates up to 55.8% for any use of illicit drugs and up to 28.0% for illicit drug use in the workplace (Frone 2006). The highest risk was seen in men aged between 18 and 30 years, working in occupations where illicit drug use is particularly prevalent (legal, food preparation and serving, building and grounds maintenance occupations).

A survey carried out in Europe, in 2007, showed that 23 million people had used cannabis in the previous year, 4.5 million had used cocaine, 3 million had used drugs of the ecstasy group, 2 million had used amfetamines and 1–2 million had used heroin (EMCDDA 2007). A UK study (Smith *et al.* 2004) reported that 13% of employed people admitted to using drugs over the previous year and 7% in the previous month. Another European survey showed that among 15 to 24 year olds using cannabis, 54% were students, 30% were employed and 12% were unemployed (Taylor 2004).

The incidence of drug-positive samples found in workplace drug testing samples ranges from 1% to 20%. In pre-employment testing about 5% of samples are positive. In the UK, some surveys of drug use have reported a particularly high incidence (25%) in junior medical doctors and dentists (Newbury-Birch *et al.* 2002).

Regulatory process in the USA

The DHHS *Guidelines* (Federal Register 1988a) were mandatory rules that governed the laboratory procedures for testing urine specimens from federal employees. They did not govern directly the testing of employees in the transportation sector, although they were incorporated into the DOT rules for such testing in 1988.

A number of professional organisations responded to the publication of the *Guidelines*. As they were somewhat restrictive in scope (e.g. covered only five drugs or drug classes), the College of American Pathologists (CAP) introduced a similar but voluntary programme in the early 1990s. This included other drugs and allowed laboratories to use procedures other than immunoassay and gas chromatography–mass spectrometry (GC-MS). Today there are approximately 40 laboratories certified by DHHS and 45 accredited by the CAP. In a number of US states, laboratories must be accredited by either programme before being allowed to carry out workplace drug testing. However, only those laboratories certified by DHHS are allowed to perform workplace testing on federal employees and on those covered by the DOT Regulations.

The DHHS *Guidelines* (Federal Register 1988a) consist of a number of subparts, two of which relate directly to laboratory testing; these are subpart B (Scientific and Technical Requirements) and subpart C (Certification of Laboratories Engaged in Urine Drug Testing for Federal Agencies). Within subpart B are included sections on drugs to be tested, specimen collection procedures, laboratory personnel, testing procedures, quality assurance and quality control, and the reporting and review of results. Subpart C includes sections on performance testing, the certification process and inspections. These sections of the *Guidelines* have become models for other accreditation programmes, particularly those of the CAP and the American Board of Forensic Toxicology (ABFT; American Academy of Forensic Sciences and the Society of Forensic Toxicologists 1991). Each of the important sections is discussed in more detail below and, where applicable, discussed in the light of today's practices in the USA and Europe.

Legislation in Europe

A survey of the legislation on workplace drug testing in Europe was published by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA 2006). Three countries have specific legislation: Finland (2003), Norway (2005) and Ireland (2005). Much of the legal framework, where it exists at all, comes from interpretations of a combination of various national laws, including those on Labour Codes, privacy, data protection, and health and safety at work. A multinational company, therefore, may not be able to implement the same procedure in all its offices. Some common patterns can be discerned.

There is an *agreement on some basic principles*, e.g. the European Convention on Human Rights and the EU directives on data protection and health and safety at work. National data protection authorities have made clear statements on workplace drug testing in some countries

Many countries state that testing can take place when there is a health, safety or security risk, or when it is 'necessary', 'proportionate', 'justified' or 'reasonable', or when there is a suspicion of drug taking.

The *emphasis is on the health aspects*, rather than on the possible illegality of drug taking: in many countries the occupational doctor can inform the employer only whether an employee is 'fit for work', rather than revealing the full results of the test. There are statements that testing should form part of an overall health policy and that one should test whether the person is fit for duty (as opposed to detecting any traces of drugs). The employer has a legal duty to provide a safe place of work.

Regarding the *employment aspects*, countries vary considerably in their emphasis on testing before or during employment. Testing is permitted for job applicants in some countries in certain situations; changes in contracts including a clause agreeing to testing must be negotiated with unions or workers' associations; employees must give prior informed consent. In some countries even a contractual agreement clause is not considered to be voluntary consent.

Sanctions: some countries specifically penalise unjustified testing with criminal fines, either as a breach of workers' privacy or as a breach of privacy generally. However, the EU directive on data protection leaves it to the Member States to define any sanctions for breach of data protection.

In Finland, the Act on Workplace Drug Testing (759/2004) permits workplace drug testing, paid for by the employer, for successful job applicants or current employees. This occurs in certain defined circumstances, where intoxication or addiction may endanger life, health, national or traffic safety, security of information in the public interest, or business or professional confidentiality. The State Council Decree on Good Practice in Workplace Drug Testing (218/2005) outlines the details of the testing procedures. The Occupational Health Care Act (1383/2001) requires that the need for a test to be assessed by a health care professional, not by the employer. Only a general conclusion on the health of an employee (fit, fit with restrictions or not fit) can be given to the employer. The employer is also obliged to prepare a written comprehensive prevention programme on alcohol and drugs policy in the enterprise in cooperation with the workers. Tests must follow the Act on Patients' Rights (785/1992): this requires the 'informed consent' of the patient. Thus, an employee has the right to refuse testing.

In Ireland, the Safety, Health and Welfare at Work Act 2005 requires employees not to be under the influence of drugs at work, and to submit to drug tests if reasonable; it is an offence, punishable by a fine or imprisonment, to fail to do this. The employer can ask a doctor to check an employee's medical fitness to work. The doctor must tell the employer of the decision, and the employee the reason for the decision. The employer is obliged to provide a safe place of work.

In Norway, Act No. 62 of 17 June 2005 relating to Working Environment, Working Hours and Employment Protection states that the employer can demand medical examinations (e.g. drug testing) only when pursuant to law or regulation:

- for positions which are associated with special risk
- when the employer finds it necessary in order to protect the life or health of employees or a third party.

These provisions apply both in relation to employees and to job applicants. Section 9-4 is exhaustive in the sense that the consent of the employee or job applicant does not provide a sufficient legal basis for drug testing. The testing must also comply with the general provisions in section 9-1, which state that all controlling measures must be objectively justified and not place a disproportionate burden on the employee. The employer must discuss controlling measures with the elected representatives of the employees as early as possible and provide information to the employees themselves about certain aspects of the controlling measures.

Workplace policies on drugs and alcohol

In many European countries, testing is only possible in the framework of a comprehensive alcohol and drugs policy. Such a policy is an important instrument in preventing and dealing with alcohol- and drugs-related job performance problems.

A comprehensive alcohol and drugs policy should include several elements:

- Prevention of alcohol and drugs problems through information, education and training.
- Education to explain the need for the policy, and to help the supervisors and managers who will have to deal with issues arising from the policy.
- The policy statement which explains the rules, and the consequences if the rules are broken.
- Procedures for intervention in cases of acute or chronic use of alcohol or other drugs.
- Measures to prohibit or restrict the availability of alcohol in the workplace.
- Assistance for employees, available both before and after drug/alcohol problems develop.
- Ways of monitoring and measuring the success of the policy, which is where testing fits in.

Successful drug and alcohol policies must reflect the interests and concerns of the entire organisation. A workplace policy has to provide for responses both to immediate impairment and to anyone developing dependency problems. The workplace is an excellent environment for promoting the sensible use of alcohol, awareness of the side-effects of medicines, and an understanding of the harmful effects of illegal drugs. Having an Employee Assistance Programme in place can reduce many of the home and work stress factors that could contribute to excessive drug and alcohol use.

If testing is included in the policy, the prospect brings the objectives of the company's drug and alcohol policy sharply into focus. Employees will know the policy is there, and that they are expected to comply with it. Testing provides a deterrent to irresponsible use, and helps people with problems to face up to them.

Drugs to be tested

The DHHS *Guidelines* clearly define the drugs that can be tested under the regulatory programmes and the cut-offs (or thresholds) to be used for both the initial testing by immunoassay and confirmation testing by GC-MS. These two procedures are the only ones allowed for under the *Guidelines*.

Table 3.1 includes details of the drugs tested and the cut-offs used in the USA, and those adopted for testing in the European Union (EU; European Workplace Drug Testing Society (EWDTS 2002)), the UK (London Toxicology Group 2001) and Australia/New Zealand (Standards Australia 2001). The EWDTS guidelines have been accepted by the European Accreditation (EA) body as a benchmark for workplace drug testing.

There are two points to make regarding Table 3.1:

- When the DHHS and European programmes are compared, significant differences are found between the cut-offs used for the opiates and amfetamines. Initially, the DHHS included a cut-off of 300 ng/mL for opiates, but this was raised to 2000 ng/mL in 1998 to resolve some of the issues associated with the medical review of opiate positives following poppy seed ingestion and prescription codeine use. On the other hand, the cut-off for amfetamines has remained at 1000 ng/mL since 1989. That proposed by the European programmes is more realistic, considering the increasing use of the so-called 'designer amfetamines' throughout the 1990s.
- Phencyclidine (PCP) has always been included in the DHHS *Guidelines*, even though it is not widely used in the USA. It is even less widely used in Europe and therefore its exclusion from the EU proposal is not surprising.

The majority of testing in the USA is performed in the non-regulated workplace and, although a large number of these programmes follow the federal guidelines, a number include drugs or drug classes other than the five listed in Table 3.1 or test these five drugs or drug classes at different cut-offs. Table 3.2 lists the other drugs or drug classes that are tested and the differing cut-offs used. It can be seen that laboratories that perform workplace drug testing in the USA need to be prepared to offer a variety of tests and cut-offs if they are to satisfy fully the demands of the marketplace.

The current DHHS *Guidelines* require that all confirmations be performed by GC-MS using the cut-offs listed in Table 3.3. There are a number of points to be made regarding Table 3.3:

- Currently, the DHHS *Guidelines* do not require certified laboratories to confirm the presence of methylenedioxymethamfetamine (MDMA), methyldioxyamfetamine (MDA) and methylenedioxyethamfetamine (MDE), whereas the European guidelines do. In addition, the UK and Australia/New Zealand guidelines use cut-offs for the confirmation of ephedrine and pseudoephedrine. Draft proposals (Federal Register 2004b) to amend the DHHS *Guidelines* require the inclusion of MDMA, MDA and MDE.
- The DHHS *Guidelines* require a laboratory report of metamfetamine as positive only if its concentration is 500 ng/mL or greater, and if that of amfetamine is 200 ng/mL or greater. This reporting criterion was introduced in 1991 after several specimens were reported as positive for metamfetamine when the specimen contained large amounts of pseudoephedrine or ephedrine. It was discovered subsequently (Hornbeck *et al.* 1993) that these hydroxylated sympathomimetics could convert to metamfetamine in either the extraction or chromatographic stages of the analysis. None of these specimens contained amfetamine when tested, and therefore the introduction of the reporting rule prevented the reporting of 'false-positive' metamfetamine results. Today, the vast majority of laboratories use a pre-oxidation step with periodate (Klette *et al.* 2000; Paul *et al.* 1994) to prevent the possibility of this happening.
- As with the initial testing cut-offs, there is a difference in the thresholds used for opiate confirmations. The DHHS cut-off is 2000 ng/mL for morphine and codeine, whereas the European ones are 300 ng/mL (the UK and EU). There is also a difference across the three regions in the opiates to be tested. All require morphine

Table 3.1 Initial testing cut-offs (μg/L) for those drugs and/or drug classes included in regulatory programmes in urine

Drug or drug class	*USA DHHS*	*EU*	*UK*	*Australia/NZ*
Amfetamines	1000	300	300	300
Cannabis	50	50	50	50
Cocaine	300	300	300	300
Opiates	2000	300	300	300
Phencyclidine	25	Not tested	25	Not tested

Table 3.2 Initial testing cut-offs (μg/L) for those drugs and/or drug classes included in non-regulatory programmes in urine

Drug or drug class	*Cut-off in the USA (μg/L)*
Amfetamines	300 or 500
Barbiturates	100 or 200
Benzodiazepines	100, 200 or 300
Cannabis	20, 25 or 100
Cocaine	150
Methadone	100 or 300
Methaqualone	100 or 300
Opiates	300
Propoxyphene	100 or 300

Table 3.3 Confirmation cut-offs (μg/L) for those drugs and/or drug classes included in regulatory programmes in urine

Drug or drug class	*Drugs tested*	*US DHHS*	*EU*	*UK*	*Australia/NZ*
Amfetamines	Amfetamine	500	200	200	300
	Metamfetamine	500	200	200	300
	MDMA, MDE		200	200	300
	Pseudoephedrine			200	500
	Ephedrine			200	500
Cocaine	Benzoylecgonine	150	150	150	150
Cannabis	THCA	15	15	15	15
Opiates	Morphine	2000	200	300	300
	Codeine	2000	200	300	300
	6-AM	10	10	10	10
	Dihydrocodeine			300	
Phencyclidine	PCP	25	Not tested	25	Not tested

and 6-acetylmorphine (6-AM) under certain circumstances in some cases; the DHHS, Australia/New Zealand and UK guidelines also require codeine, and the UK one requires dihydrocodeine. The DHHS *Guidelines* require that certified laboratories analyse the specimen for 6-AM (with a cut-off of 10 ng/mL) if the morphine concentration exceeds 2000 ng/mL.

- Cannabis (marijuana) use is confirmed by quantifying one of the major urinary metabolites of tetrahydrocannabinol (THC), 11-carboxytetrahydrocannabinol (THCA) and the cut-off value of 15 ng/mL has been also adopted by the European programmes.

Table 3.4 details the other drugs that may have to be confirmed in non-regulated programmes and the different cut-offs that may be used for the five drug classes tested for in the regulated programme. Although several compounds are listed under some of the drug classes, this does not imply that all these tests are performed on every non-regulated specimen with a positive initial testing result.

Lysergide testing

Over the years there has been discussion regarding the usefulness of incorporating lysergide (LSD) into workplace drug testing programmes. There are many reasons for its exclusion, one of the major ones being that demographically those included in workplace drug-testing programmes are not in the age group expected to be users. Nevertheless, certain populations, such as inductees into the US military, are being tested using cut-offs for screening and confirmation of less than 1 ng/mL for LSD. A second major reason is the analytical challenge presented. Today, both enzyme and microparticle immunoassays are available for screening urine specimens, and LSD can be confirmed using tandem mass spectrometry. However, there has been concern regarding testing

Table 3.4 Confirmation cut-offs (μg/L) for those drugs and/or drug classes included in non-regulatory programmes in urine

Drug or drug class	*Drugs tested*	*USA*	*EU*	*UK*	*Australia/NZ*
Amfetamines	Amfetamine	300 or 500	200	200	300
	Metamfetamine	300 or 500	200	200	300
	MDMA, MDA	300 or 500	200	200	300
Barbiturates	Amobarbital	100 or 200	150	150	
	Butalbital	100 or 200	150	150	
	Pentobarbital	100 or 200	150	150	
	Phenobarbital	100 or 200 (or higher)	150	150	
	Secobarbital	100 or 200	150	150	
Benzodiazepines	α-Hydroxyalprazolam	100 (or lower)			
	Nordiazepam	100	100	100	200
	Oxazepam	100, 200 or 300	100	100	200
	Temazepam	100, 200 or 300	100	100	200
Cocaine	Benzoylecgonine	100 or 150	150	150	150
Cannabis	THCA	10 or 15	15	15	15
Methadone	Methadone	100 or 300	250	250	
Methaqualone	Methaqualone	100 or 300	300	300	
Opiates	Morphine	300	300	300	300
	Codeine	300	300	300	300
	Hydrocodone	300			
	Hydromorphone	300			
	6-AM	10	10	10	10
Propoxyphene	Propoxyphene	100 or 300	300	300	

for unchanged LSD, and work published by Klette *et al.* (2000) and Poch *et al.* (2000) shows clearly that the 2-oxo-3-hydroxy LSD metabolite is the preferred target analyte.

Testing procedures

As already indicated, the DHHS *Guidelines* mandate the use of immunoassay as the initial test and GC-MS as the confirmatory procedure. These are also the testing procedures generally used for non-regulated testing, although some laboratories may be using MS-MS technology or liquid chromatography–mass spectrometry (LC-MS, or LC-MS-MS) for the confirmation of the non-regulated analytes.

The immunoassay methods have to have been approved by the Food and Drug Administration (FDA), which in the USA has regulatory authority for approval of diagnostic reagents. Although workplace drug testing is not being performed for diagnostic purposes, it was believed that requiring FDA approval would bring at least some standardisation to the reagents used. However, no regulations were introduced that required the immunoassay kits to use antibodies directed towards certain members of a drug class, and if the kits used for regulated and non-regulated testing are compared there is some variation in the target antigen (Liu 1995).

Nearly all drug-testing laboratories use either reagents based on enzymes (e.g. EMIT or CEDIA), microparticles (KIMS) or fluorescence polarisation (TDx). Table 3.5 shows the calibrators used in each of the immunoassays for the drugs tested today.

Given the difference in target antigens and calibrators, different detection rates on specimens that contain the same drug(s) and/or metabolites might be expected. For example, the CEDIA and EMIT assays for amfetamine have almost 100% cross-reactivity to *d*-amfetamine, whereas the KIMS assay has little cross-reactivity to *d*-metamfetamine. In fact, the latter was designed to comply with the Reporting Rule issued by DHHS for metamfetamine positives, and therefore is effective in detecting 'real-life' specimens that contain metamfetamine and its metabolite, amfetamine. Conversely, if a proficiency-testing specimen contained only metamfetamine it would give a negative result. Similar dichotomies exist in examining the benzodiazepines, for which the detection of the more traditional members of the group is not a problem, but the assays vary widely in their ability to detect some of the later members, such as lorazepam and flunitrazepam metabolites (Drummer 1998). Even when the procedures are targeted towards the same antigen and use the same calibrators, there can be variation in their ability to detect positive specimens. For example, immunoassays that have greater specificity towards the target urinary metabolite of THC (THCA), may not be as efficient as those that cross-react more widely to the THC metabolites in detecting urine specimens from cannabis users, particularly when cut-offs are used.

One of the major concerns in the past few years has been the ability of the amfetamine assays to detect MDMA, MDA and MDE. Some of the manufacturers have introduced special kits, whereas others have relied on the inherent cross-reactivity of their existing amfetamine assay. From existing data (Zhao *et al.* 2001), it appears that either approach is satisfactory, with the most variation being seen in specimens with concentrations close to the cut-off. In an attempt to resolve this problem, the draft proposals (Federal Register 2004b) from the DHHS on the new guidelines require that '*d*-metamfetamine be the target analyte and the test kit must cross-react with MDMA, MDA and MDE (approximately 50–150% cross-reactivity)'. Whether this requirement can be satisfied, given the vagaries of immunoassays, remains to be seen.

Some of the issues that surround initial testing by immunoassay have been discussed already. Far fewer issues relate to confirmation testing by GC-MS. Numerous GC-MS procedures have been published for the identification and quantification of the drugs and their metabolites in urine specimens, and increasingly so for their detection in hair, oral fluid and other specimens. Confirmation analyses of drugs and metabolites in hair, oral fluid and sweat will require procedures with more sensitivity than those currently used for urine specimens, and the draft proposals (Federal Register 2004b) from the DHHS recognise this in that they permit the use of MS(-MS) technology with GC and LC interfaces. The use of these technologies is likely to raise issues similar to those that were debated two decades or so ago, when GC-MS was mandated for confirmation of drugs and metabolites in urine. Some of these issues are set out and discussed below:

- How reliable is the use of ion ratios and how many ions should be monitored? Experience has certainly shown that the monitoring of three ions for the analyte and two for the deuterated internal standard, and the use of a 20% range for ion ratios, result in satisfactory positive identification of the analyte.
- How diagnostic are the ions? Most toxicologists believe that using ions of higher mass is more appropriate than using ones of lower mass. For example, using *m/z* 91 for amfetamine is not recommended. On the other hand, there are a number of assays in use today that routinely use isotopes for identification and ratio purposes. Perhaps the best example is the confirmation of PCP, for which large numbers of laboratories use *m/z* 242 and its carbon isotope, *m/z* 243. This has become an accepted practice for this drug because, apart from *m/z* 205, there are no other diagnostic ions. Another example of a drug class for which isotopes may be used, with less justification, is the benzodiazepines (halogen isotope ratios are used).
- What is an acceptable chromatographic peak shape? Two integral parts of the DHHS *Guidelines* are the need for proficiency testing (the *Guidelines* use the term performance testing) and regular inspections of the certified laboratories. The latter has become known as the National Laboratory Certification Program (NLCP) and requires that each laboratory be inspected at 6-monthly intervals. There are allowances within the *Guidelines* for other inspections, specifically special inspections that can be performed at the direction of DHHS and outside the normal cycle. A number of accepted practices have evolved as a result of the inspections. Two examples are the detailed quality-control requirements and the definition of acceptable peak shape. The acceptance criteria for these practices, and others, are not included in the DHHS *Guidelines* themselves, but are included in the Resource Manual for the NLCP. Within this

Table 3.5 Calibrators used in commercial immunoassays

Drug or drug class	*CEDIA*	*EMIT*	*OnLine (KIMS)*	*TDx/ADx*
Amfetamines	*d*-Metamfetamine	*d*-Metamfetamine	*d*-Amfetamine	*d*-Amfetamine
Barbiturates	Secobarbital	Secobarbital	Secobarbital	Secobarbital
Benzodiazepines	Nitrazepam	Lormetazepam	Nordiazepam	Nordiazepam
Cannabis	THCA	THCA	THCA	THCA
Cocaine	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine
Methadone	Methadone	Methadone	Methadone	Methadone
Methaqualone	Methaqualone	Methaqualone	Methaqualone	Methaqualone
Opiates	Morphine	Morphine	Morphine	Morphine
Phencyclidine	PCP	PCP	PCP	PCP
Propoxyphene	Propoxyphene	Propoxyphene	Propoxyphene	Propoxyphene

manual, an acceptable chromatographic peak shape has been defined as having greater than 90% resolution and symmetry.
- What are the limits of detection and quantification and how are they determined? The definition of these criteria has also evolved since 1988. Initially, the majority of certified laboratories used the traditional definitions based on the analysis of drug-free specimens and, although this is still acceptable, the favoured definitions are now ones based on serial dilutions and satisfying quantitative and ion-ratio criteria.

Although it is straightforward to define these criteria for GC-MS analysis of urine specimens, it is difficult to imagine the same criteria being applied to MS(-MS) technology, particularly if chemical ionisation or electrospray are used.

Quality control

The DHHS *Guidelines* stress the importance of appropriate quality control and quality assurance programmes, but do not define specific requirements. Within the DOT Regulations (Federal Register 2000) is the requirement that 1% of samples referred to testing laboratories by employers and other bodies that manage drug-testing programmes should be blind quality-control specimens. These must be double-blind to the laboratory and must include negatives, positives and specimens that may be adulterated or substituted.

In addition to these double-blind specimens, the laboratory itself is required to analyse a further 1% as blind specimens through the initial immunoassay testing. These specimens are best described as 'single blinds' in that their identity is known before the specimens are released for reporting. The DHHS *Guidelines* indicate the following requirements for open quality control (when the identity is known to the analyst):

- Initial testing: each analytical run of specimens to be screened shall include:
  - sample(s) certified to contain no drugs;
  - positive controls fortified with drug or metabolite;
  - at least one positive control with the drug or metabolite at or near the cut-off;
  - a minimum of 10% of the total specimens in each analytical run shall be controls.
- Confirmation testing (in which each analytical run of specimens to be confirmed) shall include:
  - sample(s) certified to contain no drug;
  - positive controls fortified with drug or metabolite;
  - at least one positive control with the drug or metabolite at or near the cut-off.

Currently, there are no requirements to include 'single blinds' in confirmation batches or to confirm those 'single-blind' controls that have tested positive by immunoassay, although a number of certified laboratories do so.

More detailed guidelines were developed over time, as a result of the inspection process, and the NLCP now considers the following to be appropriate quality control for initial testing batches:

- A minimum of 10% of the total specimens and controls in each analytical run shall be controls, for example if the batch size is 50 (including controls) then at least 5 need to be open controls.
- The controls shall include:
  - sample(s) certified to contain no drug or metabolite;
  - positive controls targeted to contain drug or metabolite at no greater than 125% of the cut-off;
  - 'negative' controls targeted to contain drug or metabolite at no less than 75% of the cut-off.
- Positive controls have to test positive and negative controls negative before a batch can be released for reporting.
- Specimens have to be bracketed by acceptable controls before they can be released for reporting.

Although there are no specific requirements for the trend analysis of control values, there are expectations that a certified laboratory should monitor these to determine assay drift. In addition, when validating an immunoassay the laboratory is expected to ensure that there is adequate separation between the values of the 'negative' control and the cut-off and those of the cut-off and positive control.

The DHHS *Guidelines* for quality control of confirmation assays were also vague and required further clarification through the NLCP. The situation here was further complicated by the requirements to re-test specimens. Both the DHHS *Guidelines* and DOT Regulations recognised the possibility that drug concentrations may decrease over time in positive specimens, even when they were frozen. This was viewed as a particular problem for THCA and benzoylecgonine. To prevent a specimen that initially tested as positive from reporting as 'negative' on a re-test, it was decided that the reconfirmation test should determine only the presence or absence of the drug or metabolite, that is no cut-offs were to be used for GC-MS analyses of re-test specimens. The laboratories were therefore asked to test these specimens to their 'limits of detection' for the specific drug. The issue quickly arose that each laboratory essentially used a different limit of detection for these re-tests and there was no standardisation. In the early 1990s, guidance issued by the NLCP required certified laboratories to have 'limits of detection' of at least 40% of the confirmation cut-off for these re-tests. For THCA and benzoylecgonine this meant that they had to confirm at 6 and 60 ng/mL, respectively, using the standard acceptance criteria for ion ratios and chromatographic peak shape. To ensure that they can do so, the NLCP challenges laboratories with re-test specimens as part of the performance testing programme and requires that a 40% of cut-off control be included in all re-test batches.

The NLCP considers the following to be an acceptable quality control programme for confirmation analyses:

- Each batch contains a certified negative sample.
- Each batch contains one cut-off control, targeted to be 125% of the cut-off.
- Each re-test batch contains a control targeted to be 40% of the cut-off.
- The acceptable range for the control is plus or minus 20% of a validated mean concentration for that control pool.
- If the laboratory prepares its calibrators and controls in-house, these need to be prepared from at least two different lot numbers of drug or metabolite.

Certification of data is an integral part of the operation of a drug-testing laboratory and is covered by the DHHS *Guidelines*. All results, including negatives, have to be reviewed by a certifying scientist before they can be reported. The larger laboratories have two different categories of certifying scientist, those trained to review negative results and those trained to review positives. The major difference between the two is in the level of expertise and experience; those who can report positives must have knowledge of GC-MS and of the quality control of GC-MS procedures. Both groups need to have knowledge of accessioning (the receipt of specimens), chain-of-custody protocols, immunoassays and quality control of these assays. The certification process includes a review of testing data, quality-control results, chain-of-custody documentation (external and internal) and any other documentation associated with the specimen being reported. A certifying scientist with the experience and training to release positives is also required to compare the initial immunoassay and GC-MS results for consistency (when this can be done) and may be asked to provide technical assistance to medical review officers (MROs) and others who receive drug testing results.

Testing procedures, quality control and quality assurance have been discussed in some detail, with the emphasis being placed on the protocols expected of DHHS-certified laboratories. Laboratories are expected to follow similar protocols for CAP accreditation, and these have been used as a framework for accreditation in other programmes. This is not necessarily because they are ideal, but because they have become the standard of care for accepted laboratory practice in drug testing and increasingly so in forensic toxicology. It is worthy of comment that the majority of laboratories that perform comprehensive forensic toxicology now have a quality-control programme, not necessarily for all analytes but certainly for those that they test routinely. This was not the case in the early 1990s.

Table 3.6 Proposed initial testing cut-offs for alternative specimens

Drug or drug class	*Hair (pg/mg)*	*Oral fluid (μg/L)*	*Sweat (ng/patch)*
Amfetamines	500	50	25
Cocaine	500	20	25
Cannabis	1	4	4
Opiates	200	40	25
Phencyclidine	300	10	20
MDMA	500	50	25

Proposed changes to the DHHS *Guidelines*

The DHHS is considering expanding the *Guidelines* to include specimens other than urine and to lower the cut-offs in urine. Although these proposals (Federal Register 2004b) have not been adopted, it is probably worthwhile to summarise the proposed cut-offs for these alternative specimens. The three new specimens being considered are hair, oral fluid and sweat (Tables 3.6 and 3.7). Today, each of these specimens is being used outside the federally regulated workplaces for workplace and/or criminal justice testing.

Within the proposal there are a number of footnotes to Tables 3.6 and 3.7.

Hair

- It will be permissible to test initially all specimens for 6-AM using a 200 pg/mg cut-off.
- For a specimen to be positive for cocaine, both cocaine and benzoylecgonine must confirm positive and the ratio of metabolite to parent cocaine must be equal to or greater than 0.05.
- If the specimen confirms positive for 6-AM, it must also contain morphine at a concentration equal to or greater than 200 pg/mg.
- For a specimen to be positive for metamfetamine it must contain amfetamine at a concentration equal to or greater than 50 pg/mg.

Oral fluid

- It will be permissible to test initially all specimens for 6-AM using a 4 ng/mL cut-off.
- For a specimen to be positive for metamfetamine it must contain amfetamine at a concentration equal to or greater than the limit of detection.

Standards Australia has now published guidelines for specimen collection and the detection and quantification of drugs in oral fluids (Standards Australia 2006). The guidelines were developed by a committee made up of practising toxicologists, pathologists, industry representatives, unions, and government officials with input from the accreditation authority. They cover the collection, on-site testing, transport and laboratory testing, and confirmation for drugs in the fluid.

The standard focuses on testing for amfetamine-related stimulants, cocaine, cannabis and opiates. It describes the training of persons collecting oral fluids and performing on-site drug screens, the conduct of quality control testing and proficiency testing on-site as well as the ISO/IEC 17025 or 15189 requirements for laboratory-based testing. A referee specimen should be available if a result is disputed. The standard uses the concept of 'target concentration' rather than 'cut-off' to indicate a concentration sufficient for detection of drug use, since international agreement for cut-offs has not yet been reached for any application. These concentrations are those that should be detectable in order to identify relatively recent drug use. The standard recommends the use of quality controls not only in a laboratory setting but also on site. For on-site tests one negative and one positive control should be run each day and one quality control sample should be analysed for every 25 specimens. Quality control samples are prepared in oral fluid at or within 50% of the concentration nominated by the manufacturer to detect the drug reliably and for which performance data are available.

Sweat

- For a specimen to be positive for metamfetamine it must contain amfetamine at a concentration equal to or greater than the limit of detection.

Clearly, there will be a dramatic increase in the complexity of the analyses needed to test these alternative specimens for workplace drug testing, and in the proficiency testing and inspection programmes involved in certifying laboratories to do such testing.

Although no mention has yet been made regarding the specimen volume to be collected, it is important to realise that the current urine testing programme requires the collection of at least 45 mL, of which 30 mL is placed in bottle A and 15 mL in bottle B. Bottle B is considered to be the donor's and can be opened only at the request of the donor. This request is generated by the MRO and the bottle is normally opened for re-test purposes by a second certified laboratory. Given the sensitivity of current technology available for testing urine specimens, there are no concerns regarding specimen availability during this process. However, that may not be the case for the alternative specimens, for

Table 3.7 Confirmation cut-offs for alternative specimens

Drug or drug class	*Drugs tested*	*Hair (pg/mg)*	*Oral fluid (μg/L)*	*Sweat (ng/patch)*
Amfetamines	Amfetamine	300	50	25
	Metamfetamine	300	50	25
	MDMA	300	50	25
	MDA	300	50	25
	MDE	300	50	25
Cocaine	Benzoylecgonine	50		
	Cocaine	500		
	Cocaine or benzoylecgonine		8	25
Cannabis	THCA	0.05		
	THC		4	1
Opiates	Morphine	200	40	
	Codeine	200	40	
	Morphine, codeine or 6-AM			25
	6-AM	200	4	
Phencyclidine	PCP	300	10	20

which the minimum specimen sizes are recommended to be hair 100 mg (2×50 mg), oral fluid 2 mL (1.5 mL for the primary specimen and 0.5 mL for the split specimen) and sweat (two FDA-approved patches worn for 7–14 days).

More than 2000 comments were received from 285 responders representing drug-testing laboratories, manufacturers of testing kits, federal employees who will be drug tested, agencies that may choose to use new drug testing methods, private sector employers and members of the public at large. The proposed changes have not been adopted yet. Their implementation will require laboratories to be proficient in the use of more sensitive immunoassays (particularly enzyme-linked immunosorbent assay (ELISA)) and tandem mass spectrometry (probably with an liquid chromatography interface).

Table 3.8 provides a comparison of hair, oral fluid and sweat as specimens for drug detection.

Point-of-collection tests

The proposed changes to HSS *Guidelines* also included a section on point-of-collection tests (POCTs). This establishes the criteria for POCT devices that may be used as part of the Federal Workplace Drug Testing Program, i.e. when federal agencies may use a POCT, what the responsibilities are of a federal agency that chooses to use a POCT, and the procedures that must be followed in using a POCT.

A POCT device that may be used in a Federal Workplace Drug Testing Program is one that:

- is FDA-cleared; and
- effectively determines the presence or absence of drugs and determines the validity of a specimen, either as an integral feature of the POCT device or as a set of compatible devices or procedures.

POCT devices may be:

- *non-instrumented*, for which the endpoint result is obtained by visual evaluation (i.e. read by human eye); or
- *instrumented*, for which the result is obtained by instrumental evaluation (e.g. a densitometer, a spectrophotometer, a fluorometer).

Oral fluid (saliva) and urine specimens may be tested using a POCT.

For drug POCTs the quality control requirements are as follows:

- Each day testing is performed using devices with visually read endpoints, each individual performing drug tests using these devices must test at least one negative control and one positive control (with the concentration of the drugs or metabolites in the range of 25% above the cut-off concentration) before donor specimens are tested.
- Each day testing is performed using devices with semi-automated or automated testing devices with machine-read endpoints (i.e. spectrophotometer); at least one negative control and one positive control must be tested on each device used.

For validity POCTs, for each day that testing is performed, at least one control that is normal for the specific validity test and one control that is abnormal must be tested. The results must be correct before donor specimens are tested.

At least one specimen out of every 10 specimens that test negative must be submitted to an DHHS-certified laboratory as part of a quality assurance programme. None of these proposed changes has been adopted.

Adulterated and substituted specimens

Over the past decade, increasing attention has been paid to attempts to 'beat the drug test'. There have always been donors who have attempted this through diuresis, through substitution of the specimen (with clean urine or another fluid) or through deliberate adulteration of the collected specimen. The DOT first issued guidance on this in 1992 and recommended that laboratories identify adulterants in a 'forensically acceptable manner'. A 'dilute urine specimen' was defined as one that contained less than 20 mg/dL creatinine and had a relative density (specific gravity) of less than 1.003. At that time regulations allowed an observed urine collection on a donor producing dilute urine at their next scheduled collection, and at the request of the employer. In practice this was hardly ever done. Collection guidelines also required the collector to perform an observed collection if there were suspicious circumstances during collection or if the urine temperature was out of range (the acceptable range being 32–38°C).

In recent years use has been made of the Internet to sell products that are supposedly designed to beat drug tests. While a number of these include the instructions to drink copious amounts of water before taking the test (i.e. diuresis), others are designed specifically to oxidise the THCA metabolite of cannabis and thereby reduce the ability of the laboratory to confirm cannabis use. Some examples include nitrite, chromate (chromium(VI)), halogens and peroxide. Although

Table 3.8 Comparison of hair, oral fluid and sweat as specimens for drug detection

Specimen	*Detection window*	*Advantages*	*Disadvantages*
Hair	Months – dependent to some extent upon the drug	Can be used as a long-term measure of drug use	Not a suitable specimen for detecting recent drug use
		Relatively non-invasive collection	May be an invasive collection if head hair is unavailable
		Can obtain a second specimen for re-testing (if necessary)	Requires sensitive immunoassays and MS-MS technology
		Relatively resistant to adulteration	Deposition of drug and/or metabolite in hair is reported to be dependent upon hair colour
			Potential environmental contamination
Oral fluid	Hours or days – dependent to some extent upon the drug	Relatively non-invasive collection	Short detection window for some drugs
		An 'observed' collection and therefore resistant to adulteration and substitution	Requires sensitive immunoassays and MS-MS technology
		For some drugs correlates to free drug concentration in plasma	Collection methods can dilute the specimen, which makes drug detection more difficult
			After cannabis use, THC in the buccal cavity is the detected material, THCA is not detected
Sweat	Up to weeks – dependent to some extent upon the drug	Cumulative measure of drug use Can monitor drug use for a period of weeks with a sweat patch	Requires sensitive immunoassays and MS-MS technology High inter-subject variability For workplace drug testing, application of sweat patch for several days is impractical

accurate data are difficult to obtain, recent data from Quest Diagnostics show that the rate of substituted specimens in 2007 was 0.05% for workers covered by federal regulations and 0.01% for those in the general workplace. The comparable rates for adulterated specimens were 0.01% and 0.0%, respectively.

The *CFR Part 40* (Federal Register 2000) requires certified laboratories to detect adulterated and substituted specimens. The DHHS has issued detailed proposed rules (Federal Register 2004b) for such testing, which include definitions and the testing and quality control procedures to be used. Steps that the MRO can use for review of these non-negative specimens are also included. These rules are for federal employees only. The DOT issued a Notice of Proposed Rule Making in November 2005 which differed from the rules covering federal employees in the handling of substituted specimens. Under the DHHS rules the definitions for a dilute, substituted and adulterated specimen are as follows:

- Dilute: creatinine greater than or equal to 2 mg/dL, but less than 20 mg/dL and relative density is greater than 1.0010 but less than 1.0030, except when the definition of a substituted specimen is met.
- Substituted: creatinine less than 2 mg/dL and relative density less than 1.0010 or equal to or greater than 1.0200.
- Adulterated:
  - Nitrite is greater than or equal to 500 µg/mL.
  - pH is less than 3 or greater than or equal to 11.
  - The presence of chromium(VI) is verified.
  - The presence of a halogen is verified.
  - The presence of glutaraldehyde is verified.
  - The presence of pyridine is verified.
  - The presence of a surfactant is verified.
  - The presence of any other adulterant is verified.

With these Rules, DHHS required the laboratories to verify the presence of an adulterant using a test different from the one used to detect it. For example, the laboratories could use a general oxidant screening test, but if this was positive it would have to verify the presence of chromium(VI) by a specific analytical procedure. If they did not do this then the specimen would be reported as invalid and the MRO would then have to interview the donor and, if there was no medical explanation, require a recollection under direct observation. This has led to an increase in the number of invalids reported by the laboratories (Quest Diagnostics 2008). In the federal workplace the rate has risen from 0.08% in 2004 to 0.11% in 2007, and in the non-federal workplace from 0.10% to 0.13%.

The following definitions are now used for invalid specimens:

- Inconsistent creatinine concentration and relative density (i.e. creatinine concentration is less than 2 mg/dl and relative density is greater than 1.0010 but less than 1.0020, or relative density is less than or equal to 1.0010 and creatinine concentration is greater than or equal to 2 mg/dL).
- The pH is greater than or equal to 3 and less than 4.5 or greater than or equal to 9 and less than 11.
- The nitrite (or nitrite equivalent) concentration is greater than or equal to 200 µg/mL but less than 500 µg/mL.
- The possible presence of chromium(VI) is determined
- The possible presence of a halogen is determined.
- The possible presence of glutaraldehyde is determined.
- The possible presence of an oxidising agent is determined.
- The possible presence of a surfactant is determined.
- Interference occurs with the immunoassay drug results.
- Interference occurs with the GC-MS confirmation tests.
- The physical appearance of the specimen is such that it may damage the laboratory's instruments.

Under the proposed rules for DOT, the definition of a substituted specimen remains the same but includes a requirement that the MRO order an immediate re-collection under direct observation for a negative dilute with a creatinine concentration greater than or equal to 2 mg/dL but equal to or less than 5 mg/dL. This requirement obviously means that the laboratory reports both the creatinine concentration and the relative density to the MRO.

These rules are interesting for a number of reasons. First, a workplace drug-testing laboratory now has to perform tests that, historically, have not been performed in toxicology laboratories. Second, the toxicologist and/or MRO may be called upon to defend these specimen validity testing procedures and their results when legally challenged, for example in an arbitration hearing. Defending a non-negative result may present new challenges in terms of the interpretation of the data. Third, they expand the duties of the laboratory to include the identification of new and existing adulterants, which is a major analytical challenge. Finally, they are the first such rules to prescribe quality-control protocols for diluted, adulterated or substituted specimens.

This brief overview of some of the newer requirements and of the expansion of the programmes to include alternative specimens demonstrates that the range and complexity of workplace drug testing has increased dramatically and will continue to do so.

Collection of specimens

The proper collection, packaging and transporting of urine specimens is a crucial part of a drug-testing programme. Although this is the most frequently challenged aspect of the process in any legal proceeding, it is also the least regulated one. Until the introduction of the updated *CFR Part 40* (Federal Register 2000), there were no requirements for training or certification of collectors, and there are still no requirements for certification. The training programme is one that depends upon the 'train the trainer' concept and requires an evaluation of the individual's ability to collect specimens and complete the appropriate chain-of-custody documentation in certain situations. A collector who makes errors is required to undergo error-correction training. These errors are documented either by the laboratory when receiving specimens or by the MRO when reviewing the chain-of-custody documentation.

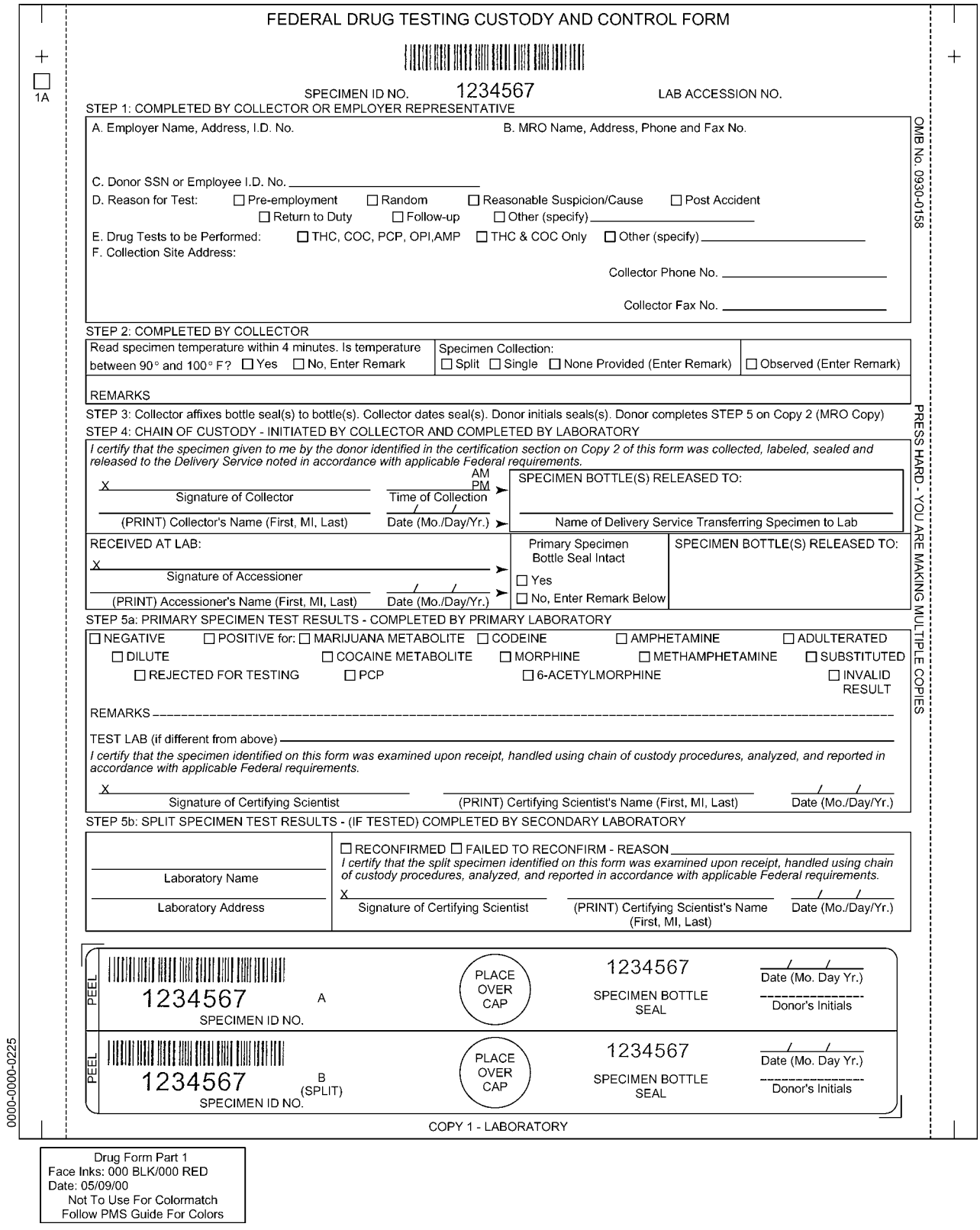
It has been accepted practice, and is now a requirement, in the industries regulated by the DOT that a urine specimen be collected and split into two portions (sometimes referred to as bottles A and B) and that the minimum volume should be 45 mL. As described above, at least 30 mL is placed in bottle A and 15 mL in bottle B. During the collection process the collector is required to monitor the temperature of the urine (within 4 min of the collection), ensure that the donor does not substitute or adulterate the specimen and complete the necessary chain-of-custody documents. This process is itemised into the following major steps:

- Preparation of the collection area, for example adding a blue dye to the toilet water and taping of the taps.
- Confirmation of the donor's identity using photographic evidence and requesting him or her to remove outer clothing and to empty pockets.
- Having the donor randomly select a collection container and two bottles, which should be wrapped separately (only the collection container should be taken into the toilet enclosure).
- Checking the temperature of the sample within 4 min of voiding and noting the result on the chain-of-custody form. The collector then has to ensure that there is at least 45 mL of urine in the container and that the specimen has no unusual odour, colour or physical properties (e.g. excessive frothing) that may indicate attempted adulteration.
- Pouring at least 30 mL into bottle A and 15 mL into bottle B in the presence of the donor, and immediately closing the bottles and applying tamper-evident seals across the lids or caps of the bottles.
- Ensuring that the donor initials the seals and completes his or her section of the chain-of-custody form. The collector then completes the remaining sections of the form.
- Preparing the specimen bottles for shipment to the laboratory.

Although these are the recommended steps for the completion of a regulated chain of custody, it is also the general procedure used to collect all urine specimens. The steps most often challenged are that the

collector did not complete the process in the presence of the donor and that more than one specimen was being collected at the same time (i.e. there were multiple specimens and chains of custody in various stages of collection).

Completion of the chain-of-custody form is an important part of this process, and in the regulated arena a five-part document is used (see Fig. 3.1 for Copy 1 of this document). There are several sections to the form:



FEDERAL DRUG TESTING CUSTODY AND CONTROL FORM

1A

SPECIMEN ID NO. 1234567 LAB ACCESSION NO.

STEP 1: COMPLETED BY COLLECTOR OR EMPLOYER REPRESENTATIVE

A. Employer Name, Address, I.D. No.

B. MRO Name, Address, Phone and Fax No.

C. Donor SSN or Employee I.D. No. _____

D. Reason for Test: ☐ Pre-employment ☐ Random ☐ Reasonable Suspicion/Cause ☐ Post Accident ☐ Return to Duty ☐ Follow-up ☐ Other (specify) _____

E. Drug Tests to be Performed: ☐ THC, COC, PCP, OPI,AMP ☐ THC & COC Only ☐ Other (specify) _____

F. Collection Site Address:

Collector Phone No. _____

Collector Fax No. _____

OMB No. 0930-0158

STEP 2: COMPLETED BY COLLECTOR

Read specimen temperature within 4 minutes. Is temperature between 90° and 100° F? ☐ Yes ☐ No, Enter Remark	Specimen Collection: ☐ Split ☐ Single ☐ None Provided (Enter Remark)	☐ Observed (Enter Remark)

REMARKS

STEP 3: Collector affixes bottle seal(s) to bottle(s). Collector dates seal(s). Donor initials seals(s). Donor completes STEP 5 on Copy 2 (MRO Copy)

STEP 4: CHAIN OF CUSTODY - INITIATED BY COLLECTOR AND COMPLETED BY LABORATORY

I certify that the specimen given to me by the donor identified in the certification section on Copy 2 of this form was collected, labeled, sealed and released to the Delivery Service noted in accordance with applicable Federal requirements.

X _____ Signature of Collector

_____ (PRINT) Collector's Name (First, MI, Last)

AM PM Time of Collection

/ / Date (Mo./Day/Yr.)

SPECIMEN BOTTLE(S) RELEASED TO:

_____ Name of Delivery Service Transferring Specimen to Lab

RECEIVED AT LAB:

X _____ Signature of Accessioner

_____ (PRINT) Accessioner's Name (First, MI, Last)

/ / Date (Mo./Day/Yr.)

Primary Specimen Bottle Seal Intact

☐ Yes

☐ No, Enter Remark Below

SPECIMEN BOTTLE(S) RELEASED TO:

STEP 5a: PRIMARY SPECIMEN TEST RESULTS - COMPLETED BY PRIMARY LABORATORY

☐ NEGATIVE ☐ POSITIVE for: ☐ MARIJUANA METABOLITE ☐ CODEINE ☐ AMPHETAMINE ☐ ADULTERATED ☐ DILUTE ☐ COCAINE METABOLITE ☐ MORPHINE ☐ METHAMPHETAMINE ☐ SUBSTITUTED ☐ REJECTED FOR TESTING ☐ PCP ☐ 6-ACETYLMORPHINE ☐ INVALID RESULT

REMARKS _____

TEST LAB (if different from above) _____

I certify that the specimen identified on this form was examined upon receipt, handled using chain of custody procedures, analyzed, and reported in accordance with applicable Federal requirements.

X _____ Signature of Certifying Scientist

_____ (PRINT) Certifying Scientist's Name (First, MI, Last)

/ / Date (Mo./Day/Yr.)

STEP 5b: SPLIT SPECIMEN TEST RESULTS - (IF TESTED) COMPLETED BY SECONDARY LABORATORY

_____ Laboratory Name

_____ Laboratory Address

☐ RECONFIRMED ☐ FAILED TO RECONFIRM - REASON _____

I certify that the split specimen identified on this form was examined upon receipt, handled using chain of custody procedures, analyzed, and reported in accordance with applicable Federal requirements.

X _____ Signature of Certifying Scientist

_____ (PRINT) Certifying Scientist's Name (First, MI, Last)

/ / Date (Mo./Day/Yr.)

PEEL

1234567 A SPECIMEN ID NO.

PLACE OVER CAP

1234567 SPECIMEN BOTTLE SEAL

/ / Date (Mo. Day Yr.)

_____ Donor's Initials

PEEL

1234567 B (SPLIT) SPECIMEN ID NO.

PLACE OVER CAP

1234567 SPECIMEN BOTTLE SEAL

/ / Date (Mo. Day Yr.)

_____ Donor's Initials

COPY 1 - LABORATORY

PRESS HARD - YOU ARE MAKING MULTIPLE COPIES

0000-0000-0225

Drug Form Part 1
Face Inks: 000 BLK/000 RED
Date: 05/09/00
Not To Use For Colormatch
Follow PMS Guide For Colors

Figure 3.1 Federal drug testing custody and control form.

- Copy 1 includes five sections:
  - Information on the company requesting the test, the name, etc., of the MRO, the donor's social security or identification number (in the regulated programmes the donor's name cannot be used to identify the donor on Copy 1), the reason for testing and the drugs to be tested
  - Information on the collector and the collection (noting the temperature and any remarks concerning the collection itself)
  - Chain-of-custody information to be completed by the collector
  - Documentation of receipt at the laboratory
  - Certification of laboratory test results and analysis of split samples (if performed).
- Copies 2–4 carry essentially the same information, except that Copy 2 includes a section for the MRO to complete after receiving results from the laboratory and additional information on the donor (name and contact information).

Most laboratories have incorporated barcode bottle seals attached to the bottom of Copy 1. These are the seals used in the collection process and the barcode number on these matches that on the chain-of-custody form.

Within this process there are obvious opportunities for inadvertent errors (e.g. failure of the collector to complete all the necessary parts of Steps 2–4). Over time, the DOT has defined a number of so-called fatal errors (which if present require the testing of the specimen to be cancelled) and a number of correctable errors, which require the laboratory or the MRO to obtain a statement of correction from the collector. The 'fatal errors' are as follows:

- The barcode numbers on the bottle seals and the chain-of-custody form do not match.
- The collector's printed name and signature are both missing from Step 4.
- Bottle A's seal is broken, absent or shows obvious signs of tampering.
- Bottle A contains less than 30 mL of urine.

The correctable flaws are:

- The collector's signature is omitted.
- There is no donor's signature and there is no remark explaining why the donor failed to sign.
- The Certifying Scientist's signature is missing from a non-negative specimen.
- A non-DOT chain-of-custody form has been used for a DOT-mandated test.

The MRO is required to cancel the test when a statement of correction cannot be obtained. Although the steps outlined above are those required for a regulated specimen collection, similar procedures are used outside the regulated industries. A number of laboratories have simpler chain-of-custody procedures, which resemble requisitions used in medical testing, but the confirmation of identity, preparation of the collection area, completion of the chain-of-custody form and sealing of the specimen bottles in the presence of the donor, and, increasingly, the collection of split specimens are normal practices today.

Almost all drug testing programmes in the USA have provisions for 'shy bladder syndrome'. In this situation the donor fails to provide an adequate urine volume after remaining at the collection site for up to 3 h and being provided with 1.25 L of liquid. The donor is deemed to have a 'shy bladder' and is required to undergo a medical examination. If there is a reasonable medical explanation, the test is cancelled. If the donor requires a negative test for employment, specimens other than urine may be used, for example hair or oral fluid.

In the regulated industries, observed collections have to be performed by same-gender collectors and can currently be performed only in five special cases:

1. When the specimen temperature is out of range at the collection site. In this case there has to be an immediate observed collection.
2. Where the collector has identified an apparent tampering with the specimen at the collection site, for example the addition of bleach to the urine. Again, there has to be an immediate observed collection.
3. If the previous specimen has been declared invalid by the laboratory and when there is no obvious medical explanation for this. The most obvious example of this is where the laboratory has proof of adulteration, but cannot specifically identify the adulterant.
4. When the MRO has cancelled the test because the bottle B specimen was unavailable or had been adulterated and was so identified by the laboratory performing the re-confirmation.
5. In return-to-work and follow-up tests, for which the employer can, in certain circumstances, decide to conduct observed collections.

These general guidelines are also followed outside the regulated industries, although some employers and some sectors may have more rigorous ones. For example, the US military requires observed collections for all specimens.

Despite these safeguards, donors are still able to adulterate urine specimens by adding oxidising agents after voiding. The amount of such material added is extremely small (a vial of it can be hidden easily in a shoe or sock) and, if it is liquid, the volume is insufficient to alter the temperature of the specimen. Alternative specimens to urine have advantages in that their collection can be considered non-invasive and can be observed. For example, collection of oral fluid is usually performed using a pad or swab and the donor can do this themselves in the presence of the collector. Collection of hair is also 'observed', although some questions remain as to the invasive nature of this process, particularly when non-head hair has to be collected.

Role of the medical review officer

The concept of the MRO was introduced in the DHHS *Guidelines* (Federal Register 1988a). The MRO's initial role was to receive the testing results from the laboratory, contact the donor to determine whether a positive drug result could have arisen from the legal use of a drug and, if not, to verify the result as positive and contact the employer. Where the legal use of a medication explained the result, the MRO would report the result as negative and communicate this to the employer.

Increasingly, MROs serve as gatekeepers for drug-testing results and as administrators of functions ancillary to the process, such as the collection and storage of all documentation. Given the breadth of these tasks and the extent of drug testing in the USA, some physicians practise as MROs full time. Many others act as MROs in the course of their duties as occupational health physicians and corporate medical directors.

Before the introduction of the federal programmes, the laboratory toxicologists had filled the interpretative role, and few issues had arisen. However, within the federal programmes, which introduced random and post-accident testing to a large percentage of the workforce, there was a need for additional safeguards. It was anticipated that MROs would serve as the final quality-assurance check on the laboratory result and, indeed, they have done so. For example, some vigilant MROs who could not rationalise the laboratory result with the donor's demographics and medical history first raised the phenomenon of the conversion of ephedrine and pseudoephedrine to metamfetamine. They questioned the findings and requested the re-tests in which the second laboratory could not confirm the findings. Another reason for their introduction was a purely legal one; in the USA only licensed physicians can access prescription records, and it was obvious that this could be essential to determine the legitimacy of a donor's claim.

When the MRO receives a positive result, he or she has to contact and interview the donor, obtain prescription records (if necessary) and then verify the laboratory result as positive or negative before reporting the result to the employer. These reviews are generally straightforward; for example, there is no legal reason for a donor to test positive for PCP, whereas a large majority of specimens that test positive for codeine do so because of prescription use or, in countries such as the UK, because of the extensive use of codeine in over-the-counter medicines. Questions arise in other areas, particularly in the interpretation of metamfetamine and morphine positives; these are discussed in more detail later in the chapter.

The MRO is also responsible for reviewing non-negative results from donors. These include adulterated, substituted and invalid specimens.

Before the new *CFR Part 40* (Federal Register 2000) was issued, MROs had very little role in the review of substituted and adulterated specimens; they simply received the results from the laboratory, reviewed the paperwork and reported them to the employer. There was no requirement to contact the donor and no ability for the donor to request a re-test. The new *CFR Part 40* changed that and incorporated a requirement for the MRO to contact the donor to determine whether there was a medical reason for the laboratory findings, although this would be extremely unlikely, and allowed the donor to request a re-test. These particular re-tests were to be performed using the same criteria for defining a specimen as substituted or adulterated as used in the initial set of tests. For example, if a specimen was determined to be substituted with creatinine readings of 1.8 and 1.7 mg/dL, with relative density readings of 1.0008 on bottle A, but bottle B had a creatinine reading of 2.1 mg/mL and a relative density of 1.0008, the re-test specimen would be reported as 'Failed to Confirm: Substituted Specimen'. Some of the interpretative issues relevant to adulterated and substituted specimens are discussed later in the chapter.

Interpretation of amfetamine-positive results

This particular drug-testing result has caused confusion among the MRO community and continues to do so. Some of this stems from the reporting rule for metamfetamine. This rule requires that for metamfetamine to be reported as positive there must be at least 200 ng/mL of amfetamine present, in addition to at least 500 ng/mL of metamfetamine. If both amines are present in concentrations greater than 500 ng/mL by GC-MS, both are reported as positive and there is no confusion. Difficulty arises when the amfetamine concentration determined by the laboratory is between 200 and 500 ng/mL (i.e. less than the amfetamine confirmation cut-off) but the metamfetamine concentration is above 500 ng/mL. In this situation, the MRO receives only a metamfetamine-positive result, rather than one that indicates that both metamfetamine and amfetamine are positive. Once the MRO understands that the laboratory must comply with this reporting rule, the confusion disappears.

The second area of confusion is in the differentiation of *d*- and *l*-metamfetamine. *l*-Metamfetamine is found in the USA in Vicks Inhaler (an over-the-counter decongestant product) and, although its use can result in the detection of metamfetamine and amfetamine in urine, the concentrations are generally low (Fitzgerald *et al.* 1988). Moreover, given the low cross-reactivities of immunoassays to the *l*-isomers of metamfetamine and amfetamine, a positive result is unlikely following normal use. However, a metamfetamine user may still claim that the positive is because of his or her use of Vicks Inhaler and, in such cases, the MRO can request a GC-MS separation of the isomers. These are usually qualitative analyses and reported as *x*% of the *d*-isomer and *y*% of the *l*-isomer. Where the *l*-isomer is greater than 80%, the MRO reports the result as negative.

Although some drugs can metabolise to metamfetamine and amfetamine (e.g. benzphetamine and selegiline), these are available only on prescription in the USA and the interpretation of positive amfetamine results is relatively straightforward. In other countries, this may not be the case. Cody (2002) has reviewed the issue of precursor medications.

Interpretation of opiate-positive results

This is another area that has caused confusion in the MRO community. From 1989 to 1998 the cut-off used for the opiates was 300 ng/mL, both in the initial testing and in the confirmation of codeine and morphine by GC-MS. During this period there was no requirement that a certified laboratory should be able to confirm the presence of the characteristic metabolite of heroin, 6-AM, although several did so routinely. In 1998 the cut-offs were raised to 2000 ng/mL and the laboratories were required to confirm the presence of 6-AM if the morphine concentration exceeded 2000 ng/mL. The GC-MS cut-off for 6-AM was set at 10 ng/mL. The major reason for raising the opiate cut-offs was associated with MRO reviews of opiate-positive samples. Use of the 300 ng/mL cut-off criterion resulted in a large number of positive results that derived from either poppy-seed ingestion or therapeutic doses of codeine, and which were subsequently verified as negative by the MROs.

Where there was a prescription for codeine, the MRO's review presented no problems. However, in the absence of a prescription for codeine (or morphine) the MRO was required to observe 'clinical signs of opiate use' before reporting a verified opiate-positive result back to the employer. This requirement remains for some results, even within the new *CFR Part 40*, unless the specimen has tested positive for 6-AM, which is conclusive evidence of heroin use.

Under the new *CFR Part 40*, if the morphine concentration is greater than 15 000 ng/mL the donor has the responsibility for providing evidence that the presence of morphine was the result of a legitimate use of a drug, and not the illicit use of heroin or morphine. There are certainly no reports that indicate such urinary morphine concentrations from poppy-seed ingestion, even under extreme conditions (Selavka 1991), so if the donor cannot provide this evidence the MRO verifies the result as a positive.

Passive exposure

Passive exposure is an issue that still causes confusion to some MROs; it essentially deals with the passive inhalation of cannabis smoke and the unwitting ingestion of cocaine or cannabis. Although passive exposure to crack (free-base cocaine) smoke has been claimed as an excuse for a positive urine benzoylecgonine, there is no evidence that this is valid. In two studies that investigated this (Baselt *et al.* 1991; Cone *et al.* 1995), no urine specimens gave immunoassay readings above 300 ng/mL.

However, unwitting ingestion of cocaine may result in positive urine specimens with the cut-offs currently used. There is no doubt that an individual who ingests a drink fortified with cocaine can test positive. In one study in which a volunteer consumed 25 mg cocaine hydrochloride (Baselt, Chang 1987), urine benzoylecgonine concentrations were greater than 300 ng/mL for 48 h. The question most often raised is the use of decocainised coca teas. As with decaffeinated coffee, these teas contain small amounts of cocaine; one study found an average of 4.8 mg per tea bag (Jackson *et al.* 1991). After the ingestion of one cup of this tea, immunoassay results were positive for 21–26 h. Obviously, the more regular the use, the more likely these specimens are to be positive and to be so for longer periods. From the MRO's viewpoint, these are not legitimate uses of cocaine and in the regulated industries would be validated as positives. Outside these industries, MROs may be more flexible in their interpretation after consulting with the donor.

Passive inhalation of cannabis smoke continues to be an excuse offered by donors for their positive results. There is no doubt that environmental exposure to cannabis smoke can occur through passive inhalation, but the question is whether it produces measurable concentrations in urine and if so for what time. In a number of early studies (Law *et al.* 1984; Mørland *et al.* 1985; Perez-Reyes *et al.* 1982) there was evidence for the presence of THCA in urine after passive exposure, but the concentrations measured were orders of magnitude lower than the cut-offs in use in workplace drug testing. In a more extreme study by Cone *et al.* (1987), volunteers were exposed to 4 and 16 cannabis cigarettes for 1 h for 6 consecutive days. After exposure to 4 cigarettes few urine specimens were positive, while after 16 cigarettes many more specimens were positive with a maximum THCA concentration of 87 ng/mL reported by GC-MS. The authors stressed, however, that these were extreme conditions, in that exposure was in a non-ventilated, tightly sealed small room and that it occurred for multiple days. In fact, the smoke was of such intensity that goggles were required by a number of the volunteers. It is generally accepted that social exposure to cannabis smoke (at parties, in outdoor arenas) will not result in a positive urine specimen.

Interpretation of adulterated and substituted specimens

Nearly all the adulterants that are commonly used today to oxidise THCA are listed in the Federal Register (2004a) and the provision

for new adulterants. Nitrite can be detected in urine as a result of bacterial infection, the ingestion of certain foodstuffs and the use of some medications. In one study that considered these sources and the presence of nitrite in normal urine (Urry *et al.* 1998), the highest urinary concentration was approximately 130 μg/mL; the cut-off used today is 500 μg/mL, almost four times this concentration. Some of the other adulterants include chromium(VI), peroxide, iodine and bleach; none of these should be detected as a result of normal physiology and metabolism. However, a donor may claim that their presence is the result of workplace exposure or the ingestion of vitamins or herbal material, and the MRO needs to be prepared to refute these claims.

The interpretation of substituted results has generated considerable discussion, with some individuals arguing that urine specimens with creatinine concentrations less than 5 mg/dL can be provided under rare conditions. Barbanel *et al.* (2002) reviewed over 800 000 urine specimens. In this population both creatinine and relative density measurements were taken from over 13 000 specimens, and none of these satisfied the criteria for a substituted specimen. These authors also examined the medical records of patients who satisfied one of the criteria and reported that these were neonatal, moribund or so severely ill that essentially none could have been in the working population.

The DOT has also provided further information (Edgell *et al.* 2002). In a study in which 12 volunteers (5 men and 7 women) consumed 2.5 L of water over a 6-h period and urine specimens were collected for the measurement of creatinine and specific gravity, none of the 500 specimens collected was identified as substituted using the criteria.

In 2003 at a conference organised by the Federal Aviation Authority, information was presented that indicated that a small number of female flight attendants produce urine with creatinine readings between 4 and 5 mg/dL and this led to the introduction of the updated DHHS and DOT rules.

Conclusion

This chapter focuses on the DHHS *Guidelines* and the DOT *Regulations*, as these are used to test almost 25% of specimens and have become the standard of care for the industry in the USA. Without question they are detailed, and some toxicologists may argue that, in certain aspects, they are unreasonable. However, their introduction and evolution through the NLCP have resulted in a dramatic improvement in the quality of drug testing carried out, including the quality of the laboratory testing. The future challenge for all involved is to balance the desire to proscribe procedures against the need to allow laboratories to select their own technology, procedures and quality-control programmes. The expansion of the programme into the testing of alternative specimens and into specimen validity analyses will be a test of this challenge.

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CHAPTER

4 Driving Under the Influence of Alcohol

AW Jones

Introduction

Historical background

An editorial published in the July 1904 issue of the *Quarterly Journal of Inebriety* drew attention to the potential danger of driving an 'automobile wagon' after consumption of alcohol (Editorial 1904). Although nothing was mentioned about the amounts of alcohol consumed before driving, this editorial gave an early warning signal that driving after drinking was a hazardous business. Scores of experimental and epidemiological studies have subsequently appeared providing unequivocal evidence of the role played by alcohol abuse and alcohol-related impairment in road traffic crashes. Statistics from many nations confirm that 20–50% of all fatal crashes involve an alcohol-impaired driver (Evans 1990; Kennedy *et al.* 1996; Logan, Schwilke 1996; Zador *et al.* 2000). Indeed, alcohol consumption represents a well-known risk factor in all kinds of transportation accidents, including boating (Smith *et al.* 2001), bicycling (Li *et al.* 2001), pedestrians (Margolis *et al.* 2000) and use of snowmobiles (Ostrom, Eriksson 2002).

Drinking driving legislation

The UK Licensing Act of 1872 made it an offence to be 'drunk in charge on any highway or other public place of any carriage, horse, cattle or steam engine'. It took until 1925 before the offence was extended to cover 'any mechanically propelled vehicle'. The evidence needed to charge a person with driving under the influence of alcohol (DUIA) depended to a large extent on the testimony of an eye-witness, the arresting police officer and in particular the results of a clinical examination of the suspect (Wayne 1965).

Alcohol and impaired driving laws were initially not very effective because they contained wording such as 'drunk and incapable', which was not easy to prove in a court of law. This was later changed to 'being under the influence of drink or a drug to such an extent as to be incapable of having proper control of a vehicle'. Successful prosecution for DUIA was not always easy to obtain and much depended on the conclusions of the clinical test made by a physician or police surgeon. During this examination, questions were asked about recent use of alcohol and/or medication and orientation in time and place, and simple cognitive and psychomotor tests were made to assess performance.

Proof beyond a reasonable doubt that a person was unfit to drive through drink or drugs was not always readily available, which meant that many suspects were acquitted if the case went to trial. The concentration of alcohol in a specimen of blood or urine from the accused was not given sufficient weight by the courts. The blood alcohol concentration (BAC) served only as supporting evidence of alcohol influence. All this changed after the British Road Traffic Act of 1967, which stipulated a punishable limit of alcohol concentration in blood (80 mg/100 mL) and urine (107 mg/100 mL), above which it was an offence to drive or to be in charge of a motor vehicle on the public roads.

When prohibition was abolished in the USA in 1933, attention was drawn to the problem of alcohol consumption and transportation safety. Driving while intoxicated (DWI) was thought to pose a serious public health problem because the ownership and use of motor vehicles had increased appreciably during a decade of prohibition. Representatives of the US federal government (Department of Justice, Bureau of Prohibition) wrote to the leading researcher in medical–legal aspects of alcohol, Professor Erik MP Widmark at the University of Lund in Sweden, for advice (Andreasson, Jones 1996a). The US Department of Justice was anxious to find a more objective and scientific way to test for drunkenness. They described the situation as follows:

> Such tests in our various states vary from smelling the offender's breath to making him walk a chalk line, but no scientific test apparently is applied. We are particularly anxious to know what the alcoholic content of the blood must be before a person can be described as being under the influence of alcohol. Some median line must have been established on one side of which an offender is not under the influence of liquor and on the other side of which he may be said to be intoxicated. Just what that line is, we would like to know.

The concentration of alcohol determined in a specimen of blood was considered a more objective way to establish whether a person was under the influence of alcohol. Widmark (1889–1945) had already developed a method to measure the concentration of alcohol in a small volume (0.1 mL) of fingertip blood (Widmark 1922). He also showed that a person's BAC could be translated into the amount of alcohol consumed, which often made more sense to a judge and jury than the BAC itself (Widmark 1941).

Finding a high concentration of alcohol in blood or urine meant that the person's unusual behaviour could be attributed to heavy drinking rather than a medical condition or injury that might mimic the signs and symptoms of drunkenness (Smith 1960). Injuries sustained in a car crash, such as skull trauma, might account for the person's unusual or irrational behaviour and be mistakenly confused with overconsumption of alcohol. Low blood sugar (hypoglycaemia) causes cognitive and behavioural impairment resembling that seen after acute alcohol intoxication (Saunders 1992; Service 1995). Knowledge of whether an apprehended driver suffered from type 1 or type 2 diabetes and might recently have taken too much insulin is obviously important (Daneman 2006). People with neurological disorders, such as epilepsy or Parkinson's disease, show hand tremor and ataxia, which might be confused with the influence of alcohol. A person's strange or erratic behaviour might be attributed to a psychiatric problem, such as mania or schizophrenia, and have nothing to do with drink or drugs.

Alcohol and traffic safety literature

Much has been written on the subject of traffic safety and the role played by overconsumption of alcohol as well as other psychoactive substances. Major developments in the field of alcohol and traffic including legislation, enforcement strategies, epidemiology, crash statistics, countermeasures, police procedures, as well analysis of alcohol in body fluids can be gleaned from the proceedings of 18 international conferences devoted to the subject. The first meeting was held in Stockholm in 1950 and the nineteenth was in Oslo in 2010. The proceedings from the most recent meetings (since 2000) are available on CDs and many of the articles can be downloaded as pdf documents from the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) website, which can be located

at the address www.icadts.com. Another useful website for information and publications about alcohol and traffic safety is that of the US National Highway Traffic Safety Administration (NHTSA), which contains a treasure trove of information on the subject of alcohol, drugs and driving. Scores of reports, monographs and brochures dealing with all aspects of traffic safety can be downloaded gratis as pdf documents from NHTSA's website www.nhtsa.dot.gov.

Articles and reports on the subject of alcohol and alcohol-related impairment appear in a wide variety of scientific journals devoted to legal medicine and toxicology as well as those specialising in biomedical alcohol research and substance abuse. Some scientific journals that focus specifically on aspects of transportation safety including human factors are listed under Further reading at the end of the chapter.

Statutory alcohol limits for driving

Different countries have their own structure and traditions when it comes to dealing with the problem of alcohol and road traffic safety. This is reflected in, among other things, the setting of statutory alcohol limits for driving, the methods used to enforce the law and the sanctions imposed for people convicted of this crime. Statistics about road traffic fatalities, the attitude of the media and the public towards drinking and driving, the activity of temperance movements and various political influences, as well as the way the courts interpret the law, are also important considerations. Other factors that deserve attention in connection with DUIA are the relative cost and availability of alcoholic beverages, opening hours of bars and restaurants, and the resources available to the police for apprehending and prosecuting offenders. The punishable alcohol limits for driving seem to depend as much on politics as on traffic safety research. The BAC limit chosen needs to balance the traffic safety hazard posed by alcohol-impaired drivers, the methods available for enforcement and public acceptability.

Blood alcohol concentration limits

In 1936 Norway introduced a statutory BAC limit for driving of 0.50 mg/g blood (~50 mg/100 mL), which is now set at 0.20 mg/g (~20 mg/100 mL). This was followed by Sweden, where in 1941 a BAC limit of 0.80 mg/g (~80 mg/100 mL) was introduced as well as a more serious offence, which carried enhanced penalties above a BAC of 1.50 mg/g (~150 mg/100 mL). These alcohol limits were subsequently lowered to 0.50 mg/g (~50 mg/100 mL) in 1956 and 0.20 mg/g (~20 mg/100 mL) in 1990. The upper limit representing aggravated drunken driving is now 1.0 mg/g (since 1994) and more severe sanctions are imposed for those convicted. Most EU nations, apart from the UK and the Republic of Ireland, enforce a punishable BAC limit of 50 mg/100 mL and operationally also define higher limits above which there are enhanced penalties.

Lowering the statutory alcohol limit for driving does not solve the problem of DUIA although this might help to draw attention to the problem and hopefully have a pedagogic influence on young people learning to drive. Besides lowering BAC limits, a more effective law enforcement will require that the police be allowed to conduct random alcohol testing of drivers, otherwise limits of 20 or 50 mg/100 mL will be difficult to enforce.

After the British Road Safety Act of 1967, a police officer in uniform was able to stop a driver and request a roadside breath alcohol test if (a) there was a reasonable cause to believe the driver had been drinking alcohol, (b) the driver had committed a moving traffic offence or (c) the vehicle concerned was involved in a collision. Obtaining a positive roadside breath alcohol test meant that the suspect was arrested and obliged to provide a specimen of blood or urine for determination of the ethanol content. The concentration of alcohol in the blood or urine became the sole deciding factor for a successful prosecution after the 1967 Act of Parliament.

In the early 1970s, a committee within the UK Department of the Environment was commissioned to review the situation after the passing of the 1967 Road Traffic Act and also to recommend improvements (Blennerhassett 1976). Among other things, this committee felt that the science and technology of breath alcohol analysis had advanced to such an extent that this test could be made at a police station and the results subsequently used as evidence for prosecution. Sidestepping the need to obtain specimens of blood or urine would therefore simplify and speed up police procedures. Obtaining immediate results to confront the suspect that he or she was in breach of the law was another advantage of evidential breath alcohol testing. Hitherto, breath alcohol tests had only been used for orientation purposes, as a preliminary screening test, and positive results always had to be followed up by blood or urine samples analysed at a forensic laboratory, which took more time.

Breath alcohol concentration (BrAC) limits

Because statutory limits of BAC existed in most countries before instruments suitable for breath alcohol analysis were developed, it became the standard practice to convert the suspect's BrAC into the coexisting BAC by means of a calibration factor (Jones 1996). This factor was known as the blood-to-breath ratio of alcohol (BBR) and had been derived empirically from experiments in which volunteers had consumed known amounts of alcohol. The results of many such blood–breath correlation studies pointed to a mean BBR of 2100 : 1 and this factor was used for law enforcement purposes in the USA and Canada (Jones 2000b).

The Breathalyzer instrument was developed in the mid-1950s and was widely used for testing drunken drivers throughout the USA and Canada (Borkenstein, Smith 1961). Although the Breathalyzer instrument was calibrated on the assumption of a 2100 : 1 BBR, evidence was mounting to show that this factor varied both between and within individuals and was closer to 2300 : 1 than to 2100 : 1(Begg *et al.* 1964; Wright *et al.* 1975; Emerson *et al.* 1980). Nevertheless, use of a BBR of 2100 rather than 2300 : 1 meant that the results of a breath alcohol test were to the benefit of the suspect because the venous BAC was underestimated by about 10%. This was deemed acceptable for law enforcement purposes because it gave a suspect the benefit of the doubt.

The need to convert BrAC into BAC was, however, becoming increasingly troublesome as more and more studies showed that the BBR varied as a function of time after the end of drinking (Jones 1978). This problem was solved by introducing a statutory definition of the concentration of alcohol in a specimen of breath as evidence of DUIA. In the USA this threshold was set at 0.08 g/210 L breath, which was equivalent to 0.08 g/100 mL blood (Mason, Dubowski 1976). When European countries began using evidential breath alcohol instruments in the 1980s, there was a lack of consensus about the BBR and values ranging from 2000 : 1 to 2400 : 1 were adopted when the threshold BrAC were set according to

$$\text{Statutory BrAC} = (\text{existing BAC})/\text{BBR}$$

The British Road Transport Act of 1981 stipulated alcohol concentrations of 35 µg/100 mL breath, 80 mg/100 mL blood or 107 mg/100 mL urine (Cobb, Dabbs 1985). This implies a BBR of about 2300 : 1 ([80/35] × 1000 = 2286 : 1, which rounds to 2300 : 1). The Republic of Ireland, the Netherlands and Belgium adopted the same 2300 : 1 ratio when their threshold BrAC limits were introduced.

Other European nations, such as France and Spain, assumed a BBR of 2000 : 1, which gives an advantage to the suspect if the mean BBR in reality is closer to 2300 : 1. In Germany and the Nordic countries, the BAC for legal purposes is reported as mass/mass units (e.g. 0.50 mg/g), which is the same as 0.527 mg/mL (density of blood = 1.055 g/mL). Dividing 0.527 mg/mL by 2100 (BAC/BrAC ratio) gives a threshold BrAC of 0.25 mg/L ([0.50/2100] × 1000 = 0.25 mg/L). A wide variety of concentration units are used to report BAC and BrAC for legal purposes as well as BBR ratios, which is the cause of confusion when alcohol and traffic safety statistics are compared between countries (Jones 1992a).

Table 4.1 lists the statutory limits of alcohol concentration in blood and breath for driving in various countries, the operational BBR, the name of the breath analyser used for evidential purposes and the analytical principle on which the instrument operates (Harding, Zettl 2008; Jones 2010a).

Table 4.1 Statutory BAC and BrAC limits in various countries, the blood/breath ratio (BBR) that operates, the evidential breath alcohol instrument approved and the principle for analysis of ethanol

Country	*Statutory BAC*[a]	*Statutory BrAC*[a]	*BBR*	*Evidential breath alcohol analyser*[b]	*Operating principle*[c]
Australia	0.05 g/100 mL	0.05 g/100 mL[d]	2100	Alcotest 7110	Infrared (9.5 μm) and EC
				DataMaster[e]	Infrared 3.4 μm
Austria	0.50 g/L	0.25 mg/L	2000	Alcotest 7110	Infrared (9.5 μm) and EC
Belgium	0.50 g/L	0.22 mg/L	2272	Ethylometer	Infrared 9.5 μm
				Alcotest 7110	Infrared 3.4 μm and EC
				Alcotest 8510	Infrared 9.5 μm
Canada	80 mg/100 mL	80 mg/100 mL[d]	2100	Intoxilyzer 5000C	Infrared 3.4 μm
				Intoxilyzer 8000C	Infrared 9.5 μm
				DataMaster C[b]	Infrared 3.4 μm
Denmark	0.50 mg/g	0.25 mg/L	2100	Not yet decided	Probably infrared
Finland	0.50 mg/g	0.22 mg/L	2400	Alcotest 7110	Infrared and EC
France	0.50 g/L	0.25 mg/L	2000	Ethylometer	Infrared 9.5 μm
Germany	0.50 mg/g	0.25 mg/L	2100	Alcotest 7110	Infrared 9.5 μm and EC
Greece	0.50 mg/mL	0.25 mg/L	2000	Alcolmeter SD-400	EC
Iceland	0.50 mg/g	0.22 mg/L	2000	Intoxilyzer 5000	Infrared 3.4 μm
Italy	0.50 g/L	0.50 g/L[c]	2000	Alcotest 7110	Infrared (9.5 μm) and EC
				Intoxilyzer 8000C	Infrared 9.5 μm
				Ethylometer	Infrared 9.5 μm
New Zealand	80 mg/100 mL	400 μg/L	2300	Ethylometer	Infrared 9.5 μm
				Intoxilyzer 5000	Infrared 3.4 μm
Norway	0.50 mg/g	0.25 mg/L	2100	Intoxilyzer 5000N	Infrared 3.4 μm
Poland	0.20 g/L	0.10 mg/L	2000	AlcoSensor IV	EC
				Alcotest 7410	EC
Portugal	0.50 g/L	0.50 g/L[c]	2300	Alcotest 7110	Infrared (9.5 μm) and EC
Republic of Ireland	80 mg/100 mL	35 μg/dL	2300	Intoxilyzer 6000	Infrared 3.4 μm
Spain	0.50 g/L	0.25 mg/L	2000	Alcotest 7110	Infrared (9.5 μm) and EC
Sweden	0.20 mg/g	0.10 mg/L	2100	Evidenzer	Infrared 3.4 μm
Netherlands	0.50 mg/mL	220 μg/L	2300	DataMaster[e]	Infrared 3.4 μm
United Kingdom	80 mg/100 mL	35 μg/100 mL	2300	Intoxilyzer EC-IR	Infrared 3.4 μm
				Intoxilyzer 6000	Infrared 3.4 μm
United States of America	0.08 g/100 mL	0.08 g/210 L	2100	Intoxilyzer 8000	Infrared 9.5 μm
				Intoximeter EC-IR	EC and Infrared
				DataMaster[e]	Infrared (3.4 μm)
				Alcotest 7110	Infrared (9.5 μm) and EC

[a]Most countries also enforce a higher limit defining a more serious offence carrying enhanced penalties.
[b]Instruments carry a country code, such as Intoxilyzer 6000 Ire for Ireland or N for Norway.
[c]Those infrared instruments operating at 3.4 μm are equipped with several wavelength filters to give enhanced selectivity.
[d]In these countries, ethanol analysed in the breath is reported as BAC by pre-calibration with an assumed BBR.
[e]Also known as BAC DataMaster.
BAC, blood alcohol concentration; BrAC, breath alcohol concentration; EC, electrochemical.

Apprehending offenders

The first suspicion of driving under the influence of alcohol or drugs usually arises from observations made about the suspect's driving by the police authorities. The vehicle is then stopped and contact is made with the driver. The smell of alcohol on the breath and the person's general appearance and behaviour are among the first things that arouse suspicion that the driver might be under the influence of alcohol (Moskowitz *et al.* 1999). Next a roadside breath alcohol test is conducted and, if the result is above the legal limit for driving, the suspect is arrested and transported to a police station or other location where an evidential breath alcohol instrument is available.

Under some circumstances, a physician is called to examine the driver and to determine the degree of alcohol influence or whether other drugs might have been involved (Penttila, Tenhu 1976). This is followed by sampling blood for analysis of alcohol and even other drugs that might be present. The results from the clinical examination formed the main thrust of the prosecution case before the introduction of laws on alcohol concentration itself (Jones 1988). The clinical examination is still important in some countries to furnish evidence of impairment caused by drugs other than alcohol (Bramness *et al.* 2003).

Figure 4.1 presents a flow diagram showing the events and tests made during a traffic stop and suspected DUIA. A negative roadside breath alcohol test might mean that the driver is allowed to continue to drive or instead questioned further if evidence exists that drugs other than alcohol were present. Many countries now enforce zero-tolerance laws for driving after use of illicit drugs and toxicological analysis of the concentration in a blood sample is the key piece of evidence in such cases (Jones 2005).

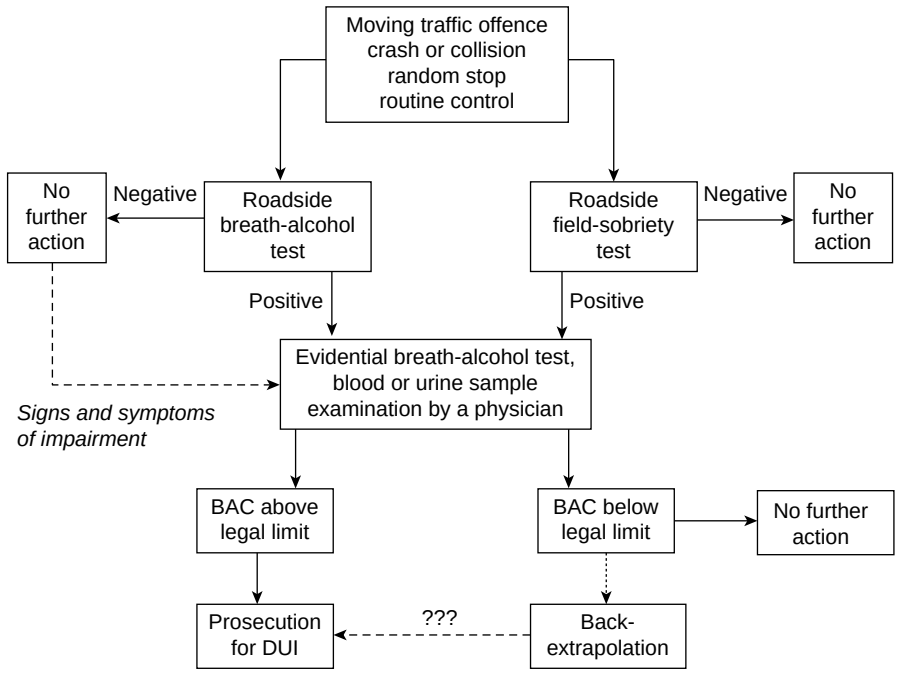

Figure 4.1 Flow chart of the events leading to the apprehension, testing and arrest of alcohol- and drug-impaired drivers.

Depending on how the law is written, the prosecution might want to know the BAC or BrAC that existed at the time of driving and not at the time of sampling, which is usually 30–120 min later (Montgomery, Reasor 1992; Anderson 2005). This requires back-calculating the BAC to compensate for metabolism occurring between time of driving and time of sampling. Such an exercise is controversial, especially if the BAC was below the legal limit at the time the blood was sampled (Ferner 1996). Expert evidence is necessary whenever a back-extrapolation is made and the court needs to be informed about the many variable factors and assumptions made (Lewis 1987; Gullberg 1994).

Effects of alcohol on performance and behaviour

Ethanol-induced impairment

The signs and symptoms of drunkenness are common knowledge, although the mechanisms by which ethanol exerts its effect on the brain are less well known. Ethanol acts as a depressant of the central nervous system and its effects are felt almost immediately after drinking begins, which testifies to rapid uptake from the gut, transport to the brain and passage through the blood–brain barrier. Different areas of the brain react differently to alcohol depending on the amounts ingested, the speed of absorption and the concentration gradient in the various brain regions.

The cerebral cortex is influenced at relatively low concentrations of alcohol in the blood, as seen by altered thought processes and the loss of inhibitions and self-control. The limbic system (hippocampus) is the area of the brain that controls memory and emotions, which succumb as the BAC increases further. The cerebellum is the brain region important for muscular coordination and fine manual skills and excess alcohol alters a person's posture, perception and articulation. The hypothalamus controls, among other things, the hormones of the pituitary gland, including vasopressin (antidiuretic hormone), the release of which is inhibited by alcohol, as seen by an increase in the production of urine after drinking. On reaching a very high BAC, the medulla (brainstem) suffers from alcohol narcosis, which impacts on heart rate and body temperature drops and breathing becomes more difficult. The impairment of such vital body functions often results in unconsciousness or alcoholic coma with circulatory collapse and a grave risk of death from asphyxia.

Molecules of ethanol interact with various brain receptors and neurotransmitter systems in a similar way to the general anaesthetic gases (Eckardt *et al.* 1998). Examples of drugs that share some of the same receptor sites as ethanol are sedative hypnotics, such as benzodiazepines or barbiturates. These depressant drugs act as agonists for the major inhibitory neurotransmitter γ-aminobutyric acid type A ($GABA_A$, De Vries *et al.* 1987). $GABA_A$ is a so-called ligand-gated ion-channel and its configuration in the cell membrane is altered when an agonist binds (Ulrichsen *et al.* 1988). Binding to the receptor opens an ion channel, facilitating the flow of chloride ions into the cell, which damps or stops the firing of adjacent neurons (Wallner *et al.* 2006). This suggests that impairment of body functioning is worsened if a person combines alcohol with a sedative drug, such as diazepam or some other depressant; such drug–alcohol interactions are sometimes life threatening. Even therapeutic doses of diazepam impair a person's ability to perform skilled tasks such as driving, the effect being exaggerated after consumption of small amounts of ethanol (Kerr *et al.* 1991).

On reaching a BAC of 20–30 mg/100 mL, the individual usually becomes less inhibited and more talkative and relaxed, and experiences a mild euphoria. As more alcohol is consumed, and higher BACs are reached (50–80 mg/100 mL), the ethanol-induced effects on performance and behaviour become more obvious. A person's self-control and judgement are impaired, which means that people are more likely to take risks. As the BAC reaches and exceeds 100 mg/100 mL, the classic signs of drunkenness are seen, including slurred speech, unsteady gait and slower reaction times.

The speed of onset and intensity of the effects of ethanol depend to a large extent on the quantities consumed, whether it is in the form of beer or spirits, the speed of drinking, and also the rate of gastric emptying. The individual's previous experience with alcohol and the development of tolerance and degree of habituation are also important points to consider when the effects of a given BAC are interpreted (Kalant 1998). After a single acute dose of ethanol, the behavioural impairment is greater at a given BAC on the ascending limb of the BAC curve compared with the same BAC on the descending limb several hours later (Martin, Earleywine 1990; Earleywine, Martin 1993; Martin, Moss 1993). The brain can seemingly adapt to the impairment effects of alcohol during a single exposure; this phenomenon is known as acute tolerance or the Mellanby effect, named after a British pharmacologist, Sir Edward Mellanby (1884–1955), who first made the observation, mainly through experiments in dogs (Mellanby 1920).

Table 4.2 Clinical signs and symptoms of alcohol influence shown as a function of increasing BAC

BAC (mg/100 mL)[a]	*Signs and symptoms of alcohol influence*[b]
<20	Sobriety, lack of outward signs, possible smell of alcohol on breath
20–50	Loss of inhibitions, more talkative, impairment of certain cognitive skills, especially those requiring divided attention
50–100	Lack of judgement and control, more rowdy and daring, slowed reaction time, especially in choice situations
100–150	Lack of coordination, unsteady gait, slurred speech, prolonged reaction to sights and sounds
150–200	Obvious drunkenness, aggression, ataxia, slowed reaction time even when relatively simple tasks are performed, nausea and vomiting especially after rapid absorption
200–300	Confusion, inability to stand upright or walk without help, slurred speech, motor areas of the brain are now markedly impaired, distorted perception of time and judgement risk lapsing into a comatose state
300–400	Stupor or comatose state with shallow breathing, risk of respiratory arrest, loss of gag reflex and danger of inhalation of vomit leading to asphyxia and death
400–500	Profound risk of death from respiratory paralysis and cardiopulmonary arrest

[a]The subjective and objective effects of alcohol are more pronounced on the rising part of the BAC (blood alcohol concentration) curve close to the C_{max} compared with several hours after passing C_{max}; this phenomenon is known as the development of acute tolerance.
[b]Large inter-subject variations exist within a given BAC interval depending on age, previous experience with alcohol speed of drinking, and central nervous system tolerance.

Table 4.2 lists typical signs and symptoms of alcohol influence as a function of the person's BAC at or near the peak concentration (Jones 2009a). Note that these observations do not apply to a specific individual, but are intended to present a general picture of the effects of alcohol on vital body functions and performance and behaviour. Both acute (Mellanby effect) and chronic tolerance to alcohol depend on the age and sex of the individual as well as on the pattern of drinking and the amounts consumed daily (Kalant 1998). This tends to skew the relationship between BAC and the signs and symptoms of alcohol influence shown in Table 4.2 and accordingly great care is need when this information is interpreted in any individual case.

A typical example of central nervous tolerance and outward signs of alcohol influence is provided by the driver (Mr Henri Paul) of the car in which Diana Princess of Wales was killed in a single-vehicle crash in Paris in 1997. According to postmortem reports, the driver had a BAC of 170 mg/100 mL (0.17 g%). This high BAC stood in sharp contrast to CCTV footage from the Ritz Hotel shortly before the crash showing Mr Paul walking around the hotel. He showed no obvious indications of alcohol influence, such as an unsteady gait or unusual movement or behaviour and even had occasion to bend down and tie his shoelace. The disparity between the postmortem BAC and the CCTV footage is easily explained by the relative insensitivity of such tests to disclose the influence of alcohol or drugs. The long investigation of the case produced evidence to suggest that Mr Paul had begun drinking alcohol earlier the same day (his day off) and it is also well documented that he took two further drinks after returning to the Ritz Hotel before the fatal drive.

If the vehicle had been fitted with an ignition interlock device, this might have prevented the crash or, at the very least, Mr Paul would not have been able to start the engine. Consumption of alcohol over a lengthy period of time allows the brain to adapt to the effects of this drug so that an individual might seem perfectly normal on CCTV. However, when performance of skilled tasks and divided attention are required, such as driving at high speed and trying to avoid the paparazzi, reaction time and performance are seriously affected. Accordingly, tolerance to the effects of alcohol can account for the discrepancy between the postmortem report and CCTV footage prior to the crash.

Clinical tests of drunkenness

A critique of the traditional clinical tests of alcohol influence is the subjective nature of such tests. Much depends on the enthusiasm and experience of the examining physician, including his or her skill and training for this purpose. Up until 1982, every driver apprehended in Sweden underwent a clinical examination in conjunction with sampling of blood for determination of alcohol. This provided a mass of information about the relationship between a driver's BAC and the conclusions from clinical tests of alcohol influence (Bonnichsen *et al.* 1967). A physician examines the driver about one hour after arrest and administers a standardised questionnaire and simple cognitive and psychomotor tests are made on the suspect. The physician then has to conclude whether the individual is under the influence of alcohol or whether some medical condition or use of other drugs might be involved.

Figure 4.2 presents the results of a clinical examination of apprehended drivers by physicians or police surgeons (Bonnichsen *et al.* 1967). The results from examination of more than 7000 apprehended drivers is shown as a function of their BACs. The examining physician did not know the driver's BAC, although some bias might have existed through knowledge that the person examined was suspected of DUIA and had failed a roadside breath alcohol test (BAC > 50 mg/100 mL). The conclusion of the examination – whether the driver was not under the influence of alcohol, or slightly, moderately or heavily impaired – was arranged according to the BACs. Although the degree of alcohol influence was more evident at higher BACs, there were large discrepancies, with many people being judged as not under the influence of alcohol despite a high BAC (upper left plot in Figure 4.2). Studies of this nature underscore the problem of relying on subjective criteria as evidence to assess whether a person was under the influence of alcohol or drugs for legal purposes.

One obvious explanation for discrepancies between results of the clinical examination and the actual BAC is the degree of skill and training on the part of the physicians or police surgeons who perform the tests. But even when the same physician performs the clinical examination, the results leave much to be desired, as evidenced by Table 4.3. Here a study was made of 244 alcohol-impaired drivers subsequently examined by the same physician and in this way eliminating variability between observers (Bonnichsen *et al.* 1967). Nevertheless, even with this study design, there was still considerable overlap between the results of the examination in terms of degree of alcohol impairment at the same BAC interval. This dichotomy can be accounted for, at least in part, by individual differences in tolerance to alcohol and the ability of some people to pull themselves together in a critical situation. Furthermore, the relative simplicity of the tests conducted, plus the driver's demeanour, educational standard and ability to converse politely, verbal skills and ability to perform simple mental tasks can account for the differences within similar BAC ranges shown in Figure 4.2.

In many European nations, the police are allowed to perform random breath testing of motorists without having any prior suspicion of DUIA. By contrast, such testing is not permitted under the fourth amendment of the constitution of the USA, which protects people from unreasonable search and seizure. However, the US Supreme Court has ruled that sobriety checkpoints entail such a minimal intrusion that they should be permitted and are thus used in many states under certain circumstances (Voas 1991). However, in most US states the arresting police officer still needs probable cause that a driver was under the influence of alcohol before a blood or breath alcohol test can be made. Hence the development of so-called standardised field sobriety tests (Burns 2003), which involve three behavioural tests at the roadside.

Standardised field sobriety tests

Much has been written about the pros and cons of standardised field sobriety tests (SFSTs) (Hlastala *et al.* 2005; Stuster 2006). Such tests were developed and evaluated to assist police officers in making a decision whether to arrest a person for driving under the influence of alcohol or

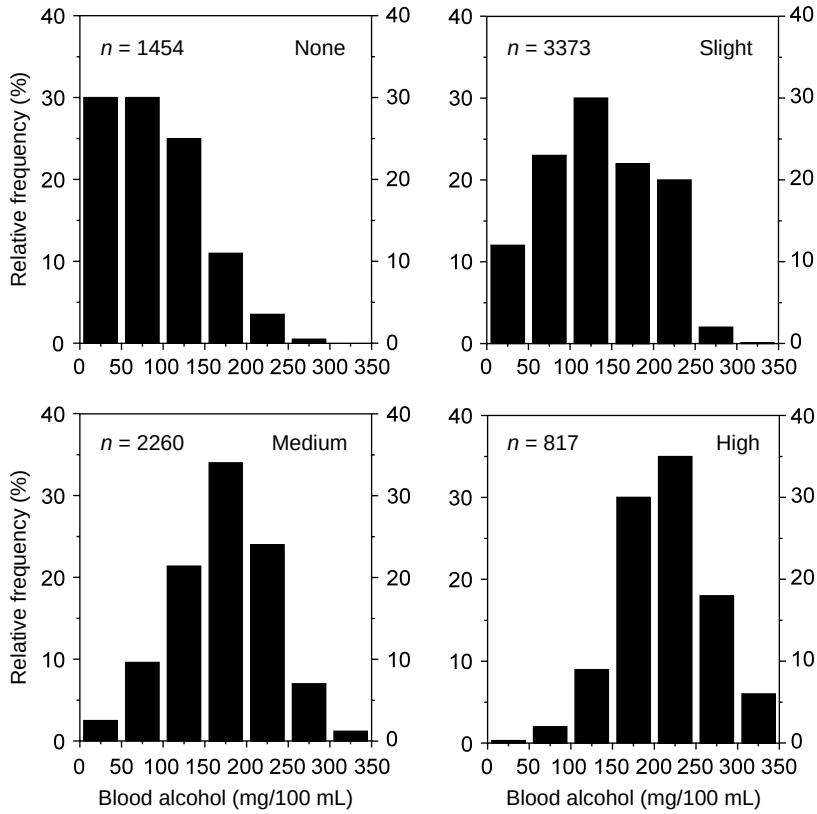

Figure 4.2 Relative frequency distributions comparing the results of clinical tests of drunkenness made by physicians with the suspect's blood alcohol concentration (BAC). The results were sorted according to whether the suspects were judged not under the influence or slightly, moderately or highly influenced as a function of the BAC.

Table 4.3 Numbers of apprehended drivers judged as not under the influence of alcohol or slightly, moderately or severely influenced after clinical examination and questionnaire in relation to blood alcohol concentration (BAC; all 244 individuals were examined by the same physician)

BAC (mg/100 mL)	*Degree of alcohol influence*				*Total (%)*
	None	*Slight*	*Moderate*	*Severe*	
0–49	3	26	0	0	29 (12)
50–99	2	37	0	0	39 (16)
100–149	2	41	10	1	54 (22)
150–199	1	24	17	5	47 (19)
200–249	0	20	22	5	47 (19)
250–299	0	7	11	7	25 (10)
300–349	0	1	1	1	3 (1)
Total	8 (3)	156 (64)	61 (25)	19 (8)	244 (100)

drugs (Brick, Carpenter 2001). SFSTs are usually carried out at the roadside in close proximity to the traffic stop. Failing in these tests will usually lead to arrest and further testing with a more objective blood or breath alcohol analysis (Rubenzer 2008). The SFSTs were developed by psychologists to be administered by highway patrol or other police officers who undergo training in the theory and practice of behavioural and impairment effects of alcohol (Burns 2003). The battery of SFSTs comprises three types of test of the driver: horizontal gaze nystagmus (HGN), disturbances in balance and coordination, and ability to divide attention among several tasks at once.

The first test, and the one considered to be the most sensitive of the three, is HGN, which is an involuntary jerking or bouncing of the eyeball in the lateral direction when the eyes gaze to the side (McKnight *et al.* 2002). When under the influence of ethanol and some other drugs, the brain's ability to properly control eye muscles is impaired, which makes the eyeball jerk or bounce back and forth. An alcohol-impaired person will have difficulty in tracking a moving object placed before the eyes and the degree of jerking increases as a function of the BAC (Chiang, Young 2007).

The second SFST is the walk-and-turn test, which is a kind of divided-attention task. The suspect has to listen to instructions given by the police officer and then take nine steps forward walking heel-to-toe along a straight line, before turning on one foot and then walking back in the same direction. Stepping off the line or losing balance is counted as evidence that the person is under the influence of alcohol or some other impairing drug.

The third SFST is called the one-leg stand test, which requires the motorist to stand with one foot held approximately 15 cm (6 inches) above the ground while counting in thousands (1001, etc.) for approximately 30 s. The police officer has to score the number of mistakes made, such as swaying and using the arms to maintain balance or putting the foot down during the 30 s duration of the test. The results from the three tests are then averaged into a combined error score.

It must be fairly obvious that many sober people will have difficulties in passing the above-described tests, whether because of old age and infirmity, problems with blood pressure, other medical conditions, failure to understand instructions or the fact that they might be using certain prescription drugs. The environmental conditions, such as weather, the road surface, the traffic intensity and the type of shoes worn can all play a role in the outcome of SFSTs. Of the three SFSTs, HGN is considered to be most sensitive and objective and positive results have been reported at BACs as low as 40 mg/100 mL (McKnight *et al.* 2002). Others have strongly criticised the HGN test on physiological grounds and the fact that ophthalmic problems or use of drugs other than alcohol can skew the results (Booker 2001, 2004). Furthermore, the police officer who

administers SFSTs is often expected to testify in court as to how the tests were conducted and the way the suspect responded to questions, which opens the officer to cross-examination by the lawyers hired by the defence.

Controlled laboratory experiments

Much more is known about the dose–concentration relationships for the effects of ethanol on sensory, cognitive and psychomotor functioning than for other licit or illicit drugs. Goldberg (1943) made the first quantitative studies of the effects of alcohol on performance and behaviour and also interpreted the results in relation to BAC and the drinking habits of volunteer subjects. He established BAC thresholds for onset of and recovery from impairment using a battery of sensory, cognitive and psychomotor tests in non-drinkers, moderate drinkers and alcoholics. This work laid the foundation for many later studies aimed at establishing the time-course of alcohol-induced effects on performance and behaviour (Drew *et al.* 1958; Evans *et al.* 1974; Franks *et al.* 1976).

A host of other types of experimental approaches have been developed to measure the effects of alcohol on skills resembling driving (Moskowitz *et al.* 1985). The effects of alcohol on visual functions, cognitive tasks, sedation, drowsiness, vigilance and psychomotor performance, such as standing steadiness, reaction time (simple and choice) and hand steadiness, have been well studied (Fillmore, Vogel-Sprott 1995, 1998; Farquhar *et al.* 2002). The results show that divided attention tasks are more sensitive to the effects of alcohol (Ferrara *et al.* 1994). Examples of the types of experimental approach to document the effects of alcohol on human performance and behaviour and risk of a crash are given in Table 4.4.

The international scientific literature dealing with the effects of alcohol on driving-related skills was the subject of an in-depth review by Moskowitz and Fiorentino (2000). These investigators made a detailed evaluation of 112 articles published between 1981 and 1997 on the subject of alcohol-related impairment. Results were evaluated according to the BAC of the person tested and the type of cognitive, psychomotor or behavioural test used. One major aim of the review was to establish the lowest BAC at which impairment could reliably be detected for each particular driving-related task. Furthermore, an attempt was made to determine the thresholds of impairment in terms of BAC for each of 12 separate tasks performed. The authors reached the following conclusions (BAC units of g/dL given in the original report are also shown as mg/100 mL):

- Alcohol impairs some driving skills beginning with any significant departure from zero BAC. By BACs of 0.05 g/dL (50 mg/100 mL) the majority of the experimental studies examined reported significant impairment. By 0.08 g/dL (80 mg/100 mL), more than 94% of the reviewed studies showed impairment in the skills that they measured.
- Specific performance skills are differentially affected by alcohol. Some skills are significantly impaired by BACs of 0.01 g/dL (10 mg/100 mL), while others do not show impairment until BACs of 0.06 g/dL (60 mg/100 mL).
- Discrepancies among the reported BAC thresholds of impairment within a behavioural area reflected a lack of standardisation of testing methods, instruments and measures in the studies reviewed.
- All drivers can be expected to experience impairment in some driving-related skills by 0.08 g/dL (80 mg/100 mL) or less.

Table 4.4 Methods used to establish the relationship between blood alcohol concentration (BAC) and alcohol-related impairment

Type of testing	*Examples of the type of measurements made*
Physiological measures	Pulse rate, respiratory rate, blood pressure, nerve conduction velocity, body temperature, etc. before and after intake of a known amount of alcohol
Subjective feelings and objective measures of alcohol influence	Administration of a questionnaire about mood changes and subjective feelings of intoxication after consumption of alcohol and comparison with objective assessment by trained observers or a physician
Controlled laboratory studies of performance decrement after subjects drink known amounts of alcohol	Use of a battery of tests of cognitive and psychomotor functioning including mental arithmetic, accuracy in number recall, etc.
	Eye movements (horizontal gaze nystagmus)
	Standing steadiness (body sway)
	Hand steadiness (tremor)
	Tracking
	Divided attention tasks
	Tests of simple and choice reaction time
Driving simulators	Video-controlled driving tasks both interactive and non-interactive or use of highly sophisticated driving simulators including a dummy car and other equipment
On-the-road driving tests	Assessment of mistakes made during actual driving performance using specially constructed vehicle, e.g. monitoring of steering, speed, braking and response to critical situations
Roadside surveys and case–control studies	Epidemiological studies of crash-risk assessment as function of BAC; so-called case–control studies of drivers involved in a crash compared with carefully matched non-accident control drivers

Blood alcohol and crash risk

Statistics derived from postmortem reports from road traffic fatalities in various countries show that 20–50% of drivers had been drinking alcohol before the crash and their BAC was above the legal limit for driving (Brewer *et al.* 1994; Margolis *et al.* 2000; Baker *et al.* 2002). The most compelling evidence of the effects of alcohol on driving performance comes from so-called on-the-road driving tests. These types of studies involve the use of specially adapted vehicles and take advantage of closed-off roads or tracks to evaluate steering, emergency braking and evasive manoeuvres around fixed objects, and also the response of the driver to critical situations (Laurell 1977). Along the same lines, various computer-controlled driving simulators have been developed with steering wheel and dummy car to test the effect of alcohol and drugs on driving performance. Some such driving simulators are highly sophisticated and can be programmed to generate a wide range of hazardous conditions likely to be encountered in the real world, such as variations in speed, light–dark environment, different road conditions, weather (rain, snow, ice) and traffic intensity in various combinations.

Perhaps the most convincing evidence of the role of alcohol consumption and elevated BAC for the probability of causing a crash comes from the so-called roadside surveys and case–control studies. Several controlled epidemiological studies have documented that the risk of a crash increases as the BAC increases. The most widely cited study of this nature was conducted in the city of Grand Rapids (Michigan, USA) in the early 1960s and the results were published in 1965 (Borkenstein *et al.* 1974; Hurst *et al.* 1994). Roughly 6000 drivers involved in collisions had their BACs determined indirectly by analysis of breath and this was compared with about 7500 control drivers. The crash and control groups were regrettably not matched for crash location, time of day, day of week and direction of travel, although other variables were considered, such as age of the driver, experience at the wheel, sex, race,

education, marital status, drinking frequency, occupation of the driver, etc. This enormous undertaking was possible thanks to full cooperation from the police authorities and use of trained interviewers who designed and administered a detailed questionnaire to crash and control groups.

In the Grand Rapids study, the breath samples were stored in specially constructed plastic bags and transported for later analysis with a Breathalyzer instrument. The stability of alcohol in breath on storage is sometimes suspect and tends to give lower concentrations than expected if breath had been analysed directly. Losses of ethanol from the breath would tend to underestimate the true blood alcohol level in the accident and control drivers. Nevertheless, a relative risk curve of the probability of a crash showed a slight dip at BAC between 20 and 40 mg/100 mL, but a sharp increase in risk as BAC passed 80 mg/100 mL. The results of the Grand Rapids study were a strong motivating factor when countries such as the UK introduced statutory alcohol limits for driving in the 1960s.

Because of certain methodological problems with the data collected in the Grand Rapids study, including the method of breath alcohol analysis, two further case–control studies have been undertaken, one in Florida and the other in California (Compton *et al.* 2002; Moskowitz *et al.* 2002). Advantage was taken of modern hand-held instruments for breath alcohol analysis using electrochemical detection. The results provided immediate information on whether the people tested had consumed alcohol and also gave an estimate of their BAC. Attempts were also made to correct problems from the earlier studies such as 'missing cases', that is people leaving the crash scene before they could be tested. These so-called hit-and-run drivers had a major impact on the relative risk scores for the crash group because it is very likely that these drivers in many cases had been drinking alcohol (Moskowitz *et al.* 2002).

In the latest roadside surveys, hit-and-run drivers were actively pursued by the police and, whenever possible, breath alcohol tests were performed. Even some of the control group refused to provide a breath alcohol test and the study investigators attempted to obtain a sample without consent by using a passive alcohol sensor. This device, which is shaped like a flashlight, was kept close to the driver's face when he or she was engaged in conversation (Voas *et al.* 2006; Fell, Compton 2007). Activating the device draws a sample of the breath into the instrument for determination of alcohol. Although the readings are expected to be lower than if a proper deep lung end exhalation had been made, the presence or absence of alcohol can be established (Lestina, Lund 1992).

These and other methodological improvements give much credence to the relative risk curve shown in Figure 4.3 (Compton *et al.* 2002). The material comprised 5000 crash-involved drivers and 10 000 control non-accident-involved drivers sampled at the same site, time, day of the week and duration of travel as the crash driver. Of the crash drivers, 12% drove away from the crash site, but were pursued by police; of these 20% had consumed alcohol and could be included in the statistical analysis.

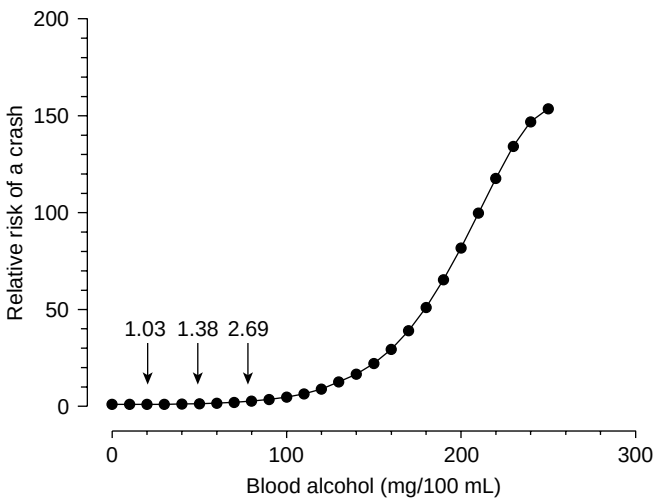

Figure 4.3 Relative crash risk as a function of the driver's blood alcohol concentration (BAC), estimated indirectly by analysis of breath (zero BAC = risk 1.0).

The numbers above the arrows shown on this plot indicate the relative risk of a crash at BACs of 20 mg/100 mL, 50 mg/100 mL and 80 mg/100 mL. Although there is an increase in risk as the BAC increases, the major effect of alcohol occurs when the BAC passes 80 mg/100 mL, in confirmation of the Grand Rapids study. The confidence intervals for the relative risk scores at various BAC levels have not yet been reported. It also needs to be made clear in a multiple-vehicle crash in which one of the drivers had been drinking whether it was this person who was responsible for the crash. Culpability assessment is an important feature of this kind of controlled epidemiological study of the role played by alcohol and drugs in traffic crashes (Robertson, Drummer 1994; Stewart 2005).

Analysis of alcohol in body fluids

Concentration units

No standardisation exists of the way in which blood and breath alcohol concentrations are reported for legal or scientific purposes as is obvious from information presented in Table 4.1. This sometimes leads to confusion when scientific articles and reports are compared and contrasted and results are reviewed in legal proceedings. The word 'concentration' has little meaning when used alone because it can be expressed in different ways depending on the choice of units. In the UK, BAC for legal purposes is reported as mg/100 mL, which often appears as the ambiguous mg% (Emerson 2004). In the USA, the concentration unit for blood ethanol in law enforcement is g/100 mL (% w/v), whereas most European countries present results as mg/mL or g/L.

For historical reasons, Germany and Scandinavian countries report BAC as mass/mass units, either mg/g or g/kg, because the blood aliquots analysed were originally measured by weight rather than by volume (Widmark 1922). The density of whole blood averages 1.055 g/mL, which means that a BAC expressed as mass/mass is not exactly the same as mass/volume, the difference being 5.5% (Lentner 1981). Accordingly, a person with a BAC of 100 mg/100 mL (mass/volume) has a mass/mass concentration of 95 mg/100 g and this difference should be considered when international comparisons are made. For example, a difference of 5.5% can determine whether a person is classified as being above or below the legal limit for driving and is a confounding factor when alcohol crash statistics are compared between nations.

Other ways of reporting BAC often appearing in scientific journals include mg/dL, g/L or μg/L. By the time most EU countries enacted statutory blood alcohol limits for driving, the aliquots of blood were measured by volume and the concentrations of alcohol were therefore reported as mass per unit volume; whether grams, milligrams or micrograms of ethanol in a volume of millilitres, decilitres or litres seemed arbitrary.

In hospital clinical chemistry laboratories the use of SI units has gained wide acceptance (Powsner 1984). On this scale the amount of substance is 'mole' rather than mass, and litre is the preferred unit of volume. The molecular weight of ethanol is 46.06, which means that a concentration of 1 mol/L corresponds to 46.06 g/L and 1.0 mmol/L is 46.06 mg/L, and so on (Stewart, Watson 1983). Many publications in the field of clinical and biomedical alcohol research report concentrations of ethanol and other drugs in terms of mmol/L. A simple calculation shows that a blood ethanol concentration of 100 mg/100 mL is the same as 21.7 mmol/L.

Many clinical chemistry laboratories at general hospitals need to measure concentrations of ethanol in blood and other body fluids because casualty patients often arrive for treatment after heavy drinking. Their BAC needs to be known before surgical treatment is given (Watt *et al.* 2005; Cherpitel 2007). The specimen used for analysis in hospital laboratories is usually plasma or serum and not whole blood, which is the body fluid used in forensic science and toxicology (Winek, Carfagna 1987). Ethanol is not evenly distributed between plasma and erythrocyte fractions of whole blood and this fact needs to be considered if and when

hospital laboratory results find their way into courts of law (Wright 1968; Charlebois *et al.* 1996). Careful measurement of the water content of body fluids by dessication showed that plasma was ~92% w/w water compared with ~80% w/w for whole blood and ~70% w/w for red cells (Jones 1992b).

A multicenter study from Germany reported 1.16 : 1 as the mean distribution ratio of water between serum and whole blood with a standard deviation (SD) of 0.0163 (Iffland *et al.* 1999). This implies that 95% of people will have a distribution ratio of water (and therefore of ethanol) within 1.16 ± 2 SD or from 1.12 to 1.19. For forensic purposes, if the concentration of ethanol had been determined in plasma or serum by mistake, the concentration in whole blood needed for use in a criminal trial on impaired driving is obtained by dividing serum alcohol by 1.2 (mean ± 2 SD). This calculation would provide a conservative estimate of the concentration of ethanol that would be expected in whole blood had that been the specimen analysed.

Determination of alcohol in blood and urine

Few, if any, substances can be determined in body fluids with such a high degree of accuracy, precision and specificity as ethanol (Moriya 2005). The older analytical methods required that ethanol be removed or separated from the biological matrix prior to analysis by taking advantage of its volatility. This required a pre-analytical stage involving distillation, diffusion or aeration; alternatively proteins in the blood were removed by precipitation (e.g. with perchloric acids) and the aqueous supernatant was then used for analysis of ethanol. One of the first analytical methods reliable enough to be used for legal purposes and for prosecution of drunken drivers was the microdiffusion method developed by Widmark (Widmark 1922). This involved microdiffusion in special flasks and chemical oxidation of ethanol with a mixture of potassium dichromate and sulfuric acid in excess. The amount of oxidising agent used up in the oxidation reaction was determined by iodometric titration with sodium thiosulfate and a starch indicator. Wet chemistry methods of analysis lacked selectivity, which meant that if other volatile materials were present in the blood specimen (e.g. methanol, acetone or ether) these would be oxidised and resulted in falsely high ethanol concentrations.

The selectivity for identification of ethanol was improved in the early 1950s when methods utilising milder oxidation conditions with enzymes (alcohol dehydrogenase) were developed. (Bonnichsen, Theorell 1951; Bücher, Redetzki 1951). At alkaline pH (~8.8) in a phosphate buffer, the coenzyme nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH as ethanol is oxidised to acetaldehyde, which is trapped with semicarbazide to drive the reaction to completion. The quantitative analysis of ethanol was achieved by measuring the formation of the reduced coenzyme (NADH) by ultraviolet (UV) photometry at a wavelength of 340 nm.

The gold standard method in use today for the qualitative and quantitative analysis of blood ethanol concentrations in blood and urine for legal purposes is headspace gas chromatography (HS-GC) with automated sampling with a flame ionisation detector (FID) as outlined below (Jones, Schuberth 1989). The principles of GC applied to quantitative and qualitative analysis of drugs and poisons is dealt with elsewhere in this book and will not be repeated here. The advantage of GC over other analytical methods is that the target analyte (ethanol) is separated from non-volatile components of blood at the injection port and potential interfering compounds (e.g. acetone, methanol) are separated on the column prior to quantitative analysis. Accordingly, GC provides both a qualitative and a quantitative analysis in a single analytical run (Tagliaro *et al.* 1992).

All determinations for legal purposes should be done in duplicate by two technicians working independently with different sets of equipment. Aliquots of blood (100 µL) are removed from evacuated tubes by means of diluter-dispenser equipment and immediately diluted 11 times (1 + 10) with *n*-propanol (0.08 g/L) as the internal standard. If the difference between the two independent determinations exceeds some preestablished threshold value, the blood samples should be re-analysed. If the average BAC is less than 10 mg/100 mL, the result is reported back to the police as negative, so a concentration of 10 mg/100 mL serves as the limit of quantitation. The FID response shows good linearity for ethanol and other alcohols over a wide range of concentration from 10 mg/100 mL to 500 mg/100 mL that might be encountered in forensic blood samples. An analytical procedure for blood ethanol analysis that has proved robust and reliable over many years is described in brief below:

1. The tubes of blood are placed in racks and mechanically inverted for about 5–10 min to ensure homogeneity of the specimen. This step is important because sedimentation of red cells occurs on standing and without proper mixing there is a risk that a plasma-rich aliquot of blood is removed from the tube and analysed.
2. The stoppers are removed from a batch of evacuated tubes and aliquots of blood (100 µL) are diluted 1 + 10 with an aqueous solution of the internal standard (*n*-propanol), the concentration of which is usually about 8–10 mg/100 mL. This dilution step requires a high degree of precision and accuracy and automated diluter-dispenser equipment is available for this purpose. The blood specimen and internal standard are immediately ejected into a glass vial, which is then fitted with a Teflon-lined stopper and made airtight by crimped-on aluminium caps in readiness for HS analysis.
3. Equipment for HS-GC analysis is available from several manufacturers, although the first instruments (since the early 1970s) were produced by the Perkin-Elmer company. The quantitative analysis of ethanol is accomplished by measuring the peak area ratios of the response on the gas chromatogram for ethanol and the internal standard after electronic integration. When this EtOH : PrOH ratio is plotted as a function of the concentration of ethanol in the known standards, a linear calibration plot is obtained. The concentrations of ethanol in the unknown blood samples are found by interpolation using computer software and linear regression analysis for the relationship between peak area ratio (ethanol to internal standard) and known concentration of ethanol in the standards.
4. The calibration standards are made from 99.5% v/v ethanol by weighing to give a 10% w/v stock solution and volumetric dilution from this to give working standards spanning the concentration range of interest (e.g. 10–500 mg/100 mL). Within each analytical run it is useful to include ethanol standards prepared from another source or purchased from an independent manufacturer. The ethanol standards of known strength are dispersed among the blood samples within each analytical run.
5. It is also advisable to include a blank (ethanol-free) specimen of blood diluted with internal standard in the same way as the unknowns. Each analytical run might also include an aqueous mixture of potential interfering substances (e.g. acetaldehyde, methanol, acetone, isopropanol) to check adequate resolution from the ethanol and internal standard peaks.

The standard deviation of alcohol analysis by HS-GC tends to increase as the concentration of ethanol in the blood samples increases. In a recent study, the critical differences between duplicate determinations for acceptable agreement were 1.6 mg/100 mL (BAC 20 mg/100 mL), 3.6 mg/100 mL (BAC 50 mg/100 mL) and 6.9 mg/100 mL (BAC 100 mg/100 mL) (Jones 2007c). For blood specimens that show acceptable agreement, a deduction is usually made from the mean ethanol concentration and the reduced value is used for prosecution. The amount deducted needs to be higher at higher concentrations of ethanol to ensure the same legal security for all suspects regardless of their BAC. After making the appropriate deduction, the prosecution BAC is not less than the true concentration with 99% or 99.9% certainty.

The identity of an unknown volatile in blood can be established by comparing the retention time (RT) with that of known reference substances (Jones, Pounder 2007; Emerson 2004). Use of different chromatographic conditions for analysis makes it extremely unlikely that two compounds will have the same RT on both stationary phases, especially if these are chosen to have differing polarity. Alternatively, enhanced selectivity can be achieved by analysis using different methods, such as GC and enzymatic oxidation, as is customary in Germany and Austria. Urine samples can be treated and analysed in exactly the same way as blood specimens.

Table 4.5 Retention times of ethanol and other low-molecular-weight volatile substances analysed by headspace gas chromatography on four different stationary phases used in forensic science and toxicology laboratories (retention times relative to *n*-propanol as internal standard are shown in parentheses)

Volatile substance	*Retention time (min) Carbopak C*[a]	*Retention time (min) Carbopak B*[b]	*Retention time (min) Rtx-BAC1*[c]	*Retention time (min) Rtx-BAC2*[d]
Acetaldehyde	0.56 (0.38)	0.53 (0.29)	1.19 (0.53)	0.82 (0.34)
Acetone	1.00 (0.68)	0.86 (0.46)	2.05 (0.91)	1.36 (0.57)
n-Butanol	4.68 (3.16)	4.11 (2.22)	4.63 (2.06)	5.28 (2.20)
Ethanol	0.72 (0.49)	0.98 (0.53)	1.32 (0.59)	1.27 (0.53)
Methanol	0.49 (0.33)	0.67 (0.36)	1.06 (0.47)	0.96 (0.40)
Methyl ethyl ketone	2.45 (1.66)	1.49 (0.81)	3.08 (1.37)	2.49 (1.04)
Isopropanol	1.16 (0.78)	1.31 (0.71)	1.66 (0.74)	1.48 (0.62)
n-Propanol	1.05 (1.00)	1.85 (1.00)	2.25 (1.00)	2.40 (1.00)
t-Butanol	1.90 (1.28)	1.68 (0.91)	1.98 (0.88)	1.65 (0.69)

[a]Column: glass with 0.2% Carbowax (polyethylene glycol) 1500 (2 m × 0.5 mm i.d.).
[b]Column: glass with 5% Carbowax (polyetheylene glycol) 20M (2 m × 0.5 mm i.d.).
[c]Column: capillary (30 m × 0.53 mm i.d.).
[d]Column: capillary (30 m × 0.53 mm i.d.).

Table 4.5 presents the absolute retention times (min) as well as retention times relative to the internal standard (*n*-propanol) seen during a typical HS analysis of volatiles in blood (Jones 2009b). The results are shown for two types of glass-packed columns with stationary phases – Carbopak C and Carbopak B – as well as more modern capillary columns (Rtx-BAC1 and Rtx-BAC2).

Urine–blood alcohol relationship

The functioning of the kidney and the physiology of ethanol excretion including analysis and interpretation of urine alcohol concentration have been reviewed (Jones 2006). Ethanol passes by diffusion from the renal artery blood with the glomerular filtrate into the urine, which is stored in the bladder until voided. Controlled drinking experiments have shown that the concentration–time profile of ethanol in urine differs from that of blood alcohol (Jones 1992b). The urine alcohol concentration (UAC) curve is displaced in time and runs on a higher level compared with that for the corresponding BAC. Furthermore, if a pool of residual alcohol-free urine were present in the bladder before drinking, this would dilute the alcohol concentration in freshly produced urine (Biasotti, Valentine 1985). Urine is almost 100% w/w water compared with 80% w/w water in blood, so the UAC : BAC ratio after equilibration is expected to be greater than unity or approximately 1.25 : 1 (100/80).

Urine is produced at a rate of approximately 1 mL per minute or 60 mL per hour, resulting in a total volume of 1–2 litres per 24 h. Much depends on total intake of fluids and the intensity of diuresis, which is higher after drinking alcohol, sometimes by 10-fold for short periods (Bendtsen, Jones 1999). However, alcohol-induced diuresis is only evident during the absorption stage of the blood alcohol curve and close to the C_{max} value (Jones 1990a). During the post-absorptive period, the UAC always runs above the BAC, mainly because of the difference in water content of the two fluids.

Figure 4.4b shows typical UAC and BAC profiles after subjects drank a bolus dose of alcohol (0.85 g/kg) as neat whisky in 25 min (Jones 2009a). The subjects emptied their bladders before drinking and urine was collected thereafter at hourly intervals and blood samples were taken at 30 to 60-min intervals. In Figure 4.4a, the median volumes of urine collected are shown as a function of sampling time for up to 8 h post drinking. Medians are plotted because of large inter-subject variation in urinary volumes, which are not normally distributed. The shift or displacement in UAC and BAC profiles is evident from Figure 4.4b. The UAC is less than BAC for the first 60 min after drinking (absorption phase) and always higher than BAC for the rest of the time alcohol is in the body (post-absorptive phase).

The shapes of UAC and BAC profiles during repetitive drinking over several hours, as might be expected in a typical drink–driving scenario, have not yet been published.

The threshold concentration limit of alcohol in urine in the UK and Republic of Ireland is 107 mg/100 mL urine, which was arrived at from the statutory BAC of 80 mg/100 mL on the assumption of a UAC : BAC

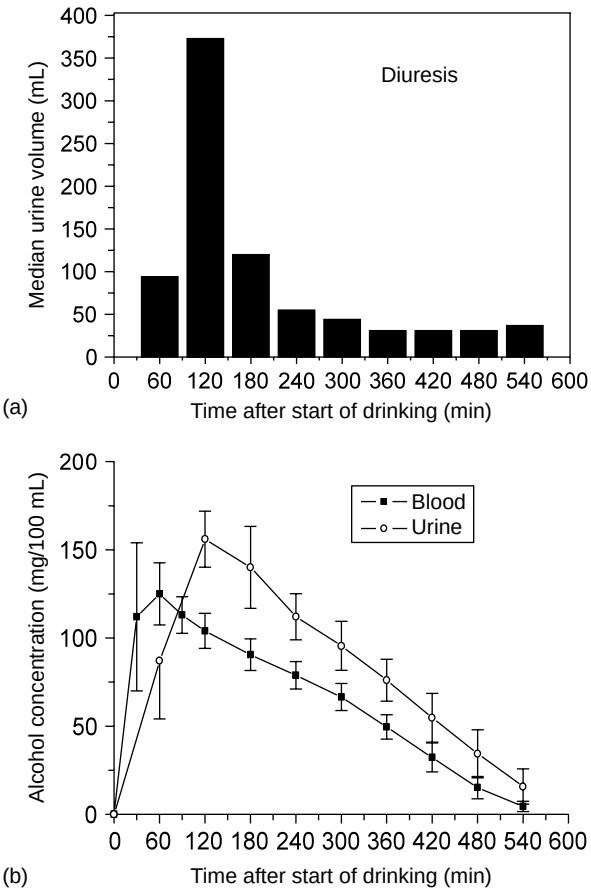

Figure 4.4 Concentration–time profiles of alcohol (mean curves, $n = 16$) in venous blood and urine after a bolus dose (0.85 g/kg ethanol) as neat whisky taken on an empty stomach (b). Plot (a) shows the volumes of urine collected at hourly intervals after drinking alcohol over a period of 25 min.

ratio of 1.33 : 1(Payne *et al.* 1967). When drink–driving law is interpreted by the courts, there is no need to consider the UAC : BAC ratio or make any inter-conversion between the two because both are defined by statute (Curry 1972).

Urine is produced in batches so that the UAC reflects the average concentration of alcohol in the blood passing through the kidney during the period that urine is formed and stored in the bladder. The storage time of urine in the bladder is not known in cases of DUIA and the UAC might therefore reflect the BAC prevailing several hours earlier, even before the time of driving. During the storage time of urine in the bladder, the concentration of ethanol in the blood decreases appreciably through metabolism in the liver. Ethanol-metabolising enzymes do not exist in the bladder. Under these circumstances, the UAC : BAC ratio of alcohol is expected to be abnormally high.

This pooling effect of ethanol in the bladder is avoided by having the suspect first empty the bladder of any 'old urine' and then provide a fresh specimen about 30–60 min later. It is the concentration of alcohol in this second of two voids that determines whether the person exceeds the statutory UAC limit of 107 mg/100 mL. Another important requirement is to include a chemical preservative, sodium fluoride (1% w/v), in the specimen container used to store and transport the urine specimen for analysis (Jones *et al.* 1999). The fluoride ions act as an enzyme inhibitor to prevent the production of ethanol, which might otherwise occur if there was sugar in the urine and the person suffered from a urinary tract or yeast infection. The inclusion of urine as an alternative body fluid to blood in the prosecution of impaired drivers was intended for people who might refuse to provide blood for religious reasons or claim that they suffer from blood injury phobia.

Both inter- and intra-individual variations in the urine-to-blood relationship exist as demonstrated by the scatter plot in Figure 4.5 (Jones 2002). The venous BAC was plotted against the UAC for the second of two urine voids collected from several hundred drunken drivers. The Pearson correlation coefficient ($r = 0.978$) indicates a strong positive association between the two variables. However, the scatter of the individual data points around the regression line (residual standard deviation) is 21 mg/100 mL, which suggests that there is considerable uncertainty in using the regression equation to predict BAC from UAC or vice versa.

The relative frequency distribution of UAC : BAC ratios is seen in Figure 4.6 and resembles a normal distribution of values with mean of 1.23 : 1 and median 1.22 : 1 (Jones 2002). Both ratios are slightly lower than 1.33 : 1, which is the ratio used to set the statutory UAC limit in the UK. Accordingly, a ratio of 1.33 : 1 instead of 1.23 or 1.22 for setting the UAC limit gives a slight benefit to the person who opts to give a urine specimen compared with blood. The mean UAC : BAC ratio of 1.23 : 1 would lead to a threshold UAC of 80 × 1.23 or 98 mg/100 mL instead of 107 mg/100 mL.

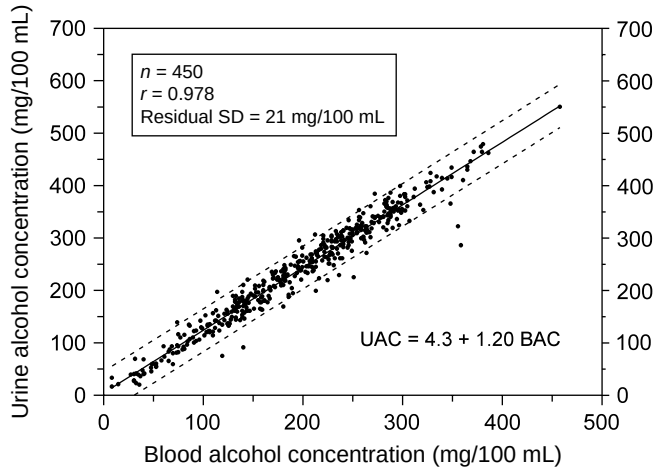

Figure 4.5 Scatter plot and correlation–regression analysis of the relationship between venous blood alcohol concentration (BAC) and urine alcohol concentration (UAC) for the second of two consecutive voids of the bladder ~60 min apart.

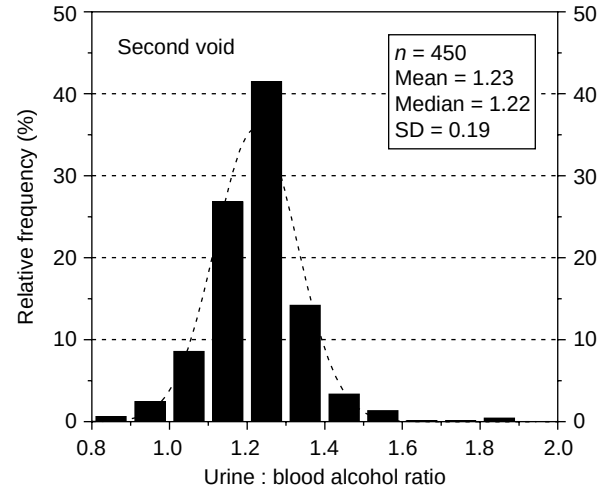

Figure 4.6 Relative frequency distribution of UAC : BAC ratios of alcohol in apprehended drivers for analysis of the second of two voids ~60 min apart.

Determination of alcohol in breath

The smell of alcohol on the breath probably represents the oldest, albeit primitive biomarker for overindulgence in alcoholic beverages. The first scientific investigations measuring exhaled alcohol date back to the 1870s when it was shown that only a very small fraction of the total amount of ethanol consumed was recoverable in the breath collected for several hours after the end of drinking (Jones 1996). In an important paper from the 1930s, the distribution ratio of ethanol between blood and air was investigated under *in vitro* and *in vivo* conditions (Liljestrand, Linde 1930). This work showed that the concentration of alcohol in alveolar air was about 2000 times less than that in an equal volume of blood, which indicates a blood : breath alcohol ratio of approximately 2000 : 1.

Methods of breath alcohol analysis can be classified and discussed according to whether the results are intended as a qualitative screening test or will be used as evidence for prosecution (Harding, Zettl 2008). The first practical breath alcohol screening test used by police for testing drivers was the chemical tube and bag device. The task of the police officer was to judge whether a change in colour (yellow to green) of crystals (potassium dichromate on silica) had occurred. The chemical reagent was packed into a narrow tube; one end of this was attached to a plastic bag (~1 L) and the suspect exhaled through the other end of the tube to inflate the bag in about 15 s. The production of a green stain up to a red graduation mark on the middle of the tube indicated that the suspect's BAC was above the legal limit for driving. Later, much-improved devices for breath alcohol testing became available and these are used worldwide for testing motorists at the roadside or even as they sit behind the wheel of their car. Current generation hand-held devices for breath alcohol screening are based on the electrochemical oxidation of alcohol, and widely known instruments for this purpose are Alcolmeter, Alcotest and AlcoSensor. These instruments have been manufactured in a wide range of models with manual or automated sampling, and with analogue or digital display or simply showing pass or fail results of the test.

Evidential breath alcohol testing is performed under more controlled environmental conditions and a special room is sometimes made available at the police station. The procedure follows strict rules and regulations that differ slightly between different jurisdictions or countries. Information about the suspect is recorded and the test procedure must start with an instrument calibration, which involves analysis of a known strength air–alcohol vapour standard from a wet bath simulator or a compressed dry gas standard (Dubowski, Essary 1992, 1996).

Another requirement is that the air from the room is analysed as a blank test and this is done before and after the subject provides breath samples. In most instances, two consecutive samples of breath are analysed 2–6 min apart. The results of all tests, including date and time, are printed out in real time and also stored in computer memory for verification later if necessary. With the latest generation of evidential instruments, such as those based on infrared technology (see Table 4.1), the entire breath ethanol exhalation profile is recorded and stored for later inspection and further analysis if necessary (Jones 2009b). Quality assurance aspects of evidential breath alcohol testing have been reviewed elsewhere (Dubowski 1994; Gullberg 2000).

Key developments in methods for the determination of alcohol in breath are summarised in Table 4.6 (Jones 1996, 2000a). The first device (the Breathalyzer) sufficiently practical to be used by police officers appeared in the mid-1950s and gained wide acceptance throughout North America (Borkenstein, Smith 1961). Instruments employing physiochemical methods of analysis, such as GC, infrared spectrometry and electrochemical oxidation appeared in the 1970s (Jones 1996). Today's instruments for breath alcohol testing are considerably automated and the police operator has only to observe and encourage the suspect to provide end-expired breath samples at the appropriate time in the test programme.

Infrared (IR) and electrochemical (EC) methods of analysis differ in several ways, including selectivity for different volatile materials and long-term stability of calibration. The IR method is non-destructive analysis, which means that the breath specimen can be stored for a later verification analysis if necessary. However, neither IR nor EC is completely specific for measuring ethanol in breath. The EC detectors respond to other alcohols (e.g. methanol, *n*-propanol and isopropanol), and also to acetone, which is an endogenous volatile exhaled in breath. Indeed, the concentrations of acetone can increase considerably under some conditions, such as in untreated diabetes or after prolonged fasting (Flores, Franks 1987). The hand-held instruments fitted with EC detectors are mainly used for roadside screening of motorists (Zuba 2008).

Table 4.6 Chronological developments in methods used for the analysis of ethanol in breath that have been used for legal purposes

Period	*Method for analysis of ethanol in breath*
1930–1950	Chemical oxidation with potassium permanganate (Drunkometer and Intoximeter), iodine pentoxide (Alcometer), trapping a portion of the breath on an absorbent (e.g. silica gel) allowing later confirmatory analysis at a laboratory (Intoximeter)
1954	Chemical oxidation of ethanol with a mixture of potassium dichromate and sulfuric acid contained in specially designed glass ampoules and with the quantitative analysis done by photometry (Borkenstein Breathalyzer)
1969	Use of compact and portable instruments for gas chromatography (GC) with thermal conductivity detector (AlcoAnalyzer) or flame ionisation detector (GC Intoximeter)
1970	Single-wavelength infrared analyser operating at 3.39 μm (Intoxilyzer 4011) measuring C–H stretching in ethanol molecules
1975–1980	Hand-held electrochemical (fuel cell) instruments, such as Alcolmeter, AlcoSensor and Alcotest mainly intended for roadside screening analysis as replacement for the chemical tube and bag device from the mid-1950s
1990–present	Multiple -wavelength infrared spectrometry at wavelengths of 3.39–3.42 μm for (C–H) bond stretching (Intoxilyzer 5000 and DataMaster) or at 9.5 μm for (C–O) bond stretching (Alcotest 7110 and Ethylometer)
	Dual-sensor technology combining electrochemical oxidation (fuel cell) and infrared t 9.5 μm (Alcotest 7110). Intoxilyzer 8000 measures the absorption of infrared energy at both 3.4 and 9.5 μm wavelengths
2000–present	Small compact mass spectrometers for determination of ethanol and other volatiles.[a] Fourier transform infrared spectroscopy (FTIR) analysers.[a]

[a]Not yet used for legal purposes.

Breath analysers fitted with multiple IR detectors have emerged as the instruments of choice for evidential testing, mainly because this technique is more robust and the calibration remains stable over longer periods (Harding, Zettl 2008). The first IR breath analysers measured the C–H stretching frequencies in ethanol molecules that occur in the wavelength range 3.3–3.4 μm (Harte 1971). Most of the instruments listed in Table 4.1 are equipped with several IR wavelength filters to ensure a higher selectivity for identifying ethanol if there happened to be interfering substances present that contain C–H bonds in the molecule, such as acetone. Instruments are equipped with between three and five narrow-band filters covering the wavelengths 3.3–3.5 μm. Some IR breath analysers are equipped with IR technology designed to measure the C–O bond stretching frequency, which is prominent at a wavelength of ~9.5 μm. Other analysers are equipped with both 3.4 and 9.5 μm wavelength detectors (Intoxilyzer 8000) and even dual technology such as ~3.4 μm IR detector and an EC detector to offer enhanced selectivity of analysis (Alcotest 4011).

The physiological principles of gas exchange in the lungs have been reviewed elsewhere (Jones 1990b; Hlastala 1998). Ethanol rapidly equilibrates between the pulmonary blood and the alveolar air by diffusion across the alveolar capillary membrane in a fraction of a second. However, during a prolonged exhalation alcohol and other volatiles undergo re-equilibration with the watery mucous surfaces covering the upper airways that receive ethanol from the bloodstream. Moreover, during exhalation the alveolar air cools from about 37°C at the interface to 34°C when the breath leaves the mouth (Jones 1982c). Because the solubility coefficient of ethanol in plasma and water is about 6.5% per degree Celsius, the exhaled concentration of ethanol is lower than the alveolar concentration (Jones 1983a). How much lower depends on factors such as body temperature, breathing pattern, and the temperature and humidity of the ambient air breathed, as well as the completeness of ethanol equilibration between the mucous surfaces of the upper airway, nose and mouth, and the expired air (Hlastala 2002, 2003).

Unlike respiratory gases (N_2, O_2 and CO_2), ethanol is highly soluble in water and it seems likely that early proponents of breath alcohol testing for legal purposes failed to appreciate the importance of re-equilibration of ethanol between the exhaled air and the airway surfaces (Wright *et al.* 1975; Hlastala 2002). Such interactions probably account for much of the variability in BBRs of ethanol observed in practice. The final end-expired BrAC also depends on pre-exhalation manoeuvre, body temperature, lung size as well as the temperature and humidity of ambient air breathed (Jones 1982a; Jones 1982b; Hlastala, Anderson 2007). Studies have shown that hyperventilation lowers and hypoventilation raises the expired BrAC compared with a normal inhalation and exhalation (Jones 1982b; Mulder, Neuteboom 1987). Abnormalities in breathing pattern influence equilibration of ethanol between airway mucus and respired air. People with lung dysfunction, such as thickening of the mucus as seen in asthma or chronic obstructive pulmonary disease, were shown to have higher BBRs than an age-matched control group of men with healthy lungs (Hahn *et al.* 1991).

The concentrations of ethanol in exhaled breath increase after re-breathing the initial exhalation a number of times (Jones 1983b). During the process of re-breathing, more time is available to achieve equilibration of ethanol between the air phase and the watery surfaces covering the upper airway. Furthermore, the temperature of the breath increases after re-breathing and ethanol concentration increases by ~6.5%/°C (Jones 1983a).

Blood–breath alcohol relationship

The concentrations of ethanol in near-simultaneous samples of blood and breath are highly correlated according to controlled drinking experiments and in tests made with apprehended drivers (Jones, Andersson 1996b; Jones, Andersson 2003). A physiological relationship

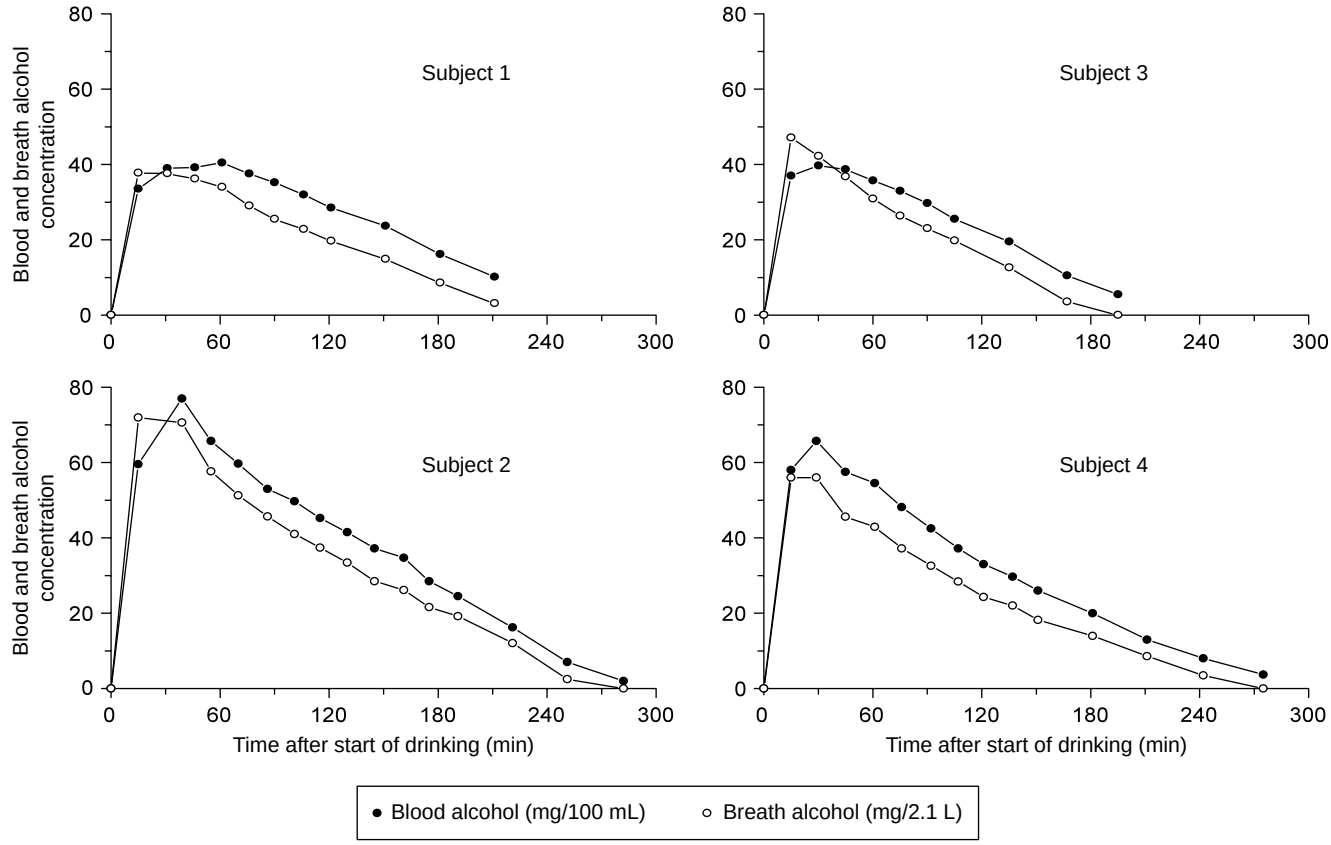

Figure 4.7 Comparison of the concentration–time profiles of ethanol in venous blood and end-expired breath derived from controlled drinking studies involving four volunteers who drank a moderate dose of ethanol starting 2 h after lunch.

between BAC and BrAC means that resulting concentrations are highly correlated, although breath contains about 2000 times less ethanol than an equal volume of blood (Harding, Field 1987; Harding *et al.* 1990). This difference in concentration should be considered when blood and breath alcohol concentration curves are plotted on the same coordinates, such as by appropriate choice of units for BAC (mg/100 mL or g/100 mL) and BrAC (mg/2.0 L or g/210 L).

Figure 4.7 compares BAC and BrAC profiles from an experiment in which four healthy men consumed a moderate dose of alcohol in the morning after an overnight fast (Jones 2010a). The alcohol (96% v/v) was diluted with orange juice and alcohol consumption was finished in 15 min. Thereafter, near simultaneous specimens of venous blood and end-expired breath were taken for analysis repeatedly for up to 6 h.

These plots demonstrate that the relationship between alcohol concentration in blood and breath varies as a function of time after the end of drinking. During the first 60 min, the breath level (mg/2 L) might be slightly higher than the concentration in blood (mg/100 mL) but at all later times the breath instrument readings are lower than the coexisting BAC. The closeness of agreement between BAC and BrAC also depends on whether BrAC was reported as mg/2 L or mg/2.3 L, and therefore the assumed BBR. Studies originating from several countries have shown that a BBR of 2300 : 1 gives closer agreement when sampling is done in the post-absorptive phase (>60 min post drinking). Most alcohol-impaired drivers are tested 1–2 h after their last drink, making it a reasonable assumption that they have reached the descending limb of the blood alcohol curve.

One reason for the discrepancy between BAC and BrAC time profiles is the use of venous blood for the determination of alcohol concentration. The time course of alcohol in breath runs much closer to the arterial blood concentration rather than that of venous blood, which is the blood sample analysed for legal purposes in DUIA investigations (Jones *et al.* 2004). However, because of medical risks with sampling arterial blood, this specimen is not so practical for use in drink–driving offences. According to a recent study the mean BBR for blood from the radial artery and exhaled air was 2280 : 1 (Lindberg *et al.* 2007).

Despite many physiological sources of variation inherent in the sampling and analysis of ethanol in breath, there is a significant correlation between BAC and BrAC as shown in Figure 4.8 derived from tests with apprehended drivers (Jones 2010a). Venous BAC was determined by automated HS-GC and BrAC was analysed with an IR analyser Intoxilyzer 5000.

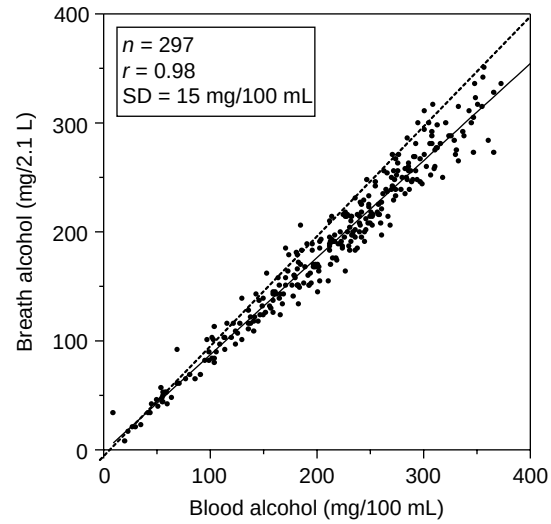

Figure 4.8 Scatter plot and correlation-regression analysis of venous BACs and end-expired BrACs in tests made with apprehended drivers.

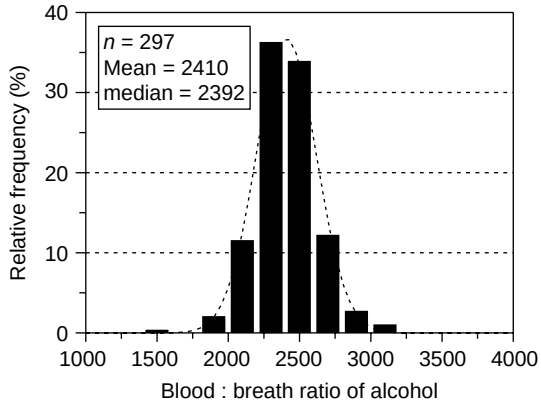

Figure 4.9 Relative frequency distribution of BAC : BrAC ratios of alcohol in apprehended drivers tested with the Intoxilyzer 5000 infrared analyser.

The magnitude of biological and analytical variation in BAC/BrAC ratio can be gleaned from the relative frequency distribution shown in Figure 4.9 (Jones 2010a). The mean and median BAC : BrAC ratios are close to 2400 : 1 in these apprehended drivers, which is considerably higher than the BBR assumed for legal purposes (2000 : 1–2300 : 1). This means that a person who submits to a breath alcohol test gains a certain advantage compared with the person who provides a blood sample. One exception to this occurs if the breath test is done shortly after the end of drinking (absorption phase) when arterial–venous differences in ethanol concentration are at their greatest. Under these circumstances, BrAC expressed as mg/2 L might be higher than BAC in units of mg/100 mL.

Allowing for uncertainty

An important consideration when physical or chemical methods are used to establish whether an analytical result complies with some legal specification, e.g. the legal BAC for driving, is the inherent uncertainty (Hibbert 2001; Andersson 2002). All measurements are subject to error and the final results, if used for criminal prosecution, need to be accompanied by an explicit quantitative statement of the amount of error or uncertainty (Brach, Dunn 2004). Uncertainty is formally defined as *a parameter associated with the result of measurement, which characterises the dispersion of the values that could reasonably be attributed to the measurand* (Hund *et al.* 2001).

The magnitudes of random errors are best determined by statistical methods to assess variations between duplicate determinations and the systematic error or bias is established by analysing known-strength standard solutions of alcohol (Gullberg 1993). By combining random and systematic errors, a statement can be made about the overall uncertainty in the analytical method, which can then be used to determine an allowance (deduction factor). In this way the final prosecution BAC can be presented with a high degree of statistical certainty as being not less than the true value, usually 99% or 99.9% (King 1999; Hibbert 2001).

The analytical precision of GC methods for determining blood ethanol depends strongly on the ethanol content in the samples analysed; the standard deviation of the measurements increases almost linearly with increasing BAC (Jones 1989). This means that the amounts deducted from the mean to allow for uncertainty also increase with the ethanol concentration in the blood specimen (Jones, Schuberth 1989). The usual practice in the UK is to deduct 6 mg/100 mL from the mean result if this less than 100 mg/100 mL or deduct 6% if the mean concentration exceeds 100 mg/100 mL (Walls, Brownlie 1985; Emerson 2004). Other countries have developed their own statistical methods to compensate for uncertainty in BAC results when these are reported to the courts for prosecution of impaired drivers (Fung *et al.* 2000).

When evidential breath alcohol instruments started in the UK, other safeguards were considered necessary (Cobb, Dabbs 1985). First, it was the lower of two breath test results that was used for prosecution. Second, although the statutory breath alcohol limit was 35 µg/100 mL, a prosecution would be brought only if the BrAC was 40 µg/100 mL or more. This safeguard was felt necessary to compensate for biological variations in the BBR. The operational BBR is therefore 2000 : 1 (80/40) instead of the BBR used to set the statutory BrAC of 2300 : 1 (80/35). Third, if the breath test result was between 40 and 50 µg/100 mL, the suspect was given the opportunity to provide a blood specimen instead and this was sent for the determination of alcohol concentration. If the blood option was taken, the result of the breath test became null and void and was not used for prosecution.

This option (which is no longer in force) to allow a suspect to provide a blood sample seems questionable without any consideration being given to the metabolism of alcohol during the time it takes for a physician or nurse to arrive and draw the specimen. An unusually long time delay could mean that the person was over the critical 40 µg/100 mL breath (guilty) at time of testing, but below the punishable BAC limit of 80 mg/100 mL (not guilty) when blood was eventually taken.

Quality assurance of analytical results of alcohol testing is imperative to ensure the legal security of the individual under a 'concentration *per se*' statute. This legal framework creates a razor-sharp difference in penalty for those who find themselves just below or just above the critical threshold, e.g. 79 mg/100 mL instead of 81 mg/100 mL. This gives strong support for making an allowance for uncertainty considering the consequences for the individual accused of driving with excess alcohol.

Alcohol in the body

Besides a thorough knowledge and expertise in analytical chemistry and statistical quality assurance, forensic scientists and others who intend to testify in court as expert witnesses in impaired driving cases also need to understand the basic principles of absorption, distribution, metabolism and excretion of ethanol in the body, as reviewed elsewhere (Jones 2008).

Alcoholic beverages are primarily composed of ethanol (CH_3CH_2OH) and water in proportions varying from 2% v/v up to 55% v/v ethanol. Also present are tiny amounts of other low-molecular-weight substances, such as other alcohols, aldehydes, lactones, etc. (<1% v/v). These other substances are known collectively as congeners and are derived as by-products from the fermentation process or might be incorporated during the manufacturing process, such as during ageing and storage in wooden casks (McAnalley 2008). The cleanest alcoholic beverage is vodka and those richest in congeners are fruit brandies, whiskies and cognacs, which usually have a characteristic colour, taste and smell (Bonte 2000).

The concentrations of congener alcohols are too low to exert their own pharmacological effect on the individual, although they are often incriminated in the unpleasant after-effects of an evening's heavy drinking, known as the alcohol-induced hangover (Greizerstein 1981; Wiese *et al.* 2000). Qualitative and quantitative analysis of congeners can help to identify the origin of a particular drink and this approach has gained forensic applications in relation to clandestine production of distilled spirits. Some scientists advocate that the profile of congeners and their metabolites in body fluids can be used as a way to identify the particular alcoholic beverage consumed (Krause, Wehner 2004). Another forensic application of congener analysis is in connection with alleged consumption of alcohol after driving, the so-called hip-flask defence, described in more detail by Iffland and Jones (2003). This defence tactic has become increasingly common in some countries (e.g. Finland, Germany and Sweden) and is often difficult for the prosecution to disprove. Identification and quantification of the major congener alcohols along with the blood ethanol concentration can help to establish whether a certain drink (usually spirits) was recently ingested and sometimes confirm or challenge alleged post-offence drinking (Bonte 2000).

Amount of alcohol consumed

The strength of alcoholic beverages is usually printed on the bottles or containers as alcohol percentage by volume (ABV, % v/v). Beers usually range in strength from 2% to 10% v/v, table wines from 6% to 14% v/v,

Table 4.7 Concentrations of ethanol in beer, wine or spirits and the amounts of ethanol contained in one standard drink. In the UK a unit of alcohol corresponds to 8 g ethanol; a standard drink in the USA is almost double, 14 g ethanol

Alcoholic beverage (volume of one drink)	*Alcohol (% v/v)*	*Alcohol (% w/v)[a]*	*Ethanol (g) in one standard drink[b]*
Beer (12 ounces or ~360 mL)	2	1.6	5.8
	3	2.4	8.6
	4	3.2	11.5
	5	4.0	14.4
Table wine (5 ounces or ~150 mL)	8	6.3	9.5
	10	7.9	11.8
	12	9.5	14.2
Fortified wine (3 ounces or ~90 mL)	16	12.6	11.3
	18	14.2	12.7
	20	15.8	14.2
Distilled liquor (1.5 ounces or ~45 mL)	35	27.6	12.4
	40	31.6	14.2
	50	39.5	17.8

[a]Volume per cent (% v/v) multiplied by 0.79 g/L to give weight per cent (% w/v).
[b]Weight per cent (% w/v) multiplied by volume of drink consumed in decilitres (100 mL) gives grams of ethanol consumed.

fortified wines (sherry and port) from 15% to 20% v/v and distilled liquors from 40% to 50% v/v. The density of ethanol at room temperature is close to 0.79 g/mL and this can be used to convert ABV (mL/100 mL) into percentage weight/volume (g/100 mL), e.g. % v/v × 0.79 g/mL = %w/v. The amount of alcohol in a drink is the volume in decilitres multiplied by percentage alcohol by weight (Brick 2006). Knowledge of the amount of ethanol consumed in grams is needed in many types of forensic blood alcohol calculation (Ferner, Chambers 2001). Examples of the amounts of pure ethanol in various alcoholic beverages in relation to ABV are given in Table 4.7.

According to the American Medical Association, moderate drinking corresponds to an intake of 1–2 standard drinks per day for men and 1 drink for women and elderly people (>65 years). The age and sex differential stems from the fact that, on average, women weigh less than men and also have less body water (Seidl *et al.* 2000). Body weight, body water and lean body mass also decrease in elderly people. For the same dose of alcohol (grams per kg body weight) a woman achieves a higher BAC than a man and the female body is therefore more exposed to the toxic effects of ethanol after excessive drinking.

The drinking pattern harmful to health varies widely between different individuals owing to factors such as age, sex, genetics, state of health and nutrition, as well as amounts of ethanol consumed and the frequency of intake (Zakhari, Li 2007). Clinicians and specialists in alcohol diseases consider that amounts exceeding 60–80 g per day (about 1 bottle of wine) for men and somewhat less for women will eventually lead to alcohol-related health problems, such as liver dysfunction and cirrhosis (Dufour 1999; Zakhari, Li 2007).

In the USA, 'one standard drink' corresponds to a 12-ounce (~360 mL) bottle of beer (5% v/v), 5 ounces (~150 mL) of wine (12% v/v) or 1.5 ounces (~45 mL) of distilled spirits (Dufour 1999). One standard drink therefore contains approximately 14 g of pure ethanol (about 0.6 fluid ounces), but the exact amount varies depending on the ethanol content of the beverage consumed (Miller *et al.* 1991). Care is needed when reading the medical literature to appreciate the difference between the US standard drink (14 g) and the British unit of alcohol.

In the UK, alcoholic consumption and patterns of drinking are often discussed in terms of 'units of alcohol' and the number of units per day has become widely used as a yardstick to document overconsumption (Wansink, Van Ittersum 2005). One unit of alcohol corresponds to a single bar measure of distilled spirits (40% v/v) or 25 mL, which contains exactly 8 g of ethanol. One unit of alcohol is consumed in half a pint (275 mL) of medium-strength beer (3–4% v/v) or 100 mL of wine (10% v/v), but glass size and alcohol content vary. One imperial pint of beer (568 mL) at 4.2% v/v contains 2.4 units and 175 mL table wine (13% v/v) contains 2.1 units of alcohol (Seabrook 2007). Keeping tabs on the quantities of alcohol consumed during social occasions is not easy and much depends on the generosity of one's host. A 125-mL glass of wine contains 1 unit (8 g) if the ABV is 8%, but many table wines are closer to 10–12% v/v. Care and consistency are needed when calculating the units of alcohol that a person has consumed or the weight of ethanol used in any blood alcohol calculation.

Absorption

After the consumption of beer, wine or spirits, the alcohol (ethanol) contained in these beverages reaches the stomach where the absorption process begins. The rate of absorption occurs much faster from the upper part of the small intestine (duodenum and jejunum), owing to the infinitely larger absorption surface area provided by the microvilli covering the surface of epithelial cells. The effects of alcohol are felt very soon after drinking begins, which is proof of rapid absorption into the bloodstream and transport to the brain. The larger the amount consumed, and the faster drinking occurs, the higher the BAC and, accordingly, the more pronounced the effects on the individual (Zakhari 2006).

The absorption rate of ethanol is highly variable and depends on many factors (Friel *et al.* 1995). Alcohol taken in the form of beer or wine is generally absorbed more slowly than alcohol ingested as whisky or vodka, not only because of the lower concentration of ethanol but also because malt and fermented beverages contain sugars and other constituents that retard stomach emptying (Jones 2008). Eating a meal before or during drinking is effective in slowing the rate of absorption by delaying gastric emptying (Jones, Jonsson 1994b). Under these conditions, peak BAC is appreciably lower and the effects on the individual are less pronounced when the same dose of ethanol is taken after a meal compared with after an overnight fast (Millar *et al.* 1992).

The nature of the food in terms of its fat, protein or carbohydrate content seems less important than the amounts or bulk eaten (Jones *et al.* 1997). Thus a large portion of mashed potatoes proves just as effective as eating a beef steak in delaying the rate of ethanol absorption. Medication with drugs that alter gastric motility can either slow or accelerate gastric emptying and alter the speed of uptake of alcohol accordingly. Smoking cigarettes seems to delay the opening of the pyloric sphincter, thereby slowing the rate of ethanol absorption into the blood as reflected in a lower C_{max} and delayed t_{max} in smokers (Johnson *et al.* 1991).

The bioavailability of ethanol after oral administration is usually never 100%, especially if drinking occurs together with or after a meal. Some of the ethanol seemingly undergoes oxidative metabolism during contact with the gastric mucosa or during the first passage of blood through the liver. The existence of this first-pass metabolism needs to be considered when blood alcohol calculations are made, but unfortunately this is not always the case and misleading information is sometimes presented to the courts. To ensure 100% bioavailability, the ethanol dose must be administered intravenously, which is not the case in a typical drink–driving situation. Drinking on an empty stomach ensures that absorption is close to 100% if a moderate dose is taken (>0.5 g/kg) because ethanol dilution experiments have been used to estimate total body water (Jones 1984). The results of ethanol dilution experiments agree well with isotope dilution techniques (Endres, Gruner 1994).

Distribution

After absorption from the gut, the molecules of ethanol enter the portal venous blood and are transported by the bloodstream throughout the body to all organs and tissues. Ethanol distributes into the total body water space; unlike many other drugs, it does not bind to plasma proteins, and solubility in fat and bone is negligible (Wilkinson 1980;

Zakhari 2006). Concentrations of ethanol are initially highest in the portal venous blood. The hepatic portal vein carries alcohol to the liver and then the hepatic vein carries it to the heart; after the blood picks up oxygen in the lungs, ethanol is distributed into all the body fluids and tissues.

The speed of distribution and equilibration in the various organs and tissues depends on the heart minute volume and on the ratio of blood flow to tissue mass. The concentration of ethanol in blood and tissues at equilibrium depends not only on the dose ingested but also on the water content of the fluid analysed and the age, body weight and sex of the individual. The proportion of fat to lean muscle tissue in the body is an important determinant of the BAC reached at equilibrium. Women have a higher proportion of adipose tissue per kilogram of body weight than men, which makes females more vulnerable to the toxic effects of ethanol; they also achieve a higher BAC for the same ingested dose (Kwo *et al.* 1998; Li *et al.* 2000; Dettling *et al.* 2007).

Because ethanol distributes into the total body water without binding to plasma proteins, it is an easy task to convert a measured BAC into the quantity of alcohol absorbed and distributed into all body fluids at the time the blood was drawn. This calculation is accomplished using the Widmark equation (see below) or some modification (Watson *et al.* 1981). If the time of starting to drink is known, then the total amount of alcohol consumed can be calculated by adding the amount destroyed by metabolism (Gullberg, Jones 1994):

$$\text{Ethanol in body (g)} = \text{BAC (g/L)} \times \text{Body weight (kg)} \times \text{Distribution factor } (\rho)$$

The distribution factor (ρ, 'rho') is an important concept to understand in all types of blood alcohol calculations (Forrest 1986). The value of ρ depends on the person's age, sex and body composition, particularly the amount of fat to lean body mass (Yelland *et al.* 2008). Because alcohol distributes solely into water and not the fat, average ρ factors for women ($\sim$0.60 L/kg) are less than for men ($\sim$0.70 L/kg). These factors represent ratios between the water content of the entire body and water content of the blood, which is the fluid analysed in forensic work relating to enforcement of alcohol-impaired driving laws (Widmark 1981; Andreasson, Jones 1996b).

Total body water in men ($\sim$60% of weight) and women ($\sim$50% of weight) in health and disease have been determined by isotope dilution experiments (Schoeller *et al.* 1980). The water content of whole blood is close to 80% w/w as determined by desiccation or freeze drying (Iffland *et al.* 1999; Seidl *et al.* 2000). Blood water is actually slightly less in women because of their lower haematocrit (35–45%, mean 41%) compared with that of men (43–49%, mean 46%). Using broadly average values for blood and body water leads to the following distribution factors for ethanol: $60/80 = 0.75$ for men and $50/80 = 0.63$ for women. Experimentally determined values in non-obese healthy individuals agree well with average values of 0.7 for men and 0.6 for women (Martin *et al.* 1991; Norberg *et al.* 2001).

The ρ factor can vary widely between different individuals and much depends on the degree of obesity (Barbour 2001). A good starting point when considering an appropriate ρ factor is to calculate the person's body mass index (BMI), which is the ratio of body weight in kilograms to the square of height in metres (kg/m^2; Jones 2007a). A BMI over 30, which is considered as being clinically obese, should raise a warning flag to consider carefully the distribution factor ρ used in blood alcohol calculations. Unfortunately, only limited research has been done on the relationship between BMI and inter- and intra-individual variations in the distribution factors, although values as low as 0.40 L/kg are not unlikely in morbidly obese individuals (Jones 2007a).

Attempts have been made to update the basic Widmark equation as defined above to take into consideration variable body composition (more obesity) of people since the time of Widmark in the 1930s (Widmark 1932). Obesity is highly prevalent in today's society and needs to be considered in blood alcohol calculations, such as when calculating the person's BMI or estimating total body water (Watson *et al.* 1981; Seidl *et al.* 2000; Barbour 2001). Several computer programs can be purchased or are available gratis over the internet to perform blood alcohol calculations. Although some of these programs plot graphs showing the hypothetical blood alcohol concentration–time plots, the final results are seldom much of an improvement over use of a handheld calculator. The most important point to remember is uncertainty in the ρ factor, such as $0.7 \pm 20\%$ for men and $0.6 \pm 20\%$ for women. There might be a definitive advantage in computing a ρ factor by taking into consideration total body water (TBW) or BMI based on anthropometric information (age, height and weight), which has the advantage that the values obtained are tailored to the specific individual concerned.

Metabolism and excretion

The bulk of the dose of alcohol ingested undergoes oxidative metabolism by enzymes mainly located in the liver (Zakhari 2006). The proximate metabolite of ethanol is acetaldehyde, which is a highly toxic substance that is fortunately rapidly oxidised further to acetate (Meier-Tackmann *et al.* 1990). Most of the acetate leaves the liver with the hepatic venous blood and enters into intermediary metabolism, being converted into acetyl-coenzyme A, which then participates in the citrate cycle. Acetate is metabolised mainly in peripheral tissues to give the end-products carbon dioxide and water. After administration of ethanol labelled with ^{14}C, the end-product $^{14}CO_2$ is almost immediately detectable in the exhaled breath.

A small fraction of ethanol (<0.1%) is removed from the body by non-oxidative metabolism involving hepatic conjugation reactions to produce ethyl glucuronide and ethyl sulfate. These water-soluble metabolites are transported to the kidney where they are excreted into the urine. Compared with ethanol itself, ethyl glucuronide and ethyl sulfate are excreted in urine for a considerably longer time, thus making analysis of these metabolites a useful biomarker to disclose recent drinking. Other examples of non-oxidative metabolites include fatty acid ethyl esters, which are formed by the reaction of ethanol with short-chain fatty acids, and an enzyme-catalysed reaction with certain phospholipids to produce phosphatidylethanol. Although these non-oxidative metabolites account for only a small fraction (<0.1%) of the dose of ethanol administered, their analysis in blood, other body fluids and hair has attracted considerable research interest, both clinical and forensic. They are increasingly being used as biomarkers of acute and chronic alcohol ingestion.

Between 2% and 6% of the dose of alcohol ingested is excreted unchanged together with urine, expired air and in perspiration. Ethanol is easy to measure in urine, breath and sweat, and these alternative specimens are widely used in clinical and forensic work to test whether a person has recently consumed alcohol. The fate of ethanol in the body, including the excretion in breath, urine and sweat (2–6% of dose), oxidative metabolism into acetaldehyde and acetate (95%) and the formation of non-oxidative metabolites ($\sim$0.1%), is illustrated in Figure 4.10.

The liver is well equipped with enzymes (biological catalysts) specialising in detoxification of drugs, poisons and environmental chemicals that might enter the body with the food eaten or the air breathed. The oxidative enzyme mainly responsible for metabolism of ethanol is known as alcohol dehydrogenase (ADH) class I, which is located in the cytosol or soluble fraction of the hepatocytes (Ashmarin *et al.* 2000; Jornvall *et al.* 2000). ADH converts ethanol into acetaldehyde and this proximate metabolite is rapidly converted into acetate by low-K_m aldehyde dehydrogenase (ALDH), found in the mitochondria (Goedde, Agarwal 1987; Vasiliou, Pappa 2000).

Both ADH and ALDH display racial and ethnic differences in their catalytic activity including polymorphisms that have clinical consequences and determine a person's sensitivity to alcohol (Yamamoto *et al.* 1993). Many people of East Asian descent (e.g. Japanese and Chinese) inherit an inactive form of the enzyme that metabolises acetaldehyde, the low-K_m mitochondrial enzyme (Mizoi *et al.* 1983). These individuals are rendered highly sensitive to drinking small amounts of

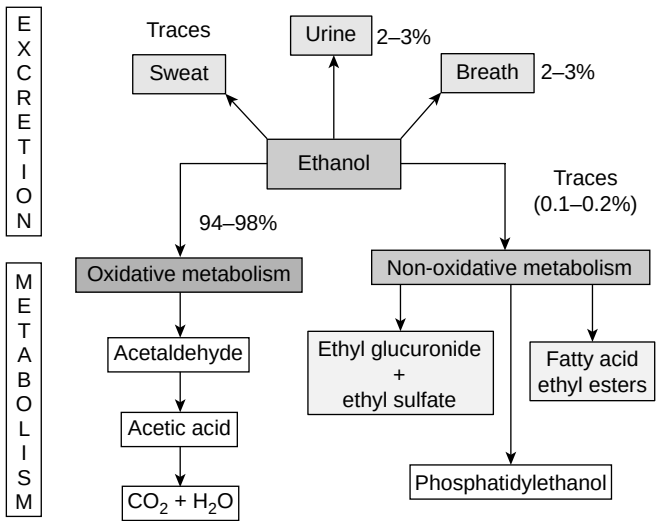

Figure 4.10 Fate of alcohol in the body, illustrating the amounts disposed of by excretion unchanged in urine, breath and sweat, and through metabolism in the liver by oxidative and non-oxidative mechanisms.

alcohol because the concentration of acetaldehyde in blood is higher, which triggers a range of unpleasant effects, including nausea, facial flushing, hypotension and tachycardia (Agarwal, Goedde 1987; Thomasson *et al.* 1993). This defect in the ALDH enzyme in East Asians creates a genetic aversion to drinking alcohol and protects them from becoming alcoholic or for that matter from DUIA. The prescription drug Antabuse is used clinically for the treatment of alcoholism and this aversion therapy works by blocking the metabolism of acetaldehyde, resulting in adverse effects due to the build-up of acetaldehyde after drinking alcohol (Karamanakos *et al.* 2007).

Besides involvement of the ADH pathway in the metabolism of ethanol, another important enzyme is located in the microsomal fraction of the hepatocytes, belonging to the cytochrome P450 (CYP) family, namely CYP2E1 (Lieber 1997, 2004). The cytochrome enzyme has a higher K_m for oxidation of ethanol (60–80 mg/100 mL) compared with ADH (5–10 mg/100 mL). This makes CYP2E1 more important for clearing ethanol from the bloodstream after a period of heavy drinking, as might occur in impaired drivers, who reach high BACs, e.g. 170 mg/100 mL on average. Another important feature of CYP2E1 is the phenomenon of enzyme induction, which is the property of metabolising substrate more effectively after continuous exposure, as occurs in binge drinkers (Teschke, Gellert 1986; Oneta *et al.* 2002). The rate of elimination of alcohol from the bloodstream might be as high as 36 mg/100 mL per hour in alcoholics when they enter detoxification (Pach *et al.* 1977; Jones, Sternebring 1992). However, after a few days of abstinence, this enhanced capacity to metabolise ethanol is seemingly lost as enzyme protein is newly synthesised (Keiding *et al.* 1983).

The disappearance rate of ethanol from blood has been investigated in hundreds of controlled dosing studies and 15 mg/100 mL per hour still remains a good average value for moderate drinkers (Widmark 1981; Jones 1984; Jones 1993). In alcohol-impaired drivers the average rate of ethanol elimination from blood is slightly higher, being closer to 19 mg/100 mL per hour because in this population of drinkers there is an overrepresentation of binge drinkers and alcoholics (Jones, Andersson 1996a). Another useful rule of thumb is that rate of elimination of ethanol from the whole body is close to 0.1 g/kg body weight per hour, which means that a person weighing 80 kg eliminates 8 g ethanol (100%) per hour or one unit of alcohol. Accordingly, in an elapsed time of 6 h after the start of drinking before sampling blood in a drink–driving case, ~48 g of pure ethanol is destroyed by metabolism (6×0.1 g/kg per h $\times 80 = 48$). Over the same period of time, the BAC decreases by 6×15 mg/100 mL per hour or 90 mg/100 mL.

Blood alcohol curves

The BAC reached after drinking alcohol depends on the quantity consumed, the speed of drinking, the efficacy of absorption into the blood, the distribution into body water and the removal of ethanol through metabolism and excretion. The fate of ethanol in the body is usually displayed in the form of a blood concentration–time (C–t) profile that depicts absorption, distribution and elimination processes. The shapes of BAC curves are likely to show considerable inter-individual variations, especially the parameters C_{max} and t_{max}, which denote the maximum concentration in blood and the time of its occurrence. Typical examples of such curves are shown in Figure 4.11 for nine healthy men who drank a bolus dose of ethanol (0.68 g/kg) as neat whisky on an empty stomach (Jones 1984).The C_{max} and t_{max} read from the curves are representative pharmacokinetic parameters and the ratio C_{max}/t_{max} is sometimes used to indicate the rate of absorption of ethanol into the bloodstream.

The magnitude of inter-individual variations in BAC curves is well illustrated by Figure 4.12, which shows the concentration–time data plotted on the same x–y coordinates. These curves were generated from drinking experiments with 12 healthy men who consumed 0.80 g ethanol per kg body weight in the morning after an overnight fast (Jones, Jonsson 1994a). The alcohol that they consumed was made from pure ethanol (95% v/v) after dilution to 20% v/v with sugar-free orange juice and the drink was finished in 30 min.

The experiment outlined above was expanded to include 53 male volunteers and mean and range of C_{max} and t_{max} as well as the rise in BAC after end of drinking before reaching C_{max} are shown in Table 4.8. The C_{max} ranged from 76 mg/100 mL to 145 mg/100 mL (two-fold difference) even for standardised drinking conditions, namely same beverage and drinking time and with the dose adjusted for differences in body weight. The time necessary to reach C_{max} was also highly variable and ranged from 0 min to 120 min, although most volunteers had peaked by 60 min post drinking. The mean rise in BAC measured from end of drinking to C_{max} was 33 mg/100 mL (range 0–77 mg/100 mL). This large inter- and intra-individual variation seen in controlled drinking experiments underscores the need for caution when expert witnesses and others testify in court about BACs expected based on various drinking scenarios.

Forensic pharmacokinetics of ethanol

Knowledge about the pharmacokinetics of ethanol is important in forensic science and toxicology because a person's BAC can be interpreted in terms of the amount of alcohol in the body or the total quantity consumed (Wagner *et al.* 1990; Brick 2006). Another common request in forensic casework is the estimation of the BAC at some time other than when blood was taken, such as the time of driving. This requires making a back-extrapolation or back-tracking of BAC, which is often a controversial practice owing to the many uncertainties involved, although certain guidelines can be given when this information is needed by the courts (Lewis 1987; Stowell, Stowell 1998).

As mentioned previously, Widmark administered known amounts of alcohol to volunteers and determined the concentrations (C) in capillary blood at various times (t) post drinking to establish the shapes of the resulting C–t plots (Widmark 1932). These experiments showed that the BAC first rose fairly rapidly as alcohol was being absorbed from the gut and a peak or C_{max} was reached after about 30–60 min post drinking. Thereafter, on reaching the post-absorptive phase, the BAC decreased at a more or less constant rate per unit time, which suggests that the elimination followed zero-order kinetics.

Figure 4.13 shows a typical blood alcohol curve for one male subject who drank 0.68 g ethanol per kg body weight as neat whisky in 30 min on an empty stomach (Jones 2010b). Pharmacokinetic parameters of ethanol are derived and shown on this plot using the method first proposed by Widmark (Andreasson, Jones 1996b; Widmark 1981). It is important to establish the linear segment in the post-absorptive declining phase so that this line is extrapolated back to the time of starting to drink, which gives the y-intercept (C_0). The intercept is interpreted as the BAC if

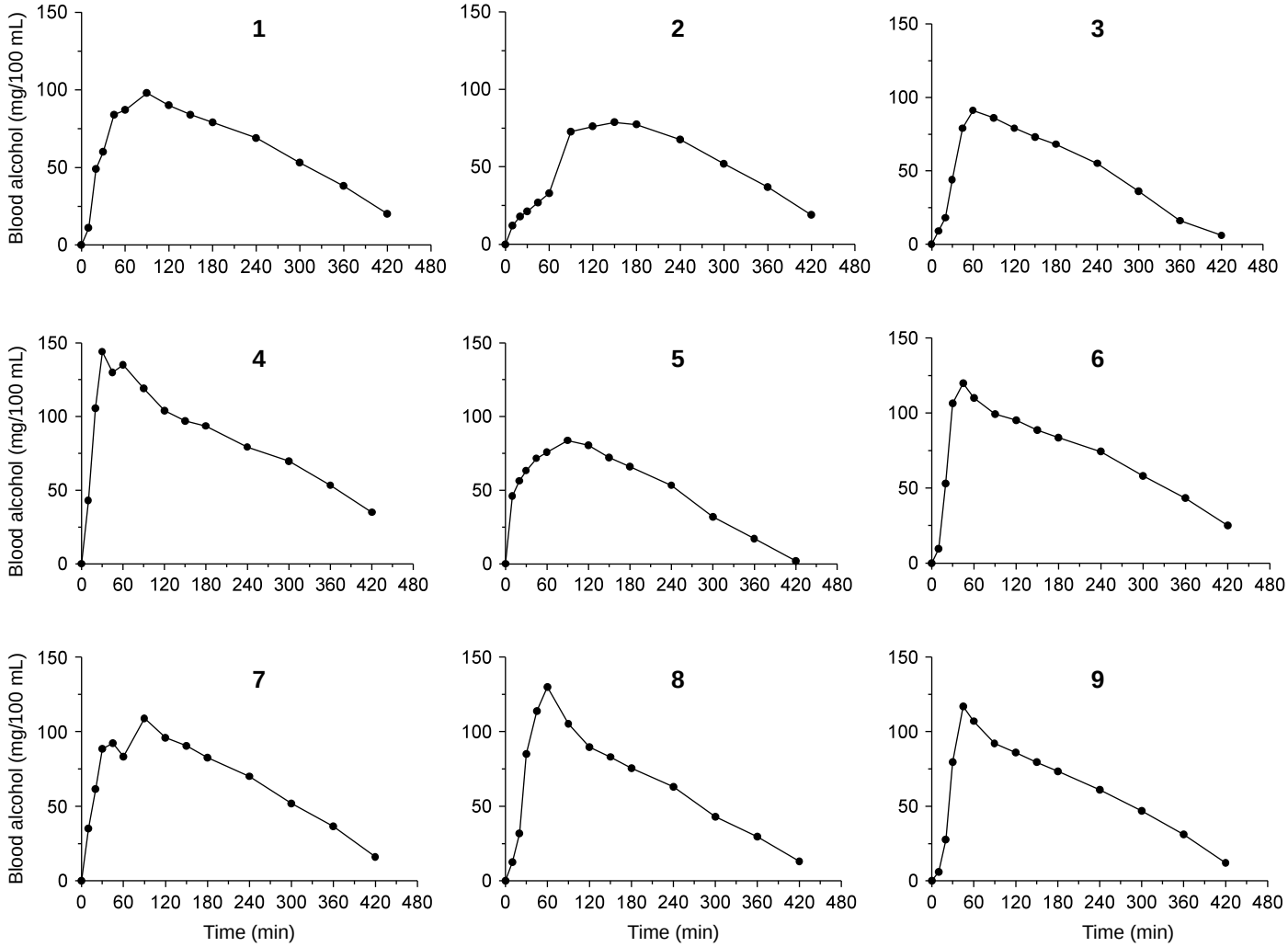

Figure 4.11 Individual concentration–time profiles of ethanol in nine healthy volunteers who drank a bolus dose of ethanol (0.68 g/kg) as neat whisky in 20 min on an empty stomach.

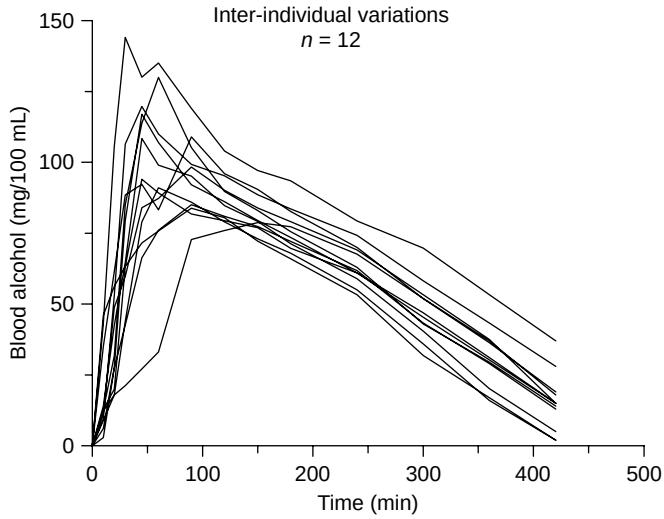

Figure 4.12 Inter-individual variations in blood alcohol curves in 12 healthy volunteers who drank a bolus dose (0.80 g/kg) as pure ethanol diluted with orange juice to 20% v/v and consumed in 30 min after an overnight fast.

absorption and distribution of the entire dose occurred instantaneously without any metabolism. The value of C_0 is used in the calculation of the slope of the elimination phase and other pharmacokinetic parameters, such as the volume of distribution (ρ factor). Widmark denoted the linear post-peak elimination phase by the Greek letter β and it is the best-fitting straight line to *C–t* data. This slope is sometimes reported as k_0 to denote a zero-order reaction. The value of β or k_0 is best derived by linear regression analysis of *C–t* data points and the method of least squares. The regression coefficient, which is the negative slope in the regression equation, $C_t = C_0 - \beta t$, is the elimination rate of alcohol from the bloodstream in concentration per unit time, such as mg/100 mL per hour.

Two different models are generally used to describe the pharmacokinetics of ethanol. The simplest and most practical for forensic purposes is that described above, namely zero-order kinetics. This method is appropriate provided that the BAC is above 20 mg/100 mL so that the hepatic metabolising enzymes are saturated with substrate. In almost all alcohol-related case work, whether impaired driving, sexual assault or alcohol-related deaths, forensic scientists are dealing with people having much higher BACs, as evidenced by an average BAC of 170 mg/100 mL in impaired drivers.

At blood ethanol concentrations below 20 mg/100 mL, the elimination rate of ethanol decreases and the *C–t* curve levels off, becoming

Table 4.8 Peak blood alcohol concentration (BACs; C_{max}), times needed to reach the peak and rate of increase in BAC to peak level after 53 subjects drank 0.80 g ethanol per kg body weight in 30 min after an overnight fast. The drink was made of ethanol solvent (95% v/v) and diluted with a sugar-free orange drink to a concentration of 20% v/v

N	*BAC (mg/100 mL)*		*Number of subjects reaching peak BAC*						*Rise in BAC (mg/100 mL)[a]*		
	C_{max}	*Range*	*0 min*	*15 min*	*23 min*	*60 min*	*90 min*	*120 min*	*Mean*	*Median*	*Range*
53	103	76–145	3	14	16	16	3	1	33	32	0–77

[a]Increment in the BAC after end of drinking and before peak (C_{max}) was reached.

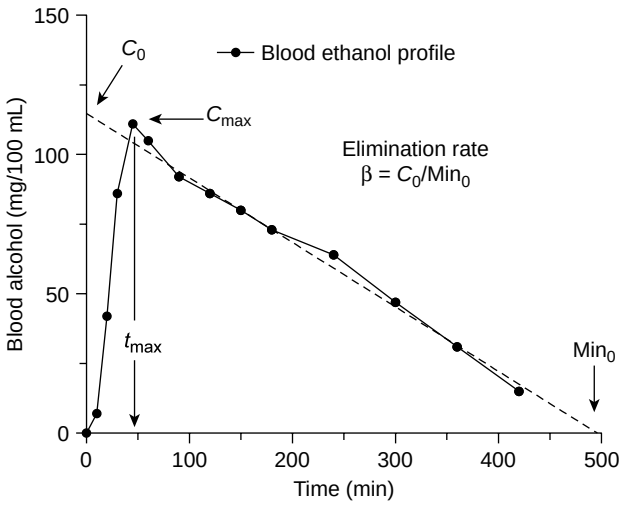

Figure 4.13 Representative blood alcohol curve for a male subject who drank a bolus dose of ethanol (0.80 g/kg) on an empty stomach with pharmacokinetic parameters derived according to Widmark's method.

curvilinear. The entire post-absorptive phase looks more like a hockey stick than a straight line (Figure 4.14). Pharmacologists generally consider that ethanol is metabolised by capacity-limited or saturation kinetics and not zero-order kinetics. Accordingly, the entire post-absorptive portion of the *C*–*t* curve is best described by the Michaelis–Menten equation, which also applies to other drugs such as γ-hydroxybutyrate, phenytoin and salicylic acid (Wagner *et al*. 1985; Wagner 1989; Rowland, Tozer 1995).

In the case of ethanol, the linear part changes into a curvilinear part as BAC drops below 20 mg/100 mL ($\sim 2 \times K_m$ of ADH). However, a pseudo-linear segment exists for about two-thirds of the post-absorptive elimination phase and below 20 mg/100 mL first-order kinetics operates, with an elimination half-life of about 15–20 min. The well-known rate equation for drugs with saturation kinetics is that developed by Michaelis and Menten:

$$-\frac{dC}{dt} = \frac{V_{max} \times C}{K_m + C}$$

where V_{max} is the maximum velocity of the enzymatic reaction, C is the substrate concentration and K_m is called the Michaelis–Menten constant and is a measure of the affinity of the enzyme for its substrate. The lower the K_m, the higher the affinity. The above equation collapses into two limiting forms depending on the concentration of substrate. When C is much greater than K_m, the Michaelis constant in the denominator can be eliminated from the equation and C cancels, so the elimination rate ($-dC/dt$) approaches its maximum velocity, $-dC/dt = V_{max}$ as expected for zero-order kinetics. When C is much less than K_m (at low BAC) the elimination rate ($-dC/dt$) becomes proportional to the substrate concentration; $-dC/dt = (V_{max}/K_m) \times C$, where V_{max}/K_m represents the reaction rate constant for first-order kinetics (k_1). This breakdown of the Michaelis–Menten equation is a consequence of capacity-limited kinetics and does not reflect any sudden change in the order of the biochemical reaction.

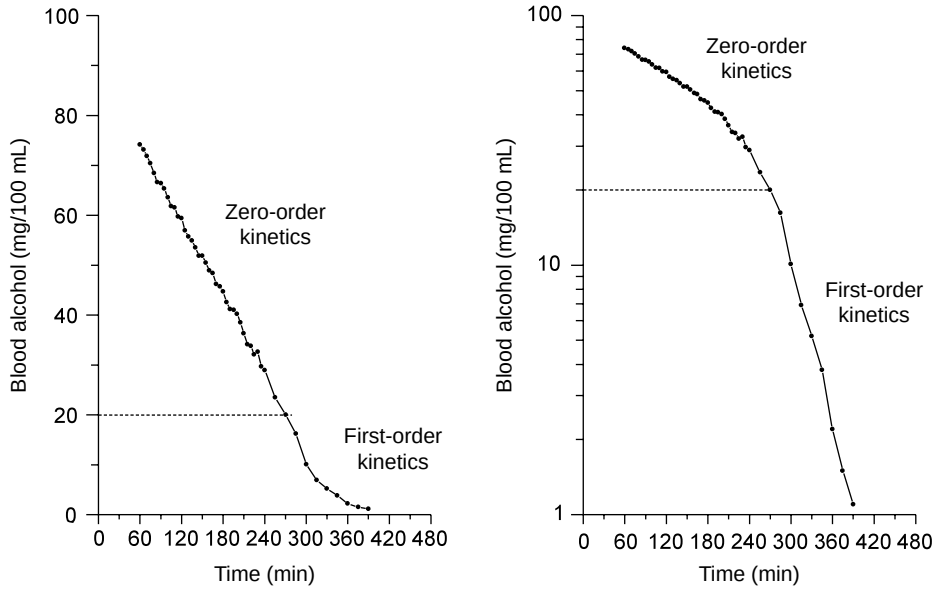

Figure 4.14 Blood alcohol concentration (BAC) curves demonstrating saturation kinetics of ethanol involving a zero-order model (BAC >20 mg/100 mL) and a first-order kinetic model at lower concentrations. Graph (a) is plotted on Cartesian coordinates and illustrates the hockey-stick shape of the curve; graph (b) shows the corresponding semi-logarithmic plot.

Absorption and elimination kinetics

Much less is known about the absorption kinetics of ethanol than about the elimination kinetics. The mathematical modelling of the absorption phase is complicated because uptake from the gut is sometimes so fast that insufficient *C–t* data points are available for curve fitting. Furthermore, ethanol is a drug absorbed from both the stomach and the upper part of the small intestines. Indeed, the rate of uptake of alcohol after passing through the pyloric sphincter is much faster than from the stomach. The speed of absorption and time of occurrence of C_{max} therefore depend to a large extent on factors that influence gastric emptying, such as fed or fasting state before drinking.

Unlike many prescription drugs, the dose of ethanol is not swallowed immediately, but is instead ingested in portions often over several hours. In most experiments dealing with the pharmacokinetics of ethanol, a single moderate dose is consumed in 5–15 min as a bolus. In the real world, people more often consume spirits, beer and/or wine repetitively in divided doses, which makes it much more difficult to model the shape of the BAC curve. Unlike most drugs, ethanol is absorbed to some extent through the gastric mucosa and ~20% of the dose enters the bloodstream in this way, although empirical support for this is not easy to find in the literature.

A mathematical model of the entire BAC curve is possible if it is assumed that absorption occurs according to first-order kinetics and that elimination, which occurs simultaneously, is a zero-order process. Under these assumptions, the entire concentration–time profile of ethanol can be fitted to the equation:

$$C_t = C_o(1 - e^{-kt}) - k_o t$$

where C_t = BAC at some time t after administration; C_0 = initial back-extrapolated BAC at the time of starting to drink; k = first-order absorption rate constant; k_0 = zero-order elimination rate constant; and t = time after drinking.

Factors that influence gastric emptying, such as food in the stomach, will delay the rate of absorption resulting in a lower C_{max} and delayed t_{max}. With a rapid absorption, the C_{max} might coincide with time of last drink or occur before the first blood sample is taken. With delayed absorption, as might occur under some circumstances, a plateau in the *C–t* profile is likely, which implies that the rates of absorption and elimination are the same. Other evidence exists that, even with delayed absorption, the BAC reaches about 80% of the final C_{max} within 15–30 min of the end of drinking (Jones, Neri 1991).

Because ethanol exhibits saturation-type kinetics, the peak BAC and the area under the curve (AUC) increase more than proportionally with increase in the dose administered, which is particularly evident after very small doses (Wagner *et al.* 1985). When the rate of delivery of ethanol to the liver is slow, the AUC is smaller for a given dose and vice versa (Levitt, Levitt 1994). The systemic availability (bioavailability) of ethanol must be considered when blood alcohol calculations are made for legal purposes. If some part of the dose of ethanol fails to reach the systemic circulation, owing to first-pass metabolism or other reason, then the results of calculations of BAC are obviously incorrect.

If only the post-absorptive phase of the *C–t* profile is considered, then the equation widely used in forensic science practice is:

$$C_t = C_o - \beta t$$

where C_t is the blood alcohol concentration, C_0 is the extrapolated concentration of alcohol in blood at the time of starting to drink (*y*-intercept) and β is the zero-order slope of the declining phase, sometimes denoted as k_0.

Disappearance rate of alcohol from blood

The slope of the pseudo-linear declining phase of the blood alcohol curve represents the rate at which ethanol is eliminated from the bloodstream. People who drink excessively over long periods of time develop a higher capacity to metabolise ethanol compared with moderate or occasional drinkers. This phenomenon, known as metabolic tolerance, is reflected in a steeper slope in the *C–t* plot during the post-absorptive phase and is often seen in alcoholics during detoxification after a period of binge drinking. Elimination rates of alcohol up to 35 mg/100 mL per h have been reported in these individuals. However, after a few days of abstinence, the rate returns to the expected 15 mg/100 mL per h (range 10–20 mg/100 mL per h) seen in moderate drinkers. The faster rate of metabolism is considered to reflect induction of the microsomal enzyme CYP2E1 as discussed earlier, and this occurs after heavy drinking lasting several days or weeks without an alcohol-free period. The quantities of alcohol necessary and the duration of drinking required to cause induction of the microsomal enzymes have not been determined in humans.

The information in Table 4.9 gives a likely physiological range of elimination rates of ethanol from blood in humans and the circumstances under which such rates might be encountered (Jones 2010b). Accordingly, for the vast majority of people, the rate of ethanol elimination is likely to vary three-to-four fold or from 10 per 35 mg/100 mL per h. In blood alcohol calculations for research and legal purposes, back-extrapolation with an alcohol burn-off rate of 10–25 mg/100 mL per h is appropriate (mean 15 mg/100 mL per h). In apprehended drunken drivers, because of the many alcoholics in this population, the average elimination rate of alcohol from blood is 19 mg/100 mL per h, being slightly faster in women than in men (Jones, Andersson 1996a).

The information about elimination rate of ethanol in impaired drivers has been determined by taking two blood samples about 1 h apart in over 1000 offenders. Under the assumptions of zero-order

Table 4.9 Rates of ethanol elimination from blood that might physiologically be observed under various conditions or circumstances

Elimination rate	*Expected values (mg/100 mL per h)*[a]	*Conditions or circumstances in which such rates might be expected*
Very slow	8–10	Malnourished individuals or people eating low-protein diets. Medical conditions such as advanced liver cirrhosis or portal hypertension and reduced hepatic blood flow. Administration of a drug (e.g. 4-methylpyrazole or fomepizole) that blocks the action of alcohol dehydrogenase
Slow	10–13	Healthy individuals after overnight (10 h) fast and a single bolus dose of ethanol (<1 g/kg)
Moderate	13–17	Regular drinkers after consumption of alcohol in the fed state and consumption of a moderate dose to reach BAC of 1.0 g/L
Rapid	17–25	Typical values seen in many apprehended drivers according to evaluation of double blood samples
Ultra-rapid	25–35	Heavy binge drinkers or alcoholics during detoxification with high starting BAC of 200–300 mg/100 mL. Caused by induction of microsomal enzyme (CYP2E1) through chronic exposure to ethanol or other drugs. People with a genetic predisposition for rapid metabolism of ethanol. Medical condition leading to a hypermetabolic state (e.g. burn trauma)

Modified from Jones (2010b, 2008).
[a]Values below 8 mg/100 mL and above 35 mg/100 mL per hour should be considered suspect and details of the study carefully examined, including dose of alcohol administered and the shape of the resulting BAC curve.

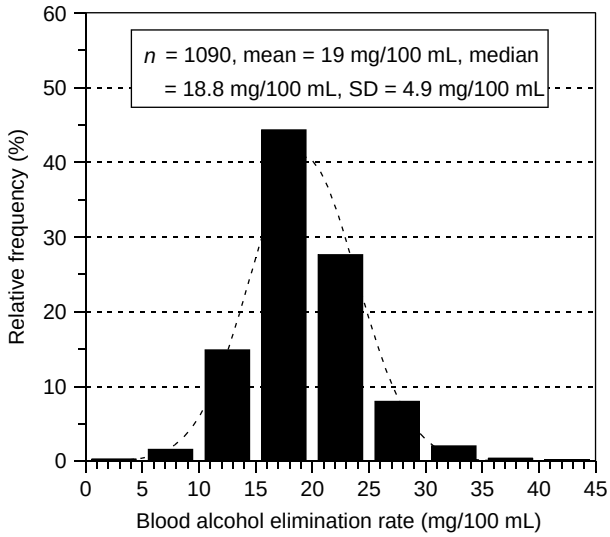

Figure 4.15 Relative frequency distribution of the elimination rates of ethanol from blood determined by taking two blood samples about 1 h apart from apprehended drunken drivers.

kinetics and the existence of the post-absorptive limb of the BAC curve when the first blood sample was taken, the elimination rate (β) of ethanol is given by:

$$\beta(\text{mg/100 mL per hour}) = (\text{BAC}_1 - \text{BAC}_2)/t_{\text{diff}}$$

In the above equation, BAC_1 is the concentration of ethanol in the first blood sample, BAC_2 is the concentration in the second sample and t_{diff} is the time difference between sampling in hours. Support for this method was also obtained when three consecutive blood samples were available from suspected drunken drivers.

A relative frequency distribution of the elimination rates of ethanol from blood in apprehended drivers derived from double blood samples is shown in Figure 4.15 (Jones, Andersson 1996a). This bell-shaped curve seems a good approximation to a normal distribution, which permits calculation of the proportion of individuals having defined elimination rates of alcohol in the entire population of offenders. These rates are given by multiples of the SD (9 mg/100 mL per h) such that 95% of people are expected to be between 9 and 29 mg/100 mL per h. Extreme values, such as less than 9 mg/100 mL per h, probably reflect individuals with unusually slow rates of absorption of ethanol and as such might not have reached their peak BAC when the first blood sample was taken. Extremely rapid rates of elimination are more difficult to explain. Some very high values might be artefacts caused by problems in sampling blood or incorrect recording of the blood-sampling times on the tubes. Enhanced metabolic activity in some individuals gives them an ultra-rapid rate of ethanol elimination and values as high as 35 mg/100 mL per h have been documented.

Back-extrapolation

The process of back-extrapolation (back-tracking) the blood or breath alcohol concentration from the time of sampling to the time of driving has many proponents and many critics (Lewis 1987; Al-Lanqawi *et al.* 1992; Ferner 1996). With only a single blood or breath sample available, nothing is known about the pharmacokinetic profile of ethanol, such as the stage of ethanol metabolism or whether the BAC was rising, declining or on a plateau at the time of the traffic offence (Jackson *et al.* 1991). Neither is the disappearance rate of ethanol from blood known in any individual cases, although two blood samples taken 60 min apart can give useful information on this point (Jones, Andersson 1996a). Much work has been published on the rate of metabolism of ethanol in controlled drinking studies and in alcoholics during detoxification. The results demonstrate that the slope of the descending limb of the BAC curve varies as much between different subjects as it does within the same subject on different occasions (Jones, Jonsson 1994a; Yelland *et al.* 2008). This makes it rather pointless to perform alcohol drinking studies to determine that person's capacity to eliminate alcohol from the bloodstream. A more pragmatic approach is to work with a range of values such as 10–25 mg/100 mL per h if and when back-extrapolation is required by the courts. If evidence of the offence of drink driving involved breath alcohol tests, then the elimination rates of alcohol from breath should be utilised in the back-tracking calculations, such as 4.4–10.9 μg/100 mL per h for low and rapid elimination, respectively. These breath alcohol elimination rates were derived from BACs of 10 and 25 mg/100 mL per h and assuming a 2300 : 1 BAC : BrAC ratio as practised in the UK.

Examples of back-calculating a measured blood or breath alcohol concentration from the time of sampling to the time of driving are given in Table 4.10. Slow, medium and rapid rates of alcohol elimination are assumed and used in the calculation along with elapsed times of 1–5 h between driving and blood sampling or analysis of breath. One necessary assumption for back-calculating is that the person had reached the post-peak part of the concentration–time profile at the time of the offence and at the time of sampling of blood or breath. Support for this assumption requires careful questioning and consideration of the prior drinking pattern, such as the type and time of last drink and the amount of alcohol it contained in relation to the time of driving or an accident. If 60 min or more had elapsed after the end of drinking to the time of the offence, this makes it highly unlikely that the person was still absorbing alcohol. Studies have shown that, after drinking alcohol over several hours, the small amounts contained in the last drink before driving have no marked influence on C_{max} or time to reach peak BAC (Jones *et al.* 2006).

Drunk-driving defence challenges

The enforcement and prosecution of alcohol–driving laws in most nations is nowadays based almost entirely on the concentration of

Table 4.10 Examples of back-calculation of BAC and BrAC from time of sampling to time of driving. The calculation assumes a measured BAC of 60 mg/100 mL or a BrAC of 26 μg/100 mL and low, median and high rates of alcohol elimination for times (*t*) of 1–5 h

Rate of ethanol elimination	*Blood (mg/100 mL per h) Breath (μg/100 mL per h)*	*$BAC_1 = BAC_2 + (\beta \times t)$ $BrAC_1 = BrAC_2 + (\beta \times t)$*				
		t = 1 h	*t = 2 h*	*t = 3 h*	*t = 4 h*	*t = 5 h*
Slow	Blood = 10.0	70	80	90	100	110
	Breath = 4.4	31	35	39	44	48
Medium (most probable)	Blood = 15.0	75	90	105	120	135
	Breath = 6.6	33	39	46	52	59
High	Blood = 25.0	86	110	135	160	185
	Breath = 10.9	37	48	59	70	81

alcohol determined in a specimen of blood or breath from the suspects. Such laws are known as 'alcohol concentration *per se*' laws and represent a considerable simplification over the older 'under the influence' or impairment-based DUIA laws. Under a *per se* statute, the prosecution are not required to prove that a driver was unfit to drive as a result of excess alcohol and instead needs to show that only the concentration of ethanol was at, or above, a certain statutory concentration (Voas 1991). The *per se* law is therefore a much more pragmatic way to prosecute offenders, but at the same time draws considerable attention to the reliability of the sampling and the analysis of specimens to arrive at the concentration of ethanol (Jones, Logan 2007).

The use of evidential breath alcohol testing commenced in European countries in the early 1980s when more automated and reliable technology had become available. Breath alcohol testing cannot totally replace the need for blood sampling because some people are genuinely unable to provide a proper sample for various health or medical reasons. Lung dysfunction in some people of short stature or in smokers may result in them being incapable of providing a continuous forced exhalation for a sufficiently long time, such as 6 seconds, at a fixed pressure and flow rate. However, in this connection, a breath sample captured after an exhaled volume of only 500 mL gives a good indication of the concentration of alcohol in end-exhaled breath. Some people refuse to cooperate with the police in providing a proper breath sample, whereas a blood sample can be taken by force if necessary. Furthermore, a person injured in a traffic crash might need emergency hospital treatment, so that obtaining blood is more practical than breath sampling at a police station. The advantages and limitations of blood versus breath alcohol testing are summarised in Table 4.11.

Punishments and sanctions for driving under the influence of alcohol are becoming increasingly burdensome and include immediate revocation of the driving licence for 1–2 years, monetary fines, house arrest and community sentences. A person with a BAC or BrAC considerably over the legal limit is liable to receive enhanced penalties for a DUIA conviction. A person with a BAC over 100 mg/100 mL in Sweden risks losing the driving permit for 2 years and is also expected to re-take the driving test. Before re-taking the test, the authorities require proof that the drinking habits of the individual concerned are moderate. This is achieved by medical examination, questionnaire and psychological assessment including use of biomarkers for overconsumption of alcohol.

The widespread use of alcohol concentration *per se* laws means that analysis of alcohol in body fluids provides the gold standard evidence for prosecution and other information about the person's behaviour and driving ability is not needed to win the case. A *per se* law is easier to enforce because of the clear-cut definition of when an individual is breaking the law. Such a law makes challenging the correctness of the results of the chemical analysis of alcohol in blood, breath or urine the major factor in a defence and a host of imaginative ideas have been tested and documented in drink–driving case law.

The legal framework behind a concentration *per se* law places special demands on the reliability of the methods used to determine alcohol in blood and breath and quality assurance of the entire process. The accuracy, precision and specificity of the methods of analysis must conform to high standards and the elements of analytical quality control must be rigorously enforced. This should include regular external proficiency tests of laboratory performance or reliability of the evidential breath analyser by testing needs to be verified by analysis of known-strength simulator solutions of ethanol. Other necessary scientific safeguards to boost the quality of the results are well-constructed rules and regulations governing sampling, labelling, transport, preservation and storage of body fluids. Finally an allowance should be made to compensate for uncertainty in the analysis and to ensure that a suspect is not over the legal limit because of inherent analytical errors.

In the USA many lawyers and law firms specialise in defending alcohol-impaired drivers and have developed a high level of expertise in the science and pitfalls of alcohol testing in biofluids.

Blood and urine samples

Examples of the top 10 challenges directed against the results of blood alcohol analysis when used as evidence for the prosecution of DUIA offenders are given in Table 4.12 and their strengths and weaknesses have been reviewed elsewhere (Jones, Logan 2007). Experience has shown

Table 4.11 The advantages and disadvantages of blood (BAC) and breath (BrAC) alcohol analysis for prosecuting alcohol-impaired drivers

BAC	*BrAC*
Less influenced by sampling and physiological variations – higher overall precision	More prone to breath sampling and physiological variations
Blood specimens can be taken from living, dead and uncooperative persons	Only feasible with people willing to cooperate with the sampling procedure
Blood sampling is possible regardless of state of health, e.g. hospitalised victims of traffic crashes	Some individuals, owing to respiratory dysfunction (asthma or chronic obstructive pulmonary disease), genuinely fail to meet sampling requirements of some instruments
Blood ethanol concentration is relatively stable during storage decreases by 3 mg/100 mL per month	Storage of breath samples is more problematic and long-term stability of ethanol is questionable
Confirmatory analysis of blood sample is easily done because specimens are usually stored for up to 12 months at 4°C	It is possible to store breath for later analysis but this procedure is more troublesome
Drugs other than alcohol can be determined in the same blood sample	Not possible with breath samples
Blood samples can be used to analyse for biomarkers of heavy drinking and damage to the liver	Not possible with breath samples
Longer time to obtain results of blood alcohol analysis as samples must be shipped to a laboratory	Use of breath alcohol analysis gives on-the-spot results of alcohol influence
Sampling of blood is an intrusive procedure	Breath sampling is non-intrusive
A physician, nurse or phlebotomist, for a fee, takes the blood specimens	Trained police officers operate the instruments used for breath alcohol analysis
Time delay of about 30–120 min after the time of driving until sampling blood	Breath analysis can in principle be made at the roadside much closer to the time of the driving offence
Less subject to pre-analytical variations, such as the method or equipment used to take blood	Results of breath alcohol analysis are influenced by the subject's breathing pattern just before or during delivery of a sample
Blood samples can be taken any time after drinking and still give valid results	Breath analysis is suspect if done within the first 15 min after end of drinking
The concentration of ethanol in blood depends to some extent on sampling site, whether from an artery, vein or capillary	The time course of breath alcohol runs much closer to the arterial blood rather than the venous blood alcohol concentration

Table 4.12 Examples of the types of challenges aimed at discrediting the admissibility or validity of blood alcohol test results when alcohol-impaired drivers are prosecuted

Rank	*Challenges to blood test results*	*Brief description and references*
1	Hip-flask defence[a]	The suspect maintains that alcohol was consumed after driving or involvement in a crash but before the blood or breath sample was obtained (Iffland and Jones 2002; Simic *et al.*, 2004)
2	Laced drinks	Above the legal alcohol limit for driving but without intent; someone had spiked beer with additional alcohol (spirits) without the drinker's knowledge (Langford *et al.* 1999)
3	Rising blood alcohol defence	Driving shortly after the last drink, possibility of being below the legal limit at the time of driving but above the limit 1–2 h later when the blood sample was taken (Jones *et al.* 1991)
4	Unintentional intake of alcohol	Driving after being engaged in wine tasting or after use of medication containing alcohol, such as cough syrups or vitamin preparations or possibly a drug–alcohol interaction (Franklin and Stephens 2000)
5	Contamination of blood with alcohol during collection	Swab used to clean the skin before sampling blood contained alcohol (Peek *et al.* 1990)
6	Blood specimen was coagulated or diluted or wrong tubes were used	Evacuated tubes contained incorrect chemical preservative or the volume of blood specimen was deficient, or might be diluted with intravenous fluids during sampling (Riley *et al.* 1996; Jones and Fransson 2003)
7	Discrepancy in analytical results reported by an independent laboratory	Large discrepancy between analytical result from the government laboratory and that from a private laboratory (Oliver *et al.* 1975)
8	Mix-up of specimens[b]	Alleged mix-up of the blood specimen either at the police station or the forensic laboratory
9	Endogenous production of alcohol	Alcohol produced naturally in the body by the action of yeasts or bacteria on dietary sugars (Logan and Jones, 2000; Al-Awadhi *et al.* 2004)
10	Phobia of blood sampling	Alleged phobia of the sight of blood and needles, genuine failure to provide a specimen (Gudjonsson and Sartory 1983)

[a]Also a common argument against results of breath alcohol testing.
[b]Resolved by re-analysis of ethanol content or by DNA testing.

that most challenges are bogus and are simply intended to confuse the non-scientist and hopefully raise a reasonable doubt in the eyes of the judge or jury. Issues related to blood sampling procedure (pre-analytical), the chain of custody, the way the laboratory analysis was performed or whether the suspect suffered from some medical condition that meant that BAC was higher than expected for the amount consumed are often raised (Jones, Logan 2007).

When a venous blood sample is taken for the determination of ethanol, obviously the skin should not be swabbed with an alcohol-containing liquid. Studies have shown, however, that when evacuated tubes are used to draw blood the risk of carry-over and contamination of the blood specimen with alcohol from the swab is negligible (Goldfinger, Schaber 1982). If an alcohol swab had inadvertently been used to disinfect the skin, this raises a suspicion in the mind of a jury that the blood might have been contaminated (Peek *et al.* 1990).

The convictions of a large number of DUIA offenders were quashed after they had pleaded guilty to the charge (*Regina v Bolton Magistrates Court Ex parte Scally*). Evidence surfaced after the trials that the swabs used to sterilise the skin before sampling blood were impregnated with alcohol. According to the analytical reports, all suspects were above the legal limit for driving (80 mg/100 mL) and as such they pleaded guilty to the charge of driving with excess alcohol in blood. Interestingly, the convictions were quashed not because of scientific evidence showing that blood *de facto* might have been contaminated with alcohol, but because the suspects were not informed by the prosecution that alcohol swabs had been used when they were asked to enter a plea of guilty or not guilty. In another UK case, a deficient volume of blood in the tubes sent for analysis was alleged to give a higher than expected concentration of alcohol when analysed by HS-GC because of salting out. This notion was tested experimentally and no such increase was observed; rather, there was a small decrease in BAC when the volume of blood in the tube was deficient (Jones, Fransson 2003; Miller *et al.* 2004). However, at trial the prosecution did not call expert evidence to rebut the defence argument about salting out and the suspect was duly acquitted.

As mentioned previously, an important safeguard when urine is the specimen used for determination of alcohol is to ensure that the tube contained sodium fluoride (1% w/v) as a preservative (Jones *et al.* 1999). In the UK it is recommended that the fluoride concentration should be in excess of 1.5%. Secretion of glucose in the urine is likely in diabetics or in those with other disturbance in carbohydrate metabolism. If these individuals also have a urinary tract or yeast (*Candida albicans*) infection, without fluoride alcohol might be produced post-sampling by fermentation of glucose (Alexander *et al.* 1988; Saady *et al.* 1993). The question of residual urine having a higher concentration of alcohol than was present in the blood at the time of sampling is avoided by collecting two voids 60 min apart. The well-known physiological variation in urine : blood ratios of alcohol is a non-issue because of a statutory definition of 107 mg/100 mL urine as being the same as 80 mg/100 mL blood (Curry 1972).

Breath samples

In terms of analytical precision, accuracy and specificity, blood alcohol determinations by GC are far superior to the results of breath alcohol analysis using the latest generation of instruments. A major reason for this is the dominance of physiological variation inherent in sampling and analysis of ethanol in the end-exhaled air, whereas a blood alcohol analysis is essentially devoid of physiological sources of variation (Gullberg 2003).

Among many quality assurance and procedural aspects of evidential breath alcohol testing, none is more important than the 15-min observation and deprivation period (Gullberg 1992; Wigmore, Leslie 2001). The result of a breath alcohol test is not considered valid if this is made within 15 min (20 min in some countries) after a person last consumed alcohol or had some alcohol-containing liquid in the mouth, such as cough medicine or an oral hygiene product (Fessler *et al.* 2008). Some of the latest breath alcohol instruments are capable of monitoring the authenticity of the exhalation profile of ethanol from its slope and waviness. The existence of an abnormal curve is one indication of contamination with 'mouth alcohol'. However, the slope detector is not foolproof, especially when the oral mucosa is contaminated with relatively low concentrations of alcohol (Buczek, Wigmore 2002).

Table 4.13 Examples of the types of challenges directed against the admissibility or validity of breath alcohol test results when alcohol-impaired drivers are prosecuted

Rank	*Challenges to breath test results*	*Brief description and references*
1	Erroneous or impossible result	Denial of drinking and claiming that a medical condition or a legally prescribed drug was responsible for the excess alcohol. Alternatively, a medication being used led to abnormally slow metabolism of alcohol (Gomm *et al.* 1990; Logan *et al.* 1998)
2	Alcohol in the mouth (mouth alcohol effect)	False high result caused by 'mouth alcohol' from recent drinking or regurgitation of stomach contents containing alcohol (Kechagias *et al.* 1999; Wigmore and Leslie 2001)
3	Interfering volatile substances present in the breath sample	Inhalation of organic solvent vapours by exposure in the workplace, uptake into the body via the lungs and lack of selectivity of the breath analyser (Denney 1990; Gill *et al.* 1991a, 1991b, 1991c)
4	Faulty instrument	Large difference between duplicate determinations of breath alcohol, error in calibration or failure to obtain zero results from room air
5	Inability to provide a proper breath sample	Lung dysfunction, (e.g. asthma or chronic obstructive pulmonary disease) prevented the person from providing a sufficient volume of breath to satisfy the requirements of the instrument or use of inhalers containing alcohol (Gomm *et al.* 1991; Ignacio-Garcia *et al.* 2005)
6	False high reading	Alcohol trapped under dentures or denture adhesives or production in the oral cavity by yeast infection (*Candida)* in mouth or upper airway. Blood (and alcohol) in the mouth during breath testing from injuries sustained in a crash (Harding *et al.* 1992; Trafford and Makin 1994; Chu *et al.* 1998)
7	Medical condition	Subject suffers from a medical condition or metabolic disturbance that allegedly produces volatiles in the breath indistinguishable from ethanol, e.g. acetone, methane, hydrogen (Marks 1984; Logan *et al.* 1994)
8	Elevated body and breath temperature	Suffering from a fever at the time of the breath alcohol test; alcohol concentration increases by 6.5% per 1°C rise in body temperature (Fox, Hayward 1989)
9	Blood : breath ratio	Tests were made during the absorption phase when the blood : breath ratio of alcohol was abnormally low owing to arterial–venous differences (Jones 1978; Jones *et al.* 2004)
10	Instrument malfunction	Printout from the instrument corrupted in some way, showing wrong date or time of the test (Denney 1998)

This might occur if a person suffers from gastric reflux and regurgitates stomach contents just before providing the breath sample (Jones 2007b). Stomach contents contain appreciable amounts of alcohol if the absorption rate is unusually slow and prolonged.

Table 4.13 gives examples and supporting literature citations to many well-documented challenges to the validity of results of breath alcohol analysis for legal purposes. As already mentioned, some people, especially those suffering from lung dysfunction, might be incapable of providing a proper sample owing to low forced expiratory volumes (Honeybourne *et al.* 2000; Stephens and Franklin 2001). This makes it advisable to allow a blood or urine option in these special cases, which is a better approach than allowing a police officer or instrument operator to decide whether the suspect was purposely failing to provide a specimen.

Concluding remarks

Road traffic crashes caused by drunken drivers will always plague society for the simple reason that alcohol is a legal drug and social drinking is an accepted part of normal life. Alcohol is readily available for purchase almost without restriction and is enjoyed by the vast majority of people in public places, such as restaurants and bars, making it necessary to obtain transport home afterwards. Many factors contribute to the cause of traffic crashes, not least of which is the human element and impairment of the driver caused by irresponsible use of alcohol or other psychoactive drugs.

This overview of DUIA has focused on many aspects of the problem including different types of legislation, alcohol-related impairment, methods used for analysis of alcohol in blood, breath and urine, the disposition and fate of alcohol in the body, and a plethora of defence arguments challenging the use of BAC or BrAC as evidence for prosecution of offenders. Alcohol-impaired drivers in most countries are typically men (85–90%) aged 35–45 years, which suggests that campaigns to improve road traffic safety and deterring DUIA should be directed specifically at this risk group of individuals. Teaching about the health hazards of excessive drinking and ethanol-induced impairment of skilled tasks, such as driving, should start during adolescence, such as in high schools, before young people reach the age to take a driving test.

The high average BAC values found in apprehended drivers in most countries (160–170 mg/100 mL) make it abundantly clear that these individuals are not moderate drinkers. Many apprehended drivers, especially repeat offenders, suffer from a substance abuse problem and they might be diagnosed as being clinically alcohol-dependent. This suggests that more attention should be given to the underlying alcohol-use disorder when offenders are sentenced. Instead of the more conventional penalties for this traffic offence (fines or imprisonment), it might prove more beneficial to focus on psychological counselling and rehabilitation for alcohol abuse.

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CHAPTER

5 Driving Under the Influence of Drugs

BK Logan and MD Osselton

Introduction

Forensic aspects of alcohol, drugs and driving

The impact of the toll caused by driving under the influence of drugs (DUID) became more and more apparent over the decade ending in 2010. Numerous initiatives in Europe and the USA have led to drug testing of drivers stopped at survey or enforcement checkpoints (Beirness, Beasley 2010), arrested during random or for-cause traffic stops (Senna *et al.* 2010), and killed in motor vehicle crashes (Schwilke *et al.* 2006); this has demonstrated an incidence of drug use in the driving population that mirrors or in some cases exceeds the rates of alcohol use. With this increased recognition of its prevalence, additional efforts have been directed into drug-impaired driving enforcement (Boorman, Owens 2009) and comprehensive testing, and a variety of legislative approaches have been adopted to enable prosecution of drug-impaired drivers (Walsh *et al.* 2002). Also of concern is the issue of combined drug and alcohol use, as studies including those cited above suggest that as many as 50% of impaired driving deaths involving alcohol may also be positive for drugs. Drugs and alcohol together can act in either an additive or a synergistic manner, causing greater impairment than would be expected when either substance is taken on its own.

Recognition of the role played by alcohol in impairment and crash causation has led to the development of effective processes to legislate against it, strategies to detect and document alcohol impairment, and provisions to sanction, treat and rehabilitate offenders. The resources for addressing the drug-impaired driving problem have been slower to develop but are gaining more attention as the scale of the problem becomes more widely known. Forensic toxicologists play an important role in many phases of this problem. In the area of research, they assist with study design, contributing insight into the drugs most frequently encountered in the driving population, and implementing novel analytical techniques such as oral fluid (OF) testing, as a convenient means of obtaining a timely, less-invasive sample. Toxicologists also participate in the training of law-enforcement personnel to ensure that they appropriately apply the use of clues or indicators of drug use in assessment tools, and understand the dangers and adverse effects of those drugs on driving. Increasingly this training is also being provided to the lawyers who try the cases, and to the judges who have to decide these complex scientific issues, and toxicologists play a key role in such educational programmes. In the laboratory, the toxicologist will design and validate methods for the detection and quantification of drugs in biological specimens and also ensure that analytical procedures meet national or international standards of good practice and that test results are appropriately reported and legally defensible. The toxicologist typically consults with the lawyers in the trial preparation phase, providing education and perspective on the strengths and weaknesses of the test results and any limits to their interpretation. At trial, the toxicologist provides unbiased and objective evidence about the chain of custody and the analytical results. The toxicologist also has a role in educating the jury as to the likely effects. The consequences to the offender (arrest, detention, trial, legal expenses, fines, loss of licence, imprisonment) are serious, and defendants deserve assurances that the laboratory employs appropriate analytical safeguards and standards, and that the individual who interprets the results has the appropriate education and qualifications.

This chapter will consider the roles of the toxicologist in all these aspects of DUID and some of the considerations that help to ensure that valid and meaningful results and testimony can be provided to the court.

Enforcement and prosecution

There are two main approaches to legislating against drivers who drive while under the influence of drugs. The first is an impairment standard, under which a driver is guilty of the crime of driving under the influence (DUI) or driving while intoxicated/impaired (DWI) if it can be shown that their driving ability is affected by drug or alcohol use. The statutory language used varies widely. This important variable is often overlooked in the quest to derive thresholds for impairment associated with blood drug concentrations. As discussed later, there are several barriers to establishing these thresholds, not the least of which is that legislative standards vary by state and country. For example, in the USA individual jurisdictions use language such as 'impaired to the slightest degree', 'such that the person cannot exercise the same standard of care as a sober person', 'so that the person is less able than the person ordinarily would have been' and so on, which seem to require different standards of evidence. This 'affected by' approach is the most straightforward, and relates the person's behaviour to the crime of impaired driving. Evidence to support these charges may include:

- Appearance (flushed face, bloodshot eyes, pinpoint or dilated pupils)
- Speech (slow, slurred, mumbling, erratic, confused, etc.)
- Response to questions
- Ability of the suspect to produce a driver's licence from their purse or wallet
- Coordination on exiting the vehicle
- Ability to stand without swaying or using a support for balance
- Performance of simple tests of coordination
- The presence of gaze nystagmus (an involuntary jerking of the eye)
- Divided attention and balance.

All of the above provide cumulative evidence that a subject may be in a state in which the cognitive and psychomotor skills needed for safe driving are to some degree and for some reason deficient. Having established the presence of impairment, the investigation then proceeds to confirming by chemical tests that the reason for the deficit is use of alcohol and/or drugs.

Alcohol is generally the first substance to be tested for, as reliable portable breath tests allow testing for alcohol use in the field at the point of contact or arrest (Dubowski, 1994). If elevated, the breath alcohol concentration may help to explain the person's behaviour, and if low or negative it may provide reasonable suspicion that the person is under the influence of a drug, leading to collection of a blood or oral fluid sample. In the USA these indicators of intoxication may be needed legally to provide 'probable cause' (a higher standard than reasonable suspicion) to place a subject under arrest in order to allow the collection of a biological sample for testing. In the UK a blood specimen can be collected only if a medical practitioner (the forensic medical examiner or FME) is prepared to state that he or she believes that the driver is impaired through the use of drugs. In some European countries and Australian states, biological samples can be collected simply by demand of the law-enforcement officer on the basis of suspicion of driving or a random traffic stop.

The second approach to prosecuting impaired driving behaviour is the so-called 'per se approach'. Under this construction, the government,

based on its obligation to preserve public health and welfare and in consideration of the risks to its citizens of sharing the roadways with impaired drivers, has moved to outlaw driving after having consumed a drug with potentially intoxicating properties. This approach is called 'per se' if a quantitative standard in blood, oral fluid or urine is set above which driving is prohibited, or as 'zero tolerance' if the law defines the offence as having any detectable amount of a proscribed substance present in the body. Generally these laws apply only to scheduled substances, but would include prescription medications in situations where the subject does not have a legitimate prescription. Having a valid prescription is generally an absolute defence to a 'per se' charge, but the individual in most jurisdictions could still be charged under the 'affected by' standard, even if they have a prescription, if they show evidence of impairment. Examples of the diversity of statutory language are shown in Table 5.1.

At trial, the prosecution must generally show either that the subject had a proscribed drug present in their body or that they were impaired by their drug use, depending on the violation that is charged. In the first instance, the toxicologist's testimony can be quite straightforward, and relates to their qualifications, the chain of custody, the proper handling and analysis of the specimen, and the analytical results.

In cases where there is an element of impairment in the charge, the toxicologist's testimony becomes more complex, and must relate the individual's drug consumption to observed patterns of driving or other behavioural or physiological indicators. With appropriate education, qualifications and training, the toxicologist can consider the observations of the arresting officer or other witnesses concerning behavioural effects, such as balance, speech, motor sway, pupil size, wakefulness, performance in field sobriety tests, and other factors to arrive at opinions about whether the observed effects could be accounted for by the class or classes of drugs present. Blood drug concentrations can also be helpful in assessing whether drug consumption reflects therapeutic, recreational or overdose use and the likelihood of drug–drug or drug–alcohol interactions, and may sometimes be helpful in indicating recent drug use. It is generally agreed, however, that for most drugs there is no good quantitative correlation between blood drug concentrations and specific degree of impairment or signs and symptoms exhibited by the driver. Nor can blood drug concentrations be reliably expressed in terms of an equivalent blood alcohol concentration (BAC). Oral fluid and urine are currently considered to be good indicators of drug exposure or consumption, but are even more difficult to relate to a degree of impairment. The toxicologist must recognise the limitations of their expertise and should not be tempted to step outside their area of expertise by providing opinions about medical diagnosis, appropriateness of prescribing practices, law-enforcement procedure or other peripheral issues.

Assessing the effect of a drug on driving performance

It is important to understand the means by which drugs affect individual components of the driving task and how they can affect their overall performance. This may be achieved in a number of ways, and three are considered here.

Behavioural domain analysis and assessment

Before a new drug is approved for use, it should be evaluated for its cognitive and psychomotor effects. This allows the prescribing physician not only to reach informed patient care decisions based on the patient's medical condition, and the efficacy of the drug to treat it, but also to balance those needs with the patient's need to drive or engage in other physically or cognitively challenging tasks.

For purposes of this assessment, driving can be analysed on a functional level. Five functional domains can be described, and may be tested in a study or research environment at no risk to the driver, while providing information about the potential of a drug to cause impairment. Various analyses are possible, but Table 5.2 describes an approach using five domains:

1. Alertness/arousal
2. Attention and processing speed
3. Reaction time/psychomotor functions

Table 5.1 UK and selected US statutory language for DUI involving drugs

United Kingdom

Road Traffic Act 1988 (c. 52) Part I (4)

1. A person who, when driving or attempting to drive a motor vehicle on a road or other public place, is unfit to drive through drink or drugs is guilty of an offence.
2. Without prejudice to subsection (1) above, a person who, when in charge of a motor vehicle which is on a road or other public place, is unfit to drive through drink or drugs is guilty of an offence
3. For the purposes of subsection (2) above, a person shall be deemed not to have been in charge of a motor vehicle if he proves that at the material time the circumstances were such that there was no likelihood of his driving it so long as he remained unfit to drive through drink or drugs.

United States

Colorado Revised Statutes Section 43-4-1301(1)

(f) 'Driving under the influence' means driving a vehicle when a person has consumed . . . one or more drugs . . . which . . . one or more drugs alone . . . affects the person to a degree that the person is substantially incapable, either mentally or physically, or both mentally and physically, to exercise clear judgment, sufficient physical control, or due care in the safe operation of a vehicle.

(g) 'Driving while ability impaired' means driving a vehicle when a person has consumed . . . one or more drugs . . . which . . . one or more drugs alone . . . affects the person to the slightest degree so that the person is less able than the person ordinarily would have been, either mentally or physically, or both mentally and physically, to exercise clear judgment, sufficient physical control, or due care in the safe operation of a vehicle.

Florida Statutes Annotated Section 326.193

1. A person is guilty of the offense of driving under the influence and is subject to punishment as provided in subsection (2) if the person is driving or in actual physical control of a vehicle within this state and
   (a) The person is under the influence of . . . any chemical substance . . . or any controlled substance . . . when affected to the extent that the person's normal faculties are impaired.

Indiana Code Annotated Section 9-13-2-86

'Intoxicated' means under the influence of . . .

2. a controlled substance
3. a drug other than alcohol or a controlled substance so that there is an impaired condition of thought and action and the loss of mental control of a person's faculties to an extent that endangers a person.

Table 5.2 Classification of psychological/behavioural domains related to skills needed for safe driving

Domain	*Description*	*Assessment tools*	*Examples of effects on driving*
Alertness/arousal	Level of consciousness, ranging from full alertness, to drowsiness, somnolence, stupor and coma	Self-report (e.g. visual analogue scales and sleepiness scales); laboratory performance measures (e.g. psychomotor vigilance testing); and sleep laboratory measures of daytime wakefulness	Drifting out of lane, driving off the road, rear-end collisions, failure to obey traffic signals
Attention and processing speed	Capacities or processes involved in being receptive to internal or external stimuli	Tests of focused or selective attention (i.e. concentration); sustained attention or vigilance; divided attention; shifting or alternating attention; and working memory	Rear-end collisions, driving unsafely for conditions, weaving, poor emergency response
Reaction time/psychomotor functions	Speed of response and coordination of skilled movement	Choice reaction time; driving simulator or over-the-road driving tasks; psychomotor tasks (e.g. upper motor speed and coordination	Rear-end collisions, dangerous merging, unsafe lane changes
Sensory–perceptual functioning	Visual, auditory, proprioceptive and sensorimotor abilities that enable the critical driving tasks	Ability to perceive vehicle movement and roadway conditions, and to provide appropriate feedback to the operator on the consequences of motor movement	Collisions, dangerous manoeuvres, risky driving
Executive functions	Planning, approaching, organising, monitoring, prioritising and carrying out various cognitive activities	Mental flexibility; adaptive problem solving; abstract reasoning; impulse control; risk taking, organisational ability and planning	Risky, aggressive driving, last-minute lane changes, poor performance in high-demand situations

4. Sensory–perceptual functions
5. Executive functions.

A behavioural assessment protocol can be designed to evaluate all five of these domains, and a drug that impairs performance in one or more domain at a magnitude known to be associated with increased crash risk can be presumed to have a negative impact on driving safety. This approach allows the assessment of the impact of drug or combined alcohol and drug use on skills essential to safe driving, in a laboratory environment.

Empirical toxicological assessment

When considering a newly developed drug, or one about which relatively little has been published behaviourally, the compound's known pharmacology should be considered. Sedative hypnotic drugs, narcotic analgesics, central nervous system (CNS) depressants, anaesthetics, dissociative drugs and hallucinogens all have clear detrimental effects on the above domains. Stimulants increase wakefulness and attentiveness and improve reaction time; however, overstimulation leads to sleep disturbance and fatigue, anxiety, irritability, impulsiveness and increased risk taking. Under conditions of abuse, stimulants might be expected to have a different effect from that when used under a doctor's supervision. Nevertheless, comparing a new drug with other drugs with known effect profiles can inform opinions about the relative risk of a drug for causing impairment.

Epidemiological monitoring

Once a drug is available on the market, epidemiological data can be reviewed to identify over-representation of certain drugs in crash-risk statistics and relative risk of impairment for drugs or drug classes based either on comparison with control populations or through culpability assessment (Longo *et al.* 2000).

Based on all of the above considerations, several European nations (France, Belgium, Spain and Germany) have developed graduated warning systems regarding the potential of a drug to cause impairment (ICADTS Working Group 2007; ICADTS Drugs List 2007). Under these schemes, panels of psychologists, safety experts, pharmacologists and toxicologists have evaluated drugs for their potential effects under different dosing intervals and amounts, and rated the drugs for their relative risk for impairment. These systems provide prescribing physicians with drug alternatives based on the likelihood of impairing effects or side-effects, and help the patient make good decisions about timing of dose and when and whether to drive.

Identifying the drug- or alcohol-impaired driver

Operating a motor vehicle is a complex task that requires integration of many psychomotor and cognitive tasks. If the processes involved in driving are analysed, it becomes clear that the operator is called upon to steer the vehicle, maintain speed consistent with signage and road conditions, maintain headway between vehicles, maintain lane position, monitor the activity of other drivers, pedestrians and animals, monitor rear view and side mirrors, operate other controls in the vehicle and execute goal-directed driving. At the same time, the operator may also deal with other potential distractions such as passenger activity, operating the radio or CD player, eating, smoking, talking on a mobile phone, engaging in conversation, and so on. The demands of these tasks can vary, requiring adaptation, and can be further influenced by the driver's familiarity with the vehicle and locality, the vehicle's condition, road conditions and/or construction, and lighting or weather, among other things. As demanding as any of these single tasks might be, their coordination represents an additional demand, namely the ability to prioritise, shift and divide attention appropriately between these tasks while performing each competently.

The integration of all the tasks can be compromised easily by the presence of drugs or chemicals that impact cognition, information processing or psychomotor control. Particularly sensitive are divided-attention tasks, of which driving is clearly a good example. Drugs that fall into this category are any that act on the CNS, and those with CNS depressant, sedative or hallucinogenic effects. An additional complication is seen with stimulants and opioids, which not only have important effects acutely but have potential late-phase or withdrawal effects that can also impair performance. Table 5.2 lists some of the general driving behaviours and how they can be related to the driving domains discussed previously. There is no basis for assigning specific types of impairment to specific classes of drugs, however, as each domain may be affected in different ways by a given class of drugs but with the same net deterioration in performance.

Once a driver has been stopped by the police for demonstrating suspicious driving behaviour, the officer then looks for indicators of possible intoxication that would lead to an arrest or to a request for blood, breath or urine samples for chemical testing. On this first contact through the car window, clues that might indicate drug use include:

- Smell (principally the smell of burning marijuana; cannabis)
- Confusion, disorientation or lack of engagement

- Unusual behaviour, statements or responses by the driver to an officer's questions (including slow or slurred speech and physical responses), inappropriate answers to simple questions, unusual nervousness
- Hyperreflexive head body or hand movements, and general motor restlessness
- Flat, dull or overexcited demeanour
- Unusually large or small pupils, unusual eye movements
- Impairment of fine motor skills and balance
- The presence of tablet containers, drug wraps or drug paraphernalia, while not evidence of drug use as such, should not be overlooked.

Generally, after noting the presence of some of these indicators or clues, the officer escalates the investigation to determine the cause of the suspected impairment. The subject is first asked to exit the vehicle. This provides the officer an opportunity to observe some gross motor skills and balance. The subject may be asked to perform some tasks designed to test his or her psychomotor and cognitive abilities, and divided-attention skills. If drug influence is suspected, documenting the physiological parameters influenced by the use of these drugs (pulse, blood pressure, muscle tone, eye movement, pupil size and reaction to light, etc.) can assist in identifying the class or classes of drugs that might account for the observed impairment.

Tests of impairment

Historically, a variety of roadside impairment tests has been used to assist with the assessment of whether a driver is impaired. Picking up coins, counting backwards, serial addition or subtraction, alphabet recitation, standing on one leg and so on have all had their proponents, but many are not suitable for use because of their dependence on the subject's fluency in a particular language, or baseline ability to count or perform simple arithmetic. Other tests require specialised equipment or the active participation of the officer, which can jeopardise the officer's safety and can bring unnecessary physical risk to an impaired subject. By far the most popular test battery now employed in North America and many other countries involves three tests: the walk-and-turn test, the one-leg-stand test and an assessment of horizontal gaze nystagmus (HGN). This battery of standardised field sobriety tests is derived from work funded by the National Highway Traffic Safety Administration (NHTSA) in the 1970s and 1980s (Burns, Moskowitz 1977). The first two tests are classic divided-attention tests that require the subject to listen to a set of straightforward directions (a cognitive task) while performing a separate balance (psychomotor) test. In the walk-and-turn test, the subject places his or her feet on a straight line and must maintain balance without use of the arms while receiving the test instructions. During the second phase of the test, the subject must walk the requisite number of steps, heel to toe, down the line, according to the initial instructions, turn in a prescribed manner and return the same number of steps down the line, while maintaining balance. The person assessing the test is watching for inability to remember or follow instructions, use of arms for balance, stepping off the line, not walking heel to toe or taking the wrong number of steps. In the one-leg-stand test, the subject must again maintain balance while receiving the instructions, then execute the test according to the instructions, which are to raise the foot 15–25 cm (6–10 inches) off the ground and maintain it there while counting out loud, by thousands, to 30 000. This again divides the subject's attention between a cognitive (counting) and a psychomotor (balancing) task. The officer is watching for ability to remember and follow instructions, use of arms for balance, putting the raised foot down or hopping to maintain balance. While neither of these tests is a test of driving itself, they are arguably more challenging and models driving better than a test of, say, simple reaction time. When used individually, these tests have been shown to have an overall efficiency (the percentage of time the test returned a correct answer to the question, and the subject's breath alcohol was >1.0 g/L) of 75.5% and 75.1% for the walk-and-turn and one-leg-stand, respectively (Burns, Moskowitz 1977).

HGN is not a divided-attention test but rather takes advantage of the CNS-depressant properties of alcohol and other drugs, which produce among other things an observable lateral jerking of the eye, or nystagmus, that can be observed by asking the subject to track a stimulus such as a fingertip or penlight across the field of vision while keeping the head still. While pronounced nystagmus can result from other causes (brain damage, inner-ear disturbances, thermal imbalance in the inner ear, rotation, etc.), it is produced reliably by alcohol at blood concentrations known to impair driving ability. Further, other CNS-depressant drugs can also elicit HGN (e.g. depressants, phencyclidine (PCP) and volatile solvents), which makes the test a useful indicator of use of some other classes of drugs in addition to alcohol.

Only limited evaluation of these field sobriety tests has been done for different categories of drug use. In investigations of suspected drug-impaired driving, the value of these and similar tests is to demonstrate an inability on the part of the subject to integrate simple psychomotor and cognitive skills, and as such to demonstrate evidence of impairment. The Drug Evaluation and Classification Program (DECP) that has been established in some jurisdictions in the USA, and modified for use in other countries such as Germany and Australia, seeks to classify subjects as under the influence of a specific class or classes of drugs based on certain psychomotor and physiological measurements. Table 5.3 shows a matrix that relates these effects to the classes of drugs recognised in the DECP. In some European countries, such as the UK and Norway, impairment assessment is done by a physician.

On-site chemical tests

Of increasing interest to the law-enforcement and traffic safety community is the use of 'point of contact' or 'on-site' drug testing. The use and acceptability of such tests vary in different countries. Urine was initially used for this purpose in some countries, and lateral-flow immunoassay-based dipstick devices or test cups were commercially produced and have since proliferated. The devices test for common drugs of abuse including cocaine, marijuana and opiates and are generally sensitive and specific enough for this purpose, although there are quality differences between manufactured devices. Four such devices showed sensitivity ranging from 82.9% to 100% for cannabinoids and from 82.5% to 100% for cocaine, and all showed 100% for opiates. Accuracy ranged from 94.0% to 98.3% for marijuana, from 97.4% to 98.0% for cocaine, and from 99.7% to 100% for opiates (Buchan *et al.* 1998). The benefit of the on-site urine test was the ability to confirm the officer's suspicions about drug use at the time of the arrest, and the devices had great potential for use in enforcement checkpoints or during random screening in jurisdictions where 'per se' laws were in effect. The limitations of the devices included the limited scope of drugs that could be detected (typically five to seven drug classes on any given device, with variable cross-reactivity to compounds within the class) and, on a practical level, the need to transport the subject to a private location to provide the urine sample. Often observed collection was not possible owing to the unavailability of a same-sex observer, creating the potential for substitution or adulteration. While urine is a good specimen for screening, it is essentially 'out of the body' and drugs may be detected in urine after the effects on driving ability have worn off; hence this is not a good specimen for interpretative purposes.

More recently, on-site oral fluid drug testing has gained in popularity. OF testing offers the same benefits of an immediate result to corroborate the officer's opinion regarding drug use, but has the advantages over urine of being capable of being conducted at the roadside, eliminating the cost and time of transporting the subject. It also offers the opportunity of obtaining a sample proximate to the time of driving, it provides a non-invasive, clean sample, and collection can be directly observed irrespective of the sex of the suspect or the arresting officer. OF also has an advantage over blood of not requiring a licensed collector or phlebotomist, and the associated cost of that procedure. Following the result of a field test, OF can also be collected and sent to a laboratory for the confirmatory test needed for forensic purposes. OF test devices currently have a limited scope compared with a laboratory test but they can cover the major drug

Table 5.3 Symptomatology chart for drug effects (IACP, DECP Program)

Categories	*Depressants*	*Stimulants*	*Hallucinogens*	*PCP*	*Narcotic analgesic*	*Inhalants*	*Cannabis*
HGN	Present	None	None	Present	None	Present	None
Vertical nystagmus	Present (high dose)	None	None	Present	None	Present (high dose)	Present
Lack of convergence	Present	None	None	Present	None	Present	Present
Pupil size[(a)]	Normal	Dilated	Dilated	Normal	Constricted	Normal	Dilated
Reaction to light	Slow	Slow	Normal	Normal	Little or non-visible	Slow	Normal
Pulse rate[(b)]	Down	Up	Up	Up	Down	Up	Up
Blood pressure[(c)]	Down	Up	Up	Up	Down	Up/down or Normal, DOS	Normal
Body temperature	Normal	Up	Up	Up	Down		
Muscle tone	Flaccid	Possibly rigid	Possibly rigid	Rigid	Normal or flaccid	Flaccid	Normal
General indicators	Uncoordinated Disoriented Sluggish Thick slurred speech Drowsiness Drunk-like behaviour Fumbling Impaired vision Droopy eyes Body tremors will be evident with methaqualone	Restlessness Body tremors Excited Talkative Exaggerated reflexes Anxiety Grinding of teeth Insomnia Dry mouth Irritability Runny nose Redness to nasal area	Dazed appearance Body tremors Synaesthesia Paranoia Hallucinations Disoriented Memory loss Flashbacks Piloerection Perspiring Uncoordinated Speech difficulty	Perspiring Warm to touch Repetitive speech Cyclic behaviour Speech difficulty Incomplete verbal responses Increased pain threshold Possibly violent	On the nod Dry mouth Facial itching Droopy eyelids Low raspy slow speech Track marks Euphoria Nausea Fresh puncture wounds	Odour of substance Traces on clothes or face Bloodshot watery eyes Confused Disoriented Lack of muscle control Nausea Flushed face Headaches	Odour of cannabis Cannabis debris in mouth Eyelid tremors Reddening of conjunctiva Body tremors Disoriented Relaxed inhibitions Possible paranoia

[(a)]Pupil size normal range, 3.0–6.5 mm.
[(b)]Pulse normal range, 60–90 beats/s.
[(c)]Blood pressure normal range, 120–140 mmHg systolic, 70–90 mmHg diastolic.

classes encountered in the driving population. OF on-site tests have been deployed in a large-scale roadside random testing programme in Australia, with some success (Boorman, Owens, 2009), and on-site OF testing has recently been trialled by police in the UK. Studies conducted as part of the Driving under the Influence of Drugs and Alcohol (DRUID) project in the European Union have evaluated available on-site devices and found them to be significantly improved over their earlier versions (DRUID 2009).

The merits of OF as a sample for confirmatory testing are discussed below. Any on-site drug test needs to take account of the fact that not all drugs that can cause impairment will be detected in an on-site test and that the primary consideration in whether to release a driver would be his or her appearance of sobriety and fitness to drive.

Choice of specimen for drug testing in impaired driving cases

The three options for sample collection for DUI enforcement purposes are blood, OF and urine. Each has its advantages and disadvantages.

Urine has the advantage of being easily collected (privacy issues around observed collection have been discussed above). The concentration of drugs in urine is dependent on the volume of liquid consumed, the degree of hydration of the subject and the time of ingestion, as it is on the amount of drug ingested. Urine drug concentrations are often several orders of magnitude higher than blood drug concentrations, making urine an ideal sample for preliminary screening. Depending on the drug, it or its metabolites may be detected for days or occasionally weeks after administration. Shorter-acting drugs may be detected in the urine even after the effect of the drug has disappeared. This is true, for example, of some of the short-acting benzodiazepines, γ-hydroxybutyric acid (GHB) and zaleplon.

Urine drug concentrations can be normalised for creatinine concentration, which corrects for dilution caused by the ingestion of large volumes of water, but such a correction is of limited value in a single urine specimen, and does not add to an interpretation of the degree of intoxication. Urine is, therefore, an excellent specimen for answering the question 'Did the donor at some time prior to providing the specimen use or ingest this drug?', but it is of little use in determining whether the subject was impaired or intoxicated at the time that the sample was collected. The investigator must consider the result of the urine drug test in the light of objective evidence of impairment (slurred speech, staggering gait, inappropriate conduct or response, poor coordination, glassy stare, agitation, restlessness, etc.) to reach any conclusion about the subject's fitness to drive. The toxicologist can then use his or her specialised knowledge to assess whether the observed effects are consistent with the known properties of the drugs present. Another limitation of urine is that, following drug use, it may take some time before a drug is metabolised and excreted. If ingestion had been very recent, the urine may test negative for the presence of the drug, even though the drug is present in the body and exerting an effect on the subject.

In spite of these limitations, urine has been a popular specimen for drug testing in impaired driving cases because of the ease with which it can be collected, the essentially unlimited volume for testing, and the ease with which urine can be screened in the laboratory by immunoassay on automated instruments.

Blood has emerged as the preferred specimen for collection and analysis in impaired-driving investigations. The presence of drugs in the circulating blood, in equilibrium with the brain, provides the most direct measure of a person's likelihood of intoxication. From an interpretive standpoint, blood drug concentrations can usually be related to some degree to published concentrations associated with known doses

or patterns of therapeutic or recreational use. Blood drug concentrations are a function of the dose, time since last use, and whether the use is acute or chronic. They can be useful in distinguishing drug use from abuse and assessing the likelihood of drug interactions, and can be related to behavioural studies of effects on critical abilities related to driving, although relationships between blood drug concentration and any specific degree of impairment or effect on driving have not been established for many drug substances. Apart from alcohol, the exception to this may be other members of the depressant drug class, which have been studied to a greater degree (Bramness *et al.* 2003, 2004). Hysteresis or varying early phase and late phase effects for a given concentration as a result of acute tolerance or withdrawal make this relationship more complex for stimulant drugs, and issues of chronic tolerance can impact on the interpretation of opiate concentrations. Consequently, the interpretation of blood drug concentrations still needs to be treated with care and the full range of possible ingestion patterns, and likely effects, must be considered.

Blood must be collected by a medical professional, which can add expense and time to the collection process. The time factor is a consideration for drugs such as zolpidem, tetrahydrocannabinol (THC) and some of the synthetic cannabinoids (JWH-018 and JWH-250), which are distributed and metabolised out of the blood rapidly. Consequently the concentrations in the blood at the time of collection may not reflect the concentrations present at the time impairment is first observed or during driving.

Typical volumes of blood available for testing are around 10 mL which is usually adequate for a sensitive broad-spectrum screen, confirmation and quantification. It can be difficult to collect an adequate volume from some individuals with collapsed veins. Drugs are typically quite stable in blood provided that it is collected over an enzyme inhibitor such as sodium fluoride. Collection with an anticoagulant such as heparin, citrate or oxalate is important in order to prevent clotting and inhomogeneity in sampling.

As noted above there is increased interest in OF testing for drugs in driving investigations. OF (a mixture of salivary, parotid and crevicular ultrafiltrates and mucus) has gained popularity as an alternative to blood because of the ease and low cost of collection proximate to the time of driving, and the increasing reliability of on-site test devices. In the USA, 16 states now allow OF collection in their DUI statutes, and others are following suit. In some European countries, OF is also accepted in court for traffic enforcement cases subject to appropriate laboratory confirmation. Several large-scale surveys of drivers have also recently assessed the utility of OF for traffic investigation, and have found generally good correlation between the presence of drugs in OF and the presence in a subsequently collected blood sample (Diplock, Plecas, 2009). This meets the requirement in 'per se' jurisdictions where the mere presence of a proscribed drug in a body fluid constitutes the offence. OF drug concentrations are, however, more difficult to interpret quantitatively than blood concentrations. Partition of drugs into OF is a function of many factors: salivary flow, oral pH, drug polarity and pK_a, contamination of the oral cavity with drug (especially an issue for smoked drugs such as cocaine or marijuana), and the degree of protein binding of the drug in blood (only non-bound drug partitions into the OF). Consequently, it is inadvisable to try to predict blood drug concentrations based on OF concentrations, and there are currently limited data available to determine whether impairment can be predicted based on quantitative OF test results (Wille *et al.* 2009). Analytically, the target compounds in OF can be different from whole blood, as the less polar parent drug is often the major analyte present in OF as is the case with THC and cocaine. More polar highly bound drugs, such as the benzodiazepines, can be present at much lower concentrations in OF.

In summary, blood remains the specimen of choice in DUID investigations, but OF is emerging as a valuable alternative within the correct statutory framework.

Laboratory approaches to drug testing in DUID cases

At the heart of every DUID investigation is a forensically defensible drug test. This generally means a test with appropriate scope to identify the range of drugs that are known to affect driving and are prevalent in the impaired driving population at large, supplemented with other drugs that may be an issue locally. Prescription drugs, over-the-counter drugs and drugs of abuse should all be included. Two sets of guidelines with largely overlapping recommendations for scope and limit of detection in blood and urine, for both screening and confirmation, are shown in Table 5.4 (Farrell *et al.* 2007; Walsh *et al.* 2008).

Most laboratories begin the analytical process with a test for volatiles to establish any role for alcohol in the subject's impairment. This can also disclose the presence of volatiles including anaesthetic gases, and solvents subject to abuse such as toluene, xylene or difluoroethane.

The volatiles screen is usually followed by an immunoassay screen. The immunoassay provides a valuable role in screening for the presence of certain drug classes, for example identifying the presence of amfetamines or benzodiazepines but not the identity of the specific amfetamine or benzodiazepine. The variable cross-reactivity of the assay to members within a class must be kept in mind. For example, many benzodiazepine immunoassay tests do not cross-react well with lorazepam (Ativan), so a chromatographic screen will be needed to exclude those drugs with poor cross-reactivity.

Appropriate cut-offs need to be established depending on the matrix being tested (see Table 5.4). It is not appropriate to apply most urine drug screening cut-offs to blood analysis, since urine drug concentrations may exceed those in the blood by several orders of magnitude, and the presence of some drugs might be subsequently missed.

Table 5.4 Recommended scope and analytical cut-offs of toxicological analysis in DUID investigations

	Blood (ng/mL)		*Urine (ng/mL)*	
Target analyte	***Screen***	***Confirmation***	***Screen***	***Confirmation***
DRE category – cannabis				
THC	–[(a)]	2	–[(a)]	2
Carboxy-THC	10	5	20[(b)]	5
11-OH-THC	–[(a)]	2	–[(a)]	2
DRE category – CNS stimulants				
Metamfetamine	20	20	200	50
Amfetamine	20	20	200	50
MDMA	20	20	200	50
MDA	20	20	200	50
Cocaine	–[(a)]	10	–[(a)]	20
Benzoylecgonine	50	50	300	50
Cocaethylene	–[(a)]	10	–[(a)]	20

Table 5.4 continued

	Blood (ng/mL)		Urine (ng/mL)	
Target analyte	***Screen***	***Confirmation***	***Screen***	***Confirmation***
DRE category – CNS depressants				
Alprazolam	–[c]	10	–[c]	50 total[b]
Chlordiazepoxide	–[c]	50	–[c]	50 total[b]
Clonazepam	–[c]	10	–[c]	50 total[b]
7-Aminoclonazepam	–[c]	10	–[c]	50 total[b]
Diazepam	–[c]	20	–[c]	50 total[b]
Nordiazepam	50	20	100	50 total[b]
Lorazepam	–[c]	10	–[c]	50 total[b]
Oxazepam	50	50	100	50 total[b]
Temazepam	–[c]	50	–[c]	50 total[b]
Trazodone	–[d]	25	–[d]	50
Amitriptyline	–[d]	25	–[d]	50
Nortriptyiline	–[d]	25	–[d]	50
Diphenhydramine	–[d]	25	–[d]	50
Carisoprodol	–[d]	500	–[d]	500
Meprobamate	–[d]	500	–[d]	500
Zolpidem	–[d]	20	–[d]	20
Butalbital	–[a]	100	–[a]	100
Phenobarbital	–[a]	100	–[a]	100
Secobarbital	100	100	200	100
Phenytoin	–[d]	500	–[d]	5000
Carbamazepine	–[d]	500	–[d]	5000
Topiramate	–[d]	1000	–[d]	1000
γ-Hydroxybutyrate	–[d]	5000	–[d]	10 000
DRE category – narcotic analgesics				
Codeine	–[e]	10	–[e]	50
6-Acetylmorphine	–[e]	10	–[e]	10
Hydrocodone	–[e]	10	–[e]	50
Hydromorphone	–[e]	10	–[e]	50
Methadone	50	10	300	50
Morphine	20 free[f]	10	200	50 total[b]
Oxycodone	–[e]	10	–[e]	50
Propoxyphene	50	50	300	50
Tramadol	–[d]	20	–[d]	20
DRE category – dissociative drugs				
Dextromethorphan	–[d]	20	–[d]	50
Phencyclidine	10	10	25	10

THC, Δ^9-tetrahydrocannabinol; carboxy-THC, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; MDMA, 3,4-methylenedioxymetamfetamine; CNS, central nervous system.
[a]Immunoassay screening not targeted to this analyte.
[b]Combination of free and conjugated analyte.
[c]Immunoassay screening targeted to nordiazepam, oxazepam or both; not an effective tool for screening all drugs in this class.
[d]Not routinely screened for by immunoassay.
[e]Immunoassay screening targeted to morphine; not an effective tool for screening all drugs in this class.
[f]Free drug, not conjugated.

Preliminary drug screening

Many different types of immunoassay screening tests are available commercially. The techniques in most widespread use include enzyme multiplied immunoassay technique (EMIT), ELISA, kinetic interaction of microparticles in solution (KIMS) and fluorescence polarisation immunoassay (FPIA; see Chapters 3, 18 and 32). When applied appropriately, with the correct calibrators and controls, any of these techniques is suitable for drug screening in DUID cases. ELISA is in most widespread use, and has the advantage as a heterogeneous assay with a separation step of being readily applied to blood in addition to other matrices. Homogeneous systems such as EMIT typically require some pretreatment (protein precipitation or extraction) prior to analysis of samples other than urine or serum. An immunoassay panel for blood or urine typically includes cocaine metabolites, opiates, amfetamines, methylenedioxyamfetamines, cannabis metabolites, methadone, PCP, propoxyphene, barbiturates and benzodiazepines. For OF, the same general targets would be included, with the exception of using a THC-specific assay for cannabinoids, and a cocaine-specific assay rather than a metabolite assay. Immunoassay is only a presumptive technique, and should not be

considered proof of the identification of a compound without a complementary confirmatory analysis. Immunoassay screening covers only a partial range of drugs, and typically would not detect other potentially impairing compounds such as novel analgesics, tramadol and tapentadol, or anticonvulsant drugs such as gabapentin, topiramate or leveteracitam, or partial γ-aminobutyric acid (GABA) agonists such as zolpidem or zopiclone. All these drugs may play a role in driver impairment and underscore the need for a comprehensive chromatographic screen (GC-MS or LC-MS) in addition to the immunoassay.

Chromatographic screening

The 'gold standard' for confirmatory identification of drugs in biological samples is GC-MS or LC-MS. Typically, two fractions, one for basic drugs and the second for weakly acidic or neutral drugs, are obtained.

Basic drugs

Drug extraction

The procedure described below efficiently extracts basic drugs, including those commonly encountered in DUID cases such as those indicated in Table 5.4. The procedure described below includes a back -extraction into acid, which produces a very clean extract and reduces the amount of time required to analyse the GC-MS data, and the time spent on system maintenance. The back extraction, however, eliminates weak bases or amphoteric compounds, notably some benzodiazepines, which must be analysed for using a separate confirmatory procedure that does not include the back-extraction step.

1. Add 1 mL of the sample to a 20-mL disposable glass culture tube.
2. To each tube add 50 µL internal standard.
3. Add 1 mL pH 9.0 borate buffer to each tube and vortex mix for approximately 10 seconds.
4. Add 3 mL *n*-butyl chloride to each tube, cap, and rotate on a tube rotator for a minimum of 5 minutes.
5. Centrifuge until the organic and biological matrix layers are separated.
6. Transfer the *n*-butyl chloride organic layer to a clean, labelled, 10-mL, conical, disposable centrifuge tube.
7. Back extract by adding 200 µL 3 mol/L HCl to the *n*-butyl chloride.
8. Cap and rotate for at least 5 minutes.
9. Centrifuge until separated; aspirate and discard the (upper) *n*-butyl chloride layer.
10. Remove any remaining butyl chloride by blowing air over the lower layer.
11. Add 100 µL concentrated ammonium hydroxide and 100 µL saturated ammonium carbonate to each tube to make the extract alkaline.
12. Vortex mix for 15–30 seconds.
13. Add 150 µL of chloroform and vortex mix for 15 seconds.
14. Centrifuge until the organic and aqueous layers are separated.
15. Using a serological disposable pipette, transfer approximately 50–75 µL of the lower organic layer into two labelled autosampler vials for GC-MS analysis.

GC-MS analysis

Many different conditions of flow rate, columns and temperature programming can be used for the GC-MS analysis of basic drugs to emphasise or optimise certain separations for frequently encountered compounds that might co-elute or interfere. The conditions for optimum separation have to be balanced against the run time so as to effectively use the laboratory's resources and keep up with casework. The following method is an example of such a system that allows the chromatography of approximately 250 commonly encountered basic drugs and metabolites.

Simultaneous analysis is performed on a dual-column gas chromatograph with a nitrogen phosphorus detector (NPD) on column 1 and a mass-selective detector (MSD) on column 2. Use of identical columns and simultaneous injection permits the comparison of the mass- and nitrogen-specific chromatograms, which assists with data interpretation and peak identification. In this example, both columns are 5% phenylmethylsiloxane column (DB-5 or equivalent) 30 m × 0.25 mm i.d., 0.25 mm film thickness. The temperature programme starts at 90°C, rises by 15°C/min for 12 minutes (to 180°C), then rises by 8°C/min for 15 minutes (to 300°C) and holds for 4 minutes. The total run time is 31 minutes.

Weakly acidic and neutral drugs

Extraction

The procedure described here isolates drugs with acidic and neutral character, such as non-steroidal anti-inflammatory compounds and paracetamol, carbamates (such as carisoprodol and meprobamate), barbiturates and anticonvulsants (such as carbamazepine and phenytoin; Logan *et al.* 2000).

1. Add approximately 1 g XAD resin (washed in ethyl acetate) to disposable glass culture tubes (20 mL).
2. Add 5 mL deionised water and 50 µL internal standard to each tube.
3. Add 1 mL of sample to the appropriately labelled tubes.
4. Thoroughly vortex mix each tube for approximately 60 seconds and allow to stand for at least 60 seconds to allow settling.
5. Aspirate the blood–water layer from each tube, leaving the XAD in the tube.
6. Add 6 mL ethyl acetate to each tube and vortex thoroughly for 60 seconds.
7. Centrifuge until separated, and transfer the ethyl acetate to an appropriately labelled, clean, conical, disposable tube.
8. Evaporate to dryness at approximately 50°C under air until very dry (approximately 20 minutes).
9. Reconstitute using 75 µL acetonitrile and wash with 500 µL heptane.
10. Vortex mix for about 15 seconds and centrifuge until separated.
11. Aspirate the heptane layer (top), and transfer the acetonitrile layer to labelled autosampler vials for GC-MS analysis.

GC analysis

As with basic drugs, many GC protocols are suitable for this screen. Since the number of compounds being tested for is considerably smaller (~30), the run time is correspondingly shorter. GC is performed on a gas chromatograph equipped with a flame ionisation detector (FID, for screening) or a: MSD (for confirmation). Helium is used as the carrier gas. The column is a 5% phenylmethylsilicone column (DB-5 or equivalent), 30 m × 0.32 mm i.d., 0.25 mm film thickness. The temperature programme starts at 60°C, rises by 10°/min for 16 minutes, then rises by 20°C/min to 295°C and holds for 1 minute. The total run time is 21 minutes.

Determining an appropriate protocol for your laboratory

While attention should be paid to the opinion of the arresting officer about what drugs are suspected in any given case, the toxicologist should apply a good broad-spectrum drug screen to be able to exclude the possibility of other drugs with similar effects. For example, as discussed later, an individual withdrawing from intravenous amfetamine use may appear drowsy and have constricted pupils, poor psychomotor performance and injection marks. This constellation of symptoms is very similar to those caused by a narcotic analgesic, and consequently a test only for opiates would not disclose the actual drug responsible for the person's intoxication.

The class of CNS-depressant drugs is probably the most challenging analytically, since it includes some chemically very diverse drugs, from solvents and inhalants, to long- and short-acting benzodiazepines, antidepressants, antihistamines and novel recreational agents such as GHB and butane-1,4-diol. Practically speaking, an escalation approach is the most economical and effective, beginning with the commonly encountered drugs and expanding and adding assays for drugs that could account for the symptoms observed on the basis of review of the preliminary data.

It is critical that laboratories performing this work observe accepted standards for forensic analysis, including: maintaining security and chain of custody, maintaining written protocols for commonly performed

Table 5.5 CNS-depressant drugs associated with impaired driving

Antidepressants	*Anxiolytics*	*Sedative hypnotics*	*Analgesics*	*Antipsychotics*	*Anticonvulsants*	*Muscle relaxants*	*Antihistamines*
Amitriptyline Nortriptyline Amoxapine Clomipramine Imipramine Desipramine Doxepin Meprobamate Trazodone Trimipramine	Alprazolam Clonazepam Diazepam Oxazepam Triazolam	γ-Hydroxybutyric acid (GHB) Flunitrazepam Lorazepam Temazepam Zolpidem Zopiclone Zaleplon	Tramadol Tapentadol Codeine Morphine Hydrocodone Dextropropoxy-phene Pentazocine Fentanyl Methadone Pethidine Hydromorphone Oxycodone	Chlorpromazine Mesoridazine Thioridazine Thiothixene Loxapine	Phenobarbital Carbamazepine Chlordiazepoxide Phenytoin Clonazepam Topiramate	Butalbital Carisoprodol Diazepam Cyclobenzaprine	Diphenhydramine Chlorphenamine (Chlorpheniramine) Brompheniramine

procedures, validating method accuracy through use of appropriate standards and controls, verifying the identity of any drugs reported (using MS wherever possible), implementing the scientific review of data, participating in proficiency testing programmes and ensuring that staff have appropriately documented qualifications and training.

Priority drugs associated with driving impairment

CNS depressants

Many drugs have CNS-depressant properties either as a targeted effect or as a side-effect of the drug. While not intended to be a comprehensive list, Table 5.5 presents some common drugs with CNS-depressant activity that have been implicated in impaired driving cases. Most of these drugs exert their effects through the GABA pathways, with the exception of the opiates which cause sedation through the opioid μ and κ receptors.

Alcohol is the best-known example of a CNS-depressant, and the general presentation of alcohol impairment is seen with these drugs, as discussed below. Performance in divided-attention tests, such as field impairment tests, is affected similarly by many CNS-acting drugs and observable nystagmus can be seen using the HGN test. Driving has been affected demonstrably in driving simulators, in on-road driving studies and from epidemiological and anecdotal reports for many drugs, and monographs concerning the benzodiazepines, GHB and carisoprodol are included in two special issues of *Forensic Science Reviews* (Vol. 14, 2002, and Vol. 15, 2003). By virtue of their common effect, combining alcohol with prescription or recreational drugs causes an additive or synergistic effect. Drugs with CNS-depressant properties are frequently prescribed in combination for the legitimate treatment of a constellation of effects associated with illness. One of the most commonly encountered examples in DUID casework is that of chronic pain patients. A patient who suffers from chronic debilitating back pain might be prescribed one or two centrally acting muscle relaxants such as carisoprodol or cyclobenzaprine, a barbiturate such as butalbital, an opioid analgesic such as propoxyphene, hydrocodone or oxycodone, a sleeping aid such as zolpidem, and often an antidepressant drug to treat the depression that typically accompanies chronic pain. The resultant combined effect makes some decrement in driving performance almost inevitable, and the patient, pharmacist and physician have an obligation to monitor the effectiveness of the treatment, select drugs with the least likelihood for impairment, use minimum effective doses, advise the patient of the risks to driving, and take care when adjusting the dose, changing the dosing schedule or adding new medications. The patient needs to be similarly advised that fatigue, alcohol and his or her illness itself can lead to unexpected changes in the risk of impairment. Whether the impairment is a result of legitimate, compliant, prescription use or not is immaterial from a public safety standpoint, and a driver impaired by use of prescription drugs must be subject to the same removal from the road as a recreational drug user.

As noted earlier, there is currently insufficient information to relate blood drug concentrations to a specific degree of impairment; however, CNS-depressant drugs do display a qualitative dose–response relationship between concentration and effect, with higher concentrations being associated with more obvious symptoms, progressing from mild impairment to unconsciousness, even in subjects with some tolerance. Effects on driving can occur with the therapeutic use of a single drug. Blood drug concentration data can help to establish whether a subject is following his or her prescribed course of medication.

A subject under the influence of a CNS-depressant will typically display signs that are familiar from impairment through alcohol. Consistently noted are problems with fine or coarse motor control, staggering gait (ataxia), loss of balance, impairment in divided-attention tests, poor concentration and slurred speech. Subjects often have a sleepy or dazed appearance, may have difficulty understanding questions or responding appropriately, and may be disoriented to time and place. Readily observable HGN is a consistent and common feature of CNS-depressant intoxication. Driving behaviours commonly noted include weaving within or between lanes, failure to notice and obey traffic signals, failure to obey posted speed limits, wide turns, rear-end collisions, no use of turn signals and driving at night without lights.

Marijuana

Marijuana (cannabis) is the most popular recreational drug after alcohol in most jurisdictions. Obtained from the plant *Cannabis sativa*, the leaves and buds contain a variety of cannabinoids that posses psychoactive effects, the predominant one being Δ^9-THC. Cannabinoids have significant behavioural and physiological effects that contribute to changes in a person's ability to drive safely. The drug is popular for its relaxation-promoting and euphoric properties, accompanied by sedation and changes in perception. Accompanying effects include altered time and distance perception, poor concentration, impaired learning and recall, increased appetite and mood changes. Associated physiological effects include increased pulse and blood pressure, and bloodshot conjunctivae. A loss of convergent vision is also reported.

Beginning in 2008, synthetic cannabinoid agonists developed as investigational drugs started to appear in the recreational drug market. Sold as 'Spice', 'Pep-spice', 'K2' and by many other names, they are marketed as incense products or 'legal highs', and often labelled not for human consumption. A very diverse range of structural families has been identified with significant binding and agonist effects at the cannabinoid CB1 receptor, responsible for the intoxicating effects of THC. Preliminary data suggest that these compounds have very similar effects to marijuana, and will probably become important to consider in DUID investigations. The compounds most frequently reported to date are JWH-018, JWH-019, JWH-073, JWH-250, CP47,497, HU-210, WIN55212, RCS-4 among many others. These drugs generally do not cross-react with cannabinoid immunoassays, and will probably require detection levels of less than 1 ng/mL in blood, necessitating LC-MS(-MS) or other high-sensitivity mass-spectrometric techniques.

The predominant effects of concern with respect to the effects of cannabinoids on driving are sedation, vigilance, and the effects on concentration, divided attention, perception, and temporal and spatial orientation. The sedative effects can be similar to those of CNS depressants, and the associated driving behaviours are similar also, resulting in weaving, slowed responses and inattentiveness, frequently resulting in collisions.

The epidemiological literature with respect to cannabis has been reviewed extensively by Huestis (2001). Many of the underlying studies, however, suffer from a lack of appropriate control groups, and confounding factors (time, dose, duration, last use, combined use of cannabis with alcohol or other drugs, laboratory test cut-offs, etc.) make comparisons between these studies difficult. The ability to compare and compile study data is another reason for the international efforts towards standardised methods for drug testing in DUID research (Walsh *et al.* 2008).

In concert, however, the behavioural, epidemiological and toxicological studies that have been undertaken to date point towards a general picture of an overrepresentation of cannabinoid-positive blood, OF or urine samples (indicating use) in drivers involved in accidents or arrested for impaired driving, when compared with the general population. This has been further demonstrated in the recent roadside surveys discussed earlier (Bierness *et al.* 2010).

The second source of evidence for a relationship between cannabis use and driving impairment comes from laboratory-based tests of psychomotor performance. These studies often suffer from limitations on what dose of drug can ethically be administered to subjects and which subjects can be included. Studies may also be subject to observation effects in which the participants can exert greater effort because of the motivating factor of being under observation in the study. The value of the studies is that they can isolate and demonstrate decrements in specific measures of psychomotor and cognitive performance (e.g. tracking, reaction time, error rate, vigilance, learning, recall, divided attention). By extension, the evidence for impairment in the driving task after cannabis use, which incorporates many of these components, is verified. Laboratory-based studies suffer from a practical limit to which subjects can be dosed with recreational drugs; consequently, they probably underestimate the potential effect in chronic or frequent recreational users.

Finally, and perhaps most convincingly, a limited number of studies have been performed in which subjects have been administered cannabis and then tasked with driving in electronic driving simulators, or actually operating vehicles on open or closed driving courses. These studies have also been reviewed by Huestis (2001), and reveal evidence of significant mild-to-moderate decrements in driving performance at the doses given. In an early study, Klonoff (1974) tested the effects of low doses of THC (5–8.5 mg) on subjects who were then asked to undertake closed-course and on-street driving in an urban setting in Vancouver, Canada. The authors reported impairment of attentiveness (increased distractibility), problems with braking, bad judgement and difficulty in concentrating. These effects were most pronounced on city streets when the demands of the driving were greatest. Illustrating some of the contradictions of these studies, however, some drivers actually improved in performance after the cannabis dose. This highlights one of the mitigating effects of cannabis intoxication, whereby some subjects have demonstrated an ability to focus concentration on tasks for short periods of time and perform quite well. This has led to the conclusion that sustained attention, or vigilance, rather than attention in general is most affected by cannabis. This finding can explain why it may be difficult to demonstrate impairment in field sobriety tests, which by their nature are of short duration. A research group in Australia has studied the effects of marijuana smoking on standardised field sobriety tests (Papafotiou *et al.* 2005a). They found that there was a dose-dependent increase in errors in the field sobriety tests, with between 30% and 40% of subjects showing clear indicators of impairment. The same doses of marijuana produced impairment in driving performance (Papafotiou *et al.* 2005b).

On-road driving studies performed in the Netherlands (Robbe, O'Hanlon 1993, 1999) have produced other evidence of impairment. Volunteers smoked cannabis in doses of 100, 200 and 300 μg/kg (the latter being consistent with a 'user-preferred dose') and were assessed in an on-road driving task 40 and 100 minutes later. Drivers showed evidence of decrements in maintaining lateral vehicle position (weaving) at all doses, equivalent to blood alcohol levels of between 0.3 and 0.7 g/L. Importantly, no correlation was found between the blood THC concentrations and the degree of effect. Using similar methodology, the authors showed that, when cannabis was combined with alcohol, the effects of both drugs on driving were increased.

In summary, cannabis is a psychoactive drug that can influence mood, concentration and judgement, in addition to its sedating properties, all of which contribute in a dose-related fashion to impairment of vehicle operation. Compared with many other drugs, the level of impairment after mild-to-moderate single-dose recreational use is low, equivalent to a BAC in the range 0.3–0.7 g/L. Combining alcohol and cannabis produces a greater impairment than either alone, and cannabis use should be considered in determining the likely impairment in subjects with low BACs. The blood THC and metabolite concentrations are not well correlated with effect, although some authors have explored the possibility of relating these concentrations to time since last use (Huestis *et al.* 2005).

CNS stimulants

In contrast to the sedative drugs discussed above, stimulants generally increase neural activity, which, in moderation, may not be entirely detrimental. Caffeine can revive the drowsy driver, and patients with attention deficit hyperactivity disorder (ADHD) or narcolepsy can benefit from appropriate doses of methylphenidate or amfetamine. Just as these drugs can act to restore some balance in these individuals, they can upset the delicate neural homeostasis in a healthy individual. Stimulants, principally cocaine and the amfetamines, are used recreationally for their excitatory, euphorigenic properties. In this context they produce intense, overwhelming and distracting effects. After acute administration, users report feeling elated or powerful, having superior intellect and insight, and sexual arousal and stimulation. Time may appear to pass more quickly, speech becomes faster and less coherent, and users can become impatient and agitated, sometimes to the point of violence. These perceptual changes are accompanied by increases in pulse and blood pressure, pupillary dilatation, sweating and psychomotor restlessness, manifested as pacing, fidgeting and scratching. Simple reaction time may be improved under the influence of stimulants, but this is only one component of driving; in fact, complex reaction time, which demands impulse control, intelligent decision-making and appropriate response, may be affected adversely. The intensity of these effects depends on the dose, on the route of administration, and to some extent on the user's experience with, and tolerance of, the effects of the drug. Inevitably, these effects, when combined, are detrimental to complex task performance and make drivers less attentive, while the psychomotor excitation demands greater focus on muscle control and vehicle operation. These opposing effects result in poorer driving. As with any other drugs, the effects are likely to become more apparent when demands are high, such as when driving in bad weather, in heavy traffic, in an unfamiliar environment or in a defective vehicle.

In addition to these acute effects, humans display both acute and chronic tolerance of the effects of stimulants. With cocaine and the amfetamines, the initial excitatory, euphoric phase can be followed by a withdrawal phase, the intensity of which depends on the duration and intensity of drug use. Binges of metamfetamine use are generally longer, of the order of days, than those for cocaine use, for which binges typically run only to hours, and amfetamine withdrawal is often more severe. This is in part a reflection of the different half-lives of the drugs, with cocaine having a 1–2 h half-life, while for amfetamines it is of the order of 7–15 h. During excitation, there is some downregulation of the dopamine receptors, reduction in dopamine transporter activity and reduction in concentrations of the enzyme tyrosine hydroxylase, responsible for dopamine synthesis. This decline in dopamine activity has the opposite effect to that experienced during excitation. Subjects are typically fatigued, lack energy, can be irritable and depressed, and are anhedonic and dysphoric. Sometimes delusions and pseudohallucinations can occur, and the subject can become psychotic. Often this is called the 'crash' phase, during which the subjects sleep a fitful, restless sleep, sometimes for days in the case of metamfetamine withdrawal. During this phase, subjects can appear as if under the influence of a CNS-depressant or opiate, and a poorer complex task performance is expected, such as in driving a vehicle. Drivers have been reported as being lethargic, sleepy, with very poor lane travel (weaving), and frequently drive at high speed, drive off the road or drive into oncoming traffic.

Isenschmid (2001) and Logan (2001) reviewed the available epidemiological, psychomotor performance and anecdotal evidence for impairment in driving after stimulant use, from the effects of both the acute excitation and later withdrawal phases, and concluded that these effects are real and significant. Blood cocaine and amfetamine drug concentrations must be interpreted with caution, since a single blood drug measurement does not predict what phase of intoxication the subjects may be experiencing, and consequently what pattern of effects are likely to predominate. Nevertheless, blood drug concentrations can show whether drug use is consistent with recreational doses in the case of cocaine, and may help distinguish between legitimate dosing of amfetamines in the treatment of narcolepsy, ADHD or eating disorders, and recreational use. The doses of stimulant drugs required to produce the sought-after euphoric effects are accompanied inevitably by deterioration in driving performance.

Hallucinogens

Drugs with hallucinogenic properties have an obvious deleterious effect on driving. Inability to distinguish illusion from reality results in poor decision-making and consequently poorer driving. Drugs such as psylocibe mushrooms, mescaline, lysergide (LSD), ketamine and PCP can produce fully formed hallucinations, seeing objects, shapes or individuals that are not present, and synaesthesia or blending of sensory information such as 'seeing' sounds, or 'hearing' colours. Ketamine and its psychomotor effects on driving have been evaluated (Mozayani 2001). Many other drugs can produce milder hallucinations, including, as noted earlier, cannabis and stimulants.

Methylenedioxy-substituted amfetamines, such as MDMA (ecstasy), methylenedioxyamfetamine (MDA) or methylenedioxyethamfetamine (MDEA; Eve) can also produce hallucinations, particularly tactile ones, that enhance sensitivity to touch. However, the predominant impairing effects of that class of compounds appear to be more related to their excitatory and stimulant properties (Logan, Couper 2001).

Interpretation of results

In many jurisdictions, the law requires that the presence of drugs in blood should be quantified and reported to the court. This then poses the question 'If you have a quantitative measurement, what does it mean?'. Unlike alcohol, where a large body of information is available that enables the toxicologist to offer a generalised statement about the effects that might be expected when an individual has a known BAC, it is not possible to relate blood drug concentrations to drug effects or degrees of intoxication. Drugs affect different people in different ways and it is unlikely that anybody who has used a particular psychoactive drug will exhibit all of the signs and symptoms listed in the sections above to a noticeable degree. One approach to using quantitative results is to employ population profiles or cumulative frequency plots (Figure 5.1) to allow the results of a particular analysis to be compared with those of a population of drivers who have previously been classified as unfit to drive by qualified medical personnel. Examination of data plotted in this way allows the toxicologist to compare an analytical result against the frequency with which it has been encountered in a casework population. Although impairment and symptoms cannot be correlated directly with blood drug concentrations, cumulative data plots enable the toxicologist to assist the court by comparing specific case results against a population of drivers who have previously been certified as unfit to drive. For example, Figure 5.1 shows population profiles for diazepam, nordiazepam and temazepam in 354 cases where all three drugs were detected. According to this figure, a subject with a blood diazepam concentration of 2 μg/mL would have a higher concentration than 75% of the 354 cases previously reviewed. This provides the court with some context for understanding the drug concentration reported in any individual case.

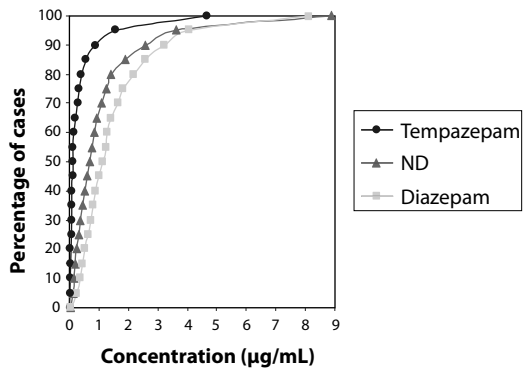

Figure 5.1 Cumulative frequency profiles for diazepam, nordiazepam (ND) and temazepam in 354 cases where all three drugs were detected.

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CHAPTER

6 Drug Testing in Human Sport

DA Cowan

Introduction

Drug abuse in sport is often called doping, the international word 'dope' being used both as a noun and as a verb. This is thought to originate from the Dutch word 'doop' which means 'Christian baptism'. It seems likely that the religious fervour associated with this ceremony resulted in the use of the same word, in a somewhat cynical and contemptuous way, to describe the state of intoxication and euphoria induced by certain drugs. The word does not appear in this context before the twentieth century despite the practice of horse 'nobbling', which was known well before this time and is described separately in Chapter 7. The word appears to have come into use early in the twentieth century, and it is probably associated with the rise of the pharmaceutical industry.

The abuse of drugs in an attempt to enhance performance in human sporting competitions is not new. For example, the Greek authors Phylostratos and Galen commented on the ethics of competitors in the Olympics who would take any preparation to improve their performance. Roman gladiators were often drugged to make their fights more lusty and bloody as demanded by the spectators.

The effect of drugs on performance is often extremely difficult to determine, and there is little definitive published work for any species. The results that have been published are often conflicting; some workers suggest an increase in the competitor's performance and others suggest no improvement. The test system may not adequately relate to the appropriate sporting performance, such as increase in muscle strength and sprint running. Changes in speed of less than 1% cannot be demonstrated with statistical significance because of the many variable and uncontrollable factors, yet an improvement of only 1% represents a lead of 1 metre in a 100-metre sprint or 17.6 yards over 1 mile, which is an enormous lead in either race. Furthermore, athletes may take far larger amounts of drugs than would be ethically acceptable in most human experiments.

The toxic side-effects of drugs are less difficult to ascertain, but the conclusions drawn from the available data are often circumstantial (e.g. the possible links between taking anabolic steroids and liver cancer). Nevertheless, there is sufficient evidence of the harmful effects when certain drugs are misused to justify their prohibition from sports competitions.

Rules

In human sports, the main controlling body is the International Olympic Committee (IOC). However, since 2004 doping issues have been taken over by the World Anti-Doping Agency (WADA; originally called the International Anti-Doping Agency). The IOC and WADA jointly issued the Olympic Movement Anti-Doping Code, which defined doping as:

> The use of an expedient (substance or method) that is potentially harmful to athletes' health and/or capable of enhancing their performance, or
> The presence in the athlete's body of a prohibited substance or evidence of the use thereof or evidence of the use of a prohibited method.

The stated intention of the Medical Commission of the IOC was to ban those drugs that are likely to be harmful when misused, with the minimum of interference with the normal therapeutic use of drugs. Thus, a competitor who is undergoing a legitimate course of treatment is not disqualified.

However, this creates many problems in deciding which drugs should or should not be prohibited, and whether or not a competitor who requires a particular drug treatment should be allowed to compete.

WADA has recently published the new World Anti-Doping Code (WADA 2009h). In this document doping is defined as 'the occurrence of one or more of the anti-doping rule violations set forth in Article 2.1 through Article 2.8 of the Code'. WADA has published criteria that it uses when deciding what should be prohibited. Among these is Article 4.3.1.2 of the Code, which gives as one of the criteria for prohibiting a substance as 'Medical or other scientific evidence, pharmacological effect or experience that the *use* of the substance or method represents an actual or potential health risk to the *athlete*' (note that the italicised words are WADA-defined terms).

The IOC and now WADA list examples of prohibited drugs according to their pharmacological classification (Table 6.1), but the entries are often far from explicit, using the words 'and other substances with similar chemical structure or similar biological effect(s)'. For most substances, the mere presence of the substance or a diagnostic metabolite in the biological fluid sampled constitutes an offence, but for some substances (Table 6.2) there is a reporting threshold. Furthermore, certain methods are prohibited (Table 6.3); one category of which is to attempt to prevent detection of doping. WADA states that 'The use of any drug should be limited to medically justified indications'. It has a system in place (WADA 2009d) whereby athletes being treated with certain medications such as β_2-agonists may obtain a 'Therapeutic Use Exemption' certificate that will allow them to use that medication without contravening the WADA Code. WADA maintains a comprehensive set of guidance documents to help with the exemption process to permit needed medical treatment. Also WADA permits the use of certain substances (Table 6.4) other than during competitions. Finally, some sports prohibit additional substances (Table 6.5) and WADA publishes a comprehensive Prohibited List (WADA 2010b) each year.

Reported analytical findings

Data for human sports have been available since 1987 and are presented for the years 1987, 1990, 1995, 2000 and 2005 in Table 6.6. Figure 6.1 shows the proportion of samples analysed by WADA-accredited laboratories in the years 1988 to 2008 reported for the three most commonly found prohibited substances. Note the marked increase in the reporting of testosterone in recent years following the reduction of the reporting threshold by WADA. Note also the decrease in the reporting of salbutamol in the last 2 years following the increase in the reporting threshold by WADA.

Sampling

Sample collecting procedures must take into consideration both scientific and legal aspects:

- The health of the individual being sampled must be safeguarded.
- Incorrect labelling, contamination or sample switching must be avoided at the time of sample collection and subsequently.

Table 6.1 Substances prohibited by the World Anti-Doping Agency at all times

Category	Substance
Anabolic agents	1. Anabolic androgenic steroids (AAS)
	(a) Exogenous[(a)] *AAS, including:*
	1-androstendiol (5α-androst-1-ene-3β,17β-diol)**; 1-androstendione** (5α-androst-1-ene-3,17-dione)**; bolandiol** (19-norandrostenediol)**; bolasterone; boldenone; boldione** (androsta-1,4-diene-3,17-dione)**; calusterone; clostebol; danazol** (17α-ethynyl-17β-hydroxyandrost-4-eno[2,3-*d*]isoxazole)**; dehydrochlormethyltestosterone** (4-chloro-17β-hydroxy-17α-methylandrosta-1,4-dien-3-one)**; desoxymethyltestosterone** (17α-methyl-5α-androst-2-en-17β-ol)**; drostanolone; ethylestrenol** (19-nor-17α-pregn-4-en-17-ol)**; fluoxymesterone; formebolone; furazabol** (17β-hydroxy-17α-methyl-5α-androstano[2,3-*c*]-furazan)**; gestrinone; 4-hydroxytestosterone** (4,17β-dihydroxyandrost-4-en-3-one)**; mestanolone; mesterolone; metenolone; methandienone** (17β-hydroxy-17α-methylandrosta-1,4-dien-3-one)**; methandriol; methasterone** (2α, 17α-dimethyl-5α-androstane-3-one-17β-ol)**; methyldienolone** (17β-hydroxy-17α-methylestra-4,9-dien-3-one)**; methyl-1-testosterone** (17β-hydroxy-17α-methyl-5α-androst-1-en-3-one)**; methylnortestosterone** (17β-hydroxy-17α-methylestr-4-en-3-one)**; methyltrienolone** (17β-hydroxy-17α-methylestra-4,9,11-trien-3-one)**; methyltestosterone; mibolerone; nandrolone; 19-norandrostenedione** (estr-4-ene-3,17-dione)**; norboletone; norclostebol; norethandrolone; oxabolone; oxandrolone; oxymesterone; oxymetholone; prostanozol** (17β-hydroxy-5α-androstano[3,2-*c*] pyrazole)**; quinbolone; stanozolol; stenbolone; 1-testosterone** (17β-hydroxy-5α-androst-1-en-3-one)**; tetrahydrogestrinone** (18α-homo-pregna-4,9,11-trien-17β-ol-3-one)**; trenbolone** and other substances with a similar chemical structure or similar biological effect(s)
	(b) Endogenous[(b)] *AAS when administered exogenously:*
	androstenediol (androst-5-ene-3β,17β-diol)**; androstenedione** (androst-4-ene-3,17-dione)**; dihydrotestosterone** (17β-hydroxy-5α-androstan-3-one)**; prasterone** (dehydroepiandrosterone, DHEA)**; testosterone**
	and the following metabolites and isomers:
	5α-androstane-3α,17α-diol; 5α-androstane-3α,17β-diol; 5α-androstane-3β,17α-diol; 5α-androstane-3β,17β-diol; androst-4-ene-3α,17α-diol; androst-4-ene-3α,17β-diol; androst-4-ene-3β,17α-diol; androst-5-ene-3α,17α-diol; androst-5-ene-3α,17β-diol; androst-5-ene-3β,17α-diol; 4-androstenediol (androst-4-ene-3β,17β-diol); **5-androstenedione** (androst-5-ene-3,17-dione); **epi-dihydrotestosterone; epitestosterone; 3α-hydroxy-5α-androstan-17-one; 3β-hydroxy-5α-androstan-17-one; 19-norandrosterone; 19-noretiocholanolone**
	2. Other anabolic agents, including but not limited to:
	Clenbuterol, selective androgen receptor modulators (SARMs), tibolone, zeranol, zilpaterol
Hormones and related substances	The following substances and their releasing factors are prohibited: 1. **Erythropoiesis-stimulating agents (e.g. erythropoietin (EPO), darbepoietin (dEPO), haematide)** 2. **Growth hormone (GH), insulin-like growth factors (**e.g. **IGF-1), mechano growth factors (MGFs)** 3. **Chorionic gonadotrophin (CG) and luteinizing hormone (LH) in males** 4. **Insulins** 5. **Corticotrophins** and other substances with similar chemical structure or similar biological effect(s)
β_2-Agonists	**All β_2-agonists including their D- and L-isomers are prohibited.**
	Therefore, formoterol, salbutamol, salmeterol and terbutaline when administered by inhalation also require a Therapeutic Use Exemption in accordance with the relevant section of the International Standard for Therapeutic Use Exemptions
	Despite the granting of a Therapeutic Use Exemption, the presence of salbutamol in urine in excess of 1000 μg/L will be considered as an *Adverse Analytical Finding* unless the *Athlete* proves, through a controlled pharmacokinetic study, that the abnormal result was the consequence of the use of a therapeutic dose of inhaled salbutamol
Hormone antagonists and modulators	The following classes are prohibited: 1. **Aromatase inhibitors** including, but not limited to: **anastrozole, letrozole, aminoglutethimide, exemestane, formestane, testolactone** 2. **Selective oestrogen receptor modulators (SERMs)** including, but not limited to: **raloxifene, tamoxifen, toremifene** 3. **Other anti-oestrogenic substances** including, but not limited to: **clomiphene, cyclofenil, fulvestrant** 4. **Agents modifying myostatin function(s)** including but not limited to: **myostatin inhibitors**
Diuretics and other masking agents	**Masking agents are prohibited**. They include: **Diuretics, probenecid, plasma expanders** (e.g. IV administration of **albumin, dextran, hydroxyethyl starch** and **mannitol**) and other substances with similar biological effect(s) Diuretics include: **acetazolamide, amiloride, bumetanide, canrenone, chlorthalidone, etacrynic acid, furosemide, indapamide, metolazone, spironolactone, thiazides** (e.g. **bendroflumethiazide, chlorothiazide, hydrochlorothiazide**), **triamterene**, and other substances with a similar chemical structure or similar biological effect(s) (except drosperinone and topical dorzolamide and brinzolamide, which are not prohibited)

[(a)] 'Exogenous' refers to a substance that is not ordinarily capable of being produced by the body naturally.
[(b)] 'Endogenous' refers to a substance that is capable of being produced by the body naturally.

Table 6.2 WADA Code. Summary of urinary concentrations above which WADA-accredited laboratories must report findings for specific substances (WADA 2009f)

Substance	*Urinary concentration to be reported*
Carboxy-THC[a]	>15 µg/L
Cathine[b]	>5 mg/L
Ephedrine	>10 mg/L
Epitestosterone[c]	>200 µg/L
Methylephedrine	>10 mg/L
Morphine[d][e]	>1 mg/L
19-Norandrosterone[c]	>2 µg/L
Salbutamol[d][f]	>1 mg/L
testosterone/epitestosterone (T/E) ratio[g]	>4

[a] 11-Nor-delta 9-tetrahydrocannabinol-9-carboxylic acid.
[b] Unless it may be as a metabolite of a permitted substance such as pseudoephedrine.
[c] Threshold adjusted if specific gravity >1.020.
[d] Sum of glucuronide conjugate and free drug concentrations.
[e] Unless it may be as a metabolite of a permitted substance such as codeine.
[f] Concentrations greater than 500 µg/L and less than 1 mg/L should be reported as consistent with the use of a β_2-agonist.
[g] Testosterone/epitestosterone ratio. Although a report must be issued for samples with a T/E ratio greater than 4, samples with lower ratios must also be reported if there is evidence of an exogenous origin of testosterone.

Table 6.3 Methods prohibited by the World Anti-Doping Agency at all times

Enhancement of oxygen transfer

The following are prohibited:

1. Blood doping, including the use of autologous, homologous or heterologous blood or red blood cell products of any origin
2. Artificially enhancing the uptake, transport or delivery of oxygen, including but not limited to perfluorochemicals, efaproxiral (RSR13) and modified haemoglobin products (e.g. haemoglobin-based blood substitutes, microencapsulated haemoglobin products)

Chemical and physical manipulation

1. Tampering, or attempting to tamper, in order to alter the integrity and validity of Samples collected during Doping Controls is prohibited. These include but are not limited to catheterisation, urine substitution and/or alteration
2. Intravenous infusions are prohibited except in the management of surgical procedures, medical emergencies or clinical investigations

Gene doping

The transfer of cells or genetic elements or the use of cells, genetic elements or pharmacological agents to modulate expression of endogenous genes having the capacity to enhance athletic performance is prohibited **Peroxisome proliferator activated receptor** δ (PPARδ) **agonists** (e.g. GW 1516) and **PPARδ-AMP-activated protein kinase** (AMPK) **axis agonists** (e.g. AICAR) **are prohibited**

Table 6.4 Substances prohibited by the World Anti-Doping Agency only in-competition

Stimulants	All stimulants (including both their D- and L- optical isomers where relevant) are prohibited, except imidazole derivatives for topical use and those stimulants included in the 2009 Monitoring Program[a] Stimulants include: *(a) Non-specified stimulants:* Adrafinil; amfepramone; amiphenazole; amfetamine; amfetaminil; benzphetamine; benzylpiperazine; bromantan; clobenzorex; cocaine; cropropamide; crotetamide; dimethylamfetamine; etilamfetamine; famprofazone; fencamine; fenetylline; fenfluramine; fenproporex; furfenorex; mefenorex; mephentermine; mesocarb; methamfetamine (D-); methylenedioxyamfetamine; methylenedioxymethamfetamine; *p*-methylamfetamine; modafinil; norfenfluramine; phendimetrazine; phenmetrazine; phentermine; 4-phenylpiracetam (carphedon); prolintane A stimulant not expressly listed in this section is a Specified Substance *(b) Specified stimulants (examples):* Adrenaline[b]; cathine[c]; ephedrine[d]; etamivan; etilefrine; fenbutrazate; fencamfamin; heptaminol; isometheptene; levmetamfetamine; meclofenoxate; methylephedrine[d]; methylphenidate; nikethamide; norfenefrine; octopamine; oxilofrine; *p*-hydroxyamfetamine; pemoline; pentetrazol; phenpromethamine; propylhexedrine; selegiline; sibutramine; strychnine; tuaminoheptane and other substances with a similar chemical structure or similar biological effect(s)
Narcotics	The following narcotics are prohibited: **Buprenorphine, dextromoramide, diamorphine (heroin), fentanyl and its derivatives, hydromorphone, methadone, morphine, oxycodone, oxymorphone, pentazocine, pethidine**
Cannabinoids	Cannabinoids (e.g. hashish, marijuana) are prohibited
Glucocorticosteroids*	All glucocorticosteroids are prohibited when administered by oral, intravenous, intramuscular or rectal routes In accordance with the International Standard for Therapeutic Use Exemptions, a declaration of use must be completed by the Athlete for glucocorticosteroids administered by intra-articular, periarticular, peritendinous, epidural, intradermal and inhalation routes, except as noted below Topical preparations when used for auricular, buccal, dermatological (including iontophoresis/phonophoresis), gingival, nasal, ophthalmic and perianal disorders are not prohibited and require neither a Therapeutic Use Exemption nor a declaration of use

[a] The following substances included in the 2009 Monitoring Program (bupropion, caffeine, phenylephrine, phenylpropanolamine, pipradol, pseudoephedrine, synephrine) are not considered as Prohibited Substances.
[b] **Adrenaline** associated with local anaesthetic agents or by local administration (e.g. nasal, ophthalmological) is not prohibited.
[c] **Cathine** is prohibited when its concentration in urine is greater than 5 mg/L.
[d] Each of **ephedrine** and **methylephedrine** is prohibited when its concentration in urine is greater than 10 mg/L.
[e] WADA use the term 'glucocorticosteroid' for what is more conventionally known as a corticosteroid.

- The rights of the individual or team must be safeguarded against error by the analyst.

Samples in human sport are now usually collected by agencies certified in accordance with the ISO 9001 standard (www.iso.org/iso/iso_catalogue/management_standards/iso_9000_iso_14000.htm) and using doping control officers who have been appropriately trained to collect the samples. Apart from its Code (WADA 2009h), WADA publishes an International Standard for Testing (WADA 2009c), which incorporates elements from ISO/PAS 18873 (which was withdrawn in 2005) and the ISO 9000 series of quality management system standards. WADA and most international federations always provide a second portion of the sample for defence use. This is to be opened only after the first sample has been found to contain a banned drug, and after the competitor has been notified and invited to attend the second analysis, with his or her own expert if the competitor so wishes.

Table 6.5 Substances prohibited by particular sports and only in-competition	
Alcohol	Alcohol (ethanol) is prohibited in-competition only, in the following sports. Detection will be conducted by analysis of breath and/or blood. The doping violation threshold (haematological values) is 0.10 g/L. ■ Aeronautic (FAI) ■ Archery (FITA, IPC) ■ Automobile (FIA) ■ Boules (IPC bowls) ■ Karate (WKF) ■ Modern Pentathlon (UIPM) for disciplines involving shooting ■ Motorcycling (FIM) ■ Ninepin and Tenpin Bowling (FIQ) ■ Powerboating (UIM)
Beta-blockers	Unless otherwise specified, beta-blockers are prohibited in-competition only, in the following sports. ■ Aeronautic (FAI) ■ Archery (FITA, IPC) (also prohibited Out-of-Competition) ■ Automobile (FIA) ■ Billiards and Snooker (WCBS) ■ Bobsleigh (FIBT) ■ Boules (CMSB, IPC bowls) ■ Bridge (FMB) ■ Curling (WCF) ■ Golf (IGF) ■ Gymnastics (FIG) ■ Motorcycling (FIM) ■ Modern Pentathlon (UIPM) for disciplines involving shooting ■ Ninepin and Tenpin Bowling (FIQ) ■ Powerboating (UIM) ■ Sailing (ISAF) for match race helms only ■ Shooting (ISSF, IPC) (also prohibited Out-of-Competition) ■ Skiing/Snowboarding (FIS) in ski jumping, freestyle aerials/halfpipe and snowboard halfpipe/big air ■ Wrestling (FILA) Beta-blockers include, but are not limited to, the following: **acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, bunolol, carteolol, carvedilol, celiprolol, esmolol, labetalol, levobunolol, metipranolol, metoprolol, nadolol, oxprenolol, pindolol, propranolol, sotalol, timolol**

Urine

Urine is the preferred body fluid. Its collection is non-invasive, it is generally available in sufficient quantity, and the drugs or their metabolites tend to be present in relatively high concentrations. The disadvantages are that a drug may be present as its metabolites or in a conjugated form, and the parent drug may be present only in a relatively low concentration. Furthermore, the relationship with the concentration in blood is very imprecise.

Substitution of samples is clearly a possibility that must be avoided and particular care is required during the period of waiting before a sample is obtained to balance this risk against the desire for privacy on the part of a person. It has been reported that racing cyclists have carried a rubber bladder of (negative) urine under their arm, connected by a rubber tube to the appropriate discharge point.

Blood

The principal advantage of a blood sample is that its integrity is easier to safeguard because it is usually collected by a doctor or phlebotomist experienced in the procedure. In addition, drug concentrations in blood are interpreted more easily than those in urine and certain drugs that are not excreted in urine in significant quantities (e.g. human growth hormone) can be detected in blood. Since the 2000 Olympic Games in Sydney, blood has been collected routinely by some federations (e.g. the International Cycling Union) as a 'health check'. Any competitor whose haematocrit is above 50% is not permitted to compete. This test is intended to limit the use of erythropoietin (EPO) to stimulate red cell production. However, this haematocrit test is readily circumvented and depends on too many factors; the use of haemoglobin concentration is preferred. Furthermore, blood samples may also be collected for more sophisticated tests to indicate the use of EPO. In addition, the administration of small doses (so-called micro-dosing) of EPO has been shown (Ashenden *et al.* 2006) to reduce the chance of detection of EPO use and yet still raise haemoglobin concentrations, hence the need to collect blood samples. Recently, tests for recombinant human growth hormone (rhGH) administration and for blood transfusion have been developed (see later) that rely on the use of blood samples.

Other matrices

At the present time, WADA does not permit the use of alternative biological matrices such as oral fluid or hair to counter an analytical finding obtained from either a urine or blood sample. Nevertheless, Kintz has reviewed hair testing and doping control in human sport (Kintz 1998) and an interesting case report of its use in detecting a variety of prohibited substances in body builders has been published (Dumestre-Toulet *et al.* 2002) (see Chapter 19).

Table 6.6 Prohibited substances most commonly reported by WADA-accredited laboratories, in order of frequency[(a)]

Number of reports									
Substance	***1987***	***Substance***	***1990***	***Substance***	***1995***	***Substance***	***2000***	***Substance***	***2005***
Nandrolone	262	Nandrolone	192	Testosterone	293	Salbutamol	367	Testosterone	1132
Pseudoephedrine	100	Testosterone	171	Cannabis	224	Nandrolone	325	Cannabis	503
Testosterone	83	Pseudoephedrine	123	Nandrolone	212	Testosterone	306	Salbutamol	357
Ephedrine	58	Stanozolol	79	Methandienone	132	Cannabis	295	Nandrolone	298
Phenylpropanolamine	57	Phenylpropanolamine	64	Salbutamol	132	Pseudoephedrine	136	Stanozolol	233
Methenolone	42	Ephedrine	43	Pseudoephedrine	102	Ephedrine	129	Amfetamine	194
Stanozolol	37	Codeine	32	Ephedrine	78	Stanozolol	116	Terbutaline	171
Methandienone	27	Methenolone	25	Stanozolol	78	Terbutaline	110	hCG	143
Codeine	26	Amfetamine	24	Methenolone	39	Methandienone	75	Budesonide	116
Amfetamine	24	Methandienone	23	Clenbuterol	31	Lidocaine	64	Ephedrine	93

[(a)] 1987 was first year of available data.

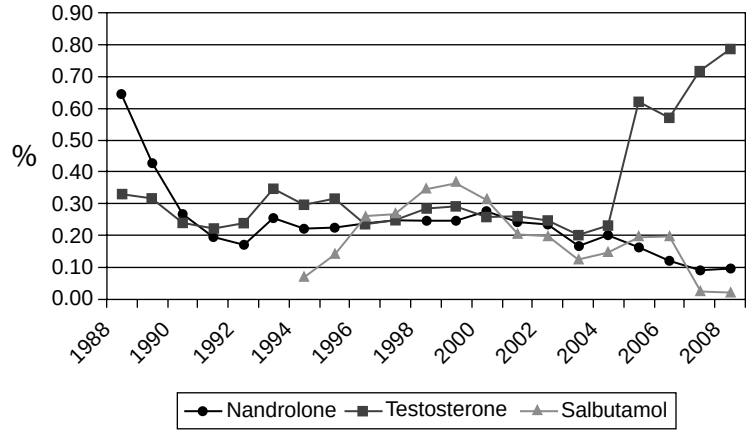

Figure 6.1 The proportion of human sports samples analysed by WADA-accredited laboratories in the years 1988 to 2008 reported for the three prohibited substances most commonly found.

Analytical approach

With the exception of anabolic steroids, prohibited substances are generally administered at or near the therapeutic dose, which results in relatively low concentrations in biological fluids. The laboratory is provided with a coded sample to preserve the anonymity of the athlete. They may also be given a declaration of any drug that has recently been taken by the athlete but, apart from that, there is usually no evidence whether or not a drug has been administered, or what sort of drug it might be. As with equine testing, any drug used in human treatment or in veterinary practice may be found. Thus, screening procedures are designed to be both sensitive and of wide coverage. The material analysed is usually in a fairly fresh condition. The analyst thus has a clearer picture of a normal sample than does the forensic or hospital chemist, who may be required to examine a wide variety of materials in various states of decomposition. Any sample that fails a screening test is invariably submitted to rigorous confirmatory testing (see below) before an adverse report is issued. The WADA-accredited laboratory must reliably be able to detect and confirm the presence of prohibited substances or their metabolites at least down to a minimum required performance level (MRPL; see Table 6.7) (WADA 2009f).

However, WADA states that 'for non-threshold substances prohibited in-competition only, it is not recommended that laboratories report below 10% of the MRPL'. This refers to stimulants, narcotics and beta-blockers only (the latter being prohibited only by particular sports) since, for glucocorticoids, WADA states that 'laboratories are not to report below the MRPL'.

Although, with some exceptions, the parent drug is the entity that appears in the Prohibited List, screening procedures rely upon the detection of either the unchanged drug or its metabolites. The identification of the corresponding metabolites is often useful supplementary evidence to support the identification of the parent drug, and indeed WADA expects the laboratory to identify as many of the presumptive analytical findings from the screening procedures as possible. In addition, the presence of metabolites in the appropriate concentrations relative to the parent drug helps to support the conclusion that a drug has been administered. Conversely, the absence of any expected metabolites is possible evidence that a sample has been contaminated; this should be refutable by a proper chain of custody. Occasionally, the parent drug is not excreted in urine at a detectable concentration and a knowledge of the metabolic pathways is thus essential. An example of this is the identification of 19-norandrosterone and 19-noretiocholanolone (Fig. 6.2) in the urine of humans as evidence of the administration of the anabolic steroid nandrolone or a 19-norsteroid precursor.

Drugs can be used either to improve or to impair athletic performance, though in human sport the latter category of drug is

Table 6.7 Minimum required performance levels

Prohibited class	*Specific examples/ exceptions*	*Concentration*
Stimulants		0.5 mg/L
	Strychnine	0.2 mg/L
Narcotics		0.2 mg/L
	Buprenorphine	10 µg/L
Anabolic agents		10 µg/L
	Clenbuterol	2 µg/L
	Methandienone	2 µg/L
	Methyltestosterone	2 µg/L
	Stanozolol	2 µg/L
	Epitestosterone	2 µg/L
Hormone antagonists and modulators	Aromatase inhibitors, SERMs and other anti-oestrogenic substances	50 µg/L
Beta$_2$-Agonists		100 µg/L
Beta-blockers		0.5 mg/L
Diuretics		0.25 mg/L
Glucocorticosteroids		30 µg/L
Peptide hormones	hCG	5 IU/L

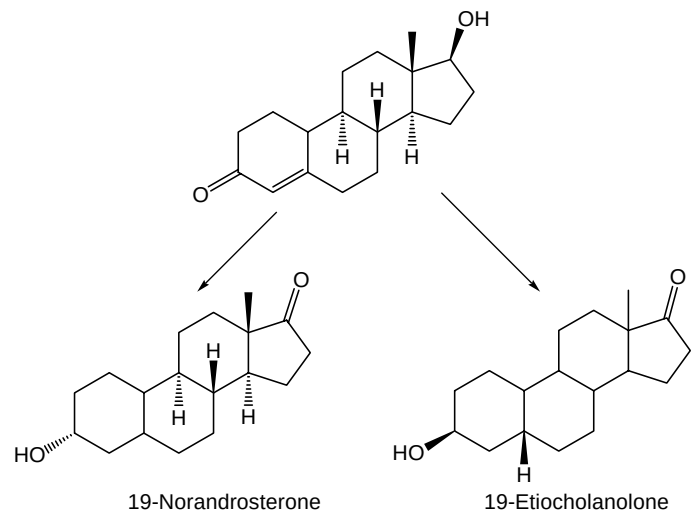

Figure 6.2 Main metabolites of nandrolone (19-norandrosterone).

unlikely to be used. No single analytical scheme will suffice to cover so many different types of compound; various approaches have evolved in sports drug testing laboratories to address this challenge.

Some drugs are notable for being excreted in urine almost entirely in conjugated form as, for instance, most anabolic steroids. When the presence of these drugs is suspected, hydrolysis before extraction is essential, although liquid chromatography–tandem mass spectrometry (LC-MS(-MS)) of intact conjugates is starting to be used.

Liquid–liquid extraction may be used, for example with alkalinisation of the urine, to prepare samples for the detection of basic drugs by gas chromatography (GC) with nitrogen-selective detection. Alternatively, drugs have been extracted on the styrene–divinylbenzene copolymer XAD-2 resin. The development of solid-phase extraction (SPE) in the cartridge format in the late 1970s (Shackleton, Whitney 1980) and the rapid advances made in the technology associated with the technique have provided an attractive alternative to liquid–liquid extraction in many drug-screening programmes. Immunochemical methods that covered anabolic steroids were first introduced into human drug screening programmes in the 1970s (Brooks *et al.* 1979). However, in the late 1980s, these were largely replaced by GC-MS methods. Unlike many horseracing drug testing laboratories, human sports drug testing laboratories have not employed thin-layer chromatography (TLC) and do not use enzyme-linked immunosorbent assay (ELISA) extensively, but have used XAD-2 resin in columns for sample extraction and now, more commonly C_8 and C_{18} SPE.

Instrumental methods based on GC-MS, GC–high resolution mass spectrometry (GC-HRMS), GC-MS(-MS) and LC-MS(-MS) are preferred by most laboratories.

Solvent extraction

In general, the choice of solvent is dictated by the wide range of drugs to be covered, or the need to extract a specific drug as in confirmatory analysis procedures.

In many sports drug testing laboratories throughout the world, SPE has replaced liquid–liquid extraction for the isolation of drugs from both urine and plasma. Based upon the studies of Shackleton and Whitney (1980), the use of C_{18} or C_8 bonded-phase cartridges for the isolation of anabolic steroids and their metabolites is the most common approach for sample extraction.

Sample preparation

Extraction method for drugs and metabolites

Note the initial pH and specific gravity of each sample.

Basic drugs

1. Take an aliquot (4.0 mL) of each urine sample in a 10 mL centrifuge tube.
2. Add 0.5 mL of 5 mol/L potassium hydroxide solution, approximately 3 g of sodium chloride, 100 µL of diphenylamine as a reference standard solution (80 mg/L, final concentration 2 mg/L) and 1.6 mL of *t*-butyl methyl ether.
3. Vortex thoroughly and then mix for at least 10 min on a rotary mixer.
4. Centrifuge to separate the phases at 800*g* for 5 min.
5. Remove about 1 mL of the ethereal layer and transfer to an autosampler vial containing approximately 20 mg of anhydrous sodium sulfate to dry the extract (take care that none of the lower aqueous layer is transferred).
6. Examine 2 µL of this extract by GC using a cross-linked 5% phenylmethylsilicone-fused silica column (12.5 m × 0.32 mm i.d., 0.52 µm) using nitrogen–phosphorus detection.

The use of sodium chloride to increase the ionic strength of the aqueous phase increases the extraction of many of the compounds into the ether, which obviates the need for solvent evaporation. Although some polar compounds, such as ephedrine, are readily extracted in this procedure, it is best suited to less polar compounds.

Anabolic steroids and corticosteroids

1. Condition C_{18} or C_8 SPE cartridges with methanol and then water. Add 4 mL of the urine sample.
2. Wash with an equal volume of water and then elute the adsorbed steroids with an equal volume of methanol.
3. Remove the methanol under nitrogen, dissolve the residue in 1 mL of 0.1 mol/L phosphate buffer (pH 6.2) containing β-glucuronidase from *Escherichia coli* (approximately 1.4 units using 4-nitrophenyl-β-D-glucuronide as the substrate at 37°C).
4. Incubate for either a minimum of 2 h at 50°C or overnight at 37°C.
5. Cool and add approximately 100 mg potassium carbonate and extract the steroids with 5 mL distilled diethyl ether.
6. Divide the ether into two portions.
7. Evaporate each portion under nitrogen, dry the residue in a vacuum desiccator over phosphorus pentoxide–potassium hydroxide.
8. To one portion, add 200 µL of LC mobile phase, transfer to an autosampler vial and immediately cap the vial.
9. Examine the solution by LC-MS(-MS) for corticosteroids (and anabolic steroids such as tetrahydrogestrinone that are not amenable to trimethylsilylation for GC-MS analysis).
10. Derivatise the other portion by the addition of 100 µL N-methyl-N-trimethylsilyl-trifluoroacetamide–ammonium iodide–ethanethiol (1000 : 2:6) and heating at 60°C for at least 15 min.
11. Examine the solution by capillary column GC-MS.

Mass spectral and chromatographic data for many anabolic steroids and their metabolites have been published by Ayotte *et al.* (1996) and are given as system GAI (see Chapter 40):

- Column: methylsilicone fused silica (25 m × 0.2 mm i.d., 0.11 µm), connected to a mass spectrometer.
- Temperature programme: 180°C for 1 min to 280°C at 8°/min.
- Carrier gas: He.

Diuretics

1. Take an aliquot (2.0 mL) of each urine sample in a 10 mL centrifuge tube.
2. Add 100 µL mefruside internal standard (10 µg/mL) and 2 mL 0.1 mol/L acetate buffer (pH 5.2).
3. Vortex thoroughly for 30 s.
4. Decant the urine onto a cross-linked polymeric sorbent cartridge with hydrophilic and lipophilic moieties (such as Abselut Nexus) and allow it to pass through the column. Do not let the bed dry out.
5. Add 1 mL of purified water onto each cartridge and allow it to pass through the column. Do not let the bed dry out.
6. Add 1 mL of methanol–water (20 : 80) onto each cartridge and allow it to pass through the column. Apply vacuum to the column to dry the bed.
7. Release the vacuum and place a labelled 10 mL glass centrifuge tube under the cartridge to collect the sample eluent.
8. Elute the adsorbed compounds with 3 mL methanol under gravity and then with the aid of a vacuum for a minimum of 30 s to dry the bed and achieve maximum recovery.
9. Evaporate the methanolic solution to dryness using oxygen-free nitrogen at 60°C.
10. Reconstitute the samples by adding 200 µL of LC mobile phase.
11. Vortex; transfer the reconstituted extract to a 0.2 mL tapered autosampler vial and cap the vial securely.
12. Examine 10 µL of the solution by LC-MS(-MS) for diuretics.

Gas chromatography and gas chromatography–mass spectrometry

The GC procedures referred to above detect a wide range of compounds in urine samples, at concentrations in the order of 0.1 mg/L. They depend on the fact that all the compounds of interest contain at least one nitrogen atom and produce a signal in an alkali flame-ionisation

detector. The methods of extraction and the selectivity of the detector ensure minimal interference from other compounds that do not contain nitrogen, although certain plasticisers that contain phosphorus, such as tributyl phosphate, may produce signals. In addition, other nitrogen-containing compounds that are not prohibited in human sport (e.g. antihistamines) produce interfering peaks.

Identification is based on the retention index; alternatively, retention time (relative to a standard) may be used. Details of retention indices or relative retention times of compounds in the systems described below are given in Chapter 40 and in the index of Gas Chromatographic Data.

The identity of a substance should be confirmed using derivative formation and GC-MS, comparing the data obtained with reference material analysed contemporaneously (see Criteria for identification below).

Liquid chromatography–mass spectrometry

With the development of atmospheric-pressure ionisation (API) techniques, LC-MS has found increasing application in doping control for both the qualitative and quantitative analysis of drugs. Screening methods have been developed for corticosteroids (Deventer, Delbeke 2003; Mazzarino, Botré 2006) and diuretics (Deventer *et al.* 2002; Ventura *et al.* 2008).

In human sports drug testing, LC-MS(-MS) has been used more frequently over the last 10 years. For example, Barrón *et al.* (1996) developed a direct method to determine anabolic steroids in human urine by on-line SPE LC-MS with a particle beam interface. Bean and Henion showed that it was possible to determine intact, i.e. conjugated, anabolic steroids using LC-electrospray ionisation (ESI)-MS(-MS) (Bean, Henion 1997). Thevis and colleagues used LC-MS(-MS) for the rapid screening of samples for beta-blockers (Thevis *et al.* 2001). Diuretics have been screened by LC-MS techniques (Ventura *et al.* 1991) and by LC-MS(-MS) (Thieme *et al.* 2001).

Recently, the use of sub-2 μm porous particles to enable faster separations and greater separation power has become possible (Mazzeo *et al.* 2005). High-pressure pumping systems (greater than the conventional 400 bar [40 MPa] limit) needed to obtain the linear velocities desired are now readily available. Although the frictional heating caused by the velocity of the mobile phase through the column can limit the benefit that can be obtained using small particles, the use of 2.1 mm and narrower internal diameter columns has minimised this potential difficulty. Thörngren and colleagues have used ultra-performance liquid chromatography (UPLC)-MS(-MS) to screen 130 different substances (diuretics, masking agents, central nervous system stimulants and opiates) in urine (Thörngren *et al.* 2008) in approximately 6 min.

Isotope ratio mass spectrometry

Combustion isotope ratio MS (CIRMS) is now used routinely by several WADA-accredited laboratories as an additional tool to help distinguish an individual whose testosterone : epitestosterone ratio may be naturally beyond the normal range from one who was administered testosterone. This technique relies on the fact that synthetic testosterone has a different proportion of ^{13}C to the more abundant ^{12}C than the normal endogenous steroid (de la Torre *et al.* 2001). The extracted steroids are separated by GC and then converted into CO_2 and the relative amounts of ^{12}C to ^{13}C as CO_2 is determined for each eluting steroid in turn. Typically, the testosterone metabolites androsterone and etiocholanolone or androstanediols are monitored (Aguilera *et al.* 2000), or the metabolites 5α-androstanediol and 5β-androstanediol (Aguilera *et al.* 2001; Shackleton *et al.* 1997b), often comparing the results with pregnanediol or pregnanetriol as endogenous internal standards (Aguilera *et al.* 1999; Shackleton *et al.* 1997a). Flenker and colleagues and Cawley and colleagues have published reference isotope ratios for endogenous steroids (Cawley *et al.* 2009; Flenker *et al.* 2008) and Cawley has also published a method for the direct analysis of testosterone rather than its metabolites (Cawley *et al.* 2009). Grosse and colleagues have shown that, in some urine samples, 19-norsteroids may be produced in small quantities from endogenous steroids (Grosse *et al.* 2005). Hebestreit and co-workers have published a method to distinguish this or the minute amounts that may be produced, especially in females, from the administration of nandrolone (Hebestreit *et al.* 2006). Buisson and colleagues (2009) have also used GC-CIRMS to detect exogenous hydrocortisone administration. Cawley and Flenker have published a tutorial article that reviews the use of CIRMS in doping control (Cawley, Flenker 2008).

Methods

In human sports drug testing, MS is essential for the definite identification of a prohibited substance, with the exception of peptide hormones and glycoproteins, such as human chorionic gonadotrophin (hCG) for which a validated immunoassay is required for detection and quantification. For confirmation of hCG, a second different immunoassay is required. Specific techniques and methodologies for other peptide hormones and glycoproteins such as EPO have been described by WADA (WADA 2009a) and one for recombinant hCG (rhGH) is currently being considered.

Criteria for identification

WADA requires laboratories to be accredited to ISO 17025 to be eligible for WADA accreditation. ISO/IEC 17025 : 2005 requires traceability of measurements and for sports drug testing this is considered to be met by WADA, when identifying a prohibited substance (WADA 2003), by the direct comparison with a reference material or reference collection analysed in parallel or in series with the test sample. A reference material is generally accepted as a homogeneous, stable chemical with a well-established structure. The material may be characterised structurally within the laboratory using appropriate techniques or validated against a certified reference material or by comparison with uncontroversial published data. WADA permits the use of a reference collection 'obtained from a verified administration study in which scientific documentation of the identity of metabolite(s) can be demonstrated' (WADA 2009b). Certified reference materials issued by organisations accredited for compliance with ISO Guide 34 : 2000 are often not available and hence there is the need to use authenticated administrations for comparison purposes. Many of the most common substances are now available with certificates of analysis, and thereby fully meet the generic traceability requirements of ISO/IEC 17025 : 2005. This is particularly important for quantitative analysis, where it is usually extremely difficult for an individual laboratory to determine the purity of a non-certified material to a sufficient standard to be able to establish the measurement uncertainty.

WADA generally expects a chromatographic retention time match between the analyte and a reference collection sample (see above) or reference material analysed using the same procedure in the same assay. This retention time difference must be not more than 1% or ±0.2 min, whichever is the smaller, and three diagnostic ions in the electron impact (EI) and chemical ionisation (CI) mass spectra must also match to within 20% of the relative abundance of each ion (Table 6.8). Figure 6.3 illustrates the relative abundance criteria that, paradoxically, show a step change for the data obtained using CI, LC or tandem MS. These standards generally meet or exceed those required by the Substance Abuse and Mental Health Services Administration (SAMHSA) for the US federal employment drug testing programmes (see also Chapter 3). Although library data may be useful in the early phase of substance identification, especially in generic screening procedures, they are not considered sufficiently reliable for the final identification. Similarly, published data are used more to assure reliability than directly for substance identification.

The required documentation for the analytical certificate is clearly set out in ISO/IEC 17025 : 2005 but merely requires a statement as to the substance found. However, WADA also sets out a

Table 6.8 Maximum relative ion intensity tolerances for substance identification using MS

Relative abundance (% of base peak)	*EI-GC-MS*	*CI-GC-MS; GC-MS^n; LC-MS; LC-MS^n*
>50%	±10% (absolute)	±15% (absolute)
25–50%	±20% (relative)	±25% (relative)
<25%	±5% (absolute)	±10% (absolute)

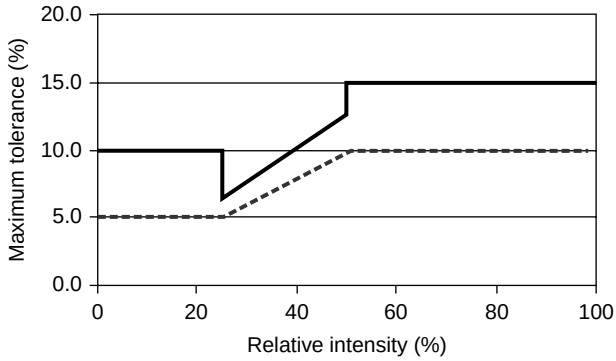

Figure 6.3 Graphical representation of WADA Maximum Tolerance Windows for relative abundances. Dashed line, EI-GC/MS data; solid line, CI-GC/MS, GC/MS^n, LC/MS and LC/MS^n.

standardised documentation package (WADA 2009e) to be provided in accordance with the International Standard for Laboratories (WADA 2009b) in support of a so-called Adverse Analytical Finding to avoid grounds for claims by lawyers that data are being concealed.

Threshold analysis

Thresholds may be set either in the rules for laboratory reporting (WADA 2009f) or as a penalty threshold (WADA 2009h). In either case, the laboratory needs a protocol to determine whether the specified threshold has been exceeded. Fully validated assays are used in these cases, with suitable standards and quality control samples run concurrently with the sample being assessed to assure the assay validity. Measurement uncertainty must be determined as part of the assay and validation (see Chapter 20 and Chapter 23). This uncertainty must be stated together with the mean result from three determinations whenever a quantitative result is included in the analytical report. Furthermore, the mean result must be greater than the reporting threshold, having taken into account the measurement uncertainty with the coverage factor, *k*, at a 95% confidence level. WADA has recently published a 'guard band' approach, whereby the group measurement uncertainty of its accredited laboratories has been assessed for the determination of 19-norandrosterone and used to determine whether a measured concentration exceeds the reporting threshold (WADA 2009g).

Drugs misused in sport

As described earlier, drugs misused in sport include anabolic steroids, stimulants, narcotics, corticosteroids and diuretics. Of particular interest are the anabolic steroids and corticosteroids; not only may synthetic analogues of endogenous substances be misused, but also testosterone or hydrocortisone (i.e. chemically or biosynthetically produced). This use of a pseudoendogenous compound that is either indistinguishable, or distinguishable with difficulty, from that which is produced naturally by an individual presents interesting analytical problems. Other examples of pseudoendogenous substances prohibited in sport include rhGH and rhEPO.

Dietary supplements

In recent years and probably since the passing of the Dietary Supplement and Health Education Act (DSHEA) by the United States Congress in 1994, which requires the US Food and Drug Administration to treat supplements as harmless food products, a large number of food supplements that contain anabolic steroids either deliberately or by contamination have become readily available. This may have given rise to inadvertent violation of the anti-doping rules. Nevertheless, most governing bodies of sport work on the principle of strict liability, and it is the responsibility of the competitor in human sport to avoid the administration of a prohibited substance.

Anabolic steroids

In human sport, anabolic steroids are used as bodybuilding drugs, sometimes in very large quantities, in events such as weightlifting and the shot-put. There are broadly two chemical types based upon the androstane and estrane ring systems: those with a 17α-alkyl group, which are active orally but are hepatotoxic, and 19-nor derivatives administered by injection. Although steroidal oestrogens and some stilbenes reputedly possess anabolic activity and are employed in beef production, they do not appear to be used as doping agents.

The metabolism of anabolic steroids in humans has been documented quite well, especially by Schänzer and co-workers (Schänzer 1996; Schänzer, Donike 1993). Synthetic anabolic steroids used in humans may be detected readily by the use of GC-MS analysis. Detection of the administration of pseudoendogenous compounds (i.e. compounds that are virtually identical to those of endogenous origin, such as testosterone) presents a more complex problem. There are several methods to indicate that testosterone has been administered to a male human. Natural testosterone production is controlled by a feedback mechanism that involves the pituitary gland, and administration of testosterone suppresses the natural production of pituitary hormones, such as luteinising hormone (LH) and follicle-stimulating hormone (FSH).

GC-MS analysis may be used to measure the testosterone concentration in urine, and immunoprocedures to determine LH and hence the urinary ratio of total (free plus conjugated) testosterone to LH. Testosterone is measured in nanomoles/litre, and LH in International Units of Human Menopausal Gonadotropin 2nd International Reference Preparation per litre (HMG-IR2/L). A ratio in excess of 200 is abnormal (Brooks *et al.* 1979).

In the human female, whose pituitary hormones may be suppressed by the use of oral contraceptives, the testosterone : LH ratio is of little evidential value. Instead, a method suitable for both males and females is to measure the ratio of testosterone (T) to epitestosterone (E) using GC-MS. In this method, the bis(trimethylsilyl) derivative is formed and the intensity of the molecular ions at *m*/*z* 432, under EI conditions for both of the steroid derivatives, is used to determine the ratio (Donike, Zimmermann 1980). WADA requires its accredited laboratories to report an 'atypical finding' whenever the measured ratio significantly exceeds 4. The increase in reported findings in 2005 reflects the reduction in the threshold for reporting from 6 to 4. Samples with a T/E greater than 4 may be submitted to CIRMS (see earlier). A method to detect the administration of dihydrotestosterone, the more active metabolite of testosterone, has also been proposed (Kicman *et al.* 1995) based on the perturbation of the endogenous hormone profile following administration. Isotope ratio MS, as described earlier, is also now used to assist detection of the administration of testosterone and related compounds.

Sampling carried out only at the time of competition has enabled the misuser to switch from using a preferred synthetic anabolic steroid to testosterone or to a synthetic anabolic steroid that is eliminated rapidly in sufficient time to escape detection. Thus sampling out-of-competition and with minimum notice as well as at competitions is now accepted as the optimal approach to detecting, and thereby limiting, the use of anabolic steroids in humans. A comprehensive

review of the detection of anabolic steroid administration has recently been published (Kicman *et al.* 2009).

Diuretics

Furosemide and hydrochlorothiazide are the diuretics most commonly reported by WADA-accredited laboratories. They are used in weightlifting and other competitions classified by weight such as boxing. The dilution of the urine, which results from diuretic use, can render detection of some other drugs more difficult. Diuretics have a wide variety of chemical structures, which makes it difficult to find a single suitable screening procedure. Diuretics can be detected in urine by HPLC using system HN (Chapter 41). A method applicable to human urine using extractive alkylation and GC-MS (Lisi *et al.* 1991, 1992), or LC with tandem MS (Thieme *et al.* 2001) may be used. A rapid screening method using automated SPE and LC-ESI-MS(-MS) that is capable of detecting 35 diuretics and related compounds at a concentration significantly below the WADA minimum required performance level in about 12 min per sample has been described (Goebel *et al.* 2004). Thörngren *et al.* (2008) have published a UPLC-MS(-MS) method that requires just 6 min per sample. The disadvantage of such rapid methods is that care needs to be taken to avoid ion suppression that might result in a false-negative result.

Protein hormones

Currently, in human sports drug testing, methods are accepted for the analysis of hCG and for EPO only. Tests for hCG are based on immunoprocedures (Cowan *et al.* 1991; Laidler *et al.* 1994). A method has been developed to identify the different isoform pattern of recombinant EPO from that of endogenous material using isoelectric focusing (Lasne 2001; Lasne, de Ceaurriz 2000), whereas a method based on the perturbation of a number of blood parameters (haematocrit, reticulocyte haematocrit, percentage macrocytes, serum EPO and soluble transferrin receptor [Parisotto *et al.* 2001]) was required by the IOC as part of the test. Subsequently, WADA accepted the isoelectric focusing method to be used alone to evidence EPO administration. New forms of EPO are now reaching the marketplace. Catlin and colleagues (2002) have published the detection of the administration of darbepoetin, a novel erythrocyte-stimulating protein, using the isoelectric focusing method of Lasne and de Ceaurriz (2000). This recombinant EPO-related substance has a half-life that is about three times longer than that of endogenous EPO. Recently a pegylated form of EPO (epoeitin-β) has been marketed that has an even longer half-life, and relatively little appears in urine. This foreign substance has a molar mass of about 60 kDa. Although an immunoassay may be used for screening purposes, the isoelectric focusing method is used for confirmation. WADA has recently updated a technical document that describes the confirmation of this and other forms of EPO that may include the use of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (WADA 2009a). Interestingly, although natural equine and canine EPOs do not cross-react with the antibodies used to detect human EPO, so that the use of an immunoassay to detect EPO may be sufficient, confirmation using immunoblotting may be desirable and indeed has been undertaken (Bartlett *et al.* 2006).

A method has been published for detection of insulin administration in plasma (Thevis *et al.* 2005) and in urine (Thevis *et al.* 2006b), and also for detection of administration of the corticotrophin tetracosactide (Synacthen) in plasma (Thevis *et al.* 2006a).

A test to prove GH administration has been available since the Olympic Games in Athens in 2004. This test is based on the fact that, since recombinant GH does not contain the 20 kDa isoform, the absence of this isoform is the basis of the so-called isoform test (Bidlingmaier *et al.* 2009; Wu *et al.* 1999). An alternative biomarker approach is based on using a discriminant function with the GH-sensitive substance IGF-1 produced by the liver and procollagen type III (P-III-P) (Cowan, Bartlett 2009; Powrie *et al.* 2007; Sönksen 2001).

To support screening on the basis of immunochemistry methods, LC-MS(-MS) methods for IGF-1 determination in the horse have been developed (Bobin *et al.* 2001; De Kock *et al.* 2001). In addition, LC-MS has been shown to provide a viable approach for the quantification of IGF-1 in the horse (Popot *et al.* 2001). Recently, IGF-1 has been quantified from human serum (Bredehöft *et al.* 2008) and also compared with immunoassay data (Kay *et al.* 2009).

Blood doping

Tests for blood doping have been in place since the Olympic Games in Athens in 2004 based on the quantification of blood group antigens (Nelson *et al.* 2002, 2003). Currently, no method exists to evidence autologous blood transfusion. With the advent of blood substitutes based on cross-linked haemoglobin, methods have recently been developed also to detect their use (Goebel *et al.* 2005; Lasne *et al.* 2004; Thevis *et al.* 2003). A method to detect the use of efaproxiral (RSR13), an agent that enhances oxygen uptake, was published (Breidbach, Catlin 2001) even before the substance reached the market.

The future

Over the past few years, analytical methods to detect the use of more and more of the substances and methods prohibited in sport have been added to our armoury. Gaps still remain such as the detection of autologous blood transfusion and gene doping. The field is developing extremely rapidly, with many new analytical techniques and approaches being used. The use of high temperatures and high pressure with small-particle (sub-2 μm) LC columns seems likely to find increased use to provide fast chromatography with increased resolving power (Plumb *et al.* 2007). In addition, mass spectrometry is likely to be used more for the identification and quantification of protein hormones, e.g. IGF-1 and hCG, which is currently only possible using immunoassays. Furthermore, although the detection of many endogenous substances makes use of population data, there is a move towards a so-called athlete's passport (WADA 2010a) enabling the use of the individual athlete's normal data. A Bayesian approach to evaluating the data has been published (Sottas *et al.* 2008) and reviewed (Kicman, Cowan 2009).

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CHAPTER

7 Drug Testing in Animal Sport

P Teale

Introduction

The use of drugs to affect the performance of horses in competition predates the twentieth century: so-called horse 'nobbling' was known well before this time. For example, the account of the famous trial of Daniel Dawson, publicly hanged at Cambridge, England, in 1812 for poisoning racehorses with arsenic, reveals that drinking troughs in some stables were padlocked. 'Exciting substances' were also banned in races in Worksop in 1666.

In human sport the abuse of drugs is almost entirely intended to enhance performance by one means or another. The effect of drugs on performance is often extremely difficult to determine, and there is little definitive published work for any species. Changes in performance of less than 1% cannot be demonstrated with statistical significance because of the many variable and uncontrollable factors, yet an improvement of only 1% represents a lead of about six lengths in a horse race over 1 mile (1.6 km).

While an improvement in performance through the use of drugs may be difficult to measure, or to achieve, there are other considerations in animal sports, particularly racing. Historically, the most important of these has been that gambling is central to the funding and, for many, to the enjoyment of horse and dog racing. There is no doubt that numerous drugs are available with the capability of adversely affecting athletic performance: sedatives, tranquillisers, anaesthetics and beta-blockers being some of the most obvious candidates. It is interesting to note that some of these classes of drugs could, at lower doses, have the contrary effect and potentially improve performance. This explains the relatively early introduction of drug testing into animal sports, which was well established in several European countries by the early 1920s and saw the establishment of the Association of Official Racing Chemists (AORC) in the USA by 1947.

While the prevention of the use of drugs to affect performance remains important to maintaining the credibility of animal sports, welfare considerations have become increasingly important. There is a clear need to allow veterinarians to administer legitimate medication to treat an animal so as to ensure its long-term well-being. At the same time, it must be ensured that such administrations have no effect on racing performance. In horseracing; this has led to a narrowing of the gap in the philosophy adopted by racing authorities across the world between those with a 'zero tolerance' of drugs (predominantly European and Asia/Australia) and those that allow certain medications under controlled conditions (predominantly the USA).

The result of these diverse requirements is that animal sports testing requires the capability of detecting a hugely diverse range of substances, from small molecules such as carbon dioxide, through conventional pharmaceutical drugs, to large molecules such as proteins. These requirements have led to a steady development in the testing technologies applied.

Rules

In horseracing, the International Agreement on Breeding and Racing, published by the International Federation of Horseracing Authorities (IFHA), provides guidance to maintaining the integrity of the sport for recognised racing and breeding authorities (IFHA 2001). Article 6 of this agreement addresses prohibited substances and provides guidance on sampling, sanctions, trainers' responsibilities, raceday regulations, prohibited substances, thresholds and laboratory services. The objective of Article 6 is to protect the integrity of horseracing through controlling the use of substances capable of giving a horse an advantage or making it disadvantaged in a race contrary to the horse's merits. Section 10 of Article 6 defines prohibited substances on the basis of pharmacological action (Table 7.1).

The finding of a prohibited substance means a finding of the substance itself or a metabolite of the substance or an isomer of the substance or an isomer of a metabolite. The finding of any scientific indicator of administration of or exposure to a prohibited substance is also equivalent to the finding of the substance.

As of 2010, 40 countries were signatory to Article 6 in full and use the above definitions as the basis for their national rules of racing. Another 16 countries are signatory in part to Article 6 with a further 2 non-signatories. The basis of such rules is to ban the use of any prohibited substance in racehorses at the time of competition – that is, a policy of 'zero tolerance' except for substances controlled by thresholds. Article 6 states that thresholds can only be adopted for:

- substances endogenous to the horse
- substances that arise from plants traditionally grazed or harvested as equine feed
- substances in equine feed that arise from contamination during cultivation, processing or treatment, storage or transportation.

The list of threshold substances taken from the 2009 publication of the International Agreement on Breeding and Racing is shown in Table 7.2.

Substances included in Table 7.2 detected below the thresholds are not actionable. For any finding of an endogenous substance above the threshold, the Horseracing Authority may decide itself or at the trainer's or owner's request to examine the horse further. In authorities applying the 'zero tolerance' approach, a positive finding of a drug or metabolite almost invariably results in disqualification.

In contrast to the policy of 'zero tolerance' of drugs currently adopted by racing authorities that are full signatories to Article 6, some other jurisdictions permit certain drugs at the time of racing. For example, in North America, in an attempt to harmonise sanctions, the Racing Commissioners International (RCI) produced *Uniform Classification Guidelines for Foreign Substances and Recommended Penalties and Model Rules* (RCI 2002). Drugs are placed in categories 1 to 5, with class 1 agents having a high potential to affect the performance of the horse. Class 1 agents, with a high potential to affect performance, have no place in racing and include opiates, central nervous system (CNS) stimulants and psychoactive drugs. Class 2 agents include psychotropics, certain stimulants, depressants, neuromuscular blocking agents and local anaesthetics that could be injected as nerve-blocking agents. These drugs also have the potential to affect racing performance, but less significantly than class 1 agents. Class 3 agents include some drugs registered for equine veterinary use and have less potential to affect performance than class 2 agents. Racing authorities in certain states in North America have set thresholds for some of the widely used non-steroidal anti-inflammatory drugs (NSAIDs): phenylbutazone, its major metabolite oxyphenylbutazone, flunixin and meclofenamic acid. The use of furosemide is subject to a threshold in many authorities in North and South America and other authorities have set 'decision levels' in urine for some

Table 7.1 Prohibited substances according to Article 6 of the International Agreement on Breeding and Racing (IFHA 20011)

Substances capable at any time of acting on one or more of the following mammalian body systems:

- Nervous system
- Cardiovascular system
- Respiratory system
- Digestive system
- Urinary system
- Reproductive system
- Musculoskeletal system
- Blood system
- Immune system except for licensed vaccines
- Endocrine system

Endocrine secretions and their synthetic counterparts
Masking agents

substances accepted for equine veterinary use (acepromazine, albuterol, atropine, benzocaine, mepivacaine, procaine, promazine and salicylates).

At the time of writing, both the 'zero tolerance' and 'allowed medication' approaches are under reconsideration. The 'zero tolerance' approach is largely dictated by the sensitivity of analytical methods used by laboratories to test for drugs in body fluids which has increased markedly over the past two decades. Major reasons for this increased sensitivity are enzyme-linked immunosorbent assays (ELISAs) and instrumental screening methods, such as mass spectrometry coupled to gas chromatography (GC-MS) and liquid chromatography (LC-MS). As a result of this available sensitivity, it is possible that some therapeutic medications may be detected in urine for periods of time beyond the point at which the drug continues to exert a pharmacological action. Various approaches have been adopted or proposed to address this issue. In Canada, the racing authorities have adopted a policy of the 'deliberate non-selection of unnecessarily sensitive testing methods for specific substances' (Stevenson 1995). Tobin *et al.* (1999) proposed the development of threshold values for therapeutic substances based upon the determination of the highest no-effect dose for their primary pharmacological effect, such as the use of the heatlamp–abaxial sesamoid block model for local anaesthetics (Harkins *et al.* 1996). Similarly, Smith (2001) questioned the continued adherence to the policy of zero tolerance for drugs registered for equine veterinary use and suggested the use of pharmacologically determined reporting levels. Smith drew an analogy with other fields – veterinary drug residues in feed, food packaging contaminants, and food additives in which the levels of chemicals of no concern for a biological effect have been determined using pharmacological (toxicological) and pharmacokinetic parameters. For example, maximum residue limits (MRLs) and the acceptable daily intake (ADI) have been determined for many drugs used in veterinary practice for the treatment of livestock.

This change in attitude to the 'zero tolerance' approach is being mirrored by modifications to the Uniform Classification Guidelines adopted in most of the USA. In particular the designation of anabolic steroids as class 4 drugs has been changed to class 3 drugs, the use of which is more tightly controlled or prohibited. Model rules recently proposed by the Racing Medication and Testing Consortium (RMTC) and supported by the RCI have banned the use of most of the synthetic anabolic steroids and limited the use of nandrolone, boldenone, testosterone and stanozolol. As of 2008, the majority of racing authorities in the USA have either introduced these rules or undertaken to introduce them in the near future.

In addition to horseracing, a wide range of other equine sports is popular around the world. Many of these, such as show jumping, eventing and dressage, are under the control of the Fédération Equestre Internationale (FEI). Drugs prohibited under FEI rules are broadly similar to those prohibited by the IFHA.

No internationally agreed list of prohibited substances exists for canine sports. Where testing is carried out in an organised manner, prohibited substances are specified locally or nationally. In most instances the substances prohibited are broadly similar to those banned in horseracing.

Table 7.2 List of internationally agreed threshold substances for horseracing

Substance name	*Threshold*
Arsenic	0.3 μg total arsenic per mL in urine
Boldenone	0.015 μg free and conjugated boldenone per mL from male horses (other than geldings)
Carbon dioxide	36 mmol available carbon dioxide per L in plasma
Dimethyl sulfoxide	15 μg dimethyl sulfoxide per mL in urine, or1 μg dimethyl sulfoxide per mL in plasma
Estranediol in male horse (other than geldings)	0.045 μg free and glucurono-conjugated 5α-estrane-3β, 17α-diol/mL in urine
Hydrocortisone	1 μg hydrocortisone per mL in urine
Methoxytyramine	4 μg free and conjugated 3-methoxytyramine per mL in urine
Salicylic acid	750 μg salicylic acid per mL in urine, or 6.5 μg salicylic acid per mL in plasma
Testosterone	0.02 μg free and conjugated testosterone per mL in urine from geldings, or 0.055 μg free and conjugated testosterone per mL in urine from fillies and mares (unless in foal)
Theobromine	2 μg theobromine per mL in urine

Reported analytical findings

The drugs most commonly detected in horseracing in Europe (France, Germany, the UK, Ireland and Italy) over the period 1993–1997 are shown in Table 7.3 and Table 7.4 (Smith 2001). The majority of the reported findings (77%) were for substances registered for equine veterinary use (Table 7.3). The NSAIDs accounted for 28% of the reported findings and isoxsuprine for 22%. Isoxsuprine, a vasodilator, is widely used in veterinary practice for the treatment of navicular disease and can be detected in urine for long periods of time after dosing has ceased (Pompa *et al.* 1994). Moreover, when used as a powder for oral administration through admixture with feed, it can produce serious contamination problems in the stable environment (Russell, Maynard 2001). Modifications to assay sensitivity along with changes in veterinary practice have largely eliminated the reporting of low levels of isoxsuprine in racing animals. For the drugs without market authorisation (Table 7.4), anabolic steroids accounted for 35% of the reported findings, xanthines for 24%, and CNS-stimulant active drugs for 12%.

It is interesting to compare these findings with those for reported findings in the USA and Canada over a similar period. If a prohibited substance is reported in a post-race sample from a horse, the International Agreement on Breeding and Racing recommends that the horse be disqualified. However, the USA and Canada are not signatories to this aspect of Article 6. Rather, the 'model rules' discussed in the previous section are applied. Carter (2001) summarised the combined reported findings of substances in classes 1, 2 and 3 over the period 1995–1999 for racing in California, Canada, Florida, Kentucky, Maryland, New York and Ohio in thoroughbred, standardbred and quarter horses (horses bred for maximum speed over a quarter of a mile). For comparison with the European findings, the results for quarter horses have been omitted. Findings for class 1 agents included morphine (15), cocaine (presumably as the major metabolite benzoylecgonine) (15), strychnine (3), oxymorphone

Table 7.3 Reported findings in Europe for prohibited substances with veterinary market authorisation (Smith [2001]; reproduced with the kind permission of Professor RL Smith)

Drug	*Number of reports*
Isoxsuprine	73
Phenylbutazone	66
Caffeine	28
Dexamethasone	25
Lidocaine	24
Procaine	22
Flunixin	14
Acepromazine	13
Clenbuterol	10
Dembrexine	9
Salicylate	8
Dipyrone	7
Furosemide	6
Hyoscine	5
Naproxen	4
Methylprednisolone	3
Altrenogest	3
Other drugs[a]	<3

[a]Other drugs with fewer than three reports each are diphenhydramine, detomidine, xylazine, prednisolone, mepivacaine, meclofenamic acid, ketoprofen, butorphanol and atropine.

Table 7.5 Findings of Class 3 agents in California, Canada, Florida, Kentucky, Maryland, New York and Ohio during the period 1995–1999 (Carter 2001)

Drug	*Number of reports*
Clenbuterol	55
Promazine	35
Pyrilamine	30
Albuterol	28
Procaine	22
Detomidine	12
Atropine	10
Bumetanide	10
Glycopyrrolate	10
Butorphanol	9
Phenylpropanolamine	6
Pentoxyfylline	4
Hyoscine	4
Acepromazine	3
Theophylline	3
Xylazine	3
Pentazocine	2
Other drugs[a]	1

[a]Drugs with one report each are ketorolac, metoprolol, nefopam and terbutaline.

(2), apomorphine (1), dextromoramide (1) and oxycodone (1). Findings for class 2 agents were lidocaine (28), caffeine (21), mepivacaine (8), buspirone (7), ephedrine (4), nalbuphine (3), imipramine (2), chlorprothixene (1), ketamine (1) and tramadol (1). The findings for class 3 agents are shown in Table 7.5.

Within Europe, 49 substances were detected a total of 431 times in a 4-year period with a total of 97 451 samples tested, an incidence of reported findings of 0.44%. Carter (2001) estimated that the number of samples tested per annum in Canada and seven states within the USA was 200 000. Thus, for class 1, 2 and 3 agents, within a 4-year period, 39 substances were detected a total of 389 times in an estimated 800 000 samples, an incidence of reported findings of 0.049%.

Table 7.4 Reported findings in Europe for prohibited substances without veterinary market authorisation (Smith [2001]; reproduced with the kind permission of Professor RL Smith)

Drug/drug type	*Number of reports*
Xanthines	24
Testosterone	16
Nandrolone	10
Boldenone	7
Morphine	6
Heptaminol	5
Promazine	4
Pyrilamine	3
Ketorolac	3
Propranolol	2
Nikethamide	2
Methandriol	2
Diclofenac	2
Cortisol	2
Chlorpromazine	2
Other drugs[a]	1

[a]Drugs with one report each are salbutamol, propionylpromazine, phenylpropanolamine, nalbuphine, mephenesin, hydroxyzine, bumetanide and amitriptyline.

The significant difference in the incidence of reported findings in the two geographical areas can almost certainly be explained by the absence of data for class 4 and 5 agents in the American study. Many of the substances reported in Europe would fall into these classes within the USA. For example, in the USA, NSAIDs and isoxsuprine are class 4 agents controlled by the application of threshold levels, the concentration of phenylbutazone and/or oxyphenylbutazone in the reporterd samples in Europe would be well below the US threshold. European racing authorities also place significant importance on the detection of anabolic steroids, whereas at the period covered by these findings anabolic steroids were class 4 agents in the USA and limited testing was applied. The difference in the incidence of reported findings reflects, in part, the difference in philosophical approaches to doping control currently adopted on the two continents at that time: 'zero tolerance' versus permitted medication.

It is interesting to note the influence that the introduction of new or more sensitive tests can have upon reporting statistics. Carter (2001) reported 24 findings of class 1 agents in standardbred racing in the USA over the period 1995–1999; 23 of the findings were for the drug metaraminol. These findings were all reported in one state, Louisiana, and occurred over a short period and resulted from the introduction of a new test. A similar situation arose with the drug clenbuterol (see Table 7.5) when a more sensitive test was introduced in 1998.

Sampling

Sample collecting procedures must take into consideration both scientific and legal aspects:

- The health of the animal being sampled must be safeguarded.
- Incorrect labelling, contamination or sample switching must be avoided.
- The rights of interested parties, generally the owner and trainer of an animal, must be safeguarded against error by the analyst.

Article 6 of the *International Agreement on Breeding and Racing* (IFHA 2009) states that a sample collected under a secure chain of custody shall be split into an A sample and a B sample, and this policy is adopted by racing jurisdictions worldwide. If the A sample is reported to contain prohibited substances, the B sample can be analysed for those substances, either automatically or at the trainer's request. The analysis is carried out either in the primary laboratory or in a nominated secondary laboratory. Within greyhound racing, a split sampling policy generally is not adopted because sometimes only a small volume of urine is available.

In animal sport the vast majority of samples taken are post event, with the majority being urine. In fact, until relatively recently, post-event sampling was the only approach applied to the control of drug misuse. This is entirely suitable for controlling agents, the presence of which could affect the outcome of the competition. However, it has been recognised that it is inappropriate for controlling the misuse of drugs that continue to offer a benefit after they become undetectable – anabolic steroids, erythropoietin (EPO), etc. For this reason many authorities now undertake additional sampling out-of-competition. Also, a number of authorities undertake pre-race testing, mostly for the presence of alkalising agents, but more extensive pre-race testing is undertaken by a small number of authorities, for example in Hong Kong.

Urine

Until relatively recently, urine was the preferred body fluid in all species. Its collection is non-invasive, it is generally available in sufficient quantity and the drugs or their metabolites tend to be present in relatively high concentrations. The disadvantages are that a drug may be present as its metabolites or in a conjugated form and the parent drug may be present only in a relatively low concentration or be entirely absent, as is the case with several protein-based therapeutic agents.

Urine is collected almost invariably by voiding naturally. Greyhounds urinate very readily after being released from their transporter and 96% of horses in Britain urinate within 1 hour of racing. Urine samples from greyhounds are caught directly in a bowl held under the animal. For horses, a container held on the end of an extending handle is generally used (e.g. a net held on a metal ring into which is inserted a polythene bag). Metal ladles are unsuitable because the noise produced by the urine that falls into them frequently inhibits the horse from urinating further.

Blood

The principal advantage of a blood sample in equine sport is the ease with which it can be collected. In addition, drug concentrations in blood are interpreted more easily than those in urine and certain drugs that are not excreted in urine in detectable quantities (e.g. reserpine) can be detected in blood. Blood is rarely collected from the greyhound because of the relative ease of urine sampling and the limited volume of blood available. Blood is sampled increasingly in horseracing as a second choice when urine is not obtainable or as an additional matrix for further testing. Blood is the body fluid of choice for pre-race and testing in training samples because of the relative ease of collection compared with that of obtaining urine samples.

Saliva

The principal disadvantages of saliva are that it is difficult to obtain a useful volume and few drugs are present at a concentration higher than that in plasma. Non-ionised drugs and drugs not protein bound in plasma diffuse passively into saliva. Thus, alkaline saliva (as in the horse) tends to concentrate acidic drugs but, because the percentage of unbound acidic drug in plasma is generally very low, concentrations remain lower than the corresponding total plasma concentrations. For drugs of low lipid solubility, and for high salivary flow rates, equilibrium is not established, which results in concentrations even lower than those predicted on theoretical grounds. The principal value of saliva, therefore, is in the detection of topical exposure that results from recent oral ingestion.

Hair

Hair analysis has value within animal sports in that it can provide a historical record of drug administrations; this information would be particularly useful for those drugs with no legitimate therapeutic use and long-lasting effects (e.g. anabolic steroids). Reported studies (Boyer *et al.* 2007; Jouvel *et al.* 2000; Popot *et al.* 2001b; Whittem *et al.* 1998) have addressed the detection of morphine, diazepam, clenbuterol and steroids in horse hair. The development of instrumental systems with ever-increasing sensitivity has the potential to further enhance the effectiveness and applicability of hair testing in animal sport.

Analytical approach

Screening methods

Drugs are generally administered at or near the therapeutic dose, which results in relatively low concentrations in biological fluids. There is usually no obvious outward evidence as to whether a drug has been administered, or what sort of drug it might be. Any drug used in human treatment or in veterinary practice may be found. Thus, screening procedures must be both sensitive and of wide coverage. However, the sports chemist enjoys the advantage of examining relatively constant material, usually in fairly fresh condition. He or she thus has a clearer picture of a normal sample than does the forensic or hospital chemist who may be required to examine a wide variety of materials in various states of decomposition. Any sample that fails a screening test is invariably submitted to rigorous confirmatory testing (see below) before an adverse report is issued.

Although the parent drug is the entity normally described in the rules, screening procedures rely upon the detection of either the unchanged drug or its metabolites. The identification of the corresponding metabolites is often useful supplementary evidence to support the identification of the parent drug. In addition, the presence of metabolites in the appropriate concentrations relative to the parent drug helps to support the conclusion that a drug has been administered. Occasionally, the parent drug is not excreted in urine at a detectable concentration and knowledge of the metabolic pathways in the particular species is thus essential. An example of this is the identification of 19-norepiandrosterone and 19-noretiocholanolone in the urine of dogs as evidence of the administration of the anabolic steroid nandrolone or a 19-norsteroid precursor. Some drugs are notable for being excreted in urine almost entirely in the conjugated form as are, for instance, apomorphine, fentanyl, nefopam and pentazocine in the horse. Hydrolysis or other appropriate pretreatment prior to extraction is therefore required if instrumental screening methods are to be effective. The use of hydrolysis significantly increases the complexity of the matrix, particularly in the case of horse urine.

No single analytical scheme will suffice to cover so many different types of compound; various approaches have, therefore, evolved in racing chemistry laboratories to address this challenge.

Immunochemical methods that cover glucocorticosteroids (Chakraborty *et al.* 1976; Dumasia *et al.* 1973) and anabolic steroids (Jondorf 1977; Jondorf, Moss 1978) were first introduced into equine drug-screening programmes in the 1970s. However, in the late 1980s, ELISAs were developed specifically for equine drug-testing programmes (e.g. Woods *et al.* 1988). A range of ELISA tests is now available and their use remains widespread, though far from universal. Many laboratories, primarily in the USA, make use of ELISA tests to provide coverage of a range of drugs.

For more general screening, liquid–liquid extractions designed for group separation of drugs followed by thin-layer chromatography (TLC) were almost universally employed, but their use is now in decline. In many laboratories around the world, while specific analytes are targeted using ELISA, broad coverage is increasingly provided by instrumental screening techniques. For this purpose the use of GC with nitrogen–phosphorus detection (GC-NPD) and high performance liquid chromatography using ultraviolet detection (HPLC-UV) was almost entirely superseded by GC-MS through the 1980s and 1990s. In the last decade, LC-MS has been increasingly used in animal sports drug testing

laboratories. This is reflected in the fact that the number of analytical papers submitted to the International Conference of Racing Analysts and Veterinarians (ICRAV) relating to the use of LC-MS increased from 11% in 1998 to 88% by 2006. The balance of use of immunoassays and instrumental methods is a complex equation between relative costs of consumables, staff and capital equipment that varies widely depending upon geography.

Confirmatory methods

In animal sports testing, substances detected using screening techniques are subsequently subjected to further analysis intended to unambiguously confirm the screening finding. This usually involves an analyst taking a second aliquot from the primary (A) sample and the use of a suitably selective technique to provide the required confidence in the analytical finding. Industry guidelines for horseracing laboratories state that, for regulatory identification, MS or a similarly definitive technique, if applicable to the analyte in question, must be included. An important aspect of this confirmatory analysis is that the chain of evidence must be maintained from the primary sample to the final analytical result. In most instances, a second confirmatory analysis is either required or available upon request. Where a B bottle sample has been obtained, this second confirmatory analysis is undertaken with that sample, normally in the presence of a witnessing analyst or at a second laboratory.

Laboratories are required to have established and documented chromatographic and mass-spectrometric criteria that the analyte of interest in the test sample must have in common with the reference material for identification purposes. Industry guidelines for low-resolution mass spectrometry have been introduced by the AORC. These are similar to those applied by World Anti-Doping Agency (WADA) laboratories. In the 1990s, discussions within the IFHA identified the desirability for accreditation to International Standards Organization (ISO) standard 17025. While many laboratories have attained accreditation, this is not universally applied. To assist in the process of accreditation, the racing industry, through the efforts of the AORC, has produced the guidelines *Accreditation Requirements and Operating Criteria for Horseracing Laboratories*, published by the International Laboratory Accreditation Co-operation (ILAC 1997). Further development of this document to maintain its applicability is ongoing. ISO 17025 (ISO 2003) requires traceability of measurements and in the sports fields this is considered to be met, when identifying a prohibited substance, by the direct comparison with a reference material analysed in parallel or in series with the test sample. A reference standard is generally accepted as a chemical with a well-established structure. The material may be characterised structurally within the laboratory using appropriate techniques or validated against a certified reference material or by comparison with uncontroversial published data. A reference material may also be an isolate from a urine or blood sample after an authenticated administration, provided that the analytical data from it is sufficient to justify fully its identity as a metabolite of the substance administered. Chemical reference standards are often not available and hence there is the need to use authenticated administrations for comparison purposes. Many of the most common substances are now available with certificates of analysis, and thereby fully meet the generic traceability requirements of ISO 17025 (ISO 2003).

Sample extraction

In many racing chemistry laboratories throughout the world, solid-phase extraction (SPE) has augmented or replaced liquid–liquid extraction for the isolation of drugs from the sample matrix. Based upon the studies of Shackleton and Whitney (1980), the early applications of SPE to racing chemistry involved the use of C_{18} bonded-phase cartridges in the isolation of anabolic steroids and their metabolites from equine urine (Dumasia *et al.* 1986). With the development of a range of more polar phases and, more importantly, ionic and particularly mixed-mode phases, the technique evolved to provide a simple, rapid, robust and efficient approach to the fractionation of drugs from biological fluids. In an early example of this, Dumasia and Houghton (1991) reported the application of lipophilic/cationic mixed-mode cartridges for the extraction of β-agonists, β-antagonists and their metabolites in horse urine prior to their analysis by GC-MS. Following the application of a urine sample at pH 6.0 to the cartridge, basic drugs are retained primarily by cation-exchange processes, whereas acidic and neutral drugs are retained by hydrophobic interactions. Washing the cartridge with acetic acid (1 mol/L) fortifies the ionic interaction for the basic drugs. The acid and neutral drugs can then be eluted with an organic solvent such as ethyl acetate or dichloromethane. As a result of the retention of basic drugs through ionic interaction, the cartridges can be washed with methanol to remove interfering substances. Subsequently, the basic drugs are eluted by suppression of ionisation using a solvent such as ethyl acetate containing concentrated ammonia solution or triethylamine (2–4%). The ability to use cationic mixed-mode cartridges to fractionate acid/neutral and basic drugs is widely exploited by many laboratories, although the details of the procedure often differ markedly.

Polymeric phases are now also available, the surface chemistry of which can provide a variety of advantages compared with the traditional silica-based phases. These properties can be further modified by combining two classes of polymeric sorbents or by using co-polymeric and functionalised polymers. The treatment of the functionalised polymer with sulfonic acid provides cation-exchange characteristics. These cartridges have been shown to be effective in the extraction of a number of basic drugs from equine urine (Stanley *et al.* 2001).

One of the major problems encountered in the SPE of horse urine, particularly if automated procedures are used, is cartridge blockage. This is primarily because of the viscous nature of horse urine and, to a lesser extent, suspended material. This problem has been addressed (Wynne *et al.* 2001a, 2001b) using non-conditioned SPE (NC-SPE). The sorbents used in this technique are highly cross-linked with a unique combination of hydrophilic and lipophilic moieties and thus do not require conditioning. They have good flow characteristics and can be applied to the extraction of a wide range of drugs. Wynne *et al.* (2001b) suggested the application of NC-SPE for the pre-extraction of horse urine before application of the recovered extract to mixed-mode cartridges. Other laboratories favour the use of a protease, which significantly decreases the viscosity of the urine sample. A comprehensive fractionation procedure using multiple SPE cartridges has been described (Wynne *et al.* 2004) and the application of SPE to veterinary drug abuse has been reviewed by the same author (Wynne 2000).

Gas chromatography–mass spectrometry

A major advantage of GC-MS for general broad-range screening has been the availability of extensive electron-impact mass spectral libraries. Typically, derivatisation is employed to render analytes containing polar and active functionalities amenable to chromatography. Derivatisation methods widely applied include trimethylsilylation, acetylation and alkylation, for example to form pentafluoropropionyl or methyl derivatives. No single procedure is appropriate for derivatising all analytes. However, it is possible to develop methods capable of detecting very large numbers of drugs and metabolites. Arguably, the major issue with full-scan GC-MS screening is the complexity of the resulting data-sets and difficulty of producing meaningful and accurate analytical reports in an automated manner.

GC-MS is also widely used for target analysis using selected-ion monitoring (SIM) approaches. An excellent example of the use of this approach is in the detection of anabolic steroids in urine. GC provides excellent separation of isomers, an important consideration in steroid chemistry where multiple isomers are possible, and SIM provides the required sensitivity to detect the target analytes in the low nanogram per millilitre range. The selection of derivatisation reagent is an important consideration in this application. For example, formation of *t*-butyldimethylsilyl derivatives where fragmentation results in the production of intense high-mass ions (Teale, Houghton 1991).

GC-MS has also been widely applied for confirmatory analysis, in which the identity of a substance should be confirmed using derivative formation and comparison of the data obtained with reference material analysed contemporaneously.

Liquid chromatography–mass spectrometry

An early application of LC-MS to drug testing in equine sports demonstrated the use of the moving belt interface in the confirmatory analysis of synthetic corticosteroids (Houghton *et al.* 1981). Other techniques employed in animal sports testing have included direct liquid introduction (Henion, Maylin 1980) and particle beam-MS (Stanley *et al.* 1995). With the development of atmospheric-pressure ionisation (API) techniques, LC-MS has found increasing application in doping control in animal sports for both the qualitative and the quantitative analysis of drugs. The technique has been applied to the analysis of intact steroid conjugates isolated from horse urine with particular reference to the quantification of testosterone sulfate (Dumasia *et al.* 1996). Methods developed for other individual drugs include hydrocortisone (Samuels *et al.* 1994), triamcinoloneacetonide (Koupai-Abyazani *et al.* 1994b), levodopa and its metabolites (Koupai-Abyazani *et al.* 1994a), clidinium bromide (Ryan *et al.* 1996) and glycopyrrolate (Matassa *et al.* 1992).

Many of the initial screening methods developed using API LC-MS were based upon ion-trap systems that were popular owing to their full-scan sensitivity and flexibility. Examples include methods for diuretics (Eenoo *et al.* 2001), also a broad screen detecting a range of diuretics, some cyclooxygenase-2 (COX-2) inhibitors and anti-ulcer drugs in a single analysis (Woodward *et al.* 2003). Product-ion scan MS-MS has facilitated the development of screening procedures for large numbers of compounds in a single analysis. Screening methods have been developed for corticosteroids (Tang *et al.* 2001), while You *et al.* (2003) developed a screening procedure for simultaneous analysis of 60 basic drugs isolated from horse urine with detection limits at low microgram per litre levels. The drugs were isolated from enzyme-hydrolysed urine by SPE and analysed by LC-MS in the positive-atmospheric-pressure chemical ionisation (APCI) mode. The method enabled the analysis of 60 drugs within 10 min using a combination of full-scan MS and product-ion MS-MS. LC-MS is also increasingly being employed in the analysis of anabolic steroids in equine blood (Guan *et al.* 2005) and urine (Ho *et al.* 2006).

With the development and commercial availability of high-sensitivity, triple-quadrupole, LC-MS systems, the application of LC-MS to screening has become increasingly important. The duty cycle of modern systems and the development of improved software systems for instrument control have significantly increased the number of analytes that can be included in a single screening run. Further advances include the development of hybrid triple-quadrupole/ion-trap systems that allow generation of full-scan data triggered by the detection of a multiple reaction monitoring (MRM) signal. However, triple-quadrupole screening is by its nature and definition a highly targeted approach. Significant interest in the application of full-scan, high-resolution, accurate mass techniques for general screening has been expressed by a number of laboratories around the world (Hewitt *et al.* 2007). Technological advances in appropriate analyser technologies, such as time-of-flight (TOF) and Orbit rap, are making this significant change in analytical approach more likely (Hudson *et al.* 2007). In addition, developments such as ultra-performance liquid chromatography and improvements in source design allowing the use of higher flow rates with greater reliability will serve to extend the applicability of LC-MS to sport testing.

Drugs misused in sport

As listed above, drugs misused in animal sports include the anabolic steroids, the corticosteroids, NSAIDs, diuretics, sedatives, bronchodilators, protein-based therapeutic agents and alkalinising agents. Of particular interest are the anabolic steroids and corticosteroids; not only may synthetic analogues be misused, but also testosterone or hydrocortisone. This use of a pseudo-endogenous compound that is either indistinguishable, or distinguishable with difficulty, from that which is produced naturally by an individual presents interesting analytical problems. Other examples include recombinant growth hormones and recombinant EPO.

Anti-inflammatory agents

The use of anti-inflammatory agents is considered a major welfare issue by many racing authorities. Loosely they can be categorised as steroidal (based upon the naturally occurring corticosteroid, hydrocortisone) or NSAID. A number of corticosteroids are available, and they have a range of uses and routes of administration including: topical treatment of skin conditions, inhalation for treatment of respiratory tract disorders, and intra-particular administration to treat joint inflammation. The high potency of the corticosteroids, combined with difficulties with derivatisation and extensive fragmentation in electron-impact ionisation, make their detection by mass spectrometry difficult, although confirmatory methods based upon negative-ion chemical ionisation GC-MS have been developed (Houghton *et al.* 1982). A number of commercial ELISAs capable of detecting either a range of corticosteroids or a specific corticosteroid are available. More recently, the introduction of API LC-MS has significantly simplified their analysis by instrumental techniques and a number of methods have been published (Guan *et al.* 2003; Ho *et al.* 2006; Tang *et al.* 2001).

The NSAIDs are a much more structurally diverse group of compounds, including acidic drugs such as ibuprofen and neutral to weakly acidic compounds such as phenylbutazone. Many of the NSAIDs are detectable using GC-MS following methylation and this is a common approach to screening in many laboratories. Many of these drugs are also administered at comparatively high doses. The relatively recent introduction of the COX-2 inhibitor class of NSAIDs has further complicated screening for NSAIDs as most of these are extensively metabolised and not readily amenable to detection by GC-MS, although LC-MS methods are available (Woodward *et al.* 2003).

Anabolic steroids

The use of anabolic/androgenic steroids (AASs), either at the time of racing or as a training aid, is banned by most racing authorities. In most European, Asian and Australasian jurisdictions, the use of these agents is completely prohibited. In the USA the use of selected AASs (nandrolone, testosterone, boldenone and stanozolol) is allowed for legitimate veterinary purposes; all other agents are subject to a complete ban. In addition to the expected improvement in musculature, AASs are said to improve a horse's appetite and to produce behavioural changes of a masculinising type in geldings and mares, though they may also be used in stallions.

In the greyhound, the use of AASs as anabolising agents is also banned. A complication in this arena was the use of testosterone and methyltestosterone as oestrus suppressants (Biddle *et al.* 2005). In many authorities this practice is now banned.

Structurally, there are broadly two chemical types of AASs based upon the androstane and estrane ring systems: those with a 17α-alkyl group, which are active orally but possess hepatotoxicity, and 19-nor derivatives administered by injection.

Studies have shown the metabolism of the veterinary anabolic agents nandrolone, testosterone, boldenone and trenbolone in the horse to be complex, with both phase I and phase II processes playing important roles (Dumasia, Houghton 1981, 1984; Dumasia *et al.* 1983; Houghton 1977; Houghton, Dumasia 1979, 1980). Nandrolone and boldenone have been shown to be endogenous to the male horse (Ho *et al.* 2004; Houghton *et al.* 1984) and along with testosterone are controlled by threshold values (see Table 7.2). A review of the presence and metabolism of endogenous steroid hormones in the horse has recently been published (Scarth *et al.* 2009). Radioimmunoassay and subsequently ELISA procedures have been developed for these veterinary anabolic agents (Jondorf 1977; Jondorf, Moss 1977) and commercial assays are currently available. Also, the identification of the major metabolites for these steroids has enabled the development of multi-residue GC-MS screening procedures (Dumasia *et al.* 1986; Houghton *et al.* 1986). Reduction of the 4-ene-3-one group and epimerisation at C-17 are major phase I pathways. Phase II processes also differ markedly, with sulfate conjugation playing an important role in horses but a minor role in dogs. Thus, the developed methods for screening and confirmatory analysis by MS methods require steps for cleavage of both glucuronic acid and sulfate conjugates.

Metabolism and detection of a number of synthetic steroids, including their esters, have also been studied in the horse (Ho 2005; Ho *et al.* 2007a, 2007b; Kim *et al.* 2000; Lampinen-Salomonsson *et al.* 2006; McKinney *et al.* 2001, 2004; Schoene *et al.* 1994) and both aspects have recently been reviewed (McKinney 2009; Teale, Houghton 2010). A number of studies have been carried out in the dog (Biddle 2008; Brockwell *et al.* 1992; Ralston *et al.* 1992; Stewart *et al.* 2009; Williams *et al.* 2000). These studies have thus been essential to identify key analytes for the development of screening and confirmatory analysis methods.

Diuretics

Furosemide, a loop diuretic, is widely used in horse racing due to the claimed efficacy in reduction of exercise-induced pulmonary haemorrhage (nose bleeds), a relatively common occurrence in some animals. Because of this, the diuretic furosemide is permitted at the time of racing by a number of racing jurisdictions around the world. Some concerns have been raised that the dilution of the urine that results from diuretic use could render detection of some other drugs more difficult. For this reason, in many countries, diuretic drugs are banned as potential masking agents. Diuretics have a wide variety of chemical structures, which make it difficult to find a single suitable screening procedure, although LC-MS is increasingly used. Many of the diuretics are neutral to weakly acidic compounds that are difficult to analyse by GC-MS as they cannot be derivatised reliably.

Basic drugs

A number of classes of drugs of interest to animal sports administrators fall into the general classification of basic drugs. These include, in no particular order, sedatives (such as the phenothiazines), antidepressants (tricyclics such as amitriptyline), local anaesthetics (lidocaine, procaine, etc.), β-agonists and antagonists (clenbuterol, propranalol, etc.). Immunoassays are available for a range of these analytes, particularly those that have high potency and offer particular threats to the sport. Instrumental screening methods offering the ability to detect a number of these substances in a single analytical method are popular and GC-MS is widely used, often following extraction using mixed-mode cartridges as described earlier. LC-MS is also highly applicable and offers the advantage of high sensitivity and rapidity, although this is frequently at the expense of breadth of coverage, particularly where triple-quadrupole systems are employed.

Protein hormones

Rumour concerning the abuse of growth hormone (GH) in horseracing has been circulating for a number of years, but interest in developing methods for its control intensified in 1998 with the approval, in Australia, of recombinant equine GH for veterinary use. Insulin-like growth factor-1 (IGF-1) has been confirmed as a universal marker to detect abuse of the three commercially available recombinant GH variants (equine, porcine and bovine GH) that were shown to be active in the horse (De Kock *et al.* 2001c). However, as IGF-1 is endogenous to the horse, the current international rules of racing would require its control through a threshold value.

In a collaborative study, IGF-1 levels in normal serum samples of horses in South Africa, Australia and the UK were determined by an immunoradiometric assay (IRMA) and were shown to have a close to normal distribution (De Kock *et al.* 2001a). Also, significant increases in IGF-1 levels in serum have been demonstrated after GH administration (De Kock *et al.* 2001c; Faustino-Kemp *et al.* 2001; Noble, Sillence 2001; Popot *et al.* 2001a, 2001d).

To support screening on the basis of immunochemistry methods, LC-MS methods for IGF-1 determination have been developed (Bobin *et al.* 2001; De Kock *et al.* 2001b). In addition, LC-MS has been shown to provide a viable approach for the identification and quantification of IGF-1 (Popot *et al.* 2001c, 2008). Means of directly detecting exogenous GH, based upon the difference in primary amino sequence, have also been reported (Bailly-Chouriberry *et al.* 2008b), as has the use of techniques to detect the immune response of the body to the exogenous protein (Bailly-Chouriberry *et al.* 2008a).

Misuse of recombinant human erythropoietin (rhEPO) in horses, supposedly to improve performance, has received wide publicity. However, the horse is a natural blood doper in that, under exercise, contraction of the spleen can increase the haematocrit by 33%. Thus, misuse of rhEPO presents a serious threat to the welfare of the horse, as its administration coupled with splenic contraction could result in a marked increase in blood viscosity. This increase in an animal undergoing severe exercise could have serious health implications. Some physiological implications of administration of rhEPO to horses were reviewed by (McKeever *et al.* 1996).

ELISA tests to detect rhEPO administration to horses and greyhounds have been evaluated and shown to be effective in serum (Tay *et al.* 1996) and in serum, plasma and urine (Roberts *et al.* 2003). The double western blotting technique developed by Lasne and de Ceaurriz (2000) for EPO analysis in urine has also been evaluated in the horse (Lasne *et al.* 2005) and shown to be effective. As the natural equine EPO does not cross-react with the antibody used in the test, the test provides a better distinction between positive and negative samples when applied to equine urine as opposed to human urine (Roberts *et al.* 2003). In addition an LC-MS method for detection of rhEPO and darbepoetin in equine plasma has been reported (Guan *et al.* 2007, 2008). A number of positive findings in racing horses have been confirmed using both of these techniques.

A further approach to the detection of the misuse of rhEPO has been to screen blood samples for the presence of antibodies, and several ELISA systems have been developed for this purpose (Birks *et al.* 2007). A number of animals with an immune response have been identified in the USA/Canada and subjected to the sanction of not being allowed to compete until the antibody titre decreases to normal levels.

New technologies and future threats

Modern animal sports testing laboratories employ many of the most up-to-date techniques and technologies to address the issues of drug use, including the detection of doping and maintaining the welfare of the athlete. These abilities are particularly well developed in the area of small-molecule drug analysis, but this has come at the cost of increasing complexity of the analytical processes and data handling requirements. A significant challenge facing testing laboratories is to simplify these processes while maintaining the required breadth of coverage. A further challenge in small-molecule analysis is addressing the possible use of 'designer drugs', such as tetrahydrogestrinone, specifically intended to evade detection. The emergence of affordable, robust, high-resolution ($>$20 000) and accurate mass LC-MS instrumentation is considered by many to offer the potential to address many of these issues. Screening based upon full-scan accurate mass LC-MS is potentially both broad ranging and highly selective and could replace more limited targeted screens such as those based upon triple-quadrupole systems. Inclusion of additional analytes is straightforward and, at least for the purpose of gathering information on previous use, retrospective. The use of chemometric tools either to directly detect novel agents or to identify metabolite patterns indicative of abuse may also offer a solution to designer drugs.

A potentially more serious threat is posed by the switch to biological drugs and therapies emerging from the pharmaceutical industrial and medical research laboratories. Development of methods to detect the misuse of GH and EPO presented both the analytical laboratories and the sporting authorities with significant challenges. Rumours of the abuse of more exotic protein-based agents such as snake and cone snail venoms have circulated around the world, although seized materials claiming to contain these materials have often proved to be counterfeit. While these challenges are far from trivial, developments in protein MS, transcriptomics and metabolomics provide the promise that detection methods will become available, either directly or indirectly via biomarkers (Teale *et al.* 2009). It should also be noted that, for the most part, animal sport testing laboratories have the advantage of dealing with protein therapeutics that are clearly endogenous. For example, all the pharmaceutical preparations of EPO currently available are based upon human EPO, the primary structure of which is significantly different from equine or canine EPO.

A more difficult area to address is likely to be the use of therapies that do not directly involve administration of a drug. Examples of these include shock wave therapy and gene doping. Direct detection of these therapies may prove difficult, although significant research into the detection of gene doping has been undertaken and various targets for detection have been identified. A highly attractive possibility is the use of biomarkers. If successful, appropriate biomarkers could provide evidence of misuse of a wide range of agents and possibly replace traditional means of screening.

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CHAPTER

8 Drug-facilitated Sexual Assault

MD Osselton

Drug-facilitated assault (DFA) has emerged as a major area of forensic work during recent years as a consequence of media publicity attributed to a handful of drugs such as flunitrazepam, gamma-hydroxybutyrate (GHB) and ketamine. Although drug-facilitated sexual assault (DFSA) has attracted much attention, drug-related assault may also be encountered in cases of robbery or uncharacteristic and antisocial behaviour by an individual. DFSA has been loosely described by the popular media as 'date rape' and linked with drink spiking, particularly in club venues. Despite media publicity, drink-spiking cases probably make up only a small percentage of DFSA cases and the majority of cases are associated with recreational drug and alcohol use by the victim. In a typical drink-spiking scenario, a potential sexual offender surreptitiously spikes the drink of an unsuspecting person with a sedative substance for the purpose of 'drugging' and subsequently sexually assaults the victim while he or she is under the influence of this substance. Victims often report loss of memory during and after these incidents and may wake up in unfamiliar places, inappropriately dressed and often with the sense but not the actual recollection of having had sex. DFSA is not limited to male assaults on females but may involve female assault on males as well as homosexual assault. DFSA is not a new crime and appears to be documented in the Bible where Lot's daughters are recorded to have used wine to drug him before having sexual intercourse with him in order to procreate. Both daughters became pregnant and subsequently produced sons Moab and Benammi (Genesis 19 : 30-38).

This chapter will consider the terminology used in association with drug-related assault, the types of substances most frequently encountered, and the approaches that may be taken when investigating such cases.

Definitions

'Assault' may be defined as 'an intentional act by one person that creates an apprehension in another of an imminent harmful or offensive contact'. In the UK the Sexual Offences Act 2003 came into force in May 2004 and essentially repealed most of the existing statute law relating to sexual offences. Under the auspices of the UK Sexual Offences Act 2003 a person A is guilty of a sexual assault if: (i) he/she intentionally touches another person (B); (ii) the touching is sexual; (iii) B does not consent to the touching; and (iv) A does not reasonably believe that B consents. It is up to the court to decide whether 'belief' is reasonable after having regard to all the circumstances, including any steps A has taken to ascertain whether B consents. This law covers any kind of intentional sexual touching of another person without their consent. It includes touching any part of their body, clothed or unclothed, either with the body or with an object (www.homeoffice.gov.uk/crime/sexualoffences/legislation/act.html). The Act therefore makes it an offence for any male or female to intentionally touch another person sexually without his or her consent. Under the same Act, rape occurs if a male penetrates with his penis the vagina, or the anus or mouth of a female or male without their consent. The issue of consent is therefore a key issue. The Sexual Offences Act 2003 for the first time in UK law provides a clear definition of consent, making it easier for courts to decide upon evidence put before them. Section 74 of the Act defines consent as follows: 'A person consents if he/she agrees by choice and has the freedom and capacity to make that choice.'

Date rape, also referred to as 'acquaintance rape', defines a sexual assault/rape or attempted assault/rape by an acquaintance who may have been previously known to the victim or whom the victim has met socially for the first time. Although the terms 'date rape' and 'drug-assisted rape' have been used interchangeably, the two should not be confused. Drug-facilitated sexual assault occurs when a person is subjected to non-consensual sexual acts while their capacity to provide consent is impaired (or they are unconscious) owing to the effect(s) of ethanol, a drug and/or other intoxicating substance, and are therefore prevented from resisting and/or unable to consent. The drug can be any substance that induces changes to the physical state of the intended victim or that exerts mind-altering properties. While UK law has been cited above as an example of legislation, the definitions of rape, sexual assault and drug-facilitated sexual assault are not dissimilar in other countries. In the USA, sexual assault may be referred to in different terms in the laws of different states (sexual battery, criminal sexual assault, rape, etc.); however, the underlying principles of law remain essentially the same.

Frequency/statistics of DFSA

The extent of DFSA cases has not been officially recorded and only estimates of its prevalence are available in the literature. The extent of DFSA is difficult to gauge because some incidents are not reported to the police and others are not reported within a time frame that enables drug exposure to be identified by the analysis of blood or urine specimens. The European Monitoring Centre for Drugs and Drug Addiction stated that between 1997 and 2007 there had been a rise in the number of reports in which drugs and alcohol had been used to immobilise victims for the purposes of sexual assault (www.emcdda.europa.eu). It further reported that surveys in six European Union countries suggested that up to 20% of women had experienced some form of sexual assault in their adult lifetime. In 2001 the British Crime Survey estimated the total number of rapes of females to be between 11 000 and 39 000 (Walby, Allen 2004); however, the proportion of cases in which drugs were implicated is unknown. Figures taken from the British Crime Survey 2010 relating to male and female rape and sexual assault between 2004/2005 and 2009/2010 are summarised in Table 8.1, which shows an apparent decrease in the number of cases. The way the statistics have been collected has changed, however, and therefore the latest results are not directly comparable with those of earlier years. As with the earlier reported figures by Walby and Allen, there is no indication in the British Crime Survey 2010 of what proportion of cases were implicated as involving drug use. It is notable that during the same period the number of drug-related offences rose by 62% from 145 837 to 234 998. Only a small number of studies have been published in the scientific literature that provide information on the extent of alcohol and drug use in DFSA cases. For example, the number of reported sexual assaults in the USA according to the FBI Uniform Crime Report is presented in Table 8.2. Both the number and the rate of reported rapes declined throughout most of the 1990s, reaching a low in 1999. It is also well known that the number of reported rapes is significantly lower than the actual number. In 1999, for example, the Bureau of Justice Statistics estimated that there were over 141 000 cases of sexual assault, 58% more than the number actually reported to the police.

Table 8.1 Rapes and sexual assaults reported between 2005 and 2010, abstracted from the British Crime Survey 2010

Year	*Rape – female over 16 years*	*Rape – female under 16 years*	*Sexual assault on females over 13 years*	*Rape – males over 16 years*
2005/2006	8786	3153	17 158	460
2006/2007	8247	2853	16 883	431
2007/2008	7731	2413	15 780	344
2008/2009	7950	2538	15 503	339
2009/2010	9102	2926	15 713	372

Elsohly and Salamone (1999) surveyed 1179 urine samples from suspected DFSA victims collected from 49 states, Puerto Rico and the District of Columbia over a 26-month period. The urine specimens were screened using immunoassay for amfetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite (benzoylecgonine), methaqualone, opiates, phencyclidine and propoxyphene. In addition, samples were screened for flunitrazepam metabolites and GHB by gas chromatography–mass spectrometry and for ethanol by gas chromatography using a flame ionisation detector. No drug substances were detected in 468 of the samples; 451 specimens tested positive for ethanol, 218 for cannabinoids, 97 for benzoylecgonine, 97 for benzodiazepines, 51 for amfetamines, 48 for GHB, 25 for opiates and 12 for barbiturates. Of the samples shown to contain drugs, 35% contained multiple drugs. A similar study undertaken in the UK analysed the toxicology results from 1014 cases submitted to the Forensic Science Service London laboratory between January 2000 and December 2002 (Scott-Ham, Burton 2005). Alcohol was reported to be present in 470 of all cases (46%) either alone or in combination with drugs. Drugs of abuse were detected in 344 cases (34%), with cannabis being the most commonly detected in 260 cases (26%), cocaine in 110 cases (11%), MDMA in 47 cases (5%), amfetamine in 23 cases (2%), diamorphine in 12 cases (1%) and ketamine in 3 cases (0.5%). Potentially stupefying drugs were detected in 187 cases (18%), although in the vast majority of these cases the drugs had been taken therapeutically by the victim or taken after the incident. Only 21 cases (2%) were attributed to involuntary ingestion, i.e. as a result of deliberate drink spiking. During the 3-year period of the survey no evidence for the use of flunitrazepam was observed. As in the ElSohly study, only a few cases were encountered in which a sedative drug was detected and its presence could not be attributed to voluntary use by the complainant. Scott-Ham and Burton further analysed the blood and urine alcohol concentrations in cases of alleged DFSA (Scott-Ham, Burton 2006). Of the 1041 cases scrutinised, 391 had blood and/or urine samples collected within 12 h of an alleged incident. The authors back-calculated the alcohol concentrations in these 391 samples to estimate the possible alcohol concentration at the time of the alleged incident. Following back-calculation, 60% of cases were estimated to have a blood alcohol concentration in excess of 150 mg alcohol per 100 mL of blood at the time of the alleged incident. Hall *et al.* (2008) surveyed blood alcohol concentrations (BACs) determined in cases of alleged DFSA in Northern Ireland between 1999 and 2005 and reported that the estimated average BAC (218 mg/100 mL) remained broadly similar during the period of the study. Jones *et al.* (2008) analysed blood and urine specimens from 1806 female victims of alleged non-consensual sexual activity. No alcohol or drugs were reported to be present in 559 cases (31%) and ethanol alone was detected in 772 cases (43%). In 215 cases (12%) ethanol occurred together with at least one other drug. The mean, median and highest concentrations of ethanol in blood ($N = 806$) were 124, 119 and 370 mg/100 mL, respectively. Amfetamine and cannabinoids were reported to be the most common illicit drugs detected. Elliott and Burgess (2005) reported on 169 clinical requests relating to surreptitious drug administration over a 2-year period between 2002 and 2004. Approximately half of the cases analysed were negative for alcohol or drugs, but in the cases that were positive alcohol and common drugs of abuse were found to be present. Neither GHB nor flunitrazepam was detected in any of the cases. These studies indicate that the patterns of drug and alcohol use in DFSA cases is similar in different countries and that alcohol is the most frequently encountered substance in these cases.

Table 8.2 Number of reported sexual assaults – FBI Uniform Crime Report

Year	*Number of sexual assaults*	*Rate per 100 000*
1990	102 560	41.2
1991	106 590	42.3
1992[a]	109 060	42.8
1993	106 010	41.1
1994	102 220	39.3
1995	97 460	37.1
1996	96 252	36.3
1997	96 153	35.9
1998	96 144	34.5
1999[b]	89 411	32.8
2000	90 178	32.0
2001	90 863	31.8
2002	95 136	33.0
2003	93 433	32.1

[a] The highest.
[b] The lowest.

Toxicological approach to DFSA

Delay in collecting samples can be a critical factor for drugs that exhibit short elimination half-lives, e.g. GHB, as these may not be detectable at concentrations above endogenous levels after around 10–12 h of administration. In the UK, the Association of Chief Police Officers (ACPO) produced the report *Operation MATISSE – Investigating drug facilitated sexual assault* (Gee *et al.* 2006), which assessed the time periods between alleged incidents and the collection of urine samples. They found that, out of a total of 117 samples collected, 25.6% of samples had been collected within the first 6 h, 47.8% within 11.59 h, 84.5% within 23.59 h and 94.8% within 47.59 h.

It is essential that specimens for analysis should be collected as soon as possible after the victim becomes aware that a drug-related sexual assault has taken place. This of course depends on the victim reporting the incident to the appropriate authorities and is outside the toxicologist's control. Laboratories can, however, take a proactive role in working with their local authorities, colleges, health departments, police forces and forensic medical examiners to make them aware of the need to educate the population at large to report incidents and of the need to collect specimens as soon as possible after an alleged offence is suspected to have taken place. Urine specimens can be collected without the need for trained medical personnel to be present and without the need for observed supervised collection, thus facilitating the obtaining of an early evidence specimen while waiting for medical examiners to arrive and collect blood specimens. In order

- Name of subject
- Age
- Sex
- Weight
- Height
- Build (proportional/stocky or muscular/obese/slim etc.)
- Date of incident
- Time of incident
- Date samples taken
- Time samples taken
- Occupation of complainant
- Name and contact details of investigating officer
- Name and contact details of the doctor that examined the victim
- Details of the incident – full case history
- What led the victim to suspect that a DFSA offence had taken place?
- Did the victim fall unconscious? If so provide approximate times, e.g. how long was he/she unconscious?
- Does the victim suffer from any medical condition requiring treatment from a physician?
- Does the victim suffer from any medical condition, e.g. diabetes, depression?
- Did the victim empty his/her bladder prior to reporting the incident?
- Had complainant taken any alcohol, drugs or medication within 48 hours prior to the incident? If so what? How much? and when?
- If alcohol was consumed in the period leading up to the incident was this more than would have normally been consumed? If yes give details of times and amounts consumed.
- Were alcohol or any drugs consumed after the incident? If yes what, how much and when?
- Were any symptoms experienced, e.g. drowsiness, dizziness, nausea, vomiting, impairment of memory, thirst, sweating or shivering, unusual taste, hallucinations?
- Were any of the following symptoms observed by police officers or the medical examiner? Drowsiness, poor coordination, unsteadiness, shivering, sweating, nystagmus, hyperactivity, abnormal pupil size
- Is the complainant a known drug user? If so obtain details
- Is the identity of the suspect known? If so have blood or urine specimens been obtained from the suspect for analysis?
- What is the occupation of the suspect?
- What are the hobbies of the suspect, e.g. making model aircraft
- Have any other exhibits been collected and submitted for analysis, e.g. cups, glasses, tablets, etc.?

Figure 8.1 Information required in cases of suspected drug-facilitated sexual assault.

to avoid irretrievable loss of forensic evidence, it is recommended that a urine specimen should be collected before the commencement of any interviews with the victim. At least 20–25 mL urine should be collected as soon as possible after an incident has been reported. Ideally the urine should contain sodium fluoride as preservative at a minimum final concentration of around 1.5% w/v. There have been anecdotal reports of concerns being expressed in some areas of the USA that supervised collection should be enforced, as in workplace drug testing, to ensure that alleged victims do not add drug substances to their urine specimens in order to make malicious accusations against an innocent party.

As soon as the forensic medical examiner is available, a specimen of blood should be collected. The volume of blood collected should ideally be of a minimum volume of 10 mL and should be collected into a sealed glass container with a minimum final concentration of 1.5% w/v sodium fluoride as preservative. A second urine specimen (20 mL) should also be collected into a container with sodium fluoride preservative as above. All specimens should be clearly labelled with the name of the victim as well as the time and date that the specimen was collected. Specimen containers should not be over-filled. This avoids breakage if the sample is frozen at a later time. Once the early evidence specimens have been obtained, the case history and information regarding the events and circumstances should be recorded. Many police forces and clinics will have their own protocols for collecting details of the case circumstances. However, it is important that the case history should be as detailed and comprehensive as possible. The information that should be collected is summarised in Figure 8.1. Information can provide the toxicologist with important clues as to what, or what not, to look for and in cases where limited sample volumes have been submitted for analysis can make the difference between detection of an incapacitating agent and a negative finding.

In addition collection of a detailed case history and blood, hair and urine from the alleged victim, any suspected tablets, powders, drinks, containers or residues in cups, etc. should be collected and submitted for possible analysis. While this may be impracticable if the alleged drink spiking occurred in a bar, many alleged incidents take place in the home where exhibits are less likely to have been disposed of. The examination of drinking vessels may reveal drug or tablet residues. In cases where gelatin capsules have been added to hot drinks it is not uncommon to be able to see the melted capsule adhering to the base of the cup. If the subject has vomited and vomit stains are available, these may also be considered for analysis. Alleged incidents in public bars may be recorded on security video systems and examination of these can also provide useful evidence of an alleged incident.

Samples and sample collection

Blood, urine and hair are recommended for collection wherever possible, although each has its merits and limitations. Blood is the tissue of choice for analysis because it provides potentially interpretable results and helps place drug consumption within a fairly narrow time frame (up to 24–36 h depending on the drug consumed and the amount taken). Many drugs may be detected in blood for 24–36 h after consumption, although GHB is unlikely to be present in concentrations greater than endogenous concentrations approximately 6–8 h following consumption (Andresen *et al.* 2010). If it is suspected that GHB has been consumed, urine should be collected in addition to blood. Blood and urine should be stored in a refrigerator prior to submission to the forensic laboratory for analysis.

As well as being easy to collect, urine is usually plentiful and easy to analyse, contains higher concentrations of drugs or their metabolites than blood, and provides a wider window of detection than blood. Depending on the drugs consumed, urine may enable the detection of drugs of abuse, benzodiazepines, flunitrazepam metabolites, trichlorinated compounds and many basic drugs for up to 2–3 days after consumption and hence is particularly useful in cases where a long delay in reporting an incident has occurred. The window of detection for GHB in urine, although greater than that of blood, is still short and is unlikely to yield meaningful results for this compound if more than 12 h has elapsed between ingestion and specimen collection. Alcohol may usefully be analysed in urine and used for back-calculations up to around 24 h after consumption but after this time the results are likely to provide only limited information. The major flunitrazepam metabolite (7-aminoflunitrazepam) can be detected in urine for up to 72 h following the ingestion of a single therapeutic dose (Negrusz *et al.* 2000; Snyder *et al.* 2001). Table 8.3 summarises the approximate detection times for alcohol and some common drugs in blood and urine after single or therapeutic doses.

In cases where the time elapsed between an incident and reporting exceeds 24–48 h, hair analysis should be considered. Hair grows, on average, at a rate of approximately 1 cm per month and therefore a hair specimen should be collected at the time of the first examination and the victim advised to return to provide a second hair specimen after approximately 6 weeks. The first hair specimen taken after the incident may then be used as a control and any drugs taken around the time of the incident will hopefully show up in the second sample. Protocols for hair collection and analysis are provided in Chapter 19. If it is proposed to collect hair specimens, a clear explanation should be given to the alleged victim of the potential outcomes in terms of the results of the analysis. The subject should be advised that where he or she is a voluntary drug user but has not declared the fact, the ramifications of this being disclosed in court could be potentially damaging.

Table 8.3 Approximate detection times for alcohol and common drugs following the consumption of single or therapeutic doses

	Detection time (h)	
Drug	***Blood*[a]**	***Urine*[a]**
Alcohol	8–10	24
Amfetamines	12	24–48
Barbiturates	24	242–48
Benzodiazepines (including metabolites)	48	48–72
Cannabinoids	4–12[b]	12–48
Cocaine	12	12
Benzoylecgonine	12–24	24–48
Flunitrazepam	12–24	48–72
GHB	<8	10–12
Morphine/opiates	12	24–48
Methadone	24	24–48
MDMA	12–24	24–48
Ketamine	4–10	12–24

[a]Dependent on limit of detection of assay and quantity consumed.
[b]May not detect occasional use.

Sample analysis

Beverages, cups and tablets

Commercial 'Quick Test' kits have been marketed for use by the public to test drinks for adulteration *in situ* in a nightclub scenario. Beynon *et al.* (2006) reported on the evaluation of two such kits – 'Drink Guard' and 'Drink Detective' – and concluded that 'whilst drink spike testing kits may be a useful public health tool, neither kit demonstrated high levels of sensitivity, specificity or utility' under the laboratory protocols used for the evaluation. They further stated that 'in practice this could leave the public feeling falsely reassured or overplay the magnitude of drink spiking'.

Liquids submitted for analysis should be carefully examined against control samples for signs of adulteration, which might be indicated by cloudiness, opacity, colour changes (the manufacturers of flunitrazepam incorporated a blue dye into the tablets to provide an indication of drink spiking), particles floating on the surface or residues in the bottom of the container. If a liquid preparation of an adulterant such as GHB is added to a beverage there may be no easily recognisable sign of adulteration. However, if a liquid herbal preparation is added, e.g. valerian tincture, distinct colour changes may be observed. Herbal preparations such as crushed herbal sleep aid tablets often yield floating debris that is easily recognisable. The addition of coloured tablets or capsules containing oily drug formulations may be indicated by a coloured scum or immiscible oily globules floating on the surface of the drink. Most tablets comprise bulking agents in addition to the active pharmaceutical ingredient and these may sometimes be left as visible residues in the bottom of a drinking container. Figures 8.2–8.5 illustrate, respectively, a crushed tablet showing parts of coating prior to addition to a beverage, an Arthrotec tablet partially dissolved in water, a residue in a tea cup following removal of the beverage by decanting and floating waxy particles following the addition of crushed tablet with wax coating.

In some cases there may be sufficient residue present in a container that it can be scraped from the bottom and analysed directly using colour tests, ultraviolet spectrophotometry (UV), Fourier transform infrared spectrometry (FTIR), thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS). In cases where only a slight residue may be indicated, the receptacle should be washed with a small quantity of ethanol, which can be retrieved, evaporated and subsequently tested by immunoassay screening or instrumental analysis such as high

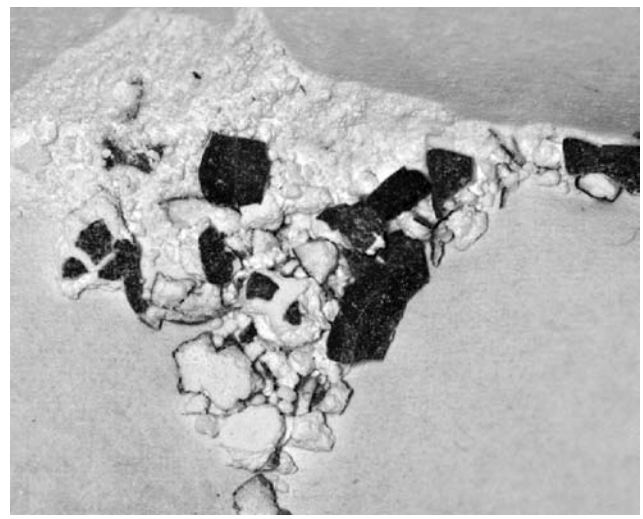

Figure 8.2 Crushed tablet material showing parts of tablet coating.

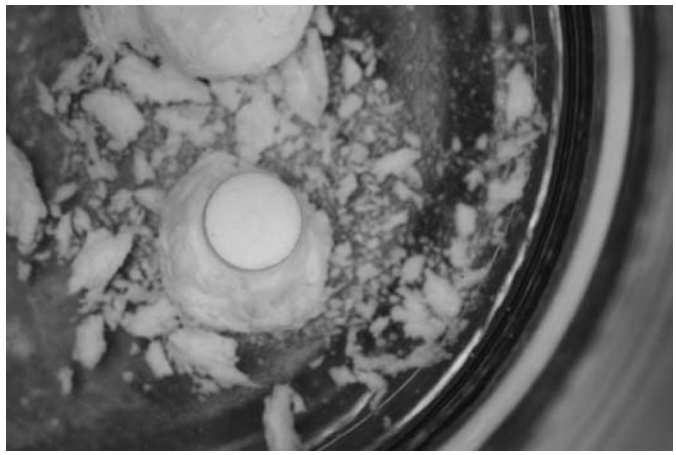

Figure 8.3 Arthrotec tablet added to water showing undissolved residue and inner tablet.

performance liquid chromatography–mass spectrometry (LC-MS). Procedures for the identification of intact tablets are given in Chapter 13 and colour tests are provided in Chapter 30. HPLC is a particularly valuable technique for the analysis of beverages suspected to have been adulterated with drugs since the beverage may be injected directly into the HPLC instrument and the elution profile compared against an unadulterated control. Figure 8.6 shows the HPLC analysis of Pepsi Max adulterated with ketamine following direct injection of the beverage into the HPLC diode array system. Figure 8.7 provides a checklist of points for consideration when analysing an exhibit suspected to contain an adulterated beverage. Olsen *et al.* (2005) added eight potentially sedating medicinal drugs (flunitrazepam, oxazepam, zopiclone, carisprodol, clonazepam, morphine, diazepam and alprazolam) to water, beer, Coca-Cola and 12% ethanol, and measured the drug concentrations at 5, 10, 20 and 40 min after adding the drugs. They concluded that, although a sufficient amount of each drug dissolved in the beverages to potentially cause impairment, adulteration would be likely to be apparent to the consumer from the altered appearance and/or taste of the beverages.

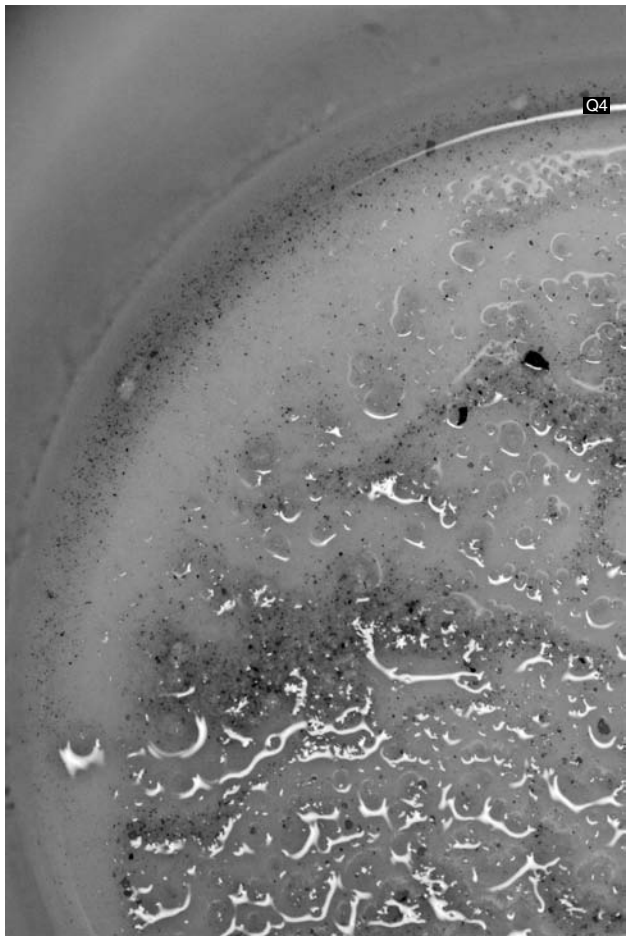

Figure 8.4 Residue of crushed tablet in bottom of cup that had contained tea.

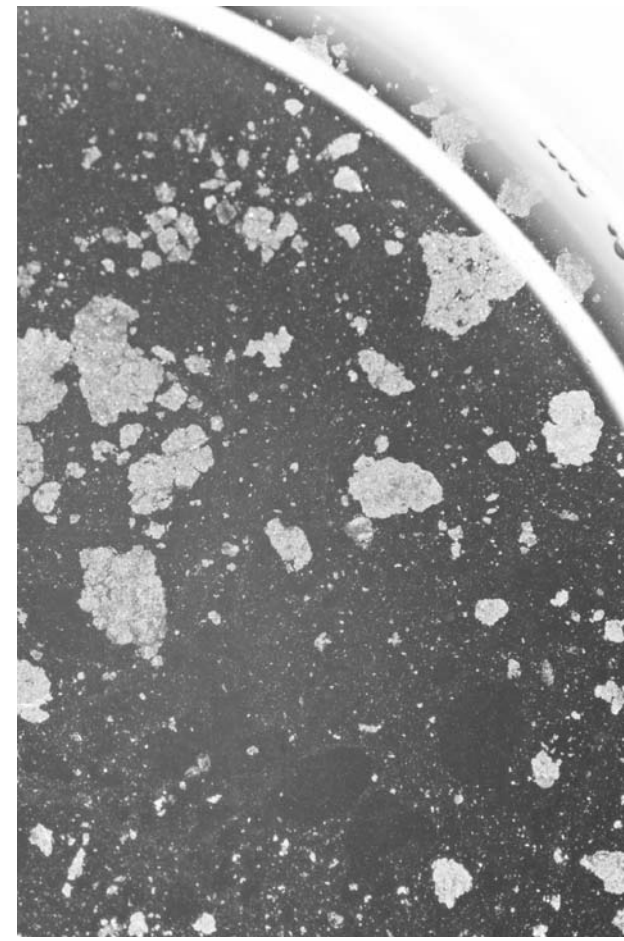

Figure 8.5 Wax film floating on the surface of adulterated tea.

Blood and urine

As a consequence of the wide range of drugs that may be used to facilitate DFSA there is no single analytical method that can be applied for the detection of possible adulterants. Blood and urine should always be analysed for the presence of alcohol (*see* Chapter 4) followed by immunoassay screening for the presence of drugs of abuse. At the time of writing, the availability of immunoassay screening tests for drugs other than common drugs of abuse is limited. However, suppliers of commercial immunoassay reagents are beginning to develop screening test panels for substances that may be encountered in DFSA cases, e.g. GHB, ketamine, meprobamate, the 'Z' drugs (zaleplon, zolpidem and zopiclone), flunitrazepam metabolites, phenothiazines and chloral metabolites. The Randox microarray technology (Figure 8.8) offers a sensitive means of screening up to 12 drugs simultaneously on a single drop of blood or urine (www.Randox.com). Immunoassay screening in DFSA cases should be carried out using appropriate 'cut-off' concentrations. Laboratories should strive to employ the lowest cut-off concentrations that are reliable and should recognise that the high cut-offs

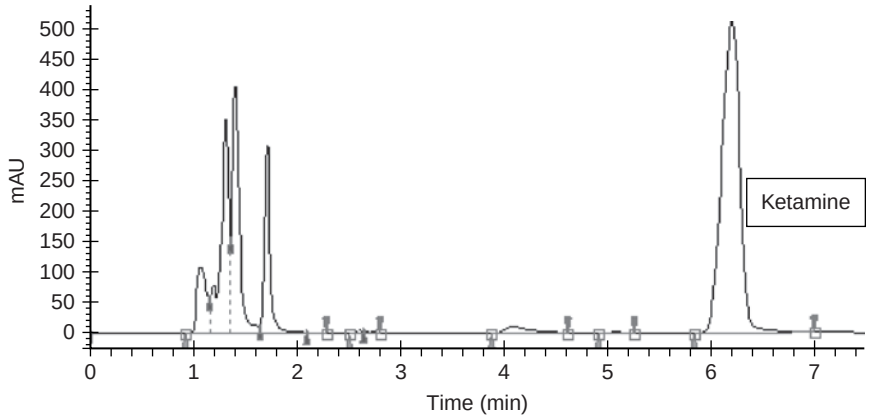

Figure 8.6 HPLC chromatogram of Pepsi Max adulterated with ketamine. Varian C_{18} column (150 mm × 2.1 mm); isocratic 5 mmol/L ammonium acetate–acetonitrile (50 : 50 v/v) monitored at 210 nm.

recommended by SAMHSA for workplace drug testing (see Chapter 3) are totally inappropriate for use in DFSA case screening. Analysts should also be aware of the limits of detection and quantification of their methods used in wider GC or HPLC screening procedures. The lower the limit of detection of an assay, the greater the window of drug detection will be. The Society of Forensic Toxicologists (SOFT) formed an expert committee to provide guidance for its members on the way cases should be approached. The SOFT expert committee published a list of recommended minimum

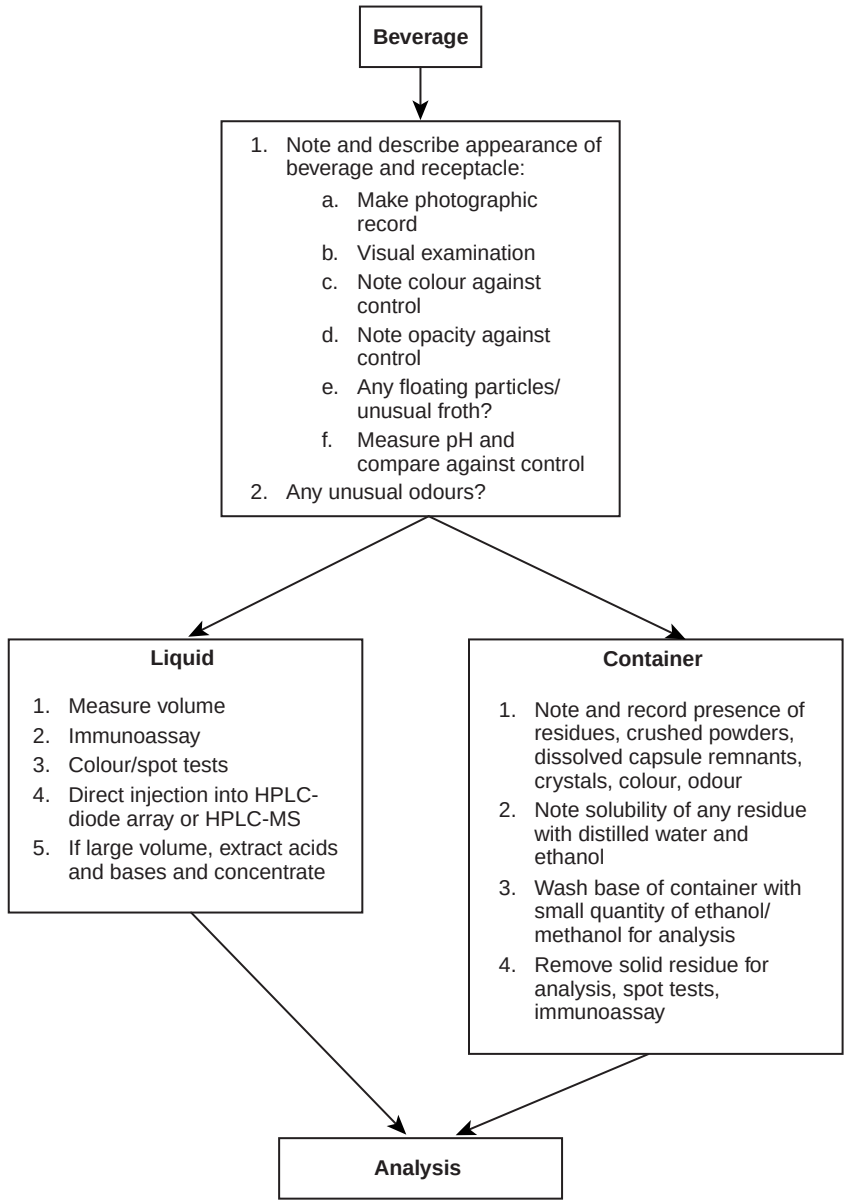

Figure 8.7 Examination checklist for adulterated beverages.

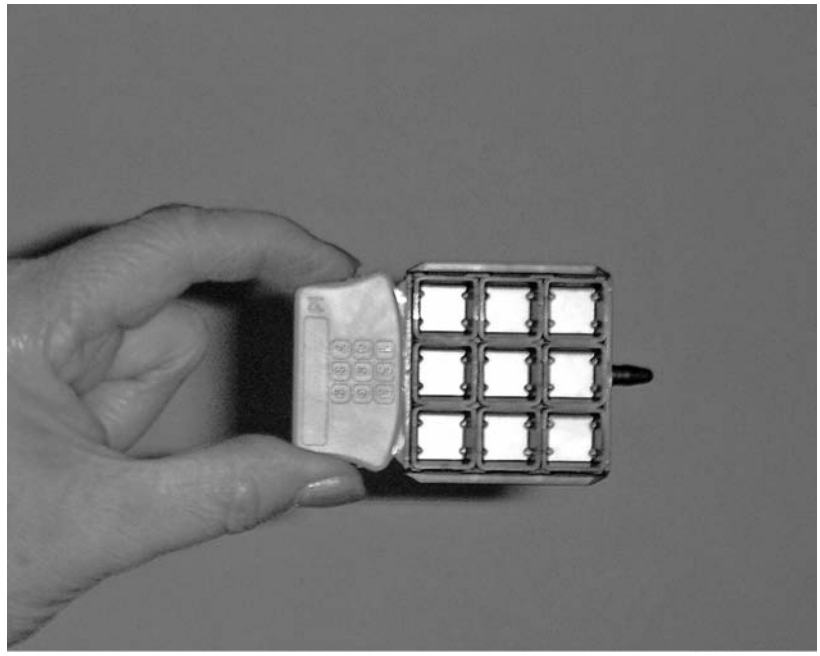

Figure 8.8 Randox Microarray for drug screening. Each of the nine squares shown in the figure is capable of detecting up to 12 different drugs from 1–2 drops of urine, blood or tissue suspension.

limits of detection for drugs that laboratory methods should achieve when screening DFSA cases. The list of recommended limits of detection is presented as Table 8.4 courtesy of SOFT. Comprehensive methods for drug screening of blood and urine can be found in Chapter 1.

A method described by Adamowicz and Kala (2010) for the simultaneous screening of 128 drugs associated with DFSA cases in urine using GC-MS is summarised as follows. To urine (2 mL) add diazepam-d_5 and methadone-d_4 internal standards to achieve final concentrations of 2.5 μg/mL for each internal standard. Add 1 mL of 0.1 mol/L acetate buffer (pH 5.5) and β-glucuronidase/arylsulfatase (50 μL). Incubate for 60 min at 55°C. Precondition Oasis HLB SPE cartridges using 2 mL of methanol followed by 2 mL of deionised water. Add the hydrolysed urine specimens to the preconditioned SPE cartridges and allow the urine to drain slowly through the column under a mild vacuum. Rinse the column with 2 mL of deionised water and discard the water wash before drying the columns under vacuum for 5 min. Elute retained analytes using 0.8 mL of methanol and 0.8 mL of a mixture of methanol–isopropanol (3 : 1) under gravity. Evaporate the eluate to dryness under nitrogen at 37°C. Derivatise the dry column eluates using 50 μL of BSTFA + 1%TMCS in 40 μL of ethyl acetate–acetonitrile (1 : 1) at 60°C for 30 min. Derivatised samples may be analysed by GC-MS.

If urine is available it should also be screened for trichlorinated compounds, e.g. trichloroethanol, which is formed in the body after the consumption of chloral/chloral hydrate using the Fujiwara test. To 2 mL of urine in a boiling tube add 2 mL freshly prepared 20% sodium hydroxide solution followed by 1 mL of pyridine. Place into a bath containing boiling water and leave for 5–10 min. The presence of trichlorinated substances is indicated by the formation of a pink/violet coloration in the upper pyridine layer. A blank sample should always be tested when using the Fujiwara test as the presence of chloroform vapour in the laboratory atmosphere can also give a positive result.

Drugs with potential for use in DFSA cases

The ideal drug properties for substances used in DFSA include rapid onset of action, reduced inhibitions, drowsiness, reduced ability to resist unwanted advances, anterograde amnesia, tastelessness, potency such that effects are obtained after consumption of low doses, short elimination half-life for rapid elimination from the body, and ease of administration. Substances that have been associated with DFSA are all psychoactive, sedative or hypnotic drugs exerting their action on the central nervous system (CNS). The list of drugs that may potentially be used in sexual assault is long and may include alcohol and sleep aids that are readily available in over-the-counter preparations in supermarkets and health shops, as well as medicinally prescribed hypnotics, anxiolytics, antidepressants, phenothiazines, antihistamines and illegal drugs of abuse. To make the analyst's role even more challenging, we must also be aware that, as a consequence of the high frequency of international travel, drugs that are not commonly encountered in the victim's country of residence may be obtained while individuals are on holidays or business trips abroad. Proprietary drug names used in different parts of the world may be obtained from sources such as *Martindale's Complete Drug Reference* (Sweetman 2007) and the *Physicians Desk Reference*.

Alcohol

Alcohol is the most commonly encountered substance in DFSA cases probably because it is taken voluntarily, is inexpensive and is easy to obtain. Alcohol misuse has drawn considerable attention in recent years because many young people engage in binge drinking. There are even venues in some major cities where alcohol may be purchased via intravenous administration to enable users to experience rapid and intense intoxication. Alcohol is a CNS depressant that in low doses may produce enhanced sociability, mild euphoria, talkativeness and relaxation of inhibitions. Higher BACs may induce drowsiness, impaired coordination, impaired information processing, impaired judgement, memory loss, reduced attention span, increased reaction times and impaired muscular function. The physical signs of alcohol intoxication include nystagmus, loss of balance and impaired coordination, impaired speech, vomiting and incontinence. A full account of alcohol abuse is provided in Chapter 4. Unlike most other drugs possessing psychoactive properties and inducing behaviour changes, it is possible to broadly estimate the effects that a given BAC might exert on a person (see Table 4.2, Chapter 4).

Antihistamines

Antihistamines are widely available as both prescription and over-the-counter products for the treatment of allergies, as cold remedies and as sleep aids. The most commonly encountered antihistamines include bromodiphenhydramine, brompheniramine, chlorpheniramine, cyclizine, diphenhydramine, doxylamine, ethopropazine and

Table 8.4 Recommended maximum detection limits for common DFSA drugs and metabolites in urine samples

Target analytes	*Parent drug*	*Trade names/ `street names'*	*Recommended maximum detection limit*
Ethanol			
Ethanol	Ethanol	Alcohol, ethyl alcohol, 'booze'	10 mg/dL
GHB and analogues			
Gamma-hydroxybutyrate	Gamma-hydroxybutyrate	Xyrem, 'GHB', 'Easy Lay', 'G', 'Georgia Home Boy', 'Grievous Bodily Harm', 'Liquid Ecstasy', 'Liquid E', 'Liquid G', 'Liquid X', 'Salty Water', 'Scoop', 'Soap'	10 µg/dL
	1,4-Butanediol	'1,4-BD', 'Enliven', 'Inner G', 'Revitalize Plus', 'Serenity', 'SomatoPro', 'Sucol B', 'Thunder Nectar', 'Weight Belt Cleaner', 'White Magic'	
	Gamma-butyrolactone	'GBL', 'Blue Nitro', 'G3', 'Gamma G', 'G.H. Revitalizer', 'Insom-X', 'Invigorate', 'Remforce', 'Renewtrient', 'Verve'	
Benzodiazepines			
Many benzodiazepines are biotransformed into glucuronide-conjugated metabolites. To improve detection limits and times, it is recommended that laboratories use hydrolysed urine specimens to free the conjugate before extraction. Instrumental techniques will detect the glucuronide metabolites *or* hydrolyse urine specimens to free the conjugate before extraction.			
Alprazolam	Alprazolam	Xanax, Nirava	10 ng/mL
α-Hydroxy-alprazolam			
Chlordiazepoxide	Chlordiazepoxide	Librium, Libritabs	10 ng/mL
Clonazepam	Clonazepam	Clonapin, Klonopin, Rivotril	5 ng/mL
7-Aminoclonazepam			
Diazepam	Diazepam	Valium, Diastat, Dizac	10 ng/mL
Flunitrazepam	Flunitrazepam	Rohypnol	5 ng/mL
7-Flunitrazepam			
Lorazepam	Lorazepam	Ativan	10 ng/mL
Nordiazepam	Diazepam, chlordiazepoxide		10 ng/mL
Oxazepam	Oxazepam, diazepam, chlordiazepoxide, nordiazepam, temazepam	Serax	10 ng/mL
Temazepam	Temazepam, diazepam	Normison, Restoril	10 ng/mL
Triazolam	Triazolam	Halcion	5 ng/mL
4-Hydroxy-triazolam			
Marijuana			
11-Carboxy-THC	Tetrahydrocannabinol (THC)	Marinol, Dronabinol, 'Marijuana', *Cannabis sativa*	10 ng/mL
Barbiturates			
Amobarbital	Amobarbital	Amytal	25 ng/mL
Butalbital	Butalbital	Esgic, Fioricet, Fiorpap, Fiorinal	
Pentobarbital	Pentobarbital, thiopental	Nembutal	
Phenobarbital	Phenobarbital, primidone		
Secobarbital	Secobarbital	Seconal, Tuinal	20 ng/mL
Over-the-counter medications			
Brompheniramine	Brompheniramine	Alatapp, Bromaline, Bromanate, Bromfed, Bromphen, Dimetane, Dimetapp, Myphetane, Polytine, Puretane	10 ng/mL
Desmethylbrompheniramine			
Chlorpheniramine	Chlorpheniramine	Aller Chlor, Chlor-Trimeton, Coricidin, Deconamine, Efidac, Kronofed, Teldrin	
Desmethylchlorpheniramine			
Dextromethorphan	Dextromethorphan	Benylin, Romilar, Delsym	
Diphenhydramine	Diphenhydramine	Banophen, Belix, Benadryl, Dermarest, Excedrin PM, Hydramine, Sleepinal, Sleep-Eze 3, Tylenol PM, Unisom Sleep Gels	
Doxylamine	Doxylamine	Unisom, Bendectin	
Desmethyldoxylamine			

Table 8.4 continued

Target analytes	*Parent drug*	*Trade names/`street names'*	*Recommended maximum detection limit*
Antidepressants			
Amitriptyline	Amitriptyline	Elavil, Endep	10 ng/mL
Nortriptyline			
Citalopram	Citalopram	Celexa, Cipramil	
Desmethylcitalopram			
Desipramine	Desipramine, Imipramine	Norpramin, Pertofrane	
Doxepin	Doxepin	Sinequan, Adapin, Zonalon, Prudoxin	
Desmethyldoxepin			
Fluoxetine	Fluoxetine	Prozac, Sarafem	
Norfluoxetine			
Imipramine	Imipramine	Tofranil	
Paroxetine		Asimia, Paxil	
Sertraline	Sertraline	Zoloft	
Norsertraline			
Narcotic and non-narcotic analgesics			
Codeine	Codeine		
Fentanyl	Fentanyl	Actiq, Duragesic, Sublimaze, Innovar	
Hydrocodone	Hydrocodone	Anexsia, Hycodan, Lorcet, Lortab, Norco, Panacet, Vicodin, Zydone	
Hydromorphone	Hydromorphone	Dilaudid, Palladone	
Meperidine	Meperidine	Demerol, Mepergan	
Normeperidine			
Methadone EDDP	Methadone	Dolophine	10 ng/mL
Morphine	Morphine	Avinza, Astramorph, Duramorph, Kadian, MSIR, MS Contin, Oramorph, Roxanol	
Oxycodone	Oxycodone	OxyContin, Oxyir, Roxicodone, Percodan, Percocet, Percolone, Roxicet, Tylox	
Propoxyphene	Propoxyphene	Darvocet, Darvon, Wygesic	
Norpropoxyphene			
Miscellaneous drugs			
Carisoprodol	Carisoprodol	Som	50 ng/mL
Clonidine	Clonidine	Catapres, Combipres, Clorpres, Duraclon	1 ng/mL
Cyclobenzaprine	Cyclobenzaprine	Flexeril	10 ng/mL
Ketamine	Ketamine	Ketalar	1 ng/mL
Norketamine			
Methylenedioxyamfetamine	Methylenedioxyamfetamine		10 ng/mL
Methylenedioxymethamfetamine	Methylenedioxymethamfetamine		
Meprobamate	Meprobamate, carisoprodol	Equagesic, Equanil, Micrainin, Miltown	50 ng/mL
Phencyclidine	Phencyclidine		10 ng/mL
Scopolamine	Scopolamine	Isopto Hyoscine, Scopace, Transderm Scop	
Valproic acid	Valproic acid	Depacon, Depakene, Valproate	50 ng/mL
Zolpidem	Zolpidem	Ambien	10 ng/mL
Stimulants			
While the drugs below do not possess the pharmacological effects typically associated with DFSA drugs, owing to their popularity it is recommended that screens for these drugs and metabolites be conducted at the detection limits listed or better.			
Amfetamine	Amfetamine, metamfetamine	Adderall	50 ng/mL
Cocaine	Cocaine		50 ng/mL
Benzoylecgonine			
Metamfetamine	Metamfetamine	Desoxyn	50 ng/mL

tripelennamine. Most antihistamines induce drowsiness or sleep and their sedative effects may be potentiated when taken in combination with alcohol, sedative hypnotics and narcotics.

Benzodiazepines

The benzodiazepines are the most widely used and prescribed class of sedatives, anxiolytics and hypnotics in medical practice. The benzodiazepines replaced the barbiturates as a consequence of their greater safety and lower toxicity. The first benzodiazepine to be synthesised was chlordiazepoxide, which was introduced into medical practice in 1960, followed in 1963 by the introduction of diazepam. Diazepam became the most commonly prescribed anxiolytic drug in the latter part of the 20th century and is still widely prescribed today. Over 3000 benzodiazepine compounds have been synthesised, although only around 35 are in use worldwide today. All benzodiazepines are capable of inducing sedation, memory impairment, reduced inhibitions, reduced anxiety, muscle relaxation, anticonvulsant and antidepressant activity, anterograde amnesia and reduced nocturnal gastric secretions. The properties of the benzodiazepines are governed by the dose administered, the half-life and the mode of administration. Benzodiazepines are classified either by their chemical structures or by their half-lives, i.e. short, intermediate and long half-life. Table 8.5 provides examples of benzodiazepines classified as either hypnotics or anxiolytics and Table 8.6 provides examples of benzodiazepines classified by half-life. Some benzodiazepines, e.g. diazepam and midazolam, are administered intravenously to facilitate anterograde amnesia during unpleasant short-term medical procedures, e.g. gastroscopy, endoscopy and dental treatment, that may require patient cooperation. There have been reports that some female patients may experience sexual fantasies when sedated with IV diazepam or midazolam, although these appear to be dose related (Brahams 1989; Dundee 1990). This observation is of potential importance since a number of allegations of sexual assault have been made against dentists and medical staff by female patients who have received IV benzodiazepine treatment. The benzodiazepines exert their pharmacological action by reacting with a benzodiazepine binding site on the $GABA_A$ receptor. The metabolism of most benzodiazepines is associated primarily with the cytochrome P450 system, and in particular with isoenzymes CYP2C19 and CYP3A4. Thus, adverse effects may be produced by use of benzodiazepines with drugs that inhibit, induce or are metabolised by these isoenzymes. Enhanced sedation may occur when benzodiazepines are taken in combination with other drugs that exert CNS-depressant properties, such as alcohol, antidepressants, sedative antihistamines, antipsychotics, other hypnotics or sedatives and, opioid analgesics. Despite widespread indications in the popular press, flunitrazepam has not been widely encountered in DFSA cases either in the UK or in the USA. Flunitrazepam is not available on prescription in the UK but can be obtained via internet sites.

Cannabis

After alcohol, cannabis is the most frequently encountered drug in DFSA cases and it is usually present as a consequence of the victim having smoked cannabis or cannabis resin prior to the alleged incident. Cannabis is most widely used for its euphoriant and psychotropic effects, the intensity and duration of which are influenced by susceptibility of the person taking the drug, the quantity of active components such as Δ^9-tetrahydrocannabinol (THC) reaching the brain, and the frequency of use. Cannabis induces a feeling of well-being, euphoria, relaxation and distortion in the perception of space and time. It can also cause deterioration in coordination and disturbance of judgement, loss of concentration, drowsiness, sleepiness, lethargy and slowed reactions. The perceived and euphoric effects of cannabis may last for between 2 and 4 hours after smoking a single cigarette. However, there is considerable evidence that some unwanted behavioural effects may persist for much longer. The term 'temporal disintegration' has been used to describe the inhibitory effects that cannabis exerts on short-term memory and the impairment of ability to undertake memory-dependent, goal-directed behaviour (Leonard 1992). Subjects suffering from temporal disintegration have a tendency to confuse the past, the present and the future, and to feel depersonalised. The sensation of depersonalisation is more pronounced after higher doses of cannabis are consumed. High doses of cannabis are also associated with hallucinations, anxiety, acute panic reactions, acute delirium (characterised by marked memory impairment), delusions, acute paranoia, schizophrenic behaviour and acute mania. The effects of cannabis may be enhanced when taken in combination with alcohol and the relaxant effects of cannabis might be expected to be enhanced when the drug is taken in combination with other CNS depressants.

The unknowing consumption of a pharmacologically significant quantity of cannabis via a 'spiked drink' is unlikely. The main active ingredient, Δ^9-THC, is insoluble in water; hence the addition of herbal cannabis or cannabis resin to a cold beverage such as beer or cola would not release into the liquid sufficient active drug to exert any pharmacological effect. Addition of herbal cannabis and crushed resin yields floating herbal material on the surface of drinks and resin also leaves a residue in the bottom of containers. Giroud *et al.* (2000) prepared water and milk decoctions from 5 g of cannabis containing 35 mg THC and administered them to two groups of six volunteers. The water decoction was found to contain approximately 1.6 mg free THC per 200 mL, while the milk decoction contained approximately 23.2 mg free THC per 200 mL, reflecting the greater lipophilic nature of milk. They demonstrated that the whole blood of volunteers who had consumed the water decoction of cannabis contained only trace quantities of THC and 11-hydroxy-THC and that only THC-carboxylic acid reached any significantly measurable concentration (~3 μg/L). The THC-acid remained detectable for approximately 10 h. After the consumption of the milk decoction, however, measurable quantities of both THC (~4 μg/L) and 11-hydroxy-THC (~4 μg/L) were present in whole blood and the whole blood concentration of THC-acid attained concentrations of approximately 25 μg/L. No subjective effects of cannabis were described for volunteers after consumption of the water decoction, but effects were noted after the consumption of the milk decoction. It should also be remembered that oral doses of cannabis must be significantly higher than smoked doses to obtain equivalent effects. Therefore the quantity of insoluble debris that would be needed to be added to a drink to achieve a pharmacological effect would not be disguisable. Cannabis oil would likewise not yield significant quantities of Δ^9-THC into solution and, although it might be easier to mask the presence colour-wise in a dark beverage such as cola, it would settle as a tar on the base of the receptacle to which it was added and would therefore be unlikely to be consumed.

Table 8.5 Examples of anxiolytic and hypnotic benzodiazepines

Anxiolytic	*Hypnotic*
Alprazolam	Estazolam
Chlordiazepoxide	Flunitrazepam
Clonazepam	Flurazepam
Diazepam	Nitrazepam
Lorazepam	Quazepm
Medazepam	Temazepam
Oxazepam	Triazolam

Table 8.6 Examples of short, intermediate and long half-life benzodiazepines

Short half-life	*Intermediate half-life*	*Long half-life*
Alprazolam	Lorazepam	Chlordiazepaoxide
Flunitrazepam	Temazepam	Diazepam
Midazolam	Oxazepam	Nordiazepam
Triazolam		Medazepam

Elsohly and Jones (1995) measured the Δ^9-THC content of cannabis teas and found that when 1 g of cannabis (total Δ^9-THC content of 2%) was boiled for 1 min in water and then allowed to steep for a few minutes, only 5% of the total Δ^9-THC content was released into the tea and that 85% of this was in the form of the acid precursor of Δ^9-THC. They further experimented by boiling cannabis for 45 min and still found only minute quantities of Δ^9-THC in the final solution. Elsohly and Jones reported that it is necessary to heat cannabis at 90–100° to achieve the quantitative conversion of THC-acids into free THC.

Cooking with cannabis has been well established in some cultures and there is no shortage of recipes for cannabis culinary practices. Elsohly and Jones (1995) conducted experiments by simmering cannabis in spaghetti sauce for 90 min and demonstrated that under these conditions Δ^9-THC was stable and hence could enter the body systems of individuals who consumed such preparations. Cone *et al.* (Cone *et al.* 1988; Cone 1990) measured the urinary concentrations of Δ^9-carboxy-THC in subjects who had consumed cannabis-laced brownies. Cone demonstrated that oral doses of THC equivalent to one and two cigarettes (approximately 28.5 and 57 mg THC, respectively) could produce peak urinary carboxy-THC concentrations between 108 and 436 µg/L. The peak urinary carboxy-THC concentrations measured in subjects who had smoked equivalent amounts of THC (one to two cigarettes) ranged between 9 and 152 µg/L. Cone (1990) also demonstrated that the time span for the detection of cannabis metabolites in urine was dependent upon the method of analysis and the cut-off or limit of detection used. Using EMIT and a cut-off of 20 µg/L the mean detection time for THC metabolites in urine was 121 h after consumption of a brownie containing THC equivalent to one cigarette (~28.5 mg THC) and 142 h after consumption of a brownie containing THC equivalent to two cigarettes (~57 mg THC). Using GC-MS with a cut-off of 5 µg/L the mean detection times increased to 149 and 156 h respectively. It follows therefore that THC metabolites may be present in urine at concentrations above the cut-offs normally used for workplace drug testing following the unknowing oral consumption of cannabis.

Hempen Ale is a proprietary beer brewed with hemp seeds. Although the seeds do not contain THC, they do contain other cannabinoids found in the cannabis plant. Kunsman *et al.* (1999) organised a study in which 10 subjects consumed Hempen Ale and subsequently provided urine specimens for analysis. Nine of the volunteers were allowed to drink Hempen Ale as desired throughout the day (the amount of the ale consumed varied between 5 and 14 bottles (12 ounce)) while the tenth volunteer drank two bottles each day over an 8-day period. A total of 146 urine specimens were collected and screened for cannabinoids using a selection of immunoassays including fluorescence polarisation immunoassay (FPIA), kinetic interaction of microparticles in solution assay (KIMS), enzyme immunoassay (EIA) and radioimmunoassay (RIA), and any immunoassay positives were further analysed by GC-MS for the presence of Δ^9-THC-carboxylic acid. With the exception of 10 specimens analysed by RIA all immunoassay results were negative and the 10 samples indicated to be positive by RIA were shown to be negative by GC-MS.

Chloral

Chloral, often known as the traditional 'Micky Finn' when mixed with alcohol, is available as chloral hydrate and chloral betaine. Chloral is an effective sedative hypnotic that produces only minimal respiratory and circulatory depression when administered in therapeutic doses. Although not widely used today, chloral hydrate and chloral betaine are used in the short-term treatment of insomnia, inducing sedation and as a premedicant in children. Chloral hydrate is rapidly converted into trichloroethanol in the body and may also be converted into trichloroacetic acid by the enzyme chloral hydrate dehydrogenase. Chloral hydrate is rapidly absorbed from the gastrointestinal tract and its effects begin to be noticed after approximately 30 min. The sedative hypnotic effects of chloral are potentiated by alcohol and other drugs with CNS-depressant properties, e.g. barbiturates and sedatives. Chloral is said to possess an unpleasant taste. Recorded side-effects associated with the consumption of chloral include drowsiness, light-headedness, ataxia, headache, paradoxical excitement, hallucinations, nightmares, delirium and confusion (sometimes with paranoia). Chloral is available as Chloraldurat (Germany), Chloraldurat, Nervifene (Switzerland), Welldorm (UK) and Somnote (USA).

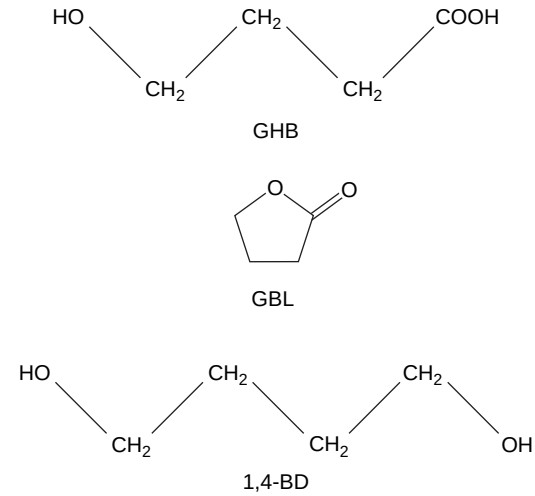

Figure 8.9 Chemical structure of gamma-hydroxybutyrate (GHB), gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD).

Gamma-hydroxybutyrate

Gamma-hydroxybutyrate (synonym gamma-hydroxybutyric acid) and related compounds that are converted to GHB, such as gamma-butyrolactone and 1,4-butanediol (1,4-BD), are shown in Figure 8.9. Figure 8.10 shows the metabolic transformation of GBL and 1,4-BD to GHB by alcohol dehydrogenase, aldehyde dehydrogenase and lactonase. GHB is sold on the streets under names such as Salty Water, Scoop, Soap, Liquid X, Natural Sleep-500, Liquid Ecstasy. GHB is rapidly metabolised and eliminated from the body. The detection window for blood is about 6–8 h and for urine is about 8–10 h. GHB is endogenously produced in the human body and in some foods, and this needs to be taken into account when carrying out an analysis for GHB. It is used in DFSA because it is effective rapidly,

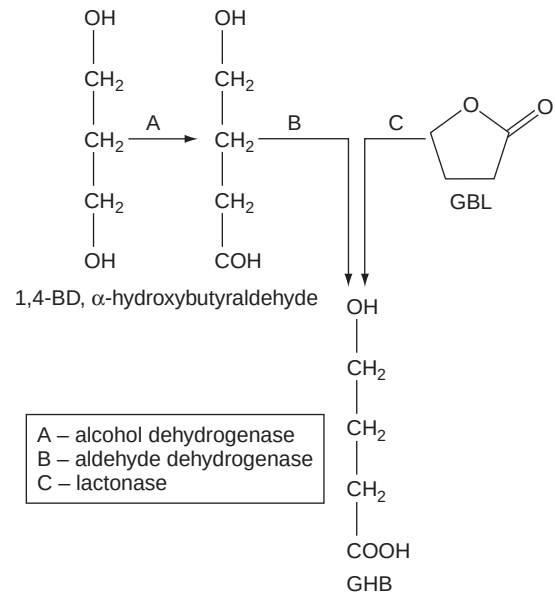

Figure 8.10 Metabolic transformation of GBL and 1,4-BD to GHB.

is relatively easy to manufacture and obtain, and is alleged to have aphrodisiac properties. Possession, sale and clandestine manufacturing of GHB are illegal in many countries including the USA, the UK and Japan. In 2003 GHB became a class C controlled drug in the UK, although it is approved for treatment of narcolepsy in the USA.

Ketamine

Ketamine has become increasingly popular as a drug of abuse in the 'club scene' owing to its ability to produce psychedelic effects. Ketamine is a dissociative anaesthetic that exerts potent analgesic, hypnotic and amnesic properties. These properties confer suitability on the drug for use as an anaesthetic in painful procedures where muscle relaxation is not essential, e.g. the manipulation of fractures, minor gynaecological surgery, dental procedures, changing burn dressings and cardiac catheterisation in children. Because ketamine is not a muscle relaxant, subjects under the effects of the drug do not require assisted respiration and the drug is therefore particularly useful in situations where anaesthesia has to be performed single-handedly by a surgeon with limited resources such as in disaster or battlefield conditions. Ketamine is structurally related to phencyclidine (PCP) and exerts activity at multiple sites in the brain. It acts primarily as a glutamate antagonist by non-competitive binding to the PCP receptor located in the ion channel of the *N*-methyl-D-aspartate (NMDA) receptor complex. Ketamine sterically blocks the cation channel gated by the NMDA receptor, impeding the flow of Na^+ and Ca^{2+} ions into the neuron, resulting in disruption of glutamate-mediated transmission at these sites throughout the brain. The *S*-isomer is more potent in displacing the NMDA ligand from its receptor than (*R*)-ketamine. In addition, ketamine facilitates monoamine transmission by inhibiting the reuptake of dopamine, noradrenaline (norepinephrine) and serotonin, resulting in an accumulation of these neurotransmitters in synapses. It also acts on the opiate system as an agonist at the μ-opiate receptor. When taken in low doses, ketamine may produce hallucinations and 'out-of-body' or 'near-death experiences'. A state of helplessness may be induced in which the user loses awareness of the environment and experiences severe loss of coordination, pronounced analgesia and total amnesia. Because of its ready solubility in water and alcohol, ketamine has the potential to be used in 'date rape' situations and should always be considered in the analysis of specimens from subjects claiming to have been victims of DFSA. Most street ketamine is prepared from diverted medical or veterinary preparations by heating the injectable solution to yield ketamine hydrochloride powder. Ketamine powder is most commonly taken by nasal insufflation in quantities of between 25 and 50 mg. Ketamine is readily water soluble and may also be injected, or taken orally or anally. It has an unpleasant taste and is usually mixed with a flavoured drink such as orange juice when prepared for oral consumption. The presence of ketamine in adulterated beverages may be readily demonstrated by HPLC with diode array detection – see Figure 8.6.

'Z' drugs: zopiclone, zolpidem and zaleplon

Zopiclone, zaleplon and zolpidem, known as the 'Z' drugs, are all potent, low-dose, short-acting hypnotics characterised by a fast onset of action and act in a similar way to the benzodiazepines. As with the benzodiazepines the 'Z' drugs induce drowsiness and sleep, as well as anterograde amnesia. All have very short elimination half-lives and users of these drugs are subsequently less likely to experience drowsiness and other after-effects the morning after they have been taken. The peak plasma concentrations of the 'Z' drugs are low (5 mg oral zaleplon ~10–20 μg/L; 20 mg oral zolpidem ~250 μg/L; 7.5 mg oral zopiclone ~ 60–90 μg/L). Zopiclone is extensively metabolised and undergoes *N*-demethylation, *N*-oxidation and decarboxylation in the liver. The metabolism of zaleplon is complex with the formation of a number of intermediate metabolites including desethylzaleplon prior to glucuronidation.

'Legal highs'

The term 'legal high' is used to describe a range of synthetic and plant preparations that are marketed primarily for the 'club scene' but for which, at the time they are sold, there are no legal controls relating to their sale or possession. These substance are claimed to offer users similar psychedelic and hallucinatory experiences to those of controlled drugs such as THC, methylenedioxymethylamfetamine (MDMA), methylamfetamine (MA) and phencyclidine (PCP), and may be encountered in DFSA cases. The use of the term 'legal high' implies to many who purchase these preparations that because there are no legal controls on the substance it is safe to use. This is a total misconception and many of the chemicals that have been marketed as legal highs have no legitimate use and may be orders of magnitude more potent than some of the substances that they are purported to mimic. Because of the unknown and untested properties of many of these substances, those who use them are putting their health at considerable potential risk.

It is a difficult task for legislators to control all newly introduced chemicals and their identification is difficult since there are often no control substances available for analysts to compare these newly introduced compounds against in order to ascertain their identity and composition.

Substances marketed as legal highs are wide ranging and include substances originating from herbal materials such as cathinones and *Salvia divinorum* to chemically synthesised substances such as mephedrone (4-methylmethcathinone), which was recently classified as a Class B drug under the Misuse of Drugs regulations in the UK, and the piperazines. All exert stimulant properties and many behave similarly to MDMA by also exerting enactogenic properties. The piperazines, *N*-benzylpiperazine (BZP), *m*-chlorophenylpiperazine (mCPP) act by elevating the serotonin and dopamine neurotransmitters in the brain by blocking synaptic reuptake of transmitters. Trifluoromethylphenyl-piperazine (TFMPP) acts as a substrate for dopamine and serotonin transporters, resulting in increased concentrations of serotonin and dopamine in the brain's synapses. The piperazines exert stimulant and euphoriant effects in users. Other stimulants marketed as 'legal highs' include naphthylpyrovalerone, also known as naphyrone and NRG-1.

Summary

Although much has been made of the drug-facilitated sexual assault problem in the popular media and, to some extent in the scientific literature, the true extent of the problem is not yet known. Literature surveys published to date suggest that although 'drink spiking' does occur, recreational consumption of alcohol and other drugs is the major contributor in the majority of reported cases. Numerous drug substances have properties that convey upon them the potential to be used in DFSA cases. There is, therefore, a clear need for toxicologists to keep informed about new products that might be used and to develop appropriately sensitive methods for detection of these drugs in the body fluids and/or hair of complainants.

Considerable progress has been made during recent years in the standardisation of sexual assault evidence collection protocols and devices. These efforts have been coordinated in many places by groups with representation from police, prosecutors, clinicians, victim support services, advocates and forensic laboratories. Continued education of, and communication between, those agencies involved with DFSA cases is essential, particularly with respect to the collection of appropriate evidence and dissemination of information concerning new drug findings. The DFSA committee of the Society of Forensic Toxicologists has taken an international lead in providing information for toxicologists by placing updated reports on the society's website (www.soft-tox.org). Toxicologists who might be involved with DFSA cases are encouraged to adopt the recommendations of professional groups such as SOFT and the International Association of Forensic Toxicologists (TIAFT) and to maintain current awareness of new trends by visiting the websites of these organisations on a regular basis.

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CHAPTER

9 Forensic Toxicology

MD Osselton, AC Moffat and B Widdop

> Learning toxicology is easy! It only takes 2 lessons to become an expert; each lesson takes 10 years. Irving Sunshine (1910–2006)

Introduction

This chapter is dedicated to the memory of three of the most eminent toxicologists of the twentieth century: John Jackson (1924–1984), the author of this chapter in the first and second editions of 'Clarke'; Dr Allan Curry (1925–2007); and Dr Irving Sunshine (1910–2006). The basic principles of forensic toxicology have remained unchanged since the first edition of Clarke and accordingly much of John Jackson's original chapter has been retained. The content of this chapter has been bought up to date and integrated with the remaining chapters where appropriate. The philosophical style, attention to detail and excellent science taught by these eminent toxicologists have been preserved by the editors as a tribute to them.

The science of toxicology relates to the investigation of poisons and derives its name from the Greek words *τοξικόζ or toxikon (poison)* and *λογία* or *logia (a discourse or study)*.

Paracelsus (1493–1541) is attributed with stating that 'All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy' (Amdur *et al.* 1991). Orfila (1787–1853) refined the definition of a poison as 'any substance which, when taken inwardly in a very small dose, or applied in any kind of manner to a living body, depraves the health, or entirely destroys life'. The first complete work of international importance on the subject of forensic toxicology was written by Orfila in 1813 (*Traité des poisons tirés des regnes minéral végétal et animal, ou toxicologie générale, considerée sous les rapports de la pathologie et de médecine légale*; see Orfila (1818)). It was an immediate success and won him the title of 'Father of Toxicology'. 'The chemist', said Orfila, 'horrified by the crime of homicidal poisoning, must aim to perfect the process necessary for establishing the case of poisoning in order to reveal the crime and to assist the magistrate punish the guilty'. It is interesting to note that he realised the necessity of adequate proof of identification, emphasised the importance of what we now call quality assurance (purity of standards, etc.) and anticipated the need for pharmaceutical, clinical, industrial and environmental toxicology.

The term 'forensic toxicology' covers any application of the science and study of poisons to the elucidation of questions that occur in judicial proceedings. The subject is usually associated with work for the police, the coroner and the criminal law courts. However, the analysis and identification of medicines and the maintenance of agricultural, environmental, industrial and public health legislation (to ensure clean air, pure water and safe food supplies) are all aspects of forensic toxicology, although associated with civil courts rather than criminal courts. Like the forensic toxicologist in criminal cases, analysts employed in these civil areas may at times find their work the subject of severe public scrutiny in a law court, and both groups should be aware of the strengths and limitations of each other's methodology.

During the first half of the twentieth century, accidental self-poisoning and attempted suicide cases used to fall largely within the domain of the forensic toxicologist, but as sensitive analytical methods such as ultraviolet (UV) spectrometry, gas chromatography (GC), high performance liquid chromatography (HPLC) and gas chromatography linked to mass spectrometry (GC-MS) became more widely available, such cases increasingly became the responsibility of the clinical toxicologist or the hospital biochemist. A small proportion of these cases is still referred to the forensic toxicologist, usually because of an allegation of malicious poisoning, or because the patient died under suspicious circumstances and a coroner's inquest is ordered. The preliminary analysis may have been carried out already, and close cooperation between the forensic and hospital laboratories is obviously desirable.

The rapid development of more sensitive methods in the latter part of the 20th century and since 2000 also resulted in a change to the types of samples that can now be analysed with a movement away from organs such as the liver, where large quantities of tissues were required. Improvements in analytical capabilities also enabled toxicologists to analyse alternative samples such as oral fluid (Chapter 18), eye fluid, sweat (Chapter 28) and hair (Chapter 19), thus widening the scope of analyses that toxicologists can offer to their customers. What follows is an account of the principles, methodology and special problems encountered in forensic toxicology for all workers in these related fields, and for chemists and pharmacists in more remote areas of the world who may be faced with the request 'test for poisons'.

Principles of forensic toxicology

The forensic toxicologist is expected to detect and identify poisons, but if 'poison' is defined as a chemical substance harmful to living organisms, it is obvious that 'harmfulness' is not a property that can be measured by any chemical method of analysis. Toxicity is a biological concept, usually determined by some form of bioassay, but bioassays are hardly ever used now in forensic toxicology. Chemical analyses are used to detect the presence of the poison, measure its concentration and relate this to its known toxicity. If the poison is not specified by name, the request to 'test for poisons' becomes a major problem for the chemist, because no single chemical method of analysis is able to detect all the various poisons. At least seven different analytical schemes are required to exclude even the most commonly encountered poisons (Figure 9.1). Compared with toxicologists in academic research or industry, the task of the forensic toxicologist is made more difficult because the analytical material, the available time and the resources are all severely limited. The forensic toxicologist has scarcely any control over the sampling time or the selection of material submitted for analysis, and has no certain knowledge that a poison is present. Dr Alan Curry was renowned for saying 'if you do not look for it you will not find it'.

Forensic toxicology demands an overall analytical system designed to exclude or indicate the presence of any poison in each of the chemical groups shown in Figure 9.1. Most of the numerous screening procedures reported in the literature are too limited to permit a confident negative report. All too often these procedures are drug oriented, even though many criminal poisonings result from compounds other than drugs. For example, Trestail (2000) estimated that out of 679 documented classical homicidal poisonings, 31% resulted from arsenic, 9% from cyanide, with only 6% from strychnine and 2% from morphine.

Apart from these analytical problems, the legal aspect of the work demands a scrupulous attention to detail. Failure to make full descriptive notes on the items received, a simple error in the date on which the analysis was performed, or neglecting to record weighings or to check

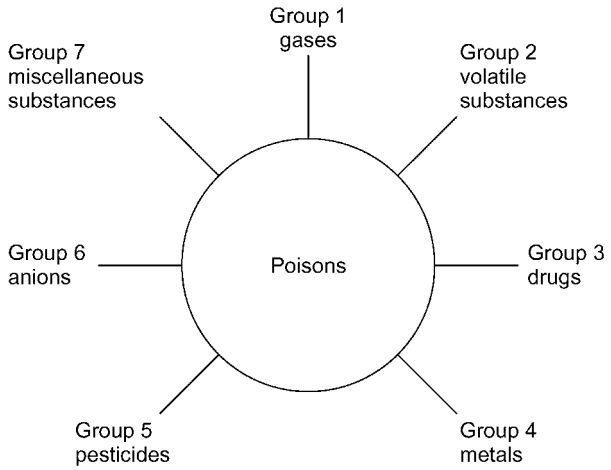

Figure 9.1 The seven major groups of poisons.

one's calculations or reagent purity can become evidence of careless work in the hands of an astute lawyer. The lawyer may, with justification, explore the extent of the toxicologist's experience and knowledge, demand a detailed account of the analytical methods and challenge the integrity of any opinion. The crucial evidence of identification and quantification of the poison may be faultless and the conclusions may be correct, but if the court's confidence in the forensic toxicologist as an unbiased scientific expert is destroyed, the case may be lost. A secure chain of custody of all the exhibits submitted also has to be demonstrated.

Orfila was well acquainted with this aspect of forensic toxicology, and the guiding principles he established nearly 200 years ago are still applicable. These may be summarised as follows:

- All chemists who undertake this work must have toxicological experience.
- The analyst must be given a complete case history that contains all the information available.
- All the evidential material, suitably labelled and sealed in clean containers, must be submitted and examined.
- All the known identification tests should be applied and adequate notes made at the time.
- All the necessary reagents used for these tests should be pure, and blank tests should be performed to establish this fact.
- All tests should be repeated, and compared with control samples to which the indicated poison has been added.

Strict adherence to these principles makes forensic toxicology one of the slowest and most expensive forms of analysis. However, this must be accepted not only to ensure justice for the alleged poisoning victim and for the accused, but also to protect the integrity and reputation of the analyst and the laboratory that he or she represents. In today's economic climate, where police or other customers are required to pay for analysis and where competitive tendering for suppliers of laboratory services is becoming commonplace, the toxicologist is often limited by what a prospective customer is willing to pay for and must therefore be cautious that the scope and quality of his work are not compromised. If such restrictions are imposed on the toxicologist, the case notes should be amended to reflect customer instructions. A comparison of the principles above with the modern requirements of quality control and quality assurance may be made by reference to Chapters 21 and 22.

Range of cases submitted

Historically, forensic toxicology gained notoriety because the cases that caught the public eye were invariably associated with allegations of murder – e.g. the cases of Dr William Pritchard (antimony; 1865), Mary Ann Cotton (arsenic; 1873), Eugene Chantrelle (opium; 1878), Dr Lamson (aconitine; 1882), Florence Maybrick (arsenic; 1884), Adelaide Bartlett (arsenic; 1886), Roland Molineux (mercury cyanide; 1899), Hawley Harvey Crippen (hyoscine; 1910), Donald Griggs (arsenic; 1928), John Christie (carbon monoxide; 1953), Kenneth Barlow (insulin; 1957), Graham Frederick Young (thallium; 1971), Harold Shipman (diamorphine; 1999) to name but a few. More details of historically interesting cases are provided in the Further reading list at the end of this chapter. The birth of forensic toxicology as a profession arose as a consequence of the widespread use of arsenic as a poison in the early nineteenth century during the Victorian era and much has to be attributed to the work of the early pioneers Mathiue Orfila (1787–1853), Robert Christison (1797–1882), James Marsh (1794–1846), Hugo Reinsch (1841) and Alfred Swaine Taylor (1806–1880), who were the founding fathers of this new field of medical science. During the early nineteenth century analytical techniques used in toxicology were limited to colour tests, crystal tests and taste tests. The twentieth century saw the advent of chromatography and mass spectrometry, although it was not until the introduction of the microchip that the boundaries of analytical toxicology suddenly opened up, permitting mass spectrometry to become the daily workhorse in most forensic toxicology laboratories (Figure 9.2). The scope of work undertaken by forensic toxicologists has since broadened and, in addition to the investigation of sudden deaths and cases of alleged criminal poisoning, forensic toxicology now extends to areas such as driving under the influence of drugs and alcohol (Chapters 4 and 5), drug-facilitated sexual assault (Chapter 8) and workplace drug testing (Chapter 3).

The ultimate objective of any forensic scientist is to attempt to provide answers to questions that may arise during coronial, criminal or tribunal investigations or in subsequent court proceedings. In cases

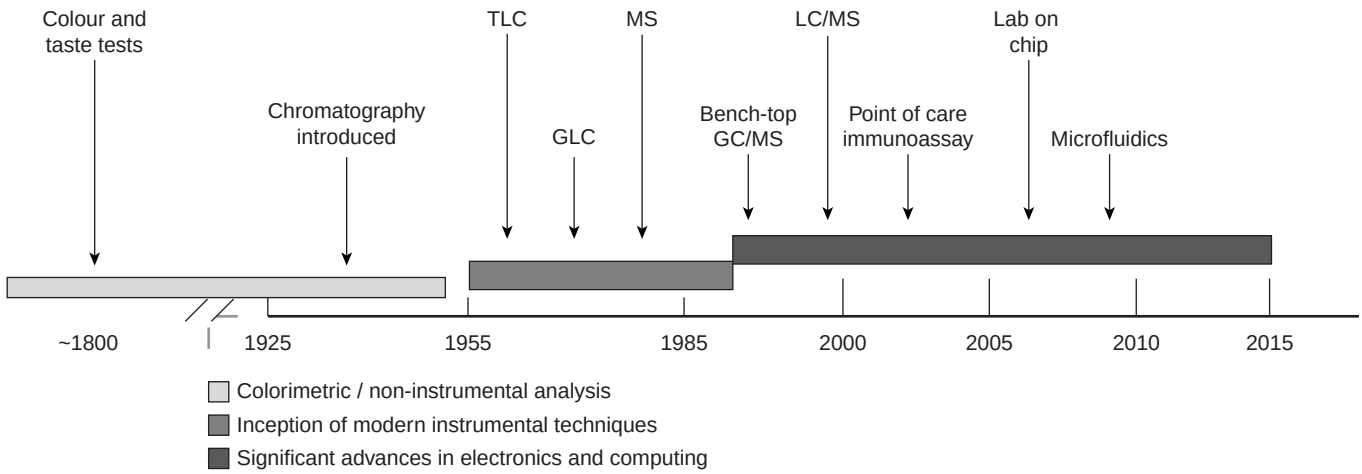

Figure 9.2 Landmarks in drug and poison analysis. (Reproduced with permission of MD Osselton.)

associated with criminal and coronial investigations the traditional question that must be answered is 'Has this person been poisoned?', together with the supplementary queries that follow if the result is positive, such as 'What is the identity of the poison?', 'How was it administered?', 'What are its effects?' and 'Was it a dangerous or lethal amount?'. Whereas early forensic toxicologists were largely employed as analysts, interpretation of results has become a firmly established part of their role in all types of cases and today's toxicologist consequently requires in-depth knowledge of analytical chemistry, pharmacology, pharmacokinetics and biochemistry.

Laws that govern the possession and use of narcotic and stimulant drugs, and legislation concerned with the influence of drink or drugs on driving skills, have increased the workload of many forensic laboratories. The widespread recreational use of psychoactive drugs has also led to a significant increase in allegations of drug-related sexual offences. In cases associated with drug use and the workplace, the principal challenges will be related to the analytical findings in relation to the workplace drug policy adopted by an employer (Chapter 3). These will often be focused around the screening and confirmation cut-offs, whether an analytical result may have been affected by attempts to dilute or adulterate the specimen submitted for analysis, and the analytical methods employed by the laboratory. Workplace drug testing, although not traditionally regarded as forensic work, has now become accepted as an area of forensic toxicology and has seen significant growth.

Modern analytical methods can give the forensic toxicologist the ability to answer questions that previously were considered either hopeless or not worth considering because the results were so often negative. Methods that are sensitive to nanogram or picogram amounts of drugs and poisons make it worthwhile to undertake an analysis, even when a plate, cup or container has apparently no food or drink left in it.

Drugs may be detected in blood and hair at therapeutic concentrations or lower, so it is possible to obtain clues to the clinical history of the deceased, the victim or the accused, even when they are unable or unwilling to provide this information themselves. Thus, the discovery of drugs used in the treatment of epilepsy, diabetes, etc., in a blood sample taken from an unidentified body may start a new train of enquiries that leads to successful identification of the body. Similarly, allegations of doping prior to rape or robbery may be refuted or confirmed.

A now routine area of forensic toxicology concerns the analytical checking of statements made by witnesses during the course of a police inquiry. Provided that a blood or urine sample is taken within about 12 hours of an event, there is a good chance of checking the truth of statements such as 'I don't remember what happened because I was high on drugs at the time', 'I used to be an addict, but I haven't taken anything for over a year' or 'I killed him in self-defence because after taking LSD he went berserk and attacked me with a knife'.

Stains can also be examined successfully for drugs and poisons (Hammond *et al.* 1979). For example, if the victim notices a nasty taste and spits out a drink, the allegation that someone tried to poison him or her can be investigated if the stained garment is submitted for analysis.

In most cases, the results obtained in the various types of cases mentioned above can be proved conclusively, i.e. the identity of the poison can be confirmed by more than one method and it can be quantified. Even when specific identification is not feasible, an opinion as to whether the suspect is most probably telling the truth or lying can be of value to the investigator.

Case investigation

Most cases that enter a forensic toxicology laboratory start with the suspicion that a drug or poison may be present. A fatality might be an accident, suicide or murder, but a toxicological examination must be carried out to assist the investigating officer to decide which of these it might be. Often the investigating officer will not know whether any offence has been committed until the results of the forensic toxicological analyses are available, so that forming the correct questions for him to ask is vital if accurate and useful answers are to be given. Details of the circumstances that lead to the conclusion that a criminal action might have taken place must be supplied to the toxicologist so that the analyses can be planned. There is no universal test for a poison and without the provision of relevant information intuitive considerations or 'guesswork' is likely to predominate. This is not only costly and time-consuming but may also result in valuable samples being used up before the drug(s) or poison(s) present can be identified. Information concerning possible access to drugs or poisons either by the deceased or by a suspect can provide a starting point for toxicological analysis. National information relating to death or prescription statistics may also be useful to toxicologists if it is available. For example, in England and Wales about 80% of fatal overdose poisoning cases are associated with 20 or fewer poisons; hence, in drug overdose cases, and in the absence of any other clues, these substances might provide a starting point for analysis. Table 9.1 lists the drugs associated with 80% of 2668 deaths attributed to poisoning in 1980 in England and Wales. Table 9.2

Table 9.1 Drugs associated with 80% of 2668 poisoning-associated deaths in 1980 (England and Wales)[a]

Drug	*Number of cases*
Paracetamol	559
Dextropropoxyphene	348
Amylobarbital	287
Secobarbital	237
Amytriptyline	172
Aspirin	141
Pentobarbital	92
Chlormethiazole	88
Diazepam	88
Butobarbital	77
Nitrazepam	77
Dothiepin	76
Flurazepam	75
Phenobarbital	60
Imipramine	48
Orphenadrine	40
Chlorpromazine	36
Dipipanone	31
Cyclizine	27
Trimipramine	27

[a] From Osselton *et al.* (1984); see also Osselton *et al.* (1980).

Table 9.2 Drugs associated with 80% of 2928 drug-related poisoning deaths in 2008 (England and Wales)[a]

Drug	*Number of cases*
Diamorphine and morphine	897
All antidepressants	381
Methadone	378
Paracetamol	242
Cocaine	235
All benzodiazepines	230
All amfetamines	99
Tramadol	83
Dextropropoxyphene	48
Zopiclone/zolpidem	36
Aspirin	15

[a] From Wells C (2009). Deaths related to drug and poisoning in England and Wales, 2008. *Health Statistics Quarterly* 43: 48–55.

summarises the principal drugs associated with 80% of poisoning-related deaths in 2008 in England and Wales. Deaths associated with barbiturates, dextropropoxyphene and paracetamol (acetaminophen) have decreased markedly since 1980, while in 2008 there appears to be a trend associated with drugs of abuse (diamorphine, morphine, cocaine).

Knowledge of the apparent volume of distribution (V_d), the therapeutic index and the potentially lethal doses of drugs and poisons may also provide valuable assistance to the toxicologist in selecting appropriate tissues for analysis as well as suitable analytical methods. The *apparent volume of distribution* is defined as the volume of fluid that would be required to contain the amount of drug in the body if it were uniformly distributed at a concentration equal to that in the plasma. This provides an indication whether the drug is likely to be retained within the blood ($V_d < 1.0$) or distributed into tissues ($V_d > 1.0$). A list of potentially fatal doses for some common drugs and poisons is provided in Table 9.3, showing that the likely amounts of poisons ingested may vary by several orders of magnitude (grams to g $\times 10^{-12}$), so that screening methods must take this into account.

The *therapeutic index* of a drug is defined as the ratio of the toxic dose to the therapeutic dose. Hence, drugs with a low therapeutic index may require only a small increase in dose to produce toxic effects. Consideration of weight, body mass index (BMI), concomitant drug therapy, renal function or drug levels in the plasma is usually necessary before the prescribing of drugs with a low therapeutic index. As well as indicating that some therapeutic drugs are more inherently dangerous than others, knowledge of the therapeutic index can assist the analyst in selecting appropriate analytical methods. The lower the dose difference between therapeutic benefit and initiation of toxic effects, the more accurate and precise an assay must be to provide confidence in interpreting results. Drugs with low therapeutic indices include digoxin, phenytoin, warfarin, carbamazepine and ciclosporin.

Table 9.3 Likely fatal oral doses of some common drugs and poisons

Drug	*Possible oral fatal dose*
Alcohol	200 g (consumed over a short period of time)
Paracetamol	15 g
Carbon tetrachloride	10 g
Chlorate	10 g
Amylobarbital	1.5 g
Phenobarbital	1.5 g
Thallium	1 g
Amitriptyline	1 g
Codeine	800 mg
Amfetamine	200 mg
Fluoroacetate	700 mg
Dextropropoxyphene	500 mg
Amfetamine	200 mg
Cyanide	200 mg
Arsenic	200 mg
Morphine	200 mg
Strychnine	100 mg
Nicotine	80 g
Cantharadin	60 mg
Aconitine	3–6 mg
Ricin	1 mg
Botulinum toxin	50 ng
Polonium 210	1 pg

Table 9.4 The fatal/therapeutic dose ratios of some common drugs

Drug	*Fatal/therapeutic dose ratio*
Morphine	1
Cocaine	2
Digoxin	3
Strychnine	5
Amylobarbital	10
Aspirin	35
Paracetamol	70
Diazepam	100

Table 9.4 lists the fatal/therapeutic dose ratios for some common medicinal compounds. Pharmacokinetic information relating to therapeutic index and V_d is provided in the individual monographs where available.

Every case submitted should be accompanied by a form similar to that shown in Figure 9.3. This information will assist the toxicologist to use the most directly useful methods of analysis, and to interpret the results in the context of the case at a later stage.

The information required for each type of case will differ and various forms have been suggested for use and are reproduced in this book: information to be submitted with the exhibits in all cases where there is suspicion of poisoning or doping (Figure 9.3); drug-facilitated sexual assault request form (see Chapter 8); request form for toxicological analysis in hospital cases (see Chapter 1); and the postmortem toxicology request form (see Chapter 10, Figure 10.1). Every effort must be made to obtain the information requested on the relevant form. If possible, a personal consultation with the investigating officer should be arranged, either in person or by telephone. A few minutes talking with the investigating officer can save many hours, or even days, of analysis time.

Samples

It is essential that the appropriate samples be collected as soon as possible, and be correctly and informatively labelled and stored appropriately. Their acquisition, storage and transport to the laboratory should be documented adequately (with dates and times) to ensure a safe chain of custody. A list of suggested postmortem samples for routine toxicological screening is given in Chapter 10, those for drug-facilitated sexual assault in Chapter 8 and those for particular anions and metals in Chapter 17.

The containers used for the samples may vary depending on the analysis to be performed, and it is vital that the correct types are used. For example, a fluoride oxalate sample tube for blood is useless if a fluoride estimation is required and gamma-hydroxybutyrate (GHB) may be destroyed by citrate. Blood requiring analysis for alcohol should ideally contain fluoride as a preservative at a concentration of at least 1.5% and ideally 2% in the final sample volume. Glass containers with gas-tight seals should be used for collection of samples requiring analysis of volatile substances. If containers are to be examined for fingerprints, this should be carried out before any toxicological examination. In cases where the tissue may also be required for DNA analysis it is essential that the toxicologist and biologist communicate before any analysis is undertaken in case the sequence of analysis could interfere with the work of other experts; for example, the biologist might open the only available blood container before alcohol or volatiles have been analysed.

Victim's information

Name .....
Gender .....
Age .....
Weight or height .....
Nationality .....
Recent foreign travel? .....
Occupation (details of end-product of factory or firm) .....

Medical history

Did victim suffer from viral hepatitis or any other infectious disease? .....
Any recent illness or chronic disease? What drugs were prescribed? .....
Was victim an alcoholic, drug addict or smoker? .....
What poison is suspected? How much? (tablet bottles, syringes, etc., found near the body should be submitted) .....
Give names of any drugs or poisons to which victim or associates had access (apart from any mentioned above) .....

Timings

Date and time victim last seen to have been in normal health .....
Date and time of illness or death, and where victim found (e.g. at work, in bed, outdoors) .....
If these times are not known, when was the victim found? .....
Time and details of last meal .....

Treatment

Any medical attention given after the suspected poisoning or doping? .....
Time of hospital admission. Date and time discharged .....
Details of any treatment given (volume of stomach wash, time when blood/urine samples taken) .....
Hospital analysis: supplied/not done/not available .....
Tick any of the following symptoms that apply:

Diarrhoea	☐	vomiting	☐	thirst	☐	blindness	☐	constipation	☐	cyanosis (blue tinge to skin)	☐
jaundice	☐	loss of weight	☐	shivering	☐	convulsions	☐	eye pupils dilated	☐	eye pupils constricted	☐
delirium	☐	coma	☐	sweating	☐	renal failure	☐				

Name of pathologist or doctor .....
Autopsy report sent: Yes/No
Date of autopsy and possible cause of death .....
Any further information that could be useful to the laboratory, such as victim pregnant, details of suspect (especially occupation and end-product of factory or firm, comments made by victim, witnesses or suspect) .....

Samples required

If victim alive:
Vomit, stomach aspirate or wash, blood and urine

If victim dead:
Stomach contents. All available (no preservative); enquire if stomach wash or vomit is available. If no contents, submit stomach

Blood

Femoral vein	30 mL unpreserved and 5 mL preserved (for alcohol analysis); identify source; do not mix
Heart	All available from intact heart chambers; avoid body cavity samples; enquire if antemortem samples are available
Urine	All available, however small a volume (preserved); enquire if antemortem samples are available. If no urine, submit kidney and bile
Liver	250 g; gall bladder should not be included with this sample

If the suspected poison is a volatile substance, brain and lungs will be required. Bone and hair are needed if metal poisoning is suspected. Brain should always be submitted if the body is decomposed.
If in doubt, consult the laboratory.
Glass jars should be used whenever possible. Samples should be properly labelled and sealed. The label should include the name of the victim, signature of pathologist and the date. Antemortem samples should also specify time of sampling.

Figure 9.3 Information to be submitted with the exhibits in all cases in which there is suspicion of poisoning or doping.

Analytical approaches

General methodology

The forensic toxicologist should remember Orfila's maxim: 'The presence of a poison must be proved in the blood and organs before it can be considered as a cause of death.' There are five stages in any toxicological examination:

1. Detection – to detect any drugs or poisons in the samples submitted by means of screening procedures.
2. Identification – to identify conclusively any drugs, metabolites or poisons present by means of specific relevant physicochemical tests.
3. Use of corroborative tests, where available, to confirm the identify and amount of any substance found.
4. Quantification – to quantify accurately those drugs, metabolites or poisons present.
5. Interpretation and reporting – to interpret and report the analytical findings in (2) and (4) in the context of the case, the information given and the questions asked by the investigating officer.

The detection of the drug or poison is the most difficult part, since the nature of the poison may not be known. Screening tests for any possible drugs or poisons should be used where there is no information about the possible identity. Immunoassays are available for screening blood, urine and vitreous samples for a wide range of drug substances (Chapter 31). Immunoassays provide a sensitive means of testing for a wide range of drugs in small samples. However, it is important to ensure that appropriate 'cut-off' concentrations are selected for the types of cases under investigation. For example, the use of workplace drug testing cut-offs in drug-facilitated sexual assault cases would be inappropriate and could result in many potential drugs being undetected.

General screening methods are usually more flexible than special methods and can therefore be applied to a wide variety of materials. They are essential for the investigation of unknown poisonings and have some advantages even when the toxic agent is known or suspected. The bulk of this chapter gives the philosophy of applying such tests to the seven classes of poisons shown in Figure 9.1. Since the last edition of this book was published there has been growing awareness among toxicologists concerning bioterrorism and the use of chemical and radioactive threats against individuals or sectors of society. The notable case of Alexander Litvinenko (see http://archive.student.bmj.com/issues/07/09/education/324.php), who was poisoned with polonium in London in November 2006, the sarin gas attack on the Tokyo subway system (March 1995) and the anthrax attacks in the USA (in 2001) exemplify the need for toxicologists to maintain an open mind when dealing with the 'unknown' types of cases. Consideration should also be given to the growth in international travel for holidays and business since this opens the opportunity for the importation from different parts of the world of drugs or poisons that are not normally available in the home country.

Once a toxic agent has been detected, specific analytical procedures can be used to identify it conclusively. Such procedures are given in Chapters 1–19 in this book. Each of these chapters looks at the analysis of drugs and poisons from a different analytical standpoint, which depends on the area of toxicology being described. Particular techniques to be used in such analyses are given in Chapters 30–44, and the individual monographs of drugs and poisons in Part 2 give further physicochemical information to be used for identification, such as for mass spectrometric data. Methods for the quantification of groups of drugs and individual drugs and poisons are also given in Chapters 1–19 and each monograph also contains references to established quantitative methods in various tissues using a variety of techniques. The interpretation of the results is explained in Chapter 26 and use of the disposition and toxicity data, etc., given in the relevant monographs in Part 2.

General guidance

Analyses may be required to answer specific questions concerning, for instance, the amount of alcohol in blood. Alternatively, the enquiry may be more general, such as 'Was this food poisoned?'. This latter type of case is termed a 'general unknown' (i.e. those cases in which the analyst knows nothing about the nature of the poison or, indeed, whether any is present). All seven groups of poisons shown in Figure 9.1 should be excluded before a negative report can be given. Fortunately, group tests are available for five of these groups (i.e. gases, volatile compounds, drugs, metals and pesticides). Specific tests must be used to detect anions and miscellaneous compounds. Obviously, the choice of which group should be eliminated first is critical if a rapid answer is expected.

Most forensic toxicology cases involve substances that act on the central nervous system (e.g. alcohol, hypnotics, narcotics, sedatives, or tranquillisers). However, it may be that these are the most commonly identified poisons because they are sought more often than other toxic substances and because they are the poisons for which most comprehensive screening systems have been published. There are inherent dangers in this pragmatic approach because there is no differentiation between self-poisonings and criminal poisonings. On the other hand, a completely systematic and comprehensive analytical scheme that covers all the poison groups is extremely time-consuming and costly. Much will depend on the requirements of the investigating officer.

The first essential task of the analyst must be to ensure that the so-called 'general unknown' case is an authentic unknown poisoning. Only a small proportion of submitted cases have no guiding features; these cases consist mainly of unidentified drowned, burnt or decomposed bodies, or of the submission of some untasted, but allegedly poisoned, food samples. In most cases there are some facts available that provide clues to the most likely group of poisons to be excluded as a first action. Thus, if the time of onset of the symptoms was less than 1 hour, and there is no evidence of injected material, the sample should be examined for gases and volatile poisons before other groups. Severe vomiting and diarrhoea indicate possible metal poisoning, although food poisoning or too much alcohol should be ruled out.

The age and occupation of the victim or the site of the incident may guide the choice of the first group screen to be employed. For instance, few elderly people indulge in solvent abuse; farm workers have easier access to the more toxic pesticides; and a death in an electroplating factory certainly requires cyanide to be eliminated. These facts are not always freely available and therefore must be sought.

It is impossible to design a single analytical scheme that is both able to detect all the drugs and poisons now available and suitable for all the various purposes mentioned above. The forensic toxicologist needs a repertoire of standard methods that can be modified according to the nature of the investigation, the type and amount of material for analysis, and the time and resources available. This collection of standard methods should include about a dozen general screening methods and as many special methods as can be acquired and practised.

Special methods detect, or exclude, only a limited number of related chemical compounds. Their more limited purpose means that they can be made more efficient, rapid and selective than general methods, but in certain circumstances they waste both time and material. The temptation to screen for poisons by employing a whole series of special methods should be resisted. Some of the specialised methods designed to detect a particular poison or a chemically related group of drugs (e.g. benzodiazepines or phenothiazines) are not as selective as they purport to be. This is because the extraction procedures have been simplified for rapid processing, because they utilise a non-specific identification technique or, more frequently, because they are designed to quantify the drug rather than identify it. For example, many methods based on a colour reaction only require the preparation of a protein-free filtrate. Consequently, the drug or poison is not really separated from most of the endogenous material and the only evidence of identification involves the dangerous assumption that the positive response is from the compound sought. If possible, non-selective special methods should be avoided in forensic toxicology. Unfortunately, they are often the only types that the manufacturer of the compound can supply.

Classification of poisons

Drugs and poisons can be classified alphabetically, pharmacologically (antidiabetic, anticonvulsant, etc.) or by chemical structure (barbiturates, phenothiazines, etc.). However, for analytical purposes it is more useful to classify poisons according to the method used for extraction. Five major groups are usually considered:

- Gaseous and volatile substances isolated by diffusion or distillation
- Organic non-volatile substances isolated by solvent extraction (drugs and pesticides)
- Metallic poisons isolated by ashing, by wet oxidation of the organic matter or by enzymic hydrolysis of the tissue
- Toxic anions isolated by dialysis
- Miscellaneous poisons that require immunoassays or special extraction techniques, such as ion-exchange columns, formation of derivatives or ion pairs, freeze-drying and continuous extraction with a polar solvent.

Some of these groups have been subdivided because they are too large or because alternative methods of extraction are available. The seven groups so formed are illustrated in Figure 9.1.

Most analyses require several unit operations, namely separation of the poison and its metabolites from the biological material, isolation, purification, concentration, identification, confirmation of identity and quantification. The most useful methods are those that combine two or more of these unit operations. Thus, colour tests (see Chapters 1 and 30), which can be applied to the sample directly without the need for any isolation or purification processes, are indispensable in the initial stages of an analysis (e.g. Fujiwara and Reinsch tests). Immunoassay techniques (see Chapter 31) also eliminate the need for many separate operations and, like colour tests, can provide a tentative identification and approximate quantification of the poison. However, a disadvantage of both these methods is that a negative result eliminates only a few of the possible toxic substances. Consequently, additional colour tests or immunoassays are required before that particular group of poisons can be excluded. This type of sequential testing can waste much time.

A broad-spectrum screen, able to detect or eliminate most of the poisons in a group, usually requires a combination of three or more of the available techniques. For the drug and pesticide groups, the only combination potentially able to encompass all the required steps is mass spectrometry (MS) coupled with either GC or HPLC. However, a simple, direct solvent-extraction scheme is generally employed to eliminate endogenous substances that might otherwise reduce the efficiency of the system. Completion of the following analytical probing tests will, in many cases, provide clues to the nature of the poison.

Analytical probing

Medicaments and other materials near the body

Empty, or nearly empty, tablet containers found in the home or close to a body can provide a valuable first clue as to what to look for, particularly if accompanied by a suicide note. It is a common mistake, however, to spend too much time identifying every tablet or capsule found at the scene (they may have nothing to do with the event or may have been placed there to try to draw attention from another direction). However, a quick attempt at identification before analysing body fluids can be valuable (see Chapter 13) for the identification of solid dosage forms). Other materials may include containers of household or industrial products (e.g. cleaning agents, disinfectants, gas-lighter refills, pesticides) and syringes. Their chemical content is usually obvious from the container labels and in situations where a syringe is found it makes sense to analyse any residual content.

Food or drink residues near the body

The priority given to this aspect depends on the case history, but a rapid initial examination at an early stage is often useful (see Examination of food and drink below).

Body fluids

A number of preliminary tests should be carried out to confirm or exclude the most common toxic agents.

Urine The following tests, details of which are given in Chapter 1 (Fast limited screening), should be performed on urine samples: Trinder's test for salicylates; cresol–ammonia test for paracetamol; iron(III) chloride–perchloride acid–nitric acid (FPN) reagent for phenothiazines; and Fujiwara test for trichloro compounds. In addition, the content of alcohol should be determined by GC (see Chapter 4).

Stomach contents Stomach contents should be examined visually for colour and the presence of tablet residues or excipients (often maize starch). The odour should be noted as this may indicate the presence of alcohols, aldehydes, ketones, phenols, cyanide, ethchlorvynol, nicotine, etc. Caution should be applied when smelling stomach contents in case of the presence of HCN (approximately 50% of the population are unable to smell cyanide). The following tests, details of which are given in Chapter 1 (Fast limited screening) should be performed: Trinder's test for salicylates; cresol–ammonia test for paracetamol; FPN reagent for phenothiazines; and Fujiwara test for trichloro compounds. In addition, the diphenylamine test for oxidising agents and the Reinsch test for heavy metals (Chapter 30) should be carried out and a diluted, filtered extract examined by direct ultraviolet (UV) spectrophotometry for drugs with high absorbance (A_1^1) values. A small aliquot of stomach contents may also be rapidly screened by thin-layer chromatography (TLC) following alkaline solvent extraction to indicate the presence of basic drugs or alkaloids.

Blood In many laboratories, and in the light of human tissue legislation in the UK, blood may be the principal sample submitted for toxicological screening. Tests on the blood sample should include screening, a quantification of alcohol by GC (Chapter 4), and detection and quantification of carbon monoxide by spectrophotometry (Chapter 1). Visual examination of the blood may immediately indicate carbon monoxide poisoning (bright cherry-red specimen), and brown blood as a result of methaemoglobinaemia may signify poisoning by oxidising agents such as nitrite and chlorate. Immunoassay provides a rapid and efficient way of screening for the presence of many drugs using small tissue samples. New immunoassay technologies such as the Randox biochip enable a wide range of drugs to be screened on a single drop of blood, urine, vitreous and even homogenised tissue.

The precise order in which these tests are carried out depends on the information available, but most of them need to be done at some time, unless the evidence overwhelmingly indicates a specific poison (e.g. cyanide). Even in these cases, ancillary questions may arise in court. For instance, was the victim doped or drunk prior to the administration of the fatal poison? A toxicologist should, therefore, carry out most of the above plus a routine drug screen in every case. For example, not all carbon monoxide cases are suicidal or accidental, even if the circumstantial evidence seems to indicate a non-criminal cause of death.

The flow chart in Figure 9.4 shows how the information obtained from the questionnaire and the suggested analytical probing are combined to obtain a probable identity, or at least the type of poison involved. If the analytical probing suggested above is compared with the poison group classification shown in Figure 9.1, it can be seen that every group has been checked by at least one test designed to confirm or exclude one or more of the most common toxic agents within that classification, with the exception of group 7, miscellaneous substances.

A simple drug screen, such as those in Chapter 1, of the urine and stomach contents may be undertaken as part of this probing phase. Alternatively, a more traditional multi-fractional screen can be carried out (see Chapter 10). Both approaches are valid, depending on the type of case that predominates in the laboratory. If most of the work is undertaken for the coroner and pathologist and concerns mainly the investigation of suspected suicidal overdoses, the simple drug screen is probably better. However, if a large number of antemortem investigations (i.e. non-fatal doping, poisoning, traffic and drug offences) are undertaken, the traditional multi-fraction screen is more economic of both sample and time in the long term. Moreover, there is an increasing demand for the forensic laboratory to investigate the possibility of drug involvement in murder, manslaughter and rape cases. In these cases, proof is required of ingestion of a drug rather than of the consumption of an overdose, so that a simple drug screen might not suffice.

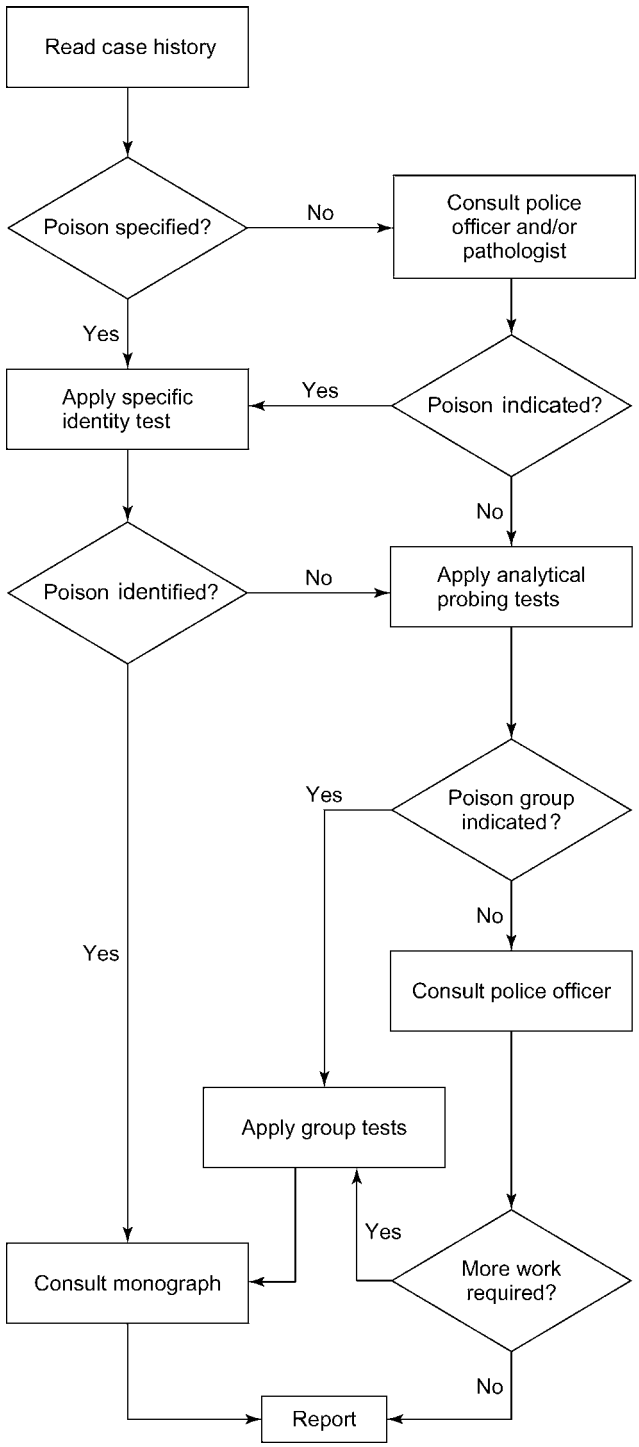

Figure 9.4 Analytical probing flow chart.

Only a small number of qualitative tests utilise the blood sample. This is because it is generally the most limited sample, its matrix is not compatible with simple tests, and it is also the most valuable for the confirmation and quantification phases of a toxicological analysis. The methods employed for drugs-driving cases (Chapter 5) may be of assistance here.

The analysis of tissue samples was formerly a prerequisite of any toxicological examination, but it is less important now provided that the laboratory has sensitive instrumental methods in routine use. However, analysis of the liver is essential when a full range of body fluids cannot be obtained, or if the detailed analysis of these fluids fails to provide an answer. For these reasons, liver should still be requested as a routine specimen in every postmortem case. It is doubtful whether the supply and analysis of a full set of body organs, advocated by many classic toxicology textbooks, can now be justified, for there is little evidence that such routines have uncovered homicidal poisonings that would otherwise have been overlooked. In general, the analysis of liver specimens need be considered only in those cases in which the circumstantial evidence conflicts strongly with the analytical results. For example, toxic concentrations of some drugs (e.g. thioridazine) in blood are not significantly higher than therapeutic concentrations. This is not generally true of liver concentrations, which, in postmortem specimens, may be over 10 times higher than those obtained from patients on therapeutic dose regimens. Liver analysis in these cases may provide a more certain indication of a fatal overdose. Liver tissue analysis is essential when analysing drugs that undergo postmortem redistribution. This term describes the movement of drugs within the body after death, resulting in postmortem blood concentrations far in excess of those present when death occurred. Major causes of this phenomenon are the release and diffusion of drugs from organs such as the liver and lungs into adjacent blood vessels and simple diffusion from gastric contents into the abdominal aorta and the iliac vein. A full discussion is given in Chapter 10 under Interpretation of postmortem toxicology results.

Tissue analysis is obviously a necessity for exhumed or decomposed bodies, or when the victim has survived for several days after ingestion of the poison. In all these cases the routine samples (stomach contents, blood and urine) may not be available or, if they are, may not produce meaningful results. In an exhumed body, thigh muscle and hair may be the most appropriate specimens to collect.

Group tests

In this section, each classified group of poisons is discussed in turn. The group tables summarise the type of circumstantial evidence usually characteristic for the majority of poisons, list possible spot or probing tests to confirm or refute the circumstantial indications, and indicate the analytical methods required for the group. A general method is suggested where this is available. Further details about specific groups of drugs and poisons are given in other chapters. The figure given under 'number of compounds to be excluded' is an estimate of the total number of poisons within that particular group.

Group 1 poisons: gases

The presence of this group of poisons is usually indicated by the circumstances of the incident (provided that they have been reported in full to the analyst). Furthermore, the identity of the actual gas involved is often indicated by the circumstantial evidence. This is fortunate, since no single-stage group test or analytical method is available for this group (Table 9.5). However, a few points need careful investigation. For instance, a portable heater may have been removed from the room where the death occurred. In one case, a devoted mother warmed her son's cold bedroom before he was awake using a defective paraffin heater, which was subsequently transferred to warm the bathroom before he took a bath. He was found dead in bed with no visible source of carbon monoxide in the room and so the laboratory was only requested to analyse for drugs. (The classic pink skin colour from carboxyhaemoglobin is not always apparent.) Fortunately, a routine test for carbon monoxide established the cause of death before too much time had been wasted looking for drugs. The analyst should also enquire whether oxygen was administered or artificial respiration was attempted before death, since these measures can bring about a significant reduction in carboxyhaemoglobin concentrations. As a result, carboxyhaemoglobin may be found in a postmortem blood sample at a non-fatal concentration, but no-one may think to inform the analyst of the first aid that had been given.

Some compounds that are gases at normal temperature and pressure can be detected by headspace GC using systems GA or GI (Chapter 40). In blood samples, system GI detects butane, ethane, methane, nitrous oxide, propane and also several of the fluorocarbons with low boiling points. In Chapter 14 a summary of the GC systems for gases and volatile compounds is given in Table 14.2 together with a description of the use of such systems.

Table 9.5 Group 1 poisons: gases

Indications	
Symptoms	Apnoea, asphyxia, dyspnoea, vomiting; pink or red skin colour (carbon monoxide or cyanide)
Onset of symptoms	Very rapid onset of illness or death
Scene	Hospitals and dental surgeries (anaesthetic gases), industrial sites, laboratories, mines
	Victims found in bathrooms, boats, caravans, cars, fires, kitchens
	Presence of fire extinguishers, gas fires, geysers, portable heaters
Occupation	Chemical industry, electroplating, fumigation, furnaces, glue factories, industrial tank cleaning, jewellery, metal treatment, mines, photography, sewers, tanneries
Additional investigations	
	Examination of equipment or vehicle
	Examination of clothing if stained or if odours are noted
	Postmortem examination of lung and brain specimens
Analysis	
Odour	Cyanide
Colour tests	Direct-reading colorimetric indicator tubes (Dräger tubes)
Group tests	Not available
General methods	Diffusion in Conway cell (cyanide), headspace GC
Most common poisons	Carbon monoxide, cyanide
Number of compounds to be excluded	30

Only two poisons in this group require routine exclusion: cyanide and carbon monoxide. The main exceptions to this general recommendation are cases that arise from incidents on industrial premises, or the use and abuse of compressed solvent fuels and aerosols. In the former case, a list of the industrial gases used or available on site should be requested. In the latter case, most of the relevant compounds are covered by headspace GC, as mentioned above.

Carbon monoxide

The detection and measurement of carbon monoxide in blood samples is necessary to investigate deaths in fires, cars, caravans, etc., and it is essential for all routine screening schemes. The forensic toxicologist infrequently undertakes scene visits and those that are most likely to require a visit tend to involve carbon monoxide accumulation linked to a blocked flue/chimney or faulty gas heating appliance. These visits often occur in the mid/late autumn when heating systems are fired up for the first time after a prolonged period of non-use over the warmer summer months and when birds have nested in the chimney stack or when an unserviced boiler is started. When visiting such a scene it is essential to have a qualified gas board or appliance engineer present as well as coloured smoke bombs. Placing a coloured smoke bomb in the fireplace or grate of a solid fuel burner will rapidly demonstrate a blocked chimney or flue. The gas appliance engineer will be able to safely inspect the appliance for evidence of faults or lack of servicing. The detection of carbon monoxide at the scene may be achieved by use of either colorimetric tests, e.g. the Dräger gas detection tubes or via electronic measurement devices that sample the atmosphere. The introduction of the catalytic converter into car exhaust systems has significantly reduced the number of carbon monoxide fatalities associated with the inhalation of vehicle exhaust fumes. The most rapid and convenient method to detect and quantify carbon monoxide in blood as a primary or secondary cause of poisoning is by a spectrophotometric method, preferably using a co-oximeter (Chapter 1) or by GC with flame ionisation detection (FID).

Cyanide

Cyanide is most frequently encountered in fire deaths or following the ingestion of sodium or potassium cyanide. In most cases where cyanide has been ingested the cyanide is self-indicating by its odour of bitter almonds. However, as previously stated, the ability among the population to detect cyanide by its odour is very variable, so a colour test is useful (Chapter 30). Ingestion of sodium nitroprusside is likely to be indicated by the presence of dark blue/black staining around the stomach wall. For blood and tissue specimens it is convenient to use quantitative methods that involve Conway micro-diffusion cells.

Group 2 poisons: volatile compounds

The rapid onset of symptoms followed by serious illness or death is the most valuable clue to the presence of this group. The probable identity of the particular poison may be indicated by the nature of the symptoms (Table 9.6). Physical evidence at the scene (bottles, residues in cups

Table 9.6 Group 2 poisons: volatile compounds

Indications	
Symptoms	Abdominal pain (especially with phenols), convulsions (especially with glycols), delirium, drunken behaviour (drowsiness, ataxia, speech and vision disturbance), jaundice (aniline, nitrobenzene), tremors, vomiting
Onset of symptoms	Rapid onset of illness or death when inhaled, slower if taken orally
Clinical history	Alcoholism; volatile substance abuse (more common in children and teenagers)
Scene	Domestic locations, hospitals and research laboratories, industrial locations
	Presence of liquor, methylated spirit or surgical spirit; glues or polishes associated with plastic bags; unusual siting of antifreeze or other domestic products
Occupation	Degreasing plants, dry cleaners, chemists, printers; manufacturers of adhesives, dyes, paints, petroleum products, plastics, polishes, perfumes, rubber
Additional investigations	
	Bottles or containers found near victim, even if empty
	Examination of clothing if stained or if odours are noted
	Antemortem examination of blood or urine samples
	Postmortem examination of lung and brain specimens; also vitreous humour, especially if the body is decomposed
Analysis	
Odour	
Colour tests	Dichromate test for ethanol, aldehydes and ketones; colorimetric indicator tube; Fujiwara test for trichloro compounds
Group tests	Headspace GC
General methods	GC
Most common poisons	Ethanol, methanol, toluene, hydrocarbons and halocarbons
Number of compounds to be excluded	120

etc., tubes of adhesives and plastic bags with characteristic odours) frequently leaves little doubt as to the nature of the intoxicant, if the material is submitted within 48 hours. If death has been delayed for several days by hospital treatment, it is imperative to obtain for analysis any antemortem samples taken on admission to hospital, no matter how small.

If several days have elapsed before the body is discovered, there is a tendency to think that analysis for volatile poisons would be futile, but in such cases it should be one of the first groups to be checked. The presence of alcohols, toluene and halogenated hydrocarbons has been shown in many such samples, although the interpretation of the detected concentrations may present problems. In all cases of suspected volatile substance involvement, it is essential that samples are collected and stored in glass containers with a gastight stopper, preferably lined with metal foil. It is also advisable to leave as little headspace as possible in the sample container. However, if the sample is to be stored in a freezer, care should be taken to leave sufficient space for expansion to avoid breakage of the tube.

Special care is needed if the samples have been frozen. Volatile poisons may be missed if the analytical material is not allowed to thaw, preferably at room temperature, before examination, although care must be taken to ensure that the container is properly sealed to avoid loss during warming.

Solvent abusers now turn their attention to a wide variety of industrial solvents. A list of products that may be abused by inhalation is given in Chapter 14. It follows that a good general screening method backed up with a large database is required to detect and identify the agent(s) involved. GC is the only way to achieve this coverage, since all other methods require the application of separate colour tests or chemical reactions for each volatile substance, although the Fujiwara test, which detects the presence of trichloro compounds remains a useful tool (Chapters 1 and 30).

Headspace GC (Chapter 40) is probably the most widely used method for the general screening of volatile poisons; systems GA or GI (Chapter 40) may be used. A comprehensive screening and identification system is given in Chapter 14, Table 14.3.

Group 3 poisons: drugs (solvent soluble)

In general, postmortem stomach contents, blood, vitreous, hair and urine samples may be analysed by immunoassay (see Chapter 31), and by the same methods as those detailed for patients' samples in Chapter 1, provided that the objective is identical, i.e. to obtain evidence of a drug overdose (Table 9.7). However, in some suspicious deaths or criminal cases, stomach contents, blood and urine samples cannot be obtained and alternative tissue samples have to be examined (Chapter 28). Most screening systems for biological fluids are modified easily by the introduction of a few additional clean-up procedures to permit the use of homogenates of liver or other tissues. There is much to be said for having a few routine extraction methods of general applicability, rather than a large number of detailed methods each designed for a specific purpose. The inherent defects of a few routine systems are soon discovered through constant use, but this is preferable to the unknown defects of a multiplicity of systems that are used rarely. Enzyme digestion allows the extension of stomach content, blood and urine extraction routines to samples of solid tissue.

Tissue analysis

Direct solvent extraction, or solid-phase extraction, before or after acidic or enzymatic digestion is the most widely used sample preparation method. The sensitive detection methods of GC and MS require only small amounts of tissue to be processed and any emulsion problems that arise are easily resolved.

Enzymatic digestion

Enzymatic digestion is usually the best choice for general screening of solid tissue (Osselton *et al.* 1978; Osselton 1979, 1981). It is simple, readily adaptable and gives a protein-free filtrate from which any drugs present may be extracted. It also gives enhanced extraction of many drugs compared with classic methods.

Table 9.7 Group 3 poisons: drugs (solvent soluble)

Indications	
Symptoms	Effects are variable, but the following information may be used as a guide:
Analgesics	Gastric irritation, haematuria, tinnitus, sweating, coma, convulsions
Opiates and synthetic narcotics	Contracted pupils, muscle twitching, slow respiration, hypotension, coma
Sedatives and hypnotics	Ataxia, slurred speech, drowsiness, stupor, coma
Stimulants and antidepressants	Dilated pupils, dry mouth, headache, tachycardia, tremors, convulsions
Onset of symptoms	Can be relatively slow unless injected (i.e. 1–48 h)
Age	Illicit drugs more common in the group 16–30 years of age; the elderly use their own prescribed drugs
Scene	Illegal lodgings, colleges, discos, clubs, residential homes
Additional investigations	
	Antemortem samples if the victim is admitted to hospital
	Postmortem examination of nasal swabs, injection marks and a control area of skin
Analysis	
Colour tests	No general tests, only selective tests for salicylates, phenothiazines, sulfonamides, etc.
Group tests	Direct solvent extraction and examination by TLC and immunoassay
	Confirmation and quantification by UV, GC, HPLC, MS
Number of compounds reported	200
Number of compounds to be excluded	>2000

A suitable procedure is as follows:

1. Macerate 10 g of liver or other tissue with 40 mL of 1 mol/L tris-(hydroxymethyl)methylamine.
2. Add 10 mg of Subtilicin A and incubate in a water bath at 50–60°C for about 1 h, with agitation.
3. Filter the digest through a small plug of glass wool to remove undissolved connective tissue.

Aliquots of this digest may be substituted for the specified biological fluid in most routine screening procedures. The filtered digest has a pH of 8.0–9.5.

This method is very useful for the analysis of sectioned injection marks. The superficial fatty skin layer is removed and the remaining muscle layer is analysed as above. If the injection was intramuscular and of recent origin, the drug concentrations should be greater than in a similar tissue sample that does not show an injection mark. If the injection was intravenous, such a distinction cannot be expected.

The method's superb ability to 'liquidise' solid tissue can be used for many purposes apart from drug analysis. The recovery of shotgun pellets and small bomb fragments in body tissues is one such application. The preparation of solutions for direct aspiration into atomic absorption spectrophotometry instruments to detect some metals is useful, as is their use in the detection of toxic anions and some pesticides.

General drug screens

A multipurpose drug screen may be applied to samples of body fluids, especially when there is a limited amount of sample. A suitable TLC screening procedure is given in Chapter 1 (Toxicological screening by thin-layer chromatography), as is a GC one (Gas chromatographic screening for drugs) and an HPLC one (HPLC screening using the systematic toxicological identification procedure).

Drug screening in non-fatal criminal cases and traffic offences

Many forensic enquiries entail analytical investigations that require detection of therapeutic drug concentrations rather than toxic concentrations. For example, in driving and sexual assault offences a screen is required for drugs that can impair memory, judgement and psychomotor activities (e.g. sedatives, tranquillisers, stimulants and narcotics). This type of analysis may also be needed to check allegations that the victim or accused is a drug addict or was under the influence of drugs at the time of the crime. The amount of blood and urine available for analysis in these cases is usually very limited and irreplaceable; additional blood or urine samples taken several days after the event are useless, although hair may be considered (Chapter 19).

Background information is of paramount importance, and the following should be requested as a matter of routine:

- Were there any drugs in the possession of the victim or the defendant?
- What was the subject's condition (e.g. drowsy, asleep, confused, agitated)?
- Did the police doctor certify impairment of driving ability?
- Was the subject prescribed any drugs by his or her own doctor and, if so, when was the last dose taken?
- Is the subject a known drug addict?

Further information on the analysis of such samples is given in Chapters 5 and 8.

Blood and urine samples should be obtained whenever possible in this type of investigation: when the blood sample is small, the chance of detecting and confirming residual traces of an antidepressant or stimulant drug may be low. If there is no urine sample, pre-screening by immunoassay methods is essential to provide an analytical guide to the nature of any drug present in the blood sample.

For forensic purposes, at least two uncorrelated methods of identification are required (e.g. HPLC and GC, HPLC and MS, or GC and MS). Immunoassay methods provide good exclusion evidence but poor confirmation of identity, although they are vital for the detection of insulin, lysergide and the cannabinoids.

When drug concentrations are liable to be low, the extraction of separate aliquots of urine or stomach contents is recommended for acidic and neutral drugs and for basic drugs (Chapter 10).

Group 4 poisons: metals

Historically, poisons in this group (Table 9.8) have been used frequently, probably because in general they are potent, tasteless, readily available and produce symptoms similar to many common diseases. Consequently, suspicion is rarely aroused until it is too late. For example, the substitution of arsenious oxide for the contents of one or two capsules of a prescribed medicine can have fatal results with all the appearance of a straightforward drug overdose suicide. A drug-screen analysis in these circumstances will result in a very perplexed analyst and much wasted time and case material.

Group 4 poisons should be checked as a matter of routine if vomiting and diarrhoea are noted as symptoms, no matter what other poisons may be suggested by medical opinion or circumstantial evidence. Simple qualitative tests should be applied first, e.g. the Reinsch test (Chapter 30). Although this test detects only seven toxic metals, it can be applied to almost any material (body fluids, homogenised tissue, food and drinks) without any elaborate preparation, and it is sensitive enough to detect toxic concentrations of the most common poisonous metals in a few minutes. However, it misses too many metals for it to be considered as a complete group-exclusion test. Another simple test is the Gutzeit test for arsenic (Chapter 30).

Table 9.8 Group 4 poisons: metals

Indications	
Symptoms	Anaemia, cramps, diarrhoea, gastric pain, hair loss (thallium and selenium), jaundice, metallic taste, paralysis, peripheral neuritis, salivation, urine retention, vomiting, weight loss
Onset of symptoms	Usually after several hours; death may occur within 24 h, but more commonly after several days (with acute rather than chronic poisoning)
Scene	Industrial locations, laboratories
Occupation	Electroplating, smelting; manufacture of agricultural chemicals, alloys, batteries, ceramics, glass, paint, petroleum products
Additional investigations	
	Antemortem examination of blood and urine
	Postmortem examination of kidney, intestinal contents, bone, hair, nails
Analysis	
Colour tests	Reinsch test, Gutzeit test
Group test	Atomic emission spectroscopy
Most common poisons	Arsenic, antimony, lead, lithium, mercury, thallium
Number of compounds to be excluded	20

A satisfactory routine metal-screening method must be able to analyse any biological material, without drastic modifications and with detection limits that provide reliable exclusion of minimum quoted toxic concentrations. Fortunately, for most metals the lower threshold of toxicity is at least an order of magnitude higher than the normal level.

The most widely used methods for the analysis of metals in biological fluids are electrochemical (anodic stripping voltammetry), electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry, inductively coupled plasma emission spectrometry and inductively coupled plasma MS. Details of the identification and quantification of the most common metal poisons by these methods are given in Chapters 17 and 43.

Group 5 poisons: pesticides (solvent soluble)

Pesticides may be classified as insecticides, herbicides, rodenticides, molluscicides and acaricides, depending on their use. They may also be from a variety of chemical classes (e.g. organophosphorus compounds, carbamates, chlorinated hydrocarbons, pyrethroids and substituted ureas). Most of the published work on the analysis of pesticides makes the assumption that the analyst knows the identity of the actual compound or that they have some knowledge of the particular type of pesticide present in the analytical sample. This is rarely true in forensic work and, until this information has been obtained, many excellent published methods are not suitable. Indications associated with pesticide poisoning are provided in Table 9.9 and Chapter 16 gives detailed information on the analysis of pesticides.

In Western countries, pesticides are not used commonly by those with suicidal tendencies. However, this is not true of poisoners; in fact many of the chemicals used for homicidal poisoning have been pesticides. Arsenic, thallium, phosphorus and strychnine preparations have been used as rodenticides, and nicotine and mercury salts as insecticides and fungicides. The criminal use of the herbicides sodium chlorate and paraquat has been relatively common. The solvent-soluble pesticides should always be considered in the forensic investigation of a malicious food poisoning, sudden illness or suspicious death.

The detection of pesticides is best accomplished by applying simple presumptive colour tests as the preliminary screening tests followed by a

Table 9.9 Group 5 poisons: pesticides (solvent soluble)

Indications	
Symptoms (the additional symptoms below may be used as a guide)	Principal features are vomiting and convulsions
Chlorinated hydrocarbons	Dizziness, headache, muscular weakness, tremors
Chlorinated phenoxyacetic acids	Burning sensation, low blood pressure (convulsions are not a main feature for these substances)
Organophosphates	Contracted pupils, salivation, sweating, dyspnoea, anoxia, cyanosis
Phenols and cresols	Fever (main symptom), thirst, sweating, anoxia, haematuria, jaundice
Onset of symptoms	Rapid (within 30 min) if product contains a petroleum solvent or is inhaled; otherwise, slow (1–6 h)
Scene	Farms and horticultural nurseries, food-processing factories, domestic premises
Occupation	Manufacture of agricultural chemicals, farm workers, gardeners, pesticide officers
Additional investigations	
	Stained clothing, vomit, drink, stomach contents
Analysis	
Visual appearance	Abnormal colours in food, drink or stomach contents (pesticides often contain dyes)
Odour	Hydrocarbon solvents or odours that are fishy, sulfide or urine-like
Colour tests	Ammonium molybdate test for phosphorus and phosphides
	Furfural test for carbamates
	Phosphorus test for organophosphorus compounds
	Dithionite test for diquat and paraquat
	Test for cholinesterase inhibitors (Chapter 16)
Group tests	Colour tests, UV spectrophotometry, GC (Chapter 16)
General methods	Solvent extraction; most pesticides extract into acid and neutral groups
Common poisons	Chlordane, dichlorophenoxyacetic acid, dinitro-*o*-cresol, lindane, paraquat, parathion
Number of compounds to be excluded	>1000

comprehensive screening procedure by means of TLC or GC. GC-MS is the recognised standard technique for confirming identity (see Chapter 16 for details).

Group 6 poisons: anions

Poisoning from this group of poisons is fairly rare in Western countries, but cases still occur. Therefore, an attempt at isolation, identification and quantification in blood and tissues should be made to assist in the interpretation of an atypical case in which symptoms and circumstances do not provide the usual clues. Systematic analysis is difficult, and individual tests for anions are often necessary. Chapters 17 and 30 describe tests for individual anions.

The toxic dose of most of the chemicals in this group is relatively large, about one or two teaspoonfuls. This may explain why, with the exception of chlorates and fluorides, they have not featured in many homicidal poisonings. However, anions are used in malicious poisoning cases, so food, drink, vomit, stomach washings, gastric aspirates and urine should always be examined for their presence. In non-fatal cases, blood samples are usually inadequate and should be reserved until the results of the work on other samples are known. Table 9.10 summarises the common features of this group.

Table 9.10 Group 6 poisons: anions

Indications	
Symptoms	Violent vomiting, diarrhoea, abdominal pain, cyanosis (methaemoglobin formed with oxidising agents), stained skin and mucosa (permanganate, oxalate, iodide and bleaching agents)
	Exceptions to these symptoms are fluoride and bromide
Onset of symptoms	Usually within 1 h, death may occur within several hours (note that the toxic dose of this group is relatively large, >10 g)
Scene	Agricultural sites (nitrate, chlorate, fluoride and fluoroacetate)
	Industrial sites (nitrite, oxalate and sulfite)
	Domestic sites, drain and lavatory cleaners (hypochlorite and persulfate), weed killers (chlorate), insect powders (fluoride)
	Laboratories
Occupation	Sewer workers, rat catchers (fluoroacetate), factory workers
Additional investigations	
	Vomit, stained clothing, cups, etc., found near victim, stomach contents or washings, antemortem blood
Analysis	
Visual evidence	Chocolate-coloured blood, blood-stained vomit or urine, crystalline residues in food and drink
Colour test	Diphenylamine test for oxidising agents, which covers about 50% of the group
Group tests	Not available
Number of compounds to be excluded	15

The diphenylamine test for oxidising agents (Chapter 30) has a sensitivity of about 1 ppm and, if negative, excludes most of the toxic anions in this group.

In non-fatal poisoning cases, the sample of food, vomit, etc., may be very limited. If this is the case, the sequential method described next can be used.

Examination of food and drink

Most malicious poisoning cases, and many extortion attempts, involve the addition of toxic substances to food or drink. The analysis of suspected poisoned food or drink presents problems different from those in the detection of drugs and poisons in body fluids. The concentration of the toxic agent is usually higher than that found in blood, urine or postmortem tissue samples, but the available material for analysis is often more limited, and of much greater variety. The routine analysis of blood and urine samples soon ensures recognition of endogenous substances (e.g. urea, tryptamine, cholesterol) and of common exogenous chemicals (e.g. caffeine and nicotine) when they occur in extracts, spectra and chromatograms. But one scarcely knows what to expect from the analysis of chicken curry or chocolate gateau. Packet labels demonstrate that even simple foodstuffs may contain several unfamiliar chemical additives (e.g. so-called E numbers, see

Indexes of Analytical Data). The parallel analysis of a purchased sample of the same food may be necessary to check that inexplicable reactions or unfamiliar UV spectra are normal or abnormal for that type of material.

Before starting the analysis, every effort should be made to obtain details of the circumstances that led to the complaint or suspicion of doping or poisoning. As mentioned above, every case must be accompanied by an enquiry form relevant to the alleged crime. Figure 9.2 gives the information required in a general poisoning or doping case, and Chapter 8 the information required in a date-rape case. This information requirement may be compared with that in a postmortem toxicology request form (Chapter 10, Figure 10.1). The information should be supported, when possible, by an interview with the police officer involved. Was the suspect material tasted? If not, what aroused suspicion? Is the victim ill? What are the symptoms? Without clues to guide the selection of tests and screening systems, the sample is a 'general unknown'.

Depending on the case history, it is sometimes useful to request a urine and a blood sample from the victims; they may have been poisoned, but not by the food or drink submitted for analysis! Alternatively, the food may contain a poison, but there may be no evidence of the poison in their blood or urine. Fake poisonings are not uncommon, especially in marital cases or extortion attempts. Another type of fake poisoning is the misguided joke of putting some obnoxious material (e.g. urine, aloes, soap, mustard) into a person's food or drink.

In date-rape cases, the victim may not remember accurately what went on prior to the alleged incident. However, it is most likely that a hypnotic or sedative drug would have been used and the analysis should concentrate on those classes of drugs most likely to have caused unconsciousness. It is recommended that a urine specimen be collected as soon as possible after a victim of suspected date rape reports the incident, even before the police surgeon arrives. The UK Forensic Science Service has issued a special 'early evidence' kit for just this purpose, which includes a container with fluoride preservative in which to collect an early urine specimen. The reason for this is that victims of date rape often report the incident several hours after it has taken place and drugs with short half-lives (e.g. γ-hydroxybutyrate) may not be detectable after approximately 10 h. Blood and urine alcohol concentrations should be measured as well as those of drugs.

About 10 g of food or 10 mL of drink (which may be all that is available) is adequate, provided that there is some guide to the nature of the poison. However, this would not be sufficient for 20 or 30 individual colour tests. A drug screen alone is not sufficient, unless circumstantial evidence indicates that the intention was to dope rather than to poison the victim. The method must utilise techniques that are non-destructive and eliminate large numbers of compounds.

The method described below is designed to obtain the maximum amount of analytical data from the minimum quantity of material. It is very flexible and can be applied to tea, coffee, milk, alcoholic drinks, sandwiches, cakes, pies, sausages and numerous other types of food. Vomit and food stains may also be examined.

Analytical procedure

Make a full description of the exhibit, noting the type of container, labels, seals, stains or identification marks on the outer surface and total volume or mass of the contents. Note the colour, odour, possible presence of injection marks, presence or absence of suspended solids or sediment, pH and whether material has a tendency to form a stable froth on shaking. Note also the type and materials comprising the suspected container; one of the authors has encountered a case in which hydrofluoric acid had been placed in a plastic aftershave bottle.

Colour

The appearance of abnormal colours may indicate the presence of inorganic pigments (e.g. copper, nickel or cobalt salts) or dyestuffs from tablets, capsules, medicines, pesticides and rodent baits, especially those that contain warfarin, reserpine, chloralose or diphenadione. Common rat-bait colours are blue, green or red, and are usually associated with oatmeal or cereal grain.

Odour

Although odour is often very characteristic, and therefore smelling an exhibit can be very revealing, there is always the inherent danger of inhaling toxic vapours and this should be borne in mind when conducting such tests. Material taken straight from the refrigerator should be warmed gently prior to examination for odour. If possible, the opinions of several colleagues should be obtained on any abnormal odour. Many poisons can be detected in this way, but the test is very subjective, and some people have a poor sense of smell (e.g. cyanide detection by odour is an inherited ability). In favourable circumstances, smell is a very sensitive test, e.g. cyanide, chloroform and toluene can be detected at about 1 ppm.

Apart from the usual characteristic odours of sulfides, aldehydes, ketones, esters, etc., the analyst should be familiar with the smell of chloral hydrate, ethchlorvynol, methylpentynol, phenelzine, thiamine, penicillamine, penicillins and other common antibiotics.

These cases can be most frustrating to the analyst, for a negative report cannot be given when there is a most obvious detectable abnormal odour, the source of which has not been identified. If the material has a distinct odour, a headspace GC screen is indicated.

Sediments and suspensions

Sediments may result from insoluble or sparingly soluble tablet or capsule excipients (e.g. talc, starch or calcium phosphate), certain poisons (e.g. arsenious oxide) or sometimes from an interaction of the added contaminant with the beverage or drink (e.g. battery acid produces a coagulation of protein material). Microscopic examination of the sediment or filtered suspension may give a clue to the nature of the material, e.g. herbal or plant material.

The presence of insoluble inorganic crystalline sediments is often caused by the addition of cleaning powders (especially when accompanied by a faint odour of bleach or ammonia). Nevertheless, an analysis for metals should be undertaken at some stage (see group 4 poisons and Chapters 17 and 43). Gelatin capsules often remain as undissolved residue in the base of cups or drinking vessels. When crystalline material can be isolated from the material under examination, X-ray diffraction and infrared (IR) spectrophotometry are valuable non-destructive techniques if they are readily available and are backed up by good spectral libraries.

Frothing

The presence of a stable froth when the sample is shaken (with water if necessary) may indicate contamination with soap or detergents. Acidify the sample, shake again and, if froth still forms, a detergent rather than soap is present. Although of low toxicity, soap, detergents and cleaning powders are frequently the cause of complaints of poisoning or adulteration.

Direct ultraviolet spectrophotometry

In poisoned food or drink samples, concentrations of 0.1–10% of the toxic agent are not uncommon, so that direct UV spectrophotometry is often used. This is in contrast to biological samples, for which prior solvent extraction is required because of the very low concentrations (<0.01%).

If a sample of food contains a foreign substance with an A^1_1 >200, and it is present at a concentration of 0.1%, the material may be diluted 50 times and still show the characteristic spectrum for that substance when examined by direct UV spectrophotometry. This is a very useful, rapid and non-destructive exclusion method for the large number of drugs, disinfectants and pesticides with high specific absorbances.

Direct UV spectrophotometry is invaluable as an initial screening technique for all alcoholic drinks, and for beverages such as tea, coffee, milk and soup. It may also be applied to solid foods (cakes, sweets, bread, etc.) and to complete meals (meat, stews, vegetables), either by application to a diluted homogenate or by examination of a copious water-wash of the food material. The latter method is preferred, as most poisoners tend to sprinkle the poison on the surface of the food rather than add it during the cooking process. A note of the mass of the material used and the volume of added water should be made to facilitate calculation of the approximate quantity of poison present.

If possible, a control sample of the particular food or drink should be examined at the same time. If the spectra obtained with the control sample are similar to those obtained with the sample, a large number of substances are excluded without loss or destruction of what might be an extremely limited amount of original material.

Colour tests

After UV spectrophotometry, the diluted liquid or washing is available for further preliminary tests, using a small aliquot for each test:

1. Apply the Fujiwara test for trichloro compounds (Chapter 1, Fast limited screening).
2. Apply the diphenylamine test for oxidising agents (Chapter 30).
3. Test for the presence of halides with silver nitrate (Chapter 17).

Warm the main bulk of the sample on a water bath; note any odour and test the sample with appropriate test papers (pH, lead acetate, silver nitrate, starch). Apply the Reinsch test for metals (Chapter 30). After the application of the Reinsch test, filter the sample and retain the solids and the filter paper. Use small aliquots of the filtrate to test for borate and phosphate.

Extraction scheme

The preliminary observations often allow the tentative identification of a poison, so that specific tests can be made. If this is not so, the extraction procedure outlined in Figure 9.5 should be applied to the filtrate obtained above. Immunoassay screening may be undertaken on a small aliquot extract. Details of the TLC systems used to screen basic nitrogenous drugs are given in Chapter 39 (systems TA, TB and TC), as are those for three TLC systems used to screen acidic and neutral drugs (systems TD, TE and TF).

Analysis of tablets and capsules

Most tablets and capsules are complex mixtures that contain a small amount of one or more drugs together with a larger amount of excipients. A combination of visual and microscopic examination, colour tests, chromatography and UV and IR spectroscopy is employed in the analysis. Chapter 13 gives the details for these examinations. If the preparation is fairly common and does not contain a complex mixture of drugs, two or three of these techniques usually succeed in establishing the identity of the tablet or indicating the nature of the drug or drugs that it contains. The first step is to make careful notes about the container and its contents.

- The container may be a tube, envelope, folded paper packet, box or bottle. Note any seals and labels. Make full notes of the information given on the label.
- For the contents, note the total number of tablets or other preparation in the container, together with an estimate of the total number that the container could hold if full. Note the shape (flat, bevelled, biconvex, etc.), whether compression coated, film coated, enteric coated or sugar coated, markings (numbers, letters, symbols or score marks), exterior and interior colour, layered and core tablets, mean mass, diameter and thickness.

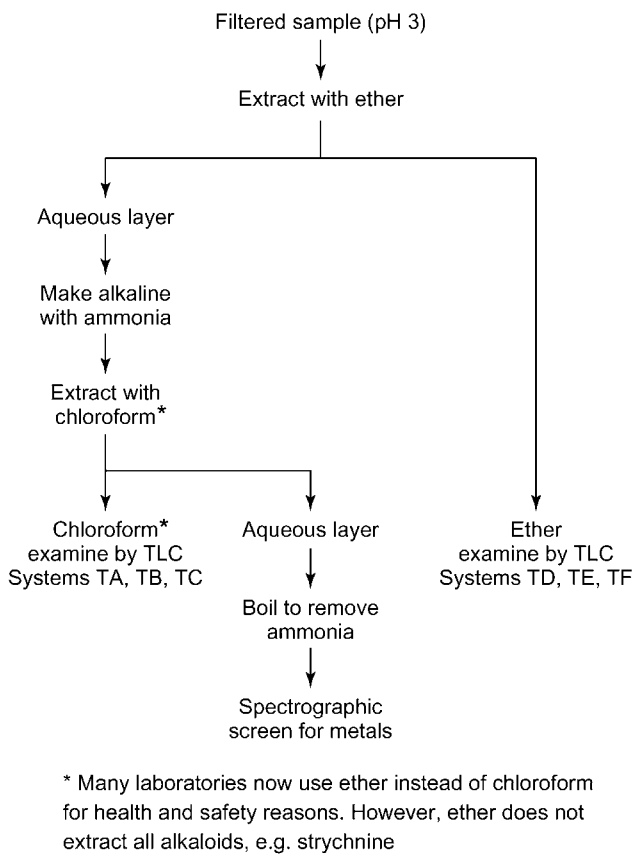

Figure 9.5 Scheme for the extraction of toxic substances in food and drink.

Visual recognition

Visual recognition can be regarded only as a useful clue; even tablets with well-known brand names may be faked, and placebo tablets are sometimes made. Chapter 13 gives methods for identifying solid dosage forms from their visual appearance. Especial care is required with capsules because their contents may be changed readily (arsenic, cyanide, strychnine and lysergide have been substituted in the past). Counterfeit preparations may occasionally be encountered (see Chapter 12, Counterfeit medicines).

Homoeopathic preparations

Homoeopathic preparations are usually round, flat–flat tablets with rough surfaces, 5–6 mm in diameter and contain mainly lactose.

Herbal remedies and health food preparations

Herbal remedies may contain active therapeutic compounds as well as some components that may be toxic. Health foods usually contain vitamins, minerals, herbal extracts, yeast, etc. Further information is given in Chapter 12, Herbal and homoeopathic products).

Sex-shop preparations

Sex-shop preparations usually contain substances reputed to be aphrodisiacs, such as vitamin E, ginseng and amyl nitrite (poppers).

Non-medicinal preparations

Non-medicinal preparations include a variety of preparations such as sweetener tablets, fertiliser tablets, stink bombs, indoor fireworks and slug pellets. Sweets may often be confused with medicinal products as they are often very similar in appearance.

`Legal highs'

A wide variety of chemicals are currently available via the internet purporting to be 'legal highs'. The term 'legal high' is used to describe substances that are designed to mimic the effects of recreational drugs such as cocaine, metamfetamine, amfetamine and MDMA but that are not controlled by legislation. The term 'herbal high' is also used, sometimes interchangeably with 'legal high' to describe products of herbal origin that are not controlled by legislation but may produce stimulant or psychotropic effects. These preparations are produced by chemists trying to stay ahead of drug control legislation and may contain a wide range of substances including synthetic cannabinoids and chemicals such as mephadrone (dimethycathinone), methylone, butylone and ephedrine, or herbal material such as 'Spice' (synthetic cannabinoids), *Salvia divinorum* and yohimbine. This is a constantly changing scene and toxicologists must ensure that they keep abreast of local developments in their country. One of the problems confronting forensic toxicologists in cases where so-called legal highs are involved is the ability to obtain reference standards. Although some reagent suppliers are responding to this need, there is inevitably a delay between a new substance becoming available and its identification and subsequent availability for use as a reference compound.

Analytical procedure

Chapter 12 gives details of the analysis of medicinal products with the identification of the active ingredient. If the identity of the drug is unknown, a screening procedure (TLC, GC, HPLC) followed by MS confirmation is often the most efficient method of analysis.

The presence of plant material indicates that the tablet is probably a herbal remedy. These are frequently very complex mixtures and are unlikely to be identified by simple methods. However, examination of an alcohol extract by UV spectrophotometry and TLC is worth attempting.

Odour

A few drugs have distinctive odours, which can provide a clue to the compound or at least to the type of preparation. A yeast-like or meaty smell may indicate a harmless vitamin preparation; an odour of peppermint may indicate a non-toxic indigestion remedy. Antibiotics, especially of the penicillin group, sometimes have a rather unpleasant sulfide-type smell. Other drugs with characteristic odours are clomethiazole, phenelzine, amitriptyline, chloral hydrate, methylpentynol and ethchlorvynol.

Size

The size of the tablet, which should be measured with callipers having a vernier reading to 0.1 mm, can provide a rough guide to the general class of preparation. Tablets of large diameter (i.e. >10 mm) are unlikely to contain potent alkaloids (except codeine); conversely, tablets with diameters less than 5 mm could not contain drugs with medicinal doses of 200 mg or more.

There is an approximate correlation between tablet diameter and the amount of drug contained in the tablet (Table 9.11), which may be used as a rough guide.

Extraction of tablets and capsules

Elaborate solvent-fractionation schemes are not usually necessary for solid dosage preparations. Most drugs are soluble in methanol and most of the excipients are not. Therefore, the preparation of a simple methanol extract is all that is required.

Thin-layer chromatography

The great majority of drugs present in pharmaceutical products are nitrogenous bases. The extract should be examined, therefore, using systems TA, TB or TC (Chapter 39). If these fail to reveal any positive reacting spots with the suggested location reagents, the possible identity of the unknown substance is reduced from a large number of basic drugs to a few hundred acidic and neutral drugs. The extract should then be examined using system TD, TE or TF (Chapter 39). Alternatively, one of the GC or HPLC screening procedures could be used, as given in Chapters 40 and 41, respectively.

Ultraviolet spectrophotometry

A portion of the methanol extract (see above) is diluted with water and examined at both acid and alkaline pH by UV spectrophotometry. The features of any resultant spectrum should be checked in the index to UV absorption data in Part 3. If the identity is suspected, the spectrum may be compared with that given in the appropriate monograph in Part 2.

Table 9.11 Relationship between tablet diameter and probable drug content

Tablet diameter (mm)	*Probable drug content (mg)*
<6	<5
<7	<15
<8	15–100
<9	30–200
<10	200–300
>11	250–500

Infrared spectroscopy

Extract a small portion of the crushed tablet or capsule contents with chloroform; to a portion of the chloroform extract add solid potassium bromide, dry under an IR lamp, prepare a compressed disc and examine by IR spectroscopy. Further details are given in Chapter 33. The features of any resultant spectrum should be checked in the index of IR peaks in Part 3. If the identity is suspected, the spectrum may be compared with that given in the appropriate monograph in Part 2.

For the majority of compounds, a mixture of 1 mg of drug with 150 mg of dry potassium bromide produces a good spectrum. It is not too difficult to obtain a rough estimate of the amount of sample needed to achieve this concentration if the product has been partially identified by other means. However, when the identity is completely unknown, it may require several attempts to obtain a disc with the correct concentration.

Mass spectrometry

Running the methanolic extract on a temperature-programmed gas chromatograph with a non-polar column connected to a mass spectrometer should detect most drugs present. Non-volatile drugs may be examined by LC–MS. The likely identity may then be found by using the index of MS data in Part 3. If the identity is suspected, the spectrum may be compared with that given in the appropriate monograph in Part 2.

Analysis of powders

The analysis of powders and similar articles suspected of containing drugs of abuse is described in Chapter 11.

Examination of syringes

A syringe found at the scene of an incident or in the possession of the victim or suspect should always be examined by the toxicologist, even if it has been stated that it is clean or empty. The usual safety measures regarding needlestick injury and possible microbial contamination should be taken.

If the syringe contains a few drops of an aqueous solution, remove a portion with a small TLC-spotting pipette and examine by the usual routine GC, GC-MS, LC-MS or TLC system. There is a high probability that a narcotic drug will be present, but amfetamine or methylphenidate may also be abused using a syringe. Wash the syringe with 1 mL of water and use this solution for UV spectrophotometry in acid and at pH 10. If a drug is indicated, a pure sample of the substance should be examined in conjunction with the original undiluted aqueous solution.

If the syringe is dry, introduce one or two drops of methanol into the syringe and proceed as described above.

The volumes of water used and any dilutions necessary for the UV examination must be noted to give a quantitative estimate of any drug present. Direct solvent extraction can be applied to the extracts and dilutions used for UV spectrophotometric analysis. This may also be necessary if the syringe is heavily blood stained. It is important to remember that some of the drugs encountered in syringes are susceptible to alkaline hydrolysis (e.g. diamorphine and methylphenidate). Thus, the addition of strong sodium hydroxide solution to the syringe should be avoided.

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CHAPTER

10 Postmortem Toxicology

G Jones

Postmortem toxicology is used to determine whether alcohol, drugs or other poisons may have caused or contributed to the death of a person. It differs fundamentally from clinical toxicology, including therapeutic drug monitoring and emergency toxicology, which is used to assist in the clinical management of a living patient. While drug analysis in clinical toxicology shares some common approaches with postmortem analysis, such as the use of immunoassay, chromatography and mass spectrometry (MS), clinical assays usually need to be modified to give acceptable results with the unique fluids and tissues encounusuatered in postmortem cases. Compared with serum or plasma, and certainly with urine, whole blood contributes a large number of endogenous compounds that, although present in serum and plasma, are at much lower concentrations (e.g. fatty acids, cholesterol and other sterols). However, it is the greater difficulty of interpreting postmortem results that principally differentiates postmortem toxicology from clinical toxicology.

Specimens and other exhibits

Request, receipt and storage

It is the responsibility of the laboratory to advise its clients (e.g. coroner, medical examiner, lawyers, pathologist) what types and amounts of specimens are required for postmortem toxicology testing, and what preservative, if any, should be used. At least one tube of whole blood preserved with a minimum of 1% sodium fluoride should be provided, to be reserved for testing for ethanol and drugs such as cocaine. Stomach contents and most tissues are usually provided unpreserved. A recommended list of specimens is given in Table 10.1.

The laboratory should provide guidelines on specimen collection and storage as well as a requisition form to be completed by the submitter, which should be sent with the specimens to the laboratory. The requisition serves five primary purposes:

1. It identifies the deceased and gives appropriate demographic information and case history (e.g. circumstances of death, relevant medical history, autopsy findings).
2. It identifies the specific specimens and exhibits submitted.
3. It provides space to identify the testing required.
4. It identifies the submitter and serves as a chain-of-custody document.
5. It provides directions and information for packaging and transport of the specimens.

Each specimen must be labelled uniquely to identify the deceased from which the specimen was obtained (i.e. name or case number), and the specimen type. Figure 10.1 gives an example of a requisition for postmortem specimen analysis.

The extent of information requested depends on the jurisdiction in which the toxicologist is working. Where most samples are transferred internally within a medical examiner facility or department of forensic medicine, a less detailed case history or autopsy summary may be required if it can be readily obtained later.

Upon receipt in the laboratory, the specimens submitted must be checked against the information given on the requisition. Where there is more than one specimen of the same type, each container should be labelled uniquely (e.g. A, B, C, ...) since, with the exceptions of urine, vitreous humour and bile, postmortem specimens are not homogeneous and different containers of the same specimen type (e.g. blood) can sometimes have different drug concentrations. Receipt of the specimens must be recorded, on paper or electronically. That log should include appropriate demographic information, an adequate description of the specimen and its site of sampling (e.g. femoral blood), the approximate volume or mass, the type of container (e.g. grey-stoppered tube; tub) and any abnormal appearance of the specimen (decomposed, heat denatured, bloody urine, etc.).

All laboratories that undertake postmortem toxicology should document the chain of custody. At a minimum, the laboratory should document what was received, from whom, by what means (by hand, courier, mail) and when. Storage of the specimens and exhibits should be secure, and access to specimens and case files should be limited to authorised laboratory personnel. Blood and other tissue specimens should be stored under refrigerated conditions between receipt at the laboratory and analysis. Where there may be a significant delay between receipt and analysis, frozen storage should be considered, especially if potentially unstable substances may be involved. A record should be kept of each occasion on which the specimen is opened to remove an aliquot. The date when specimens are discarded or returned to the submitter should also be recorded. The length of time of retention of tissues by the laboratory may be a set period (e.g. 3–12 months agreed with the client), or the time required to complete any legal proceedings.

Specimen types

The specimens available for analysis in postmortem cases may be numerous or may be limited to blood or a single tissue, depending on the case history and preferences of the submitter. In a relatively recent death, blood, vitreous humour, at least one organ tissue (usually liver) and the gastric contents are commonly collected. However, in a severely decomposed case found outdoors, muscle, hair and bone may be the only specimens available. Although toxicology testing can theoretically be performed on almost any specimen, it is usually limited to those for which there is an appropriate database available to assist with interpretation of the results. Proper collection and preservation of postmortem specimens are critical, since there is usually no opportunity to go back for re-collection of specimens at a later date as the body may well have been cremated or buried.

Table 10.1 List of recommended postmortem specimens for routine toxicology examination

Specimen	*Quantity*
Heart blood	25 mL
Peripheral blood	10 mL
Brain	50 g
Liver	50 g
Vitreous humour	All available
Bile	All available
Urine	All available up to 50 mL
Gastric contents	All available (or 100 g and record total present in stomach)

Blood

In living patients the dose of a drug is most closely correlated with its concentration in blood or plasma. Historically, therefore, blood has been used as one of the primary specimens in postmortem toxicology. In most cases postmortem blood is relatively fluid and typically has numerous small clots. Sampling can usually be achieved with a syringe and large-gauge needle.

It used to be assumed that postmortem blood was more or less homogeneous: it is not. Postmortem blood concentrations of many drugs may vary from site to site (see later). As a result, much attention has been focused on the site of collection of postmortem blood samples. A word of caution may be appropriate about specimen labelling. Samples simply labelled 'blood' may have been collected from almost anywhere in the body. Even samples labelled as 'heart blood' may not have been collected from the heart itself but drawn blind through the chest wall, and may include pleural or chest fluid, pericardial fluid and even gastric contents if the death was traumatic. On occasion, it might be collected outside the body following a traumatic accident, as pooled blood in a body bag. As the awareness of postmortem redistribution grows, great faith is being placed in the analysis of subclavian and femoral blood rather than cardiac blood. However, the toxicologist should be wary of the anatomical purity of these specimens when large volumes are supplied, and should take this into account when offering an interpretation. Unless the femoral or subclavian veins are ligated, it is very likely that some blood will be drawn from other vessels. For example, a skilled pathologist or technician can sometimes draw as much as 50 mL of blood from the femoral vein. However, the femoral vein is relatively small and it is highly likely that much of that volume will be drawn down from the larger iliac vein and inferior vena cava. It is difficult to collect more than 5–10 mL from a ligated femoral vein, unless the leg is massaged. However, massaging the leg may promote release of drug from the skeletal muscle, potentially increasing the concentration of drug in the blood specimen.

Vitreous humour

Vitreous humour has been used for many years as the preferred specimen for postmortem confirmation of the ingestion of ethanol, since

POSTMORTEM
TOXICOLOGY REQUEST

JUSTICE
Office of the Chief Medical Examiner

47601

SEE REVERSE FOR SPECIMEN AND HANDLING GUIDE

MEDICAL EXAMINER	M.E. LOCATION	NAME OF DECEDENT	SEX
PATHOLOGIST	**DATE OF EXTERNAL/AUTOPSY**	**DATE OF DEATH**	**AGE**
YOUR AUTOPSY NUMBER	**EXTERNAL/AUTOPSY LOCATION**	**PLACE OF DEATH**	**CME NUMBER**

OPERATOR OF VEHICLE/MACHINE? ☐ NO ☐ YES HISTORY OF ALCOHOL ABUSE? ☐ NO ☐ YES HISTORY OF OTHER SUBSTANCE ABUSE? ☐ NO ☐ YES

MEDICATION AVAILABLE/ PRESCRIBED:

MEDICAL HISTORY & CIRCUMSTANCES OF DEATH:

IS THERE AN ANATOMIC C.O.D. PRESENT? ☐ NO ☐ YES IS DEATH LIKELY DRUG OR SUBSTANCE RELATED? ☐ NO ☐ YES IS CAUSE PENDING TOXICOLOGY? ☐ NO ☐ YES

ANALYSIS REQUIRED: TOXICOLOGIST MAY EXTEND OR LIMIT ANALYSIS (SEE OVER...)

☐ BLOOD ALCOHOL ☐ OTHER (SPECIFY) _____ NOTE: **MINIMUM** 10 ML BLOOD REQUIRED FOR DRUG SCREEN

☐ CARBON MONOXIDE ☐ NO TESTS REQUESTED (SPECIMENS WILL BE STORED FOR 6 MONTHS)

SPECIMENS SUPPLIED KEY: C COLLECTED NC NOT COLLECTED NA NOT AVAILABLE

C NC NA URINE C NC NA LIVER ☐ ANTEMORTEM SPECIMENS (FOR DELAYED DEATHS): _____

C NC NA VITREOUS C NC NA STOMACH CONTENTS

C NC NA BILE C NC NA BLOOD: SPECIFY COLLECTION SITE: _____

CHAIN OF CUSTODY	INITIALS	DATE
MEDICAL EXAMINER/ PATHOLOGIST		
TRANSPORTED/ DELIVERED BY		
RECEIVED BY		

☐ OTHER SPECIMEN OR PARAPHERNALIA: _____

Send with specimen(s) to Toxicology

Figure 10.1 Example of a postmortem toxicology requisition from a medical examiner office. CME, Chief Medical Examiner; C.O.D., cause of death.

postmortem formation of ethanol does not occur to any significant extent in vitreous humour. Even in the presence of elevated concentrations of glucose, fermentation does not occur because the interior of the eye is a sterile medium until the most advanced stages of decomposition. For this reason, vitreous humour is particularly useful for ethanol estimation in decomposing bodies. Vitreous humour has also been used increasingly for the measurement of drugs. For example, digoxin concentrations increase markedly in postmortem cardiac blood, but do not increase significantly in vitreous humour (Vorpahl, Coe 1978). Accordingly, vitreous digoxin concentrations give a better indication of perimortem concentrations than does heart blood. It has also been shown that monoacetylmorphine and cocaine may be more stable in vitreous humour than in blood (Lin *et al.* 1997). This presumably results from the relative lack of esterases in the eye, as compared with blood. The main disadvantage of vitreous humour is its relatively small volume – about 3 mL in each eye. Another disadvantage is that there is relatively little information in the literature on the concentrations expected after therapeutic doses for most drugs. While the vitreous : blood ratio for some drugs is close to unity, it is considerably less than unity for many drugs. The concentrations of highly lipid-soluble drugs, such as benzodiazepines, are relatively low in vitreous humour compared with whole blood; concentrations of highly protein-bound drugs, such as the tricyclic antidepressants, also tend to be much lower (Evenson, Engstrand 1989; Scott, Oliver 2001). The use of vitreous humour is therefore limited by the volume available, and the difficulty of interpretation of the results for many drugs.

Urine

Urine is a useful fluid for toxicology testing as it comprises more than 99% water and contains relatively few endogenous substances that interfere with chromatography or immunoassay tests. However, there are three disadvantages with urine in postmortem work. First, urine is available in only about 50% of deaths, as it is fairly common for the bladder to be voided during the dying process. It is therefore unwise to develop an analytical protocol for postmortem testing that relies solely on the presence of urine for the detection of drugs. Second, many drugs are metabolised so extensively that the parent drug is not detected in urine, or is present only at a relatively low concentration. However, if the metabolites are searched for, urine can be a useful fluid, especially for inexpensive methods such as thin-layer chromatography (TLC), in which the metabolite patterns of some drugs, such as the tricyclic antidepressants and phenothiazines, can be diagnostic. The third disadvantage of urine is that urinary concentrations of most drugs are difficult, if not impossible, to interpret. The correlation between the concentration of drugs in urine and blood is extremely poor. The primary reason for this is that urine is not a circulating fluid, but is a waste product collected in the bladder. The concentrations of drugs and metabolites in urine therefore depend on the time of urine formation relative to sampling and drug ingestion.

Liver

While many tissues are collected and analysed in postmortem toxicology, liver is the most important. The main reasons are the large amount of tissue available, its ease of collection and the relative ease of sample preparation compared with other tissues. There is also a relatively large database of liver drug concentrations available in the literature compared with the amount of data for other tissues. Concentrations of many basic drugs are also higher in the liver than in blood, making detection easier. For example, concentrations of the tricyclic antidepressants are roughly 10–50 times greater in the liver than in the blood, partly because of the absorption of drugs from the small intestines by the hepatic portal system. Today, with more sensitive analytical methods, the majority of drugs are detected readily in the blood, and it is not necessary to rely on the liver for their detection. However, liver is an extremely valuable tissue for the analysis of drugs that undergo postmortem redistribution because concentrations in the liver are relatively stable after death. As a result of the increased stability of drugs in liver, analysis of this tissue can be a valuable aid in the interpretation of postmortem toxicology results. Liver concentrations can fall slightly after death through diffusion, although this effect is quantitatively minor (Hilberg *et al.* 1994). It has also been demonstrated that some local increases in drug concentration can occur because of postmortem diffusion of drugs from the stomach (Pounder *et al.* 1996a, 1996b). The only major disadvantage of the liver as a specimen is that it tends to be fatty and can putrefy faster than blood. It is therefore important that analytical methods incorporate some type of clean-up step and are robust enough to minimise the matrix effect of the tissue.

Stomach contents

Stomach (or gastric) contents are valuable for two primary reasons. After overdosage, drug concentrations in the stomach may be quite high, even after the majority of the drug has passed into the small intestine. Analysis of the stomach contents is uncomplicated by metabolism, so drugs that are metabolised extensively in the body may be detected unchanged. Similarly, drugs that may be difficult to detect in the blood because of extensive distribution in the body might be detected readily in the stomach. The disadvantage of stomach contents is their composition, which varies from a thin watery fluid to a semi-solid, depending on the amount and type of food present. The interpretative value of stomach contents is in confirming the consumption of an oral overdose. If the total amount of drug detected in the stomach contents is significantly greater than the prescribed dose, the possibility of drug abuse or an overdose should be considered. There are, however, two important caveats. First, stomach contents are rarely homogeneous, and therefore it is difficult to measure accurately the representative concentration of drug in the volume of stomach contents received, unless the contents are homogenised. Most chromatographic assays are based on volumes as small as 1 mL or less, and therefore the potential for sampling errors is great. The second reason for the difficulty of accurate estimation of a dose in gastric contents is that the total stomach contents are often not sent to the laboratory. Results should therefore be reported as the amount of drug present in the volume or mass of stomach contents received. Specimen collection guidelines should therefore encourage pathologists to submit the complete stomach contents rather than an aliquot.

There are two misconceptions regarding interpretation of drug concentrations in the stomach. First, the concentration (as distinct from the amount) of a substance in the stomach contents is virtually meaningless by itself. Shortly after a therapeutic dose, the concentration of a drug in the stomach may be very high, even if the total amount is not. Second, the absence of a large amount of residual drug in the stomach does not necessarily rule out an oral overdose. It may take several hours to die from a drug overdose, during which most or all of the drug could have passed from the stomach to the small intestine, or even have been largely absorbed. On the other hand, consumption of an oral overdose of medicine can result in a formation of a medicine 'mass' or bezoar in the stomach, which may take several hours or even a day or more to dissipate (Ong, Achkar 2001). High concentrations of some drugs can delay gastric emptying. It must therefore be accepted that gastric drug concentrations should never be interpreted on the same basis as those for blood. The detection of a drug or metabolite in the stomach contents does not necessarily mean that the drug was taken orally. Gastric juice is constantly being secreted into the stomach; it in turn is formed from extracellular fluid, which may contain significant amounts of basic drugs and metabolites circulating in the blood. Active back extraction of basic drugs from the circulation into the acidic environment of the stomach may also occur. It is also important to bear in mind that gastric juice may have been contaminated with bile from retching or vomiting. In overdose patients administered oral charcoal, large amounts of charcoal in the stomach lead to an underestimation of the total amount of drug present.

Other fluids, tissues and organs

Bile has been collected historically, although its usefulness is limited. Previously, bile was valuable because it contains high concentrations of drug conjugates, most notably morphine. Detection of morphine and many other drugs (e.g. benzodiazepines, colchicine and buprenorphine) is therefore more likely in the bile than in the blood, in which

concentrations may be as much as 1000 times lower. The possibility that drugs in the bile may undergo enterohepatic re-circulation should not be overlooked. With the widespread use of sensitive immunoassays and other techniques, the use of bile as a screening specimen is therefore less valuable than it once was. In addition, bile, like urine, is a waste fluid and, with the possible exception of ethanol, the correlation between blood and bile concentrations of drugs is generally poor.

Brain, kidney and spleen have been used to determine and interpret the concentrations of drugs or other toxins. Brain, and indeed other organ tissues, can be useful in assessing the overall body burden of the drug, although the database of reference values that may assist interpretation is limited. The brain offers the additional advantage that it is a relatively isolated organ and should be unaffected by trauma to the abdomen and chest, although concentrations of some drugs vary considerably from one region of the brain to another (Jones, Pounder 1987; Pounder, Jones 1990). The measurement of drugs in the brain may therefore be misleading unless the origin of the tissue analysed is identified and there is an adequate database regarding concentrations in that anatomical region.

Drug concentrations in the kidney and spleen have little intrinsic significance, other than as part of the overall assessment of the body burden of a toxin, although the kidney has been found to be useful in determining heavy metal concentrations. Spleen has been used as a secondary specimen for toxins such as carbon monoxide and cyanide that bind to haemoglobin.

Injection sites

Forensic folklore indicates that injection sites may be valuable for determining whether or not someone has been injected with a drug or poison. However, proof of intravenous injection through the analysis of excised tissue around the suspected injection site is unlikely to be convincing, because the drugs will probably be swept away rapidly by the blood circulation. Arguably, a botched injection might leave an extravascular residue. Subcutaneous injection sites offer a better chance of detection, since absorption is considerably slower. In either case, it is critical that a control site be excised, for example from the opposite side of the body. It is easily forgotten that most drugs and other toxins are distributed to virtually every tissue and fluid in the body.

Nasal swabs

Some pathologists collect intranasal samples using cotton-tipped swabs in an attempt to demonstrate nasal administration. However, the same principles apply as those for proving that a drug was injected at a particular site, and interpretation should be undertaken with caution. Using cocaine as an example, if the drug is used one would expect to find small amounts of cocaine, and certainly cocaine metabolites such as benzoylecgonine, in the nasal passage, just through normal secretions. Therefore, without a difficult quantitative assessment, the simple detection of cocaine or its metabolites in nasal swabs does not prove that cocaine was snorted.

Syringes and other items

Detection of some drugs, and particularly non-drug poisons, may be considerably easier in items found at the scene than through analysis of blood alone, and might also assist interpretation of the results. For example, residues of partially dissolved medications found in a drinking glass at a scene of death can be a strong indicator of suicidal intent. Other containers used to mix poisons prior to suicidal consumption (or homicidal administration) can also be useful to the toxicologist. Some pesticides are not detected readily in blood using routine screening procedures but can be detected much more easily in the concentrated residue in a container. Similarly, some potent drugs that are difficult to detect in blood or tissues may be detected in syringes. For example, the interpretation of blood morphine concentrations may be influenced by whether they resulted from use of morphine or diamorphine (heroin). Since diamorphine rapidly breaks down in blood, and monoacetylmorphine is only slightly more stable, proof of the use of diamorphine may depend on circumstantial evidence, such as detecting a residue in a syringe. Similarly, the finding of insulin in a syringe near a person who was not a known diabetic is useful circumstantial evidence of insulin administration. It is otherwise extremely difficult to prove hypoglycaemia in a dead person.

Antemortem specimens

Not infrequently, victims of an accident or overdose may be admitted to hospital, albeit sometimes surviving only briefly before they die. When that occurs, it is common for blood and sometimes urine and gastric contents to be collected as part of the medical evaluation and treatment. There are at least two reasons why collection and analysis of antemortem specimens can be invaluable, even if death occurs fairly soon after admission to hospital. First, analysis of hospital admission specimens gives a good idea of the circulating blood concentration at the time of admission to the hospital, which by definition is unaffected by postmortem redistribution and may provide the only reliable indicator of dosage. Second, the antemortem specimen may provide the sole opportunity to perform meaningful toxicology if the person survives long enough for alcohol or drugs to be cleared from the body prior to death or to be diminished to a concentration of limited or no forensic value. Even if blood or plasma collected on admission is not available, clearly timed specimens drawn several hours later may still be useful if allowance is made for clearance and for the presence of drugs administered as part of treatment.

There are some caveats. Blood collected for clinical purposes is usually centrifuged to separate serum or plasma for testing on clinical analysers. However, once separated, the serum or plasma may not be resealed after analysis, and this will allow ethanol and other solvents to evaporate. This should be taken into account if the sample is analysed subsequently for forensic purposes, sometimes several days later. Another problem that can occur with clinical samples is the deterioration of some drugs in unpreserved serum, most notably cocaine. Intuitively, a serum or plasma specimen collected in the casualty or emergency department might be expected to contain higher concentrations of unchanged cocaine than a postmortem blood sample collected later. However, this is often not the case, because postmortem blood is often collected in tubes that containing sodium fluoride, which inhibits cocaine hydrolysis, whereas clinical samples are typically unpreserved.

Analytical toxicology

Scope of testing

One of the reasons why postmortem toxicology is so challenging is that it can require a search for any drug or poison of toxicological significance. However, this approach, while idealistic, is not practical for any laboratory that receives many hundreds or thousands of cases each year. A more practical approach includes a search for the common drugs of abuse, prescription and non-prescription drugs, followed, as necessary, by specific analyses as indicated by the case history. Any laboratory that claims to perform general 'drug screening' should, at a minimum, have protocols that include gas chromatography (GC) and/or liquid chromatography (LC) combined with MS and that are not just limited to a panel of immunoassay assay screens (e.g. for drugs of abuse). Other substances may be included, depending on the history. Carbon monoxide should be tested for in garage-related deaths, or circumstances in which malfunction of fossil-fuel devices is a possibility (e.g. house furnace, propane-powered devices). Deaths caused by cyanide are often occupationally related (e.g. metal plating, geology, agriculture, academic chemistry staff or students).

In practice, the laboratory investigation is directed by information received from the submitter of the specimens, including basic details of the circumstances of death and the key autopsy findings. No matter how good the laboratory is, some relatively common prescription and non-prescription drugs are not readily detected by commonly used methods. Also, in some geographical regions, particularly those devoted to large-scale agriculture and with relatively limited access to drugs, deaths caused by pesticides and rodenticides may be common, which necessitates a modified approach to drug screening (see Chapter 16). The scope of laboratory testing may vary considerably with case history and is often progressive. Initial negative findings after preliminary testing

may prompt further discussions with investigators or pathologists. The death may involve drugs or other poisons that are not detected readily by the screening methods used by the laboratory.

Specimen preparation and extraction

The first stage of the analytical process involves separation of the drug or compound of interest from the biological matrix in which it is contained. Urine and other non-viscous fluid specimens do not usually require treatment prior to extraction. However, even for relatively fluid blood samples, volumetric measurement with a positive displacement pipette designed for viscous samples, or gravimetric sampling, is preferred. The use of standard glass pipettes is discouraged as being inaccurate with viscous samples. Liquid–liquid extraction or solid-phase extraction (SPE) are both appropriate procedures for extracting drugs from urine and blood. Clotted blood may be homogenised in water or buffer prior to analysis. A typical analytical scheme for the screening of drugs in blood is given in Chapter 1.

The extraction of drugs from solid tissues requires that the tissue matrix be broken down to release drugs into an environment from which they are accessible for solvent extraction or SPE. This can be achieved by homogenisation, acid or alkaline hydrolysis, or enzyme digestion. Direct solvent extraction, with or without acid or enzymatic digestion, has almost superseded the classic protein-precipitation methods. The more sensitive detection methods of GC-MS and LC-MS mean that much smaller amounts of tissue can be processed. Consequently, any emulsion problems that arise are resolved more easily than in the past, when several hundred grams of liver and large volumes of solvent were required. The use of protein-precipitation reagents, such as barium chloride, zinc sulfate and tungstic acid, is discouraged for quantitative work because a significant portion of the analyte may be co-precipitated with the coagulated protein and therefore lost to the analysis.

Homogenisation

Tissues may be homogenised in water or buffer (e.g. Tris [(hydroxymethyl)methylamine] buffer). A dilution of one part tissue plus three parts water is common and gives a homogenate sufficiently thin to be pipetted easily, although some laboratories use one part tissue to nine parts water. Use of an efficient homogeniser, such as those that have a 'probe' design that blends, shears and cuts, is preferable. Older-style Waring (food-processor style) blenders or 'stomachers' are less efficient. Tissue homogenates may be analysed without further treatment if they have been prepared with an efficient homogeniser and the assay uses a good internal standard. Care should be taken to prevent operator exposure to aerosols that might be formed during the homogenisation process.

A typical procedure is as follows. Weigh 5 g liver or other tissue, and cut into small pieces with scissors or a scalpel. Place the tissue in a suitable tube or small beaker and add 15 mL distilled water. Homogenise the tissue to a uniform consistency. For liver and most other tissues, 1–2 mL of whole homogenate can subsequently be extracted directly using the protocol shown in Figure 10.2 for basic drugs. The protocol will extract basic drugs but leave behind lipids and sterols such as cholesterol. The protocol described in Figure 10.3 may be applied for acidic and many neutral drugs. For difficult tissues, e.g. those with a large amount of connective tissue, the enzymatic digestion described later may be useful. It is important to emphasise that, for quantitative work, whole homogenate must be extracted, not just the supernatant. Drugs that are protein bound are typically present at much higher concentrations in the uncentrifuged homogenate than in the supernatant.

Enzymatic digestion of tissues

Enzymatic digestion involves the use of a robust proteolytic enzyme, such as subtilisin Carlsberg, to digest the tissue to yield an essentially aqueous matrix for extraction; it is suitable for general screening as well as for more specific analysis (Osselton 1977, 1979; Osselton *et al.* 1978). It is simple, readily adaptable and provides a protein-free filtrate from which any drugs present may be extracted. It also provides enhanced extraction of many drugs compared with the tungstate, ammonium sulfate or Stas–Otto methods.

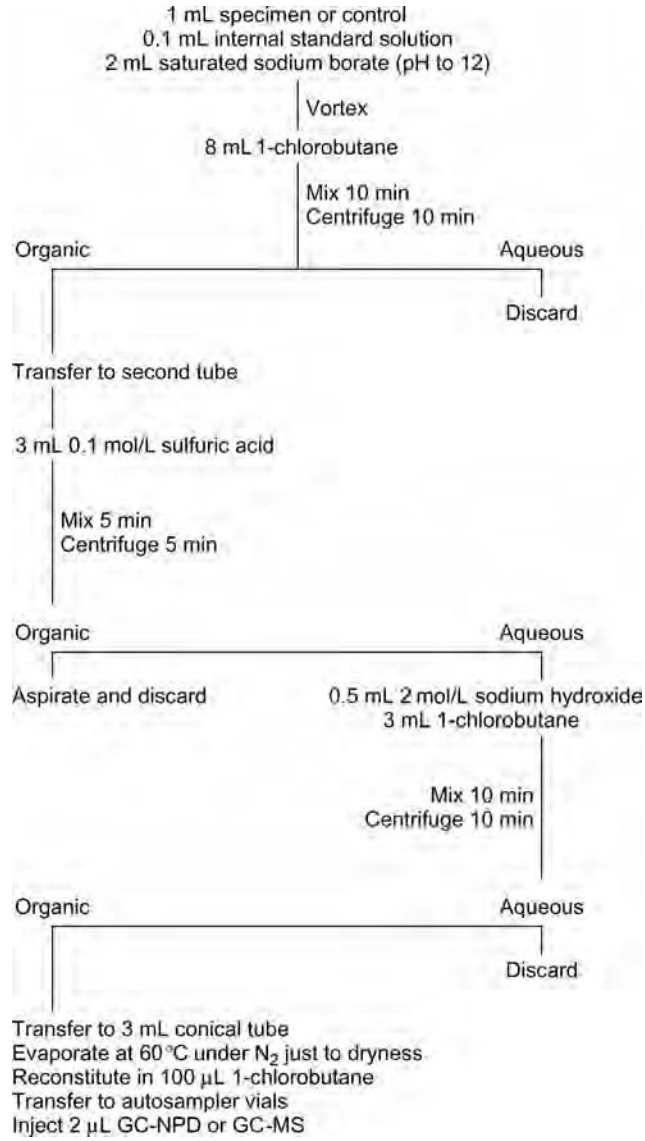

Figure 10.2 Extraction pathway for strong bases.

A suitable procedure is as follows. Macerate 10 g of liver or other tissue with 40 mL of 1 mol/L Tris; add 10 mg of subtilisin Carlsberg and incubate in a water bath at 50–60°C for about 1 h, with agitation. Filter the digest through a small plug of glass wool to remove undissolved connective tissue. Aliquots of this digest may be substituted for the specified biological fluid in most routine screening procedures. The filtered digest has a pH of 8.0–9.5.

This method is very useful for the analysis of sectioned injection marks. The superficial fatty skin layer is removed and the remaining muscle layer analysed as above. If the injection was intramuscular and of recent origin, the drug concentrations should be greater than in a similar tissue sample that does not show an injection mark. If the injection was intravenous, such a distinction cannot be expected. The method's superb ability to 'liquidise' solid tissue can be used for many purposes apart from drug analysis. The recovery of shot-gun pellets and small bomb fragments in body tissues is one such application. The preparation of solutions for direct aspiration into atomic absorption instruments for the detection of some metals has also been studied (Lock *et al.* 1981), and its use for the detection of toxic anions and some pesticides also seems feasible.

Hydrolysis of glucuronide conjugates

Drugs that form glucuronide conjugates may be hydrolysed with β-glucuronidase prior to extraction. For example, the determination

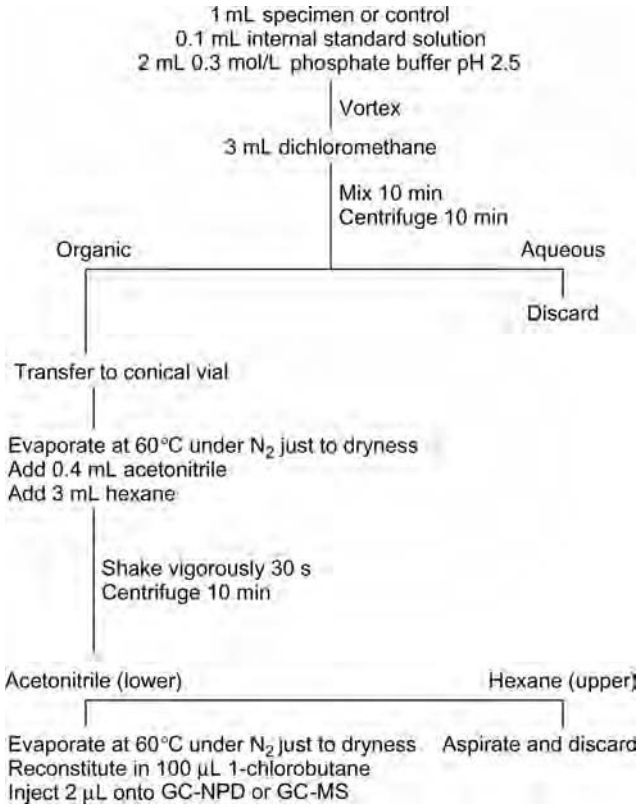

Figure 10.3 Extraction pathway for acids and neutrals.

of morphine in blood is often performed with and without the addition of β-glucuronidase to estimate the unconjugated (free) and 'total' (conjugated plus unconjugated) drug present, and therefore to aid interpretation. Other applications include enhanced detection of benzodiazepines and other drugs in blood and urine.

A typical procedure for the enzymatic hydrolysis of glucuronides is to mix 1 mL blood or urine with internal standard and 1.5 mL buffer and then to add 100 µL of β-glucuronidase obtained from *Helix pomatia*. Mix the solution and allow to incubate at 37°C overnight (~16 h). After incubation, the pH of the solution is adjusted appropriately for solvent extraction or SPE of the drugs of interest.

Acid hydrolysis may be used to cleave glucuronide conjugates; however, this method is restricted to acid-stable analytes such as morphine in urine. Acid hydrolysis of blood dramatically increases the amount of potentially interfering substances and may produce a denatured protein mass that reduces analyte recovery.

Extraction

Liquid–liquid extraction still predominates in most laboratories. The choice of an appropriate solvent is often a matter of experience or tradition. The chosen solvent should ideally extract as much of the target analyte as possible, while minimising the extraction of endogenous substances. Ideally, a solvent should extract the target analyte with an efficiency of at least 50%, and preferably much higher.

The pH of the specimen influences the extent to which acidic and basic drugs are extracted. Addition of a weakly basic buffer, such as sodium borate (pH 9), favours the extraction of weakly basic drugs, as well as most neutral substances. Similarly, the addition of an acidic buffer, such as sodium dihydrogenphosphate, favours the extraction of acidic as well as neutral drugs. The majority of drugs of forensic interest are 'basic' in character, but are often present at relatively low concentrations in blood. It is therefore desirable to have an extraction scheme that incorporates a back-extraction step to eliminate or minimise the extraction of endogenous molecules. An example extraction scheme is shown in Figure 10.2. The sodium hydroxide will force the basic drugs into the lipid-soluble unionised form, allowing extraction into the chlorobutane. The chlorobutane is transferred to a fresh tube and the drugs back extracted into sulfuric acid. Neutral or acidic substances will remain in the upper chlorobutane layer, which may be pipetted or aspirated to waste. The remaining acid layer is then made basic by addition of sodium hydroxide, and the now unionised basic drugs re-extracted with chlorobutane. The upper solvent layer may then be removed and concentrated under nitrogen, prior to analysis by a suitable chromatographic method. This extraction scheme will give extracts that are relatively free of interfering substances. However, it should be noted that morphine and other amphoteric drugs cannot be detected by this method since the phenolic functional group will ionise at high pH, therefore precluding extraction into the solvent. For amphoteric drugs, the basic phase should be less than pH 9.0, and preferably pH 8.0–8.5.

While a similar extraction scheme to that used for basic drugs (but with the additions of acid and base reversed) could be used for strongly acidic drugs, such a method does not efficiently extract weakly acidic drugs, such as the barbiturates, and neutral drugs, such as meprobamate. Conversely, simple addition of an acidic buffer to whole blood and extraction with a solvent results in the co-extraction of large amounts of endogenous lipid substances. Such extracts may be 'cleaned up' by partitioning between immiscible solvents of different polarities, such as acetonitrile and hexane, as shown in Figure 10.3. The more polar drugs tend to partition into the acetonitrile, whereas the endogenous lipids (fatty acids, sterols) tend to partition into the hexane.

SPE has been used for many years in clinical toxicology, although to a lesser extent for postmortem work. SPE usually results in better extraction efficiencies than liquid–liquid methods, especially for more polar analytes. One of the major obstacles to general acceptance in this field has been the difficult nature of postmortem specimens, which are often clotted and laden with solid material that can easily plug the fine material in SPE columns. However, improved sample preparation techniques and SPE column technology have largely overcome these problems. Use of a good internal standard, appropriate dilution of the sample and centrifugation of residual solid material usually results in a solution that does not plug the column and for which quantitative determination has not been compromised. Despite the inclusion of wash steps, the higher extraction efficiency of SPE columns compared with liquid–liquid extractions can sometimes result in dirtier extracts, although this need not be a problem if specific (e.g. MS) detection methods are used. Manufacturers of SPE columns readily provide sample extraction protocols.

Screening and detection

Postmortem toxicological analysis usually starts with a drug screen. Certainly, a drug screen can never be a single test, and most commonly is an open-ended panel of tests designed to detect the maximum number of substances of toxicological interest. This approach has often been called a search for the 'general unknown' (see Chapter 9). Careful distinction must be made between this open-ended approach and a panel or targeted approach, in which the testing protocol detects only specific substances or classes of substances (e.g. drugs of abuse). Such an approach should not be referred to as a drug screen because this misleads the reader of a report into believing that a broader range of substances has been tested for than can possibly be the case.

Targeted testing is sometimes justified where the case history strongly indicates that a specific substance is involved, particularly where that substance is not detected by the methods usually employed in the 'general unknown' approach. However, most experienced toxicologists have encountered instances in which the suspected drug was not found, with an entirely different substance detected in a clearly fatal amount. There can be several reasons for this. It may be that the person has consumed someone else's medication. Or it may be that the medications reportedly found at the scene were not those taken. In suicidal deaths, it is not uncommon for the victim or family to try to hide evidence of the overdose.

In most forensic laboratories a drug screen consists of a panel of immunoassay tests and headspace analysis for alcohol and other

volatiles, combined with one or more broad-based GC or high performance liquid chromatography (HPLC) procedures, and sometimes TLC and other techniques. The GC screening tests frequently use a nitrogen–phosphorus detector (NPD), since the vast majority of drugs contain nitrogen and therefore give a response in the detector, whereas non-nitrogenous compounds, such as fatty acids, cholesterol and other lipids, do not. Similarly, most HPLC systems use ultraviolet (UV) or diode array detectors, since the vast majority of drugs absorb light in the region between 210 and 350 nm. However, increasingly, as technology improves and prices decrease, MS is replacing NPDs and electron-capture detectors in GC and even in HPLC systems.

Forensic identification and confirmation

The forensic toxicology profession and the courts have increasingly demanded that the identification of a substance be beyond reasonable scientific doubt. The principle has long been established that forensic identification of an analyte requires the use of two techniques that employ different physical and chemical principles (SOFT/AAFS Guidelines Committee 2006). This approach has the advantage that two completely different scientific techniques are used that are supportive in arriving at a positive result. It has been argued that forensically acceptable detection and identification of an analyte can be achieved by a single extraction of a postmortem sample followed by GC-MS or LC-MS analysis of that extract. The argument is that GC-MS (or LC-MS) methods are a combination of two very different analytical methods – separation of the mixture and determination of retention time being one, and production of the mass spectrum being the other. While this approach is reasonable, it produces a forensically acceptable confirmation only if laboratory contamination and, if possible, contamination of the original specimen can be ruled out. Therefore, at a minimum, the drug should be detected using two different extracts of the same specimen. This is often accomplished incidentally, because separate extracts may be prepared for the initial drug screen and for a subsequent quantitative analysis. Even better is detection and identification of the substance in two different specimens. An example might be detection and identification in a urine specimen, followed by quantification in blood and one or more other tissues. This latter approach increases confidence in the result by ruling out a false-positive finding through contamination from glassware or, indeed, one of the specimens.

Identification of a drug by the use of two similar methods, such as two different immunoassays, is not acceptable, even though such tests may employ different endpoint reactions (e.g. fluorescence polarisation immunoassay (FPIA) and enzyme immunoassay (EIA)). The reason is that the antibodies used may have similar cross-reactivities, even though the designs of the immunoassays as a whole differ. Similarly, identification of a substance on the basis of different retention times (or different relative retention) on two different GC columns is rarely acceptable unless it can be shown clearly that the columns differ markedly in their retention and discrimination characteristics.

A caveat to this approach is simultaneous detection and quantification of ethanol and other specific analytes, such as carbon monoxide. Although the use of two independent methods may be used to identify ethanol, it is still generally accepted that a single GC method is forensically acceptable. Analysis of alcohol using two different non-correlating column-packing materials and internal standards provides much greater confidence. The quality of GC methods used in most laboratories and the fact that few other compounds are likely to be present at such a high concentration mean that erroneous identification of ethanol is unlikely. The few other compounds include methanol, isopropanol and acetone, for which separation from ethanol in the analytical system must be demonstrated. Similar arguments can be made for carbon monoxide, especially when the history clearly indicates that carbon monoxide poisoning is likely. However, in rare circumstances grossly elevated carbon monoxide concentrations may be detected where there is not an obvious source of the toxin. In such instances, confirmation by an independent technique is highly desirable (e.g. headspace GC or palladium chloride via a Conway diffusion cell).

Specific analytical techniques

The principal analytical techniques used to screen, confirm and quantify drugs that have been isolated from tissues include immunoassay, GC, HPLC, TLC and MS. All of these are described in detail in other chapters of this book and the reader is recommended to refer to these chapters as appropriate.

Quantification of drugs and other toxins

The vast majority of drugs, metabolites and other toxins are quantified by GC or HPLC, increasingly in combination with MS detection. Simple GC or HPLC detection is usually based on the total peak area produced by the detector (e.g. NPD, flame ionisation detection (FID), UV). MS quantification, while it can be based on the total ion signal, is more usually based on the peak area for specific ion fragments – called selected-ion monitoring (SIM). By its nature, SIM GC-MS quantification is considerably more specific and often more sensitive than use of the more traditional GC or HPLC detection. This is of particular importance in postmortem work, in which endogenous lipids and putrefactive products can produce significant interference. Increasingly, LC-MS and especially LC-MS(-MS) methods are being used for the quantification of drugs and metabolites in postmortem specimens. LC-MS(-MS) methods in particular can be highly specific and produce very clean chromatograms. The quantification of benzodiazepines creates many problems for some laboratories because of large differences in the blood concentrations and the thermal instability of some drugs and metabolites. Use of LC-MS (MS) allows all benzodiazepines and the major metabolites to be confirmed and quantified in a single run (Smink *et al.* 2004; Marin *et al.* 2008).

Other than detection techniques, there are two other major considerations in quantitative postmortem work. The first is the reproducibility and robustness of the extraction procedure. The second is the choice of an appropriate calibration method. Use of an appropriate assay design and sound extraction and calibration methods can minimise the effect of the matrix, especially if the calibrators are prepared in a similar matrix to that being analysed.

Calibration methods

Choice of an appropriate calibration method is critical to obtaining reliable results. Single-point calibrations are generally unacceptable for postmortem toxicology, unless it can be shown that the calibration is stable and linear over the desired range, and that appropriate controls are used to validate the calibrations when specimens are being analysed. Multipoint calibrations are preferred, unless the calibration is known to be very stable and linear (e.g. GC-FID headspace ethanol analysis). A minimum of three calibration points is usually recommended, although five or more are preferred, depending on the linearity and precision of the method. An appropriate internal standard is considered almost essential for all GC- or HPLC-based methods to help minimise matrix effects and also to correct for other variables, such as slight differences in transfer volumes when using liquid–liquid extraction or SPE. An internal standard should ideally be similar in chemical structure to the target analyte (e.g. an alkyl analogue). Use of stable isotope analogues (e.g. deuterated analogues) is becoming quite common, where available. A good internal standard can provide much greater accuracy and precision than would be possible otherwise with difficult postmortem specimens. Quantitative results are only acceptable if the analyte concentration lies within the validated calibration range. If the concentration lies outside that range, it may be reported as either greater than or less than the calibration range, as appropriate, or additional calibrators or controls could be run to validate the calibration in that range. Standard curves should not be extrapolated and, if an accurate result is required when the initial quantification lies outside the scope of the standard curve, the specimen should be diluted and re-analysed.

Ideally, an assay calibration should be linear and produce a good correlation coefficient (e.g. better than 0.98). Not infrequently the calibration line will be non-linear and may require a quadratic fit.

Some GC or HPLC assays are inherently non-linear. For example, at very high or low concentrations, ions common to both the analyte and internal standards may cause a deviation from linearity. A large deviation from linearity usually indicates the use of an inappropriate internal standard, poor chromatography or poor analyte recovery during the extraction, or that the dynamic range of the detector has been exceeded.

The method of standard addition can be useful in quantitative postmortem analysis. In essence, the specimen being analysed is used as the matrix to prepare calibrators. Multiple calibrators are prepared by adding known amounts of the analyte to tubes that contain the target specimen. An internal standard should be used; if possible, these should be deuterated analogues in MS techniques. The analyte : internal standard ratio for the sample can be read off the *y*-axis and the concentration of the analyte calculated. This method can be used with some success for difficult matrices. The concentrations of the spiked calibrators should not be too high above the anticipated concentration of the analyte since linearity of the procedure cannot be guaranteed much below the concentration of the lowest calibrator. Ideally, another person in the laboratory should also independently prepare a control (in the same sample matrix).

Method validation

It is almost universally accepted that any method used in forensic work must undergo some type of validation (see Chapter 20). However, there is considerable disagreement as to the extent of validation required. Perhaps, arguably, the extent of validation depends on the specificity and sophistication of the assay, whether the assay is in routine use and the potential consequences of producing an inaccurate result. For example, the legal consequences of a quantitative error in measuring an endogenous analyte, such as adrenaline (epinephrine), in a homicide case are obviously more serious than for, say, strychnine, which ordinarily should not be present in any amount. Similarly, to report an amfetamine analogue as present instead of a decongestant such as pseudoephedrine can have serious consequences.

Qualitative methods, such as immunoassay, should be validated for specificity and limit of detection (LOD). It is accepted that most immunoassays cross-react to some extent with analytes other than those targeted, and it is important to know the extent of that cross-reactivity, particularly for structurally related compounds. LOD is important because a class assay (e.g. opiates, amfetamines) may be far less sensitive for some drugs than for others, which allows the possibility of a false negative. Where an assay is used to analyse matrices other than those for which it was designed, appropriate validation should be performed. For GC-based and other chromatographic drug screens, it is not usually practical to determine the LOD for every analyte expected to be detected, but the LOD for representative examples can be determined. If the laboratory is asked to determine whether a particular drug is present in a specimen, the laboratory should have some idea what the sensitivity of the assay is, and that it can at least detect potentially toxic concentrations.

A quantitative assay should be validated for accuracy, precision, linearity and LOD. However, it can be argued that some assays are, by their design, self-validating. For example, if a GC-MS-SIM assay uses a good internal standard, numerous (e.g. six) matrix-matched calibrators, at least one independently prepared matrix-matched control and appropriate acceptance criteria for the calibrators, the calibration as a whole and to the control, the assay could be described as self-validating. If the assay was not accurate, the control result would be out of range. If the assay has poor precision, one or more of the calibrators would not read within an acceptable percentage when read against the calibration. Specificity can be demonstrated by the appropriate choice of ion ratio qualifiers and lack of chromatographic interference with those ion chromatograms. For some analytes, determination of LOD or limit of quantification (LOQ) is irrelevant if a cut-off is used, or where the analyte concentration is accepted only if within the valid calibration range. Demonstration of that cut-off may be satisfied if it represents the value of the lowest calibrator or control.

Quality control and quality assurance

Quality assurance (QA) deals with all aspects of laboratory practice that might influence the accuracy of the final analytical result, and is dealt with elsewhere (see Chapter 22). Quality control (QC) refers to the inclusion of material spiked with a known amount of a target analyte. The independently prepared material should be included in an assay to verify that the calibration is accurate within acceptable, defined limits. However, while the routine inclusion of proper QC material should be considered essential, the practice has not been adopted widely in postmortem toxicology testing. With the exception of some very common analytes, such as ethanol and a few therapeutic drugs, control material has not been available commercially; when it is available, it is only by custom order, tends to be relatively expensive and is often available only in serum or plasma rather than whole blood. Although time-consuming, spiking analytes into drug-free whole blood with storage at $-40^{\circ}C$ is an acceptable practice. Ideally, if the control material is prepared in-house, it should be prepared by a person other than the one running the assay, and from different stock material or at the very least from a different weighing of the same powdered stock material. It is not acceptable for an analyst to spike calibrators and then spike the same solution into separate tubes and call these the controls. Any errors made in the preparation of the standard solution or in spiking calculations would not be uncovered using this approach. It is important for quantitative work that acceptance criteria for controls be set and that they be realistic – neither too loose nor unrealistically strict. Failure to meet these criteria should invariably result in corrective action and, as necessary, repeat of the assay. Generally, controls for drugs and other toxins should read within 20% of their nominal value. For some analytes, such as ethanol, criteria such as $\pm 10\%$ or tighter are more appropriate. If a control is targeted close to the LOQ for the assay, $\pm 30\%$ may be acceptable for drugs and other toxins.

Participation in at least one suitable proficiency-test programme is another vital component of a good-quality assurance programme and goes a long way to demonstrating competence, as is the accreditation of a laboratory; these inevitably enhance the quality of postmortem toxicology analyses.

Unusual specimen matrices

One of the unique aspects of postmortem toxicology work is that often specimens are received in various states of decomposition or putrefaction. Specimens may be denatured by heat, or mummified. All of these sample types create problems. Samples that are heat denatured are probably the easiest to deal with because the lipid content and concentrations of any putrefactive amines are not much higher than those in relatively fresh postmortem specimens. Heat-denatured samples usually require homogenisation, and for accurate quantitative work may require some type of protease treatment, since a proportion of the analyte may be occluded by coagulated protein. Decomposed and mummified tissues probably create the biggest challenge, because the presence of high concentrations of lipids and putrefactive amines may obscure or interfere with the detection or accurate quantification of target analytes. Even if there is no obvious interference using a specific MS method, there may be a sufficient matrix effect to influence quantitative measurement adversely. Overcoming the effects of specimen decomposition is very difficult and often has limited success. Finding a matrix-matched sample to act as a blank for the preparation of calibrators or controls is difficult, because samples vary tremendously in the nature or extent of decomposition. The quantitative determination of analytes in decomposed or other deteriorated samples is invariably less accurate than that in fresher samples. Robust, well-validated methods inevitably produce more reliable results than those that are not.

Other matrices, such as bone, nails and hair, have been analysed successfully for a variety of substances (Drummer 2008; Garside 2008; Kintz 2008). As with any other matrix, the appropriate use of internal standards (at least for chromatographic assays) and calibrators is important. The more difficult issue may be interpretation of the quantitative results. More detailed discussion concerning oral fluid and hair are given in Chapters 18 and 19.

Limited specimen volume

For a variety of reasons, the volume of postmortem material available for analysis may be very limited. The problem faced by the toxicologist is how to make the best use of that material. With the widespread use of sensitive MS techniques, or even other GC and LC detection methods, it should seldom be necessary to base a single assay on more than 1 mL of specimen. However, even if the volume of specimen used per sample used per test does not exceed 1 mL, the total amount of specimen available for a case may not permit the usual range of tests, or allow the normal level of sensitivity. Therefore, when such results are reported, it is important to reflect any such shortcomings in the final report, such as a higher LOD, or the inability to perform certain screening tests that otherwise might imply a false-negative result.

Interpretation of postmortem toxicology results

When attempting to interpret drug concentrations, forensic toxicologists traditionally have placed a great deal of faith in the assumption that the postmortem concentration of the substance at least approximates that present at the moment of death. Over the years, we have learned that such faith is often misplaced. Even for ethanol, we continue to learn more about its kinetics and disposition during life and changes that occur after death. A thorough understanding of what happens to drugs in the body after death is still lacking, and even for living patients there is a poor correlation between blood concentration and effects. So-called 'therapeutic ranges' have been established for only a relatively small number of drugs, and patient-to-patient variability can be considerable even for these. Some patients exhibit unacceptable side-effects with drug plasma concentrations well within the therapeutic range, whereas plasma concentrations above the therapeutic range are necessary to obtain the desired control with minimal side-effects in others. The problems of interpretation are even greater with postmortem specimens.

Ethanol

Although ethanol is a common and relatively well-understood intoxicant, interpretation of postmortem results can be complex. Ethanol can be formed by postmortem fermentation, degraded by bacterial action and redistributed within the body through trauma and other processes.

The postmortem formation of ethanol in the blood, urine and tissues has been well described (Corry 1978). Under appropriate conditions, ethanol can be formed in concentrations up to, and exceeding, those set as the statutory limit for driving a motor vehicle in many countries (e.g. 50–100 mg/100 mL blood). What is poorly understood is that concentrations as high as 200–400 mg/100 mL can be formed in exceptional circumstances (Harper, Corry 1988). Conversely, ethanol can also serve as a substrate for many microorganisms such that ethanol concentrations in blood and tissues may initially increase and later decrease. There is no known correlation between the degree of putrefaction of a specimen and the production of postmortem ethanol. Many severely decomposed specimens may contain no ethanol at all, whereas others that appear less severely decomposed may contain concentrations of 80 mg/100 mL or higher.

Other factors can also cause ethanol to be present in postmortem blood as an artefact. It has been demonstrated that, when the stomach contains a sufficiently large amount of ethanol, the ethanol may diffuse through the stomach wall and diaphragm and eventually enter into the heart and central blood vessels (Backer *et al.* 1980; Pounder, Smith 1995; Iwasaki *et al.* 1998). Severe trauma, sufficient to rupture the stomach and diaphragm, may allow gastric contents to pass into the chest cavity. In such cases it may be difficult to obtain blood from the usual peripheral vessels. The presence of a small amount of beer or wine, such as might be left after a single drink with lunch, could produce an enormously elevated, but artefactual, chest blood ethanol concentration. Another mechanism by which blood alcohol may be elevated is the agonal, or postmortem, movement of gastric contents into the trachea and lungs (Pounder, Yonemitsu 1991). This can lead to elevated blood ethanol concentrations in the major central pulmonary and cardiac vessels and subsequently to erroneous interpretation.

For these reasons, analysis of a second alternative specimen in postmortem cases is recommended. Vitreous humour is the specimen of choice because it remains sterile for a period of days after death and therefore postmortem fermentation does not take place. Only in the case of very severe putrefaction, in which the eye dries out and little fluid is available, is a slight increase in ethanol concentration seen, but the extent of this effect is rarely, if ever, above 20 mg/100 mL (Zumwalt *et al.* 1982; Gilliland, Bost 1993). Although an equilibrium between ethanol in the vitreous humour and that in blood is attained quickly, there may be a lag period during absorption when the vitreous ethanol concentration is slightly lower than that in the blood (Fernandez *et al.* 1989). After equilibrium has been attained, the concentration of ethanol in vitreous humour is about 1.15 times higher than that in blood (Garriott 2008). This is because ethanol is distributed in the body according to water content and, while vitreous humour contains more than 98% water, whole blood contains on average approximately 85–88% water. Cerebrospinal fluid may be similarly useful, but is more difficult to collect and therefore is less often available for analysis than vitreous humour.

Urine is also a useful fluid for the corroboration of ethanol concentrations in blood. Although concentrations of ethanol in urine average about 1.3 times those in blood, there is considerable variability. Urine is a waste fluid stored in the bladder and, once formed, is largely unaffected by the circulating blood ethanol concentration, unlike ethanol in vitreous humour, which is in equilibrium with the circulating blood. Ethanol can be present in urine and not in blood if sufficient time has elapsed between its consumption and death to allow for clearance from the blood into the bladder. Ethanol can also be detected in urine (sometimes at high concentrations) but not in blood if the donor is a poorly controlled diabetic and if high concentrations of glucose are present. The coexistence of a urinary tract infection (yeast or bacterial) can allow considerable *in vitro* postmortem fermentation to occur in the bladder (Alexander *et al.* 1988; Saady *et al.* 1993). It is virtually impossible to have significant concentrations of ethanol in blood, but not in the urine, except as an artefact caused by postmortem fermentation or contamination.

Other postmortem specimens are less useful for ethanol measurement. Bile ethanol concentrations are roughly comparable to those in blood for uncontaminated specimens (Winek *et al.* 1983). However, because of the proximity of the gallbladder to the stomach and liver, bile is of little value where postmortem fermentation or postmortem diffusion is of concern. Similarly, liver and other tissues can undergo postmortem fermentation when bacteria are present. In relatively fresh postmortem tissue, concentrations of ethanol are approximately 50–85% of the corresponding blood concentrations, because of the lower water content of solid tissues. The average liver : blood ratio for alcohol is approximately 0.6 (Jenkins *et al.* 1995).

Other alcohols and volatiles

The presence of volatiles other than alcohol in postmortem specimens generally, but not always, indicates exposure to, or ingestion of, such compounds. An example is acetone, which can be present in poorly controlled diabetics at concentrations up to, and sometimes exceeding, 80 mg/100 mL. Acetone is also sometimes present at lower concentrations (e.g. below 10 mg/100 mL) in chronic alcoholics, malnourished individuals and those who suffer from severe stress. Isopropanol can also be present in trace amounts, and is probably formed as a postmortem artefact from acetone (Davis *et al.* 1984). The presence of both isopropanol and acetone can also result from solvent ingestion (acetone is the major metabolite of isopropanol).

In more northerly climates, methanol is readily available and accidental or suicidal methanol poisonings are common, but methanol can also be present as an artefact of postmortem change. Methanol is a major ingredient in many embalming fluids, and therefore is present in most embalmed bodies. Some forensic examinations take place in funeral homes, especially in rural areas, and blood or other fluids collected may be contaminated inadvertently with embalming fluid. Less obvious is contamination of motor vehicle accident victims with methanol contained in windshield washer fluid, in which concentrations can be 30%

or higher. Blood collected from any site other than from an intact blood vessel has the potential for contamination. It is even possible for the vitreous fluid to be contaminated after the eye has been splashed with windshield washer fluid (Jones *et al.* 2007).

Drugs and other toxins

The interpretation of the concentrations of drugs in postmortem blood and tissue specimens is complicated because many drugs are unstable *in vivo* and *in vitro*. Interpretation may also be affected by tolerance, inter-individual variation in pharmacological response, drug interactions, the presence or absence of natural disease, and the circumstances under which death occurred. For example, cocaine is hydrolysed readily before and after death. It is thought that serum cholinesterase is responsible for the hydrolysis of cocaine to ecgonine methyl ester, while the formation of benzoylecgonine may arise from spontaneous non-enzymatic hydrolysis (Isenschmid *et al.* 1989). Interpretation must therefore depend not only on the concentration of cocaine measured but also that benzoylecgonine and ecgonine methyl ester. Even when postmortem blood is collected into a tube that contains sodium fluoride (to retard *in vitro* hydrolysis of the cocaine), the degree of hydrolysis that occurs *post mortem* compared with that prior to death is almost impossible to determine. In other words, if relatively high concentrations of cocaine metabolites are detected, it can be difficult to determine whether they resulted from an acute overdose of cocaine or from chronic heavy consumption (bingeing) over a period of several hours or even days. Even if that question could be answered, it is known that cocaine can cause serious and even fatal cardiac arrhythmias at high concentrations, but it is also known that regular cocaine users can snort or inject large doses of cocaine without apparent, serious, short-term toxicity. Some individuals can develop excited delirium syndrome and die after relatively small doses of cocaine (Wetli, Fishbain 1985; Pollanen *et al.* 1998).

Some benzodiazepines (e.g. flunitrazepam, nitrazepam and clonazepam) are known to be unstable *in vitro* and back calculation of the perimortem concentration is not practical (Robertson, Drummer 1995, 1998). Many other drugs are known to have poor stability in postmortem blood (e.g. chlordiazepoxide, phenelzine, olanzapine, zopiclone). Morphine glucuronide may be converted back into unconjugated morphine in postmortem blood in circumstances in which sufficient bacterial contamination is present to release glucuronidase (Carroll *et al.* 2000). The extent to which glucuronide metabolites can be formed is poorly understood for most drugs. For example, while it is well known that morphine readily undergoes glucuronidation, it is much less well known that codeine also forms a glucuronide metabolite that can be found abundantly in both blood and urine (Wang *et al.* 2006). While glucuronide formation is thought to occur primarily on functional groups that have active hydrogens (e.g. phenols, alcohols), extensive glucuronide formation of tertiary amines such as amitriptyline is rarely appreciated (Breyer-Pfaff 2004).

One of the most important factors to affect the interpretation of postmortem drug concentrations is the phenomenon of postmortem redistribution. The term 'postmortem redistribution' has been used to describe the movement of drugs within the body after death with the result that the blood concentration of a drug is significantly higher at autopsy than that immediately after death. Postmortem redistribution is a complex phenomenon, and probably involves at least three mechanisms to a greater or lesser degree. The first, and probably major, contributor in most cases is the release and diffusion of the drug after death from tissues or organs that contain high concentrations (usually the lungs and liver) into nearby cardiac and pulmonary blood vessels. This mechanism has been clearly identified for several drugs, including amitriptyline, for which the concentration in the liver and lungs may be 20–100 times that in the blood. The exact mechanism at a molecular level has not been identified, but it is known that changes in pH and protein structure occur after death, and thereby disrupt the protein-binding characteristics of drugs. Therefore, drugs such as the tricyclic antidepressants, which concentrate in the major organs through binding to protein and other molecules, are more likely to undergo redistribution by diffusion along nearby blood vessels. It is worth noting that, although toxicologists have referred to postmortem redistribution from the heart, the bulk of the redistribution occurs from the lungs and liver (Hilberg *et al.* 1994). In contrast to the tricyclic antidepressants, the benzodiazepines undergo very little postmortem redistribution because they are not highly concentrated in the major organs relative to blood. In one case study in which blood was collected from 10 separate ligated venous and arterial sites, the marked site dependence in the concentrations of

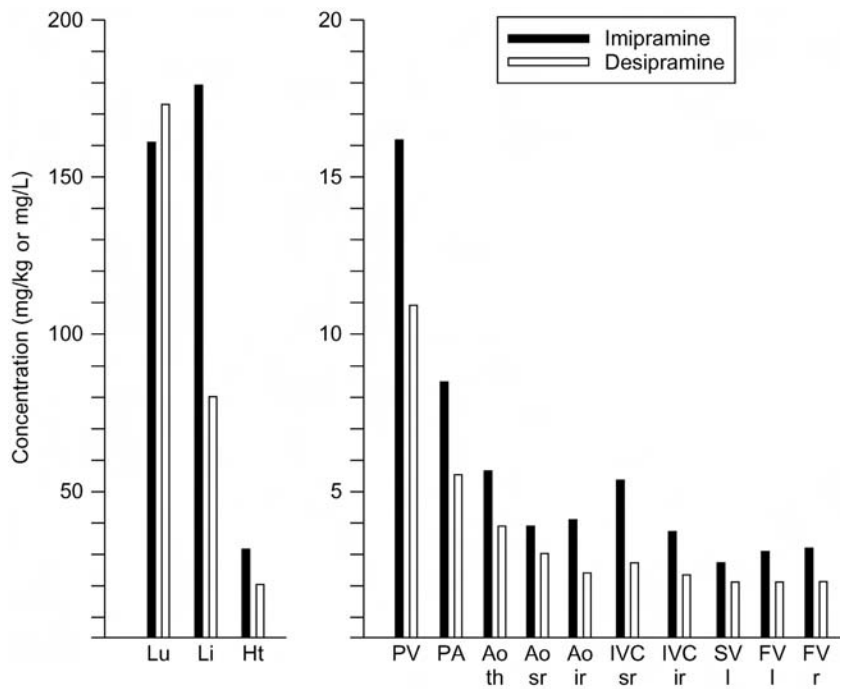

Figure 10.4 Comparison of concentrations of imipramine and the metabolite desipramine in several specimens from the same case. Lu, lung tissue; Li, liver tissue; Ht, heart tissue; PV, pulmonary vein blood; PA, pulmonary artery blood; Ao th, thoracic aorta blood; Ao sr, suprarenal aorta blood; Ao ir, infrarenal aorta blood; IVC sr, suprarenal inferior vena cava blood; IVC ir, infrarenal vena cava blood; SV l, left subclavian vein blood; FV l, left femoral vein blood; FV r, right femoral vein blood. Based on data from Jones and Pounder (1987).

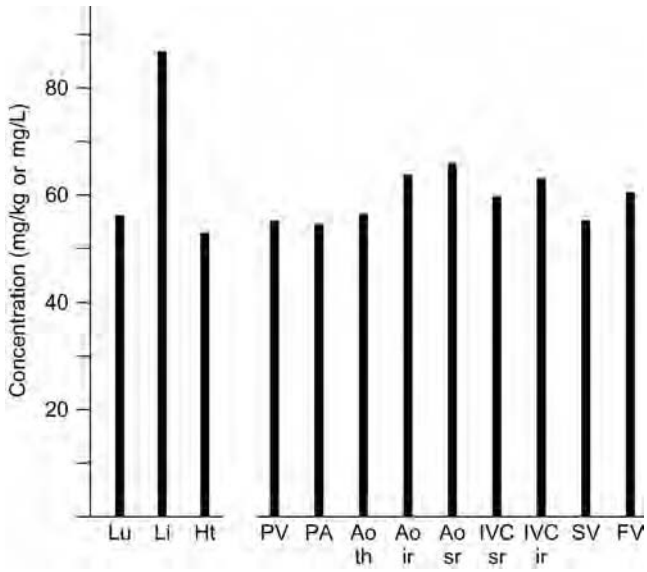

Figure 10.5 Comparison of concentrations of paracetamol in several specimens from the same case. Lu, lung tissue; Li, liver tissue; Ht, heart tissue; PV, pulmonary vein blood; PA, pulmonary artery blood; Ao th, thoracic aorta blood; Ao sr, suprarenal aorta blood; Ao ir, infrarenal aorta blood; IVC sr, suprarenal inferior vena cava blood; IVC ir, infrarenal vena cava blood; SV, subclavian vein blood; FV, femoral vein blood. Based on data from Jones and Pounder (1987).

some drugs, but not others, was demonstrated clearly (Jones, Pounder 1987). Figure 10.4 shows the marked site dependence of imipramine and desipramine in the case study, and Figure 10.5 shows the relative lack of site dependence of paracetamol in the same case.

The second mechanism is simple diffusion after death from a drug depot such as the gastric contents. This is unrelated to release from the major organs because of changes in protein binding. At least two, and possibly three, situations have been identified where this can occur: diffusion or traumatic release from the stomach, agonal aspiration of the stomach contents into the lungs and continued release from a drug-delivery system. If a drug is present in sufficient concentration in the stomach, diffusion through the stomach wall can occur. Such diffusion can potentially elevate concentrations of drugs in the abdominal blood vessels, such as the abdominal aorta and iliac vein, as well as in tissues such as the liver and kidney (Parker *et al.* 1971; Pounder *et al.* 1996a, 1996b). The extent of postmortem diffusion is directly related to both the concentration and the total amount of drug in the stomach. Rupture of the stomach in a traumatic accident or suicide can cause artificially elevated drug concentrations by allowing gastric contents to spill into the chest cavity. Under such circumstances even the residue of a single therapeutic dose could produce an erroneous chest blood concentration equivalent to 10–100 times that expected after therapeutic doses. In many jurisdictions a postmortem examination is not performed if the cause of death is obvious, and a less-experienced coroner or medical examiner might attempt to draw blood from the heart by a blind stick through the chest wall (Logan, Lindholm 1996). Agonal or postmortem movement of the gastric contents into the trachea and lungs can occur after vomiting at the time of death, or as a consequence of handling of the body after death. Marked increases in the concentration of ethanol and drugs can occur through this mechanism, especially into the pulmonary and aortic blood (Pounder, Yonemitsu 1991). In the third situation, markedly increased blood concentrations can occur with at least two different drug delivery devices. Transdermal patches left on a body after death give rise to locally high concentrations of the drug (e.g. fentanyl, nicotine). Transdermal devices rely on passive diffusion across a rate-limiting membrane for drug delivery and therefore, if not removed, concentration of the medication in the local area of the patch continues to rise after death, albeit at a slower rate. As there is no blood circulation through the skin after death, the drug is no longer transported away (except by diffusion), which results in a local accumulation of the drug. The concentration gradient between the drug-containing medium (e.g. gel, adhesive) that contains the medication in the patch and the skin is so high that even modest postmortem diffusion can raise postmortem tissue and blood concentrations up to several centimetres away from the patch. The magnitude of such effects depend on the proximity of the patch to the site from which blood was drawn and the postmortem interval. Perhaps more obvious is the situation in which someone dies while receiving analgesics or other medication from an intravenous delivery device (e.g. syringe driver). Intravenous solutions may continue to be pumped into the patient after death, and potentially cause a large local increase in blood concentration (Jenkins *et al.* 1999). While most of these devices are external and readily switched off, some are internal and not obvious until the autopsy is conducted.

The third mechanism is incomplete distribution at the time of death. Even for drugs for which little or no redistribution is thought to occur, marked site-to-site differences in blood drug concentration can occur following an overdose. Since clinical pharmacokinetic studies have shown that a significant arterial–venous difference in concentrations can occur after therapeutic doses, it is reasonable to conclude that even larger differences are likely after massive oral or even intravenous overdoses. In the case of an oral overdose, localised high concentrations are likely in the portal vein, inferior vena cava, and right heart and pulmonary vessels. The existence of this phenomenon has been suggested as an explanation for site-to-site differences in unconjugated blood morphine concentrations in diamorphine-related deaths, even though morphine was shown by the same work not to undergo redistribution as such (Logan, Smirnow 1996). It is therefore important to bear in mind that demonstrating site-to-site differences in the blood concentrations of a particular drug does not necessarily prove that the drug undergoes postmortem redistribution. The distinction is an important one. By the nature of postmortem redistribution and postmortem diffusion, increases in blood concentration are time dependent. However, simply to demonstrate that blood samples from two different sites in the body contain different concentrations of a particular drug does not prove that redistribution is likely to occur for that drug in all circumstances. The reader is also referred to more detailed reviews of the possible mechanisms of postmortem redistribution (Pelissier-Alicot *et al.* 2003; Yarema, Becker 2005).

Blood and/or plasma distribution

One issue that is often overlooked in comparing postmortem data with that from living patients is that most postmortem laboratories analyse whole blood, whereas clinical laboratories invariably analyse serum or plasma. Many drugs are not evenly distributed between plasma and erythrocytes, and therefore concentrations may be misrepresented by a factor of up to 2 or more. For example, digitoxin is primarily distributed into plasma, with virtually none in the red cells, with a blood : plasma ratio of 0.5, whereas digoxin has a blood : plasma ratio of close to 1.0 (Abshagen *et al.* 1971; Lukas, Peterson 1966). Similarly, Δ^9-tetrahydrocannabinol (THC) has a blood : plasma ratio approaching 0.5, which indicates that most of the drug is distributed in the plasma, with little in the erythrocytes (Mason, McBay 1985). The significance of this is again that most postmortem measurements are conducted on whole blood, whereas much of the pharmacokinetic data are based on plasma measurement. Although the blood : plasma distribution is not likely to have as big an influence on interpretation as postmortem redistribution for some drugs, it is a factor that should not be overlooked, particularly if the analytical results are being compared with those obtained from plasma and/or serum specimens.

Blood and/or tissue distribution

Liver and brain have been used extensively for the postmortem measurement of drugs. Initially, tissues were used because many drugs are present at concentrations up to 10–50 times that in blood, and because tissues provided a large volume of material for extraction and analysis. This was essential in the days when physical isolation of the poison, crystallisation and pharmacological testing were a goal. As analytical methods improved, analyses for most drugs could be performed on blood, leading to a trend in the analysis of blood alone. However, with

the recognition that redistribution and related phenomena could seriously decrease confidence in postmortem blood drug concentrations, analysis of tissues is regaining importance. While concentrations of some drugs can increase by as much as 2- to 10-fold after death in postmortem blood, concentrations in tissues such as liver remain relatively stable. The problem lies with the interpretation of tissue drug concentrations. Unlike in blood, reference ranges for drugs in tissues are not obtainable from clinical studies, and animal data are not directly transferable to humans. Forensic toxicologists have to rely on empirical data from other cases in their own laboratories, or on published material. To be useful, the postmortem data must include cases in which drugs are likely to have been taken in therapeutic doses, not just overdose cases.

The question can be posed., 'How do you convert a tissue concentration into a blood concentration?' You do not. Organs are anatomically distinct entities with different kinetics from blood. Although it is generally true that very high blood concentrations tend to be associated with high tissue concentrations and that a useful relationship can be demonstrated, there is too much variation to attempt mathematical conversion for any single case. Although virtually no studies have examined the dose–time–concentration relationship in tissues, and certainly none in humans, some studies have demonstrated marked variation in drug concentrations within an organ, such as the liver and the brain. This further demonstrates why caution must be exercised in attempting to convert tissue concentrations into an equivalent blood concentration. A tissue concentration provides an additional piece of the puzzle. Multiple tissue concentrations provide additional pieces, and help to build a picture of the body burden of the drug in a qualitative sense. This information, together with blood concentrations, information from the autopsy and the circumstances of death, can help formulate informed conclusions about the role of the drugs (if any) in a death.

Pharmacokinetics

Postmortem toxicologists tend to interpret drug concentrations simply in terms of 'What role, if any, did these substances play in the death of this person?'. While this question is important, lawyers, judges and the public frequently ask 'How many tablets did the person take and when did they take them?' The non-scientist readily understands the concept of 'dose' (the number of tablets a person took, compared with the prescribed dose). However, the concept of 'blood concentration' and its relationship (or lack thereof) with time and dose is more difficult to understand. Pharmacokinetics is, in theory, a scientific tool that could bridge the link between the concentration of a drug in the blood and dosage. However, pharmacokinetics can be misapplied in postmortem cases.

Pharmacokinetics is an invaluable tool to help understand the time course of drugs in the body. In the living, it can be used to determine duration of action, inter-individual differences in peak plasma concentrations and clearance, and the likely effectiveness of different pharmaceutical formulations. However, rarely can pharmacokinetics be applied successfully to postmortem toxicology. When clinical pharmacokinetic studies are performed, the dose and time of dose are controlled, and often multiple plasma samples are collected to determine the pharmacokinetic parameters for a drug. For living persons, determining the dose from a single plasma or blood concentration is fraught with uncertainty. The problem is even more complex for postmortem cases. The most common equation applied is:

$$\text{Dose (g)} = C\,(\text{g/L}) \times \text{body mass (kg)} \times V_d\,(\text{L/kg})$$

where C is the concentration of drug in plasma or blood and V_d is the volume of distribution. For postmortem cases, only the body mass of the deceased can be ascertained with certainty. The V_d for any given drug typically varies over a range of at least two fold in the general population, and frequently more. Theoretically, the concentration of a drug can be determined with reasonable accuracy in the blood sampled. In postmortem cases, there is always uncertainty whether the concentration of drug in the sample was the same, or similar, at the time of death. Furthermore, the use of calculations that involve V_d assumes that absorption of the drug is complete and the drug is in equilibrium throughout the body. In postmortem cases this assumption may be invalid, especially when dealing with acute intoxications or overdoses. As a result, dose calculations may overestimate a dose by as much as 10-fold or more.

Forensic toxicologists have occasionally used analysis of multiple tissue samples from various organs in the body in attempt to overcome the errors inherent in the use of V_d calculations. The approach requires quantitative analysis of tissue from multiple organs and sites to estimate the total body burden of a drug – that is, the total amount in the body. At the very least, the major organs such as liver, lungs and brain must be analysed, in addition to skeletal muscle and adipose tissue. Masses measured at autopsy can be used to calculate the total drug in the organs sampled. However, the masses of skeletal muscle and adipose tissue can be estimated realistically only from historical data (Butler 1971; Diem, Lentner 1971) and may poorly reflect the actual tissue masses in the victim. As previously discussed, the concentration of a drug in the piece of organ or other tissue measured may or may not reflect the average for all of that organ or tissue. Another often overlooked factor is that, after chronic dosing, steady-state levels for some drugs, especially those with a large V_d and long half-life, may be several-fold higher after chronic therapeutic dosing than after a single dose. To summarise, therefore, pharmacokinetic calculations should be attempted with extreme caution, if at all, and any assumptions made should be stated clearly. In most instances, pharmacokinetic calculations using postmortem blood measurements are rarely defensible forensically.

Metabolism and pharmacogenetics

Although the primary pathways of drug metabolism have been understood for at least 20–25 years, the extent and mechanisms of drug interactions and pharmacogenetic influences on blood concentrations have really been elucidated only in the past few years. A detailed discussion of pharmacogenetics and drug metabolism is beyond the scope of this chapter; however, some aspects should be highlighted. It is known that 7–8% of the white population is deficient in cytochrome P450 2D6 (CYP2D6), one of the major enzymes responsible for many important oxidative pathways, such as alkyl hydroxylation. That deficiency is determined genetically. As a result, the ability of those affected to metabolise and clear many drugs may be affected seriously. In many instances, even though drug clearance is significantly slower than for those not deficient in CYP2D6, it is not as slow as might be predicted because other pathways may compensate. Even if a person is not specifically identified as being deficient in CYP2D6, the person or physician may be indirectly aware of it because of an unusual sensitivity to some drugs and the higher prevalence of side-effects. Even in people without deficiencies in drug-metabolising enzymes, drug–drug interactions can result in dangerously elevated concentrations. It has also been recognised that many drugs can inhibit their own metabolism by saturating the primary metabolising enzyme systems. For example, the dose–plasma concentration curve for phenytoin can rise almost exponentially at high therapeutic doses. These variabilities in the relationship between dose and plasma or blood concentration can therefore introduce even more error into any attempt to apply conventional pharmacokinetics to estimate dosage (Richens 1979).

There is a great temptation to categorise blood levels in black-and-white terms as being the result of therapeutic doses, or of a suicidal overdose, or perhaps of abuse. Other possibilities are sometimes overlooked when high concentrations of drugs are encountered. One of the simplest ways to determine compliance with prescribed dosage, although not foolproof, is to conduct a medication count. Knowing when the medication was prescribed, how much was dispensed, the dose and the number of days between dispensing and death can often provide a powerful indicator of patient compliance, and whether an overdose is likely or not. Information regarding compliance over a longer period may often be obtained by a review of the pharmacy or medical records.

The slowing of drug metabolism with age has been well documented, but this can be overlooked as an explanation for elevated postmortem drug concentrations in the elderly.

Caution against using reference tables

Interpretation of postmortem toxicology results can be very challenging and should be done only with a thorough knowledge of the case history, including autopsy findings, information from the scene and relevant medical history. It is not difficult to interpret a high blood strychnine concentration in a person found dead in a farmhouse together with an open container of strychnine-containing rodent poison and a suicide note. However, how should the toxicologist interpret a moderately high blood concentration of imipramine in an adolescent prescribed the drug for attention-deficit disorder. Could the drug have accumulated? Was the subject suffering from depression? Was he or she complaining of side-effects? Can any medication that remains be accounted for by the time since the prescription was filled and dosage (i.e. a medication count)? Did the autopsy reveal any significant natural disease? Was the behaviour of the subject observed in the immediate period leading to death? Was the death a sudden, witnessed collapse? Was there a period of emergency hospitalisation leading up to death? If so, what did the medical assessment reveal? Are there any antemortem plasma or blood specimens still available? These are the types of questions that the forensic toxicologist should ask in cases that are anything less than straightforward.

There is a great temptation for forensic toxicologists and others to refer to tables of therapeutic, toxic and fatal concentrations. While these tables may be of some use in clinical toxicology, they are of very limited value for the interpretation of postmortem toxicology results and can be very misleading. Such tables are often drawn extensively from clinical data, seldom take into account tolerance and do not allow for phenomena such as postmortem redistribution. For example, interpretation of morphine and other narcotic concentrations may be very dependent on how long the person has been prescribed the drug and at what dosage. The inappropriate use of tables can result in over- and underestimation of the potential toxicity of a drug depending on the degree of tolerance developed, natural disease and whether other substances are present. The ranges given may not take into account the circumstances of drug use. For example, the therapeutic range for fentanyl when used intravenously as an adjunct to anaesthesia may be greater than 10-fold that after use for analgesia via a transdermal patch. Data compendia may also include cases in which there was a prolonged survival time and the person died from the sequelae of the intoxication (e.g. hypoxia, organ failure), but after medical intervention had prolonged life, resulting in lower blood concentrations. Specific references to case data and further information are lacking in most instances. Experienced postmortem toxicologists rely first on their own case experience, supplemented by compilations of drug monographs, where references to the original published work are available, and the circumstances of the case (Baselt 2008).

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CHAPTER

11 Drugs of Abuse

SD McDermott

Introduction

Definitions

One definition of the term 'drug of abuse' is any substance that, because of some desirable effect, is used for some purpose other than that intended. The intended use of the substance could be for a therapeutic effect with, for example, benzodiazepines, or a practical use in the case of the solvent abuse by 'glue-sniffing'.

Another definition of 'drug of abuse' is any substance whose possession or supply is restricted by law because of its potential harmful effect on the user. Such drugs are known as controlled or scheduled substances. For example, in the United Kingdom (UK), such 'Controlled Drugs' are those listed in Schedule 2 of the Misuse of Drugs Act 1971. The Schedule is divided into three parts (or classes) largely on the basis of decreasing order of harmfulness: Part I (Class A), Part II (Class B) and Part III (Class C). This division into three classes is solely for the purpose of determining penalties for offences under the Act. The drugs comprise licit materials (i.e. those manufactured under licence), the illicit products of clandestine factories and some natural products. Although many plant-based drugs have been self-administered for thousands of years (e.g. coca leaf, cannabis (marijuana and hashish), opium, peyote cactus), the imposition of criminal sanctions is mostly a product of the 20th century. Many of the drugs currently abused were once not only on open sale but often promoted as beneficial substances by the food and pharmaceutical industries. A pattern developed whereby initial 'misuse' of pharmaceutical products such as heroin, cocaine and amfetamine led to increasing legal restrictions and the consequent rise of an illicit industry. Nowadays, nearly all serious drug abuse involves illicit products. Most abused drugs fall into just a few pharmacological groups, e.g. central nervous system (CNS) stimulants, narcotic analgesics, hallucinogens and hypnotics. It is still true that the most prevalent drugs are plant-derived or semi-synthetic substances (e.g. cannabis, cocaine and heroin), but the view of the United Nations (UN) Drug Control Programme is that wholly synthetic drugs (e.g. amfetamine, 3,4-methylenedioxymetamfetamine (MDMA) and related designer drugs) are likely to pose a more significant social problem in the future.

This limited definition of drug abuse excludes those pharmaceutical products that may be misused in the sense that they could lead to accidental or deliberate overdose or that they could contribute to vehicle accidents or are banned by sporting organisations. Also excluded are drugs such as alcohol, tobacco and caffeine, which either are foodstuffs, with or without nutritional value, or whose use is socially acceptable. According to the World Health Organization (WHO), scheduled drugs are 'abused' rather than 'misused'. Drugs of abuse may or may not lead to physical or psychological 'dependence', a term used by WHO in preference to 'addiction'.

Legislation

Drug laws have been a common feature of human law for several hundred years. Today's 'war on drugs' bears many similarities to earlier drug laws, particularly in motivation. The motivation behind many earlier drug laws was based on religious observance, allegations of violence related to the consumption of the drug and concern for public health. The first law prohibiting outright the use of a specific drug in the United States of America (USA) was a ban on the smoking of opium in San Francisco in 1875. Cocaine was prohibited in the first part of the 20th century. In 1914 The Harrison Act was passed in the USA. This law required sellers of opiates and cocaine to have a licence. While originally intended to require paper trails for drug transactions, it soon became a prohibitive law. The Marijuana Tax Act of 1937 was introduced in the USA to counter the loss of revenues associated with the purchase of the drug from an unlicensed source as no legal licence to sell the drug was in existence.

Narcotic drugs are classified and placed under international control by the 1961 UN Single Convention on Narcotic Drugs, as amended in 1972. The Single Convention limits 'exclusively to medical and scientific purposes the production, manufacture, export, import, distribution of, trade in, use and possession of drugs' (Table 11.1).

Psychotropic substances are placed under international control by the 1971 UN Convention on Psychotropic Substances. The objectives of this Convention are again to limit the use of these substances to medical and scientific purposes (Table 11.2). While some psychotropic substances may have therapeutic value, they also present a dangerous risk of abuse.

The Controlled Substances Act (CSA, 1970) in the USA places all substances that were in some manner regulated under existing federal law into one of five schedules. This placement is based upon the substance's medical use, potential for abuse, and safety or dependence liability. The Act also provides a mechanism for substances to be: controlled, or added to a schedule; decontrolled, or removed from control; and rescheduled or transferred from one schedule to another.

The following items are indicators within the terms of the CSA that a drug or other substance has a potential for abuse:

- There is evidence that individuals are taking the drug or other substance in amounts sufficient to create a hazard to their health or to the safety of other individuals or to the community.
- There is significant diversion of the drug or other substance from legitimate drug channels.
- Individuals are taking the drug or other substance on their own initiative rather than on the basis of medical advice from a practitioner licensed by law to administer such drugs.
- The drug is a new drug so related in its action to a drug or other substance already listed as having a potential for abuse as to make it likely that the drug will have the same potential for abuse as such drugs, thus making it reasonable to assume that there may be significant diversions from legitimate channels, significant use contrary to or without medical advice, or that it has a substantial capability of creating hazards to the health of the user or to the safety of the community. Of course, evidence of actual abuse of a substance is indicative that a drug has a potential for abuse.

In determining into which schedule a drug or other substance should be placed, or whether a substance should be decontrolled or rescheduled, certain factors are required to be considered. The body with the responsibility for this in the USA is the Drug Enforcement Agency (DEA) and the factors to be considered include:

1. The drug's actual or relative potential for abuse.
2. Scientific evidence of the drug's pharmacological effects. The state of knowledge with respect to the effects of a specific drug is, of course, a major consideration. For example, it is vital to know whether or not a

Table 11.1 1961 UN Single Convention on Narcotic Drugs

Schedules	*Harmfulness*	*Degree of control*	*Examples of listed drugs*
I	Substances with addictive properties, presenting a serious risk of abuse	Very strict; 'the drugs in Schedule I are subject to all measures of control applicable to drugs under this Convention' (Article 2.1)	Cannabis and its derivatives, cocaine, heroin, methadone, morphine, opium
II	Substances normally used for medical purposes and given the lowest risk of abuse	Less strict	Codeine, dihydrocodeine, propiram
III	Preparations of substances listed in Schedule II, as well as preparations of cocaine	Lenient; according to the World Health Organization, these preparations present no risk of abuse	Preparations of codeine, dihydrocodeine, propiram
IV	The most dangerous substances, already listed in Schedule I, which are particularly harmful and of extremely limited medical or therapeutic value	Very strict, leading to a complete ban on 'the production, manufacture, export and import of, trade in, possession or use of any such drug except for amounts which may be necessary for medical and scientific research' (Article 2.5.b)	Cannabis and cannabis resin, heroin

drug has a hallucinogenic effect if it is to be controlled because of that effect. The best available knowledge of the pharmacological properties of a drug should be considered.

3. The state of current scientific knowledge regarding the substance. Criteria (2) and (3) are closely related. However, (2) is primarily concerned with pharmacological effects and (3) deals with all scientific knowledge with respect to the substance.
4. Its history and current pattern of abuse. To determine whether or not a drug should be controlled, it is important to know the pattern of abuse of that substance, including the socio-economic characteristics of the segments of the population involved in such abuse.
5. The scope, duration and significance of abuse. In evaluating existing abuse, the DEA administrator must know not only the pattern of abuse but also whether the abuse is widespread. In reaching a decision, the administrator should consider the economics of regulation and enforcement attendant on such a decision. In addition, the administrator should be aware of the social significance and impact of such a decision upon those people, especially the young, who would be affected by it.
6. What, if any, risk there is to public health. If a drug creates dangers to the public health, in addition to or because of its abuse potential, then these dangers must also be considered by the administrator.
7. The drug's psychological or physiological dependence liability. There must be an assessment of the extent to which a drug is physically addictive or psychologically habit forming, if such information is known.
8. Whether the substance is an immediate precursor of a substance already controlled. The CSA allows inclusion of immediate precursors on this basis alone into the appropriate schedule and thus safeguards against possibilities of clandestine manufacture.

After considering the factors listed above, the administrator must make specific findings concerning the drug or other substance. This will determine into which schedule the drug or other substance will be placed.

In the European Union (EU), when a new psychoactive substance is first detected, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) together with Europol conduct a risk assessment on the health and social risks, caused by the use of, the manufacture of and traffic in the new psychoactive substance, the involvement of organised crime and the possible consequences of control measures. An early warning system exists to fast track the collection of information and the subsequent control of the substance.

Various offences are defined under the legislation, and in the UK offences under the Act include:

- Possession of a controlled substance unlawfully.
- Possession of a controlled substance with intent to supply it.
- Supplying or offering to supply a controlled drug (even where no charge is made for the drug).
- Allowing premises you occupy or manage to be used unlawfully for the purpose of producing or supplying controlled drugs.

Drug trafficking (supply) attracts serious punishment including life imprisonment for Class A offences. To enforce this law the police have special powers to stop, detain and search people on 'reasonable suspicion' that they are in possession of a controlled drug.

Legal status of cannabis

The legal status of cannabis for personal use is one of the most controversial policy issues in the EU and indeed worldwide. Although cannabis is a classified narcotic drug placed under control by the UN and by all EU Member States, the measures adopted to control it at national level vary considerably. Cannabis extracts – marijuana, hashish and cannabis oil – are classified as narcotic drugs under both Schedules I and IV of the UN Single Convention on Narcotic Drugs

Table 11.2 1971 UN Convention on Psychotropic Substances

	Harmfulness	*Degree of control*	*Examples of listed drugs*
I	Substances presenting a high risk of abuse, posing a particularly, serious threat to public health which are of very little or no therapeutic value	Very strict; use is prohibited except for scientific or limited medical purposes	Lysergide (LSD), MDMA (ecstasy), mescaline, psilocybin, tetrahydrocannabinol (THC)
II	Substances presenting a risk of abuse, posing a serious threat to public health which are of low or moderate therapeutic value	Less strict	Amfetamines and amfetamine-type stimulants
III	Substances presenting a risk of abuse, posing a serious threat to public health which are of moderate or high therapeutic value	These substances are available for medical purposes	Barbiturates, including amobarbital, buprenorphine
IV	Substances presenting a risk of abuse, posing a minor threat to public health with a high therapeutic value	These substances are available for medical purposes	Tranquillisers, analgesics, narcotics, including allobarbital, diazepam, lorazepam, phenobarbital, temazepam

(1961). Article 36 requests State Parties to 'adopt such measures as will ensure that . . . possession . . . of drugs contrary to the provisions of this Convention . . . shall be punishable offences when committed intentionally'. The active principles of cannabis, the cannabinoids THC (tetrahydrocannabinol) and specifically dronabinol (Δ^9-THC) are classified as psychotropic substances under Schedules I and II respectively of the UN Convention on Psychotropic Substances (1971). Article 22 of this convention echoes the terms of the 1961 Convention mentioned above, stating that 'each Party shall treat as a punishable offence, when committed intentionally, any action contrary to a law or regulation adopted in pursuance of its obligations under this Convention. . .'. The EU Member States have transposed the UN precepts concerning the penal or administrative control of cannabis, and have applied them according to their own local or regional circumstances. This has resulted in a heterogeneous 'legal map' regarding cannabis offences: some countries or regions tolerate certain forms of possession and consumption; other countries apply administrative sanctions or fines; and still others apply penal sanctions. A study of the decriminalisation of cannabis in Switzerland also examined the situation in Australia and the USA and found varying applications of the law relating to cannabis use (Magg 2003).

Fines, cautions, probation, exemption from punishment and counselling are favoured by most European justice systems. It is of interest to note that cannabis in particular is frequently distinguished from other substances and given special treatment in these cases, in the law, by prosecutorial directive, or by the judiciary. Nevertheless, police arrests for drug offences, mainly those involving cannabis and mainly use-related offences, are increasing in several countries.

Legal status of hallucinogenic mushrooms

Mushrooms containing psilocin or psilocybin are known as hallucinogenic or 'magic' mushrooms. Psilocin and psilocybin are controlled substances under Schedule 1 of the 1971 UN Convention on Psychotropic Substances, so all Member States control them accordingly. However, the control of the mushrooms themselves is interpreted in many different ways across Europe – this may reflect the extent to which they grow freely in certain conditions, and the fact that they appear to be a somewhat regional phenomenon.

In some countries, the law specifically lists hallucinogenic mushrooms themselves as a controlled substance and forbids their sale or possession. Some will simply treat the mushrooms as being the controlled substances of psilocin or psilocybin in compound form. Some look at the intent of the act; they ban cultivation, possession or sale only when for the purposes of abuse. Their condition is also considered – fresh mushrooms might not be considered illegal, but prepared or treated mushrooms are illegal – again perhaps reflecting the intent. Interpretation of the term 'prepared' or 'treated' may become a complex matter for the courts. Others use a catch-all phrase in the law ('cultivation of any plant for the purposes of making a psychoactive substance'). Finally, a number of countries remain with unclear legislation, simply because so few cases have come to court.

Penalties for drug offences

Penalties for drug offences vary considerably across the world. There is generally a distinction between possession for personal use (smaller quantities) and possession with intent to supply to others.

In Europe, in general, the penalty associated with possession of a small quantity of a controlled drug for the first time is a fine. Further convictions even for possession of a relatively small quantity can lead to jail sentences, and possession of large quantities of controlled drugs generally attracts a jail sentence. Some countries have mandatory minimum jail sentencing for drugs above a certain quantity or monetary value.

In the USA the Anti-Drug Abuse Act of 1988 allows the government to punish minor drug offenders without giving the offender a criminal record if the offender is in possession of only a small amount of drugs. This law is designed to impact the 'user' of illicit drugs, while simultaneously saving the government the costs of a full-blown criminal investigation. Under this Act, the government has the option of imposing only a civil fine on individuals possessing only a small quantity of an illegal drug. Possession of this small quantity, identified as a 'personal use amount' carries a civil fine of up to $10 000. In determining the amount of the fine in a particular case, the drug offender's income and assets will be considered. This is accomplished through an administrative proceeding rather than a criminal trial, thus reducing the exposure of the offender to the entire criminal justice system, and reducing the costs to the offender and the government.

Congress has imposed two limitations on this Act's use. It may not be used if (1) the drug offender has been previously convicted of a Federal or state drug offence or (2) the offender has already been fined twice under this Act.

Analysis of seized drugs

Items suspected of containing drugs occur in four principal forms: (i) powders; (ii) tablets and capsules; (iii) living plants or dried vegetable matter; and (iv) liquids. Apart from situations where the analyst has made extracts from clothing or other matrices, the last category could include solvents, aqueous solutions (e.g. γ-hydroxybutyric acid (GHB)), injection solutions, alcoholic solutions (e.g. cocaine in liquor) and hash oil, many of which will have a characteristic appearance or packaging. Powders are unlikely to show any clear visual clues to their identity and will often be presented in paper or plastic wrapping. Although the analytical approach to each may differ, there are six basic components, not all of which will be needed in every case: physical examination; sampling; screening; qualitative analysis; quantitative analysis; profiling/comparison (Fig. 11.1). The particular techniques used will depend on available equipment, staff skills and the objectives of the analysis, but there are certain minimum criteria that need to be satisfied, particularly when the results are presented as evidence in court.

Physical examination

A natural starting point in any analysis procedure is the physical examination of the item in question. This may involve making a sketch or taking a photograph of the item. It will invariably involve either taking some physical measurements such as length or diameter, or making a record of the number of items (e.g. number of tablets) or the weight of the item in question. It may involve taking detailed notes on the type of wrapping material used. On many occasions, details such as these can take on a large significance.

While the physical appearance of a suspect material can sometimes give an early indication of the possible drug(s) present, it is only after chemical analysis that the full picture can emerge.

Sampling

Drugs submitted to forensic science laboratories can vary enormously in the manner of presentation. One exhibit may be a quantity of ecstasy tablets that are well made and essentially clean to handle; the next exhibit may be packs of heroin or cocaine that had been concealed internally by a drug user. The initial approach to each scenario will depend on such matters as:

- Health and safety
- Linking of packaging material
- Fingerprint analysis
- Other evidence.

It is also true that, because of time constraints and costs, not all of the items submitted will be analysed and therefore a sampling policy must be established.

The number of items tested will be determined by a number of factors such as the number of items seized, their physical appearance and the need to satisfy a court of law.

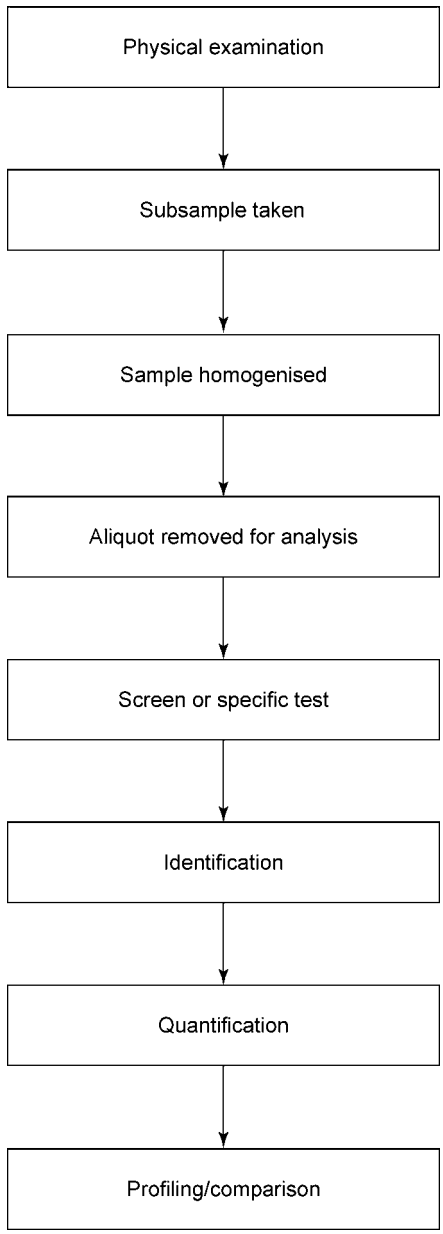

Figure 11.1 Flowchart for the examination of an unknown substance.

Exhibits of controlled substances are received by forensic laboratories in large numbers and a representative sampling plan must be established. The benefits of a sampling plan are to

- Reduce the number of analytical determinations.
- Reduce overall workload.
- Decrease exposure to controlled substances.
- Reduce handling of biologically contaminated evidence.

With any sampling plan, an initial visual examination of all the units in the population is conducted. If all the units are similar in appearance, the population can be considered as homogeneous and a sampling plan can be implemented (Aitken 1999; Clarke, Clark 1990; Colon *et al.* 1993; Frank *et al.* 1991; Tzidony, Ravreby 1992).

The most frequently used sampling procedures are as follows:

(1) Take a sample of size equal to the square root of the population size ($n = \sqrt{N}$); e.g. from a population of 400 take a sample of 20.
(2) Take a sample equal to 10% of the population size ($n = N/10$); e.g. from a population of 400 take a sample of 40.

Although experience has shown that the square-root method produces reliable results, it does not provide a statistical foundation to the sampling problem.

(3) Another popular method of sampling is the use of a hypergeometric distribution. This procedure involves statistical tables that can be used to determine how many samples should be taken for analysis. Table 11.3 is an example of such a table. If these analysis tests are positive, an inference can be made for the whole population. For example, if 1000 suspect tablets are seized and sent to the laboratory, by testing 28 tablets and if the tests are positive for MDMA, then there is a 95% probability that 90% of the tablets contain MDMA.

In relation to sampling from single items, it is important that a correct sampling procedure be put in place. The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Most methods – qualitative and quantitative – used in forensic science laboratories for the examination of drugs require very small aliquots of the material, so it is vital that these small aliquots are entirely representative of the bulk from which they are drawn.

However, because of various legal requirements, it may not always be possible to render a sample into a homogeneous state before sampling. This could be the case if the sample was to be separately tested by another laboratory or if keeping the sample in its original 'heterogeneous' state was an important piece of physical evidence.

As a rule, though, items should be in a homogeneous state prior to sampling, and this is especially important in quantitative analysis. This might involve the simple grinding of a small amount of powder (or tablet) in a mortar with a pestle, or might involve taking numerous samples from a larger quantity of powder and grinding them together.

In many instances the amount of drug submitted to the forensic analysis is too large to get an accurate weight of the total and therefore an estimate of the weight may be used. The simplest way to get such an estimate is to accurately weigh a subsample and get average/standard deviation information and extrapolate this from the subsample to the total population. The forensic report produced should reflect the fact that the total weight of the drug is based on an estimate. Generally the court will accept this as a legitimate scientific practice.

Table 11.3 Hypergeometric sampling table (UN 1998)

Total number of items in exhibit	*Number of items to be tested*
10–12	9
13	10
14	11
15–16	12
17	13
18	14
19–24	15
25–26	16
27	17
28–35	18
36–37	19
38–46	20
47–48	21
49–58	22
59–77	23
78–88	24
89–118	25
119–178	26
179–298	27
299–1600	28
>1600	29

Screening tests

Colour/spot tests provide a valuable indication of the content of any particular item tested, but it must be stressed that positive results to colour tests are only presumptive indications of the possible presence of the drug. Colour tests have the advantage that they can be used as field tests by unskilled operators, with the obvious need for follow-on analysis in the laboratory. One of the most important and widely used colour tests is the Marquis reagent test.

Another important screen is thin-layer chromatography (TLC). This has many advantages as an analysis/screening tool. It is quick, easy to use and has a low cost; it is relatively sensitive and can give a good degree of discrimination.

Solvent systems TA and TB are suitable for many drugs (see Table 11.4 and Chapter 39).

Visualisation of many of the drugs may be achieved by a variety of methods. However, spraying with acidified potassium iodoplatinate reagent is suitable for many drugs.

Qualitative analysis

Using a capillary column gas chromatograph and a suitable temperature programme coupled to a mass spectrometer (GC-MS; see Chapter 37), the drug components of most samples can be separated and identified. Because of the reduced capital outlay of such instruments in recent times, it is not uncommon for laboratories to have several such instruments working with automatic samplers (possibly on a 24-hour basis). The use of GC-MS has become the routine method of identification of most drugs. A general GC-MS screen method (GAK, Table 11.4) can be used to separate and/or identify most of the drugs encountered in exhibits. Figure 11.2 shows the separation achieved, by this method, of a mixture of the main drugs described in this chapter.

Identification of the various components of a suspect mixture can be made by library searching against commercial libraries but it is important to run a standard of the specific drug being tested, e.g. pure heroin. This obviously will need to give a retention time and mass-spectral match.

High performance liquid chromatography (HPLC) is a simple and reliable method of analysis of most drugs. It is both accurate and precise and thus lends itself to quantitative analysis. It is especially useful for compounds that are thermally labile. HPLC has some advantages over GC because of the variety and combinations of mobile phases that can be chosen. There is also a choice of detectors available for specific applications. HPLC can, however, involve significantly more method development than GC and the resolution of GC is greater.

No one system is suitable for optimum separation of all the different drug types, so different systems are used to give optimum separations for specific analysis. The system that is best for the separation of heroin/acetylcodeine/noscapine/papaverine (Huizer 1983) will not be the same as that which separates cocaine from its impurities and processing by-products (Moore, Casale 1994).

A general screening method such as HBC (Table 11.4) can be used for the separation of heroin (diamorphine), cocaine, amfetamine and metamfetamine (Fig. 11.3).

Using this system, metamfetamine and MDMA co-elute. However, by changing the method to HBD, amfetamine, metamfetamine, MDMA and methylenedioxyethamfetamine (MDEA) may be separated (Fig. 11.4). This illustrates the versatility of HPLC. Separations can occur between compounds that co-elute by altering the elution system. A system such as the above could be used as a screen, but identification would necessitate some spectroscopic method such as MS or infrared (IR) spectroscopy.

Most modern laboratories are now equipped with Fourier transform IR (FTIR) spectrometers (see Chapter 33). These have many advantages over traditional IR instruments. They are faster and can work with smaller samples. When they are coupled with a microscope, tiny samples can be analysed. The difficulty with IR analysis of drug samples is the presence of other material that will interfere with the spectrum. These interfering compounds could be other drugs that occur naturally in the samples (or from the synthetic process) or could be adulterants such as caffeine and paracetamol. IR analysis can, however, give valuable information on chemicals that are not suitable for GC-MS analysis.

Another technique that is popular is GC-FTIR. Because of the speed of scanning of the FTIR it can be used to obtain a spectrum of the compounds that have been separated by GC.

In practice, neither spectra nor pure reference samples may be available for comparison for the more unusual substances. In this situation, nuclear magnetic resonance (NMR) spectroscopy (see Chapter 36) is the method of choice.

Quantitative analysis

For most controlled drugs there are no minimum quantities below which an offence does not occur. The quantitative analysis of drugs is therefore not carried out routinely on all exhibits. The main reason for determining purity/drug content of powders and tablets is to enable a court to establish a monetary value of the seizure or when sentencing structures are based on equivalent pure drug content. In some countries the death sentence can apply if one is convicted of possession/supply of greater than a specified quantity of a substance.

In some situations, information on drug purity will be used for intelligence purposes such as to assess trends in the illicit drug market or for use in drug comparison and profiling.

Having already identified the powder as, for example, heroin, quantitative analysis may be carried out by GC using flame ionisation detection (FID) or by HPLC. In performing quantitative analysis it is always desirable to include an internal standard in the analysis. This has the advantages of ease of use, increased accuracy, no need for volumetric glassware, no need to measure injection volume and easy determination of reproducibility, and can be used as a monitor for GC or HPLC systems.

An internal standard must meet the following criteria: it must be absent from the sample; it must be readily available (and not too costly); and it must be pure, show good chromatographic behaviour, be reproducible and be soluble in the solvent used. Straight-chain hydrocarbons (for GC) fulfil all of these requirements and they elute as a homologous series, so they are a popular choice as internal standard. It has been suggested that the internal standard that one chooses should be chemically related to the compound being analysed. However, provided that it fulfils the above criteria, any compound can be chosen.

A general approach to quantification by GC could be as follows. The GC conditions for the qualitative analysis can again be used. A standard curve is established by preparing up to five standard solutions of the drug being quantified. A range from 1 mg/mL to 5 mg/mL is prepared using solvent containing an internal standard. A concentration of internal standard of 0.5 or 1 mg/mL will normally be adequate. A test sample is prepared that will have a concentration between 1 and 5 mg/mL, i.e. within the range of the standard curve. If the test sample is outside the range, a second sample is prepared based on the information from the first sample. In general it is suggested that at least two samples of the powder being tested be taken for quantitative analysis and an average of these be taken as the true result. The amount of the drug in the test sample can now generally be calculated by the data-analysis function of the instrument.

Both GC and HPLC are used extensively for quantitative analysis and it is useful to compare the results obtained by one method with the other, for a given drug.

Profiling and comparison

A more detailed analysis of drug samples can be used to provide 'collective' information. This is generally called profiling when it involves the chemical analysis of powders, or is known as characterisation when the physical properties of tablets and other dosage forms are measured. Chemical profiling has been the technique most widely used and is often based on the chromatographic separation of impurities and precursors (as in the case of amfetamine and metamfetamine) or other naturally occurring components and adulterants (e.g. heroin, cocaine,

Table 11.4 GC, HPLC and TLC conditions

Condition code	*Source*	*Details*
Gas chromatography		
GAK	SD McDermott (unpublished)	Column: HP Ultra-1 cross-linked methylsiloxane (12.5 m × 0.2 mm i.d., 0.33 μm)
		Carrier gas: He, 1 mL/min, 50 : 1 split ratio
		Temperature programme: 60°C for 2 min to 180°C at 15°C/min to 290°C at 25°C/min for 3 min
		MS conditions: low mass 40, high mass 550 with a solvent delay of 1.5 min
GAL	Clarke (1989)	Column: OV-1 (12 m × 0.32 mm i.d., 0.52 μm)
		Carrier gas: He, 1.5 mL/min, 25 : 1 split ratio
		Temperature: 260°C
GAM	McDermott (unpublished)	Column: HP Ultra-1 (12 m × 0.2 mm i.d., 0.33 μm), split ratio 50 : 1
		Temperature programme: 150°C (no hold) to 295°C at 15°C/min
GAN	Lee (1995)	Column: HP-1 (12 m × 0.22 mm i.d., 0.33 μm)
		Temperature programme: 100°C for 2 min to 300°C at 15°C/min for 5 min
GAO	Blackledge, Miller (1991)	Column: HP-1 (25 m × 0.2 mm i.d., 0.33 μm)
		Temperature programme (lactone): 70°C for 1 min to 300°C at 20°C/min for 3 min
		Temperature programme (BSTFA derivative): 100°C for 1 min to 300°C at 20°C/min for 7 min
GAP	SD McDermott (unpublished)	Column: HP Ultra-1 cross-linked methyl siloxane (12.5 m × 0.2 mm i.d., 0.33 μm)
		Carrier gas: He, 1 mL/min, 50 : 1 split ratio
		Temperature programme: 70°C for 2 min to 210°C at 20°C/min for 1 min
		MS conditions: low mass 40, high mass 550 with a solvent delay of 1.5 min
GAQ	SD McDermott (unpublished)	Column: HP 101 cross-linked methylsiloxane (12 m × 0.2 mm i.d., 0.33 μm)
		Carrier gas: He, 1 mL/min, 50 : 1 split ratio
		Temperature programme: 200°C for 1 min to 270°C at 5°C/min to 295°C at 20°C/min for 3 min
High performance liquid chromatography		
HBC	McDermott (unpublished)	Column: Spherisorb 5 ODS-1 (150 × 4.6 mm i.d.) at 30°C
		Mobile phase: acetonitrile: TEAP[a] (50 : 50), flow rate 1.5 mL/min
		Detector: DAD (λ = 254 nm)
HBD	McDermott (unpublished)	Column: Spherisorb 5 ODS-1 (150 × 4.6 mm i.d.) at 30°C
		Mobile phase: acetonitrile: TEAP[a] (20 : 80), flow rate 1.0 mL/min
		Detector: DAD (λ = 254 nm)
HBE	Clarke (1989)	Column: Supelcosil 5 LC-18 (250 × 4.6 mm i.d.)
		Mobile phase: phosphate buffer[b] : methanol (60 : 40)
		Detector: UV (λ = 309 nm)
HBF	Borner, Brenneisen (1987)	Column: Spherisorb ODS-1 (250 × 4.6 mm i.d., 10 μm)
		Mobile phase: 0.3 mol/L ammonium acetate in water (buffered to pH 8 with ammonia): 0.3 mol/L ammonium acetate in methanol (100 : 0 for 2 min to 5 : 95 at 14 min)
		Detector: UV (λ = 269 nm)
HBG	Mesmer, Satzger (1998)	Column: Bondapak C_{18} (300 × 3.9 mm i.d., 10 μm)
		Mobile phase: phosphate buffer[c] : methanol (70 : 30), flow rate 1 mL/min
		Detector: UV (λ = 215 nm)
HBH	Japp *et al.* (1988)	Column: ODS (250 × 5 mm i.d., 5 μm)
		Mobile phase: methanol : water : phosphate buffer[d] (pH 7.25, 55 : 25 : 20), flow rate 1 mL/min
		Detector: UV (λ = 240 nm)
HBI	Japp *et al.* (1988)	Column: ODS (250 × 5 mm i.d., 5 μm)
		Mobile phase: methanol : water : phosphate buffer[d] (pH 7.67, 70 : 10 : 20), flow rate 1 mL/min
		Detector: UV (λ = 240 nm)
HBJ	CND Analytical (1989)	Column: Bondex C_{18} (300 × 3.9 mm)
		Mobile phase: methanol : water (70 : 30), flow rate 1 mL/min
		Detector: UV (λ = 254 nm)
HL	Baker *et al.* (1980)	Column: Spherisorb ODS silica (250 × 4.6 mm i.d., 5 μm)
		Mobile phase: 0.01 mol/L sulfuric acid : methanol : acetonitrile (7 : 8 : 9)

table continued

Table 11.4 continued

Condition code	*Source*	*Details*
Thin-layer chromatography		
TAH		Silica gel plates with hexane : diethyl ether (4 : 1)
TAI		Silica gel plates with acetone
TAJ		Silica gel plates with *n*-butanol : acetic acid : water (2 : 1 : 1)

[a]TEAP, triethylammonium phosphate buffer made up by preparing a 1.0 mol/L phosphoric acid (65 mL of 85% phosphoric acid to 1 L of water) titrated to pH 2.5 with triethylamine (approximately 10 mL triethylamine per 100 mL 1.0 mol/L phosphoric acid). A 10 mL quantity of this solution is made up to 1 L of water to give the working solution of 10 mmol TEAP.
[b]Phosphate buffer is prepared by adding 10 mL of phosphoric acid to 1 L of water, followed by the addition of sufficient 2 mol/L sodium hydroxide to raise the pH to 6.5.
[c]Phosphate buffer is 10 mmol potassium dihydrogen phosphate, adjusted to pH 3 with phosphoric acid.
[d]0.1 mol//L phosphate buffer is prepared by dissolving 14.35 g sodium dihydrogen phosphate dihydrate and 1.14 g disodium hydrogen phosphate in 1 L of water.

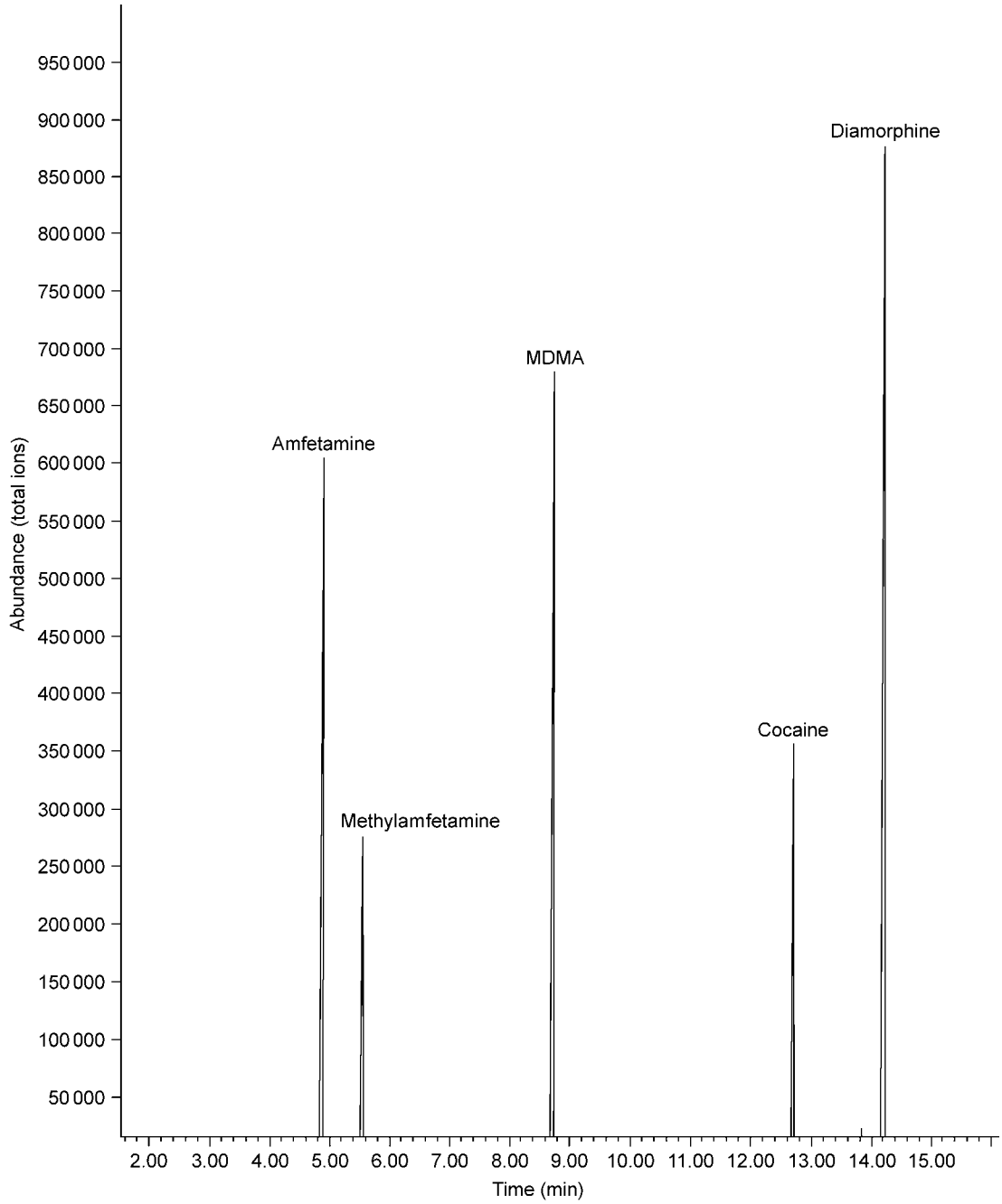

Figure 11.2 Gas chromatographic separation.

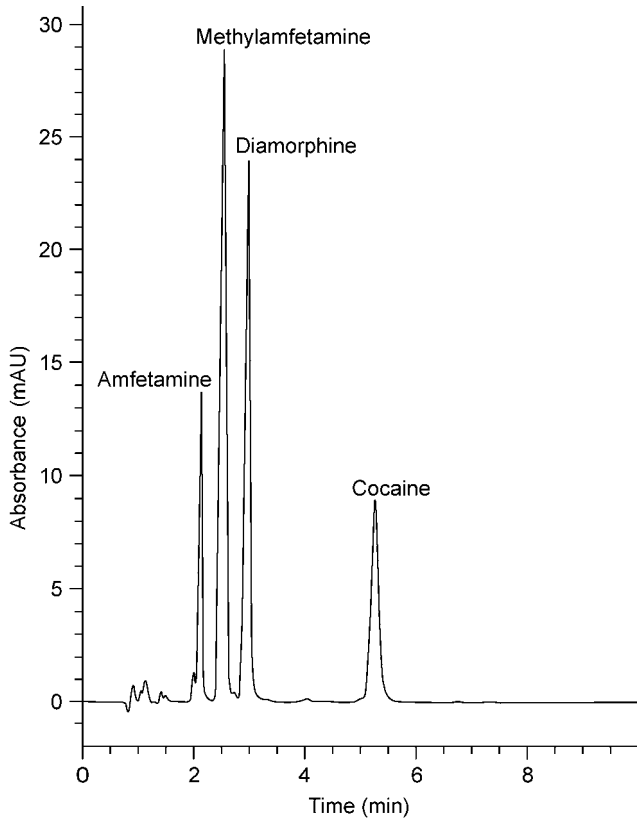

Figure 11.3 HPLC separation.

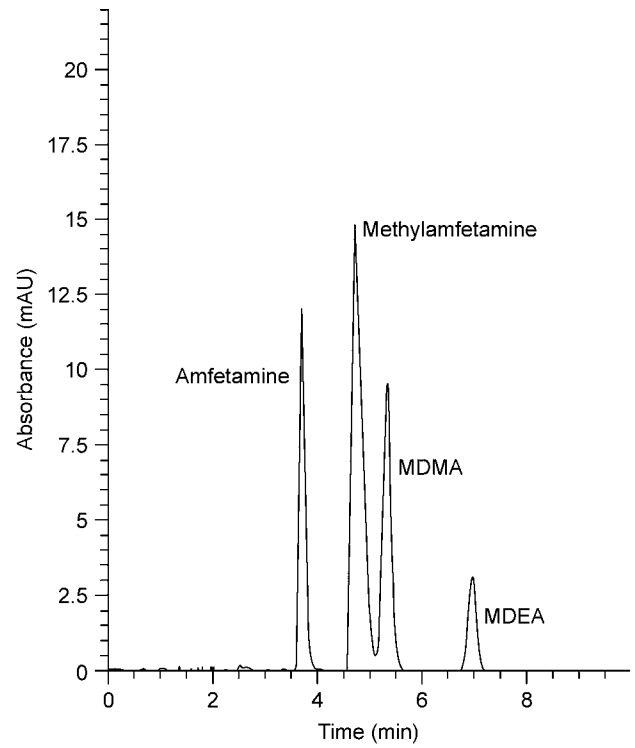

Figure 11.4 HPLC separation.

cannabis resin). Detection may range from flame-ionisation to isotope-ratio mass spectrometry. Non-separation methods, for example using IR, Raman or X-ray diffraction spectroscopy, have only limited scope.

Drug profiling may be used for two quite separate purposes. In the first case, it can establish connections between a number of exhibits that are suspected of being linked as part of a local distribution chain. This is known as comparison or tactical profiling and may be carried out as a routine requirement in forensic casework. A second stage (intelligence or strategic profiling) exists where answers to wider questions may be sought. These will depend on the drug concerned, but include estimating the number of different profile types in circulation and relating them to the number of active laboratories and how long they have been in operation; determining the extent of importation by comparing the profiles of police and customs seizures; identifying the route of synthesis and types of precursors used; creating large-scale maps of drug distribution and identifying the country or region of origin.

Tablet comparisons using general physical features, gross drug content and microscopic examination of defects and punch marks (so-called ballistic analysis) can be of some value, but they suffer from the fact that at any one time a large fraction of illicit tablets in circulation may be almost identical. Such small differences as may exist could simply reflect inherent differences in the punches and dies of a multiple-stage tabletting machine. This can be illustrated by the Mitsubishi logo (Fig. 11.5), which was found on over half of all MDMA tablets seized in Europe in the late 1990s. A similar pattern was also found in the UK for amfetamine in the early 1990s, when nearly half of all samples belonged to one profile type. In these circumstances, any connection between two separate seizures of otherwise identical tablets or powders may be purely fortuitous.

This, in turn, raises other problems with profiling. It is necessary to maintain a database of profiles such that the significance of any 'match' or 'non-match' can be critically assessed. However, in a situation where profiles may change with time, what constitutes a 'current' database is not always clear. In the case of determination of country of origin, authentic samples are required in order to provide a statistical 'training set', yet such samples may be difficult to obtain and their true provenance uncertain.

A general approach to the analysis of unknown substances

A general approach to the analysis of unknown substances has been outlined in Fig. 11.1. Different approaches will be required depending on whether the exhibit is a powder, vegetable matter, tablet/capsule or liquid.

Powders

When a powder is submitted for analysis, the most likely drugs to be present include heroin, cocaine, amfetamine and metamfetamine. Although others may be present, these are the most common. Initial examination will involve describing/detailing of the packaging material. If there are multiple packs present then a subsample may be removed for analysis. The powder must be weighed before analysis.

The powder is then homogenised and an aliquot is taken for analysis. A screen (colour test, TLC, HPLC, GC) will indicate the drug(s) present. Identification of the drug can be achieved using GC-MS or FTIR spectroscopy. GC-MS has an advantage over FTIR spectroscopy because of retention time and a mass-spectral comparison with a known standard.

Figure 11.5 Impressions on illicit MDMA tablets.

Identification of the other components in the powder can be achieved by using a range of analytical techniques including FTIR, X-ray fluorescence (XRF), X-ray diffraction (XRD), NMR spectroscopy and others.

The drug content can be quantified by GC or HPLC by preparation of a curve using a range of concentrations of the drug in question. In some circumstances comparison may be required between powders for links in a specific case or for intelligence purposes, and GC and HPLC can be employed to examine some of the minor ingredients of the powder.

Vegetable material

This includes cannabis plants, cannabis resin, khat and psilocybe mushrooms. A physical examination would include a description of the material followed by a measurement of the weight of material or the height of the plant. A subsample of the population may be chosen and an aliquot taken for analysis. Homogenisation may be necessary depending on the material. The physical appearance will generally give a very good idea of the drug present, for example cannabis plant or psilocybe mushrooms. We can therefore go directly to a specific test rather than use a screening technique. Identification can be carried out by a combination of microscopic and chemical techniques.

The drug content can be quantified by GC or HPLC and comparison/profiling carried out by chemical and physical comparisons.

Tablets and capsules

Tablet or capsules submitted to the laboratory include ecstasy (MDMA, MDEA, etc.), benzodiazepines, steroids, LSD squares and others. An initial examination will include a description of any markings or logos, counting of the items, measurement of the tablet/capsule and possibly a weight. An examination of a tablet/capsule identification database such as TICTAC may give an indication of the drug present (see Chapter 13). Subsampling followed by homogenisation will lead to essentially a powder sample and the procedure for analysis of powder samples may then be followed.

For comparison/profiling a physical comparison of the logo or mark may be the most informative piece of information available.

Liquids and other forms

These include GHB, steroid oils, cocaine liquor and others. A physical examination may give an indication of the drug likely to be present. The physical measurements to be noted would include the volume, colour, odour and general appearance of the liquid. A subsample may be taken and an aliquot removed for analysis. It may be necessary to (base) extract the drug from the liquid into an organic solvent prior to analysis. The physical examination may allow the analyst to proceed to the identification stage, otherwise a screen may be used to indicate the presence of a certain drug. Identification, quantification and comparison/profiling can be carried out along the lines outlined for powders.

Clandestine laboratories

Because of the increase in abuse of synthetic drugs, clandestine laboratories have become an increased part of forensic investigations. The investigation of such sites is very interesting as they reveal (*in situ*) the synthetic processes, intermediates, and often notes and chemical equations describing the various reactions used. These laboratories, however, are also very hazardous sites to investigate. The use of the word 'laboratory' disguises the more usual scenario of a garage, shed or kitchen.

Forensic scientists frequently become involved in an advisory capacity in the initial stages of a clandestine laboratory investigation. Information may come to light about certain chemicals being used at a premises and the scientist is responsible for formulating an opinion as to whether a controlled substance is being produced. The police can then act on the basis of this opinion.

Many countries have specially trained police and scientists to deal with the specific problems that clandestine laboratories pose. These problems could be in the form of hazardous chemicals (acids, bases, solvents and reagents) and fire and explosion potentials.

Ultimately, if the seizure results in a court case, the testimony in these cases can be technically demanding for the scientist. In many situations, only a small amount (or none) of the final product (i.e. the controlled substance) may be found. In these instances, detailed explanations of the synthetic routes may be required. An explanation of the role of each of the chemicals found at the scene could be required. The scientist must also be aware of alternative explanations for the presence of the chemicals, as this is the likely defence in such cases. In many instances the precursor chemicals themselves are controlled. The forensic investigation of clandestine laboratories has been well documented (Christian 2003) and many of the complications associated with such laboratories have been explored.

In the USA, the number of clandestine metamfetamine laboratories has increased enormously in recent times. Illegal seizures increased from 7438 in 1999 to 12 484 in 2005. The hazards and contamination issues associated with such laboratories pose difficulties for those involved in the process and for public health in general (Martyny *et al.* 2007). Capillary electrophoresis (CE) has been employed to characterise phosphorus species used in the manufacturing process (Knops *et al.* 2006).

Concealment of drugs

The internal concealment of illicit drugs to smuggle quantities across borders has been a phenomenon for decades (Fineschi *et al.* 2002; Gill, Graham 2002; Takekawa *et al.* 2007). The 'body packer' or 'mule' carries quantities of drugs that are generally well wrapped to withstand the biological hazards of internal concealment. Deaths related to such activities are not uncommon. However, it is not known how widespread the practice is as the detection is frequently associated with instances that have adverse medical consequences.

Impregnation of items with cocaine is also a novel method of concealment. Cocaine-impregnated silicone in baseball cap parts were detected and chloroform was used to extract the cocaine (Microgram 2003). Items of clothing impregnated with cocaine were extracted with methanol (McDermott, Power 2005) and in this instance 'moth balls' containing camphor were added to the suitcase to deter detection by dogs. An unusual way of smuggling cocaine is the use of a cocaine–poly (methylmethacrylate) solid solution (Gostic, Klemenc 2007).

Analysis of the main drugs of abuse

In this section, methods are described for the analysis of the main drugs of abuse. In all cases a number of analysis methods are described. The methods chosen will depend on the aim of the analysis, the apparatus/equipment available, legal aspects and the number of analyses to be performed, and may depend on other details associated with the specific drugs seizure.

The analytical data for the various drugs are shown in Tables 11.5 and 11.6.

Amfetamine and metamfetamine

Amfetamine (α-methylphenethylamine; 1-phenylpropan-2-amine) and metamfetamine (*N*-methyl-α-methylphenethylamine; *N*-methyl-1-phenylpropan-2-amine) in free base form are both liquids. Amfetamine is normally produced as amfetamine sulfate or phosphate and is common in Europe. Metamfetamine is normally produced as metamfetamine hydrochloride and is more popular in North America and Japan.

Street-level amfetamine and metamfetamine are normally submitted to the laboratory as white/off-white powders with relative low purity (e.g. 5%).

Synthesis of amfetamine and metamfetamine

Many methods are available for the illicit synthesis of amfetamine, but the Leuckart reaction has been the most popular. This method is simple and rapid, gives a good yield and does not involve any particularly hazardous chemicals or procedures. It may be considered as a three-step reaction involving the condensation of phenyl-2-propanone (P-2-P) with formamide followed by a hydrolysis of the *N*-formylamfetamine and finally purification by steam distillation.

Table 11.5 Analytical data using GC

Drug	*Retention time (min)*	*GC system*
Amfetamine	4.85	G1
Methamfetamine	5.52	G1
MDMA	8.70	G1
Heroin	14.19	G1
Cocaine	12.68	G1
LSD	7.28	G2
LAMPA	8.06	G2
Psilocin	4.88	G3
Psilocybin	6.90	G3
Cathine	4.06	G4
Cathinone	3.93	G4
GHB	5.60	G5
GBL	3.98	G5
Diazepam	13.51 (4.47)	G1 (G6)
Flunitrazepam	14.02 (5.24)	G1 (G6)
Flurazepam	14.59 (6.14)	G1 (G6)
Nitrazepam	14.48 (5.94)	G1 (G6)
Fluoxymesterone	9.04	G7
Nandrolone	7.16	G7
Testosterone	7.98	G7
Methyltestosterone	8.33	G7
Cannabinol	13.86	G1
Tetrahydrocannabinol	13.64	G1
Cannabidiol	13.32	G1

Table 11.6 Analytical data using HPLC

Drug	*Retention time (min)*	*HPLC system*
Amfetamine	2.06 (3.68)	H1 (H2)
Methamfetamine	2.42 (4.67)	H1 (H2)
MDMA	2.42 (5.31)	H1 (H2)
Heroin	2.88	H1
Cocaine	5.21	H1
LSD	5.34	H3
LAMPA	5.66	H3
Psilocin	6.80[(a)]	H4
Psilocybin	3.20[(a)]	H4
Cathine		
Cathinone		
GHB	3.5	H5
GBL	4.0	H5
Diazepam	2.29[(a)] (10.41)[(a)]	H6 (H7)
Flunitrazepam	0.86[(a)] (3.34)[(a)]	H6 (H7)
Flurazepam	3.12[(a)] (12.98)[(a)]	H6 (H7)
Nitrazepam	0.87[(a)] (3.22)[(a)]	H6 (H7)
Fluoxymesterone	5.5	H8
Nandrolone	6.0	H8
Testosterone	7.5	H8
Methyltestosterone	9.5	H8
Cannabinol	11.77[(a)]	HL
Tetrahydrocannabinol	13.35[(a)]	HL
Cannabidiol	7.47[(a)]	HL

[(a)] k values.

Metamfetamine can be made by the Leuckart reaction using methylamine and formic acid or *N*-methylformamide in the condensation step.

Analysis of amfetamine and metamfetamine

Because many of the street-level samples submitted to the laboratory are relative low in purity (5%), pre-concentration of samples may be required for the analysis to be successful. This may be achieved by base extracting the amfetamine/metamfetamine into ether and evaporating to dryness in an airflow without heat. A few drops of methanol can then be added and the methanolic solution transferred to a plastic insert prior to analysis.

Colour test Marquis test gives an orange colour for both amfetamine and metamfetamine.

TLC

- TA: amfetamine $R_f = 0.43$; metamfetamine $R_f = 0.31$
- TB: amfetamine $R_f = 0.15$; metamfetamine $R_f = 0.28$
- Visualisation is with acidified iodoplatinate solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

In addition, it is common practice with primary amines to prepare derivatives such as *N*-methylbis(trifluoroacetamide) (MBTFA) or trifluoroacetic anhydride (TFAA) derivatives. It is good practice to analyse both derivatised and underivatised samples since *N*-hydroxylamines may give the same product as the parent amines.

Using a concentrated/base-extracted sample, the molecular ion peaks *m/z* 134 and *m/z* 148 for amfetamine and metamfetamine, respectively, can readily be achieved in an underivatised sample.

Both amfetamine and metamfetamine have one asymmetrical carbon atom, resulting in a pair of enantiomers in each case. Depending on the synthetic route *l*-, *d*- and *dl*-amfetamine or metamfetamine could be encountered in samples submitted to the laboratory for analysis. These optical isomers differ in their pharmacological activity and are subject to different regulatory measures in certain countries. In those countries where the specific optical isomer needs to be identified, chiral analysis can be undertaken by derivatisation/GC, by the use of chiral columns (GC and HPLC) and by the use of CE (Anastos *et al.* 2005; Fanali *et al.* 1998; Lebelle *et al.* 1995; Sellers *et al.* 1996). Enantiomeric analysis of metamfetamine samples (Lee *et al.* 2007) showed that up until 1997 the vast majority of the metamfetamine encountered in the Republic of Korea was the *S*-(+)-enantiomer, but from 1997 onwards the *R*-(−)-enantiomer began to appear and increased continuously until 2005 when 50% of the metamfetamine samples seized contained the *R*-(−)-enantiomer.

Quantitative analysis and profiling of amfetamine/metamfetamine

Amfetamine and metamfetamine can be quantified by HPLC or GC. Normally if GC is used the samples are base extracted into an organic solvent and either run directly or derivatised and then run. Using HPLC there is no need to extract and, in many cases, this is the preferred method for quantitative analysis of amfetamine/metamfetamine.

Amfetamine produced illicitly often contains impurities that are a result of the manufacturing process. The presence of these impurities can be used to compare and distinguish samples of amfetamine since material used in the same manufacturing batch would almost certainly have the same number and relative amount of identical impurities. Samples from the same illicit laboratory produced at different times may show strong similarities, whereas samples from unrelated laboratories are expected to show major qualitative and quantitative differences.

Basic extracts into organic solvents are subjected to GC or GC-MS analysis. Samples are compared by visual inspection of the GC trace and by quantitative comparisons.

Metamfetamine impurity profiling is also carried out by GC analysis, with the impurities also giving information on the synthetic route (Seta *et al.* 1994). A recent study (Lee *et al.* 2006) of the impurities present as a result of the synthetic process showed that the selected impurity peaks may be utilised as the indicators of synthetic conditions and analysis of their patterns can supply valuable information for understanding the

conditions of clandestine synthesis of metamfetamine. The use of thermal desorption followed by GC-MS was found to be an efficient method for impurity profiling of metamfetamine (Kuwayama *et al.* 2007).

The purpose of the comparison/profiling is: to identify dealer–user links; to establish possible sources, i.e. the clandestine laboratory; and to build up databases to allow interpretation in comparison casework. This approach has been used in Australia to examine samples over the period 1998–2002 (Qi *et al.* 2006).

A series of studies were undertaken to develop a harmonised method for the profiling of amfetamine synthesised by three different methods. The study covered the sample preparation, extraction procedure, optimisation of GC-MS method, selection of target compounds and numerical comparison of amfetamine impurity profiles (Aalberg *et al.* 2005a, b; Andersson *et al.* 2007a,b,c; Lock *et al.* 2007). This inter-laboratory study found that the variation from laboratory to laboratory was affected by such factors as homogeneity of samples and concentration effects due to dilution.

Cannabis, cannabis resin and cannabinoids

Herbal cannabis (marijuana) means all parts of the plant *Cannabis sativa L.*, but excludes the seeds and mature/woody stalk material. *Cannabis sativa L.*, which can be grown in all parts of the world, is an annual plant and attains a height of 1–5 m. When it is planted for the production of hemp fibre, the stalks are crowded and without foliage except near the top. The wild growing plant, in contrast, has numerous branches. The resin of the plant occurs mainly in the flowering area, the leaves and the stem, particularly at the top of the plant. The highest amount of resin is found in the flowering part. Up to the time of flowering, the male and female plants produce resin almost equally. After shedding their pollen the male plants soon die. The resin is found in the glandular trichomes.

The leaves of *Cannabis sativa L.*, are compound and consist of 5 to 11 separate leaflets. Each leaflet is characteristically hair covered and veined and has serrated edges (Fig. 11.6).

Cannabis herbal material may be encountered in blocks of dried flowering tops and dried leaves. Cannabis resin (hashish) is a compressed solid made from the resinous parts of the plant and is usually produced in 250 g blocks. Herbal cannabis imported into Europe may originate from West Africa, the Caribbean or South East Asia, but cannabis resin derives largely from either North Africa or Afghanistan. Cannabis and cannabis resin are normally mixed with tobacco and smoked, but can be ingested. The average 'reefer' 'joint' cigarette contains around 200 mg of herbal cannabis or cannabis resin.

The main psychoactive compound in cannabis and cannabis resin is Δ^9-THC. Cannabinol (CBN) and cannabidiol (CBD) are among the other main components.

Analysis of cannabis/cannabis resin

Colour test The presence of cannabinoids in suspect material can be indicated by the Duquenois–Levine test.

Figure 11.6 Cannabis plant.

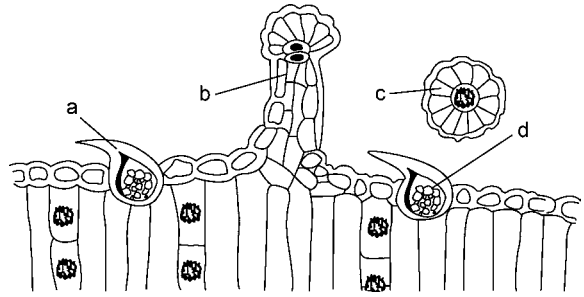

Figure 11.7 Microscopic examination of cannabis: (a) cystolith hair; (b) large glandular hair with several cells in the head and stalk; (c) head of one of the large glandular hairs; (d) calcium carbonate.

A sample of the material is extracted with petroleum ether. The petroleum ether is removed and evaporated to dryness. The addition of Duquenois reagent followed by concentrated hydrochloric acid will yield a purple colour after a few minutes. The addition of chloroform should result in a purple colour in the chloroform layer. This result can be taken positive for cannabinoids.

TLC

- TAH: THC $R_f = 0.50$; CBD $R_f = 0.60$; CBN $R_f = 0.45$
- Visualisation is by fast blue BB with THC showing a red colour, CBD showing an orange colour and CBN showing a purple colour.

Microscopic examination of cannabis/cannabis resin

The most characteristic botanical feature visible under the microscope is the hairs. There are two types of hair:

- Cystolithic hairs
- Glandular hairs.

The cystolithic hairs contain a deposit of calcium carbonate at their base. These hairs are mostly single celled. The glandular hairs are most important since they contain and secrete the resin. They are short and may be unicellular or multicellular. The bigger glandular hairs have a multicellular stalk with heads containing 8–16 cells (Fig. 11.7).

The microscopic test is carried out by putting a small portion of the dry material (cannabis herbal material or cannabis resin) on a microscope slide. A few drops of Duquenois reagent are added followed by a few drops of concentrated hydrochloric acid. The cystolithic hairs contain a deposit of calcium carbonate at their base and a characteristic effervescence can be observed. The heads at the end of the glandular hairs will become a red/purple colour.

An alternative method is to add a few drops of chloral hydrate solution to the dry material. This is particularly useful for getting more detailed information on the structure of the plant tissue since it removes coloured materials such as chlorophyll.

Quantitative analysis and comparison of cannabis/cannabis resin

As already stated, cannabis resin is normally produced in 250 g blocks. Frequently these blocks carry an impression, e.g. a number or a letter or a symbol. Comparison can be made between different blocks on the basis of similar impressions, though unrelated blocks often have the same impression. The street-level deal of cannabis resin is typically a finger-sized portion (normally 1–10 g) possibly wrapped in tinfoil or plastic. It may be possible to link a smaller piece of cannabis resin to its original block by a physical fit between the smaller and larger pieces.

GC or HPLC may be used to obtain a chemical profile of the cannabis/cannabis resin (see Tables 11.5 and 11.6). The THC content can be calculated and comparison can be made on that basis. It must be noted that variations can occur in the THC content of a single block of cannabis resin as the THC content decreases with age and storage conditions. The outer material in a block of cannabis resin can differ from that in the centre. The variation of distribution of cannabinoids within blocks of resin was studied and it was noted that composition of the resin changed even within a 12-month period (Lewis *et al.* 2005). The main chemical constituents of cannabis have been well documented (Elsohly, Slade 2005).

The THC contents of the various forms of cannabis can vary enormously. For example, recent seizures of cannabis in the UK had the following THC contents: sensemilla (indoor intensively cultivated herbal cannabis), mean 16.2%, range 4.1–46%; imported herbal material, mean 8.4%, range 0.3–22%; resin (mostly from North Africa), mean 5.9%, range 1.3–28% (Hardwick, King 2008).

Short tandem repeat (STR) DNA markers have been used to indicate the likely origin of a cannabis crop and STR markers can permit the identification of hydroponically propagated clonal drug lines, providing evidence to link illegal operators (Gilmore *et al.* 2007).

Cocaine

Cocaine is a naturally occurring alkaloid found in certain varieties of plants of the genus *Erythroxylum*. Coca cultivation is distributed throughout the central and northern Andean Ridge, with approximately 60% in Peru, 30% in Bolivia, and the remainder in Columbia, Ecuador, Venezuela, Brazil, Argentina and Panama.

Cocaine production

Production of illicit natural cocaine involves three steps:

1. Extraction of crude coca paste from the coca leaf.
2. Purification of the coca paste to cocaine base.
3. Conversion of cocaine base to cocaine hydrochloride.

Cocaine is normally encountered in the laboratory in paper or plastic packs of white powder and can be analysed without extraction.

Analysis of cocaine

Colour test Cobalt thiocyanate test or modified cobalt thiocyanate test (Scott test): a blue colour indicates the presence of cocaine.

Odour test A 5% methanolic solution of sodium hydroxide added to the test sample and warmed gives a characteristic odour in the presence of cocaine.

TLC

- TA: $R_f = 0.65$; TB: $R_f = 0.47$
- Visualisation is with acidified iodoplatinate solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

In addition to GC and HPLC, IR spectroscopy is routinely used in cocaine cases if a distinction is to be made between cocaine as a salt, e.g. cocaine hydrochloride, and cocaine in base form. The cocaine base is known as crack and, unlike cocaine hydrochloride, can be consumed by smoking. The differences in their IR spectra are shown in Fig. 11.8.

The differences in the spectra at 1736 and 1709 cm^{-1} for the base and 1729 and 1711 cm^{-1} for the hydrochloride are explained (Elsherbini 1998) by the effect of the hydrochloride ion on the C=O stretching bands.

A simple laboratory test also exists for the determination of the chemical form of cocaine (Logan *et al.* 1989).

Quantitative analysis and profiling of cocaine samples

Quantitative analysis of cocaine samples may be carried out by GC or HPLC. The general quantitation method previously described may also be used for cocaine. In some jurisdictions, preparations containing less than 0.1% cocaine base are exempt from certain controls and analytical procedures must be designed to accommodate this.

Because of the unsophisticated nature of the cocaine manufacturing process, a multitude of trace-level alkaloid impurities are present in illicit cocaine. Many of these impurities are naturally occurring alkaloids that originate from the coca leaf and are carried through the manufacturing process. In addition, cocaine is also contaminated with a variety of manufacturing by-products. The relative amounts of these compounds can be used to compare cocaine samples (Moore, Casale 1998).

The alkaloid impurities, which originate in the coca leaf, include *cis*- and *trans*-cinnamoylcocaine, tropacocaine, truxillines and hydroxycocaines.

Manufacturing by-products found in illicit cocaine include hydrolysis products such as benzoylecgonine, ecgonine methyl ester, ecgonine and benzoic acids.

Oxidation by-products also arise and these include *N*-norcocaine and *N*-norecgonine methyl ester. New impurities arising from the oxidation of crude cocaine base have been reported recently (Casale *et al.* 2007).

In addition to the above, solvent residues may be detected by NMR or headspace GC. The solvents detected include acetone, methyl ethyl ketone, benzene, toluene and diethyl ether (Cole 1998).

A comparison of cocaine samples can be achieved by a combination of qualitative analysis for the presence/absence of certain trace impurities and by quantitative analysis of the cocaine and other ingredients. Isotope ratio analysis has also been used in the profiling of cocaine (Benson *et al.* 2006). A recent study explored the optimisation and harmonisation of a profiling method for cocaine in two separate laboratories using eight main alkaloids as the comparators (Lociciro *et al.* 2007).

Heroin

Street-level heroin (diamorphine, diacetylmorphine) is normally encountered in the laboratory in paper or plastic packs containing typically 100–200 mg of brown (and sometimes white) powder. The street-level purity varies depending on availability and other factors, but values of 40–60% are common.

Production of heroin

The raw material for the production of heroin is opium. Opium is a naturally occurring product of the plant *Papaver somniferum* L. (opium poppy).

Opium is purified to form crude morphine. The morphine is acetylated with acetic anhydride to produce diacetylmorphine, the primary constituent of illicit heroin samples. Sometimes known as 'Chinese heroin', heroin from south east Asia is a white powder consisting of heroin hydrochloride and minor amounts of other opiate alkaloids, but adulterants are unusual. This material is ideally suited to injection. Heroin from south west Asia is a much cruder product. Typically seen as a brown powder containing heroin base, it has variable amounts of other opiate alkaloids (e.g. monoacetylmorphine, noscapine, papaverine and acetylcodeine) as well as adulterants such as caffeine and paracetamol. It is believed that these cutting agents are added to heroin either at the time of manufacture or during transit. Brown heroin may be 'smoked' by heating the solid on a metal foil above a small flame and inhaling the vapour. Those intending to inject brown heroin must first solubilise it with, for example, citric acid or ascorbic acid.

Analysis of heroin

Colour test Marquis reagent gives a purple/violet colour.

Other opiate alkaloids (morphine, codeine, monoacetylmorphine and acetyl codeine) give the same positive reaction to the Marquis test.

TLC

- TA: $R_f = 0.47$. TB: $R_f = 0.15$
- Visualisation is with acidified iodoplatinate solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Quantification and profiling of heroin

Heroin may be quantified by either GC or HPLC. A problem associated with GC analysis is that heroin may hydrolyse to 6-*O*-monoacetylmorphine and/or transacetylation may occur of the common cutting agent paracetamol by heroin in the injection port of the GC column. The use of fresh samples and of chloroform as solvent can avoid these problems.

By examining the amount of heroin, papaverine, noscapine and acetylcodeine in the samples it is possible to discriminate between or show a link between samples (Besacier, Chaudron-Thoxet 1999; Dufey *et al.* 2007; Seta *et al.* 1994; Stromberg *et al.* 2000). This method of comparison was used successfully on 500 heroin samples that were divided into nine groups (Zhang *et al.* 2004). It may be further possible to examine heroin samples and show potential links between samples by the presence (and amount) of adulterants such as caffeine or by the presence of less common adulterants such as diazepam, phenobarbital

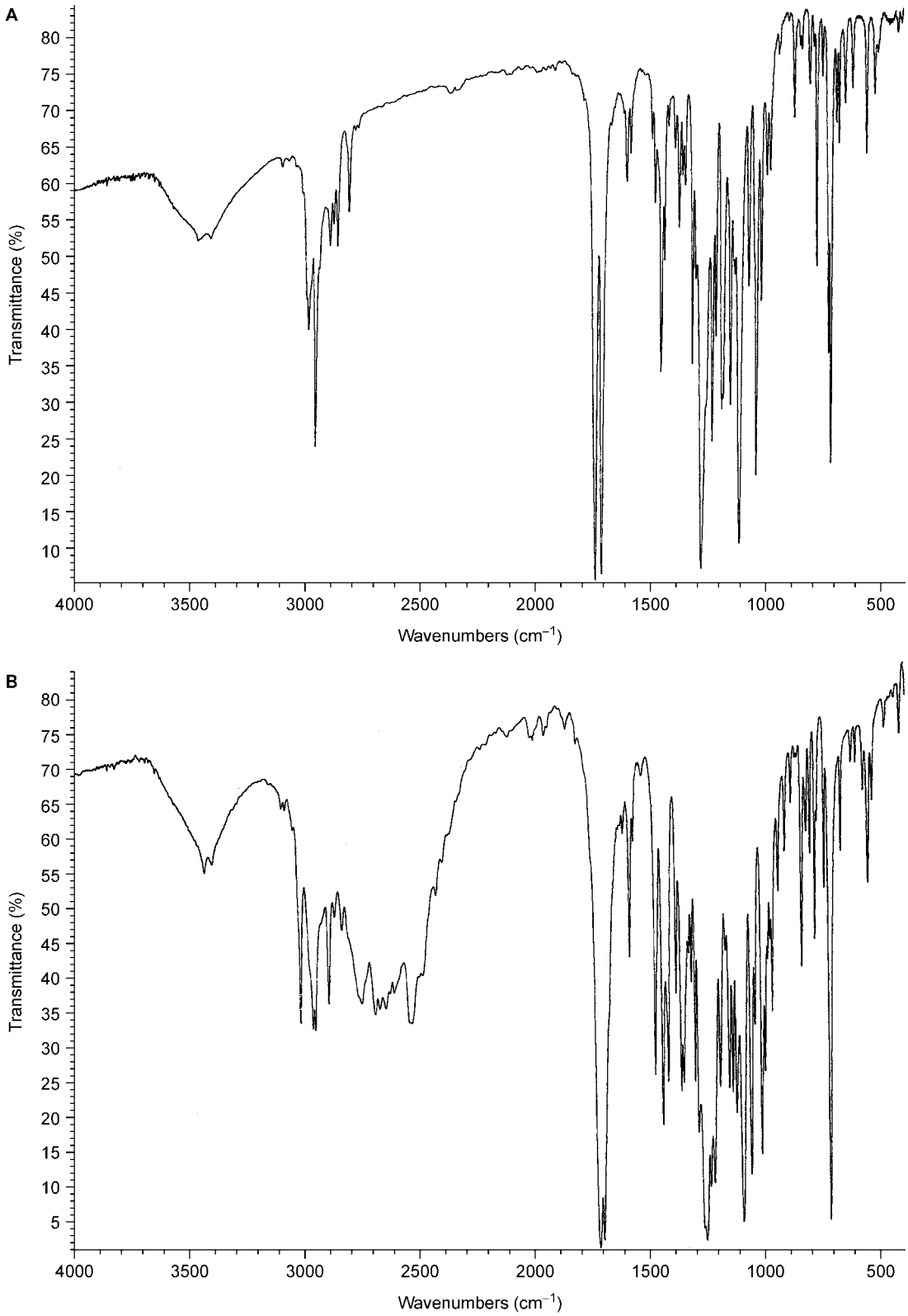

Figure 11.8 Infrared spectra of (A) cocaine base and (b) cocaine hydrochloride.

or mannitol hexa-acetate (El Haj *et al.* 2004). In large seizures, differences may be found between various samples originating from the seizure that indicate that it includes more than one batch of heroin. Chemical profiling was used to determine the country of origin of heroin samples in Australia (Collins *et al.* 2006) and stable isotope analysis was used to complement the chemical profiling on the same samples (Casale *et al.* 2006). Isotope ratio analysis has increasingly been used as a comparison tool for heroin (Carter *et al.* 2005; Zhang *et al.* 2005).

In addition to examining the relative ratios of the main components, it is possible to analyse for solvent residues (Cole 1998; Dams *et al.* 2001).

LSD

LSD is one of the most potent hallucinogenic substances known. Its properties were first discovered in the 1930s and its popularity as a drug of abuse was very high during the 1960s and 1970s when it was associated with the hippy movement.

Synthesis of LSD

LSD can be produced by several different methods, the majority of which use lysergic acid as the starting material. Lysergic acid itself is also produced in clandestine laboratories using, most commonly, ergometrine or ergotamine tartrate as the starting material. Ergotamine refluxed with potassium hydroxide and hydrazine in an alcohol–water mixture produces lysergic acid.

The methods used for the production of LSD yield a crude product, which is then cleaned up and converted to a more stable form, e.g. the tartrate salt.

In the past, LSD was encountered in a variety of substrates including powder in gelatine capsules, gelatine squares, sugar cubes and 'microdots'. Nowadays LSD is encountered mostly in paper dose form. The paper dosages are produced by soaking pre-printed paper in a solution of LSD. These sheets are then perforated into squares (typically 5×5 mm) with each square ('tab') containing approximately 50 µg of LSD.

The designs on the paper can vary from one design per square to one large design covering many squares (Fig. 11.9).

Analysis of LSD

The only analogue of LSD to receive widespread interest is lysergic acid *N*-(methylpropyl)amide (LAMPA) and any analytical technique should be capable of separating LAMPA from LSD.

The presence of LSD may be signalled early by placing the suspect paper under long-wavelength UV light. The presence of LSD is indicated by a blue fluorescence.

Colour test Van Urk reagent gives a purple colour.

TLC

- TAI: LSD $R_f = 0.58$; LAMPA $R_f = 0.49$
- Visualisation: observe the plate under UV light (254 and 365 nm)
- Spray with Van Urk reagent and heat to give a blue spot.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Some difficulty may be encountered in obtaining an unequivocal identification of LSD is because of its low dosage (50 µg or less). However, if the sample is concentrated, a satisfactory analysis can be achieved.

Place a suspect LSD square in a glass vial and cover with methanol. After soaking (or sonication) for 10–20 min, the methanol can be transferred to a plastic insert for analysis. Another method is to add concentrated ammonia (~2 drops) to the methanol.

In addition to chromatographic separation, LSD can be discriminated from other ergot alkaloids by its MS fragmentation pattern; for example, the presence in the LSD spectrum of a *m/z* 100 fragment nearly as intense as the as the *m/z* 111 fragment differentiates LSD from other di-substituted amides (Clarke 1989).

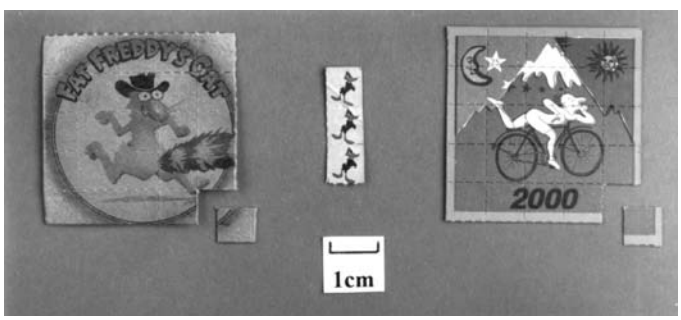

Figure 11.9 Examples of LSD paper squares.

Quantitative analysis and comparison of LSD

HPLC is the method of choice for quantitative analysis of LSD. Using the solvent mixture methanol–water (1 : 1), quantitative extraction of LSD from paper squares is normally achieved after sonication for 20 min (McDonald *et al.* 1984).

In some instances, a comparison is requested between one square of LSD and a large sheet of perforated squares. This can be an easy matter if the design on the large sheet spreads over the whole sheet and the 'missing' square fits neatly into the pattern. In other instances, the design may be on each individual square (or there may be no design). In such a case it is necessary to examine the colour/design/dimensions of the squares and the perforation pattern.

Chemical comparisons can also be undertaken, but squares from the same larger sheet can vary in the amount of LSD on each square.

MDMA

MDMA is the prototypical member of a large series of phenethylamine designer drugs and has become one of the main drugs of abuse in many countries in northern Europe. Clandestine production is largely centred in Europe. A number of homologous compounds with broadly similar effects, e.g. MDA (methylenedioxyamfetamine), MDEA (methylenedioxyethamfetamine) and MBDB (*N*-methyl-1-[1,3-benzodioxol-5-yl]-2-butanamine) have also appeared, but have proved less popular. These substances are collectively known as the 'ecstasy' drugs.

MDMA is the most common drug encountered in 'ecstasy' tablets. The tablets are typically 10 mm in diameter, either flat or biconvex, and weigh approximately 300 mg. The MDMA content varies but is generally in the range 80–100 mg per tablet. The tablets normally carry a characteristic logo/imprint. These designs are not restricted to MDMA tablets, but may be found on amfetamines and other illicit products. In other words, the logo and other physical characteristics provide no reliable information on the drug content. Many hundreds of different impressions have been found and several examples are shown in Fig. 11.5.

Synthesis of MDMA

Several methods of synthesis can be employed including:

1. Amine displacement method using safrole as the starting material.
2. Via the intermediate 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP2P) with isosafrole or a nitrostyrene as starting material.

Analysis of MDMA

Colour test Marquis test gives a blue/black colour.

TLC

- TA: $R_f = 0.31$; TB: $R_f = 0.23$
- Visualisation is with acidified iodoplatinate solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Base extraction into an organic solvent and/or derivatisation prior to GC-MS analysis is common with MDMA.

Quantitative analysis and profiling/comparison

In order to perform a quantitative analysis on 'ecstasy' tablets they must first be ground to produce a homogeneous powder and the MDMA content determined by either GC (either directly or base extracted) or HPLC.

Chemical profiling of tablets containing MDMA involves the examination/quantification of the drug and main adulterants present such as caffeine, sugars and binding agents. In addition to the main ingredients, many trace-level impurities from the synthetic process can be present and these can be used for comparison (Bohn *et al.* 1993; Renton *et al.* 1993). A cross-laboratory study on organic impurity profiling of MDMA tablets based on 46 organic impurities yielded separation between population of samples from the same synthesis batch and samples from different batches (Weyermann *et al.* 2008). Trace metal analysis has proved to be a useful method for comparing MDMA tablets (Koper *et al.* 2007; Waddell *et al.* 2004). The metals arise in the tablets as a result of the catalyst or reducing agent in the synthesis. Isotope ratio analysis has also been used to discriminate between different tablets

containing MDMA (de Korompay *et al.* 2008; Palhol *et al.* 2004). Recent advances in impurity profiling in MDMA tablets have employed a variety of techniques, with multiple techniques being used in many situations (Waddell-Smith 2007).

As already mentioned, tablet comparisons can also be made using (so-called) ballistic analysis. In this, general physical features and microscopic examination of defects and punch marks are used for comparison. The difficulty is that, at any one time, a large fraction of illicit tablets in circulation may be almost identical.

Anabolic steroids

Anabolic steroids may be abused by 'body builders' and athletes. In the UK, 48 steroids are listed specifically and generic legislation covers certain derivatives of 17-hydroxyandrostan-3-one or 17-hydroxyestran-3-one. Methandienone, nandrolone, oxymetholone, stanozolol, and testosterone and its esters account for most cases. Further non-steroidal anabolic compounds are also controlled, i.e. human chorionic gonadotrophin (HCG), clenbuterol, non-human chorionic gonadotrophin, somatotropin, somatrem and somatropin. Certain anabolic steroids are scheduled in the US Controlled Substances Act, but these drugs are not listed in the UN Conventions. A large number of the anabolic steroids encountered in seizures have been found in counterfeited packaging and the drug content may differ qualitatively or quantitatively from what is indicated in the information on the product label. This mislabelling can be particularly frustrating to the forensic chemist trying to identify the particular steroid in the product. Formulations may be either as tablets or as steroid esters dissolved in vegetable oil suitable for injection. The oils may be extracted using a steroid : hexane : methanol ratio of 1 : 2 : 1 (Chiong *et al.* 1992) with the methanol layer being used for analysis.

Because of the large number of steroid products available, analytical information is presented here on only four: fluoxymesterone, nandrolone, testosterone and methyltestosterone. Further information on the analysis of anabolic steroids in urine is to be found in Chapters 6 and 7 on Drugs in sport.

Analysis of steroids

TLC

- TP: fluoxymesterone $R_f = 0.51$; nandrolone $R_f = 0.87$; testosterone $R_f = 0.60$; methyltestosterone $R_f = 0.70$
- TQ: fluoxymesterone $R_f = 0.09$; nandrolone $R_f = 0.48$; testosterone $R_f = 0.07$; methyltestosterone $R_f = 0.16$
- Visualisation is with ethanol–sulfuric acid or *p*-toluene sulfonic acid solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Benzodiazepines

There are 34 benzodiazepines listed in Schedule 4 of the UN 1971 Convention. Most are now rarely prescribed and abuse is largely restricted to pharmaceutical preparations containing diazepam, flunitrazepam, nitrazepam, flurazepam and temazepam. They may be used in conjunction with opiates (e.g. heroin) or in their own right. A particular problem occurred in Scotland during the mid-1990s when the contents of gel-filled temazepam capsules were injected. Abuse of temazepam declined following the withdrawal of capsules from the market and their replacement with tablets. In other countries, flunitrazepam became the most widely abused benzodiazepine. This drug also gained a certain notoriety for its association with 'date-rape'. For these reasons, flunitrazepam was moved to Schedule 3 of the UN 1971 Convention and is therefore subject to more stringent controls.

Analytical information is presented here only for diazepam, flunitrazepam, nitrazepam and flurazepam.

Analysis of benzodiazepines

Colour test Zimmerman's test: reddish-purple or pink indicates the possiblility of some of the benzodiazepines.

TLC

- TA: diazepam $R_f = 0.75$; flunitrazepam $R_f = 0.63$; nitrazepam $R_f = 0.62$; flurazepam $R_f = 0.68$
- TB: diazepam $R_f = 0.23$; flunitrazepam $R_f = 0.10$; nitrazepam $R_f = 0.30$; flurazepam $R_f = 0.00$
- Visualisation is with acidified iodoplatinate solution.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

GHB and analogues

γ-Hydroxybutyric acid (GHB) was originally developed as an anaesthetic drug and is still used for that purpose in some countries. It acts as a central nervous system depressant and hypnotic, and is chemically related to the brain neurotransmitter GABA (γ-aminoxybutyric acid). Synonyms include sodium oxybate, γ-OH, somatomax, 'GBH' and 'liquid ecstasy'. The effects of GHB have been likened to those produced by alcohol and there are claims that it has anabolic properties. GHB is easily manufactured by adding aqueous sodium hydroxide to γ-butyrolactone (GBL) to leave a weakly alkaline solution. Not only is the precursor GBL widely used as an industrial solvent, it can also be ingested directly to produce the same effects as GHB. Illicit GHB is normally sold in solution as a clear liquid in 30 mL opaque plastic bottles. The typical dose is around 10 mL equivalent to about 1 g or more of GHB. The sodium and potassium salts of GHB are hygroscopic. This property mean that GHB is almost never found as a powder or in tablets.

Analysis of GHB

The legal distinction between GHB and GBL, coupled with the potential for GBL to undergo interconversion with GHB, raises important issues in the analytical approach to GHB analysis. The potential exists for aqueous-based GBL products to undergo conversion to GHB in the time between manufacture and consumption. Some of the factors affecting this interconversion have been explored (Ciolino *et al.* 2001).

Colour test With 1% cobalt nitrate a pink to violet colour is indicative of GHB.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

GC analysis of GHB samples will result in conversion of GHB to GBL, thus necessitating the need for derivatisation prior to analysis (Blackledge, Miller 1991).

Test samples are taken to dryness under a stream of dry air. Samples are then derivatised with 99 : 1 *N*,*O*-bis(tri-methylsilyl)trifluoroacetamide (BSTFA) : trimethylchlorosilane (TMCS) in the presence of pyridine and incubated at 70°C for 30 min.

GHB is detected as the di-trimethylsilyl (TMS) derivative and GBL does not form a silyl derivative.

HPLC can be used without derivatisation (Mesmer, Satzger 1998).

In a study of the reaction of GHB with alcohol it was found that an ester was formed under certain conditions and this can be separated from both GHB and GBL by HPLC and GC (Hennessy *et al.* 2004). Capillary electrophoresis has been used to separate GHB, GBL and 1,4-butanediol (Dahlen, Vriesman 2002). Carbon isotope ratio analysis has been used to examine the discrimination between endogenous and exogenous GHB (Saudan *et al.* 2007).

Khat

Catha edulis is a flowering evergreen shrub cultivated in East Africa and the Arabian Peninsula. The leaves and fresh shoots are commonly known as khat, qat or chat. Khat can be used by chewing the leaves or by brewing as a 'tea' and daily consumption can be up to several hundred grams. Khat has stimulant effects similar to those of amfetamine. Alcoholic extracts (tinctures) of khat have been noted especially in 'herbal high' sales outlets and at music festivals.

The active components of khat, cathinone ((−)-1-aminopropiophenone) and cathine ((+)-norpseudoephedrine), are usually present at around 0.3–2.0% (Lee 1995). Both substances are close chemical

relatives of synthetic drugs such as amfetamine and methcathinone. Khat must be used fresh as the more active cathinone begins to deteriorate rapidly after harvesting. Both cathine and cathinone are scheduled under the UN 1971 Convention, but khat itself is only specifically listed in a few jurisdictions.

Analysis of khat

Approximately 5–6 g of plant material is cut into small pieces. Methanol (15–20 mL) is added and sonicated for 15 min. The green methanolic solution is filtered/decanted and condensed to near dryness. Approximately 20 mL of 0.2 mol/L sulfuric acid is added and the solution acquires a reddish hue. A chloroform extract will remove the neutral organic compounds. The aqueous layer (red layer) is basified with saturated sodium bicarbonate solution. Methylene chloride (20 mL) is added to extract the cathinone and cathine. A stream of air is used to reduce the volume to approximately 1 mL.

Colour test Cathinone gives no reaction with Marquis reagent, but does produce a slow-forming yellow/orange colour with Chen's reagent.

TLC

- TE: cathinone $R_f = 0.46$; cathine $R_f = 0.25$
- Visualisation: UV (254 nm) and 0.5% ninhydrin with cathinone showing an orange colour and cathine a purple colour.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Psilocybe mushrooms

The hallucinogenic substances psilocin and its phosphate ester psilocybin occur in a number of fungi, particularly those of the genus *Psilocybe*. These are small grey mushrooms which grow wild over large areas. Although such material is not in itself controlled, and neither is its cultivation, it has been held in UK case law that the deliberate drying or processing of these mushrooms constitutes preparation of a controlled drug.

Analysis of psilocybe mushrooms

A small quantity (approximately 1 g) of the dried mushrooms is sonicated with methanol (approximately 5 mL) for 10 min. The liquid is removed and reduced in volume at room temperature in an air flow. Psilocybin can be converted to psilocin by heating. This conversion can also occur if the mushrooms are not dried prior to or when they arrive into the laboratory.

Colour test Ehrlich reagent: a violet colour is indicative of psilocybin and psilocin

TLC

- TAN: psilocybin $R_f = 0.34$; psilocin $R_f = 0.59$
- TA: psilocybin $R_f = 0.05$ psilocin $R_f = 0.39$
- Visualisation is with Van Urk's reagent, with both compounds showing a blue/violet colour.

Separation/identification

(For analytical data see Tables 11.5 and 11.6.)

Direct injection of psilocybe mushroom extracts onto a GC column will convert psilocybin to psilocin by thermal dephosphorylation and only psilocin will be detected. Thus prior derivatisation is necessary if psilocybin is to be detected.

To eliminate sugars that may interfere with derivatisation, 1 mL of acetone is added to the methanolic solution and the mixture allowed to stand for 30 min and then filtered. The solution is taken to dryness in a stream of air. Pyridine (15 µL), TMCS (15 µL) and BSTFA (100 µL) are added and heated at 100°C for 30 min.

Psilocin is converted to psilocin di-TMS and psilocybin to psilocybin tri-TMS. LC-MS and LC-MS-MS have also been used to analyse the constituents of magic mushrooms (Kamata *et al.* 2005).

'Designer Drugs'

Although a few ring-substituted phenethylamines (e.g. 2,5-dimethoxy-4-bromoamfetamine (DOB), 4-bromo-2,5-dimethoxyamfetamine) had been subject to limited abuse since the 1960s, it was not until the 1980s that the phenomenon of so-called designer drugs was fully recognised.

Starting in the late 1980s, a large series of designer drugs began to appear, all of which were based on the phenethylamine nucleus. Just as with the production of the major illicit phenethylamines (e.g. MDMA), much of this synthetic activity took place in Europe.

Table 11.7 lists a number of designer drugs that have appeared in Europe and the USA since the mid-1990s. This list, which may not

Table 11.7 Designer drugs reported in Europe and the USA since the mid-1990s

Compound/drug	*Acronym*	*UN/UK*
Ring-substituted phenethylamines		
3,4-Methylenedioxyamfetamine	MDA	+/+
3,4-Methylenedioxymetamfetamine	MDMA	+/+
3,4-Methylenedioxyethylamfetamine	MDE(A)	+/+
4-Bromo-2,5-dimethoxyamfetamine	DOB (Bromo-STP)	+/+
4-Methoxyamfetamine	PMA	+/+
N-Hydroxy-MDA	N-OH MDA	+/+
3,4-Methylenedioxypropylamfetamine	MDPA	−/+
N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine	MBDB	−/+
1-(1,3-Benzodioxol-5-yl)-2-butanamine	BDB	−/+
4-Bromo-2,5-dimethoxyphenethylamine	2C-B	−/+
3,4-Methylenedioxydimetamfetamine	MDDM	−/P
2,5-Dimethoxy-4-(*n*)-propylthiophenethylamine	2C-T-7	−/+
4-Allyloxy-3,5-dimethoxyphenethylamine	AL	−/+
3,5-Dimethoxy-4-methylallyloxyphenethylamine	MAL	−/+
N-Hydroxy-MDMA	FLEA	−/P
2,5-Dimethoxy-4-chloroamfetamine	DOC	−/+
4-Methylthioamfetamine	4-MTA	−/P
2,5-Dimethoxy-4-ethylthiophenethylamine	2C-T-2	−/+
4-Methoxy-*N*-metamfetamine	Me-MA	−/+
6-Chloro-MDMA	—	−/+
N-(4-Ethylthio-2,5-dimethoxyphenethyl)-hydroxylamine	HOT-2	−/+
2,5-Dimethoxy-4-iodo-phenethylamine	2C-I	−/+
4-Methoxy-*N*-ethylamfetamine	—	−/+
***N*-Substituted amfetamines without ring substitution**		
N-Hydroxyamfetamine	N-OHA	−/P
N,N-Dimetamfetamine	—	−/−
N-Acetylamfetamine	—	−/−
Di-(1-phenylisopropyl)amine	DIPA	−/−
Tryptamines		
N,N-Dimethyl-5-methoxytryptamine	5-MeO-DMT	−/+
N,N-Di-(n)-propyltryptamine	DPT	−/+
4-Acetoxy-*N,N*-di-isopropyltryptamine	—	−/−
α-Methyltryptamine	α-MT	−/−
Other phenylalkylamines and miscellaneous		
1-Phenethylamine	1-PEA	−/−
N-Methyl-1-phenethylamine	N-Me-PEA	−/−
4-Methyl-1-phenethylamine	4-Me-PEA	−/−
1-Phenyl-3-butanamine	—	−/−
N-Benzylpiperazine	BZP	−/−
Methcathinone	—	+/+

necessarily be complete, shows that the phenethylamines comprise the largest group. Ring-substituted compounds were more common than *N*-substituted homologues without ring substitution. The substances shown in Table 11.7 have appeared both as powders and as tablets, often manufactured, packaged or marked in such a way that they may appear to the user to be amfetamine or MDMA. Considerable scope exists to develop further series of phenethylamine-related 'designer drugs'. Thus ring-substituted analogues of cathinone and methcathinone might have MDMA-like activity.

As mentioned earlier, the EU, via Europol and EMCDDA, carry out risk assessments on new synthetic drugs. Since 1997 ten risk assessments have been undertaken on the following drugs: MBDB, 4-MTA, GHB, ketamine, *p*-methoxymethamphetamine (PMMA), 2C-1, 2C-T-2, 2C-T-7, TMA-2 and benzylpiperazine (BZP). Various studies have outlined the analytical methods available to separate and identify these and other designer drugs (Blachut *et al.* 2002; Furnari *et al.* 1998; Poortman, Lock 1999; Tsai *et al.* 2006) A detailed chemical identification of DOB has been described using CE, IR spectroscopy and GC-MS (da Costa *et al.* 2007). Raman spectroscopy has been used as a screen for tablets containing DOB (Bell *et al.* 2007).

Piperazines have increasingly become an alternative to amfetamine-derived drugs of abuse (EMCDDA 2005). Among the most prominent of these have been *N*-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and 1-(3-chlorophenyl)piperazine (mCPP). The legal status of piperazines is very inconsistent and many European countries are considering control measures, while others already control mCPP (EMCDDA 2005). In the USA, BZP and TFMPP were temporarily placed into Schedule 1 of the Controlled Substances Act in 2002 followed by final placement of BZP into Schedule 1 2004. In 2003 both BZP and TFMPP were controlled in Japan.

The analysis of aryl-piperazines has been carried out by HPLC and GC-MS with and without derivatisation (de Boer *et al.* 2001; Tsutsumi *et al.* 2005). Analytical profiles covering a range of techniques have been reported for a number of tryptamines (Spratley *et al.* 2005).

The approach to the analysis of these compounds, especially the phenethylamine-related 'designer drugs', could be in line with the general procedure outlined for powders and with specific reference to the analytical procedures employed to analyse MDMA or amfetamine.

Those substances listed in UN 1971 or which are controlled in UK by the Misuse of Drugs Act 1971 are shown by (+) in Table 11.7. Substances pending control in the UK are shown by 'P'. In the USA, unscheduled substances may still be deemed to be controlled by virtue of the Controlled Substances Analogue Enforcement Act 1986.

Conclusion

Many other compounds are encountered in the laboratory as 'drugs of abuse' such as opium, phencyclidine and analogues, tryptamines, barbiturates, methadone, morphine, dihydrocodeine, ephedrine, ketamine and alkyl nitrites. However, the general approach to the analysis of an unknown substance previously outlined should pose no difficulty to the identification of any of these drugs. Analytical information and background information on many of the drugs can be found in some of the general texts in the area (CND Analytical 1994; Cole, Caddy 1995; Gough 1991; Karch 1996, 1998; Klein *et al.* 1989; Redda 1989; Shulgin, Shulgin 1991; Smith 2005; UN 1994; Weaver, Yeung 1995).

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CHAPTER

12 Medicinal Products

AC Moffat and AG Davidson

Introduction

Medicinal products should be safe and efficacious. Manufacturers of medicinal products are required by law to possess marketing authorisations from government regulatory agencies in countries in which their products are marketed and to manufacture their products in compliance with current Good (Pharmaceutical) Manufacturing Practice (cGMP) standards. These requirements include conducting appropriate quality control tests to check that the product conforms to a specification that assures its safety and efficacy. Typical specifications include tests to verify the chemical composition and physical properties of the medicine and to ensure that the medicine is not contaminated by microorganisms or other substances.

Although authentic medicinal products are subject to quality control testing by the manufacturer, they may have been counterfeited, adulterated or stored poorly, and therefore need to be checked for the following purposes:

- Determine or confirm their composition
- Assess their suitability for use
- Investigate defects
- Identify unknown medicinal products
- Identify contaminants
- Determine whether the products have been adulterated
- Establish whether the products are counterfeit.

Independent quality checks of medicinal products may be carried out by official medicines control laboratories as part of a government surveillance programme, and by hospital quality control laboratories, public analyst laboratories and forensic laboratories.

This chapter describes the philosophies, strategies and methodologies for the analysis and testing of medicinal products by laboratories that may not have access to the manufacturers' research and development data or to the manufacturers' test methods, specifications and reference materials for the products. It focuses on the information about the medicinal product that can be derived from other sources and on the conclusions that can then be made about the quality of the product.

Submission of samples and choice of tests

A wide range of tests that utilise a variety of analytical techniques are available to laboratories. Samples submitted for testing should therefore be accompanied by a clear written request about the nature of the investigation required. The provision of relevant background information about the sample, including the reason for the request, allows the laboratory to choose the most appropriate tests, analytical techniques and, where relevant, acceptance criteria (i.e. criteria that allow an objective assessment to be made about the quality of the product).

In most cases that involve checking of the quality of a medicinal product, it is preferable to use the test methods in the product specification defined in the manufacturer's marketing authorisation for the product. This is because the product specifications, including the test methods and acceptance criteria, have been assessed by the relevant government regulatory agency as being valid and justified. Some manufacturers publish their analytical methods in the scientific literature. Consequently, it may be worthwhile to check the literature or maintain a library of published methods for widely used medicinal products. Alternatively, in certain circumstances, manufacturers may provide the laboratory with the test methods, acceptance criteria and reference materials.

If the laboratory does not have access to the authorised finished product specification, including the test methods and acceptance criteria, pharmacopoeial monographs can be used, where applicable (see later). In the absence of a pharmacopoeial monograph, alternative approaches are required to obtain as much information as possible about the quality of the medicinal product. This requires the development of valid test methods to measure relevant quality parameters and the application of generally accepted criteria to assess the quality. However, if the laboratory uses analytical techniques different from those used by the manufacturer (e.g. a high performance liquid chromatographic (HPLC) technique instead of an ultraviolet (UV) spectrophotometric technique), the results may differ significantly from those obtained by the manufacturer, particularly if high levels of impurities are present. This is because analytical techniques differ in their accuracy, precision, selectivity, and/or specificity and sensitivity. It is therefore important to consider the findings in relation to the technique used.

This chapter describes the tests that should be carried out to provide information about different aspects of medicinal products. A summary of the principal tests and techniques for checking known products and investigating unknown products is given in Table 12.1.

Counterfeit medicines

The World Health Organization (WHO) describes counterfeit medicines as part of the broader phenomenon of substandard pharmaceuticals – medicines manufactured below established standards of quality and therefore dangerous to patients' health and ineffective for the treatment of diseases. The difference is that counterfeits are deliberately and fraudulently mislabelled with respect to identity or source. Counterfeiting occurs with both branded and generic products.

Counterfeit medicines may:

- contain no active ingredient
- contain the wrong active ingredient (e.g. a cheap antibiotic instead of an expensive antibiotic)
- contain an incorrect (usually low) quantity of the active ingredient
- be in low-quality packaging
- be manufactured using low-quality active ingredient or excipients
- be manufactured under poor standards of cGMP compliance.

Counterfeit products that contain no active ingredient and those that contain the wrong active ingredient or the correct active ingredient in the wrong amount can be detected by carrying out appropriate identification and quantitative tests (see below). In situations where resources for accurate and precise quantitative testing are limited, for example in developing countries, basic tests, including semi-quantitative tests, may be sufficient to detect these types of counterfeit medicines (see below).

It may also be possible to identify products as being counterfeit by their general appearance (colour, markings, etc.), particularly when the appearance differs from a genuine batch of the product. For this reason, laboratories that regularly undertake checks for counterfeit medicines maintain a stock of genuine reference products for comparison. Examination of the labelling should also be carried out to check the

Table 12.1 Summary of tests and techniques

Purpose	*Test(s)*	*Analytical technique(s)*[a]	*Comment*
1. General check of quality	Test to full specification		Refer to typical monographs for dosage forms in pharmacopoeias
	Identity	See 2 below	
	Assay	See 5 below	
	Homogeneity	Uniformity of content	
	Contaminants	HPLC, GC, TLC, CE, etc.	
	Release of active ingredient	Dissolution test	
	Microbial quality	TVAC, specific microorganisms, sterility test	
2. Confirm identity of product	Instrumental tests for active ingredient (s)	NIR, IR or combination of UV, HPLC, TLC, colour reaction, melting point, etc.	Instrumental checks provide greater assurance about identity
	WHO basic tests	Colour reactions, TLC	
3. Rapid identification of unknown product	Visual comparison of physical characteristics (size, colour, shape, etc.) against library	TICTAC, Identidex, etc.	See Chapter 13
	Simple screening tests	UV, TLC, HPLC, colour tests	See Chapters 32, 39, 41 and 30
4. Unambiguous identification of unknown products	Specific identification tests	IR, NMR, LC-MS, GC-MS, CE, chiral HPLC (for enantiomeric substances), AAS (for inorganic moieties)	See relevant chapters on these techniques
5. Quantification of active ingredient(s)	Assay	HPLC, GC, UV, CE	See relevant chapters on these techniques
6. Homogeneity of active ingredient	Uniformity of content (or mass)	Assay of several (e.g. 10) dosage units or subsamples of the product	Uniformity of content preferred, particularly for unit doses with a content of active substance <25 mg or 25% of the total mass
7. Investigation of reduced efficacy	Assay Dissolution	HPLC, GC, CE	Stability-indicating assay[b] and discriminating dissolution test[c] required
8. Investigation of side-effects or contamination	Drug-related impurities	HPLC, GC, CE, TLC, etc.	
	Solvent residues	GC, GC-headspace	
	Non-drug-related contaminants	Screening techniques, e.g. HPLC, GC, TLC, LC-MS, GC-MS	
	Dissolution (to detect 'dose-dumping' of the active ingredient)[d]		
	Microbial contamination	TVAC, specific microorganisms	
9. Investigation of counterfeit medicines	Rapid identification test	See 3 above	
	NIR and impurity profiling	See 2 above	
	Identification of unknown drugs	See 4 above	
	Identification of manufacturer		

AAS, atomic absorption spectroscopy; CE, capillary electrophoresis; GC, gas chromatography; HPLC, high performance liquid chromatography; IR, infrared; LC, liquid chromatography; MS, mass spectrometry; NIR, near infrared; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography; TVAC, total viable aerobic counts; UV, ultraviolet.
[a]Most tests require extraction of the active ingredient before application of the analytical technique.
[b]A stability-indicating assay is selective for the active ingredient in the presence of decomposition products.
[c]A discriminating dissolution test distinguishes between batches with different bioavailability.
[d]Dose-dumping is release of the active ingredient at a rate faster than intended.

accuracy of the information presented. Labels of products made by counterfeit manufacturers may contain errors that are evident to an expert. The quality of the packaging materials and of the printing should also be inspected carefully and, if possible, compared with that of a genuine batch of the product. Genuine products may contain anti-counterfeiting devices (e.g. holograms) that are difficult to copy.

Many counterfeit products are manufactured under unsatisfactory conditions, which may result in defects that are detectable visually. For example, black marks on sugar-coated tablets may arise from the use of an excessive temperature during the coating process, which chars the sugar. A high level of particulate matter or fibres in parenteral and ophthalmic preparations may indicate poor environmental control during the manufacturing process. Inadequate or faulty sealing of the container or packaging may indicate the use of inappropriate equipment. Physical defects alone may not prove that the product is counterfeit, but their presence may corroborate other indicators of the counterfeit nature of the product.

Direct comparison of products

The direct comparison of an authentic sample and the questioned product is a quick and easy way to detect counterfeit medicines. In this respect, near-infrared (NIR) spectroscopy (see Chapter 34) is rapidly becoming an indispensable analytical technique used by many manufacturers to detect counterfeits of their products. The power of the technique lies in its ability to fingerprint intact products qualitatively and, in favourable circumstances, quantitatively. Anti-counterfeiting application of the technique involves the preparation of a library of

NIR spectra of genuine batches of the product within a pre-defined range of variables. With the correct mathematical transformations, NIR spectra are specific for the medicinal product. This is because NIR spectra depend not only on the active ingredient but also on the nature of the excipients. It may be possible to put the correct amount of active ingredient in a product, but getting the right grade and percentage of excipients in the product is very difficult. The spectra are also very sensitive to changes in particle size, moisture levels and other variables. The authenticity of a batch of a product can be proved by obtaining a good spectral match with the reference library within the defined range of variables. Conversely, failure of a suspect sample to provide a good spectral match indicates that it may be a counterfeit product.

Figure 12.1 shows an example of the use of NIR spectroscopy to compare two questioned batches of Plavix tablets 75 mg (containing clopidogrel as hydrogensulfate) with tablets from an authentic batch. Figure 12.1a shows the authentic and the first questioned batch to be the same, whereas Fig. 12.1b clearly shows the second questioned batch to be very different from the authentic batch and therefore counterfeit.

NIR spectroscopy is not very good for aqueous samples, but ultraviolet (UV) spectrophotometry can easily be used for direct comparison of medicinal products in these cases. Figure 12.2 shows a comparison of an authentic and a question batch of Cosopt Eye Drops which contain: dorzolamide as the hydrochloride 2%, timolol as the maleate 0.5% and benzalkonium chloride 0.0075%. Both spectra show peaks for the two active pharmaceutical ingredients and are almost superimposable, demonstrating that the questioned product is almost certainly authentic.

The usual questions concerning a medicinal product are: does it contain the correct active ingredient and is it at the right amount? Methods to answer these questions are given in the next two sections and basic tests are covered in the following section. Methods for

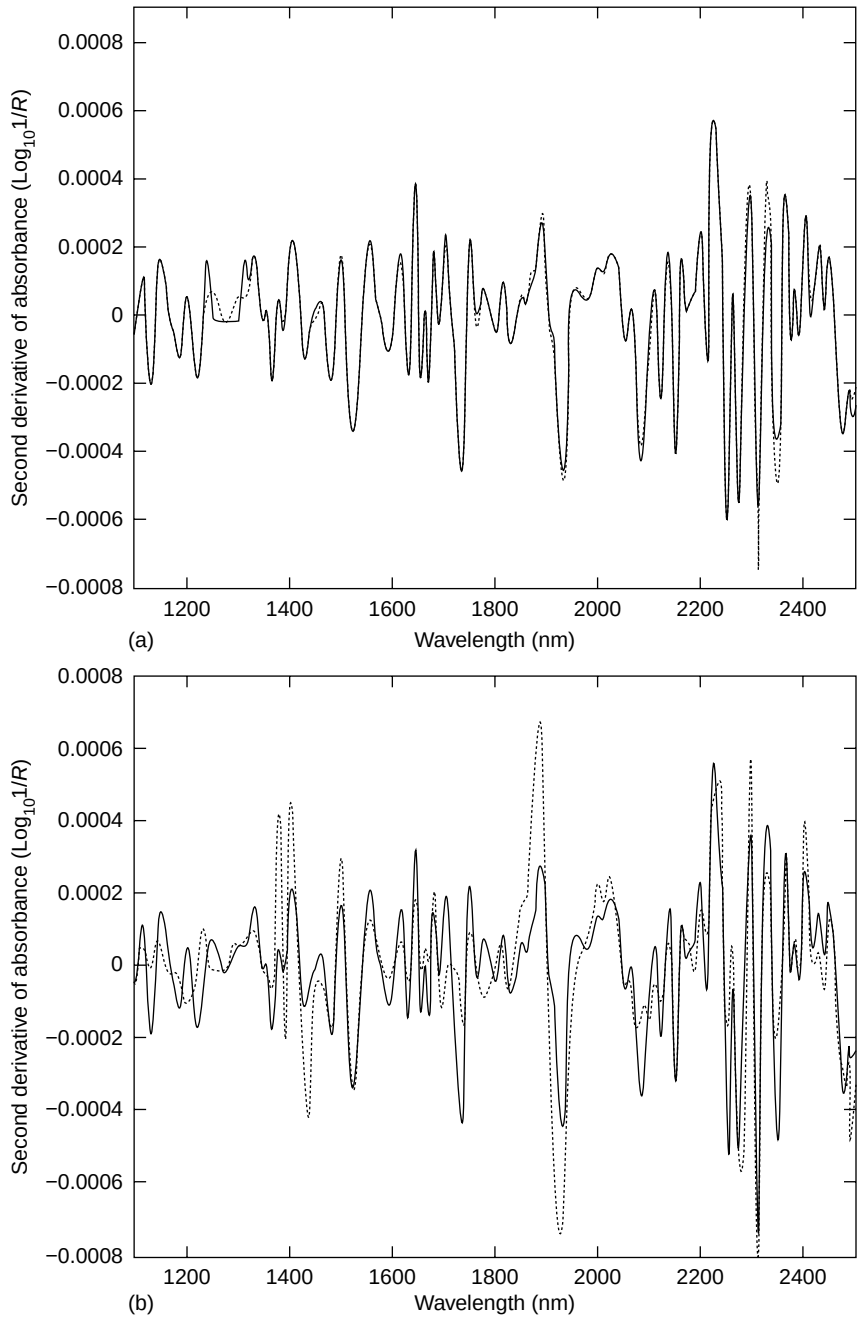

Figure 12.1 Second derivative near-infrared spectra of Plavix tablets 75 mg. (a) Authentic tablets (solid line) and questioned tablets of a different batch which turned out to be authentic (dotted line); correlation coefficient 0.970. (b) Authentic tablets (solid line) and counterfeit tablets (dotted line); correlation coefficient 0.718.

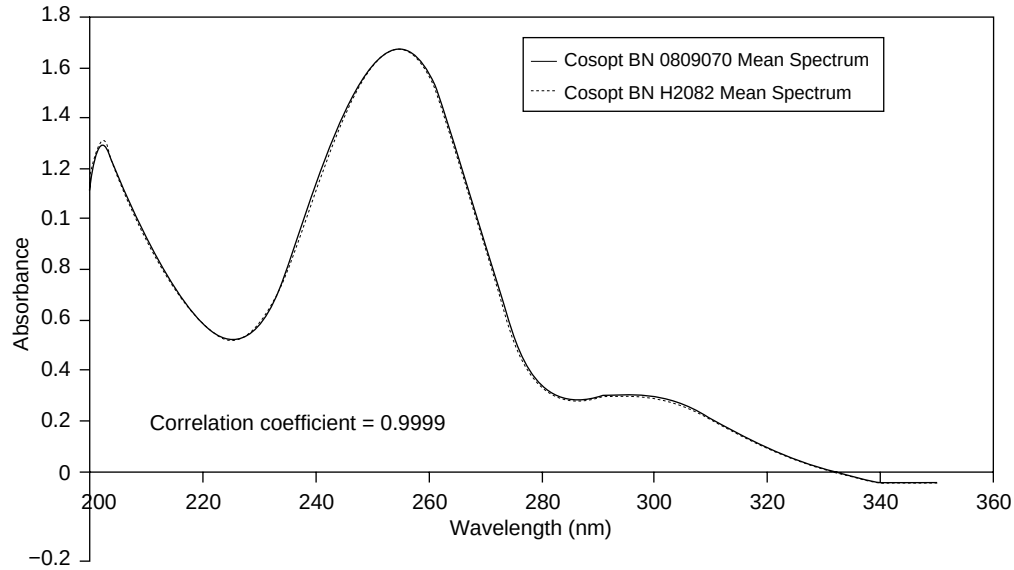

Figure 12.2 Ultraviolet spectra of Cosopt Eye Drops diluted 1 : 1000 in ethanol: authentic eye drops (solid line); questioned eye drops of a different batch which turned out to be authentic (dotted line), correlation coefficient 0.9999; the two spectra are therefore almost totally superimposable. Cosopt Eye Drops contain dorzolamide as the hydrochloride 2%, peak at 255 nm; timolol as the maleate 0.5%, peak at 295 nm; and benzalkonium chloride 0.0075%, no significant absorption due to its low concentration.

profiling medicines in order to compare them, e.g. to substantiate a charge of trafficking, follow.

Identification of active ingredients

The first step in the analysis of a medicine is usually to verify that it does contain the correct active ingredient(s). A visual examination of the medicine should be made (colour, size, shape, marking, etc.) to verify the identity of the product and the label should be examined for information about the active ingredient(s) (see Chapter 13).

The identity tests in the registered finished product specification or pharmacopoeial specification generally are suitable for verifying the identity of the active ingredient. Identity tests in pharmaceutical specifications are not necessarily intended to be absolutely specific, but should be able to distinguish between similar active ingredients. For example, the identity tests in the European Pharmacopoeia (Ph. Eur.) monograph for glycerol now include a test for refractive index to differentiate glycerol from diethylene glycol (DEG). An incident in Haiti in the mid-1990s, in which DEG had been substituted for glycerol in a paediatric product, resulted in the deaths of over 100 children. In 2007 the Food and Drug Administration (FDA) reported the detection of batches of counterfeit toothpaste containing DEG.

Infrared (IR) spectroscopy is one of the most commonly applied analytical techniques for identification. The IR spectrum of the drug substance extracted from the medicinal product should be concordant (in terms of the position and relative intensities of the absorption bands) with a reference spectrum of the substance or with the spectrum of a reference substance (see Chapter 33). For substances with a highly characteristic 'fingerprint' spectrum in the 1600–400 cm^{-1} region, concordance of the spectra provides a high level of assurance about the identity of the active ingredient. Alternatively, a combination of non-selective identity tests can provide a similar level of assurance. For example, in the Ph. Eur. monograph for the drug substance atenolol, verification of the identity of the drug is carried out by IR spectroscopy alone or, alternatively, by a combination of melting point test, thin-layer chromatography (TLC) and UV spectrophotometry. When using these three tests, none of the tests alone is sufficiently selective, but the combination of the three tests provides the level of assurance required. The British Pharmacopoeia (BP) monograph for atenolol injection uses only an IR spectrophotometric test carried out on the residue obtained by extracting the active ingredient from the injection into chloroform followed by drying and evaporation of the solvent. It is more difficult to obtain a pure residue of atenolol from atenolol oral solutions (e.g. syrups) because colouring and flavouring agents in the formulation may co-extract with atenolol and interfere in the IR spectrum. The BP monograph for atenolol oral solution, therefore, uses both TLC and HPLC to verify the identity of the active ingredient.

Identity tests should also be able to distinguish optical isomers and different salts and polymorphic forms of the drug substances. An optical rotation test often suffices to distinguish enantiomers from each other and from a racemic mixture of the two enantiomers. Chiral HPLC (see Chapter 41) may be used to verify the enantiomeric nature of the wanted enantiomer and quantify the unwanted enantiomeric impurity.

The presence of counter-ions of acidic and basic drugs can be confirmed in the bulk substance by standard pharmacopoeial tests for chloride, phosphate, citrate, sodium, calcium, aluminium, etc. When IR and/or melting point tests do not distinguish different polymorphic forms of a drug substance, it may be necessary to use a radiographic diffraction technique.

When the identity of the medicinal product is not stated on the label or on documentation that accompanies the sample, or when the appearance of the medicine does not correspond to that stated on the label, its identity might be determined in a number of ways.

The combination of colour, size, shape, breakbars, markings, etc., of solid oral dosage forms is reasonably characteristic of the medicine, and several systems are available to identify the medicine based on its physical characteristics (see Chapter 13).

When it is not possible to identify the medicine from its appearance (e.g. because of the absence of distinctive colour, shape and markings), physicochemical tests should be carried out to determine the qualitative and quantitative composition of the active ingredients. The chromatographic screening techniques described in this book (TLC, HPLC and gas chromatography (GC)) are particularly useful for this purpose.

Identification of 'rogue' tablets in a container may help to determine whether the rogue tablets are the result of poor manufacturing practices or of malicious adulteration of the product after it has been released onto the market. If the rogue tablets prove to have been manufactured at the same site as the 'correct' tablets, a breakdown of manufacturing standards is indicated. However, if another producer made the rogue tablets, malicious contamination may be more likely.

Medicinal products that are simple aqueous solutions containing active ingredients, with or without excipients, may be injected directly,

or after appropriate dilution with the mobile phase, into a liquid chromatograph. The relative retention time or retention index may allow the active ingredient(s) and excipients to be identified tentatively. Excipients in solid dosage forms and semi-solid dosage forms (e.g. creams and ointments) and in viscous liquids, which are likely to be present in high concentrations in relation to the amount of active ingredient, can interfere in identification tests. Consequently, separation of the active ingredient(s) from the excipients should be carried out to 'clean up' the sample prior to analysis. The choice of solid-phase extraction (Chapter 29) or solvent extraction depends on the relative polarities of the active ingredients and excipients. Extraction of drugs from solid dosage forms can often be carried out with aqueous acid, aqueous alkali or methanol alone, followed by filtration of the extract.

If the extracts show absorption bands in the UV or visible region, reference to the wavelengths of maximum absorption in the individual drug monographs or the Index of Ultraviolet Absorption Maxima may provide a rapid tentative identification of the active ingredient. Confirmatory identification tests should then be carried out by other test methods such as IR spectroscopy, TLC, HPLC and melting point determination.

For viscous liquids and semi-solid dosage forms, it may be necessary to carry out a liquid–liquid extraction to separate the active ingredient(s) from the excipients. The screening extraction procedure described in Chapter 29 (solvent extraction procedure) should be used, followed by a range of screening spectroscopic and/or chromatographic tests.

If forensic standards of proof of identity are required, for example to support litigation or regulatory or enforcement action, a specific identification test should be employed, such as mass spectrometry (MS; see Chapter 37).

Quantification of active ingredients

The content of the active ingredient(s) in a medicinal product is one of the most important quality parameters that assure its efficacy. As with other tests in a specification, if the laboratory does not have access to the assay method specified in the marketing authorisation or if there is no relevant pharmacopoeial monograph for the product, a suitable assay should be developed. It may also be necessary to quantify excipients that have a critical bearing on the safety or efficacy of the product (e.g. preservatives).

Commonly used techniques for the assay of pharmaceuticals that offer the optimum combination of accuracy, precision, selectivity, sensitivity, robustness, speed and accessibility are HPLC (Chapter 41), GC (Chapter 40) and UV spectrophotometry (Chapter 32). Capillary electrophoresis (CE; Chapter 42) and NIR spectroscopy (Chapter 34) are used increasingly for certain applications. Although NIR methods are usually published for the assay of active ingredients in intact proprietary medicines, there is an increasing use of NIR applications for generic medicines, e.g. paracetamol tablets (Shek *et al.* 2006) and ibuprofen tablets (Lo *et al.* 2007). Reference should be made to the relevant chapters in this book and to the Further reading list of this chapter.

As stated previously, the selectivity of different spectrometric and chromatographic techniques may differ markedly, particularly when there are high levels of impurities that are chemically related to the active ingredient. Consequently, care is required in the interpretation of results in relation to the test method employed.

Basic tests for drug substances and products

Some developing countries do not have access to fully equipped laboratories and the use of rapid, basic tests offers a cost-effective alternative approach to the detection of substandard medicines, including counterfeit products. The WHO has published two books to assist such laboratories: *Basic Tests for Drugs* (WHO 1998), which includes pharmaceutical substances, medicinal plant materials and dosage forms, and *Basic Tests for Pharmaceutical Substances* (WHO 1986). The basic tests described, which are designed to verify the identity of drug substances and medicinal products and to detect gross contamination, use a limited range of readily available reagents and equipment.

Tests to verify identity are based on a combination of organoleptic checks and simple physicochemical tests, such as colour reactions ('test-tube methods') and melting point determinations.

Basic tests for degradation products should be carried out for drug substances and drug products that are unstable (e.g. adrenaline (epinephrine) and ampicillin), particularly under conditions of high temperature and humidity, which may occur during transportation and storage. These tests, which are generally based on simple colour reactions, detect only gross contamination.

Semi-quantitative TLC methods have been developed as basic tests using a limited number of solvent systems and detection systems. References to these tests are given in the WHO (1998) text. The tests involve the extraction of one unit dose of the product. For quantification, the spots are compared with reference spots that represent acceptable upper and lower limits (e.g. 80% and 120% of the nominal strength). A single test is, therefore, sufficient to verify (or disprove), with reasonable certainty, the identity of the product and to indicate whether its content is within acceptable limits. Portable test kits based on this principle have been developed for use under non-laboratory conditions (e.g. for testing at importation centres).

It should be emphasised that basic tests are intended not to replace pharmacopoeial requirements but to provide a rapid, inexpensive means to verify identity and strength and possibly detect poor-quality counterfeit and other substandard products. When suspect products are detected, they should be tested for compliance with pharmacopoeial requirements (see below).

Profiling

The intention of this kind of analysis is to compare two medicinal products to show that they have a common origin, identify the synthetic route or determine the site of manufacture. NIR spectroscopy is particularly good at comparing medicinal products because it looks at the whole matrix of the product and not just at the active ingredient. It is relatively easy to compare authentic and counterfeit products (see above), but it is also possible to differentiate generic products containing the same amount of active ingredient (Fig. 12.3) or to identify the site of manufacture of proprietary products (Yoon et al. 2004).

'Impurity profiling' involves the determination of the pattern of drug-related impurities present in a medicinal product. The impurity profile (e.g. by HPLC or GC) of the active ingredient in a genuine product is reasonably consistent from batch to batch because of the consistency of the manufacturing process. If a sample of a product shows a different impurity pattern, this may be through the use of drug substance produced by a different synthetic route and/or active ingredient manufacturer and may indicate that the product is counterfeit.

Impurity profiling may also allow the manufacturer of the drug substance to be identified. This involves developing a separation method that distinguishes ('fingerprints') the impurity profiles of a drug substance from different manufacturers. The chromatographic profiles from a number of genuine batches from each manufacturer are expressed quantitatively by an appropriate mathematical technique, such as cluster analysis. The same technique can also be applied to products that contain the drug substance, provided that the excipients do not significantly interfere with the impurity pattern of the drug substance. If a sample of a medicinal product has an impurity profile that does not match that of the authorised manufacturer for the product, it may be a counterfeit product or may have been manufactured using a non-authorised source of the active ingredient.

In an actual case that involved antibiotic oral preparations from different manufacturers, the drug-related impurity profiles were similar because all the manufacturers used the same synthetic route. However, the 'fingerprint' of solvent residues detected by headspace-GC with a nitrogen–phosphorus detector was sufficiently characteristic to allow the synthetic process and the manufacturer to be identified.

When the use of a single technique is insufficient to differentiate all the manufacturers of a drug substance, a combination of analytical

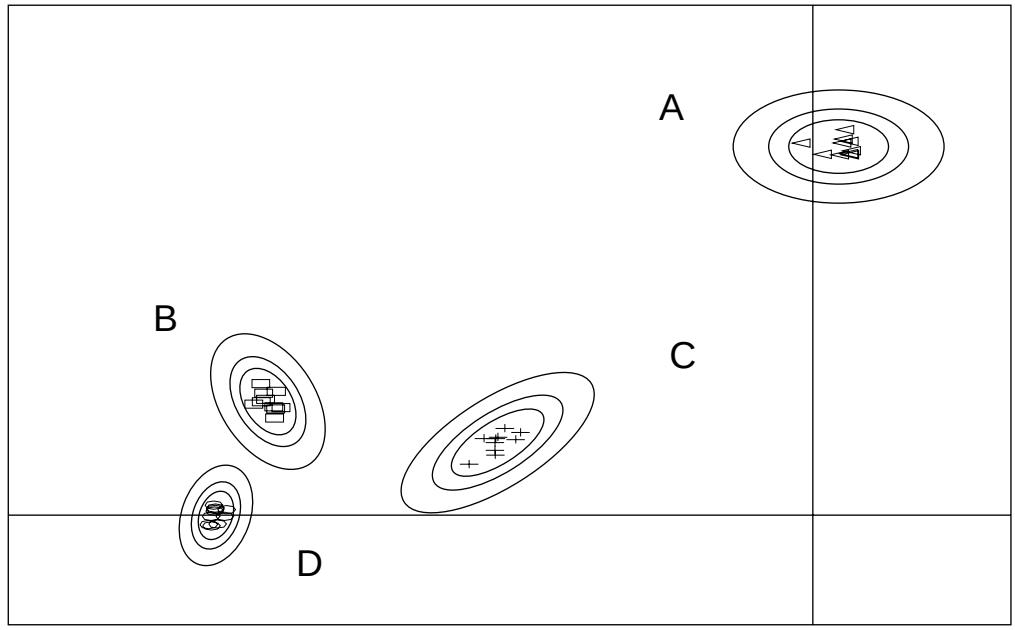

Figure 12.3 Near-infrared centre of gravity plots of four different manufacturers of allopurinol tablets 100 mg. The equal frequency ellipses for a given manufacturer are 0.95, 0.99 and 0.999 (inner, middle and outer respectively). The scales for the axes are $X_{min} = -0.015\,760$, $X_{max} = 0.073\,238$; $Y_{min} = -0.027\,013$, $Y_{max} = 0.108\,089$. Range = 1750–850 nm.

techniques may be used, for example HPLC for drug-related impurities and headspace-GC for volatile impurities. When the drug substance contains inorganic elements, inductively coupled plasma (ICP) spectroscopy and/or atomic absorption spectroscopy (AAS) can be used to quantify the pattern of inorganic impurities.

General tests

In addition to tests for identity and assay (see above), and for contaminants (see below), specifications may include tests to check the uniformity of the batch of medicinal product and its fitness for use. Examples of these tests include:

- Uniformity of mass and uniformity of content of solid dosage forms
- *In vitro* disintegration and dissolution testing of tablets and capsules
- Particle size testing of medicines in which particle size of the active ingredient can affect the bioavailability of the product
- Viscosity tests on creams and ointments
- Sterility tests for parenteral and ophthalmic products
- Tests for microbiological quality of non-sterile products
- Friability testing of tablets to ensure that they will withstand normal handling during transportation of the product and use by the patient
- Aerodynamic assessment of fine particles in inhalation products (e.g. pressurised metered-dose inhalation preparations).

These tests are described in pharmacopoeias (see below).

Defective samples

Defective medicines may be reported to a laboratory because they do not work as they should, or the tests described above may indicate a counterfeit or substandard medicine. Medicines may be defective in their chemical composition, physical properties (which may affect the safety and efficacy of the product) or microbiological quality. When medicines are suspected of being defective, appropriate tests should be carried out to investigate the defect.

Low potency

If a batch of a medicine is suspected to have reduced efficacy, it should be tested for content (see above) and release of active ingredient by a suitable dissolution test. The assay should be able to indicate stability (i.e. it should be selective in the presence of degradation products, to detect loss of potency that arises from decomposition of the active ingredient in the dosage form).

The pharmacopoeias describe dissolution tests for tablets, capsules, suppositories and transdermal patches. These may be used to compare a complaint sample suspected of having low efficacy with a sample from a 'good' batch of the medicinal product. Immediate-release tablet and capsule preparations that comply with the general criteria of a pharmacopoeia (e.g. the BP requirement of not less than 75% of the content of active ingredient being released within 45 min) are usually satisfactory. A significant difference in dissolution rates between a complaint sample and the 'good' sample may explain the observation of reduced efficacy, particularly if the complaint sample releases less than 75% in 45 min.

If the results of the assay and dissolution test do not confirm the suspicion of reduced potency, then further tests should be carried out to investigate:

- Polymorphism of the active ingredient
- Uniformity of the content of the active ingredient
- Particle size of the active ingredient.

Contaminants

Products with a high incidence of unwanted side-effects should be investigated initially for their content of active ingredient and impurities, as high concentrations of these may result in an unacceptable toxicity and account for the observed effects.

Impurities arise in medicinal products from a variety of sources, which include the synthetic starting materials, reagents, reaction by-products, synthetic intermediates and decomposition products. These are often referred to as 'related substances' and can usually be detected chromatographically (HPLC, GC, CE or TLC) using suitable detection systems. If samples of the impurities are not available from the manufacturer or other sources, it may be possible to synthesise them. For example, decomposition products of the active ingredient can be generated by hydrolytic, oxidative or photochemical reactions. Process-intermediate impurities can be synthesised if the synthetic route of the active ingredient is known. Alternatively, a comparison of the chromatographic profile of the complaint sample can be made with

that of a sample of a 'good' batch of the product. Unknown impurities found to be present may be identified by coupling the chromatographic separation with mass spectrometry (Chapter 37).

Residual solvent impurities may be present as a result of incomplete solvent removal during synthesis and recrystallisation. The presence of some solvent residues (e.g. isopropyl ether) may impart an unpleasant taste or smell to the medicinal product, which may discourage patient compliance. Some solvents are toxic even at very low levels, or are environmental hazards, and are controlled to very low concentrations in medicinal substances. For example, benzene and other International Conference on Harmonisation (ICH) Class 1 solvents (see Further reading) should be avoided in pharmaceutical manufacture. If their use is unavoidable, they should be controlled to very low levels in drug substances (e.g. benzene, 2 ppm; carbon tetrachloride, 4 ppm; and 1,1-dichloroethene, 8 ppm). Less toxic solvents (Class 2) and those with low toxic potential (Class 3) should still be controlled to a level that depends on the permitted daily exposure of the solvent. GC, with or without a headspace analyser, is used frequently to determine residual solvent residues (see Chapter 40).

A much more difficult analytical problem is the identification of organic impurities, present in small quantities, that are unrelated to the active ingredient and its synthetic route. These may arise from accidental cross-contamination with other drug substances or products manufactured at the same site (an indication of poor compliance with cGMP) or, in extreme cases, by malicious adulteration or contamination of the active ingredient or finished product. A full screening programme, involving chromatographic and spectrometric instrumentation, including 'hyphenated' techniques (e.g. HPLC-MS), should be used to isolate, identify and quantify the unknown contaminant.

Mineral impurities, including simple inorganic ions and heavy metals, may give rise to problems of safety and quality if they are present in certain types of products. For example, heavy metals and certain other ions (e.g. aluminium and nickel) may be toxic when administered parenterally in high doses or over long periods. Metallic ions (e.g. Fe^{3+}, Cu^{2+}, Co^{3+}) may catalyse oxidative decomposition reactions of the active ingredient (e.g. adrenaline, morphine and phenothiazine drugs), which can result in the formation of toxic and/or coloured impurities.

Inorganic impurities are determined by flame atomic emission spectrometry or AAS, or by the general pharmacopoeial test methods for heavy metals and sulfated ash. For more selective and/or sensitive measurements, electrothermal AAS, ICP spectroscopy, ion chromatography or atomic fluorescence spectrometry may be employed. (see Chapter 17).

Acceptable limits for impurities that assure the drug's safety depend on the toxicity of the impurity, daily dose and duration of treatment of the drug, and its route of administration. The very wide range of toxicities means that acceptable limits vary from the parts per million level to several per cent. For example, the limit for the highly toxic impurity hydrazine in povidone BP is 1 ppm. In contrast, the total limit for related impurities in vincristine sulfate BP is 5%. Typical limits for individual impurities are 0.1–0.5% and for total impurities 0.2–1%.

If an unknown impurity is found in a significant quantity, say greater than 0.1% expressed on an area percentage basis by HPLC or GC, an attempt should made to identify it. HPLC or GC, coupled with MS, may be used for this purpose (see Chapter 37).

For samples in which there are high levels of known impurities or in which new impurities or contaminants have been identified, it may be necessary to carry out a toxicological assessment of the impurities before the reported side-effects can be attributed conclusively to the impurities. If the results of the assay and tests for impurities do not explain the observation of side-effects, the dissolution profile of the complaint sample should be compared with that of a 'good' batch of the product. In some products, for example digoxin tablets (Beckett, Cowan 1973) and carbamazepine tablets (Davidson 1995), the use of micronised active ingredients resulted in very fast dissolution rates of the active ingredient ('dose dumping') and in reported incidents of adverse reactions in patients.

Physical defects

The laboratory should undertake a thorough visual examination for physical defects as part of the sample receipt procedure. If one or more physical defects are detected in a batch of medicinal product, the batch is usually withdrawn or recalled from the market, even if the visual defect does not directly affect the safety and efficacy of the product. If visual defects are observed, appropriate tests should be carried out to investigate the cause. Some examples are given below.

If the colour of a batch of a medicinal product differs from that of other batches of the product, or if the colour is uneven throughout the batch, the most likely explanations are the use of the wrong active ingredient or colouring agent (which indicates a serious breakdown of the manufacturer's quality management system), formation of coloured decomposition products, lack of homogeneity or microbiological contamination.

Verification of the identity of the active ingredient and/or colouring agent can be checked as described above. The formation of coloured decomposition products should be investigated by chromatographic tests for related impurities and a stability-indicating assay of the medicinal product. The former test is likely to yield the greater amount of information because even trace amounts of some intensely coloured decomposition products can impart a significant colour to medicinal substances and products.

Gross microbial contamination may be evident visually from microbial growth in aqueous or semi-solid products or from the appearance of light or dark areas on solid products. The pharmacopoeial tests for total viable aerobic counts (TVACs) and specified microorganisms are much more sensitive than visual detection and should be carried out whenever microbial contamination is suspected or as a general check of microbiological quality. If significant numbers of organisms are found, it may be necessary to carry out microbial identification tests to determine whether the microorganisms are harmful.

A significant proportion of broken tablets may indicate a manufacturing defect. The pharmacopoeial test for friability of uncoated tablets is intended to determine the extent of breakage or surface damage under defined conditions of tumbling the tablets in a drum. A maximum loss of 1% of the tablet mass after 100 revolutions is considered acceptable for most products.

The pharmacopoeial test for resistance to crushing of tablets (hardness) can also be used to measure the force needed to disrupt the tablets by crushing. The hardness of tablets is a property of the tablets, but the value measured depends on the type of instrument used to measure the hardness. Acceptable limits, expressed in newtons, are wide. Nevertheless, a comparison of the hardness of the complaint batch with a 'good' batch of the same product may indicate significant differences, which may account for the damage observed.

Breakage of the tablets may also be the result of the ingress of moisture. This can be investigated by comparison of the moisture level in the complaint sample with that of a 'good' batch of the product. If a significantly higher moisture level is found, the effect on the stability of the active ingredient should be investigated by using a chromatographic test for decomposition products (see Contaminants).

Foreign bodies

Visual examination of the medicine may reveal the presence of extraneous matter, which may be inorganic, organic or biological in origin. The appearance of the extraneous matter, including its microscopic appearance, may give a clue to its general identity. Visible fragments of glass, metal and entomological parts are generally easy to isolate from the sample, which allows a more specific identification to be carried out.

To investigate the origin of glass or metal fragments, the inorganic composition should be compared with that of the container and, if possible, the equipment used in the manufacture of the medicinal product (e.g. mills, tablet presses).

Vegetable, animal and insect fragments can usually be identified microscopically, which may also indicate the source of the contamination. In an actual case that involved the presence of insect fragments in a

medicinal product manufactured in the UK from vegetable drugs imported from Asia, the insect was entomologically identified as one indigenous only to Asia. This proved that the source of the extraneous matter was the vegetable drug ingredient and not the local environment at the UK manufacturing site.

The identity of organic extraneous matter can be determined by the usual techniques for the identification of contaminants (see under Contaminants). The task is relatively straightforward if crystals of the organic contaminant can be isolated from the bulk of the sample prior to identification.

Pharmacopoeial specifications and methods

Pharmacopoeias are good sources of the specifications and standards with which a wide range of commonly used drug substances, excipients, packaging materials and finished products must comply throughout their shelf-life. The principal pharmacopoeias are the European Pharmacopoeia (Council of Europe 2007), British Pharmacopoeia (Medicines and Healthcare products Regulatory Agency 2008) and the United States Pharmacopeia (USP; US Pharmacopeial Convention 2006). National pharmacopoeias of some countries, for example Japan, China and individual European countries, may provide specifications for pharmaceuticals that are available only in those countries. The International Pharmacopoeia (Ph. Int.) published by the World Health Organization (WHO 2006) is intended for use by WHO member states that wish to establish pharmacopoeial requirements for pharmaceuticals used in their country.

The specifications in monographs of pharmacopoeias are intended to be applicable to drug substances, excipients and finished products from different producers. Although the monographs contain the essential tests that provide verification of the suitability of the product for use, the monographs do not attempt to control unique aspects of individual products. Consequently, drug substance and finished product specifications in marketing authorisations of individual products may be based on the requirements of a relevant pharmacopoeia, but may also contain additional tests or tighter acceptance criteria. This is particularly true of finished product specifications for the release of batches of products onto the market (release specifications are used in Europe, but not in the USA). For these, tighter acceptance criteria may be required at the time of release to ensure that the product remains within specification during its shelf-life. Since samples tested by a laboratory are often obtained from the marketplace, the shelf-life acceptance criteria in pharmacopoeias provide a suitable means by which the quality of medicinal products can be judged.

Pharmacopoeial monographs for medicinal products provide good models upon which the testing of non-pharmacopoeial products should also be based when a general check of their quality is required. Typical pharmaceutical specifications for dosage forms include the following types of tests to provide assurance of their quality, safety and efficacy:

- Identity test to confirm the identity of the active ingredient(s) and, if appropriate, other important constituents (e.g. preservatives)
- Assay of the active ingredient(s) and, if appropriate, other constituents
- Homogeneity (e.g. uniformity of content for tablets and capsules)
- Release of the active ingredient from the formulation
- Drug-related impurities and other contaminants
- Microbiological tests (e.g. sterility tests for injectable and ophthalmic products
- Other relevant tests that assure quality.

Sources of information

Pharmacopoeias contain useful chemical, physical and microbiological information about drug substances, excipients and dosage forms. Examples include molecular structures and formulae, relative molecular masses, melting and boiling points, spectroscopic data and solubilities in various solvents.

The appendices in the Ph. Eur. and BP and the general chapters in the USP are useful texts that provide information on, for example:

- Methods of sterilisation
- Production of vaccines
- Containers
- Materials for the manufacture of containers
- Storage conditions to ensure stability of the product.

Supplementary chapters of the BP and general information chapters of the USP provide information on a range of diverse subjects, such as:

- Pharmacopoeial philosophy on the control of impurities
- Systems for nomenclature
- Guidance on the interpretation of results of microbiological assays
- *In vivo* bioequivalence guidelines
- Biotechnology-derived articles.

Reference standards

Approximately 3000 different pharmaceutical reference materials are available from the BP, Ph. Eur., USP and WHO for a variety of different purposes. Chemical reference substances (CRSs) are used, for example, for the following:

- Comparison of spectra in identification tests
- Standards in comparative assays
- Identification and quantification of specific impurities
- Calibration of instruments (e.g. dissolution apparatus and melting point apparatus).

The suffix CRS (e.g. BPCRS, EPCRS, USPCRS or ICRS (International Pharmacopoeia)) indicates that the CRS has been issued by the relevant pharmacopoeia. Similarly, certain biological reference preparations (BRPs) are available (e.g. from the Ph. Eur. as European Pharmacopoeia Biological Reference Preparations (EPBRP)) for the biological assay of antibiotics (microbiological assay) and hormones, blood products, and immunological and other biological products.

It is important to emphasise that a pharmacopoeial reference substance should be used only for the purpose for which it is described in the pharmacopoeia. For example, a pharmacopoeial CRS used only to confirm the identity of a drug substance in the bulk material or in dosage forms by IR spectroscopy or TLC may not have been purified exhaustively, although it will be satisfactory for use in the identity test. Such a standard should not be used in a selective assay (e.g. an HPLC assay) without first assigning a purity value by an appropriate method.

Sources of chemical and biological reference materials are listed at the end of this chapter.

Test methods

Pharmacopoeial methods should always be considered when the testing of pharmacopoeial articles is required. Even if the test methods in the marketing authorisation differ from those in the relevant pharmacopoeia, the article must also comply with the standards of the pharmacopoeia, including the use of the pharmacopoeial test methods, if it is covered by a monograph in the pharmacopoeia.

In most circumstances, pharmacopoeial methods can be assumed to be valid for the materials covered by the monographs. This is because they are intended for use by a wide variety of laboratories, sometimes on an infrequent basis. One of the criteria for the selection of the methods is that they should be robust. Nevertheless, it is good practice to carry out additional tests (e.g. a recovery test) to demonstrate that the method is suitable for the sample.

If a test method is required for a pharmaceutical product for which there is no pharmacopoeial monograph, it may be possible to use or adapt the test methods in a monograph of a similar material. In these circumstances, however, relevant validation tests should be carried out, such as recovery, precision, robustness, selectivity and/or specificity and (for trace amounts, e.g. impurities) limits of detection and quantification, to provide confidence in the suitability of the adapted method.

Pharmacopoeial monographs generally do not rely on a single analytical technique, but instead contain a number of tests based on different analytical methodologies (e.g. identity tests that use spectroscopic techniques and colour reactions, tests for impurities using chromatographic techniques and assays using a non-selective titrimetric or UV spectrophotometric technique). Pharmacopoeial authorities consider that this approach provides the optimum level of assurance about the quality of the product. For many substances and products it is possible to combine the identity test, tests for impurities and assay into a single chromatographic test. Indeed, many manufacturers, including those of pharmacopoeial products, adopt this approach for economic reasons. For similar reasons, a laboratory may choose to develop a single chromatographic test to check identity, impurities and assay in surveillance programmes that involve large numbers of samples. However, it is important to weigh the cost benefits of this approach against the risks of failure to detect substandard products (e.g. those that contain impurities not detected by the single test).

Pharmacopoeial monographs may contain, in addition to product-specific test methods, a number of general tests applicable to a wide variety of materials in the pharmacopoeia. Examples of these are melting point and boiling point determination, heavy metals test, sulfated ash test (test for residue on ignition in the USP), disintegration testing of solid dose products, tests for microbial contamination, preservative efficacy tests, sterility tests and the Karl Fischer determination of moisture. These pharmacopoeial methods have been accepted as standard test methods and, consequently, can be applied by laboratories to a wide range of non-pharmacopoeial materials, provided that appropriate validation has been carried out.

Instrument performance standards

Pharmacopoeial standards for the performance of analytical instruments and testing equipment in most circumstances provide adequate assurance about the general suitability of the equipment for the testing of non-pharmacopoeial products also. These standards include:

- Wavelength, absorbance and resolution checks for spectrophotometers
- Accuracy of pH meters
- Repeatability and symmetry factors in HPLC and GC.

Acceptance criteria

Acceptance criteria in some pharmacopoeial tests are specific to the article covered by the monograph; for example, limits for specific, named impurities in drug substances and dosage forms and limits for water in hydrated substances. However, the acceptance criteria in many other tests are general and are not dependent on the article; for example:

- Limits of 95–105% for the content of active ingredients in medicinal products that are not subject to significant decomposition
- A disintegration time of not more than 15 min for uncoated tablets
- A limit of not less than 75% of the content of active ingredient released after 45 min from tablets or capsules under the conditions of a discriminating dissolution test
- Limits for uniformity of mass for tablets, capsules, suppositories and powders for injection
- Limits for microbial contamination of different types of pharmaceutical preparations
- Limits for the efficacy of antimicrobial preservatives.

These general limits are useful for assessing the quality of non-pharmacopoeial articles as well.

Biological and biotechnological products

Biological products are medicines that contain biological substances that cannot be characterised fully by physicochemical tests. Consequently, in addition to physicochemical tests, biological tests (e.g. a biological assay) are needed to characterise the product. Biological medicines are classified according to their modes of activity:

- Vaccines
- Hormones
- Antisera
- Coagulation factors.

Biotechnological medicinal products are proteins, polypeptides or their derivatives generated by living organisms. They are classified according to their mode of preparation:

- Monoclonal antibodies derived from the fusion of homologous or heterologous cells
- Products derived from recombinant DNA (rDNA) technology
- Products derived from transformed continuous cell lines.

Examples of biotechnological products are human insulin, erythropoietin and growth factor (which are natural products), and novel products (e.g. proteins with modified sequences and gene therapy).

Biotechnological and biological products are generally more complex than chemically synthesised small-molecule drugs, which have a well-defined structure and can be characterised readily by physicochemical tests in terms of their composition, purity and content. The production of biological and biotechnological products from living systems requires very careful control to ensure safety, homogeneity throughout the batch and batch-to-batch consistency. Factors such as the type and stability of the cell systems, culture conditions, the use of continuous or batch processes, and purification conditions are critical to assure the safety, quality and efficacy of the product. Even minor changes in the production process can have profound effects, for example the introduction of potent contaminants or changes in the stability of the product. The use of a new strain of microorganism and a minor process change in the fermentation production of L-tryptophan resulted in over 30 deaths and hundreds of cases of eosinophilia–myalgia syndrome in 1989. The process change introduced a new, highly toxic impurity, 1, 1-ethylidene bis-tryptophan (Belongia *et al.* 1990), which was not controlled by the specification in use at that time.

The pharmacopoeias contain monographs for several biological and biotechnological substances and products including:

- Alteplase for injection (a tissue plasminogen activator)
- Erythropoietin (a mixture of glycoproteins indistinguishable from naturally occurring erythropoietin)
- Human insulin and interferon alfa-2.

All of these are derived from rDNA technology. In a case study, a batch of somatrophin (a protein produced by rDNA technology, having the same structure as the main component of the growth hormone produced by the human pituitary) was shown to be counterfeit by application of the Ph. Eur. monograph for somatrophin (Charlton *et al.* 1998).

Characterisation of biological and/or biotechnological products includes the determination of physicochemical properties, biological activity, immunological properties, tests for purity and impurities, and quantity.

Physicochemical tests used to characterise biological substances and products include:

- Determination of amino acid sequence
- Amino acid composition
- Terminal amino acid sequence
- Peptide mapping
- Number and position of sulfhydryl groups and disulfide bridges
- Carbohydrate structure.

Other physicochemical tests may provide additional information about the properties of the materials, including determination of relative molecular mass, isoform pattern (e.g. by isoelectric focusing), molar absorptivity, and electrophoretic, liquid chromatographic and spectrometric profiles. Identity tests in specifications should be highly specific and focus on unique aspects of the molecular structure and other physicochemical, biological and/or immunochemical properties.

The biological character of the material should be defined in terms of its biological effects and, for antibodies, its immunochemical effects. Biological activities are measured by bioassays, such as animal-based bioassays (which measure the response of an organism to the product), cell culture-based bioassays (which measure biological responses at the cellular level) or biochemical assays (e.g. measurement of enzymatic reaction rates).

The potency (expressed in units) of the material, as determined by bioassay, is calculated relative to the biological response of an official reference preparation (e.g. EPBRP) or a well-characterised in-house reference preparation.

Tests for process impurities and other contaminants include immunoassay for protein impurities derived from the host organism. For the control of product-related impurities, including degradation products, tests based on chromatographic, electrophoretic, MS and circular dichroism techniques may be employed. An appropriate combination of tests to control impurities should be included in the specifications.

Testing of biological and biotechnological medicines requires special instrumentation and analytical expertise that may not be available in laboratories normally engaged in the testing of conventional medicines. It may be necessary, therefore, to refer the testing of such products to specialist institutes that have the ability to undertake the appropriate tests. Further information on biological testing is contained in the BP and the USP.

Herbal and homeopathic products

Herbal remedies have seen a revival in recent years. They comprise a wide range of botanical materials (roots, leaves, seeds, bark, etc.), herbal preparations (extracts, tinctures, oils, resins, etc.) and herbal medicinal products (tablets, creams, etc.).

Herbal remedies are difficult to characterise fully because the production of the active constituents is dependent on natural processes. Genetic and environmental factors, therefore, may result in a wider variation of the constituents of herbal products compared with those of biotechnological products, the manufacturing processes of which are strictly controlled.

As few herbal products have been subjected to rigorous safety (toxicological) and efficacy (clinical) studies, protection of the public depends on adequate measures to control the quality of the products. Monographs of quality standards for herbal products are now included in the *British Pharmacopoeia* 2010 (Medicines and Healthcare products Regulatory Agency 2008) and have been published in the *British Herbal Pharmacopoeia* (British Herbal Medicine Association 1996), the *European Pharmacopoeia* (Council of Europe 2007) and the *Escop Monographs* (European Scientific Co-operative on Phytotherapy 2003). Regulatory guidance on the setting of specifications for herbal medicinal products has been published by the European Medicines Agency Committee on Herbal Medicinal Products (2007). These monographs should be used whenever possible.

Identification tests

Identification tests for herbal products should discriminate between related botanical species and potential adulterants, and should include verification of the microscopic and macroscopic characters, chromatographic (e.g. TLC or HPLC) tests and chemical reactions.

Tests for potential contaminants

General pharmacopoeial tests for foreign matter in vegetable drugs, ash and acid-insoluble ash are useful for determining the presence of adulterants such as inorganic matter and dirt. A test for water content should be included when the herbal drug is known to be hygroscopic. Pesticide residues should be checked using the method described in the European Pharmacopoeia.

Microbial contamination levels generally are higher in herbal products than in synthetic materials because plants have their own microbial flora and are able to sustain microbial growth. It is important, therefore, to carry out tests for total viable organisms (bacteria, yeasts and moulds). The limits given in guidelines for microbiological quality of pharmaceutical preparations (category 4) in the Ph. Eur. can be used to assess this aspect of the quality. The microorganisms detected by the test for TVAC generally are not pathogenic, but the number provides an indication of the general microbiological 'cleanliness' of the product. From a safety point of view, the presence of specific pathogenic microbes, such as *Escherichia coli*, *Salmonella* spp. and enterobacteria, may be more relevant. The test methods for these organisms and the acceptance criteria of the Ph. Eur. should be used to assess this parameter.

Although high levels of bacteria and of yeasts and moulds generally do not present a health risk, their presence under certain circumstances can lead to contamination by microbial toxins. Bacterial endotoxins, which are released from the cell walls of Gram-negative bacteria during the sterilisation of parenteral products by autoclaving, can induce pyrogenic symptoms such as fever, pain and headache. High levels of certain mycotoxin-producing fungi, in particular aflatoxin-producing strains (e.g. *Aspergillus spp.*), may lead to high levels of the mycotoxins being formed during storage under warm, humid conditions. Aflatoxins are particularly dangerous as they are mutagenic and carcinogenic and can cause hepatitis. Tests for bacterial endotoxins (limulus amoebocyte lysate (LAL test)) are described in the pharmacopoeias.

Quantitative tests

The active chemical constituents of herbs vary widely and include alkaloids, glycosides, tannins and volatile oils. However, the active chemical constituents of some medicinal herbs (e.g. valerian) have not been elucidated fully. Most herbs contain several active constituents that vary in composition, depending on the cultivation conditions. To ensure that herbs are of an appropriate quality, quantitative tests should be sufficiently selective and sensitive for the active constituents, requirements usually met by TLC or HPLC.

Ethnic herbal remedies

The availability of ethnic herbal remedies, for example traditional Chinese medicines (TCMs) and ayurvedic medicines, presents additional challenges to the regulatory authorities and to laboratories. The problems associated with controlling TCMs result mainly from the large number of ingredients in many TCM formulae and from the risk of contamination and confusion about nomenclature, which may result in the accidental or deliberate substitution of one herb by another. Substitution is particularly important when a relatively non-toxic herb can be confused with, or substituted by, a more toxic herb. The incorporation of herbal ingredients into products should be subject to adequate quality control, including identification tests, to ensure the use of the correct ingredient and the absence of adulteration.

In a serious incident in Belgium in 1993, over 100 women who had been prescribed a TCM at a slimming clinic developed progressive renal failure, which resulted in renal replacement therapy in 60% of the women and in renal carcinomas. The nephrotoxicity of the slimming treatment was traced to inadequate quality control: 80–90% of the *Stephania tetranda* powder (Pin Yin name *han fang ji*) in the slimming preparation was inadvertently substituted by *Aristolochia fangchi* powder (Pin Yin name *guang fang ji*). The toxic elements of *Aristolochia* species are aristolochic acids (substituted nitrophenanthrene carboxylic acids), which are potent immunostimulant, mutagenic, carcinogenic and nephrotoxic agents (Vanherweghem 1998). Many countries have now banned the use of *Aristolochia spp.* and other botanical species, such as certain *Stephania* spp. and *Akebia* spp., with which *Aristolochia* may be confused.

Another problem encountered is the deliberate addition of synthetic drug substances to TCMs, an example of which is the addition of potent corticosteroids (e.g. triamcinolone, dexamethasone and fluocinolone acetonide) to Chinese herbal cream formulations used for eczema. These can be investigated by a HPLC screening test for corticosteroids using UV detection at 240 nm (see Chapter 41).

A particular problem with ayurvedic medicines and some TCMs is the presence of high levels of toxic heavy metals. These may arise from the general contamination of the environment with pollutants (cadmium, lead, mercury), but more usually from the deliberate addition of heavy metals and/or arsenic to the formulation. Tests for these elements can be carried out by a multielement technique, such as ICP spectroscopy (see Chapter 17).

Homeopathic preparations

The nature of homeopathic products precludes tests for the identity and content of the active ingredient because of their high dilution. Consequently, only general tests can be performed on homeopathic products (e.g. uniformity of mass of solid dosage forms and tests for microbial contamination). However, quality standards and tests for the homeopathic stocks and mother tinctures from which these products are made are increasingly becoming available in pharmacopoeias, e.g. the BP 2010. Identification tests rely predominantly on macroscopical description and TLC. Tests for foreign matter are included where there is a hazard for contamination.

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Pharmacopoeial reference substances

For Chemical Reference Substances produced for use in physical and chemical tests in pharmacopoeias, see below.

British Pharmacopoeia. www.bpclab.co.uk

European Pharmacopoeia: www.edqm.eu/site/Ph_Eur_Reference_Standards-627.html

The International Pharmacopoeia: www.apl.apoteket.se/Svenska/who/index.htm

United States Pharmacopeia: www.usp.org/referenceStandards

Pharmaceutical, chromatographic and forensic reference materials

LGC Standards, Queens Road, Teddington, Middlesex, TW11 0LY, UK; Tel: +44(0) 20 8943 7565; Fax: +44(0)20 8943 7554; URL: www.lgcstandards.com

CHAPTER

13 Solid Dosage Form Identification

J Ramsey

Introduction

History

Tablets and capsules are a surprisingly modern development. Thomas Brockedon was granted a patent in 1843 'for manufacturing pills and medicinal lozenges by causing materials, when in a state of granulation, dust or powder, to be made into form and solidified by pressure in dies'. In 1884, Burroughs Wellcome & Co. applied for the term 'tabloid' to be made a registered trademark. It is said that Henry Wellcome (1853–1936) derived this word from 'tablet' and 'alkaloid' and used it to mean highly effective drugs in a compressed, concentrated form. The British Pharmacopoeia (BP) of 1885 included a monograph for Glyceryl Trinitrate Tablets. No others appeared until 1945 when the Seventh addendum to the BP of 1932 still contained only 35 tablets. Tablets and later dosage forms, such as gelatin or starch capsules, and sugar-coated tablets (dragées), proved to be particularly well suited to mass production.

Tablets and capsules are now commonplace and a large number of consumer products are available in this form, not only medicines but also products such as sweeteners (sugar substitutes), confectionery (mints), detergents and even toys (indoor fireworks, jumping beans).

Legitimate medicines supplied as tablets and capsules, such as the barbiturates (Tuinal, Seconal), methaqualone (Mandrax, Quaalude) and amfetamines (Drinamyl) were commonly misused in the 1960s. Legitimate capsules were also emptied and used to conceal heroin in the 1970s. Illicit drugs were rarely seen as tablets or capsules until the early 1990s, when the drugs now commonly known as ecstasy flooded the club–dance scene, first in Europe and later the USA. Currently, many millions of illicitly made tablets that contain methylenedioxymetamfetamine (MDMA) or its homologues and analogues are consumed each week. This 'normalisation' of tablets as an illicit dosage form has resulted in an expansion of illicitly produced tablets that contain other drugs, principally amfetamine and metamfetamine, in other countries, particularly in South East Asia (Thailand, Myanmar).

The need for tablet and capsule identification arises for many reasons in both health care and law-enforcement practice. Treatment of the poisoned patient may be assisted by identification of the product responsible, even though most patients are treated symptomatically. The agent responsible for the poisoning, if a tablet or capsule, may often be identified more rapidly than the diagnosis can be made by the analysis of body fluids. However, the tablets found alongside the patient are not necessarily the same as those that were taken. Pharmacy practice generates many identification enquiries, such as the rogue tablet found in a tablet bottle or the customer who wants 'some more of these' but does not know what they are. In Europe, 'parallel imports' are common, because it is cheaper to import some medicines from other countries. The imported drugs, even if manufactured by the same company, usually have a different physical appearance from the familiar native product. Patients, when prescribed a generic form of a medicine, might receive an unfamiliar product of foreign origin and request confirmation that an error has not been made. A constantly changing melange of products in circulation also results from takeovers and mergers within the pharmaceutical industry (when the physical appearance of products is changed to meet new corporate identity standards). Consequently, patients, pharmacists and law officers are continually faced with unfamiliar products.

Changes in legislation may also generate new reasons to perform identifications; for example, when anabolic androgenic steroids came under the Misuse of Drugs Act in the UK in 1996 both the Police and Her Majesty's Customs and Excise (now HM Revenue and Customs) were suddenly required to be able to recognise them.

The misuse of pharmaceutical products, such as the benzodiazepines (particularly temazepam and diazepam), and an illicit drug scene in which users like to experiment, results in solid dosage forms being much more prevalent. Drug-facilitated sexual assault may also involve drugs supplied as tablets and capsules (e.g. flunitrazepam). The trading across national boundaries via the Internet of so-called 'lifestyle drugs' used to treat obesity (e.g. phentermine, orlistat), impotence (sildenafil, vardenafil, tadalafil), baldness (finasteride) or smoking cessation (bupropion) also results in enquiries to identify unfamiliar products.

The emergence of 'herbal highs' or 'party pills' sold on the Internet and containing mostly piperazine derivatives, many of which are not subject to legal controls, has resulted in many more recreational drugs available as tablets or capsules.

The current 'harm minimisation' strategy to counteract drug misuse, which involves informing users, potential users and parents honestly about the risks of drug misuse, has alerted parents and those responsible for the welfare of youngsters (e.g. teachers) to the possibility that those in their charge might be in possession of illicit (illegal) drugs. In turn, this has meant that many more enquiries are made to health care professionals regarding the identification of suspect materials. Many enquiries are made to community (retail) pharmacists regarding 'tablets' found in the possession or proximity (bedrooms, school playgrounds) of young people.

Some confectionery may coincidentally resemble medicines and some may even be deliberately designed to resemble drugs (such as sugar cigarettes). Small white tablets marked with a P, an O or an L purported to be the holes from the centre of Polo Mints caused much concern when they were found out of context, such as in school playgrounds (Ramsey 2001; Young 1997).

Dosage forms

Pills

Pills are an outdated dosage form seldom encountered in modern pharmacy practice. Technically, a pill is a small rounded mass, usually handmade, and is intended to be swallowed whole. However, the word is in common usage as a generic term for all solid dose forms, e.g. ecstasy pills, slimming pills or contraceptive tablets ('the pill'). Some branded products still use the word, but usually referring to small sugar-coated tablets (Beechams Pills, Ex-Lax Pills).

Tablets

Tablets are compressed dosage forms that contain both active ingredients and inactive materials called excipients, which include diluents, adhesives, binders, fillers, lubricants, disintegrants and colours. Tablets need to be strong enough to withstand transport and handling yet still disintegrate and release the active ingredients when consumed. The behaviour of the tablet depends on the production methods, the various excipients and their effect on the active ingredient. The physical form of the active ingredient (particle size) and the particular salt are also important.

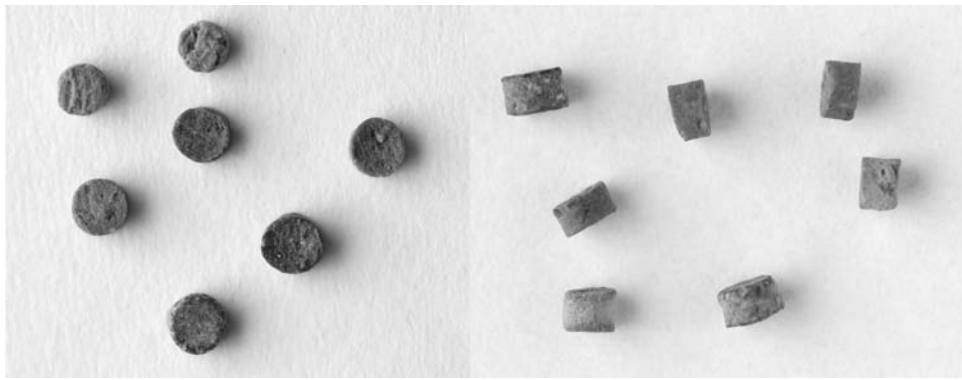

Figure 13.1 LSD 'microdots' made using perforated metal sheet. Reproduced with permission from TICTAC Communications.

The identification of a tablet is usually based on the physical appearance or the analytical determination of the active drug and seldom depends upon the nature of the excipients. However, examination of the gastric contents, after gastric lavage (now less favoured) or *post mortem*, with a polarising microscope for the presence of starch granules may indicate the recent consumption of tablets or capsules.

Illicit tablets

Seizures of millions of illicit tablets, mostly of the drug 'ecstasy', have been made in many countries, (e.g. the UK, The Netherlands, USA, China, Poland). These tablets are usually marked with distinctive logos that often belong to large commercial organisations (automobile manufacturers, mobile phone makers, fashion designers), or represent cartoon characters or simple text or currency symbols.

The investigation of the illicit production of the synthetic phenethylamines (MDMA, amfetamine) may require the examination of the clandestine 'laboratories' that produce the active ingredient as well as tablet-making facilities. Information may be gleaned on the synthetic route to the active substance and batch characteristics by analysis of the impurity profile of the finished tablet.

Moulded tablets

Small numbers of tablets may be made by moulding, a similar process to that once used in pharmacies. The active ingredient, a diluent and some form of binder (gum or adhesive) are pressed into a mould and allowed to set. Perforated metal sheet or even pegboard may be used as the mould. This dose form was common for lysergide (LSD) in the UK in the 1970s. Small 'microdots' (2.4 mm in diameter and 1.3 mm thick, weighing 5 mg: Fig. 13.1) were produced in large numbers using sheets of perforated metal.

The product shown in Fig. 13.2 is from a London club's 'amnesty bin'. The tablet is made from plaster-of-Paris to resemble an ecstasy tablet, but it contains no active ingredients.

Figure 13.2 Fake ecstasy tablet made from plaster-of-Paris, taken from a London club's 'amnesty bin'. Reproduced with permission from TICTAC Communications.

Compressed tablets

Legitimate products

Modern commercial compressed tablets are made by highly automated machines that use multiple sets of dies and punches in clean-room conditions. Strict quality-assurance procedures ensure that the product is manufactured to close tolerances, so there is little variation in physical appearance (size, weight, colour), content of active ingredient and bioavailability.

Illicit tablets

Small numbers of tablets may be made using drilled metal plates and hammer-driven punches or by screw-driven hand presses. Most illicit tablets are made by similar machines to those used by the legitimate manufacturers, but in very different conditions (Fig. 13.3). The equipment needed to satisfy the demand for ecstasy tablets (estimated at one

Figure 13.3 Commercial tablet machine used to produce illicit tablets. Reproduced with permission from TICTAC Communications.

Figure 13.4 Diazepam tablets that appear to be professionally made but are of unknown origin. Reproduced with permission from TICTAC Communications.

million each week in the UK) has to be highly automated. The machines may be in poor condition and the dies and punches may be damaged and leave characteristic visible imperfections on the tablets that they produce, which may provide valuable intelligence. The so-called 'ballistic' examination of tablets for these imperfections (using techniques like those for the examination of firearms and ammunition) by forensic laboratories was once common, but is no longer carried out routinely (in the UK).

The variability of dimensions and dose of illicitly produced tablets is often much greater than that found in legitimate tablets, despite the use of similar machinery. Tablets pressed in the same die must all have the same diameter, but can vary greatly in thickness depending on the fill weight and the degree of compression. This may affect both the dose and the disintegration time and hence the bioavailability of the active compound.

Coated tablets

Tablet cores may be coated with layers of sucrose, a thin film of resin or a soluble wax. This may be: to make the medicine more palatable; to protect the active ingredient from exposure to air, light or moisture; to delay the absorption until it reaches the small intestine; or to extend the duration of action. Products may also be coated to give them a more attractive appearance. Sugar-coated tablets vary more in dimensions (both size and weight) than uncoated or film-coated products. Markings printed on sugar-coated tablets may often rub off quite easily. The sugar coating also tends to 'round off' the edges of the core, so that sharp edges become rounded. Sugar-coated tablets may closely resemble confectionery in both appearance and taste, at least initially, and may be consumed inadvertently by children who believe them to be sweets (candy).

Counterfeits

There are many examples of counterfeit or substandard pharmaceutical products that are fraudulently labelled with respect to their source or content. Their use has resulted in failure of treatment or even poisoning (Reidenberg, Conner 2001). Less surprisingly, products of uncertain origin appear on the drug-abuse scene. Diazepam tablets that appear to be professionally made and of foreign origin are commonly in circulation in the UK (Fig. 13.4), but neither the manufacturer nor the importer is known.

Anabolic agents in ampoules and tablets, purportedly from reputable manufacturers, have been detected that do not contain the active ingredient stated on the packaging. For example, the counterfeit product shown in Fig. 13.5 was found to contain testosterone and not boldenone as stated on the packaging, where the word steroid was incorrectly spelled.

Illicit tablets of similar appearance, probably made in the same machine, may also contain different active ingredients. Examples of the tablet shown in Fig. 13.6 have been found to contain methyltestosterone, methandrostenalone or clenbuterol.

Individual legitimate pharmaceutical tablets may also be modified by hand to look like other products and so increase their value. The half-scored tablet in Fig. 13.7 had a Y-shaped marking (similar to the Mercedes motor manufacturer's logo) carved on to make it resemble an ecstasy tablet.

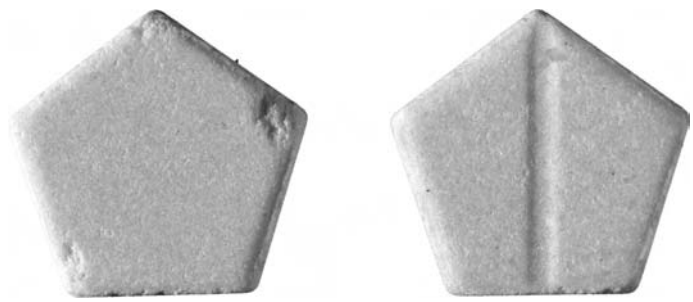

Figure 13.6 Illicit tablet seen with different active ingredients (methyltestosterone, methandrostenalone or clenbuterol). Reproduced with permission from TICTAC Communications.

Figure 13.5 Counterfeit anabolic steroid (Boldebal-H) packaging with a spelling mistake. Reproduced with permission from TICTAC Communications.

Figure 13.7 A legitimate tablet modified to look like an ecstasy tablet. Reproduced with permission from TICTAC Communications.

Capsules

Capsules are a popular consumer dosage form because their slender shape makes them easy to swallow, and they effectively mask any unpleasant taste or odour of their contents. Most capsules consist of two parts, a cap and body, and are made from gelatin. These shells are made using precision-machined moulds, to which the liquid gelatin adheres, forming both the cap and body sections. High-speed machinery removes the gelatin shell layers from the moulds and then joins, prints and packages them. The two parts may be the same or different colours, or may be transparent.

Capsule filling

Small numbers of capsule shells may be filled by hand; highly automated filling machines are able to fill many thousands of capsules per hour. The contents may be powder, controlled-release granules, herbal material or, less commonly, liquid.

Capsule printing

Capsules are usually marked, although the printing may rub off, particularly on oil-filled soft gelatin products. The printing may not be consistently on the cap or body (Fig. 13.8). This is particularly noticeable when the cap and body are different colours.

Special capsules

Special capsules made from cellulosic (hydroxypropylmethylcellulose) raw materials and printed with natural printing inks satisfy vegetarian and cultural (e.g. kosher) needs. Wider-diameter capsules are designed for double-blind clinical trials to allow containment and blinding of large-diameter or uniquely shaped tablets.

Very small gelatin capsules are used for oral dosage to rodents in preclinical studies and are specially designed for performing preclinical trials, such as pharmacokinetic, pharmacodynamic and safety studies.

Liquid-filled capsules

Soft gelatin capsules are commonly used to contain liquid or semi-liquid preparations (Fig. 13.9). Some have abuse potential. Encapsulated liquid preparations of temazepam had to be modified and subsequently discontinued because the contents were being withdrawn with a syringe and needle and injected intravenously, intramuscularly or even intra-arterially, which caused severe lesions (Dodd *et al.* 1994).

Figure 13.8 Examples of capsule showing marking reversed on cap and body. Reproduced with permission from TICTAC Communications.

Figure 13.9 Soft gelatin capsule containing liquid. Reproduced with permission from TICTAC Communications.

Special two-piece hard gelatin capsules may be sealed for secure containment of liquids and semi-solids. The overlapping portion is moistened during the filling process and the cap and body effectively weld together as they dry. Two-piece capsules (Fig. 13.10) may be secured with a sealing band of shellac to prevent leakage of, or tampering with, the contents.

Transdermal devices

Potent drugs, with suitable solubility characteristics, that require extended dosing may be given in preparations designed to deliver the drug through the intact skin (transdermally; Table 13.1). The dosage forms may be creams, sprays or patches that resemble adhesive wound dressings (Elastoplast, Band Aid). Patches are usually either transparent or in colours to match skin tones. Some are marked with names or codes, while others are completely unmarked.

The misuse of transdermal patches that contain fentanyl has been reported; fentanyl may be extracted from these patches and used as a source of the drug for injection or they may even be dried and smoked (Marquardt, Tharratt 1994; Purucker, Swann 2000).

Novel preparations

There are many novel dosage forms, some of which are used when the fast or slow controlled release of an active ingredient is required.

Melts

The product Maxalt Melt Wafer 10 mg contains the antimigraine drug rizatriptan. Although approximately 13 mm in diameter, it weighs only 64 mg and is intended to be taken without water (Fig. 13.11).

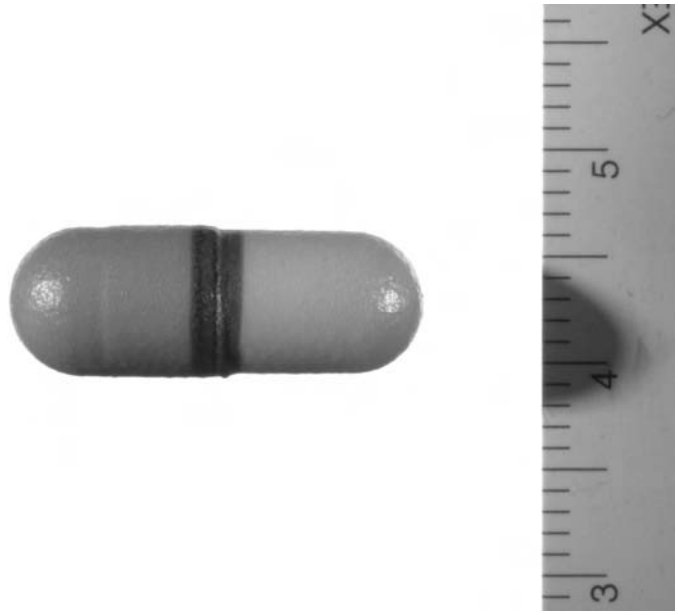

Figure 13.10 Cap and body capsule with sealing band (scale in centimetres). Reproduced with permission from TICTAC Communications.

Table 13.1 Preparations designed to deliver the drug through the intact skin

Preparation	*Function*
Drugs delivered in transdermal devices	
Estradiol	Hormone replacement
Testosterone	Hormone replacement
Norethisterone (norethindrone)	Hormone replacement
Nicotine	Tobacco withdrawal
Glyceryl trinitrate (nitroglycerin)	Antianginal
Fentanyl	Analgesic
Hyoscine (scopolamine)	Motion sickness
Other compounds in patches	
Citronella oil	Insect repellent
Tropical orchid aroma	Appetite control
Capsicum (cayenne pepper)	Counter irritant
Arnica montana (leopard's bane)	Counter irritant
Methyl salicylate	Analgesic
Ferrite magnet	Pain relief?

Figure 13.11 Maxalt Melt Wafers, anti-migraine treatment designed to be taken without water. Reproduced with permission from TICTAC Communications.

Slow-release products may have cores that do not disintegrate in the gut and may be found intact at postmortem examination in stomach contents. These cores are also found in other circumstances, such as in homes for the elderly and psychiatric institutions, with the outer coating removed (presumably after having been spat out).

The Adalat tablet (Fig. 13.12) has such a core (Fig. 13.13); it is a complex device with a laser-drilled hole that is clearly visible in a yellow layer. The core is revealed when the coating, which is pink, is removed; this is a common identification enquiry.

Figure 13.12 Adalat tablet (coated). Reproduced with permission from TICTAC Communications.

Figure 13.13 The Adalat tablet shown in Fig. 13.12 with coating removed. Reproduced with permission from TICTAC Communications.

Figure 13.14 LSD-impregnated paper sheet with a pattern that covers multiple doses (the small square is a single dose). Reproduced with permission from TICTAC Communications.

Veterinary products

Relatively few veterinary medicines for use in large animals are supplied as tablets or capsules because it is usually easier for a veterinary practitioner to administer an injection to an animal. However, treats and worming preparations and medicines for domestic animals are commonly sold as tablets. Animal treats may have markings reminiscent of those on ecstasy tablets.

Some products may be very large: Coppinox 27 copper supplement capsules for cattle are 24 mm long and weigh over 25 g.

Impregnated paper

LSD is commonly supplied impregnated into small absorbent paper 'stamps', which usually carry a distinctive marking. Large sheets are perforated so that they may be torn into individual doses on quarter-inch or 7 mm squares. They are impregnated by dipping the sheet into a solution of the drug in a solvent, often vodka or ethanol. More rarely the solution of the drug may be applied to the individual paper squares on the sheet with a dropper. The pattern may be complete for each square or may extend across multiple squares or the whole sheet (Fig. 13.14). The patterns are classified by law enforcement agencies by a Prototype number. LSD has also been impregnated in gelatin squares (window panes) or on sugar lumps and supplied as moulded tablets.

Other very potent illicit drugs (e.g. 4-bromo-2,5-dimethoxyamfetamine (DOB)) may also be seen, though rarely, impregnated into paper.

Confectionery

Some confectionery is commonly mistaken for illicit drugs. Sweets, mainly mints, may look like medicines or illicit drugs, particularly when out of the original packaging (Fig. 13.15).

Figure 13.15 Confectionery that resembles ecstasy tablets. Reproduced with permission from TICTAC Communications.

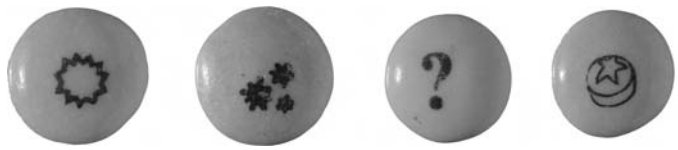

Figure 13.16 Sugar-coated chocolate drops. Reproduced with permission from TICTAC Communications.

Figure 13.17 Sweeteners that are mistaken for drugs. Reproduced with permission from TICTAC Communications.

Sugar-coated chocolate drops (Smarties, M&Ms) may also be confused with sugar-coated tablets, particularly when they bear markings for special promotions (Fig. 13.16). The TICTAC database (see later) contains 120 different Smarties.

Sweeteners (sugar substitutes) in tablet form regularly result in identification enquiries when they are found in or near food or out of context in schools or public places (Fig. 13.17).

Examination of unknown products

Unnecessary submissions and expensive laboratory time may be saved if solid dosage forms can be recognised from their physical appearance. They may be identified with a high degree of confidence if they have sufficiently characteristic features, if the appearance can be accurately described and if an appropriate database is available.

The method of identification used depends upon why the identification is required, the speed with which a result is needed, the nature of the unknown and the resources available. When a rapid identification is required for clinical purposes, and if the unknown is likely to be a legitimate pharmaceutical, it is usually sufficient just to locate it in a commercial database, particularly if it carries a distinctive marking. The treatment of the patient is likely to be symptomatic and is unlikely to depend solely on the identification of the product. In contrast, if the identification is for forensic purposes and the product is likely to be illicit or has no distinctive features, a chemical analysis is required. However, a preliminary search of a database can save much time and expense and can guide the subsequent analysis.

Solid dosage form identification databases

History

The legitimate pharmaceutical products available vary greatly from country to country. National pharmacopoeias define medicines available in each country, but generally do not provide much data on physical appearance. The physical appearance of branded medicines usually differs when marketed in different countries and manufacturers are seldom familiar with their own products from other countries. Several identification systems have been developed in the UK over the years. There are no international databases of the physical appearance of legitimate solid dosage forms. Paradoxically, there is more uniformity in illicit tablets found in different countries – the same designs and logos on ecstasy tablets are seen all around the world.

TICTAC (The Identification CD-ROM for Tablets And Capsules)

TICTAC (http://www.tictac.org.uk) is a UK commercial database supplied every 13 weeks on CD-ROM, with interim updates provided via the Internet. It is used by both health care and law-enforcement professionals. TICTAC includes data on over 23 000 solid dosage forms from the UK and Eire and contains both legitimate and illicit drugs. It includes over 65 000 pictures of the products, chemical structures of the active ingredients and their reactivity with immunoassay reagents. An index of street slang for drugs and drug-related activities is also included (1992; Ramsey, Woolley 2001).

IDENTIDEX

IDENTIDEX is a commercial text-only database of largely American products. It is supplied as part of the Micromedex suite of medical information programmes on CD-ROM (IDENTIDEX System 2001).

Drug Identification Bible

The *Drug Identification Bible* has an alphabetical index of markings (imprints) on almost 14 000 American tablets and capsules; it also has almost 900 pictures of controlled prescription drugs. It is published annually (Marnell 2001). The data on American marked tablets and capsules is also available on CD-ROM as RxID, which is updated quarterly.

The Compendium of Pharmaceutical Specialities

The *Compendium of Pharmaceutical Specialities* (CPS) is a Canadian compendium with pictures of most pharmaceuticals (Canadian Pharmacists Association 2001). It is available in hard copy and on CD-ROM.

Identification for clinical purposes

Community

Community pharmacy practice sometimes requires the identification of tablets and capsules. Customers may become anxious when dispensed unfamiliar generic products, particularly when they are dispensed mixed batches. Parallel imported products may also cause anxiety and confusion. Patients on large amounts of medication may become confused and may report the presence of 'rogue' tablets.

Anxious parents may also rely on community pharmacists for advice on suspected 'drugs' found around the home. While abused drugs are more likely to be in tablet form than ever before, many such enquiries result from the discovery of confectionery and sweeteners in unlikely circumstances.

Residential nursing homes for elderly people often receive medications in drug trays containing all the medication required daily for each resident. The quality management of dispensing these trays is often carried out with the aid of a database that contains details of the physical appearance of the medicines.

Hospital

The treatment of acute poisoning rarely depends upon the identification of a tablet or capsule. However, confirmation of the identity of medicines brought in with the patient may be of value. Those who treat drug abusers also frequently require identifications. Many abused drugs are in tablet form and most drug abusers use multiple drug types. Enquiries for identification of the benzodiazepines (particularly temazepam and diazepam) and opiate/opioid analgesics (codeine and dihydrocodeine in the UK; dihydrocodeinone in the USA) are particularly common.

Pharmaceuticals

The pharmaceutical manufacturers need to be able to recognise their own products as a public service and also, from time to time, to check proposed markings for new products to ensure that they are not currently in use. They also need to investigate allegations that batches of their products contain 'rogue' tablets.

The practice of workplace drug testing requires Medical Review Officers to investigate whether the medication declared by the testee is consistent with the results of the subsequent laboratory analysis of a urine sample. The medication is frequently declared in insufficiently precise terms that require clarification (e.g. 'Anadin', or is described as 'pink headache tablets'). Suspicious tablets may also be found in the workplace (e.g. gymnasia, food production plants, off-shore oil rigs).

Forensic

Identifications are carried out in the law-enforcement sector for many reasons by many different agencies. HM Revenue and Customs need to be able to recognise suspected contraband in luggage, particularly now that controls on anabolic agents are in force. Prisons need to be able to screen drugs in the possession of remand prisoners and to investigate contraband and enforce the status of drug-free wings. Police benefit from ready access to identification resources to make decisions about items discovered during searches and to prevent unnecessary submissions for laboratory analysis.

The international proliferation of ecstasy (MDMA and similar compounds) has created the requirement to investigate the illicit production of these tablets. This is an international trade in which the same or similar tablets are found all over the world. They are commonly marked with logos from all manner of consumer products and companies, particularly those that produce trendy products of appeal to the youth market. The TICTAC database currently has over 1300 logos, most from illicit products.

New synthetic drugs sold as 'legal' alternatives to the amfetamines or 'ecstasy' sold openly on the Internet and in 'head shops' as tablets or capsules have recently emerged as an international problem. Initially piperazine derivatives (BZP, *N*-benzylpiperazine; TFMPP, 1-[3-trifluoromethylphenyl] piperazine; mCPP, 1-[*m*-chlorophenyl]piperazine) were seen, but as legal controls have been introduced other novel compounds (glaucine; D2PM, diphenyl-2-pyrrolidinemethanol, diphenyl prolinol) have emerged. Tablets containing these compounds are usually marked with logos similar to, or the same as, those found on ecstasy tablets.

Another aspect is the identification of solid dosage forms from the scene of a fatality at which death by an overdose of drugs is suspected.

The identification process

The identification process is illustrated using the UK TICTAC CD-ROM database. Examples using the other databases are given when appropriate. The software–user interface uses a filing card analogy (Fig. 13.18), with a card appropriate for each type of identification enquiry. The procedure to identify an unknown tablet or capsule is shown in Fig. 13.19.

If a product is distinctively marked or is unusual in some way, an unambiguous identification may be achieved, but there are limitations if the product has few distinguishing features. For example, a plain white unmarked tablet is unlikely to be identified from a physical description

Figure 13.18 The TICTAC user interface showing the filing card analogy. Reproduced with permission from TICTAC Communications.

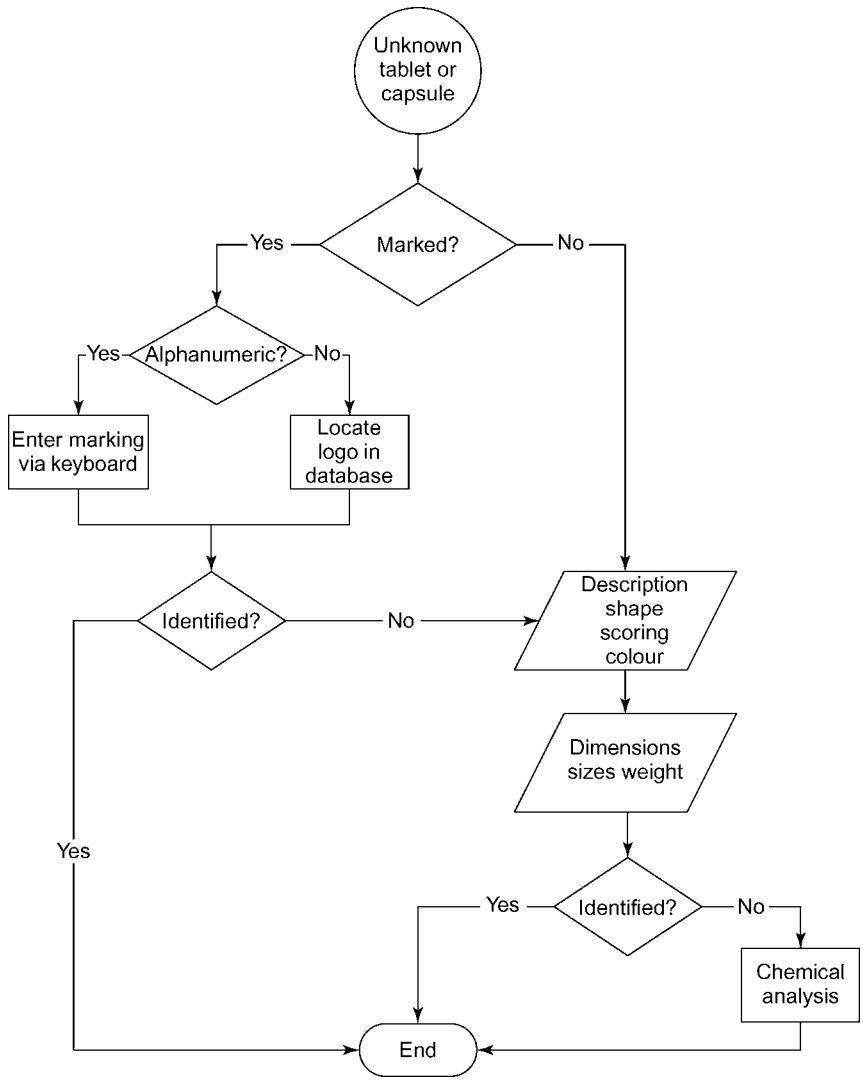

Figure 13.19 Flow diagram showing the procedure to identify an unknown tablet or capsule. Reproduced with permission from TICTAC Communications.

unless it is unusually large or small, so chemical analysis will be required. If a unique identification is not possible, TICTAC can present candidates in order of best fit to the data entered. It may be necessary to confirm the tentative identification made from a physical description by chemical analysis if the identification is required for legal purposes.

Distinctively marked product

TICTAC

Although the product shown in Fig. 13.20 is actually pink and quarter scored, it is also distinctively marked and can be identified simply by entering the marking 4Z1 into the appropriate card (*Product, Tablet*).

The software retrieves information on any products that match the description. The pictures and sizes and weights are presented to confirm that the identification is correct (Fig. 13.21). Further details of the ingredients (chemical structure, therapeutic category) and the supplier (contact details, website) can be obtained by pressing the appropriate button.

The TICTAC database contains alternative markings for products for which the marking might be considered ambiguous. Examples of marking that require care are shown in Table 13.2.

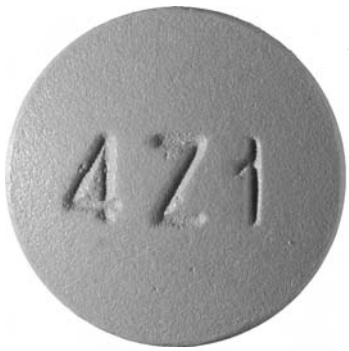

Figure 13.20 Example of a distinctively marked tablet. Reproduced with permission from TICTAC Communications.

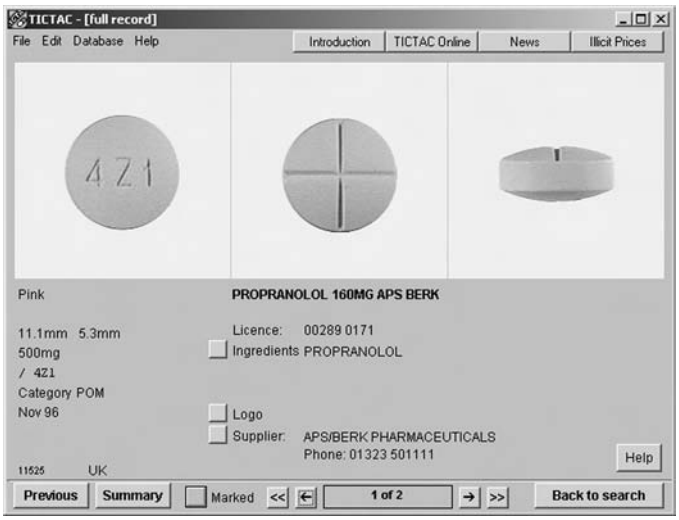

Figure 13.21 TICTAC output screen showing the result of a successful identification. Reproduced with permission from TICTAC Communications.

Table 13.2 Examples of markings that are easily confused

Numbers or letters		*Lower case L or upper case I*		*Depending which way up the product is viewed*	
1	0	L	u	Z	W
I	O	I	n	N	M

Figure 13.22 IDENTIDEX data input screen.

IDENTIDEX

The same identification may be performed using the IDENTIDEX database from Micromedex. The marking is entered in the *Enter Search Term* box and the *Search* button pressed (Fig. 13.22). A list of markings that match or are similar to those in the query is retrieved (Fig. 13.23). The matching entry is double clicked to retrieve the final detailed data; there are no images of the products (Fig. 13.24).

Unmarked product

TICTAC is the only database available to address the identification of unmarked products. Plain unmarked products may be located by entering the description in the appropriate card (*Tablet, Capsule, Patch* or *Stamp*). The *Patch* card deals with the identification of transdermal devices, and the *Stamp* card with illicit drugs supplied impregnated in paper 'stamps'.

Figure 13.23 IDENTIDEX intermediate data output screen.

Figure 13.24 IDENTIDEX final data output screen.

Figure 13.25 Example of an unmarked white tablet. Reproduced with permission from TICTAC Communications.

The tablet in Fig. 13.25 is 6.4 mm in diameter and 2.6 mm total thickness, and it weighs 90 mg. In this case the data entered need to be quite detailed:

- Unmarked (no alphanumeric marking or logo)
- Half-scored no marking (no marking other than a break-line which divides it in two)
- Shape in elevation – biconvex
- Shape in plan – round
- Colour – white
- Colour type – solid (as opposed to mottled or layered)
- Dimensions (measured in millimetres with a ruler or digital calipers).

The data are entered by selecting options using checkboxes or pull-down menus (Fig. 13.26). The dimensions are entered in order, largest first. Pressing the *Retrieve* button initiates a search for products in the database that match the data within the specified precision (10% by default) and, if the sort box is checked, the products found are sorted in order of best fit to the data entered. The default precision is appropriate unless the product is damaged or the measurements are imprecise. Sugar-coated and illicit tablets vary more in size and weight, so the precision may be widened when searching for these.

This enquiry retrieves about 20 similar products sorted in order of best fit to the sizes and weights entered. Many have the same active ingredient (prednisone) and may be identical products marketed by different companies, or may be manufactured to the same British Pharmacopoeia specification.

This identification cannot be achieved using IDENTIDEX, which deals with marked products only.

Products marked with a logo

TICTAC

A different strategy is required for products marked with a logo (defined as a marking that cannot be entered from a computer keyboard). The product in Fig. 13.27 may be identified by first searching the *logo database* for all the logos that have the description *cat* (Fig. 13.28), selecting the appropriate one and then searching the *products database* for those that bear the cat marking (Fig. 13.29).

Figure 13.27 Example of a tablet marked with a logo. Reproduced with permission from TICTAC Communications.

Products that are an unusual shape (those not shown in the shapes on the tablet or capsule forms) are also located in a similar manner, for example the cat's head shape in Fig. 13.30.

Capsule identification

Capsules are identified in a similar manner to tablets, but using the *Capsule* form. This form, although similar to the tablet form, has the appropriate descriptors for shape and allows two colours to be chosen (Fig. 13.31). As discussed above, the printing on capsules may not be consistent on the cap or body.

Unsealed capsules should be checked to ensure they have not been tampered with. It is useful to check the total weight of any capsule during identification, as this will almost certainly be different from that of the intact product if it has been interfered with. The capsule in Fig. 13.32 has had its contents removed and replaced with a crushed ecstasy tablet.

Confirmation of identity

Confirmation of identity may be required if the product is not identified uniquely or if the consequences of an error demand that identification be confirmed. The analysis can be quite challenging for some products,

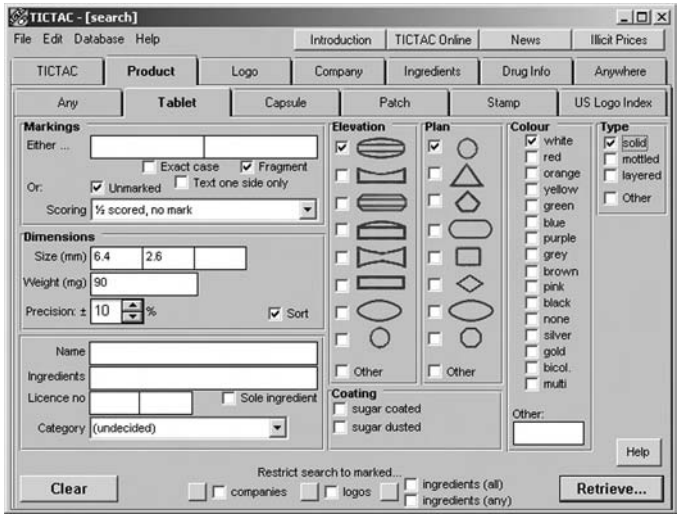

Figure 13.26 TICTAC data input screen with information required for the unmarked white tablet in Figure 13.25. Reproduced with permission from TICTAC Communications.

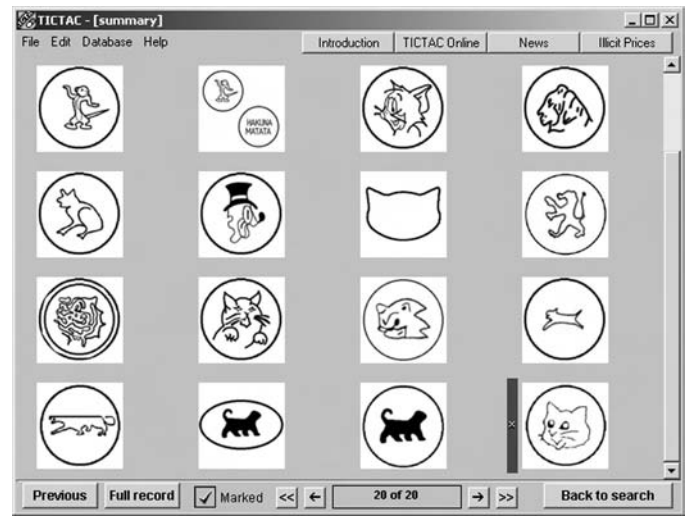

Figure 13.28 TICTAC output screen showing the results of searching for a cat logo. Reproduced with permission from TICTAC Communications.

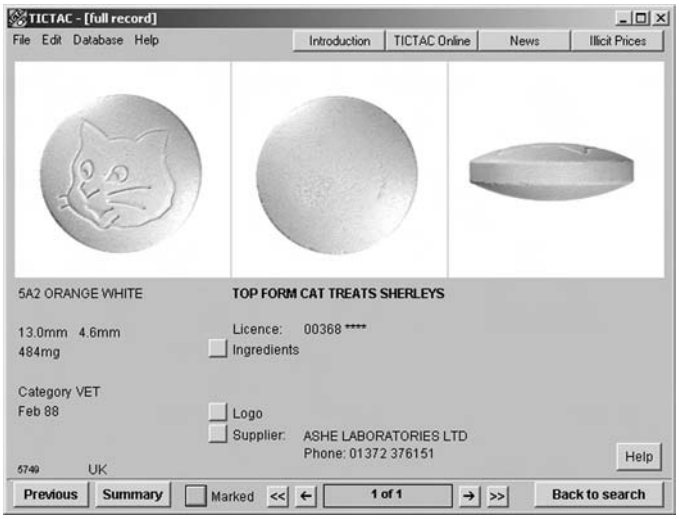

Figure 13.29 TICTAC output screen showing the result of searching for the tablet marked with the cat logo. Reproduced with permission from TICTAC Communications.

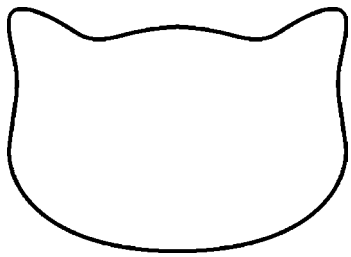

Figure 13.30 An example of a tablet that is an unusual shape. Reproduced with permission from TICTAC Communications.

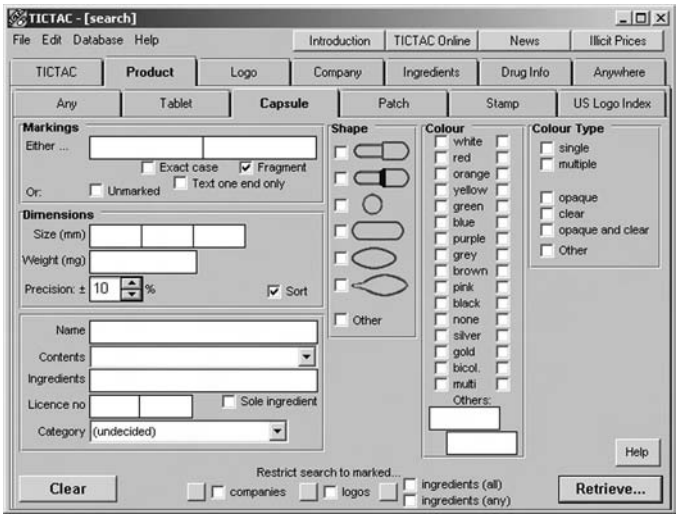

Figure 13.31 TICTAC data-input screen used to identify capsules. Reproduced with permission from TICTAC Communications.

such as multivitamins and minerals, herbal products, homeopathic remedies and steroids

Colour tests have been promoted for use by purchasers of ecstasy tablets at dance venues, either by organisations that offer a service or by the users themselves (Spruit 2001; Winstock *et al.* 2001). The Marquis reagent and more recently the Mecke or modified Mecke reagent and Mandelin's reagent are available to the public for this purpose on the Internet (Spruit 2001; Winstock *et al.* 2001).

Figure 13.32 A capsule that has had the contents removed and replaced with a tablet. Reproduced with permission from TICTAC Communications.

Confirmation of identity may be required for a variety of reasons:

- Identification of illicit products
- Unequivocal identification for forensic purposes
- Detection of adulteration
- Detection of the substitution of capsule contents
- Identification of products not in commercial databases
- Investigation of counterfeit or substandard products.

This may often be achieved by suspending a whole tablet or a scraping from a tablet or a portion of a capsule contents in a solvent, such as ethanol or methanol, and analysing it by spectroscopic methods (UV, IR, MS) or chromatographic methods (TLC, GLC, HPLC, CE or a combination of both (GC-MS, HPLC-diode array, UV). Commercial or in-house libraries of IR and mass spectra may be of great value in identifying unknown products. However, new synthetic illicit drugs, particularly when reference materials are not available, such as 4-methylthioamfetamine and those described in *PiHKAL* (e.g. 2C-T-7) (Shulgin, Shulgin 1991) can present significant analytical challenges and may require structural elucidation using NMR spectroscopy. Identification by diamond attenuated total reflectance (ATR) IR of the scrapings of a tablet or the contents of a capsule may be achieved providing the active ingredients are present as major components.

The analysis of legitimate pharmaceutical products is discussed in Chapter 12.

The analysis of non-active ingredients (excipients in legitimate medicines or diluents in illicit drugs) may be of value in investigating counterfeit medicines or the origin of illicit drugs. Near-IR spectroscopy, particularly when linked with microscopy, is a valuable technique for this purpose (see Chapter 34). Visible polarised-light microscopy may be used to identify excipients such as the starches (e.g. maize, potato) used as disintegrants.

The confirmation of identity of herbal products is difficult and may require microscopic examination for plant fragments by a specialist and chromatographic analysis (often TLC) for indicator compounds (Barnes *et al.* 2007). The genetic characteristics of traditional Chinese herbal drugs are under investigation at the Royal Botanic Gardens at Kew and may be of value for the confirmation of their identity in the future.

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CHAPTER

14 Volatile Substances

RJ Flanagan

If anaesthesia is excluded, acute poisoning with volatile substances usually follows the deliberate inhalation of gas or solvent vapour in order to become intoxicated ('glue sniffing', solvent abuse, volatile substance abuse (VSA)). Solvents from adhesives, notably toluene, and from certain print-correcting fluids and thinners, hydrocarbons such as those found in cigarette lighter refills, halocarbon aerosol propellants and fire extinguishers, 'air dusters', and anaesthetic gases are among the products/compounds that may be abused in this way (Table 14.1).

Those who ingest, or more rarely inject, solvents or solvent-containing products, and the victims of clinical, domestic and industrial accidents, may also be poisoned by volatile substances. In addition, chloroform, diethyl ether and other volatiles are still used occasionally in the course of crimes such as rape and murder (Flanagan, Fisher 2008). Ingestion of the solvent γ-butyrolactone (GBL), and also of 1,4-butanediol, which are converted rapidly in the body into γ-hydroxybutyric acid (GHB), has become prevalent in recent years. A further volatile compound, chlorobutanol (chlorbutol), sometimes employed as a sedative, a plasticiser and a preservative, has been used in doping greyhounds. Inhalation of asphyxiant gases such as helium has been used as a means of suicide (Field-Smith *et al.* 2009).

Inhalation of a range of other volatile substances ranging from mothball vapour (naphthalene or 1,4-dichlorobenzene) to overtly toxic gases such as chlorine in order to experience particular effects has also been reported, but such practices are very uncommon. Isobutyl and isopentyl ('amyl') nitrites ('poppers', 'snappers') may also be inhaled in order to experience their vasodilator properties. In the USA, abuse of these compounds is discussed along with abuse of other volatiles under the term 'inhalant abuse' (Williams *et al.* 2007), but in other countries these substances are normally discussed separately. Isobutyl nitrite is now listed as a Class 2 carcinogen.

Solvents and many other volatiles can produce dose-related central nervous system effects similar to those of ethanol. Small doses can lead rapidly to euphoria and other behavioural disturbances, and may also induce more profound effects such as delusions and hallucinations. Heightened sexual (self-) gratification may also be a feature, sometimes in association with partial asphyxia (Musshoff *et al.* 2006). Once exposure ceases, rapid recovery normally ensues – rapid recovery after exposure may be a factor in the continuing popularity of VSA among children of high-school age (13–18 years old) in some countries. On the other hand, psychological dependence is common in chronic users. VSA has now been reported from most parts of the world, mainly among adolescents, individuals living in remote communities and those with occupational access to abusable volatiles.

The major risk associated with VSA is that of sudden death and, although the UK is the only country to collect VSA-related mortality data systematically, there have been many reports of 'sudden sniffing deaths' worldwide. There were at least 2308 (1967 male) UK sudden VSA-related deaths in the period 1971–2007, with some 47% in youngsters aged 7–17 years (Field-Smith *et al.* 2009). Some 5% of all VSA-related deaths were thought to have been suicides. Deaths occurring from 'indirect' causes such as inhalation of vomit (14.2% of deaths), asphyxia associated with use of a plastic bag (11.1%), and trauma (10.9%) were frequently associated with abuse of products containing toluene, usually adhesives (glue). However, most deaths (60.3%) were attributed to 'direct toxicity' (Field-Smith *et al.* 2009). Nine hundred and twenty-three deaths (40 per cent of all USA-related deaths) were associated with abuse of liquefied petroleum gas (LPG) cigarette-lighter refills. Annual numbers of deaths have been decreasing in the UK in recent years (58 deaths, 2007).

Chronic toxicity from exposure to volatile substances has also been described both in abusers and after occupational use of certain compounds. Although sale of adhesives and spray paints containing more than 0.1% (v/v) toluene to the general public was prohibited within the European Community from June 2007, industrial use of toluene remains unaffected. Deposition of titanium-containing granules in the lung after presumed inhalation of a toluene-based spray paint has been noted (Byard *et al.* 2007). Chronic toxicity such as lead poisoning from abuse of leaded gasoline has been reported in less developed countries or societies (e.g. among aboriginal populations, but is much less common nowadays). In addition to the risk of fire and explosion from abuse of LPG in confined spaces, hydrofluoric acid burns have been observed following ignition of 1,2-difluoroethane from compressed air cleaners (Foster *et al.* 2003).

Role of the analytical toxicology laboratory

The analytical toxicology laboratory may be asked to perform analyses for solvents and other volatile compounds in biological samples and related specimens for the following purposes:

1. To assist in the diagnosis of acute poisoning including the investigation of deaths where poisoning by volatile compounds (including anaesthetic agents and asphyxiant gases) is a possibility.
2. To confirm a suspicion of chronic VSA in the face of denial from the patient and/or a person responsible for the care of the patient such as a parent or guardian.
3. To aid investigation of rape or other assault, or other offences such as driving a motor vehicle or operating machinery, which may have been committed under the influence of volatile substances, or in which volatile substances may have been administered to a victim.
4. To help investigate fire or explosion where VSA might have been a contributory factor.
5. To assess occupational or environmental exposure to anaesthetic or solvent vapour. However, other techniques such as ambient or expired air monitoring or, in some instances, the measurement of urinary metabolite excretion may be more appropriate in this context.

The analysis of volatile substances presents particular problems. First, collection, storage and transport of biological samples must be controlled as far as practicable in order to minimize loss of analyte – quantitative work is futile if very volatile compounds such as propane are encountered unless precautions are taken to prevent losses from the sample prior to the analysis. Second, many of the compounds of interest occur commonly in laboratories and this necessitates precautions against contamination/interference. Third, many volatile compounds are excreted unchanged via the lungs and thus blood (and/or other tissues in fatalities), and not urine, is usually the sample of choice. Finally, the interpretation of results can be difficult, especially if legitimate exposure to solvent vapour is a possibility.

A diagnosis of VSA should be based on a combination of circumstantial, clinical and analytical evidence rather than on any one factor alone. It is especially important to consider all circumstantial evidence in cases of possible VSA-related sudden death since suicide or even homicide cannot be excluded simply on the basis of the toxicological

Table 14.1 Some products/compounds that may be abused by inhalation[a]

Product	*Major volatile components*
Adhesives	
Balsa wood cement	Ethyl acetate
Contact adhesives	Butanone, hexane[b], toluene and esters
Cycle tyre repair cement	Toluene and xylenes[c]
Polyvinylchloride (PVC) cement	Acetone, butanone, cyclohexanone, trichloroethylene
Woodworking adhesives	Xylenes[c]
Aerosols	
Air freshener	Purified LPG[d], dimethyl ether (DME) and/or fluorocarbons[e]
Deodorants, antiperspirants	Purified LPG[d], DME and/or fluorocarbons[e]
Fly spray	Purified LPG[d], DME and/or fluorocarbons[e]
Hair lacquer	Purified LPG[d], DME and/or fluorocarbons[e]
Paint	Purified LPG[d], DME and/or fluorocarbons[e] and esters
Anaesthetics/analgesics	
Inhalational	Nitrous oxide, cyclopropane[f], diethyl ether[f], halothane, enflurane, desflurane, isoflurane, methoxyflurane[f], sevoflurane, xenon
Topical	Ethyl chloride, fluorocarbons[e]
Cigarette lighter refills	Purified LPG[d]
Commercial dry cleaning and degreasing agents	Carbon tetrachloride[g], dichloromethane, methanol, 1,1,2-trichlorotrifluoroethane (FC 113), 1,1-dichloro-1-fluoroethane (FC 141b), methanol, propylene dichloride[g], 1,1,1-trichloroethane[g], tetrachloroethylene, toluene, trichloroethylene
Domestic spot removers and dry cleaners; surgical plaster/chewing gum remover	Carbon tetrachloride[g], dichloromethane, 1,1,1-trichloroethane[g], tetrachloroethylene, trichloroethylene
Dust removers ('air brushes', 'air dusters')	DME, fluorocarbons[e],[g]
Fire extinguishers	Bromochlorodifluoromethane (FC 12B1, BCF)[f], trichlorofluoromethane (FC 11)[f], dichlorodifluoromethane (FC 12)[f]
Hydrocarbon fuels/solvents	Acetylene, 'butane'[h], petrol (gasoline)[i], petroleum ethers[j], 'propane'[k]
Industrial/laboratory solvents	Chloroform[f], methyl acetate, methyl isobutyl ketone (MIBK), methyl *tert*-butyl ether (MTBE)
Injected oxidant (drag racing, blow torches)	Nitrous oxide
Paint/paint thinners	Acetone, butanone, esters, hexane[b], toluene, trichloroethylene, xylenes[c]
Paint stripper	Dichloromethane, methanol, toluene
Starting fluid	Diethyl ether, hydrocarbons (heptane, LPG[d]), DME
Typewriter correction fluids/thinners (some)	1,1,1-Trichloroethane[f]
Vasodilators	Butyl nitrite, isobutyl nitrite ('butyl nitrite'), isopentyl nitrite (isoamyl nitrite, 'amyl nitrite')[l]
Whipped cream dispenser bulbs/cylinders	Nitrous oxide

[a]The composition of some products varies with time and country of origin.
[b]Commercial 'hexane' mixture of hexane and heptane with small amounts of higher aliphatic hydrocarbons.
[c]Mainly *m*-xylene (1,3-dimethylbenzene).
[d]Liquified petroleum gas (LPG; butane, isobutane, propane; if unpurified also butenes, propenes, sulfur compounds, etc.).
[e]Nowadays often 1,1,1,2-tetrafluoroethane (FC 134a), but chlorodifluoromethane (FC 22), 1,1-difluoroethane (FC 152a), difluoromethane (FC 32), pentafluoroethane (FC 125), perfluoropropane (FC 218) and 1,1,1-trifluoroethane (FC 143a) might also be encountered.
[f]Rarely used for this purpose nowadays.
[g]May also contain denatonium.
[h]LPG.
[i]Mixture of aliphatic and aromatic hydrocarbons with boiling range 40–200°C.
[j]Mixtures of pentanes, hexanes, etc. with specified boiling ranges (e.g. 40–60°C).
[k]Propane, butanes, etc.
[l]Commercial 'amyl nitrite', mainly isopentyl nitrite but other nitrites also present.

examination. There have been a number of reports of the use of inhalational anaesthetics for suicidal purposes, and there has been one example in the UK of a serial homosexual rapist who murdered his victims and disposed of the bodies by setting fire to them in garden sheds in circumstances that suggested that the victim had caused the fire accidentally while indulging in VSA (Flanagan, Fisher 2008). The possibility of VSA should also be considered in individuals giving very high readings on evidential breath alcohol instruments (Gill *et al.* 1991). A result of 333 µg alcohol in 100 mL breath has been recorded in one instance after alcohol ingestion and butane inhalation; a contemporary specimen for blood ethanol measurement was not available for analysis (Flanagan, Fisher 2008). Modern instruments may be less prone to such interference, however.

Analytical methods

The analysis of biological samples for solvents and other volatiles that may be abused by inhalation has similarities to the analysis of methanol, ethanol and 2-propanol. Static headspace gas chromatography (GC) often provides a convenient and easily automated mode of analysis for

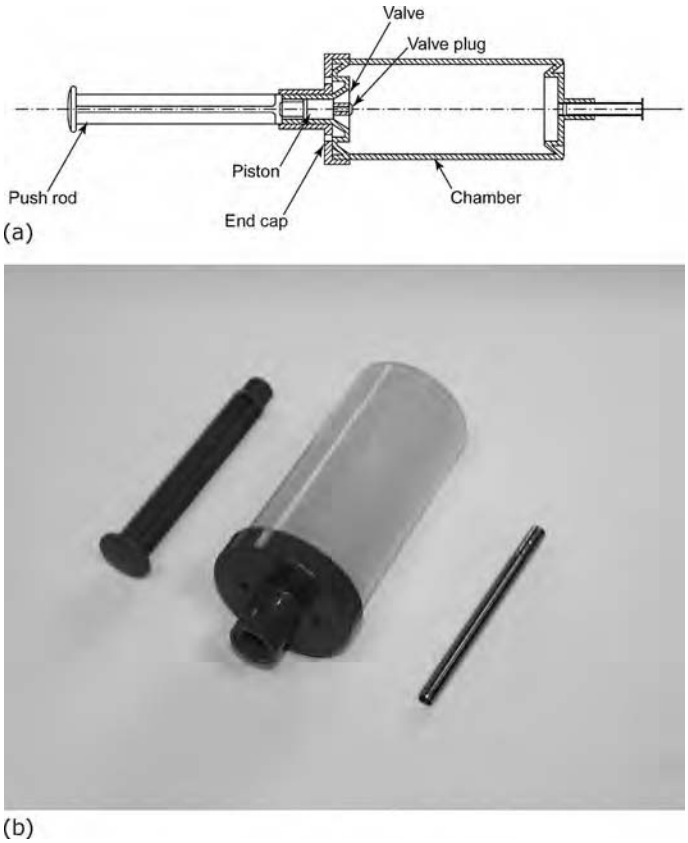

Figure 14.1 Device for capturing breath samples for solvent analysis: (a) schematic; (b) components. (Dyne *et al.* 1997; illustrations courtesy of Dr J Cocker, Health & Safety Laboratory, Buxton, UK.)

blood and other biological specimens that may be obtained without using special apparatus in order to collect the sample (Kolb, Ettre 1997). Many analyses can be accomplished using flame ionisation and/or electron capture detection (FID and/or ECD). Nitrous oxide and most halogenated compounds give a response with ECD, although thermal conductivity detection (TCD) may be used as an alternative if nitrous oxide poisoning is suspected. GC methods for xenon would require TCD or mass spectrometry (MS) (Miyano *et al.* 1993).

Use of expired air collected into either a Tedlar (polyvinylfluoride, PVF) bag or via a special device constructed from a polytetrafluoroethylene (PTFE)-like polymer (Fig. 14.1) (Dyne et al. 1997) with subsequent GC or GC-MS analysis can facilitate the analysis of a number of compounds. Direct MS of expired air can also detect many compounds several days post exposure. However, the use of these techniques is limited by the need to take breath directly from the patient and the specialist equipment required. This being said, helium has been detected in gas samples from the lungs of a helium-related suicide by GC-MS using nitrogen as carrier gas (Auwaerter et al. 2007). Vapour-phase infrared (IR) spectrophotometry may be useful in the analysis of abused products or ambient atmospheres. High performance liquid chromatography (HPLC) is useful in the analysis of polar metabolites of certain solvents.

Sample collection, transport and storage

If the analyte is very volatile (e.g. helium, propane, butane) direct sampling, for example of air in the lungs, may be indicated. If a quantitative analysis is required, a blood sample should be collected directly into the vial in which the analysis will be carried out (Gill *et al.* 1992). Many other volatile compounds are relatively stable in blood and other tissues if simple precautions are taken. In the case of blood, the container used for the sample should be glass, preferably with a cap lined with metal foil; greater losses may occur if plastic containers are used. The tube should be as full as possible and should be opened only when required for analysis and then only when cold (4°C) (Gill *et al.* 1988). If the sample volume is limited it is advisable to select the container to match the volume of blood so that there is minimal headspace. An anticoagulant should be used (sodium ethylenediamine tetraacetate (EDTA) or lithium heparin). Specimen storage between 2 and 8°C is recommended and 1% (w/v) sodium fluoride should be added to minimise enzymic activity.

If a necropsy is to be performed, tissues (approximately 10 g each of brain, lung, liver, kidney and subcutaneous fat) should be obtained in addition to standard toxicological specimens (femoral blood, urine, stomach contents, vitreous humour). Tissues should be stored before analysis in the same way as blood. No preservative should be added. Products implicated in the incident (and stomach contents if ingestion is suspected) should be packed, transported and stored entirely separately from (other) biological specimens to avoid cross-contamination. Investigation of deaths occurring during or shortly after anaesthesia should include analysis of the inhalational anaesthetic(s) used in order to exclude an administration error (Uyanik 1997). The stability of chloroform, diethyl ether, ethanol and toluene in formalin-fixed rabbit tissue (brain, lung, liver, kidney and skeletal muscle) has been studied (Takayasu *et al.* 1994). It was concluded that these compounds could be detected for up to 14 days after fixation even though there was marked loss of toluene, especially from brain (84%), even after 1 day.

Gas chromatography

Summary details of some GC methods for volatile compounds in biological specimens are given in Table 14.2. Packed columns, for example 2 m × 2 mm i.d. 0.3% (w/w) Carbowax 20M on Carbopack C programmed from 35°C to 175°C, have been used with headspace sample preparation (Ramsey, Flanagan 1982). Disadvantages include the poor resolution of some very volatile substances, a long total analysis time, and variation in the peak shape given by alcohols between batches of column packing. Retention index data for a range of solvents and other volatile compounds of interest on both 0.3% and 6.6% (w/w) Carbowax 20M are available (De Zeeuw et al. 1992).

Porous layer open tubular (PLOT) columns give good retention and thus resolution of compounds with similar relative molecular mass, but peak shapes of polar compounds are poor and it is difficult to screen for compounds of widely different volatility in one analysis. However, bonded-phase wide-bore capillary columns permit relatively large-volume septum injections and can offer advantages of improved efficiency, reproducibility and reliability. A 60 m × 0.53 mm i.d. fused silica capillary coated with the dimethylpolysiloxane SPB-1 (5 µm film) programmed from 40°C to 200°C offers many advantages over packed column and PLOT systems (Flanagan *et al.* 1997). Improved resolution of very volatile compounds is obtained and, even with an initial temperature of 40°C, the total analysis time can be reduced to 26 min, and good peak shapes can be obtained, even for alcohols. Moreover, splitless septum injections of up to 300 µL headspace can be performed with no noticeable effect on column efficiency; hence sensitivity is at least as good as that attainable with a packed column. Retention data on this system for some commonly used compounds are given in Table 14.3.

The use of a capillary column together with two different detectors (FID and ECD) confers a high degree of selectivity, particularly for low-molecular-mass compounds where there are relatively few alternative structures. If more rigorous identification is required, GC combined with MS or Fourier transform IR spectrometry (FTIR) may be used. However, GC-MS can be difficult at high sensitivity when the fragments produced are less than *m/z* 40, particularly if the instrument is used for other purposes as well as solvent analyses. In particular, the available sensitivity and spectra of the low-molecular-weight alkanes renders them very difficult to confirm by GC-MS. Inertial spray MS allows introduction of biological fluids directly into the mass spectrometer without prior chromatographic analysis and has been used in the analysis of halothane in blood during anaesthesia.

GC-FTIR may be more appropriate than GC-MS in the analysis of volatiles, but sensitivity is poor particularly when compared with ECD.

Table 14.2 Summary of some GC methods for volatile compounds in biological fluids published 1989–1998 (adapted from Pihlainen, Ojanpera 1998)

Application	*Sample*	*Extraction*[a]	*GC column and/or conditions*	*Detection*	*LoD*[b]	*Reference*
Toxicology	Blood, tissue	HS	DB-1701, 40 m × 0.25 mm i.d., 1.0 µm, 30–120°C	MS (ion trap)	~1 mg/L	Weller, Wolf (1989)
Anaesthetics	Blood (1 mL)	HS	Porapak S, 80/100 mesh, 1.9 m × 2 mm i.d., 165°C	FID	~2 mg/L	Watts *et al.* (1992)
Environmental	Blood (10 mL)	PT	DB-624, 30 m × 0.32 mm i.d., 1.8 µm, 0–190°C	MS	0.05 µg/L[c]	Ashley *et al.* (1992)
Toxicology	Blood (1 mL), tissue (1 g)	HS	0.2% Carbowax 1500, 80/l00 mesh Carbopak C, 1.8 m, l00°C	FID	—	Kuhlman *et al.* (1993)
Toxicology	Blood (1.5 mL)	HS	DB-1, 30 m × 0.25 mm i.d., 1.0 µm, 40–250°C	MS (ion trap)	—	Schuberth (1994)
Workplace monitoring	Urine (0.5–1 mL)	HS	Porapak Q, 80–100 mesh, 2 m × 1.8 mm i.d., 100°C	MS	0.7 µg/L[d]	Imbriani *et al.* (1995)
			HP-5, 25 m × 0.2 mm i.d., 25°C	MS	0.1 µg/L	Imbriani *et al.* (1995)
Toxicology	Blood, CSF (1 mL)	HS	Porapak Q, 80/l00 mesh, 1 m × 2.5 mm i.d., 50–l70°C	MS	0.02 mg/L[e]	Maruyama *et al.* (1995)
Workplace monitoring	Urine (50 mL)	HS	DB-5, 30 m × 0.25 mm i.d., 40°C	MS	—	Schaffernicht *et al.* (1995)
Toxicology	Blood, plasma	PH	GS-Q, 30 m × 0.53 mm i.d., 70–230°C (screening)	MS	—	Takayasu *et al.* (1995)
	Serum (1–3 µL)	PH	Porapak Q, 1 m × 2.6 mm i.d.,180°C (screening)	FID	—	Takayasu *et al.* (1995)
		PH	DB-17, 15 m × O.53 mm i.d., 1.0 µm, 50°C (quantification)	MS	0.02 mg/L[e]	Takayasu *et al.* (1995)
Anaesthetics	Blood (3 µL)	PH	GS-Q, 30 m × 0.53 mm i.d., 160°C	MS	0.2 mg/L	Saito *et al.* (1995)
	Blood (0.5 mL)	HS	DB-1, 30 m × 0.53 mm i.d., 5 µm, 60°C	MS	—	Saito *et al.* (1995)
Toxicology	Blood (0.5 mL)	HS-SPME	DB-WAX, 30 m × 0.32 mm i.d., 0.25 µm, 35–230°C	FID	2 µg/L	Lee *et al.* (1995)
Toxicology	Blood (0.5–5 mL)	PT	PoraPLOT Q, 25 m × 0.32 mm i.d., l0 µm, 30–240°C	FTIR, FID	0.05 mg/L[f]	Ojanperä *et al.* (1996)
Toxicology	Blood, brain (2–32 mg)	HS-FE	DB-1, 30 m × 0.25 mm i.d., 1.0 µm, 40–250°C	MS (ion trap)	0.01 mg/L[g]	Schuberth (1996)
Toxicology	Blood	HS-SPME	HP-1, 30 m × 0.53 mm i.d., 2.65 µm, 50°C	FID	1 µg/L	Tytgat, Daenens (1996)
Toxicology	Blood (0.5–5 mL)	PT	PoraPLOT Q, 25 m × 0.32 mm i.d., l0 µm, 30–250°C	FTIR, FID	0.01 mg/L[f]	Ojanperä *et al.* (1998)
Toxicology	Blood, urine (0.5 mL)	HST	DB-624, 30 m × 0.32 mm i.d., 1.8 µm, 5–110°C	FID	1 µg/L	Lee *et al.* (1998)
Anaesthetics	Blood (0.1 mL)	HS	DB-5, 60 m × 0.25 mm i.d., 0.25 µm, 35–120°C	MS	20 mg/L[f]	Yang *et al.* (2001)

[a]HS, headspace; HS-FE, headspace full evaporation; HS-SPME, headspace solid-phase microextraction; HST, headspace low-temperature trapping; PH, pulse heating; PT, 'purge and trap'.
[b]LoD, limit of detection (in some cases limit of identification or limit of accurate measurement).
[c]1,1,1-Trichloroethane.
[d]Nitrous oxide.
[e]Toluene (3 µL sample).
[f]Isoflurane.
[g]Methyl *t*-butyl ether (30 mg sample).

Moreover, the apparatus is expensive and not widely available. In addition, interference, particularly from water and carbon dioxide in the case of biological specimens, can be troublesome. 'Purge and trap' and multiple headspace extraction offer ways of increasing sensitivity and, although not needed for most clinical and forensic applications, 'purge and trap' has been used in conjunction with GC-FTIR and FID in forensic case work (Ojanpera *et al.* 1996, 1998). Pulse heating has also been employed in the analysis of volatiles in biological specimens (see Table 14.2). This method involves the use of a Curie point pyrolyser employing a ferromagnetic alloy that can accurately attain temperatures in the range 150–1040°C very rapidly (4 s or so) (Nagano et al. 1989;Saito et al. 1995;Takayasu et al. 1995). Advantages of this latter technique include use of a small sample volume (0.5–5 µL), short extraction time and lack of matrix effects. Headspace solid-phase microextraction (HS-SPME) has also been used in the analysis of volatile compounds in biological samples (Junting et al. 1998; Walker et al. 2006).

Chiral GC methods are available should enantiomer separation of anaesthetics prove important (Schurig, Grosenick 1994; Shitangkoon *et al.* 1993).

Qualitative analysis

The procedures described below are based on those of Flanagan *et al.* (1997) using a 60 m × 0.53 mm i.d. fused-silica capillary coated with the dimethylpolysiloxane SPB-1 (5 µm film) with split FID/ECD detection and manual sample preparation and headspace injection. Note that the

Table 14.3 Retention and relative detector response data on the SPB-1 column system (Flanagan *et al*. 1997)[a]

Compound	*Retention index*							
	RT	*RRT*	*ECD*	*Calc.*	*Lit.*	M_r	*BP*	*CAS*
Acetaldehyde	3.59	0.192	0	352	372	44.1	21	75-07-0
Acetone (dimethyl ketone, propanone)	5.66	0.303	1	460	469	58.1	57	67-64-1
Acetonitrile (methyl cyanide)	5.22	0.279	0	443	455	41.1	82	75-05-8
Acetylene	2.63	0.141	0	165	—	26.0	−81	74-86-2
Benzene	14.39	0.770	0	655	660	78.1	80	71-43-2
Benzonitrile	25.16	1.347	0	965	—	103.1	191	100-47-0
Bromochlorodifluoromethane (BCF; FC 12B1)	4.07	0.217	2	398	405	165.4	−3	353-59-3
Bromomethane (methyl bromide)	4.47	0.239	2	414	—	94.9	−94	74-83-9
Butane	4.09	0.219	0	*400*	*400*	58.1	−1	106-97-8
1-Butanol	14.08	0.754	0	650	651	74.1	117	71-36-3
2-Butanol	10.80	0.578	0	585	624	74.1	100	78-92-2
Butanone (methyl ethyl ketone, MEK)	10.18	0.545	1	572	579	72.1	80	78-93-3
1-Butene	3.94	0.211	0	386	390	56.1	−6	106-98-9
2-Butoxyethanol (butyl cellosolve)	23.39	1.244	0	889	—	118.2	171	111-76-2
Butyl acetate	20.36	1.090	0	795	794	116.2	125	123-86-4
Butyl nitrite	12.41	0.663	2	617	608	103.1	78	544-16-1
Carbon disulfide	8.03	0.429	1	527	524	76.1	47	75-15-0
Carbon tetrachloride (tetrachloromethane)	14.70	0.785	2	661	659	153.8	77	56-23-5
Chloral hydrate	16.60	0.887	2	698	705	165.4	98	302-17-0
Chlorobutanol (chlorbutol)	25.44	1.359	2	976	949	177.5	167	57-15-8
2-Chloro-1,1-difluoroethane	3.41	0.182	1	335	—	100.5	−10	75-68-3
2-Chloro-1,1-difluoroethylene	3.46	0.185	1	339	—	98.5	−18	359-10-4
Chlorodifluoromethane (FC 22)	2.90	0.155	1	258	—	86.5	−41	75-45-6
Chloroform (trichloromethane)	11.65	0.622	2	603	605	119.4	61	67-66-3
2-Chloro-1,1,1-trifluoroethane	3.73	0.199	1	365	375	118.5	7	75-88-7
Cyclohexane	14.91	0.798	0	666	664	84.2	81	110-82-7
Cyclohexanol	22.77	1.219	0	874	899	100.2	161	108-93-0
Cyclohexanone	22.90	1.226	1	878	875	98.1	156	108-94-1
Cyclopropane (trimethylene)	8.29	0.444	0	533	—	42.1	−33	75-19-4
Dibromodifluoromethane	6.12	0.327	2	477	—	209.8	25	75-61-6
Dichlorodifluoromethane (FC 12)	3.18	0.170	2	313	305	120.9	−30	75-71-8
1,1-Dichloroethane	9.57	0.511	2	559	563	99.0	58	75-34-3
1,2-Dichloroethane	13.09	0.699	2	630	631	99.0	83	107-06-2
1,1-Dichloroethylene	7.28	0.389	2	512	—	96.9	61	156-59-2
1,2-Dichloroethylene (both isomers)	9.19	0.491	2	552	556	96.9	60	540-59-0
Dichloromethane (methylene chloride)	7.45	0.398	2	515	515	84.9	40	75-09-2
1,2-Dichloropropane	15.87	0.848	1	684	—	113.0	96	78-87-5
1,3-Dichloropropane	19.20	1.026	2	765	—	113.0	122	142-28-9
1,2-Dichlorotetrafluoroethane (FC 114)	3.59	0.192	2	352	361	170.9	4	76-14-2
Diethyl ether	6.69	0.358	0	499	515	74.1	35	60-29-7
1,1-Difluoroethane (FC 152a)	2.84	0.152	1	242	—	66.1	−25	75-37-6
1,1-Difluorotetrachloroethane	16.77	0.896	2	702	785	203.8	92	76-11-9
1,2-Difluorotetrachloroethane (FC 112)	16.90	0.903	2	704	730	203.8	93	76-12-0
Diisopropyl ether	11.43	0.612	0	598	594	102.2	69	108-20-3
Dimethyl ether (DME)	3.34	0.179	0	328	—	46.1	−24	115-10-6
N,N-Dimethylformamide (DMF)	18.47	0.989	0	746	—	73.1	153	68-12-2
Dimethyl sulfoxide (DMSO)	19.97	1.069	0	784	—	78.1	191	67-68-5
Dioxane	16.16	0.865	0	690	696	88.1	101	123-91-1
Enflurane	6.14	0.328	2	478	462	184.5	57	13838-16-9
Ethane	2.69	0.144	0	*200*	*200*	30.1	−88	74-84-0

Table 14.3 continued

Compound	*Retention index*							
	RT	*RRT*	*ECD*	*Calc.*	*Lit.*	M_r	*BP*	*CAS*
Ethanol	4.80	0.257	0	427	421	46.1	79	64-17-5
2-Ethoxyethanol (ethyl cellosolve)	16.38	0.877	0	694	701	90.1	135	110-80-5
2-Ethoxyethyl acetate	22.83	1.222	0	876	874	132.2	156	111-15-9
Ethyl acetate	11.42	0.611	0	598	596	88.1	77	141-78-6
Ethylbenzene	22.38	1.198	0	861	860	106.2	136	100-41-4
Ethylene	2.63	0.141	0	165	—	28.1	−104	74-85-1
Ethylene glycol[(b)]	15.15	0.811	0	670	772	62.1	198	107-21-1
Fluorotrichloromethane (FC 11)	6.13	0.327	2	478	484	137.4	24	75-69-4
Halothane	8.76	0.468	2	543	533	197.4	50	151-67-7
Heptane	16.70	0.894	0	*700*	*700*	100.2	98	142-82-5
2-Heptanone	22.79	1.220	0	874	880	114.2	152	110-43-0
Hexanal	19.82	1.061	0	781	—	100.2	130	66-25-1
Hexane	11.51	0.616	0	*600*	*600*	86.2	69	110-54-3
2,5-Hexanedione	23.25	1.242	1	890	894	114.1	188	110-13-4
1-Hexanol	22.12	1.184	0	852	860	102.2	157	111-27-3
2-Hexanol	19.95	1.068	0	784	786	102.2	140	626-93-7
2-Hexanone (methyl butyl ketone)	19.45	1.041	0	771	787	100.2	127	591-78-6
Isobutane (2-methylpropane)	3.61	0.193	0	354	370	58.1	−12	75-28-5
Isobutyl nitrite	10.37	0.555	2	576	—	103.1	67	542-56-3
Isoflurane	5.52	0.295	2	454	—	184.5	49	26675-46-7
Isopentyl nitrite ('amyl nitrite')	15.87	0.848	2	684	680	117.2	98	110-46-3
Isopropyl nitrate	14.84	0.793	2	664	693	105.1	103	1712-64-7
Methane	2.52	0.135	0	*100*	*100*	16.0	−161	74-82-8
Methanol	3.60	0.192	0	353	491	32.0	65	67-56-1
2-Methoxyethanol (methylcellosolve)	12.38	0.663	0	617	616	76.1	124	109-86-4
Methoxyflurane	17.04	0.910	2	617	701	165.0	105	76-38-0
Methyl acetate	7.30	0.391	0	512	513	74.1	57	79-20-9
Methyl *t*-butyl ether (MTBE)	9.60	0.514	0	560	—	88.2	55	1634-04-4
Methyl isobutyl ketone (MIBK)	17.60	0.942	1	723	724	100.2	118	108-10-1
Methyl isopropyl ketone	13.71	0.734	1	642	650	86.0	93	563-80-4
2-Methylpentane	9.90	0.530	0	566	610	86.2	60	107-83-5
3-Methylpentane	10.61	0.568	0	581	—	86.2	64	96-14-0
2-Methyl-2-pentanol	17.45	0.934	0	719	725	102.2	124	590-36-3
3-Methylpent-1-yn-3-ol (methylpentynol)	16.42	0.879	0	695	715	98.1	121	77-75-8
2-Methyl-1-propanol (isobutanol)	12.22	0.654	0	614	619	74.1	108	78-83-1
2-Methyl-2-propanol (*t*-butanol)	7.14	0.382	0	509	512	74.1	82	75-65-0
Monochloroethane (ethyl chloride)	4.77	0.255	2	426	447	64.5	12	75-00-3
Nitrous oxide	2.66	0.142	2	182	—	44.0	−88	10024-97-2
Octane	20.57	1.101	0	*800*	*800*	114.2	126	111-65-9
Paraldehyde	19.28	1.032	0	767	771	132.2	124	123-63-7
Pentane	6.72	0.360	0	*500*	*500*	72.2	36	109-66-0
Perfluoropropane (FC 218)	6.08	0.325	2	476	—	188.0	−39	76-19-7
Propane	3.05	0.163	0	*300*	*300*	44.1	−42	74-98-6
1,2-Propanediol[(b)]	17.09	0.915	0	710	745	76.1	187	57-55-6
1,3-Propanediol[(b)]	20.29	1.086	0	793	820	76.1	214	504-63-2
1-Propanol	8.61	0.461	0	539	571	60.1	97	71-23-8
2-Propanol (isopropanol)	6.04	0.323	0	474	530	60.1	83	67-63-0
Styrene	23.18	1.241	0	887	890	104.1	145	100-42-5
Sevoflurane	4.32	0.231	1	409	—	200.1	24	28523-86-6
1,1,2,2-Tetrabromoethane	25.50	1.362	2	978	—	345.7	229	79-27-6

table continued

Table 14.3 continued

Compound	*Retention index*							
	RT	*RRT*	*ECD*	*Calc.*	*Lit.*	M_r	*BP*	*CAS*
1,1,1,2-Tetrachloroethane	21.85	1.167	2	843	870	167.9	131	630-20-6
1,1,2,2-Tetrachloroethane	23.33	1.246	2	892	905	167.9	146	79-34-5
Tetrachloroethylene (perchloroethylene)	20.89	1.116	2	811	807	165.9	121	127-18-4
1,1,1,2-Tetrafluoroethane (FC 134a)	2.76	0.148	1	219	—	102.0	−27	811-97-2
Tetrahydrofuran (THF)	12.31	0.659	0	615	638	72.1	66	109-99-9
Toluene	19.14	1.025	0	763	768	92.1	111	108-88-3
1,1,1-Trichloroethane (methylchloroform)	13.56	0.724	2	639	634	133.4	74	71-55-6
1,1,2-Trichloroethane	18.72	1.000	2	752	727	133.4	113	79-00-5
2,2,2-Trichloroethanol	22.29	1.191	2	858	859	149.4	151	115-20-8
Trichloroethylene	16.25	0.868	2	691	710	131.4	87	79-01-6
1,1,1-Trichlorotrifluoroethane (FC 113)	8.01	0.428	2	527	530	187.4	46	354-58-5
1,1,2-Trichlorotrifluoroethane	7.96	0.425	2	526	555	187.4	48	76-13-1
2,2,2-Trifluoroethanol	5.17	0.276	1	441	580	100.0	75	75-89-8
m-Xylene	22.62	1.211	0	869	871	106.2	138	108-38-3
o-Xylene	23.32	1.250	0	892	895	106.2	144	95-47-6
p-Xylene	22.66	1.213	0	870	870	106.2	138	106-42-3

[a]RT, retention time (min); RRT, retention time relative to 1,1,2-trichloroethane (on the ECD channel for compounds responding on that channel); ECD, relative ECD response (0 = nil, 1 = poor, 2 = good); Calc., calculated retention index on the SPB-1 system; Lit., literature retention index on SE30/OV-1/OV-101; M_r, relative molecular mass; BP, boiling point (°C, atmospheric pressure); CAS, Chemical Abstracts Registry number.
[b]Compounds injected as liquids.

pulsed discharge (PD) detector (Forsyth 2004) can be used as an ECD. A few per cent of an additional gas ('dopant') is added, which is ionised and provides electrons to produce a standing current. Several dopants have been tested – the best appear to be methane and xenon. The PD-ECD has a sensitivity (minimum detectable quantities of 10^{-15} to 10^{-12} g) similar to, or better than, ^{63}Ni-ECD, but does not require licensing, wipe tests, and other administrative or safety requirements.

Preparation of internal standard solution Approximately 50 mg ethylbenzene and 50 mg 1,1,2-trichloroethane (both checked for purity by gas chromatography before use – common impurities are toluene in ethylbenzene and 1,1,1-trichloroethane in 1,1,2-trichloroethane) are measured into 50 mL glass volumetric flasks containing outdated, 'volatile-free', blood-bank whole blood. After thorough mixing, 1.0 mL of the 1,1,2-trichloroethane solution and 2.5 mL of the ethylbenzene solution are diluted to 100 mL using outdated, blood-bank, whole blood–deionised water (1 + 24) to give the working internal standard solution. The final ethylbenzene and 1,1,2-trichloroethane concentrations are approximately 25 and 10 mg/L, respectively. This solution remains usable for not less than 2 years if stored in 3 mL portions at −5°C to −20°C in screw-topped glass vials (3.5 mL, Merck 215/0073/23) or equivalent.

Sample preparation

Blood and urine Internal standard solution (200 µL) is added to a 12 mL glass septum vial (Chromacol 12-CV or equivalent) using an air displacement pipette. The vial is then sealed with a crimped-on, Teflon-lined, silicone rubber septum (Chromacol 20-ST3 or equivalent) and disposable aluminium vial cap. The vial is incubated (65°C, heating block) and, after 15 min, a portion (100–300 µL) of the headspace is withdrawn using a 0.5 mL gas-tight glass syringe (Supelco 500F-GT or equivalent) that has been warmed by being placed on the heating block (10 min), and injected onto the gas chromatography column over 2–3 s.

Subsequently, the sample (whole blood, plasma, serum or urine) (200 µL) is added via the septum to the same vial using a 1.0 mL hard plastic (polystyrene) disposable syringe fitted with a 2.5-cm (1-inch), 25-gauge Luer needle and, after at least 15 min, a further portion of the headspace is taken using the 0.5 mL gas-tight syringe described above and injected onto the GC column. After each injection, the plunger should be removed from the gas-tight syringe and the assembly placed on the heating block until the next injection to ensure evaporation of any remaining analyte. The syringe should also be rinsed occasionally with methanol to remove deposits and dried by purging with compressed air.

Tissues A 'blank' internal standard analysis should be performed as described above. Samples of solid tissues should be analysed in a separate vial after adding a proteolytic enzyme to the incubation mixture to digest the sample. Thus, 20–50 mg wet weight of tissue is dissected from the centre of the frozen specimen. Duplicate portions of the specimen are incubated (65°C, 15 min) with internal standard solution (200 µL) and approximately 1 mg of solid protease (Sigma Type VIII (Subtilisin Carlsberg; Subtilopeptidase A; EC 3.4.21.62), P 5380) or equivalent, prior to the analysis of 100–300 µL headspace as described above.

Products Aerosols and fuel gases can be analysed after releasing a portion of the product into a headspace vial, and then transferring a few microlitres of the vapour to another vial for analysis. Liquids can be analysed in the same way except that it is often possible to withdraw a portion (5–50 µL) of the headspace directly from the container. In this latter case, however, the result may not be representative of the composition of the liquid as a whole. Adhesives and other liquid or semi-liquid products can be introduced into a glass vial. The vial is sealed and, after incubation (65°C, 15 min), a portion (50–100 µL) of the headspace is transferred to a sealed pre-incubated vial (65°C, 15 min) containing internal standard solution. After re-equilibration (65°C, 5 min) 100–200 µL of headspace is taken for analysis.

Quantitative analysis

Quantitative assays should be performed in duplicate either isothermally or on a temperature programme using the appropriate detector. If concentrations of ECD-responding compounds are very high, it is sometimes more convenient to use FID. Assay calibration should be by analysis of standard solutions prepared as described below; the same solutions are used in the analysis of blood and of tissue digests. Analyte concentrations in the ranges 0.1–10 or 0.5–50 mg/L are usually adequate in cases of acute poisoning. Calibration graphs of peak height or area ratio to the internal standard are usually used, although absolute calibration in terms of amount of analyte injected should be possible, especially if an automated headspace analyser is employed. Such apparatus not only permits unattended operation, but also gives much better reproducibility in quantitative work.

Liquid and solid analytes Calibration solutions are prepared by adding a known volume of the liquid analyte to a volumetric flask containing 'volatile-free' blood by using a positive displacement pipette and ascertaining the exact amount added by weighing. Solid analytes are weighed in directly. After allowing time for equilibration, appropriate dilutions are performed, taking care to minimize loss of analyte by handling reagents and glassware at 4°C and storing samples and standards at 4°C with minimal headspace. Small (3.5 or 7.0 mL) glass vials (Merck 215/0073/23 or 25, or equivalents) with screw caps lined with aluminium foil are convenient for performing standard dilutions. Portions of the standards are transferred to headspace vials for analysis as described above and a calibration graph of peak height or area ratio of analyte to internal standard against analyte concentration is prepared. Often, either 1,1,2-trichloroethane or ethylbenzene can be used as the internal standard.

Gaseous analytes Calibration solutions of gaseous analytes have been prepared from a solution of the analyte in methanol with subsequent dilution in blood (Broussard *et al.* 2001). Alternatively, calibration mixtures for gaseous analytes may be prepared directly into headspace vials (Gill *et al.* 1992). Septum vials of approximately 30, 50 and 100 mL nominal capacity (Supelco 33106, 33108-U, 33110-U; or equivalent) are calibrated by weighing when filled with deionised water. Each vial is then dried and filled with nitrogen. A piece of aluminium foil (approximately 1 cm^2) is added to aid mixing and the vial is sealed with a crimped-on, Teflon-lined, silicone rubber septum identical to those used in sample analyses. A 125 mL gas sampling bulb fitted with two grease-free Teflon stopcocks and a central cylindrical septum (Supelco 2-2146 or equivalent) is filled with pure analyte, usually purchased in a small cylinder ('lecture bottle'), at atmospheric pressure. The selected volume of vapour is then taken from the gas sampling bulb via the septum using a gas-tight syringe and added through the septum of a calibrated, nitrogen-filled, sealed septum vial. Care should be taken to ensure that the contents of this latter vessel remain at atmospheric pressure. Thus, if the volume of gas transferred is greater than 0.1 mL, a short vent needle should be inserted through the septum well away from the point of the gas-tight syringe needle. After thorough mixing of the gas transferred to the septum vial, further dilutions into additional calibrated, nitrogen-filled, sealed septum vials may be prepared as required. Finally, measured volumes of diluted analyte vapour are transferred using a gas-tight syringe into headspace vials containing the same volume of 'blank' blood as used in sample analyses. A constant volume of appropriately diluted internal standard vapour (2,2-dimethylpropane in the case of butane) prepared as above is also added to the sample headspace vial and to the headspace vials containing the calibration mixtures prior to the analysis.

Pharmacokinetics and the interpretation of results

The UK maximum exposure limit (MEL) or occupational exposure standard (OES; Table 14.4) provides information on the relative toxicities of different compounds after chronic exposure to relatively low concentrations of vapour. Inhaled compounds may rapidly attain high concentrations in well-perfused organs (brain, heart) while concentrations in muscle and adipose tissue may be very low. Should death occur, this situation is 'frozen' to an extent, but if exposure continues the compound will accumulate in less-accessible, poorly perfused tissues, only to be slowly released once exposure ceases. Thus, the plasma concentrations of some compounds may fall monoexponentially, while others may exhibit two (or more) separate rates of decline (half-lives).

The solubility of a volatile compound in blood is an important influence on the rate of absorption, tissue distribution and elimination of the compound. The partition coefficients of a number of compounds between air, blood and various tissues have been measured *in vitro* using animal tissues, and some *in vivo* distribution data have been obtained from postmortem tissue measurements in humans (Table 14.4). However, these latter data must be used with caution since there are many difficulties inherent in such measurements (sampling variations, analyte stability, external calibration, etc.). Published data on the plasma half-lives of volatile substances (Table 14.4) are not easily comparable, because either too few samples have been taken or the analytical methods used did not have sufficient sensitivity to measure the final half-life accurately.

Many volatile substances, including butane, dimethyl ether, most fluorocarbon refrigerants/aerosol propellants, isobutane, nitrous oxide, propane, tetrachloroethylene and 1,1,1-trichloroethane, are largely eliminated unchanged in exhaled air. Others are partly eliminated in exhaled air and also metabolised in the liver and elsewhere, the metabolites being eliminated in exhaled air or in urine, or incorporated into intermediary metabolism. After ingestion, extensive hepatic metabolism can reduce systemic availability ('first-pass' metabolism) of certain compounds. Table 14.5 gives details of examples where blood or urinary metabolite measurements have been used to assess exposure to volatiles.

Interpretation of qualitative results

The likelihood of detecting exposure to volatile substances by headspace-GC of blood is influenced by the dose and duration of exposure, the time of sampling in relation to the time elapsed since exposure, and the precautions taken in the collection and storage of the specimen (Broussard 2000; Gill *et al.* 1988; Willie, Lambert 2004). In a suspected VSA- or anaesthetic-related fatality, analysis of tissues (especially fatty tissues such as brain) may prove useful since high concentrations of volatile compounds may be present even if very little is detectable in blood.

Analysis of metabolites in urine may extend the time after which exposure may be detected but, of the compounds commonly abused, only toluene, the xylenes and some chlorinated solvents, notably trichloroethylene, have suitable metabolites (Table 14.5). Chronic gasoline 'sniffing' has been diagnosed by the measurement of blood lead concentrations or detection of aromatic components such as toluene and ethylbenzene. With some petrols and other complex mixtures such as petroleum ethers (see Table 14.1), however, the blood concentrations of the individual components are often below the limit of detection of HS-GC methods even after significant inhalational exposure. Abuse of methoxyflurane has been detected by measuring serum and urine fluoride ion concentrations.

Detection of a volatile compound in blood does not always indicate VSA or occupational/environmental exposure to solvent vapour. Acetone and some of its homologues may occur in high concentrations in ketotic patients. Large amounts of acetone and butanone may also occur in blood and urine from children with acetoacetyl-coenzyme A thiolase deficiency, for example, and may indicate the diagnosis. In addition, acetone is the major metabolite of 2-propanol in humans. Conversely, 2-propanol has been found in blood from ketotic patients. Other ketones may also give rise to alcohols *in vivo* (Table 14.5). Other volatile compounds such as halothane or chlorobutanol may be used in therapy or inadvertently added to the sample as a preservative.

Use of aerosol disinfectant preparations when collecting specimens may contaminate the sample if an aerosol propellant is used. Contamination of blood samples with ethanol or 2-propanol may also occur if an alcohol-soaked swab is used to cleanse skin prior to venepuncture. In 20 subjects studied after drinking alcohol, 2-propanol (0.13 g/L) was detected in one sample by HS-GC, while in two others the measured ethanol concentration increased from 0.58 g/L (dry cotton swab or 70% 2-propanol swab) to 1.2 g/L (pure ethanol swab) and from 0.12 g/L (dry swab and 2-propanol swab) to 1.26 g/L, respectively, with the swab being used to exert pressure on the venepuncture site upon withdrawal of the needle (Carter, McConnell 1990). Higuchi *et al.* (2005) also reported a definite risk of sample contamination from use of ethanol-containing swabs during sample collection from hospital patients, an extreme blood ethanol concentration of 9.6 g/L being recorded if the swab was used to wipe the syringe needle after sampling. Other studies in volunteers have, however, found at worst minimal contamination using either ethanol- or 2-propanol-soaked swabs (Goldfinger, Schaber 1982; Malingre *et al.* 2005; McIvor, Cosbey 1990; Peek *et al.* 1990). It seems likely that variation in sampling technique underlies these differences, the danger being that blood not drawn

Table 14.4 Physical properties and pharmacokinetic data of some volatile compounds (data summarized from Baselt 2004; Pihlainen, Ojanperä 1998)

Compound	*MEL/OES[a] (mg m^{-3})*	*Vapour pressure[b] (20°C, mmHg)*	*Inhaled dose absorbed (%)*	*Proportion absorbed dose (%)*		*Half-life[c] (h)*	*Brain:blood distribution ratio (deaths)*	*Partition coefficient (blood:gas) (37°C)*
				Eliminated unchanged	*Metabolised*			
Acetone	1210	183	—	—	—	3–5[d]	—	243–300
Benzene	16	75	46	12	80	9–24	3–6	6–9
Butane	1450	(1554)	30–45	—	—	—	—	—
Isobutane	1750[e]	(2282)	—	—	—	—	—	—
Butanone	600	75	70	99+	0.1	0.5	—	116
Carbon disulfide	32	294	40	<30	50–90	<1	—	2.4
Carbon tetrachloride	13	90	—	50?	50?	48	—	1.6
Chlorodifluoromethane	3590	(6701)	—	—	—	—	1.9	—
Chloroform	9.9	157	—	20–70 (8 h)	>30	—	4	8
Cyclopropane	—	(4701)	—	99	0.5	—	1.5–3.6	0.55
Desflurane	—	669	—	—	0.02	—	1.29[f]	0.42
Dichlorodifluoromethane	5030	(3639)	35	99	<0.2	—	1.4	0.15
Dichloromethane	350	350	—	50?	<40	0.7	0.5–1	5–10
Diethyl ether	310	438	—	>90	—	—	1.1	12
Enflurane	383	172	90+	>80 (5 days)	2.5	36	1.4[f]	1.9
Ethyl acetate	1460	72	—	—	—	—	—	—
Halothane	82	244	90+	60–80 (24 h)	<20	2–3	2–3	2.57
Hexane	72	122	—	—	—	—	—	—
Isoflurane	383	240	—	—	0.2	—	1.57[f]	1.38
Methoxyflurane	—	23	—	19 (10 days)	>44	—	2–3	13
Methyl isobutyl ketone	208	15	—	—	—	—	—	—
Nitrous oxide	183	(39800)	—	>99	—	—	1.1	0.47
Propane	1750[e]	(6269)	—	—	—	—	—	—
Sevoflurane	—	157	—	—	3	20	1.7[f]	0.68
Styrene	430	4	—	1–2	>95	13	—	32
Tetrachloroethylene	345	14	60+	>90	1–2	72	9–15	9–19
Toluene	191	22	53	<20	80	7.5	1–2	8–16
1,1,1-Trichloroethane	555	98	—	60–80 (1 week)	2	10–12	2	1–3
Trichloroethylene	550	58	50–65	16	>80	30–38	2	9.0
Trichlorofluoromethane	5710	667	92	89	<0.2	1.5	2.5	0.87
'Xylene'	220	6	64	5	>90	20–30	—	42.1

[a]UK maximum exposure limit/occupational exposure standard (8 h time-weighted average; Health & Safety Executive 2002).
[b]Figures in parentheses indicate compound gas at 20°C.
[c]Terminal phase plasma half-life.
[d]Longer after high doses.
[e]As components of LPG.
[f]Experimental: 37°C.

initially for blood ethanol measurement and with due precautions is later used for this purpose.

Gross contamination with technical xylene (a mixture of *o*-, *m*- and *p*-xylene together with ethylbenzene) has been found in blood collected into Sarstedt Monovette Serum Gel blood collection tubes; contamination with toluene (up to 22 mg/L), 1-butanol, ethylbenzene and xylene has been found in more recent batches of these same tubes (Dyne *et al.* 1996). Contamination with 1-butanol or 2-methyl-2-propanol occurs commonly in blood collected into tubes coated with EDTA. Carbon disulfide has been detected in blood collected in tubes sealed with soft-rubber stoppers (Weller, Wolf 1989). Care should be taken when handling frozen tissues prior to analysis as any compounds present in ambient air may condense on the cold surface and give rise to false positives. Processing blank frozen tissue can control for this possibility.

The interpretation of case data involving chloroform is particularly difficult, especially since this compound is still sometimes used with criminal intent (Flanagan, Pounder 2010). In addition to sometimes being present in drinking water at low concentrations as a result of chlorination, chloroform is found in a variety of medicinal preparations, in cigarette smoke, soft drinks, margarines, and in swimming pools if a chlorination plant is in operation. A further possible source of chloroform on HS-GC is from thermal decomposition of trichloroacetic acid (Aggazzotti *et al.* 1987). Trichloroacetic acid is a metabolite of several

Table 14.5 Metabolites of some solvents and other volatile substances that may be measured to assess exposure

Compound	M_r	*Parent compound*	*Fluid*[a]	'*Normal*'[b]	'*High*'[c]	*Comment*
Acetaldehyde	44.1	Ethanol	Blood	0.2 mg/L	(Not known)	
Acetone	58.1	2-Propanol	Blood	10 mg/L	—	Blood/urine acetone concentrations can rise to 2 g/L in ketosis. 2-Propanol also acetone metabolite
			Urine	10 mg/L	80 mg/L	
4-Aminophenol	109.1	Aniline	Urine	—	10 mg/L	
Bromide ion	79.9	Bromomethane, other organobromines	Serum	10 mg/L	40 mg/L	The bromide concentrations associated with toxicity are lower after exposure to organobromines than when bromide salts given orally
Butoxyacetate	132.2	2-Butoxyethanol	Urine	—	100 mg/L	
Carbon monoxide	28.0	Dichloromethane	Blood	<5% HbCO	>20% HbCO	Carboxyhaemoglobin blood half-life 13 h breathing air, atmospheric pressure (carboxyhaemoglobin half-life 5 h after inhalation of CO). Blood carboxyhaemoglobin useful indicator of chronic exposure
Cyanide ion	26.0	Acetonitrile, acrylonitrile, other organonitriles	Blood	0.2 mg/L (non-smokers)	2 mg/L	Cyanide metabolised to thiocyanate; both compounds may accumulate during chronic exposure
Cyclohexanol	100.2	Cyclohexane, cyclohexanone	Urine	—	5 mg/L	Additional cyclohexane metabolites: *t*-1, 2-cyclohexanediol, *t*-1, 4-cyclohexanediol (alcohol metabolites excreted mainly as glucuronides in adults)
Cyclohexanone	98.2	Cyclohexane, cyclohexanol	Urine	—	0.5 mg/L	See entry for cyclohexanol above
2,5-Dichlorophenol	163.0	1,4-Dichlorobenzene	Urine	—	100 mg/L	
Dimethylsulfone	94.1	Dimethylsulfoxide (DMSO)	Urine	—	(Not known)	
Ethanol	46.1	Ethyl acetate	Blood	0.1 g/L	0.8 g/L	0.8 g/L legal UK driving limit
Ethoxyacetate	104.1	Ethoxyethanol, 2-ethoxyethyl acetate	Urine	—	50 mg/L	
β-Hydroxyethoxyacetate (HEAA)	120.1	1,4-Dioxane	Urine	—	0.5 g/L	Dioxane-2-one also found in urine
Fluoride	19.0	Methoxyflurane and some other organofluorines	Serum	0.2 mg/L	2 mg/L	
			Urine	1 mg/L	2 mg/L	
Formate	46.0	Formaldehyde, methanol, methyl formate	Urine	20 mg/L	30 mg/L	
2,5-Hexanedione + 4, 5-dihydroxy-2-hexanone	—	Hexane, 2-hexanone (MBK)	Urine	—	5 mg/L	2-Hexanol and 2-hexanone additional hexane metabolites
Hippurate	179.2	Toluene	Urine	0.2 g/L	2 g/L	Not ideal indicator of toluene exposure as there are other (dietary, pharmaceuticals) sources of benzyl alcohol/benzoate hence hippurate
Mandelate	152.1	Ethylbenzene, styrene	Urine	0.005 g/L	2 g/L	Phenylglyoxylic acid also urinary metabolite
Methanol	32.0	Methyl acetate, methyl formate	Urine	—	30 mg/L	
N-Methylacetamide (NMA)	73.1	*N*,*N*-Dimethylacetamide (DMAC)	Urine	—	65 mg/L	

table continued

Table 14.5 continued

Compound	M_r	*Parent compound*	*Fluid*[a]	'*Normal*'[b]	'*High*'[c]	*Comment*
N-Methylformamide (NMF)	59.1	*N*,*N*-Dimethylformamide (DMF)	Urine	—	15 mg/L	Hydroxymethyformamide and formamide additional metabolites
4-Methyl-2-pentanone	100.2	Methyl isobutyl ketone	Urine	—	3.5 mg/L	
2-Methylphenol (*o*-cresol)	108.1	Toluene	Urine	—	3 mg/L	Hippurate and other methylphenols additional toluene metabolites
t,*t*-Muconate (*t*,*t*-MA)	142.1	Benzene	Urine	—	2 mg/L	Phenol and *S*-phenylmercapturic acid additional benzene metabolites
Nitrite ion	46.0	Butyl nitrite, isopentyl nitrite, other organonitrites	Plasma	2.5 mg/L	(Not known)	
			Urine	—	10 mg/L	
Oxalate	90.0	Ethylene glycol	Urine	2.5 mg/L	4 mg/L	Glycolate and glyoxylate also plasma and urinary ethylene glycol metabolites
Phenol	94.1	Benzene	Urine	0.1 mg/L	50 mg/L	Excreted as sulfate and glucuronide conjugates. Other metabolites include catechol and hydroquinone. Urinary phenol excretion not a reliable indicator of benzene exposure. *t*,*t*-Muconic acid and *S*-phenyl-mercapturic acid excretion have been used to assess exposure
S-Phenylmercapturate (*S*-PMA)	239.2	Benzene	Urine	—	45 µg/L	*t*,*t*-Muconic acid and phenol additional benzene metabolites
2-Propanol	60.1	Acetone	Blood	—	2.5 g/L	Acetone also 2-propanol metabolite
Thiocyanate ion	58.1	Acetonitrile, acrylonitrile, other organonitriles	Plasma	4 mg/L (non-smokers), 20 mg/L (heavy smokers)	120 mg/L	Cyanide/thiocyanate may accumulate during chronic exposure.
2-Thiothiazolidine-4-carboxylate (TTCA)	163.2	Carbon disulfide	Urine	—	8 mg/L	TTCA glutathione conjugate of carbon disulfide. Urinary TTCA excretion reliable indicator of exposure
Tolurates (methylhippurates)	193.2	Xylenes	Urine	0.01 mg/L	1.5 g/L	
Trifluoroacetate	114.0	Halothane and some other fluorinated anaesthetics	Urine	—	2.5 mg/L	
Trichloroacetate	163.4	Trichloroethylene	Urine	—	100 mg/L	Metabolite of 2,2,2-trichloroethanol
2,2,2-Trichloroethanol	149.4	Trichloroethylene	Plasma	10 mg/L	50 mg/L	Also metabolite of chloral hydrate, dichloralphenazone and triclofos

[a]Urinary excretion often expressed as a ratio to creatinine.
[b]Upper limit of normally expected or 'non-toxic' concentration.
[c]Lower limit of concentration associated with toxicity/occupational exposure action limit.

compounds, including the solvent trichloroethylene and the drugs chloral hydrate, dichloralphenazone and triclofos. Trichloroacetic acid has a half-life in blood of 3–5 days and thus may be detected for a relatively long time after exposure to, or ingestion of, a precursor. Trichloroacetic acid plasma concentrations of up to 40 mg/L have been reported after occupational exposure to trichloroethylene vapour.

In 25 adult white women in Florida, USA, over a period of 6 months average plasma chloroform concentrations were generally less than 25 µg/L, but in two plasma chloroform concentrations of 2.9 and 4.0 mg/L, respectively, were found during routine sampling (Pfaffenberger, Peoples 1982). All subjects were carefully screened to exclude occupational and recreational exposure to chloroform and other compounds that could give rise to chloroform on headspace-GC. Nevertheless incidental acute exposure to chloroform or a trichloroacetic acid precursor must have occurred to give rise to these results. At the other extreme, postmortem blood chloroform concentrations in fatalities involving this agent have been reported as 5–800 mg/L (Baselt 2004).

It is well known that ethanol may be both produced and metabolised by microbial action in biological specimens. Propanols and butanols may also be produced by microbial action. 'Congener alcohols' such as methanol, propanol, butanol, 2-butanol, 2-methylpropanol, 2-methylbutanol and 3-methylbutanol (and ketone metabolites of secondary alcohols) may arise from ingestion of alcoholic drinks (Bonte 2000). Methanol or 2-methyl-2-propanol (*t*-butanol) may be used together with pyridine or denatonium benzoate (Bitrex, Aversion) to denature alcohol, although this may not stop the abuse of products containing denatured alcohol (Carnahan *et al.* 2005). Butyraldehyde, dimethyl disulfide, isovaleraldehyde and valeraldehyde may arise from putrefaction (Weller, Wolf 1989). Small amounts of hexanal may arise from degradation of fatty acids in blood on long-term storage even at −5°C to −20°C (Gill *et al.* 1988). Hexanal may co-elute with toluene on some GC systems, but interference from this source is only likely to be important if very low concentrations of toluene (0.1 mg/L or less) are to be measured.

In some deaths attributed to the abuse of LPG, only butane, isobutane and propane are detected on headspace-GC of postmortem samples. In other cases 2-propanol, acetone, 2-methyl-2-propanol, 2-butanol and/or butanone is also present. These latter compounds probably arise from the metabolism of the butanes and propane; using HS-SPME butanol, 2,3-butanediol, 3-hydroxy-2-butanone and 2,3-butanedione have also been detected in postmortem samples from LPG abusers (Walker *et al.* 2006).

The alkyl nitrites that can be abused by inhalation (isobutyl nitrite, isopentyl nitrite) are a special case in that (i) they are extremely unstable and break down rapidly *in vivo* to the corresponding alcohols (Kielbasa *et al.* 1999; Tytgat, Daenens 1996), and (ii) they usually also contain other isomers (butyl nitrite, pentyl nitrite) (Baselt 2004; Osterloh 1984). Any products submitted for analysis will usually contain the corresponding alcohols as well as the nitrites. The profound methaemoglobinaemia arising particularly from ingestion of these compounds can be detected easily.

Interpretation of quantitative results

In very general terms, soon after acute exposure, blood concentrations of volatile substances of 5–10 mg/L and above may be associated with clinical features of toxicity. In other words, pharmacologically effective concentrations of volatile substances are similar to those of inhalational anaesthetics (Baselt 2004; Flanagan 1998), and are thus an order of magnitude lower than those observed in poisoning with relatively water-soluble compounds such as ethanol.

There may be a considerable overlap in the blood concentrations of volatile compounds attained after workplace exposure and as a result of deliberate inhalation of vapour. In the occupational setting, blood toluene concentrations after exposure to up to 127 ppm toluene (the UK occupational exposure limit at the time was 100 ppm) for 8 h ranged between 0.4 and 6.7 mg/L (Campbell *et al.* 1987). After brief exposure only signs of moderate intoxication (e.g. slurred speech, unsteady movements) have been associated with blood toluene concentrations as high as 30 mg/L (Garriott *et al.* 1981). However, blood toluene concentrations above 9.2 mg/L were associated with impairment or probable impairment in car drivers arrested in Norway in 1983–87 (Gjerde *et al.* 1990).

Blood toluene concentrations in samples from 132 patients who were thought to have engaged in VSA ranged from 0.2 mg/dL to 70 mg/L, and were above 5 mg/L in 22 of 25 deaths (Meredith *et al.* 1989). On the other hand, 13 patients with blood toluene concentrations greater than 10 mg/L were either asymptomatic or only mildly intoxicated (headache, nausea, vomiting and/or drowsiness), although these manifestations of toxicity sometimes prove fatal. Similar findings have been reported from Japan (Miyazaki *et al.* 1990). Aside from individual differences in tolerance and possible loss of toluene from the sample prior to analysis, sample contamination, etc., the lack of a strong correlation between blood concentrations and clinical features of poisoning is probably due to rapid initial tissue distribution.

Some 80% of a dose of toluene is converted to hippuric acid, which is excreted in urine. Similarly, more than 90% of a dose of xylene is metabolised to methylhippuric (toluric) acids. The principal isomer found in urine is 3-methylhippurate since *m*-xylene is the principal component of technical-grade xylene (see Table 14.1). Methylhippurates are not normal urinary constituents, but hippuric acid may arise from the metabolism of benzoates used as preservatives in foods and medicines. Hippurate and methylhippurate excretion is often expressed as a ratio to creatinine since this obviates the need for 24-h urine collections. Occupational exposure to toluene can give rise to ratios of up to 1 g hippurate per g creatinine or more; in patients suspected of VSA a ratio of more than 1 g hippurate per g creatinine strongly suggests, but does not prove, toluene exposure (Meredith et al. 1989).

Conclusion

The analysis of volatile substances falls into two main areas: the diagnosis of acute poisoning and monitoring of occupational/environmental exposure to solvents and other volatiles. The Montreal Protocol and subsequent legislation aimed at limiting the use of certain chlorinated and brominated hydrocarbons has had a marked impact on the range of volatile substances available in many Western countries. The next few years are likely to see far fewer changes. As regards assessing occupational/environmental exposure, use of breath analysis via simple collection devices such as that of Dyne *et al.* (1997) and/or urinary metabolite excretion (see Table 14.5) in conjunction with ambient air monitoring is probably the best way forward. However, analysis of blood, and other tissues if available, will remain important in other applications. HS-GC or variants thereof used with appropriate detectors is ideally suited to the analysis of unchanged volatiles and is likely to remain predominant except when the analysis of polar metabolites possessing an appropriate chromophore suggests the use of HPLC.

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CHAPTER

15 Natural Toxins

J F de Wolff and F A de Wolff

Introduction

The term 'natural toxins' usually refers to potentially toxic organic compounds of natural origin, in contrast to mineral poisons and synthetic drugs. Sources of these toxins range from simple microorganisms to highly developed vertebrates, and their chemical structures are correspondingly diverse. Exposure to natural toxins may lead to acute as well as long-term symptoms that affect almost any organ system. The highly varied chemical, biological and clinical nature of this class of poisons means that the contribution of the analytical toxicologist to the diagnosis, therapy and follow-up of 'naturally poisoned' patients is limited.

An extensive number of substances that comply with the definition of natural toxins have been used for therapeutic purposes. Classic examples are ergotamine, salicylic acid and the cardiac glycosides, such as digoxin, and penicillin. More recent examples are ciclosporin and botulinum toxin. Most of these are not discussed in this chapter. Similarly, commonly abused substances of natural origin, such as cannabis, cocaine and morphine, are covered elsewhere in this book.

The diversity in chemical composition of natural toxins prevents an arrangement according to chemical structures. Therefore, a biological classification has been chosen. Substances are discussed that originate from:

- Bacteria
- Fungi
- Higher plants
- Invertebrates
- Vertebrates.

Bacteria

The impact of pathogenic bacteria on human health mostly, if not always, results from their ability to produce microbial toxins. For this chapter, a selection has been made of three common potent bacterial toxins: tetanus toxin, botulinum toxin and verotoxin. The former two are related neurotoxic proteins produced by several *Clostridium* spp. strains; verotoxin is produced by certain *Escherichia coli* strains.

Bacteria may also be the source of toxins previously attributed to other organisms, such as tetrodotoxin (TTX), which is found in puffer fish but is most probably produced by commensal microorganisms. For reasons of convention, TTX is discussed in the section on fish poisoning. The same holds for those freshwater cyanobacteria that produce saxitoxins, which are described in the section on mollusc poisoning.

Clostridia (botulinum and tetanus)

Botulinum and tetanus neurotoxins are produced by strictly anaerobic bacteria belonging to the genus *Clostridium* and cause the neuroparalytic syndromes of botulism and tetanus. The botulinum toxins consist of at least four peptides with molecular sizes that range from 150 kDa to 900 kDa. The tetanus toxin has two disulfide-linked peptide chains of molecular size 50 kDa and 100 kDa. The clostridial neurotoxins are the most potent toxins known, with an estimated median lethal dose of about 1 ng/kg intravenously. Botulinum toxins are food poisons, whereas the tetanus toxin is not. Tetanus follows the contamination of necrotic wounds with spores of *Clostridium tetani*. Tetanus is rare in countries with immunisation programmes, but it has a high case fatality rate of 24%. An estimated 800 000 newborns die from neonatal tetanus worldwide each year. Treatment with antitoxin and intravenous administration of penicillin soon after infection may reduce mortality. Generally applicable analytical methods for the identification and quantification of tetanus toxin have not been reported (Cherington *et al.* 1995).

Classic food-borne botulism occurs after ingestion of food contaminated by preformed toxin of *C. botulinum*. The clinical presentations are stereotypical. Within 12–36 h of ingestion, the patient develops diplopia and ptosis, followed by a descending pattern of weakness that affects the upper and then the lower limbs, and respiratory paralysis in severe cases. There is no specific treatment for botulism; recovery is not uncommon but it requires the regeneration of new motor endplates, which takes weeks. Food-borne botulism remains a matter of concern. Effective treatment depends on provision of intensive care and rapid administration of botulinum antitoxin based on clinical presentation, and should not await the results of time-consuming laboratory diagnosis (Sobel 2005). Owing to its extreme neurotoxicity and easy production, botulinum toxin has also been suggested as a possible agent in bioterrorism (Villar *et al.* 2006).

Laboratory proof of botulism requires the detection of the toxin or bacterial deoxyribonucleic acid (DNA) in the patient's blood or stools. If it is still available, the suspected food should also be tested for the toxin. With an old toxin identification method using a mouse toxicity test with antitoxin neutralisation, laboratory results are not always confirmatory. A number of immunoassay methods have been reported for the detection of botulinum toxins. A radioimmunoassay has been replaced by a more sensitive 'double sandwich' enzyme-linked immunosorbent assay (ELISA) method based on application of three antibodies. An ELISA amplification technique may generate a 10-fold increase in sensitivity, rendering it suitable for diagnostic purposes (Hatheway and Ferreira 1996). A promising new method for the detection of bacterial spores and toxins uses a biosensor based on electrochemiluminescence. Toxins and bacterial spores are captured on antibody-conjugated micrometre-sized magnetic beads, followed by binding of ruthenium(II)-trisbipyridal chelate-labelled reporter antibodies. Immunomagnetically captured target materials are then collected on a magnet. Electrochemiluminescence is evoked from the Ru-tagged reporter antibodies by application of an electric potential. Using a commercially available analyser (ORIGEN), a femtogram sensitivity level is obtained for a number of toxins, including botulinum toxin. For *Bacillus anthracis*, this method yields a limit of detection of 100 spores (Gatto-Menking *et al.* 1995). Even more promising are laboratory methods based on the measurement of clostridium DNA by polymerase chain reaction (PCR) in combination with capillary or gel electrophoresis or other detection methods. Combined with ELISA or probe hybridisation, detection limits as low as 0.1 spore per gram of faeces or food have been reported (Lindström and Korkeala 2006).

Escherichia coli

Several strains of the common intestinal bacterium *E. coli* may cause diarrhoeal disease in humans. One such strain, which occurs naturally in the gut of cattle and other animals, produces a potent cytotoxin: verotoxin or verocytotoxin. This *E. coli* strain, usually referred to as VTEC, is

geographically widespread and is associated with life-threatening human diseases that range from bloody diarrhoea to the haemolytic uraemic syndrome and thrombocytopenic purpura. Young children, especially those from urban areas, are a particularly vulnerable group because of their age and immunological naivety to farmhouse infections (Chattopadhyay *et al.* 2001; Parma *et al.* 2000; Pritchard *et al.* 2000).

The chemical nature of verotoxin has not yet been elucidated. Verotoxin-producing *E. coli* strains are characterised through PCR and DNA hybridisation, and by a Vero-cell cytotoxicity assay (Leung *et al.* 2001).

Other *E. coli* strains (O157:H7) and other bacterial species produce Shiga toxins, which may produce disease in humans after consumption of undercooked contaminated beef. In humans these toxins may cause haemorrhagic colitis and haemolytic uraemic syndrome. Enzyme immunoassays are available that detect Shiga toxins in diarrhoeal stool samples from humans and in contaminated beef (Atalla *et al.* 2000; Hyatt *et al.* 2001).

Fungi

Mycotoxins are a group of potentially toxic substances produced by species of the botanical class Fungi. Remarkably, only a very few mushroom species are deadly after ingestion. Many more fungi that grow on food may produce toxins that do not, generally speaking, cause symptoms of acute intoxication, but that may cause adverse effects after chronic ingestion of small quantities. In this section, a selection is made of fungal toxins from *Aspergillus*, *Fusarium* (*Gibberella zeae*) and *Claviceps* genera that have a possible impact on human health, where the analytical toxicologist may have an important role in the prevention of food-borne diseases. In addition, the toxic principles from some of the most common toxic mushroom species are described.

Aspergillus

Aflatoxin

Aflatoxin (Fig. 15.1) is a family of mycotoxins, produced by the mould *Aspergillus flavus*, that are found as contaminants in both human foodstuffs and animal feed, particularly in maize, groundnuts and other nuts such as pistachios. The aflatoxin-producing *Aspergillus* species are ubiquitous in areas with a hot, humid climate. Foodstuffs may be contaminated with aflatoxin both pre- and post-harvest. Exposure occurs through consumption of contaminated food and also during the handling and processing of aflatoxin-contaminated crops. Aflatoxins have been detected in human milk, urine and blood samples (IARC 1993a).

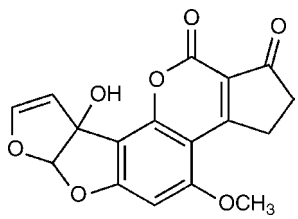

Figure 15.1 Aflatoxin-M_1.

Aflatoxin-B_1 is a very potent human carcinogen that reacts with DNA once it has been bioactivated to the epoxide. It is thought to cause hepatocellular carcinoma.

Aflatoxins have been measured in human blood by high performance liquid chromatography (HPLC) with fluorescence detection (Abdulrazzaq *et al.* 2002). In 201 samples of umbilical cord blood, aflatoxins were detected in 110 and there was a significant negative correlation between birth weight and the aflatoxin concentrations found. Kussak *et al.* (1995) applied a liquid chromatography–tandem mass spectrometry (LC-MS[-MS]) system to measure aflatoxins in spiked urine samples and achieved detection limits of 2–10 pg. A number of analytical methods have been described for the analysis of aflatoxins G_1, G_2, B_1 and B_2 in foodstuffs. High performance thin-layer chromatography (HPTLC) and ELISA procedures have been described, but HPLC with fluorescence detection is considered to be the method of choice (Boenke 1998; Gilbert 1993; Holcomb *et al.* 1992; Kok 1994). A review of these methods is presented in Jaimez *et al.* (2000). Sample preparation and extraction depend greatly on the physicochemical properties of the infected matrix; aflatoxins are soluble in slightly polar solvents, such as a mixture of acetone, chloroform or methanol. Non-polar solvents can be applied for fat extraction. Further purification can be accomplished by solid-phase extraction followed by concentration for chromatographic analysis. Reversed-phase HPLC with methanol–water or acetonitrile–water results in a good separation of the aflatoxins; a disadvantage of these aqueous media is that the natural fluorescence, which renders them so readily detectable by TLC, is quenched. This can be overcome by post-column derivatisation with iodine, a procedure finally adopted as an official method by the Association of Analytical Communities (AOAC) and the International Union of Pure and Applied Chemistry (IUPAC) in 1991 (AOAC 1991). Although much more is known about aflatoxins, ochratoxin is gradually being recognised as a fungal toxin of equivalent importance (Bayman and Baker 2006).

Ochratoxin

Ochratoxin-A (OTA, Fig. 15.2) is a widespread mycotoxin produced mainly by the fungi *A. ochraceus* and *Penicillium verruculosum* during the storage of cereals, cereal products, herbs, spices (IARC 1993b; Petzinger and Ziegler 2000) and other plant-derived products such as coffee (Viani 1996). OTA is chlorinated, which is unusual for a natural product. Ochratoxin-B, which is not chlorinated, and ochratoxin-C, the ethyl ester of OTA, are less toxic. OTA has been reported to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic in animal studies. However, as with aflatoxins, it is often not clear to what extent animal studies are applicable to toxicity in humans.

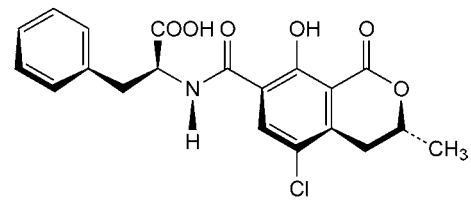

Figure 15.2 Ochratoxin-A.

Ochratoxin-A has been found primarily in northern temperate barley- and wheat-growing areas, but the presence of OTA in green and processed coffee has received much attention recently. As the list of OTA-producing fungi has expanded, so has the list of foods that can be contaminated with ochratoxins (Bayman and Baker 2006).

Consumption by pigs of mouldy pig feed may result in detectable levels of OTA in pork-derived products. Since OTA is hydrolysed rapidly by ruminal flora, it is unlikely to be found in milk or meat from cattle (Scudamore 1996). When ingested by humans, OTA is very persistent, with an elimination half-life of about 35 days attributed to very strong binding to plasma proteins. During the 1950s, a fatal chronic renal disease was identified in several countries in the Balkans and became known as Balkan endemic nephropathy (BEN). BEN has a striking similarity to porcine nephropathy induced by OTA. The causative factor is thought to be high OTA levels in the diet. The toxin is also a possible carcinogen, a teratogen and an immunotoxic agent. It appears to exert its toxic effects by promoting an increased level of lipid peroxidation, by inhibition of an amino-alkylation reaction and possibly by conversion into metabolites that are capable of binding DNA (Marquardt *et al.* 1990).

For the analysis of OTA in blood, serum and milk samples, Zimmerli and Dick (1995) used immunoaffinity column clean-up and HPLC with fluorescence detection and were able to obtain a limit of quantification (LOQ) of 5 pg/g. In a more recent report, Vatinno *et al.* (2007) applied solid-phase microextraction (SPME) to samples of human urine and analysed the extracts by HPLC with fluorescence detection. The LOQ and limit of detection (LOD) values were 0.05 μg and 0.01 μg respectively.

Several analytical procedures have been proposed for the identification and measurement of OTA in food products, as reviewed by Van

Egmond (1991) and Valenta (1998). Although TLC analysis gives satisfactory results in the absence of sophisticated instrumentation, C_{18} reversed-phase HPLC with fluorescence detection is the method of choice, with an excitation wavelength of 330 nm and an emission wavelength of 460 nm. More recently, Noba *et al.* (2009) have described a method based on immunoaffinity clean-up followed by LC-MS(-MS) for the analysis of OTA in ready-to-drink coffee. The LOQ was reported as 0.0065 ng/mL. OTA was detected in 30 samples of coffee available commercially in Japan. A review of LC-MS methods for the analysis of OTA and related substances has been given by Zollner and Mayer-Helm (2006), and it is likely that LC-MS will be the method of choice for ochratoxins in the near future.

Fusarium

Deoxynivalenol

Deoxynivalenol (DON) is a mycotoxin belonging to the trichothecene type of toxins produced by *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum*. These fungi grow on cereal crops, mainly wheat and maize (IARC 1993c). As wheat and maize products form a considerable part of the diet in many regions, the toxicity and the content of DON are important issues in food safety control.

Deoxynivalenol can affect the immune system, and both suppression and activation have been reported, but the toxin is not considered to be mutagenic or carcinogenic.

Two outbreaks of human disease related to trichothecenes have been reported, one in China in 1984 to 1985 and one in India in 1987. Consumption of mouldy wheat or maize results within 5–30 min in nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache. DON has been detected in the cereals in a range of 0.34–92.8 mg/kg, together with 0.004–0.587 mg/kg zearalenone (IARC 1993c) (see later). The tolerable daily intake (TDI) in humans is considered to be 0.5 μg/kg body mass per day (Health Council of The Netherlands 2001). An overview of some of the major mycotoxins, including DON, has been given by Richard (2007).

Black *et al.* (1986) developed a gas chromatography–mass spectrometry (GC-MS) procedure for the measurement of trace quantities of trichothecene mycotoxins in samples of blood and urine. The method involved purification by reversed-phase Sep-Pak C_{18} cartridges and derivatisation to the heptafluorobutyl esters. LODs for a range of mycotoxins ranged from 2 ppb to 7 ppb. Meky *et al.* (2003) explored urinary measurement of DON as a potential biomarker of human exposure. Urine samples were treated with β-glucuronidase and sulfatase enzymes to release conjugated DON, which was then analysed by an immunoaffinity-column–HPLC technique. Positive results were confirmed by LC-MS. Urine samples collected from females living in a potentially high-exposure region of China and a low-exposure region were compared. Mean levels in samples from the suspected high- and low-exposure regions were 37 ng/mL (range 14–94 ng/mL) and 12 ng/ml (range 4–18 ng/ml), respectively.

A method for the quantification of DON in wheat using immunoaffinity-column isolation and HPLC with ultraviolet (UV) detection has been published (Cahill *et al.* 1999). It compares very well with the GC–electron capture detection (ECD) method published by Tacke and Casper (1996). Nowadays, HPLC with UV detection is the recommended method for residue analysis in food (Stroka *et al.* 2006). However, a more sophisticated method combining LC-MS with atmospheric pressure photoionisation has been recently described by Tanaka *et al.* (2009) for the detection of nivalenol and DON in wheat. This reduced the matrix effects seen with other methods and gave LODs of 0.2 and 0.39 ng/g for nivalenol and DON, respectively.

T-2 toxin

Another cereal mycotoxin, the trichothecene congener T-2 toxin, is produced by *F. sporotrichiodes*. T-2 toxin has been suggested as a potential chemical warfare agent (Hodgson *et al.* 1998). A disease known as alimentary toxic aleukia is associated with T-2 toxin. In severe outbreaks of poisoning this disease has a fatality rate as high as 50%. Aleukia is characterised by hyperaemia of the oral mucosa, weakness, fever, nausea and vomiting, and in severe cases by gastroenteritis. In later stages of the disease leukopenia, granulopenia and progressive lymphocytosis occur, followed by severe haemorrhagic diathesis, necrotic pharyngitis and laryngitis, and anaemia. In an outbreak in Kashmir in 1987, T-2 toxin concentrations in flour samples were found to be 0.55–0.8 mg/kg (IARC 1993d). The analytical aspects of T-2 toxin are discussed in the next section, which describes general methods for the analysis of trichothecenes

Zearalenone

Zearalenone is a benzoxacyclotetradecin derivative produced in *F. graminearum* (*Gibberella zeae*) and related species, and is primarily associated with maize (*Zea mais*). It is among the most widely distributed mycotoxins. Toxic effects in humans are extremely difficult to assess as, in cases of contaminated cereals, several mycotoxins are present simultaneously. Endocrine-disrupting effects occur in animals, but these were not reported in the two outbreaks described above, in which both DON and zearalenone were involved (IARC 1993c). A recent review on zearalenone toxicity was published by Zinedine *et al.* (2007).

Zearalenone is a metabolite of zeronal, a non-steroidal oestrogenic growth promoter that is banned for use in food-producing animals in the European Union. Consequently, methods have been devised for determination of the parent compound and its metabolite in bovine urine. Schmidt *et al.* (2008) applied LC-MS(-MS) to screen for stilbenes and zeronal and zearalenone in urine, and were able to detect and quantify these substances at levels below 1.5 μg/L.

As humans are usually exposed to a number of fusarium toxins through contaminated cereals, most analytical methods for use on these foodstuffs have been developed to identify and quantify a number of mycotoxins in a single procedure. The application of TLC in mycotoxin analysis has been reviewed (Lin *et al.* 1998). TLC is useful for the rapid screening of mycotoxins in food with detection limits as low as 1 ng/kg for aflatoxin-B_1 in feed, 0.3 μg/kg ochratoxin-A in pork kidney and 20 μg/kg zearalenone in cereals. Most quantitative methods have been based on GC-ECD, but HPLC has also been applied. Krska (1998) described clean-up procedures for the analysis of zearalenone in maize and trichothecenes in wheat in the microgram per kilogram range, based on immunoaffinity and multifunctional clean-up columns. A method for the direct qualitative and quantitative GC-MS analysis of eight different mycotoxins, including DON, T-2 toxin and zearalenone, was described by Onji *et al.* (1998), with limits of detection of 0.1–0.5 μg/kg and recoveries of around 90%. Analytical methods for the fusarium toxins DON and T-2 toxin were reviewed by Koch (2004). With the considerable advances in LC-MS technology since that time, the latter is becoming the major technique for multianalysis of mycotoxins in foodstuffs. For example, Sulyok *et al.* (2006) were able to develop a LC-MS (-MS) method for the determination of 39 mycotoxins in wheat and maize, including zearalenone and related derivatives. Because of the chemical diversity of the analytes, both positive- and negative-ion electrospray ionisation (ESI) modes were applied in two consecutive chromatographic runs. It was possible by extracting samples with a mixture of acetonitrile, water and acetic acid (79 : 20 : 1) to inject raw extracts diluted 1+1 without further clean-up. More recently, Diana Di Mavungu *et al.* (2009) reported on the use of LC-MS(-MS) with positive-ion ESI for the multianalysis of mycotoxins in foodstuffs such as soy isoflavones, St John's wort, garlic, *Ginko biloba* and black radish. They found the optimum extraction solvent to be a mixture of ethyl acetate and formic acid (95 : 5) and purified the extracts by a combination of liquid–liquid partition with hexane and solid-phase extraction cartridges. LODs and LOQs were in the range 0.3–30 and 1–100 ng/g, respectively.

Claviceps purpurea

Poisoning by ergot alkaloids produced by the mould *Claviceps purpurea* is usually referred to as *ergotism*, and is probably the oldest recorded food-borne disease of fungal origin. *Cl. purpurea* grows on food grain, particularly rye, during wet seasons. Symptoms of ergotism include erythema, diarrhoea, vomiting, a burning sensation of the limbs and, eventually, gangrene. Central nervous system (CNS) effects are

convulsions, catalepsy, dullness or maniacal excitement. These symptoms can be explained by the pharmacological properties of the ergot alkaloids, which may cause an α-adrenergic blockade, as well as serotonin antagonism. The ergot alkaloids ergotamine (the best known, Fig. 15.3) and ergometrine, or ergonovine, have been used for centuries as therapeutic agents to stimulate uterine contractions and in the treatment of migraine attacks. Overdose with these agents, and intoxication with ergot alkaloids from other sources, can be treated symptomatically with potent vasodilator drugs, such as sodium nitroprusside, and by maintaining adequate circulation.

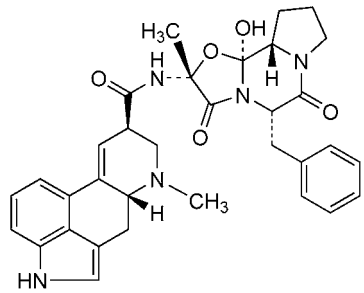

Figure 15.3 Ergotamine.

The method of choice for analysis of ergot alkaloids in serum is HPLC with fluorescence detection after liquid–solid extraction (Moubarak *et al.* 1996). The structural chemistry and a comparison of the available analytical techniques for ergot alkaloids have been reviewed extensively by Flieger *et al.* (1997).

Krska *et al.* (2008) published a review on the analysis of ergot alkaloids in cereal samples by means of LC with diode array detection and LC-MS(-MS).

Mushrooms

Amanita muscaria

A number of basidiomycetes contain hallucinogenic principles, the best-known examples being the *Amanita muscaria* and the *Psilocybe* species types. The fly agaric (*Am. muscaria)* is the European archetypical 'mother of all mushrooms' in legends and fairy tales, with a red hood and white spots. It is indigenous to the northern hemisphere, but is also found in some parts of South Africa, South America, Australia and New Zealand. Despite popular belief, no fatalities have been reported in humans. The most important toxin in *Am. muscaria* is *not* muscarine (which does occur in trace amounts) but ibotenic acid and its decarboxylation product muscimol. Poisoning with *Am. muscaria* usually results from deliberate ingestion to obtain a psychoactive response and symptoms occur within 20–180 min. Muscimol (Fig. 15.4) is a γ-aminobutyric acid (GABA) receptor agonist. It causes CNS depression that results in drowsiness and dizziness, followed by elation, increased motor activity, tremor, agitation and hallucinations. There are no specific antidotes and recovery is complete upon awakening.

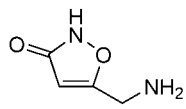

Figure 15.4 Muscimol.

There is no validated procedure available for analysis of ibotenic acid and muscimol in blood or urine. For their determination in mushrooms, an ion-pair HPLC method with UV detection after methanol extraction has been described (Tsunoda *et al.* 1993). More recently, methods have been published for the analysis of ibotenic acid in dried mushrooms using GC-MS (Tsujikawa *et al.* 2006) and LC-MS (Tsujikawa *et al.* 2007).

Psilocybe type

The 'psilocybe syndrome' is caused by consumption of one of the many species of the cosmopolitan genera *Psilocybe*, *Panaeolus*, *Copelandia*, *Gymnophilus* or *Stropharia.* These mushrooms contain the indole-alkylamine hallucinogen psilocybin (Fig. 15.5) and its dephosphorylated congener psilocin, which is about 1.5 times as potent as psilocybin. These hallucinogens are about 100 times less potent than lysergic acid diethylamide (LSD, lysergide). Intoxication with psilocybin-containing mushrooms is almost always intentional. Some of the somatic reactions to psilocybin resemble anticholinergic effects, for example mydriasis and urinary retention, which may be reversed successfully with physostigmine (De Wolff and Pennings 1995). An 18-year-old man who used *Psilocybe semilanceata* developed seizures, Wolff–Parkinson–White syndrome, arrhythmia and myocardial infarction (Borowiak *et al.* 1998).

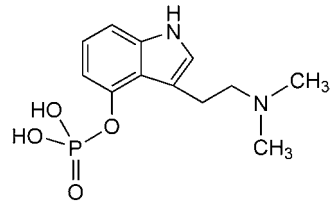

Figure 15.5 Psilocybin.

Measurement of psilocybin and related indole alkaloids in mushroom samples or patient material may assist the diagnosis and treatment of patients with suspected poisoning. A TLC method has been described (Stienstra *et al.* 1981). A 20 mL urine sample is made alkaline with 25% ammonia to pH 10, concentrated on an Extrelut column, and eluted with 30 mL diethyl ether. The dried residue is dissolved in 50 µL ethanol and chromatography is carried out on F_{254} silica gel plates with methanol–ammonia 25%. Psilocybin and psilocin are visualised under 254 nm UV light and after spraying with Van Urk's reagent for indole groups (grey–violet and faint blue, respectively). Quantitative procedures have been described using HPLC (Christiansen and Rasmussen 1983; Hasler *et al.* 1997; Koike *et al.* 1981; Kysilka and Wurst 1989; Lindenblatt *et al.* 1998) or ion-mobility spectrometry and GC-MS (Keller *et al.* 1999). In clinical and forensic practice, the HPLC method with electrochemical detection, as described by Hasler *et al.* (1997), seems to be most useful. The high instability of the analytes requires special measures to prevent oxidation during the clean-up procedure. Solid-phase extraction with reversed-phase material gives unsatisfactory results. Microdialysis yields lower recoveries, but improved reproducibility. Psilocin concentrations in human plasma can be measured in the microgram per litre range.

Amanita phalloides

Toxins from *Amanita phalloides* and related Agaricales are among the most lethal natural substances. In countries where the consumption of wild mushrooms is popular, as in middle and eastern Europe and some Mediterranean countries, hundreds of fatalities are reported every summer and autumn. The toxic principles are cyclic polypeptides: the phallotoxins are bicyclic heptapeptides; the virotoxins are monocyclic heptapeptides; and the amatoxins are bicyclic octapeptides (Fig. 15.6).

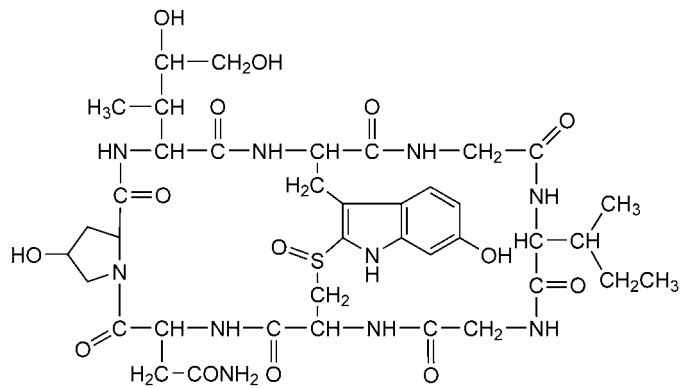

Figure 15.6 α-Amanitin.

The most important biochemical effect of the amatoxins is an irreversible inhibition of ribonucleic acid (RNA) polymerase II; the phallotoxins stimulate the polymerisation of G-actin and stabilise the F-actin filaments (De Wolff and Pennings 1995; Vetter 1998).

Symptoms of *Am. phalloides* poisoning can be roughly divided into three phases, the first appearing over 6 h after mushroom consumption and characterised by violent emesis and cholera-like diarrhoea. This phase is ascribed to the action of the phalloidins, and can usually be treated successfully with fluid and electrolyte replacement. The second phase occurs after 2–3 days as a transient remission, which is, however, fallacious, since the most dangerous third phase may then begin to take place. This is characterised by hepatorenal symptoms from the effect of amatoxins on RNA polymerase. Liver injury is evident by immensely elevated serum aminotransferase activities. Numerous antidotes against amatoxins have been proposed, but none has been proved to be successful, as hepatorenal symptoms appear only after irreversible binding of the toxins to its target enzyme. Liver transplantation seems to be an option for effective symptomatic treatment (De Wolff and Pennings 1995).

Identification and measurement of the toxins in blood for diagnostic purposes have proved a considerable challenge because of their complicated chemical structures and the low plasma concentrations. A rapid radioimmunoassay with a detection limit of 3 ng/mL was published by Faulstich *et al.* (1982). Since then, a number of HPLC methods have been described, as reviewed by Dorizzi *et al.* (1992), but most often with complicated sample pretreatment procedures. Electrochemical detection can achieve a sensitivity of 2 μg/L. An LC-MS procedure for α- and β-amanitin described by Maurer (1998) has a detection limit of 10 μg/L. Filigenzi *et al.* (2007) have reported a method for quantifying α-amanitin in serum and homogenised liver samples which involves precipitation of proteins with acetonitrile and subsequent cation-exchange SPE and analysis on a linear ion trap LC-MS system. The method has a limit of detection of 0.26 μg/kg in serum and 0.50 μg/kg in liver. In 11 of a series of 45 patients admitted to hospital with *Am. phalloides* intoxication, plasma levels ranged from 8 μg/L to 190 μg/L for α-amanitin and from 15.9 μg/kg to 162 μg/kg for β-amantin. Amatoxins were generally detectable in plasma samples taken within 36 h of admission, but were still present in urine samples for up to 4 days (Jaeger *et al.* 1993).

Higher plants

In this section a limited selection of lesser-known plant toxins has been made. For the more familiar toxins, such as atropine and hyoscine from the Jimson weed *Datura stramonium*, or digoxin from the foxglove *Digitalis purpurea*, the reader is referred to the monographs section in this book and to the specialist pharmacognostic literature. For an extensive overview of the analysis of plant toxins, see Gaillard and Pepin (1999).

Ricinus communis

The beans of *Ricinus communis* are the source of castor oil and consist mainly of the triglyceride of ricinoleic (12-hydroxyoleic) acid. Castor oil is well known for its purgative action, which is so strong that it may lead to colic and dehydration. *R. communis* also contains the extremely toxic peptide ricin, which is present in the seed husks. Ricin is a lectin that consists of two polypeptide chains of 34 kDa each, connected through a disulfide bond. One of the chains (the A-chain) is toxic because it inhibits protein synthesis. The B-chain is a galactose- or *N*-acetylgalactosamine-binding lectin and is responsible for the binding of ricin to cell surface receptors (Narang *et al.* 1997; Wellner *et al.* 1995). Ricin is insoluble in lipids; it therefore does not occur in medicinal castor oil. Ricin may have a therapeutic use as an immunotoxin in the treatment of leptomeningeal carcinomatosis (Muraszko *et al.* 1993; Woo *et al.* 1998, 2001).

Animal experiments indicate that the liver is the primary target organ in ricin poisoning (Balint and Halasz 1972). Intravasal aggregation of erythrocytes, and acute adrenocortical and renal insufficiency have also been demonstrated (Balint 1978).

The extreme toxicity of ricin first came to public attention after the Markov case in 1978 in the UK. Georgi Markov, a Bulgarian journalist in exile, was stabbed in the thigh with an umbrella and within a few hours developed high fever and a high leukocyte count. This was followed by a fall in blood pressure and body temperature, and he died on the third day. At the postmortem examination, a small platinum–iridium sphere with tiny holes was found in the subcutaneous tissue. Although the presence of ricin in the bullet was not confirmed analytically, the symptomatology led to the conclusion that ricin was the poison responsible. Oral ingestion of ricin is also very dangerous and may cause erythrocyte agglutination, haemorrhagic gastrointestinal irritation, vomiting, profuse watery or bloody purging, fever, convulsions and death. Death after the consumption of a single castor bean has been reported (Knight 1979), although others have survived ingestion of as many as 10–15 beans (Aplin and Eliseo 1997). There is evidence that ricin has been recommended for use in terrorist attacks. The fact that ricin can be absorbed through the lungs and its lethal inhalatory toxicity estimated at only 5–10 μg/kg mean that ricin presents a serious threat in this regard. Ricin toxicity has been reviewed by Bradberry *et al.* (2003) and Audi *et al.* (2005). Vaccines to treat ricin poisoning are currently being developed. The most promising approach has been to genetically engineer the A-subunit to eliminate both its enzymatic activity and its ability to induce vascular leaking. The non-toxic subunit is reported to elicit antibodies in animals, subsequently protecting them against the effects of large doses of ricin.

Administration of the vaccine is planned to be by an oral or intranasal route (Doan 2004). Godal *et al.* (1981) devised a radioimmunoassay for ricin that was applied subsequently in a patient who ingested 30 castor beans in a suicide attempt. The maximum plasma level on the first day was 1.5 μg/L, with a biological half-life of approximately 8 days. In urine, ricin became detectable only on the third day in low concentrations – around 0.3 μg/L (Kopferschmitt *et al.* 1983). Methods for the identification and quantification of ricin in tissues based on ELISA have also been reported (Griffiths *et al.* 1986; Leith *et al.* 1988). With avidin–biotin immunoperoxidase complex amplification, a limit of detection as low as 0.2 μg/L of tissue extract could be reached. Using a similar immunological procedure with chemiluminescence detection, Poli *et al.* (1994) achieved a limit of quantification in the 0.1–1.0 μg/L range.

A sensitive and accurate method to measure ricin in fluids is that of Narang *et al.* (1997), using a fibreoptic fluorescence biosensor and an avidin–biotin sandwich immunoassay scheme. The linear quantification range is 0.1–200 μg/L. A recent review on the analysis of potential warfare agents including ricin, staphylococcal toxin B and T-2 toxin has been published by Ler *et al.* (2006). The mechanistic, historical and analytical aspects of ricin have been reviewed by Musshoff and Madea (2009).

Lawsonia inermis (henna)

Henna is a greenish-brown vegetable colouring made from the leaves of *Lawsonia inermis*, a flowering plant from the family of the Lythraceae. The main pigment is the aromatic compound 2-hydroxy-1,4-naphthoquinone, also known as lawsone or hennotannic acid, and this can cause adverse reactions after application of henna to the skin.

Severe intravascular haemolysis has been reported following the topical application of henna in children with glucose-6-phosphate dehydrogenase deficiency (Katar *et al.* 2007).

Reactions to lawsone are uncommon, however, and most adverse effects from henna application result from reactions to *p*-phenylenediamine (PPD), which is sometimes added to henna to increase the intensity and longevity of a tattoo and to accelerate its drying. The most common reaction is type IV cutaneous hypersensitivity causing a contact allergy. Sensitisation to PPD is lifelong, and PPD may cross-react with a number of pharmacological compounds, such as the sulfonylureas and the thiazides. PPD sensitivity can be determined by patch testing. There have been calls to restrict the use of PPD in henna products (Jacob *et al.* 2008). Oral ingestion causes severe mucosal oedema, rhabdomyolysis and haemolysis (Anuradha *et al.* 2004).

Lawsone can be assayed in henna by HPTLC (El Shaer *et al.* 2007); for the analysis of PPD the reader is referred to the monographs section.

Catha edulis (khat)

Khat is a small evergreen shrub that grows in the horn of Africa and in the western Arabian peninsula. The chewing of khat leaves is practised in Ethiopia, Somalia and Yemen as well as in immigrant communities elsewhere in the world (Pennings *et al.* 2008). Regulatory control is not consistent around the world. The predominant active compounds in khat are the alkaloids (2*S*)-2-amino-1-phenylpropan-1-one (cathinone) and (1*S*,2*S*)-2-amino-1-phenylpropan-1-ol (norpseudoephedrine or cathine); both bear a structural relationship to amfetamine and have predominantly sympathomimetic effects. Cathinone produces more of the positive effects, while the negative systemic effects are attributable to cathine (Cox and Rampes 2003). Khat use has an anorexic effect similar to that of amfetamines (Pennings *et al.* 2008).

Khat use has been linked to physical and mental illness. Cox and Rampes (2003) found an increased risk of schizophreniform and manic psychosis, usually in brief episodes, and depression was more common after cessation of frequent use. Khat also has a number of systemic effects, which include cardiac arrhythmias, hypertension, constipation, and an increased risk of head and neck cancer as well as upper gastrointestinal tract cancer. The risk of myocardial infarction is markedly increased in heavy users (Pennings *et al.* 2008). In absolute terms, the health risk of khat is fairly low (Pennings *et al.* 2008).

Khat metabolites may be detected in urine as norephedrine up to 48 h after consumption, by immunoassay or GC (Cox and Rampes 2003). Quantification of cathinone along with eight other herbal phenalkylamines in plasma has been described by Beyer *et al.* (2007) using SPE and analysis by a LC-MS(-MS) system. The LOQ was 10 µg/L for all the analytes.

Umbelliferae

The Umbelliferae or Apiaceae family (named after their umbrella-shaped inflorescence) includes a large number of extremely toxic species, together with many species that are fit for human consumption, such as carrot, parsley and parsnip. The most best-known toxic species are the water hemlock *Cicuta virosa* and the poison hemlock *Conium maculatum*. The former contains the polyin cicutoxin as the active principle, whereas the latter contains the piperidine alkaloid coniine (Fig. 15.7). *C. maculatum* was the source of the poison used for the execution of Socrates in 399 BC. Cicutoxin affects primarily the CNS causing seizures, whereas coniine affects the neuromuscular junction and may lead to respiratory failure. Both plants have a very high mortality rate after ingestion. In both cases, treatment is symptomatic and does not depend on the result of toxicological analysis. Beyer *et al.* (2009) published a review of methods for the analysis of a number of alkaloids, including coniine, in biological samples.

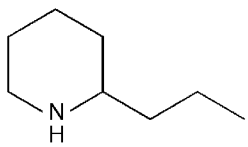

Figure 15.7 Coniine.

Another class of toxins produced by umbellifers are the furocoumarins or psoralens, the active principles of the hogweed or common cow parsnip *Heracleum sphondylium* which may cause serious dermal phototoxicity after skin contact with the juice of this plant and exposure to sunlight. The best-known psoralen from hogweed is 8-methoxypsoralen (8-MOP; Fig. 15.8), which is also used in the therapy of psoriasis in combination with UV-A irradiation (De Wolff and Thomas 1986). Psoralens can be measured in biological matrices with HPLC (Herfst *et al.* 1980; Stolk *et al.* 1987). Both the toxic and therapeutic action is based on binding of reactive products from 8-MOP, formed by irradiation with UV light, to DNA in the basal cells of the epidermis.

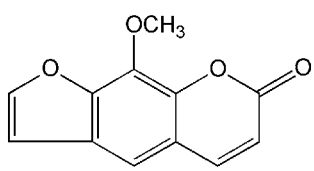

Figure 15.8 8-Methoxypsoralen.

A very recent application of psoralen phototoxicity is the inactivation of pathogens in donated blood as pretreatment for transfusion of blood products. The furocoumarin used for this application is amotosalen (Wollowitz 2001).

Lathyrus sativus

Lathyrus sativus contains the excitatory amino acid β-*N*-oxalylamino-L-alanine (L-BOAA), which is the cause of a neurodegenerative disorder, lathyrism, that occurs in many parts of the world. A likely mechanism of action of L-BOAA is that as an excitant amino acid of motor neurons it causes neurotoxicity by overstimulation of glutamate receptors. This action is stereospecific: D-BOAA and the α-isomer of BOAA seem to lack a neurotoxic effect.

Human consumption of 400 g *L. sativus* daily of seeds ground to flour to make bread for a prolonged period leads to symptoms of lathyrism, which is characterised by spastic paresis of the lower limbs and, in a more advanced stage, by loss of control of the bladder and rectum, and by impotence. Young males form the most sensitive part of the population. Treatment is limited to reduction of muscular spasm, with some success. For reviews on lathyrism, see Spencer (1995) and Barceloux (2009), which includes data on the analysis of the toxin.

Cycas circinalis

Toxins from the false sago palm *Cycas circinalis* have been associated with a neurodegenerative disorder that occurs on the Western Pacific island of Guam, on the Japanese Kii peninsula and in Irian Jaya (West Papua). The female cones of this tree have been used as a dietary source of starch for centuries and consumption is thought to be related to the incidence of a peculiar neurological disease, Guamanian motor neuron disease (GMND), which occurs in two clinical forms. The first form resembles amyotrophic lateral sclerosis and the second form is characterised by dementia and extrapyramidal manifestations. Cycad seed contains two classes of substances with a neurotoxic potential – amino acids and azoxyglycosides. As a result of its chemical analogy with BOAA (see above) and the clinical similarities between lathyrism and cycadism, the amino acid β-*N*-methylamino-L-alanine (BMAA) has long been thought to be causal in GMND, but this has been questioned in recent years. Excitotoxic amino acids such as BOAA have a dicarboxylic structure in common, which does not occur in BMAA. A more likely candidate for the instrumental neurotoxin is cycasin (methylazoxymethanol-β-D-glucoside), which is metabolised to methylazoxymethanol by β-glucosidase. Methylazoxymethanol is a direct alkylating agent with hepatotoxic, mutagenic, carcinogenic and teratogenic properties. It may also alter the function of post-mitotic neurons and so precipitate a slowly progressive degeneration of cells (Spencer *et al.* 1991). There is strong epidemiological support of a role for cycasin in the pathogenesis of GMND, probably in combination with inherited susceptibility (Roman 1996). Methods for the analysis of both BMAA and cycasin in cycad flour have been published (Kisby *et al.* 1992). For a review of cycadism, see De Wolff and Bruyn (1995).

Blighia sapida

Jamaican vomiting sickness affects mainly young children and is a form of toxic hepatitis associated with ingestion of the arilli of the unripe fruit of the ackee tree, *Blighia sapida* (Sherratt 1995). The ackee tree was imported into the West Indies, but is native to West Africa and deaths among children have been reported in the Ivory Coast (Foungbe *et al.* 1986) and in Burkina Faso (Meda *et al.* 1999). The disease presents with severe vomiting and hypoglycaemia followed by neurological symptoms, which include convulsions, coma and death (Tanaka 1979). The toxin responsible is hypoglycin, L-(*S*)-2-amino-3-methylenecyclopropylpropionic

acid. Seeds of the common sycamore (*Acer pseudoplatanus*) and of the lychee fruit (*Litchi sinensis*) also contain hypoglycin. Most of the metabolic effects of hypoglycin are caused by its metabolite, methylenecyclopropylacetyl-coenzyme-A (MCPA-CoA). It inhibits the β-oxidation of fatty acids, induces organic aciduria and inhibits gluconeogenesis. Sherratt (1995) has reviewed the biochemical mechanism of hypoglycin in detail.

Administration of intravenous glucose immediately after diagnosis may help to prevent a fatal outcome, and administration of riboflavin, glycine and L-carnitine might also be effective (Sherratt 1995). Kupfer and Idle (1999) have suggested methylene blue as an antidote to hypoglycin poisoning. No methods are available that detect hypoglycin in body fluids to confirm a suspected intoxication. However, hypoglycin exposure may be confirmed indirectly by measuring dicarboxylic acids in urine (ethylmalonic, glutaric and adipic acids) by GC with flame-ionisation detection (FID) after derivatisation with bis (trimethylsilyl)trifluoroacetamide (Meda *et al.* 1999). A rapid HPLC method for the analysis of hypoglycin-A in ackee fruit was described by Golden *et al.* (2002).

Invertebrates

Many species of animals without backbones produce venoms or contain toxins that may be harmful to humans, either externally by stinging or after ingestion (Mebs 2002). A selection of the most relevant types of toxins has been made here, with emphasis on mollusc species that usually cause human food poisoning by transmitting accumulated toxins produced by protozoal organisms. Analytical toxicology may be instrumental in the prevention and management of these food-borne diseases. Animals often reported to cause envenomation (representatives of the Cnidaria and Arthropoda) are also mentioned briefly.

Cnidaria (formerly Coelenterata)

Cnidaria may be stationary creatures (e.g. 'fire coral' or *Millepora alcicornis*, which is associated with reefs in, for example, the Caribbean and Australia) or free swimming (e.g. jellyfish, such as the Portuguese man-of-war or *Physalia physalis*). They have stinging cells or nematocysts (40 μm spheres that contain a viscous fluid composed of multiple toxins) and a long thread-like coiled tube (nema). When physical, osmotic or chemical stimuli trigger the nematocyst, its internal pressure produces an explosive inversion of the nema and the contents of the capsule are injected into the victim (Schwartz and Meinking 1997).

The Portuguese man-of-war jellyfish is particularly dangerous and can cause death. Its nemocysts contain a wide range of biologically active substances, including histamine, haemolytic toxins, phospholipases, proteases and a powerful neuromuscular toxin – hypnotoxin. Acute reactions involve stinging and burning, followed by the development of painful and pruritic linear papules and weals. Acute systemic reactions to physalia envenomation may include hypotension, shock, seizures, ataxia, haemolysis and acute renal failure. Other possibly lethal jellyfish include *Stomolphus nomurai*, which occurs in the waters around China; eight deaths from pulmonary oedema or cardiac arrest following envenomation by this species have been recorded (Burnett *et al.* 1996). The box jellyfish *Chironex fleckeri*, found in the Indo-Pacific region, is reputed to be the world's most venomous marine animal and has been responsible for at least 80 recorded deaths in Australia. The venom has a range of autonomic effects, such as vasospasms, cardiac irregularities, peripheral neuropathy, aphonia, ophthalmic abnormalities and parasympathetic dysautonomia. Related species, such as *Carukia barnesia*, produce a toxin that mimics extensive catecholamine release and targets the myocardium (Burnett *et al.* 1998). For purification of coelenterate venoms, capillary electrophoresis (CE), followed by binding to high-titre commercial ovoid hyperimmune serum, is the technique of choice (Burnett *et al.* 1996). For some jellyfish stings, rapid treatment of the affected area with household vinegar, which inactivates the toxins, is recommended, followed by administration of an antivenom (Tibballs 2006).

Molluscs

Mollusc poisoning or 'shellfish poisoning' is caused by the consumption of bivalve molluscs that accumulate toxins of protozoal or algal origin. Toxins from algal sources are also referred to as phycotoxins (Quilliam 1999), analogous to the mycotoxins from fungal sources (see above). Mollusc poisoning caused by algal toxins is usually classified according to the symptoms that they cause in humans (Baden 1983, 1995; Daranas *et al.* 2001; Sierra-Beltran *et al.* 1998):

- Paralytic shellfish poisoning (PSP, saxitoxins)
- Diarrhoetic shellfish poisoning (DSP, e.g. okadaic acid)
- Neurotoxic shellfish poisoning (NSP, brevetoxin)
- Amnestic shellfish poisoning (ASP, domoic acid).

Intoxication with venoms (the conotoxins) from snails belonging to the genus *Conus* is another form of mollusc poisoning.

Paralytic shellfish poisoning

Paralytic shellfish poisoning (PSP) is caused by saxitoxins (STX; Fig. 15.9) produced by marine 'red tide' dinoflagellates and freshwater blue–green algae such as *Alexandrium* spp., *Gymnodinium catenatu* and *Pyrodinium bahamense*. The chemistry and biosynthesis of the STX group of toxins have been described by Moore (1999) and Daranas *et al.* (2001). STX are potent agents that can block sodium channels in nerves and muscles at the extracellular side of the channel, which leads to conductivity disturbances and paralysis. About 1600 cases of poisoning are estimated to occur every year. In severe cases, the neurological symptoms spread to the extremities and respiratory muscles and, without ventilatory support, patients die between 2 and 12 h after ingestion. STX has become both an invaluable research tool and an internationally regulated chemical weapon. Apart from its activity as a calcium channel blocker, in recent times STX has also been found to bind to potassium channels, neuronal nitric oxide synthase, STX-metabolizing enzymes and two circulatory fluid proteins, namely a transferrin-like family of proteins and a unique protein found in the blood of pufferfish (Llewellyn 2006). Lawrence *et al.* (1996) described a fast and reliable analytical method to detect STX in molluscs, based on HPLC with fluorescence detection after pre-chromatographic oxidation. Shellfish samples were extracted by boiling with 0.1 mol/L HCl and purified by solid-phase extraction on C_{18} and strong ion-exchange cartridges, respectively. The samples were then oxidised with periodate and separated on a Supelcosil LC_{18} column. A number of PSP toxins, including STX, can be detected and quantified using fluorimetric detection at wavelengths of 330 nm (excitation) and 400 nm (emission). Garcia *et al.* (2004) applied a HPLC method with post-column derivatisation and fluorescence detection to the quantitative analysis of STX in a wide range of tissues and biological fluids taken from two fishermen who died 3–4 h after consuming a small number of toxic mussels. A comprehensive list of the findings is to be found in the STX monograph.

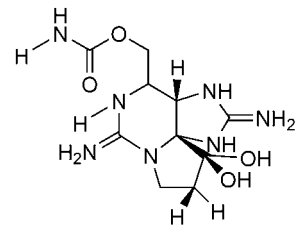

Figure 15.9 Saxitoxin.

Diarrhoetic shellfish poisoning

The major causative agent of diarrhoetic shellfish poisoning (DSP) is okadaic acid (Fig. 15.10), which is produced primarily by 'red tide' dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum* (Aune 1997; Daranas *et al.* 2001). DSP toxins are lipophilic and accumulate in the digestive gland of mussels. Okadaic acid is a potent inhibitor of protein phosphatases 1 and 2A. In humans, consumption of contaminated molluscs leads almost exclusively to gastrointestinal

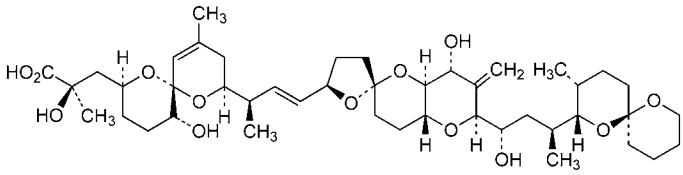

Figure 15.10 Okadaic acid.

symptoms – diarrhoea, nausea, vomiting and abdominal pain – which appear between 30 minutes and a few hours after the meal and can be caused by as little as 40 μg of toxin. Treatment is supportive and recovery is complete after a few days. Several methods for the analysis of okadaic acid in shellfish have been reported by, for example, HPLC (Quilliam 1995), LC-MS (Paz *et al.* 2007) and CE (Consoli and Damerval 2001). However, no simple analytical methods are available as yet to detect okadaic acid in these materials (Quilliam 1999).

Neurotoxic shellfish poisoning

Neurotoxic shellfish poisoning (NSP) is caused by a toxin produced by another 'red tide' dinoflagellate, *Gymnodinium breve*, which has been observed on the west coast of Florida, in the Gulf of Mexico, Japan and New Zealand.

The active principle is the lipid-soluble polyether brevetoxin (Fig. 15.11), which has a molecular weight of around 900 and is one of the most potent neurotoxins known. The mode of action of brevetoxin resembles that of ciguatoxin (CTX; see later). It is a depolarising substance in the cholinergic system that opens membrane channels permeable to sodium ions. This alters the membrane flow of excitable cell types, and results in an increase in inward flow of sodium ions, which can be blocked by tetrodotoxin (TTX; see later).

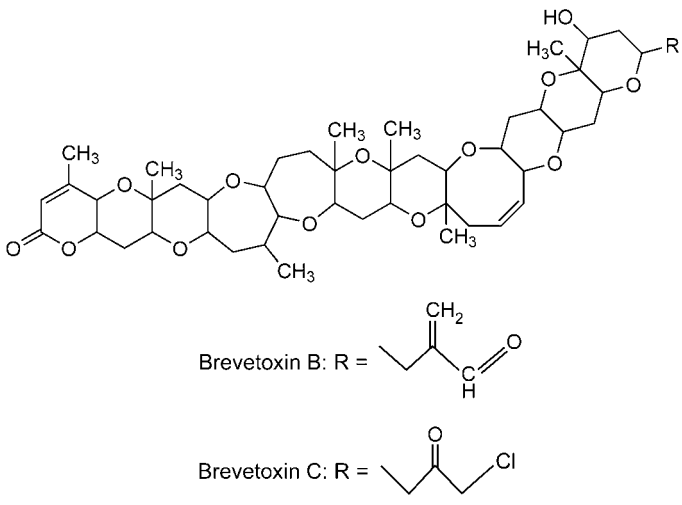

Figure 15.11 Brevetoxin.

In humans, ingestion of brevetoxin-contaminated shellfish can result in gastroenteritis with neurological symptoms. Within 3 h, nausea and vomiting, paraesthesias, reversal of hot/cold sensation, throat tightness and ataxia may occur. There is no paralysis. There is complete recovery from these symptoms within 2 days without specific treatment. No human deaths have been reported with brevetoxin poisoning. No simple and rapid analytical techniques for the detection of the toxins from *G. breve* have been described, although ELISA methods offer some promise (Quilliam 1999). Brevetoxin may also be detected with the LC-MS(-MS) method for CTX described by Lewis *et al.* (1999) (see later). Recent developments in the toxicology and analysis of brevetoxins have been reviewed by Watkins *et al.* (2008).

Amnestic shellfish poisoning

Amnestic shellfish poisoning (ASP) was identified as a marine toxin disease in 1987 in Canada, when more than 150 people were affected by the consumption of cultured blue mussels, which resulted in the deaths of three patients. The toxin responsible is domoic acid, a tricarboxylic acid that is formed by certain species of the diatom genus *Pseudonitzschia*. Domoic acid (Fig. 15.12) is a neurotoxic agent that binds competitively to the kainic acid and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-subtype glutamate receptors in the CNS, leading to an elevation of intracellular free calcium and resulting in cellular damage.

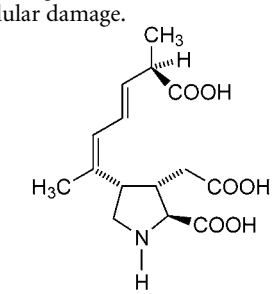

Figure 15.12 Domoic acid.

An estimated concentration of 200 μg domoic acid per gram of mollusc flesh may produce symptoms in humans. Acute symptoms include vomiting and diarrhoea and, in some cases, are followed by confusion, memory loss, disorientation, coma or death. Permanent neurological sequelae, especially cognitive dysfunction, are most likely in patients who develop neurological illness within 48 h (Baden *et al.*1995).

A large number of different analytical methods have been described for domoic acid, probably because of its relatively straightforward chemical structure. Quilliam *et al.* (1998) analysed domoic acid semi-quantitatively in shellfish using TLC. Tissues were extracted and concentrated using a strong ion-exchange resin. The extracts were applied to silica-gel TLC plates and developed with a butanol–acetic acid–water mixture (3 : 1 : 1, $R_f = 0.45$ for domoic acid). As little as 10 μg domoic acid per gram of tissue can be detected in this way. Several HPLC methods for domoic acid have been described. Lawrence *et al.* (1994) compared a reversed-phase HPLC method with an existing radioimmunoassay. Both methods appeared to be useful for the measurement of domoic acid in seafood, spiked rat serum and urine. A new pre-column derivatisation reagent, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, was applied by Sun and Wong (1999) before reversed-phase HPLC with fluorescence detection. This method, which was employed in phytoplankton samples, has a mass limit of detection as low as 0.001 ng.

James *et al.* (2000) described another fluorimetric HPLC method for the determination of domoic acid in seafood and marine phytoplankton. The analyte was derivatised with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) prior to isocratic reversed-phase HPLC with fluorescence detection with excitation at 470 nm and emission at 530 nm. Using a strong ion-exchange solid-phase extraction column for clean-up, the method detection limit is 6 ng domoic acid per gram of mussel tissue. This detection limit seems high when compared with the indirect competitive enzyme immunoassay published by Kawatsu *et al.* (2000). With this procedure, based on an anti-domoic acid monoclonal antibody, domoic acid concentrations were found in Japanese blue mussels in the range 0.11–1.81 ng per gram of tissue.

Capillary electrophoresis with UV absorbance detection has also been described for the analysis of domoic acid (Zhao *et al.* 1997). This has advantages over HPLC in that, in addition to domoic acid, several of its isomers can be separated. The method detection limit of 150 ng per gram of tissue, however, is higher than those obtained with HPLC and enzyme immunoassay (EIA). A new, sensitive method for the measurement of domoic acid in seawater and plankton based on HPLC was described by Mafra *et al.* (2009), with detection limits as low as 42 ng/L with UV detection and 15 ng/L by LC-MS. Domoic acid has been measured in serum by LC (Blanchard and Tasker 1990).

Conotoxin snails

Gastropods (snails) of the genus *Conus* feed on other molluscs, fish or worms, which they hunt by injecting venoms through a harpoon and proboscis. Stings of some species of these molluscs are known to cause

human fatalities. There are about 50 000 different conotoxins present in species of the genus *Conus*; about 1% have been pharmacologically characterised. The venom of *Conus geographus* contains several peptides. α-Conotoxin is a 13-amino-acid peptide with two disulfide bonds, which has a potent nicotinic receptor antagonist activity; μ-conotoxin is a sodium channel blocker, like tetrodotoxin. The neurotoxins from *Co. textile neovicarus*, which feeds specifically on molluscs, have been reviewed by Spira *et al.* (1993). A peptide that causes a characteristic tremor in experimental animals (*ω*-conotoxin GVIA) is now used as a pharmacological tool in neuroscience (Olivera and Cruz 2001). Conotoxin peptide toxins can be isolated from snail extracts by Sephadex G-50 column extraction followed by HPLC purification on C_{18} columns (Spira *et al.* 1993). The conotoxin ziconotide is now approved by the US FDA for the treatment of severe chronic pain by intrathecal administration (Williams *et al.* 2008).

Arthropods

Ticks, spiders and scorpions (Arachnids) and bees, wasps and ants (Hymenoptera) are part of this group; they usually transmit their toxins by stinging. Their venoms form a diverse group of chemicals with a wide range of toxicological mechanisms: for a review, see Piek and Leeuwin (1995). The role of the analytical toxicologist in the management of stinging incidents is limited and, therefore, only a small selection of the most important venomous arthropods is included in this section.

Hymenoptera

Isolated stings from bees, wasps and ants are usually not life threatening, although death may occur through anaphylactic shock. Mass stinging events do take place and are life threatening via the toxic action of the venom injected in large amounts. The rapid spread of the very aggressive Africanised subspecies of the honey bee *Apis mellifera* – which is held responsible for hundreds of human deaths – may become responsible for an increase in mass stinging. The major bee venom fractions consist of peptides (peptide-401, apamin and mellitin) and enzymes (phospholipase A_2 and hyaluronidase). Mellitin, which is able to hydrolyse cell membranes, is the primary cause of pain after a honey bee sting. Allergic reactions, mainly through phospholipase A_2 as the antigen, are IgE mediated. A sting interval of less than 2 months is a risk factor for the development of hymenoptera allergy. Wasp venom contains similar enzymes to bee venom, but with additional cholinesterases and phospholipase A_1 instead of A_2. The major peptides in wasp venom are the mastoparans, which induce histamine release. The enzymes, however, are responsible primarily for allergic reactions; they are usually not cross-reactive with bee venom allergens. The venom from the fire ant *Solenopsis invicta* also contains phospholipase A_1 and other allergens related to vespid venom.

Systemic clinical signs caused by multiple hymenoptera stings include acute renal failure, rhabdomyolysis, optic neuritis, atrial flutter, hepatic dysfunction, diffuse intravasal coagulation and respiratory distress. Wasp and ant stings may cause neurological symptoms as well. About 150–1000 bee stings and 20–200 wasp stings are thought to induce a critical situation. In all cases of allergic and mass stinging events, the victim should be transported to the emergency ward of the nearest hospital (Ewan 1998; Gelder *et al.* 1996; King and Spangfort 2000; Vetter *et al.* 1999).

Arachnids

Although tick bites are nowadays most notorious as transmitters of bacterial infections (*Borrelia* causes Lyme disease), several tick species, such as *Ixodus holocyclus* (Australia) and *Dermacentor andersoni* (North America), produce holocyclotoxins, which can result in a lethal paralysis through acetylcholinesterase inhibition at the neuromuscular junction.

A serious bite from the black widow spider *Latrodectus mactans* produces α-latrotoxins. These are proteins of about 1000 amino acid residues that bind irreversibly with presynaptic cell membranes, to produce cation-sensitive channels and interfere with the endocytosis of vesicle membranes. Local clinical signs are negligible; systemic clinical signs range from pain and muscle cramps to hypertension, pulmonary oedema and CNS involvement. Specific antivenom and muscle relaxants are administered in treatment (Piek and Leeuwin 1995; ME Peterson 2002, personal communication). Constituents of the black widow spider venom are now under investigation for possible use as insecticides (Rohou *et al.* 2007). The Australian funnel web spider *Atrax robustus*, found around Sydney, is far more dangerous, with a bite capable of causing death within 15 min. The toxin, robustotoxin, acts by opening sodium channels in axons of the victim. Administration of an antivenom has been shown to be life saving and, since its introduction, no more fatalities have been reported. The related species *Hadronynche versutus*, which is found in the southern regions of Queensland, Australia, produces versutoxin, the effects of which can also be treated with *At. robustus* antivenom (Hawdon and Winkel 1997). A different symptomatology is caused by the brown recluse spider *Loxosceles* spp., of which five species are known in the southern United States. Their venom contains a range of enzymes which are responsible for necrotic arachnidism at the biting site and a wide range of systemic symptoms, from fever and arthralgia to diffuse intravasal coagulation and renal failure (ME Peterson 2002, personal communication).

Scorpions constitute a serious public health concern in warm climates. Worldwide, 30 species produce potentially fatal toxins; the estimated yearly number of human fatalities that result from scorpion stings is 5000. Examples of scorpion species are: *Centruroides infamatus*, which is responsible for the highest envenomation rate in Mexico; *Tityus serrulatus*, which causes thousands of envenomations each year in Brazil; and *Buthus occitanus*, the most venomous scorpion, which is common in North Africa. Scorpion venom contains at least 85 different peptide neurotoxins. These disrupt sodium channel function (Becerril *et al.* 1997; Possani *et al.* 1999), which causes an increased activity of the pre-synaptic neuromuscular junction, and so affects the parasympathetic, adrenergic and adrenal medulla system. This explains the wide range of clinical symptoms in human victims. The sting site is usually very painful; pain may migrate proximally and hyperaesthesia may occur. Systemically, the cardiovascular system is affected predominantly, which results in hypertension, arrhythmias, myocardial damage, pulmonary oedema and respiratory failure. Other symptoms include hyperthermia, mydriasis, salivation, opisthotonus and roving eye movements. CNS dysfunction is seen in children, but rarely in adults, and may be secondary to cerebral hypertension (Gueron *et al.* 1992; Ismail 1995). Antivenom administration and treatment of hypertension are the recommended treatment (Ismail 1995). Murthy and Hase (1994) have suggested that insulin and glucose should be given to counteract the inhibition of insulin secretion brought about by the massive release of catecholamines during envenomation.

Vertebrates

Fish

Poisoning by the consumption of fish flesh (ichthyosarcotoxism) usually occurs in warm climates but is also observed in moderate climate zones when hygiene measures are ignored (Lipp and Rose 1997). Tetrodotoxic and ciguatoxic fish poisoning are caused by ingestion of fish that accumulate toxin-producing organisms, such as bacteria or protozoa, without being affected themselves. Scombroid poisoning is an example of a toxin produced by improper storage after death, and other fish produce poisonous stings; for a review, see Mebs (2002).

Ciguatera fish poisoning

Ciguatera fish poisoning (CFP) is caused by two groups of toxins, of which the principal one includes the lipid-soluble ciguatoxin (CTX) (Fig. 15.13) and gambierol, produced by the epiphytic dinoflagellate *Gambierdiscus toxicus.* The other group comprises the water-soluble maitotoxin. CTX is a group of heat-stable, lipid-soluble, highly oxygenated cyclic polyether molecules, which appear in the food chain through coral reef-fishes that have become toxic through their diet (Baden 1995; Daranas *et al.* 2001; Lehane 2000; Lewis 2001; Rein and Borrone 1999). CTX-containing dinoflagellates live on solid surfaces of macroalgae and are consumed during fish grazing. The mechanism of CTX toxicity is through its direct effects on excitable membranes. Its potent depolarising action, caused by a selective increase in sodium

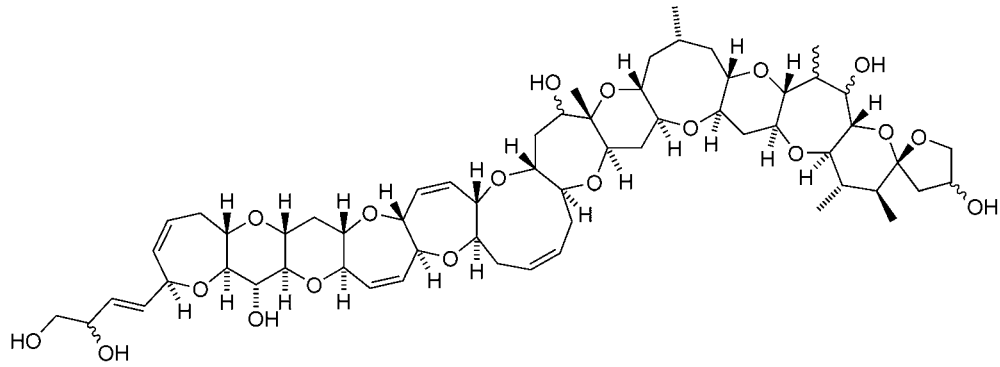

Figure 15.13 Ciguatoxin.

permeability in nerve cells and striated muscle, is based on its affinity to the 'polyether ladder toxin-binding site' of the ion channel receptor, through which sodium and calcium channels are activated. CTX activity can be counteracted by calcium ions and tetrodotoxin (see below). The ciguatera poisoning syndrome is a direct result of the stimulation of both the sympathetic and parasympathetic nervous system through opening of the voltage-dependent sodium channels in nerve cell membranes. Consumption of contaminated fish leads to gastrointestinal disturbances (nausea, vomiting and diarrhoea) within a few hours, followed by neurological symptoms. The latter include paraesthesias, tooth pain and reversal of hot/cold temperature sensation. The third symptom is pathognomonic for CFP. Paraesthesia and weakness may persist for months after the acute illness (Baden 1995). In some cases, the neurological sequelae may last for years (Lewis 2006).

Ciguatera fish poisoning is the most common cause of poisoning with marine toxins. Of those who live in or visit subtropical and tropical areas, an estimated 10 000–50 000 people per year suffer from ciguatera. The fatality rate, however, is probably less than 1%.

There is no specific therapy for CFP; prevention of consumption of contaminated fish is essential, and a simple and sensitive assay for CTX would be useful in this context. Analysis is difficult because of the low toxin level in fish and the complex structure of the CTX group of toxins. A method based on HPLC and tandem electrospray MS offers the required sensitivity (less than parts per billion; Lewis *et al.* 1999). This assay measured the Caribbean type of CTX (C-CTX) in fish flesh in concentrations of around 2.0 ng per gram of tissue. Non-toxic flesh samples spiked with C-CTX, Pacific-CTX (P-CTX) and brevetoxin yielded limits of detection of 0.2, 0.1 and 0.5 ng per gram of tissue, respectively. However, this method is unlikely to be cost-effective for routine screening. A membrane immuno-bead assay appears to be a simple, rapid, sensitive and specific detection method for CTX and its related polyethers, but further validation of this test is required (Hokama *et al.* 1998; Lehane and Lewis 2000; Lewis 2001); for a review, see Quilliam (1999).

Puffer fish poisoning

Puffer fish poisoning (PFP), or tetrodotoxin (TTX) (Fig 15.14) intoxication, causes gastroenteritis with severe neurological manifestations similar to those of PSP or saxitoxin intoxication. TTX intoxication constitutes a public health problem in subtropical and tropical regions. It is not limited to Japan, where consumption of Fugu – a local puffer fish – is popular. Fugu is harmless if prepared by a qualified chef who discards the organs in which TTX accumulates – the ovaries, roe, liver, intestines and skin. TTX can also be found in *Tetraodontiformes* spp., but these can usually be distinguished by their peculiar morphology and their ability to inflate themselves when in danger (Baden 1995; de Kom 2001). In recent years, it has been shown that a large variety of organisms besides puffer fish possess TTX (Noguchi and Arakawa 2008). TTX is produced by bacteria that colonise the gut and skin mucosal layers of the fish, and the toxin is sequestered in the gonads and the liver and, in some species, in muscle. Other marine organisms, such as the Japanese ivory shell, the trumpet shell and the blue-ringed octopus, may contain TTX. The toxin is also found in the skin of certain frogs.

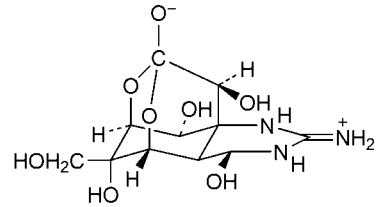

Figure 15.14 Tetrodotoxin.

Tetrodotoxin is a neurotoxic agent that blocks axonal excitability by reducing the inward flow of sodium ions, much like the saxitoxins (see above). It acts on conduction of both somatic and sensory nerves and it affects the emetic chemoreceptor. As opposed to saxitoxin, it causes hypotension through paralysis of the blood vessels. TTX is one of the most potent of the natural toxins; the lethal ingested dose for humans is 5–30 mg/kg wet tissue. The symptoms of TTX poisoning are comparable to those of PSP (see above), except for marked hypotension, and are apparent within 5–30 min after consumption. They include gastroenteric effects, paraesthesias, motor paralysis, hypotension and respiratory paralysis. In Japan the mortality rate is reported to be about 60%. Therapy consists of supportive measures, such as artificial ventilation and management of hypotension. Bioassays for PSP and PFP have been reported (Yasumoto 1991, cited in de Kom 2001). In addition, an HPLC method with fluorimetric detection has been described (Yotsu *et al.* 1989). TTX and its analogues are separated after clean-up on a Develosil ODS-5 column with 0.06 mol/L heptafluorobutyric acid in 0.001 mol/L ammonium acetate buffer (pH 5.0). The eluate is mixed with 4 mol/L aqueous NaOH solution and passed through a stainless steel column in an oil bath at 135°C. The fluorescence emission of the reaction products is measured at 500 nm, after excitation at 375 nm. The limit of detection is reported to be of the order of 2 µg/g, which is claimed to be sufficiently sensitive to measure TTX and its analogues in puffer fish liver. Tsai *et al.* (2006) have measured TTX in blood by GC-MS and by LC-MS; the latter method had a limit of sensitivity of 15.6 nmol/L. Using LC with post-column derivatisation with sodium hydroxide and fluorescence detection, O'Leary *et al.* (2004) were able to quantify TTX in serum with an LOQ of 5 µg/L. Yu *et al.* (2010) described a rapid method for measuring TTX in urine and plasma samples from poisoned patients by use of commercial SPE cartridges (C_{18} and weak cation-exchange columns) followed by analysis by HPLC with UV detection. The detection limit for both urine and plasma was 10 µg/L. They made the interesting observation that urine TTX levels measured during the first 24 h of admission to hospital showed a better correlation with the severity of poisoning when adjusted according to variations in concomitant creatinine excretion. TTX has also been measured in serum and urine samples as well as in puffer fish tissues by LC-MS(-MS). The detection limit in both urine and serum was 0.1 µg/L (Akaki and Hatano 2006). Examples of cases of puffer fish poisoning where TTX was measured in specimens of blood, serum and urine are described in detail in the tetrodotoxin monograph.

Scombroid fish

Scombrotoxin poisoning is the most commonly reported fish poisoning and occurs in many parts of the world. Scombrotoxin is formed in improperly stored fish, and its name derives from the type of fish in which it was described originally. Scombroid fish (*Scombridae*) are dark-fleshed migratory species, such as mackerel and tuna. Cases of poisoning have also been described after ingestion of non-scombroid species, such as herring, sardines and salmon.

'Scombrotoxin' is generally thought to be identical to histamine. The flesh of scombroid fish has a high histidine content, which is readily decarboxylated to histamine by enteric bacteria (*Proteus morgani*, *P. vulgaris*, *Clostridium* spp., *Escherichia coli*, *Salmonella* spp. and *Shigella* spp.) when fish stored for as little as 2–3 h at temperatures above 20°. Despite a widely reported association between histamine and scombroid food poisoning, histamine alone appears to be insufficient to cause food toxicity. Putrescine and cadaverine have been suggested to potentiate histamine toxicity (Al Bulushi *et al.* 2009). Prolonged cooking does not effectively destroy scombrotoxin and, therefore, it may also be present in canned products.

Clinical manifestations of the disease occur rapidly (10–30 min) after ingestion, and include acute gastrointestinal symptoms (vomiting, cramps, diarrhoea) associated with erythema, urticarial patches and oedema. The disease is distressing but seldom, if ever, fatal and recovery is spontaneous within 24 h. Treatment with intravenous cimetidine resolves most symptoms very quickly.

In suspect fish samples, histamine concentrations can be measured with standard methods for the analysis of histamine in food (Clark *et al.* 1999; Grant 1997; Trevino 1998).

Pfiesteria-*associated syndrome*

An illness that affects fish and humans was recently observed in the mid-Atlantic region and shown to be caused by a dinoflagellate, *Pfiesteria piscicida.* It is lethal to fish; in humans, it causes deficiencies in learning and memory, acute respiratory and eye irritation, and acute confusional syndrome (Grattan *et al.* 1998; Morris 1999). The toxins from *P. piscicida* have been identified as ligated copper compounds (Moeller *et al.* 2007).

Stonefish and weeverfish

Several species of fish with venom containing spines have been reported to cause fatalities. The stonefish *Synanceja horrida*, *S. trachynis* and *S. verrucosa*, belonging to the family Scorpaenidae, are located in temperate and tropical seas that extend across South Africa, Japan, the Pacific and Indian Oceans, Australia and New Zealand. Their venoms contain cholinesterase, alkaline phosphatase and phosphodiesterase. Stonefish stings are severely painful and patients may suffer collapse, cyanosis or pulmonary oedema (Burnett 1998).

Weeverfish (*Trachinus draco*, *T. vipera*, *T. radiatus* and *T. araneus*) occur in European coastal waters. Their venom contains serotonin, a kinin-like substance, adrenaline (epinephrine) and histamine, as well as several enzymes. Weeverfish sting causes an intense burning pain and death can occur rapidly through severe pulmonary oedema (Borondo *et al.* 2001).

Haff disease

Haff disease, a muscular condition, was first described in Germany in 1924. Lentz described muscular rigidity and some of the classic features of rhabdomyolysis in people who had consumed fish from the Haff coast off the Baltic Sea (Lentz 1925). Over subsequent years, about 1000 cases were described. There have been few reports of Haff disease since then, although scientists based at the US Centers for Disease Control described a small cluster of six cases discovered in 1997; they were able to trace the source to buffalo fish (*Ictiobus cyprinellus*) from Louisiana or Missouri. The toxin could not be isolated, could not be inactivated by cooking and was soluble in hexane, and fish from the same source fed to mice produced myopathy (Buchholz *et al.* 2000).

Amphibians

Of the amphibians, the toads are of major interest to the toxicologist because a number of species produce noxious substances in their dermal glands. These compounds include amines, peptides, proteins, steroids, and both water-soluble and lipid-soluble alkaloids. With the exception of the last, these substances are produced by the toad itself rather than bio-accumulated; for reviews, see Daly (1995) and Mebs (2002). The genus *Bufo* exudes the alkaloid bufotenine (*N*,*N*-dimethyl-5-hydroxytryptamine; Fig. 15.15), and there have been reports of attempts to gain psychedelic effects by licking the toad or smoking its venom, although Lyttle *et al.* (1996) point out that the psychedelic effects of bufotenine cannot be confirmed by objective studies. The substance may also be abused by intravenous injection, which has led to death (Kostakis and Byard 2009). Bufotenine is also a product of the 5-hydroxytryptamine (serotonin) degradation pathway, and its presence in urine has been suggested as a diagnostic indicator of psychiatric disorders (Takeda *et al.* 1995). These workers devised a three-dimensional HPLC method with electrochemical detection to measure bufotenine in urine. A method for the determination of bufotenine and other potentially hallucinogenic *N*-dimethylated indoleamines in human urine has also been described by Forsström *et al.* (2001). This used SPE followed by LC-MS, with identification based on retention data and two fragment ions produced by triple-quadrupole MS. Quantification was based on ESI and multiple reaction monitoring (MRM) to improve the sensitivity.

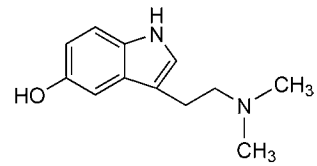

Figure 15.15 Bufotenine.

Reptiles

Gila monsters

These venomous lizards belong to the genus *Helodermatidae* (*Heloderma suspectum*, the Gila monster, *H. horridum*, the Mexican bearded lizard and their subspecies) and are indigenous to the south-western USA and Mexico. They have venom glands on the mandible and deliver the venom along grooved teeth into a bite. The venom contains hyaluronidase and proteases, in addition to gilatoxin, a 35 kDa glycoprotein with serine protease and kallikrein-like activity. The bite can lead to anaphylactoid syndrome (Hooker *et al.* 1994).

Snakes

Three main families of poisonous snakes exist – the Elapidae (cobras), the Viperidae (vipers) and the Crotalidae (pit vipers). The elapids comprise about half the world's species of venomous snakes and include the cobras and the mambas. Genera of the elapid family are found in Asia, the Pacific, the Americas and Africa (Mebs 2002). The true vipers (Viperidae), of which the common viper is the best known, inhabit Europe, Asia and Africa. The pit vipers (Crotalidae) are mainly found in North, Central and South America and include the rattlesnake genera *Crotalus* and *Sistrurus.* An estimated 30 000 lethal cases of snakebite occur annually in Asia and 1000 in Africa and South America (WHO 1995). The role of the analytical toxicologist in the diagnosis and management of snakebites is limited and the selection of information presented here aims to provide some basic data to facilitate consultative tasks.

The effects of snake venoms can be divided roughly into two major groups – neurotoxic and coagulotoxic. The neurotoxins from Elapidae and Viperidae act either at the postsynaptic level (α-neurotoxins) by preventing the binding of acetylcholine on its receptor, or at the presynaptic level by affecting neurotransmitter release. These are either non-enzymatic potassium channel blockers or β-neurotoxins, which are characterised by phospholipase A_2 activity (Bon *et al.* 1994); see also the Hymenoptera above. The dendrotoxins are small proteins from mamba venom with such specificity to potassium channels that they have become a probe for studying the physiological function of these channels (Harvey 2001). Mamba venom has also been shown to contain

toxins with high specific affinity for muscarinic acetylcholine receptors (Bradley 2000).

The haematotoxic principles of snake venoms may interfere with a number of components of the haemostatic system. These proteins and peptides are usually found in the venom of the Viperidae and Crotalidae. There are enzymes that cause fibrinogen coagulation, and enzymes that degrade fibrin or fibrinogen, there are factor V and factor X activators, substances with haemorrhagic activity, and platelet aggregation inducers and inhibitors. The haemorrhagins are of special interest; these are zinc-containing metalloproteases that act by degrading the component proteins of the basement membrane underlying capillary endothelial cells. Two reviews on snake haematotoxicity are by Markland (1997) and Hati *et al.* (1999).

The symptoms of snakebites in humans vary greatly depending on the species, but in general consist of local pain, oedema, blistering and necrosis. Systemic effects of neurotoxic species include blurred vision, ptosis and respiratory paralysis. Haemostatic toxins, such as the haemorrhagins, cause spontaneous bleeding in the gingival sulci, nose, skin and gastrointestinal tract. Fatalities result from cerebral haemorrhage or massive retroperitoneal bleeding (Warrell and Fenner 1993). Renal lesions include glomerulopathy, vasculopathy, tubular necrosis and interstitial nephritis, and often accompany snakebites with haematotoxic and neurotoxic venoms (Sitprija and Chaiyabutr 1999).

The only specific and effective treatment for systemic and severe local envenomation is the administration of antivenom, a hyperimmune immunoglobulin (Moroz 1998; Warrell and Fenner 1993). For administration of the proper antivenom, the species that has bitten the patient must be known, and panacea antivenom is not and will not be available. Purification and characterisation of snake venoms is not only important for the elucidation of their mechanism of action, but also for the preparation of these antivenoms (Bhat *et al.* 1991; Chang *et al.* 1997; Gasanov *et al.* 1997; Stiles *et al.* 1994). A future development in reducing snakebite fatalities may be active immunisation of populations at risk (Chippaux and Goyffon 1998). An exhaustive state-of-the-art review on available antivenoms, not only against snake bites but also against bites by spiders and scorpions, was recently published by Espino-Solis *et al.* (2009).

Birds

Coturnism is a peculiar type of poisoning, resulting in the development of rhabdomyolysis and acute renal failure after the ingestion of quail (*Coturnix coturnix*). Reactions to the consumption of quail have been known since antiquity, especially in the Middle East. Some authors attribute the death after ingestion of quail of the Israelites in the Bible (*Numbers*, chapter 11) to coturnism (Rizzi *et al.* 1991). It has been presumed that a toxin ingested by the birds during migration is directly toxic to striated muscle. The toxin has been postulated to be coniine from hemlock (*Conium maculatum*) (Rizzi *et al.* 1991) or red hempnettle (*Galeopsis ladanum*) (Tsironi *et al.* 2004).

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CHAPTER

16 Pesticides

M Kała

More than a thousand pesticides are available and widely used in the world today. In addition, several hundred compounds that are no longer manufactured or marketed for crop protection still remain in people's houses. Both these groups of substances play an important role in clinical and forensic toxicology as causes of suicidal, homicidal and accidental poisonings. The immense variety of chemical compounds with pesticidal properties means that the identification of an unknown substance is complicated. The screening procedure presented is based on three methods: thin-layer chromatography (TLC), gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS). Gas chromatography with tandem mass spectrometry (GC-MS[-MS]), high performance liquid chromatography (HPLC) and liquid chromatography combined with single-stage or tandem mass spectrometry (LC-MS or LC-MS[-MS]) are also used as confirmation and quantification methods. These methods can be used to identify the active component(s) in commercial preparations and household products and cosmetics, and to examine waters, drinks and foodstuffs to which a pesticide has been added either for criminal purposes or accidentally. They are also used to determine these compounds in biological samples and other materials taken from or found near a patient or body – so-called scene residues.

Classification of pesticides

The term 'pesticide' encompasses a wide variety of substances used to destroy unwanted life forms. Pesticides are applied in agriculture for crop protection and pest control, and in human and animal hygiene. They are classified as insecticides, herbicides, rodenticides, fungicides, nematocides, molluscicides and acaricides on the basis of their field of use. Commercial formulations can be mixtures of pesticides from different classes. It should be noted that some superseded materials are known to be still in use for non-agricultural purposes.

Most pesticides have common names agreed by the International Organization for Standardization (ISO) through its Technical Committee 81 (ISO/TC 81), for which the secretariat is the British Standards Institution (BSI). The principles for coining these common names are explained in ISO 257 : 2004. If the molecule is, for example, an acid, it may be used in a salt or ester form. Usually the common name is assigned to the parent acid; from this name, the salt or ester name can be derived in a straightforward manner by adding the name of the ester or ion (e.g. dichlorprop and dichlorprop, methyl). These common names are used throughout this chapter for convenience and brevity, but their equivalent systematic chemical names can be ascertained easily according to the rules of the International Union of Pure and Applied Chemistry (IUPAC) and the Chemical Abstracts Service Registry Number (CAS RN) (Merck Index 2006; Tomlin 2006). Approximately 880 active substances are currently formulated in pesticide products (Tomlin 2006). These substances belong to more than 100 chemical subclasses. Often the type of chemical is also indicated by a stem in the common name (e.g. 'uron' for ureas, and 'carb' for carbamates).

Structures of various pesticides are given in Fig. 16.1. Table 16.1 gives an alphabetical list of approximately 500 compounds together with their uses, chemical classification and analytical data.

Insecticides

Insecticides may be classified into eight chemical groups, of which the following five are the most important:

- Organophosphorus (OP) compounds, which have the general structure **1** in Fig. 16.1 with subclasses listed in Table 16.2
- Carbamates (structure **2** in Fig. 16.1 where R^1 = methyl, R^2 = H or methyl and R^3 = aryl, heterocyclic or oxime groups) (e.g. aldicarb, structure **3** in Fig. 16.1)
- Chlorinated hydrocarbons, which include dichlorodiphenyltrichloroethane (DDT) and its analogues (e.g. methoxychlor), hexachlorocyclohexane isomers (e.g. lindane) and bridged polycyclic chlorinated compounds (e.g. endosulfan, structure **4** in Fig. 16.1)
- Pyrethroids, both natural (e.g. pyrethrin II, structure **5** in Fig. 16.1) and synthetic (e.g. deltamethrin, structure **6** in Fig. 16.1)
- Substituted ureas (e.g. diflubenzuron, structure **7** in Fig. 16.1)

Other insecticide groups are organotin (e.g. cyhexatin, structure **8** in Fig. 16.1) and heterocyclic compounds (e.g. dazomet, structure **9** in Fig. 16.1).

Herbicides

Herbicides may be classified into at least 12 groups, but the seven most important are:

- Chlorinated phenoxy acids (e.g. 2,4-dichlorophenoxyacetic acid (2,4-D), structure **10** in Fig. 16.1)
- Substituted ureas (e.g. metobromuron, structure **11** in Fig. 16.1)
- Triazines (e.g. atrazine, structure **12** in Fig. 16.1)
- Uracils (e.g. lenacil, structure **13** in Fig. 16.1)
- Quaternary ammonium compounds (e.g. paraquat, structure **14** in Fig. 16.1, and diquat)
- Carbamates, which include not only the carbamates (e.g. propham), but also thiocarbamates (e.g. tri-allate, structure **15** in Fig. 16.1)
- Carboxylic acids and esters (e.g. dicamba).

Other herbicides include amides, chloroacetanilide, OP and organoarsenic compounds.

Fungicides

Chemicals from many groups belong to this class: benzimidazoles (e.g. carbendazim), dithiocarbamates (e.g. thiram, structure **16** in Fig. 16.1), acylalanines (e.g. metalaxyl) and OP compounds (e.g. pyrazophos). The most important are:

- Dithiocarbamate complexes with manganese, nickel and zinc.
- Organic and inorganic compounds of copper and mercury.

Rodenticides

Three types of compounds are notable in this category:

- Phosphines (derived by the reaction of moisture with magnesium, aluminium and zinc phosphides)
- Thallium salts, usually sulfates
- Coumarin anticoagulants (e.g. brodifacoum, bromadiolone, coumatetralyl, difenacoum; Fig. 16.2).

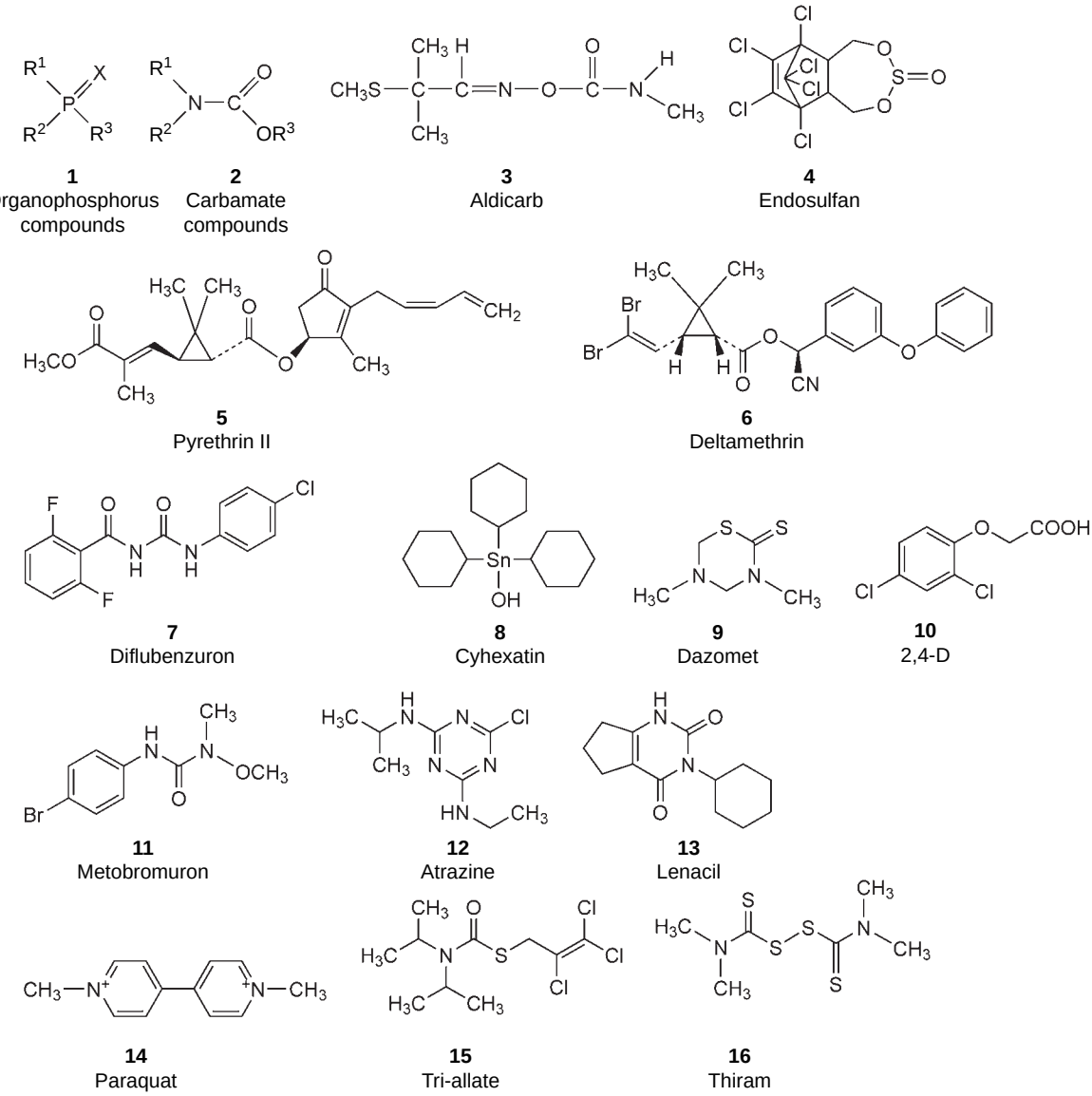

Figure 16.1 Structures of pesticides.

Acaricides, molluscicides and nematicides

These include organotin (acaricide), niclosamide (molluscicide) and phorate (nematicide). Some of these compounds that have more than one application can be found among those mentioned above.

Toxicity

The large variety of chemical compounds that show pesticide properties means that there is a very wide range of toxicity in humans. It is believed that an oral dose of only several drops (100 mg) of terbufos is fatal to most adults, whereas another pesticide (amitrole) is non-toxic in humans even when several hundred grams are ingested. Within a particular class of pesticide the lethal dose may vary considerably. Moreover, the metabolites of many pesticides (e.g. oxygen analogues of phosphorothionates) are much more toxic than the parent compounds. The commercially available preparations usually contain an active substance mixed with filler (solids) or dissolved in an organic solvent (liquids). Although certain pesticides are unlikely to cause acute toxicity, the vehicle in which they are formulated (toluene, xylenes, butan-1-ol, cyclohexanone, farbasol and solvent naphtha) (Chłobowska *et al*. 1996) may itself be toxic and, in some cases, can be the main causative agent for the symptoms observed.

The pesticides have been classified into five groups according to the World Health Organization (WHO) toxicity classification for estimating the oral acute toxicity of pesticides (Tomlin 2006) (Table 16.3). Toxicity was determined on the basis of the LD_{50} of the active ingredient for the rat and the estimated lethal doses related to a 70 kg person. However, realistic human lethal doses of pesticides can be estimated only on the basis of well-documented cases of poisoning.

Ideally, all the toxicological data for each formulation should be available from the manufacturer. However, if such data are not obtainable, then the classification may be based on proportionate calculations from the LD_{50} values of the technical ingredient(s) according to the following formula (WHO 2004):

$$\frac{LD_{50}\text{ active ingredient} \times 100}{\text{percentage of active ingredient in formulation}}$$

If the formulation contains more than one ingredient (including the solvent, wetting agents, etc.) that has significant toxicity-enhancing properties, then the classification should correspond to the toxicity of the mixed ingredients.

Analytical techniques

The chemical and physical properties of pesticides may differ considerably. There are several acidic pesticides; others are neutral or basic. Some

Table 16.1 Classification of pesticides and analytical data

Compound[a]	*Use*[b]	*Class*[c]	*TOX*[d]	*TX*[e]	*TY*[e]	*TZ*[e]	*TAA*[e]	*TAB*[e]	*TAC*[e]	*GA*[f]	*Col.*[g]	*GK*[h]	*HAO*[i]	*HAP*[i]	*LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)*		*Typical ions and intensities*[j]		
Acephate	I	OP	III	0	0					1470	c/p				184→143 (25)	184→125 (13)	136 (100)	94 (57)	183 (5)
Acephate, M, -C_2H_2O (methamidophos)	A, I	OP	Ib	1	0					1190	c/p	—					94 (100)	141 (30)	64 (25)
Acephate, -C_2H_2O, TFA	—	—	—	—	—					1110	p	—					96 (100)	125 (55)	237 (15)
Acetochlor	H	Misc.	III	—	—					1845	c	—					45 (100)	188 (23)	237 (6)
Alachlor	H	Misc.	III	40	45					1876	c	—			270→238 (15)	270→162 (25)	160 (100)	188 (94)	269 (10)
Aldicarb	I, A, N	CB	Ia	18	12	65	12	4	3	1320	p	—	7.63	0.69	208→89 (21)	208→116 (13)	86 (100)	144 (55)	100 (47)
Aldrin[j]	I	CH	—	89	98			67	52	1943	p[m]	0.88	0	33.86	—	—	66 (100)	263 (39)	362 (1)
Allethrin	I	PY	III	—	—	—					p	1.04					123 (100)	79 (34)	302 (2)
Allidochlor[j]	H	Misc.	—	—	—					1140	p	—					56 (100)	138 (31)	173 (4)
Ametryn	H	TR	III	26	23					1775	p	—			228→186 (25)	228→96 (35)	58 (100)	68 (91)	227 (86)
Amitraz	A, I	Misc.	III	—	—					2570	c	—			294→163 (21)	294→122 (41)	293[o]	162[o]	132[o]
Amitraz	—	GCDP	—	—	—					1570	c	—					120 (100)	149 (63)	162 (29)
Amitraz	—	GCDP	—	—	—					2570	c	—					121 (100)	106 (18)	252 (14)
Amitrole	H	TR	U	0	0					0000[n]	c	—			85→58 (29)	85→57 (23)	84 (100)	57 (14)	75 (3)
Amitrole	H	TR	U	—	—					1312	p	—					84[k]	—	—
Amitrole, 2ME	—	—	—	—	—					1050	p	—					98 (100)	56 (75)	112 (25)
Amitrole, AC	—	—	—	—	—					1010	p	—					84 (100)	57 (35)	126 (18)
Anilazine[j]	F	TR	U	—	—					2030	c	—			275→153 (33)	275→178 (33)	239 (100)	178 (31)	143 (26)
Aramite[j]	A	Misc.	—												352→191 (19)	352→105 (57)	185 (100)	319 (37)	334 (23)
Asulam	H	CB	U	—	—			1	0	<1000	p	—	99.99	0			230[k]	—	—
Asulam, HY, artefact, -$C_2H_2O_2$	—	—	—	—	—					2175	c/p	—					65 (100)	172 (56)	156 (55)
Atrazine	H	TR	U	—	—			4	8	1714	c/p	0.79	10.75	1.24	216→174 (25)	216→104 (27)	58 (100)	200 (75)	215 (44)
Atrazine, M (desethyl)	—	—	—	—	—					1680	c	—			188→146 (25)	188→104 (33)	58 (100)	172 (72)	187 (22)
Atrazine, M (desethyl, deschloro, methoxy)	—	—	—	—	—					1670	c	—					58 (100)	168 (95)	183 (49)
Azamethiophos	I	OP	III	—	—	—					—	—			325→183 (21)	325→139 (33)	215[o]	1550	125[o]
Azamethiophos	—	GCDP	—	—	—					1655	c	—					184 (100)	101 (26)	64 (21)
Azinphos, ethyl	I, A	OP	Ib	24	48					2560	c/p	—			346→132 (21)	346→160 (15)	132 (100)	186 (10)	345 (1)
Azinphos, methyl	I	OP	Ib	20	42					2473	c/p	1.6			318→132 (21)	318→160 (13)	77 (60)	160 (34)	317 (1)
Aziprotryne[j]	H	TR	—	42	50					1765	c/p	0.85					225 (100)	139 (85)	68 (75)
Azocyclotin	A	OM	II	1	0					2730	c	—			369→205	369→287	436[k]	—	—
Barban[j]	H	CB	—	—	—			39	16	2126	c/p	1.24	0.42	2.56			51 (100)	153 (76)	87 (66)
Barban, ME	—	—	—	—	—					2335	c	—					256 (100)	111 (26)	271 (25)
Barban, M (Cl-benzamine), 2ME	—	GCDP	—	—	—					1180	p	—					155 (62)	154 (100)	75 17
Barban, M (Cl-benzamine), ME	—	GCDP	—	—	—					1110	p	—					140 (100)	141 (74)	77 (31)
Barban, M (Cl-benzamine), TFA	—	GCDP	—	—	—					1125	p	—					223 (100)	154 (100)	111 (55)
Barban, M (HOOC-), ME	—	GCDP	—	—	—					1500	c	—					185 (100)	140 (87)	59 (69)
Beflubutamid	H	Misc.	—												373→91 (47)	373→162 (39)	355[o]	221[o]	176[o]
Benalaxyl	F	Misc.	U												326→148 (27)	326→208 (21)	266[o]	234[o]	206[o]
Benazolin	H	Misc.	U	—	—					2055	c/p	1.07					170 (100)	243 (61)	198 (59)
Benazolin, ME	—	—	—	—	—					2000	c	—					170 (100)	257 (61)	198 (59)
Benazolin, ethyl	—	—	—	—	—					2045	c	—					170 (100)	198 (54)	271 (40)
Bendiocarb	I	CB	II	—	—					1640	c	—			224→167 (13)	224→109 (21)	151 (100)	126 (47)	223 (6)
Bendiocarb, -C_2H_3NO (2,2-dimethyl-1,3-benzodioxol-4-ol)	—	GCDP	—	—	—					1110	p	0.31					126 (100)	151 (92)	166 (42)
Bendiocarb, -C_2H_3NO, TFA	—	GCDP	—	—	—					<1000	p	—					247 (100)	125 (51)	262 (47)
Bendiocarb, TFA	—	—	—	—	—					1560	p	—					247 (100)	69 (88)	319 (52)
Benfluralin	H	Misc.	U	—	—					1672	c	—			—	—	292[o]	264[o]	187
Benfluracarb	I	CB	II												411→195 (31)	411→252 (19)	190[o]	164[o]	163[o]
Benomyl	F	Misc.	U	—	—					—	—	—			291→160 (35)	291→192 (17)	290[k]	—	—
Benomyl, M (aminobenzimidazole), 3ME	—	GCDP	—	—	—					1715	c	—					160 (100)	146 (80)	175 (78)
Benomyl (desbutylcarbamoyl), 2ME	—	GCDP	—	—	—					1875	c	—					160 (100)	219 (37)	132 (23)
Bensulfuron, methyl	H	SU	U												411→149 (27)	411→119 (51)	—	—	—
Bentazone, ME	H	Misc.	III	—	—					1910	c	—			239→132 (−32)	239→197 (−24)	212 (100)	105 (83)	254 (23)

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Bentazone	—	GCDP	—	—	—					1675	c	—					120 (100)	92 (43)	178 (38)
Benzoximate	A	Misc.	U												364→199 (17)	364→105 (35)	213[o]	198[o]	170[o]
Benzoylprop, ethyl[j]	H	Misc.	—	—	—	—					p	1.42					105 (100)	292 (10)	51 (7)
Benzthiazuron[j], 2ME	H	SU	—	8	3					1985	c	—					72 (100)	235 (8)	136 (4)
Bifenox	H	Misc.	U												359→309 (17)	359→189 (35)	341 (52)	173 (37)	189 (28)
Bifenthrin	I, A	PY	II												440→181 (21)	440→166 (55)	182[o]	181[o]	167[o]
Bioallethrin	I	PY	II	48	52					2105[m]	c	—					123 (100)	79 (34)	302 (2)
Bioresmethrin	I	PY	U	—	—					2300[m]	c	—					123 (100)	171 (50)	338 (7)
Biphenyl	F	Misc.	III	—	—					1320[m]	p	—			—	—	154 (100)	76 (25)	63 (16)
Bitertanol	F	Misc.	III	10	3					2656	c	—			338→70 (25)	338→269 (15)	170 (100)	57 (27)	337 (2)
Boscalid	F	Misc.	—												343→307 (27)	343→140 (27)	344[o]	342[o]	142[o]
Brodifacoum	R	CA	Ia	—	—	—				—	—				523[p]	521[p]	121 (100)	360 (57)	518 (55)
Bromacil	H	UR	U	—	—					1900	c/p	1.15			261→205 (19)	261→188 (37)	205 (100)	188 (16)	260 (4)
Bromacil, ME	—	—	—	—	—					—	p	1.08					219 (100)	221 (68)	190 (40)
Bromadiolone	R	CA	Ia	—	—	—				—	—						527[k]	—	—
Bromadiolone, ME	—	GCDP	—	—	—					1985[m]	c	—					260 (100)	258 (100)	76 (39)
Bromofenoxim	H	Misc.	III	—	—	—				—	—			461[k]	525[p]	527[p]	461[k]	—	
Bromofenoxim (2,4-dinitrophenol)	—	GCDP	—	—	—					1480	c/p	—					184 (100)	63 (88)	91 (45)
Bromofenoxim (bromoxynil)	H	GCDP	—	—	—					1688	c	—					277 (100)	88 (59)	275 (56)
Bromofenoxim (bromoxynil), ME	—	GCDP	—	—	—					1650	c	—					86 (100)	72 (59)	289 (15)
Bromfenvinfos	I	OP	—			80	16										404[k]	323 (56)	295 (18)
Bromophos[j]	I	OP	—	58	92					2001	c/p	1.05					331 (100)	329 (74)	361 (4)
Bromophos impurity (2,5-dichloro-4-bromophenol)	—	—	—	—	—					—	p	0.31					242 (100)	240 (62)	244 (48)
Bromophos, ethyl[j]	I	OP	—	69	93					2077	c/p	1.09			395→339 (23)	395→367 (17)	97 (100)	359 (79)	357 (60)
Bromopropylate	A	Misc.	III							2425[m]	c	—			—	—	341 (100)	183 (73)	426 (1)
Bromoxynil	H	Misc.	II	8	16					1690	c	—			276→79 (−36)	278→81 (−40)	277 (100)	88 (59)	275 (56)
Bromoxynil, ME	H	Misc.	II	—	—					1650	c/p	0.65					86 (100)	202 (26)	289 (15)
Bromoxynil octanoate	H	Misc.	II	—	—			47	39	—	p	1.31	0.19	31.65			57 (100)	127 (83)	43 (78)
Bromuconazole	F	TR	II												378→159 (37)	378→161 (35)	297[o]	295[o]	175[o]
Bupirimate	F	Misc.	—	—	—					2165	c	—			317→166 (33)	317→108 (35)	273 (100)	208 (92)	316 (34)
Buprofezin	I, A	Misc.	U												306→201 (17)	306→116 (21)	175[o]	172[o]	105[o]
Butafenacil	H	Misc.	III												492→180 (59)	492→331 (29)	333[o]	332[o]	331[o]
Butocarboxim	I	CB	Ib	15	11					1580	c	—			208→75 (15)	208→116 (11)	87 (100)	55 (56)	144 (39)
Butoxycarboxim	I, A	CB	Ib	3	2					1415	p	—			240→106 (19)	240→166 (13)	85 (100)	165 (16)	222[k]
Butoxycarboxim	—	GCDP	—	—	—					1405	c	—					85 (100)	108 (7)	149 (1)
Buturon[j]	H	Misc.	—	20	29					2135	p	—			237→84 (21)	237→126 (37)	56 (100)	236 (28)	75 (19)
Butylate	H	CB	U												218→57 (29)	218→156 (17)	217[o]	174[o]	146[o]
Cadusafos	N	OP	Ib												271→159 (19)	217→97 (47)	270[o]	214[o]	159[o]
Captafol	F	Misc.	1a	—	—					2355	c/p	1.44			—	—	79 (100)	311 (6)	347 (3)
Captafol (cyclohexenedicarboxylic acid), 2ME	—	GCDP	—	—	—					1190[m]	p	—					79 (100)	138 (47)	198 (1)
Captafol (cyclohexenedicarboximide)	—	GCDP	—							1450	c	—					79 (100)	80 (63)	151 (41)
Captan	F	Misc.	U	43	42			25	5	2020	c/p	1.13	0.54	99.99			79 (100)	264 (5)	299 (1)
Captan (cyclohexenedicarboxylic acid), 2ME	—	GCDP	—	—	—					1190[m]	p	—					79 (100)	167 (18)	198 (1)
Captan (cyclohexenedicarboximide)	—	GCDP	—	—	—					1450	p	—			—	—	79 (100)	80 (63)	151 (41)
Carbaryl	I	CB	II	18	25	68	17	12	4	1865	c	—	2.39	0.97	202→145 (15)	202→127 (35)	144 (100)	115 (59)	201 (2)
Carbaryl, TFA	—	—	—	22	—					1785	c	—					69 (100)	240 (54)	297 (11)
Carbaryl, M (1-naphthol)	—	GCDP	—							1490[m]	c/p	0.47					144 (100)	115 (65)	—
Carbatyl-M (1-naphthol) AC	—	GCDP	—	—	—					1555[m]	c	—					144 (100)	115 (60)	186 (4)
Carbendazim	F	Misc.	U	—	—					—	—	—			192→160 (25)	192→132 (41)	151 (100)	191 (57)	103 (38)
Carbendazim, -$C_2H_2O_2$	—	GCDP	—	—	—					1930	c	—					133 (100)	105 (26)	—

table continued

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Carbetamide	H	CB	U	—	—					1975	c	—			237→192 (13)	237→118 (19)	119 (100)	93 (35)	236 (12)
Carbetamide, 2ME	—	—	—	—	—					1965	c	—					58 (100)	158 (30)	264 (3)
Carbetamide, TFA	—	—	—	—	—					1870	c	—					332 17	196 73	119 100
Carbofuran	I	CB	Ib	17	20	74	8	5	4	1690	c/p	—	5.93	0.87	222→165 (17)	222→123 (29)	164 (100)	149 (56)	221 (5)
Carbofuran-C_2H_3NO (2,3-dihydro-2, 2-dimethyl-benzofuran-7-ol)	—	GCDP	—	—	—					1060	c/p	0.23					164 (100)	149 (85)	131 (35)
Carbophenothion[j]	I, A	OP	—										0	15.26					
Carbosulfan	I	CB	II	49	66					2432	c	—			381→118 (25)	381→160 (21)	164[o]	163[o]	160[o]
Carboxin	F	Misc.	U	—	—					2410	c	—			236→143 (21)	236→87 (33)	143 (100)	235 (59)	87 (33)
Carfentrazone, ethyl	H	Misc.	III												412→346 (31)	412→366 (25)	340[o]	330[o]	312[o]
Cartap hydrochloride	I	Misc.	II												238→73 (37)	238→150 (19)	—	—	
Chinomethionat	F, A	Misc.	III	—	—										235→207 (21)	235→163 (39)	234[o]	206[o]	144[o]
Chloralose[j], 3AC	R	Misc.	II							2260[m]	c	—					115 (100)	272 (88)	399 (2)
Chloralose, M (destrichloroethylidenyl), 5TFA	—	GCDP	—	—	—					1795[m]	c	—					69 (100)	109 (76)	479 (13)
Chloralose	—	GCDP	—	—	—					2155[m]	c	—					71 (100)	247 (11)	349 (1)
Chloramben[j], ME	H	Misc.	U	—	—					1730	c	—					188 (100)	219 (66)	124 (55)
Chloramben isomer-1, 2ME	—	—	—	—	—					1795	c	—					233 (100)	202 (57)	174 (26)
Chloramben isomer-2, 2ME	—	—	—	—	—					1815	c	—					188 (100)	233 (75)	161 (49)
Chlorbromuron[j], 2ME	H	SU	U	22	30					1880	p	1.09	1.35	2.05	293→182 (23)	293→204 (21)	248 (100)	220 (97)	306 (9)
Chlorbufam[j]	H	CB	U	—	—					1720	c/p	0.83			241→172 (17)	241→154 (27)	53 (100)	127 (35)	223 (29)
Chlorbufam, TFA	—	—	—	—	—					1510	c	—					53 (100)	154 (9)	319 (2)
Chlordane	I	CH	II	—	—			64	44	2020	c	—	0	99.99	—	—	373 (100)	374 (91)	410 (4)
Chlordimeform[j]	A	Misc.	—	—	—					1655	c	—					196 (80)	181 (67)	89 (61)
Chlorfenethol[j]	F	Misc.	—	—	—					1853	c	—					139 (100)	251 (75)	267 (8)
Chlorfenprop, methyl[j]	H	Misc.	—	—	—					—	p	0.55					125 (100)	165 (92)	232 (4)
Chlorfenson[j]	A	Misc.	—	—	—					2150[m]	c	—			—	—	111 (100)	175 (72)	302 (13)
Chlorfenvinphos	I, A	OP	Ia	26	26	85	42			2030[m]	c/p	1.08			359→155 (19)	359→99 (43)	81 (100)	109 (49)	358 (1)
Chlorfenvinphos, M	—	GCDP	—	—	—					1495[m]	p	—					173 (100)	145 (24)	222 (1)
Chlorflurenol, ME	H	Misc.	U	—	—					2095[m]	c	—					215 (100)	152 (65)	274 (9)
Chlorflurenol impurity(deschloro), ME	—	—	—	—	—					1950[m]	c	—					181 (100)	152 (32)	240 (6)
Chloridazon	H	Misc.	U	—	—					—	p	1.1			222→104 (31)	222→92 (35)	77 (100)	221 (60)	88 (37)
Chloridazon, TFA	—	—	—	—	—					1170	p	—					77 (100)	317 (52)	69 (24)
Chlormephos	I	OP	Ia	64	91					1399[m]	p	—			235→97 (33)	235→143 (19)	121 (100)	97 (96)	234 (28)
Chloroaniline (p)	H	Misc.	—	—	—					1210	p	—					127 (100)	129 (32)	65 (16)
Chloroneb[j]	F	Misc.	IV[l]	—	—					—	p	0.54					191 (100)	206 (52)	141 (31)
Chlorophacinone	R	Misc.	Ia	0	1					3280	c	—					173 (100)	165 (22)	374 (21)
Chlorotoluron	H	SU	U	15	9					—	p	0.92			213→72 (33)	213→140 (33)	72 (100)	44 (17)	212 (14)
Chlorotoluron (3-chloro-4-methylaniline)	—	GCDP	—	—	—					—	p	0.23					141 (100)	106 (68)	140 (37)
Chlorotoluron (3-chloro-4-methylphenylisocyanate)	—	GCDP	—	—	—					—	p	0.13					167 (100)	132 (97)	169 (29)
Chlorotoluron, ME	—	—	—	—	—					1695	c	—					226[k]	—	—
Chloroxuron[j]	H	SU	—	10	7					1895	p	—			291→72 (41)	291→218 (33)	72 (100)	290 (45)	105 (21)
Chloroxuron, ME	—	—	—	—	—					2430	c	—					72 (100)	304 (25)	85 (19)
Chloroxuron (4-[4-chlorophenoxy]aniline)	—	GCDP	—	—	—					—	p	1.01					108 (100)	219 (61)	80 (31)
Chloroxuron (4-[4-chlorophenoxy] phenylisocyanate)	—	GCDP	—	—	—					—	p	0.9					245[k]	245	—
Chlorpropham	H	CB	U	—	—					—	p	0.63	0.41	2.68	—	—	43 (100)	127 (71)	213 (31)
Chlorpyrifos	I	OP	II	64	95					1955	c/p	1			350→97 (41)	350→198 (25)	197 (40)	97 (37)	349 (3)
Chlorpyrifos, methyl	I	OP	U	56	89			60	35	1847	c	—	0	6.18	322→125 (27)	322→290 (25)	125 (100)	286 (82)	321 (2)
Chlorthal, di-methyl	H	Misc.	U	—	—					<1000	c/p	1			—	—	301 (100)	299 (75)	330 (24)
Chlorthiamid	H	Misc.	III	—	—					1870	c	—			206→119 (55)	206→154 (45)	170 (100)	205 (43)	75 (27)

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Chlorthiamid (dichlobenil)	H	GCDP	U	—	—					1303	c/p	—					171 (94)	100 (32)	136 (20)
Chlorthiophos isomer-1[(j)]	I	OP	—	55	91					2210[(m)]	c	—			361→305 (21)	361→333 (17)	97 (100)	222 (61)	360 (16)
Chlorthiophos isomer-2[(j)]	I	OP	—	55	91					2230[(m)]	c	—					97 (100)	325 (15)	360 (3)
Chlorthiophos isomer-3[(j)]	I	OP	—	—	—					2260[(m)]	c	—					97 (100)	269 (77)	360 (26)
Cinerin I (pyrethrins)	I	PY	II	—	—					—[(m)]	p	1.17					123 (100)	121 (27)	107 (27)
Cinerin II (pyrethrins)	I	PY	II	—	—					—[(m)]	p	1.5					123 (100)	107 (100)	121 (53)
Clethodim	H	Misc.	III												360→164 (25)	360→268 (17)	—	—	
Clopyralid, ME	H	Misc.	U	0	0					1320	p	—			192→146 (29)	192→110 (47)	147 (100)	110 (50)	205 (15)
Coumachlor[(j)], ME	R	CA	—	—	—					2775[(m)]	c	—					313 (100)	356 (21)	125 (19)
Coumachlor[(j)]	—	GCDP	—	—	—					1575[(m)]	c	—					165 (100)	102 (51)	180 (32)
Coumaphos	I	OP	Ia	27	61					2573[(m)]	c	—			363→227 (35)	363→307 (23)	109 (100)	97 (100)	362 (73)
Coumatetralyl	R	CA	Ib	14	30					2635[(m)]	c	—			291[(p)]	292[(p)]	292 (100)	121 (69)	188 (68)
Coumatetralyl, HY	—	—	—	—	—					2250[(m)]	c	—					130 (100)	248 (13)	266 (3)
Coumatetralyl, HYME	—	—	—		—					2300[(m)]	c	—					130 (100)	135 (70)	280 (4)
Coumatetralyl isomer-1, ME	—	—	—	—	—					2655[(m)]	c	—					306 (100)	175 (72)	291 (47)
Coumatetralyl isomer-2, ME	—	—	—	—	—					2690[(m)]	c	—					306 (100)	291 (28)	175 (27)
Coumatetralyl, M (HO-), isomer-1, ME	—	—	—	—	—					2910[(m)]	c	—					203 (100)	303 (91)	322 (11)
Coumatetralyl, M (HO-) isomer-2, 2ME	—	—	—	—	—					2925[(m)]	c	—					336 (100)	205 (62)	232 (34)
Coumatetralyl, M (HO-) isomer-3, 2ME	—	—	—	—	—					2935[(m)]	c	—					321 (100)	336 (62)	175 (60)
Coumatetralyl, M (HO-) isomer-4, 2ME	—	—	—	—	—					2990[(m)]	c	—					336 (100)	205 (61)	232 (50)
Coumatetralyl, M (di-HO-) isomer-1, 2ME	—	—	—	—	—					3005[(m)]	c	—					333 (100)	205 (11)	352 (1)
Coumatetralyl, M (HO-methoxy), 2ME	—	—	—	—	—					3070[(m)]	c	—					366 (100)	262 (42)	351 (37)
Coumatetraryl, M (di-HO-) isomer-2, 2ME	—	—	—	—	—					3085[(m)]	c	—					333 (100)	205 (15)	352 (2)
Coumatetraryl, M (di-HO-) isomer-3, 3ME	—	—	—	—	—					3105[(m)]	c	—					366 (100)	351 (92)	232 (33)
Coumatetraryl, M (tri-HO-), H_2O, 2ME	—	—	—	—	—					3175[(m)]	c	—					350 (100)	205 (28)	335 (11)
Cyanazine	H	TR	II	16	12					1956	p	1.1			241→214 (23)	241→104 (41)	68 (100)	225 (45)	240 (24)
Cyanofenphos[(j)]	I	OP	—	—	—					2236	c	—			304→157 (27)	304→276 (17)	157 (100)	185 (22)	303 (11)
Cyanophos	I	OP	II	—						1710	c	—			261→125 (27)	261→212 (27)	109 (100)	125 (53)	243 (35)
Cycloxydim	H	Misc.	U	—						2580	c	—			326→280 (17)	326→180 (25)	178 (100)	108 (21)	279 (7)
Cycloxydim, ME	—	—	—	—						2380	c	—					192 (100)	95 (27)	293 (9)
Cycluron[(j)]	H	SU	—	13	5					1760	c	—					72 (100)	127 10	198 3
Cycluron[(j)], ME	—	—	—	—						1720	c	—					72 (100)	141 (13)	212 (2)
Cyfluthrin	I	PY	II	37	89					2749	c	—			451→191 (21)	451→127 (41)	163 (100)	226 (35)	433 (1)
Cyhexatin	A	OM	III	0	0					—	—	—			369→205 (23)	369→287 (17)	272 (100)	270 (96)	390 (5)
Cypermethrin	I	PY	II	41	91					2794	c/p	1.76			433→191 (21)	433→127 (39)	163 (100)	209 (15)	415 (3)
Cypermethrin, M (HOOC-), ME	—	GCDP	—	—		95	72			1170[(m)]	p	—					91 (100)	187 (64)	222 (4)
Cypermethrin, M (desacyl), HCH	—	GCDP	—	—						1700[(m)]	p	—					198 (100)	77 (68)	169 (64)
Cypermethrin, M (desacyl), ME	—	GCDP	—	—						2590	c	—					197 (100)	141 (30)	239 (2)
Cyphenothrin	I	PY	II	—						2960	c	—			393→151 (21)	393→123 (33)	123 (100)	81 (30)	375 (6)
Dalapon	H	Misc.	U										99.99	0					
Dazomet	H, F, I	Misc.	III	—						1660	c/p	0.86	2.19	0.47			89 (100)	162 (54)	57 (35)
2,4-D (dichlorophenoxyacetic acid)	H	CP	II	0	2			2	3	1800[(m)]	c	—	99.99	0	219→161 (−14)	219→125 (−21)	162 (100)	220 (55)	155 (29)
2,4-D, isoctyl	H	CP	II	—	—					—[(m)]	p1.12, 1.18	71 44					43 (100)	57 (96)	
2,4-D, ME	H	CP	II	—	—					1605[(m)]	p	0.65					199 (100)	234 (54)	175 (51)
2,4-D, DB, ME (dichlorophenoxybutyric acid)	H	CP	III[(l)]	—						1835[(m)]	c/p	0.87			247→161 (−12)	247→125 (−34)	101 (100)	59 (43)	262 (2)

table continued

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
DDT (*op′*)	I	CH	II	76	98	90				2218[m]	p	1.24	0	31.72	—	—	235 (100)	165 (65)	352 (1)
DDT (*pp′*)	I	CH	II	—						2299[m]	p	1.32	0	29.2	—	—	235 (100)	165 (66)	352 (1)
Deltamethrin (decamethrin)	I	PY	II	40	90	95	72			2900	c	—			523→281 (23)	523→181 (51)	181 (100)	253 (57)	503 (1)
Deltamethrin, M (HOOC-), ME	—	GCDP	—	—	—					1540[m]	c	—					91 (100)	253 (46)	310 (3)
Deltamethrin, M (desacyl-)-HCN	—	GCDP	—	—	—					1700[m]	c	—					198 (100)	77 (68)	169 (64)
Deltamethrin-M (desacyl), ME	—	GCDP	—	—	—					2590[m]	c	—					197 (100)	77 (19)	239 (2)
Demeton (-O, -S)[j]	I	OP	—	17, 83	17, 81					1576	c	—					—	—	—
Demeton-*S*-methyl	I	OP	Ib	18	13					1628[m]	c/p	0.7			248→89 (17)	248→61 (47)	88 (100)	142 (12)	230 (3)
Demeton-*S*-methylsulfon[j]	I	OP	—	1	3					1866[m]	c/p	—			263→169 (37)	263→109 (21)	169 (100)	109 (87)	262 (1)
Demeton-*S*-methylsulfoxide[j]	I	OP	—	—	—					1860[m]	c	—					109 (87)	169 (60)	218 (1)
Desmedipham, TFA	H	CB	U	32	22					2460	c	—			318→182 (19)	318→136 (33)	277 (100)	205 (100)	396 (52)
Desmedipham, M (phenol)	—	GCDP	—	—	—					1740	c	—					109 (100)	122 (62)	181 (60)
Desmedipham, M (phenol), TFA	—	GCDP	—	—	—					1540	c	—					277 (100)	205 (92)	218 (70)
Desmedipham, M (phenylcarbamic acid), 2ME	—	GCDP	—	—	—					1190	c	—					165 (100)	106 (56)	120 (40)
Desmedipham, M (phenylcarbamic acid), ME	—	GCDP	—	—	—					1320	c	—					151 (100)	106 (76)	119 (56)
Desmedipham, M (phenol), 3ME	—	GCDP	—	—	—					1560	c	—					195 (100)	136 (47)	108 (34)
Desmedipham, M (phenol) 2ME	—	GCDP	—	—	—					1640	c	—					209 (100)	108 (57)	150 (52)
Desmetryn[j]	H	TR	III	21	16					1795	c/p	0.95					58 (100)	213 (89)	198 (64)
Dialifos[j]	I	OP	Ia	—	—					2523	c/p	1.61			394→208 (23)	394→187 (17)	208 (100)	76 (21)	357 (4)
Di-allate[j]	H	CB	—	—	—					1690	c/p	0.64			270→86 (23)	270→109 (37)	86 (100)	234 (35)	254 (1)
Diazinon (dimpylate)	A, I	OP	II	47	50	76	21	30	20	1758	c/p	0.79	2.13	4.92	305→169 (29)	305→97 (41)	137 (100)	152 (64)	304 (27)
Diazinon	—	GCDP	—	—	—					1140	p	—					151 (100)	138 (50)	166 (41)
Diazinon	—	GCDP	—	—	—					1400[m]	p	—					111 (100)	138 (95)	198 (26)
Diazinon	—	GCDP	—	—	—					1685	p	—					137 (100)	152 (49)	84 (38)
Dicamba	H	Misc.	III	41	70			4	5	1795[m]	c/p	0.49	99.99	0	219→175 (−6)	219→145 (−14)	173 (100)	220 (62)	73 (41)
Dicamba (2,5-dichloromethoxybenzene)	—	GCDP	—	—	—					1200[m]	p	—					176 (100)	133 (72)	63 (37)
Dicamba, ME	H	Misc.	—	—	—					1525[m]	c	—					203 (100)	188 (23)	234 (22)
Dichlobenil	H	Misc.	U	—	—					1303[m]	c/p	0.31			—	—	171 (94)	100 (32)	136 (20)
Dichlobenil, M (HO-)	—	—	—	—	—					1540[m]	c	—					187 (100)	159 (62)	86 (57)
Dichlofenthion	N	OP	Ib	—	—					1857[m]	c/p	0.88			315→259 (21)	315→287 (15)	97 (100)	279 (72)	314 (1)
Dichlofluanid	F	Misc.	U	30	64					1950	c/p	1			350→123 (41)	350→224 (21)	123 (100)	224 (24)	332 (7)
Dichlorophen	F	Misc.	III	12	16					2140[m]	p	—					269[k]	—	—
Dichlorophen, 2AC	—	—	—	—	—					2250[m]	c	—					268 (100)	128 (32)	352 (11)
Dichlorophen, 2ET	—	—	—	—	—					2225[m]	c	—					324 (100)	309 (44)	289 (30)
Dichlorophen, 2ME	—	—	—	—	—					2245[m]	c	—					121 (100)	296 (51)	155 (47)
Dichlorprop	H	Misc.	III	3	2			3	4	1840[m]	c	—	5.84	0	233→161 (−14)	233→125 (−36)	162 (100)	234 (19)	133 (11)
Dichlorprop, M (2,4-dichlorophenol)	—	—	—	—	—					1320	c	—					162 (100)	63 (64)	164 (58)
Dichlorprop, isoctyl	H	CP	III	—	—					—[m]	p	1.05, 1.11					43 (100)	57 (83)	162 (41)
Dichlorprop, methyl	H	CP	III	—	—					1630[m]	c/p	0.48					162 (100)	189 (43)	248 (34)
Dichlorvos (DDVP)	I	OP	Ib	20	20	75	36	0	0	1275[m]	p	0.23	5.22	0.86	221→127 (27)	221→145 (25)	109 (100)	79 (26)	220 (4)
Diclofop, methyl	H	Misc.	III	43	76					2362[m]	c	—					253 (100)	120 (59)	340 (48)
Dicofol	I	CH	III	43	80					2485[m]	c	—			—	—	139 (100)	251 (59)	368 (1)
Dicofol (*pp*-dichlorobenzophenone)	—	GCDP	—	—	—					2340[m]	c/p	1.02					139 (100)	111 (38)	250 (25)
Dicrotophos	A, I	OP	Ib	—	—					1645	c	—			238→112 (17)	238→127 (23)	127 (100)	67 (46)	237 (7)
Dieldrin[j]	I	CH	—	65	87			51	27	2110[m]	p	1.13	0.11	12.04	—	—	79 (100)	82 (32)	265 (12)
Difenacoum	R	CA	Ia	—	—					—	—	—			443[p]	444[j]	444 (100)	167 (94)	282 (72)
Difethialone	R	CA	Ia	—	—										539[p]	537[p]	—	—	—
Diflubenzuron, ME	I	SU	U	18	24					2290	c	—			309→156 (−12)	309→289 (−8)	141 (100)	113 (19)	338 (8)
Diflufenican	H	Misc.	U	—	—					2670	c	—			395→266 (33)	395→246 (45)	266 (100)	246 (12)	394 (9)
Dimethachlor	H	Misc.	III	—	—					1565	c	—			256→224 (19)	256→148 (33)	134 (100)	210 (10)	255 (1)

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Dimethirimol methyl ether[j]	F	Misc.	U	—	—					1705	p	0.5					180 (100)	223 (23)	181 (10)
Dimethoate	I	OP	II	4	4	37	8	3	1	1725	c/p	0.86	0.81	0.46	230→199 (13)	230→125 (29)	87 (100)	93 (62)	229 (7)
Dimethoate, M (HO-)	—	—	—	—	—					1430	p	—					93 (100)	125 (45)	245 (19)
Dimethoate, M (HOOC-), ME	—	—	—	—	—					1400[m]	p	—					93 (100)	125 (67)	230 (49)
Dinobuton	A, F	Misc.	II	—	—					2060	c/p	1.1			—	—	211 (100)	240 (12)	267 (4)
Dinocap	A, F	Misc.	III	—	—					2460	c	—					69 (100)	364 (1)	197 (1)
Dinoseb[j]	H	Misc.	—	40	81			51	18	1790	c	—	0.17	0.79	239→134 (−52)	239→193 (−30)	211 (100)	163 48	240 16
Dinoterb	H	Misc.	Ib	—	—					1760	c/p	0.86			239→207 (−32)	239→176 (−48)	225 (100)	177 (40)	240 (12)
Dioxacarb[j]	I	CB	Ib	7, 16	—	38	5			1825	c	—					121 (100)	166 (63)	193 (1)
Dioxacarb, -C_2H_3NO (2-[1,3-dioxolan-2-yl]phenol)	—	GCDP	—	—						1325[m]	p	0.28					121 (100)	166 (30)	73 (28)
Diphenamid	H	Misc.	III	—						1970	c/p	1.1					167 (100)	72 (95)	239 (28)
Diphenylamine	F	Misc.	—	—						1595	c/p	—			170→93 (37)	170→152 (37)	169 (100)	168 (74)	84 (24)
Diquat dibromide	H	QA	II	—				0	0	0	c	—	99.99	0	184→168	184→157	344[k]	—	—
Diquat (reduction product I, monoene)	H	QA	—	—						—	p	0.4					83 (100)	108 (73)	192 (44)
Diquat (reduction product II, diene)	H	QA	—	—						—	p	0.49					54 (100)	108 (89)	190 (31)
Disulfoton	A, I	OP	Ia	58	89			52	35	1778[m]	c/p	0.86	0.1	6.48	275→89 (17)	275→61 (43)	88 (100)	97 (28)	274 (15)
Disulfoton, M (sulfone)	—	—	—	—						2077	c	—					213 (100)	153 (81)	97 (71)
Disulfoton, M (sulfoxide)	—	—	—	—						1303	c	—					29 (100)	153 (32)	213 (22)
Ditalimfos[j]	F	Misc.	—	—						2095	c	—					130 (100)	299 (39)	243 (31)
Diuron, ME	H	SU	U	15	10					1850	c	—			233→72 (31)	233→160 (33)	72 (100)	246 (14)	174 (5)
Diuron, M (3,4-dichloroaniline)	—	GCDP	—	—						1420	p	0.36					161 (100)	99 (20)	126 (14)
Diuron, M (3,4-dichlorophenyl isocyanate)	—	GCDP	—	—						—	p	0.13					187 (100)	124 (74)	159 (30)
Diuron, M (3,4-dichloroaniline), AC	—	—	—	—						1990	c	—					161 (100)	203 (28)	63 (26)
DNOC (dinitro-orthocresol)	I, H	Misc.	Ib	6	38					1660	c	—			197→137 (−24)	197→109 (−28)	198 (100)	121 (60)	53 (60)
DNOC, methyl ether	I, H	Misc.	—	—						—	p	0.74					182 (100)	165 (74)	212 (48)
Dodemorph	F	Misc.	U	—						2020	c/p	0.86			282→116 (29)	282→98 (39)	154 (100)	55 (30)	281 (10)
Drazoxolon[j]	F	Misc.	—	—		—				—	—	237[k]					—	—	
Endosulfan	I	CH	II	40	77	77	95			2085[m]	p	1.1					195 (100)	237 (84)	339 (17)
Endosulfan sulfate	I	CH	II												421→97 (−38)	421→80 (−130)	272 (100)	227 (76)	
Endothal	H	Misc.	II[l]	—						1370[m]	p	—					68 (100)	100 (29)	140 (9)
Endrin[j]	I	CH	Ib	71	90			55	35	2183	c/p	1.19	0.2	11.24	—	—	81 (100)	263 (53)	345 (6)
Esfenvalerate	I	PY	II	54	68					—	—	—			437→167 (23)	437→125 (51)	125 (100)	167 (86)	419 (54)
Ethiofencarb	I	CB	Ib	21	25					1820	c	—			226→107 (21)	226→164 (13)	107 (100)	168 (62)	225 (4)
Ethiofencarb, M (descarbamoyl) (2-ethylthiomethylphenol)	—	GCDP	—	—						1390	c/p	0.29					107 (100)	168 (54)	77 (44)
Ethion	A, I	OP	II	—						2230[m]	c/p	—			385→199 (17)	385→171 (23)	97 (100)	231 (67)	384 (5)
Ethirimol[j]	F	Misc.	IV[l]	—						2080	c	1.06			210→140 (31)	210→98 (37)	166 (100)	96 (37)	209 (13)
Ethirimol, methyl ether	F	Misc.	IV[l]	—						—	p	0.56					180 (100)	223 (23)	85 (14)
Ethofumesate	H	Misc.	U	25	50					1985[m]	c/p	1.02			304→121 (27)	304→161 (31)	207 (100)	161 (87)	286 (23)
Ethoprophos	I, N	OP	Ia	33	28					1700[m]	c	—			243→131 (29)	243→97 (41)	97 (100)	158 (83)	242 (12)
Ethoxyquin	F	Misc.	III[l]	—						1720[m]	c	—			218→160 (43)	218→174 (37)	202 (100)	174 (56)	217 (11)
Etridiazole	F	Misc.	III	—						1480	c/p	—			—	—	211 (100)	183 (83)	246 (11)
Etridiazole (deschloro)	—	GCDP	—	—						1320	c/p	—					149 (100)	184 (79)	212 (25)
Etrimfos[j]	I	OP	—	3, 52	0, 67					1815	c/p	0.87			293→125 (33)	293→265 (21)	56 (100)	125 (61)	292 (40)
Fenamiphos	N	OP	Ib	—	—					2020	c	—			304→217 (31)	304→202 (45)	303 (100)	154 (43)	217 (27)
Fenarimol	F	Misc.	U	16	12					2590	c/p	1.58			331→268 (31)	331→81 (47)	139 (100)	107 (80)	330 (38)
Fenchlorphos[j]	I	OP	—	61	67	85	78			1900[m]	c/p	—			—	—	125 (100)	285 (94)	320 (12)

table continued

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Fenfuram	F	Misc.	U	—	—					1900	c	—			202→109 (27)	202→120 (21)	109 (100)	201 (26)	65 (7)
Fenitrothion	I	OP	II	32	76	82	65	50	17	1905	c/p	1.01	0.22	2.86	278→125 (29)	278→109 (25)	125 (100)	109 (90)	277 (39)
Fenoprop[j]	H	CP	—	1	1					1760[m]	c	—					196 (100)	97 (33)	268 (17)
Fenoprop, isoctyl[j]	H	CP	—	—	—					—	p	1.27					57 (100)	196 (63)	198 (59)
Fenoprop, ME	—	—	—	—	—					1720	c/p	0.72					196 (100)	59 (92)	281 (31)
Fenoprop, M (2,4,5-trichlorophenol)	—	—	—	—	—					1440[m]	c/p	—					196 (100)	198 (93)	97 (64)
Fenoxaprop, ethyl	H	CP	U	—	—					2615	c	—			362→288 (23)	362→121 (37)	288 (100)	361 (77)	76 (58)
Fenoxaprop, ethyl, M (phenol)	—	GCDP	—	—	—					1630[m]	c	—					110 (100)	137 (64)	210 (30)
Fenpropathrin	A, I	PY	II	62	69	95	74			2450	c	—			350→125 (19)	350→97 (43)	97 (100)	265 (15)	303 (9)
Fenthion	I	OP	II	41	81	90	68			1938[m]	c	—			279→169 (23)	279→247 (17)	278 (100)	125 (77)	109 (63)
Fentin, acetate	F	OM	II	—	—					2718	c	—			351→197 (39)	351→120 (41)	409[k]	—	—
Fenuron[j], ME	H	SU	—	11	7					1405	p	0.73					72 (100)	106 (28)	178 (13)
Fenuron (aniline)	—	GCDP	—	—	—					1158	p	0.06					93 (100)	66 (52)	94 (6)
Fenuron (phenyl isocyanate)	—	GCDP	—	—	—					—	p	0.02					119 (100)	91 (62)	64 (34)
Fenvalerate	I	PY	II	42	92		75			—	—	—			437→167 (23)	437→125 (57)	125 (100)	167 (79)	419 (49)
Fenvalerate isomer-1	A, I	PY	II	41	92					2890	c	—					125 (100)	167 (72)	419 (5)
Fenvalerate isomer-2	A, I	PY	II	41	92					3839	c	—					125 (100)	167 (73)	419 (5)
Flamprop, isopropyl	H	Misc.	U	29	35			9	10	2235	c/p	1.26	4.29	5.71	364→105 (23)	364→77 (71)	105 (100)	276 (4)	363 (1)
Flamprop, methyl	H	Misc.	II	22	29					2165	c/p	1.23			336→105 (21)	336→77 (67)	105 (100)	335 (1)	276 (1)
Fluazifop, butyl	H	Misc.	III	46	66					2203	c	—			384→282 (27)	384→328 (23)	91 (100)	146 (97)	383 (11)
Fluchloralin[j]	H	Misc.	III	—	—					1800	c	—			—	—	63 (100)	326 (30)	355 (1)
Fluotrimazole[j]	F	Misc.	—	—	—					—	p	1.38					165 (100)	311 (51)	233 (31)
Fluquinconazole	F	Misc.	—	—	—					—	—	—					376	—	—
Flurenol, ME	H	Misc.	—	—	—					1950[m]	c	—			244→181 (29)	244→209 (15)	181 (100)	152 (31)	240 (7)
Flurenol	—	GCDP	—	—	—					1790[m]	c	—					180 (100)	152 (60)	76 (31)
Fluroxypyr, 2ME	H	Misc.	U	—	—					1890	c	—			255→209 (21)	255→181 (31)	209 (100)	282 (68)	181 (52)
Fluroxypyr, ME	—	—	—	—	—					1830	c	—					209 (100)	181 (51)	268 (40)
Folept	F	Misc.	U	—	—					2015	c/p	—			315→130 (39)	315→163 (19)	76 (100)	260 (91)	295 (19)
Fonofos[j]	I	OP	Ia	59	89					1750[m]	c	—			247→109 (25)	247→137 (15)	109 (100)	137 (48)	246 (34)
Formetanate	A, I	CB	Ib	1	1					2105	c	—			222→165 (19)	222→120 (35)	221 (100)	163 (84)	149 (79)
Formetanate	—	GCDP	—	—	—					1660	c	—					109 (100)	80 (28)	166 (9)
Formothion[j]	A, I	OP	II	—	—					1820	c/p	0.97					93 (100)	125 (89)	257 (9)
Formothion, -CO_2	—	GCDP	—	—	—					1705	c	—					87 (100)	93 (62)	229 (7)
Fuberidazole	F	Misc.	II	9	8					1940	c	—			185→157 (31)	185→65 (59)	184 (100)	155 (24)	129 (13)
Furalaxyl	F	Misc.	III	—	—					1960	c	—					95 (100)	242 (30)	301 (11)
Glyphosate	H	Misc.	U	—	—			0	0	—	—	—	99.99	0	168→150		83 (100)	69 (89)	57 (46)
Heptachlor	I	CH	II	84	97			69	51	1880[m]	c/p	0.85	0	20.83	—	—	100 (100)	272 (15)	370 (1)
Heptenophos	I	OP	Ib	20	18					1555[m]	c	—			251→127 (19)	251→109 (37)	124 (100)	89 (91)	250 (2)
Hexachlorobenzene	F	CH	Ia	—	—					1690[m]	c	—			—	—	284 (100)	282 (53)	142 (15)
Hexaconazole	F	Misc.	U	—	—					—	—	—			314→70 (39)	314→159 (37)	231[o]	216[o]	214[o]
Hexazinone	H	Misc.	III	4	2					2305	c/p	1.46			253→171 (21)	253→71 (47)	171 (100)	83 (57)	252 (4)
Hymexazol	F	Misc.	U	—	—					1300	p	—					99 (100)	71 (18)	54 (14)
Imidacloprid	I	Misc.	II	—	—					—	—	—			256→209 (21)	256→175 (25)	255[k]	—	—
Iodofenphos[j]	I	OP	III	—	—			62	31	2150	c/p	1.18	0	11.45			377 (100)	125 (37)	412 (2)
Ioxynil, ME	H	Misc.	II	4	19					1885	c	—					385 (100)	370 (43)	88 (26)
Ioxynil, isoctyl[j]	H	Misc.	—	—	—					—	p	1.54					127 (100)	57 (96)	371 (16)
Ioxynil, methyl ether[j]	H	Misc.	—	—	—					—	p	0.75					385 (100)	243 (56)	370 (41)
Isofenphos	I	OP	Ib	41	67					2020	c	—			346→217 (33)	346→245 (17)	58 (100)	255 (18)	345 (2)
Isofenphos, M (HOOC-), ME	—	GCDP	—	—	—					1980	c	—					58 (100)	227 (57)	317 (2)
Isoprocarb	I	CB	II	—	—					1481	c	—			211→95 (25)	211→137 (17)	137[o]	136[o]	121[o]
Isoproturon, ME	H	SU	III	—	—					1685	c/p	0.99			207→165 (19)	207→72 (33)	72 (100)	148 (8)	220 (8)
Isoxaben	H	Misc.	U	—	—					2910	c	—					165 (100)	150 (8)	332 (1)
Isoxathion	I	OP	Ib	—	—					2133	c	—			314→105 (21)	314→170 (19)	313[o]	177[o]	105[o]
Jasmolin I (pyrethrins)	I	PY	II	—	—					—[m]	p	1.23					132 (100)	164 (23)	107 (20)
Jasmolin II (pyrethrins)	I	PY	II	—	—					—[m]	p	1.54					107 (100)	121 (58)	163 (48)
Lambda-cyhalothrin	I	PY	—	40	—	95	74			—	—	—					450[k]	—	—
Lenacil	H	UR	U	15	8					2285	c	1.52			235→153 (21)	235→136 (43)	153 (100)	53 (24)	110 (13)
Lenacil, 2ME	—	—	—	—	—					2280	c	1.41					181 (100)	67 (21)	262 (3)

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Lenacil, ME	—	—	—	—	—					2260	c	—					167 (100)	67 (22)	248 (2)
Lindane (gamma-HCH)	I	CH	II	51	92					1745	p	0.76			—	—	181 (100)	109 (48)	288 (2)
Linuron	H	SU	U	22	31					1927	c	0.97			249→160 (23)	249→182 (21)	248[o]	163[o]	161[o]
Linuron, M (3,4-dichloroaniline)	—	GCDP	—	—	—					1323	p	0.36					161 (100)	99 (20)	126 (14)
Linuron, M (3,4-dichlorophenylisocyanate)	—	GCDP	—	—	—					—	p	0.13					187 (100)	124 (74)	159 (30)
Linuron, ME	—	—	—	—	—					1785	c	—					174 (100)	202 (87)	262 (11)
Malathion	I	OP	III	31	53	82	33	24	14	1925[m]	c/p	1.03	0	0	331→127 (17)	331→99 (29)	127 (100)	173 (86)	330 (2)
Malathion, M (malaoxon)	I	OP	—	—	—					1890[m]	c	—					127 (100)	268 (16)	314 (1)
MCPA (4-chloro-2-methylphenoxy) acetic acid)	H	CP	III	0	0			4	2	1580[m]	c/p	0.54	0.78	0	199→141	(−18)	200 (100)	141 (100)	77 (58)
MCPA, ME	—	—	—	—	—					1525[m]	c	—					214 (100)	141 (85)	125 (49)
MCPB 4-(4-chloro-2-methylphenoxy butyric acid)	H	CP	III	8	6			6	2	1845[m]	c/p	1.24, 1.28	0.47	0	227→141 (−10)	227→105 (−36)	142 (100)	107 (35)	228 (13)
MCPB, ME	—	—	—	—	—					1760[m]	c/p	0.79					101 (100)	59 (76)	242 (8)
Mecoprop	H	CP	III	5	2			3	4	1540[m]	c/p		7.67	0	213→141	(−14)	142 (100)	107 (36)	
Mecoprop, ME	H	CP	—	—	—					1500[m]	c/p	0.45					228 (100)	169 (81)	142 (64)
Mephosfolan[j]	I	OP	Ia	—	—					—	p	1.24					269[k]	41	
Metalaxyl	F	Misc.	III	—	—					1890	c	—			280→220 (19)	280→160 (31)	206 (100)	160 (68)	279 (17)
Metamitron	H	TR	III	5	4					2185	c/p	1.36			203→175 (29)	203→104 (29)	104 (100)	202 (85)	174 (40)
Metazachlor	H	Misc.	U	—	—					2260	c	—			278→134 (29)	278→210 (15)	209 (100)	133 (93)	277 (13)
Methabenzthiazuron	H	SU	U												222→165 (23)	222→150 (43)	164[o]	136[o]	135[o]
Methabenzthiazuron, ME	H	SU	U	18	22					1985	c	—					72 (100)	235 (8)	109 (4)
Methabenzthiazuron (2-methylaminobenzthiazole)	—	GCDP	—	—	—					—	p	0.72					164 (100)	136 (84)	135 (81)
Methamidophos	A, I	OP	Ib	1	0					1190	c/p	—			142→94 (19)	142→125 (19)	94 (100)	141 (30)	64 (25)
Methidathion	A, I	OP	Ib	29	56			25	13	2120	c	1.19	0.81	1.84	303→145 (15)	303→85 (27)	145 (100)	85 (67)	302 (3)
Methiocarb	A, I	CB	Ib	26	28	76	19	14	5	—	—	—	1.93	1.63	243→169 (17)	243→121 (125)	168 (100)	153 (84)	109 (37)
Methiocarb (4-methylthio-3,5-xylenol)	—	GCDP	—	—	—					—	p	0.53					168 (100)	153 (70)	107 (14)
Methomyl	I	CB	Ib	6	6		9	4	1	1525	c	—	12.64	0.34	163→88 (13)	163→106 (13)	58 (100)	105 (64)	162 (1)
Methomyl (*N*-hydroxythioacetimidate)	—	GCDP	—	—	—					—	p	0.05					45 (100)	47 (91)	105 (71)
Methoprotryne[j]	H	TR	—	19	11					2098	p	—					256 (100)	212 (34)	271 (17)
Methoxychlor (*op′*)	I	CH	U	—	—					2417[m]	p	1.4			—	—	121 (100)	227 (94)	228 (15)
Methoxychlor (*pp′*)	I	CH	U	—	—					—[m]	p	1.46					227 (100)	228 (15)	212 (4)
Methoxychlor	I	CH	U	43	84		65			2445[m]	c	—					227 (100)	212 (5)	344 (2)
Methoxychlor, -HCl	—	GCDP	—	—	—					2340[m]	c	—					308 (100)	238 (80)	223 (28)
Methyl bromide	I	Misc.	FM	—	—					—	—	—					95[k]	—	—
Metobromuron[j]	H	SU	III	21	27					2040	c/p	0.79			259→170 (25)	259→148 (21)	61 (100)	172 (9)	259 (2)
Metobromuron (4-bromoaniline)	—	GCDP	—	—	—					—	p	0.2					171 (100)	65 (99)	173 (93)
Metobromuron (4-bromophenyl isocyanate)	—	GCDP	—	—	—					—	p	0.08					199 (100)	197 (97)	90 (72)
Metobromuron, ME	—	—	—	—	—					1735	c	—					212 (100)	184 (70)	272 (11)
Metobromuron, M (HOOC-), ME	—	GCDP	—	—	—					1800	c	—					229 (100)	91 (93)	63 (71)
Metolachlor	H	Misc.	III	—	—					—	—	—			284→252 (19)	284→176 (35)	240[o]	238[o]	162[o]
Metoxuron	H	SU	U	7	4					—	p	0.49			229→72 (35)	229→156 (31)	72 (100)	183 (23)	228 (22)
Metoxuron (3-chloro-4-methoxyaniline)	—	GCDP	—	—	—					—	p	0.49					142 (100)	157 (35)	144 (32)
Metoxuron (3-chloro-4-methoxyphenylisocyanate)	—	GCDP	—	—	—					—	p	0.35					168 (100)	183 (72)	140 (67)
Metoxuron, ME	—	—	—	—	—					1855	c	—					72 (100)	242 (22)	85 (12)
Metoxuron (HOOC-), ME	—	GCDP	—	—	—					1515	c	—					215 (100)	59 (83)	200 (52)
Metribuzin	H	TR	II	24	31					1870	c/p	0.75			215→187 (25)	215→84 (29)	198 (100)	57 (21)	214 (4)
Mevinphos	I	OP	Ia	12	10			3	2	1450[m]	p	0.5	1.37	0.45	225→127 (21)	225→193 (13)	127 (100)	192 (25)	224 (1)

table continued

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Molinate	H	CA	II												188→183 (25)	188→126 (19)	187[o]	127[o]	126[o]
Monocrotophos	I	OP	Ib	1	1					1665	c	0.9			224→127 (21)	224→98 (17)	127 (100)	67 (49)	223 (3)
Monocrotophos, TFA	—	—	—	—	—					1540	c	—					127 (100)	193 (27)	319 (1)
Monolinuron	H	SU	U	23	30					1910	c/p	0.78			215→126 (25)	215→148 (19)	61 (100)	99 (10)	214 (9)
Monolinuron (4-chloroaniline)	—	GCDP	—	—	—					1100	p	0.19					127 (100)	65 (48)	129 (27)
Monolinuron (4-chlorophenyl isocyanate)	—	GCDP	—	—	—					—	p	0.08					90 (100)	153 (89)	125 (82)
Monolinuron, ME	—	—	—	—	—					1675	c	—					168 (100)	140 (69)	228 (12)
Monolinuron, M (HOOC-), ME	—	GCDP	—	—	—					1690	c	—					185 (100)	153 (90)	140 (63)
Monuron[j]	H	Misc.	—	13	7					1100	c	—			199→72 (19)	199→126 (25)	—	—	—
Monuron, ME	—	—	—	—	—					1610	c	—					72 (100)	212 (6)	140 (4)
Naled	I	OP	II	—	—					1625[m]	c[l]	—			398→127 (25)	398→109 (53)	109 (100)	145 (83)	301 (17)
2-(1-Naphthyl)acetic acid, ME	H	Misc.	U	—	—					—	p	0.76					141 (100)	200 (51)	115 (24)
2-Naphthyloxyacetic acid, ME	H	Misc.	III	—	—					—	p	0.91					216 (100)	127 (27)	157 (20)
Napropamide	H	Misc.	U	—	—					2135	c/p	1.18			272→129 (21)	272→171 (23)	72 (100)	100 (58)	271 (12)
Naptalam, ME	H	Misc.	U	21	52					—	p	1.64					273 (100)	76 (53)	228 (35)
Naptalam, -H_2O	—	—	—	—	—					2545	c	—					273 (100)	76 (49)	228 (42)
Neburon, ME	H	SU	U	26	24					2070	c	—			275→88 (23)	275→114 (21)	57 (100)	114 (63)	288 (10)
Niclosamide, ME	Mol	Misc.	U	—	—					2920	c	—					169 (100)	126 (15)	340 (8)
Nicotine	I	Misc.	Ib	4	1					1348	c/p	0.31			163→132 (21)	163→84 (25)	84 (100)	133 (26)	162 (16)
Nicotine, M (cotinine)	—	—	—	—	—					1715	c	—					98 (100)	176 (35)	118 (12)
Nitrofen[j]	H	Misc.	—	—	—					2190	c/p	1.24			—	—	283 (100)	202 (51)	139 (25)
Nitrothal, isopropyl	F	Misc.	U	—	—					2005	c	—			—	—	236 (100)	194 (79)	295 (8)
Nuarimol	F	Misc.	III	—	—					2390	c/p	1.37			315→252 (31)	315→81 (43)	107 (100)	235 (51)	314 (34)
Omethoate	A, I	OP	Ib	0	0					1595	c/p	0.73			214→125 (29)	214→109 (35)	110 (100)	156 (90)	213 (7)
Oryzalin	H	Misc.	U	—	—					2680	c	—					317 (100)	275 (50)	346 (8)
Oryzalin	—	GCDP	—	—	—					2025	c	—					238 (100)	196 (64)	367 (12)
Oxadiazon	H	Misc.	U	—	—					2140	c/p	1.17			362→220 (31)	362→177 (45)	175 (100)	258 (58)	344 (32)
Oxadixyl	F	Misc.	III	—	—					2280	c	—			279→219 (17)	279→133 (29)	105 (100)	163 (75)	132 (54)
Oxamyl	I	CB	Ia												237→72 (21)	237→90 (13)	—	—	—
Oxamyl, -C_2H_3NO	I	CB	—	—	—					1630	c	—					72 (100)	162 (13)	145 (13)
Oxydemeton-*S*-methyl	I	OP	Ib	18	0					1860[m]	c/p	1.1			247→169 (19)	247→109 (35)	109 (100)	169 (60)	218 (1)
Paraquat dichloride	H	QA	II	—	—			0	c	—	99.99	0	257[k]	—	186→170	186→144	257[k]	—	—
Paraquat (reduction product I, monoene)	H	QA	—	—	—					—	p	0.36					96 (100)	150 (23)	194 (14)
Paraquat (reduction product II, diene)	H	QA	—	—	—					—	p	0.58					96 (100)	192 (35)	148 (35)
Parathion-ethyl	A, I	OP	Ia	41	84					1950	c/p	1.04					109 (100)	97 (96)	291 (49)
Parathion, ethyl, M (amino-)	—	—	—	—	—					1892	c/p	—					125 (100)	109 (100)	261 (84)
Parathion, ethyl, M (*p*-nitrophenol)	—	—	—	14	20					1525	c/p	—					139 (100)	65 (57)	109 (22)
Parathion, ethyl, M (*p*-nitrophenol), AC	—	—	—	—	—					1500	c	—					139 (100)	109 (78)	181 (67)
Parathion, ethyl, M (*p*-nitrophenol). ME	—	—	—	—	—					1455	p	—					153 (100)	77 (72)	92 (55)
Parathion, ethyl, M (paraoxon)	I	OP	—	—	—					1895	c/p	—					109 (100)	149 (53)	275 (52)
Parathion, methyl	I	OP	Ia	30	73					1815	p	0.96			264→125 (25)	264→232 (23)	109 (100)	263 (95)	125 (88)
Parathion, methyl oxygen analogue	—	—	—	—	—					1747	c	—					—	—	—
Parathion, methyl, M (*p*-nitrophenol)	—	—	—	14	20					1525	c/p	—					139 (100)	65 (57)	109 (22)
Parathion, methyl, M (*p*-nitrophenol), AC	—	—	—	—	—					1500	c	—					139 (100)	109 (75)	181 (67)
Parathion, methyl, M (*p*-nitrophenol), ME	—	—	—	—	—					1455	p	—					153 (100)	77 (72)	92 (55)
Pencycuron, ME	F	Misc.	U	—	—					2575	p	—					125 (100)	77 (22)	342 (4)
Pendimethalin	H	Misc.	III	60	92					2020	c/p	1.06			282→212 (15)	282→194 (23)	252 (100)	281 (37)	162 (22)
Pentanochlor	H	Misc.	U	—	—					1935	c/p	0.94					141 (100)	71 (60)	239 (15)

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Permethrin	I	PY	II	61	94	95	68	63	40	—	p	1.62	0	51.96	408→183 (25)	408→153 (63)	183 (100)	77 (13)	390 (4)
Permethrin isomer-1	I	PY	—	—	—					2640	c	—					183 (100)	77 (8)	390 (4)
Permethrin isomer-2	I	PY	—	—	—					2670	c	—					183 (100)	127 (6)	390 (4)
Phenmedipham	H	CB	U	11	17					512	c/p	0.52			301→136 (25)	301→168 (15)	133 (100)	104 (54)	165 (31)
Phenmedipham, M (3-methoxycarbonylaminophenol)	—	GCDP	—	—	—					—	p	0.91					167 (100)	135 (93)	122 (49)
Phenmedipham, M (*m*-tolyl isocyanate)	—	GCDP	—	—	—					—	p	0.06					133 (100)	104 (46)	132 (45)
Phenmedipham, M (HOOC-), ME	—	GCDP	—	—	—					1370	c	—					165 (100)	133 (75)	77 (61)
Phenmedipham, M, ME (phenol)	—	GCDP	—	—	—					1560	c	—					195 (100)	136 (47)	108 (34)
Phenmedipham, 2ME (tolylcarbamic acid)	—	GCDP	—	—	—					1340	c	—					179 (100)	120 (52)	91 (51)
Phenothrin	I	PY	U	—	—					2835[m]	c	—					123 (100)	183 (75)	350 (7)
Phenthoate	I	OP	II	—	—					1993	c	—			321→163 (17)	321→79 (51)	320[k]	—	—
Phorate	A, I, N	OP	Ia	—	—					1675[m]	c/p	0.71			278→75 (23)	278→171 (21)	75 (100)	121 (38)	260 (26)
Phosalone	I	OP	II	31	67	83	50	39	17	2527	c/p	1.52	0.35	5.94	368→182 (21)	368→111 (51)	182 (100)	367 (59)	121 (54)
Phosmet	A, I	OP	II	—	—					2378	c	—			318→160 (19)	318→133 (49)	160 (100)	77 (30)	317 (21)
Phosphamidon	A, I	OP	Ia												300→127 (27)	300→174 (19)	264[o]	158[o]	138[o]
Phosphamidon isomer-1	A, I	OP	Ia	7	2			4	2	1820	c	—					127 (100)	264 (63)	72 (42)
Phosphamidon isomer-2	A, I	OP	Ia	—	—					1900	c	—					127 (100)	72 (76)	264 (67)
Phosphine	I, R	Misc.	FM	—	—					<1000[m]	p	—					34 (100)	31 (33)	—
Phoxim	I	OP	II	42	86					1987	c	—			299→129 (17)	299→77 (41)	109 (100)	81 (57)	298 (16)
Phoxim, M	—	GCDP	—	—	—					1400[m]	p	—					143 (100)	171 (96)	196 (9)
Phoxim	—	GCDP	—	—	—					1670	c	—					194 (100)	278 (24)	306 (5)
Picloram, ME	H	Misc.	U	0	—					1892	c/p	1.08			241→197 (−14)	241→123 (−30)	196 (100)	254 (23)	168 (17)
Picloram, -CO_2	—	GCDP	—	—	—					1440	c	—					196 (100)	161 (33)	134 (22)
Pindone	R	Misc.	—	17	45					1817[m]	c	—					173 (100)	89 (49)	230 (29)
Piperonyl butoxide	I	Misc.	U	—	—					—	—	—					338[k]	—	—
Pirimicarb	I	CB	II	26	17	54	5	6	5	1823	c/p	0.92	99.99	1.13	239→72 (31)	239→182 (21)	166 (100)	72 (49)	238 (36)
Pirimicarb (desmethyl)	—	—	—												225→72	225→168	224[o]	207[o]	179[o]
Pirimiphos, ethyl[j]	I	OP	Ib	—	—					—	p	1.05			334→198 (29)	334→182 (27)	168 (100)	318 (94)	333 (69)
Pirimiphos, methyl	I	OP	III	50	75					1943	c/p	0.76			306→164 (29)	306→108 (39)	290 (100)	226 (85)	305 (78)
Prochloraz	F	Misc.	III	—	—					2405	c	—			376→308 (17)	376→70 (23)	143 (100)	235 (50)	310 (3)
Procymidone	F	Misc.	U	—	—					2065	c	—			301→256 (29)	—	96 (100)	283 (53)	67 (43)
Procymidone (deschloro)	—	GCDP	—	—	—					1935	c	—			—	—	96 (100)	249 (54)	67 (41)
Profenofos	I	OP	II	—	—					2155[m]	c	—			373→303 (25)	373→97 (43)	372 (27)	139 (83)	97 (100)
Promecarb[j]	I	CB	—	—	—					1665	c	—			208→109 (21)	208→151 (13)	135 (100)	150 (69)	207 (1)
Promecarb, M (descarbamoyl)	—	GCDP	—	—	—					1290[m]	c	—					135 (100)	150 (67)	91 (42)
Prometryn	H	TR	U	32	31					1930	c	—			242→158 (31)	242→200 (25)	241 (100)	184 (97)	58 (70)
Propachlor	H	Misc.	III	—	—					1604	c/p	0.58			212→170 (21)	212→94 (37)	120 (100)	77 (44)	211 (6)
Propamocarb	F	CB	U	—	—					1875	c	—			189→102 (23)	189→144 (17)	58 (100)	129 (3)	188 (2)
Propamocarb, TFA	—	—	—	—	—					1290	c	—					58 (100)	69 (9)	284 (1)
Propanil	H	Misc.	III	—	—					—	p	0.94					161 (100)	163 (70)	219 (9)
Propazine	H	TR	U	—	—					—	—	—			230→146 (29)	230→188 (23)	214 (100)	172 (73)	229 (61)
Propham	H	CB	U	39	57	86	45			1444	c/p	0.35			180→138 (13)	180→120 (25)	93 (100)	179 (50)	137 (46)
Propiconazole	F	Misc.	II	18	11					2335	c	—			342→159 (37)	342→69 (33)	69 (100)	259 (60)	340 (1)
Propoxur	I	CB	II	20	21	66	13			1572	c/p	—			210→111 (19)	210→168 (11)	110 (100)	152 (17)	209 (1)
Propoxur (4-isopropylphenyl-isocyanate)	—	GCDP	—	—	—					—	p	0.13					146 (100)	161 (30)	161 (30)
Propoxur, M (*o*-isopropoxyphenol)	—	GCDP	—	—	—					1070[m]	c/p	0.09					110 (100)	152 (13)	81 (7)
Propoxur, HYAC	—	—	—	—	—					1390[m]	c	—					110 (100)	52 (10)	194 (2)
Propoxur, HYME	—	—	—	—	—					1380[m]	c	—					110 (100)	64 (43)	166 (5)
Propoxur, TFA	—	—	—	—	—					1530	c	—					69 (100)	206 (62)	305 (8)
Propoxur, M (*O*-desalkyl), HY	—	—	—	—	—					<1000[m]	c	—					110 (100)	64 (42)	81 (15)

table continued

Table 16.1 continued

Compound[a]	Use[b]	Class[c]	TOX[d]	TX[e]	TY[e]	TZ[e]	TAA[e]	TAB[e]	TAC[e]	GA[f]	Col.[g]	GK[h]	HAO[i]	HAP[i]	LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)		Typical ions and intensities[f]		
Propoxur, M (HO-), HY 2AC	—	—	—	—	—					1680[m]	c	—					126 (100)	168 (42)	252 (8)
Propyzamide	H	Misc.	U	33	52					1776	c	—			256→190 (19)	256→173 (31)	173 (100)	145 (34)	255 (26)
Propyzamide (deschloro)	—	GCDP	—	—	—					1645	c	—					139 (100)	111 (29)	221 (24)
Prothiofos	I	OP	II	—	—					2190[m]	c	—			345→24 (27)	345→133 (69)	113 (100)	63 (92)	344 (1)
Pyrazophos	F	OP	II	32	47					2590	c	—			374→222 (29)	374→194 (43)	221 (100)	232 (34)	373 (10)
Pyrethrin I (pyrethrins)	I	PY	II	—	—					—[m]	p	1.27					123 (100)	91 (58)	105 (45)
Pyrethrin II (pyrethrins)	I	PY	II	—	—					—[m]	p	1.59					91 (100)	133 (70)	107 (47)
Pyridaphenthion	I	OP	III	—	—					2364	c	—			341→189 (29)	341→205 (27)	340[o]	204[o]	199[o]
Pyridate	H	Misc.	III	47	64					2985	c	—			379→207 (21)	379→351 (17)	205 (100)	350 (6)	378 (2)
Pyrifenox	F	Misc.	III												295→93 (31)	295→263 (25)	264[o]	262[o]	227[o]
Quinalphos	I	OP	II	—	—					2070	c	—			299→147 (29)	299→163 (29)	146 (100)	118 (41)	298 (18)
Quinomethionate[j]	A	Misc.	III	70	82					2073	c/p	1.14					116 (100)	206 (90)	234 (70)
Quintozene	F	CH	U	86	95					1790	c	0.72			—	—	237 (100)	293 (47)	212 (47)
Quizalofop-P	H	Misc.	II														372	301	299
Resmethrin	I	PY	III	—	—					2300[m]	c	1.37			356→171 (21)	356→128 (57)	123 (100)	171 (50)	338 (7)
Rotenone	I	Misc.	II	—	—					3195	c	—			395→213 (29)	395→192 (31)	394 (100)	192 (43)	379 (20)
Sethoxydim	H	Misc.	III	50	48					2394	c	—			328→178 (25)	328→282 (17)	178 (100)	149 (62)	281 (8)
Simazine	H	TR	U	22	18					1690	c/p	0.79			202→132 (27)	202→124 (25)	201 (100)	68 (83)	186 (67)
Simazine, M (desethyl)	—	—	—	—	—					1730	c	—					173 (100)	158 (97)	68 (78)
Sulfotep	A, I	OP	Ia	50	84					1657[m]	c	—			323→115 (39)	323→97 (45)	322 (100)	97 (48)	202 (30)
Sulprofos	I	OP	II	—	—					2260[m]	c	—			323→247 (17)	323→219 (21)	156 (100)	139 (93)	322 (90)
2,4,5-T[j] (trichlorophenoxyacetic acid)	H	CP	III	—	—			4	2	1850[m]	c	—	99.99	0			196 (100)	254 (60)	167 (30)
2,4,5-T, M (trichlorophenol)	—	—	—	—	—					1440[m]	c/p	—					196 (100)	198 (93)	97 (64)
2,4,5-T-isoctyl[j]	H	CP	III	—	—					2320[m]	c/p	1.24, 1.31					71 (100)	254 (78)	366 (59)
2,4,5-T, methyl[j]	H	CP	III	—	—					1740[m]	c/p	0.8					233 (100)	268 (46)	209 (32)
Tebuthiuron, ME	H	SU	III	—	—					1900	c	—					72 (100)	126 (23)	242 (5)
Tebuthiuron, C_2H_3NO, ME	—	—	—	—	—					1500	c	—					170 (100)	185 (29)	156 (19)
Tecnazene	F	Misc.	U	—	—					1605	c/p	0.55			—	—	203 (100)	108 (64)	259 (41)
Temephos	I	OP	U	—	—					3205[m]	c	—					466 (100)	125 (22)	203 (20)
Terbacil	H	UR	U	—	—					1825	c/p	1.02			217→161 (17)	217→144 (35)	161 (100)	160 (73)	216 (4)
Terbacil, ME	H	UR	—	—	—					—	p	0.95					56 (100)	174 (79)	175 (31)
Terbufos	I, N	OP	Ia	63	90					1778[m]	c	—			289→103 (15)	289→57 (35)	57 (100)	231 (38)	288 (2)
Terbumeton	H	TR	II	—	—					1790	c	—			226→170 (23)	226→114 (33)	210 (100)	169 (78)	225 (39)
Terbuthylazine	H	TR	U	—	—					1805	c	—			230→174 (23)	230→104 (43)	214 (100)	173 (40)	229 (33)
Terbutryn	H	TR	U	32	32			7	12	1940	c/p	0.99	12.09	1.68	242→186 (25)	242→68 (57)	226 (100)	185 (74)	241 (72)
Tetrachlorvinphos[j]	I	OP	T.5	25	29					2083	c	—			367→127 (21)	367→241 (27)	109 (40)	329 (36)	364 (1)
Tetrachlorvinphos, M	—	GCDP	—	—	—					1710[m]	c	—					207 (100)	179 (26)	256 (5)
Tetradifon	A	Misc.	U	—	—					2484[m]	c	—			—	—	159 (100)	111 (68)	354 (19)
Tetramethrin	I	PY	U	50	52	95	45			2735	c	—					164 (100)	123 (31)	331 (1)
Tetramethrin ([1*R*]-isomers)	I	PY	U												349→164 (29)	349→135 (23)	164[o]	139[o]	135[o]
Tetrasul[j]	A	Misc.	—	—	—					2310[m]	c/p	1.28					252 (100)	324 (55)	322 (44)
Thiabendazole	F	Misc.	U	—	—					2040	p	1.18			202→175 (35)	202→131 (43)	201 (100)	174 (72)	63 (12)
Thiazafluron[j], ME	H	SU	—	10	4					1560	c	—					72 (100)	126 (7)	254 (1)
Thiocyclam[j]	I	Misc.	II	26	11					1495	p	—					71 (100)	135 (31)	181 (1)
Thiofanox	I	OP	Ib	20	17					1300	c/p	0.72			219→57 (17)	219→61 (15)	57 (100)	68 (39)	115 (31)
Thiofanox, C_2H_3NO (3,3-dimethyl-1-(methylthio) butan-2-one oxime)	—	GCDP	—	—	—					1085	c/p	0.18					55 (100)	61 (69)	161 (28)
Thiometon	I	OP	Ib	—	—					1675[m]	c/p	0.72			247→89 (17)	247→61 (49)	88 (100)	60 (46)	246 (4)
Thiophanate, methyl, 4ME	I	CB	U	—	—					2600	c	—			343→151 (25)	343→192 (21)	88 (100)	351 (13)	398 (1)
Thiophanate, 4ME	—	—	—	16	8					2575	c	—					88 (100)	379 (10)	426 (1)
Thiram	F	CB	III	19	51	86	37			2256	c/p	1.19					88 (100)	76 (43)	240 (13)
Tolclofos, methyl	F	OP	U	52	89					1872[m]	c	—			301→175 (35)	301→269 (23)	265 (100)	125 (27)	300 (1)
Totylfluanid	F	Misc.	U	33	67					2050	c	—			364→238 (19)	364→137 (37)	137 (100)	181 (33)	346 (10)
Triadimefon	F	Misc.	III	22	23					1983	c	—			294→197 (21)	294→225 (19)	57 (100)	208 (31)	293 (6)

Table 16.1 continued

Compound[a]	*Use*[b]	*Class*[c]	*TOX*[d]	*TX*[e]	*TY*[e]	*TZ*[e]	*TAA*[e]	*TAB*[e]	*TAC*[e]	*GA*[f]	*Col.*[g]	*GK*[h]	*HAO*[i]	*HAP*[i]	*LC-MS (-MS) transitions (m/z→m/z) and collision energy (V)*		*Typical ions and intensities*[f]		
Triadimenol	F	Misc.	III	11	3					2058	c	—			296→70 (19)	296→227 (15)	112 (100)	238 (2)	295 (1)
Tri-allate	H	Misc.	III	82	90					1817	c/p	0.77			304→143 (35)	304→86 (23)	86 (100)	268 (41)	303 (1)
Triamiphos[j]	F, I	OP	—	—	—					2200	c	—					160 (100)	294 (48)	135 (38)
Triazophos	A, I, N	OP	Ib	21	38					2258	c	—			314→162 (25)	314→119 (47)	161 (100)	172 (62)	313 (19)
Tributylphosphate[j]	I	OP	—	—	—					1485[m]	c	—					99 (100)	155 (13)	211 (3)
Trichlorfon	I	OP	II	7	2	15	5	2	1	1438[m]	c/p	0.27	99.99	99.99	257→109 (31)	257→221 (21)	79 (100)	109 (96)	221 (3)
Trichlorfon, ME	—	—	—	—	—					1395[m]	p	—					93 (100)	205 (36)	235 (3)
Tridemorph[j]	F	Misc.	II	—	—					1875	c/p	0.73			298→130 (35)	298→116 (33)	128 (100)	70 (5)	297 (1)
Trietazine	H	TR	U	43	42					1754	c/p	0.83			230→132 (29)	230→99 (33)	200 (100)	229 (54)	186 (52)
Trifenmorph[j]	Mol	Misc.	—	58	73					2105	c	—					243 (100)	165 (62)	329 (1)
Trifluralin	H	Misc.	U	72	97					1674	c/p	0.58					306 (100)	264 85	335 7
Vamidothion	I	OP	Ib	—	—					2055	c/p	1.26			288→146 (17)	288→118 (31)	87 (100)	58 69	287 2
Vinclozolin	F	Misc.	U	42	76					1891	c/p	0.88					285 100	212 84	53 84
Warfarin	R	CA	Ib	12	11					0	c	—					265 (100)	308 (32)	121 (24)
Warfarin (phenylbutenone)	—	GCDP	—	—	—					1435[m]	p	—			161[p]	250[p]	103 (100)	145 (58)	146 (56)
Warfarin, ME	—	—	—	—	—					2540[m]	c	—					279 (100)	322 (18)	91 (15)
Warfarin, M (dihydro), -H_2O	—	—	—	—	—					2550[m]	c	—					292 (100)	263 (72)	249 (43)
Warfarin, M (HO-) isomer-1, 2ME	—	—	—	—	—					2810[m]	c	—					309 (100)	352 (21)	91 (14)
Warfarin, M (HO-) isomer-2, 2ME	—	—	—	—	—					2830[m]	c	—					309 (100)	352 (32)	121 (56)
Warfarin, M (HO-) isomer-3, 2ME	—	—	—	—	—					2870[m]	c	—					309 (100)	352 (21)	91 (21)
Warfarin, M (dihydro), ME	—	—	—	—	—					2660[m]	c	—					91 (100)	291 (62)	324 (18)

[a]AC, acetylated for GC analysis; -C_2H_3NO, desmethylcarbamoyl GCDP; -CO_2, GCDP formed by decarboxylation; ET, ethylated for GC analysis; -HCl, GCDP formed by the elimination of hydrochloric acid; -H_2O, GCDP formed by dehydration; HY, acid hydrolysed; M, metabolite; M (HO-), hydroxy metabolite; M (HOOC-), carboxy metabolite; ME. methylated for GC analysis; TFA, trifluoroacetylated for GC analysis.
[b]Classification of pesticides according to use: A, acaricide; F, fungicide; H, herbicide; I, insecticide; Mol, molluscicide; N, nematicide; R, rodenticide.
[c]According to chemical classes: CA, coumarin anticoagulant; CB, carbamate; CH, chlorinated hydrocarbon; CP, chlorinated phenoxy acid; GCDP, gas chromatography decomposition product; Misc., miscellaneous; OM, organometallic; OP, organophosphorus; PY, pyrethroid; QA, quaternary ammonium compound; SU, substituted urea; TR, triazine; UR, uracil.
[d]See Table 14.3.
[e]The R_f values were taken from Erdmann *et al*. (1990), De Zeeuw *et al*. (1992b) and Osselton and Snelling (1986) with kind permission of the authors and publishers.
[f]The retention indices and the typical ions and their intensities were taken from Pfleger *et al*. (2007) and De Zeeuw *et al*. (1992a) with kind permission of the authors and publishers.
[g]col., column; c, index is measured on capillary column; p, index is measured on packed column.
[h]Retention times relative to caffeine in system GK were taken from Fysh and Whitehouse (1986) with kind permission of the authors.
[i]HPLC data were taken from Osselton and Snelling (1986) with kind permission of the authors and publishers.
[j]Superseded entries.
[k]Relative molecular mass.
[l]Toxicity class according to Environmental Protection Agency (EPA).
[m]Compound without nitrogen.
[n]This compound could not be detected by GC.
[o]The transition ions and the typical ions were taken from Alder *et al*. (2006) and from www.bfr.bund.de/cd/5832 with kind permission of the author and publishers.
[p]Recorded ions were taken from Adamowicz and Kała (2009) with permission of the authors and publisher.

Table 16.2 Subclasses of organophosphorus compounds (structure 1 in Fig. 16.1)

Subclass	R^1	R^2	R^3	*X*	*Example*
Phosphorodithioates	*O*-alkyl	*O*-alkyl	*S*-alkyl, *S*-aryl	S	Phosalone
Phosphorothionates	*O*-alkyl	*O*-alkyl	*O*-alkyl, *O*-aryl	S	Fenitrothion
Phosphorothioates	*O*-alkyl	*O*-alkyl	*S*-alkyl, *S*-aryl	O	Omethoate
Phosphates	*O*-alkyl	*O*-alkyl	*O*-alkyl, *O*-aryl	O	Dichlorvos
Phosphonates	*O*-methyl	*O*-methyl	alkyl, aryl	O	Trichlorfon
Phosphonodithioates	alkyl	*O*-alkyl	*S*-aryl	S	Fonofos
Phosphoroamidothioates	*O* or *S*-alkyl	*O*-alkyl	amine	O or S	Methamidophos

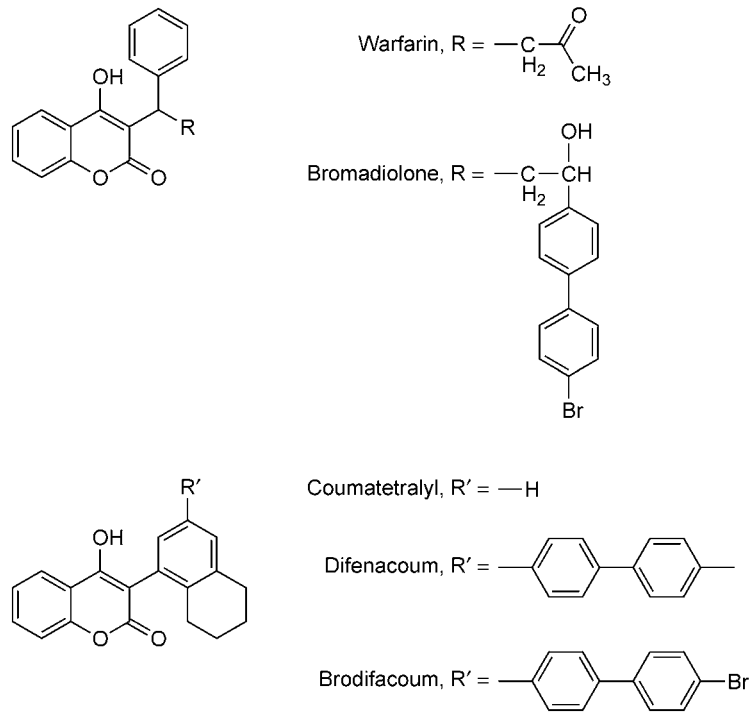

Figure 16.2 Chemical structures of the 4-hydroxycoumarin anticoagulant rodenticides. Reproduced with permission from Felice *et al*. (1991).

Table 16.3 WHO toxicity classification (Tomlin 2006)

Class	*Description*	*Oral LD_{50} for the rat (mg/kg body mass)*		*Estimated lethal dose (for a 70 kg person)*
		Solids	**Liquids**	
Ia	Extremely hazardous	≤5	≤20	≤30 drops, a taste
Ib	Highly hazardous	5–50	20–200	≤a tablespoon
II	Moderately hazardous	50–500	200–2000	≤a teacup
III	Slightly hazardous	≥501	≥2001	≥a teacup
U	Product unlikely to present acute hazard in normal use	≥2000	≥3000	

compounds contain halogens and others phosphorus, sulfur, or nitrogen. These heteroatoms may have relevance for the detection of pesticides. A number of compounds are very volatile, but several do not evaporate at all. This diversity causes serious problems in the development of a universal analytical method, which should have the widest scope possible.

The next sections describe a range of methods that can be applied to the detection of pesticides, from simple presumptive colour tests and TLC through to complex instrumental techniques. These can form part of a systematic analytical protocol that uses standardised extraction techniques and chromatographic conditions.

Preliminary screening tests

Primary references to particular methods are given, sometimes to the original methods even though many tests have been modified over the years.

Colour tests

Some colour tests can be very useful preliminary indicators of the class of compound and can confirm the constituents of a proprietary formulation. Simple quantification of compounds that belong to specific groups is also possible. To reduce false positives from artefactual sources,

a blank solution should be subjected to the same procedure as the sample. It is also essential to check the viability of the reagents by analysing a reference compound.

Ammonium molybdate test

The ammonium molybdate test is used for phosphorus and phosphides in stomach contents and non-biological materials (Flanagan *et al.* 1995). Impregnate a strip of filter paper in a saturated methanolic solution of silver nitrate and dry. Impregnate a second strip of filter paper in 10% (w/v) lead acetate solution and dry. Place 5 mL of sample in a test tube fitted with a cork with two slits cut on opposite sides. Insert the test paper into the slits, close the tube and heat in a water bath at 60°C for 20 min. If only the silver nitrate paper is blackened, phosphorus or phosphides may be present. If both papers are blackened, sulfides may be present and the result is inconclusive.

To test more specifically for phosphorus, place the silver nitrate paper on a glass microscope slide and cover with calcium hypochlorite powder. Leave in a moist chamber for 15 min to allow oxidation of phosphide to phosphate. Remove excess hypochlorite by careful washing with a small amount of water and dry the test paper by blotting with absorbent tissue. Add 50 µL of 0.05% (v/v) *o*-toluidine solution in 10% (v/v) glacial acetic acid and expose the paper to ammonia fumes from concentrated ammonium hydroxide in a fume cupboard. A blue colour confirms phosphorus.

Furfuraldehyde test

The furfuraldehyde test is used for carbamates in stomach contents and non-biological materials (Flanagan *et al.* 1995). Dissolve the dry residue after carrying out a standard liquid–liquid extraction (LLE; see the section Liquid–liquid extraction below) in 0.1 mL of methanol, apply a spot of the solution to a filter paper and allow to dry. Apply 0.1 mL of freshly prepared furfuraldehyde solution in methanol (1 : 10 v/v) to the spot, allow to dry and expose the paper to concentrated hydrochloric acid fumes for 5 min in a fume cupboard. Carbamates (pesticide and non-pesticide) give a black spot.

Phosphorus test

The phosphorus test is used for organophosphorus compounds. Extract each sample (several drops of proprietary preparation or 2 mL of other specimens) with 5 mL of a mixture of toluene–hexane (1 : 1 v/v). After separation, the organic layer is dried over sodium sulfate for 10 min and evaporated to dryness. To the dry residue add concentrated acids (1 mL nitric and 0.2 mL sulfuric acid), heat at 120°C in a heating block for 30 min, allow to concentrate and then cool. Add 1 mL of 10% (w/v) solution of ammonium molybdate in 5 mol/L nitric acid and replace in the water bath at 100°C for 10 min. A bright yellow solution or precipitate indicates the presence of phosphate ions, which may derive from an OP pesticide. The test has a sensitivity of approximately 5 µg per sample and is therefore not able to detect OP compounds in blood.

Sodium dithionite test

The sodium dithionite test is used for diquat and paraquat (Tompsett 1970). To 1 mL of urine or stomach contents add 1 mL of a freshly prepared 1% (w/v) solution of sodium dithionite in 1 mol/L sodium hydroxide solution. A blue colour indicates the presence of paraquat. A green colour is given by diquat, but paraquat may also be present. The limit of detection (LOD) for paraquat is 1.0 mg/L. Diquat may be differentially extracted from examined samples in the presence of paraquat using butan-1-ol (Minakata *et al.* 1990). Kuo *et al.* (2001) described a quantitative method for measuring paraquat and diquat in biological fluids and tissues that involved protein precipitation with trichloroacetic acid and subsequent purification of the supernatant by solid phase extraction. When both paraquat and diquat were present, their concentrations could be determined by the peak amplitudes of their respective second-derivative spectra after the addition of alkaline dithionate reagent. Interference was negligible when the diquat/paraquat concentration ratio was within the range 5.0–0.2 (Kuo *et al.* 2001).

Test for cholinesterase inhibitors in plasma or serum

To each of three tubes add 2 mL of 0.02% (w/v) dithiobisnitrobenzoic acid in 0.1 mol/L sodium dihydrogenphosphate buffer, pH 7.4 solution and 1.0 mL of 0.5% (w/v) aqueous acetylthiocholine iodide solution (Flanagan *et al.* 1995). To the first tube add 20 µL of control plasma and to the second 20 µL of sample plasma. To the third tube add 20 µL of 20% (w/v) aqueous pralidoxime chloride solution (reverses the inhibitor activity) and 20 µL of test plasma. Vortex mix the contents of all three tubes and allow to stand at room temperature for 2 min. If a cholinesterase inhibitor is present the yellow colour in the control tube will be deeper than that in the sample tube. Further confirmation is provided if the depth of colour in the pralidoxime tube is similar to that in the control tube.

Comprehensive screening procedure

This procedure consists of two initial screens by means of TLC or GC. TLC is a very useful screening and identification technique for pesticides in commercial preparations added to beverages or foodstuffs and in body fluids (stomach contents, urine) and tissues. Testing blood samples for these compounds requires the use of a more sensitive GC technique. The method of choice, regarded as the reference method for identification and confirmation of the presence of pesticide(s) in different materials, is GC-MS. More recently, GC-MS(-MS), LC-MS and LC-MS(-MS) methods have been developed for screening and determination of particular classes of pesticides in biological and non-biological matrices (Botitsi *et al.* 2007; Fernandez Moreno *et al.* 2008; Frenich *et al.* 2007; Pang *et al.* 2009; Walorczyk 2007). All these instrumental methods need special sample preparation procedures for biological materials.

Reference materials

For all the methods described it is very important to use good-quality reference materials, although in some situations, such as when using TLC screening methods, commercial preparations can be an adequate substitute. Many pesticide standards are available prepared in sealed ampoules from LGC Standards (http://lgcstandards.com/home/home_en.aspx), which is Radian's international distributor of certified analytical reference materials. Although an expiry date is provided with each reference material, dilute solutions of many pesticides will not remain stable for more than a few months after the ampoules are opened.

Sample preparation

A broad spectrum of pesticides in heterogeneous matrices (non-biological and biological samples) may be submitted for toxicological analysis. Depending on the pesticide class, biological sample preparation may involve denaturation of proteins with either organic solvents (methanol, acetonitrile) or acids (acetic, formic, trichloroacetic or perchloric), addition of solid urea, zinc salts, saturated sodium chloride solution or mixing with sodium sulfate, cleavage of conjugates, isolation from the matrix, volume reduction, clean-up steps and/or derivatisation of the pesticides and/or their metabolites. For some derivatives antioxidants, e.g. sodium disulfite, can be used (Ueyama *et al.* 2006). Each step can be a source of artefacts or decomposition products as a result of light sensitivity, atmospheric oxidation, hydrolysis and heat. The major metabolites of many pesticides (e.g. carbamates and organophosphates) are sulfate and glucuronide conjugates. Cleavage of conjugates by enzymatic or acid hydrolysis is necessary before extraction. Analytical data for pesticides after acid hydrolysis are given in Table 16.1. However, deconjugation of pesticides by acid hydrolysis drastically increases the formation of artefacts and can destroy analytes completely. Therefore, the gentle enzymatic method is recommended. The extraction of body fluids is further complicated because certain pesticides are decomposed readily by acids or alkalis. Moreover, the decomposition products of some subclasses of pesticides (e.g. substituted ureas) can react with the extraction solvent (e.g. ethanol or acetone) used for reconstitution of the dried residue after extraction. Many pesticides can undergo degradation during the chromatographic process. Accordingly, the possibility of formation of artefacts during all these processes must always be taken into account and minimised wherever possible. Derivatisation of pesticides can often improve chromatographic separation. Moreover, in

some cases, chromatography of the extract before and after derivatisation increases the identifying power of GC, and for GC-MS methods changes in the fragmentation pattern can yield additional information for characterisation.

Standard and special sample preparation procedures are described below. Pesticides can be isolated either by LLE and manual or by automated SPE. LLE is still regarded as the more universal method for screening, whereas SPE is preferred for quantification of certain pesticides in blood samples or for extraction of a particular chemical class of pesticides, such as coumarin anticoagulants (Maurer, Arlt 1998).

Other methods that should be considered include LLE followed by purification by gel permeation chromatography; this has been developed for the analysis of 660 pesticides in various tissues (Pang *et al.* 2006). Solid-phase microextraction (SPME) and headspace solid-phase microextraction (HS-SPME) have been used for the isolation of organophosphorus compounds from blood (Wollersen, Musshoff 2007) and Hernández *et al.* (2004) have described the direct injection of human urine samples for the determination of free, glucuronide and sulfate metabolites of parathion, methyl-parathion and fenitrothion by LC-MS(-MS).

Previously reported methods for extraction dialkylphosphate metabolites of organophosphorus pesticides as lyophilisation or azeotropic distillation were extremely time consuming and labour intensive.

The quantity of the sample taken for extraction depends on its type and is restricted by its availability. Samples may be proprietary formulations (solid or liquid in an amount of several drops to several millilitres or grams), beverages or foodstuffs, soil samples (20 g), river or lake water (up to 500 mL) and body fluids – stomach contents, urine (5 mL), blood (1–2 mL, but more often less than 1 mL) or tissues (5 g). Sundberg *et al.* (2006) described an extraction procedure followed by GC–electron-capture detection (ECD) for the analysis of organochlorine pesticides and polychlorinated biphenyls from 0.1 to 1 mL samples of avian serum with absolute recoveries of 74–101%.

It should be remembered that many pesticides are not stable during storage even when refrigerated. Moreover, when collecting samples to detect organophosphorus compounds, sodium fluoride should not be added to liquid specimens. For example, the presence of this common preservative in drug-free fresh blood samples (pH 7.4) caused complete degradation of dichlorvos over 15 min, and chlorpyrifos-methyl also became very unstable (Moriya, Hashimoto 1999).

Enzyme hydrolysis (urine)

To 5 mL of urine specimen are added 1 mL of 1 mol/L acetate buffer (pH 5) and 40 µL of β-glucuronidase plus arylsulfatase (30 U/mL plus 60 U/mL), and the mixture is incubated overnight at 37°C in a closed test tube. Incubation for 45 min at 56°C is less time-consuming, but the quantitative results are more variable – this shorter process can be used in emergency cases.

Liquid–liquid extraction

Solid or semi-solid samples are homogenised with an equal mass of water. Foodstuffs, stomach contents (especially with undigested food residues) or stomach tissues homogenised with water are treated with saturated calcium chloride solution, allowed to stand overnight, and filtered or centrifuged.

An aliquot of the filtered sample or homogenate is shaken for 5 min with an equal volume of a mixture of hexane–toluene (1 : 1, v/v) without adjusting the pH. After organic layer separation by centrifugation, the organic phase is retained. The aqueous fraction is adjusted to pH 2 by the addition of 1 mol/L sulfuric acid and then extracted with an equal volume of diethyl ether. The aqueous fraction is retained for later examination.

The hexane–toluene and ethereal fractions are combined and then divided into three equal portions. Each portion is evaporated to dryness at 40°C under a stream of nitrogen. One dry residue is reconstituted in 50 µL of toluene or hexane. A 5–10 µL aliquot is spotted on a TLC plate and a 1–2 µL aliquot is injected into the GC or the GC-MS apparatus. The two other residues and the aqueous phase are retained for derivatisation and/or additional tests, which are a part of screening or confirmation procedures.

Derivatisation procedures Table 16.1 gives retention indices, typical ions and their abundances for methyl (ME in Table 16.1), trifluoroacetyl (TFA in Table 16.1) and acetyl derivatives (AC in Table 16.1) of pesticides. Trifluoroacetylation and acetylation processes can be carried out for 5 min under microwave irradiation at about 400 W (Kraemer *et al.* 1997).

Acetylation Acetylation can be used interchangeably with trifluoroacetylation; 100 µL of pyridine and 100 µL of acetic anhydride are added to the second part of the dried extract (see above). The samples are vortex mixed, heated at 70°C for 20 min in a heating block, dried under nitrogen and reconstituted in 50 µL of ethyl acetate. Then, 1–2 µL are injected into the GC or the GC-MS apparatus. Alternatively, the acetylation process can be carried out with 50 µL of an acetic anhydride–pyridine mixture (3 : 2, v/v) for 5 min under microwave irradiation (Kraemer *et al.* 1997).

Methylation Methylation is used for System GK (see later) (Fysh, Whitehouse 1986). The third retained portion of extract, dissolved in 50 µL of methanol, is methylated for 2 h at room temperature with 200 µL of an ethanol-free solution of diazomethane in diethyl ether (Eistert *et al.* 1968) and then the methylation mixture is evaporated to dryness. The residue is dissolved in 50 µL of methanol and 1–2 µL are injected into the GC or the GC-MS apparatus. It should be noted that Diazald, a reagent for preparing diazomethane, is available (Sigma-Aldrich Co. Ltd.).

Methylation is also used for system GA (see later) (De Zeeuw *et al.* 1992a). The dried residue is dissolved in 0.5 mL of toluene and 0.5 mL of dimethyl sulfoxide. After addition of 50 mg of sodium hydride and 0.5 mL of methyl iodide, the sample is derivatised for 10 min at room temperature. After addition of 2 mL of hexane, the sample is shaken for 1 min. To remove the excess dimethyl sulfoxide, 2 mL of water is added and gently shaken, and then the organic layer is transferred and the aqueous phase is extracted again with 2 mL of hexane and then with 2 mL of ethyl acetate. The combined extracts are dried over sodium sulfate for 15 min and then concentrated to 0.1 mL (Rüssel 1986).

Trifluoroacetylation Trifluoroacetylation can be used interchangeably with acetylation. The trifluoroacetate derivative is prepared by reconstituting the dried extract with 50 µL of trifluoroacetic anhydride and 50 µL of ethyl acetate, and incubating at 60°C for 15 min (Catlin *et al.* 1987). After evaporation of the derivatisation mixture, the residue is dissolved in 50 µL of ethanol and dried ethyl acetate (1 : 1, v/v) and 1–2 µL are injected into the GC or the GC-MS apparatus.

Special procedure for quaternary ammonium herbicides A portion of the previously retained aqueous phase, filtered stomach contents or urine sample can be tested for the presence of paraquat and/or diquat by the colour test described above. Other assays to confirm the presence of paraquat or diquat can be carried out as follows (Draffan *et al.* 1977). The remaining aqueous phase after LLE or filtered stomach contents or urine sample is adjusted to pH 10 by the addition of 5% (v/v) ammonia solution and then mixed with 50 mg of sodium borohydride. After allowing to stand at room temperature for 30 min, the reaction mixture is extracted with an equal volume of diethyl ether. The ether extract contains the monoene and diene reduction products, which can be analysed by GC and/or GC-MS.

Thin-layer chromatography

Four TLC systems are used; each consists of an independent mobile phase and a sequence of different spray reagents, widely used for pesticide visualisation. General systems TZ and TAA (Kała, Chacia 1994) are used to reveal any pesticide in an examined sample and to enable presumptive chemical classification. Two more systems, TX and TY (De Zeeuw 1992b; Erdman *et al.* 1991; Erdmann *et al.* 1990), are used to identify the type of pesticide. The TX and TY systems give good reproducibility of R_f values with an error window of 7 calculated on the basis of interlaboratory results. The R_f values of the reference compounds chosen for the four screening systems are derived using 5–10 µg of each substance. Each extract is spotted onto a TLC plate in an amount corresponding to 2 g of the biological material being analysed. For

additional information, two other solvent systems (TAB and TAC) can be applied (Osselton, Snelling 1986).

The chromatographic process uses silica-gel plates of 0.25 mm layer thickness (e.g. Art. No 5721 from Merck), without fluorescent indicator and four mobile phases in saturated chambers in ascending mode. Seven spray reagents are suggested, which produce a variety of colours to facilitate differentiation. A large number of pesticides react with more than one reagent. The reagent sequences chosen allow the plates to be oversprayed (Kała, Chacia 1994). The LOD for most pesticides is 10 µg after reagent overspraying and 2–5 µg after single-reagent spray detection. After drying, all the chromatograms are first examined under ultraviolet (UV) light (366 and 254 nm) and then sprayed successively with the location reagents appropriate for each system. The plate is sprayed with a location reagent and dried, and a note is taken of any colours. The plate is then oversprayed with another reagent and again any changes are noted.

In addition to the spray reagents indicated below, useful individual detecting agents include anisaldehyde, 4-aminopyridine, 2-methylthioacridone or the Griess reagent (Patil, Shingare 1993) for organophosphorus compounds and also, used separately, trichlorobenzoquinoneimide and zinc hexacyanoferrate for the identification of carbamates. A sequence of 4-aminoantipyrine followed by potassium ferricyanide is recommended especially for carbaryl, propoxur and carbofuran as intense red spots develop (Sevalkar *et al.* 2000). Other reagents reported as selective and very sensitive are: tautomeric phloroglucinol for methomyl (Mali *et al.* 2006) and 2% diphenylamine and 5% formaldehyde in alcoholic solutions, which detect carbaryl and 1-naphthol as blue-green spots on heating (Daundkar *et al.* 2006).

Spray reagents

Silver nitrate ($AgNO_3$) The plates are sprayed with a 0.1 mol/L aqueous solution of $AgNO_3$. After spraying, the dry plates are exposed to UV radiation (254 nm) for 10 min. Many pesticides give white, grey and brown spots on a bright brown background.

Rhodamine B and sodium hydroxide (RHB-NaOH) A 0.02% (w/v) solution of rhodamine B (RHB) in ethanol and a saturated solution of NaOH in ethanol are used as the spray. After both the RHB and NaOH solutions have been sprayed, compounds are located as navy-blue spots by examination under UV light.

Diphenylamine and zinc chloride (DPA-$ZnCl_2$) The spray comprises 0.7% (w/v) diphenylamine (DPA) and 0.7% (w/v) $ZnCl_2$ solution in acetone. After spraying, the plates are exposed to UV radiation for 10 min and then heated at 100°C until no further colour change is observed. Light blue, blue, green and pink spots are observed on a white background.

2,6-Dibromoquinone-4-chlorimide and sodium hydroxide (DBQ-NaOH) A 0.2% (w/v) solution of 2,6-dibromoquinone-4-chlorimide (DBQ) in acetone and a saturated solution of NaOH in ethanol are used to spray the plates. After spraying, the plates are heated at 100°C for 10 min. Navy blue, pink and violet spots on a light blue background are observed.

Palladium chloride ($PdCl_2$) To make the spray, dissolve 0.5 g of $PdCl_2$ in 2.5 mL of 35% (v/v) hydrochloric acid and carefully dilute with water to 100 mL. After spraying, yellow and brown spots are observed.

4-(4-Nitrobenzyl)pyridine and tetraethylenepentamine (NBP-Tetren) To make the spray, dissolve 5 g of 4-(4-nitrobenzyl)pyridine (NBP) in 100 mL of acetone and dilute 1 : 5 (v/v) of tetraethylenepentamine (Tetren) with acetone (Brose *et al.* 1992). Spray the plate with the NBP solution and dry at 110°C for 10 min. After cooling, spray the plate with the dilute Tetren solution and observe the blue–violet spots on a white background. The colours are not stable. The stability of the colours can be enhanced by spraying the plate with a 20% (v/v) solution of acetic acid and drying at room temperature and at 110°C before using Tetren (Fodor-Csorba, Dutka 1986). The reagents should be freshly prepared.

Dragendorff spray, ferric chloride, iodine and hydrochloric acid The reagents are Dragendorff spray, a 5% (w/v) solution of $FeCl_3$, 1 g of iodine and 4 g of KI dissolved in 100 mL of ethanol, and finally a 25% (v/v) solution of concentrated hydrochloric acid made up in ethanol. Spray the reagents consecutively and examine any spots and colour changes.

Chromatography systems

System TX
- Mobile phase: hexane–acetone (4 : 1).
- Reference compounds: trichlorfon (hR_f 7), carbofuran (hR_f 17), methoxychlor (hR_f 43), dieldrin (hR_f 65) and quintozene (hR_f 84).
- Location systems: DPA-$ZnCl_2$, NBP-Tetren and DBQ-NaOH.

System TY
- Mobile phase: toluene–acetone (95 : 5).
- Reference compounds: thiophanate (hR_f 8), 2,4-D (hR_f 10), desmedipham (hR_f 22), captan (hR_f 42), tetramethrin (hR_f 52) and fenitrothion (hR_f 76).
- Location systems: $AgNO_3$ and $PdCl_2$.

System TZ
- Mobile phase: chloroform–acetone (9 : 1).
- Reference compounds: trichlorfon (hR_f 15), dimethoate (hR_f 37), propoxur (hR_f 66) and DDT (hR_f 90).
- Location systems: $AgNO_3$, RHB-NaOH, DBQ-NaOH and $PdCl_2$.

System TAA
- Mobile phase: chloroform.
- Reference compounds: methomyl (hR_f 9), dichlorvos (hR_f 36), chlorfenvinphos (hR_f 42), methoxychlor (hR_f 65) and fenvalerate (hR_f 75).
- Location system: Dragendorff-$FeCl_3$-I_3 in KI–HCl.

System TAB
- Mobile phase: dichloromethane.
- Reference compounds are any compounds examined in the TAB system from Table 16.1.

For location systems, see later.

System TAC
- Mobile phase: ethyl acetate–isooctane (15 : 85).
- Reference compounds are any compounds examined in the TAC system from Table 16.1.

For location systems, see later.

Location reagents for TAB and TAC Compounds are located with the DPA-$ZnCl_2$ reagent. A second spray system is as follows:
- Reagent A (fluorescein in dimethylformamide): dilute 1 mL of a 0.25% (w/v) solution of fluorescein in dimethylformamide to 50 mL with ethanol.
- Reagent B (silver nitrate and phenyl cellosolve): dissolve 1.7 g of silver nitrate in 5 mL of water and mix with 10 mL of phenyl cellosolve and 185 mL of acetone.
- To develop the colours, expose the developed plates to an atmosphere of bromine vapour for 1 min and then spray sequentially with reagents A and B. Yellow spots on a pink background appear which, after exposure to UV radiation, produce yellow spots on a black background.

Other spray reagents

The following reagents can provide additional confirmatory information.

Chlorine and *o*-toluidine Dissolve 1 g of *o*-toluidine in 10 mL of anhydrous acetic acid and 4 g of potassium iodide in 10 mL of distilled water. Mix the two solutions and dilute with distilled water to 1 L. To develop the colours, put the plate in a closed tank with chlorine gas (prepared by adding 2 mL of concentrated hydrochloric acid to 1 g of potassium permanganate) for 1 min. Remove excess chlorine from the plate under a stream of air in a fume cupboard. Dip the plate in the reagent for about 3 s. Yellow-orange spots appear against a white–blue background.

***N*,*N*-Dimethyl-*p*-phenylenediamine hydrochloride** Dissolve 0.1 g of *N*,*N*-dimethyl-*p*-phenylenediamine hydrochloride (MPA) in 10 mL of ethanol, and mix with 10 mL of sodium methoxide just before spraying. Spots develop against a grey–black background when the plate is irradiated with UV light at 366 nm for 10 min.

Ammonium molybdate with sodium antimony tartrate and ascorbic acid

- Reagent A (ammonium molybdate with sodium antimony tartrate): dissolve 10 g of ammonium molybdate in 40 mL of 2 mol/L H_2SO_4. Separately, dissolve 0.1 g of sodium antimony tartrate in 50 mL of 2 mol/L H_2SO_4. Mix both reagents and make up to 500 mL with the same acid.
- Reagent B (ascorbic acid): dissolve 2 g of ascorbic acid in 100 mL of water. This reagent can be used for 1 week if stored at 4°C.
- To locate any spots, remove the plate from the solvent chamber, air dry and heat for 2 h at 110°C. After cooling, lightly spray the plate first with reagent A and then with reagent B. The decomposition product of OP compounds (inorganic phosphate) reacts with ammonium molybdate and then phosphomolybdate is reduced to a blue complex by ascorbic acid (Murty *et al*. 1980).

Chromatographic data

The hR_f values for 167 pesticides in the TX, TY, TAB and TAC systems are listed in Table 16.1. Detection characteristics and limits of detection for chemical classes of pesticides with the spray reagents are given in Table 16.4.

Gas chromatography

Two GC systems are described. In both systems GC should be carried out with temperature programming and with an effluent splitter fitted to a dual flame ionisation–nitrogen-phosphorus detection (FID-NPD) system. Separation on the GA system (see below) can be carried out using packed or capillary columns, but for the GK system only packed columns are used. Nitrogen at a flow rate of 50 mL/min is used as a carrier gas. As reference substances, for calculation of the retention index (RI) of an unknown substance and for daily checking of the chromatographic performance, a mixture of *n*-alkanes with an even number of carbon atoms or other mixtures of typical drugs that cover a broad range of RI or retention time values should be used. One hundred nanograms of each substance in an aliquot of 1 µL of a reference mixture should be easily detectable by FID and 10 ng/µL of each using NPD. Peaks should be sharp and clearly separated (De Zeeuw *et al*. 1992a).

System GA

For packed columns

- Column: 3% SE-30 or 3% OV-1 on 80/100 mesh Chromosorb G HP.
- Temperature: 200–280°C.
- Reference compounds comprise 1 µL of methanolic solution of the following compounds (name, RI): barbital, 1489; pentobarbital, 1735; diphenhydramine, 1870; phenobarbital, 1953; methaqualone, 2135; codeine, 2375; morphine, 2445; nalorphine, 2572; quinine, 2800; haloperidol, 2930; strychnine, 3116.

For capillary columns

- Column: dimethylsilicone, fused silica, 10–15 m × 0.32 or 0.53 mm i.d., 1.5–3.0 µm.
- Temperature programme: 135°C for 4 min, to 200°C at 13°/min, to 240°C at 8°/min and to 312°C at 6°/min for 6 min.
- Reference compounds comprise 1 µL of ethyl acetate solution of each of the following compounds (name, RI): ethosuximide, 1205; ethinamate, 1365; barbital, 1489; aprobarbital, 1618; secobarbital, 1786; phenobarbital, 1953; heptabarbital, 2055; primidone, 2250; phenylbutazone, 2367; bis-(ethylhexyl)phthalate, 2507; prazepam, 2648.

System GK

- Column: 3% OV-17 on 80/100 mesh Gas Chrom Q, 1 m × 6 mm i.d.
- Temperature programme: 100°C to 260°C at 10°/min.
- Reference compounds comprise 1 µL of a solution of caffeine in ethyl acetate containing 1 mg/mL. Inject 1–5 µL of the sample extract, together with 1 µL of the caffeine solution.

Identification of pesticides

Gas chromatography–mass spectrometry

GC-MS is the recognised standard for systematic toxicological analysis and the GC-MS system described below is part of the most comprehensive GC-MS screening procedure for the detection of drugs and poisons, among which are pesticides (Pfleger *et al*. 2007). GC-MS systems are equipped with comprehensive libraries that contain reference spectra for many pesticides, their metabolites and decomposition products. The latest, most up-to-date library is the Wiley Registry, 8th edition/NIST 2008 which is a combination of the Wiley Registry of Mass Spectral Data and the NIST 2008 Library. The W8/N08 contains 562 000 EI spectra, 5308 precursor ions for MS-MS and, in addition, more than 2 million chemical names and synonyms, 350 000 searchable structures and 43 000 entries of GC retention indices. An integrated part of this library is the Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) (http://eu.wiley.com). The most useful library for use in analytical toxicology is the Pfleger, Maurer and Weber library (3rd edn, 2007), which contains more than 7800 entries (Pfleger *et al*. 2007; Maurer 2006). Both of these libraries contain data for many pesticides, their metabolites and artefacts derived from different sources. For screening, the best GC-MS strategies are based on full-scan spectra in EI mode, reconstructed mass chromatography, and identification via library searches. Although evaluation of the data is greatly facilitated by the use macros, the final decision on the identity of a given compound

Table 16.4 Detection and limits of detection (µg) for chemical classes of pesticides with spray reagents

Spray reagent[a]	*Chemical class of pesticides*[b]							
	CB	*CH*	*CP*	*OP*	*PY*	*SU*	*TR*	*Misc.*
$AgNO_3$	5	2	5	2	5	5	5	±
RHB-NaOH	5	5	5	5	5	10	5	±
DPA-$ZnCl_2$	2	2	5	5	5	–	–	±
DBQ-NaOH	1	–	–	3	–	–	–	±
$PdCl_2$	–	–	–	1	–	–	+	±
NBP-Tetren	–	–	–	1	–	–	–	–
FDF-$AgNO_3$ + PC	+	–	–	+	–	–	–	±
Dragendorff-$FeCl_3$-I_3-KI-HCl	5	–	–	5	2	5	5	±
Chlorine–*o*-tolidine	+	–	+	+	+	+	+	+
MPA	+	+	+	+	+	+	+	±
AMSA	–	–	–	0.1	–	–	–	–

[a]RHB-NaOH, rhodamine B with sodium hydroxide; DPA-$ZnCl_2$, 2,6 dibromoquinone-4-chloromide and sodium hydroxide; NBP-Tetren, 4-(4-nitrobenzyl)pyridine and tetraethylenepentamine; FDF-$AgNO_3$ + PC, fluorescein in dimethylformamide and silver nitrate with phenyl cellosolve; MPA, *N,N*-dimethyl-*p*-phenylenediamine hydrochloride; AMSA, ammonium molybdate with sodium antimony tartrate and ascorbic acid.
[b]+, positive; –, negative; ±, positive and negative; CB, carbamates; CH, chlorinated hydrocarbons; CP, chlorinated phenoxy acids; Misc., miscellaneous; OP, organophosphorus compounds; PY, pyrethroids; SU, substituted ureas; TR, triazines.

should always rest in the hands of the experienced toxicologist or analyst who undertakes a visual comparison between the EI full mass spectrum of the measured compound and the reference spectra and at the same time considers other chemical, analytical and toxicological evidence to support the identification. For forensic purposes ultimate identification should be performed by analysis of the analyte and the reference substance under the same analytical conditions. GC-MS methods can be used not only for simultaneous screening of pesticides and polychlorinated biphenyl compounds in biological samples, but also for solid environmental media (Liu, Pleil 2002).

System GC-MS

- Column: cross-linked methylsilicone capillary HP-1, 12 m × 0.2 mm i.d., 0.33 µm.
- Temperature programme: 100°C for 3 min to 310°C at 30°/min for 5 min.
- Injection port temperature, 270°C.
- Carrier gas: He, 1 mL/min.
- Ionisation mode, electron impact (EI).
- Ionisation energy, 70 eV.
- Ion source temperature, 200°C.
- Scan rate, 1 scan/min.
- Reference compounds comprise 1 µL of a methanolic solution containing 50 ng/mL of each of following compounds (name, RI): metamfepramone, 1355; acetylated amfetamine, 1505; pentobarbital, 1740; diphenhydramine, 1870; phenobarbital, 1935; methaqualone, 2155; codeine, 2375; morphine, 2455; nalorphine, 2620; quinine, 2800; haloperidol, 2940; strychnine, 3120; and the hydrocarbon C_{40}, 4000.

Analytical data The relative retention times for pesticides in the GK system (Fysh, Whitehouse 1986), the retention indices for the GA system (De Zeeuw *et al*. 1992a) and the GC-MS system (Pfleger *et al*. 2007), and the typical ions and their intensities are listed in Table 16.1. Despite different chromatographic conditions for the GA system with capillary column and the GC-MS system, in most cases the RI values do not differ by more than 50–60 RI units for the same pesticide.

No chromatographic conditions can ensure complete separation of all pesticides and therefore the possibility of peaks overlapping should be considered, even among the standards (Fig. 16.3).

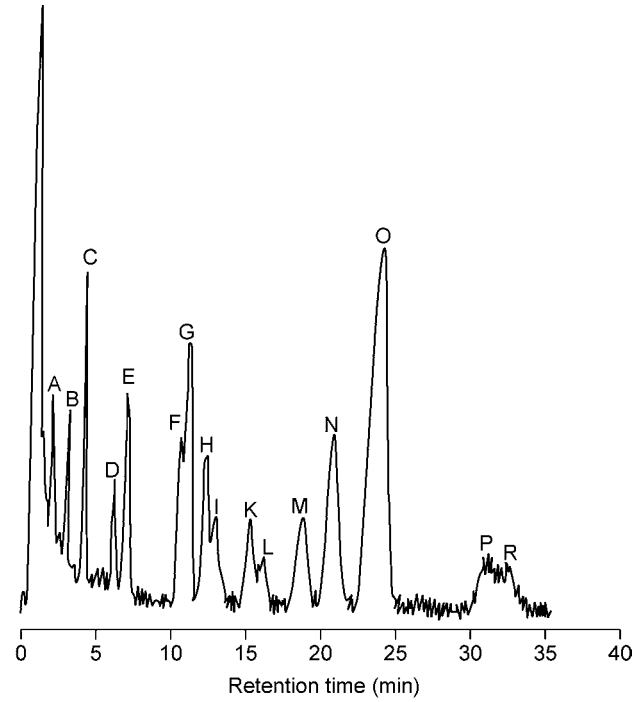

Figure 16.3 Gas chromatogram of some pesticides using an FID detector (column 10% SE-30 on Gas Chrom Q, column temperature 230°C). Relative retention times (RRTs) are given in parentheses: A, diuron (0.13); B, chloroneb (0.19); C, propachlor (0.27); D, hexachlorobenzene (0.40); E, diazinon + lindane + quintozene (0.45); F, fenchlorphos (0.69); G, fenitrothion + tetrachlorvinphos (0.72); H, fenthion (0.80); I, dicofol (0.85); K, captan (external standard, 1.00); L, chlorfenvinphos (1.05); M, α-chlordane (1.22); N, γ-chlordane (1.36); O, *pp′*-dichlorodiphenyldichloroethylene (DDE) + dieldrin (2.03); P, *op′*-dichlorodiphenyltrichlorethane (DDT); R, tetrasul (2.11). Reproduced with permission from Chłobowska *et al*. (1996).

High performance liquid chromatography

HPLC is less useful than GC for screening, since it tends to have less sensitivity and the retention times are not as reproducible. This technique can be used for those pesticides that are not stable at higher temperatures or are too polar for GC analysis. Several HPLC methods have been described, either for a specific pesticide (Meyer *et al*. 1998) or for a group of pesticides, e.g. dialkylphosphates, dialkylthiophosphates (Abu-Qare, Abou-Donia 2000, 2001; Aprea *et al*. 2002; Bardarov, Mitewa 1989). Spectrophotometric detection (UV or diode array detection [DAD]) is useful for aryl organophosphate esters, which show good UV absorption at higher wavelengths, whereas the corresponding alkyl derivatives have their absorption maxima below 250 nm. Fluorescence detection is suitable only for a few compounds that show natural fluorescence after alkaline hydrolysis, which can enhance fluorescence, and/or after derivatisation. Maximum native fluorescence intensity was observed at basic pH solutions for 3-indolyl acetic acid (Calatayud *et al*. 2006) and for asulam (Súbová *et al*. 2006). The effect of native cyclodextrins upon the UV-Vis and fluorescence properties of poorly fluorescent *N*-methyl carbamates such as bendiocarb and promecarb has been examined (Pacioni, Veglia 2007).

Five carbamate pesticides (aldicarb, carbaryl, 3-hydroxycarbofuran, carbofuran, methiocarb) were analysed by HPLC with fluorescence detection after *o*-phthalaldehyde derivatisation (King, Zhang 2002). Propoxur, carbofuran and carbaryl were coupled after alkaline hydrolysis with diazotised sulfanilic acid (Tena 1992). The application of *m*-periodate transgenic tobacco peroxidase, as a chemiluminescent label, and subsequent coupling to 2,4-dichlorophenoxyacetic acid (2,4-D) allowed determination of the conjugate by ELISA within the range 30 pg and 500 ng/mL (Dzgoev *et al*. 1999). Data for two HPLC systems are given in Table 16.1 and the chromatographic conditions are described below (Osselton, Snelling 1986).

System HAO

- Column: ODS-Hypersil, 160 × 5 mm stainless steel, 5 µm.
- Mobile phase: acetonitrile–water (60 : 40, v/v), 2 mL/min.

System HAP

- Column: Spherisorb S5W silica, 250 × 5 mm.
- Mobile phase: dichloromethane–isooctane (60 : 40, v/v), 2 mL/min.

Analytical data

In Table 16.1 retention data are expressed as capacity factors *k*, which are defined by $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the substance under investigation and a non-retained compound, respectively.

Liquid chromatography–mass spectrometry

HPLC has become a valuable technique for the screening, identification and/or multianalyte quantification of pesticides when coupled with single-stage or tandem mass spectrometry with electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI). The best LC-MS strategies for screening are based on tandem MS in multiple-reaction monitoring (MRM) mode. Only few LC or LC-MS(-MS) libraries have been developed so far because of the rather poor inter-apparatus reproducibility of ESI spectra. Therefore, before applying data from different sources, the apparatus should be standardised using certain test compounds. The ESI and APCI mass spectra library for identification of pesticides developed by Schreiber is commercially available (www.chemicalsoft.de.index-ms.htm). Another source of ion transitions from precursor to product ions for LC-MS(-MS) in SIR mode is the review by Alder *et al*. (2006). Complete acquisition parameter sets for positive

and negative electrospray, using an API 2000 mass spectrometer, in combination with single-quadrupole mass spectra and product ion scans are available from the Federal Institute for Risk Assessment in Berlin and can be downloaded from www.bfr.bund.de/cd/5832. These data are also available as a CD-ROM free of charge from the institute. All tuning parameters were also tested for a triple-quadrupole API 4000 apparatus. Tuning of other types of instruments may be assisted by product ion spectra, which were recorded at three fixed collision energies. The sensitivity of LC-MS(-MS) instruments was assessed by the injection of 20 μL of an analytical standard on a reversed-phase column (Phenomenex Aqua, 50 × 2 mm, 5 μm), using a gradient of methanol–water containing 5 mmol/L ammonium formate. Two ion transitions and collision energies obtained under the above-mentioned conditions have been added to the list of pesticides in Table 16.1.

Quantification of pesticides

Measurements of pesticide concentration in biological samples from acutely poisoned patients can have an immediate bearing on treatment, particularly when active elimination procedures, such as diuresis or haemodialysis, are contemplated. In fatal cases of suicidal or homicidal poisoning, it is easier to detect and identify pesticides by examining suspect materials, such as liquids, food, clothing and soil, in which the concentrations are likely to be quite high. Thereafter, if there is strong evidence that the substance detected may have been responsible for the death, quantitative examination of postmortem tissues may provide conclusive proof of poisoning.

Quantitative analyses also have an important role in monitoring pesticide concentrations in soil, water supplies, rivers, lakes and foodstuffs; in some countries, legislative control of permissible levels has been introduced. Their application is likely to increase with the growing public concern about the release of pesticides into the environment. GC-MS and LC-MS(-MS) are the techniques most commonly applied in multianalyte methods for pesticides at present. Many guidelines for quality assurance and method validation have been developed by individual scientists (Peters *et al.* 2007; Simonelli *et al.* 2007) and scientific organisations and several national and international proficiency testing schemes are now in operation. Numerous analytical procedures for the quantitative analysis of pesticides in various media are described in the National Institute for Occupational Safety and Health (NIOSH) *Pocket Guide to Chemical Hazards* (available on-line at www.cdc.gov/niosh/npg). Other publications have described original and sensitive GC-MS and LC-MS methods using standardised sample preparation procedures for the detection and quantification in human biological matrices of 61 pesticides of toxicological significance (Lacassie *et al.* 2001); a GC-negative chemical ionisation (NCI)-MS method for determining 37 organophosphorus pesticides in human tissues (Russo *et al.* 2002); and a multianalyte method for the quantification of 29 contemporary pesticides in human serum and plasma using isotope dilution gas chromatography–high-resolution mass spectrometry (Barr *et al.* 2002). More recently, validated methods for 229 pesticides in fruits and vegetables using GC-MS and LC-MS have been reported (Lehotay *et al.* 2005), and details of LC-MS(-MS) with MRM transitions for the determination of 660 pesticides in animal tissues have been published (Pang *et al.* 2006).

Both GC-MS- and LC-MS-based methods reveal a significant variation of sensitivity, covering a range of at least three to four orders of magnitude, depending on the pesticide. On the basis of review of the literature, it can be concluded that GC-MS achieves better performance only for one substance class, the organochlorine pesticides.

Capillary electrophoresis with titania-based columns has also been used to determine phosphorus-containing amino acid-type herbicides and their metabolites, e.g. glyphosate, aminomethylphosphonic acid, gluphosinate and 3-methylphosphonico-propionic acid, in human blood (Tetsuya *et al.* 2007).

Stability experiments for pesticides during the various stages of analysis (including storage prior to analysis) are essential. Ageda *et al.* (2006) studied the effect of temperature on the stability of 14 organophosphorus insecticides in fresh blood. Short- and long-term stability studies have been carried out for various chemical classes of pesticides by Simonelli *et al.* (2007). Moriya *et al.* (1999) found that the addition of sodium fluoride to blood samples caused the complete degradation of dichlorvos within 15 min and that chlorpyrifos-methyl also became very unstable. Organophosphates are degraded more rapidly by esterase activity than by chemical mechanisms, whereas organophosphorothioates are hydrolysed chemically in aqueous solutions but are very stable in biological specimens and not affected by esters.

Specific pesticides

Organophosphorus compounds

Organophosphorus compounds are by far the most important class of pesticides, in terms of both worldwide usage and their toxicity to humans. They act by the irreversible inhibition of cholinesterases, which are responsible for hydrolysing, and thereby deactivating, the neurotransmitter acetylcholine (ACh). Build-up of ACh at the neural junction leaves the muscles, glands and nerves in a constant state of stimulation, which produces a wide range of acute symptoms. These include dizziness, confusion and blurred vision, excessive salivation and sweating, nausea and vomiting, and muscular weakness. Severe poisoning leads to coma, flaccid paralysis, breathing difficulties, cyanosis and cardiac arrhythmias. Atropine and pralidoxime are effective antidotes in severe cases. In acute clinical poisoning, diagnostic tests for depressed cholinesterase activity are the most crucial. Detecting, identifying and quantifying the particular agent responsible has less bearing on immediate treatment, although some of the lipophilic diethyl phosphothiolates can be sequestered in the tissues for several days and patients who appear to have recovered may suffer a recurrence of toxic effects. Identification of the agent involved can alert clinicians to this possibility.

Quantification of cholinesterase activity in plasma or serum and whole blood Two types of cholinesterases exist in the body. Acetylcholinesterase (AChE), which is also known as true cholinesterase, is found in red cells, nerve endings, lungs and brain tissues. Its main function is to hydrolyse ACh at cholinergic nerve endings. The second type is usually known as pseudocholinesterase (ChE) and occurs in the plasma in addition to other body tissues. The exact physiological function of ChE is unknown, but it has the ability to hydrolyse a variety of esters in addition to cholinesterase. Depression of ChE can also be caused by non-pesticide chemicals, liver diseases and other factors (physiological, pharmacological or genetic). Measurement of red-cell AChE is therefore a more specific indicator of cholinesterase inhibition caused by OP or carbamate pesticides. Moreover, the depression of red-cell AChE activity can be demonstrated for up to 2–6 weeks after exposure, whereas that of plasma ChE returns to normal much more quickly. Nevertheless, in practice, plasma ChE activity is a useful indicator of exposure, since normal values effectively exclude acute poisoning by these substances.

Some carbamate herbicides and fungicides, such as the dithiocarbamates, do not inhibit cholinesterases to any significant degree and are relatively non-toxic in humans. Postmortem specimens for AChE assay must be kept in cold storage and analysed as soon as possible to minimise the effects of spontaneous reactivation of the enzyme (Geldmacher-v.Mallinckrodt *et al.* 1974).

It must be emphasised that the normal ranges for AChE activity are highly dependent on the type of assay system used and wide variations exist.

Determination of plasma or serum cholinesterase activity Adjust the temperature of 3 mL of 0.02% (w/v) dithiobisnitrobenzoic acid in 0.1 mol/L sodium dihydrogen phosphate buffer solution (pH 7.4) to 25°C; add 20 μL of sample serum and 0.1 mL of 5% (w/v) acetylthiocholine iodide solution, mix well, and record the absorbance of a 1 cm thickness at 405 nm at 0.5 min intervals for 2 min. If the change in absorbance value exceeds 0.2 when developing within 30 s, dilute the sample (one in ten) with normal saline and repeat the measurements (the readings must then be multiplied by 10). The cholinesterase activity is calculated as:

$$\text{Cholinesterase (munits/mL or mU/mL, at 25°C)} = \text{change in absorbance in 30 s} \times 23\,400$$

Normal values of ChE activity in serum range from 1900 mU/mL to 4000 mU/mL. Commercial kits for the determination of ChE activity in plasma and serum are available (Sigma Chemical Co., St Louis, MO, USA; Biotron Diagnostics, Inc., Hemet, CA, USA; Lovibond, Tintometer GmbH, Dortmund, Germany; Dade Behring, Paris).

Determination of whole-blood acetylcholinesterase activity The reagents for this are (Fleisher, Pope 1954):

1. Phosphate buffer (0.134 mol/L, pH 7.2), prepared by mixing 7 parts by volume of a solution of 23.752 g of $Na_2HPO_4{\cdot}2H_2O$ per litre and three parts of a solution of 18.156 g of KH_2PO_4 per litre; pH adjusted to 7.2 if necessary.
2. ACh (0.04 mol/L), prepared by dissolving 0.7266 g of acetylcholine chloride in 100 mL of 0.001 mol/L acetate buffer (pH 4.5); stable indefinitely in the cold.
3. ACh (0.004 mol/L), prepared by diluting Solution 2 with 9 volumes of phosphate buffer (Solution 1); made daily in the quantity required for the analyses.
4. Hydroxylamine hydrochloride (2 mol/L), made by dissolving 27.8 g in water to 200 mL.
5. NaOH (3.5 mol/L), made by dissolving 28 g in water to 200 mL.
6. Alkaline hydroxylamine prepared from equal volumes of Solutions 4 and 5, mixed shortly before use in a quantity required for the samples being analysed, and made up freshly for each set of samples analysed.
7. HCl (concentrated acid, specific gravity 1.18), diluted with 2 volumes of water.
8. Ferric chloride ($FeCl_3$, 0.37 mol/L), made with 10 g of $FeCl_3{\cdot}6H_2O$ dissolved in 100 mL of 0.1 mol/L HCl.

Prepare three test tubes. To the first tube (E), add 0.95 mL of 0.01% (v/v) saponin solution, 50 µL of heparinised blood sample and then 1 mL of 0.004 mol/L ACh; mix well and incubate at 25°C for 10 min. For the control, 1 mL of 0.004 mol/L ACh solution is incubated in a second tube (C) alongside the experimental sample. After exactly 10 min the reaction is stopped by the addition of 4 mL of alkaline hydroxylamine reagent (with vigorous shaking) to both experimental and control samples. After a wait of at least 1 min, 0.95 mL of saponin and 50 µL of blood sample are added to the control solution of ACh. Then 2 mL of HCl reagent is added to each sample, followed by 2 mL of $FeCl_3$ reagent, with mixing after each addition. The solutions are filtered through Whatman filter paper, and the absorbance of a 1 cm layer at 520 nm is recorded 10 min after the addition of the $FeCl_3$. The absorbance (A_E, in mmol/L) is measured against a reagent mixture that consists of 4 mL of alkaline hydroxylamine, 2 mL of HCl and 2 mL of $FeCl_3$ reagent. The measured value of absorbance of the control sample (A_C) should be in the range 0.3–0.4. The activity of AChE in blood is calculated as follows:

$$4 - (4A_E/A_C) \times 2000$$

= AChE activity in international units per millilitre (IU/mL)

The precision of the method is 210 IU/mL.

Normal values for AChE activity in whole blood range from 3500 IU/mL to 8000 IU/mL. Commercial kits for the determination of AChE activity in red blood cells, whole blood and plasma are available (EQM Research, Inc., Cincinnati, OH, USA).

Determination of organophosphates in urine Most compounds of this chemical class are hydrolysed rapidly by plasma and tissue enzymes with the production of many metabolites. Metabolites and their conjugates are excreted in urine and are known to be unstable in stored specimens. To derive data that accurately represent the true degree of exposure, as indicated by the concentration of OP compounds, it is essential to obtain and analyse samples as soon as possible after an incident. Urine samples should be analysed within a week of obtaining the sample and kept at −20°C prior to analysis (Comer *et al.* 1976).

Colorimetric procedure In the colorimetric procedure (Namera *et al.* 2000), to 1 mL of urine (pH 5–8) add 0.1 mL of 45% (w/v) of 4-(4-nitrobenzyl)pyridine (NBP) solution in acetone, vortex for 30 s and heat at 100°C on a heating block for 20 min. After cooling to room temperature, add 0.1 mL of Tetren and 1 mL of diethyl ether, then close the tube and vortex mix for 3 min. Measure the absorbance of the ethereal layer at 520 nm against a reagent mixture. Construct a calibration graph for the analysis of the standard OP compound solutions and calculate the concentration in the sample. The limits of detection range from 0.1 mg/L to 3.0 mg/L for 24 OP compounds and 10 mg/L for acephate, isofenphos and vamidothion.

Chromatographic analysis ?A3B2 twb=.28w?>The OP compounds can be analysed without prior derivatisation. Screening for these compounds by GC is assisted greatly by a dual FID–NPD system. Detection by NPD gives a 10-fold increase in sensitivity for phosphorus-containing compounds compared with those detected using nitrogen only. This is very important because many OP pesticides are highly toxic and their concentrations in biological samples are low. Mass fragmentation of these compounds to typical ions (which vary widely in intensity) allows the chemical class to be determined. Molecular ions of OP compounds can be monitored using the thermospray LC-MS technique (Niessen 1999). Both protonated and ammoniated molecules are detected in the positive-ion mode without any fragmentation. Phosphates and phosphorodithioates appear to give a better response than other chemical classes. Phosphorothioates with chlorine, bromine or nitrate substitution on the ring are especially intense. The thermospray mass spectra obtained by operating in the negative-ion mode are more compound dependent.

Human exposure to OP pesticides is often assessed by measuring general dialkyl phosphate metabolites of OPs in urine. Six common urinary dialkyl metabolites can be quantified by GC-MS to confirm cumulative exposure to OP pesticides (De Alwis *et al.* 2006).

An experiment on animals demonstrated the possibility of using hair analysis by GC-NCI-MS for monitoring low-level exposure to diazinon (Tutudaki, Tsatsakis 2005). Hair was also shown to be a promising material by documenting non-fatal intoxication in two men, one exposed to alachlor a year before sampling and the other exposed to carbofuran 14 days before sampling. The concentrations of alachlor in five analysed hair segments were between 12 and 136 pg/mg. Carbofuran and its main metabolite (3-hydroxycarbofuran) were detected in the hair strand (global analysis) at concentrations of 207 and 164 pg/mg, respectively (Dulaurent *et al.* 2008).

Blood and tissue levels Pre-treatment plasma concentrations (mg/L) in cases of non-fatal organophosphate poisoning are summarised below (Kłys *et al.* 1989, 1991, 1992).

Chlorphenvinphos	Fenitrothion	Phosalone
n = 20	*n* = 15	*n* = 16
0.1–10.6	0.096–0.35	0.005–0.39

Many studies have been carried out on the distribution of organophosphate in cases of fatal poisoning with this group of pesticides. Their concentrations (mg/L or mg/kg) in various body fluids and tissues can help to determine the specimens of choice in postmortem toxicological analysis. The following data have been reported:

For chlorphenvinphos (Kłys *et al.* 1989):

Blood	Liver	Kidney	Lung	Brain
n = 7	*n* = 7	*n* = 5	*n* = 7	*n* = 6
0.30–15.0	0.32–15.94	0.16–15.0	0.80–15.94	0.1–4.3

For fenitrothion (Kłys *et al.* 1992):

Blood	Liver	Kidney	Brain
n = 6	*n* = 5	*n* = 2	*n* = 5
1.9–14.8	0.8–11.5	3.9–4.5	0.04–1.15

Values reported in single cases are given below.
For dimethoate (Tarbah *et al.* 2007):

Blood	Urine	Brain	Liver	Lung	Myocardial muscle	Skeletal muscle	Kidney	Gall bladder	Stomach contents
38	0.47	2.2	4.6	7.6	7.6	21	55	31	104

In this case the blood alcohol concentration was 2.85 g/L and the blood was also positive for cyclohexanone and cyclohexanol, a mixture used as a vehicle in the commercial product.

For omethoate (Pavlic *et al.* 2002):

Cardiac blood	Urine	Bile	Liver	Kidney	Stomach contents
208	225	524	341	505	48 223

For dichlorvos (Moriya *et al.* 1999):

Thoracic aorta blood	Thoracic inferior vena cava blood	Cerebro-spinal fluid	Peri-cardial fluid	Vitreous humour	Bile	Spleen	Stomach contents
0.043	0.082	0.027	0.438	0.067	8.99	0.542	2929

In this case no dichlorvos was detected in blood from the left and right cardiac chambers, the pulmonary arteries and veins or the right femoral vein. Dichlovos was also absent from the urine, cerebrum, lung, kidney and right femoral muscle.

For chlorpyrifos-methyl (Moriya *et al.* 1999):

Blood						
Left cardiac chambers	Right cardiac chambers	Pulmonary arteries	Pulmonary veins	Thoracic aorta	Thoracic inferior vena cava	Right femoral vein
1.01	1.71	4.15	2.83	0.99	2.24	0.62
Cerebrospinal fluid	Pericardial fluid	Vitreous humour	Lung left hilus	Myocardium	Right kidney	Right femoral muscle
0.01	0.01	0.01	8.60	0.49	0.47	0.39
Liver deep right lobe	Spleen	Cerebrum	Stomach contents	Bile	Urine	
1.41	0.666	0.38	2041	ND	ND	

ND – not detected.

Concentrations of other OP compound in postmortem blood have also been reported:

Compound	Concentration (mg/L)	Reference
Chlorpyrifos	0.21–2.05 ($n = 6$)	(Park *et al.* 2009)
Diazinon	0.24–2.82 ($n = 4$)	(Park *et al.* 2009)
Diazinon	0.4–277 ($n = 3$)	(Poklis *et al.* 1980)
Malathion	0.35–1.32 ($n = 4$)	(Park *et al.* 2009)
Malathion	175–517 ($n = 6$)	(Jadhav *et al.* 1992)
Parathion	0.21–19.64 ($n = 17$)	(Park *et al.* 2009)
Phosalone	0.024–0.19 ($n = 3$)	(Kłys *et al.* 1991)

Carbamates

Analysis These compounds can be divided into various subclasses, characterised by their different thermal stabilities. *N*-Methylcarbamates give thermal decomposition products, mainly substituted phenols. The molecular ions that arise from these products are more abundant in mass spectra. The compounds from other subclasses are more thermally stable. The molecular ions are of low intensity, but together with diagnostic fragments they enable identification to be made. In LC-MS methods, the carbamates do not present a serious problem. Positive ion detection with a soft ionisation technique is the method of choice (Niessen 1999). Some compounds, e.g. carbofuran and promecarb, can be determined by a chemiluminescence method (on-line conversion into methylamine by irradiation with UV light, and then reaction with tris(2,2′-bipyridine)ruthenium(III), which was generated through the on-line photooxidation of tris-2,2′-bipyridineruthenium(II) with peroxydisulfate) (Perez-Ruiz *et al.* 2002).

Toxicity Carbamate pesticides have a similar action to that of the OP compounds in causing a decrease in cholinesterase activity, but the binding to the active site of the cholinesterase enzyme is reversible. Consequently, although the symptoms are practically identical to those of organophosphorus poisoning, they have a shorter duration.

Blood and tissue levels Blood carbofurane concentrations in 15 non-fatally poisoned patients ranged from 0.15 to 2.78 mg/L, and in seven fatal cases concentration ranges were as follows (Kłys, Bialka 1990):

Blood	Liver	Kidney
0.31–3.90	0.09–9.34	0.54–6.29

In a case of oral ingestion of thiodicarb (which is unstable under acidic condition and rapidly hydrolysed to methomyl), the parent compound was detected only in gastric contents at a concentration of 24.3 mg/L. In all other fluids and tissues, various concentrations of methomyl – its metabolite – were detected. Methomyl concentrations (mg/L or mg/kg) were as follows (Hoizey *et al.* 2008):

Stomach contents	Peripheral blood	Urine	Bile	Liver	Kidney	Lung	Brain	Heart
19.9	0.7	8.5	2.7	0,7	1.7	1.5	9.3	3.6

Traces of methomyl were also detected in vitreous humour. Moreover, blood concentrations (mg/L) of zolpidem (2.87), bromazepam (2.39), nordazepam (4.21) and levopromazine (0.64) were above their common therapeutic ranges. In contrast to this case, blood and liver methomyl concentrations following methomyl ingestion in several published fatal case reports varied between 0.003 and 63.5 mg/L, and between 'not detectable' and 1.2 mg/kg, respectively (Hoizey *et al.* 2008). Blood methomyl levels in nine fatal cases ranged from 8 mg/L to 56 mg/L (Baselt 2004, pp. 700–701).

Blood methomyl concentrations (mg/L) in a case reported by Moriya and Hashimoto (1999) were site-dependent as shown below:

Blood						
Left cardiac chambers	Right cardiac chambers	Pulmonary arteries	Pulmonary veins	Thoracic aorta	Thoracic inferior vena cava	Right femoral vein
4.89	1.08	4.75	4.09	7.00	0.56	3.91
Cerebrospinal fluid	Pericardial fluid	Vitreous humour	Lung left hilus	Lung right hilus	Right kidney	Right femoral muscle
5.37	4.75	3.67	1.17	1.86	ND	2.61
Liver deep right lobe	Spleen	Cerebrum	Stomach contents	Bile	Urine	Myocardium
ND	ND	2.26	340	5.68	4.76	0.08

ND, not detected.

In three cases involving fatal ingestion of carbaryl, concentration ranges in blood and urine (mg/L) and tissues(mg/kg) were as follows (Baselt 2004, pp. 167–169):

Blood	Liver	Kidney	Brain	Urine
6–27	12–29	1.9–25	4.6	31

In a lethal poisoning with ethiofencarb and ethanol, concentrations of the parent carbamate compound and its two major metabolites (mg/L) in antemortem (taken on admission to hospital) and postmortem biological fluids (death occurred 3 h after admission, samples were taken at autopsy 2 days after death) were measured with the following results (Al Samarraie *et al.* 2009):

	Antemortem blood	Postmortem peripheral blood	Postmortem urine
Ethiofencarb (mg/L)	18.8	26.4	1.7
Ethiofencarb sulfoxide (mg/L)	25.6	37.9	41.2
Ethiofencarb sulfone (mg/L)	0.5	0.9	0.4
Ethanol (g/kg)	Not analysed	1.2	2.6

Chlorinated hydrocarbons

Analysis The chlorinated hydrocarbons chemical class of pesticides may be analysed intact using chromatography with a dual FID-NPD detection system, but greater sensitivity can be achieved using an ECD. Among hyphenated techniques, GC-MS is the method of choice for this class of pesticide. The methods applied in clinical and forensic cases do not need to be highly sensitive because most compounds that belong to this class are only slightly toxic and severe symptoms of poisoning are observed only after ingestion of large quantities (several grams). Moreover, the symptoms often result from the solvents in which the chlorinated hydrocarbons are formulated. Useful references for the determination of chlorinated hydrocarbons in human or avian serum are Brock *et al.* (1996) and Sundberg *et al.* (2006) using GC after SPE, and López *et al.* (2007) using GC-MS or GC-ECD.

Toxicity Chlorinated hydrocarbons are neurotoxins that also damage the liver and kidneys. Major clinical features of poisoning are headache, disorientation, paraesthesia and convulsions.

Blood and tissue levels A blood lindane concentration of 0.13 mg/L was measured in a patient who developed seizures after ingesting about 250 mg (Burton *et al.* 1991). An adult male who ingested 57 g of 1% lindane lotion and became comatose had a blood lindane concentration of 1.3 mg/L on the first day of admission to hospital and died 7 days later with a postmortem blood concentration of 0.02 mg/L (Kurt *et al.* 1986).

In a fatal case of endosulfan poisoning in a woman, the blood endosulfan concentration was 30 mg/L (Bernardelli, Gennari 1987).

In a case of endrin suicide poisoning, its concentrations (mg/L or mg/kg) in different postmortem materials were as follows (Moriya, Hashimoto 1999):

Blood					
Left cardiac chambers	Right cardiac chambers	Thoracic aorta	Thoracic inferior vena cava	Right femoral vein	Kidney
0.615	0.568	0.542	0.453	0.353	ND
Cerebrospinal fluid	Pericardial fluid	Vitreous humour	Lung left hilus	Lung right hilus	Myocardium
0.515	1.00	1.67	6.20	1.19	0.467
Liver deep right lobe	Urine	Cerebrum	Stomach contents	Bile	Right femoral muscle
13.8	ND	1.93	1100	2.06	2.08

ND, not detected.

Pyrethrins and pyrethroids

Analysis The term 'pyrethrins' is used collectively for the six insecticidal constituents present in extracts of the flowers of *Pyrethrum cinerariaefolium* and other species. Pyrethrins comprise esters of the natural stereoisomers of chrysanthemic acid (pyrethrin I, cinerin I and jasmolin I) and the corresponding esters of pyrethric acid (pyrethrin II, cinerin II and jasmolin II). Their low photochemical stability leads to synthetic analogues (pyrethroids), which are highly toxic to insects. In recent years pyrethroids have been manufactured and used in large quantities. These contain no nitrogen and therefore both GC-FID and GC-MS are appropriate detection systems. The compounds can be analysed easily by chromatography, either without derivatisation or after methylation (Bissacot, Vassilieff 1997; Cherstniakova *et al.* 2006; Fernández-Gutiérrez *et al.* 1998).

Toxicity Pyrethrins and pyrethroids have relatively low toxicity to humans, but exposure to these compounds by inhalation can cause localised reactions to the upper and lower respiratory tract, which leads to oral and laryngeal oedema, coughing, shortness of breath and chest pain. In acutely exposed sensitised patients a serious asthmatic-type reaction can be triggered that can prove fatal within a few minutes.

Nitrophenols and nitrocreosols

Analysis Dinitro-*o*-creosol (DNOC) can be measured in blood specimens by colorimetry (Smith *et al.* 1978).

Toxicity Dinitrophenol, dinitro-*o*-creosol and dinoseb stimulate oxidative metabolism in the mitochondria and cause profuse sweating, headache, tachycardia and fever.

Blood levels Blood and urine measurements are useful as an aid to diagnosis and treatment. Symptoms of headache and malaise are noted with DNOC blood concentrations of around 40 mg/L and serious intoxication has been observed in workers with concentrations of 44–60 mg/L (Bidstrup *et al.* 1952). In a fatal case, the postmortem blood concentration was 75 mg/L (Bidstrup *et al.* 1952). In two deaths attributed to the

use of dinitrophenol, its concentrations in blood were 28 and 36.1 mg/L (Miranda *et al.* 2006). In another case involving ingestion of dinitrophenol together with medicinal drugs, the postmortem blood contained 48.4 mg/L of dinitrophenol, 1.2 mg/L of 2-amino-4-nitrophenol, citalopram and its desmethylated metabolite (in toxic concentrations), and olanzapine, desalkylflunitrazepam and nordiazepam (in therapeutic or subtherapeutic concentrations) (Politi *et al.* 2007).

Chlorinated phenoxy acids

Analysis Substituted phenoxy acids occur in commercial products as salts or esters. Conversion of salts by extraction and derivatisation to the corresponding methyl esters improves their chromatographic properties. The presence of isooctyl (2,2,4-trimethylpentyl) esters of chlorinated phenoxy acid herbicides can be indicated using mass chromatography with ions of *m/z* 41, 55, 57, 69, 71 and 85, as well as high-mass ions of significant intensity (Fysh, Whitehouse 1986).

Toxicity Chlorinated phenoxy acids are corrosive chemicals that damage the skin, eyes, and respiratory and gastrointestinal tracts. Ingestion of large doses causes vomiting, abdominal pain, diarrhoea, metabolic acidosis, pulmonary oedema and coma. Some herbicide preparations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been shown to contain a contaminant, dioxin (2,3,7,8-tetrachlorodibenzodioxin), which is a potent teratogenic agent. Alkalinisation of the urine to increase the excretion of 2,4-D and other chlorophenoxy compounds after poisoning has proved an effective therapy.

Blood and tissue levels A patient who survived after taking 7 g of 2,4-D had an initial plasma concentration of 400 mg/L (Park *et al.* 1977). In another non-fatal case, a maximum 2,4-D plasma level of 1031 mg/L was recorded (Rivers *et al.* 1970). In five fatal cases, blood 2,4-D concentrations have ranged from 58 mg/L to 826 mg/L (Baselt 2004, pp. 323–325). A patient who died 30 h after taking a mixture of 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP; 44 g), 2,4-D (39 g) and chlorpyrifos had blood concentrations of 389 µg/g MCPP and 325 µg/g 2,4-D. Postmortem tissue concentrations were also reported (Osterloh *et al.* 1983).

Ingestion of 4-chloro-2-(methylphenoxy)acetic acid (MCPA) led to the following concentrations of MCPA and its metabolite, *p*-chloro-*o*-cresol (mg/L or mg/kg) in postmortem materials (Takayasu *et al.* 2008):

	Heart blood	Peripheral blood	Urine	Brain	Left lung	Right lung	Liver	Left kidney	Right kidney	Stomach contents
MCPA	888.3	578.1	52.2	770.9	2864	1 362	1 135	867.1	755.5	10 200
p-Chloro-*o*-cresol	2.16	1.92	0.24	1.64	9.06	7.41	10.36	5.56	4.85	16.45

Triazines

Analysis Triazines contain several nitrogen atoms and show enhanced NPD and FID ratios. Most triazines, which are readily amenable to GC-MS, exhibit highly characteristic mass spectra of the parent compounds and yield the important degradation products hydroxy- and desalkyl triazines. By using LC-MS with APCI and ESI, and optimising the in-source parameters, the protonated triazine molecule can be seen without fragmentation (Niessen 1999). Quantification of atrazine metabolites, e.g. atrazine mercapturate, desethyl and desisopropyl atrazine, as markers of atrazine exposure, in urine has been achieved using isotope-dilution calibration of an LC-MS(-MS) method (Nguyen *et al.* 2007).

Toxicity Ingestion of about 100 g of atrazine can lead to coma, circulatory collapse, metabolic acidosis and gastric bleeding. This may be followed by renal failure, hepatic necrosis and a disseminated intravascular coagulopathy that may prove fatal. Haemodialysis is recommended for severe cases.

Blood levels An adult died 3 days after taking approximately 100 g of atrazine together with ethylene glycol and formaldehyde; 1 h after ingestion the plasma concentration was 2 mg/L (Pommery *et al.* 1993).

Quaternary ammonium compounds

Analysis Paraquat and diquat are not extractable by conventional LLE. A recently published comparison study of different extraction procedures showed that a chloroform–ethanol 7 : 3 (v/v) solvent mixture was the most effective extraction solvent (Baeck *et al.* 2007). The diene or monoene reduction products of paraquat and diquat produced by sodium borohydride can be extracted by diethyl ether from alkaline solution for chromatography (Draffan *et al.* 1977). Very limited data are available for the mass spectral characterisation of these compounds (de Almeida, Yonamine 2007). Colorimetric determination of paraquat and diquat after reduction with sodium dithionite under alkaline conditions is probably the most widely used technique. Both of the bipyridylium reduction products have absorbance maxima at 396 and 379 nm. Using an ion-pairing extraction technique, a lower limit of measurement of 50 µg/L can be achieved (Jarvie, Stewart 1979). However, it is not possible to include an internal standard in this method. Radioimmunoassay (Fatori, Hunter 1980) and fluorescence polarisation immunoassay (Colbert, Coxon 1988) methods for the determination of paraquat in serum are very sensitive and require only small sample volumes, but they are not widely available. Paraquat can also be determined in serum by HPLC-UV after extraction onto a disposable cartridge of end-capped octadecyl silica (Fig. 16.4) (Croes *et al.* 1993) down to a concentration of 25 µg/L. Diquat may be analysed in biological specimens by most of the procedures described for paraquat. Specific HPLC procedures for paraquat and/or diquat have also been described (Ameno *et al.* 1995; Arys *et al.* 2000; Hara *et al.* 2007).

Difenzoquat, diquat and paraquat can be quantified in human whole blood in addition to other quaternary ammonium compounds by LC-MS(-MS) with positive ESI following SPE and ion-pair chromatography (Ariffin, Anderson 2006).

Toxicity Ingestion of concentrated paraquat formulations causes burning of the mouth, oesophagus and stomach, and after massive absorption patients die of multiple organ failure. Absorption of smaller amounts can lead to renal damage followed by a progressive pulmonary fibrosis that causes death from respiratory failure, in some cases after 2–3 weeks of ingestion. Treatments to reduce absorption or increase elimination have not been effective. A strongly positive urine test with the dithionite test in a sample collected more than 4 h after ingestion indicates a poor prognosis. Measurement of the plasma paraquat concentration is a more accurate prognostic guide. Diquat is also an irritant poison that causes vomiting, diarrhoea and epigastric pain. In severe cases, liver and renal failure, convulsions and coma may ensue, but diquat ingestion does not lead to progressive pulmonary fibrosis.

Blood levels A fatal outcome is usually associated with plasma paraquat concentrations greater than 0.2 mg/L at 24 h after ingestion and 0.1 mg/L at 48 h after ingestion (Scherrman *et al.* 1983). In a fatal case of accidental paraquat poisoning a blood concentration of 0.64 mg/L of paraquat was reported (Ariffin, Anderson 2006). In fatal cases of diquat poisoning, plasma diquat concentrations ranging from 0.45 mg/L to 4.5 mg/L have been recorded (Vanholder *et al.* 1981).

Phosphides

Analysis The ammonium molybdate test and commercially available detector tubes (Guale *et al.* 1994) may be used as qualitative and quantitative procedures for stomach contents and non-biological materials. Phosphine can also be determined in biological samples using GC and NPD detection (Chan *et al.* 1983; Musshoff *et al.* 2008).

Toxicity Hydrogen phosphide is widely used as an insecticide and rodenticide (agricultural fumigant) and is usually generated by the action of water on metallic phosphides (aluminium, magnesium or zinc). Inhaled

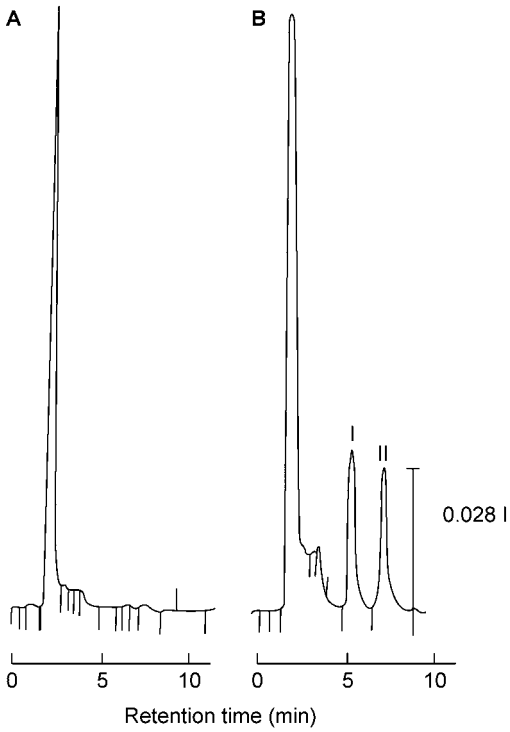

Figure 16.4 HPLC chromatograms of extracted human serum with UV detector (Microspher C18 column, λ = 258 nm). A, blank serum; B, spiked serum sample with (I) 500 µg/L paraquat, RRT 0.75, and (II) 2500 µg/L 1,1-diethyl-4,4′-bipyridyl chloride (internal standard), RRT 1.00. Reproduced with permission from Croes *et al*. (1993).

phosphine is readily absorbed by the lungs. Following the ingestion of metallic phosphides, phosphine is generated in the stomach and the gas acts on the gastrointestinal and central nervous systems. In severe cases abdominal pain, vomiting, convulsions and coma develop rapidly and death usually ensues within 2 h.

Blood and tissue levels Postmortem blood in a man who ingested an unknown number of aluminium phosphide tablets contained 0.5 µg/L of phosphine. The tissue phosphine concentrations were 3 µg/kg in liver and 3000 µg/kg in stomach contents (Chan *et al.* 1983). In another case after accidental ingestion of novel rodenticide pills also containing aluminium phosphide, the following phosphine concentrations (mg/kg) were found:

Stomach contents	Nose smear	Small intestine
0.2	0.56	0.28

Urine, femoral and heart blood, liver, kidney, bile and brain samples tested negative (Musshoff *et al.* 2008).

Thallium

Analysis Several convenient colorimetric methods can be applied to determine thallium in urine, stomach contents and suspect preparations. One of these methods (Flanagan *et al.* 1995) is based on measuring the absorbance of a chloroform-extractable pink–red thallium–dithizone complex from an alkaline solution that contains potassium, sodium and cyanide ions to mask interference from other metal ions. It indicates the presence of thallium in urine at concentrations of 1 g/L or more. However, the method is not specific and atomic absorption spectrophotometry is a much more reliable technique (see Chapter 43).

Toxicity Poisoning with thallium salts leads to delayed damage to the peripheral and central nervous systems. The cardiorespiratory system is also affected. Loss of body hair 1–2 weeks after ingestion is a characteristic sign of thallium poisoning. The most reliable diagnostic procedure is a quantitative thallium determination in serum and/or urine. Treatment with potassium ferrohexacyanoferrate (Prussian or Berlin blue) to absorb thallium re-circulated in the bile is effective if instituted early enough; serum and urine thallium measurements are useful in monitoring the progress of the therapy.

Blood levels Blood thallium concentrations in fatal cases of poisoning have ranged from 0.5 mg/L to 11 mg/L (Baselt 2004, p. 1092). The following tissue concentrations (mg/kg) of thallium were determined in three people who died after ingestion of this poison (Sadlik 1997):

	Woman, age 63	Man, age 58	Boy, age 17
Liver	81.0	59.2	12.1
Kidney	62.5	38.5	11.8

In a 15-year-old boy who survived the intentional ingestion of thallium sulfate, blood and urine concentrations (mg/L) examined during treatment were as follows (Lech, Sadlik 2007):

	Day 14	Day 24	Day 31
Blood	0.88	0.44	0.36
Urine	2.35	3.35	5.00

Uracils

Analysis Uracils are pyrimidine herbicides that can be analysed intact and also after derivatisation. HPLC with UV detection is a good technique for confirmation (Lawrence, Turton 1978). Quantities of 200 ng or 20–50 ng of these compounds at 254 nm or 270–280 nm, respectively, give a response equivalent to 0.01 absorbance units (Lawrence, Turton 1978). The use of HPLC and atmospheric pressure ionisation (API, both ESI and APCI) MS in positive- and negative-ion mode for qualitative analysis of uracils (e.g. bromacil) has also been published (Schreiber *et al.* 2000).

Toxicity No serious cases of human poisoning with these compounds have been reported.

Substituted ureas

Analysis These herbicides, especially phenyl- and sulfonylureas, contain nitrogen and respond to both FID and NPD. They are very thermolabile and cannot be derivatised readily. Analysis by GC is complicated, as it is impossible to avoid the formation of their numerous decomposition products because of the high injection port temperature. To reduce the formation of additional artefactual products, inert solvents (ethyl acetate or hexane) should be used to reconstitute the extracts. Protonated substituted ureas without fragmentation have been observed by LC-MS in APCI mass spectra. The appearance of spectra obtained by thermospray LC-MS is highly dependent on the analytical conditions (Niessen 1999).

LC-MS(-MS) methods with positive ion and MRM modes for measurement of dichlorophenyl urea, dichlorophenylmethyl urea, diuron and linuron as markers of phenylurea herbicide exposure and of dimethoxypyrimidine, dimethylpyrimidine and methoxymethyl triazine as markers for sulfonylurea herbicide exposure in urine have been developed by Nguyen *et al.* (2007).

Toxicity No serious cases of human poisoning as a result of ingesting this class of pesticides have been reported.

Coumarin anticoagulants

Analysis Warfarin and the superwarfarin anticoagulant rodenticides (brodifacoum, bromadiolone, coumatetralyl and difenacoum; Fig. 16.5) can be analysed either intact or after derivatisation, by either GC or GC-MS methods, these being the most sensitive and selective. For biological samples (plasma and urine), extractive methylation using the phase-transfer reagent tetrahexylammonium hydrogensulfate, methyl

iodide and then SPE has been developed as part of a systematic toxicological system (Maurer, Arlt 1998). Using MS with ions of *m/z* 291, 294, 295, 309, 313, 322, 324, 336, 343 and 354, the presence of 4-hydroxycoumarin anticoagulants and even the hydroxy metabolites of coumachlor, coumatetralyl and warfarin can be detected (Maurer, Arlt 1998). Five of the 4-hydroxycoumarin anticoagulants (brodifacoum, bromadiolone, coumatetralyl, difenacoum and warfarin) can also be resolved and determined in serum by HPLC with fluorimetric detection (Felice *et al*. 1991), using the following method.

1. To 2 mL of serum add 4 mL of acetonitrile, vortex for 10 s and then centrifuge for 5 min at 700*g*.
2. Decant the supernatant liquid to another test tube, add 8 mL of diethyl ether and vortex mix for 2 min and then centrifuge.
3. Separate the ethereal phase (approximately 7 mL); evaporate to dryness at 50–55°C under a stream of nitrogen.
4. Dissolve the residue in 150 µL of methanol and add 50 µL of mobile phase.
5. Inject an aliquot of 50 µL into the HPLC apparatus:
   - Detector: fluorescence (λ_{ex}=318 nm, λ_{em}=390 nm).
   - Column: C_{18} Ultrasphere, 250 × 4.6 mm.
   - Mobile phase: gradient mixture of 0.05 mol/L ammonium acetate buffer (with addition of 2 mL of glacial acetic acid and 2 mL of triethylamine in 1 L of aqueous phase) and methanol (38 : 62 for 2 min to 18 : 82 at 5 min for 8 min to 10 : 90 at 13.5 min for 1.5 min to 38 : 62 at 17 min).

Recoveries average from 68% (warfarin) to 98% (bromadiolone). Bromadiolone and warfarin can be quantified with a precision of at least ±10% at serum concentrations of 20 µg/L, and brodifacoum, coumatetralyl and difenacoum at serum concentrations of 10 µg/L.

A series of procedures for the screening, confirmation and quantification of superwarfarin anticoagulant rodenticides in serum or blood by LC-MS(-MS) has been published recently. Grobosch *et al.* (2006) developed a method for the simultaneous determination of five superwarfarins (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) and five other vitamin K antagonists (acenocoumarol, coumatetralyl, coumachlor, phenprocoumon and walfarin) in 0.5 mL serum samples. Jin *et al.* (2007) determined bromadiolone in 0.2 mL whole blood specimens.

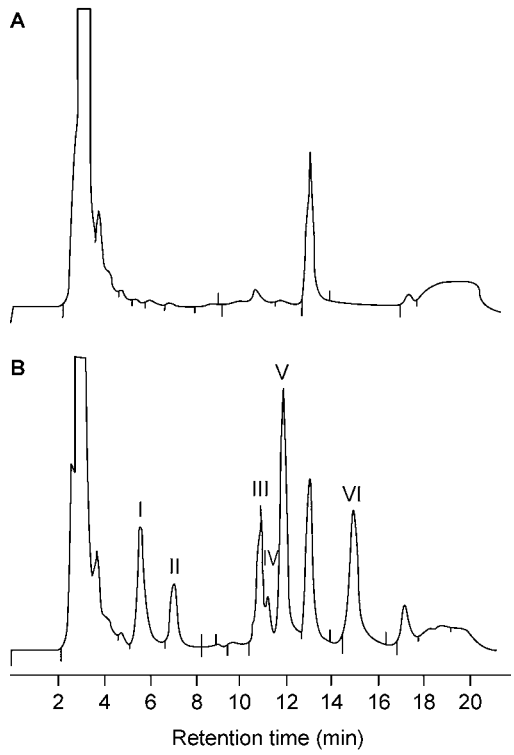

Figure 16.5 HPLC chromatograms of extracted canine serum (fluorescence detector, Ultrasphere C_{18} column). A, blank canine serum; B, canine serum spiked with 50 µg/L of coumatetralyl (I), difenacoum (V) and brodifacoum (VI) and 100 µg/L of warfarin (II) and bromadiolone (III, IV). Reproduced with permission from Felice *et al*. (1991).

Extraction Adamowicz and Kała (2009) evaluated the following LC-ESI-MS method for the detection and quantification of six anticoagulants:

1. To 1 mL of blood add 50 µL (10 µg/mL) of nimesulide (IS), 1 mL of acetate buffer (pH 5.5) and 6 mL of chloroform–acetone (1 : 1, v/v).
2. Shake the mixture for 20 min and then centrifuge for 5 min at 6000*g*.
3. Transfer 5 mL of lower organic layer to a clean tube and evaporate to dryness at 50°C on a heating block under a stream of nitrogen.
4. Reconstitute the dry residue in 100 µL of mixture consisting of acetonitrile–water (1 : 1, v/v).
5. Inject an aliquot of 20 µL into the HPLC using an autosampler.

Chromatography

- Column: LichroCart Purospher RP-18e, 125 × 3 mm, Merck.
- Mobile phase, gradient mixture of 0.1% (v/v) of formic acid in water–acetonitrile (90 : 10 to 0 : 100 at 10 min for 5 min to 90 : 10 at 16 min for 4 min).
- Detector, triple-quadrupole mass spectrometer, operated in ESI negative-ion and selected-ion recording (SIR) modes
- Recorded ions (*m/z*): warfarin, 161 and 250; nimesulide (IS), 229; coumatetralyl, 291 and 292; bromadiolone, 525 and 527; difenacoum, 443 and 444; brodifacoum, 523 and 521; difethialone, 539 and 537.
- Validation parameters: 9-point (0, 10, 50, 100, 200, 500, 1000, 2000 and 5000 µg/L) calibration curves were linear from limit of quantifications (LOQs) up to 5000 µg/L; LOQs (S/N>10) (µg/L) were 10 for coumatetralyl, 15 for warfarin, 50 for bromadiolone, 60 for brodifacoum and difenacoum, and 200 for difethialone.
- Retention times are shown in Fig. 16.6.

Other parameters were determined at blood concentrations of 100 µg/L. Recoveries averaged from 65% (warfarin) to 81% (brodifacoum). Within-day and between-day precision was less than 15% RSD. Specificity was checked using ten blank blood samples (including post-mortem samples) from different sources and no interfering peaks were observed.

The same extraction procedure and HPLC-DAD method was successfully applied for the identification of above-mentioned active anticoagulant components in commercial rodenticide preparations and waters, drinks and foodstuffs (soups, sausages, ground coffee) to which a rodenticide has been added for criminal purposes, and in stomach contents taken from animals (dogs, seals, spoonbills) suspected of having been poisoned and stomach washings in emergency cases of severe poisoning (Adamowicz, Kała 2005).

Toxicity Accidental and intentional ingestion of 4-hydroxycoumarin rodenticides can lead to serious poisoning manifested by bleeding in multiple organ sites. Treatment consists of supplements of vitamin K (mild cases) and, for serious cases, infusions of fresh frozen plasma or purified clotting factors until the prothrombin time returns to the normal range.

Blood and tissue levels Cases of serious poisoning are generally associated with serum concentrations greater than 5 mg/L, but for some derivatives (e.g. acecoumarol and brodifacoum) toxicity appears at much lower concentrations (Geldmacher-v.Mallinckrodt 1997).

A limited number of cases of non-fatal intoxications by coumarin anticoagulants have been reported where serum concentrations (µg/L) were measured. These can be summarised as follows (Grobosch *et al.* 2006):

Brodifacoum	Difenacoum	Bromadiolone
n = 3	*n* = 2	*n* = 2
630, 710, 731	600, 970	40, 440

In a case of bromidiolone, poisoning the whole-blood concentration was 51 µg/L (Jin *et al.* 2007). In a fatal poisoning following ingestion of a

rodenticide containing 0.005% of brodifacoum, its concentrations (μg/L or μg/kg) were as follows:

Heart blood	Femoral blood	Bile	Liver	Spleen	Lung
2240	3919	4276	50	34	31

Brodifacoum was not detected in vitreous humour or in brain tissue. It is worth adding that in liver preserved by formalin and in formalin solution after preservation concentrations of brodifacoum were 820 μg/kg and 5440 μg/L, respectively. The most reasonable explanation for such high levels of brodifacoum in the fixed liver and formalin fixative was significant release of brodifacoum from hepatic tissues (Palmer *et al.* 1999).

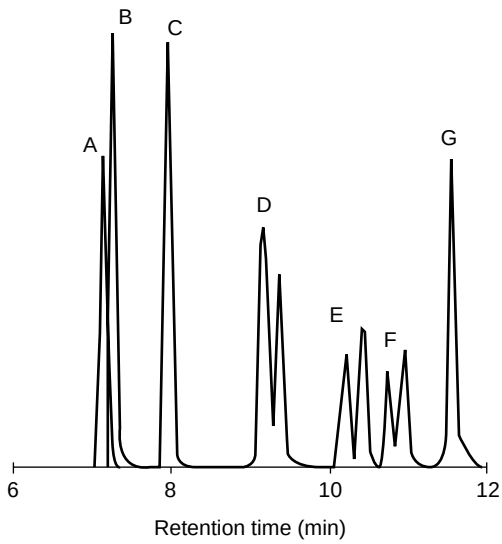

Figure 16.6 LC-MS chromatogram (mass detector, operated in ESI negative-ion and SIR modes; Purospher RP-18e). The extract of control blood spiked with: A,100 μg/L of warfarin; B, 500 μg/L of nimesulide (IS); C, 100 μg/L of coumatetralyl; D, 200 μg/L of bromadiolone; E, 200 μg/L of difenacoum; F, 200 μg/L brodifacoum; G, 400 μg/L of difethialone. Reproduced with permission from Adamowicz and Kała (2009).

Organic and inorganic metallic compounds

A wide range of organic and inorganic metallic compounds is found in agricultural use. Inorganic and organometallic compounds are used as acaricides (organotin), herbicides (organoarsenic), fungicides (dithiocarbamate compounds of nickel and dithiocarbamate complexes with manganese and zinc, organic and inorganic compounds of copper and mercury) and rodenticides (magnesium, aluminium and zinc phosphides, and thallium sulfate). For some compounds, exposure to the organic form results in more serious toxicity and the features of poisoning may differ significantly from those of the inorganic compound. For example, whereas inorganic tin compounds are relatively innocuous, the organic forms (ethyl, butyl or phenyl derivatives), used mainly as molluscicides, present a serious hazard because of their lipid solubility, which allows rapid uptake into the central nervous system. This can lead to partial paralysis, visual and psychic disturbances, convulsions and death from respiratory or cardiac failure. Ingestion of inorganic mercury compounds causes gastrointestinal problems (vomiting, diarrhoea) and renal failure, whereas the lipid-soluble organic mercurials (e.g. the dimethyl and diethyl derivatives) concentrate in the central nervous system and lead to ataxia, chorea and convulsions. Numerous techniques, based on atomic absorption spectrophotometric detection and colorimetric detection, can be used to analyse these compounds (see Chapters 30 and 43). Since most methods require prior destruction of the organic matrices (wet or dry), LC-MS techniques have evolved, in particular for the analysis of organotin compounds (Niessen 1999).

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CHAPTER

17 Metals and Anions

R Braithwaite

General introduction

Metals and anions form an important, but disparate, group of agricultural, household and industrial poisons that present many difficulties in their systematic chemical analysis (Baldwin and Marshall 1999; Yeoman 1985). Severe acute poisoning with these agents is rare in most developed countries, but remains common in many developing parts of the world. Chronic poisoning, as a result of occupational or environmental exposure to heavy metals and pesticides, occurs in many countries and can be a cause of ill-health in both adults and children.

The signs and symptoms of acute poisoning may differ from those associated with chronic toxicity. Some metallic (e.g. arsenic) and anionic (e.g. cyanide) substances undergo extensive metabolism after ingestion. These factors have a significant bearing on analytical investigations applied to biological materials and their interpretation. It is important in individual cases, therefore, to know whether poisoning resulted from acute, chronic or acute-on-chronic exposure. Of equal importance is the time of specimen collection in relation to the alleged time of ingestion or exposure.

The wide range of metallic or anionic poisons that might be involved in any case of suspected poisoning means that great care is required in the collection of appropriate specimens and the selection of toxicological and other tests. There is no simple systematic way to investigate cases for which the history is uncertain and the identity of the poison unknown. The investigation is often led by a process of elimination of the more likely causes of poisoning (e.g. pharmaceuticals and illicit drugs), and then a careful examination of the detailed history of the patient or deceased, in particular any access to compounds associated with industrial and agricultural use, or specific household products.

Considerable advances in analytical techniques for measuring metals in biological fluids have been made since the early 1980s, particularly in electrothermal atomic absorption spectrometry (ETAAS) (Halls 1984; Slavin 1988) and inductively coupled plasma–mass spectrometry (ICP-MS) (Hsiung *et al.* 1997). These techniques are discussed in detail in Chapter 43. The use of ICP-MS has expanded rapidly in the last 5 years and it is now the technique of choice for sensitive multi-element analysis, particularly in the investigation of suspected poisoning of 'heavy metals' or less common inorganic poisons. When investigating chronic exposure to toxic metals, great care must be taken to control the accuracy and precision of biological analyses. This calls for ready access to a supply of reliable internal quality-control materials and regular participation in external quality-assurance programmes. These are often not available in developing countries, but much can be done at relatively low cost with cooperation between laboratories (Braithwaite and Girling 1988; Halls 1984).

The development of analytical techniques for anions in biological fluids has historically been limited. The increased availability of liquid chromatography–mass spectrometry (LC-MS) techniques has greatly improved matters. However, a number of simple methods for measuring selected poisons remain valid and have a role to play where access to more sophisticated equipment is restricted (Flanagan *et al.* 1995).

Specimen collection and analysis

In cases of suspected poisoning admitted to hospital, specimens of blood and, where possible, urine should be taken (see Chapter 1). These should be labelled carefully (full name of patient, admission number and date and time of collection) and stored in a refrigerator (4°C) pending any delay in transport to the laboratory. When blood specimens are received in the laboratory and analyses are not required immediately, it is useful to separate off plasma or serum from red cells, prior to deep freezing. However, for toxins that have a significant distribution into the red cells (e.g. lead, cadmium, mercury and cyanide), it is essential to conserve samples of anticoagulated whole blood. In postmortem examinations it is important to undertake a more systematic specimen collection and great care is required in the selection of sampling sites, method of collection and use of appropriate specimen containers (see Chapter 10).

Where an industrial accident has occurred there may be access to 'scene residues' or materials used in a chemical process. Analysis of these materials can yield invaluable clues when the precise nature of the chemical agent is unknown. However, prior to transportation to the laboratory, separate packaging from any biological specimens is advisable to avoid the risk of contamination.

Stomach contents

Vomit, stomach aspirate and washout fluid are now rarely available from cases of acute poisoning admitted to hospital. Stomach washout procedures (except in rare cases) have been replaced by the administration of oral activated charcoal in most developed countries. In fatal cases, the whole stomach and its contents can be removed *post mortem* for detailed examination.

When dealing with the initial examination of stomach contents, or vomitus, it is helpful to note any unusual smell, colour or other appearance, such as the presence of fresh or altered blood (coffee grounds; see Chapter 1). Great care should be taken when dealing with cases that involve the oral ingestion of cyanide salts or phosphides, as the contents of the stomach may represent a serious hazard and risk of secondary poisoning (a fume cupboard or safety cabinet must be used in these circumstances). The total volume of the stomach contents should be recorded. These can then be homogenised in a blender to allow two or three representative smaller specimens to be taken for both qualitative and quantitative analysis.

Homogenised specimens can be filtered directly using a coarse-grade filter paper or diluted with distilled water. The clear supernatant or a representative sample of the homogenised stomach contents can be used for analysis. Quantitative analysis of the stomach contents is generally of interest only in medicolegal cases in which the quantity of chemical poison present in the stomach contents may provide useful forensic evidence.

Blood

Venous blood (10 mL) should be collected in all cases of suspected poisoning; for metallic poisons and anions, such as cyanide, a potassium ethylenediamine tetraacetic acid (K-EDTA) container is the most appropriate. A wide range of blood-collection tubes is available commercially; to avoid the possibility of contamination, the use of products certified as suitable for trace-element analyses is strongly recommended, particularly when dealing with environmental or subclinical exposure to agents such as lead and cadmium. Blood-collection tubes that contain gel separation barriers should not be used. Alternatively, the laboratory can purchase relatively large batches of standard blood-collection tubes

(all with the same batch number) and carefully test a few tubes from each batch to ensure that they are free of contamination with those metals of clinical interest (e.g. lead, cadmium and aluminium). When blood specimens are received in the laboratory in an unusual container, it may be useful to request a 'blank' container that can be analysed for the presence of any contaminating substance.

It is equally important to ensure that reliable blood-specimen containers are used for postmortem examinations. These can be supplied to the pathologist ahead of any postmortem examination as part of the standard specimen collection kit. It is recommended that, at any postmortem examination, blood should be taken from an identified anatomical site (preferably the femoral vein) for quantitative analysis, since after death significant diffusion or redistribution of poisons may occur (see Chapter 10). Other sites (e.g. the heart) may be useful for qualitative 'screening' purposes.

Blood should never be taken from the body cavity at the end of the postmortem examination. The main problem is that blood samples taken from different sites, e.g. femoral, subclavian, aorta, at postmortem examination show different concentrations. In cases of rapid death, where there may be large quantities of unabsorbed poison present in the stomach, or aspirated into the lungs, this can undergo significant diffusion after death, leading to very high concentrations in adjacent blood vessels.

Urine

An early specimen of urine (20 mL in a plain Sterilin-type container with no preservatives added) should be collected, with care taken to ensure that the sample is not contaminated during the collection process. If patients are undergoing chelation therapy, it can be useful to collect sequential 24 h urine specimens into acid-washed plastic urine containers. The total volume of urine should be recorded carefully prior to taking aliquots of specimens for analysis to determine the excretion of chelated elements and the efficacy of treatment.

Other specimens

Hair analysis for trace elements has often been used for diagnostic purposes in cases where an individual complains of symptoms for which no cause can be found by routine medical or pathological investigations. However, experience and published studies show that the results can sometimes be misleading (Poon *et al.* 2004; Seidel *et al.* 2001; Taylor 1986). Hair analysis has more application in surveys of population exposure and in investigations of suspicious deaths, such as those that involve arsenic or mercury. The long persistence of metals and many other chemical poisons in hair and nail samples compared with their relatively short duration in blood or urine specimens can be a major advantage in forensic cases (Daniel *et al.* 2004). Analysis of hair and nail sections can also sometimes yield an important chronological forensic record of when doses were administered.

Specimens such as tissues and bone may sometimes be collected at postmortem examination as part of the investigation of complex medicolegal cases (Benes *et al.* 2000; Lech and Trela 2005; Warren *et al.* 2002). As with blood specimens, great care is required in selecting the site of collection and to avoid contamination. In addition, interpretation of the results without access to comparable reference specimens can be very difficult.

Quality assurance

Access to appropriate 'internal' control or other reference materials is very important for the analysis of trace metals. A wide variety of certified reference materials, including blood, serum, urine and bone, is available commercially. Where these cannot be obtained easily, an inexpensive alternative is to prepare such materials from bovine blood, or any suitable large animal as a cooperative effort between experienced laboratories (Braithwaite and Girling 1988). This entails the preparation of large batches of samples with trace metal concentrations at, for example, normal, borderline toxic levels and toxic levels, which can then be analysed repeatedly by the laboratories so that the pooled results can be used to assign concentration ranges. It is equally important for laboratories to subscribe to national or international external quality-assurance schemes where these are available.

Table 17.1 Common methods of analysis for trace elements in biological fluids and tissues

Colorimetric
Fluorimetric
Electrochemical (anodic stripping voltametry and ion-selective electrodes)
Flame atomic absorption spectrometry
Electrothermal atomic absorption spectrometry
Inductively coupled plasma–emission spectrometry
Inductively coupled plasma–mass spectrometry

Metals

Methods of analysis

A wide range of techniques is available to laboratories for metals analysis (Table 17.1) and these are described in Chapter 43. A few simple qualitative and quantitative tests (e.g. the Gutzeit and Reinsch tests) can be useful, particularly where laboratory resources are severely limited (Flanagan *et al.* 1995).

Aluminium

Introduction

Aluminium is the most abundant non-essential element in the earth's crust, but its role in human health and disease became understood only recently (Krewski *et al.* 2007; Martin 1986). The normal intake of aluminium from food, water and beverages is probably 2–3 mg per day, but its absorption from the gut is relatively poor and depends on the speciation of the element and the presence of other substances (e.g. phosphate and citrate) in the diet (Klein 2005). Aluminium is a powerful neurotoxin, and it is well established that excessive intravenous exposure in patients undergoing dialysis results in its significant accumulation in the body, particularly in the brain, which can cause 'dialysis dementia', a type of encephalopathy that can be rapidly progressive and lead to death within a few months (Alfrey *et al.* 1976). Use of aluminium sulfate as a flocculating agent in domestic water supplies is the major source of the metal in these patients, particularly if the water used for dialysis is not purified. The large quantities of oral aluminium salts that may be given to some renal patients to reduce the intestinal absorption of phosphate may also cause toxicity. Aluminium may also be a suspected risk factor in Alzheimer's disease (Flaten 2001; Miu and Benga 2006). More recently, concern has developed about the harmful effects of occupational exposure to aluminium (Akila *et al.* 1999; Iregren *et al.* 2001; Meyer-Baron *et al.* 2007; Polizzi *et al.* 2002; Rifat *et al.* 1990). Attention has also been drawn to the possible harmful effects of aluminium present in infant formulae (Freundlich *et al.* 1985; Sedman *et al.* 1985).

Specimen collection

Specimens are collected as follows:

- Blood/serum/plasma – 5 mL, plain or lithium–heparin tube
- Water – 20 mL, plastic universal container
- Dialysis fluid – 20 mL, sterile plastic universal container
- Urine – 20 mL, sterile plastic universal container.

It is important to check that all specimen containers are 'aluminium free'. Glass containers should never be used to collect specimens for aluminium analysis.

Analysis

Constant vigilance is required to avoid contamination of specimens, reagents, tubing and equipment from aluminium in dust in laboratory areas. Cleaned glass containers should be used only to prepare concentrated stock solutions of aluminium salts. Great care is required to avoid

Table 17.2 Recommended water and dialysate aluminium concentrations

Solution	*Concentration*
Maximum allowable concentration (MAC) for potable water	200 µg/L (7.4 µmol/L)
Guideline concentration for potable water	50 µg/L (1.9 µmol/L)
MAC for water for preparation of dialysis fluid	10 µg/L (0.4 µmol/L)
Recommended upper limit for dialysis fluid	30 µg/L (1.1 µmol/L)

contamination when separating plasma or serum from blood specimens. Storage conditions of specimens are important (Wilhelm and Ohnesorge 1990). Aluminium is most commonly measured in plasma using ETAAS with background correction. The use of matrix-matched calibration standards and control material is essential (Taylor *et al.* 1994). Other methods have been described based on colorimety and fluorimetry but these are more suitable for water analysis. More recently there has been the increased use of ICP-AES and ICP-MS to measure aluminium in plasma, water and dialysate or other more complex matrices (Murko *et al.* 2007; Zhang *et al.* 2000, 2002).

Interpretation

The recommended (European) guidance values for water and dialysate aluminium concentrations are shown in Table 17.2. In patients on haemodialysis, in which a water purification system (such as reverse osmosis) is in operation, it is important to monitor aluminium in pre- and post-water samples to detect any breakdown in the purification process that would allow contamination of the dialysis solution used to treat the patient (Berend *et al.* 2001).

Plasma aluminium concentrations should be monitored routinely in all patients in end-stage renal failure who receive dialysis therapy, to ensure that absorption of aluminium is kept to an absolute minimum. In addition, regular testing is needed of water supplied to patients who have home dialysis. European guidelines for plasma aluminium concentrations in patients who receive dialysis therapy are given in Table 17.3. The body burden of aluminium in dialysis patients can be obtained from the measurement of plasma aluminium concentration before and after the administration of a standard dose of an aluminium-chelating agent such as desferrioxamine (Chazan *et al.* 1989; Marumo *et al.* 1987).

Where renal function is normal, aluminium is excreted rapidly from the body and there is little possibility of accumulation. The reference value for urine aluminium in non-exposed healthy adults is less than 15 µg/L (0.5 µmol/L). In situations of acute or chronic occupational or environmental exposure to aluminium, measurement of blood (plasma) and/or urine aluminium is an effective way to assess the degree of exposure (Ljunggren *et al.* 1991; Pierre *et al.* 1995). Where occupational exposure involves inhalation of fine particles or dusts, aluminium may be stored in the lung tissue and leach out very slowly over several months. As a result, plasma and urine aluminium concentrations can remain elevated for several weeks or months (Ljunggren *et al.* 1991).

Table 17.3 Recommended action limits for plasma aluminium concentrations

Situation	*Concentration*
Normal (no history of chronic renal failure)	<10 µg/L (<0.4 µmol/L)
Desirable in chronic renal failure	≤60 µg/L (<2.2 µmol/L)
Excessive accumulation	>60 µg/L (>2.2 µmol/L)
Cause for concern; high risk of toxicity in children	>100 µg/L (>3.7 µmol/L)
Urgent action required; high risk of toxicity in all	>200 µg/L (>7.4 µmol/L)

There is also evidence of a dose-dependent association between increased aluminium body burden and central nervous system effects in these workers (Akila *et al.* 1999). Suggested thresholds for these effects in aluminium welders are 108–160 µg/L (4–6 µmol/L) in urine and 7–10 µg/L (0.25–0.35 µmol/L) in plasma (Riihimaki *et al.* 2000).

Aluminium accumulates in the bone of dialysis patients and bone biopsy samples can be analysed to estimate the body burden (Tang *et al.* 1996). Extensive data are available on the aluminium content of bone in dialysis patients with aluminium-related bone disease. Bone aluminium concentrations of up to 300 mg/kg have been reported in these patients, whereas concentrations in non-exposed reference populations are very low (<13 mg/kg). De Wolff *et al.* (2002) recently reported blood and tissue aluminium concentrations in four patients who had died as a result of the use of dialysate contaminated with aluminium, which subsequently resulted in the criminal prosecution of hospital staff who had been involved in the incident (Berend *et al.* 2004). A major chemical incident involving the contamination of public drinking water by aluminium occurred in north Cornwall, in the UK, in 1988. Approximately 20 000 people were exposed to very excessive amounts of aluminium over a relatively short period of time. Although there were no fatalities, or apparent serious toxicity, the long-term consequences of this exposure were uncertain. However, a recent postmortem examination carried out on a woman who was exposed to aluminium and subsequently developed a rare form of Alzheimer's disease showed elevated brain concentrations of aluminium (Exley and Esiri 2006).

Antimony

Introduction

Various salts of antimony (e.g. tartar emetic, antimony potassium tartrate) have a long history of use as medicines (McCallum 1999) and continue to be used to treat tropical parasitic diseases such as schistosomiasis (bilharziasis) and leishmaniasis. Antimony compounds are also used industrially in the manufacture of lead batteries, semiconductors, paints, ceramics and pewterware. Other modern industrial compounds include antimony oxychloride ($Sb_6O_6Cl_4$), widely used as a fire retardant on fabrics and mattresses. It was postulated by Richardson in 1994 that the formation of antimony hydride (stibine, SbH_3) might be a cause of sudden infant death syndrome (SIDS) in babies who sleep on mattresses with polyvinyl chloride (PVC) covers manufactured using antimony-based fire retardants. It was claimed that fungal growth (*Scopulariopsis brevicaulis*) on older soiled mattresses converted the antimony fire retardant into stibine, which was inhaled by the babies and led to unexpected death. This was subsequently disproved by a government enquiry that showed that this hypothesis had been based on erroneous experimental work (Cullen *et al.* 2000; Delves *et al.* 1997; Warnock *et al.* 1995).

The fatal dose of antimony in the form of tartar emetic is about 1 g in an adult, but there is much inter-individual variability. The signs and symptoms of acute antimony poisoning include metallic taste, dysphagia, epigastric pain, violent vomiting, diarrhoea, abdominal pain and circulatory collapse (Stemmer 1976). These symptoms are almost indistinguishable from those of acute arsenic poisoning, but larger doses are required. Similarly, the effects of exposure to stibine are similar to those of arsine (AsH_3). Chronic effects of occupational antimony exposure include 'antimony spots' on the skin and pneumoconiosis (Stemmer 1976).

Specimen collection

Specimens are collected as follows:

- Bloods – 10 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container.

Analysis

A number of methods have been described for measuring antimony in biological fluids, including the use of hydride generation and ETAAS. ICP-MS has been applied to the analysis of a wide range of fluids and tissues, particularly those from young infants and babies (Delves *et al.* 1997; Dezateux *et al.* 1997; Miekeley *et al.* 2002).

Interpretation

Reference values for antimony in body fluids and tissues are below 1 µg/L (8 nmol/L). In a study reported by Smith *et al.* (1995) concerning the analysis of urine antimony concentrations in occupationally exposed battery workers, values of up to 150 µg/L (1.2 µmol/L) were reported. A male AIDS patient who received by accident 10 times the prescribed dose of sodium stibogluconate for treatment of visceral leishmaniasis (6.5 g instead of 650 mg) and survived had a blood antimony concentration of 1.38 mg/L 20 h after dosing (Reymond and Desmeules 1998). A female patient who died 48 h after ingesting antimony trichloride solution had tissue concentrations of 4.6 mg/L (blood), 6 mg/kg (brain), 6 mg/kg (lung), 45 mg/kg (liver), 404 mg/kg (bile) and 32 mg/kg (kidney) (Baselt 2005).

Arsenic

Introduction

Arsenic is widely distributed in the environment, particularly in rocks, sediments and some water supplies. A number of inorganic arsenicals have a particularly long historical use as drugs and poisons. In the eighteenth century, Fowlers solution (1% w/v potassium arsenite) was used as a 'cure all' and remained in use until the twentieth century. Many thousands of arsenic-containing organic compounds were synthesised in the late nineteenth and early twentieth centuries as pigments (e.g. Emerald, or Paris Green, copper aceto-arsenite), chemical warfare agents (Lewisite, chlorovinyldichloroarsine) and pharmaceuticals (Salvarsan, arsphenamine; Gorby 1994). Arsenic trioxide (white arsenic or sugar of arsenic) was particularly popular as a homicidal poison up until the late nineteenth century, but its use declined following the development of sensitive techniques for its detection in body fluids and tissues such as the Marsh, Reinch and Gutzeit tests (Rosenfeld 1985). In most countries arsenic compounds are now rarely encountered in cases of suspected murder or suicide, but they have not disappeared from use (Duenas-Laita *et al.* 2005; Gorby 1988; Lech and Trela 2005; Mackell *et al.* 1985; Poklis and Saady 1990). It has been shown to be useful to screen for the presence of arsenic and other inorganic poisons where there are signs and symptoms of unexplained gastrointestinal illness in unexplained or suspicious deaths.

Organic forms of arsenic (e.g. arsenosugars, arsenobetaine, arsenocholine) occur naturally in seaweed, fish and shellfish, but are relatively non-toxic (Francesconi *et al.* 2002; Le *et al.* 1994). Arsenic is metabolised in the liver to mono- and dimethylated species as a means of detoxification, but the various enzymes and pathways are far from being fully understood (Aposhian and Aposhian 2006) Different forms of arsenic have widely differing human toxicity. They are also handled by the body in different ways, which causes problems in their determination and in the interpretation of laboratory findings, particularly when the source of arsenic is unknown (Aposhian and Aposhian 2006; Buchet *et al.* 1981; Johnson and Farmer 1991; Lovell and Farmer 1985; Pott *et al.* 2001; Vahter 1994).

Arsenic has three common valence or oxidation states: 0 (metalloid), 3+ (arsenite) and 5+ (arsenate). The trivalent inorganic salts of arsenic (e.g. sodium arsenite; $NaAsO_2$) are the most toxic and may cause serious toxicity or death after acute ingestion of relatively small doses (<200 mg). Inhalation of arsine gas (AsH_3) may cause massive haemolysis, renal and other organ failure and rapid death, as in industrial accidents (Pakulska and Czerczak 2006). In such cases plasma exchange appears to be the only effective method of treatment, but should be applied as soon as possible (Song *et al.* 2007). The signs and symptoms of chronic arsenic poisoning are extremely complex, affect different organ systems (Table 17.4) and differ from those of gastroenteritis, with bloody diarrhoea, vomiting, excruciating abdominal pain, circulatory collapse and coma associated with acute poisoning (Fowler and Weissberg 1974). In addition, some of the early cutaneous signs, such as melanosis, may go unnoticed (Saha 2003). Skin, liver and respiratory cancer is another major feature of chronic arsenic exposure; huge populations are affected in parts of India, Bangladesh and Vietnam because of the consumption of contaminated well-water (Ahsan *et al.* 2000; Berg *et al.* 2001; Chowdhury *et al.* 2000; Piamphongsant 1999; Smith *et al.* 2000; Yoshida *et al.* 2004). The main sources of the problem are wells sunk into land that contains rocks with a high content of arsenic salts, with subsequent contamination of the underground aquifers, although the precise mechanism of release of arsenic into water is not fully understood.

Table 17.4 Signs and symptoms of chronic arsenic poisoning (arsenic oxide)

Anorexia
Weight loss
Malaise
Hyperpigmentation of skin (diffuse or spotted melanosis)
Hyperkeratosis
Mee's white transverse lines on nails
Peripheral neuropathy
Liver damage
Haematological change (pancytopenia)
Hypertension
Ischaemic gangrene (black foot)
Increased risk of cancer (skin, liver lung)

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container.

Analysis

Simple tests such as the Reinsch test may identify the presence of arsenic in stomach contents tissue digests or 'scene residues'. The Gutzeit test may also be applied to the quantitative analysis of arsenic in stomach contents, water, food and other materials (Crawford and Tavares 1974; Kneip *et al.* 1977). This technique is relatively specific because it is based on the conversion of arsenic into arsine. Other simple spectrophotometric methods to measure arsenic in biological fluids, and other materials such as water, have been described (Lakso *et al.* 1979; Pillai *et al.* 2000). However, the most popular modern approach involves hydride generation following acid digestion and then ETAAS, which can be applied to the measurement of arsenic in a variety of matrices. Analysis of arsenic in urine can be problematic in view of the presence of several different inorganic and organic species (Feldman 1991) (Table 17.5). A major issue is the removal of dietary sources of organic arsenic compounds, particularly those that result from the recent ingestion of fish or shellfish. The limit of detection of this procedure is 5 µg/L (67 nmol/L) of arsenic. More sophisticated techniques include ICP-MS or the separation and measurement of individual arsenic species using gas chromatography (GC)-MS (Claussen 1997) or ion-exchange chromatography linked to ICP-MS (Brima *et al.* 2006; Hughes 2006;

Table 17.5 Measurement of arsenic species in biological fluids

Sequence	*Arsenic species*	*Group*
1	Arsenite (As^{3+})	Inorganic arsenic species
2	Arsenate (As^{5+})	
3	Monomethyl arsonic acid (MMAA)	Methylated arsenic metabolite species
	Methyl arsenate	
4	Dimethyl arsinic acid (DMAA; cacodylic acid)	
5	Complex organo-As compounds (e.g. arsenobetaine, arsenocholine)	Dietary organic species (e.g. fish)

Lindberg *et al.* 2007; Milstein *et al.* 2003). However, the separation of different species of arsenic can be very time-consuming in the investigation of acute or chronic arsenic poisoning, although reference materials are available for urine analysis (Yoshinaga *et al.* 2000).

Interpretation

Normal values for arsenic are <10 μg/L (0.1 μmol/L) in blood and urine. Elevated values can be obtained, depending on the type of methodology and the ingestion of seafood prior to specimen collection or occupational exposure (Apostoli *et al.* 1999). The current American Conference of Government Industrial Hygienists (ACGHI) Biological Exposure Index (BEI) for arsenic (at the end of the working week) is 50 μg/g creatinine (as inorganic arsenic or its metabolites; note that creatinine correction has recently been abandoned because arsenic is excreted by diffusion). Special care is required in the interpretation of results in situations of chronic occupational or environmental exposure. However, in cases of acute symptomatic inorganic arsenic poisoning, concentrations above 2000 μg/L (28 μmol/L) may be seen in blood and urine (Duenas-Laita *et al.* 2005; Lech and Trela 2005). Hair and nail analysis has also been used in the diagnosis and evaluation of chronic arsenic poisoning, particularly suspected homicides (Poklis and Saady 1990). However, there may be problems distinguishing external contamination from ingested arsenic (Hindmarsh 2002).

Barium

Introduction

The inorganic compounds of barium are very widely used as welding fluxes, pigments and glazes in industry and in the manufacture of paint, glass, explosives and ceramics. Soluble salts are used as pesticides and depilatory agents. The insoluble sulfate salt is widely used as radiographic contrast medium. Most (insoluble) barium salts are relatively non-toxic. However, pneumoconiosis related to the inhalation of barium dusts (baritosis) has been recognised in the mining industry. By contrast, the soluble salts of barium, particularly the acetate, hydroxide and chloride, are extremely toxic if ingested orally or given intravenously. Less-soluble salts, such as the carbonate, may be converted to the chloride at low pH in the stomach. Toxicity is associated with the ingestion of as little as 200 mg of soluble barium salts and the oral fatal dose of soluble salts may be as little as 800 mg (Boehnert 1988; Deng *et al.* 1991). Signs and symptoms of barium poisoning may show within 1 h of ingestion and include abdominal pain, diarrhoea, vomiting, muscular weakness and a tingling around the mouth (Johnson and VanTassell 1991; Schorn *et al.* 1991). Severe hypokalaemia may also develop from a shift of extracellular potassium into muscle. This may cause cardiac rhythm disturbances, which require close monitoring, and the administration of large doses of potassium chloride intravenously to correct the hypokalaemia. Haemodialysis has been used successfully in the treatment of barium poisoning (Bahlmann *et al.* 2005).

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container.

Note that some gel-containing blood tubes may be contaminated with barium.

Analysis

Barium can be measured in plasma, urine and tissue digests using ETAAS or ICP-MS (De Boer *et al.* 2004; Dinya *et al.* 2005).

Interpretation

Reference concentrations of barium in plasma are less than 1 μg/L (<7 nmol/L) and urine excretion is below 10 μg/24 h (<0.70 nmol/24 h). In non-fatal cases of barium poisoning, plasma barium concentrations of up to 8 mg/L (58 μmol/L) have been reported (Boehnert *et al.* 1985). Other useful investigations in cases of acute barium poisoning include radiography of the abdomen for the presence of radiopaque material in the gut. Careful monitoring of serum potassium concentrations in any case of suspected barium poisoning is of vital importance. A death in a 49-year-old pharmacist who deliberately ingested barium chloride was associated with a postmortem blood concentration of 10 mg/L (site of collection unknown). Barium concentrations in bile and urine were 8.8 mg/L and 6.3 mg/L, respectively (Jourdan *et al.* 2001). Analytical data from other poisoning cases are given in the barium monograph.

Beryllium

Introduction

Beryllium is used in the manufacture of corrosion-resistant and high-strength alloys. Such products are commonly used in the nuclear, aerospace and weapons industries. Beryllium alloys with other metals, such as aluminium, copper, magnesium and nickel, are used in diverse products such as springs, gears, electrical contacts and other engine components.

Beryllium itself is a highly toxic element and is associated with a characteristic occupational disease (Kolanz 2001). However, chronic occupationally related disease associated with beryllium exposure may go unrecognised (Infante and Newman 2004) The major target organ is the lung, where it causes granulomatous disease (berylliosis) and an increased risk of lung cancer. The pathological and clinical features of chronic beryllium disease may resemble those of sarcoidosis (Infante and Newman 2004). Other organ systems may also be affected, including the lymphatic system, liver, heart, kidney, skin and bone (Stiefel *et al.* 1980).

Most of an absorbed dose of beryllium is excreted in urine over a period of several days, but lung deposits may leach beryllium so slowly that urine excretion continues for several years.

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container
- Lung – postmortem examination of beryllium-related deaths.

Analysis

Beryllium is most often measured in urine using ETAAS. Newer procedures based on ICP-MS are increasingly being used, particularly when dealing with low-level exposure (Apostoli and Schaller 2001; De Boer *et al.* 2004; Xia *et al.* 2004).

Interpretation of results

Normal values for beryllium in urine are generally accepted as less than 1 μg/L (0.1 μmol/L). A recent large study of urine beryllium concentrations in a group of 65 exposed workers reported values in the range 0.12–0.15 μg/L (13–17 nmol/L), with control subjects having values below 0.63 μg/L (70 nmol/L) (Apostoli and Schaller 2001).

Beryllium has also been measured in lung tissues from workers who were known to have had beryllium-related lung disease.

Bismuth

Introduction

Bismuth, a heavy metal, produces toxicity that can sometimes mimic that associated with lead and mercury. For this reason, it can be useful to include bismuth in any heavy-metal screening procedure undertaken in patients with unexplained neurological symptomatology or acute renal failure. Bismuth salts have been used in medicine for more than 200 years as topical salves and in the treatment of gastrointestinal (GI) disorders, and are available in over-the-counter preparations. Because most orally administered inorganic salts of bismuth are relatively insoluble in water and are poorly absorbed from the GI tract, they cause minimal toxicity. Other compounds, particularly lipid-soluble organic compounds, are known to accumulate in the body after excessive dosing and can cause severe neurotoxicity, such as a progressive encephalopathy (Playford *et al.* 1990). Water-soluble compounds of bismuth are more likely to cause renal damage, including oliguria and acute renal failure (Huwez *et al.* 1992). A number of deaths have been reported after acute and chronic overdose with various bismuth medicinal products.

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, K-EDTA
- Urine – 20 mL, sterile plastic universal container.

Analysis

Historically, there have been problems with the reliable measurement of bismuth in biological fluids. The method of choice is ETAAS, which may be carried out by direct determination or following hydride generation (Froomes *et al.* 1988). Methods based on ICP-MS have also been reported (Mauras *et al.* 1993; Vanhoe *et al.* 1993).

Interpretation

Reference values for bismuth in blood are extremely low (<1 μg/L [5 nmol/L]). Acceptable 'therapeutic concentrations' are generally up to 50 μg/L (239 nmol/L) and concentrations in excess of 100 μg/L (480 nmol/L) are generally associated with toxicity (Serfontein and Mekel 1979; Slikkerveer and De Wolff 1989). Extremely high (>1000 μg/L [4.8 μmol/L]) blood bismuth concentrations may be found in patients with severe neurological symptoms such as encephalopathy. Monitoring of blood bismuth concentrations is useful in patients who develop acute renal failure as a result of bismuth toxicity. Reference concentrations of bismuth in urine are <1 μg/L (5 nmol/L). Measurement of urinary bismuth concentrations may be useful diagnostically or after treatment of poisoning using oral chelating agents, which can greatly increase urinary clearance of bismuth (Basinger *et al.* 1983; Slikkerveer *et al.* 1993).

Cadmium

Introduction

Cadmium and its salts and alloys are used in the manufacture of nickel–cadmium batteries, pigments, anticorrosion coatings and special alloys. Acute or chronic ingestion of cadmium salts is extremely rare, but reported signs and symptoms include nausea, vomiting, haemorrhagic gastroenteritis and general organ failure.

Most of the toxicological problems associated with cadmium are the result of occupational and environmental exposure and have been known for many years. These include pneumonitis and emphysema from the acute inhalation of cadmium fumes and, more long term, renal impairment (Jarup *et al.* 1988; Nordberg 2004). Water supplies contaminated with industrial cadmium in Japan led to accumulation of the metal by rice and other dietary sources, with subsequent human poisoning on a vast scale. Those affected developed renal damage, skeletal deformities caused by disturbances of calcium and phosphate metabolism, and severe back and leg pain (Friberg *et al.* 1971; Nordberg 2004). This painful condition became known as 'itai-itai' ('ouch-ouch') disease. For those not occupationally exposed, the major source of cadmium remains the diet, although it is absorbed poorly from the gut. However, inhalation of tobacco smoke is an important source of cadmium exposure, as the bioavailability of cadmium via the lung is very high. Following oral or pulmonary absorption, cadmium is stored mainly in the liver and kidney, where it is bound to metallothionein and stays in the body for decades. The most important toxic effect is on the kidney, with proximal renal tubular necrosis; this can be detected by a characteristic increase in the excretion of low-molecular-weight proteins (Lauwerys and Bernard 1986; Lauwerys *et al.* 1994). Both occupational and environmental exposure to cadmium now appears to be a determinant for the subsequent development of end-stage renal disease (Hellstrom *et al.* 2001).

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, K-EDTA
- Urine – 20 mL, sterile plastic universal container.

It is very important to ensure that specimen tubes are free from cadmium contamination; use of certified trace element tubes is strongly recommended. Strict precautions against contamination should be taken when handling and processing specimens.

Analysis

Routine flame AAS techniques lack sufficient sensitivity for cadmium analysis in blood and urine, but techniques employing special specimen introduction devices, such as the Delves cup, have proved successful, particularly in the assessment of occupational exposure or high levels of environmental exposure (Delves 1977). Most current methods are based on ETAAS with Zeeman background correction (Halls *et al.* 1987). ICP-MS has increasingly become a more suitable approach to analysis particularly in the assessment of occupational cadmium exposure (Botta *et al.* 2006).

Interpretation

Reference values for cadmium in blood and urine are extremely low. Blood cadmium concentrations in non-smokers are generally less than 1 μg/L (9 nmol/L) and in smokers less than 6 μg/L (53 nmol/L). Urine concentrations of cadmium are generally below 1 μg/L in both smokers and non-smokers. Recent occupational guidance values for cadmium in the assessment of cadmium exposure are 5 μg/L (45 nmol/L) in both blood and urine or 10 nmol/mmol creatinine in urine. Thus, heavy smokers may have blood cadmium concentrations at the limit of current occupational guidance values. Cadmium-induced renal tubule impairment is generally related to urine cadmium concentrations in excess of 15 μg/L (134 nmol/L) but these may be much lower for those who were exposed to cadmium many years earlier (Bernard 2004). Blood cadmium concentrations are an index of both recent and past exposure. Individuals who have a long history of excessive occupational cadmium exposure can have a high body burden, which persists into old age (Mason *et al.* 1999). Elevated blood and urine cadmium concentrations may be observed many years after the cessation of exposure in such individuals. Large amounts of cadmium are stored in the liver and kidney, and this can result in a marked redistribution of cadmium into blood after death. This may result in significantly raised postmortem blood cadmium concentrations, depending on the site of blood collection, which could be mistaken for the presence of 'suspiciously elevated' blood cadmium concentrations prior to death.

Copper

Introduction

Copper is an essential, but potentially very toxic, trace element with a recommended intake of approximately 1.53–3.0 mg per day in adults (Aggett 1999; Piscator 1979). The concentration of copper in drinking water may vary greatly, depending on the source of water and the use of copper piping. Recommended concentrations are usually <1.0 mg/L. Copper can be tasted in water at concentrations above 1 mg/L, and there may also be a blue–green coloration at water concentrations >5 mg/L. Copper salts, particularly the water-soluble sulfate, are still used as fungicides and algicides, and may sometimes be found in children's chemistry sets. Total body concentrations of copper are relatively low, with the highest quantity found in the liver, which controls copper homeostasis. Most (~95%) of the circulating copper found in blood is bound to a specific copper-binding protein, ceruloplasmin. Generally, only small amounts of copper are excreted in urine, unless there is the presence of disease. Wilson's disease (hepatolenticular degeneration) is an important genetically inherited disorder of copper metabolism in which there is an inability to transport and excrete copper from the liver into bile (Aggett 1999). This leads to 'copper overload' and associated liver and neurological damage that can cause death unless treated early. It is also known that a deficiency of copper in the diet is associated with pathologically 'low' plasma copper concentrations, which may cause anaemia and low white cell counts, particularly in patients receiving long-term enteral feeding, or taking excessive amounts of zinc, or copper-chelating agents (Lang *et al.* 2004; Oliver *et al.* 2005; Todd *et al.* 2004).Thus copper measurements may be useful in investigating certain types of unexpected death in patients receiving enteral feeding or taking medication that reduces copper absorption.

There is some evidence that excess environmental exposure to copper in drinking water or diet (from copper or brass cooking pots)

may lead to copper-related liver disease in babies and young children (Müller *et al.* 1996; Sethi *et al.* 1993; Spaziano *et al.* 2002; von Muhlendahl and Lange 1994). Copper toxicity has also been found in patients who undergo dialysis with defective copper-containing dialysis membranes (O'Donohue *et al.* 1993). Occupational exposure to copper compounds is relatively rare. The main risk is associated with copper fumes in smelting furnaces that lead to respiratory illness and metal-fume fever. Copper-related liver and respiratory disease has also been reported in vineyard workers who spray copper-containing fungicides (Bordeaux mixture). Toxicity has also been reported following occupational exposure during the application and removal of antifouling paints in ship repair facilities.

Acute copper poisoning may sometimes be seen in cases of accidental and suicidal ingestion of copper salts and solutions, particularly water-soluble salts, such as the sulfate, chloride or acetate (Saravu *et al.* 2007). The signs and symptoms of acute (oral) copper poisoning are metallic taste, GI irritation, abdominal pain, nausea, vomiting, diarrhoea and GI bleeding (Chuttani *et al.* 1965; Patel *et al.* 1976; Saravu *et al.* 2007). In severe cases this can lead to hypotension, shock, liver failure, jaundice, cardiac and multiorgan failure, and death (Gulliver 1991; Walsh *et al.* 1977). The fatal dose of copper sulfate is thought to be approximately 0.2 g/kg body mass.

Specimen collection

Specimens are collected as follows:

- Blood – 2 mL, K-EDTA tube
- Urine – aliquot of 24 h collection in acid-washed urine container for the investigation of copper-related disease and assessment of chelation therapy
- Stomach contents – may be useful in cases of fatal accidental or suicidal poisoning, particularly if they have a blue coloration
- Water – 20 mL, sterile plastic universal container for the investigation of suspected contaminated drinking water.

Analysis

In the investigation of acute copper poisoning, measurement of plasma copper may be useful to assess the severity, although it is unlikely to change clinical management. Analysis can be carried out using colorimetric analysis, or by flame AAS (Taylor 1996) or ICP-MS (De Boer *et al.* 2004; Venelinov *et al.* 2004).

Urine copper can be measured by ETAAS, ICP-AES or ICP-MS, and is generally only of value in the investigation of chronic copper-related liver disease (da Costa *et al.* 1991; Taylor 1996). Measurement of copper in liver biopsy specimens is best undertaken using ICP-MS.

Interpretation

The reference range for plasma copper is wide and depends on age, pregnancy and any underlying disease state. In healthy adults the reference range is 0.8–1.6 mg/L (12–25 μmol/L). Lower values are seen in neonates, copper deficiency and Wilson's disease. Somewhat higher reference values are seen in pregnancy or in patients with inflammatory or neoplastic disease (up to 2.8 mg/L [44 μmol/L]) (Taylor 1996).

The urinary excretion of copper in healthy adults is less than 50 μg/day (0.8 μmol/24 h). Significantly raised values are associated with patients with hepatobiliary disease (50–100 μg/day) or Wilson's disease (>100 μg/day). Much higher values for copper excretion are seen after a challenge with a chelating agent (penicillamine) in both healthy adults (<760 μg/day) and patients with Wilson's disease (>1600 μg/day) (da Costa *et al.* 1991). Other investigations of chronic copper-related liver disease include standard liver function tests and the measurement of plasma ceruloplasmin (Walshe 2003). Copper may also be measured in liver tissue samples after needle biopsy in the investigation of certain types of liver disease. In cases of fatalities following ingestion of soluble copper salts there may be large differences between ante- or perimortem blood/plasma copper concentrations and postmortem blood copper concentrations. These may be caused by postmortem diffusion of unabsorbed copper remaining in the stomach (Gulliver 1991).

Iron

Introduction

Iron poisoning was historically often seen in young children after the accidental ingestion of iron tablets and was until recently one of the leading causes of death in those under 6 years of age (Bosse 1995; Robotham and Lietman 1980). The introduction of unit-dose packaging of iron supplements in 1997 lead to a significant fall in fatalities (Tenenbein 2005). Accidental or suicidal ingestion of iron preparations in adults is relatively rare. Most over-the-counter products contain various ionic iron salts (e.g. sulfate, fumarate, gluconate) and many are slow-release preparations. More recently a number of non-ionic iron preparations have been introduced (e.g. carbonyl iron and polysaccharide iron) that contain a much greater proportion of iron, which is only slowly converted to ferrous iron. The amount of elemental iron in any particular brand of tablet can range from 12% w/v to 98% w/v, which makes assessment of the actual dose of iron ingested very difficult, particularly if the formulation is unknown (Chyka and Butler 1993). The signs and symptoms of iron poisoning can be difficult to assess if the time of ingestion is uncertain. Peak plasma levels of iron are generally seen within about 5 h of ingestion, but this is strongly influenced by the formulation. Initial signs and symptoms include nausea, vomiting and abdominal pain. In more severe cases of overdosage this may lead to intestinal ulceration, small-bowel infarction, haematemesis and melaena. In the most severe cases there may be the subsequent development of metabolic acidosis, hepatic failure and coma. GI symptoms are generally associated with the ingestion of >10–20 mg/kg body mass elemental iron. Serious toxicity after acute ingestion is generally seen at doses of more than 50 mg elemental iron/kg. Life-threatening signs and symptoms and fatalities are more common when the ingested dose of iron is above 100 mg/kg. The clinical presentation of iron poisoning and its time course can be quite complex and go through different phases, particularly when dealing with young children if the ingested dose is very high.

Measurement of serum iron can be useful in the diagnosis and management of patients, but it is not an infallible guide. Early single measurements may be difficult to assess, particularly when iron is still being absorbed from the gut. Measurement of serum iron is of greatest value when considering the intravenous use of chelation therapy with desferrioxamine (Cheney *et al.* 1995; Schauben *et al.* 1990). The measurement of iron in urine is mainly used to assess the efficacy of iron removal using chelation therapy, but it can be of interest in dealing with cases of acute poisoning and in the management of iron overload. In cases of suspected fatal iron poisoning, measurement of iron in body fluids and tissues can be helpful.

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, plain tube
- Urine – aliquot of 24 h collection of value in the assessment of chelation therapy
- Vomit/stomach contents – generally only of value in forensic cases.

Analysis

Iron is traditionally measured in unhaemolysed serum by colorimetric assay. The two major approaches are coloured complex formation followed by spectrophotometry or AAS. Colorimetric procedures to quantify serum iron are usually available in hospital pathology departments and are suitable for rapid emergency analyses, although they are not reliable if desferrioxamine has been administered. More sensitive and specific ETAAS and ICP-MS procedures can be applied to the analysis of iron in serum, plasma, whole blood and urine, particularly in postmortem cases

Interpretation

Interpretation of serum iron concentrations in acute poisoning can be quite complex, particularly as the absorption of iron from the gut may be delayed or prolonged because of the ingestion of a sustained-release formulation.

Reference ranges for serum iron are dependent on age and sex, but typical values are:

Males 0.6–1.7 mg/L (10–30 μmol/L).

Females 0.5–1.5 mg/L (9–27 μmol/L).

On initial presentation a serum iron concentration above 3 mg/L (54 μmol/L) is associated with the presence of GI symptoms; clear signs and symptoms of toxicity are associated with concentrations between 3 and 5 mg/L (54–90 μmol/L); pronounced systemic toxicity is seen at concentrations between 5 and 10 mg/L (90–180 μmol/L); significant morbidity and mortality are associated with concentrations >10 mg/L (>180 μmol/L). However, if chelation therapy has been carried out using desferrioxime, it is not possible to interpret subsequent plasma iron concentrations. A range of serum iron concentrations of 2.8–25.5 mg/L (50–457 μmol/L) has been reported in children who survived the ingestion of up to 10 g ferrous sulfate (Baselt 2002). Children who died 3–5 days after ingesting 6–15 g of ferrous sulfate had serum iron concentrations of 19–50 mg/L (340–900 μmol/L) on the first and second days (Baselt 2002).

Lead

Introduction

The toxic effects of (inorganic) lead have been known since antiquity, but this metal still presents a significant public health problem in many parts of the world (Laraque and Trasande 2005; Needleman and Gatsonis 1990; Tong *et al.* 2000). Lead compounds (and other heavy metals) continue to be used as components of some traditional medicines (Bayly *et al.* 1995; Fisher and Le Couteur 2000; Hardy *et al.* 1998; Moore and Adler 2000). Acute lead poisoning is relatively uncommon, however, and most symptomatic cases result from chronic ingestion, or inhalation of lead fumes or dusts during occupational exposure, or use of lead-containing 'traditional' medicines and ingestion of lead paint chips or dust from old or substandard housing in children with pica (Braithwaite and Brown 1988; Carton *et al.* 1987). Other sources include the ingestion of lead objects such as fishing sinkers by children or adults with psychiatric or learning difficulties (Newton *et al.* 2005; St Clair and Benjamin 2008; Treble and Thompson 2002). Lead poisoning can also arise from retained bullets or lead fragments from gunshot wounds (McQuirter *et al.* 2001). Lead and its compounds are very rarely involved in suspected homicides, which may therefore go unrecognised, or the signs and symptoms may be confused with those of natural disease (Lech 2006).

In adults, barely 10% of ingested lead is absorbed from the GI tract; in children this proportion may be much higher. However, the bioavailability of ingested lead may be influenced substantially by the individual's diet and nutritional status (for example, iron and calcium deficiency will increase lead absorption from the GI tract). Lead absorbed by inhalation has a much greater bioavailability, but this may depend on factors such as respiratory rate, particle size, the atmospheric concentration of lead and the duration of exposure. Thus there can be a significant risk of lead poisoning in demolition workers using cutting tools, or those refurbishing old houses or other buildings by burning off lead paint, without using appropriate protection.

The clinical diagnosis of lead poisoning can be difficult when there is no clear history or apparent source of exposure, since many of the signs and symptoms of chronic lead poisoning are relatively non-specific (e.g. tiredness, abdominal pain, anorexia) (Cullen *et al.* 1983; Newton *et al.* 2005; Visvanathan 2001). Wider ranging laboratory investigations, therefore, play an essential part in the diagnosis and management of lead poisoning and also in the assessment of occupational and environmental lead exposure (Delves 1998).

Specimen collection

The recommended specimen collection for lead analysis is:

- Blood – 5 mL, K-EDTA tube (2 mL in children); check that the tube is lead free (certified lead-free tubes are commercially available)
- Urine – 20 mL, sterile plastic universal container (only useful after organolead exposure)
- Urine – 24 h collection (aliquot) only in the case of chelation therapy
- Water –20 mL, plain universal container.

Analysis

Published procedures to determine lead in blood, urine and other fluids, such as tap water or tissues (bone, teeth), are numerous. One of the most successful historical methods for blood lead measurement used flame AAS with the blood specimen introduced into the air–acetylene flame in a small nickel cup (Delves cup), and is still in routine operation in several parts of the world (Delves 1970). This has been superseded by ETAAS methods, which can determine blood lead concentrations accurately (<100 μg/L [0.5 μmol/L]) in 'non-exposed' populations (Bannon *et al.* 1994; Shuttler and Delves 1986). More recently, ICP-MS has been employed to measure lead in situations of low-level lead exposure. ICP-MS techniques are able to determine blood lead concentrations below 10 μg/L (0.05 μmol/L); also by measuring lead isotope ratios in biological specimens it is possible to correlate these with the likely sources of exposure that might be found in a chemical incident or poisoning from an unusual source of lead (Delves and Campbell 1988, 1993).

Interpretation

As mentioned, symptoms of lead poisoning are relatively non-specific and this can make its clinical diagnosis very difficult, particularly where the source of lead exposure is not known (Braithwaite and Brown 1988). Anaemia (haemoglobin <110 g/L) may be present at high blood lead concentrations (>1 mg/L [50 μmol/L]). Children with blood lead values of this order due to the ingestion of lead paint or use of traditional medicines may also develop severe symptoms of encephalopathy, although this is uncommon.

The best-understood toxic effect of lead is its influence on haemoglobin synthesis. leading to anaemia. Lead inhibits the enzyme ferrochelatase, which is involved in iron transport in the bone marrow and catalyses the introduction of ferrous iron (Fe^{2+}) into the porphyrin ring to form haem (Sakai 2000) (this is the last stage of haemoglobin synthesis). Chronic lead exposure leads to the incorporation of zinc (rather than iron) into the porphyrin ring to produce erythrocyte zinc protoporphyrin (ZPP). The assay of ZPP is relatively simple and is used as an inexpensive screening test for chronic lead exposure. However, raised levels of ZPP may also be found in those with iron deficiency anaemia (Solé *et al.* 2000). Monitoring the reduction in blood haemoglobin and the elevation in erythrocyte ZPP helps to assess chronic lead poisoning (Braithwaite and Brown 1988).

About 98% of the lead in blood is associated with the erythrocytes and has a half-life of a few months. Constant exposure results in the accumulation of lead in blood and tissues until a 'steady state' is reached. Provided that the degree and type of exposure are relatively constant, blood lead concentrations in environmentally, as well as in some occupationally, exposed individuals may be stable over long periods of time. Although lead can be found in most tissues of the body, over 90% of the body burden is deposited in the skeleton as insoluble lead phosphate. Following chronic exposure over many years, as occurs in some industrial workers, tissue stores such as bone become substantial. This can have a profound effect on the toxicokinetics of lead and associated long-term health risks. As a result, after cessation of exposure, blood concentrations may decline slowly with an elimination half-life of up to a year. This causes problems in occupational surveillance programmes where the benefits of reduced exposure of lead workers, as monitored by a decline in blood lead concentrations, may be underestimated by the continued contribution from bone and tissue stores (Mason and Williams 2005). Lead is poorly excreted from the body, the most important route being via the kidney. Normal urinary output of lead is less than 10 μg/day (50 nmol/day), but this can be increased greatly by chelation therapy. However, studies in rats suggest that chelating agents do not have access to bone lead stores; thus the skeleton may become a permanent internal reservoir of lead that is slowly released to soft tissues, causing toxicity over the lifetime of the individual.

Extensive studies have demonstrated the harmful effect of lead exposure on child development, behaviour and intelligence (Needleman

Table 17.6 Blood lead concentrations following occupational exposure as applied in the UK (Control of Lead at Work Regulations 1998)

Situation	*Concentration*[a]
Reference values in non-occupationally exposed adults (and children)	<10 μg/L (<0.5 μmol/L)
Action level in occupationally exposed females capable of bearing children	250 μg/L (1.2 μmol/L)
Suspension level in occupationally exposed females capable of bearing children	300 μg/L (1.5 μmol/L)
Action level in occupationally exposed males (400 μg/L in those under 18 years old)	500 μg/L (2.4 μmol/L)
Suspension levels in occupationally exposed males (500 μg/L in those under 18 years old)	600 μg/L (2.9 μmol/L)

[a]Reference values not given in regulations, but shown for comparison.

2006; Needleman and Gatsonis 1990; Shannon *et al.* 2005; Woolf *et al.* 2007). In adults and children a maximum blood lead concentration of 100 μg/L (about 0.5 μmol/L) is currently internationally recommended (Bellinger *et al.* 1992). However, recent evidence suggests that there may be intellectual impairment in children (from either pre- or postnatal exposure) with blood lead concentrations below 100 μg/L (Canfield *et al.* 2003; Shnaas *et al.* 2006). Somewhat higher levels may be acceptable in adults who are occupationally exposed to lead, but careful monitoring of exposure is essential. Occupational exposure limits apply in many countries; the current UK guidance is shown in Table 17.6. However, there is concern that current occupational limits are far too high (Schwartz and Hu 2007), particularly concerning occupational limits in female workers. There is evidence to show that pregnancy and lactation may cause mobilisation of lead from bone stores leading to an increased risk of lead toxicity to both the mother and her newly born child.

Lithium

Introduction

Lithium salts are used in the acute treatment of mania and in the prophylaxis of bipolar (manic–depressive) psychiatric disorders. These are usually prepared from lithium carbonate or citrate, and most are in the form of sustained-release preparations. The drug is well absorbed from the gut, with peak plasma lithium concentrations observed within 2 h for instant-release preparations and between 6 and 12 h for sustained-release formulations. Lithium is not bound to plasma proteins and is eliminated only via the kidneys. Plasma concentrations can accumulate to toxic levels if renal impairment develops. It is recommended that patients who receive therapy with lithium have regular monitoring to maintain plasma lithium concentrations in the therapeutic range. Nevertheless, overdosage with lithium, whether acute, acute on chronic or chronic, is relatively common (Bailey and McGuigan 2000). In acute overdosage the initial symptoms are largely of GI upset; signs and symptoms of central nervous system disturbance may then develop over the next 24 h as lithium is slowly distributed into the brain. Because of the delay in peak plasma lithium concentrations following ingestion of sustained-release preparations, toxicity may sometimes be unrecognised (Borras-Blasco *et al.* 2007). Most cases of chronic overdosage are due to renal impairment or interaction with other drugs and the main features are of central nervous system disturbance.

Signs and symptoms of toxicity include ataxia, tremor, dysarthria, slurred speech, drowsiness and coma. In assessing any case of suspected lithium poisoning it is important to obtain a full medical history, including dosage regime, use of other drugs and the results of any previous lithium measurements (Tyrer 1996).

Specimen collection

- Blood (5 mL) – a plain or K-EDTA tube. It is important to ensure that specimens are not taken into a lithium–heparin tube.

Analysis

Lithium can be quantified in biological fluids by various techniques including simple flame photometry, colorimetric assays, ion-selective electrodes, flame AAS and ETAAS. The specimens of choice are plasma or serum. Whole-blood measurements are possible using flame AES, although the presence of potassium in haemolysed specimens can cause falsely elevated values.

Interpretation

Recommended therapeutic concentrations of lithium in plasma or serum in the treatment of acute mania can be up to a maximum of 1.3 mmol/L; in the prophylaxis of bipolar disorders recommended values are generally 0.5–0.8 mmol/L. These may be associated with some side-effects such as gastrointestinal upset, polyuria, thirst and fatigue. Plasma lithium concentrations in cases of acute and chronic poisoning can often be very difficult to interpret clinically. Signs and symptoms of toxicity are generally associated with values above about 1.4 mmol/L, and values greater than 2 mmol/L require urgent medical attention. Lithium concentrations above 3 mmol/L, particularly in cases of chronic toxicity, can be life threatening and may require the use of active elimination therapy such as haemodialysis, which appears to be the therapy of choice (Eyer *et al.* 2006; Leblanc *et al.* 1996). Signs of toxicity following chronic or acute-on-chronic overdosage do not always correlate with the quantity of lithium ingested, with the serum lithium concentrations on admission to hospital, or with a fatal outcome (Waring *et al.* 2007).

Mercury

Introduction

Mercury has been used in the manufacture of thermometers and a range of other scientific and medical instruments for hundreds of years, but this use has now declined. Elemental mercury continues to be used widely across the world in a number of developing countries by subsistence gold miners using the mercury amalgamation process (Spiegel *et al.* 2005). Mercury and its compounds have also been used widely in the chemical industry and in the manufacture of drugs and pesticides. Some compounds, such as ethyl mercury derivatives, have been historically used as preservatives (thiomersal) in the manufacture of vaccines used in infants and young children. This has come under intense scrutiny, particularly regarding the aetiology of certain neuropsychiatric disorders such as autism. However, detailed follow-up studies in children do not support any causal link (Clements and McIntyre 2006; Hviid *et al.* 2003; Pichichero *et al.* 2002; Thompson *et al.* 2007). Dental amalgam fillings containing mercury are still used in many countries, although their use, particularly in children, does not appear to be associated with a risk of neurotoxicity (Bellinger *et al.* 2006; DeRouen *et al.* 2006; Needleman 2006).

The toxicity of mercury and its compounds is influenced greatly by the chemical form and valence state (Broussard *et al.* 2002; Clarkson *et al.* 2003; Nuttall 2004). The most toxic forms of inorganic mercury are its divalent (Hg^{2+}) water-soluble salts, particularly mercuric chloride, which was used as a disinfectant in earlier times. The fatal (oral) dose of mercuric chloride is less than 5 g in an adult, which led to its popularity as a homicidal and suicidal poison and gave it its popular name, corrosive sublimate. There is significant toxicity associated with both acute and chronic inhalation of (elemental) mercury vapour, which has a very high vapour pressure at normal room temperature (Broussard *et al.* 2002; Clarkson *et al.* 2003; Houeto *et al.* 1994; McNeil *et al.* 1984). Broken mercury thermometers can constitute a serious risk in children through inhalation of vapour, particularly in a home environment such as a bedroom (Velzeboer *et al.* 1997). Organic forms of mercury, such as methylmercury, are strongly neurotoxic and, being relatively lipid soluble, can accumulate in fatty tissues of the body such as the brain (Aschner and Syversen 2005; Bakir *et al.* 1973). Large-scale environmental disasters have occurred, such as that involving the Minimata Bay area in Japan where thousands of people died or became severely incapacitated. This

was caused by the factory discharge of industrial waste that contained mercury, which settled into the sediments of the bay and river and was methylated by microorganisms. The methylmercury became incorporated into the fish diet on which the residents of Minimata, a fishing village in Japan, largely existed, and this resulted in so-called Minimata disease (Ekino *et al.* 2007; Hachiya 2006). Mercury poisoning may also be seen after the use of traditional medicines and cosmetics (Ho *et al.* 2003; Kang-Yum and Oransky 1992; Soo *et al.* 2003; Weldon *et al.* 2000).

The signs and symptoms of acute and chronic mercury poisoning mainly involve the central nervous system, kidney or skin (Clarkson *et al.* 2003; Winship 1985). Characteristic symptoms in children include acrodynia ('pink disease'), which include signs such as pink hands and feet, which was historically associated with the use of mercurous chloride in teething remedies (Velzeboer *et al.* 1997; Warkany 1966). Mercury species may be retained in the body for a long time after the cessation of exposure, and blood elimination and urine excretion rates show long half-lives (Barregård *et al.* 1992; Nuttall 2004; Roels *et al.* 1987). Mercury may be excreted in the urine for 6–12 months after cessation of exposure, which makes urine measurements an attractive way to assess historical exposure and has advantages over use of other matrices such as hair or nails (Ritchie *et al.* 2004).

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, K-EDTA tube.
- Urine – 20 mL; a random 'spot' specimen in a sterile plastic universal container or aliquot of a 24-h collection (must be collected in a hard plastic container without preservative) is of greatest value in assessing chelation therapy). Preservation of mercury in stored specimens requires acidification and freezing.
- Hair – can be useful in environmental studies or unusual clinical cases.

Analysis

In the investigation of any suspected poisoning by or exposure to mercury, collection of both blood and urine specimens can be useful. In the case of acute symptomatic poisoning or recent exposure, blood specimen collection is particularly useful. In the case of 'historical poisoning' or the assessment of occupational exposure or that in asymptomatic patients, a 'spot' urine specimen is the initial sample of choice, although the stability of mercury in urine specimens is poor on prolonged storage.

Mercury is often determined in biological specimens (urine, blood and hair) using 'cold vapour' AAS, and a large number of reliable methods have been published (Ngim *et al.* 1988; Pineau *et al.* 1990). In recent years a number of commercial systems have been developed using flow cells that greatly increase sensitivity. The wider availability of ICP-MS has resulted in its use for mercury analysis of biological fluids, particularly blood, at very low levels of exposure (White 1999).

Interpretation

Reference values for mercury in non-exposed populations in blood are less than 4 μg/L (20 nmol/L) and less than 5 μg/L (25 nmol/L) in urine.

With chronic exposure there is generally a good association between exposure to mercury fumes and urine concentration, particularly where there is significant exposure. Blood and urine mercury concentrations can be more difficult to interpret at low levels of alleged exposure, where other sources of mercury from diet or mercury amalgams may contribute significantly. The current recommended BEI of the ACGHI (USA) for occupational exposure assessment is a pre-shift urine mercury (total) concentration of 35 μg/g creatinine or an end-of-shift, end-of-working-week blood mercury concentration of 15 μg/L (75 nmol/L).

In individuals with mercury amalgam fillings, urine mercury concentrations are rarely more than 8 μg/L (40 nmol/L). However, post-mortem studies have reported significantly higher concentrations of mercury in tissues such as brain in subjects with a greater number of amalgam fillings (Guzzi *et al.* 2006). Mercury from the diet (particularly fish) contributes to the concentration of mercury found in blood, but is usually well within the reference limits (see above). Populations who consume unusually large quantities of certain fish (e.g. swordfish) or of whale meat may accumulate high concentrations of (methyl) mercury (Kales and Goldman 2002). There is some evidence that such exposure presents a serious risk in pregnancy and early child development (Grandjean *et al.* 2003) or, in certain cases, loss of visual acuity or impairment of colour vision (Saldana *et al.* 2006). Hair mercury concentrations are generally <1 μg/g in individuals who do not eat fish, but elevated concentrations in hair can often be difficult to interpret and do not always correlate with blood mercury concentrations, or symptoms of toxicity (Nuttall 2006).

In cases of severe poisoning it is useful to monitor both blood and urine mercury concentrations, particularly when chelation therapy is given. Measurement of 24 h mercury excretion may be used as a guide to the efficiency of removal of chelated metal. Use of certain chelation challenge tests to assess the body burden of mercury in patients with ill-defined symptoms of alleged mercury toxicity may, however, be unreliable or associated with serious side-effects (Archbold *et al.* 2004).

Selenium

Introduction

Selenium is now firmly established as an essential trace element, although historically it was associated only with toxicity as a result of occupational or environmental exposure (Glover 1970; Yang *et al.* 1983). Selenium is found in nature mainly in the form of metal selenides in sulfide ores, sometimes in association with arsenic.

Most of the earliest reports of selenium toxicity are associated with poisoning in grazing livestock and the cause of 'alkali disease' and 'blind staggers' (Wilber 1980). The sources of selenium in such cases are particular plants that concentrate selenium when growing in selenium-rich soils. There are also well-documented early reports of chronic selenium toxicity (selenosis) in populations living in areas where there are excessive amounts of selenium in soil and water supplies, such as in parts of rural China.

There are many industrial uses of selenium, including the manufacture of solar and photoelectric cells, pigmented glass and ceramics. Selenium-compounds (e.g. selenium sulfide) are also used as anti-dandruff agents in shampoos and in gun-blueing compounds, which contain selenous acid. In the last decade there has been an increased interest and promotion of the value of selenium-containing nutritional supplements in the prevention of a wide range of disorders including degenerative illness, viral infections and various forms of cancer. Some supplements may contain relatively high concentrations of selenium, which may cause toxicity if ingested in excess (Clark *et al.* 1996).

Selenium is a metalloid placed in group VI of the periodic table. Common oxidation states of selenium are 2+ (selenide), 4+ (selenite) and 6+ (selenate). There are also a number of important organoselenium compounds in which selenium is able to substitute for sulfur (e.g. selenocysteine and selenomethionine). Recent years have seen a large increase in our understanding of the essential biological role of selenium, and important selenoproteins and glutathione peroxidases (GSHPx) are found in most tissues of the body (Thompson 1998). Selenium has been shown to have an important role in thyroid function, fertility (particularly sperm motility), mood regulation and immunity to infectious disease, and as a cellular 'antioxidant', acting in association with vitamins E and C (Rayman 2000; Reilly 1993). Deficiency of selenium is associated with a number of disorders, such as Keshan disease (China), and more recently with an increased risk of cancer (Bjelakovic *et al.* 2004). Selenium can also have a protective role in ameliorating human exposure to mercury (Hansen 1988).

In cases of chronic occupational or environmental exposure, selenium is associated with signs and symptoms such as skin irritation, garlic breath, painful and discoloured nails, brittle hair and alopecia. Toxicity may occur after ingestion or inhalation of different selenium compounds. However, elemental selenium is poorly bioavailable and organoselenium compounds, although very bioavailable, are relatively non-toxic. In comparison, soluble inorganic selenium compounds, particularly the selenites, selenious acid and selenious oxide and dioxide (following their *in vivo* conversion to selenious acid) are extremely toxic if ingested and are linked to severe intoxications or fatalities (Alderman and Bergin 1986; Brighthope 2007; Gasmi *et al.* 1997; Handsacker *et al.*

2005; Köppel *et al.* 1986; Lombeck *et al.* 1987; Matoba *et al.* 1986; Quadrani *et al.* 2000; Schellmann *et al.* 1986; Spiller and Pfiefer 2007).

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, K-EDTA tube
- Urine – aliquot of 24 h collection in severe cases of acute poisoning
- Stomach contents – useful in fatalities
- Scene residues – useful for identification purposes.

Analysis

Most earlier methods for measuring selenium in blood were based on fluorimetry. Techniques recently described include LC and GC, ETAAS by direct analysis or after hydride generation and ICP-MS, which can also measure selenium in tissues (Sheehan and Halls 1999). Reference materials suitable for internal accuracy control are commercially available, and a number of external quality-assessment schemes include the analysis of selenium in plasma and blood.

Interpretation

Reference ranges for selenium in whole blood and plasma vary from country to country because of differences in dietary sources of selenium. In the UK, adult plasma reference ranges are generally between 70 and 130 μg/L (0.9 and 1.7 μmol/L), with a median population value of approximately 90 μg/L (1.2 μmol/L).

The reference range for children is much lower than that of adults, particularly in babies and younger children (serum selenium approximately 35–115 μg/L). Reference values for whole-blood selenium are somewhat higher and are generally within the range 80–180 μg/L in adults. Values for plasma and blood selenium are greatly influenced by diet, particularly the intake of foods high in selenium content, such as Brazil nuts, or use of supplements containing selenium. Urine excretion of selenium may vary widely, depending on intake, but is usually within the range 20–200 μg/L. Measurement of urine selenium can be useful in monitoring occupational exposure to selenium compounds.

In cases of acute poisoning, very high concentrations of selenium may be detected in whole blood, plasma and urine. Blood selenium values below 1000 μg/L (12 μmol/L) indicate minimal toxicity, whereas values above 2000 μg/L (25 μmol/L) predict serious complications (Gasmi *et al.* 1997).

Thallium

Introduction

Thallium salts, particularly the sulfate, were introduced into medicine in the late nineteenth century for the treatment of several disorders, including the treatment of syphilis, but were soon discarded because of their toxicity. A somewhat later and lasting therapeutic application was as a depilatory agent in the treatment of ringworm of the scalp, particularly in children. This continued up until the 1940s, despite frequent problems associated with thallium toxicity, including many deaths. Thallium salts were also introduced as rodenticides in the 1920s and continue to be applied in some countries. Modern industrial uses of thallium salts include the manufacture of cosmetic jewellery, pigments, semiconductors, fireworks and optical lenses, and in producing special alloys for seawater batteries.

Most thallium compounds are colourless, tasteless and odourless. The water-soluble salts of thallium include the sulfate, acetate and carbonate, all of which are highly toxic (Aoyama *et al.* 1986; Moore *et al.* 1993; Saddique and Peterson 1983). The fatal oral dose in adults is thought to be 1–2 g. The clinical picture of thallium poisoning is highly complex, and poisoning can be very difficult to diagnose in its early stages and may be confused with colchicine poisoning, food poisoning or Guillain–Barré syndrome (Hoffman 2003; Matthews and Dubowitz 1977). Initial signs and symptoms of acute thallium poisoning may not be particularly severe but usually include abdominal pain, nausea, vomiting and diarrhoea, sometimes followed by constipation (Hoffman 2003; Villanueva *et al.* 1990; Wainwright *et al.* 1988). Neurological symptoms may develop over about 1–2 weeks and include both peripheral and central neurological changes (Cavanagh 1991; Cavanagh *et al.* 1974). The most characteristic feature is that of a rapidly progressive ascending peripheral neuropathy. Cardiovascular changes, such as an increase in blood pressure and tachycardia, may also develop after 1–2 weeks. The most characteristic signs associated with thallium toxicity are dermatological changes, which may take up to 4 weeks to develop fully, and include within the first week an initial dark brown or black pigmentation of the roots of the hair (only visible under a microscope) followed by a gradual loss of body hair (mainly scalp hair), which can result in total alopecia after about one month. Nail growth may also be impaired, with the development of white transverse lines, similar to those seen with arsenic poisoning. Thallium salts have a high oral bioavailability but the metal's pharmacokinetics are quite complex, with a high distribution volume and long terminal elimination half-life of between 1 and 4 weeks (Hoffman 2003). The mechanism of thallium toxicity is poorly understood. Because thallium has a similar ionic radius to that of potassium, it is thought that cell membranes are unable to differentiate between the two elements. As a consequence, thallium is able to accumulate in areas of the body with a high potassium content, such as the central and peripheral nervous systems. At high concentrations, thallium will also inhibit potassium-dependent enzyme systems, including those involved with energy production within the citrate cycle. Accidental poisoning with thallium is relatively uncommon in those countries that no longer permit the use of thallium as a rodenticide. However, thallium poisoning has been reported in some historical cases involving the adulteration of illicit drugs such as diamorphine and cocaine and, more recently, in suspected homicides, including political assassinations.

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container (24 h collection only of value in monitoring oral antidotal therapy with Berlin [Prussian)] blue). Additional specimens that may be of forensic interest include hair and nails.

Analysis

Historically, the most commonly used approach to the analysis of thallium in blood or urine involves the use of ETAAS. More recently, ICP-MS has become the technique of choice, being easily applied to the analysis of thallium in a wider range of matrices including hair and nails (Hann *et al.* 2005; Yoshinaga *et al.* 1993).

Interpretation

Reference values for thallium in blood and urine are below 1 μg/L (5 nmol/L), and concentrations in excess of 100 μg/L (0.5 μmol/L) in blood and 200 μg/L (1 μmol/L) in urine are associated with toxicity. In particularly severe cases of thallium poisoning, extremely high levels of thallium in blood (>300 μg/L) and urine (>600 μg/L) may be recorded within the first few days after ingestion. Fatalities are associated with blood thallium concentrations in excess of 2500 μg/L (Hologgitas *et al.* 1980). A recent study has indicated that, in the case of short-term thallium intoxications, dematological findings appear to correlate with thallium concentrations. Measurement of thallium excretion in urine is useful in cases for which oral antidotal therapy with Berlin (Prussian) blue (potassium ferric hexacyanoferrate) is instituted (Heath *et al.* 1983; Hoffman 2003; Pedersen *et al.* 1978). This dye forms an insoluble complex with thallium in the gut, which leads to enhanced faecal excretion. Tissue concentrations of thallium have been reported in fatal cases of thallium poisoning, ranging up to 178 μg/g (wet weight) (Davis *et al.* 1981).

Anions

The systematic analysis of anions in body fluids presents a major analytical challenge. Historically, the main approach was the analysis of stomach contents and scene residues using classic colorimetric methods of analysis (Yeoman 1985). However, many anions are unstable and undergo rapid breakdown in the stomach and gut after absorption. The analysis of metabolites and various hydrolysis products in blood or urine using modern methods is also not well described. In some cases,

the investigation of poisoning caused by anions that are oxidising agents (e.g. chlorates) is best carried out by measurement of changes in blood chemistry, such as the formation of oxidised haemoglobin (methaemoglobin).

The development of analytical techniques such as ion chromatography to separate and measure anions in biological fluids has been slow. However, increased application of ICP-MS techniques has greatly enhanced the ability to detect the presence of elevated concentrations of particular elements (e.g. bromine, boron and iodine) in biological fluids and tissues that may indicate the ingestion of particular anions. Classic analytical chemical methods (colour tests) may sometimes still be applicable for cases in which stomach contents or scene residues are available (Yeoman 1985).

Borates

Introduction

Boric acid has been used as an antiseptic for external use for more than 100 years but has generally been superseded by modern agents. Sodium borate (borax) is still used in cleaning agents, contact lens solutions, wood preservatives, pesticides and fungicides. Boric acid has also been used as a preservative in urine specimens requiring bacteriological examination. Boron is widely distributed in the environment and is a normal constituent of the diet, including drinking water (Murray 1995). Boron is an essential element in plants, but its role as an essential trace element in animals is less well established. A number of clinical studies suggest that boron deficiency in humans might be significant in a wide range of disease states such as osteoporosis, arthritis and dementia. Boron-containing supplements have been promoted by the complementary health sector and there is the potential for excessive self-medication and toxicity.

Compounds of boron have relatively low toxicity and reports indicate that following a single acute ingestion symptoms are often very mild However, a number of cases of acute and chronic boron poisoning have been reported in children and adults (Hamilton and Wolf 2007; Linden *et al.* 1986; O'Sullivan and Taylor 1983; Wong *et al.* 1964). Signs and symptoms have generally included nausea and vomiting, diarrhoea and abdominal pain. In some cases patients may develop a generalised erythoderma sometimes described as a 'boiled lobster' appearance. Seizures, coma and renal dysfunction have been described in more severe cases.

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, K-EDTA tube
- Urine – 20 mL, sterile plastic universal container
- Stomach contents – useful in postmortem examination
- Collection of hair specimens may be useful in cases of chronic non-accidental administration.

Analysis

Transfer a portion of the sample to an evaporating basin, add 1 mL sulfuric acid and 3 mL of ethanol, and ignite. A green border around the flame indicates the presence of borate.

Quantitative methods Historically, borate has been measured in biological fluids and tissues by colorimetric procedures using carminic acid as a reagent. More recently, ETAAS has been used to investigate cases of suspected acute poisoning where the blood boron concentrations are high enough to compensate for the poor sensitivity of the methods. ICP-MS is far more sensitive and has been used successfully to measure the concentration of boron in blood and tissues in patients who receive treatment with boron-containing drugs such as boronphenylalanine (BPA) as part of boron neutron capture therapy of certain cancers (Morten and Delves 1999; Goulé *et al.* 2005).

Interpretation

Extremely high concentrations of boron have been detected in cases of acute poisoning with borates, particularly in children (Linden *et al.* 1986). Plasma boron concentrations ranging from 200 mg/L to 1600 mg/L have been reported in fatal cases. Survival has been reported in cases of adult poisoning despite admission boron concentrations in excess of 1000 mg/L. Reference values in healthy adults show blood boron concentrations between 14 and 44 μg/L, plasma concentrations between 19 and 79 μg/L, urine concentrations between 282 and 2072 μg/L and hair values between 0.26 and 1.87 μg/L (Goulé *et al.* 2005). There are no data on children or populations consuming food or water supplies with a high boron content that could greatly influence urine excretion of boron.

Bromide

Introduction

Bromide salts (e.g. ammonium, potassium and sodium bromides) were first introduced into medicine in the nineteenth century and were used extensively as anticonvulsants and sedatives. With the exception of organic bromides such as carbromal (a sedative), their use is now limited. However, potassium bromide may still be found in traditional medicines used in the treatment of epilepsy. Bromates have been used as preservatives and detonators. Methyl bromide is a colourless gas most commonly used as an insecticidal fumigant for grain stores and soil, but has also been used historically as a refrigerant and in fire extinguishers. Its use should eventually be phased out under the Montreal Protocol on Substances that Deplete the Ozone Layer, but is currently still in use in many parts of the world. Methyl bromide releases bromide ion as a metabolite and is associated with accidental poisoning, particularly within an occupational setting (Yamano and Nakadate 2006).

Following ingestion of bromide salts, absorption of bromide ion takes place rapidly from the stomach and proximal small intestine by passive diffusion. Bromide ions behave like chloride ions and are distributed mainly in extracellular fluid. The most important route of elimination is via the kidney. Their elimination half-life is relatively long, being of the order of 10 days after acute dosing, or several weeks following the cessation of chronic intake, particularly in cases of bromide intoxication.

Bromide poisoning may cause complex neurological symptoms (e.g. tremor, ataxia, autonomic disturbance, cognitive impairment) and clinical diagnosis can be difficult if the use of bromide salts is not suspected. Methyl bromide intoxication has been associated with a reversible symmetrical brain-stem and cerebellar MRI lesion, indicating that bromide exposure can produce an acute 'toxic energy deprivation syndrome' that is normally associated with nutritional and genetic disorders such as Wernicke's encephalopathy (Geyer *et al.* 2005). In contrast, bromate intoxication is associated with the development of irreversible hearing loss due to damage to the cochlea.

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, heparinised or EDTA tube
- Urine – 2- mL, sterile plastic universal container
- Stomach contents – useful in postmortem examination of fatalities
- Scene residues – useful for identification purposes.

Analysis

For the analysis of bromide ions in blood, plasma or urine, colorimetric procedures, ion-selective electrodes, GC or ICP-MS has been described (Corina *et al.* 1979; Quinones *et al.* 2006; Sunshine 1975). Routine clinical biochemistry methods for plasma chloride analysis can show spuriously elevated values when high levels of bromide ion are present (Wenk *et al.* 1976). One of the older colorimetric methods based on the reaction with gold chloride (Sunshine 1975) is still a useful technique for the rapid measurement of bromide ions in serum in cases of acute intoxication. The limit of detection of this method is 50 mg/L using 1 mL of specimen. A suitable calibration range is 100–2000 mg/L bromide ion. More recently, a number of ion-chromatographic methods have been developed to separate bromide and bromate in water disinfection by ozonisation (Schminke and Seubert 2000). The wider availability of ICP-MS makes analysis of bromide ion in biological fluids and tissues relatively easy, with a very low limit of detection.

Table 17.7 Plasma bromide concentrations in relation to 'therapeutic' use and intoxication

Situation	*Plasma bromide ion (mg/L)*	*Equivalent plasma bromide ion (mmol/L)*
'Normal' reference values	<5	0.05
Peak concentration after single oral dosage of 100 mg Br/kg	230	2.9
'Therapeutic' concentrations in adult epileptics	750–1000	9.3–12.5
Signs of intoxication	>1000	12.5
Signs and symptoms of severe intoxication	>3000	37.5

Interpretation

Although there is a complex relationship between bromide ion concentration and signs and symptoms of toxicity, measurement of bromide is helpful in the diagnosis and management of cases of intoxication. Table 17.7 shows reference values of bromide and typical concentrations after therapeutic use and for cases of bromide intoxication (Maes *et al.* 1985; Vaiseman *et al.* 1986). Elimination of bromide from the body may be extremely slow in cases of intoxication without the use of active elimination therapy (e.g. diuresis, haemodialysis or haemofiltration).

Chlorate

Introduction

Sodium chlorate ($NaClO_3$) is an effective, inexpensive, non-selective herbicide. Potassium chlorate is used in the manufacture of matches and some explosives. Both compounds are powerful oxidising agents. Early signs and symptoms of chlorate poisoning include nausea, vomiting and abdominal pain (Steffen and Seitz 1981; Vaiseman *et al.* 1986). Systemic absorption leads to substantial oxidation of haemoglobin to form methaemoglobin, which may cause cyanosis, dyspnoea, anuria and coma (Ellenhorn 1997). Intravascular haemolysis and severe metabolic acidosis may also occur (Ellenhorn 1997). Ingestion of potassium chlorate may cause hyperkalaemia.

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, lithium–heparin tube
- Urine – 20 mL, sterile plastic universal container
- Stomach contents – useful in investigation of fatalities
- Scene residues – useful for identification purposes.

Analysis

In clinical cases of chlorate poisoning the diagnosis rests on measurement of methaemoglobin, which is easily carried out as part of a routine biochemical analysis.

A method that involves ion chromatography for the direct analysis of chlorate in body fluids and tissues has been described by Eysseric *et al.* (1999). Ion chromatography can resolve chlorate from closely related anions such as nitrate, bromate and phosphate.

Interpretation

Serious and sometimes fatal poisoning can occur after the ingestion of 15 g or more of sodium or potassium chlorate. The case described by Eysseric *et al.* (1999) involved a 49-year-old man who was admitted to hospital with severe features associated with chlorate poisoning. He had acute haemolysis with a plasma haemoglobin of 3.9 g/L and 30% methaemoglobin formation. High concentrations of chlorate ion were found in the admission plasma (54 mg/L), gastric contents (1300 mg/L) and urine (4300 mg/L). The patient died 12 h after admission.

Cyanide

Introduction

Severe or fatal cyanide poisoning is relatively rare and mostly involves suicidal ingestion of cyanide salts. Hydrogen cyanide (HCN; prussic acid) is a highly toxic volatile liquid. Fumes of hydrogen cyanide are given off when cyanide salts are mixed with acids or produced in the stomach following oral ingestion. Although HCN has a characteristic almond-like odour, up to 50% of the population are unable to smell it. Surprisingly, it was not a characteristic feature *post mortem* of a large series of cyanide suicide deaths, although this could have been related to air-flow ventilation systems in postmortem rooms (Gill *et al.* 2004). Soluble salts of cyanide include potassium and sodium cyanide, which are used industrially in electroplating and metal processing and as laboratory reagents. Less soluble salts of cyanide include silver and gold cyanide, and mercuric cyanide, which also release HCN on contact with strong acids. As a general rule most cyanide suicide deaths are reported in those individuals, mostly male, who have occupational access to cyanide salts. HCN may also be formed as a combustion product in fires from nitrogen-containing materials such as wool and silk or synthetic polymers such as polyurethanes, polyamides and polyamides (Barillo *et al.* 1994; Baud *et al.* 1991; Chaturvedi *et al.* 2001). Less common sources of cyanide include the accidental or intentional ingestion of cyanogenic plants or their seeds.

The signs and symptoms of cyanide toxicity appear rapidly after inhalation of HCN or ingestion of cyanide salts; the estimated fatal doses are approximately 100 mg HCN or 300 mg potassium cyanide (Ellenhorn 1997). Early neurological signs include headache, dizziness, anxiety and confusion. In severe cases there may be a rapid loss of consciousness, respiratory failure and convulsions that lead to cardiorespiratory arrest and death. The mechanism of cyanide toxicity is to block electron transport in the cytochrome a-a_3 complex; this leads to a dramatic fall in oxidative metabolism and cellular hypoxia, which most directly affects the brain and heart. Cyanide is metabolised rapidly in the liver by an enzyme (rhodanase) to thiocyanate (SCN), which is largely non-toxic. As a consequence, blood cyanide concentrations decline rapidly after exposure or ingestion, with an estimated elimination half-life of 1–2 h.

A number of antidotes are useful in the treatment of cyanide poisoning (e.g. cobalt EDTA and hydroxocobalamin). The measurement of blood cyanide concentrations is rarely useful in a medical emergency when dealing with the acute management of a patient. It can be useful in the retrospective evaluation of the severity of cyanide ingestion and in assessment of the efficacy of different antidotes. Measurement of blood cyanide is most useful in the investigation of suspected cyanide poisoning and in fire-related deaths, including aircraft accidents.

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, lithium–heparin or K-EDTA container
- Stomach contents – useful as part of postmortem examination, but great care is required because of the risk of secondary cyanide exposure
- Cyanide in blood is stable if kept at 4°C for up to 1 week, but is unstable if frozen or kept at room temperature. However, contradictory findings regarding cyanide stability in whole blood have often been reported (Lindsay *et al.* 2004).

Analysis

Numerous methods have been described for the analysis of cyanide in biological fluids. These involve colorimetric, fluorimetric or chromatographic techniques (Cruz-Landeira *et al.* 2000; Dunn and Siek 1990; Felscher and Wulfmeyer 1998; Gambaro *et al.* 2007; Lindsay *et al.* 2004; Lundquist *et al.* 1987; Odoul *et al.* 1994; Suzuki *et al.* 1982). Some of these methods can be laborious and are generally unsuited to the emergency diagnosis and management of patients (Lindsay *et al.* 2004). Many described methods are devoted to the forensic examination of postmortem blood and stomach contents. Some methods are based on the formation of the purple-coloured complex 2-nitrophenylhydroxylamine (Guilbart and Kramer 1966).

This complex is formed by the reaction of cyanide in alkaline solution with 4-nitrobenzaldehyde and 1,2-dinitrobenzene, and the reaction has been adapted for quantitative Conway diffusion methods (Dunn and Siek 1990; Flanagan *et al.* 1995). As this method has an unstable endpoint, great care is required in standardising the analytical procedure to ensure accurate results. However, a significant innovation described by Vesey *et al.* (1999b) has made major improvements. The new procedure is very much faster (<30 min) and has a stable endpoint that makes it suitable for the rapid analysis of blood cyanide in clinical cases.

A useful fluorimetric method has been described by Suzuki *et al.* (1982), which is based on the reaction of cyanide with pyridoxal 5-phosphate to produce highly fluorescent 4-pyridoxic acid 5-phosphate in acid solution. This method can be applied to both clinical and forensic cases, requires only 50 µL of blood and has a limit of detection of 0.1 mg/L. Analysis of plasma or urine thiocyanate may help to assess 'chronic' cyanide exposure, including the use of cyanogenic compounds (Haque and Bradbury 1999; Vesey *et al.* 1999a). This may be important in patients with renal impairment, in whom thiocyanate may accumulate to high levels and cause toxicity. The presence of thiocyanate and thiosulfate at concentrations of up to 50 mg/L does not interfere with the determination of cyanide (R Braithwaite, unpublished findings).

Interpretation

Very small quantities of cyanide are found in blood from normal metabolic processes and also from smoking. Reference values are somewhat variable. A recent review of published studies showed that reference values in non-smokers ranged between <0.01 and 0.08 mg/L, whereas in smokers values ranged between <0.01 and 0.18 mg/L (Lindsay *et al.* 2004).

Minor signs and symptoms of cyanide toxicity are associated with blood cyanide concentrations up to 1 mg/L. Severe symptoms are generally associated with higher concentrations, which may be as high as 20 mg/L on admission to hospital after suicidal ingestion or industrial exposure (Singh *et al.* 1989).

In postmortem cases after suicidal ingestion of cyanide salts, blood cyanide concentrations above 10 mg/L are generally observed. However, it is important to ensure that blood specimens are taken from reliable peripheral sites, such as the femoral vein. In cases of oral ingestion of cyanide salts, elevated (>50 mg/L) blood cyanide concentrations may sometimes be found in samples taken from the heart or other central sites; the cause is postmortem diffusion of unabsorbed cyanide from the stomach. In a study of 17 cases of suicide by cyanide reported from New York, postmortem blood cyanide concentrations ranged between <1 and 185 mg/L, with a mean value of 38 mg/L. However, the site of blood collection was not stated. The amount of cyanide detected in gastric contents ranged between 2 and 2218 mg, with an average of 232 mg (Gill *et al.* 2004). Fires can also generate considerable quantities of HCN (as well as carbon monoxide and a range of other toxic gases) and this may result in relatively high concentrations of cyanide in the blood of fire victims (Alarie 2002; Canfield *et al.* 2005; Lindsay *et al.* 2004; Yeoh and Braitberg 2004). In a study of fire victims who survived, blood cyanide concentrations ranged from 'not detected' to 3.9 mg/L, while fatal cases had levels of up to 7.4 mg/L (Lindsay *et al.* 2004). It seems clear that there is a synergistic effect between carbon monoxide and cyanide in causing toxicity and possible survival in accidents. This issue has been studied in some detail in the case of aviation accidents, where it has been shown useful to take into account the concentration of both carboxyhaemoglobin and cyanide in the blood (Canfield *et al.* 2005). An additional factor that may need to be taken into account in the interpretation of blood cyanide concentrations in fire victims is the amount of methaemoglobin present, which is able to sequester cyanide in the form of cyanomethaemoglobin (Moriya and Hashimoto 2003). There is evidence that administration of cyanide antidotes such as hydroxocobalamin at the fire scene, prior to hospitalisation, may save lives and that the benefits outweigh the risks (Boron *et al.* 2007; Fortin *et al.* 2006).

Where there is evidence of putrefaction, particularly in postmortem specimens, it is possible for bacterial or fungal contamination to either break down or generate cyanide in the body or in specimens. This can be reduced by collecting blood into anticoagulated tubes that contain sodium fluoride (Curry *et al.* 1967; Lokan *et al.* 1987).

Fluoride

Introduction

Fluoride is present in variable amounts in the soil and natural water supplies. It is also found in almost all plant and animal food products and is regarded as an essential trace element. Fluoride may also be added to domestic water supplies as a prophylaxis against dental caries, generally at a concentration of 1 mg/L where the 'natural' water content is low. Inorganic salts of fluoride have widespread use in industry, for example in smelting aluminium, and are also applied as insecticides and rodenticides. Fluoride is an effective enzyme inhibitor that has found extensive use in the preservation of biological specimens. The highly corrosive hydrofluoric acid (HF) is widely used industrially, for example in cleaning processes such as rust removal and in surface etching techniques. Different strengths of HF may be used industrially and the anhydrous form of the acid is particularly dangerous, following even minor exposure to skin (Huisman *et al.* 2001). Following ingestion, HF is rapidly absorbed from the stomach, causing extensive internal damage to tissues. Surface acid burns can also be particularly painful following deep penetration into tissues.

Acute and chronic poisoning caused by the ingestion of fluoride salts have been well documented (Augenstein *et al.* 1991; Gessner *et al.* 1994; Poklis and Mackell 1989; Sandy and Rose 1988). Fluoride compounds are available in tablet form to prevent dental decay in infants and children. They are also prescribed in the treatment of osteoporosis and other bone disorders. Signs and symptoms of acute fluoride poisoning by different routes of absorption include nausea, vomiting, diarrhoea and abdominal pain. In cases of oral ingestion of dilute concentrations of HF there may only be minimal damage to the oral mucosa but extensive damage to the lower GI tract. In severe cases there may be significant erosion of tissue and the development of hypocalcaemia, hypomagnesaemia and hyperkalaemia. This may also be associated with signs of cardiotoxicity including heart rhythm disturbances (Martinez *et al.* 2007). Chronic exposure to fluoride compounds has been associated with the development of osteofluorosis. Concern has also been expressed regarding the amount of fluoride in drinking water and possible association with hip fractures in the UK, but a large case–control study found no evidence of increased risk (Hillier *et al.* 2000).

Specimen collection

Specimens are collected as follows:

- Blood – 5 mL, plain or K-EDTA tube (avoid fluoride and/or oxalate anticoagulated containers)
- Urine – 10 mL, sterile plastic universal container
- Water – 10 mL, plastic universal container.

Analysis

Numerous colorimetric and spectrophotometric methods for the analysis of fluoride in biological fluids have been described (Cowell 1975). Some of these methods are very laborious to perform, particularly if fluoride has to be separated first by a diffusion process. A more convenient approach is to use an electrode specific to fluoride ions (Ekstrand 1977; Kissa 1987; Ohlson and Sheridan 1991; Speaker 1976). However, great care must be taken in the use and calibration of these electrodes (Kissa 1987; Martinez *et al.* 2007).

Interpretation

Reference ranges for serum, blood and urine vary widely, depending on dietary intake, access to fluoridated water and the use of fluoridated dental products. Reference ranges of 6–42 µg/L (serum) and 20–60 µg/L (blood) have been reported. For urine, a reference range of 0.2–3.2 mg/L has been reported, depending on dietary intake. Administration of multiple doses of 3–4.5 mg of fluoride yield steady-state plasma fluoride concentrations between 54 and 145 µg/L (Ekstrand *et al.* 1977). The therapeutic range for plasma fluoride concentrations in the treatment of osteoporosis is between 100 and 200 µg/L, with side-effects occurring at concentrations >300 µg/L. In fatalities, a wide range of antemortem and postmortem blood and urine fluoride concentrations have been reported. Fluoride concentrations in blood have ranged between 0.5 and 85 mg/L, whereas urine concentrations have ranged between 5 and 320 mg/L (Martinez *et al.* 2007) In a recent case of a suicidal

ingestion of rust remover by a 33-year-old psychiatric patient who died within 3 h of ingestion, the peripheral blood fluoride concentration was 19 mg/L, urine 670 mg/L, liver 40 mg/kg and kidney 60 mg/kg. The estimated dose of fluoride ingested was estimated to be 2.5–5 g (Martinez *et al.* 2007).

Nitrites and nitrates

Introduction

Sodium nitrate finds uses in artificial fertilisers, food preservatives and explosives (as do the potassium salts). Sodium nitrite is also used as a food preservative and in the manufacture of explosives, but has the additional property of causing vasodilatation, which has led to its application in treating angina pectoris. Organic nitrates (e.g. glyceryl trinitrate, isosorbide mononitrate and dinitrate) release nitrite ion when ingested and are also used as vasodilators. Intravenous injection of sodium nitrite to induce methaemoglobinaemia has been used to treat cyanide poisoning. Nitrite ion is rapidly converted into nitrate ion by the body. Symptoms of nitrate and/or nitrite poisoning include nausea, vomiting, diarrhoea, abdominal pain, confusion and coma. These strong oxidising agents can cause a life-threatening methaemoglobinaemia to which infants are particularly susceptible. Methaemoglobin measurement is the most useful test in the diagnosis and clinical management of poisoning cases. Treatment with oxygen and methylene blue to reduce methaemoglobin can be an effective therapy.

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL K-EDTA tube
- Urine – 20 mL sterile plastic universal container.
- Stomach contents and scene residues may also be useful.

Analysis

A number of methods are used to quantify nitrate and nitrites in biological fluids, based on spectrophotometric or chromatographic techniques (El Menyawi *et al.* 1998; Giovannoni *et al.* 1997; Kage *et al.* 2002b; Moshage *et al.* 1995).

Interpretation

Plasma nitrite and nitrate concentrations in unexposed subjects are about 0.2 mg/L and 1.2 mg/L, respectively. Serum nitrite levels of 0.5 and 0.6 mg/L were measured in two men who became cyanotic after accidentally ingesting about 1 g of sodium nitrite, but survived after treatment (Sevier and Berbatis 1976). A child who absorbed sodium nitrite from a liniment solution applied to the skin developed a severe cyanosis and died some days later with a serum nitrite concentration of 1.0 mg/L and a blood methaemoglobin level of 76% (Saito *et al.* 1996). In a fatal suicide, nitrite concentrations were 0.5 mg/L (blood), 0.3 mg/kg (kidney), 8.7 mg/L (urine) and 3.9 mg/L (gastric contents) (De Beer *et al.* 1975).

Phosphine and phosphides

Introduction

Phosphine (hydrogen phosphide; PH_3) is a highly toxic colourless gas with a strong garlic or fishy smell; it is used in a number of industrial processes (e.g. the production of acetylene gas and manufacture of semiconductors). It is also generated by the action of moisture on phosphides. Aluminium phosphide is used extensively as a cheap and effective grain fumigant and rodenticide in developing countries. Aluminium phosphide poisoning has a high mortality and the 1990s have seen a dramatic increase in the number of poisoning cases and deaths caused by suicidal ingestion, particularly in India (Christophers *et al.* 2002). Poisoning cases involving inhalation of phosphine gas or ingestion of phosphides have also occurred in many European countries (Anger *et al.* 2000; Bayazit *et al.* 2000; Gregorakos *et al.* 2002; Memis *et al.* 2007; Popp *et al.* 2002).

Signs and symptoms of poisoning include headache, nausea, vomiting, cyanosis, coma, non-cardiogenic pulmonary oedema and heart rhythm disturbance, which may progress to hepatic and renal failure (Guale *et al.* 1994; Lakshmi 2002; Popp *et al.* 2002; Singh *et al.* 1996). Methaemoglobin formation has also been reported (Lakshmi 2002). Histological changes in liver and brain following postmortem examination of phosphine-related deaths have recently been reported (Saleki *et al.* 2007; Tripathi and Pandey 2007).

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL, lithium–heparin or K-EDTA tube (use of special trace element tubes is recommended if the analysis of aluminium is to be carried out; glass containers should not be used)
- Urine – 20 mL, sterile plastic universal container
- Stomach contents – useful in postmortem cases
- Scene residues – may be useful for identification purposes.

Analysis

A quantitative test for phosphine in gastric contents can be carried out using a commercial detector tube (Guale *et al.* 1994). Phosphine can be measured in body fluids and postmortem tissues by headspace analysis and GC with either nitrogen–phosphorus detection or mass-selective detection (Anger *et al.* 2000; Chan *et al.* 1983; Chugh *et al.* 1996). Alternative approaches include the measurement of aluminium in blood using ETAAS or ICP-MS (Anger *et al.* 2000; Garry *et al.* 1993). Phosphorus can also be measured by ICP-MS (Anger *et al.* 2000).

Interpretation

A detailed case report by Anger *et al.* (2000) concerned the suicidal ingestion of aluminium phosphide by a 39-year-old man in whom phosphine was found in the brain (94 mg/g), liver (24 mg/g) and kidneys (41 mg/g), but was absent in the blood and urine. The blood contained a high concentration of phosphorus (76.3 mg/L) and 8.22 mg/g was found in the liver. The aluminium concentration in the blood was 1538 μg/L, compared with reference values of less than 15 μg/L. Raised aluminium concentrations were also found in the brain (36 μg/g) and liver (75 μg/g). Where there has been inhalation of phosphine gas, rather than oral ingestion of phosphides, it can sometimes be difficult to detect the presence of phosphine in blood, or in postmortem tissues, such as brain. This may be due to the rapid elimination of phosphine, particularly if patients are hospitalised prior to death.

Sulfide

Introduction

Many organic and inorganic sulfide compounds are used in industry, but the most common cause of sulfide poisoning is by inhalation of hydrogen sulfide (sewer) gas, particularly in industrial or waste disposal sites, fisheries, tanneries, dye works and sewer networks (Christia-Lotter *et al.* 2007; Guidotti 1994; Jappinen and Tenhunen 1990; Kage *et al.* 2002a; Knight and Presnell 2005). The gas has a very characteristic foul odour of rotten eggs and has a very low odour threshold (0.03 ppm). However, very high sulfide concentrations (>100 ppm) may cause paralysis of the olfactory nerves and failure to detect the characteristic smell that would normally be a clear hazard warning (Guidotti 1994). At even higher sulfide concentrations (>700 ppm) there may be a rapid loss of consciousness, apnoea and respiratory failure leading to a rapid death (Knight and Presnell 2005).

Hydrogen sulfide is unstable and is metabolised rapidly in the body, such that it may be difficult to detect in biological samples from cases of suspected poisoning. It is metabolised into thiosulfate, which presents a useful approach to the investigation of acute or chronic sulfide poisoning.

Specimen collection

Specimens are collected as follows:

- Blood – 10 mL of anticoagulant blood (K-EDTA)
- Urine – 20 mL, sterile plastic universal container
- Stomach contents – useful in postmortem examination of fatalities
- Scene residues – useful for identification purposes.

Analysis

Early methods used microdiffusion followed by colorimetric measurement. Ion-selective electrodes have also been used (Jappinen and Tenhunen 1990). More recently, GC methods have been described using derivative formation and mass spectrometric detection (Kage *et al.* 2002a, 2004). A number of methods for measuring the main metabolite thiosulfate in blood and urine have been reported, including GC, LC and GC-MS (Kage *et al.* 1991, 1997, 2004; Kangas and Savolainen 1987).

Interpretation

Reference values for sulfide concentrations in biological fluids are very low (<10 μg/L). In a series of cases of hydrogen sulfide poisoning from a sulfate pulp mill reported by Jappinen and Tenhunen (1990), sulfide concentrations ranged from 30 μg/L to 130 μg/L in blood samples taken 0.5–2 h after exposure. Similar concentrations have been reported by Kage *et al.* (2002a) in fatal and non-fatal poisonings. In cases of suspected acute or chronic exposure to hydrogen sulfide, blood specimens must be collected as soon as possible because of its rapid metabolism. Measurement of blood or urine thiosulfate concentration may be the most viable investigation in some cases (Durand and Weinstein 2007; Kage *et al.* 1991, 1997).

Postmortem case reports after industrial accidents have shown blood sulfide concentrations of 0.9–3.8 mg/L (Goodwin *et al.* 1989). In a recent report of four acute adult fatalities that occurred in a Japanese dye works, postmortem blood sulfide concentrations ranged between 0.3 and 9.4 mg/L and thiosulfate concentrations ranged between 0.1 and 0.2 mmol/L (Kage *et al.* 2004).

Acknowledgements

I thank Dr Brian Widdop for his invaluable advice and assistance in the preparation of this manuscript.

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CHAPTER

18 Drugs in Saliva

V Spiehler

Introduction

Definitions

Saliva is the secretion product of the salivary glands of the head and mouth. The fluids found in the oral cavity are a mixture of predominantly saliva, with lesser amounts of gingival crevicular fluid, cellular debris and blood. For this reason the New York Academy of Sciences meeting on saliva testing in 1993 agreed to use the term 'saliva' for glandular secretions that are collected directly from the salivary glands (most often the parotid glands), and the term 'oral fluid' for fluid collected by placing absorbents in the oral cavity or by expectoration (Malamud 1993).

Advantages of saliva drug testing

The advantages of saliva drug testing are twofold. First, saliva drug concentrations can be related to plasma free drug concentrations and pharmacological effects of drugs. Second, collection of saliva or oral fluid is non-invasive and simple, and can be done on-site under observation.

Saliva drug concentrations are related to blood or plasma concentrations of the unbound, unionised parent drug or its lipophilic metabolites (Haeckel, Hanecke 1996). Since it is the free lipophilic drug and drug metabolites that cross cell membranes, such as the blood–brain barrier, and cause physiological effects, free drug in plasma, and its reflection in saliva, can be correlated with drug effects. The presence and concentration of drugs in saliva therefore provide much of the same information as determination of drug presence and drug concentrations in blood or plasma. Saliva drug concentrations can be used to determine pharmacokinetic parameters. Additionally, saliva or oral fluid collection is much easier than venepuncture.

The collection of saliva or oral fluid is simple and non-invasive. It can be carried out by the specimen donor him- or herself by having the donor place a cotton swab or absorptive material attached to a stick into his or her mouth for a few minutes. The oral fluid absorbed on the material can then be processed for testing. Saliva can be collected at the site of the incident in accident or crime investigation. If necessary, saliva flow can be stimulated with citrate hard candy or citrate salts or by chewing on gum or rubber to ensure adequate sample volume. Because saliva collection is non-invasive and can be carried out by the donors themselves in most situations, it is more acceptable to most people than providing urine, blood or hair (Fendrich *et al.* 2002), and can be achieved while the donor is under observation by the collector. An exception may be when the donor is unconscious or sedated such that he or she is unable to follow instructions. Finally, it is difficult to adulterate or substitute oral fluid specimens in an attempt to avoid detection of drug use as any adulterating substances held in the mouth will dissipate, be swallowed or be spat out during the 10-minute observation period before collection of the specimen (Jehanli *et al.* 2001).

A simple non-invasive collection finds many applications in toxicology. For example, investigation of the involvement of drugs in impaired driving is facilitated by a roadside test for drugs or alcohol in saliva. The feasibility of detecting drugs in saliva samples obtained from impaired drivers was first investigated by Peel *et al.* (1984). They found that the presence of drugs in saliva correlated well with officers' judgements of driving while intoxicated. This was confirmed by better correlation of drugs in oral fluid or serum with driving and impairment symptoms observed by police officers and medical officers than of drugs in urine (Toennes *et al.* 2005). Saliva testing has also been compared with urinalysis in a population of arrested persons (Yacoubian *et al.* 2001) and in drugged drivers (Steinmeyer *et al.* 2001). The greatest advantage of saliva in roadside testing is the possibility of having the sample collected by the donor under observation shortly after the time of the incident.

Disadvantages of saliva drug testing

Like any biofluid from human, oral fluid may transmit infectious agents and should be handled with the appropriate universal precautions for human biological fluids. Saliva contains mucopolysaccharides and mucoproteins, which make it less fluid and less easily poured or pipetted than urine. Some drugs, medical conditions or anxiety can inhibit saliva secretion and cause a dry mouth; accordingly, saliva may not be available from all individuals at all times. Finally, because saliva drug concentrations depend on plasma drug concentrations, drugs that have a short plasma half-life and are rapidly cleared from the body will be detectable in saliva only for a short time. This is a potential disadvantage against detection of drugs in hair, sweat or urine. Saliva and blood will have the shortest detection windows. Drugs or their long-lived metabolites will be detectable in urine and sweat for several days to a week after use. Drugs will be detectable in hair for months or even years after use depending on the length of the hair. In general, drugs enter saliva from the blood and will be detectable in plasma and saliva from the time that the drug enters the general circulation until approximately four half-lives after administration. It is important to bear in mind that some drugs may be detected in oral fluid as a consequence of external contamination (see later) and that the measured drug concentration may not accurately reflect that in the blood.

Anatomy and physiology of saliva

The saliva glands

The human salivary glands produce between 0.5 and 1.5 L of saliva daily. During resting conditions, most mixed saliva is supplied by the submandibular glands (70%), with a lesser amount (25%) from the parotid glands and the remainder (5%) from the sublingual and other minor glands. During stimulation saliva output from the parotid gland increases to about half of the total. Saliva is composed of 99% water, 0.3% protein (largely amylase) and 0.3% mucins. The parotid gland produces mostly serous fluid. The submandibular and sublingual glands excrete both serous fluid and mucins. The salivary glands, like the liver, kidney and brain, are well supplied with arterial blood.

Salivary glands comprise two regions: the acinar region, which contains the cells that are capable of secretion, and the ductal region, lined with water-impermeable cells that carry the secretions to the outlets in the mouth (Turner 1993). Similarly, saliva formation occurs in two

stages. Water and exocrine proteins are secreted by the secretory cells in the acinar region. The fluid that collects in the acinar lumen is isotonic with plasma. As the fluid travels down the saliva ducts, sodium and chloride are reabsorbed while potassium and bicarbonate are secreted. Therefore, when saliva moves rapidly through the ducts, less time is available for sodium reabsorption and the pH of the saliva is higher (Dawes, Jenkins 1964). When the fluid reaches the mouth it is hypotonic to plasma.

Salivary glands are activated by the autonomic nerves. Generally, sympathetic stimulation via noradrenaline causes low levels of fluid and high concentrations of protein, while parasympathetic stimulation via acetylcholine induces large amounts of fluid secretion.

Movement of drugs into saliva

Excretion and diffusion

Some drugs such as digoxin, steroids and hormones are actively excreted into saliva by the acinar cells. Most drugs enter saliva by simple diffusion across the phospholipid bilayer of the acinar cells or though the cell membranes of the ductal cells in the tubules. Diffusion across cell membranes requires that the molecules are lipid soluble, non-ionised and unbound. For this reason the concentrations of drugs in saliva represent the free, non-ionised fraction in the blood plasma.

Henderson–Hasselbach equation

At equilibrium, drug and lipophilic metabolite concentrations in saliva are a function of a drug's pK_a, plasma and saliva pH, and the fraction of drug bound to saliva and plasma protein as shown by the following form of the Henderson–Hasselbach equation for saliva (S/P is the saliva : plasma ratio):

$$S/P = \frac{1+10^{(pK_d - pH_S)}}{1+10^{(pK_d - pH_p)}} + \frac{f_p}{f_s} \text{ for basic drugs}$$

$$S/P = \frac{1+10^{(pH_s - pK_a)}}{1+10^{(pH_p - pK_a)}} + \frac{f_p}{f_s} \text{ for acidic drugs}$$

where S is the drug concentration in saliva and P is the drug concentration in plasma; pK_d is the log of the ionisation constant for basic drugs; pK_a is the log of the ionisation constant for acidic drugs; pH_s is the pH of saliva and pH_p is the pH of the plasma; f_p is the fraction of drug protein bound in plasma and f_s is the fraction protein bound in saliva.

The pH value that governs this equilibrium is the pH of the saliva in the acinar lumen and in the duct at the moment of secretion. When the fluid enters the mouth, carbon dioxide is lost and the pH increases. Dawes and Jenkins (1964) demonstrated that saliva pH is inversely proportional to flow rate and the reabsorption of sodium in the salivary tubules. At faster flow rates, less sodium is reabsorbed in the tubules on the way from the salivary glands to the saliva outlets in the mouth, and the pH rises. For this reason, unstimulated saliva has a low pH and stimulated saliva has a higher pH. The pH of unstimulated saliva is fairly constant. The normal pH of saliva at low flow rates is between pH 6.0 and pH 7.0. The pH of stimulated saliva can reach as high as pH 8.0.

Since human saliva normally has a lower pH than human plasma, the saliva : plasma ratios for acid drugs are generally less than unity and the saliva : plasma ratios for basic drugs are greater than unity, providing an amplification of basic drug levels in saliva. For drugs that have a pK_a between 5.5 and 8.5, the saliva/plasma ratio can vary between stimulated and unstimulated saliva. This is true of many drugs of abuse. For this reason it is more conservative to use a cut-off value for drugs of abuse in saliva rather than to determine the absolute concentration. The most common example given is that of cocaine, which has a pK_a of 8.6 (Haeckel 1993; Schramm *et al.* 1992). As the saliva pH varies from 5.0 to 7.8, the saliva : plasma ratio for cocaine varies from 273 to 0.44. The theoretical range of saliva : plasma ratios over a saliva pH range of 6.4–7.6 have been calculated for cocaine, amfetamine, metamfetamine, 6-monoacetylmorphine (6-MAM), morphine, codeine, methadone and diazepam, and compared with published saliva : plasma ratios (Spiehler *et al.* 2000).

The protein binding of drugs is mainly to albumin or α-acid glycoprotein in plasma. Saliva mucoproteins have very little binding capacity for drugs. Oral fluids may contain albumin from the gingival crevicular fluid.

Oral deposition: smoked drugs and sublingual or buccal routes of administration

The deposition into mouth tissues of drugs taken by smoking, snorting or oral routes of administration is an additional source of drugs in oral fluid. For example, Jenkins *et al.* (1995) showed that the saliva : plasma ratio for smoked diamorphine was 100–400 times that of diamorphine administered intravenously. After smoking of diamorphine, it was detected in oral fluid up to 24 h compared with up to 30 min after intravenously administration.

O'Neal *et al.* (2000) reported codeine saliva : plasma ratios of 75–2580 in the first 15–30 min after and of 13–344 for several hours after oral administration of liquid codeine phosphate, despite efforts at decontamination by having people brush their teeth and vigorously rinse their mouth prior to saliva collection.

Similarly, Jenkins *et al.* (1995) reported that when cocaine was smoked the saliva : plasma ratio was 5–300 times that found after cocaine administered intravenously. The pyrolysis product of cocaine, anhydroecgonine methyl ester (AEME), was detected in oral fluid collected after smoking cocaine but not in plasma. Similarly, the cannabinoids found in oral fluid are almost totally due to oral deposition of cannabinoids from smoked marijuana rather from secretions or diffusion into saliva (Ohlsson *et al.* 1986).

The formation of oral mucosa depots of drug that are rapidly absorbed into the blood circulation is used for drug administration. Sublingual or buccal absorption of drugs such as nitroglycerin, buprenorphine or fentanyl has the advantage of very rapid delivery that bypasses the liver and gastrointestinal first-pass metabolism. Drugs administered by this route also produce large concentrations of the parent drug in oral fluid with a short detection window as the drugs are rapidly absorbed.

Individual drugs in saliva

Opiates: diamorphine, 6-MAM, morphine, codeine and related drugs

The major metabolite found in saliva following diamorphine use is 6-MAM, which has a saliva : plasma ratio of 6 (Cone 1993; Cooper *et al.* 2005; Jenkins *et al.* 1994, 1995; Moore L *et al.* 2001; Presley *et al.* 2003). Diamorphine parent drug was found in oral fluid for up to 24 h after smoking and up to 60 min after injection of diamorphine with a saliva : plasma ratio after intravenous administration of 2.13, range 0.12–7.2 (Jenkins *et al.* 1995). If diamorphine is snorted (Cone 1993) or smoked (Jenkins *et al.* 1995) then very high concentrations of diamorphine in the milligram per litre range may be detected in oral fluid for several hours after use owing to deposition of parent drug in the oral cavity. In addition to diamorphine and 6-MAM, morphine may be found in saliva after diamorphine use. An average saliva : plasma ratio of 0.67 (range 0.1–1.82) for morphine in oral fluid after intravenous administration of diamorphine was reported by Jenkins *et al.* (1995). Euphoria after diamorphine use occurs rapidly and diminishes within the first hour, paralleling the time course of diamorphine in saliva and blood. Miosis caused by diamorphine peaks approximately 15 min after administration by smoking or intravenous injection and persists for up to 4 h. Miosis parallels the time course of saliva and blood concentrations of 6-MAM and morphine (Jenkins *et al.* 1994).

Morphine appears in saliva after administration of morphine sulfate with a saliva : plasma ratio of 0.2 (Cone 1993). Leute *et al.* (1972) detected

morphine in saliva at concentrations exceeding 200 μg/L using a free-radical immunoassay. Gorodetzky and Kullberg (1974) reported the detection of morphine in saliva by radioimmunoassay (RIA) for 6 h after dosing in patients receiving 30 mg morphine sulfate subcutaneously. Cone (1990) reported that morphine concentrations in saliva ranged from 0.6 μg/L to 10.9 μg/L after intramuscular administration of 10 mg morphine sulfate and 37.8 μg/L in a single individual who received 20 mg morphine sulfate by the same route. Saliva concentrations peaked at 0.5 h after dosing and were no longer detectable after 24 h. Miosis was noted within 15 min of dosing and declined to baseline after 24 h. Morphine is also found in oral fluid after diamorphine use (Cone 1993; Jenkins *et al.* 1994, 1995; Moore L *et al.* 2001).

Hydromorphine is found in saliva with a saliva : plasma ratio in the elimination phase ranging from 0.25 μg/L to 2.32 μg/L for eight subjects with both inter-individual and intra-individual variation (Ritschel *et al.* 1987). Hydromorphone was detected in saliva for up to 10 h by RIA after administration of 2–5 mg intravenous hydromorphone. The terminal elimination half-life for saliva (2.12 h) was not significantly different from that for plasma (2.36 h). Hydromorphone confirmation in oral fluid by gas chromatography–mass spectrometry (GC-MS) using a methoximine derivative has been reported (Jones *et al.* 2002).

Pholcodine is found in saliva in the range 1.5–350 μg/L after oral doses of 20 and 60 mg (Chen *et al.* 1988). The saliva : plasma ratio was 3.6 calculated from the mean areas under the concentration–time curves for plasma and saliva. The elimination half-life for pholcodine from saliva was 51.2 ± 11.9 h. Pholcodine was detectable in saliva for 20 h after the last dose on day 11 of chronic dosing.

After codeine administration, codeine is found in saliva with a saliva : plasma ratio of 3.3 (Cone 1993). O'Neal *et al.* (1999) reported a mean saliva : plasma ratio of 3.7 ± 0.28 when measured 2–12 h after oral administration of 30 mg codeine and concluded that saliva codeine concentrations could be used to estimate plasma concentrations through use of saliva : plasma ratios. Chen *et al.* (1991) determined saliva and plasma concentration–time profiles for codeine following single and chronic oral doses of 30 mg codeine. The codeine saliva : plasma ratio was 3.0 from the area under the concentration–time curves. Chen *et al.* (1991) concluded that pharmacokinetic parameters for codeine for individuals and their individual status as a poor or extensive metaboliser can be determined from saliva measurements if a 1 h clearance time is allowed after oral dosing. Codeine 6-glucuronide was not found in saliva (Chen *et al.* 1991).

O'Neal *et al.* (2000) reported that saliva codeine concentrations collected by expectoration or draining of unstimulated of oral fluid were 1.3–2.0 times higher than codeine concentrations in oral fluid collected by swabbing with absorptive devices (OraSure, Salivette and Finger Collector containing AccuSorb foam). As expected from the Henderson–Hasselbach equation above, codeine concentrations in unstimulated saliva collected by expectoration were 3.6 times higher than concentrations in specimens collected by expectoration after acidic stimulation by citric acid. In unstimulated saliva collected by expectoration into test tubes, O'Neal *et al.* (2000) found codeine in 15 of 22 specimens at 24 h after oral administration of 30 mg liquid codeine phosphate but in only 20–40% of specimens collected with the absorptive swabs. Jehanli *et al.* (2001) reported that three of five volunteers' oral fluid specimens were positive for codeine with the Cozart RapiScan Saliva collection and testing system at 24 h after oral administration of 16 mg codeine. These were confirmed by GC-MS using a cut-off of 5 μg/L (Fig. 18.1). The clinical sensitivity and specificity for codeine were 91% and 98% respectively (Fig. 18.2).

Other prescription opiates and opioids reported in oral fluid include hydrocodone, oxycodone (Jones *et al.* 2002), dihydrocodeine (Skopp *et al.* 2001) and dextromethorphan (Lutz *et al.* 2004; Rodrigues *et al.* 2008).

The sensitivity and specificity of a qualitative analytical test are functions of the decision threshold cut-off employed in the test. If the saliva : plasma ratio values are multiplied by the lower limit plasma levels for therapeutic or recreational effects from Uges (1996), the resulting suggested cut-offs for opiates in saliva are: 6-MAM 30 μg/L, morphine 40 μg/L, codeine 66 μg/L, methadone 50 μg/L, cocaine 60 μg/L, amfetamine 56 μg/L, metamfetamine 40 μg/L and diazepam 3.6 μg/L (Spiehler *et al.* 2000). The proposed US workplace cut-off for opiates in saliva is 40 μg/L morphine equivalents by immunoassay and the confirmation cut-offs are 40 μg/L morphine, 40 μg/L codeine or 4 μg/L 6-MAM (SAMHSA 2001).

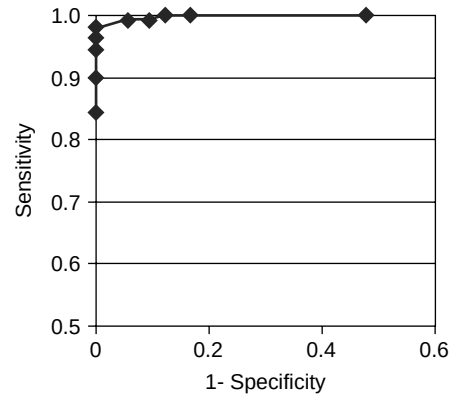

Figure 18.1 Receiver operating curve for saliva opiates using the Cozart Microtiterplate Opiates test for putative cut-offs of 1 to 100 ng/mL morphine equivalents in diluted oral fluid.

A laboratory-based oral fluid opiate test (Intercept, OraSure Technologies, Bethlehem, PA, USA) used a cut-off of 10 μg/L in dilute oral fluid (threefold dilution) and found a correlation of 93.6% with urine specimen results in a population of diamorphine abusers (Niedbala *et al.* 2001b). Ingestion of 5.2–40 g of poppy seeds produced saliva opiate positive results at 15 min but not at 1 h. However no 6-MAM was found in saliva after poppy seed ingestion.

Speckl *et al.* (1999) evaluated a collection device (ClinRep) consisting of a treated cotton roll which was then centrifuged and the oral fluid collected and filtered before extraction, derivatisation and analysis by GC-MS. They reported that the concordance of the analytical results for opiates of the saliva samples with urine was 93% for a decision limit of 100 μg/L and 98% for a decision limit of 300 μg/L.

An on-site lateral-flow immunoassay for opiates in oral fluid employing up-converting phosphor antibody labels (UPLink System, OraSure Technologies, Inc.) was reported to have a sensitivity (true positive rate) for opiates vs GC-MS(-MS) of 62% using a decision cut-off of 40 μg/L morphine equivalents and a specificity (true negative rate) of 84% (Niedbala *et al.* 2002). The assay had a cross-reactivity of 87% with 6-MAM, greater than 73.7% with diamorphine, 60% with codeine, hydrocodone and hydromorphine, and 30–40% cross-reactivity with oxycodone, oxymorphone and normorphine.

The cut-off of the Cozart RapiScan Oral Fluid Drug Testing System (Cozart BioScience Ltd, Abingdon, UK), was established by receiver operating curve (ROC) analysis, as 10 μg/L morphine equivalents in diluted oral fluid or 30 μg/L morphine equivalents in neat saliva (Moore L *et al.* 2001). The Cozart RapiScan antibody is equally cross-reactive with morphine, 6-MAM, diamorphine, dihydrocodeine and codeine. Saliva opiate-positive results with the Cozart RapiScan were confirmed by GC-MS in 18 of 22 specimens collected from volunteers at a substance abuse treatment clinic and negative results in 49 of 49 specimens (Moore *et al.* 2001).

		Opiates by Cozart RapiScan	
		+	–
Codeine dose given	yes	40	4
	no	1	42

Sensitivity	40/44	90.9 %	3.0%
Specificity	42/43	97.7 %	1.6%

Figure 18.2 Clinical sensitivity and specificity of the Cozart RapiScan after 60 mg codeine administered orally (from Jehanli 2001).

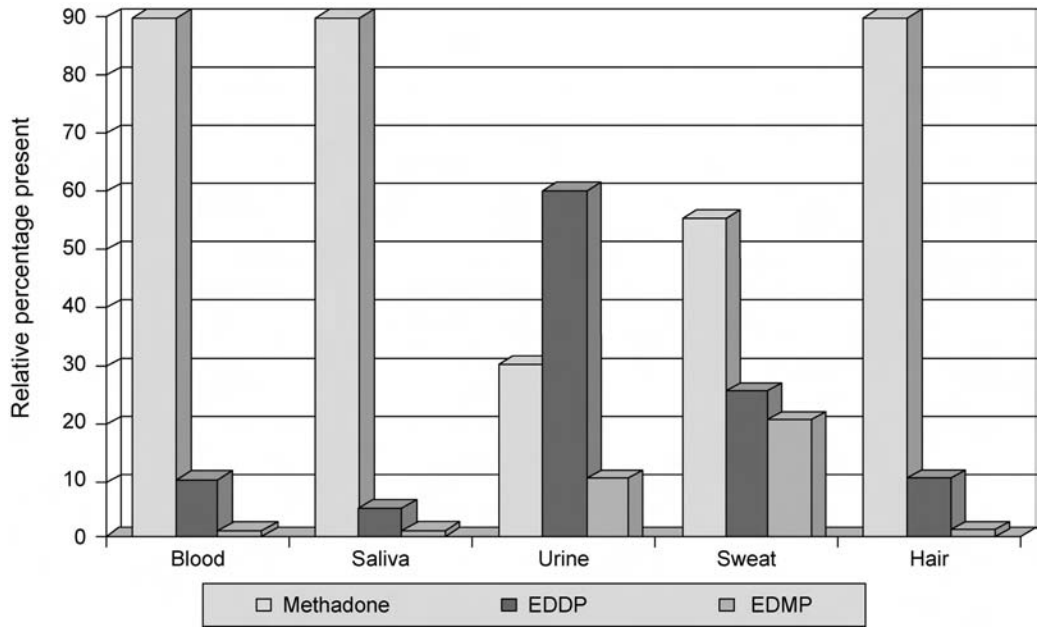

Figure 18.3 Metabolic profile of methadone in blood, saliva, urine, sweat and hair.

Methadone

Lynn *et al.* (1975) reported that methadone was found in saliva after parenteral administration of methadone. Kang and Abbott (1982) reported a GC-MS method for methadone and 3-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in saliva. The relative metabolic profile for methadone in blood, saliva, urine, sweat and hair is shown in Fig. 18.3.

Wolff *et al.* (1991) found that saliva methadone concentrations could be used to estimate plasma methadone concentrations to monitor consumption of methadone. Patient volunteers from an addiction treatment unit were asked to expel stimulated mixed saliva into plastic tubes before consumption of the daily dose of methadone linctus. Saliva and plasma methadone and EDDP were determined by high performance liquid chromatography (HPLC). A correlation coefficient of 0.8 was found by linear regression. Wolff *et al.* (1991) reported a saliva : plasma ratio of 1.30. This was greater than the ratio of 0.51 found by Kang and Abbott (1982), but less than the value of 4 reported by El Guebaly *et al.* (1981).

Since methadone has a pK_a of 8.3, the saliva : plasma ratio is a function of saliva pH. Bermejo *et al.* (2000) reported that the methadone saliva : plasma ratio as a function of saliva pH ranged from 0.6 to 7.2 with an average of 3.7 ($n = 10$) over a measured saliva pH range of 5.0–7.0. In the same specimens, the saliva : plasma ratio of EDDP ranged from 0.2 to 1.8 with an average of 0.89 and was not a function of saliva pH. Malcolm and Oliver (1997) reported that the saliva : whole blood ratio ranged from 0.45 to 3.4. Saliva was collected using the Omni-SAL collection device and analysed by microplate enzyme immunoassay and GC-MS.

Moore L *et al.* (2001) reported that saliva methadone positive results with the Cozart RapiScan Saliva Test were confirmed by GC-MS in 37 of 37 specimens collected from volunteers at a substance misuse treatment clinic and negative results in 34 of 34 specimens. The Cozart RapiScan oral fluid drug testing system cut-off is 10 μg/L methadone equivalents in diluted oral fluid or 30 μg/L methadone equivalents in neat saliva. The correlation to microtitre plate enzyme immunoassay for methadone for different saliva methadone cut-offs is shown in Fig. 18.4.

Cocaine

Cocaine parent drug is the major analyte found in saliva after cocaine use. In unstimulated saliva, cocaine is ion-trapped in saliva and the saliva : plasma ratio may be 5 or greater (Schramm *et al.* 1992, 1993a). In stimulated saliva, the saliva : plasma ratio ranges from 0.5 to 3.0 (Cone, Weddington 1989; Cone *et al.* 1988, 1997; Jenkins *et al.* 1995). Cocaine appears in saliva immediately after administration of the drug by intravenous injection. Benzoylecgonine and ecgonine methyl ester appear in saliva within 15 min of cocaine administration and are found in saliva at concentrations similar to those found in blood. Schramm *et al.* (1993a) reported a saliva : plasma ratio of approximately 2.5 for benzoylecgonine. Jenkins *et al.* (1995) reported saliva : plasma ratios for benzoylecgonine ranging from 0.02 to 0.66. Norcocaine and *p*-hydroxycocaine may be found in saliva after cocaine administration. Jufer *et al.* 2000 reported a saliva : plasma ratio of 8.7 (range 3.8–13.2) for cocaine, 3.7 (range 2.3–5.1) for ecgonine methyl ester, 0.4 (range 0.3–0.5) for benzoylecgonine, 10.3 (5.6–13.6) for norcocaine and 6.1 (range 2.4–10.8) for *p*-hydroxycocaine. Cocaethylene is found in saliva when ethanol is ingested concurrently with cocaine. In a rat model, Barbieri *et al.* (1994) reported a saliva : plasma ratio of 1.3 for cocaethylene after cocaethylene administration. Jenkins *et al.* (1995) reported that a pyrolysis product of cocaine, anhydroecgonine methyl ester (AEME), was detected in oral fluid collected after smoking cocaine but not in plasma.

Inaba and Kalow (1975) reported that radiolabelled cocaine given orally was detectable for up to 5 h in saliva. Thompson *et al.* (1987) reported that cocaine was found in saliva immediately after intravenous

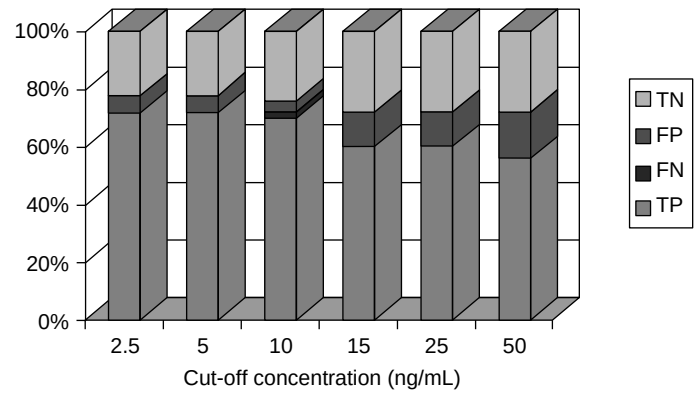

Figure 18.4 True negatives, false positives, false negatives and true positives for the Cozart RapiScan Saliva Methadone Test for different cut-offs versus a microplate enzyme immunoassay.

doses of cocaine and that saliva and plasma cocaine concentrations paralleled each other. Saliva cocaine concentrations correlated with the physiological and behavioural effects of the drug. Cocaine saliva : plasma ratios ranged from 0.36 to 9.74. Cone and Weddington (1989) reported detection of cocaine equivalents by immunoassay for 5–10 days after abstinence in heavy cocaine addicts and GC-MS confirmation for 1–2 days. In a more recent study of cocaine and metabolite elimination patterns, Moolchan *et al.* (2000) measured cocaine, benzoylecgonine and ecgonine methyl ester in saliva of admitted cocaine abusers for 12 h. In the later specimens, metabolites predominated, as is consistent with their longer half-lives in the body. Saliva terminal half-lives were 7.9 h for cocaine, 9.2 h for benzoylecgonine and 10 h for ecgonine methyl ester. Cone and Menchen (1988) reported the correlation of saliva and plasma cocaine concentrations after intravenous cocaine administration with an average half-life of cocaine of 34.7 min in saliva and 34.9 min in plasma. Jenkins *et al.* (1995) reported a detection time for cocaine in saliva of up to 8 h after intravenous injection and 12 h after smoking of cocaine. Cone *et al.* (1997) reported mean detection times of cocaine in saliva of 3.92 h after intravenous administration, 5.67 h after insufflation and 3.17 h after smoking of cocaine, which correlated with pupil diameter, heart rate and feeling of drug changes, but not with drug-induced euphoria and feeling high, which were of much shorter duration. Jufer *et al.* (2000) reported a prolonged terminal elimination half-life for cocaine metabolites from saliva after chronic (16-day) administration of cocaine due to accumulation of lipophilic drug.

Kato *et al.* (1993) compared cocaine in unstimulated with that in stimulated saliva. Unstimulated saliva collected by expectoration contained, on average, 5.2 (range 3.0–9.5) times as much cocaine as saliva collected under stimulated conditions (citric acid sour candy). The mean ratios of unstimulated to stimulated area under the concentration–time curve for benzoylegconine and ecgonine methyl ester were 6.0 (range 3.3–9.0) and 5.5 (range 2.5–8.8), respectively.

Jenkins *et al.* (1995) followed cocaine and metabolites in saliva after smoked and intravenous administration. It was shown that, after smoking, cocaine and pyrolysis products of cocaine persisted in saliva for up to 6 h. Cone *et al.* (1997) compared cocaine appearance in saliva after intravenous, intranasal and smoked administration, and found that both intranasal and smoked routes of administration produced elevated saliva : plasma ratios of cocaine due to contamination of oral fluids, which cleared within 2 h after administration.

Niedbala *et al.* (2001a) established an optimum screening cut-off of 10 μg/L benzoylecgonine equivalents by immunoassay by ROC analysis of clinical trial saliva results using GC-MS with a cut-off of 10 μg/L benzoylecgonine as the reference standard. The Cozart RapiScan Oral Fluid Drug Testing System uses a cut-off of 10 μg/L benzoylecgonine or 25 μg/L cocaine in diluted oral fluid, which is equivalent to 30 and 75 μg/L, respectively, in saliva (Cooper *et al.* 2004). The Cozart RapiScan antibody has a 40% cross-reactivity with cocaine. The proposed US workplace screening cut-off is 20 μg/L benzoylecgonine equivalents by immunoassay. The proposed US workplace confirmation cut-off is 8 μg/L benzoylecgonine or cocaine (SAMHSA 2001). Jufer *et al.* (2000) reported detection times in saliva of 15 h, 45 h and 35 h, for cocaine, benzoylecgonine and ecgonine methyl ester, respectively, at a 10 μg/L cut-off, and detection times of 85, 93 and 93 h respectively at a 1 μg/L cut-off threshold.

Amfetamines: amfetamine, metamfetamine, MDMA, MDA and MDEA

Amfetamine, metamfetamine, 3,4-methylenedioxymethamfetamine (MDMA), 3,4-methlyenedioxyamfetamine (MDA) and other amfetamine class drugs can be found in saliva. Parent drug rather than amfetamine metabolites is found in saliva. The saliva : plasma ratio for amfetamine is 2.76 and for metamfetamine is 3.98. After administration of 10 mg amfetamine, plasma levels ranged from 1 μg/L to 20 μg/L and saliva concentrations ranged from 10 to 60 μg/L (Wan *et al.* 1978). When amfetamine was administered to subjects as a racemic mixture, both *d*- and *l*-isomers were found in saliva (Wan *et al.* 1978). Saliva has been found to be positive for metamfetamine as long as 50 h after dosing (Suzuki *et al.* 1989). Cook *et al.* (1993) and Huestis and Cone (2007) compared metamfetamine concentrations in saliva and plasma of volunteers administered drugs by smoking and intravenous routes. Because of the high concentrations of amfetamines in oral fluid, amfetamines are easily detected in reduced volumes of oral fluid using rapid extraction and automated assays (Moore *et al.* 2007a).

The excretion profile of MDMA and its metabolites in saliva and in plasma after ingestion of a single 100 mg dose has been reported (Navarro *et al.* 2001). In eight healthy volunteers, salivary concentrations peaked at 1.5 h after ingestion and the peak values ranged from 1728.9 μg/L to 6510.6 μg/L. These peak values corresponded to an average ± standard deviation saliva : plasma ratio of 18.1 ± 7.9. The time profile of the saliva : plasma ratio was also reported, with the peak occurring at 1.5 h followed by decline to a plateau between 7.3 and 6.4 at 10 and 24 h after dosing. MDA was found in saliva at concentrations approximately 4–5% of MDMA concentrations (relative area under the curve [AUC]) with highest concentrations between 1.5 and 4 h after dosing. 4-Hydroxy-3-methoxymethamfetamine (HMMA) was detected in trace amounts in saliva. Pichini *et al.* (2002) reported that MDMA could be reliably detected in oral fluid using a Drugwipe only if oral fluid was pipetted directly onto the collector.

Samyn and van Haeren (2000) also found MDMA saliva : plasma ratios exceeding theoretical values. Samyn *et al.* (2002) quantified MDMA, MDA and MDEA concentrations in 50 μL of oral fluid by LC-MS(-MS). They reported detection of MDMA in oral fluid of volunteers dosed with 75 mg of MDMA for 5 h duration, which paralleled impairment in psychomotor skills important for car driving (Laloup *et al.* 2005).

After administration of 100 mg *N*-methyl-1-(3,4-methylenedioxypheny)-2-butanamine (MBDB) to a single patient, saliva concentrations ranged from 14 μg/L to 1082 μg/L (Kintz, Samyn 1999). Peak saliva concentrations were observed at 2 h and both MBDB and the desmethylated metabolite, BDB, were detected in saliva for 17 h.

Based on the therapeutic range of 20–150 μg/L for amfetamine (Uges 1996), saliva positives at a cut-off value of 56 μg/L amfetamine or greater would indicate pharmacologically significant levels of amfetamine drugs in blood. The proposed US workplace screening cut-off in saliva is 50 μg/L amfetamines by immunoassay and the confirmation cut-off is 50 μg/L metamfetamine, MDMA, MDA or amfetamine (SAMHSA 2001). The Cozart RapiScan Oral Fluid Drug Testing System has a cut-off of 10 μg/L amfetamine in diluted oral fluid or 30 μg/L amfetamine or MDA in saliva. This would correspond to a plasma concentration of approximately 10 μg/L amfetamine or MDA in plasma. The metamfetamine/MDMA cartridge has a cut-off of 50 μg/L MDMA or metamfetamine or 150 μg/L metamfetamine or MDMA in neat saliva. The Securitec Drugwipe on-site saliva sampling and test strip has a cut-off of 80 μg/L *d, l*-amfetamine sulfate. The Cozart DDS testing system detects as much as 45 μg/L amfetamine and metamfetamine in neat oral fluid.

Analysis of 81 matching saliva and urine clinical specimens (57 from self-reported metamfetamine users and 24 from non-users), using a cut-off of 30 μg/L saliva for screening with a microtitre plate enzyme immunoassay (EIA) and a confirmation cut-off of 10 μg/L by GC-MS, found 97.5% correlation for positive results and a 92.7% correlation for negative results between oral fluid and urine. Metamfetamine concentrations ranged from 10 μg/L to over 10 000 μg/L (Simer *et al.* 1998).

Barbiturates and antiepileptic drugs

Wilson (1993) reviewed the attempts to use saliva for therapeutic drug monitoring of anticonvulsant drugs, including barbiturates. These drugs generally have a neutral or acidic pK_a and many are highly protein bound in blood. Their saliva : plasma ratios are usually less than unity. For example, carbamazepine saliva levels correlate well with dose and blood concentrations. The saliva : plasma ratio for carbamazepine is 0.13–0.33 and intra-individual variation is about 5% (Miles *et al.* 1991).

However, phenytoin has been reported to have saliva : plasma ratios varying from 0.01 to 0.25, average 0.09, for blood total and from 1.06 to 2.22 for blood-free phenytoin. Miles *et al.* (1991) reported an inter-individual variation of 5–10%. Phenytoin saliva concentrations do not

correlate with blood concentrations or therapeutic effect. They do show compliance or non-compliance and elevated levels are related to toxicity. Kamali and Thomas (1994), using atropine-induced reductions in saliva flow rate, demonstrated that the saliva : plasma ratio and saliva phenytoin concentrations were dependent on saliva flow rate but that this did not account for all of the intra-individual variance in the saliva : plasma ratio for phenytoin.

Phenobarbital saliva : plasma ratios of 0.31–0.63 have been reported (Mucklow *et al.* 1978; Nishihara *et al.* 1979; Wilson 1993). Dilli and Pillai (1980) reported a half-life for pentobarbital in saliva of 17–19 h and for amobarbital 22–26 h. Sharp *et al.* (1983) reported saliva secobarbital concentrations of 210 ± 40 μg/L at 3 h after oral administration of 50 mg secobarbital. The saliva : plasma ratio was 0.30 ± 0.04. Van der Graaff *et al.* (1986) reported pharmacokinetics of hexobarbital in plasma and saliva. Wilson (1993) listed the following therapeutic ranges for saliva: carbamazepine 1.4–3.5 mg/L, phenobarbital 5.0–15 mg/L and diphenylhydantoin 1.0–2.0 mg/L.

Benzodiazepines

Benzodiazepines have an unfavourable saliva : plasma ratio (0.01–0.08) owing to acid pK_a values and a high percentage protein binding in plasma (95–99%), so that sensitive methods must be used for detection of benzodiazepines in saliva (Tjaden *et al.* 1980). Immunoassays have been reported that detect various subgroupings or clusters of the very large class of benzodiazepine drugs (Kintz *et al.* 2005; Smink *et al.* 2006). One or more immunoassays may be needed to screen for the most common benzodiazepines in a certain region. A number of LC-MS and LC-MS(-MS) methods have been reported for benzodiazepines in oral fluid (Moore *et al.* 2007b; Ngwa *et al.* 2007; Quintela *et al.* 2005; Smink *et al.* 2006) and, because of the wide spectrum of benzodiazepines detected, LC-MS(-MS) has been suggested as a screening tool as well as a confirmation method for benzodiazepines (Allen *et al.* 2005).

Lucek and Dixon (1980) reported a mean saliva : plasma ratio for chlordiazepoxide of 0.027 ± 0.011. Saliva concentrations were found to be equal to the concentrations of unbound drug in plasma. The drug half-lives determined from plasma and saliva concentration–time curves after intravenous administration of 35 and 50 mg and chronic dosing of 10 mg three times a day for 4 days were 17.2 and 17.8 h for the 35 mg dose, 12.2 and 11.6 h for the 50 mg dose, and 21.9 and 19.9 h after cessation of chronic dosing.

Diazepam (pK_a 3.3) and its metabolites are found in saliva at concentrations of 2–5 μg/L with a saliva : plasma ratio of 0.02 (Giles *et al.* 1977). Di Gregorio *et al.* (1978) reported a ratio of 0.035 ± 0.0047 for parotid saliva : plasma and a mixed saliva : plasma ratio of 0.029 ± 0.0048 after a single 10 mg oral dose. There were no significant differences between diazepam concentrations or time course in parotid saliva and mixed saliva. Giles *et al.* (1980) reported saliva desmethyldiazepam levels in patients receiving chronic diazepam therapy. Hallstrom and Lader (1980) reported a saliva : plasma ratio of 0.016 ± 0.003 for diazepam and 0.029 ± 0.01 for nordiazepam in chronic diazepam users. de Gier *et al.* (1980) reported saliva : plasma ratio of 0.017 ± 0.003 for diazepam, but failed to accurately predict plasma free diazepam levels from saliva diazepam concentrations. The Cozart Rapiscan oral fluid cut-off is 10 μg/L temazepam or 20 μg/L diazepam in diluted oral fluid or 30 and 60 μg/L, respectively, in saliva. The Cozart DDS testing system has a cut-off of 20 and 50 μg/L for temazepam and diazepam in neat oral fluid, respectively.

Concheiro *et al.* (2005) reported windows of detection of tetrazepam in urine, oral fluid and hair after drug-facilitated sexual assault. Laloup *et al.* (2007) compared tetrazepam and diazepam in oral fluid, urine and hair after oral dosing with Myolastan (tetrazepam) and Valium (diazepam).

Methods for detection of midazolam and its hydroxymetabolites have been reported for plasma and oral fluid. Midazolam is a useful probeol drug for CYP3A phenotyping (Link *et al.* 2007). Oral fluid is a more convenient specimen for multiple samplings in pharmacokinetic studies or in phenotype screening.

Kangas *et al.* (1979) and Hart *et al.* (1987) reported that saliva concentrations of nitrazepam were considerably lower than the protein unbound fraction in serum and that monitoring of saliva nitrazepam was of no clinical value. However, Hart *et al.* (1988) reported that, while diazepam, nordiazepam and clonazepam are stable in saliva, nitrazepam is unstable in saliva and is rapidly converted to 7-aminonitrazepam on standing in saliva at room temperature.

Samyn *et al.* (2002) reported detection of flunitrazepam and 7-aminoflunitrazepam in oral fluid using chemical ionisation GC-MS for up to 6 h after giving four volunteers an oral dose of 1 mg Rohypnol (flunitrazepam). Maximum concentrations, reached 2–4 h after dosing, were less then 1 μg/L for flunitrazepam in oral fluid and 1–3 μg/L for 7-aminoflunitrazepam. A cut-off concentration of 0.25 μg/L of 7-aminoflunitrazepam was proposed as corresponding to the duration of substantial impairment in motor abilities (6 h). Flunitrazepam and its metabolites were not stable in oral fluid specimens even after treatment with sodium fluoride and refrigeration.

Kintz *et al.* (2004) reported the windows of detection of lorazepam in urine, oral fluid and hair.

Studies of the effect of benzodiazepines on psychomotor performance and driving performance have compared saliva and plasma concentrations (de Gier *et al.* 1981; Jansen *et al.* 1988; Linnoila *et al.* 1983). Results indicated good correlation between saliva and plasma concentrations, but poor correlation with psychomotor impairment.

Cannabis

Δ^9-Tetrahydrocannabinol (THC), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA) and conjugated 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA-glucuronide) have been reported in oral fluid (Day *et al.* 2006; Moore *et al.* 2006a, 2007c), as well as the pyrolytic precursor Δ^9-tetrahydrocannabinolic acid-A, cannabinol and cannabidiol (Moore *et al.* 2007d). Because of the lipophilicity of THC, early methods of collection of oral fluid sometimes suffered false negatives owing to problems of recovery from and stability of cannabis components on collector absorbents (Dickson *et al.* 2007; Kauert 2000; Langel *et al.* 2007). Since more than half of the THCA content of oral fluid may be present as the glucuronide conjugate, false negatives can occur if the specimen is not hydrolysed before analysis or analysed by a method such as LC-MS that is able to detect glucuronide conjugates. Moore *et al.* recommended base hydrolysis (average 63.4% increase in THCA detected) or β-glucuronidase enzymatic hydrolysis (48.2% increase) (Moore *et al.* 2007c).

Idowu and Caddy (1982) calculated a theoretical saliva : plasma ratio of 0.099–0.129 for THC and of 0.060–0.099 for 11-OH-THC. However, the measured saliva : plasma ratio for THC after smoking marijuana is 10 and is a function of the time since smoking (Cone 1993). Cannabinoids in saliva are often due to residual material left in the mouth during ingestion or smoking of cannabis or cannabis products (Niedbala *et al.* 2001c). For this reason, concentrations are highest immediately after smoking and decline rapidly over the first 2–4 h. In contrast, cannabinoids may not appear in urine or sweat for several hours after smoking (Fig. 18.5). Niedbala *et al.* (2001c) reported an average lag time to urine appearing positive for cannabinoids of 4 h (GC-MS) to 6 h (EIA). The advantage of measuring cannabinoids in saliva is that it is an indication of recent use of cannabis. By detecting THCA and THCA-glucuronide in oral fluid, passive contamination by environmental cannabis smoke can be ruled out (Moore *et al.* 2007c).

Just *et al.* (1974) detected THC by two-dimensional thin-layer chromatography and mass spectrometry in saliva extracts for 2 h after smoking a single tobacco cigarette that contained 2.8 mg of THC. Gross *et al.* (1985) reported that saliva THC levels by RIA were greater than 400 μg/L at 0.5 h after smoking, rapidly dropped to less than 10 μg/L, and were undetectable 5 h after smoking. Maseda *et al.* (1986) detected THC in saliva using capillary GC with electron capture detection (ECD) (limit of detection 1 μg/L) for 4 h after smoking cannabis containing approximately 10 mg THC. Concentrations in saliva ranged from 250 μg/L 1 h after smoking to 34 μg/L at 4 h. Maseda *et al.* (1986) reported that saliva Δ^9-THC concentrations at 1 h were lowered by drinking 200 mL of beer immediately after the cannabis smoking. Thompson and Cone (1987) reported THC in saliva using

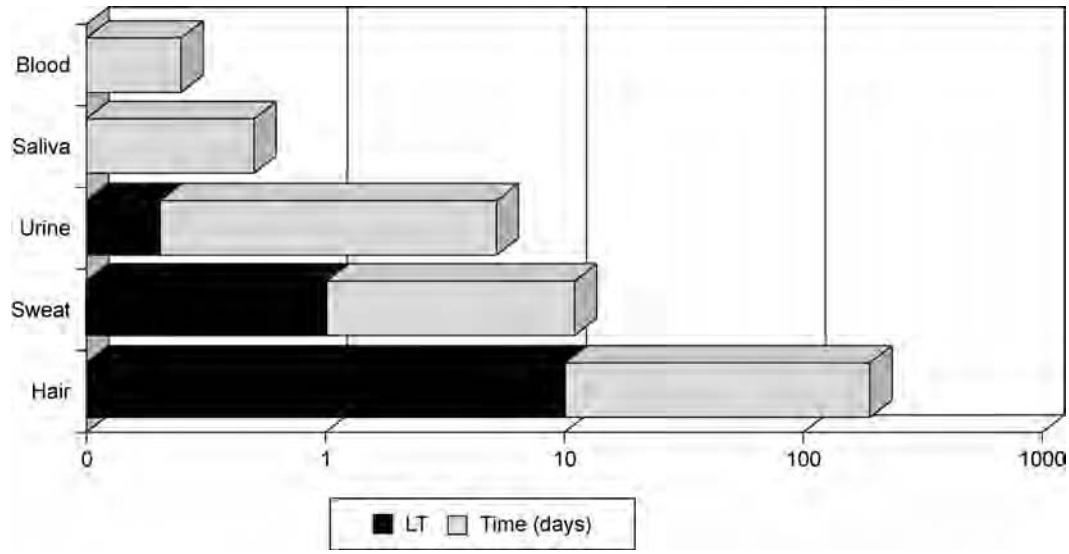

Figure 18.5 The lag time and window of detection for cannabinoids in blood, saliva, urine, sweat and hair after smoked marijuana. LT, lag time; time days, window of detection.

HPLC with electrochemical detection (limit of detection 1 μg/L). Saliva was collected by sour candy stimulation and stored at −20°C. Concentrations reached greater than 500 μg/L THC immediately after smoking one or two cannabis cigarettes and fell to 10 μg/L at 7 h. Saliva concentrations were greater than serum concentrations throughout. Cone (1993) found saliva levels of greater than 100 μg/L for the first 1 h after smoking a 3% THC cigarette, which rapidly dropped to less than 1 μg/L within several hours.

Niedbala *et al.* (2001c) found saliva concentrations of 0.3–216 μg/L THC from 1 to 72 h after smoking a cannabis cigarette, and saliva concentrations of 6–684 μg/L at 15 min to 1 h after smoking. Following oral ingestion of cannabis concentrations were from 0.12 μg/L to 21.3 μg/L for 1–48 h. There were no detectable cannabinoids in saliva after passive exposure to cannabis smoke. There were no significant differences in cannabinoid concentration or detection for oral fluid collected simultaneously from the right and left sides of the mouth. Using GC-MS(-MS) with a 0.5 μg/L THC cut-off concentration, Niedbala *et al.* (2001c) detected THC in oral fluid of 10 participants who smoked 20–25 mg THC for an average of 34 h.

Menkes *et al.* (1991) found that saliva levels of THC correlated with rapid heart rate and psychological feelings of a 'high'. Saliva THC concentrations were measured after smoking a cigarette containing 11 mg THC. Subjective intoxication was measured using a visual analogue scale and heart rate, and correlated significantly with the log of the saliva THC concentration.

Using ROC analysis, Laloup *et al.* (2006) found that the optimal cut-off value for THC in oral fluid in order to predict a positive plasma result for THC was 1.2 μg/L THC. They reported that a cut-off value of 5.2 μg/L THC in oral fluid by LC-MS(-MS) corresponded to the serum legal cut-off for driving in Belgium of 2 μg/L. Kintz *et al.* (2000) recommended a screening cut-off concentration of 2 μg/L THC in oral fluid when oral fluid is used to detect the presence of drugs in impaired drivers. Niedbala *et al.* (2001c) used a screening cut-off of 1 μg/L in diluted oral fluid (0.4 mL specimen and 0.8 mL preservative solution) collected by swabbing with citrate stimulation and a confirmation cut-off of 0.5 μg/L by GC-MS(-MS) for a laboratory-based saliva testing system. However, the Epitope absorbant that they used has been shown to retain THC. The proposed US workplace cut-off for opiates in saliva is 4 μg/L THC or THC equivalents by immunoassay and the confirmation cut-off is 2 μg/L THC (SAMHSA 2001). The Cozart RapiScan Oral Fluid Drug Testing System cut-off is 10 μg/L THCA in diluted oral fluid or 30 μg/L THCA in saliva. The Cozart DDS oral fluid drug testing system detects at least 30 μg/L THC.

Hall *et al.* (1998) applied solid-phase microextraction (SPME) to the determination of cannabidiol, Δ^8-THC, Δ^9-THC and cannabinol in human saliva by quadrupole ion-trap GC-MS. SPME allowed analysis of small saliva samples and eliminated the use of organic solvents. Samyn and van Haeren (2000) reported on-site testing of drivers with the DrugWipe (Securitec, Munich, Germany) and confirmation by GC-MS after solid-phase extraction and derivatisation. Cannabinoids were confirmed in 10 of 15 subjects; THC concentrations ranged from 1.4 μg/L to 42 μg/L saliva.

LC-MS and LC-MS(-MS) methods have been published for confirmation of THC, THCA and other cannabinoid metabolites in oral fluid (Laloup *et al.* 2006; Teixeira *et al.* 2004).

Phencyclidine and ketamine

Phencyclidine (PCP) has low protein binding in plasma (less than 10%) and a pK_a of 9.43. From the Henderson–Hasselbach equation, the saliva : plasma ratio would be expected to be greater than unity. After giving oral (1 mg) and intravenous (0.1 or 1 mg) doses of radiolabelled PCP to healthy male volunteers, Cook *et al.* (1982a) reported that the parent drug was found in saliva at concentrations higher than would be expected from the pH differential between plasma and saliva and the binding of drug in plasma and saliva. The saliva : plasma ratio ranged from 1.5 to 3.0. Saliva was collected by expectoration into glass vials. Saliva pH averaged 6.7 ± 0.17.

PCP is primarily abused by smoking tobacco or cannabis cigarettes that have been dipped into PCP-containing solvents. Inhaled PCP would be trapped in the tissues of the mouth. Cook *et al.* (1982b) also reported that both PCP and phencyclohexene (PC) were present in plasma after volunteers smoked 100 μg of radiolabelled PCP. The persistence of PCP or PC in saliva after smoking of PCP was not reported.

McCarron *et al.* (1984) analysed paired serum and saliva samples from 100 patients suspected of PCP intoxication. Both serum and saliva tests were positive for PCP by RIA in 70 of the cases. Both were negative in 7 cases. In 21 cases with no clinical evidence of PCP intoxication, both serum and saliva RIA were negative in 17 cases, and positive in 3 cases. Saliva PCP concentrations ranged from 2 μg/L to 600 μg/L.

The proposed US workplace cut-off for PCP in saliva is 10 μg/L PCP equivalents by immunoassay and the confirmation cut-off is 10 μg/L PCP (SAMHSA 2001).

Ketamine is a structurally related dissociative anaesthetic. Methods for detection and the pharmacokinetics of ketamine in oral fluid have been reported (Cheng *et al.* 2007).

Collection of saliva

The greatest advantage of saliva is the possibility of having the sample collected by the donor while under observation. In addition, in on-site

testing such as close to the patient or roadside testing, the specimen can be collected shortly after the time of the incident. Saliva collection is non-invasive and can be done by donors themselves in most situations. An exception may be when the donor is unconscious or so sedated as to be unable to follow instructions. Oral fluid has been collected from donors by spitting, draining, absorption and suction. A number of devices are available for saliva and oral fluid collection.

Collection devices

Peel *et al.* (1984) asked 'driving while intoxicated' suspects to spit into a test tube with or without sour candy stimulus. A similar approach was taken by Cone (1993) at the Addiction Research Center in their many controlled-administration drug studies. The Greiner Bio-One Saliva Collection System requires the donor to rinse his or her mouth with Saliva Extraction Solution (pH 4.2) and spit the resulting mixed liquid into a collection beaker. This is then emptied into a graduated saliva transfer tube for measurement of the volume. The Bio-One Saliva Extraction Solution contains a food dye that serves as an internal standard for photometric determination of the saliva concentration. In a study with 102 donors, the recovered fluid volume ranged between 4.4 and 9.8 mL with a saliva content of 39–89% (median 66%) (Schmidt *et al.* 2007).

The Salivette collector (Sarstedt, Germany) employs a dental cotton roll, which is chewed by the donor for 30–45 s with or without citric acid stimulation. After the oral fluid is collected, it is placed in a container that fits into a centrifuge tube. During centrifugation the saliva passes from the cotton roll into the lower part of the tube. Cellular particles are retained at the bottom of the tube. The cotton roll reliably absorbs 1–1.5 mL of oral fluid. The disadvantage of the cotton roll is that it may adsorb analyte(s) or give off compounds that may interfere in hormone and drug assays.

The Finger collector (Avitar, Canton, MA, USA) uses a proprietary dental absorbent (AccuSorb foam) that absorbs saliva when placed in the mouth for a few minutes. Saliva is expressed from the absorbent using finger pressure. Volume recovery ranges from 83% (1.86 mL collected and 1.48 mL recovered, Salivette) or 78% (0.82 mL collected and 0.64 mL recovered, Intercept) to 18% (1.69 mL collected 0.3 mL recovered, Hooded Collector) (Crouch 2005).

The Drugwipe (Securitec, Munich, Germany) saliva test attempts to wipe the tongue with a wiping pin and then transfer the oral fluid collected by washing it onto a test strip (Frontline urine test strip, Boehringer Mannheim GmbH, Germany) developed for testing surfaces for traces of drugs. Pichini *et al.* (2002) reported that the Drugwipe wiping pin holds at most 2 μL of fluid. They also reported that placing 2 μL saliva on the collection pin produced the best accuracy, but that wiping the tongue with the pin produced inadequate sample for reliable results.

The Intercept saliva sampling device comprises an absorbent paper pad impregnated with buffered salts on a plastic rod. The pad is placed in the mouth for 2–5 min. After collection, the paper is placed in a tube with preservative liquid and shipped to the laboratory for analysis. The Intercept pad is estimated to absorb approximately 0.4 mL of oral fluid, which is diluted 1 : 3 with 0.8 mL preservative fluid. Several studies have shown that some drugs such as THC are poorly eluted from the absorbent paper pad (Kauert 2000; Langel *et al.* 2007). Crouch (2005) reported losses of greater than 40% at room temperature from the Intercept pad.

Collection indicators

Modern oral fluid collection devices generally use an absorbent material on a plastic rod with a colour indicator to signal when a certain volume has been collected. It is essential to know the volume collected if the sample is to be diluted with buffer and reagents for point-of-collection testing or with preservatives for shipment to a laboratory for testing.

For example, the Cozart DDS Oral Swab (Cozart BioScience, Abingdon, UK) consists of a cotton bud on a plastic stem. The mouth of the oral fluid donor is swabbed with the cotton bud until the sample presence indicator turns blue. This usually occurs within less than a minute (mean time 34 s for drug-free subjects and 44 s for drug users) and indicates collection of 0.34 ± 0.06 mL average volume (Speedy *et al.* 2007). Drug recovery was greater than 90% for THC, amfetamine, cocaine, methadone, metamfetamine, morphine and temazepam (Speedy *et al.* 2007).

Other collectors (RapiScan, Cozart Bioscience, Abingdon, UK; Omnisal, SDS, StateSure Diagnostics Systems, Framingham, MA, USA; Quantisal, Immunoalysis, Pomona, CA, USA) use a detachable absorbent cotton pad on a plastic handle containing a sample volume adequacy indicator. After the indicator has turned from white to blue, indicating that 1 mL of saliva has been collected on the pad, the pad can be detached and kept in a test tube containing preservative buffer until analysis. Drug recovery and stability have been reported for the SDS type collector (Dickson *et al.* 2007; Langel *et al.* 2007; Moore *et al.* 2006b) and depends on the drug and the buffer/preservative solution used.

Schramm and colleagues (Schramm, Smith 1991; Schramm *et al.* 1993b) developed a small plastic sack (SalivaSac, BioQuant) composed of semipermeable membranes which contains high-molecular-weight sugars. When it is placed in the mouth, osmotic pressure drives an ultrafiltrate of saliva into the interior of the sack. The drawback with this system is that the sack requires from 10 to 20 min (depending on the sack size) for collection of sufficient fluid for testing.

Foley *et al.* (2000) developed an aspirator (LifePoint, Rancho Cucamonga, CA, USA) that would draw saliva directly from the mouth of the test subject through a tube with a disposable individual sterile mouthpiece. For on-site testing the saliva passed directly into the analyser, eliminating the need to elute saliva from the collector pad with buffer and transfer it into an immunoassay cartridge.

For collection of saliva (generally parotid saliva), small intraoral cups (Schaeffer cup, Curby cup) that can be placed over the Stensen's duct of the parotid gland are available. The collection cup is placed in the buccal vestibule with the opening over the duct orifice. Gentle pressure on the cheek over the cup causes air to be expressed from the cup and creates a slight negative pressure that keeps the cup in place until it has collected sufficient saliva.

The collection device or method has been shown to influence the pH of the saliva by stimulating saliva flow and hence the drug content of oral fluid according to the Henderson–Hasselbach equation (Kintz, Samyn 2002; O'Neal *et al.* 2000) Collection devices can also affect recovery of drugs from oral fluid due to retention of oral fluid in the absorbent, and adsorption of drug on device components and drug recovery from device buffers (O'Neal *et al.* 2000). After controlled administration of codeine, oral fluid codeine concentrations were higher in specimens obtained by spitting than those obtained using absorptive devices (O'Neal *et al.* 2000).

Dealing with adulterants

One of the advantages of saliva testing is that the sample is collected under direct observation. Before collection of saliva or oral fluid, the collector should observe the donor for a 10–15 min period in which the donor should not smoke, consume food or drink. Experience with saliva and breath alcohol testing is that contaminants, such as ascorbic acid from foods or drink, will clear from the oral cavity either by swallowing of saliva or by dissipation into the general circulation within 10–15 min. Also, a simple experiment shows that a person cannot hold saliva, especially saliva containing liquid or solids, in the mouth for more than 3 min without swallowing or dribbling (Jehanli *et al.* 2001). Rinsing of the mouth is not required for collection of saliva nor does it reduce the levels of drugs found in oral fluid. Wong *et al.* (2005) studied the effects of adulterants and foodstuffs on oral fluid THC and opiates and found no false positives or negatives.

Sample treatment

Cone *et al.* (1997) stored collected saliva samples at -20°C. The OraSure System (OraSure Technologies, Bethlehem, PA, USA) provides for shipping the oral fluid collected with the Intercept pad to a central laboratory

for analysis following the addition of a preservative fluid. The process requires no special refrigeration and no sample treatment is required for immunoassay screening of the saliva or its dilutions. If the analyte of interest is unstable in aqueous solutions (e.g. cocaine) or subject to changes by oral fluid bacteria or enzymes (e.g. nitrazepam, flunitrazepam) then further preservation efforts may be required. Freezing of the collected oral fluid reduces interference from mucins in pipetting and liquid–liquid extractions for chromatographic confirmation testing. Solid-phase extraction or solid-phase microextraction reduces the need for freezing the sample (e.g. dos Santos Lucas *et al.* 2000; Hall *et al.* 1998). Moore *et al.* (2006b) described losses of 10% and 20% for THC when the Quantisal oral fluid collections device was stored in the refrigerator or at room temperature, respectively.

Analysis of saliva for drugs

Screening tests

Immunoassays for detection of drugs in saliva must target or have significant cross-reactivity with the parent drug and lipophilic metabolites. For example, cocaine parent drug and ecgonine methyl ester, heroin and 6-MAM, and THC predominate in saliva owing to their lipophilicity. When drugs are leached into saliva from buccal depots, which is the case for smoked drugs such as cannabis, smoked cocaine or diamorphine, parent drug and pyrolysis products will predominate in saliva. Immunoassays have been developed that are appropriate for saliva screening. In addition, clinical trials and analysis of the sensitivity, specificity and predictive value of different putative cut-off values for saliva screening assays as illustrated in Figs. 18.1, 18.2 and 18.4 have been published for specific immunoassays for saliva drug screening.

On-site testing

A number of on-site test systems have been developed for drugs in oral fluid. These tests are immunochromatographic screening tests (see also Chapter 31). They generally employ lateral diffusion of the oral fluid sample mixed with labelled antibodies in buffer across a linear array of immobilised drugs. When drugs are present in the oral fluid, they bind to the anti-drug antibodies, and the antibodies then pass by and do not bind to the corresponding test line containing the immobilised drug conjugate. Visualisation of the antibody label (using colloidal gold, phosphor or other indicator) reveals a lack of response from the array location corresponding to the drug(s) present.

The first on-site saliva test was the Cozart Rapiscan Oral Fluid Drug, Testing System (Cozart BioScience Ltd, Abingdon, UK) in 1998, which uses lateral transfer immunoassay with colloidal gold-labelled anti-drug antibodies. The procedure is typical of those used for on-site oral fluid testing. The saliva specimen is collected using a collection pad and placed in a test tube containing 2 mL running buffer. When placed in the mouth, the collection pad absorbs 1 mL of saliva. This is indicated by the development of a blue colour in the indicator section of the handle (Fig. 18.6). The pad is then placed in the tube, where it is diluted with 2 mL of buffer fluid. The cellulose pad is separated from the plastic handle along a perforated edge. After removing the cap and plastic collector handle, the cotton pad is compressed using a dispensing filter which is used to dispense six drops of the saliva–buffer mixture onto the cassette by directing the tip of the dispensing filter tube into the cassette well and gently squeezing the tube.

For each test, a fresh disposable cassette and collection kit are used. The cassette or cartridge is inserted into the hand-held instrument for incubation, reading and reporting. The saliva/run fluid rehydrates the gold-labelled anti-drug antibodies contained within the cartridge. This mixture travels by capillary action across an array of immobilised drug sites (3–5 min for single-panel and two-panel tests; 12 min for a five-panel test). The absence of colour development at an immobilised drug position indicates presence of the drug. The quality control position contains anti-mouse IgG to ascertain that complete lateral transfer of specimen has been achieved.

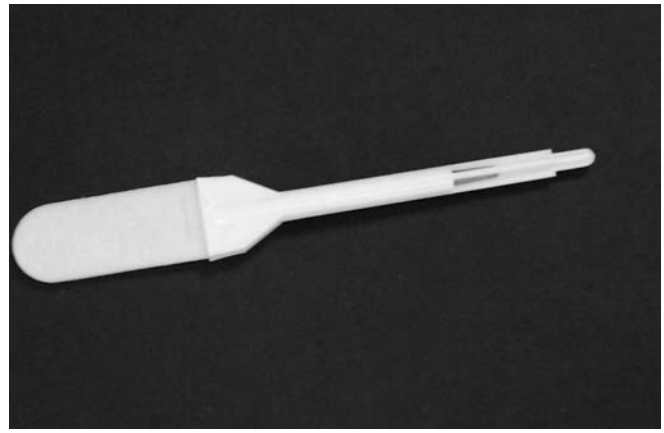

Figure 18.6 The Cozart RapiScan collection device.

The cassette result (binding of gold-labelled antibody to immobilised drug in the absence of drug in sample) is monitored by the portable, battery-powered reader and reported on the display screen. In addition, if one or more positive results are obtained, then a light on the face of the reader will appear red in colour. If all results are negative the light will appear green. The results can be printed out on an optional battery-powered printer to provide a permanent record of results. Results are sent to the printer via the multifunctional port, which also serves for charging the instrument's batteries and to up-load new versions of the instrument software, new drug combinations, etc. via an internet interface module.

If the saliva screening test is positive, the remainder of the sample (2.8 mL fluid) and the whole of a second sample of saliva collected at the same time as the first or at the time of the positive reading (10–15 min later) may be capped, tamper-proof tape placed across the cap and the samples sent to the designated laboratory for confirmation.

Alternatively, a urine or blood sample may be collected and sent with the remainder of the positive saliva to the laboratory, depending on the preference of the contracting laboratory.

Recently developed point-of-collection tests have shortened the time of the immunochromatographic test by using multiple test strips with only two drug tests per strip (Cozart DDS, Abingdon, UK; Drager Drug Test 5000, Drager Safety AG, Lubeck, Germany). For example, the Cozart Drug Detection System (DDS) shown in Fig. 18.7, displays results for up to six different drugs in under 5 min and two drugs in under 90 s. Other on-site immunochromatographic oral fluid drug tests that use colloidal gold antibody labels are ORALScreen System (Avitar, Canton, MA, USA) (Barrett *et al.* 2001) and Drugwipe and Drugread (Securitec GmbH, Ottobrun bei Munchen, Germany) employing the Frontline urine dipstick (Boehringer-Manheim GmbH, Manheim, Germany).

Figure 18.7 The Cozart DDS Saliva Test reader and cassette.

An on-site immunochromatographic assay has been developed by Orasure Technologies that uses an up-converting phosphor based on lanthanide particles that absorb infrared light and emit visible light (up-conversion) as the antibody label (Up-Link Rapid Detection system, OraSure Technologies, Inc., Bethlehem, PA, USA). Biological matrices do not up-convert, eliminating test background from autofluorescence. As in the immunochromatographic procedure described above, oral fluid specimens are collected in a device that indicates sample adequacy and retains oral fluid sample for confirmation testing if required. Specimens are mixed with buffer and introduced to a test cassette. Antibodies labelled with up-converting phosphor microparticles contained on a lateral flow membrane in the cassette are mobilised when liquid sample flows across the pad. The presence of increasing amounts of drug in the sample decreases the amount of antibody-bound label bound to the corresponding test line containing the immobilised drug conjugate. A 10 min incubation is required. The test simultaneously detects amfetamine, metamfetamine, PCP and opiates in oral fluid with 40% or better displacement at 10 µg/L drug (Niedbala *et al.* 2001b). A reader utilising an excitation infrared laser (980 nm) and photomultiplier tube with a filter to determine visible light visualises the location of the bound phosphor-labelled antibody, which indicates whether drugs are present in the oral fluid. Up-converting phosphors that emit visible light at 475, 505, 550 and 720 nm are available. Different phosphors can be used as labels for different anti-drug antibodies. This allows close spacing of the drug conjugate lines on the immunochromatography strip.

Confirmation testing

When immunoassays are used as screening tests for drugs in saliva, chromatographic tests should be used for confirmation (Spiehler *et al.* 1988). Like screening tests, confirmation tests for drugs in saliva must be able to detect the parent drug or lipophilic metabolites. They must also be able to detect the levels of drugs that appear in saliva. GC-MS methods for confirmation of prescription opiates (Jones *et al.* 2002), opiates and methadone (Moore L *et al.* 2001), diamorphine (Jenkins *et al.* 1995), cocaine (Wang *et al.* 1994) and cannabinoids (Kintz *et al.* 2000) have been reported. A tandem immunoaffinity chromatography/HPLC procedure for cannabinoids was published by Kircher and Parlar 1996. GC-MS(-MS) methods have been published for confirmation and quantification of cannabinoids in saliva (Hall *et al.* 1998; Niedbala *et al.* 2001c). Many LC-MS and LC-MS(-MS) methods are published for confirmation of drugs in oral fluid.

Interpretation of saliva drug results

The foremost question in the application of saliva testing to forensic casework is 'What is the relationship of saliva positive results to blood drug concentrations?'. Drug concentrations in saliva reflect the free, unbound parent drug and lipophilic metabolites circulating in the blood. Since these are the forms of the drug that cross the blood–brain barrier and affect performance and behaviour, saliva is a good specimen for detecting patient compliance with medication, drug involvement in driving behaviour, fitness for duty or impairment of performance for many drugs. However, efforts to use saliva concentrations to predict blood free-drug concentrations, cerebrospinal fluid (CSF) concentrations or degree of performance impairment have not reached the accuracy of blood measurements. Without knowledge of the instantaneous saliva pH, saliva drug concentrations may not be extrapolated to blood drug concentrations. When appropriate cut-off concentrations are employed, saliva drug presence may be associated with recent drug use and in some cases with being under the influence of the drug.

Saliva concentrations can be exceptionally high when the route of administration is via inhalation, such as occurs with cannabis, cocaine, metamfetamine and diamorphine, sublingual or buccal adsorption or snorting of the drug. In cases involving these routes of administration, 2–4 h must elapse before the contamination of saliva by the remains of ingested drug is cleared from the mouth and saliva concentrations reflect plasma levels. Cannabis is an example. For the first hour or so, THC found in saliva is most likely coming from cannabinoids deposited in the oral mucosa as a result of smoking the drug. However, saliva concentrations of THC follow the same time course as the appearance and decline of physiological indices of cannabis's pharmacological effects. The rate of clearance of THC from the oral tissues appears to match the binding and clearance of THC from the central nervous system site of action of cannabinoids.

Conclusion

For many drugs the saliva drug concentrations are related to the blood concentration of the unbound, unionised parent drug or its lipophilic metabolites. For these drugs, saliva concentrations are a function of circulating drug blood levels. For many patient populations the ease of obtaining oral fluid and avoiding venepuncture outweighs the inaccuracy of estimation of drug levels from saliva. This includes clinical uses in infants and children, elderly people, and HIV-positive patients. Saliva is useful in pharmacokinetic studies since multiple specimens can be obtained over time with minimal discomfort to the subject. In forensic practice, saliva drug collection and testing, unlike venepuncture, can be carried out in non-clinical settings, provides information about the presence of drugs, indicates recent ingestion and may be correlated to psychomotor impairment for some drugs such as cannabis. Finally, in large-scale testing such as the US workplace drug-testing programmes, collection and testing of oral fluid provide specimens that can be collected under direct observation and that are not easily diluted or adulterated. For this reason, saliva drug testing has been proposed for US federal workplace testing as a specimen for random testing, for testing triggered by reasonable suspicion or cause and for post-accident testing. Both laboratory-based and on-site saliva drug testing are anticipated in the workplace.

Saliva alcohol

Ethanol is a low-molecular-weight compound that passes through cell membranes and does not ionise or bind to plasma proteins. Ethanol distributes to all body fluids in proportion to their water content. The measured saliva : plasma ratio for alcohol, 1.10, is slightly higher than the calculated value, perhaps because of the high blood flow to the salivary glands; saliva ethanol is in equilibrium with arterial blood rather than venous blood collected for analysis. Saliva equilibrates rapidly with blood ethanol.

Pharmacology

The passage of ethanol into saliva and the close correlation between saliva and blood alcohol concentrations was reported in the 1930s (Friedemann *et al.* 1938). Jones (1979b) reported an ethanol saliva : plasma ratio of 1.077 with a range of 0.84–1.36 in 48 men between 1 and 3 h after ingestion of 0.72 g/kg ethanol in a fasting condition. Variation was determined by analysis to be equally due to inter- and intra-individual components. Individual variation in saliva : plasma ratios showed no systematic variation through absorption, distribution and elimination phases of ethanol metabolism. Jones (1993) confirmed this value with a measured saliva : plasma ratio of 1.094 (range 0.88–1.36) in 21 male volunteers. McColl *et al.* (1979) found a highly significant linear correlation between blood ethanol concentrations and those in mixed saliva obtained before and after rinsing and drying the mouth and parotid saliva. McColl pointed out that this applies only if the saliva ethanol is determined in saliva obtained more than 20 min after ingestion of ethanol.

Haeckel and Bucklitsch (1987) reported that ethanol concentrations reached higher peak concentrations in saliva than in peripheral blood. It was noted that saliva ethanol concentrations, like breath ethanol concentrations, more closely correlate with capillary blood than with venous blood. Haeckel and Peiffer (1992) reported a saliva : plasma ratio of 0.85 (if related to the aqueous compartment of blood) in saliva and breath samples taken from motorists detained by the police.

Newman and Abramson (1942) correlated saliva alcohol concentrations with ethanol's effects on performance. Jones (1993) compared

saliva, breath and blood concentration–time profiles with subjective feelings of intoxication, body sway, hand tremor, positional nystagmus and roving ocular movement after ingestion of 0.68 g/kg ethanol by fasting men. Saliva concentrations were higher than blood and breath concentrations. All three correlated equally well with measures of alcohol effects. Maximum impairment was reached at the same time as peak saliva, blood and breath concentrations. The mean elimination rate of ethanol from saliva of 13 ± 2.5 mg/100 mL per h was not significantly different from that of blood, 12 ± 1.1 mg/100 mL per h and paralleled the recovery of baseline function in the physiological tests.

Analysis

Ethanol can be analysed in saliva by the same headspace chromatographic (Jones 1978) or enzymatic methods (Jones 1979a) as used for blood. Dipstick or reagent strip tests for alcohol have been reported (Pate *et al.* 1993; Tu *et al.* 1992) but were found to be too unreliable for use in determining blood alcohol content (Lutz *et al.* 1993; Pate *et al.* 1993; Wong 2002). Current enzymatic tests have proven more reliable as quantitative tests (Bendtsen *et al.* 1999; Christopher, Zeccardi 1992; Jones 1995; Smolle *et al.* 1999) and several commercial tests for on-site or point-of-collection testing of saliva alcohol are available.

An example of a point-of-collection quantitative test is the QED Saliva Alcohol Test (OraSure, Bethlehem, PA, USA) (Fig. 18.8). The saliva or oral fluid is collected by the donor with a cotton swab which is applied to the test pad.

As the saliva moves along the reagent bar by capillary action, any ethanol present is oxidised by alcohol dehydrogenase to acetaldehyde with the simultaneous reduction of nicotinamide adenine dinucleotide (NAD). This results in a cascade of electron donor–acceptor reactions catalysed by diaphorase and involving FeCN and a tetrazolium salt, which proceed to production of a purple-coloured endpoint. The length of the resulting purple-coloured bar on the QED device is directly proportional to the concentration of ethanol in the specimen. The alcohol concentration can be read directly from the height of the coloured reaction bar from a printed scale in mg/dL or mg% ethanol just as in reading a thermometer (Fig. 18.8). To get an accurate reading the capillary must draw saliva all the way to the top of the device. This is signalled by development of a purple colour within 5 min at the QA spot at the top of the 'thermometer' (Fig. 18.8).

Since the saliva moves along the reagent bar, and reacts directly with the indicator chemicals, any oxidant in the saliva can cause a false-positive result. The most common oxidant found in saliva is ascorbic acid, which is commonly added to fruit juices, sodas and soft drinks as a preservative. Ascorbic acid is absorbed in the gums and is still found in the mouth in amounts sufficient to give a false positive with the QED Saliva Alcohol Test for up to 10 min after drinking some soft drinks and sodas.

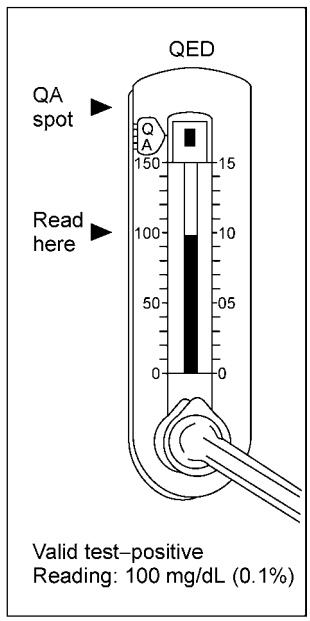

Figure 18.8 The QED Saliva Alcohol Test.

The QED Saliva Alcohol Test comes in two ranges: 0–150, which can be read from 10 mg/100 mL to 150 mg/100 mL, and 0–350, which can be read from 20 mg/100 mL to 350 mg/100 mL. The first, lower, range is for Department of Transport and driving-under-the-influence applications, the second is for hospital and overdose applications.

An example of a headspace enzymatic assay is the On-Site Alcohol Assay for qualitative detection of alcohol in urine and saliva (Roche Diagnostics, Nutley, NJ, USA; Ansys Technologies Inc., Lake Forest, CA, USA). The On-Site Saliva Alcohol test is similar to the QED Saliva Alcohol Test in that saliva is collected by the donor from his or her own mouth with a cotton swab and then the swab is applied to the specimen well. Since alcohols are volatile, alcohol vapours will diffuse from the sample pad to the reaction pad where they react with alcohol dehydrogenase and diaphorase. The hydrogen released is transferred to a tetrazolium salt to produce a highly coloured formazan dye. The presence of alcohol is indicated by the appearance of a purple plus sign (+) in the result pad. The On-Site Saliva Alcohol test does not have a control spot, so an external control must be run in each testing session on an additional test unit. Since the test detects alcohol vapours from the saliva, the saliva sample does not come into contact with the reagents. Therefore, there is no possibility of false positives from oxidising agents such as ascorbic acid in the saliva. However, the result is only qualitative. The cut-off is 0.02 g%, so a purple plus sign (+) indicates only the presence of alcohol at greater than 0.02 g%.

Interpretation

Saliva ethanol concentrations are an accurate reflection of blood alcohol concentrations. Saliva alcohol can be used to estimate blood alcohol concentrations, to estimate pharmacokinetics of ethanol in an individual, as evidence of impairment and to determine fitness for duty in the workplace.

In the US workplace testing, the Department of Transportation (DOT) has codified saliva ethanol testing (DOT 1994). The screening cut-off is 0.02 g% saliva or 0.02 g/210 L of breath. Because the saliva alcohol tests are non-specific, chemical screening tests that may react with oxidising agents other than alcohol and with ketones and alcohols other than ethanol, it is necessary to confirm any positive results greater than 0.02 g% with an independent test based on a different chemical principle. Breath alcohol tests are usually either fuel cells or infrared spectrometers with optical filters and computer software safeguards for specificity and sensitivity. Specifically, they must be able to distinguish acetone from ethanol at the 0.02 g% alcohol level. A time limit is put on confirmation of saliva ethanol results since ethanol is rapidly metabolised by the liver. Initially the confirmation breath test had to be performed within 20 min. The DOT now requires the confirmation test to be done within 30 min.

The DOT (1994) requires a 15 min waiting period before saliva or breath alcohol tests. The DOT describes this as a safeguard against false high readings due to accumulation of mouth alcohol, but the 15 min waiting period will also protect against false-positive readings from food or drink components, such as ascorbic acid, which might cause false-positive readings in saliva tests. To obtain a correct test, the most important factor is that a waiting period be observed in which the donor does not eat, drink, smoke or belch (i.e. nothing by mouth).

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CHAPTER

19 Hair Analysis

P Kintz

Introduction

In the 1960s and 1970s, hair analysis was used to evaluate exposure to toxic heavy metals such as arsenic, lead or mercury; this was due to the availability of atomic absorption and neutron activation analysis that allowed detection in the nanogram range (Curry, Pounds 1977) At this time, examination of hair for organic substances, especially drugs, was not possible because analytical methods were not sufficiently sensitive. Examination by means of drugs marked with radioactive isotopes, however, established that these substances can move from blood to hair and are deposited there (Lindquist, Ullberg 1974). Ten years after these first investigations, it was possible to denominate various organic drugs by means of radioimmunoassay (RIA). In 1979, Baumgartner and colleagues (Baumgartner, Hill 1992) published the first report on the detection of morphine in the hair of heroin abusers using RIA. They found that differences in the concentration of morphine along the hair shaft correlated with the time of drug use. Today, gas chromatography coupled with mass spectrometry (GC-MS) is the method of choice for hair analysis and this technology is routinely used to document repetitive drug exposure in forensic sciences, traffic medicine, occupational medicine, clinical toxicology and more recently in sports drug testing.

The major practical advantage of hair testing compared with urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2–4 days for blood and urine). For practical purposes, the two tests complement each other. Urinalysis and blood analysis provide short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. While analysis of urine and blood specimens cannot distinguish between chronic use or single exposure, hair analysis can offer the distinction.

Biology of hair

Hair is a product of differentiated organs in the skin of mammals. It differs in individuals only in colour, quantity and texture. Hair composition is primarily protein (65–95%, essentially keratin), as well as water (15–35%) and lipids (1–9%). The mineral content of hair is from 0.25% to 0.95%. The total number of hair follicles in an adult is estimated to be about 5 million, with 1 million found on the head (Harkey *et al.* 1991). Hair follicles are embedded in the epidermal epithelium of the skin, approximately 3–4 mm below the skin's surface.

Hair growth

The hair shaft begins in cells located in a germination centre, called the matrix, found in the base of the follicle. Hair does not grow continually, but in cycles, alternating between periods of growth and quiescence. A follicle that is actively producing hair is said to be in the *anagen* phase. Hair is produced during 4–8 years for head hair (<6 months for non-head hair) at a rate of approximately 0.22–0.52 mm/day or 0.6–1.42 cm/month (Saitoh *et al.* 1969) for head hair (the growth rate depending on hair type and anatomical location). After this period, the follicle enters a relatively short transition period of about 2 weeks, known as the *catagen* phase, during which cell division stops and the follicle begins to degenerate. Following the transition phase, the hair follicle enters a resting or quiescent period, known as the *telogen* phase (10 weeks), in which the hair shaft stops growing completely and the hair begins to shut down. Factors such as race, disease states, nutritional deficiencies and age are known to influence both the rate of growth and the length of the quiescent period. On the scalp of an adult, approximately 85% of the hair is in the growing phase and the remaining 15% is in a resting stage.

Types of hair

Pubic hair, arm hair and axillary hair have been suggested as alternative sources for drug detection when scalp hair is not available. Various studies have found differences in concentrations between pubic or axillary hair and scalp hair. Comparison of methadone, cocaine, morphine and phenobarbital concentrations provides the highest values in the axillary hair, followed by pubic hair and the hair of the head. In contrast, the highest morphine concentrations were found in pubic hair (0.80–1.34 ng/mg), followed by head hair (0.62–27.10 ng/mg) and axillary hair (0.40–24.20 ng/mg). The significant differences of the drug concentrations in these studies were explained by a better blood circulation, a greater number of apocrine glands, a totally different telogen/anagen ratio and a different growth rate of the hair (axillary hair 0.40 mm/day, pubic hair 0.30 mm/day). Beard hair grows at about 0.27 mm/day and is considered a suitable alternative, as it can be collected on a daily basis with an electric shaver and can be used to evaluate the incorporation rate of drugs (Mangin 1996).

Mechanisms of drug incorporation into hair

It is generally proposed that drugs can enter into hair by two processes: adsorption from the external environment and incorporation into the growing hair shaft from blood supplying the hair follicle. Drugs can enter the hair from exposure to chemicals in aerosols, smoke or secretions from sweat and sebaceous glands. Sweat is known to contain drugs present in blood. Because hair is very porous and can increase its weight up to 18% by absorbing liquids, drugs may be transferred easily to hair via sweat. Finally, chemicals present in air (smoke, vapours, etc.) can be deposited onto hair. Drugs appear to be incorporated into the hair during at least three stages: from the blood during hair formation, from sweat and sebum, and from the external environment. This model is more able than a passive model (transfer from blood into the growing cells of the hair follicle) to explain several experimental findings; for example, (1) drug and metabolite ratios in blood are quite different from those found in hair; and (2) drug and metabolite concentrations in hair differ markedly between individuals receiving the same dose. Evidence for the transfer of the drug via sweat and sebum can be supported as drugs and metabolites are present in sweat and sebum at high concentrations and persist in these secretions longer than they do in blood. The parent drug can be found in sweat long after it has disappeared from the blood (Henderson 1993; Cone 1996).

The exact mechanism by which chemicals are bound into hair is not known. It has been suggested that passive diffusion may be augmented by binding of drug to intracellular components of the hair cells such as the hair pigment melanin. For example, codeine concentration in hair after oral administration is dependent on melanin content (Kronstrand *et al.* 1999). However, this is probably not the unique mechanism since drugs are trapped into the hair of albino animals, which lack melanin. Another proposed mechanism is the binding of drugs to

sulfhydryl-containing amino acids present in hair. There is an abundance of amino acids such as cystine in hair that form cross-linking S–S bonds to stabilise the protein fibre network. Drugs diffusing into hair cells could be bound in this way. The course of appearance of drugs in hair was evaluated in beard hair. A time lag of various duration between the administration and appearance in hair was observed in all cases: 1 day for codeine to 8 days for morphine and codeine (Mangin 1996). This time lag is probably a result of the growth time necessary for the hair shaft to emerge from the bulb area in the follicle to a height above the skin surface sufficient for collection.

It has been demonstrated from various studies that, after the same dosage, black hair incorporates greater quantities of drugs than blond hair (Henderson *et al.* 1998; Höld *et al.* 1999). This has resulted in discussion of a possible racial or genetic bias of hair analysis, and the matter is still under evaluation.

Specimen collection and procedures

Collection procedures for the analysis of drugs in hair have not yet been standardised. In most published studies, the samples are obtained from random locations on the scalp. Hair is best collected from the area at the back of the head, called the *vertex posterior* (Figure 19.1). Compared with other areas of the head, this area has less variability in the hair growth rate, the number of hairs in the growing phase is more constant, and the hair is less subject to age- and sex-related influences. Hair strands are cut as close as possible to the scalp, and the location of the root-tip must be indicated. Storage is achieved at ambient temperature in aluminium foil, an envelope or a plastic tube. The sample size required for analysis varies considerably between laboratories and depends on the drug to be analysed and the test methodology. For example, when fentanyl or buprenorphine is investigated, a 100 mg sample of hair is recommended. Sample sizes reported in the literature range from a single hair to 200 mg, cut as close to the scalp as possible. As a guide, many laboratories recommend that a hair sample equivalent to the thickness of a pencil should be collected and submitted for analysis. When sectional analysis is performed, the hair is cut into segments of about 1, 2 or 3 cm, which correspond to about 1, 2 or 3 months' growth.

Stability of drugs in hair

The presence of opiates was detected in five hair shafts (about 7.5 cm in length) from the Victorian poet John Keats 167 years after his death (Baumgartner *et al.* 1989). It is believed he took laudanum (opium) to control the pain of tuberculosis. The scalps of eight Chilean and Peruvian mummies dating from 2000 BC to AD 1500 also tested positive for benzoylecgonine (Cartmell *et al.* 1991). All of these studies indicate that drug incorporation is very stable in hair and that modern medical technology can assist other disciplines. Clearly, organic substances are capable of surviving in hair for thousands of years under favourable conditions (ambient temperature, dry atmosphere).

Decontamination procedures

Contaminants of hair would be a problem if they were drugs of abuse or their metabolites or if they interfered with the analysis and interpretation of the test results. It is unlikely that anyone would intentionally or accidentally apply anything to their hair that would contain a drug of abuse. The most crucial issue facing hair analysis is the avoidance of technical and evidentiary false positives. Technical false positives are caused by errors in the collection, processing and analysis of specimens, while evidentiary false positives are caused by passive exposure to the drug. Various approaches for preventing evidentiary false positives due to external contamination of the hair specimens have been described.

Most but not all laboratories use a wash step; however, there is no consensus or uniformity in the washing procedures. Among the agents used in washing are detergents such as Prell shampoo, surgical scrubbing solutions, surfactants such as 0.1% sodium dodecylsulfate, phosphate buffer, or organic solvents such as acetone, diethyl ether, methanol, ethanol, dichloromethane, hexane or pentane of various volumes for various contact times. Generally, a single washing step is employed; sometimes a second identical wash is performed. If external contamination is found by analysing the wash solution, the washout kinetics of repeated washing can demonstrate that contamination is rapidly removed. According to Baumgartner and Hill (1992), the concentration of drug in the hair digest after washing should exceed the concentration in the last wash by at least 10 times. Moreover, the total concentration in

Figure 19.1 Sampling a specimen of hair.

the three phosphate washes should be greater than 3.9 times the concentration in the last wash and the concentration in the hair digest must be greater than one-third of that of the three phosphate washes.

Detection in hair of drug metabolite(s), the presence of which could not be explained by hydrolysis or environmental exposure, would unequivocally establish that internal drug exposure had occurred (Cone *et al.* 1991). Cocaethylene and norcocaine would appear to meet these criteria, as these metabolites are formed only when cocaine is metabolised. Because these metabolites are not found in illicit cocaine samples, they would not be present in hair as a result of environmental contamination, and thus their presence in hair could be considered as a marker of cocaine use. This procedure must be extended to other drugs.

However, there is still great controversy about the potential risk of external contamination, particularly for crack, cannabis and heroin when smoked, as several authors have demonstrated that it is not possible to fully remove the drugs (Blank, Kidwell 1995; Kidwell, Blank 1996). In conclusion, although it is highly recommended to include a decontamination step, there is no consensus on which procedure performs best. Therefore, each laboratory must validate its own technique.

Effects of cosmetic treatments

An important issue of concern for drug analysis in hair is the change in the drug concentration induced by the cosmetic treatment of hair. Hair is continuously subjected to natural factors, such as sunlight, weather, water, pollution, that affect and damage the cuticle, but hair cosmetic treatments enhance that damage. Particular attention has been focused on the effects of repeated shampooing, 'perming', relaxing and dyeing of hair. Repeated shampooing was found to have no significant action on the drug content of hair (Baumgartner, Hill 1992). After cosmetic treatments, drug concentrations decline dramatically, decreasing from 50% to 80% of the original concentration. The products used for cosmetic treatments, such as bleaching, permanent waving, dyeing or relaxing, are strong bases. They will cause hair damage and affect drug content (by loss) or directly affect drug stability (Cirimele *et al.* 1995a).

Drug solubilisation

To determine the amount of a drug remaining in hair after washing, it is necessary to solubilise the drugs in the hair. Solubilisation must be done such that the analytes are not altered or lost. For example, care is necessary to prevent the conversion of cocaine to benzoylecgonine or of 6-monoacetylmorphine to morphine. Prior to testing, the hair sample can be pulverised in a ball-mill, cut into segments or dissolved/digested to yield a uniform matrix for subsequent extraction.

The preparation techniques are generally based on one of the following procedures:

- Drug incubation in an aqueous buffer and analysis with immunological techniques, mostly RIA
- Drug incubation in a chemical (acid or base), liquid–liquid extraction or solid-phase extraction and analysis with chromatographic techniques, mostly GC-MS
- Drug incubation in an organic solvent (generally methanol with or without hydrochloric acid), liquid extraction or solid-phase extraction and analysis with chromatographic techniques, mostly GC-MS
- Hair digestion in an enzymatic solution, liquid extraction or solid–phase extraction and analysis with chromatographic techniques, mostly GC-MS.

Incubation of the hair sample in sodium hydroxide completely destroys the protein matrix. Parameters to be determined are the molarity of NaOH, the time of incubation and the temperature of incubation. The alkaline hydrolysis of hair is not suitable for the extraction of chemically unstable compounds, such as cocaine, 6-monoacetylmorphine, benzodiazepines or esters of anabolic steroids, which are hydrolysed under strong alkaline treatment. To provide extraction of hair containing cocaine or 6-monoacetylmorphine, Jurado *et al.* (1995) and Girod and Staub (2000) proposed the use of acid hydrolysis. The samples can be incubated in 0.1 mol/L HCl overnight at room temperature, at 45°C or at 56°C, or in 0.6 mol/L HCl for 30 min at 120°C. The organic solvent incubation method is the simplest. It involves placing hair samples in methanol or ethanol and then putting them in an ultrasound bath at 45°C for several hours. GC-MS analysis can be carried out directly following the evaporation of the organic solvent (Rothe, Pragst 1995; Pragst 2007). With this method, it is possible to detect heroin in the hair of heroin addicts. Next to chemical hydrolysis and direct extraction of hair with organic solvents, various types of enzymatic digestion of keratin matrices have been proposed (Höld *et al.* 1998). Hair samples can be treated with pronase solution, glucuronidase-arylsulfatase or proteinase K. The enzymes act upon the keratin without altering the chemical structure of the analytes present in the hair. This fact seems to be particularly important when the substances are chemically unstable, such as heroin or cocaine (Sachs, Kintz 1998). An interesting extraction procedure has been developed using supercritical fluid extraction with CO_2 (Edder *et al.* 1994). Adding polar modifiers such as water, methanol or triethylamine leads to a subcritical fluid with high extractive properties. The high speed of the extraction (30 min) and the possibility of connecting on-line with a GC-MS system are advantages that have to be paid for high instrumental costs compared with solid-phase or liquid–liquid extraction. Only small amounts of non-halogenated organic solvents are needed, which result in low environmental pollution (Edder *et al.* 1994).

Drug analysis

The first publication dealing with the analysis of morphine in hair for determining opiate abuse histories reported on analysis with RIA (Baumgartner *et al.* 1979). This paper was followed by a great number of procedures, which mostly used RIA and/or GC-MS. Chromatographic procedures seem to be a more powerful tool for the identification and quantification of drugs in hair owing to their separation ability and their detection sensitivity and specificity, particularly when coupled with MS. Proposed cut-off concentrations and expected concentrations for drugs of abuse in hair are presented Table 19.1.

Immunological methods

Immunoassays are used as screening tests in several laboratories because of their sensitivity, speed and convenience. The procedure must be compatible with the screening test used, i.e. detergents or hair-digestion products must not interfere with the assay. Neutralisation, in the case of chemical hydrolysis, is always necessary. The destruction of the organic

Table 19.1 Proposed cut-off concentrations (when tested by GC-MS) and expected concentrations for drugs of abuse in hair

Drug	*GC-MS cut-off concentration*	*Expected concentrations*
Heroin	0.5 ng/mg for 6-acetylmorphine	0.5–100 ng/mg, in most cases <15 ng/mg
Cocaine	0.5 ng/mg for cocaine	0.5–100 ng/mg, in most cases <50 ng/mg, in crack abusers >300 ng/mg is possible
Amfetamine, MDMA	0.5 ng/mg for both drugs	0.5–50.0 ng/mg
Cannabis	0.05 ng/mg for THC 0.5 pg/mg for THC-COOH	THC: 0.05–10 ng/mg, in most cases <3 ng/mg THC-COOH: 0.5–50 pg/mg, in most cases <5 pg/mg

MDMA, methylenedioxymethamfetamine; THC, tetrahydrocannabinol; THC-COOH, tetrahydrocannabinol-11-oic-acid.

protein matrix of hair must be done under sufficiently mild conditions that will not damage the entrapped analyte or the protein antibodies subsequently added for the immunoassay. Quantification by immunoassay is difficult to achieve as the specificity of most kits is directed to a group of drugs and drug metabolites rather than to a single substance.

Radioimmunoassay

RIA is the most common screening test for hair. Kits, generally proposed for urine, can be used without any modification, at pH greater than 7.0. Calibration curves are obtained either from the control urines in the kit or from extracts of drug-free hair samples spiked with the drugs. Duplicate determinations are recommended. The RIA results should be confirmed by GC-MS. In the absence of a second independent method, RIA detection will thus be interpreted with caution. Sometimes, however, the sensitivity of GC-MS is not sufficient, especially with a small amount of material. For these reasons, it is necessary to carry out immunological drug analysis in hair only with specific RIA kits for the selective estimation of a determined drug, such as fentanyl, LSD or buprenorphine.

Enzyme-multiplied immunoassay

Enzyme-multiplied immunoassay (EMIT) (*see* Chapter 31), based on spectroscopic measurement, is subject to interferences by colour and turbidity, and therefore should not be used to analyse hair samples.

Fluorescence polarisation immunoassay

First reported in 1987 (Franceschin *et al.* 1987), fluorescence polarisation immunoassay (FPIA) seems to be correlated to GC-MS, at least when the antibody has a good cross-reactivity with the parent drug, such as for dextropropoxyphene, amfetamine or methadone. FPIA can be proposed for toxicological screening in hair samples, but results must always be confirmed (Kintz *et al.* 1992).

Chromatographic methods

Chromatographic methods have been used as screening and confirming tests. Moreover, they allow quantification of drugs and drug metabolites present in hair as well as confirmation of drug identity. Some classic procedures are given in detail Table 19.2.

Thin-layer chromatography

In 1980, Klug reported a thin-layer chromatography (TLC) method to detect morphine in the hair of drug abusers in which drug detection and quantification were achieved using fluorimetry (Klug 1980). A high performance TLC method was used to determine morphine in human hair with quantification by densitometry (Jeger *et al.* 1991).

High performance liquid chromatography

High performance liquid chromatography (HPLC) methods were used in some studies for the detection of morphine, haloperidol, β-blockers, some antiepileptic or psychotropic drugs, and buprenorphine (Kintz *et al.* 1994; Couper *et al.* 1995). Different kinds of detectors were involved, including ultraviolet, fluorimetry and coulometry. The latter two detectors allow enough sensitivity for low drug concentrations (Sachs, Kintz 1998). At this time, most of the recent literature is focused on the use of LC-MS(-MS) owing to its excellent specificity and its extraordinary sensitivity, which allow, in some special cases, the detection of a single exposure to a specific drug (Müller *et al.* 2000; Huang Y *et al.* 2008; Huang DK *et al.* 2009; Stout *et al.* 2010).

Gas chromatography

Gas chromatography coupled to flame ionisation or nitrogen detection procedures is less useful for the analysis of drugs in hair since the great number of interfering substances (exogenous and endogenous compounds) makes the interpretation of chromatograms very difficult. GC-MS is the most powerful tool for the detection of drugs in hair. In 1992, Moeller presented a screening procedure in which amfetamines, cocaine, benzoylecgonine, codeine, morphine and tetrahydrocannabinol-H-oic acid (THC-COOH) were analysed in one procedure (Moeller *et al.* 1992a). More recently, Kauert and Rohrich (1996) presented a screening procedure based on methanolic incubation that was suitable for opiates, cocaine, amfetamines, methadone and cannabis. Today, this procedure is considered as the standard in hair testing.

Other methods

Capillary electrophoresis (CE) has been proposed for the quantitative determination of cocaine and morphine in the hair of cocaine and heroin users (Tagliaro *et al.* 1997). Infrared (IR) microscopy can delineate passive exposure from drug use by analysing only the central core of the unextracted hair shaft with either cross-sectionally microtomed or laterally microtomed hair. IR spectra can be obtained of the medulla, cortex and cuticle of the single hair with a nominal spatial resolution of 10 μm (Kalasinsky *et al.* 1994). Fourier transform-IR (FTIR) microscopy was presented to be more sensitive than classic GC-MS procedures by Kalasinsky *et al.* (1994). Fluorescence microscopy has been used to

Table 19.2 Screening procedures for the detection of illegal drugs in hair[a]

Method	*Kauert*	*Moeller*	*Kintz*
Analytes	Heroin, 6-MAM, dihhydrocodeine, codeine, methadone, THC, cocaine, amfetamine, MDMA, MDEA, MDA	6-MAM, dihhydrocodeine, codeine, methadone, THC, cocaine, amfetamine, MDMA, MDEA, MDA	6-MAM, codeine, methadone, cocaine, amfetamine, MDMA, MDEA, MDA, most pharmaceuticals
Decontamination step	Ultrasonic 5 min each	20 mL H_2O (2×)	5 mL CH_2Cl_2
	5 mL H_2O	20 mL acetone	(2 × 5 min)
	5 mL acetone		
	5 mL petrol ether		
Homogenisation	100 mg hair cut into small sections in a 30-mL vial	Ball mill	Ball mill
Extraction	4 mL methanol Ultrasonic 5 h, 50°C	20–30 mg powdered hair, 2 mL acetate buffer + β-glucuronidase/arylsulfatase, 90 min/40°C	50 mg powdered hair, 1 mL 0,1 mol/L HCl, 16 h/56°C
Clean-up	None	$NaHCO_3$; SPE (C_{18}), elution with 2 mL acetone–CH_2Cl_2 (3 : 1)	$(NH_4)_2HPO_4$;extraction 10 mL $CHCl_3$–2-propanol–*n*-heptane (50 : 17 : 33); organic phase purified with 0.2 mol/L HCl; HCl phase to pH 8.4; re-extraction with $CHCl_3$
Derivatisation	Propionic acid anhydride	1000 μL PFPA–75 μL PF-*n*-propanol; 30 min/60°C; N_2/60°C; 50 μL ethyl acetate	40 μL BSTFA–1%TMCS or HFBA; 20 min/70°C

BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; HFBA, heptafluorobutyric acid; PF, pentafluoro; PFPA, pentafluoropropionic anhydride; SPE, solid-phase extraction; THC, tetrahydrocannabinol; TMCS, trimethylchlorosilane.
[a]From Sachs, Kintz (2000).

detect organic substances in hair and presents a good alternative to chromatographic procedures (Pötsch and Leithoff 1992).

Drug identification

Opiates

In 1979, Baumgartner and colleagues (1979) published the first results on morphine concentrations in the hair of 25 heroin users (Baumgartner *et al.* 1979). Morphine was in the range 0.3–6.6 ng/mg. Since heroin samples always contain codeine, codeine is also detected in cases of heroin abuse. Morphine is a metabolite of codeine and can be detected when codeine is abused. The quantification of both drugs was proposed to differentiate between codeine and heroin abuse (Sachs and Arnold 1989). If the morphine concentration is clearly higher than the codeine concentration in the examined hair sample, heroin or morphine abuse is highly probable. If the codeine concentration is clearly higher than that of morphine then it may be assumed that codeine has been ingested. However, the discrimination of heroin users from individuals exposed to other sources of morphine alkaloids can be achieved by directly identifying heroin or 6-monoacetylmorphine (Nakahara *et al.* 1992; Sachs, Uhl 1992). In this case, no alkaline hydrolysis must be performed so as to avoid hydrolysis. In most samples, it was demonstrated that the concentration of 6-monoacetylmorphine exceeded that of morphine, which is a less lipophilic compound. Other opioids have been detected in hair including dihydrocodeine, pholcodine, ethylmorphine, dextromoramide, methadone, fentanyl, sulfentanyl, pentazocine, zipeprol and buprenorphine (Sachs, Kintz 1998). A comprehensive review concerning the analysis of opioids in hair has been published by Yegles and Wennig (2006).

Cocaine

Procedures for the detection of cocaine have been published in several papers (Sachs, Kintz 1998; Moore *et al.* 2007; Barroso *et al.* 2008; Hoelzle *et al.* 2008; Vogliardi *et al.* 2009). There is considerable variety in the work-up and derivatisation conditions. In most cases, cocaine is found in higher concentrations than benzoylecgonine and methylecgonine (Moeller *et al.* 1992a; Kidwell 1993; Henderson *et al.* 1996). The determination of the pyrolysis product of cocaine, anhydroecgonine methyl ester (AEME), is helpful in distinguishing cocaine smoking from crack use. Kintz found AEME in the range 0.2–2.4 ng/mg for seven crack users (Kintz *et al.* 1995b).

An important study with controlled doses of cocaine-d_5 was published in 1996 (Henderson *et al.* 1996). The deuterium-labelled cocaine was administered intravenously and/or intranasally in doses of 0.6–4.2 mg/kg under controlled conditions. A single dose could be detected for 2–6 months; the minimum detectable dose appeared to be between 22 and 35 mg.

As an example, the procedure for simultaneously testing for opiates and cocaine is as follows (from Kintz, Mangin 1995).

Decontamination

- Take a hair strand (~100 mg)
- Wash with 5 mL methylene chloride, for 2 min
- Dry with adsorbent paper
- Second wash in 5 mL methylene chloride, for 2 min.

Homogenisation/pulverisation

- Pulverise the sample in a ball mill for 10 min at 100 cycles/min.

Solubilisation

- Take 30–50 mg of powdered hair and add:
  - 1 mL phosphate buffer pH 8.4
  - 100 ng of deuterated opiates and cocaine derivatives
- Incubate for 16 h at 56°C.

Extraction

- Homogenize with 10 mL dichloromethane–isopropanol–*n*-heptane (50 : 17 : 33, v/v)
- Agitate on a horizontal shaker for 20 min at 95 cycles/min
- Centrifuge for 15 min at 3000 rpm
- Purify the organic phase by acid extraction (5 mL 0.2 mol/L HCl), then alkaline back extraction (1 mL NaOH 1 mol/L plus 2 mL phosphate buffer pH 8.4 in dichloromethane)
- Collect the organic phase and evaporate to dryness.

Derivatisation

- Dry the extract using 30 µL BSTFA (bis(trimethylsilyl)trifluoroacetamide) plus 1% trimethylchlorosilane (TMCS)
- Incubate for 30 min at 70°C.

Analysis

- Inject a 1.5-µL portion in splitless mode onto a HP5-MS capillary column (30 m × 0.25 mm)
- GC parameters:
  - Flow rate, helium N55: 1.0 mL/min
  - Injector temperature: 270°C
  - Temperature programme: 60°C for 1 min, 30°C/min to 295°C for 6 min.

Amfetamines

Papers dealing with amfetamine and methamfetamine (MA) have mostly been published by Japanese authors (Nakahara *et al.* 1990). The work-up (liquid–liquid extraction after acid or alkaline hydrolysis) and derivatisation procedures (trifluoroacetic anhydride, TFA) are similar in most of the publications.

After intake of methamfetamine, its major metabolite, amfetamine, can be detected in hair samples, and the differentiation of intake is shown by the ratio between the drugs.

In 1992, methylenedioxy-*N*-methamfetamine (MDMA or ecstasy) was first detected in the hair of an abuser at a concentration of 0.6 ng/mg (Moeller *et al.* 1992b). In Europe, MDMA is one of the most frequently identified amfetamines. Since the first screening procedure in 1995, different methods have been published (Kikura *et al.* 1997; Rothe *et al.* 1997). These procedures allow the determination of amfetamine, methamfetamine, methylenedioxyamfetamine (MDA), MDMA, methylenedioxyethamfetamine (MDEA) and *N*-methylbenzodioxolylbutanamine (MBDB) by electron impact GC-MS.

As an example, the procedure for testing for several stimulants is as follows (from Sachs, Kintz 1998).

Decontamination

- Take a hair strand (~100 mg)
- Wash with 5 mL methylene chloride, for 2 min
- Dry with adsorbent paper
- Wash again in 5 mL methylene chloride, for 2 min.

Homogenisation

- Pulverise the sample in a ball mill for 10 min at 100 cycles/min.

Solubilisation

- To 30–50 mg of powdered hair, add 1 mL 1 mol/L NaOH and 200 ng by weight of deuterated drugs
- Incubate for 10 min at 95°C.

Extraction

- Homogenise and add 5 mL ethyl acetate
- Agitate on a horizontal shaker for 20 min at 95 cycles/min
- Centrifuge for 15 min at 3000 rpm
- Collect organic phase, addition of 20 µL methanol–HCl (99 : 1 v/v) and evaporate to dryness.

Derivatisation

- Dry the extract and add 150 µL ethyl acetate–heptafluorobutyric anhydride (1 : 2 v/v)
- Incubate for 30 min at 60°C
- Re-evaporate to dryness.

Analysis

- Dissolve the derivatised extract in 25 µL ethyl acetate. Inject a 1.5-µL portion in splitless mode into a HP5-MS capillary column (30 m × 0.25 mm)

- GC parameters:
  - Flow rate, helium N55: 1.0 mL/min
  - Injector temperature: 240°C
  - Temperature programme: 60°C for 1 min, to 295°C at 30°C/min for 6 min.

Cannabis

In 1995, the first results on cannabis in hair using GC-MS and the determination in the same run of Δ^9-tetrahydrocannabinol (THC) and its major metabolite 11-nor-Δ^9-THC carboxylic acid (THC-COOH) were reported (Cirimele *et al.* 1995b; Jurado *et al.* 1995). The measured concentrations were low, particularly compared with other drugs. Some authors suggested the use of negative-ion chemical ionisation (Kintz *et al.* 1995b) to target the drugs or the application of tandem mass spectrometry (Cirimele *et al.* 1996b). More recently, a simpler method was proposed (Uhl 1997), based on the simultaneous identification of cannabinol (CBN), cannabidiol (CBD) and THC. This procedure is a screening method that is rapid and economic and does not require derivatisation prior analysis. To avoid potential external contamination (since THC, CBD and CBN are present in smoke), the endogenous metabolite, THC-COOH, should be shown to be present in order to confirm drug use. The concentrations measured are very low, particularly for THC-COOH, which was identified in the picogram/milligram range.

The simple procedure to test for cannabinoids is as follows (from Cirimele *et al.* 1996b).

Decontamination

- Take a hair strand (~100 mg)
- Wash with 5 mL methylene chloride for 2 min
- Dry with adsorbent paper
- Wash again in 5 mL methylene chloride for 2 min.

Homogenisation

- Pulverise the sample in a ball mill for 10 min at 100 cycles/min.

Solubilisation

- Take 50 mg of powdered hair
- Add1 mL 1 mol/L NaOH plus 100 ng of deuterated THC
- Incubate for 10 min at 95°C.

Extraction

- Homogenise with 5 mL *n*-hexane–ethyl acetate (9 : 1, v/v)
- Agitate for 20 min at 95 cycles/min
- Centrifuge for 15 min at 3000 rpm
- Collect the organic phase and evaporate to dryness.

Analysis

- Dissolve the extract in 20 µL cyclohexane
- Inject a 1.5-µL portion in splitless mode into a HP5-MS capillary column (30 m × 0.25 mm)
- GC parameters:
  - Flow rate, helium N55: 1.0 mL/min
  - Injector temperature: 250°C
  - Temperature programme: 60°C for 1 min, to 295°C at 30°C/min for 6 min.

Analytical data The data are given in Table 19.3; ions with the *m/z* values shown in italic type are used for quantification.

Table 19.3 Analytical data for cannabinoids. The ions with *m/z* values shown in italic type are used for quantification[a]

Compound	R_t *(min)*	*Ions* (m/z)
THC	9.59	*299*, 314, 271
THC-d_3	9.58	*302*, 317, 274
Cannabidiol	9.28	*231*, 314, 246
Cannabinol	9.84	*295*, 310, 238

[a]From Cirimele *et al.* (1996b).

Benzodiazepines

Surprisingly, until 1995 the chromatographic detection of benzodiazepines, the most commonly used class of pharmaceuticals in the world, did not appear to be documented. Large series of results were obtained using incubation in buffer, such as Sörensen buffer (Cirimele *et al.* 1996a; Kintz *et al.* 1996). In the early stages of benzodiazepine determination, GC-MS in either electron impact (EI) or negative chemical ionisation (NCI) mode was used; however, to detect diazepam, nitrazepam, oxazepam or alprazolam, liquid chromatography with UV detection (HPLC-UV), gas chromatography with electron capture detection (GC-ECD) or liquid chromatography with diode array detection (HPLC-DAD), respectively, were employed. An extensive review of analytical procedures to test for benzodiazepines, particularly flunitrazepam, was published in 1998 (Sachs, Kintz 1998). As demonstrated by the quantitative levels found, benzodiazepine concentrations are generally low, in the picogram/milligram range. This is why today LC-MS (-MS) represents the state of the art for testing benzodiazepines in human hair.

As an example, the procedure for screening for most benzodiazepines is as follows (from Villain *et al.* 2005).

Decontamination

- Take a hair strand (~100 mg)
- Wash with 5 mL methylene chloride for 2 min
- Dry with adsorbent paper
- Wash again in 5 mL methylene chloride for 2 min.

Homogenisation

- Pulverise the sample in a ball mill for 10 min at 100 cycles/min.

Solubilisation

- Take 20 mg of powdered hair
- Add 1 mL phosphate buffer pH 8.4
- Add 5 ng of diazepam-d_5
- Incubate for 12 h at 40°C.

Extraction

- Homogenise using 5 mL diethyl ether–dichloromethane (80 : 20, v/v)
- Agitate on a horizontal shaker for 20 min at 95 cycles/min
- Centrifuge for 15 min at 3000 rpm
- Collect the organic phase and evaporate to dryness.

Analysis In the source paper (Villain *et al.* 2005), the residue was reconstituted by adding 50 µL of methanol. A 10-µL aliquot of the extract was injected onto the column (XTerra MS C_{18} 3.5 µm, 100 × 2.1 mm i.d.), protected by a 1-mm C_{18} frit. Each 20 min chromatographic run was carried out with a gradient (5% acetonitrile–95 % formic acid to 80 : 20 at 10 min), at a flow rate of 200 µL/min. The HPLC system was a Waters Alliance 2695.

Detection was carried out by a Micromass Quattro Micro tandem mass spectrometer equipped with an ion spray atmospheric pressure interface (APCI). The instrument was operated in the positive ionisation mode. Best results were obtained with a capillary voltage of 1 kV, source block temperature of 120°C and desolvation gas (N_2) heated to 350°C delivered at 550 L/h. Collision cell pressure was 300 Pa (3 mbar) of argon. Data were recorded in the multiple reaction monitoring (MRM) mode.

Analytical data The data are given in Table 19.4; ions with the *m/z* values shown in italic type are used for quantification.

Doping agents

Very few papers dealing with the identification of anabolic steroids in hair have been published and only a few studies are available (Kintz 1998; Rivier 2000). In a fatal case of a male bodybuilder who was known to abuse various anabolic steroids regularly and who died of cardiac infarction at the age of 32, testosterone esters, nandrolone decanoate, metenolone and metandienone were identified in hair (Thieme *et al.* 2000). In a forensic case involving French customs, two bodybuilders were arrested with 250 ampoules and more than 2000 tablets of anabolic steroids. In both subjects, hair tested positive for stanozolol (135 and

Table 19.4 MRM transitions for the detection of 17 benzodiazepines and hypnotics and IS by LC-MS(-MS). The transition for quantification is shown in italic type[a]

Compound	*Retention time (min)*	*Parent ion (m/z)*	*Daughter ions (m/z)*	*Cone (V)*	*Collision energy (eV)*
Alprazolam	10.9	309.1	205.2	45	40
			274.2	45	26
7-Aminoclonazepam	7.5	286.1	222.2	40	25
			250.2	40	20
7-Aminoflunitrazepam	8.4	284.2	*135.1*	40	28
			227.2	40	25
Bromazepam	9.6	316.0	*182.3*	35	30
			209.3	35	25
Clobazam	11.7	301.1	224.2	30	33
			259.1	30	20
Diazepam	12.1	285.2	*154.2*	40	25
			193.3	40	30
Lorazepam	11.0	321.1	229.1	30	27
			275.1	30	22
Lormetazepam	11.7	335.1	177.1	28	40
			289.1	28	20
Midazolam	9.3	326.1	244.1	44	25
			291.2	44	28
Nordiazepam	11.1	271.2	*140.1*	40	25
			165.1	40	28
Oxazepam	10.8	269.1	163.1	45	32
			241.2	45	20
Temazepam	11.5	301.1	283.1	30	40
			255.2	30	20
Tetrazepam	11.2	289.2	225.2	40	26
			253.2	40	22
Triazolam	11.0	343.1	*308.1*	45	26
			315.1	45	27
Zaleplon	10.4	306.2	*236.2*	40	28
			264.2	40	20
Zopiclone	7.9	389.0	*245.1*	35	15
			345.1	35	9
Zolpidem	8.4	308.2	*235.3*	40	35
			263.2	40	26
Diazepam-d_5	12.1	290.2	*154.1*	40	30
			198.3	40	30

[a]Data from Villain *et al.* (2005).

156 pg/mg), nandrolone (196 and 260 pg/mg) and testosterone (46 and 71 pg/mg), clearly indicating chronic exposure to anabolic drugs (Kintz *et al.* 1999). During the last few years, various other performance-enhancing drugs have been identified in hair, such as β-adrenergic drugs (agonists and antagonists) (Machnik *et al.* 1999; Kintz *et al.* 2000b), ephedrine (Dumestre, Kintz 2000) and corticosteroids (Bévalot *et al.* 2000; Cirimele *et al.* 2000).

Miscellaneous drugs

Several other drugs have been identified and quantified in human hair samples, including antidepressant drugs, antiepileptics, neuroleptics, cardiovascular drugs, barbiturates, caffeine, phencyclidine, fentanyl, pentazocine, methadone, chloroquine, digoxin, buprenorphine (Tracqui *et al.* 1997), nicotine and fenfluramine (Kauert, Rohrich 1996; Sachs, Kintz 1998).

Little has been published concerning the analysis of pesticides in hair (Cirimele *et al.* 1999). This seems to be due to a lack of interest on the part of physicians.

Sectional analysis

Multisectional analysis involves taking a length of hair and cutting it into sections to measure drug use during shorter periods of time. The hair must be cut as close as possible to the scalp. Particular care is also required to ensure that the relative positions of the sections of cut hair are retained. The further away from the hair root, the more cautious the interpretation of the quantitative findings of the individual hair sections has to be. It has been claimed that this technique can be applied to provide a retrospective calendar of an individual's drug use. For example, one can perform multisectional analysis for people who test positive on an initial screen. This information can be used to validate an

individual's claim of prior drug use but of abstinence during the most recent several months. Baumgartner and colleagues have proposed that another use of such information is to compare the results with the individual's self-reported drug use history, to establish the level of denial prior to referring the individual to rehabilitation (Baumgartner *et al.* 1989). The most extensive study on sectional analysis for drugs of abuse involved patients in rehabilitation centres. Segmental hair analysis was used to verify both their previous drug history and their recent enforced abstinence. In cases where abstinence has been observed, the lowest drug concentrations are found in the segments nearest the root, thus confirming decreased drug use or recent abstinence. Abstinence from tobacco can be demonstrated by sectional analysis. The switch from heroin to another drug (codeine, ethylmorphine, dihydrocodeine) can be established with accuracy. Given the variation of hair growth rates and the long half-life of particular drugs (cannabinoids) that can be retained in the body for weeks or months after last use, results from a multisectional analysis should not be used to determine a precise period of drug abuse or to compare individuals.

Dose–concentration relationship

A critical question about hair analysis that remains controversial is the relationship between intake dose and resultant concentration in hair. In cases of chronic abuse, daily doses vary significantly from day to day, and the establishment of a dose–response relationship requires a large amount of data to take individual differences into consideration. Weak dose–concentration relationships can be explained by the following points: the drug dose of abusers is uncertain, the purity of illicit compounds is unknown and the uptake of the drug from blood to hair varies with the individual. On the other hand, some papers report that a significant dose–concentration relationship exists for digoxin, cocaine, phencyclidine, cannabinoids, morphine, meprobamate, haloperidol and amitriptyline. These results strongly suggest that a dose–response relationship exists between drug levels in hair and the administered dose, and this seems particularly true in controlled studies in which a drug was taken for the first time or under close supervision (Kintz 1996).

The possibility of ethnic/genetic bias due to differences in melanin concentrations or those due to hair porosity is still under discussion. Melanins are responsible for the colour of hair. Two types of melanin are present, eumelanin (with low sulfur content) and pheomelanin (with high sulfur content). Black and brown hair contain more eumelanin than red and blond hair. It appears that it is not simply the concentration of drugs in blood that determines the concentration in hair. Numerous factors may influence the incorporation of drugs into hair, such as the nature of the compound (pK_a, lipid solubility, metabolism pattern) and variation in hair growth cycles. Until these mechanisms are elucidated, the quantitative results and extrapolation to the amount of drug intake of such a hair analysis should be considered with extreme caution (Harkey *et al.* 1991; Nakahara *et al.* 1992).

Applications

Comparison with urine testing

There are essentially three types of problems with urinalysis drug testing: false positives when not confirmed with GC-MS, embarrassment associated with observed urine collection and evasive manoeuvres, including adulteration. These problems can be greatly mitigated or eliminated through hair analysis. It is normally possible to obtain a fresh, identical hair sample if there is any claim of a specimen mix-up or breach in the chain of custody. This makes hair analysis essentially fail-safe, in contrast to urinalysis, since an identical urine specimen cannot be obtained at a later date.

Another potential use of hair analysis is to verify accidental or unintentional ingestion of food or drink that has been laced with drugs. In case of a single exposure, the hair will not test positive using the Society of Hair Testing cut-offs. Ingestion of poppy seeds appears to be sufficient for the creation of a positive urine result, while ingestion of up to 30 g of poppy seeds did not result in a positive hair identification (H. Sachs, personal communication, 1994). Its greatest use, however, may be in identifying false negatives, since neither abstaining from a drug for a few days nor trying to 'beat the test' by diluting the urine will alter the concentration in hair. Urine does not indicate the frequency of drug intake in subjects who might deliberately abstain for several days before biomedical screening. While analysis of urine specimens cannot distinguish between chronic use or single exposure, hair analysis can make this distinction. Table 19.5 illustrates the differences between hair and urine analysis.

Table 19.5 Comparison between urine and hair

Parameter	*Urine*	*Hair*
Major compound	Metabolites	Parent drug
Detection period	2–5 days	Weeks, months
Type of measure	Incremental	Cumulative
Screening	Yes	Difficult
Invasiveness	High	Low
Storage	−20°C	Ambient temperature
Risk of false negative	High	Low
Risk of false positive	Low	Undetermined
Risk of adulteration	High	Low
Control material	Yes	Needed

Verification of history of drug use

By providing information on exposure to drugs over time, hair analysis may be useful in verifying self-reported histories of drug use in any situation in which a history of past, rather than recent, drug use is desired, such as in pre-employment and employee drug testing or patient compliance monitoring as required in therapeutic drug monitoring. In addition, hair analysis may be especially useful when a history of drug use is difficult or impossible to obtain, such as from psychiatric patients. During control tests of hair fragments, a drug addict will not be capable of hiding the fact of drug abuse. Hair analysis will even detect the case of an addict who takes drugs only every few days; this behaviour cannot be proved by means of urine and blood tests, even when the tests are repeated.

Determination of gestational drug exposure

Maternal drug abuse is a health hazard for the foetus, and the effects of cocaine, phencyclidine, nicotine and other compounds are well documented. In 1987, Parton and colleagues first reported quantitation of foetal cocaine exposure by RIA of hair obtained from 15 babies (Parton 1987). Others studies have demonstrated placental transfer of maternal haloperidol and the presence of nicotine, morphine, amfetamine and benzodiazepines in neonatal hair (Klein *et al.* 2000). It has been suggested that foetal accumulation of cocaine and its metabolites follows a linear pattern within the clinically used doses (Forman *et al.* 1992) and that there is a dose-dependent transfer of maternal nicotine to the baby (Kintz *et al.* 1993). Analysis of newborn hair may overcome the disadvantages of the methods currently used to verify drug abuse, such as maternal self-reported drug history, maternal urinalysis (risk of false negatives), and analysis of the urine or the meconium of the baby at time of delivery (risk of false negative information during the preceding 1–3 days).

Applications in forensic science

Numerous other forensic applications have been described in the literature where hair analysis was used to document the case: differentiation between a drug dealer and a drug consumer, chronic poisoning, crime under the influence of drugs, child sedation and abuse, suspicious circumstances associated with death, child custody, abuse of drugs in

jail, body identification, survey of drug addicts, chemical submission, obtaining a driving licence and doping control (Moeller *et al.* 1993; Sachs 1996a).

To date, more than 600 articles concerning hair analysis have been published reporting applications in forensic toxicology, clinical toxicology, occupational medicine and doping control. The major practical advantage of hair for testing drugs, compared with urine or blood, is its larger detection window, which is weeks to months, depending on the length of hair shaft analysed, versus a few days for urine. In practice, detection windows offered by urine and hair testing are complementary: urine analysis provides short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. Although there is reasonable agreement that the qualitative results from hair analysis are valid, the interpretation of the results is still under debate because of some unresolved questions, such as the influences of external contamination or cosmetic treatment, and possible racial bias.

Specific problems associated with doping control using hair

Although hair is not yet recognised as a valid specimen by the International Olympic Committee (IOC) for sports testing, it is accepted in most courts of justice. The credibility of hair testing in sport has been associated with uncertainty because of some conflicting results observed, involving athletes who tested positive in urine in accredited IOC laboratories and negative in hair in forensic certified laboratories. A great deal of experience has been acquired in the detection of opiates and cocaine in hair. In contrast, there is a serious lack of suitable references with which to interpret the analytical findings for doping agents. In hair, doping agent concentrations, such as those of anabolic steroids, corticosteroids or β_2-agonists, are in the range of picograms/milligram, whereas cocaine, amfetamines and opiates are generally found in the range of several nanograms/milligram.

It is clear that there is a great deal of research to be performed before the scientific questions and curiosity surrounding hair drug testing is satisfied. Some of this uncertainty is due to a lack of consensus among the active investigators on how to interpret the results of an analysis of hair. Among the unanswered questions, five are of critical importance:

1. What is the minimal amount of drug detectable in hair after administration?
2. What is the relationship between the amount of the drug used and the concentration of the drug or its metabolites in hair?
3. What is the influence of hair colour?
4. Is there any racial bias in hair testing?
5. What is the influence of cosmetic treatments?

Several of these specific topics were addressed by Kintz *et al.* (2000a).

Alcohol abuse

Considering the large scale of alcohol-associated problems in society, the diagnosis of excessive alcohol consumption is an important task from a medical point of view. The methods used for this purpose are based on indirect alcohol markers such as increased liver enzyme activity (γ-GT or GPT), increased erythrocyte mean cell volume or the presence of carbohydrate-deficient transferrins, which can also originate from other pathological causes. Ethyl glucuronide, phosphatidylethanol and fatty acid ethyl esters (FAEEs) are all markers of ethanol consumption. The first investigations of a marker of alcohol consumption in hair were reported by Sachs and colleagues and focused on ethyl-glucuronide (Sachs 1997). Detection of ethyl glucuronide in hair is always associated with alcohol consumption, whereas a negative result does not unambiguously exclude alcohol abuse (Jurado *et al.* 2004; Yegles *et al.* 2004). Recently, new investigations on the FAEEs were proposed by Pragst and colleagues to monitor alcohol consumption (Pragst *et al.* 2001). FAEEs are formed in the presence of ethanol and free fatty acids, triglycerides, lipoproteins or phospholipids by a FAEE synthetase found in the liver as well as in hair roots. FAEE determination is of interest as the esters appear to be formed in cases of alcohol-induced organ damage. In blood, FAEEs can be used as a marker of an actual or recent alcohol intake at least 24 h after completion of alcohol intake. Hair concentrations of four FAEEs (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) found in the hair of children, adult teetotallers and social drinkers in comparison with FAEE concentrations found in hair of alcoholics led the authors to conclude that FAEEs are suitable markers for the detection of heavy alcohol consumption. Segmental hair analysis in a case of alcohol withdrawal treatment showed a decrease in FAEE content from the distal to the proximal root segment (Pragst *et al.* 2001; Yegles *et al.* 2004).

Driving licence regranting

The major practical advantage of hair testing compared with urine and blood testing for drugs is the longer detection window, which is weeks to months depending on the length of the analysed hair shaft, against a few days for urine. It is generally agreed that the qualitative results obtained from hair analysis are valid and that long-term histories are accessible through hair analysis. This approach is being used in Italy and Germany in cases where applications are submitted for the regranting of driving licences after suspension (Sachs 1996b; Tagliaro *et al.* 2000). Persons whose driving licence has been refused, revoked or suspended for addiction to psychoactive drugs or for 'driving under the influence', and who claim to have quit all drugs of abuse, can obtain a licence after a medical committee has confirmed the actual and complete abstinence from illicit drugs and excluded any additional risk of future relapse of drug abuse. To provide objective evidence of abstinence from drugs with an acceptable chronological window in order to support the clinical decision of this medical committee, hair analysis has been included in a panel of clinical and laboratory tests aimed at investigating retrospectively the toxicological behaviour of subjects. Comparison between hair analysis and urinalysis shows a much higher diagnostic sensitivity for hair tests.

Drug-facilitated crime

The use of a drug to modify a person's behaviour for criminal gain is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery, incapacity, etc.) has caused alarm in the general public. Drugs involved can be pharmaceuticals such as benzodiazepines (flunitrazepam, lorazepam), hypnotics (zopiclone, zolpidem), sedatives (neuroleptics, some anti-H_1) or anaesthetics (GHB, ketamine); drugs of abuse such as cannabis, ecstasy or LSD; or more often ethanol. Owing to their low dosage, except for GHB, a surreptitious administration into beverages such as coffee, soft drinks (cola) or even better alcoholic cocktails is relatively simple Most of these substances possess amnesic properties and the victims are therefore less able to accurately recall the circumstances under which the sexual offence occurred. As they are generally short-acting, they impair an individual rapidly. In these situations, blood or even urine can be of little interest. To address this important deficiency, hair was suggested as a valuable specimen in situations where, as a result of a delay in reporting the crime, natural processes have eliminated the drug from typical biological specimens. The use of LC-MS(-MS) today allows the detection of a single dose of most sedatives (Kintz *et al.* 2004; Villain *et al.* 2004a, 2004b).

Discrimination between a single exposure and long-term use can be documented by multisectional analysis. With the concept of absence of migration along the hair shaft, a single spot of exposure must be present in the segment corresponding to the period of the alleged event, using a growth rate for hair of 1 cm/month. As this growth rate can vary from 0.7 to 1.4 cm/month, the length of the hair section must be calculated accordingly. A delay of 3–5 weeks between the offence and hair collection for sectional analysis (2-cm segments) can be considered as satisfactory to have the hair shaft including the spot of exposure. The hair must be cut as close as possible to the scalp. Particular care is also required to ensure that the individual sections of the hair strand retain their relative positions.

Table 19.6 Recommended cut-off concentrations (when tested by GC-MS) and expected concentrations for drugs of abuse in hair

Drug	*GC-MS cut-off concentration*	*Expected concentrations*
Heroin	0.2 ng/mg for 6-acetylmorphine	0.5–100 ng/mg, in most cases <15 ng/mg
Cocaine	0.5 ng/mg for cocaine and 0.05 ng/mg for benzoylecgonine and cocaethylene	0.5–100 ng/mg, in most cases <50 ng/mg, in crack abusers >300 ng/mg is possible
Amfetamine, MDMA	0.2 ng/mg for both drugs	0.5–50.0 ng/mg
Cannabis	0.1 ng/mg for THC 0.2 pg/mg for THC-COOH	THC: 0.05–10 ng/mg, in most cases <3 ng/mg THC-COOH: 0.5–50 pg/mg, in most cases <5 pg/mg

MDMA, methylenedioxymethamfetamine; THC, tetrahydrocannabinol; THC-COOH, tetrahydrocannabinol-11-oic-acid.

Conclusions

The value of hair analysis for the identification of drug users is steadily gaining recognition. This can be seen from its growing use in pre-employment screening, in forensic sciences and in clinical applications. Hair analysis may be a useful adjunct to conventional drug testing in toxicology. Specimens can be more easily obtained with less embarrassment, and hair can provide a more accurate history of drug use.

Although there are still controversies about how to interpret the results, particularly concerning external contamination, cosmetic treatments, ethnic/genetic bias or drug incorporation, pure analytical work in hair analysis has reached a sort of plateau, having solved almost all the analytical problems.

GC-MS is the method of choice in practice, although GC-MS(-MS) and LC-MS(-MS) are today used in several laboratories, even for routine cases, particularly to target low-dosage compounds such as THC-COOH, fentanyl, flunitrazepam or buprenorphine. Electrophoretic/electrokinetic analytical strategies, chiral separation or application of ion mobility spectrometry constitute the latest new developments of the analytical tools reported to document drug testing in hair.

Today, quality assurance is a major issue of drug testing in hair. Since 1990, the National Institute of Standards and Technology (Gaithersburg, MD, USA) has developed inter-laboratory comparisons, more recently followed by the Society of Hair Testing (Strasbourg, France). The Society of Hair Testing has recommended cut-off concentrations (Table 19.6) together with the expected concentrations.

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CHAPTER

20 Method Development and Validation

FT Peters

Introduction

Reliable analytical data are of utmost importance in the analysis of drugs and poisons. In drug registration, they support decision-making of pharmaceutical companies and regulatory authorities from pre-clinical phase I studies to post-registration bioequivalence studies. In therapeutic drug monitoring (TDM) and clinical and forensic toxicology, they are the prerequisite for the correct interpretation of the respective cases. In these areas, unreliable results might not only lead to wrong treatment of the patient but also have unjustified legal consequences for the defendant or be contested in court.

Reliable analytical methods are the basis for the acquisition of high-quality analytical results. Therefore, the quality of analytical methods must be ensured throughout their lifetime. In this continuous process of quality assurance, method validation stands at the pivotal point between method development and quality control (QC) during routine application of the final method. It includes all analytical experiments and statistical procedures required to demonstrate that a particular analytical method is reliable for the intended purpose. It is important to note that the method's inherent quality is a result of the method development process. Method validation can only objectively document this inherent quality but cannot enhance it.

This chapter gives an overview of general aspects of analytical method development and of the state of the art in analytical method validation. Experimental designs and statistical procedures for estimation of validation parameters will be presented and discussed, using examples from the literature where appropriate. Readers not familiar with basic statistical procedures are referred to the *Handbook of Chemometrics and Qualimetrics* (Massart *et al.* 1997) for further reading.

Method development

The most appropriate strategy for the development of an analytical method depends very much on the type and purpose of the method. It is obvious that there is a big difference between the development of an assay for determining the content of a pharmaceutical substance and an assay for determining ng/mL concentrations of a drug in a complex matrix such as blood, urine or hair. A detailed presentation and discussion of the best strategies for all or even only a few different method development situations is of course far beyond this chapter. However, there are some general principles that apply to many different situations and these are discussed below.

When developing a new analytical method, it is reasonable to first consider a number of factors relating to the analytical problem to be addressed, on the one hand, and different options of approaching the problem, on the other. As illustrated in Figure 20.1, these factors will influence the design of the new analytical method. In a first step, it is important to clearly define the analytical purpose, i.e. which analyte is supposed to be determined in which matrix and whether qualitative or quantitative analysis is required. It may seem trivial to define the analyte (s) and in fact it is, if only a single analyte is to be covered. However, in many situations it will be reasonable not to look at just one compound but also to include others related to the analytical problem to be addressed. This could involve impurities in an assay for a drug in pharmaceutical product, allowing assay and purity testing in one step. In clinical and forensic toxicology, it is often reasonable to include many or all compounds from one drug class in a single analytical method. This will allow application of the same method, if the presence of a compound from a certain drug class is suspected but it is not known exactly which one in particular. Moreover, this approach saves time and resources during method validation and routine QC, because calibrators and control materials containing all analytes have to be analysed only once rather than it being necessary to use a different method for each analyte. In analysis of pharmaceutical products, the matrix is predetermined by the product to be analysed. In bioanalytical methods to be used in pharmacokinetic, bioavailability and bioequivalence studies, the analytical matrix is generally blood plasma or serum. In analytical toxicology, however, the choice of an appropriate sample matrix is pivotal for successful application of the method in routine work. For example, whole blood or serum is generally the most appropriate matrix if the extent of impairment by certain drugs is to be estimated, but they would be poorly suited for the determination of drugs supposedly taken more than a couple of days or even weeks before. In the latter case, hair analysis is generally a more appropriate solution. For toxicological screening analysis, urine is usually the matrix of choice, because large sample volumes can be obtained non-invasively, most analytes are physiologically concentrated in urine and the presence of urinary metabolites can corroborate findings of the parent drug.

Once the analytical purpose has been defined, the separation and detection systems must be chosen. In a first step, it is reasonable to check whether one of the analytical methods already established in the laboratory can be adapted for the analysis of a new analyte with minor or major modifications. If this is not the case, the value of a thorough literature search can hardly be overestimated. Often, analytical methods for the same or structurally very similar analytes can be found in the literature. If identical analytical equipment is available, one of these methods may theoretically be used, but this is hardly ever the case in practice, at least in the author's experience. Nevertheless, methods from the literature are generally a good starting point for method development and often they can be established with a few modifications of equipment and analytical parameters. A point that should be considered when developing new methods is that there should always be a balance between the difficulty of an analytical problem and the equipment used. Expensive high-end equipment should be used only if required by the analytical problem.

When starting development of the separation system, a high analyte concentration may be used and hence highly sensitive detectors may not be needed at this stage. For example, separation systems for a liquid chromatography–(tandem) mass spectrometry (LC-MS)(-MS) method may well be developed on a high performance liquid chromatography (HPLC) system with UV detection, provided that the mobile phase buffers used are compatible with a later transfer to LC-MS(-MS). Once separation of the analyte(s) has been achieved, analyte concentrations corresponding to those expected in samples or extracts analysed in the final method should be used. If these can be detected with a simple low-cost detector, and the selectivity or identification power of the latter is sufficient for the intended analytical purpose, the development of sample preparation can continue. If not, the separation system should be coupled with a more sensitive/selective detector. After separation and detection have been established, the penultimate step of method development is sample preparation. This may involve pre-extraction steps such a homogenisation of tissue samples in postmortem toxicology or

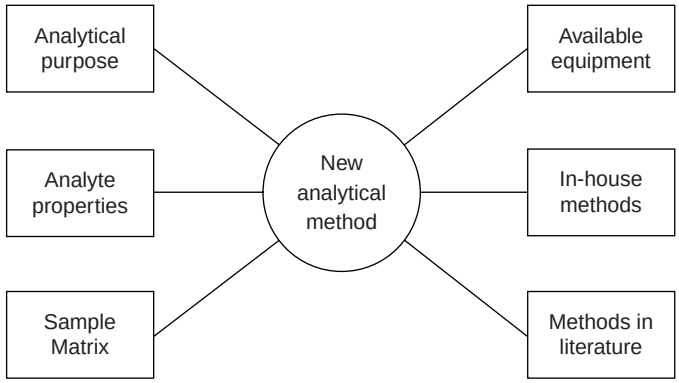

Figure 20.1 Factors to be considered in the development of a new analytical method.

cleavage of glucuronide metabolites present in urine samples. With respect to extraction, the most appropriate technique very much depends on the method's purpose. If a method is developed for systematic toxicological analysis, i.e. if it is supposed to cover a large number of compounds with a wide spectrum of physicochemical properties, then it is important to use a non-selective extraction technique, because part of the analytes might otherwise be lost during workup. If the method is developed for the analysis of a particular compound, or a class of compounds with similar physicochemical properties, differences between these properties and those of the matrix compounds may be exploited to achieve clean sample extracts. For example, basic drugs may be effectively isolated from blood plasma by solid-phase extraction (SPE) with a cation-exchange sorbent retaining the analytes while most matrix compounds can be washed from the extraction cartridges. Thereafter, the cleaned-up analytes can be eluted to yield very clean extracts.

The last step of method development is optimisation of the method. It may be necessary to modify chromatography to separate an analyte from a co-eluting matrix compound. Also, it may become necessary to increase the sensitivity of detection or the sample volume used for extraction to compensate for analyte loss during sample workup. The optimisation process should be continued until the results of the optimisation experiments strongly indicate that the final method is applicable for the intended purpose. Then, and only then, the final method should be taken to method validation.

Method validation

Analytical method validation is a process in which a series of validation experiments are performed and (statistically) evaluated to objectively demonstrate the method's applicability for the intended purpose. Since the type and extent of validation experiments depend very much on the purpose of the method, various guidance documents specifically addressing analytical method validation in different fields of analysis have been issued by various organisations.

Guidelines and literature on validation

The most widely accepted guidance documents on validation of methods in pharmaceutical analysis of drugs, drug preparations or drug products (henceforth called pharmaceutical analysis) were developed by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and approved by the regulatory agencies of the European Union, the United States of America and Japan. The first, approved in 1994, concentrated on the theoretical background and definitions (ICH Topic Q2A 1994). The second, approved in 1996, on methodology and practical issues, concerned validation of methods used to acquire data for drug approval submissions (ICH Topic Q2B 1996). In 2005, the second document was incorporated into the first, resulting in the current guidance document Q2(R1) *Validation of Analytical Procedures: Text and methodology* (ICH Topic Q2(R1) 2005), which addresses validation of the following types of analytical methods: identification tests, quantitative tests for impurities content, limit tests for the control of impurities, and quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. It can be downloaded from the ICH homepage free of charge (www.ich.org). Very helpful guidance on the validation of high performance liquid chromatography methods for analysis of pharmaceutical products can also be found in a review article by Epshtein (2004).

The validation of bioanalytical methods for bioavailability, bioequivalence and pharmacokinetic studies (henceforth called pharmacokinetic studies) was extensively discussed by experts from the pharmaceutical industry and regulatory authorities during a conference on 'Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies' held in Washington DC in 1990. The consensus of this conference was summarised in a report by Shah *et al.* (1991) (Conference Report I), which soon became one of the most important guidance papers in the field of drug analysis in biological matrices. Ten years later, the experience and progress since the first conference were discussed in the first follow-up workshop 'Bioanalytical Method Validation – A Revisit with a Decade of Progress', the results of which were published in a report by (Shah *et al.* 2000) (Conference Report II). Like the previous document, this report set the standard for bioanalytical method validation in the area of drug registration studies; its impact is underlined by the fact that it was used as a template for the respective regulatory guidance for the pharmaceutical industry published by the US Food and Drug Administration (FDA) that is still valid today (FDA 2001). In 2007, the second follow-up workshop 'Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays' was held. This workshop focused mainly on ligand-binding assays used in the analysis of macromolecular drugs, but also dealt with topics relevant to validation of analytical methods for analysis of low-molecular-weight drugs and poisons. Analysis of incurred samples (biological samples from persons or animals after the administration of a drug), carryover issues, and the evaluation of matrix effects in the validation of methods employing LC-MS(-MS) were the most important topics in this respect. The outcome of this workshop was reported by Viswanathan *et al.* (2007) (Conference Report III) and it can be expected that this paper will have as much impact as the two previous documents. In an excellent review on the validation of bioanalytical chromatographic methods, Hartmann *et al.* provided not only a detailed overview and critical discussion of Conference Report I and related papers, but also helpful information on the design of validation experiments and statistical analysis of the analytical results (Hartmann *et al.* 1998).

In the field of analytical toxicology, no widely accepted validation guideline currently exists. Specific guidance documents on the validation of analytical methods have been published by the Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) (Peters *et al.* 2009) and by the Société Française de Toxicologie Analytique (SFTA) (Nicolas *et al.* 2004), but their widespread use is hampered by the fact that they are currently available only in German and French, respectively. Besides these validation guides, The International Association of Forensic Toxicologists (TIAFT) (De Zeeuw *et al.* 1994) and the Society of Forensic Toxicology/American Academy of Forensic Sciences (SOFT/AAFS) (SOFT/AAFS Guidelines Committee 2006) have published laboratory guidelines aimed at ensuring the high quality of routine toxicological analyses. Both of these documents acknowledge the need for method validation, but apart from listing the parameters that should be evaluated they contain only little or no specific information or acceptance criteria that might be used as guidance in a validation study. Owing to this lack of widely accepted guidance documents for the validation of bioanalytical methods in analytical toxicology and because of the close relationship to bioanalysis in the context of bioavailability, bioequivalence and pharmacokinetic studies, many toxicologists have validated their bioanalytical methods according to the recommendations of Conference Reports I and II (Shah *et al.* 1991, 2000), which have

also clearly influenced the guidance documents of the GTFCh (Peters *et al.* 2009) and SFTA (Nicolas *et al.* 2004). Specific aspects of method validation in forensic and clinical toxicology have also been covered in two review articles and a book chapter by Peters and colleagues (Peters, Maurer 2002; Peters 2006; Peters *et al.* 2007a).

In addition to the above-mentioned documents and reviews, several other guidance papers on analytical methods have been published by various organisations. Although these documents do not have a particular focus on the analysis of drugs and poisons, they are worth considering in the context of analytical method validation. The EURACHEM guide *The Fitness for Purpose of Analytical Methods* published in 1998 (EURACHEM/CITAC 1998) provides definitions of validation parameters as well as useful practical guidance on performance and evaluation of validation experiments. It is available free of charge on the EURACHEM website (www.eurachem.org). More recently, the International Union of Pure and Applied Chemistry (IUPAC), the International Organization for Standardization (ISO) and the Association of Official Analytical Chemists (AOAC) developed a *Harmonized Guideline for Single-Laboratory Validation of Methods of Analysis* (Harmonized Guide) (Thompson *et al.* 2002). It provides guidance on principles of method validation, but practical aspects such as experimental designs are not included. Analytical method validation in general has been focused on two further review articles. One, by Taverniers *et al.* (2004), gives an excellent overview on the position of analytical method validation in the greater context of QC, accreditation and proficiency testing. This paper includes a table listing definitions, expressions (calculations), requirements for acceptance and practical assessment (experimental design) for the most important validation parameters. The other focuses on validation of qualitative analytical methods (Trullols *et al.* 2004).

A common drawback of many validation guidelines is that they often address general concepts and acceptance criteria rather than providing detailed guidance on how to perform and evaluate validation experiments. In order to overcome this problem, the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) has issued guidance documents specifically addressing experimental designs and statistical concepts in establishing quantitative parameters in analytical method validation (Hubert *et al.* 2003, 2006). Most importantly, it proposes combining bias and precision into so-called accuracy profiles as opposed to their separate consideration (see below). The concept described is not limited to a particular field of analytical chemistry but can rather be seen as an integrative approach that is in line with the majority of the above-mentioned validation guidelines. Hence, it is a valuable tool for all analysts looking for practical and statistically sound guidance on how to design and evaluate their validation experiments. The document itself is available only in French, but the concepts have been described and discussed extensively in English in several articles that may be used as guidance by analysts not speaking French (Boulanger *et al.* 2003; Hubert *et al.* 2004, 2007a,b; Rozet *et al.* 2007).

Validation parameters

Which validation parameters have to be evaluated depends on the purpose of the analytical method. The validation parameters to be evaluated for different types of analytical methods in pharmaceutical analysis according to ICH (ICH Topic Q2(R1) 2005) are reported in Table 20.1.

Bioanalytical methods in drugs analysis are generally used for identification and/or quantification of drugs, poisons and/or their metabolites in biological fluids or tissues. For quantitative bioanalytical procedures, there is general agreement that at least the following validation parameters should be evaluated: selectivity, calibration model (linearity), stability, accuracy (bias and precision) and the lower limit of quantification (LLOQ). Additional parameters that might have to be evaluated include the limit of detection (LOD), recovery, reproducibility and ruggedness (robustness) (Shah *et al.* 1991, 2000; De Zeeuw *et al.* 1994; Hartmann *et al.* 1998; Peters *et al.* 2007a, 2009; Viswanathan *et al.* 2007).

Table 20.1 Parameters to be evaluated depending on the type of analytical procedure according to the ICH (ICH Topic Q2(R1) 2005)

Parameter	*Type of analytical procedure*			
	Identification	*Testing for impurities*		*Assay (dissolution, content)*
		Quantitative	*Limit test*	
Bias	−	+	−	+
Precision				
Repeatability	−	+	−	+
Intermediate precision	−	+	−	+
Selectivity/specificity	+	+	+	+
Limit of detection (LOD)	−	−	+	−
Lower limit of quantification (LLOQ)	−	−[(a)]	−	−
Linearity	−	+	−	+
Range	−	+	−	+

[(a)]May be needed in some cases.

Qualitative bioanalytical methods are often used in forensic and clinical toxicology, especially for so-called systematic toxicological analysis. A general validation guideline is currently not available for such qualitative procedures (Trullols *et al.* 2004). However, there seems to be agreement that at least selectivity and the LOD should be evaluated and that additional parameters such as precision, recovery and ruggedness (robustness) might also be important (Jimenez *et al.* 2002; Rivier 2003; Trullols *et al.* 2004).

Nowadays, LC-MS(-MS) is by far the most widely employed technique for the quantitative analysis of drugs and/or their metabolites in the context of pharmacokinetic studies. It is also of tremendous and still increasing importance in TDM as well as forensic and clinical toxicology. The most important drawback of this analytical technique is the problem of so-called matrix effects, i.e. ion suppression or enhancement, which must therefore be adequately covered in the validation of any LC-MS(-MS) method, particularly of those employing electrospray ionisation (ESI) (Shah *et al.* 2000; Annesley 2003; Matuszewski *et al.* 2003; Maurer 2005; Taylor 2005; Viswanathan *et al.* 2007).

Before moving on to detailed discussions of the various validation parameters, it must be pointed out that in the literature on method validation different sets of terminology have been employed by different guidelines and authors. This may lead to confusion and misunderstandings. A detailed discussion of this topic is beyond the scope of this chapter but can be found in the review of Hartmann *et al.* (1998).

Selectivity (specificity)

In Conference Report II (Shah *et al.* 2000), selectivity was defined as 'the ability of the bioanalytical method to measure unequivocally and to differentiate the analyte(s) in the presence of components which may be expected to be present. Typically, these might include metabolites, impurities, degradants, matrix components, etc.'. This definition is very similar to that used in the ICH document (ICH Topic Q2(R1) 2005), although that used the term specificity rather than selectivity. At this point it should be noted that specificity is often used interchangeably with selectivity, although in a strict sense specificity refers to methods that produce a response for a single analyte, whereas selectivity refers to methods that produce responses for a number of chemical entities, which may or may not be distinguished (Karnes *et al.* 1991). Selective multi-analyte methods (e.g. for the analysis of different drugs in blood) should of course be able to differentiate all interesting analytes from each other and from the matrix. One approach to establishing method selectivity is to prove the lack of response in blank matrices, i.e. that there are no signals interfering with the signal of the analyte(s) or the internal

standard (IS) (Shah *et al.* 1991, 2000; Hartmann *et al.* 1998; Taverniers *et al.* 2004; Peters *et al.* 2007a). The second approach is based on the assumption that, for merely quantitative procedures, small interferences can be accepted as long as accuracy (bias) and precision at the LLOQ remain within certain acceptance limits (EURACHEM/CITAC 1998; Hartmann *et al.* 1998; Thompson *et al.* 2002; Taverniers *et al.* 2004). This is usually the case in pharmaceutical analysis, TDM or pharmacokinetic studies. However, in forensic and clinical toxicology, analysis is often mainly performed to prove the intake of an (illicit) substance and qualitative data are therefore also important. Here, the approach to proving selectivity by absence of interfering signals seems much more reasonable (Peters, Maurer 2002; Peters *et al.* 2007a).

Establishing selectivity by demonstrating absence of interfering signals The selectivity experiments should be performed very early in the validation study, when it is still possible to switch back to the development phase without having spent a lot of time and expense on further validation experiments.

In pharmaceutical analysis, demonstration of the absence of interfering signals can be used to establish selectivity of identification and impurity tests (Epshtein 2004; ICH Topic Q2(R1) 2005). The aim is to demonstrate that other compounds likely to be present in the samples to be analysed do not interfere with the compound of interest. If the potentially interfering compounds (impurities and/or excipients) are available, appropriate concentrations of the compounds of interest should be analysed. Sufficient separation of the latter from the spiked impurities/excipients demonstrates sufficient selectivity/specificity. If potentially interfering impurities are not available, samples stored under stress conditions, i.e. exposure to light, heat, humidity, acid/base hydrolysis and oxidation, should be included in the selectivity studies. Such samples might be expected to contain at least a certain amount of impurities that may be formed during storage of real samples and hence may be used to study impurity profiles and their potential interference with the analytes of interest (Epshtein 2004; ICH Topic Q2(R1) 2005).

In bioanalysis, absence of interference from other compounds is much more complex, because not only endogenous matrix compounds but also co-ingested exogenous compounds and/or their metabolites may interfere with the analyte(s) of interest. The requirement established by Conference Report I (Shah *et al.* 1991) to analyse at least six different sources of blank matrices has become state of the art during the last decade and this number has been used in the validation of many published bioanalytical methods. However, Hartmann *et al.* (1998) stated from statistical considerations that, with analysis of such a small number of matrix blanks, relatively rare interferences will remain undetected with a rather high probability. For the same reason, Dadgar and colleagues (Dadgar, Burnett 1995) proposed the evaluation of at least 10–20 sources of blank samples, which seems to be a good compromise between reducing the workload during method validation and lowering the risk of unexpected interferences during routine application. In Conference Report II (Shah *et al.* 2000), however, the required number of matrix sources was even reduced to a single source for methods using hyphenated mass spectrometric methods such as LC-MS(-MS) for detection. This confinement does not seem reasonable for toxicological applications because of the great importance of selectivity in this field. Furthermore, it increases the risk of falsely assuming sufficient selectivity of the tested procedure. Some working groups, including the author's, have taken such considerations into account and checked matrix samples from at least 10 or even 20 sources for absence of interfering matrix peaks (Peters *et al.* 2003, 2007b; Pichini *et al.* 2003; Sottani *et al.* 2003; Kratzsch *et al.* 2004; Maurer *et al.* 2004).

In contrast to samples from pharmacokinetic studies, where usually only a single drug or a very limited number of drugs are administered under controlled conditions, samples from forensic or clinical toxicology cases often contain many different drugs, poisons and/or their metabolites. In this field, it is therefore also important to check for possible interferences from other xenobiotics that may be expected to be present in authentic samples (Peters, Maurer 2002). This can be accomplished by analysing blank samples spiked with possibly interfering compounds at their highest expectable concentrations. The number and type of compounds to be used in such spiking experiments depend on the purpose of the procedure. For example, Pichini *et al.* (2003) checked interference from various amfetamines, cannabinoids, benzodiazepines and antidepressants (altogether 23 compounds) during the selectivity experiments of an assay for the determination of opiates and cocaine in meconium. Crommentuyn *et al.* (2004) studied possible interference from over 30 often coadministered drugs in an assay for atazanavir and tipranavir, two human immunodeficiency virus (HIV) protease inhibitors used in the treatment of acquired immune deficiency syndrome (AIDS). At first sight, this may look exaggerated, but it is not, considering that the treatment of AIDS usually involves at least three different anti-HIV drugs and coadministration of further drugs is the rule rather than the exception. In addition, spiking mixtures of possibly interfering compounds rather than single compounds allows effective simultaneous investigation of interference from many compounds, while the workload is kept at a minimum.

Another way to exclude interference from other drugs or their metabolites is to check authentic samples containing these, but not the analyte of interest. This approach is preferable if the possibly interfering substance is known to be extensively metabolised as it also allows for exclusion of interferences from such metabolites, which are usually not available as pure substances. An example can be found in Streit *et al.* (2004), which describes an assay for the immunosuppressant mycophenolic acid. Here, the authors used 30 samples from transplant recipients not treated with mycophenolic acid but treated with a number of other immunosuppressive drugs and other drugs commonly prescribed in transplant recipients.

It must be noted that metabolites of the target analyte can also be an important source of interference. This is of particular relevance for methods employing less selective detectors such as ultraviolet/visual wavelength (UV/VIS) or diode array detection (DAD), because metabolites with an unchanged chromophore often have spectral properties very close to those of the parent drug. However, interference from metabolites may also occur in highly selective LC-MS(-MS) analysis, because metabolites that are in principle amenable to this type of analysis, such as glucuronides or *N*-oxides, can convert back to the parent drug by in-source collision-induced dissociation (CID) and thus lead to cross-talk in the channel of the parent drug (Ackermann *et al.* 2002; Jemal *et al.* 2002; Sun, Naidong 2003; Zhang *et al.* 2004). Similarly, labile prodrugs may be converted to the active metabolite, in this case the target analyte, by in-source CID and lead to cross-talk in the metabolite channel. As such phenomena occur only when there is insufficient separation between the respective compounds, they can be avoided by optimising the chromatographic conditions. However, this type of interference must be detected in the first place to be avoided. If the metabolite is available, this can be achieved by spiking experiments as described above. If not, the only way to check for this type of interference is by the analysis of incurred samples, i.e. samples from persons (or animals) who have ingested the parent drug (Jemal *et al.* 2002; Sun, Naidong 2003). A useful strategy for performance of such studies is presented in Jemal *et al.* (2002).

Stable-isotope-labelled analogues of the target analytes are often used as the internal standard (IS) in MS-based methods. Owing to their similar physicochemical properties, they can ideally compensate for variability during sample preparation and measurement but can still be differentiated from the target analyte by mass spectrometric detection. However, isotopically labelled compounds may contain the non-labelled compound as an impurity, or their mass spectra may sometimes contain fragment ions with the same mass-to-charge ratios (m/z) as the monitored ions of the target analyte. In both cases, the peak area of the analyte peak would be overestimated, thus compromising quantification. The absence of such interference caused by the IS can be checked by analysing so-called zero samples, i.e. blank samples spiked with the IS, as exemplified in Janda *et al.* (2002), Peters *et al.* (2003), de Jonge *et al.* (2004), Kratzsch *et al.* (2004) and Zhang *et al.* (2004). The importance of this is underlined by the findings of Janda *et al.* (2002), who found a low signal of non-labelled ethyl glucuronide in a blank hair sample spiked only with ethyl glucuronide-d_5, most probably caused by traces of the non-labelled drug in the stable-isotope-labelled standard. These authors

stated that it may be useful to reduce the total amount of IS to eliminate this interference.

In a similar way to that described above, the analyte might interfere with a stable-isotope-labelled IS. This can become a problem even with deuterated analogues when the number of deuterium atoms of the analogue or one of its monitored fragments is three or less (Bogusz 1997). Blank samples spiked with the analyte at the upper limit of the calibration range, but without IS, can be used to check for absence of such interferences (Zhang *et al.* 2004).

Establishing selectivity by acceptable bias and precision data In the ICH guideline (ICH Topic Q2(R1) 2005), this approach is recommended for assay and impurity tests. If potentially interfering compounds are available, the compound of interest can be analysed in the presence and absence of appropriate concentrations of the potentially interfering compounds and the results can be compared. If they do not show relevant differences, the analytical method can be considered sufficiently selective. If potentially interfering compounds for such spiking experiments are not available, the quantitative results obtained with the method to be validated can be compared with results obtained with an established validated method, e.g. from a pharmacopoeia. Such studies should also include samples previously stored under stress conditions (see previous section). If the results obtained with the method to be validated are in good agreement with those of the alternative method, sufficient selectivity can be assumed.

In bioanalysis, the approach of establishing selectivity by acceptable bias and precision was preferred by Dadgar and Burnett (1995) and Hartmann *et al.* (1998). These authors proposed the analysis of up to 20 different blank samples spiked with analyte at the LLOQ and with possibly interfering compounds at their highest likely concentrations, if available. In this approach, the method can be considered sufficiently selective if precision and accuracy (bias) data for these LLOQ samples are acceptable. For a detailed account of experimental designs and statistical methods for establishing selectivity, see Dadgar and Burnett (1995).

An important disadvantage of this approach is that it requires quantification and can therefore be performed only in the main validation phase. If the selectivity of the method is found to be insufficient at this late stage of validation, the method needs further development and validation experiments may have to be repeated. In their spiking experiments discussed above Crommentuyn *et al.* (2004) used an intermediate approach and defined the maximum acceptable area of interfering peaks to be 20% of the peak area of the analyte at the LLOQ. This semiquantitative approach can be used in the early phases of validation, because it requires no calibration or precision estimate.

Calibration model (linearity)

The choice of an appropriate calibration model is necessary for reliable quantification. Therefore, the relationship between the concentration of analyte in the sample and the corresponding response (in bioanalytical methods mostly the area ratio of analyte versus IS) must be investigated. The range of the calibration curve depends on the purpose of the analytical method. In pharmaceutical analysis, the ranges should be: 80–120% of the test concentration in the analysis of drugs or drug products; from 70% to 130% for methods to be used in content uniformity testing; ±20% of the specified range for methods to be used in dissolution testing of drug products; and from the reporting level to 120% of the specification in quantitative impurity testing (ICH Topic Q2(R1) 2005). In bioanalysis, the calibration range should cover the majority of concentrations to be expected in the samples to be analysed. This might be the concentration range expected after a certain dosage in pharmacokinetic studies, the therapeutic concentration range of the respective drug in TDM, or even wider ranges for methods to be used for quantitative analysis in clinical or forensic toxicology.

The calibration curve can be established by analysing (spiked) calibration samples and plotting the resulting responses versus the corresponding concentrations. A plot of an exemplary dataset and the corresponding calibration line obtained from simple linear regression is shown in Figure 20.2a. The resulting standard curves can then be further evaluated by graphical methods such as residual plots or by mathematical methods. The latter also allow statistical evaluation of the response functions. Residuals are the deviations of the observed values from the values predicted by the applied calibration model. They can be calculated according to equation (20.1). In residual plots, these residuals are plotted against the respective concentrations, which ideally results in a random distribution of residuals around zero. A residual plot corresponding to the calibration plot in Figure 20.2a is shown in Figure 20.2b. The plotted residuals are clearly not ideally distributed; the reasons for this phenomenon will be discussed below.

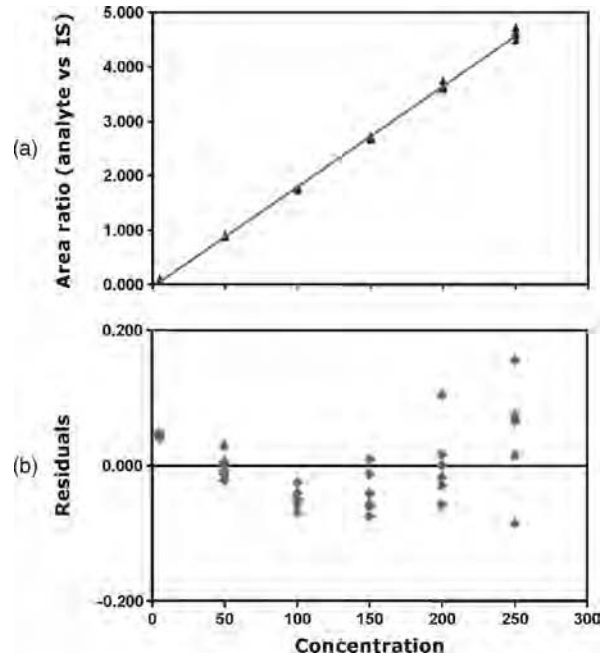

Figure 20.2 Regression plot of an exemplary data set with calibration curve calculated using simple linear regression (a) and the corresponding residual plot (b).

$$e_{ij} = y_{ij} - \widehat{y_j} \tag{20.1}$$

where e_{ij} = the ith residual at the jth concentration level; y_{ij} = the ith observed value at the jth concentration level; $\widehat{y_j}$ = the predicted value at the jth concentration level.

There is general agreement that, for bioanalytical methods, calibrators should be matrix-based – i.e. prepared by spiking the blank matrix – and that calibrator concentrations must cover the whole calibration range (Dadgar *et al.* 1995; Hartmann *et al.* 1998; Lindner, Wainer 1998; Shah *et al.* 2000; Peters *et al.* 2007a). However, recommendations on how many concentrations should be studied with how many replicates per concentration differ significantly in the literature on analytical method validation (Dadgar *et al.* 1995; EURACHEM/CITAC 1998; Hartmann *et al.* 1998; Lindner, Wainer 1998; Shah *et al.* 2000; Thompson *et al.* 2002; Taverniers *et al.* 2004; Peters *et al.* 2007a). In Conference Report II (Shah *et al.* 2000), 'a sufficient number of standards to define adequately the relationship between concentration and response' was demanded. Furthermore, it was stated that at least five to eight concentrations should be studied for linear and maybe more for non-linear relationships. However, no information was given on how many replicates should be analysed at each level. The guidelines established by the ICH (ICH Topic Q2(R1) 2005) also required at least five concentrations, but again no specific requirements for the number of replicates at each level were given. In the validation guide by EURACHEM (EURACHEM/CITAC 1998) and in the Harmonized Guide (Thompson *et al.* 2002), at least six concentrations are stipulated. The latter has further specified that the concentrations should be evenly spaced over the concentration range and that at least two and preferably three or more replicates should be

analysed at each concentration (Thompson *et al.* 2002). On the basis of studies by Penninckx *et al.* (1996), Hartmann *et al.* (1988) proposed in their review to use rather fewer concentrations with a greater number of replicates, e.g. four evenly spread concentrations with nine replicates, in order to achieve more reliable variance estimations while still having enough concentrations for a preliminary indication of possible non-linearity. These different recommendations probably explain why almost every thinkable combination of concentrations and replicates per concentration has been used for individual assays in the literature. However, two important points need to be considered. First, with a decreasing number of concentrations, the evaluation of non-linear models becomes increasingly unreliable and, thus, their evaluation requires more concentrations than for linear models (Shah *et al.* 1991, 2000; Penninckx *et al.* 1996; Hartmann *et al.* 1998). Second, assessing the behaviour of variance across the calibration range becomes increasingly difficult with a decreasing number of replicates (Penninckx *et al.* 1996).

The initial step of studying the calibration function is to check for outliers. This can first be done visually, e.g. by evaluation of residual plots. In Figure 20.2b, the highest data point of the second highest concentration level might be suspected to be an outlier. Such suspected outliers should be checked by appropriate statistical procedures such as the Grubbs test, and eliminated if found to be significant. The suspected outlier in Figure 20.2b was found to be not significant and was left in the dataset. The presence of more than two outliers in a complete data set of a calibration model experiment may indicate serious problems with the method that require further investigation (Penninckx *et al.* 1996).

The next step is to check for homogeneity of variance (homoscedasticity) over the calibration range. Again, this can first be done visually using residual plots. For example, in the residual plot in Figure 20.2b, one can clearly see that the scatter of the replicates increases with concentration, indicating inhomogeneous variances (heteroscedasticity) over the calibration range. In such cases, homoscedasticity should be checked by an appropriate statistical procedure, e.g. by a simple one-sided *F*-test between the variances at the highest and the lowest concentration levels. For a more detailed account and alternative statistical procedures, see Penninckx *et al.* (1996).

The evaluation of the behaviour of variance is very important for the choice of the correct regression model. Ordinary least-squares regression models are applicable only for homoscedastic datasets, whereas in a case of heteroscedasticity the data should mathematically be transformed or a weighted least-squares model should be applied (Penninckx *et al.* 1996; EURACHEM/CITAC 1998; Hartmann *et al.* 1998; Shah *et al.* 2001; Thompson *et al.* 2002). It should be noted that, for calibration ranges spanning more than one order of magnitude, heteroscedasticity is the rule rather than the exception. Regarding the usually wide concentration range of assays used in analytical toxicology or in the area of pharmacokinetics, it is not surprising that weighted regression models have been used in the vast majority of the validated bioanalytical assays published in the scientific literature. The weighting factors $1/x$ and $1/x^2$, i.e. the inverse of the concentration or the inverse of the squared concentration, respectively, are by far the most often used. Only few authors have used $1/y$ or $1/y^2$, i.e. the inverse of the response or the inverse of the squared response, respectively.

Theoretically, the suitability of a weighting factor can easily be checked by comparing the variances of the weighted residuals. Figure 20.3a shows the calibration line obtained by weighted linear regression with the weighting factor $1/x^2$ of the same data as in Figure 20.2. Figure 20.3b shows the corresponding plot of the weighted residuals. It can clearly be seen that the scatter of the weighted residuals is comparable at all concentration levels, indicating that the weighting factor used was appropriate. Such a finding can again be tested statistically by performing an *F*-test on the variances of the weighted residuals at the highest and lowest concentrations. However, some authors chose the weighting factor in a more practical way (Pereira *et al.* 2000; Breda *et al.* 2004; de Jonge *et al.* 2004). They evaluated alternative weighting schemes and back-calculated the concentrations of the calibrators using the respective regression models. Then they added the relative deviations of these back-calculated values from the nominal values and chose the weighting scheme as the most appropriate with which the smallest total relative deviation had been obtained.

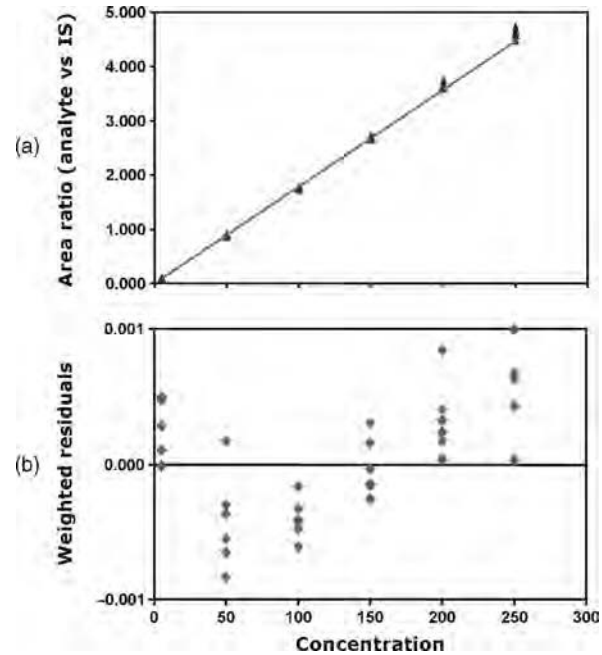

Figure 20.3 Regression plot of an exemplary data set with calibration curve calculated using a weighted ($1/x^2$) linear regression model (a) and the corresponding plot of the weighted residuals (b).

After significant outliers have been purged from the dataset and, if applicable, an appropriate weighting factor for regression has been found, a mathematical model has to be found that adequately describes the relationship between analyte concentration in the sample and response. Usually, linear models are preferable, but, if necessary, the use of non-linear models is not only acceptable but even recommended. Examples of the use of second-order polynomial (quadratic) (Chang *et al.* 1999; Jemal *et al.* 1999; Mortier *et al.* 2002b; Li *et al.* 2004) and more complicated non-linear regression models (Egge-Jacobsen *et al.* 2004) have been described in the literature. The model-fit can again first be evaluated visually using residual plots. For example, the plot of weighted residuals in Figure 20.3b corresponds to a weighted linear regression model applied to the same data as in Figure 20.2. One can see that the residuals at the lowest and the two highest concentration levels are above the zero line, while the majority of those at the intermediate concentration levels are below the zero line. Such patterns are typically obtained when linear models are fitted into curved datasets. This indicates that, in the example presented, a non-linear model might be more appropriate to describe the data. In any case, the model-fit should also be tested by appropriate statistical methods (Shah *et al.* 1991, 2000; Penninckx *et al.* 1996; Hartmann *et al.* 1998; Thompson *et al.* 2002; ICH Topic Q2(R1) 2005). For example, the fit of simple regression models (homoscedastic data) can be tested by the analysis-of-variance (ANOVA) lack-of-fit test (Penninckx *et al.* 1996; Hartmann *et al.* 1998). Testing the fit of weighted regression models is somewhat more complicated. For example, deviation from a weighted linear model can be tested by evaluation of an alternative weighted second-order regression model. If the quadratic term describing the curvature of the second-order model is significantly different from zero, non-linearity has to be assumed. Such significant non-linearity was also found for the data in Figure 20.3. The regression line of the alternative second-order model and the corresponding plot of the weighted residuals are shown in Figure 20.4a and 20.4b, respectively. It can be seen that the weighted residuals are now randomly distributed around zero, indicating that the weighted second-order model adequately describes the dataset. A detailed discussion of alternative statistical procedures for testing the model-fit of both unweighted and weighted calibration models can be found in Penninckx *et al.* (1996).

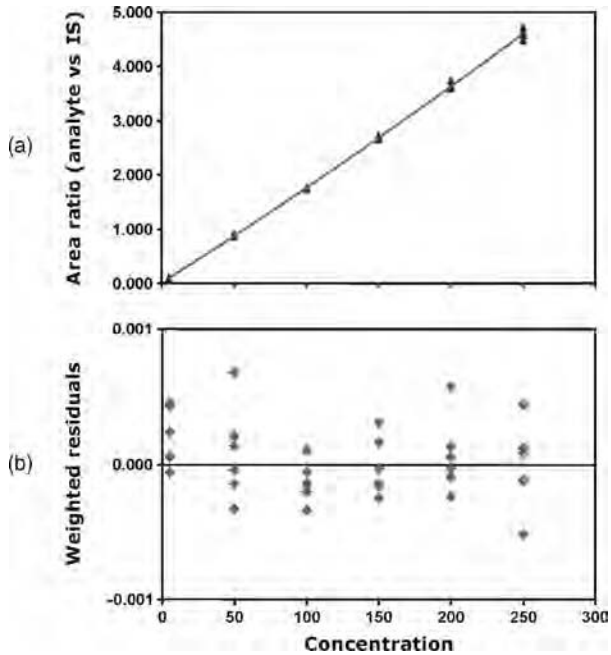

Figure 20.4 Regression plot of an exemplary data set with calibration curve calculated using a weighted ($1/x^2$) second-order regression model (a) and the corresponding plot of the weighted residuals (b).

The widespread practice of evaluating a calibration model via its coefficients of correlation or determination is not acceptable from a statistical point of view (Hartmann *et al.* 1998; Hubert *et al.* 2007b).

One important point should be kept in mind when statistically testing the model fit: the better the precision of a method, the higher the probability of detecting a statistically significant deviation from the assumed calibration model (Hartmann *et al.* 1998). Therefore, the practical relevance of the deviation from the assumed model should also be taken into account. If the accuracy (bias) and precision data or accuracy profiles are within the required acceptance limits or an alternative calibration model is not applicable, slight deviations from the assumed model may be ignored (Hartmann *et al.* 1998; Hubert *et al.* 2004, 2007a,b).

Besides the above-mentioned classic way of evaluating the calibration model, an integrated approach is suggested by the SFSTP guidance documents (Hubert *et al.* 2003, 2006). This approach is based on statistical considerations, but practical aspects such as the purpose of the method as well as the experience of the analyst are also considered. A full description is beyond the scope of the present chapter, but is available and is discussed in great detail by Hubert *et al.* (2003, 2004, 2006, 2007a, b). These references should be valuable sources of information, particularly for experienced analysts with advanced understanding of statistical concepts in method validation.

Once a calibration model has been established, the calibration curves for other validation experiments (precision, bias, stability, etc.) and for routine analysis can be prepared with fewer concentrations and fewer or no replicates (Hartmann *et al.* 1998; Hubert *et al.* 2004, 2007a,b).

Accuracy

The accuracy of a method is affected by systematic (bias) as well as random (precision) error components (Hartmann *et al.* 1994, 1998). This fact has been taken into account in the definition of accuracy as established by the ISO (1994). However, it must be mentioned that accuracy is often used to describe only the systematic error component, i.e. in the sense of bias (Karnes *et al.* 1991; Shah *et al.* 1991, 2000; ICH Topic Q2(R1) 2005).

Bias

According to the ISO, bias is 'the difference between the expectation of the test results and an accepted reference value' (ISO 1994). It may consist of more than one systematic error component. Bias can be measured as a percentage deviation from the accepted reference value. The term 'trueness' expresses the deviation of the mean value of a large series of measurements from the accepted reference value. It can be expressed in terms of bias. Owing to the high workload of analysing such large series, trueness is usually not determined during method validation, but rather from the results of a great number of QC samples during routine application or in inter-laboratory studies. It should be noted that trueness and bias are sometimes reported in terms of (analytical) recovery of an acceptance reference value (Thompson *et al.* 2002). This must not be confused with the validation parameter recovery (RE), in the sense, for example, of extraction efficiency (see below).

Precision

According to the ICH, precision is 'the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions and may be considered at three levels: repeatability, intermediate precision and reproducibility' (ICH Topic Q2(R1) 2005). Precision is usually measured in terms of imprecision expressed as an absolute or relative standard deviation (RSD) and does not relate to reference values.

Repeatability 'Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision' (ICH Topic Q2(R1) 2005). Within-run or within-day precision is also often used to describe repeatability.

Intermediate precision 'Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc.' (ICH Topic Q2(R1) 2005). The ISO definition uses the term 'M-factor different intermediate precision', where the M-factor expresses how many and which factors (time, calibration, operator, equipment or combinations of those) differ between successive determinations (ISO 1994). In a strict sense intermediate precision is the total precision under varied conditions, whereas so-called inter-assay, between-run or between-day precision measures only the precision components caused by the respective factors (see below). However, the latter terms are not clearly defined and are obviously often used interchangeably with each other and also with the term intermediate precision.

Reproducibility 'Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology)' (ICH Topic Q2(R1) 2005). Reproducibility has to be studied only if a method is supposed to be used in different laboratories.

Unfortunately, some authors also used the term reproducibility for within-laboratory studies at the level of intermediate precision (Causon 1997; Lindner, Wainer 1998). This should be avoided in order to prevent confusion.

Bias and precision experiments and calculations As already mentioned, precision and bias can be estimated from the analysis of QC samples under specified conditions. Both bias and precision can vary substantially over the calibration range. It is therefore generally recommended to evaluate these parameters at least at three concentration levels: low, medium and high relative to the calibration range (Karnes *et al.* 1991; Shah *et al.* 1991, 2002; Hartmann *et al.* 1998; Taverniers *et al.* 2004; ICH Topic Q2(R1) 2005). In Conference Report II (Shah *et al.* 2000), it was further defined that the concentration of the low QC sample must be within three times the LLOQ. Four concentration levels (LLOQ, low, medium, high) were studied in the experimental design proposed by Wieling *et al.* (1996). Owing to the often higher concentration ranges in the fields of forensic and clinical toxicology, it might be reasonable also to validate the analysis of QC samples containing concentrations above the highest calibration standard after dilution or after reduction of sample volumes, as it has been described by Dadgar and Burnett (1995). These authors also described the use of QC samples with concentrations below those of the lowest calibration standard using greater sample volumes. The Harmonized Guide (Thompson *et al.*

2002) recommends studying precision at the extremes of the working range while the EURACHEM guide (EURACHEM/CITAC 1998) recommends various concentrations across the working range without any further specification. Neither of these two guides contains any specific recommendations concerning the number of concentration levels for studying bias.

The acceptance criteria for bias and precision are very much dependent on the purpose of the method, i.e. which level of accuracy is required during routine use of the method. This is probably the reason why most validation guidelines intended only for a specific type of analytical procedure (including the ICH document (ICH Topic Q2(R1) 2005) do not contain specific acceptance criteria for these two parameters. However, acceptance limits for bias and precision in bioanalysis of drugs were specified in the Conference Reports (Shah *et al.* 1991, 2002; Viswanathan *et al.* 2007) and have been widely accepted throughout the field of bioanalytical analysis of drugs, poisons and/or their metabolites. In these documents, bias was required to be within ±15% of the accepted true value, except at the LLOQ where ±20% was accepted. Precision was required to be within 15% RSD except at the LLOQ where 20% RSD was accepted. These requirements have been subject to criticism in the analysis of Conference Report I by Hartmann *et al.* (1994). These authors concluded from statistical considerations that it is not realistic to apply the same acceptance criteria at different levels of precision (repeatability, reproducibility) because RSD under reproducibility conditions is usually considerably greater than under repeatability conditions. Furthermore, if precision and bias estimates are close to the acceptance limits, the probability of rejecting an actually acceptable method (β-error) is quite high. These concerns were shared by the expert group of the SFSTP and were one of the main reasons for introducing the concepts of accuracy profiles in the respective guidance documents (Hubert *et al.* 2003, 2004, 2006, 2007a,b) (see below).

Again, the proposals on how many replicates at each concentration should be analysed vary considerably. In pharmaceutical analysis, analysis of triplicates may be acceptable (ICH Topic Q2(R1) 2005), while the Conference Reports (Shah *et al.* 1991, 2000) required at least five replicates at each concentration level. However, one would assume that these requirements apply only to repeatability studies; at least no specific recommendations were given for studies of intermediate precision or reproducibility.

The EURACHEM guide (EURACHEM/CITAC 1998) recommends performing for each concentration level 10 independent determinations under each repeatability, M-factor- different intermediate precision, and reproducibility conditions. In this straightforward approach, the corresponding precision data can simply be calculated as RSDs of the values obtained under these stipulated conditions. Examples of this approach can be found in Tracqui *et al.* (1996), Mortier *et al.* (2002b), He *et al.* (2004) and Nordgren and Beck (2004).

Other authors have proposed an approach whereby replicates are analysed on a number of different occasions (e.g. different runs, days) (Wieling *et al.* 1996; Hartmann *et al.* 1998; Thompson *et al.* 2002; Hubert *et al.* 2003, 2006, 2007a). In their experimental design, Wieling *et al.* (1996) analysed three replicates at each of four concentrations on each of 5 days. Similar approaches were suggested by Causon (1997) (six replicates at each of four concentrations on each of four occasions), Hartmann *et al.* (1998) (two replicates at each concentration level on each of 8 days), and the Harmonized Guide (Thompson *et al.* 2002) (duplicates in a number of successive runs). The SFSTP documents do not recommend a fixed number of days and replicates, but rather recommend variable numbers of days and replicates depending on the results of preliminary bias and precision estimates as derived from the pre-validation used to determine the calibration model (Hubert *et al.* 2003, 2004, 2006, 2007a,b).

Using one-way ANOVA with the varied factor (e.g. day) as the grouping variable, such experimental designs allow separate calculation of repeatability and of the precision component caused by the grouping variable from the same datasets, as well as the calculation of factor-different intermediate precision as the combination of the previous two (Krouwer, Rabinowitz 1984; Massart *et al.* 1997; NCCLS 1999). Thus, repeatability, expressed as RSD in per cent, can be calculated from equation (20.2):

$$\mathrm{RSD_r}[\%] = \frac{\sqrt{\mathrm{MS_{wg}}}}{\overline{X}} \times 100 \qquad (20.2)$$

where $\mathrm{RSD_r}$ = repeatability, within-group precision, expressed as percentage RSD; $\mathrm{MS_{wg}}$ = mean square within groups, obtained from ANOVA table; $\overline{X}$ = grand mean of all observations.

The precision component caused by the varied factor or grouping variable corresponds to the standard deviation (SD) of the group means after subtraction of the contribution from within-group variability. Expressed as RSD, this precision component can be calculated from equation (20.3). From a strictly statistical point of view, this is the between-group precision (component). Unfortunately, this term is often used for total precision or factor-different intermediate precision, which is not correct.

$$\mathrm{RSD_{bg}}[\%] = \frac{\sqrt{\frac{\mathrm{MS_{bg}} - \mathrm{MS_{wg}}}{n}}}{\overline{X}} \times 100 \qquad (20.3)$$

(set $\mathrm{RSD_{bg}} = 0$ if $\mathrm{MS_{bg}} < \mathrm{MS_{wg}}$)

where $\mathrm{RSD_{bg}}$ = between-group precision (component), expressed as percentage RSD; $\mathrm{MS_{bg}}$ = mean square between groups, obtained from ANOVA table; $\mathrm{MS_{wg}}$ = mean square within groups, obtained from ANOVA table; n = number of observations in each group; $\overline{X}$ = grand mean of all observations.

The factor-different intermediate precision or total precision can be calculated according to equation (20.4) as a combination of within- and between-group effects:

$$\mathrm{RSD_{I(F)}}[\%] = \frac{\sqrt{\frac{\mathrm{MS_{bg}} + (n-1)\mathrm{MS_{wg}}}{n}}}{\overline{X}} \times 100 \qquad (20.4)$$

where $\mathrm{RSD_{I(F)}}$ = factor-different intermediate precision, expressed as percentage RSD and other symbols are as before.

This value corresponds to the intermediate precision estimate obtained with the previously mentioned alternative design proposed by EURACHEM (EURACHEM/CITAC 1998). However, in the author's opinion, the ANOVA approach should be preferred, because more information can be derived from this approach with a comparable number of analyses. For example, in case of unacceptably high intermediate precision values, the ANOVA approach allows easy comparison of the precision components. This can be very helpful in finding the cause of the problem.

The ANOVA approach has been used in many publications, e.g. Bennett *et al.* (1997), Yao *et al.* (1998), Jemal *et al.* (2003), Peters *et al.* (2003, 2007b), Xue *et al.* (2003, 2004), Kratzsch *et al.* (2004). In the majority of these publications, four to six replicates were analysed on 3–4 days, i.e. a total number of 15–20 samples (Bennett *et al.* 1997; Yao *et al.* 1998; Jemal *et al.* 2003; Xue *et al.* 2003, 2004). With such designs, repeatability is estimated with quite a large number of degrees of freedom (12–16), while there are only a few degrees of freedom for the between-day component (2–3), making its estimation quite unreliable. In the designs proposed by Hartmann *et al.* (1998) and in the Harmonized Guide (Thompson *et al.* 2002), the degrees of freedom for both estimations are more balanced, e.g. 8 for repeatability and 7 for the between-day component in case of the Hartmann design. For this reason the latter design is preferred by the author's (former) working group (Kratzsch *et al.* 2003, 2004; Maurer *et al.* 2004). As already mentioned, optimum numbers of days and replicates are recommended for certain combinations of expected bias and precision in references (Hubert *et al.* 2003, 2004, 2006, 2007a,b).

Bias experiments usually include a number of replicate measurements from which a mean value is calculated. The EURACHEM guide

(EURACHEM/CITAC 1998) recommended 10 replicates. The mean value is then compared with a certain reference value, e.g. the target value of a certified reference material, the results obtained for the same sample using a reference method, or the theoretical concentration of a spiked sample (EURACHEM/CITAC 1998; Shah *et al.* 2000; Thompson *et al.* 2002; Taverniers *et al.* 2004; ICH Topic Q2(R1) 2005). Bias is then usually calculated as the percentage deviation of the observed mean value from the respective reference value according to equation (20.5).

$$\text{Bias}[\%] = \frac{\overline{X} - \mu}{\mu} \times 100 \qquad (20.5)$$

where $\overline{X}$ = observed mean value; μ = accepted reference value.

Bias experiments can be carried out together with the precision experiments, i.e. the same results are used for the calculation of both. In the approach using separate experiments for the evaluation of precision under repeatability and intermediate precision conditions, this will yield two bias values, one from the mean value of the repeatability experiment (within-day or within-run bias), and one from the mean of the factor- different intermediate precision experiment (between-day or between-run bias). Using the ANOVA approach, usually only a single bias value is obtained from the grand mean of all observations.

It is important to note that daily variations of the calibration curve can influence bias estimation. Therefore, bias estimations should be based on data calculated from several calibration curves (Hartmann *et al.* 1998). In the experimental design of Wieling *et al.* (1996), the results for QC samples were calculated via daily calibration curves. Therefore, the overall means from these results at the different concentration levels reliably reflect the average bias of the method at the corresponding concentration level.

The concept of accuracy profiles An alternative approach to separate evaluation of bias and precision, the concept of accuracy profiles, has been proposed by the SFSTP (Hubert *et al.* 2003, 2006). A detailed discussion of this concept is beyond the scope of this chapter, where only the most important aspects can be addressed, but it is available in references (Hubert *et al.* 2004, 2007a,b). On the basis of the argument that for individual measurements the total error (i.e. the combination of systematic and random error) is more important than the two error components, the SFSTP document proposes using acceptance intervals for accuracy, i.e. the combination of bias and precision, rather than separate acceptance criteria for the two error components. Consequently, a larger bias can be partly compensated by good precision and vice versa. The combined error at each concentration level can be expressed as confidence intervals calculated from the respective bias and intermediate precision data. Plotting these confidence intervals against the respective concentrations, the so-called accuracy profile is obtained. If the entire accuracy profile lies within the predefined acceptance interval, the method can be considered sufficiently accurate. A major advantage of this approach is that accuracy profiles provide at-a-glance information about the method's performance with respect to the expected accuracy of individual measurements. However, it must be noted that, in the accuracy profile approach, the above-mentioned acceptance criteria of $\pm$15% for bias and 15% cofficient of variation for intermediate precision would correspond to an acceptance interval of approximately 45% for the accuracy profile. The GTFCh document (Peters *et al.* 2009) also recommends the use of accuracy profiles with acceptance intervals of $\pm$30% ($\pm$40% near the LLOQ).

Incurred sample reanalysis In bioanalysis, standard and QC samples prepared by spiking blank matrix are not necessarily representative of the samples from dosed subjects (incurred samples) that are analysed during routine application of the analytical method. For example, re-conversion of metabolites to the parent compound, different protein binding in samples from patients with certain diseases, etc. may influence the results. In Conference Report III (Viswanathan *et al.* 2007), it was therefore recommended to check as early as possible whether bias and precision data obtained by repeated analysis of incurred samples are in line with the respective findings obtained during the validation study. Although Conference Report III left the decision on the extent and nature of the incurred sample analysis to the analytical investigator, it recommended that analyte concentrations and special study populations (e.g. renally impaired patients) should be considered in sample selection. A more detailed discussion of incurred sample analysis for validation purposes can be found in Rocci *et al.* (2007).

Limits

Lower limit of quantification The LLOQ is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy (bias) (Shah *et al.* 2000; ICH Topic Q2(R1) 2005). There are different approaches for the determination of LLOQ.

LLOQ based on precision and accuracy (bias) data This is probably the most practical approach and defines the LLOQ as the lowest concentration of a sample that can still be quantified with acceptable precision and bias (Shah *et al.* 1991, 2000; Hartmann *et al.* 1998; ICH Topic Q2(R1) 2005). In the Conference Reports (Shah *et al.* 1991, 2000), the acceptance criteria for these two parameters at LLOQ are 20% RSD for precision and $\pm$20% for bias. When using the concept of accuracy profiles, the LLOQ corresponds to the lowest concentration at which the accuracy profile is completely inside the acceptance interval (Hubert *et al.* 2003, 2006). It should be pointed out, however, that bias and precision or accuracy profiles must be determined using an LLOQ sample independent of the calibration curve. The advantage of this approach is that the estimation of LLOQ is based on the same quantification procedure as used for real samples. In order to include the possible influence of matrix differences into the LLOQ evaluations, many authors used 6–10 different sources of blank matrix to prepare QC samples at the LLOQ (Bennett *et al.* 1997; Yao *et al.* 1998; Chang *et al.* 1999; Jemal *et al.* 1999; de Jonge *et al.* 2004; Li *et al.* 2004; Xue *et al.* 2004).

LLOQ based on signal-to-noise ratio This approach can be applied only if there is baseline noise, e.g. in chromatographic methods. The signal-to-noise ratio (*S/N*) can then be defined as the height of the analyte peak (signal) and the amplitude between the highest and lowest point of the baseline (noise) in a certain area around the analyte peak. For LLOQ, *S/N* is usually required to be $\geq$10 (ICH Topic Q2(R1) 2005). While this approach should be applicable in pharmaceutical analysis, estimation of baseline noise can be quite difficult for bioanalytical methods if matrix peaks elute close to the analyte peak.

LLOQ based on the standard deviation of the response from blank samples Another definition of LLOQ is the concentration that corresponds to a response that is k times greater than the estimated SD of blank samples (ICH Topic Q2(R1) 2005). From the response, the LLOQ can be calculated using the slope of the calibration curve using equation (20.6) (for blank corrected signals).

$$\text{LLOQ} = k\frac{\text{SD}_{\text{bl}}}{S} \qquad (20.6)$$

where k = factor, usually 10; SD_{bl} = standard deviation of blank response; S = slope of the calibration curve.

This approach is applicable only for methods where SD_{bl} can be estimated from replicate analysis of blank samples. It is, therefore, not applicable for most quantitative chromatographic methods, as here the response is usually measured in terms of peak area units, which of course cannot be measured in a blank sample analysed with a selective method.

LLOQ based on a specific calibration curve in the range of LLOQ In this approach, a specific calibration curve is established from samples containing the analyte in the range of LLOQ (ICH Topic Q2(R1) 2005). The calibration curve must not be used over the whole range of quantification for this determination, because this may lead to overestimation of the LLOQ. The SD of the blank can then be estimated from the residual SD of the regression line or the SD of the *y*-intercept.

Upper limit of quantification (ULOQ) The ULOQ is the maximum analyte concentration of a sample that can be quantified with acceptable

precision and accuracy (bias). In general, the ULOQ is identical with the concentration of the highest calibration standard (Shah *et al.* 2000).

Limit of detection Quantification below LLOQ is by definition not acceptable (Shah *et al.* 1991, 2000; Dadgar *et al.* 1995; Hartmann *et al.* 1998). Therefore, below this value a method can produce only semi-quantitative or qualitative data. However, it can still be important to know the LOD of the method. According to the ICH, it is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value (ICH Topic Q2(R1) 2005). According to Conference Report II (Shah *et al.* 2000), it is the lowest concentration of an analyte in a sample that the bioanalytical procedure can reliably differentiate from background noise.

In many bioanalytical methods to be used in pharmacokinetic studies, the LOD is not evaluated because of their purely quantitative character. However, authors from the field of toxicology often report the LOD of their analytical methods because, in this field, qualitative data can also be very important. The approaches most often applied for estimation of the LOD are basically the same as those described for LLOQ with the exception of the approach using precision and accuracy data, which cannot be used here for obvious reasons. In contrast to the LLOQ determination, for LOD a *S/N* or *k*-factor ≥ 3 is usually chosen (EURACHEM/CITAC 1998; Hartmann *et al.* 1998; Thompson *et al.* 2002; Taverniers *et al.* 2004; ICH Topic Q2(R1) 2005). If the calibration curve approach is used for determination of the LOD, only calibrators containing the analyte in the range of LOD must be used to avoid overestimation of the LOD. All these approaches evaluate only the pure response of the analytes. In toxicology, however, unambiguous identification of an analyte in a sample requires more complex acceptance criteria to be fulfilled. Such criteria were reviewed by Rivier (2003). In forensic toxicology and doping control, it would certainly be more appropriate to define the LOD as the lowest concentration of analyte in a sample at which specific identification criteria can still be fulfilled.

Stability

The definition of stability according to Conference Report II (Shah *et al.* 2000) was 'the chemical stability of an analyte in a given matrix under specific conditions for given time intervals'. Stability of the analyte during the whole analytical procedure is a prerequisite for reliable quantification. Therefore, full validation of a method must include stability experiments for the various stages of analysis, including storage prior to analysis, unless such data are already available in the literature.

Long-term stability The stability in the sample matrix should be established under storage conditions, i.e. in the same vessels, at the same temperature and over a storage period at least as long as the one expected for authentic samples (Karnes *et al.* 1991; Shah *et al.* 1991, 2000; Dadgar *et al.* 1995; Dadgar, Burnett 1995; Hartmann *et al.* 1998).

Freeze/thaw stability As samples are often frozen and thawed, e.g. for reanalysis, the stability of analyte during several freeze/thaw cycles should also be evaluated. The Conference Reports (Shah *et al.* 1991, 2000) require a minimum of three cycles at two concentrations in triplicate, which has also been accepted by other authors (Dadgar, Burnett 1995; Wieling *et al.* 1996; Hartmann *et al.* 1998).

In-process stability, bench-top stability The stability of analyte under the conditions of sample preparation (e.g. ambient temperature over time needed for sample preparation) is evaluated here. There is general agreement that this type of stability should be evaluated to find out whether preservatives have to be added to prevent degradation of analyte during sample preparation (Dadgar, Burnett 1995; Hartmann *et al.* 1998; Shah *et al.* 2000).

Processed sample stability Instability may occur not only in the sample matrix but also in processed samples. It is therefore important also to test the stability of an analyte in the prepared samples under conditions of analysis (e.g. autosampler conditions for the expected maximum time of an analytical run). The stability should also be tested in prepared samples under storage conditions, e.g. in a refrigerator, in case prepared samples have to be stored prior to analysis (Dadgar *et al.* 1995; Dadgar, Burnett 1995; Wieling *et al.* 1996; Hartmann *et al.* 1998; Shah *et al.* 2000).

Stability experiments and evaluations A detailed account of experimental designs and statistical evaluations of stability experiments can be found in Dadgar *et al.* (1995), Dadgar and Burnett (1995) and Hartmann *et al.* (1998). Stability can be tested by comparing the results from QC samples analysed before (comparison samples) and after (stability samples) being exposed to the conditions for stability assessment. It has been recommended to perform stability experiments at least at two concentrations (low and high) (Dadgar *et al.* 1995; Dadgar, Burnett 1995; Wieling *et al.* 1996; Hartmann *et al.* 1998). For both comparison and stability samples, analysis of at least six replicates was recommended (Hartmann *et al.* 1998). Ratios between comparison samples and stability samples of 90–110% with 90% confidence intervals within 80–120% or 85–115% (Dadgar, Burnett 1995) have been regarded as acceptable. Alternatively, the mean of the stability samples can be tested against a lower acceptance limit corresponding to 90% of the mean of the comparison samples (Causon 1997; Hartmann *et al.* 1998). An alternative approach for statistical testing of processed samples in the autosampler was used in the experimental design proposed by Wieling *et al.* (1996). A sample extract was injected repeatedly at certain intervals for a total time period corresponding to the time required to measure a batch of samples under routine conditions. The peak areas of the analytes were then plotted against the respective injection times. Subsequently, regression analysis was performed to check for a significantly negative slope, which would indicate instability.

The importance of stability is obviously widely accepted in the bioanalytical community, which can be deduced from the fact that the majority of papers published in this area in recent years included descriptions of stability experiments. Because of the many different experimental designs used in these papers, a comprehensive overview over all of them would be beyond the scope of this chapter. Exemplary stability studies can be found in Jemal *et al.* (1999, 2000), Jemal and Mulvana (2000), Lin *et al.* (2001), Skopp and Pötsch (2002), Kratzsch *et al.* (2003, 2004), Egge-Jacobsen *et al.* (2004), Maurer *et al.* (2004), Naidong and Eerkes (2004), Ramakrishna *et al.* (2004), Scheidweiler and Huestis (2004) and Shou *et al.* (2004). The papers of the group of Maurer and colleagues (Kratzsch *et al.* 2003, 2004; Maurer *et al.* 2004) describe studies on bench-top, freeze/thaw, long-term, and autosampler stability including statistics and acceptance criteria. The last are generally missing in most other papers. The papers by the group of Jemal and colleagues (Jemal *et al.* 1999, 2000; Jemal, Mulvana 2000) describe stability studies including the effect of modifications introduced to stabilise labile analytes. Egge-Jacobsen *et al.* (2004) performed a comprehensive stability study of various anti-HIV drugs including stability under conditions usually applied for HIV inactivation in blood samples. Skopp and Pötsch (2002) studied the stability of the labile 11-nor-Δ^9-carboxy-tetrahydrocannabinol glucuronide in plasma and urine at various temperatures ranging from -20°C to 49°C. Lin *et al.* (2001) described a comprehensive stability study including the stability of stock solutions at -20°C, which should also be of special interest for forensic and clinical toxicologists because this paper deals with the analysis of cocaine and benzoylecgonine, two potentially unstable compounds relevant in these fields of toxicology. In another interesting paper, Scheidweiler and Huestis (2004) studied hydrolysis of cocaine and 6-monoacetylmorphine during sample preparation of an LC-MS (-MS) assay for quantification of opiates, cocaine and metabolites in hair. They spiked blank hair with cocaine and 6-monoacetylmorphine and estimated their hydrolysis by quantification of the degradation products benzoylecgonine and morphine after sample preparation. All these examples show that, besides the above-mentioned types of stability studies, which should be performed in any validation study, additional experiments might be necessary for certain analytes.

A serious problem encountered during stability testing for bioanalytical methods in forensic and clinical toxicology is that there are a great number of different sampling containers. Furthermore, the anticoagulants used also differ. Both factors make it difficult to assess long-term stability, as the workload to analyse all possible combinations of

containers and anticoagulants is far too great. However, for some analytes relevant to forensic and clinical toxicology (e.g. cocaine), stability problems with different sampling containers have been reported (Toennes, Kauert 2001), which shows that at least for some analytes such extensive studies may have to be considered.

Recovery

As already mentioned above, recovery is not among the validation parameters regarded as essential for method validation and it is not covered by the ICH guidance document for method validation in pharmaceutical analysis (ICH Topic Q2(R1) 2005). In bioanalysis also most authors agree that the value for recovery is not important, as long as the data for LLOQ, (LOD), precision and bias are acceptable (Dadgar *et al.* 1995; Hartmann *et al.* 1998; Shah *et al.* 2000). It can be calculated as the percentage of the analyte response after sample workup compared with that of a solution containing the analyte at a concentration corresponding to 100% recovery. Therefore, absolute recoveries usually cannot be determined if the sample workup includes a derivatisation step, as the derivatives are often not available as reference substances. Nevertheless, some guidance documents request the determination of the recovery at high and low concentrations (Lindner, Wainer 1998; Peters *et al.* 2009) or even specify that the recovery should be greater than 50% (Lindner, Wainer 1998; Peters *et al.* 2009).

In LC-MS(-MS) analysis, a different experimental design must be used to determine recovery, because part of the change of the response in prepared samples in comparison with respective standard solutions might be attributable to matrix effects. In the validation of LC-MS (-MS), it is therefore more appropriate to perform the recovery experiments together with ion suppression/enhancement experiments as described below.

Robustness/ruggedness

According to ICH Topic Q2(R1) (2005), robustness is a measure of an analytical method's capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In other words, it measures the effects that small changes of 'internal' method parameters such as buffer pH, mobile phase compositions, etc. have on the analytical results. Ruggedness refers to a method's capacity to remain unaffected by changes encountered when transferring a method from one laboratory to another, meaning that it focuses more on 'external' parameters of the method such as analysts, instruments, laboratory environment, etc. However, in practice it is difficult to separate robustness and ruggedness, because these parameters do of course interact. It is therefore not surprising that the terms robustness and ruggedness are often used interchangeably.

Full validation does not necessarily include ruggedness testing; it can, however, be very helpful during the method development/prevalidation phase, as problems that may occur during validation are often detected in advance. Ruggedness should be tested if a method is going to be transferred to another laboratory (Karnes *et al.* 1991; Hartmann *et al.* 1998; Vander-Heyden *et al.* 2001; ICH Topic Q2(R1) 2005; Dejaegher, Heyden 2007). A detailed account and helpful guidance on experimental designs and evaluation of ruggedness/robustness tests can be found in Vander-Heyden *et al.* (2001) and Dejaegher and Vander-Heyden (2007).

Matrix effects (ion suppression/enhancement)

Suppression or enhancement of analyte ionisation by co-eluting compounds is a well-known phenomenon in LC-MS(-MS) analysis, depending mainly on the sample matrix, the sample preparation procedure, the quality of chromatographic separation, mobile phase additives and ionisation type (King *et al.* 2000; Ackermann *et al.* 2002; Muller *et al.* 2002; Annesley 2003; Matuszewski *et al.* 2003; Dams *et al.* 2003; Liang *et al.* 2003; Mallet *et al.* 2004; Souverain *et al.* 2004). While ESI has been reported to be much more prone to such effects, they may also occur with atmospheric pressure chemical ionisation (APCI) (King *et al.* 2000; Ackermann *et al.* 2002; Annesley 2003; Dams *et al.* 2003; Liang *et al.* 2003; Matuszewski *et al.* 2003; Mallet *et al.* 2004; Souverain *et al.* 2004). It is obvious that ion suppression as well as ion enhancement may affect validation parameters such as LOD, LLOQ, linearity, precision and/or bias. Sojo *et al.* (2003) demonstrated that mutual suppression of analytes and their stable-isotope-labelled IS in the ESI mode should not affect quantification, but they were concerned about a negative influence on the LOD if too high a concentration of IS was used. In principle, these findings were confirmed by Liang *et al.* (2003), who also reported mutual suppression of analytes and IS in the ESI mode resulting in negative effects on LOD and LLOQ. They also confirmed the findings concerning linearity, but only for certain IS concentrations. For the APCI mode, they reported mutual ionisation enhancement for parts of the analytes and their IS, possibly improving LOD and LLOQ data. Furthermore, the calibration curves were linear when an appropriate concentration of the IS was chosen. From these two studies, one might conclude that, while the limits might be affected by ion suppression/enhancement, quantification would not as long as a stable-isotope-labelled IS was used. However, this is not always true, as could be demonstrated by Jemal *et al.* (2003) who reported, for certain batches of urine, matrix effects that lead to changes in the response ratio (analyte vs IS) that could have a negative influence on quantification. Worse problems have to be expected when no stable-isotope-labelled IS is available.

All this clearly shows that studies of ion suppression/enhancement should be an integral part of the validation of any LC-MS(-MS) method. This is also in line with Conference Report II (Shah *et al.* 2000), which in the case of LC-MS(-MS) procedures explicitly recommends studies on possible matrix effects 'to ensure that precision, selectivity and sensitivity will not be compromised'. However, no specific experimental design was proposed in this document. In the literature, two approaches have been used extensively to study ion suppression/enhancements. In the first approach, a solution of the analyte is constantly infused into the eluent from the column via a post-column tee connection using a syringe pump. The continuous post-column infusion leads to a constant signal in the detector unless compounds that elute from the column suppress or enhance ionisation, which would respectively lead to decreased or increased detector response. Thus, monitoring of the detector response after injection of blank matrix extracts can be used to check for possible ion suppression/enhancement of blank matrix compounds and for their retention times. Applications of this approach can be found in Muller *et al.* (2002), Naidong *et al.* (2002), Dams *et al.* (2003), Liang *et al.* (2003) and Streit *et al.* (2004). Strategies for the second, the so-called post-extraction addition approach, were published by Matuszewski *et al.* (2003). This paper provides excellent guidance on how to perform and evaluate studies on matrix effects (ME) in LC-MS(-MS) analysis. The principal approach involves determination of peak areas of analyte in three different sets of samples: one consisting of neat standards (set 1), one prepared in blank matrix extracts from different sources and spiked after extraction (set 2), and one prepared in blank matrix from the same sources but spiked before extraction (set 3). From these data, the ME (ion suppression/enhancement), RE and process efficiencies (PE) can then be calculated according to equations (20.7)–(20.9), respectively (Matuszewski *et al.* 2003).

$$\mathrm{ME}[\%] = \frac{B}{A} \times 100 \tag{20.7}$$

$$\mathrm{RE}[\%] = \frac{C}{B} \times 100 \tag{20.8}$$

$$\mathrm{PE}[\%] = \frac{C}{A} \times 100 = \frac{\mathrm{MR} \times \mathrm{RE}}{100} \tag{20.9}$$

where A = peak area in sample from set 1(neat standard); B = peak area in sample from set 2 (spiked blank matrix extract); C = peak area in sample from set 3 (extract of spiked blank matrix); ME = matrix effect (ion suppression/enhancement); RE = extraction efficiency (extraction recovery); PE = process efficiency.

The full experimental design proposed in Matuszewski *et al.* (2003) involves analysis of 105 samples (3 sets with 7 concentration levels and

$n = 5$ per level). Thus a tremendous amount of data is acquired that can provide valuable information concerning the performance of the assay studied. As analysis of such large numbers of samples is time-consuming and expensive, a number of simplified experimental designs are also described in Matuszewski *et al.* (2003). Other examples using the same or very similar approaches for evaluation of matrix effects can be found in Matuszewski *et al.* (1998, 2003), Mortier *et al.* (2002a,b), Naidong *et al.* (2002), Jemal *et al.* (2003), Pichini *et al.* (2003), Breda *et al.* (2004), Crommentuyn *et al.* (2004), He *et al.* (2004), Li *et al.* (2004), Ramakrishna *et al.* (2004) and Scheidweiler and Huestis (2004).

Recently, the post-extraction addition approach was adapted in Conference Report III (Viswanathan *et al.* 2007). In this document the matrix effect was termed matrix factor (MF) and expressed as a factor rather than a percentage, but the calculations were essentially the same as described above for ME. It was further specified that MF should be evaluated for at least six individual lots of blank matrix and that its variability (expressed as RSD) should be less than 15%. Based on the assumption that stable-isotope-labelled IS normalised the MF to the theoretical value of 1 (or 100%), evaluation of six individual blank matrix sources was not considered necessary for methods using such stable-isotope-labelled IS (Viswanathan *et al.* 2007). However, this seems highly questionable considering two recent reports of incomplete compensation of matrix effects despite the use of stable-isotope-labelled IS (Wang *et al.* 2007; Lindegardh *et al.* 2008).

Carryover and contamination

Carryover and contamination can seriously affect the precision and bias of an analytical procedure, particularly in samples containing low analyte concentrations. This potential problem was addressed in Conference Report III (Viswanathan *et al.* 2007), which states that carryover should be assessed during validation by injecting one or more blank samples after a high-concentration sample or standard. A standard acceptable magnitude of carryover was not specified in this paper, but it is obvious that for methods to be used in pharmacokinetic studies carryover might be tolerated as long as quantification near the LLOQ is not compromised to a relevant extent. In toxicological analysis, however, where qualitative information about the presence or absence of the analyte can be crucial, even minimal carryover might be unacceptable.

Experimental design for method validation

In the following, a rational experimental design for method validation will be described, which is summarised in Table 20.2. It is based on the experimental design proposed by Wieling *et al.* (1996), the modification introduced by the author of this chapter (Peters *et al.* 2003) and, of course, the considerations presented above. Similar experimental designs have been used in several publications from the group of Maurer (Peters *et al.* 2002; Kratzsch *et al.* 2003, 2004; Peters *et al.* 2003, 2005, 2007b; Maurer *et al.* 2004).

It is recommended to start the validation studies with its selectivity and, in the case of LC-MS(-MS) methods, with ion suppression/enhancement experiments; if either of these two parameters is not acceptable, major changes of the method might be required. For the reasons outlined above, 10 instead of only 6 sources of blank matrix are evaluated to exclude interference from the blank matrix. The zero samples (blank + IS) are analysed to exclude interference from the IS. If appropriate, spiking experiments with compounds likely to be present in real samples should also be performed. If no interferences are detected during these selectivity experiments, the next set of validation experiments can be performed. For LC-MS(-MS)-based methods these are the experiments on matrix effects. A post-extraction addition approach as proposed by Matuszewski *et al.* (2003) is best suited for this purpose, because it allows simultaneous evaluation of ME, RE and PE. In the design proposed in Table 20.2, six sources of blank matrices are used to account for variability between matrix source. If selectivity (and ME) results are satisfactory, processed sample stability should be assessed next to ensure stability of processed samples under the conditions on the autosampler tray during analysis of large batches of samples. For this purpose, 10 extracts each at low and high concentration can be pooled, thoroughly mixed, and divided again into aliquots which are then injected at certain time intervals until the maximum expected run-time is reached. If the processed samples are stable, the linearity experiments follow. If not, the processed samples must be stabilised, e.g. by cooling the autosampler tray, or further optimisation of the method must be undertaken. For the evaluation of the calibration model (linearity experiments) six concentration levels are analysed in replicates of six. This allows for checking for outliers and for studying the behaviour of variance across the calibration range as well as the evaluation of

Table 20.2 Experimental design for analytical method validation

Selectivity	*ME/RE/PE*[a]	*Processed sample stability*	*Linearity*
10 different sources of blank matrix, 2 zero samples, *x* spiked samples	36 samples (6 neat standards, 6 spiked blank extracts, 6 extracts of spiked blanks, 2 concentrations each)	16 injections of extracts (at certain time intervals, 8 at each of 2 concentrations)	36 samples (6 concentration levels, 6 replicates each)

Main validation phase

Run	Calibration 6 levels	Validation samples								
		Low			Medium	High			Optional	
									LLOQ	Dilution
		P & B	F/T	RE[b]	P & B	P & B	F/T	RE[b]	P & B	P & B
1	6	2	6		2	2	6		2	2
2	6	2		10	2	2			2	2
3	6	2			2	2		10	2	2
4	6	2	6		2	2	6		2	2
5	6	2			2	2			2	2
6	6	2			2	2			2	2
7	6	2			2	2			2	2
8	6	2			2	2			2	2

F/T, freeze/thaw stability; LLOQ, lower limit of quantification; ME, matrix effect; P & B, precision and bias; PE, process efficiency; RE, extraction efficiency. For details see text.
[a]LC-MS(-MS) methods only.
[b]Not for LC-MS(-MS) methods.

non-linear models, at least in a preliminary fashion. After an appropriate calibration model has been established, the early validation phase is completed and the main validation phase can begin.

During the main validation phase, bias and precision as well as freeze/thaw stability are evaluated. For methods not employing LC-MS(-MS), recovery experiments may also be performed in the main validation phase. For determination of bias and precision, duplicate QC samples are analysed at a minimum of three concentration levels on each of 8 days. Where applicable, a QC sample at very high concentrations can be analysed in the same fashion after an appropriate dilution to check whether reliable quantification is possible after dilution of real samples containing very high concentrations. In addition, QC samples corresponding to the LLOQ can be analysed in the same way to investigate whether precision and bias are acceptable at such low concentrations. From the data obtained during these experiments, the precision components and intermediate precision can be calculated using one-way ANOVA as described above. The bias values can be calculated as the percentage deviations of the observed mean values from the nominal concentrations of the respective QC samples. It should be noted that a calibration curve is run on each of the 8 days to include variability of daily calibration curves into the precision and bias estimates. Performing the freeze/thaw experiments together with the precision and bias experiments allows use of the daily calibration curves for the stability evaluations also. Freshly prepared control samples are analysed on the first day to obtain the control value. Samples from the same pools are then frozen and thawed three times, before they are analysed on the basis of the calibration curve of the fourth day. The initial mean values and those obtained after storage are then compared as described above. If the stability samples are kept at room temperature for the time needed to prepare a batch of samples before they are frozen again, the freeze/thaw stability evaluation also includes benchtop stability. Recovery samples at low and high concentrations (five controls and five extracted samples, each) can be run with the precision and bias samples on days 2 and 3, respectively, of the main validation phase.

Method development and validation example

In the following, the strategies described above for method development and validation will be illustrated using an example from the author's research work. An assay for enantioselective analysis of amfetamine, metamfetamine, 3,4-methylenedioxyamfetamine (MDA), 3,4-methylenedioxymetamfetamine (MDMA) and 3,4-methylenedioxyethylamfetamine (MDEA) in oral fluid is used as model method (Peters *et al.* 2007b). Method validation will be exemplified using validation results obtained for the analyte (*S*)-(+)-amfetamine.

Two methods already established in the laboratory were used as starting points for method development. The first was a method for enantioselective analysis of amfetamine and metamfetamine (Peters *et al.* 2002), the second a method for enantioselective analysis of MDA, MDMA and MDEA (Peters *et al.* 2005) in human plasma samples. Both methods employed mixed-mode SPE followed by chiral derivatisation with optically pure (*S*)-heptafluorobutyrylprolyl chloride transforming enantiomers to diastereomeric derivatives. The latter could be separated on standard achiral gas chromatography (GC) column and sensitively detected by negative-ion chemical ionization (NICI) MS allowing the use of small volumes of plasma (200 µL). These methods for highly sensitive enantioselective determination of these analytes from small sample volumes were chosen as starting points, because often only small sample volumes of 50–100 µL are available for oral fluid analysis.

In the first development step, a new GC oven temperature programme was developed that allowed separation of the enantiomers of all five analytes from each other and from those of the other analytes within 16 minutes. Considering that the derivatisation reaction had been carried out under aqueous alkaline conditions (Peters *et al.* 2002, 2005) and that oral fluid consists mainly of water, the next step was to study the feasibility of derivatisation directly in the oral fluid samples. It was found that this indeed worked very well and so the sample preparation could be considerably simplified by eliminating the SPE step. During the final optimisation prior to method validation, the electron multiplier voltage (EMV) settings in the different time windows were set to values that led to comparable signal intensities for all analytes. Thereafter, the method was taken through the validation process.

In the selectivity experiments with blank oral fluid samples from 10 healthy volunteers, no matrix peaks interfering with the detection of the analytes were detected. Results from a blank oral fluid sample and low QC samples spiked with high concentrations of other sympathomimetic amines (ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, phentermine, gepefrine, and pholedrine) gave no indication of potential interference of these drugs with detection or quantification of the analytes.

Processed sample stability was evaluated by injecting 10 aliquots of QC sample extracts at intervals of 2 hours covering a total of 18 hours. Regression analysis of the absolute peak areas for (*S*)-amfetamine yielded a slope not significantly different from zero for the low QC sample and a slight but significant positive slope for the high QC samples. The latter might be explained by evaporation of part of the reconstitution solvent. In any case, there was no indication of degradation of the analyte in processed samples.

The calibration model for (*S*)-amfetamine was evaluated by the analysis of six calibrators, the concentrations of which were evenly distributed over a range of 25–1250 ng/mL. Six replicates of each calibrator were analysed. One outlier was identified at the highest concentration level and eliminated. A weighting factor of $1/x^2$ was found appropriate to compensate for heteroscedasticity across the calibration range. Comparison of linear (straight line) and a polynomial second-order (quadratic) fit using an *F*-test showed that the response function was best described by the linear calibration model ($R^2 = 0.996$) with a slope of 0.0041 ng/mL and *y*-intercept of −0.005, the latter not being significantly different from zero.

Besides QC levels containing low (LOW), medium (MED) and high (HIGH) analyte concentrations relative to the calibration curve, two additional QC levels containing analyte concentrations below the calibration range (BCR) and above the calibration curve (ACR) were included in the bias and precision experiments. This was done to account for the extremely wide range of analyte concentrations to be expected in authentic oral fluid samples. While 50 µL volumes were used for analysis of LOW, MED and HIGH samples, an increased volume of 250 µL was analysed for the BCR samples and a reduced volume of 10 µL was used for the ACR samples. All QC samples were analysed in duplicate on each of 8 days and bias and precision data were calculated using the ANOVA method. The results are listed in Table 20.3. Bias values were acceptable for all QC samples lying well within ±10%. Precision data were all below 15% for all QC samples with the exception of the ACR, where a value of 15.7% was obtained. While in a strict sense this is outside the acceptance limit of 15%, it must be considered that this acceptance limit was established for methods to be used in pharmacokinetic studies, for which the sample matrices are usually blood, plasma or serum. Because the absolute concentrations in oral fluid samples are influenced by many parameters including oral fluid collection (Crouch 2005), and because of their weak correlation with plasma concentrations, the slightly lesser precision obtained for the ACR samples should still be sufficient for quantitative analysis in oral fluid samples.

Another modification to the above-described experimental design was that six rather than three freeze/thaw cycles were used in the respective stability experiments. This was done to account for the fact that repeated freezing and thawing is often used to reduce the viscosity of oral fluid samples. QC samples at two concentrations were analysed before and after six freeze/thaw cycles with six replicates at each time point. The ratios between (*S*)-amfetamine concentrations in control and stability samples were 99.2% for the LOW QC samples and 96.1% for the HIGH QC samples and hence well within the acceptance interval of 90–110% for this parameter. The respective 90% confidence intervals were 94.3–104.1% and 82.5–109.6% and thus also within their acceptance interval of 80–120%. These results showed that up to six freeze/thaw cycles have no relevant effect on (*S*)-amfetamine concentrations in oral fluid samples.

Table 20.3 Nominal QC concentrations, samples volumes, bias, repeatability and intermediate precision data for the determination of (*S*)-amfetamine in oral fluid

QC sample	*Nominal concentration (ng/mL)*	*Sample volume (μL)*	*Bias (%)*	*Repeatability (CV %)*	*Intermediate precision (CV %)*
BCR	10	200	−9.4	2.7	3.2
LOW	50	50	−6.1	7.9	10.6
MED	500	50	0.4	6.1	7.0.
HIGH	1000	50	3.4	6.0	8.8
ACR	5000	10	−0.7	15.7	15.7

ACR, QC sample with analyte concentrations above the calibration range; BCR, QC sample with analyte concentration below calibration range; CV, coefficient of variation; HIGH, QC sample with high analyte concentrations relative to the calibration range; LOW, QC sample with low analyte concentrations relative to the calibration range; MED, QC sample with medium analyte concentrations relative to the calibration range; QC, quality control.

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CHAPTER

21 Quality Control in the Pharmaceutical Industry

P Graham

Introduction

A pharmaceutical industry quality control laboratory has the important function of testing raw materials, packaging components, materials being processed and finished products for quality. It is important to recognise that quality control plays an important role in the quality assurance of pharmaceuticals all the way from research and development on investigational medicinal products, through to scale-up and commercial manufacture. Key decisions are made from the analytical data generated and so the reliability of the results is paramount. The safety of patients depends upon the body of knowledge generated by analytical chemists on active pharmaceutical ingredients (APIs) and drug products during product research and development, process validation studies, stability testing, in-process control and finished product testing. When problems occur, the data generated by the quality control laboratory will help to determine the root cause and improve process and product quality.

Testing laboratories involved in the generation of data for product development, marketing authorisation and batch release of medicinal products face the challenge of undertaking their activities in a heavily regulated environment. In addition, as a functional and costly part of the business, testing laboratories must run their operations as efficiently as possible.

Regulatory authorities such as the Medicines and Healthcare products Regulatory Agency (MHRA), the Food and Drug Administration (FDA) or the European Agency for the Evaluation of Medicinal Products (EMEA) enforce national and international regulations. In order to comply with these, pharmaceutical companies are required to put appropriate quality systems in place. Maintaining the quality system in good working order draws heavily on resources, and costs can be high. Regulatory compliance versus lean and efficient operational costs can be the dichotomy that every manufacturer faces.

The balance, however, favours compliance for two reasons. First, serious lack of compliance may put a company out of business by regulatory action, and the remedial actions to re-instate compliance could prove very costly. Second, adequate compliance can help to achieve 'right first time', contributing to the efficient operation of the testing laboratory; this is because regulations and guidelines are broadly based on good scientific and business practices.

The reliability of the results generated depends upon a solid foundation of interdependent quality systems in operation within the quality control laboratory and with their roots firmly embedded in good manufacturing practice (GMP) (MHRA 2007a,b). The pharmacopoeias play an important part in establishing and maintaining standards of testing and control that can be used by all pharmaceutical quality control laboratories, not only for established drugs but also as a good starting point for setting specifications for new chemical entities.

This chapter lays out the various quality systems embedded within the quality control laboratory operating to assure the reliability of the data produced.

Equipment qualification, calibration and maintenance

Before generating reliable analytical data it is essential that all equipment is operating correctly (Bedson, Sargent 1996). The requirements for the qualification of equipment are set out in the US Code of Federal Regulations, 21 CFR Part 211 §211.160(b)(4), §11.10(a), and the European Annex 15 and Annex 11. The purpose of the qualification of laboratory equipment is to ensure that the instrumentation has been installed correctly, that the instrument functions according to specifications and that the overall performance is satisfactory for its intended use. This is usually achieved through a qualification plan containing a Design Qualification, an Installation Qualification, an Operational Qualification and a Performance Qualification. It is for good business, financial and compliance reasons that the analytical instruments perform as intended. The procurement of equipment should be a well-structured and documented process, leading to adequate testing during the qualification in the testing laboratory. The instrument configuration and validation status should be managed throughout its life cycle: from installation to retirement.

The Design Qualification (DQ) must be written and approved before the purchase of the equipment. The DQ documents the specification of the equipment that will meet all expected needs and encompass all regulatory, business, methodological and pharmacopoeial requirements. For example, if an ultraviolet/visible (UV-Vis) spectrophotometer is to be purchased, it is necessary to specify in the DQ the wavelength range to cover all the assays made now as well as those that are likely to be made in the future in the UV and visible ranges. Compliance with the British Pharmacopoeia UV-Vis spectrophotometer monograph (or similar) regarding wavelength and absorbance accuracy is usual.

Once the equipment has been purchased, an Installation Qualification (IQ) is performed. This checks that all the parts of the equipment are present and undamaged, all the serial numbers are recorded, including firmware/software revision numbers, and the equipment is appropriately located in the laboratory with all required services and that it powers up.

Next an Operational Qualification (OQ) is performed wherein the instrument is checked to ensure that it is set up correctly and calibrated to the manufacturer's specification and national standards where appropriate. This is typically undertaken by the supplier, although this must be overseen and approved by laboratory personnel.

Finally, a Performance Qualification (PQ) is carried out to verify and document the ongoing performance of the equipment. This is typically carried out periodically at an appropriate frequency. This can be daily (e.g. pH meters) to 6 monthly (e.g. high performance liquid chromatography (HPLC) systems). PQ is documented in log books or pro-forma worksheets. For larger equipment, the PQ will follow routine maintenance activities to ensure that the equipment is within calibration following maintenance. This is because the maintenance may have involved intervention to replace worn parts that could have rendered the instrument out of calibrated status.

Maintenance records must be maintained and signed off by laboratory personnel. These records will demonstrate that appropriate care is being given to the equipment and are often requested as part of regulatory inspections.

One aspect that must not be forgotten in the pharmaceutical laboratory is the grade of volumetric glassware such as pipettes and volumetric flasks. These must be of the appropriate grade (Grade A) that guarantees the accuracy of the glassware for quantitative work.

Analytical development and validation

The testing methods should be developed to comply strictly with the overall purpose of the method. This may seem obvious, but, frequently, methods are not appropriate for the purpose of the testing, for example because of lack of specificity or sensitivity. The subject of development of testing methods is far too complex to discuss here. Nevertheless, in the same way that equipment is designed for a certain purpose, the development of testing methods should be preceded by a careful consideration of objectives, purpose, intended use and the capability of the techniques employed.

The validation of the testing methods is a well-established requirement in the pharmaceutical industry. This legal aspect is documented in *Rules governing medicinal products in the European Union*, Vol. 4, chapter 6, section 6.15 and *21 Code of Federal Regulation* (CFR) part 211 §211.194(a)(2). The International Conference on Harmonisation (ICH) guidelines, *Validation of Analytical Procedures: Terminology* (Q2A) and *Validation of Analytical Procedures: Methodology* (Q2B) have been in place for several years. Adherence to these guidelines is not mandatory, although the rationale for deviating from these guidelines should be clearly documented in the method validation protocol.

As it is established in the guideline Q2A, 'The objective of the validation of the testing method is to demonstrate that it is suitable for its intended purpose'. This objective is achieved by demonstrating that the results of the validation characteristics obtained during the validation are within the acceptance criteria included in the validation protocol, which has to be written and approved before the validation exercise is started.

ICH Q6A and Q6B specifications can provide significant assistance for the establishment of rational, sound acceptance criteria.

The development and validation of analytical methods and procedures for the stability testing of medicines are a particular and extremely important requirement; 21 CFR 211, §211.166 demands the existence of such testing. Adequate guidance for the conduct of these tests can be found in the ICH guidelines Q1a, Q1b, Q1c and Q1d.

The ICH guideline Q2(R1) on *Validation of Analytical Procedures: Text and Methodology* defines eight characteristics to be considered when validating testing methods (ICH Topic Q2(R1) 1994). These are:

1. Specificity
2. Accuracy
3. Precision:
   - — Repeatability
   - — Intermediate precision
   - — Reproducibility
4. Detection limit
5. Quantification limit
6. Linearity
7. Range
8. Robustness.

Table 21.1 shows which characteristics should be evaluated for identity tests, impurity tests (quantitative and limit tests) and assay methods. Before the publication of the ICH guidelines on analytical validation there was a plethora of validation terms used by analysts. The guidelines have therefore helped to standardise the terminology. The terms used in analytical validation information are now clear to regulators, academia and the industry.

The following sections describe the ICH validation characteristics that may be applied in pharmaceutical analysis. For a description of the validation of methods for the analysis of drugs and poisons in biological materials see Chapter 20.

Specificity

Specificity is defined as the ability of a method to selectively respond to the analyte of interest. When developing methods for drug products it is important to consider related substances, including synthesis or fermentation impurities (by-products) and degradants as well as excipients.

Specificity is built into a chromatographic analytical method during its development by systematically varying the system variables (column, mobile phase composition, temperature, flow rate, etc.) until satisfactory resolution is achieved. The use of expert systems for automatic optimisation of chromatographic separations is commonplace in research and development analytical departments. The use of diode array detectors and peak deconvolution software can aid the analytical chemist in demonstrating that the analytical method is specific for the analyte of interest. Closely eluting impurities should be used in a system suitability test solution and appropriate limits for resolution set. System suitability checks are performed each time an assay is performed to verify that the test was set up correctly to generate meaningful data.

In early drug development, the potential routes of degradation of a drug substance may not be known. Nevertheless, the analytical chemist must develop a stability-indicating assay procedure to determine the

Table 21.1 Parameters to be evaluated during method validation

Characteristics	*Type of analytical procedure*			
	Identification	*Testing for impurities*		*Assay*
		Quantification	*Limit*	■ *Dissolution (measurement only)* ■ *Content/potency*
Accuracy	–	+	–	+
Precision				
Repeatability	–	+	–	+
Intermediate precision	–	+[a]	–	+[a]
Selectivity/specificity[b]	+	+	+	+
Limit of detection (LOD)	–	–[c]	+	–
Limit of quantification (LOQ)	–	+	–	+
Linearity	–	+	–	+
Range	–	+	–	+

– signifies that this parameter is not normally evaluated.
+ signifies that this parameter is normally evaluated.
[a]In cases where reproducibility has been performed, intermediate precision is not needed.
[b]Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).
[c]May be needed in some cases.

inherent stability of the drug substance. This is done by subjecting the drug substance to extremes of temperature, light, pH and oxidation, and then examining the resulting solutions using the proposed chromatographic conditions.

Absence of excipient interference should be verified by preparing a placebo mix of a drug product formulation and taking this through the analytical procedure. In the case of a spectroscopic method, no absorbance reading should be observed at the quantification wavelength for the analyte. However, for chromatographic procedures, a peak from an excipient may be acceptable provided that it is well resolved from the analyte(s).

It is also advisable to consider the effects of ageing of the excipients on the analytical method where decomposition products may interfere. Accelerated stability studies on placebo formulations are useful in this respect.

Accuracy

Accuracy expresses the closeness of agreement between the value that is accepted, either as a conventional true value or an accepted reference value, and the value found. Expressing this another way, it is a measure of how close, on average, the results of a method come to the true value of sample. It is sometimes called 'bias' or 'systematic error'.

Accuracy is usually demonstrated by carrying out recovery experiments through the spiked addition of active drug to a placebo formulation. The experiments must be planned so that the results may be statistically evaluated. It should be shown that the recoveries are independent of the concentration of the active, over a reasonable concentration range, typically 50–150% of the nominal concentration. The range should cover different dose levels if this is appropriate. The experiments will involve adding known amounts of API to a given weight (or volume) of the placebo formulation as required by the method. Each spiked sample must be taken through the whole analytical process, from sample preparation, through extraction to measurement.

The results are expressed as percentage recovery. A typical acceptance criterion for chromatographic methods is a recovery of 98–102% with a relative standard deviation of less than 2%.

Precision

This is a measure of the variation or scatter between replicate measurements as a result of random errors. It is usually expressed quantitatively as the relative standard deviation (or coefficient of variation) of the method.

Precision is an important parameter especially when investigating the homogeneity of a product. It is important that the precision of a method be sufficiently low that it does not affect any interpretation of the results for the degree of uniformity of a batch. As with accuracy, it is important to take the sample through the whole procedure. It is not sufficient, for example, to quote simply the precision of injection or the repeatability of a spectrophotometer reading, as the precision of a method must include the effects of sample preparation.

Precision is considered to have three components: repeatability, intermediate precision and reproducibility.

Repeatability

This is the intra-assay precision of the method obtained under the same operating conditions, i.e. the precision obtained by one analyst on the same HPLC equipment on the same day. It is determined by replicate analysis (at least six times) of a homogeneous sample and calculation of the standard deviation. The homogeneity of the sample is crucial.

Intermediate precision

This is the within-laboratory precision of the method, i.e. the variability obtained by different analysts on different days using different equipment but within the same laboratory. As with repeatability, it is usually expressed as the standard deviation (or coefficient of variation).

Reproducibility

This expresses the precision of the method between laboratories. It is determined by inter-laboratory collaborative studies using homogeneous samples split between the laboratories. It is important that each laboratory follows the method exactly. Reproducibility is an important parameter for regulatory and control purposes.

As might be expected, the repeatability of the method is always less than the reproducibility. Studies have shown that the ratio of the repeatability to the reproducibility is usually in the range 0.5–0.7. A ratio considerably less than 0.5 indicates a very 'personal' method. This may indeed indicate that the method requires considerable personal skill (for example, counting particles in an ophthalmic solution), or that the method instructions need to be clarified.

A survey by Horwitz of the data from over 150 collaborative studies showed that the precision varied with the concentration of the analyte (Royal Society of Chemistry 2004). A curve (Fig. 21.1) was generated relating the coefficient of variation to concentration of analyte. From this it was determined that assays of the active in a formulation at the 1–10% w/w level would be expected to have a reproducibility of about 1–2%, while analysis of trace impurities at the parts per million (ppm) level would be expected to have a coefficient of variation of about 15–20%.

Detection limit/quantification limit

Detection limits and quantification limits must be determined when quantifying trace impurities such as process impurities and degradants.

Detection limit is expressed as the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. It is often quoted as three times the relative standard deviation of the method.

Quantification limit, on the other hand, is the lowest amount of analyte that can be quantitatively determined with acceptable accuracy and precision. It is often quoted as 10 times the relative standard deviation of the method.

A quantification limit check standard is usually prepared and run during tests for trace impurities. In this way, meaningful results can be reported; for example, impurity X: not detected less than 0.05% w/w.

Linearity

It is important to demonstrate that the response of the detector system is linear over the range of interest of the method. This is determined by means of plotting a calibration curve using increasing amounts of a standard solution and plotting a graph of response vs concentration.

The data can be evaluated statistically by linear regression. If the linearity is confirmed, then it is satisfactory to make calculations against a single standard during routine use of the method. The demonstration of linearity of response is an important criterion for all methods of analysis.

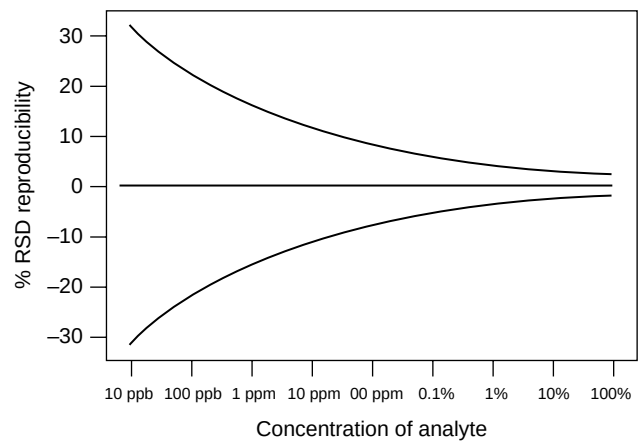

Figure 21.1 The 'Horwitz Trumpet' showing how reproducibility deteriorates with decreasing concentration of the analyte. Reproduced from AMC Technical Brief No.17, Thompson M, ed. Royal Society of Chemistry, July 2004

Range

The range of an analytical method is the interval between the upper and lower concentrations of the analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. It is usually demonstrated during recovery experiments. This is particularly relevant to, say, dissolution testing where a dissolution profile is being determined. The analytical method must be capable of accurately and precisely quantifying the analyte over a range of 10–110% of the nominal concentration of the analyte in the dissolution vessel during the course of the experiment.

Robustness

It is not unknown, when an analytical method is transferred to another site, that problems are encountered and equivalent results are not obtained during the technology transfer process.

There can be many reasons for this; for example, the procedure may not be written clearly or may be ambiguous, or the use of different equipment, standards, reagents, solvents, etc. may have an adverse effect on the operation of the method.

These problems can be overcome if, at the completion of the method development programme, the robustness of the procedure is formally checked. This can be done by examination of the effect of making small changes in the experimental conditions. The changes made should be of the order expected in normal daily operation (slight changes in temperature, reagent concentration, reaction time, etc.). If significant effects are noted, then critical limits can be specified in the method. By obtaining a better insight into the effects of method variables, a much smoother handover process can be achieved, and considerable effort can be saved in investigating discrepancies and avoiding problems that may occur at a later stage.

Youden (in the *Statistical Manual of the Association of Official Analytical Chemists*) has put forward a simple experimental plan in which the effect of seven different factors can be studied (Youden, Steiner 1984). If the factors were varied individually this would result in the need for 128 individual experiments. By adopting a 'partial factorial' approach that allows several changes to be made at once by careful choice of these subsets, it is possible to examine the effect of seven factors in only eight experiments.

When performed, this approach can highlight method variables that must be carefully controlled such as mobile phase composition or pH of test solutions.

Note, however, that this procedure is intended only to study the effects of minor changes in the factors. The assumption is that minor changes in any one factor have no effect on the others. The procedure should not be used as an optimisation procedure during method or process development. Substantial changes in factors seldom act independently and the situation is more complex, for which this procedure is not suitable.

Sampling and retained sample management

Sampling is a crucial step in obtaining meaningful results. The sample must be representative of the batch and the sample integrity must be maintained prior to testing. If this is not the case, erroneous conclusions on the quality of the batch will result.

The taking of samples should be performed in such a manner as not to contaminate the batch being sampled. For this reason, raw materials (APIs and excipients) are usually sampled in laminar airflow booths using clean or disposable sampling implements by staff trained to do so.

Samples must be collected in accordance with approved procedures using an accepted sampling plan (British Standards Institution 1999) such as BS6001-1 : 1999/ISO 2859-1 : 1999. Each container of an API is sampled for identity and a composite sample is made for testing. Full testing may be relaxed if a supplier certification programme is in place that rates suppliers on the basis of audit, quality history of the material being sourced and skip lot testing.

Each container of some excipients with a high-risk profile is sampled to provide adequate assurance of quality; for example, each received container of glycerol is sampled to verify the absence of diethylene glycol due to recent contamination alerts.

The sample container needs to be adequately labelled so that the laboratory can identify it when commencing testing.

The sample size is important when assessing certain attributes such as blend uniformity. Here the sample size should be the same as the single dose size so that it is representative of the single dose unit for solid dose products.

Sufficient sample of a batch needs to be kept for re-test purposes should a quality problem be uncovered during the shelf-life of a product. This can then be re-examined in order to help identify the root cause and extent of the problem. Finished packed products are retained and visually examined periodically in order to highlight any product deterioration, e.g. leakage or degradation.

Reagent and reference standard management

Reagents are routinely used in the analytical laboratory, from titrations to preparing HPLC mobile phases. The analytical procedure should specify the required grade of reagent which is usually analytical reagent (AR) grade. The suitability of the reagents will have been verified during the validation of the analytical procedure. Some trace analysis work will require high-purity reagents to reduce interference from impurities.

The shelf-life of the reagents must be considered. In one laboratory, a storeman performing a stock take/clearout asked an analyst about a bottle of picric acid that he had found in the stores and read out the label which stated 'explosive when dry'. It was so old it had dried out completely to the extent that picric acid crystals could clearly be seen around the lid, ominously waiting to explode on any unsuspecting person innocently opening the container. The Bomb Squad calmly dealt with that one with a controlled explosion in a nearby field.

Shelf-life must take into account not only the chemical stability, but also microbial spoilage and potential cross-contamination when the reagent container is continuously opened and closed to remove aliquots for use. For these reasons, it is good practice to assign a shelf-life, both closed and opened, by attaching an expiration date label to the container. The supplier can (and often does) specify a shelf-life and required storage conditions for the reagent. If not, then experience must be used to assign a realistic shelf-life. The opened shelf-life should not exceed the unopened shelf-life.

A standard operating procedure (SOP) documenting the management of reagents (receipt, storage, use and shelf-life) is useful.

Laboratory-prepared reagents and solutions such as buffers, diluents and HPLC mobile phases will have a short shelf-life, e.g. one week to one month. This is to ensure that the effects of microbial spoilage, evaporation and uptake of CO_2 are minimised.

The reference standards need to be fully characterised to ensure their correct identity and purity. Purity is usually expressed as % w/w 'as is' or on the dry basis. For the latter, the reference standard needs to be dried prior to use or the moisture determined and factored into the assay calculation. The reference standard of a new chemical entity (NCE) will be characterised by the manufacturer using spectroscopic techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy to confirm the structure. Purity is determined by a primary method such as non-aqueous titration or by subtraction of related substances, moisture and volatile organic substances (e.g. recrystallisation solvents) from 100%.

If the drug has a pharmacopoeial monograph, it usually has an associated chemical reference standard from that pharmacopoeia, e.g. British Pharmacopoeia (BPCRS), European Pharmacopoeia (EPCRS) and United States Pharmacopeia (USPCRS). These are available from LGC Standards: www.lgcstandards.com.

Once received, the standards must be stored as directed to prevent degradation that would alter the purity and cause bias in results calculated using the reference standard.

Cleaning of laboratory glassware

It is rare to use dedicated glassware for particular tests so the glassware that is to be used must be cleaned prior to next use to prevent cross-contamination.

A variety of glass washing methods are available from hand washing to specialist laboratory glassware washers. Irrespective of the method employed to clean the glassware, it is good practice to validate the washing method to ensure that traces of the previous drug have been removed and also that no residues of cleaning agents that may interfere with subsequent tests have remained on the glassware following rinsing.

This can be achieved by first defining the cleaning regime in a protocol; for example, specify cleaning agents and concentrations to be used, reference the cleaning cycle to be used, and so on.

The protocol will then define a 'marker drug' to be used that can be quantified. This will typically be a 'difficult to clean' drug that is used in the laboratory. A variety of glassware will be spiked with the drug and then subjected to the defined washing process. Following washing and drying, the cleaned glassware is rinsed with a suitable solvent and the rinse solutions are assayed for residual marker drug. Residual cleaning agent can also be checked with purified water as the solvent by using a specific method from the supplier, if available, or using non-specific but sensitive methods such as conductivity or total organic carbon (TOC) using the input purified water as the reference. The non-specific methods can be calibrated by setting up a calibration curve using serial dilutions of the cleaning agent.

Recording of the UV spectrum of the rinse solutions as well as the pH can also be employed to show that no interfering substances are present in the rinse solutions.

Once the cleaning procedure has been validated, it needs to be documented in an SOP to ensure that compliance with the validated procedure is maintained during routine use.

The cleaning of pipettes and burettes is particularly important since a build-up of grease can alter the volume through incomplete drainage. Pipette washers can be used that soak the pipettes in a suitable cleaning agent which removes grease. This is followed by a rinse cycle with potable water followed by a final rinse in purified water.

Training and compliance

High on the list of critical requirements in the testing laboratory is to have and to follow written procedures and to have an educated, trained workforce. The *Rules governing medicinal products in the European Union*, chapter 1, section 1.4; chapter 2, chapter 6, and the 21 CFR, part 211, §211.22(d) §211.25 establish this as a legal requirement.

Analysts and operators must be trained and knowledgeable in all necessary procedures to carry out their job duties effectively. They must have the appropriate education and experience to carry out these in a responsible manner.

Training of laboratory personnel is a critical element in the generation of valid analytical results. New starters, whether from school or university, will need to be trained in the laboratory procedures, equipment and test methods so that procedures are followed accurately (Pharmaceutical Analytical Sciences Group 2009).

Best practice involves talking the trainee through the procedure, SOP or method and then assessing competency through either a knowledge assessment or a skill assessment. A knowledge assessment could involve multiple-choice questions on a procedure, while a skill assessment will involve the trainer observing the trainee performing the method: for example, correct set-up, calibration and use of an IR spectrometer or following a test method and obtaining the correct results.

It is also important to give new starters GMP training at induction and then provide periodic refresher training to all laboratory staff on at least an annual basis. Training packages and videos are available to provide variety to this element of training and also to focus on areas to improve as identified in out-of-specification investigations and periodic reviews of laboratory performance.

Recording of laboratory data

21 CFR Part 211 and, in particular, §211.194 and *Rules governing medicinal products in the European Union*, Vol. 4, chapter 1, section 1.4 (iv) (vi); chapter 6 establish the regulatory requirements for documentation. Adequate procedures should be in place in order to generate, preserve and archive the laboratory documentation. Part 11 and Annex 11 requirements must be taken into consideration because most of the documentation created in modern laboratories is electronic. There are important demands to be placed on the documentation systems:

- Records must be complete, containing the necessary information and signed off by the person in charge.
- Records must be traceable.
- Records must be cross-referenced.
- Amendments made to a record must be duly documented and an audit trail must exist to ensure that information such as by whom, why and when the record was amended is available.
- Records must be kept for the relevant period; appropriate measures must be taken to ensure that records are preserved through time, use and disaster.

Recording laboratory data legibly and accurately is of crucial importance. The data will be checked before the results are used and they can also be reexamined in years to come in the event of a customer complaint, during regulatory inspections and customer audits.

The data can be recorded in a laboratory notebook or on a pro-forma template, but both must be controlled to ensure that data are not intentionally or accidentally discarded. Laboratory note books must have a unique identifying number and each page must be numbered. A proforma must be version controlled and issued to laboratory personnel in a controlled manner. The reason for the version number is to ensure that any superseded copies in the laboratory can be identified and destroyed. For example, a test method may have been changed, requiring a change to the calculation in the proforma.

The recorded data should form a permanent record with full traceability to verify the validity of the results. The following is an example of what needs to be recorded:

- Product name and strength
- Test method and specification number and version
- Name of the person performing the test
- Date
- Instrument numbers
- Reference standard lot number, expiration date and purity
- Weighings for the test article and reference standard(s), ideally with printouts
- Reagent lot numbers and expiration date
- HPLC column number
- Microbiological media lot number
- Variable instrument settings if not recorded on raw data printouts
- Calculation of results
- Name of the person checking the data.

From this information it is possible to trace back to verify that the person conducting the test was trained, that the instruments were maintained and calibrated, that the reagents were within their expiration date, and so on.

Out-of-specification investigations

As with any failure investigation, an out-of-specification (OOS) result is an opportunity to improve. The caveat is that the investigation must be conducted thoroughly in order to determine the root cause.

First and foremost, the OOS investigation is designed to determine whether the OOS is a result of a laboratory error, a processing error or a product problem (Food and Drug Administration 2006). At the outset there must be a standard methodology to investigate the OOS result in a structured manner in order to correctly determine whether the result is valid. Some years ago the manner in which the industry handled OOS investigations was inconsistent and ranged from performing repeat testing until an in-specification result was obtained to following a systematic approach to assign root cause. This has now ceased and robust procedures to investigate OOS results are routine.

There are three reasons why an OOS result is obtained:

1. Laboratory error, e.g. a 250 mL volumetric flask was used instead of a 200 mL flask.

2. Processing error, e.g. the stirrer in the production vessel stopped due to a mechanical problem when homogenising a suspension.
3. A flaw in the process or product design, e.g. the process when run correctly did not result in a uniform blend.

Use of decision trees is commonplace in investigating OOS results (Fig. 21.2). The first stage of an OOS investigation involves the analyst informing the supervisor that an OOS has been obtained and performing an initial investigation. At this stage it is crucial to retain all test solutions, reference standard solutions and glassware so that these can be examined during the investigation, if required.

The following aspects will be checked at this stage:

- The calculations are correct and there are no arithmetic errors.
- The correct method has been used.
- The analyst is trained and is competent in performing the test.
- The correct weighings, dilutions and glassware have been used.
- The test solutions and reference standard solutions are examined to show that they are typical.
- The instruments used during the test have been calibrated and are working correctly.
- The chromatograms and spectra are examined to highlight any anomalous or suspect information. The response factors of reference standards are determined to see whether these are anomalous, which could point to incorrect preparation.
- The correct reagents, solvents and reference standards have been used and these are within their expiration dates.
- Anything anomalous that the analyst observed or noted during the conduct of the test.

The test solutions may be reanalysed and dilutions re-performed to verify a hypothesis formed during the initial investigation. For example, if it is suspected that the diluted test sample was not mixed thoroughly, then the dilution can be performed again from the primary test solution, ensuring thorough mixing, and the result compared with the original OOS result to take the investigation forward.

If the OOS result can be directly attributed to an issue identified during this initial review, the OOS result can be invalidated and the corrective action will be to repeat the test on the same sample. It is important that preventive actions are considered that could stop the problem recurring.

Should this initial investigation not highlight any laboratory error, a full-scale OOS investigation is initiated. This involves a detailed manufacturing review. Here, the batch records and associated documentation will be reviewed to determine any atypical events that occurred during the manufacture of the batch that could explain the OOS result. If this is the case, the OOS investigation may be terminated and the batch rejected. However, the laboratory may assist in performing further testing to establish appropriate preventative actions in the manufacturing process.

If the manufacturing review shows no atypical event, and all in-process results are satisfactory and all process critical control points have been achieved, then the laboratory investigation proceeds to the next stage.

Here, a re-test plan is developed to identify the source of the OOS, which will involve re-testing and if justified re-sampling (if, for example, the integrity of the initial sample is suspect for reasons such as inappropriate storage or non-compliance with the approved sampling plan).

At the end of the investigation, if the initial result is invalidated then it should not be used to evaluate the quality of the batch for release or rejection. However, if a scientifically justified reason cannot be found to invalidate the initial result, even if all repeat results are within specification, then the initial result must be included in the data set used by the qualified person (within the European Union) or the quality control unit responsible for the release/reject of the batch.

One final word on OOS. The procedure is also applicable to atypical or out-of-trend results. Such results can signal an alarm that not all is well with the batch and further re-testing in an OOS investigation could reveal this and prevent a poor-quality batch being released to the market.

Management of SOPs, specifications and test methods

Laboratory SOPs are best written by staff who understand the equipment or process. SOPs must have a unique identifier number and version number (e.g. SOPXXXX/YY) and they must be periodically reviewed to ensure that they reflect current practice and are in the current format. Sometimes events supersede the review period, such as a firmware upgrade for a piece of laboratory equipment that affects the output screen or sequence of operation. Such changes require an immediate update of the SOP. Probably the biggest cause of SOP update is the high level of mergers and acquisitions in the pharmaceutical industry, with the resulting changes in company names and logos.

When well written, SOPs can have a positive impact on laboratory compliance by encouraging their use as a result of their being user friendly. The use of photographs and process flow diagrams can help to reduce human error. A picture is worth a thousand words, so the saying goes. The SOP should ideally be laid out into chronological steps and segmented to encourage staff dipping into the relevant section. The use of flowery language or too many words should be avoided as staff will be reluctant to seek out the information that they require if it is buried deep in a huge SOP.

Turning to specifications, these also need to be uniquely numbered and version controlled and the product to which the specification relates should be clearly documented in the title. The units of measurement must be included and particular attention needs to be paid to the correct number of decimal places.

Test methods must be well written in manageable steps so that it is easy to keep track of where you are should the reader be distracted. Human error can occur through skipping or repeating steps, and a good document layout can help to reduce this.

During robustness testing of the method, critical limits such as mobile-phase pH limits and the grade of reagents should have been defined.

Trending and periodic reviews

In a pharmaceutical quality control laboratory, numerical data should be trended to flag up potential problems before they occur. Sometimes processes drift and, if the laboratory can highlight that a process is going out of control, then an investigation may identify the root cause before a situation is reached requiring batch rejection. There are a variety of trending methods available that highlight whether a process is drifting. These include Acceptance Control Charts (see ISO 7966), Control Charts with Arithmetic Average and Warning Limits (see ISO 7873), Cumulative Sum Charts (see ISO 7871) and Shewhart Control Charts (see ISO 8258).

Periodic product reviews are useful in spotting step changes in processes that may be linked to, say, a planned and validated process change. The validation of the change may have verified that the resulting product was within the specification limits. However, the step change may not have been identified at the time of the change approval.

Periodic equipment reviews in the laboratory are useful in verifying that the equipment has been adequately maintained and calibrated. It will highlight whether the calibration frequency is appropriate or whether it needs to be shortened or can be lengthened, thereby freeing up valuable laboratory resources. It can also highlight when a piece of equipment is coming towards the end of its serviceable life if the review picks out that the level of breakdowns has increased.

Stability testing of pharmaceuticals: ICH conditions and shelf-life prediction

Stability testing of pharmaceuticals is an integral part of the development programme. Initially, stability results will assist in selecting appropriate primary packaging materials such as amber vials to protect light-sensitive products and aluminium blister strips to protect hygroscopic products.

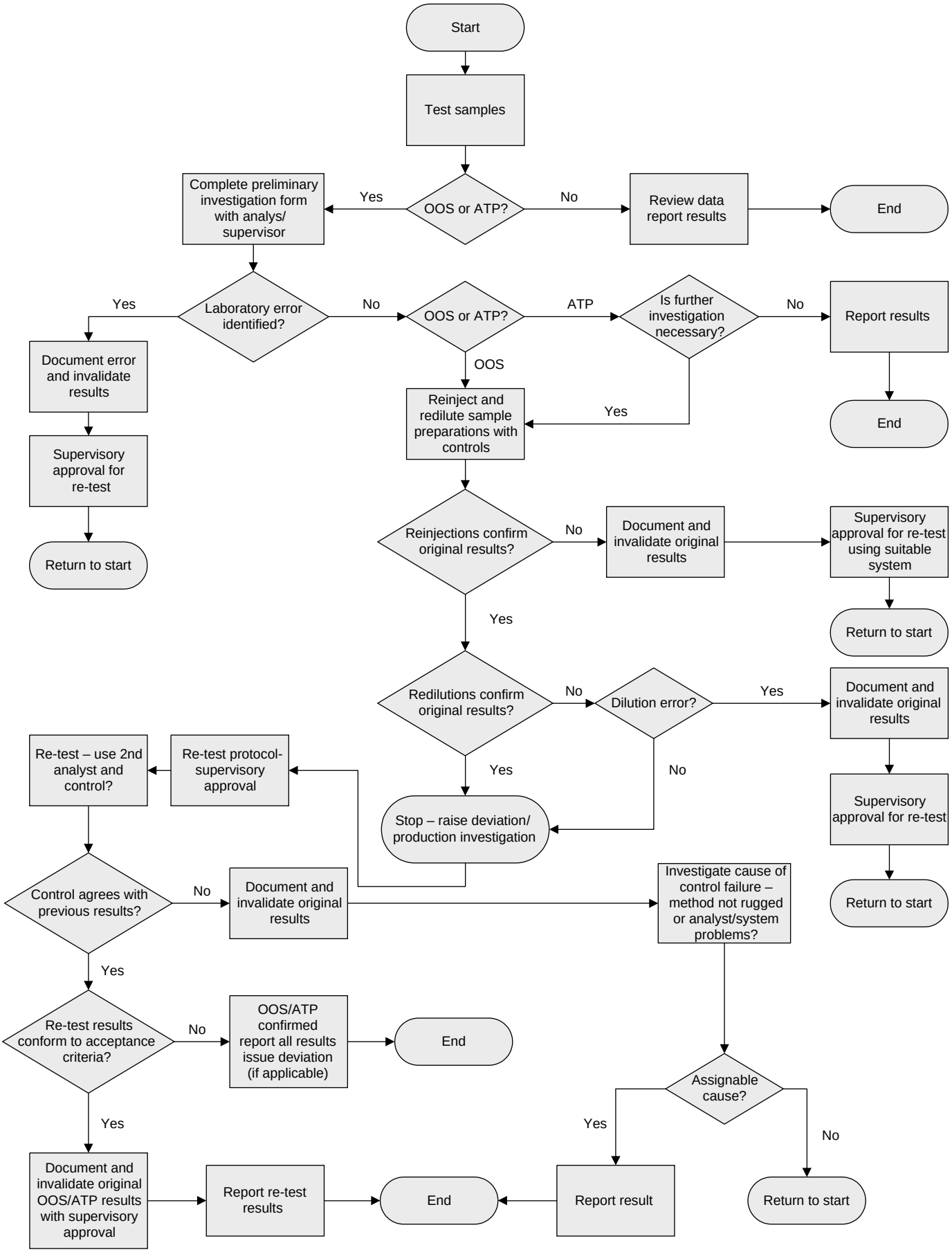

Figure 21.2 Typical out-of-specification (OOS) investigation decision tree.

Once the pack configuration has been selected, a stability programme can be initiated. ICH have published standardised storage conditions in the guideline Q1A (R2): *Stability Testing of New Drug Substances and Products* (Second Revision) (ICH Topic Q1A(R2) 2003) These are as follows:

Product intended for storage in a freezer:

$-20°C \pm 5°C$
$5°C \pm 3°C$ (accelerated storage condition).

Product intended for storage in a refrigerator:

$5°C \pm 3°C$
$25°C \pm 2°C$ and 65% relative humidity (RH) $\pm$ 5% RH (accelerated storage condition).

Product intended for room-temperature storage:

$25°C \pm 2°C$ and 60% RH $\pm$ 5% RH or
$30°C \pm 2°C$ and 65% RH $\pm$ 5% RH or
$40°C \pm 2°C$ and 75% RH $\pm$ 5% RH (accelerated storage condition).

A typical stability protocol will involve testing at initial, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, etc. with testing of the accelerated storage samples up to 3–6 months.

Testing will depend upon the dosage form, but the following tests are typically performed:

- Appearance, assay, degradants, functionality tests such as dissolution for tablets or fine particle dose for metered dose inhalers.
- Once sufficient real-time stability data are available, extrapolation can be used to estimate shelf-life as shown in Fig. 21.3. Up to four times the real-time data can be granted for investigational medicinal products provided that this is supported by data extrapolation; for example, 3 months' real-time data can support up to 12 months' shelf-life.
- Stability studies are also performed on a sample of routine production batches to determine whether any change in stability characteristics occur over time. Here, the long-term storage condition is employed with no accelerated conditions used. Some product recalls have occurred because stability testing has predicted that the product will not meet the labelled expiration date.

Functionality testing

Quality control is not only about determining the concentration and identity of the active ingredient and potential impurities in a dosage form. It is important also to control the manufacturing process to ensure that the delivery of the drug will meet the design characteristics of the formulation so that a patient receives the right dose over the correct duration. For instance, if a patient has a blinding headache, the analgesic tablet must dissolve rapidly in the stomach to release the active ingredient into the bloodstream as rapidly as possible. Similarly, an enteric-coated tablet is designed to release the active ingredient in the duodenum and not in the stomach, so it is essential to check that the enteric coat works and the drug is not released in the acidic environment of the stomach where it may be degraded before it is released into the bloodstream.

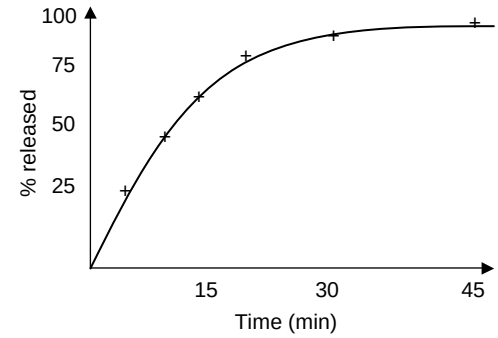

Figure 21.4 Dissolution curve showing percentage released against time.

Standardised methods are employed to confirm the correct functioning of the dosage form, and these are described in the pharmacopoeias. The testing of tablet functionality involves the use of dissolution equipment.

The basic equipment consists of a glass pot with a rotating basket (or paddle) immersed in a water bath at $37 \pm 0.5°C$. The pot is filled with a specified volume of de-aerated medium and a tablet is added to each of six pots. The paddle is rotated at a fixed speed and samples are taken for quantitative analysis at the prescribed time(s). The samples are then assayed and the results are expressed as percentage released (Fig. 21.4).

The equipment is highly standardised with tight tolerances in order to ensure consistent results test to test (Fig. 21.5). Regular calibration is undertaken to ensure that the equipment remains within tolerance. The checks include dimensional checks, speed of rotation of the paddle, degree of wobble of the paddle and temperature of the medium. For calibration of the dissolution apparatus the US Pharmacopeia uses calibrator tablets of salicylic acid and prednisolone with predefined acceptance criteria for release, although these rely heavily on the consistency of the batch of calibrator tablets, which has been a problem over the years. For this reason, it is proposed to discontinue the use of calibrator tablets. The sample location is important for reliable results, so the sampling tube is fixed at a defined height and distance from the vessel wall.

A typical specification for a rapid release tablet is not less than 70% released in 45 min.

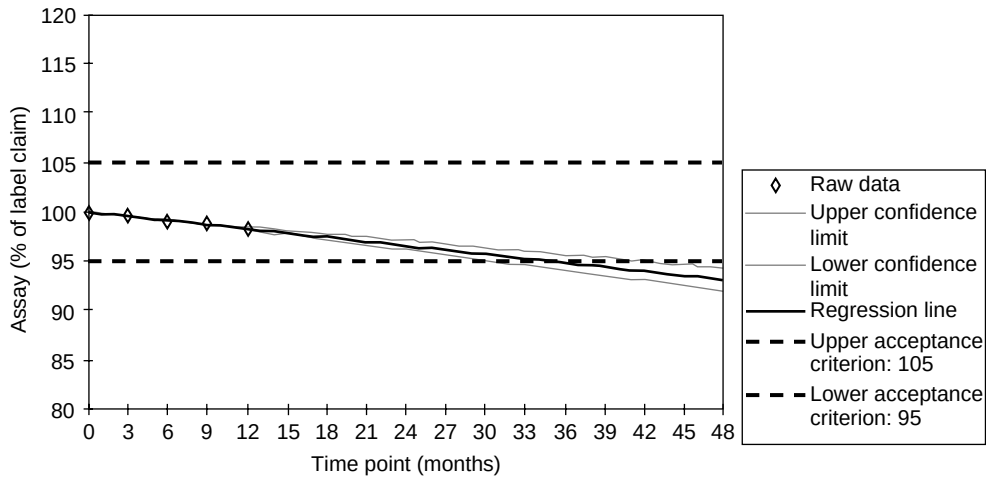

Figure 21.3 Extrapolation of stability data to estimate shelf-life. Reproduced from ICH Q1E.

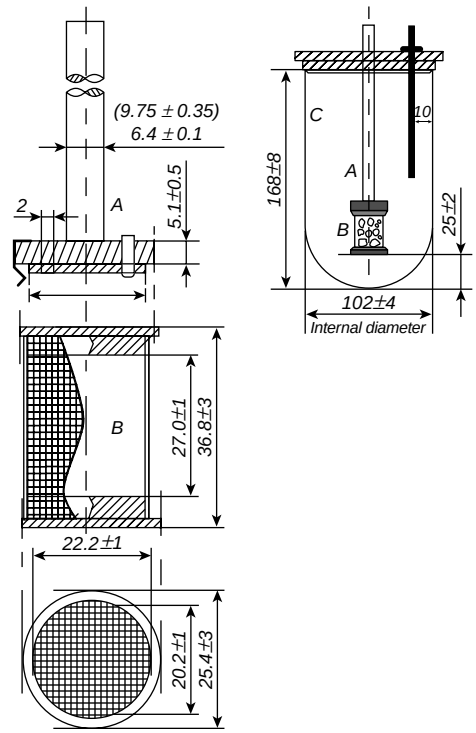

Figure 21.5 Dissolution pot and basket configuration with dimensions for Apparatus I. Reproduced from the British Pharmacopoeia.

Functionality testing is also important for inhalation products such as metered-dose inhalers. The correct dose delivered to the patients lungs is dependent upon the correct operation of the metered-dose valve, the correct particle size of the aerosol cloud and the homogeneity of the drug in the propellant.

For inhalation products, an impinger device such as a glass impinger (Fig. 21.6) is used to confirm that the correct dose is delivered to the lungs. Note that, as with the dissolution apparatus for solid dose products, the glass impinger has similarly tight tolerances specified for the apparatus.

Testing for impurities

The control of impurities in an API and drug product is important to ensure the safety and quality of the drug product for the patient. Impurities in APIs can be classified as organic impurities (also referred to as related substances) such as starting materials and by-products, inorganic impurities such as reagents and catalysts, and residues of solvents used in the synthesis or purification steps.

Acceptable limits for residual solvents in APIs are defined in ICH Q3C (ICH Topic Q3C 1997). Quantification is usually by GC.

Inorganic impurities are normally detected and quantified using pharmacopoeial methods, often as limit tests. The limits are established from known safety data on the particular impurity.

The ICH guideline Q3A(R2) *Impurities in New Drug Substances* (ICH Topic Q3A(R2) 2002; ICH Topic Q3B(R2) 2003) gives a standardised approach to impurity specifications. For organic impurities, the guideline specifies thresholds for reporting, identification and qualification of impurities based on the maximum daily dose as shown in Table 21.2.

For example, a drug that has a maximum daily dose of less than or equal to 2 g per day must have all impurities present at a level greater than 0.05% w/w reported. Of these, any impurity found to be present at a level greater than 0.10% w/w (or has a daily intake greater than 1.0 mg, whichever is the lower) must be identified. Furthermore, if the impurity is present at a level greater than 0.15% w/w (or 1.0 mg per day intake, whichever is lower) then it must be qualified. Qualification in this context is the process of obtaining data to establish the biological safety of the impurity.

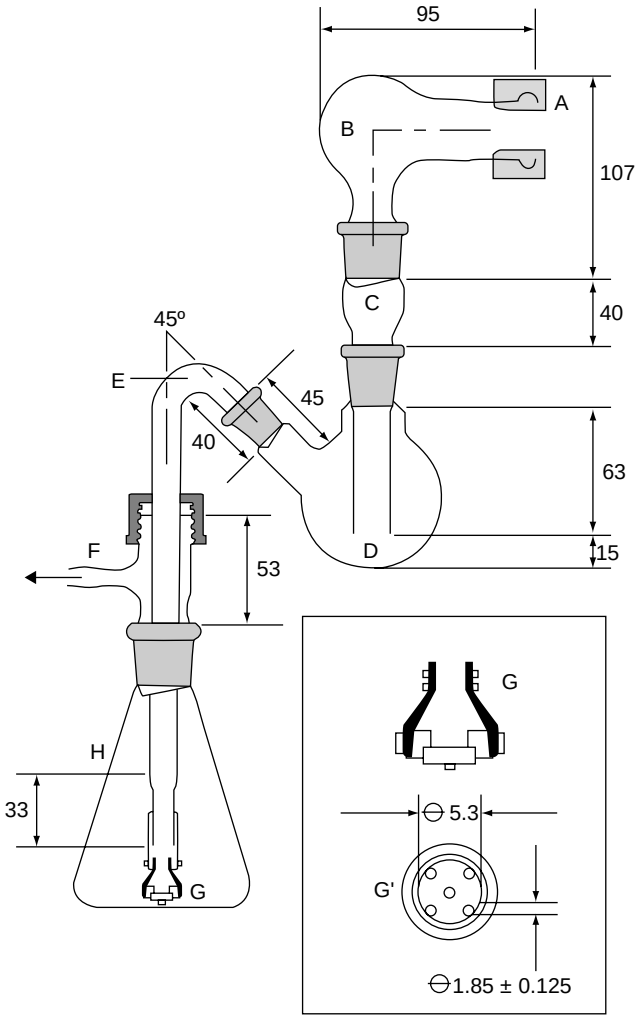

Figure 21.6 Glass impinger for metered dose inhaler functional testing. Reproduced from the British Pharmacopoeia.

As an example, a new drug substance that is to be administered at 100 mg per day has the following impurities by HPLC:

Impurity A	0.12% w/w
Impurity B	0.03% w/w
Impurity C	0.07% w/w
Impurity D	0.19% w/w

ICH guideline Q3A (R2) would require that impurities A, C and D be reported, that impurities A and D be identified (i.e. the structure elucidated), and that impurity D be qualified before clinical trials can take place.

It is also a requirement to set a total impurity specification.

For the example above, a typical related-substances specification and results would be as shown in Table 21.3.

For drug products, the ICH guideline Q3B (R2) *Impurities in New Drug Products* applies, which follows the same principles of thresholds as for new drug substances. Since impurities in the drug substance are already controlled by the drug substance specification, such impurities need not necessarily be controlled in the drug product. A drug product specification only needs to control impurities that are formed during the manufacturing process or degradation products formed during storage. It could well be that an impurity in the drug substance is also a degradant in the drug product and so will need to be controlled in both the drug substance and drug product specifications.

So how does one go about setting a specification for an API or a drug product? This is usually done at an early stage of the development process during initial validation studies of the analytical method,

Table 21.2 Thresholds for reporting, identifying and qualifying impurities

Maximum daily dose[a]	*Reporting threshold*[b,c]	*Identification threshold*[c]	*Qualification threshold*[c]
≤2 g	0.05% w/w	0.10% w/w or 1.0 mg per day intake (whichever is lower)	0.15% w/w or 1.0 mg per day intake (whichever is lower)
>2 g	0.03% w/w	0.05% w/w	0.05 w/w

[a]The amount of drug substance administered per day.
[b]Higher reporting thresholds should be scientifically justified.
[c]Lower thresholds can be appropriate if the impurity is unusually toxic.

Table 21.3 Typical related substances specification and results

Related substance	*Specification*	*Result*
Specified impurity A	Not more than 0.15% w/w	0.12% w/w
Specified impurity C (unidentified)	Not more than 0.10% w/w	0.07% w/w
Specified impurity D	Not more than 0.30% w/w	0.19% w/w
Unspecified impurities	Not more than 0.10% w/w	Detected[a] <0.05% w/w
Total impurities	Less than 1.00% w/w	0.38% w/w

[a]Impurity B (unspecified impurity) was detected but below the reporting limit of 0.05%.

usually HPLC with UV detection, to control the assay and purity of the drug.

Known reference materials of synthesis impurities/degradants, if available, can be used to develop the chromatographic separation so that the active is resolved from its impurities. Forced degradation studies are also performed to understand the potential degradation pathways of the drug substance. These will look at the effect of pH, light and oxidation on the drug substance. (See the section on Analytical development and validation, Specificity.) Major degradants will be identified and characterised as this information may be useful in selecting an appropriate container/closure system and in developing the formulation to prevent or reduce the degradation of the drug to provide a usable shelf-life of the product. The retention times of the impurities are recorded. If unknown impurities are observed during the method development, these can be identified using HPLC with MS detection. It is usual then to confirm the correct identification by synthesising the impurity and verifying that the retention time and spectral characteristics match. It can occur that an impurity is closely related to the drug substance and co-elutes owing to its similar chromatographic behaviour. It is important during the development of the method that the chromatography is challenged by checking the peak purity using deconvolution software and varying the chromatographic conditions to tease out any co-eluting impurities. If this occurs, the chromatography is modified to ensure that resolution is achieved in order to control the impurity. Closely eluting impurities are often used in chromatographic system suitability resolution checks to demonstrate that the method is performing as intended on the day of testing.

Quantification of impurities can be performed by calculation against a known concentration of a reference standard of the impurity with a known purity. However, if a reference standard is not available then percentage normalisation against the drug substance peak can be employed. The assumption is that the impurity has the same or similar response factor as the drug substance. If this is not the case, then a response factor of the impurity can be determined against the drug substance and then used in the calculation.

The use of mass balance calculations when assessing the purity of a drug substance can give the analyst a clue that there is a response factor difference between the drug substance and the impurity. For example, if the assay (on a dry basis) of a drug substance is 97.5% w/w and the sum of the impurities is 5.5% w/w, then this suggests that an impurity has a greater response factor than the drug substance which required further investigation. This may reveal that an impurity has twice the response factor in comparison to the drug substance, which will need to be taken into consideration for accurate quantification of the impurity. This may be achieved by quantification of the impurity against a reference standard of the same response factor or by employing response factor compensation in the calculation.

As drugs become more and more potent, the accurate quantification of low levels of impurities becomes increasingly more difficult and more sensitive methods must be employed to control them. Analytical instrument manufacturers must develop detectors with increasing sensitivity, or the analysts must devise strategies to quantify impurities down to the required specification with currently available technology. For genotoxic impurities for which a toxicological threshold level cannot be determined, the regulatory expectation is that the analyst must develop a method sufficiently sensitive to control the level to as low as is reasonably practicable (European Medicines Agency 2006) (the so-called ALARP principle).

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CHAPTER

22 Quality Control and Accreditation in the Toxicology Laboratory

AC Moffat

Introduction

It is important in many spheres to have reliable, interchangeable data on the recovery, identification and quantification of drugs. In the pharmaceutical industry, quality control and assessment are required to monitor production and assess the quality, safety and efficacy of its products. In clinical analysis, it is vital to the quality and safety of patient care, to the diagnosis and control of therapy for the individual patient, and for research and public health purposes. For toxicologists and pathologists, it helps to distinguish between therapeutic and overdose levels and to determine the cause of death. In law enforcement, it is used to provide information to link drugs offences, to identify drugs distribution networks and to provide evidence of possession or misuse for the courts. It is also essential for monitoring individuals on drug rehabilitation programmes and for workplace testing of employees in certain occupations.

To produce reliable and interchangeable data, toxicology laboratories have to employ competent analysts and use validated methods. These analysts need appropriate accommodation and environmental conditions in which to work and access to all the equipment and other materials required to carry out their analyses safely and to an acceptable standard. They must also have systems that monitor and maintain the quality of their results and establish the uncertainty of their measurements, and they should participate in relevant external quality assessment (inter-laboratory proficiency testing) exercises (ISO/IEC 1997).

These requirements all fall within the ambit of the quality assurance programme to which laboratories must work if they seek accreditation to national or international quality standards or to protocols relevant to the field of drugs testing, such as ISO/IEC 17025:2005 (ISO/IEC 2005), ISO 15189 (ISO 2007), good laboratory practice (GLP; OECD 1998) or the Mandatory Health and Human Services Guidelines for Federal Workplace Drug Testing Programs (DHHS 2004).

Accreditation to ISO 17025 is now becoming the international standard for forensic toxicology laboratories. For example, in Australia all laboratories must be accredited to ISO 17025 to undertake any kind of forensic toxicology testing. Also, in the UK the Forensic Science Regulator has made it a firm requirement that all forensic science providers shall gain accreditation to his draft published standard and to ISO 17025 for the scope of their work (Forensic Science Regulator 2009a). There was overwhelming support from the forensic science community that providers with a laboratory function should be accredited to ISO 17025 and agreement that the standard be applied rigorously to all providers, including those working for the defence and in-house provision by law enforcement bodies (Forensic Science Regulator 2009b). In addition, the World Anti-Doping Agency (WADA) requires laboratories to be accredited to ISO 17025 to be eligible for WADA accreditation (see Chapter 6).

Quality assurance terminology

Quality is the totality of features and characteristics of a product or service that bears on its ability to satisfy stated or implied needs. It is its fitness for the purpose or use specified by the customer. It is determined by the competence of the people who produce the product or provide the service, the facilities available to them and the processes and procedures that they employ.

Quality assurance (QA) is a global term to describe those means of ensuring that the results and interpretations issued by a laboratory are dependable and sufficiently unbiased and precise to allow decisions to be taken with confidence. It is an internal management tool concerned with the prevention of quality problems through planned and systematic activities. These include the establishment of a documented *quality system* that sets out the organisational structure, responsibilities, procedures, processes and resources to implement quality management, assessment of the adequacy of this quality system, audit of its operation and review of the system itself.

Quality control (QC) is that aspect of QA concerned with the practical activities and techniques employed to achieve and maintain the product or service quality. It thus also involves monitoring the processes and procedures used, and the identification and elimination of causes of quality problems so that the requirements of the customer are met continually. The aim of QC is to ensure compliance with the quality specification and to provide economic effectiveness by eliminating causes of unsatisfactory performance at the relevant stages of the operation.

Internal audit is a process of critical review of the laboratory's processes. It is conducted by quality managers and experienced laboratory staff. Audits usually aim to monitor the accuracy, timeliness and cost of work and compliance with the quality management system, and to identify for action areas where errors can occur.

External audit involves customers or other stakeholders in the evaluation of the quality and usefulness of the laboratory's services. It facilitates comparison of methods, working practices, costs and workload between laboratories (testing sites), and forms part of the cyclic process of setting standards, examining compliance and re-examining the standards.

External quality assessment (EQA) is a way to benchmark the performance of one laboratory with that of other testing sites by comparing the results of their analyses of an identical specimen with each other's results and with the 'correct' answer. It allows laboratories to identify areas for improvement and best practice.

Assessment is the process by which an independent accrediting body inspects and evaluates the operation of an organisation that produces the product or provides the service against a relevant national or international quality standard. The factors considered vary from one standard to another, but may include the management structure, the numbers, qualifications and training of staff, the facilities available, the methods and processes adopted, the QA and QC arrangements, documentation, reporting procedures, communications within the laboratory and with users, safety and participation in EQA schemes. In essence, the accrediting body has to ensure that the organisation's quality system is sufficiently comprehensive for the operation being assessed and that the organisation consistently does what the quality system says it should. If the assessment is satisfactory, the organisation may be *accredited* for the particular operation.

Accreditation is thus one way to demonstrate the quality of an organisation's products or services to its customers. Accreditation may be voluntary or may be linked to a formal system of licensing, whereby only accredited laboratories are legally entitled to practise (or to receive payment for their services).

Quality systems

The quality system should address all stages of an operation that could affect the reliability of the end result and the monitoring of performance, so that timely corrective action can be taken where necessary. The technical requirements of ISO 17025 requires specification, determination and documentation of the: personnel; accommodation and environmental conditions; methods, method validation and measurement uncertainty; equipment; measurement traceability; sampling, handling and storage; assuring quality; and reporting results. These requirements are described below.

Personnel

Staff in toxicology laboratories should demonstrably be competent to do the range of work for which they are employed. This requires a code of ethics to which the individual must conform, clearly stated expected standards of performance and behaviour, a programme of training to help the individual attain those standards, and a mechanism in place to assess independently that they are working to the standards in the workplace. Training itself does not guarantee competence, so the assessment stage is vitally important. As soon as possible, the individual should be formally certified as competent by an appropriate authority and the scope of his or her competence should be recorded with the date on which competence was confirmed. Any extensions to scope should require the individual to go through the same process for the new area of work, and his or her competence should be reassessed regularly through task-related performance monitoring and participation in internal and external proficiency testing programmes. Job descriptions should be continuously reviewed. Continuing professional development is essential to keep up to date with developments in the field of work.

Accommodation and environmental conditions

Laboratory facilities and conditions should be suitable for the purposes for which they are being used. Contamination risks should be avoided by the use of appropriate air handling systems, designated areas for particular tasks and cleaning regimens. Attention should be given to features such as humidity, electrical supply, temperature, sound and vibration levels, and so on, and appropriate records made. Staff should have appropriate protective clothing as well as access to isolation facilities when known contaminated material, e.g. from an AIDS patient, is being analysed.

Methods, method validation and measurement uncertainty

Methods

Analytical methods should be characterised and documented fully by the use of manuals, standard operating procedures, checklists, reference data, etc. Their reliability in the specified area of application should be demonstrated before they are brought into use. Method validation ensures that methods are under statistical control and are fit for their intended purpose. Validation should cover all stages through sample selection and preparation, analyte recovery, calibration of equipment, the analysis protocol, and the assessment, interpretation and reporting of results. The measurement of uncertainty should form part of the method and reporting procedure.

Data should be controlled to ensure that they are not lost, or corrupted, and that confidentiality is maintained. Thus, computers should be adequately maintained and software validated before use. This is especially true where software is developed in house. Care must also be exercised when critical observations are made, calculations are performed and data are transcribed, to ensure that the results are recorded correctly and attributed to the right sample. It is best practice to use standard forms to facilitate these processes and to demonstrate that these records have been checked independently (e.g. by double-entry systems), where appropriate. The use of automated processes under the control of laboratory information management systems reduces the risks of error significantly.

Method validation

Validation of methods for the quantitative analysis of drugs involves determining, as a minimum:

- Selectivity
- Calibration model (linearity)
- Accuracy (bias and precision)
- Limit of quantification.

Additional parameters that might be evaluated include: limit of detection, working range, recovery, reproducibility and ruggedness under the conditions, and with the typical sample matrices that will be met in practice. For qualitative analysis, usually only the selectivity, limit of detection and ruggedness are important. Where there is a predefined threshold concentration for reported results, the accuracy and precision should be determined at the threshold level. For methods that are to be used by more than one laboratory, each laboratory should verify the method, and the inter-laboratory variation should be determined. These data should be used to define how the performance of the method is to be monitored through QC and to specify what performance is fit for purpose. If it is necessary to compare the results with those from other methods, the compatibility of data from the different methods should also form part of the validation (see Chapter 20 on method validation for a description of how to achieve this).

Any subsequent change at any stage of the method, or in the sample matrix or concentration range, will require revalidation of the method. The extent and requirements of revalidation will depend on what changes have been made.

Where a laboratory adopts an already validated method, it should demonstrate that the performance characteristics it can achieve are fit for the intended purpose of the method. Particularly important in this respect are selectivity (if the sample matrix is different), limit of detection, accuracy and precision.

In addition to analytical validation, it may also be necessary to carry out clinical validation. This requires:

- Determination of expected concentrations
- Differences associated with age, sex or other factors
- Cut-off values to classify results as 'normal' or 'abnormal', or as decision points in screening
- Clinical sensitivity ('positivity in users')
- Clinical specificity ('negativity in non-users').

Measurement uncertainty

The uncertainty associated with any measurement is made up of contributions from sample selection, sample preservation/transport, sample preparation, sample analysis and data evaluation. Those from sample selection are discussed below. During sample preparation, the uncertainty may arise from preferential selection, because of the different hardness of particles when crushing the sample, for example, through incomplete dissolution or through density separation during the attempted homogenisation of liquids. It may also result from the loss of sample material or analyte by adsorption or degradation, interference or contamination, or chemical changes in the material composition through oxidation or various other factors. Analytical measurement errors can be caused by breakdown of the analyte in the process, incomplete reaction processes, instrumental errors or maladjustments, poor calibration or matrix effects. Data evaluation errors can result from the use of incorrect or incomplete algorithms and the response can be additive, multiplicative or non-linear.

The errors that affect analytical measurements are of four types:

- Random errors, which are manifested as a spread of results of repeat determinations to higher and lower values around a mean for the sample, and determine the reproducibility or precision of the measurements.
- Systematic errors, which displace the results of measurement to higher or lower values and the existence and magnitude of which characterise the trueness of the measurement.
- Outliers, which are random errors of such large deviation that they would distort the mean if they were not eliminated.

- Gross errors, which are caused by human mistakes, or instrumental or mathematical problems, and may have either a random or systematic character.

A comprehensive guide to analytical uncertainty is available (EURACHEM 2000; also see Chapter 23 for a practical guide).

Random errors are a fundamental characteristic of the design of the processes and analytical method chosen. They can be minimised but not eliminated, and are usually characterised in terms of a confidence interval in an assumed normal distribution of results about the mean value. If this is determined simply by repeat measurement in the analytical method, the confidence interval represents only the error of the result caused by measurement. If the total error of the procedure is to be reflected in the confidence interval, parallel samples have to be selected and taken through the whole process.

Systematic errors can be recognised only if the results fall outside the random error confidence interval on one side of the mean, but they can be eliminated once their cause is known. They are best identified by analysis of chemical reference materials (where these are available), by comparison with results from independent validated methods or by EQA. The observed mean result is said to be correct if its confidence interval includes the true value.

Gross errors and outliers are usually, but not always, easy to identify and correct if other aspects of QA are working effectively.

Equipment

All equipment should be maintained and calibrated according to the manufacturer's recommendations and laboratory requirements. This may include ensuring traceability to calibration reference materials (e.g. for mass and volume measurements). Records should include:

- The name of the equipment and associated software
- Manufacturer's name, model and serial number
- Date of commissioning and verification of design qualification
- Location
- Manufacturer's instructions
- Name of person responsible for its maintenance
- Maintenance plan and records: calibration certificates, maintenance records, QC records, etc.
- Damage, malfunction or repair to the instrument.

Measurement traceability

All measurements made by a laboratory should be traceable to the international system of units (SI units). Thus, all equipment used in testing procedures should be calibrated before use and periodically tested with QC samples to prove that it is still working to specification. This is usually accomplished by the use of reference standards and reference materials.

Reference standards used for the calibration of instruments should be traceable to international standards. These may be obtained from a competent supplier of such materials to give reliable physical or chemical characterisation of the standard. Alternatively, the value of the standard may have been established by a consensus value obtained from the results of inter-laboratory trials.

Reference materials used for identification or quantification should also be traceable to international standards. There are ISO guidelines for companies providing reference materials (ISO 2000) and those so accredited should be chosen as providers of reference materials. One major supplier of forensic, clinical and pharmaceutical reference materials is LGC Standards (http://lgcstandards.com/home/home_en.aspx) and pharmacopoeial authorities provide drugs and impurities as well.

Sampling, handling and storage

The laboratory should have a plan to encompass sampling, handling and storage to ensure the integrity of the samples at all times. Records should be maintained of who was in possession of the samples for chain-of-custody purposes.

Selection

Ideally, the entire item of interest is made available for examination. However, in most circumstances this is not possible and only a sample can be provided and it is important to ensure that the correct sample is obtained in the right way by competent personnel. The use of a formal toxicology request form detailing the laboratory's requirements can be an invaluable aid to getting the correct samples.

A variety of sampling protocols from which meaningful statistical inferences can be drawn is available. Which protocol is used depends on the size of the whole material involved, its characteristics, access and location, and the sampling objective. These protocols include random sampling, systematic sampling, stratified sampling and sequential sampling.

The objective is that the results for the analytical sample can be considered as representative of the entire population or subject from which the sample originated. This primarily involves consideration of any non-homogeneity in the material being sampled. For illicit drugs seizures and biological specimens, non-homogeneity can result from segregation or separation, and from variable analyte content and distribution in the population. In drugs manufacture it can arise from periodicity in continuous processes. It can be reduced by good mixing, small particle size and taking large samples, or by repeated sampling in small increments in continuous processes.

Where drugs are encountered as discrete packages, tablets or capsules, large numbers may be available, so it would be impracticable to consider analysing them all. The issue is then to select a meaningful composite sample to obtain a result representative of the whole, to estimate either the proportion that contains drugs or the quantity of drug present. The customer or the analyst can carry out this selection, but it should be undertaken only by agreement between the two. A common approach is to select not fewer than 10 samples to form the composite if the number of packages, tablets or capsules is less than 100, and the square root of the total if there are more than 100. In postmortem toxicology, drugs may be distributed unevenly between tissues, or even within one particular tissue, and may also undergo redistribution after death. Careful consideration with respect to sampling and subsequent interpretation of results is therefore required (see Chapter 10 for a more in-depth discussion).

Collection

Samples should be collected in such a way as to avoid both loss of analyte and the introduction of contaminating substances that could interfere with the analysis and interpretation of the analytical results. The provision and use of quality-assured sampling kits, sampling materials and collection protocols is helpful in this respect, as is a comprehensive standard operating procedures policy.

For forensic purposes, it is often a requirement not only to identify what is present in a sample but also to compare this with the results from another sample, to establish that they came from the same source. It is thus vital that such samples should not come into direct contact with each other and that steps are taken to ensure that no secondary transfer of material occurs, for example via the person taking the samples or the sampling equipment.

It may be necessary in some circumstances (e.g. obtaining samples of urine for workplace drugs testing or from patients in addiction clinics) for the collection process to be supervised, to remove the risk of substitution or adulteration and thus false-negative or false-positive results (see Chapter 3). In procedures to detect doping in human sport, most international federations always provide a second portion of the urine sample from the athlete for defence use, so the sampling procedure must involve splitting the urine sample (see Chapter 6).

Preservation

Drugs and the sample matrix itself can, in some circumstances, deteriorate prior to analysis and subsequently interfere with analysis and the interpretation of the results unless precautions are taken to avoid this. It is important that the analyst is made aware if any preservative has been added so that the level present, and thus its effectiveness, can be determined, if necessary, and an appropriate analytical protocol can be used

in which the preservative does not interfere. In some areas, such as analysis for ethanol, different preservative concentrations may be needed for clinical and for forensic applications.

Packaging

The choice of sample container depends on its intended purpose and should be prescribed by the analyst. Special considerations are required to avoid loss of analyte or leakage of sample where volatile and liquid materials are involved. Additional packaging requirements may also need to be specified to protect sample containers against damage during transport to the laboratory for examination or to comply with regulations that apply when body fluids are involved.

For forensic purposes, samples must also be sealed in such a way that any evidence of tampering would be evident.

Identification

It is essential that sample containers and any intermediate containers used to carry the sample be labelled in sufficient detail to remove any doubt about the sample origin. It is necessary to have documentation as to who took the sample, where it came from, and when and how it was obtained. A unique identification number that accompanies the sample at all stages is a valuable safeguard. Transposition errors in clinical analysis can prove harmful or even fatal to the patient. In the legal context, it must also be possible to demonstrate the chain of evidence through a record of who had possession of the sample, what they did with it and when.

Storage

If the sample cannot be analysed immediately, it must be stored at an appropriate temperature in a safe and suitable environment, accessible only to authorised staff, so as to ensure its security and integrity. In some circumstances, these considerations may also have to be applied to the transport and continued storage of samples after analysis.

Where the laboratory deals with both trace amounts of material (e.g. illicit drugs on balance pans or other paraphernalia) and bulk drugs, it is essential prior to analysis to separate spatially or temporally those activities that may result in contamination. It is also important for QA purposes to have effective cleaning regimes in place before, between and after the examination of separate samples, and to control and monitor the environment carefully (clothing, benches, equipment, etc.) in the trace analysis laboratory.

Transport

Transport is normally the responsibility of the customer, but there will be constraints on what can be transported or posted legally. Where drugs are involved, even in the smallest quantities, transportation across national frontiers is a criminal offence without appropriate import/export licences. Temperature and other environmental factors may also need to be controlled.

Sample disposal

There must be a clear policy in place for the disposal of materials on completion of examination and analysis. In some instances, this involves returning what remains of the samples to the customer, subject or legal custodian. In other cases, the samples need to be destroyed safely, immediately or after a specified time period, and statutory requirements may have to be observed in doing this. However, in no circumstances should anything be disposed of without the customer's or other relevant authority's consent, and records should be maintained of what has been disposed of and what has been retained.

Assuring quality

Appropriate QC procedures should be in place to monitor calibrations and the results of analyses to ensure their accuracy.

In-process performance monitoring

Internal QC assesses, in real time, whether performance is sufficiently similar to the individual laboratory's own previous performance for the results to be reported. It helps control reproducibility (precision) and facilitates continuity of service over time. Most internal QC procedures employ analysis of a control material and comparison of the results obtained on this with preset limits of acceptability, which thus allows unsatisfactory sets of results to be identified and corrected before release to the customer.

For qualitative testing, it is important to include characterised positive and negative control materials with each batch of analyses. In both qualitative and quantitative analysis, it is essential that these QC samples be taken through as much of the process as is possible, otherwise errors may go undetected. Sample collection may represent a major source of variation that is not possible to control using internal QC measures and so must be minimised through careful training and supervision procedures.

Some internal QC procedures can be simple and inexpensive to implement, and include:

- Recording lot numbers of all reagents, calibrants and controls used, with particular attention when reagent and/or calibrant lots change
- Recording and monitoring instrument readings (e.g. absorbance for the calibration material) as a check on reagent and instrumental drift
- Recording and monitoring assay properties (e.g. non-specific binding and signal for zero and highest standards in immunoassays) as a check on drift
- Analysis of one or more samples (at different concentrations) from the previous analytical batch, as a check on assay stability (provided that the samples are stable for the intervening period).

These procedures do not require sophisticated statistical techniques or expensive materials, but can provide invaluable information on assay performance.

Replication

The validity of results is sometimes checked by repeating the analysis of a proportion of the samples. This can be achieved in a variety of ways, such as by:

- The laboratory itself carrying out the repeat analyses, either openly or blindly
- Comparison of results obtained by the laboratory with those from samples submitted independently to reference laboratories
- Selecting an appropriate percentage of samples reported as negative and as positive by the laboratory and re-examining these in a reference laboratory.

Statistical monitoring of performance

Most in-process performance monitoring procedures rely on introducing QC samples into each batch of analyses. These samples must be stable and of known, reproducible composition, and the results obtained on them must be able to reflect the assay's performance with test samples. Graphical or statistical analyses can then be applied to the results to confirm whether or not the analytical process is 'in control' and thus whether or not the results can be reported. It is important that different materials are used for the calibration and in-process performance monitoring functions, or the monitoring will not be effective.

The number of control samples included in each batch processed depends on the size of the batch and its homogeneity. For the routine analysis of large numbers of samples of a similar type it is common to have 5–10% as controls, with a minimum of two per batch. For smaller, inhomogeneous batches this level may need to be increased to 25% or even more.

Shewhart QC charts are plots of measurements on the control samples over time in relation to the assigned value for the analyte, with upper and lower warning limits related to the customer requirement or the expected standard deviation (SD) when the process is under control. These charts can be used to plot single values of measurements, means, medians, blank values, SDs, ranges, etc. Decision making on whether to accept a batch of results or to take corrective action is facilitated if horizontal lines are drawn at the target value and at 2 and 3 SD above and below this value. If the analysis is in control, the results are scattered randomly above and below the assigned value, with a distribution such that only 5% are more than 2 SD from this value and only 1% more than 3 SD from it. Bias causes a shift to higher or lower values, while loss of precision yields a wider scatter of results. A result of

more than 2 SD should act as a warning to investigate the method to avoid future problems, and a result of more than 3 SD should prompt rejection of the batch and investigation of the problem before the analyses are repeated. Trends away from the mean should particularly be investigated.

More complex and effective control rules have been formulated, with validation of their power to reject unsatisfactory batches and accept satisfactory batches. The so-called 'Westgard rules' are based on the analysis of two controls in each batch (usually one with 'normal' and one with 'abnormal' values). If both results fall within 2 SD of the target value (the 'warning' limit) the batch is accepted, but otherwise the remaining rules are evaluated in turn and the batch is rejected if any fail. If none fails, the batch is accepted but the situation should be investigated before the next batch is analysed.

Where automated systems incorporate internal QC software, this should be reviewed to confirm its appropriateness to the application.

Cusum control charts provide an alternative sensitive method of identifying when processes are out of control by displaying cumulative deviations from the assigned value.

Work undertaken on analytical goals in relation to biological variation provides a rational basis for limits of acceptability. Initial studies addressed primarily precision criteria, with the general guidance that analytical imprecision should not exceed half the within-individual variation, but the scope has been broadened to include assessment of factors such as bias, interference and exogenous analytes such as drugs. Continued development may be expected to provide a rational basis for future performance standards.

Materials for internal quality control and external quality assessment

The materials used as controls for internal QC or EQA purposes must be selected to behave in the analyses as similarly as possible to routine samples. They must also be homogeneous, stable, safe, available in sufficient quantity and affordable.

Many QC materials are obtained from commercial sources and are produced in large quantities to specified quality standards. However, these standards may not always cover the properties of interest and care still has to be taken to ensure that they are fit for the particular analysis required. For example, materials may be guaranteed to be 95% pure, but the impurities may not be specified; these impurities could interfere with the analysis required. Also, biological materials may be stabilised by lyophilisation and their behaviour may therefore not mimic that of the test samples (e.g. matrix effects). It is therefore essential when using commercial materials to be aware of their often limited range of suitability (sometimes to one system only) and to test their suitability. It is good practice to use third-party controls (i.e. from a different manufacturer from those of the reagents and instrument used).

An alternative is to prepare the QC materials locally. However, reproducibility of preparation and homogeneity of such materials is sometimes difficult to achieve and, where a biological fluid matrix is involved, the addition of pure analyte may not produce a control sample directly comparable with a routine sample. In some circumstances, it may be possible to obviate this by obtaining samples from volunteer donors (e.g. serum or urine known to contain the analyte of interest) and to use these singly or pooled to achieve the concentration levels required. However, both ethical and safety issues need to be considered in this approach.

Proficiency testing

Participation in appropriate proficiency testing programmes is now becoming mandatory in many areas of toxicology, see below.

Reporting the results

Once analytical results are obtained, the analyst needs to be certain of their validity. This involves checking that the results of the in-process performance monitoring fall within the limits of acceptance and that the test results can be demonstrated to relate to the appropriate sample. The effects of transposition or transcription errors can often be severe.

It is also advisable to apply a common-sense test of plausibility to the results in the circumstances known about the sample or against previous experience of samples of the same type or from the same source. Specimen source (e.g. femoral or cardiac blood *post mortem*) and sampling time may also be relevant. An 'unusual' result may not be wrong, but usually merits further investigation.

A report should be accurate, clear, unambiguous and objective to answer the question(s) posed by the customer. A standard report format is very helpful to the customer and results should be reported in appropriate units, preferably written out in full to avoid confusion (e.g. between mU/L and μU/L), with appropriate reference data or information to help the customer understand their significance. This may, for example, be a reference to legislation, production criteria, published details in the scientific literature or the expected values for clinical investigations. It may also be a requirement to indicate the measurement uncertainty.

Reports should contain the following:

- Title
- Name and address of the laboratory where the tests were performed
- Unique identification of the report, unique page numbering and identification of its end
- Name and address of the customer
- The methods used
- Identification and description of the items received and tested, together with their date of receipt
- Sampling plan
- Test results with appropriate units of measurement
- Name(s), function(s) and signature(s) of those authorising the report
- A statement explaining that the results relate only to the items tested.

Any opinions or interpretations should be clearly differentiated from the test results in the report.

Management requirements

Although meeting the technical requirements for laboratory work is vital, having appropriate management systems is just as important. The management requirements of ISO 17025 encompass: organisation; management system; document control; review of requests, tenders and contracts; subcontracting; purchasing; service to the customer; complaints procedure; control of non-conforming testing and/or calibrations; improvement; corrective action; preventive action; control of records; internal audits; and management reviews. These requirements are described below.

Organisation

The laboratory should be organised to continually satisfy the agreed needs of the customer and the accrediting authority. The staff of the laboratory should have the authority and resources to accomplish this. In addition, the staff should not have any undue internal and external commercial, financial and other pressures that may adversely affect their work. In this regard there should be appropriate policies to prevent any loss of confidence in the work of the laboratory. There should be an organisational chart defining the relative positions of quality management, technical operations and support services within the laboratory and any parent organisation. Associated with this should be specified the responsibilities and authorities of all staff who carry out, or are associated with, the tests. In addition, there should be adequate supervision of testing and calibration staff.

The position of quality manager is crucial within the organisation and their position should be clearly shown in the organisation chart. It is their job to ensure that the management system is working at all times and it is vital that they have direct access and the support of the highest level of management in order to do this. In their turn, the highest level of management must support the staff in every way possible to carry out their jobs and have excellent communication with them. The quality manager's main tasks are: ensuring that processes needed for the management system are established, implemented, maintained and kept

current; monitoring compliance of the provider's staff with the management system; ensuring appropriate validation/verification of new technical procedures; selecting and training internal auditors and evaluating their performance; reporting to top management on the performance of the management system and any need for improvement; recommending training to improve the quality of the provider's staff; and ensuring the promotion of awareness of the customer requirements throughout the organisation (Forensic Science Regulator 2009a). The quality manager needs to have the trust and support of the top management and staff alike and needs to be diplomatic in nature as well as having sufficient experience, knowledge, communication skills, seniority and authority to successfully carry out the job.

Management system

The management system must be fit for the tasks carried out by the laboratory and its policies should be documented in a quality manual. Among the most important contents of the quality manual is the top management's commitment to the development and implementation of the management system and to continually improving its effectiveness. The manual gives the structure of the documentation as well as a list of the individual documents used in the management system such as the supporting procedures and the technical procedures for testing.

Document control

All the quality management documents should be controlled by having authorised personnel to approve them. These personnel sign and date each version of a document, with a future review date, and ensure that old copies are replaced by the new version at all appropriate locations of the laboratory where they are to be used.

Review of requests, tenders and contracts

It is useful to review the requests made by customers to ensure that their requirements may be adequately met by appropriate methods and that the laboratory has the resources to meet these needs. The tender contents may be subject to negotiation with the customer before the contract is signed but, on signing, both laboratory and customer should agree its contents. If changes to the contract are made as the work progresses, these too should be agreed and documented and all relevant personnel should be informed. Where there is deviation from the contract, the customer should be informed promptly.

Subcontracting

Laboratories often contract work because another specialist laboratory has a specific expertise or when a larger than normal workload makes it effective to have some of the work temporarily carried out by another laboratory. A register should be maintained of all such subcontractors, who should also be compliant with ISO 17025. Customers should be informed that some of the work is being carried out by a subcontractor.

Purchasing

A laboratory needs a policy and procedures for the selection and purchasing of services and supplies used that affect the quality of its tests. Procedures need to exist for the purchase, reception and storage of reagents and laboratory consumable materials relevant for the tests and calibrations.

Service to the customer

Communicating with the customer to clarify their requests and to monitor the laboratory's performance in meeting their requirements should be continuous. The use of standard forms is very useful in ensuring that the analyst has all the relevant information before the analyses are made. This is especially important when interpretations of the results of the analyses are to be made. Getting the customer's feedback, whether it is positive or negative, is particularly helpful to continuously improve the laboratory's performance.

Complaints procedure

This is particularly important to allow managers of laboratories to monitor how well the laboratory is satisfying its customers. Complaints should be documented, speedily resolved and reviewed on a regular basis.

Control of non-conforming testing and/or calibrations

There needs to be policy and procedures for the situation when non-conforming work, i.e. work not complying with the laboratory's procedures or agreed customer requirements, has been carried out. Corrective action should be made immediately. If such non-conforming work has already been reported, the customer should be informed and the relevant work/report recalled.

Improvement

Laboratory personnel should have continuous improvement in their minds at all times. This applies to all aspects of the laboratory's performance and regular reviews of performance at all levels are useful in this respect. Among methods of formally examining a laboratory's efficiency are:

- *Lean approach* – a systematic approach for identifying and eliminating waste through continuous improvement (Chalice 2007)
- *Six sigma* – a disciplined data management approach and methodology for eliminating defects and variability (driving towards 6 SD between the mean and the nearest specification limit) in any process (Pande, Holpp 2002)
- *Total quality management* – to identify the root causes of defects and implement solutions to stop their recurrence (Oakland 2003).

Corrective action

When non-conforming work has been identified, suitable corrective action should follow. When the root cause(s) of the problem have been determined, the actions to rectify the problem and prevent a recurrence should be put in place and documented. Learning from errors through improvement and corrective actions is an important element of any QA regime. Such changes need to be reinforced through staff training and audited post implementation to ensure that they are effective.

If issues related to sample identity or integrity are identified, the problem is likely to be caused by poor sample collection or identification, data transposition, sample handling errors or contamination, and changes to the process should be introduced to prevent recurrence.

Where issues are indicated through the statistical monitoring of performance, the nature of the problem depends on whether it has resulted in the analysis becoming imprecise (random errors) or biased (systematic errors). Loss of precision can be caused by:

- Specimen non-homogeneity (uneven particle size, poor mixing, clots, etc.)
- Inconsistent amounts of sample or reagents being taken
- Instability in the instrumentation employed or the analysis conditions.

Possible causes of bias include:

- Incorrect sample or reagent volumes being routinely used
- Deterioration of calibration material
- Incorrect settings on the instrumentation
- Incorrect analysis conditions
- Systematic calculation errors.

Preventive action

It is obviously better to prevent problems occurring than to have to put them right after they have happened. To do this, there should be a proactive process to identify opportunities for improvements, and potential weaknesses and sources of non-conformities.

Control of records

It is vital that all records of all types are maintained adequately. This includes procedures for identification, collection, indexing, access, filing, storage, maintenance and disposal of these records. A regular back-up system for electronic records is essential to prevent the loss of information. When a mistake is made in a record, it should be crossed out and the correct entry put alongside together with the initials of the person making the correction, so that it is obvious who made the correction and what it was.

Internal audits

Regular audits of a laboratory's activities should be made to ensure that the operations are conforming to all the requirements of the management system. It is best that these audits are carried out by competent people who are independent of the activity being audited to provide an objective view of that activity. If the audit indicates that there is non-compliance in an activity, the normal procedure for corrective action should be undertaken, which could include informing the customer of the situation if that is relevant.

Management reviews

The top management of a laboratory should carry out a regular review of the laboratory's management system and all its testing activities to determine that everything is working properly or that some improvements or changes are necessary. These reviews are normally conducted annually as they are at such a high level. The review should take into account the outcomes of audits, corrective and preventive actions, assessments by outside bodies, results of inter-laboratory or proficiency tests, customer feedback and complaints. Any actions flowing from such reviews should have agreed timescales attached to them to ensure that they are completed in a timely manner.

Quality standards

There are a number of quality standards that are used internationally by laboratories to ensure that their results may be relied upon and as a basis for proving their competence by accreditation bodies. In addition, there are guidelines produced by professional bodies and learned societies to assist laboratories in their work. The main quality standards and protocols used in the analytical sector are ISO 9000, ISO/IEC 17025, ISO 15189, and Good Laboratory Practice and Compliance Monitoring. These standards are described below.

ISO 9000

ISO 9000 'Quality management' (ISO 2009) is a series of 18 standards that represents an international consensus on good quality management practices. It consists of standards and guidelines relating to quality management systems and related supporting standards. Of these standards, ISO 9001:2008 is the standard that provides a set of standardised requirements for a quality management system, regardless of what the user organisation does, its size, or whether it is in the private or public sector. It is the only standard in the family against which organisations can be certified, although certification is not a compulsory requirement of the standard. The other standards address fundamentals and vocabulary, performance improvements, quality plans, projects, configuration management, measurement processes and measuring equipment, system documentation, realising financial and economic benefits, training, the selection and use of consultants, quality and/or environmental management systems auditing, complaints handling, codes of conduct for customer satisfaction and dispute resolution.

ISO/IEC 17025

ISO/IEC 17025: 2005 'General requirements for the competence of testing and calibration laboratories' (ISO/IEC 2005) specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. The general requirements have already been given above under the sections on quality systems and management requirements. The standard covers testing and calibration performed using standard methods, non-standard methods and laboratory-developed methods. It is applicable to all organisations performing tests and/or calibrations. This includes subcontracted laboratories and laboratories where testing and/or calibration forms part of inspection and product certification. The standard is applicable to all laboratories regardless of the number of personnel or the extent of the scope of testing and/or calibration activities. When a laboratory does not undertake one or more of the activities covered by ISO/IEC 17025:2005, such as sampling and the design/development of new methods, the requirements of those clauses do not apply. ISO/IEC 17025:2005 is for use by laboratories in developing their management system for quality, administrative and technical operations. Laboratory customers, regulatory authorities and accreditation bodies may also use it in confirming or recognising the competence of laboratories. The standard is not intended to be used as the basis for certification of laboratories. Compliance with regulatory and safety requirements on the operation of laboratories is not covered by ISO/IEC 17025:2005.

ISO 15189

ISO 15189:2007 'Medical laboratories – particular requirements for quality and competence' (ISO 2007) specifies requirements for quality and competence particular to medical laboratories. It is for use by medical laboratories in developing their quality management systems and assessing their own competence, and for use by accreditation bodies in confirming or recognising the competence of medical laboratories. The standard requires an ISO 9000 quality system and includes the consultative and interpretative elements as well as responsibilities for point-of-care testing.

Good Laboratory Practice and Compliance Monitoring

The Principles on Good Laboratory Practice (GLP) guidance (OECD 1998) is one of the Organization for Economic Development and Cooperation (OECD) series on Good Laboratory Practice and Compliance Monitoring, which evolved in response to the need to regulate the design, conduct and reporting of studies carried out in support of the licensing of pharmaceuticals and other chemicals for human or animal use. It is recommended for the setting up and maintenance of a laboratory QA system; compliance with its principles is applied mainly to analytical chemical laboratories concerned with genetic, clinical, pharmacological, toxicological and other biochemical studies, in particular where laboratory animals are used. Its main function is to enable licensing authorities to reconstruct research studies exactly and to internationally recognised standards.

There is no conflict between GLP and ISO/IEC 17025, and the GLP regulations can be considered as complementary to the general criteria for specialised chemical laboratories. However, on its own, GLP does not provide an assurance of fitness for purpose.

Guidelines

Specific guidelines have also been produced in some areas of drugs testing: Mandatory Guidelines for Federal Workplace Drug Testing Programs; SOFT/AAFS Forensic Toxicology Laboratory Guidelines; UK Laboratory Guidelines for Legally Defensible Workplace Drug Testing; and TIAFT Laboratory Guidelines for Toxicological Analysis. These guidelines are described below.

Mandatory Guidelines for Federal Workplace Drug Testing Programs

These guidelines were developed by the Department of Health and Human Services (DHHS) in the USA and contain comprehensive standards for laboratory testing for drugs in the workplace. They were first

published in the Federal Register in 1988, revised several times with the latest version published in 2008 (DHHS 2008). The DHHS also developed the National Laboratory Certification Program to certify compliance with the standards by laboratories that test specimens collected for the federal agency drug testing programmes. The National Laboratory Certification Program includes a requirement to participate in a performance testing programme that includes blind sample submissions.

SOFT/AAFS Forensic Toxicology Laboratory Guidelines

The Society of Forensic Toxicologists (SOFT) and the Toxicology Section of the American Academy of Forensic Sciences (AAFS) first produced these guidelines in 1991. They have been revised four times since then and the current version is the 2006 version (SOFT/AAFS 2006). They cover both postmortem forensic toxicology and human performance forensic toxicology, with the exclusion of forensic urine drugs testing (which is covered by the DHHS Guidelines and College of American Pathologists' Accreditation Program), and provide detailed guidance for laboratory practices. They also originally contained a checklist for self-evaluation and preparation for accreditation, but this was removed when it was adopted by the American Board of Forensic Toxicology as the basis of their Forensic Toxicology Accreditation Program in 1996.

UK Laboratory Guidelines for Legally Defensible Workplace Drug Testing

These guidelines have adopted the general principles established internationally and represent an overview of best practice in the UK. They were prepared by a steering group representing UK analytical laboratories and other parties (Workplace Drug Testing Forum 2001). The guidelines are also now being used as the basis for new European guidelines for best practice. They focus on urine specimens, but are equally applicable in principle to all specimen types.

TIAFT Laboratory Guidelines for Toxicological Analysis

The International Association of Forensic Toxicologists (TIAFT) was one of the first organisations to produce guidelines for toxicology laboratories when they produced their Laboratory Guidelines for Toxicological Analysis in 1993 (TIAFT 1993), which were intended to serve as a basis on which adequate working practices and methodologies could be developed. The detection and identification sections of these guidelines were expanded in 2003 (TIAFT 2003).

External quality assessment

External quality assessment addresses differences between testing sites, for comparability of results. It usually involves the analysis of identical samples at many laboratories and comparison of the results with each other and the 'correct' answer. The process is necessarily retrospective. Tests where the intention is to determine whether the laboratory is proficient in carrying out particular analyses should be differentiated from those inter-laboratory trials where the intention is to set a value for a new reference standard or to investigate the capabilities of a new technique or method.

Purpose of external quality assessment

EQA is concerned primarily with the assessment of an individual laboratory's performance and is one means for the laboratory to identify areas for improvement. However, this is only truly possible if the EQA samples have been processed in the same way as routine samples.

It is essential that the EQA scheme provider respects the confidentiality of the laboratory's performance data, but EQA can give information on the following:

- Overall inter-laboratory standard of performance and best practice
- Influence of variations in analytical procedures (e.g. methods, reagents, instruments and calibration)
- Quality of the samples provided.

Such information can be useful to participants (e.g. in identifying more reliable procedures and encouraging adoption of best practice).

Participation in an EQA scheme should thus be seen as essential to the maintenance of professional standards and a requirement for accreditation. Results should not be used to police or license laboratories, and the level of confidentiality of data must be agreed between the scheme and participant laboratories.

Selection of an external quality assessment scheme

Where alternative EQA schemes are available, laboratories may need to make a choice between them, though participation in more than one can provide complementary information. Factors to be taken into account when making the selection include:

- Scheme design validity, especially sample appropriateness and concentration range, report and scoring quality
- Independence from manufacturing and marketing interests
- Scheme service and responsiveness, including turnaround time and response to enquiries
- Reputation and previous experience
- Accreditation status
- Cost (value for money).

Scheme design

The theoretical and practical principles of EQA across all analytical sectors have been comprehensively reviewed in ISO/IEC Guide 43 (ISO/IEC 1997). The World Health Organization has also provided recommendations for EQA scheme design and operation in the clinical sector.

Participants should have confidence in the scientific validity of the scheme design and the reliability of its operation, or they will not take action on information from the scheme. Experience with many schemes indicates the essential design criteria to be:

- Provision of EQA samples that are stable, homogeneous and behave like routine samples
- Reliable qualitative and/or quantitative data and target values for the analytical results
- Appropriate frequency of distribution of the EQA samples
- Rapid feedback on initial performance to participants after analysis
- A scoring system that is clear, robust, reliable and amenable to trend analysis
- Consistent and reliable assessment
- Well-structured, informative and intelligible reports.

Scoring systems

Suitable scoring systems are available for qualitative and quantitative investigations and were introduced to make EQA information more comprehensible to participants. Combination of information for an analyte, which includes results within a rolling time window, should permit appraisal of performance relative to other participants at that time or to the individual laboratory's past performance. For the latter, the score must be independent of other participants' performance.

The deviations from target value may be scaled to reflect the general standard of performance and to yield scores in a 'common currency'. This is important where attainable performance is concentration related (in many cases agreement is substantially worse at low drug concentrations) and a combination of percentage deviations across the full concentration range is inappropriate.

Report format

The most helpful scoring system will fail in its objective if the scores are not presented in such a way as to simplify the interpretation. Each

participant has only limited resources (in terms of time, effort and ability) to devote to this interpretation, and experience suggests that those in most need to act as a result of EQA data devote the least resources. A well-designed combination of scoring system and report format can assist considerably, and thus contribute not only through stimulation of improvement, where this is indicated, but also by removing the need for unnecessary investigation.

Target values

If participants are to have confidence in taking action based on EQA data, the targets against which performance is assessed are critical. The primary purpose of EQA is bias assessment, so the target should ideally be traceable.

The main approaches to determine target values are:

- From the known amount of analyte added during preparation (added analyte value)
- From analysis in one or more laboratories using a reference method (reference method value)
- From analysis in one or more reference laboratories (reference laboratory value)
- From the mean or median derived from participants' results (consensus value).

Target values for drug assays cannot always be predetermined from the amount of added analyte because of differences in behaviour between endogenous and added analyte and the effects of metabolites or adulterants. Depending on assay specificity, metabolites may constitute interferents or part of the measured analyte.

Reference method values are theoretically the best to use, but such methods are not available for all analytes and the cost of obtaining them may be prohibitive. Also, the EQA materials may not behave exactly as routine samples and they may produce different values by different methods through matrix effects. This can lead to incorrect conclusions being drawn about method performance.

Reference laboratory values take account of method variation and the laboratories are usually selected on the basis of past reliable performance. They can thus provide a practical benchmark for which other participants can strive.

There is no scientific reason for consensus values to be accurate, but practical experience shows that in most cases the mean of many participants' results is sufficiently reliable to be used as the target value, given robust procedures for elimination of outliers. They are also convenient to obtain, available when required and essentially free. However, their validity must not be assumed, and should be demonstrated through repeatability, recovery and comparison studies with other EQA schemes wherever possible. A subset of participants who satisfy these criteria may be used.

How to use external quality assessment data

When assessing its performance in an EQA scheme, the laboratory should always:

- Check that the report is the correct one for the laboratory (i.e. it has the correct laboratory code)
- Check that the results are those obtained and reported by the laboratory (i.e. there have been no transcription or transposition errors)
- Examine the deviation from target for each sample and analyte in the distribution
- Compare current performance scores with those of other participants and particularly other users of the same method
- Examine its performance scores for trends
- Compare the relationship of the mean value for the method used by the laboratory against the target value
- Examine the relationship between the laboratory's results and target values for several previous distributions.

As with all quality system elements, the outcome of this assessment should be recorded. Where an apparent problem is noted, it should be investigated and both the investigation and conclusion (whether action or inaction) documented. Accreditation inspectors can learn more about a laboratory's competence from their record of resolving problems than from current performance scores. EQA data should always be considered in conjunction with current internal QC performance.

Use of new techniques

The use of information gained from the use of new techniques has always been of concern to courts as the new techniques may not have been proven to be capable of giving accurate results by the scientific community. Thus laboratories should have a robust policy of introducing new techniques into the laboratory and using the results from such techniques in evidence presented in court.

In the USA, the challenging of the admissibility of evidence gained from the use of new techniques has a long history and a major milestone was the *Frye* judgement in 1923 (*Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923)).The D.C. Circuit Court held that scientific evidence was admissible only if it was based on a scientific technique generally accepted as reliable by the scientific community. Thus, an expert witness testimony was admitted in court based on the expert's credentials, experience, skill and reputation. It was believed that any deficiencies or flaws in the experts' conclusions would be exposed through cross-examination. This decision became known as the *Frye* test or the general acceptance test.

In 1993 the US Supreme Court ruled that the Federal Rules of Evidence passed by the US Congress in 1975 (Act to Establish Rules of Evidence for Certain Courts and Proceedings, Pub.L. 93-595, 88 Stat. 1926. (1975)) in effect had overturned the *Frye* standard and a new standard known as the *Daubert* standard was introduced (*Daubert v. Merrell Dow Pharmaceuticals*, Inc. (1993) 509 U.S. 579, 589). There are four major points to a *Daubert* standard concerning the admissibility of the results of a new technique in court:

1. Has the technique been tested in real conditions?
2. Has the technique been subject to peer review and publication?
3. What is the known or potential rate of error?
4. Has the technique been generally accepted within the relevant scientific community? Which is effectively the same as the Frye test.

Although the *Daubert* standard was developed in the USA, it has had an influence around the world. For example, in the UK, the House of Commons Science and Technology Committee recommended the creation of a Forensic Science Committee to regulate forensic science in the UK and further recommended (House of Commons Science and Technology Committee 2005) that

> The absence of an agreed protocol for the validation of scientific techniques prior to their being admitted in court is entirely unsatisfactory. Judges are not well-placed to determine scientific validity without input from scientists. We recommend that one of the first tasks of the Forensic Science Advisory Council be to develop a 'gate-keeping' test for expert evidence. This should be done in partnership with judges, scientists and other key players in the criminal justice system, and should build on the US *Daubert* test.

It is likely that other countries will follow this lead and forensic scientists across the world will have to have in place a robust procedure for the introduction of new techniques in their laboratories as part of their quality management system.

The accreditation process

A laboratory that seeks accreditation must first develop a documented quality management system in a form that addresses all the requirements of the relevant quality standard for the range of activities that it wishes to be included in its scope of accreditation. Assessors from the accrediting body review this and may make a pre-assessment visit to the laboratory to help identify areas that still need to be addressed. Once the necessary follow-up actions have been completed, the assessors

return to carry out the formal assessment. Any further deficiencies in the quality management system and any perceived non-compliances of practice in relation to the documented quality management system are brought to the laboratory's attention. If the laboratory responds satisfactorily within the specified timescale, accreditation is awarded. The awarding body carries out regular surveillance visits to monitor continued compliance with the standard and there is normally a process for formal re-accreditation after a few years.

In many countries there are established accreditation bodies that carry out the accreditation of laboratories and regularly audit them afterwards to maintain the accreditation. In addition, there is the International Laboratory Accreditation Cooperation (ILAC) which is an international cooperation of laboratory and inspection accreditation bodies formed more than 30 years ago to help remove barriers to trade. They have provided Guidelines for Forensic Science Laboratories to be used by accreditation bodies to provide appropriate criteria for the assessment and accreditation of laboratories providing forensic services (ILAC 2002). Individual laboratories can be kept up to date by organisations such as the European Network of Forensic Science Institutes (ENFSI) who raise awareness of member laboratories on international accreditation standards and provide the guidance available to achieve accreditation. Sometimes governments of countries consider accreditation so important that they conduct the process themselves. For example, in Belgium, the accreditation of clinical chemistry laboratories is carried out by the Belgian Ministry of Public Health who lay down the regulation requirements.

In some countries this accreditation will now be checked by the regulators of forensic science. For example, in the UK, the Forensic Science Regulator has made arrangements for the United Kingdom Accreditation Service to carry out audits on his behalf to the Standard set out by him and to advise him of failure to comply with its requirements (Forensic Science Regulator 2009a).

The future

There is no doubt that forensic scientists and laboratories will more and more have to prove to their customers that they get the correct answers. Accreditation by third parties to international standards will become increasingly the case and in some countries is already mandatory. This can only improve the quality of work of laboratories and should be viewed as a benefit and not just an extra cost.

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CHAPTER

23 Measuring and Reporting Uncertainty

MA LeBeau

Introduction

To the layperson, uncertainty suggests doubt. To others it may suggest error. This confusion can create problems in the area of forensic chemistry and forensic toxicology when the term is first introduced to judges, attorneys and jurors. This chapter will address the concept of measurement uncertainty, explain some of the more common approaches to estimating uncertainty, introduce a simple eight-step approach for this estimation, and provide examples of how this approach can be applied in forensic chemistry and toxicology laboratories.

The choice of the term 'uncertainty' is unfortunate because the true intent of the process of determining measurement uncertainty is to express the level of certainty or confidence that can be placed on a given measurement. The International Organization for Standardization (ISO) defines *uncertainty* (*of measurement*) as 'a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand' (ISO/IEC 2007).

To evaluate the uncertainty associated with the determination of the amount of an analyte – whether it be expressed as mass, concentration, purity or volume – the analyst must carefully consider all the possible sources that contribute to the uncertainty in that particular measurement. While this may at first take a great amount of effort, once established, it can easily be applied to subsequent measurements obtained by the same method in the same laboratory provided that neither the procedure nor the instrumentation changes. Additionally, a good estimate of a method's uncertainty can be made by focusing on the most significant sources of uncertainty within that method.

On this note, it is very important to understand that the true uncertainty of a measurement will never really be known. To establish the range of values within which the true measurement result is expected to lie at a given level of confidence, reasonable approximations must be made. Therefore, it is useful to remember that measurement uncertainty is a good-faith estimate of this range of possible values and is not an absolute range. This is because of the vast number of variables that contribute to measurement uncertainty (e.g. sampling, matrix effects, interferences, environmental sources, equipment, instrumentation, assumptions and random variations). Later in this chapter, real-life examples will be presented to allow the reader to begin to recognise the most significant sources of uncertainty in their measurements.

Error and measurement uncertainty

A common mistake is to confuse measurement uncertainty with error. Uncertainty is not error. Error is the difference between a true value and the value that is measured by a given technique. So to determine error, the true value of the measurand must be known. Obviously, the true value of the substance being measured in forensic chemistry or forensic toxicology is usually not known, otherwise laboratories would not need to perform the analysis in the first place.

In contrast, uncertainty is a calculated range that does not require the true value of the measurand to be known. In fact, when uncertainty calculations are performed correctly, they allow the analyst to say, with a given confidence level, that the true value has a high probability of falling somewhere within the calculated range. Further, uncertainty calculations allow for the reliability of a result to be assessed or for different measurement results (e.g. by different techniques within the same or different laboratories) to be compared. Most importantly, measurement uncertainty allows for an assessment of the confidence that can be placed on a result when that result is to be used in decision-making (evaluation of drug concentrations, courts of law, compliance evaluations, etc.).

A practical example can demonstrate the importance of this concept. Say that two different laboratories perform a quantitative analysis for ethanol in the same blood sample. The first laboratory reports a result of 910 mg/L The second laboratory reports a result of 760 mg/L. To the average person looking at these results, they would likely conclude that one result is correct and the other is incorrect. If both laboratories were instead to report their results as 910 ± 140 mg/L (95% confidence level) and 760 ± 30 mg/L (95% confidence level), it becomes very easy for the results to be properly compared and evaluated for credibility. When results are reported as the measured value with the estimated uncertainty at a defined confidence level, it is known as the *expression of uncertainty*.

Traceability and uncertainty

Traceability is another important concept in that it allows results from different laboratories (or results from the same laboratory conducted at different times) to be compared. It is established through a chain of calibrations that can be directly linked to a primary national or international standard.

Traceability provides the means of placing all related measurements on a consistent measurement scale, while uncertainty characterises the strength of the links in the chain and the agreement to be expected between laboratories making similar measurements.

There are many ways to help establish traceability in a measurement. One way is to ensure that the standards used to calibrate the measurement equipment can be linked back to a primary national or international standard, whenever possible. Another complementary or separate technique is to use a standardised method in performing the measurement or, alternatively, to compare the results of a method with those of a standardised method. Pure reference materials or appropriate matrix-matched certified reference materials can be used. The use of accepted, closely defined procedures will help establish traceability in a measurement as well.

Establishing traceability is particularly important when making critical measurements that will be used in determining an individual's guilt or innocence or will play a role in an individual's sentencing by a court. This is less of an issue in postmortem toxicology where the primary goal in most cases is to determine whether the decedent had traces of a drug or metabolite, as opposed to a therapeutic or lethal amount.

Methods for estimating the uncertainty associated with a quantitative method

Many forensic chemists and toxicologists envision that there will be a great degree of rigor and difficulty involved in estimating the uncertainty in a quantitative method, which causes reluctance to embrace the process. The reality is that there are many different options in *how* measurement uncertainty can be estimated. Some are very time-consuming and should be left only to statisticians. Others are overly simplistic and will mislead the laboratory's customers. A good rule of thumb is to base the amount of effort put into the uncertainty estimate on the intended use of the results and the requirements of the laboratory's customers.

Some laboratories choose to rely solely on professional judgement in estimating the measurement uncertainty of a method. Others may consider their repeatability data associated only with controls. Both of these techniques may be too simplistic for many quantitative measurements in forensic laboratories, but again, will be based on the intended use of the results.

Conversely, there are very advanced techniques that require sophisticated computer programs or an extensive amount of work and effort for each individual case that is undertaken by the laboratory. Obviously, this is likely to be far more than what is needed for most quantitative work in forensic laboratories with, perhaps, the exception of those that calibrate breath alcohol devices.

There are numerous reference texts on the subject of measurement uncertainty, but the 'gospel' on uncertainty is the *Guide to the Expression of Uncertainty in Measurement* or the 'GUM' (ISO/IEC 1995). In reality, the GUM is much more complicated than is necessary for anything other than the work performed in national laboratories such as the National Institute of Standards and Technology (NIST). While providing the basic theory behind measurement uncertainty, the GUM uses a substantial amount of mathematical language that is difficult for non-mathematician scientists to understand. Additionally, it introduces new terminology that is not well defined. Conversely, the GUM approach does establish a universally accepted method for determining measurement uncertainty.

Using a simplified GUM approach to estimating measurement uncertainty

Over the years, various authors and international groups have made the GUM approach more straightforward (EURACHEM/CITAC 2000; UKAS 2007). Using the GUM to estimate uncertainty leads to a better understanding of the analytical method and helps identify the major causes of uncertainty. It also ensures that an internationally agreed technique is used in making the estimate. On the other hand, it is likely to require the development of some new skills for many forensic chemists and toxicologists and, if interpreted too rigidly, will become burdensome and very costly to the laboratory.

A simplified GUM approach can be broken down into eight steps (Fig. 23.1). Step 1 involves the specification of what is being measured. The sources of uncertainty in each stage of the measurement are listed in step 2. Step 3 allows for removal of components that are adequately accounted for by other data (usually reproducibility data). In step 4, the uncertainty components are separated into categories of type A and type B data and quantified. Conversion of these quantified uncertainties into standard uncertainties occurs in step 5 so that they can be combined in step 6. Finally, the combined uncertainties are expanded on the basis of a chosen confidence level in step 7 and reported in step 8. An *uncertainty budget sheet* (Fig. 23.2) allows these steps to be easily documented and retained for auditing purposes.

Examples at the end of this chapter provide clarification for each of these steps. The reader may wish to refer to these examples as they learn about each of the steps below.

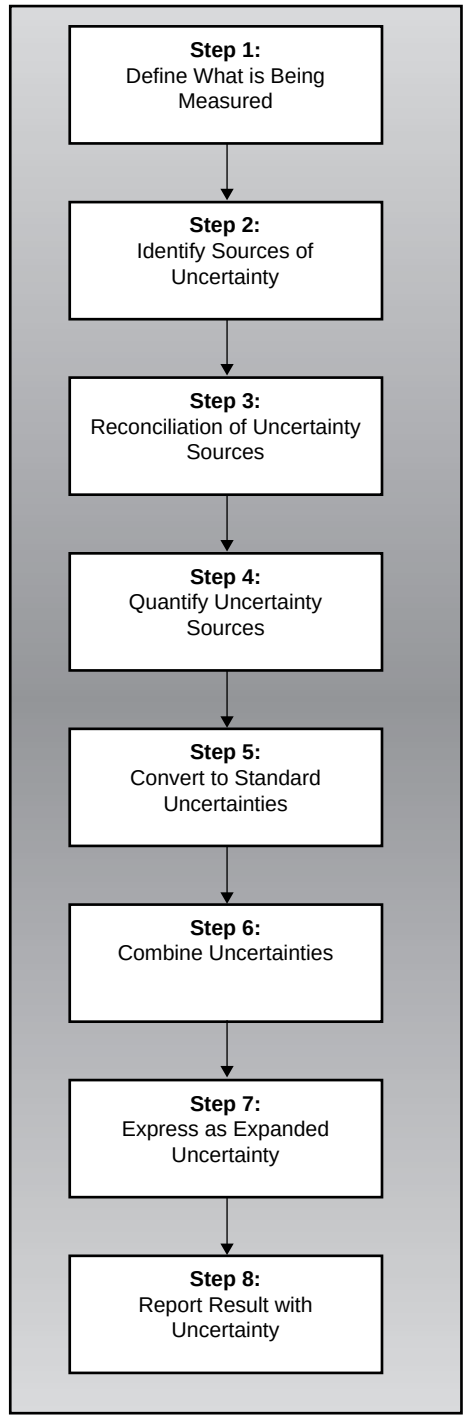

Figure 23.1 Simplified GUM approach.

Step 1: Define what is being measured

The first step is the easiest – clearly define what is being measured by the quantitative method for which the uncertainty is being determined. It is also important to include the date on which the uncertainty budget sheet is prepared, as well as identifying its author. Some sources also recommend generating a mathematical expression relating the quantitative result to the parameters on which that result depends: this may help the user better identify the sources of uncertainty in the next step. Examples 1 and 2 later in this chapter demonstrate how a quantitative expression may be used.

Step 2: Identify sources of uncertainty

Possible sources of uncertainty associated with making the measurement should be assembled into a reasonably comprehensive list. Table 23.1 contains sources of uncertainty that may be considered in this process.

If a quantitative expression was generated in step 1, it is usually best to start with this expression, as all of its parameters are potential sources of uncertainty. Once all potential sources of uncertainty are listed, the quantitative expression can be reworked to represent each effect that contributes to the overall uncertainty.

Another way to identify sources of uncertainty is a *cause-and-effect diagram* (Fig. 23.3). This tool helps visualise how different sources of uncertainty relate to one another and minimises the chances of counting the same source of uncertainty more than once.

There are four steps in creating a cause-and-effect diagram. The first involves the quantitative expression that was generated in step 1 above

Uncertainty Budget Form :					
Method:					
Analyst:			Date:		
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
Combined Uncertainty:					
Confidence Level :					
Expanded Uncertainty:					

Figure 23.2 Sample uncertainty budget form.

Table 23.1 Possible sources of uncertainty

Sampling	*Sample preparation*	*Use of CRM and other control*	*Calibration curves*	*Analysis*	*Data processing*
■ Homogeneity ■ Sampling strategy effects ■ Physical state (solid, liquid, gas) ■ Environmental effects ■ Sampling process	■ Homogenisation ■ Drying ■ Milling ■ Dissolution ■ Extraction ■ Derivatisation ■ Dilution ■ Concentration	■ Uncertainty of CRM and controls ■ Ability to matrix match	■ Uncertainty of reference materials ■ Matrix matching of calibrators ■ Instrument precision	■ Systematic errors ■ Subjective evaluations by analyst ■ Assumed stoichiometry ■ Environmental effects ■ Matrix interferences ■ Reagent purity ■ Instrument parameters and settings ■ Run-to-run precision	■ Averaging results ■ Rounding and truncating ■ Statistics ■ Processing algorithms

(or a suitable alternative). The parameters of the expression form the main branches of the diagram. Next consider each step of the method and add any additional factors to the diagram using Table 23.1 to assist in this brainstorming endeavour. For each branch, add contributory factors. Do not add contributing factors that seem only remotely possible. Resolve duplications using the rules explained in Table 23.2. Examples 2, 3 and 5 show how such diagrams may be useful in setting up an uncertainty budget.

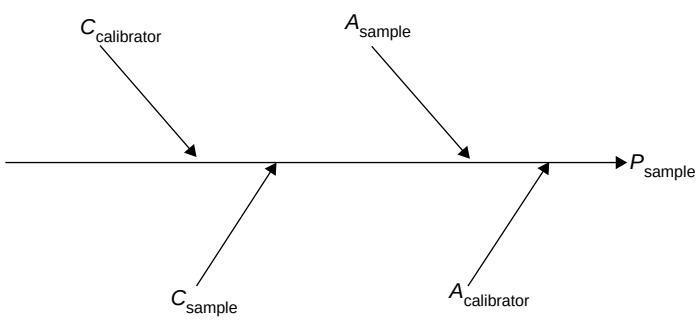

Figure 23.3 Sample cause-and-effect diagram.

Step 3: Reconciliation of uncertainty components

Reconciliation is another useful tool to simplify the uncertainty budget (Ellison 1998). In this step, a review is conducted to determine whether a listed uncertainty source is adequately accounted for by existing data (usually reproducibility data) or by small, planned experiments. The basis for this step lies in the fundamental assumption that, if an effect is representatively varied during the course of a series of observations, the uncertainty associated with that effect is adequately accounted for in the standard deviation of those observations. Of course, it is important that those uncertainty components that are reconciled in this step are truly represented through the existing data or planned experiments. See examples later in this chapter.

Step 4: Quantifying the uncertainty components

Once the sources of uncertainty have been identified and reconciled, the size of each must be measured or estimated. An easy way to start this process is to first determine what information is readily available for each of the listed sources. Not only does this help in the organisation of information, but it also allows for the recognition of sources of uncertainty for which existing data are not adequate. The later situation may require that further literature research or planned experiments be undertaken. Once the available information has been collected, each

Table 23.2 Rules for resolving duplication in lists of uncertainty sources

Cancelling effects, remove both

When the uncertainty source's effect is shown to have no net effect on the result, both sources can be cancelled or removed from the list. A common example occurs when determining a mass by difference. Two masses are determined, both subject to the balance 'zero bias'. The zero bias will cancel out of the mass by difference and can be removed from the branches corresponding to the separate weighings.

Same effect, same time

When a similar source is uncovered on multiple branches of a cause-and-effect chart, they can be combined into a single input since the effect is happening at the same time. For example, reproducibility will likely occur on many of the input branches, but they can be combined into a single overall reproducibility branch on the diagram.

Different instances, relabel

When similarly named effects that refer to different instances of similar measurements (i.e. cannot be combined into one effect) are found, these effects should be re-labelled to clearly distinguish them from each other. For example, 'calibration' may appear as an effect on more than one intermediate measurement. These must be clearly distinguished before proceeding.

potential source of uncertainty is categorised as either *type A* or *type B* uncertainty data.

Type A uncertainty

Uncertainty data determined by repeating a measurement a number of times and performing a statistical analysis on the results are categorised as type A. In general, these data are usually already available to laboratories through quality control data, validation studies, collaborative studies and/or results from proficiency tests. Occasionally, a laboratory may find that individual components of uncertainty must be evaluated when few or no method performance data are available. Means of using these data in each of these scenarios are described briefly below.

Use of historical quality control (QC) reproducibility data

Accredited laboratories performing quantitative measurements demonstrate good method performance through the use of QC samples and an effective quality assurance programme. The QC samples may be certified reference materials or purchased spiked samples, or may be prepared in-house as spiked matrix controls.

The data contained in the historical quantification of QC samples provide useful information about the reproducibility of the analytical method. These data capture the day-to-day capability of the laboratory, take into account many variables (e.g. different working calibration standards, various instruments, several operators and assorted environmental conditions), and tend to capture the method's performance over an extended period of time. Examples 1 to 4 demonstrate how historical QC data can be used to assist in estimating a procedure's uncertainty.

Use of in-house validation study data

Newer methods or methods that are used infrequently will be unlikely to have an extensive amount of QC sample data generated. In these instances, the validation studies that were conducted to bring the method on-line provide another source of data to assist in uncertainty determinations. For example, precision and bias studies are typically conducted on QC samples for which the samples are analysed several times over a period of different days, resulting in data that can be statistically analysed. For guidance in conducting appropriate validation experiments, the reader is referred to Chapter 20. Example 5 shows how in-house validation data can be useful for uncertainty determinations in a non-routine quantitative analysis.

Use of collaborative study data

Occasionally, collaborative studies are carried out to develop and validate a published method. In doing so, valuable validation and/or reproducibility data are generated that capture an even broader range of variables than those represented in a single laboratory's historical QC data. Therefore, the multi-laboratory validation and QC data may provide useful information to support the uncertainty estimate.

Use of proficiency test data

Some of the more common analytes encountered by a laboratory are routinely included in the proficiency tests that are analysed as part of the laboratory's quality assurance programme. A laboratory's results on these proficiency samples can be valuable for uncertainty estimates when the assigned values of the analyte are traceable to an appropriate value with an uncertainty that is small compared with the distribution of results. The reproducibility of the reported values can assist in uncertainty estimates. The only difficulty is that there must be a sufficient number of proficiency tests analysed and reported for the specific analyte measured by the procedure for this approach to outweigh other type A approaches.

Statistical analysis for type A uncertainty data

No matter which of the above type A data are used in the uncertainty estimation, the statistical analysis of these data can be quite simple. Since measurement values should generally be randomly scattered around the mean, it follows that the distribution should be 'normal' or gaussian-shaped (Fig. 23.4) provided that a sufficient number of measurements (n) have been made. The above data sources all allow for the laboratory to statistically calculate the mean ($\overline{X}$) measured value of the spiked sample (x) using equation (23.1):

$$\overline{X} = \frac{\text{sum of all measured values}}{\text{number of measurements}} = \frac{\sum_{i=0}^{n} x_i}{n} \tag{23.1}$$

Since the data used will only be samples of a larger population, the standard deviation of the population is estimated from the standard deviation of the sample data (s) using equation (23.2):

$$s = \sqrt{\frac{\sum_{i=0}^{n} \left(x_i - \overline{X}\right)^2}{n-1}} \tag{23.2}$$

Throughout the remainder of this chapter, s and σ are used interchangeably with the understanding that the population standard

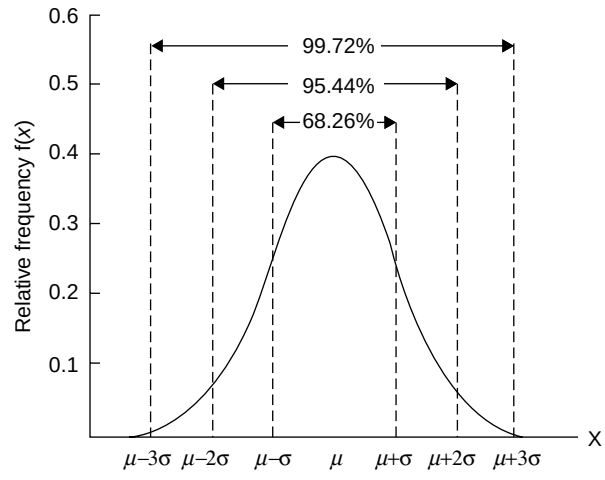

Figure 23.4 Normal distribution model.

deviation (σ) is better characterised by the sample standard deviation (s) as the number of measurements increase. Likewise, the sample mean also becomes a better estimation of the population mean as the number of measurements increase. The reader is referred to basic statistical textbooks for more information on the relationship between a sample and the population that it represents.

With sufficient measurements (usually more than 100) of a spiked sample, a Gaussian-shaped distribution of the results can generally be assumed such that $\pm 1\sigma$ should include 68% of the measurement results, $\pm 2\sigma$ should include 95% of the measurement results and 99.7% of the results should be distributed within $\pm 3\sigma$ (Fig. 23.4). With this distribution assumption applied to the historical measurement results for a quantitative QC sample, standard deviation and confidence level can easily be related. For example, if 200 measurements have been made on a QC sample over the course of a year and the mean measured value is 748 units with a standard deviation of 23.1 units, to express this at a 95% confidence level these data would be written as $748 \pm 2\sigma$, or 748 ± 46 units. Similarly, it may also be represented as $748 \pm 3\sigma$ (748 ± 69 units) at a 99.7% confidence level.

In instances where there are not a great deal of historical data to rely upon (e.g. newly validated quantitative methods or non-routine methods), compensation for the greater uncertainty associated with estimates based on small data samples should be made using a Student's t distribution table (Table 23.3). Using this table and the degrees of freedom (the number of measurements of the spiked sample that are available minus one $(n-1)$), a correction factor is provided for the selected confidence interval. This concept becomes more important and will be revisited in step 7 when expanded uncertainties are calculated.

Table 23.3 Student's *t* distribution table. The cover factor (*k*) is determined by the degrees of freedom ($n-1$) and desired confidence level.

$n-1$	95% confidence level	99.75% confidence level
1	12.7	318.3
2	4.3	22.3
3	3.2	10.2
4	2.8	7.2
5	2.6	5.9
6	2.5	5.2
7	2.4	4.8
8	2.3	4.5
9	2.3	4.3
10	2.2	4.1
11	2.2	4.0
12	2.2	3.9
13	2.2	3.9
14	2.1	3.8
15	2.1	3.7
16	2.1	3.7
17	2.1	3.6
18	2.1	3.6
19	2.1	3.6
20	2.1	3.6
30	2.0	3.4
40	2.0	3.3
50	2.0	3.3
60	2.0	3.2
70	2.0	3.2
80	2.0	3.2
90	2.0	3.2
100	2.0	3.2
∞	2.0	3.1

It goes without saying that, in general, the more replicate analyses that are made on a material, the more confidence can be placed on the final result. The uncertainty associated with that measurement will also be improved. In other words, when a measurement result is expressed as the mean of a number of measurements, the improvement in the uncertainty is found from the number of measurements that went into the mean. This is known as the *standard deviation of the mean* (σ_{mean}) in which the standard deviation of the historical data is divided by the square root of the number of measurements (p) made on the material (equation 23.3).

$$\sigma_{\text{mean}} = \frac{\sigma}{\sqrt{p}} \tag{23.3}$$

For example, assume a laboratory is asked to determine the concentration of morphine in a blood sample collected from a patient. This laboratory has performed this analysis for years, so they have a record of measuring a 500 µg/L morphine control in blood. These data show that the standard deviation of measuring the morphine control is 52 µg/L, so, if they were to quantitatively measure the patient's blood sample only once, the standard deviation associated with the reproducibility data remains 52 µg/L. If they instead quantify the patient's sample three times and report a mean result of those three measurements, the standard deviation is then calculated as $\sigma_{\text{mean}} = (52\ \mu g/L)/\sqrt{3} = 30\ \mu g/L$. For additional clarification, the reader is referred to examples 2 and 5.

Type B uncertainty data

Uncertainty data that are derived from techniques other than repeated analyses and statistical calculations are called *type B uncertainty*. Simply put, type B uncertainty data are those that are not measured statistically by the laboratory and usually must be considered individually. These arise because of a particular error in the measurement method and can be minimised by optimising the system, but they can never be completely eliminated. Some examples include reference standards calibrated by an external laboratory, environmental parameters that cannot be readily measured, instrument resolution and personal bias when performing visual measurements.

The values associated with type B sources of uncertainty data may be obtained from a number of sources such as calibration certificates of laboratory standards or equipment, manufacturer's specifications, or reference data from handbooks or certificates. These documented sources of uncertainty from other processes are sometimes reported at a 95% confidence level, so to derive the standard uncertainty the reported uncertainty may need to be divided by 2.

In other instances, data are not available from a certified laboratory. On other occasions a laboratory must rely on experience or general knowledge of the behaviour and properties of the relevant materials and instruments to make a good-faith estimate of the worse-case effect, absent blunders. In both of these scenarios, the laboratory should take these data or personal experiences to establish the outside limits ($\pm a$) for a given source of uncertainty. A standard deviation can then be computed based on a *rectangular distribution* or *triangular distribution*.

Rectangular distribution

If a measurement has an equal chance of being any value within the outside limits ($\pm a$), it follows a rectangular (or uniform) distribution. The standard deviation for this type of distribution is calculated using $\sigma = a/\sqrt{3}$. For example, if a drug sample must be diluted into a 10 mL volumetric flask in order to perform the purity determination, it may be that the manufacturer's specification of the flask is ± 0.3 mL. The standard deviation for the measurements using this flask is $0.3\ \text{mL}/\sqrt{3} = 0.17$ mL. In general, assuming a rectangular distribution for many type B uncertainty data sources will introduce a conservative 'safety' factor into the uncertainty estimation.

Triangular distribution

If there is reason to expect that the extreme outside values associated with $\pm a$ are unlikely to occur, it is acceptable to assume a triangular distribution. The standard deviation for a triangular distribution is determined using $\sigma = a/\sqrt{6}$. If the same 10 mL volumetric flask used in the above example has been subject to routine in-house checks to demonstrate that the extreme values are rare, the standard deviation for measurements with the flask could alternatively be expressed as 0.3 mL/$\sqrt{3} = 0.12$ mL.

Step 5: Conversion to standard uncertainties

For discussions of uncertainty, the standard deviation is also known as the *estimated standard uncertainty*. In this step, all previously calculated standard deviations are now simply expressed as standard uncertainties. However, to facilitate the next step in the simplified GUM approach to determining measurement uncertainty, it is also useful to ensure that common units are used throughout the budget. If this is not the case, then all units are converted into percentages, keeping in mind that the reverse conversion will be necessary later in the process.

For example, if measuring 7.0 mL has a standard deviation of 0.2 mL associated with it, the percentage standard deviation of the volumetric measurement is calculated as $(0.2\text{ mL}/7.0\text{ mL}) \times 100 = 2.9\%$.

Step 6: Combining standard uncertainties

At this point it should be understood that not all of the listed sources of uncertainty will have a significant contribution to the measurement's overall uncertainty. In most cases, only a few of the sources actually contribute significantly. As a general rule of thumb, components with standard uncertainty values that are less than one-third of the component with the largest standard uncertainty value can be ignored at this point. Simply cross a line through each of these minor contributors on the uncertainty budget form to document that the component was considered but was determined to be insignificant. This will also simplify the calculations involved in combining uncertainties.

Another important task is to determine whether any of the remaining sources of uncertainty are correlated. If sources of uncertainty are related to one another, they may cancel each other out or, if working in concert, have a larger impact on the final combined uncertainty. If a cause-and-effect diagram was used in step 2, it is easy to determine any correlated uncertainties that may exist with the method. To combine correlated uncertainties (U_{corr}) the individual correlated uncertainties are added to or subtracted from one another using equation (23.4):

$$U_{\text{corr}} = (U_1 U_2 U_3 U_4 \cdots) \tag{23.4}$$

Only those uncertainties determined to be dependent on one another should be combined in this fashion.

Fortunately, most sources of uncertainty are independent of one another. This allows for an assumption to be made that uncertainties combine in a random fashion and that small components will have an insignificant impact on the overall uncertainty. The mechanism of calculating uncorrelated uncertainties (U_{uncorr}) is the *root sum square* (RSS) technique. Each of the remaining standard uncertainty values (as well as any combined correlated uncertainty) is squared and then added together prior to taking the square root of the sum as expressed in equation (23.5):

$$U_{\text{uncorr}} = \sqrt{\left(U_{\text{corr}}^2 + U_1^2 + U_2^2 + U_3^2 + U_4^2 + \cdots\right)} \tag{23.5}$$

As the procedures above are meant to be a simplified GUM approach, the reader is referred to the reference list for more detailed discussions on calculating combined standard uncertainties.

Step 7: Expressing expanded uncertainties

The GUM approach requires that a coverage factor (k) be applied in the final step of the uncertainty estimate. The coverage factor is a number that, when multiplied by the combined standard uncertainty, produces an interval around the measurement result that is expected to include a large specified percentage (usually 95% or 99.7%) of the values. Usually k is set to a value of 2 to represent a 95% confidence level and 3 to represent a 99.7% confidence level. The reader will recognise that the k value is the equivalent to the number of standard deviation values in a normal distribution pattern (see Fig. 23.4).

When the type A uncertainty component is dominant *and* the number of measurements used to calculate the standard deviation is small, there will be reduced confidence in that calculated standard deviation. The normal distribution model indicates that results close to the mean are more probable than results far from the mean. So if only a few measurements are made in determining the standard deviation, it will likely be an underestimate of the true standard deviation. To account for this, a correction factor can be applied based on the Student's t distribution. Table 23.3 shows the corrected values for k when fewer than 100 measurements have been made in determining the standard deviation for type A uncertainty data. In contrast, the value of k is not affected by type B uncertainties since they are considered reliable given that outer limits are used to estimate these components. Examples 2 to 5 demonstrate how a corrected coverage factor is used in expressing expanded uncertainties.

Finally, it is important to remember that, if standard uncertainties were converted to percentages in step 4, the final combined uncertainty should be converted back to the units of measurement of the material in question.

Step 8: Reporting results with uncertainties

The most important rule in reporting uncertainties is to use common sense. Since an uncertainty range is only an estimate, it should generally never exceed two significant figures. Furthermore, it is good practice to round up, as opposed to normal rounding conventions.

The reported measurement should be reported only to the level of precision to which the uncertainty is reported. For example, if the 99.7% confidence level for the uncertainty of a measurement of metamfetamine in blood is 29 ng/mL, it would not make sense to report the result as 498.23 ng/mL. Instead it should be reported as 498 ng/mL.

When reporting uncertainties, it is equally important to include the confidence level. The above metamfetamine example should be reported as 498 ± 29 ng/mL (99.7% confidence level). Failure to report the confidence level will leave the user of the information without any basis for evaluating the estimated uncertainty.

Conclusion

This chapter has presented a simplified, eight-step approach to the GUM technique for determining and reporting measurement uncertainty. Laboratories should not fear measurement uncertainty, as it helps both internal and external recipients of the information to better understand that a measurement is not an absolute. Instead, the true value falls within an estimated range of values with the centre of that range serving as the most probable place that the true value will fall.

The examples that follow are meant to assist in the understanding of measuring and reporting uncertainty. The reader is encouraged to follow through the examples in order. Each one offers a unique approach to making a fair estimate of the uncertainty associated with the presented measurements. Furthermore, a laboratory performing similar types of examinations should be able to use these examples as templates for determinations of uncertainty in their own quantitative measurements.

Examples

Example 1: Measurement uncertainty associated with the determination of the mass of a sample of cannabis

A laboratory is asked to determine the mass of a seized sample of suspected cannabis. The laboratory's standard operating procedure calls

for the mass to be determined as the difference in the packaging containing the cannabis inside and the packaging with the cannabis removed. The laboratory records each mass once. The mass of the packaging containing the cannabis (m_{p+c}) is 479.20 g. The mass of the empty packaging (m_p) is 4.10 g. Therefore, it is determined that there is 475.10 g of suspected cannabis.

The laboratory also conducts a quality check on the balance used for these mass determinations utilising a 100 g certified weight before and after each measurement. These quality check readings are recorded in a logbook and the historical performance is re-evaluated every 6 months. They have 210 separate measurements of the 100 g certified weight recorded in a logbook.

Step 1: Define what is being measured

A simple but clear title for the uncertainty budget might be 'Uncertainty Associated with the Mass Determination of Cannabis Samples'. A simple quantitative expression for such a measurement might be $m_c = m_{p+c} - m_p$, where m_c is the mass of the cannabis.

Step 2: Identify sources of uncertainty

As explained earlier, the quantitative expression provides a starting point for identifying potential sources of uncertainty. In this case, the most likely sources of uncertainty come from the calibration of the balance and the reproducibility of performing mass measurements. The balance calibration can be further defined as it is affected by both its bias and its linearity. Both measurements (m_{p+c} and m_p) contribute to the measurement uncertainty, but, since the measurement is a weight by difference, the biases of each measurement cancel each other out (see Rules for resolving duplication, Table 23.2). The uncertainties associated with the balance linearity are still considered as two separate contributions because of the possibility of a non-linear balance response. This leaves four sources of uncertainty: reproducibility of each weighing on the balance in question (U_r and U_{12}) and the linearity of the balance for each measurement (U_{c+p} and U_p).

The uncertainty budget form at steps 1 and 2 is shown in Fig. 23.5.

Step 3: Reconciliation of uncertainty components

The reproducibility data are based on weighing a 100 g certified weight but probably do not take into account the linearity of the balance. So, for this example, reconciliation is not justified.

Step 4: Quantifying the uncertainty components

The laboratory's practice of performing quality checks before and after each measurement provides a means of effectively evaluating the reproducibility of the procedure. The mean and standard deviation for the historical performance of these measurements on the balance used for determining the cannabis mass are 100.00 g and 0.08 g, respectively. As these values were determined statistically within the laboratory, they become categorised as type A uncertainty data. The balance linearity (0.02 g; 95% confidence level) is provided on the balance calibration certificate, thus falling into the category of type B uncertainty data. For this example, it is assumed that the calibration certificate is from a certified vendor.

Step 5: Conversion to standard uncertainties

Standard deviations from the information collected in step 3 are then written as standard uncertainties. The standard deviation associated with the reproducibility data is already expressed as $\pm 1\sigma$, but the balance's linearity is expressed as $\pm 2\sigma$ (95% confidence), so it must be divided by 2 to convert it to a $\pm 1\sigma$ value (Fig. 23.6).

Step 6: Combining standard uncertainties

Before combining the standard uncertainties, remember to use the one-third rule and cross out those sources that do not serve as significant contributors. Clearly the standard uncertainty associated with the calibration linearity of the balance is far less than the uncertainty associated with the type A data related to reproducibility. Both are crossed out, leaving just the uncertainty associated with reproducibility. Since these are uncorrelated, the root sum square equation is used to combine the uncertainty components (Fig. 23.7).

Step 7: Expressing expanded uncertainties

Since the uncertainty related to the type A data for reproducibility was the only surviving source of uncertainty, it is relied upon as a guide in determining the value for the coverage factor (k). There were 210 separate measurements of the 100 g certified weight, therefore a well-established normal distribution pattern is assumed by the laboratory. This laboratory reports uncertainty to their customers at a 95% confidence level, so $k = 2$.

Step 8: Reporting results

Remembering the rules of rounding and significant figures, the laboratory reports the weight as 475.10 ± 0.22 g (95% confidence level).

Uncertainty Budget Form :

Method: Uncertainty Associated with the Mass Determination of Cannabis Samples

Analyst: John Smith — Date: 11-Apr-2008

Sources of Uncertainty	Type A or Type B?	Std Dev or Outside Limits (units)	Distribution Model	Divisor	Standard Uncertainty (1σ)
U_{r1} - Reproducibility					
U_{r2} - Reproducibility					
U_{c+p} - Linearity					
U_p - Linearity					
Combined Uncertainty:					
Confidence Level :					
Expanded Uncertainty:					

Figure 23.5 Uncertainty budget – steps 1 and 2 for Example 1.

Uncertainty Budget Form :					
Method:	Uncertainty Associated with the Mass Determination of Cannabis Samples				
Analyst:	John Smith		Date:	11-Apr-2008	
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
U_{r1} - Reproducibility	Type A	0.08 g	Normal	1	0.08 g
U_{r2} - Reproducibility	Type A	0.08 g	Normal	1	0.08 g
U_{c+p} - Linearity	Type B	0.02 g	Normal	2	0.01 g
U_p - Linearity	Type B	0.02 g	Normal	2	0.01 g
Combined Uncertainty:					
Confidence Level :					
Expanded Uncertainty:					

Figure 23.6 Uncertainty budget – steps 3, 4 and 5 for Example 1.

Uncertainty Budget Form :					
Method:	Uncertainty Associated with the Mass Determination of Cannabis Samples				
Analyst:	John Smith		Date:	11-Apr-2008	
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
U_{r1} - Reproducibility	Type A	0.08 g	Normal	1	0.08 g
U_{r2} - Reproducibility	Type A	0.08 g	Normal	1	0.08 g
U_{c+p} - Linearity	Type B	0.02 g	Normal	2	0.01 g
U_p - Linearity	Type B	0.02 g	Normal	2	0.01 g
Combined Uncertainty:		0.11 g			
Confidence Level :		95% (k = 2)			
Expanded Uncertainty:		0.22 g			

Figure 23.7 Uncertainty budget – steps 6 and 7 for Example 1.

Example 2: Measurement uncertainty associated with the determination of the purity of a street sample of cocaine base

A laboratory is requested to determine the purity of a 1 kg seized drug sample identified as cocaine base. The laboratory's standard operating procedure calls for the purity to be determined by taking two sets of samples from throughout the bulk of the material. Each sample set consists of 10 random samples of ~10 mg each that are thoroughly homogenised together. For each homogenised sample set of the cocaine base, 10 mg is weighed directly into 10 mL volumetric flasks and then diluted to volume with methanol. Likewise, a single 10 mg reference standard of cocaine hydrochloride is weighed (correcting for the hydrochloride salt) and diluted in methanol in a 10 mL volumetric flask to serve as a calibration solution.

Table 23.4 Results for the purity of sample of cocaine base

	Calibrator	*Sample 1*	*Sample 2*	*QC*
Weighed amount	10.1 mg (after salt correction)	9.8 mg	10.4 mg	9.6 mg
Dilution volume	10 mL	10 mL	10 mL	10 mL
Concentration of prepared sample	1.01 mg/mL	0.98 mg/mL	1.04 mg/mL	0.96 mg/mL
Area of 1st injection	21 139	11 198	12 498	9487
Area of 2nd injection	20 948	12 050	12 613	9532
Area of 3rd injection	21 004	11 482	12 266	9554
Average area	21 030	11 577	12 459	9524
Standard deviation of area	98	434	177	34
Calculated purity	100%	56.7%	57.5%	47.6%
Average purity	100%	57.1%		47.6%
Standard deviation of purity	–	0.6%		–
Differences in purity	–	±0.4%		–

A QC sample of cocaine base that was prepared in-house has been in use in this laboratory for about 10 months. It is mixed with a common cutting agent and targeted to contain about 50% of cocaine base. The QC sample is sampled only once in a similar fashion as for the calibrator sample. This QC sample has been previously analysed eight times over a 10-month period with a mean result of 48.6% and a standard deviation of 6.3%.

The samples, control and calibrator are each injected three times into a high performance liquid chromatograph. The areas of cocaine peaks in the samples and control are individually averaged and compared with the average area of the cocaine calibrator to determine the purity of cocaine base in each sample (Table 23.4). Finally, the two sample concentrations are averaged and reported to the case contributor.

Step 1: Define what is being measured

The title for the uncertainty budget may be 'Uncertainty Associated with the Purity Determination of Cocaine Base'. The quantitative expression for this measurement is:

$$P_{\text{samp}}(\%) = \frac{C_{\text{cal}}}{C_{\text{samp}}} \times \frac{A_{\text{samp}}}{A_{\text{cal}}} \times 100 \qquad (23.6)$$

where P_{samp} is the sample purity, C_{cal} and C_{samp} are the concentrations of the prepared solutions of the calibrator and samples, and A_{cal} and A_{samp} are the integrated HPLC peak areas for the calibrator and sample, respectively. Multiplying by 100 allows for the result to be expressed as a percentage.

Step 2: Identify sources of uncertainty

To visualise the possible uncertainty sources, the laboratory constructs an initial cause-and-effect diagram (Fig. 23.8).

To further define the cause-and-effect diagram, the laboratory recognises that both C_{cal} and C_{samp} are calculated as the mass of the calibrator (m_{cal}) or sample (m_{samp}), respectively, divided by its related dilution volume (V_{cal} or V_{samp}). So equation (23.6) can be rewritten as:

$$P_{\text{samp}}(\%) = \frac{m_{\text{cal}}/V_{\text{cal}}}{m_{\text{samp}}/V_{\text{samp}}} \times \frac{A_{\text{samp}}}{A_{\text{cal}}} \times 100 \qquad (23.7)$$

The laboratory further recognises that the calibration of the flask (*flask cal*), the ambient temperature (*vol temp*) and the repeatability of filling the flask (*flask repeat*) are all potential sources of uncertainty related to V_{cal} and V_{samp}. Sources related to m_{cal} and m_{samp} include the balance calibration (*balance cal*) and linearity (*balance lin*), as well as the reproducibility of determining mass on the balance (*balance repeat*). Additionally, since the laboratory places the volumetric flask on the balance and tares it prior to weighing the calibrator or sample directly into the flask, it considers this as one distinct measurement for each. Additional potential sources of uncertainty include the certified purity of the cocaine hydrochloride used to prepare the calibration solution ($purity_{\text{cal}}$) and the uncertainty associated with the homogeneity of the cocaine base sample ($Homogen_{\text{samp}}$). This leads to a modified cause-and-effect diagram (Fig. 23.9).

Using the rules for resolving duplication (see Table 23.2), the diagram is restructured (Fig. 23.10). From this diagram, the uncertainty budget form can be populated.

Step 3: Reconciliation of uncertainty components

An important step in simplifying the GUM process is to reconcile those sources of uncertainty that are fairly represented in data from reproducibility studies or other experiments. Figure 23.11 demonstrates the usefulness of this step. The laboratory has historical data of its QC sample that cover more than a 10-month period. Presumably these QC data represent separate analytical runs on different days, perhaps even by different analysts. Therefore, the uncertainty associated with the

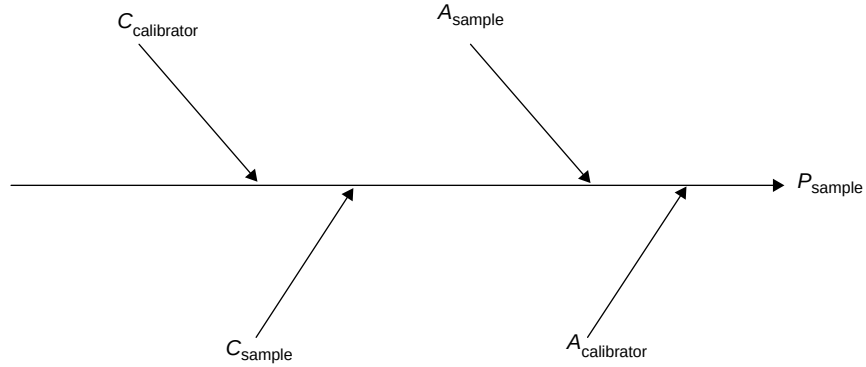

Figure 23.8 Initial cause-and-effect diagram for Example 2.

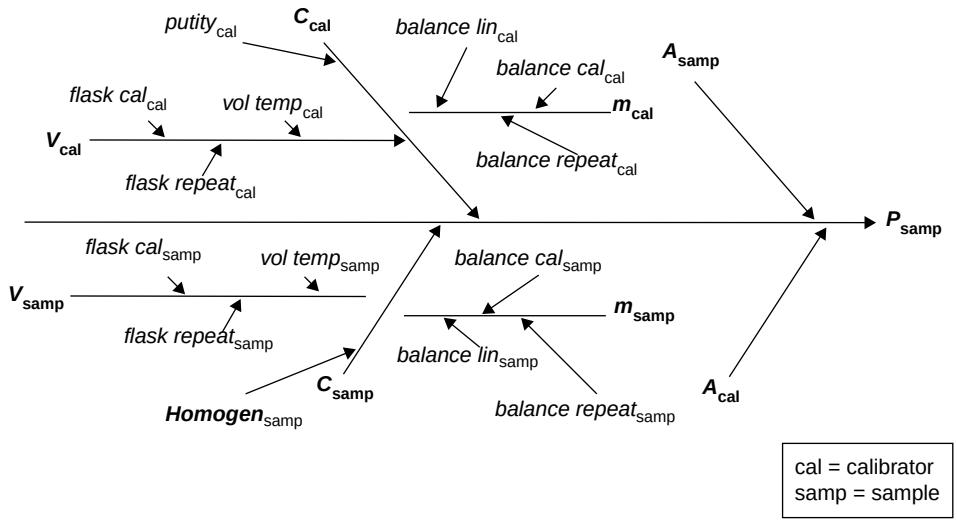

Figure 23.9 Modified cause-and-effect diagram for Example 2.

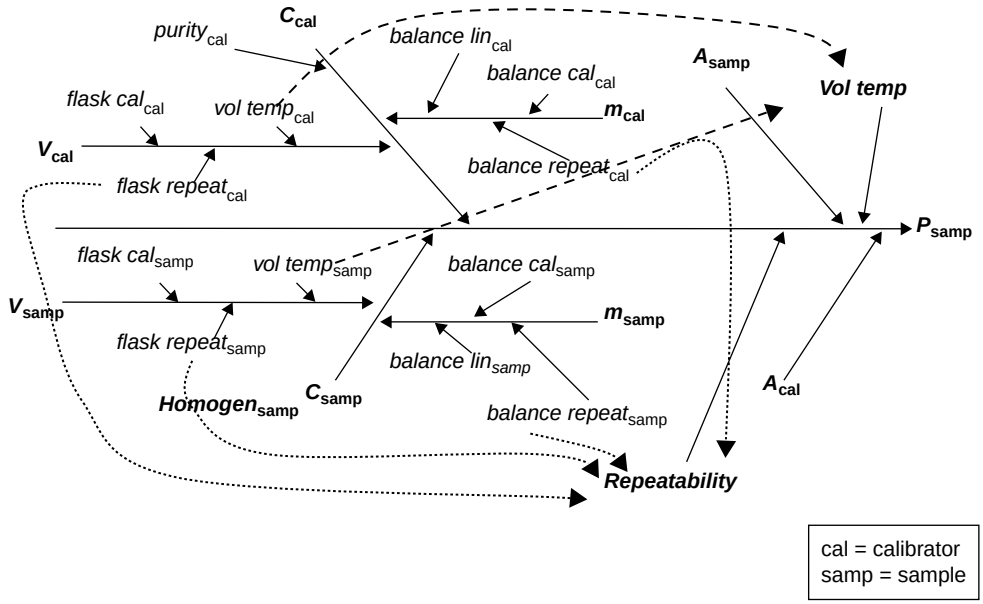

Figure 23.10 Combined cause-and-effect diagram for Example 2.

effects of temperature on volume (*Vol temp*) is likely accounted for in the historical QC data. Likewise, the variabilities in peak integration (A_{samp} and A_{cal}) are also captured by this reproducibility data. Unlike the first example, the laboratory does not rely on quality checks of a certified weight to demonstrate the reproducibility of mass determinations. Instead, if it is assumed that the laboratory is using the same balance when weighing the calibrator, QC sample and the cocaine base, the historical QC data may be considered as capturing the reproducibility associated with these mass determinations. Additionally, since the measured amounts are all relatively the same (unlike the previous example), these data also account for the *balance lin* and *balance cal* (if it is assumed that the balance used for the historical QC determinations is the same balance used for the calibrator and cocaine base samples). The reproducibility data do not, however, adequately account for $purity_{cal}$ (which varies from lot to lot), *flask cal* (assuming different flasks are used) or the potential inhomogeneity of the sample.

Step 4: Quantifying the uncertainty components

Although there are some historical QC data upon which to rely, they are somewhat limited in that only eight measurements of the cocaine QC have been made. None the less, they remain a valuable source for a type A assessment of reproducibility. In this example, samples are taken in duplicate and each is injected three times. The reported purity is actually an average of the two samples. The calibrator and QC are each sampled only once, but are also injected three times. Therefore, the standard deviation of the mean applies here as $\sigma_{mean} = 6.3\%/\sqrt{2} = 4.5\%$. Remember, the standard deviation that is used is the historical standard deviation from reproducibility data and not the standard deviation of the measurements used to determine the mean. While there were a total of six injections of the two sample aliquots, because the QC data are based on an average of three injections and the average values are the source of the historical standard deviation, the improved certainty afforded by the standard deviation of the mean should be based only on the fact that two aliquots of the sample were taken, as opposed to a single sample. The remaining sources of uncertainty data require type B assessments (Table 23.5).

Determination of sample homogeneity can be challenging in forensic chemistry and toxicology. Given the legal need to retain a significant portion of the sample for future testing, it is important that sampling of any potentially inhomogeneous material be done in a random fashion. When possible, the sample should be homogenised to the greatest extent possible. To account for this uncertainty component, a laboratory may

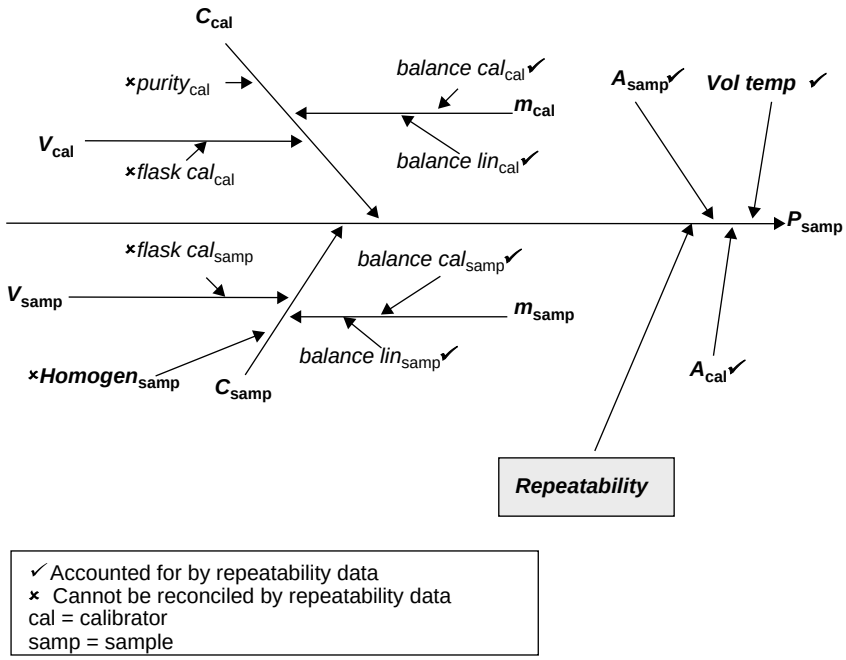

Figure 23.11 Reconciliation of cause-and-effect diagram is Example 2.

rely on a good-faith estimate of the inhomogeneity of the matrix, rely on the standard deviation of multiple measurements or use the differences between multiple measurements of the random samples. For this example, the latter method is demonstrated. The laboratory took steps to ensure that random samples were homogenised prior to taking the two 10 mg samples that were ultimately analysed. The laboratory considers the difference of the two measured purities as representative of the inhomogeneity of the entire sample (Table 23.4). As these are the 'extreme' values, the laboratory treats these data as fitting into a rectangular distribution.

Step 5: Conversion to standard uncertainties

Figure 23.12 shows the uncertainty budget with standard deviations converted to standard uncertainties. The standard deviation for the *flask cal* is obtained from the manufacturer's data and is considered the outside limit. Therefore, a rectangular distribution model is assumed.

Step 6: Combining standard uncertainties

It is clear that the standard uncertainty associated with $purity_{cal}$ and $Homogen_{samp}$ are minor in comparison to the others listed in Fig. 23.12. Therefore, these sources are removed from the uncertainty budget to simplify the combination of the standard uncertainties. Technically, the standard uncertainties for both *flask cal* values could also be removed using the one-third rule (each is approximately one-eighth of the largest standard uncertainty). However, given that these three sources combined do account for about a quarter of the largest standard uncertainty, this laboratory has decided to include these in their uncertainty budget to demonstrate how they affect the combined standard uncertainty.

Table 23.5 Data for uncertainty assessment

Uncertainty source	*Data source*	*Mean*	*Standard deviation*
Repeatability	QC data	48.6%	6.3%
$purity_{cal}$	Certificate from drug standard (95% confidence)	99.98%	0.04%
flask cal	Manufacturer's specifications	10.0 mL	0.1 mL
$Homogen_{samp}$	Experiment	57.1%	0.6%

The remaining standard uncertainties are uncorrelated, so the root sum square technique is employed as follows:

$$U = \sqrt{\left((4.5\%)^2+(0.6\%)^2+(0.6\%)^2\right)} = 4.6\%$$

Step 7: Expressing expanded uncertainties

Since the type A uncertainty data dominate, they are relied upon as a guide in determining the value for the coverage factor (k). However, the relatively small number of measurements ($n = 8$) of the QC sample does limit the confidence in the calculated standard deviation, so the Student's t distribution table (see Table 23.3) is consulted for the appropriate coverage factor. Since this laboratory also reports uncertainty to its customers at a 95% confidence level, $k = 2.4$ ($n = 8$; $n - 1 = 7$). This leads to an expanded uncertainty of 11.1%, but this value will be rounded up to 12% as suggested earlier.

Step 8: Reporting results

The laboratory reports the purity of the cocaine base as $57 \pm 12\%$ (95% confidence level).

Example 3: Measurement uncertainty associated with the quantification of ethanol in a blood sample

A laboratory routinely analyses whole-blood samples for ethanol concentration. Following a thorough mixing, they aliquot 0.25 mL of the blood sample, calibrator or QC sample directly into a headspace sample vial. To this, 0.25 mL of an internal standard solution and about 1 mL of a saturated aqueous solution of sodium chloride are added. The vials are capped and then incubated for 30 min at 60°C before 1.0 mL of the headspace is injected into a gas chromatograph with a flame ionisation detector. Quantification is based on the ratio of the integrated peak area for ethanol versus the peak area for the internal standard. This ratio is converted to a concentration on the basis of the equation of a straight line generated from a 6-point calibration curve.

Two QC samples (800 mg/L and 1500 mg/L) are analysed with each batch of samples. There are 74 entries for each of these QC samples recorded. Statistics for the first QC sample indicates a mean result of 780 mg/L with a standard deviation of 20 mg/L. The second QC sample has a mean result of 1510 mg/L with a standard deviation of 30 mg/L.

Uncertainty Budget Form: Uncertainty Associated with the Purity Determination of Cocaine Base					
Method: SOP #23					
Analyst: J. Smith			Date: 02-Apr-2008		
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
Repeatability	A	6.3%	Student's *t*	$\sqrt{2}$	4.5%[(a)]
~~$Purity_{cal}$~~	~~B~~	~~0.04%~~	~~Normal~~	~~2~~	~~0.02%~~
Flask cal_{cal}	B	1%	Rectangular	$\sqrt{3}$	0.6%
Flask cal_{samp}	B	1%	Rectangular	$\sqrt{3}$	0.6%
~~Sample Homogeneity~~	~~B~~	~~0.4%~~	~~Rectangular~~	~~$\sqrt{3}$~~	~~0.2%~~
Combined Uncertainty:		4.6%			
Confidence Level:		95% Confidence Level (k = 2.4)			
Expanded Uncertainty:		12%			

[(a)]Refer to Student's *t* table.

Figure 23.12 Uncertainty budget for Example 2.

A request comes into the laboratory to analyse a whole-blood sample collected from a suspected drink driver. Two aliquots of the whole blood are analysed and the lower result of the two (BAC_{samp}) values is reported to the contributor.

Step 1: Define what is being measured

An appropriate title for the uncertainty budget would be 'Quantification of Ethanol in Whole Blood'.

Step 2: Identify sources of uncertainty

The laboratory lists the potential sources of uncertainty (Table 23.6) and then constructs a cause-and-effect diagram to visualise the relationships (Fig. 23.13). At first, it may seem that there should be six instances for each of the sources of uncertainty related to the calibration curve, but the same effect, same time rule (see Table 23.2), allows all to be combined into single branches off the *Cal* branch. A similar situation occurs with the duplicate analysis of the whole-blood sample, as well as the pipette delivery volume of the internal standard (Vol_{IS}) and its integrated peak area (A_{IS}).

Now that all of the potential sources of uncertainty are identified, some of these sources can be eliminated by logic alone. For example, while the laboratory initially considered that sample homogeneity might be an important source of uncertainty, the fact that they thoroughly mix the blood samples prior to pipetting most likely eliminates this source as a major contributor to the overall uncertainty. Similarly, since the amount of saturated sodium chloride added is only meant to be an approximate amount, it seems logical that all sources related to it can be eliminated. Also, since the laboratory prepares a calibration curve with each batch of samples, the uncertainty associated with the calibration of the internal standard is irrelevant. With regard to the internal standard, the important aspect is that the same volume (Vol_{IS}) is added to each sample, calibrator and QC sample.

Table 23.6 Potential sources of uncertainty

Reproducibility of method (*Reprod*)
Calibration curve (*Cal*)
- Certification of calibrator solutions (Cal_{cal})
- Pipetting calibration solutions (Vol_{cal})
- Integration of calibrator peaks (A_{cal})

Samples (*Samp*)
- Homogeneity (H_{samp})
- Pipetting samples (Vol_{samp})
- Integration of sample peaks (A_{samp})

Internal standard (*IS*)
- Certification of internal standard stock (Cal_{IS})
- Preparation of internal standard solution ($Prep_{IS}$)
- Pipetting internal standard solution (Vol_{IS})
- Integration of internal standard peaks (A_{IS})

Sodium chloride (*NaCl*)
- Preparation of sodium chloride solution ($Prep_{NaCl}$)
- Pipetting of sodium chloride solution (Vol_{NaCl})

Step 3: Reconciliation of uncertainty components

The laboratory evaluated the sources of uncertainty for which its historical reproducibility data should sufficiently account (Fig. 23.13). They felt that it was reasonable to consider that the uncertainty associated with the individual integrated areas for the peaks related to ethanol and the internal standard were represented by the reproducibility data. The same cannot be said for the remaining sources of uncertainty (Cal_{cal}, Vol_{cal}, Vol_{IS}, and Vol_{samp}), so they should still be considered in developing the uncertainty budget.

Step 4: Quantifying the uncertainty components

Given that two separate QC samples have been in use and that each has its own established standard deviation, there are two different approaches that can be taken for determining the standard deviation for these QC data. The first is to convert the standard deviation for each of the QC levels into percentages (2.6% and 2.0%, respectively) and then, since there are equal numbers of entries for each QC level, take the average of the two percentages as the standard deviation for the historical data (in this case, 2.3%).

Second, and easier, is to simply use the higher of the two percentages (2.6%) as the reproducibility standard deviation. For this example, the latter is demonstrated, but since only 74 QC measurements are reflected in this value, the laboratory must remember to refer to the Student's *t*-test distribution table when expanding the combined uncertainty. Additionally, since the laboratory reports the lower of the two quantitative measurements, it should not take the standard deviation of the mean for the type A assessment of the reproducibility data.

Calibration certificates for pipettes used in the delivery volumes were referenced for the uncertainty budget. Additionally, a certificate was obtained for each pre-made calibration solution and the worst of the listed standard deviations of the calibrators was used to represent the uncertainty on the budget form (Fig. 23.14).

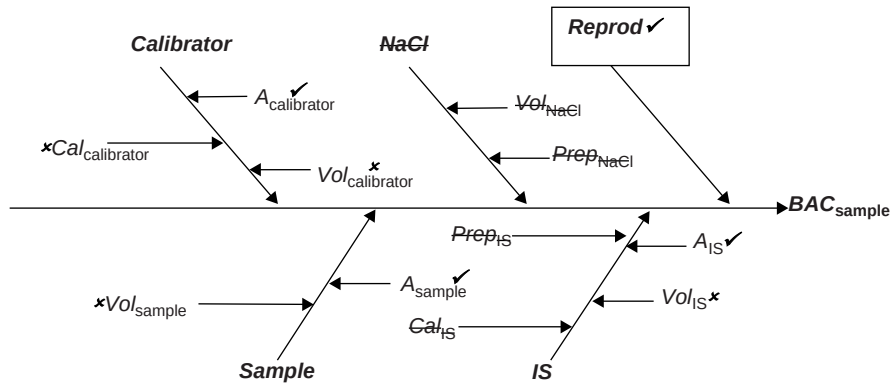

Figure 23.13 Cause-and-effect diagram for Example 3.

Uncertainty Budget Form: Quantification of Ethanol in Whole Blood					
Method: SOP #3					
Analyst: D. Trump			Date: 09-May-2008		
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
Repeatability	A	2.6%	Student's *t*	1	2.6%[(a)]
Vol_{samp}	B	1.3%	Normal	2	0.7%
~~Vol_{IS}~~	~~B~~	~~0.2%~~	~~Normal~~	~~2~~	~~0.1%~~
Vol_{cal}	B	0.9%	Normal	2	0.5%
Cal_{cal}	B	2.0%	Normal	2	1.0%
Combined Uncertainty:		2.9%			
Confidence Level:		99.7% Confidence Level (k = 3.2)			
Expanded Uncertainty:		9.3%			

[(a)]Refer to Student's *t* table.

Figure 23.14 Uncertainty budget for Example 3.

Step 5: Conversion to standard uncertainties

At this point, it is useful to remember that most calibration certificates should provide the standard uncertainty at a 95% confidence level. To convert these values back to $\pm 1\sigma$ value, it must be divided by 2. When it is unclear whether the calibration is actually done by a certified laboratory, a rectangular distribution (divide by $\sqrt{3}$) is a more conservative option.

Step 6: Combining standard uncertainties

Using the one-third rule, the uncertainty associated with the volume of the internal standard added to the samples (Vol_{IS}) can be eliminated from the uncertainty budget. Others could also be excluded, but the laboratory decides to include the rest in the combined uncertainty.

The RSS technique will be used to combine the uncertainties as none appears correlated. Doing so will result in a combined uncertainty value of 2.9%.

Step 7: Expressing expanded uncertainties

The type A uncertainty data appear to have the largest influence on the overall uncertainty, so they are relied upon in determining the k value. Once again, due to the relatively small number of measurements ($n = 74$) of the QC samples, the Student t distribution table (see Table 23.3) is consulted for the coverage factor. This laboratory reports uncertainty to its customers at a 99.7% confidence level, so $k = 3.2$, resulting in an expanded uncertainty of 9.3%.

Step 8: Reporting results

The laboratory's first measurement of the ethanol in the whole-blood sample was 1210 mg/L. Their second aliquot measured 1240 mg/L. The lower of the two values (1210 mg/L) is reported to the customer. The expanded uncertainty associated with this measurement must be converted from a percentage into mg/L. So the 9.3% expanded uncertainty correlates to 110 mg/L at a 99.7% confidence level. The customer should receive a report that indicates that the concentration of ethanol in the whole blood sample was 1210 ± 110 mg/L (99.7% confidence level).

Example 4: Measurement uncertainty associated with the quantification of alprazolam and α-hydroxyalprazolam in a blood sample

A toxicology laboratory routinely analyses blood samples for common benzodiazepines and provides quantitative results of their findings to their customers. Their procedure calls for 1 mL of a matrix-matched calibrator, control or sample to be mixed with 0.1 mL of a deuterium-labelled internal standard and 4 mL of an aqueous buffer. Following vortexing and centrifugation, solid-phase extraction is utilised. The resulting eluent is taken to dryness, reconstituted, and analysed by liquid chromatography–tandem mass spectrometry. Quantification is based on the ratio of the integrated peaks for the benzodiazepine (or metabolite) versus its corresponding internal standard. Each ratio is converted to a concentration based on the equation of a straight line generated from a 5-point calibration curve.

The laboratory has prepared a set of in-house calibration standards in whole blood that contain common benzodiazepines and their metabolites. Similarly, their internal standard solution contains a mixture of deuterium-labelled benzodiazepines and their metabolites.

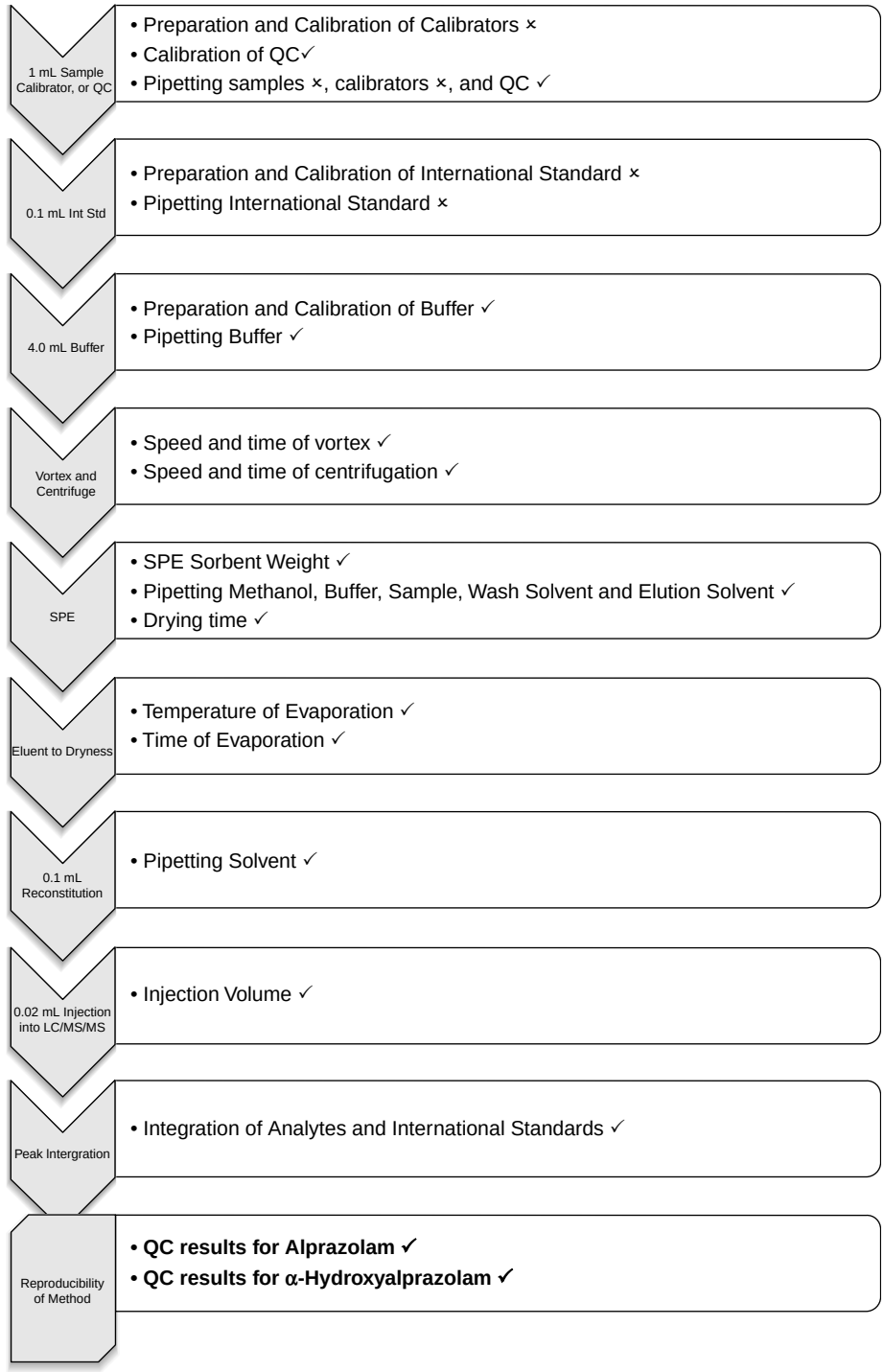

Figure 23.15 Sources of uncertainty in Example 4.

The laboratory uses a commercially prepared QC sample that contains the same common benzodiazepines and metabolites in whole blood. Although the QC sample has been analysed in over 200 batch runs, the laboratory documents only the QC results of individual benzodiazepines and metabolites if they are actually quantified in one of the samples within that batch run. Therefore, there are far fewer actual QC results recorded for individual benzodiazepines and metabolites.

A blood specimen arrives in the laboratory for analysis. Screening results suggest that a benzodiazepine may be in the sample, so confirmatory and quantitative analysis is undertaken using the above procedure. The unknown specimen, as well as the calibrators and QC specimens, are analysed by taking single samples of each.

The results confirm the presence of alprazolam at 0.08 mg/L and its metabolite, α-hydroxyalprazolam, at 0.10 mg/L in the blood sample. The laboratory's QC data for alprazolam (0.03 mg/L) and α-hydroxyalprazolam (0.03 mg/L) show standard deviations of 0.002 mg/L and 0.003 mg/L, respectively ($n = 49$).

Step 1: Define what is being measured

Since two analytes are being quantified, there will actually be two uncertainty budgets developed. The titles could reasonably be 'Quantification

of Alprazolam in Blood' and 'Quantification of α-Hydroxyalprazolam in Blood'.

Step 2: Identify sources of uncertainty

At first glance, it may seem that there is a large number of uncertainty sources in quantitative procedures such as these (Fig. 23.15). But the rules of Table 23.2, as well as common sense (and the reconciliation performed in step 3), help keep things manageable.

Step 3: Reconciliation of uncertainty components

As can be seen in Fig. 23.15, the vast majority of the listed sources of uncertainty can be removed as they are adequately taken into account in the reproducibility data. In reality, most sources of uncertainty become irrelevant once the internal standard is added to the samples, calibrators and controls. Provided that the same volume of the internal standard solution is added to all samples, the uncertainty associated with most of the remaining steps should be compensated by the fact that the quantification is based on the ratio of analyte to internal standard. After all, this is the very reason for using internal standards in quantitative methods. So, after reconciliation, the sources of uncertainty can be limited to those listed in Table 23.7.

Step 4: Quantifying the uncertainty components

Table 23.7 also includes the sources for the standard deviations of each listed uncertainty source. Of particular note for this example is that the laboratory prepares a working calibrator solution of alprazolam and α-hydroxyalprazolam in whole blood at 1.00 mg/L. This solution is then further diluted to make additional calibrators by mixing with whole blood in the actual extraction tube. To include the potential source of uncertainty into these dilutions, a low calibrator is chosen to represent the entire calibration curve dilution. In this example, the laboratory prepares 5-point calibration curves in the concentration range 0.01–1.00 mg/L. Choosing the 0.25 mg/L calibrator (prepared by mixing 250 µL of the 1.00 mg/L working calibration solution with 750 µL of drug-free whole blood) allows the laboratory to apply the uncertainties of two pipettors into the uncertainty budget.

Step 5: Conversion to standard uncertainties

Table 23.7 explains the calculations performed for this step. Figures 23.16 and 23.17 shows how these values are applied to the two uncertainty budgets.

Step 6: Combining standard uncertainties

Once again, the one-third rule helps simplify the calculations involved in combining the standard uncertainties (Figures 23.16 and 23.17). The RSS technique is utilised, as none of the remaining standard uncertainties is correlated.

Step 7: Expressing expanded uncertainties

As usual, the type A uncertainty data are the largest contributor to the overall uncertainty and are relied upon in determining the k value. With only 49 measurements of each of the controls, the Student's t distribution table (see Table 23.3) provides the coverage factor. This laboratory uses a 99.7% confidence level in reporting uncertainty to its customers, so $k = 3.3$, resulting in an expanded uncertainty of 27% and 36% for alprazolam and α-hydroxyalprazolam respectively.

Step 8: Reporting results

The final step before reporting the uncertainty to the customer is to convert the expanded uncertainty back into the units of measurement. For alprazolam, this conversion involves multiplying 27% (0.27) by the measured concentration of the drug in the blood sample (0.08 mg/L). Similarly for the metabolite, the measured amount of α-hydroxyalprazolam (0.10 mg/L) is multiplied by 36%. This results in the following

Table 23.7 Sources of uncertainty

Uncertainty source		*% standard deviation*
Reproducibility of method		
■ QC data for alprazolam ($n = 49$)		6.7%[a]
■ QC data for α-hydroxyalprazolam ($n = 49$)		10.0%[b]
Calibration curve		
■ Calibration of alprazolam stock calibrator		0.20%[c]
■ Calibration of α-hydroxyalprazolam stock calibrator		0.30%[d]
■ Pipetting stock calibration solutions to prepare working calibrator solution in whole blood		0.12%[e]
■ Volumetric flask used to prepare working calibrator solution in whole blood		1.15%[f]
■ Pipetting various volumes of working calibrator solutions to whole blood for calibration curve	250 µL	0.09%[g]
	750 µL	0.08%[g]
Internal standard		
■ Calibration of stock of alprazolam-d_3 internal standard		0.30%[h]
■ Calibration of stock of α-hydroxyalprazolam-d_3 internal standard		0.40%[i]
■ Pipetting stock internal standard solutions to prepare working internal standard solution		0.23%[j]
■ Volumetric flask used to prepare working internal standard solution		2.3%[k]
■ Pipetting 0.01 mL of working internal standard solution into samples, controls and QCs		3.8%[l]
Sample		
■ Pipetting 1.0 mL of sample		0.23%[j]

[a] From QC data: $(0.002/0.03) \times 100$.
[b] From QC data: $(0.003/0.03) \times 100$.
[c] From certificate of analysis [1.003 mg/mL ± 0.004 mg/mL (95% confidence)]: $[(0.004/1.003) \times 100)/2]$.
[d] From certificate of analysis [0.996 mg/mL ± 0.006 mg/mL (95% confidence)]: $[((0.006/0.996) \times 100)/2]$.
[e] From specified tolerance of pipettor (50.0 µL ± 0.1 µL): $[(0.1/50.0) \times 100]/\sqrt{3}$.
[f] From manufacturer's specifications (50.0 mL ± 1.0 mL): $[(1.0/50.0) \times 100]/\sqrt{3}$.
[g] Choose low calibrator to represent this source (see text). From specified tolerance of pipettor (250 µL ± 0.4 µL) and (750 µL ± 1.1 µL). For 250 µL pipettor: $[(0.4/250) \times 100]/\sqrt{3}$. For 750 µL pipettor: $[(1.1/750) \times 100]/\sqrt{3}$.
[h] From certificate of analysis [0.995 mg/mL ± 0.006 mg/mL (95% confidence)]: $[(0.006/0.995) \times 100]/2$.
[i] From certificate of analysis [0.998 mg/mL ± 0.008 mg/mL (95% confidence)]: $[(0.008/0.998) \times 100]/2$.
[j] From specified tolerance of pipettor (1000.0 µL ± 3.9 µL): $[(3.9/1000.0) \times 100]/\sqrt{3}$.
[k] From manufacturer's specifications (10.0 mL ± 0.4 mL): $[(0.4/10.0) \times 100]/\sqrt{3}$.
[l] From specified tolerance of pipettor (10.00 µL ± 0.66 µL): $[(0.66/10.0) \times 100]/\sqrt{3}$.

Uncertainty Budget Form: Quantification of Alprazolam in Whole Blood					
Method: SOP #355-3					
Analyst: J. Miller			Date: 16-Jun-2008		
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
Repeatability - Alpraz QC Data	A	6.7%	Student's *t*	1	6.7%[(a)]
~~Calibration of Alpraz Stock Std~~	B	0.40%	Normal	2	0.20%
~~Pipet 50 µL of Alpraz Stock Std~~	B	0.20%	Rectangular	$\sqrt{3}$	0.12%
~~50-mL Vol Flask (for Working Alpraz Cal Soln)~~	B	2.0%	Rectangular	$\sqrt{3}$	1.15%
~~Pipet Cal Soln (250 µL)~~	B	0.16%	Rectangular	$\sqrt{3}$	0.09%
~~Pipet Cal Soln (750 µL)~~	B	0.14%	Rectangular	$\sqrt{3}$	0.08%
~~Calibration of Alpraz-d_3 Int Std~~	B	0.60%	Normal	2	0.30%
~~Pipet 1000 µL of stock Alpraz-d_3 Int Std (for Working Int Std Soln)~~	B	0.40%	Rectangular	$\sqrt{3}$	0.23%
10-mL Vol Flask (for Working Alpraz-d_3 Int Std Soln)	B	4.0%	Rectangular	$\sqrt{3}$	2.3%
~~Pipet 1.0 mL of sample~~	B	0.40%	Rectangular	$\sqrt{3}$	0.23%
Pipet 0.1 mL of Working Alpraz-d_3 Int Std Soln samples	B	6.6%	Normal	2	3.8%
Combined Uncertainty:		8.04%			
Confidence Level:		99.7% Confidence Level (k = 3.3)			
Expanded Uncertainty:		27%			

[(a)]Refer to Student's *t* table.

Figure 23.16 Uncertainty budget for alprazolam for Example 4.

Uncertainty Budget Form: Quantitation of α-Hydroxyalprazolam (α-HA) in Whole Blood					
Method: SOP #355-3					
Analyst: J. Miller			Date: 16-Jun-2008		
Sources of Uncertainty	**Type A or Type B?**	**Std Dev or Outside Limits (units)**	**Distribution Model**	**Divisor**	**Standard Uncertainty (1σ)**
Repeatability - α-HA QC Data	A	10.0%	Student's *t*	1	10.0%[(a)]
~~Calibration of α-HA Stock Std~~	B	0.60%	Normal	2	0.30%
~~Pipet 50 µL of α-HA Stock Std~~	B	0.20%	Rectangular	$\sqrt{3}$	0.12%
~~50-mL Vol Flask (for Working α-HA Cal Soln)~~	B	2.0%	Rectangular	$\sqrt{3}$	1.15%
~~Pipet Cal Soln (250 µL)~~	B	0.16%	Rectangular	$\sqrt{3}$	0.09%
~~Pipet Cal Soln (750 µL)~~	B	0.14%	Rectangular	$\sqrt{3}$	0.08%
~~Calibration of α-HA-d_3 Int Std~~	B	0.80%	Normal	2	0.40%
~~Pipet 1000 µL of stock α-HA-d_3 Int Std (for Working Int Std Soln)~~	B	0.40%	Rectangular	$\sqrt{3}$	0.23%
~~10-mL Vol Flask (for Working α-HA-d_3 Int Std Soln)~~	B	4.0%	Rectangular	$\sqrt{3}$	2.3%
~~Pipet 1.0 mL of sample~~	B	0.40%	Rectangular	$\sqrt{3}$	0.23%
Pipet 0.1 mL of Working Alpraz-d_3 Int Std Soln samples	B	6.6%	Rectangular	$\sqrt{3}$	3.8%
Combined Uncertainty:		10.7%			
Confidence Level:		99.7% Confidence Level (k = 3.3)			
Expanded Uncertainty:		36%			

[(a)]Refer to Student's *t* table.

Figure 23.17 Uncertainty budget for α-hydroxyalprazolam for Example 4.

reported result to the laboratory's client: 'Alprazolam and its metabolite, α-hydroxyalprazolam, were identified in the blood sample at 0.08 ± 0.02 mg/L and 0.10 ± 0.04 mg/L, respectively, using a 99.7% confidence level.'

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CHAPTER

24 Pharmacokinetics and Metabolism

OH Drummer

Introduction

An important part of any investigation involving drugs or poisons is the interpretation of toxicological data. The onset, duration and intensity of action of a drug after administration are controlled by the rate at which the drug reaches its site of action, by the concentration of the drug and by the sensitivity of the individual to the drug. Hence, a good understanding of the basic concepts of pharmacokinetics and metabolism is essential to enable an informed comment to be made on the degree and timing of the exposure, and a likely response to the substance(s) under question. These answers are always predicated by the amount of information available to the toxicologist. However, the health of the subject, particularly disease processes capable of affecting the pharmacokinetics, and whether drug exposure is acute or chronic are essential in any interpretation.

Basic concepts of pharmacokinetics

The disposition of a drug includes the processes of absorption, distribution into and out of tissues, and the metabolism and elimination from the individual. Pharmacokinetics describes the time course of the blood and tissue concentration profile, while pharmacodynamics refers to the relationship between dose and the intended pharmacological response.

The pharmacokinetics of a drug's concentration in blood or plasma consists of the absorption, distribution and elimination phases. The absorption phase relates to the entry of drug from the absorption site. This may be relatively slow, i.e. from the gastrointestinal tract for oral absorption, or rapid if given intravenously. During the absorption phase, drugs are distributed by the circulation to all parts of the body. The uptake of drugs into the various tissues is a time-dependent process and will differ between tissues and from drug to drug. Shortly after entry into tissues, drugs are subject to both metabolism and elimination from the body. These two processes are often the more important for toxicologists and are also often the most variable.

Most drugs given intravenously or orally will give blood (or plasma) concentration–time curves of the type shown in Fig. 24.1A and Fig. 24.1B, respectively. Following intravenous administration, there is a rapid decrease in plasma-drug concentration in the early period (α phase) when distribution is the major process, followed by a slower and more constant rate of decrease in the elimination phase (β phase). After oral administration, plasma concentrations initially increase while the drug is being absorbed and then decrease when elimination becomes the major process. The drug distribution phase is often not considered since it tends to be more rapid than either the absorptive or elimination phases. However, in some situations it will need to be considered.

Absorption

Drug absorption is an important process of drug pharmacokinetics. The route of administration is an important factor in determining the rate and extent of absorption. Routes of administration can include oral, rectal, ocular, inhalation through the nose or mouth absorption, through the skin and other body surfaces, and by injection into muscle or veins. All of these routes have different rates and extents of absorption.

Most drugs are administered orally, hence an understanding of the mechanism of absorption by this route is most important for the toxicologist.

Absorption from the gastrointestinal tract

Drugs are absorbed either by passive diffusion of the un-ionised drug or by active transport. Passive diffusion is by far the most common mechanism. Drug absorption occurs predominantly in the small intestine primarily because of its large surface area and the relatively long contact time.

Absorption is possible throughout the gastrointestinal tract, from stomach to rectum, although the major site is the upper small intestine. This has high peristalsis, a high surface area, high blood flow and optimal pH for the absorption of most drugs, all of which result in a high absorption rate. Drug absorption tends to be much less rapid from other parts of the gastrointestinal tract.

Some drugs are absorbed to a small extent in the stomach, although these are largely acidic drugs that are un-ionised in the low pH environment in this organ. These drugs include aspirin, non-steroidal anti-inflammatory drugs and some angiotensin-converting enzyme inhibitors.

Absorption also occurs if drugs are given rectally as suppositories. In this situation absorption may be less efficient than oral administration. For example, oxycodone suppositories require a higher dose to achieve the desired response than oral administration. However, those drugs subject to large first-pass metabolism via the liver may have higher absorption when administered rectally since they will bypass the liver by this route.

Absorption of ionised drugs also occurs. For example, paraquat is highly ionised but appears to be absorbed slowly from the gastrointestinal tract throughout its length and over a considerable time from the moment of ingestion.

Poisoning by orally active drugs can be treated effectively by the prompt administration of an oral adsorbent, such as charcoal, which prevents further absorption of the drug. This is effective only if the drug has not passed significantly down the gastrointestinal tract. Figure 24.2 shows the factors that influence gastrointestinal absorption of drugs.

Absorption from other sites

Absorption through the lungs occurs for substances that are smoked or inhaled, e.g. smoked cocaine or heroin, nicotine smoked from cigarettes, tetrahydrocannabinol smoked from cigarettes and compounds involved in volatile substance abuse. The lungs are an efficient organ for transporting drug from the air into the blood supply, such that the rate of absorption approaches intravenous injection.

Absorption of drugs through membranes is also common. This includes nasal insufflation of cocaine ('snorting'), sublingual and buccal absorption of nitroglycerin-like vasodilators and buprenorphine, as well as absorption of drugs through skin patches, such as oestrogens, fentanyl or nicotine. Less commonly, vaginal absorption and ocular absorption of drugs are also encountered by toxicologists. The rate of absorption can vary significantly from one site to another. Sublingual absorption is very rapid, with drug effects noticeable within minutes, while drugs are absorbed relatively slowly through the skin.

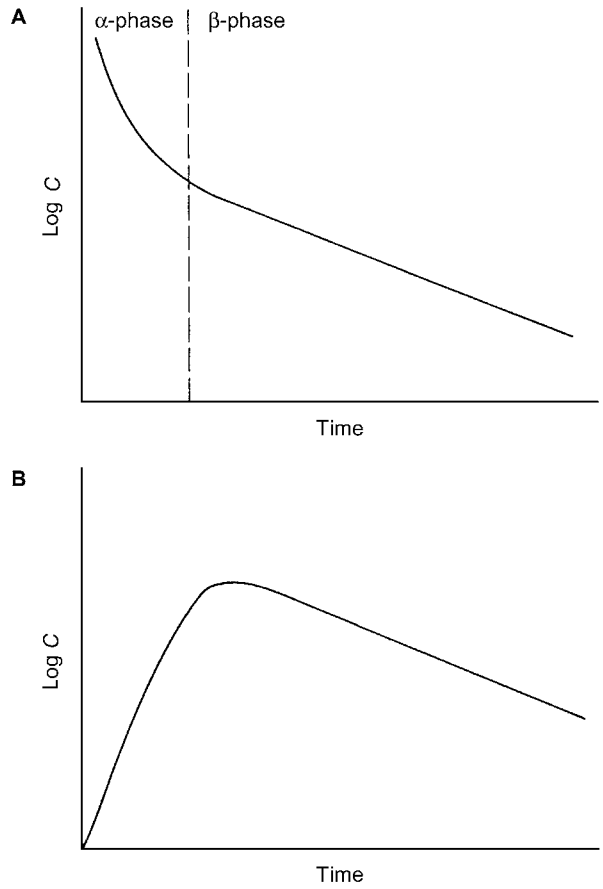


Figure 24.1 Typical semilogarithmic plots of plasma concentration (*C*) versus time for a drug given (A) by intravenous injection and (B) orally. The terminal rate of decline of plasma concentration is the same irrespective of the route of administration.

Drugs injected into the spinal canal, and into muscle or surface tissues (intramuscularly, intraperitoneally), usually exhibit relatively rapid absorption. However, absorption of even intramuscularly injected drugs can be slow if the site of injection is poorly perfused by a blood supply and the site is very fatty.

Drugs that are absorbed and bypass the gastrointestinal tract will not be subject to first-pass metabolism (see next section). These drugs will therefore show higher bioavailability than the same drug delivered through the gastrointestinal tract.

First-pass metabolism and bioavailability

Drugs absorbed following oral ingestion pass through the mesenteric circulation into the liver before entering the systemic circulation. In the 'first pass', drugs can be substantially metabolised by the liver before ever having a pharmacological effect. The proportion of the drug reaching the systemic circulation following oral administration to that obtained following intravenous dosing is the oral (or absolute) bioavailability (F). This is measured by comparing the area under the curve (AUC) for the oral and intravenous doses from zero to the time point at which most or all of the drug is finally eliminated by the body.

This bioavailability is given by the formula:

$$F = \frac{\text{AUC (oral route)}}{\text{AUC (intravenous route)}}$$

where AUC (the area under the plasma concentration–time curve) represents the amount of drug entering the systemic circulation from zero to infinite time.

The relative bioavailabilities of drugs can be determined by comparing other routes of administration with oral or another reference route of administration, or even between drug formulations.

The oral bioavailabilities of selected drugs are shown in Table 24.1. In general, drugs that are readily metabolised by liver enzymes have lower bioavailabilities than drugs that are not as readily metabolised by the liver. While the effect of first-pass metabolism is to reduce the action of the parent drug, drugs that are metabolised to active forms will have a different profile of activity than when the drug is given parenterally (not through the gastrointestinal system). For example, the activity of cannabis is increased substantially by the presence of the 11-hydroxy metabolite of tetrahydrocannabinol (THC) in addition to THC. This 11-hydroxy metabolite is present only in blood (and tissues) in relevant amounts when cannabis is consumed orally (cookies), but not when it is smoked. This is due to the actions of the liver on absorbed THC as the drug passes through the liver from the mesenteric circulation. Smoking (of cannabis) bypasses this 'first-pass' effect allowing little 11-hydroxy metabolite to be produced.

Prodrugs activated by liver metabolism are preferentially taken orally to maximise the activity of the drug. An example is enalapril, which is converted to enalaprilic acid. The latter form is much more active than the parent drug. Drugs given rectally will be subject to only a small degree of first-pass metabolism since only about one-third of the contents of the lower part of the gastrointestinal system passes though the liver.

Enterohepatic circulation

Drugs and metabolites present in the liver are often also excreted into bile. These pass into the jejunum and may be reabsorbed back into the mesenteric circulation or passed into the faeces. The process of biliary excretion and re-absorption may occur a number of times before drug is completely eliminated by the body. This recycling of drugs is known as enterohepatic circulation.

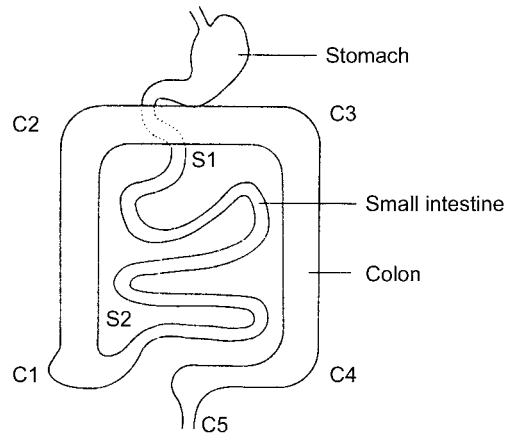
Common examples include the glucuronide conjugates of drugs that are excreted in significant amounts in the bile, such as morphine. Enterohepatic circulation prolongs the persistence of a drug in the body and may lead to delayed toxicity. Drugs that undergo enterohepatic circulation may be detected in the faeces even if they have been administered by a parenteral route. Drugs may even be detected in gastric contents (small amounts) following reflux or vomiting. It is therefore important that small amounts of drug in the gastric contents are not automatically assumed to be from oral administration.

Drugs are found in bile both as parent species and as metabolites such as the glucuronide conjugates. In the absence of urine, bile can be a useful body fluid, e.g. for opioids, the benzodiazepines and colchicine.

Distribution of drug into tissues

The uptake of drugs into tissues depends on a number of factors. These include the blood flow to the tissues, the partition coefficient of the drug between blood and the tissue, the degree of ionisation of the drug at the pH of plasma, the molecular size of the drug, and the extent of tissue and plasma protein binding. For example, the distribution of plasma protein-bound drugs such as the warfarin-type anticoagulants is restricted to plasma and extracellular fluid, whereas alcohol distributes equally into the total body water.

The approximate volumes of the body water compartments for a person of average weight are intracellular water 25 L and extracellular water 17 L (of which 3 L is plasma water). An intravenous dose of a drug that is distributed immediately into the total body water (approximately 42 L) would give an initial plasma concentration (dose divided by 42) approximately two-fifths of that obtained if the same dose were distributed only into extracellular water (dose divided by 17). If the drug is extensively bound to tissue proteins, an even lower initial plasma concentration would be obtained and the volume term relating the dose to the plasma concentration could exceed the volume of the body. The approximate proportions of drugs with nominated volumes of distribution in the plasma water are given in Table 24.2.



Organ	pH	Surface area	Blood flow*	Motility and transit time of contents
Stomach†	1 to 3	$<5\,m^2$	Rises on stimulation of gastric secretions	Relatively 'quiet'. Transit time of contents depends on their chemical and physical nature, including pH and osmotic pressure. Placed in order of increasing transit times, liquids < solids; carbohydrates < proteins < fats. Times vary from 0.5 h for a small amount of liquid carbohydrate to 20 h for a large fatty meal
Small intestine, S	S1, 5 to 7 S2, 7 to 8	$200\,m^2$	Very fast counter-current flow in villi. Blood transit time – 1 s after meals; 5 s resting; 30 s in shock	Food churned, and propelled by very active movements in waves, by both peristalsis and segmentation Transit time – about 2 h
Colon, C	7 to 8	$<5\,m^2$	Average	Residues of meals found at C1 after 4 h, C2 after 6 h, C3 after 9 h and C4 after 12 h. Residues are held up in the rectum (C5) and then egested in faeces over a period of time. Earliest traces may be egested within 6 h, the last may take 1 week. On average, 75% of residues are egested by the third day

* Average is 36 mL/min per 100 g intestine, 50 to 75% of which circulates in the mucosal layers.
† The volume of the stomach can change markedly: 50 mL during fasting and up to 2 L when full.

Figure 24.2 Factors that influence the passage and absorption of drugs in the gastrointestinal tract.

Table 24.1 Bioavailabilities and volumes of distribution for selected drugs

Drug	*Bioavailability (%)[a]*	*Volume of distribution (L/kg)*	*Clearance (mL/min per kg)*
Alprazolam	90	~0.7	~0.7
Amitriptyline	30–60	~15	11.5
Diazepam	100	0.5–2.5	0.3–0.5
Ethanol	50–80	~0.6	—
Flunitrazepam	80–90	3.4–5.5	~2.0
Morphine	20–30	3–5	15–20
Oxazepam	93	0.5–2	1–2
Pentobarbital	95	0.7–1.0	0.3–0.5
Temazepam	>80	~1.0	1–2
Tetrahydrocannabinol	6–20	~10	14
Thioridazine	—	18	—
Zaleplon	30	1.3	0.9
Zolpidem	70	0.5–0.7	0.26
Zopiclone	80	1.3–1.6	13.9

[a]Oral bioavailability compared with an intravenous injection.

Table 24.2 Proportion of a drug in the body water compartment

Volume of distribution (L/kg)	*Proportion in water compartment (%)*
0.1	40
0.15	27
0.6	6.7
1.0	4
10	0.4

This has implications in cases involving loss of blood and when trauma victims are given fluid replacements (artificial blood replacements, transfusions, etc.). Provided that some re-equilibrium has occurred and elimination time is not too long, the drug concentration after blood replacement will be quite similar to the concentration seen before blood loss for drugs with volumes of distribution greater than about 0.5 L/kg.

The instantaneous equilibrium of drug concentrations throughout the body as considered above does not necessarily require that the concentrations are equal throughout the body. In fact, drug concentrations in tissues are rarely equal to those in plasma. For example, the tissue : plasma concentration ratio will be very low immediately following intravenous administration. As time progresses, the amount of drug

in the tissue compartment will increase and, like that in the plasma compartment, will eventually reach a plateau. If the drug is actively stored in a particular tissue compartment, then the ultimate concentration ratio between the tissue and plasma will be relatively high. It should also be noted that the tissue : plasma concentration ratio depends not only on the processes of distribution but also to a large extent on the route of drug administration and on whether single or multiple doses have been given.

Blood and plasma concentrations

Many drugs show differences in concentration between whole blood and plasma (or serum). This occurs because uptake of drugs into red blood cells (erythrocytes) can be limited by the physiochemical properties of the drug and its ability to move through cellular membranes. For example, THC is almost absent in red blood cells, hence the plasma concentration will be twice that of whole blood, assuming a haematocrit of 0.5. In contrast, chloroquine has a much higher red blood cell concentration than plasma.

Toxicologists should be wary of performing this calculation in haemolysed specimens, since haemolysis will liberate the contents of red blood cells into the plasma (serum), and thereby affect this equilibrium. This applies particularly to postmortem specimens.

Since most of the pharmacokinetic data have been obtained in plasma, it may be necessary to make some allowances for blood concentration data for drugs that exhibit different blood and plasma concentrations.

Binding of drugs to plasma proteins

Many, if not most, drugs bind to proteins in plasma with sufficient affinity to effectively remove that portion of the drug from having biological activity. For example, if a drug is 90% bound to plasma proteins, only 10% can exhibit biological activity. Plasma-binding data for drugs are given in the monographs.

The binding sites for drugs in plasma are predominantly albumin, although β-globulin and α_1-acid glycoprotein are also significant sites for some drugs.

The significance of protein binding of drugs is that the 'free' or unbound fraction in plasma may be affected by illness and by the use of other drugs. In disease states, particularly kidney and liver dysfunction, protein binding can be markedly reduced, often increasing the apparent effects of drugs. Since binding sites are saturable, other drugs can compete with binding and reduce the net binding of drugs. This can cause a net increase in drug action for some drug combinations, although in practice this is rarely of clinical significance.

Volume of distribution

The apparent volume of distribution (V_D) is the amount of drug in the body (A_P) divided by the plasma concentration (C_p) after distribution equilibrium has been established:

$$V_D = A_P / C_p$$

An estimate of V_D can be obtained by calculating the concentration before elimination has occurred by extrapolating the concentration versus time curve for intravenous doses to time zero (C_0) and dividing this value into the dose delivered.

If oral doses are used, the dose must be adjusted for the bioavailability (F). For example,

$$V_D = FD_o / C_o$$

where D_0 is the dose at time 0.

Drugs that are taken up into body fat or bind to cellular structures will have a higher V_D. It is not uncommon for volumes of distribution to be over 10. Morphine has a V_D of 2–5 L/kg. A range of 0.5–5 is seen for most of the amfetamines and many of the benzodiazepines. The highly lipid-soluble THC has a volume of distribution of approx. 10. Drugs with high octanol/buffer partition coefficients will generally have high volumes of distribution. Examples of these parameters are contained in the monographs in Volume 2.

The drug concentration in body fluids other than plasma may be used, e.g. whole blood, but different values for V_D are obtained for each. It is important, therefore, to note which fluid is being used. In the monographs, volumes of distribution, unless otherwise stated, are based on plasma concentrations.

The value of the volume of distribution is determined mainly by the physiological processes of perfusion and protein binding, but it seldom has a true physiological meaning. For example, the volume of distribution of highly protein-bound furosemide is of the order of 15 L, and that of alcohol is about 35 L. However, the value for digoxin, which is extensively distributed and bound in extravascular tissues, is of the order of 450 L.

After distribution equilibrium has been established, knowledge of the volume of distribution allows the amount of drug in the body (D) to be estimated from a single measured blood concentration:

$$D = V_D \times C$$

If the time elapsed since drug administration (t) is known, together with some pharmacokinetic data for the drug, then it should be possible to estimate the original dose (D_0) of the drug. Thus, for a drug given by intravenous injection:

$$D_o = V_D C e^{k_{el} t}$$

where k_{el} is the elimination rate constant (see later).

However, if the drug is given orally, a much more complex relationship applies. It is necessary to know the bioavailability (F) and the absorption rate constant (k_a). Then the dose is given by the expression

$$D_o = \frac{V_D C (k_a - k_{el})}{F k_a (e^{k_{el} t} - e^{k_a t})}$$

If a drug were distributed instantaneously throughout the body, then the volume of distribution would be constant at all times and the decrease in plasma concentration could be attributed solely to elimination of the drug. However, in practice, there are time-dependent changes in tissue concentration including absorption and distribution. In a drug overdose, non-linear pharmacokinetics occur, i.e. the plasma concentration does not increase in proportion to dose since many of the pharmacokinetic processes have reached saturation. Hence, calculation of dose from the volume of distribution can be substantially wrong and misleading. It is recommended to use volume of distribution only when an overdose has not been taken and there is a reasonable chance of equilibrium.

Elimination of drugs

Most drugs are eliminated from the body by metabolism in the liver and/or by excretion of the drug and its metabolites by the kidneys. Other mechanisms for drug metabolism and excretion apply for some drugs and poisons. For example, ethanol and other volatile substances are partially removed by transpiration through the lungs, although other mechanisms may also apply (e.g. via sweat and faeces).

Two terms, clearance and half-life, are frequently used to quantify the rate and extent of drug removal from the body.

Clearance

Overall, the efficiency of elimination by an organ can be expressed as the proportion of drug entering the organ that is eliminated from the plasma in a single passage; this is called the *extraction ratio*. The other major factor that controls the overall ability of an organ to remove drug from the body is the rate of delivery of the drug (i.e. blood flow) to the

organ. Drug elimination can be represented as the product of this rate of delivery and the extraction ratio. This product gives the volume of plasma from which drug is completely removed per unit time, and is given the name clearance (Cl). Clearances by different organs are additive. Although the reference fluid normally used is plasma, whole blood may also be used.

In the drug monographs, clearance has been included wherever possible and usually refers to the total plasma clearance (or total whole-blood clearance) after intravenous administration. In some instances, the total clearance calculated after oral administration has been included if the drug is known to be well absorbed and is not subject to significant first-pass metabolism.

The concept of clearance has found particular application in clinical work as it offers a simple relationship between dose rate (dose divided by the time interval between doses, D/τ), and the average plasma concentration (C_{av}) of the drug:

$$C_{av} = (D/\tau)/\mathrm{Cl}$$

Renal clearance is often measured with creatinine. Creatinine is a metabolic by-product of protein metabolism that is neither reabsorbed nor secreted by the tubules. Its concentration can therefore be used to measure the degree of concentration of urine from the glomerular filtrate.

The efficiency of an eliminating organ in removing drug from plasma depends on the health of the organ. Thus, the net change in clearance is proportional to the extent of renal impairment.

Despite its clinical utility, the concept of clearance has certain limitations for the forensic toxicologist because it does not give an immediate indication of the persistence of a drug in the body. For example, although gentamicin and digoxin have similar clearances (about 100 mL/min), digoxin will stay in the body much longer than gentamicin. This is because the volume of distribution of digoxin is several times that of gentamicin, hence there is a much greater volume of fluid from which the drug must be cleared before it is all eliminated. It is therefore of some advantage to the toxicologist to be able to relate clearance to the persistence of a drug in the body.

This can be done by expressing clearance as a fractional clearance, i.e. clearance divided by the volume of distribution. Fractional clearance (Cl/V_D) has the dimension reciprocal time and represents the proportion of drug removed from the body per unit time, i.e. it is a first-order rate constant for drug elimination (k_{el}). This rate constant is given by the gradient of the terminal part of the concentration–time curves shown in Fig. 24.3.

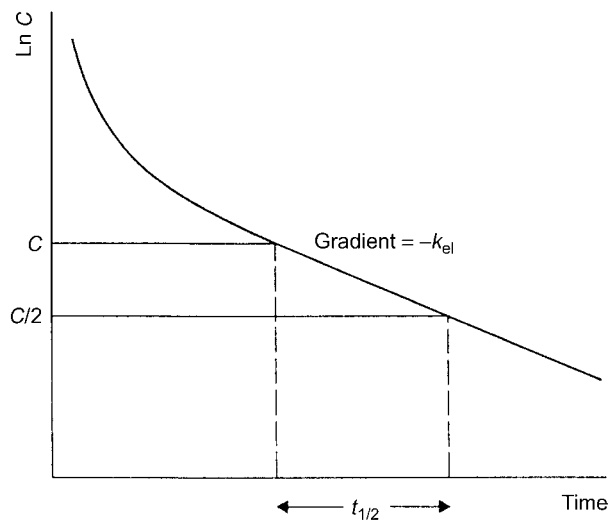


Figure 24.3 Plot of the natural logarithm of plasma drug concentration (ln *C*) versus time (*t*) after intravenous administration. The gradient of the linear part of the curve is equal to the elimination rate constant ($-k_{el}$).

Half-life

The half-life of a drug ($t_{1/2}$) is the time required for plasma concentrations to decline by 50%, provided that elimination occurs by a first-order process (Fig. 24.3). It is related to the elimination rate constant (k_{el}) by the equation:

$$t_{1/2} = 0.693/k_{el}$$

The half-life of a drug provides a measure of the rate of drug loss from the blood. If the dose is known, the half-life of a drug can be used together with information on the volume of distribution, and bioavailability where necessary, to estimate the time elapsed since administration. Conversely, if the elapsed time is known, the half-life can be used to estimate the drug dose, subject to the limitations discussed earlier. The half-life is a function of volume of distribution, clearance and the proportion of drug elimination in unit time. This last term depends on both the extent of its distribution and the efficiency of its elimination. Thus the half-life of a drug may differ between children and adults because of size and weight differences, even though the clearances are equivalent.

For many drugs, the estimated half-life will depend on how the elimination phase is determined. All too often half-lives are calculated while significant absorption and/or tissue distribution is occurring, underestimating the true terminal elimination half-life. It is therefore essential that sufficient time points are used beyond the absorption and distribution phases. For example, the apparent half-life of THC can be an hour or so if only the first several hours of plasma concentrations are determined following smoking of marijuana. Sensitive analytical methods that can detect plasma concentrations below 1 ng/mL indicate that the terminal elimination half-life of THC is actually 2–3 days.

Zero-order processes are best described as a loss of drug per unit time. For example, ethanol elimination is approximately zero order over 200 mg/L. The rate of elimination is expressed as a loss of ethanol per unit time, i.e. 0.10–0.25 (g/L/h) (mean 0.17 (g/L)/h)).

Excretion

Drugs and metabolites are excreted by the kidneys into urine (Fig. 24.4). This process is the single most important mechanism for excretion of most drugs. Renal clearance can result either from glomerular filtration or through tubular secretion. In some cases reabsorption occurs, reversing the secretion process.

The drug is brought to the kidneys with a total plasma flow for both kidneys of approximately 1.4 L/min. Plasma is filtered at the rate of 125 mL/min in the glomeruli, which are the principal sites of excretion. Filtration is passive and only the non-protein-bound drug in the plasma is eliminated by this pathway. A considerable amount of filtered drug may be reabsorbed into the plasma by diffusion back across the tubule wall (which is permeable to un-ionised, lipid-soluble species). The filtrate (125 mL/min) is gradually concentrated as it passes down the tubule to give a final production of urine of about 1 mL/min. About 575 mL/min of plasma circulates in intimate contact with the proximal and distal renal tubules.

The renal tubule may contribute to elimination by active secretion (tubular secretion), and in such cases protein-bound drug may also be eliminated from the plasma.

The extent of elimination by the kidneys can be extremely variable depending on which of the three processes of filtration, secretion and reabsorption predominates for the drug in question. Thus, procainamide is eliminated partly by metabolism and partly as unchanged drug through the kidney. Its renal clearance is of the order of 450 mL/min, indicating major involvement of tubular secretion. By contrast, digoxin has a renal clearance of about 120 mL/min and this could be explained either by filtration alone or by the fact that secretion is balanced by reabsorption. In practice, it is known that filtration accounts for almost all of the renal clearance of digoxin. A further example is methaqualone,

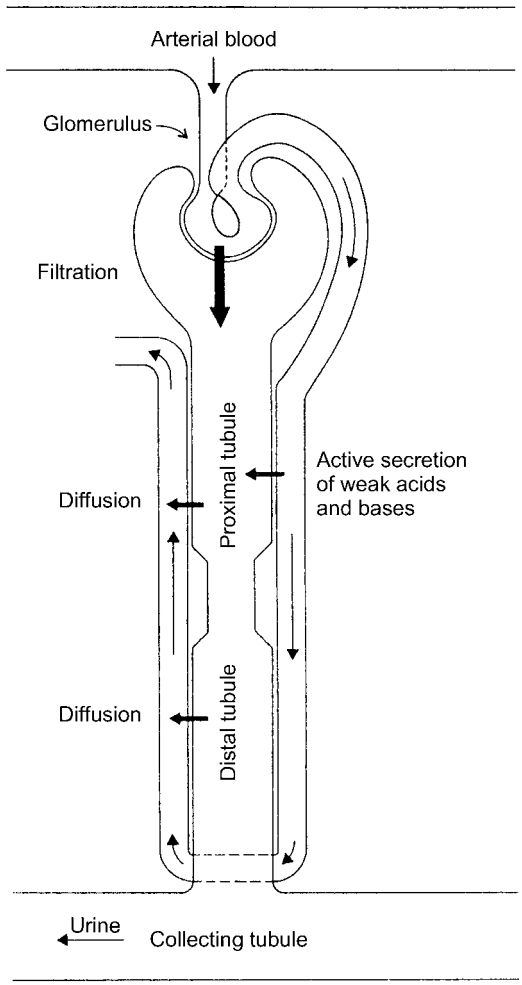


Figure 24.4 Drug elimination by the kidneys. Schematic of a nephron to illustrate the sites of filtration, diffusion and active secretion of drugs.

which has a renal clearance of ~1 mL/min, indicating extensive reabsorption of filtered drug.

One of the major physiological factors that determine the variability in the rate of drug excretion into the urine is the pH of the urine. Only un-ionised species are available for reabsorption by the tubules along the concentration gradient. Thus, acidic drugs (e.g. barbiturates, salicylates) are excreted more rapidly at high pH than are basic drugs (e.g. amfetamines). Conversely, basic drugs are excreted more rapidly at low pH. For example, about 85% of a dose of aspirin is excreted as free salicylic acid in alkaline urine, but only about 5% is excreted when the urine is acidic. Conversely, about 75% of a dose of amfetamine is excreted unchanged in acidic urine but less than 5% if the urine is alkaline.

The effect of varying urinary pH has been used in the treatment of drug overdoses by applying forced alkaline diuresis as an adjunct to the treatment of salicylate or phenobarbital poisoning. The success of the treatment is limited by the extent to which these drugs are distributed and by the presence of alternative pathways of elimination. Unfortunately, drugs with a high volume of distribution will often have a relatively long half-life; hence any increase in clearance will not make much difference to its pharmacological or toxicological effect.

Persons who abuse amfetamines have used the effect of urinary pH on excretion to advantage by simultaneously ingesting bicarbonate. This produces alkaline urine which delays elimination of the amfetamine and therefore prolongs its stimulant effect. Conversely, substances that acidify urine have been taken to enhance elimination of amfetamine-like stimulants in the hope of avoiding detection in routine doping-screening procedures. Exercise in itself can also decrease urinary pH somewhat and thus increase the renal clearance of basic drugs.

Although the quantity of drug in a urine sample is the product of the renal clearance of the drug, the average plasma concentration of the drug during the interval that the urine was produced, and the duration of that interval, the calculation of a likely plasma concentration or even a dose is ill advised since urinary flow rate and the degree of metabolism must also be considered. Many drugs also show non-linear pharmacokinetics, i.e. their excretion rate and degree of metabolism are subject to dose dependency.

Chronic dosing

Drugs accumulate in plasma or tissues if more than one dose is administered and the interval between doses is less than the time taken to eliminate the previous dose. Under these circumstances, there will also be a change in the shapes of the plasma concentration–time curve and the tissue concentration–time curve. In all cases, accumulation is controlled by the size of the dose, the dose interval and the terminal elimination phase for loss of drug from the body (k_{el}). The problems of drug accumulation are of particular interest to the toxicologist because the resulting high drug concentrations may lead to a progressive and insidious toxicity.

The extent to which a drug will accumulate in multiple dosing can be estimated. After each successive dose, the maximum, minimum and average plasma concentrations will be higher than those for the previous dose. This is so in the early stages, but, since drug elimination is often a first-order rate process, the total amount of drug in the body will increase only until the amount eliminated during a dose interval equals the amount taken in (total injected dose or the net absorbed dose after oral dosing). This is called steady state. The clearance (Cl) can be related to dose rate ($DR = D/\tau$) divided by steady-state plasma concentration (C_{ss}).

$$Cl = DR/C_{ss}$$

A good example of the importance of this concept is the use of methadone to treat opioid dependency. Methadone has a long pharmacokinetic half-life of approx. 1 day. With once-a-day dosing the plasma concentrations of methadone will accumulate for at least 5 days. Accordingly, the effects of methadone increase during the first 5 days of therapy. If the dose consumed is too high, or dose increases are made during these 5 days, potentially life-threatening respiratory depression can set in.

Similarly, for other drugs steady state is achieved after about five terminal elimination half-lives.

Drug metabolism

Metabolism is an integral part of drug elimination. As well as facilitating excretion of a drug, it may also affect the pharmacological response of a drug by altering its potency and/or duration of action. With few exceptions, metabolites of drugs are more polar (and water soluble) than the parent drug and are therefore more likely to be excreted from the body.

Metabolites may be pharmacologically inactive (e.g. salbutamol sulfate) or they may be active. This is the case with many drugs of toxicological interest. For example, glucuronidation of morphine on the 6-hydroxyl moiety yields an opioid with more activity than morphine itself. The hydroxylation of THC to the 11-hydroxy form yields an active cannabinoid. Hydroxylation and demethylation of the benzodiazepine diazepam give the metabolites temazepam and oxazepam, both of which are also available as drugs. Similarly, amitriptyline, a tricyclic antidepressant, is demethylated to yield another antidepressant, nortriptyline. Diamorphine is deacetylated to 6-acetylmorphine and morphine, both potent opioids.

Active metabolites may also have different modes of action and different potencies; thus dealkylation of the antidepressant drug iproniazid gives the tuberculostatic drug isoniazid, while the anticonvulsants primidone and methylphenobarbital are both metabolised to phenobarbital, another anticonvulsant with a much longer duration of action.

Clearly the formation of active metabolites changes the profile of drug action.

Pathways of drug metabolism can be divided into two types. Phase I reactions include oxidation, hydroxylation, *N*- and *O*-dealkylation, and sulfoxide formation as well as reduction and hydrolysis reactions. Phase II processes involve conjugation reactions, such as with glucuronic acid, as well as acetylation, methylation and conjugation with amino acids and sulfate. Phase II reactions remove or mask functional groups (amino, carboxyl, hydroxyl, sulfhydryl, etc.) on the drug or phase I metabolites by the addition of an endogenous substrate.

Many drugs undergo a combination of phase I and phase II reactions. The major phase II reaction is conjugation of glucuronic acid with the phenolic or alcoholic hydroxyl groups that are common products of phase I reactions. Thus, chlorpromazine gives rise to at least twenty metabolites by its three major routes of metabolism, i.e. hydroxylation, *N*-demethylation and sulfoxidation. Fortunately, such complicated patterns of metabolism are not a major problem to the analyst since at most only one or two key metabolites are usually targeted.

A new generation of hypnotics and sedatives belong to different structural classes from the benzodiazepines – the azapirones (buspirone), the imidazopyridines (alpidem, zolpidem), the cyclopyrrolone class (suriclone, zopiclone) and the pyrazolopyrimidine zaleplon. These are metabolised by a variety of different pathways to inactive metabolites.

Many of the critical pathways are catalysed by microsomal membrane-bound enzymes in the hepatocytes (parenchymal cells of the liver). For example, the cytochrome P450 mixed-function oxidase system that catalyses oxidations and glucuronyltransferase, and the enzyme responsible for conjugation with glucuronic acid, are both located on microsomal membranes.

Metabolism can occur in tissues other than the liver. The major additional sites are the gastrointestinal tract, kidneys and lungs. Their contribution will clearly depend on the route of administration. For example, many metabolic reactions occur in the gastrointestinal tract before an orally administered drug is absorbed, carried out by enzymes in the mucosal lining or by microflora. Most of these reactions involve reduction and hydrolysis because of the anaerobic environment. Plasma esterases cause extensive hydrolysis of drugs such as diamorphine, cocaine and procaine.

In postmortem cases, anaerobic bioconversion occurs by endogenous enzymes active in such situations or by invading gastrointestinal bacteria. The nitrobenzobenzodiazepines nitrazepam, clonazepam and flunitrazepam are subject to reduction to the 7-amino metabolite.

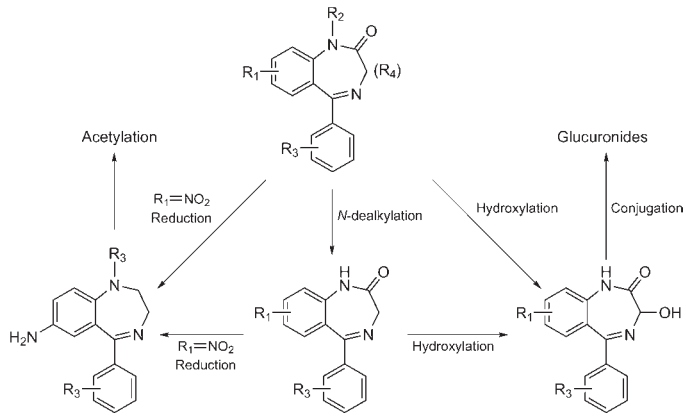
Using drugs principally of forensic interest, a number of examples are given below to illustrate the variety of metabolic routes that can be followed in humans and the effects that these might have on disposition and pharmacological activity. The list is not intended to be exhaustive with regard to either the pathways or the drugs covered.

All the major oxidative mechanisms can be illustrated by considering the metabolism of the benzodiazepines, amfetamines, antidepressants and opioids.

Benzodiazepines and other sedatives

The benzodiazepines are one of the most widely prescribed groups of drugs and are frequently found in toxicological cases. They undergo extensive metabolism by *N*-dealkylation, hydroxylation and conjugation pathways (Fig. 24.5). Many of the metabolites of the prototype benzodiazepine, diazepam, show pharmacological activity, including desmethyldiazepam (nordazepam), 3-hydroxydiazepam (temazepam) and desmethyl-3-hydroxydiazepam (oxazepam).

Ring-substituted benzodiazepines based on the triazolam structure show much higher potency than the first-generation benzodiazepine based on chlordiazepoxide and diazepam (Fig. 24.6). These also include midazolam and alprazolam. For example, triazolam is the most potent member in active use, with daily doses starting at 0.125 mg. By comparison, a typical dose for diazepam is 5–20 mg, and for chlordiazepoxide 100 mg.



R^1	R^2	R^3	R^4	Drug
Methyl	Hydrogen	Hydrogen	Hydrogen	Diazepam
Hydrogen	Hydrogen	Hydrogen	Hydrogen	Nordiazepam
Hydrogen	Hydrogen	Hydrogen	Hydroxy	Oxazepam
Methyl	Hydrogen	Hydrogen	Hydroxy	Temazepam
2-Nitro	Hydrogen	2-Chloro	Hydrogen	Clonazepam
2-Nitro	Methyl	2-Fluoro	Hydrogen	Flunitrazepam
2-Nitro	Hydrogen	Hydrogen	Hydrogen	Nitrazepam
2-Chloro	Hydrogen	2-Chloro	Hydroxy	Lorazepam
2-Chloro	Diethylamino-ethyl	2-Fluoro	Hydrogen	Flurazepam

Figure 24.5 Metabolic scheme for 1,4-benzodiazepines.

Benzodiazepines that do not belong to these two classes are still likely to be metabolised by the same routes (Fig. 24.7). The atypical benzodiazepine chlordiazepoxide is metabolised by demethylation and deamination to desmethylchlordiazepoxide and demoxepam. Demoxepam is further metabolised to nordiazepam by hydrolysis and cleavage of the lactam ring.

The pharmacokinetic half-lives of benzodiazepines are largely used to determine their principal medical use. Benzodiazepines with a relatively short half-life are used predominantly as hypnotics and as supplements to preoperative anaesthesia, whereas the longer-acting benzodiazepines (such as diazepam) are used as minor tranquillisers (anxiolytics).

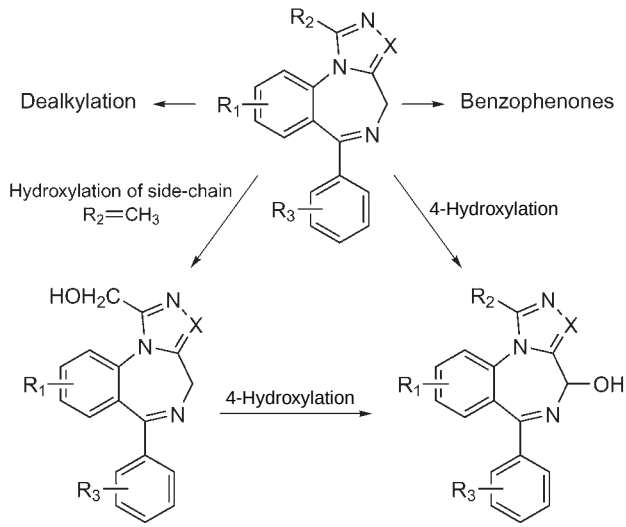
The urine usually contains extensive metabolites of benzodiazepines, often with little parent drug present. It is essential that individual metabolites of target benzodiazepines are known when assessing the urine of persons exposed to this class of drug.

The clearance of benzodiazepines is decreased by liver disease, although the greatest effects occur with those drugs metabolised by the P450 system. Lorazepam and oxazepam and other similar drugs metabolised by glucuronidation are least affected. Kidney disease particularly affects benzodiazepines metabolised to active drugs and those showing a high degree of protein binding.

Advanced age has similar effects to liver and kidney disease owing to the reduction in output of major organs and changes in the volume of distribution. Doses of sedatives are usually halved in the elderly (>65 years), although those benzodiazepines that are primarily conjugated such as oxazepam, lorazepam and temazepam are least affected by age.

Amfetamines and other stimulants

The amfetamines are metabolised by a combination of hydroxylation of the ring and the side-chain carbon atom adjacent to the ring, and



R^1	R^2	R^3	X	Drug
2-Chloro	Methyl	Hydrogen	Nitrogen	Alprazolam
2-Chloro	Methyl	2-Fluoro	Hydrogen	Midazolam
2-Chloro	Methyl	2-Chloro	Nitrogen	Triazolam
2-Chloro	Dimethylamino-methyl	Hydrogen	Nitrogen	Adinazolam
2-Chloro	Hydrogen	Hydrogen	Nitrogen	Estazolam

Figure 24.6 Metabolic scheme for diazolo- and triazolo-benzodiazepines.

removal of the nitrogen (Fig. 24.8). Drugs with alkyl groups on the nitrogen are dealkylated, e.g. metamfetamine and methylenedioxymetamfetamine (MDMA) to other active amfetamines (amfetamine and methylenedioxyamfetamine (MDA), respectively) (Fig. 24.8). Methylenedioxyethylamfetamine (MDEA) and MDMA are both metabolised to MDA as well as other metabolites. The methylenedioxy amfetamines are also transformed to dihydroxy compounds (catechols) following opening of the ring. These hydroxy metabolites can be either monomethylated or conjugated with sulfate esters or with glucuronic acid.

The side-chain of non-*N*-substituted amfetamines is oxidised to form benzoic acid derivatives (e.g. amfetamine), which are excreted as the glycine conjugate, or the sulfate or glucuronide conjugate. Amfetamine and metamfetamine are also oxidised at the beta-carbon to form the pharmacologically active ephedrine analogues.

A number of legal stimulant drugs are metabolised to metamfetamine or amfetamine. These include fenethylline, clobenzorex, mefenorex, fenproporex and benzfetamine. The antiparkinsonian drug selegiline is metabolised to the weakly active L-isomer of metamfetamine. Detection of the parent drug and possibly the conduct of chiral analyses are essential to determine the source of the amfetamine.

As expected from its different structure, cocaine undergoes substantially different routes of metabolism than the amfetamine class. Cocaine is rapidly hydrolysed by ubiquitous enzymes to the inactive benzoylecgonine. This is the main metabolite in both blood and urine. Other significant metabolites are ecgonine methyl ester (EME) and ecgonine (Fig. 24.9).

Anhydroecgonine methyl ester (AEME, also known as methylecgonidine) is a pyrolytic substance formed by smoking cocaine. Cocaethylene is also found as a metabolite in persons co-consuming alcohol. Metabolism of cocaine to norcocaine allows the oxidation of the nitrogen to *N*-hydroxynorcocaine. *N*-Nitrosonorcocaine and the *N*-oxide are also produced in small amounts.

Antidepressants

Modern antidepressants can be divided into several chemical classes. The traditional tricyclic antidepressants include amitriptyline, clomipramine, dothiepin, doxepin and imipramine. The newer-generation antidepressants include the selective serotonin reuptake inhibitors (SSRIs): citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. Other antidepressants include the monoamine oxidase inhibitor moclobemide and other mixed uptake inhibitors venlafaxine, nefazodone and mitazepine.

The tricyclic antidepressants are metabolised by three major pathways: *N*-oxidation, hydroxylation of the alicyclic ring and of the aromatic ring, and *N*-dealkylation of the dialkylamino group. The last route gives rise to the most important metabolites since the *N*-demethylated metabolites are themselves pharmacologically active. Amitriptyline is metabolised to nortriptyline, and imipramine to desipramine; both metabolites are also available as therapeutic agents (Fig. 24.10).

When monitoring concentrations of tricyclic antidepressants for their therapeutic effect, it is important to determine both the parent drug and the desalkyl metabolites as the latter may be present in a significant quantity. These can be added to provide an estimate of therapeutic activity. The hydroxy metabolites predominate in the urine, usually occurring as glucuronide conjugates.

The other classes of antidepressants have varied chemical structures, hence their fate is very much dependent on the drug concerned (Fig. 24.11).

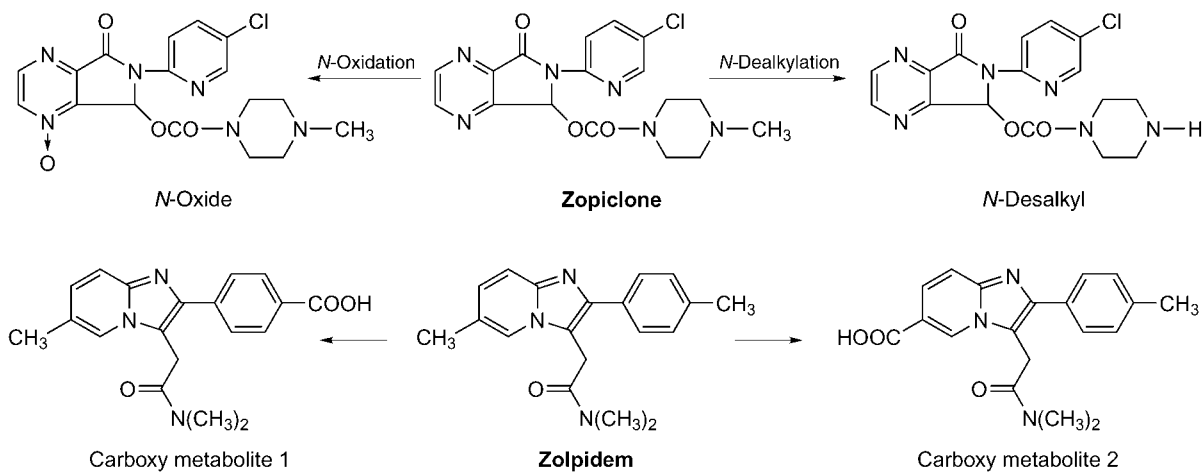


Figure 24.7 Metabolic pathways for zopiclone and zolpidem.

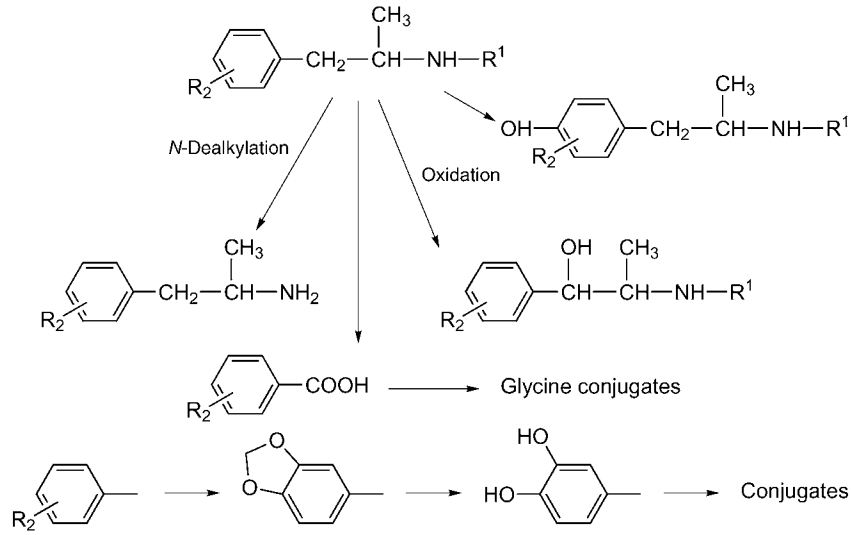


Figure 24.8 Major routes of amfetamine metabolism.

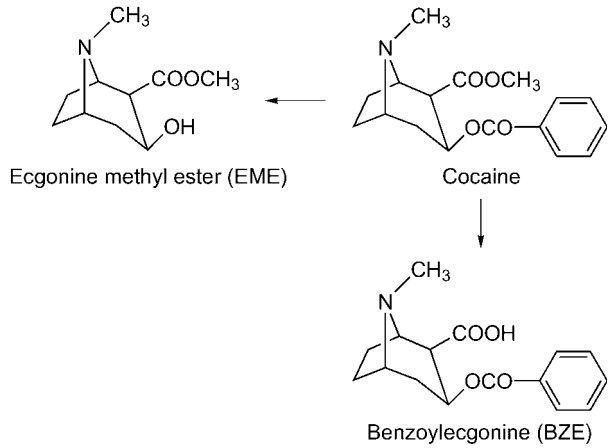


Figure 24.9 Main cocaine metabolic pathways.

Antipsychotic drugs

The antipsychotic drugs represent diverse classes of chemical compounds.

The first drugs of this type were largely of the phenothiazine type represented by thioridazine and chlorpromazine. They undergo sulfoxidation to yield sulfoxides and sulfones. In addition, oxidation at the nitrogen, hydroxylation of one or both of the aromatic rings, *N*-dealkylation of the side-chain and fission of the side-chain may also occur. The phenolic metabolites are then conjugated with glucuronic acid or sulfate and excreted in both the urine and the bile.

The number of different metabolic routes that are possible results in a complex mixture of metabolites for many phenothiazines. For example, many of the drugs that contain an *N,N*-dialkylaminoalkyl side-chain (e.g. chlorpromazine) are extensively metabolised by *N*-oxidation, together with hydroxylation, sulfoxidation and *N*-dealkylation. Thioridazine is predominantly oxidised on the side-chain sulfur to active sulfoxide and sulfone metabolites (Fig. 24.12).

Haloperidol is metabolised by side-chain oxidation to a propionic acid derivative (Fig. 24.13), which is then conjugated, or by reduction of the keto group. Clozapine is metabolised to the active desmethyl form, norclozapine, which is often measured with the parent drug in therapeutic drug monitoring situations. Olanzapine is metabolised by *N*-demethylation and oxidation to a 2-hydroxymethyl metabolite and *N*-glucuronidation. Risperidone is metabolised to the active 9-hydroxy metabolite (see below under Prodrugs) and inactive 7-hydroxy- and *N*-dealkyl forms.

Opiates and centrally active analgesics

The opiates include the analogues of morphine, such as codeine, ethylmorphine and diamorphine, as well the synthetic opiates methadone,

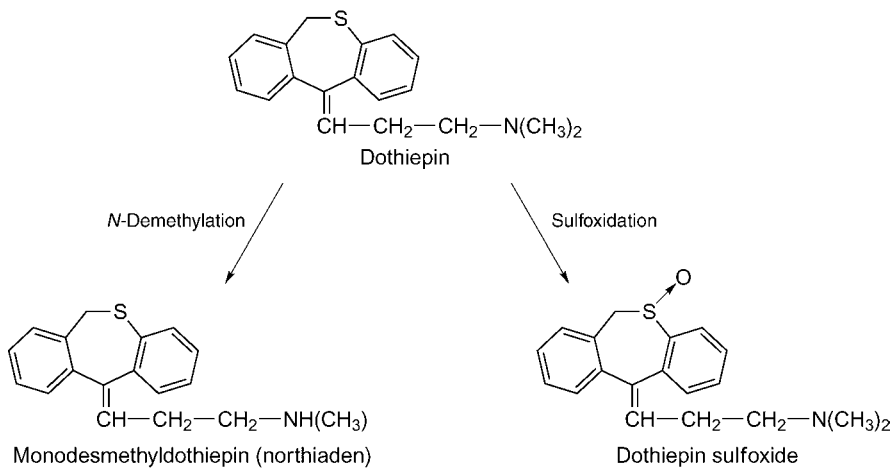


Figure 24.10 Major pathways of dothiepin metabolism.

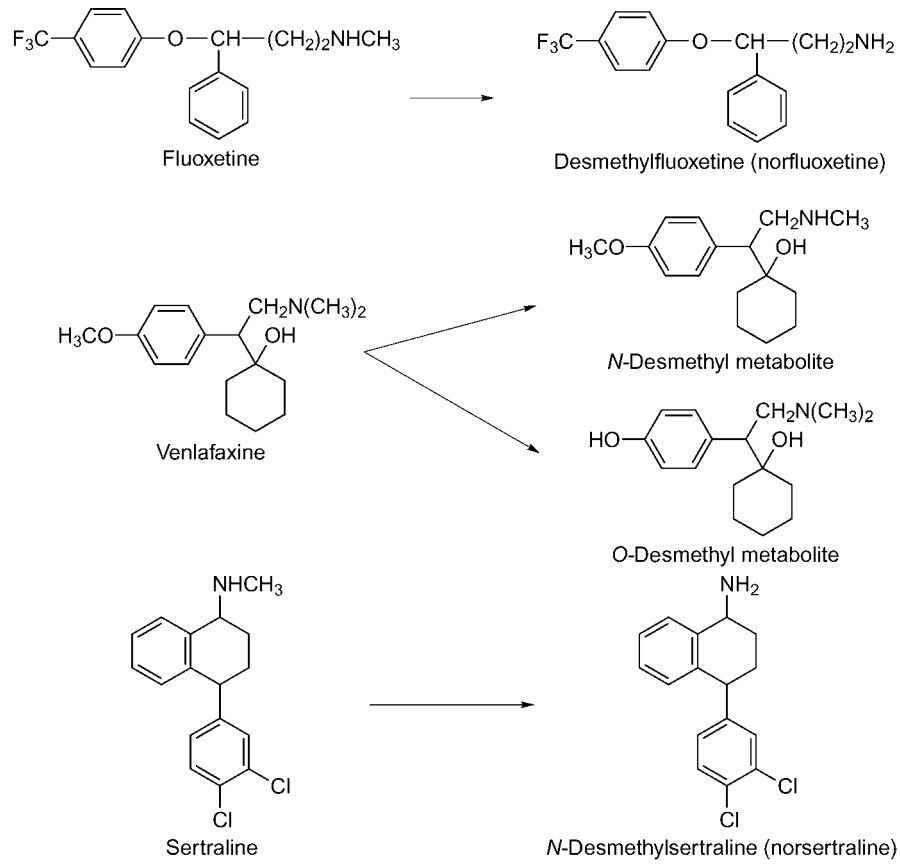


Figure 24.11 Key metabolic pathways for selective serotonin reuptake inhibitors.

pethidine, propoxyphene and the highly potent fentanyl derivatives. Depending on their structural features, the metabolism of opiates can vary widely.

The morphine analogues are metabolised by *O*-dealkylation or de-esterification and conjugation with glucuronic acid. Thus diamorphine (heroin) is rapidly hydrolysed in the body to 6-acetylmorphine, which is further and more slowly hydrolysed to morphine. The morphine so formed is excreted largely as the 3- and 6-glucuronides together with some free morphine. Codeine and ethylmorphine are conjugated and metabolised by *O*-dealkylation to morphine. Morphine is also metabolised to a minor extent by *N*-demethylation to normorphine (Fig. 24.14).

Oxycodone is subject to demethylation and conjugation (Fig. 24.15).

Methadone, propoxyphene and pethidine are largely dealkylated. In the case of methadone, a cyclisation product known as EDDP is formed (Fig. 24.16).

The non-opioid centrally active analgesic tramadol is metabolised to *N*- and *O*-demethylated products followed by sulfation and glucuronidation of the phenol. The *N*-demethyl metabolite (known as M1) is the active form of tramadol (Fig. 24.17).

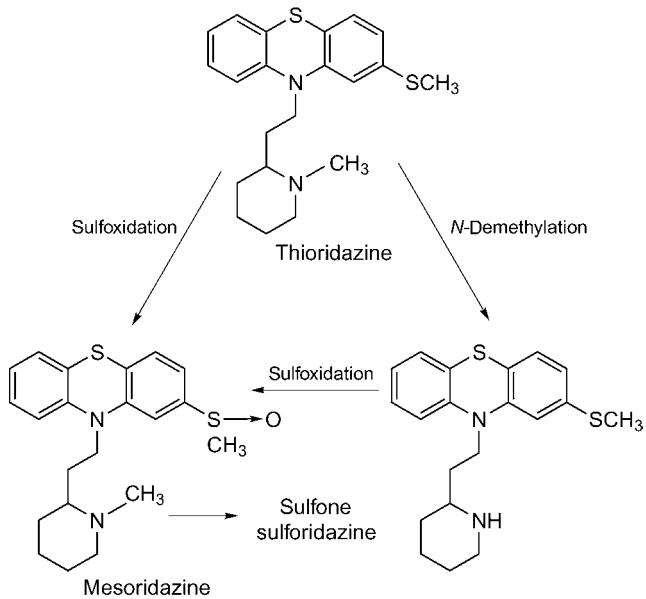


Figure 24.12 Thioridazine metabolic pathways.

Activation of drugs

Drugs can be activated or the pharmacological activity can be modified by metabolism, by design (prodrugs) or through the formation of active metabolites.

Prodrugs

A number of drugs have been modified such that metabolism is required to produce an active species. This is often done to facilitate oral absorption or to reduce toxicity, although for some drugs the active form was not established until after clinical use. Examples include: the esters of many angiotensin-converting enzyme (ACE) inhibitors, e.g. enalapril and quinapril, which are hydrolysed to potent di-acid forms; azathioprine, which is metabolised to mercaptopurine; and zidovudine, which is metabolised to zidovudine triphosphate.

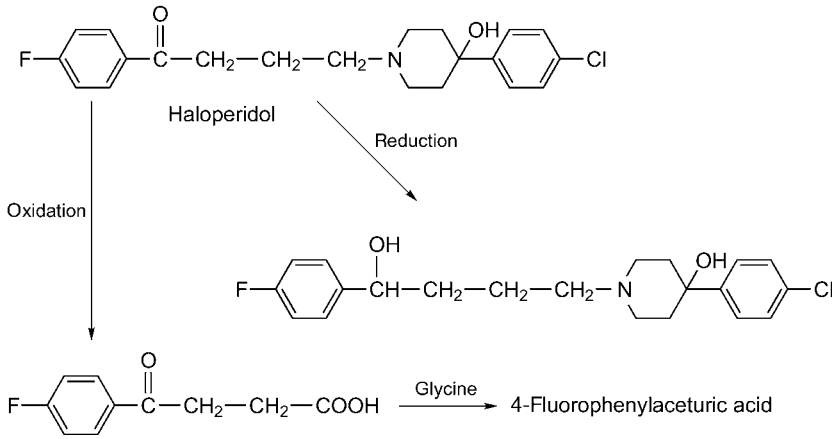


Figure 24.13 Haloperidol metabolic pathways.

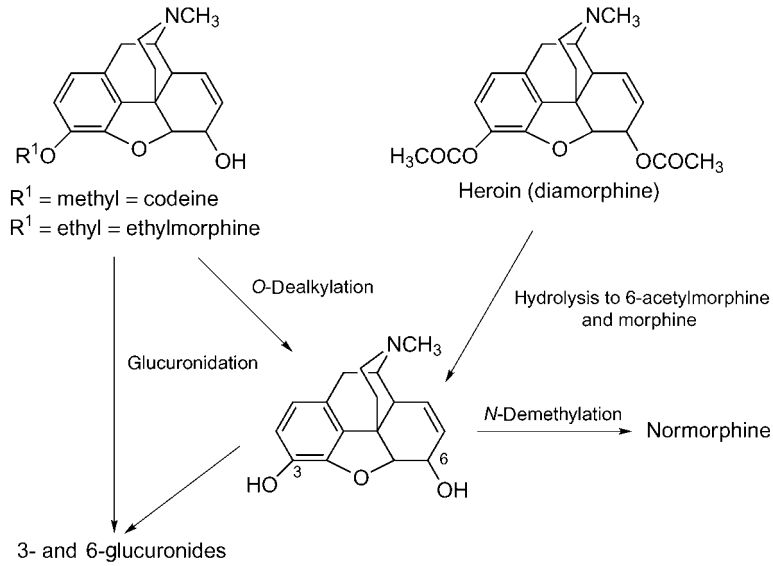


Figure 24.14 Metabolic pathways of morphine analogues.

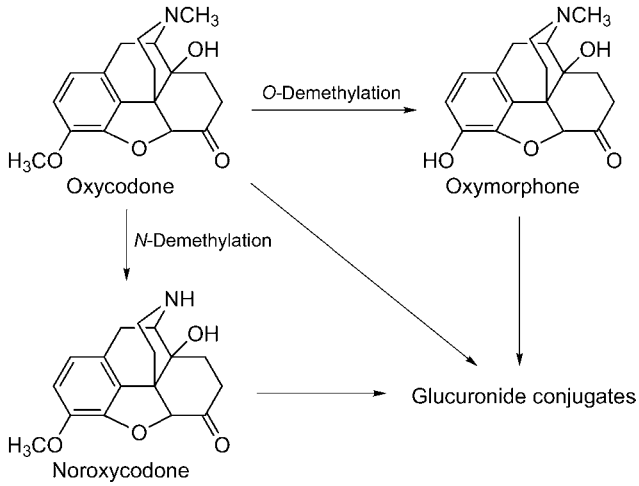


Figure 24.15 Oxycodone metabolism.

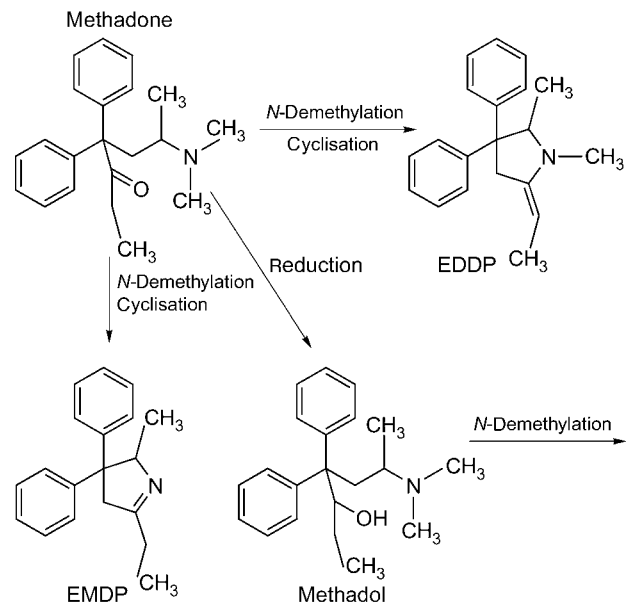


Figure 24.16 Methadone metabolism.

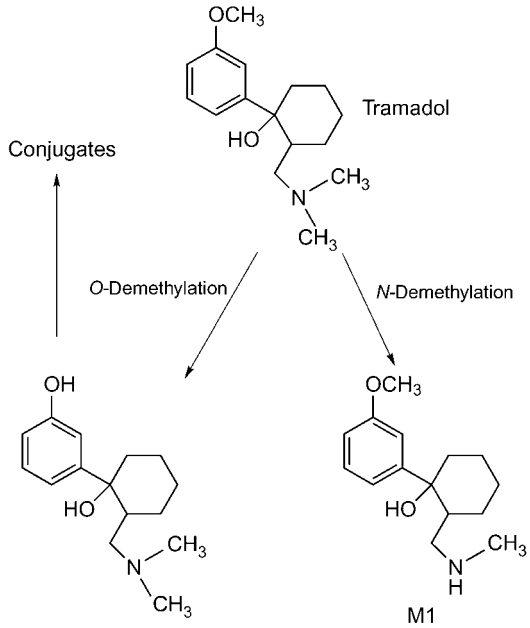


Figure 24.17 Tramadol metabolism.

Some metabolites of earlier antihistamines are now used as drugs in their own right. For example, fexofenadine is a carboxy metabolite of terfenadine, and cetirazine is the carboxy metabolite of hydroxyzine.

A more recent example is the activation of risperidone by the formation of 9-hydroxy metabolite, now known as paliperidone.

Active metabolites

When an active metabolite makes an important contribution to the overall pharmacological response, the interpretation of toxicological data is further complicated. Toxicological situations involving such metabolites (e.g. oxazepam, nortriptyline, desipramine and phenobarbital, derived from diazepam, amitriptyline, imipramine and methylphenobarbital, respectively) can be misinterpreted if only the parent drugs are assayed. The concentrations of active metabolites must be taken into account. Although there is controversy over the best way to evaluate the contribution of metabolites, the individual concentrations of drug and metabolites are often added together to provide an estimate of the total amount of active drug species present in the sample. This assumes that their relative pharmacological activities are equal, which is not generally true.

Reactive metabolites

Toxic metabolites can occur in the same way that pharmacologically active and/or inactive metabolites are produced. For example, deacetylation of phenacetin yields *p*-phenetidine, the precursor of substances believed to be responsible for methaemoglobinaemia.

Similarly, paraoxon, the oxygenated metabolite of parathion, is responsible for the severe toxicity observed after the ingestion of parathion.

Changes in the pathways of metabolism of a drug can also result in toxicity. In paracetamol intoxication, the pathways responsible for sulfate and glucuronide conjugation become saturated and the concentrations of cysteine and mercapturic acid metabolites increase. When the production of the latter two metabolites increases sufficiently to deplete stores of glutathione, the active intermediate can no longer be conjugated and is thought to bind irreversibly to cellular macromolecules such as DNA, RNA and proteins, resulting in a dose-related hepatic necrosis. It is believed that the toxic molecule arises from the oxidation of paracetamol to *N*-acetyl-*p*-benzoquinoneimine (Fig. 24.18).

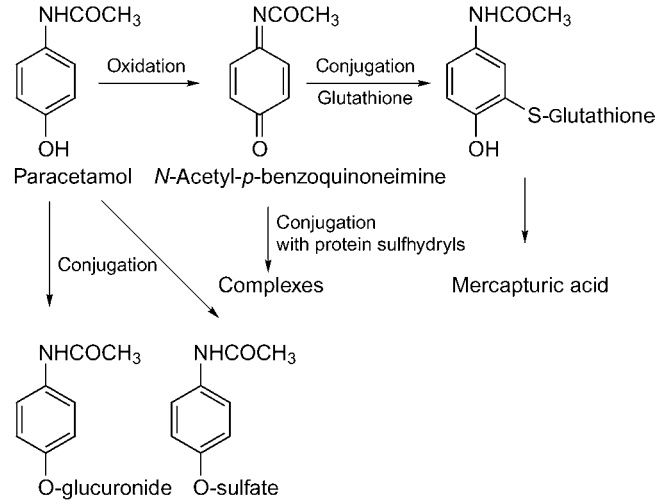


Figure 24.18 Paracetamol metabolism.

Whatever the actual pathway, the administration of compounds containing sulfhydryl groups has been shown to be effective in paracetamol intoxication, presumably because they are able to bind to the electrophilic species in the same way as glutathione.

Another example of drug toxicity induced by metabolism is that associated with acetylation. The rate of acetylation is controlled by an *N*-acetyltransferase that shows genetic polymorphism; about 60% of white people are classified as 'slow' acetylators. The extent of acetylation is related to the toxic effects of certain drugs. For example, the *N*-hydroxy metabolite of acetylated isoniazid is thought to cause isoniazid-related hepatotoxicity. This toxicity is more severe in 'rapid' acetylators than in 'slow' acetylators but, as with paracetamol, some protection can be given by sulfhydryl compounds.

In contrast, 'slow' acetylators appear to show a greater incidence of systemic lupus erythematosus following administration of hydrazine drugs than do 'rapid' acetylators as this toxic reaction is related to the parent drug. Fortunately, modern drugs are not frequently acetylated, so this is no longer an important problem in clinical pharmacology and toxicology.

Factors affecting the pharmacokinetics of drugs

Metabolic effects

There are many examples of drugs affecting the metabolism or pharmacology of other drugs. For example, cimetidine (an anti-ulcer drug) and a number of the newer generation of antidepressants inhibit the metabolism of many of the benzodiazepines by a subtype of the cytochrome P450 enzymes, CYP3A. This occurs either by competitive inhibition of the enzyme(s) involved in their mutual metabolism or by inhibition of the enzyme(s) by the interfering drug. Many of the newer SSRIs, including fluoxetine, paroxetine and sertraline, as well as some of the antifungal drugs, such as fluconazole and antiviral drugs, are relatively potent inhibitors of this enzyme.

The activity of another P450 enzyme, CYP2D6 (debrisoquine hydroxylase), has activity that is genetically determined. For example, the main metabolic pathway for codeine, dextromethorphan and ethylmorphine is the metabolism to morphine through *O*-dealkylation. Some 7% of white people are deficient in this enzyme and are unable to produce significant amounts of morphine. In these people, codeine and ethylmorphine appear to be far weaker analgesics than in those who are able to produce morphine. The same enzyme is involved in the bioconversion and activation of oxycodone to oxymorphone, of hydrocodone to hydromorphone, and of risperidone to 9-hydroxyrisperidone, and metabolism of olanzapine. The efficacy and toxicity of these drugs will therefore be affected by this genetic difference.

Cimetidine (used in the treatment of gastric and duodenal ulcers) will inhibit metabolism of opioids requiring microsomal cytochrome enzymes.

Monoamine oxidase inhibitors nialamide, phenelzine and tranylcypromine also inhibit cytochrome P450 enzyme metabolism and have been shown to increase the effects of alcohol, amfetamines, appetite suppressants, barbiturates, pethidine and other opioids. The analgesic propoxyphene may have similar activity.

A further variable is that drugs such as the barbiturates and the anticonvulsants phenobarbital and phenytoin enhance the production of the enzymes and therefore induce metabolism of drugs metabolised through this and related enzyme systems. In fact, barbiturates also induce their own metabolism, causing a time-dependent increase in clearance as the liver produces more enzymes.

Effect of age

Neonates and elderly people generally have a lower metabolic capacity than subjects between these extremes of age. The enhanced sensitivity of the very young to drugs is related to immaturity in the development of microsomal enzymes within the first several months of birth. Hence neonates can have quite different clearance rates from adults. Differences may also occur in older children.

Furthermore, very young children do not have the necessary plasma-binding proteins which help to compartmentalise drugs. Infants (over 1 year old) require lower doses to produce comparable effects because the drugs are distributed into a smaller volume. It is important that the known pharmacokinetics of a drug in question are examined when neonates and even children generally are the focus of an investigation relating to drug effects, since some drugs may behave differently than in adults.

In elderly subjects (over 65 years old) there is a decreasing capacity for drug metabolism as a consequence of a gradual decline in overall physiological function of organs. This includes effects on volume of distribution, protein binding and both hepatic and renal clearances. The change in the pharmacokinetics is an explanation of the increased sensitivity to drug effects in the elderly. For example, doses of benzodiazepines are reduced in the elderly to avoid excessive sedation and adverse effects on cognition.

Effect of disease

Diseases can affect all the processes by which a drug is absorbed, distributed, and eliminated from the body. A drug may be poorly absorbed during gastrointestinal disturbance; the rate of uptake of drugs that rapidly cross tissue membranes may be altered in cardiovascular diseases that alter blood flow to critical organs such as the liver, kidney, lungs and heart, and of course diseases that fundamentally affect metabolic and excretory pathways of drugs.

Diseases that affect the liver or kidneys probably have the greatest effect on drug concentrations because normal functioning of these organs is essential for efficient metabolism and excretion. The liver has a large metabolic reserve; however, severe disease such as cirrhosis or drug-induced necrosis will cause the terminal elimination half-life to increase dramatically.

Renal disease leads to a decreased ability to excrete drugs and/or their metabolites. A drug will accumulate in plasma or tissues if the interval between doses is such that not all of the previously administered drug is removed before the next dose. Even for those drugs where excretion into the urine does not normally appear to be an important route of elimination, there can be a risk of increased toxicity during disease if significant drug accumulation takes place. This is especially true where potentially serious interactions can occur with the accumulated drug or metabolite. For example, metabolites can displace their parent drugs from binding sites on plasma and tissue proteins if their concentrations build up sufficiently.

Drug concentration and pharmacological response

The relationship between drug action and the processes of absorption, distribution and elimination has been successfully applied in clinical pharmacology for the optimisation and individualisation of therapy for many drugs. This is termed therapeutic drug monitoring (TDM). In clinical and forensic toxicology, similar relationships can be applied in the interpretation of analytical results.

For most drugs there is a correlation between the dose given, the concentration of the drug in blood and the duration and intensity of the biological effect. In general, as blood concentrations rise above those associated with a therapeutic effect, the frequency and severity of toxic side-effects increase. It should be stressed that this correlation is at best poor for most drugs and there is considerable individual variability. Hence any prediction of response from a drug concentration may be poor.

The significance of toxicological data is assessed by attempting to explain the clinical or toxicological effects in terms of the drug concentrations found. Before this can be done, the toxicologist must be satisfied that the clinical and analytical data are valid.

In some situations drugs have long-lasting or even irreversible biochemical effects such that there is little correlation between concentration and response. For example, reserpine and some monoamine oxidase inhibitors still have clinical effects long after drug administration has stopped and when plasma concentrations of the drug are negligible. Similarly, unless the time of ingestion is known with reasonable accuracy, it is almost impossible to relate drug concentrations with the secondary and potentially fatal responses to substances such as paracetamol (liver damage) and paraquat (lung necrosis). Incorporation of drugs or chemicals into endogenous metabolic cycles may result in a toxicity (lethal synthesis) that is not related to blood concentrations of the drug.

Finally, assessment of drug pharmacokinetics is made difficult or impossible when underlying disease has substantially altered the pharmacokinetics and even pharmacological action of the drug.

It is therefore essential that any assessment of drug concentrations considers as far as reasonably possible the factors that may affect drug clearance.

Further reading

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CHAPTER

25 Pharmacogenomics

SHY Wong

Introduction

From Gregor Mendel to pharmacogenomics, personalised medicine, forensic toxicology and personalised justice

Gregor Mendel characterised his early scientific genetic findings, to his contemporary, Eichling, as a 'little trick – long story'; the continuation of that 'story' may be partially developing now in pharmacogenomics and personalised medicine (PM), and in the emerging field of personalised justice. Some of the landmarks in pharmacogenetics are summarised in Figure 25.1 (Weber 2002), which has been modified to include nutritional genomics and proteomics. After Mendel established early genetics in 1865, pharmacogenetics emerged in 1955, followed by pharmacogenomics in the early 1990s. According to Weber, PM may be achieved by 2015, when drugs will be prescribed according to a patient's genetic profile. Further, personalised justice complements the practice of PM, and may be defined as the applications of genomic and other molecular biomarkers in the interpretation of the effect of genetic variations and other epigenetic factors on the efficacy of drug therapy and, therefore, on individual behaviour (Wong 2007). Thus, translational pharmacogenomics will contribute to various clinical and forensic practices in the near future.

The emerging practice of pharmacogenomics as part of genomic medicine (Linder *et al.* 1997; Weber 1997; International Human Genome Sequencing Consortium 2001; Venter *et al.* 2001; Jicinio, Wong 2002; Evans, McLeod 2003; Weinshilboum 2003; Guttmacher *et al.* 2004; White, Wong 2005; Wong *et al.* 2006a) and PM is one of the most tangible benefits as a result of the completion of the human genome project (Personalized Medicine e-Symposium 2006). This was recently addressed by the September 2008 report of the President's Council of Advisors on Science and Technology, 'Priorities for Personalised Medicine' (President's Council 2008). The report focused on three inter-related areas: technology and tools, regulation and reimbursement as follows:

- To develop a strategic, long-term plan coordinating public and private sector efforts for advancing research and development (R&D) relevant to PM
- To stimulate and facilitate modernisation of the regulatory process affecting PM. These processes would be transparent, systematic and iterative approaches utilised in the regulation of PM technologies and tools
- To achieve cost-containment objectives so that health care should not arbitrarily hinder the application of innovative PM products
- To establish a Department of Health and Human Services office to specifically coordinate activities related to PM.

In the 15 December 2008 issue of *Newsweek* the future of PM was illustrated in three areas: HIV, lung cancer and depression (Newsweek 2008). These areas will be described in more detail later in this chapter. In a recently published commentary in the *Journal of the American Medical Association*, the benefit of genomic medicine was demonstrated by reviewing its use in preventive medicine (Offit *et al.* 2006). With preimplantation genetics for cancer, the molecular diagnosis may be regarded as one of the assisted reproductive technologies. Embryos without a familial variation are chosen for implantation. Another example is in the treatment of lung cancer. *UGT1A1*28* homozygosity is associated with developing neutropenia, while slow partial response might be attributed to *GSTP1 1105 G/A* or *G/G* genotypes (Pillot *et al.* 2006). In another study the combination of five gene biomarkers (*DUSP6, MMD, STAT1, ER BB3, LCK)* was proposed as an independent predictor of relapse-free and overall survival (Chen *et al.* 2007). The accompanying editorial (Herbst, Lippman 2007) proposed four phases of applying molecular signatures of lung cancer, along with other biomarkers – proteomic and molecular imaging – in enabling PM for lung cancer, as shown in Figure 25.2. For example, phase 2 prospective studies should be performed to demonstrate improved benefit–risk ratios. This was also suggested by Eichelbaum *et al.* (2006).

Pharmacogenomic biomarkers and personalised medicine

> *If it were not for the great variability among individuals, medicine might as well be a science and not an art.* Sir William Osler 1892 (Roden *et al.* 2006).

Pharmacogenomic biomarkers, in combination with other well-accepted biomarkers such as therapeutic drug monitoring (TDM) and other functional testing, the emerging proteomic biomarkers and possibly molecular imaging, may be used to assess inter-individual variability, thus enabling PM. The combination of these biomarkers identifies the right patient, with the right diagnosis/treatment, matching with the right drug, the right dose and the right time, thus achieving clinical efficacy with little or no toxicity.

Although the terms 'pharmacogenomics' and 'pharmacogenetics' are used interchangeably in practice, pharmacogenetics is the study of the effects of individual genetic variations (e.g. single nucleotide polymorphism (SNP), gene duplications and deletions, and others) on drug therapy such as an individual's ability to metabolise a drug or compound, while pharmacogenomics is the study of the whole genome and its effect on drug metabolism, transport, receptor sensitivity, signalling pathways and target modulators, and the resultant effects on drug efficacy. This is shown in Figure 25.3 and further defined in the glossary at the end of this chapter (Roden *et al.* 2006).

In bringing about translational pharmacogenomics, various groups and networks in the USA and Europe have been organised to provide coordinated planning and developments. For example, an authoritative review article (Roden *et al.* 2006) outlined the various investigations of the Pharmacogenetics Research Network (PGRN, a group of researchers), including the mechanisms resulting in variable drug responses, the possible contribution of genetic factors, and the current and emerging approaches to identifying these genetic factors. A recent update of PGRN (Giacomini *et al.* 2007) described interest in correlating drug response with genetic variation for disorders including depression, cardiovascular disease, addiction to nicotine and cancer, and in studies involving drug protein transporters and phase II drug-metabolising enzymes. The report summarised recent developments in informatics, cardiovascular, pulmonary, addiction, cancer, transport and metabolism. Long (2007) reaffirmed that PGRN's ultimate goal was to identify clinically significant sequence variations for PM, and to implement the findings for health-care improvement.

Complementing these efforts, colleagues in pharmaceutical research and development areas also embraced the integration of chemistry and biology, recognising the important contributions to pharmacogenomics of drug metabolism enzymes as well as transporters (Yengi *et al.* 2007).

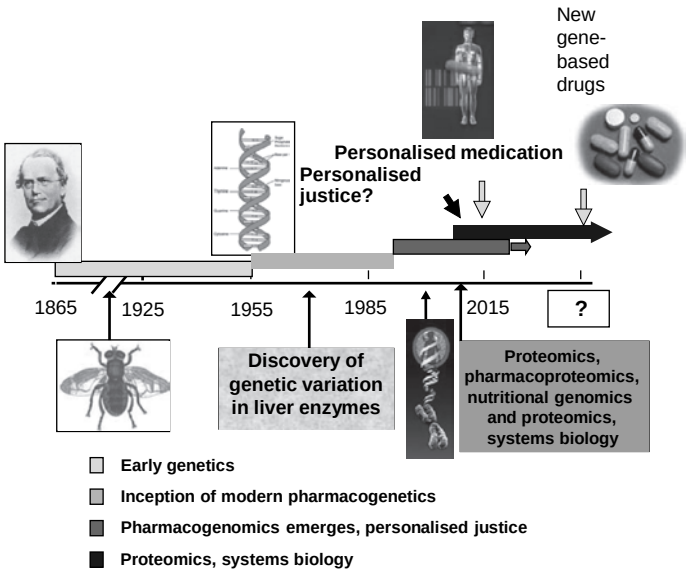

Figure 25.1 Landmarks in pharmacogenetics and proteomics according to Weber at 2002, modified by Wong in 2007 (Weber 2002; Wong 2007).

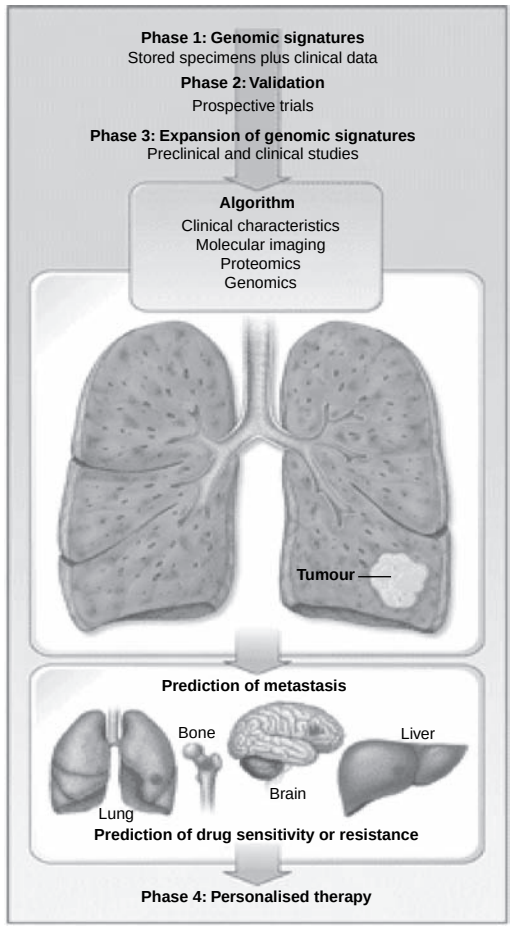

Figure 25.2 Development of personalised drug for lung cancer, from identification of genomic signatures (biomarkers) to prospective trials of personalised therapy (personalised medicine) (Herbst, Lippman 2007).

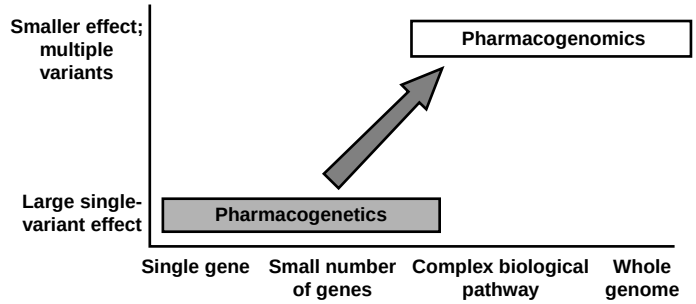

Figure 25.3 Pharmacogenetics and pharmacogenomics. (Roden *et al.* 2006).

Other efforts included the HapMap project (O'Shaughnessy 2006) and a proposed Genomic Prescribing System (GPS; Ratain 2007). The practice of PM (O'Shaughnessy 2006) would be enhanced by understanding the effect of genetic variations beyond pharmacokinetics to include pharmacodynamics – variations in receptors and their signalling molecules, enabled by data from the human genome sequence and the HapMap project. In achieving PM, Ratain proposed the concept of a GPS, which will result in a better outcome and lower drug cost (Ratain 2007). This might be achieved through lower genotyping cost, research and informatics. However, the market might be limited because the genotyping is needed only once in the lifetime of a patient, because there is a difficulty in patenting alleles, and because unanticipated regulatory requirements might become more rigorous in the future. Whether a new pharmacogenomics industry will develop is questionable. However, the public should support it, possibly through federally funded agencies. For example, when Barack Obama was still a senator he proposed the 'Genomics and Personalised Medicine Act of 2006', in support of the formation of a GPS foundation. Another perspective – The art and science of PM' (Piquette-Miller, Grant 2007) – traced the origin to a quote from Sir William Osler as shown at the beginning of this section. From this historical perspective, authors described the earlier development of pharmacogenetics of drug metabolism, followed by drug target polymorphisms. Further, the transitioning of pharmacogenetics to pharmacogenomics for effects of polygenic variations on pharmacokinetics and pharmacodynamics would enable targeted drug therapy for patient subgroups. The authors also included the following challenges: interpretation, regulatory compliance, testing cost and education of health-care professionals. However, error-free PM might not be achievable because of pharmacogenomic complexities and gene expression changes that are time dependent (Kalow 2006). Thus, PM would require the use of bioinformatics to interpret multiple gene modifiers (Davies 2006).

As a result, the Personalised Medicine Coalition (Abrahams *et al.* 2005) was founded as a non-profit umbrella organisation of pharmaceutical, biotechnology, diagnostic and information technology companies, health-care insurers/financers, patient advocacy groups, industry policy organisations, major academic institutions and government agencies. It defines PM as the delivery of individual medical care based on genomics and molecular profile and uses various strategies to achieve PM.

Translational pharmacogenetics and pharmacogenomics have been enabled by the availability of several Food and Drug Administration (FDA)-approved genotyping methodologies and platforms, their inclusions in scientific and clinical national and international meetings, and a recently introduced 2007 pharmacogenomic survey programme offered by the College of American Pathologists. All of these positive developments, however, should be interpreted with some probing questions of the available evidence-based studies to support clinical pharmacogenomic applications. The emerging reality is one of pharmacogenomics serving as an 'adjunct' to other testing and practice. The term 'convergence' is often mentioned in its use in combination with functional testing such as therapeutic drug management and, in the future,

pharmacoproteomics (Turck 2005) and pharmacometabonomics (Nebert, Vesell 2006) biomarkers.

Pharmacogenomics, forensic toxicology and personalised justice

In furthering the applications of genome, proteome and other molecular discovery and development, Wong proposed the definition of personalised justice as the inclusion of molecular analysis – genomic and proteomic – in criminal and forensic proceedings in the deliberation of possible genetic and proteomic contributions to adverse behaviour/outcome. This complements the current forensic applications such as DNA fingerprinting.

In the September 2008 Santorini conference, Wong proposed the complementary and balanced relationship of personalised medicine and personalised justice – both enabled by the understanding provided by molecular biomarkers such as pharmacogenomics (Wong *et al.* 2008). Whereas PM strikes to optimise drug therapy, personalised justice focuses on the application of molecular/pharmacogenomics biomarkers in legal proceedings such as driving and working under the influence of drugs. For example, pharmacogenomics (PGx) may be useful in explaining an elevated drug concentration in a driver involved in an accident, or in workers medicated for pain management and involved in an accident at work. The concept is depicted in Figure 25.4, showing the complementary nature and check-and-balance relationship.

Thus, this chapter characterises pharmacogenomics not only as an emerging, interdependent discipline but as a complementing field in optimising drug therapy and as an adjunct to forensic pathology/toxicology. Following this introduction, a section is devoted to the pharmacogenomics 'space' and the enabling drivers, including the FDA, professional organisations and academic centres. Then, the principles of pharmacogenetics/pharmacogenomics will be introduced, with an update on the candidate pharmacogenomics tests. References are provided for readers interested in the details of the clinical findings of the applications of pharmacogenomics and the technical details of pharmacogenomics protocol. In applying pharmacogenomics for purposes of forensic pathology/toxicology, a previously published algorithm emphasises the integral use of PGx with case history including drug use/abuse, scene investigation, and postmortem findings. Recent PGx opioids and antidepressants cases are included.

Historically, DNA fingerprinting – genetic policing – is the use of molecular diagnostics/genetic testing for identity testing in the forensic sciences. More recently, pharmacogenomics as molecular autopsy involves the assessment of genetic contributions to drug toxicity in postmortem forensic toxicology. Subsequent findings will add to the understanding of disease mechanisms of action and optimisation of drug therapy by lowering/eliminating drug toxicity due to genetically predisposed impaired drug metabolism. These findings may be 'back extrapolated' for the benefits of optimisation of 'live, antemortem' drug therapy. Thus, PGx in forensic toxicology would result in better interpretation, indirectly enabling the emerging personalised medicine. The chapter will conclude with the projected development of the application of pharmacogenomics biomarkers in personalised justice.

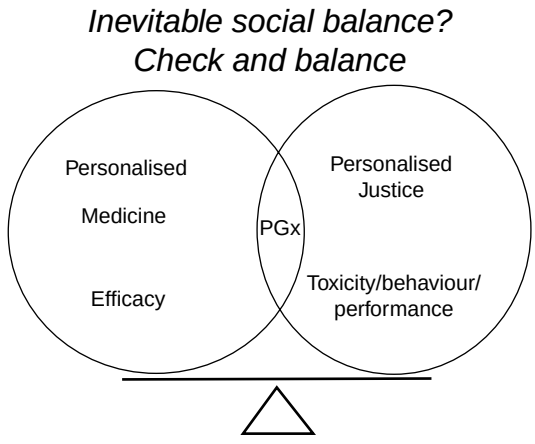

Figure 25.4 Complementary relationship of personalised medicine and personalised justice. PGx, pharmacogenomics.

Proactive roles of governmental agencies, pharmaceutical corporations, academic centres and professional organisations

> The FDA in the next century will be at the forefront of changes, just like you have been in the last century. FDA personnel will be playing a leading role in the transformation of the critical path to medical product development, finding better ways to rapidly develop and improve safety in the treatments that we all look forward to, treatments that are highly personalised, that will come at lower cost and with a greater degree of success. That transformation of the critical path will be a larger part of the transformation toward personalised medicine, one that will improve the health of this nation in ways that Roosevelt and Wiley could have never imagined. Michael O. Leavitt, Secretary of Health and Human Services. Remarks delivered at the FDA Centennial, 30 June 2006. (Available at www.hhs.gov/news/speech/2006/060630.html.)

In assessing the status of pharmacogenomics for drug discovery and development and clinical adaptation, a recent market analysis was completed by O'Dell and Doyle (2004). The market analysis was conducted by surveying 53 out of a 200-person contact database. These individuals were representatives from pharmaceutical and diagnostic companies, regulatory and clinical colleagues, and others. The market was small: $800 million in 2002 compared with the pharma market of $433 billion in 2003. Some of the key findings included the encouraging and engaging roles of the FDA, and the need for physician education. It concluded that PGx approached a 'tipping point' in 2003–04. With the recent development of several new FDA approvals of PGx testing/devices, PGx 'tipped' forward in the clinical PGx/PM space, in part owing to the proactive roles of the FDA in collaboration with other professional organisations for the past several years.

In 2005, Woodcock stressed the proactive roles of the FDA in bringing about the use of pharmacogenomics for PM (Woodcock 2005). She outlined the FDA outreach to pharma, National Institutes of Health (NIH) and scientific/academic communities through workshops and meetings. The FDA envisioned the increase of drug application with more PGx information. She predicted that the then-limited submission would be greatly increased by 2010.

In a state-of-the-art article about PM, Lesko concluded that the practice and reality of PM will emerge but was currently an eventual dream that has not yet been achieved in reality (Lesko 2007). The vision of the FDA for PM was addressed by Michael Leavitt, Secretary of Health and Human Services in June 2006, as shown at the beginning of this section. With recognition of the limitations of the traditional model of 'one size fits all' for dosing drugs with therapeutic index such as warfarin, the molecular and genetic biomarkers of the patients may be used to individualise therapy. PM examples included: targeted therapies – trastuzumab (Herceptin) for treatment of *HER2*-positive metastatic breast cancer, imatinib (Gleevec) for Philadelphia chromosome-positive (Ph+) chronic myeloid leukaemia, and erlotinib (Tarceva) for non-small-cell lung cancer – and targeted dosing – warfarin dosing guided by *CYP2C9* and *VKORC1* genotypes, and treatment of acute lymphoblastic leukaemia by thiopurine methyltransferase (*TPMT*) genotype. Five driving forces shaping the emergence of PM were:

- Availability of low-cost and point-of-care testing genotyping technologies
- Decrease in productivity and innovation in drug discovery
- The increasing use of genomics in drug development towards a 'mini-blockbuster model'

- The desire to increase drug safety
- Recent trends pointing to eminent practice of PM.

In addition, other health-care changes point to an evolving infrastructure for PM including clinician education and PM test interpretation, the emerging 'concierge' or 'boutique' medical practice, and electronic medical records. In enabling PM, pharmacologists will be key as they were in the practice of TDM in the 1980s and 1990s. The evidence for PM would be dependent on prospective, randomised controlled trials and prospective or retrospective observational studies and clinical end points. Dose–exposure–response relationship for dosing adjustment would be based on genotypes. Thus, pharmacometrics or quantitative clinical pharmacology would be used to develop the scientific basis and application of 'personalised medicine'.

Another recent study funded by the European Commission examined the status of pharmacogenetics and the challenges for applications (Hopkins *et al.* 2006). It reviewed the science and industry base in the USA, Europe and Japan. Pharmacogenomics is regarded as interdisciplinary in 60 research institutions. The countries with more than 10 institutions are as follows: USA 73, Germany 35, UK 27, Japan 25, the Netherlands 21, Sweden 14, Italy and Switzerland 13, and France 12. The US institutions are usually better funded by the NIH. European institutions received funding from national governments but not from the European Union. The major areas of research study are drug metabolism, disease mechanisms and disease predisposition. These institutions collaborated more with other research groups than with companies, with the possible outcome of limiting the clinical applications. The commercial sector comprised about 47 companies, mostly small to medium in size with a high turnover rate of about 40%. However, the influx of new companies seemed to have maintained the total number. The business model may be divided into 12 options under the areas: drug discovery, drug safety in development, drug efficacy in development, marketed drug safety, and stratification of diseases and infectious agents. The top five stratified areas are:

- CNS
- Drug metabolism/toxicity
- Cardiovascular disease
- Cancer
- Infection.

The FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) are both proactive but they follow different paths. While the FDA provides guidance documents, EMEA conducts meetings with sponsors. The established clinical pharmacogenomics tests for the European countries would include *HER2* testing for breast cancer and *TPMT* for acute lymphoblastic leukaemia (ALL). The recent study concluded that many interdependent variables would contribute to clinical applications of pharmacogenomics.

The FDA has proactively outreached to scientific colleagues in pharmaceutical companies, partially for the purpose of developing a rational approval process. A series of workshops were held in the Washington DC area (Pharmacogenomics: 1st workshop May 2002, 2nd workshop November 2003 and 3rd workshop April 2005; Pharmacogenomic drug-diagnostic co-development workshop July 2004; Application and Validation of Genomic Markers October 2005). This was followed by the publication of guidance documents (First draft November 2003 and final draft March 2005), and white/concept papers (Critical Path Initiative March 2004, and Drug-diagnostic co-development April 2005; Salerno 2004; Salerno, Lesko 2004a, 2004b; www.fda.gov/downloads/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm085716.pdf 2005; Lesko *et al.* 2003; Lesko, Woodcock 2004; Leighton *et al.* 2004; Gualberto *et al.* 2004; Trepicchio *et al.* 2004, 2011; Wang 2011).

The topics included co-development of drug and diagnostics, sometimes regarded as 'theranostics', and the voluntary genomics data submission (VGDS) process. More recently, VGDS was modified to VXDS – voluntary 'X' data submission, with 'X' representing diagnostic proteomic and other 'omics' biomarkers in the future. In September 2006, the Center for Clinical Device and Radiological Health (FDA-CDRH) issued a draft of a guidance document, 'In Vitro Diagnostic Multivariate Index Assays', that addressed test systems and data processes, with implications for genetic testing. For practitioners of clinical pharmacogenomics, it will be important to follow the outcome of the final draft of this and other documents. Two recent chapters by the FDA-CDRH (Tezak, Hackett 2006) and FDA-Center for Drug Evaluation and Research (Goodsaid *et al.* 2006) provided guidance on the various regulatory issues related to use of PGx biomarkers. In summary, the FDA workshops, the guidance documents and publications of concept and white papers serve as enabling tools towards the practice of clinical pharmacogenomics.

As a result of the decreasing number of submissions, the Critical Path has been advocated by the FDA to facilitate the co-development of drugs along with genomic and proteomic diagnostic biomarkers (Feigal *et al.* 2006).

As an example and extension of that concept and practice, the Critical Path Institute (C-Path), founded by the University of Arizona as part of the Arizona Biosciences Roadmap in July 2005, is an independent, neutral, community-funded, non-profit/tax-exempt organisation. Other key members are SRI International and the FDA. Funding sources include public sector, foundation, the FDA and the Agency for Healthcare Research on Quality. It also has partnerships with universities such as George Washington University, and professional organisations such as the Drug Information Association, the American College of Clinical Pharmacology and others. It has a consortium of 13 pharmaceutical companies – Merck, Johnson & Johnson, Pfizer, Novartis, GlaxoSmithKline, Schering, Roche, AstraZeneca, Boehringer-Ingelheim, Amge, Sanofi-Aventis, Bristol-Myers Squibb and Abbott.

As shown by the model of collaboration in Figure 25.5, the C-Path Institute will share methods, data and strategies in order to ensure the safety of newly marketed drugs. Some of the projects enlisted a consortium of diagnostic and pharmaceutical companies to develop better treatments of lung cancer, to develop an approach for stroke treatment and to lower the incidence of death due to embolism via warfarin dosing therapy, all possibly by using PGx biomarkers. According to the article by Feigal *et al.* (2006), C-Path is currently undertaking 76 projects in 6 main categories:

- Better evaluation tools
- Streamlining clinical trials
- Harnessing bioinformatics
- Modernising manufacturing
- Developing products to address urgent public health needs
- Specific at-risk populations – paediatrics.

Another major factor in adapting clinical pharmacogenomics is the educational outreach to both patient and clinician. To that end, the International Society of Pharmacogenomics recently published a position paper recommending the education effort to the deans of schools of medicine, pharmacy and allied health care (Gurwitz *et al.* 2005). The following 10 recommendations are proposed:

- Encouragement of the deans to include the teaching of pharmacogenomics
- Global outreach to policy makers and government leaders to educate physicians, pharmacists and nurses

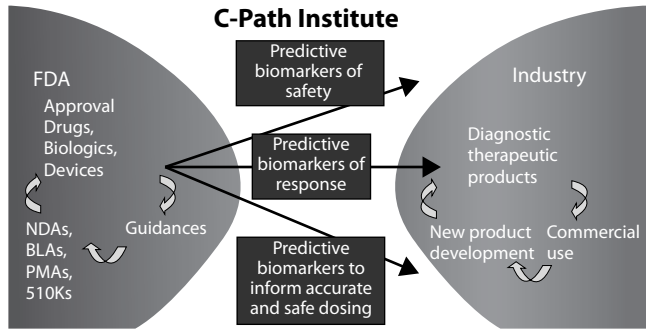

Figure 25.5 FDA–C-Path–industry collaboration to solve common roadblocks in medical product development (Feigel et al. 2006).

- Basic medical teaching to include 4–8 hours of lectures
- Graduate medical and pharmacy schools
- Continuing medical education
- Pharmacogenomics for oncology
- Pharmacogenomics update
- Pharmacogenomics-dedicated issues in journals
- Educational tools using web-based learning
- Better general education for outreach to patient and general public.

If these recommendations were adopted by the deans, they would pave the way to preparing graduating physicians for clinical pharmacogenomics. For example, a PM e-Symposium held on 21 June 2006 addressed the various issues (Personalized Medicine e-Symposium 2006).

The need for developing information for educating physicians and patients on the possible benefit of pharmacogenomics was demonstrated by a recent German study (Rogausch *et al*. 2006). Of the invited 328 patients and 378 general practitioners, 60% and 28%, respectively, consented to participate. There were 96% patients and 52% physicians who were also in favour of the availability of pharmacogenetic tests for a disease such as asthma. Patients worried about the results and privacy, while the physicians worried about putting the patient under pressure and the possible negative impact on obtaining health insurance. Another major educational effort in enhancing the practice is the Laboratory Medicine Practice Guidelines for Clinical Pharmacogenetics, prepared by the National Academy of Clinical Biochemistry (NACB) and the academy of the American Association for Clinical Chemistry (AACC; Nebert, Vesell 2006). The document was drafted with input from NACB and AACC members as well as selected colleagues from other professional societies and regulatory agencies both in the USA and in Europe. There are 10 sections: introduction, pharmacology and populations genetics, methodologies and quality assurance, services consideration, reporting, clinical considerations, TDM and PGx interface, and ancillary considerations – dose and forensic, regulatory and glossary.

Principles of pharmacogenetics/pharmacogenomics

The basic principles of pharmacogenetics and pharmacogenomics have been reviewed in depth (Linder *et al*. 1997; Weber 1997; Jicinio, Wong 2002; Evans, McLeod 2003; Weinshilboum 2003; White, Wong 2005; Wong *et al*. 2006a). Additionally, a more recently published primer provided a quick review of the central dogma of molecular biology, followed by descriptions on genetics, genomics and proteomics (Tsai, Tsao 2007). Other topics included structure, evolution, genetic variation such as SNP alleles, haplotype and linkage disequilibrium, enabling technologies (genomics, proteomics and bioinformatics), implications for gene discovery and mechanisms of human disease, therapeutic and predictive medicine, and dermatology and dermatopathology. Identifying gene clusters responsible for biological processes, the primer suggested that the individual genomic signatures would enable the practice of predictive medicine.

According to the central dogma, the genetic code is passed, through transcription, from DNA to mRNA, and subsequently, through translation, from mRNA to protein synthesis. These proteins may be drug-metabolising enzymes, transporters, receptors, target modulators and other biomolecules involved in signalling pathways. Thus, DNA genetic variations would change the enzyme activity, transporters and receptor sensitivity. The discovery of RNA interference and the emerging evidence for epigenetics have impacted on this simple relationship.

Genetic variations of drug-metabolising enzyme genes can result in normal, deficient or higher enzyme activities. Variations include SNPs, deletions, duplications and others. The polygenic determinants of drug response are illustrated in Figure 25.6 (Evans, McLeod 2003).

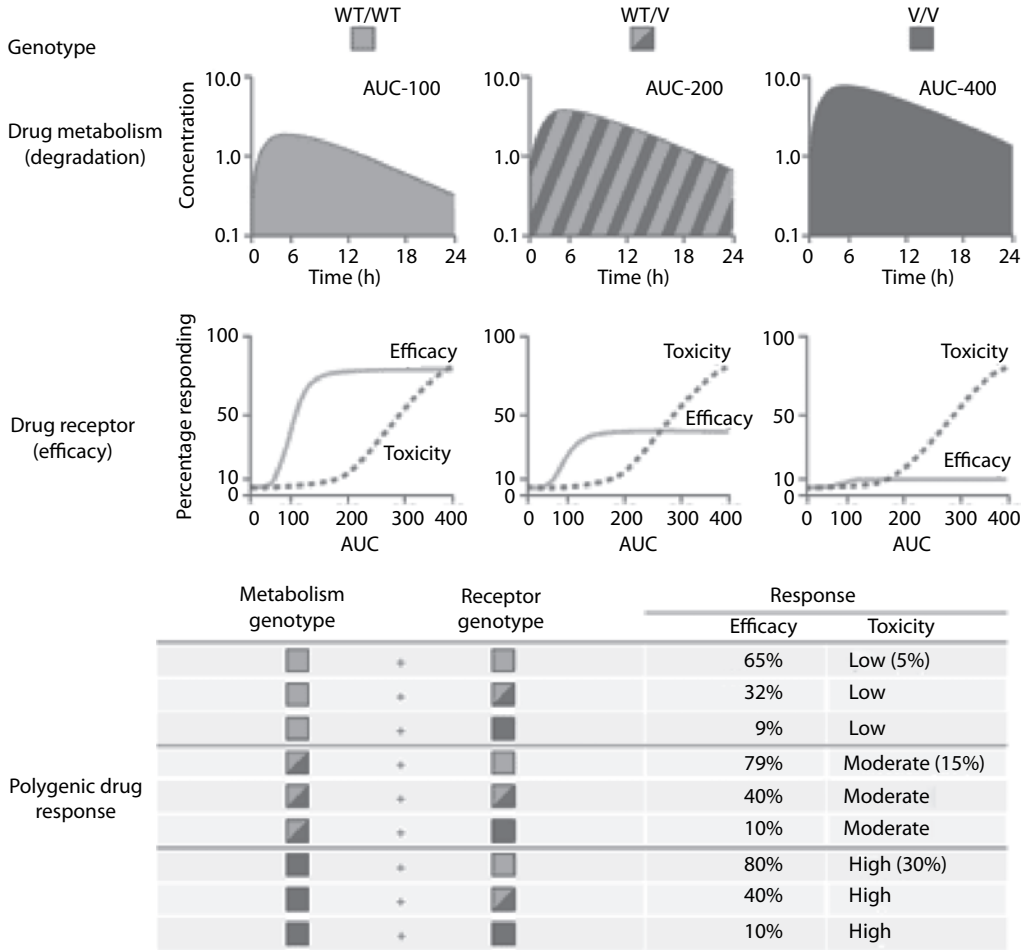

Figure 25.6 Polygenic determinants of drug response (Evans, McLeod 2003).

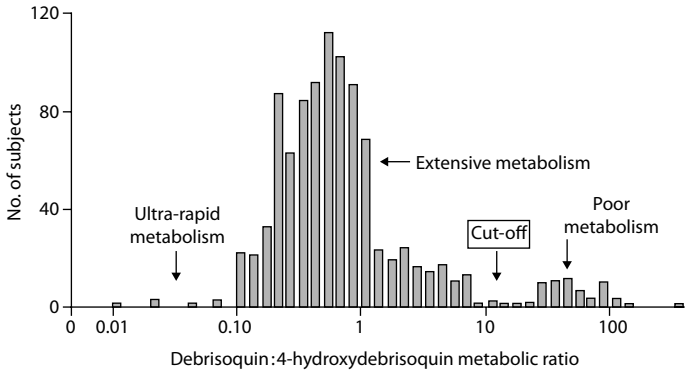

Figure 25.7 Pharmacogenetics of CYP2D6 (Bertillsson *et al.* 1992).

An individual with two wild-type alleles, an extensive metaboliser, when compared with an individual with two variant alleles, a poor metaboliser, has higher enzyme activity and consequently, a lower area under the curve (AUC) with minimal toxicity and adequate efficacy. The heterozygous individual in the middle with one variant allele, an intermediate metaboliser, achieves AUC, toxicity and efficacy intermediate between those of the extensive and poor metabolisers. The possible combination of nine metabolism and receptor genotypes would result in a therapeutic index ranging from 13 to 0.125. Further, individuals with multiple copies of the genes, not shown in Figure 25.6, correspond to ultra-rapid metabolisers. These individuals, with increased enzyme activities, often overmetabolise drugs, sometimes with adverse consequence such as the overconversion of codeine to morphine. Figure 25.7 shows the debrisoquine metabolic ratios of these four phenotypes (Bertillsson *et al.* 1992).

This relationship might be readily further conceptualised by a pharmacology triangle, proposed by Linder and Valdes (1999) (Figure 25.8). Pharmacogenetics provides the fundamental basis, the independent variable for the two interrelated, dependent variables – pharmacokinetics (drug metabolism) and pharmacodynamics (drug action).

Weinshilboum (2003) reviewed the pharmacogenetics of phase 1 drug-metabolising enzymes (including CYP2D6, -2C9, -2C19, dihydropyrimidine dehydrogenase and butyrylcholinesterase) and phase 2 enzymes (*N*-acetyltransferase 2, uridine diphosphate-glucuronosyltransferase 1A1, thiopurine *S*-methyltransferase and catechol *O*-methyltransferase). Weinshilboum and Wang (2006) asserted that, while pharmacogenomics studies are rapidly expanding to include entire signalling pathways, targets and whole genome expressions, it may also be characterised as translational for emerging clinical application and drugs. Evans and McLeod (2003) reviewed the influence of polymorphism of drug target genes on drug effect (angiotensin-converting enzyme, arachidonate 5-lipoxygenase, β_2-adrenergic receptor, bradykinin B_2 receptor, dopamine receptors, oestrogen receptor α, glycoprotein and serotonin transporter), and the effect of polymorphism in disease- or treatment-modifying genes (adducin, apolipoprotein E, HLA, cholesterol ester transfer protein, ion channels, methylguanine methyltransferase, parkin, prothrombin and factor V, and stromelysin-1). Wong and Jannetto (2006) supplemented the information in a recent book chapter.

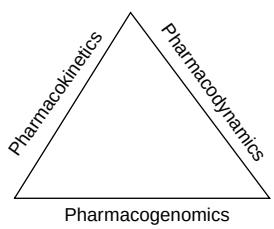

Figure 25.8 The pharmacology triangle (Linder and Valdes 1999).

Several recent reviews have addressed the current status in accounting for race, ethnicity, and ethical and social issues. Race was not an ideal proxy for PGx owing to genome variation, environment–gene interactions and definitions of 'race' in the USA (Jones, Perlis 2006). Another study asserted that race and ethnicity were social constructs reflecting culture, behaviour, diet, environment and socioeconomic status such as income, education and occupation, which cannot be measured by genetic variations (Doyle 2006). Combining the AmpliChip CYP450 prototype microarray assay and other assays, 222 African–Americans (healthy controls, $n = 131$, and psychiatric patients, $n = 91$) were genotyped. This revealed 3 poor metabolisers and 10 poor metabolisers, a new *CYP2D6* allele (*58) and two new duplicated *CYP2D6* alleles (*17xn and *2Lxn) (Cai *et al.* 2006). These latter variations would require dosage adjustment for African–Americans. In a Chinese population (Wang *et al.* 2006), *CYP2D6*10* was most prevalent, ranging from 51% to 70%. Of the 70 cancer patients recovering from gastrectomy, the numbers of patients without *CYP2D6*10*, heterozygous for *CYP2D6*10* and homozygous for *CYP2D6*10* were 17, 26 and 20, respectively. The last group required more tramadol in 48 hours, possibly explaining variable response to pain management to these three groups. A commentary was concerned with race being a complex genetic and non-genetic biomarker (Nguyen *et al.* 2007). In order to optimise pharmacogenetic therapy based on race, non-genetic biomarkers should be used in combination. A paper by Paul and Fangerau (2006) attempts to understand the specific, crucial ethical and social questions surrounding depression in order to assess patients' situations, disease entities and phenotypes, and how they relate to genetic variations.

In assessing the current situation of application of clinical pharmacogenomics, Roden *et al.* (2006) outlined the challenges as shown in Table 25.1.

The scarcity of pharmacogenomic information available from prescription drug package inserts was evident from a recent study (Zineh *et al.* 2006). Of the top 200 prescribed drugs in 2003, there was

Table 25.1 Challenges in pharmacogenomics (Roden 2006)

Challenge	*Potential approaches*
Establishing that drug responses are heritable	Twin studies; family studies
	Linkage between drug response and genomic loci in cell lines, or model organisms
Defining candidate genes	Pharmacokinetic
	Pharmacodynamic:
	Drug targets Biological milieu in which drugs act Disease genes and pathways
	Whole-genome approaches
Defining drug responses	Biomarkers
	Surrogates
	'Hard' end points
Data management, including uniform representation of phenotypical data	Improved informatics
	Centralised, web-accessible public database relating genetic variants and drug responses: www.PharmGKB.org
Reproducibility	Replication sets
	Large study populations
Statistical analysis of associations	New statistical methods, including consideration of haplotypes
Interrogating very large sets of polymorphisms in large numbers of patients	New platforms (e.g. chip or bead -based)
Moving to practice	Reproducible study results Cost-effectiveness Health-care provider education

Table 25.2 Updated top 12 pharmacogenomics tests[a]

Abbreviation	*Name and function*
1. *CYP2D6*	Cytochrome P450 (CYP) 2D6, phase I drug-metabolising enzyme
2. *TPMT*	Thiopurine *S*-methyltransferase, phase II drug-metabolising enzyme
3. *CYP2C9*	CYP2C9, phase I drug-metabolising enzyme
4. *CYP2C19*	CYP2C19, phase I drug-metabolising enzyme
5. *NAT*	*N*-Acetyltransferase, phase II drug-metabolising enzyme
6. *CYP3A5*	CYP3A5, phase I drug-metabolising enzyme
7. *UGT1A1*	Urindine diphosphate glucuronosyltransferase 1A1, phase II drug-metabolising enzyme
8. *MDR1*	Multidrug resistance (P-glycoprotein), drug protein transporter
9. *CYP2B6*	CYP2B6, phase I drug-metabolising enzyme
10. *MTHFR*	Methylenetetrahydrofolate (CH_2THF) reductase, converts CH_2THF to 5-methyltetrahydrofolate
11. *VKORC1*	Vitamin K epoxide reductase complex protein 1, mediates vitamin K reduction
12. *CYP4F2*	CYP4F2, phase I drug-metabolising enzyme

[a]*Clin Lab News*, May, 2005.

Table 25.3 Methodologies for pharmacogenetics testing

Method	*Company*	*FDA approval*
Sequencing	Abbott	Yes
Real-time PCR	ABI	–
PCR arrays	Autogenomics	Yes[a]
PCR arrays (DMET for 1228 SNPs)	Affymetrix	–
Sequencing	Bayer	Yes
Pyrosequencing	Qiagen (Biotage)	–
Real-time PCR	Celera	–
Real-time, allele-specific PCR	DxS	–
RT-PCR	ParagonDx	Yes[a]
RT-PCR (*S*witch *M*echanism *A*t the 5′ end of *R*everse *T*ranscript (SMART™) amplification process – SMAP) – POCT 1 hour	–	–
User-developed PCR arrays	Nanogen	–
Nanoparticles	Nanosphere	Yes[a]
PCR electronic arrays	Osmetech	Yes[a]
Real-time PCR	Roche	–
PCR arrays	Roche	Yes
Invader assay	Thirdwave	Yes
eQPCR LC	TrimGen	Yes[a]
PCR bead-based detection	TM Biosciences	Yes
dHPLC	Transgenomics	–
FISH	Abbott	Yes
MALDI-TOF	Applied Biosystems	–

Modified from Payne (2006).
[a]Warfarin.

discordance between pharmacogenetic package insert information. Pharmacogenetic data were available for 71%, with drug-metabolising enzyme genes accounting for 34.5% and drug targets and transporters for 65.5%. Package inserts for only three drugs among the top 200 – celecoxib, fluoxetine, pantoprazole – contained pharmacogenetic information for prescribing. The lack of pharmacogenetic information might be due to uncertainty about clinical relevance.

Pharmacogenomics tests and methodologies

Current clinical testing is primarily pharmacogenetics, i.e. SNPs, gene deletions, duplications and, to a much lesser extent, gene expression, which has been used in drug discovery, and screening of candidate genes. A 2005 AACC survey of the top 10 tests is now supplemented by three more tests: *VKORC1* (Reider *et al.* 2005) and *CYP4F2* as a result of recent studies for genotype-based warfarin dosing, and HLA (human leukocyte antigen gene) for drug sensitivity such as the development of Steven–Johnson syndrome in some Asians medicated with carbamazepine; see Table 25.2.

CYP and other phase 2 enzymes such as UGT1A1 account for the majority of drug/substrate metabolism for drugs approved in the USA, about 75% involving CYP3A4 and CYP2D6 enzymes. According to the draft of Laboratory Medicine Practice Guidelines by NACB (listed in the website: www.nacb.org/lmpg/LMPG_Pharmacogenetics.pdf), the proposed alleles to be initially included for clinical pharmacogenetics are: *CYP2D6 *1* to **12, *17* and **2A, 2C9 *1* to **6*, and *2C19 *1* to **8* and **17*. The final recommendations are pending. During the third FDA-DIA workshop, O'Kane presented an assessment of possible routine pharmacogenomics testing for medical care (O'Kane 2005). He summarised some of the barriers including the current findings of more than 160 alterations for *CYP2D6* genes. Assay problems include allelic drop-out, intra-allelic recombination, the need for specific assays not affected by pseudogenes *CYP2D7* and *CYP2D8*, and the need to address gene conversion of *CYP2D6* from *CYP2D7*. Caution was recommended that *CYP2D6* genotyping might not be purely routine. The top pharmacogenomic tests are readily performed either by 'home-brew' assay or by some commercially available, FDA-approved test/platform. Payne (2006) reviewed how to choose a method. Jannetto *et al.* (2006), Weber (2006) and other recent publications (e.g. Wong *et al.* 2006a) have also summarised the currently available technologies. Table 25.3 shows an updated list of the methodologies, companies and status of FDA approval/clearance. According to Payne, the methodologies might be readily classified as non-amplification (e.g. fluorescent *in-situ* hybridisation (FISH)), target and signal amplification methods including end-point polymerase chain reaction (PCR) detection, allele-specific primers, length analysis using restriction fragment length polymorphism (RFLP) and Oligonucleotide Ligation Assay (OLA), real-time PCR, signal amplifications, limited sequencing, and new methods including solid-phase 'low-density' microarrays and fluorescence-based bead assay (liquid microarray) and nanotechnology.

Some laboratories performing genotyping have adapted the PCR liquid-bead-based detection (personal communications). Platform and assay choices depend, similar to the selection of clinical chemistry analysers and tests, on the experience of the personnel, FDA approval, the ease of 'home-brew' assay development, and the cost of the instrument and reagents.

Several articles updated on the PGx testing methodologies. An example is the comparison of genotyping 159 samples from *CYP2D6* by Amplichip and *RFLP* (Heller *et al.* 2006), and Amplichip correctly identified gene duplications in 7 discordant samples. The authors concluded that it was a fast and efficient procedure. Using an electronic array by Toshiba, N-acetyltransferase2 *(NAT2)* gene polymorphisms (*C481T G590A G857A*) were determined for 38 samples and the results were in complete concordance with the RFLP results (Nakamura *et al.* 2007). The electrochemical DNA chip and the compact, automated DNA detection system offered fast turnaround time of about 90 minutes. An array-based allele-specific primer extension (AsPEX) was used to detect about 14 variations in 8 genes (*TPMT*, *NQO1*, *MTHFR*, *GSTP1*, *CYP1A1*, *CYP2D6*, *ABCB1* and *SLC19A1*) from 371 specimens of cord blood. In comparison with RFLP, concordance was 99% (Lu *et al.* 2007). Using oligonucleotide ligation/PCR and capillary electrophoresis, the SNPlex Genotyping System detected 48 SNPs simultaneously (Tobler *et al.* 2005). By analysing 521 SNPs in 92 individuals, 99.84% concordance was achieved in comparison to the TaqMan probe-based assays.

Clinical applications

Wong *et al.* (2006a) classified the clinical applications of pharmacogenomics according to drug group, specialties and diseases, including opioids, pain management, nicotine addiction, HIV treatment, immunosuppressants, TPMT for ALL, psychiatry, and clinical and forensic toxicology. A previous publication by Jicinio and Wong (2002) offered extensive basic and clinical information for pharmacogenomics. Readers are directed to these references for further details.

Several reviews are recommended for inter-related developments in pharmacogenomics. In addition to reviewing the uses of predictive genotype *UGT1A1*28*, haplotypes such as *VKORC1* haplotype A, and somatic mutations such as epidermal growth factor receptor, it also demonstrated a genotype-guided thymidylate synthetase (TYMS) TSER in the treatment of rectal cancer (Marsh, McLeod 2006). An extensive review of CYP pharmacogenetics in drug metabolism emphasised that prospective studies should be performed prior to adapting genotype-based dosing (Kirchheiner, Seeringer 2007). Polymorphisms of the neurotransmitter transporters (Kirchheiner *et al.* 2006) serotonin, noradrenaline (norepinephrine), dopamine and P-glycoprotein showed limited effect on selective serotonin reuptake inhibitor (SSRI) response, suggesting that transporter genotypes are not yet proven to be contributory to predictive therapy. However, a later review article (Roots *et al.* 2007) demonstrated that there was evidence to support the use of genotype-based dosing for drug transporters such as P-glycoprotein (ABCB1) and OATP-C (SLC21A6), and other CYP enzyme genes. Table 25.4 shows important and emerging areas including AIDS, cancer, cardiovascular disorders, infectious diseases, haematology and psychiatry (Roden *et al.* 2006).

In complementing pharmacogenomics, TDM and toxicology play a pivotal role as global phenotypic indexes in contributing pharmacokinetic, pharmacodynamic and drug–drug interactions, epigenetics and other environmental factors. As such, pharmacogenomic biomarkers serve as adjuncts to enable the practice of PM. The following recent examples are summarised: warfarin therapy, the treatment of colorectal cancer by irinotecan, the use of carboplatin and irinotecan for non-small-cell lung cancer, alcoholism, psychiatric disorders and opiates.

Warfarin

Warfarin, an antithrombotic agent, has a narrow therapeutic index and large interindividual variation. Recent publications proposed a new dosing regimen based on pharmacogenetics of genes of *CYP2C9* and vitamin K epoxide reductase complex protein 1 *(VKORC1;* Higashi *et al.* 2002; Rieder *et al.* 2005; Sconce *et al.* 2005). Warfarin is racemic, with the active enantiomer, (*S*)-warfarin metabolised by CYP2C9. Variants *CYP2C9*2* and **3* correspond to decreased enzyme activities. For white people, the prevalence of extensive, intermediate, poor and ultra-rapid metabolisers are 58%, 38%, 4% and 4–18% respectively. *CYP2C9* genotype accounts for 6–10% of warfarin dosing variability (Higashi *et al.* 2002; Sconce *et al.* 2005). VKORC1 mediates the reduction of vitamin K, and its genetic variations account for 25% of warfarin dose variability. Mean doses for *VKCORC1 A/A*, *A/B*, and *B/B* genotypes are 2.7, 4.8 and 6.1 mg/day (Nguyen *et al.* 2007). The additional contribution from *CYP2C9* and non-genetic factors accounts for up to 60% of warfarin variability (Marsh, McLeod 2006). A dosage adjustment model is proposed along with INR measurement with dosage reduction to 33% for *CYP 2C9*3/*3* genotype. Recently, Caldwell and colleagues showed that CYP4F2 variations contributed about 5% of the warfarin dosing requirement (Caldwell *et al.* 2008). Increased warfarin dosing is needed on the basis of *CYP4F2* genotyping (TT > CT > CC), with an increase of 1 mg/day for TT homozygotes. Cole recently reported on warfarin resistance due to *VKORC1*5* haplotype, which includes g.−1320G>A, g.−679A>G and a missense SNP, p.Asp36Tyr (Cole 2008). This haplotype is <5% for Europeans, but higher in Ashkenazi Jews and eastern Mediterranean populations. Other resistance genotypes might be identified in the future.

Colorectal cancer

The latest example in the use of pharmacogenomics for cancer is the FDA-approved test for uridine diphosphate-glucuronosyltransferase 1A1 (Third Wave Technologies) for stratifying patients undergoing colorectal cancer treatment with irinotecan. UGT1A1 mediates the conjugation of irinotecan active metabolite, SN-38, to a glucuronide metabolite (Araki *et al.* 1993; Rivory *et al.* 1996; Iyer *et al.* 1999, 2002). Individuals homozygous for the *UGT1A1*28* allele would have reduced enzyme activity, and therefore require a lower dose.

Table 25.4 Examples of associations between drug response and genetic variants (Roden *et al.* 2006)

Drug	*Variable clinical effect*	*Genes with associated variants*	*Possible mechanism*
Azathioprine and mercaptopurine	Bone marrow aplasia	*TPMT*	Hypofunctional alles
	Reduced therapeutic effect at standard doses		Wild-type alleles
Some antidepressants and β-blockers	Increased side-effect risk	*CYP2D6*	Hypofunctional alleles
	Decreased efficacy		Gene duplication
Omeprazole	*Helicobacter pylori* cure rate	*CYP2C19*	Hypofunctional alleles
Irinotecan	Neutropenia	*UGT1A1*	Decreased excretion due to regulatory polymorphism
HIV protease inhibitors	Central nervous system levels	*MDR1*	Altered P-glycorotein function
β-Blockers	Blood pressure lowering and heart rate slowing	*ADRB1*	Altered receptor function or number
Diuretics	Blood pressure lowering	*ADD1*	Altered cytoskeletal function by adducin variants
Warfarin	Anticoagulation	*VKOrC1*	Variant haplotypes in regulatory regions leading to variable expression
		CYP2C9	Coding region variants causing reduced (*S*)-warfarin clearance
Abacavir	Immunological reactions	*HLA* variants	Altered immunological responses
QT-prolonging antiarrhythmics	Drug-induced arrhythmia	Ion-channel genes	Exposure of subclinical reduction in repolarising currents by drugs
General anaesthetics	Malignant hyperthermia	*RYR1*	Anaesthetic-induced increased release of sacroplasmic reticulum calcium by mutant channels
Inhaled steroids	Bronchodilatation	*CHCR1*	Unkown
HMG-CoA reductase inhibitors (statins)	Low-density lipoprotein	*HMGCR*	Altered HMG-CoA reductase activity

Non-small-cell lung cancer

In a phase II study (Pillot *et al*. 2006) of patients with non-small-cell lung cancer treated with carboplatin and irinotecan, pharmacogenomic analysis included *ABCB1, CYP3A4*1B, ERCC2, GSTP1, UGT1A1*28* and *XRCC1*. Common haematological toxicities included neutropenia and thrombocytopenia. Grade 4 neutropenia was more prevalent among patients who were homozygous *UGT1A1*28 (7/7)* than among *6/6* or *6/7* genotypes. Patients with *GSTP1 I105V G/A* or *G/G* genotypes achieved partial response compared with no response for *A/A* genotype. It was concluded that future studies should include pharmacogenomics analysis as possible biomarkers for response and toxicity.

Alcohol

A recent review on alcoholism (Enoch 2003), a complex psychiatric disorder with high heritability (50–60%) showed the lifetime prevalence of alcohol dependence to be 20% in men and 8% in women in the USA. Type 1 alcoholism is characterised by later onset with feelings of anxiety, guilt and high harm avoidance, while type 2 alcoholism is associated with early age of onset (usually in men), impulsive and antisocial behaviour, and low levels of brain serotonin (Enoch 2003). Genetic variations of alcohol-metabolising genes might affect drinking behaviour owing to the accumulation of acetaldehyde. The unpleasant 'flushing' sensation might result in decreased alcohol consumption. Thus, therapeutic targets may include genes of neurotransmitter 'reward pathways' (serotonin, dopamine, GABA, glutamate and β-endorphin) and the behavioural stress response system (corticotrophin-releasing factor and neuropeptide Y). Furthermore, the type 1/2 systems enhance the understanding of the pharmacogenomic response to the current pharmacotherapy. Alcohol inheritability is attributable to genetic variations of alcohol dehydrogenase 1B and aldehyde dehydrogenase 2 (Oroszi, Goldman 2004). COMT Val158Met mutation affects executive cognitive function, stress/anxiety response and opioid function. Opioid receptor micro1 (OPRM1) Asn40Asp, a gatekeeper molecule affecting naltrexone, and HTTLPR, possibly affecting serotonin transporter function, stress response and anxiety/dysphoria, are contributory to initial vulnerability, addiction and relapse.

Psychiatric disorders

In reviewing the current psychiatric practice of using polypsychopharmacology for the treatment of depression, psychosis, anxiety and mood disorders, Preskorn (2006) indicated that the scientific literature had provided clinicians with more on patient safety than on efficacy. With the advent of pharmacogenomics and bioinformatics, this will change as the pathophysiology of these illnesses becomes more established. A review (Black *et al*. 2007) of phase 1 antidepressant-metabolising enzyme genes focused on the CYP superfamily polymorphism and the impact on antidepressant metabolism, antidepressant substrates, inhibitors and inducers, and others. Prathikanti and Weinberger (2005) reviewed the literature and noted that schizophrenia-associated genes included *NRG-1*, *DISC1*, *RGS4*, *COMT*, *PRODH*, *DTNBP1*, *G72*, *DAAO* and *GRM3*, while *5-HTTLPR* and *BDNF* are associated with affective disorders. Along with advances in pharmacogenomics, these developments will enable PM. Murray (2006) reviewed the impact of CYP pharmacogenomics and drug–drug interaction for antipsychotic therapy. Besides CYP2D6, CYP1A2 and CYP3A4 also mediate antipsychotic metabolism as shown in Table 25.5. Since CYP1A2 is inducible, individuals with *CYP1A2* variants and some SNP combinations (haplotypes) in the 5′-regulatory regions may demonstrate variable responses. The author cautioned that, in addition to genotyping of CYP, phenotyping may complement and may be complicated by drug–drug interactions.

A report by Yu *et al*. (2006) examined the relationship between serotonin receptor polymorphisms and fluoxetine treatment. Desensitisation of somatodendritic 5-hydroxytryptamine 1A (HTR1A) autoreceptors was implicated in the SSRI response. Variation of HTR1A Gly272Asp and linkage disequilibrium between C-1019G were analysed in 222 Chinese major depressive patients treated with fluoxetine. These two variations showed strong linkage disequilibrium. Response to fluoxetine was established for the HTR1A haplotype of the two polymorphisms. Female patients with − 1019C/C genotype had a better response than – 1019G carriers. Provisional recommendations (de Leon *et al*. 2006) were drafted for identifying and treating CYP2D6 poor metabolisers with tricyclic antidepressants (TCAs), venlafaxine, typical antipsychotics and risperidone; CYP2C19 poor metabolisers with TCAs and perhaps citalopram, escitalopram and sertraline; and CYP2D6 ultra-rapid metabolisers. For patients taking "new" drugs – aripiprazole, duloxetine and atomoxetine – the efficacy of genotyping *CYP2D* was pending. Another review (Kirchheiner 2001) developed recommendations for dosing 14 antidepressants based on either *CYP2D6* or *2C19* genotype or phenotype. A dose reduction of about 50% of tricyclic antidepressants was recommended for poor metabolisers of these two enzymes. A review on pharmacogenetics for antipsychotics (Bondy, Spellmann 2007) again confirmed the emerging importance of CYP polymorphism, the pending response prediction by candidate genes, and the possible link between genes of the serotoninergic system and weight gain. With the rapid development of pharmacogenomics, the authors concluded that new and cost-effective assays and research findings will advance the understanding and treatment of schizophrenia. Since CYP2D6 is often involved in antipsychotic metabolism, Dorado *et al.* (2006) reviewed and summarised the impact of physiological and environmental factors on CYP2D6 hydroxylation, CYP2D6 inhibition, the use of metabolite ratio (MR) for evaluating CYP2D6 hydroxylation, and the influence of CYP2D6 on drug levels and QTc interval elongation.

Table 25.5 Cytochrome P450s (CYPs) with major roles in the *in-vivo* clearance of antipsychotic agents (Marsh, McLeod 2006)

CYP	*Antipsychotic drug*	*Altered drug substrates and inhibitors that may be used in psychotic patients*	*Inducers*	*Numbers of allelic variants*
1A2	Clozapine	Omeprazole	Omeprazole	24 plus wild-type (also 9 predicted haloptypes)
	Olanzapine		Cigarette smoke	
			Barbecued meats	
2D6	Risperidone	Dextromethorphan	None	94 plus wild-type
	Chlorpromazine	Codeine		
	Thioridazine	Imipramine		
		Nortriptyline		
		Paroxetine		
3A4	Ziprasidone	Erythromycin	Rifampicin	38 plus wild-type
	Quetiapine	Diltiazem	Carbamazepine	
	Aripiprazole	Ciclosporin	Phenytoin	
	Haloperidol	Ethinylestradiol	Dexamethasone	

Olanzapine therapy and P-glycoprotein (PGP) polymorphisms were investigated in 41 schizophrenia patients (Lin *et al*. 2006). The rationale of the study was based on the effect of three common PGP polymorphisms – C1236T, G2677TA and C3435T – which result in a decrease in olanzapine transport out of the CNS. 3435T allele carriers demonstrated possible effects on the penetration of olanzapine into the CNS, as shown by olanzapine plasma levels, and by the positive symptom reduction. Baumann *et al*. (2006) gave a case report of a 14-year-old girl treated for obsessive–compulsive disorder who suffered a generalised tonic–clonic seizure after daily doses of 200 mg/day of sertraline, 10 mg/day of olanzapine and lorazepam (0.5–1.5 mg/day). On day 65, sertraline and olanzapine plasma concentrations were 352 μg/L and 20 μg/L respectively (recommended range, 20–80 μg/L). Since CYP2C9, CYP2B6, CYP2C19, CYP2D6 and CYP3A4 contribute to the *N*-demethylation of sertraline, the patient was genotyped and phenotyped, showing genetic deficiency of *CYP2D6* (**4/*4*) and *CYP3A5 (*3/*3)*, and homozygosity for the allele **6* of *CYP2B6*. The authors concluded that her rare pharmacogenetic profile and the co-medication with olanzapine could have contributed to the epileptic seizure.

Opioids

A review on pharmacogenomics and addiction to opiates (Lichtermann *et al*. 2000) summarised association studies of 11 candidate genes – both pharmacokinetics (CYP2D6) and pharmacodynamics (transporters and receptors) as shown by Table 25.6. While findings of animal studies with opioid, cannabinoid or dopamine receptor-disrupted genes were not confirmed in humans, whole-genome expression in animals will be used with more success in the future.

Table 25.6 Association studies of 11 candidate genes in probands with opiate dependence (Lichtermann *et al*. 2000)

Protein	*Gene symbol*	*Cytogenetic location*	*Number of cases*	*Number of control subjects*	*Results*
Cytochrome P450 subfamily II	*CYP2D6*	22q13.1	83 (oral opiate)	276	Protective effects of homozygote deletion
			93 (multi-drug)		(no poor metabolisers in 83 opiate abusers)
					[$P = 0.05$, OR = 7.2 (95% CI, 0.4–124.1)]
Dopamine receptor D_2	*DRD2*	11q23	232 (poly-substance)	56	Alleles B1 and A1 ∧ in cases ($P < 0.01$, $P < 0.05$)
			40 (opiate preferring)	119	No association
			465 (heroin addicts)	298	141 D17 Ins/Del (insertion/deletion polymorphism) associated in nasal inhalers ($P = 0.006$)
Dopamine receptor D_3	*DRD3*	3q13.3	54 (opiate addicts)	70	Ser9Gly homoz.∧ in sensation seekers ($P = 0.034$)
			193 (heroin addicts)	134	No association with Srin9Glycin polymorphism
			121 (heroin addicts)	180	No association with Srin9Glycin polymorphism
Dopamine receptor D_4	*DRD4*	11p15.5	141 (heroin addicts)	110	Allele 7 ∧ [$P = 0.001$,RR 2.5(95% CI, 1.4–4.4)]
			121 (heroin addicts)	154	Long allele ∧ [$P = 0.02$, OR 2.3(95% CI, 1.1–4.9)]
			55 (opiate dependent)	144	No association with four coding polymorphisms
			285 (heroin addicts)	197	No association (allele 7, $P = 0.19$)
			111 parent–offspring trios		Transmission/disequilibrium test (TDT), $P = 0.74$
Solute carrier family 6, member 3	*SLC6A3*	5p14.3	32 (opiate preferring)	38	No association with 40 base-pair variation in the number of tandem repeats polymorphism the in 3′-untranslated region
Dopamine transporter	*DAT1*				
Solute carrier family 6, member 4	*SLC6A4*	17q11.1-q12	63 (heroin addicts)	72	Allele 10∧ [$P = 0.005$, OR = 3.5(95% CI, 1.4–8.6)]
5-Hydroxytryptamine transporter	*5-HTT*		186 (heroin addicts	217	No association with promoter variant ($P > 0.1$)
5-Hydroxytryptamine receptor 2A	*HTR2A*	13q14-q21	121 (heroin addicts)	180	No association with A-1438G and T102C single nucleotide polymorphisms
γ-Aminobutyric acid receptor γ-2	*GABRG2*	5q31.1-q33.1	121 (heroin addicts)	180	No association with G3145A single nucleotide polymorphism
Cannabinoid receptor 1	*CNR1*	6q14-q15	29 (opiate users)	114	No association with AAT triplet repeat
Opioid receptor μ-1	*OPRM1*	6q24-q25	113 (heroin addicts)	39	No association with C17T ($P = 0.054$) or A118G ($P = 0.16$; except in Hispanics, $P = 0.004$)
Opioid receptor δ-1	*OPRD1*	1p36.1-p34.3	103 (heroin addicts)	115	T921C:CC homoz ∧[RR 4.4 (95%, 1.8–10.8)]
			233 (heroin addicts)	173	T921C: no association ($P = 0.30$)
			90 parent–offspring trios		Transmission/disequilibrium test (TDT), $P = 0.68$

Opioid pharmacogenomics was reviewed by Somogyi *et al.* (2007), showing that codeine and other opioids are metabolised by CYP2D6, with poor metabolisers experiencing reduced antinociception. Since P-glycoprotein is an opioid transporter, the influence of the *ABCB1* genotypes on opioid pharmacodynamics and dosage requirements was highly variable. SNPs of the μ-opioid receptor gene were correlated with increasing morphine sensitivity.

Since codeine is metabolised by CYP2D6 to morphine (Kirchheiner *et al.* 2007), ultra-rapid metabolisers may suffer exaggerated and toxic opioidergic effects. A study compared the pharmacokinetics of 11 ultra-rapid, 10 extensive and 3 poor metabolisers, and the results showed that there was a significantly higher morphine area under the curve and higher frequency of sedation for ultra-rapid metabolisers than for extensive metabolisers. The authors suggested that genotyping ultra-rapid metabolisers would be helpful for codeine dosing. In a case report (Gasche *et al.* 2004) an ultra-rapid CYP2D6 metaboliser developed life-threatening opioid-morphine toxicity following the ingestion of a therapeutic dose of codeine. The toxic morphine concentration was partially attributed to the ultra-rapid metaboliser genotype, drug–drug interaction and metabolite accumulation due to acute renal failure as shown in Figure 25.9.

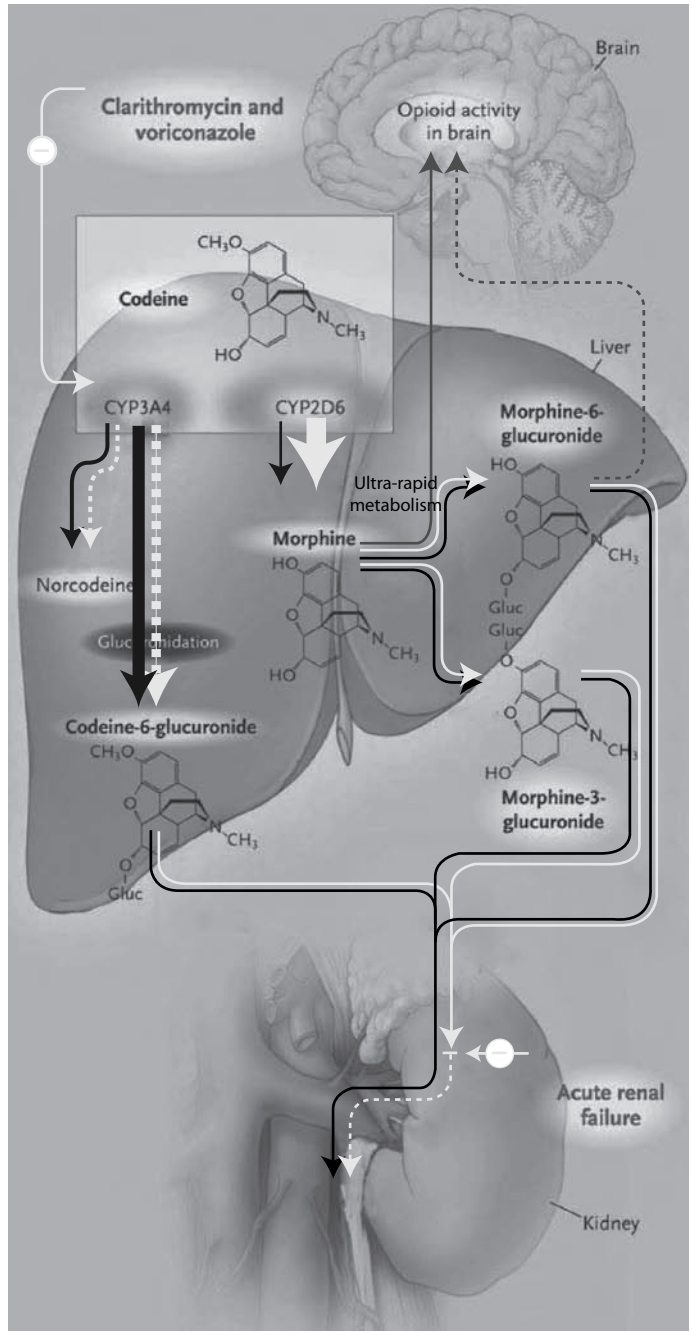

Figure 25.9 Metabolic pathways of codeine biotransformation. The conversion of codeine into norcodeine by CYP3A4 and into codeine-6-glucuronide by glucuronidation usually represents 80% of codeine clearance, and conversion of codeine into morphine by CYP2D6 represents only 10% of codeine clearance (black arrows). Morphine is further metabolised into morphine-6-glucuronide and into morphine-3-glucuronide. Morphine and morphine-6-glucuronide have opioid activity (grey arrows). Glucuronides are eliminated by the kidney and are thus susceptible to accumulation in cases of acute renal failure. The patient (white arrows) had ultra-rapid CYP2D6 metabolism, inhibition of CYP3A4 as a result of treatment with clarithromycin and voriconazole, and glucuronide accumulation due to acute renal failure. White arrows with dotted lines indicate low levels of drug conversion or elimination, grey arrows with dotted lines indicate low levels of brain penetration and thick arrows indicate high levels (Gasche *et al.* 2004).

Forensic applications

The application of pharmacogenomics to postmortem forensic toxicology was initially demonstrated by Druid, Holmgren, Carlsson and Ahlner in a 1999 Swedish study (Druid *et al.* 1999), followed by other studies from Finland and the USA. The experiences and observations were summarised in three chapters of a book on pharmacogenomics (Holmgren, Ahlner 2006; Sanjantila *et al.* 2006; Wong *et al.* 2006b). Collectively, these publications emphasised pharmacogenomics as an adjunct to forensic pathology, complementing information including postmortem findings, case history including medication and scene investigation. The molecular autopsy is proposed as an adjunct, similar to the use of molecular analysis of *SCN5A* variant as a contributing factor to sudden infant death syndrome (Ackerman *et al.* 2001).

The selective application of pharmacogenomics as contributing to the molecular autopsy is demonstrated in Figure 25.10, the proposed Milwaukee pharmacogenomic algorithm for forensic toxicology.

Case selection is initiated during the forensic pathology review (Jannetto *et al.* 2002). The algorithm assesses various co-variables such as acute or chronic toxicity, postmortem findings, sample collection sites, postmortem intervals, co-administered drugs, case/medical/medication histories, scene investigations and possible intent. As the case review continues with developing toxicological findings, prime selection criteria include elevated drug concentrations and identification of known and 'unknown' and potentially interacting drugs/metabolites. The postmortem intervals that possibly result in changes in drug concentrations are also considered in case selection and in interpretation. For the selected cases, whole blood samples are then transferred, with chain of custody, for pharmacogenomics testing by the molecular and pharmacogenomics laboratory. Using pyrosequencing, genotyping included *CYP2D6*2-*8*, *CYP2C9*2*3*, *CYP2C19*2-*4*, *CYP3A4*1B* and *CYP3A5*3*. The forensic applications are illustrated by three opioid cases from recent publications: methadone and antidepressants, oxycodone and fentanyl.

Methadone and antidepressants

The decedent was a 41-year-old woman 6 months into her pregnancy (Wong *et al.* (2003), and this is also case 1 in Wong (2009)). She had a heart murmur and rheumatoid arthritis treated with methadone. In addition, amitriptyline was prescribed for her depression. She celebrated New Years' Eve with her husband. On the following morning, she was found dead in her living room. Scene investigation revealed her ingestion of 9×50-mg tablets of amitriptyline within 17 days, and $2–3 \times 95$-mg doses of methadone. Several years previously, she had attempted suicide by drug ingestion. Toxicological analysis of iliac blood showed the following drug concentrations in mg/L: methadone, 0.7; amitriptyline, 1.5; nortriptyline, 2.2; diazepam, 0.19; *N*-desmethyl diazepam, 0.13. No alcohol was detected. The elevated antidepressant concentrations of iliac, peripheral blood would not be due to postmortem drug redistribution and are more likely attributable to acute drug ingestion. Molecular autopsy by pharmacogenomics showed that she was

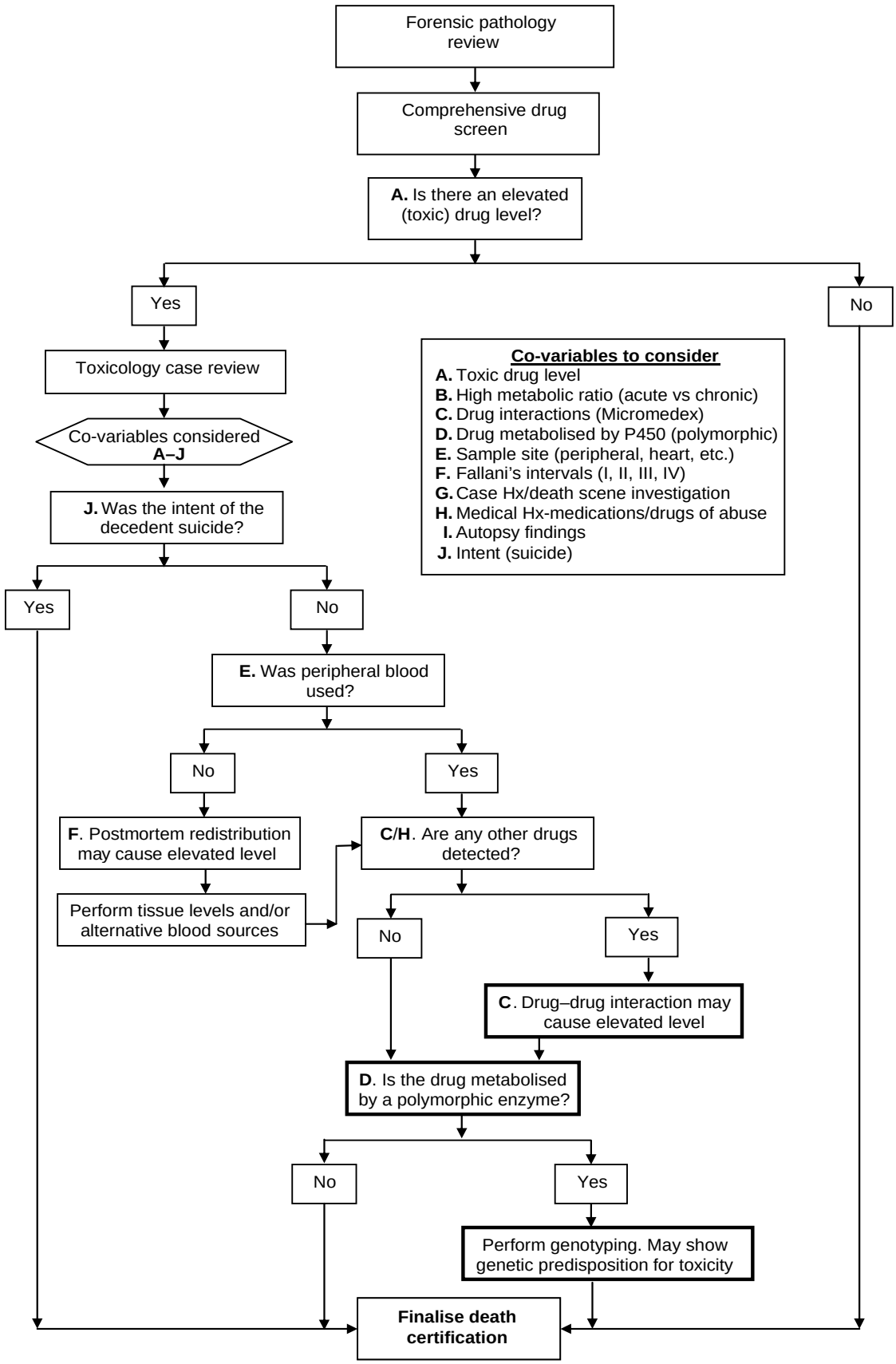

Figure 25.10 A proposed pharmacogenomics algorithm (Jannetto *et al.* 2002).

homozygous for *CYP2D6*4,* corresponding to the poor metaboliser phenotype. This would result in the lack of hydroxylation of methadone, amitriptyline and nortriptyline, thus resulting in elevated parent drug concentrations. Death certification was: cause of death, mixed drug toxicity; manner of death, accident.

The use of pharmacogenomics was shown in a doxepin fatality possibly due to *CYP2D6* variations with resultant poor metaboliser phenotype (Koshi *et al.* 2007). In 2002, a 43-year-old Finnish man was found dead at home. His health record showed that he had recently been involved in an accidental fall, suffered from alcohol withdrawal and demonstrated suicidal tendencies. His medications included disulfiram, fluoxetine, diazepam, and drugs of abuse – hashish and heroin. He had also recently been anxious and aggressive. Postmortem findings showed numerous bruises and abrasions, anthracosis, left-sided bronchopneumonia in the lungs and stasis in the spleen, and haematoma in the internal scalp, possibly due to the fall. Toxicology showed femoral venous blood concentrations of doxepin, 2.4 mg/L (therapeutic range: 0.0–0.15 mg/L) and nordoxepin, 2.9 mg/L. These were the highest when compared with the Finnish database, with concentrations ranging from 0.2 mg/L to 1.4 mg/L for 34 cases. The MR was 0.83, lowest of the range from 0.83 to 75. For 20 fatal doxepin cases in 2002, the lowest MR was 3.8, with an exception of one case with MR of 2.

Molecular autopsy pharmacogenetics showed that the decedent was *CYP2D6*3/*4*, corresponding to a poor metaboliser due to enzyme deficiency, and *CYP2C19*1/*1*, corresponding to an extensive metaboliser. The CPY2D6 enzyme deficiency resulted in the lack of adequate hydroxylation of both doxepin and nordoxepin. Cause of death was doxepin toxicity due to repeated use, and manner of death was undetermined. The authors urged the combined use of toxicology and pharmacogenomics with other postmortem findings.

In a multicentre study on the effect of ABCB1 and cytochrome P450 genotypes on methadone plasma concentrations and treatment response, 245 patients were genotyped for *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *ABCB1* and *UGT2B7*, and CYP3A activity was phenotyped with midazolam (Crettol *et al.* 2006). CYP3A4 and CYP2B6 contributed significantly to methadone metabolism, with CYP2D6 to a minor extent. ABCB1 accounted for minor pharmacokinetic variability. Other studied CYP genes did not affect methadone metabolism. Further, a recent study of 179 patients receiving racemic methadone (Eap *et al.* 2007) showed that CYP2B6 poor metabolisers were more likely to develop QT elongation. Since PGP is encoded by an ABCB1 gene, a methadone transporter, a study of 60 opioid-dependent individuals was performed by detection of SNPs at positions 61, 1199, 1236, 2677 and 3435 (Coller *et al.* 2006). Higher doses were needed for individuals with two copies of the wild-type haplotype, and lower doses for AGCTT haplotype. Thus, haplotyping may be used to individualise methadone therapy.

Oxycodone (Jannetto et al. 2002)

A 49-year-old man with a history of alcoholism and chronic lower back pain was prescribed OxyContin and Percocet (this is case 2 in Wong 2009). He also had depression and post-traumatic stress disorder and had previously attempted suicide once. The scene investigation revealed only 12 of the 60 oxycodone tablets that had been obtained the day before. Last seen by his roommate in the morning, the decedent was found unresponsive later that afternoon. Toxicological analysis of subclavian blood showed methadone 0.437 mg/L; no alcohol was detected. Molecular autopsy by pharmacogenomics showed that he was *CYP2D6*4* homozygous, corresponding to a poor metaboliser phenotype. Postmortem also showed that he had hepatic cirrhosis and atherosclerotic heart disease. Given the short postmortem interval and subclavian blood source, the elevated oxycodone was not due to postmortem drug redistribution. It might be due to the poor metaboliser phenotype and hepatic cirrhosis, both contributing to impaired drug metabolism. Death was certified as follows: cause of death, oxycodone overdose; manner of death, accident.

Fentanyl (Jin et al. 2005)

A 44-year-old white woman complained about her knee pain and was treated with Duragesic fentanyl patches (this is case 3 in Wong 2009).

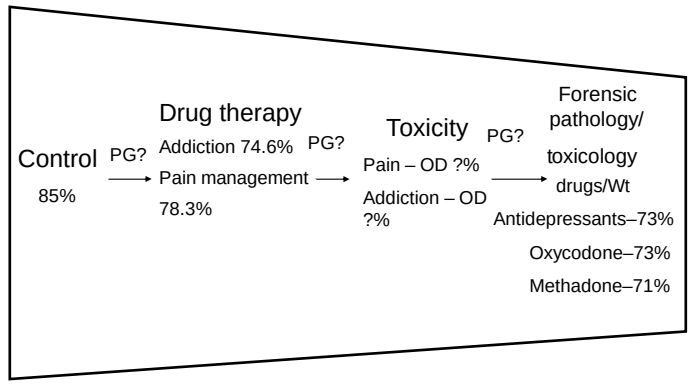

Figure 25.11 A pharmacogenomics converging continuum? (Wong *et al.* 2006b).

She appeared to be 'goofy' and went to bed. She was found dead 24 hours later. The decedent was a drug abuser with a psychiatric history. She had previously cut her arm to obtain drugs and also expressed suicidal ideation. Toxicological analysis of subclavian blood showed the following drug concentrations in mg/L: fentanyl, 0.019; norfentanyl, 0.008; cyclobenzaprine, 0.16; tramadol, 0.06; diphenhydramine, 0.08; citalopram, 0.22. Olanzapine was detected but not quantified and no alcohol was detected. The MR of fentanyl/norfentanyl was 2.5. Molecular autopsy by pharmacogenomics showed that she was heterozygous for *CYP3A4*1B* and *CYP3A5*3*, different from the majority of caucasians *CYP3A4, WT* and *CYP3A5*3 HM*. In this study with a limited number of fentanyl cases, the MR of this case is lower than the MRs of majority of the cases with high fentanyl concentrations. Together, these findings suggested, for the first time, that CYP3A5 co-mediated with CYP3A4 the metabolism of fentanyl to norfentanyl. Death certification for the above case was: cause of death, mixed drug toxicity; manner of death, accident.

From these studies, the wild-type prevalence defined as non-*CYP2D6 *2, *3, *4 and *5*, showed the following trend: antidepressants and oxycodone 73% and methadone 71%, lower than the 85% in controlled group and 78.3% in pain management study. Together, the genetic contribution to drug metabolism impairment may be interpreted as a gene dose effect, resulting in more pronounced drug toxicity. Such an effect might be readily demonstrated as a pharmacogenomics converging continuum as shown by Figure 25.11, and is helpful to understand the effect in a 'live antemortem' population.

Codeine/morphine

A full-term baby died from morphine intoxication (Koren *et al.* 2006) as a result of breast-feeding by the mother, who had been prescribed codeine for episiotomy pain, as well as paracetamol (acetaminophen). The mother was later genotyped to have *CYP2D6*2X2* gene duplication, corresponding to ultra-rapid metaboliser phenotype. Breast milk and postmortem blood concentrations of the baby were 87 and 70 μg/L, respectively, compared with expected concentrations of 2–21 and 0–2.2 ng/mL. The authors suggested management strategies for codeine use by breast-feeding mothers.

Conclusions

Gregor Mendel's 'long story' may continue in personalised medicine and personalised justice

Pharmacogenomics as an adjunct to forensic pathology/toxicology adds to the understanding of one of the contributing factors of drug toxicity. The clinical and forensic applications point to the integrative use with other pertinent clinical information, case history and postmortem findings. The proposed algorithm serves as a preliminary model to initiate the use of pharmacogenomics and would need validation by other centres and future studies. In the meantime, the use of

pharmacogenomics as a molecular autopsy is already showing the translational applications as shown in the two cases of morphine toxicity due to ultra-rapid metabolism of codeine. The postmortem report produced tangible benefits, such as guidelines on the use of drugs for breast-feeding mothers. As shown by the WHO data, warfarin fatalities are listed among the highest worldwide. Thus, the potential application of genotyping for warfarin dosing would achieve the benefit of a more rational dosing strategy, as well as a possible decrease in warfarin fatalities. Perhaps a retrospective study for warfarin-related death would further verify the merit of this protocol. In contrast with other pharmacogenomics applications, the use of pharmacogenomics in forensic pathology/toxicology, according to the proposed algorithm, has been initiated by toxicological findings. This points to the recognition of toxicology and hence of TDM as possible global phenotypic indexes, accounting for other factors such as drug–drug interactions, diet and other epigenetic factors. This convergence – pharmacogenomics with toxicology/TDM – merits prospective pharmacoeconomics studies in the future. In extending the use of molecular diagnostics, other emerging areas such as proteomics, metabolomics, RNA interference, epigenetics and others are rapidly being integrated in clinical investigations and translational medicine. Forensic scientists should be vigilant about their potential applications in death certifications, such as suicide and gene expression. These findings have potential for beneficial extension to decedents' living family members.

Personalised justice

The above experience may be helpful in applying pharmacogenomics in the emerging practice of personalised justice, such as the use of pharmacogenomic biomarkers for the interpretation of possible 'side-effects/behaviour/impaired performance' of drivers arrested in cases involving driving under the influence of drugs. Drivers' impaired driving performance may be partially explained on the basis of genetically predisposed impairment of drug metabolism and hence accumulation, which might result in driving impairment. Similar to the use of DNA fingerprinting in identity testing, the use of pharmacogenomics and other molecular biomarkers in the future as adjunct biomarkers in the above context may constitute a rational approach to understanding and ruling on the driver's liability, hence offering the possibility of personalised justice (Figure 25.12).

As the public gains awareness of the benefits of PM and the role of pharmacogenomics, the public media and scientific groups such as the White House Council of Advisors on Science and Technology are proactive forces. Other encouraging developments have included the availability of a proficiency survey programme by the College of American Pathologists in 2007, and quality assurance/control from commercial sources, indicating increasing clinical adaptations by clinical laboratories. NACB guidelines would certainly pave the way. Challenges include adequate reimbursement, clinical interpretation, ethical guidelines, and education of patients and healthcare professionals. Thus, Gregor Mendel's 'little trick – long story' will continue in new applications of the human genome project and rapid advances in molecular biology and pharmacogenomics.

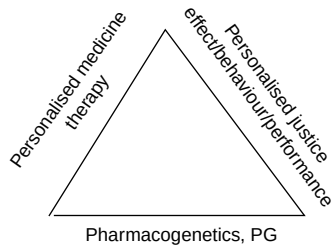

Figure 25.12 Personalised medicine and personalised justice. A new social paradigm? Complementary, tandem, overlapping and inevitable?

Glossary (Roden et al. 2006)

***Alleles*:** Alternate sequences of the same gene, one inherited from each parent.

***Association*:** A statistical finding that the frequency of one or more genetic variants is significantly different in patients with a given phenotype than in those without the phenotype. Often polymorphisms in candidate genes are studied. A more recent method, whole genome association, seeks to identify new genes involved in variable phenotypes; the technique uses new methods to compare genotypes at hundreds of thousands of polymorphic sites in large numbers of patients with and without a specific phenotype.

***Biological pathway*:** A set of proteins that interact to produce normal and abnormal physiology.

***Candidate gene*:** A gene in which variants could plausibly explain a given phenotype, such as severity of disease or variable response to a drug. Methods to identify candidate genes include basic science studies, identifying DNA sequences conserved across species, human genetics or genome-wide analyses. Candidate genes may be in pharmacokinetic or pharmacodynamic pathways.

***Genome*:** The collection of all DNA in an organism. Only a small proportion (probably 3%) of the human genome encodes proteins.

Genetic variant: A difference in DNA sequence compared with a reference sequence.

Genotype: The genetic make-up of an individual, which may refer to the whole genome or to specific genes or regions of genes.

Haplotype: A set of genetic variants that are inherited together. Polymorphisms that are co-inherited more often than by chance alone are in linkage disequilibrium. Haplotype blocks may include many individual polymorphisms in high linkage disequilibrium; as a result, establishing genotype at any single polymorphic site with such a block may establish genotypes at linked sites within the block. Individual single-nucleotide polymorphisms (SNPs) that can be used to establish genotype within a haplotype block are termed tag SNPs.

Heterozygous: Different alleles in a specific region of DNA.

Homozygous: The same alleles in a specific region of DNA.

Mutations: Rare variants, most often in coding regions, which are often associated with genetic diseases, such as cystic fibrosis or sickle cell anaemia.

Pharmacodynamics: The study of the relationship between drug concentrations and drug effects.

Pharmacogenetics: The study of the relationship between individual gene variants and variable drug effects.

Pharmacogenomics: The study of the relationship between variants in a large collection of genes, up to the whole genome, and variable drug effects.

Pharmacokinetics: The study of the relationship between drug dose and drug concentrations (often as a function of time) in plasma or tissue.

Phenotype: Measurable characteristics of an organism. These may derive from genotype, environment or their combination. Organisms with the same phenotype can have different genotypes.

Polymorphisms: DNA variants that are common, often defined as greater than 1% in a given population (although rare polymorphisms are increasingly being recognised). Polymorphisms can be in coding regions (where they may synonymous or non-synonymous) or, more commonly, in non-coding regions, and often vary by ethnicity. The most common type of polymorphism is a change

in one nucleotide (base pair) in a DNA sequence, referred to as an SNP. Other polymorphisms are insertion and deletion of multiple sequential nucleotides ('indels'); variable numbers of repeats, such as doublets or triplets; or large-scale duplications or deletions. Although some genetic variants are known to alter protein abundance or function, the functional consequences of most polymorphisms are unknown.

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CHAPTER

26 Interpretation of Toxicological Data

OH Drummer and S Karch

Introduction

Toxicology has a range of applications, as other chapters of this book have shown. One of the main applications of forensic toxicology has been death investigations, i.e. postmortem toxicology. However, investigating a possible drug-affected driver or an alleged assailant and investigations of drug-facilitated sexual assault are important and growing applications.

The assessment of blood or tissue drug results is not always straightforward. Many factors must be considered, and a truly accurate assessment is likely to require discussions between all the relevant specialists involved, and perhaps even with experts from outside, who can bring with them additional expertise.

The purpose of this chapter is to provide information about how interpretation should be carried out and detail the range of factors that must be considered. It is hoped providing readers with actual examples from selected real cases will improve their ability to properly interpret toxicological information.

Initial acquisition of information

Scene information is vital. A toxicologist will often get some initial information from the police investigators or from his or her colleagues in the medical examiner's office. At the very least, the toxicologist must know all the relevant circumstances, i.e. the driver having been seen to be driving erratically and thought to be drug affected by a central nervous system (CNS) depressant, or a possible victim of rape with no clear recollection of the incident. This provides the toxicology laboratory some useful preliminary information on the types of tests that need to be targeted. It goes without saying that all medication bottles at the scene of any suspicious death should be collected at the time of the initial investigation. When specific substances are suspected, these should be clearly stated or, if drugs are collected at a scene, these should be clearly listed and provided to the laboratory. Without this information, the laboratory will not be able to target its screening tests relevant to the case. This limitation becomes particularly important if suspected substances are not detected by routine screening tests.

A case conference with the laboratory helps both the investigators and the laboratory personnel to maximise the effectiveness of the testing process. A 'sit-down' conference may not always be possible in every case, but a telephone call may suffice to exchange information. At a minimum, the investigators should be speaking with each other. The following sections contain illustrations showing how the nature of the information provided determines the extent to which a proper interpretation of any results is obtained.

Toxicology investigations

To some degree the choice of toxicology test to be performed is determined by the client (chiefly the police, the forensic physician or the medical examiner) and unfortunately by the budget. This consideration is particularly true when it comes to expensive procedures such as hair and DNA testing (see below). Depending on the circumstances, testing may be requested for one or more specific substances. Preferably, a full screen for unknown substances should be ordered. There will be instances where only targeted testing is warranted, i.e. alcohol and some illicit drugs such as when drivers are suspected of driving under the influence or following an accident. However, it is the experience of the authors that multiple drug use is common and often the drugs finally detected were not the ones suspected. Consequently, whenever possible, the laboratory should be asked to conduct a reasonably comprehensive drug screen (or series of drug screens) for common drugs.

Each jurisdiction will have their own specific drugs of interest and it is important for the toxicologist to be acquainted with the drugs abused in their jurisdiction. Generally, an adequate screen will include alcohol (ethanol), a range of common illicit drugs (e.g. amfetamines, benzodiazepines, cocaine, opiates and cannabinoids) and a range of common prescription and over-the-counter (OTC) drugs (antidepressants, antipsychotic drugs, etc.). Depending on the circumstances, other drugs or poisons may also need to be targeted, i.e. volatile substances, such as gamma-hydroxybutyrate (GHB). It is impossible to perform a comprehensive toxicology investigation without first discussing with the relevant investigators the type of substances likely to be involved.

It is also vital that all information pertaining to the specimen itself is provided to the analyst. In the living, this would include the name of the person, date of birth, type of specimen type (serum, blood, etc.) and time and date of sampling. In postmortem cases, it is mandatory to specify the site of blood collection (see Chapter 10) and the conditions of storage. The following questions may also need to be answered:

1. Was the body refrigerated, or at room temperature, and for how many hours?
2. How was the body positioned? Was the body prone or supine? Had the body been moved?
3. Had putrefaction begun and did the specimens show any signs of decomposition? If it did, that might affect the concentration of substances detected.
4. Was there any trauma to the body? This may have affected the integrity of the specimens (postmortem alcohol formation is a particular problem).

As mentioned above, death investigators need to examine the scene in order to determine what medications were present (including OTC preparations and unregulated herbal supplements), record the relevant data and bring the medicine bottles back to the office for further investigation if warranted. If drug abuse is suspected, then neighbours and/or friends may be useful sources of information. Neighbouring rubbish bins should be searched and obvious surfaces within the dwelling chemically tested. In poor areas frequented by narcotic users, it is reasonable to assume that any remaining narcotics have been stolen before investigators arrive. In colleges and in the homes of the affluent, it is likely that attempts will be made at concealment, though they generally will not be very sophisticated.

The following data should be recorded about each medical prescription found at the scene or located nearby:

1. Details on the packaging including label, nature of substance, name of pharmacy, name of person to whom prescriptions were made out, date filled and number of tablets/volume of solution/syrup dispensed.
2. Number of tablets/volume of solution/syrup remaining unused.
3. If appropriate, each tablet/capsule can be identified by the laboratory, as they nearly all have unique markings. Chemical analyses can be conducted if identity or strength is unknown.

4. Subject to privacy and legal issues, medical practitioners may also be requested to provide details of prescriptions issued to their patient under investigation.
5. Again, subject to privacy and legal issues, a report of dispensed drugs can also be obtained from nominated pharmacists.
6. Residues in glasses or containers suspected of containing drugs/poisons, or powders located at the scene should be collected and sent to the laboratory for chemical analysis if deemed appropriate.

The final laboratory report should: (1) clearly show exactly what tests have been conducted; (2) indicate substances that can reasonably be excluded; (3) list the substances that were detected in the relevant specimen; and (4) if appropriate, list the results of an analysis performed on physical exhibits (tablets, powders, etc.). The end user of the report must be able to understand exactly which tests were conducted; testing limitations, if any (including the provision of an inadequate sample for whatever reason), must be stated; if postmortem blood samples have been tested, the origin of the samples should be stated as well (see below) and, if the origin of the sample was a particularly problematic site, such as cavity or heart blood, this problem must be indicated in the final report. The end user must also be told what other tests may be needed if more information comes to hand.

Interpretation: principal considerations

Other chapters explore the use of toxicology in various settings. In both clinical and forensic settings a range of factors can affect the concentration of a drug in a particular specimen. This, in turn, influences the final interpretation. The range of factors that are of most importance in interpreting a drug or poison testing result is shown in Table 26.1.

It may not always be possible to answer precisely all the questions raised in Table 26.1. However, the less that is known, the more likely it is that the conclusions reached are either mistaken or misleading. Each of the 10 factors in Table 26.1 is considered in turn below.

Inherent pharmacological activity and toxicity of a substance

As Paracelsus was reported to have said: 'All substances are poisons, there is none which is not a poison. The right dose differentiates a poison from a remedy.' This is still an apt proverb in modern toxicology.

Not all substances found in toxicology investigations are toxic, and their presence may not even be particularly relevant to a particular case. What is one to make of paracetamol (acetaminophen) detected in the body of a dead diamorphine user? Paracetamol is unlikely to be a factor in the death unless it can be demonstrated that liver necrosis is present. It would be a grave mistake to presume that just because a drug is present and anatomical lesions are absent the drug was responsible for death even if it were to be present in higher than normal concentrations.

Many widely used OTC preparations and herbal supplements produce a sort of 'background noise'. Their detection in postmortem specimens merely acts as an indicator of the prevalence of their use. A report describing the occurrence of a common disorder such as myocardial infarction or cardiac arrest, in a subject taking a common medication such as paracetamol, is much less cause for concern than an agent like digitalis. For example, survey data suggest that 20% of all US citizens, or nearly 60 million people, used paracetamol in 2004. The accepted rate for acute myocardial infarction in the USA is of the order of 0.52% per year. Even assuming that only one half of the paracetamol users were seriously at risk of infarction (i.e. over age 35 years), then more than 312 000 (0.52% of 30 million episodes of myocardial infarction) should occur in individuals taking paracetamol each year. The accepted rate for cardiac arrest resulting in sudden death in the USA is 0.1% per year, which means that another 30 000 paracetamol users should have died suddenly, if only by chance. The rates for stroke are much higher than for sudden cardiac death, with nearly 0.25% per year, or 75 000 episodes, bringing the total number of cardiovascular events occurring in US paracetamol users to well over half a million per year. Yet we know very well that disease, and not paracetamol, was the cause of death. Drawing any other conclusion commits the well-known legal fallacy, *post hoc ergo propter hoc* (literally, 'after this, therefore because of this').

Thus, the mere detection of paracetamol in a decedent's blood, particularly a decedent who has suffered an infarction, or other identifiable natural disease, does not prove that the drug played any role in that individual's death unless, of course, type 3 zonal necrosis liver injury was present.

In the same manner, the mere detection of non-steroidal inflammatory drugs does not prove that they have played a role in the death, unless some pathology relevant to the use of this substance is seen (i.e. gastric erosions). These situations can occur, but they are uncommon and are usually not particularly important from a forensic perspective. It is far more likely that other substances detected are more relevant, but each substance detected must be considered on its own merits. The inherent toxicity of a substance can also be gleaned from epidemiological studies. This type of information is generally obtained by dividing the number of deaths associated with a drug by the number of prescriptions or defined daily doses of that substance (King, Moffat 1983; Buckley, McManus 1998; Frey *et al.* 2002).

Table 26.1 Principal factors that require consideration in interpreting toxicological information

1.	Is the substance capable of exerting significant harmful or toxic effects? If so, how does it work (i.e. by what mechanisms does it exert its unwanted effect)?
2.	What is the most likely source of the drug and the route(s) of administration?
3.	Are the toxicology results obtained on postmortem specimens? If so, what artefacts need to be considered?
4.	Did death occur shortly after administration of substance or after a substantial delay?
5.	Has the substance been used as a single dose or did death follow multiple doses?
6.	What is the age of the person and does the person affected have significant kidney, liver or heart disease (including inapparent heart disease)?
7.	Does the person affected have significant injuries?
8.	Is there a possibility of a genetically related disorder of drug metabolism (pharmacogenomics)?
9.	Is there a possibility that tolerance to the substance(s) has developed?
10.	If other substances are present in the case, how do they interact with each other, or did they even interact at all?

Case example 1

A young woman was admitted to hospital with abdominal pains and possible liver failure. Markedly elevated transaminases were detected together with 300 mg/L of paracetamol in blood. The patient died 2 days later, despite having received appropriate treatment for a paracetamol overdose. Toxicological testing also confirmed the presence of sertraline and diazepam at concentrations of approximately 1 mg/L in blood taken on admission to hospital. Clearly, this patient died from paracetamol-induced necrosis. The other drugs were unlikely to have contributed to the development of liver disease and would not be considered as significant contributors to her death even if they are regarded as being somewhat elevated.

Sometimes the presence of a substance can be used to substantiate other historical information about the circumstance of death; for example, the presence of a drug used to treat hypertension suggests an underlying diagnosis of hypertensive cerebrovascular disease. In the same way the presence of an antiepileptic drug suggests, but does not prove, treatment for seizure-related disorders, as some antiepileptic medications are being used to treat other types of disorders (carbamazepine, valproate, etc.).

Case example 2

A woman was found dead with multiple stab wounds. The accused was detained shortly after the event and was examined medically as well as toxicologically for the drugs. Drug testing disclosed the presence of methamfetamine at a concentration of 1.1 mg/L, amfetamine at 0.2 mg/L, alprazolam at 0.6 mg/L and alcohol at 1.6 g/L, respectively. The presence of alprazolam and alcohol in significant concentrations can be associated with disinhibition, while use of methamfetamine (and its metabolite, amfetamine) is associated with aggression and violence. The accused was a long-term abuser of amfetamines and benzodiazepines and had become increasingly violent and unpredictable with episodes of paranoia. While the drug use and the behaviour associated with it are no legal defence, it does help explain how a simple argument led to a tragic outcome.

The difficulty here is not to become too enamoured with the idea that correlations can be established between blood concentrations in specific behavioural states. A driver weaving erratically in traffic, later found to have sedatives in his blood, was no doubt intoxicated at the time, but a dead body found to contain traces of cocaine metabolite is just a dead body containing a small amount of drug. There is no method by which inferences about actual behaviour could be drawn from just a blood or tissue concentration, except to indicate a range of likely behavioural changes associated with use of this drug.

This situation comes about for two reasons: tolerance to most abused drugs occurs quickly, making it impossible to correlate specific behaviours and specific drug levels. The other problem is that blood drug concentrations change after death, and there is no guarantee that the blood concentration measured at autopsy bears any predictable relationship to blood concentrations in the immediate antemortem period (see below). In such cases there is little that a pathologist can do except to note the presence of the drugs detected.

Behavioural changes associated with substances may also be relevant to the case. For example, a person taking amfetamine may be involved in a serious assault, and amfetamine will be detected in a forensic drug screen. Amfetamines, as with many other substances taken recreationally, produce unpredictable effects on behaviour. They are certainly associated with criminal behaviour but, among those with attention deficit disorder, they are associated with better impulse control (see Case example 2). The net effect of the drug on any individual, however relevant to the case, is still subject to the considerations and limitations listed in Table 26.1. The same considerations apply to drugs used to facilitate sexual assault ('date-rape' drugs). These drugs are given to render a person incapable of effectively resisting assault, and include many of the CNS depressants, as well as ketamine and some of the amfetamines, e.g. MDA and MDMA.

Source of drug and route of administration

In some cases, simple facts give a clear indication of the route of administration. The presence of residual drug in the stomach contents or gastrointestinal tract suggests oral ingestion. A needle mark in the arm may indicate intravenous injection, just as high concentrations of drug found in muscle tissue point to intramuscular injection. However, in the majority of cases it is not reasonably possible to determine with any certainty the route or routes of administration from toxicological data considered in isolation. Many drug takers use multiple routes. Polydrug abuse is an increasing problem. In the USA it is not uncommon to see crack cocaine smokers who also inject diamorphine. It is not uncommon to find both a crack pipe and a diamorphine syringe at a decedent's side.

In reality, toxicology testing alone is rarely able to provide answers about the route of ingestion. The route of administration is best determined from the circumstances of the case and by ensuring forensic examinations.

Substances can be far more toxic if given intravenously rather than orally. The rate at which a drug reaches its site of action is a critical factor governing the duration and the severity of the pharmacological response. Intravenously administered diamorphine, cocaine or amfetamine almost instantaneously reaches high concentrations in the brain and heart, capable of causing serious harm, as will the smoked form of these drugs, but the same dose taken orally may well be comparatively safe. For more information on the pharmacokinetics and metabolism of drugs see Chapter 24. A drug injected as a depot intramuscularly, or applied on a patch on the skin, usually provides a controlled delivery of drug to the circulation. However, when the contents of the patch are injected intravenously or, even worse, volatilised and smoked, they can be dangerous. As the black market for fentanyl patches has grown, so has the number of bizarre deaths reported after their use. Several deaths have been reported in users who have heated the patches and inhaled the vapours, and others have occurred in young women who have inserted the patches intravaginally. These patches will continue to release drug after death, but it is unclear how much will continue to be absorbed after death.

The collection of the gastric contents can provide a useful way to establish possible oral exposure (for death investigations) and even to establish intent, i.e. deliberate ingestion of a large amount of substance. However, when small amounts of substance are present in gastric contents (a fraction of a dosage form), the possibility of contamination from bile during the agonal phase of death must always be considered. Persons in a coma, or persons exposed to drugs that inhibit intestinal motility (opioids and anticholinergic substances), will show delayed transit times in the gastrointestinal system and ion trapping occurs. Drugs injected parenterally may appear in the gastric secretions, as may cocaine that has been insufflated. Once in the circulation, drugs that have been injected can still become trapped in the stomach.

The presence of needle marks in the arm may suggest intravenous drug use. However, attempts at resuscitation by paramedics can also lead to injection marks. These artefacts must be excluded if the route is to be determined accurately. Often the presence of 'resuscitation drugs' can give a clue to paramedical activities. These drugs include lidocaine, diazepam and phenytoin. Subjects undergoing emergency surgery may also have anaesthetics such as propofol, thiopental and ephedrine, not to mention dopamine and other pressor agents. The situation is becoming somewhat problematic as cocaine producers seek alterative cutting agents. The American Drug Enforcement Agency has reported the seizure of several different cocaine shipments that have been diluted with popular medical drugs (one of the most recent is diltiazem and another is levamisole). The explanation seems to be that if the substance is white, and can be stolen, it can serve as a diluent for illicit drugs.

In some cases the measurement of metabolites can assist in determining the route of administration. For example, when diazepam is taken orally, first-pass metabolism produces significant amounts of nordiazepam. This means that blood will contain both substances. Following intravenous administration of diazepam (intravenous abuse of benzodiazepines or after emergency medical administration), little or no nordiazepam (*N*-desmethyl diazepam) is detectable for at least a few hours after administration. This difference in the production of the *N*-desmethyl metabolite also occurs for other drugs that undergo this form of metabolism, or other forms of liver-mediated metabolism for drugs with a high liver extraction ratio (amitriptyline, dothiepin, dextropropoxyphene, codeine, etc.). However, each case needs to be considered on its merits since the rate of *N*-desmethylation may be relatively slow for some drugs and require days of treatment orally before significant amounts are found in blood (e.g. MDMA).

The route-dependent variability of drug disposition into tissues may also provide useful information relating to the route of administration. Because of the physiological processes involved, substantially smaller amounts of an intravenously administered drug are partitioned into the liver than when the same drug is given orally. If death resulted rapidly following a large overdose by intravenous injection, the liver-to-blood drug concentration ratio would therefore be lower than that observed had the drug been given by mouth. Thus, liver : blood ratios of 2.5 and 5.0 have been observed in pentobarbital fatalities involving intravenous and oral administration respectively, and ratios of 1.3 and 4.2 have been found for intravenous and oral fatalities involving morphine.

Anhydroecgonine is produced only when cocaine has been pyrolysed, which means, by definition, that the route of administration was smoking. Pyrolysis products have also been identified after the use of methamfetamine. Cocaethylene is produced only when alcohol and cocaine are consumed at the same time, while ethyl glucuronide and ethyl sulfate are produced after simple alcohol consumption. While of little direct evidential value (the rate-limiting step of the reaction forming cocaethylene is the alcohol concentration, which needs to be so high that it is sure to be detected), cocaethylene has a half-life many hours longer than that of cocaine, so that cocaethylene may persist for many hours after cocaine is long gone from the body.

Case example 3

A 46-year-old man had been found dead in his bed. He had a history of depression and had been on amitriptyline (150 mg daily) for many years. Toxicology showed the presence of amitriptyline in femoral blood at 1.6 mg/L, in liver at 35 mg/kg and in gastric contents at approximately 300 mg. These values indicated the presence of excessive, and potentially fatal, concentrations of amitriptyline in both blood and liver. Concentrations of this drug in blood and liver following therapeutic use usually range up to 0.8 and 10 mg/kg respectively. The presence of several tablets (4×75 mg) of unabsorbed drug in the stomach confirmed oral ingestion of a much higher than normal dose. No significant pathology was otherwise detected at autopsy.

It is also worth noting that oral dosage forms come in a variety of formulations, some of which have modified-release characteristics. For example, most solid oral formulations (e.g. tablets, capsules) release drug almost immediately on impact with the stomach. Sustained-release formulations will slowly release drug over many hours, leading to a slow but sustained absorption. Examples include verapamil, morphine and oxycodone. Enteric coated tablets have a protective coating that dissolves only at pH values higher than that found in the stomach. Accordingly, the contents are released in the small intestine and this avoids gastric irritation. Examples include aspirin and potassium chloride. As a result, the blood concentration versus time profile will be shallower than with immediate-release formulations. Toxicity will also be delayed in the event of an overdose.

Case example 4

A 35-year-old woman was admitted to the emergency department of a major public hospital suffering from a possible opioid overdose. She presented with pin-point pupils and shallow laboured breathing. She was given naloxone (an opioid antagonist), which rapidly reversed the CNS depressant effects. Four hours later she developed laboured breathing and lost consciousness. Further naloxone was given with almost immediate effects. Investigation established that she had taken sustained-release morphine, which continued to be absorbed well after a few hours, resulting in sustained blood concentrations over many hours. In this situation a short-acting opioid antagonist would need to be administered repeatedly until the offending opioid was finally cleared from the body.

Postmortem results

Alcohol

Specimens taken *post mortem* are problematic for many drugs and the situation with alcohol is probably the most difficult. Obvious decomposition or putrefaction will affect the quality of the specimens. When this occurs, any interpretation of quantitative results should be treated with much more caution than when specimens have been taken from a body not long after death. Indeed in most 'decomposed' cases it is best to report only qualitative or semi-quantitative data. The state of the specimen (or tissue) could mean that substances have significantly changed their concentrations, though some substances are surprisingly resistant to postmortem degradation, and some poisons are actually preserved by the embalming process.

In some instances the drug will be lost owing to inherent chemical or bacterial instability. Examples of drugs that are unstable *post mortem* are the benzodiazepines, drugs that contain a sulfur atom (dosulepin, thioridazine, etc.), drugs containing acetyl or ester functionalities (e.g. diamorphine, cocaine) and even drug conjugates (morphine glucuronides and salbutamol sulfate, etc.). The nitrobenzodiazepines (e.g. clonazepam, flunitrazepam) are almost completely converted to their corresponding 7-amino derivatives after death, requiring these forms to be targeted in postmortem screening rather than the parent drug (Roberton. Drummer 1995).

Alcohol can be produced *post mortem* by the action of certain bacteria. This process is also dependent on time and temperature; production is almost predictable under some circumstances. Aircraft crash investigators are well aware that separated body parts are extremely prone to bacterial colonisation and artefactual elevation of the blood alcohol concentration, though the extent of formation cannot be reliably estimated. It is believed that alcohol can be formed *post mortem* up to at least 2 g/L under the right circumstances, even in the collecting tube. It is therefore essential that blood is collected in tubes containing fluoride, which will act as a preservative. Experience suggests that, when dealing with alcohol or cocaine, containers with higher concentrations of sodium fluoride than normally used in the hospital (1% vs 2%) should be used. It is recommended whenever alcohol concentrations are important in a case that vitreous humour concentrations also be measured. If vitreous humour is unavailable, then urine analysis should be performed. And, if the option exists, ureteral or even calyceal urine should be used. This is much more likely to reflect the blood alcohol in the perimortem period than is the pooled urine found in the bladder. In cases of putrefactive formation of alcohol, the vitreous humour will contain little if any alcohol.

Urine measurement can provide useful information, but it too can be affected by prolonged postmortem intervals. Urinalysis is problematic in diabetics, because of the presence of glucose in their urine, which may lead to fermentation. Urine may not always be available (approximately one-third of the time the bladder is empty and perhaps even more often when death is due to trauma). Thus the credibility of results obtained with urine testing depends largely on the source of the urine. Urine from the bladder accumulates over a period of many hours and therefore represents an average concentration, not necessarily the concentration at the time of death. On the other hand, concentrations measured in urine collected from the ureters, or pelvis of the kidney, closely reflect concentrations measured in the vitreous humour.

When no other competing factor is present, both vitreous humour and urine will contain somewhat more alcohol than blood owing to their higher water content. Increases of about 15% and 30%, respectively, occur on average and it is important to raise this issue in court since this may affect the interpretation.

Blood alcohol concentrations may also be increased by diffusion into the bloodstream from neighbouring tissues, e.g. gastric or intestinal tissues, in the dead and in the living. Animal studies have shown that, in experimentally induced haemorrhagic shock followed by fluid resuscitation, alcohol will diffuse from the tissue back into the bloodstream. In jurisdictions where *per se* laws apply, the movement may be sufficient to qualify a patient as intoxicated, even though their blood concentration may have been much lower at the time of the accident.

After death, a person who had recently ingested a large amount of alcohol could have blood concentrations increased *post mortem* owing to diffusion. The collection of blood peripherally can avoid this phenomenon. Even more likely, the decedent will vomit and aspirate as he or she is dying. If the alcohol concentration in the vomitus is high, and the person aspirates on the left side, alcohol may diffuse through the bronchi into the left side of the heart, leading to spuriously high alcohol concentrations. Pathologists should avoid sampling the liver adjacent to the stomach so as to avoid diffusion from the stomach leading to falsely high concentrations in the liver.

Drugs

The postmortem redistribution of drugs is hardly confined to ethanol. Whenever the process has been systematically studied, it has been found that postmortem drug concentrations (obviously excluding drugs like cocaine that continue to break down after death) are consistently higher after death than they were in the immediate perimortem period (Pounder, Jones 1990; Drummer 2004).

In a study assessing postmortem redistribution of MDMA, antemortem and postmortem MDMA concentrations were measured and compared in five patients who died in hospital, having been admitted for MDMA toxicity. Admission plasma concentrations ranged from 0.55 mg/L to 4.33 mg/L for MDMA and 0 mg/L to 0.10 mg/L for MDA. At autopsy, postmortem MDMA concentrations ranged from 0.47 mg/L to 28 g/L, while concentrations for MDA ranged from 0.02 mg/L to 1.33 mg/L. The antemortem-to-postmortem ratios ranged from 0.02 to 1.33 (Elliott 2005). Similar findings have been observed with both abused and therapeutic drugs.

The diffusion of drug from a tissue containing a high concentration into blood at a lower concentration is relatively common since most drugs tend to have higher tissue concentrations than blood concentrations. This process is termed redistribution. Substances most subject to an elevation of blood concentrations after death are those with a high fat solubility. These drugs usually have volumes of distribution (V_d) greater than 2 L/kg. In general, the higher the volume of distribution, the more likely this change is to occur, but the degree of intra-individual variability is very high. For example, in a recent study of methamfetamine users confined to a metabolic ward, the V_d ranged from 2 L/kg to 11 L/kg (Schepers *et al.* 2003). There is, of course, no way to know what the correct V_d is in any individual without actually measuring it in that individual. Table 26.2 summarises the approximate extent of these changes for selected drugs. It must be emphasised that these increases are not predictable and will vary from case to case depending on a range of factors including time, temperature and other variables that cannot be estimated at all, or crudely estimated at best. If ambient room temperature and body core temperature are recorded at the scene, there is a good chance of estimating the time of death with reasonable accuracy. If the time of death is known with some certainty, inferences made about blood concentrations and redistribution have much more credibility.

Postmortem increases in drug concentration can be minimised by taking peripheral blood and by limiting the size of the blood sample. A femoral blood sample of greater than 25 mL is almost certain to contain hepatic blood. For many years the preferred drawing site has been the femoral vessels, but only if the superior portion of the vein or artery is tied off to avoid collection of abdominal blood. Newly published evidence suggests that ligation may not be mandatory since clamped femoral blood concentrations and blind-stick femoral blood gave concentrations similar across all drug classes with ratios around 1.0 (Hargrove, McCutcheon 2008). Even so, drugs with a high volume of distribution will have muscle concentrations higher than those in the blood, allowing diffusion of muscle-bound drug to the pooled blood. When deaths occur in hospital it is prudent, if not imperative, to obtain and analyse specimens taken at the time of admission, as these are more likely to be relevant than postmortem specimens and they will be free of postmortem artefacts.

Antemortem specimens are collected on behalf of the physicians for diagnostic purposes, and are likely to consist of plasma and not whole blood (which could have bearing depending on the distribution of the drug within the blood). They can be valuable for toxicologists and can provide better insight into the 'true' concentration of substances of interest than can be obtained *post mortem*. They do need to be requested soon after death to avoid routine disposal by hospitals.

Table 26.2 Examples of common drugs subject to substantial postmortem redistribution

Amfetamines
Chloroquine/hydroxychloroquine
Digoxin
Methadone
Phenothiazines (e.g. thioridazine, chlorpromazine)
Dextropropoxyphene
Propranolol
Pethidine
Tricyclic and tetracyclic antidepressants

Case example 5

A 63-year-old woman died some hours after admission to hospital for heart rhythm abnormalities. She had a significant history of heart disease with one previous episode of myocardial infarction. She had been on digoxin. Postmortem toxicology on femoral blood disclosed a concentration of 5.6 µg/L, a concentration that is potentially fatal. An antemortem blood specimen taken shortly after admission was found to contain digoxin at 2.5 µg/L, a concentration consistent with normal therapeutic use. In this case, elevation of the postmortem redistribution was the likely explanation given the drug's high volume of distribution. It would have been a mistake to rely on the postmortem blood concentration while at the same time ignoring heart lesions (fibrosis in the zone of infarction) that are known to be associated with rhythm abnormalities and sudden death.

Case example 6

A chronic schizophrenic, treated with 10 mg of fluphenazine twice a day, also suffered from multiple somatic disorders, including decubitus ulcers, poorly controlled diabetes and severe coronary artery disease. When he was found dead in a nursing home, the medical examiner duly noted all the medical conditions, as well as a reported postmortem blood fluphenazine concentration of 72 µg/L. Fluphenazine has an extremely high volume of distribution (of the order of 11 L/kg) which means that redistribution is all but guaranteed. The medical examiner ruled that the level of fluphenazine was a significantly elevated concentration relative to therapeutic concentration and would be contributory if not the actual cause of death. The argument was (quite properly) not accepted by the jury.

Time of exposure

The time elapsed from exposure to specimen collection is important because the concentrations of some drugs decrease substantially over time. For example, in cases of suspected drug-facilitated sexual assault, GHB can be detected at concentrations above endogenous concentrations for only a few hours. Absence of this substance some hours later may not necessarily be consistent with absence of exposure.

Moreover, a drug can cause a toxic response, e.g. death, and not be present in 'lethal' concentrations or even in any concentration at all. This situation comes about for two reasons: it may be that sufficient time has passed to allow most of the substance to be excreted. A person collapsing from the use of diamorphine can eventually become brain dead hours after the injection, at which point little or no morphine is detectable in blood or tissues. The leukoencephalopathy seen in diamorphine smokers is thought to be the result of a toxin that is rapidly absorbed by myelin. By the time neurological symptoms develop, no toxin is circulating in the bloodstream. Alternatively it may be that the drug is entirely absent. Chronic cocaine use leads to changes in heart structure that favour the occurrence of sudden death. These structural changes can cause death even in the absence of cocaine.

There is also the phenomenon of tolerance. This allows the ingestion of massive quantities of drug, leading to spectacular blood concentrations. It is not uncommon to see a living diamorphine maintenance patient with blood morphine concentrations many times higher than a drug-naive individual who died after a first diamorphine exposure. It cannot be overemphasised that the circumstances and the pathology determine the most likely cause of death, not the blood concentration of morphine. Of course, the converse can apply: when a death occurs moments after intravenous injection of diamorphine (provided that it was diamorphine injected and not fentanyl, a potent narcotic that is not detected on routine drug screens) but little or no morphine is found in

urine, that is an indication that the kidney has not had sufficient time to excrete morphine before death, though the blood concentration will often be relatively high.

Case example 7

A 28-year-old man was found lying dead on the floor in his apartment. The scene showed signs of intravenous drug use including some unused diamorphine in a foil. Autopsy disclosed highly congested lungs and viscera and extensive bronchopneumonia in his lungs. Substantial hypoxic damage was observed in the brain. Toxicology showed morphine (free) in femoral blood of 0.04 mg/L and total morphine of 0.2 mg/L, and morphine (total) in urine at 40 mg/L. Alcohol was also detected in urine (0.9 g/L) but not in blood. These data suggest a delayed death that allowed most of the morphine in blood to be eliminated. Oedema of the lungs, bronchopneumonia and ischaemic damage to the brain are consistent with this observation, as is the absence of alcohol in the blood. Ideally in drug-death investigations it is possible to correlate the pattern of toxicology findings with the pattern of anatomical changes. There are two general patterns of anoxic brain injury: the type that results in short-term global ischaemia (as in cardiac arrest survivors) and long-term partial ischaemia, a pattern seen in someone who remains in a comatose state for a prolonged time (or who experiences a problem during cardiopulmonary bypass). In this case, both the toxicological and the pathological findings are consistent and anatomically very distinct, with each helping to confirm the validity of the other. Taken in isolation, both findings are difficult to interpret.

As a rule, it is impossible to estimate with any precision time of ingestion by measuring metabolite ratios, for example the ratio of cocaine to benzoylecgonine, or that of free to total morphine. This approach will not work with cocaine, even in blood taken from the living, because cocaine has a much larger volume of distribution than benzoylecgonine. And, of course, after death cocaine will continue to break down, ruling out any possibility for comparison. The same holds true for morphine, where the metabolites, morphine-3-glucuronide and morphine-6-glucuronide, have a steady-state volume of distribution that is only a fraction of that of the parent compound, which means that most of the free morphine is in tissue and most of the metabolite in the blood.

Single or multiple doses

Many drugs will accumulate on repeated administration, leading to an increasing response until 'steady state' is achieved. Drugs accumulate when the terminal elimination half-life is similar to or greater than the dosing interval. As a simple rule, it takes about five half-lives for the drug to reach steady state, i.e. when the drug intake equals the metabolism and excretion of the drug. For example, GHB has a half-life of less than one hour. Unless repeat dosing occurs hourly (or more frequently), this drug shows no accumulation with repeated dosing. Similarly with cocaine and diamorphine, the half-lives are relatively short and with the exception of (invariably short-term) binge consumption it is quite difficult to cause an accumulation of drug with repeat dosing.

In contrast, many of the antidepressants possess half-lives in excess of one day and will accumulate with daily use. Amfetamines can have long half-lives and regular users can develop increasingly high blood concentrations over a week or two of use.

Blood concentrations following repeated doses will therefore be higher than after a single dose and could cause an unsuspecting toxicologist, who did not know the circumstance of use, to suggest that a large single dose had been administered. Tolerance to the adverse effects of the drug often develops with repeated dosing, further reducing the ability to accurately interpret the possible significance of a blood concentration. Methadone is a model drug to illustrate this concept and the mortality associated with accumulation of drug in non-tolerant persons is well known (Drummer *et al.* 1990; Caplehorn, Drummer 1999).

Case example 8

A 48-year-old woman had been receiving methadone for her chronic pain condition (spinal fusion following a car crash, with degenerative arthritic changes). She had been taking 80 mg of methadone daily for several months. She was found dead in her bed. Autopsy showed significant ischaemic heart disease, with a 90% occlusion of the left anterior descending artery and concentric left ventricular hypertrophy, evidence of either long-standing hypertension or past stimulant abuse. Toxicology testing showed a potentially fatal femoral methadone blood concentration of 1.2 mg/L. No drug was detected in her gastric contents. In this case, if abuse of the drug can be excluded through an examination of her medication and pharmacy records, the most likely cause of death is 'heart disease' and possibly death following a coronary thrombosis. The apparently high methadone concentration derives from accumulation of drug over time and postmortem increases and is accompanied by acquired tolerance.

In fact, it is virtually impossible to interpret an isolated postmortem drug measurement without knowing something about the circumstances of the death. As discussed above, there is enormous intra-individual variation of the V_d for many drugs. Using a reported average for a drug is not acceptable forensic practice. Nor is it acceptable to use the V_d formula to estimate dosage, unless, of course, all the circumstances are known. This formula was derived for use only with intravenous dosage in drug-naive individuals who have no other drug stored in any body compartments from previous doses (see below for more complete discussion). V_d is used in therapeutic drug monitoring situations where a clinical pharmacologist can determine a dose change on the basis of plasma concentration and the body weight of the person. This allows the plasma concentration of drug to be within the optimal range for pharmacotherapy provided that there are no compliance problems.

Age and natural disease

Age can affect the pharmacokinetics of drugs. The very young (under 3 years of age) can have underdeveloped drug-metabolising enzymes, e.g. cytochrome P450 enzymes, which can reduce the ability to clear drugs. For example, benzodiazepines have lower clearance in neonates and very young children because their clearance requires the action of oxidative enzymes, but these concerns do not apply to diamorphine or morphine, which are primarily metabolised by conjugation (Coffey *et al.* 1983; Barrett *et al.* 1996). Age-related sensitivity also applies to antiepileptic drugs (Perucca 2006). Conversely, an elderly person will usually have a lower capacity to metabolise drugs than the young owing to the declining function of organs (Burton *et al.* 2005). Clearance of drugs may drop by a factor of 2–3. Age is also associated with an increased volume of distribution (Noble 2003; Perucca 2006). For example, it is quite dangerous to treat the elderly with dextropropoxyphene on a chronic basis. This drug's principal metabolite is quite cardiotoxic and will accumulate if renal function is diminished.

Significant dysfunction of the major organs is often associated with reduced drug clearance. Liver disease can reduce the ability to metabolise and hence inactivate drugs (Morgan, McLean 1995). This occurs particularly for drugs subject to phase I metabolism (oxidation, *N*-desmethylation, hydroxylation, etc.) since this involves oxidative processes most severely altered by liver disease. Loss of kidney function reduces the ability of the body to remove drugs and drug metabolites from the bloodstream. Even if drugs are extensively metabolised, the accumulation of drug metabolites can cause the biological effects to continue, or even cause concentrations to rise, depending on their activity relative to the parent drug.

Heart disease leading to congestive heart failure reduces the perfusion rate of the liver and kidney and will often be associated with reduced drug clearance. Examples include cardiomyopathy, the presence of which will result in decreased liver and renal perfusion and drug accumulation (not to mention an increased propensity to cardiac arrhythmias), even in the absence of arrhythmia-inducing

Table 26.3 Effect of organ disease on reduced drug clearance

Organ	*Drug examples*
Liver	Paracetamol
	Most antidepressants
	Cannabis
Kidney	Angiotensin-converting enzyme inhibitors
	Morphine
Lung	Volatile substances
Heart	Most drugs

drugs. Table 26.3 lists some drugs for which the main effect of organ disease is to reduce their clearance rates.

Obesity will also affect drug clearance. This is mainly due to an increase in V_d because of the increase in body fat. Drugs with a higher V_d will tend to show the largest increase. For example, the highly lipophilic drugs can show increases by a factor of 3 (Cheymol 2000).

Injuries

It is patently obvious that the cause of death in a person with a life-threatening injury, e.g. gunshot wound to the head, is likely to be from that injury. What is not always recognised is that these persons may have drug concentrations that are also potentially fatal, e.g. the presence of morphine or cocaine. In the circumstance of someone being shot in the head and dying shortly after this injury, the injury was the immediate cause of death. Drug use is usually irrelevant to the cause of death owing to the temporal relationship of the injury and death. However, in other circumstances, when there are no competing causes of death, the same amount of drugs would be regarded as the cause of death. See, for example, Case example 9, in which drug use has been linked to causing death when injuries and natural disease have been excluded as relevant factors. In other cases it is quite possible to find the same amount of morphine (and methamfetamine) in a person with a fatal head wound in which the cause of death would be given as 'gunshot injury to head' or 'gunshot injury to head in a person using diamorphine'.

Case example 9

A 28-year-old man was found dead in a lane. He was a known user of diamorphine, methamfetamine and cocaine and had recently been released after 3 months in prison. A syringe containing traces of diamorphine was found under the body. He had numerous scars on the antecubital region of both arms. Pathology confirmed signs of intravenous drug abuse and signs of a fatty liver, but found no other significant pathology apart from pulmonary oedema. Comprehensive toxicology testing found morphine (free) in femoral blood at 0.3 mg/L and a trace amount of methamfetamine in the same blood specimen. The cause of death was given as 'toxic effects of diamorphine'.

This case illustrates the fact that without knowledge of the circumstances, including the pathology findings, e.g. gunshot wound to head, the role of the morphine (ex diamorphine) can be wrongly attributed. In Case example 9 the knowledge that the decedent had recently been released from prison adds credence to the diagnosis of drug death, since tolerance is lost during imprisonment owing to abstinence (usually persons in this situation become abstinent) from regular supplies of drugs.

The presence of serious injury will impact on drug clearance rates. For example, loss of large amounts of blood causes the body to go into shock. Shock together with blood loss also reduces blood pressure and perfusion of vital organs, reducing drug clearance rates. Attempts to back calculate drug concentrations are almost certain to be misleading. Short of the presence of other debilitating disease, there is no evidence that drug addiction as such makes the outcome of trauma significantly worse than the trauma itself, and several studies have demonstrated that the hearts and other organs of cocaine users are suitable for cardiac transplantation.

Pharmacogenomics

Pharmacokinetic differences resulting from polymorphism of enzymes can have potentially important clinical consequences. Genetic variation of drug-metabolising enzymes, particularly the cytochrome P450 (CYP) enzymes, is recognized as one of the major causes of differences in drug effects between persons (Wong *et al.* 2000).

CYP3A4, CYP2D6, CYP2C9 and CYP2C19 are the most important of the P450 enzymes, all of which are polymorphic. Polymorphism can lead to slow, extensive or even ultra-rapid drug metabolism. Drugs most likely to be subject to these polymorphisms are those subject to *N*- or *O*-dealkylation processes, e.g. codeine and methadone. Genotyping of enzymes is still uncommon in current practice but is likely to become accepted as a standard of care in the near future as drug metabolism polymorphism becomes increasingly important in explaining patient responses to drugs (Jannetto *et al.* 2004; Dorado *et al.* 2005). See Case examples 9 and 10.

However, the most pressing pharmacogenetic issues facing toxicologists and pathologists are mutations and polymorphisms that affect cardiac function. No matter how meticulous the autopsy, non-traumatic deaths go unexplained nearly 10% of the time. The percentage is higher in children and young adults. Advances in molecular biology and DNA technology now make it possible to explain some of those deaths, and emerging evidence suggests that the underlying cause of death in many of these cases is genetic. The problem is that death from a wide variety of genetic defects may leave no histological markers. The ability to identify these 'invisible diseases' with postmortem genetic testing has become a reality far more quickly than anyone had ever imagined. The introduction of these methods will have an enormous effect on the practice of forensic pathology and toxicology.

Long-term drug abuse produces anatomical and neurochemical changes that favour sudden cardiac death, particularly among stimulant abusers. Many of these changes are plainly visible in the heart: microfocal fibrosis, myocyte hypertrophy and hypertrophy of the media of the intramyocardial resistance vessels (Blechman *et al.* 2004; Karch 2005). However, many other changes are invisible, because they involve genetic defects. Cocaine, for example, binds to the hERG (the human ether-à-go-go-related gene) potassium channel and, therefore, has the potential of causing QT interval prolongation and, perhaps, a lethal cardiac arrhythmia called torsades de pointes (TdP). Whether or not this occurs depends on the genetic heterogeneity of the individual, because some changes in the structure of the hERG channel facilitate interaction with the cocaine molecule (Guo *et al.* 2006).

Closing of hERG potassium channels terminates the plateau phase of the cardiac action potential. Although there are many other candidate ion channels, blockade of hERG, which controls the rapid component of the delayed rectifier current, appears to be responsible for most, if not all, clinical cases of drug-induced TdP, no matter what the drug.

The reason why more people with hERG mutations do not die of TdP after taking drugs known to interact with the hERG channel is that hERG blockade is only one of many factors that must act together (the 'multiple hit' theory) to cause prolonged ventricular repolarisation. These other factors include low serum potassium concentrations, slow heart rate, other genetic factors, and the co-administration of other drugs that either block hERG channels or interfere with drug metabolism (Fitzgerald, Ackerman 2005). For example, it has been known for years that high intravenous doses of racemic methadone block the hERG channel and cause Q–T prolongation, but that normal oral dosing is seldom associated with this phenomenon because concentrations are not high enough to block the hERG channel. However, it has recently been demonstrated that, even when the drug is given orally, Q–T prolongation may occur in one special subset of patients: should the methadone user be a CYP2B6 slow metaboliser, they will not be able to break down the *S*-form and it will accumulate, reaching concentrations sufficient to interact with hERG and cause Q–T prolongation (Eap *et al.* 2007).

In other words, the circumstances must be just right, and the decedent very unlucky. The pathologist, on the other hand, needs a bit of luck to make the diagnosis. In practice, a pathologist may detect minor structural variants in cardiac structure, such as minimal perivascular

fibrosis, some interstitial fibrosis or perhaps medial hypertrophy of some small vessels, but no single obvious lesion or set of lesions that could account for death. In the not too distant future, the molecular biologists may be able to diagnose hERG mutations – indeed, mutations of all the other cardiac ion channels and proteins – and this ability is likely have a considerable effect on their final diagnosis.

Case example 10

A 9-year-old boy with multiple developmental disorders was admitted to hospital with seizures. He was under the care of a paediatrician, and was receiving methylphenidate, clonidine and fluoxetine. The seizures proved uncontrollable and the boy died after several days of treatment. Postmortem blood testing disclosed very high concentrations of fluoxetine, but no other apparent cause of death. State authorities immediately intervened, concerned that the parents were incompetent or had actually murdered the child. Several other children were removed from the household. Samples of the decedent's liver were tested using a commercial DNA array system. It was determined that the child had no functional CYP2D6 and could not form the main metabolite of fluoxetine, causing concentrations of that drug to reach toxic levels (fluoxetine is demethylated by CYP2D6).

Case example 11

A 6-month-pregnant, 41-year-old woman, diagnosed with heart murmur and rheumatoid arthritis, was treated with methadone and tricyclic antidepressants for her depression. She was found dead by her husband one morning. Toxicology analysis showed: methadone 0.7 mg/L, amitriptyline 1.5 mg/L and nortriptyline 2.2 mg/L. Pharmacogenomic testing showed CYP2D6*4 homozygously, which means that she was a ' poor metaboliser', unable to hydroxylate methadone, amitriptyline and nortriptyline, so that the active form of amitriptyline and unmetabolised methadone would have accumulated in her body. Death was attributed to an accidental mixed drug overdose.

Perhaps the most striking example yet was reported in 2008 (Case example 12).

Case example 12

The child of a breast-feeding mother being treated with codeine died unexpectedly 12 days after a normal birth. Morphine was detected in the infant's blood, and analysis of the mother's milk disclosed a morphine concentration of 70 µg/L. Genetic testing of the mother showed that she was heterozygous for a CYP2D6* allele with CYP2D6*2-2 gene duplication. In other words, she was an ultra-rapid metaboliser. Codeine is effective after *O*-demethylation to morphine. Under normal circumstances, only a small percentage of codeine is converted to morphine. In this case, nearly all of the codeine was converted and the child died (Koren *et al.* 2006).

Tolerance

The issue of drug tolerance has been raised before in this chapter. Tolerance is a phenomenon whereby a person adapts to the effects of a drug, often requiring higher doses to elicit the same effect as were experienced when use of the drug was first initiated. This process is also termed neuroadaptation. This adaptation is commonly seen with alcohol, diamorphine and cocaine, where doses used by long-term users are much higher than could be tolerated by naive individuals. The mechanisms behind tolerance can be complex but appear to be linked to a reduction in the number of receptors or to a reduced sensitivity of the receptors to a physiological response.

The average 'line' of cocaine contains roughly 50 mg of the drug, but regular abusers can consume many grams of the drug in one day because neuroadaptation has occurred over time. Similarly, diamorphine users can safely inject doses that would almost certainly kill an occasional user who has not yet developed tolerance. In the case of most drugs, tolerance constitutes the pharmacodynamic response of the body to the drug, but the pharmacokinetics of the drug is usually the same as for naive users. This means that tolerant users can survive blood concentrations that would be dangerous, if not fatal, in naive users. Morphine concentrations in otherwise healthy patients receiving the drug chronically for pain control are often higher than fatal concentrations in occasional users of diamorphine. This is because they have developed tolerance to the drug over a period of weeks to months. Consequently, without knowledge of the history of drug use it is not possible to determine whether a blood concentration is necessarily significant with respect to any adverse outcomes.

Tolerance is impossible to measure after death, although in some cases it may be estimated by measurement of hair drug concentrations. Drugs remain stable in hair (usually the parent compound) indefinitely. If a decedent is found with high blood morphine or cocaine levels, but no drug or very low levels of the drug in the hair, that would suggest that the individual was drug naive – certainly that was found to be the case in the Shipman murders where the absence of drug in their hair helped establish that the decedents were not drug users (Pounder 2003).

The disparity between hair and blood concentrations can be a useful finding both in forensic medicine and in postmortem pathology. In cases of drug-facilitated sexual assault, it will often be alleged that the victim was a regular drug user. The question can easily be resolved with simple hair testing.

However, if a person has been taking a drug over a long period of time and becomes tolerant, drug concentrations in the hair will also be high and it will not be possible to estimate the degree of this tolerance. It is possible to become dependent on a substance(s) but not develop significant tolerance. For example, occasional diamorphine users who have not injected daily for weeks at a time will not have developed a significant tolerance and therefore are at risk of an inadvertent overdose if they have used more drug than normal, perhaps owing to a higher street potency than usual.

Tolerance rapidly develops to the sedative effects of phenobarbital and carbamazepine. Indeed, it is a common feature of prolonged therapy with either drug. Epileptic patients treated with these drugs are often free from any adverse effects despite having blood drug concentrations normally associated with serious toxicity in patients not accustomed to taking the drug.

Tolerance invariably extends the upper limit of the therapeutic range of drugs, and there is a more marked overlap between concentrations associated with different clinical responses. When tolerance is suspected, some of the problems of interpreting data can best be resolved by reference to previous results from the same patient (e.g. results of a therapeutic drug monitoring programme). Unfortunately, in most forensic cases such background information is not available and in these instances blood concentrations alone are of little value. A more reliable interpretation of analytical data can be made only by comparison of blood concentrations with those measured in urine, or in liver (where concentrations can be much higher in addicts), and/or by measuring the relative amounts of unchanged drug and its metabolite(s). Documentation of the known history of drug use, prescribed or recreational, can assist in determining the sensitivity of a person to a particular drug.

Whatever tolerance an individual has acquired can change if health is affected. For example, it is likely that persons suffering from an illness as common as influenza can become more sensitive to some drugs and are therefore at risk of an adverse outcome even if the dose has not changed. Methadone metabolism is a perfect example of this phenomenon. Methadone circulates more than 90% bound to plasma protein. Many diamorphine abusers are actually dual substance abusers, and that other substance is often alcohol, a combination that leads to the production of diamorphine addicts with liver disease. As the liver fails, plasma proteins decrease, resulting in an increase in plasma methadone, and possibly even death (see Case example 13).

Case example 13

A 33-year-old woman had been placed on a methadone maintenance programme for her addiction to diamorphine. The medical practitioner assessed her starting dose to be 40 mg on the basis of her stated usage of diamorphine. The woman was found dead on the morning following her third dose. Pathology found no significant disease or injuries; some degree of pulmonary congestion was noted. Toxicology found methadone in femoral blood at 0.4 mg/L and some alprazolam (0.1 mg/L). The cause of death was determined to be mixed drug toxicity due principally to the toxic effects of methadone. If a new patient begins a methadone programme at a dose higher than 30 mg, they will be put at a higher risk of sudden death, which typically occurs while sleeping at night. This usually occurs within 2–7 days of initiation. The exclusion of other relevant causes of death is necessary before this conclusion is reached. Pulmonary oedema is common in narcotic deaths but is by no means a unique finding suggestive of methadone toxicity. The blood concentration of methadone is no help in determining a likely outcome due to redistribution *post mortem*. The alprazolam concentration would be consistent with normal therapeutic use. Access to this drug was confirmed by the treating practitioner.

Drug interactions

Polydrug use is the rule in adult clinical and forensic cases. Common associations include alcohol plus an illicit drug, two or more illicit drugs, or a benzodiazepine (or another sedative) in combination with alcohol and/or an illicit drug. When this occurs it is not surprising to find that the combination of drugs has led to greater behavioural changes or a higher risk of death than if only one psychoactive substance is taken. Impaired drivers are often found to have been using two or more psychoactive drugs.

This synergistic effect is suggested by the lower fatal dose of diamorphine in persons with alcohol concentrations at or higher than 1.0 g/L (Ruttenber *et al.* 1990), while drivers consuming alcohol are at a higher risk of a crash when also using cannabis (Drummer *et al.* 2004), although generalisation to all drugs is probably not warranted. In two-thirds of accidental cocaine-related deaths no ethanol was detected (Karch *et al.* 1999; Karch 2005).

When alcohol was present along with cocaine, no differences between control groups not drinking alcohol were identified. The findings suggest that acute cocaine toxicity is not enhanced by alcohol–cocaine interactions. However, alcohol concentrations were generally low and it is possible that increased toxicity is apparent when much larger quantities of alcohol have been consumed. The same caveat very likely applies for other drugs of abuse.

In most situations, the net effect of drug combinations is the sum of the psychoactive effects, which usually means some form of CNS depression. Occasionally the combination leads to a greater than expected effect, often termed an adverse reaction. This could be the result of a drug inhibiting an enzyme required for the metabolism of another drug, leading to a build-up of tissue concentrations, or some other pharmacokinetic or even pharmacodynamic interaction.

It is well known that the combination of a monoamine oxidase A inhibitor (e.g. moclobemide) with another drug affecting serotonin reuptake (e.g. sertraline) can lead to a potentially fatal serotonin syndrome. Inhibitors of cytochrome P450 enzymes commonly prolong the action of other drugs. Examples include many of the serotonin reuptake inhibitors and many of the antifungal agents (e.g. ketoconazole) and antiretrovirals (e.g. aciclovir). A recently reported case illustrating this issue is described in Case example 14. See also Case example 11.

Case example 14

A 42-year-old man had a history of depression and was being treated with moclobemide. He had becoming increasingly unwell on the day prior to his death: slurring his words, talking slowly, showing signs of muscular rigidity, breathing heavily and shaking, and was very tired. He went to bed but never woke up. Autopsy showed no significant natural disease. Toxicology showed moclobemide in femoral blood at 16 mg/L, citalopram in femoral blood at 0.5 mg/L and some traces of benzodiazepines. He had some days prior gone to another doctor who had prescribed the antidepressant citalopram. These symptoms and circumstances are a classic case of serotonin syndrome provoked by the combination of a monoamine oxidase inhibitor (moclobemide) and serotonin reuptake inhibitor (citalopram). Prescribing recommendations are to cease moclobemide for 2 weeks prior to starting on a serotonin reuptake inhibitor to avoid any adverse reaction.

What are therapeutic, toxic and fatal concentrations and do the terms have any application in the dead?

In this context the term 'therapeutic' is used to describe the concentrations normally expected following the recommended doses of the substance. Clearly, the term has no application for non-therapeutic substances, i.e. illicit drugs and poisons such as organophosphates. Concentrations are described as toxic when the dose of substance causes or has the potential to cause serious adverse reactions. A fatal concentration relates to those concentrations that are associated with fatal poisonings.

Large collections of data are available in various texts and in databases concerning the reported therapeutic, toxic and fatal concentrations of drugs and poisons. These can be an aid to establishing a likely response to a drug when interpreting a toxicological result. Wherever possible, appropriate data have been included in the monographs.

Unfortunately, the use of such data is subject to many restrictions and limitations. These are detailed below. It goes without saying that values derived from therapeutic drug monitoring do not necessarily apply in the postmortem setting, especially since there is no guarantee that the postmortem measurement represents the actual drug concentration at the time of death.

It is essential that, when a specific drug is suspected, the investigator consider the possible involvement of other drugs. Databases may not indicate whether a poisoning was due to that agent alone or in combination with other substances. Common examples here include the presence of alcohol in cases involving other CNS-depressant drugs such as opioids or benzodiazepines. There has, for example, been a long debate as to whether buprenorphine toxicity is enhanced in the presence of benzodiazepines (Drummer 2005). The use of cocaine or amfetamine in combination with diamorphine is alleged to be more toxic than one drug alone, though controlled data are hard to come by, partly because the simultaneous abuse of both drugs is so common as to make it impossible to gather adequate controls.

When proprietary preparations are given by the recommended route, it may occasionally be possible to extrapolate, or estimate, the dose (or range of doses) from blood concentrations because comparable data are usually available. However, when illicit drugs or preparations are involved, prediction of blood concentration is much more difficult.

Particular examples of variable and unpredicted doses include use of volatile substances through inhalation (abuse), and the smoking of cannabis, diamorphine or cocaine. In all of these cases the degree of inhalation together with the technique used greatly affects the amount of substance actually absorbed. Special devices have been devised for controlled laboratory studies; such devices may ensure the consistency of dose delivered, but their relevance to real abusers, on the street, is hard to assess.

There is an enormous amount of variability in absorption after oral dosing and, therefore, enormous variability in resultant blood concentrations. Most of the variability is related to first-pass metabolism (see earlier), a common finding in drugs with low oral bioavailability. A number of factors can influence bioavailability. These include the motility of the stomach and bowel, the pH and, for a small number of drugs, the activity of gut enzymes that metabolise the drug before it is even absorbed. Liver P450 polymorphisms are, or course, an important issue with some drugs, but even liver disease may be a factor. See Case example 15.

Case example 15

A man with a history of dual substance abuse (alcohol and diamorphine) was being treated for hepatitis B and C. He had been on a stable dose of methadone, 60 mg per day, for 23 months, did not smoke and was active in a 12-step programme for his alcoholism. Owing to an administrative error, his prescription for his hepatitis medications was not renewed. He was found dead in bed 3 days later. Autopsy disclosed moderately advanced cirrhosis of the liver, but no haemorrhage or other obvious cause of death and his methadone concentration (the only drug detected) was consistent with his dose. Blood tests drawn in his doctor's office 3 days earlier showed very low levels of serum protein. The cause of death was ruled to be natural. Methadone is highly bound to A-1 glycoprotein. A precipitous drop in glycoprotein production, secondary to his liver disease, led to an abrupt rise in free methadone, causing respiratory arrest.

Several drugs, including salicylate (in overdose), alcohol, and possibly some hydrazines and other drugs that are metabolised by acetylation have saturable elimination kinetics. With these drugs, capacity-limited elimination is complicated further by their low therapeutic index. A good example is phenytoin. A 50% increase in the dose of phenytoin can result in a 600% increase in the steady-state blood concentration and thus expose the patient to potential toxicity.

The combined effect of all of these factors is to make the task of interpreting analytical results even more difficult. Pharmacokinetic and toxicological data, such as those given in the monograph section of this book, must be used circumspectly. When a specific case is being examined there is always the possibility of misinterpretation if consideration is not given to the special circumstances of the case and the other factors discussed above.

Use of pharmacokinetics to predict time and dose

Toxicologists are often asked to use pharmacokinetic data to estimate the time since last administration and dose, but this question can be extremely difficult to answer. The reasons derive from information discussed in previous sections: the large, if not enormous, inter-individual variability, possible effects of disease states and injuries, possible effects of other drugs, frequency of use, and assumptions made about the route of administration.

Estimating the time after administration

In any situation involving the production of a legal opinion or report it is important to establish the known relevant facts. To estimate the time of ingestion it is necessary to determine the likely route of ingestion, and that can be done only by investigating the circumstances of the case, combined with any clinical or pathological data, knowledge about the physiological state of the person, and their relevant personal characteristics (weight, age and sex). Even then, under most circumstances, all that can be determined with any degree of accuracy are the likely minimum and maximum time boundaries.

One of the most useful tests in the assessment of fatal poisonings is the measurement of gastric contents and, if possible, bowel contents. The test is easily performed but must include the whole contents in order to be useful, and must show a mass amount, e.g. milligrams of drug found in the contents. The presence of substantial drug relevant to the dosage form probably indicates oral consumption and, if present in the gastric contents, relatively recent ingestion, i.e. within a few hours.

Care is needed not to over-interpret these data, since coma and certain other physiological states can lead to reduced gut motility, substantially delaying gastric emptying time and prolonging drug absorption time from the bowel. Furthermore, most drugs are excreted into bile and may be present in measurable amounts in gastric and bowel contents even after intravenous injection. For example, morphine biliary concentrations are some 20- to 100-fold higher than blood concentrations. As a consequence, milligram amounts of morphine and its conjugates may be excreted into the bowel.

Another way to assess recency of drug use is by measuring the amount of drug in the urine. This is a very useful test in cases of diamorphine overdose when death has occurred soon after injection. In such cases morphine will be present in the blood, but little or no morphine will be present in urine (<1 mg/L of total morphine). The absence of morphine in the urine indicates that death occurred within several minutes of injection, before renal excretion of morphine could occur. It is important to realise that the absence of drug in urine also indicates that there was no use of this drug in the day or two prior to the most recent dose. However, if the drug was used a day or two before death, it will be present in urine, even if death did occur very rapidly after the last dose. Despite these limitations, this test can be useful in a proportion of diamorphine deaths.

When a drug is rapidly metabolised, the relationship between time of ingestion and drug or metabolite concentrations can help to indicate whether the drug was taken recently or in the more distant past. Diamorphine is rapidly metabolised to morphine via 6-acetylmorphine, followed by a second deacetylation to yield morphine. The first step occurs in less than 5 minutes after injection, the second within 20 minutes or so. However, the half-life of 6-acetylmorphine is quite short. If survival is prolonged following intravenous injection, neither diamorphine nor 6-acetylmorphine will be detected in postmortem tissues. Thus, even if only traces of 6-acetylmorphine are detected in a postmortem sample of blood, it can only be because intravenous diamorphine was administered in the very recent past, or, alternatively, a massive dose was administered. No matter how rapidly death occurs, diamorphine itself is rarely detected because of the hydrolytic action of the plasma esterases.

When metabolism is extensive, and when drug and/or metabolite concentrations in the blood are very low, it can be profitable to determine the urinary concentrations of excretion products. For example, diazepam is metabolised either by *N*-demethylation to desmethyldiazepam (nordiazepam or nordazepam), which then undergoes 3-hydroxylation to yield oxazepam, or by hydroxylation to temazepam (*N*-methyloxazepam), which is then demethylated to oxazepam. All the urinary metabolites appear as their glucuronides.

After administration of a single oral dose of diazepam, the concentration of desmethyldiazepam in the urine reaches a maximum 6–12 h after ingestion, the temazepam concentration reaches its maximum after 12–24 h, and the oxazepam concentration reaches a maximum after 1–3 days. From the second day, oxazepam is the major urinary metabolite. Up to 24 h after ingestion, the oxazepam : desmethyldiazepam concentration ratio is 1 or less, with a corresponding oxazepam : temazepam ratio of less than 0.7. Beyond 24 h, the ratios cover a wider range of times, and estimates of the time since ingestion can be made only by comparing metabolite : metabolite ratios with absolute metabolite concentrations.

The blood concentration of free morphine also correlates well with the survival time, at least for the first 24 h. Average blood concentrations of morphine observed in cases where death from circulatory shock and respiratory arrest occur less than 3 h after administration are 3–10 times greater than those seen after deaths following prolonged coma (3–24 h).

Cannabis provides a similar example. The detection of Δ^9-tetrahydrocannabinol (THC) in blood ($>2\,\mu$g/L) indicates very recent use of the drug (within 8 h). Further, the concentrations of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid and its glucuronide increase with time, the ratio of the acid to THC increasing to over 50 after about 3 h. This metabolite can be present in blood for several days, whereas the pharmacological effects persist only for a few hours. If acute use of cannabis is known, then pharmacokinetic modelling can be used to estimate time of ingestion (Huestis *et al.* 1992), but only provided that a very short period of time has elapsed since death. THC has the largest volume of distribution of all abused drugs, which means that large amounts accumulate in tissue. As decomposition occurs, THC metabolite, and perhaps even cannabis itself, will inevitably be released from the decomposing tissue. How much will actually reach the blood depends on the sampling site and the method by which the sample

was obtained. The difficulty here is the lack of controlled experiments. The mere detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid does not permit legitimate pharmacokinetic modelling. The question to be resolved is when such calculations are legitimate, particularly in a postmortem setting.

The ratios of drug : metabolite concentrations or the ratio of one metabolite to another can provide a very rough estimate of the time since ingestion. These calculations are relatively meaningless if a drug is very rapidly metabolised (as is the case with diamorphine, cocaine and cannabis), because only very low blood concentrations of metabolites are present a short time after a single dose. Consequently, a relatively high drug : metabolite ratio can be expected in cases of very rapid death following acute overdose. Conversely, the presence of significant amounts of metabolite indicates that sufficient time has existed for metabolism to occur.

When dealing with postmortem tissue it is important to realise that the ratio of parent compound to metabolite is meaningless if the parent and metabolite have very different volumes of distribution. Cocaine is generally thought to have a V_d of 2–3, but the V_d for benzoylecgonine, when measured in human volunteers, is closer to 1. Thus the blood cocaine concentration cannot be divided by the blood benzoylecgonine level to yield a meaningful estimate about the amount of drug taken or the timing of ingestion. The same situation applies to morphine, which has a V_d more than 10 times higher than its metabolites M3G and M6G. There are known exceptions to this rule.

In fatalities due to misuse of dextropropoxyphene, the postmortem blood concentrations of dextropropoxyphene are generally high compared with those of the major metabolite, norpropoxyphene, the dextropropoxyphene : norpropoxyphene ratio being greater than 5. This indicates that death is relatively rapid (less than 1 h). As survival time is increased, the drug : metabolite concentration ratio decreases. Thus, when an individual survives the initial phase of acute intoxication associated with dextropropoxyphene, the drug : metabolite ratio falls dramatically to less than 0.5 within a few hours of ingestion. Parent drug and measurable metabolites can be useful for many of the antidepressants and benzodiazepines, particularly those undergoing *N*-demethylation, i.e. diazepam and nordiazepam, amitriptyline and nortriptyline.

As well as relating drug and metabolite concentrations in the same biological sample, the concentration of the drug in one tissue can be related to the concentration in another. For example, if a fatality occurs shortly after the oral ingestion of a drug, then the liver : blood concentration ratio is higher than if death had occurred after a more prolonged period. In fact, liver concentrations can be more reliable measures of toxicity than blood concentrations for compounds subject to significant postmortem redistribution – tricyclic antidepressants, dextropropoxyphene, phenothiazines, etc.

Toxicologists are frequently called upon to back extrapolate the amount of drug ingested on the basis of concentrations measured in blood some hours later. This can be done in living persons or in deceased cases, but only with the caveat that any such measurements be made shortly after death. If they are not, postmortem redistribution may well have occurred so that the concentration measured bears little or no relationship to the concentration at the time of death. The time of ingestion can be calculated if a reasonable estimate of half-life is available from the equation:

$$C_x = C_s e^{0.692\Delta T/\text{half-life}} \quad (26.1)$$

where C_x is the concentration required at time ΔT before the measured concentration C_t.

This equation works only if the blood concentration versus time curve is in the elimination part of the pharmacokinetic curve relevant to the half-life of first-order elimination, and of course if the half-life has not been affected by disease, injuries or saturable metabolism. It is advised that a range of likely half-life data be used to indicate a likely range of blood concentrations. This will give a much more realistic estimate than a point calculation (see Table 26.4 for example calculations).

For alcohol, Equation (26.1) does not apply since ethanol elimination is for the most part zero-order, or more accurately obeys Michaelis–Menten kinetics. Michaelis–Menten kinetics is defined by the equation:

$$C_t = K_m R/(V_{max} - R) \quad (26.2)$$

where K_m is a constant equal to the plasma concentration at which the rate is one-half of the maximum, R is the rate of metabolism and V_{max} is the maximum rate of metabolism. In practice, the rate of elimination over most of the blood ethanol concentration (BAC) range can be regarded as 0.15 g/L per h, with a range of 0.10–0.25 g/L per h. Back calculation can be done on the basis of a linear model using the point estimate and the likely extremes.

For example, a BAC of 0.12 g/100 mL (%) is likely to be $4 \times 0.015\%$ higher 4 hours earlier, with a range of 4×0.010 and 4×0.025. This calculates at 0.16% (range 0.14–0.22%). If absorption of alcohol is likely to be still occurring, then up to 1 hour can be subtracted from the estimated elapsed time. Alcoholics will be able to eliminate alcohol faster than 0.015%/hour and in some persons a very high BAC microsomal metabolism of alcohol may also occur. However, this is rarely beyond 0.025%/hour.

As a rule of thumb, one measure of alcohol raises BAC by about 0.015–0.02% in an average person. Hence BAC = number of drinks $\times$ 0.02%. For each hour after the first hour (absorption delay) about 0.015% is eliminated. For more accurate estimates use of the formula is recommended, particularly when more than a few hours is involved and the body weight is well below or above average.

For more details readers are referred to Chapter 4.

Estimating the dose

In some cases the estimation of dose may be helpful to confirm other pieces of evidence or to indicate the possibility of an accidental or suicidal death. As indicated previously, many assumptions need to be made if any realistic calculation is to be performed. Of particular importance is the knowledge of whether a single dose has occurred or multiple doses, and the overall health of the person and time elapsed since last dose. When postmortem specimens have been analysed, redistribution and other artefacts will seriously limit the ability to conduct any dose calculation.

Measurement of a blood concentration may not always allow the differentiation of multiple therapeutic doses from large accidental or suicidal doses. Thus, long half-life drugs such as methadone and thioridazine may show a marked overlap in blood concentrations following multiple doses with those seen in fatal poisonings.

In the same way that drug and metabolite concentrations can be linked to time, they can also be related to dose regimens. Thus, steady-state drug : metabolite concentration ratios are sometimes used to check drug compliance. Also, since the extent of drug metabolism tends to decrease with increasing dose, the ratio of unchanged drug to metabolite will increase with increasing dose. Examination of the relative concentrations of parent drug and its major metabolite(s) in blood,

Table 26.4 Estimation of blood concentration at an earlier time

C_1/C_t[a]	*Half-life (hours)*	*Time difference,* ΔT *(h)*
2.0	2	2
4.0	2	4
1.4	4	2
2.0	4	4
1.2	8	2
1.4	8	4
2.0	8	8

[a]Ratio of concentrations from calculated time to measured time point, i.e. if ratio is 2, then concentration at earlier time is double.

or in other tissues as necessary, can provide useful information about the likely size of the dose given. Thus, an amitriptyline : nortriptyline concentration ratio of less than 2 is consistent with steady-state drug concentrations following administration of therapeutic doses, while a ratio greater than 2 is more consistent with the ingestion of larger, potentially toxic doses.

When a drug is extensively metabolised, large acute doses can result in metabolic profiles significantly different from those seen after therapeutic doses. Thus, following administration of normal single doses of phenylbutazone, the ratio of the blood concentrations of its major metabolites oxyphenbutazone and 3′-hydroxyphenylbutazone may be as high as 10 : 1. In overdose, the pattern of metabolism can be reversed, giving ratios as low as 1 : 5.

Similarly, the metabolic profile of diazepam in urine changes dramatically with dose and the ratio of nordiazepam to oxazepam concentrations can provide useful information regarding the relative size of an ingested dose of the drug. When doses are low, demethylation of diazepam appears to be more important than hydroxylation, while hydroxylation becomes more important at higher doses.

Once interpretation of tissue drug concentrations or drug : metabolite concentration ratios has established that an overdose was or was not administered, the actual amount of drug ingested may be estimated. Ideally, the dose should be determined by measuring the total amount of drug remaining in the body (including any unabsorbed drug in the gastrointestinal tract) and adding to this the amount that has been metabolised and/or excreted. Obviously this is rarely possible. A compromise is usually made by estimating the minimum amount of drug ingested. This can be attempted in a number of ways.

Analytical results may be compared with previously recorded data in fatal cases for which drug doses are known. The next best method is the direct comparison of peripheral blood concentrations with clinical data, i.e. blood concentrations following the administration of therapeutic doses. Finally, in extreme cases drug doses may be estimated using pharmacokinetic data.

The half-life of the drug ($t_{1/2}$) and a reasonable estimate of the time elapsed between administrations and sampling (t), together with the blood concentration (C), allow the calculation of a theoretical drug concentration at time zero (C_0) which, for intravenous administration is

$$\ln C_o = \ln C_t + 0.693t/t_{1/2} \qquad (26.3)$$

This concentration can be used to estimate the dose if the volume of distribution of the drug is known (see earlier), or it may be compared with clinical data as described above.

This pharmacokinetic approach probably gives a better estimate of the actual dose administered since it takes some account of the amount of drug eliminated. However, the use of pharmacokinetic equations should be interpreted with great caution, especially if relatively accurate survival times are not available and if the kinetic characteristics of the drug following administration of large acute doses are significantly different from those observed following therapeutic doses. In reality, elimination rates of drugs following overdose are invariably slower than with normal doses due to saturation of normal metabolic and excretory mechanisms, or even drug-induced reduction in physiological state. If these formulae are applied it is advised to use a range of likely pharmacokinetic parameters to estimate a possible range of doses, rather than relying on a point estimate.

Some of these problems can be overcome in a clinical situation if sufficient samples are available to characterise the terminal elimination kinetics of a drug taken in overdose. However, unless all of the assumptions are clearly detailed and a range of possibilities are considered, estimating a dose in a postmortem situation can be misleading and is not advised.

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CHAPTER

27 Paediatric Toxicology

D Reith

Introduction

Children differ significantly from adults in their risk of poisoning, response to poisoning and circumstances of poisoning. That children are not just 'small adults' is demonstrably not just a cliché. Children form a large proportion of the population presenting with poisoning symptoms and about whom poisons information/advice is sought. Despite the large number of exposures, the mortality and morbidity of childhood poisoning are low and it is important that interventions for childhood poisoning do not confer greater risks than the exposure. There are some noteworthy issues in childhood poisoning, such as intentional poisoning by a third party and suicidality in adolescents. In addition, there are differences in the disposition of drugs and toxins in children compared with adults and also in the response to poisoning, hence the need to address children as a subpopulation with this chapter.

There are a number of stages in childhood and the clinical priorities differ depending upon the stage. These stages are represented by pre-term neonates, term neonates, infants, young children and adolescents. They are recognised by the International Conference for Harmonisation (ICH) in their recommendations for drug development in children in the following age categories: term newborn infants (0–27 days), infants/toddlers (28 days to 23 months), children (2–11 years) and adolescents (12–16/18 years) (ICH 2000). In clinical practice, pre-term infants form an additional grouping. In pre-term neonates, there are specific issues relating to dermal exposures (due to a thin stratum corneum) and to preservatives in medicines and infusion solutions. In term neonates, the primary issues are the toxicity of therapeutic medicines (e.g. aminoglycosides). In infants/toddlers the issues are exploratory ingestions of household medicines and chemicals. In children, the issues are intentional poisoning by a third party, aberrant behaviour and exploratory ingestions. In adolescents the issues relate to self-harm and substance abuse. Some specific examples of how paediatric toxicology differs from adult toxicology are given below.

Neonatal dermal toxicity

A 2-week-old male pre-term neonate developed skin pustules that appeared to be staphylococcal. In addition to oral antibiotics, he was treated with daily baths using an antibacterial skin preparation as soap. At the end of a 1-week period he was noted to have increasing episodes of apnoea. Hospital staff questioned whether the infant might have developed toxicity from dermal absorption of the antibacterial compound.

In the 1960s and early 1970s hexachlorophene was used routinely as a total body bath in newborn nurseries in order to prevent staphylococcal sepsis. Examination of postmortem specimens from patients under the age of 5 years found vacuolar encephalopathy (distinct vacuoles in the reticular system, predominantly involving the medulla in children who had been born prematurely and the mesencephalic reticular formation in children) (Shuman *et al.* 1974). These findings were similar to those in experimental animals exposed to toxic amounts of hexachlorophene. The vacuolar encephalopathy was related to the number of whole-body baths with hexachlorophene and to prematurity. It was estimated that two or more total-body baths would be required for a premature neonate to develop toxicity. Newborn infants bathed daily with 3% hexachlorophene had blood levels at discharge of 0.009–0.646 mg/L, while blood levels of 0.985–1.48 mg/L were associated with brain injury in rats (Lockhart, Simmons 1973). Seizures were also described in burn patients who had had extensive washing with hexachlorophene.

Infant: poisoning from breast milk

Cocaine

In temporal relation to maternal intranasal cocaine use, a breast-fed 2-week-old female infant developed irritability, vomiting, diarrhoea and pupillary dilatation. On examination the infant was noted to be tremulous, irritable, frequently startling, with a resting heart rate of 130–160 beats/min and a systolic blood pressure of 96 mmHg. Respiratory rate was 36 breaths/min and rectal temperature was 37.4°C. At discharge, the infant's heart rate was 128 beats/min, respiratory rate was 36 breaths/min and systolic blood pressure was 80 mmHg. Cocaine and benzoylecgonine were detected in breast milk and in the infant's urine.

Cocaine has been detected in breast milk at concentrations greater than 12 mg/L. A breast-fed neonate's fluid requirement is in the range 120–180 mL/kg per day. This would result in a daily oral dose to the neonate of approximately 1.8 mg/kg per day. In adults, the estimated minimum lethal dose is 1.2 g, but susceptible persons have died from doses as small as 30 mg when applied to mucous membranes (see the monograph on Cocaine). Hence significant exposure to cocaine can occur through breast milk. Cocaine is metabolised by hepatic and serum esterases and hepatic mixed-function oxidases. There are few data available to determine whether neonatal pharmacokinetics differ from adults. It is therefore not possible to determine whether accumulation may occur.

Codeine

A full-term healthy male infant showed signs of poor feeding and lethargy from day 7 (Koren *et al.* 2006). By day 12 the infant was noted to have grey skin and reduced intake. The infant was found dead on day 13. The postmortem blood concentration of morphine was 70 µg/L. The mother had been taking 30 mg codeine and 500 mg paracetamol for episiotomy pain, initially 2 tablets every 12 h, reduced to 1 tablet every 12 h from day 2 and continued for 2 weeks. The concentration of morphine in her breast milk was measured as 87 µg/L, the typical range normally being 1.9–20.5 µg/L at doses of 60 mg every 6 h. Genotype analysis of the cytochrome P450 enzymes (CYP) demonstrated that the mother was an ultra-rapid metaboliser of codeine to morphine (CYP2D6*2A with CYP2D6*2x2 duplication). It was proposed that the infant died from opioid toxicity because of a reduced capacity for neonates to eliminate morphine.

This case report stimulated some discussion in the literature (Bateman *et al.* 2008). It was suggested that, even if the entire maternal dose of codeine had been converted to morphine, the dose of morphine from breast milk would not have been toxic to the neonate. It was also pointed out that the infant's postmortem blood concentration of paracetamol was 5.9 mg/L and the infant's stomach contained both high levels of morphine and low levels of paracetamol. Hence there were some inconsistencies in the data that could be interpreted as indicating administration of codeine and paracetamol to the neonate. The authors responded, attributing the high blood paracetamol concentration to postmortem redistribution, and stating that the neonate is more sensitive to the respiratory depressant effects of morphine than an adult. The

plasma concentration, corresponding to 0.07 mg/L, is lower than previously reported fatalities in opioid-naive subjects (see monograph on Morphine), but an increased susceptibility for neonates to the respiratory suppressant effects of opioids would account for this discrepancy.

Most drugs are transferred to some degree into breast milk, but for the majority of agents the exposure of the breast-feeding neonate is minimal. Some agents that should be avoided during breast feeding include cytotoxic drugs (cyclophosphamide, ciclosporin, doxorubicin and methotrexate), drugs of abuse (amfetamine, cocaine, diamorphine, cannabis, phencyclidine) and radioactive compounds (American Academy of Pediatrics Committee on Drugs 2001). More complete information can be found in *Drugs in Pregnancy and Lactation* (Briggs *et al.* 2008).

Infant: poisoning from contaminated milk formula

In late 2008 it was reported that in China over 6000 infants became unwell following exposure to melamine in an infant formula. Four infants died and over 1200 required hospitalisation. The melamine had been added to the infant feeding formula in order to increase the measures of protein concentration in the formula.

Melamine causes the formation of renal calculi, which can result in urinary tract obstruction and renal failure. In infants this may manifest as crying, distress when urinating, haematuria, vomiting and failure to thrive. In animal models, the histological features include renal distal tubular lesions with polarisable crystals and striations in the distal tubules and collecting duct, inflammation and interstitial fibrosis. Acute exposure does not appear to be harmful, and trace amounts of melamine are common in some processed foods. However, chronic exposure to larger concentrations is harmful. The occurrence of widespread melamine poisoning in the infants in China highlights the importance of developing food safety standards and their monitoring and enforcement. The freeing up of trade between countries may also contribute to a lessening of food safety standards and spread of such incidents to other countries.

Toddler: caustic ingestion

A 20-month-old male infant was presented to an accident and emergency department after ingesting liquid dishwasher detergent. While briefly unobserved, he had managed to drink some of the substance while the dishwasher was being loaded. On presentation, the child was drooling and was refusing oral intake. On examination, there was some swelling of the lower lip and erythema of the tongue. The child was admitted to hospital and treated with dexamethasone, omeprazole, IV ceftriaxone and IV fluids. An oesophagoscopy was performed at 48 h and there was non-circumferential erythema, sloughing and ulceration of the mid-oesophagus. At the 6-month review, the child was described as having difficulty swallowing. A stricture was found at oesophagoscopy and dilatation of the stricture was performed.

Caustic and acidic ingestions where the pH of the substance is >12 or <2 are a significant cause of morbidity in young children. At oesophagoscopy, around 50% of cases have mucosal injury, and 10% have long-term sequelae such as oesophageal strictures. The most commonly ingested agents are dishwasher dish-cleaning agents, whether in liquid or powder form, as well as oven cleaners, degreasers and drain cleaners. These agents are commonly present in the home and require safe storage in conjunction with child-resistant packaging. Children commonly ingest those substances that are most easily available in the home, such as household chemicals and over-the-counter medicines (Reith *et al.* 2001).

Adolescent: beta-blocker

A 13-year-old girl presented to an emergency department 2 h after ingesting 4 g of propranolol. The propranolol was her own medicine, which had been prescribed for migraine headaches. On presentation, her heart rate was 25 beats/min and her systolic blood pressure was 60 mmHg. In the ambulance on the way to the hospital she had a generalised tonic–clonic seizure lasting for 5 min and on arrival her Glasgow Coma Scale score was 10. Shortly after arrival she had an asystolic cardiac arrest and did not respond to cardiopulmonary resuscitation. At postmortem examination her serum propranolol concentration was 3.8 mg/L and her liver propranolol content was 50 mg/kg.

Propranolol appears to have relatively greater toxicity than other beta-blockers, perhaps related to its potential to cause seizures in overdose, in addition to its cardiotoxicity (Reith *et al.* 1996). It is also prescribed for a younger age group than other beta-blockers because of its use as a preventer for migraine headaches. Headaches may be an indicator of stress, and therefore propranolol may be prescribed to an at-risk group to a greater extent than other beta-blockers.

The interpretation of laboratory data in adolescents is similar to that in adults. During adolescence, developmental differences in toxin disposition are on the basis of body size rather than organ maturation. The postmortem blood and organ concentrations of propranolol in the present case are in the same order as those of previously reported fatalities attributed to propranolol.

Exposures in adolescents predominantly relate to risk-taking behaviours and psychiatric morbidity. Adolescents tend to take their own medications, or household medicines, in overdose, and tend not to plan and obtain a poison on the basis of lethality. Of adolescents presenting with self-poisoning, 9% will re-present with poison exposure within 1 year of initial presentation and 16% within 5 years (Reith *et al.* 2003b). The mortality rate from poisoning in the 10- to 14-year age group is 1.20 : 100 000 per year, which is around 1 per 50 presentations. After an initial presentation with self-poisoning, overall 4% will die within 10 years and 2% will commit suicide within 10 years (Reith *et al.* 2003c). Hence, poison exposures in adolescents have greater mortality and morbidity than those in younger children because of higher doses and greater toxicity. In addition, an increasing severity of subsequent re-exposure is a marker of increased risk for subsequent suicide (Carter *et al.* 2005). Hence, there is an opportunity for psychiatric intervention whenever an adolescent presents with self-poisoning. This is not restricted to cases of deliberate self-harm, because overdoses with recreational drugs are also risk factors for suicide and accidental death and there may be underlying psychiatric morbidity.

Social aspects of paediatric toxicology

Epidemiology

The epidemiology of paediatric poisoning changes from infancy through to adolescence. The presentation rates with poisoning are highest for children aged 0–4 years at around 700 : 100 000/year, decreasing to 40 : 100 000/year for children aged 5–9 years and 70 : 100 000/year for children aged 10–14 years (Reith *et al.* 2001). These changing presentation rates most likely reflect the different motivations for poisoning in these age groupings. Exposures in the 0- to 4-year age group predominantly represent exploratory ingestions where the child is experimenting with potential food sources or flavours. In the 5- to 9-year age group, exposures represent copying/modelling of adult behaviours, aberrant development or behavioural disorder in the child. Rarely, in these groups, the exposure is intentional by a third party. In adolescents, the exposures represent self-harming behaviour or substance misuse. Admission rates vary between countries, with rates of 184 : 100 000/year for 2 year olds in the USA and 462 : 100 000/year in the UK (Woolf, Lovejoy 1993).

Most commonly, children ingest those agents that are closest to hand rather than seeking out agents on the basis of toxicity. Hence, the substances most commonly ingested by children include paracetamol, rodenticides, cough and cold preparations, pesticides and household chemicals (Reith *et al.* 2001). In developing countries, kerosene and plant poisonings are more common than medicinal poisonings. The agents resulting in death from poisoning are more commonly opioids, iron preparations, cardiotoxic drugs such as chloroquine and tricyclics, and, in coronial data, carbon monoxide.

Intentional poisoning of children

In Australia, in the 10-year period 1983–1992 for the 0- to 4-year age group there were 32 accidental deaths from poisoning and 12 homicides by poisoning. However, over the same time period in the 5- to 9-year age

group there were 3 accidental deaths from poisoning and 11 homicides by poisoning. The decrease in accidental poisonings in the older age group reflects the decreased rate of poisons exposures with age, due to increasing maturity and awareness of hazards, but the similar number of homicidal poisoning deaths indicates that the risk of intentional poisoning in children does not decrease with age.

Poisoning is an unusual cause of death in child victims of homicide (Vanamo *et al.* 2001). Head and neck injuries, suffocation and drowning are more common causes of death in intrafamilial child homicide.

Poisoning should also be considered in the investigation of apparent life-threatening events in children (Pitetti *et al.* 2008). In children under 2 years of age presenting with an apparent life-threatening event, 274 of 596 underwent toxicology testing, of whom 50 (18.2%) were positive for drugs other than the child's known medications, and 23 (8.4%) were considered clinically significant (Pitetti *et al.* 2008). Thirteen (4.7%) were positive for medications contained in over-the-counter cough and cold preparations (Pitetti *et al.* 2008).

Illness induced by poisoning in children is a rare but potentially serious presentation (Dine, McGovern 1982; McClure *et al.* 1996). There is a high mortality rate and, in around 30% of cases, the poisoning continues while the child is in a healthcare facility (Dine, McGovern 1982). The most common drugs used are anticonvulsants and opiates (McClure *et al.* 1996). Covert video surveillance may be useful in establishing the diagnosis but must be performed with regard to the local ethical and legal framework (Southall *et al.* 1997). Evidence of other injuries is present in only 20% of cases (Dine, McGovern 1982). It is important to consider the plausibility of the history of the poisons exposure in the context of the child's developmental stage: for example, could a 6 month old access a medicine or chemical?

Munchausen by proxy is a term first used by Roy Meadow in 1977 to describe a condition in which parents fabricate illness in their child for secondary gain (Meadow 1989). The condition may lead to serious illness or even death in the child as a consequence of the parents' actions. The term fell into disrepute following a General Medical Council finding that Professor Meadow gave erroneous and misleading evidence during the Sally Clark case. The General Medical Council ruling was later successfully appealed by Professor Meadow. The case against Professor Meadow was partly based on findings from Carpenter *et al.* (2005), which were themselves controversial because of potential bias in the classification of data (Carpenter *et al.* 2005; Gornall 2006). As a consequence, descriptive terms such as 'factitious', 'fabricated' and 'induced' might be more useful and accurate when describing symptoms and signs attributed to cases in which the medical condition may have been caused intentionally by a caregiver.

Diphenhydramine

In response to an emergency call, paramedics found a 5-year-old boy who had stopped breathing and who did not respond to resuscitation efforts. The paramedics noted marks on the child's wrists, ankles and feet that indicated that he had been tied up and burned, and they reported these findings to the police. A postmortem revealed a skull fracture and toxicology tests demonstrated a serum diphenhydramine level of 0.02 mg/L and diphenylmethoxyacetic acid of 1.6 mg/L from blood collected from the heart. The defendants claimed that the boy had autism and frequently threw temper tantrums with head banging. He had been tied up and burned as a punishment. The diphenhydramine had been administered so that he would sleep through the night.

The toxicologist may be asked to comment on the contribution of a poison to the cause of death. Serum concentrations of diphenhydramine in fatal cases range from 0.3 μg/mL to 119 μg/mL (Pragst *et al.* 2006). Diphenhydramine acts as a sedative at serum levels below 0.7 mg/L, and at serum concentrations above this level anticholinergic symptoms become more dominant with delirium, tachycardia and hypertension progressing to seizures and coma as plasma concentrations increase (Pragst *et al.* 2006). Diphenhydramine undergoes extensive biotransformation and the ratio of parent to metabolites may give some indication to the timing of exposure prior to death. In particular, the ratio of diphenhydramine to one of its metabolites, diphenylmethoxyacetic acid, can be used as an indicator of the time between ingestion and sampling or death. The ratio of diphenylmethoxyacetic acid to diphenhydramine increases from 0.5, prior to 5 h post ingestion, to greater than 20 after 40 h or more post ingestion (Pragst *et al.* 2006). In addition there is also extensive redistribution of diphenhydramine *post mortem*, with higher concentrations found in the heart blood than in peripheral blood (Pragst *et al.* 2006). In the above case, the ratio of diphenylmethoxyacetic acid to diphenhydramine of 80 would indicate ingestion of diphenhydramine at least 48 h prior to death. Hence the intoxication would be expected to have had a minor contribution to the child's death.

Iron

A 7-week-old male infant presented with grunting, tachypnoea and abdominal distension. Physical examination indicated dehydration and he was treated with 2 × 20 mL/kg boluses of normal saline (Black, Zenel 2003). Arterial blood gases demonstrated a metabolic acidosis with a pH of 7.09, P_{CO_2} of 32 and base deficit of −19.6. The metabolic acidosis persisted over the next few days, and in addition he developed elevations in serum transaminases (aspartate aminotransferase (AST) 1603 U/L, alanine aminotransferase (ALT) 1600 U/L) and in serum creatinine (12 mg/L). His mother was noted to be detached from the child, and investigation for physical abuse was performed. A skeletal survey and bone scan showed a posterolateral rib fracture. His mother admitted administering Formula 409 (containing 0.3% alkyldimethylbenzylammonium chloride) and crushed iron tablets to the infant. The iron level at 36 h after admission was 55 μmol/L.

Iron is one of the commonest causes of poisoning death in children but in most cases this is due to accidental exposure. In this case, the child was too young to be able to access the poison on his own and the iron must have been administered to the child. Rib fractures in a child of this age are a major indicator of non-accidental injury. Hence, the iron was administered with the intent of causing injury.

Iron is toxic from doses of greater than 20 mg/kg elemental iron (approximately 3–4 standard iron supplement tablets), and lethal from doses of 180 mg/kg (from approximately 26 tablets). Iron tablets are often attractively presented and may be present in large quantities in households with young families because of the push for iron supplementation in pregnancy. With significant exposures, initial signs and symptoms include vomiting, haemorrhagic gastritis, diarrhoea, lethargy and coma. This may be followed by a quiescent phase for 2–12 h when there may be deceptive improvement. There may be recurrence of symptoms from 12 h to 48 h, including haematemesis, melaena, gut perforation, lethargy, coma, convulsions, vasomotor collapse, pulmonary oedema, hepatorenal failure, coagulopathy and hypoglycaemia. In the case presented above, the co-ingestion of alkyldimethylbenzylammonium chloride may have contributed to the metabolic acidosis and elevation in liver enzymes. Serum iron concentrations are normally 0–18 μmol/L, possibly toxic at 18–63 μmol/L, potentially serious at 63–90 μmol/L, definitely serious at 90–180 μmol/L and potentially fatal above 180 μmol/L. Other abnormal laboratory tests results may include elevated white cell count, decreased blood sugar levels, coagulopathy, and elevated serum transaminases and creatinine.

Medical euthanasia: Dr Arthur Case

In 1980 a neonate was born with Down syndrome and was treated at Derbyshire Children's Hospital in the UK. In addition to Down syndrome the neonate may also have had an intestinal malformation and congenital heart malformations. At the request of the child's parents the treating doctor, Dr Leonard Arthur, ordered 'nursing care only'. The child died at 60 h of age after being given only water (with no other nutrition) and dihydrocodeine. A colleague reported Dr Arthur's actions to the police and he was charged with murder. The possibility of congenital heart disease resulted in the charge being changed to attempted murder. The jury found Dr Arthur not guilty.

The case confirmed the principle, previously established in the case of Dr John Bodkin Adams, that 'the administration of a drug by a doctor when it is necessary to relieve pain is a proper medical practice even when the doctor knows that the drugs will themselves cause the patient's death'. However, Dr Arthur had admitted that the drug was intended to stop the child seeking sustenance as well as being a sedative. At the time this was thought to be justifiable medical practice by an expert witness.

Subsequently, the British Medical Association (BMA) changed its ethical guidelines 'to ensure that newborn handicapped babies were treated with the same respect as normal children'. Currently the BMA offers guidance that oral nutrition and/or hydration should continue to be offered to *all* patients who are able to swallow (BMA 2009). However, 'where there is a problem with the swallowing mechanism which makes oral feeding very difficult or impossible, ... careful thought should be given to whether artificial feeding should be provided or continued'. Such cases may include those where a person has 'no or very minimal awareness of their own existence or surroundings, no ability to interact at any level and no chance of regaining any awareness'. However, with regard to euthanasia, the BMA insists that 'voluntary euthanasia should not be made legal in the UK'. The differentiation between euthanasia and withholding artificial feeding would appear to be the use of drugs in order to cause death.

Victims of chemical warfare; the Holocaust

Children are also disproportionately victims of genocide and chemical warfare (Austin 1996). An estimated 1.5 million children were murdered during the Holocaust. Children were particularly vulnerable because of their low economic value (i.e. they could not be used as forced labour). More recently, in 1988, the use of chemical warfare agents in the town of Halabja in Iraqi Kurdistan resulted in the deaths of around 5000 civilians, many (75%) of them women and children, and injury to more than 10 000 (Hawrami, Ibrahim 2004).

Exploratory ingestions

Exploratory ingestions are characterised by a small dose and lower risk of morbidity and mortality. The mortality rate for the 0- to 4-year age group is around 0.82 : 100 000/year, which is around 1/1000 presentations. The agents ingested are predominantly household medications and chemicals: paracetamol, rodenticides, cough/cold preparations, pesticides and chemicals. A lessening in morbidity and mortality has been associated with a decrease in the toxicity of generally available medicines and household chemicals and also with efforts to restrict access by young children. A smaller number of agents are responsible for mortality, such as opioids, strychnine, iron, tricyclics, propranolol and chloroquine. Hence, given the large number of low-risk exposures, management should be based upon a risk assessment, and decontamination and other possibly hazardous interventions should be restricted to those patients at a defined risk of morbidity and/or mortality from the exposure. Following a poisons exposure, or indeed any serious household accident, a hazard assessment of the child's home/environment could be offered.

Lead

A 4-year-old girl presented to hospital 6 days after her mother noted lead pellets in the family toilet bowl that were also seen over the subsequent 2 days. On questioning, the child admitted swallowing lead pellets from her father's skin-diving weightbelt. A store of such pellets was found in her bedside drawer. She had symptoms of mild intermittent abdominal pain and had vomited once. She was taken to her local general practitioner, who measured her blood lead level as 4.8 μmol/L (990 μg/L). An abdominal radiograph was taken that demonstrated seven lead pellets. The child was admitted to hospital, for treatment with PEG-ELS 4 L over 4 h, dimercaprol (75 mg/m^2 by IM injection 4-hourly for 2 days), calcium disodium EDTA 1500 mg/m^2 daily as an IV infusion, Senokot and Agarol. Her blood lead levels on days 6, 22, 42 and 117 after the initiation of treatment were 2.42, 2.37, 2.17 and 1.86 μmol/L (500, 490, 450 and 390 μg/L), respectively. An abdominal radiograph after 6 days demonstrated four pellets that were subsequently passed. She remained well and there were no observed adverse effects (Reith *et al.* 2003a).

There are a number of sources of lead exposure for children. These include lead-based paint (from flaking and renovation of lead-based paint in old houses), lead weights and sinkers, lead-based paint on toys, parents bringing home lead on clothes from occupational exposure, environmental exposure from lead smelters and contaminated soil, cosmetics and pottery glazes. Lead absorption is increased in the presence of iron deficiency. Children with pica are at increased risk of lead poisoning.

Lead is not an element essential to humans and ideally there should be no lead in the body. However, blood lead concentrations less than 0.48 μmol/L (100 μg/L) are considered acceptable. Lead abatement measures should be performed for blood lead concentrations greater than 0.72 μmol/L (150 μg/L) and, if the patient is symptomatic at concentrations greater than 1.20 μmol/L (250 μg/L), chelation should be performed. Chelation should be performed in all patients with blood lead concentrations greater than 2.40 μmol/L (500 μg/L). It is important that the patient be removed from all sources of lead exposure during chelation otherwise absorption of lead may be increased.

Self-harm/substance misuse

The patterns of substance abuse in children and adolescents are slightly different from those in adults: abuse of volatile agents (hydrocarbons/glues) is more common in children and adolescents, and also agents such as *Datura* (anticholinergic). Lead poisoning can result from inhalation of leaded petrol. Nicotine and ethanol use can occur from childhood, with cannabis, amfetamine and opioid abuse becoming more common in late adolescence. A particular issue arises when a naive opioid user is exposed to a dose intended for a habituated user, resulting in overdose and delay in seeking treatment. Methadone is a particular hazard because of delay in the maximum effect and resulting progressive, greatly reduced level of consciousness. There are legal issues with regard to supply of illicit drugs to minors.

Methadone

A 4-year-old boy was found unresponsive by his mother after he had been sleeping on a sofa for several hours. Resuscitation was performed immediately by his mother but following transportation to the local hospital he was pronounced dead on arrival. His blood and liver methadone concentrations were 0.6 mg/L and 1.6 mg/L respectively. His blood and liver EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, the main metabolite of methadone) levels were 0.09 mg/L and 0.4 mg/L respectively. It was subsequently reported that the child's grandmother was a nurse in a methadone clinic who often stole methadone to take home and sell. The night before the child died a cup containing 40 mg methadone dissolved in orange juice had been left on the dining table (Li *et al.* 2000).

Postmortem methadone concentrations can vary by 100% between samples collected from different sites, implying postmortem redistribution (Milroy, Forrest 2000). Blood methadone concentrations in children who have died consequent to methadone intoxication have been in the range 110–489 μg/L, which is within the range of methadone concentrations reported for adults in methadone maintenance programmes (Milroy, Forrest 2000). This is because of the tolerance that develops with long-term opioid exposure when patients on methadone maintenance programmes may be treated with daily doses well in excess of fatal doses for opioid-naive subjects. For example, methadone doses up to 100 mg per day are commonly used in methadone maintenance programmes. Methadone is equipotent with morphine and a dose of 100 mg morphine to a 14 kg child would be around 70 times the usual dose for that child (if they were in severe pain). A large proportion of dispensed methadone is in liquid form (Milroy, Forrest 2000). These formulations are relatively palatable and easy to ingest. Hence, naive users and children are at greater risk of fatality.

Methadone poisoning in children has high mortality and morbidity. The presentation is similar to that for adult opioid intoxication, with respiratory and central nervous system (CNS) depression, miosis, hypotension and hypoxia (Binchy *et al.* 1994). It is not uncommon for the patient to present with no history of ingestion. The pharmacodynamics differ in some respects in children: in children hyperglycaemia has been reported in association with methadone poisoning (Tiras *et al.* 2006). Children may also be more sensitive to the respiratory depressant effects of opioids.

The ratio of methadone to EDDP concentrations may be used to discriminate between acute ingestion (opioid naive) and chronic ingestion (opioid dependent) (Li *et al.* 2000).

Medication error

Cases of medical misadventure tend not to be published in the literature because of the medicolegal implications. The following fictional case study is therefore being used illustratively.

An 8-month-old infant with a history of complex congenital heart disease presents to a suburban hospital with supraventricular tachycardia. The arrhythmia had previously responded to verapamil, but this had been ceased 1 month previously. The junior doctor on duty at the suburban hospital discusses the patient with a paediatric cardiologist at a tertiary referral centre. The paediatric cardiologist subsequently states the advice given was to treat the patient with IV verapamil at a dose of 0.2 mg/kg. The patient was prescribed IV verapamil at a dose of 2 mg/kg, a total dose of 10 mg, by the junior doctor. The dose was questioned by nursing staff, but they were reassured by the statement that this was what the paediatric cardiologist had recommended. The drug was administered by the junior doctor with continuous electrocardiogram (ECG) monitoring. The patient subsequently reverts from supraventricular tachycardia (SVT) to sinus rhythm but then develops complete heart block, atrioventricular (AV) dissociation and bradycardia. This progresses to asystolic cardiac arrest and death despite cardiorespiratory support.

The case highlights a number of safety issues in the process of prescribing for children. First, dosing errors are common and potentially serious. The 10-fold dosing error is the commonest dosing error in the paediatric population (Wong *et al.* 2004). Second, there are particular hazards in the use of phone orders and in remembering drug doses for children. Phone orders should be recorded by two people, in order to avoid errors in the transmission and transcription of the order. Drug doses, unless for familiar/everyday drugs, should be checked against a printed formulary, such as the *BNF for Children* (Paediatric Formulary Committee, 2009). Ultimately, the prescription is the responsibility of the prescriber and doses given by phone order, or remembered by colleagues, even if senior, should always be checked. However, a supervisor also has the responsibility of overseeing the work of junior doctors and ensuring that standards are met.

Another issue demonstrated by the case is that of ameliorability. Once an error has been made, can steps be taken to ameliorate the effects of the error? In the case of verapamil poisoning there may be treatments available, such as IV calcium or even short-term cardiopulmonary bypass. If a medication error is identified, it is also important to treat the adverse effects promptly.

Injuries resulting from errors in the prescribing or administering of medicines to children (adverse drug events, ADEs) are common and occur at similar rate as in adults. Events where injury could have occurred as a result of medication error (but fortuitously did not) – potential ADEs – appear to be more frequent in children than in adults (Kaushal *et al.* 2001). In a hospitalised, paediatric, patient population, ADEs occurred at a rate of 0.24–2.1 per 100 medication orders, 2.3–12.9 per 100 admissions and 6.6–22.1 per 1000 patient-days (Kaushal *et al.* 2001; Holdsworth *et al.* 2003; Kunac *et al.* 2009). Potential ADEs occurred at a rate of 1.1–2.4 per 100 medication orders, 8–14.6 per 100 admissions and 9.3–29 per 1000 patient-days (Kaushal *et al.* 2001; Holdsworth *et al.* 2003; Kunac *et al.* 2009). The incidence of ADEs and potential ADEs is higher in an intensive care population, 7.9 and 12.2% respectively, than in a general paediatric population, 5.1 and 4.6% respectively (Holdsworth *et al.* 2003). Differences between studies in the rates of ADEs and potential ADEs can to some extent be accounted for by differences in the methodology used in ascertainment and classification (Kaushal *et al.* 2001; Morimoto *et al.* 2004; Kunac *et al.* 2009).

Medication errors in children can result in mortality, morbidity and additional costs to the healthcare system. From 8% to 11% of ADEs are fatal or life threatening, 13–35% are serious and 58–76% are significant (Kaushal *et al.* 2001; Holdsworth *et al.* 2003). Of potential ADEs, 0–16% are potentially fatal or life threatening, 5–45% are serious and 39–95% are significant (Kaushal *et al.* 2001; Holdsworth *et al.* 2003). However, although ADEs and potential ADEs are known to contribute to the overall costs of healthcare, there are few published data quantifying these costs in the paediatric population. In an adult population, the cost of an ADE occurring during a hospital admission is in the range US$2013–3244 and is associated with an additional length of stay of 2.2 days, with preventable ADEs having a greater unit cost of US$5857 and an additional length of stay of 4.6 days (Bates *et al.* 1997; Classen *et al.* 1997; Senst *et al.* 2001). For hospitalised children in New Zealand, the cost of a preventable ADE is NZD900 (Kunac *et al.* 2009).

Paracetamol

Master W., a 13-year-old boy with damaged femoral heads, underwent percutaneous insertion of screws into both hips. His weight was 104 kg, height was 166 cm and body mass index (BMI) was 38 (the typical weight for a child of this age and height would be about 50 kg). He received IV morphine, then paracetamol and codeine as required for analgesia. He was then transferred to a small hospital for 4 weeks' strict bed rest. Eleven days post operation, he developed a spiking temperature, nausea and some restlessness. He was given regular paracetamol for analgesia and temperature control. The next day he was started on IV flucloxacillin for a presumed chest infection. Three days later he had a low-grade fever, nausea and vomiting, and right upper quadrant tenderness. Blood was taken for liver function tests. These showed grossly elevated liver enzymes. He continued to receive paracetamol until the next day. In total, he had been given 32 g of paracetamol in a 14-day period. During transfer to a large regional hospital, he was restless and aggressive, requiring IV diazepam for sedation. Upon arrival he was found to have acute fulminating hepatic failure with acute renal failure and hepatic encephalopathy. Later that day, brain death was diagnosed and he died 18 days after his operation. The postmortem examination showed severe fatty change in the liver, consistent with the boy's obesity, and extensive hepatocellular necrosis secondary to acute paracetamol toxicity (Risk Watch 2003).

This case demonstrates a number of features of chronic paracetamol poisoning in a child and raises the issue of appropriate dosing. First, in the case of a child with obesity, dosing should be modified to reflect lean body weight rather than total body weight. Dosing in a child, when based on body weight, should not exceed the adult dose of the drug. Paracetamol should not be continued in a patient when there is evidence of hepatocellular damage. When there are indications of paracetamol toxicity *N*-acetylcysteine should be commenced immediately and continued until either the diagnosis is refuted or there is clinical evidence of improvement.

Chronic paracetamol poisoning poses a greater risk to children than acute paracetamol poisoning. Chronic paracetamol poisoning refers to multiple, cumulative (usually supratherapeutic) doses of paracetamol over several days. This type of poisoning is a particular hazard for younger children, with acute paracetamol poisoning being more common in adolescents (Mahadevan *et al.* 2006). Hepatotoxicity may occur at total doses as low as 90 mg/kg, with fulminant hepatic failure occurring at doses as low as 220 mg/kg (Mahadevan *et al.* 2006). The range of tolerable doses indicates considerable inter-individual variability in the susceptibility to paracetamol hepatotoxicity. This may relate to variability in the metabolism of paracetamol due to inter-individual/pharmacogenetic variability in enzyme kinetics (Reith *et al.* 2009). Such variability could relate to under-expression of uridine glucuronosyltransferase (UGT) and/or sulfotransferase isoforms, or alternatively to over-expression of CYP enzymes (in particular CYP2E1). Glutathione is also required for neutralising the toxic metabolite of paracetamol. Depletion of glutathione as a result of inflammation or poor nutrition may also increase the risk of paracetamol toxicity. Prodromal illness and fasting have been identified as risk factors for chronic paracetamol poisoning in children (Miles *et al.* 1999). Phenobarbital and phenytoin are inducers of the CYP system, and also inhibitors of UGT and would therefore pose a double risk in paracetamol poisoning (Mutlib *et al.* 2006).

Drug and toxin disposition in children

An understanding of how the processes of drug and toxin disposition differ in children compared with adults is important for the clinical management of childhood poisonings and in the interpretation of laboratory data. For example, the estimation of a dose or time of exposure may be made by back-calculating from serum concentrations. Knowledge of paediatric pharmacokinetics is important in making

plausible estimations. In addition, the systemic exposure to a poison will determine the likely clinical effect, and therefore the need for clinical interventions.

The systemic exposure to a toxin, and therefore the subsequent effects of the poison exposure, depends upon the amount absorbed, which in turn depends upon bioavailability. Bioavailability is influenced by binding or metabolism in the gut, metabolism in enterocytes (principally mediated by CYP3A4), transmembrane solute transporters in enterocytes (principally ABCB1) and metabolism in the liver. These processes may differ in children compared with adults, but knowledge of the extent of these differences is incomplete.

In addition there are issues of scaling. Children are smaller than adults, but, because of some physiological differences, cannot simply be considered as 'little adults'. Various methods have been proposed to scale between children and adults, ranging from simple weight-based approaches, to allometric scaling and to physiologically based models. Validation of these different methods depends upon already knowing the pharmacokinetics of the drug in children, and as yet none of these methods has been validated prospectively.

Absorption of drugs

In general, the absorption of xenobiotics in children is similar to that in adults.

Bioavailability

Contributors to first-pass metabolism include CYP3A4, sulfotransferases and P-glycoprotein in enterocytes, in addition to hepatic metabolism of xenobiotics (Chen *et al.* 2003). Enterocyte CYP3A4 expression may be decreased under the age of 6 months, and increased relative to adults around the age of 1 year (Fakhoury *et al.* 2005). This suggests that for drugs with low bioavailability, greater exposure to these drugs occurs in neonates, with lesser exposure in children around the age of 1 year. There are, however, few data on bioavailability in neonates and infants to confirm this.

ABCB1 (P-glycoprotein) expression is variable at all ages. There is some evidence of age-dependent expression of ABCB1 polymorphisms, with bioavailability of ciclosporin appearing to be influenced by polymorphisms over the age of 8 years (Fanta *et al.* 2008). However, in general, the bioavailability of ciclosporin does not appear to be influenced by age in children (Fanta *et al.* 2007).

Volume of distribution

Body composition changes from infancy to adulthood. Infants have a higher proportion of body mass as water, and boys also have a higher proportion of body weight as water than girls (Wells *et al.* 2005). In term infants, total body fat comprises around 15% of body weight in both males and females, increasing to around 25% by 120 days of age, 39% at 6 months and 29% by 14 months of age (Butte *et al.* 2000; Olhager *et al.* 2003; Tennefors, Forsum 2004). At 2 years of age total body fat comprises around 25% of body weight.

However, despite the differences in proportion of body weight as adipose tissue, this does not necessarily translate into differences in pharmacokinetics. Thiopental, which is highly lipid soluble and redistributes to adipose tissue, does not have a different volume of distribution in children compared with adults, although clearance is higher in children (Sorbo *et al.* 1984)

Renal clearance

The kidney clears xenobiotics and their metabolites through glomerular filtration and tubular secretion.

Filtration

The development of glomerular filtration through childhood has been well described. By 36 weeks' gestation the cortical nephrons are formed, but the glomerular filtration rate (GFR) is around 5% of adult values owing to decreased renal blood flow (Haycock 1998). Glomerular filtration rate per unit surface area increases rapidly in the first week after birth, and then increases at a slower rate until it peaks at 3.25 years. From that time, GFR per unit surface area declines at a steady rate throughout childhood, adolescence and adulthood (Wahl *et al.* 2003). Pre-term neonates have decreased GFR in relation to term neonates. Mean GFR per 1.73 m^2 of body surface area is around 20 mL/min at birth, increasing to 50 mL/min in the first week, peaking at 120 mL/min in the third year and decreasing slightly to 115 mL/min in adolescence.

In 2006 the Centers for Disease Control and Prevention in Atlanta, USA (CDC) and National Association of Medical Examiners conducted a review of deaths associated with cough and cold medications in infants aged $\leq$12 months in the year 2005 (CDC 2007) Three deaths in infants aged from 1 month to 6 months were identified. The infants had postmortem blood concentrations of pseudoephedrine ranging from 4.74 mg/L to 7.10 mg/L. Two infants had detectable dextromethorphan in blood: 390 and 1909 µg/L. One infant had a doxylamine blood concentration of 1.00 mg/L. One of the infants had concurrently received a prescription and an over-the-counter medication both of which contained pseudoephedrine. Two of the infants had postmortem findings of pneumonia and the third had findings of acute anoxic encephalopathy and a small fracture of the left distal tibia. During 2004–2005 it was estimated that 1519 children were treated for adverse events associated with cough and cold medications, including overdoses, in US emergency departments.

In general, cough and cold medicines are not indicated for use in children under the age of 2 years. Since 2008 these medicines have been contraindicated in this age group in the USA, Canada, the UK and other regulatory jurisdictions. In 2009 in Canada and the UK, the age limit for cough and cold medicines was increased to 6 years because of lack of data supporting efficacy, and concerns regarding safety.

Pseudoephedrine is a stereoisomer of ephedrine. It is predominantly excreted in the urine, and in adults has a half-life of 5–8 h. Infants have a reduced GFR relative to adults, and therefore reduced ability to clear pseudoephedrine. Hence with repeated dosing accumulation will occur, resulting in elevated blood concentrations of pseudoephedrine and toxicity. However, older children have relatively greater GFRs than adults and an enhanced ability to excrete pseudoephedrine (Table 27.1 gives information on comparative pharmacokinetics of drugs in children of different ages and adults). Dextromethorphan is metabolised by CYP2D6, so that although its half-life is normally 1.2–3.9 h it may be increased to up to 45 h in patients who are poor metabolisers. Neonates also have decreased expression of CYP2D6. Hence in some patients there may also be accumulation of dextromethorphan.

Renal sodium handling matures at a slower rate than GFR, and the infant kidney has reduced ability both to excrete and to retain sodium (Haycock 1998), hence infants are susceptible to both water intoxication and hypernatraemia. Pre-term infants can lose sodium relative to water, with resultant hyponatraemia, because of immature renal tubules, up to a gestation of 35 weeks. Infants are susceptible to salt poisoning as a result of inappropriate reconstitution of formula, the use of undiluted cows' milk or intentional salt poisoning. Water intoxication (hyponatraemia) may also result from dilution of infant formulas.

A male infant presented at 8 months of age with high fever, profuse diarrhoea, exacerbation of vomiting, hypotonia, weight loss and poor perfusion (Coulthard, Haycock 2003). He had been born prematurely at 28 weeks' gestation and had a history of aortic arch and bronchial anomalies and severe gastro-oesophageal reflux. He had been treated with domperidone, a compound alginate preparation (Gaviscon Infant) and feed supplements that contained glucose polymers. At presentation his serum sodium was 156 mmol/L, and his urine osmolality, sodium and creatinine were 961 mmol/kg, 152 mmol/L and 5.5 mmol/L, respectively. At presentation his fractional excretion of water was 1.04% and of sodium was 1.01%; 1.8 h later the values were 0.86 and 0.42, respectively. Administration of IV fluids dramatically improved his condition and restored his body weight.

Coulthard and Haycock (2003) stated that 'the boy was taken into foster care after he was admitted at age 8 months, because doctors believed his biochemistry results confirmed salt poisoning and because his siblings' medical histories were considered suspicious. Three siblings had become hypernatraemic under similar circumstances: one died after remaining at

Table 27.1 Comparative pharmacokinetics of drugs between children of different ages and adults

Drug/age group	*Volume of distribution*	*Clearance*
Midazolam		
Pre-term	Median (range)	Median (range)
	1.1 (0.4–4.2) L/kg	1.8 (0.7–6.7) mL/kg per min
Infants	3.8 L	0.157 L/min (median weight 9.4 kg)
Child	Mean (SD)	Mean (SD)
	1.7 (1.1) L/kg	5.8 (3.5) mL/kg per min
Adult	0.8–1.86 L/kg	4.0–12.2 mL/kg per min
Paracetamol		
Neonates <10 days	Mean (SD)	Mean (SD)
	0.7 (0.2) L/kg	0.149 (0.067) L/kg per h
Neonates >10 days	0.9 (0.1) L/kg	0.365 (0.219) L/kg per h
Children	0.86/0.89 L/kg	0.277/0.383 L/kg per h
Adult	1.29 (0.37) L/kg	0.28 (0.04) L/kg per h
Theophylline		
Neonate	Mean (SD)	Mean (SE)
	0.69 (0.095)	17.6 mL/kg per h
Infant (3–23 months)	0.34 (0.2)	1.07 (0.55) mL/kg per min
Child	0.44 (0.05)	Mean
		56 mL/kg per h
Healthy non-smoking adults	0.44 (0.053) L/kg	47.4 (10.3) mL per min
		0.65 mL/kg per h
Caffeine		
Neonate		$t_{1/2} > 50$ h
Child		$t_{1/2} = 3.5$ h
Adult	0.61 ± 0.02 L/kg	$t_{1/2} = 8$–9 h
		1.4 ± 0.5 mL/min per kg
Pseudoephedrine		
Infant	Data not available	Data not available
Child 2 to <6 years	3.6 (9%) to 4.2 (21%) L/kg (CV%)	11.4 (21%) mL/kg per min
Child 6 to <12 years	2.4 (17%) to 3.5 (20%) L/kg (CV%)	8.5 to 12.7 (17%) mL/kg per min
Adult	2.8 (15%) to 3.7 (17%) L/kg (CV%)	5.2 (26%) to 7.5 (36%) mL/kg per min
Morphine		
Pre-term neonate (24 weeks)	2.7 L/kg	1.5 mL/kg per min
Term neonate	2.7 L/kg	4.8 mL/kg per min
Neonate–2 years	2.7 L/kg	19 mL/kg per min
Adults	3.3 L/kg	24 mL/kg per min

CV coefficient of variation; SD, standard deviation; SE, standard error.

home (on the general practitioner's advice) with pyrexia, explosive diarrhoea and weight loss but without haemorrhagic encephalopathy. In addition, one sister, who was eunatraemic, died suddenly and unexpectedly. The odds of a second innocent death were suggested (incorrectly) to be 73 million to 1. A court found that the mother was not guilty of manslaughter'. The authors considered the case represented hypernatraemic dehydration secondary to gastroenteritis exacerbated by the continued administration of glucose polymers in the presence of diarrhoea.

Salt poisoning and hypernatraemic dehydration require careful differentiation. Salt poisoning is characterised by vomiting, diarrhoea, failure to thrive and in severe cases coma (Meadow 1993). Plasma concentrations of sodium are high, and may be in excess of 200 mmol/L. Urine concentrations of sodium are also high. The urine sodium : creatinine ratio is elevated above the normal of around 39. The urine sodium : potassium ratio is also elevated, the normal being around 2.8 (range 1.4–5.2). Hypernatraemia with elevated urinary sodium concentrations can also occur with hypernatraemic dehydration. Hence, high urinary concentrations of sodium alone cannot distinguish salt poisoning from dehydration (Coulthard, Haycock 2003). Fractional excretions of sodium and water, which are calculated from the sodium and creatinine concentrations of paired plasma and 'spot' urine samples, can distinguish the two situations (Coulthard, Haycock 2003). The values should be >2% in a child who has been salt poisoned and is volume replete and <1% in a dehydrated child with viable renal tubules. Renal and endocrine disease should also be excluded as explanations for the hypernatraemia. If the salt poisoning is intentional, then the hypernatraemia should resolve when the child is removed from contact from possible perpetrators.

Active transport of xenobiotics in the renal tubules

There are a number of transmembrane solute transporters expressed in the renal tubules and the development of these processes during childhood is largely unstudied (Launay-Vacher *et al.* 2006). Tubular secretion of xenobiotics (mediated by transmembrane solute transporters) appears

to be enhanced to a greater degree than GFR in infants and children, but there are limited data (Linday *et al.* 1981). Digoxin, the pharmacokinetics of which are well characterised in children, can be used as a marker of this process, specifically P-glycoprotein/MDR-1/ABCB1 activity (Ieiri *et al.* 2004). In children, the ratio of digoxin clearance to creatinine clearance is higher and decreases to adult ranges around the time of puberty, suggesting that the change is related to sexual maturation rather than size (Linday 1994) Transmembrane solute transporters may also be upregulated (induced) by some drugs (Launay-Vacher *et al.* 2006).

Hepatic clearance

Hepatic drug clearance occurs through metabolic transformation and drug transportation processes. Hepatic drug clearance becomes a function of the capacity and affinity of facilitated processes, and of hepatic blood flow. Ultimately, all of the facilitated processes are saturable, and therefore clearance is also dependent upon substrate concentration:

$$\text{Rate of metabolism} = \frac{V_{\max} \times C}{K_m + C}$$

where C is the concentration of the substrate, $V_{\max}$ is the maximum rate of metabolism and K_m is the Michaelis–Menten constant.

In children, these processes can be affected by enzyme expression (degree of enzyme expression and differential expression of enzymes and transporters during development), thus affecting $V_{\max}$ and K_m for the processes. Liver size can also affect $V_{\max}$ by influencing the total amount of enzyme or transporter. Hepatic blood flow may also vary during development.

Changes in hepatic blood flow do not appear to influence the differences in drug elimination with development (Alcorn, McNamara 2002). However, there are few data describing hepatic blood flow in children, and in physiologically based pharmacokinetic modelling hepatic blood flow has been assumed to be proportional to body surface area (BSA) (Björkman 2005). Biliary excretion appears to be reduced in infants, based on limited data mainly from animal models (Alcorn, McNamara 2002).

Cytochrome P450 development

There is differential expression of drug-metabolising enzymes among the foetus, neonate, infant and adult (Alcorn, McNamara 2002). CYP1A2 has negligible expression in the foetus, is detectable by 1–3 months of age, and reaches adult levels by 1 year of age (Alcorn, McNamara 2002). This is reflected in the metabolism of CYP1A2 substrates such as caffeine (Ginsberg *et al.* 2004). Similarly CYP2A6 is not expressed in foetal liver and is developed by 1 year of age (Alcorn, McNamara 2002). CYP2B6 appears to mature later than CYP2A6 (Tateishi *et al.* 1997). CYP2C9 has no activity in foetal liver but is detected by 1 day of age, and activity matures to 50% of adult activity by 1 month of age (Treluyer *et al.* 1997; Alcorn, McNamara 2002). CYP2C8 and CYP2C18 appear to parallel CYP2C9 (Treluyer *et al.* 1997). CYP2D6 activity is present from 23 weeks' gestation (25 weeks post-conceptional age), is not fully expressed at birth and reaches levels comparable to the adult activity by 1 year of age (Allegaert *et al.* 2005).

CYP3A expression changes through development by changes in the relative and absolute expression of CYP3A4, CYP3A5 and CYP3A7. CYP3A7 expression is greatest in foetal liver and it is highly expressed through to 6 months' postnatal age, but soon after birth its expression decreases and adult levels of expression are around 10% that of foetal expression (Tateishi *et al.* 1997; Lacroix *et al.* 2003; Stevens *et al.* 2003). CYP3A4 activity is well developed from birth (Alcorn, McNamara 2002). CYP3A4 expression is low in the foetal liver, but reaches 30–40% of adult expression by 1 month of age (Lacroix *et al.* 2003). CYP3A5 expression has high variability that appears to be independent of age (Stevens *et al.* 2003). Although there is some substrate specificity between isoforms of CYP3A, there is considerable overlap; hence overall there is similar CYP3A activity from infancy to adulthood.

A 5-week-old male infant presented with persistent tachycardia, agitation and irritability (Rivenes *et al.* 1997). On examination, the heart rate was 205–237 beats/min, respiratory rate was 60 breaths/min, blood pressure 118/65 and rectal temperature 39.5°C. The liver edge was palpable 4 cm below the right costal margin. ALT was 1079 U/L and AST was 578 U/L. A comprehensive urine toxicology screen indicated the presence of caffeine and the serum caffeine concentration was 117 mg/L (therapeutic range 5–12 mg/L). The infant had initially been treated for suspected sepsis, but subsequently a referral to Child Protection Services was made. The child was subsequently returned to the care of the family provided that there was no contact with the father. Three weeks later the child presented again following resuscitation after a cardiopulmonary arrest. The child was found to have retinal haemorrhages and subarachnoid haemorrhages. Death resulted from hypoxicischaemic encephalopathy, cerebral oedema and multi-organ system failure. At postmortem examination there was evidence of fractures of varying ages including healing rib fractures, a left spiral radial fracture and a right distal clavicular fracture. The father had admitted administering 3 × 200 mg caffeine tablets to the infant prior to the initial presentation.

The child initially presented with a clinical picture mimicking sepsis and the toxicological diagnosis was serendipitous. Caffeine would not normally arouse suspicion in a urine toxicological screen, but because of the infant's young age it was considered unusual that he would have been exposed to caffeine. Hence serum caffeine concentrations were determined. The intentional poisoning was also combined with physical abuse, which ultimately resulted in the death of the child. The case illustrates that abuse by poisoning is as serious as any other form of child abuse and requires the same level of intervention.

Caffeine metabolism is complex and changes from birth through to early childhood. Neonates and young infants have reduced clearance of caffeine compared with adults, and therefore there is a much longer half-life in neonates (Table 27.1). Caffeine is used as a respiratory stimulant in premature neonates, and is present in caffeinated beverages consumed from childhood through to adult life. A typical cup of coffee will contain 100 mg caffeine, and colas contain 97 mg/L. However, in neonates, because of the decreased metabolic clearance of caffeine, the duration of effect is much longer than for adults. In the first month post partum, 85% of identifiable caffeine and its metabolites in urine is parent caffeine (Aldridge *et al.* 1979). The half-life of caffeine in neonates is up to 30 h, while in adults it is around 8–9 h.

Glucuronidation

Glucuronidation is catalysed by a number of different UGTs. Individual processes, e.g. paracetamol glucuronidation, may be catalysed by several different UGTs (Mutlib *et al.* 2006). Glucuronidation of paracetamol is age related, being decreased in infancy and increasing to adult levels over the first 2 years after birth (van der Marel *et al.* 2003). However, it is not clear whether all the drug-metabolising UGTs mature over the same time course. There is little expression of UGTs in the foetus, and at birth there is an increase in their expression, with about 25% of the quantity of adult expression by 3 months of age (Alcorn, McNamara 2002). Morphine glucuronidation is also deficient in neonates and matures by late infancy (Alcorn, McNamara 2002) and was an important factor in the Dr Arthur Case cited above as the neonate could not glucuronidate the dihydrocodeine.

Other metabolic pathways

Sulfation is well developed in neonates (Choonara *et al.* 1990). However, paracetamol sulfation is saturated at relatively low doses/concentrations and it is not clear whether there are relative changes in capacity with age. The acetylator (*N*-acetyltransferase) phenotype undergoes maturation up to 4 years of age and in that time slow acetylators may become fast acetylators (Pariente-Khayat *et al.* 1997). Acetylation may also be decreased under the age of 3 months (Rey *et al.* 2001).

In addition to changes in the expression of enzymes, there are also changes in the relative size of the liver and possibly also in liver blood flow (Murry *et al.* 1995). Relative to total body weight, liver size decreases during childhood. For some drugs, clearance is proportional to liver size (e.g. carbamazepine, (*S*)-warfarin and lorazepam), whereas for others it is not (e.g. antipyrine, (*R*)-warfarin) (Murry *et al.* 1995; Takahashi *et al.* 2000; Reith *et al.* 2001). This may depend on whether the enzyme system is of high or low capacity, e.g. in the case of (*S*)-warfarin

CYP2C9 and (*R*)-warfarin CYP3A4 (Takahashi *et al.* 2000). There are few data on liver blood flow in children and it is not known whether liver blood flow parallels liver size. However, changes in liver blood flow might explain some of these phenomena.

Extrapolation of adult data to children

Where the pharmacokinetics/toxicokinetics of a substance in children are unknown, it may be necessary to extrapolate from adult or even animal data. The two main approaches to this are first allometric scaling and second physiologically based modelling (Björkman 2006). Allometric scaling assumes that the kinetic parameters are related to size, whereas physiologically based models take into account maturational effects, e.g. in renal or hepatic function. Hence after the age of 2 years allometric scaling may be appropriate, but prior to 2 years of age the maturation of renal and hepatic function would make physiologically based models more appropriate. The limitations of both allometric scaling and physiologically based modelling mean that such processes should be used only in the absence of observational data about the disposition of the drug in children, and the results should be interpreted with caution.

Allometric scaling

Drug doses are often scaled on the basis of weight or body surface area, but it may be more appropriate to scale by power functions of weight (allometric scaling). These power functions are usually weight$^{0.67}$ or weight$^{0.75}$ and are based on the observation that physiological processes can be scaled between species and between individuals on the basis of the relationship (Holford 1996). However, there may be some publication bias with regard to papers discussing allometric scaling and use of allometric scaling prospectively during drug development has had patchy success (Bonate, Howard 2000). Hence, allometric scaling is appropriate when the pharmacokinetics of the drug are known to be related to allometric functions of weight.

Habtemariam *et al.* published a population pharmacokinetic analysis of valsartan in children aged 1–16 years (Habtemariam *et al.* 2009). The study used allometric exponents on fat-free mass (calculated on the basis of height, weight and sex) of 0.75 for clearance and of 1 for volume of distribution. This approach fitted the data better than covariate models based on weight and age. However, there is a reliance here on having prior data. Using such models to extrapolate between species, or from adults to childrens is useful when designing dosing regimens for clinical trials but has limited applicability in clinical practice. There is a wide range of allometric coefficients between drugs (from 0.2 to 1.2) when interspecies comparisons are made (Hu, Hayton 2001).

Physiologically based models

Physiologically based models use physiological data such as body weight, organ weights, organ blood flows, body compartments (interstitial and vascular spaces), cardiac output, GFR, enzyme activities, etc. to predict pharmacokinetic parameters in children (Björkman 2005). The inclusion of data such as liver blood flow, liver microsomal protein content, portal vein flow and liver volume can be used to further refine physiologically based models (Edginton *et al.* 2006; Johnson *et al.* 2006). These models have performed well in comparison with historical data but will require further evaluation in prospective studies, such as in drug development.

Ginsberg *et al.* used physiologically based pharmacokinetic modelling applied to caffeine and theophylline in neonates and adults (Ginsberg *et al.* 2004). In this example, the model uses the known expressions of the enzymes involved in the metabolism of the two substrates (caffeine and theophylline) along with the Michaelis–Menten constants for the enzymes, compartmental volumes, organ blood flows, cardiac output and body weight in order to explain the differences in pharmacokinetics between neonates and adults. However, in order to fully explain the differences a novel metabolic pathway, expressed in neonates, but not adults, was proposed. It remains to be seen whether such models can be used to predict pharmacokinetic differences.

Dose calculations for children

In most cases drug dosage for children can be determined from published data, including the product information documents or datasheets provided by the manufacturer. In these cases the dosage guidelines have been determined from pharmacokinetic or clinical trial data. The recommendations usually base the dose on body weight, age categories or body surface area. These different methods can result in quite different mass doses, e.g. the 3rd and 97th centiles for weight for a 4-year-old male are 15 kg and 25 kg, respectively – close to a twofold difference. Hence, age-based dosing is not favoured, but for some drugs there will be clinical trial evidence to support an age-based recommendation, e.g. for omeprazole. Where a drug has a high therapeutic index, age-based dosing is acceptable, but when the therapeutic index is low, dosing by age and weight, or by surface area, is more acceptable (e.g. antineoplastic agents). A good source of information on drug dosing in children is the *BNF for Children*. Where there are no published guidelines, for repeated dosing a child's exposure to a drug relative to an adult could be determined using allometric scaling, or physiologically based models, as previously discussed. The relative exposure for a single dose is best calculated on the basis of volume of distribution, which other than for neonates can be scaled by weight alone. Some examples of the differences of drug dosing for newborn infants, infants/toddlers, children and adolescents are given in Table 27.2.

Formulations for children

Many oral formulations, such as tablets, capsules and most slow-release formulations, are not suitable for children. Even when a drug has paediatric indications, there may not be a suitable formulation (Balakrishnan *et al.* 2007). Adult dose forms may be crushed and administered with food, but this poses difficulties for accurate dosing. Extemporaneous formulations lack stability data, and also require shaking/mixing prior to dosing in order to avoid under- and over-dosing.

Other differences between children and adults

With regard to the effects of poisoning, various physiological parameters may be interpreted differently in children than in adults. This poses problems for the clinical management of children by non-paediatrically trained clinicians. In children, the normal ranges of blood pressure are lower, and heart rate is higher, than in adults. When measuring blood pressure in children, correct cuff size is crucial: specifically a bladder width that is approximately 40% of the arm circumference midway between the olecranon and the acromion (a cuff bladder that will cover 80–100% of the circumference of the arm) (National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents 1996). A practical formula for the 5th centile for systolic blood pressure, at the 50th centile for height, is 2 times age in years plus 65, and for mean arterial pressure, 5th centile at 50th height centile, 1.5 times age in years plus 40 (Haque, Zaritsky 2007). In patients who are critically ill, particularly where there is raised intracranial pressure, higher values may be needed for the lower threshold of systolic blood pressure and mean arterial pressure.

The threshold for hypertension is also lower in children and adolescents. The diagnosis of hypertension requires the use of nomograms or tables to determine thresholds by age, sex and height (National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents 1996).

A heart rate of <60 beats/min is considered bradycardia in an infant, whereas a healthy adolescent may have a resting heart rate of <40 beats/min. Hence a heart rate considered normal for an adult can represent bradycardia in an infant. This is of relevance to poisonings resulting in bradyarrhythmias, such as from beta-blockers and calcium-channel antagonists.

Children may present differently from adults following envenomation from snakes or spiders (White 1995). In a small child there may be no history of a bite, and the child may present with symptoms of neurotoxicity (such as seizures), pain (from myotoxicity) and/or coagulopathy. Alternatively, the child may present with a progressive illness

Table 27.2 Examples of differences in drug dosing[a]

Drug	*Newborn infants (0–27 days)*	*Infants/toddlers (28 days to 23 months)*	*Children (2–11 years)*	*Adolescents (12–16/18 years)*
Aminophylline (IV)	6 mg/kg then 2.5 mg/kg every 12 h	5 mg/kg followed by 1 mg/kg per h	5 mg/kg followed by 1 mg/kg per h to 9 years then 0.8 mg/kg per h	5 mg/kg followed by 0.8 mg/kg per h to 16 years then 0.5 mg/kg per h
Omeprazole (oral)	0.7 mg/kg, once daily	0.7 mg/kg up to 20 mg, once daily	10–20 kg BW: 10 mg, once daily; >20 kg BW: 20 mg, once daily	20 mg once daily
Phenytoin	18 mg/kg (IV loading), then 2.5–5 mg twice daily (oral)	1.5–2.5 mg/kg twice daily (oral)	1.5–2.5 mg/kg twice daily (oral)	75–150 mg twice daily (oral)
Cyclophosphamide (IV)		500 mg/m^2 BSA once a month	500 mg/m^2 BSA once a month	500 mg/m^2 BSA once a month
Propofol (IV)		2.5–4 mg/kg	2.5–4 mg/kg to 8 years, then 2.5 mg/kg	1.5–2.5 mg/kg
Digoxin (oral)	Neonate <1.5 kg: 25 µg/kg in 3 divided doses for 24 h then 4–6 µg/kg daily Neonate 1.5–2.5 kg: 30 µg/kg in 3 divided doses for 24 h then 4–6 µg/kg daily Neonate >2.5 kg: 45 µg/kg in 3 divided doses for 24 h then 10 µg/kg daily	45 µg/kg in 3 divided doses for 24 h then 10 µg/kg daily	Up to 5 years: 35 µg/kg in 3 divided doses for 24 h then 10 µg/kg daily From 5 to 10 years: 25 µg/kg in 3 divided doses for 24 h then 6 µg/kg daily After 10 years: 0.75–1.5 mg in 3 divided doses for 24 h then 62.5–250 µg daily	0.75–1.5 mg in 3 divided doses for 24 h then 62.5–250 µg daily

BSA, body surface area; BW, body weight; IV, intravenous.
[a]Doses taken from *BNF for Children*: these are examples of starting doses for common drugs and are not intended as a dosing recommendation; see *BNF for Children* for complete dosing information.

with generalised symptoms such as lethargy, vomiting, mild pyrexia and neurological signs (e.g. paralysis and fixed, dilated pupils). There may be discoloration of the urine due to myoglobinuria.

Children are at greater risk from envenomation because of the relatively greater dose of toxin. Fatality rates for children are higher than for adults following snake bite (McGain *et al.* 2004). The risk of incurring a snake bite may also be greater in children. Children may develop signs of envenomation sooner than adults and require a higher dose of antivenom.

Clinical aspects

Assessment

History

A thorough history should be recorded for each episode of poisoning. The history should include time of ingestion, probable dose, the poisons the child was exposed to and the circumstances at the time (including extent of supervision, where the episode happened, who was there, where the poisons were stored and whose medicines were ingested). It is important to establish whether the history is consistent with the patient's developmental age. A child who is not rolling will not be able to move towards a poison hazard. A child who is not feeding himself or herself will not be able to take a poison independently.

Preformatted charts aid in the collection of clinical data, improving both accuracy and completeness of the data (Buckley *et al.* 1999). An example of a preformatted chart is presented in Figure 27.1. Data can be collected for patient management but also for epidemiology/accident prevention purposes. Electronic databases are useful for recording the data and for performing audits. Patient management software, as is commonly found in emergency departments and hospital in-patient departments, can be modified to improve data capture for toxicology (e.g. disease-specific data entry screens).

The clinical history of an episode of poisoning in young children is extremely unreliable (Hwang *et al.* 2003). Of children presenting with poisoning, around 60% have actually been exposed to the putative poison (Sugarman *et al.* 1997; Belson *et al.* 1999; Hwang *et al.* 2003). A history from an adult or another child is not more reliable than that from the child him-or herself. Physical evidence, such as an abnormal smell of the breath, staining of the clothes and symptoms/clinical signs consistent with poisoning, increases the positive predictive value to 100%, 86% and 92%, respectively (Hwang *et al.* 2003). Although routine urine drug screens are not useful, urine drug screens may be useful in detecting toxins in symptomatic patients without a history of ingestion or when ingestion is denied (Belson *et al.* 1999), in particular where drugs of abuse, e.g. cocaine or methadone, may have been ingested by small children.

Examination

The initial step in the physical examination of the poisoned child is the measuring and recording of the vital signs: temperature, pulse rate, respiratory rate, blood pressure and oximetry. Temperature is an important component of the paediatric general examination as it may indicate alternative diagnoses, such as infection. There are some poisonings that affect temperature, such as hypothermia with organophosphate poisoning, and hyperthermia with serotoninergic syndrome or the anticholinergic toxindrome. Pulse rate in children should be interpreted in relation to age-appropriate norms (Wallis *et al.* 2005). Abnormal pulse (or heart) rate is an important sign in toxicology, indicating cardiotoxicity. Bradycardia and tachycardia are defined at relatively lower pulse rates in children than in adults. Hence, there are different intervention points. Similarly, both diastolic and systolic blood pressure are lower in children than in adults. This results in different values for defining hypotension and hypertension, and the treatment decision points. Respiration rate is also higher in infants and children than in adults (Wallis *et al.* 2005). Oximetry is useful in assessing respiratory function, particularly when the poison is a sedative or decreases neuromuscular function (e.g. organophosphate poisoning). Arguably, in the context of child poisoning, blood sugar level is also a vital sign. Children are at greater risk of hypoglycaemia than adults (hypoglycaemia may be a differentiating feature in some childhood poisonings compared with adults). Hypoglycaemia, if uncorrected, may lead to tissue/organ injury and long-term morbidity. It is therefore important to identify and correct hypoglycaemia in childhood poisoning.

An assessment of conscious state is important in any toxicological examination because many poisons impair consciousness and this may lead to secondary injury due to impairment of respiration and airway. The Glasgow Coma Scale (GCS) is the most widely applied measure of

NEWCASTLE MATER MISERICORDIAE HOSPITAL

PLEASE USE GUMMED LABEL IF AVAILABLE

Surname: | Unit Number:

Other names:

Address:

Date of birth: | Mo:

TOXICOLOGY ADMISSION

First medical contact (eg LMO/St Elsewhere's Hospital/Ambulance):

Method of transport (to Mater):

Exposure/overdose date and time:

Presentation date and time (to first contact):

Admission date and time (to Mater):

Type of admission: Deliberate self harm ☐ Premeditated ☐ Currently suicidal ☐
Recreational use ☐
Accidental poisoning ☐
Iatrogenic toxicity ☐
Other poisoning ☐
Envenomation ☐

Substances ingested or exposed to (include alcohol and note other self harm eg. wrist laceration)

Substance	No	Tablet strength	Whose medication?	Date prescribed	Dose/day

Patient's normal medication (whether taken in overdose or not)

Drug	Dose/strength	Frequency

Where was substance taken in overdose stored?

Past History

Substance abuse (Tick the 1st box for type of substance abuse and the 2nd box if abuse is current)

Cigarettes ☐☐ Alcohol ☐☐ Benzodiazepines ☐☐ Amphetamines ☐☐ Narcotics ☐☐ Cocaine ☐☐

Cannabis ☐☐ Hallucinogens ☐☐ Barbiturates ☐☐ Volatile substances ☐☐ Antihistamines ☐☐ Other ☐☐

IV drug use (past or present) ☐ Hepatitis B positive ☐ Hepatitis C positive ☐ HIV positive ☐

Past suicide attempts ☐ if yes, how many: Past psychiatric contact (inpatient or outpatient) ☐

Epilepsy ☐ Past psychiatric diagnosis:

Other medical history:

Family history:

Figure 27.1 Preformatted chart for the collection of clinical data in cases of poisoning. (Continued overleaf)

state of consciousness, and can be modified for paediatric use. However, the GCS was developed for the assessment and monitoring of patients with head injury and is less useful in poisoning:

A neurological examination focusing upon pupils, nystagmus, muscle tone and reflexes should be performed. Pupil size and response can be important indicators of poisoning: dilated pupils occur particularly in anticholinergic poisoning, while contracted pupils occur in opioid poisoning. Unresponsive pupils can indicate profound coma, such as with barbiturate-induced coma. Horizontal nystagmus is commonly seen in sedative poisoning, and vertical nystagmus may be seen in addition to this with some anticonvulsants, e.g. barbiturates and carbamazepine. An oscillation of horizontal gaze (differentiable from nystagmus by a lack of directional component) may be seen in serotonin syndrome. Hypotonia can also indicate sedative poisoning or neuromuscular blockade (such as organophosphate poisoning). Deep tendon reflexes may be increased as a result of serotonin syndrome, while they are decreased with sedative poisoning or neuromuscular blockade.

Routine examination of the chest should be performed by listening to the sounds of the heart and breathing. Abdominal examination should be performed to determine liver size and condition, and whether there is any abdominal tenderness or rigidity and presence of bowel sounds. Presence of any signs indicating blockage of the intestine or perforation of the viscera would preclude the use of any gastrointestinal decontamination. The skin, scalp and mouth should be examined for any signs of

Examination (please note or tick)

Weight: Height: Temp: Pulse: Blood pressure: Skin appearance:

CVS:

Respiratory:

Resps/min: Apnoea ☐0 Cheyne-Stokes/Biot ☐1 <12 ☐2 12-29 ☐3 29-39 ☐4 40+ ☐5

Abdominal:

Bowel sounds: Normal ☐0 Absent ☐1 Reduced ☐2 Hyperactive ☐3

CNS: Drug paralysed at time of first CNS examination ☐

Eye opening	Best verbal response	Best motor response
0☐ None	0☐ None	0☐ None
1☐ To pain	1☐ Incomprehensible	1☐ Extending (decerebrate)
2☐ To speech	2☐ Inappropriate	2☐ Flexing (decorticate)
3☐ Spontaneous	3☐ Confused/screaming	3☐ Flexing (withdrawal)
4☐ Open	4☐ Orientated/dysarthric	4☐ Localizing pain
	5☐ Conversant/clear speech	5☐ Obeys commands or spontaneous movement
		6☐ Ambulatory

Level of Coma

0☐ Alert (no signs of CNS depression)
1☐ Drowsy (all patients who fall between alert and stuporous)
2☐ Stuporous (responds to verbal or tactile stimuli)
3☐ Coma 1 (responds to painful stimuli only, normal BP and resps)
4☐ Coma 2 (no response to painful stimuli, normal BP and resps)
5☐ Coma 3 (no response to painful stimuli, abnormal respiratory pattern and/or low but adequate BP)
6☐ Coma 4 (no response to painful stimuli, apnoea or inadequate respiration and/or inadequate BP)

Pupil size:	0☐ Normal	1☐ Dilated	2☐ Small	3☐ Pinpoint	
Pupil reaction:	0☐ Brisk	1☐ Sluggish	2☐ Absent		
Nystagmus:	0☐ None	1☐ Horizontal	2☐ Vertical	3☐ Both	4☐ Rotatory
Gag reflex:	0☐ Normal	1☐ Depressed	2☐ Absent		
Tone:	0☐ Normal	1☐ Hypertonic	2☐ Hypotonic		
Tendon reflexes:	0☐ Normal	1☐ Brisk	2☐ Depressed	3☐ Absent	
Seizure activity:	0☐ None	1☐ Myoclonus	2☐ Focal	3☐ Single G Mal	4☐ Multiple G Mal

Psychosis or delirium ☐ (hallucination, confusion, depression)

Other neurological signs (if any): _____

Investigations

FBC ☐ MBA ☐ ABG ☐ ☐ O_2 saturation_____% ECG ☐ QRS width_____msec CXR ☐

Blood ethanol ☐ Breathalyser_____ Urinary drug screen ☐ Paracetamol ☐_____at 4 hrs

Other drug or toxin level (specify): _____

Other investigation (specify): _____

Management (more than one box may be ticked. Tick first box for treatment prior to Mater presentation, 2nd box for treatment at Mater)

Decontamination:	Spontaneous emesis ☐☐	Ipecac emesis ☐☐
	Gastric lavage ☐☐	Activated charcoal ☐☐
	Repeated doses activated charcoal ☐☐	Whole bowel lavage ☐☐
Intubation:	Solely for lavage ☐☐	For continued management ☐☐

Antidotes (include specific antidotes given before presentation to Mater):

Antidote	Dose	Date and time commenced

Any other therapy (specify): _____

Figure 27.1 Continued.

injury. The general state of care of the child should be recorded, e.g. lack of hygiene, general state of hair, nails and clothing.

Use of laboratory analyses

Considerations for blood sampling

Children have smaller blood volumes than adults and there is greater difficulty in collecting serum, plasma and urine samples. The amount of blood that can be sampled from a child without the need for transfusion depends upon the initial haematocrit (Lister *et al.* 2008). Recommendations for paediatric clinical trials are for no more than 3% of blood volume to be sampled on any one study day in a child with a normal red cell mass (haematocrit) (Kauffman 2000). In an infant or toddler this would represent 2.4 mL/kg body weight. As a guide, children can tolerate sampling of 0.25 mL/kg per day without a fall in haematocrit (Lister *et al.* 2008). In critically ill neonates, it is standard practice to transfuse after 10–15 mL/kg has been sampled. In critically ill patients, there may be other influences decreasing the haematocrit, such as blood loss, haemolysis, and expanded intravascular and/or extracellular volume. The potential clinical effects of the poisoning upon blood volume, haematocrit and oxygen transfer will also need to be considered.

Consequently, smaller volumes are available for analysis. More sensitive assays are therefore required for the analysis of samples from children. Capillary electrophoresis (CE), and liquid chromatography–mass spectrometry (LC-MS) are newer methods that enable highly sensitive assays to be performed on small sample volumes.

Topical anaesthetics (lidocaine, prilocaine, tetracaine or amethocaine) are effective in reducing the pain of venepuncture in children aged from the neonatal period through to adolescence. Distraction through the use of play therapy and/or music therapy is an effective means for reducing the stress of venepuncture (Caprilli *et al.* 2007). However, it should be considered whether sampling is necessary and sampling should be avoided when the results will not contribute to patient management.

Samples obtained by heel-prick sampling yield results comparable with those obtained by venepuncture (Webb *et al.* 2007). Heel-prick samples are capillary blood as opposed to venous blood. Therapeutic drug monitoring of drugs such as ciclosporin and tacrolimus can be performed using capillary blood specimens because of a high correlation between capillary and venous concentrations. A pitfall in capillary blood sampling arises if the skin is contaminated with the analyte, e.g. if investigating an exposure where the child has had the putative agent on his or her hands and then a finger-prick specimen is obtained.

In the majority of cases of paediatric poisons exposure, blood sampling will not be necessary. However, where blood concentrations are decision criteria, e.g. paracetamol poisoning, or where intentional poisoning is suspected, then blood sampling will be necessary. Blood concentrations give information about the magnitude of exposure (i.e. they are quantitative), unlike urine concentrations which give less information about the magnitude of exposure (i.e. they are qualitative). Blood concentrations can be used to determine whether to intervene, (e.g. paracetamol, lead, theophylline) or whether to monitor treatment (e.g. phenobarbital).

Urine drug screens

Urine drug screens may be used to confirm exposure to poisons but are of limited utility in the immediate management of acute poisoning. Urine can be collected by the clean catch method in young children, and by using collection bags in infants. Bacterial contamination of the sample is common, and if a sterile sample is required from an infant then a catheter or suprapubic aspirate sample may be collected. Many of the agents involved in paediatric poisons exposure may not be detectable by routine urine drug screens (Hwang *et al.* 2003), the results of the test may not be available until after the clinical presentation has resolved, and the vast majority of paediatric poisons exposures do not pose a significant hazard. Hence, performing urine drug screens routinely is not justifiable. However, where there is a diagnostic dilemma, or where deliberate poisoning by a third party is suspected, urine drug screens are warranted.

When intentional poisoning is suspected it will be necessary to inform the clinicians responsible for the care of the patient, and the police or the coroner (depending on the circumstances). In most jurisdictions there is a legal requirement to report assaults on children to either the police or child protection authorities. Intentional poisoning would constitute an assault under most legal systems. It is the responsibility of the health professional who suspects that the assault has occurred to report the incident to the police/child protection authorities. Hence, reports should be made directly to the police/child protection authorities rather than to other health professionals. The police will be responsible for documenting the chain of evidence. However, the procedures will vary between legal jurisdictions and it will be necessary for the medical scientist to be familiar with procedures in each state or country in which they work.

Saliva

The rate of production of saliva is influenced by hydration status, food, drugs and diurnal variations. In general, drugs are transferred into saliva by passive diffusion and the saliva : plasma ratio of drugs is influenced by the physicochemical properties of the drug (such as pK_a, lipid solubility, molecular weight, spatial configuration and charge), the unbound plasma concentration of the drug and the characteristics of the saliva (production rate, pH, salivary binding proteins and salivary enzymes) (Aps, Martens 2005). Hence, salivary assays may be more useful in documenting exposure than in determining management criteria or the extent of exposure (see Chapter 18).

Hair

Hair analysis can be used to document exposure and give an indication of the timing of exposure in the medium term. When the timing of sampling is late, it may not be possible to detect drugs in blood or urine samples, but it may be possible to detect their presence in hair (Kintz *et al.* 2007). This approach has been used to detect drugs such as benzodiazepines, zopiclone, barbiturates, methadone, glibenclamide, trimeprazine and diphenhydramine (Kintz *et al.* 2006, 2007; Villain *et al.* 2006). More commonly, hair analysis has been used to demonstrate exposure to arsenic or heavy metals (such as mercury, lead, cadmium, chromium, and manganese). Samples of fingernail clippings and teeth ('milk' or deciduous teeth are shed naturally in children) can also be analysed to demonstrate heavy-metal exposure (see Chapter 19).

Neonatal hair can be analysed to determine intrauterine exposure to ethanol and nicotine as well as illicit drugs such as cocaine, methamfetamine, opioids, cannabinoids and benzodiazepines (Koren *et al.* 2008).

Meconium

In utero exposure to opioids, cocaine, benzodiazepines and ethanol can be determined from the analysis of meconium passed by the newborn (Lopez *et al.* 2009; Moller *et al.* 2010; Wang *et al.* 2010). ELISA and LC-MS methods have been reported, as has good correlation between meconium and hair samples. The results have been used by child protection authorities to establish *in utero* exposure to substance misuse, and also for population studies.

Treatment of poisoning

Treatment of poisoning in children requires dose adjustment for size and development. This is complicated by the lack of paediatric dosing information for many antidotes. Dosing information can be obtained from the *BNF for Children*, as well as from electronic resources such as Toxbase, TOXINZ and Poisindex. These electronic resources require a subscription in order to obtain access, but the poisons information services will invariably have access.

Decontamination, although once standard practice, is increasingly being matched to the risk posed by the exposure. Charcoal is used for carbon-based poisons, but is not effective for metals, strong acids or alkalis. Whole-bowel lavage can be used for slow-release preparations and for metals (e.g. lithium) (Buckley *et al.* 1995). However, there is a significant risk of aspiration with paediatric decontamination. Gastric lavage can result in aspiration in around 4% of children (Tibballs *et al.* 1985). Charcoal aspiration, particularly when nasogastric or orogastric tubes are used, is a hazard and can lead to respiratory failure and death (Golej *et al.* 2001). Whole-bowel lavage, with polyethylene glycol electrolyte lavage solutions, requires large volumes of fluid, beyond the capacity of a child to ingest orally. Hence, nasogastric or orogastric tubes are required. Care is also required for the correct placement of these tubes in order to avoid aspiration. It is accordingly important to consider the risk–benefit ratio of decontamination, and to avoid decontamination when no significant toxicity is predicted.

When considering decontamination, the hazard posed by the exposure should be assessed by determining the following:

- Does the agent have known toxicity?
- Has the child been exposed to a significant amount of the poison?
- Are there any confirmatory signs of exposure?
- Are there alternative safer treatments than decontamination, e.g. antidotes, intravenous fluids?

Some treatment protocols need to be modified for children. The administration of *N*-acetylcysteine requires relatively high amounts of dextrose solutions which for a child may result in water intoxication. For children, these administration protocols need to be modified to deliver smaller volumes of dextrose.

Conversely, some antidote doses are based upon the toxin load rather than the size of the patient. For example, with snake or spider bite envenomation, the dose of antivenom is based on the bite/quantity of venom rather than the size/age of the patient. In this case the dose should not be modified for children. However, there should be a lower threshold for treatment in children because the amount of toxin delivered by a snake bite is the same as that for an adult but the dose is proportionately higher because of their smaller body size (White 1995).

When a medicine is used in an off-label or unlicensed manner, it is important to make a clinical assessment of its appropriateness. For medicines that are in common use (i.e. when it is the recognised standard of care) or when good-quality evidence is available, the medicine should be prescribed in the normal manner (Gazarian *et al.* 2006). Where such evidence is lacking, the use of the medicine could be considered experimental or innovative, and measures such as peer-review and informed consent should be conducted prior to use.

Response to poisoning (pharmacodynamics)

Pharmacodynamic differences between children and adults have been described, e.g. prepubertal children show enhanced response to warfarin compared with pubertal children and adults (Takahashi *et al.* 2000). The insulin requirements of children with established type 1 diabetes (after the remission phase) are around 0.7–1.0 units/kg per day, increasing at puberty to around 1.4–1.6 units/kg per day in boys and around 1 unit/kg per day in girls, and then decreasing a few years after puberty to adult requirements of around 0.7–0.8 units/kg per day. These insulin dosing requirements are influenced by rates of growth. Selective serotonin reuptake inhibitors (SSRIs) appear to have less efficacy in children and adolescents than in adults, but this may reflect difficulties in diagnosing depression in this age group. Differences in drug response between children and adults may also parallel different susceptibilities to poisoning, e.g. with aspirin causing Reye syndrome and SSRIs causing aggressive behaviour (see below).

Aspirin

A 14-year-old boy presented with encephalopathy following the use of aspirin for symptom control during a mild influenza-like illness. Laboratory investigations demonstrated an elevated plasma ammonium concentration (>700 μmol/L), elevated AST (3355 U/L), elevated ALT (2488 U/L), elevated serum lactate (108 mmol/L) and a prolonged prothrombin time (23.6 s). He had a serum salicylate concentration of 232 mg/L. Despite intensive support he died as a consequence of cerebral oedema and tonsillar herniation. Postmortem findings included diffuse microvesicular steatosis and some macrovesicular steatosis (He *et al.* 2007).

The presentation is consistent with a diagnosis of Reye syndrome, which has been linked with aspirin. The mechanism of the interaction between aspirin and a viral illness to produce this condition has been linked with β-oxidation of fatty acids, whereby some individuals are predisposed to aspirin-inhibiting fatty acid metabolism (Deschamps *et al.* 1991; Glasgow *et al.* 1999; He *et al.* 2007). Cytokines can also downregulate some enzymes involved in exogenous and endogenous substrate metabolism. Reye syndrome was described predominantly in children, and this resulted in recommendations to avoid aspirin in children under the age of 12 years.

Selective serotonin reuptake Inhibitors

An 11-year-old boy was initially started on atomoxetine 25 mg/day, which was subsequently increased to 60 mg/day after 2 weeks, for the treatment of aggressive behaviour and attention deficit hyperactivity disorder (ADHD). After the increase in dose, he was noted to be increasingly agitated and to have an increase in mood swings. He was reported to have thrown an object at a teacher, and was uncontrollably agitated, crying and threatening to kill himself. These behaviours ceased when his medications were withdrawn (Paxton, Cranswick 2008).

When treated with SSRIs, adolescents and young adults are susceptible to treatment-emergent suicidal ideation and suicide-related behaviour (Reith, Edmonds 2007). This risk appears to be age related, with the vulnerable window being from adolescence through to early adulthood. Although this appears to be a class effect, the risk varies between individual chemical entities. Atomoxetine has its primary mode of action via the inhibition of presynaptic noradrenaline (norepinephrine) reuptake but it also has some effects on serotonin reuptake. Treatment-emergent suicidal ideation and suicide-related behaviour have also been reported with atomoxetine.

Other examples of different susceptibilities to poisoning include response to hypnosedatives and ethanol. Children are more susceptible to the respiratory suppressant effects of hypnosedatives. Ethanol intoxication in children has a higher risk of hypoglycaemia than in adults.

Conclusion

The approach to poisoning in childhood is determined by the nature and circumstances of the exposure, the stage of development of the child and the legal jurisdiction. Apart from the approach to sample collection, the toxicological analysis is similar to that for adults. For example, in a child who presents with suspected chloroquine ingestion (see the monograph on Chloroquine), a 1 g dose can cause death in a child compared with 3 g in an adult. Exposure to chloroquine could be confirmed by clinical signs (hypotension, QRS prolongation). Analysis of blood concentrations might be used to further confirm exposure, to make an assessment of dose, to predict outcome and to inform management (duration of observation, need for elimination enhancement). In paraquat poisoning the ingested dose might be relatively small in a child because of the unpleasant taste, and larger in an adult because of suicidal intent (see the monograph on Paraquat). Serum paraquat concentrations can be used as a guide to prognosis and as intervention criteria. Hence, the interpretation of the laboratory data can be placed within the paediatric context.

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CHAPTER

28 Sampling, Storage and Stability

S Kerrigan

Specimen selection, sampling, storage and stability

Appropriate selection, sampling and proper storage of biological evidence are important, yet sometimes overlooked, steps in forensic toxicology. These factors, in combination with drug stability, can profoundly impact the interpretation of results and the outcome of forensic casework. Criteria surrounding each of these are presented and discussed in the material that follows. Further reference to tissue sampling will also be found in other chapters in this book and will be cross-referenced within the text where appropriate.

Specimen selection

Selection of the appropriate specimen is a critical component of any toxicological investigation. Circumstances surrounding the case, the availability of specimens, the nature of the investigation and even legal or statutory issues may dictate which specimens are selected, and for what purpose. Timing is an important factor in specimen collection, particularly in antemortem cases where some drugs have short detection times and therefore limited detection windows; examples include detection of an elevated concentration of Δ^9-tetrahydrocannabinol (THC) in blood from an impaired driver, or of γ-hydroxybutyric acid (GHB) following an alleged drug-facilitated sexual assault.

Postmortem specimens pose additional challenges owing to autolytic and putrefactive changes. Timing is also important in death investigations because it becomes increasingly difficult to obtain good-quality specimens as the time between death and sampling (postmortem interval) increases. Factors such as embalming of the body, decomposition or burial can further complicate interpretation if tissues have been preserved, if specimens are putrefied, or if exhumation is necessary. In order to be able to select the appropriate specimen(s) the toxicologist should have access to the case history, autopsy records/pathologist's report and all other relevant documents.

Some of the important specimen selection considerations are listed in Table 28.1.

Collection and sampling

Specimen containers

It is important that the specimen container is appropriate for the intended use and does not compromise the analytical findings. Container size should be appropriate for the volume or weight of the specimen so that headspace is minimised. Typical specimen collection quantities are given in Table 28.2. Excessive headspace in the container can increase the chance of oxidative loss, volatilisation of analyte (e.g. ethanol and other low-boiling-point compounds) or salting out, which may occur if preservatives are present. Some analytes have a tendency to adhere to plastic or glass surfaces depending on their physicochemical properties. Silanisation of glassware can reduce adsorptive losses for drugs that are present at trace levels (10 μg/L or less). Although glass containers are preferred by many, disposable plastic containers are used routinely for a wide variety of postmortem tissues and antemortem samples, particularly urine.

If glass containers are used, it is important to make use of appropriate racks for storage and transportation. One of the major disadvantages of glass is the possibility of breakage, particularly during storage at low temperatures. In order to minimise sample loss, glass containers are preferred if volatile analytes such as solvents or anaesthetic gases are suspected. Plastic containers are more susceptible to interferences by plasticisers such as phthalates that might interfere with the analysis. The use of an inert plastic such as Nalgene decreases the likelihood of chemical interference, but it is good laboratory practice to evaluate all new specimen containers prior to routine use in the laboratory. If plastic containers are chosen, their integrity at low temperatures should be evaluated. Polystyrene is more susceptible to cracking at frozen temperatures than polypropylene vessels. Rubber septa or liners in screw-cap containers should be avoided and replaced with inert liners (e.g. polytetrafluoroethylene (PTFE), or Teflon) to reduce leakage and minimise drug adsorption.

Antemortem blood samples are generally collected into evacuated glass tubes such as Vacutainer or Venoject for forensic toxicology purposes. Collection of blood into similar glass collection vessels is also good practice in postmortem blood sampling. These tubes allow the sample to be collected into a vessel that already contains necessary additives to stabilise and preserve the matrix. Proper mixing is necessary when sodium fluoride or other additives are used to ensure that dissolution is complete.

Blood

Blood is one of the most important specimens of toxicological interest as it provides unique advantages over other matrices in terms of the wide variety of analytical methodologies available, the vast amount of published reference data for both antemortem and postmortem drug concentrations, and the interpretive value of the matrix from a pharmacological standpoint. However, antemortem and postmortem blood samples are notably different, and the site of the postmortem blood draw (central or peripheral) can be of critical importance. Determination of parent drug and metabolite concentrations (and their ratios) may also yield useful information pertaining to acute or chronic use. A summary of the common advantages and disadvantages of various specimens is given in Table 28.3.

Antemortem blood

Antemortem blood is collected by venepuncture, typically from the antecubital region of the arm, using a syringe or evacuated container (e.g. Vacutainer, Venoject). Prior to collection, an antiseptic wipe is often used to clean the collection site. Non-alcohol-containing antiseptic wipes such as Betadine (povidone–iodine) are preferred to avoid any contamination that could interfere with alcohol analysis (see Chapter 4). Although evacuated blood tubes are typically glass, plastic tubes have also been evaluated (Karinen *et al.* 2010).

Postmortem blood

Postmortem blood collected at autopsy is quite different from antemortem blood collected by venepuncture from both qualitative and quantitative standpoints. Postmortem blood may be more viscous, may contain numerous small clots or sedimented cells, has a lower pH (as low as 5.5 owing to protein degradation), may contain 60–90% water, and is subject to varying degrees of haemolysis. The site of blood

Table 28.1 Specimen selection considerations

- Ease of use
- Ease of specimen collection
- Presence of interferences
- Matrix effects
- Parent drug and/or metabolites
- Detection time
- Stability of the drug(s) in the specimen
- Putrefaction
- Potential for automated analysis
- Sample volume
- Indication of short-term or long-term drug use
- Reference data
- Interpretive value

collection should be clearly identified on postmortem specimens and blood from different sources should never be combined.

Central blood

Cardiac blood samples are ideally collected following opening of the pericardial sac, removal of the pericardium, and removal of the blood from the left or right chamber after the heart has been dried. Collection of central blood by insertion of a needle through the chest wall ('blind stick') is practised but is discouraged. Although central blood collected in this manner may be identified to the laboratory as 'heart blood', it may be contaminated with pericardial fluid, fluid from the pleural cavity, or blood that has drained from the pulmonary vein or artery or the inferior vena cava (Jones 2007). Blood collected in this manner is considered non-homogeneous. Central blood may contain elevated drug concentrations as a result of postmortem redistribution or contamination (diffusion) from other body compartments (Prouty, Anderson 1990; Yarema, Becker 2005), especially following blunt force trauma. Passive drug release from reservoirs such as the gastrointestinal tract, liver, lungs and myocardium may occur immediately after death; later, cell autolysis and the putrefactive process participate in redistribution (Pelissier-Alicot *et al.* 2003). Drug properties such as volume of distribution, lipophilicity, protein binding and pK_a play a role in the site- and time-dependent mechanisms responsible for postmortem redistribution. Drugs with high volumes of distribution and basic character appear particularly susceptible to postmortem redistribution and their cardiac blood concentrations should be interpreted accordingly. Postmortem redistribution can account for central/peripheral blood drug concentrations that differ by 10-fold or more. Redistribution is time and concentration dependent and is very difficult to predict. Cardiac blood is typically more plentiful than peripheral blood. Although cardiac blood can be a very useful specimen for screening purposes, the relationship between cardiac blood drug concentrations and antemortem blood drug concentrations is complex. Many toxicologists therefore advise against the use of cardiac blood for quantitative and interpretative work.

Peripheral blood

Femoral blood is the best specimen for use in postmortem testing (Chapter 10) and should be sampled wherever possible. Blood collection from a ligated vein that has been 'tied off' is least likely to be contaminated by other sources of blood or a result of release of drug from tissues and organs. Typically, however, a 'femoral stick' involves the collection of femoral blood from an unligated femoral vein in the groin area. Only a small volume of blood should be collected to avoid 'milking' the vein and drawing blood from other sources. Typically 10–20 mL of femoral blood can be collected. Over-sampling of blood from the femoral vein will draw blood from the inferior vena cava, and hence the liver, and from the larger iliac vein. Although sampling from a ligated vein is generally preferred, a comparison of drug concentrations in clamped and unclamped femoral vessels showed good correlation for eight drugs including selective serotonin reuptake inhibitors, benzodiazepines, antihistamines and one opioid (Hargrove, McCutcheon 2008). If femoral blood is not available, subclavian or iliac blood may be an alternative.

Blood clots

Following a fall or blunt trauma to the head, a victim may survive with circulation intact for several hours. Owing to the decreased circulation in the damaged region of the brain, drug or alcohol concentrations in blood clots (e.g. subdural, subarachnoid and/or epidural) may be influenced by incomplete metabolism. It has been suggested that intracranial

Table 28.2 Typical specimen collection quantities

Postmortem		*Antemortem*	
Specimen	***Quantity***	***Specimen***	***Quantity***
Blood, heart	25 mL	Blood	10–20 mL
Blood, peripheral	10–20 mL	Urine	25–100 mL
Urine	All	Amniotic fluid	5–30 mL
Bile	All	Breast milk	10–20 mL
Vitreous humour	All	Meconium	All
Cerebrospinal fluid	All	Hair	Pen-size lock
Gastric contents	All	Saliva	1–5 mL
Liver (remote proximity from liver)	All	Sweat	Microlitres (insensible sweat); 1–5 mL (sensible sweat)
Kidney	50 g		
Spleen	50 g		
Brain	50 g		
Lung	50 g		
Hair	50 g Pen-size lock (150–200 hairs or 50 mg)		

Sources: Dinis-Oliveira *et al.* (2010); Hepler, Isenschmid (2007); Kerrigan (2002); Kidwell *et al.* (1998); Skopp (2004); SOFT/AAFS (2006).

Table 28.3 Advantages and disadvantages of antemortem and postmortem biological specimens

Specimen	*Advantages*	*Disadvantages*
Amniotic fluid	■ Determination of prenatal drug exposure ■ Not readily adulterated ■ Minimal sample preparation ■ Relatively few interferences	■ Invasive collection ■ Risk of complications ■ Limited reference data ■ Collection by medical personnel
Bile	■ Ease of detection of certain drugs (accumulation) ■ Particularly useful for conjugated drugs	■ Complex matrix ■ Interferences due to bile salts and fats ■ Requires sample preparation/pretreatment ■ Limited reference data
Blood (AM)	■ Widely accepted matrix ■ Determines recent drug use (hours–days) ■ Related to pharmacological effect ■ Not readily adulterated ■ Extensive reference data	■ Invasive collection ■ Collection by medical personnel ■ Shorter detection time
Blood (PM)	■ See above (AM) ■ Reference data widely available ■ Central/peripheral blood drug ratios known for some drugs ■ Cardiac blood typically in plentiful supply but requires caution with interpretation	■ Susceptible to postmortem redistribution (central) ■ Susceptible to postmortem artefacts and interferences ■ Susceptible to contamination (e.g. trauma) ■ Quality of specimen highly dependent on collection protocol ■ Limited volume of peripheral blood
Brain	■ Particularly useful for lipophilic drugs, volatiles and centrally acting drugs	■ Non-homogeneous matrix ■ Drug concentrations vary by region ■ Complex matrix ■ Requires sample preparation/pretreatment ■ Limited reference data
Breast milk	■ Determination of neonatal drug exposure ■ Not readily adulterated ■ Many drugs present	■ Privacy, invasive collection ■ Limited reference data ■ Interferences due to high lipid content ■ Drug content varies with milk composition ■ Variable matrix
Cerebrospinal fluid	■ Determines recent drug use (hours–days) ■ Minimal sample preparation ■ Relatively few interferences	■ Invasive collection ■ Limited reference data
Gastric contents	■ Identification of acute ingestion/delayed absorption ■ Identification of pill fragments possible ■ Particularly useful for orally administered drugs/poisons	■ Non-homogeneous matrix ■ Complex matrix ■ Requires sample preparation/pretreatment ■ Requires total specimen collection for interpretation
Hair	■ History of drug use (months) ■ Readily available, easy collection ■ Low potential for donor manipulation ■ Useful for drug and non-drug analytes, e.g. metals	■ New technology ■ Recent drug use not detected ■ Environmental contamination ■ Potential for ethnic bias ■ Limited reference data
Kidney	■ Particularly useful for non-drug analytes, e.g. metals	■ Complex matrix ■ Requires sample preparation/pretreatment
Liver	■ Ease of detection of certain drugs (accumulation) ■ Interpretive value for some drugs ■ Reference data available	■ Complex matrix ■ Requires sample preparation/pretreatment
Lung	■ Particularly important for volatile analyses	■ Complex matrix ■ Requires sample preparation/pretreatment

table continued

Table 28.3 continued

Specimen	*Advantages*	*Disadvantages*
Meconium	■ Long-term window of drug exposure ■ Non-invasive sample collection	■ Non-homogeneous matrix ■ Complex matrix (waxy) ■ Interferences ■ Requires sample preparation/pretreatment ■ Limited reference data
Nails	■ Easy collection ■ History of drug use (months) ■ Particularly useful for metals	■ Limited data ■ New technology ■ Not yet widely accepted ■ Recent drug use not detected ■ Environmental contamination
Saliva	■ Readily available, easy collection ■ Parent drug present ■ Related to free drug concentration in plasma ■ Minimal sample preparation ■ Many drugs determined ■ Indicates recent drug use	■ New technology ■ Short drug detection time ■ Small sample volume (1–5 mL) ■ Potential for oral contamination ■ Collection method influences specimen pH and drug content
Spleen	■ Particularly useful for certain analytes if no blood is available	■ Complex matrix ■ Requires sample preparation/pretreatment ■ Limited data for most analytes
Sweat	■ History of drug use (weeks) ■ Cumulative measure of drug use ■ Parent drug present ■ Non-invasive collection ■ Less frequent drug testing required ■ Not readily adulterated	■ Newer technology ■ Potential for environmental contamination ■ High intersubject variability ■ Requires special collection device ■ Skin irritation and discomfort ■ Small sample volume ■ No pharmacological interpretation possible ■ Non-homogeneous matrix (sweat/sebum)
Urine	■ Widely accepted matrix ■ Easy collection ■ Plentiful supply ■ Amenable to automated analysis ■ Longer detection window than blood (days–weeks)	■ Potential for donor manipulation ■ Minimal parent drug ■ Not useful for quantitative analysis ■ Not related to impairment or pharmacological effect
Vitreous humour	■ Determines recent drug use (hours–days) ■ Related to pharmacological effect ■ Resistant to putrefaction ■ Interpretive value for ethanol-related investigations ■ Minimal sample preparation ■ Relatively few interferences ■ Useful for postmortem chemistry	■ Limited data compared with blood ■ Small sample volume

blood clots may serve as 'time capsules' prior to death, because they may reflect drug concentrations several hours prior to death, when an injury may have taken place.

Vitreous humour

Direct aspiration of vitreous humour using a hypodermic syringe may yield 2–3 mL of fluid per eye. The needle should be placed in the central globe and aspirated with gentle suction. Preservation with sodium fluoride is generally recommended. The eye is located within the protective environment of the orbit and, being essentially outside the body, is remote from other tissues. Vitreous fluid is therefore a particularly useful specimen owing to its anatomical isolation, affording it notable resistance in terms of microbial invasion and degradation, as well as being remote from the central organs and subsequently less susceptible to postmortem redistribution phenomena. Vitreous humour is particularly useful for cases involving digoxin or hydrophilic analytes including paracetamol (acetaminophen) and salicylates. The equilibrium that exists between blood and vitreous fluid is slower than with other extracellular fluids, which can result in a slight delay in uptake. Furthermore, only free drugs are able to leave the blood and enter the vitreous humour. Since eye fluid is sterile and less susceptible to microbial contamination and hence postmortem alcohol production, it is routinely used for ethanol determination owing to its interpretive value from the standpoint of postmortem alcohol production and the determination of the pre- or post-absorptive phase of ethanol use

(Honey *et al.* 2005). Vitreous humour is particularly useful for postmortem analysis of glucose, urea nitrogen, uric acid, creatinine, sodium and chloride. These are important analytes for the evaluation of diabetes, degree of hydration, electrolyte imbalance, postmortem interval and the state of renal function prior to death (Coe 1977, 1993). Sodium, calcium and chloride concentrations in vitreous humour during the early postmortem interval can be used to estimate antemortem serum concentrations. It is therefore important that sodium fluoride is not added to specimens requiring vitreous chemistries. For that reason, vitreous humour is frequently collected into two separate containers: one preserved (for drug and alcohol testing) and one unpreserved (for clinical purposes).

Cerebrospinal fluid

Cerebrospinal fluid (CSF) can be collected either by lumbar puncture at the base of the spine using a hypodermic syringe or by withdrawal of cisternal fluid by puncturing the base of the neck. Although there are limited published reference data for quantitative drug concentrations in CSF, this clear fluid comprising mostly water is amenable to most routine methods of toxicological analysis. CSF may be of particular importance in alcohol-related cases where no vitreous humour is available, particularly if postmortem alcohol production is suspected. Like vitreous humour, CSF is anatomically isolated and less prone to contamination and bacterial invasion. Although it is more plentiful than vitreous humour, the lack of plentiful reference data limits its usefulness. However, CSF may be particularly useful in surgical death investigations.

Bile

Bile is generally aspirated from the gallbladder using a hypodermic syringe. It may be necessary to tie off the gallbladder prior to collection if contamination appears to be an issue. Bile should be collected prior to the liver specimen to avoid contamination. Many drugs of forensic interest accumulate in the bile, particularly those that are heavily conjugated, such as opiates, benzodiazepines and cannabinoids. Bile may also be used in cases where chronic heavy-metal poisoning is implicated. However, owing to the presence of bile salts and fats, drug extraction from this matrix can be complicated and extensive extraction and clean-up procedures are often required.

Gastric contents

Gastric content is a potentially valuable specimen for analysis in postmortem and clinical cases. Unabsorbed drug or tablet fragments in the gastric contents may provide valuable information concerning ingested compounds and provide an excellent material for preliminary screening (Chapter 9) owing to the potentially large amounts of drug that may be present. The absence of a drug in the gastric contents does not necessarily preclude oral administration. Odours emanating from the gastric content can provide valuable clues about what may have been consumed, e.g. pesticides and cyanides. The entire contents of the stomach should be collected and weighed. Gastric contents are non-homogeneous and should be homogenised prior to sampling. Quantitative drug determinations should be interpreted within the context of the entire contents (total quantity, rather than concentration) and it is important to take into consideration the differing absorption rates of drugs based on their physicochemical properties as well as their formulations and coatings.

The presence of a drug in gastric contents, particularly at low concentration, does not necessarily indicate oral administration. Drugs may be absorbed into the stomach via gastric juices that are in equilibrium with blood or as a result of intranasal drug use. Basic drugs are more susceptible to this because they have a tendency to become trapped in the gastric compartment owing to the low pH. If heavy metals are suspected, gastric contents should be collected, together with intestinal contents. In cases of suspected poisoning where the patient may have survived for a few days in hospital prior to death and where drugs may have been metabolised and eliminated from the body prior to death, any stomach content collected and retained by medical staff may provide valuable information concerning drugs or poisons consumed.

Urine

In antemortem settings, a mid-stream urine sample is usually collected into a plastic container containing sodium fluoride as preservative (Chapter 9). In some settings it may be necessary to take precautions against specimen adulteration. In postmortem settings, urine is collected by insertion using a hypodermic syringe directly into the bladder under visualisation. Puncture of the abdominal wall should be avoided to reduce the possibility of contamination. Urine is a valuable specimen for both antemortem and postmortem drug testing because it is a relatively uncomplicated matrix. However, the multiplicity of factors influencing urine drug concentrations (e.g. urine volume, clearance, metabolism, pH and time of last void) generally means that, in isolation, these results have limited quantitative value. Exceptions to this rule may include ethanol determination in a second void. Care must be exercised when considering the interpretation of urine GHB concentrations as GHB is present as an endogenous compound formed as a by-product of metabolism and may also be produced as a postmortem artefact as a consequence of the breakdown of succinic acid semialdehyde.

Tissues

When tissues are sampled they should be collected quickly and placed immediately into airtight containers. This is particularly important if volatiles or inhalants are suspected. Liver, kidney, brain, lung and spleen are the most frequently collected postmortem tissues.

Liver

Liver is a particularly important organ because of the very large number of drugs that undergo hepatic metabolism and the fairly extensive published reference data that exist. To reduce the possibility of drug diffusion from the small bowel, tissue from deep within the right lobe is preferred (Drummer 2004). The concentrations of drugs and metabolites in liver are often elevated, hence this specimen has limited interpretive value. However, liver is particularly useful for highly protein-bound drugs and the comparison of liver/blood drug ratios may allow the differentiation of acute overdose from chronic drug use for some drugs.

Kidney

Most drugs pass through the kidney as a result of urinary elimination. Kidney is an important specimen in cases of suspected heavy-metal poisoning owing to accumulation in this tissue. The presence of heavy metals or ethylene glycol during toxicological tests may be accompanied by structural changes to the kidney that can be documented using histological tests.

Spleen

Spleen is an important specimen for cyanide or carbon monoxide analyses, particularly in fire-related deaths where blood may be compromised or unavailable. Lung and brain are valuable specimens in cases involving volatiles or inhalants.

Brain

Brain tissue is lipid rich and has a tendency to concentrate some drugs, particularly lipophilic analytes, narcotics and halogenated hydrocarbons (Skopp 2004). If quantitative drug brain concentrations are used, it is important to know the location of the specimen because the brain is a non-homogeneous matrix. Drug concentrations within the brain may vary several-fold from one region to another owing to its complex structure and differing composition. Brain is not widely used in routine toxicological analysis.

Muscle

Muscle is not routinely encountered, despite the fact that it frequently contains relatively high drug concentrations, particularly for substances with high volumes of distribution. Perfusion rates between sites and drug concentrations are not consistent, and drug concentrations must be interpreted accordingly. Muscle is encountered more frequently for

ethanol determination in the absence of blood, or during the investigation of a suspected injection site.

Hair

Hair has been used in a variety of antemortem toxicology settings to provide a history of drug exposure and has therefore found applications in workplace drug testing, in monitoring of persons on probation or on parole for drug use, in insurance testing to verify the truthfulness of statements made by applicants relating to whether they use drugs or are smokers, in child endangerment, in drug-facilitated sexual assault and in other types of criminal casework (Nakahara 1999; Kintz *et al.* 2006; Curtis, Greenberg 2008). Further discussion relating to the scenarios in which hair testing is employed is provided in Chapters 8, 19 and 27. One of the major advantages is the long drug detection window compared with many other specimens. Hair may allow drug exposure over several weeks or months to be determined, depending on the length of the hair. Segmenting the hair by length may allow an approximate timeline for exposure to be determined based on head hair growth rates of approximately 1 cm per month (Clauwaert *et al.* 2000). Hair should be cut as close as possible to the scalp from the posterior vertex region of the head, since this region shows least variation in growth rate. Typically a lock of hair equivalent to the thickness of a pen or pencil is collected. The colour, length, sampling site and any obvious cosmetic treatment of the hair should be recorded. The root (proximal) and tip (distal) sections of the hair should be clearly identified. Although head hair is the preferred specimen, hair from other sites (e.g. pubis, axillae) may be used, but interpretation of analytical findings may be more complex. The lock of hair is typically tied, wrapped in aluminium foil and stored under dry conditions in the dark at room temperature.

Hair is also a useful specimen in postmortem investigations where arsenic or heavy metals are suspected. Although postmortem hair analysis is not yet widespread, there is growing interest because it may provide valuable interpretive information pertaining to the chronological sequence of toxin exposure (Cirimele *et al.* 2002). Hair has also proved to be useful in cases where exhumation is necessary (Tsatsakis *et al.* 2001). If hair is collected *post mortem*, it should be sampled at the very beginning of the examination to reduce the risk of contamination.

Hair can provide complementary toxicological information. Issues with drug testing in hair include external contamination, ethnicity and pigmentation, chemical treatment and the use of appropriate cut-off concentrations. Contamination of the hair with drugs from other sources (external deposition, environmental contamination, sweat or sebum) is generally minimised by pretreatment of the sample using a variety of aqueous and organic rinses or wash steps prior to analysis.

Other keratinised specimens such as nails can also be used to determine long-term exposure to drugs or poisons, in particular heavy metals such as thallium, arsenic or lead. However, drugs are deposited into nails at a much slower rate. External decontamination procedures should be performed prior to analysis (see Chapter 19).

Injection sites

Excision of skin and tissue (muscle) may be necessary in postmortem investigation of a suspected injection site. Typically a cube of muscle and skin is removed for this purpose. However, it is important to compare the drug concentrations in the suspected injection site with those in a control specimen from the same individual where there is no evidence of injection. Injection sites are not always reliable indicators of drug administration since the presence or absence of drugs in injection site tissue is dependent on the type and depth of the injection. If the injection is made directly into a blood vessel, little drug is likely to remain in the surrounding tissue.

Entomological specimens

The potential use of insects for detecting drugs and other toxins in decomposing tissues has been demonstrated and reviewed (Introna *et al.* 2001). If insects or larvae are collected from human remains they should be frozen as soon as possible. Larvae rapidly eliminate drugs when removed from the food source. Drugs, metals and pesticides have been identified in entomological specimens including larvae and pupae. Following wash steps to remove external contamination, entomological specimens are homogenised and analysed in a manner similar to that for tissues.

Saliva

Saliva or oral fluid can be collected non-invasively by expectoration, by aspiration, by vacuum or by saturation of an absorbent swab (Kidwell *et al.* 1998). Detection times are comparable to those in blood. As much as 1.5 L of saliva per day is produced by the submandibular, parotid and sublingual glands inside the mouth. Secretions from a specific gland may be collected using a special device or by cannulation, but this is uncommon. Although specific gland secretions are advantageous from a standpoint of saliva : plasma ratio and reduced oral contamination, mixed saliva is typically collected for routine drug-testing purposes. Oral fluid can be collected non-invasively, conveniently and without invasion of privacy. Chewing an inert substance, such as Teflon tape or a rubber band, may increase salivation for the purpose of specimen collection. It should be verified that no adsorption takes place between the drug and the chewed substance. Acidic sweets or citric acid has also been used to stimulate glandular secretions. Care must be taken that residual food, drink or interfering substances inside the mouth do not interfere with the analysis. This is particularly important for drugs that are ingested orally or smoked.

Owing to the ease and non-invasive nature of specimen collection, saliva is of particular interest in workplace drug testing, for insurance testing and, more recently, for roadside impairment testing. Saliva contains serous fluid derived from plasma. This ultrafiltrate of interstitial fluid contains the unbound fraction of drug at concentrations that are typically proportional to those measured in plasma. However, the predictable relationship that theoretically exists between saliva and plasma drug concentrations is influenced by many factors such as saliva flow rate, which can complicate pharmacological interpretation (Crouch 2005). A more detailed discussion on saliva and oral fluid testing is provided in Chapter 18.

Sweat

Moisture loss via the skin and elimination of insensible (non-visible) sweat take place during normal breathing at a rate of 0.3–0.7 L/day. Sensible sweat refers to perspiration that is actively excreted during stress, exercise or extreme temperature, at rates of 2–4 L/h. About half the total volume of sweat is eliminated from the trunk of the body. The remaining fluid is lost from the legs or upper extremities and head in approximately equal amounts (Kidwell *et al.* 1998).

Sweat is usually collected using an adhesive absorbent patch that is placed on the surface of clean skin or by wiping the skin with a swab or gauze. Careful preparation of the skin is necessary prior to placement of a sweat patch to minimise external drug contamination or bacterial degradation of the drug once it has been retained. Use of a semi-permeable membrane to cover the absorbent pad prevents non-volatile components in the environment from penetrating the pad externally, but allows oxygen, water and carbon dioxide to diffuse through. Salts, solids and drugs that pass through the skin are trapped in the absorbent pad, where they are temporarily stored *in situ*, until the patch is removed.

Owing to the relatively small volume (μL) of insensible sweat secreted from a small absorbent area (typically 3×5 cm), patches are typically worn for several days on the outer portion of the upper arm or back. In practice most skin wipes or sweat patches contain a mixture of sweat and sebum, the oily secretion from the sebaceous glands. As with saliva, increased flow rates can influence the quantity of drug eliminated into sweat. This specimen is particularly useful for compliance testing or monitoring long-term exposure (weeks), which might be desirable in probation or parole settings.

Amniotic fluid

Amniotic fluid has been used to investigate prenatal drug exposure. Its collection (amniocentesis) typically takes place between weeks 16 and 20

of pregnancy. A needle is inserted through the abdomen into the uterus where there is the least chance of touching the placenta or the foetus. The collection of amniotic fluid is typically performed in conjunction with ultrasound visualisation in order to reduce the risk of damaging the developing foetus. Although complications are rare, miscarriage occurs in a very small percentage of women. Typically 5–30 mL of amniotic fluid is removed during the procedure.

Breast milk

During pregnancy, oestrogen and progesterone, secreted in the ovary and placenta, cause milk-producing glands in the fatty tissue of the breasts to develop and become active. The pituitary hormone prolactin stimulates the production of fluid (600–1000 mL/day) by the milk-secreting cells. Contraction of the myoepithelial cells surrounding the alveoli allows the milk to be expressed. For specimen collection purposes, a breast pump can be used. The matrix is somewhat non-homogeneous. Colostrum, a creamy white to yellow pre-milk fluid, may be expressed from the nipples during the last trimester of pregnancy and shortly after delivery. Many drugs are excreted into breast milk and the scientific and medical literature contains numerous citations of the presence of drugs in this matrix (Chapter 27). Drugs that are extensively protein bound may not readily pass into the milk, but emulsified fats contained in the milk may concentrate highly lipid-soluble drugs. The high lipid content and natural emulsifying agents present in breast milk mean that some sample pretreatment is often required.

Meconium

Meconium formation begins between weeks 12 and 16 of gestation. As the first faecal matter passed by the neonate, it is typically collected within 1–5 days of birth. Analysis of drugs in meconium may provide a relatively long-term history of drug exposure during pregnancy, in particular the last 20 weeks of gestation. It provides more complete and long-term information on drug exposure than neonatal urine or cord blood. The specimen is complex and non-homogeneous. All available samples should be collected and homogenised prior to analysis. Meconium and other important matrices involved in maternal–foetal medicine have been reviewed (Gray, Huestis 2007; Lozano *et al.* 2007).

Sample handling

Sample handling is an important consideration during the pre-analytical phase. Unlike a clinical setting, where the time between sample collection and testing is often very short, significant delays are common in a forensic setting. The pre-analytical phase may be considerable, spanning the time of death and/or discovery of a victim, autopsy and collection of specimens, sample storage, transport to the laboratory and subsequent storage prior to analytical testing. In antemortem toxicology settings, the time delay between an alleged offence and specimen collection may be short (e.g. minutes to hours in the case of most impaired driving cases) or long (e.g. hours to days in the case of some sexual assault cases). Following collection, antemortem specimens may be subject to similar delays due to shipping or transport of specimens, requests for testing made by the submitting agency and storage of samples prior to actual testing. Although the toxicologist must consider the time delay between the event (i.e. death, or committing or being the victim of an offence) and collection of a specimen for interpretation purposes, these delays are beyond the control of the laboratory. Measures can be taken, however, to preserve and maintain the integrity of specimens after collection. Sample quality plays an important role in the validity or usefulness of subsequent analytical determinations. Inappropriate sample preservation or storage may have a deleterious effect on qualitative and quantitative determinations.

Preservation and storage

Specimens should be stored at appropriate temperatures, with adequate preservative and in an environment accessible only to authorised personnel to ensure security and integrity. Short-term storage at refrigerated temperature (4°C) is recommended for most samples, or frozen (−20°C or lower) during long-term storage (more than 2 weeks). Exceptions to this include hair, nails or dried blood swatches on filter paper, which can be stored at ambient temperatures.

Whereas clinical specimens are typically unpreserved, the use of a chemical preservative is often warranted in forensic specimens. Preservation of blood samples with sodium fluoride (2% w/v) is routine in most laboratories. Commercial evacuated blood collection tubes (e.g. grey-top tubes) contain sodium fluoride as the preservative and potassium oxalate as the anticoagulant. These are the preferred evacuated blood tubes for antemortem forensic toxicology casework. Inhibition of microorganisms and enzymes with sodium fluoride is important for commonly encountered analytes such as ethanol, cocaine and others. Fluoride acts as an enzyme inhibitor and helps prevent glycolysis.

Commercial blood tubes may contain a wide variety of additives (citrate, heparin, EDTA, thrombin, acid citrate dextrose mixtures, clot activator, etc.). Although these tubes are designed for a variety of clinical uses, they are not the preferred specimen containers for drug-testing purposes. Laboratories frequently encounter these blood tubes when they are submitted from a hospital setting and special care must be taken when interpreting their results (LeBeau *et al.* 2000; Toennes, Kauert 2001). If an anticoagulant is to be used, potassium oxalate is preferred rather than alternatives such as EDTA, heparin or citrate. Antioxidants such as ascorbic acid (0.25% w/v) or sodium metabisulfite (1% w/v) are sometimes used to prevent oxidative losses, but these agents have the potential to act as reducing agents towards some drugs, in particular *N*-oxide metabolites, which may be transformed into the parent drug. In a similar fashion, adjustment of specimen pH is not generally favoured routinely, because, just as some drugs are alkali labile (e.g. cocaine, 6-acetylmorphine), others are acid labile. Sodium azide (0.1% w/v) is sometimes used as a preservative and antimicrobial agent in urine samples. Sodium azide should not be used if samples are to be analysed by enzyme-linked immunosorbent assay because it can interfere with horseradish peroxidase-mediated colorimetric detection.

Although the addition of preservative should be routine for most antemortem and postmortem blood samples, an aliquot of unpreserved postmortem blood is sometimes collected. For example, fluoride preservatives should not be used if organophosphorus chemicals are suspected since this accelerates chemical degradation (Skopp, Potsch 2004). Some drugs are known to be photolabile (e.g. ergot alkaloids such as lysergic acid diethylamide and the phenothiazines). Specimens known to contain photolabile drugs should be stored in amber vials or foil-covered containers, or otherwise protected from direct sources of light. Storage of tightly sealed appropriate containers at low temperature further inhibits sample loss. Short-term storage at refrigerated (4°C) and frozen (−20°C) temperatures is commonplace in most laboratories and repeated freeze–thaw cycles should be avoided.

Labelling and specimen transfer

All samples should be properly marked for identification with the case number, donor name, date and time of collection, signature or initials of the collector and specimen description. Tamper-proof containers and/or tape bearing the collector's initials and date of collection should be used. Specimens should be forwarded to the laboratory in appropriate leak-proof and tamper-proof packaging/shipping materials with all appropriate documentation (chain-of-custody forms, requisitions for testing, special requests, case information, medications list, police report, donor information/identifier such as date of birth or social security number, agency case number, pathologist/police officer name and contact information). Improperly packaged or identified materials should be returned to the submitting agency. Documentation accompanying the specimen(s) should list all of the specimens that were collected or available for testing. Once received by the laboratory, the specimens should be inspected and appropriately documented in terms of condition and quantity during the accessioning process.

Contamination

There are a variety of contamination sources for both antemortem and postmortem specimens. In addition to the potential contamination issues that may result from the use of containers and external factors, a number of important exogenous and endogenous sources of contamination should be considered.

Exogenous contaminants

Specimens collected into plastic containers are sometimes susceptible to phthalate interferences. Numerous plasticiser interferences such as dibutylphthalate may co-extract and interfere with analytical detection by gas-chromatographic or mass-spectrometric techniques, yielding characteristic phthalate ions. All plastic containers should be evaluated prior to widespread implementation. It should be noted that contamination from phthalates may occur during the analytical process through use of disposable pipette tips, solvent containers, solid-phase extraction cartridges, tubing and numerous other sources. However, environmental exposure to these substances from household items, food, beverages and other sources can produce detectable quantities of phthalate esters or their metabolites in biological specimens including blood, serum, urine and breast milk (Silva *et al.* 2005; Högberg *et al.* 2008).

Embalming fluids, which typically contain a variety of alcohols and aldehydes, are a potential source of contamination in postmortem casework. These fluids not only dilute any remaining fluid in the body, but also alter drug distribution in remaining tissues. Another potential source of contamination comes from reusable syringes and containers for postmortem specimen collection. Some cleaning fluids that are used for syringes may contain alcohols that can compromise the analysis of volatiles. This highlights the importance of analysing specimens from multiple sites and using disposable syringes wherever possible.

The principal concern with antemortem contamination arises from the intentional manipulation of the sample to mask the presence of drugs.This typically involves the substitution, dilution or adulteration of the biological specimen with a foreign substance. Donor manipulation occurs most frequently with urine samples in workplace drug-testing situations (see Chapter 3). As a result, specimen validity testing is required in some drug-testing programmes such as for federal employees under US Department of Health and Human Services (DHHS) guidelines. Initially, adulteration of urine for drug-testing purposes involved the use of crude household items such as soap, bleach, vinegar, ammonia or cleaning fluids. Although these substances met with some success, a wide variety of commercial adulteration reagents and kits is now widely available (Dasgupta 2007). A summary of in-vitro adulteration agents is provided in Table 28.4. Some of the most popular commercial products contain glutaraldehyde (fixative), pyridinium chlorochromate (PCC) or chromium(VI)-containing species (oxidant), nitrite (oxidant) or peroxide/peroxidase. In general, in-vitro adulterants can interfere with presumptive immunoassay tests, with the intention of producing false-negative results. However, some agents have the potential to interfere with confirmatory tests such as gas chromatography/mass spectrometry (GC-MS) as well. Although this is less likely, studies have shown that some reagents may produce lower than expected or negative results for some analytes. Adulteration detection products are available commercially. On-site or dipstick tests are available for nitrite, glutaraldehyde, pH, specific gravity, creatinine, bleach, PCC and oxidants.

Specimen dilution or in-vivo adulteration by ingestion of a substance to mask the presence of drugs is also encountered. This is commonly achieved by the ingestion of large quantities of fluid prior to the test or by administration of a diuretic. Examples of in-vivo adulteration agents are given in Table 28.5. Urine specimen substitution or dilution can be detected if specimen validity tests are performed. A specimen may be considered invalid if the pH is between 3 and 4.5 or between 9 and 11. It may be adulterated if the pH is less than 3 or greater than 11. The normal temperature range is 32–38°C. A specimen is considered dilute if the creatinine concentration is less than 200 mg/L and the specific gravity is less than 1.003.

Other sources of contaminants or unexpected analytes include pyrolytic breakdown products due to thermal degradation of drugs. These may be present due to pyrolysis during administration of the drug (e.g. anhydroecgonine methyl ester following crack cocaine use) or occasionally *in situ* during analysis if conditions are not properly controlled or evaluated. Other sources of contamination may arise from pharmaceutical impurities or adulterants and cutting agents that are incorporated into illicit drugs prior to sale.

Table 28.4 In-vitro adulteration agents

- Ascorbic acid
- Alcohols
- Amber-13 (hydrochloric acid)
- Ammonia
- Bleach
- Clear Choice (glutaraldehyde)
- Detergent or soap (surfactant)
- Drano
- Ethylene glycol
- Gasoline
- Glutaraldehyde
- Hydrogen peroxide
- Klear (potassium nitrite)
- Lemon juice
- Liquid soap
- Lime-A-Way
- Mary Jane Super Clean 13 (surfactant)
- Salt
- Stealth (peroxide/peroxidase)
- THC-Free (hydrochloric acid)
- UrinAid (glutaraldehyde)
- Urine Luck (chromium VI, oxidant)
- Vanish
- Vinegar
- Visine
- Water
- Whizzies (sodium nitrite)

Source: Kerrigan, Goldberger (2005).

Table 28.5 In-vivo adulteration agents

Diuretics

Prescription

- Thiazides **and** thiazide-like drugs (e.g. hydrochlorothiazide, metolazone)
- Carbonic anhydrase inhibitors (e.g. acetazolamide)
- Loop diuretics (e.g. bumetanide, furosemide, torsemide)
- Osmotic diuretics (e.g. mannitol)

Over the counter (OTC)

- Aqua-Ban
- Diurex
- Fem-1
- Midol
- Pamprin
- Premsyn PMS

Other

- Alcoholic beverages
- Xanthines (e.g. caffeine, theophylline, 8-bromotheophylline)
- Herbals **and** aquaretics (e.g. golden seal root, juniper)

Source: Kerrigan, Goldberger (2005).

Medical artefacts

Clinical therapy can sometimes produce medical artefacts that complicate toxicological findings. Medical artefacts are most common in postmortem cases where infusion pumps may continue to run after death, introducing high concentrations of drug in local body compartments. Access to hospital records and case information, and collection of peripheral blood, vitreous fluid and liver are particularly important in these types of cases. Other sources of medical artefacts may include organ harvest drugs such as the calcium-channel blocker verapamil, or papaverine, which is used to inhibit vasoconstriction during transplant surgery.

If living patients are administered fluids (e.g. saline) during clinical care, blood is only contaminated (diluted) with the infusion solution if it is collected downstream from the intravenous line. Blood circulation and equilibrium with tissues is rapid, so the administration of fluids does not usually influence drug or alcohol concentrations in blood if normal precautions are taken. If downstream collection is suspected, careful review of the medical records and/or measurement of the haematocrit to determine specimen dilution may be necessary.

Endogenous contaminants, artefacts and interferences

By their very nature, all biological specimens are subject to endogenous interferences, regardless of whether or not they are derived from living or deceased persons. More complex biological specimens such as blood, tissue or meconium will require more extensive sample preparation to remove these interferences than less complex matrices such as vitreous humour, or cerebrospinal or oral fluid. In general, however, antemortem specimens are somewhat less susceptible to endogenous artefacts or contaminants. Ethanol, GHB, carbon monoxide, cyanide and other short-chain alcohols can be metabolically produced *post mortem* (Skopp 2004). The formation of toxicologically significant concentrations of cyanide in postmortem tissue (Lokan *et al.* 1987) has been attributed to the conversion of thiocyanate to cyanide and the breakdown of protein (Curry *et al.* 1967). Although in some circumstances ethanol can be produced *in situ* in unpreserved antemortem fluids, the same is true to a far greater extent in postmortem specimens, particularly blood. Likewise, GHB is present in antemortem fluids at very low concentrations in the absence of a serious genetic disorder such as GHB-uria (Knerr *et al.* 2007). Differentiation of exogenous and endogenous GHB is complicated by specimen type, storage conditions, preservative and other factors. Many laboratories use a cut-off concentration to help differentiate the two, for example 10 mg/L in urine (Kerrigan 2002; LeBeau *et al.* 2007). Concentrations of GHB may increase in urine during storage, upon collection and storage of unpreserved blood, or in citrate-buffered antemortem blood (LeBeau *et al.* 2000). Although preserved antemortem blood GHB concentrations are typically lower than those in urine, numerous studies have shown forensically significant concentrations of GHB in postmortem blood. Postmortem urine and vitreous fluid appear to be less susceptible to this increase.

Major changes that occur after death produce autolytic changes and putrefaction by microorganisms. Invasion of microorganisms, particularly from the gastrointestinal tract into tissues and body fluids, occurs within hours at ambient temperature. Lipids, carbohydrates and proteins are hydrolysed by microbial enzymes, the pH of blood steadily increases, and the putrefactive amines, tyramine, tryptamines, phenethylamines and other endogenous substances are liberated.

Trauma is a non-preventable source of contamination in postmortem forensic toxicology. Rupture of organs or compartments within the body can compromise quantitative drug analyses owing to the mixing of fluids (e.g. of gastric contents with blood) or from the microbial action that occurs as a result. Postmortem alcohol production can also result in detectable quantities of ethanol as an artefact. Glycolysis and the presence of yeasts and microorganisms can convert a variety of postmortem substrates to ethanol. Although concentrations are typically low (<0.7 g/L), concentrations of 2 g/L and higher have been reported (Zumwalt *et al.* 1982; O'Neal, Poklis 1996). Postmortem alcohol production is influenced by many factors, including the time between death and sampling, environmental conditions (temperature, humidity, location), external factors (traumatic injury, incineration), the availability of an ethanol substrate and the extent to which microorganisms are available. Vitreous humour and urine provide complementary information that may assist with the interpretation of results. This highlights the importance of collecting a variety of specimens *post mortem*. Other short-chain alcohols can be produced by microorganisms *in situ*. Isopropanol has been documented as a postmortem artefact, particularly in drowning victims. Putrefaction can also complicate carbon monoxide determination in postmortem blood. Increases in the apparent concentration of carboxyhaemoglobin have been documented owing to the formation of methaemoglobin, a decomposition product that can interfere with spectrophotometric determination. Preservation of postmortem blood (and storage in the dark at 4°C or lower) has been recommended (Skopp 2004).

Stability

Drug stability can be influenced by many factors including the physicochemical properties of the drug, characteristics of the specimen or matrix, tendency to conjugate/deconjugate, specimen collection procedure (e.g. contamination with microorganisms), container selection (e.g. oxidation, adsorption), and the use of preservatives or other additives.

The majority of published drug stability studies focus on antemortem or non-biological matrices. Scientific findings for biological specimens are complex because drug instability is often matrix dependent and influenced by factors such as specimen pH and the presence of other substances in addition to external factors. In general, drug instability in any toxicological specimen is due to metabolic degradation, chemical transformation, or a combination of both (e.g. cocaine).

Drug stability in postmortem matrices poses an added level of complexity because conditions are less controlled and degradation of analytes may be accelerated owing to putrefactive decomposition, microbial invasion and the increased presence of bacteria, for example, the bacterial enzymatic conversion of morphine glucuronides to free morphine in blood (Carroll *et al.* 2000), and the bioconversion of nitrobenzenes by enteric bacteria (Robertson, Drummer 1998).

After a specimen has been collected, enzymes may remain active and continue to degrade or transform the drug *in vitro*. This process may take place *post mortem* inside the body, or after postmortem or antemortem specimens have been collected, during transportation to the laboratory and during storage. This is particularly important with esterases, which may further hydrolyse drugs post-collection unless they are inhibited by a preservative.

In general, drug instability arises as a result of moieties or functional groups that are susceptible to transformation, such as esters (e.g. 6-acetylmorphine, cocaine, acetylsalicylic acid), sulfur-containing drugs, photolabile drugs (e.g. phenothiazines, midazolam, lysergic acid diethylamide) or those with functional groups that are readily oxidized or reduced. Although instability typically leads to decreases in drug concentration, this is not always the case. Conjugated drugs, such as the glucuronides may deconjugate under some conditions, increasing the concentration of free drug. Depending on the collection, storage conditions, use of preservative, container type, matrix and other factors, the concentration of a drug at the time of assay may not be identical to the concentration at the time of collection. All toxicological results should be interpreted within this context.

Some drugs also exhibit a degree of thermal instability. This is a consideration for drugs that are subjected to elevated temperatures during administration (e.g. by smoking) and during analysis (e.g. by GC-MS). Pyrolysis products may be indicative of smoking if it can be shown that they are not produced during analysis, for example: anhydroecgonine methyl ester (AEME) following the use of crack cocaine; 1-phenyl-cyclohexene following phencyclidine (PCP) use; and transphenyl propene following metamfetamine use.

Ideally, drug stability should be evaluated in a number of ways: long-term stability in the specimen or matrix of interest; the effect of freeze–thaw cycles; short-term stability (typically refrigerated); and bench-top

(room temperature) stability. The kinetic variables governing instability are often matrix and temperature dependent and an understanding of any one of these (e.g. long-term stability of a particular drug in blood) does not necessarily imply predictable results under different conditions (i.e. short-term storage of the drug in urine).

It should be noted that, although there are many published studies and reviews of drug stability, these tend to focus on long- or short-term storage in common matrices such as blood, urine, serum or plasma. As the number of drugs of interest continues to grow and the variety of specimens becomes more diverse, the scientific literature becomes somewhat limited in terms of drug stability. The stability of drugs in non-traditional and non-biological matrices is beyond the scope of this discussion, but investigations in this area are ongoing. These include studies of the stability drugs in dry stains of biological origin (DuBey, Caplan 1996), in formaldehyde solutions following embalming (Tracy *et al.* 2001), and in hair fibres after exposure to cosmetic treatment such as bleaching, perming or straightening (Pötsch, Skopp 1996). Drug stability data are often confounded by the fact that a considerable number of studies report instability as an incidental or anecdotal finding, rather than as part of a formalised and well-designed investigation of stability. Guidelines for conducting stability experiments and the statistical evaluation of the results have been reviewed (Peters 2007).

Of all of the most commonly encountered drugs, cocaine is certainly the most notorious in terms of instability. Nevertheless, the vast majority of drugs are relatively stable or moderately so to the extent that one can make reasonable assumptions when interpreting the results. Stability for some of the most frequently encountered drugs is summarised below.

Amfetamines

Metamfetamine and amfetamine in urine samples preserved with sodium fluoride (1% w/v) were stable during long-term storage at −20°C for at least a year (Moody *et al.* 1999). A long-term study of whole blood stored over 5 years at room temperature showed erratic but significant decreases in metamfetamine between 3 months and 5 years of storage, ranging from 9% to 38% (Giorgi, Meeker 1995). Results suggested that amfetamine was perhaps less stable than metamfetamine. Samples were collected into 10-mL grey-top Vacutainer tubes with sodium fluoride (100 mg) and potassium oxalate (20 mg). Although both drugs are considered to be moderately stable, storage of blood samples at room temperature is not advised owing to the production of interfering substances.

The overall stability of the amfetamine class extends to many of the designer amfetamines, for example, 3,4-methylenedioxymetamfetamine (MDMA), 3,4-methylenedioxyamfetamine (MDA) and 3,4-methylenedioxyethylamfetamine (MDEA). Stability of these drugs was investigated in urine, blood and water for 21 weeks at −20°C, 4°C and 20°C (Clauwaert *et al.* 2001). Although all drugs were stable at −20°C, results were compromised in blood samples at 5 and 13 weeks when stored at 20°C and 4°C, respectively, owing to matrix degradation and interfering substances.

Cannabinoids

Decreases in cannabinoid concentrations in refrigerated and frozen samples are largely attributed to oxidative losses, temperature effects or lipophilic binding to containers. THC may decompose when exposed to air, heat or light. It can undergo hydrolysis to cannabidiol, or be oxidised to cannabinol as a result of exposure to air or acidic conditions. THC has been reported to be stable in refrigerated blood for 6 months and at room temperature for 2 months (Johnson *et al.* 1984). Binding of THC to hydrophilic surfaces, such as storage containers or rubber stoppers should be considered. For example, THC stored in blood collected in unsilanised glass tubes was stable for 4 days at room temperature and 4 weeks at −20°C. By comparison, similar samples stored in polystyrene tubes showed 60–100% decreases in concentration (Christophersen 1986). The principal metabolite shows greater stability than the parent drug. In one study, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) was stable in frozen urine preserved with sodium fluoride (1% w/v) for a year (Moody *et al.* 1999). However, this study used silanised glassware for urine sample storage, which may not be typical of storage containers for actual casework. Loss of THCA in urine is largely attributed to adsorption, or from foaming of the sample, which can account for losses as high as 89% (Dextraze *et al.* 1989). The use of a de-foaming agent (e.g. 2-octanol) can reverse these losses, but use is not routine. It has been suggested that, although the adsorptive losses of THCA from urine stored in plastic containers take place quickly (within an hour of collection), they are not significant enough to compromise the analysis (Stout *et al.* 2000). Furthermore, adsorptive losses of THCA in urine may be pH dependent, with greater losses occurring at acidic pH (Jamerson *et al.* 2005).

Deconjugation of cannabinoids, such as THCA-glucuronide, to THCA should also be considered (Skopp, Potsch 2004). Hydrolysis of the acylglucuronide results in increased concentration of the unconjugated metabolite. THCA-glucuronide was unstable in urine at 4°C and above, and at increasing urinary pH (Skopp 2004). Studies suggest that THCA-glucuronide is less stable in plasma than in urine (Skopp, Potsch 2002). Although no significant losses in THCA-glucuronide were seen in plasma or urine stored at −20°C, instability was documented following storage of plasma and urine at refrigerated and room temperature, in some cases within 2 days (Skopp, Potsch 2002).

Opioids

6-Monoacetylmorphine (6-MAM) is a labile metabolite of diamorphine due to hydrolysis of the ester bonds. It may undergo deacetylation to morphine during storage. Buprenorphine, codeine, fentanyl, hydromorphone, methadone, morphine, oxycododone, oxymorphone and tramadol were all stable in frozen plasma stored for almost 3 months and subjected to two freeze–thaw cycles (Musshoff *et al.* 2006). Free concentrations of morphine, codeine and methadone were moderately stable in frozen urine preserved with sodium fluoride over a year (Moody *et al.* 1999). However, total morphine concentrations under similar conditions may be less stable (Moriya, Hashimoto 1997). Long-term storage in preserved whole blood stored at room temperature showed significant increases and decreases (Giorgi, Meeker 1995) over 1–5 years. These results suggest important differences in stability between free and conjugated species.

The stability of glucuronidated morphine is of importance because ratios of free and total morphine are sometimes used for interpretive purposes. Marked differences in glucuronide stability exist between antemortem and postmortem blood. Morphine-3-glucuronide was stable in refrigerated antemortem blood preserved with sodium fluoride, but unstable in postmortem blood under the same conditions (Carroll *et al.* 2000). Hydrolysis of the glucuronidated species to free morphine increases with temperature, storage time and degree of putrefaction. Other studies have confirmed the stability of morphine-3-glucuronide in refrigerated antemortem blood and plasma for up to 6 months (Skopp *et al.* 2001). Storage of postmortem specimens at −20°C prevented *in vitro* hydrolysis of the glucuronide.

Phencyclidine

Studies suggest that PCP is a relatively stable drug, even when stored in blood at room temperature for up to 18 months (Levine *et al.* 1983). However, significant decreases in concentration were measured in preserved blood at room temperature over 5 years (Giorgi, Meeker 1995).

Cocaine

Of all of the common drugs of abuse, cocaine is certainly notorious in terms of stability. Both chemical and enzymatic transformations occur to produce hydrolytic products. Spontaneous conversion of cocaine to benzoylecgonine (BE) via the ester linkage occurs at physiological and alkaline pH. At pH 5, there were no measurable decreases in cocaine concentration at 40°C after 21 days, compared with a decrease of 40–70% in urine at pH 8 (Baselt 1983). Ester linkages have a tendency

to be alkali labile, and as a result the chemical transformation of cocaine to BE is increasingly favourable as the pH of the matrix increases. Although the addition of preservative does not prevent chemical hydrolysis, the kinetics can be inhibited by storage of samples at low temperature, or by pH adjustment. The latter is not favoured in routine casework because of the possibility that acid-labile drugs might also be present. Liver methylesterases are largely responsible for the enzymatic transformation of cocaine to BE and plasma pseudocholinesterase for the conversion of cocaine to ecgonine methyl ester (EME). Addition of a cholinesterase inhibitor such as sodium fluoride and reduced temperature are important precautions. Both BE and EME undergo further transformation to ecgonine, a polar metabolite. In unpreserved blood, hydrolysis of the phenyl ester predominates, yielding EME. Addition of sodium fluoride inhibits the production of EME but does not prevent chemical hydrolysis of cocaine to BE. Cocaine in blood stored at 4°C was undetectable within 3 days in the absence of preservative, and only 40–60% of the cocaine was detected in preserved blood and plasma after 21 days (Baselt 1983). There have been numerous studies on the stability of cocaine and its metabolites in various media and comprehensive stability studies are available (Isenschmid *et al.* 1989). In unpreserved blood, cocaine is hydrolysed to EME, whereas transformation to BE predominates in preserved blood. Although BE exhibits greater stability than cocaine, decreases in concentration are largely due to further hydrolysis to ecgonine. BE was shown to be stable in preserved urine for a period of at least 1 year when stored at −20°C (Moody *et al.* 1999).

Chemical hydrolysis of cocaine during analysis should also be considered. Liquid–liquid and solid-phase extraction of cocaine and metabolites from biological specimens routinely employ alkaline conditions during extraction or elution steps. These conditions may result in chemical hydrolysis of cocaine to BE as an artefact of analysis. Minimising the duration of exposure and appropriate use of deuterated internal standards is recommended.

Antidepressants

Studies in serum have shown the tricyclic antidepressants to be moderately stable. Amitriptyline, imipramine, clomipramine and doxepin are relatively stable in serum samples stored at −25°C for 3–6 months or at 4°C for 7 days (Rao *et al.* 1994). Newer antidepressants, including reboxetine, sertraline and venlafaxine, were also stable in frozen plasma. Significant decreases in concentration were seen for sertraline, desmethylsertraline and reboxetine when stored at room temperature for more than 7 days (Heller *et al.* 2004). Atomoxetine, citalopram, fluoxetine, mirtazepine and paroxetine have also shown moderate long-term stability when frozen (Peters 2007).

Neuroleptics

Among the newer atypical neuroleptics, quetiapine and olanzapine have been shown to exhibit significant instability at room temperature (Heller *et al.* 2004). Although both were stable for a few days, quetiapine concentrations decreased by as much as 50% after 14 days and olanzapine was undetected in some samples. However, the majority of neuroleptic drugs, including olanzapine and quetiapine, were stable in frozen plasma at −20°C for 1 month after three freeze–thaw cycles (Kratzsch *et al.* 2003). Phenothiazines exhibit a pH-dependent photosensitivity. Furthermore, increases in chlorpromazine and thioridazine concentrations in patient samples have been documented and may be attributed to a conversion of the metabolite back to the parent drug during storage (Davis *et al.* 1977; Holmgren *et al.* 2004).

Benzodiazepines

Benzodiazepines including alprazolam, chlordiazepoxide, clonazepam, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, temazepam and triazolam were stable in plasma for 1 month at −20°C (Kratzsch *et al.* 2004). Diazepam was found to be stable in blood stored at room temperature or refrigerated over a period of 5 months (Levine *et al.* 1983). In contrast, diazepam and temazepam were unstable in postmortem blood under putrefying conditions. In general, benzodiazepines with a nitro group (e.g. clonazepam, nitrazepam, flunitrazepam) are among the most unstable owing to reduction of the nitro group. Additives that inhibit reduction (e.g. 2% w/v sodium metabisulfite) slow the degradation. Postmortem conversion of nitrobenzodiazepines to their respective 7-amino breakdown products may also occur as a result of anaerobic bacterial action (Robertson, Drummer 1995, 1998). Chlordiazepoxide, which contains an *N*-oxide functionality, is also unstable in whole blood. At room temperature, chlordiazepoxide rapidly decreased and was undetectable by day 8 (Levine *et al.* 1983). Sodium fluoride inhibits the degradation of chlordiazepoxide to nordiazepam and demoxepam, but does not completely prevent it. Storage at low temperatures, preferably frozen, is recommended (Drummer, Gerostamoulos 2002; Peters 2007).

Lysergide

Lysergide (LSD) is photolabile and specimens suspected of containing LSD should be protected from the light. Decreases in drug concentration have been documented in blood, serum and urine, with and without sodium fluoride. In one study, however, LSD concentrations in urine were stable for 4 weeks at room and refrigerated temperatures (Francom *et al.* 1988).

Ethanol

During storage, ethanol concentrations may increase or decrease. Ethanol losses are largely attributed to evaporation, chemical oxidation and microbial consumption, whereas increases are largely due to microbial conversion of substrates to ethanol. Although measured increases in blood ethanol concentrations have been documented under some conditions, this is inhibited by the addition of sodium fluoride as preservative and storage at refrigerated temperatures. Ethanol was stable in fluoridated blood for 2 months at room temperature (Glendening, Waugh 1965). Even after storage for 1–3 years at room temperature, average decreases at room temperature were 0.4 g/L. Average losses following storage of blood at room temperature for 3 and 6.75 years were 0.19 and 0.33 g/L, respectively (Chang *et al.* 1984). In one study, loss of ethanol was evident in blood contaminated by *Pseudomonas*. Although this was not prevented by 1% sodium fluoride, increasing the quantity of preservative to 2% did prevent ethanol loss (Dick, Stone 1987).

Urine is less susceptible than blood to *in vitro* ethanol production except in rare instances. Urine samples treated with microorganisms known to produce ethanol did not produce increases in ethanol concentration greater than 0.2 g/L, even following incubation at 37°C (Blackmore 1968). The use of preservative and refrigeration of urine samples is effective in terms of maintaining ethanol stability. Exceptions have been noted, but are rare. For example, a dramatic increase in ethanol concentration was documented in the urine from a diabetic patient found to contain *Candida albicans*. The increase in ethanol concentration was also accompanied by a significant decrease in glucose concentration (Ball, Lichtenwalner 1979).

γ-Hydroxybutyric acid

In addition to GHB being present in a variety of biological specimens as an endogenous substance, *in situ* production of GHB during storage has been documented and widely studied. In general, increases in GHB concentration are more pronounced in postmortem specimens. The concentration of GHB in an unpreserved postmortem blood sample stored under refrigerated conditions for 4 months approached 100 mg/L. GHB increases in postmortem urine are less pronounced and typically an order of magnitude lower, even in the absence of preservative (Berankova *et al.* 2006). Antemortem samples are much less susceptible to *in situ* production over time (Kerrigan 2002; LeBeau *et al.* 2007). Nevertheless, storage at refrigerated temperature, use of sodium fluoride as preservative and analysis at the earliest possible interval are recommended wherever possible.

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CHAPTER

29 Extraction

T Stimpfl

Introduction

The process of extraction is the first step in the qualitative and quantitative analysis of drugs and poisons from complex biological specimens. The large group of substances that are toxicologically relevant (pharmaceuticals, herbicides, pesticides, etc.) differ greatly in their physicochemical properties. They are often potent agents that are present only in very low concentrations. Therefore, a chosen extraction procedure must not only be able to separate the target substances from interferences in the specimens (purification) but also be able to increase their concentration relative to co-extracted matrix compounds (enrichment). The extraction process is often time-consuming and labour intensive, but practical experience shows that the effort is necessary because the subsequent analytical steps are based on, and benefit from, sample preparation. In short, proper extraction increases the chance of a successful analysis of drugs and poisons (e.g. there are fewer interferences in gas chromatography–mass spectrometry (GC-MS) and there is less ion suppression in liquid chromatography–mass spectrometry (LC-MS)). Cleaner extracts also reduce the 'down-times' of sensitive and cost-intensive analytical instruments by increasing service intervals.

Because of the large number of toxicologically relevant compounds and the different questions that need to be answered in the various fields of analytical toxicology, a universal, standard extraction procedure does not exist. Optimised extraction procedures are available for many drugs and poisons and these can be found in this book or through a comprehensive review of the literature. However, the toxicologist will often be confronted with compounds for which no appropriate extraction procedure has been published, necessitating the development of a new procedure based on available data of the target compound (most of which can be found in the individual monographs of this book). In a worst-case scenario, neither the physicochemical properties of the target compounds nor the composition of the specimen is known. A challenging example from the field of postmortem forensic toxicology would be the undirected search for a 'general unknown poison' in a putrefied tissue sample from a homicide case. In such a case, a non-selective extraction procedure covering a wide variety of possible target compounds would be required.

Choosing the best extraction procedure for a particular case and, when necessary, developing a new extraction method that correctly addresses specific case challenges are essential skills of the analytical toxicologist. These will be addressed in this chapter.

The fundamental principles of extraction

In order to develop a successful extraction procedure, some fundamental principles must be considered. An overview of the principles underlying extraction techniques is presented to enable the reader to make use of the data in the individual monographs for drugs and poisons that are included in this book. Comprehensive details about these principles can be found in the literature (see Further reading at the end of this chapter).

The following fundamental principles of extraction are covered in this section: solubility, partition coefficient, dissociation constant, ion-pair extraction, adsorption mechanisms, extraction efficiency recovery and the use of internal standards.

Solubility, distribution equilibrium and partition coefficient

The ability of a substance to dissolve in a particular solvent is referred to as its solubility and approximate values for individual drugs and poisons (from very soluble to insoluble) are given in the monographs. Solubility is temperature dependent and, in most cases, the solubility of organic compounds increases with an increase of temperature.

When a low concentration of a substance is dissolved in water, its extraction into an organic solvent is based on the non-ionised substance's distribution between the aqueous phase and the solvent (which may not be water miscible). Upon reaching equilibrium (according to the Nernst equation) the concentration ratio of the substance, distributed between organic solvent and water, is constant. On this basis, an apparent partition coefficient (K_p) for a particular substance in a two-phase system (e.g. octanol/water) can be defined as:

$$K_p = C_o / C_w$$

where C_o = concentration in the organic solvent and C_w = concentration in water.

The decimal logarithm of the partition coefficient for the octanol/water system gives log P values (see monographs). For ionisable substances, these values are pH dependent.

Examples:

- Tetrahydrocannabinol: log P (octanol/water) = 7.6
- Codeine: log P (octanol/pH 7.4) = 0.6.

These log P values can be a very useful indication of the likely success of extracting a drug from an aqueous medium into an organic solvent, i.e. the greater the value of log P the more likely the solute is to be extracted into an organic solvent.

Equilibrium of electrolytic dissociation, dissociation constant and state of ionisation

In the case of direct liquid–liquid extraction from an aqueous phase, the distribution behaviour of neutral substances can be estimated on the basis of their solubility in different solvents. However, a great number of drugs are acidic, basic or amphoteric compounds (mainly weak electrolytes), resulting in pH-dependent electrolytic dissociation.

At a given pH value, an equilibrium of electrolytic dissociation will be reached in the aqueous phase, which can be described by the acid dissociation constant (K_a) for acidic compounds (HA) and a corresponding dissociation constant (K_b) for basic compounds (B):

$$K_a = [H_3O^+]\ [A^-]/[HA]$$
$$K_b = [BH^+]\ [OH^-]/[B]$$

For practical reasons, the negative decimal logarithm of the dissociation constant is calculated (as $pK_a = -\log_{10} K_a$). These pK_a values are widely used and many of them can be found in the drug monographs. The pK_a values of basic compounds can easily be calculated using their corresponding pK_b values:

$$pK_a = pK_w - pK_b$$

where pK_w = negative decimal logarithm of the dissociation constant of water, which is 14 at 25°C

Examples:

- CH_3COOH: $pK_a = 4.75$
- CH_3COO^-: $K_b = 9.25$
- NH_4^+: $pK_a = 9.25$
- NH_3: $pK_b = 4.75$.

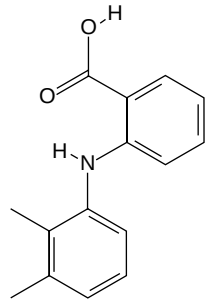
In order to estimate the distribution behaviour of acidic, basic or amphoteric substances for direct liquid–liquid extraction, their state of ionisation has to be considered. This is because only undissociated molecules are extracted into an organic phase (with the exception of ion-pair extraction, which will be discussed later).

According to the Henderson–Hasselbalch equation, an acidic or basic substance is ionised to the degree of 50% at a pH value equal to that substance's pK_a value. For acidic substances, nearly total ionisation (99%) is reached two full pH units or more above their pK_a values, and almost no ionisation (1%) is observed two full pH units or more below their pK_a values.

Conversely, for basic substances 99% ionisation is reached two full pH units or more below their pK_a values, and 1% ionisation is observed two full pH units or more above their pK_a values. Therefore, with regard to the extraction yield of weakly acidic and basic substances using direct liquid–liquid extraction it can be concluded that:

- For acidic substances, the maximum extraction yield is observed two pH units or more below their pK_a values.

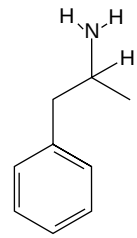
  Thus to extract mefenamic acid (pK_a 4.2) from an aqueous solution, it should be acidified with sulfuric acid to give a pH value of <1. Sulfuric acid should be preferred over hydrochloric acid when separating acidic from basic drugs as many drug hydrochlorides (e.g. methadone hydrochloride) are soluble in organic solvents.



Mefenamic acid

- For basic substances the maximum extraction yield is observed two pH units or more above their pK_a values.

  Thus to extract amfetamine (pK_a 10.1) from an aqueous solution, it should be made basic with sodium hydroxide solution to give a pH value of >13. Ammonia solution will give an aqueous solution pH of only about 10, which is not sufficiently basic for a successful extraction.



Amfetamine

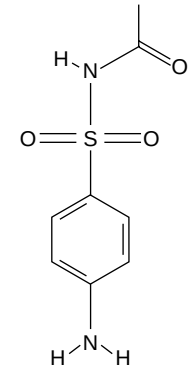
The distribution behaviour of amphoteric organic electrolytes (e.g. with one acidic and one basic function) also depends on their state of ionisation, but extraction yields are more difficult to predict.

Amphoteric substances with one weak acidic and one weak basic function ($pK_a + pK_b > 18$) can be extracted as non-ionised molecules at a pH range around their isoelectric zone, where the ionisation of the basic group has ended and the ionisation of the acidic group has not yet begun. In the special case that $pK_a + pK_b = 18$, the isoelectric zone shrinks to a particular point (isoelectric point) at which the molecule is not ionised.

Example:

- Sulfacetamide: $pK_a = 5.4$ and $pK_b = 12.2$ ($pK_a + pK_b = 17.6$).

  To a great extent the molecule is not ionised at pH = 3.6. (This value lies about two pH units above $pK_a = 1.8$, corresponding to $pK_b = 12.2$, and about two pH units below $pK_a = 5.4$.)



Sulfacetamide

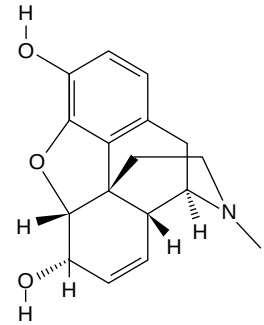
Amphoteric substances with one weaker and one stronger acidic and basic function ($pK_a + pK_b$ = between 18 and 10) can be extracted as non-ionised molecules at a pH range around their isoelectric point with a lower extraction yield because the molecule is also partly a zwitterion (i.e. the molecule has both its acidic and its basic functions ionised).

Example:

- Morphine: $pK_a = 9.9$ and $pK_b = 6.0$ ($pK_a + pK_b = 15.9$).

  The molecule is partly non-ionised at pH = 8.9 (this value lies about one pH unit above $pK_a = 8.0$, corresponding to $pK_b = 6.0$, and about one pH unit below $pK_a = 9.9$); at this pH value there also exist zwitterions that will not be extracted and which therefore reduce the overall extraction yield.

  Thus morphine is best extracted from aqueous media by using a freshly prepared, saturated sodium hydrogencarbonate solution, or similar compound, to give a pH value between 8 and 9. The high salt concentration of the saturated aqueous solution reduces the drug's aqueous solubility and therefore improves extraction efficiency.



Morphine

For amphoteric substances with one strong acidic and one strong basic function ($pK_a + pK_b = 10$ or < 10), zwitterions are to be expected, which results in a limited extraction yield.

Ion-pair extraction

Strong electrolytes (e.g. compounds with a quaternary ammonium function, such as paraquat) and very hydrophilic compounds (e.g. sulfonamides) tend to stay in the aqueous phase and will not be sufficiently extracted by organic solvents. Liquid–liquid extraction can be accomplished only by 'ion-pair' formation with appropriate counterions. These ion pairs are much more soluble in organic solvents than the drugs themselves and so assist an efficient extraction. Anionic dyes such as bromothymol blue, bromocresol green and bromophenol blue are widely used for this purpose. Moreover, solvents such as chloroform and dichloromethane can extract basic substances as hydrohalogenides, perchlorates, nitrates, phosphates, sulfates or thiocyanates. Conversely, the extraction of acidic drugs can be improved by using quaternary ammonium compounds such as cetyltrimethylammonium bromide as ion-pairing compounds.

Adsorption

Extraction of non-volatile drugs and poisons from biological specimens can also be accomplished by adsorption and the fundamental principles discussed above form the basis for adsorption methods as well. When adsorption is used, intermolecular interactions (van der Waal's forces, aromatic interaction) or electrostatic interactions will enable the isolation of target compounds from the biological matrix.

Hydrophobic interactions between neutral or non-polar substances are based on dispersion forces and are short-ranged, weak interactions between molecules or parts of molecules (bond strength 4–20 kJ/mol). They result from random fluctuations in local electron density distribution within molecules.

Hydrophilic interactions between polar functional groups or two dipoles are based either on hydrogen bonding (e.g. hydroxyls, carboxylic acids, amines), or on dipole–dipole interactions. Like dispersion forces, they too are short ranged and relatively weak forces (bond strength 10–40 kJ/mol).

Aromatic interactions are caused by attractive forces between diffuse electron clouds in π systems; their bond strength is similar to dispersion forces.

Electrostatic interactions between oppositely charged molecules have the highest bonding energies (200–1000 kJ/mol) and ionic bonding can be used for extraction via ion exchange.

Extraction efficiency, recovery and internal standards

On the basis of the fundamental principles described above, and on the data that can be found in the individual monographs of this book, extraction methods can be developed and optimised with respect to extraction efficiency. With a high extraction efficiency for the target compound, it should then be possible to create a complete analytical procedure with sufficient overall recovery (a complete procedure includes sample pre-treatment, extraction, fractionation, purification, evaporation, chromatographic separation, detection, identification and quantitative determination). The overall recovery can be calculated as the percentage of the analyte response after sample work-up compared with that of a solution containing the analyte at a concentration corresponding to 100% recovery. A low overall recovery can be tolerated only as long as the data for limit of quantification (LOQ) and limit of detection (LOD), precision and bias are acceptable (this is why recovery is not an essential part of method validation). However, it is good practice to determine recovery at high and low concentrations and ensure that it is greater than 50% to confer robustness to the analytical procedure.

For the quantitative determination of the analyte, and in order to monitor the whole procedure when biological specimens are extracted, an internal standard has to be added at the earliest possible stage. The internal standard must mimic the physicochemical properties of the analyte as closely as possible and must follow the target compound through the entire process of extraction and subsequent analytical steps in order to compensate for any loss of the target compound.

The selection of the internal standard can be a difficult task and each individual step of the whole analytical procedure should be carefully considered when making this decision. Even minor differences in the physicochemical properties between the target compound and the internal standard can result in errors, because of their different behaviour during extraction, fractionation, purification and concentration towards the applied chromatographic system, the reagents for derivatisation or their response to the detection system.

When mass spectrometry is used, stable-isotope-labelled analogues of the target compounds are the best choice as internal standards. For other analytical techniques or, if stable-isotope-labelled analogues are not available, alkyl analogues (because of their similar structure) can be used as an alternative; but it should be kept in mind that some alkyl analogues – such as morphine and codeine (methylmorphine) – can show significant differences in their physicochemical properties (the additional methyl group in codeine inactivates the phenolic function that is active in morphine). If alkyl analogues are also not available, the internal standard should be chosen on the basis of similarity in structure and functional groups (preferably from the same substance class, e.g. benzodiazepines). When glucuronides are analysed, the internal standard should also be a glucuronide. Only if there are no alternatives should active drugs be used as internal standards; in this case the presence of the active drug used as the internal standard would have to be tested for separately in the specimen. The following examples illustrate some specific considerations that should be kept in mind when choosing the internal standard: when GC is used, attention should be paid to volatility, thermal stability and reactivity with the possible derivatisation reagent; for LC, the solubility in the injection solvent (e.g. mobile phase of the LC) and the response to the detection system (e.g. UV spectrum), as well as the applicability of the ionisation technique for LC-MS should be considered. Sometimes simple problems at the beginning of the procedure can cause errors, such as the solvent used to add the internal standard to the sample (e.g. problems because of protein precipitation, or different extraction of the previously dissolved internal standard and the target compound from the sample matrix). Moreover, the added concentration of the internal standard should be similar to the expected concentration of the analyte.

In conclusion, stable-isotope-labelled analogues of the target compounds mimic the analyte very closely and therefore they should be used whenever available. But it should be kept in mind that the high separation power of modern chromatographic systems can separate stable-isotope-labelled analogues from the targeted analytes (stable-isotope-labelled analogues elute slightly earlier), leading to the possible influence of, for example, ion suppression on only one of the compounds. Moreover, stable-isotope-labelled analogues and targeted analytes can produce identical ion fragments, which can lead to problems during quantification.

To produce accurate quantitative results, the analytical procedure should be validated (see Chapter 20). If a certified reference material to validate the analytical procedure is not available (e.g. for postmortem specimens), then the 'method of standard addition' can be used, where calibration and quantification are performed directly in the sample matrix, compensating for matrix effects. In this procedure different concentrations of the target compound are spiked to aliquots of the homogeneous sample prior to work-up and the detector responses are plotted as a graph ('standard addition plot'). The initial concentration of target compound can then be calculated via extrapolation.

Practical aspects of extraction

The isolation of the compounds of interest from the biological matrix is essential for their successful detection, identification and quantification. The strategy applied and the effort invested in the development of an extraction procedure depend not only on the physicochemical properties and the expected concentration of the target compounds but also on the nature of the specimen and the available equipment in the analytical laboratory. Sometimes the physicochemical properties of the target compounds allow for their direct detection after digestion of the sample matrix (e.g. metals), or for an easy separation from the less-volatile

matrix components (e.g. gases and volatile compounds via headspace analysis). However, there is an important group of less-volatile drugs and poisons that demand more complex extraction procedures (such as liquid–liquid or solid-phase extraction) to ensure their isolation from the biological matrix.

A literature review of the *Journal of Analytical Toxicology* over the past 20 years shows that the number of publications using liquid–liquid extraction has been quite constant and has covered a wide range of analytes, whereas, for solid-phase extraction, the number of publications has continuously increased and, in recent years, the focus has switched from drugs of abuse to applications with a wider range of target analytes. It can be concluded that, although the importance of liquid–liquid extraction in analytical toxicology has already been established, solid-phase extraction is gaining increasing importance in this field.

These two important techniques for the extraction of less-volatile drugs and poisons will be discussed in more detail in this chapter. The final decision about which of these two techniques should be chosen for the particular challenge at hand is mainly based on the practical experience of the analyst and a careful consideration of the objective and the intended use of the extraction method, as well as the availability of analytical techniques.

Preconditions of extraction

Frequent problems in analytical toxicology include the lack of control over the sampling process and, sometimes, a limited availability of specimens. Such problems can result in insufficient or even inadequate samples. This challenge is partially resolved by close cooperation with the person responsible for sampling (e.g. the physician or pathologist) as well as appropriate quality control and educational measures.

In most cases, the appropriate preparation of the specimen is a fundamental precondition for successful sample extraction. Protein-free samples (such as urine) or liquid samples with low protein content (such as serum or plasma) that are frequently used in clinical toxicology are comparable to a purely aqueous phase and therefore direct extraction can be tried. If metabolites should also be detected, hydrolytic cleavage of the conjugate bond (deconjugation) with strong acids, bases or enzymes prior to extraction can increase the recovery of these metabolites from biological fluids. This approach is particularly useful for urine and essential for drugs (e.g. laxatives) that are excreted almost exclusively as conjugated metabolites. For the differentiation of the amount of conjugated and unconjugated metabolite present in the sample, either LC-MS is required or two analyses have to be performed. In the first step the unconjugated metabolite is extracted and quantified and then the sample is re-analysed after hydrolysis, resulting in 'total' metabolite concentration (conjugated plus unconjugated). To obtain reliable quantitative results, appropriate standards (also conjugated) must be carried throughout the procedure to monitor the efficiency of the hydrolysis step.

The use of an enzyme to cleave chemical bonds is the more specific of the different approaches mentioned above. It incurs additional cost and is more time intensive, but cleaner extracts can be achieved, which reduces the 'down-time' of analytical instruments. For the different preparations of purified glucuronidase and sulfatase that are available, it is crucial to pay attention to their pH and temperature optima to obtain reproducible results.

A typical procedure for the enzymatic hydrolysis of glucuronides is as follows. Mix 1 mL of blood or urine with an internal standard and 1.5 mL of appropriate buffer and then add 100 µL of β-glucuronidase obtained from *Helix pomatia*. Mix the solution and incubate it at 37°C overnight (approximately 16 h). After incubation, the pH of the solution is adjusted appropriately for solvent or solid-phase extraction of the compounds of interest (see also Chapter 10).

Acid or basic hydrolyses (Dubost, Pascal 1955), although faster and less expensive, tend to produce more artefacts due to the vigorous hydrolysis conditions and are therefore more demanding in terms of necessary clean-up procedures. Typically, strong mineral acids or alkalis are used, often with boiling or treatment in a microwave or pressure cooker. To protect the analytical instruments in subsequent analyses, the extracts must be neutralised and organic solvents have to be dried prior to injection, otherwise chromatographic performance deteriorates quickly. Moreover, care should be taken to ensure the stability of the analytes under these harsh conditions of hydrolysis. If several compounds can be hydrolysed to an identical, single compound, the accurate identification of the original substance present can be precluded. For example, both the acid and the enzymatic hydrolysis of benzodiazepines result in the cleavage of conjugates, but acid hydrolysis also converts different drugs to the same benzophenone compound (e.g. diazepam, temazepam, ketazolam, medazepam and camazepam are all converted into 2-methylamino-5-chlorobenzophenone). Although the resulting compound has good chromatographic characteristics, the approach is unsuitable for those applications (such as forensic analysis) that require absolute identification of the drug ingested (see also Chapter 40).

Biological materials that are not homogeneous, protein rich or degraded (such as tissue samples and postmortem samples) need homogenisation (e.g. with a blender to disrupt the cellular structure) and sometimes further sample preparation, such as deproteinisation, before extraction from the aqueous phase is possible.

Homogenisation can be performed directly in a buffer solution with a physiological pH of 7.4, to avoid protein precipitation. A high dilution ratio of blood (up to 1 : 10) and tissue (up to 1 : 50) results in samples that tend not to clog tightly packed extraction cartridges and are therefore suitable for direct solid-phase extraction. Automation of the extraction process will enable the analyst to handle the large sample volumes and at the same time secures a uniform and efficient extraction by providing a homogeneous flow of the sample through the extraction cartridge.

For liquid–liquid extraction of complex sample matrices, protein precipitation is generally needed before extraction. Deproteinisation can be performed with solvents such as ethanol or acetone, or with dimethylformamide, which is particularly well tolerated by most GC stationary phases. Moreover acetonitrile is frequently used for procedures where high performance liquid chromatography (HPLC) systems are applied. It is usual to use two volumes of organic phase to one volume of blood.

The following procedure of a combined protein precipitation and subsequent extraction with acetonitrile under alkaline conditions works for target analyses of neutral and basic compounds in specimens of low collagen content (such as blood or brain tissue): to 0.5 mL of blood or 0.5 g of homogeneous brain tissue, add an internal standard and a freshly prepared mixture of 1 mL acetonitrile and 0.1 mL of a saturated aqueous solution of disodium hydrogenorthophosphate. After shaking, centrifugation and evaporation of the supernatant the reconstituted residue can be extracted. For solid-phase extraction, the dilution of the supernatant (to achieve a concentration of acetonitrile below 20%) is in most cases sufficient and the resulting solution can be applied directly to the sorbent.

Protein precipitation may also be accomplished with acids (e.g. hydrochloric acid, perchloric acid, trichloroacetic acid and tungstic acid) and salts (e.g. sodium tungstate, ammonium sulfate, cupric sulfate and uranyl nitrate) (Curry 1988).

However, all of these procedures are very time-consuming and labour intensive. Protein precipitation also risks loss of the analyte from adsorption and occlusion. When perchloric, trichloroacetic or tungstic acids are applied there is a particularly high chance that the drugs being analysed may also be co-precipitated. For the extraction of trace amounts of drugs and poisons from complex matrices, dilution and homogenisation with water or buffer solutions are therefore preferred and can prevent these problems.

To prepare tissue specimens for extraction, enzymatic digestion of these samples is sometimes useful and can be achieved using pepsin, trypsin, enterokinase, lipase and β-glucuronidase. A suitable procedure is as follows: macerate 10 g of liver or other tissue with 40 mL of 1 mol/L tris(hydroxymethyl)methylamine; add 10 mg of subtilisin Carlsberg and incubate in a water bath at 50–60°C for ~1 h, with agitation. Filter the digest through a small plug of glass wool to remove undissolved connective tissue. Aliquots of this digest may be substituted for the specified biological fluid in most routine screening procedures

(Osselton 1978, 1979; Osselton *et al.* 1978). The filtered digest has a pH of 8.0–9.5 (see also Chapter 10).

The drawback of this procedure is that the resulting extracts contain undesirable by-products and artefacts, created during the process of digestion, and therefore a screening procedure will become more difficult. The presence of proteases may interfere with antibodies in immunoassay screening procedures.

Even after extensive sample preparation, a large number of matrix components are still present in the sample and these will affect the solubility and the sorption of analytes through their association with molecules as well as by their ability to change the ionic strength of a solution. Soluble matrix components are also distributed between heterogeneous phases and can influence the completeness of equilibrium adjustment as well as the overall extraction efficiency. These considerations have to be kept in mind for the subsequent extraction procedure.

Liquid–liquid extraction

Direct liquid–liquid extraction (LLE) is still predominant in many laboratories when protein-free samples (such as urine) or liquid samples with low protein content (such as serum or plasma) need to be extracted, because this technique is fast, inexpensive and efficient. LLE is based on well-defined thermodynamic relationships and has a wide dynamic range.

The extraction yield is strictly determined by:

- The distribution equilibrium (solubility)
- The equilibrium of electrolytic dissociation (pH dependent)
- The ratio of phase volumes (organic/aqueous)
- The number of extraction stages.

In practical applications, the constitution of the sample matrix has an important influence on results and appropriate sample pre-treatment (e.g. protein precipitation) is a fundamental prerequisite for successful LLE. An excess of solvent and/or repetitive extraction will simply increase the amount of co-extracted impurities. Therefore, a phase ratio (organic/aqueous) of 1 to 2 is recommended. For compounds with low extractability, it is better to switch to another solvent instead of increasing the volume of the organic phase and/or to engage in multiple extractions. In order to separate target compounds from interferences as well as from each other, their varied distribution constants and different acidic or basic properties can be used for fractionation or back extraction (see below).

Practical experience shows that, in order to achieve sufficient separation, the pK_a values of the compounds to be separated must differ by more than four units. Fractionation in more than two or three groups is therefore ineffective, because under such circumstances substances are dragged into several fractions. It should be kept in mind that neutral compounds are to be expected in the first fraction and that co-extraction of endogenous compounds is to be expected in each extract. Therefore, in every extraction scheme, sacrifices must be made when it comes to the question of the cleanliness of extracts. For the adjustment of an appropriate pH in the aqueous solution, acids or bases can be used, but in this case the stability of the analyte and the possibility of protein precipitation should be kept in mind. Thus the use of an appropriate buffer solution for the reliable adjustment of the pH is recommended.

The choice of solvent is mainly based on the solubility of the drugs and poisons that have to be extracted and follows the rule of thumb that 'like dissolves like'. Moreover, the solubility of known interferences from the sample matrix (such as lipids: fatty acids, cholesterol, etc.) that have a negative effect on the identification and/or the quantification of the analyte should also be considered in the choice of the solvent. A major criterion for the solubility of a particular substance is the solvent's polarity, which results from an unequal sharing of electrons within the molecule and depends on the electronegativity of its atoms and the asymmetry of the molecule. Polarity aside, solvents can also be chosen by their ability or inability to form hydrogen bonds (hydrogen donor or acceptor), their boiling point (solvent removal with the risk of evaporation losses), their pH stability (e.g. ester cleavage), as well as their water miscibility. A low solubility of water in the solvent facilitates drying of the extract and co-extraction of water-soluble substances (e.g. salts) can be minimised. In the case of larger amounts of water dissolved in the solvent, it is possible to put the extract in a freezer to freeze the water in order to remove it. Small amounts of water can be removed by addition of anhydrous sodium sulfate, but the possible loss of trace amounts of the analyte should be kept in mind. When choosing a solvent, the potential toxicity should also be considered (e.g. chloroform) as well as flammability and explosive potential (e.g. peroxides in diethyl ether). For practical reasons the density (top or bottom layer with water), and the solvent's UV absorbency (when spectrophotometric detection is used), as well as the grade of purity are important considerations. Before a solvent is used in a new extraction procedure, an aliquot of the solvent should be evaporated and the residue should be analysed to detect any impurities. Moreover, solvents possess different emulsifying potentials (e.g. that of chloroform is greater than that of diethyl ether), and the correct choice of solvent for LLE also includes considerations to avoid emulsion formation. The addition of neutral salts and the use of slow rotation or vortex speeds as well as using larger phase volume ratios can prevent the formation of emulsions. If emulsions are unavoidable, the phases may be separated by centrifugation, by putting the extract in a freezer and by the addition of a small amount of methanol. If emulsions are routinely obtained, the extraction method should be changed. A promising approach to avoiding emulsions, which also works well for solvents with a high density (such as dichloromethane) that are difficult to isolate from the bottom layer with water, is immobilisation of the aqueous phase (e.g. on diatomaceous earth) prior to extraction. This technique of a supported LLE can increase the extraction yield for the analyte. Some solvents can possess potential reactivity with certain analytes (e.g. some pesticides react with ethanol or acetone) and also ion-pair extraction can occur in certain solvents. This can be exploited to extract analytes (e.g. paraquat), but sometimes ion-pair extraction is an unwanted side-effect (e.g. in the case of extraction of hydrochlorides with chloroform or dichloromethane).

Finally, the analytical method applied may also influence the choice of the solvent. For example, in GC, chlorinated solvents would not be chosen if a halogen specific detector were going to be used. However, if the extract is to be evaporated to dryness and then reconstituted in another solvent before being injected into the chromatographic system (e.g. the eluent of an HPLC system), the choice of the original solvent can be wider. The boiling point and expansion volume of the solvent may also be issues (see also Table 40.6 in Chapter 40).

Adding a high concentration of neutral salts (e.g. sodium chloride) to the aqueous phase can support the extraction process (the 'salting-out effect'), depressing the mutual solubility of phases and simultaneously reducing emulsification and foaming.

The evaporation of solvents (for enrichment) demands particular care in order to avoid the loss of volatile analytes (e.g. amfetamines: b.p. 200°C). To prevent the loss of volatile analytes, a small volume of acidified methanol can be added to the extract prior to evaporation. The quality and the volume of the glassware should be chosen with care, to avoid loss of the analyte by adsorption and to enable the reconstitution of the extract in a small amount of solvent (to optimise the concentration step) for further analyses.

The appropriate solvent should extract as much of the target compound as possible while at the same time co-extracting only a minimum of interferences. To identify which solvents are frequently used in analytical toxicology, a literature review of the *Journal of Analytical Toxicology* over 5 years (2004 to 2008) was performed (Table 29.1). The most frequently used solvents were ethyl acetate, followed by 1-chlorobutane, hexane and dichloromethane. Isopropyl alcohol was mainly used as a modifier. Chloroform and diethyl ether were frequently used in the past as versatile solvents and, although the severe health risks of chloroform and the fire and explosion hazards of diethyl ether are well known today, they are still in use and have not been totally substituted by dichloromethane and methyl t-butyl ether, respectively. Finally, acetonitrile was also used on a regular basis. Other solvents (such as toluene, methanol, pentane, butyl acetate, acetone and ethanol) were used only occasionally.

Table 29.1 Number of times a solvent was used in a paper published in the *Journal of Analytical Toxicology* between 2004 and 2008 inclusive

Solvent	*Number of citations*
Ethyl acetate	38
1-Chlorobutane	28
Hexane	27
Dichloromethane	25
Isopropyl alcohol	22
Chloroform	18
Diethyl ether	15
Acetonitrile	11
Methyl *t*-butyl ether	7
Toluene	6
Isoamyl alcohol	6
Methanol	5
Pentane	3
n-Heptane	3
Octane	2
Dichlorothane	2
n-Butyl acetate	2
Diisopropylether	1
Isobutyl alcohol	1
Propyl acetate	1
Acetone	1
Cyclohexane	1
Tricholoroethanol	1
Ethanol	1
TOTAL	227

When a new extraction procedure is developed, and there are no data about an appropriate solvent available, the following solvents should be tested after adjusting the aqueous solution to an appropriate pH value (in order of increasing polarity): hexane, 1-chlorobutane, methyl *t*-butyl ether, dichloromethane, 1-butanol, ethyl acetate and acetonitrile. The basic properties of these solvents can be found in Table 29.2.

If the results are not sufficiently good using a pure solvent, then modifiers can be added, such as in chloroform–2-propanol (9 : 1; e.g. for morphine), or mixtures can be used. Although so-called all-purpose extraction solvents such as ethanol–hexane–acetone (1 : 2 : 2) or dichloromethane–2-propanol–ethyl acetate (1 : 1 : 3) are used, it should be kept in mind that, in general, the more substances with different physicochemical properties are extracted, the larger the amount of interferences from the matrix that will be co-extracted. These mixtures are therefore primarily used in the screening for a 'general unknown'. For target analysis, a toxicologist would choose a more refined procedure with a more selective solvent. If the target compound is known, data about appropriate solvent and pH value for extraction can be found in the monographs in this publication or through a literature review. A frequently used solvent that co-extracts a relatively low amount of interferences from biological samples when compared with other solvents is 1-chlorobutane. For this solvent, extraction yields of 331 toxicologically relevant compounds are available from aqueous media at pH 9.0 (Table 29.3 and in the relevant monographs). At this pH, 228 of the 331 compounds were extracted with an extraction yield equal to or higher than 80% (represented in Table 29.3 by a yield in the organic phase with a value equal to or higher than 0.8). For the remaining compounds, sufficient extraction yields could be reached by changing the pH to acidic (e.g. for phenobarbital) and/or through the addition of a modifier (e.g. for morphine). If a target compound has to be extracted, the database gives a quick overview of the extractability of the compound with 1-chlorobutane at a defined pH value. If the extraction yield from aqueous solution is good, it can be expected that the extraction is also possible from serum.

Example protocols for the extraction of various analytes can be found in various chapters of this book (e.g. Hospital Toxicology (Chapter 1), Postmortem Toxicology (Chapter 10) and High Performance Liquid Chromatography (Chapter 41)). The extraction procedure will become more complex with an increase in the complexity of the sample matrix because additional steps for purification (back extraction) have to be added.

As an example of a complete extraction scheme for the extraction of bases, neutrals and acids, and to illustrate the theoretical background discussed above, the following is reproduced from Chapter 10.

The pH of the specimen influences the extent to which acid and basic drugs are extracted. Addition of a weakly basic buffer, such as sodium borate (pH 9), favours the extraction of weakly basic drugs, as well as most neutral substances. Similarly, the addition of an acidic buffer, such as sodium dihydrogenphosphate, favours the extraction of acidic as well as neutral drugs. The majority of drugs of forensic interest are 'basic' in character, but are often present at relatively low concentrations in blood. It is, therefore, desirable to have an extraction scheme that incorporates a back-extraction step to eliminate or minimise the extraction of endogenous molecules. An example extraction scheme is shown in Fig. 29.1. The saturated sodium borate solution will force the basic drugs into the lipid-soluble un-ionised form, allowing extraction into the chlorobutane. The chlorobutane is transferred to a fresh tube and the drugs are back extracted into sulfuric acid. Neutral or acidic substances will remain in the upper chlorobutane layer, which may be pipetted or aspirated to waste. The remaining acid layer is then made basic by addition of sodium hydroxide, and the now un-ionised basic drugs are re-extracted with chlorobutane. The upper solvent layer may then be removed and concentrated under nitrogen, prior to analysis by a suitable chromatographic method. This extraction scheme will give extracts that are relatively free of interfering substances. However, it should be noted that morphine and other amphoteric drugs cannot be detected by this method since the phenolic functional group will

Table 29.2 Properties of solvents

Solvent	*Density (g/mL)*	*Boiling point (°C)*	*Solubility (g/100 mL water at 20°C)*	*Polarity index (water = 10.2)*	*Hydrogen acceptor*	*Hydrogen donor*
Hexane	0.66	69	0.01	0.1	No	No
1-Chlorobutane	0.89	78	0.07	1.0	No	No
Methyl *t*-butyl ether	0.74	55	0.5	2.5	Yes	No
Dichloromethane	1.34	40	2	3.1	No	No
1-Butanol	0.81	118	7.9	3.9	Yes	Yes
Ethyl acetate	0.90	77	8.6	4.4	No	Yes
Acetonitrile	0.78	82	Miscible	5.8	No	Yes

Table 29.3 Extraction yields of 331 compounds of clinical or forensic interest using 1-chlorobutane from water at pH 9 (multiple observations in different laboratories were averaged)

Compound	*Extraction yield*
10-Hydroxycarbazepine (metabolite of oxcarbazepine)	0
2-(2,3-Methylenedioxyphenyl)butane-1-amine	0.8
2-(2,3-Methylenedioxyphenyl)propane-1-amine	0.7
2-(3,4-Methylenedioxyphenyl)2-methylpropane-1-amine	0.9
2-(3,4-Methylenedioxyphenyl)butane-1-amine	0.8
2-(3,4-Methylenedioxyphenyl)propane-1-amine	0.5
2-Methylamino-1-(3,4-methylenedioxyphenyl)butane (MBDB)	1
2,3-Methylenedioxyamfetamine	0.6
2,3-Methylenedioxymethamfetamine	0.9
2,3-Methylenedioxo-*N*-methylphenethylamine	0.6
2,3-Methylenedioxo-*N*-phenethylamine	0.3
2,4,5-Trimethoxyamfetamine (TMA-2)	0.2
2,4,6-Trimethoxyamfetamine (TMA-6)	0.5
2,5-Dimethoxy-4-brom-phenethylamine (2C-B)	0.8
2,5-Dimethoxy-4-metamfetamine (DOM)	0.8
2,5-Dimethoxy-4-methyl-phenethylamine (2C-D)	0.5
2,5-Dimethoxy-phenethylamine (2C-A)	0.3
3-(2,3-Methylenedioxyphenyl)pentane-2-amine	0.95
3,4-Methylenedioxyamfetamine (MDA)	0.6
3,4-Methylenedioxymetamfetamine (MDMA)	0.7
3,4-Methylenedioxy-*N*-ethylamfetamine (MDE)	0.9
3,4-Methylenedioxy-*N*-methylphenethylamine	0.5
3,4-Methylenedioxy-*N*-phenethylamine	0.3
4-Methoxy-2-pyrrolidinopropiophenone	1
4-Methoxyamfetamine (PMA)	0.5
4-Methyl-2-pyrrolidinopropiophenone (MPPP)	1
4-Methylthioamfetamine (4-MTA)	0.6
N-(1-Phenylcyclohexyl)-3-ethoxypropylamine (PCEPA)	1
N-(1-Phenylcyclohexyl)-3-methoxypropylamine (PCMPA)	1
N-(1-Phenylcyclohexyl)-1-propylamine	1
N-(1-Phenylcyclohexyl)-2-methoxyethylamine (PCMEA)	1
N-Methyl-4-methoxyamfetamine (PMMA)	0.7
N,*N*-Diethyltryptamine (DET)	0.95
Acebutolol	0.05
Acetaminophen (paracetamol)	0
Adenosine	0
Ajmaline	0.5
Alfentanil	1
Alimemazine	1
Alprazolam	0.95
Alprenolol	1
Amantadine	0.5
Amfebutamone	1
Amfepramone	1
Amfetaminil	1
Amiodarone	0.95
Amisulpride	0.6
Amitriptyline	1
Amitriptyline oxide	0.10
Amlodipine	1

Table 29.3 continued

Compound	*Extraction yield*
Amfetamine	0.5
Apomorphine	0.8
Aprindine	0.95
Articaine	1
Atenolol	0
Atropine	0.6
Azathioprine	0
Azinphosethyl	1
Azinphosmethyl	1
Benperidol	1
Benserazide	0
Benzatropine	1
Benzoylecgonine	0
Betaxolol	1
Biperiden	1
Bisacodyl	0.7
Bisoprolol	0.9
Bromazepam	0.9
Bromocriptine	1
Bromophosethyl	1
Bromophosmethyl	1
Bromperidol	1
Brotizolam	1
Budipine	1
Bupivacaine	1
Bupranolol	1
Buprenorphine	1
Buspirone	0.95
Caffeine	0.3
Carazolol	0.9
Carbamazepine	0.95
Carbamazepine epoxide	0.6
Carbidopa	0
Carteolol	0.06
Carvedilol	1
Cathine	0.07
Celiprolol	0.10
Chlordiazepoxide	0.95
Chlormezanone	0.9
Chloroquine	0.95
Chlorovinphos	1
Chlorpromazine	1
Chlorprothixene	1
Citalopram	1
Clobazam	1
Clobutinol	1
Clomethiazole	1
Clomipramine	1
Clonazepam	1
Clopamide	0.06
Clopenthixol	1

Table 29.3 continued

Compound	*Extraction yield*
Clotiazepam	1
Clozapine	1
Cocaine	1
Codeine	0.8
Colchicine	0.13
Cotinine	0.10
Cyamemazine	1
Deanol	0.4
Demelverine	1
Desipramine	1
Detajmium	0.9
Dialifos	1
Diazepam	0.95
Dibenzepin	1
Dichlorvos	1
Diclophenac	0.2
Dihydrocodeine	0.7
Dihydroergocryptine	1
Diltiazem	1
Dimethoate	0.7
Dimetindene	0.98
Diphenhydramine	1
Disopyramide	0.8
Dosulepin (dothiepin)	1
Doxazosin	0.95
Doxepin	1
Doxylamine	1
Droperidol	0.9
Enalapril	0
Entacapone	0
Ephedrine	0.2
Esmolol	0.8
Ethosuximide	0.2
Etomidate	1
Felodipine	0.95
Fenethylline	0.9
Fenofos	1
Fentanyl	1
Fenthion	1
Flecainide	0.95
Fluconazole	0.10
Flumazenil	0.8
Flunitrazepam	1
Fluoxetine	0.8
Flupenthixol	1
Fluphenazine	1
Flupirtine	1
Flurazepam	0.95
Fluspirilene	0.9
Fluvoxamine	0.8
Furosemide	0

table continued

Table 29.3 continued

Compound	*Extraction yield*
Gabapentin	0
Gallopamil	1
Gamma-hydroxybutyric acid	0
Glibenclamide	0.2
Glutethimide	1
Haloperidol	1
Heptenophos	0.8
Hydrochlorothiazide	0
Hydromorphone	0.10
Hydroxyzine	1
Ibuprofen	0
Imipramine	1
Indometacin	0
Ipratropium	0
Isofenphos	0.95
Kavaine	0.7
Ketamine	1
Lamotrigine	0.17
Levetiracetam	0
Levodopa	0
Levomepromazine	1
Lidocaine	1
Lisinopril	0
Lofepramine	0.9
Oprazolam	1
Lorazepam	0.85
Lormetazepam	1
Loxapine	1
Lysergide (LSD)	0.95
Maprotiline	1
Medazepam	1
Mefenorex	0.95
Melperone	1
Mepindolol	0.4
Mepivacaine	1
Meprobamate	0.10
Meptazinol	0.9
Mesuximide	1
Metamizole	0.4
Methadone	0.95
Methamfetamine	0.7
Methaqualone	1
Methohexital	0.95
Methylphenidate	0.8
Metixene	1
Metoclopramide	0.9
Metoprolol	0.8
Mevinphos	0.9
Mexiletine	0.9
Mianserin	1
Midazolam	0.9

table continued

Table 29.3 continued

Compound	*Extraction yield*
Mirtazapine	0.9
Moclobemide	0.9
Modafinil	0.4
Morphine	0
Nadolol	0
Nalbuphine	0.8
Nalorphine	0.3
Naloxone	0.9
Nefazodone	1
Nefopam	0.95
Nicotine	0.9
Nifedipine	1
Nicotinamide	0
Nimodipine	1
Nisoldipine	1
Nitrazepam	1
Nordiazepam	0.95
Nortriptyline	1
Noscapine	1
Olanzapine	1
Opipramol	1
Orciprenaline	0
Oxazepam	0.85
Oxcarbazepine	0.9
Oxitriptan	0
Oxprenolol	0.9
Oxycodone	0.95
Paraoxon	0.9
Parathion ethyl	1
Parathion methyl	1
Paroxetine	1
Pemoline	0
Penbutolol	1
Pentazocine	0.8
Pentobarbital	0.2
Pentoxyverine	1
Perazine	1
Perphenazine	1
Pethidine	1
Phenazone	0.4
Phencyclidine	0.9
Phenobarbital	0.10
Phenolphthalein	0.7
Phenprocoumon	0.2
Phenytoin	0.5
Pholedrine	0
Phosphamidon	0.8
Phoxime	1
Pimozide	1
Pindolol	0.4
Pipamperone	1
Pirimiphos	1

Table 29.3 continued

Compound	*Extraction yield*
Piritramide	0.9
Piroxicam	0
Prajmalium	0.9
Pramipexole	0
Prazepam	1
Prilocaine	0.95
Primidone	0
Procainamide	0.10
Procaine	0.9
Procyclidine	1
Promazine	1
Promethazine	1
Propafenone	0.95
Propofol	0.95
Propoxyphene	1
Propranolol	1
Propyphenazone	1
Prothipendyl	0.95
Pseudoephedrine	0.2
Quetiapine	1
Quinidine	0.95
Quinine	1
Ranitidine	0
Reboxetine	1
Remifentanil	1
Risperidone	1
Ropivacaine	1
Salicylate	0
Scopolamine (hyoscine)	0.7
Sertindole	1
Sertraline	1
Sildenafil	1
Sotalol	0
Strychnine	0.9
Sulfentanil	1
Sulfotep	1
Sulpiride	0
Sultiame	0
Talinolol	0.2
Temazepam	1
Terbufos	1
Tertatolol	1
Tetrazepam	0.95
Theobromine	0
Theophylline	0
Thiopental	0.9
Thioridazine	1
Tiagabine	0.5
Tiapride	0.4
Ticlopidine	1
Tilidine	1
Timolol	0.6

Table 29.3 continued

Compound	*Extraction yield*
Tocainide	0.3
Tolperisone	1
Topiramate	0.2
Tramadol	1
Tranylcypromine	1
Trazodone	1
Triazolam	1
Trichlorophos	1
Trifluperidol	1
Triflupromazine	1
Trihexyphenidyl	1
Trimipramine	1
Tryptophan	0
Valproic acid	0.07
Venlafaxine	0.95
Verapamil	1
Vigabatrin	0
Viloxazine	0.85
Zaleplon	1
Ziprasidone	1
Zolpidem	1
Zopiclone	0.9
Zotepine	0.95
Zuclopenthixol	1

Extraction yield = extraction yield in the organic phase (e.g. 0.3 representing an extraction yield in 1-chlorobutane of 80%).
The database was compiled by the Committee on Extraction of the Society of Toxicological and Forensic Chemistry under the supervision of Dr U. Demme, and is reproduced here with the kind permission of the Society of Toxicological and Forensic Chemistry.

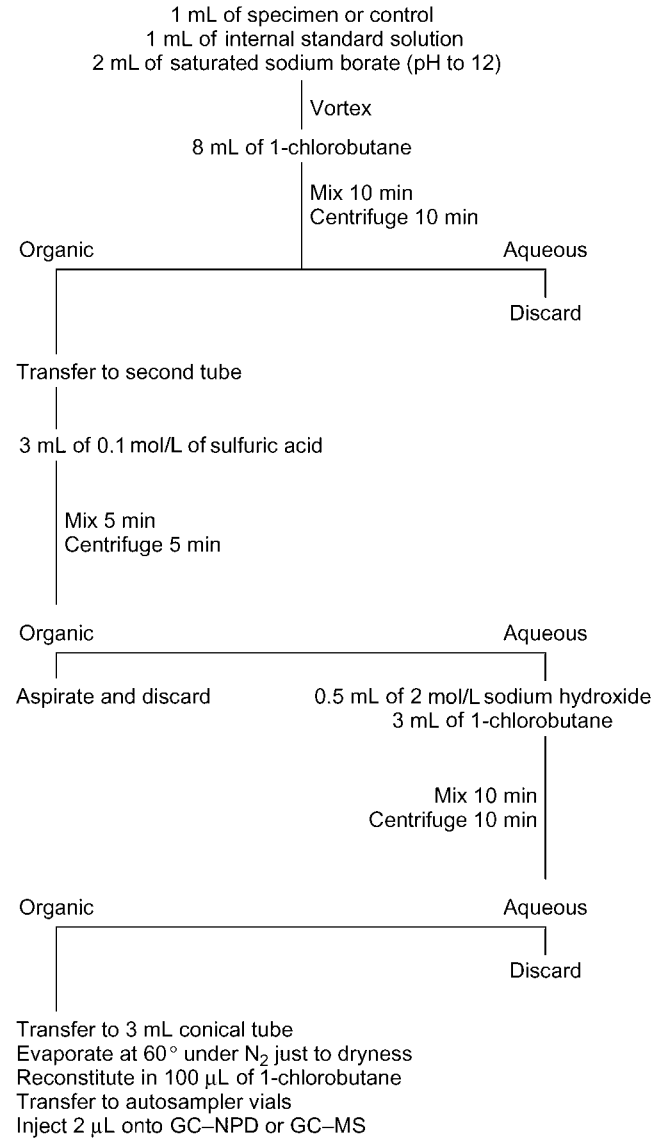


Figure 29.1 Extraction scheme for strong bases.

ionise at high pH, therefore precluding extraction into the solvent. For amphoteric drugs, the basic phase should be less than pH 9.0, and preferably pH 8.0–8.5.

Although a similar extraction scheme to that used for basic drugs (but with the additions of acid and base reversed) could be used for strongly acidic drugs, such a method does not efficiently extract weakly acidic drugs, such as the barbiturates, and neutral drugs, such as meprobamate. Conversely, simple addition of an acidic buffer to whole blood and extraction with a solvent results in the co-extraction of large amounts of endogenous lipid substances. Such extracts may be 'cleaned up' by partitioning between immiscible solvents of different polarities, such as acetonitrile and hexane, as shown in Fig. 29.2. The more polar drugs tend to partition into the acetonitrile, whereas the endogenous lipids (fatty acids, sterols) tend to partition into the hexane.

Solid-phase extraction

Although method development for solid-phase extraction (SPE) is not as straightforward as for LLE, this technique offers appealing advantages. Because of SPE's high extraction efficiency, even very small sample sizes are sufficient, thereby reducing solvent consumption. Additionally, there is no emulsion formation.

Past problems with inconsistent quality of the extraction cartridges have been overcome by manufacturers' implementation of extensive quality control measures. The increasing interest in SPE by toxicological laboratories lies mainly in its compatibility with automation. The need for automated extraction procedures is directly related to the expectation that toxicological laboratories become economically self-sufficient as well as an increased demand for quality assurance and reproducibility and, therefore, comparable results between different laboratories.

In SPE the analytes are isolated from the aqueous sample by adsorption onto a solid sorbent, followed by washing and elution steps. In each of these steps a mechanism of total retention or total release of the target compounds is desired and, as with LLE, this technique is based on the fundamental principles of extraction discussed above. Depending upon the choice of sorbent, the extraction of toxicologically relevant compounds is achieved via hydrophobic, hydrophilic, aromatic or electrostatic interactions; often a combination of several mechanisms is involved. In the field of analytical toxicology non-modified silica, surface-modified silica and polymer resins are used, and a more detailed description of these sorbents can be found in Chapter 41 (packing materials). To date, both selective procedures (for target analysis) and non-selective SPE procedures (for the screening for a 'general unknown') have been developed. Because a wide variety of different analytes can be extracted using a combination of hydrophobic and electrostatic interactions, these so-called mixed-mode sorbents (or functionalised sorbents, or hybrid extraction sorbents) are widely used in analytical toxicology. A practical

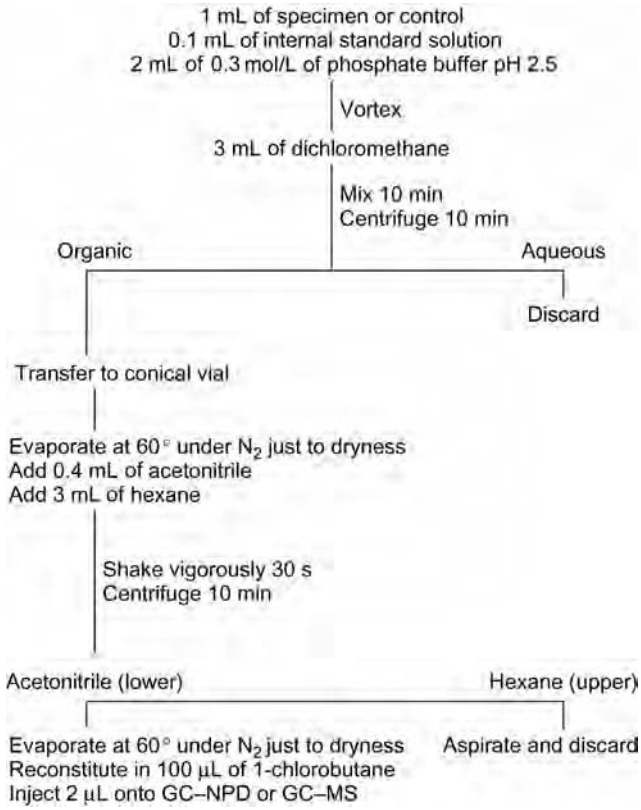


Figure 29.2 Extraction scheme for acids and neutrals.

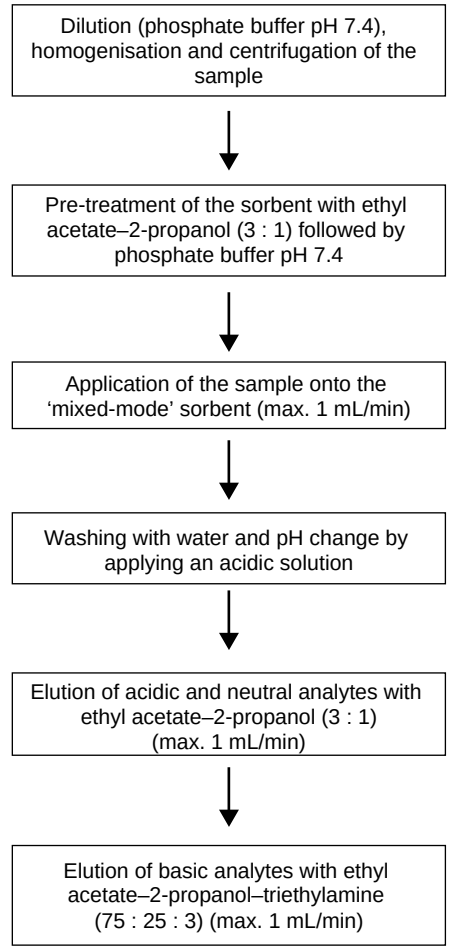


Figure 29.3 Overview of a 'mixed-mode' solid-phase extraction procedure.

example of such a procedure for the extraction and fractionation of neutral and basic metabolites of cyclizine in the urine of racehounds can be found in Chapter 7.

To illustrate the fundamental principles and possible pitfalls of SPE, the following section includes a step-by-step discussion of the development of a non-selective 'general unknown' screening procedure for a wide variety of analytes. An overview of the procedure can be found in Fig. 29.3.

The first step is the appropriate pre-treatment of viscous specimens so that the samples do not cause flow problems when passing through the tightly packed cartridges and choosing the sorbent. Tissue homogenates, especially, tend to clog the polyethylene frit (pore diameter ~20 µm) holding the sorbent in place. Practical experience has shown that diluting the specimens with ten times the amount of phosphate buffer before homogenisation provides samples that can be extracted with either negative or positive pressure. Some tissues (e.g. putrefied liver) have to be diluted even more (up to 50 times). A physiological pH of 7.4 prevents protein precipitation in biological specimens and therefore avoids an unpredictable loss of analytes. After homogenisation (e.g. with a blender), and before the sample is applied to the extraction cartridge, remaining particles and cellular structures must be removed by centrifugation (cooling can prevent the loss of volatile analytes). For the handling of the sometimes large sample volumes, automation of the whole extraction process is highly recommended. The controlled and therefore homogeneous flow of the sample through the extraction cartridge in such an extraction device secures a uniform and efficient extraction, leading to more reproducible results.

For this 'general unknown' screening procedure, a mixed-mode sorbent based on a combination of a polar modified polystyrene resin and a cationic exchanger is used. In this way analytes can be extracted by non-selective hydrophobic and aromatic interactions and, in a second step, acidic and neutral compounds can be separated from basic compounds that are retained by electrostatic interactions through their amine functionality. Polystyrene resins do not possess residual silanol groups like silica-based sorbents do (due to varying degree of endcapping) Polystyrene resins have the additional advantage of being stable across the entire pH range, in contrast to the limited stability (pH 2–8) of silica-based sorbents. Moreover, polystyrene resins offer a higher capacity than silica-based sorbents and sufficient retention of a wide variety of different analytes can be achieved at a pH of 7.4. At this pH value an equilibrium of electrolytic dissociation will be reached for acidic and basic analytes in the aqueous phase. By adsorption of the non-ionised analyte to the sorbent, equilibrium must be continuously re-established and a nearly complete extraction will be reached if sufficient contact time with the sorbent is allowed and if the capacity of the sorbent is high enough. Additionally, the porous structure of polystyrene resins can be used to exclude large molecules (e.g. proteins and lipids bound in micelles) from the extraction process. The amount of sorbent that has to be used is determined by the amount of interferences that are expected in the sample matrix analysed.

The second step is the pre-treatment of the sorbent. It should be washed with the strongest solvent applied in the procedure and then it must be conditioned – in the procedure described here, with a phosphate buffer of pH 7.4 – in order to make the sorbent compatible with the sample. Silica-based bonded sorbents should not dry out between the applications of different solutions. This is not an issue when working with polar modified polystyrene resins because of their additional hydrophilic properties.

The third step is the application of the sample. Competitive influences of matrix components displacing the analytes from the limited surface area of the sorbent have to be considered and 'overloading' the extraction cartridge must be avoided. Because the mass transfer of analyte to sorbent is determined by kinetic functions and, because the intermolecular interactions (van der Waals' forces, aromatic interactions, etc.) are short-ranged and relatively weak forces, sufficient contact

Table 29.4 Possible pitfalls in the process of developing a mixed-mode solid-phase extraction procedure

Steps of the mixed-mode procedure	*Possible pitfalls*
Specimen pre-treatment	Protein precipitation (loss of analytes), flow problems
Choice of sorbent	Capacity, undesired secondary interactions, stability
Washing and conditioning sorbent	Improper conditioning (drying of silica-based sorbents, capacity problems)
Sample application	Insufficient contact time, 'break-through', clogging
Washing and changing pH to acidic	Protein precipitation on the sorbent, insufficient acidic capacity
Elution of acidic and neutral compounds	Insufficient contact time, inappropriate solvent (solvent strength, water miscibility)
Elution of basic compounds	Insufficient contact time, inappropriate solvent (solvent strength, water miscibility), insufficient basic capacity, sorbent stability (silica-based sorbents)

time is a key factor for ensuring reproducible results. To avoid 'break-through' due to ineffective retention of analytes, the adsorption flow rate should be kept below 1 mL/min.

In the fourth step, protein and other interferences are washed away with water (to avoid protein precipitation on the sorbent when subsequently applying organic solvents) and the pH is changed by applying an acidic solution so that analytes with amino functions are protonated and establish their electrostatic interactions with the charged sorbent.

In the fifth step, because ionic interactions are relatively strong forces, acidic and neutral analytes as well as hydrophobic and polar interferences can be eluted with strong organic solvents (with different polarity and/or solvent strength; the first solvent should be miscible with water to be able to reach the 'inner surface' of the sorbent, which is loaded with water comparably to a sponge; flow rates should be kept below 1 mL/min). In the procedure described here, ethyl acetate–2-propanol (3 : 1) is used.

In the sixth and final step, all bonding mechanisms for the remaining basic analytes (ionic and hydrophobic interactions) have to be disrupted simultaneously and this can be done using an appropriate basic organic solvent (in the procedure described, ethyl acetate–2-propanol–triethylamine (75 : 25 : 3) is used). Elution will be incomplete if the basic solution is not strong enough to completely disrupt the electrostatic interaction. Again, flow rates should be kept below 1 mL/min, because ion-exchange binding kinetics are even slower than with hydrophobic and aromatic interactions. Volatile organic bases (such as triethylamine) have the advantage that they can be removed easily from the extract by evaporation.

After evaporation of the eluent from step 5, the extract can then be analysed for neutral and acidic compounds (in the case of a very complex matrix and/or a very low concentration of the analyte, a further back-extraction step might be necessary). Simultaneously, after evaporation, the extract from step 6 can be analysed for basic compounds. The aqueous phase of the sample should not be discarded in this 'general unknown' screening procedure, because strong electrolytes (e.g. quaternary ammonium compounds) will not be sufficiently extracted onto the sorbent. Extraction of these compounds can be accomplished by ion-pair formation (see Ion-pair extraction above). The possible pitfalls in using a mixed-mode SPE procedure are given in Table 29.4.

Microextraction

Miniaturisation of the extraction process simplifies the entire analytical procedure by having the extraction carried out in one vessel, eliminating the evaporation step and possible loss of volatile materials, reducing solvent use, and considerably saving in extraction time. It usually involves vigorous mixing of a small volume of solvent with a large volume of biological material (e.g. urine), centrifuging to separate the solvent layer and direct injection into a chromatograph. For example, amfetamines and related compounds may be extracted from 5 mL of urine into 100 µL of chloroform and this is injected into a GC apparatus (Ramsey, Campbell 1971).

Some microextraction methods such as hollow fibre-based liquid-phase extraction (Esrafili *et al.* 2007) and fibre-in-tube solid-phase microextraction (Yazdi *et al.* 2008) have been successfully used for the analysis of antidepressant drugs in biological fluids.

Microextraction by packed sorbent (MEPS) with on-line connection to GC or LC has been applied for the analysis of local anaesthetics in plasma samples (Abdel-Rehim 2004) and the analysis of amfetamines in hair (Miyaguchi *et al.* 2009).

Conclusions

The ongoing development of more powerful and sensitive analytical instruments can only be fully utilised after the successful isolation of the target compounds from the biological specimens. Without sufficient sample extraction, either these sensitive instruments cannot be used at all or the results are unreliable owing to interferences. Although many optimised procedures for the extraction of specific target compounds (in most cases from urine or blood) can be found in the literature, non-selective, reliable and robust procedures for the simultaneous extraction of a wide range of analytes with different physicochemical properties (e.g. for the 'general unknown' screening) are rare, especially when more complex matrices have to be extracted. Unfortunately, the greatly desired 'universal standard extraction procedure' for all possible constellations of target compounds and specimens does not (yet) exist.

The selection or development of a proper extraction procedure and the procedure's adaptation to specific cases – based on analytical data of targeted compounds, available specimens, advantages and disadvantages of the various extraction techniques, and the time and resources at hand – are key tasks of the analytical toxicologist. Special knowledge and experience are needed to meet these challenges. Analytical results usually have serious medical or legal consequences: toxicologists therefore carry a high level of responsibility. To ensure the integrity of analytical results, stringent quality control measures have been implemented in modern toxicological laboratories. But such measures should not lead to overly rigid structures that limit the toxicologist to a few strictly defined extraction methods and target analytes, as this would narrow a laboratory's flexibility to an unacceptable level.

The analytical toxicologist must stay open to new extraction technologies, improvements in sample preparation and developing trends. Among these improvements is automation, which enables the unattended, reproducible extraction of samples; advances in this application of technology are expected soon.

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CHAPTER

30 Colour Tests

B Widdop

Introduction

The bulk of the material in this chapter derives from that contributed by the late HM Stevens to the second edition of this publication, which was modified and expanded by Wayne Jeffrey in the third edition. The main addition in this latest version is an appendix dealing with tests for metals and anions that are not covered by the general scheme.

Colour tests (sometimes referred to as chemical spot tests) provide toxicologists and drug analysts with one of the first tools for the presumptive identification of drugs and poisons. These colour tests are most usefully applied to pharmaceuticals and scene residues, and to a lesser extent to biological fluids such as stomach contents, urine. They are used to place the unknown into a specific class of compounds or to eliminate categories or classes of compounds. These colour tests remain popular for many reasons. They are simple to perform, use minimal reagents, are inexpensive and give results that can be viewed by the naked eye. They appeal particularly in parts of the developing world where laboratory facilities tend to be very limited. In many instances they can also be used as thin-layer chromatography (TLC) location reagents applied by spraying or dipping (see Chapters 1, 11, and 39). This chapter describes the colour tests cited in the monographs. For some substances, the colour reaction with a particular chemical reagent may be quite specific, but it is much more common for the colour to be produced by a class of compounds. Moreover, compounds that do not fall into the class may also give colours. For some of the tests, the colour reactions can be correlated with certain aspects of the chemical structure of a compound or group of compounds. However, anomalous responses often occur that cannot be explained on that basis. Some of these are noted in the colour tests described below, but it should be borne in mind that many others may be found. It follows that colour tests are only an indication of the presence of a compound or class of compounds and that all tests must be confirmed by more specific methods. This is especially important in forensic cases!

The colour tests included here range from those that rely on reactions with certain functional groups (e.g. Folin–Ciocaltaeu for phenols), those that are almost specific for a given group (e.g. FPN reagent for phenothiazines) through to those that give diagnostic colours with a wide range of compounds (e.g. Mandelin's test and the Marquis test).

Interpretation of colour tests

Colour descriptions

Colours exhibited by these tests cannot be described with any accuracy. They may vary in intensity or tincture with the concentration of compounds in the test samples and the presence of extraneous material. In addition, their assessment is always a subjective one, even in people with normal colour vision. Some of the complexes formed are unstable such that the colour changes or fades with time.

Effects of ionic form

Salts may give colours different from those of the corresponding acid or base. In general, free acids or bases that have been isolated from the test material by an extraction process give better colours than their salts. The colour of a salt may be modified by the nature of the other ions present. For example, all hydrochloride salts give a red colour in Mandelin's test and a blue colour with Koppanyi–Zwikker reagent (prior to adding pyrrolidine). Basic salts of weak acids may produce different colours because of a change in pH. Where a compound has been extracted from biological material, these factors should not create any difficulty, since it will be present in the form of the base. However, when applying the tests to pharmaceutical preparations, where the compounds are usually present as salts, this can cause problems. To overcome this, the material can be extracted in much the same way as for biological samples to derive the free base. Bromide and iodide salts can be converted into the nitrate before testing, which gives the same colour as the base, by the following method:

- To 0.5 mL of a 1% (w/v) solution of the salt in dilute acetic acid, add one drop of an 8% (w/v) solution of silver nitrate followed by one drop of a 2% (w/v) solution of sodium chloride to remove excess silver.
- Centrifuge to separate the precipitated silver halide and use the supernatant liquid, either as a solution or evaporated to dryness where necessary, for the colour tests.

The colours that are recorded in the tables and monographs are usually those obtained by testing either the free acid or the free base.

Use of the colour tests lists

The system adopted uses ten basic colours: the spectral colours (red, orange, yellow, green, blue and violet), together with pink, brown, grey and black. Where there is a variation in hue, this is indicated by combining two colours (e.g. red–brown). The second-named colour is considered to be the dominant one and is the main colour used in the lists. For example, red–brown is listed under brown, whereas brown–red is listed under red. When interpreting results, it is often necessary to search the lists given under two main colours (e.g. for red–brown, the lists under both red and brown should be consulted). This takes account of the subjective nature of colour assessment. An arrow between two colours (e.g. red→brown) indicates that the colour changes during the course of the test. In the monographs, the notation brown/red is used where there are two parts to a test that produce two colours. Occasionally, the colour displayed by a test solution in reflected light may be different from that in transmitted light, in which case the solution is described as dichroic. A combined colour may be obtained when more than one drug is present or the drug itself is coloured, which limits the value of the tests for biological samples.

Practical points

Performing the colour tests

The tests are carried out either in clear glass test-tubes or on white glazed porcelain tiles (spotting tiles), which give a uniform background against which the colours can be assessed. For drugs, the tests are designed to work on about 1 mg, either as the solid form or as a dried extract of this amount (see below), unless stated otherwise. Solutions should be made in water unless otherwise stated. Where an instruction, time, temperature, etc., appears in brackets after the drug name, such as (add water), (15 s) or (slowly at 100°C), this indicates a change in the test procedure for that particular drug.

The following recommendations are most important:

- A sample known not to contain the compound of interest should be tested at the same time as the test sample. This enables a comparison of the colours produced by the sample and by the reagent blank. Ideally, the blank sample should have the same matrix as the test sample (e.g. for urine tests use analyte-free urine), since this takes account of the effects of extraneous materials. Otherwise, water is usually adequate.
- Before making a final decision on the result of a test, the reaction of the unknown should be compared with that of a reference substance tested under exactly the same conditions.

Validation of a colour test

It is essential to validate all tests and test reagents for sensitivity and specificity; O'Neal *et al.* (2000) have outlined a suitable method for a chemical spot test.

Application of colour tests to sample extracts

Several solvent extraction schemes have been devised to fractionate compounds on the basis of their acidic, neutral or basic characteristics (see Chapters 1 and 10). The tests listed in Table 30.1 can be applied to the evaporated extracts.

Table 30.1 Tests that can be applied to the evaporated extracts

Fraction	*Test*
Strong acid	Aromaticity
	Ferric chloride
	Folin–Ciocaltaeu reagent
	Liebermann's reagent
	Millon's reagent
	Nessler's reagent
Weak acid	Aromaticity
	Coniferyl alcohol
	Diazotisation
	Ferric chloride
	Folin–Ciocaltaeu reagent
	Koppanyi–Zwikker reagent
	Liebermann's reagent
	Mercurous nitrate
	Millon's reagent
	Nessler's reagent
Neutral	Aromaticity
	Furfuraldehyde
	Koppanyi–Zwikker reagent
	Liebermann's reagent
	Mercurous nitrate
	Nessler's reagent
Basic	Amalic acid test
	p-Dimethylaminobenzaldehyde
	Ferric chloride
	Formaldehyde–sulfuric acid
	Forrest reagent
	FPN reagent
	Liebermann's reagent
	Mandelin's reagent
	Marquis reagent
	Nessler's reagent
	Sulfuric acid

Table 30.2 is formulated to give a quick lead to those tests that can be applied to detect some of the most important drug groups and other poisons.

Table 30.2 Indication of which tests can be applied to detect some of the most important drug groups and other poisons

Substance/functional group	*Useful tests*
Alcohols	Potassium dichromate
Alkaloids and nitrogenous bases	Dragendorff's reagent
Amides (aliphatic)	Nessler's reagent
Aldehydes (aliphatic)	Schiff's reagent
Amfetamines	See Appendix 30.1
	Sodium nitroprusside–acetone
Antidepressants	Marquis test
Barbiturates	Dille–Koppanyi reagent
	Koppanyi–Zwikker reagent
	Mercurous nitrate
	Vanillin reagent
	Zwikker reagent
Benzodiazepines	Formaldehyde–sulfuric acid
Cannabis	Duquenois reagent
Carbamates (non-aromatic)	Furfuraldehyde
Cocaine	Cobalt thiocyanate
	p-Dimethylaminobenzaldehyde
	Mandelin's test
	Scott's test
Chlorinated phenols	Nitric acid (fuming)
Chlorinated hydrocarbon insecticides	Nitric–sulfuric acid
Cyanide	Ferrous sulfate (B)
	Sodium picrate
Cyanide groups	Sodium picrate
Dithiocarbamates	Sodium nitroprusside
Ergot alkaloids	*p*-Dimethylaminobenzaldehyde
Halogenated hydrocarbons	Fujiwara test
Imides	Koppanyi–Zwikker test
Ketones	Sodium nitroprusside
Methadone	Cobalt thiocyanate
	Mandelin's test
	Marquis test
	Tetrabromophenolphthalein ethyl ester
Mono-substituted pyridine ring	Cyanogen bromide
Nitrates and nitrites	Ferrous sulfate
Opiates	See Appendix 30.2
Oxidising agents	Diphenylamine
Paraquat/diquat	Sodium dithionate
Phencyclidine	Cobalt thiocyanate
	p-Dimethylaminobenzaldehyde
	Tetrabromophenolphthalein ethyl ester
Phenols	*p*-Dimethylaminobenzaldehyde
	Ferric chloride
	Folin–Ciocaltaeu reagent
	Millon's reagent

Table 30.2 continued

Substance/functional group	*Useful tests*
Phenothiazines	Ferric chloride
	Formaldehyde–sulfuric acid
	Forrest reagent
	FPN reagent
Phenylpyrazolines	Nitrous acid
Primary aromatic amines	Coniferyl alcohol
	Diazotisation
Primary and secondary amines	Dragendorff's reagent
	Simon's test
Propoxyphene	Cobalt thiocyanate
	Froehde's reagent
	Liebermann's test
	Tetrabromophenolphthalein ethyl ester
Quaternary ammonium compounds	Tetrabromophenolphthalein ethyl ester
Quaternary amines	Dragendorff's reagent
Quinines	Cobalt thiocyanate
	Thalleioquin
Quinones	Methanolic potassium hydroxide
Reducing agents	Benedict's reagent
Salicylates	Ferric chloride
	Trinder's reagent
Steroids	Antimony pentachloride
	Naphthol sulfuric acid
	Sulfuric acid
Sulfonamides	Copper sulfate
	Koppanyi–Zwikker reagent
	Mercurous nitrate
	Nitrous acid
Sulfur containing	Palladium chloride
	Sodium nitroprusside
Tertiary amines	Dragendorff's reagent
	Tetrabromophenolphthalein ethyl ester

Additional information

Colour reactions given by narcotics and amfetamines with four of the so-called 'alkaloid colour reagents' (Marquis, Mecke's, Froedhe's and Mandelin's) are listed in Appendix 30.1 and Appendix 30.2. Three colour tests have been developed for gamma-hydroxybutyric acid (GHB) and its precursor gamma-butyrolactone (GBL; see Appendix 30.3).

Colour test methods

Caution: the following lists of colour tests and drugs tested are not exhaustive; the omission of a compound from a list does not indicate that no response is given, but that it may not have been tested.

Amalic acid test (test for xanthines)

Method

Add to the sample a few drops of 10 mol/L hydrochloric acid followed by a few crystals of potassium chlorate, and evaporate the mixture to dryness. Observe the colour of the residue then add 2 or 3 drops of 2 mol/L ammonium hydroxide and again observe the colour.

Table 30.3 Colours with amalic acid

Colour	*Compound*
Red (→violet)	Bufylline, caffeine
Pink (→violet)	Pentoxifylline
Orange (→violet)	Acepifylline piperazine, bamifylline, xanthinol nicotinate
Pink–orange (→violet)	Fenetylline, pentifylline
Yellow (→pink)	Etamiphylline
Yellow (→violet)	Diprophylline, proxyphylline, theobromine, theophylline

Indications

A red, pink, orange or yellow residue, which changes to pink, red or violet after the addition of ammonium hydroxide, indicates the presence of a xanthine (Table 30.3).

Ammoniacal silver nitrate

Reagent

To 20 mL of 0.1 mol/L silver nitrate add sufficient strong ammonia solution to dissolve the initial precipitate.

Method

Dissolve the sample in a minimum amount of water, with the addition of ethanol if necessary, add an equal volume of the reagent and note any colour that develops. Heat the mixture in a water-bath at 100°C for 30 s.

Indications

Red, yellow, brown or black colours (especially at room temperature) indicate potent reducing power, which occurs when adjacent carbon atoms in a ring each bear a hydroxyl group (Table 30.4). There is no response when the hydroxyl groups are *meta* to each other, but there is some restoration of reducing power when they are *para* to each other. Some colour production is also obtained with ethynyl bonds, but not with ethylenic bonds. Ethchlorvynol and ethinylestradiol both give a white precipitate that turns yellow on heating. Carbidopa gives a silver mirror on heating.

Table 30.4 Colours with ammoniacal silver nitrate

Colour at room temperature	*Compound*	*Colour at 100°C*
Red	Isoetharine	Brown–orange
	Hexoprenaline (→brown→black)	
	Isoprenaline (→red–brown)	Brown
	Rimiterol	Brown
Yellow	Ethinamate	Brown
	Levodopa (→brown)	Black
Grey–yellow	Hydroquinone	Brown
Brown		
Red–brown	Adrenaline	
	Methyldopa	Black
Orange–brown	Dopamine	Black
	Methyldopate	Orange–brown
Grey		
Red–grey	Protokylol	Brown
Black	Ascorbic acid	
	Benserazide	–
	Dobutamine	–
	Dodecyl gallate	–
	Noradrenaline	–

Antimony pentachloride

Reagent

Dry some antimony trichloride over phosphorus pentoxide, melt the dried material (m.p. 73°C), and pass dry chlorine gas into the melt until a yellow fuming liquid is obtained. Add this liquid to about 10 times its volume of chloroform, filter the solution into a dark glass-stoppered bottle and store in a desiccator.

Method

Place a drop of an ethanolic solution of the sample on a filter paper, add a drop of the reagent and dry in a current of warm air. Alternatively, the test may be carried out by adding a drop of the reagent to the sample on a white tile.

Indications

Various colours are obtained with the cardiac glycosides, their aglycones and certain oestrogens and corticosteroids (Table 30.5). No colour is obtained with beclometasone, cortisone, fluocinolone, fludroxycortide, prednisolone, prednisone, progesterone, testosterone or triamcinolone.

Aromaticity

Method 1

Place a portion of the sample in each of two ignition tubes, and to one tube add some solid sodium hydroxide. Heat both tubes carefully, allow the water vapour to escape, insert into the vapours in each tube an open capillary tube that contains Marquis reagent, and observe the colour of the reagent.

Indications

Red or orange colours indicate that the sample is aromatic in nature. The colours probably result from the liberation of traces of aromatic hydrocarbons, phenols, etc. Colours obtained after heating with sodium hydroxide generally indicate the presence of aromatic acids. Colours obtained after heating without sodium hydroxide generally indicate the presence of phenols, phenolic acids and aldehydes that contain more than one hydroxyl group.

A negative result does not necessarily imply that the substance is non-aromatic.

Method 2

Add 2 or 3 drops of concentrated nitric acid to the sample, heat in a water-bath at 100°C for 1 min, cool the mixture, dilute 3–4 times with water and make the solution alkaline by the addition of a 40% (w/v) solution of sodium hydroxide.

Table 30.5 Colours with antimony pentachloride

Colour	*Compound*
Red	Dienestrol, diethylstilbestrol
Orange	Cholesterol (→brown), desoxycortone, dydrogesterone, fludrocortisone, hydrocortisone, hydroxyprogesterone, strophanthin-K (→red)
Yellow	Alfadolone
(→brown)	Androsterone, digitoxigenin, digoxigenin
(→brown→black–violet)	Digitoxin, digoxin, lanatoside C, ouabain (very weak)
Green–yellow	Fluocortolone
Green	Betamethasone (→brown), dexamethasone, mestranol, pancuronium
Brown	Carbenoxolone, dimethisterone, estradiol, estriol, estrone, ethinylestradiol (→black), fluoxymesterone, norethandrolone, norethisterone, oxymetholone, rotenone
Orange–brown	Enoxolone (→violet)
Green–brown	Noretynodrel

Table 30.6 Colours with aromaticity test, method 2, obtained on addition of alkali to acid solution

Colour of acid solution	*Colour after addition of alkali*	*Compound*
Colourless	Red	Clobazam (heat for 3 min with acid)
	Orange	Butanilicaine, tolazoline
	Yellow	Amprolium, atropine methobromide, hyoscine butylbromide, hyoscine methobromide, ketoprofen, pipazethate, tetrahydrozoline, tetramisole, trimetaphan
	Violet	Atropine methonitrate (transient), hyoscine methonitrate (transient)
	Brown	Isopropamide
Yellow	Red	Aminacrine, benzonatate, tetracaine (amethocaine), trimethoprim
	Orange–red	Dextromethorphan, haloperidol
	Orange	Amicarbalide, carbocromen, dyclonine, glibenclamide, levallorphan, metocurine, padimate, propanidid, quinuronium, salinazid
	Brown	Dibromopropamidine, dichlorophen tubocurarine
Red	Brown–violet	Dequalinium

Indications

A change from colourless or yellow in acid solution to darker colours (e.g. orange or red–orange) after the addition of sodium hydroxide indicates the presence of a benzene ring in the molecule, probably though the production of a nitrophenol or other nitro compound.

Certain compounds (e.g. diazepam, methaqualone) give a negative result. Orange colours are given by certain non-aromatic corticosteroids (e.g. cortisone), by substances that contain sulfur and by compounds that already contain an aromatic nitro group (e.g. nifursol).

Colour changes are given in Table 30.6.

Certain substances give distinct colours with cold nitric acid, but the colours fade on heating; these are listed in Table 30.7.

Benedict's reagent

Reagent

Dissolve 1.73 g of copper sulfate in 10 mL of water. Dissolve 17.3 g of trisodium citrate and 10 g of anhydrous sodium carbonate in 80 mL of water with the aid of heat; pour this solution into the copper sulfate solution and dilute the mixture to 100 mL.

Method

Add 0.5 mL of the reagent to the sample and heat in a water-bath at 100°C for 3 min.

Indications

The formation of red cuprous oxide occurs with strong reducing agents, such as ascorbic acid, dithionites, certain phenolic compounds that contain two hydroxyl groups *para* to each other, and compounds that contain at least four hydroxyl groups on a non-aromatic ring (e.g. glucose, tetracyclines).

Table 30.7 Colours with aromaticity test, method 2, obtained on addition of cold nitric acid to sample, which fade on heating

Colour	*Compound*
Red	Aminacrine (15 s), clozapine, dropropizine, medazepam, trimethoprim
Brown	Metocurine
Pink–brown	Diethylthiambutene (changing to green)
Black	Tubocurarine

A weak response (orange–brown or brown colours) is given by streptomycin, hydroxylamine and substituted hydrazines (e.g. phenelzine). No colour is obtained with beclometasone, cardiac glycosides and estriol (two hydroxyl groups) or clindamycin (three hydroxyl groups).

Carbon disulfide

Method

Mix the sample with 1 mL of water and 0.1 mL of a 1% (w/v) solution of sodium tetraborate, add 0.2 mL of a 10% v/v solution of carbon disulfide in ethanol and heat in a water-bath at 100°C for 3 min; cool the solution and add 3 drops of 0.1 mol/L silver nitrate.

Indications

A brown colour indicates the presence of a dithiocarbamate, which suggests that the original substance was an aliphatic or heterocyclic primary or secondary amine. The original sample should be tested to ensure that it does not give a brown colour with silver nitrate alone.

Chromotropic acid

Reagent

1. Dissolve 20 mg of chromotropic acid in 10 mL of concentrated sulfuric acid.
2. Dissolve 1 g of sodium nitrite in 10 mL of concentrated sulfuric acid.

Method 1

Add a small amount of sample, either solid or in solution, to 1 mL chromotropic acid reagent. Note any colour that may be produced, and then add the solution dropwise to 0.5 mL of water, with cooling. Substances that give a colour with cold sulfuric acid must be excluded.

Indications

See Table 30.8.

Table 30.8 Colours with chromotropic acid

Colour	*Compound*
Red (before dilution)	Formaldehyde, paraformaldehyde (reacts slowly)
Violet (after dilution)	Hydrochlorothiazide, hydroflumethiazide

Method 2 (for chlorophenoxy herbicides)

Add 1 mL of 1 mol/L hydrochloric acid to 10 mL of sample and extract with 20 mL of toluene for 5 min. Centrifuge for 5 min and remove the toluene layer. Repeat with a further 20 mL of toluene and evaporate the combined extracts to dryness. Dissolve the residue in 0.2 mL of concentrated sulfuric acid and divide between two wells of a spotting tile. Add 0.1 mL of sodium nitrite solution to one well and 0.1 mL of chromotropic acid reagent to the other. Heat the tile at 80°C and observe any colour development.

Indications

See Table 30.9.

Table 30.9 Colours with sodium nitrite and chromotropic acid

Compound	*Sodium nitrite*	*Chromotropic acid*
2,4-Dichlorophenoxyacetic acid	Brown	Purple
2,4-Dichlorophenoxypropionic acid	Dark brown	Light purple
4-Chloro-2-methylphenoxyacetic acid	Light brown	Light purple
2-(4-Chloromethylphenoxy)propionic acid	Light brown	Purple
2,4,5-Trichlorophenoxyacetic acid	No reaction	Purple
2-(2,4,5-Trichlorophenoxy)propionic acid	No reaction	Light pink/ purple

Cobalt thiocyanate (see Scott's test)

Reagent

1. A 2% (w/v) solution of cobalt thiocyanate in water
2. Phosphoric acid
3. 1 g $H_2PtCl_6 \cdot 6H_2O$ in 20 mL of H_3PO_4.

Mix 9 parts of solution 1 and 3 parts of solution 2, add 1 part of solution 3 and mix well. Add 9 parts distilled water and mix. When the solution turns pink it is ready for use.

Method

Add a few drops of the reagent to the sample to be tested.

Indications

See Table 30.10.

Limit of detection (LOD): cocaine-HCl 60 µg, methadone-HCl 15 µg.

Table 30.10 Colours with cobalt thiocyanate

Colour	*Compound*
Blue (flaky precipitate)	Cocaine
Brilliant greenish blue	Benzfetamine-HCl, brompheniramine maleate, chlordiazepoxide-HCl, chlorpromazine-HCl, doxepin-HCl, hydrocodone tartrate, methadone-HCl, methylphenidate-HCl
Strong greenish blue	Diacetylmorphine-HCl, ephedrine-HCl, phencyclidine-HCl, procaine-HCl, propoxyphene-HCl, pseudoephedrine-HCl
Strong blue	Quinine-HCl

Coniferyl alcohol (primary aromatic amines)

Reagent

Warm 0.1 g of coniferyl alcohol until it melts (m.p. 74°C), dissolve in 3 mL of ethanol and dilute to 10 mL with ethanol.

Method

Place 1 drop of a solution of the sample on a filter paper, add 1 drop of the reagent and expose the paper to hydrochloric acid fumes.

Indications

An orange colour indicates the presence of an aromatic primary amine in which the amino group is attached directly to a benzene ring.

An anomalous reaction is obtained with diphenylamine (bright orange).

Copper sulfate

Method 1

Dissolve the sample in a minimum volume of 0.1 mol/L sodium hydroxide and add a 1% (w/v) solution of copper sulfate, drop by drop, until the colour change is complete.

Indications

Green, blue or brown colours indicate the presence of a sulfonamide (Table 30.11).

Table 30.11 Colours with copper sulfate, method 1

Colour	*Compound*
Green	Phthalylsulfathiazole, succinylsulfathiazole (→violet), sulfachlorpyridazine, sulfadimethoxine, sulfadimidine (→brown), sulfadoxine, sulfaethidole, sulfamerazine (→brown), sulfamethizole, sulfamethoxazole, sulfametopyrazine, sulfapyridine (→brown–green), sulfaquinoxaline, sulfasomidine
Blue	Phthalylsulfacetamide, sulfacetamide, sulfaguanidine, sulfanilamide, sulfaphenazole, sulfaurea, sulfinpyrazone, sulthiame
Brown	Sulfafurazole
Orange–brown	Sulfasalazine
Green–brown	Sulfamethoxypyridazine, sulfamoxole
Violet–brown	Sulfadiazine, sulfamethoxydiazine, sulfathiazole

Method 2
Add 1 or 2 drops of a 1% (w/v) solution of copper sulfate to the sample on a white tile.

Indications
A blue colour indicates the presence of an alkali salt of a fatty acid, such as sodium cromoglicate (1–2 min) or valproate. The colours are not produced by a change of pH (some of the alkali salts will change the pH), as negative results are obtained with sodium bicarbonate.

Cyanogen bromide

Reagent
1. Decolorise bromine solution by the addition of solid potassium cyanide and then add more bromine solution until the solution is pale yellow.
2. Prepare a saturated solution of aniline in water.

Solutions 1 and 2 are stable for 1 week. Mix equal volumes of the two solutions immediately prior to the test.

Method
Add 1 drop of the mixed reagent to the sample on a white tile.

Indications
Red, orange or yellow colours indicate the presence of a mono-substituted pyridine ring. Increasing chain length of the substituent group weakens the response; a delayed response is obtained when the pyridine ring is substituted by nitrogen adjacent to the ring nitrogen; a weak response is obtained where there is a C=O substituent adjacent to the ring nitrogen. There is no response to the test if the pyridine ring is bound to another ring, if it is substituted in more than one position or if the nitrogen in the ring is substituted. Anomalous results are obtained with azatadine (pink), bisacodyl (no response) and tropicamide (violet–pink) (Table 30.12).

Diazotisation

Method
Dissolve the sample in 2 mol/L hydrochloric acid, and to 1 drop on a white tile add 1 drop of a 1% solution of sodium nitrite, and 1 drop of a 4% solution of naphth-2-ol in 2 mol/L sodium hydroxide.

Indications
A bright red or orange–red colour indicates the presence of a primary aromatic amine. Diphenylamine does not give a reaction; aminonitrothiazole (solid) gives a violet colour.

Dille–Koppanyi reagent modified (a general test for barbiturate-like compounds)

Reagent
1. Dissolve 0.1 g of cobalt(II) acetate dihydrate in 100 mL of methanol. Add 0.2 mL of glacial acetic acid and mix.
2. Add 5 mL of isopropylamine to 95 mL of methanol.

Table 30.12 Colours with cyanogen bromide[a]

Colour	*Compound*
Red	Zimeldine (30 s)
Pink	Azatadine
Orange–pink	Carbinoxamine, dimetindene, doxylamine, iproniazid, phenyramidol, triprolidine (1–2 min)
Violet–pink	Tropicamide
Orange	Azaperone, brompheniramine, chlorphenamine, isoniazid, metyrapone, nicametate, nicotinamide, nicotine, nicotinic acid, nifenazone, nikethamide, pheniramine, xanthinol nicotinate
Red–orange	Benzyl nicotinate
Yellow	Halopyramine, mepyramine, tripelenamine

[a]Anomalous results are obtained with azatadine (pink), bisacodyl (no response) and tropicamide (violet–pink).

Method
Add 2 drops of solution 1 to the drug, followed by 1 drop of solution 2.

Indications
A light purple (blue–violet) colour indicates the presence of a barbiturate. Other reacting compounds are hydantoins, sulfonamides, pyrimidine, piperidine, methyprylon. The LOD is 25 μg or lower.

p-Dimethylaminobenzaldehyde (Wasicky reagent or Van Urk reagent; a general test for ergot alkaloids)

Reagent
Dissolve 2.0 g of *p*-dimethylaminobenzaldehyde (p-DMAB) in 50 mL of 95% ethanol and 50 mL of concentrated hydrochloric acid. The reagent should be freshly prepared.

Method
Add the reagent to the sample in a test-tube, warming if necessary. Observe any colour produced, then carefully dilute with water or spray dried spots on filter paper and heat.

Indications
Colours are given by a number of substances, which include ergot alkaloids, dimethyltryptamine, psilocin, psilocybine (gives a violet colour), cannabinols and certain indoles in which the indole ring is not bonded to another conjugated ring (red changing to violet on dilution), and certain phenols and phenolic amines (red or orange, usually changing to violet on dilution). Some other types of compound also respond. See Table 30.13.

The LOD for lysergide (LSD) is 6 μg.

Diphenylamine test

Reagent
Mix 0.5 g of diphenylamine in 20 mL of water and dilute to 100 mL with concentrated sulfuric acid.

Method
Apply the reagent to the sample on a white tile or in a test-tube.

Indications
A blue colour indicates the presence of an oxidising agent such as bromate, chlorate, chromate, dichromate, iodate, lead(IV), manganese (III, IV, VII), nitrate, nitrite, permanganate or vanadate.

This test has been modified for use on blood samples to detect ethchlorvynol (Caughlin 1991). Blood (0.5 mL) is mixed with 1.0 mL of acetone and vortex mixed. The sample is centrifuged and 50 μL of the supernatant is added to 50 μL of diphenylamine reagent and 25 μL of chloroform. The mixture is vortex mixed and allowed to stand. A pink colour that develops in the chloroform layer indicates ethchlorvynol.

Dragendorff reagent (a general reagent for nitrogenous bases)

Reagent
Dissolve 1 g of bismuth subnitrate in 3 mL of 10 mol/L hydrochloric acid with the aid of heat. Dilute to 20 mL with water and dissolve 1 g of

Table 30.13 Colours with *p*-dimethylaminobenzaldehyde

Colour	*Compound*
Red (changing to violet on dilution)	Cannabinols, phenazone (100°C, 5 min), pindolol, psilocin, psilocybine, tryptamine
Red (no violet on dilution)	Benserazide, cocaine (100°C, 3 min), feprazone, harmine, phencyclidine (100°C, 3 min)
Orange (changing to violet on dilution)	Dobutamine, dopamine, diamorphine, morphine, orciprenaline, phenol, terbutaline, tyramine
Violet	Ergot alkaloids (dihydroergotamine, ergometrine, ergotamine, ergotoxine, lysergide, methysergide), dimethyltryptamine, psilocin, psilocybine
Yellow	Primary aromatic amines, e.g. aminosalicylic acid, anileridine, aniline, procaine, benzocaine

potassium iodide in the mixture. If black bismuth triiodide separates, add 2 mol/L hydrochloric acid and more potassium iodide to dissolve it.

Method
Dissolve the sample in 3 drops of 2 mol/L hydrochloric acid, add 2–3 mL of the reagent and dilute to 10 mL with water.

Indications
An orange, red–orange or brown–orange precipitate suggests the presence of an alkaloidal base (precipitated as the alkaloidal bismuth iodide). Primary, secondary, tertiary and quaternary amines give positive results. This reagent is commonly used as a spray or locating agent to detect alkaloids on TLC plates.

Duquenois reagent, modified

Reagent
1. Add 2.5 mL of acetaldehyde and 2.0 g of vanillin to 100 mL of 95% ethanol
2. Concentrated hydrochloric acid
3. Chloroform.

Method
Place the solid sample, or an evaporated petroleum ether (or other organic solvent) extract of the sample, in a test-tube and add 3 drops of solution 1. Shake for 1 min and add 3 drops of solution 2. Agitate gently and observe the colour produced. Add 9 drops of solution 3, vortex mix gently and note whether the colour is extracted from the mixture.

Indications
A colour change from grey to green through blue to violet–blue suggests the presence of cannabis, but differentiation from roasted coffee and patchouli oil is required. The colour change is best seen with fresh drug material. The violet colour is extracted into the chloroform layer only when cannabis is present (Table 30.14). The LOD is 350 µg of tetrahydrocannabinol (THC). No colour is obtained with other natural products, such as basil, bay leaf, eucalyptus oil, mace, marjoram, rosemary, sage, thyme or tobacco.

Ferric chloride (general reagent for phenols, e.g. salicylates)

Reagent
Dissolve 5 g of anhydrous ferric chloride, or 8.25 g of ferric chloride hexahydrate, in 100 mL of distilled water.

Method
Add ferric chloride solution to the sample or an ethanolic solution of the sample.

Indications
Red, orange, green, blue, violet or brown colours indicate the presence of a phenolic compound, fatty acid or a phenylpyrazoline. High quantities of phenothiazines can also cause this test to be positive. Salicylates give a violet colour. Many phenols give no colour with ferric chloride when water is used as a solvent, but give positive tests when anhydrous solvents such as chloroform are used. Aspirin (acetylsalicylic acid) does not give a positive result unless first hydrolysed with concentrated sodium hydroxide to give salicylate. Colours are listed in Table 30.15.

Table 30.14 Colours with modified Duquenois reagent

Compound	*Initial colour*	*Colour extracted by chloroform*
Cannabis	Violet–blue	Violet
Coffee (roasted)	Violet–brown	Nil
Nutmeg	Pale reddish purple	Nil
Patchouli oil	Violet	Nil
Tea (leaves)	Green–blue	Nil

Table 30.15 Colours with ferric chloride

Colour	*Compound*
Red	Acetates, phenazone, propionates
Brown–red	Nifenazone
Orange	Chlorpromazine, hexoprenaline, propyphenazone, valproate
Yellow	
Green–yellow	Paracetamol
Green	Adrenaline, betanaphthol, dobutamine, dopamine, etamivan, ethylnoradrenaline, hexylresorcinol, hydroquinone, hydroxyquinoline, isoetarine, isoprenaline, levodopa, methyldopa, methyldopate, noradrenaline, paraphenylenediamine, phenothiazine, protokylol, rimiterol, thioridazine
Blue–green	Chlorquinaldol
Blue	Apomorphine, dodecyl gallate, gallic acid, morphine, parachlorophenol, pethidine, phenol, tannic acid
Violet	Aminosalicylic acid, diflunisal, dipyrone, hexachlorophene (transient), labetalol, salicylaldehyde, salicylamide, salicylic acid, salicyluric acid
Blue–violet	Aminophenazone, salicylamide (after hydrolysis), salicylic acid
Brown	Aloin, carbidopa
Yellow–brown	Salinazid
Green–brown	Benserazide
Black	
Violet–black	Ethyl gallate (→blue–black)

Ferrous sulfate A (test for nitrates and nitrites)

Reagent
To 1 volume of a 10% (w/v) solution of ferrous sulfate ($FeSO_4$,$7H_2O$) add 5 volumes of concentrated sulfuric acid with cooling.

Method
Add the sample to 0.5 mL of the reagent.

Indications
A red or pink colour is given only by nitrates and nitrites (e.g. glyceryl trinitrate).

Ferrous sulfate B (test for cyanide)

Reagent
Dissolve 10 g of ferrous sulfate in 100 mL of freshly boiled and cooled water (prepare fresh).

Method
Dilute 1 mL of sample with 2 mL of 10% (w/v) sodium hydroxide solution and add 2 mL of ferrous sulfate solution. Add sufficient 10% (v/v) hydrochloric acid to dissolve the ferrous hydroxide precipitate.

Indications
A blue colour is given by cyanide. There are no common sources of interference.

Folin–Ciocaltaeu reagent (test for phenolic compounds)

Reagent
For the stock solution, dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 800 mL of water in a 1500 mL flask, add 50 mL of phosphoric acid and 100 mL of concentrated hydrochloric acid, and reflux for 10 h. Cool, add 150 g of lithium sulfate, 50 mL of water and 4–6 drops of bromine, and allow to stand for 2 h. Boil for 15 min to remove the excess bromine, cool, filter and dilute to 1000 mL with water.

This stock solution should be stored at a temperature not exceeding 4°C and used within 4 months of its preparation; it has a yellow colour and must not be used if any trace of green colour is present.

For use, dilute 1 volume of this stock solution with 2 volumes of water.

Method
Add the diluted reagent to the sample and make the mixture alkaline with 2 mol/L sodium hydroxide.

Indications
A blue colour indicates the presence of a phenolic compound. The reaction is progressively inhibited with increased halogenation of the phenol nucleus.

Formaldehyde–sulfuric acid

Reagent
To 4 volumes of concentrated sulfuric acid add 6 volumes of formaldehyde solution (using a pipette with the tip below the surface of the acid) with stirring and adequate cooling. When the reagent is warm it remains clear for about 1 h. If turbidity develops, this may be dispelled by heating in a water-bath at 100°C for about 1 min (note that this reagent is not the same as that used in the Marquis test).

Method
Mix the sample with the reagent and heat at 100°C for 1 min.

Indications
Benzodiazepines generally give an orange colour with the exception of bromazepam and clozapine (a benzodiazepine-like compound), which both give yellow, and flurazepam (pink). Other indications include phenothiazines, tetracyclines and thioxanthenes. Tryptamine (brown) and zomepirac (red) also react. Those marked with an asterisk in Table 30.16 fluoresce orange under ultraviolet (UV) light ($\lambda = 350$ nm). No response is obtained with chlordiazepoxide, dimethoxanate or proquamezine. Some of the newer benzodiazepines have not been tested.

Table 30.16 Colours with formaldehyde–sulfuric acid

Colour	*Compound*[(a)]
Red	Chlorprothixene*, clopenthixol*, flupentixol*, fluphenazine, metopimazine, pericyazine, promazine, thiothixene, trifluprom azine, zomepirac
Brown–red	Lymecycline, oxytetracycline, tolmetin
Pink	Flurazepam, thioproperazine, trifluoperazine
Orange	Clonazepam, clorazepic acid, demeclocycline (→brown–red), demoxepam, diazepam, flunitrazepam, ketazolam, lorazepam, lormetazepam, medazepam (add water), metixene, nitrazepam, nordazepam, oxazepam, prazepam, temazepam, tetrazepam
Red–orange	Methacycline
Yellow	Bromazepam, clozapine, dimethothiazine, doxycycline
Green–yellow	Rolitetracycline (→yellow–brown), tetracycline (→yellow–brown)
Green	Thiethylperazine
Blue	Carphenazine, levomepromazine, thioridazine
Violet	Mesoridazine, perphenazine
Red–violet	Alimemazine, chlorpromazine, diethazine, mequitazine, methdilazine, perazine, phenothiazine, prochlorperazine, profenamine, thiopropazate
Blue–violet	Acepromazine, acetophenazine, piperacetazine, promethazine, propiomazine
Brown–violet	Thiazinamium
Brown	Tryptamine
Orange–brown	Chlortetracycline, clomocycline

[(a)]Compounds giving colours that fluoresce under UV light ($\lambda = 350$ nm) are indicated by an asterisk.

Table 30.17 Colours with Forrest reagent

Colour	*Compound*
Red	Acepromazine, carfenazine, chlorpromazine, diethazine, dimetotiazine, mequitazine, mescaline, mesoridazine, piperacetazine, prochlorperazine, promethazine, propiomazine, thiazinamium, thiopropazate, thioproperazine, thioridazine
Violet–red	Perphenazine
Brown–red	Alimemazine
Pink	Profenamine
Orange	Fluphenazine, phenothiazine, trifluoperazine, triflupromazine
Pink–orange	Acetophenazine
Red–orange	Methdilazine
Brown–orange	Perazine, pericyazine
Green	
Blue–green	Clomipramine, desipramine, imipramine, ketamine, opipramol, thiethylperazine, thioridazine, trimipramine
Violet	Levomepromazine, proquamezine (→red→orange)
Brown	Metopimazine
Red–brown	Promazine

Forrest reagent

Reagent
Mix together equal volumes of a 0.2% (w/v) solution of potassium dichromate, a 30% (v/v) solution of sulfuric acid, a 20% (w/w) solution of perchloric acid and a 50% (v/v) solution of nitric acid.

Method
Dissolve the sample in a minimum volume of 2 mol/L hydrochloric acid and add an equal volume of the reagent. To test urine, add 1 mL of reagent to 0.5 mL of urine.

Indications
Red, pink, orange, blue or violet colours are obtained with phenothiazines. A blue colour is obtained with certain dibenzazepines. The blue colour is inhibited by the presence of phenothiazines, so an excess of reagent must be added to overcome this. Colours are listed in Table 30.17.

FPN reagent (general reagent for phenothiazines)

Reagent
Mix together 5 mL of 5 % (w/v) ferric chloride solution, 45 mL of a 20% (w/w) solution of perchloric acid and 50 mL of a 50% (v/v) solution of nitric acid.

Method
Dissolve the sample in a minimum volume of 2 mol/L hydrochloric acid (or use 1 mL of urine) and add an equal volume of the reagent.

Indications
A variety of colours, from pink, red, orange, violet to blue, indicate the presence of phenothiazines (Table 30.18).

Froehde reagent

Reagent
Dissolve 1.0 g of molybdic acid or sodium molybdate in 100 mL of hot concentrated sulfuric acid.

Method
Add a drop of the reagent to the sample on a white tile.

Indications
Colours are listed in Table 30.19.

Table 30.18 Colours with FPN reagent

Colour	*Compound*
Red	Chlorpromazine, dimetotiazine, mesoridazine, methdilazine, prochlorperazine, thiazinamium, thiopropazate
Orange–red	Mequitazine
Violet–red	Perphenazine, proquamezine (→red→orange)
Brown–red	Alimemazine
Orange	Acetophenazine, diethazine (→yellow), fluphenazine, metopimazine, morphine, pericyazine, phenothiazine, profenamine, promethazine, thioproperazine, trifluoperazine, triflupromazine
Red–orange	Carfenazine
Pink–orange	Propiomazine (→red→fades)
Brown–orange	Acepromazine, perazine, piperacetazine
Blue	Clomipramine, imipramine, thiethylperazine, thioridazine, trimipramine
Violet	Levomepromazine
Brown	
Red–brown	Promazine

Table 30.19 Colours with Froehde reagent

Colour	*Compound*
Yellow	Hydrocodone, pethidine
Blue–yellow	Oxycodone-HCl
Orange	Diphenhydramine, flurazepam, promazine, trifluoperazine, triflupromazine
Green	Chlorphentermine, codeine, mescaline, oxycodone, phenyltoloxamine
Yellow–green	Lysergide
Blue	Pentazocine
Red	Amfetamine, chlorpromazine-HCl
Grey–red	Propoxyphene-HCl
Purple–red	Alimemazine, diacetylmorphine, promethazine, propylhexadrine, salicylic acid, tetracycline, thioridazine
Brown	Ephedrine, mescaline
Red–brown	Doxepin-HCl
Black	
Brown–black	Opium
Green–black	Methylenedioxyamfetamine (MDA)-HCl

Fujiwara test (general reagent for halogenated hydrocarbons)

Reagent
Freshly prepared 20% (w/v) sodium hydroxide solution.

Method
Mix together 2 mL of the reagent and 1 mL of pyridine. Add the sample (1 mL of urine) and heat in a water-bath at 100°C for 2 min with shaking.

Indications
A red–pink colour in the pyridine layer indicates the presence of compounds that possess at least two halogen atoms bound to one carbon atom. These include chloramphenicol, chlorbutanol, chloroform, dichlorophenazone, trichloroethane, trichloroethanol, trichloroacetic acid and trichloroethylene. Chloral hydrate and dichlorophenazone do themselves react but are excreted in urine as trichloroacetic acid. No colour is given by dicophane (DDT) or carbon tetrachloride, although massive exposure to the latter solvent may lead to a positive urine test because of the presence of chloroform as a contaminant. 2,2,2-Trichloroethanol gives a yellow colour. The LOD is 1 mg/L.

Furfuraldehyde (general reagent for carbamates)

Reagent
A 10% (v/v) solution of furfuraldehyde in ethanol.

Method
Dissolve the sample in ethanol, place a drop of the solution on a filter paper, add 1 drop of the reagent and expose the paper to hydrochloric acid fumes for 2–3 min.

Indications
A black spot indicates the presence of non-aromatic carbamates. *N*-Substituted carbamates do not react. The LOD is 1 μg.

Iodine test

Method
Mix the sample with an equal volume of manganese dioxide and heat the mixture carefully to dull redness over a small flame. Repeat the test by heating the sample alone.

Indications
The appearance of violet vapour indicates the presence of iodine in the molecule. Better results are sometimes obtained when the manganese dioxide is omitted (e.g. with amiodarone).

Iodoplatinate test (general test for alkaloids and nitrogenous heterocyclic compounds)

Reagent
Add 2 mL of a 5% (w/v) solution of platinic chloride and 5 g of potassium iodide to 98 mL of water and shake until dissolved. This reagent is often used as a locating agent in TLC.

Method
Dissolve the sample in 2 drops of 2 mol/L hydrochloric acid, add 2–3 mL of the reagent and dilute to 10 mL with water.

Indications
A violet, blue–violet, brown–violet or grey–violet precipitate suggests the presence of an alkaloidal base (precipitated as the alkaloid–iodoplatinate complex). The clearest colours are obtained with tertiary and quaternary amines; primary amines give indistinct colours and amines of small relative molecular mass generally do not react.

Koppanyi–Zwikker test

Reagent
A 1% (w/v) solution of cobalt nitrate in ethanol.

Method
Dissolve the sample in 1 mL of ethanol, add 1 drop of the reagent followed by 10 μL of pyrrolidine and agitate the mixture.

Indications
A violet colour is given by substances that contain the following structures:

- Imides, in which C=O and NH are adjacent in a ring (e.g. barbiturates, glutethimide, oxyphenisatine and saccharin).
- Sulfonamides and other compounds with free $–SO_2NH_2$ on a ring (e.g. clopamide, furosemide, sulfanilamide and thiazides), or with $–SO_2NH_2$ in a side-chain (e.g. chlorpropamide), or with $–SO_2NH_2$ that links a benzene ring with another ring other than a pyrazine, pyridazine, pyridine or pyrimidine ring (e.g. sulfafurazole and sulfamethoxazole). These latter structures give pink or red–violet colours (e.g. sulfadiazine and sulfadimethoxine).

No response is obtained with compounds with other substituents on the nitrogen atom. Anomalous responses are obtained with parametha-dione and theophylline (violet), and with cycloserine, idoxuridine, mephenytoin, niridazole and riboflavin (no response). Note that hydrochlorides give a blue colour before the addition of pyrrolidine.

Liebermann's reagent

Reagent
Add 1 g of sodium or potassium nitrite to 10 mL of concentrated sulfuric acid with cooling and swirling to absorb the brown fumes.

Method
Add 2 or 3 drops of the reagent to the sample on a white tile. Occasionally it is necessary to carry out the test in a tube and heat in a water-bath at 100°C. Many substances give colours with sulfuric acid alone and the test should be repeated using sulfuric acid instead of the reagent.

Indications
This test was originally developed to give intense colours with phenols:

- Orange colours are given by substances that contain a monosubstituted benzene ring not joined to C=O, N–C(=O)– or to a ring that contains a C=N–O– group.
- Orange or brown colours are given by some substances that contain two monosubstituted benzene rings (or some disubstituted compounds in which fluorine is the second substituent) that are joined either to one carbon atom or to adjacent carbon atoms.
- A wide range of colours is given by compounds that contain –OH, O–alkyl or –O–CH_2O– groups attached to a benzene ring or to a ring in a polycyclic structure that contains a benzene ring. The benzene ring must not bear –NO_2, or be halogenated, or contain an –O– substituent *ortho* to the oxy groups. Compounds that contain ring sulfur give a similar range of colours.

Colours are listed in Table 30.20. Note that a yellow colour is given by a variety of other compounds.

Mandelin's test (useful test for amfetamines and antidepressants)

Reagent
Dissolve 1.0 g of ammonium vanadate in 1.5 mL of water and dilute to 100 mL with concentrated sulfuric acid.

Method
Add a drop of the reagent to the sample on a white tile.

Indications
When interpreting the result of this test, account should be taken of the colour given by sulfuric acid and by Liebermann's test. Hydrochlorides give a red colour with this reagent. When the colours differ from those given with sulfuric acid or Liebermann's test, this indicates an aromatic ring together with a saturated 5-, 6- or 7-membered ring that contains only one nitrogen atom. The heterocyclic ring must not contain a second nitrogen atom or an oxygen atom. It must not be substituted or bound by –CONH– to the aromatic ring. The aromatic ring must not have –CF_3 as a substituent. Colours are also produced if sulfur is in a ring, provided that the ring does not contain more than one nitrogen atom (Table 30.21).

LOD values are: codeine sulfate 5.0 μg, amphetamine-HCl 10.0 μg, diamorphine-HCl 20 μg, metamfetamine 150 μg, morphine 5 μg and strychnine 0.05 μg.

Marquis test

The Marquis test is a useful broad-spectrum test used mostly for opium alkaloids and amfetamines.

Reagent
Carefully mix 100 mL of concentrated sulfuric acid with 1 mL of 40% (v/v) formaldehyde solution (stable for several weeks if protected from light).

Method
Add a drop of the reagent to the sample on a white tile.

Indications
Various colours that represent the whole of the visible spectrum are given by a large number of compounds. Structures that tend to maintain the response to the reagent at the violet end of the spectrum are, in decreasing order of efficacy: ring sulfur (with or without aromatic ring); ring oxygen (with aromatic ring); extra-ring oxygen or sulfur (with aromatic ring); aromatic compounds that consist entirely of C, H, N. Thus, there is a tendency for the response to the Marquis reagent to move gradually towards longer wavelength (i.e. through green to orange and red) as the ratio of C, H, N to the other groups in the molecule rises (Table 30.22).

The LOD values are: 1 μg for codeine sulfate, mescaline sulfate, methadone-HCl; 5 μg for lysergide tartrate, metamfetamine-HCl and morphine; 10 μg for amphetamine-HCl and diamorphine-HCl.

McNally's test

Reagents
1. A 0.5% solution of copper sulfate in 10% acetic acid.
2. A freshly prepared 2% (w/v) solution of sodium nitrite.

Method
Dissolve the sample (1 mg) in a few drops of acetone, and add 1–2 mL of water. Add 3 drops of solution 1 and an equal volume of solution 2. Shake and heat in a water-bath at 100°C for 3 min.

Indications
A red colour indicates the presence of free salicylic acid. Aminosalicylic acid gives a brown precipitate, and diflunisal gives a violet colour. Certain acids produced during the putrefaction of tissues also give red colours in this test: *p*-hydroxyphenylacetic acid, *p*-hydroxyphenylpropionic acid and *p*-hydroxyphenyl-lactic acid.

Mecke's reagent (useful test for opium alkaloids)

Reagent
Dissolve 1.0 g of selenious acid in 100 mL of concentrated sulfuric acid.

Method
Add a drop of the reagent to the sample on a white tile.

Indications
An immediate blue or green colour is indicative of opiates (see Table 30.23).

Melzer's reagent (general reagent for hallucinogenic mushrooms)

Reagent
Dissolve 1.5 g of iodine in 100 mL of an aqueous solution that contains 5 g of potassium iodide and 100 g of chloral hydrate.

Method
Place a few drops of the reagent on the mushroom spores or mushroom tissue to be tested.

Indications
A blue, bluish-grey or black–grey colour indicates amyloid mushrooms. A slight yellow or no change indicates that the mushrooms are non-amyloid. Psilocybes are always non-amyloid.

Mercurous nitrate (general reagent for barbiturate-like compounds)

Reagent
To a saturated solution of mercurous nitrate, add solid sodium bicarbonate until effervescence ceases and the precipitate formed becomes yellow. The precipitate then changes to a biscuit colour. This reagent should be freshly prepared and should be shaken immediately before use, and should not be kept for more than 1 h.

Method
Dissolve the sample in the minimum amount of ethanol, add 1 drop of the opaque reagent, shake and examine at intervals during 2 min. A blank solution that contains only ethanol and reagent should be treated similarly at the same time.

Table 30.20 Colours with Liebermann's reagent

Colour	*Compound*
Red	Acepromazine, ajmaline, alprenolol, aminacrine (100°C), antazoline, brucine, chlorprothixene, clopenthixol, flupentixol, mestranol, oxytetracycline, prajmalium, thiazinamium, tiotixene, tolmetin (100°C), trifluoperazine, xylazine
Violet-red	Indapamide
Brown-red	Methylchlorophenoxyacetic acid
Pink	Trichlorophenoxyacetic acid (→brown)
Brown-pink	Prazosin (100°C →red-orange)
Orange	Aletamine, alverine, ampicillin, atropine methobromide, atropine methonitrate, baclofen, benactyzine (→brown), bethanidine (→brown), broxyquinoline, butanilicaine, chloroquine (100°C), clidinium (→brown), cyclandelate, cyclizine, dazomet, decoquinate (slow), diethylthiambutene (100°C), dimefline, diuron, doxapram, dyclonine (100°C), fenclofenac (100°C, →brown), fenitrothion, fenpipramide, glibenclamide (100°C, 15 s), hyoscine butylbromide, hyoscine methonitrate, linuron, loxapine (50–60°C), metindizate (→brown), methylphenidate, metolazone (→green-brown), monolinuron, nomifensine, phenazone (100°C), phenelzine, propham, salinazid, sulfinpyrazone, tolazoline, trimetaphan, tripelennamine (→brown), triprolidine, xipamide, zomepirac (100°C)
Red-orange	Acetanilide, amfetamines, aniline, atropine, bamipine, beclamide, benethamine, caramiphen, carbetapentane, chlorcyclizine, cinchophen, cycrimine, diphenylpyraline, doxylamine, dropropizine, ephedrines, famprofazone, fencamfamin, glutethimide, hyoscine, hyoscyamine, isoaminile, isocarboxazid, levamisole, meclozine, mephentermine, methoin, methyl benzoquate, methylphenobarbital, metixene, metomidate, morazone, nialamide, pentapiperide, pethidine, phenacemide, phenbutrazate, phendimetrazine, phenglutarimide, pheniramine, phenmetrazine, phenobarbital, phensuximide, phenylmethylbarbituric acid, phenytoin, prolintane, tofenacin, tranylcypromine, triamterene, triphenyltetrazolium, warfarin
Brown-orange	Ambutonium, bumetanide, diphenhydramine, fenuron, feprazone (100°C, →brown), ibuprofen, labetalol, mepivacaine, methadone, nefopam (→brown), tetrahydrozoline
Yellow	Amicarbalide (100°C), clonidine (100°C, →orange), dequalinium (100°C, →orange), diethylpropion, diloxanide, ethoxzolamide, fenfluramine (100°C), flavoxate, gliclazide, metoclopramide, nifenazone (100°C), piroxicam, propachlor, tropicamide
Brown-yellow	Amiodarone
Green	Bialamicol, chlorotrianisene, colchicine, dextromoramide (100°C), diamthazole, hydrastine, mequitazine, naphthols, phenol, phenothiazine, thiocarlide
Blue-green	Hydrochlorothiazide, hydroflumethiazide, pindolol
Brown-green	Cyclopenthiazide
Grey-green	Azapropazone
Black-green	Naproxen
Blue	Amidopyrine (100°C), bendroflumethiazide, benzonatate (100°C), chromonar (100°C, 3 min), clomipramine, diphenylamine, dipyrone (100°C), imipramine, mefenamic acid, mefruside, oxypertine, padimate (100°C), procarbazine (100°C, 15 s), propyphenazone (100°C; red with water), tetracaine (100°C), yohimbine
Green-blue	Amiphenazole (100°C)
Violet	Methocarbamol, mianserin, paracetamol, penthienate methobromide, phenacetin, propiomazine, resorcinol, timolol (100°C), trazodone (100°C; transient)
Red-violet	Chloroxuron
Black-violet	Methoxychlor
Brown	Acepromazine, acetophenazine, adiphenine, azacyclonol, barban, benzilonium, benzyl nicotinate, biperiden, clemastine, clofenotane, clomifene, cyclothiazide, dextropropoxyphene, dichlorprop, diperodon, diphemanil, difenidol, emepronium, etenzamide, fenpiprane, flurbiprofen, haloperidol, mepenzolate, methylpiperidyl benzilate, mexiletine, nadolol, penfluridol, phenaglycodol, phenylbutazone (100°C), phosalone, pimozide, pipazethate (100°C, →red), pipoxolan, pyrrobutamine, rotenone, sotalol (100°C), sulindac, veratrine, zimeldine
Red-brown	Benzthiazide, bisacodyl, carfenazine, chlorpromazine, diclofenac, dosulepin, profenamine, etisazole, fenbufen, fenoprofen, methapyrilene, perphenazine, polythiazide
Pink-brown	Metoprolol
Orange-brown	Benazolin, diphenadione, maprotiline, methiocarb, piperidolate
Green-brown	Methdilazine, norbormide, promazine, thiopropazate
Violet-brown	Bamethan, clofibrate, dichlorophen
Black-brown	Mecoprop
Grey	Isopropamide
Black	Acetomenaphthone, aloin, aminophenols, amodiaquine, apomorphine, atenolol, benorilate, benzquinamide, buprenorphine, butorphanol, carbaryl (→green), carbidopa, cephaëline, chloroxylenol, chlorphenesin, clomocycline, clorgyline, codeine, cotarnine, cresol, cyclazocine, dextromethorphan, diamorphine, dibromopropamidine, diprenorphine, doxepin, emetine, ethamivan, ethinylestradiol, estradiol, estriol, estrone, etilefrine, furosemide, glycopyrronium, guaiphenesin, hexobendine, hydroxyephedrine, hydroxystilbamidine, ibogaine, indometacin, levallorphan, mebeverine, mescaline, methylchlorophenoxyacetic acid, methylenedioxyamfetamine, morantel, morphine, naloxone, 1-naphthylacetic acid, narceine, nicergoline, normetanephrine, noscapine, noxiptiline, octafonium, oxprenolol, oxyphenisatine, papaverine, pholcodine, pizotifen, practolol, profadol, propanidid, protokylol, pyrantel, rimiterol, ritodrine, rotenone, salbutamol, terbutaline, tetrabenazine, tetracycline, thymol, trimethobenzamide, trimetozine, tubocurarine, verapamil, viloxazine

Table 30.21 Colours with Mandelin's test

Colour	*Compound*
Red	Ajmaline, amfetamine, azacyclonol, chlorprothixene, diperodon (→green), dofamium (→brown), flupentixol, gelsemine (→green), indapamide, mequitazine, methotrexate, nialamide, pericyazine, prajmalium, prolintane, sodium cromoglicate, tiotixene, xylometazoline
Brown-red	Diacetylmorphine-HCl, doxepin-HCl, nadolol, propoxyphene-HCl
Orange	Brompheniramine, dropropizine (slow), ethylnoradrenaline, hydrastinine (→green), lachesine (→green), levamisole (→grey-green), methanthelinium, metixene, methyldopa, methyldopate, methylpiperidyl benzilate (→brown→green), noradrenaline, orphenadrine, pipenzolate (→green), poldine metilsulfate (→green→violet), procaine-HCl, propantheline, proquamezine (→violet), solanidine (→violet→blue), solanine (→violet→blue), strychnine (blue→purple→violet→red→red-orange), sulindac, thenalidine (→brown)
Red-orange	Cotarnine (→brown), doxepin
Green-orange	5-Methyltryptamine
Brown-orange	Mexiletine
Yellow	Azaperone, benzatropine, broxaldine, chelidonine (→green), conessine, deptropine, desipramine (→blue), dihydralazine, diphenhydramine, difenidol, diphenylpyraline, dropropizine (→orange), halquinol, homidium, lidoflazine, methacycline (→orange-violet), paraphenylenediamine, penicillamine, protokylol (→brown), tofenacin, tylosin (→yellow-brown), veratrine (→orange→violet-brown), viprynium
Orange-yellow	Cocaine-HCl, hexoprenaline, methaqualone, methylphenidate-HCl
Green-yellow	Methoxamine, oxycodone-HCl
Yellow-brown	Mescaline-HCl
Green	Acepromazine (→red), adiphenine (→blue), amfetamine, benorilate, bephenium hydroxynaphthoate, bibenzonium, buclosamide (blue rim), bunamidine, chlorpromazine (→violet), clefamide (→brown), codeine (green→blue), colchicine, cyclazocine, cyclomethycaine (→brown), debrisoquine, diaveridine, dibenzepin, diethazine (→violet with excess reagent), diethylthiambutene (→green-blue), dimethindene, dimethoxanate (→brown), dimoxyline, dipipanone (→blue), dosulepin, doxorubicin, doxycycline (→yellow), fenpiprane, guanoxan, harman, hydroxyephedrine, isoxsuprine, metanephrine, methadone (→blue), methdilazine (→violet), methocarbamol, methoxyamfetamine, methylenedioxyamfetamine (→blue), α-methyltryptamine (→orange), metopimazine, monocrotaline, niclosamide, nitroxoline, norharman (→yellow), normetanephrine, obidoxime (→blue), oleandomycin, oxymetazoline, paracetamol, pecazine (→violet), pentazocine, perazine (→violet), phenazone, phenazopyridine, phenformin, phenindamine, phenoxybenzamine (→violet), phenyltoloxamine, pindolol, piperacetazine (→red→violet), pipoxolan (→brown), prenylamine, profenamine (→violet), proflavine, promazine (→violet), promethazine (→violet), propranolol, reserpine, ritodrine, salicylic acid, thenium, thenyldiamine, thiocarlide (→yellow), tranylcypromine (→violet), trifluomeprazine (→red-violet), trihexyphenidyl
Yellow-green	Benzfetamine-HCl, metamfetamine-HCl, normethadone, opipramol
Blue-green	Amfetamine-HCl, benzoctamine, berberine (→brown), edrophonium, hydroxystilbamidine, ketobemidone, methoxyphenamine, phentolamine, profadol (→green), viloxazine
Brown-green	Benzydamine, chlorphenesin
Grey-green	Alverine, azapropazone, diamphenethide, diethyltryptamine (→yellow), dihydrocodeine, guaifenesin, hordenine, levomethadyl acetate, normorphine, oxyphencyclimine, papaverine, terbutaline, trihexyphenidyl
Blue	Bamethan (→green), clomipramine, deserpidine (→green), desferrioxamine (→violet), doxapram, droperidol (→green), harmine (→green), imipramine (add water), maprotiline, mebhydrolin, metaraminol, phenaglycodol, phenyramidol, pyridoxine (→grey-green), salbutamol (blue rim→brown rim), strychnine (blue→purple→magenta→red→red-orange→orange), thioridazine (→violet), trimipramine (add water), triphenyltetrazolium (slow), xipamide, xylazine, yohimbine (→green)
Green-blue	Chlophedianol, labetalol
Violet	Alimemazine, amidefrine, benperidol, bezitramide (→orange), bisacodyl, captodiame, cefaloridine, chloropyrilene (→orange), clomifene (→orange-brown), clomocycline (→brown), denatonium, dipyridamole, guanoclor (→orange→brown-yellow), guanoxan, hexobendine, hydromorphone (→orange), mepacrine (→yellow), mepyramine, metisazone (→yellow), mianserin, morantel, naloxone (→brown), oxyclozanide (→orange), oxyphenisatine, oxytetracycline (→red→orange), penthienate, perphenazine, phenylbutazone, pizotifen (→green), prilocaine, primaquine (→orange), propiomazine, pyrantel, pyrrobutamine, rolitetracycline (→red→orange), tetracycline (→red→orange), thiethylperazine, thiopropazate, triacetyloleandomycin (slow), tridihexethyl, trimetazidine
Red-violet	Antazoline, carfenazine, dimethothiazine, histapyrrodine, thonzylamine
Blue-violet	Alcuronium, hexocyclium, levomepromazine
Brown-violet	Alprenolol, bitoscanate, butaperazine, naphazoline
Grey-violet	Methadone-HCl, methoserpidine, oxprenolol, tricyclamol
Black-violet	Methylenedioxyamfetamine-HCl, methapyrilene
Brown	Amitriptyline (→green), azapetine, bamipine, carbetapentane (slow), clidinium (→green), cyclopentolate, diphemanil, dipyrone, doxepin, embutramide, fluanisone, fluphenazine, isoetarine, isometheptene, isoprenaline, metindizate, methyl benzoquate, methysergide, metoclopramide, norpipanone (→blue), nortriptyline (→green), opium, phenelzine, phenylephrine, pimozide, piperidolate, prochlorperazine (→violet), propoxycaine, rescinnamine, salinazid, stanozolol, tetrabenazine, thioproperazine (→green→violet), tolnaftate, tolpropamine, tramazoline, tubocurarine
Red-brown	Benzthiazide, clioxanide, cycrimine, decoquinate, diclofenac, ethomoxane, hydrastine (→red), trifluoperazine, triflupromazine
Pink-brown	Metoprolol
Orange-brown	Rifampicin, spiramycin, thebaine
Yellow-brown	Clemastine, clofazimine, physostigmine, rifamycin SV, trimethoprim, tripelennamine
Green-brown	Etenzamide, harmaline, lysergic acid, mesoridazine, narceine, pentazocine, phenyltoloxamine, syrosingopine
Violet-brown	Chlortetracycline (→yellow), cyproheptadine, demeclocycline, dihydroergotamine, ergotamine, lymecycline (→yellow), methylergometrine, nicergoline (→brown), octaphonium, oxethazaine, protriptyline, trimethobenzamide
Grey-brown	Dextropropoxyphene, mephenesin carbamate

Table 30.21 continued

Colour	*Compound*
Grey	Dihydromorphine, diprenorphine, etilefrine (→green→brown), ibogaine (→violet), indometacin, lobeline, lysergide, morphine, oxypertine, propranolol, trazodone (→violet)
Blue-grey	Alphaprodine, diamorphine, morphine
Black	Procyclidine
Grey-black	Flurazepam

Table 30.22 Colours with the Marquis test

Colour	*Compound*
Red	Alprenolol, benzylmorphine (→violet), buphenine, dimethothiazine, etenzamide, etilefrine, fenclofenac (slow), fenpiprane, fluphenazine, flurbiprofen, hexoprenaline, labetalol (→brown-red), maprotiline, mephenesin carbamate, mequitazine (slow), mesoridazine (→violet), methoxyphenamine, metopimazine, mexiletine, nadolol, pentazocine (→green), pericyazine, phenazopyridine, phenoperidine, phenylephrine, piperacetazine, prenylamine, thebaine (→orange), thiethylperazine (→green), thioproperazine, tiotixene, tolpropamine, tranylcypromine (→brown), vinblastine
Orange-red	Alverine, amfetamine-HCl, bethanidine, diphemanil, flupentixol, metamfetamine-HCl
Violet-red	Thioridazine (→blue-green)
Black-red	Doxepin-HCl
Brown-red	Alphaprodine, doxepin, trihexyphenidyl
Pink	Alimemazine, fenoprofen, fluopromazine, metoprolol, promazine, promethazine, trifluoperazine
Orange	Adrenaline (→violet), aletamine, amfetamine (→red→brown), anileridine (slow), benactyzine (→green→blue), benzethonium, benzilonium (→green→blue), benzfetamine, bunamidine (→red), carbetapentane (slow), carfenazine (→red-violet), chlorphentermine, clidinium (→blue), cyclandelate (slow), cycrimine (→red), dehydroemetine, dimethyltryptamine, dipyridamole, ethacridine (→red), ethoheptazine, ethylnoradrenaline (→brown), famprofazone, fenbufen (→brown), fencamfamin, fenethylline, fentanyl, harmine, indapamide (→violet), indometacin, isothipendyl, ketobemidone, lachesine (→green→blue), lymecycline, mepenzolate (transient), mephentermine (→brown), mescaline, metamfetamine, metanephrine (→violet-brown), methacycline, methanthelinium, methindizate (→green), methylphenidate, methylpiperidyl benzilate (→green→blue), 5-methyltryptamine (→brown), α-methyltryptamine (→brown), *N*-methyltryptamine, nefopam (→brown), nomifensine (slow), normetanephrine (→violet-brown), oxeladin, oxytetracycline, pentapiperide, pethidine, phenethylamine, phenformin (→brown), phentermine, piminodine, pipenzolate (→green→blue), piperidolate, pizotifen (→red), poldine methylsulfate (→green→blue), primaquine, profadol (→red-brown), prolintane (→brown), propantheline, prothipendyl, psilocybine, rolitetracycline, spiramycin, tetracycline, trimethoprim, trimethoxyamfetamine, tryptamine, veratrine, xylometazoline
Red-orange	Chlorprothixene
Pink-orange	Diuron
Yellow-orange	Orphenadrine, pipradrol
Brown-orange	Amitriptyline
Yellow	Acriflavine (→red), amiloride, azacyclonol, benzquinamide, benzatropine, bromazine, broxaldine, broxyquinoline, caramiphen, chlordiazepoxide, chlorphenoxamine (→green), chlortetracycline (→green), chlortalidone, cinchophen, clefamide, clemastine (green rim), colchicine, conessine (→orange), cyclizine, demeclocycline (→green), deptropine, diethyltryptamine (→brown), 2,5-dimethoxy-4-methylamfetamine, diphenhydramine, diphenidol, diphenylpyraline, doxycycline, ethoxzolamide, ethylmorphine (→violet→black), furaltadone, halquinol, hydrocodone (→brown→violet), hydromorphone (→red→violet), hydroxyephedrine, isoetarine (→orange), lidoflazine, lorazepam, mepacrine, methyldopa (→violet), methyldopate (→violet), norcodeine (→violet), orciprenaline, oxycodone (→brown→violet), oxyphenbutazone, phanquone, phenbutrazate (slow), phentolamine, phenyramidol, pindolol (→brown), pramoxine (→green), proflavine (→orange), salbutamol, salinazid, sodium cromoglicate, solanine (→violet), terbutaline, tetrabenazine, thebacon (→violet), tofenacin, triamterene, trimetazidine (fades), vancomycin, viprynium embonate, zomepirac (100°C, →orange)
Orange-yellow	Methylphenidate-HCl, stanozolol
Pink-yellow	Methadone-HCl
Green	Berberine, carbaryl, chelidonine, harman, norharman, oleandomycin, propranolol, protriptyline, pseudomorphine, sulindac (slow)
Yellow-green	Acepromazine (→red), verapamil (→grey)
Blue-green	Tolnaftate
Brown-green	Harmaline
Grey-green	Cyproheptadine, deserpidine, naphazoline, oxypertine, phenindamine, protokylol, rescinnamine, reserpine (→brown)
Blue	Clofibrate, embutramide, nicergoline (→grey)
Grey-blue	Mebhydrolin, 1-naphthylacetic acid
Violet	Alimemazine, apomorphine (→black), azatadine, benorilate, bisacodyl, buprenorphine, butriptyline, captodiame, chloropyrilene, chlorpromazine, clofazimine, codeine, diamorphine, diethylthiambutene, dihydrocodeine, dimethindene (→blue), dimethoxanate, doxorubicin, doxylamine, etoxazene, guaifenesin, guanoxan, hexocyclium metilsulfate, mepyramine, 6-monoacetylmorphine, morphine, nalorphine, normorphine, oxprenolol, oxycodone-HCl, oxyphenisatine, pecazine, penthienate, pentazocine, perazine, perphenazine, phenoxybenzamine, phenyltoloxamine, pholcodine, pimozide, pipoxolan (→grey), prochlorperazine, procyclidine, profenamine, promazine, promethazine, proquamezine, solanidine, thenium, thiopropazate, tricyclamol, viloxazine
Red-violet	Acetophenazine, benzoctamine, bephenium hydroxynaphthoate, cefaloridine, chlophedianol (→brown), dihydromorphine, ethomoxane, isoxsuprine, lobeline, methdilazine, propiomazine, tramazoline, trifluomeprazine, trifluoperazine, triflupromazine, trimeperidine
Blue-violet	Methocarbamol, levomepromazine, morantel, neopine, noscapine (fades), pyrantel

table continued

Table 30.22 continued

Colour	*Compound*
Brown–violet	Butaperazine, dopamine, methylenedioxyamfetamine, tridihexethyl
Grey–violet	Diprenorphine, oxymorphone, pyrrobutamine, thenalidine, trihexyphenidyl
Black–violet	Dextropropoxyphene (→green), methapyrilene, thenyldiamine
Brown	Bibenzonium, carbidopa, cyclazocine (→green), diclofenac (slow), dimoxyline, dosulepin, doxepin, ergometrine, ergotamine, erythromycin, hordenine (→green), ibuprofen (100°C, →orange), isoprenaline (→violet), lysergamide, methadone, naloxone (→violet), naproxen, narceine (→green), noradrenaline, pethidine-HCl, phentermine, phenazocine, rimiterol (→black), serotonin (slow), syrosingopine, tyramine (→green)
Red–brown	Biperiden, debrisoquine, methyl benzoquate, oxetacaine, phenprobamate, trimetozine, tripelennamine
Orange–brown	Benethamine (→brown), clomocycline, nortriptyline
Yellow–brown	Moxisylyte, ritodrine, triacetyloleandomycin, tylosin
Green–brown	Alcuronium, bufotenine, psilocin
Violet–brown	Clomifene, diethazine, levomethadyl acetate (→grey–green), methoxamine (→green)
Grey–brown	Dihydroergotamine, methylergometrine, octafonium
Grey	Butorphanol, diaveridine (→violet–brown), ibogaine (→orange), lysergide, methoserpidine, methysergide, pholedrine (→green)
Blue–grey	Acetorphine (→yellow–brown), etorphine (→yellow–brown)
Black	Methylenedioxyamfetamine
Blue–black	Methylenedioxyamfetamine
Green–black	Lysergide

Table 30.23 Colours with Mecke's reagent

Colour	*Compound*
Green	Diacetylmorphine, mescaline-HCl, morphine, oxycodone-HCl
Blue–green	Codeine, diacetylmorphine-HCl, hydrocodone tartrate, methylenedioxyamfetamine-HCl
Brown–green	Methadone
Orange	Alimemazine, diphenhydramine, fluopromazine, pethidine, phenyltoloxamine, promazine, promethazine, propoxyphene, trifluoperazine, triflupromazine
Yellow	Amfetamine, procaine
Red	Doxepin-HCl
Black–red	Chlorpromazine-HCl
Purple–red	Tetracycline
Violet (dark blue)	Methylenedioxyamfetamine, thioridazine
Brown	Ephedrine
Red–brown	Propoxyphene-HCl
Black	
Green–black	Lysergide, opium

Indications

A dark grey or black colour indicates a ring imide group or sulfonamides with an additional ring. The speed and intensity of the reaction varies between different compounds. The following ring imides react in decreasing order of intensity: barbiturates, bemegride, phenytoin > benperidol, cycloserine, pimozide > glutethimide, oxyphenisatine > saccharin, sulfinpyrazone. In the case of sulfonamides, succinylsulfathiazole, sulfamoxole, sulfanilamide, sulfasomidine and sulfathiazole react with greater intensity than all others. Chlorpropamide and tolbutamide give a moderate response. If used as a spray, the LOD is 1–5 µg for barbiturates.

Methanolic potassium hydroxide

Reagent

A 20% (w/v) solution of potassium hydroxide in methanol.

Method

Add a few drops of the reagent to a solution of the sample in methanol and heat if necessary to boiling point to develop the colour.

Indications

A change from colourless or from a pale colour to red, orange, yellow, green or blue is given by quinones, diones that possess an aromatic ring, phenols with adjacent hydroxy groups and compounds that contain nitro groups on a ring (Table 30.24). Many of these compounds are coloured already and give pale or colourless solutions in methanol.

Millon's reagent (general reagent for phenols)

Reagent

Dissolve 3 mL of mercury in 27 mL of fuming nitric acid and add an equal volume of water with stirring.

Method

Add 0.5 mL of reagent to the sample and warm the mixture.

Table 30.24 Colours with methanolic potassium hydroxide

Colour	*Compound*
Red	Benserazide, isoetarine (→orange→yellow), metronidazole, nitrofurazone, phenindione
Orange–red	Fenitrothion, quintozene, tecnazene, trifluralin
Pink	Levodopa (→red–brown)
Orange–pink	Rimiterol
Brown–pink	Dobutamine
Orange	Acinitrazole, barban, carbidopa, dinitro-orthocresol (100°C), dinobuton, dinoseb, dodecyl gallate, hexoprenaline (→brown), isoprenaline (→yellow), nifedipine, nifuratel, nifursol, obidoxime, protokylol (→yellow)
Pink–orange	Adrenaline(→brown)
Yellow–orange	Nitrofurantoin
Yellow	Acebutolol, diphenadione, methyldopa (→orange), metolazone, niclosamide, niridazole, nitroxoline, nitroxynil, phanquone (→brown–violet), sodium cromoglicate
Green–yellow	Phytomenadione (→violet→brown)
Green	Apomorphine (→red), dinitolmide
Blue	Dopamine (→orange→brown), methyldopate (→orange), noradrenaline (→orange)
Violet	Dimetridazole (when boiled)

Indications
A red or orange–red colour indicates the presence of a phenolic substance. Primary aryl amines also react. Some basic compounds that contain a phenolic group do not react to this test; a combination of this test with the Folin–Ciocaltaeu reagent is therefore advised for phenolic compounds. Phenols that contain more than one hydroxyl group do not give the typical red colour. This reagent does not react with phenols substituted with Cl, Br or I.

Naphthol-sulfuric acid

This test should be carried out in conjunction with the sulfuric acid test.

Reagent
Mix 1 g of naphth-2-ol with 40 mL of concentrated sulfuric acid and heat in a water-bath at 100°C, with occasional stirring, until the naphth-2-ol is dissolved.

Method
Mix the sample with 1 mL of the reagent, heat in a water-bath at 100°C for 2 min and note any colour produced. Cool, add 1 mL of water and note the colour again.

Indications
A range of colours is obtained with steroidal structures (Table 30.25). A positive response to this test combined with a positive response to the sulfuric acid test is indicative of the presence of a steroid.

Compounds other than steroids that give colours with this test include chloral hydrate and chloramphenicol (brown–yellow), starch and tartaric acid (green).

Nessler's reagent

Reagent
1. Dissolve 50 g of mercuric chloride and 35 g of potassium iodide in 200 mL of water and cool.
2. Dissolve 50 g of sodium hydroxide in 250 mL of water and cool.

Add the cold solution 2 to the cold solution 1 and make up to 500 mL. Allow the mixture to stand and decant the clear supernatant (stable for many months) for use. Store in dark brown bottles away from the light.

Method
Add the reagent (3 drops) to the sample (3 drops), agitate and heat the mixture to 100°C in a water-bath, examining it every minute for 10 min. A blank solution should be treated similarly at the same time.

Indications
A brown–orange colour is produced quickly by aliphatic amides and thioamides. The presence of an aromatic ring slows the reaction. The nearer the amide group is to the ring, the more the reaction is inhibited. Substituents in the ring may cause a weak reaction. An immediate black colour is produced by substances that contain *ortho*- or *para*-hydroxy groups and by substances that contain an –NH–NH– or –NH–NH_2 group in an aliphatic side-chain. Some compounds must be heated to 100°C to produce blackening. Colours are given in Table 30.26.

Ninhydrin

Reagent
Dissolve 0.5 g of ninhydrin in 40 mL of acetone.

Method
Dissolve the sample in methanol, place a drop of the solution on a filter paper, add 1 drop of the reagent and dry in a current of hot air.

Indications
A violet colour that appears rapidly indicates the presence of an aliphatic primary amine or an amino acid group. The presence of an aromatic ring inhibits the response, and the inhibition increases the nearer the amino group is to the ring, as for amfetamine (pink–orange), procainamide and proxymetacaine (both yellow). If the amino group is associated with a saturated ring, a positive but weak pink–violet colour is obtained (amantadine, rimantadine). Gentamicin gives a violet colour after heating for 4 min.

Table 30.25 Colours of steroids with naphthol-sulfuric acid

Colour with hot reagent	*Steroid*	*Colour after dilution*
Red	Mestranol	Red
Orange-red	Desoxycortone	Blue-black
	Dydrogesterone	–
	Hydroxyprogesterone	Blue, violet (dichroic)
	Noretynodrel	Brown-red
Brown-red	Ethinylestradiol	Pink
Orange	Norethisterone	Orange-brown
Orange, green (dichroic)	Norethandrolone	Red-orange
Yellow	Diethylstilbestrol	Orange
	Testosterone	Green, brown (dichroic)
Green-yellow	Fluoxymesterone	Yellow
Green	Beclometasone	Brown-yellow
	Fluocinolone	Yellow
Yellow-green	Dexamethasone	Yellow
Green, yellow (dichroic)	Estriol	Orange
	Estrone	Orange
	Triamcinolone	Yellow
Green, brown (dichroic)	Fludroxycortide	Yellow
Blue-green, yellow (dichroic)	Estradiol	Orange
Violet	Fludrocortisone	Brown
Brown	Oxymetholone	Pink-orange
	Prednisolone	Brown
	Prednisone	Orange
	Progesterone	Yellow
Red-brown	Dimethisterone	Brown-green
	Enoxolone	Orange
	Fluocortolone	Red-brown
Orange-brown	Alfadolone	Orange
	Androsterone	Orange
	Cortisone	Orange
	Dienestrol	Yellow
Yellow-brown	Carbenoxolone	Orange
	Cholesterol	Violet
	Hydrocortisone	Yellow-brown
Green-brown	Betamethasone	Orange-brown

Nitric acid, fuming

Method
Mix the sample with 3 drops of fuming nitric acid, heat at 50°C for 30 s and observe any colour produced. Cool the mixture, add 2 drops of it to 2 mL of concentrated sulfuric acid and observe the colour. To the remainder of the cooled mixture, add 2 mL of water followed by 2 mol/L sodium hydroxide, dropwise, until pH 8 is reached (use an indicator paper).

Indications
Chlorinated phenols give a series of colours in the three parts of this test (Table 30.27).

Nitric acid-sulfuric acid (Erdmann's reagent)

Mix 1 mL of concentrated nitric acid with 30 mL of concentrated sulfuric acid.

Table 30.26 Colours with Nessler's reagent

Colour	*Compound*
Orange	Acebutolol (slow), carbidopa (→black), methotrexate
Brown–orange	Acetylcarbromal (slow), bromvaletone, carbromal, chloramphenicol, dinitolmide, etenzamide (weak), ethionamide, fluoroacetamide, nicotinamide, phenacemide (slow), pheneturide (slow), protionamide, pyrazinamide, salicylamide (weak), urea
Yellow	Dihydrostreptomycin (→brown), penicillamine
Brown	Demeclocycline, mebutamate (slow), nadolol, paracetamol (slow)
Yellow–brown	Atenolol (slow)
Black (immediate)	Adrenaline, apomorphine, ascorbic acid, benserazide, dihydralazine, dobutamine, dodecyl gallate, dopamine, ethylnoradrenaline, hexoprenaline, hydralazine, iproniazid, isocarboxazid, isoetarine, isoniazid, isoprenaline, levodopa, mebanazine, methyldopa, methyldopate, nialamide, noradrenaline, phenelzine, procarbazine, protokylol, rimiterol
Black (at 100°C)	Cimetidine, gentamicin, labetalol, meprobamate (grey–black), methallibure, salinazid, thiacetazone

Table 30.27 Colours with fuming nitric acid

Colour			
Part 1	*Part 2*	*Part 3*	*Compound*
Orange–red	Orange	Orange–brown	Hexachlorophene
Red	Red	Brown–violet	Pentachlorophenol

Method

Dissolve the sample in 1 mL of ethanol, add a pellet of potassium hydroxide and evaporate to dryness (100°C in a water-bath). To the residue add 0.5 mL of water and 1 mL of carbon tetrachloride, shake and allow to separate. Decant the lower carbon tetrachloride layer and shake it with 1 mL of the reagent.

Indications

A red colour in the acid layer suggests the presence of clofenotane or its metabolite, dichlorodiphenyldichloroethylene (DDE). The red colour changes to orange and then to green. Weak pink colours are given by aldrin, dieldrin and endrin. A red colour is also given by dichlorodiphenyldichloroethane (DDD, mitotane), but the colour does not change.

Note that the substance should be tested to ensure that it does not give a colour with sulfuric acid alone.

Nitrous acid

Method

Dissolve the sample in a minimum volume of water, and add an amount of solid sodium nitrite equal in volume to the sample followed by a few drops of 2 mol/L hydrochloric acid.

Indications

Orange or yellow colours are given by certain sulfonamides, and green, blue or violet colours by certain phenylpyrazolines (Table 30.28).

No response is obtained with succinylsulfathiazole, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfaquinoxaline, sulthiame or propyphenazone.

Palladium chloride

Reagent

Dissolve, with the aid of heat, 0.1 g of palladium chloride in 5 mL of 2 mol/L hydrochloric acid and dilute the solution to 100 mL with water. Mix together equal volumes of this solution and 2 mol/L sodium hydroxide. The mixed reagent is stable for several weeks.

Table 30.28 Colours with nitrous acid

Colour	*Compound*
Orange	Sulfafurazole
Yellow	Sulfadoxine, sulfachlorpyridazine, sulfadimidine, sulfaethidole, sulfamethizole, sulfamethoxazole, sulfamethoxydiazine, sulfamethoxypyridazine, sulfametopyrazine, sulfamoxole, sulfaphenazole, sulfapyridine, sulfasomidine, sulfathiazole, sulfinpyrazone
Green	Phenazone
Blue	Dipyrone (transient)
Violet	Amidopyrine (transient)

Method

Mix the sample with 1 mL of the reagent and heat at 100°C in a water-bath for 2 min. A blank solution should be treated similarly at the same time.

Indications

Red, orange, yellow, brown or black colours are given by aliphatic compounds that have a sulfur atom in the chain, and by aromatic compounds that have a sulfur atom in the side-chain. However, no colour is given when an *S*-alkyl chain is present, unless the chain is terminated by an halogenated group.

No response is obtained if the sulfur is in a group that links two rings. Reducing agents such as ascorbic acid, chloral hydrate, chloroform and glucose, and compounds that contain a chain with a hydrazine link (–NH–NH–, –NH–NH_2), give a translucent dark-grey or black colour, but do not give the gradual yellow to orange to brown colour seen with sulfur-containing compounds. Compounds that contain adjacent hydroxyl groups on an aromatic ring give orange colours that turn brown (Table 30.29).

Phosphorus test

Method

To the sample add 0.5 mL of concentrated nitric acid and 0.2 mL of concentrated sulfuric acid and heat at 100°C in a water-bath for 30 min. Cool, add 1 mL of a 10% (w/v) solution of ammonium molybdate and replace in the water-bath at 100°C for 5 min. A blank solution should be

Table 30.29 Colours with palladium chloride

Colour	*Compound*
Red	Gloxazone
Orange	Adrenaline (→brown), benserazide (→brown), bitoscanate, captopril, carbidopa (→brown), carbimazole, disulfiram, dobutamine (→brown), ecothiopate, isoetarine (→brown), levodopa (→brown), methallibure, thiamazole, polythiazide, rimiterol, thiacetazone, thiopental
Brown–orange	Demeton-S
Yellow	Clindamycin, dazomet, dimercaprol, dimethoate, methisazone (→orange→brown), penicillamine
Orange–yellow	Thialbarbital
Brown	Ambazone, azinphos-methyl, dihydrostreptomycin (slow), ethionamide, malathion, noxythiolin, parathion, phosalone, protionamide, spironolactone, thiram
Orange–brown	Chlorthiamid, diazinon, disulfoton, fenitrothion, formothion, phorate, vamidothion
Black–brown	Di-allate, dichlofluanid, tri-allate
Grey	Chlorfenvinphos
Black	Ascorbic acid, captan, chloroform, cloral hydrate, mebanazine, nifuratel, phenelzine, procarbazine, sulfasalazine, sulfaurea, trichlorfon

Table 30.30 Colours with potassium dichromate

Colour	*Compound*
Red	Carbidopa
Yellow (→brown)	Phenol (2 min)
Green (→brown)	Adrenaline, dopamine, hexoprenaline, isoetharine, isoprenaline, levodopa, methyldopa, methyldopate, noradrenaline, rimiterol
Blue–green	Aniline (2 min)
Brown	Benserazide, *o*-cresol (30 s), *m*-cresol (2 min), orciprenaline (slow), protokylol (→red–brown on warming), terbutaline (slow)
Green–brown	Dobutamine

treated at the same time. For some compounds, the reaction may occur after shorter heating times than those stated above.

Indications
A bright yellow solution or precipitate indicates the presence of phosphorus and suggests an organophosphorus pesticide, especially if the sample is a water-immiscible liquid. Cyclophosphamide and triclofos also react.

Potassium dichromate

Method 1
Dissolve the sample by shaking in 0.5 mL of 2 mol/L hydrochloric acid and add a few crystals of potassium dichromate.

Indications
An immediate brown colour, or a green colour that changes to brown, indicates the presence of an aminophenol or of a phenol that has two or more hydroxyl groups in adjacent positions on the ring (Table 30.30). Monophenols, halogenated phenols and phenols with hydroxyl groups *meta* to each other react more slowly or not at all.

Method 2
If the sample is a liquid, add 1–2 drops to 1 mL of water followed by 1 mL of a saturated solution of potassium dichromate in 50% v/v sulfuric acid.

Indications
A green colour is given by acetaldehyde, ethanol, methanol, propan-1-ol and propan-2-ol.

Schiff's reagent

Reagent
Dissolve 0.2 g of basic magenta (fuchsin, CI 42510) in 120 mL of hot water, cool, add 20 mL of a 10% (w/v) solution of sodium hydrogen-sulfite and 2 mL of concentrated hydrochloric acid, and dilute to 200 mL. Store at 4°C and protect from light.

Method
Add the sample to 1 mL of the reagent.

Indications
A violet colour indicates the presence of an aliphatic aldehyde. The longer the carbon chain, especially if it is branched, the weaker the response to the test.

Scott's test (see also Cobalt thiocyanate)

Reagent
1. Cobalt thiocyanate dissolved in water (2% w/v) and then diluted 1 : 1 with glycerine
2. Concentrated hydrochloric acid
3. Chloroform.

Method
Add a small amount of the sample to be tested to a test-tube, add 5 drops of solution 1 and shake. If cocaine is present a blue colour develops at once. Add 1 drop of solution 2 and shake (the blue colour disappears and a clear pink solution develops). Add several drops of solution 3.

Indications
The chloroform layer develops an intense blue colour if cocaine is present. Methadone also reacts.

The LOD is 60 µg cocaine-HCl and 15 µg methadone-HCl.

Simon's test (modified sodium nitroprusside test)

Reagent
1. Dissolve 1 g of sodium nitroprusside in 100 mL of water and add 2 mL of acetaldehyde to the solution with thorough mixing.
2. Freshly prepared 2% sodium carbonate in distilled water.

Method
Add 1 drop of solution 1 to the sample, followed by 2 drops of solution 2.

Indications
A dark-blue colour indicates a secondary amine (e.g. metamfetamine, ephedrine, 3,4-methylenedioxymetamfetamine (MDMA)) or an unsubstituted heterocyclic amine as its free base. A deep blue colour indicates the presence of metamfetamine. Primary amines (e.g. amfetamine, methylenedioxyamfetamine (MDA)) yield a slow pink to cherry-red colour.

Sodium dithionite

Reagent
A 5% (w/v) solution of sodium dithionite in a 10% (w/v) solution of sodium hydroxide.

Method
Apply the reagent to the sample, either on a white tile or as a solution in a test-tube. A blank solution should be treated similarly at the same time.

Indications
Colours are produced by bis(pyridyl) compounds (Table 30.31). Dark colours are likely to be given by certain metallic solutions because of reduction.

Sodium hypobromite test (for carbamazepine)

Reagent
Dissolve 0.5 mL of bromine in 5 mL of a 40% w/v solution of sodium hydroxide with shaking and cooling. (this should be freshly prepared).

Method
Add 1 mL of 2 mol/L hydrochloric acid to 5 mL of sample and 5 mL of chloroform. Vortex mix for 1 min and centrifuge for 5 min. Remove the upper layer, add 1 mL of the chloroform extract to 0.2 mL of sodium hypobromite reagent and mix for 30 s.

Indications
Carbamazepine forms a blue to violet colour in the chloroform layer. The test has a sensitivity of 250 mg/L.

Sodium nitroprusside

Reagent
A 1% (w/v) solution of sodium nitroprusside.

Method 1
Add the sample to 2 mL of the reagent followed by a drop of 2 mol/L sodium hydroxide.

Indications
Orange colours are given by ketones and red colours by acetaldehyde.

Table 30.31 Colours with sodium dithionate

Colour	*Compound*
Green	Diquat
Blue	Paraquat

Method 2
Mix the sample with a minimum volume of 2 mol/L sodium hydroxide, evaporate to dryness, dissolve the residue in 2 drops of water and add 0.5 mL of the reagent.

Indications
A violet colour is given by substances that contain labile sulfur in the molecule and by unsubstituted dithiocarbamates.

Method 3
Carry out Method 2 above, but after evaporation to dryness heat the residue until it is yellow or orange in colour before proceeding.

Indications
A violet colour is given by certain substances that contain labile sulfur and do not react to method 2 (e.g. clomethiazole, lincomycin and monosulfiram).

Sodium nitroprusside–acetone

Reagents
1. Dissolve 2 g of sodium nitroprusside in 5 mL of water and add 45 mL of methanol
2. 2% (w/v) sodium carbonate
3. Acetone
4. 10% acetaldehyde.

Method 1
Add a drop of solution 1 followed by a drop of solution 2 to 3–4 mg of sample dissolved in solution 3 on a spot plate. A purple colour is indicative of amfetamine. The LOD is 30 μg.

Method 2
Add a drop of solution 1 followed by a drop of solution 4 to 1–2 mg of sample dissolved in solution 3. An immediate blue colour is indicative of metamfetamine. The LOD is 5 μg.

Sodium picrate (Steyn test)

Reagent
Prepare a solution of 5 g sodium bicarbonate and 0.5 g picric acid in 100 mL of water.

Method
Mix the sample with a few drops of chloroform and concentrated sulfuric acid to hasten the reaction while holding a piece of filter paper, impregnated with the reagent, in the vapours that issue from the tube, and heating the contents to 30°C.

Indications
The yellow colour of the filter paper changes from orange to brown–orange and then to orange–red or red in the presence of cyanide. Positive results are given by compounds that contain cyanide groups (e.g. cimetidine, diphenoxylate and isoaminile).

Sulfuric acid

Method
Apply concentrated sulfuric acid directly to the sample on a white tile or in a test-tube.

Indications
A range of colours is obtained with compounds of various types. Steroids give orange or yellow colours, many of which fluoresce under UV light ($\lambda = 350$ nm) either immediately or after dilution (Table 30.32). Thioxanthenes give red or orange colours that fluoresce under UV light ($\lambda = 350$ nm) (Table 30.33).

Sulfuric acid–fuming sulfuric acid

Reagent
Mix together 7 mL of concentrated sulfuric acid and 3 mL of fuming sulfuric acid.

Method
Dissolve the sample in a minimum volume of toluene and add 1 or 2 drops of the reagent.

Indications
A red colour that appears in the lower acid layer indicates the presence of dieldrin (colour develops quickly) or aldrin (colour develops slowly). A pink–orange colour is obtained with endrin.

Tetrabromophenolphthalein ethyl ester

Reagent
Dissolve 50 mg tetrabromophenolphthalein ethyl ester (TBPE) in 100 mL chloroform, shake the solution for 2 min with 1 mL of 10% (v/v) hydrochloric acid and discard the aqueous phase. Dry the organic layer with anhydrous sodium sulfate. Separate the drying agent by filtration. Store the reagent in an amber bottle at 4°C.

Method
Place 0.5 mL of sample to be tested in a conical test tube, add 100 μL phosphate buffer (10 mmol/L, pH 8.0) and vortex mix. Add 50 μL of the TBPE reagent and vortex mix. After 2–3 min note the colour of the chloroform layer. If the sample to be tested is a solid, dissolve 1–2 mg of the material in 0.5 mL of buffer and proceed.

Indications
A deep blue colour indicates quaternary ammonium compounds. An orange, brown, red or purple colour indicates the presence of basic drugs. This test is most sensitive to tertiary amines (e.g. tricyclics, propoxyphene, phenothiazines, diphenhydramine, phencyclidine, methadone, pethidine). Its LOD is 1 mg/L.

Thalleioquin test

Method
Dissolve the sample in a minimum volume of 2 mol/L hydrochloric acid, add 2 drops of bromine solution, place 1 drop of the mixture on a piece of filter paper and expose the paper to ammonia fumes.

Indications
A green colour indicates the presence of a quinine-type structure (e.g. hydroquinidine, hydroquinine, quinidine, quinine). Cinchonidine and cinchonine do not respond.

Trinder's reagent (see Ferric chloride)

Reagent
The solution is prepared as follows: 40 g of mercuric chloride and 40 g of ferric nitrate are dissolved in 850 mL of distilled water; 10 mL of concentrated HCl is added and the solution is diluted to 1 L. This solution is stable for 1 year.

Method
A few drops of the reagent are added to a few drops of urine. A purple colour indicates the presence of a salicylate. This test was devised for the quantitative assay of salicylates in serum, with the mercuric chloride serving as a protein precipitant. The ferric chloride test has been modified for use on blood samples (Asselin, Caughlin 1990). Blood (0.5 mL) is mixed with 1.0 mL of acetone and vortex mixed. The sample is centrifuged, and 50 μL of the supernatant is added to 50 μL of ferric chloride. A purple colour at the interface indicates salicylates.

Vanillin reagent

Reagent
Dissolve 1 g of vanillin in 20 mL of concentrated sulfuric acid, warming if necessary.

Method
Add 2 drops of the reagent to the sample, heat in a water-bath at 100°C for 30 s and note any colour that is produced. Dilute the cooled mixture by adding a few drops of water and note any change of colour.

Indications
Many compounds of different chemical structure react with this reagent. However, for barbiturates, the reaction appears to be a steric

Table 30.32 Reactions of steroids with sulfuric acid

Initial colour	*Compound*	*Fluorescence at 350 nm*	*Fluorescence after dilution*
Red			
Orange–red	Dienestrol	Nil	Nil
	Dimethisterone	Nil	Yellow
	Mestranol	Yellow	Orange (pink in daylight)
Pink			
Orange–pink	Dexamethasone	Nil	Nil
	Prednisolone	Nil	Green (red in daylight)
Orange	Beclometasone (slow)	Nil	Nil
	Cholesterol	Nil	White
	Dydrogesterone	Green–yellow	Green–yellow
	Fludrocortisone	Green	Green (dichroic in daylight)
	Norethandrolone	Green–yellow	–
	Norethisterone	Orange	Orange (violet in daylight)
	Norethynodrel	Orange	Orange
	Oxymetholone	Nil	Nil
	Spironolactone (→yellow–green)	Yellow–green	Green
	Diethylstilbestrol	Nil	Nil
	Triamcinolone	Nil	Nil→green (slow)
Red–orange	Ethinylestradiol	Orange	Orange (red in daylight)
Pink–orange	Betamethasone	Nil	Nil
Green, orange (dichroic)	Hydrocortisone	Green	Green
Yellow	Alfadolone	Nil	Nil
	Androsterone	Nil	White
	Carbenoxolone	Nil	Yellow
	Cortisone	Green	Green
	Desoxycortone	Green–yellow	Yellow (violet in daylight)
	Enoxolone	Nil	Green–yellow
	Fluocinolone	Green	Quenched
	Fluoxymesterone	Green	Quenched
	Fludroxycortide	Green	Quenched
	Hydroxyprogesterone	Green	Quenched
	Estradiol	Green	Green (orange in daylight)
	Prednisone	Green	Green
	Progesterone	Green	Quenched
Orange–yellow	Fluocortolone	(Weak)	(Weak)
Green–yellow	Estrone	Green	Green–yellow (orange in daylight)
No colour	Estriol	Yellow–green	Quenched (orange in daylight)
	Testosterone	Green	Nil

phenomenon that depends on the structure of the side-chain at the 5-position. Dark colours, which are either dispelled or changed to violet, blue or green by dilution, are produced when either side-chain is greater than two carbon atoms in length or contains a cycloalkene ring. Branching can be proximal to the pyrimidine ring, but not distal. No colour is obtained if both side-chains are less than three carbon atoms in length or if either is branched distally or contains an aryl nucleus. Long, straight, saturated chains also appear to hinder reaction.

Hydroxybarbiturates give positive responses (Table 30.34), but bemegride, glutethimide, phenytoin and primidone do not respond. No response is obtained with amobarbital, aprobarbital, barbital, butobarbital, enallylpropymal, hexethal, ibomal, idobutal, metharbital, methylphenobarbital, nealbarbital, phenobarbital or phenylmethylbarbituric acid. With cold reagent, an orange colour is produced by pentobarbital, secobarbital and thiopental, and a brown colour by cyclopentobarbital.

Zwicker reagent (alkaline cobalt test)

This is a general test for barbiturate-like compounds.

Reagent

1. Dissolve 0.5 g of copper(II) sulfate pentahydrate in 100 mL of distilled water.
2. Add 0.5 mL of pyridine to 95 mL of chloroform.

Method

Add a few drops of solution 1 to the sample to be tested, followed by a few drops of solution 2 and then heat.

Indications

The presence of a violet–blue colour indicates barbiturates (Table 30.35). The LOD is 1000 μg for phenobarbital.

Table 30.33 Colours of thioxanthenes with sulfuric acid

Colour	*Compound*[(a)]
Purple	Oxytetracycline, tetracycline
Red	Caramiphen (when warmed), dantron, fenitrothion, methenamine (when warmed), mequitazine (slowly at 100°C), methacycline, methylprednisolone (after 1 min), metopimazine, nuarimol, ouabin, phenothiazines, prednisolone (after 1 min), pipoxolan
Orange–red	Oxprenolol*, quinomethionate
Violet–red	Morantel, oxytetracycline
Pink	Doxylamine, indapamide (slow)
Orange	Alprenolol, amitriptyline, benactyzine, benzilonium, benzquinamide, benzyl nicotinate, chlorprothixine*, clidinium, clopenthixol*, cyclothiazide (→red–brown), diethylthiambutene, diphemanil, diphenhydramine, diphenidol, doxepin, flupentixol*, indometacin, mazindol, mebanazine, mecoprop, methapyrilene, methindizate, methixene*, methyclothiazide, methylpiperidyl benzilate, naproxen, nefopam, nifedipine, nortriptyline, orphenadrine, penthienate methobromide, polythiazide, pyrantel (→violet), rotenone, tiotixene*, tofenacin
Yellow	Acebutolol, amiloride, amiodarone, benzthiazide, broxaldine, broxyquinoline, cinchophen, clefamide, clemastine (green rim), cyclopenthiazide, diphenadione, doxycycline, enoxolone, fenbufen, furosemide, halquinol, hydroquinidine*, hydroquinine*, lorazepam, methyl benzoquate, 5-methyltryptamine, α-methyltryptamine, *N*-methyltryptamine, metolazone, minocycline, piperacetazine (→red), procyclidine, quinidine*, quinine*, rimiterol, salbutamol, salinazid, sodium cromoglicate, trichlormethiazide, veratrine (→violet), zomepirac
Orange–yellow	Ethyl biscoumacetate, hexoprenaline, pizotifen (→violet)
Green	Bromodiphenhydramine, cyclizine, diphenhydramine, diphenylpyraline, phenothiazine, protriptyline
Blue	
Brown–blue	Chlortetracycline, demeclocycline, thioridazine
Violet	Bendroflumethiazide, chlorotrianisene, chromonar*, clofazimine, cyproheptadine, dosulepin, mesoridazine (→blue), methylenedioxyamfetamine, nicergoline, perazine, phenindione, rolitetracycline, tetracycline
Red–violet	Trifluomeprazine
Brown	Chelidonine, sulindac
Red–brown	Lymecycline
Orange–brown	Biperiden, ouabain (slow)
Yellow–brown	Tylosin (slow)
Grey–brown	Octaphonium
Black	
Blue–black	Clomocycline

[(a)]Compounds giving colours that fluoresce under UV light (λ = 350 nm) are indicated by an asterisk.

Basic tests for drug substances and products

The WHO has published texts *Basic Tests for Drugs* (WHO 1998), which includes pharmaceutical substances, medicinal plant materials and dosage forms, and *Basic Tests for Pharmaceutical Substances* (WHO 1986). The basic tests described, which are designed to verify the identity of drug substances and medicinal products and to detect gross contamination, use a limited number of readily available reagents and equipment. Overall, the combined texts offer compound-specific tests for approximately 500–600 products that are based on a combination of organoleptic checks and simple physicochemical tests, such as colour reactions and melting-point determinations.

Table 30.34 Reactions of barbiturates with vanillin reagent

Colour after heating	*Compound*	*Colour after dilution*
Red	3′-Hydroxybutobarbital	Violet (transient)
Violet–red	Heptabarbital	Colourless
	3′-Hydroxyamylobarbital	Colourless
Brown–red	Cyclobarbital	Green
	Cyclopentobarbital	Green
	Pentobarbital	Violet
	Secobarbital	Violet
	Thiopental	Violet
Orange	Butalbital (weak)	Colourless
	Secbutobarbital (weak)	Violet
Brown–orange	Allobarbital	Violet (transient)
	Brallobarbital	Brown–orange
	Talbutal	Violet
	Thialbarbital	Violet (transient)
Brown	Hexobarbital	Violet
Violet–brown	Methohexital	Colourless
	Vinbarbital	Colourless
	3′-Hydroxypentobarbitone	Colourless

Table 30.35 Colours with Zwicker reagent

Colour	*Compound*
Blue	Diphenylhydantoin
Green	Diacetylmorphine, pseudoephedrine
Yellow	Tetracycline

Semi-quantitative TLC methods have been developed as basic tests using a limited number of solvent systems and detection systems. References to these tests are given in (WHO 1998).

It should be remembered that basic tests are not, in any circumstances, intended to replace pharmacopoeial requirements, but should be used as a rapid, inexpensive means to verify identity and strength of drugs and medicinal products, and possibly to detect poor-quality counterfeit and other substandard products. In the event that suspect products are detected, these should be tested for compliance against pharmacopoeial requirements.

Colour reagents and thin-layer chromatography

Many common colour-test reagents are used routinely as spray locating reagents in TLC (e.g. acidified iodoplatinate solution, Dragendorff's reagent, Marquis reagent, Van Urk's reagent). It is worth noting that the preparation of the spray equivalent of a colour reagent may differ slightly from that of the colour reagent preparation itself (see Chapters 1 and 11 and Index of Reagents).

It is also true that the reaction and resultant colour that marks the presence of a certain substance obtained from spraying a reagent on TLC plates may differ from that obtained from a direct colour test and, in some cases, will not yield any results. This is because of pH effects – that is, whether the TLC plate has been dipped in 0.1 mol/L sodium hydroxide and the substance tested is acidic in character (i.e. the free acid of a salt post extraction). However, this aspect of colour reagents and their use in TLC as sprays can often give clues as to the drug or substance's chemistry.

A list of substances and their colour reactions to various TLC spray reagents is given in the Index of Analytical Data.

Appendix 30.1. Colour reactions of amfetamine-like compounds

Compound	*Marquis*	*Mecke*	*Froehde*	*Mandelin*
D-Amfetamine	R/O→R/Br	Y	Css	O→R→G/Br
Benzfetamine	R→Br	G→Y	Y→G	
Diethyltryptamine	Y→Br	B→G/Bk		
2,5-Dimethoxyamfetamine	lrY→R/Br	Br/G	ltG	ltG
Dimethoxymethylamfetamine	Y/G	Y→G→dkR/Br	Y/G	Y→G→Br
2,3-Dimethylamfetamine	M R/V	V dkV	V Gy/V	V Gy/G
2,4-Dimethylamfetamine	V O/R	G G/Br	Y Br/Y	Y Br/Y
2,5-Dimethylamfetamine	Y/G	Br/G	Y/G	Y/G
2,6-Dimethylamfetamine	Css	Br/G	Css	Br/Y
3,4-Dimethylamfetamine	Y/Br	G→Br/G	Lt G	NR
3,5-Dimethylamfetamine	Y	O/Br→Br/G	Css	Gy/V
Dimethyltryptamine	Y→G→Br	Y→G/Bk	ltY	G→Br
Ephedrine	Css	Css	Css	ltBr
Mephentermine	O→RBr	G		
Mescaline	O/R	O→G/Br	ltY	Y→G→Br
Metamfetamine	R/O→R/Br	NR	NR	G→B/G
3-Methoxy-4,5-methylenedioxyamphetamine	O→M	dkB	B→dkB	O→O/R
3,4-Methylenedioxyamfetamine	B/BK→dkV	G→dkB	G→dkV	R/V→dkV
3,4-Methylenedioxymethylamfetamine	B→V→Bk	G→dkB	Y/G→dkB	B→V→Bk
Paramethoxyamfetamine	Effervescence	ltG	G→R/Br	
Phendiamine	V→Bk	ltG	ltV	G
Phendimetrazine	NR	NR	NR	NR
Pheniramine	NR	NR	NR	NR
Phenmetrazine	NR	ltY	NR	NR
Phentermine	ltO	ltO	Br	G
Phenylephrine	O	O/Br	B	G
Phenylpropano lamine	ltY	ltY	Br	NR
Pseudoephedrine	Css	Css	Css	ltBr
3,4,5-Trimethoxyamfetamine	R/O→O	G/Br→Br	Y	NR

B, blue; Bk, Black; Br, brown; Css, colourless; dk, dark; G, green; Gy, grey; lt, light; M, magenta; NR, no reaction; O, orange; R, red; V, violet; Y, yellow.

Appendix 30.2. Colour reactions of narcotics

Compound	*Marquis*	*Mecke*	*Froehde*	*Mandelin*
Anileridine	O	NR	NR	NR
Codeine	V	dkG	ltG	ltG
Dihydromorphinone	V/Br	Br	V	Css
Ethylmorphine	O→V	dkG	Y→ltG→B	G
Fentanyl	O→Br	G		
Diamorphine	V	ltG	M	NR
Hydrocodone	V	G→B	ltY	NR
Hydromorphine	Y→R→V	Y→O→G	dkB→Gy	
Levorphanol	Gy→Bk	ltGy→Bk	B	G
Meperidine (pethidine)	O(slow)	ltY(slow)	NR	NR
Methadone	ltV	G→Br	B	
Morphine	M→V	G→B	V→Gy	ltGy
Nalorphine	O→R→V	ltG→dkG	M	G
Oxycodone	O/Y→ltV	Y→dkG	Y→YBr	Y→G
Oxymorphone	M→V	Y→Br	B→V	Bk
Papaverine	M	dkG	G	G/Br
Pentazocine	M	ltGy→V	dkB	G→Br
Thebaine	V/Br	Br	Br	V/Br

B, blue; Bk, black; Br, brown; Css, colourless; dk, dark; G, green; Gy, grey; lt, light; M, magenta; NR, no reaction; O, orange; R, red; V, violet; Y, yellow.

Appendix 30.3. Chemical test for gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL)

Reagents

Chlorophenol red
0.04 g chlorophenol red in 100 mL water, adjust to pH 7 with 0.01 mol/L sodium hydroxide.

Modified Schweppes
1. 2 g of dextrose in 20 mL of water
2. 2.4 g of aniline hydrochloride in 20 mL of ethanol.

Mix solutions 1 and 2 together and dilute to 80 mL with methanol.

Bromocresol purple
0.04 g bromocresol purple in 100 mL of water. Adjust to pH 7 with 0.01 mol/L sodium hydroxide.

Bromothymol blue
0.04 g bromothymol blue in 100 mL of water. Adjust to pH 7 with 0.01 mol/L sodium hydroxide.

Bromocresol green
0.03 g bromocresol green in 100 mL of methanol–water 4 : 1. Adjust to pH 7 with sodium hydroxide 0.01 mol/L.

Methyl orange
0.01 g methyl orange in 100 mL of methanol. Adjust to pH 7 with sodium hydroxide 0.01 mol/L.

Colour test 1

Reagent
Mix chlorophenol red and modified Schweppes reagent in a 3 : 1 ratio.

Method
Place 0.5 mL of a liquid sample in a test tube. Adjust to pH 5–8 with 0.01 mol/L sodium hydroxide. Add 2 drops of the test reagent and swirl. An immediate colour change (orange–red to dark red) indicates GHB. GBL gives a yellow colour.

Colour test 2

Reagent
Mix bromocresol purple and bromothymol blue in a 1 : 1 ratio and mix the combined reagent with modified Schweppes reagent in a 7 : 1 ratio.

Method
Same procedure as for colour test 1. GHB gives a purple colour and GBL gives a yellow colour.

Colour test 3

Reagent
Mix bromocresol green and methyl orange in a 1 : 1 ratio and mix the combined reagent with modified Schweppes reagent in a 3 : 1 ratio.

Method
Adjust the pH of the test solution to neutral if necessary and add 2 drops of test reagent. A dark-green colour indicates GHB; GBL gives a yellow–orange colour.

Colour test 4

Reagent
1% cobalt nitrate solution.

Method
Place 0.5 mL of a liquid sample in a test tube. Add a few drops of the test reagent. A pink-to-violet colour indicates GHB.

Appendix 30.4. Tests for metals and anions not covered by the general scheme

Metals

Reinsch test (for antimony, arsenic, bismuth and mercury)

Method
Use a 5 × 5 mm piece of copper foil or mesh. Clean the copper in 50% (v/v) nitric acid until it develops a shiny surface, rinse with water and proceed as follows:

1. Place 20 mL of sample and 10 mL of concentrated hydrochloric acid in a 100 mL conical flask and add the copper foil or mesh.
2. Heat on a boiling water-bath for 1 h and add as necessary dilute hydrochloric acid to maintain the volume of the solution.
3. Cool, remove the copper, wash gently with water and examine the surface.

Indications
- A purple–black stain indicates antimony.
- A dull black stain indicates arsenic.
- A shiny black stain indicates bismuth.
- A silvery deposit suggests mercury.

Note that other elements, (e.g. selenium and tellurium) also give black deposits and sulfur compounds may give a speckled discoloration.

Confirmatory test
This is applied to the stained copper foil or mesh derived as described above.

Reagents
1. Aqueous potassium cyanide solution (100 g/L)
2. Freshly prepared aqueous sodium sulfite solution (50 g/L)
3. 3 mol/L nitric acid
4. Quinine/potassium iodide reagent. Dissolve 1 g of quinine in 100 mL of water containing 0.5 mL of concentrated nitric acid. When the quinine has dissolved add 2 g of potassium iodide.

Method
1. Leave the copper in potassium cyanide solution for 10 min.
2. Wash any undissolved stain with water and add 1 mL of sodium sulfite solution followed by 1 mL of 3 mol/L nitric acid.
3. Shake the mixture frequently for 5 min and add 1 mL of water and 1 mL of potassium iodide reagent.

Indications Stains caused by the presence of arsenic dissolve in potassium cyanide solution whereas stains due to antimony and bismuth remain. The slow formation of an orange–brown suspension is seen with the quinine/potassium iodide reagent if a stain due to bismuth is present. The LOD for arsenic is about 5 mg/L and for antimony and bismuth is 2 mg/L. A more specific test for arsenic, the 'Gutzeit test', involves the conversion of arsenic to arsine and subsequent reaction of the gas with reagents such as silver diethyldithiocarbamate to give a coloured product. A modified version of the Gutzeit apparatus is available from Fischer Scientific which allows quantitative measurement of arsenic in stomach contents, food, water and other materials.

Confirmatory test for mercury
This is applied to the silver stained foil or mesh from the Reinsch test.

Reagent Cuprous iodide suspension. Dissolve 5 g of copper sulfate and 3 g of ferrous sulfate in 10 mL of water with continuous stirring and add 7 g of potassium iodide dissolved in 50 mL of water. Allow the cuprous iodide precipitate to form, filter, and wash with water. Transfer the precipitate as a suspension in water to a brown glass bottle.

Method Add 0.1 mL of copper(I) iodide suspension to a filter paper, place the foil on the suspension, cover and leave for 1–12 h.

Indications A salmon-pink colour due to the formation of cuprous mercuric iodide suggests the presence of mercury and positive results may appear within 1 h, but with low concentrations colour development may take up to 12 h. The LOD for mercury is about 5 mg/L.

Barium

Reagents
1. Concentrated hydrochloric acid
2. Platinum wire.

Method
1. Dip the end of the platinum wire into the hydrochloric acid and then into the test material.
2. Insert the wire into the hot area of a micro-burner flame and observe any changes in the flame colours.

Indications
Barium salts impart a green flame; copper and thallium salts also give a green flame in this test.

Confirmatory test

Reagents
1. 1 mol/L sulfuric acid
2. 100 g/L aqueous lead acetate solution
3. 50 mL/L aqueous acetic acid
4. Solid ammonium acetate.

Method
1. To a mixture of 2 mL of lead acetate solution and 2 mL of dilute sulfuric acid add enough ammonium acetate to dissolve the lead acetate precipitate.
2. Add 0.1 mL of dilute acetic acid to 1 mL of sample followed by 1 mL of the lead sulfo-acetate solution (from step 1) and vortex mix for 5 s.
3. Centrifuge for 2 min and observe the tube against a dark background.

Indications Barium salts yield either a white turbidity or white precipitate. Calcium and strontium salts interfere. The LOD for barium is approximately 100 mg/L.

Copper

Reagents
1. 10 g/L solution of dithiooxamide in methanol
2. Concentrated ammonium hydroxide.

Method
Place 0.1 mL of sample onto a filter paper to give a spot of around 1 cm in diameter. Expose the paper to ammonia fumes and add 0.1 mL of the dithiooxamide reagent to the spot.

Indications
An olive green stain is seen with copper salts. A green stain due to chromium is usually visible before addition of the dithiooximide reagent. Yellow–brown or yellow–red colours are given by several other metals. The LOD for copper is approximately 1 mg/L.

Iron

Reagents
1. 2 mol/L hydrochloric acid
2. 10 g/L aqueous potassium ferricyanide solution
3. 10 g/L aqueous potassium ferrocyanide solution.

Method
1. Add 0.1 mL of 2 mol/L hydrochloric acid and 0.05 mL of potassium ferricyanide solution to 0.1 mL of sample.
2. Add 0.1 mL of 2 mol/L hydrochloric acid and 0.05 mL of potassium ferrocyanide solution to a further 0.1 mL of sample.
3. Agitate both mixtures for 5 s, leave for 5 min at room temperature and centrifuge for 5 min.

Indications
Ferrous salts give a deep blue precipitate with potassium ferricyanide and ferric salts give a deep blue precipitate with potassium ferrocyanide. The LOD for both ferrous and ferric salts is about 10 mg/L.

Thallium

Test 1

Reagents
1. Cyanide reagent: dissolve 1.6 g of sodium hydroxide, 1.2 g of potassium sodium tartrate and 1.36 g of potassium cyanide in 10 mL of water.
2. Prepare a fresh solution of dithizone (250 mg/L) in chloroform.

Method Add 1 mL of cyanide reagent to 5 mL of urine in a stoppered glass test-tube and vortex mix for 20 s. Then add 2 mL of dithizone solution, vortex mix for 1 min and centrifuge (5 min).

Indications The presence of thallium is indicated by a pink–red colour in the chloroform layer. The test will detect thallium at 0.1 mg/L. A number of other metal ions give colours with this test.

Test 2

Reagents
1. Bromine water (saturated)
2. 6 mol/L hydrochloric acid
3. 20% (w/v) aqueous sulfosalicylic acid solution
4. 0.1% (w/v) aqueous methyl violet solution
5. Toluene.

Method To 1 mL of urine carefully add 2 drops of hydrochloric acid and 5 drops of bromine water. Leave to stand for 5 min, add 5 drops of sulfosalicylic acid solution and 0.5 mL of toluene, and shake gently.

Indications A transient blue–green colour in the toluene layer suggests the presence of thallium. The LOD for thallium is 1 mg/L.

Anions

Borate

Reagents
1. 10 g/L solution of turmeric in methanol
2. 1 mol/L hydrochloric acid
3. 4 mol/L ammonium hydroxide.

Method
1. Prepare turmeric test papers by soaking strips of filter paper (1×5 cm) in the turmeric solution and drying at room temperature.
2. Acidify a portion of the sample with the dilute hydrochloric acid and apply to a strip of turmeric paper.
3. When the paper is dry moisten it with dilute ammonium hydroxide.

Indications
A positive sample will impart a red–brown colour to the turmeric paper which strengthens as the paper dries. When the paper is moistened with dilute ammonium hydroxide a green–black colour is produced. Oxidising agents that bleach turmeric (e.g. bromates, chlorates, iodates and nitrites) interfere with the test. The LOD for borate is 50 mg/L.

Confirmatory test

Reagent 0.5 g/L solution of carminic acid in concentrated sulfuric acid.

Method Add 0.5 mL of filtered stomach contents or scene residue to a 10 mL glass tube and slowly pour 0.5 mL of carminic acid solution down the inside of the tube so that a layer is formed underneath the sample.

Indications Borate is indicated by the formation of a blue–violet ring at the junction of the two layers. Note that strong oxidising agents (e.g. bromates, chlorates, iodates and nitrites) also give positive results.

Bromides

Reagents
1. 2 mol/L nitric acid
2. 10 g/L solution of silver nitrate
3. Concentrated ammonium hydroxide.

Method
1. To 1 mL of clear tests sample add 0.5 mL of 2 mol/L nitric acid and mix for 5 s.

2. Add 0.1 mL of silver nitrate solution.
3. Centrifuge any precipitate and decant off the supernatant.
4. Add 0.1 mL of concentrated ammonium hydroxide.

Indications

Bromide gives an off-white precipitate that is slightly soluble in ammonium hydroxide. A white precipitate that dissolves in ammonium hydroxide indicates chloride and a yellowish precipitate insoluble in ammonium hydroxide suggests iodide. The LOD for bromide is 50 mg/L.

Confirmatory test

Reagents

1. Saturated solution of fluorescein in aqueous acetic acid (600 mL/L)
2. Concentrated sulfuric acid
3. Solid potassium permanganate.

Method

1. Soak a strip of filter paper in fluorescein solution.
2. Transfer 2 mL of test solution to a 10 mL glass tube and add about 2 mg of potassium permanganate.
3. Add 0.2 mL of concentrated sulfuric acid and hold the fluorescein paper at the mouth of the tube.

Indications Any bromide is oxidised to free bromine, which then reacts with the fluorescein dye (yellow) to give tetrabromofluorescein (eosin) which has a pink–red colour. The LOD for bromide is 50 mg/L.

Chlorate

Chlorates and other oxidising agents can be detected by the diphenylamine test described previously. The following tests can also be applied.

Test 1

Reagents

1. 6 mol/L sulfuric acid
2. 1% w/v aqueous solution of indigo carmine
3. Solid sodium sulfite.

Method To 1 mL of sample add 4 mL of 6 mol/L sulfuric acid followed by 1 mL of indigo carmine reagent.

Indications A deep blue colour indicates the presence of chlorates. The colour fades after adding several crystals of sodium sulfite. The same reactions are given by bromates and hypochlorites.

Test 2

Reagents

1. Manganous sulfate reagent: a 1 : 1 mixture of saturated aqueous manganous sulfate and orthophosphoric acid
2. A 10 g/L solution of diphenylcarbazide in methanol.

Method

1. Add 0.2 mL of manganous sulfate reagent to 0.1 mL of test sample and warm the mixture gently over a burner.
2. Cool and add 0.1 mL of diphenylcarbazide solution.

Indications Chlorate yields a purple colour that intensifies after cooling and adding diphenylcarbazide. A similar reaction is given by persulfates and periodates.

Note: after ingestion of chlorate the blood turns brown owing to the formation of methaemoglobin by oxidation of the ferrous ion of haemoglobin. Add 0.2 mL of a 10% (w/v) of aqueous potassium cyanide solution to 1 mL of blood and an immediate red colour confirms the presence of methaemoglobin. Exposure to a wide range of other substances (e.g. aniline, nitrites, nitrates, aniline, dapsone, benzocaine, urea herbicides) causes methaemoglobinaemia.

Fluoride

Test 1

Reagents

1. A 50 g/L aqueous solution of sodium chloride
2. Concentrated sulfuric acid
3. Calcium chloride (solid)
4. Powdered silica.

Method

1. To 5 mL of sample in a porcelain crucible add 100 mg of calcium chloride and gently evaporate to dryness over a burner.
2. Destroy the organic material by strong heating to leave a white ash.
3. Mix the residue with 200 mg of powdered silica.
4. Apply a drop of sodium chloride solution to a microscope slide, add 1 mL of concentrated sulfuric acid to the contents of the crucible and immediately position the slide such that the sodium chloride drop is suspended over the crucible.
5. Rest a small beaker of ice on the slide and heat the crucible gently for 5 min over a burner.
6. Remove the slide after 5 min and examine the sodium chloride solution under a microscope.

Indications In the presence of fluorine-containing compounds, silicon tetrachloride is produced, which dissolves in the sodium chloride solution to form sodium silicon tetrafluoride. This forms small hexagonal crystals as the water evaporates from the slide, which sometimes have a pink hue. The crystals are seen at the edge of the drop and appear before the larger cubic crystals of sodium chloride.

Test 2

Reagents

1. Concentrated sulfuric acid
2. Calcium hydroxide (solid)
3. Paraffin wax.

Method

1. Repeat steps (1) and (2) described for test 1.
2. Smear a film of paraffin wax on one side of a glass microscope slide and expose part of the surface by making an identifiable sign on the paraffin film.
3. Add 5 mL of concentrated sulfuric acid to the crucible and cover it with the slide, with the paraffin film on the inside.
4. Heat the crucible gently for 20 min and then remove the slide.
5. Remove the paraffin film with toluene and examine the slide.

Indications Hydrogen fluoride is generated from fluorine-containing compounds and etches the glass to give a mark corresponding to that made in the paraffin film.

Both tests have a LOD of approximately 100 mg/L of fluoride.

Hypochlorite

Test 1

Reagents

1. Glacial acetic acid
2. 50 g/L aqueous lead acetate solution.

Method

1. To 1 mL of test solution add acetic acid dropwise to reach a pH of approximately 6 (test with universal indicator paper).
2. Add 0.5 mL of lead acetate solution and boil for 3 min.

Indications Hypochlorite forms a brown precipitate. An immediate brown to black precipitate is given by sulfides with lead acetate.

Test 2

Reagents

1. 100 g/L aqueous potassium iodide solution
2. Glacial acetic acid
3. Solid starch.

Method

1. To 0.1 mL of test solution add 0.1 mL of acetic acid followed by 0.1 mL of potassium iodide solution.
2. Mix and add about 20 mg of starch.

Indications Hypochlorite gives a blue colour. (*Note:* hypochlorite also gives a positive reaction in the diphenylamine test for oxidising agents described above.)

Iodides

Reagents
1. 10 g/L aqueous silver nitrate solution
2. 2 mol/L nitric acid
3. Concentrated ammonium hydroxide.

Method
1. To 1 mL of clear test solution add 0.1 mL of nitric acid and 0.1 mL of silver nitrate solution.
2. Centrifuge down any precipitate, remove the supernatant and add to it 0.1 mL of ammonium hydroxide.

Indications
A curdy yellow precipitate that is insoluble in ammonium hydroxide is given by iodides. Chlorides give a white precipitate that dissolves in ammonium hydroxide, and an off-white precipitate that is sparingly soluble in ammonium hydroxide suggests bromides.

Confirmatory test

Reagents
1. 2 mol/L hydrochloric acid
2. 100 g/L freshly prepared sodium nitrite solution
3. Solid starch.

Method Mix thoroughly about 20 mg of starch with 0.1 mL of test solution, 0.1 mL of hydrochloric acid and 0.1 mL of sodium nitrite solution in a test-tube.

Indications A blue colour confirms the presence of iodide.

Oxalates

Reagents
1. 100 g/L aqueous calcium chloride solution
2. 30% (v/v) solution of acetic acid
3. 2 mol/L hydrochloric acid.

Method
1. Add 1 mL of calcium chloride solution to 2 mL of test solution and mix.
2. If a precipitate forms, add 1 mL of acetic acid.
3. If the precipitate does not dissolve, separate it by centrifugation and add 1 mL of dilute hydrochloric acid.

Indications
A white precipitate that is insoluble in acetic acid but dissolves in hydrochloric acid indicates the presence of oxalates.

Confirmatory test

Reagents
1. Thiobarbituric acid
2. Concentrated ammonium hydroxide.

Method
1. Add 50 µL of test solution to 100 µL of ammonium hydroxide in a micro test-tube and mix thoroughly.
2. Gently evaporate the mixture to dryness over a micro-burner.
3. Add about 200 mg of thiobarbituric acid and reheat gently to about 150°C.

Indications Oxalates give a bright red product that is soluble in methanol. The LOD for oxalate is 250 mg/L.

Sulfides

Reagents
1. 10% (v/v) aqueous solution of sulfuric acid
2. 10% (w/v) solution of lead acetate in boiled and purified water
3. 2 mol/L acetic acid.

Method
1. Immerse a strip of white filter paper in a mixture of 10 volumes of lead acetate solution and 1 volume of acetic acid and allow to dry.
2. To 1 mL of test sample in a test-tube add 3 mL of sulfuric acid and insert the lead acetate paper into the neck of the tube.
3. Heat the tube in a boiling water-bath for 5–10 min.

Indications
Hydrogen sulfide fumes that turn lead acetate paper black are produced by sulfides. The LOD for sulfide is 50 mg/L.

Thiocyanates

Reagent
A 50 g/L aqueous solution of ferric chloride.

Method
Mix 0.5 mL of ferric chloride solution with 0.5 mL of sample.

Indications
A deep red colour is given by thiocyanates. The LOD for thiocyanate is 50 mg/L.

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CHAPTER

31 Immunoassays

RS Niedbala and JM Gonzalez

Introduction

The first immunoassay principles were published decades ago (Yalow, Berson 1959). Since that time immunoassays have become a routine part of day-to-day life for scientists in various disciplines as well as for general consumers. For example, every over-the-counter pregnancy and fertility test is based upon immunoassay results. The impact has been even greater in broader fields where analysts use immunoassays for the routine screening of samples for a host of target analytes. Fields such as veterinary medicine, environmental testing, anti-terrorism and human clinical diagnostics all use various types of immunoassays (Huckle, Wild 2005).

For instance, all the applications that exist, immunoassays have limitations that are generally created by the antibodies used. Antibodies may have poor specificity that results in cross-reactivity with structurally similar compounds, creating false results. Antibodies may also be affected by the matrix used. For example, hair testing for drugs of abuse is challenged by the treatments that consumers use on their hair and scalp. It is also difficult to generate antibodies reliably against target compounds. It requires a 'make it and test it' scenario that can take years.

Nevertheless, immunoassays have become an integral part of routine clinical testing. When developed carefully, immunoassays can detect minute amounts of target analyte in complex samples. They can be robust and capable of working in harsh conditions along the roadside or in remote areas. Immunoassays will remain an important tool for anyone who needs to detect analytes reliably in a cost-effective and rapid manner.

The focus of this chapter will be to explain the principles of immunoassays and practical considerations in applying them in the field of toxicology.

Immunoassays can generally be constructed for target molecules with molecular weights as low as 250, as well as for large, complex proteins and cellular components. Each immunoassay is formatted to the usage situation and its analytical requirements. Some are designed to be highly sensitive in detecting and quantifying extremely small amounts of target analyte. Others are designed only for qualitative analysis to detect the presence or absence of analytes. Thus, researchers and developers have refined immunoassays to become reliable, robust, accurate tests that can be manufactured on a large scale.

Parallel to its development, and in response to it, societies have embraced the use of immunoassay technologies. For example, many countries have enacted legislation defining cut-offs as well as specific targets for immunoassays aimed at the detection of drugs of abuse in various situations where individuals or the general public may be at risk (US Department of Health and Human Services 2006; US Department of Transportation 2000). These legislated assay parameters regulate the implementation of millions of tests that use urine, saliva, hair or blood as matrices. The results from an immunoassay test followed by confirmation using alternative technologies can be used as forensic evidence in a court of law. In several countries individuals are tested for drug use prior to starting a new job, for suspicion of use during employment (Chapter 3) or following treatment or incarceration. Other countries are seeking to allow police to perform roadside testing of suspected drivers who may be under the influence of drugs of abuse (Parliament of Victoria, Australia 2003) (Chapter 5). Efforts are under way to develop and validate robust on-site immunoassays that can meet specific requirements of law and technical performance. Although they are not yet available for all desired target drugs, much progress has been made (Maes *et al.* 1999; Moeller *et al.* 1999; Samyn *et al.* 1999b; Verstraete, Puddu 2000; Walsh *et al.* 2004).

Immunoassays may take a variety of formats, although they all contain common elements regardless of design (Table 31.1)

The first element is an antibody that has been targeted against a specific analyte. A target analyte can be a large molecule, an infectious agent or a small molecule that can elicit an immune response. The quality of the antibody will ultimately determine the potential performance of any immunoassay. The antibody is relied upon to be specific for the desired target analyte, insensitive to the test matrix being used, and stable over long periods of storage when packaged into an immunoassay test kit. The second element in an immunoassay is the reporter that will generate a signal that can be used to determine the immunoassay results. A variety of systems based on radioactive, enzymatic and chemiluminescent labels as well as colloidal particles are available for this task (Table 31.2). Each has advantages and disadvantages in immunoassay design and use. These strengths and weaknesses will be discussed later in this chapter.

Once a quality antibody and a reporter have been identified, the platform for performing the immunoassay may be selected. There are many commercial platforms that are proprietary to corporations. However, basic researchers can utilise microtitre plates or rapid test formats such as lateral flow (Peace 2000; Niedbala *et al.* 2000; Perrigo, Joynt 1995; The Walsh Group 2002; Verheijen 2002; Volkov *et al.* 2009).

Finally, the buffers and packaging for any immunoassay must meet the requirements for stability and storage of the test. Buffers also serve another key purpose, which is to prepare the sample matrix to be tested for compatibility with the antibody and test format being used.

Various issues confront an immunoassay test developer when trying to use samples as diverse as urine or saliva. Each matrix has unique characteristics that can interfere with an immunoassay causing false-negative or false-positive results. Immunoassay developers have the challenge first of developing and then of thoroughly validating any test before it is used by collaborative researchers or the general public.

When specifically evaluating the performance of a toxicology assay it is worth examining what makes a good immunoassay test. First of all the purpose of the test should be considered. For instance, is the test to be used for qualitative screening or for monitoring concentrations of a particular drug in biological fluids? The difference in analytical requirements between, for example, a test for therapeutic drug monitoring (TDM) purposes and a qualitative screening test can be substantial. A TDM test is performed to optimise the level of drug therapy and therefore the immunoassay test result must be very accurate. In some cases, changing the dose of the drug incorrectly can result in serious or fatal outcomes (Baselt 2005; Hardman *et al.* 2001; Porter 2006; Uhlenhuth *et al.* 1990) (Chapter 2)

Conversely, a screening test must simply detect the presence or absence of a target compound or a class of compounds reliably above or below a designated cut-off concentration. In such tests the cut-off concentration is specified; however, the actual performance of the test will have some variation around the cut-off. It is worth discussing two aspects of immunoassay design and performance. The terms sensitivity and specificity are often used and require some explanation. These terms relate to analytical sensitivity/specificity or clinical sensitivity/specificity. The term 'analytical sensitivity' is used to describe the assay's ability

Table 31.1 Elements of an immunoassay

Element	*Purpose*	*Representation*
Antibody	Targets analyte	
Reporter	Amplifies result	
Hapten derivative	Drug derivative to link to reporter	
Analyte	Target for assay	
Buffers	Conditions, pH Sample	
Solid phase	Location to immobilise antibody	
Matrix	Describes type of sample being analysed	

to detect the lowest level of target drug while 'analytical specificity' describes how precise it is at targeting a compound. 'Clinical sensitivity' describes how reliably a test identifies positives among a tested population, while 'clinical specificity' is a measure of whether or not identified positives are truly positive. For example, in the case of a screening test for drugs of abuse, a test that was clinically 100% sensitive and specific would have correctly identified all presumptive positives and all of them would have been confirmed positive for the target drug by an alternative and completely specific technique.

The reality is that no screening immunoassay test is 100% accurate in identifying positives or negatives. In the case of drugs of abuse assays, positives are confirmed by other techniques such as gas chromatography–mass spectrometry (GC-MS). This is often expensive, but necessary, since antibody-based immunoassays are often subject to interferences or lack target analyte specificity. A well-designed screening immunoassay will therefore have a high degree of clinical specificity, meaning that positive immunoassay results are likely to be confirmed quickly and cheaply by GC-MS, thereby minimising costs by identifying samples that do not contain the analyte of interest. A final consideration before exploring the specifics of immunoassay is the metabolism and disposition of any target analyte drug and the matrix in which it is to be detected or quantified. Toxicological assays are increasingly performed on a variety of matrices including hair, saliva, blood or urine. The reason for choice of matrix may be ease of collection (saliva), a long window of detection (hair), compliance with regulations (urine) or forensics (stool or blood). Prior to developing an assay, it is critical to know which metabolites are the most prevalent and reliable as target analytes in a given type of sample. References are available to help any immunoassay developer outline their strategy for development (Baselt 2005).

As immunoassay use has expanded, so have the tools and the understanding of the principles for developing them. Application of the principles described below should allow researchers to achieve optimal performance for any immunoassay they may develop or evaluate.

Table 31.2 Various reporters used in immunoassays

Reporter	*Sensitivity achieved in immunoassay (molecules detected)*
Enzymes	10^7
Fluorescence	10^6
Chemiluminescence	10^5
Radioimmunoassay	10^5

Basic principles and issues of immunoassays

Antibody development and production

The development of an immunoassay for opiates (diamorphine, morphine, etc.) serves as a good example with which to describe in detail the principles and issues of immunoassay development. Diamorphine, morphine and related opiates are used throughout the world and their benefits and deleterious effects on health are well known (Baselt 2005; Hermes 1993). However, developing an immunoassay to identify specific opiates can be tedious and requires an appropriate strategy.

The first step when developing such an assay is to consider the target molecule and the human matrix that will be tested. Diamorphine and morphine have been thoroughly studied and their pharmacokinetics are well understood (Moore *et al.* 1984). Figure 31.1 shows the metabolism of diamorphine and the major metabolites formed. It can be seen that morphine is a primary metabolite in urine, saliva and blood. It also has a relatively long half-life and is known to be stable in biological matrices. Therefore, morphine becomes the target analyte against which antibodies will be needed.

Antibodies are proteins in mammals with a primary purpose to fight infection. They are generated by beta-lymphocytes following exposure to an immunogen. Immunogens are materials such as foreign proteins or cells that trigger the immune response. There are several antibody subtypes including IgG, IgA, IgM, IgE and IgD. Each functions within various compartments of the human body and may act to attack new infections or as sentinels if an infection reappears. For our purpose, antibodies are deliberately generated by introducing a designed immunogen (or antigen) into a host cell or animal. Beta cells within the host respond to the antigen by producing antibodies with affinity for the target antigen.

Antibodies may be generated using a number of methods. The two major techniques produce monoclonal or polyclonal antibodies (Howard, Kaser 2007; Levine 2003). Polyclonal antibodies are often produced in rabbits, sheep, chickens and even llamas (Frenken *et al.* 2000). Each species has particular characteristics. Sheep, for example, may be bled to yield substantial quantities of blood for large-scale needs. Chickens are easy to use since antibodies may be isolated from eggs. No matter what the choice of species, the disadvantage of polyclonal antibodies is that there may be a mixture of antibody subtypes isolated with varying performance. In contrast to polyclonal antibodies, monoclonals are produced following fusion of a polyclonal cell with bacterial cell lines (Kohler, Milstein 1975). The fused line will produce a highly specific antibody that is immortalised in the cell line. Thus, supply is never an issue as long as the cell line is carefully maintained.

Newer methods that are alternatives to traditional polyclonal and monoclonal antibody production include phage display as well as targeted engineering of protein domains with antibody-like attributes (Binz *et al.* 2005; Chiswell, McCafferty 1992; Ryan 2003). These newer techniques have primarily been used against large proteins and have not yet been developed sufficiently for use against small drug molecules.

Following the example of morphine, an immunogen must first be developed that will be used to trigger production of antibodies. Morphine is a small molecule and, by itself, is not immunogenic. Usually a compound must have a molecular weight greater than 2000 before it will trigger an immune response. To solve the problem of immunogenicity, a morphine derivative is first linked to a carrier protein by a process known as haptenisation. The aim of this haptenisation process is to conjugate multiple, chemically modified, or derivatised, drug molecules (the hapten) to the carrier protein.

Figure 31.2 shows the structure of morphine. Examining the chemical structure, it can be seen that morphine has amino or hydroxyl groups that can be used as sites for conjugation. The specificity of the antibodies generated can be determined by the position on the molecule used for conjugation. The site of conjugation is usually hidden from the immune system, so that changes made to the molecule at this position will have no effect on antibody binding. In the case of morphine, if an

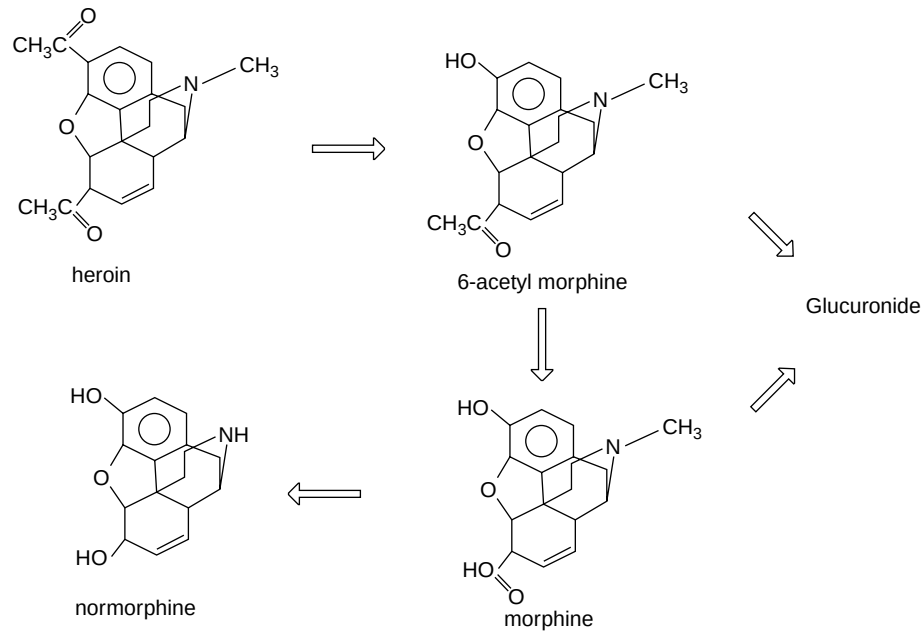

Figure 31.1 Structure of diamorphine (heroin) and its major metabolites.

immunogen is produced via conjugation with the hydroxyl group at position 3, this ceases to be a determinant against which antibodies are generated. As a result, the antibodies are likely to have cross-reactivity towards the major urinary metabolite of both diamorphine and morphine, morphine-3-glucuronide, and they will also recognise codeine (3-*O*-methylmorphine). Urine screening immunoassays to detect abuse of diamorphine or morphine usually employ antibodies raised by this means. Conversely, conjugation via the hydroxyl group at position 6 will yield antibodies that have a greater specificity towards morphine relative to morphine-3-glucuronide and that also display good cross-reactivity towards 6-monoacetylmorphine and morphine-6-glucuronide. These antibodies are well suited to formulating saliva assays for detecting diamorphine abuse, since this sample contains large amounts of 6-monoacetylmorphine as well as of morphine itself. Various methods are available to covalently couple through these groups or to add an extension linker prior to linking it to a carrier protein (Van Regenmortel *et al.* 1988). Carriers include large proteins such as bovine serum albumin (BSA), bovine gamma globulin or keyhole lymphocyte cyanin. A plethora of other possible carrier proteins exists, but in general the selected carrier proteins are dissimilar in structure to the reporter to be used in the assay and large enough to trigger the immune response.

Once antibodies are generated they are usually purified using simple precipitation techniques or by isolation using protein-A. Protein-A is a lectin that specifically binds to antibodies and allows rapid isolation of the purified proteins (Hober *et al.* 2007).

Immunoassay design

The antibody is a key element in the construction of any immunoassay and requires detailed consideration. Once an antibody is available it must be evaluated in the format in which it will be used. Assay formats are generally divided into two categories. The first is called a heterogeneous immunoassay, in which the differentiating characteristic is the requirement of a step to separate bound from free material. The alternative is called a homogeneous immunoassay and is distinguished from heterogeneous assays by the fact that it does not require a separation step. Figure 31.3 shows the basic elements of a heterogeneous immunoassay.

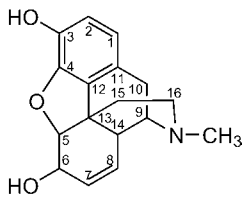

Figure 31.2 Structure of morphine.

Heterogeneous immunoassay/examples

The simplest form of heterogeneous immunoassay involves a competitive assay using a solid phase. Most commonly, a microtitre plate coated with immobilised antibodies against the target drug can be used. In this assay format, sample, buffer and a hapten–drug–reporter conjugate are added to a microtitre well. The mixture is incubated for some time during which the conjugate and free drug compete to bind to the solid phase. The solid phase is then washed to remove unbound material. Substrate is added, after which the signal is measured in each reaction well. The signal generated is inversely proportional to the concentration of free drug in the sample.

Enzyme-linked immunosorbent assay (ELISA) is the most common format used for the detection of large molecules or proteins, but it has also been adapted to detect small-molecule drugs.

These types of heterogeneous assay are sometimes called 'sandwich' assays because the target analyte is captured between two antibodies.

Figure 31.4 shows a diagram of a typical heterogeneous sandwich assay.

In the first step, an aliquot of sample or calibrator is mixed with buffer and incubated with the capture antibody that has been conjugated or adsorbed onto a solid surface such as a microtitre plate. After

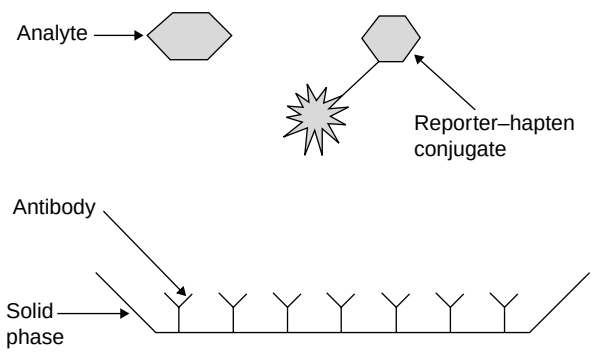

Figure 31.3 Basics of an immunoassay.

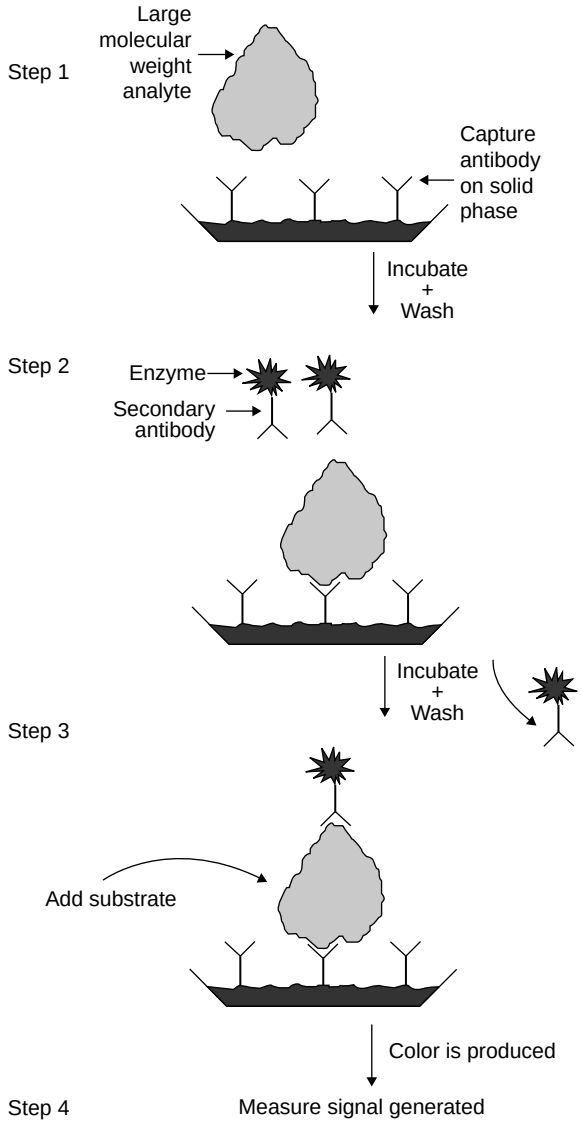

Figure 31.4 The basic scheme for an ELISA assay. Note the wash between steps. This is the distinctive feature of a heterogeneous assay.

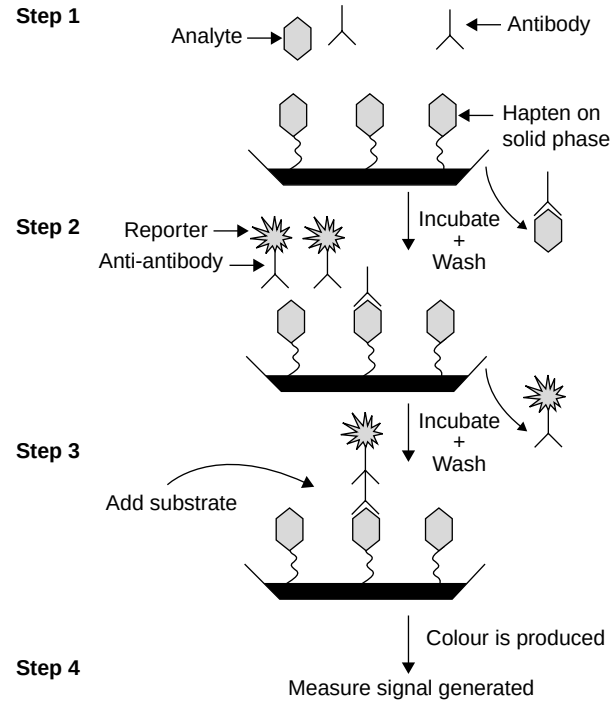

Figure 31.5 The scheme shows a typical competitive solid-phase immunoassay commonly used to detect drugs of abuse. It is a heterogeneous format using washing steps to remove materials that did not bind immunologically. Signal is inversely proportional to the concentration of free drug in the sample.

incubation, the surface is washed to remove whatever material did not specifically bind to the antibodies on the solid surface. In the second step, another solution is added that contains a secondary antibody, often labelled with a reporter. Following this incubation, the surface is again washed to remove unbound material. Next, a substrate solution or developer solution is added to generate a signal. Once sufficient signal has been generated, the reaction is stopped and the signal is measured.

The amount of signal generated is proportional to the amount of target material that was captured on the surface. Heterogeneous assays are often extremely sensitive and may be titred by adjusting the concentration of reagents and sample. The disadvantages of heterogeneous assays include the long incubation periods and multiple wash steps.

In the field of toxicology a number of heterogeneous assay techniques have become available and are in routine use. These techniques include ELISA, radioimmunoassay (RIA), chemiluminescent immunoassay (CIA), fluorescent immunoassay (FIA) and finally lateral flow assay (LFA).

Figure 31.5 shows a scheme for a typical heterogeneous assay targeting a small-molecule drug. In the first step, sample and buffer are mixed with anti-drug antibodies. If target drug is absent, the antibody will bind to the drug linked to the solid phase. The solid phase can be a microtitre plate, bead or membrane made of nitrocellulose, polymers or glass. Once this initial incubation is complete, the surface is washed and a second reagent is added containing reporter-labelled antibodies against the first antibody targeting the drug of interest. A sandwich is formed between the drug conjugate on the solid phase and the secondary antibody–reporter conjugate. After incubation, unbound secondary antibody is washed away and substrate or signal is measured in the reaction mix. The signal detected is inversely proportional to the concentration of drug in the sample.

There are a number of variations on the two assay schemes described above. In most cases the differences are in either the reporter used or the solid surface (see Table 31.2). Various reporters used include radioactive (RIA), fluorescent (FIA) and chemiluminescent (CIA) labels. The key to each heterogeneous assay is the wash step that removes excess reagent and lowers the background signal from the sample or other interferents. In this way heterogeneous assays achieve maximum analytical sensitivity.

Lateral flow is a type of heterogeneous assay that has become prevalent in emergency rooms and laboratories and in law enforcement to test on-site for drugs of abuse (Ulti-Med 2002). These tests are capable of multiplexed detection of a panel of drugs of abuse from a single aliquot of urine, blood or saliva (Inoue, Seta 1992; The Walsh Group 2002).

The basic scheme for a multiplexed lateral flow assay is shown in Fig. 31.6. A lateral flow assay is constructed from various materials that are assembled to form a test strip. A sample pad is the first component and is where a small aliquot, usually 50–100 µL, of sample is placed onto the strip. The sample pad often contains buffer salts to condition the sample to the correct pH and sometimes to remove cells or debris. These pads are often glass fibre or cellulose (Verheijen 2002; Volkov *et al.* 2009). The next material beyond the sample pad is the conjugate pad. It will usually hold the reporter–antibody conjugate targeting each drug of interest. The reporter most commonly used is colloidal gold. Gold particles are small, usually 10–100 nm in size, but are easily seen with the naked eye as they accumulate in a detection zone on a strip. Purified antibodies can easily be adsorbed onto the surface (Verheijen 2002; Volkov *et al.* 2009). Once a sample is added to a test strip it flows through the sample pad, the conjugate pad and then onto a nitrocellulose strip. Nitrocellulose as a raw material acts to bind capture

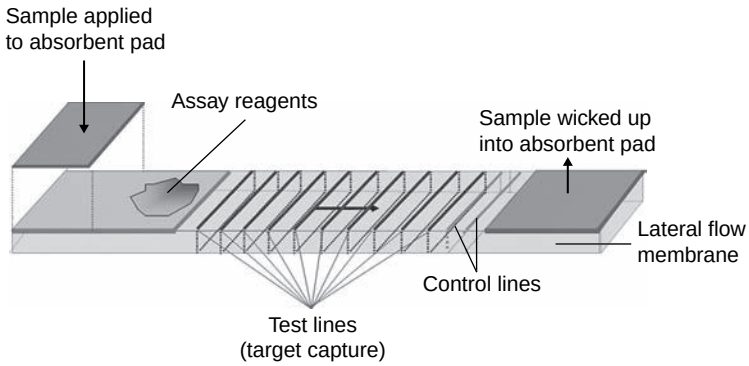

Figure 31.6 The figure shows the basic design of a lateral flow assay. It contains a series of material in linear order designed to contain reagents that flow along with the sample past capture zones. The immunological result is read in each capture zone usually by visual interpretation.

reagents and to allow liquids to flow by capillary action along a test strip. The nitrocellulose strip can vary in length according to the desired goals of the test. For example, a longer strip will allow multiple analyte test lines (see Fig. 31.6). However, it will take longer for fluids to move along the strip, thus increasing the time of the assay. The nitrocellulose is also where the immunological reaction takes place. Capture zones at the control line and test lines are created by spraying a solution of the capture protein derivative onto the nitrocellulose. The proteins then adsorb onto the nitrocellulose and become immobilised (see Fig. 31.6). The usual capture zone material is target hapten derivatised and conjugated to a carrier protein such as BSA. As the buffered sample and reporter antibody reach the reaction zone there is competition between drug in the sample and hapten conjugate on the strip to bind the reporter antibody. If there is a large concentration of drug, there will be little reporter conjugate available to bind to the reaction zone and the line will have no colour. If the sample is negative for drug, the line at the capture zone will be intense and easily seen with the naked eye. Use of lateral flow assays has grown exponentially in the last decade.

Once the basic chemistry has been developed, the system must be packaged into a housing (Fig. 31.7). The housing may be integrated into a urine cup, dip stick or a saliva collector (Draeger Safety UK Ltd 2008; Orasure Technologies 2008).

The challenge for lateral flow assays used in toxicology is that they must reliably detect a panel of drug targets using dynamic flow, and binding of reagents all moving through a series of striped materials is governed solely by capillary forces. From a purely analytical perspective, lateral flow assays are often imprecise and insensitive. It is not uncommon for lateral flow assays to have relative standard deviations (RSDs) of 15–30% when testing replicates of sample or calibrator. Also, numerous field evaluations have highlighted performance shortcomings (Peace 2000; The Walsh Group 2002). Even so, lateral flow is an accepted method used by those performing on-site testing of drugs of abuse. It is expected that, as commercial use continues to grow, new instrument-interpreted methods will improve the performance of lateral flow assays (Alverix Inc. 2010; Faulstich *et al.* 2007).

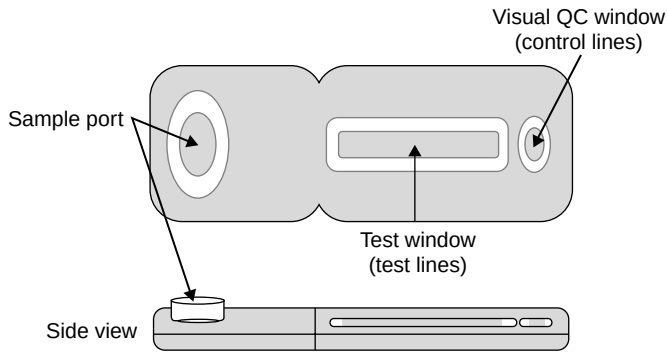

Figure 31.7 Lateral flow assay in a housing.

Homogeneous immunoassay/examples

Homogeneous immunoassays, by definition, do not require a step to separate bound from free target analytes and reagents. In most cases, homogeneous assays are used on automated platforms that require little operator interaction. This is something of a disadvantage since such equipment is not easily portable. Thus, homogeneous assays are used routinely on large laboratory-based instrument platforms capable of analysing hundreds or thousands of samples per day. The availability of these assays at a very low cost has revolutionised immunoassay-based drugs of abuse testing.

Homogeneous drug assays are often competitive assays where free drug in a calibrator or sample competes with a reporter conjugate to bind to a specific antibody on the target. After a brief incubation, the change in signal is measured spectrophotometrically, fluorescently or through chemiluminescence. The following are descriptions for some homogeneous assay methods available for drugs of abuse testing.

The EMIT (enzyme-multiplied immunoassay technique) has been a staple in drug testing since the 1970s when the method was first developed (Kabakoff, Greenwood 1981; Rubenstein *et al.* 1972; Ullman 1994; Ullman, Maggio 1980). An EMIT assay is a competitive format wherein hapten-labelled glucose-6-phosphate dehydrogenase (G6PDH) competes with free drug in a sample or calibrator to bind to a specific antibody raised against the hapten target. If the hapten-G6PDH binds to the antibody, there is a conformational change in the enzyme that leads to a decrease in activity (Fig. 31.8). This change in activity is proportional to the concentration of free drug present in the sample.

EMIT assays have been developed for a large number of drugs of abuse and TDM analytes. Over time a wide variety of instrumentation has also been made available to laboratory workers, allowing EMIT to quickly become a method of choice. However, it is worth noting that, as with all immunoassays, EMIT assay performance is dependent upon the antibody used. In addition, EMIT assays are subject to interference from sample adulteration and non-specific cross-reactivity (Colbert 1994; Rollins *et al.* 1990; Rossi *et al.* 2006).

Somewhat comparable in operating principles, CEDIA (cloned enzyme donor immunoassay) relies on the modulated activity of mutant beta-galactosidase. (Henderson *et al.* 1986) developed a genetically engineered form of beta-galactosidase which exists as two components. One fragment is called the enzyme acceptor (EA) and the other is called the enzyme donor (ED). The mutant enzyme becomes enzymatically active when both the ED and the EA are present. A homogeneous assay can be developed by conjugating a hapten to the ED. The hapten-ED will compete with free drug in the presence of antibody against the hapten (Fig. 31.9). When high concentrations of target hapten-drug are present, the hapten-ED is available to bind to the EA and an increase in beta-galactosidase will be detected. Thus, like EMIT, the amount of enzymatic activity is proportional to the concentration of free drug in the sample. Additionally, CEDIA assays can be run on the same automated spectrophotometric equipment that also runs EMIT assays.

Other types of homogeneous assays exist and are based upon reporters that are non-colorimetric. In this category, fluorescence

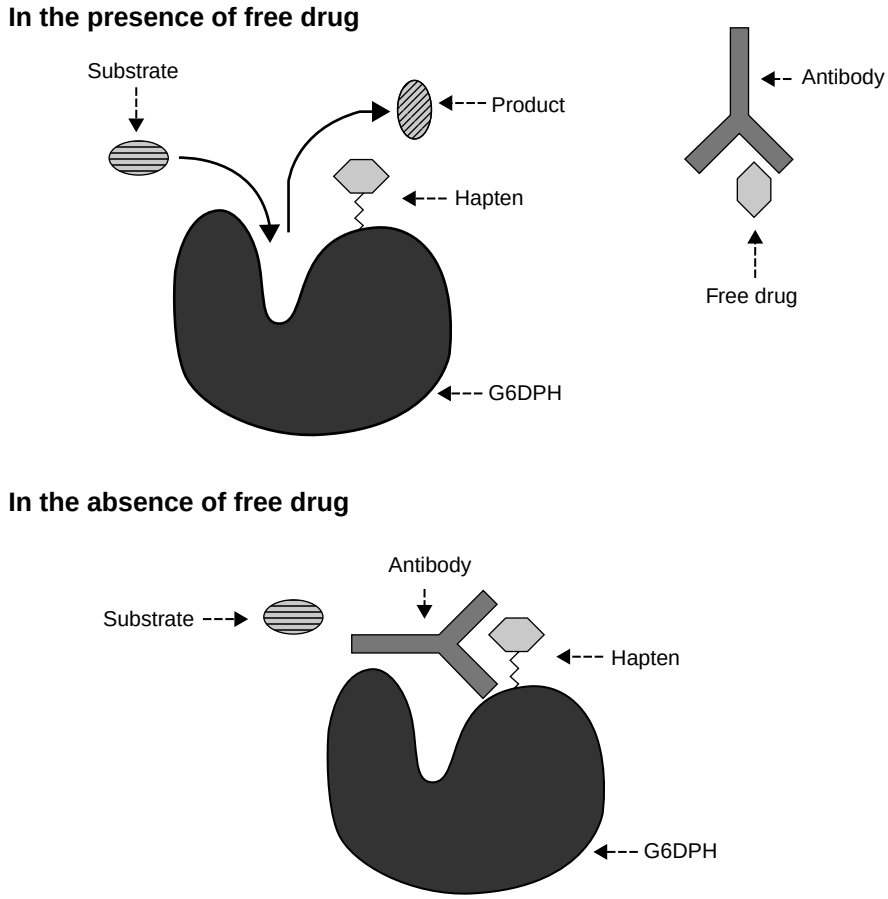

Figure 31.8 EMIT assay scheme; signal is directly proportional to the concentration of analyte.

polarisation, microparticle agglutination and new techniques such as LOCI (luminescent oxygen channelling immunoassays) (Ullman 2005) have all been demonstrated and used routinely in commercial laboratories.

Fluorescence polarisation is a relatively simple technique that utilises the competitive binding to antibodies between a hapten–fluoroscein conjugate and free drug (hapten; Fig. 31.10) (Abbott Laboratories 2005; Colbert *et al.* 1985; Dandliker *et al.* 1973). The hapten–fluorescein conjugate rotates rapidly when not bound to an antibody. When it is bound to the antibody, the rotation is slowed dramatically compared with the unbound molecule. To generate the assay signal, a fluorimeter shines light at the excitation wavelength for fluorescein through a vertical polarising filter. Rapidly rotating unbound hapten–fluorescein molecules emit light in a different plane to the incident light. The

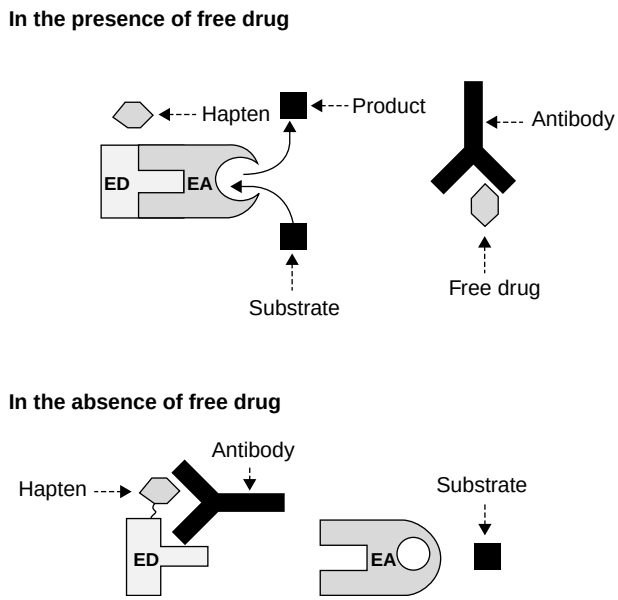

Figure 31.9 CEDIA assay scheme; signal is directly proportional to the concentration of analyte.

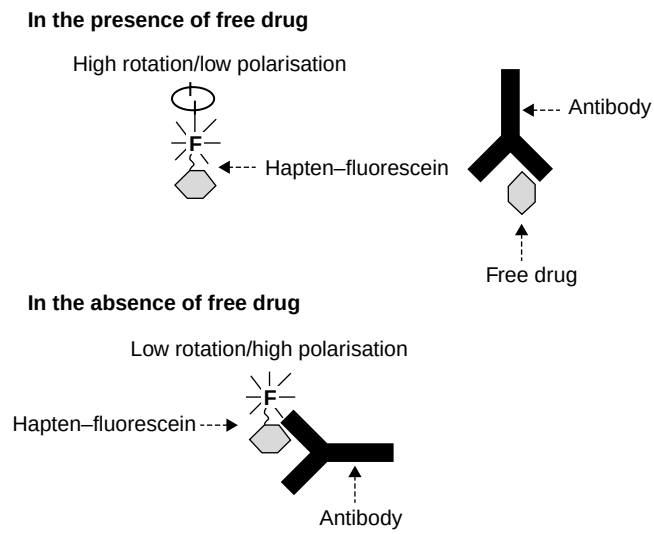

Figure 31.10 Fluorescence polarisation immunoassay assay scheme; signal is inversely proportional to the concentration of analyte.

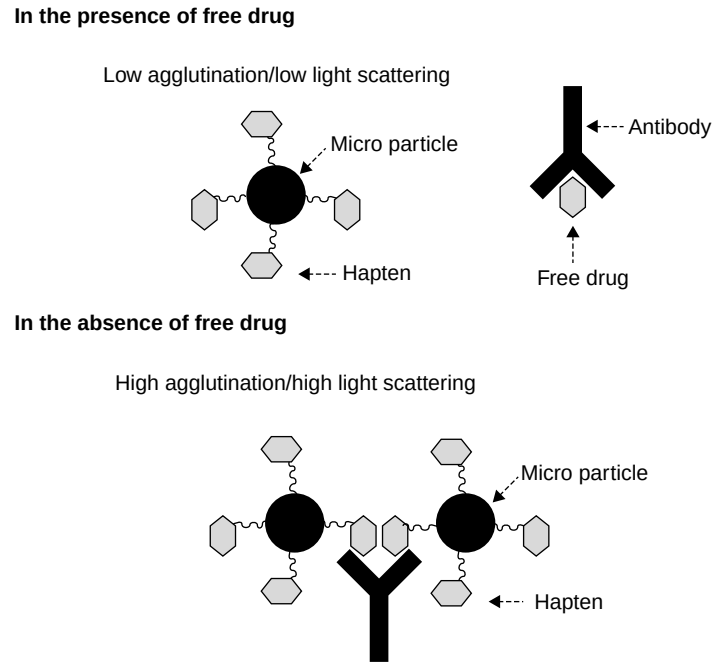

Figure 31.11 KIMS assay scheme signal is inversely proportional to the concentration of analyte.

relatively stationary antibody-bound hapten–fluorescein molecules, however, return light in a similar plane. This is detected via the polarising filter. Drug added via the sample competes for binding to the antibody with the fluorescein-labelled hapten, thereby reducing the amount of fluorescein bound to the antibody, resulting in less emitted fluorescence being detected via the polarised filter. Fluorescence polarisation assays require specialised equipment and are the basis of the Abbott ADx system. The background fluorescence found with many biological samples means that it is necessary to take a blank reading of the sample and reagents before the addition of the fluorescent tracer to the mixture.

Another popular homogeneous technique used for drugs of abuse assays is based upon microparticle agglutination. The KIMS assay (kinetic interaction of microparticles in solution) relies on the old but reliable principles of latex particle agglutination (Fig. 31.11) (De Giovanni, Fucci 2006; Feldman *et al.* 2004). In the KIMS technology, polystyrene latex microparticles have been coated with a drug-conjugate. These particles are dispersed in solution, after which sample and antibodies to the drug (hapten)-conjugate are added. In the presence of high drug concentrations, the antibody-binding sites are occupied by free drug and a low amount of agglutination takes place. In the presence of low target drug concentrations, the antibodies bridge between the particles and begin to agglutinate particles into large clusters. These clusters scatter light. Therefore, the change in signal is inversely proportional to the concentration of free target drug in the sample. KIMS is the basis of the Roche Abuscreen Online system, which involves monitoring the rate of agglutination by spectrophotometric means.

The challenge to any homogeneous assay technique is sensitivity. These assays usually take seconds to minutes to perform and are often limited by kinetics or antibody quality. LOCI is a chemiluminescent system that overcomes many limitations of other assay reporters (Ullman 2005). The reagents used to produce a signal in the assay are encapsulated in latex spheres which prevent interference from sample matrices. One population of latex spheres contains a chemiluminescer and the second a photosensitiser capable of exciting oxygen when exposed to 680 nm light. When the two beads are in close proximity, excited oxygen diffuses out of the bead and into the chemiluminescer bead, producing detectable photons. The LOCI assay has been tested with a variety of analytes and each one has shown superior sensitivity to other competing technologies (Ullman 2005).

Immunoassay optimisation

Earlier in this chapter the methods used to develop an antibody against a chosen hapten drug target were reviewed. Assuming that a viable candidate antibody has been generated and an assay format chosen, the next step is to begin developing and optimising the immunoassay. The goal of this section is to outline some of the key experiments followed by an explanation of important assay parameters that should be considered.

Again the target analyte to be used as an example in this section will be morphine. The structure for morphine is shown in Fig. 31.1. Morphine is one of a large class of opiate compounds that are often abused. Therefore, the goal of this hypothetical assay is to detect a broad class of compounds in a single sample. The format for the assay to be developed is lateral flow and the sample matrix will be saliva. Studies of the metabolism of opiates have shown that both the parent drug and its major conjugated metabolites appear in oral fluids and these have previously been shown to be detectable using a laboratory-based immunoassay (Niedbala, Kardos 2005). This suggests that a lateral flow assay is plausible.

To start developing the lateral flow assay, morphine is derivatised through the hydroxyl group before being conjugated to BSA (Verheijen 2002; Volkov *et al.* 2009).

Using a Kinematic Linomat striper, the morphine–BSA conjugate can be adsorbed onto the surface of a 10 µm pore size nitrocellulose sheet by sequentially dispensing small amounts of conjugate to form a capture zone (Verheijen 2002; Volkov *et al.* 2009). Additionally, a reporter such as colloidal gold, fluorescent latex particles or upconverting phosphors can be labelled with purified anti-morphine as described elsewhere (Niedbala *et al.* 2000, 2001). Finally, phosphate-buffered saline, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes), or an alternative buffer may be chosen as long as it adequately controls the pH of the final assay mixture without negatively affecting the assay.

Once all the reagents are prepared and a standard curve showing a response over various concentrations of morphine has been run, experiments can be conducted to optimise the concentration of each assay component. Usually the components that are varied are the loading of reagents onto the capture zone, the level of reporter conjugate to control detectable signal, and finally the sample volume to obtain the best precision and optimal dynamic range.

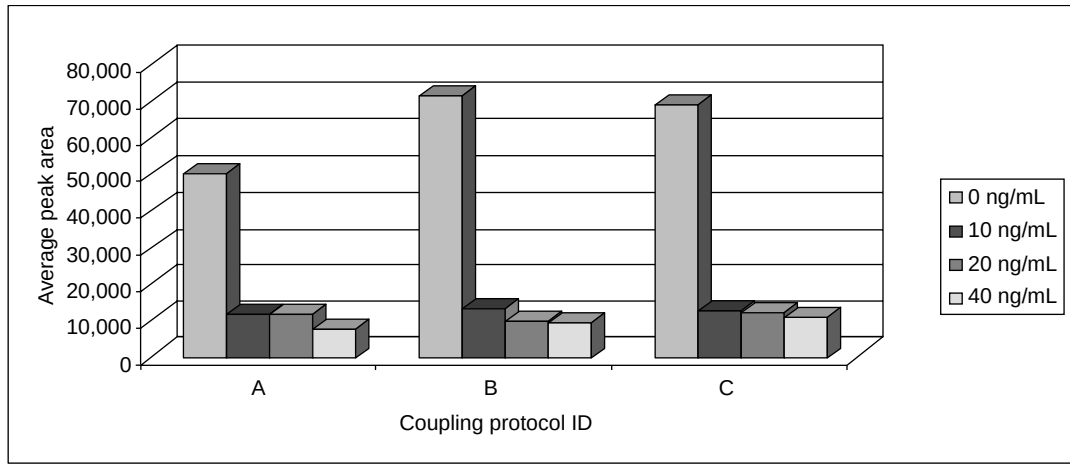

Figure 31.12 This graph shows the results from three experiments loading various amounts of morphine-BSA onto a lateral flow strip. The optimum level was reached when the signal at zero concentration of morphine was highest.

For example, Fig. 31.12 shows the effect of three different line striping protocols for the capture zone on the nitrocellulose strip. As the loading of morphine-BSA increased there was a maximum amount of signal that could be generated. Note, however, that the overall displacement between zero and the various concentration of free drug remained about the same.

Next, the concentration of reporter conjugate may be optimised. Figure 31.13 shows an example of a lateral flow assay utilising up-converting phosphor conjugates as the reporter. Note that, as the level of reporter increased, so did the signal at 0 ng/mL free morphine. This large separation helps to ensure discrimination between negative and positive samples.

Finally, the assay sample size was adjusted and the assay was tested for precision by running replicates at a number of morphine levels. As shown in Fig. 31.14, the assay developed was capable of easily discriminating between 0 and 40 ng/mL morphine, the target concentrations appropriate for an oral fluid-based test.

Immunoassay performance parameters

Having discussed the major aspects of test reagents and development, it is appropriate now to turn to parameters used to evaluate immunoassay performance. It is important to note that there are a number of ways to determine each of the parameters considered. Those discussed here are relevant to scientists developing or evaluating commercial immunoassays for toxicological investigations (Green, Isenchmid 1995; Kwong *et al.* 1988; Linnet, Brandt 1986).

Parameter 1: Precision

Precision is a measure of the variation that occurs either when replicate samples are tested in a single run (intra-assay precision) or when replicate samples are tested and compared between runs (inter-assay precision). Whether intra- or inter-assay precision, the value is always reported as the RSD. It is important to plot the RSDs of any test and look for the deviation around each point tested. In the author's laboratory it is usual to plot the mean plus 2 standard deviations above and below each assay point on the curve. If it overlaps the standard deviation from the calibrator above, optimisation of the assay is continued to improve performance. It is important that precision data should be examined as either the precision of the signal generated in an assay or transformed numbers on a standard curve. Most toxicology assays are qualitative and therefore use signal only. However, transformed numbers are more stringent and are an excellent way to show robust assay performance.

Parameter 2: Limit of detection

Many toxicology screening assays require cut-off levels that challenge the development of any immunoassay. Analytes such as LSD, buprenorphine or tetrahydrocannabinol (THC) must be detected at extremely low concentrations in oral fluids. The assay parameter that indicates the lowest level detectable is the limit of detection (LOD). The LOD for an

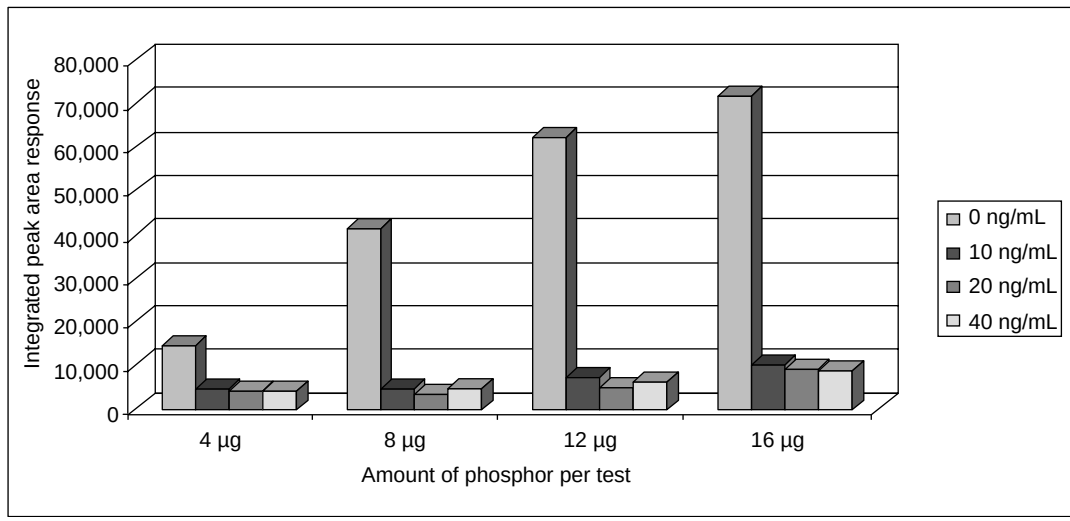

Figure 31.13 This graph shows results of varying the conjugate reporter in the lateral flow assay being designed to detect morphine. Signal increased as more conjugate was added. This produced a steeper curve and improved the performance of the assay.

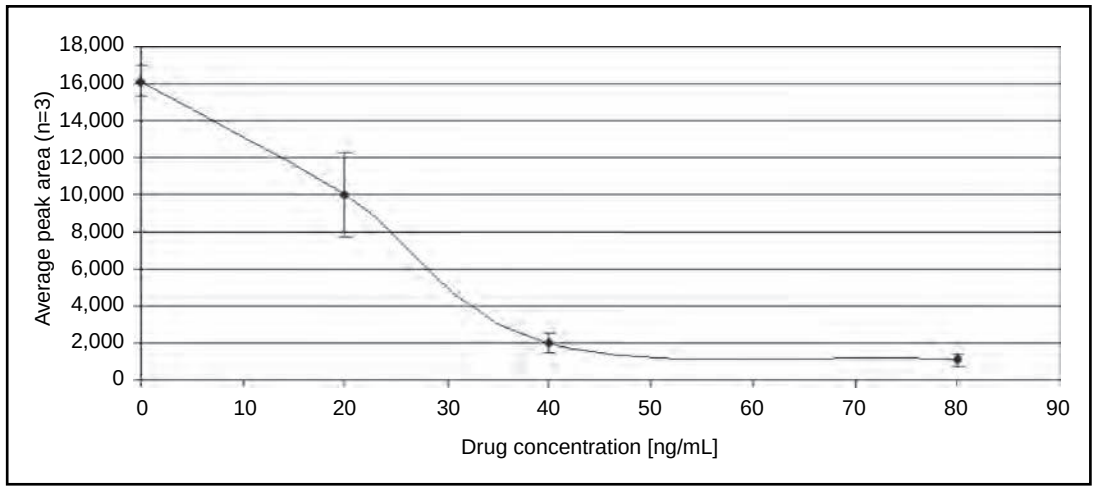

Figure 31.14 Once optimised, the morphine lateral flow assay was tested for precision. This graph shows that the optimised assay had little variation around each calibrator concentration. There was also a large separation in signal between calibrators.

immunoassay is often determined in one of two ways. The first approach is to run replicates of the zero calibrator in an assay and then take the mean plus 2 or 3 standard deviations and plot it onto the assay standard curve. This concentration is then assigned as the LOD. The second method, which is more robust, is to run replicates of a variety of concentrations along the assay curve and, similarly to determining precision, plot 2 standard deviations above and below each point. The lowest concentration that can separate 2 standard deviations above zero and 2 standard deviations below the replicate concentration is then denoted the LOD.

Parameter 3: Specificity/cross-reactivity

This parameter is extremely important for toxicology assays. As stated earlier, some assays are targeted for certain compounds, such as THC, while others will ideally detect a broad class of compounds, such as benzodiazepines. In addition, assays should also be free from interference from more common over-the-counter medications. Using the example of a morphine assay, Table 31.3 lists a number of opiates of interest along with their cross-reactivities in an immunoassay. In addition to cross-reactivity, the table also shows calculations for morphine equivalents and the concentration that may produce a positive result. Each of these values for cross-reactivity, equivalents and concentration that may produce a positive result is derived by running various concentrations of potential cross-reactants in the assay of interest and comparing the results against the cut-off calibrator, which, in this case, is morphine. Each of the parameters can be determined by comparing concentrations against the cut-off.

Parameter 4: Interferences/adulteration

Even if an immunoassay is performed correctly, it can provide an incorrect result if the sample was somehow adulterated or contained an interfering substance. The literature or product package inserts for assays that use blood or urine will frequently warn that haemolysis, lipaemia or high levels of protein in a sample can interfere with homogeneous immunoassays. More recently, orally based diagnostic tests have looked at the food or drinks that can interfere with assays, while hair testing has considered the effects of various treatments, soaps or hair colorants. In all of these cases the assay designers must be prepared to realistically evaluate such interferences. At a minimum, immunoassay developers should document and inform users of their assays about potential interferents.

Adulterants are related to, but different from, interfering compounds. Interferents are defined as normal day-to-day materials used by individuals that can cause aberrant results. Adulterants are materials wilfully added to a sample in order to disrupt a toxicology immunoassay. Such materials have been used in recent years to target many of the homogeneous assays used to initially screen urine samples for drugs of abuse (Wu *et al.* 1999). Materials such as bleach (sodium hypochlorite), salts or detergents have been reported to be very effective and their potential for disruption should be evaluated in any newly developed assay.

Parameter 5: Stability

Any immunoassay is expected to perform over a relatively long period. Commercial immunoassay kits are usually stable from 6 months to

Table 31.3 Morphine immunoassay cross-reactants

Compound	*Concentration (ng/mL)*	*Morphine equivalents (ng/mL)*	*Percentage cross-reactivity (%CV)*	*Concentration that may produce a positive result (ng/mL)*
6-Acetyl morphine	40	34.8	87.0	46
Codeine	40	24.1	60.3	66
Diacetylmorphine	100	>73.7	>73.7	54
Hydrocodone	100	60.5	60.5	66
Hydromorphone	100	>55.9	55.9	72
Meperidine	10 000	68.8	0.7	5,714
β-Morphine-3-glucuronide	100	80.0	80.0	50
Nalorphine	1	36.8	>100	1
Normorphine	100	34.3	34.3	117
Oxycodone	100	43.1	43.1	93
Oxymorphone	100	36.3	36.3	110

several years. Laboratory-based tests are somewhat easier to maintain and control, since cold-room storage is not a problem. Point-of-care tests that are used in remote locations without room temperature control or perhaps no facilities at all face some of the greatest challenges to stability. In these cases the stability of a test must always be related to the functional temperature range that can be tolerated. Any immunoassay developer should field test their immunoassay under various conditions of humidity, temperature and even altitude. Limitations can then be reported to potential users of the test. Such studies are difficult and often highlight the limitations of an immunoassay. However, they are essential so that the incidence of false results is minimised in the field or in the laboratory.

Parameter 6: Quality control

There are various ways to approach quality control for any immunoassay. Some laboratory methods look at the precision of controls from one run to another. Other immunoassays use the standard curve generated to see whether the spread between calibration points is appropriate. These internal assay controls are all good measures that ensure that the final result is correct.

Additional quality control steps should include external controls. Some vendors sell urine, blood or saliva samples that have been spiked at various concentrations.

Proficiency testing programmes are a very useful means by which an individual laboratory can ensure that its assays are performing correctly and for detecting any problems with a particular method. These schemes regularly send samples to a large number of participant laboratories to be tested on a blind basis. A number of organisations exist that can supply urine, saliva, hair or sweat proficiency samples. Examples include RTI International, the College of American Pathologists or Cardiff Bioanalytical Services Ltd. Once tested, the results for each proficiency sample are returned to the co-coordinators of the scheme and each participant laboratory receives a report showing how its findings have compared with those of the others (Cone 1992).

Immunoassay automation

Great strides have been made over the last few decades in the automation of immunoassays. The use of homogeneous assays such as EMIT, CEDIA or KIMS for the rapid analysis of large numbers of samples for drugs of abuse has been described above. In addition, specialised instrumentation has been developed by commercial companies to utilise reporters such as fluorescence, radiolabels and chemiluminesence. In every case these immunoassay platform instruments allow for precise pipetting and incubation of samples. This degree of control is essential in assuring consistent and reliable results. It is interesting to see the overall sensitivity of each system (see Table 31.2). Future systems will be required not only to perform rapid analyses on large numbers of specimens but also to test different specimen types. This is an enormous challenge for immunoassay design and matching instrumentation. Future systems may be as much as 1000 times more sensitive than current techniques.

Alternative fluids for toxicology immunoassays

The latest area of drug immunoassay innovation has been concerned primarily with applications to alternative matrices. Traditionally, toxicological assays for drugs of abuse have been developed for use with urine, whereas TDM assays almost always utilise blood, serum or plasma. Urine testing remains the dominant matrix for drugs-of-abuse screening, although it is now recognised that this sample can be easily adulterated, requires private collection facilities, and is affected by overhydration (Cone *et al.* 2003). In recent years, more assays are being performed using fluids or matrices such as hair, sweat and oral fluid. Although considered several years ago, it is only recently that interest in using alternative fluids for routine screening of drugs of abuse has arisen (Cone 1992; Malamud, Niedbala 2007; Samyn *et al.* 1999a; Schramm *et al.* 1992; Wong 2008) There are several reasons behind this trend and they vary for different countries. For some there is a desire to allow law enforcement to screen for drugs of abuse at the roadside (Parliament of Victoria, Australia 2003). This is not practical with any specimen except an oral sample (Verstraete *et al.* 1999c). In other situations, the drug concentration in the sample collected must have some correlation with that in blood so that some idea of impairment can be demonstrated. Again, this has led to the increased use of saliva testing (Cone *et al.* 2002; Thompson *et al.* 1987; Toennes *et al.* 2005) (Chapter 18). Where the goal is to have the longest window of detection to identify drug abuse, hair is the specimen of choice (Chapter 19).

Conclusions

Immunoassays for the detection or measurement of drugs and their metabolites are routinely used in commercial laboratories worldwide. Automated immunoassay instrumentation capable of analysing thousands of samples per hour has now become routine. Although there are ways in which immunoassays can be flawed, the great majority perform reliably when used correctly. Future directions in immunoassay development are expected to improve upon methods to develop antibodies and format assays. However, these improvements will still utilise the basic principles outlined in this chapter.

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CHAPTER

32 Ultraviolet, Visible and Fluorescence Spectrophotometry

J Cordonnier and J Schaep

Introduction and theoretical background

General introduction

Analytical absorption spectroscopy in the ultraviolet (UV) and visible regions of the electromagnetic spectrum has been widely used in pharmaceutical and biomedical analysis for quantitative purposes and, with certain limitations, for the characterisation of drugs, impurities, metabolites and related substances. By contrast, luminescence methods, and fluorescence spectroscopy in particular, have been less widely exploited, despite the undoubted advantages of greater specificity and sensitivity commonly observed for fluorescent species. However, the wider availability of spectrofluorimeters able to present corrected excitation and emission spectra, coupled with the fact that reliable fluorogenic reactions permit non-fluorescent species to be examined fluorimetrically, has led to a renaissance of interest in fluorimetric methods in biomedical analysis.

UV and visible spectrophotometry: theoretical background

General considerations

Molecular absorption in the UV and visible regions arises from energy transitions that involve the outer orbital or valence electrons. Spectra in liquid media are usually broad, relatively featureless bands, a result of the large number of closely spaced vibrational and rotational transitions. The fundamental band shape approximates Gaussian or log-normal Gaussian curves. Given the broad, overlapping profiles commonly encountered, the shape and precise location of individual bands are of limited usefulness in qualitative analysis. However, any fine structure detected in the spectra, coupled with solvent and pH effects, can be of diagnostic value. More informative spectra can be obtained for some volatile molecules of toxicological interest, such as benzene and polynuclear aromatic hydrocarbons; when examined in the vapour phase, vibrational and rotational fine structure can readily be seen superimposed on the broad spectral profiles. This is illustrated in Fig. 32.1 for 1,2,4,5-tetrazine. However, most drugs, metabolites and related compounds are relatively non-volatile; their spectra are observed necessarily in solution, or possibly in the solid phase by reflectance, or by compression to form a KBr disc, as used in infrared spectrophotometry.

UV and visible spectrophotometry find their primary application in quantitative analysis. The scope of absorption spectroscopy can be extended significantly by the use of colour reactions, often with a concomitant increase in sensitivity and/or selectivity. Such reactions are used to modify the spectrum of an absorbing molecule so that it can be detected in the visible region, well separated from other interfering components in the UV spectrum. Moreover, chemical modification can be used to transform an otherwise non-absorbing molecule into a stable derivative that possesses significant absorption.

Spectral selectivity can be enhanced further by a number of chemical or instrumental techniques, which include difference, higher-derivative and dual-wavelength spectrophotometry. Such methods, and certain graphic techniques such as the Morton–Stubbs method, can contribute in different ways to reducing the general problem of spectral interference in quantitative spectroscopy. Spectral interference can arise from so-called 'irrelevant' non-specific absorption, and also from absorption by other materials and impurities that may be present. When interference arises specifically from the spectral overlap of two or more well-defined components, a number of methods can be applied to measure the individual concentrations. These methods include the Vierordt multi-wavelength technique, least-squares deconvolution and second- or higher-derivative spectrophotometry.

Spectral selectivity, and in some cases detection sensitivity, can be enhanced significantly by the various chemical and instrumental techniques outlined above. Such methods should, of course, be validated by applying the conventional analytical criteria of accuracy (against a reference method), linearity, precision and independence from interfering substances.

The scope of UV and visible spectrophotometry can be further extended when combined with a chromatographic separation step, such as high performance liquid chromatography (HPLC). The development of rapid-scanning detectors based on the linear photodiode array permits spectra to be acquired during the elution of peaks. Computer-aided manipulation of these spectra has led to new strategies for the examination of chromatographic peak homogeneity, based on classic techniques in spectroscopy. The use of microcomputers enables the development of archive-retrieval methods for spectral characterisation (Fell *et al.* 1984).

Nomenclature

In the UV and visible spectrum, the energy of photons associated with electronic transitions lies in the range 147–630 kJ/mol. This energy (ΔE) can be expressed in terms of the principal parameters that define electromagnetic radiation, namely frequency μ (Hz), wavelength λ (nm) and wavenumber $\overline{\nu}$ (cm^{-1}):

$$\Delta E = h\mu = \frac{hc}{\lambda} = hc\overline{\nu} \tag{32.1}$$

where h is Planck's constant and c is the velocity of radiation *in vacuo*.

The positions of peaks are sometimes described in terms of wavenumber, which has the advantage of being a linear function of energy, but this term is much more frequently used in infrared spectrophotometry. The practical unit most often used in UV and visible spectrophotometry is wavelength, usually expressed in nanometres (nm). The units that have previously been used for wavelength – millimicron (mμ) and ångström (Å) – are not recommended terms. The position of maximum absorbance of a peak is designated λ_{max}.

The wavelength span is conventionally divided into two ranges: the UV extends from 200 nm to about 400 nm; the visible range extends from about 400 nm to 800 nm. Outside these limits, the 'far UV' or 'vacuum UV' extends from 100 nm to 200 nm, and the 'near infrared' from 1 μm to about 3 μm.

A molecular grouping specifically responsible for absorption is described as a chromophore, and is usually a conjugated system with extensive delocalisation of electron density. Any saturated group with little or no intrinsic absorption of its own, but that modifies the absorption spectrum when attached directly to a chromophore, is described as an auxochrome, examples being –OR, $–NR_2$, –SR. Auxochromes are considered to exert their effect through partial conjugation of their polarisable lone-pair electrons with those of the adjacent chromophore. If, however, the lone pair of electrons is involved in bonding as, for example, in the case of a protonated quaternary ammonium group, the

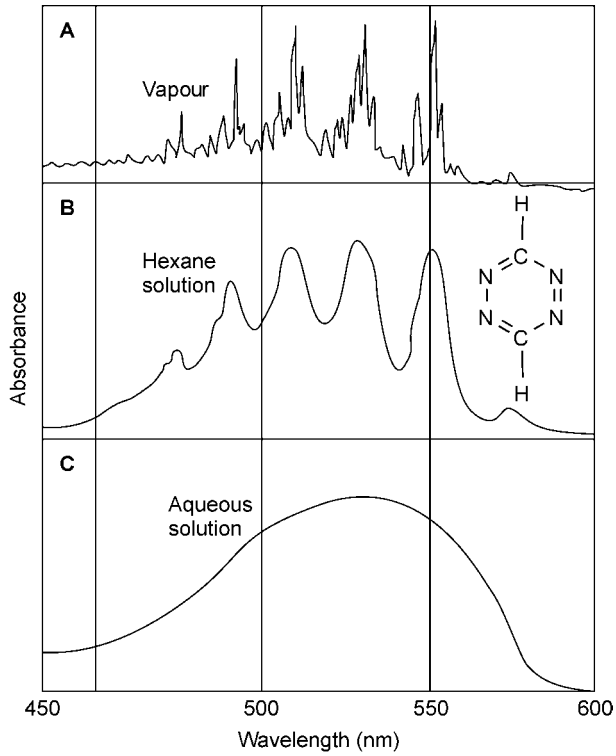

Figure 32.1 UV absorption spectra of 1,2,4,5-tetrazine.

auxochromic effect vanishes. This property can be used for molecular characterisation, as discussed below.

Laws of absorption spectrophotometry

The extent of absorption of radiation by an absorbing system at a given monochromatic wavelength is described by the two classic laws of absorptiometry, which relate the intensity of radiation incident on the absorbing system (I_0) to the transmitted intensity (I) (Fig. 32.2). Lambert's (or Bouguer's) law concerns instrumental factors, and states that, at a given concentration (c) of a homogeneous absorbing system, the transmitted intensity (I) decreases exponentially with increase in path length (b). The complementary Beer's law deals with concentration and states that, for a layer of defined path length (b), the transmitted intensity (I) decreases exponentially with the increase in concentration (c) of a homogeneous absorbing system. Combination of these observations gives the familiar Beer–Lambert law:

$$\log \frac{I_0}{I} = cb \tag{32.2}$$

where ε is the molar absorptivity or molar extinction coefficient of the system, defined as 'the absorbance of a one molar solution in a cell of 1 cm path length'. The concentration c is here expressed in mol/L.

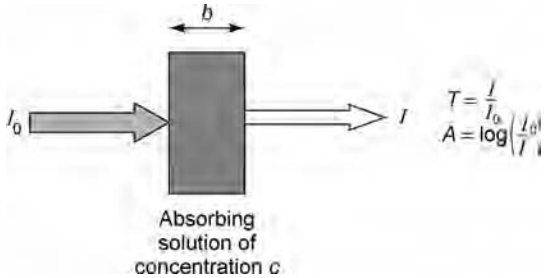

Figure 32.2 Attenuation of a beam of radiation by an absorbing solution.

The logarithmic term is linearly related to concentration and path length, and is referred to as absorbance (A). The older terms extinction (E) and optical density (OD) are not recommended, although they are often found in the literature. Transmittance ($T = I/I_0$) and percentage transmittance ($\%T = 100(I/)_0$) are not linear functions of concentration and path length, and can be related readily to absorbance:

$$A = \log \frac{I_o}{I} = \log \frac{1}{T} = 2 - \log(\%T) \tag{32.3}$$

The molar absorptivity, ε, is a fundamental property of a molecule that tells how much light is absorbed at a particular wavelength. It has two connotations in European usage, and a third according to American convention. If the concentration is expressed in g/L, ε is described as the absorptivity (k, L/g per cm).

When concentration is expressed in g/100 mL, k is described as the specific absorbance and given the symbol $A_{1\ \mathrm{cm}}^{1\%}$ or A(1%, 1 cm), defined as 'the absorbance of a 1% w/v solution in a cell of 1 cm path length'. It is usually written in the shortened form A_1^1 and is widely used in analytical chemistry. It was formerly known as the 'specific extinction coefficient', symbol $E_{1\ \mathrm{cm}}^{1\%}$ or E(1%, 1 cm).

American convention recognises the constant k as 'absorptivity' (a, L/g/cm) defined as 'the absorbance of a 1 g/L solution in a cell of 1 cm path length'. These terms for absorptivity can readily be interconverted:

$$a = \frac{A_1^1}{10} = \frac{\varepsilon}{M_r} \tag{32.4}$$

where M_r is the relative molecular mass. Thus, a compound with an M_r of 100 and absorptivity a of 20 at wavelength λ in a particular solvent at a defined pH (if aqueous) and at a specified temperature, has a corresponding specific absorbance A_1^1 of 200 and a molar absorptivity ε of 2000.

Absorbance and absorptivity are often expressed in logarithmic form in cases where spectra are to be compared. The logarithmic form of the Beer–Lambert law expresses the effects of the molar extinction coefficient (ε), concentration (c) and path length (b) as additive terms

$$\log A = \log\varepsilon + \log c + \log b \tag{32.5}$$

The value for $\log\varepsilon$ is typically in the range 1–5. Since only the molar extinction coefficient (ε) is a function of λ, the shape of a logarithmic absorption curve is independent of concentration and path length. Their only effect is to shift the $\log A$ spectrum along the $\log A$ axis. A disadvantage of the $\log A$ plot is that fine structure near the top of the peak is compressed.

Validity of the Beer–Lambert law

The validity of the Beer–Lambert law is affected by a number of factors. If the radiation is non-monochromatic, i.e. if its spectral bandwidth is greater than about 10% of the drug absorption bandwidth at half-height, the observed absorbance will be lower than the 'true' limiting value for monochromatic radiation. Thus, sharp bands are more susceptible than broad bands to absorbance error on this account. Moreover, if the absorbing species is non-homogeneous, or if it undergoes association, dissociation, photodegradation, solvation, complexation or adsorption, or if it emits fluorescence, then positive or negative deviations from the Beer–Lambert law may be observed. Stray-light effects and the type of solvent used may also lead to non-compliance with the Beer–Lambert law.

Stray-light effects

Stray light is radiation at wavelengths different from those desired. It may arise from light scattering or other defects within the instrument, or it may be caused by external radiation. If the stray light is not absorbed, the observed absorbance tends to a constant value as the concentration of drug is increased, yielding a negative deviation from the Beer–Lambert law.

Stray-light errors are more likely to be observed near the wavelength limits of an instrument, at which the radiation intensity of the source and the efficiency of the optical system are reduced, especially below 220 nm and at the crossover point between the UV and the visible lamps (about 320–400 nm). Errors may become serious if the solvent absorbs strongly or if a strongly absorbing sample is measured by difference spectrophotometry.

Solvent effects

The solvent often exerts a profound influence on the quality and shape of the spectrum. For example, many aromatic chromophores display vibrational fine structure in non-polar solvents, whereas in more polar solvents this fine structure is absent because of solute–solvent interaction effects (see also Fig. 32.1). A classic case is phenol and related compounds, which have different spectra in cyclohexane and in neutral aqueous solution. In aqueous solutions, the pH exerts a profound effect on ionisable chromophores because of the differing extent of conjugation in the ionised and the non-ionised chromophore.

The quality of spectral measurement is affected directly by the type and purity of the solvent used. Each solvent has a cut-off wavelength (which corresponds to about 10% transmittance) and this varies with solvent purity (Table 32.1). A solvent should not be used below its cut-off wavelength, even though reference-cell compensation is employed, because of the greater risk of stray-light effects. The UV spectra of some solvents are illustrated in Fig. 32.3.

Some cautionary comments may be appropriate at this point. It is better to use single- or double-distilled water, and to avoid deionised water, which can be contaminated with absorptive fragments of ion-exchange resin or contain bacterial metabolites; these can contribute significantly to non-specific absorption at low wavelengths. Ethanol is normally used as the 96% v/v strength, since dehydrated alcohol is usually contaminated with traces of benzene added to form the azeotropic mixture for distillation. Acetonitrile can vary noticeably in quality, depending on the supplier; the grade supplied for use in HPLC is usually to be recommended. Acetone, sometimes used to clean cells, is highly absorptive and not always easily removed, despite its volatility and aqueous solubility. Chloroform and carbon tetrachloride absorb strongly at about 250 nm and should therefore be used only for measurements at wavelengths above about 280 nm. Given the safety considerations of chlorinated solvents, use of these is best avoided if possible. Ether, although transparent down to 220 nm, presents particular problems because of its volatility (unstable standard solutions) and inflammability. Although absorptivity is considered to be relatively insensitive to temperature changes, organic solvents in general suffer from high temperature coefficients of expansion, so that for ultimate precision a cell provided with a thermostat may be required.

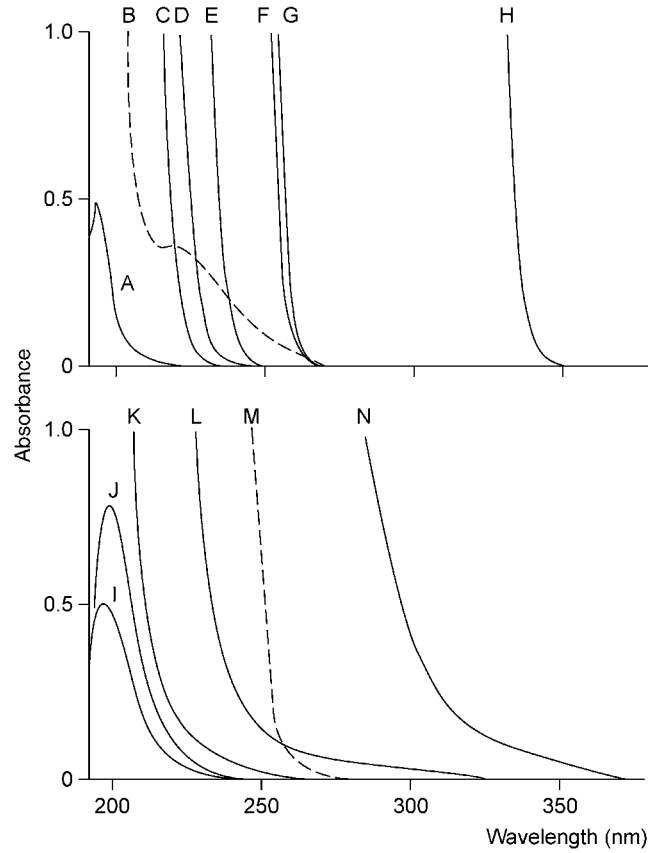

Figure 32.3 UV absorption of solvents (HPLC grade unless otherwise stated): A, acetonitrile (far-UV grade); B, methyl *t*-butyl ether; C, acetonitrile; D, 1-chlorobutane; E, methylene chloride; F, acetic acid (AR grade); G, ethyl acetate; H, acetone; I, hexane; J, iso-octane; K, methanol; L, tetrahydrofuran; M, chloroform; N, diethylamine (AR grade).

Table 32.1 Cut-off points equivalent to 10% transmittance for spectroscopic solvents

Solvent	*Wavelength (nm)*
Water (distilled) or dilute inorganic acid	190
Acetonitrile (HPLC, far-UV grade)	200
Acetonitrile	210
Butyl alcohol	210
Cyclohexane	210
Ethanol (96% v/v)	210
Heptane	210
Hexane	210
Isopropyl alcohol	210
Methanol	210
Ether	220
Sodium hydroxide (0.2 mol/L)	225
Ethylene dichloride	230
Methylene chloride	235
Chloroform (stabilised with ethanol)	245
Carbon tetrachloride	265
N,*N*-Dimethylformamide	270
Benzene	280
Pyridine	305
Acetone	330

Fluorescence spectrophotometry: theoretical background

General considerations

Molecular fluorescence is an emission process in which molecules are excited by the absorption of electromagnetic radiation. The excited species then relax to the ground state, giving up their excess energy as photons.

There are several ways in which an excited molecule can give up its excess energy and relax to its ground state. Two of the most important of these mechanisms are non-radiative relaxation and fluorescent relaxation.

Non-radiative relaxation can occur through collisions between excited molecules and molecules of the solvent, giving excess energy to solvent molecules. When relaxation takes place by fluorescence, bands of radiation are produced as the excited molecules relax to several energy states, which are very close in energy level and thus in wavelength (Fig. 32.4). Fluorescence occurs only from the lowest vibrational level of an excited electronic state.

Note that molecular fluorescence bands are made up largely of lines that are longer in wavelength (lower in energy) than the band of absorbed radiation responsible for their excitation. This shift to

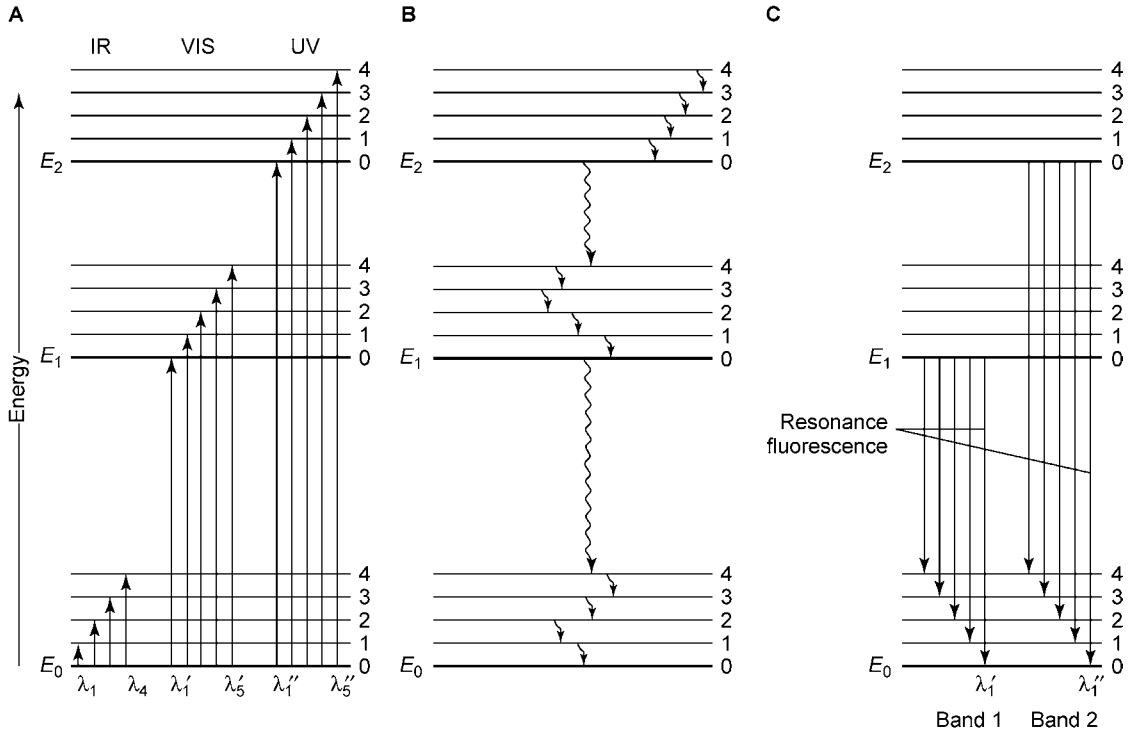

Figure 32.4 Energy-level diagram showing some of the energy changes that occur during (A) absorption, (B) non-radiative relaxation and (C) fluorescence by a molecular species.

longer wavelength is sometimes called the Stokes shift. For that reason, the absorption or excitation spectrum and the fluorescence spectrum for a compound often appear as approximate mirror images of each other. The most useful region for the fluorescence technique is 200–800 nm.

Fluorescence spectrophotometry is usually the method of choice for quantitative analytical purposes if applicable. It has assumed a major role in analysis, particularly the determination of trace contaminants in our environment, industries and bodies, because for applicable compounds fluorescence spectrometry gives high sensitivity and high specificity. The selectivity of fluorescence methods is greater than that of absorption methods, as fewer substances fluoresce than absorb radiation in the UV or visible region. Furthermore, fluorescence is more selective because both the emission and the absorption spectra can be obtained. Fluorescence is usually also more sensitive than absorption methods, as it is always easier to measure a small signal against a very small zero background than to measure a small difference between large signals. However, the phenomenon of fluorescence itself is subject to more rigorous constraints on molecular structure than is absorption.

Nomenclature

The term quantum efficiency used in fluorescence is quantified by the quantum yield (i.e. the ratio of the number of molecules that fluoresce to the total number of excited molecules). Highly fluorescent molecules can have quantum efficiencies that approach unity. Many drugs possess rather high quantum efficiencies for fluorescence, such as quinine and lysergic acid diethylamide (LSD).

All absorbing molecules have the potential to fluoresce. They do so if fluorescent emission occurs at a greater rate than relaxation by non-radiative pathways. The kind of relaxation process is highly dependent on the molecular structure. Compounds that contain aromatic rings give the most intense and most useful fluorescence emission. Substitution on an aromatic ring causes shifts in the excitation wavelength spectrum and in fluorescence efficiency. Substituents such as $-NH_2$, –OH, $-OCH_3$ and $-NHCH_3$ groups often enhance fluorescence, while –Cl, –Br, –I, $-NO_2$ or –COOH are electron-withdrawing groups that can lead to reduction or absence of fluorescence (e.g. aniline fluoresces whereas nitrobenzene does not). The molecular grouping responsible for fluorescence is sometimes described as a fluorophore.

Fluorescence is particularly favoured in rigid molecules, as molecular rigidity reduces deactivation by non-radiative processes (there being fewer internal vibrations). This is also why certain organic chelating agents are more fluorescent when complexed with a metal ion.

Laws of fluorescence spectrophotometry

The power of fluorescent radiation I_f is proportional to the radiant power of the excitation beam absorbed by the system:

$$I_f = K'(I_0 - I) \tag{32.6}$$

The constant K' depends upon the quantum efficiency of the fluorescence. Beer's law can be used to relate I_f to the molar concentration c of the fluorescing molecule:

$$\frac{I}{I_0} = 10^{-bc} \tag{32.7}$$

Substituting equation (32.7) into (32.6) we obtain:

$$I_f = K' I_0 \left(1 - 10^{-bc}\right) \tag{32.8}$$

After expansion of the exponential term, and provided that $\varepsilon bc < 0.05$, we can write:

$$I_f = 2.3K' bcI_0 \tag{32.9}$$

or at constant I_0, with the factor K composed of all constants:

$$I_f = Kc \tag{32.10}$$

Thus, a plot of the fluorescence power of a solution versus the concentration of the emitting species should be linear at low concentrations. Limiting factors for linearity are not only the concentration of the solute

but also factors such as the blank fluorescence, quenching and absorption of exciting radiation by the solvent.

Quenching and other special effects

When the fluorescence of a species is attenuated as a result of its reaction with an analyte, the signal decreases. This effect is called quenching and can be used for quantification purposes, primarily for the determination of anions. Quenching can also be an unwanted effect in the case of dissolved oxygen (see later).

Also, if the analyte is too concentrated, self-quenching may occur when fluorescing molecules collide and lose their excitation energy by radiationless transfer. The fluorescence versus concentration curve may have a maximum and then actually show a decrease in fluorescent power with increasing concentration. It is imperative in quantitative determinations to be aware of this problem, since a given fluorescent power can correspond to two values of concentration.

Instrumentation

General considerations

The basic components of analytical instruments for absorption and fluorescence spectroscopy are alike in function and general performance requirements. Most spectroscopic instruments are made up of five components:

- Stable source of radiant energy
- Wavelength selector that permits the isolation of a restricted wavelength region
- Sample container
- Radiation detector, which converts radiant energy to a measurable signal (usually electrical)
- Signal processor and readout.

Ultraviolet and visible spectrophotometry

Colorimeters

Colorimeters usually employ a single tungsten radiation source in combination with broad-band (~30 nm) optical filters of nominal wavelength, or narrow-bandwidth interference filters with a defined wavelength for use in the visible range. The range of linearity of the colorimeter may be constrained by the relatively broad spectral bandwidths employed, and therefore should be checked carefully for each type of assay.

Single-beam spectrophotometers

These differ from the colorimeter in using a prism or a high-quality diffraction grating monochromator, together with an additional intense source of UV radiation, usually a deuterium (or hydrogen) lamp. They are capable of high precision, particularly in the optimum absorbance range (0.3–0.6 absorbance units). The reference and sample cells must be moved manually in and out of the radiation beam at each wavelength, so it is not practicable to scan a spectrum using such a device.

Double-beam spectrophotometers

Double-beam spectrophotometers use similar high-quality optical components to those in the single-beam instrument. However, the radiation from the monochromator is split into two identical beams by a rotating mirror. One beam passes through the sample and the other through the reference cell, before being recombined to focus on the detector. Each signal is processed appropriately by the detector electronics to measure the absorbance 10–20 times per second, which gives full compensation for cell and solvent absorption. A scan motor drives the monochromator to give a constant wavelength change per second, which is synchronised with a recorder or digital plotter to present the spectrum. For broad bands, scan speeds up to 2 nm/s can be employed. However, some computer-controlled spectrophotometers with fast data-processing capabilities can scan at rates approaching 20 nm/s and still maintain spectral fidelity even for sharp peaks.

A relatively new type of energy source is the xenon lamp. This lamp is used as a pulsed lamp source as the lamp emits light in a discontinuous fashion (i.e. the lamp emits light only at the time of light measurement). The xenon lamp covers a wavelength range of 200–1100 nm. Advantages of a xenon lamp include the following:

- There is no transition region between UV and visible range.
- There is no need for a warming-up period to ensure stabilisation.
- The lamp has a substantially longer lifetime than a conventional lamp source.

Diode-array spectrophotometers

Diode-array spectrophotometers employ multichannel detectors. The most commonly encountered detector of this type is the linear photodiode array. The reversed-optics mode is employed, so that radiation is passed through the sample or reference cell, then dispersed by a diffraction grating polychromator and detected by a device that comprises several hundred diodes. Each photodiode registers the integrated intensity of radiation incident on it, which is determined by the spectral dispersion : photodiode ratio. For example, if a 200 nm bandwidth of radiation is dispersed across 256 photodiodes, the nominal resolution per photodiode is 0.78 nm.

A spectrum in a specified range is acquired within 20 ms. The analogue signals from each photodiode are digitised and transferred to a computer, where they are corrected for dark-current response and transformed to absorbance. A number of digital techniques are available to increase sensitivity, extending the use of rapid-scanning detectors to multicomponent analysis, reaction kinetics, tablet dissolution tests, process control and detection in HPLC (Fell *et al.* 1982).

Microplate readers

Microplate readers make no use of conventional cuvettes in which light runs through the walls of the cuvette containing the sample. Microplates are transmissible to light and typically have 96 wells (8×12) containing small sample volumes. The light is conducted from under the plate and the detector is mounted above the plate. As the plate moves, the absorbance of all 96 well positions can be read.

By making use of optical fibres the light from the source can be divided over eight samples, thus speeding up the reading process.

Automatic thin-layer chromatography

UV-visible spectrophotometry can also be applied to quantify organic toxic substances, separated by thin-layer chromatography (TLC). By moving the TLC plate, the absorbance of each spot can be scanned to give a two-dimensional absorption spectrum. For fluorescing molecules this technique can also be applied to obtain a fluorescence spectrum.

Fluorimetry

Single-beam fluorimeters

A single-beam spectrofluorimeter consists of a radiation source (usually a mercury or xenon lamp), a primary filter (excitation), a sample cell, a secondary filter (emission) and a fluorescence detection system. In most such fluorimeters the detector is placed on an axis at 90° from that of the exciting beam. This right-angle geometry permits the exciting radiation to pass through the test specimen and not contaminate the output signal received by the fluorescence detector. However, the detector unavoidably receives some of the exciting radiation as a result of the inherent scattering properties of the solutions themselves, or if dust or other solids are present. Filters are used to eliminate this residual scatter. The primary filter selects the short-wavelength radiation able to excite the test specimen, while the secondary filter is normally a sharp cut-off filter that allows the longer-wavelength fluorescence to be transmitted but blocks the scattered excitation. Most fluorimeters use photomultiplier tubes as detectors. The photocurrent is amplified and read out on a meter or recorder.

Scanning spectrofluorimeters

When at least one monochromator (grating or prism) is used instead of a filter, the instrument is called a spectrofluorimeter. The use of gratings instead of filters makes the instrument superior in wavelength selectivity, flexibility and convenience. More complex spectrofluorimeters employ two diffraction gratings (or prisms) to select the fixed excitation

wavelength (λ_{ex}) and the fixed wavelength (λ_f), together with a high-intensity xenon source, scanning motors and electronic compensation for variations in source intensity as the wavelength is varied.

Coupled techniques

Where large numbers of samples must be analysed quickly, the use of automatic instruments becomes viable. One path is to use the automated sample handling in the flow injection analysis technique.

Flow injection analysis (FIA) is an example of a continuous flow system: the sample becomes part of a flowing stream in which the unit operations of the analysis take place as the sample is carried from the injection point to a flow-through measuring device (such as a photometer) and finally to waste.

The intensity of the radiation that reaches the detector is recorded continuously; when an absorbing species is passing through, a sharp peak is generated, with the height of the peak proportional to the analyte concentration. Sample sizes are mostly in the range 10–30 μL.

Instruments are available with the ability to carry out manipulations such as in-line heating, in-line distillation, in-line UV digestion or in-line extraction, so that UV and/or visible spectroscopy can be used for a wide variety of compounds.

In multicomponent systems, the limited specificity for spectroscopic methods can be an important drawback and a restriction for analysis. With the technique of liquid chromatography, a wide variety of macromolecules and ionic species in complex mixtures can be separated. Detection systems depend upon the nature of the component of interest, but the most widely used detectors in liquid chromatography are based upon UV or visible radiation. Photometers often make use of the 254 nm and 280 nm lines from a mercury source, because many organic functional groups absorb in this region. However, to achieve an adequate specificity, often diode arrays are used, which are able to display an entire spectrum as an analyte exits the column (liquid chromatography with diode array detector (LC-DAD)). Compared with single-wavelength detection, which provides no information about peak purity, the diode array's full spectral comparison provides results with a far greater confidence level.

Generally, the LC-DAD technique is applied for toxicological screening. Substances are identified on the basis of both retention time and UV spectrum (Bogusz, Wu 1991; Elliott, Hale 1998; Lambert *et al.* 1995; Tracqui *et al.* 1995). The diode array can also be connected to a mass spectrometer (LC-DAD-MS), which increases the sensitivity and gives an extra confirmation of the component's identity.

For components of interest that fluoresce, the chromatographic system can be equipped with a fluorimetric detector (LC-FL). In the case of weakly fluorescent or non-fluorescent drugs, a number of well-characterised derivatisation reactions are available. These include dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) for primary and secondary amines and phenolic hydroxyl groups, and fluorescamine (4-phenylspiro-[furan-2(3*H*),1′-isobenzofuran]-3,3′-dione), and *o*-phthalaldehyde for primary amines. Derivatisation reactions of this type have extended the scope of fluorescence detection in HPLC significantly.

The same type of detectors (DAD/fluorescence) can be coupled with capillary electrophoresis (CE-DAD or CE-FL) or ion chromatography to determine charged components in complex mixtures.

Data processing and presentation of results

Ultraviolet and visible spectrophotometry

Single-component systems

Where only one component in the sample absorbs significantly, the wavelength is chosen to coincide with the centre of a broad maximum in the spectrum to minimise wavelength-setting errors. If the spectrum has no suitable maximum, a flat absorption minimum can be used, provided that the consequent loss of sensitivity is acceptable. Wavelengths near the extremities of the UV and visible ranges must be avoided, because of the effects of stray-light errors.

Accurate measurements of a drug in solution may be difficult because of non-specific absorption. In these circumstances, the geometric correction devised by Morton and Stubbs is sometimes applied. This assumes that the non-specific absorption varies linearly with wavelength over the range measured. Taking a solution of pure drug, two equi-absorptive points are selected, one at a lower wavelength (λ_1) and the other at a higher wavelength (λ_3) than that of the peak maximum (λ_2). Any irrelevant absorption in the sample increases the observed absorbance of one equi-absorptive point (usually λ_1) more than the other (λ_3). A simple geometrical calculation involving absorbances at λ_1 and λ_3 enables the absorbance at λ_2 to be corrected for the non-specific absorption (Donbrow 1967). The assumption of linearity of the irrelevant absorption can be tested by subtracting the theoretical curve for the calculated quantity of pure material and inspecting the residual difference spectrum.

The classic example of a pharmacopoeial assay based on the Morton–Stubbs correction is that for vitamin A alcohol and the ester (The Stationery Office 2002). Other techniques proposed for the correction of non-specific absorption include difference spectrophotometry, second-derivative spectrophotometry, the use of orthogonal polynomials, and chemical or physical transformation of the drug to give absorption at a longer wavelength.

Multicomponent systems

The absorption spectra of two or more drugs of interest often overlap. Subject to certain conditions, the Vierordt method of simultaneous equations can be employed to obtain the individual concentrations (Glenn 1960). If each of n drugs obeys the Beer–Lambert law over the concentration range of interest, and if the law of additivity of absorbances applies, then the total absorbance, A_T^λ, observed at any wavelength λ is given by the sum:

$$A_T^\lambda = \sum_{i=1}^{n} A_i^\lambda = \sum_{i=1}^{n} k_i^\lambda c_i b \qquad (32.11)$$

where the subscript i denotes each component in the system. The term k_i^λ represents the absorptivity a (L/g/cm), the specific absorbance ($A_{1\ cm}^{1\%}$), or the molar absorptivity ε (L/mol/cm), as determined by the units selected for concentration c_i.

For a two-component system, two wavelengths λ_1 and λ_2 are selected (as discussed below) and two corresponding simultaneous equations are set up:

$$A_T^\lambda / b = k_1^{\lambda_1} c_1 + k_2^{\lambda_1} c_2 \qquad (32.12)$$

$$A_T^{\lambda_2} / b = k_1^{\lambda_2} c_1 + k_2^{\lambda_2} c_2 \qquad (32.13)$$

Equations 32.12 and 32.13 readily yield the concentration of each component, c_1 and c_2, by conventional algebra.

The selection of appropriate wavelengths and the use of accurate absorptivity values are clearly crucial. Generally, λ_1 is the λ_{max} for component 1, while λ_2 is the λ_{max} for component 2, provided that at these wavelengths the absorptivity of the overlapping component is small. If the spectra of both components are very similar, the errors of the method increase appreciably as the difference between the absorptivity ratios tends to zero.

Although this method should apply to the analysis of three or more components, in practice it is often difficult to select wavelengths that fulfil all the requisite conditions. However, computer-aided spectrophotometers exploit the 'principle of overdetermination', in which the number (m) of observation wavelengths exceeds the number (n) of components known to be present. This gives an $n \times m$ matrix of data that can be solved readily by standard matrix algebra.

The limit test for amphotericin A (a tetraene, λ_{max} 300 nm) in the antifungal antibiotic amphotericin (consisting primarily of amphotericin B, a heptaene, λ_{max} 380 nm) is an example of such a two-component analysis (The Stationery Office 2002).

Difference spectrophotometry

Difference spectrophotometry is a method of compensating for the presence of extraneous materials in a sample that would otherwise interfere with the spectrum of the drug being determined. It involves the measurement of the absorbance difference, at a defined wavelength, between two samples in one of which a physical or chemical property of the drug has been changed. It is assumed that the spectrum of the drug can be changed without affecting the spectrum of the interfering material. Alternatively, the absorbance difference may be measured between the sample and an equivalent solution without the drug. Difference spectrophotometry is sometimes described as 'differential spectrophotometry', but this term is not recommended because of its possible confusion with derivative spectrophotometry.

Many suitable methods for physical and chemical modification of the drug absorbance have been reported. For example, the bathochromic effect (also discussed later) is used in the difference spectrophotometric assay of barbiturates. The absorbance of the sample at about pH 10 (A_{10}), to which the mono-anionic species contributes (A_M), is used to compensate for the absorption of interfering endogenous materials (A_{EM}) that have been carried through the extraction procedure. The sample absorbance at pH 13 (A_{13}), to which the di-anionic species (A_D) contributes, is measured at about 260 nm with reference to the sample absorbance at pH 10 (A_{10}), so that

$$A_{13} = A_D + A_{EM} \tag{32.14}$$

$$A_{10} = A_M + A_{EM} \tag{32.15}$$

Thus,

$$\Delta A = A_{13} - A_{10} = A_D - A_M \tag{32.16}$$

If

$$\Delta = (_D - _B) \tag{32.17}$$

then

$$\Delta A = \Delta bc \tag{32.18}$$

Thus, the difference absorbance can be related readily to concentration by prior calibration of the constant $\Delta\varepsilon$, or the concentration may be found by simple proportion:

$$\frac{\Delta A_{test}}{\Delta A_{standard}} = \frac{c_{test}}{c_{standard}} \tag{32.19}$$

It should, however, be established that ΔA is a linear function of concentration (c) over the range required. It is convenient to select for the analytical wavelength a value that corresponds to a maximum in the difference spectrum, obtained by scanning the sample and reference solution over an appropriate wavelength range.

Difference spectrophotometry can be used for quality control in cases where the interfering material is well defined, because an appropriate dilution of a suitable reference solution can be used in the reference cell. The difference absorbance is, however, susceptible to systematic error when there is uncertainty in the concentration of interfering materials in the samples to be assayed. This error increases in proportion to the ratio of the molar absorptivity of the interference to that of the drug.

A further technique to correct for absorptive interferences by difference measurement is based on *dual-wavelength spectrophotometry*. In this method, two monochromatic beams at different wavelengths are passed through the same sample. One wavelength (λ_1) is generally characteristic of the drug, while the other (λ_2) is selected carefully so that the absorbance is equivalent to the level of absorptive interference (A_m^{λ}) anticipated at the analytical wavelength (λ_1). Thus, the second radiation beam is analogous to the reference cell employed in conventional difference spectrophotometry, and the difference in absorbance at the two wavelengths (ΔA) represents the absorption of drug (A_m^{λ}) corrected for interference:

$$A^{\lambda_2} = A_n^{\lambda_1} + A_m^{\lambda_2} \tag{32.20}$$

and since

$$A^{\lambda_2} = A_m^{\lambda_1} \tag{32.21}$$

then

$$\Delta A = A^{\lambda_1} - A^{\lambda 2} = A_n^{\lambda_1} \tag{32.22}$$

A classic application of this method is the correction of Rayleigh scattering in samples of biological origin.

Derivative spectrophotometry

In derivative spectrophotometry, the absorbance (A) of a sample is differentiated with respect to wavelength (λ) to generate the first-, second- or higher-order derivatives:

$A = f(\lambda)$ zero order

$dA/d\lambda = f'(\lambda)$ first derivative

$d^2A/d\lambda^2 = f''(\lambda)$ second derivative

and so on.

Derivative spectra often yield a characteristic profile, in which subtle changes of gradient and curvature in the normal (zero order) spectrum are observed as distinctive bipolar features (Fig. 32.5). The first derivative of an absorption spectrum represents the gradient at all points of the spectrum and can be used to locate 'hidden' peaks, since $dA/d\lambda = 0$ at peak maxima (Fig. 32.5). However, second- and higher-even-order derivatives are potentially more useful in analysis.

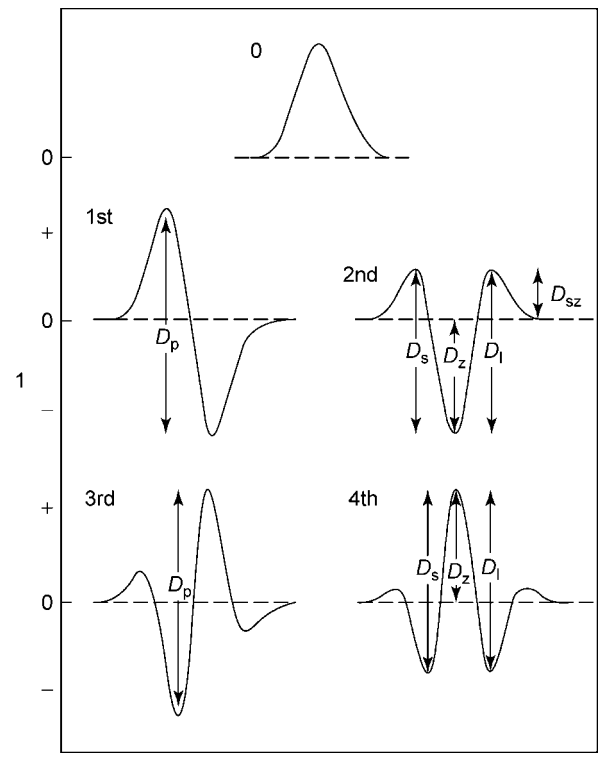

Figure 32.5 First to fourth derivatives of a Gaussian peak, and some graphic measures of derivative amplitude (D). D_p, peak-to-peak; D_s, peak-to-satellite at short wavelength; D_z, peak-to-derivative zero; D_l, peak-to-satellite at long wavelength; D_{sz}, satellite peak-to-derivative zero.

The even-order derivatives are bipolar functions of alternating sign at the centroid (i.e. negative for second, positive for fourth, etc.), whose position coincides with that of the original peak maximum (Fig. 32.5). To this extent, even-derivative spectra bear a similarity to the original spectrum, although the presence of satellite peaks that flank the centroid adds a degree of complexity to the derivative profile. A key feature is that the derivative centroid peak width of a Gaussian peak decreases to 53%, 41% and 34% of the original peak width in the second, fourth and sixth orders, respectively. This feature can increase the resolution of overlapping peaks. However, the increasingly complex satellite patterns detract from resolution enhancement in higher-derivative spectra.

An important property of the derivative process is that broad bands are suppressed relative to sharp bands. This effect increases with increasing order of the derivative, since the amplitude (D_n) of a Gaussian peak in the nth derivative is inversely related to the original peak width (W), raised to the nth power:

$$D_n \sim W^{-n} \tag{32.23}$$

Thus, for two coincident peaks of equal intensity, the nth derivative amplitude of the sharper peak (X) is greater than that of the broader peak (Y) by a factor that increases with derivative order:

$$\frac{D_{n,\,\mathrm{X}}}{D_{n,\,\mathrm{Y}}} = \left[\frac{W_\mathrm{Y}}{W_\mathrm{X}}\right]^n \tag{32.24}$$

This property leads to the selective rejection of broad, additive spectral interferences, such as Rayleigh scattering.

If the Beer–Lambert law is obeyed, that is:

$$A = bc \tag{32.25}$$

then:

$$\frac{\mathrm{d}A}{\mathrm{d}\lambda} = \frac{\mathrm{d}}{\mathrm{d}\lambda} bc \tag{32.26}$$

$$\frac{\mathrm{d}^2A}{\mathrm{d}\lambda^2} = \frac{\mathrm{d}^2}{\mathrm{d}\lambda^2} bc \tag{32.27}$$

and similarly for higher derivatives, where ε is the molar absorptivity (L/mol per cm), b is the cell path length (cm) and c is the concentration (mol/L).

For quantitative work, the amplitude of a derivative peak can be measured in various ways (Fig. 32.5). Although the true derivative amplitude is that measured with respect to the derivative zero, the most common practice is to record the amplitude with respect to a satellite in the spectrum, which affords an extra degree of suppression of interference from extraneous substances. It is essential to run standards in bracketing sequence with the samples, and thus subject both to the same experimental conditions. It should be established that the graphic derivative adopted fulfils the analytical criteria of linear response with concentration, regression through or close to the origin, independence from interfering substances and optimum precision.

In general, methods for generating derivative spectra fall into two classes. These are optical methods, which operate on the radiation beam itself, and electronic or digital methods, which operate on the photometric detector output. The electronic analogue device generates the required derivative as a function of time as the spectrum is scanned at constant speed ($\mathrm{d}\lambda/\mathrm{d}t$), and therefore the derivative amplitude varies with the scan speed, slit width and gain. Moreover, the signal-to-noise ratio has been reported to decrease by approximately a factor of 2 in each successive derivative order.

Alternatively, a microcomputer, employing one of a number of digital algorithms, can be used to produce smoothed derivative spectra. This is carried out in real time or by post-run processing of the digitised spectrum. The digital approach is employed commonly in contemporary spectrophotometers, because of the widespread adoption of microprocessors for instrument control and data handling, coupled with the addition of powerful software for further data processing.

Although transformation of a spectrum to its second- or higher-order derivative often yields a more highly characteristic profile than the zero-order spectrum, the intrinsic information content of the data is not increased; indeed, some data, such as constant 'offset' factors, are lost. However, the derivative method tends to emphasise subtle spectral features by presenting them in a new and visually more accessible way. The method is generally applicable in analytical chemistry and can be used equally for resolution enhancement of electrochemical, chromatographic or thermal analysis data.

Derivative spectrophotometry has found significant application in clinical, forensic and biomedical analysis (Gill *et al.* 1982). In forensic toxicology, the suppression of the absorbance from interfering substances by second-derivative spectrophotometry is well demonstrated in studies on amfetamine in a homogenised liver extract (Fig. 32.6).

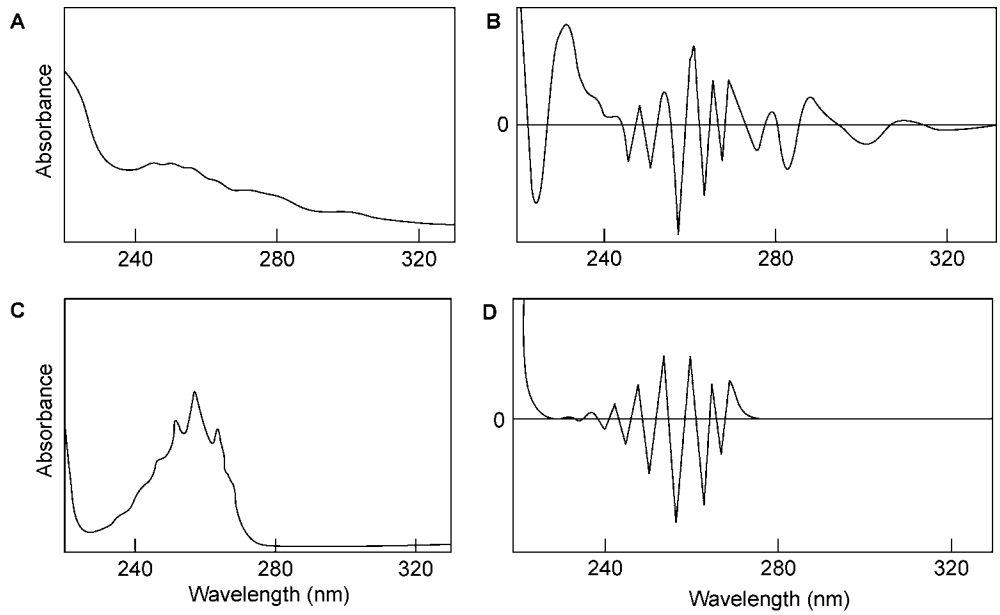

Figure 32.6 Detection of amfetamine in homogenised liver extract. Standard zero-order (A) and second-derivative (B) spectra of the liver extract, compared with the standard zero-order (C) and second-derivative (D) spectra of amfetamine; solutions were in 0.1 mol/L sulfuric acid. (From Gill *et al.* 1982].)

Transformation of the zero-order spectrum (A) to its second derivative (B) using a rapid-scanning multichannel spectrophotometer permits the characteristically sharp benzenoid peaks of amfetamine to be detected and compared with an authentic standard (D), while the interfering background absorption is reduced substantially. The second- and fourth-derivative method for biological background correction can give a tenfold increase in the detection limit of serum paraquat in cases of poisoning (Fell *et al.* 1981). The derivative method can be combined successfully with difference spectrophotometry to give second-derivative difference spectra, in which enhanced discrimination against interfering substances and sharpened fine structural features are observed.

Analytical fluorimetry

The fluorescence properties of a compound are characterised by two spectra. An excitation spectrum is obtained by monitoring the fluorescence at a convenient fixed wavelength λ_f, while scanning the excitation monochromator at a fixed speed up to a wavelength no higher than λ_f. The excitation spectrum should, in principle, be comparable with the absorption spectrum. The fluorescence spectrum is obtained by illuminating the sample at a convenient fixed excitation wavelength λ_{ex} and scanning the emission monochromator at a fixed speed over a wavelength range no lower than λ_{ex}. For interlaboratory comparisons, corrected fluorescence and excitation spectra should be obtained using one of the generally available digital or instrumental techniques.

Some spectrofluorimeters are able to scan the excitation and emission monochromators synchronously to yield fluorescence spectra that are generally simpler and considerably sharper than the conventional fluorescence spectrum. With computer-aided spectrofluorimetry, the acquisition and digital storage of fluorescence spectra are possible. These can then be manipulated in various ways to give the derivative spectrum in which fine structure is accentuated, the difference spectrum or multiwavelength spectral deconvolution, to calculate the concentration of known overlapping components (Winfield, Williams 1984).

In another digital technique, a series of fluorescence spectra is acquired while sequentially stepping up the excitation wavelength. When these spectra are combined to give a matrix of (I_f, λ_f, λ_{ex}), a three-dimensional isometric projection is presented. This type of graphic presentation is described as an 'emission–excitation matrix' or 'fluorogram' (Fig. 32.7). The data can also be plotted as the equivalent two-dimensional plot of isointensity contours in the (λ_f, λ_{ex}) plane (Fig. 32.8). Three-dimensional graphics are increasingly used for the qualitative comparison of fluorescent molecules, as in the example of promethazine and its principal degradation product, promethazine sulfoxide (Figs 32.7 and 32.8).

Instrument performance checks

Although instruments with conventional optics may require frequent calibration because they have many moving parts, diode array spectrophotometers with no moving parts are extremely reproducible and stable, in both the short and the long term.

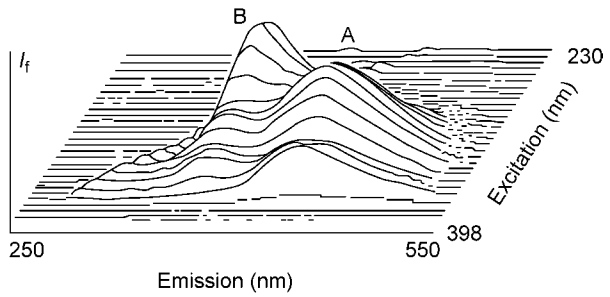

Figure 32.7 Isometric projection of the emission–excitation matrix of fluorescence intensity (I_f) for promethazine hydrochloride (A) and promethazine sulfoxide (B) in buffer at pH 3.0. (From Fell *et al.* 1981.)

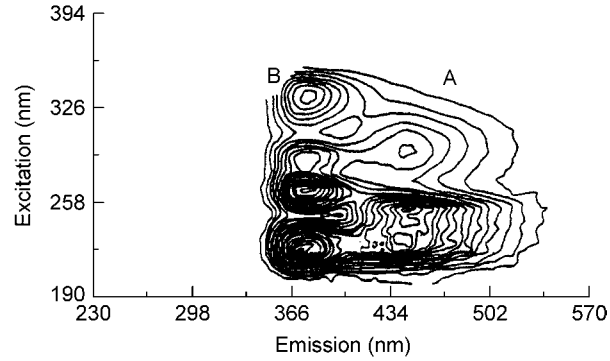

Figure 32.8 Contour plot of emission–excitation matrix of promethazine hydrochloride (A) and promethazine sulfoxide (B) in buffer at pH 3.0. (From Clark *et al.* 1985.)

Following the recommendations of the European Pharmacopeia (Council of Europe 2007) for UV and visible spectrophotometry, the wavelength and the absorbance must be calibrated. The wavelength scale must be verified using the absorption maxima of holmium perchlorate solution, the line of a hydrogen or deuterium discharge lamp, or the lines of a mercury vapour lamp. The permitted tolerance is ±1 nm for the UV range and ±3 nm for the visible range. The absorbance should be checked using suitable filters or a solution of potassium dichromate of 60 mg/L in 0.005 mol/L sulfuric acid at the wavelengths indicated in Table 32.2.

The level of stray light should be assessed, since it increases with instrument age. It may be detected at a given wavelength with suitable filters or a 1.2% w/v potassium chloride solution. The European Pharmacopeia (Council of Europe 2007) requires that the absorbance be greater than 2 at a wavelength between 198 and 202 nm when compared with water as compensation liquid.

In some assays it is necessary to specify the minimum desirable resolution, since changes in the spectral bandwidth (or monochromator slit width) can seriously affect the observed absorbance of sharp peaks. The European Pharmacopoeia requires that the spectral bandwidth employed should be such that further reduction does not lead to an increase in measured absorbance. This is particularly important for drugs that have aromatic or strongly conjugated systems (e.g. diphenhydramine, phenoxymethylpenicillin, and amphotericin A and B). In such cases, a spectral bandwidth of more than 1 nm leads to a reduction in observed absorbance at the peak maximum (and conversely an increase in absorbance at a peak minimum), since the recorded absorbance is the mean of that over the whole bandwidth at that wavelength. Although increasing the slit width gives a better signal-to-noise ratio, a slit width of 2 nm is adequate for most bands, with 1 or 0.5 nm being used for very sharp peaks.

For qualitative analysis the resolution can be measured by recording the spectrum of a 0.02% solution of toluene in hexane. The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monographs of the European Pharmacopoeia (Council of Europe 2007).

According to the principles of good laboratory practice (GLP), the apparatus should be periodically inspected, cleaned, maintained and calibrated according to the laboratory's standard operating

Table 32.2 Wavelengths at which absorbance should be checked

Wavelength (nm)	*Specific absorbance* (A_1^1)	*Maximum tolerance*
235	124.5	122.9–126.2
257	144.5	142.8–146.2
313	48.6	47.0–50.3
350	107.3	105.6–109.0

procedures. Records of procedures should be maintained. Calibration should, where appropriate, be traceable to national or international standards of measurements.

Examples of interesting options on the system are a logbook database in which lamp changes and defects are registered. Also, a key-lock function is now a common part of a quality system to avoid effects from unwanted keystrokes.

In fluorescence the calibration of excitation and emission monochromator wavelengths should be checked regularly by the use of sharp lines from the instrument's own radiation source (e.g. xenon lines at 450.1, 462.4, 467.1 and 473.4 nm) or the use of sharp fluorescence peaks in solutions or glasses of trivalent lanthanide ions (tellurium, europium). Other fluorescence standards in common use are ovulene and other polycyclic aromatic hydrocarbons with fine vibrational structures such as naphthalene and anthracene. In practice, it is necessary only to calibrate one monochromator, since the other can then be calibrated by the Rayleigh scattered radiation using a sample of colloidal silica in the sample position. The use of an auxiliary light source, such as a mercury light pen, is the least satisfactory method for wavelength calibration.

It is recommended that the performance of each computerised system be verified for the proper functioning of data acquisition, method-related calibration and reporting. Usually, test packages are commercially available from the supplier.

Sample preparation and sample presentation

The most frequent mode of sample presentation is as a dilute solution, although gases and solid surfaces can also be examined. Combinations of UV and visible spectrophotometry or spectrofluorimetry with HPLC are particularly advantageous for sensitive and selective detection of chromophores and/or fluorophores.

Ultraviolet and visible spectrophotometry

Cells

In the visible region, a matched pair of glass cells can be used, but these are inappropriate for the UV region because of the poor transmission properties of glass in this range (Fig. 32.9). Fused-silica or quartz cells have high transmittance from 190 nm to 1000 nm, and are therefore the cells of choice. The path length employed is usually 1.00 cm; cells with longer path length are used for poorly absorptive drugs and/or where the concentration is low. Flow cells designed to minimise turbulent flow through the cell are used to monitor changes in absorbance during a reaction, for tablet dissolution studies or for HPLC; care should be taken that the cell walls do not block the radiation beam, otherwise variable errors are introduced. Cells provided with a thermostat are used for studies on enzymatic and other processes in which temperature is a key parameter.

The meticulous handling and care of cells is a necessary condition for precise and accurate measurement. Cells should be cleaned carefully, filled with an appropriate solvent and matched for absorbance to less than 1%. Each pair of cells should be marked on the base in soft pencil to identify the set and its normal orientation. The tolerance on the path length of the cells used is ± 0.005 cm (Council of Europe 2007).

When filled with the same solvent, the cells intended to contain the solution to be examined and the compensation liquid must have the same transmittance. If this is not the case, an appropriate correction must be applied. It is convenient to designate the more strongly absorbing cell as the 'sample' cell, the other cell being coded as 'reference'. In this way the cell constant (i.e. the difference in absorbance at the measurement wavelength when filled with solvent) will be positive and can thus be subtracted from each absorbance reading. Moreover, the possibility of 'oscillating error' introduced by randomly changing the cell orientation during a series of measurements is eliminated. The 'cell constant' should be checked regularly at the measurement wavelength when filled with an appropriate solvent, or by scanning the baseline over the wavelength range.

Cells should be cleaned scrupulously after use. If they have contained aqueous solutions, they can be cleaned readily by repeated rinsing with distilled water or by soaking overnight in a very dilute solution of detergent; special detergents should be used to clean cells contaminated with biological material. Periodically, it is good practice to soak cells and stoppers thoroughly in a fresh solution of chromic acid, followed by copious rinsing with distilled water, to restore their matched performance. Cells that have been used with organic solvents require special care, with a sequence of solvents ending in spectroscopic ether being convenient to obtain dry, clean cells. In all cases, the manufacturer's instructions should be followed, when available. Sharp glass or metal objects should not be introduced into a cell, lest the internal surface be scratched. The outside optical surfaces should be polished before use with a soft cloth or photographic lens tissue. Cells should be stored in pairs, dry and in a protective container.

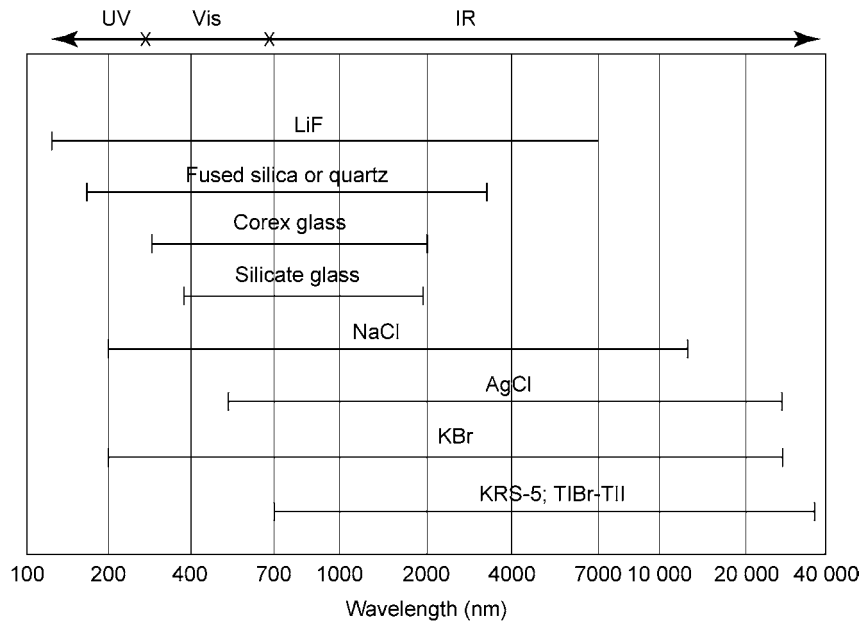

Figure 32.9 Transmittance ranges for various optical materials. (From Skoog *et al*. 1996, p. 529.)

Solvents

Solvents used in spectrophotometry must meet certain requirements to ensure successful and accurate results. The solvent chosen must dissolve the sample, yet be compatible with cuvette materials. The solvent must also be relatively transparent in the spectral region of interest. To avoid poor resolution and difficulties in spectrum interpretation, a solvent should not be used for measurements near or below its UV cut-off (i.e. the wavelength at which absorbance for the solvent alone approaches one absorbance unit). The cut-offs of common solvents are listed in Table 32.1.

Once a solvent is selected on the basis of its physical and spectral characteristics, its purity must be considered. The absorbance curve of a solvent, as supplied, should be smooth (i.e. have no extraneous impurity peaks in the spectral region of interest). Solvents specially purified and certified for spectrophotometric use are available from suppliers.

Good laboratory practice

According to GLP, chemicals, reagents and solutions should be labelled to indicate identity (with concentration if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available. The expiry date may be extended on the basis of documented evaluation or analysis.

Records, including test-item and reference-item characterisation, date of receipt, expiry date, and quantities received and used in studies should be maintained. Handling, sampling and storage procedures should be identified in order that the homogeneity and stability are assured to the degree possible, and that contamination and mix-up are precluded. Storage containers should carry information on identity, expiry date and specific storage instructions. The stability of test and reference items under storage and test conditions should be known for all studies.

Fluorimetry and spectrofluorimetry

Cells

Specimen cells used in fluorescence measurements may be round tubes or rectangular cells similar to those used in absorption spectrophotometry, except that they are polished on all four vertical sides. A convenient test specimen size is 2–3 mL, but some instruments can be fitted with small cells holding 100–300 µL or with a capillary holder that requires an even smaller amount of specimen.

Temperature regulation is often important in fluorescence spectrophotometry. For some substances, fluorescence efficiency may be reduced by as much as 1–2% per degree of temperature rise. In such cases, if maximum precision is desired, temperature-controlled sample cells are useful. For routine analysis, it may be sufficient to make measurements rapidly enough that the specimen does not heat up appreciably from exposure to the intense light source (US Pharmacopeial Convention 2002).

Solvents

Change of solvent may markedly affect the intensity and spectral distribution of fluorescence. It is inadvisable, therefore, to alter the solvent specified in established methods without careful preliminary investigation. Many compounds are fluorescent in organic solvents, but virtually non-fluorescent in water; thus, a number of solvents should be tried before it is decided whether or not a compound is fluorescent. In many organic solvents, the intensity of fluorescence is increased by elimination of dissolved oxygen, which has a strong quenching effect. Oxygen may be removed by bubbling an inert gas, such as nitrogen or helium, through the test specimen.

Interpretation of spectra and qualitative analysis

Ultraviolet and visible spectrophotometry

Spectrophotometric measurements with UV or visible radiation are useful for detecting components that contain unsaturated groups or atoms such as sulfur or halogens. A drug, its impurity or a metabolite can also be transformed selectively so that the spectrum is shifted to the visible region and away from interference caused by another drug, formulation components or biological substances, and thereby confer a further degree of specificity.

However, specific identification of a compound can rarely be made on the basis of UV spectral evidence alone. Often, the spectrum serves as confirmatory evidence of identity, in support of other analytical data. The general approach usually followed in qualitative applications is first to establish by independent means (e.g. chromatography) that the material consists substantially of one absorbing component. Spectra are then recorded in aqueous acidic, basic, and ethanolic or methanolic solution. The wavelengths of the principal peaks and the corresponding absorptivity values are noted for each solvent system. By comparison with data tabulated in ascending wavelength order (see Ultraviolet Absorption Maxima in Indexes of Analytical Data), a number of compounds with absorbing properties similar to the test substance are selected (using a wavelength window of ± 2 nm). One must be aware that, in general, the spectrum of metabolites of a component matches closely the spectrum of the component itself.

Further evidence can be deduced from the absorptivity ratios of peaks within a spectrum; moreover, the change in these ratios together with the shift in peak positions as the pH is changed can be diagnostic. If a drug molecule ionises reversibly (i.e. without degradation), the family of curves for a constant concentration in acidic and basic solvents displays one or more isosbestic points at characteristic wavelengths, at which the absorbance is constant at all values of pH.

Spectral shifts are among the most useful diagnostic features in drug molecules that possess ionisable groups. A marked *bathochromic shift* (or 'red' shift) to longer wavelengths in alkaline solution is observed not only for most of the phenolic drugs, such as the phenolic oestrogens, but also in the case of hydroxypyridines, ketones, benzodiazepines, pyridones and nitro-compounds. The bathochromic shift is often large ($<$10 nm) and accompanied by an increase in molar absorptivity (*hyperchromic effect*) and loss of any fine structure.

This effect has been exploited, both for qualitative and for quantitative purposes, for the analysis of barbiturates. In acidic or neutral solution, barbiturates show little absorption above 230 nm (Fig. 32.10), but in 0.05 mol/L borax buffer (pH 9.2) ionisation yields an intense conjugated chromophore (Figs 32.10 and 32.11) with a well-defined maximum near 240 nm ($A^{1}_{1} = 400$–450). In sodium hydroxide solution (pH 13), a second stage of ionisation occurs (except in *N*-substituted derivatives) to extend further the conjugation and give a peak maximum near 255 nm (Figs. 32.10 and 32.11). However, solutions in alkali are unstable through ring opening, so that measurements must be made rapidly.

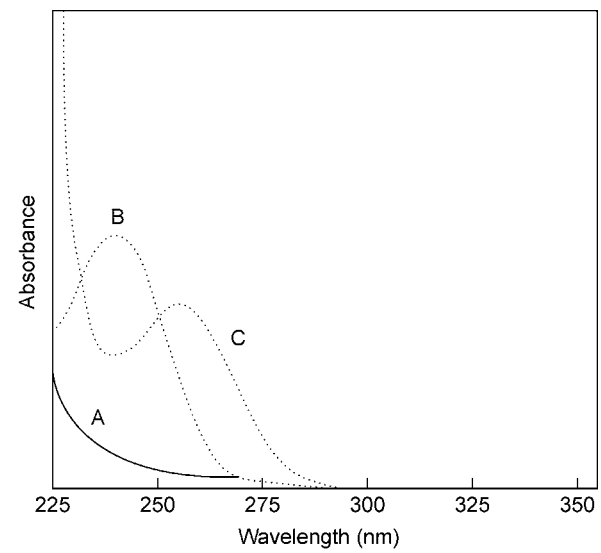

Figure 32.10 Effect of pH on the UV spectrum of phenobarbital: A, non-ionised barbiturate in 0.1 mol/L hydrochloric acid; B, mono-anion in 0.05 mol/L borax buffer pH 9.2; C, di-anion in 0.5 mol/L sodium hydroxide pH 13.

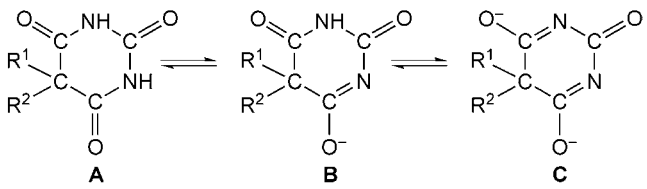

Figure 32.11 Dissociation of C5-substituted barbituric acids: (A) undissociated free acid; (B) mono-anion; (C) di-anion.

The *hypochromic* or 'blue' shift to shorter wavelengths is shown by aromatic amines in acid solution and is highly characteristic for many drugs. On acidification, the protonated quaternary ammonium group no longer participates in the chromophore so that the spectrum is shifted to lower wavelengths, sometimes by as much as 30 nm, with a sharp fall in absorptivity (*hypochromic effect*).

In addition to their use in characterising a chromophore, pH-induced shifts can also be exploited to shift a spectrum along the wavelength scale to obtain an interference-free window for measurement of an ionisable species in a mixture.

Solvent effects

A solvent for UV and visible spectroscopy must be transparent throughout the region of interest and should dissolve a sufficient quantity of the sample to give well-defined peaks. Moreover, consideration must be given to possible interactions with the absorbing species. For example, polar solvents, such as water, alcohols, esters and ketones, tend to suppress vibrational fine structure and should thus be avoided when spectral detail is desired. Non-polar solvents, such as cyclohexane, often provide spectra that more closely approach those of a gas (see also Fig. 32.1). In addition, the polarity of the solvent often influences the position of absorption maxima. Consequently, a common solvent must be employed when comparing spectra for the purpose of identification.

When using an HPLC-UV system, any effects on the spectral characteristics of a substance brought about by various mobile phases must be considered when comparing spectra generated in this manner with those recorded from acidic, neutral and alkaline solutions. Moreover, potential effects brought about during gradient elution HPLC, in which the composition of the mobile phases is constantly changing, should be borne in mind. With acetonitrile, methanol, ethanol, isopropanol and other water-miscible solvents, no essential changes in the spectra may occur and direct comparison with other databases is feasible. However, changes in pH can have a significant effect on the UV spectra of compounds involved in an acid–base equilibrium (e.g. carboxylic acids, phenols, thiophenols) and compounds with basic nitrogen atoms. Direct comparison of spectra with other compendia of data, including those listed in this volume, is valid only when the mobile phases have the same pH.

Spectrofluorimetry

For compounds with appropriate fluorescence properties, this technique gives high sensitivity and high specificity. High sensitivity results from the difference in wavelengths between the exciting and fluorescence radiation. This results in a signal contrasted with an essentially zero background; it is always easier to measure a small signal directly than a small difference between two large signals, as is done in absorption spectrophotometry. High specificity results from dependence on two spectra, the excitation and emission spectra, and the possibility of measuring the lifetimes of the fluorescent state. Although in biological samples the fluorescence intensity of interfering substances may be relatively high, the sensitivity and selectivity of the method are generally such that fluorescent drugs and their metabolites can be analysed more readily than by conventional spectrophotometry. Two compounds that are excited at the same wavelength, but emit at different wavelengths, are readily differentiated without the use of chemical separation techniques. Likewise, two compounds may fluoresce at the same wavelength but require different excitation wavelengths. Also, a fluorescent compound in the presence of one or more non-fluorescent compounds is readily analysed fluorimetrically, even when the compounds have overlapping absorption spectra. Non-fluorescent or weakly fluorescent compounds can often be reacted with strong fluorophores, which enables them to be determined quantitatively.

Many fluorescent species contain ionisable groups with fluorescent properties sensitive to pH. In some cases only one of the ionised species may be fluorescent. Examples are the barbiturates, which fluoresce only at an elevated pH in the di-anionic form. Phenol fluoresces at pH 7, but at pH 12, when it is converted into its anion, there is no fluorescence. Therefore, the relationship of fluorescence intensity with pH should always be examined as part of the method development.

Fluorescence intensity and wavelength often vary with the solvent. In most molecules, fluorescence decreases with a decrease in solvent viscosity as the probability of intermolecular energy transfer tends to be enhanced. The same effect occurs with an increase in temperature.

Quantitative analysis: ultraviolet and visible spectrophotometry

Fundamental basis

The basic principle of most spectrophotometric measurements involves comparing, under well-defined conditions, the absorption of radiation by the substance in an unknown amount with the same absorption of radiation by a known amount of the material being determined. In general, to obtain the maximum sensitivity it is best to work with radiation of a wavelength that is approximately equal to that for which the solution exhibits a maximum selective absorption.

Assuming that the linear range for compliance with the Beer–Lambert law has been established and that the drug concentration has been adjusted within the optimum range for the type of instrument concerned, two approaches to quantification may be employed. If an acceptable reference standard of the drug is available, and if the calibration graph passes through zero, measurement of replicates of the standard (at a comparable concentration) and of the tests are performed in bracketing sequence (i.e. each group of samples is preceded and followed by the standard), under identical conditions of solvent and temperature and using the same pair of matched cells. Each result should be corrected for the cell constant; the concentration of the test sample is then found by reference to the results from the standards.

Alternatively, the specific absorbance is used to calculate the sample concentration, using the absorbance measured in the specified solvent. A check on the accuracy of the absorbance scale is clearly essential. Wavelength accuracy is not so important.

The practical usefulness of reference-specific absorbance values (A_1^1) clearly depends on a number of factors. These include the state of purity of the substance, the solvent conditions originally used to establish the reference data, the precise conditions employed in the reference instrument and the extent to which they correspond with those of a particular test laboratory. It is therefore wise to ascertain the status of any absorptivity data in the literature. In the monographs in Volume 2, the reliability of all A_1^1 values has been assessed and indicated (see General Notices). However, if a sample of the drug concerned is available in pure form, it is good practice to establish periodically a 'local' value of the absorptivity and to use this in calculating sample concentrations.

Linearity issues

The validity of the Beer–Lambert law should be established for each drug under the measurement conditions to be used over an appropriate concentration range. For single-beam instruments, the absorbance range for precise measurements is between about 0.3 and 0.6 absorbance units, the optimum being at 0.43 absorbance units. For double-beam spectrophotometers, the optimum range lies between 0.6 and 1.2 absorbance units. Five or more standard solutions, with absorbances that span the working range, should be measured in duplicate in a matched pair of cells against the solvent as reference;

the residual absorbance difference between the cells when filled with solvent (the cell constant) should be subtracted from each individual measurement and checked regularly.

The linearity of an analytical method is determined by mathematical treatment of the absorbance data of the standard solutions across the claimed range of the method. The treatment is normally a calculation of a regression line $y = ax + b$, with y being the absorbance and x the concentration, by the method of least squares. The linearity is usually expressed by means of a correlation coefficient r, where

$$r = \frac{\sum_i (c_i - \bar{c})(A_i - \bar{A})}{\sqrt{\left(\sum_i (c_i - \bar{c})^2\right)\left(\sum_i (A_i - \bar{A})^2\right)}} \quad (32.28)$$

for all n points with i from unity to n. When $r = 1$, there is perfect correlation. Usually, r values better than 0.995 can be obtained.

The intercept should not differ significantly from zero. If a significant non-zero intercept is obtained, it should be demonstrated that there is no effect on the accuracy of the method.

Frequently, the linearity is evaluated graphically in addition or alternatively to a mathematical evaluation. The evaluation is made by visual inspection of a plot of absorbance A as a function of analyte concentration c. If any systematic positive or negative deviation is found, additional points should be inserted and the linear working range established.

Deviations from linearity are sometimes difficult to detect, and two additional graphic procedures can be used. The first is to plot the deviations from the regression line against the concentration, or the logarithm of the concentration if the concentration range covers several decades. For linear ranges the deviation should be distributed equally between positive and negative values.

Another approach is to plot the function A/bc (the absorptivity) on the y-axis and the corresponding concentrations on the x-axis. The line obtained should be horizontal over the full linear range. Parallel horizontal lines are drawn on the graph that correspond to, for example, 95% and 105% of the plotted line. The method is then linear up to the point where the plotted relative response line intersects one of these two lines.

International Conference on Harmonisation validation criteria

The main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. The objective of an analytical procedure should be understood clearly, since this will govern the validation characteristics that need to be evaluated. Typical validation characteristics that should be considered according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantification limit, linearity and range. All these characteristics are also listed and explained in the USP 25 (US Pharmacopeial Convention 2002). For the quantitative determination of impurities, all these criteria should be evaluated, while for limit tests the specificity and detection limit are most relevant. For assays, all characteristics except detection limit and quantification limit are normally evaluated.

For linearity, the ICH recommends a minimum of five concentrations to be measured. The range is allocated the following limits:

- For the assay of a drug substance or a finished (drug) product – normally from 80% to 120% of the test concentration.
- For content uniformity – covering a minimum of 70–130% of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g. metered-dose inhalers), is justified.
- For dissolution testing – ±20% over the specified range.
- For the determination of an impurity – from the reporting level of an impurity to 120% of the specification.
- If assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities to 120% of the assay specification.

To determine the accuracy, use of a minimum of nine determinations over a minimum of three concentration levels that cover the specified range is recommended.

Repeatability should be assessed using a minimum of nine determinations that cover the specified range for the procedure (e.g. three concentrations with three replicates each) or a minimum of six determinations at 100% of the test concentration.

To determine the detection limit, the ICH recommendations correspond with the USP 25 and can be determined, for example, on the basis of signal-to-noise ratios, where 2 : 1 or 3 : 1 is generally accepted, or on the standard deviation of the blank.

To calculate the quantification limit, both ICH and USP 25 recommend a signal-to-noise ratio of 10 : 1. Both USP 25 and the ICH recommend explicitly validation of the quantification limit by the analysis of a suitable number of samples known to be near or prepared at the quantification limit.

Quantitative analysis: fluorimetry

Fundamental basis

Excitation spectra are usually used to confirm the identity of components and to select an optimum excitation wavelength for quantitative analysis. The emission spectrum is then used for analytical applications.

Spectrofluorimetry differs from absorption spectrophotometry in not yielding an absolute scale of values, as it depends on the number of excited molecules and their method of relaxation. For this reason it is essential to employ a reference standard for quantitative measurements.

The linear relationship between the power of fluorescence and the concentration of the fluorescing solute forms the basis for quantification, the linearity constant for which may be established by calibration with standards. A plot of fluorescence readings against the concentration of the reference solutions furnishes the calibration curve. Glass reference filters are also suitable as a calibration standard.

Linearity and baseline issues

In dilute solution, the fluorescence intensity (I_f) for defined values of λ_{ex} and λ_f is related linearly to molar concentration (c), according to the approximate relationship:

$$I_f = Kc \text{ at } \lambda_{ex},\ \lambda_f$$

under constant instrumental conditions.

Sensitivity can be increased by working at high excitation powers to give larger signal-to-noise ratios. Since the source intensity can change from time to time, fluorescence signals are not measured as absolute parameters. They are expressed rather in terms of relative fluorescence. All measurements are made relative to reference standards of known concentration. All readings must be corrected for background fluorescence.

A necessary condition is that the total absorbance ($=\varepsilon bc$) of the system should not exceed 0.05 absorbance units, otherwise progressively greater negative deviations from linearity are observed. At high drug concentrations, fluorescence intensity reaches a plateau. Beyond this, fluorescence intensity actually decreases with increasing concentration, because of inner-filter effects, in which ground-state molecules absorb the fluorescence emitted by excited molecules.

It is essential to establish the range of linearity of the calibration curve *of* I_f versus c, using at least five standard solutions, for which the condition holds that absorbance at the wavelength of maximum excitation is <0.05 absorbance units. Samples are usually analysed by single-point bracketing, taking a standard conveniently close to the anticipated sample value and calculating the result by simple proportion.

The sequence of standard measurements before and after measuring the sample permits any baseline drift to be compensated. For additional assay security, two-point bracketing can be employed, in which two standard solutions, one higher and the other lower than the concentration observed for the sample, are used in bracketing sequence.

Instrumental limitations caused by instability of the radiative source can be overcome by the ratio mode operation. A small fraction of the exciting radiation is directed to a reference photodetector, which is chosen primarily for wide wavelength response. The output signal is used as a monitor and, as the excitation radiation increases or decreases in power because of fluctuations in the source, there is a corresponding increase or decrease in relative fluorescence.

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CHAPTER

33 Infrared Spectroscopy

RD Jee

Introduction

The absorption of infrared (IR) radiation is a very characteristic property of a substance and is widely used for identification purposes. Infrared spectroscopy is the study of the scattering, reflection, absorption or transmission of IR radiation in the spectral range 800–1000 000 nm (0.8–1000 μm). In older literature (pre-1970), IR radiation was referred to in terms of its wavelength in units of microns (micrometres, μm). Nowadays, the wavenumber ($\tilde{\nu}$) is used almost exclusively. The relationship between wavenumber in units of reciprocal centimetres (cm^{-1}) and wavelength (λ) in micrometres (μm) is given by:

$$\tilde{\nu} = \frac{10^4}{\lambda}$$

The IR spectrum can be divided into three subregions: 12 500–4000 cm^{-1} (0.8–2.5 μm; near IR), 4000–400 cm^{-1} (2.5–25 μm; mid-IR) and 400–10 cm^{-1} (25–1000 μm; far IR). Only the mid-IR region (often referred to simply as infrared) is considered here because it is the region widely used in the analysis of drugs and pesticides. However, some modern instruments can scan from 15 000 cm^{-1} to about 50 cm^{-1}; the extension to the far IR is useful for halogenated compounds and for inorganic substances.

The energy associated with electromagnetic radiation is given by Planck's equation:

$$E = \frac{hc}{\lambda}$$

where E is the energy, h is Planck's constant, c is the speed of light and λ is the wavelength of light.

IR radiation can excite molecular vibrations (and associated molecular rotations). At room temperature, a molecule is generally in its ground electronic state and lowest vibrational state. Provided that the incoming IR radiation has the appropriate energy (wavelength, wavenumber), resonant absorption occurs to excite the molecule to a particular higher vibrational state. Vibrational transitions give rise to an absorption spectrum characteristic of the compound. Several factors characterise this absorption spectrum: the number of absorption features and their associated wavenumbers, the strength (intensity) of the absorption features and the sharpness of these features.

Energy and wavenumber of infrared absorption by molecular vibration

Molecules can undergo two types of vibrations, namely stretching vibrations that involve changes in bond length and bending vibrations that involve changes in bond angles. The vibrational modes associated with the methylene CH_2 group are illustrated in Fig. 33.1.

Theoretically, a non-linear molecule has $(3N-5)$ such modes of vibration, where N is the number of atoms in the molecule. A linear molecule has $3N-6$ modes of vibration. These are called fundamental modes and require IR energy in the range 4000–400 cm^{-1} (mid-IR) to become excited. Not all vibrations in a molecule are assignable; generally, only the most prominent are readily assigned to a given vibrational mode. These characteristic vibrations are a good way to detect the existence of functional groups in a chemical compound. The precise wavenumber at which a particular vibration absorbs light is associated with bond strength and the atomic masses of the atoms in the bond. The wavenumbers required for the excitation of typical vibrations are given in Table 33.1.

In addition, there are overtones (the excitation of a vibration to a double or higher frequency) and combinations that are the sum or difference of two or more fundamental bands. No fundamental vibrations require energy greater than 4000 cm^{-1} to become excited. All vibrations in the near IR are therefore overtones or combination bands. The reader is referred to spectroscopic texts for a more detailed explanation of the origin of IR bands (e.g. Williams, Fleming 2007, and others listed in the Further reading).

Changes in the wavenumber of a band can be related to changes in either the structural environment or the physical state of the molecule. However, many bands in the complex region from 1800 cm^{-1} to 400 cm^{-1}, which is usually referred to as the 'fingerprint region', remain of unconfirmed origin. Many of the bands are characteristic of the molecule as a whole and cannot be assigned directly to particular bonds. Nevertheless, inspection of IR spectra can form the basis of qualitative analytical work in IR spectroscopy to confirm the identity of a sample. A complete molecular structure cannot be deduced directly from an IR spectrum. Rather, functional groups are identified and total molecular identity is confirmed by comparison with IR spectra in a spectral library.

Strength of molecular vibration absorption

Traditionally, an IR spectrum is reported as a plot of percentage transmittance ($T\%$) against wavenumber $\tilde{\nu}$. Increasingly, spectra are reported as a plot of absorbance against wavenumber. The IR transmission spectrum of a polystyrene film used to calibrate the wavenumber scale is given in Fig. 33.2.

The absorption of light in Fig. 33.2 is registered as the transmission of light. The simple ratio of the transmitted intensity to the incident intensity is known as the transmittance. The percentage transmittance is 100 times the transmittance (Fig. 33.3).

The absorption of light is quantified through Beer's law as:

$$A = \log(I_0/I_t) = cl$$

where A is the absorbance, c is the molar concentration (mol/L), l is the path length (m) of the sample and ε is the molar extinction coefficient (L/mol per cm). Absorbance is the log of the inverse of transmittance:

$$A = \log(I_0/I_t) = \log(1/T) = -\log T = 2 - \log T\%$$

Not all vibrational modes are 'active' in the IR region. For absorption of radiation, the act of vibration must cause the dipole moment to change. The carbonyl (C=O) stretch at around 1650 cm^{-1} has a particularly strong transition electric dipole moment (i.e. large change in dipole moment), and therefore ε is large for a vibration (approximately 100 mol/L per cm) and the carbonyl absorption is a very prominent feature. The C=O stretch is said to be allowed spectroscopically. The C–H stretching mode generates a lower transition electric dipole

moment and the ε value is smaller at approximately 10 mol/L per cm. However, there are usually many C–H bonds in a molecule and the additive absorption of these makes the C–H vibration a prominent feature.

The stretches of symmetrical bonds, such as H–H (hydrogen gas), –C–C– (ethane), O–O (oxygen) and N–N (nitrogen), do not have a transition dipole moment and therefore ε→0 mol/L per cm and these stretches are not observed and are said to be forbidden. Similar vibrations in complex molecules do absorb, but extremely weakly. On the other hand, gases such as NO, NO_2, CO, CO_2, SO_2, CH_4 and H_2O (water vapour) do have IR-active vibrations that can be measured to monitor environmental levels. The low value of ε and low concentrations of these gases mean that gas cells for IR spectroscopy are very long (up to 1 m or more). Water vapour and CO_2 present in the normal atmosphere produce absorption effects in an IR spectrometer in the absence or presence of a sample. High-precision work therefore benefits from flushing with dry air or nitrogen. The water vibrations centred at 3782 cm^{-1} and 1587 cm^{-1} show a fine structure associated with the rotations of the water molecule.

The actual movement of charge during a vibration is in practice very small and a value of ε of approximately 200 mol/L per cm is a practical upper limit. In tables of IR data, the intensity of vibrational bands is designated as 'vs' (very strong), 's' (strong), 'm' (medium) and 'w' (weak) to reflect the variation in extinction coefficient (intensity of spectral feature). For older designs of instrument the measurement of IR intensity was unreliable, which made quantitative work in the IR unreliable. This is not true for modern instruments.

The spectroscopic character of overtones and combinations is less well defined. Accordingly, ε in the near IR is low and absorption is detected only for concentrated solutions with an absorption that may be too strong for spectral regions in which ε is larger. The molar extinction coefficient, ε, is largely responsible for setting the sensitivity of an analysis.

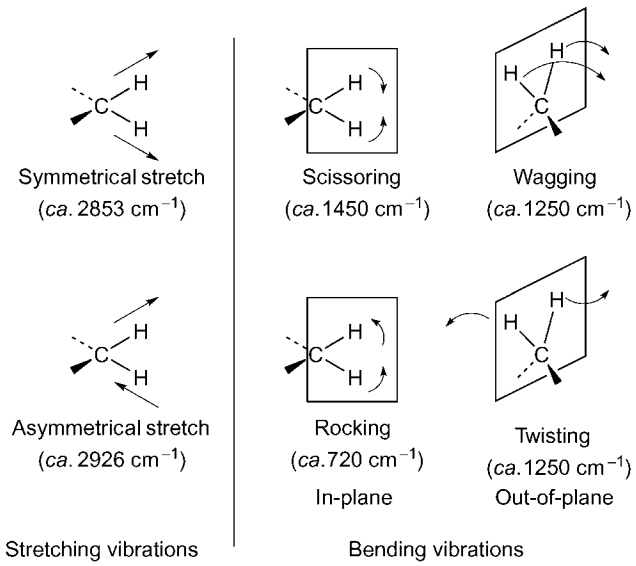

Figure 33.1 Molecular vibrations of the methylene CH_2 group (courtesy of Pavia *et al*. 1996).

Table 33.1 Important vibrations and IR frequencies	
Wavenumber (cm^{-1})	***Vibration***
3600–2500	O–H stretch, broad, strong (prominent)
3400	N–H stretch, broad, strong (prominent)
3000	C–H aromatic stretch
2900	C–H aliphatic stretch
1800–1650	C=O stretch strong (prominent)
	Ester R–O–C=O, ca. 1740 cm^{-1}
	Ketone C=O, ca. 1715 cm^{-1}
	Carboxylic acid HO–C=O, ca. 1705 cm^{-1}
	Amide H_2N–C=O, ca. 1650 cm^{-1}
1300–1000	C–O stretch, strong
1800–400	Forest of vibrations – fingerprint region

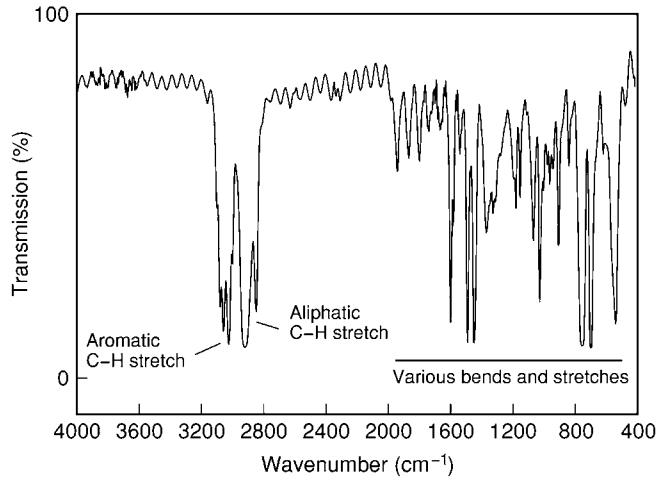

Figure 33.2 Transmission spectrum of a polystyrene film.

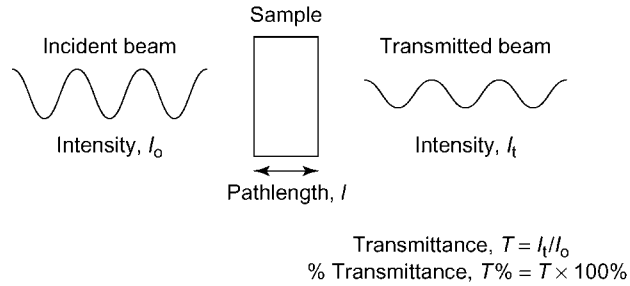

Figure 33.3 Transmission of light by a sample.

Width of an infrared absorption band

In ultraviolet (UV) and visible electronic absorption spectroscopy, absorption is limited to the excitation of a single electron in a chromophore, albeit to one of several excited states. An electronic spectrum is rarely composed of more than three prominent spectral features. However, the energy required to excite an electron is enough also to excite associated vibrational and rotational states, and the absorption profile is broad. The UV-visible spectrum is characterised by only a few broad features. A typical half-height width of a UV-visible absorption is between 2000 and 5000 cm^{-1}.

The IR spectrometer sees the excitation of bond vibrations and associated rotational motions. The IR spectrum comprises many relatively sharp features that provide an excellent fingerprint for identification. A typical half-height width of an IR absorption band is 10–20 cm^{-1}.

Instrumentation

IR spectrometers consist of three basic components: radiation source, monochromator or interferometer, and detector. Fourier transform IR (FTIR) spectrometers are the most widely used and no major instrument manufacturer provides dispersive instruments any longer.

Dispersive spectrometers

Conventional spectrometers start with an appropriate light source focused onto the entrance slit of a monochromator that splits the light up into its wavelength components. The monochromator exit slit selects a particular emerging wavelength. The monochromatic light passes through a sample, where it may or may not be absorbed before being detected by a light detector (photomultiplier or photodiode). This type of spectrometer is known as a dispersive instrument. To measure transmittance or absorbance, both the incident intensity I_0 and the transmitted intensity I_t need to be measured at every wavelength.

Single-beam dispersive spectrometers

With a single-beam instrument, the I_0 spectrum is measured first with air (or solvent) as a reference in the light beam. In a separate measurement the I_t spectrum of the sample is recorded. Interfacing with a computer allows data to be stored and processed automatically. To ensure accuracy and precision, all components in the instrument (light source, detector and electronics) need to be very stable to ensure that I_0 does not drift.

Nowadays, single-beam dispersive IR spectrometers are likely to be found only in monitoring processes (e.g. environmental pollution). The FTIR spectrometer described below normally operates as a single-beam instrument.

Double-beam spectrometer

Double-beam spectrometers are designed to compensate for instrument drifts and the need to determine I_0 and I_t in separate measurements. The layout of a typical double-beam spectrometer is illustrated in Fig. 33.4. Dispersive instruments in the infrared have the sample located next to the light source before the monochromator, unlike their UV-visible counterparts.

IR light from the source, typically an electrically conducting element such as a Globar maintained at about 1300 K, illuminates equally two mirrors M_1 and M_2. The light from mirror M_1 acts as the reference beam; the light from M_2 is the sample beam. Mirrors M_3 and M_4 send the light beams to mirrors on a mechanical chopper. The mechanical chopper is a rotating disc carrying mirrors that alternately reflect the reference and sample beams into the monochromator. After passage through the monochromator, monochromatic light from the sample and reference beams is detected alternately by the single detector as the wavelength drive changes the wavelength that passes through the system. In the reference-beam chopper period the detector registers I_0 and in the sample-beam chopper period I_t is measured. The alternating signals are amplified and their ratio is calculated to give $I_t/I_0 = T$ or $\log(I_0/I_t) = A$. The preferred detector, certainly by the 1980s, was deuterated triglycine sulfate (TGS). This is a pyroelectric detector with an electrical resistance very sensitive to heat (IR intensity).

All measurements can be made simply with air in the reference beam. Placing a cell with solvent in the reference beam enables compensation for unwanted absorption (e.g. from solvents). The measurement cell path lengths in the reference and sample beams must be identical. A computer interfaced with the spectrometer enables post-measurement subtraction of a reference spectrum from the sample spectrum.

The amount of polychromatic IR light that hits the detector from the natural room-temperature black-body radiation of the cell compartment walls can be 10-fold or more than the desired monochromatic IR radiation from the reference or the sample. This background radiation is stray light that severely affects the accuracy of the I_0/I_t value. Locating the sample before the monochromator ensures that the 'chopped' signal selected by the detector system is related almost exclusively to light derived from either the sample or the reference beams. Artificial reduction of absorbance values is thereby greatly reduced. However, locating a sample close to an IR source can cause deleterious heating effects.

Interferometric spectrophotometers

FTIR spectrometers have the sample next to the detector after wavelength selection. This reduces heat effects from having a sample in proximity to an IR source. Locking into the mirror oscillation frequency coupled with the signal filtering associated with the Fourier transform and the improvements in optics and detectors makes this preferred sample position viable.

The FTIR spectrometer incorporates an interferometer in place of a monochromator. The way a FTIR spectrometer operates is presented here with reference to Fig. 33.5.

If the mirror M_1 is set to oscillate along the optic axis, the distance travelled by beam (B + D) varies, while the distance travelled by (C + E) remains unchanged.

Identical (B + D) and (C + E) distances means that D and E are in phase and recombination is fully constructive. As mirror M_1 moves towards the beam splitter, the beam D arrives 'ahead' of beam E;

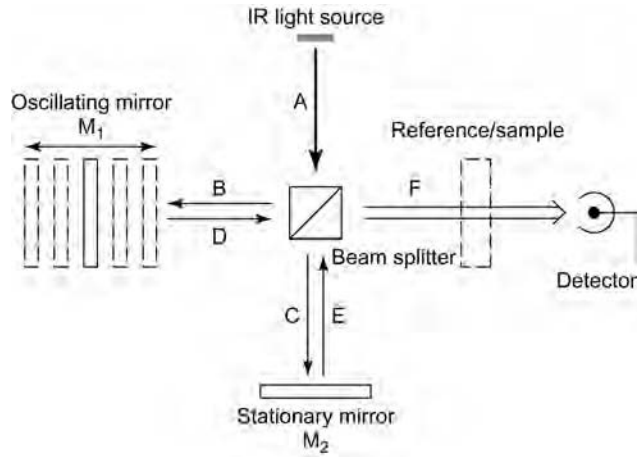

Figure 33.5 Layout of an IR interferometer (FTIR spectrometer).

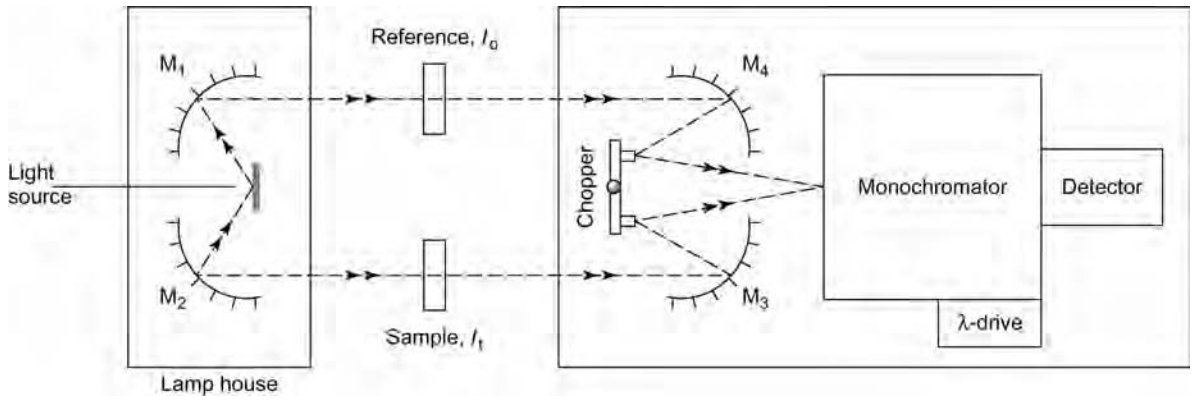

Figure 33.4 Double-beam dispersive IR spectrometer.

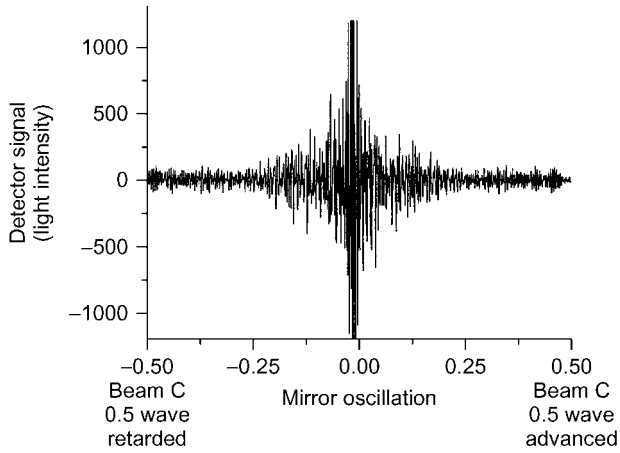

Figure 33.6 Interferogram produced by a single mirror oscillation.

recombination is not fully constructive and the intensity of beam F is reduced and its phase changes. Eventually the mirror movement of M_1 leads to beam D being a half-wave ahead of beam E. The recombination is now fully destructive and the intensity of beam F becomes zero. At this point, the movement of M_1 is reversed past the oscillation mid-point to eventually make D retarded compared with E to give zero intensity at half-wave retardation before returning to the mid-point of the oscillation.

- As the mirror moves back and forth through a single mirror oscillation period, the intensity of a single wavelength of IR light varies considerably. In practice, all wavelengths are passing through the system simultaneously. Accordingly, the total IR light intensity registered as falling on the detector during a single mirror oscillation period is very complicated and takes the form illustrated in Fig. 33.6.
- The signal illustrated in Fig. 33.6 is now subjected to a mathematical procedure called a Fourier transform. This extracts the light intensity versus wavelength (wavenumber) information.
- With no reference or sample in the beam, this measurement is the background or I_0 spectrum, which is stored in the computer to be used as the I_0 for all subsequent transmittance (I_t/I_0) or absorbance [$\log(I_0/I_t)$] measurements during the working session.
- A measurement is now made with the sample in place. A similar interferogram is created. This is also subjected to a Fourier transformation to produce a sample light-intensity throughput spectrum.
- Subsequent data manipulation in the computer produces the transmission or absorption spectrum of the sample.

Technical details

The IR source is a Globar or similar proprietary 'hot' element that operates at approximately 1300 K. The detector needs to have a fast response and low inherent noise. The TGS detector remains the most widely employed for routine use. Pyroelectric devices based upon lithium tantalate are becoming popular as they are less expensive, have greater ordinate linearity and present better temperature stability (TGS linearity falls off above approximately 32°C). For high-precision work with lower noise, liquid-nitrogen-cooled semiconductor detectors are available based on indium–antimonide (In–Sb), indium–gallium–arsenide (In–Ga–As) or mercury–cadmium–telluride (Hg–Cd–Te).

FTIR spectrometers have advantages over dispersive instruments. The interferometer offers greater light collection and throughput. More light means less noise and greater sensitivity. The multiplex advantage concerns the very nature of the interferometric measurement. All wavelengths are observed for a single scan at the same time. In a dispersive instrument, only one wavelength is detected at any one time. In the interferometer, more time is spent effectively measuring each wavelength, even though the total scan time may be the same. More time to measure each wavelength means lower noise and greater sensitivity.

Two options are available to the FT technique: either a spectrum can be scanned much faster than with a dispersive instrument or the same time as for the dispersive instrument can be spent measuring a spectrum to present lower noise results. In practice, a single mirror oscillation in the interferometer produces a spectrum scan in a fraction of a second. A dispersive instrument scan can take up to 10 min. Taking 10 min over an FTIR measurement allows the averaging of very many scans. The signal-to-noise (S/N) ratio in an IR spectrometer is proportional to the square root of the number of scans. Accumulating and averaging 1, 4, 16, 64, 256 or 1024 scans produces a signal-to-noise improvement of 1-, 2-, 4-, 8-, 16- or 32- fold, respectively.

- The level of stray light associated with FTIR spectroscopy is low, typically less than 0.02%, because the technique is devoid of imperfect gratings and the signals selected are associated only with the oscillating mirror movement. Absorbance values remain linear up to two absorbance units, which in turn leads to more accurate quantitative measurements, even with strongly absorbing bands.
- Resolution is generally excellent over the whole spectrum, with an effective measurement spectral bandwidth (SBW) of 1 cm^{-1} being achieved readily.

Data processing

All modern IR spectrometers are computer controlled, with measured data stored digitally. Computer control simplifies the process of running instruments and allows the easy implementation of standard operating procedures (SOPs). Although software is usually manufacturer specific, companies such as Galactic Industries among others have produced software (GRAMS/AI from Thermo Galactic, 395 Main Street, Salem, NH 03079, USA) that operates many instruments and certainly accepts data from effectively all spectrometers on the market.

Computers readily allow changes between spectral units. In the early days, the IR spectrum of a compound was reported as percentage transmittance as a function of wavelength in microns; by the 1970s percentage transmittance as a function of wavenumber became the preferred form. The computer allows the ready conversion between transmittance and absorbance and between microns and wavenumber. Presentation in terms of absorbance/wavenumber is likely to become increasingly more familiar.

Computers readily allow accumulation of spectra. The spectrum of a weak sample can be scanned repeatedly to give an averaged spectrum with appreciably reduced noise and an improvement in sensitivity. For example, there is little difference between the spectra of carbon disulfide and of benzocaine in carbon disulfide (Fig. 33.7), but with spectrum manipulation, a good spectrum of benzocaine is obtained readily

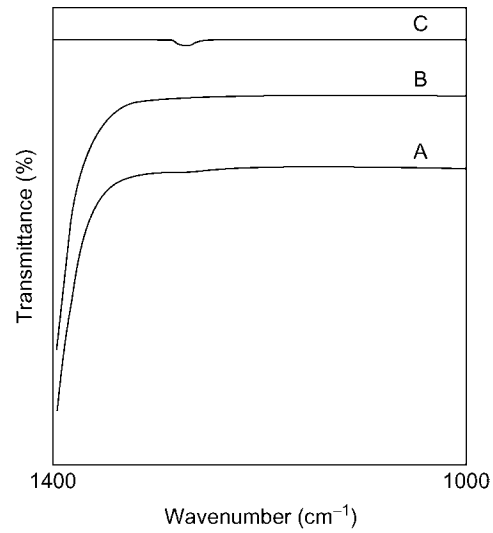

Figure 33.7 IR spectra of benzocaine in carbon disulfide (A) and of carbon disulfide (B). (C) is the the difference spectrum (A – B). (Courtesy of Perkin-Elmer Ltd.)

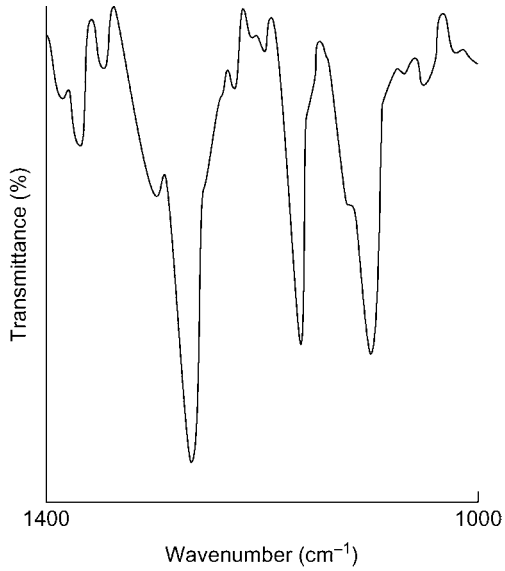

Figure 33.8 The difference spectrum of benzocaine shown in Fig. 33.7 smoothed, corrected for baseline contribution and with percentage transmittance scale expanded 200 times. (Courtesy of Perkin-Elmer Ltd.)

(Fig. 33.8). The amount of benzocaine in the cell was approximately 4 μg but only about a quarter of this was in the IR beam. Each spectrum was recorded in less than 30 s.

Digitised spectra can be corrected easily for solvent absorption or the presence of impurities. Various mathematical procedures can be applied, which include baseline corrections and levelling, smoothing, the determination of peak bandwidths and the calculation of absorption band areas. Derivative spectra can be produced to help distinguish the contributions of overlapping components in an absorption band.

The identification and interpretation of spectra are assisted greatly by computer analysis. Spectra can be readily overlaid for comparison. The spectrum of a sample can be compared with a library of spectra (database) and a list of the compounds of best fit can be either displayed on a screen or printed out. The presence of certain functional groups can also be confirmed.

Six factors need to be considered when calibrating an IR spectrometer:

- Wavelength (wavenumber) scale
- Absorbance scale
- Stray light
- Spectral resolution
- Data resolution
- Time scale of measurement (time constant).

Wavenumber (wavelength) scale

A card carrying an accredited transparent polystyrene film approximately 35 μm in thickness is used to calibrate the wavenumber (wavelength) scale, which should have the form illustrated in Fig. 33.2 and Fig. 33.9. Peak locations and tolerances recommended in the *British Pharmacopoeia* (2008) are given in the inset in Fig. 33.9.

Absorbance scale

Unfortunately, there are no internationally recognised standards for IR spectra. The absorbance scale is set in the factory with the aid of complicated optics. Filters are available in the 4000–2000 cm^{-1} range. The high background black-body radiation that falls on the detector from sources other than the sample means that the ideal absorbance for good signal-to-noise ratio is approximately 0.4.

Stray light

Stray light can be tested by introducing a neat solvent or very high-concentration sample into the sample beam so that at the wavelength (wavenumber) of interest the expected absorbance is in excess of $A = 5$. Effectively all the relevant light at this wavelength (wavenumber) has been absorbed.

Spectral resolution (spectral bandwidth)

In dispersive instruments, the SBW is set by the entrance and exit slits of the monochromator. In the FTIR spectrometer, the 'depth' of the Fourier transformation sets SBW with typical values of 1 cm^{-1}, 2 cm^{-1} and 4 cm^{-1} available in the spectrometer control software. The narrower the SBW the more faithfully are sharp absorption bands registered; however, the noisier the spectrum (less light), the more time (number of accumulations) is required to keep the noise level down.

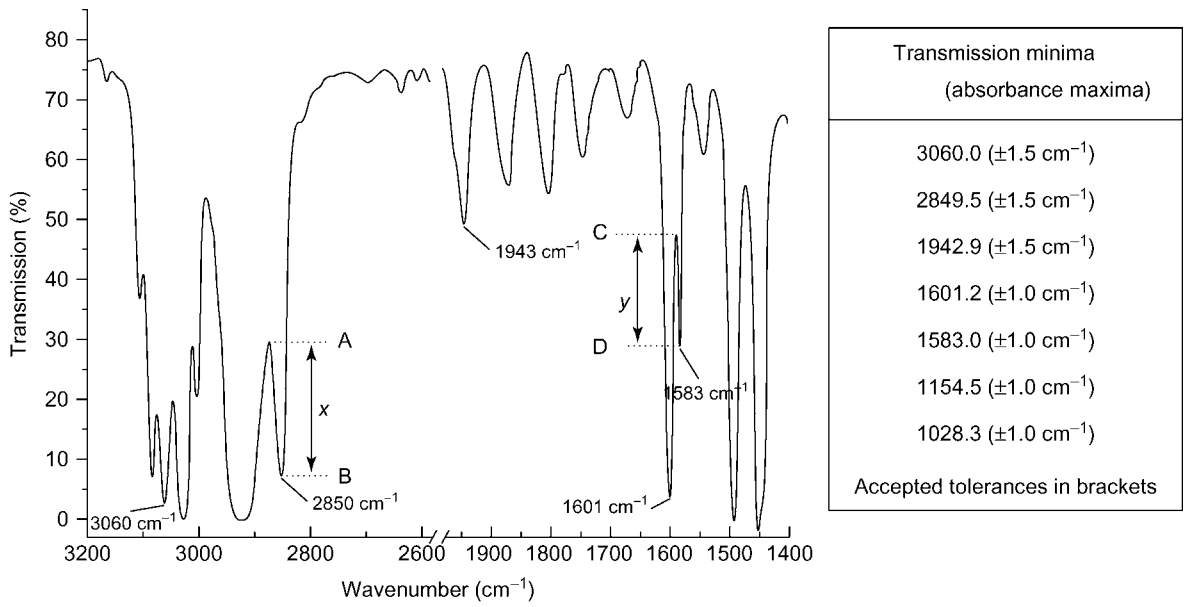

Figure 33.9 Zoomed-in regions of the IR spectrum of a 40 μm thick polystyrene film indicating the location of critical parameters. A whole spectrum is illustrated in Fig. 33.2.

The *British Pharmacopoeia* (2008) recommends the following to ensure good spectral resolution:

> For instruments having a monochromator, record the spectrum of a polystyrene film approximately 35 µm in thickness. The difference x [Fig. 33.9] between the percentage transmittance at the transmission maximum A at 2870 cm^{-1} and that at the transmission minimum B at 2849.5 cm^{-1} must be greater than 18. The difference y between the percentage transmittance at the transmission maximum C at 1589 cm^{-1} and that at the transmission minimum D at 1583 cm^{-1} must be greater than 10.
>
> For Fourier-transform instruments, use suitable instrument resolution with appropriate apodisation prescribed by the manufacturer. The resolution is checked by suitable means, for example by recording the spectrum of a polystyrene film approximately 35 µm in thickness. The difference between the absorbances at the absorption minimum at 2870 cm^{-1} and the absorption maximum at 2849.5 cm^{-1} is greater than 0.33. The difference between the absorbances at the absorption minimum at 1589 cm^{-1} and the absorption maximum at 1583 cm^{-1} is greater than 0.08.

This recommendation is very specific for a 35 µm thick film of polystyrene.

Data resolution

In a computer, spectra are stored as lists of wavenumber and transmittance (absorbance) data pairs. These X,Y data pairs are plotted on demand. A sufficient number of data points are required to give an undistorted picture. Too many points may be unnecessary and require excessive memory allocations. Figure 33.10 illustrates the effects of spectral and data resolution on the spectral integrity of the IR spectrum of a 40 µm thick polystyrene film.

The terms spectral resolution and data resolution are often confused. Some instruments, in modifying the Fourier transform mathematics to reduce the SBW, also produce a change in the data resolution of the spectra computed. The 1154 cm^{-1} band of polystyrene is reproduced in Fig. 33.10 measured with different SBW and data resolutions. Inspection of the spectra presented in Fig. 33.10 indicates that data resolution can be a more important issue than spectral resolution. In general, for typical drug molecules, a SBW of 4 cm^{-1} is probably sufficient to describe faithfully the natural bandwidth of a vibrational absorption band. However, a data resolution of 1 cm^{-1} is preferred to ensure that the measured data are a faithful representation of the capability of the spectrometer. Increasing SBW, reducing data resolution, or both, can reduce noise.

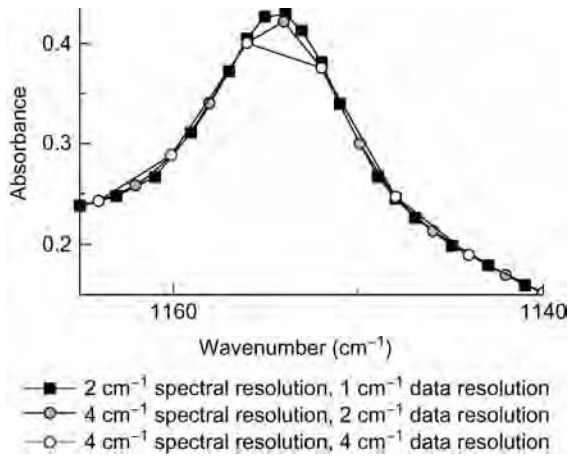

Figure 33.10 Zoomed-in region of the IR spectrum of a 40 µm thick polystyrene film illustrating the effect of SBW and data resolution.

Time scale of measurement (time constant, averaged scans)

In analogue dispersive instruments, the scan speed refers to the rate of rotation of the monochromator diffraction grating, which in turn controls the rate of change of the wavelength (wavenumber) that comes from the monochromator exit slit. The instrument has an inherent controllable response time (time constant). Scanning too fast means that measured peaks are distorted and flattened (reduced intensity); the instrument is being run too fast for the electronics to cope with the changing signal from the detector. A scan should be fast enough to avoid time wastage, yet slow enough to leave the spectrum undistorted. The lower the response time (time constant), the lower the noise but the longer the time required to measure spectra. The choice of time response versus scan speed is sample dependent and needs to be selected on the basis of experience.

In an FTIR spectrometer, the scan speed is set by the oscillation rate of the moving mirror and the computer Fourier transformation speed. A typical spectrum can be accumulated in a second or so. The instrument operator has no control of this. The time to measure an FTIR spectrum is set by the number of scans accumulated and averaged, and the speed of the computer (see Technical details earlier).

Sample preparation

A major advantage of IR spectroscopy is the ability to measure relatively heterogeneous materials and poorly characterised samples, particularly in condensed phases (e.g. creams, powders, crystalline materials). By their nature, these samples are often not chemically pure and IR spectroscopy can identify or confirm the existence of major constituents. IR spectroscopy is often used to demonstrate that a sample is concordant with expectation.

Nevertheless, it is essential to have pure samples to act as standards for IR spectroscopy. A major difficulty can be that of purifying and handling a few micrograms of material without substantial losses, although these problems have largely been overcome by using fractional crystallisation or chromatography as a prelude to IR spectroscopy. On-line FTIR spectroscopy is possible, but is largely a research tool. Nowadays, the identification of samples in minute amounts is achieved by other techniques, such as nuclear magnetic resonance (NMR) spectrometry (see Chapter 36) and mass spectrometry (MS) (see Chapter 37). This is particularly the case with 'hyphenated' techniques, which involve the use of spectrometric methods on-line with a chromatographic process. Nevertheless, IR spectroscopy has an important role to play in identifying functional groups.

However, the isolation of pure samples of an analyte for IR spectroscopy can still be an important issue. When the starting material is a residue from the evaporation of a solvent extract of urine, blood, tissue or other material, the most suitable method of purification is some form of chromatography.

Thin-layer chromatography

Suitable systems for thin-layer chromatography (TLC) are described in Chapter 39. Any of these systems is potentially useful when it is required to elute a spot, but reversed-phase systems should be avoided because it is difficult to remove the spot without the stationary phase, which would interfere with the IR spectrum. Furthermore, location reagents must be chosen with care, and a destructive reagent, such as the Marquis reagent for alkaloids, should not be used. Non-destructive reagents, such as iodoplatinate solution, can be used because the coloured complex is decomposable to yield the original compound. However, even this procedure may introduce extraneous peaks into the spectrum and, ideally, location reagents are best avoided. If the compound cannot be detected under UV light, it could be applied to the thin-layer plate twice and only a portion of the chromatogram sprayed, which thus allows the unsprayed portion to be eluted.

The use of aqueous acid or alkali to elute the compound from the thin-layer plate, followed by solvent extraction of the aqueous solution, is more efficient than direct solvent extraction of the adsorbent. In one

method of direct extraction, the adsorbent is scraped from around the spot, the glass adjacent to the spot is carefully cleaned and the adsorbent is eluted *in situ* directly onto a wall of potassium bromide (KBr) built around the tip of the spot. The KBr is then pressed into a disc. This technique is suitable only for well-resolved spots. Elution of the spot sideways reduces contamination from compounds that are not resolved as well. The recovery of material from chromatograms varies from nil to over 70%. Compounds that contain hydroxyl and carboxyl groups, which can readily form hydrogen bonds with the solid support, tend to be recovered in low yield. Considerable interference in the 1100 cm^{-1} region is found with some adsorbents and compounds.

In a variation of this method, the thin-layer adsorbent is placed in the bottom of a glass vessel together with a triangular 'wick' of compressed KBr. Solvent is added and it rises up the wick and evaporates from the upper region. The compound is conveyed up the wick by the solvent and accumulates at the tip of the triangle, which is then cut off, dried and used to prepare a disc. About 10 μg of compound is required to produce a satisfactory spectrum. The advantage of this technique is that the lower part of the KBr wick acts as a filter and removes finely divided adsorbent, which can give rise to spurious peaks.

In a further method, the thin-layer adsorbent is scraped onto a small amount of KBr powder in the hub of an 18-gauge metal hypodermic needle. A 1 mL glass syringe is filled with pure solvent and connected to the needle, and the compound is eluted dropwise onto a mound (10 mg) of dry KBr powder. Each drop of solvent is allowed to evaporate completely before the elution of the next drop. The powder and solute are then mixed and pressed into a disc.

Eluted material almost always includes unwanted extraneous matter co-extracted from the thin-layer chromatogram. Thus it is advisable to use the eluent from a 'blank' area as a reference solution. Contamination from plasticisers, solvents and dirty glassware can also be a serious problem when a spectrum has to be obtained from a few micrograms of a compound. Even momentary contact of dry adsorbent with plastic tubing can remove appreciable quantities of plasticisers. Hence the following precautions should be taken:

- Use the minimum amount of the purest adsorbent available.
- Elute with less than 1 mL of a solvent that contains <0.0001% (1 ppm) of non-volatile residue.
- Keep sample handling to a minimum.
- Clean all glassware with an efficient detergent in an ultrasonic bath.
- Avoid contact of materials and samples with plastics.

Gas chromatography

Gas chromatography (Chapter 40) can provide a very convenient method of obtaining pure samples for IR spectroscopy. However, the sample can still be contaminated with impurities eluted from the stationary phase. The effluent from the chromatograph is a hot vapour and the problem is to obtain small quantities in a form suitable for presentation to the spectrometer. The spectrum of the vapour can be recorded directly or the compound can be trapped and then its spectrum recorded. Unfortunately, there is no entirely satisfactory method for the direct coupling of a gas chromatograph to a standard dispersive IR spectrometer. The outlet of the gas chromatograph can be split and one part connected to a heated cell (or light pipe) placed in the beam of an IR spectrometer. The gas flow is then stopped, trapping the sample in the cell, and the spectrum is recorded in the vapour phase. This technique can provide acceptable spectra of volatile compounds such as butyl acetate, which has a strong carbonyl band, but spectra of less-volatile compounds such as caffeine and phenylbutazone are more difficult to obtain. The temperatures of the connecting pipe and cell are clearly of great importance to keep the compounds as vapours. The coupling of a gas chromatograph to an FTIR instrument is much more satisfactory because the speed of scanning is sufficiently rapid to enable the spectrum of a compound to be recorded as it is eluted. Nevertheless, the temperatures of the cell and pipework are still of critical importance.

The method used to trap a compound depends on whether it is a solid or a liquid and, if the latter, on its volatility. Ways in which small samples can be obtained from a gas chromatograph in a form suitable to present to the spectrometer are given below. The main difficulty, common to all these methods of collecting fractions, is to determine the optimum temperature of the outlet tube from the chromatograph and the temperature of the collecting device. This problem can be resolved only by trial and error.

Cooled tubes

Most techniques for collecting the effluent employ cooled tubes of glass or metal, but it is difficult to obtain good recoveries of a few micrograms of compounds of different volatilities by any one technique. Drugs such as the barbiturates and phenothiazines can be recovered in 50–70% yields in glass or metal capillary tubes held at room temperature, whereas more volatile drugs, such as the amfetamines, need to be cooled in liquid nitrogen or solid carbon dioxide (Curry *et al.* 1968; De Leenheer 1972).

Alkali halide tubes

A straight tube that contains a plug of powdered alkali halide is connected to the outlet of the chromatograph. The effluent condenses on the halide, which can then be pressed into a disc. This technique is most useful for compounds that are solid at room temperature.

High performance liquid chromatography

High performance liquid chromatography (Chapter 41) provides a very convenient method of purification, particularly if gas chromatography is inapplicable or derivatisation of the compound is necessary. Unlike gas chromatographs, liquid chromatographs are usually operated at or slightly above ambient temperature, and most types of detector are non-destructive. Thus, the appropriate fraction of eluate can be collected by holding a test-tube under the exit port.

The method used to retrieve the sample from the eluate for presentation to the spectrometer depends upon whether the compound is a solid or a liquid and, if the latter, on its volatility and the quantity present. All the common solvents absorb in the IR region. However, with the data-processing facilities of modern IR spectrometers, this is not a great disadvantage. The spectrum of the solvent can be recorded and then subtracted from the combined spectra of the compound and solvent to give a difference spectrum. If the concentration of the sample is low, the difference spectrum can be enhanced either by repetitive scanning and signal averaging or by expansion of the ordinate scale. In many cases, however, the amount of material is too small to enable the compound to be collected and transferred to standard cells.

Alternatively, the compound can be recovered by evaporation of the solvent. However, evaporation also concentrates any non-volatile impurities in the solvent, so the use of pure solvents is essential. Another possible source of contamination is the packing material used in liquid chromatography columns. Many of these materials are based on silica gel and appreciable amounts of silica may be dissolved by certain solvents.

Microsublimation

This simple technique can be highly effective in purifying certain compounds (Fig. 33.11). Drugs may be sublimed from an evaporated solvent extract in the tube onto the cold finger of the apparatus, and the sublimate transferred by grinding the KBr powder gently with the cold finger.

Sample presentation

IR spectra can be measured in the gas, liquid or solid phase. However, most compounds of interest are solids at room temperature. In principle, an IR spectrum can be obtained from as little as 1 μg of a compound. From a practical point of view, quantities of the order of 200–1000 μg are much easier to handle. Very small quantities require greater sensitivity to be achieved with micro-cells, placing as much material as possible in the IR beam. Micro-cells require beam-condensing optics to focus as much light as possible through the microcell assembly.

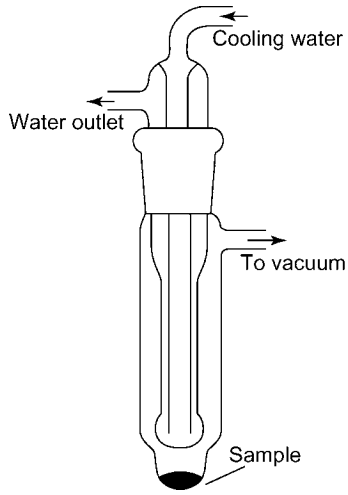

Figure 33.11 Apparatus for microsublimation.

Glass and silica contain SiO–H and Si–O bonds that can vibrate and strongly absorb IR radiation. Therefore, cell windows need to be fabricated from ionic materials with bonds that do not have vibrations. Crystal lattice vibrations cause absorption in the far IR. Barium fluoride (and calcium fluoride) are excellent for aqueous media, although window absorption prevents measurements below 1000 cm^{-1}. Silver chloride windows are water resistant and will allow transmission down to 400 cm^{-1}, but are friable. The most popular IR window material is sodium chloride, which is water soluble but transparent down to 600 cm^{-1}.

Gases

In normal laboratory experiments, IR spectroscopy is used only rarely for analysis in the gas phase. Gases are likely to be at a very low concentration and special long-path, airtight gas cells are required. These cells normally have sodium chloride windows, and mirrors may be used to reflect the light through the gas cell several times to achieve a very long path length. The detection of environmental gases is a typical application of gas-phase IR spectroscopy.

Liquids and solutions

Liquids have a very high molarity. Thus chloroform, with a relative molecular mass of 119.4 and density of 1.48 g/mL, can be said to be 12.4 mol/L. According to Beer's law, to detect the C–H stretch ($\varepsilon \approx 10$ L/mol per cm) with absorption $A = 0.4$, the path length required, given by $l = A/(\varepsilon c)$, is approximately 3×10^{-3} cm.

Non-volatile, neat liquids can be measured simply by placing a drop between two IR transparent plates and pressing the plates together to ensure a narrow (<0.1 mm) measurement path length (Fig. 33.12). Volatile liquids may need proper sealed liquid cells.

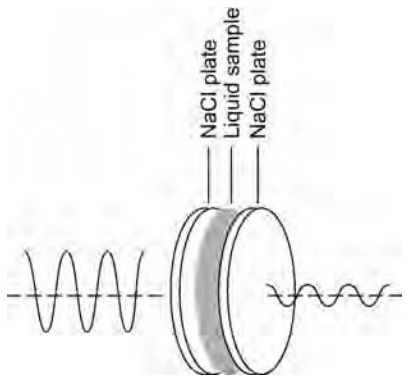

Figure 33.12 Liquid sample or Nujol mull sandwiched between two NaCl plates.

Liquid cells usually consist of two parallel transparent windows (0.1–0.01 mm apart) separated by a precise gasket made of Teflon or lead and fitted with inlet and outlet ports. Cells with variable path lengths are also available, in which one window is retained on a screw that can be finely adjusted to give a precise path length in the 0.1–0.01 mm range. These cells are particularly useful to vary path length to accommodate for solvent absorption and variations in concentration ranges. Solvent absorption can be accounted for in a computer by comparison of the solution and solvent spectra.

The number of solvents suitable for IR spectroscopy is limited. The measurement of the IR absorption of a solute is possible only in a spectral range for which the solvent is relatively transparent. Carbon tetrachloride and carbon disulfide, which lack hydrogen and contain a minimal number of bond types, are often suggested as the most useful solvents because they have relatively few absorption bands in the IR region. However, they have poor solubilisation characteristics. In practice, solvent choice is based upon solubility and IR transparency at the wavenumber of interest. Deuterated solvents can help open regions for analysis. Chloroform–deuterochloroform, acetonitrile, water–deuterated water, toluene and dioxane are good solvents to consider.

To overcome the inherent absorption of solvents and the relatively low extinction coefficient of a vibration, path lengths are short and concentrations are high. The concentration of the test compound is usually about 5–10%, but concentrations up to 20% (w/v) can be employed. With these high concentrations, hydroxyl and amino compounds often exhibit bands caused by intermolecular hydrogen bonding. Interactions between the compound and the solvent can occur, which may result in changes in the intensity and wavenumber of bands in different solvents and the breakdown of Beer's law.

IR solvents are often volatile and require very short path lengths. This combination can lead to solvent evaporation, which produces large concentration changes. In the older-style IR spectrometers, the heat of IR radiation could cause evaporation – this is less of a problem with FT systems with the sample placed after the interferometer. An example is presented here of the determination of the amount of dimeticone in a cream formulation.

Dimeticone can be extracted from creams with 4% (w/w) liquid paraffin in toluene and quantified by reference to standard solutions based upon the Si–O stretching vibration at 1260 cm^{-1} in a 0.1 mm sodium chloride liquid cell (Fig. 33.13).

Solids

Solids are generally examined either as thin films or as dispersions in either liquids or solids. The ideal sample for IR transmission measurements is clear, visually transparent and homogeneous. This can be difficult to achieve with solids. Heterogeneous samples that are optically poor with large sample particles can introduce light scattering and the Christansen effect. Light scattering becomes significant when the particle size is more than 1/20 of the wavelength of the incident light. Light scattering produces a spectrum offset that is curved, with high light scattering at shorter wavelengths/higher wavenumbers and lower light scattering at longer wavelengths/lower wavenumbers. Samples must be ground until particle sizes are less than 1 μm. The Christansen effect, which results from severe refractive index changes at the sample surface, leads to distorted band shapes. Peaks take on an S-shape with an apparent reduced (or even negative) absorption at the longer wavenumber edge. As a result, asymmetrical bands may be observed that vary in position and intensity from true values. For transmission measurements, the sample must ideally have the appearance of a 'perfect glass'. To reduce the light scattering and the Christansen effect, all components of the sample must have a very small particle size ($\leq$1 μm) and they must be dry.

The polymorphic form of a solid sample can affect the IR spectrum. This is an important issue in the pharmaceutical industry, as the rate of dissolution of a solid drug can depend upon its crystal morphology. Success has been achieved by simply crushing the sample between two diamond windows in a device known as a diamond anvil (Fig. 33.14).

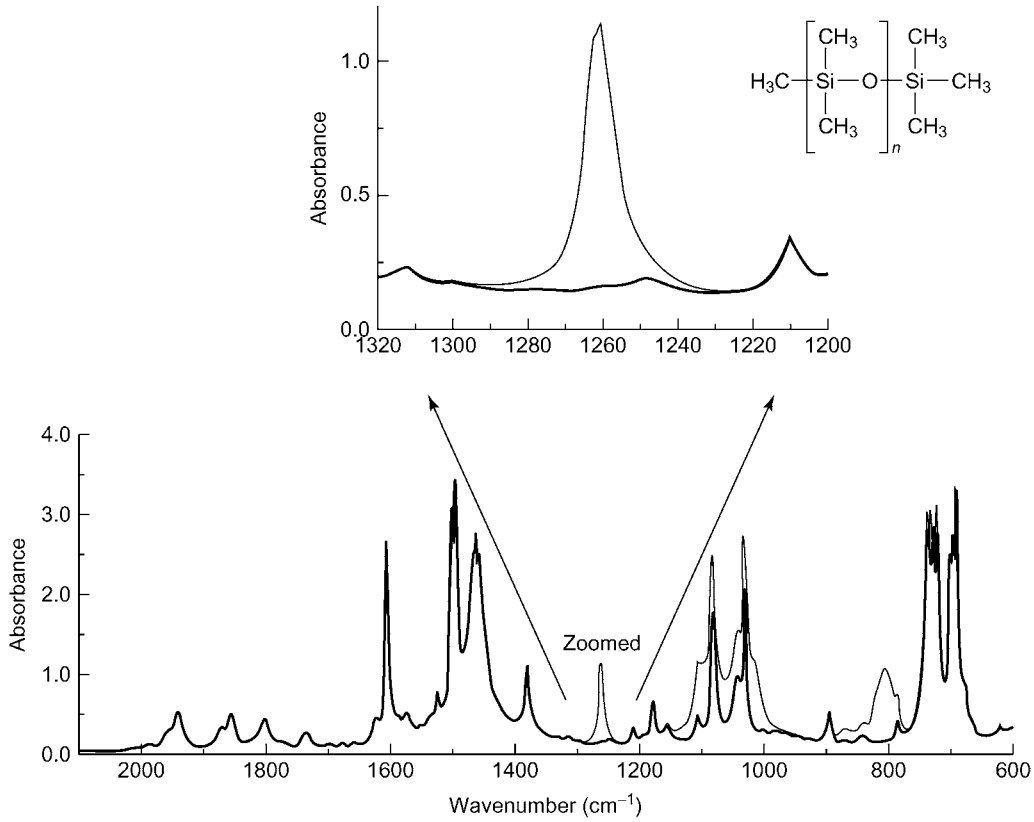

Figure 33.13 The IR spectrum of dimeticone dissolved in 4% (w/w) liquid paraffin in toluene: (heavy line) 4% (w/w) liquid paraffin in toluene; (light line) 1.5% (w/v) dimeticone dissolved in 4% (w/w) liquid paraffin in toluene.

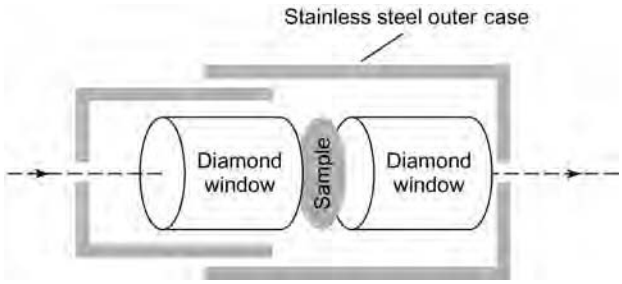

Figure 33.14 A diamond anvil.

Mulls

Solid compounds are dispersed in a liquid, such as liquid paraffin (Nujol). The finely powdered compound (about 1–10 mg) is mixed with one drop of the liquid and ground in an agate mortar. The test sample must have the constituency of a smooth, thin cream. The mull is spread onto an alkali halide plate, usually sodium chloride or KBr, and another plate is placed on top, taking care to exclude air bubbles. The plates are pressed together strongly. A disadvantage of this method is that the spectrum of the mulling agent is superimposed upon that of the sample. Consequently, liquid paraffin cannot be used if the C–H stretching vibrations are to be examined, and a halogenated liquid, such as 'Fluorolube' (a fluorinated hydrocarbon) or hexachlorobutadiene, must be employed.

Alkali halide discs

The technique of dispersing the compound in an alkali halide has been used widely in the identification of drugs. Originally, KBr was used and the technique is still often referred to as the 'KBr technique'. However, potassium chloride (KCl) is superior to KBr because it is less hygroscopic. Storage of the alkali halide in an 80°C oven helps to ensure anhydrous conditions.

The finely powdered, dry, test compound (about 1 mg) is mixed with the alkali halide (about 250 mg) and ground either mechanically in an agate ball mill or by hand in an agate mortar. A texture approaching that of talcum powder is a good consistency. The mixture is pressed under approximately 10 tons pressure in a purpose-designed press to produce an optically good, thin disc (Fig. 33.15). A vacuum helps to retain dry conditions and smooth disc formation. The pressure is applied for 10 min.

If only small quantities of the compound are available (about 200 μg), a thin cardboard mask with a slot in the centre can be used. The mask is placed in the die and the slot is filled with the mixture before pressing. A mask is often employed routinely because it provides a support for the alkali halide and so enables the disc to be handled more easily. Microdiscs of diameter down to 0.5 mm can be prepared by using

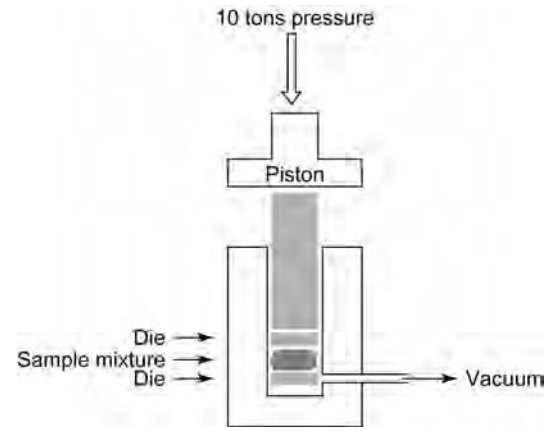

Figure 33.15 A KBr disc press.

metal (lead or stainless steel) discs of 13 mm diameter with a hole of the appropriate size in the centre. The hole is filled with KBr (about 1 mg) that contains from 0.05% to 0.2% of the sample, which is then pressed in the usual way. The metal discs should be washed before use in both polar and non-polar solvents and finally in good-quality acetone to remove traces of oil and grease, which may produce artefacts in the C–H region of the spectrum. The method may fail if excessive pressure is used, as this causes deformation of the lead disc.

Another useful technique consists of dissolving the compound in a small volume of chloroform and drawing it into a Hamilton-type syringe held in a repeater holder. A small cluster of fine KBr particles is picked up on the end of the needle by a trace of chloroform expressed from the needle. The solvent is evaporated gently and the rest of the solution is fed into the KBr from the syringe as it evaporates. A disc is then made from the powder. It is important that the end of the needle be cut at right angles to the shaft and ground flat; needles supplied for use with liquid chromatographs are suitable. For bases one must decide whether to evaporate solvents without the addition of hydrochloric acid, and accept the consequent loss of certain amines by volatilisation, or to add hydrochloric acid and accept the reduced solubility of the amine hydrochlorides in chloroform. Considerable losses of the sample by evaporation may occur for other types of compound (e.g. phenols), particularly when dilute solutions are used.

KBr is hygroscopic, which means that it is sometimes difficult to remove the last trace of water, and so AgCl may be used instead. An indentation about 0.8 mm deep and slightly wider is made in the centre of a small piece of silver chloride sheet, and a solution (about 0.1 μL) that contains as little as 500 ng of substance is placed in the indentation and gently warmed to evaporate the solvent. The sheet is then placed in a die, which produces a cone of silver chloride with the sample embedded in it. A similar cone of plain silver chloride is mounted in the reference beam. Excellent spectra can be obtained with this technique.

The alkali halide discs can be stored in a dry environment and give good spectra several years after preparation. A well-prepared disc should have over 80% transmittance in regions where the sample does not absorb, although it will not necessarily be visually clear. It is not always easy to obtain a good disc when a very small amount of a recovered drug is available. In these circumstances, attenuation of the reference beam can 'sharpen' the spectrum. Another technique is to heat the alkali halide disc to about 80°C for 30–60 min with an IR lamp to evaporate any absorbed water. However, the high temperature accentuates the disadvantages of the alkali halide disc technique. In addition, the following artefacts have been observed:

- Formation of anhydrides from carboxylic acids
- Reversion of ketals and cyanohydrins to the parent ketone
- Loss of water from secondary alcohols.

Several disadvantages are inherent in the alkali halide disc technique. The alkali halides that are generally used are hygroscopic, and it is very difficult to exclude all traces of water. This often results in an O–H band in the spectrum. A number of compounds that contain O–H groups either form hydrogen bonds with the alkali halide or are adsorbed on its surface, so the method is unsuitable if the O–H band is to be examined. In such cases, polytetrafluoroethylene (PTFE) powder can sometimes be used in place of the alkali halide. Polymorphism occurs in many compounds and the grinding and pressing can alter the crystal form and consequently the spectrum. Splitting of bands also frequently occurs. Another disadvantage is the possibility that chemical changes will occur during the preparation of the disc. For example, double decomposition can occur:

$$\text{Base HCl} + \text{KBr} \rightarrow \text{Base HBr} + \text{KCl}$$

Hence, preferably hydrochlorides should be examined in KCl. Bromide may be oxidised to bromine by some compounds, particularly strong oxidising agents, which may result in a disc becoming either discoloured or having yellow–brown spots. If the sample is a potential oxidising agent, other techniques of sample preparation should also be used to check the reliability of the spectra obtained from the alkali disc.

Organic compounds that contain nitrogen in a functional group should not be used with plates that are made of thallium bromide and thallium iodide as they appear to react with the plates.

Despite these disadvantages, the technique is still very useful for solid drugs. The advantages are that, besides being easy to use, the absorption of the alkali halide is very low and the quantity of compound required is small. The discs can easily be stored for reference purposes or the compound can be recovered if required.

Thin films

This method is of use if it is necessary to obtain spectra free from the dispersing media. The film can be prepared either by melting the solid and pouring it onto a suitable plate or by evaporation of a solution on an IR transparent plate.

Measurement of strongly absorbing or strongly light-scattering samples

IR light incident on solids, powders or other materials such as creams is transmitted only poorly, if at all, because of the long path lengths and light scattering. Neat liquids and solutions need very narrow path lengths to overcome solvent absorption. However, scattered light or reflected light can be monitored in these cases and these techniques can be used to examine intact pharmaceutical preparations.

Light scattering

IR light falling on a powder can be reflected in two ways. The light can be reflected truly in the sense of mirror reflection (angle of incidence equals the angle of reflectance); this is known as specular reflectance. Alternatively, the IR light can be scattered, in the Rayleigh scattering sense, over all angles with a scattering intensity related to particle size; this is often referred to as diffuse reflectance. Specular reflectance is related to the refractive index of the sample, and intensity versus wavenumber data are difficult to interpret. Diffuse reflectance, on the other hand, is more simply related to light intensity; if the light is absorbed at the surface, it cannot be scattered back. A typical accessory, often given the acronym DRIFT (diffuse reflectance IR Fourier transform spectroscopy), is illustrated in Fig. 33.16. The sample is placed on a sample tray located beneath two ellipsoidal mirrors M_3 and M_4. Heterogeneous powders and fibres often benefit from being ground and 'diluted' with

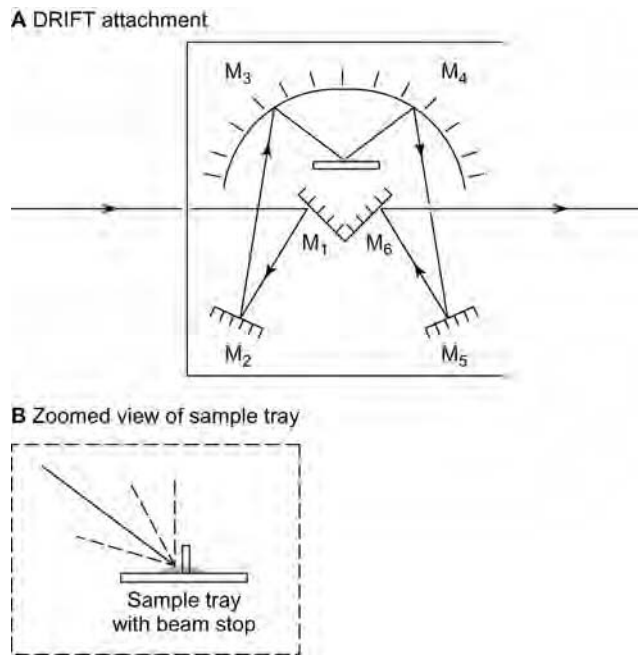

Figure 33.16 DRIFT attachment: IR light from the interferometer strikes mirror M_1 and is directed onto the sample tray by mirrors M_2 and M_3. In (A) the pure reflection path is highlighted. In practice, a beam stop as in (B) blocks the specular reflectance and only the diffuse reflected (scattered) beam is collected by mirrors M_3 and M_4 and directed towards the detector by mirrors M_5 and M_6.

KBr. Pure KBr can be used as the reference material in a separate measurement for the reference spectrum. A good sample can be produced by rubbing a solid sample with silicon carbide abrasive paper (68 μm) to produce a sample of approximately 150 μg over a 35 mm^2 area.

Diffuse reflectance is a measure of intensity versus wavenumber data, normally in a single-beam configuration. To ensure that measurements are at least approximately proportional to concentration, a Kubelka–Munk $f(R)$ transformation can be applied (Kubelka, Munk 1931):

$$f(R) = \frac{(1-R)^2}{2R}$$

where R is the reflectance of the sample. This transformation may be applied at an individual wavenumber or across the complete spectrum.

Alternatively, data can be presented as $-\log(R)$ versus wavenumber.

Attenuated total reflectance

Light that arrives at an appropriate angle to the boundary between two media (or materials) with appropriate refractive indices n_1 and n_2 can be reflected back into the first medium (Fig. 33.17A). This is known as internal reflectance. For this to happen the light beam must have at least sampled the second medium, if only to a depth of approximately 10 μm. The light in this fine slice of the second medium is referred to as an evanescent wave. If the second medium has absorption properties, this is sensed by the evanescent wave and the reflected beam has a reduced (attenuated) intensity, the attenuated total reflectance (ATR). The detected beam now provides intensity versus wavenumber characteristics that are effectively the absorption spectrum of the second medium.

Several proprietary attachments on the market are based upon a single rhomboid prism, of which an example is illustrated in Fig. 33.17. Suitable optical materials for medium 1 are zinc selenide (ZnSe), germanium and diamond. A sample well is created on the side of the rhomboid optical block. A typical ZnSe block is 50 mm × 1 or 2 mm, which gives 15–45 reflections, depending on rhomboid angles. In this case, 5–10 μm sections of medium 2 are sampled 7 to 22 times, which results in an effective optical path length of the order of 35–220 μm. The path length is reproducible and samples are easy to change in comparison with the equivalent simple transmission spacer cell, although the latter may be preferred for simple solutions. Any material that forms a good optical contact with the prism can, in principle, be measured (solutions, oils, waxes, creams, pastes, powders and films).

More sophisticated devices exist, such as such the DuraSamplIR attachment (Fig. 33.18) supplied by SensIR Technologies, (Warrington, UK). The IR radiation from the interferometer is directed into a ZnSe prism by mirror M_1. Subsequent internal refection directs the IR radiation through a diamond prism, where the evanescent wave is reflected back through the ZnSe prism and then onto the detector via mirror M_2. In principle, a powder sample, with no sample preparation, is placed on the diamond prism surfaces where it is compacted by a plunger. Powders, films, solutions, etc., can all be measured with equal ease and no sample pre-treatment. This device produces excellent results; baselines are flat as the technique is not dependent on light scattering.

Interpretation of spectra

A non-linear molecule has $(3N-5)$ fundamental (normal) modes of vibration (this excludes overtones and combinations). Thus a molecule such as paracetamol (see Fig. 33.21) with the formula $C_8H_9NO_2$ has $(3 \times 20 - 5) = 55$ fundamental (normal) modes of vibration. Assigning 55 peaks in an IR spectrum is a daunting task at the very least. Therefore, the total molecular structure of a drug is unlikely to be determined directly from IR spectral information alone.

There are three aspects to identifying a chemical entity. In the first instance, the properties (biological, chemical and spectroscopic) of a

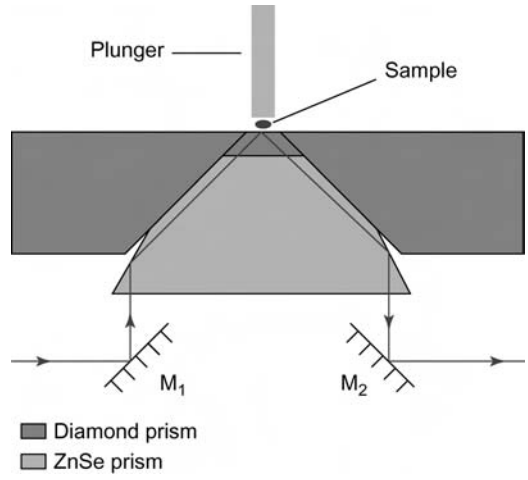

Figure 33.18 The DuraSamplIR attachment.

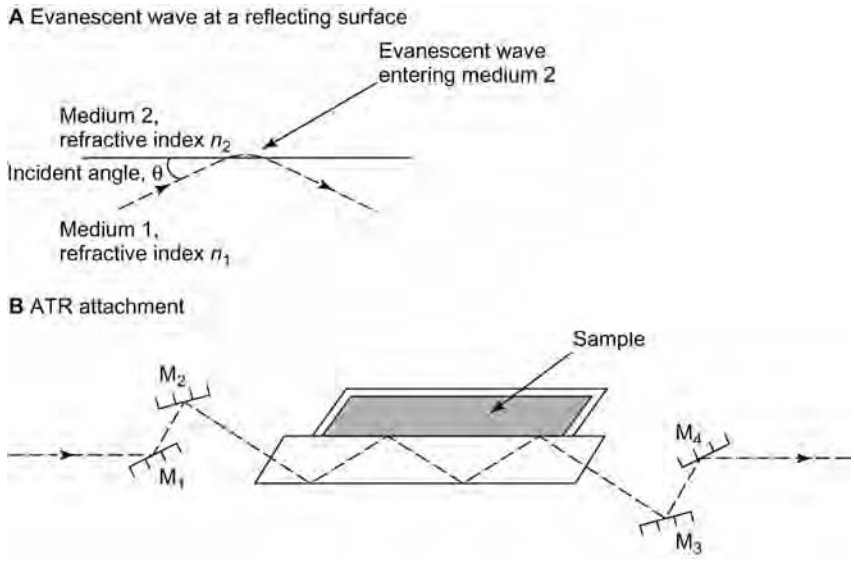

Figure 33.17 (A) The evanescent wave senses medium 2. (B) The ATR attachment, with light directed by mirrors M_1 and M_2, enters the rhomboid prism at 90°. Here the sample is measured twice (two internal reflections) before the light exits the prism at 90°, and directed to the detector by mirrors M_3 and M_4. In practice, there can be as many as 45 reflections.

drug are assessed and the drug is classified according to its type (e.g. non-steroidal anti-inflammatory drug, barbiturate, steroid). The analyte may be a previously characterised compound, in which case a comparison of data from the unknown with reference data, often termed fingerprint identification, confirms the identity of the compound. This may be possible through computer matching of the spectra. The molecular structure of a new chemical entity will most likely need to be determined by NMR spectroscopy, perhaps in combination with MS. However, information such as the existence of specific functional groups or the elimination of putative structures is of great help in processing the NMR information.

Functional group identification

Table 33.1 lists the important IR vibration frequencies. The precise location of a band often gives an indication of the structural environment of the group (e.g. the C=O group in cyclohexanone and cyclopentanone, the amide bond in an α-helix or β-sheet polypeptide chain). More substantial lists of peak assignments are found in the standard texts on IR spectroscopy (see Further reading).

Qualitative analysis

Infrared spectra matching and fingerprint identification

In the simplest case, two spectral printouts – one of the reference and the other of the analyte – can be overlaid on a light box and the spectral features related by eye. Overlaying spectra on the computer screen achieves the same objective.

When the spectrum of a substance being examined is compared with a reference spectrum, such as those in the *British Pharmacopoeia*, the positions and relative intensities of the absorption bands of the spectrum of the substance being examined should conform to those of the reference spectrum. The sample spectrum must be recorded under the same operating conditions that were used to obtain the reference spectrum, i.e. dispersive or FT instrument, and same resolution (*British Pharmacopoeia* 2008).

When a chemical reference substance is available, the substance being examined and the chemical reference substance should be prepared by the same procedure before recording the spectra (see later under Polymorphism). The transmission minima in the spectrum obtained with the substance being examined should correspond in position and relative size to those in the spectrum obtained with the reference substance (*British Pharmacopoeia* 2008).

In recent years, IR spectral databases have been created and stored electronically in databases and/or libraries. The spectrum of the analyte is presented to the database and the computer attempts to match the spectrum with one already held in the database. A report is made of the best matches. The computer program lists the most likely hits in order of a closeness of fit. Many compilations of spectra (databases) are private collections, held typically by individual pharmaceutical companies; some can be purchased and a few are in the public domain.

The number of compounds for which IR spectra have been measured is now massive. Potentially, the greater the number of spectra in a database, the greater is the probability of making a good match for the unknown sample. However, the probability of making a mismatch is also greater, as more spectra with fine differences are available for comparison. The computer is simply matching 'pictures' by the number of peaks, their positions and their relative intensities. The best that the computer fitting can do is to indicate a mathematical similarity. It is important to qualify a computer search:

- A visual overlay of the test compound spectrum and the hit spectrum ensures that the search has not chosen a match that is mathematically acceptable, but chemically not acceptable.
- Knowledge of the class of a compound can help restrict the search to a more refined reference set (database).
- Other properties of the sample and the reference compound should match, such as chromatographic retention times, chemical and colour reactions and functional group assignments.
- The computer can select only spectra that are in its library and, if the spectrum of the compound under investigation is absent, then it will select those that give the next-best fit.
- Different forms of the same compound give different IR spectra (different polymorphs, racemate and/or enantiomer, ionisation status, cations and anions).
- If the spectra have been recorded on different instruments, they may, superficially at least, appear very different. In this case a more detailed study of band frequencies and relative intensities must be undertaken.

If the matching procedure fails, and in cases where the type of compound is unknown or can be allocated only to a certain class (e.g. a phenothiazine or a barbiturate), reference may be made to the index of IR peaks in the Indexes of Analytical Data and to the information in the individual monographs. Comparison of the spectrum of the unknown with that of the suspected compound should either confirm or disprove the tentative identification. If the two spectra were recorded under similar conditions on the same type of instrument, they should be very similar in appearance. Some examples of the identification of drugs are given below.

Infrared spectra of amfetamines

The IR spectra of amfetamine base and the hydrochloride have many similarities, but the hydrochloride spectrum shows much finer detail (Fig. 33.19A and B). The IR spectra of the hydrochloride and mandelate salts show differences (Fig. 33.19B and C) because of the absorption of the mandelic acid. However, the spectra of the hydrochloride and sulfate salts (Fig. 33.19B and D) are very similar since they both have inorganic anions. The only major difference is the absorption band caused by the sulfate at 1110 cm^{-1}.

Infrared spectra of barbiturates

Important derivatives of malonylurea (barbituric acid) have two substituents at position 5. Others are also substituted at position 1 and in others the oxygen atom attached to position 2 is replaced by sulfur to form thiobarbiturates (Fig. 33.20).

The barbiturates can be classified chemically into three classes: 5,5-disubstituted barbituric acids, 1,5,5-trisubstituted barbituric acids and 5,5-disubstituted thiobarbituric acids. These classes can be further divided depending on whether the substituents in position 5 are alkyl, alkenyl, aryl or cycloalkenyl. In most common barbiturates, one of the 5-substituents is either ethyl or allyl and the other is either a straight- or a branched-chain alkyl or alkenyl group with five or fewer carbon atoms. Some barbiturates are available as sodium salts. The IR spectrum of a barbiturate therefore depends on the class of compound, the nature of the substituents and whether it is the free acid or the sodium salt.

With the exception of phenobarbital and barbituric acid, the free barbiturates do not absorb appreciably above 3300 cm^{-1} (e.g. barbital, Fig. 33.20A), a feature that distinguishes them from the ureides; a weak band of unknown origin sometimes occurs between 3500 and 3400 cm^{-1}. All the barbiturates have two bands that occur near 3200 and 3100 cm^{-1} and are caused by N–H stretching vibrations. In the 5,5-disubstituted compounds, the relative intensities of the two bands are similar, although that at 3100 cm^{-1} is usually slightly less intense. In compounds substituted on the nitrogen atom at position 1, the intensity of the band at 3100 cm^{-1} may be greatly reduced and is often present only as a shoulder on the band at 3200 cm^{-1}, e.g. metharbital. Methylphenobarbital appears to be an exception in that the band at 3100 cm^{-1} is the most intense one in the region. A similar phenomenon occurs with the sodium salts, since here again one of the hydrogen atoms in either position 1 or 3 has been replaced.

A series of up to four medium-to-intense bands occurs in the region 3000–2800 cm^{-1} and is caused by alkyl C–H stretching vibrations of the substituents in positions 1 and 5. The intensity of the bands gives a very approximate indication of the number of C–H bonds and hence the number of carbon atoms in the chain. This does not appear to apply to the sodium salts, in which the band that occurs at 3000–2950 cm^{-1} is usually increased in intensity, compared with that of the free acid, and

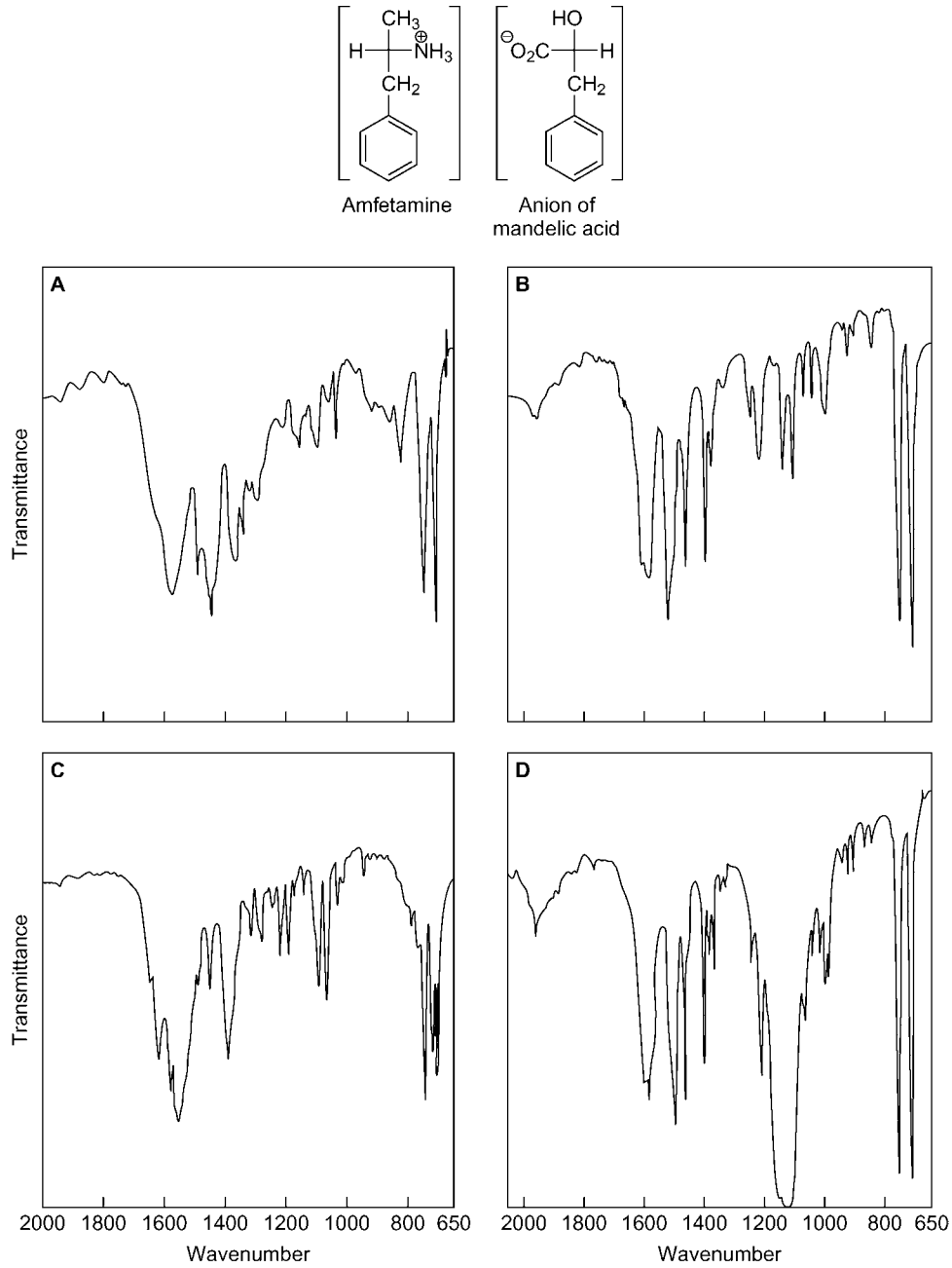

Figure 33.19 IR spectra of (A) amfetamine base; (B) amfetamine hydrochloride; (C) amfetamine mandelate; (D) amfetamine sulfate.

becomes the strongest band. Compare, for example, the spectra of barbital (Fig. 33.20A) and barbital sodium (Fig. 33.20B).

The barbiturates have up to three strong bands in the region 1765–1670 cm^{-1}, which result from C=O stretching vibrations. Knowledge of the origin of these bands helps to understand the differences in the spectra of the various types of barbiturate.

In symmetrical molecules, the three bands are all of similar intensity. In asymmetrical molecules, the band at the highest frequency is often less intense than the other two, particularly so when the molecule is substituted in position 1. The sodium salts of the barbiturates have only two bands in this region, since the molecule is no longer symmetrical, and these occur at a lower frequency, between 1700 and 1650 cm^{-1}. In addition, a broad strong band occurs between 1600 and 1550 cm^{-1}; the free barbiturates show practically no absorption in this region. The sodium salts of the thiobarbiturates exhibit only the lowest of the three C=O vibrations in the region 1700–1680 cm^{-1}. They do, however, exhibit the broad, strong band that occurs between 1650 and 1600 cm^{-1}. Therefore, the number, position and intensity of the bands between 1800 and 1500 cm^{-1} give a very good indication of whether the barbiturate is the free acid, the salt or a thiobarbiturate.

Most barbiturates have a number of strong bands between 1460 and 1250 cm^{-1}, and some of these result from C–H deformation and C–N stretching vibrations. The sodium salts of the thiobarbiturates have a broad strong band between 1500 and 1480 cm^{-1}, which is believed to be caused by C–N stretching vibrations of the carbon atom attached to sulfur. This band is not present in the ordinary barbiturates and therefore provides another way to distinguish those that contain sulfur. Many barbiturates exhibit a few weak-to-medium intensity bands in the region 1150–900 cm^{-1}. The 1-substituted barbiturates exhibit a greater number of sharp bands of medium intensity. Those compounds that contain an allyl group exhibit bands at about 1000 to 960 cm^{-1}, which probably result from C–H deformation vibrations. The sodium salts of the thiobarbiturates show a band of medium intensity between 1020 and 1000 cm^{-1}. Finally, many barbiturates, but not the thiobarbiturates,

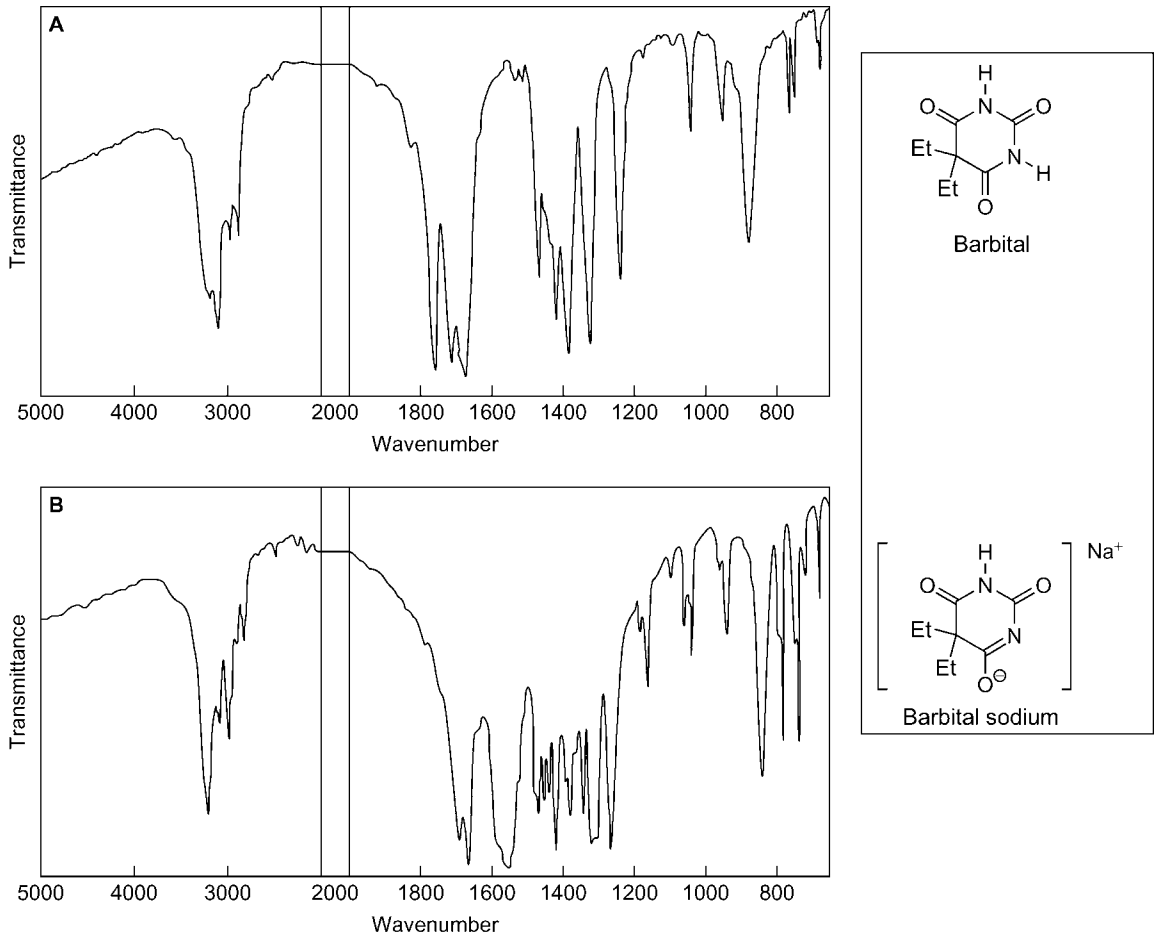

Figure 33.20 IR spectra of (A) barbital; (B) barbital sodium.

exhibit a broad band of medium-to-strong intensity between 900 and 800 cm^{-1}.

Infrared spectra of aspirin, Nujol and paracetamol

The spectra of aspirin, Nujol and paracetamol are given in Fig. 33.21, which illustrates the differentiation of N–H, O–H, ester, carboxylic acid and amide groups. In particular, the effect of Nujol on the drug spectra is apparent.

Polymorphism

Many drugs exist in polymorphic forms and have different IR spectra for each different crystalline form. IR spectroscopy can therefore be used to distinguish between different polymorphic forms, to identify them and also to measure quantitatively the proportions of each in a mixture.

If a test compound gives a different spectrum to the corresponding chemical reference substance, and polymorphism is suspected, both should be treated in the same manner so that they crystallise or are produced in the same form. This can often be achieved by dissolving them in a suitable solvent and evaporating to dryness.

The barbiturates are notable for the extent to which they exhibit polymorphism, including many metastable forms found only in mixtures. Spectral differences between polymorphs are associated with different types of hydrogen bonding, and there is a correlation between hydrogen bond strength and duration of action of the barbiturates on the central nervous system. The crystalline structure of barbiturates can be affected by grinding with an alkali halide or in preparing a mull, but, if precautions are taken to ensure reproducibility, the spectra of the barbiturates are sufficiently different to be used for identification purposes.

Interferences

Spurious bands can occur readily in IR spectra, particularly when a biological sample has undergone several purification procedures. Traces of plasticisers, surfactants and oils left on glassware can all give rise to spurious IR bands. A useful list has been compiled by Szymanski (1971), part of which is given in Table 33.2.

Infrared data in monographs

Modern spectral identification by reference to computer databases involves sophisticated chemometric algorithms to compare all the digitised points in a test spectrum with a set of reference spectra. Spectra that are judged to be most similar are said to be a match, which allows the identity of the test spectrum to be established. This type of work requires specialised database reference sets and computer programs. Much of it is proprietary and related to the software of the spectrometer being used.

However, it has been shown (Curry *et al.* 1969; Ingle, Mathieson 1976) that an IR spectrum of a particular substance can be retrieved from a collection, with some degree of confidence, by reference to its six major absorption bands. This forms the basis for a system of identification.

Data that consist of six major absorption bands have been selected from the recorded spectrum over the range 2000–650 cm^{-1} (5–15 μm) and are included in the monographs in Part 2 in Volume 2. In many cases, the spectrum is also reproduced in a reduced size. The selected peaks are the six most intense peaks, except those in the region where Nujol absorbs (1490–1320 cm^{-1}, 6.7–7.6 μm) have been omitted. The peaks are arranged in descending order of amplitude. It should be noted that, because of variations in instruments and conditions, other determinations of the spectrum might not give peaks with the same relative intensities.

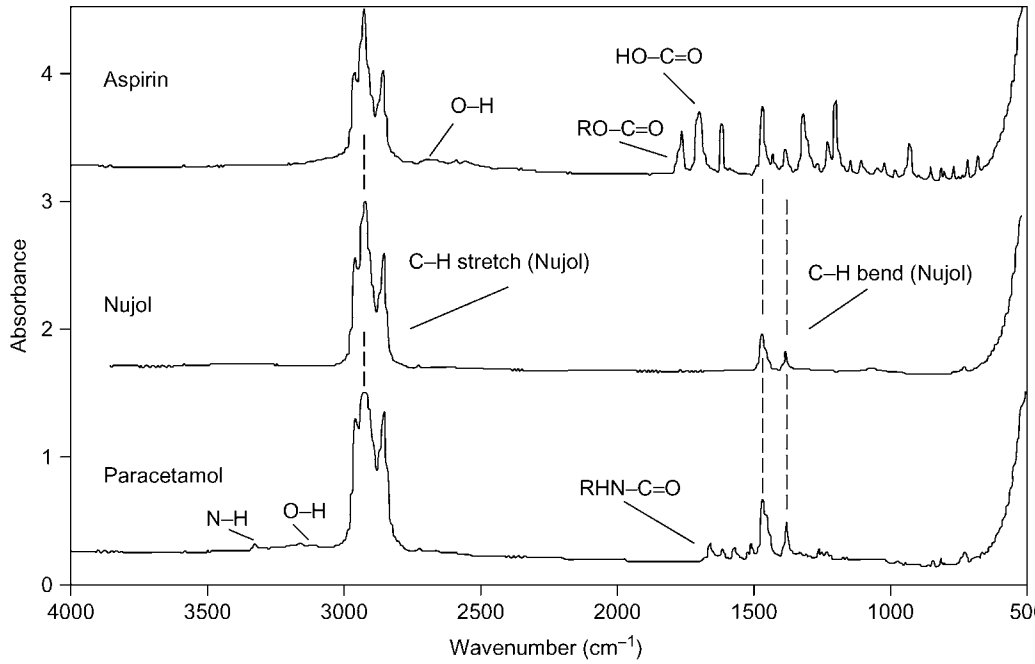

Figure 33.21 The IR spectra of aspirin, Nujol and paracetamol. The drug spectra were measured as Nujol mulls.

The principal peaks are listed in descending order of amplitude and details of how to use the data are given in the Indexes of Analytical Data.

Quantitative analysis

Concentration of molecular species

FTIR spectrometers are now very stable instruments and, coupled with computer control and data manipulation, should be as easy to operate as UV-visible spectrophotometers. They can operate routinely in the absorbance mode, which is required for concentration determinations. However, relatively high concentrations are required given the restriction of solvent absorption, the need for narrow path lengths and the low extinction coefficients of vibrations. Assuming that Beer's law is obeyed, absolute concentrations can be determined in solution from specific bands in windows of solvent transparency. In the solid state, the relative amounts of two components can be estimated readily from the relative intensities of two specific absorption bands:

$$A_{1\lambda_1} = {}_{1\lambda_1}c_1 l$$

$$A_{2\lambda_2} = {}_{2\lambda_2}c_2 l$$

$$\frac{A_{1\lambda_1}}{A_{2\lambda_2}} \propto \frac{c_1}{c_2}$$

where $A_{1\lambda_1}$, $A_{2\lambda_2}$, ${}_{1\lambda_1}$ and ${}_{2\lambda_2}$ are, respectively, the absorbances and extinction coefficients of species 1 and 2 at the corresponding wavelengths λ_1 and λ_2. The concentrations of species 1 and 2 are c_1 and c_2, and the path length is l.

Collections of data

General collections

Compilations of IR spectral data are available in two forms, either as pictures or as digital absorbance/wavenumber data in electronic databases. Pictures are available in book or computer form and are suitable for visual inspection. Spectral characteristics can be determined with the aid of a ruler. Electronic databases are available in computer memory for data manipulation and spectral matching. Third-party software for the computer manipulation of IR spectral data include:

- GRAMS/AI version 8 and Spectral ID available from Thermo Galactic, 395 Main Street, Salem, NH 03079, USA.
- Aldrich Spectral Viewer available from Sigma-Aldrich Company Ltd, The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT, UK, or via their web site www.sigmaaldrich.com.
- Thermo Nicolet OMNIC FTIR available from Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454, USA.

Table 33.2 Spurious peaks in IR spectroscopy (after Szymanski 1971)

Wavenumber (cm^{-1})	*Assignment*	*Comments on sources*
3800–2500	H_2O	Bound or unbound water in a molecule can give rise to sharp or broad bands. In alkali halide discs a water band at 3350 cm^{-1} may appear
3300–3000	NH_3^+	Lens tissues
1810–1600	C=O	Impurities that contain the carbonyl group, e.g. phosgene in chloroform, plasticisers
1750–1500	H_2O	Bound or unbound water can give rise to sharp or broad bands
1610–1515	COO^-	Alkali salts (which also have a weaker band at 1425 cm^{-1}) can be produced from alkali halides
1400	NH_3^+	Lens tissues
1265	$Si–CH_3$	Stopcock grease or silicone oil
1110–1050	Si–O–Si	Glass or hydrolysed Si compounds
730, 720	Polyethylene	Polyethylene laboratory ware
700	Polystyrene	Polystyrene laboratory ware

Other instrument manufactures can provide suitable software.

The most comprehensive collection of published spectra is that of the Sadtler Research Laboratories. This is available through Bio-Rad, Informatics/Sadtler Group, 3316 Spring Garden Street, Philadelphia, PA 19104–2596, USA. The total database covers 220 000 spectra, which are divided into over 100 spectral databases. Subgroups are preferred to ensure more secure compound matching. Bio-Rad also supplies appropriate database software.

A collection of 3000 FTIR spectra of standard chemical compounds is available from E. Merck, Darmstadt (Pachler KGR *et al.* (1988). *Merck FTIR Atlas: Eine Sammlung von FTIR-Spektren Teil I/II (A Collection of FTIR Spectra Part I/II)*. Wiley, 1988). In this:

- Absorbance spectra are presented, breaking with tradition, with the advantage that band intensities are directly proportional to concentration.
- Spectra of solid compounds are recorded in a KBr matrix. Hence, no interfering peaks from the matrix are included.
- All spectra are augmented with the following data: list of nine strongest bands, structure, molecular formula, relative molecular mass, melting and boiling points, CAS registry numbers, sample preparation and Merck–Schuchardt catalogue number.

The Sigma-Aldrich Company offers several collections of IR spectral data from printed books to computer-based systems. Details of Sigma-Aldrich products are available from Sigma-Aldrich Company Ltd, The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT, UK, or their website www.sigmaaldrich.com. Their products include:

- The *Aldrich Library of FTIR Spectra*, 2nd edn, three-volume set, 1997; contains over 18 000 IR spectra. (See also: Pouchert CJ (2008). *Aldrich Library of FT-IR Spectra*, 3 volumes, 2nd edn. Wiley Blackwell.)
- Pouchert CJ (1989). *Aldrich Library of FTIR Spectra: Vapor Phase*, Vol. 3. Milwaukee: Aldrich Chemical Co.; contains 6550 vapour-phase spectra.
- Pouchert CJ (1981). *Aldrich Library of Infrared Spectra*, 3rd edn, Milwaukee, Aldrich Chemical Co., 1981; contains more than 12 000 spectra classified by functional group and structural presentation.
- A set of over 10 400 FT-IR spectra of biochemicals and related organics is given in: Keller RJ (1986). *Sigma Library of FTIR Spectra*, Vols 1 and 2. St Louis: Sigma Chemical Co.
- The computer-based system *Aldrich Spectral Viewer*, includes an IR collection of over 11 000 spectra (Product Number Z540285) and an ATR-IR library of over 18 000 compounds (Product number Z547492).
- 667 spectra of commercial materials with fragrance or flavour-enhancing properties are included in the *Nicolet/Aldrich Flavor & Fragrances Vapor Phase Library*, Product number Z273031.
- 5010 gas-phase spectra collected by Aldrich using a GC interface to ensure chromatographically pure samples: *Nicolet/Aldrich Vapor Phase Library*, Product number Z27296-5.
- A spectral collection of 10 411 of the most common chemicals found in the Sigma Chemical catalogue represents a wide range of chemical classes of particular interest to those in biochemical research or quality control: *Nicolet/Sigma Biochemical Condensed Phase Library*, Product Number Z272973.

Other sources include:

- The Coblentz Society Special Collection of over 9500 IR spectra: *The Coblentz Society Infrared Spectra Collection for ACD/Labs*. www.coblentz.org/
- A collection of nearly 400 spectra in the *British Pharmacopoeia Collection*, Vol. IV of the *British Pharmacopoeia*. London: The Stationery Office, 2008.
- Fiveash Data Management Inc. offers software and reference spectra databases through their web site www.fdmspectra.com. The databases include the FTIR Spectra of Drugs/Canadian Forensic Spectra (3750 spectra).
- The spectra of 1044 organic compounds are presented as pictures in: Schrader B (1989). *Raman/Infrared Atlas of Organic Compounds*, 2nd edn, New York: Wiley.
- Lin-Vien D *et al.* (1991). *Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*. London: Academic Press.
- Nyquist RA *et al.* (1997). *The Handbook of Infrared and Raman Spectra of Inorganic Compounds and Organic Salts*, 4 volumes. London: Academic Press. (Also a CD-ROM version.)
- NIR, IR, Raman and UV-visible spectra of organic compounds, polymers and surfactants are presented in: Workman J Jr (2000). *Handbook of Organic Compounds*, 3 volumes. New York: Academic Press.
- Tables and charts of IR spectra can be obtained from: Socrates G (2001). *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*, 3rd edn. New York: Wiley.
- NICODOM Ltd, Hlavni 2727, CZ-14100 Prague 4, Czech Republic (www.ir-spectra.com). FTIR spectra Database of over 140 000 infrared spectra.

Specialised collections

Drugs

Collection of spectra (includes IR, UV, NMR, mass spectra, etc.) of 1750 illicit and prescription drugs: Mills T *et al.* (2005). *Instrumental Data for Drug Analysis*, Vols 1–6, 3rd edn. Boca Raton, FL: CRC Press.

Alkaloids

1000 spectra with other physical data: Holubek J, Strouf O (1965–1973). *Spectral Data and Physical Constants of Alkaloids*. London: Heyden.

Antibiotics

A comprehensive collection published by the International Centre of Information on Antibiotics: Lenzen C, Delcambe L *ICIA Inf Bull* 1972, *10:* 78–160; 1973, *11*: 1–157; 1975, 12: 1–178; 1976, 13: 1–178; 1977, 15: 1–208; 1977, 16: 1–212; 1978, 17: 1–248; 1979, 18: 1–268.

Barbiturates

Solution spectra of 41 barbiturates: Sucharda-Sobczyk A (1970). *Roczn Chem* 44: 1435–1445. Also Mesley RJ (1970). *Spectrochim Acta* 26A: 1427–1448.

Carbohydrates

Spectra of 79 carbohydrates: Kuhn LP (1950). *Anal Chem* 22: 276–283.

Excipients

Spectra of 300 commonly used excipients: Bugay DE, Findlay WP (1999). *Pharmaceutical Excipients Characterization by IR, Raman, and NMR Spectroscopy*. New York: Marcel Dekker.

Narcotics and related bases

Levi L *et al.* (1955). *Bull Narcot* 7: 42–84.

Farmilo CG, Genest K (1963). Narcotics and related bases. In: Stolman A, ed. *Progress in Chemical Toxicology*, Vol. 1. London: Academic Press, 199–295.

Natural products

Spectra of alkaloids, amino acids, carbohydrates, carotenoids, steroids and terpenes: Yamaguchi K (1970). *Spectral Data of Natural Products*, Vol. 1. Amsterdam: Elsevier.

Organophosphorus compounds

Shagidullin RR *et al.* (1990). *Atlas of IR Spectra of Organophosphorus Compounds*. Moscow: Nauka.

Pesticides and related compounds

Spectra of 76 pesticides: Gore RC *et al.* (1971). *J Assoc Off Anal Chem* 54: 1040–1082.

Spectra of 478 pesticides, disinfectants, fumigants, fungicides, etc.: Giang P (1977). Spectroscopic methods of analysis. In: Zweig G, ed. *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. 9. London: Academic Press, 153–290.

Spectra for 68 substances which may occur as impurities, residues or adjuvants in commercial pesticides: Collerson RR *et al.* (1976). *NPL Report Chem*. 48, National Physical Laboratory, Division of Chemical Standards.

Pharmaceutical spectra of 335 substances: Sammul OR *et al*. (1964). *J Assoc Off Agric Chem* 47: 918–991.

Over 400 FTIR spectra of commonly used pesticides and related metabolites: Visser T (1993) *Infrared Spectra of Pesticides*. London: Taylor and Francis (CRC).

Phenothiazines

Spectra of 19 phenothiazines: Scapini G, Gardini GP (1964). *Ateneo Parmense* 35: 328–334.

Phenothiazines and their sulfoxides: Kreyenbuhl B *et al*. (1979). *Pharm Acta Helv* 54: 197–205.

Turner LK (1963). *J Forensic Sci Soc* 4: 39–49.

Plasticisers

Spectra of 284 plasticisers and additives: Craver CD, ed. (1977). *Plasticisers and Other Additives*. Kirkwood: The Coblentz Society.

Steroids

Spectra of 900 steroids: Neudert W, Roepke H (1965). *Atlas of Steroid Spectra*. Berlin: Springer-Verlag.

Also: Arzamastsev AP, Yashkina DS (1975). *Ultraviolet and Infrared Spectra of Drugs*, No. 1, Steroids. Moscow: Meditsina.

Ketosteroids: Hodosan F *et al*. (1971). *Studii Cerc Chim* 19: 191–210.

Levchuck YN *et al*. (1971). *Zh Prikl Spektrosk* 14: 735–738.

Corticosteroids and contraceptive steroids: Mesley RJ (1966). *Spectrochim Acta* 22: 889–917.

Fluorinated corticosteroids: Bellomonte G (1973). *Ann Ist Super Sanita* 9: 121–128.

Surfactants

More than 1000 spectra of surfactants: Hummel DO (1996). Analysis of surfactants. In: *Atlas of FTIR-Spectra with Interpretations*. Cincinnati, OH: Hanser Gardner.

Sulfonamides

Spectra of 10 sulfonamides: Edwards D (1971). *J Pharm Pharmacol* 23: 956–962.

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Williams DH, Fleming I (2007). *Spectroscopic Methods in Organic Chemistry*, 6th edn. Maidenhead: McGraw-Hill Education Europe.

Further reading

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Bellamy LJ (1980). *Infrared Spectra of Complex Molecules*, Vol 2, 2nd edn. London: Kluwer Academic Publishers.

Chalmers J, Griffiths PR (2001). *The Handbook of Vibrational Spectroscopy.* Chichester: Wiley.

Griffiths PR, De Haseth JA (2007). *Fourier Transform Infrared Spectrometry*, 2nd edn. New York: Wiley.

Günzler H, Gremlich H-U (2002). *IR Spectroscopy: An Introduction.* Weinheim: Wiley VCH.

Mayo DW *et al.* (2004). *Course Notes on the Interpretation of Infrared and Raman Spectra*. Chichester: Wiley.

Nakamoto K (1997). *Infrared & Raman Spectra of Inorganic & Coordination Compounds*, 5th edn. New York: Wiley.

Nyquist RA (2001). *Interpreting Infrared, Raman, and Nuclear Magnetic Resonance Spectra*. Amserdam: Elsevier.

Pivonka DE *et al.* (2007). *Application of Vibrational Spectroscopy in Pharmaceutical Research and Development*. Chichester: Wiley.

Smith BC (1998). *Infrared Spectral Interpretation – A Systematic Approach*. Boca Raton, FL: CRC Press.

Stuart BH (2004). *Infrared Spectroscopy: Fundamentals and applications*. Chichester: Wiley Blackwell.

Williams DH, Fleming I (2007). *Spectroscopic Methods in Organic Chemistry*, 6th edn. Maidenhead: McGraw-Hill Education Europe.

Workman J Jr, Springsteen A (1998). *Applied Spectroscopy, A Compact Reference for Practitioners*. San Diego: Academic Press.

CHAPTER

34 Near-infrared Spectroscopy

RD Jee

Introduction

The near-infrared (NIR) region of the electromagnetic spectrum extends from about 780 nm to 2500 nm (or 12800 to 4000 cm^{-1}). It is therefore the part of the spectrum that exists between the red end of the visible spectrum and the beginning of the mid-infrared (IR) region. Its discovery by Herschel in 1800 was the first indication that radiation other than visible radiation existed.

The region is subdivided into two regions: 780–1100 nm and 1100–2500 nm, the first of which is named the Herschel region. Although discovered some 200 years ago, it is only in the past 35 years with the advent of computers and chemometrics that its analytical potential has been exploited. For the qualitative analysis of solid samples, invariably NIR spectra are measured by reflectance. Spectra are often complex with many overlapping peaks (Fig. 34.1A). As a result of this, the technique was viewed originally with some scepticism until the easy application of chemometric methods became possible. Although the reflectance spectrum of a material is complex, its second-derivative spectrum is very suitable for identification purposes (Fig. 34.1B).

NIR spectra are associated with molecular vibrations and consequently provide information similar to that obtained by mid-IR spectrophotometry. Although NIR spectra are much more difficult to interpret than mid-IR spectra, it is their fingerprint nature that makes the technique useful for identification purposes.

NIR spectroscopy is not a trace analytical technique nor is it generally suited to the identification of individual substances in multicomponent mixtures. Its strength lies in its ability to identify relatively pure samples rapidly or to identify a matrix of nearly fixed composition, such as tablets. Although it is used for the quantitative assay of solids in the pharmaceutical industry, setting up a calibration model is usually a time-consuming and expensive process that precludes its use for 'one-off' quantitative determinations. Spectra are particularly simple to measure, and the technique is non-destructive and generally requires no sample preparation. A further advantage of NIR spectra is that they contain information about the physical properties of the sample, such as particle size, compaction density, polymorphs, etc., which often makes it possible to differentiate between samples of the same chemical identity but of different grades or from different sources.

Theory

NIR absorbances correspond to overtones and combinations of molecular vibrations that have their fundamentals in the mid-IR region of the spectrum. Figure 34.2 illustrates the potential energy curve for a simple diatomic molecule.

The allowed energy levels, E, for such an anharmonic oscillator are given by:

$$E \approx (v+0.5)hf - (v+0.5)^2 hfx$$

where h is Planck's constant, v is the vibrational quantum number, f is the equilibrium frequency of oscillation and x is the anharmonicity constant. The vibrational quantum number may take values 0, 1, 2, ... The selection rules for an anharmonic oscillator are $\Delta v = \pm 1, \pm 2, \pm 3, \ldots$, and consequently transitions such as $\nu_{1\leftarrow 0}$, $\nu_{2\leftarrow 0}$, $\nu_{3\leftarrow 0}$, $\nu_{2\leftarrow 1}$, etc., are allowed (in practice, only transitions starting at $v = 0$ are important for spectra measured at room temperature). These transitions are referred to as the fundamental frequency of vibration, first overtone, second overtone, and so on. As the anharmonicity constant is typically small ($x < 0.05$), these spectroscopic transitions occur approximately at frequencies close to f, $2f$ and $3f$, and so on.

For most molecular vibrations the fundamental frequency of vibration occurs in the mid-IR region of the spectrum; however, the overtones occur in the NIR region, which is the basis of NIR spectroscopy.

Polyatomic molecules may exhibit simultaneous changes in the energies of two or more vibrational modes: the frequency observed will be the sum ($f_1 + f_2$, $2f_1 + f_2$, ...) or the difference ($f_1 - f_2$, $2f_1 - f_2$, ...) between the individual frequencies (note that $2f$ represents the first overtone and the subscripts 1 and 2 refer to different vibrational modes). This results in very weak bands called combination and subtraction bands – the latter are possible, but rarely observed in spectra measured at room temperature. Combination bands have a very low probability of occurrence unless they arise from two vibrations that involve a common atom or arise from bonds connected through multiple chemical bonds. The transition probabilities for overtones and combination bands are 10^{-1} to10^{-3} those for the fundamental frequency and, consequently, such absorbances are weak. This weakness of absorbances can be put to good advantage in that samples may be measured without any need for sample dilution or preparation.

Reflectance and transmission

NIR spectra may be measured by either reflectance, R, or transmission, T. Reflectance is the ratio of the intensity of radiation reflected by the sample, I_r, to the intensity of the radiation impinging on it, I_0; hence $R = I_r/I_0$.

Similarly, transmission is the ratio of the intensity of radiation that passes through the sample, I_t, to that impinging on it, I_0; hence $T = I_t/I_0$.

Reflected radiation is made up of two main components – specular and diffuse radiation. Specular reflection is radiation that is simply reflected directly from the surface of the sample and contains little useful information about the sample for identification purposes.

Diffuse reflection refers to radiation that has penetrated into the particles of the sample, undergone multiple reflections within the substance and re-emerged after the various characteristic absorptions of the substance have occurred.

Figure 34.3 shows a schematic illustration of diffuse reflectance, I_r. Such reflected radiation gives rise to chemical information. The path length of the radiation is dependent on numerous factors, such as particle size, particle shape and sample compaction, and therefore also contains information about the physical state of the sample. The exact path taken by the radiation is difficult to describe mathematically and consequently there is no totally satisfactory theory for diffuse reflectance. However, the Kubelka–Munk theory is a useful approximation for NIR reflectance measurements.

The Kubelka–Munk function, $f(R_a)$, relates the absolute reflectance, R_a, of the sample to its absorption coefficient, k, and scattering coefficient, s, according to:

$$f(R_a) = \frac{(1 - R_a)^2}{2R_a} = \frac{k}{s}$$

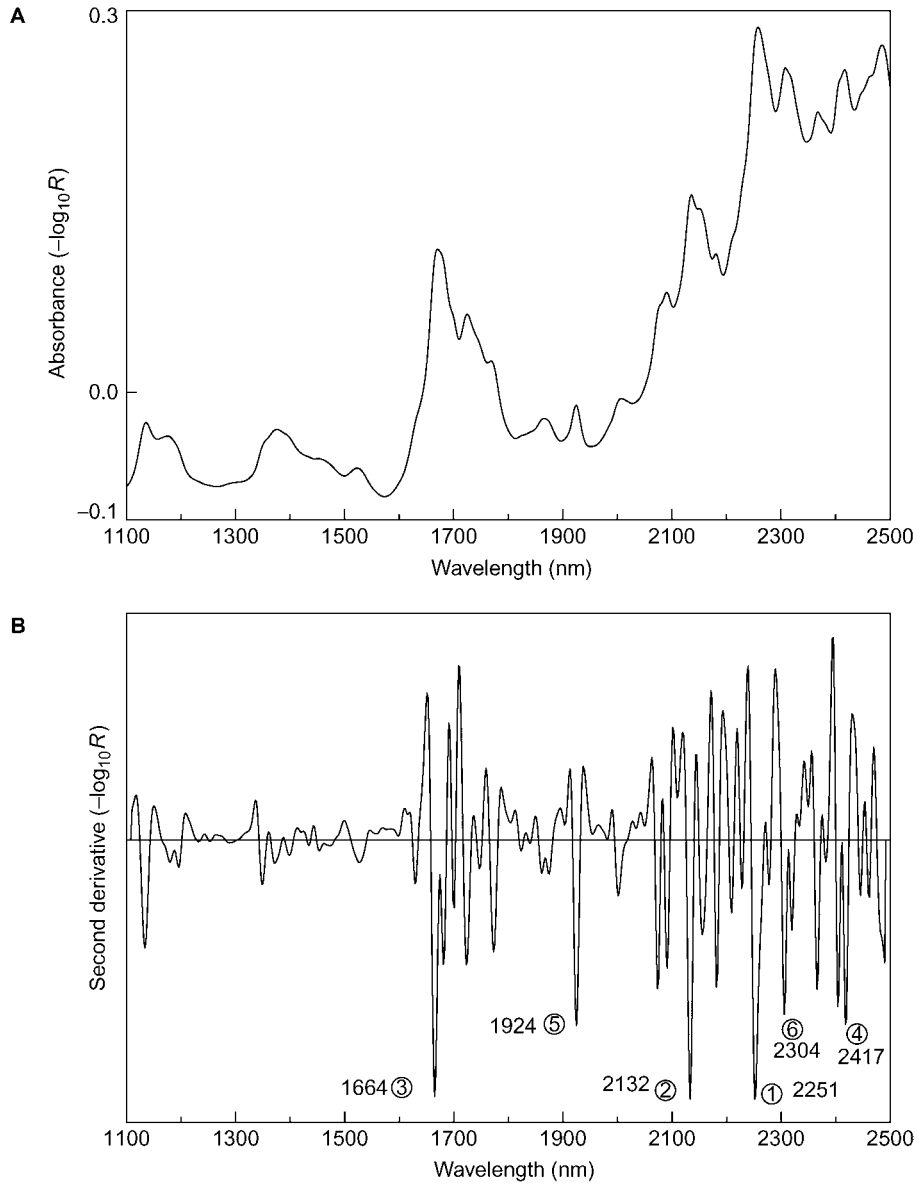

Figure 34.1 (A) Near-infrared spectrum of acetomenaphthone ((4-acetyloxy-2-methylnaphthalen-1-yl) acetate) measured by reflectance; (B) the second-derivative spectrum of (A). Circled numbers indicate the six most intense negative peaks in order of decreasing intensity.

The absorption coefficient is equal to the absorptivity, ε (as defined by the Beer–Lambert law), multiplied by the concentration, c, and hence the Kubelka–Munk function is related to concentration according to:

$$f(R_a) = \frac{\varepsilon c}{s}$$

Recently Dahm and Dahm (2007) have developed a new theory for interpreting diffuse reflectance and transmittance.

Practical reflectance measurements are made with respect to some standard reflecting material. A measure of practically measured reflectance (R_m, although R is commonly used for this function) is the ratio of the intensity of light reflected from the sample to that reflected from a background or reference surface. The dependence of reflectance on concentration is often no better described by the Kubelka–Munk function than by using the apparent absorbance ($A = -\log_{10} R$) and Beer's law:

$$A = -\log_{10} R = a' c$$

where a' is a proportionality constant.

The passage of radiation through a solid or powdered sample occurs by what is termed diffuse transmission. The radiation undergoes many internal reflections within the particles of the sample and emerges in all directions (Fig. 34.3). Because of the many internal reflections, the actual optical path length can be surprisingly large; for a 2–5 mm thick tablet it is about 20–25 cm (Johansson *et al.* 2002).

The transmission of a 2–5 mm thickness of compact powder (e.g. a tablet) when measured with respect to air will typically be 10^{-4} to 10^{-6}. Transmission measurements are commonly limited to the wavelength region below 1900 nm because of the poor signal-to-noise ratio at longer wavelengths. Compared with reflectance spectra, transmission spectra tend to lack detail and are therefore less suited for identification purposes.

Instrumentation

Spectrometers that record NIR spectra are generally based on filter, grating or interferometer designs. Instrumentation is similar to that used for ultraviolet and/or visible absorption spectroscopy and is based on fixed wavelengths, scanning or diode array systems. Tungsten–halogen lamps serve as energy sources, while lead sulfide and/or indium gallium

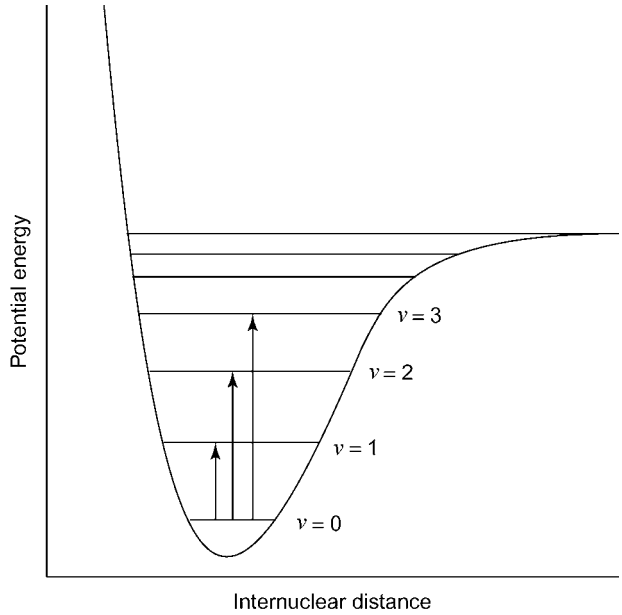

Figure 34.2 Vibrational energy levels for a diatomic molecule, illustrating the transitions that correspond to the fundamental frequency ($v = 0 \rightarrow v = 1$), the first overtone ($v = 0 \rightarrow v = 2$) and the second overtone ($v = 0 \rightarrow v = 3$).

arsenide detectors are used. Present-day instruments are computer controlled, which enables spectra to be measured in a matter of seconds and saved to a computer file.

A typical spectral file for a grating instrument consists of 700–4000 data points at 2- to 0.5-nm intervals over the wavelength range 1100–2500 nm. Fourier transform (FT) instruments normally give outputs in wavenumbers and a typical file might have 500–3000 data points at 12–2 cm^{-1} intervals over the range 4000–10 000 cm^{-1} (i.e. 2500–1000 nm). Saved spectra are generally the average of a number of scans so as to reduce noise levels. Measurements are made by reflectance or transmission. Details of sample presentation methods are given in the section Sample preparation and sample presentation.

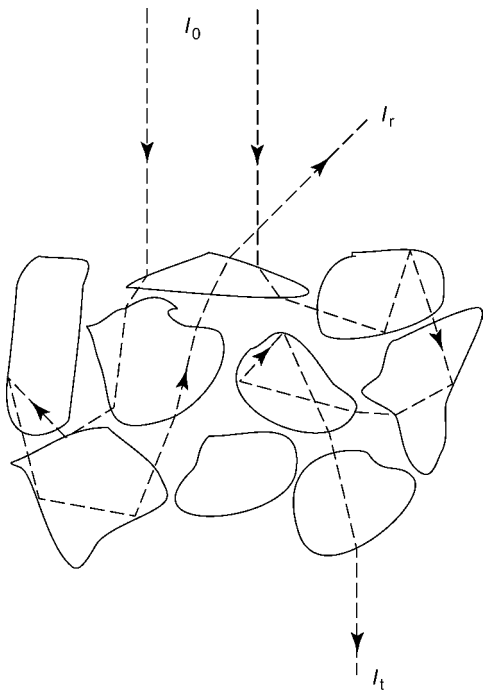

Figure 34.3 Diffuse reflectance and transmittance. I_0, intensity of impinging radiation; I_r, intensity of diffuse reflected radiation; I_t, intensity of diffuse transmitted radiation.

Data processing and presentation of results

Although the original NIR reflectance spectrum of a substance is complex and often relatively featureless, its second-derivative spectrum is very suitable for identification purposes. Taking the second derivative of the spectrum largely removes the effects of baseline offsets and baseline slopes. This is illustrated in Fig. 34.4A, which shows a spectral absorption peak with:

1. Horizontal background
2. Linearly sloping background
3. A curved background plus zero offset.

Taking the first derivative with respect to wavelength removes any offset and reduces a linearly sloping background to a simple offset (Fig. 34.4B). Taking a further derivative (i.e. second derivative) removes this offset and all three spectra are now almost identical (Fig. 34.4C).

The positions of the negative peaks in the second-derivative spectrum correspond to the positions of peaks in the original spectrum. This can be seen for acetomenaphthone in Fig. 34.1A and B. Derivative spectra can be calculated by a variety of methods. The simplest is to calculate the difference between blocks of data points on either side of the wavelength at which the derivative is required. This point is replaced by the difference between the two blocks (Fig. 34.5).

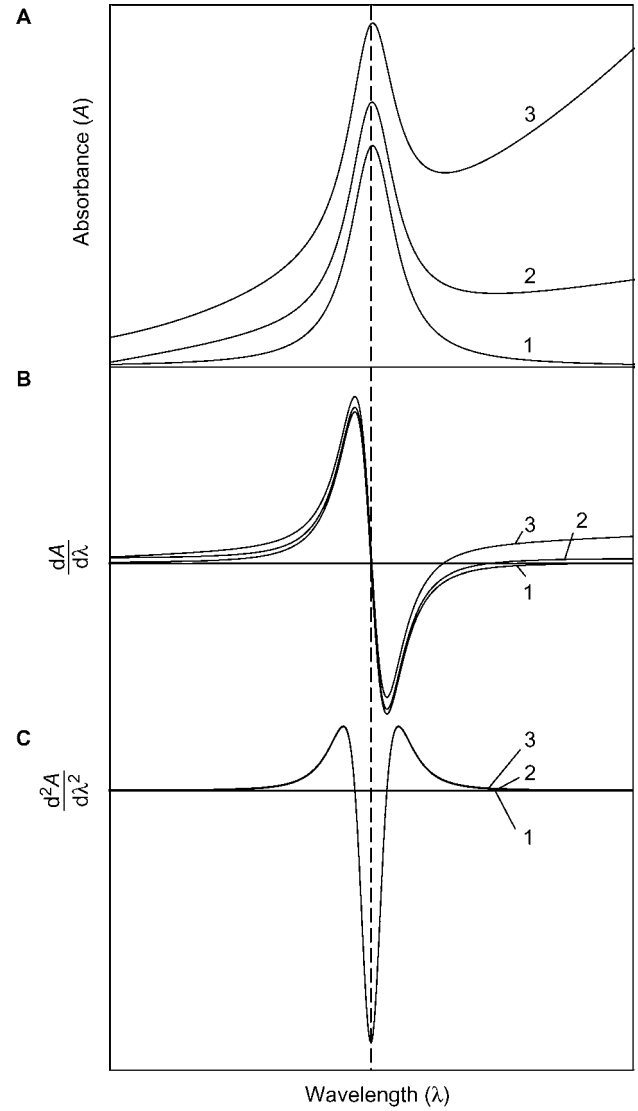

Figure 34.4 Advantages of using second-derivative spectra: (A) original spectra, (B) first-derivative spectra and (C) second-derivative spectra. 1, zero baseline; 2, linearly sloping baseline; 3, curved (quadratic) baseline with zero offset.

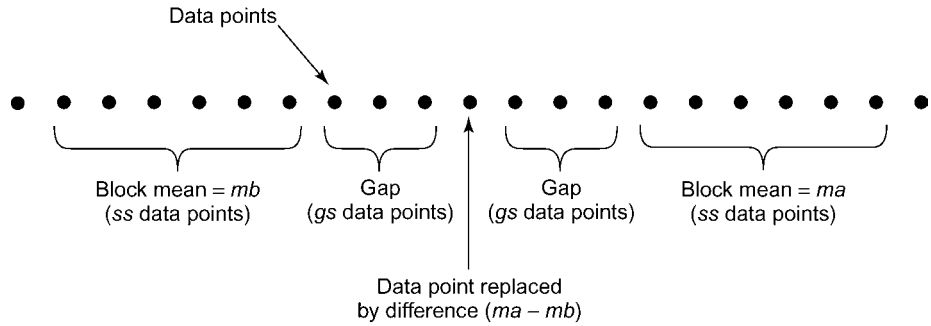

Figure 34.5 Simple difference method for calculating derivative spectra. *ss* is the number of data points in the sample block; *gs* is the number of data points in the gap block.

The process is repeated across the whole spectrum, moving along one data point each time. To calculate a second derivative, etc., the process is simply repeated the required number of times. By suitable selection of the number of data points in the sample block (*ss*) and the number of points in the gap block (*gs*), the degree of spectral smoothing can be adjusted. Values of $ss = 3$ to 10 and $gs = 0$ are commonly used. Sample spectra and reference spectra must be treated equally.

Another commonly used method is that of Savitzky and Golay (1964). In effect, a polynomial of specified order is fitted by least squares to the data using a specified number of data points before and after the point at which the derivative is required. The estimated derivative is then the derivative of the resultant fitted polynomial. The process is repeated across the whole spectrum, moving one data point each time. These many-least-squares fits could be laborious and time-consuming. However, provided that the data points are all equally spaced, they can be achieved very efficiently using a Savitzky–Golay filter. Typical parameters used are 3–10 data points with a polynomial fitting order of 2–4. The Savitzky–Golay method has the advantages that it is a published method and gives more control over the smoothing parameters (number of data points and order of polynomial).

Sample compaction and changes in particle size of samples can have a pronounced effect upon the spectrum of a sample. Figure 34.6A shows spectra for samples of lactose monohydrate of different particle sizes.

Various data pre-treatments may be applied if it is required to reduce such effects. The best one for a particular application is usually found by trial and error. The standard normal variate (SNV) transformation is one of several normalisation processes that may be used. The ordinate (absorbance, second-derivative absorbance, etc.) of a spectrum at each wavelength, i, is replaced by z_i according to:

$$z_i = \frac{(y_i - \overline{y})}{s}$$

where $\overline{y}$ is the mean spectral value over the complete spectrum, s is the standard deviation of the spectral values over the complete spectrum and y_i is the spectral value at wavelength i. Figure 34.6B shows the lactose monohydrate spectra after SNV transformation, which demonstrates that most of the effects of particle size have been removed.

Multiplicative scatter correction (MSC) is another transformation commonly applied to minimise light-scattering effects. For each spectrum in a set (e.g. calibration set) a linear regression of the ordinate values on those for the mean spectrum of the set is performed. The intercept, a, and slope, b, are then used to generate a new spectrum according to:

$$y_{\mathrm{MSC},I} = (y_i - a)/b$$

where y_i is the original ordinate value at wavelength i and $y_{\mathrm{MSC},i}$ is the corresponding corrected value. In effect, each spectrum is adjusted so that it fits the mean spectrum as closely as possible. Unlike the SNV transformation, the values of a and b calculated from the mean spectrum must be stored so that the same transformation may be applied to the spectra of test samples. An advantage with respect to SNV, however, is that the corrected spectra have an ordinate scale similar to that of the original spectra.

Systems suitability test

Most NIR instrument and/or software systems prevent sample measurements being taken until a systems suitability test has been run. Checks typically include photometric noise, wavelength accuracy, band-pass, etc., and are normally carried out once per day. The *British Pharmacopoeia* (2008) and *European Pharmacopoeia* (Council of Europe 2007) provide a number of guidelines for performance checking, as also does the Pharmaceutical Analytical Sciences Group (see Further reading). A number of independent tests for noise and wavelength accuracy, and so on, can be carried out easily.

Wavelength accuracy

Numerous wavelength standards for the NIR spectral region are available (Burgess, Hammond 2007). Standard reference materials (SRMs) are available from the United States National Institute for Standards and Technology (NIST) for wavelength accuracy measurements. The present preferred NIST standard for reflectance measurements is SRM 2036 which is a poly(tetrafluoroethylene)-backed glass made from a mixture of holmium, samarium, ytterbium and neodymium oxides in a zirconia-stabilised borate matrix (Choquette *et al.* 2005). This standard provides seven well-defined nearly symmetrical peaks in the 975–1946 nm range with a wavelength accuracy of better than 0.1 nm. Most instrument manufacturers can also supply standards that are traceable to the NIST standards. The reflectance spectrum of an equal mixture by mass of dysprosium, erbium and holmium oxides is commonly used as a standard and exhibits 37 reflectance minima in the NIR wavelength range (Weidner *et al.* 1986). Above 2000 nm there are few useful peaks in the mixed oxide standard. However, the addition of talc to the mixture provides a useful reference peak at 2313.5 nm. Figure 34.7A shows the reflectance spectrum of a mixed lanthanide oxides/talc standard over the wavelength range 1100–2500 nm, with the major minima marked. The reference wavelength values are slightly band-pass dependent and the values shown are for an instrument with a band pass of 10 nm. The reference values given by NIST for this mixed oxide standard have an uncertainty of ±1 nm. As some minima are associated with a sloping background, it is important to display the spectrum in terms of reflectance rather than absorbance ($-\log_{10} R$), this being the condition under which NIST documented the wavelength values. Pure (>99.99%) samples of the lanthanide oxides are readily available and may be used instead of expensive NIST wavelength SRMs. Examples of reflectance spectra obtained for the three separate oxides measured on a FT-NIR instrument are shown in Fig. 34.7B. As the band pass of a FT-NIR instrument varies in terms of wavelength across the spectrum, care is needed in selecting the reference values. In Fig. 34.7B, reference values for a 10 nm band pass were used in the range 4000–7000 cm^{-1} and 5 nm band-pass values for above 7000 cm^{-1}. A typical commercial grating instrument (data stored at 2 nm intervals) might have a wavelength

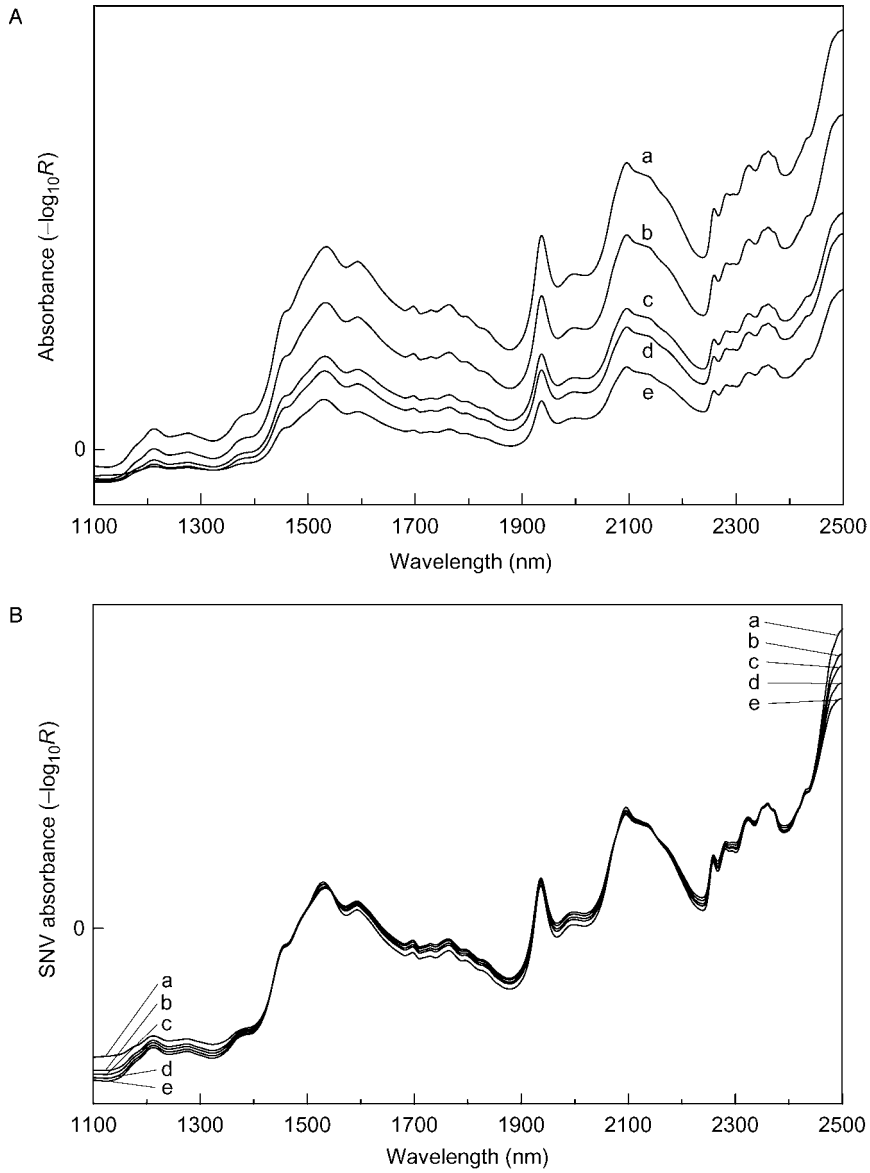

Figure 34.6 Effect of particle size on the reflectance spectrum of lactose monohydrate. Sieve fractions: (a) >150, (b) 93, (c) 63, (d) 45 and (e) 32 µm. A, original spectra; B, spectra after standard normal variate (SNV) transformation.

accuracy of ±0.3 nm, while an FT instrument (data stored at 12 cm^{-1} intervals) has an accuracy of ±2 cm^{-1}. Some form of wavelength interpolation between stored data points is essential when locating the position of minima (see Wavelength repeatability).

NIST and NIST-traceable wavelength standards for transmission measurements are also available; however, an inexpensive alternative is to use trichloromethane, which exhibits sharp absorption peaks at 1152.1, 1410.2, 1619.9 and 1861.2 nm (Busch *et al.* 2000). The *British Pharmacopoeia* recommends the use of methylene chloride (dichloromethane) as a standard. Figure 34.8 shows the spectrum of methylene chloride with the important absorption peaks labelled. For instruments in which transmittance or transflectance measurements are not possible, a spectrum may be measured by adding titanium dioxide to a few millilitres of methylene chloride and recording the diffuse reflectance spectrum.

Wavelength repeatability

Wavelength repeatability can be checked by recording a sample spectrum a number of times (e.g. more than 12 times, without moving the sample between scans) and calculating the standard deviation of the wavelengths of minima or maxima in the second-derivative absorbance spectra. Materials with well-defined narrow second-derivative peaks, such as the mixed lanthanide oxides wavelength standard (suitable for wavelengths below 2200 nm), are ideal. Alternatively, compounds such as ascorbic acid, aspartame, benzoic acid, salicylic acid, sucrose and talc may be used. Some form of wavelength interpolation between stored data points is essential when locating the position of peaks. Fitting a quadratic curve to three consecutive data points that encompass a peak and calculating the exact position from the equation works well. Let the fitted equation be $A = a + b\lambda + c\lambda^2$, where A is the second-derivative absorbance and a, b and c are the fitted coefficients; then $p_{eak} = -b/2c$. Typical standard deviations for peak wavelengths are <0.01 nm for a grating instrument that stores data points at 2 nm intervals and <0.5 cm^{-1} for a FT instrument that stores data at 12 cm^{-1} intervals.

Photometric noise

Photometric noise can be determined by measuring the absorbance of a single sample a number of times (e.g. >12) and calculating the standard deviation at each wavelength. To obtain an estimate of the instrument noise, as distinct from the repeatability of sample measurements, the

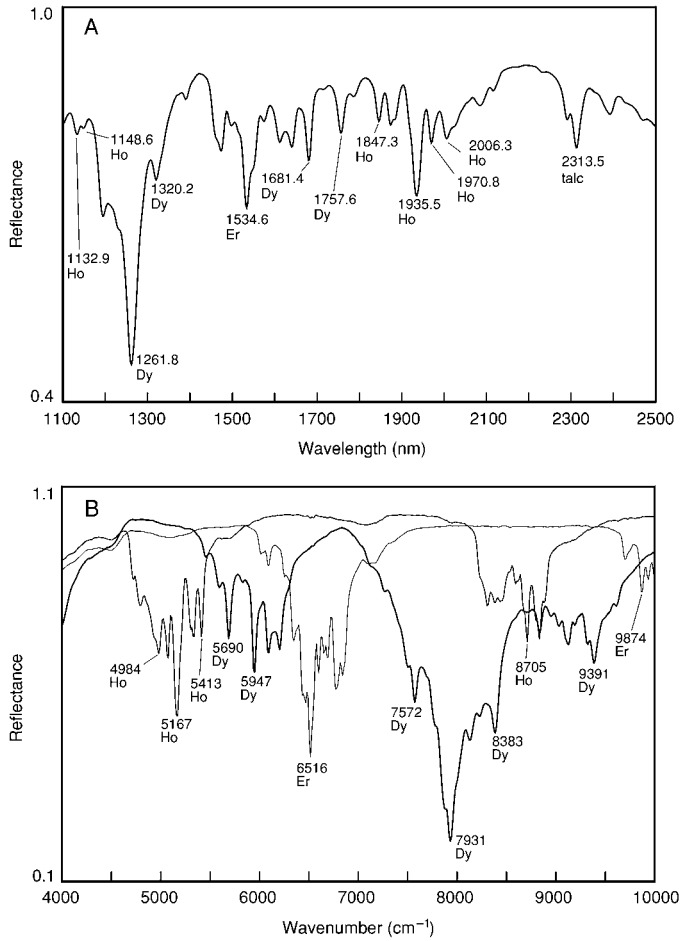

Figure 34.7 (A) Reflectance spectrum of a three lanthanide oxides (Ho_2O_3, Dy_2O_3 and Er_2O_3) and talc wavelength standard (wavelength scale); (B) reflectance spectra of holmium, dysprosium and erbium oxides (wavenumber scale).

sample should not be moved between scans. To reduce the effects of wavelength repeatability errors, the sample used should ideally have a flat spectral response. Rapid repeat scanning of a sample can result in sample heating and introduce unwanted variations in the spectra. For this reason, sintered perhalopolyethylene cake and/or carbon black reflectance standards are not recommended for this test. The noise level should be checked over the full wavelength range of the instrument and for both low and high absorbances. For low-absorbance samples the instrument reference standards, barium sulfate and titanium dioxide, are ideal. As strong absorbers, substances such as carbon black, finely ground sucrose and butylated hydroxytoluene, may be used. Although far from spectrally flat, the mixed lanthanide oxides wavelength standard is thermally stable and works well. Noise levels depend upon the number of primary scans averaged. However, under normal recommended operating conditions, the noise level is generally very low and differences between repeat spectra are not easily visible without expanding the display scale. For typical grating instruments, repeat measurements can be expected to give a standard deviation of <0.05 milli-absorbance units (mAU) across the wavelength range 1100–2500 nm and absorbance range zero to unity. The typical noise level for a FT-NIR instrument would be <0.5 mAU.

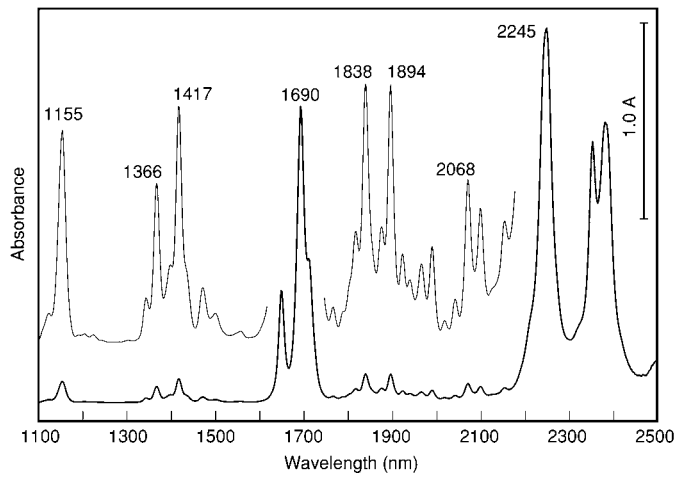

Figure 34.8 Absorbance spectrum of methylene chloride measured by transflectance with a 1 mm optical path length. The ordinate scale for the inserts has been magnified 10-fold.

Photometric accuracy, linearity and specular and/or stray radiation

All reflectance measurements made using a sample stage include specular radiation from the sample stage itself and from the sample bottle (Fig. 34.9A and B). The effect of this is to make a plot of measured absorbance ($-\log_{10} R_m$) versus true (absolute) absorbance ($-\log_{10} R_a$) curved and hence limit the photometric range. To check photometric linearity, and measure the extent of specular radiation, samples of known absolute reflectance are required. Suitable standards with absolute reflectances of 0.02–0.99 are available from NIST and various instrument manufacturers. The standards typically are carefully prepared mixtures of sintered perhalopolyethylene cake and carbon black and should regularly be sent for re-certification. Tables of actual reflectance versus wavelength are provided for each standard. The measured reflectance for a given standard (relative to the instrument standard) can be expressed as follows:

$$R_m = \frac{(I_D + I_s) + I_{ss}}{I_{oc}} = \frac{R_a(I_o - I_{ss}) + I_{ss}}{I_{oc}}$$

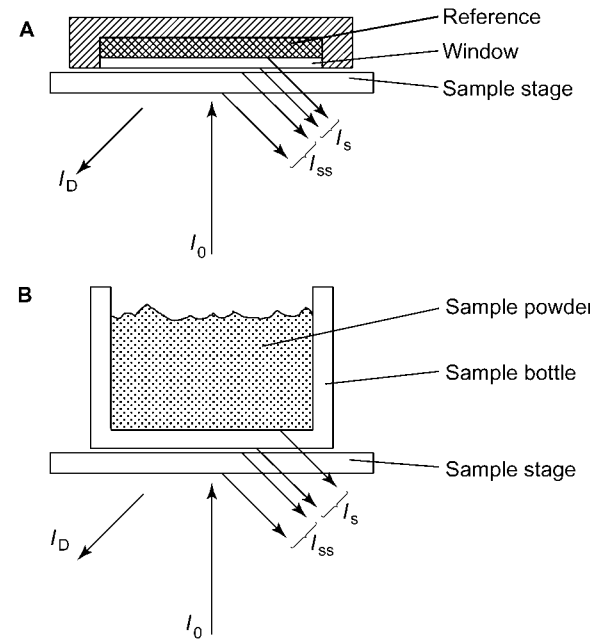

Figure 34.9 Measurement of reflectance using a sample stage: (A) reference measurement; (B) sample measurement. I_0 is the intensity of impinging radiation, I_D is the intensity of diffuse reflected radiation from the reference/sample, I_s is the intensity of specular radiation reflected by the sample bottle or reference window and I_{ss} is the intensity of specular radiation reflected by the sample stage.

or

$$R_m = R_a k + k'$$

where I_0, I_D, I_s and I_{ss} are as defined in Fig. 34.9 and k and k' are constants (and therefore $I_r = I_D + I_s + I_{ss}$). I_{0c} represents the intensity of radiation reflected by the instrument reflectance standard. The above assumes that the collection efficiency and/or geometry is the same for both the reference and the sample.

A plot of R_m versus R_a at a given wavelength should therefore give a straight line if the photometric response is linear. However, in practice, a small degree of curvature is usually observed. The value of k' represents the specular and/or stray radiation expressed as a fraction of radiation reflected by the instrument reflectance standard. Values for k' vary from one instrument to another and change with time as the surface conditions of the sample stage, etc. age ($k' < 0.01$ is typical). Measuring the signal without a sample on the stage also gives a simple method of accessing the background specular and/or stray radiation, provided that external light and internally reflected radiation can be avoided. Similarly, measuring an empty bottle (no top) gives a measure of $I_s + I_{ss}$ relative to the instrument standard.

Sample preparation and sample presentation

NIR spectra can be measured by either reflectance or transmission. Reflectance measurements are particularly easy to make. Common glasses (borosilicate, soda, etc.) are virtually transparent to NIR radiation and powdered samples may be measured in such sample bottles directly (Fig. 34.10A and B). Transmittance measurements of solids, e.g. tablets, require custom-made holders so as to minimise radiation leakage around the edge of the tablet. Absorbances for tablets by transmittance are typically 3–5 absorbance units and restricted to below 1900 nm.

The radiation reflected by the sample is measured as a function of wavelength and with respect to the reflectance of a suitable standard, such as a flat disc of ceramic or Spectralon. Spectra are clearly dependent upon the reference chosen and the reproducibility of such standards can be a problem when it comes to transferability of spectra from one instrument to another. This problem has not been completely resolved at present. Fibre-optic probes that can be inserted directly into the sample can be attached to many instruments (Fig. 34.10B). Liquids can be measured using conventional cuvettes or by transflectance, again using a fibre-optic probe fitted with a transflectance tip (Fig. 34.10C), or directly on a sample stage in a cup with a suitable reflector (Fig. 34.10D). Transflectance reflectors may be gold plated, stainless steel, PTFE, etc., and constructed with various path lengths. The optical path length for transflectance is twice the physical path length as the radiation passes through the sample twice. For most liquid samples an optical path length of 1–5 mm is suitable.

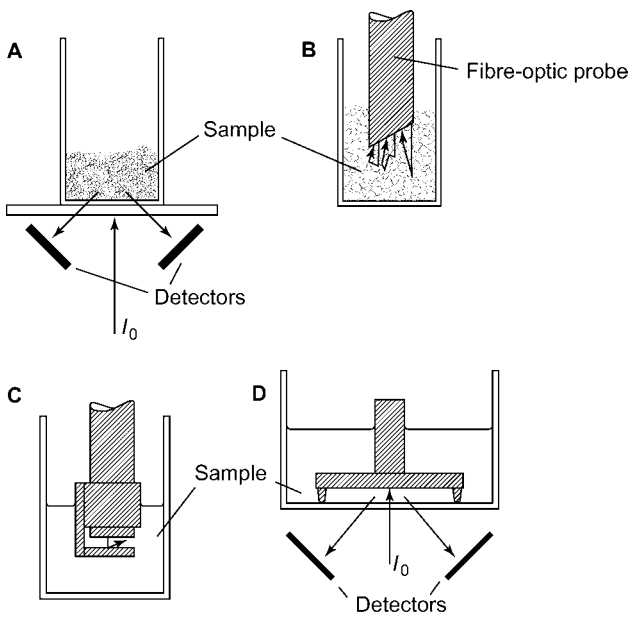

Figure 34.10 Sample presentation methods: (A) sample bottle on sample stage; (B) fibre-optic probe; (C) liquid sample using a fibre-optic probe fitted with transflectance adapter; (D) liquid sample measured by transflectance.

For the measurement of powders by reflectance, it has been recommended that sample bottles should be filled by 'pouring' the powder into the bottle without tapping (Yoon *et al.* 1998). Tapping causes compaction of the powder and was found to result in poor sample-measurement repeatability. For reflectance measurements, the sample thickness should be sufficient that any further increase in sample thickness has no effect upon the spectrum. Typically, NIR radiation penetrates 1–3 mm into a powdered sample and a sample thickness of >10 mm is recommended to give an effective 'infinite thickness'. For a compact material, such as a tablet, the reflected radiation will penetrate only 0.1–1 mm. The sample bottle diameter should exceed that of the radiation beam. For many instruments this means a bottle of diameter of about 10 mm or greater. Sample bottles should be chosen that have smooth bases, otherwise specular reflectance varies from one bottle to the next, as well as with the orientation on the sample stage. A typical sample bottle contributes 2–10% of specular radiation to the reflected signal. The same type of sample bottle should be used for both test samples and reference materials.

Sample-measurement repeatability is very dependent upon the nature of the sample. Liquids and solutions are highly reproducible because of the homogeneous nature of the sample. Crystalline powders usually show poor repeatability because of varying specular reflection from the crystal surfaces. Specular reflection distorts the 'absorbance' spectrum and limits the usable photometric range. Identification algorithms can be affected markedly by the crystallinity of samples. Careful crushing of the material to reduce the particle size can help to minimise the effects. Vibrational patterns are affected by differences in the crystal lattice and hence different polymorphs show significant differences in their NIR spectra (Blanco *et al.* 1998; Patel *et al.* 2001).

Solvents for the preparation of solutions are somewhat limited. Only carbon tetrachloride and carbon disulfide among the common solvents are transparent throughout the entire NIR region. Methylene chloride, dioxane, heptane, acetonitrile and dimethyl sulfoxide have regions below 2200 nm that can be used.

Water has a particularly strong absorbance spectrum in the NIR region and samples must be protected from uptake of water from or loss of water to the atmosphere. The presence of water can be recognised easily by the characteristic absorption peak in the range 1900–1940 nm. The exact peak position depends on the nature of the water – free water or water of crystallisation – and the extent of hydrogen bonding. The water peak in the spectrum of lactose monohydrate (see Fig. 34.6) and the residual water in the chlortetracycline hydrochloride samples (see Fig. 34.16) can be easily seen.

Intact tablets may be measured as easily as any other sample. A sample bottle that contains tablets randomly packed may be placed on the sample stage, or single tablets may be measured directly. The depth of penetration of reflected radiation from compact materials, such as tablets, is limited to only a few tenths of a millimetre. Markings on tablets can affect the spectra and a decision to measure one side or both needs to be made. With coated tablets it is possible for the reflectance spectrum to be dominated by the coating material, though some radiation will penetrate into the core. Tablets can be crushed and the powder measured in a sample bottle if required.

With grating instruments, spectra commonly exhibit a small anomalous peak known as Wood's peak at about 1520 nm. The magnitude of the peak is dependent upon the difference in diameters of the sample and reflectance standard used: the greater the difference, the larger the peak (Fig. 34.11). It can be quite pronounced in the spectra of poorly absorbing substances and care is required not to confuse Wood's peak with peaks from the material. Tablets, which generally have a small diameter, often show a prominent Wood's peak when measured by placing them directly on the sample stage.

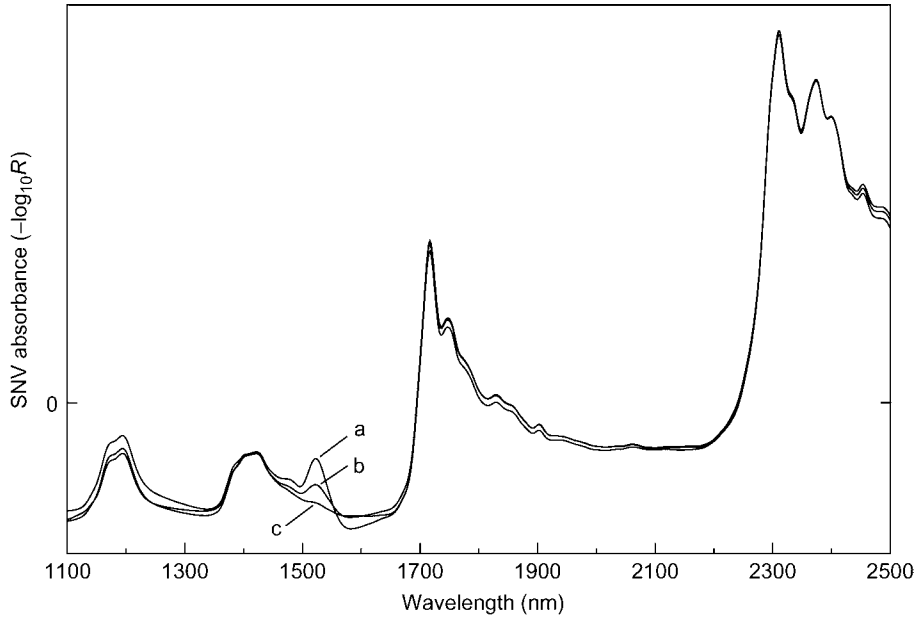

Figure 34.11 Wood's anomaly observed with grating monochromator instruments. Reflectance spectra of poly(vinyl chloride) powder measured in sample bottles of different diameter. Bottle diameter: (a) 8 mm, (b) 15 mm, (c) 25 mm. Reference diameter 50 mm.

Interpretation of spectra

Functional groups such as X–H, where X is C, N, O or S, have a small reduced mass and hence high fundamental frequency of vibration, and are particularly important for NIR spectroscopy – the first and second overtones appear in the NIR region. Groupings such as C–Cl, C–F, C=O, etc., with high reduced masses, are of less importance in NIR spectroscopy as their fundamental frequencies are low and their overtones generally also appear in the mid-IR region. For organic molecules, the C–H bond is the most important and for alkanes its fundamental stretching vibration in the mid-IR region at about 2960–2850 cm^{-1} gives rise to first and second overtones at approximately 1700–1730 and 1150–1170 nm in the NIR region. The C–H group stretching and deformation vibrational modes give rise to combination bands in the region 2000–2500 nm. Water is a particularly strong absorber in the NIR region, with the O–H first overtone of the stretching vibration occurring at 1450 nm (second and third overtones at 970 and 760 nm, respectively). Alcohols similarly show absorptions around 1450 nm. A very intense absorption occurs in the region 1900–1940 nm for water and has been assigned to the combination band between the fundamental stretching and deformation vibrations of the O–H bond; in the case of alcohols this vibration occurs at a somewhat longer wavelength. Temperature can have a marked effect on the spectra of compounds with hydrogen bonds. The spectrum of water is particularly sensitive to temperature, with changes in both the intensities and the band positions. Similar shifts are observed for different states of adsorbed water.

Although characteristic wavelengths, such as those mentioned above, can often be identified readily in the NIR spectrum of a material, the general complexity of NIR spectra precludes any easy interpretation and identifications are based on comparison with reference spectra. Figure 34.12 gives a summary of the positions of some of the more important NIR absorptions. More extensive lists of NIR vibrations are given by Osborne *et al.* (1993) and Burns and Ciurczak (2001).

Qualitative analysis

Identification of samples depends in one way or another on comparing sample spectra to spectra of known standards. Three simple classification procedures are described below.

Peak positions

The six-peak method used for mid-IR spectra (see Chapter 33) is particularly simple and can be easily applied to NIR spectra. Figure 34.1B shows the second-derivative spectrum of a sample of acetomenaphthone. The positions of the six most intense peaks (negative-going peaks) are noted and then compared with a database of peak positions of compounds. An example of part of such a database is shown in Table 34.1, in which the spectroscopic peaks of the compounds are listed in decreasing order of intensity. It is a simple matter to compare the peaks of the unknown sample with those in the database.

The peaks of the spectrum shown in Fig. 34.1B give a very good match to acetomenaphthone. Although the process of locating peak positions and finding the best match to those in a database can be carried out manually, it can also be computerised easily. Peak order is often different between sample and reference spectrum because of differences in the physical properties of the sample and those used to construct the database. Consequently, care is required when searching for the best match. An initial simple search without regard to peak intensities is preferred. To obtain maximum discrimination between different substances, numerous parameters should be optimised. Important parameters are the number of peaks, spectral range, degree of spectral smoothing, number of data points used to calculate the second-derivative spectra and the window size within which peaks are considered to match. Decreasing the window size improves the ability to distinguish between materials, but conversely tends to increase the false-negative identification rate. Increasing the number of peaks and/or calculating a score that takes the relative peak intensities into account can improve the discrimination still further. However, not relying on relative peak intensities makes the method more robust towards changes in the physical properties of the samples.

Peak positions are relatively unaffected by the physical properties of the sample. Although particle size, in particular crystallinity, can have a marked effect upon the relative peak amplitudes, there is little effect on wavelength position. Peak positions commonly vary by only a few tenths of a nanometre for different batches of pure compounds. Greater variation is observed for naturally occurring materials, but variations are still generally less than 1 or 2 nm. The position of the peak minima of the two spectra of salicylic acid (crystalline sample and solidified

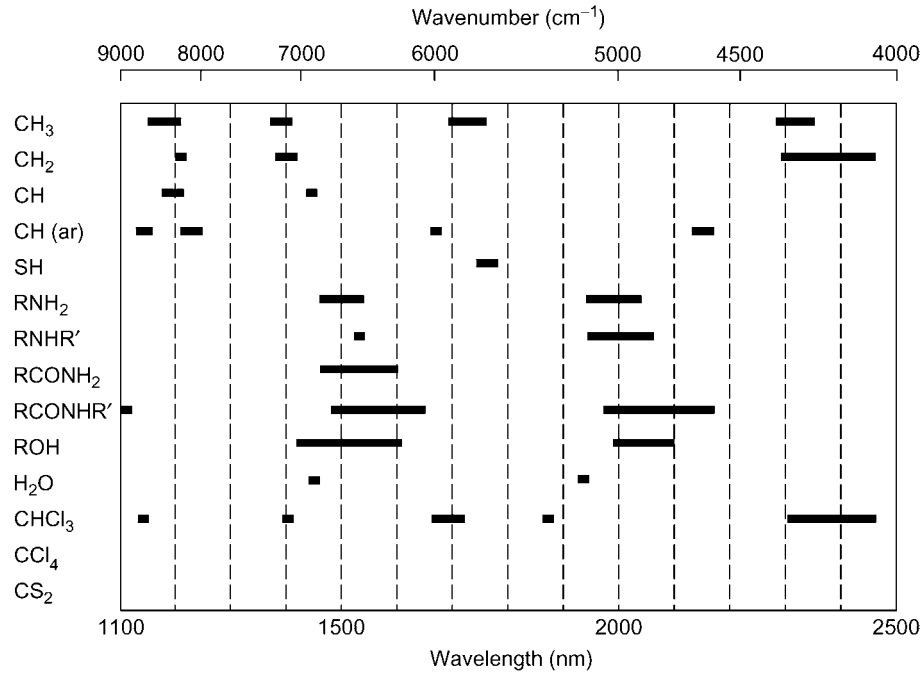

Figure 34.12 Wavelengths of important near-infrared absorbances. Black lines show regions of absorption.

sample after melting) shown in Fig. 34.13 do not deviate by more than ±0.5 nm from each other, even though there are major differences in peak intensities. A window size of ±2 nm has been found suitable for identification by peak matching.

Although increasing the number of peaks compared for each spectrum increases the ability to distinguish between substances, it is important to check that the peaks are genuine and not noise. With powders this is usually not a problem, as most second-derivative NIR spectra contain in excess of 20 usable negative peaks. Modern NIR spectrophotometers have very good signal-to-noise ratios and spectra are very reproducible. With liquids the spectra are commonly much simpler and it may not be possible to find even six peaks. When comparing such spectra with a database it is important not only to record the number of matches but also to check for missing or unaccountable peaks.

Correlation in wavelength space

In this method of identification, the sample, *S*, and reference, *R*, spectra are compared by calculating a correlation coefficient between them. If two spectra rise and fall in perfect synchronisation with each other (Fig. 34.14A), then plotting the amplitude of *S* against that of *R* at each wavelength results in a straight line (correlation plot, Fig. 34.14B).

A numerical measure of the correlation can be obtained by calculating the dot-product, r_d, or product-moment, r_p, correlation coefficient:

$$r_d = \frac{\sum S_i R_i}{\sqrt{\sum S_i^2 \sum R_i^2}}$$

$$r_p = \frac{\sum (S_i - \overline{S})(R_i - \overline{R})}{\sqrt{\sum (S_i - \overline{S})^2 \sum (R_i - \overline{R})^2}}$$

S_i and R_i are the ordinate values of the two spectra being compared at wavelength i, while $\overline{S}$ and $\overline{R}$ are the mean values across the selected wavelength range. The summations are performed over the selected wavelength range, which is usually the complete measured spectrum. An r value of unity represents a perfect match. However, in practice r is always less than unity because of noise. There is little to choose between these two correlation coefficients. For second-derivative spectra they give almost identical results as the mean value for a second-derivative spectrum is usually close to zero. When original absorbance ($-\log_{10} R$) spectra are being used, the product-moment correlation coefficient often gives better discrimination. However, because of the general increase in absorbance with increase in wavelength observed for typical NIR spectra, correlation values are all very close to unity and are of little use for discrimination purposes. The product-moment correlation coefficient is less affected by data pre-treatment than the dot-product correlation coefficient, but sample and reference spectra should always be

Table 34.1 Example of part of a database of second-derivative peak positions (negative peaks). Values in bold correspond to the best match to the peaks for the spectrum given in Fig 34.1B

Compound	*Peaks in decreasing order of intensity, left to right (nm)*					
Ascorbic acid	2249.7	1456.9	2480.3	1749.5	2096.7	2144.8
Diethylcarbamazepine citrate	2249.9	2394.0	1697.5	2462.3	2294.9	2307.2
Doxepin	2250.3	2267.6	2353.5	2176.3	1669.7	2320.3
Acetomenaphthone	**2250.8**	**2131.8**	**1664.3**	**2416.8**	**1923.6**	**2304.3**
Dextromethorphan	2251.0	2302.3	2338.8	2438.0	2285.0	1756.6
Glycine (base)	2251.2	2321.9	2454.5	2431.8	1669.1	2262.6

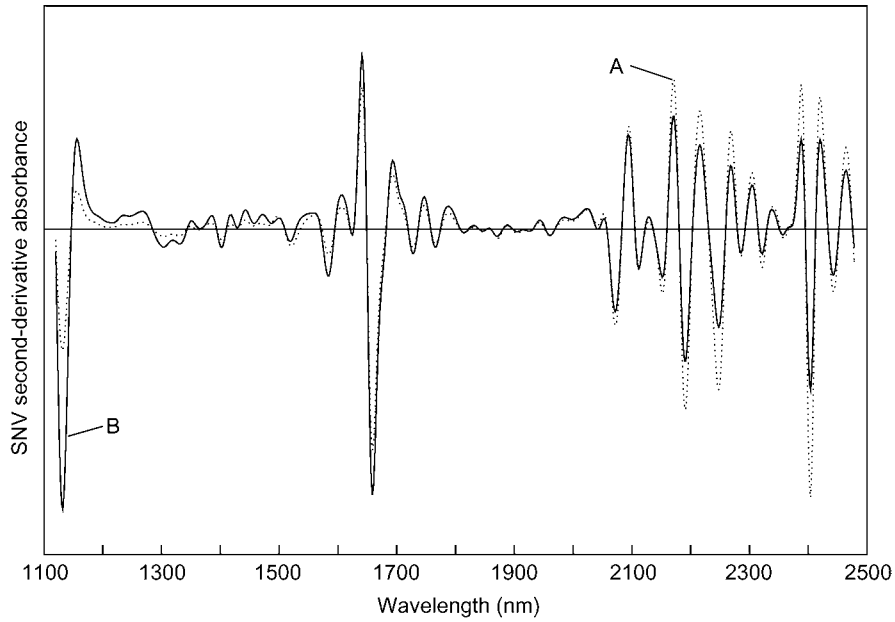

Figure 34.13 Effect of crystallinity on the NIR reflectance of salicyclic acid: (A, dotted line) original crystalline sample; (B, solid line) sample after melting and allowing to solidify. Both spectra are shown as SNV-transformed second-derivative absorbance ($-\log_{10} R$) spectra.

treated equally. A critical r value of 0.95 has commonly been used as a cut-off point; r values greater than 0.95 indicate a positive identification, and values below 0.95 indicate a mismatch. The exact value needs to be found by trial and error (see Creating a database).

Crystallinity has a marked effect on identification by correlation in wavelength space (and other identification algorithms). The relative magnitudes of peaks are very dependent upon the presence of specular radiation in the reflectance signal. Differences in crystallinity can cause r to fall to very low values and a correlation plot characteristically shows a number of intersecting straight lines rather than the ideal single line. This is illustrated for the salicylic acid sample spectra of Fig. 34.13 in the correlation plot (Fig. 34.15), which shows that different small wavelength regions of the spectra show good correlations (i.e. the individual lines), though the overall correlation coefficient is only 0.9 because the lines are not coincident. Careful visual inspection of spectra is vital when trying to identify substances.

Small shifts in wavelengths between sample and reference spectra can have a disastrous effect on identification by correlation. For a typical second-derivative spectrum, a shift of 1 nm might cause the correlation coefficient to fall to 0.97. This needs to be kept in mind if sample and reference spectra are measured on different instruments.

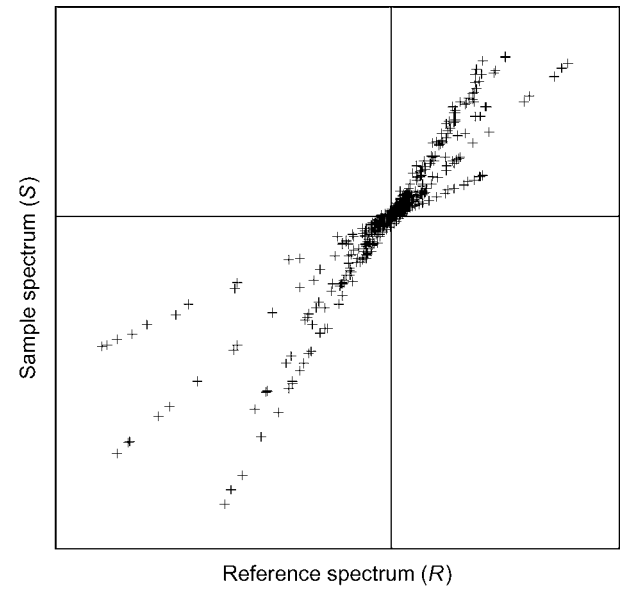

Figure 34.15 Effect of crystallinity on correlation in wavelength space. Reference spectrum, original salicylic acid sample; sample spectrum, melted and solidified sample (spectra shown in Fig. 34.13). Second-derivative spectra, correlation coefficient, $r = 0.904$.

Maximum wavelength distance

This algorithm compares spectra by testing whether the sample spectrum falls within an 'acceptance envelope' centred on a mean reference

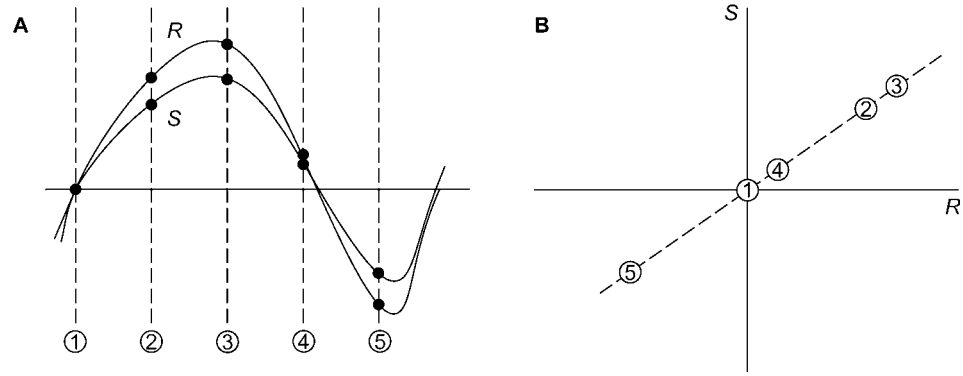

Figure 34.14 Correlation in wavelength space: (A) plot of spectra R (reference) and S (sample); (B) plot of S amplitude versus R amplitude.

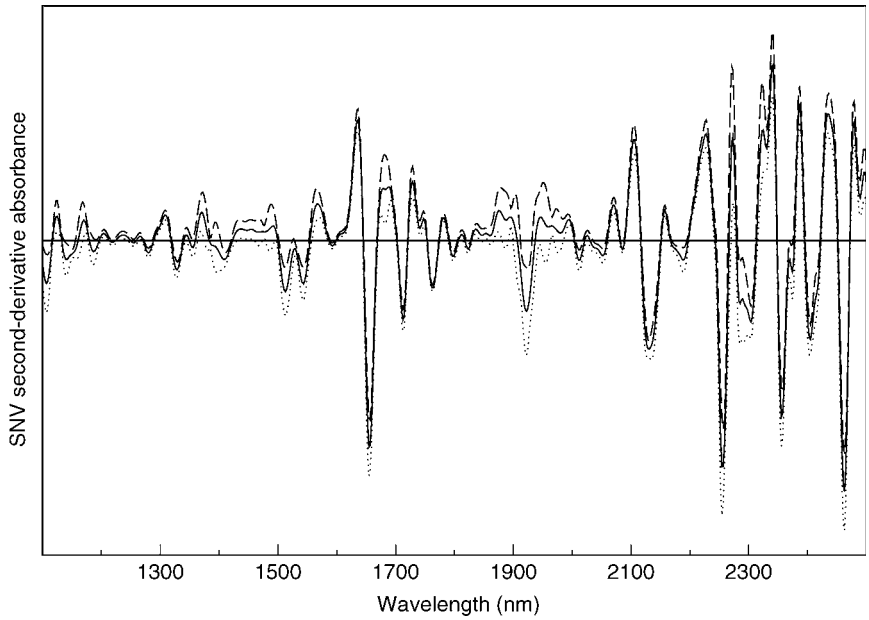

Figure 34.16 Spectra illustrating the method of maximum distance in wavelength space. Mean spectrum (solid line) with ±5.04 standard deviations envelope (dashed lines) for chlortetracycline hydrochloride (20 spectra, 690 wavelength data points). There is a 95% probability that another spectrum from the same population will fall within the envelope.

spectrum. A representative number of samples of the reference material, which should include all acceptable variations in parameters such as particle size, crystallinity, water content and purity, are measured and the mean spectral value and standard deviation at each wavelength calculated. An acceptance envelope on either side of the mean reference spectrum is then calculated, corresponding to the standard deviation multiplied by some critical value. The amplitude of the envelope changes across the spectrum, which reflects that some regions are more variable than others (Fig. 34.16). Spectral regions associated with water, for example, typically show greater variation. The size of the envelope (i.e. critical value) depends upon the number of reference spectra, number of wavelengths and the probability level required.

The mean, $\overline{X}$, and standard deviation, s_i, of the n reference spectra at each wavelength i are calculated:

$$\overline{x}_i = \frac{1}{n}\sum_{j=1}^{n} x_{ij} \quad s_i = \left[\frac{1}{n-1}\sum_{j=1}^{n}(x_{ij}-\overline{x}_i)^2\right]^{1/2}$$

The test statistic, t, is given by:

$$t = \max\left[\frac{(y_i-\overline{x}_i)}{s_i}\left\{\frac{n}{(n+1)}\right\}^{1/2}\right]_{\text{over all } i}$$

where y_i is the spectral value of the test sample spectrum at wavelength i.

The test is simply a two-sampled Student's t test carried out at each wavelength over the whole spectrum or part of the spectrum of interest. Critical values of t need to be calculated according to the number of wavelengths used as well as the reference sample size. Table 34.2 lists the critical values for some commonly used reference sample sizes, number of wavelengths and probability levels (with fewer than 10–12 reference samples, the critical t values are unreasonably large). Further details of the method and how critical values can be calculated are given in Gemperline and Boyer (1995). A sample spectrum with a t value that exceeds the critical value (i.e. outside the acceptance envelope) is considered to have come from a different population from that to which the reference spectra belong. For many purposes it is sufficient to select a critical value by trial and error.

Table 34.2 Critical Student's *t* values for maximum wavelength distance method

Number of samples in reference set, n	*Number of data points in spectrum*			
	500		*700*	
	p = *0.05*	p = *0.01*	p = *0.05*	p = *0.01*
5	15.44	23.30	16.82	25.35
8	7.85	10.09	8.28	10.62
10	6.57	8.10	6.87	8.44
12	5.90	7.09	6.14	7.36
15	5.35	6.28	5.54	6.49
20	4.89	5.62	5.04	5.78
30	4.50	5.08	4.62	5.21

Unlike correlation in wavelength space, this algorithm is sensitive not only to the shape of the spectrum but also to its magnitude; therefore, some form of data pre-treatment is commonly applied before comparing spectra. The use of derivatives and/or SNV normalisation to remove unwanted baseline shifts, etc., is typical. The algorithm is very sensitive to small spectral differences and can be used not only for identification but also to distinguish between different grades or sources of materials. The high sensitivity of the procedure makes it difficult to transfer between instruments.

Other classification procedures

There are numerous chemometric procedures that can be used for the classification of NIR spectra. Methods based on principal component analysis (PCA), such as soft independent modelling of class analogies (SIMCA) or simple visual inspection of PCA score plots, can be very useful for distinguishing between the spectra of closely related materials. Textbooks on chemometrics should be consulted for further details (Adams 1995; Martens, Næs 1991; Næs *et al.* 2002; Otto 1999). The

tutorial review by Downey (1994) is a useful guide. The software supplied with many NIR instruments commonly includes a range of chemometric algorithms. NIR spectroscopy without chemometrics is unimaginable, but its incorrect use is very common. Readers are strongly recommended to consult a number of papers describing the 'Use and abuse of chemometrics' (Pretsch, Wilkins 2006).

Creating a database

When setting up a database it is important to include all substances closely related to those of direct interest, and other likely substances, so that the optimum parameters (e.g. data pre-treatment, wavelength range) for distinguishing between the various materials can be found. As the NIR spectra of powders are sensitive to the physical characteristics of the samples, it is wise to include spectra from different batches of material to increase the probability of correct identification. The first step in validating a database is to see whether it is possible to distinguish between all the compounds within it, which is achieved most easily by comparing all possible pairs of spectra and displaying the test statistic values (e.g. number of peaks matched, *r*, *t* value) as a histogram. Ideally, a critical value can be selected below (or above) which all different pairs of compounds fall and above (or below) which all batches of the same material occur. In practice, unless the database is very small, it is unlikely that all compounds will be distinguishable and a compromise value for the test statistic must be made. To test the robustness of the identification procedure the database should be challenged with samples not used in its construction. The importance of visually inspecting spectra and not relying only on the test statistic value cannot be too strongly stressed.

Problem compounds

Compounds with long aliphatic chains, such as hydrocarbons, stearates and waxes, are difficult to distinguish as the spectrum becomes dominated by the $–CH_2$ groups. Starches and related compounds, such as maltodextrins, gums and other materials with polysaccharide groupings, are difficult to distinguish from each other reliably. With inorganic compounds, which often have little or no genuine absorbance, care is required that any apparent spectral matching is not from absorbances caused by residual moisture, Wood's peak and/or the sample bottle itself. Mid-IR and other spectroscopic techniques are most probably no better for these problem compounds – the extremely good signal-to-noise level in NIR spectra means that often the chemometric classification techniques are able to distinguish more easily between closely related compounds than they can for other spectra.

Detection of counterfeit materials

The ability of NIR spectroscopy to detect both physical and chemical differences between samples means that it can used like fingerprinting. For example, batch-to-batch variation of tablets, or of tablets manufactured at different sites, can often be distinguished. Similarly, the detection of counterfeit products is possible (Scafi, Pasquini 2001). Spectra of a representative sample of the genuine product are compared with those of the suspected counterfeit. Although spectral comparison methods, such as maximum wavelength distance, may be used, methods such as PCA are often to be preferred. A simple visual plot (two- or three-dimensional) of the principal component scores is often sufficient. Different data pre-treatments (use of derivatives, SNV, MSC, etc.) should be investigated to remove unwanted differences in spectra. Similar samples form clusters of points on the plots, while differences in scores indicate differences in samples (Fig. 34.17). More complex methods of classification (such as SIMCA, etc.) may be usefully applied and allow probability levels to be set.

Materials and/or products must be handled with care. Tablets and the like often rapidly exchange moisture with the atmosphere and an apparently suspect product might simply have a different moisture content. If moisture differences are not important, then genuine and suspect samples should be allowed to equilibrate to ambient humidity before measurement. When differences in moisture are important, measurements must be made as soon as the samples are opened (e.g. tablets must be measured immediately they are removed from a blister pack).

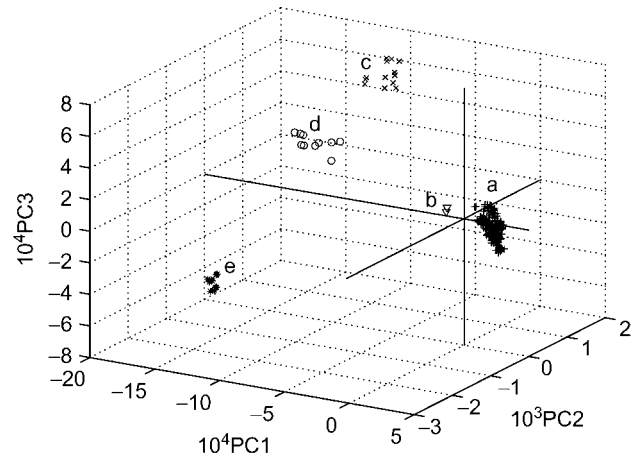

Figure 34.17 PCA score plot (first three principal components, PC1, PC2 and PC3) illustrating the detection of counterfeit tablets. (a) and (b) genuine product; (c), (d) and (e) counterfeit samples.

Examination of the principal component loadings plots can sometimes indicate possible causes for the differences between samples, especially those with just one major cause of difference. For example, when the largest difference is due to moisture content (genuine and test samples well separated along the first principal component), the loading plot resembles the spectrum of water.

Quantitative analysis

Quantitative methods of NIR analysis are generally time-consuming to set up and not suited to 'one-off' assays. However, once developed, a quantitative assay takes little more than the time to record a sample spectrum. The purpose of calibration is to construct a model that relates the sample concentration of one or more analytes to the spectral data from training samples (those samples used to create the model) and then to use the model to predict the analyte values of future new samples. NIR is best suited to measuring major components (10–90% by mass (m/m)), though with strongly absorbing substances useful calibrations as low as 1% have been achieved. The strong absorption exhibited by water often allows it to be determined at $<0.1\%$ m/m levels in solvents by transmittance. Transmittance NIR spectroscopy generally gives better results for solids than reflectance, as it is less sensitive to the inhomogeneity of the sample and gives results more representative of the bulk material.

The complex nature of NIR spectra makes it unlikely that, even for simple mixtures, a unique wavelength for the analyte (unaffected by other compounds) can be found. Some form of multivariate calibration procedure, such as multiwavelength linear regression (MLR), or whole-spectrum methods, such as principal component regression (PCR) and partial least-squares regression (PLSR), will be required. Details of these methods can be found in numerous textbooks (Adams 1995; Burns, Ciurczak 2001; Martens, Næs 1991; Næs *et al.* 2002; Otto 1999). A review of multivariate calibration has been written by Brereton (2000), while more recently Small (2006) has written about some of the pitfalls of multivariate calibration. Software provided with most spectrophotometers usually includes these calibration methods. Specialised software packages are also readily available, such as PLS-Toolbox (Eigenvector) for MATLAB (MathWorks), Pirouette (Infometrix), Unscrambler (Camo ASA) and SIMCA-P (Umetrics).

Calibration involves a number of steps:

- Selecting/preparing a set of samples
- Measuring their spectra and determining their reference values
- Developing and selecting the best model
- Validating the model.

Sample selection for constructing the model is extremely important. Methods such as PCR and PLSR are essentially statistical, and therefore it is vital that the calibration samples cover all possible sources of variation likely to be encountered in the samples that are later to be predicted. All possible chemical components and their range of concentrations, along with all variations in physical properties (e.g. particle size, powder compaction) must be included if a robust model is to be produced. Samples should be designed such that there is a minimum of correlation between all the concentrations or other properties of the components. The generation of such samples to cover a sufficiently wide range of compositions for products such as tablets, which are normally manufactured to within very tight limits, presents numerous problems. Useful guidelines are provided by the Pharmaceutical Analytical Sciences Group (2001). Blanco *et al.* (2001) investigated a number of strategies to extend the composition range. Synthetic samples, prepared by mixing weighed quantities of the pure components, and doped samples, made by adding known amounts of active component or excipients to powdered production samples, may be used. Neither approach is entirely satisfactory, though sample sets that contain production, doped and synthetic samples gave acceptable models. Reference values for the analytes of interest must be measured for the samples using a reliable analytical technique, such as HPLC, UV–visible spectrophotometry or titration. The accuracy of the model cannot be better than that for the reference values. The samples should be divided at random into ideally three sets: a calibration set, an optimisation set (a ratio of 2 : 1 or 3 : 1 is suitable) and a test set. The calibration set is used to construct the model, while the optimisation set is used to optimise the model (e.g. select the number of principal components, etc. required). Finally, the test set is used to demonstrate that the model is robust and predicts correctly for real samples. These three data sets must be independent, e.g. samples from the same batch of a product should appear in only one of the sets. Although there are no fixed recommendations for the number of calibration samples required (it depends upon the complexity of the samples and calibration model), a minimum of 30–60 is not atypical. Six to ten samples for each wavelength and/or principal component used in the model are a minimum requirement.

Before applying MLR, PCR, etc., spectra generally require some form of pre-treatment to remove baseline shifts, particle size effects, and so on. Commonly applied pre-treatments are first- or second-derivative, SNV, MSC or other normalisations. Combinations of such transformations are often applied. There are no definite rules for selecting the best pre-treatment to use, apart from trial and error. Similarly, the choice of model (MLR, PCR, PLSR, etc.) is also largely a matter of trial and error until the best is found, PLSR being the most popular.

With simple samples, such as solutions or solids in which only a single component is variable in a nearly constant matrix, it is often possible to construct a calibration model based on MLR. The concentration, c, of the analyte is modelled to an equation of the form:

$$c = b_0 + b_1 A_1 + b_2 A_2 + \cdots$$

where A_1, A_2, ... are the spectral values at wavelengths 1, 2, ..., respectively, and b_0, b_1, b_2, ... are the coefficients found by least-squares regression. The number of wavelengths required depends upon the complexity of the samples. Finding the optimum number of wavelengths and their values is not a trivial problem. Ideally, a full search of all possible combinations of wavelength values from the whole spectrum (i.e. perhaps >500 data points) should be carried out. While this is possible with two or three wavelengths, the time taken for larger numbers of wavelengths makes this impractical. For complex samples, in which many components vary, whole-spectrum techniques, such as PCR and PLSR, are preferred. Care must be taken not to over-fit the calibration data by using too many parameters in the model. The final step, validation, is the most important. The model produced is used to predict the values for the test set that can then be compared against their reference values. Only if the values predicted for this independent data set are satisfactory can the model be considered acceptable.

Plots of NIR predicted values versus reference values for both the calibration and test sets (e.g. Fig. 34.18) should be examined. Visual inspection easily reveals any problems with the data sets and model, such as outliers or curvature. Ideally, both plots should be straight lines with slope unity and intercept zero. A good calibration plot but poor validation plot suggests over-fitting of the model. Numerically, the fit can be assessed by calculating the standard error of calibration (SEC) and standard error of prediction (SEP):

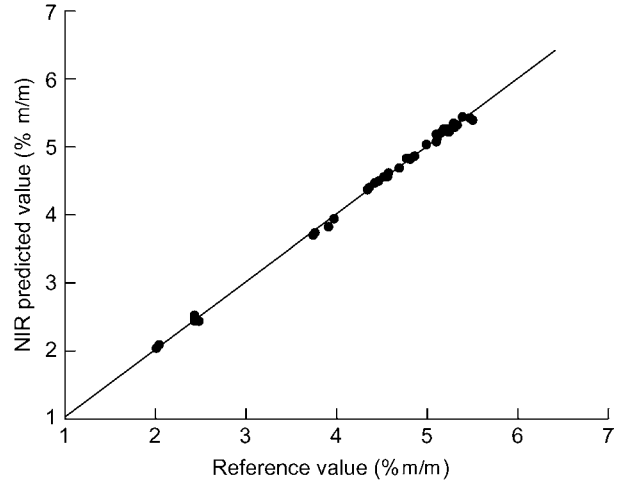

Figure 34.18 Typical validation plot of near-infrared predicted value against reference value.

$$\mathrm{SE} = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \hat{X}_i)^2}{D}}$$

where X_i is the reference value and $\hat{X}$ is the NIR predicted value for the ith sample of the calibration set (for SEC) or validation set (for SEP), n is the number of samples in the data set being considered, and D is the number of degrees of freedom (for the calibration set this will be n minus the number of parameters fixed in the model, e.g. number of wavelengths used for MLR plus 1, number of components used plus 1 (if data centred) for PCR or PLSR). For the validation set, $D = n$.

For a good model, SEC and SEP should be similar and not much larger than the standard error of the reference method. SEP is a measure of the accuracy (how close the NIR predicted values are to the reference values) for the method and, of course, needs to be compatible with the intended purpose to which the procedure is to be put. Moffat *et al.* (2000) have shown how quantitative NIR can meet the requirements of the International Conference on Harmonisation's Guidelines on Validation of Analytical Procedures for the assay of an intact pharmaceutical product in terms of specificity, linearity, range, accuracy, precision and repeatability.

Near-infrared imaging

The coupling of an NIR spectrometer and scanning microscope allows spectroscopic imaging of surfaces. Not only can small samples be identified, but information about the distribution of different chemical components and their particle size can be obtained. Commercial reflectance NIR microscopy mapping systems are based on one of three data acquisition methods. In point mapping, a single detector is used to measure a spectrum at a single point on the sample surface and then the sample position is moved and the process is repeated until the complete area has been scanned. Line mapping works in a similar way except that a linear array of detectors is used so that data are more quickly acquired. In global mapping, the whole sample area is measured simultaneously using some form of tuneable filter to control the wavelength selection. Typically spectral resolution of 4–16 cm^{-1} and spatial resolution of >10 μm can be achieved. Because of the nature of NIR diffuse reflection, it is most probably not meaningful to work at spatial

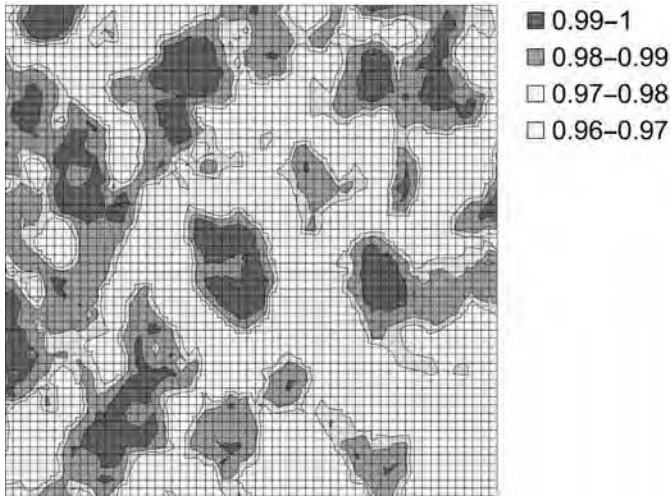

Figure 34.19 NIR image of a cross-section of a ranitidine tablet. Each pixel is $30 \times 30\,\mu m^2$. The image is 60×60 pixels (i.e. $1.8 \times 1.8\,mm^2$). The map shows the value (coded in different shades of grey) of the correlation coefficient of the NIR spectrum at each pixel when compared with that of a pure sample of ranitidine. (Reproduced with permission of RA Watt and A Duszyńska.)

resolutions of $<20\,\mu m$. A surface area of 1–10 mm^2 of the sample is commonly analysed by recording a spectrum for, say, each $20 \times 20\,\mu m^2$ area of sample, which allows a 'grid' of spectral information to be constructed – this gives many thousands of spectra. Spectral information comes from the top surface layers down to a depth of approximately 30–100 μm and represents 10–100 ng of sample. With use of suitable chemometrics the spectra may be processed to determine the chemical identity at each 'grid' position. Chemical image plots to show the distribution of the various chemical species may then be created for the whole surface. Clarke *et al.* (2001) have described such a system using both NIR and Raman spectroscopic data to give a chemical image of pharmaceutical formulations.

Figure 34.19 shows an image of a $1.8 \times 1.8\,mm^2$ cross-section of a ranitidine tablet (∼40% m/m active). The image is 60×60 pixels (each pixel is $30 \times 30\,\mu m$). The NIR spectrum from each pixel has been correlated against the NIR spectrum of a pure sample of ranitidine and the different correlation ranges are assigned different colours to give the map shown. Images are useful for comparison purposes, comparing genuine and counterfeit samples, good and bad blends, etc. However, any attempted physical interpretation of what the image represents needs to be carried out with considerable care.

Collections of data

Several commercially available NIR spectral libraries have been developed. NICODOM Ltd have a library containing 696 spectra of actives and excipients used in the pharmaceutical industry as well as a library of 660 NIR polymer spectra. Thermo Scientific have a FT-NIR spectral library collection of 385 active and inactive pharmaceutical substances. Fiveash Data Management, Inc. can provide 330 NIR spectra of food and agricultural materials. A small selection of printed NIR spectra is found in the *Handbook of Organic Compounds, NIR, IR Raman, and UV-Vis Spectra Featuring Polymers and Surfactants* (Workman 2001). The third edition of *Infrared and Raman Characteristic Group Frequencies – Tables and Charts* (Socrates 2001) has a section on NIR. The *Handbook of Pharmaceutical Excipients* (Rowe, Shesky 2009) has NIR spectra for many excipients.

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Resources

Camo ASA. *Unscrambler*. Camo ASA, Oslo. www.camo.no.

Eigenvector Research Inc. *PLS-Toolbox*. Eigenvector Research Inc., Manson, WA. www.eigenvector.com.

Fiveash Data Management, Inc. *FDM Reference Spectral Databases*. Fiveash Data Management, Inc., Madison, WI. www.fdmspectra.com.
Infometrix. *Pirouette.* Infometrix Inc., Bothell, WA. http://www.infometrix.com.
NICODOM Ltd, Hlavni 2727, CZ-14100 Praha 4, Czech Republic. www.ir-spectra.com.
Pharmaceutical Analytical Sciences Group. *Guidelines for the Development and Validation of Near Infrared (NIR) Spectroscopic Methods*. www.pasg.org.uk/NIR/NIR_Guidelines_Oct_01.pdf.
Thermo Scientific. Thermo Fisher Scientific Inc. www.thermo.com.
Umetrics. *SIMCA-P.* Umetrics, Umeå. www.umetrics.com.

CHAPTER

35 Raman Spectroscopy

DE Bugay, PA Martoglio Smith and FC Thorley

Vibrational spectroscopy has been an integral tool for the identification and characterisation of drugs. When one typically thinks of vibrational spectroscopy, infrared (IR) techniques come to mind, not Raman spectroscopy. However, over the last 25 years a renaissance of the Raman technique has been seen, mainly due to development of instrumentation. These developments have led to unique applications in the pharmaceutical and forensic industries where drug identification and characterisation are necessary. This chapter presents to the reader the theory, instrumentation, sampling techniques and applications of Raman spectroscopy as applied to drugs.

Introduction and theory

Raman spectroscopy is a form of vibrational spectroscopy that has widespread use in pharmaceutical investigations. Applications include chemical structure elucidation, routine chemical identification and solid-state characterisation such as that of polymorphism. Raman spectroscopy is also applicable to drug product characterisation including solid form analysis of the drug incorporated into the formulation, contaminant analysis, drug–excipient interaction and problem-solving. A distinct advantage of pharmaceutical analysis by Raman spectroscopy is the ease of the technique and its broad range of applicability. Analysis can be performed on virtually any type of sample such as single crystals, bulk materials, slurries, creams, particulates, films, solutions (aqueous and organic), oils and on-process streams (the last through the use of fibre-optic probes). Additionally, Raman spectroscopy is typically non-destructive in nature, allowing recovery of the material for further characterisation. Since Raman spectroscopy measures the vibrational motions associated with a molecule, it is complementary to IR spectroscopy as well as other characterisation techniques. Finally, under proper sampling conditions, Raman spectroscopy is a quantitative technique.

When a compound is irradiated with monochromatic radiation, the radiation is transmitted, absorbed or scattered by the molecule. Of the scattered radiation, a majority of the photons are scattered at the same frequency as the incident radiation frequency. This form of scattering has been termed *elastic* or *Rayleigh scattering*. If the scattered radiation is passed into a spectrometer, a strong Rayleigh line is detected at the unmodified frequency of radiation used to excite the sample. Additionally, a very small percentage of photons (about one per million) is scattered at frequencies arrayed above and below the frequency of the Rayleigh line. The *differences* between the incident frequency of radiation and arrayed frequencies correspond to the frequency of molecular vibrations present in the molecules of the sample. These wavelength-shifted frequencies are termed *inelastic scattering* and a collection of these wavelength-shifted frequencies is termed a Raman spectrum. For example, a Raman line at $\pm 2980\ cm^{-1}$ may be obtained on either side of the Rayleigh line and thus the sample possesses a vibrational mode at this frequency. The frequencies of molecular vibrations are typically 10^{12}–10^{14} Hz. A more convenient unit, which is proportional to frequency, is wavenumber (cm^{-1}) since fundamental vibrational modes lie between 3600 and 50 cm^{-1}.

As shown in Fig. 35.1, when a molecule is irradiated with monochromatic radiation, a number of different transitions may occur. If the radiation is of sufficient energy, an absorption process may occur representing an electronic transition ($S_o \rightarrow S_1$, UV/visible spectroscopy). If a slightly less energetic source is used, the molecule will be promoted to a *virtual state*. The virtual state represents a distortion of the electron distribution of a covalent bond within the molecule. After promotion to the virtual state, the molecule will immediately relax back to the original ground electronic state by emitting a photon. If the molecule relaxes back to the original vibrational state, the emitted photon is of the same frequency as the incident radiation. This represents Rayleigh or elastic scattering. If the molecule relaxes back to a higher vibrational energy state, the emitted photon represents less energy than the incident radiation. The inelastically scattered photon will have a longer wavelength (lower frequency) than the incident radiation. This energy transition represents Stokes Raman scattering. Conversely, if the molecule relaxes back to a lower vibrational energy state, the emitted photon represents greater energy than the incident radiation. The inelastically scattered photon will have a shorter wavelength (higher frequency) than the incident radiation. This energy transition represents anti-Stokes Raman scattering. Generally, the anti-Stokes lines are less intense than the Stokes lines because these transitions arise from higher vibrational energy levels containing fewer molecules, as described by the Boltzmann distribution. Hence, the Stokes portion of the spectrum is generally used.

A Raman spectrum is normally represented as a plot of Raman scattering intensity (ordinate) versus wavelength (abscissa). Normally, the abscissa of the spectrum is labelled as wavenumber shift or Raman shift (cm^{-1}) and the negative sign (for Stokes shift) is omitted (Fig. 35.2). The wavenumber or Raman shift represents the shift in frequency of a photon from the exciting wavelength.

Pharmaceutically relevant molecules are typically covalently bound organic molecules. The chemical bonds within these molecules consist of an electron cloud. In a Raman experiment, the electromagnetic radiation incident on the molecule consists of oscillating electric and magnetic fields. Interaction of the electromagnetic radiation with the chemical bond causes the electron cloud to oscillate. The oscillation in turn causes a photon to be emitted. This is called scattering. In Rayleigh scattering, the energy from the incident electromagnetic radiation causes electron oscillation and the emitted photon is observed at the same frequency. In Raman scattering, an additional energy transition occurs. The polarisability of the electron cloud may change as the positions of the atoms making up the chemical bond change. In other words, a vibrational mode may cause atoms to be displaced, in turn affecting the polarisability of the electron cloud of the chemical bond. In this case, the incident radiation causes the electron cloud to oscillate, but the electron cloud oscillation is also affected by the change in polarisability caused by a change in the position of the atoms during the molecular vibration. Oscillation causes a photon to be emitted, but the frequency is perturbed by the change in polarisability of the chemical bond. For a vibrational mode to be Raman active, a change in polarisability must take place during the vibration. This condition is termed the Raman selection rule. Since the polarisability of a chemical bond is dependent upon the atoms making up that bond, as well as atoms in close proximity, Raman spectroscopy is a probe into the chemical or physical structure of a molecule. This is why Raman spectroscopy is an important tool for pharmaceutical analysis.

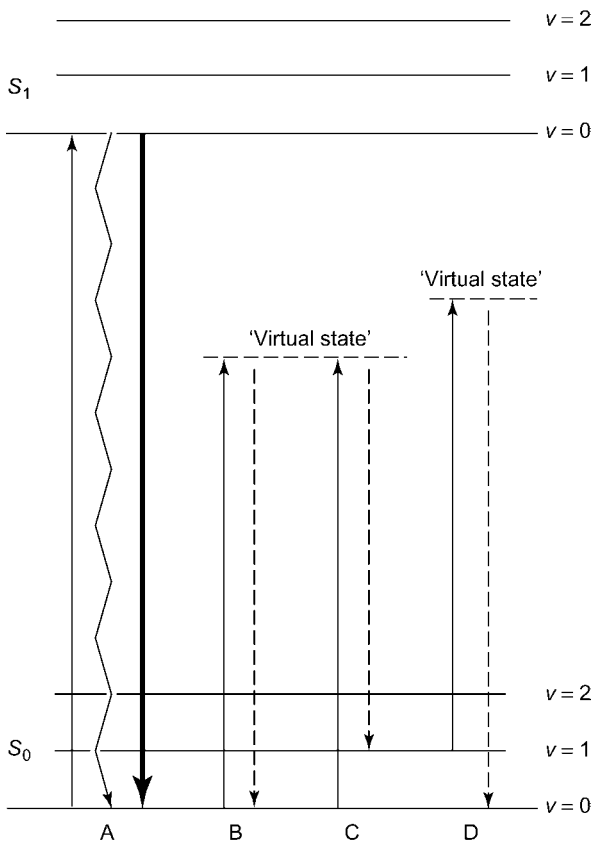

Figure 35.1 Jablonski energy level diagram illustrating possible transitions; solid lines represent absorption processes and dotted lines represent scattering processes. A, electronic transition with non-radiative decay (heat: ↯) or radiative decay (fluorescence: ⇓); B, Rayleigh scattering; C, Stokes Raman transition; D, anti-Stokes Raman transition. S_0 is the singlet ground state, S_1 the lowest singlet excited state and *v* represents vibrational energy levels within each electronic state.

Instrumentation

Although the Raman effect was discovered in 1928, the first commercial Raman instruments did not start to appear until the early 1950s. These instruments did not use laser sources, but used elemental sources and arc lamps. In 1962, laser sources started to become available for Raman instruments and the first commercial laser Raman instruments appeared in 1964–65. The first commercial Fourier transform (FT) Raman instruments were available starting in 1988 and, by the next year, FT-Raman microscopy was possible.

Dispersive spectrometers

The basic configuration and components of a dispersive spectrometer are shown in Fig. 35.3. The source of monochromatic radiation is a laser. Typically helium–cadmium (325, 354 or 442 nm), air-cooled argon-ion (488 or 514 nm), doubled continuous-wave neodymium–yttrium –aluminium garnet (Nd : YAG or Nd : $Y_3Al_5O_{12}$; 532 nm), helium–neon (633 nm) or stabilised diode (785 nm) lasers are used for dispersive Raman spectrometers. The stability of the emitted radiation from a laser is one of the key attributes of a good spectrometer. Frequency stabilisation of the laser under standard laboratory conditions (slight temperature fluctuations, vibrational effects, etc.) is required. Laser lifetimes and cost are also considerations for the choice of the laser to be used. One additional consideration for laser selection in dispersive Raman systems is the generation of fluorescence. As stated previously, the Raman signal is fairly weak. For many organic systems, fluorescence may occur when the helium–neon laser frequency of 632.8 nm is used and, instead of promoting the molecule of interest to a virtual state, an electronic transition occurs with subsequent radiative decay (fluorescence, see Fig. 35.1). The fluorescence background signal can be so intense as to mask the Raman scattered photons. Fluorescence is also wavelength dependent, so a sample that fluoresces with one laser source may not with another. If fluorescence does not pose a problem, lower-frequency lasers can be used (532, 514 nm) for enhanced sensitivity, as the efficiency of Raman scattering is proportional to $1/(\lambda)^4$, where λ is wavelength of excitation.

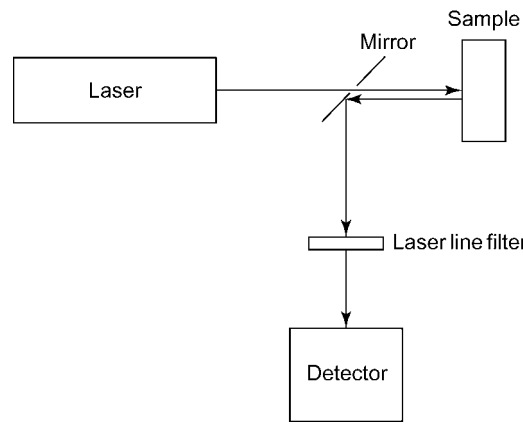

Figure 35.3 Schematic representation of a dispersive Raman spectrometer.

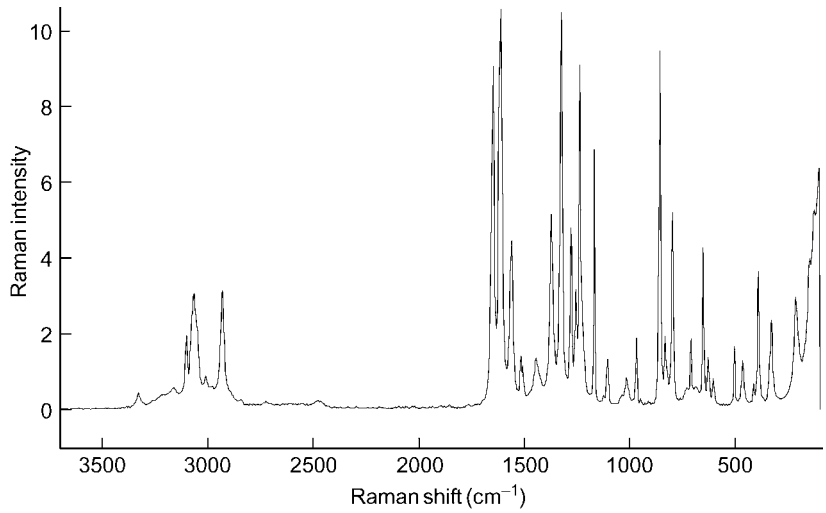

Figure 35.2 FT-Raman spectrum of paracetamol (acetaminophen).

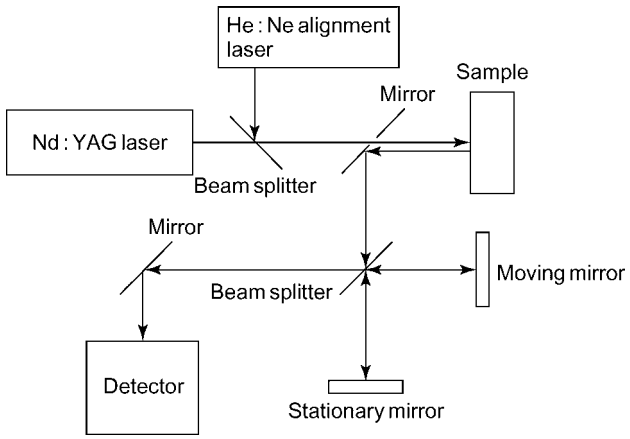

Figure 35.4 Schematic representation of an FT-based Raman spectrometer.

If fluorescence is a problem when using these high-energy sources, then lower-energy sources, such as those used in FT-based Raman spectroscopy, can be used to minimise the fluorescence effects (see below).

In a dispersive Raman spectrometer, the sample is positioned in the laser beam and the scattering radiation is collected in either a 180° backscattering or a 90° right-angle scattering configuration (see Fig. 35.3). Recent developments also offer transmission Raman geometries, which can be useful for analysing bulk tablets or capsules, as well as on-line process monitoring (Matousek, Parker 2007). After irradiation of the sample, a laser-line rejection filter is put in place to filter out the Rayleigh scattering. Finally, a detector is positioned in the spectrometer. For dispersive systems, typically a charge-coupled device (CCD) is utilised. Silicon CCD detectors are normally used for Raman spectrometers in which visible wavelength lasers are used. Previously, photomultiplier tubes (PMTs) were used for detection, but, since the advent of CCDs and their inherently better performance, PMTs are not normally used today. All commercial spectrometers are controlled digitally by a computer system.

Interferometric spectrometers

Figure 35.4 displays the configuration of an FT-based Raman spectrometer. Advantages of an FT-Raman spectrometer are wavelength accuracy and the use of a near-infrared laser which typically eliminates fluorescence. In the FT-based system, a $Nd:YVO_4$ laser, 1064 nm, is used to irradiate the sample. Analogous to the dispersive system, the sample is positioned in the laser beam and the scattering radiation is collected in either a 180° backscattering or a 90° right-angle scattering configuration. The scattered photons are then passed into an interferometer with laser line filtering. Detection of the scattered photons from systems that utilise lasers emitting light with wavelengths greater than 1000 nm are of the single-element type, either high-purity p-type germanium or indium/gallium/arsenic (InGaAs) detectors. These detectors are noisier than CCDs or PMTs, but do exhibit high quantum efficiencies. By cooling the germanium (Ge) detector to 77 K, the frequency response is extended to $\sim 3500\,cm^{-1}$.

By utilising the longer-wavelength $Nd:YVO_4$ laser in an FT-Raman spectrometer, fluorescence is minimised. The reason for fluorescence minimisation is that the longer-wavelength excitation has less likelihood of inducing an electronic transition with subsequent fluorescence than the relaxation mechanism.

Coupled techniques

Microscopy

Raman spectra can be acquired on small amounts of material through the use of a Raman microprobe. Utilising the microscope, the Raman scattered photons are collected in a 180° backscattering configuration that allows the operator to optically view the sample, focus the incident radiation and subsequently collect the Raman spectrum. Most commercial Raman microscope systems utilise confocal microscopy to increase axial resolution (*z*-axis). Confocal points are defined as the point source, the in-focus sample location and the focused image of the sample point. Axial resolution, defined as the distance away from the focal plane in which the Raman intensity from the sample decreases to 50% of the in-focus intensity, can be approximated from the numerical aperture (NA) used in the microscope. When utilising a 0.95 NA objective on a confocal microscope system, the axial resolution is proportional to the square of the NA, in this case 0.9025 μm.

An additional advantage of Raman microscopy is spatial resolution as opposed to axial resolution. The spatial resolution (*xy*-plane) is dependent upon the NA of the collecting objective and the wavelength of the laser radiation. Larger NA values and shorter wavelengths provide higher spatial resolution, often down to 1 μm. Since a high-intensity of monochromatic radiation from the laser is focused upon a small amount of sample, sample degradation by the laser must be monitored. Otherwise, the Raman microprobe is ideal for investigating polymorphism (single crystals), particulate contamination and small amounts of samples. Using an apparatus similar to those used for IR microspectroscopy, variable temperature and humidity studies can also be performed with a Raman microprobe.

Fibre-optics

Fibre-optics have been used in Raman spectroscopy since the early 1980s. Solids and liquids can be analysed with an arrangement of optical fibres on the end of a probe. Today, much of the research in the use of fibre-optics in FT-Raman spectroscopy centres around fibre and fibre bundle design, as well as collection angle. The number, type and arrangement of the fibres in a fibre bundle are all factors that are varied to produce fibre bundles for different applications. Fibre systems include single fibre, where the laser excitation and collected scattered radiation travel along the same fibre, and multi-fibre, where laser excitation is transmitted along one (or multiple) fibres and the scatter is transmitted to the detector along different fibres. The arrangements of the fibres in a multi-fibre system can also vary. Two examples include an arrangement in which one excitation fibre is surrounded by several collection fibres, or an arrangement in which several excitation and collection fibres are randomly mixed in a bundle. The collection process can occur via traditional backscatter, spatially offset Raman spectroscopy (SORS) or transmission. The greatest single advantage of the use of fibre-optics in Raman spectroscopy is the ability to sample remotely. No longer does the spectrometer have to be brought to the sample or vice versa. Fibre-optics can link the spectrometer to the sample, typically at distances of tens of metres. Common applications include monitoring of process streams or hazardous reactions.

Data processing and presentation of results

All contemporary dispersive and FT-based Raman spectrometers are controlled through a digital computer that handles instrument control, data collection, data processing and presentation of the spectral results. Utilisation of a FT-Raman spectrometer requires one extra step of data processing that is not required for dispersive-based Raman spectrometers. In the FT system, the original data are collected on a time scale and are subsequently Fourier transformed to obtain a frequency-domain spectrum. After this point, data processing for both systems is analogous.

Since the Raman spectrum of a particular sample is represented digitally, various additional processing applications can be performed. Spectral subtraction is one commonly used data processing technique as well as spectral smoothing, spectral searching and resolution enhancement. Figure 35.5 displays some of these processing techniques on one original data file. Since Raman spectroscopy can be utilised for quantitative measurements, digital representation of the spectrum allows for numerous means of measuring the analytical response of an analyte's signal and relating it to concentration. Electronic integration of

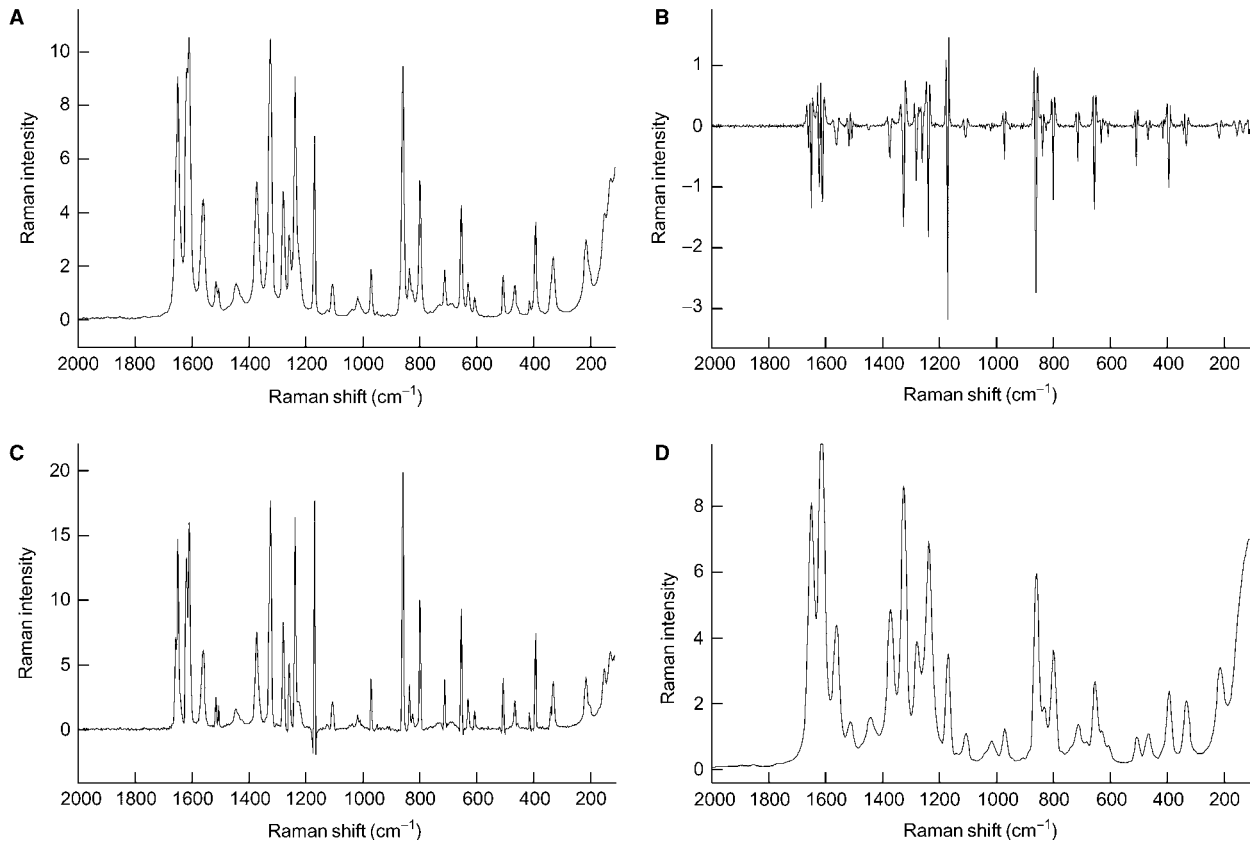

Figure 35.5 Examples of digitally processed spectral files: (A) original Raman spectrum; (B) spectral result after calculating the second derivative of the original Raman spectrum; (C) spectral result after resolution enhancement by Fourier deconvolution of the original Raman spectrum; (D) spectral result after Fourier smoothing the original Raman spectrum by 80%.

the peak area, curve fitting of the Raman spectrum and chemometric approaches can be utilised (Fig. 35.5).

System suitability tests

In a current good manufacturing practices (cGMP) environment, all laboratory instrumentation must be adequately inspected, cleaned and maintained. Additionally, instruments used for the generation, measurement or assessment of data must be adequately tested, calibrated and/or standardised. Based upon these regulatory agency requirements, the accuracy of Raman spectrometers needs to be assessed before usage via performance qualification (PQ). Typically, for the calibration testing of any instrument regulatory agencies require the use of a recognised standard material distributed by an official agency. In 2007, the American Society for Testing and Materials (ASTM) established a series of Raman shift frequency standards (ASTM E 1840) for calibration use. An attractive feature of using shift standards as opposed to wavelength standards (such as atomic emission lines) is that they are not dependent on laser wavelength. Standard Raman shifts for eight different materials are included in the ASTM standard. Included within these eight options are cyclohexane and sulfur, both of which have been recognised by the spectroscopy community for years as appropriate calibration standards. The Raman spectrum of sulfur displays vibration bands at 153, 219 and 472 cm^{-1}, whereas the spectrum of cyclohexane displays bands at 802, 1267, 1444, 2853, 2924 and 2938 cm^{-1} (Fig. 35.6). Silicon is another possible sample recognised by the spectroscopic community, and has a very strong peak at 520 cm^{-1}. A Raman intensity standard is still under consideration by the ASTM. One possible approach was proposed by Ray and McCreery (1997). Their approach uses the known luminescence of standards that fluoresce in response to laser irradiation to correct for the instrumental response function. An example of this type of correction can be found with Thermo Electron's background correction software function, which uses the white light source for the correction function. By collecting the fluorescence or white light source in the same configurations as the Raman sample of interest, sensitivities that vary across the Raman spectrum can be normalised.

System suitability has a deeper meaning than simply PQ when considered for solid-state method development and validation. In this context, system suitability testing ensures that the system (e.g. the Raman spectrometer) is working properly at the time of analysis. A good system suitability test ensures that the instrument is capable of providing appropriate data according to the developed method. For example, consider a solid-state Raman limit test or quantitative method for polymorphic analysis. Maintenance of the limit of detection (LOD) or minimal quantifiable limit (MQL) is essential if the method is to provide reliable data. Therefore, in addition to wavenumber accuracy, the signal-to-noise ratio (SNR) is also important. Because the Raman signal can be affected by sample focus and density, a reliable SNR measurement is best obtained from a solid (not powdered) material. A polystyrene puck is a useful material to use for system suitability in this regard. Polystyrene is also listed in the ASTM document noted above as a Raman shift standard.

Sample preparation and sample presentation

Sampling techniques for Raman spectroscopy are relatively simple since the only requirements are that the monochromatic laser beam irradiates the sample of interest and the scattered radiation is focused upon the detector.

Raman spectroscopy may be performed on very small samples (e.g. a few nanograms). Powders do not need pressing into discs or diluting with KBr as, in IR spectroscopy, the material simply needs to be irradiated by the laser beam. Solid samples are often examined in stainless-steel or glass sample holders generally requiring ~25–50 mg of material. Typically, liquid samples are analysed in quartz or glass cuvettes, which may have mirrored rear surfaces to improve the signal intensity. Glass is

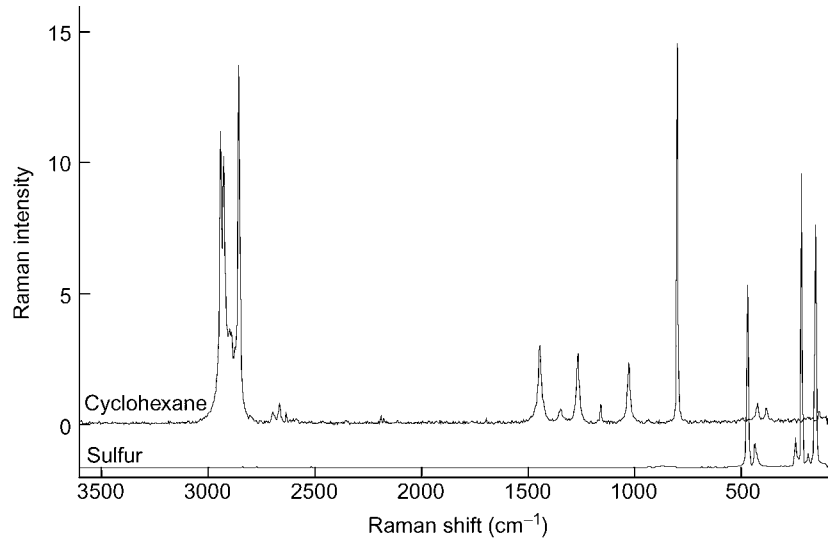

Figure 35.6 FT-Raman spectra of wavelength calibration standards cyclohexane and sulfur.

a very weak Raman scatterer, so many samples (liquid and solid) can be simply analysed in a bottle or, for example, in a nuclear magnetic resonance (NMR) tube, although fluorescence from some glasses can be problematic. Capillary tubes are also possible sample holders, but these should be used only when the sample is limited as the SNR on spectra obtained from capillary tubes can be poor. Water is a good solvent for Raman studies since the Raman spectrum of water is essentially one broad, weak band at 3500 cm^{-1}. One type of sample that may pose a problem is any material that is darkly coloured. Often, these samples absorb excessive heat and burn, causing sample and spectral degradation. Amorphous materials also have a tendency to absorb heat in the laser beam. To avoid sample burning, it may be necessary to dissipate the heat. This dissipation can be accomplished by reducing the laser power or by using an accessory that spins the sample, avoiding the irradiation of a single point in the sample. Further reduction of the laser power can also be accomplished through a neutral density filter. Sometimes, the sample can be diluted in KBr to help reduce sample burning.

The complete Stokes Raman spectrum covering shifts in the range 100 to ~3500 cm^{-1} can be obtained and the intensity of Raman scattering is directly proportional to the concentration of the scattering species, an important factor for quantitative analysis. However, the Raman effect is relatively weak and hence a material needs to be present at a concentration of at least about 1% for accurate assessments, whereas IR can be used to detect materials down to a concentration of approximately 0.01%. Fluorescence can also be problematic in Raman studies, but is typically due to additives in the glass sample tubes, or impurities within the sample of interest. Data massaging techniques can sometimes blank out Raman spectral contributions due to fluorescent materials. Photobleaching is another means of suppressing fluorescence. This technique involves irradiating the sample for a prolonged period of time (seconds to hours) with the laser. During this time the fluorescence may decrease due to the destruction of the fluorescing component as a result of the prolonged exposure to the laser irradiation. The spectrum is then acquired after photobleaching is complete. Of course, it is also possible that the laser radiation may change the component of interest. For example, a solvated crystalline material may desolvate with exposure to laser radiation. It would be wise to establish sample integrity by comparing spectra acquired with short acquisition times and those acquired with long acquisition times. Additionally, another technique, such as IR spectroscopy or X-ray powder diffraction (XRPD) could be used to check for sample changes after Raman analysis.

Variable-temperature studies in Raman spectroscopy provide a wealth of information. Because a Raman spectrum typically covers a wavelength range that extends beyond the range normally associated with mid-infrared spectroscopy (typically 4000–400 cm^{-1}), information about lattice vibrations of organic compounds is readily available. Varying the temperature of a sample changes the lattice energies of the compound, allowing for interpretation of the nature of the crystal lattice. In addition, information similar to that obtained in IR variable-temperature studies (crystal form changes and the nature of solvate association) can be achieved through variable-temperature Raman investigations.

Interpretation of spectra

The number of fundamental vibrational modes of a molecule is equal to the number of degrees of vibrational freedom. For a non-linear molecule of n atoms, $3n - 6$ degrees of vibrational freedom exist. Hence, there are $3n - 6$ fundamental vibrational modes. Six degrees of freedom are subtracted from a non-linear molecule since (a) three coordinates are required to locate the molecule in space and (b) an additional three coordinates are required to describe the orientation of the molecule based upon the three coordinates defining the position of the molecule in space. For a linear molecule, $3n - 5$ fundamental vibrational modes are possible since only 2 degrees of rotational freedom exist. Thus, in a total vibrational analysis of a molecule by complementary IR and Raman techniques, $3n - 6$ or $3n - 5$ vibrational frequencies should be observed. It should be noted that in complex molecules (such as pharmaceuticals) it may not be possible to observe every vibration due to overlap and relative intensity differences.

Regarding spectral interpretation, two of the best textbook sources are *Introduction to Infrared and Raman Spectroscopy* (Colthup *et al.* 1990) and *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules* (Lin-Vien *et al.* 1991). Both of these texts contain chapters dedicated to functional groups (e.g. methyl and methylene groups) and chemical compound types (e.g. ethers, alcohols and phenols). By the examination of peak location and intensity in Raman spectra, functional groups (and chemical compound types) can be determined. Discussed below are some guidelines for interpreting a Raman spectrum. It should be emphasised that this section is to serve as general background information. Any complete spectral interpretations should be made with the guidance of one of the referenced texts.

Raman spectroscopy provides information about the molecular bonding of a molecule. Certain functional groups will give rise to fundamental vibrational modes. For example, the C=C stretch in ethylenes will occur near 1650 cm^{-1}. The C–N stretch (amide III peak) for primary amides occurs between 1430 and 1390 cm^{-1}. A C–S stretch usually appears strongly in the 735–590 cm^{-1} region. There are many other functional groups that give rise to Raman peaks in specific spectral regions. Infrared bands will also occur in the same spectral regions, but because of selection rules the band intensities will differ, often dramatically. In the most basic terms, a vibrational mode is Raman

active when there is a change in polarisability during the vibration. Conversely, a vibrational mode is infrared active when there is a change in the molecular dipole moment during the vibration. Hence, vibrational modes that give rise to strong Raman peaks often give weak infrared bands and vice versa. It is because of this characteristic that infrared and Raman spectroscopy used together to examine the molecular bonds in a compound are described as 'complementary'. Some of the strongest Raman peaks will come from functional groups such as C=C, N=N, S–S, C–H, S–H, C=N, C=S and C–S, which have low polarity and high polarisability. These functional groups tend to occur in rather constant frequency ranges, although some shifts can occur based on other substituents in the molecule.

Vibrational modes can be separated into two classes: those in which the molecular bond is stretching and those in which it is bending. It takes a specific amount of energy for each of these actions to occur. The energy required depends on the atoms involved and the strength of the bond. In the theory section of this chapter, the nature of Raman excitation of a molecule was discussed. The location of the vibrational mode (its peak wavelength position in the spectrum) is related to the frequency of the excitation source (the laser) and the frequency of the scattered light. The equation defining this relationship is $h\nu = h\nu' + \Delta E_{\text{vibration}}$, where h is Planck's constant, ν is the excitation frequency, ν' is the scattered light frequency and ΔE is the vibrational energy. The vibrational energy is directly related to the strength of the bond and the amount of energy required to make that bond stretch or bend. These actions are elaborated below.

A stretching vibration is the motion that a molecular bond undergoes when the two atoms involved in the bond move apart and then contract. The stretch can be a simple contraction/expansion between two atoms such as the C=C stretch of ethylene. This type of motion occurs as a peak in the Raman spectrum at approximately 1650 cm^{-1}. When three atoms are involved, there are two types of possible stretches: symmetrical and antisymmetrical. Using a methylene group as an example, a symmetrical CH_2 stretch would occur when the two hydrogen atoms move apart from the carbon atom at the same time. This type of stretch occurs near 2853 cm^{-1}. The antisymmetrical CH_2 stretch occurs when one hydrogen atom is moving away from the carbon atom while the other hydrogen atom is moving closer to the carbon atom. This vibrational mode appears near 2926 cm^{-1}. The symmetrical stretches are typically stronger in Raman spectra (a greater change in polarisability), whereas the antisymmetrical stretches are stronger in infrared spectra. When many atoms are involved in the stretching, such as with aromatic rings, the types of stretches become more complicated. For example, a benzene ring monosubstituted with a halogen has 30 vibrational modes. Of these modes, some are strictly stretches, some are bends and some are combinations of both. Perhaps the simplest stretching mode for benzene rings is the ring-breathing mode, in which the two, four and six carbons move outwards. This mode occurs as a very strong Raman peak near 1000 cm^{-1} for mono-, *m*- and 1,3,5-trisubstituted benzenes. Clearly, many types of stretches can occur.

In a bending vibration, the molecular bond bends instead of stretching. Many types of bends can occur: antisymmetrical, symmetrical, torsion, scissor, wag, twist and rock. Using a CH_3 group as an example, antisymmetrical, symmetrical, rock and torsion bends are possible (Fig. 35.7). In the antisymmetrical bend, two of the CH bonds bend towards each other in a pinching motion while the third bends outwards and away from the pyramid. This mode occurs between 1470 cm^{-1} and 1430 cm^{-1}. In the symmetrical bend, the three CH bonds all bend inwards, similar to a grasping type of action. This mode occurs from 1395 cm^{-1} to 1365 cm^{-1}. In the CH_3 rock, the three CH bonds all bend in one direction in a sort of 'sweeping' mode. Finally, the CH_3 torsion involves the three CH bonds all bending in a clockwise direction in a twisting motion.

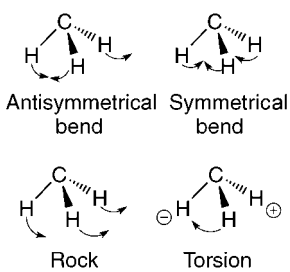

Figure 35.7 Bending vibrational motions associated with CH_3 (⊕ represents movement above the plane, ⊖ represents movement below the plane).

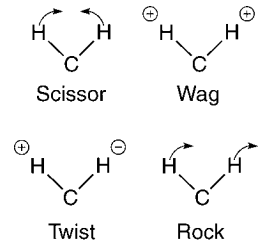

Figure 35.8 Bending vibrational motions associated with CH_2 (⊕ represents movement above the plane, ⊖ represents movement below the plane).

The CH_2 group can bend in slightly different ways (Fig. 35.8). A CH_2 scissor bend occurs when the two hydrogen atoms move towards each other in a scissoring motion. For a CH_2 wag, the two hydrogen atoms are bent towards the carbon atom. If the two hydrogen atoms alternately twist around one another, this is a CH_2 twist. Finally, if the two hydrogen atoms move back and forth, in line with the carbon atom, this is a CH_2 rock. As was seen with the CH_3 modes, each of these different types of motions will appear in different portions of the spectrum because they each require a different and unique amount of energy to occur. The scissor mode appears near 1465 cm^{-1}. The rocking, wagging and twisting modes are more complicated, falling in the range 1422–719 cm^{-1}. Depending on the substituents in the molecule, these modes can each be narrowed down to tighter ranges. It is this dependence of certain modes on substituents that gives rise to correlation tables. These are discussed in the next section.

Although the general location for a certain type of mode, for example a C=C stretch, can be listed, the exact location of the peak will vary slightly with the type of substituent present. For example, a vinyl C=C stretch (monoalkyl) will occur from 1650 cm^{-1} to 1638 cm^{-1}. A vinylidine C=C stretch (1,1-dialkyl) will occur from 1660 cm^{-1} to 1640 cm^{-1}. A *cis*-dialkyl substituted C=C stretch will occur from 1662 cm^{-1} to 1631 cm^{-1}, whereas a *trans*-dialkyl substituted C=C stretch will occur from 1676 cm^{-1} to 1665 cm^{-1}. Finally, trialkyl and tetraalkyl substituted C=C stretches appear from 1680 cm^{-1} to 1665 cm^{-1}. Some modes are very substituent sensitive, whereas others appear relatively consistently near a certain wavenumber.

Over the years, many types of compounds have been studied in great detail. When a spectrum of a single compound is interpreted such that every peak or band is assigned to a type of motion, this is called vibrational assignment. Through the examination of many vibrational assignments it was possible to draw correlations between types of vibrational modes and types of substituents. The culmination of this work is detailed in correlation tables that an investigator can use to aid in spectral interpretation (Colthup *et al.* 1990; Dollish *et al.* 1974; Lin-Vien *et al.* 1991).

The most efficient way to use correlation tables is to look for certain peaks that will quickly narrow down the type of compound. For example, if peaks are found at 3000 cm^{-1} or slightly higher, the compound is aromatic or olefinic. However, if the CH_2 stretches appear below 3000 cm^{-1}, the compound is aliphatic. Once that determination is made, then the correlation tables can be consulted to look for other confirmatory modes, such as CH wags for aromatic compounds. This type of process is continued until a compound class can be either verified or discounted.

It should be noted that for complex molecules it may not be possible to determine the structure from the Raman spectrum alone, or even if the infrared spectrum is available as well. Other techniques such as

nuclear magnetic resonance and mass spectrometry provide important information to aid in structure elucidation.

Qualitative analysis

Chemical identity testing of compounds is one role of the pharmaceutical spectroscopy laboratory. Testing can be accomplished with methods utilising Raman spectroscopy. An FT-Raman method has been developed to identify the two active components (tegafur and uracil) in formulated capsules (Petty *et al.* 1996). The Raman spectrum of the formulated product displayed a spectral region in which Raman bands unique to uracil and tegafur are observed. The presence of these bands allowed the analyst to confirm that both components were present in the formulated product. Owing in part to the ease of use and chemical specificity inherent with Raman spectroscopy, it has become an essential chemical and physical identification tool for the pharmaceutical spectroscopist.

Polymorphism (the ability of a molecule to crystallise in different three-dimensional structures) is a very important aspect of the drug development process. Raman spectroscopy is also used for the qualitative and quantitative characterisation of polymorphic compounds of pharmaceutical interest. For the sake of brevity, the term 'polymorphs' will encompass polymorphs, pseudopolymorphs, hydrates and solvates throughout the remainder of this chapter. The term 'polymorphs' is more commonly used and defined as the collection of different crystal structures that can exist for a chemical entity. The terms 'hydrates' and 'solvates', sometimes referred to as 'pseudopolymorphs', refer to the collection of different crystal structures that result from varying degrees of solvation. These materials differ chemically only by the degree of solvate incorporation (monohydrate, dihydrate) into the crystalline lattice, but physically they display different crystal structures. Since solid-state vibrational spectroscopy (Raman and IR) can be used to probe the nature of polymorphism on the molecular level, these methods are particularly useful in instances where full crystallographic characterisation of polymorphism is not found to be possible. An example is shown in Fig. 35.9, where spectra of different polymorphs of carbamazepine are displayed. An added advantage of using Raman spectroscopy for polymorphic investigations is the ease of the technique and the ability to measure low-frequency vibrations (500–50 cm^{-1}) that are attributed to lattice modes. In many cases, two different solid-state forms of a pharmaceutical entity will display spectral differences in the low-frequency region of the Raman spectrum. These spectral differences are also noted in the spectral comparison of crystalline versus amorphous material, a significant advantage for amorphous/crystalline characterisation of the drug. In addition, no sample preparation is required, providing a significant advantage over other techniques.

Raman microspectroscopy is well suited for *in situ* analysis of contaminants found in pharmaceutical processes. Owing to the non-destructive nature of the analysis, further experiments such as energy dispersive X-ray analysis or IR microspectroscopy may be performed on the same sample. A consideration for contaminant analysis by Raman spectroscopy is the axial and spatial resolution of the technique compared with IR microspectroscopy. In general, IR microspectroscopy is diffraction limited to investigating samples typically larger in size than 5 μm. As previously discussed, with a 0.95 NA objective, 1 μm spatial resolution can be achieved with a Raman microscope, permitting the analysis of very small contaminants.

Real-time monitoring of pharmaceutically relevant processes is an exciting application for Raman spectroscopy. Real-time monitoring by Raman spectroscopy has been utilised to examine synthetic organic reaction schemes to investigate kinetics, as well as to identify non-isolated reaction intermediates. Distinct advantages for Raman spectroscopy in this area are: (1) the ability to work with aqueous-based systems with little spectral interference from water, (2) utilisation of a fibre-optic probe for direct and/or remote sampling, (3) collection of the Raman spectrum directly through the glass vessel with little or no spectral interference, and (4) the ability to quantitatively analyse the spectrum. Other recent applications of real-time process monitoring by Raman spectroscopy include polymorphic interconversion under slurry conditions and crystallisation monitoring (Findlay, Bugay 1998).

Another exciting application of Raman spectroscopy is chemical mapping and imaging. With the incorporation of a microscope into a Raman spectrometer it is possible to investigate the spatial distribution of different compounds or polymorphic forms within a sample. By using a mapping stage, a sample such as a microtomed tablet can be moved in the *x* and *y* directions, obtaining spectra at each step. If the sample requires refocusing at different locations, the *z* direction can be automated as well. When the stage is moved to collect each individual spectrum, the resulting data set is referred to as a map. Spectral imaging occurs when spectra from the field of view are collected simultaneously by using an array detector.

In Raman mapping applications, multiple spectra can be collected at user-defined step sizes (as small as 1 μm) at several discrete points (a point map), along a line (a line map) or over an area (an area map)

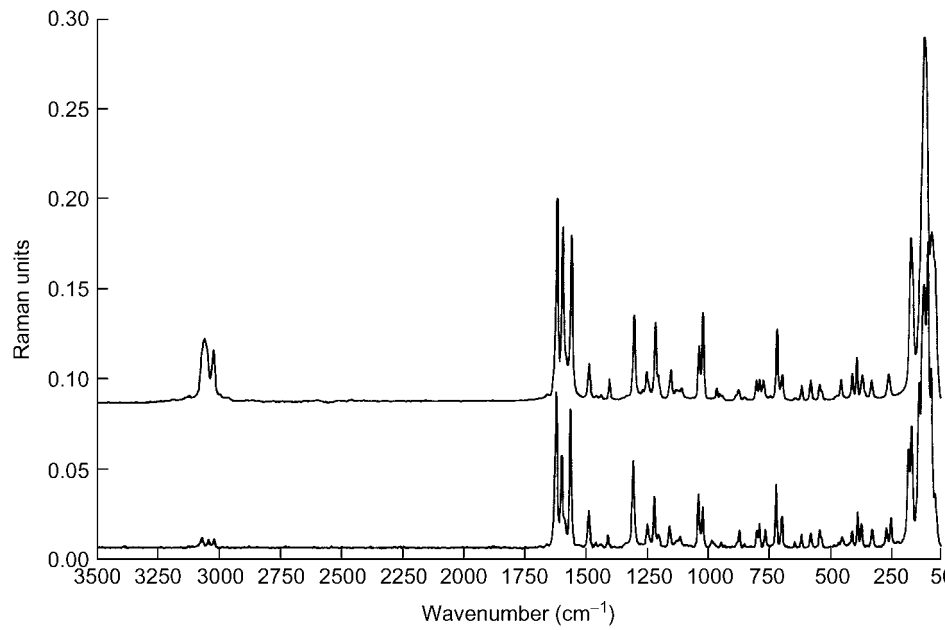

Figure 35.9 FT-Raman spectra of the polymorphs of carbamazepine (*top*, polymorph I; *bottom*, polymorph III).

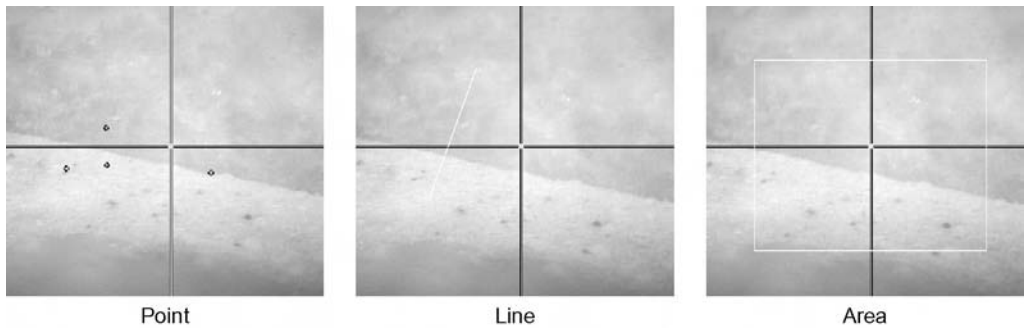

Figure 35.10 Examples of defined point, line and area maps.

(Fig. 35.10). A point map provides several different areas of a sample to be analysed consecutively, but the spectra are not related to each other spatially. A point map can be considered as a type of autosampler. An example of a point map application is a 96-well plate that contains combinatorial beads in each well. The second map type (the line map) defines a series of spectra to be obtained along one dimension. In line maps, chemical changes that occur along this dimension are investigated. An example of a line map sample is a cross-sectioned pharmaceutical beadlet that has several different layers exposed. One of the more practical reasons that line maps are so popular is that they can provide detailed information regarding the chemical changes that occur across a sample without the need to collect as many spectra as are required for area maps. The final map type (the area map) defines a series of spectra to be collected in two dimensions (i.e. over an entire region). This type of map provides a Raman image that can be directly compared with the visual image, often allowing features that are not visible to the eye to be identified. A tablet is common area map sample. The tablet can be mapped and the various ingredients can be monitored as to content uniformity, for example.

A series of images demonstrating the dispersion of several excipients in a tablet is displayed in Fig. 35.11. This particular area map was obtained with a step size of 4 μm in both the x and y directions, a sampling spot size of approximately 1 μm, and a total sampling area of 87×52 μm. The time required to collect this map was approximately 26 h. It should be noted that not all Raman mapping experiments require this amount of time. For example, if a map is being performed to search for a particular component, the step size need only be of the order of the particle size of that substance. A good approach would be to first obtain a larger area map on the sample with larger step sizes and shorter sampling times per point. Once an area of interest is defined by analysing the data from the first map, a smaller, higher-resolution map can then be defined. Another approach would be to use a separate technique to determine the area of interest and then collect a Raman map from that area. For example, near-infrared imaging (NIR) would be a good starting technique. Instrumental advances have also increased the speed of map acquisition. The instances of high-powered diode lasers required optics in order to spread the power across a line rather than onto a spot on the sample to avoid burning or heating of the sample. The line allows for several spectra to be collected simultaneously, reducing the number of times that the stage has to be moved to cover the area required and thereby reducing experimental time. Additional progress has been made over recent years in this approach (Hutchings *et al.* 2008). This improvement allows for the collection of many spectra across a whole sample in a shorter time by using larger CCDs in conjunction with a faster readout of the CCD. This configuration obviously reduces the time required to analyse samples without forgoing spatial and spectral resolution.

Raman imaging, as opposed to Raman mapping, involves the simultaneous collection of multiple responses from a larger area on a sample using CCD array cameras and liquid crystal tuneable filter (LCTF) technology. This approach greatly reduces the amount of time required to collect a chemical image (Zugates, Treado 1999). In one case, the time required to collect a chemical image decreased by approximately 4 h after switching from a CCD camera (>5 h experiment) to LCTF technology (<1 h). During an imaging experiment, the LCTF (or another form of bandpass filter) is tuned to a specific wavelength. The choice of wavelength is normally based on the spectrum of the component of interest. The laser, which has been defocused onto the sample to illuminate a greater area, irradiates the sample. The signal obtained is based on the intensity response at the given wavelength. The response also contains spatial information obtainable through mapping. A complete spectrum of a given area of the sample may be obtained by collecting an image, retuning the bandpass filter to the next wavelength and collecting a subsequent image until the whole spectral range has been obtained. This, however, would be a long process and is not typically used. If a complete spectrum is required, a fast mapping set-up is preferable.

There are instruments available nowadays that have the capability of mapping and imaging using one system. This allows the user to

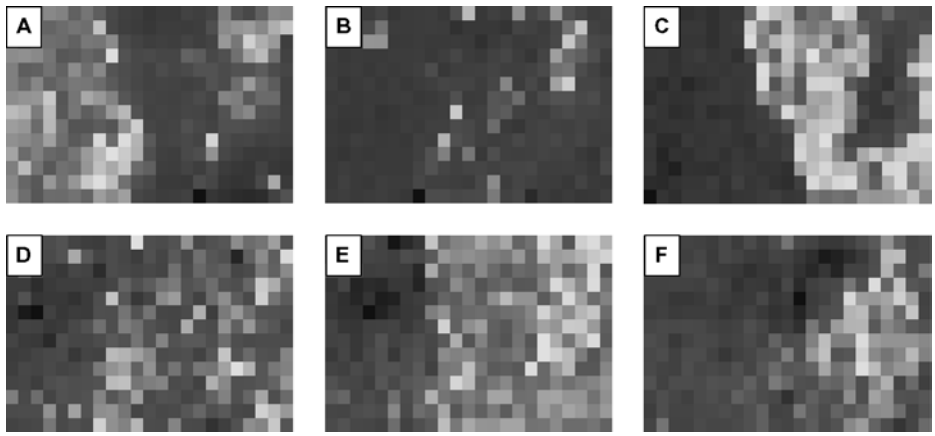

Figure 35.11 Peak area profiles (images) representing (A) mannitol, (B) aspartame, (C) cellulose, (D) magnesium stearate, (E) corn starch and (F) monoammonium glycyrrhizinate.

determine which set-up is preferable depending on the sample and data required. There are also instruments that may be interfaced with an atomic force microscope (AFM) tip that enables yet another dimension of the sample to be probed (Schmidt *et al.* 2005).

Once line or area mapping experiments have been performed, profiles are created that enable certain spectral features to be monitored spatially on the sample. The data sets generated by mapping can consist of hundreds or even thousands of spectra, and viewing all of these spectra concurrently is unrealistic. Profiles can aid in this task by reducing the data set into a more easily viewed format (an image). A profile is a representation of map data in which a measurement of spectral intensity or some other characteristic is shown for each sample point. A profile will verify the presence, location and extent of a defined spectroscopic feature in the sample. There are many types of profiles. Some profiles, such as chemigram or component profiles, compare an entire reference spectrum with every spectrum in the map, with the resulting image showing spectral similarity across the mapping region. Other images can be created based on profiling a specific peak area, peak height, peak area ratio or peak height ratio. Profiles can also be performed based on a group of peaks specific for certain functional groups (e.g. alkanes) or even on a quantitative method.

Literature references of other mapping applications of relevance to the pharmaceutical industry include some of the following: solid dispersions of ibuprofen in PVP (polyvinylpyrrolidone) (Breitenbach *et al.* 1999), crystal formation in hormone replacement therapy patches (Armstrong *et al.* 1996), particle size analysis in mixtures (Theophilus, Lancaster 2000), pharmaceutical matrix determination of dosage formulations (Clarke *et al.* 2000) and characterisation of drug substances in low dosages (Henson, Zhang 2006). An overview of examples is also given by Clark *et al.* (2007).

Quantitative analysis

The ability to perform quantitative analysis by Raman spectroscopy is a significant advantage of the technique. Through mathematical treatments, it has been shown that the Raman scattering intensity is proportional to the number of molecules being irradiated. The intensity of scattered radiation is also proportional to the intensity of the incident radiation and the fourth power of the difference in frequencies between the laser frequency and the molecular vibrational frequency. Thus, increased Raman scattering intensity, and potentially lower limits of detection, can be achieved by increasing the intensity of the laser radiation and/or increasing the frequency of the laser irradiation. This quantitative relationship between Raman scattered intensity and concentration can be expressed as:

$$I_R = (I_L \sigma K) PC$$

where

I_R = measured Raman intensity, in photons per second
I_L = laser intensity, in photons per second
σ = absolute Raman cross-section, in cm^2 per molecule
K = measurement parameters
P = sample pathlength, in cm
C = concentration, in molecules per cm^3.

The constant K represents lumped measurement parameters such as utilising the same spectrometer (collection optics efficiency), sample positioning and overall efficiency of the Raman spectrometer.

In the past, quantitative analysis was not often performed with Raman spectroscopy because of problems inherent to dispersive systems. When FT-Raman spectrometers became popular, the feasibility of quantitative applications greatly improved (Walder, Smith 1991). Some of the advantages of FT that lend themselves well to quantitative applications are Jacquinot's advantage, Fellgett's advantage and Connes' advantage. Jacquinot's advantage, also known as the throughput advantage, depends on the fact that the entrance aperture of the interferometer is large. The larger aperture makes the sampling geometry less sensitive to absolute repositioning of the sample cell. Fellgett's advantage, otherwise known as the multiplex advantage, concerns the increased speed with which spectra can be collected because all wavelengths of the spectrum are collected simultaneously. This rapid collection implies that FT-Raman spectroscopy may be less sensitive to instrument drift. Finally, Connes' advantage, also called the precision advantage, arises because there is only one moving part in an FT system (one mirror in the interferometer). An FT system is therefore very rugged, allowing excellent day-to-day wavelength precision.

Although FT-Raman spectroscopy is more applicable to quantitative applications than dispersive Raman spectroscopy, there are still some issues. These mainly concern the optimisation of sampling conditions. FT-Raman spectroscopy typically samples a relatively small area (e.g. a 1–2 mm spot) of the total sample. As such, it is important that any spectra collected are truly representative of the bulk sample. For solution studies, homogeneity of the multicomponent samples presented for quantitative analysis is not an issue. In contrast, solid-phase analysis can present significant inhomogeneity issues for quantitative analysis. One way to improve sample uniformity is to prepare all solid mixtures by geometric mixing. Slurry mixing is another good technique if the sample can withstand contact with the slurry solvent. For spectral acquisition, sample-spinning accessories should be used to spin the sample cup containing the mixture during spectral acquisition. Use of this type of accessory results in the collection of a more representative sample spectrum (a ring of data is collected, instead of data just at a single point). To effectively minimise any sample heterogeneity concerns, different rings of data can be collected for each sample cup. The individual spectra from each sampled area can then be co-averaged into groups to create representative spectra for the sample in the cup.

Another factor to consider when using Raman spectroscopy quantitatively is that the power output of the Raman laser can vary from day to day, thereby affecting the intensity of spectral peaks. It is advisable to normalise any data before using it in a quantitative manner. One possible method for normalising spectra is to ratio the spectral response of the analyte against a peak response for a non-changing component (e.g. an excipient in a drug product or an internal standard). Owing to difficulties with sample homogeneity, the use of an internal standard is usually discouraged. Alternatively, a ratio can be measured using a peak response of a component changing in an opposite direction to that of the component being monitored. For example, in a quantitative method used to monitor the amount of crystalline drug substance in the presence of the amorphous form, a crystalline peak response (e.g. peak area or peak height) can be normalised by dividing it by an amorphous peak response.

An excellent reference for Raman quantitative analysis was published in 2003 by MJ Pelletier (Pelletier 2003). This article first discusses the fundamental basis for quantitative analysis using Raman spectra, and then follows with in-depth discussions about the value and utilisation of noise, useful preprocessing operations, the use of both peak position and peak shape for method development, and univariate and multivariate calibrations. The reader is referred to this publication for more in-depth discussions beyond the scope of this chapter.

When developing a quantitative method, it is very important to follow regulatory agency guidelines. Important quantitative issues include:

- *System suitability* – an overall test of system function
- *Specificity* – the ability of Raman spectroscopy to differentiate the analyte from the matrix
- *Working range* – the concentration range over which the method is validated
- *Linearity* – demonstration of a direct relationship between a measured analytical response and concentration over the working range of the method
- *Precision* – the repeatability with which a number can be represented
- *Accuracy* – degree of conformity of a measurement to a standard or true value
- *Limit of detection* – the lowest concentration that an analyte can be detected
- *Minimum quantifiable limit* – lowest concentration at which an analyte can be quantified with acceptable accuracy and precision

- *Robustness* – demonstration of the reliability of an analysis with respect to deliberate variations in method parameters.

One literature example of quantitative Raman analysis addresses the question of amorphous/crystalline content of indometacin samples (Taylor, Zografi 1998). The paper highlights the quantitative nature of Raman spectroscopy, the need to produce homogeneous calibration/validation samples, and difficulties associated with collecting a Raman spectrum that is truly representative of the concentration. A linear correlation curve was constructed in which low concentrations of both amorphous and crystalline material could be detected and predicted in mixtures. The authors felt that the largest source of error in the measurements arose from inhomogeneous mixing of the amorphous and crystalline components in the blends. For solid-state analysis, this conclusion points out the need for a sampling device that collects a Raman spectrum that is truly representative of the sample, in this case a mixture.

Chemometrics

In the above literature example of quantitative analysis, a univariate approach (a single peak response) was used to create the calibration curve or predictive model. Chemometrics represents a multivariate approach to creating a predictive model for quantitative analysis. Chemometrics may be defined as the 'use of statistical and mathematical techniques to make either quantitative or qualitative measurements on chemical data'.

A chemometric approach is useful when there are very few spectroscopic differences between the compounds in a mixture, which is often the case for polymorphic studies. Partial least-squares and discriminant analysis are examples of two types of chemometric approaches that can be taken for quantitative and semiquantitative work. These methods are capable of identifying one or more regions of complex overlapping spectra and monitoring their differences by looking at peak position, peak shape, and first-derivative and second-derivative spectra. They represent the differences between the calibration spectra as linear combinations (factors). These factors account for the differences between the spectra, with the first factor representing the strongest differences and subsequent factors representing more subtle differences. Eventually, a point is reached where the factor simply represents changes in random noise between the spectra.

Partial least squares

In the article 'Quantitative analysis of vitamin A using Fourier transform Raman spectroscopy' the authors used partial least squares (PLS) to obtain a method quantified to a concentration of 0.005% w/w (Hancewicz, Petty 1995). One of the concerns that they addressed is that Raman spectra need to be standardised to account for changes in the overall intensity of the spectrum. Possible sources of error include changes in sample positioning, laser power fluctuations and sample wall thickness variations. It was mentioned above that forming peak response ratios with an internal or external standard could normalise the data, but these authors took another approach. They used multiplicative scatter correction (MSC) to compensate for the random multiplicative and additive contribution to the spectra caused by sampling. MSC is traditionally used for diffuse reflectance, but can be used on any data that are collected with unknown or changing pathlengths.

Discriminant analysis

Discriminant analysis is a chemometric technique that allows it to be stated that an unknown sample most resembles a certain class, where each class represents sample 'X', 'Y' or 'Z'. For example, X could be 0% crystalline material in amorphous material, Y could be 5% crystalline material and Z could be 15% crystalline material. In this example, a discriminant analysis method is a pattern recognition model that can be semiquantitative.

There are three main parts to developing a discriminant analysis method. First, decisions must be made regarding the number of spectra (or standards) needed for each class. Once all of the spectra have been collected, some are used to create a calibration (training) model. This training model is 'tweaked' with regard to the spectral regions examined, the number of factors (or principal component (PC) scores) used, baseline treatments, and so on. Once the training model is defined such that it correctly predicts all of the spectra within it (i.e. it is calibrated), the model is tested by internal cross-validation. This validation consists of omitting one standard at a time from the data set, using the depleted set to obtain a new calibration model, and applying this new model to the omitted standard. This process is repeated for each standard. If each spectrum is accurately predicted, then the method is validated. The internal cross-validation also aids in determining whether the model is over-fitted. All these steps may be performed automatically by a given software package.

Overall, chemometrics provides an excellent approach to quantitative method development by enhancing method accuracy and precision, and allowing lower limits of detection and quantification. The reader is referred to Further reading for further publications regarding chemometrics.

Collections of data

By far the quickest method for identifying unknown materials is to search the spectrum against spectral libraries. Often an answer can be found within seconds. However, care must be taken when performing spectral searching.

Computer search programs determine the difference between a sample spectrum and the reference spectra in a library. There are several possible algorithms that can be used to compare spectra, some based on intensity and others based on peak position. For example, difference algorithms accentuate peak intensity over location. The *absolute difference* algorithm puts more weight on the small differences between the unknown spectrum and library spectra, meaning that impurities will have a larger effect on the search results. The *squared difference* algorithm emphasises the large peaks in the unknown spectrum, making it useful when searching a noisy spectrum. *Derivative* algorithms accentuate peak position over intensity. The *absolute derivative* algorithm gives small peaks and peak shifts an increased effect in the search results. This algorithm removes any differences between the unknown and library spectra caused by an offset in the unknown spectrum and so it is useful for analysing spectra with distorted baselines. The *squared derivative* algorithm emphasises large peaks as well as peak shape and works best for noisy spectra with distorted baselines. Perhaps the most versatile search algorithm is *correlation*, which balances the contributions of both intensity and peak position. This algorithm normally gives the best results and is recommended for most applications.

When a search is performed, a hit list is produced. The reference spectra are ranked in order of match quality. Some programs assign a value of 100 to a perfect match, others use 0. In either case, a good hit would obviously be one that is closest to perfection. In the best-case scenario, the top hit (if 100 is perfect) is above 90 and all other hits are significantly lower. In cases of spectral mixtures, however, the best hit may not even be 50. In those cases, the best reference spectra are compared with the sample spectrum to determine whether they could represent a portion of the sample. If so, a spectral subtraction may be performed to remove the reference component from the sample spectrum and the resulting spectral subtraction can be searched again to look for additional components. Often, if strong peaks are being subtracted from each other, regions of over- or under-subtraction will occur, producing derivative-shaped peaks in the subtraction spectrum. In these cases, it may be advantageous to 'blank-out' these regions before searching for lesser components. The process of spectral searching and subtraction can be repeated until the S/N ratio of the subtraction spectrum yields unusable results and no more components can be identified.

There are several commercial libraries that are useful for drug analysis (see Further reading). Aldrich has a comprehensive library containing over 14 000 FT-Raman spectra. Thermo Scientific markets two useful libraries. One is a Raman forensic library containing 175 spectra of drug compounds, excipients, precursors and metabolites. The other is a pharmaceutical excipients library that contains 300 reference spectra.

For this second library, there is also a matched set of corresponding FT-IR spectra. Galactic Industries and Bio-Rad are two other popular suppliers of spectral libraries. Of course, sometimes the most useful libraries are ones that analysts create themselves.

A final note on the use of spectral libraries: the results from a hit list should always be verified by (1) comparing the reference spectrum with the sample spectrum, (2) ensuring that the reference spectrum is accurately named in the library (no library is perfect), and (3) determining that the suggested hit is a logical component to be expected in that particular sample.

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CHAPTER

36 Nuclear Magnetic Resonance Spectroscopy

JC Lindon and JK Nicholson

Introduction

Since its first observation in bulk phases in 1945, nuclear magnetic resonance (NMR) spectroscopy has become one of the foremost methods for molecular identification, for evaluating detailed molecular structures, for understanding conformations and for probing molecular dynamics, such as molecular tumbling and diffusion in solution. If the measurements are carried out under appropriate conditions, NMR spectroscopy can also be used for quantitative analysis. Although powerful in its own right, NMR spectroscopy can be regarded as complementary to other analytical chemical techniques. For example, it can provide information on substances with no ultraviolet (UV) chromophores, such as carbohydrates. It is a universal detector in that, if the molecule under study contains NMR-active nuclei, these should be detectable. However, it is only possible to infer details of parts of the molecule that do not contain NMR-active nuclei, such as the presence of a sulfate conjugate of a drug metabolite, in which case the definitive loss of mass 80 seen in mass spectrometry (MS) is complementary.

The vast majority of NMR spectroscopic experiments are carried out in solution to identify the structures of small chemical molecules, including natural products, but a wealth of high-resolution applications is found in other areas, such as determination of the three-dimensional structures of proteins and nucleic acids and the analyses of complex biological mixtures. This last application has seen a dramatic expansion since 2001, and is now known as metabonomics or metabolomics, with diverse studies covering drug toxicity, disease diagnosis, drug therapeutic effects, and nutrition and lifestyle effects. In addition, much effort has been devoted to solid-state NMR spectroscopy in which special techniques have to be used to overcome very broad NMR peaks and to recover useful chemical information. One application in this area of interest to pharmaceutical scientists is the determination of polymorphic forms of drugs in the solid state.

Finally, NMR spectra can be obtained from living humans and animals and *in vivo* NMR or magnetic resonance spectroscopy (MRS) has found a use in disease diagnosis. The same technology and principles lie behind magnetic resonance imaging (MRI), which is now widely available in hospitals for clinical diagnosis.

Theory

Atomic nuclei in a magnetic field

The phenomenon of nuclear magnetic resonance arises because the positively charged nuclei of certain atoms possess a quantised property called spin, denoted by a spin quantum number I. This spin is associated with a nuclear magnetic moment, also quantised, such that in a magnetic field it is possible for the nuclear magnetic moment to take up various orientations with respect to the field. Each orientation is associated with a discrete energy state and in the presence of the magnetic field these states have different energies. I can have half-integer or integer values, including zero. For nuclei with $I = 0$, there is no magnetic moment, which is the case when both the atomic number and the atomic weight are even, such as in ^{12}C and ^{16}O. In general, there are $2I + 1$ energy states or levels and so for the simplest magnetic nuclei, with $I = {}^1/_2$, there are just two levels. As a consequence of the differing energies of the states, and because of the Boltzmann distribution, the populations of spins in the two states are not equal and an excess of nuclear spins will occur in the lower energy level. It is possible to induce transitions of nuclear spins between these levels by applying an oscillating magnetic field; for commercially available NMR magnets, these transitions are in the radiofrequency region of the electromagnetic spectrum. There is a linear relationship between the magnitude of the nuclear magnetic moment and the observation frequency of the NMR phenomenon for a given applied magnetic field strength. There is also a linear relationship between observation frequency and applied magnetic field strength for a given nucleus. Because of electron shielding effects in molecules, not all nuclei of a given atomic isotope have the same resonance frequency (known as the Larmor frequency), depending on their chemical environment, and hence the NMR phenomenon gives rise to a range of resonance frequencies that correspond to peaks in an NMR spectrum.

Many good textbooks describe the theory of NMR spectroscopy in more detail, a selection of which are listed in Further reading at the end of this chapter.

Important NMR-active nuclei

Although all nuclei have at least one isotope that is, in principle, NMR active, most NMR spectra are based on just a few nuclear types. There are several reasons for this. One is that nuclei with $I > {}^1/_2$ have a property called a nuclear quadrupole moment that, in general, results in short lifetimes in the excited spin states and a rapid return to the low-energy state, and this gives very broad NMR lines. Second, many NMR-responsive nuclei exist at low natural abundances and so are difficult to detect without isotopic enrichment. Third, the strength of the NMR response is related to the size of the nuclear magnetic moment; many nuclei have rather small values of the magnetic moment and so have low detectability. Finally, some nuclei, once excited to the upper level, are slow to relax back to the ground state, which must occur before another scan can be added. This then incurs a time penalty for acquiring the summed scans necessary to improve detection limits. Sometimes these difficulties of low sensitivity, low natural abundance and long relaxation times come together.

The principal nuclei of interest for pharmaceutical and biochemical studies are given in Table 36.1, together with their NMR properties. The common isotopes of carbon and oxygen, ^{12}C and ^{16}O, do not have magnetic moments and so do not give rise to NMR spectra. The ubiquitous ^1H nucleus, or proton, has one of the highest relative sensitivities, surpassed only by its radioactive isotope tritium, ^3H. The ^{13}C isotope is useful for characterising the carbon skeleton of organic molecules and, with a natural abundance of about 1.1%, the chance of finding two ^{13}C nuclei in a given molecule is only about 0.01%, which simplifies the spectra considerably because of the absence of spin–spin interactions between carbon atoms. Many spectroscopic methods have been developed to allow the routine observation of ^{13}C NMR spectra of organic molecules. The ^{19}F nucleus is almost as sensitive as the ^1H nucleus (about 83%) in NMR terms, and ^{19}F NMR spectroscopy is used extensively in studies of the metabolism of fluorine-containing drugs. More limited use is made of other nuclei in pharmaceutical and biochemical research, and nuclei such as ^{15}N have been used extensively for protein-structure determination after isotope enrichment. The use of ^{31}P NMR spectroscopy is widespread in biochemistry and medicine as a means of investigating the various phosphorylated molecules

Table 36.1 NMR properties of some nuclei of interest for pharmaceutical studies

Nucleus	Spin quantum number	Natural abundance (%)	NMR Larmor frequency (MHz)	Relative sensitivity
^{12}C	0	98.89	—	—
^{16}O	0	>99	—	—
^{1}H	1/2	99.98	600.00	1.00
^{3}H	1/2	0.0	639.98	1.21
^{13}C	1/2	1.11	150.86	1.59×10^{-2}
^{15}N	1/2	0.37	60.80	1.04×10^{-3}
^{19}F	1/2	100.0	564.46	8.30×10^{-1}
^{29}Si	1/2	4.7	119.19	7.84×10^{-3}
^{31}P	1/2	100.0	242.88	6.63×10^{-2}
^{2}H	1	0.015	92.10	9.65×10^{-4}
^{14}N	1	99.63	43.34	1.01×10^{-1}
^{23}Na	3/2	100.0	158.71	9.25×10^{-2}
^{35}Cl	3/2	75.53	58.79	4.70×10^{-3}
^{17}O	5/2	0.037	81.34	2.91×10^{-2}

important in biology, including many studies *in vivo*. Many other spin-$^1/_2$ nuclei, such as ^{29}Si, ^{119}Sn, ^{129}Xe, ^{195}Pt and ^{199}Hg, have found much use in specialist applications. Nuclei with $I > {}^1/_2$ are quadrupolar and, in general, give broad NMR lines, but in some cases useful information can be gleaned. Examples include ^2H NMR in liquid crystals, ^{14}N NMR in heterocyclic chemistry, and ^{23}Na NMR studies of intra- and extracellular sodium ions.

Parameters from an NMR spectrum

Chemical shifts

As mentioned earlier, not all nuclei of a given isotope resonate at exactly the same frequency. This is because, in a molecule, a given atomic nucleus is surrounded by electrons which also possess a magnetic moment, and these provide a fluctuating magnetic field that opposes the main field of the NMR magnet. As a consequence, the nuclei are shielded from the main magnetic field and require a higher field to bring them to resonance and thus they can be considered to have higher Larmor frequencies. The degree of shielding depends on the electron distribution around the nucleus and hence on the chemical environment. The different degrees of shielding are known as chemical shifts. Thus, interpretation of chemical shift values allows identification of molecular structural fragments. Chemical shifts are measured relative to that of a reference substance usually placed into the sample. For ^1H and ^{13}C shifts in organic solvents, this is tetramethylsilane (TMS). The chemical shift is then defined as $\delta(H) = (\text{difference in the resonance frequency in hertz between the analyte and TMS}) \times 10^6/(\text{operating frequency of the spectrometer})$. Chemical shifts are thus quoted in parts per million (ppm) and are independent of the operating frequency of the spectrometer, which allows comparisons irrespective of magnetic field strength. For aqueous samples, an alternative reference compound is used, of which trimethylsilyl [2,2,3,3-$^2H_4$]propionic acid sodium salt (TSP) is the most common example. The chemical shifts for TMS and TSP are set arbitrarily to zero. Typical ^1H and ^{13}C NMR chemical shifts of a variety of important molecular fragments are shown in Tables 36.2 and 36.3, respectively.

Indirect (J) spin–spin coupling

The resonance lines of individual nuclei can show further splitting because of indirect spin–spin coupling. Given the symbol *J*, this is measured in hertz and is independent of the observation frequency. Such spin coupling arises from a magnetic interaction between NMR-active nuclei and is transmitted via the intervening electrons, hence the term 'indirect'. Coupling is observed only within a molecule. Thus for two spin-$^1/_2$ nuclei, such as protons, the resonance line for each proton is split into a doublet, the two lines corresponding to the two possible orientations of the adjacent proton relative to the magnetic field. For extended coupling chains, each component of a doublet can be split further into doublets of doublets and so on. If a given proton is adjacent to two equivalent other protons (as in a CH_2 group) then, of the four possible spin orientations of the two protons, two of them are identical and a 1 : 2:1 triplet results. For such 'first-order' systems, the multiplicity can be deduced on the basis of Pascal's triangle according to the number of equivalent coupled nuclei. In situations where the chemical shift difference between the protons is large compared with the *J*-coupling, this simple rule applies. For situations where the chemical shift in hertz between coupled partners is not large compared with the magnitude of the coupling constant ($\delta/J <$ ca. 10), or in symmetrical molecules, more complex rules have to be applied and sometimes the only way to

Table 36.2 The range of ^1H NMR chemical shifts for protons in various molecular fragments

Fragment	$\delta(^1H)$
Cyclopropyl CH_2	−0.2–0.8
C·CH_2·C	0.4–2.4
CH_3·C	0.5–2.0
C·NH	0.5–3.0
C·OH	0.5–5.0
CH_3·C=C	1.5–2.3
C·CH_2·C=C	1.6–2.0
CH_3S; CH_3CO	1.7–2.7
CH_3·Ph	2.0–3.0
CH_3N	2.2–3.1
C·CH_2·S	2.3–3.0
C=C·CH_2·C=C	2.3–3.0
C·CH_2·CO	2.3–3.5
C·CH(Ph)·C	2.5–3.0
C·CH_2·Ph	2.5–3.2
C·CH_2·N	2.5–3.3
C=C·CH_2·CO	2.8–3.7
C=C·CH_2·Ph	3.0–3.8
CH_3O	3.2–4.1
N·CH_2·CO	3.2–4.5
C·CH_2Cl; C·CH_2O	3.4–4.3
CH_3·O·CO	3.5–3.8
CO·CH_2·Ph	3.5–4.2
CH·N·CO	3.5–4.3
C·CH_2·OCO	3.5–4.5
N·CH_2·N	3.5–5.1
N·CH_2Ph	3.6–4.6
C=CH	3.6–8.5
C=C·CH_2O	4.0–5.2
CO·CH_2O	4.0–5.5
OCH_2O	4.5–6.3
OCH_2Ph	4.7–5.6
N·CH_2O	4.8–5.9
NH·CO	5.0–9.0
Aromatic H	6.0–9.0
O·CHO	8.0–8.3
C=N·OH	8.0–11.0
CHO	9.5–10.0
COOH	9.5–13.0

Table 36.3 The range of ^{13}C NMR chemical shifts for carbon atoms in various molecular fragments

Fragment[(a)(b)]	$\delta(^{13}C)$	*Multiplicity*[(c)]
CH_3·C; **C**H_3,C=C	0–30	q
CH_3·S	7–20	q
C·**C**H_2·C	10–70	t
CH·$(C)_3$	18–68	d
CH_3·COX	18–30	q
Acetylenic **C**	20–100	d, s
CH_3·N	25–50	q
C·**C**H_2·COX	25–60	t
C·$(C)_4$	30–80	s
$(C)_2$·**C**H·COX	35–75	d
C·**C**H_2·N	35–75	t
$(C)_2$·**C**H·N	40–90	d
$(C)_3$·**C**·COX	45–100	s
CH_3O	50–62	q
$(C)_3$·**C**·N	50–100	s
C·**C**H_2O	57–90	t
$(C)_2$·**C**H·O	65–100	d
$(C)_3$·**C**·OC	70–110	s
CH_2=C	80–135	t
Aromatic **C**H	80–140	d
C=C	80–160	d, s
O**C**O	85–110	t, d, s
Aromatic **C** (not CH)	90–160	s
Nitrile	115–125	s
C·**C**OX	165–180	s
C·**C**OOH	175–185	s
C·**C**HO	195–205	d
C·**C**O·C	205–220	s
C·CS·C	220–240	s

(a) Designated carbon is shown in bold face.
(b) X = C, O, N.
(c) Indicates peak multiplicity in a ^{1}H-coupled spectrum; s, singlet; d, doublet; t, triplet; q, quartet.

interpret a spectrum is via a computer simulation. For ^{1}H–^{1}H interactions, the coupling does not normally extend beyond three bonds, with four-bond couplings being quite small, if resolvable. Three-bond ^{1}H–^{1}H couplings provide valuable information on the dihedral angles between C–H vectors in aliphatic compounds through an empirical equation known as the Karplus equation. Typically, for CH–CH fragments, if the CH vectors have a dihedral angle of 180°, the coupling is of the order of 10 Hz, for 90° it is close to zero and for 0° it is about 6 Hz. In olefinic systems, the three-bond coupling across a C=C double bond is about 6–10 Hz for a *cis* arrangement and 12–16 Hz for a *trans* arrangement. All of these values are modified by the presence of substituents with varying electronegativities. Hence the *J*-coupling is a valuable parameter for distinguishing between isomers and for measuring molecular conformations. Compilations of coupling constants have been made and empirical models for calculating them in various conformations have been proposed (Pretsch *et al.* 1989).

Peak areas

If the NMR data are acquired under conditions in which each scan is acquired on a fully relaxed spin system in which the state populations are those given by the Boltzmann distribution, the areas under the NMR peaks are directly proportional to the number of nuclei contributing to that peak and to the concentration of the molecule in the sample. If an internal standard of known concentration is added to the sample, absolute concentrations can be determined.

Relaxation times

Two 'times' define how fast a nuclear spin interacts with the rest of the sample as a whole (known as the lattice) and how nuclear spins interact with each other in a pair-wise fashion. These are designated T_1 and T_2. T_1 is known as the spin–lattice or longitudinal relaxation time, and is the characteristic time for the exponential process of nuclear spins to reach equilibrium populations in the spin states. For small molecules in mobile solutions, ^{1}H T_1 values are usually in the range 1–10 s. The other relaxation time is known as T_2, the spin–spin or transverse relaxation time, and is related to the rate of magnetisation loss caused by spin–spin flips. For small molecules in free solution $T_1 = T_2$. However, macromolecules and exchanging species have short T_2 times, typically in the range 10–100 ms, even though T_1 may be much longer. Determined values of T_1 and T_2 can be used to deduce information on molecular dynamics in solution and the shorter T_2 values of macromolecules can be exploited to edit NMR spectra so as to attenuate their peak intensities, better revealing small-molecule NMR resonances.

Diffusion coefficients

The molecular self-diffusion coefficient is a whole-molecule property that does not normally appear in NMR spectra. However, it is a valuable measure of molecular mobility and in free solution is related directly to molecular size. It is possible to measure diffusion coefficients using a specially designed NMR experiment, which includes the application of magnetic field gradients.

Direct (dipolar) spin–spin coupling

Another important interaction in NMR spectroscopy is called the dipolar coupling. This is a direct magnetic interaction between nuclei through space, not through bonds, as for *J*-coupling; it is proportional to the inverse cube of the internuclear distance. This dipolar coupling can be several orders of magnitude larger than *J* couplings. However, because it depends on the angle that the internuclear vector makes with the magnetic field, it is averaged to zero by fast isotropic molecular tumbling in liquids, but in solids is largely responsible for the observed very broad resonance bands. In semi-solids, such as tissues, the dipolar couplings between nuclei are partially averaged out by the considerable molecular freedom and the residual couplings, and hence the line broadening can be removed by the technique of magic-angle spinning (MAS). However, for molecules tumbling in solution, the fluctuating dipolar interaction is an important relaxation mechanism and, even though its average value is zero, it can be used to interpret nuclear Overhauser enhancements (NOEs). These are peak intensity changes caused by selective perturbation of the spectrum, or a two-dimensional experiment (see later), and the sign and magnitude of NOEs can be related to internuclear distances and hence molecular structural information.

Instrumentation

Practical aspects of ^{1}H NMR spectroscopy

NMR spectroscopy is used mostly to identify molecular structures, usually as pure compounds, although, because it is such a high-resolution technique, it is possible to characterise individual components in chemical and biological mixtures. Of course, it is increasingly difficult to identify minor components (e.g. less than 5 mol%) in mixtures, in which case concentration by solid-phase extraction may improve detection. It is also possible to couple high performance liquid chromatography (HPLC) directly to NMR spectrometers (see below). Nevertheless, it is possible to quantify lower levels of impurities given a clearly resolved NMR resonance for the impurity. Each of the two natural-abundance ^{13}C satellite signals of the main component (ca. 0.55 mol%) can provide a useful quantification standard, if resolved. Thus, impurity detection limits depend on the resolution of a suitable NMR resonance, but a detection level of 0.1 mol% is not unreasonable.

Almost all spectrometers used for chemical structural studies are now based on a superconducting magnet with an operating frequency for ^{1}H observation that ranges from 300 MHz to 1000 MHz. Both resolution and detection are improved with increased magnetic field strength. For routine chemical studies in which compound availability is not limited,

300 MHz and 400 MHz observation is quite usual, but 500 MHz and 600 MHz systems are now common (in 2010). Higher observation frequencies are available (700, 750, 800, 900, 950 and 1000 MHz) and are used for specialised research studies, mainly in the field of protein-structure investigation and for complex biological mixtures such as biofluids. Historically, and still generally, a sample for high-resolution NMR spectroscopy is placed in an expensive, precision-machined, 5 mm glass tube sealed with a plastic cap, which is inserted into the detector (known as the probe) of an NMR spectrometer. Other tube diameters are also used, including 10 mm for larger samples and 3 mm when sample quantity is limited. More recently, robotic systems for injecting samples directly into a flow probe using about 500 μL from individual sample vials or 96-well plates have been developed. A wide range of capillary flow probes is also available from the main manufacturers and from accessory suppliers that allow sample volumes typically down to 10 μL, and, if the sample can be concentrated down to small volumes, this represents a way of producing the best sensitivity and hence lowest detection limits.

The development of cryogenic probes with the detector coil and preamplifier (but not the samples) cooled to around 20 K has provided a marked improvement in spectral signal-to-noise ratio (SNR) by reducing the thermal noise in the electronics of the spectrometer. An improvement of up to 500% can be obtained for samples in organic solvents, but, for strongly ionic solutions such as biofluids, the improvement is less dramatic. Conversely, because the NMR SNR is proportional to the square root of the number of co-added scans, data acquisition times shorter by up to a factor of 25 become possible for the same amount of sample, broadening the feasibility of chemical kinetics studies.

Tissue studies use a technique called high-resolution ^{1}H MAS NMR spectroscopy, employing small (∼10–20 mg) pieces of intact tissues with no pre-treatment. Rapid spinning of the sample (typically at ∼4–6 kHz) at an angle of 54.7° relative to the applied magnetic field serves to reduce the loss of information caused by line broadening effects seen in non-liquid samples such as tissues (caused by sample heterogeneity, and residual anisotropic NMR parameters that are normally averaged out in free solution where molecules can tumble isotropically and rapidly). MAS NMR spectroscopy has straightforward, but manual, sample preparation. NMR spectroscopy on a tissue sample in an MAS experiment is the same as solution-state NMR and all common pulse techniques can be employed in order to study metabolic changes and to perform molecular structure elucidation and molecular dynamics studies.

Sample preparation

As spectrometers have become more sensitive, it is now possible to provide high-quality ^{1}H NMR spectra for small organic molecules on less than 1 mg of sample using widely available 400 MHz instruments. For organic chemical samples, a dilute solution in a fully deuterated solvent is usually used. Typical solvents are chloroform-*d* and dimethylsulfoxide-d_6, but occasionally more expensive alternatives have to be used, such as dimethylformamide-d_7, cyclohexane-d_{12} or acetone-d_6. A small amount of the volatile TMS is added as a chemical-shift reference. It is also possible to use D_2O as a solvent for water-soluble materials, in which case TSP is usually used for a chemical-shift reference. For biological samples, such as biofluids, which largely comprise H_2O, it is possible to freeze-dry these and reconstitute them into D_2O, but this risks the loss of volatile components. Alternatively, it is possible simply to add a small amount of D_2O and suppress the huge water NMR resonance by using one of a number of solvent-suppression NMR pulse sequences. These either suppress the water signal selectively or have the effect of exciting the whole of the NMR spectrum except for the water peak. The deuterated solvent, or the added D_2O, provides a ^{2}H NMR signal for the spectrometer to use as a field-frequency lock signal, so that all scans are co-registered exactly.

Acquisition of NMR data

The radiofrequency circuits of the spectrometer are tuned and matched to the sample so that the reflected power is minimised and then the magnetic field homogeneity is optimised by adjusting currents in small ancillary coils around the sample, either manually or under computer control. This process is known as 'shimming' and yields the best peak heights and hence the sharpest NMR peaks. The historical method of acquiring an NMR spectrum by slowly sweeping either the magnetic field or the radiofrequency (the continuous wave or CW method) is now obsolete and all spectra are obtained using the pulse-Fourier transform (FT) approach. In this method, all nuclei are excited simultaneously using a short, powerful radiofrequency pulse that covers all frequencies of interest. A typical pulse width is ∼10 μs and, if it has sufficient power to equalise the populations of the two spin states, it is termed a 90° pulse. As the nuclei relax back to equilibrium, an oscillating, decaying voltage known as a free-induction decay (FID) is induced in the receiver coil. This signal is converted into a digital form using an analogue-to-digital converter (ADC) and stored in computer memory as many thousands of data points. The total data-acquisition time for a single scan is typically in the range 0.1–4 s.

Data processing

This amplitude-against-time signal is treated as a series of decaying cosine waves and is converted into an amplitude-against-frequency spectrum by the mathematical process of Fourier transformation. Multiple scans are usually co-added to improve the SNR, since this is proportional to the square root of the number of scans. The resultant FID is usually multiplied by some function to improve SNR or reduce the spectral line widths, and hence increase resolution. For example, it is possible to enhance SNR by multiplying the FID by a negative exponential, $\exp(-At)$, where t is the acquisition time and A is a positive constant, but this also has the effect of increasing line widths. Alternatively, it is possible to enhance resolution by multiplying by a positive exponential, $\exp(+At)$, but this has a large deleterious impact on SNR. In addition, if the acquisition time is too short, the FID is truncated before it has decayed into the noise, and artefacts are seen as oscillations on each side of the NMR peaks. A good compromise to overcome truncation artefacts and to improve resolution without too large a compromise on SNR is to carry out a Lorentzian–Gaussian transformation. This is achieved by multiplying by a positive exponential and a negative exponential squared, $\exp(+At - Bt^2)$. The value of A is chosen to match the experimental line width and B is selected to give a Gaussian line shape of the desired reduced width and acceptable SNR. Since the delays in the electronics are frequency dependent, not all peaks will appear phased (upright). This has to be corrected either manually or automatically.

System tests

A number of standard system tests are used to define the performance of NMR spectrometers to meet the specifications guaranteed by the manufacturers.

A good line shape is an important requirement of modern NMR spectroscopy. The line-shape test is often called the 'hump' test and comprises an examination of the ^{1}H NMR peak from 1% chloroform in acetone-d_6, degassed and sealed. In addition to an acceptable value for the peak width at half height (50%), a good line shape is also defined by the width at the height of the ^{13}C satellites (0.55%) and at one-fifth of this height (0.11%). NMR peaks should have a Lorentzian line shape and therefore the widths at 0.55% height and 0.11% height should be 13.5 and 30 times the width at 50% height; deviations from these values should be avoided and typical values for a 500 MHz spectrometer might be 3.5 Hz and 8.5 Hz. The spectrum is measured in both spinning and non-spinning conditions. The standard resolution test for ^{1}H NMR spectroscopy now usually uses the same sample, giving a typical value of about 0.3 Hz full width at half height.

The sensitivity test for ^{1}H NMR spectroscopy is based on a degassed and sealed sample of 0.1% ethylbenzene in $CDCl_3$. A single 90° pulse is used with a spectral width of 10 ppm and a 1 Hz line-broadening function is applied to the FID. The region of the spectrum between δ4 and δ6 is expanded vertically, typically by a factor of 16, to show the full

extent of the noise band, and this is compared with the height of the peak from the methylene group. The SNR is defined as the peak height multiplied by 2.5 divided by the peak-to-peak noise value in that specified region. A typical value for a 500 MHz spectrometer could be about 700 : 1, while for a cryoprobe on an 800 MHz spectrometer the value could be in the region of 6000 : 1.

There are two main tests of ^{13}C NMR performance. The American Society for Testing and Materials (ASTM) approach uses a solution of 60% benzene-d_6 in 1,4-dioxane, degassed and sealed, and tests only the ^{13}C performance. The other test uses a solution of 10% ethylbenzene in $CDCl_3$, degassed and sealed, and tests both the ^{13}C sensitivity and the ^1H decoupling efficiency since ^{13}C NMR spectra are usually acquired with all $J(^{13}C–^1H)$ splittings removed by secondary irradiation at the ^1H frequency. For the ASTM test, a single 90° pulse is applied to the sample with the ^1H decoupler switched off. The FID is zero-filled to 65 536 (64k) points, weighted by a line-broadening function of 3.5 Hz, and the full spectrum is plotted. The region between δ_C80 and δ_C120 is expanded vertically by a factor of 4 to allow good visualisation of the noise band, and this height is compared with that from the triplet signal from the C_6D_6. A typical value for a 500 MHz spectrometer might be 120 : 1. When using the ethylbenzene test sample, a single 90° pulse is applied to the sample, the FID is zero-filled to 65 536 (64k) points, a line broadening of 0.3 Hz is applied and the full spectrum is plotted. The noise height is taken, as before, from the region between δ_C80 and δ_C120 and compared with the peak height of the aromatic CH ^{13}C NMR signals, with a typical value of ~80 : 1 for a 500 MHz spectrometer. A similar test is used for ^{31}P NMR sensitivity and comprises 0.0485 mol/L triphenylphosphate in acetone-d_6. Another test covers water peak suppression efficiency in aqueous samples, and this uses 2 mmol/L sucrose in 10% D_2O–90% H_2O.

Two-dimensional NMR spectroscopy

One-dimensional NMR spectroscopy gives rise to one frequency axis and this is achieved by Fourier transformation of an FID obtained as the response after a radiofrequency pulse or pulse sequence. By incorporating a second incremented time period into an NMR pulse sequence, it is possible to carry out Fourier transformation with respect to both time periods to give two frequency axes that result in two-dimensional NMR spectra. This spreads out the NMR spectrum in a variety of ways, and so makes interpretation simpler. Extension to the use of three or more time periods leads to the concept of three (or more)-dimensional FT-NMR spectroscopy.

All two-dimensional NMR experiments can be considered to have a preparation period, which might simply be a relaxation delay or could be some way of preparing the spin system for further manipulation. Next, the evolution period contains the first variable time period, t_1, which is incremented in equal time steps. Then it is possible to include a mixing period to allow the various spins to interact in some non-equilibrium condition. However, not all pulse sequences use this period. This is followed by the detection of the FID during the second time period, t_2.

To see how this works in practice, it is possible to consider one of the simplest two-dimensional pulse sequences. This is correlation spectroscopy (or COSY), which uses two, typically 90°, radiofrequency pulses with an incrementable time delay between them. The time-domain data comprise a series of FIDs for a range of inter-pulse delays. After the first Fourier transformation with respect to t_2, the data consist of an array of spectra on a frequency axis, each of which corresponds to a given inter-pulse delay, but with the phase of the peaks modulated as a result of the various values of t_1. After a second Fourier transformation with respect to t_1, the data array comprises NMR intensity as a function of two frequency axes and is shown as a three-dimensional plot. This is quite difficult to interpret, especially if it is complicated by many signals. The data are visualised more easily as a contour plot 'looking down' on the peaks. In the COSY spectrum the peaks of the normal spectrum appear along the diagonal and the presence of off-diagonal cross-peaks indicates two nuclei that have a common indirect spin–spin (J) coupling. Hence, this experiment correlates nuclei that are spin-coupled together and are thus close together in bond terms. The frequency axes are labelled F1 and F2, deriving from t_1 and t_2, respectively.

There are many two-dimensional NMR pulse sequences in the literature, most of which are for very specialised applications but a few very useful experiments are used widely for molecular structural studies (Croasmun, Carlson 1994). Two-dimensional NMR experiments can be classified into two types – resolved and correlation. The former resolve a particular type of NMR parameter into the second dimension. For high-resolution solution-state studies, this type is restricted largely to the J-resolved experiment (JRES), which separates the chemical shifts and spin-coupling multiplets into the two dimensions. This can be carried out in a homonuclear mode for ^1H chemical shifts resolved from $^1H–^1H$ couplings and in a heteronuclear mode in which, usually, ^{13}C chemical shifts are resolved from $^1H–^{13}C$ couplings.

Most two-dimensional NMR experiments are of the correlation type, which includes the COSY experiment that provides a correlation between ^1H chemical shifts that have a direct J coupling. The total correlation spectroscopy (TOCSY) pulse sequence provides a correlation as off-diagonal peaks, also based on the J coupling, but now along an unbroken chain of couplings. The nuclear Overhauser enhancement spectroscopy (NOESY) experiment provides a correlation between ^1H chemical shifts connected by an NOE. This experiment can also be used to investigate spin systems of molecules undergoing chemical exchange.

It is possible to obtain heteronuclear correlation two-dimensional NMR experiments, usually the correlation of ^{13}C and ^1H chemical shifts, although the use of other nuclei such as ^{15}N or ^{31}P is possible. Direct detection of the heteronucleus is possible and historically was the main approach. However, this is usually a low-sensitivity experiment because of the lower inherent sensitivity, or natural abundance, or both, of most heteronuclei compared with ^1H. To overcome this problem, experiments have been devised that use indirect detection of the heteronuclei attached to the protons (see ^{13}C NMR spectroscopy below).

A summary of the principal, useful, two-dimensional, NMR pulse sequences is given in Table 36.4. This also lists the typical length of time required for data acquisition and the quantity of material required for each experiment.

^{13}C NMR spectroscopy

Much molecular structural information is available by measuring the ^{13}C NMR spectrum of organic molecules at natural abundance. As this nucleus is less abundant and inherently less sensitive than ^1H, larger amounts of sample are needed (typically 10 mg for a material of molecular weight 250 at a ^{13}C observation frequency of 125 MHz, which corresponds to 400 MHz for ^1H measurement) and usually more FIDs need to be accumulated to build up the SNR. However, some factors alleviate these difficulties. These include the broadband decoupling of all $^1H–^{13}C$ coupling patterns using special pulse sequences, so that each carbon signal is a singlet. This also has the effect of boosting the SNR through the NOE from the ^1H spins, this being most effective for CH, CH_2 and CH_3 carbons. One disadvantage of ^{13}C NMR spectroscopy is that some carbons, particularly quaternary carbons (which have at best only weak NOEs), also have long relaxation times and are saturated easily and thus give weak peaks. For these reasons, quantitative studies based on ^{13}C resonances are more prone to error than those based on ^1H resonances. The undesirably long ^{13}C relaxation times can be shortened by the addition of a paramagnetic agent, such as the acetylacetonate complex of chromium, $Cr(acac)_3$. The identification of the carbon resonances as C, CH, CH_2 or CH_3 can be achieved through off-resonance decoupling of the protons, which leaves a residual characteristic coupling pattern of singlet, doublet, triplet and quartet, respectively (see Table 36.3). Alternatively, a number of NMR pulse sequences, such as the attached proton test (APT) or the distortionless enhancement by polarisation transfer (DEPT) approach, can be applied to give the same information.

Directly observed ^{13}C NMR spectra have now been surpassed largely by a technique that involves indirect ^{13}C detection through ^1H NMR spectroscopy. This has come about through the advent of inverse-geometry NMR probes and the development of NMR pulse sequences that

Table 36.4 Two-dimensional NMR experiments useful for small molecules

Experiment	*F2*	*F1*	*NMR interaction*	*Information*	*Time/quantity*[(a)]	*Comments*
COSY	δ_H	δ_H	$^2J_{HH}$ and $^3J_{HH}$	H–H coupling connectivity	0·25/1	Easy
DQF–COSY	δ_H	δ_H	$^2J_{HH}$ and $^3J_{HH}$	H–H coupling connectivity	0·25/1	Easy
TOCSY	δ_H	δ_H	$^2J_{HH}$ and $^3J_{HH}$	All H in a spin system	0·25/1	Easy
NOESY	δ_H	δ_H	H–H dipolar	r_{HH}, conformation	5/5	10–100 times weaker than COSY
ROESY	δ_H	δ_H	H–H dipolar	r_{HH}, conformation	5/5	10–100 times weaker than COSY
HETCOR	δ_C	δ_H	$^1J_{CH}$	C–H	5/10	^{13}C detected
HMQC	δ_H	δ_C	$^1J_{CH}$	C–H	1/5	^{1}H detected
HSQC	δ_H	δ_C	$^1J_{CH}$	C–H	1/5	^{1}H detected
HMBC	δ_H	δ_C	$^2J_{CH}$ and $^3J_{CH}$	C–C–H and C–C–C–H	3/5	^{1}H detected
*J*RES (homo)	δ_H	J_{HH}	J_{HH}	Measurement of J_{HH}	0·25/1	Easy
*J*RES (hetero)	δ_C	J_{CH}	J_{CH}	Measurement of J_{CH} and number of attached Hs	5/10	Moderate
INADEQUATE	δ_C	$\delta_{Ca} + \delta_{Cb}$	$^1J_{CC}$	C–C connectivity	15/100	1 in 10^4 molecules, difficult

[(a)] Time in hours and quantity in milligrams for a molecule of M_r 500 on a 400 MHz NMR spectrometer.

allow transfer of magnetisation between ^{13}C and ^{1}H. In an inverse experiment, usually conducted in the two-dimensional mode: ^{1}H magnetisation is generated and then transferred to ^{13}C; the ^{1}H and ^{13}C spins are allowed to interact for a variable time based upon the one-bond ^{1}H–^{13}C coupling constant of about 125–160 Hz; and then the magnetisation is transferred back to ^{1}H for detection. The resultant two-dimensional NMR spectrum has ^{1}H chemical shifts on the detection axis (F2), ^{13}C chemical shifts on the indirect axis (F1), and a peak at the intersection of the ^{1}H and ^{13}C chemical shifts for CH, CH_2 and CH_3 groups. In addition, all ^{1}H NMR signals from hydrogens bonded to^{12}C also have to be suppressed by the pulse sequence. A number of pulse sequences achieve this correlation, the two best known being heteronuclear multiple quantum coherence (HMQC) and heteronuclear single quantum coherence (HSQC). Quaternary carbons do not appear in such a spectrum and have to be detected in a separate experiment based on a longer-range ^{1}H–^{13}C spin coupling, usually that over three bonds with a typical value of 6–10 Hz. For long-range coupling correlation, the experiment usually used is heteronuclear multiple bond correlation (HMBC). Full details of these methods are given by Croasmun and Carlson (1994), in particular, and in the books listed in Further reading. Such inverse-detected correlations have rendered obsolete the conventional ^{13}C-detected two-dimensional methods such as HETCOR.

The ^{13}C isotope has a natural abundance of only around 1.1%, so ^{13}C–^{13}C correlation spectroscopy has to rely on the observation of only 0.01% of the sample, and at the same time suppress the ^{13}C resonances of all singly labelled molecules that are 100 times larger. However, such a method does exist, given the rather ridiculous acronym INADEQUATE, and provides a correlation between carbons separated by one bond, the ^{13}C–^{13}C coupling constant in this case being around 40 Hz. Typical sample requirements for two-dimensional NMR studies that involve ^{13}C are given in Table 36.4.

Quantitative analysis

Quantification is possible using NMR spectroscopy (Holzgrabe *et al.* 2005). In this case, it is necessary to ensure that, for the summation of successive scans, the spin system is fully relaxed at the start of each scan. This is achieved by waiting between scans five times the longest T_1 for the signals of interest. This ensures that 99.3% of the signal is recovered. This is reasonable for ^{1}H NMR spectroscopy, in which T_1 values are usually less than 4–5 s, but for ^{13}C NMR spectroscopy (see above) T_1 can be much longer. As shown for ^{13}C NMR spectroscopy, the peak areas are, in general, not related quantitatively to concentration, and steps have to be taken to overcome this. This can be achieved by adding a relaxation agent and/or by modifying the pulse sequence to remove the NOE, which can affect signal intensity.

Quantification is done by measuring peak areas in the usual fashion, either by adding a known amount of a pure internal standard or by the method of standard addition. For a peak from an analyte with relative molecular mass M_{rA}, arising from N_A equivalent nuclei having an area I_A, and for a corresponding peak from an internal standard with relative molecular mass M_{rS}, from N_S nuclei with area I_S, the mass of the analyte in the sample is given by:

$$\frac{m_A}{m_S} = \frac{I_A M_{rA} N_S}{I_S M_{rS} N_A}$$

where m_S is the mass of the standard in the sample. Peak areas can be measured by integration, i.e. by summing the intensity of data points over a defined region of a peak or by curve fitting to the peak shape.

The two enantiomers of a chiral compound give identical NMR spectra in a non-chiral solvent because the magnetic environments of the nuclei are identical in both isomers. However, addition of a chiral paramagnetic shift reagent or use of a chiral solvent allows resolution of the NMR spectra of the two forms and hence integration of peak areas and measurement of the enantiomer proportions. Several applications of quantitative NMR spectroscopy to substances in international pharmacopoeias are given later.

An example of molecular structure determination: ibuprofen

The various types of NMR spectra often used to confirm a molecular structure are illustrated by the example of the non-steroidal anti-inflammatory drug ibuprofen dissolved in dimethyl sulfoxide-d_6 (DMSO-d_6). Figure 36.1 shows the 600 MHz ^{1}H NMR spectrum of this substance; the molecular structure and numbering system are also shown, along with the assignments of the various NMR signals.

Thus, the two doublets at δ0.85 and δ1.33 arise from the methyl groups with typical chemical shifts, that from CH_3(12,13) being of double intensity, with both showing spin coupling to a single proton. The resonance at δ1.80 comprises nine lines in a binomial intensity pattern because it arises from CH(11), which is coupled to eight adjacent hydrogens. The 1 : 3:3 : 1 quartet at δ3.61 is from CH(7). The doublet at δ2.40 is the resonance from CH_2(10) and arises from coupling to CH(11) exclusively. The small multiplet at δ2.49 is from the residual DMSO-d_5 in the DMSO-d_6 solvent and comprises a 1 : 2:3 : 2:1 quintet because the residual CHD_2 proton is coupled to two deuterium nuclei that have a spin quantum number of unity, each giving rise to a 1 : 1:1 splitting, and the two splittings overlap to give the resultant multiplet. Finally, the aromatic protons give rise to the multiplets at δ7.09 and δ7.17. These are not simple doublets, as can be seen by the partially

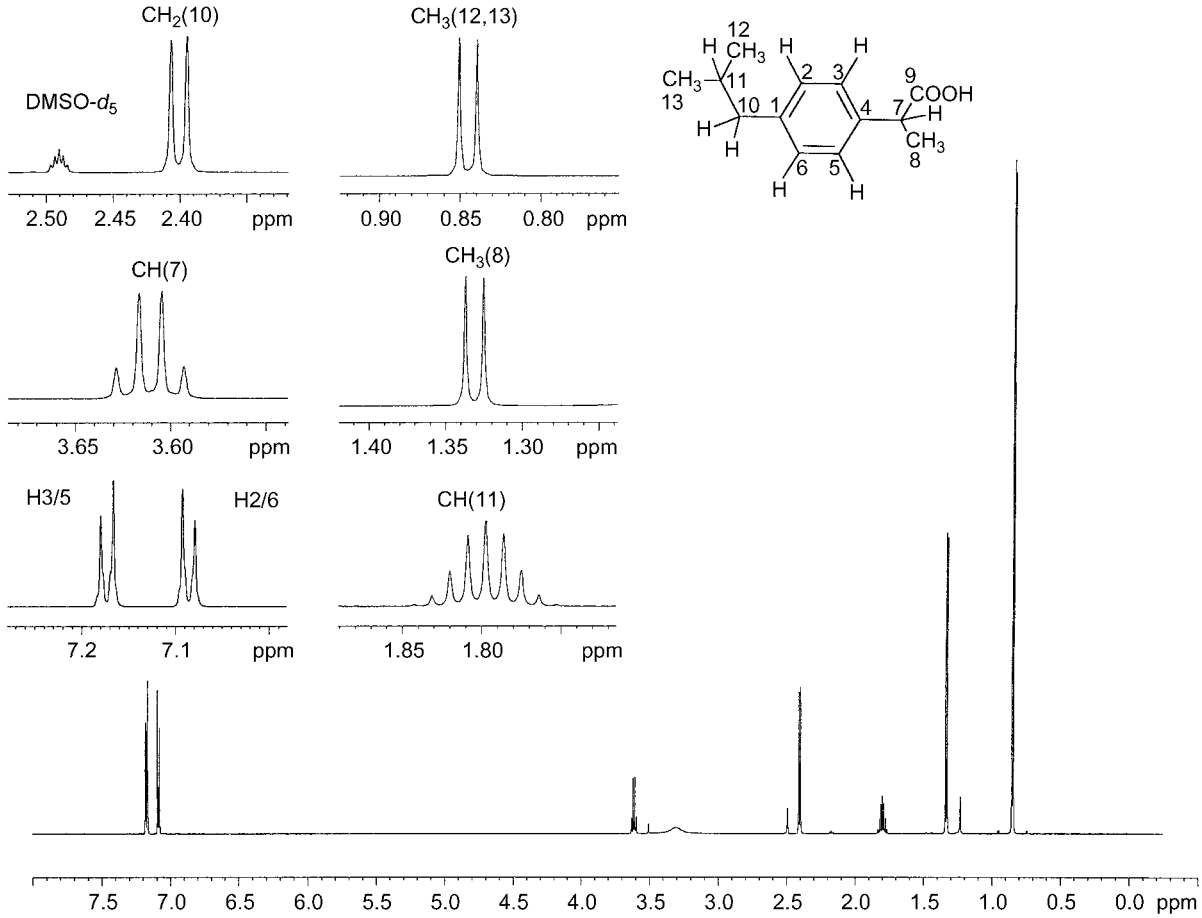

Figure 36.1 600 MHz ^{1}H NMR spectrum and molecular structure with numbering system of the non-steroidal anti-inflammatory drug ibuprofen dissolved in DMSO-d_6. Assignments are as marked. Expansions of the various resonances are shown.

resolved lines in the wings of the main peaks. These patterns arise as a consequence of the symmetry of the aromatic ring, with H2/H6 being chemically equivalent, but not magnetically equivalent (e.g. H2 will have a different spin coupling from H3 and H5). The broad peak near δ3.3 arises from the residual water in the sample and solvent.

The 600 MHz ^{1}H–^{1}H COSY spectrum of ibuprofen is shown in Fig. 36.2. Dotted lines are used to indicate the off-diagonal peaks that connect two chemical shifts arising from spin–spin-coupled protons. For example, the peak at δ0.85 from CH_3(12,13) is connected by an off-diagonal peak to the chemical shift at δ1.80 arising from CH(11), which in turn is connected to the peak at the chemical shift of δ2.40 corresponding to CH_2(10).

The 125 MHz ^{13}C NMR spectrum of ibuprofen in DMSO-d_6 is given in Fig. 36.3. Since all spin–spin couplings between protons and ^{13}C are removed by decoupling, each non-equivalent carbon gives a single resonance peak. The assignments of the peaks are also given on Fig. 36.3. Thus, the carboxyl carbon appears at 175.3 ppm and the two quaternary aromatic carbons are at 139.6 and 138.4 ppm; the assignments to C1 and C4, respectively, are based on a two-dimensional experiment, known as HMBC. This correlates ^{13}C and ^{1}H peaks via long-range spin–spin couplings (see ^{13}C NMR spectroscopy). Next come the other aromatic carbons, C2/6 at 129.0 ppm and C3/C5 at 127.0 ppm. These peaks are more intense because each arises from two equivalent carbons, and also, because of the decoupling of the ^{1}H–^{13}C spin–spin interactions, each carbon experiences an NOE that boosts the intensity of its NMR resonance. The resonances from CH(7) and CH_2(10) appear very close together, the assignments again being based on an HMBC spectrum. The 1 : 3:6 : 7:6 : 3:1 septet at 39.5 ppm arises from the carbons in the solvent DMSO-d_6 and the splitting is caused by ^{13}C–^{2}H spin–spin coupling, which is not affected by decoupling the ^{13}C–^{1}H interactions. The resonance for CH(11) is at 29.6 ppm and the methyl carbons appear at 22.1 ppm (CH_3(12,13)) and 18.4 ppm (CH_3(8)), one being twice as large as the other, as expected.

To illustrate the use of indirectly detected ^{1}H–^{13}C two-dimensional NMR spectroscopy, the ^{1}H–^{13}C HSQC spectrum of ibuprofen is shown in Fig. 36.4. This provides the connectivity between ^{13}C nuclei and directly attached ^{1}H nuclei. Hence, given the partial assignment of both the ^{1}H and ^{13}C NMR spectra based on inspection, it is often possible to complete the assignment of both spectra using this type of data. The two-dimensional contour plot shows a number of peaks, each of which occurs at the intersection of the ^{1}H and ^{13}C NMR chemical shifts of a given CH, CH_2 or CH_3 group. Quaternary carbons do not appear in this spectrum because they have no directly attached hydrogens to provide the one-bond ^{1}H–^{13}C spin–spin coupling used for the correlation. For ease of interpretation, the ^{1}H and ^{13}C one-dimensional NMR spectra, assigned earlier in Figs 36.1 and 36.3, are plotted along the appropriate axes.

Thus, in summary, all of the acquired NMR data are consistent with the known chemical structure of ibuprofen. To assign the proportions of the two enantiomers that may be present, it would be necessary to add a chiral chemical shift reagent, as described above. Even then, the absolute stereochemistry of the two forms could not be determined from such a spectrum, and recourse would need to be made to other techniques, such as circular dichroism.

Directly coupled HPLC-NMR-MS

The identification of components in complex mixtures has been rendered much more efficient by the ability to couple HPLC directly with NMR spectroscopy. The on-line coupling of HPLC with MS has already

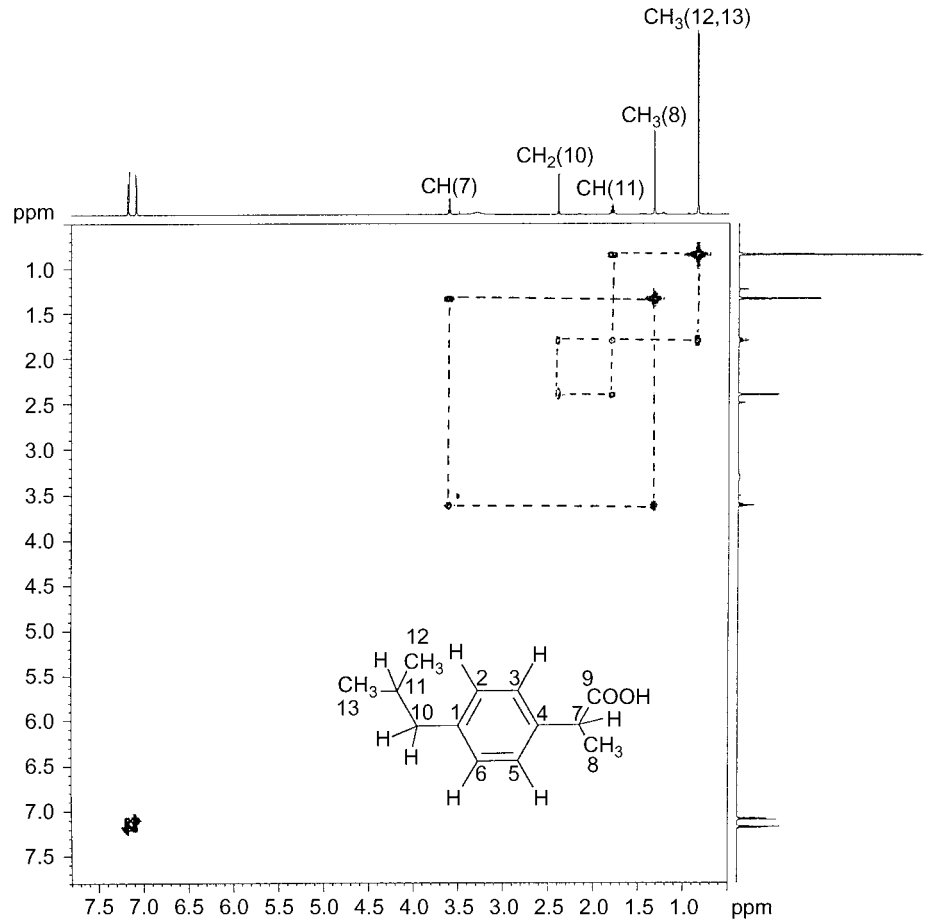

Figure 36.2 Two-dimensional 600 MHz ^{1}H–^{1}H COSY NMR spectrum and molecular structure of the non-steroidal anti-inflammatory drug ibuprofen dissolved in DMSO-d_6. The spin–spin coupling connectivities for the $CH_2(10)$–$CH(11)$–$CH_3(12,13)$ and $CH(7)$–$CH_3(8)$ spin systems are shown as dotted lines. Assignments are as marked.

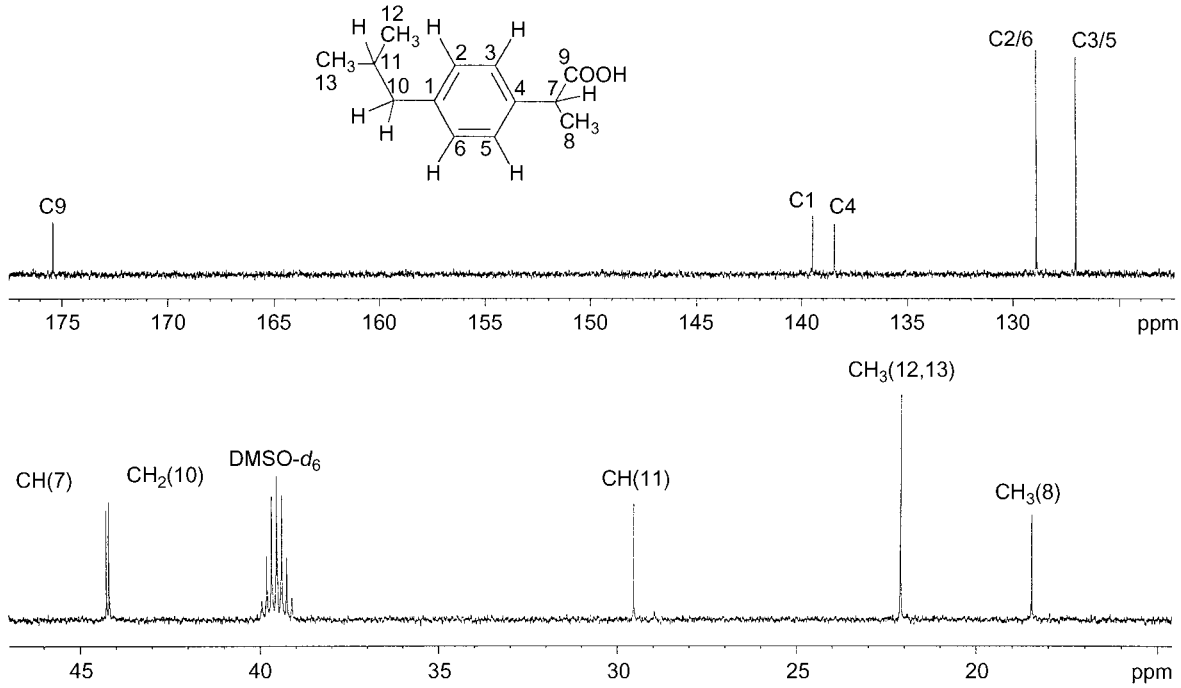

Figure 36.3 125 MHz ^{13}C NMR spectrum with broadband ^{1}H decoupling and molecular structure of the non-steroidal anti-inflammatory drug ibuprofen dissolved in DMSO-d_6. Assignments are as marked.

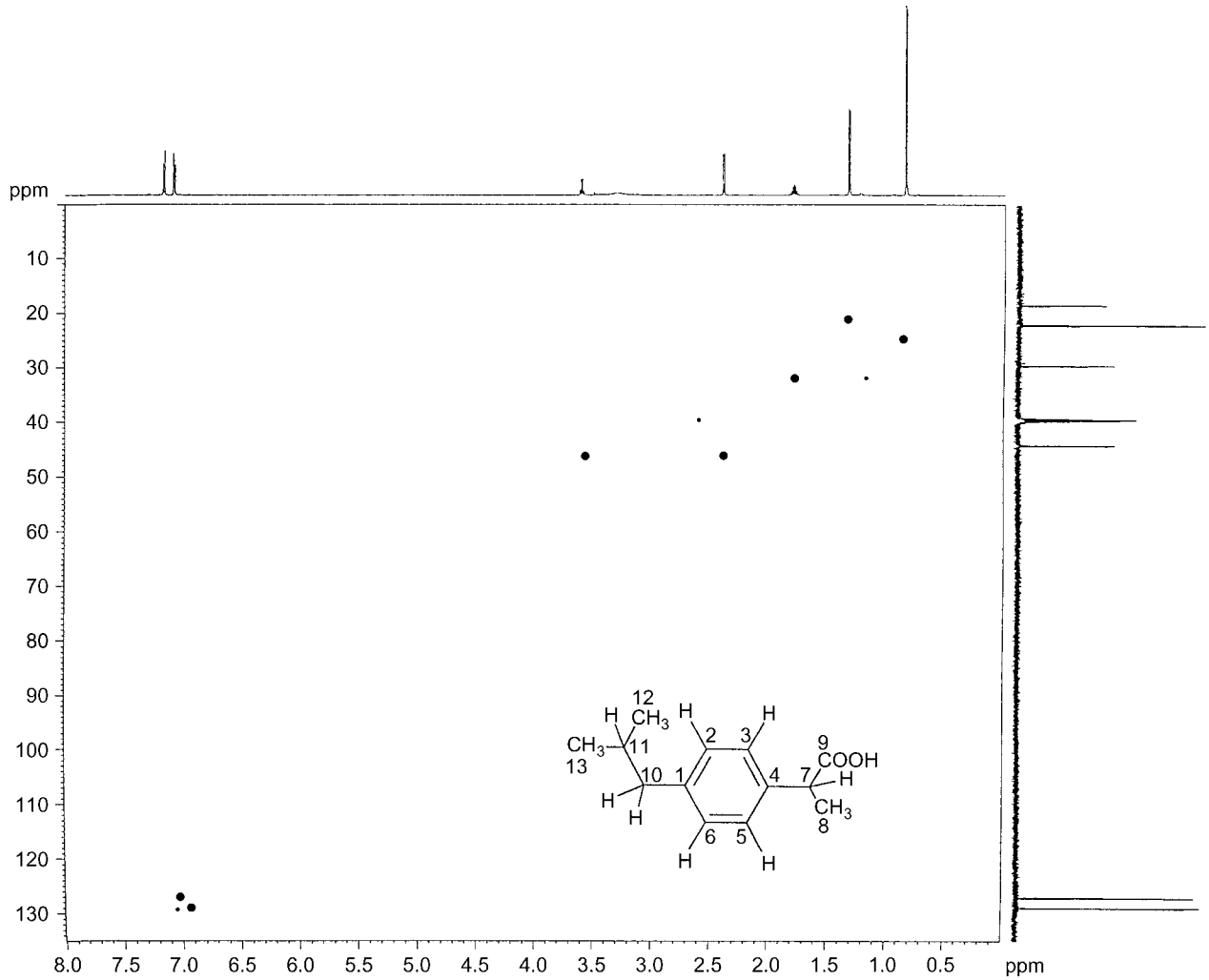

Figure 36.4 Two-dimensional ^{1}H–^{13}C HSQC NMR spectrum and molecular structure of the non-steroidal anti-inflammatory drug ibuprofen dissolved in DMSO-d_6. A peak is seen at the intersection of the ^{1}H and ^{13}C NMR chemical shifts of each CH, CH_2 and CH_3 group. Assignments are as marked by the peaks on the axes.

proved to be of immense value, and the complementary structural capability of NMR spectroscopy has meant that complete molecular identification can now be very fast. Most applications to date have focused on the identification of drug metabolites in biofluids and similar matrices, but there are also applications in drug impurity analysis and drug degradation profiles (Lindon *et al.* 2000). The general scheme for HPLC–NMR–MS is shown in Fig. 36.5, which indicates that the typical configuration is to have the HPLC apparatus situated on the bench as normal. After the column and UV (possibly diode-array) detection the eluate is split, usually in the ratio 95 : 5, to the NMR and mass spectrometers, respectively. The length of the capillary lines to these spectrometers can be adjusted so that either the NMR spectrometer or MS detects the eluate first. For example, the MS could occur first to identify characteristic ions, such as those relating to the isotope patterns of chlorine or bromine. Alternatively, the NMR spectrometry could occur first, e.g. to pick up the presence of a ^{19}F NMR signal.

Currently, five main options can be employed for HPLC–NMR, using either isocratic or gradient elution. The simplest of these is continuous-flow detection, in which NMR spectra are collected in real time as chromatographic peaks elute, but this is only practical when using ^{1}H or ^{19}F NMR for detection, unless isotopically enriched compounds are available. Where continuous-flow NMR detection is used for gradient elution, the NMR resonance positions of the solvent peaks shift with the changing solvent composition and, for effective solvent suppression, these solvent resonance frequencies must be determined as the chromatographic run proceeds.

If there is a method for detecting retention time on-line (UV, MS or radioactivity), stop–flow HPLC–NMR can be carried out using all the usual techniques available for high-resolution NMR spectroscopy. In particular, these include two-dimensional NMR experiments. In practice, it is possible to acquire NMR data on a number of peaks in a chromatogram using a series of stops during elution without on-column diffusion causing an unacceptable loss of chromatographic resolution.

There are two further categories of stop-flow experiment. First, in an increasingly popular approach, fractions eluted from the column can be stored in capillary loops for later off-line NMR study ('peak picking'). Second, the flow can be halted at short intervals during the passage of the eluting peak through the NMR flow cell ('time-slicing'), in a manner analogous to the use of a diode-array UV detector, to obtain spectra from various portions of the peak. This allows chromatographic peak purity to be estimated. Time slicing is most useful if the separation is poor, or if the compounds under study have weak or no UV chromophores, which makes it difficult to determine the retention times.

Fully automated analysis is now possible and, in this mode, automatic detection of UV peaks in the chromatogram based on predetermined time-windows or peak intensities is allowed under software control. The successful detection of each UV peak triggers the system to stop the flow at an appropriate time to isolate the peak in the NMR flow probe. This automatic NMR operation includes field homogeneity optimisation, setting and optimisation of all NMR acquisition parameters, and the pre-definition of the resultant SNR required in the spectrum. Two-dimensional NMR spectra can also be measured. With

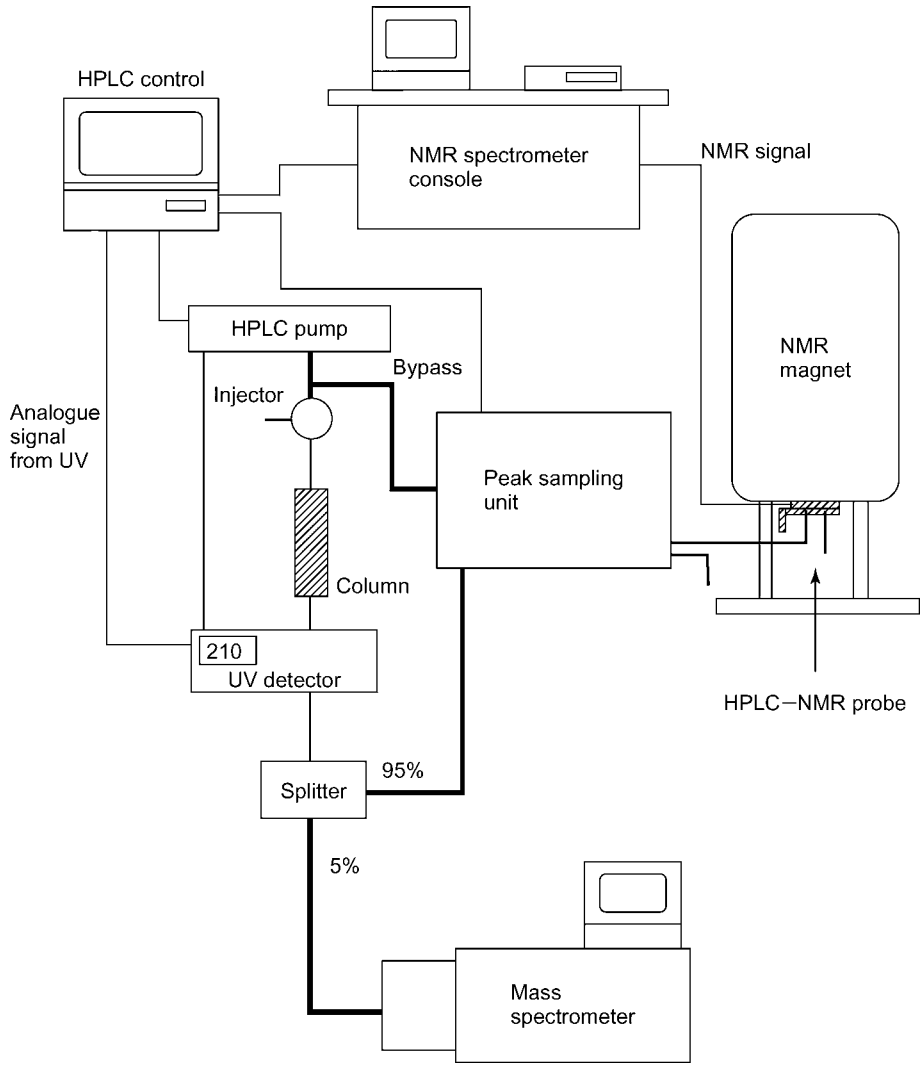

Figure 36.5 The experimental arrangement for directly coupled HPLC–NMR–MS. The bold lines indicate sample flow and the thinner lines are the electronic control and data signals.

currently available commercial software, the automated run can be halted at any time with reversion to manual control if desired.

Usually, for reversed-phase HPLC separations, a gradient eluent system of acetonitrile–water is used. To reduce the solvent suppression demands, the aqueous phase is made up of D_2O, but to save on cost any organic solvents are used in their conventional protio forms. In some cases, it is preferable to use deuterated acetonitrile, for example, as this aids the detection of NMR peaks near the methyl peak of the solvent. The increase in cost is outweighed easily by the time saving in carrying out the study and the improvement in the chance of success. Apart from this, it is not usually necessary to make any compromises in a desired chromatographic procedure to accommodate the various types of HPLC–NMR experiment. The powerful structural elucidation capabilities of NMR spectroscopy often ensure that complete chromatographic separation is not necessary for full characterisation of the peak.

In the case of HPLC–NMR–MS experiments, there are some additional considerations. So far, the principal MS ionisation method used has been electrospray in either positive- or negative-ion mode (using either single quadrupole or ion-trap mass spectrometers), which places further constraints on the chromatographic solvent systems. It is also possible to mix the eluent just prior to the mass spectrometer with a non-deuterated solvent to back exchange any deuterium atoms in exchangeable situations (e.g. NH and OH groups) for hydrogens. Comparison of the MS data with and without D_2O present enables the number of exchangeable hydrogens in any compound to be determined. In addition, MS can be used to search for particular diagnostic groups or fragments, such as an increase in m/z 16 for phase I hydroxylated drug metabolites or an increase of m/z 196 for a glucuronide.

The use of an ion-trap design allows fragmentation of the initial ions to be carried out in tandem experiments, such as MS–MS, MS–MS–MS, ... (denoted MS^n), etc. The use of time-of-flight or FT mass spectrometers provides very high resolution that allows good predictions of empirical molecular formulae from accurate ion masses, including those from MS^n experiments. Finally, some studies using inductively coupled plasma MS have been carried out. This technique provides no molecular information, as the sample is reduced to atomic form in the plasma, but it yields atom-specific chromatographic detection, which can be valuable for identifying the retention times of molecules of interest, such as those that contain heavy atoms or halogens such as chlorine and bromine.

By far the largest body of work to date using HPLC–NMR and HPLC–NMR–MS is in the field of drug metabolism, in which the methodology has been used extensively to identify metabolites in studies from clinical trials that involve human subjects, in the investigation of model drugs in animals *in vivo*, and also in *in vitro* systems such as liver microsome incubations.

Many marketed drugs are either natural products or modifications of such substances. Hence considerable effort has been spent in isolating and characterising chemicals from natural sources so that they can be tested in a variety of biological screens. Often it is necessary to carry out laborious extraction and purification steps, so the advent of directly coupled HPLC–NMR–MS has been explored as an alternative technique for natural product identification (Wolfender *et al*. 1998).

The use of NMR spectroscopy in the *British Pharmacopoeia*

NMR spectroscopy has been incorporated into a number of tests in the *British Pharmacopoeia* (BP) 2010, *European Pharmacopoeia* and *US Pharmacopoeia*.

These include assays for the peptide analogues buserelin and goserelin, in which the ^{1}H and ^{13}C NMR spectra, respectively, are compared with corresponding spectra of reference standards. Low-molecular-weight heparin samples can also be characterised by comparison with a reference sample using ^{1}H NMR spectroscopy. Medronic acid, used for radiopharmaceutical preparations, is assayed for two known impurities using the same method by comparison of an ^{1}H NMR spectrum of the material with those from reference impurity materials. In a similar fashion, an impurity in tetra-*O*-acetyl-mannose triflate is assayed using ^{19}F NMR spectroscopy.

The test for the degree in the quantification approach, the test for the degree of molar substitution (MS) in a modified cyclodextrin (hydroxypropylbetadex) is based on the relative integrals of the ^{1}H NMR peaks of the methyl groups (A1) (part of hydroxypropyl group modification) and the glycosidic protons (A2). MS = A1/(3 × A2) and the number of hydroxypropyl groups per molecule of β-cyclodextrin is 7 × MS.

Similarly, poloxamers are synthetic block copolymers of ethylene oxide and propylene oxide and thus contain oxyethylene (–O–CH_2–CH_2–O–) units and oxypropylene (–O–CH·CH_3–CH_2–O–) units. The characterisation of poloxamers includes the use of ^{1}H NMR spectroscopy to determine the ratio of the two types of subunit, based on integration of the ^{1}H NMR resonances at δ1.08 from the methyl group of the oxypropylene units and at δ3.2 from methylene groups from both types of subunit.

An assay for the positional distribution (β(2)-acyl) of different types of fatty acid in farmed salmon and cod liver oils has been developed based on a ^{1}H-decoupled ^{13}C NMR spectrum of a sample dissolved in $CDCl_3$. The main components are $C_{22:6}$, $C_{20:5}$ and $C_{18:4}$ fatty acids. By comparison with peaks from a reference sample, and by integration of the peaks in the region δC 171.5–173.5 from TMS (carbonyl carbons), it is possible to determine the overall proportions of α to β fatty acids.

Solid-state NMR spectroscopy of polymorphs

Polymorphism occurs widely in solid molecular systems, and polymorphs can differ in many of their properties, for example in the rate of dissolution and solubility of pharmaceuticals, and this can affect bioavailability. Similarly, the presence of solvates (also called pseudo-polymorphs), especially hydrates, can cause problems in pharmaceutical development. MAS NMR spectroscopy is now a useful technique for understanding the structure of polymorphs and solvates of pharmaceutical significance. The principal technique used is ^{13}C NMR spectroscopy, but other nuclei are also important, especially ^{19}F. Many drug-related studies are now in the literature since NMR spectroscopy can address problems involving amorphous systems, dynamics and non-stoichiometry that cannot readily be solved by diffraction techniques (Harris 2006). Solid-state NMR spectroscopy can provide quantitative information down to the level of ~1%.

NMR spectroscopy in metabonomics

Metabonomics encompasses the comprehensive and simultaneous profiling of metabolite levels in whole organisms through the study of biofluids and tissues. Metabolite levels undergo systematic and temporal changes, with factors such as diet, lifestyle, environment, genetic effects and pharmaceutical effects (both beneficial and adverse) having impact (Lindon *et al*. 2007). The principal analytical techniques used are NMR spectroscopy and mass spectrometry. Metabonomic studies generally use biofluids or cell or tissue extracts. Urine and plasma are obtained essentially non-invasively, and hence can be used more easily for disease diagnosis and in a clinical trials setting for monitoring drug therapy. However, a wide range of fluids have been studied, including seminal fluids, amniotic fluid, cerebrospinal fluid, synovial fluid, digestive fluids, blister and cyst fluids, lung aspirates and dialysis fluids. In addition, a number of metabonomic studies have used analysis of tissue biopsy samples and their lipid and aqueous extracts, as well as *in vitro* cell systems. Tissue studies use high-resolution ^{1}H MAS NMR spectroscopy.

In the post-genomic era, there is much emphasis on the study of the consequences of changes in gene expression (functional genomics), by examination of either the protein complement (proteomics) or the small-molecule metabolite composition of a biological system (metabonomics). There is a need to integrate information at the transcriptomic, proteomic and metabonomic levels to provide a full systems biology understanding, and this is at best only partially fulfilled.

In all cases the problem involves the detection and identification of low amounts of analytes in a very complex matrix with many potential interferences, all obscured by inter-subject biological variation. High-resolution ^{1}H NMR spectroscopy is particularly appropriate for investigating abnormal body fluid compositions, as a wide range of metabolites can be quantified simultaneously with no sample preparation and without preselection. However, high-resolution one-dimensional and multidimensional NMR spectra measured on biofluids can be extremely complex, and contain many thousands of resonances. In many cases, visual inspection of such spectra releases only a small percentage of the information available in the data. For this reason, the use of computer-based methods to extract the maximum information from such complex spectra is the general approach used. One recent development that has already proved popular is the use of correlation techniques to monitor peak changes across large cohorts of spectra. This has proved to be very useful for biomarker identification. Because it gives molecular fragment connectivity information somewhat analogous to the two-dimensional TOCSY experiment described earlier, it has been termed statistical TOCSY or STOCSY (Cloarec *et al*. 2005).

To illustrate the complexity of biofluid NMR spectra, Fig. 36.6 shows the 800 MHz ^{1}H NMR spectrum of a control human urine sample, with successive horizontal and vertical expansions. It has been shown that each biofluid has its own characteristic fingerprint.

A number of studies have used metabonomics to characterise normal metabolic variation in experimental animals such as mice and rats, caused by a range of inherent and external factors. Such differences may help explain differential toxicity of drugs between strains and inter-animal variation within a study. Many effects can be distinguished, including male/female differences, wild-type and genetically modified animal models, age-related changes, oestrus cycle effects in females, diet, diurnal effects, and interspecies differences and similarities. Analogous studies have also been undertaken in humans. The importance of the symbiotic relationship between mammals and their gut microfloral populations has been studied extensively.

The minimisation of the occurrence of drug adverse effects is one of the most important aims of pharmaceutical research and development, and metabonomic classification of the target organ or region of toxicity, the biochemical mechanism of that toxin, the identification of combination biomarkers of toxic effect and evaluation of the time-course of the effect, e.g. the onset, evolution and regression of toxicity, can all be determined.

Many examples exist in the literature on the use of NMR-based metabolic profiling to aid human disease diagnosis. A promising use of NMR spectroscopy of urine and plasma, as evidenced by the number of publications on the subject, is in the diagnosis of inborn errors of metabolism in children.

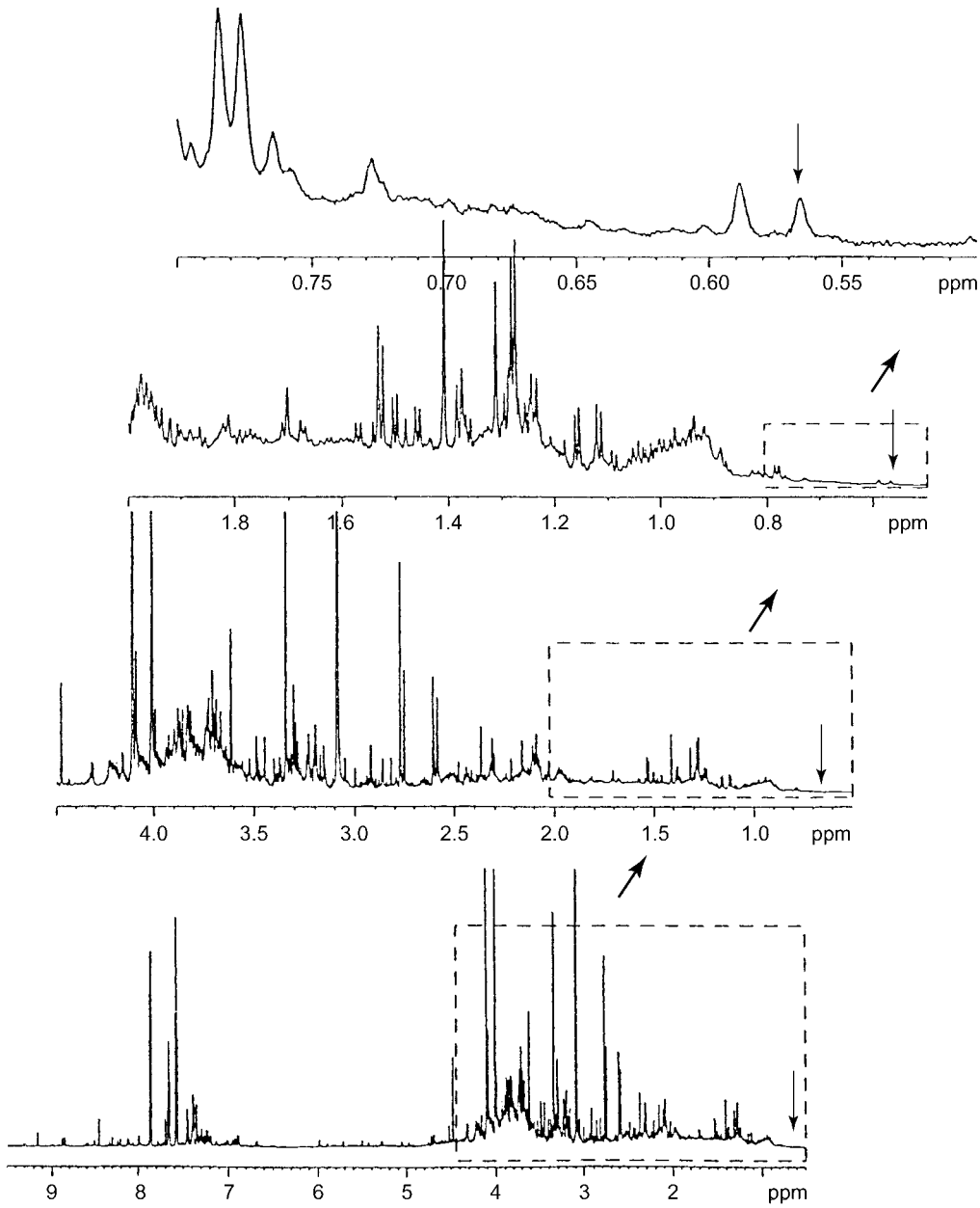

Figure 36.6 800 MHz ^{1}H NMR spectra of a typical control human urine sample showing successive horizontal and vertical expansions, and illustrating the complexity of the biochemical profile. The vertical arrow points to a very minor peak from the axial methyl group of a bile acid, a substance known to be elevated in certain types of hepatotoxicity. This demonstrates the ability of ^{1}H NMR spectroscopy of biofluids to detect very subtle changes in minor components.

An alternative approach to understanding inter-subject variability in response to drug treatment has been developed using a combination of multivariate metabolic profiling and chemometrics to predict the metabolism and toxicity of a dosed substance, based solely on the analysis and modeling of a pre-dose metabolic profile. This approach, termed pharmacometabonomics, is sensitive to both the genetic and environmental influences that determine the basal metabolic fingerprint of an individual, since these will also influence the outcome of a chemical intervention.

Integration of metabonomic data with that from other multivariate techniques in molecular biology, such as from gene array experiments or proteomics, is also feasible. It is now clear that characterising the relationships between genomic and phenotypic variation is an essential step in understanding disease processes and, to this end, the first real transcriptomic–metabonomic cross-correlations have been achieved.

Other areas where major expansion is expected are nutritional studies, sports medicine and lifestyle studies, including the effects of diet, exercise and stress, and evaluation of the effects of interactions between drugs, and between drugs and diet.

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CHAPTER

37 Mass Spectrometry

D Watson

Introduction

A mass spectrometer works by generating charged molecules or molecular fragments either in a high vacuum or immediately before the sample enters the high-vacuum region. Instruments typically maintain vacuums of about 10^{-6} mmHg (1.33×10^{-4} Pa), since ionised molecules have to be generated in the gas phase to enable them to be manipulated using magnetic or electrostatic fields. In classic mass spectrometry (MS) only one method could produce the charged gaseous molecules, but now quite a number of alternatives are available. Once the molecules are charged and in the gas phase, they can be manipulated by the application of either electric or magnetic fields to enable the determination of their relative molecular mass and the relative molecular mass of any fragments produced by the molecules breaking up. A number of useful introductory texts that describe mass spectrometers and mass spectral interpretation are available (Dass 2007; de Hoffman, Stroobant 2007; Gross 2004; McLafferty, Turecek 1993; Smith, Busch 1999; Throck Watson, Sparkman 2007; Williams, Fleming 1995).

Theory

Magnetic sector mass spectrometers

A magnetic sector mass spectrometer is illustrated in Fig. 37.1. For MS that follows chromatographic separation, other methods of ion separation are generally preferred on the basis of the cost of separation in a magnetic field.

One method of introducing a sample into a mass spectrometer is to insert it on the end of a heated probe so that it is volatilised with assistance from the high vacuum in the instrument. Once the analyte molecules are in the vapour phase, the source provides energy to cause them to ionise. The original method for producing ionisation, which is still used for routine analyses, is to bombard the analyte with electrons produced by a rhenium or tungsten filament. The electrons produced by the filament are accelerated towards a positive target with an energy of, most commonly, 70 eV. The analyte vapour is introduced into the instrument between the filament and the target. Since the electrons used to promote ionisation are of much higher energy than the strength of the bonds within the analyte (which are of the order of 4–7 eV), extensive fragmentation of the analyte usually occurs. In magnetic sector mass spectrometers the ions are separated by application of an electrostatic field followed by a magnetic one, or the reverse in reverse-geometry instruments.

In a straight geometry magnetic sector mass spectrometer the ions generated in the source are pushed out of the source by a repeller potential, which has the same charge as the ions. These ions are then accelerated and focused into a beam using a series of electrostatic lenses, which can be tuned to give optimum sensitivity. As the ions leave the ion source they have a range of kinetic energies because they were formed at different points in the source. The kinetic energies of the ions are focused into a narrow range using an electrostatic field applied at right angles to their direction of travel. As a result they take a circular path through the electrostatic analyser. Only ions with a narrow range of velocities then pass through the slit into the magnetic analyser. The width of the slits in the instrument can be varied to control its resolving power. Having focused the ion beam, the ions are then separated in the magnetic analyser according to equation (37.1). By varying the magnetic field B, ions with a range of mass/charge (m/z) values can be collected and detected using either an electron-multiplier or photomultiplier. A typical sweep time for the magnetic field across a mass range of 50–800 atomic mass units (amu) would be 1 s, but faster speeds are required if high-resolution chromatography is being used.

$$\frac{m}{z} = \frac{B^2 r^2 2}{2V} \tag{37.1}$$

where B is the magnetic field strength, r is the radius of the path through the magnet and V is the accelerating velocity. It is also possible to vary the potential of the electrostatic field and keep B constant. In this case, the mass range is limited by the range of kinetic energies of the ions generated in the source and scans may be only over a mass range of 5–10% of the mass of the ion being measured. However, the electrostatic region is not subject to hysteresis in the same way as the magnetic field and better high-resolution measurements can result from varying the electrostatic field. Although magnetic sector instruments are still widely used to determine the elemental compositions of ions, they may be superseded eventually by time-of-flight (TOF) instruments.

Relative molecular masses and elemental composition

The nitrogen rule

Most relative molecular masses of analytes are even numbers, unless the molecule contains a nitrogen atom. Compounds that contain a single nitrogen atom have odd-number relative molecular masses; two nitrogen atoms in a structure produce an even relative molecular mass, three an odd relative molecular mass and so on. Other elements (e.g. boron) can produce odd-number relative molecular masses, but nitrogen is the most common within the structures of drug molecules.

Isotopes

Table 37.1 summarises the isotope abundances of some elements commonly found in drug molecules. Chlorine and bromine have abundant isotopes; the presence of ^{35}Cl and ^{37}Cl or ^{79}Br and ^{81}Br produces characteristic 3 : 1 or 1 : 1 molecular ion patterns, respectively. These isotope patterns are very distinctive and are particularly so if a molecule contains more than one chlorine or bromine atom. The ^{13}C isotope has quite a high abundance, 1.1% of the abundance of the ^{12}C isotope. For instance, androstenedione has a molecular ion at m/z 286 (Fig. 37.2) and, since the molecule contains 19 carbon atoms, it has an isotope peak at m/z 287 that is about 21% ($19 \times 1.1\%$) of the ion at m/z 286, which is the probability of the occurrence of one ^{13}C atom in its structure. By the same logic, there is an ion at m/z 288 that is about 4% of the m/z 286 ion and results from two ^{13}C atoms in androstenedione. Androstenedione also contains 26 hydrogen atoms, so its mass will be higher by about 0.2 amu from its nominal mass, based on hydrogen having an exact mass of 1.0078 (Table 37.2). These two effects have to be taken into account when dealing with molecules of very high relative molecular mass, such as proteins. The deviation of the mass of hydrogen from unity makes a significant difference to the exact mass of a protein, and the abundance of ^{13}C in protein molecules makes the isotope patterns of protein spectra complex. The ^{15}N isotope and the ^{34}S isotope also add to the complexity of the isotope pattern of protein spectra. Often, the average mass of a molecule is quoted; this is the average of the contribution of all the

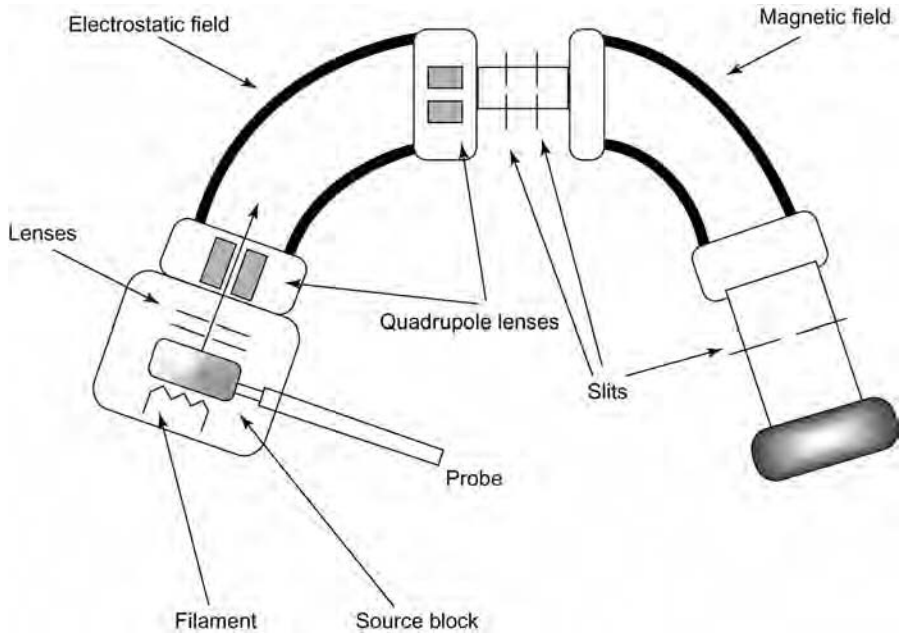

Figure 37.1 Schematic diagram of a double-focusing magnetic sector mass spectrometer.

isotopes towards its mass. Thus, HCl has an average mass of 36.5, since it contains 75% of ^{35}Cl and 25% of ^{37}Cl.

The relatively new technique of gas chromatography–combustion–isotope ratio MS (IRMS) to evaluate the content of ^{13}C or ^{15}N has potential for forensic toxicology, such as in the differentiation of endogenous and exogenous testosterone in sports drug testing. Figure 37.3

Table 37.1 Abundancee of isotopes commonly found in drug molecules

Isotope	*Relative atomic mass*	*Natural abundance (%)*
^{1}H	1.0078	99.985
^{2}H	2.014	0.015
^{12}C	12.000000	98.9
^{13}C	13.003	1.1
^{14}N	14.003	99.64
^{15}N	15.0001	0.36
^{16}O	15.995	99.8
^{17}O	16.999	0.04
^{18}O	17.999	0.2
^{19}F	18.998	100
^{28}Si	27.977	92.2
^{29}Si	28.977	4.7
^{30}Si	29.974	3.1
^{31}P	30.974	100
^{32}S	31.972	95
^{33}S	32.971	0.8
^{34}S	33.968	4.2
^{35}Cl	34.969	75.8
^{37}Cl	36.966	24.2
^{79}Br	78.918	50.5
^{81}Br	80.916	49.5
^{127}I	126.904	100

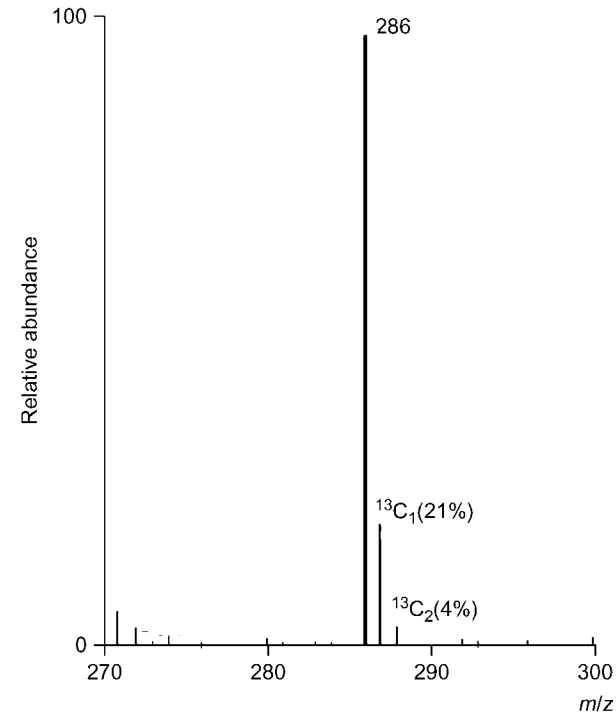

Figure 37.2 Molecular ion of androstenedione with associated isotope peaks.

Table 37.2 Masses relative to ^{12}C mass 12

Species	*Exact mass*
^{1}H	1.00782
^{14}N	14.0031
^{16}O	15.9949
CO	27.9949
CH_2 CH_2	28.0313
N_2	28.0061

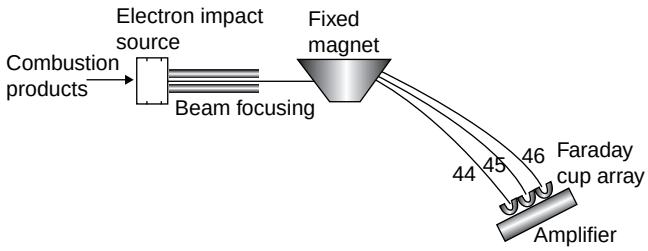

Figure 37.3 Schematic diagram of an isotope ratio mass spectrometer.

shows a schematic diagram of an isotope ratio mass spectrometer. The sample is burnt before being introduced into the mass spectrometer, generating gases such as CO_2, N_2, H_2O and SO_2 along with small amounts of these compounds incorporating stable isotopes – ^{15}N, ^{13}C, ^{2}H, ^{18}O and ^{34}S. The gaseous compounds are ionised by the electron impact mass spectrometry, and the ions are focused and then separated by a fixed magnet, i.e. without scanning. Thus ions are collected over a narrow range, e.g. CO_2 and its isotopomers. The ions are collected by an array of Faraday cups so that ion collection is continuous. Such a system produces high sensitivity and precision in the determination of isotopomers. The use of IRMS in forensic science has been reviewed by Benson *et al.* (2006) and applications include determination of geographical or synthetic origin or illegal narcotics, doping in sport, origin of explosives and flammable liquids.

Accurate mass measurement

Magnetic sector instruments can be used to determine the mass of a molecule to several decimal places. Based on the convention that carbon (^{12}C) has an exact mass of 12, the atoms of other elements do not have exact masses, as seen in Table 37.1. Thus, a high-resolution mass spectrometer would be able to distinguish CO, CH_2CH_2 and N_2, which all have a rounded mass of 28 amu The difference in mass between CH_2CH_2 and N_2 is 0.0252 amu, which relative to a mass of 28 is 900 parts per million (ppm; 1 ppm relative to 28 = 0.000028). Most double-focusing magnetic sector instruments should be able to measure masses to 1 ppm accuracy. To carry out accurate mass measurements, the instrument usually has to be calibrated at the same time as the sample is being measured.

Figure 37.4 shows the mass spectrum of chloroquine phosphate obtained using fast atom bombardment (FAB) ionisation. FAB is a soft ionisation technique, so that the spectrum is quite simple and the molecular ion for chloroquine (+H) is seen at *m/z* 320 for the chlorine-35 isotope and *m/z* 322 for the chlorine-37 isotope. A feature of FAB is that matrix peaks are usually also present in the spectrum. Two clear peaks can be seen at *m/z* 277 and *m/z* 369; these result from the formation of cluster ions from the glycerol matrix in which the sample is dissolved. These two peaks, and other glycerol cluster ions, have a known elemental composition/exact mass ratio and can be used to calibrate the mass axis of the instrument with high precision, so that an elemental composition for the two molecular ions of chloroquine can be obtained. Other commonly used calibrants are volatile ones used in heated probe work (perfluorokerosene (PFK) and perfluorotributylamine (PFTBA)) and poly(ethylene glycol) and Ultramark (used in calibrating the masses of high-molecular-weight compounds).

Resolution

Resolution in MS is rather analogous to the separation of peaks in chromatography and is of similar importance when quantitative measurements are being made, since overlap can produce quantitative inaccuracies. Unit resolution implies that two masses are completely separated (0% peak/valley ratio). For example, if 100 is separated completely from 101, this is termed 100 resolution. Magnetic sector instruments can effect resolution of up to 20 000 at 50% peak/valley, i.e. they can resolve masses of 20 000 and 20 001, so there is a valley of

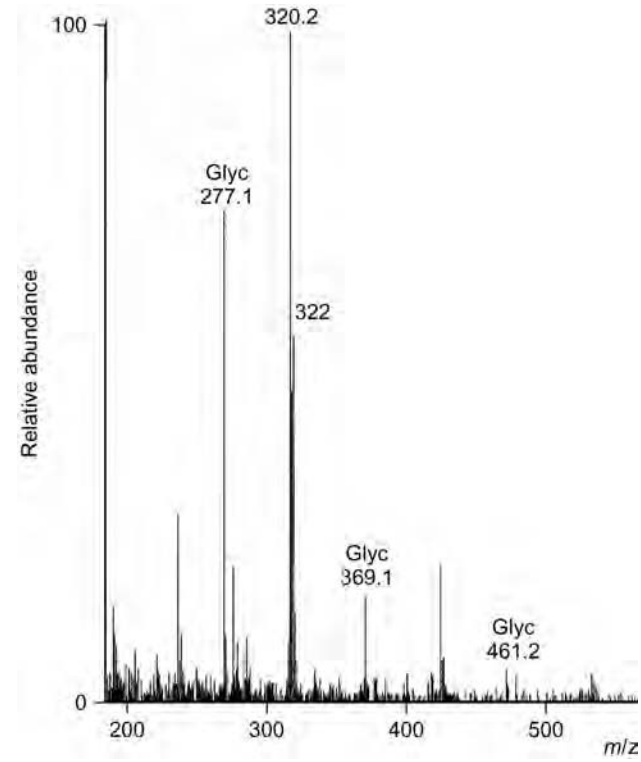

Figure 37.4 Fast-atom bombardment mass spectrum of chloroquine in a glycerol matrix.

50% of the peak height between them. However, greater resolution is achieved by narrowing the slits in the instrument, which means lower sensitivity because fewer ions pass through. Quadrupole instruments provide resolution of only about 500. Time-of-flight instruments are now beginning to approach the level of the resolving power of magnetic sector instruments. Fourier transform instruments afford the highest resolution with resolutions of up to 100 000.

Instrumentation

A prominent feature of MS is the range of instrumentation available, a range that is still rapidly expanding.

Magnetic sector instruments

The magnetic sector instrument is discussed above under Theory (and see Fig. 37.1).

Quadrupole instruments

Figure 37.5 illustrates a quadrupole mass spectrometer. For many years, quadrupoles were the only alternative to magnetic sector instruments. The principal features of quadrupole instruments are summarized below:

- Two varying electrostatic fields, one direct current (DC) and one at varying radiofrequency, are applied at right angles to each other via the four rods of the quadrupole. This creates a resonance frequency for each *m/z* value in a mass spectrum. The full mass range is scanned by varying the resonant frequency of the quadrupole.
- A simple quadrupole mass spectrometer gives only about 500 resolution and thus masses of ions separated by a quadrupole are only measured to one decimal place.
- Quadrupole instruments are of higher sensitivity than magnetic sector instruments, as they do not require all the ions that enter the quadrupole to have the same kinetic energy, and thus ion transmission is not reduced by slits.

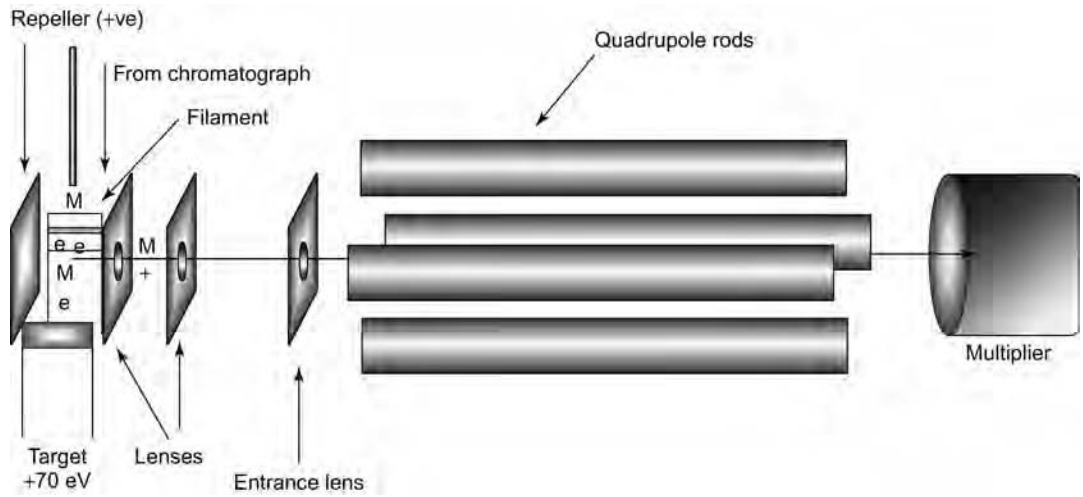

Figure 37.5 Schematic diagram of a quadrupole mass spectrometer.

- Since quadrupoles do not suffer from hysteresis, as magnetic sector instruments do, they are ideal for the rapid switching between ions required in selected-ion monitoring mode. In this mode a few significant ions in the mass spectrum of an analyte are monitored to achieve high sensitivity by ignoring background ions not derived from the analyte of interest.
- In a quadrupole instrument, larger-diameter rods produce increased sensitivity. Narrower or longer rods increase the resolving power.

Tandem mass spectrometry

The most common form of tandem MS started with three quadrupole mass spectrometers linked together. It a powerful mass spectrometric technique because it enables analytes to be separated rapidly by using a mass spectrometer rather than chromatography. It is particularly valuable in conjunction with electrospray ionisation, the most commonly used ionisation technique used in liquid chromatography-MS, since this soft ionisation technique produces few diagnostic fragments. In a triple quadrupole instrument, three quadrupoles are linked in series. Q1 (Fig. 37.6) separates the ions in a mass spectrum in the usual way. Then a selected ion (or ions) may be transferred to the second quadrupole Q2, which simply acts as an ion guide rather than producing separations, in which they are allowed to collide with atoms of a heavy gas, such as argon or nitrogen, to produce additional fragmentation. The fragmentation pattern produced is analysed using Q3. In Fig. 37.6 the MS spectrum of chlorpromazine is shown on the left and the spectrum resulting from the selection of the molecular ion at *m/z* 319 and its fragmentation in the second quadrupole is shown on the right. The fragmentation pattern obtained for chlorpromazine by tandem MS differs considerably from the spectrum of the drug obtained under electron impact conditions where the ion at *m/z* 58 dominates the spectrum.

The most common method for promoting fragmentation of an analyte is collision with an inert gas. High-energy collisions are promoted by acceleration of the ions prior to their entering the collision chamber; this is the approach used in ion-trap analyses. In this case, the gas used to promote fragmentation is usually helium and the fragmentation is fairly reproducible because the kinetic energy of the ions is controlled. Low-energy collisions rely on the inherent kinetic energy of the analyte ions and are less reproducible because the kinetic energy of the ions is variable. In this instance, either argon or nitrogen is used as collision gas and the fragmentation varies according to gas type, temperature and pressure. Photodissociation may also be used to promote ion fragmentation, the photoenergy being provided by a laser. This

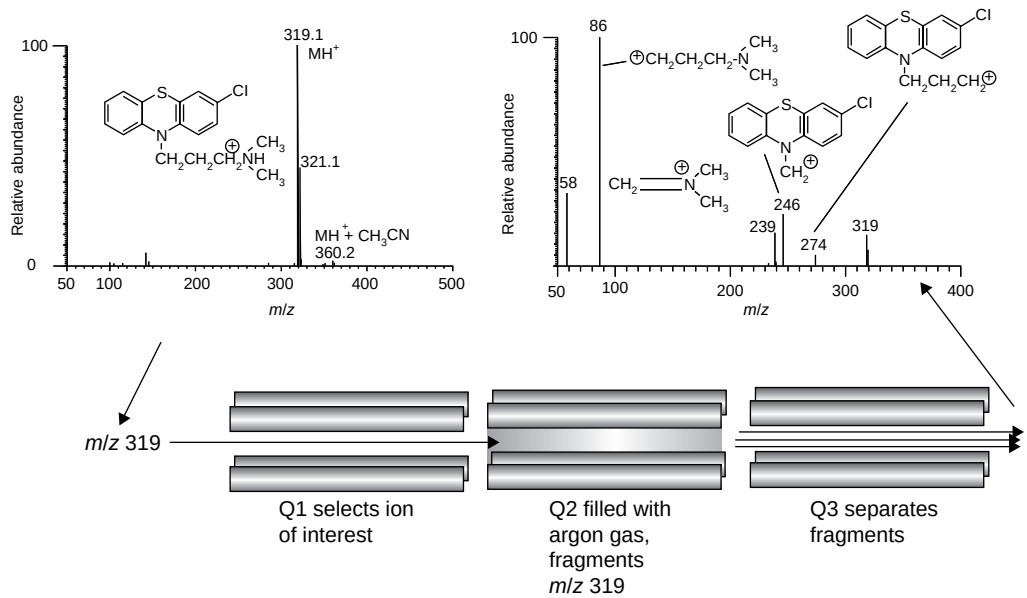

Figure 37.6 Illustration of the tandem MS process in the analysis of chlorpromazine.

technique gives very reproducible fragmentation and is particularly applicable to ion-trap analyses in which the ions can be trapped and irradiated.

Tandem mass spectrometry has become the standard method for the determination of drugs in biological fluids. The combination of high ion throughput obtainable with quadrupole ion separation and the high specificity available from selection of an ion of interest followed by quantitative analysis based on monitoring of a fragment or fragment ions of interest delivers the highest available level of sensitivity. It is important to note that monitoring of fragment ions produced by in-source fragmentation does not deliver the same level of sensitivity.

Ion-trap instruments

Ion traps (Ghosh 1995) have been developed in the past 20 years only and their performance has improved greatly in the past 5 years. The analyte is ionised using one of the available methods and the ions are transferred into the trap in which they are then confined by application of a radiofrequency voltage to the circular electrode (Fig. 37.7). The energy of the ions in the trap is quenched by helium, which is introduced into the trap to give a pressure of about 1 mmHg (133 Pa), so that they focus near the centre of the trap (i.e. their centrifugal energy is reduced). The ions can then be mass-selectively ejected by increasing a radio-frequency potential applied to the endcap electrodes (which also have a DC potential applied to them) to produce a mass spectrum as the ions are detected by the electron or photomultiplier tube. Since the ions in the trap are quenched by helium, there tends to be a greater degree of fragmentation in ion-trap spectra.

The power of the ion-trap technique resides in the amount of control that can be exerted on the ions in the trap. The ions can be confined within the trap and excited by changing the radiofrequency potential applied to the ring electrode so that their kinetic energy increases and they fragment more extensively through collision with the helium atoms in the trap. Selectivity can be introduced by ejecting all ions apart from, for instance, the molecular ion of the analyte of interest. The analyte is thus freed from any interfering peaks in the background and can be subjected to additional fragmentation by changing the radiofrequency potential of the trap. In theory, repeat fragmentation can be carried out several times on selected ions, making ion-trap mass spectrometry very useful where structure elucidation is required, such as in drug metabolism studies. Such mass spectrometric experiments are termed MS^n experiments, where n indicates the number of times that the mass spectrum has been produced. Figure 37.8 shows the MS^3 spectrum of chlorpromazine. In this case the ion at m/z 274 was selected from the initial MS^2 fragmentation and subjected to further fragmentation producing the ions at m/z 246 and 239. It can be seen that the signal-to-noise level is lower with the repeat fragmentation. Ion-trap instruments exhibit very good sensitivity in full scan mode and a wide dynamic range and can be used in quantitative work, although their ultimate sensitivity is at least an order of magnitude less than that of a top-of-the range tandem instrument.

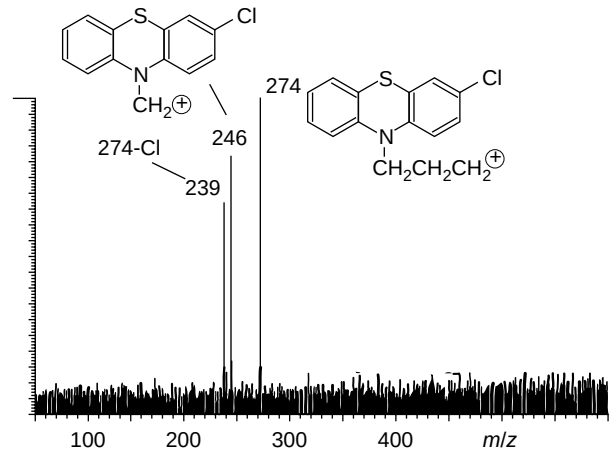

Figure 37.8 MS^3 spectrum for chlorpromazine obtained on an LCQ instrument at 30 V fragmentation energy for both stages.

Ion cyclotron resonance mass spectrometer

This instrument operates in a similar way to an ion trap. The ions are formed and then introduced into a trap and orbit within a circular magnetic field. As they circulate within the trap they pass close to two opposing electrode plates and thus induce a small alternating electric current within the circuit attached to the two plates. The amplitude of this current is proportional to the number of ions in the trap and its frequency is the same as the cyclotron frequency of the ions. The ions are

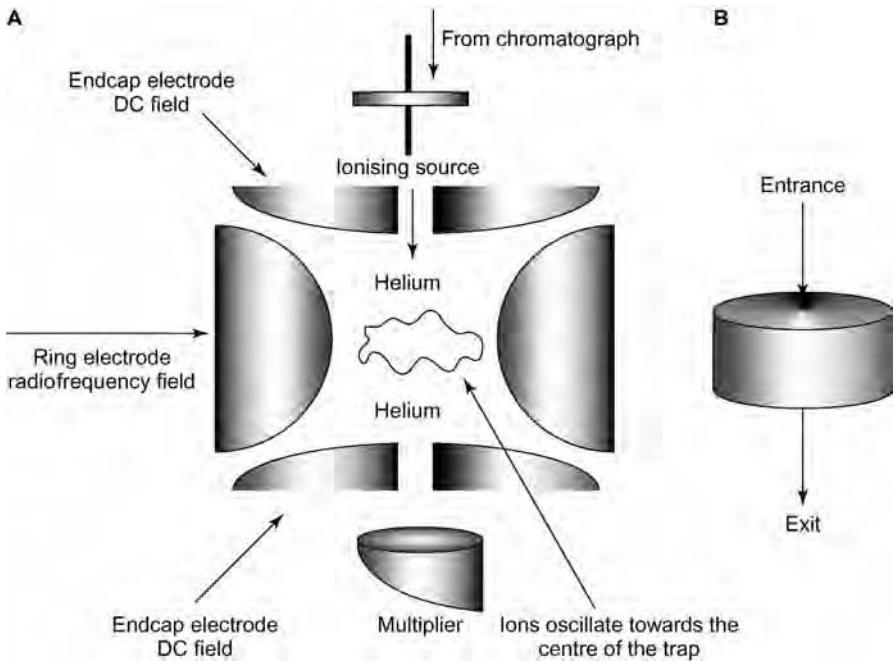

Figure 37.7 (A) Thermo Fisher LCQ ion-trap mass spectrometer. (B) View of the trap from outside.

thus detected without colliding with the detector via the current (the image current) that they induce. The ions in the trap can be excited by application of a radiofrequency pulse of radiation, which boosts the image current, which in turn falls off as the ions return to their unexcited state. The fall-off in the current can yield frequency information through the use of Fourier transformation, which leads to the construction of a mass spectrum. This process is analogous to Fourier-transform nuclear magnetic resonance spectroscopy, and can yield extremely high-resolution mass measurement, about ten times greater than the best performance of a magnetic sector instrument. The resolving power of this type of instrument is good enough to allow the accurate mass measurement of proteins. Recent developments in this field have resulted in the production of a new type of Fourier transform trap, the ThermoFinnegan Orbitrap. The design of the trap is a completely new concept but the data yielded from it are almost equivalent to that yielded by the older type of magnetic field-based FT-MS, giving accurate mass measurement to 5 decimal places and routinely mass accuracies to <2 ppm. This instrument has significant advantages over the older type of trap: it is considerably cheaper, the trap is electrostatic rather than magnetic and can be run with water cooling rather than expensive liquid helium cooling, and the instrument is more readily compatible with liquid chromatography.

Time-of-flight instruments

Time of flight (TOF) (Cotter 1997) was an early technique that produced ion separations. It is a very good technique for the analysis of compounds of high relative molecular mass such as proteins. The principle of TOF is quite simply that the larger an ion, the longer it will take for it to reach the detector of a mass spectrometer after acceleration through an electric field. For the technique to be effective, the time period during which the ions leave the ion source has to be well defined.

One ionisation process, matrix-assisted laser desorption ionisation (MALDI), uses short pulses of laser energy focused on the sample dissolved in a matrix that absorbs ultraviolet (UV) light. Alternatively, electrospray ionisation (ESI; see below) may be used in conjunction with a gating mechanism that allows ions to enter the separation field for only a very short period of time. The ions formed in the source have varying kinetic energies and, to avoid broad mass peaks, a device called a reflectron may be used to focus the kinetic energies of the ions that enter the TOF analyser. The greater the kinetic energy of an ion, the further it penetrates into the reflectron, and thus the faster ions are retarded by the reflectron, which allows the slower ions to catch up. Improvements in reflectron focusing, which include the use of double reflectron configuration and gating mechanisms, have enabled TOF to become capable of accurate mass measurement.

Hybrid quadrupole TOF (QTOF) instruments have become very popular for applications such as impurity profiling and drug metabolite identification and are a lower-cost alternative to FT-MS. In the QTOF type of instrument the third quadrupole in a tandem instrument is replaced with TOF separation. These types of instruments have tended to be used for qualitative work because of the limited quantitative dynamic range of TOF ($\sim 10^3$), although recent refinements in gating mechanisms have aimed to improve this.

MALDI-TOF has also advanced. MALDI-TOF^2 instruments have become available allowing tandem MS experiments to be conducted. Interfacing LC with MALDI is still not straightforward, however. MALDI-MS is increasingly used as an imaging technique in order, for example, to determine the distribution of a drug within a section of tissue.

Coupled techniques

Introduction

The most powerful mass spectrometric techniques involve the coupling of separation techniques with mass spectrometers. The development of ionisation techniques is linked intimately to the development of coupled techniques. Thus, in the majority of applications the ionisation technique has been developed and refined in conjunction with a separation technique. This is particularly true of the ionisation techniques used in conjunction with liquid chromatography (LC).

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS; Kitson *et al.* 1996; Message 1984; Neissen 2001) was the earliest technique in which chromatography and MS were coupled. The original type of gas chromatograph had a packed GC column with a gas flow rate passing through it of about 20 mL/min, so the major problem was how to interface the GC without losing the mass spectrometer vacuum. This was solved by use of molecular separators. In one of these, known as the jet separator, the column effluent was passed across a very narrow gap between two jets and the highly diffusible gas was largely removed, whereas the heavier analyte molecules crossed the gap without being vented. This problem of how to dispose of the GC carrier gas no longer exists, since GC capillary columns provide a flow rate of 0.5–2 mL/min of gas, which can be introduced directly into the mass spectrometer without a loss of vacuum. The GC-MS interface is very simple and no special design is required. The GC effluent is simply introduced in the same way as a direct insertion probe would be introduced into the instrument. If the same carrier gas pressure and column are used when a GC method is transferred to a GC-MS one, the retention times produced by the GC-MS method are shorter because of the effect of the vacuum at the mass spectrometer end of the column. Most GC software incorporates a flow calculator that enables an estimate to be made of the effect of a mass spectrometer vacuum on the linear velocity of the GC carrier gas.

Although many analyses are now carried out by LC-MS, GC-MS is still important for many drug analyses. In the negative-ion mode, it is probably the most sensitive analytical technique available for analytes that are strongly electron capturing. The main areas of application for GC-MS are in the characterisation of volatile compounds, such as flavourings, fatty acids and unidentified residual solvents. In the forensic field many narcotics can be analysed by GC-MS with a high degree of selectivity, as can other abused drugs such as anabolic steroids. An advantage of GC-MS is that electron impact (EI) spectra can be obtained, which are correlated readily to library spectra that have been built up over many years. The disadvantage of the technique is that more sample preparation is required in comparison with LC-MS techniques.

Liquid chromatographic interfaces with mass spectrometers

The interfacing of a liquid chromatograph to a mass spectrometer proved much more difficult than for a gas chromatograph, since each mole of solvent produces 22.4 L of solvent vapour even at atmospheric pressure. The technique has made huge advances in the past ten years and many types of interface are available, the most successful of which are the electrospray and atmospheric pressure ionisation sources (Neissen 1998). The advances in technique have meant that splitting of the eluent flow from a high performance liquid chromatography (HPLC) column is now unnecessary and instruments can cope with flow rates of up to 1 mL/min, although mobile phases with a high water content may need to be introduced at flows of less than 1 mL/min.

Electrospray ionisation mass spectrometry and atmospheric pressure chemical ionisation mass spectrometry

Electrospray ionisation (ESI) (Cole 1997; Snyder 1996) and atmospheric-pressure chemical ionisation (APCI) are the most widely used interfaces between liquid chromatography and mass spectrometers. They are carried out using the same source. In the ESI mode the sample is sprayed in solution into the source via a needle held at a high potential of 3–5 kV. The formation of an aerosol from the sample is assisted by a flow of heated nitrogen, which enters the instrument along a direction co-axial with the needle. The sample enters the instrument through a narrow orifice in a metal cone, which leads to a chamber at an intermediate level of vacuum; it then passes through a second orifice into a high-vacuum region. Figure 37.9 illustrates the production of the electrospray. At the tip of the capillary, positive ions are separated from their negative counter ions, which are pulled towards the capillary. The

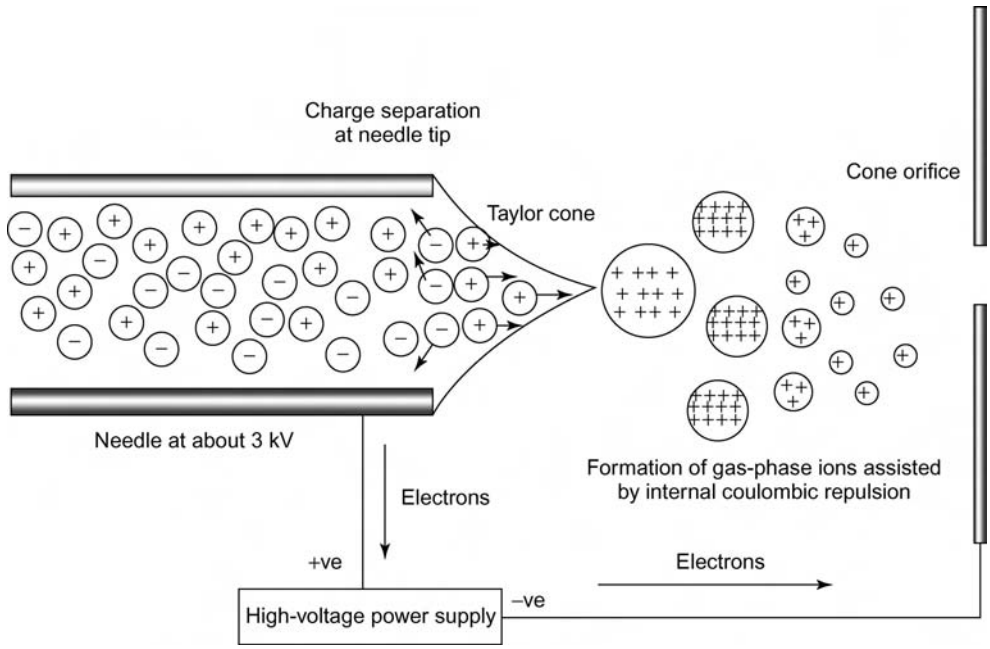

Figure 37.9 The electrospray process.

isolated positive ions generated are repelled by the capillary. This force breaks up the surface tension of the liquid in which the sample is dissolved, to generate a cone (the Taylor cone) that breaks up into charged droplets. This process is charge dependent and, since the analyte is usually of low concentration, electrolytes must also be in the liquid at a minimum concentration of 10^{-5} mol/L to assist in promoting ESI. Once the sample has formed into charged droplets, the excess of positive charges in the droplets produces repulsion, which causes the droplets to break up further. This is assisted by evaporation of solvent from the droplets, which increases their charge and thus further promotes their break up. At the final stage, the analyte is believed to abstract one or more protons from the solvent (or to donate a proton if a negative ion is formed) to give a positively charged gas-phase ion.

Figure 37.10 shows the positive ESI spectrum for morphine; it also illustrates an addition point about electrospray, which is that adducts can form with a variety of components present in the chromatographic mobile phase. Thus the ion at *m/z* 308 is due to addition of Na^+ to the molecular mass; *m/z* 327 is due to the addition of acetonitrile, which is present in the mobile phase; and *m/z* 349 is due to addition of both Na^+ and acetonitrile. Other common additions in the positive-ion mode include: K^+, NH_4^+ and methanol. In negative-ion mode the mobile phase additives acetic acid and formic acid are often added to the molecular ion.

If analytes can be protonated at multiple sites, they will carry several charges. Thus, a protein of molecular weight 10 000 amu that carries 10 charges appears to the mass analyser to have a mass of 1000 amu. Figure 37.11 shows the electrospray mass spectrum of porcine insulin. The charge on a given ion may be obtained from two adjacent ions in the mass spectrum according to equation (37.2):

$$n = \frac{M_A - 1}{M_A - M_B} \tag{37.2}$$

where n is the charge on M_B, and M_A and M_B are the masses of adjacent ions A and B with A the higher in mass. For the spectrum of porcine insulin shown in Figure 37.11:

$$n = \frac{1157 - 1}{1157 - 964} = \frac{1156}{193} = 5.99 \tag{37.3}$$

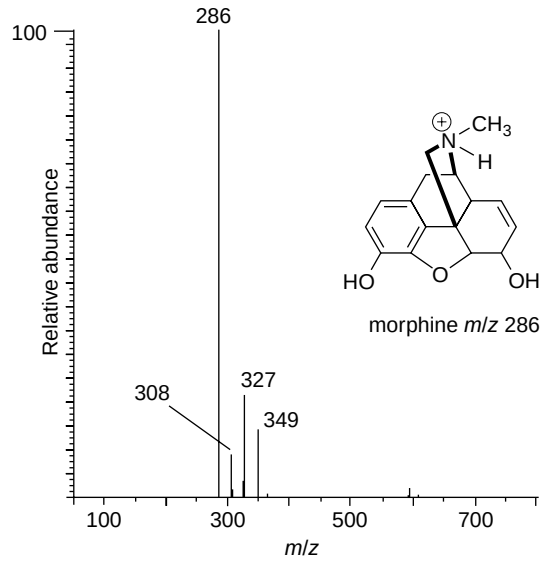

Figure 37.10 Positive-ion ESI spectrum of the MH^+ ion for morphine, *m/z* 286.

Thus the charge on the ion at *m/z* 964 is 6+, giving a relative molecular mass of 5784 for hexaprotonated porcine insulin. Insulin has six basic centres and, in this case, the predominant ion is that with all six centres protonated. Protonation of all the basic centres of a protein does not always occur.

To carry out APCI the instrument used for ESI simply has to be reconfigured to introduce a corona discharge pin at the point where the stream of solvent that contains the analyte enters the instrument. Although APCI is carried out on the same instrument as ESI, it is quite a different process. It does not depend on the production of ions by evaporation, rather it uses chemical species to promote the ionisation process in a manner analogous to the production of ions under positive-ion chemical ionisation conditions (PICI; see below). In this case the reagent ions that promote the ionisation include N_2^+ and H_3O^+. APCI tends to be less sensitive than ESI, but it is useful for molecules that will not ionise readily (e.g. neutral drugs such as steroids).

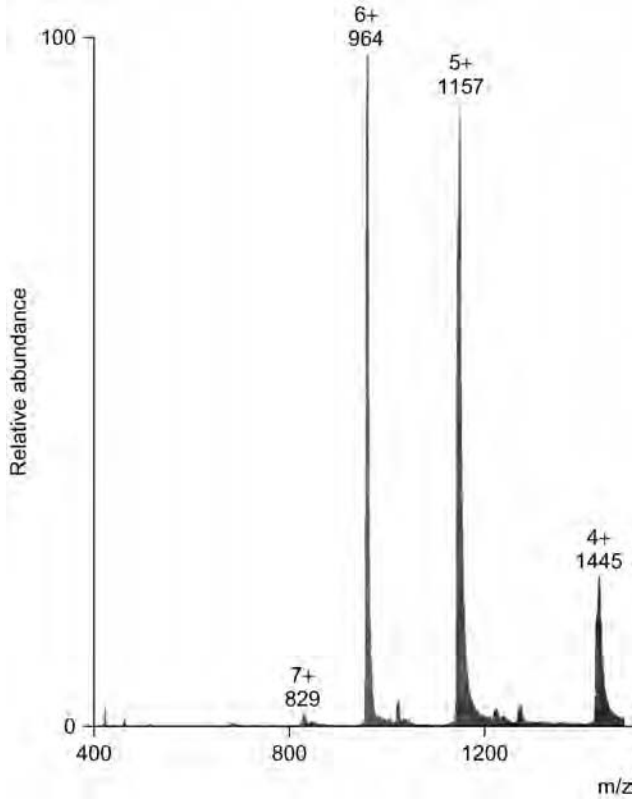

Figure 37.11 Electrospray mass spectrum of porcine insulin.

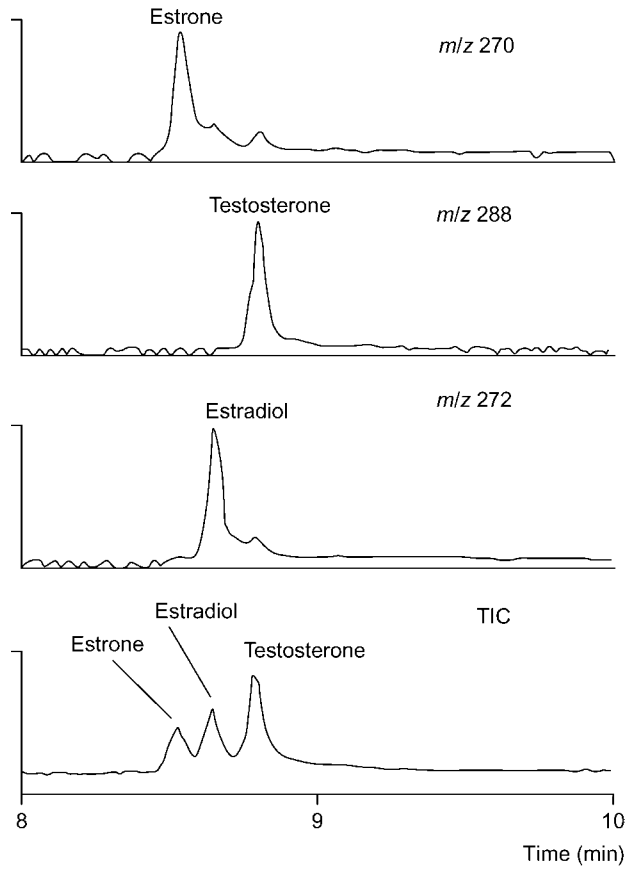

Figure 37.12 GC-MS trace of a mixture of estradiol, estrone and testosterone showing extracted ion traces for their molecular ions and the total ion current.

Capillary electrophoresis–mass spectrometry

The preferred mode of ionisation for interfacing a capillary electrophoresis (CE) instrument with a mass spectrometer is ESI. The technique has been improved by the development of a method to introduce a sheath flow of mobile phase, which augments the nanolitre per minute flows through the CE column to form a stable electrospray. During CE, a potential of 25–30 kV is applied across the capillary between the inlet and the outlet, the outlet being at ground potential. In ESI, the needle is normally held at a potential of 3–5 kV so that the potential applied across the CE has to be increased to compensate for the increase in potential at the outlet end. The CE interface is one of the most recent developments in MS and it is expected to have a wide range of applications in obtaining impurity profiles of both conventional synthetic drugs and biotechnological drugs.

Data processing

At a basic level the considerations for processing data obtained from mass spectrometers overlap those involved in the processing of chromatographic data obtained using other types of detector. Thus, from the chromatographic standpoint, the correct settings of integration parameters, such as peak width and threshold, are important to ensure that processing parameters do not confound the accuracy of the data obtained.

The difficulties of fused peaks in total ion current (TIC) chromatograms can often be circumvented by the use of selected ions that are characteristic of the compound of interest. For example, in the chromatogram shown in Figure 37.12, the peaks for testosterone, estradiol and estradione partially overlap. However, it is possible to extract the molecular ions for each individual compound and Figure 37.12 shows the extracted ion traces for the molecular ions of the three analytes, with very little interference between them. However, interference is not necessarily completely eliminated, as the testosterone peak produces some response in the selected ion trace for estrone since *m/z* 270 occurs in the mass spectrum of testosterone through the loss of water from its molecular ion at *m/z* 288.

The use of extracted ion traces is common in LC-MS data processing used for the screening of drug metabolites. The background produced by ions derived from the LC solvent in LC-MS mode means that when metabolites are present only in low amounts the molecular ions of potential metabolites can be predicted and used to generate extracted ion traces to check for their presence. However, chromatograms such as these do not show the same sensitivity as single-ion monitoring does.

Data processing in the generation of accurate mass data is a little more complex since the mass spectrum of the compound of interest may have to be calibrated against a standard, which is introduced into the mass spectrometer at the same time as the sample. For example, three of the calibrant ions are picked manually by the operator and the computer can construct a calibration curve based on the known masses of all the major calibrant ions (see Fig. 37.4). However, increasingly at the high price end of the market, instruments are capable of retaining mass accuracy after tuning for several days and thus the introduction of a calibrant at the same time as the sample is not necessary.

Beyond these basic considerations there have been extensive developments of data processing software aimed at facilitating the elucidation of drug metabolites or trace analytes in an automated fashion. Mass spectrometry manufacturers all sell versions of software that will predict the mass shifts caused by metabolic transformations for drugs and associated isotope patterns and predict fragmentation patterns. Also extensive work has been done in the proteomics field on the identification of peptide sequences produced by tryptic digestion followed by tandem MS and database matching of these sequences in order to identify the protein.

System suitability tests

Chromatographic tests

Chromatographic systems interfaced with mass spectrometers are subjected to the same system suitability tests as used in conjunction with other types of chromatographic detectors (see Chapters 39, 40, 41, 42).

Calibration of the mass axis

A test of fundamental importance in MS is calibration of the mass axis of the mass spectrometer with a suitable tuning compound or mixture. In GC-MS systems the most popular tuning compound is perfluorotributylamine (PFTBA). The use of a fluorinated tuning compound has two advantages. On the carbon-12 scale of relative atomic mass, fluorine is very close to its nominal mass of 19, whereas the mass of hydrogen is considerably greater than its nominal mass of unity. Second, since PFTBA is electron capturing, it can be used to tune in the negative-ion chemical ionisation mode. In the EI mode, PFTBA produces a number of abundant ions below a mass of *m/z* 219 and weaker ions above this value. Tuning should be carried out on a minimum of three ions that cover the mass range of interest. Typically, the ions at *m/z* 69, 219 and 502 generated by PFTBA are used to calibrate the mass axis in EI mode. These ions are also used to determine resolution between masses. There is always a trade-off between resolution and sensitivity and, if the mass window is narrowed to reduce peak width, sensitivity is lost. The PFTBA ions at *m/z* 69, 219 and 414, and 452, 595 and 633 are suitable for instrument tuning in PICI and negative-ion chemical ionisation (NICI), respectively. Instruments can be tuned automatically or manually. With quadrupole mass spectrometers and other low-resolution instruments, such as ion traps, the ions used in tuning are assigned masses to the nearest whole number. When high-resolution calibration is being carried out, masses of four or five decimal places are assigned to calibration ions; thus, for instance, the CF_3^+ ion at *m/z* 69 would be assigned its exact mass of *m/z* 68.9952. All the major ions in the spectrum of PFTBA would be used to carry out a high-resolution calibration and thus enable accurate mass assignment.

A mixture of caffeine, the tetrapeptide methionine–arginine–phenylalanine–alanine and Ultramark (a polyfluorinated fluorinated amine giving a series of ions differing by CF_2) is a popular choice for tuning electrospray instruments. Horse myoglobin is also used, as well as poly(ethylene glycol)s. In negative electrospray mode, salts of acids such as sodium dodecyl sulfate and sodium taurocholate can be used for tuning.

Sensitivity checks

There is no specific test for checking the sensitivity of a mass spectrometer. The counts provided by the tuning compound provide an indication of sensitivity, but beyond that the usual checks are of the sensitivity of the system when interfaced with chromatography. Manufacturers usually have their own favourite compounds for assessing sensitivity, such as methyl stearate to assess EI sensitivity in GC-MS mode or octafluoronaphthalene to assess NICI sensitivity in GC-MS mode. However, these particular analytes are not typical of the average drug molecule for which chromatographic factors contribute to the overall sensitivity. Sensitivity checks are usually carried out by diluting the compound of interest until it is no longer detectable by the particular system being used.

Sample preparation and presentation

The preparation of samples for GC-MS and LC-MS analysis does not differ from the types of preparation that would be carried out prior to any other chromatographic method, i.e. is, extraction to remove interferences by the sample matrix as far as possible. In the case of biological fluids, urine may not require much pre-treatment although, if it is not going to be diluted to a reasonable extent, it might be wise to precipitate out some of the salt content by dilution with acetonitrile. The most popular method for pre-treating plasma (due to its speed) is to precipitate out the protein prior to analysis by adding acetonitrile. This has been facilitated recently by the introduction of protein crash plates that allow the protein to be removed by filtration following addition of the solvent. If extraction is required, then a wide range of solid-phase extraction cartridges is available. Many drugs are bases and the cleanest extracts are produced by using strong cation exchange as the mode of extraction.

There is a tendency, because MS is a selective technique, to think that the cleanliness of the extracts and the chromatographic separation prior to introduction into the instrument is less important. In the case of ESI mode it may often be wrong to go for rapid analytical methods, since interfering ions in the matrix can often cause ion suppression of the analyte of interest. Plasma phospholipids and poly(ethylene glycol) are major culprits when it comes to ionisation suppression since they accumulate on HPLC columns and can then start to bleed off the column in subsequent runs. If the analyte is too close to the void volume of the HPLC column, this can also cause ionisation suppression. Ionisation suppression presents the greatest problem in the case of ion-trap mass spectrometers where matrix ions can cause suppression of the signal in the trap as well as during the electrospray process. It has been recommended that ionisation suppression should be checked by comparing the response factor for the analyte in water against the same amount of analyte spiked into a blank extracted matrix (Matuszewski *et al.* 2003).

Some additional considerations apply, for instance in GC-MS mode, as very often derivative formation may be necessary. For example, trimethylsilyl derivatives provide fairly abundant $[M-15]^+$ ions, which may be useful. Also, fluoroacylated derivatives may be prepared so that the sample is suitable for analysis in the negative-ion mode. If a chromatographic step is not used in conjunction with MS, it is important that the sample be relatively free of non-volatile materials, such as salts that would prevent it from evaporating from the direct insertion probe. The presence of non-volatile salts in samples may also interfere in LC-MS in the ESI or APCI mode, since the non-volatile salt will elute from the column and deposit on the cone, and so reduce instrument sensitivity quite rapidly. The cone can be continually washed to remove buffer salts, but, despite this, sensitivity tends to fall quite rapidly with time.

Data interpretation

Introduction

The interpretation of a mass spectrum (Dass 2007; de Hoffman, Stroobant 2007; Gross 2004; Lee 1998; McLafferty, Turecek 1993; Throck Watson, Sparkman 2007; Williams, Fleming 1995) is intimately bound up with the type of ionisation used to generate the mass spectrum. With EI MS, the fragmentation patterns for molecules are generally quite complex and many of the fragmentations do not fit the rules laid down by organic chemists for simpler organic molecules. Under other types of ionisation, fragmentation is less extensive and more predictable, as described below. As described above, many manufacturers of mass spectrometers now sell software that can help in the interpretation of mass spectra. In general in forensic analysis the order of importance for identification of a drug is: molecular ion, chromatographic retention time and fragmentation pattern. Identification of the fragments is of secondary importance and is required only for a particularly difficult problem.

Electron-impact mass spectrometry

EI ionisation uses high-energy electrons at 70 eV, which produce extensive fragmentation of the bonds within the analyte. It is still very commonly used in standard chemical composition analyses, but is not as readily applicable if the molecule being analysed is very non-volatile or unstable. In these cases, a range of other ionisation techniques can be applied.

Figure 37.13 shows a generalised scheme for decomposition of a molecule under EI conditions:

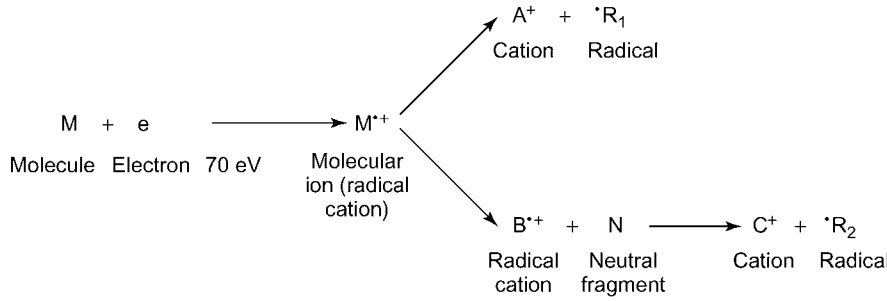

Figure 37.13 Electron-impact ionisation.

- Mrad$^+$ represents the molecular ion that bears one positive charge, since it has lost one electron, and the unpaired electron that results from the loss of one electron (represented by a dot).
- Mrad$^+$ may lose a radical that, in a straightforward fragmentation not involving rearrangement, can be produced by the breaking of any single bond in the molecule. The radical removes the unpaired electron from the molecule to leave behind a cation A$^+$.
- This cation (A$^+$) can lose any number of neutral fragments, such as H_2O or CO_2, but no further radicals.
- The same process can occur in a different order with a neutral fragment (H_2O, CO_2, etc.) being lost to produce the radical cation Brad$^+$; since this ion still has an unpaired electron, it can lose a radical to produce C$^+$, which can thereafter lose only neutral fragments.

An EI spectrum is shown for heroin in Fig. 37.14 and the fragmentation pathways that give rise to the spectrum are shown in Fig. 37.15. The molecular ion can be seen at *m/z* 369 (and therefore must contain a nitrogen atom) and the fragments at *m/z* 327 and 310 correspond to ions B and A in Fig. 37.13. The ion at *m/z* 268 corresponds to ion C in Fig. 37.13, and can also be derived via loss of acetate from ion B. The ion at *m/z* 284 results from the loss of two neutral fragments. Like many molecules the simply explained losses occur only within about 100 amu of the molecular ion, and below *m/z* 268 the fragment ions are derived from complex rearrangements of the structure of the molecule. An advantage of EI is that the complex fragmentation pattern produced can be used as a fingerprint to identify the molecule, for instance to confirm the identity of traces of heroin in a forensic sample or the identity of an anabolic steroid in a urine sample. Thus, with EI spectra there is plenty of scope to identify an unknown via interpretation. Table 37.3 shows some of the common losses from molecular ions that may be used in the interpretation of an EI mass spectrum.

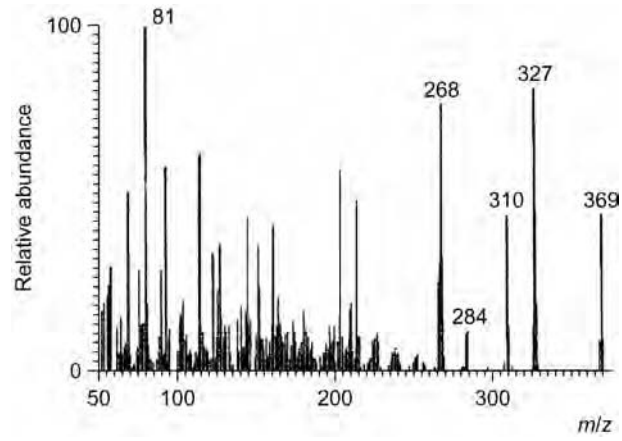

Figure 37.14 Electron-impact mass spectrum of heroin.

The fragmentation patterns of large molecules may be difficult to interpret. For instance, steroid molecules give particularly complex

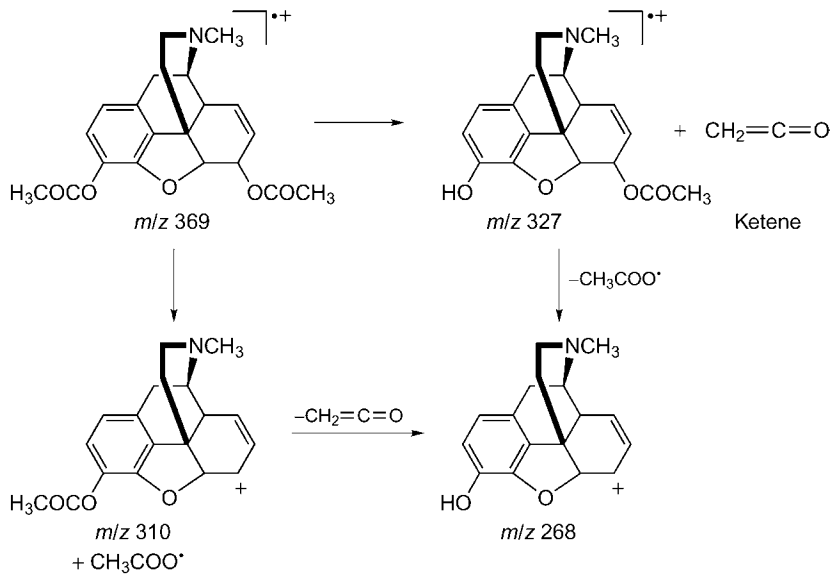

Figure 37.15 Fragmentation pathways that produce some of the ions in the EI spectrum of heroin.

Table 37.3 Some common losses from a molecular ion

Loss amu	*Radicals and/or neutral fragments lost*	*Interpretation*
1	Hrad	Often major ion in amines and aldehydes
15	CH_3rad	Most readily lost from quaternary carbon
17	OHrad or NH_3	
18	H_2O	Readily lost from secondary or tertiary alcohols
19/20	Frad/HF	Fluorides
28	CO	Ketone or acid
29	C_2H_5rad	
30	CH_2O	Aromatic methyl ether
31	CH_3Orad	Methyl ester/methoxime
31	CH_3NH_2	Secondary amine
32	CH_3OH	Methyl ester
33	$H_2O + CH_3$	
35/36	Clrad/HCl	Chloride
42	CH_2 C O	Acetate
43	C_3H_7rad	Readily lost if isopropyl group present
43	CH_3COrad	Methyl ketone or acetate
43	$CO + CH_3$rad	
44	CO_2	Ester
45	CO_2Hrad	Carboxylic acid
46	$CO + H_2O$	
57	C_4H_9rad	
60	CH_3COOH	Acetate
73	$(CH_3)_3Si$rad	Trimethylsilyl ether
90	$(CH_3)_3SiOH$	Trimethylsilyl ether

patterns. Most of the ions in the EI mass spectrum of hydrocortisone (Fig. 37.16) arise from rearrangements of the structure that involve migrations of hydrogen atoms. These types of spectra give a characteristic fingerprint and the base peak ion (most abundant ion) at *m/z* 123 occurs in many steroids. However, it may vary in mass from a hydrogen atom higher to one lower than 123. The base peak in the mass spectrum of testosterone occurs at *m/z* 124, even though it has exactly the same A and B rings as hydrocortisone. However, the mass spectrum of prednisolone, which has an additional unit of unsaturation in the A ring, is consistent with hydrocortisone and yields a base peak ion at *m/z* 121.

With many drug molecules, the use of EI ionisation results in a low abundance of the molecular ion. Thus chloroquine under EI conditions gives less than 1% abundance of molecular ion (Fig. 37.17) and its mass spectrum is dominated by an alpha cleavage fragment, which arises as shown in Fig. 37.18.

The spectra of heroin, hydrocortisone and chloroquine illustrate the three most common types of spectra observed in drug molecules. The spectra of heroin and hydrocortisone are most useful, since they are complex, provide a fingerprint, and are more open to interpretation than simple spectra such as the spectrum of chloroquine. Other dominant fragmentation modes exist, such as the formation of a tropylium ion, which occurs in benzyl compounds, but these are not as common as those mentioned above and are discussed elsewhere (Dass 2007; de Hoffman, Stroobant 2007; Gross 2004; Lee 1998; McLafferty, Turecek 1993; Williams, Fleming 1995).

If more information is required about the relative molecular mass of a drug that fragments easily, such as chloroquine, softer ionisation techniques are used. FAB ionisation is one option (the FAB spectrum of chloroquine is shown in Fig. 37.4). However, FAB combines less satisfactorily with chromatographic interfaces than other ionisation methods; quite a number of methods promote ionisation, some of which are discussed below.

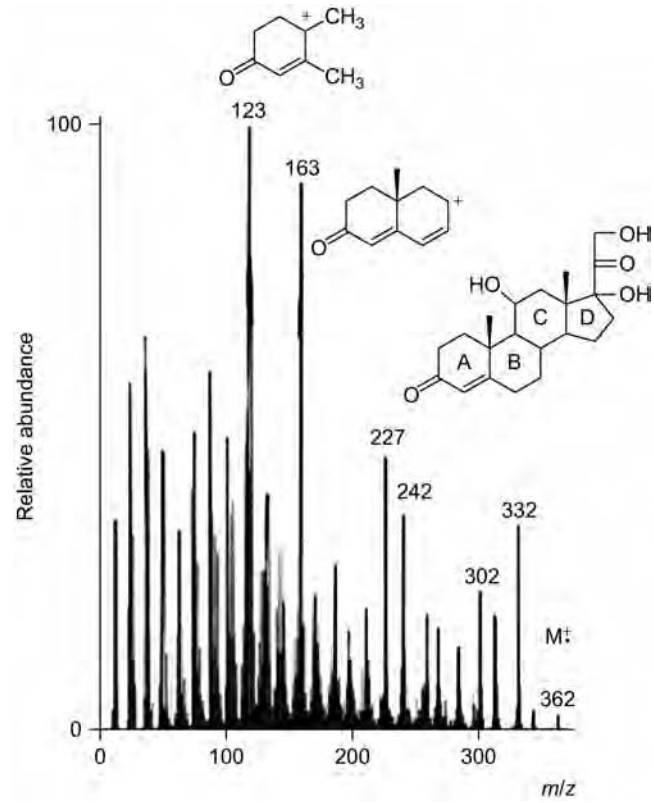

Figure 37.16 Electron-impact mass spectrum of hydrocortisone.

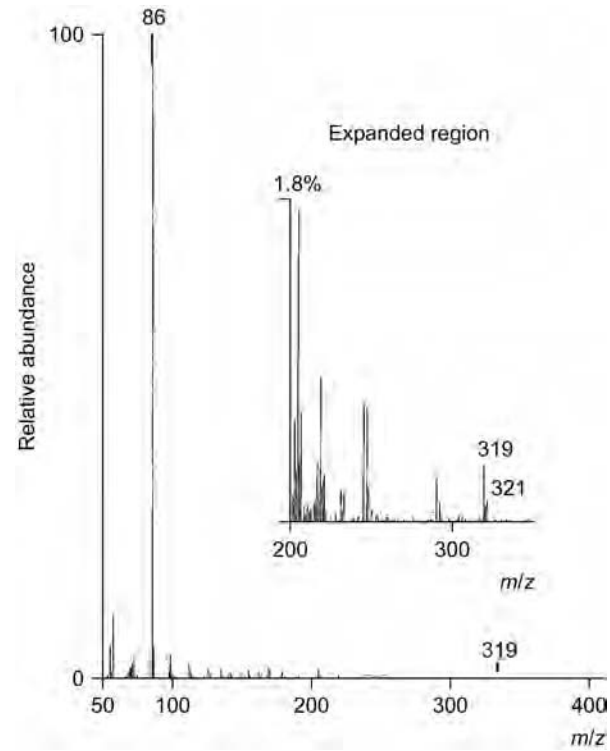

Figure 37.17 Electron-impact mass spectrum of chloroquine.

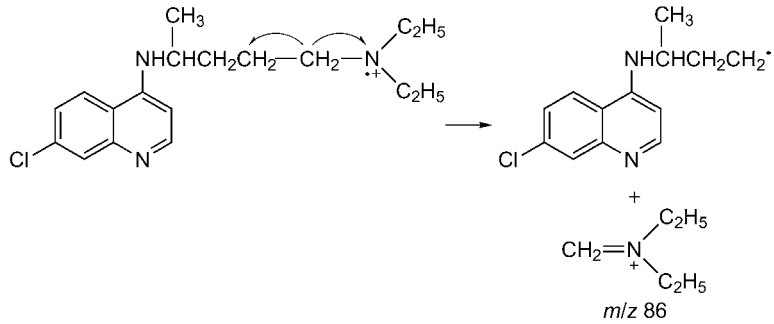

Figure 37.18 α-Homolytic cleavage of chloroquine.

Positive-ion chemical ionisation

Positive-ion chemical ionisation can be carried out with a number of reagent gases (methane, isobutene and ammonia are most commonly used), and it is most applicable in GC-MS. The gas is introduced into the source of the mass spectrometer continuously during the analysis and the source configuration is changed so that the gas is contained within a small chamber within the source. Gas is introduced to give a source pressure of about 1 mmHg (133 Pa) and bombarded with electrons produced by the same filament as used for EI ionisation at energies of about 200 eV. The electrons cause the reagent gas to ionise and further ions are produced through the gas reacting with itself. In the case of methane, three major reagent ions are produced, namely CH_5^+ (*m/z* 17), $C_2H_5^+$ (*m/z* 29) and C_3H_5 (*m/z* 41). These reagent ions can either transfer a proton to the analyte, thus ionising it, or combine with it to produce adduct ions.

When chloroquine is ionised under PICI conditions the spectrum shown in Fig. 37.19 is produced, which can be compared with the EI spectrum in Fig. 37.17. In the PICI spectrum the $[M+H]^+$ ion is the base peak and ions seen at *m/z* 348 and *m/z* 360 are from the addition of *m/z* 29 and *m/z* 41 reagent ions to chloroquine. There is also an ion at *m/z* 284, which is caused by ionisation via abstraction of the chlorine atom from chloroquine by the reagent ions. PICI is not commonly used in chromatographic analyses, since it is usually of low efficiency, but it can sometimes be a useful method to help identify impurities in drugs; it is also a useful way to generate an abundant molecular ion for elemental composition determination when using high-resolution MS.

Negative-ion chemical ionisation

Negative-ion chemical ionisation spectra are generated in the same way as PICI spectra, except that the instrument is set to focus and detect negative ions. Most negative-ion spectra are not true chemical ionisation spectra, but are rather electron-capture spectra. Molecules that contain electronegative and/or electron-rich groups, such as halogens, oxygens or conjugated double bond systems, have a high affinity for electrons and thus capture the low-energy electrons produced by collision with methane. Thus, NICI is suitable only for molecules that contain electron-capturing groups, but for this type of molecule it is an extremely efficient and selective mode of ionisation. The NICI spectrum of chloroquine is shown in Fig. 37.20. As in PICI, the spectrum is dominated by the molecular ion, in this case at *m/z* 319.

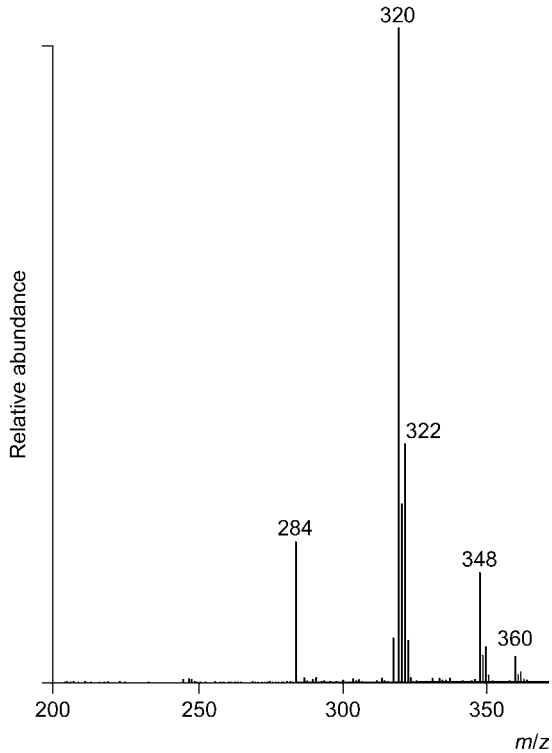

Figure 37.19 Positive-ion chemical ionisation spectrum of chloroquine.

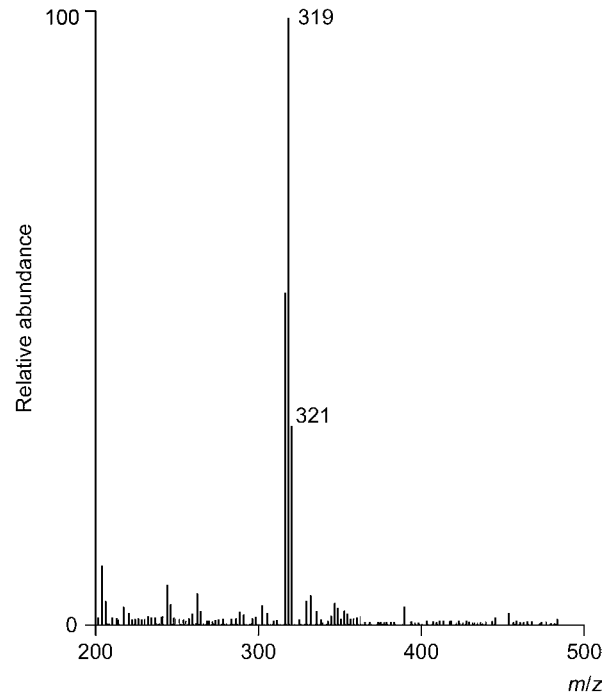

Figure 37.20 Negative-ion chemical ionisation spectrum of chloroquine.

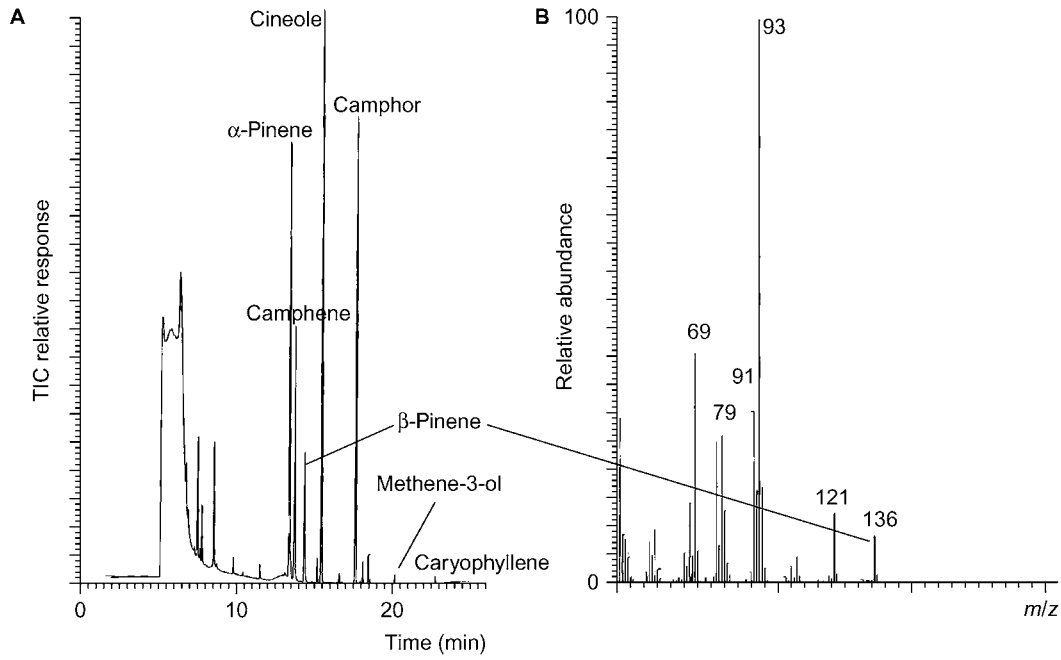

Figure 37.21 (A) Total ion current (TIC) chromatogram of rosemary oil by GC-MS. Conditions: ZB-1 column (60 m × 0.32 mm i.d., 0.5 µm film). Head pressure 130 kPa. Temperature programme: 50°C for 1 min to 80°C at 5°/min to 270°C at 10°/min. (B) The mass spectrum of the peak at 14.37 min (β-pinene).

Mass spectrometry in qualitative analysis

Some applications of GC-MS in qualitative analysis

Analysis of rosemary oil

Figure 37.21A shows the chromatogram obtained from the analysis of a sample of rosemary oil by GC-MS. The mass spectrum shown (B) is for the peak at 14.37 min. This spectrum was computer matched against the NIST library spectrum (details from www.hdscience.com) and gave two match figures of 932 and 941. Spectra are matched with the library by comparing the intensities of ions across the mass range scanned and a correlation coefficient is obtained. Any match of between 900 and 1000 is regarded as excellent. The higher second match figure in this case was obtained by matching the library spectrum to the unknown, omitting any peaks in the unknown that did not occur in the library spectrum, which is why the correlation number is slightly higher.

Library search matches are very useful for identifying unknown volatile materials; another area in which GC-MS is useful is in the identification of unknown residual solvents. Even though solvents are relatively simple molecules, there is quite a wide range of possibilities when an unknown is picked up, so library matching is very useful in such cases. In addition to solvents, many manufacturing intermediates have a relatively low molecular weight and are volatile and thus suitable for GC-MS analysis.

Impurity profiling

The Food and Drug Administration (FDA) now require that impurities of greater than 0.1% be identified in pharmaceuticals, in which MS with chromatography can assist. Such impurities can arise either from the manufacturing process or from degradation of the drug. Figure 37.22A shows a GC-MS trace for a commercial sample of the beta-blocker

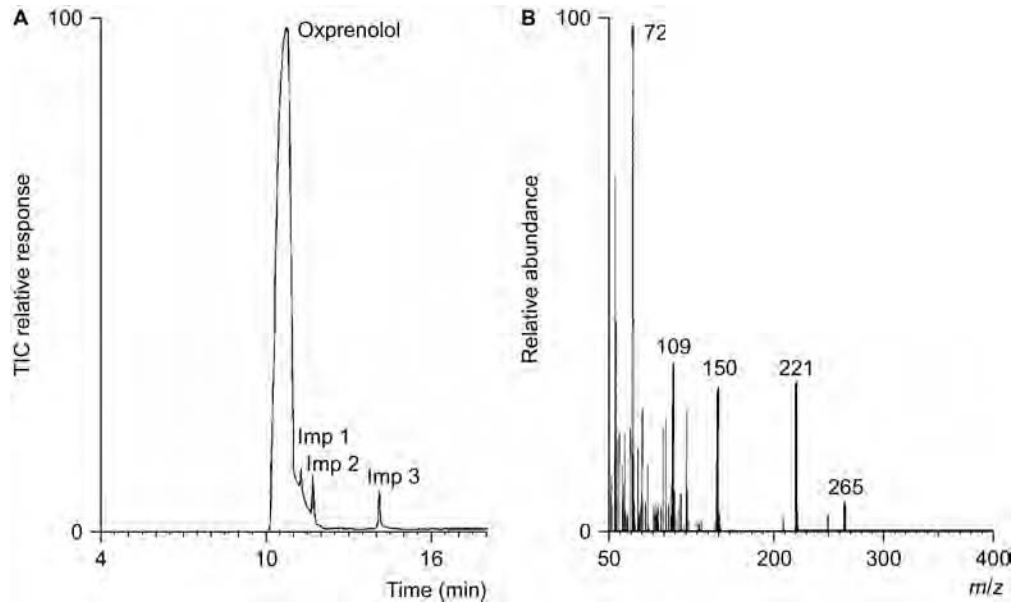

Figure 37.22 (A) GC-MS TIC trace of a commercial sample of oxprenolol and (B) the EI mass spectrum of oxprenolol.

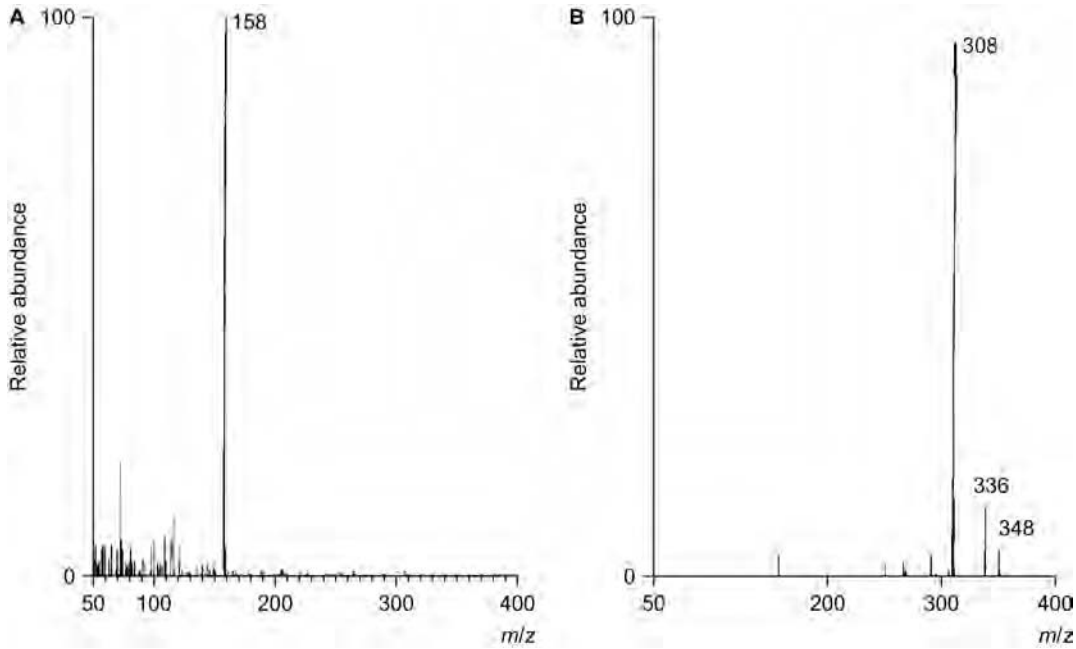

Figure 37.23 (A) EI and (B) PICI spectra of impurity 3.

oxprenolol. The compound contains three major impurities, one of which is resolved poorly from the very large peak obtained for the drug. The manufacture of beta-blockers is by fairly standard routes, which helps the identification of manufacturing impurities. The mass spectrum of oxprenolol is shown in Fig. 37.22B. Under EI conditions it gives a relatively weak molecular ion at *m/z* 265 and the spectrum is dominated by the fragment that arises from α-homolytic cleavage next to the amine in the side chain, which is typical of many amines (as in chloroquine).

Let us take, as an example, the impurity peak 3. The EI spectrum of this impurity (Fig. 37.23) shows only two major ions at *m/z* 158 and *m/z* 72. Its GC retention time is longer than that of oxprenolol and, on the non-polar GC column used, this suggests that the impurity has a higher molecular weight than oxprenolol. Under PICI conditions, the base peak of the mass spectrum of the impurity is an ion at *m/z* 308; the ion at 158 is still present, although much weaker. Taking into account the final step in the synthetic route to oxprenolol, the product possibly arises from the presence of a small amount of diisopropylamine in the isopropylamine used in the final step of the synthesis. Impurities 1 and 2 are isomers of oxprenolol; impurity 1 could arise via opening of the epoxide ring in the final step of the synthesis to form a primary alcohol, but both might also arise from isomers of the dihydroxybenzene present in the starting material for the synthesis.

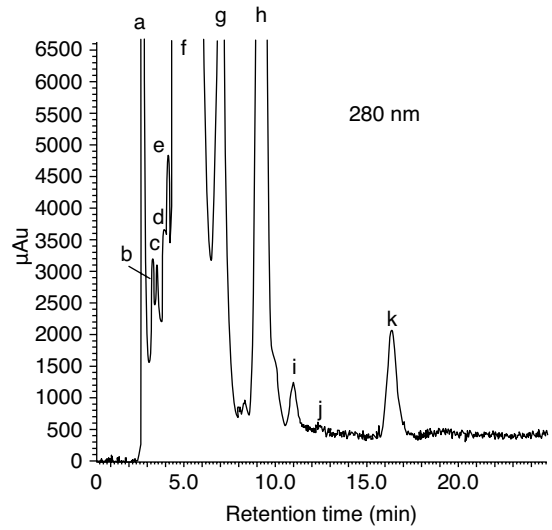

Figure 37.24 HPLC-UV chromatogram obtained from the analysis of poppy straw extract (1 g/L). Phenylhexyl column (150 × 4.6 mm); mobile phase 0.02 mol/L ammonium acetate–methanol (55 : 45), flow rate 0.4 ml/min.

Application of LC-MS in qualitative analysis

Determination of minor alkaloids in poppy straw extract

In the forensic field of analysis the most important goal in an analysis is first of all identification. The most powerful LC-MS technique available for identification is high-resolution FT-MS, whereby very precise elemental compositions can be assigned to unknown substances. Figure 37.24 shows a HPLC-UV chromatogram of morphine and co-occurring alkaloids in a poppy straw extract. Such minor congeners could be invaluable in determining the geographical origin of a sample of morphine. In the example shown the congeners are at relatively high levels (between 0.1 and 7% w/w) since the crude extract is less refined than purified morphine. However, the dynamic range of most LC-MS systems is at least 10^5 and very minor amounts of these congeners could be detected in a more refined sample. Table 37.4 shows the elemental compositions, with their deviations from the exact mass for the assigned composition, obtained on an LTQ Orbitrap, for morphine and its minor congeners and the probable identities where the elemental composition fits with known congeners. Figure 37.25 indicates the orthogonal selectivity of high-resolution mass spectrometry and compares the extracted ion traces for morphine and normorphine which occurs within the sample at ~0.08% w/w with respect to morphine itself. It can be seen that even a very low percentage of normorphine in the sample can be readily detected. Introducing patterns of congeners into chemometric software would enable forensic analysts to pinpoint geographical origin and identify factors such as time of harvesting. The presence of *N*-oxides could also give some indication of the age of the sample.

Identification of drug metabolites

Metabolism is an important component in the drug discovery and development process, and LC-MS has an important role in identifying drug metabolites (Oliveira, Watson 2000). The pathways of phase 1 and

Table 37.4 Elemental compositions determined for morphine and its congeners in poppy straw extract by LC-FT-MS with reference to Fig. 37.24

Peak	*Exact mass*	*Elemental composition*	*Deviation (ppm)*	*Identity*
a	300.15933	$C_{18}H_{22}NO_3$	−0.30	
b	302.13864	$C_{17}H_{20}NO_4$	0.17	Morphine *N*-oxide
c	194.11748	$C_{12}H_{16}NO_2$	−0.70	
d	340.11792	$C_{19}H_{18}NO_5$	−0.10	
e	316.15448	$C_{18}H_{22}NO_4$	0.46	Norreticuline
f	286.14342	$C_{17}H_{20}NO_3$	−1.43	Morphine
g	330.16968	$C_{19}H_{24}NO_4$	−0.92	Norlaudanine
h	300.15927	$C_{18}H_{22}NO_3$	−0.50	Codeine
i	344.18558	$C_{20}H_{26}NO_4$	0.20	Tetrahydropapaverine
j	354.13345	$C_{20}H_{20}NO_5$	−0.40	
k	312.15948	$C_{19}H_{22}NO_3$	0.20	Thebaine

phase 2 drug metabolism are well known, and it is possible to derive useful information even from a single quadrupole instrument by using extracted ion chromatograms to search for predicted metabolites. For example, formation of a monoglucuronide of a drug results in a shift of 176 amu from the molecular ion of the parent. However, the process of searching for metabolites is easier if tandem MS is available. The preferred mode of metabolite profiling in the preliminary analysis of metabolites is to use product-ion scanning. The predicted ion for a metabolite is selected by the first quadrupole and subjected to fragmentation in the collision cell and the fragments are analysed by the third quadrupole. This enables acquisition of clean metabolite spectra that are free from any interfering solvent background. If a quantitative analysis is required, selected-reaction monitoring may be carried out, in which a critical transition is monitored. For example, the transition produced by loss of a glucuronide moiety from a glucuronide metabolite might be monitored if it gives a very specific response for that particular metabolite. Constant neutral-loss scanning is especially useful for searching for a particular class of metabolite, since it can readily detect metabolites resulting from both phase 1 and phase 2 metabolism. For example, glucuronide metabolites for which the loss of the glucuronide moiety (−177 amu) is a major fragmentation pathway might be monitored. If the masses of the metabolites fall in a range (e.g. between 400 and 700 amu), the first quadrupole is set to scan between 400 and 700 amu and the second quadrupole to scan in the range 400–177 amu to 700–177 amu. In this way, any metabolites that are, for example, methylated or undergo additional hydroxylation followed by glucuronidation are picked out, as well as simple glucuronides.

Ion-trap instruments can also be used to good effect in drug-metabolism studies and have approximately ten times the sensitivity of triple-sector quadrupole instruments when used to examine full-scan spectra. This is advantageous in the first phase of metabolite identification when the metabolites are unknown. Another advantage of trap instruments is that fragmentation of selected ions can be carried out several times with all the ions, apart from the molecular ion of the metabolite of interest, being ejected from the trap before the next fragmentation. This process produces clean spectra for the metabolite.

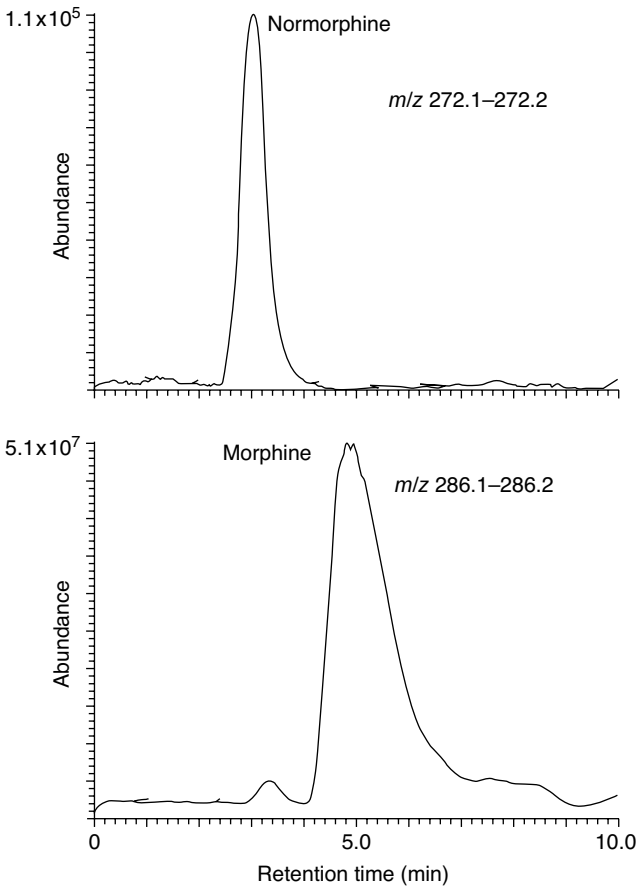

Figure 37.25 Extracted ion traces for morphine and normorphine in poppy straw extract.

Some applications of mass spectrometry in quantitative analysis

Mass spectrometric detectors are able to carry out precise and accurate quantification of analytes. However, it is generally necessary to use an internal standard in analyses, since the instrumentation is more subject to sensitivity fluctuations than simpler detectors, such as the UV-visible detectors used in HPLC analyses. The selection of an internal standard has to be made carefully so that its mass spectrometric behaviour is reproducible and closely similar to that of the analyte. The internal standards labelled with stable isotope (described below) are ideal, since they mimic the analyte very closely, but often a close structural analogue of the analyte will suffice.

The most common application of MS to quantitative analysis of biomedical samples is in the quantitative determination of drugs and their metabolites in biological fluids and tissues. The advantage of MS in this area is that its selectivity means that it is less subject to interference by other compounds extracted from the biological matrix along with the compound of interest. The greatest accuracy in such analyses is afforded by using as internal standards analogues of the compound being measured that are labelled with stable isotopes. An isotopomeric internal standard of a drug co-elutes with it from a chromatographic column (sometimes deuterated compounds elute very slightly earlier than the unlabelled compound) and should have an almost identical response factor. Figure 37.26 shows the NICI mass spectra of the trimethylsilyl oxime derivative of prednisolone and its tetradeuterated analogue. The deuterated analogue of prednisolone can be used as an internal standard in the determination of prednisolone in a biological matrix. On the basis of the mass spectra shown the ions at *m/z* 457 and 472 are monitored for prednisolone and those at *m/z* 461 and 476 for the tetradeuterated internal standard.

Since isotopomeric internal standards co-elute with the analyte, they aid in the recovery of the analyte from the chromatographic system (carrier effect). Figure 37.27 shows a selected-ion chromatogram of prednisolone methyl oxime/trimethylsilyl (MO/TMS) derivative (Knapp 1990) (monitored as the sum of the ions *m/z* 457 and 472, Fig. 37.26), which was extracted from aqueous humour after addition of 10 ng of tetradeuterated prednisolone (the MO/TMS derivative was monitored as the sum of the ions *m/z* 461 and 476, Fig. 37.26); the

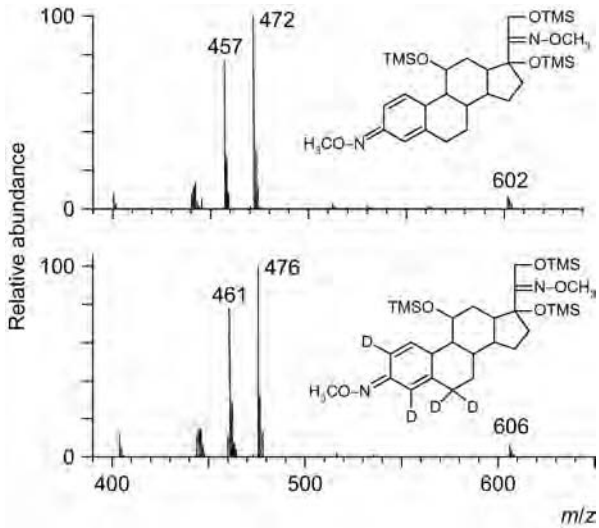

Figure 37.26 NICI spectra of trimethylsilyl prednisolone oxime and its tetradeuterated isotopomer.

analysis was carried out using GC-MS. Similar types of approaches can be taken in LC-MS analysis.

Collections of data

Electron-impact libraries

The most comprehensive collections of mass spectral data are based on EI mass spectra that have been acquired over many years and are used most commonly in conjunction with GC-MS analysis. The most popular libraries are the NIST library (details from www.hdscience.com), which contains the mass spectra of 130 000 compounds, and the Wiley Registry of Mass Spectral Data (www.wileyregistry.com), which contains 390 000 reference spectra. The mass spectrum of a molecule in the library may be called up by using either its CAS number, its name or its relative molecular mass. These libraries use peak-based matching to compare the mass spectrum of an unknown against the library spectrum. Spectra are matched in a manner analogous to the way two UV spectra are matched, except that in this case the *m/z* values and the intensity of the ions of the library spectrum and the unknown are matched. To simplify matching, a threshold can be set to eliminate, for example, ions of less than 1% intensity from the mass spectra being compared. A perfect match to a library spectrum has a value of 1000 and a value above 900 is regarded as a good match; a poor match has a value below 600. Other sources of EI mass spectra, such as the Eight Peak Index (Mass Spectrometry Data Centre 1991), and a collection of mass spectra of drugs and poisons (Pfleger *et al.* 2000) are available in book form, but the computer-based libraries offer a greater degree of convenience in most cases.

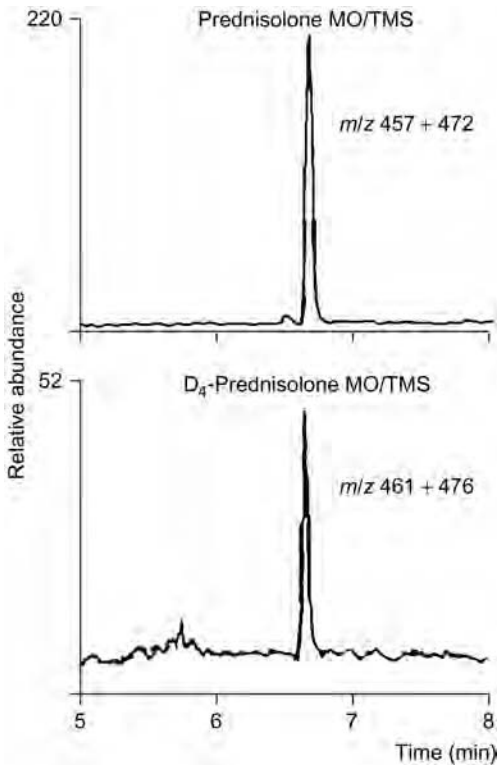

Figure 37.27 Prednisolone extracted from aqueous humour in comparison with D_4-prednisolone (10 ng) added as an internal standard (both as their trimethylsilyl oximes).

Libraries associated with LC-MS ionisation methods

Mass spectra obtained under the ionisation conditions used in LC-MS, such as electrospray, show little fragmentation, and the established libraries of EI spectra are of little use in searching such spectra. The use of tandem techniques increases the degree of fragmentation of molecules, but these do not reproduce exactly the EI spectrum of a molecule. Currently no comprehensive libraries have been developed for methods, such as ESI and APCI, used to ionise compounds after LC separation; generally, these types of library are user generated for a specific purpose.

There is one area in which comprehensive databases based on MALDI TOF or electrospray–ion-trap spectra have been built up, and this is in the field of proteomics (James 2000; Kinter, Sherman 2000). The databases built up from MALDI TOF data have been in operation longer than those based on ion-trap data. The standard approach used in conjunction with MALDI TOF is to separate proteins on gel electrophoresis, and thus obtain an approximate p*I* value (the pH of the isoelectric point of the protein), which can also be used in identification. The protein is cut from the gel, a proteolytic digest (most often using trypsin) is carried out and the peptide fragments generated are analysed using MALDI TOF. The pattern of peptides obtained can be matched against one of a number of databases by using a linking program, such as ProteinProspector (www.prospector.ucst.edu). The linking program searches one of the large protein-sequence databases, such as SwissProt, for the proteins that contain amino acid sequences most closely matching those of the unknown. Additional information can be obtained by varying the laser power during the ionisation step or by post-source decay using the reflector to produce additional fragmentation of the peptides in the digest, and this information can be used to further refine the database search. A similar process is used for the ion-trap instruments using ESI. In this case the peptide digest can be separated by HPLC prior to its introduction into the mass spectrometer. A program such as TurboSEQUEST can be used to search protein sequence databases and MS-MS spectra can be obtained from the peptides in the digest to refine the search.

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CHAPTER

38 Liquid Chromatography–Mass Spectrometry

HH Maurer

Introduction

In the 1980s, hyphenation of gas chromatography with mass spectrometry (GC-MS) had become the gold standard in all fields of analytical toxicology owing to its high identification and separation power combined with high sensitivity. It took more than 30 years to hyphenate liquid chromatography with mass spectrometry (LC-MS) for routine application. LC-MS techniques are indispensable for determination of thermolabile, polar and/or higher-molecular-weight compounds such as phase II metabolites, peptides or proteins. In contrast to GC, LC provides more flexibility in selection of stationary and mobile phases, resulting in better separation for particular analyte types. In the early 1990s, several groups started working with LC-MS in analytical toxicology. Some of them began with the transfer of existing LC–ultraviolet (LC-UV) or GC-MS procedures so that the scientific and/or practical progress was rather limited owing to problems with the new technique at that time such as rather poor spectral information (in a single-stage apparatus), the poor reproducibility of the ionisation and the susceptibility to matrix effects (ion suppression or enhancements).

In recent years, the apparatus has been improved and analysts have learned to more or less overcome the disadvantages and challenges of LC-MS analysis (Maurer 2006, 2007, 2009, 2010). For example, interfering matrix effects can often be avoided by suitable specimen clean-up, chromatographic changes, reagent modifications and effective internal standardisation. However, possible matrix effects must always be looked for during method development and validation. Furthermore, overlapping compounds such as isotope-labelled internal standards (Remane *et al.* 2010c) or analytes in multi-analyte procedures (Remane *et al.* 2010b) may lead to ion suppression or enhancement. After 15 years, single-stage or tandem LC-MS (LC-MS(-MS)) with electrospray ionisation (ESI) or atmospheric-pressure chemical ionisation (APCI) have now definitively left the development stage and are becoming increasingly standard techniques in routine toxicological analysis, especially for quantification of identified analytes (Maurer 2004, 2006, 2009, 2010). They have even opened the door to new fields of toxicological interpretation and expertise such as in the sensitive detection of chemical agents in hair in the case of drug-facilitated crimes (Kintz 2007) or the determination of chronic alcohol consumption by the analysis of ethanol conjugates in plasma, urine, or hair (see Chapter 19).

When searching for LC-MS(-MS) procedures relevant to clinical and forensic toxicology, case reports are often found with a more or less detailed description of more or less validated procedures. Here, the case information is often more useful than the analytical data. Furthermore, many toxicologists or pharmaceutical companies have published single-analyte procedures for a particular purpose, e.g. a pharmacokinetic study. However, in routine toxicological work, multi-analyte procedures for screening and/or quantification are the most useful because they allow analysis of several important compounds with a single injection, saving time and resources. Even if single analytes only have to be determined, as is often the case in clinical and forensic toxicology, such procedures provide a reliable quantification.

Sample preparation for LC-MS

At the beginning of the LC-MS era, it was thought that (extensive) sample preparation would no longer be necessary for bioanalysis by LC-MS, since LC would allow aqueous biofluids to be injected either directly or after simple deproteinisation. A series of papers describing matrix effects as a cause of invalid LC-MS analysis led to the conclusion that sample preparation was essential. With the increasing sensitivity and robustness of the apparatus, direct injection, e.g. of diluted urine for testing for drugs of abuse (Andersson *et al.* 2008) or for monitoring of psychotropic drugs in plasma after deproteinisation (Kirchherr, Kuhn-Velten 2006), was described. Such approaches enable a very high throughput of samples.

Nevertheless, for most of the currently used LC-MS procedures, a comprehensive preparative work-up of samples is needed to increase analytical sensitivity or to reduce matrix effects. All types of common sample preparation methods such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) or solid-phase microextraction (SPME) are used (Maurer 2007, 2009; Wille, Lambert 2007). Simple and fast LLE procedures are a good compromise between protein precipitation and laborious SPE and are used, for example, in multi-analyte quantification procedures (Wille, Lambert 2007; Maurer 2007, 2009; Remane *et al.* 2010a). Derivatisations that are often mandatory for GC-MS analyses are only needed in LC-MS if a particular functional group, e.g. in a metabolite, has to be confirmed. For example, Philipp and co-workers could distinguish between a carboxy and a phenol group in paynantheine metabolites by selective methylation (Philipp *et al.* 2010).

Separation systems for LC-MS

One major advantage of high performance liquid chromatography (HPLC) over GC was the flexibility in separation systems. All types of analyte (non-polar/polar, small/large molecular weight) can be separated using different stationary phases (e.g. C_{2-18} reversed phase, normal or chiral phases) and mobile phases with different buffers, solvents and modifiers in isocratic or gradient elution (for details see Chapter 41) For LC-MS using atmospheric-pressure ionisation techniques (e.g. ESI, APCI), only a limited selection of mobile phases, namely volatile phases, is suitable. In most cases, water, methanol, acetonitrile and the volatile buffers ammonium acetate or formate are used (Maurer 2007, 2009). With regard to stationary phases, a much better separation can be achieved using monolithic columns with porous channels rather than beads or so-called ultra-high pressure LC (UHPLC) systems using smaller particles and higher pressures. UHPLC is particularly advantageous for multi-analyte procedures with numerous analytes (e.g. Remane *et al.* 2010a).

Ionisation and detection modes for LC-MS

At the moment, the electrospray techniques ESI or APCI are most often used in routine applications. The principles of the ionisation and detection modes are described in Chapter 37. The question of whether ESI or APCI is more suitable for developing a new procedure cannot be answered in general terms and each must be tried for any application. APCI is more appropriate for apolar analytes. The sensitivity depends on the analyte structure and the apparatus type used and should always be evaluated. Dams and co-workers found that both ESI and APCI exhibited matrix effects, with ESI being much more susceptible than APCI (Dams *et al.* 2003). Remane and co-workers confirmed this result when studying the influence of co-eluting standards or analytes (Remane *et al.* 2010b, 2010c). Finally, it was shown that the accuracy and precision data of a plasma quantification method for toxic alkaloids were very similar when using LC-APCI-MS or LC-ESI-MS(-MS) (Beyer *et al.* 2007).

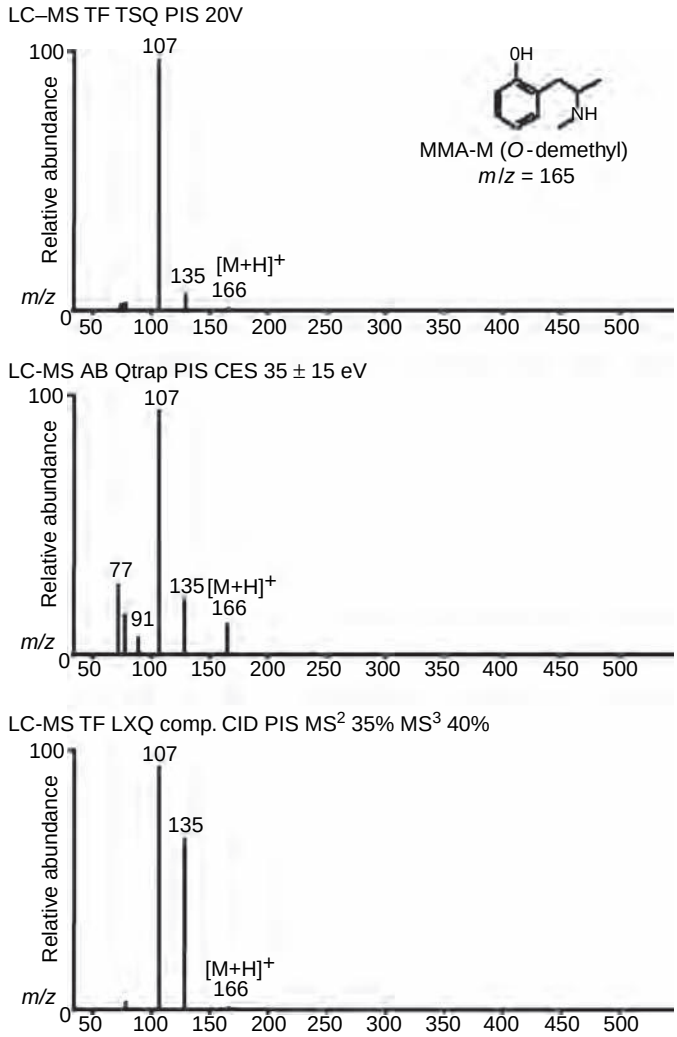

Figure 38.1 Product ion spectra of the *O*-demethyl metabolite of 2-methoxymetamfetamine (MMA) recorded using a QQQ (upper part), a QTrap (middle) and an LIT (lower part). Apparatus used: ThermoFisher (TF) TSQ; Applied Biosystems (AB) QTrap with given collision energy spread (CES); TF LXQ; composite spectrum (comp. CID) of 35% MS^2 plus 40% MS^3.

In contrast to the well-established ionisation techniques, the development of mass analysers such as the quadrupole (Q), ion trap (IT), or the high resolution (HR) time-of-flight (TOF) or Orbitrap (OT) analysers is still active. Besides single quadrupoles or TOF instruments, equipment is available that allows fragmentation to 2 (tandem MS) or *n* stages, such as triple quadrupoles (QQQ), TOFs (QTOF), IT or hybrids of quadrupoles with ITs (QTrap). They provide differing powers for quantification or for screening and identification. On the one hand, such variability is advantageous but, on the other, selection of the best apparatus for a particular application or even for all possible applications may be difficult if only one or two apparatus types can be purchased or are available. If the intention is to use LC-MS for library-based screening and identification, the major problem is the dependency of the reference spectra on the type of apparatus. In the beginning, the reproducibility of spectra was poor between different types of apparatus. Moreover, reproducibility was poor within the same type of apparatus owing to variations in the collision-induced dissociation that occurred in single-stage equipment. Only strict standardisation permitted the use of such libraries, which were mostly in-house. The reproducibility of product ion spectra in tandem MS is much better within the same apparatus family, but is still not sufficient between different analyser types (e.g. QQQ vs linear IT (LIT)).

The only ways of overcoming this limitation in future will be strict standardisation of the parameters (Hopley *et al.* 2008) and the use of sophisticated search algorithms (Mylonas *et al.* 2009). In principle, this should be possible because all fragmentation types break the molecules more or less at the same unstable bonds, but with different abundances. Figure 38.1 shows, for example, product ion spectra (PIS) of the *O*-demethyl metabolite of the doping agent 2-methoxymetamfetamine (MMA) recorded using a QQQ (ThermoFisher, TF, TSQ; upper part), a QTrap (Applied Biosystems, AB; collision energy spread, CES; middle) and an LIT (TF LXQ; composite spectrum of MS^2 and MS^3; lower part). As can be seen, the abundance varies, but most ions are present in all spectra. Similar effects are depicted in Figure 38.2 for the corresponding glucuronide. However, we are still far away from one spectral format for all types of LC-MS apparatus, as established 30 years ago for electron ionisation GC-MS spectra that can be used for Q, IT and TOF analysers coupled to GC (NIST/EPA/NIH 2008; Maurer, Peters 2006; Maurer *et al.* 2011) and are given in the monographs for individual drugs in this book.

The identification power of the different LC-MS screening and identification approaches should always be borne in mind because false-positive or false-negative results may lead to severe consequences. According to the European Union Commission Decision concerning the performance of analytical methods and the interpretation of results (European Communities 2002), identification points (IPs) are a useful criterion for assessing the identification power. Thus, single stage GC-MS or LC-MS provides a single IP per monitored ion, MS-MS methods provide 4 IPs for one precursor and two daughter ions and 5 points for two precursors and one daughter ion each, and MS-MS-MS provides 5.5

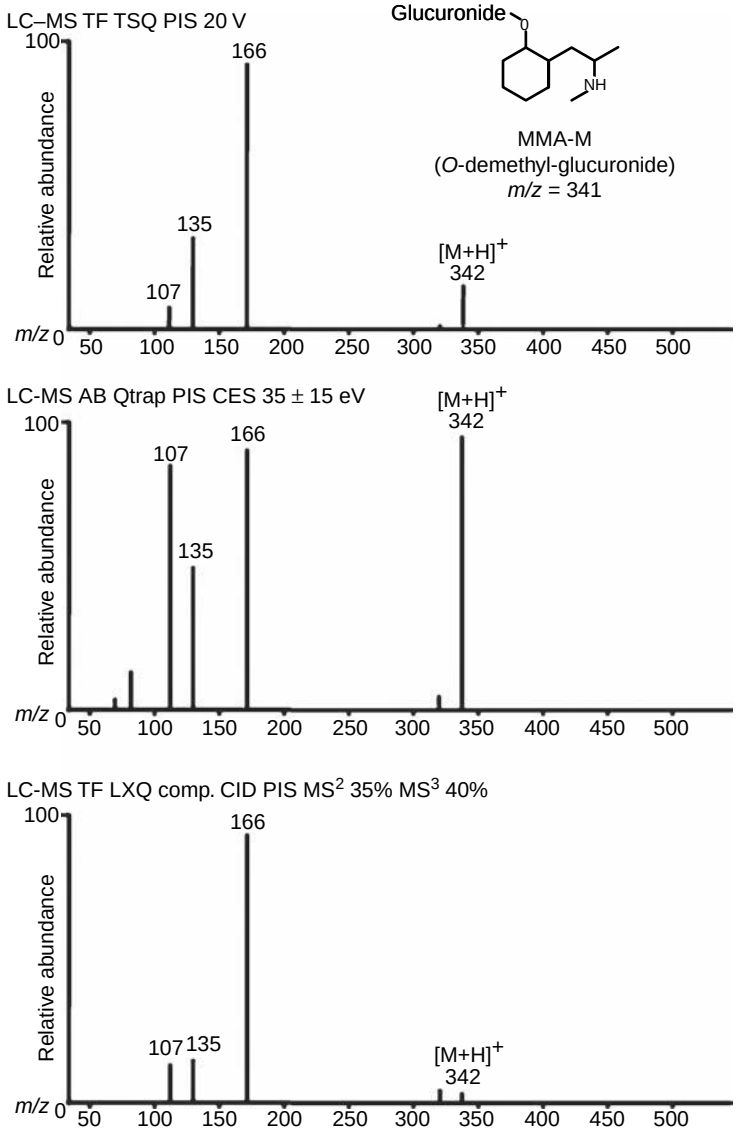

Figure 38.2 Production spectra of the glucuronide of *O*-demethyl 2-methoxymetamfetamine (MMA) recorded using a QQQ (upper part), a QTrap (middle) and an LIT (lower part). Apparatus: see Figure 38.1.

points for one precursor, one daughter and two granddaughter ions. High-resolution analysers provide double the points. Considering this recommendation, screening approaches using selected-ion monitoring (SIM) and multiple-reaction monitoring (MRM) modes are of less selectivity and are thus less reliable, as demonstrated, for example, by Allen (2006). Best identification power provides comparison of full scan spectra or full production ion spectra. The number of IPs depend on the number of ions per spectrum and on the spectral reproducibility.

Screening by LC-MS^n

Most screening procedures focus on multi-target screening rather than on systematic toxicological analysis (STA). This means that they allow a certain number of analytes to be screened for in the SIM or MRM modes, whereas analytes that are not included at the start cannot be detected. Further disadvantages, such as limited identification power, have already been discussed above. For STA, the best GC-MS strategies are based on full-scan MS, reconstructed mass chromatography and identification via library searches, while the best LC-MS strategies are based on tandem MS with a survey scan (MRM) and an enhanced product ion (EPI) scan in an information-dependent acquisition (IDA) mode and identification of the product ion spectra by library search using the authors' EPI spectra libraries (Sauvage *et al.* 2006; Dresen *et al.* 2009). LC-MS(-MS) screening procedures for drugs using a hybrid triple–quadrupole linear ion-trap mass spectrometer (QTrap), were described by the working groups of Marquet and of Weinmann. A survey scan (full scan or MRM) and an EPI scan were recorded in an IDA experiment followed by library-assisted identification using the authors' EPI spectra library. In contrast to reference libraries used for comprehensive GC-MS screening procedures (Maurer *et al.* 2011), not all currently available libraries for LC-MS screening are focused on metabolite spectra. Thus, their applicability for urine screening is limited because most toxicologically relevant compounds are excreted into urine more or less exclusively as phase I and/or II metabolites. Detection of (several) metabolites increases the selectivity, provides confirmation that the substance has passed through the body and, finally, minimises the risk of false-negative LC-MS results that might be caused by ion suppression of the target analyte. Even the risk of false-positive results can be reduced by considering the metabolite patterns. With this in mind the author's group developed a full-scan, metabolite-based screening procedure focused on urine analysis as a complement to other general unknown screening procedures (Wissenbach *et al.* 2010). Other screening procedures have recently been compared (Lynch *et al.* 2010) and reviewed (Maurer 2007, 2009; Peters 2010).

For screening of urine samples, several LC-MS strategies have been developed such as the use of classic MS-MS after extraction or direct

injection of samples or the use of accurate mass measurement with TOF mass analysers. A few years ago, Ojanpera and his colleagues started the development of urine screening procedures based on the enzymatic cleavage of conjugates and mixed-mode SPE; this used a TOF mass analyser based on measurement of the accurate mass and retention times and their comparison with reference data. They relied on a large target database of exact masses of reference drugs and their metabolites. Using liquid chromatography–Fourier transform mass spectrometry (LC-FTMS), they were able to confirm the findings with higher mass accuracy than that provided by the LC-TOF. Mass spectral identification was based on matching the measured accurate mass and isotopic pattern of a sample component with those in the database using newly developed software for automated reporting of findings in an easily interpretable form. However, it must be remembered that there are several drugs with the same empirical formula and molecular mass (e.g. morphine and hydromorphone) and that their metabolites (e.g. *N*- or *O*-demethyl metabolites) also have the same masses. This means that, in many cases, other procedures are needed for confirmation. This limitation can also be reduced by using collision-induced dissociation, which could result in some fragments that may have distinguishing isomers (Lee *et al.* 2009; Tyrkko *et al.* 2010).

A strategy for rapid and selective testing for drugs of abuse in urine as an alternative to immunoassay prescreening has been developed by the working group of Beck (e.g. Andersson *et al.* 2008). They reported multi-analyte screening methods for directly injected, diluted urine samples that were based on classic triple–quadrupole LC-MS(-MS) operated in the MRM mode. However, as such methods initially require a selection of precursor ions, the methods are limited to the target analytes. Positive screening results are considered as preliminary and are subjected to confirmation analysis. A major drawback of LC-MS procedures with direct injection of urine is the susceptibility to matrix effects. Meanwhile, corresponding studies have been performed that showed no serious matrix effects if the analytes were separated from the solvent front. Therefore, it can be concluded that this strategy may in future replace the common immunoassay prescreening approach for drugs of abuse in urine, at least in specialised laboratories with a high sample throughput where screening by LC-MS may be cost-effective.

Quantification by LC-MS^n

In analytical toxicology, quantification of compounds in urine is of less importance than in blood, because the concentrations may vary considerably depending on the hydration status of the body and the urinary pH. Moreover, compounds in urine have already been eliminated from the body, so their concentrations are of little relevance with respect to any toxic effects. Nevertheless, detection procedures are frequently developed as quantitative assays, particularly for confirmation of immunoassay results and/or for determinations that must meet designated cut-off values.

Numerous papers have been published over recent years describing the single- or multi-analyte quantification of therapeutic substances, drugs of abuse or pesticides in blood and other biosamples, mostly using QQQ in the MRM mode. Details of these developments can be found in recent review articles (Kraemer, Paul 2007; Maurer 2007, 2009; Peters 2010). The majority of these methods have targeted classic drugs of abuse and therapeutic drugs that have abuse potential or are relevant in the context of driving under the influence of drugs. Others have covered diuretics, β-blockers, stimulants, opioids, steroids, HIV medication, β-adrenergic drugs, calcium-channel antagonists, angiotensin II-receptor antagonists, antiarrhythmic drugs, muscle relaxants, antidepressants (including selective serotonin reuptake inhibitors), low-dose antipsychotics, organophosphorus pesticides and toxic plant alkaloids.

In the following section only general aspects of method development are discussed. Principles of method development and validation are described in detail in Chapter 20. An important point in early method development is the choice of appropriate internal standards (ISs). These can compensate for variability arising from sample preparation (e.g. because of differences between batches of SPE columns, chromatography, or even ion suppression/enhancement) and thus improve accuracy and precision data. As in any MS-based analytical method, a stable-isotope-labelled analogue of the analyte is an ideal IS. However, mutual ion suppression/enhancement of analytes and their stable-isotope-labelled IS has been reported. Even though this phenomenon does not affect quantification, at least as long as certain conditions are fulfilled, additional matrix effects may lead to a change of response ratios (analyte vs IS) and thus affect quantification negatively. More serious problems have to be expected when no stable-isotope-labelled analyte is available as IS, because, in such cases, ion-suppression effects on analytes and alternative IS might differ considerably. At this point it must be stressed that, if no stable-isotope-labelled IS of the analyte is available and an alternative IS must be selected, it is essential to avoid choosing a therapeutic drug for this purpose, not least in analytical toxicology. In this field, it is never possible to exclude that the patient or defendant to be monitored has taken the chosen drug. In such cases, the peak area of the IS would be overestimated, leading to underestimation of the actual analyte concentration. Therefore, if no deuterated analogue of a specific drug is available, the author recommends selecting a suitable IS from the pool of available deuterated compounds. Nowadays deuterated substances are available with a large variety of structures and with different physicochemical properties. This strategy has been used successfully in the author's laboratory for a number of GC-MS and LC-MS assays for various classes of drugs, all of which employed trimipramine-d_3 as IS. The effects of non-deuterated analytes on their respective deuterated analogues were studied with respect to concentration of the non-deuterated compounds and ionisation mode (Remane *et al.* 2010c). Again, APCI was less affected than ESI. While this may not be a problem when using the deuterated compound as IS for the respective non-deuterated analyte, it may cause serious quantification bias when the deuterated compound is also used as IS for compounds other than the non-deuterated analyte. Therefore, such ion suppression/enhancement studies are essential in such situations and the use of APCI is recommended unless there are other reasons against this, such as a large variability of ionisation.

Another important point in routine toxicological work concerns the quantification of analytes not in a large series of samples but in single cases, particularly in emergency hospital toxicology. The multi-analyte procedure concept enables the laboratory to have validated methods for a large variety of drugs. However, full calibration for a single analysis is very laborious, time-consuming and expensive. Therefore, one-point (linear through zero) calibration is often used as a compromise. Theoretically, this is applicable when the concentration–response function is linear and the y intercept is negligibly small. Considering its widespread use, surprisingly few studies have been dedicated to systematic evaluation of its applicability and performance in comparison with other calibration approaches. The most common approach is to measure the response of a single calibrator and then to calculate the concentrations of unknown samples via their responses in relation to that of the calibrator. Another strategy is very similar, but involves adjusting the absolute amount of analyte in the sample to closely match that in the calibrator by varying the amount of sample analysed. A third strategy involves transformation of exponential concentration–response functions to linear through zero functions allowing one-point calibration. To assess the feasibility of one-point calibration in multi-analyte procedures, Peters and Maurer (2007) used retrospective analysis to evaluate the validation data from six bioanalytical multi-analyte procedures. The results showed that the best one-point calibration results were obtained with the calibrator close to the centre of the full calibration range with which acceptance criteria for bias and precision were fulfilled for the majority of analytes. This study showed that, after full validation of a new approach, the one-point calibration performance should be tested statistically. If acceptable, one-point calibration can be a feasible alternative to full calibration.

Validation of LC-MS methods

Despite the maturity of the technique itself, individual LC-MS procedures must be validated before use to ensure their reliability and applicability for the intended purpose. This is of particular importance in

forensic and clinical toxicology and doping control, because reliable analytical data are a prerequisite for the correct interpretation of toxicological findings. Unreliable results might not only be contested in court but could also lead to unjustified legal consequences for the defendant or to inappropriate treatment of the patient. The importance of validation, at least for routine analytical methods, can never, therefore, be overestimated. This is especially true in the context of quality management and accreditation, which have become matters of increasing importance in analytical toxicology in recent years. At the very least the following parameters should be evaluated: selectivity, calibration model (linearity), stability, accuracy (bias), precision (repeatability, intermediate precision) and the lower limit of quantification. Additional parameters that may be relevant include limit of detection, recovery, reproducibility and ruggedness (robustness). Details can be found in Chapter 20.

Pitfalls and quality control

As mentioned previously, several pitfalls of the technique must be considered, such as matrix effects, analyte carryover via LC injection systems, poor reproducibility of spectra and limited selectivity in MRM approaches. Prevention strategies have been described for LC-tandem-MS using the selected reaction monitoring (SRM) mode (Sauvage *et al.* 2008). In this context it is important to recognise that even extensive matrix effects may be acceptable as long as they are reproducible between matrix batches and as long as the sensitivity of the method remains acceptable.

Besides suitable method validation, quality control procedures are essential in routine work. This is especially true in the context of quality management and accreditation, which have become matters of increasing relevance in analytical toxicology in recent years. A daily performance check is obligatory and should consist first of the analysis of a standard drug solution to check both the chromatographic and the MS components of the system. Figure 38.3 shows typical linear ion-trap mass analyser-merged mass chromatograms (for the chromatographic conditions see Wissenbach *et al.* 2010) with the given ions of a methanolic solution of a mixture of drugs with different retention times, polarities and molecular weights (morphine, nalorphine, codeine, strychnine, quinine, diphenhydramine, haloperidol, methaqualone and diazepam). For the system's performance to be acceptable, chromatographic separation should be obtained for compounds 3 and 4, and for compounds 8 and 9. Furthermore, symmetrical peak shapes should be obtained for at least compounds 7 and 9. Additionally, compound 1 should be clearly separated from the injection peak. This test mixture has proved suitable over the years in the author's laboratory for both GC-MS and LC-MS. Further checks are recommended by analysing blank samples between any analysis and control samples within all quantitative runs.

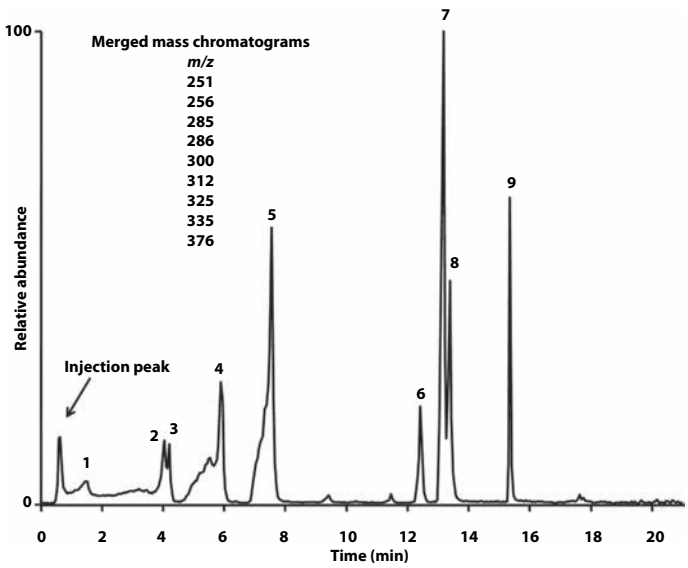

Figure 38.3 Typical linear ion-trap mass analyser-merged mass chromatograms with the given ions of a methanolic solution of drugs with different retention times, polarities and molecular weights: 1, morphine; 2, nalorphine; 3, codeine; 4, strychnine; 5, quinine; 6, diphenhydramine; 7, haloperidol; 8, methaqualone; 9, diazepam. (For chromatographic conditions see Wissenbach *et al.* (2010).)

Conclusions and perspectives

Liquid chromatography coupled with low- or high-resolution mass spectrometry is now an indispensable tool in analytical toxicology. It provides the high selectivity, sensitivity and universality needed for screening, identification and quantification of drugs, poisons and/or their metabolites in biological samples. LC-MS is a must when determining thermolabile (e.g. antiretroviral drugs (Koal *et al.* 2005)), polar (e.g. risperidone (Kratzsch *et al.* 2003)) or phase II metabolites (Ketola, Hakala 2010)) and/or higher-molecular-weight compounds such as peptides or proteins (e.g. insulins (Thomas *et al.* 2009)). Some limitations and pitfalls still exist and should always be considered when applying the technique, although it is likely that future developments will help to overcome them. While LC-MS(-MS) is the method of choice for target screening and quantification, low- and high-resolution linear ion traps are the most powerful technique for more comprehensive screening. High-resolution TOF analysers can also be useful if supplementary fragmentation increases the identification power. Finally, matrix-assisted laser desorption ionisation (MALDI, see Chapter 37) looks promising for increasing sample throughput in the routine analysis of small molecules such as most drugs or in postmortem toxicology by using direct analysis of tissues.

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CHAPTER

39 Thin-layer Chromatography

CF Poole

Thin-layer chromatography (TLC) is a widely used technique for the separation and identification of drugs. It is equally applicable to drugs in their pure state, to those extracted from pharmaceutical formulations, to illicitly manufactured materials, and to biological samples. TLC as we know it today was established in the 1950s with the introduction of standardised procedures that led to improved separation performance and reproducibility, and paved the way for its commercialisation and an increase in the number of published applications. The 1970s saw the introduction of fine-particle layers and associated instrumentation required for their correct use. In this form, TLC became known as high performance TLC, instrumental TLC or modern TLC to distinguish it from its parent, now generally referred to as conventional TLC. High performance TLC has not displaced conventional TLC from laboratory studies and the two approaches coexist today because of their complementary features (Table 39.1). Conventional TLC provides a quick, inexpensive and portable method for qualitative analysis. It requires minimal and readily available instrumentation and uses easily learned experimental techniques. High performance TLC is characterised by the use of kinetically optimised layers for faster and more efficient separations, takes advantage of a wider range of sorbent chemistries to optimise selectivity and requires the use of instrumentation for convenient (automated) sample application, development and detection. High performance TLC provides accurate and precise quantitative results based on *in situ* measurements and a record of the separation in the form of a chromatogram, such as the example in Fig. 39.1. While all modern laboratories are capable of drug analysis by conventional TLC, only those laboratories equipped with the necessary instrumentation for high performance TLC have that option.

In the basic TLC experiment, the sample is applied to the layer as a spot or band near the bottom edge of the layer. The separation is carried out in a closed chamber either by contacting the bottom edge of the layer with the mobile phase, which advances through the layer by capillary forces, or by the mobile phase being forced to move through the layer at a controlled velocity by an external pressure source or centrifugal force. A separation of the sample results from the different rates of migration of the sample components in the direction travelled by the mobile phase. After development and evaporation of the mobile phase, the sample components are separated in space, their position and quantity being determined by visual evaluation or *in situ* scanning densitometry aided by the formation of easily detected derivatives by post-chromatographic chemical reactions, as required.

Separations by column liquid chromatography (HPLC) and TLC occur by essentially the same physical process. The two techniques are frequently considered as competitors, when it would be more realistic to consider them as complementary. The attributes of TLC that support its coexistence as a complementary technique to HPLC are summarised in Table 39.2. On the basis of these attributes, TLC methods are most effective for the low-cost analysis of a large number of samples (e.g. drug screening in biological fluids and tissues, determination of the botanical origin and potency of traditional herbal medicines, stability testing and content uniformity testing), for the rapid analysis of samples that require minimal sample clean-up, or where TLC allows a reduction in the number of sample preparation steps (e.g. analysis of samples containing components that remain sorbed to the stationary phase or contain suspended microparticles). TLC is also preferred for the analysis of substances with poor detection characteristics that require post-chromatographic chemical treatment for detection. In other cases, HPLC methods are generally preferred, particularly if a large number of theoretical plates are necessary for a separation, for separations by size-exclusion and ion-exchange chromatography, and for trace analysis using selective detectors unavailable for TLC.

Stationary phases

Conventional TLC plates can be prepared in the laboratory by standardised methods, but reproducible layer preparation is easier to achieve in a manufacturing setting and few laboratories prepare their own plates today. Precoated plates for high performance, conventional and preparative TLC are available in a range of sizes and different layer thickness, supported on glass, aluminium or plastic backing sheets. To impart the desired mechanical stability and abrasion resistance to the layer a binder, such as poly(vinyl alcohol), poly(vinyl pyrrolidone), gypsum or starch in amounts from 0.1% to 10% (w/w) is incorporated into the layer. An ultraviolet (UV) indicator, such as manganese-activated zinc silicate of a similar particle size to the sorbent, may be added to the layer to visualise separated samples by fluorescence quenching. TLC plates with a narrow preadsorbent zone located along one edge of the layer are available to aid manual sample application.

Silica gel is the most important stationary phase for TLC, with other inorganic oxide adsorbents, such as alumina, kieselguhr (a silica gel of low surface area) and Florisil (a synthetic magnesium silicate), of minor importance. Most silica gel sorbents have an average pore size of 6 nm and are designed for the separation of small molecules (relative molecular mass <700). The chromatographic properties of the inorganic oxide adsorbents depend on their surface chemistry and specific surface area. For silica gel, silanol groups are the dominant adsorption sites. The complementary sample properties that govern retention are the number and type of functional groups and their spatial location (Fig. 39.2). The influence of functional group properties on selectivity is illustrated in Fig. 39.1 for the separation of ethynyl steroids. The steroids with phenolic groups are the most strongly retained, followed by hydroxyl groups, and then ketone and ester groups. Subtle separation differences through steric hindrance at a functional group and differences in ring conformations are also seen, which allow the separation of steroids with very similar chemical properties.

Chemically bonded layers are prepared from silica gel by reaction with various organosilane reagents to form siloxane bonds, with some of the silanol groups present on the silica surface (Table 39.3). Reversed-phase alkylsiloxane-bonded layers with a high level of surface bonding cannot be used with mobile phases that contain a significant amount of water ($>30\%$ v/v) because of the inadequate mobile phase velocity generated by capillary forces. Water compatibility for alkylsiloxane-bonded layers is achieved by increasing the particle size, using a reproducible although lower degree of silanisation, and by using modified binders. These layers are referred to as water wettable and are used for all types of reversed-phase separations, while layers with a high degree of silanisation are used predominantly with non-aqueous mobile phases. Alkylsiloxane-bonded phases are used primarily (but not exclusively) for the separation of water-soluble polar drugs and weak acids and bases after ion suppression (buffered mobile phase) or ion-pair formation. Water compatibility is not a problem for polar chemically bonded phases, which can be used for both normal and reversed-phase

Table 39.1 Characteristic properties of silica gel precoated TLC layers

Parameter	*High performance TLC*	*TLC*
Plate dimensions (cm^2)	10×10	20×20
Layer thickness (mm)	0.1 or 0.2	0.1–0.25
Starting spot diameter (mm)	1–2	3–6
Diameter of separated spots (mm)	2–6	6–15
Solvent front migration distance (cm)	3–6	10–15
Time for development (capillary flow) (min)	3–20	20–200
Detection limits[a]		
Absorption (ng)	0.1–0.5	1–5
Fluorescence (pg)	5–10	50–100
Nominal particle size range (μm)	3–7	5–20
Apparent particle size (μm)[b]	5–7	8–10
Minimum plate height (μm)	22–25	35–45
Optimum velocity (mm/s)	0.3–0.5	0.2–0.5
Porosity		
Total	0.65–0.70	0.65–0.75
Inter-particle	0.35–0.45	0.35–0.45
Intra-particle	0.28	0.28

[a]For drugs with favourable detection properties.
[b]Determined by chromatographic measurements. Precoated TLC layers are prepared from silica gel with a narrower particle size range than typical bulk materials available for self-made layers.

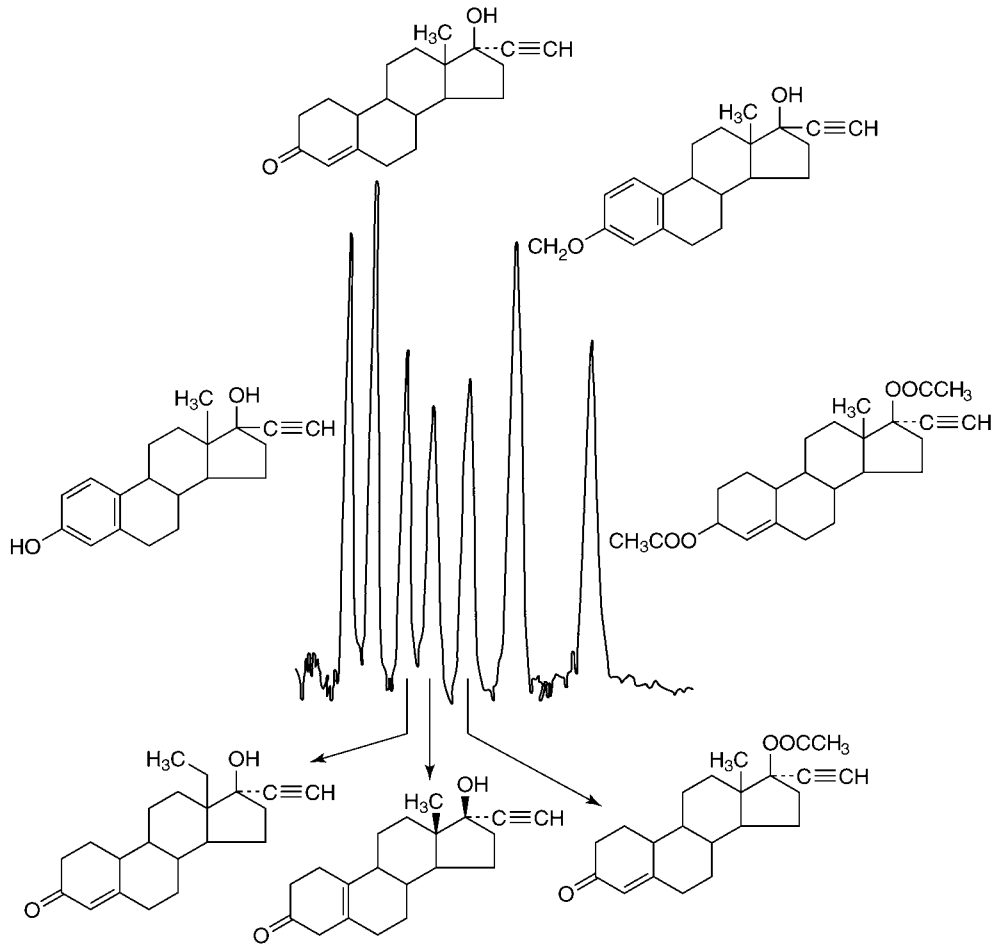

Figure 39.1 Separation of ethynyl steroids (birth-control pill components) by high performance TLC. Two 15 min developments with the mobile phase hexane–chloroform–carbon tetrachloride–ethanol (7 : 18 : 22 : 1) on a silica gel 60 high performance TLC plate. The chromatogram was recorded by scanning densitometry at 220 nm.

Table 39.2 Attributes of TLC providing the link to contemporary applications in drug analysis

Attribute	*Application*
Separation of samples in parallel	Low-cost analysis and high-throughput screening of samples requiring minimal sample preparation
Disposable stationary phase	Analysis of crude samples (minimising sample preparation requirements)
	Analysis of a single or small number of samples when their composition and/or matrix properties are unknown
	Analysis of samples containing components that remain sorbed to the separation medium or contain suspended microparticles
Static detection	Samples that require post-chromatographic treatment for detection
	Samples that require sequential detection techniques (free of time constraints) for identification or confirmation
Storage device	Separations can be archived
	Separations can be evaluated in different locations or at different times
	Convenient fraction collection for coupled column–layer chromatography
Sample integrity	Total sample occupies the chromatogram, not just that portion of the sample that elutes from the column

separations. For separations that cannot be achieved on silica gel, the polar chemically bonded phases are the most widely used stationary phases. The 3-aminopropylsiloxane-bonded layers can function as a weak anion exchanger for the separation of polyanions with a buffered mobile phase. Cellulose layers provide only weak retention of common drug substances and are used primarily to separate very polar compounds in biochemistry.

TLC has found limited use for the separation of enantiomers. The most widely used approach employs ligand-exchange chromatography on reversed-phase layers impregnated with a solution of copper acetate and (2*S*,4*R*,2*RS*)-*N*-(2-hydroxydodecyl)-4-hydroxyproline. Separations result from stability differences in diastereomeric complexes formed between the drug, copper and the proline selector. Suitable drugs for this application require an amino acid or α-hydroxycarboxylic acid group for complex formation. A more versatile approach to the separation of enantiomeric drug substances by reversed-phase TLC involves the use of chiral selectors, such as cyclodextrins or bovine serum albumin, as mobile phase additives.

Technique

The technique of TLC involves a number of separate steps: preparing the layer; applying the sample; developing the plate; and detecting the separated zones. These steps are described below.

Layer pretreatments

Prior to chromatography it is common practice to prepare the layers for use by any or all of the following steps: washing, conditioning and equilibration. Layers may also be cut to preferred sizes using scissors for plastic- or aluminium-backed plates and diamond or carbide glass-cutting tools for glass-backed plates. Newly consigned precoated layers are invariably contaminated, or quickly become so, because of residual contaminants from the manufacturing process, contact with packaging materials and adsorption of materials from the atmosphere. To remove contaminants, single or double immersion in a polar solvent, such as methanol or propan-2-ol, for about 5 minutes is generally superior to predevelopment with the mobile phase. For trace analysis, sequential immersion and predevelopment may be required to obtain the best results.

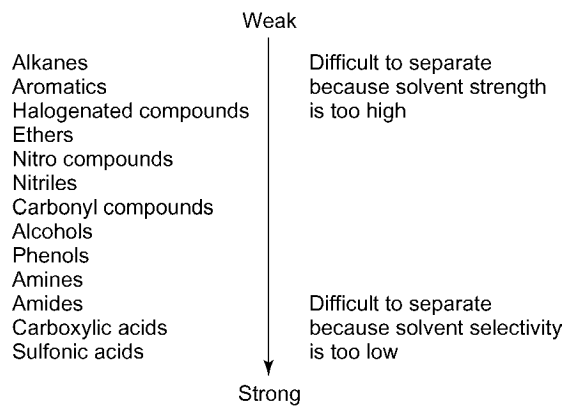

Figure 39.2 General adsorption scale for separations by silica gel TLC.

For inorganic oxide adsorbents the absolute R_f (see later) value and the reproducibility of R_f values depend on the layer activity. The latter is controlled by the adsorption of reagents, most notably water, through the gas phase. Physically adsorbed water can be removed from silica gel layers by heating at about 120°C for 30 min. Afterwards, the plates are stored in a grease-free desiccator over blue silica gel. Heat activation is not normally required for chemically bonded layers. Equilibration of activated layers by exposure to the atmosphere is extremely rapid and the step of layer activation is at times unnecessary. In modern air-conditioned laboratories, layers achieve a consistent level of activity that should provide sufficient reproducibility for most separations. Inorganic oxide layers can be adjusted to a defined activity by exposure to a defined gas phase in an enclosed chamber. Since manipulation in the atmosphere almost certainly readjusts this activity, it is best performed after application of the sample zones in a developing chamber that allows both layer conditioning and development in the same chamber (e.g. a twin-trough or automated development chamber). Atmospheres of different constant relative humidity can be obtained by using solutions of concentrated sulfuric acid or saturated solutions of various salts. Acid or base deactivation can be carried out in a similar manner by exposure to, for example, ammonia or hydrochloric acid fumes.

Sample application

Drugs are applied to TLC plates as spots or bands of minimum size with a homogeneous distribution of material within the starting zone. For high performance layers, with desirable starting spot diameters of about 1.0–2.0 mm, this corresponds to a sample volume of 100–200 nL if applied by a dosimeter (micropipette) or several microlitres if applied by a spray-on device. For conventional TLC plates, sample volumes 5- to 10-fold greater are acceptable. Desirable properties of the sample solution are summarised in Table 39.4. If scanning densitometry is used for detection, manual sample application with hand-held devices is inadequate. For densitometry, the starting position of each spot must be known accurately, which is achieved easily with mechanical devices that operate to a precise grid mechanism. Also, the sample must be applied to the layer without disturbing the surface, something that is nearly impossible to achieve using manual application.

Sample application devices for TLC encompass a wide range of sophistication and automation. The most popular devices for quantitative TLC use the spray-on technique. A controlled nitrogen-atomiser sprays the sample from a syringe or capillary, to form narrow, homogeneous bands on the plate surface. The plate is moved back and forth under the atomiser on a translational stage to apply bands of any length

Table 39.3 Retention properties of silica-based chemically bonded layers

Type of modification	*Functional group*	*Application*[a]
Alkylsiloxane	Si–CH_3	For reversed-phase separations generally, but not exclusively
	Si–C_2H_5	Separation of water-soluble polar organic compounds (RPC)
	Si–C_8H_{17}	Weak acids and bases after ion suppression (RPC)
	Si–$C_{18}H_{37}$	Strong acids and bases by ion-pair mechanism (RPC)
Phenylsiloxane	Si–C_6H_5	Of limited use for drug analysis
Cyanopropylsiloxane	Si–$(CH_2)_3CN$	Useful for both RPC and NPC
		In NPC it exhibits properties similar to a low-capacity silica gel
		In RPC it exhibits properties similar to short-chain alkylsiloxane-bonded layers (it has no selectivity for dipole-type interactions)
Aminopropylsiloxane	Si–$(CH_2)_3NH_2$	Used mainly in NPC and IEC; limited retention in RPC
		Selectively retains compounds by hydrogen-bond interactions in NPC; separation order generally different to that in silica gel
		Functions as a weak anion exchanger in acidic mobile phases (IEC)
Spacer-bonded propanediol	Si–$(CH_2)_3OCH_2$ $CH(OH)CH_2OH$	Used in NPC and RPC, but more useful for NPC because of low retention in RPC
		Polar drugs selectively retained by hydrogen bond and dipole-type interactions in NPC; more hydrogen-bond acidic and less hydrogen-bond basic than aminopropylsiloxane-bonded layers in NPC; more retentive than aminopropylsiloxane-bonded layers in RPC
		Similar retention to short-chain alkylsiloxane-bonded layers, but different selectivity for hydrogen-bonding drugs

[a]IEC, ion-exchange chromatography; NPC, normal-phase chromatography; RPC, reversed-phase chromatography.

between zero (spots) and the maximum transit length of the spray head. For analytical applications, bands are typically 0.5 or 1.0 cm in length. For preparative-scale separations, the band is usually applied for almost the complete length of the plate, leaving a 1 cm section at each edge unused. The rate of sample deposition is also adjustable to accommodate sample solutions of different volatility and viscosity. An advantage of spray-on devices is that different volumes of a single standard solution can be applied for calibration purposes and the standard addition method of quantification is carried out easily by overspraying the sample already applied to the layer with a solution of the standard. Fully automated sample applicators can be programmed to select samples from a rack of vials and deposit fixed volumes of the sample, at a controlled rate, to selected positions on the plate. The applicator automatically rinses itself between sample applications and can spot or band a whole plate with different samples and standards without operator intervention.

Glass microcapillaries for conventional TLC and fixed-volume dosimeters (which consist of a 100 or 200 nL platinum–iridium capillary sealed into a glass support capillary) for high performance TLC are also commonly used for sample application and require less sophisticated instrumentation. The capillary tip is brought into contact with the plate surface using a mechanical device to discharge its volume. A click-stop grid mechanism is used to provide an even spacing of the samples on the layer and a frame of reference for sample location during scanning densitometry.

Layers with a preadsorbent zone (a narrow zone prepared from a silica gel of low surface area with weak retention) simplify some aspects of sample application. This allows relatively large sample volumes or dirty samples to be applied to the preadsorption zone and their reconcentration to a narrow band at the interface between the preadsorbent and separation zones by a short development prior to chromatography. However, since the distribution of the sample may not be even

Table 39.4 Solution requirements for sample application

Property	*Requirements*
Sample solvent	Good solvent for the sample to promote quantitative transfer from the sample application device to the layer
	Low viscosity and sufficiently volatile to be easily evaporated from the layer (dilute viscous samples if possible with a volatile solvent of low viscosity)
	Wet the sorbent layer to provide adequate penetration of the layer by the sample (a potential problem for alkylsiloxane-bonded layers and aqueous sample solutions)
	Weak chromatographic solvent to minimise predevelopment during sample application (ideally, if used as a chromatographic solvent the least-retained sample component should have $R_f < 0.1$)
Aqueous solutions	Dilute if possible with a water-miscible solvent that forms a lower-boiling-point azeotrope
	Apply in small increments or, if spray-on techniques are used, with a slow application rate
	Use layers with a preadsorption zone and refocus the sample prior to development
Suspensions	Filter before attempting sample application
	Otherwise use layers with a preadsorption zone and an extraction solvent that mobilises the components of interest for refocusing

within the band, the quantitative accuracy of densitometric measurements may be lowered using this approach.

Development

The principal development techniques in TLC are linear, circular and anticircular, with the velocity of the mobile phase controlled by capillary forces or forced-flow conditions. In any of these modes continuous or multiple development can be used to extend the application range. Radial development is used rarely for drug analysis and is not considered further. Forced-flow development is not widely used in analytical laboratories and uses sophisticated equipment only recently available as a user-friendly system.

For linear (or normal) development, samples are applied along one edge of the plate and the separation developed for a fixed distance in the direction of the opposite edge. Viewed in the direction of development, the chromatogram consists of a series of compact symmetrical spots of increasing diameter or, if samples are applied as bands, in rectangular zones of increasing width.

In continuous development the mobile phase is allowed to traverse the layer under the influence of capillary forces until it reaches some predetermined position on the plate, at which point it is evaporated continuously. Evaporation of the mobile phase usually occurs at the plate atmospheric boundary using either natural or forced evaporation. Continuous development is used primarily to separate simple mixtures with a short development length and a weaker (more selective solvent) than employed for normal development.

In unidimensional multiple development, the TLC plate is developed for some selected distance, then either the layer or the mobile phase is withdrawn from the developing chamber, and adsorbed solvent evaporated from the layer before repeating the development process. The principal methods of unidimensional multiple development are summarised in Table 39.5. Multiple development provides a very versatile strategy for separating complex mixtures, since the primary experimental variables of development distance and composition of the mobile phase can be changed at any development step, and the number of steps varied to obtain the desired separation. Multiple development provides a higher resolution of complex mixtures than does normal or continuous development, can easily handle samples of a wide polarity range (stepwise gradient development) and, because the separated zones are usually more compact, leads to lower detection limits. Equipment for automated multiple development is commercially available.

For drug mixtures that span a wide retention range, some form of gradient development is required to separate all the components either in a single chromatogram or in separate chromatograms for successive developments. Continuous solvent-composition gradients, as commonly employed in HPLC, are used only rarely in TLC. These require experimental conditions that are less convenient than those for step mobile phase gradients. In addition, step gradients can be constructed easily to mimic a continuous linear gradient, with the added advantage that the zone-refocusing effect can be employed to minimise zone broadening. Gradients of increasing solvent strength are used to fractionate complex mixtures by separating just a few components in each step. Individual drugs are usually identified and quantified at the intermediate steps at which the drugs of interest are separated. In this way, the zone capacity can be made much larger than predicted for a complete separation recorded as a single chromatogram. However, this approach is tedious when many components are of interest and it is difficult to automate. Alternatively, if incremental multiple development is used, the sample can be separated for the shortest distance in the strongest mobile phase, with each subsequent, longer development using mobile phases of decreasing solvent strength. This strategy is most useful when the final separation is to be recorded as a single chromatogram, but it is limited in zone capacity because all the components must be located between the sample origin and the final solvent front. The two approaches for exploiting solvent-strength gradients are thus complementary and selection is made based on the properties of the sample. The decreasing solvent-strength gradient approach is the operating basis of automated multiple-development chambers.

In two-dimensional TLC the sample is spotted at the corner of the layer and developed along one edge of the plate. The solvent is then evaporated, and the plate is rotated through 90° and redeveloped in the orthogonal direction. If the same solvent is used for both developments, the sample is redistributed along a line from the corner at which the plate was spotted to the corner diagonally opposite. In this case, only a small increase in resolution can be anticipated. The realisation of a more

Table 39.5 Multiple development techniques

Method	*Features*
Multiple chromatography	Fixed development length
	Same mobile phase for each development
	The number of developments can be varied
	Drugs that are difficult to separate should be repeatedly developed with solvents that produce low R_f values corresponding to the most selective mobile phase for the separation
	The maximum zone centre separation for two drugs of similar migration properties occurs when the zones have migrated 0.63 of the solvent front migration distance
Incremental multiple development	Variable development length: (a) first development is the shortest (b) each subsequent development is increased by a fixed distance (c) last development length corresponds to the maximum useful development distance
	Same mobile phase for each development
	The number of developments can be varied
	Provides better separations than multiple chromatography
Increasing solvent-strength gradients	Uses incremental multiple development
	Fractionates sample into manageable subsets
	Optimises separation of each subset
	Complete separation of all components is not achieved at any segment in the development sequence
Decreasing solvent-strength gradients	Uses incremental multiple development
	First development employs the strongest solvent with a weaker solvent for each subsequent step
	Final separation recorded as a single chromatogram

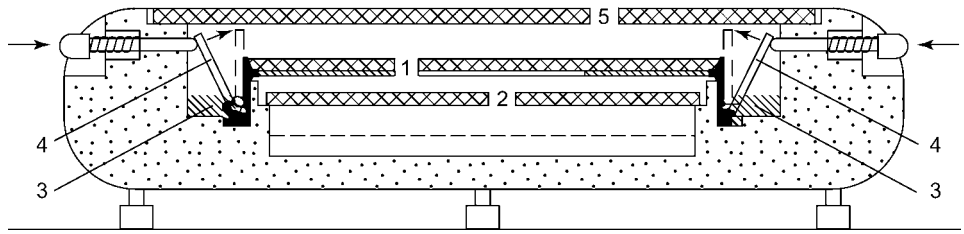

Figure 39.3 Illustration of a horizontal developing chamber (1, TLC plate; 2, removable counter plate to convert the chamber from normal to sandwich configuration; 3, mobile phase reservoirs; 4, glass slide to direct mobile phase flow; 5, glass cover plate). Raising the glass slide (4) to contact the edge of the layer (1) causes the mobile phase to flow through the layer from the solvent reservoir (3). If both glass slides are raised simultaneously, development occurs from both sides to the middle, which allows the simultaneous development of samples applied along the two edges of the layer.

efficient separation system implies that the resolved sample should be distributed over the entire plate surface. This can be achieved only if the selectivity of the separation mechanism is complementary in the orthogonal directions. Using two solvent systems with complementary selectivity is the simplest approach to implement in practice, but it is often only partially successful. In many cases the two solvent systems differ only in their intensity for a given set of properties and are not truly orthogonal. Chemically bonded layers can be used in the reversed-phase and normal-phase modes, and they enable the use of additives and buffers as a further way to adjust selectivity. Two-dimensional TLC remains a largely qualitative method, but advances in imaging analysis techniques permit quantitative evaluation of chromatograms with some restrictions.

Development chambers

The development process in TLC can be carried out in a variety of vessels that differ significantly in design and sophistication. For convenience these are often categorised under the headings of normal (N-chamber) and sandwich (S-chamber), and further subdivided according to whether the internal atmosphere is saturated (N_S or S_S) or unsaturated (N_U or S_U). Sandwich chambers have a depth of gas phase in front of the layer of less than 3 mm, with other chamber designs indicated as normal chambers. Saturation of the vapour phase is achieved by using solvent-saturated pads or filter papers as a chamber lining.

The twin-trough chamber is the most popular of the simplest TLC developing chambers. It consists of a standard rectangular developing tank with a raised, wedge-shaped bottom. The wedged bottom divides the tank into two compartments, so that it is possible either to develop two plates simultaneously or to use one compartment to condition the layer prior to development. The horizontal developing chamber (Fig. 39.3) can be used in either the normal or the sandwich configuration for either conventional edge-to-edge or simultaneous edge-to-centre development. Starting the development simultaneously from opposite edges allows the number of samples separated to be doubled in the same time. The sandwich configuration of the horizontal developing chamber is not suitable for mobile phases that contain volatile acids or bases or large amounts of volatile polar solvents, such as methanol or acetonitrile, because of the restricted access of the saturated vapour phase to the dry portion of the separation layer.

The automated developing chamber increases laboratory productivity and improves the reproducibility of separations by providing precise control of layer conditioning, mobile phase composition, solvent-front migration distance and drying conditions. This chamber can be used in the normal or sandwich configuration with all the operational features preselected on a microprocessor-based control unit and monitored by sensor technology.

The automated multiple-development chamber (Fig. 39.4) provides the necessary conditions and control for automated separations by incremental multiple development with a decreasing solvent-strength gradient. The operating parameters of layer conditioning, solvent-front migration distance, mobile phase composition and drying time for each development, and the total number of developments for the separation, are entered into the computer-based control unit. The complete separation sequence is carried out without further intervention. Each development is typically 3–5 mm longer than the previous one and, depending on the complexity of the desired mobile phase gradient, a total of 10–30 developments is used, which requires 1.5–4.5 hours for completion.

Detection

About 1–10 μg of coloured substances with a quantitative reproducibility rarely better than 10–30% can be detected by visual inspection of a TLC plate. This may be adequate for qualitative methods, but for reliable quantification *in situ* spectrophotometric methods are preferred, as their use is more accurate and far less tedious and time-consuming than excising zones from the layer for determination by conventional solution spectrophotometry. The fluorescence-quenching technique enables

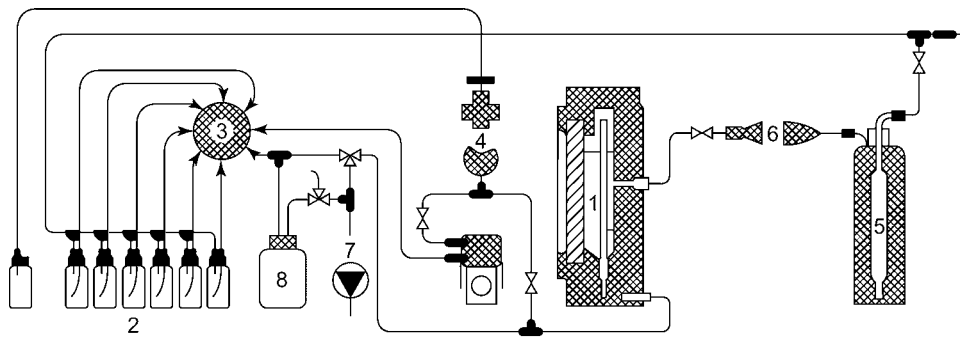

Figure 39.4 Illustration of the automated multiple-development chamber (1, developing chamber; 2, solvent reservoirs; 3, solvent-selection valve; 4, solvent mixer; 5, wash bottle for preparation of the gas phase for layer conditioning; 6, gas phase reservoir; 7, vacuum pump; 8, solvent waste reservoir). All operations of this chamber are managed by a programmable control unit and initiated as time sequences. A sequence starts with a conditioning step in which the vapour phase stored in reservoir (5) is pumped into the developing chamber (1). During conditioning the mobile phase composition is mixed by withdrawing the desired solvents from their storage bottles (2) and transferring the desired volumes to the mixer. At the selected time the mobile phase is pumped into the developing chamber and ascends the layer to the desired height, determined by an optical position monitor. The solvent is then drained from the chamber to waste (8) and the vacuum pump (7) is engaged to remove solvent vapours from the chamber. The next segment of the sequence is then commenced according to the program entered into the control unit, until all the segments are complete and the separation is finished.

visualisation of UV-absorbing drugs on TLC plates that incorporate a fluorescent indicator. The zones of UV-absorbing substance appear dark against the brightly fluorescing background of a lighter colour when the plate is exposed to UV light of short wavelength. The method is not universal, since it requires overlap between the absorption bands of the indicator ($\gamma_{max} \approx 280$ nm with virtually no absorption below 240 nm) and of the drug, but in favourable cases it is a valuable and non-destructive method for zone location.

All optical methods for the quantitative *in situ* evaluation of TLC chromatograms are based upon measuring the difference in optical response between a sample-free region of the layer and regions of the layer in which separated substances are present. Reflectance measurements can be made at any wavelength from the UV to the near infrared (185–2500 nm). The relationship between signal and sample amount in the absorption mode is non-linear, and does not conform to any simple equation. The principal method of quantification in TLC is by calibration using a series of standards that span the concentration range of the drug to be determined. The calibration curve is usually based on a second-order polynomial fit for the calibration standards, with individual samples quantified by interpolation only.

The determination of drugs that fluoresce on TLC plates is fundamentally different from absorption measurements. At low sample concentrations the fluorescence signal F is described adequately by $F = \Phi I_0 \varepsilon b C$, where Φ is the quantum yield, I_0 is the intensity of the excitation source, ε is the molar absorption coefficient, b is the thickness of the TLC layer and C is the sample amount. With the exception of the sample amount, all terms in this expression are constant, or fixed by the experiment, and therefore the fluorescence emission is linearly dependent on the sample amount over two or three orders of magnitude.

Derivatisation reactions

There is a long history of the use of derivatisation reactions in TLC to visualise colourless compounds. Many of these reactions are of a qualitative nature, which was not a problem when TLC was used rarely for quantification. Some of these reactions have been adapted to the demands of quantitative scanning densitometry, as either pre- or post-chromatographic treatments, and new reagents and methods have been added specifically for quantitative measurements in TLC.

In post-chromatographic reactions the reagents can be applied to the layer through the gas phase or by evenly coating the layer with a solution of the reagents. Gas-phase methods are fast and convenient but are restricted by the number of useful reagents. Examples include iodine, ammonia and hydrogen chloride, which are applied by inserting the layer into a tank that contains a saturated atmosphere of the reactive vapour. Spraying or dipping is used to apply reagents in solution to the layer. Spray techniques that use simple atomisers have long been used in TLC, but reagent application by this method is quite difficult to perform well. The homogeneity of the reagent distribution over the layer depends on many factors, such as the droplet size, distance between the spray device and layer, direction of spraying and discharge rate of the reagent. If ventilation of the workspace is inadequate, spray techniques can be a potential health hazard. For quantitative analysis, immersion of the layer into a solution of the reagents in a controlled manner (referred to as dipping) or the use of fully automated spray devices is the preferred technique, since neither relies on manual dexterity and produces superior results in scanning densitometry. Some spray reagents do not make good dipping solutions because they contain solvents that are too aggressive or viscous for convenient application (aqueous concentrated acids and bases, for example). Dipping solutions are usually less concentrated than spray reagents and water is often replaced by an alcohol for adequate permeation of reversed-phase layers. In general, it is necessary to reformulate dipping solutions from earlier recipes for spray solutions and, possibly, to change the reaction conditions. Automated low-volume dipping chambers provide a uniform speed and dwell time for the immersion process, which typically requires only a few seconds, and is long enough to impregnate the layer with solution but not long enough to wash sample components off the layer.

Post-chromatographic derivatisation reactions can be classified as reversible or destructive, depending on the type of interaction between the reagents and separated drugs, and as selective or universal, based on the specificity of the reaction. The most common reversible methods employ iodine vapour, water, fluorescein or pH indicators as visualising reagents. In the iodine vapour method, the dried plate is enclosed in a chamber that contains a few crystals of iodine; components on the chromatogram are stained more rapidly than the background and appear as yellow–brown spots on a light yellow background. Simple removal of the plate from the visualisation chamber to allow the iodine to evaporate can reverse the reaction. Spraying a TLC plate with water reveals hydrophobic compounds as white spots on a translucent background when the water-moistened plate is held against the light. Solutions of pH indicators (e.g. bromocresol green, bromophenol blue) are widely used to detect acidic and basic drugs.

Irreversible methods are more common for quantification and comprise hundreds of reagents based on selective chemistries reduced to standard operations over several decades of use. Some typical examples used in drug identification are summarised in Table 39.6. Reagents that are specific to functional groups or selective for compound classes can be applied to determine low levels of substances in complex matrices such as biological fluids and plant extracts.

Sometimes the fluorescence response for drugs and their derivatives on TLC layers is less than that expected from solution measurements, is observed at different excitation and emission wavelengths than in solution, and may decrease with time. Adsorption onto the sorbent layer provides additional non-radiative pathways for the dissipation of the excitation energy, which is most probably lost as heat to the surroundings and reduces the observed fluorescence signal. The extent of fluorescence quenching often depends on the sorbent used for the separation and is generally more severe for silica gel than for chemically bonded sorbents. In most cases, impregnating the layer with a viscous liquid, such as liquid paraffin or Triton X-100, before evaluating the separation enhances the emission signal (in favourable cases 10- to 200-fold). The general mechanism of fluorescence enhancement is assumed to be dissolution of the sorbed solute with enhancement in response due to the fraction of solute that is transferred to the liquid phase, where fluorescence quenching is less severe. Viscous solvents are employed to minimise zone broadening from diffusion in the liquid phase during the measurement process.

Ready accessibility of the immobilised sample after separation facilitates the application of biological reagents for detection. The inhibition of bioluminescence of the bacterium *Vibrio fischeri* is widely used to identify toxic compounds in complex mixtures. All materials necessary for the assay are available in kit form, so laboratories lacking experience in culture techniques can perform this assay as simply as any other post-chromatographic derivatisation procedure. Image analysers employed for TLC detection are suitable for monitoring the inhibition of bioluminescence. Alternative test species based on immunochemical and cellular reporting probes are in development and this aspect of biological-specific detection in TLC is expected to grow in importance and applications.

Slit-scanning densitometers

Commercial instruments for scanning densitometry usually allow measurements in the reflectance mode by absorbance or fluorescence. Most instruments employ grating monochromators for wavelength selection and spectrum recording in the absorption mode. For fluorescence measurements a filter that transmits the emission wavelength envelope but attenuates the excitation wavelength is placed between the detector and the plate. The separations are scanned at selectable speeds up to about 10 cm/s by mounting the plate on a movable stage controlled by stepping motors. A fixed sample beam is shaped into a rectangular area on the plate surface, through which the plate is transported in the direction of development. Each scan, therefore, represents a lane of length defined by the solvent-front migration distance and width defined by the slit dimensions of the source. Distorted chromatograms can be corrected by track optimisation, in which the sample zones are integrated as if the slit had moved along an optimum track from peak maximum to peak maximum. In modern TLC the relative standard deviation from all errors, instrumental and chromatographic, can be maintained below 2–3%, which makes it a very reliable quantitative tool.

Table 39.6 Some common visualisation reagents for drug identification in TLC

Name	*Reagent*	*Application*
Bratton–Marshall	Dissolve 1 g *N*-(1-naphthyl)ethylenediamine dihydrochloride in 50 mL DMB and 50 mL concentrated HCl	Aromatic and primary amines, sulfonamides, benzodiazepines
Tillman	Dissolve 0.1 g sodium salt of 2,6-dichlorophenol indophenol in 100 mL ethanol	Organic acids
Dragendorff	(a) 17% (w/v) bismuth nitrate in 20% aqueous acetic acid (b) 40% (w/v) potassium iodide in water Mix 4 parts of (a) with 1 part of (b) and 14 parts of water	Alkaloids, miscellaneous, drugs
Fast Black K	0.5% (w/v) aqueous solution of Fast Black K salt	Aliphatic primary and secondary amines, amphetamines and phenols, and heterocyclics
Fluorescamine	(a) 25 mg fluorescamine in 100 mL acetone	Primary amines, sulfonamides
FPN	(a) 0.05 mol/L ferric chloride solution (b) 5% (w/v) perchloric acid Mix 1 part (a) with 50 parts (b)	Phenothiazines, dibenzazepines
Platinic chloride	(a) 5% (w/v) aqueous platinic chloride solution (b) 10% (w/v) aqueous potassium iodide Mix 5 mL of (a) with 45 mL of (b) and dilute to 100 mL with water	Quaternary ammonium compounds, alkaloids
Mandelin	Dissolve 1.2 g ammonium monovandate in 95 mL water and carefully add 5 mL concentrated sulfuric acid	Acid drugs
Marquis	Add 0.2–1.0 mL of 37% formaldehyde solution carefully to 10 mL concentrated sulfuric acid	Alkaloids, β-blockers, amfetamines, phenothiazines
Van Urk	Dissolve 1 g *p*-dimethylaminobenzaldehyde in 100 mL ethanol and add 10 mL concentrated HCl	Indoles, amines, sulfonamides, pesticides

Image analysers

For image analysers, scanning takes place electronically using a combination of a computer with video digitiser, light source, monochromators and appropriate optics to illuminate the plate and focus the image onto a charged-coupled device video camera. The captured images are initialised, stored and transformed by the computer into chromatographic data. Background subtraction and thresholding are common data-transformation processes. Image analysers provide fast data acquisition, simple instrument design and convenient software tools that search and compare sample images. Current instruments lack the sensitivity, wavelength operating range and resolution of mechanical scanners, but the robust market for digital cameras has reduced their cost significantly. They have proved popular for less-demanding tasks, for the development of field-portable instruments and as a replacement for photographic documentation of TLC separations. A major application is in the fingerprinting of herbal drug products for quality control applications.

Other instrumental detection methods

Radioisotope-labelled drugs and their metabolites can be detected selectively with good sensitivity by imaging detectors that use windowless gas-flow proportional counters as detectors. The proportional counter is filled with a mixture of argon and methane gas, which is ionised locally by collision with beta or gamma rays produced by radioactive decay in the sample zones that contain radioisotopes. The local bursts of ionised gas molecules are sensed by a position-sensitive detector and stored in computer memory. These signals are accumulated for quantitative measurements.

Flame ionisation has been used to detect samples of low volatility that lack a chromophore for optical detection. The separation is performed on specially prepared, thin, quartz rods with a surface coating of sorbent attached by sintering. The rods are developed in the normal way, usually held in a support frame that also serves as the scan stage after the rods have been removed from the developing chamber and dried. The rods are moved at a controlled speed through a hydrogen flame and the signal processed in a similar manner to the flame ionisation detector used in gas chromatography. The linear working range of the detector is about 3–30 μg for most substances. There are few reported applications in drug analysis.

Spectroscopic detection of separated zones has moved out of the research laboratory and commercial interfaces are available for infrared, Raman and mass spectrometry. These methods offer the possibility of identification or confirmation of identity for samples in the nanogram to microgram range. These techniques are generally considered semi-quantitative since quantification often requires special calibration procedures – for example, the use of stable isotope standards in mass spectrometry. The most widely used interfaces for mass spectrometry are based on electrospray or atmospheric pressure chemical ionisation with tandem mass spectrometry or matrix-adsorption laser desorption and ionisation (MALDI) with time-of-flight mass spectrometry. Direct sampling interfaces for electrospray ionisation are based on three principles: liquid junction extraction (surface sampling probe); plunger-based extraction devices; and desorption electrospray ionisation (DESI). They differ primarily in extraction efficiency for different combinations of sample and sorbents and whether scanning of the chromatogram is possible while simultaneously recording mass spectra. The recently introduced direct analysis in real time (DART) interface has been shown to be suitable for recording mass spectra directly from TLC layers at atmospheric pressure.

Method development

Selection of the development technique is based on the number of detectable components in the mixture and their polarity range

Table 39.7 Zone capacity calculated or predicted for different conditions in TLC

Development method	*Dimensions*	*Zone capacity*
Predictions from theory		
Capillary-controlled flow	1	<25
Forced flow	1	<80 (up to 150 depending on pressure limit)
Capillary-controlled flow	2	<400
Forced flow	2	Several thousand
Based on experimental observations		
Capillary-controlled flow	1	12–14
Forced flow	1	30–40
Capillary-controlled flow (AMD)	1	30–40
Capillary-controlled flow	2	About 100

(Table 39.7). A single development with capillary-controlled flow may be too difficult or impossible for mixtures that contain more than eight to ten components of interest. In addition, if the range of polarities is too wide, multiple development techniques using mobile-phase gradients are necessary. It is only necessary to separate the components of major interest from each other and from the less important components, which need not be separated individually. Method development is easier if standards for the relevant compounds are available. Standards simplify zone tracking and enable detection characteristics and the possibility of spectroscopic resolution of incompletely separated zones to be established. Standards are also required for calibration, if quantification is required, and to construct spectral libraries for identification purposes. The expected concentration range of relevant compounds may indicate the need for derivatisation to obtain the required detection limits and to increase zone separation of neighbouring compounds if one compound is a minor component with similar migration properties to a major component.

A general guide to the selection of stationary phases for TLC separations is summarised in Fig. 39.5. Silica gel is generally the first choice to separate drugs of low molecular weight that are soluble in moderately polar organic solvents. Reversed-phase chromatography on chemically bonded layers is generally used to separate drugs that are difficult to separate on silica gel because of inadequate retention, inadequate selectivity or zone asymmetry. Ionic compounds and easily ionised compounds are frequently separated by reversed-phase chromatography using buffered mobile phases (weak acids and bases) or ion-pair reagents (strong acids and bases). Only a limited number of stationary phases are available for ion-exchange chromatography, which is not a widely used separation mechanism in TLC.

Since the solvent used for the separation is evaporated prior to detection, a wider range of UV-absorbing solvents is commonly used in TLC than is the case for HPLC. Solvents must be of high purity, since involatile impurities and stabilisers remain sorbed to the layer, which causes problems in the detection step. Multicomponent mobile phases can produce a mobile-phase gradient in the direction of development through demixing. If demixing is complete, zones with sharp boundaries are formed, which separate the chromatogram into sections of different solvent composition and, therefore, selectivity. Demixing effects are less apparent when saturated developing chambers are used. These considerations hinder optimisation strategies based on the composition of the mobile phases as popularised in HPLC.

The selection of a mobile phase to separate simple mixtures need not be difficult and can be arrived at quickly by guided trial-and-error methods. A solvent of the correct strength for a unidimensional development migrates the sample components into the R_f range 0.2–0.8, or thereabouts and, if of the correct selectivity, distributes the sample components evenly throughout this range. Solvent systems can be screened in parallel using several development chambers, as prescribed in the PRISMA model. To select suitable mobile phases, the first experiments are carried out on TLC plates in unsaturated chambers with ten solvents, chosen from the different selectivity groups, indicated by bold type in Table 39.8. After these screening experiments with single solvents, the solvent strength is either reduced or increased so that the substance zones are distributed in the R_f range 0.2–0.8. If the substances migrate into the upper third of the plate, the solvent strength is reduced by dilution with hexane (the strength-adjusting solvent). If the substances remain in the lower third of the plate with the single solvents, the solvent strength is increased by the addition of a strong solvent, such as water or acetic acid. A similar procedure is followed in the reversed-phase mode, except that solvent selection is limited to water-miscible solvents and water is used as the strength-adjusting solvent. From these trial experiments, those solvents that show the best separation are selected for further optimisation in the second part of the model.

Table 39.8 Solvent-strength parameters and selectivity groups for solvents used for separations on silica gel

Selectivity group	*Solvent*
I	*n*-Butyl ether, diisopropyl ether, methyl *t*-butyl ether, **diethyl ether**
II	*n*-Butanol, **propan-2-ol**, propanol, **ethanol**, methanol
III	**Tetrahydrofuran**, pyridine, methoxyethanol, dimethylformamide
IV	**Acetic acid**, formamide
V	**Dichloromethane**, 1,1-dichloroethane
VI	**Ethyl acetate**, methyl ethyl ketone, **dioxane**, acetone, acetonitrile
VII	**Toluene**, benzene, nitrobenzene
VIII	**Chloroform**, dodecafluoroheptanol, water

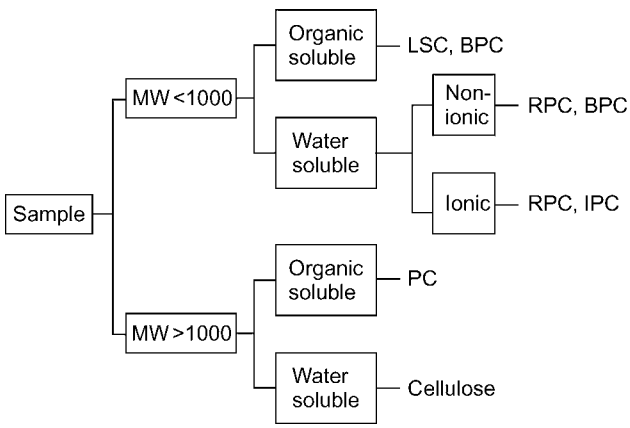

Figure 39.5 Mode selection guide for TLC. LSC, liquid–solid chromatography on an inorganic oxide layer; BPC, liquid–solid chromatography on a chemically bonded layer; RPC, reversed-phase chromatography with a chemically bonded layer and an aqueous organic mobile phase; IPC, ion-pair chromatography with reversed-phase separation conditions; PC, precipitation chromatography used to separate polymers based on solubility differences in a mobile phase solvent gradient.

Between two and five solvents can be selected to construct the PRISMA model for solvent optimisation. Modifiers required to maintain an acceptable zone shape, such as acids and ion-pair reagents, can be added in a low and constant concentration, so that their influence on solvent strength can be neglected. The PRISMA model (Fig. 39.6) is a three-dimensional geometrical design that correlates solvent strength with selectivity of the mobile phase. The model consists of three parts: the base or platform (which represents the modifier), the regular part of the prism with congruent base and top surfaces, and the irregular truncated top prism (frustum). The lengths of the edges of the prism (S_A, S_B, S_C) correspond to the solvent strengths of the neat solvents (A, B, C). Since the selected solvents usually have different solvent strengths, the lengths of the edges of the prism are generally unequal and the top plane of the prism is not parallel and congruous with its base. If the prism is cut parallel to its base at the height of the lowest edge (determined by the solvent strength of the weakest solvent, solvent C in Fig. 39.6), the lower part gives a regular prism, and the top and any planes, which represent weaker solvents diluted with a strength-adjusting solvent, are parallel equilateral triangles. The upper frustum of the model is used for mobile phase optimisation of polar drugs in normal-phase TLC, while the regular part is used to separate moderately polar drugs in normal-phase TLC and all separations by reversed-phase TLC.

For polar compounds, optimisation is always started on the top irregular triangle of the model, either within the triangle, when three solvents are selected, or along one side, for binary mobile phases. Any solvent composition on the face of the triangle can be represented by a three-coordinate selectivity point (P_S), each coordinate corresponding to the volume fraction of the solvent at that position on the triangle (Fig. 39.6). Optimisation is commenced by selecting solvent

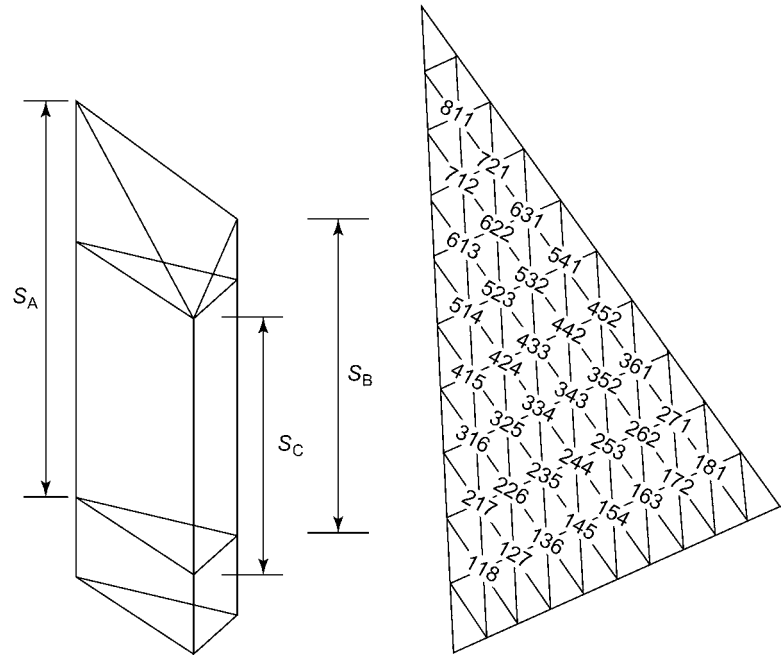

Figure 39.6 The PRISMA mobile phase optimisation model, showing the construction of the prism and the arrangement of selectivity points on the top face or horizontal plane cut through the prism.

combinations that correspond to the centre point $P_S = 333$ and three other points close to the apexes of the triangle $P_S = 811$, 181 and 118. If the separation obtained is insufficient, other selectivity points are tested around the solvent combination that gave the best separation. On changing the selectivity points on the top triangle, the solvent strength changes as well, especially when the solvent strengths of the solvents used to construct the prism are significantly different. The solvent strength should be adjusted with the strength-adjusting solvent as required to maintain the separation in the optimum R_f range. Failure to obtain the beginning of a separation requires that a new prism be constructed, using a different solvent for at least one of the edges.

For reversed-phase TLC, the solvation-parameter model provides a convenient computer-aided approach to method development. Suitable water-miscible solvents with a range of selectivity include methanol, propan-2-ol, 2,2,2-trifluoroethanol, acetonitrile (or dioxane), acetone (or tetrahydrofuran) and dimethylformamide (or pyridine). For optimisation of systems (stationary phases and binary mobile phases), preliminary results in the form of system maps (a continuous plot of the system constants against mobile-phase composition) are required. System maps are a permanent record of the system properties used in all calculations and are available for most common layers and indicated solvents for selectivity optimisation. For each computer-simulated separation a retention map is calculated from the system map and displays the computed R_f values as a continuous function of the binary mobile phase composition. A typical retention map for the computer-predicted separation of analgesics on an octadecylsiloxane-bonded layer with 2,2,2-trifluoroethanol–water mixtures as the mobile phase is shown in Fig. 39.7. Solvent compositions that result in an acceptable zone separation are identified easily by visual inspection. Computer simulation of retention maps allows those systems (defined as a combination of stationary and mobile phases) likely to provide an acceptable separation to be identified before experimental work commences. The agreement between model predicted and experimental R_f values is generally good, typically better than 0.05 R_f units. A mixture-design approach is used to extend this method to ternary solvent mixtures.

For drug mixtures of a wide polarity range, stepwise changes in solvent composition are required to achieve a satisfactory TLC separation. Models to calculate migration distances using incremental multiple development with increasing and decreasing solvent-strength gradients have been described, but are complicated and not widely used. Optimised gradients for automated multiple development are usually arrived at by more pragmatic means. Methods based on a universal gradient commence with methanol, end with hexane, and use either dichloromethane or methyl *t*-butyl ether as the intermediate solvent for separations on silica gel. By scaling and superimposing the chromatogram of the separation above the theoretical gradient profile, those regions of the chromatogram that affect the separation are identified easily. The solvent composition for the initial and final development steps is adjusted to eliminate those portions of the gradient that do not contribute to the separation. The gradient shape is modified to enhance resolution in those regions of the chromatogram that are separated poorly or to minimise regions devoid of sample zones. For moderately complex mixtures this approach is often satisfactory. If, after the above adjustments, the separation is inadequate, it is necessary to identify a

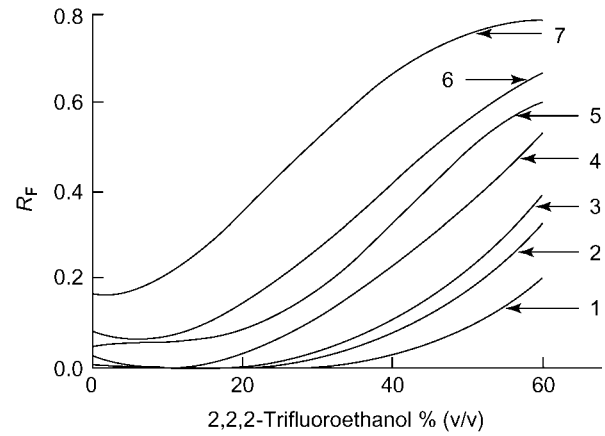

Figure 39.7 Retention map for the simulation of the separation of analgesics by reversed-phase TLC on an octadecylsiloxane-bonded layer with 2,2,2-trifluoroethanol–water as the mobile phase: 1, chlorphenamine (chlorpheniramine); 2, ibuprofen; 3, naproxen; 4, phenacetin; 5, aspirin; 6, caffeine; 7, paracetamol.

more selective solvent for problem regions in the gradient. The PRISMA model can be used at this point to identify more selective solvents to incorporate into the gradient as a replacement for the initial, terminal or base solvent.

Preparative thin-layer chromatography

Preparative TLC is used mainly to purify drugs or to isolate drug metabolites and impurities in amounts of about 1–100 mg for subsequent use as reference materials, structural elucidation, biological activity evaluation and other purposes. Scale up from analytical TLC is achieved by increasing the thickness of the layer (loading capacity increases with the square root of the layer thickness) and by increasing the plate length used for sample application. Pre-coated TLC plates for preparative chromatography vary in size from 20 × 20 cm to 20 × 40 cm and are coated with 0.5–10 mm thick layers, with the most popular thickness being 1.0–2.0 mm. As the average particle size (~25 μm) and size distribution (5–40 μm) are larger for preparative layers, and as sample overload conditions are used commonly in preparative chromatography, invariably inferior separations in a longer time (~1–2 h) are obtained compared with analytical separations. Resolution can be increased significantly by using wedge-shaped, gradient-thickness layers. These layers have a uniform increase in thickness from 0.3 mm at the bottom to 1.7 mm at the top. Sample bands are focused during migration by the negative mobile-phase velocity gradient created by the layer geometry.

Sample application is a critical step in preparative TLC, and if performed improperly can destroy all or part of the separation. The sample, usually as a 5–10% (w/v) solution in a volatile solvent, is applied as a band along one edge of the layer to give a maximum sample load of about 5 mg/cm for each millimetre of layer thickness. Sample loads are usually lower for difficult separations and for cellulose and chemically bonded layers. Any of the automated band applicators for analytical TLC are suitable for sample application in preparative TLC. Manual sample application by syringe or glass pipette must be performed carefully to avoid damaging the layer and producing irregularly shaped migrating zones. A short predevelopment, of about 1 cm with a strong solvent, is often useful to refocus manually applied bands. Preparative layers with a preadsorbent zone are useful for manual sample application, since the focusing mechanism can be used to correct for poor sample-application technique. In all cases, it is important that the sample solvent be evaporated fully from the layer prior to the start of the separation to avoid the formation of distorted separation zones. It is usual to leave a blank margin of 2–3 cm at each vertical edge of the layer to avoid uneven development.

Most of the changes in preparative TLC over the past decade have occurred in the method of development. Conventionally, ascending development in large-volume tanks that hold a number of preparative layers in a rack is used commonly. In laboratories that perform preparative TLC on a regular basis, higher resolution and shorter development times are achieved by using forced-flow development or rotation planar chromatography (accelerated development using centrifugal force). These methods allow conventional development and elution with on-line detection and automated fraction collection to be used.

After development, physical methods of zone detection are used to identify the sample bands of interest. Layers that contain a UV indicator for fluorescence quenching or the adsorption of iodine vapours are useful for this purpose. If a reactive spray reagent is used for visualisation, it should be sprayed on a small strip of the chromatogram only, so as not to contaminate the remainder of the material. Once the bands of interest are located, the zones are scraped off the plate carefully with a spatula or similar tool. A number of devices based on the vacuum-suction principle for removing the marked zones from the plate are also available. Soxhlet extraction, liquid extraction or solvent elution with a polar solvent is used to recover drugs from the sorbent. For solvent extraction, water is often added to dampen the silica gel prior to extraction with a water-immiscible organic solvent. Chloroform and ethanol (methanol is less suitable because of its higher silica solubility) are widely used for solvent elution. Colloidal silica can be removed by membrane filtration prior to vacuum stripping of the solvent.

Retardation factor

The retardation factor, or R_f value, is the fundamental parameter used to characterise the position of a sample zone in a TLC chromatogram. For linear development it represents the ratio of the distance migrated by the sample compared with the distance travelled by the solvent front:

$$R_f = Z_X/(Z_f - Z_o)$$

where Z_X is the distance travelled by the sample from its origin, $(Z_f - Z_0)$ is the distance travelled by the mobile phase from the sample origin, Z_f is the distance travelled by the mobile phase measured from the mobile phase level at the start of the separation and Z_0 is the distance from the sample origin to the mobile phase level at the start of the separation. The boundary conditions for R_f values are $1 \geq R_f \geq 0$. The R_f value is generally calculated to two decimal places. Some authors prefer to tabulate values as whole numbers, as hR_f values equivalent to $100R_f$.

Drug identification

The R_f value is affected too adversely by measurement difficulties and by variations in experimental and environmental conditions to be a useful identification parameter on its own. When standard substances are available, it is common practice to run standards and samples in the same system for improved confidence in identification based on R_f values. If scanning densitometry is used, an acceptable agreement in R_f values is generally supported by the automated matching of specific absorbance ratios or full spectra for the samples and standards.

In drug-screening programs, in which simultaneous separation of standards and samples is impractical, the certainty of drug identification is improved by simultaneous separation of a series of related standard substances that allow the experimental R_f values to be corrected to standardised R_f values for automated library searches:

$$hR_f(X)^c = hR_f(A)^c + [\Delta^c/\Delta][hR_f(X) - hR_f(A)]$$

$$\Delta^c = hR_f(B)^c - hR_f(A)^c$$

$$\Delta = hR_f(B) - hR_f(A)$$

where $hR_f(X)$ is the R_f value for substance X, $hR_f(A)$ and $hR_f(B)$ are the R_f values for the standard substances that bracket $hR_f(X)$, and the superscript c indicates the corrected value for X and the accepted values for A and B. Alternatively, a calibration curve of experimental R_f values against the accepted R_f values for the standards can be prepared and used to convert experimental R_f values to corrected R_f values. Typically, four evenly spaced standard substances with the sample origin ($hR_f = 0$) and solvent front ($hR_f = 100$) are included as additional reference points.

Database searches

Database searches are used in systematic toxicological analysis to identify suspect substances in biological fluids and postmortem tissue samples. Extracted samples are separated in one or more standard TLC systems. The corrected R_f values, often combined with the results of sequential post-chromatographic colour reactions, are then entered into the search program. The input data are automatically compared against a database of reference drugs, common metabolites, natural contaminants, etc., for identification. A number of chemometric procedures can be used for data analysis, but the most common approach is based on the mean list method.

It is assumed that the errors in individual measurements are random and can be described by a standard deviation. The precision of the separation system can then be described as the mean of the standard deviation of all substances separated in the system, called the system mean standard deviation. This allows a confidence interval or window to be assigned to the system as some multiple (typically three) of the system mean standard deviation. Each R_f value in the system database that appears in the window could be confused with the original substance. The number of substances identified as above is called the list length. Repeating the process for all R_f values in that system and averaging the individual list lengths provides the mean list length for that system. The

mean list length indicates, on average, the number of substances in the database that qualify as candidates for the identification of a single drug. The shorter the mean list length, the greater the information potential of the system. Combining the results from additional retention parameters in complementary standard separation systems, colour reactions, spectroscopic data, etc., minimises the mean list length to the point that only a small number of candidate compounds for the unknown are indicated. More specific tests can then be used to identify the unknown from among the small number of indicated possibilities. For systematic drug identification in forensic toxicology, commonly two or more

Table 39.9 TLC systems recommended by TIAFT for systematic toxicological analysis (drug database De Zeeuw 1992)

No.	*TLC system*			*Reference compounds*[(a)]	hR_f[c]	*Error window*[(b)]
	Mobile phase	*Chamber type*	*Stationary phase*			
(1)	Chloroform–acetone (4 : 1)	Saturated	Silica gel	Paracetamol	15	7
				Clonazepam	35	
				Secobarbital	55	
				Methylphenobarbital	70	
(2)	Ethyl acetate	Saturated	Silica gel	Sulfathiazole	20	8
				Phenacetin	38	
				Salicylamide	55	
				Secobarbital	68	
(3)	Chloroform–methanol (9 : 1)	Saturated	Silica gel	Hydrochlorothiazide	11	8
				Sulfafurazole	33	
				Phenacetin	52	
				Prazepam	72	
(4a)	Ethyl acetate–methanol–25% ammonia (17 : 2 : 1)	Saturated	Silica gel	Sulfadimidine	13	11
				Hydrochlorothiazide	34	
				Temazepam	63	
				Prazepam	81	
(4b)	Ethyl acetate–methanol–25% ammonia (17 : 2 : 1)	Saturated	Silica gel	Morphine	20	10
				Codeine	35	
				Hydroxyzine	53	
				Trimipramine	80	
(5)	Methanol	Unsaturated	Silica gel	Codeine	20	8
				Trimipramine	36	
				Hydroxyzine	56	
				Diazepam	82	
(6)	Methanol–*n*-butanol (3 : 2) containing 0.1 mol/L sodium bromide	Unsaturated	Silica gel	Codeine	22	9
				Diphenhydramine	48	
				Quinine	65	
				Diazepam	85	
(7)	Methanol–25% ammonia (100 : 1.5)	Saturated	Silica gel impregnated with 0.1 mol/L KOH in methanol and dried	Atropine	18	9
				Codeine	33	
				Chlorprothixene	56	
				Diazepam	75	
(8)	Cyclohexane–toluene–diethylamine (15 : 3 : 2)	Saturated	Silica gel impregnated with 0.1 mol/L KOH in methanol and dried	Codeine	6	8
				Desipramine	20	
				Prazepam	36	
				Trimipramine	62	
(9)	Chloroform–methanol (9 : 1)	Saturated	Silica gel impregnated with 0.1 mol/L KOH in methanol and dried	Desipramine	11	11
				Physostigmine	36	
				Trimipramine	54	
				Lidocaine	71	
(10)	Acetone	Saturated	Silica gel impregnated with 0.1 mol/L KOH in methanol and dried	Amitriptyline	15	9
				Procaine	30	
				Papaverine	47	
				Cinnarizine	65	

[(a)]Concentration of reference standards, 2 mg/mL of each substance.
[(b)]Error window defined as three times the mean standard deviation.
TIAFT, the International Association of Forensic Toxicologists

complementary TLC systems combined with the results from several *in situ* sequential colour reactions, are used. For drugs of toxicological interest, a mean list length from two to ten is possible.

Systematic drug identification

Systematic toxicological analysis takes advantage of the separation of an unknown substance in standard TLC systems (or other chromatographic systems) to establish the probable identity of the substance by reference to a database of candidate compounds using a statistical comparison approach, such as the mean list method. Suitable chromatographic techniques for systematic toxicological analysis must meet the following criteria:

1. The drugs must exhibit acceptable chromatographic properties in the separation system.
2. The R_f values for the drugs must be distributed evenly over the full R_f range.
3. The R_f values are standardised in such a way that good inter-laboratory reproducibility is obtained.
4. When more than one separation system is used, there must be a low correlation of R_f values in the selected systems.

TLC systems that meet these requirements are described below.

Since pH-dependent extractions are customarily used in drug extraction and work-up procedures, generally different TLC systems are used to separate acidic and basic drugs, with neutral drugs likely to occur in both fractions. The Committee for Systematic Toxicological Analysis of the International Association of Forensic Toxicologists (TIAFT) recommended 11 separation systems for drug identification (Table 39.9). Four systems (1 to 4a) are to separate neutral and acidic drugs and seven systems (4b to 10) are to separate neutral and basic drugs. Reference data are presented for about 1600 toxicologically relevant substances. For general drug screens, the use of two separation systems with a low correlation is recommended: systems 2 and 4a for neutral and acidic drugs and systems 5 and 8 for neutral and basic drugs (systems 7 and 8 are nearly as good). Combining colour reactions with the TLC data improves the certainty of identification significantly. Four colour reactions are carried out on the same plate in sequence. After each step the colour is noted and encoded by means of a colour chart (1, yellow; 2, orange; 3, brown; 4, red; 5, purple; 6, black; 7, blue; 8, green; 0, no spot observed). The sequence consists of formaldehyde vapour and Mandelin's reagent, water, fluorescence under 366 nm irradiation and modified Dragendorff's reagent. Other sequential colour reactions can be encoded and utilised in the same way. The Merck Tox Screening System (MTSS) contains the TIAFT TLC database and several other useful tools for searches using other chromatographic and spectroscopic databases and user-created databases.

Romano *et al.* (1994) presented data for 443 drugs in four TLC systems using high performance silica gel TLC plates (Table 39.10). These systems use slight modifications of the mobile phase compositions recommended by TIAFT. The UniTox system uses three TLC systems (Table 39.11). System 1 is designed to separate neutral and acidic drugs and systems 2 and 3 to separate basic, amphoteric and quaternary drugs. Two of the separation systems are based on reversed-phase separations designed to complement the more familiar silica gel separations. The database contains over 375 drugs of general toxicological interest, including a large number of amfetamines.

The Toxi-Lab system is a TLC kit for toxicological drug screening; it contains equipment for extraction, development, detection and identification. Separations are performed on unsupported, particle-embedded, glass-fibre sheets with holes punched in them to receive samples and standards as extraction or reference discs. A combination of silica gel and reversed-phase separations together with sequential colour reactions is used for identification and confirmation purposes. The database is designed for computer searches, with results entered in a standard format.

Pesticides are a further class of toxic substances of interest to systematic toxicological analysis because of their general availability, toxicity and potential confusion with drugs. Erdmann *et al.* (1990) developed a database for 170 commonly used pesticides separated in three standardised TLC systems (Table 39.12). The systems in Table 39.12 supplement those in Table 39.9, in which many common pesticides migrate with the solvent front. Systems 1 and 2 are recommended for general screening and system 3 for the identification of special compounds not distinguished in the first two systems.

General applications

Thousands of general and validated methods are available for the determination of drugs as pharmaceutical products and in biological fluids. Since the zone capacity of TLC systems is small, there are no general methods for drugs as a class, but there are a large number of methods for individual drugs defined by therapeutic or chemical categories. These

Table 39.10 The separation systems recommended by Romano *et al.* (1994) for systematic toxicological analysis by TLC (drug database Romano *et al.* 1994)

No.	TLC system			Reference compounds	$hR_f^{c(a)}$
	Mobile phase	Chamber type	Stationary phase		
(1)	Ethyl acetate–methanol–30% ammonia (17 : 2 : 3)	Saturated	Silica gel	Morphine	25
				Strychnine	44
				Aminopyrine	70
				Cocaine	85
(2)	Cyclohexane–toluene–diethylamine (13 : 5 : 2)	Saturated	Silica gel	Clobazam	15
				Aminopyrine	29
				Mebeverine	47
				Amitriptyline	60
(3)	Ethyl acetate–chloroform (1 : 1)	Saturated	Silica gel	Caffeine	09
				Ketamine	24
				Flunitrazepam	44
				Prazepam	61
(4)	Acetone	Saturated	Silica gel impregnated with 0.1 mol/L KOH in methanol and dried	Imipramine	20
				Pericyazine	37
				Aminopyrine	62
				Lidocaine	78

[(a)]Error window estimated as 7–9% R_f^c.

Table 39.11 The UniTox system for systematic toxicological analysis by TLC (drug database, Ojanpera 1995; additional compounds (amfetamines) in Ojanpera *et al*. 1991)

No.	TLC system			Reference compounds	hR_f[c]	Error window
	Mobile phase	Chamber type	Stationary phase			
(1)	Methanol–water (13 : 7)	Unsaturated	Octadecylsiloxane-bonded silica gel	Diazepam	16	4
				Secobarbital	35	
				Phenobarbital	54	
				Paracetamol	74	
(2)	Toluene–acetone–ethanol–25% ammonia	Saturated	Silica gel	Codeine	16	5
				Promazine	36	
				Clomipramine	49	
				Cocaine	66	
(3)	Methanol–water–concentrated hydrochloric acid (50 : 50 : 1)	Unsaturated	Octadecylsiloxane-bonded silica gel	Hydroxyzine	20	4
				Lidocaine	46	
				Codeine	66	
				Morphine	81	

Table 39.12 Standardised TLC systems for the screening of pesticides (pesticide database, Erdmann *et al*. 1990)

No.	TLC system			Reference compounds	hR_f[c]
	Mobile phase	Chamber type	Stationary phase		
(1)	Hexane–acetone (4 : 1)	Saturated	Silica gel	Triazophos	21
				Parathion-methyl	30
				Pirimiphos-methyl	49
				Quintozene	84
(2)	Toluene–acetone (19 : 1)	Saturated	Silica gel	Carbofuran	20
				Azinophos-methyl	42
				Methidathion	56
				Parathion-ethyl	85
(3)	Chloroform–acetone (1 : 1)	Saturated	Silica gel	Nicotine	11
				Ioxynil	39
				PCP	60
				Methabenzthiazuron	85

still represent substantial diversity driven by the need to optimise selectivity for each group of substances taken for analysis. This information can provide a useful starting point for system selection but it is no general substitute for systematic method development. For these reasons, universal methods for general drug analysis do not exist and earlier attempts at systematised approaches for different drug categories have failed to keep pace with the growth in number of drugs in those categories. In addition, systems recommended for the separation of individual drug categories rarely prove optimal for the separation of individual drugs and their impurities or metabolites.

Systems for thin-layer chromatography

The TLC systems given below are general screening methods for nitrogenous bases (systems TA, TB, TC, TL, TAE and TAF), for acids and neutral compounds (systems TD, TE, TF and TAD) with a further three general screening methods (systems TAJ, TAK and TAL). Furthermore, seven systems specific for pesticides (systems TW, TX, TY, TZ, TAA, TAB and TAC) and another 19 systems covering specific groups of drugs are also listed. The drugs are divided into chemical or pharmacological groups, but some other drugs are included with certain groups if they are chemically similar and would be extracted with that group.

There may not be one best system for a particular separation and a number of systems can be applied from those suggested. However, each of the systems described has been selected because it gives a good spread of R_f values, has high reproducibility and has low correlation with the other systems selected for that group of drugs. These systems have proved useful for a large number of groups of drugs over the years and are robust and dependable. At least three systems are given for each group, where possible. The hR_f values of the reference compounds suggested for the general screening systems have been derived using solutions of approximately 2 mg/mL of each substance.

Fluorescent plates should always be used and the absorption or fluorescence of the drug under ultraviolet light (both 254 and 350 nm) should be used as a location procedure. The suggested locating agents include general ones to visualise any drug that might be present as well as more specific ones to pick out individual classes of drugs.

Note In the tables of hR_f values, a dash indicates that no value is available for the compound.

Systems described in Table 39.9 have been assigned the following codes:

Code	Number in Table 39.9
TA	7
TB	8
TC	9
TD	1
TE	4
TF	2

table continued

Code	Number in Table 39.9
TL	10
TAD	3
TAE	5
TAF	6

Screening systems

Basic nitrogenous drugs

Stead AH *et al.* (). *Analyst* 107: 1106–1168.
de Zeeuw RA *et al.* (1992) *Thin-layer Chromatographic R_f Values of Toxicologically Relevant Substances on Standardized Systems*. Report XVII of the DFG Commission for Clinical-Toxicological Analysis, 2nd edn. Wienheim: VCH.

System TA

- *Plates*: Silica gel G, 250 µm thick, dipped in, or sprayed with, 0.1 mol/L potassium hydroxide in methanol, and dried.
- *Mobile phase*: Methanol–strong ammonia solution (100 : 1.5).
- *Reference compounds*: Atropine hR_f 18, codeine hR_f 33, chlorprothixene hR_f 56, diazepam hR_f 75.

System TB

- *Plates*: Silica gel G, 250 µm thick, dipped in, or sprayed with, 0.1 mol/L potassium hydroxide in methanol, and dried.
- *Mobile phase*: Cyclohexane–toluene–diethylamine (75 : 15 : 10).
- *Reference compounds*: Codeine hR_f 06, desipramine hR_f 20, prazepam hR_f 36, trimipramine hR_f 62.

System TC

- *Plates*: Silica gel G, 250 µm thick, dipped in, or sprayed with, 0.1 mol/L potassium hydroxide in methanol, and dried.
- *Mobile phase*: Chloroform–methanol (90 : 10).
- *Reference compounds*: Desipramine hR_f 11, physostigmine hR_f 36, trimipramine hR_f 54, lidocaine hR_f 71.

System TL

- *Plates*: Silica gel G, 250 µm thick, dipped in, or sprayed with, 0.1 mol/L potassium hydroxide in methanol, and dried.
- *Mobile phase*: Acetone.
- *Reference compounds*: Amitriptyline hR_f 15, procaine hR_f 30, papaverine hR_f 47, cinnarizine hR_f 65.

System TAE

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Methanol.
- *Reference compounds*: Codeine hR_f 20, trimipramine hR_f 36, hydroxyzine hR_f 56, diazepam hR_f 82.

System TAF

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Methanol–*n*-butanol (60 : 40) and 0.1 mol/L NaBr.
- *Reference compounds*: Codeine hR_f 22, diphenhydramine hR_f 48, quinine hR_f 65, diazepam hR_f 85.

Location reagents for systems TA, TB and TC

Ninhydrin spray Spray the plate with the reagent and then heat in an oven at 100°C for 5 min. Violet or pink spots are given by primary amines and yellow colours by secondary amines.

FPN reagent Red or brown–red spots are given by phenothiazines and blue spots by dibenzazepines. This reagent may be used to overspray a plate that has been previously sprayed with ninhydrin spray.

Dragendorff spray Yellow, orange, red–orange or brown–orange spots are given by tertiary alkaloids. This reagent may be used to overspray a plate that has been previously sprayed with ninhydrin spray and FPN spray.

Acidified iodoplatinate solution Violet, blue–violet, grey–violet or brown–violet spots on a pink background are given by tertiary amines and quaternary ammonium compounds. Primary and secondary amines give dirtier colours. This solution may be used to overspray a plate that has previously been sprayed with ninhydrin spray, FPN reagent and Dragendorff's spray.

Mandelin's reagent This reagent is preferably poured onto the plate because of the danger of spraying concentrated acid. Many different colours are given with a variety of drugs (see Chapter 30 and the Index of Colour Tests).

Marquis reagent This reagent is preferably poured onto the plate because of the danger of spraying concentrated acid. Black or violet spots are given by alkaloids related to morphine. Many different colours are given with a variety of drugs (see Chapter 30).

Acidified potassium permanganate solution Yellow–brown spots on a violet background are given by drugs with unsaturated aliphatic bonds.

R_f values R_f values for drugs in these systems will be found in drug monographs and in the Indexes of Analytical Data in Volume 2; they are also included in the systems for specific groups of drugs that follow.

Acidic and neutral drugs

Stead AH *et al.* (). *Analyst* 107: 1106–1168.
de Zeeuw RA *et al.* (1992) *Thin-layer Chromatographic R_f Values of Toxicologically Relevant Substances on Standardized Systems. Report XVII of the DFG Commission for Clinical-Toxicological Analysis*, 2nd edn. Wienheim: VCH.

System TD

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–acetone (80 : 20).
- *Reference compounds*: Paracetamol hR_f 15, clonazepam hR_f 35, secobarbital hR_f 55, methylphenobarbital hR_f 70.

System TE

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Ethyl acetate–methanol–strong ammonia solution (85 : 10 : 5).
- *Reference compounds*: Sulfadimidine hR_f 13, hydrochlorothiazide hR_f 34, temazepam hR_f 63, prazepam hR_f 81.

System TF

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Ethyl acetate.
- *Reference compounds*: Sulfathiazole hR_f 20, phenacetin hR_f 38, salicylamide hR_f 55, secobarbital hR_f 68.

System TAD

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–methanol (90 : 10).
- *Reference compounds*: Hydrochlorothiazide hR_f 11, sulfafurazole hR_f 33, phenacetin hR_f 52, prazepam hR_f 72.

Location reagents for systems TD, TE and TF

Acidic drugs

Van Urk's reagent Spray the plate with the reagent and then heat in an oven at 100°C for 5 min. Yellow spots are given by sulfonamides and by meprobamate, blue spots are given by ergot alkaloids, and pink or violet spots are given by some other compounds, e.g. phenazone.

Ferric chloride solution Blue or violet spots are given by phenols. This solution may be used to overspray a plate that has been previously sprayed with Van Urk's reagent.

Mercurous nitrate spray Barbiturates give dark spots that fade slowly; with some dilute solutions the spots fade rapidly.

Acidified potassium permanganate solution Yellow–brown spots on a violet background are given by drugs with unsaturated aliphatic bonds, e.g. secobarbital. This solution may be used to overspray a plate that has been previously sprayed with mercurous nitrate spray.

Neutral drugs

Furfuraldehyde reagent Violet to blue–black spots are given by some neutral compounds, e.g. carbamates.

Acidified iodoplatinate solution This solution may be used to overspray a plate that has previously been sprayed with furfuraldehyde reagent.

R_f values R_f values for drugs in these systems will be found in drug monographs and in the Indexes of Analytical Data in Volume 2; hR_f

values are included in the systems for specific groups of drugs that follow.

Note It is worth noting that system TE can be used for acidic, neutral and basic drugs. Furthermore, systems TC and TAD use the same mobile phase so that acidic, neutral and basic drugs can be run in the same tank, although on separate plates. It should also be noted that the above systems for basic nitrogenous drugs are also able to separate neutral drugs if the latter are present in the sample or in the basic extract thereof.

Finally, the Index of Colour Tests lists colour reactions with TLC spray reagents for approximately 250 compounds and may therefore serve as an indication of colour reactions specific to certain classes of compounds.

General screening systems

The TLC systems listed below (systems TAJ, TAK and TAL) were developed primarily by Professor George Maylin, New York State Racing Wagering Board, Drug Testing Programme, as well as system TAM listed under steroids.

System TAJ

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–ethanol (90 : 10).

System TAK

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–cyclohexane–acetic acid (4 : 4 : 2).

System TAL

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–methanol–propionic acid (72 : 18 : 10).

Location reagents for systems TAJ, TAK, TAL and TAM

Cupric chloride
Dragendorff's spray
Fearon's reagent
Ferric chloride, ethanol and sulfuric acid
Fluorescamine
Gibb's reagent
Conc. hydrochloric and ethanol
Iodine
Mandelin's reagent
Modified Ehrlich's reagent
Sodium nitrite
Ninhydrin

See Index of Colour Tests for colour reactions with TLC spray reagents.

Amfetamines, other stimulants and anorectics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Amfetamine	43	20	9	43	18	12	75	—	—	—
Bemegride	—	—	—	68	—	—	88	—	—	—
Benzfetamine	73	67	70	87	70	60	—	—	—	—
Brucine	16	—	17	—	1	5	7	4	—	64
Cathine	42	25	5	—	—	—	—	—	—	—
Chlorphentermine	44	18	17	48	8	14	77	—	—	—
Diethylpropion	76	62	63	85	64	55	56	44	2	35
Fenbutrazate	72	47	78	86	67	88	—	—	—	—
Fencamfamin	54	62	34	77	30	21	—	—	—	—
Fenfluramine	48	42	16	61	11	20	—	7	25	68
Fenproporex	—	—	—	77	—	—	—	—	—	—
Mazindol	63	7	13	53	13	46	65	4	—	24
Meclofenoxate	77	26	42	67	22	46	—	—	—	—
Metamfetamine	31	28	13	42	5	9	63	—	3	45
Methylenedioxymethamfetamine	33	24	—	39	—	8	—	3	17	57
Methylphenidate	57	35	41	66	23	40	70	11	4	70
Pemoline	60	—	23	36	40	81	81	12	14	60
Phendimetrazine	57	36	51	62	24	49	41	—	—	—
Phenmetrazine	50	14	27	46	14	34	45	20	8	60
Phentermine	46	26	24	48	12	11	78	2	5	36
Pipradrol	54	59	38	81	39	19	79	—	—	—
Prolintane	50	67	32	79	25	22	—	—	—	—
Tacrine	43	5	4	—	10	—	—	—	—	—

Anaesthetics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Benzyl alcohol	—	—	—	—	—	86	—	—	—	—
Benzocaine	67	6	57	77	66	84	87	—	—	—
Bupivacaine	69	42	73	80	65	69	79	59	5	40

table continued

Anaesthetics *continued*

	TA	TB	TC	TE	TL	TAE	TAF	TAJ	TAK	TAL
Butacaine	71	7	30	83	64	44	76	27	5	20
Butanilicaine	76	14	54	75	61	65	—	—	—	—
Butyl aminobenzoate	75	6	63	—	70	83	90	—	—	—
Chloroprocaine	59	5	23	—	37	—	—	9	—	32
Cinchocaine	63	25	34	67	35	42	—	—	—	—
Cocaine	65	45	47	77	54	35	30	13	—	2
Cyclomethycaine	58	55	36	—	25	—	—	—	—	—
Diperodon	70	15	58	—	66	—	—	—	—	—
Dyclonine	60	49	40	—	25	—	—	—	—	—
Etidocaine	91	—	—	75	—	80	—	66	19	75
Hexylcaine	1	—	—	80	—	—	—	29	15	70
Lidocaine	70	35	71	80	63	72	69	55	—	28
Mepivacaine	65	31	62	66	48	63	60	28	2	40
Methohexital	—	—	—	58	—	85	—	—	—	—
Oxetacaine	52	10	7	38	15	61	—	—	—	—
Oxybuprocaine	62	23	41	83	36	54	—	—	—	—
Piperocaine	55	53	37	76	27	24	56	—	—	—
Pramocaine	70	43	55	73	41	62	60	59	20	90
Prilocaine	77	29	64	75	60	62	79	50	22	69
Procaine	54	5	31	71	30	36	42	6	—	22
Propoxycaine	58	3	33	—	28	—	—	11	—	45
Proxymetacaine	62	26	41	—	35	—	—	—	—	—
Quinisocaine	61	55	46	—	28	—	—	25	13	66
Tetracaine	57	15	32	64	16	43	39	12	—	25
Thialbarbital	—	—	—	43	—	—	—	—	—	—

Analgesics, NSAIDs

The tabulated systems, previously described, may be used or system TG, below, which gives good separations.

System TG

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Ethyl acetate–methanol–strong ammonia solution (80 : 10 : 10).

Location reagents

The reagents for systems TD, TE and TF can be used as well as those given below.

Chromic acid solution A variety of colours are given by certain substances, e.g. diclofenac, red; diflunisal, blue–grey; feprazone, yellow; flufenamic acid, blue; indometacin, grey–brown; meclofenamic acid, violet; mefenamic acid, green; oxyphenbutazone, yellow; phenylbutazone, brown; salsalate, brown; sulindac, white.

Ludy Tenger reagent Orange or orange–brown spots are given by certain substances.

	TA	TB	TC	TD	TE	TF	TG	TL	TAD	TAE	TAJ	TAK	TAL
Acetanilide	—	—	—	45	70	45	—	—	52	80	—	—	—
Alclofenac	—	—	—	18	4	28	12	—	33	—	17	70	90
Aletamine	59	37	40	—	—	—	—	42	-	—	—	—	—
Aloxiprin	—	—	—	4	9	10	—	—	22	—	—	—	—
Aminophenazone	66	21	—	25	62	10	—	—	58	70	53	—	76
Aspirin	90	—	—	18	9	30	—	—	31	78	40	65	90
Salicylic acid	—	—	—	7	10	1	—	—	24	86	—	—	—
Salicyluric acid	—	—	—	—	—	—	—	—	—	—	—	—	—
Azapropazone	68	53	5	—	8	—	—	67	—	88	11	6	61
Benorilate	67	—	51	—	—	—	—	62	—	86	—	—	—
Benoxaprofen	—	—	—	—	—	—	14	—	—	—	56	80	99
Benzydamine	44	36	22	—	—	—	-	9	—	16	—	—	—
Bufexamac	—	—	—	11	18	19	36	—	31	—	—	—	—
Cinchophen	75	—	2	—	8	—	—	—	7	82	—	—	—

Analgesics, NSAIDs *continued*

	TA	*TB*	*TC*	*TD*	*TE*	*TF*	*TG*	*TL*	*TAD*	*TAE*	*TAJ*	*TAK*	*TAL*
Clonixin	—	—	—	—	—	—	30	—	—	—	40	14	80
Diclofenac	90	—	—	25	12	27	29	—	47	90	40	64	84
Diflunisal	—	—	—	8	16	5	37	—	18	89	6	69	69
Dipyrone	84	—	1	—	2	—	—	2	2	85	—	—	24
Etenzamide	64	3	59	—	76	—	—	55	—	87	—	—	—
Etofenamate	—	—	—	—	78	—	—	-	—	89	—	—	—
Etoxazene	65	—	56	—	—	—	—	67	—	—	57	2	70
Famprofazone	72	37	74	—	87	—	—	67	—	90	—	—	—
Fenbufen	92	—	—	18	4	30	9	—	39	—	43	68	91
Fenclofenac	—	—	—	—	—	—	20	—	—	—	—	—	—
Fendosal	95	—	—	—	22	—	—	—	—	—	5	68	83
Fenoprofen	96	—	—	42	6	38	16	—	50	—	58	78	76
Feprazone	—	—	—	—	19	—	45	—	—	92	—	—	—
Floctafenine	—	—	—	—	85	—	—	—	—	85	—	—	—
Flufenamic acid	96	—	—	—	18	—	37	—	—	84	55	78	95
Flunixin	96	—	—	—	12	—	33	—	—	—	37	20	83
Flurbiprofen	—	—	—	30	6	30	16	—	45	—	47	69	91
Glafenine	67	1	38	—	46	3	—	40	—	81	—	—	—
Ibuprofen	—	—	—	46	6	57	18	—	54	75	59	76	93
Indometacin	94	—	—	16	5	13	20	—	38	83	46	90	90
Indoprofen	—	—	—	—	—	—	08	—	—	—	—	—	—
Ketoprofen	—	—	—	27	6	25	14	—	41	85	54	82	98
Meclofenamic acid	—	—	—	—	12	43	38	—	—	—	59	77	92
Mefenamic acid	96	—	—	41	11	48	32	—	54	87	68	86	95
Methyl salicylate	96	—	—	—	84	68	—	—	—	—	95	86	—
Morazone	58	8	46	—	58	—	—	31	—	61	—	—	—
Naproxen	—	—	—	33	6	38	14	—	44	82	60	75	93
Nefopam	50	33	32	—	59	—	—	17	—	30	23	17	71
Nifenazone	57	—	—	—	36	—	—	—	—	58	—	—	—
Niflumic acid	—	—	—	3	11	3	28	—	15	88	27	56	90
Oxyphenbutazone	77	—	—	52	9	62	25	—	57	90	56	41	92
Paracetamol (acetaminophen)	95	—	—	15	45	32	—	—	26	77	30	5	73
Phenacetin	—	—	—	38	68	37	—	—	52	83	58	41	89
Phenazone	65	4	—	18	45	14	—	—	50	66	51	18	83
Phenazopyridine	59	1	50	—	70	—	—	53	—	80	46	56	91
Phenylbutazone	79	—	—	78	65	68	23	—	76	87	90	76	97
M (5-hydroxy)	—	—	—	8	3	18	—	—	—	88	—	—	—
Piroxicam	—	—	—	51	17	38	—	—	71	88	69	45	94
Propyphenazone	71	32	—	61	74	49	—	—	65	81	—	—	—
Salicylamide	—	—	—	38	50	55	—	—	43	83	—	—	—
Salsalate	—	—	—	—	—	—	23	—	—	—	—	—	—
Sulindac	—	—	—	14	4	10	13	—	34	87	39	40	92
Tenoxicam	—	—	—	—	14	6	—	—	—	87	—	—	—
Tiaprofenic acid	—	—	—	—	4	5	—	—	—	86	30	80	98
Tolfenamic acid	—	—	—	—	14	31	—	—	—	—	—	—	—
Tolmetin	—	—	—	13	5	20	10	—	30	85	20	59	83
Zomepirac	—	—	—	12	4	12	—	—	—	88	19	65	88

Antiemetics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAJ*	*TAK*	*TAL*
Benzquinamide	65	7	69	—	36	—	53	6	91
Cyclizine	57	48	41	68	16	39	23	11	72
Difenidol	61	56	45	91	51	—	24	13	67
Granisetron	—	18	—	51	—	14	—	—	—
Metoclopramide	47	1	7	51	13	17	—	—	—
Metopimazine	56	—	11	—	12	—	—	—	—
Thiethylperazine	51	30	41	52	8	27	22	11	83

Antifungals

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TAE*
Buclosamide	90	2	67	—	90
Chlorphenesin	82	—	—	62	—
Clotrimazole	—	—	—	76	80
Diamthazole	52	30	30	—	—
Econazole	80	9	61	75	78
Fenticlor	—	—	—	—	91
Fluconazole	—	—	—	35	67
Flucytosine	—	—	—	9	57
Griseofulvin	—	—	—	69	78
Hydroxystilbamidine	1	—	—	—	—
Itraconazole	—	1	—	79	87
Ketoconazole	—	—	—	50	68
Miconazole	73	11	67	80	77
Thioacetazone	78	—	—	—	—

Antibacterials

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TAE*
Amikacin	—	—	00
Aminosalicylic acid	70	—	—
Cefaloridine	42	—	—
Chloramphenicol	69	00	86
Cycloserine	44	1	—

Antibacterials *continued*

	TA	*TB*	*TAE*
Dibrompropamidine	1	—	—
Ethambutol	30	03	12
Ethionamide	65	00	—
Furazolidone	44	00	56
Hexetidine	70	48	30
Isoniazid	47	1	55
Methenamine	30	4	12
Metronidazole	58	2	75
Minocycline	—	—	88
Morinamide	54	8	—
Nalidixic acid	—	—	63
Nitrofurantoin	—	00	84
Noxytiolin	74	—	—
Oleandomycin	45	—	—
Propamidine	1	1	—
Protionamide	66	1	77
Pyrazinamide	63	3	71
Rifamycin SV	84	—	—
Salinazid	84	1	—
Thioacetazone	78	—	—
Tiocarlide	80	7	—
Tobramycin	—	—	—
Trimethoprim	55	00	45
Troleandomycin	65	—	—
Vancomycin	22	—	—

Anticholinergics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Adiphenine	64	56	60	83	51	49	—	—	—	—
Atropine	18	5	3	24	1	5	28	—	—	14
Atropine methonitrate	2	—	—	—	—	—	—	—	—	—
Benzatropine	13	26	6	—	2	6	—	—	—	—
Benzilonium bromide	3	—	—	—	—	—	—	—	—	5
Biperiden	64	68	64	83	64	45	—	37	12	73
Chlorphenoxamine	53	47	36	70	17	29	—	11	5	54
Clidinium bromide	2	—	—	1	—	3	—	—	—	—
Cyclopentolate	57	32	39	64	26	46	—	23	2	42
Cycrimine	66	67	61	—	60	—	—	—	—	—

Anticholinergics *continued*

	TA	TB	TC	TE	TL	TAE	TAF	TAJ	TAK	TAL
Dicycloverine	68	67	64	84	54	55	—	42	25	84
Diethazine	58	57	51	77	39	33	54	—	—	—
Emepronium bromide	5	—	—	2	—	3	—	—	—	—
Eucatropine	46	18	13	60	12	—	—	3	2	33
Glycopyrronium bromide	3	—	—	1	—	3	—	—	—	—
Hexocyclium metilsulfate	2	—	—	1	—	3	—	—	—	—
Homatropine	1	5	1	23	1	7	27	—	2	34
Homatropine methylbromide	—	—	—	—	—	—	12	—	—	—
Hyoscine	55	6	37	48	18	49	47	61	49	93
Hyoscyamine	18	—	—	26	—	—	—	—	1	34
Isopropamide iodide	5	—	5	3	—	3	41	—	—	19
Mepenzolate bromide	1	—	—	—	—	—	—	42	4	52
Methanthelinium bromide	2	—	—	76	—	3	—	—	—	—
Metixene	50	45	25	61	12	21	—	16	22	73
Orphenadrine	55	48	33	68	16	25	49	14	2	47
Oxyphencyclimine	2	1	3	6	—	2	18	—	1	24
Oxyphenonium bromide	3	—	1	1	—	2	36	—	—	—
Pentapiperide metilsulfate	1	—	1	—	—	—	—	—	—	—
Penthienate methobromide	2	—	—	3	—	9	—	—	—	—
Piperidolate	69	55	81	82	55	54	52	49	11	64
Procyclidine	48	62	31	74	23	20	68	—	—	36
Profenamine	67	64	47	83	66	31	55	22	10	57
Propantheline bromide	4	—	4	4	—	3	31	—	—	20
Tigloidine	42	39	21	—	7	—	—	—	—	—
Tricyclamol chloride	6	—	—	—	—	—	—	—	—	—
Trihexyphenidyl	68	66	61	83	59	43	75	38	22	80
Tropicamide	65	—	—	—	—	—	—	—	—	—

Anticonvulsants and barbiturates

The tabulated systems, previously described, together with the associated location reagents may be used or system TH, below, which gives good separations.

Systems TH

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Isopropyl alcohol–chloroform–strong ammonia solution (90 : 90 : 20).

Location reagents for systems TD, TE and TH

Mercuric chloride–diphenylcarbazone reagent White spots on a violet background are given in neutral systems, and violet spots on a pink background are given if the plate is alkaline.

Acidified potassium permanganate solution Yellow–brown spots on a violet background are given by drugs with unsaturated aliphatic bonds.

Zwikker's reagent Pink spots are given by 5,5-disubstituted barbiturates; green spots are given by thiobarbiturates; and faint pink spots are given by bromobarbiturates and by 1,5,5-trisubstituted barbiturates. The test is not very sensitive.

Fluorescein solution Spray the plates with a 10% solution of sodium hydroxide and heat at 100°C in an oven for 5 min before applying the reagent. Pink spots are given by bromobarbiturates.

Mercurous nitrate spray Barbiturates give dark spots which fade slowly; with some dilute solutions the spots fade rapidly.

	TA	TB	TC	TD	TE	TF	TH	TL	TAD	TAE	TAJ	TAK	TAL
Allobarbital	—	—	—	50	34	66	53	—	56	87	—	—	—
Alverine	66	65	39	—	—	—	—	38	—	—	—	—	—
Ambucetamide	73	5	68	—	76	—	—	61	—	76	—	—	—
Amobarbital	—	—	—	52	44	66	74	—	58	88	—	—	—
Aprobarbital	—	—	—	48	40	65	66	—	57	86	—	—	—
Barbital	—	—	—	41	32	61	51	—	57	84	—	—	—
Beclamide	65	8	65	—	—	—	—	64	—	90	—	—	—
Benactyzine	66	40	53	—	—	—	—	53	—	52	34	3	48
Brallobarbital	—	—	—	52	30	68	47	—	57	87	—	—	—
Butalbital	—	1	—	54	44	67	67	—	57	87	—	—	—
Butetamate	69	59	57	—	81	—	—	47	—	48	—	—	—

table continued

Anticonvulsants and barbiturates *continued*

	TA	TB	TC	TD	TE	TF	TH	TL	TAD	TAE	TAJ	TAK	TAL
Butobarbital	—	—	—	50	41	65	68	—	58	86	—	—	—
Carbamazepine	60	2	56	—	56	—	—	47	—	79	44	64	94
Clonazepam	72	—	53	35	67	45	—	61	56	85	50	53	91
Cyclobarbital	—	—	—	50	40	64	59	—	58	88	—	—	—
Cyclopentobarbital	—	—	—	50	39	65	62	—	59	90	66	63	90
Dimoxyline	68	16	75	—	87	—	—	58	—	—	64	10	93
Enallylpropymal	—	—	—	71	58	71	87	—	70	—	—	—	—
Ethosuximide	70	5	—	50	66	53	—	—	59	84	—	—	—
Ethotoin	88	—	—	53	71	54	—	—	60	—	61	66	91
Flavoxate	62	36	67	—	77	—	—	45	—	48	52	71	92
Heptabarb	—	—	—	50	38	64	62	—	59	88	—	—	—
Hexethal	—	—	—	53	44	67	74	—	60	—	—	—	—
Hexobarbital	—	—	—	65	53	65	85	—	69	85	—	—	—
Ibomal	—	—	—	50	32	66	61	—	56	91	—	—	—
Idobutal	—	—	—	55	41	69	71	—	59	—	—	—	—
Mebeverine	63	40	53	—	86	—	—	49	—	32	—	—	—
Mephenytoin	—	—	—	62	74	58	—	—	66	—	64	70	91
Mesuximide	76	—	—	—	86	—	—	—	—	90	85	70	98
Metharbital	—	—	—	66	54	65	86	—	69	87	—	—	—
Methylphenobarbital	—	—	—	70	41	67	72	—	70	86	—	—	—
Nealbarbital	—	—	—	58	44	68	78	—	60	92	—	—	—
Octamylamine	22	28	11	—	—	—	—	25	—	—	—	—	—
Oxcarbazepine	—	—	—	—	54	20	—	—	—	78	—	—	—
Papaverine	61	8	65	—	69	—	—	47	—	74	66	8	93
Paramethadione	86	—	—	—	7	60	—	—	56	—	87	70	94
Pentobarbital	—	—	—	55	45	66	76	—	59	90	—	—	—
Phenacemide	—	—	—	22	65	40	—	—	50	—	—	—	—
Pheneturide	76	—	—	38	71	53	—	—	59	—	—	—	—
Phenobarbital	—	—	—	47	28	65	38	—	53	85	—	—	—
Phensuximide	75	—	—	71	77	59	—	—	72	—	81	71	96
Phenytoin	—	—	—	33	41	55	—	—	53	86	48	84	96
Pipoxolan	77	53	68	—	—	—	—	56	—	—	—	—	—
Primidone	88	—	—	8	41	23	—	—	28	76	29	60	86
Secbutabarbital	—	—	—	50	48	64	69	—	57	88	—	—	—
Secobarbital	—	—	—	55	45	68	78	—	62	88	—	—	—
Sultiame	—	—	—	23	57	43	—	—	42	81	—	—	—
Talbutal	—	—	—	53	46	67	71	—	60	92	—	—	—
Thiamylal	—	—	—	—	55	75	—	—	—	—	—	—	—
Thiopental	—	—	—	77	49	74	80	—	68	—	73	71	92
Trimethadione	—	—	—	—	—	—	—	—	—	—	—	—	—
Valproic acid	—	—	—	—	—	52	—	—	—	—	—	—	—
Vinbarbital	—	—	—	50	34	65	56	—	57	89	56	62	91
Vinylbital	—	—	—	38	39	64	—	—	66	89	—	—	—

Antidepressants and antipsychotics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TL	TAE	TAF	TAJ	TAK	TAL
Acetophenazine	53	3	25	38	3	34	32	5	—	33
Amitriptyline	51	50	32	69	15	27	51	13	5	56
Benperidol	—	—	—	—	32	62	69	—	—	—
Butriptyline	59	61	48	—	38	—	—	22	8	61
Carfenazine	54	5	27	39	7	39	—	8	—	51

Antidepressants and antipsychotics *continued*

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Clomipramine	51	53	34	72	18	26	54	18	16	75
Clorgiline	67	42	70	—	59	—	—	—	—	—
Clozapine	57	4	38	55	17	42	—	—	—	—
Desipramine	26	19	11	40	3	7	71	7	23	72
Dibenzepin	54	22	35	55	14	38	22	—	—	—
Dosulepin	51	49	42	65	16	27	41	—	—	—
Doxepin	51	48	37	63	13	24	45	23	14	71
Droperidol	67	2	48	58	36	71	73	37	2	46
Fluoxetine	—	13	—	47	—	11	—	—	—	—
Fluvoxamine	—	12	—	46	—	18	—	—	—	—
Imipramine	48	48	23	67	13	21	47	7	2	52
Iprindole	47	49	34	—	16	—	—	—	—	—
Iproniazid	69	1	23	41	17	70	69	—	—	—
Isocarboxazid	71	20	74	75	61	84	86	67	67	92
Lofepramine	—	—	—	90	—	82	—	—	—	—
Maprotiline	15	18	5	36	2	6	71	—	—	50
Mebanazine	70	48	69	—	63	—	—	—	—	—
Mianserin	58	39	58	68	23	48	50	—	—	—
Moclobemide	—	1	—	52	—	65	—	—	—	—
Nialamide	70	2	25	—	4	68	64	—	—	—
Nomifensine	56	9	29	64	31	53	52	25	6	50
Nortriptyline	34	27	16	—	—	—	—	1	9	68
Noxiptiline	53	43	35	66	18	29	—	—	—	—
Opipramol	54	6	22	38	7	35	39	6	5	59
Paroxetine	—	4	—	40	—	8	—	4	4	54
Phenelzine	77	37	12	83	63	29	82	74	74	93
Protriptyline	19	18	7	38	2	6	69	4	28	76
Remoxipride	—	14	—	54	—	26	—	—	—	—
Sertraline	—	46	—	72	—	25	—	—	—	—
Tofenacin	45	26	21	48	7	14	—	—	—	—
Trazodone	63	10	58	66	37	64	61	55	—	66
Trifluoperazine	53	33	30	55	8	30	29	—	—	—
Trifluperidol	73	13	—	77	—	55	76	—	—	—
Trimipramine	59	62	54	80	37	36	56	—	—	—
Viloxazine	42	6	23	36	6	25	—	—	—	—
Zimeldine	47	27	25	48	—	20	—	—	—	—
Zuclopenthixol	56	7	32	44	11	45	—	—	—	—

Antihistamines

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Alimemazine	58	54	39	77	31	32	46	—	—	—
Antazoline	31	6	7	47	3	5	66	00	00	15
Bamipine	49	40	43	—	13	24	—	—	—	—
Bromazine	54	44	43	—	13	27	48	—	—	—
Brompheniramine	45	33	16	—	6	12	—	1	-	28
Buclizine	75	61	83	—	72	—	—	—	—	—
Carbinoxamine	48	26	19	50	4	13	16	4	—	27
Chlorcyclizine	57	42	46	67	14	35	52	21	10	70
Chloropyramine	52	41	28	63	17	22	—	—	—	—
Chlorphenamine	45	35	18	46	2	12	21	—	—	25
Cinnarizine	76	54	78	86	65	79	87	—	—	—
Clemastine	46	49	25	58	9	88	49	—	—	—

table continued

Antihistamines *continued*

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Clemizole	78	33	69	78	52	76	73	—	—	—
Cyclizine	57	48	41	68	16	39	52	23	11	72
Cyproheptadine	51	45	44	64	13	30	50	18	16	—
Deptropine	13	26	4	36	1	1	—	—	—	—
Dimenhydrinate	55 and 88	45 and 00	33 and 10	68 and 02	—	28 and 87	48 and 46	—	—	—
Dimetindene	42	36	13	47	6	10	—	—	—	40
Dimetotiazine	56	13	48	—	28	43	—	—	—	—
Diphenhydramine	55	44	33	65	15	27	48	—	—	—
Diphenylpyraline	46	42	28	68	8	23	49	61	50	92
Doxylamine	48	41	10	60	9	12	—	—	—	8
Histapyrrodine	60	—	—	75	—	32	—	—	—	—
Hydroxyzine	68	10	54	54	19	57	65	26	—	34
Isothipendyl	52	41	30	64	14	22	35	—	—	—
Levocabastine	—	—	—	12	—	76	—	—	—	—
Loratadine	—	20	—	78	—	86	—	—	—	—
Mebhydrolin	57	27	45	65	20	36	46	—	—	—
Meclozine	76	61	79	87	70	80	88	65	24	95
Mepyramine	51	39	25	58	14	22	33	—	—	—
Mequitazine	10	6	6	27	—	3	—	—	—	—
Methapyrilene	52	41	26	66	13	21	24	20	—	48
Methdilazine	29	32	15	63	6	—	—	12	19	72
Phenindamine	63	45	57	68	21	—	49	37	2	82
Pheniramine	45	35	13	46	3	14	26	—	—	—
Phenyltoloxamine	53	38	48	67	15	32	—	27	15	69
Pizotifen	48	45	—	64	—	28	—	—	—	—
Promethazine	50	36	35	65	17	30	44	—	—	—
Propiomazine	55	34	42	68	26	30	52	34	16	81
Pyrrobutamine	54	54	37	71	18	25	66	24	25	86
Thenalidine	50	38	44	52	12	20	—	—	—	—
Thenyldiamine	53	42	25	65	12	21	36	—	—	—
Thiazinamium metilsulfate	2	—	—	—	—	1	25	—	—	—
Thonzylamine	55	38	28	65	14	22	31	—	—	—
Tolpropamine	51	52	32	68	15	26	—	—	—	—
Trimethobenzamide	42	2	—	47	—	24	—	—	—	—
Tripelennamine	55	44	27	68	15	22	34	2	—	18
Triprolidine	51	11	20	55	6	19	30	95	3	69

Antimalarials

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAL*
Amodiaquine	62	8	40	74	37	38	—	9
Chloroquine	38	14	4	46	2	4	14	4
Chlorproguanil	3	—	1	—	1	—	—	—
Cinchonidine	49	6	8	44	6	24	55	70
Cinchonine	49	6	12	44	5	19	61	—
Halofantrine	—	50	—	88	—	56	—	—
Desbutylhalofantrine	—	12	—	61	—	19	—	—
Hydroxychloroquine	45	2	2	37	3	7	—	4
Primaquine	19	13	5	—	15	—	—	—
Proguanil	3	—	1	18	1	7	79	—
Pyrimethamine	61	2	31	58	21	66	—	—
Quinine	51	2	11	45	4	26	65	—

Antineoplastics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TF	TL	TAD	TAE
Aminoglutethimide	—	—	—	65	47	—	53	—
Chlorambucil	—	—	—	6	40	—	50	84
Cytarabine	5	—	1	—	—	1	—	69
Diethylstilbestrol	—	3	—	73	68	—	—	92
Doxorubicin	12	—	—	—	00	—	—	—
Epirubicin	—	—	—	—	—	—	—	—
Fluorouracil	—	—	—	4	20	—	—	—
Idarubicin	—	—	—	6	—	—	—	—
Mercaptopurine	—	—	—	2	—	—	—	77
Pipobroman	66	2	58	—	—	41	—	—
Procarbazine	49	2	10	80	—	4	68	88
Vinblastine	60	1	60	57	—	29	—	46

Antiparkinsonians

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TL	TAE	TAF
Amantadine	23	19	7	35	4	7	77
Benserazide	1	—	1	—	3	7	—
Bromocriptine	72	—	69	—	61	84	88
Levodopa	—	—	—	—	—	11	—
Selegiline	74	57	69	—	—	—	—

Antiprotozoals

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TL	TAD	TAE
Broxaldine	74	52	79	—	71	—	78
Broxyquinoline	51	—	6	—	3	—	—
Dehydroemetine	43	6	21	—	2	—	—
Hydroxystilbamidine	1	—	—	—	—	—	—
Metronidazole	58	2	36	46	40	32	75
Nifuratel	73	—	—	67	—	—	77
Nimorazole	—	3	44	58	33	—	60
Pentamidine	1	1	—	—	—	—	—

Antitussives

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TL	TAE	TAF	TAJ	TAK	TAL
Benzonatate	61	—	—	—	—	—	—	56	23	90
Bromhexine	75	67	79	—	71	84	—	98	28	78
Carbetapentane	48	48	22	—	—	—	—	—	—	—
Cephaëline	53	1	19	—	8	—	—	—	—	—
Clofedanol	52	41	37	—	29	32	—	—	—	—
Dextromethorphan	33	42	18	47	6	10	42	—	—	—
Dextrorphan	35	14	4	42	3	10	49	—	—	—
Dimethoxanate	39	18	24	49	6	21	38	10	84	49
Dropropizine	65	1	—	34	—	59	—	—	—	—
Guaifenesin	—	2	—	39	—	81	—	42	27	74
Isoaminile	68	58	54	81	55	45	—	—	—	—
Noscapine	64	21	74	78	64	72	75	65	18	93
Oxeladin	50	51	22	67	19	19	—	—	—	—
Pholcodine	36	3	18	25	2	15	—	—	—	—
Pipazetate	47	17	13	48	6	12	—	—	—	—

Benzodiazepines and hypnotics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TD*	*TE*	*TF*	*TL*	*TAD*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Acecarbromal	—	—	—	49	57	48	—	60	84	84	61	58	90
Apronal	—	—	—	33	67	52	—	—	—	—	—	—	—
Bromazepam	61	6	41	13	63	—	53	47	73	69	34	4	63
Brotizolam	72	5	52	15	52	5	27	53	72	71	—	—	—
M-6-hydroxy	68	1	35	5	28	4	13	37	76	76	—	—	—
M-α-hydroxy	72	2	46	7	45	6	31	41	78	78	—	—	—
Camazepam	76	12	73	55	75	—	65	69	82	83	—	—	—
Carbromal	—	12	—	53	75	55	—	64	85	87	—	—	—
Chlordiazepoxide	62	2	50	10	52	—	22	53	76	77	48	2	79
Clobazam	62	8	70	53	75	—	62	70	84	85	—	—	—
Clomethiazole	64	44	69	—	76	—	58	—	80	85	—	—	—
Clonazepam	72	00	53	35	67	45	61	56	85	87	50	53	91
Clorazepic acid	84	3	56	34	68	—	60	57	83	87	—	—	—
Ethchlorvynol	—	—	—	81	—	74	—	82	87	—	73	77	96
Ethinamate	76	5	—	49	76	59	—	58	86	87	58	69	91
Demoxepam	63	—	35	15	41	—	51	42	81	83	42	38	89
Diazepam	75	27	73	58	76	—	59	72	82	85	67	48	96
Flumazenil	71	3	63	30	61	14	44	61	76	72	—	—	—
Flunitrazepam	63	10	72	54	74	47	63	72	80	82	69	59	95
Flurazepam	62	30	48	3	71	3	40	41	52	45	32	8	73
Glutethimide	75	31	—	63	80	62	—	70	86	89	—	—	—
Ketazolam	66	14	64	45	74	—	66	62	83	80	—	—	—
Loprazolam	40	1	48	3	40	1	5	36	26	15	—	—	—
Lorazepam	52	1	36	23	43	—	28	42	82	82	46	42	86
Lormetazepam	52	6	61	46	59	45	50	60	82	82	—	—	—
Mecloqualone	—	25	—	—	76	—	—	—	80	—	77	68	96
Medazepam	67	41	74	54	78	—	62	73	79	83	70	12	95
Methaqualone	70	36	80	63	78	—	56	—	79	84	—	—	—
Methylpentynol	—	—	—	49	74	62	—	57	—	—	—	—	—
Methyprylon	58	—	—	31	63	25	—	55	78	—	—	—	—
Midazolam	72	6	60	13	60	5	19	53	69	70	—	—	—
Nitrazepam	68	—	36	35	64	46	55	53	84	86	53	52	92
Nordazepam	62	3	55	34	67	—	60	57	82	83	53	60	92
Oxazepam	56	—	40	22	45	—	51	42	82	91	47	47	89
Prazepam	65	36	74	64	81	—	63	72	84	89	75	69	94
Quazepam	74	27	75	78	83	71	76	78	87	96	—	—	—
M(2-oxo)	78	16	89	59	80	57	71	70	87	90	—	—	—
M(3-hydroxy-2-oxo)	71	2	55	42	69	52	59	58	88	90	—	—	—
M(3-hydroxy-*N*-dealkyl-2-oxo)	67	—	30	15	49	28	38	35	88	89	—	—	—
M(*N*-dealkyl-2-oxo)	75	2	56	34	72	45	58	60	83	88	—	—	—
Temazepam	53	8	59	51	62	47	53	65	82	82	65	54	92
Triazolam	60	1	40	5	44	2	16	41	68	65	—	—	—
Zopiclone	—	4	—	—	47	—	—	—	42	—	—	—	—

Bronchodilators

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*
Bambuterol	—	2	—	37	—	18	—
Bambuterol monocarbamate	—	—	—	21	—	19	—
Bamifylline	65	—	54	—	34	71	—
Butetamate	69	59	57	81	47	48	56

Bronchodilators *continued*

	TA	TB	TC	TE	TL	TAE	TAF
Protokylol	65	1	3	—	6	—	—
Rimiterol	—	—	—	6	—	7	—
Salbutamol	46	1	1	20	4	16	74

Cannabinoids

The tabulated systems, previously described, may be used together with the associated location reagents or systems TI and TJ, below. These systems may be used for extracts of both cannabis and cannabis resin.

System TI

- *Plates*: Silica gel G, 250 µm thick, dipped in, or sprayed with, a 10% solution of silver nitrate, and dried.
- *Mobile phase*: Toluene, using unsaturated (open tank) conditions.

System TJ

- *Plates*: Silica gel G, 250 µm thick, sprayed with diethylamine immediately before use.
- *Mobile phase*: Xylene–hexane–diethylamine (25 : 10 : 1).

Location reagents for systems TI and TJ

Fast blue B solution Cannabidiol gives an orange colour, cannabinol gives a violet colour and Δ^9-tetrahydrocannabinol gives a red colour. The colours may be intensified by overspraying with 1 mol/L sodium hydroxide or by exposing the plate to ammonia fumes.

Duquenois reagent After spraying with the reagent, overspray the plate with hydrochloric acid. Blue to violet colours are given by cannabinoids.

	TA	TE	TI	TJ	TAH	TAJ	TAK	TAL
Δ^9-THC	11	31	30	29	50	00	1	31
CBN	94	95	52	20	45	90	77	97
CBD	94	95	5	36	60	88	76	97

Cardioactive drugs

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	TB	TC	TE	TF	TL	TAE	TAF	TAJ	TAK	TAL
Ajmaline	62	7	—	56	—	—	22	—	—	—	—
Amiodarone	72	62	68	82	—	55	54	64	—	—	—
Aprindine	—	63	—	76	—	—	20	—	—	—	—
Azapetine	70	57	67	78	—	56	14	—	59	26	90
Bamethan	55	4	6	—	—	00	23	—	—	—	—
Benziodarone	—	—	—	23	58	—	—	—	—	—	—
Benzthiazide	—	—	—	9	51	—	—	—	31	6	71
Bethanidine	1	—	—	—	—	—	—	—	—	—	—
Bretylium tosilate	1	—	—	—	—	—	—	—	—	—	—
Buphenine	74	3	14	62	—	50	33	83	—	—	—
Butalamine	68	—	—	86	29	—	—	—	—	—	—
Captopril	—	—	—	—	1	—	—	—	—	—	—
Carbocromen	48	17	24	62	—	12	18	—	—	—	—
Clonidine	62	8	31	70	—	53	44	76	9	2	51
Clopamide	—	—	—	55	38	—	—	—	—	—	—
Co-dergocrine mesilate	66	1	48	—	—	29	—	—	—	—	—
Cyclandelate	—	37	—	80	77	—	87	95	81	71	95
Debrisoquine	1	—	—	—	—	00	—	—	—	3	36
Deserpidine	72	3	77	81	—	66	73	—	—	—	—
Digitoxin	—	—	—	36	10	—	88	—	35	1	78
Digoxin	—	—	—	33	05	—	85	—	—	—	—
Dihydralazine	55	34	2	18	—	01	—	—	—	—	—
Disopyramide	45	7	8	60	—	—	9	7	—	—	—
Doxazosin	—	—	—	73	—	—	71	—	—	—	—
Enalapril	—	—	—	—	—	—	85	—	—	—	—
(Enalapril)	—	—	—	—	00	—	—	—	—	—	—
(Enalaprilat)	—	—	—	—	00	—	—	—	—	—	—
Encainide	—	28	—	54	—	—	16	—	—	—	—
Felodipine	—	2	—	77	60	—	87	—	—	—	—
Flecainide	—	6	—	49	—	—	28	—	—	—	—
Glyceryl trinitrate	—	—	—	86	72	—	—	—	—	—	—
Guanethidine	01	—	2	1	—	00	3	30	—	—	16

table continued

Cardioactive drugs *continued*

	TA	*TB*	*TC*	*TE*	*TF*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Guanoclor	03	—	—	—	—	00	—	—	—	—	—
Guanoxan	01	—	—	—	—	00	3	76	—	—	—
Heptaminol	23	1	2	22	—	05	14	—	—	—	—
Hexamethonium bromide	00	—	—	—	—	—	—	—	—	—	—
Hexobendine	47	10	44	16	—	06	12	—	—	—	—
Hydralazine	51	41	11	80	—	64	73	—	1	1	25
Hydroquinidine	45	3	8	43	—	05	20	70	—	—	—
Indoramin	84	—	—	74	—	—	—	—	13	10	77
Inositol nicotinate	57	1	43	—	—	16	—	—	—	—	—
Isoxsuprine	78	3	32	62	—	53	62	81	13	5	60
Labetalol	—	—	—	29	01	—	—	32	—	—	—
Lanatoside C	—	—	—	6	—	—	—	89	—	—	—
Lidoflazine	70	11	63	70	—	36	—	70	77	—	—
Lofexidine	—	—	—	53	—	—	—	17	—	—	—
Lorcainide	—	48	—	80	—	—	—	41	—	—	—
Mecamylamine	16	51	2	—	—	04	—	—	—	—	16
Methoserpidine	72	4	77	—	—	64	—	—	—	—	—
Methyldopa	49	1	1	2	—	01	—	60	75	—	—
Mexiletine	40	17	4	55	—	09	—	25	78	—	—
Minoxidil	51	—	3	18	—	00	—	44	—	—	—
Moxisylyte	52	—	44	—	—	19	—	—	—	—	—
Naftidrofuryl oxalate	64	—	41	—	—	35	—	43	—	—	—
Nicametate	56	41	35	—	—	20	—	35	—	—	—
Nicergoline	64	—	—	73	—	—	—	43	—	—	—
Nicofuranose	61	42	70	—	—	—	—	—	—	—	—
Nicotinyl alcohol	56	4	17	—	—	22	—	74	69	—	—
Nifedipine	68	1	65	—	—	68	—	79	—	—	—
Pargyline	70	—	—	60	—	—	—	77	—	71	20
Pempidine	24	68	3	—	—	—	—	—	—	—	—
Pentaerithrityl tetranitrate	—	—	—	72	—	—	—	92	—	—	—
Pentifylline	55	6	66	66	—	46	—	72	—	—	—
Pentolonium tartrate	00	—	—	—	—	00	—	—	1	—	—
Pentoxifylline	—	—	—	55	—	—	—	64	—	49	12
Perhexiline	41	57	8	59	—	06	—	8	—	—	—
(Perindopril)	—	—	—	6	00	—	—	—	—	—	—
(Perindoprilat)	—	—	—	3	00	—	—	—	—	—	—
Phenoxybenzamine	73	63	76	87	—	68	—	84	97	65	4
Phentolamine	32	1	3	33	—	02	—	6	—	—	1
Prajmalium bitartrate	59	—	—	—	—	—	—	8	—	—	—
Prazosin	60	1	47	59	—	49	—	68	74	39	—
Prenylamine	68	55	68	84	—	56	—	43	85	47	63
Procainamide	49	1	5	39	—	09	—	17	33	—	—
Quinidine	51	4	12	49	—	06	—	30	63	—	2
Rescinnamine	73	1	75	81	—	64	—	77	79	—	—
Reserpine	69	2	74	77	—	63	—	76	80	56	6
Sotalol	53	1	3	30	—	05	—	19	—	—	—
Strophanthin-K	—	—	—	—	—	—	—	81	—	—	—
Tocainide	60	2	23	44	—	—	—	42	74	—	—
Tolazoline	13	2	2	25	—	02	—	3	55	—	—
Trimetaphan camsilate	02	—	—	—	00	—	—	—	—	—	1
Trimetazidine	22	5	4	—	—	—	—	—	—	—	—
Verapamil	59	23	70	73	—	42	—	43	61	—	—
Xamoterol	—	—	—	15	00	—	—	18	—	—	—
Xantinol nicotinate	41	—	—	21	00	—	—	26	—	—	—

Coumarins and other anticoagulants

The tabulated systems, previously described, may be used together with the associated location reagents.

	TD	*TE*	*TF*	*TAD*	*TAE*	*TAJ*	*TAK*	*TAL*
Acenocoumarol	52	16	48	60	92	68	51	92
Anisindione	—	15	—	—	—	82	70	95
Dicoumarol	18	30	32	33	88	60	80	96
Diphenadione	11	46	33	53	—	51	74	91
Ethyl biscoumacetate	4	24	32	21	—	—	—	—
Phenindione	65	21	56	70	—	—	—	—
Phenprocoumon	62	19	58	61	93	—	—	—
Warfarin	64	18	62	64	—	—	—	—

Diuretics

The tabulated systems, previously described, may be used together with the associated location reagents.

Location reagents

The reagents given for systems TD, TE and TF can be used as well as that given below.

N-(1-Naphthyl)ethylenediamine solution Spray the plate with dilute sulfuric acid, expose it to nitrogen dioxide vapour for 15 min and then spray with the reagent.

	TA	*TD*	*TE*	*TF*	*TAD*	*TAE*	*TAJ*	*TAK*	*TAL*
Acetazolamide	85	4	3	31	18	84	18	1	60
Amiloride	24	—	24	—	—	6	—	—	29
Bendroflumethiazide	—	25	52	71	30	—	38	11	72
Benzthiazide	—	14	9	51	30	—	31	6	71
Bumetanide	—	1	4	10	6	87	18	42	80
Chlorothiazide	—	2	2	16	11	—	11	—	41
Chlortalidone	—	4	42	40	23	88	17	10	63
Clopamide	79	19	55	38	39	—	—	—	—
Clorexolone	76	31	60	51	47	79	—	—	—
Cyclopenthiazide	—	21	66	62	27	—	—	—	—
Cyclothiazide	77	18	59	60	26	—	—	—	—
Epithiazide	—	13	44	62	25	88	22	5	63
Etacrynic acid	96	3	5	2	5	71	5	42	57
Ethiazide	—	11	50	50	—	—	—	—	—
Ethoxzolamide	76	43	43	65	51	—	51	48	91
Furosemide	—	1	6	7	7	86	10	25	70
Hydrochlorothiazide	—	4	34	34	11	78	9	—	40
Hydroflumethiazide	86	7	36	47	13	87	9	—	43
Indapamide	—	38	66	61	46	89	56	22	92
Mefruside	—	45	67	58	55	—	—	—	—
Methyclothiazide	87	19	53	50	27	—	30	8	69
Metolazone	—	23	57	51	33	—	34	8	75
Polythiazide	—	22	63	60	32	—	35	8	70
Quinethazone	75	4	40	21	15	—	11	6	56
Spironolactone	—	66	78	51	75	84	73	64	96
Triamterene	51	—	30	—	13	50	4	—	40
Trichlormethiazide	80	15	14	60	23	88	24	5	61
Urea	55	—	15	—	—	—	5	15	30
Xipamide	—	38	13	64	36	93	—	—	—

Drugs of abuse

The tabulated systems, previously described, may be used together with the associated location reagents. A further three systems (TAH, TAI and TAN), described below, may be used for drugs of abuse. Refer to Chapter 11 for R_f values.

System TAH

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Hexane–diethyl ether (80 : 20).

System TAI

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Acetone.

System TAN

- *Plates*: Silica gel G, 250 μm thick.
- *Mobile phase*: Butanol–acetic acid–water (2 : 1 : 1).

	TA	TB	TC	TE	TL	TAE	TAF	TAJ	TAK	TAL
5-Methyltryptamine	56	—	—	—	—	—	—	—	—	—
Amfetamine	43	20	9	43	18	12	75	—	—	—
Benzfetamine	73	67	70	87	70	60	—	—	—	—
Benzoylecgonine	21	0	1	—	—	—	—	—	—	—
Bufotenine	35	0	1	33	1	10	34	—	—	—
Cannabidiol	94	—	—	95	—	—	—	88	76	97
Cannabinol	94	—	—	95	—	—	—	90	77	97
Cocaine	65	45	47	77	54	35	30	13	0	2
Δ^9-THC	11	—	—	31	—	—	—	0	1	31
Diamorphine	47	15	38	49	4	26	33	25	5	64
Diethyltryptamine	46	15	10	63	11	14	56	2	3	41
Dimethyltryptamine	40	9	9	50	6	14	39	—	—	—
DOM	51	15	17	41	16	9	76	—	—	—
Ketamine	63	37	63	79	64	68	72	47	4	43
Lysergic acid	58	0	0	0	0	70	16	48	7	79
Lysergide	60	3	39	56	24	60	59	33	2	59
Mescaline	20	3	10	24	12	6	63	2	9	51
Metamfetamine	31	28	13	42	5	9	63	0	3	45
Methadone	48	59	20	77	27	16	60	8	0	45
Methylenedioxyamfetamine	39	18	12	42	17	10	76	—	—	—
Methylenedioxymethamfetamine	33	24	—	39	—	8	—	3	17	57
N-Methyltryptamine	18	—	—	—	—	—	—	—	—	—
p-Methoxyamfetamine	73	23	77	43	69	9	74	4	18	58
Monoacetylmorphine	46	6	19	—	—	—	—	13	2	51
Morphine	37	0	9	20	1	18	23	0	0	15
Psilocin	39	5	9	47	9	14	48	—	—	—
Psilocybin	5	0	—	0	0	80	1	—	—	—

Ergot alkaloids

Ardrey RE, Moffat AC (1979). *J Forensic Sci Soc* 19: 253–282.

The tabulated systems, previously described, may be used together with the associated location reagents.

System TL, previously described, may be used or system TM below. Note that these systems can be run in a single tank as they use the same mobile phase and have low correlation of hR_f values.

System TM

- *Plates*: Aluminium oxide, 250 μm thick.
- *Mobile phase*: Acetone.
- *Reference compounds*: Lysergide hR_f 70, ergotamine hR_f 48, ergometrine hR_f 26.

Location reagents for systems TL and TM

Naphthoquinone sulfonate solution Spray the plate with the reagent, then spray with a 10% v/v solution of hydrochloric acid and heat at 110°C for 20 min. Red–violet spots on a light pink background are given by ergot alkaloids.

Nitrosonaphthol solution Spray the plate with the reagent, then spray with a 10% v/v solution of hydrochloric acid and heat at 110°C for 20 min. Blue–black spots on a yellow background are given by ergot alkaloids.

Van Urk's reagent After spraying the plate, heat in an oven at 100°C for 5 min. Blue spots are given by ergot alkaloids.

	TA	TB	TC	TE	TL	TM	TAE	TAF	TAJ	TAK	TAL
Co-dergocrine	66	1	48	—	29	64	—	—	—	—	—
Dihydroergotamine	60	1	28	42	14	40	58	—	33	3	84
Ergometrine	57	00	12	33	08	26	62	60	17	00	37
Ergotamine	63	1	34	44	23	48	68	64	—	—	—
Ergotoxine	66	1	62	—	48	67	—	—	—	—	—
Lysergamide	60	00	19	36	6	27	57	51	—	—	—
Lysergic acid	58	—	—	—	00	00	70	16	48	7	79
Lysergide	60	3	39	3	24	70	60	59	33	2	59
Methylergometrine	62	00	14	41	12	31	69	—	—	—	—
Methysergide	65	1	21	45	12	33	—	—	23	4	66

Narcotic analgesics and narcotic antagonists

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Alphaprodine	50	30	35	62	11	28	—	23	5	60
Amiphenazole	61	2	33	62	57	—	—	—	—	—
Anileridine	73	12	56	79	51	60	66	20	—	68
Bezitramide	71	41	79	—	70	92	96	—	—	—
Buprenorphine	76	9	68	80	69	80	—	62	4	77
Butorphanol	—	—	—	—	—	—	—	16	—	36
Codeine	33	6	18	35	3	21	22	10	—	26
Cyclazocine	53	15	13	65	25	24	74	6	5	60
Dextromoramide	73	42	71	79	60	72	78	—	—	—
Dextropropoxyphene	68	59	55	—	—	—	—	33	4	51
Diamorphine	47	15	38	49	4	26	33	25	5	64
Dihydrocodeine	26	8	13	29	2	11	19	6	—	38
Dihydromorphine	25	2	3	18	1	12	—	—	—	25
Dipipanone	66	67	33	87	70	27	72	—	—	—
Embutramide	72	—	—	59	—	—	—	53	46	—
Ethoheptazine	40	45	19	55	4	12	41	8	12	65
Ethylmorphine	40	7	22	36	6	21	26	13	4	54
Fentanyl	70	43	74	78	58	70	77	59	8	84
Hydromorphone	23	3	9	18	2	12	14	—	—	—
Ketobemidone	47	2	9	37	6	26	—	3	1	40
Levallorphan	67	19	24	74	45	42	73	10	6	66
Levorphanol	35	13	7	—	—	—	—	—	—	—
Methadone	48	59	20	77	27	16	60	8	—	45
Morphine	37	—	9	20	1	18	23	—	—	15
Nalbuphine	—	—	—	34	—	58	—	19	—	20
Nalorphine	59	1	23	32	29	57	59	18	1	46
Naloxone	65	9	66	47	63	74	—	58	1	45
Naltrexone	—	—	—	—	—	—	—	49	1	40
Norcodeine	13	—	5	—	—	—	—	—	—	—
Normethadone	56	40	34	—	—	—	—	19	20	78
Normorphine	17	—	—	—	—	—	—	—	—	28
Norpipanone	68	58	50	80	38	43	—	—	—	—
Oxycodone	50	25	51	62	39	30	33	27	1	36
Oxymorphone	48	10	37	33	30	27	36	13	—	13
Pentazocine	61	16	12	70	28	34	72	2	3	57
Pethidine	52	37	34	60	11	34	40	14	6	72
Phenazocine	68	16	39	74	49	50	81	26	20	90
Phenoperidine	71	26	64	76	58	70	82	—	—	—
Piminodine	67	36	64	88	59	63	77	51	24	90
Piritramide	70	1	45	61	42	73	74	—	—	—
Profadol	42	8	6	—	8	—	—	—	—	—
Racemorphan	34	14	9	—	2	—	—	—	—	—
Thebacon	45	20	34	49	11	24	25	—	—	—
Tilidate	—	—	—	84	—	61	—	47	3	38
Tramadol	—	—	—	78	—	30	—	—	—	—
Trimeperidine	58	41	41	—	17	—	—	—	—	—

Oral hypoglycaemics and antidiabetics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TD*	*TE*	*TF*	*TAD*	*TAE*	*TAK*	*TAL*
Acetohexamide	—	39	12	43	53	—	66	91
Buformin	2	—	—	—	—	—	—	—
Carbutamide	78	—	—	—	—	87	—	—
Chlorpropamide	72	38	10	43	49	87	78	6
Glibenclamide	80	30	11	30	57	90	—	—
Glibornuride	—	40	5	60	54	92	—	—
Gliclazide	—	—	9	—	—	84	—	—
Glipizide	87	—	7	—	—	86	—	—
Glymidine sodium	76	—	5	—	—	—	—	—
Metformin	1	—	00 and 80	—	—	03 and 93	—	—
Phenformin	3	—	—	—	—	—	3	29
Tolazamide	—	52	7	50	66	86	71	95
Tolbutamide	76	51	12	55	62	88	74	93

Pesticides

System TW

Getz ME, Wheeler HG (1968). *J Ass Off Anal Chem* 51: 1101–1107.

- *Plates*: Silica gel, 250 µm thick.
- *Mobile phase*: Cyclohexane–acetone–chloroform (70 : 25 : 5).

Location reagent Allow the plate to dry in air, heat at 110°C for 2 h, allow to cool, spray with molybdate–antimony reagent, and then lightly overspray with ascorbic acid reagent.

System TX

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: *N*-Hexane–acetone (80 : 20).
- *Reference compounds*: Triazophos hR_f 20, parathion-methyl hR_f 30, pirimiphos-methyl hR_f 49, quintozen hR_f 84.

System TY

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Toluene–acetone (95 : 5).
- *Reference compounds*: Carbofuran hR_f 20, azinphos-methyl hR_f 46, methidathion hR_f 60, parathion-ethyl hR_f 85.

System TZ

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform–acetone (90 : 10).

System TAA

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Chloroform.

System TAB

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Dichloromethane.

System TAC

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Ethyl acetate–isooctane (85 : 15).

Refer to the pesticides chapter (Chapter 16, Table 16.) for hR_f values for systems TX, TY, TZ, TAA, TAB and TAC.

	TW
Azinphos-methyl	57
Diazinon	82
Dichlorvos	42
Dimethoate	19
Disulfoton	100
Malathion	74
Mevinphos	23
Oxydemeton-methyl	00
Parathion	81
Parathion-methyl	77
Phorate	100
Trichlorphon	9

Phenothiazines and other tranquillisers

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Acepromazine	48	26	24	63	12	28	—	4	—	39
Acetophenazine	53	3	25	38	3	34	32	5	—	33
Azacyclonol	10	—	3	14	1	3	—	—	—	40
Benzoctamine	59	57	52	—	43	38	—	31	14	65
Butaperazine	53	28	37	5	5	26	—	10	—	42
Captodiame	66	49	—	77	—	47	—	—	—	—
Chlormezanone	66	1	63	68	57	84	80	55	45	94
Chlorpromazine	49	45	35	70	17	25	45	11	2	47
Chlorprothixene	56	51	51	74	25	34	51	20	8	65
Clopenthixol	56	7	32	44	11	45	—	—	—	—
Clotiapine	59	41	59	—	23	—	—	—	—	—
Dixyrazine	—	—	—	49	—	47	—	—	—	—

Phenothiazines and other tranquillisers *continued*

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Ethomoxane	60	34	47	—	36	—	—	—	—	—
Fluanisone	73	39	68	82	60	67	75	—	—	—
Flupentixol	62	6	33	46	—	50	—	—	—	—
Fluphenazine	63	5	23	45	10	45	49	6	—	41
Fluspirilene	69	4	59	71	49	63	78	—	—	—
Haloperidol	67	11	27	76	33	51	75	6	2	61
Levomepromazine	57	47	38	76	46	32	49	27	19	81
Loxapine	—	36	—	54	—	49	—	45	9	78
Mebutamate	—	—	33	60	56	82	85	35	47	87
Meprobamate	75	—	32	56	58	63	87	35	29	78
Mesoridazine	38	3	6	30	1	11	—	2	2	52
Molindone	—	—	—	—	—	—	—	24	—	37
Oxypertine	68	4	65	78	58	74	—	—	—	—
Pecazine	53	47	44	65	16	27	—	—	—	—
Penfluridol	76	17	60	84	60	72	89	—	—	—
Perazine	48	25	37	47	3	21	23	—	—	—
Pericyazine	58	4	16	51	18	46	61	—	—	—
Perphenazine	55	7	29	42	9	40	40	—	3	56
Phenaglycodol	—	—	—	71	—	84	—	—	—	—
Pimozide	71	3	60	71	40	73	82	—	—	—
Pipamperone	56	1	12	43	8	33	61	—	—	—
Piperacetazine	56	6	19	—	17	—	—	4	—	30
Pipotiazine	66	3	32	53	21	40	59	—	—	—
Prochlorperazine	49	34	37	55	7	26	26	18	9	74
Promazine	44	38	30	62	11	18	35	6	2	41
Prothipendyl	47	43	23	59	9	15	29	—	—	—
Sulforidazine	—	—	—	54	—	—	—	—	—	—
Sulpiride	38	—	—	34	—	17	—	—	—	—
Tetrabenazine	69	41	78	79	67	80	—	83	33	97
Thiopropazate	61	35	53	74	42	62	59	52	36	91
Thioproperazine	46	7	34	43	6	22	22	—	—	—
Thioridazine	48	42	30	67	13	20	55	9	2	51
Tiotixene	49	10	40	44	7	26	24	19	5	71
Triflupromazine	54	47	35	75	22	32	49	—	—	—
Trimetozine	61	11	72	68	52	80	—	—	—	—
Tybamate	77	—	—	65	—	—	—	—	—	—

Psychomimetics and sympathomimetics

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TL*	*TAE*	*TAF*	*TAK*	*TAL*
Acetylcholine chloride	2	—	—	—	—	—	—	—	—
Adrenaline (epinephrine)	—	—	1	13	—	3	—	—	—
Amidefrine	15	—	1	—	2	1	—	—	—
Carbachol	—	—	—	—	—	4	23	—	—
Clorprenaline	57	18	15	—	20	—	—	—	—
Cyclopentamine	20	32	10	66	2	6	68	—	—
Dobutamine	52	—	1	49	3	87	—	—	—
Dopamine	18	—	—	43	—	14	59	—	7
Ephedrine	30	5	5	25	1	10	64	1	29
Etafedrine	44	35	9	56	15	14	—	6	49
Ethylnoradrenaline	42	1	2	15	24	—	—	—	15
Etilefrine	41	2	2	22	3	15	74	—	—

table continued

Psychomimetics and sympathomimetics *continued*

	TA	TB	TC	TE	TL	TAE	TAF	TAK	TAL
Fenoterol	76	—	1	25	4	38	81	—	—
Hexoprenaline	3	1	—	71	1	12	—	—	—
Hordenine	40	5	6	52	5	—	—	—	20
Hydroxyamfetamine	35	2	2	—	11	—	—	—	19
Isoetarine	59	—	—	36	—	73	—	—	26
Isometheptene	24	—	—	—	—	—	—	4	43
Isoprenaline	40	—	1	21	3	14	69	—	—
Mephentermine	25	34	8	40	2	6	—	3	36
Metaraminol	42	1	1	18	24	13	76	—	20
Methoxamine	55	24	4	11	38	12	73	12	50
Methoxyphenamine	23	26	4	32	2	7	—	20	64
Methylephedrine	32	—	—	35	—	12	—	—	—
Naphazoline	14	3	6	27	—	3	52	—	—
Orciprenaline	48	1	3	18	6	21	77	—	—
Oxedrine	25	4	1	—	—	—	—	—	—
Oxymetazoline	9	1	1	34	1	80	—	1	25
Phenylephrine	33	1	1	12	—	8	67	—	22
Phenylpropanolamine	44	4	4	—	—	—	—	2	29
Pholedrine	29	3	3	27	3	9	—	—	—
Pilocarpine	53	—	32	44	12	52	45	—	44
Prenalterol	47	1	9	25	—	—	—	—	—
Pseudoephedrine	33	54	4	17	63	9	—	1	30
Salbutamol	46	1	1	20	4	16	74	—	—
Terbutaline	47	1	1	21	5	18	77	—	29
Tetryzoline	13	7	2	26	2	5	60	—	—
Tramazoline	6	4	2	30	2	4	—	—	—
Tuaminoheptane	33	1	7	—	24	—	—	—	—
Xylometazoline	13	7	5	30	3	5	64	—	—

Quaternary ammonium compounds

Systems TN and TO

Stevens H, Moffat AC (1974). *J Forensic Sci Soc* 14: 141–148.

System TN

- *Plates*: Cellulose, 250 μm thick.
- *Mobile phase*: Ammonium formate–formic acid–water–tetrahydrofuran (1 : 5 : 95 : 233).

System TO

- *Plates*: Silica gel (without gypsum), 250 μm thick.
- *Mobile phase*: Methanol–0.2 mol/L hydrochloric acid (80 : 20).

Location reagents for systems TN and TO

Acidified iodoplatinate solution Violet, blue–violet, grey–violet or brown–violet spots on a pink background are given by quaternary ammonium compounds.

Cobalt thiocyanate solution Blue spots are given by quaternary ammonium compounds.

	TN	TO
Acetylcholine chloride	70	60
Atropine methonitrate	95	35
Bretylium tosilate	94	40
Cetrimide	100	50
Choline	60	60
Decamethonium bromide	56	16

Quaternary ammonium compounds *continued*

	TN	TO
Guanethidine	56	50
Hexamethonium bromide	36	10
Pancuronium bromide	80	—
Paraquat dichloride	22	10
Suxamethonium chloride	35	10
Suxethonium bromide	40	23
Tubocurarine chloride	85	40

Steroids

The tabulated systems, previously described, may be used or systems TP, TQ, TR, TS and TAM.

Systems TP, TQ, TR and TS

Lund W, ed. (1979). *Pharmaceutical Codex*, 11th edn. London: Pharmaceutical Press: 940.

System TP

- *Plates*: Silica gel G, 250 μm thick.
- *Mobile phase*: Methylene chloride–ether–methanol–water (77 : 15 : 8 : 1.2).

System TQ

- *Plates*: Silica gel G, 250 μm thick.
- *Mobile phase*: Dichloroethane–methanol–water (95 : 5 : 0.2).

System TR

- *Plates*: Kieselguhr, 250 µm thick, impregnated with a mixture of acetone–formamide (9 : 1).
- *Mobile phase*: Toluene–chloroform (3 : 1).

System TS

- *Plates*: Kieselguhr, 250 µm thick, impregnated with a mixture of acetone–propylene glycol (9 : 1).
- *Mobile phase*: Cyclohexane–toluene (1 : 1).

System TAM

Professor George Maylin: personal communication.

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: The plate is run to 5 cm in a TLC system of chloroform–ethyl acetate–methanol (50 : 45 : 5), dried and then re-run to 7 cm in the solvent composition of system TE, ethyl acetate–methanol–strong ammonia solution (85 : 10 : 5).

Location reagents for systems TP, TQ, TR, TS and TAM

DPST solution

Sulfuric acid–ethanol reagent Spray the plate and then heat at 105°C for 10 min.

p-Toluenesulfonic acid solution Heat the plate at 120°C for 15 min, cool, spray with the reagent, heat again at 120°C for 10 min, and re-spray. Refer also to the Index of Colour Tests in the Indexes of Analytical Data.

	TA	*TB*	*TE*	*TF*	*TP*	*TQ*	*TR*	*TS*	*TAE*	*TAJ*	*TAK*	*TAL*	*TAM*
Androstanolone	—	—	—	—	78	11	90	72	—	—	—	—	—
Androsterone	—	16	72	52	—	—	—	—	90	—	—	—	—
Beclometasone	—	—	—	—	75	38	89	42	—	—	—	—	—
Betamethasone	—	—	—	—	30	00	00	00	—	38	08	80	70
Betamethasone valerate	—	—	—	—	58	27	20	02	—	—	—	—	—
Cortisone	90	3	68	—	72	28	55	—	87	51	9	83	91
Desoxycortone	—	—	—	—	86	52	98	95	—	78	71	96	91
Dexamethasone	—	—	—	—	32	8	—	—	—	38	7	75	66
Dimethisterone	—	—	—	—	80	42	91	95	—	—	—	—	—
Dydrogesterone	—	—	—	—	86	53	96	98	—	—	—	—	—
Ethisterone	—	—	—	—	78	39	80	—	—	—	—	—	—
Ethylestrenol	—	—	—	—	79	50	94	99	—	—	—	—	—
Etynodiol diacetate	—	11	71	57	83	61	95	99	89	—	—	—	—
Fludrocortisone	—	—	—	—	—	—	—	—	—	55	35	91	90
Fludrocortisone acetate	90	—	86	—	58	12	30	00	—	—	—	—	—
Fluocinolone acetonide	—	—	—	—	42	8	10	1	—	—	—	—	68
Fluocortolone	—	—	50	28	—	—	—	—	—	—	—	—	—
Fluocortolone hexanoate	—	—	—	—	79	39	88	00	—	—	—	—	—
Fluocortolone pivalate	—	—	—	—	78	35	89	58	—	—	—	—	—
Fluorometholone	—	—	68	52	—	—	—	—	91	46	26	90	86
Fluoxymesterone	—	—	—	—	51	9	38	16	—	41	35	91	74
Gestonorone caproate	—	31	83	59	—	—	—	—	—	—	—	—	—
Halcinonide	—	—	—	—	—	—	—	—	—	62	58	91	—
Hydrocortisone	96	00	45	28	27	02	08	00	86	36	05	74	58
Hydrocortisone acetate	—	—	—	—	51	11	38	00	—	—	—	—	—
Hydrocortisone hydrogen succinate	—	—	—	—	08	00	00	00	—	—	—	—	—
Hydrocortisone sodium phosphate	—	—	—	—	00	00	00	00	—	—	—	—	—
Hydroxyprogesterone	—	38	85	63	—	—	—	—	86	—	—	—	—
Hydroxyprogesterone caproate	—	—	—	—	81	55	99	90	—	—	—	—	—
Lynestrenol	—	—	—	—	77	55	99	97	—	—	—	—	—
Medroxyprogesterone acetate	—	—	—	—	80	50	98	85	—	—	—	—	—
Megestrol acetate	—	—	—	—	80	50	98	85	—	—	—	—	—
Metenolone	—	—	87	62	—	—	—	—	92	—	—	—	—
Methandienone	86	—	80	—	65	10	87	61	—	44	61	92	88
Methylprednisolone	87	—	41	27	23	80	3	—	87	31	13	78	56
Methyltestosterone	89	17	73	47	70	16	91	71	86	60	65	92	92
Nandrolone	—	—	—	—	88	49	97	95	—	—	—	—	—
Norethandrolone	—	—	—	—	71	20	95	78	—	—	—	—	—
Norethisterone	—	20	76	57	71	22	87	63	86	—	—	—	—
Norethisterone acetate	—	—	—	—	87	39	98	90	—	—	—	—	—

table continued

Steroids *continued*

	TA	*TB*	*TE*	*TF*	*TP*	*TQ*	*TR*	*TS*	*TAE*	*TAJ*	*TAK*	*TAL*	*TAM*
Noretynodrel	—	—	—	—	79	32	91	71	—	—	—	—	—
Oxymetholone	95	—	9	—	69	23	85	82	—	70	74	94	86
Paramethasone	91	—	88	—	—	—	—	—	—	54	39	91	91
Prednisolone	—	—	41	24	20	—	2	—	86	19	3	65	54
Prednisone	—	—	45	28	41	—	10	—	84	33	4	74	60
Progesterone	—	36	79	56	81	20	99	95	83	76	68	95	97
Stanozolol	78	—	—	—	—	—	—	—	—	43	56	91	78
Testosterone:	—	14	70	45	60	07	90	63	85	59	63	92	92
Testosterone phenylpropionate	—	—	—	—	86	28	99	98	—	—	—	—	—
Testosterone propionate	—	—	—	—	78	12	99	98	—	—	—	—	—
Triamcinolone	79	—	27	—	09	00	00	00	—	14	6	65	33
Triamcinolone acetonide	—	—	—	—	32	00	20	06	—	—	—	—	—

Sulfonamides

Systems TT, TU and TV

De Clercq H *et al.* (1977). *J Pharm Sci* 66: 1269–1275.

Sulfonamides are difficult to separate, but these systems are effective and may be used in combination. System TF, previously described, may also be used.

System TT

- *Plates*: Silica gel G, 250 µm thick.
- *Mobile phase*: Hexanol.

System TU

- *Plates*: Aluminium oxide, 250 µm thick.
- *Mobile phase*: Acetone–ammonia solution 25% (80 : 15).

System TV

- *Plates*: Aluminium oxide, 250 µm thick.
- *Mobile phase*: Chloroform–methanol (70 : 30).

Location reagents for systems TF, TT, TU and TV

Acidified potassium permanganate solution Yellow–brown spots on a violet background are given by sulfonamides.

Copper sulfate solution This detects *N*-substituted sulfonamides.

Mercuric chloride–diphenylcarbazone reagent Blue spots are given by sulfonamides.

Van Urk's reagent After spraying, heat the plates in an oven at 100°C for 5 min. Yellow spots are given by sulfonamides.

	TF	*TT*	*TU*	*TV*
Carbutamide	—	90	27	7
Chlorpropamide	43	84	43	3
Mafenide	1	—	—	—
Phthalylsulfacetamide	00	—	—	—
Phthalylsulfathiazole	00	2	4	4
Succinylsulfathiazole	00	2	1	1
Sulfamerazine	41	33	18	7
Sulfametopyrazine	50	—	—	—
Sulfacetamide	42	53	37	4
Sulfadiazine	39	24	22	3
Sulfadimethoxine	51	85	52	34
Sulfadimidine	45	50	27	62
Sulfaethidole	35	—	—	—
Sulfafurazole	52	74	48	4
Sulfaguanidine	6	21	90	48
Sulfamethizole	23	46	36	2
Sulfamethoxazole	54	88	33	2
Sulfamethoxydiazine	43	55	17	15
Sulfamethoxypyridazine	39	53	26	50
Sulfanilamide	46	61	96	66
Sulfaphenazole	51	89	70	13
Sulfapyridine	42	47	43	73
Sulfasalazine	00	—	—	—
Sulfasomidine	16	11	49	20
Sulfathiazole	20	53	40	5
Tolbutamide	55	98	35	4

Vitamins

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TC*	*TE*	*TL*	*TAE*	*TAF*
Nicotinamide	54	21	40	27	68	66
Nicotinic acid	58	17	—	—	72	—
Pyridoxine	59	8	15	5	75	67
Thiamine	1	—	1	—	2	18

Xanthine stimulants

The tabulated systems, previously described, may be used together with the associated location reagents.

	TA	*TB*	*TC*	*TE*	*TF*	*TL*	*TAE*	*TAF*	*TAJ*	*TAK*	*TAL*
Acefylline piperazine	4	1	1	—	—	1	—	—	—	—	—
Caffeine	52	3	58	52	10	25	59	55	54	18	81
Diprophylline	48	—	12	25	—	12	70	59	—	—	—
Etamiphylline	54	12	39	74	—	17	—	—	28	2	44
Etofylline	—	—	—	38	6	—	66	—	—	—	—
Fenetylline	55	3	45	54	—	14	44	—	—	—	—
Proxyphylline	58	2	33	49	—	29	71	—	—	—	—
Theobromine	53	1	31	34	4	21	59	54	32	8	65
Theophylline	75	1	30	11	9	11	74	66	40	21	78

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CHAPTER

40 Gas Chromatography

S Dawling

The application of the principles of adsorption and partition to the technique of gas chromatography (GC) is attributed to the German scientist Fritz Prior in the late 1940s, and to Martin and Synge in the early 1950s, respectively. These discoveries undoubtedly paved the way for the adoption of GC as one of the most broadly used analytical techniques, and also earned the latter two UK scientists the Nobel Prize for chemistry in 1952. GC is applicable to a wide range of compounds of interest to toxicologists, pharmaceutical and industrial chemists, environmentalists and clinicians. If a compound has sufficient volatility for its molecules to be in the gas or vapour phase at or below 400°C, and does not decompose at these temperatures, then the compound can probably be analysed by GC.

The separation is performed in a column (containing either a solid or liquid stationary phase) that has a continuous flow of mobile phase passing through it (usually an inert carrier gas, but more recently supercritical fluids (SCFs) have been used for some applications), maintained in a temperature-regulated oven. When a mixture of substances is injected at the inlet, each component is adsorbed to some extent by the stationary phase, and also partitions between the stationary phase and the gas phase as it is swept towards the detector. Molecules that have greater affinity for the stationary phase spend more time in that phase and consequently take longer to reach the detector. The detector produces a signal proportional to the amount of substance that passes through it, and this signal is processed and fed into a recording device. Each substance that elutes from the column has a characteristic retention time, defined as the time interval from injection to peak detector response. Figure 40.1 shows a schematic of a GC system.

Identification of components was traditionally based primarily on peak retention time, but it is becoming increasingly more reliant on the nature of the response obtained from the detector. The analyst has two main goals: first, to make each different compound appear in a discrete band or peak with no overlap (or co-elution) with other components in the mixture, and, second, to make these bands uniform in shape and as narrow as possible. This is achieved partly by judicious choice of the column stationary phase and its loading, and partly by optimising the operating conditions of the column and detector. In addition, the method of introducing the sample into the chromatograph, the detector choice and chemical modification to improve the volatility of the compounds also contribute to converting a mediocre analysis into a first-class one.

There has been a continuous synergism between enhanced detector performance and column performance; each advance being mutually dependent on the other. Initially, high-sensitivity detectors permitted the development of a precise column theory that, in turn, enabled the design of columns that had much higher efficiencies. This improved efficiency, however, produced peaks of small volume compared with the sensing volume and dispersion that occurred in the collecting tubes of the contemporary detectors. Consequently, efficiency became limited by the geometry of the detector, and not by its intrinsic sensitivity. Detector design was modified to incorporate smaller tube dimensions, and the volume of the sensing cell was thus reduced greatly. The introduction of capillary columns suitable for routine use in GC in the mid-1980s provided much higher resolution efficiencies and smaller peak volumes, which provoked further modifications in detector design. The latest column developments mean that peaks of only a few milliseconds are a real possibility in both GC and liquid chromatography (LC). Since this matches the response time of the current sensor electronics, the ingenuity of the detector design engineers is again being tested. Today's biggest challenges are in the area of data manipulation as increased sensitivity and column resolution produce an enormous amount of data.

Gas chromatography columns

Packed columns made of glass or steel contain a stationary phase (see below), either loaded directly into the column if it is a solid at its operating temperature, or coated onto the surface of a solid support if it is liquid at its operating temperature. Thus, the operating principle of GC can be distinguished as either gas–solid chromatography (mainly an absorptive process by the stationary phase) or gas–liquid chromatography (mainly partition of the analytes between the mobile and stationary phases), based on the physical characteristics of the stationary phase. Capillary columns, introduced in the early 1980s, have now replaced packed columns for most applications. The original glass columns were fragile and have been superseded by fused-silica capillary columns. Fused silica is high-purity synthetic quartz, with a protective coating of polyimide applied to the outer surface. Since these columns retain their flexibility only as long as the coating remains undamaged, their operating temperature must be maintained below 360°C for standard columns (400°C for high-temperature polyimide coatings). The first capillary columns were 0.2, 0.25 or 0.32 mm in internal diameter (i.d.) and between 10 and 50 m long. Subsequently 'megabore' (0.53 mm i.d.), 'minibore' (0.18 mm i.d.) and 'microbore' (0.1 or 0.05 mm i.d.) columns have evolved. When coated with a heat-resistant polymer, these have the advantages of flexibility and strength, and can be threaded with ease through intricate pipe work. A single column can be fitted into almost any manufacturer's GC. Capillary columns provide improved resolution, sensitivity and durability, less bleed with increasing temperatures and ease of maintenance and repair, and they yield reliable and highly reproducible separations, typically over many hundreds or thousands of injections. This has reduced the number of columns required to achieve a satisfactory separation of quite complex mixtures.

The internal surface of the silica is deactivated by a variety of processes that can react silanol groups (Si–OH) on the silica surface with a silane reagent (usually a methyl or phenylmethyl surface is created). For gas–solid capillary chromatography, a fine layer (usually less than 10 μm) of stationary phase particles is adhered to the tubing (porous layer open tubular, or PLOT, columns). For gas–liquid capillary chromatography, the stationary phase may be coated or bonded directly onto the walls of the column (wall-coated open tubular, or WCOT), or onto a support (e.g. microcrystals of sodium or barium chloride) bonded to the column wall (support-coated open tubular, or SCOT). Cross-linked stationary phases have the individual polymer chains linked via covalent bonds. Both bonding and cross-linking impart enhanced thermal and solvent stability to the stationary phases (often designated 'DB'), and these should be used if they are available. Stainless-steel capillaries are usually reserved for applications that require extremes of temperature, or where the possibility of column breakage cannot be tolerated. Nowadays, the internal surface is specially deactivated chemically or by lining with fused silica, which allows for more flexibility and greater durability, and also reduces the possibility of degradation of analytes, which can be catalysed by the exposed metal surfaces.

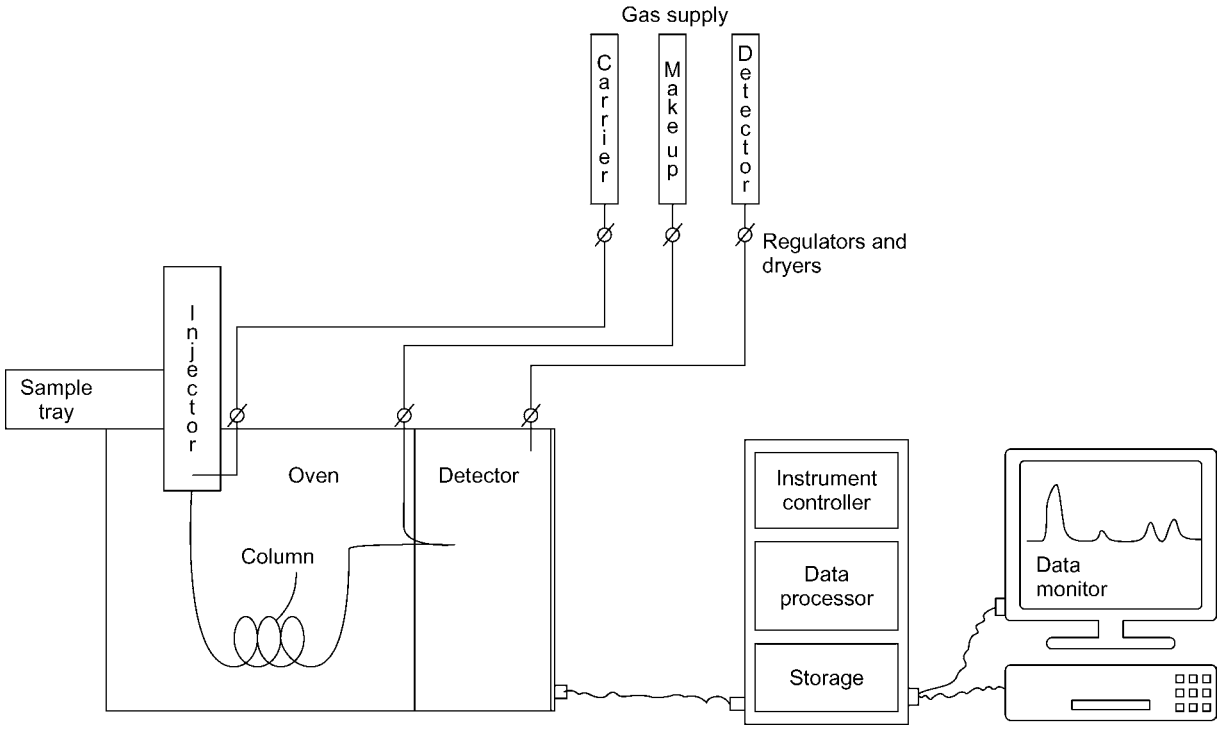

Figure 40.1 A modern GC system.

Stationary phases and support materials

Solid stationary phases

In gas–solid chromatography, the stationary phase is an active solid at its operating temperature. A conventional packed column is filled completely by stationary phase particles, but in a capillary column a fine layer (usually less than 10 μm) of particles is adhered by proprietary processes to the inner surface of the tubing, to create a PLOT column. These solid phases may be inorganic materials (e.g. aluminium oxides, molecular sieves, silica gel or graphitised carbon), or they may be organic polymers such as styrene. Both packed and capillary columns use similar solid-phase materials. Sample compounds undergo a dynamic gas–solid adsorption–desorption process with the stationary phase and, since the particles are porous, size exclusion and shape selectivity processes also occur. The carrier gas (mobile phase) merely serves to sweep towards the detector those solute molecules that are not currently adsorbed. The resultant columns are highly retentive, and separations impossible with liquid phases can be accomplished easily on PLOT columns above ambient temperature. These columns are generally reserved for the separation of low-molecular-weight materials, such as hydrocarbon and sulfur gases, noble and permanent gases, and low-boiling solvents. Since PLOT columns occasionally shed particles, their use is not advised with detectors that are affected adversely by the intrusion of particulate matter (the mass spectrometer is particularly vulnerable, as the column interface operates under vacuum).

Graphitised carbon black Carbopaks are graphitised carbon black, having adsorptive surfaces of up to 100 m^2/g. They are usually modified with a light coating of a polar stationary phase. Difficult separations of the C_1 to C_{10} hydrocarbons can be achieved rapidly. Carbopak C with 0.2% Carbowax 20 M has been used to resolve substances abused by 'glue-sniffers'. Carbopak C modified with 0.2% Carbowax 1500 and Carbopak C with 0.8% tetrahydroxyethylenediamine (THEED) are useful for the analysis of ethanol and ethylene glycol, respectively, in blood. Resolution is superior to that obtained with the Porapak and Chromosorb polymers, although the elution order is similar.

Molecular sieves Activated alumina is unique for its extremely wide pore-diameter range, and is very useful for separating most C_1 to C_4 molecules, separating light hydrocarbon saturates from unsaturates in the C_1 to C_5 range, and separating benzene, toluene and xylenes. Deactivation of alumina with potassium hydroxide reverses the elution of some molecules (acetylene and *n*-butane). Carbosieves are granular carbon molecular sieves that give good separation of C_1 to C_3 hydrocarbons. Carboxen 1006 is useful in resolving formaldehyde, water and methanol, and impurities in ethylene. Zeolites (5A, 13X) give a good general separation of inorganic gases. Carbon dioxide is irreversibly adsorbed below 160°C. Oxygen, nitrogen, carbon monoxide and methane are well separated. These columns have a tendency to adsorb water and carbon dioxide, which results in changes in retention over time.

Polymers Chromosorb 101–108 and Porapak are divinylbenzene cross-linked polystyrene copolymers. Incorporation of other functional groups, such as acrylonitrile and acrylic esters, into the polymer matrix provides moderately polar-to-polar surfaces with different pore sizes and surface areas (polarity increases with ascending number or letter). HayeSep phases are polymers of divinylbenzene and ethylene glycol dimethylacrylate. Separations range from free fatty acids to free amines, and small alcohols from methanol to pentanol. Tenax-TA is a porous polymer of 2,6-diphenyl-*p*-phenylene oxide, used both as a chromatographic phase and as a trap for volatile substances prior to analysis. It is also used for high-boiling alcohols, poly(ethylene glycol)s (PEGs), phenols, aldehydes, ketones, ethanolamines and chlorinated aromatics.

Liquid stationary phases

In gas–liquid chromatography (GLC), the stationary phase is a liquid or gum at the normal operating temperature. Components injected into the column are partitioned between the moving (mobile) gas phase and a stationary phase. Molecules that have greater affinity for the stationary phase spend more time immobilised in the column and consequently take longer to reach the detector. The process of immobilisation and subsequent release back into the mobile phase occurs thousands of times during the course of the analysis. The separation of components is dependent, to a large extent, on the chemical nature of the stationary phase. Stationary phases are essentially two types of high-boiling polymers – siloxanes (often incorrectly called silicones) and PEGs. Chiral stationary phases based on cyclodextrins (cyclic glucose chains) have

specific applications in the separation of enantiomers and are discussed separately.

Polysiloxanes Standard polysiloxanes (PSXs) are characterised by their repeating siloxane backbone in which each silicon atom has the potential to attach two functional groups, the type and amount of which distinguish the stationary phase and its properties (Fig. 40.2). The basic PSX is 100% methyl substituted so, when other groups are present, the amount is indicated as a percentage of the total substituent groups. For example, if 5% of Si atoms contain two phenyl groups and the remaining 95% of Si atoms are methyl substituted, this may be written as (5% diphenyl/95% dimethyl)-PSX, (5% phenyl/95% methyl)-PSX or simply as (5% phenyl)-PSX. In some instances, two different groups are present on the same Si atom, so a (10% cyanopropylphenyl/dimethyl)-PSX contains a total of 5% cyanopropyl, 5% phenyl and 90% methyl residues (see Fig. 40.2).

While PSXs are generally less polar in nature than PEGs, the substitution of polar residues for a proportion of the methyl groups confers added polarity to the column. Polar phases retain polar compounds more effectively than do non-polar compounds, and vice versa. The 100% methyl-substituted PSX is often considered the 'standard' non-polar phase, and has been used extensively in compilations of retention indices. This column is an ideal choice for starting a new application. However, substitution by *n*-octyl groups (up to 50%) renders the column extremely non-polar, and similar to squalene. Substitution with up to 5% phenyl groups still furnishes an essentially non-polar column with improved thermal stability. This phase has also been used for retention index (RI) work and is another good column with which to start a new application. Increasing the phenyl substitution to 20%, 35% or 50% yields columns classed as intermediate in polarity, which predictably retain aromatic compounds relative to aliphatic solutes. All these are available as bonded phases that can be solvent rinsed, are not damaged by organic acids or bases, and can tolerate small injections of water if sufficiently highly loaded, but are sensitive to strong inorganic acids and bases.

Substitution of cyanopropylphenyl groups (typically 6% or 14%) creates an intermediate-polarity mixed phase with unique elution relative to simple phenyl substitution but renders the column more susceptible to damage from oxygen, moisture and mineral acids. Fifty percent substitution is specifically designed to separate *cis*- and *trans*-fatty acid methyl esters (FAMEs). However, even low-level bleeding of the stationary phase produces a high background signal with certain types of detector (nitrogen–phosphorus detector). More polar columns are

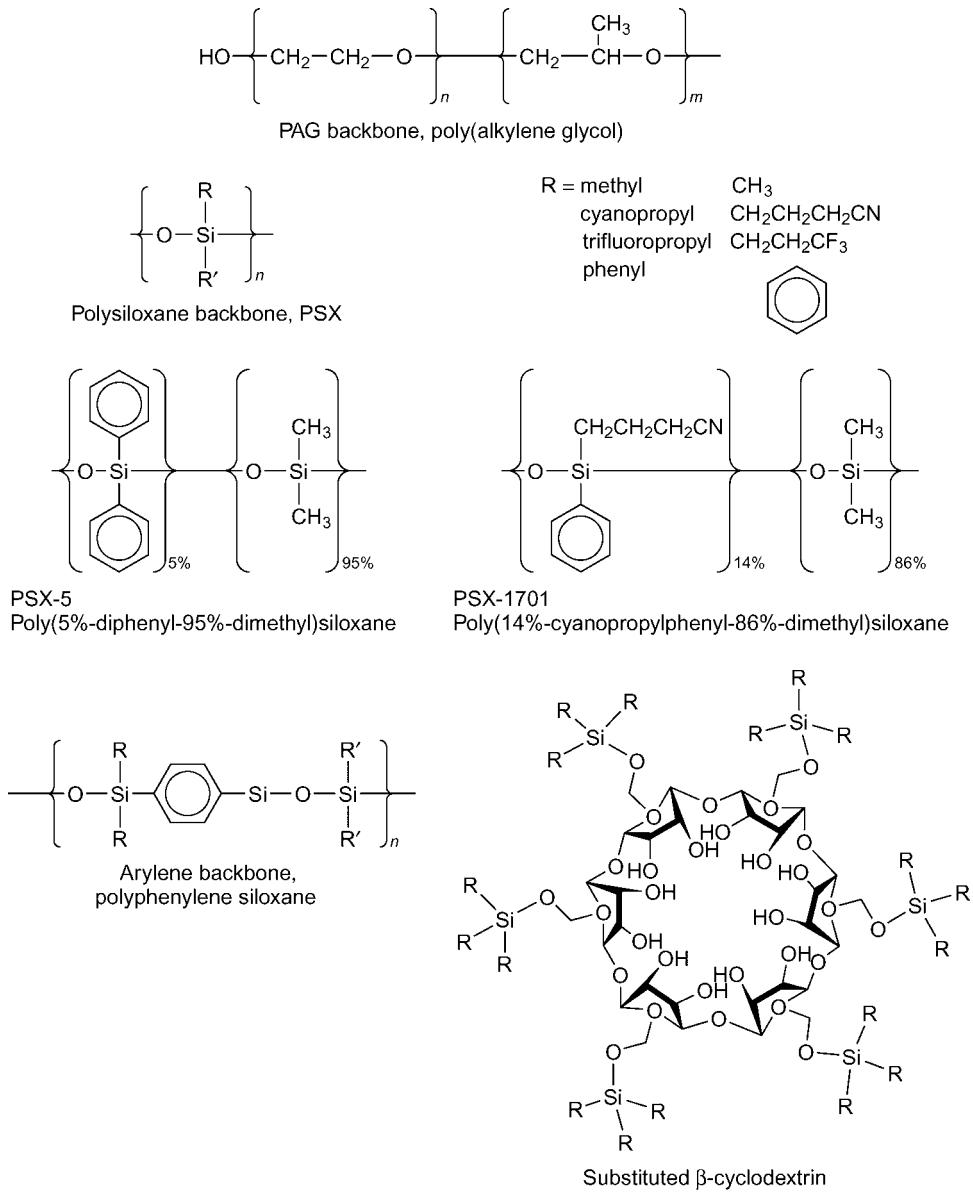

Figure 40.2 Structures of some common capillary GC stationary phases.

produced by substitution of biscyanopropyl and cyanopropylphenyl groups (80 : 20 or 90 : 10). Substitution with 100% biscyanopropyl gives the highest polarity of the PSXs. This phase can be operated at both high and low temperatures. To date, these are non-bonded columns, and therefore should not be rinsed with solvent. Trifluoropropyl-methyl-PSX is a mid-to-high polarity phase especially suited for otherwise difficult-to-separate positional isomers. Its unique interactions with nitro, halogen, carbonyl and other electronegative groups give it application in the analysis of herbicides and pesticides.

Increasing polarity in general is associated with some negative effects. Polar phases tend to have a narrower operating temperature range (higher minimum, lower maximum), are more prone to bleed at higher temperatures, are more sensitive to moisture and oxygen, and consequently have a shorter life expectancy than non-polar phases. More recently, low-bleed arylene stationary phases (sometimes designated 'mass spectrometry' or 'ms') have been introduced that have phenyl groups incorporated into the siloxane backbone (see Fig. 40.2). The incorporated phenyl groups confer additional strength to the backbone, which prevents the formation of cyclic fragments and associated 'bleed' at higher temperatures.

Poly(ethylene glycol)s PEGs are widely used polar stationary phases and their general structure (PAGs) is shown in Fig. 40.2. They are less stable, less robust (they are especially prone to oxidative damage), have lower temperature limits and a shorter life expectancy than polar PSX phases, but they have unique resolving qualities. Acid-modified PEG (FFAP) substituted with terephthalic acid is especially useful for separating acidic polar compounds, such as acids, alcohols, acrylates, ketones and nitriles. Nitroterephthalic acid-substituted PEG (e.g. Nukol) is designed for volatile fatty acids and phenols. Both are highly resistant to damage from water-based samples. Base-modified PEG (CAM) is suited to analysis of strongly basic compounds, such as primary amines, that do not chromatograph well on polar PSXs. Since this phase is usually cross-linked, it cannot be used with water or alcohol, but it can be solvent rinsed. New bonded and cross-linked PEG phases are now available to separate free fatty acids and other organic acids. These show superior inertness and can tolerate repeated injections of water, alcohols, aldehydes and acids without the need for acidification.

Chiral phases Second-generation chiral phases are based on cyclodextrins (toroidal shaped structures formed by α_{1-4} linkages of multiple glucose units). The enzyme cyclodextrin glucosyltransferase is used to cleave partially digested starch, and link the glucose units into three forms, referred to as α, β and γ, that have six, seven and eight glucose units, respectively. The mouth of the cyclodextrin molecule has a larger circumference than the base and is linked through secondary hydroxyl groups of the C2 and C3 atoms of each glucose unit. The primary hydroxyl groups are located at the base of the torus, on the C6 atoms. The number of glucose units thus determines the cavity size and electrophilic orientation (see Fig. 40.2), and affects the elution order of the enantiomeric forms. The hydroxyl groups can be functionalised selectively to provide various physical properties and inclusion selectivities. Six different cyclodextrin derivatives are manufactured: permethylated hydroxypropyl (PH), dialkylated (DA), trifluoroacetylated (TFA), propionylated (PN), butyrylated (BP) and permethylated (PM). Changes in elution order can be seen between the different derivatives, and also between cyclodextrin cavity sizes. Unlike the cyclodextrins used in high performance liquid chromatography (HPLC), these phases separate both aromatic and non-aromatic enantiomers of a wide range of chemicals, including saturated alcohols, amines, carboxylic acids, epoxides, diols, lactones, amino alcohols, amino acids, esters, pyrans and furans. Each phase has an area of specificity, as summarised in Table 40.1. Derivatised cyclodextrins are thermally stable, highly crystalline and virtually insoluble in most organic solvents. Chiral phases are fragile, however, and unless chemically bonded or cross-linked, they cannot be washed with solvent or taken to temperatures outside the 0–225°C range.

A subsequent development has been the embedding of PM cyclodextrins (usually 10% or 20% by volume) into columns that contain standard liquid stationary phases of intermediate polarity, such as 35% phenyl-PSX. Silyl-substituted cyclodextrins, such as 2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl are also available embedded (usually 25–35% by weight) in 20% phenyl-PSX, another intermediate polarity stationary phase. These columns are useful for separating positional isomers (phenols, xylenes, etc.), as well as enantiomers.

Table 40.1 Chiral stationary phases and their analytical applications

Phase	*Application*
α-PH	Small linear and saturated amines, alcohols, carboxylic acids and epoxides
β-PH	Linear and cyclic amines and alcohols, carboxylic acids, amino alcohols, sugars, epoxides and haloalkanes
γ-PH	Cyclic and bicyclic diols, steroids and carbohydrates
α-DA	Small cyclic amines, alcohols and epoxides
β-DA	Nitrogen heterocyclics, lactones, aromatic amines, sugars, amino acid derivatives, bicyclics and epoxides
γ-DA	Large cyclic diols, aromatic amines with more than one ring, and multi-ring or compounds with bulky substituents
α-TA	Small alcohols, amino alcohols, amino alkanes and diols
β-TA	Alkyl alcohols, halocarboxylic acid esters, amino alkanes, halocycloalkanes, lactones, diols, alkyl halides, furans and pyrans
γ-TA	Large range of alcohols, diols, polyols, halocarbons, lactones, amine alcohols, halocarboxylic acid esters, furans, pyrans, epoxides, glycidyl analogues and haloepihydrins
γ-PN	Epoxides, alcohols $>C_4$ and lactones
γ-BP	Amino acids, some primary amines and furans
β-PM	Largest range of acids, alcohols, barbiturates, diols, epoxides, esters, halocarbons, ketones, lactones, terpenes; first choice for chiral method development

Solute–stationary phase interactions

For liquid stationary phases, three major types of interaction determine chromatographic elution: dispersion, dipole–dipole interaction and hydrogen bonding.

Table 40.2 shows the contribution of each of these interactions for the common types of liquid stationary phases. It should be remembered that hydrogen bonding interactions are considerably stronger than dipole–dipole interactions, which are themselves stronger than dispersion interactions. Thus, although the dispersion interaction between the various stationary phases is listed as strong or very strong, and the hydrogen bonding interactions as weak or moderate, if the analyte has functional groups that can undergo hydrogen bonding with the particular stationary phase employed, the hydrogen bonding interaction is likely to be stronger than interaction by dispersion. However, the reverse is true if the analyte also has a high proportion of groups in its molecular structure that can participate in dispersion interactions, as would be the case, for example, for long-chain fatty acids.

Dispersion is the dominant interaction for all PSX and PEG stationary phases; it can be simplified into the concept that the more volatile the compound (the lower its boiling point), the more likely it is to be in the mobile phase and so the faster it elutes from the column. Although this holds true for groups of compounds with similar functional groups or

Table 40.2 Contribution of different types of interactions to solute separation on GC stationary phases

Functional group	*Type of interaction*		
	Dispersion	*Dipole–dipole*	*Hydrogen bonding*
Methyl-PSX	Strong	None	None
Phenyl-PSX	Very strong	None	Weak
Cyanopropyl-PSX	Strong	Very strong	Moderate
Trifluoropropyl-PSX	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate

within homologous series, it cannot be applied universally. In general, a difference of 30°C in boiling point is sufficient to predict and maintain elution order, but differences of less than 10°C can be overturned by the influence of other interactions.

Dipole–dipole interactions of PEG phases and the cyanopropyl- and trifluoropropyl-substituted PSXs enable these phases to separate solute molecules that have different dipole moments. Such solutes are those with positional isomers of electronegative groups, such as pesticides, halocarbons and many pharmaceuticals.

Moderate *hydrogen bonding* is exhibited by PEGs and cyanopropyl-substituted PSXs, with less marked effects shown by phenyl- and trifluoropropyl-substituted PSXs. Functional groups that exhibit strong hydrogen bonding include alcohols, carboxylic acids and amines; aldehydes, esters and ketones generally have less effect; hydrocarbons, halocarbons and ethers produce negligible hydrogen bonding. Although the amount of separation obtained through dipole–dipole interactions or through hydrogen bonding can be difficult to predict, resolution of compounds with smaller differences in dipole moments or in hydrogen bonding strengths requires larger percentages of siloxane substitution.

McReynolds constants

The retention behaviour of five carefully selected probe compounds (benzene, butanol, pentan-2-one, nitropropane and pyridine) has traditionally been used to classify stationary phases in terms of their polarity (McReynolds 1970; Rorschneider 1966). The retention indices of each of these five reference compounds are measured on the stationary phase being tested, and then compared with those obtained under the same conditions on squalene (a standard non-polar phase). The differences in the retention indices between the two phases (ΔI) for the five probe compounds are added together to give a constant, known as the *McReynolds constant*, which is used to compare the ability of stationary phases to separate different classes of compounds. Phases that provide McReynolds values of ± 4 can be substituted freely for each other; those differing by ± 10 units generally yield similar separations. Phases with McReynolds values below 100 are considered non-polar, those above 400 indicate a highly polar phase and values between 100 and 400 an intermediate polarity. Table 40.3 shows the McReynolds constants, operating temperature range and example applications for the most popular stationary phases. ΔI values for individual probes indicate the deviation from boiling point order and consequently represent the contribution of forces other than dispersion to elution for that probe. The probes are chosen to represent different functional groups as follows:

- Benzene for aromatics and olefins (π-type interactions)
- Butan-1-ol for alcohols, nitriles, carboxylic acids and diols (electron-attracting effect)
- Pentan-2-one for ketones, ethers, aldehydes, esters, epoxides and dimethylamino derivatives (dipole–dipole effect)
- Nitropropane for nitro and nitrile derivatives (electron-donating effect)
- Pyridine for bases (non-bonding electron attraction and hydrogen-bonding effects).

Moffat *et al.* (1974a) devised a system to assess the effectiveness of liquid stationary phases in packed columns by calculating the discriminating power, and examined a number of phases commonly used in toxicology

Table 40.3 Polarity (McReynolds values) of some common stationary phases, and example applications

Capillary phase[a]	*Temperature range (min./max.)*	*McReynolds values*[b]					$\sum\Delta I$	*Applications*
		x′	*y′*	*z′*	*u′*	*s′*		
SPB-octyl	−60/300	3	14	11	12	11	51	Separates by boiling point, polychlorinated biphenyls (PCBs)
*-1	−60/320	4	58	43	56	38	199	Amines, hydrocarbons, pesticides, PCBs, phenols, sulfur compounds, flavours, fragrances
*-5	−60/320	19	74	64	93	62	312	Alkaloids, drugs, FAMEs, halogenated compounds, aromatic compounds
*-1301	−20/280	69	113	111	171	128	592	Aroclors, alcohols, phenols, volatile organic acids
*-35	0/300	101	146	151	219	202	728	Aroclors, amines, pesticides, drugs
*-1701	10/280	67	170	153	228	171	789	Aroclors, herbicides, pesticides, trimethylsilyl (TMS) sugars
*-50, *-17	30/310	125	175	183	268	220	971	Drugs, glycols, pesticides, steroids
*-210	−45/250	178	204	208	305	280	1175	Aldehydes, ketones, organochlorines, organophosphates
*-225	40/230	146	238	358	468	310	1520	FAMEs, alditol acetates, neutral sterols
*-23	40/250	228	369	338	492	386	1813	*cis–trans*-FAMEs, stereoisomers
*-wax, *-20M	35/280	305	551	360	562	484	2262	Alcohols, free acids, essential oils, ethers, glycols, solvents, primary amines
*-FFAP	50/250	340	580	397	602	627	2546	Acids, alcohols, aldehydes, acrylates, nitriles
Nukol	60/200	314	569	372	578	504	2337	Alcohols, free acids, essential oils, ethers, glycols, solvents
*-2330	10/250	382	610	506	710	591	2799	*cis–trans*-FAMEs, positional isomers
*-2380	10/275	402	629	520	744	623	2918	*cis–trans*-FAMEs, positional isomers, alditol acetates
*-2340	25/250	419	654	541	758	637	3009	*cis–trans*-FAMEs, positional isomers
—	20/200	496	746	590	837	835	3504	Acids, esters, phenols, terpenoids
—	100/200	537	787	643	903	889	3759	TMS or methyl sugars, acidic drugs
TCEP	10/145	594	857	759	1031	917	4158	Flavours, fragrances, essential oils
α-Cyclodextrin in 35% phenyl-PSX	30/240	102	243	142	221	170	878	Enantiomers and isomers (see Table 40.1)
β-Cyclodextrin in 35% phenyl-PSX	30/240	119	264	154	134	187	858	Enantiomers and isomers (see Table 40.1)

[a]* is the proprietary prefix for the phase, for example: * = HP supplied by Hewlett Packard/Agilent; * = DB supplied by J&W; * = CPSil supplied by Chrompack; * = RT supplied by Resteck; * = SP supplied by Supelco; * = OV supplied by Ohio Valley. This list is not intended to be exhaustive.
[b]x′ = benzene; y′ = butan-1-ol; z′ = pentan-2-one; u′ = 1-nitropropane; s′ = pyridine.

(Moffat *et al.* 1974b). Contrary to popular belief, it was shown that one column could be used to elute all the drugs studied, and that for screening purposes a single column, either SE-30 or OV-17 (100% dimethyl-PSX or 5% phenyl-PSX capillary equivalents), was sufficient for the reliable identification of drugs.

Installing, conditioning and maintaining columns

Column installation

A GC column is attached at one end to the injector and at the other end to the detector. Attachment is typically via a nut and ferrule, the nut attaching to a screwthread on the injector and detector. As the nut is tightened, the ferrule is compressed and helps produce a gastight fitting. Fused-silica capillaries should have their ends freshly cut after insertion through ferrules, to eliminate blockages. The injector end of the column should be fitted first, adjusting the height of the protrusion above the ferrule according to the type of inlet being used, then tightening the fittings just enough to prevent leakage when tested with a proprietary leak-testing fluid (not soap solution, which leaves a residue). The detector end of the capillary column may be immersed into a small tube of methanol to ensure adequate flow, and the capillary end re-cut and then attached to the detector and checked for leaks. The detector is activated and the column tested at room temperature with an injection of 1 or 2 μL of methane, when a needle-sharp peak should be obtained. When the carrier gas pressure has been adjusted to give a flow of approximately 1–2 mL/min of carrier gas, the column may be heated and a test mixture injected. Commercial columns are invariably supplied with a chromatogram obtained from a test mixture, and it should be possible to obtain a performance at least equal to that supplied. Various test mixtures are used, including a mixture of dimethylphenol and dimethylaniline with straight-chain paraffins. Any acidity or alkalinity of the column is apparent in loss of the peak shape of the amine or phenol. The efficiency obtained is a function of the entire chromatographic system. Poor efficiency or peak shape often results from a non-swept volume somewhere in the system.

It may be necessary to add an additional gas supply to the column outlet to ensure that the detector is purged effectively, because most detectors are designed to operate with packed columns and a flow rate of about 30 mL/min, as opposed to the 1 or 2 mL/min delivered by a capillary column (see Detector systems below).

Column conditioning

Modern capillary columns require only minimal conditioning before use to remove volatile impurities that remain from the manufacturing process. For thorough conditioning, the column should be installed in the injector port only, with the detector end disconnected. With the column at room temperature, a low carrier gas pressure (14–35 kPa) should be maintained for half an hour to purge oxygen from the system. The temperature may then be raised by about 1°/min until a temperature about 10°C above the desired maximum operating temperature has been reached, and the column is maintained at this for 2 h. Care must be taken not to exceed the maximum operating temperature. After conditioning, the column is connected to the detector, and a period of further conditioning undertaken only if the background signal is excessive. Some phases are particularly oxygen sensitive and can be ruined by careless conditioning. A constrictor fitted to the detector helps prevent back diffusion of oxygen if air or oxygen is supplied to the detector.

Guard columns and retention gaps

A guard column and a retention gap are essentially the same thing, but they are installed to serve different purposes. These 1–10 m lengths of fused-silica tubing are attached to the front of the chromatography column via a press-snap connector or zero dead-volume union, and then installed into the injector port. The surface of the silica is deactivated to minimise solute interactions, but no stationary phase is added. The tubing diameter should be the same as that of the column, but if different it should ideally be of a wider bore. The function of a guard column is to trap deposits of non-volatile residues, preventing them from contaminating the analytical column. Solutes are not retained by the guard column (since there is no stationary phase) and pass directly onto the column. Portions can be cut periodically from the top of the guard column as deterioration in chromatography requires, without any appreciable loss of resolution from the analytical column. A retention gap is used to improve peak shape when poor chromatography is the result either of a large injection volume (>2 μL) or of solvent–stationary phase polarity mismatches. Greatest improvement is seen in early eluting peaks, or for solutes with similar polarity to that of the solvent.

Maximum operating temperatures

Maximum operating temperatures for stationary phases are usually quoted assuming isothermal operation with a flame ionisation detector (see Table 40.3). Other detectors may impose different limits, the mass spectrometer being much more susceptible to bleeding of the stationary phase than the thermal conductivity detector. All phases bleed at high temperatures through loss of smaller-sized (and hence lower-boiling) polymer chains, although normally this is not noticeable. Operating temperature has a profound effect on column life, particularly for capillary columns. Loss of stationary phase or breakdown of the thin film into pools exposes part of the tubing surface and results in serious loss of performance. Additionally, in columns that contain PSX phases with two different functional groups, one group (usually that which confers additional polarity) is preferentially lost. This results in a change of relative separation (or in RI – see discussion below) as well as a loss of resolution. The temperature limit of a column may be influenced by the deactivation procedure used in production, rather than by the stationary phase itself. Newer silica columns have a very low metal oxide content, thought to act as a catalyst for the degradation of both sample and stationary phase, and thus enable phases to be run at higher temperatures. Fused-silica capillary columns have a protective external coating of polyimide that is slowly degraded at elevated temperatures (maximum temperature originally 360°C, now up to 400°C), which can also limit column life. However, separations are usually achieved at much lower temperatures.

Temperature programming

For complex mixtures with components of widely varying retention characteristics, it is often impractical to choose a column temperature that allows all the components to be resolved. Increasing the column temperature throughout the analysis dramatically reduces the time taken for higher-boiling compounds to elute, and simultaneously improves the sensitivity of the assay, as the peaks are remarkably sharper. If the early eluting compounds are resolved inadequately, a lower starting temperature or slower initial ramp should be used, taking care to observe the temperature requirements of the type of injector used. All instruments currently manufactured are available with a temperature program option, and a multi-ramp programmer is particularly useful for capillary chromatography. The first ramp can be used during splitless injection (see later) to bring the column rapidly up to the initial chromatography temperature, followed by a slower analytical ramp to perform the separation. One problem with temperature programming is that the backpressure increases with temperature and reduces the carrier gas flow if a mass-flow controller is not used. For polar stationary phases, the polarity increases with temperature, which causes distortion of RI data (see discussion on the use of RIs below). Column bleed also increases, which results in a rising baseline that can be mitigated somewhat by adequately conditioning the column before use.

Evaluating column performance

A column's performance is assessed on the basis of its efficiency (the narrowness of a peak), the peak shape (whether it tails or fronts) and its ability to resolve compound mixtures. This section deals with separation theory, and the reader may find it useful to refer at intervals to Fig. 40.3.

Retention time

Retention time (t_R) is the time taken for a given solute to travel through the column, and is the time assigned to the corresponding peak on the chromatograph. It is a measure of the amount of time that the solute spends in the column, and is therefore the sum of time spent in both the stationary and the mobile phases.

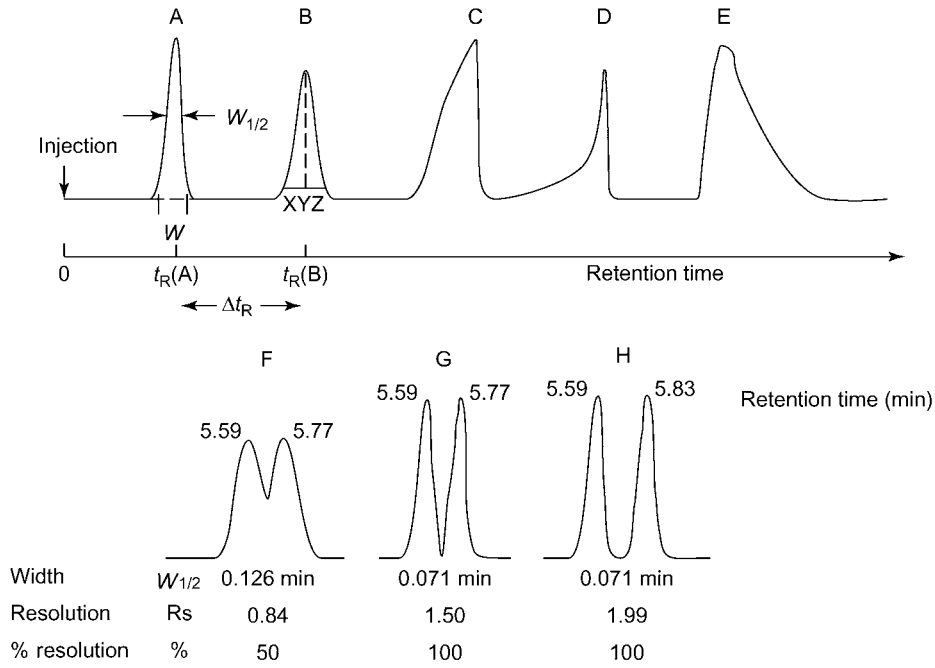

Figure 40.3 A and B are symmetrical peaks that show the measurement of significant parameters: W, width at the base of the peak; $W_{1/2}$, width at half peak height; t_R(A) and t_R(B), retention times of peaks A and B, respectively; Δt_R, the difference in retention time between A and B; XYZ, a line drawn at 10% of peak height.
Peak C is symptomatic of column overload with solute.
Peak D is symptomatic of degradation of a thermally unstable solute.
Peak E is symptomatic of adherence to active sites in the injection port or on the column.
Resolution of two compounds A and B.
F and G: peaks A and B have identical retention times (5.59 and 5.77 min, respectively), but in G the peaks are narrower ($W_{1/2}$ = 0.071 versus 0.126 min, respectively), and are fully resolved (Rs = 1.50 versus 0.84, respectively).
H: the peak widths of A and B are the same as in G, but the retention time of peak B is later (5.83 versus 5.77 min, respectively). Again, the peaks are fully resolved and Rs is larger than in G (Rs = 1.99 versus 1.50).

Retention time of a non-retained compound or hold-up time (t_M or t_0) The retention time, t_M, is the time taken for a non-retained solute to travel along the column; it represents the transit time for the mobile phase (carrier gas) in the column and is a column-specific parameter, applicable only under the prevailing conditions of gas flow and oven temperature. It is the same for all solutes on the column, and no other peak can be expected to elute earlier than this time. t_M is obtained by injecting a non-retained compound suitable for the detector system being used (butane or methane for flame ionisation detection (FID) or thermal conductivity detection (TCD); acetonitrile for nitrogen–phosphorus detection (NPD); methylene chloride for electron-capture detection (ECD); vinyl chloride for photoionisation detection (PID) or electrolytic conductivity detection (ELCD)).

Average linear velocity

The average linear velocity ($\bar{\mu}$) represents the average speed of carrier gas through the column, usually expressed in cm/s, and is considered more meaningful than measuring the flow (usually expressed in mL/min) at the column effluent, since flow is dependent on column diameter. This term directly influences solute retention times and column efficiency. Velocity is controlled by altering the column head pressure, and is calculated from equation (40.1):

$$\bar{\mu}(\text{cm/s}) = \frac{L}{t_M} \tag{40.1}$$

where L is the column length (cm), and t_M is the retention time (in seconds) of a non-retained solute.

Retention factor

The retention factor (k) is the ratio of the amount of time a solute spends in the stationary and mobile phases and is calculated from t_R and t_M using equation (40.2):

$$k = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M} \tag{40.2}$$

where t_M is the retention time of a non-retained solute, t_R is the retention time of the solute and t'_R is the adjusted retention time of the solute.

Since all compounds spend an identical time in the mobile phase, k is a measure of retention by the stationary phase. A compound with a retention factor of 4 spends twice as much time in the stationary phase (but not twice as much time on the column) as a compound with a retention factor of 2. Thus, k provides relative rather than absolute information, and is to a large degree independent of the operating conditions.

Separation factor

The separation factor (α) is a measure of the time interval between two peaks. If α equals 1, then the peaks have the same retention time and co-elute. Separation factor is calculated using the equation:

$$\alpha = \frac{k_2}{k_1} \tag{40.3}$$

where k_1 is the retention factor of the first peak, and k_2 the retention factor for the second peak.

The value of α does not indicate whether the peaks are resolved completely from one another, however. Two peaks may have only 0.01 min between them on one column but still be resolved completely, while on another column they may have 0.1 min between them but not be resolved adequately (refer to Fig. 40.3).

Number of theoretical plates or column efficiency

The theoretical plate is an indirect measure of peak width at a specific retention time. Higher plate numbers indicate greater column efficiency and narrower peaks. The number of plates per metre of column (N) is calculated from either form of equation (40.4):

$$N = 16(t_R/w_b)^2 \tag{40.4a}$$

$$N = 5.54(t_R/w_h)^2 \tag{40.4b}$$

where t_R is the time from injection to peak maximum for the solute, w_b is the peak width at base in units of time, and w_h is the peak width at half height in units of time.

Efficiency is thus a function of the column dimensions (diameter, length, film thickness or loading), the type of carrier gas and its flow, and the chemical nature of the solute and the stationary phase. For most applications in drug analysis, the chromatogram contains only two or three compounds, but an efficient column maximises the probability that a peak consists of only one compound and that it is the compound of interest. Other more complex separations (e.g. of flavours or trace residues in foods) may require two-dimensional chromatography or heartcutting (see later) to obtain sufficient separation.

Resolution

Resolution (R) takes into account both retention time and the peak width. For any pair of compounds, resolution can be calculated using either form of equation (40.5):

$$R = 1.18\left(\frac{t_{R2} - t_{R1}}{w_{h1} - w_{h2}}\right) \tag{40.5a}$$

$$R = 2\left(\frac{t_{R2} - t_{R1}}{w_{b1} - w_{b2}}\right) \tag{40.5b}$$

where suffixes 1 and 2 refer to the two peaks being evaluated; t_R = retention time; w_h = peak width at half height in units of time; w_b = peak width at base in units of time

An R value of 1.5 indicates baseline resolution, with numbers above 1.5 indicating the presence of baseline between the peaks (higher values indicate less overlap between peaks). In practice a value of about 1.2 is needed to be able to distinguish between two peaks occurring independently and a value of >1.0 to be able to quantify either peak reliably in the presence of the other. Sometimes the parameter percentage resolution is used, as this concept is easier to visualise (calculated by dividing the height of the valley between the peaks by the total peak height). equations (40.5a) and (40.5b) allow the operator to calculate resolution directly from the chromatogram, but they give little indication of the factors that contribute to it, or the parameters that can be modified by the analyst. A preferred relation is equation (40.7c), which shows that resolution is a product of three parameters, selectivity, capacity and the square root of the efficiency.

$$R = \frac{\alpha - 1}{\alpha}\left(\frac{k}{k+1}\right)\frac{\sqrt{N}}{4} \tag{40.5c}$$

where α = separation factor, k = retention factor, N = theoretical number of plates.

Phase ratio (β) The phase ratio of a column is a calculated term relating the column radius (r) in millimeters and the film thickness (d_f) in micrometres:

$$\beta = \frac{r}{2d_f} \tag{40.6a}$$

If all other conditions are held constant, then changes in phase ratio can be used to predict expected shifts in retention of solutes using equation (40.6b):

$$k\beta = \frac{kr}{2d_f} \tag{40.6b}$$

where k = retention factor.

Thus, to increase solute retention the phase ratio must be decreased, which can be brought about either by decreasing column diameter or by increasing film thickness. Sometimes it will be necessary to alter either the column diameter (e.g. to reduce flow though a detector) or to alter film thickness (e.g. to increase efficiency), but as long as the phase ratio remains constant then these changes can be accomplished without compromising separation. Table 40.8 shows calculated phase ratios for the most common sizes of capillary columns and loadings.

Peak shape or asymmetry

A well-designed GC system should give symmetrical peaks, as tailing or fronting adversely affects resolution. Tailing may result from non-swept volume in the system or from component–stationary phase or component–support interactions. Tailing of polar compounds can often be remedied by the use of a more polar stationary phase. Fronting (shark's fin peaks) is usually caused by overloading, particularly with capillary columns, and can be resolved either by making a smaller injection or by using a column with a higher stationary phase ratio. Column capacity is the maximum amount of a solute that can be chromatographed successfully without loss of peak shape. Table 40.4 shows the relationship between column capacity, film thickness and column diameter. Peak fronting caused by thermal decomposition can be reduced by either lowering the injection temperature or using a cold on-column injector system.

Table 40.4 Relationship of capacity (ng solute)[a] of GC columns with diameter and film thickness

Film thickness d_f (μm)	*Column internal diameter (mm)*			
	0.18–0.20	*0.25*	*0.32*	*0.53*
0.10	20–35	25–50	35–75	50–100
0.25	35–75	50–100	75–125	100–250
0.50	75–150	100–200	125–250	250–500
1.00	150–250	200–300	250–500	500–1000
3.00	—	400–600	500–800	1000–2000
5.00	—	1000–1500	1200–2000	2000–3000

[a]Capacity is defined as the maximum amount of solute that can be injected without peak broadening by >10% at half-height. Approximate values are given for capacity (ng) per component. Actual sample capacity depends on the operating conditions, and the polarity of the stationary phase and the solute (polar phases and solutes give lower values).

Peak shape is usually expressed by the peak asymmetry (A_s). In Fig. 40.3, the peak asymmetry factor for substance B is given by equation (40.7):

$$A_s = \frac{YZ}{XY} \tag{40.7}$$

where a vertical line is drawn through the peak maximum and XYZ is drawn at 10% of the peak height. A symmetrical peak has $A_s = 1$.

Use of retention indices

If gas chromatographic retention data are to be exchanged between laboratories, they must be independent of the instrument used. The concept of retention index (RI) has been shown to be more reliable than that of relative retention time (i.e. the retention time of the solute relative to that of a reference compound). The RI system uses a homologous series of compounds (i.e. a series of compounds that increase in size by an additional methylene unit) to provide the reference points on the scale. The most commonly used is the system described by Kovats (1961) using straight-chain saturated hydrocarbons (*n*-paraffins or *n*-alkanes). For any column temperature and stationary phase, the elution times of members of a series of *n*-alkane homologues are assumed to increase by an index of 100 for each additional methylene unit. On this scale, H_2 has an index of zero, methane has an index of 100, ethane 200, and so on up the scale of alkanes. The RIs of unknown substances are measured against this scale, obviating the need to correct data between laboratories because of variations in retention time. The method is illustrated in Fig. 40.4, in which phenobarbital has a retention time of 4.5 min and an RI of 1957.

$$\mathrm{RI} = 100(P_{z+n} - P_z) \times \frac{\log t_R(x) - \log t_R(P_z)}{\log t_R(P_{z+n}) - \log t_R(P_z)} \tag{40.9a}$$

where t_R is the retention time, P_z is the carbon number of the smaller *n*-alkane, P_{z+n} is the carbon number of the larger *n*-alkane and x is the unknown solute.

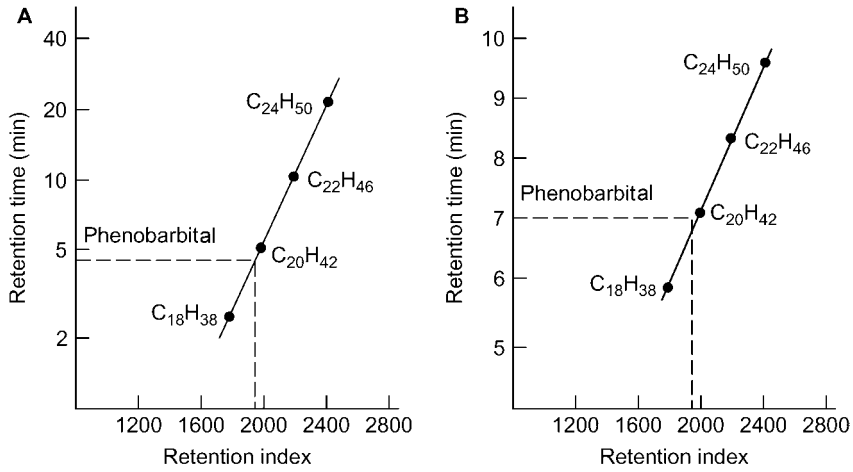

Figure 40.4 Calculation of the RI of phenobarbital from a plot of a series of homologous *n*-alkanes. (A) Packed column, isothermal conditions, retention time for phenobarbital is 4.5 min, RI = 1953; (B) capillary column, temperature programmed to run at 10°/min, retention time for phenobarbital is 7.01 min, RI = 1960.

Retention indices collected from many sources show remarkable agreement, even when measurements were made on different (though equivalent) phases and at different temperatures (Ardrey, Moffat 1981).

Most capillary GC is performed in temperature-programmed mode and the relationship between the retention time and chain length is almost linear, provided that the ramp rate is constant throughout the run and $n > 7$. The simplified equation (40.8b) is used to calculate RI (see Fig. 40.4 for an illustration).

$$\mathrm{RI} = 100(P_{z+n} - P_z) + \frac{t_R(x) - t_R(P_z)}{t_R(P_{z+n}) - t_R(P_z)} \qquad (40.8b)$$

Other homologous series have been proposed and good results were found with the alkan-2-ones and *n*-aldehydes. Use of other series (*n*-alkyl esters, *n*-alkylbenzenes, *n*-alkyl iodides) has been less successful. When using a specific detector to identify compounds, the accuracy is increased by splitting a small percentage of the column effluent to a flame ionisation detector to give a 'real-time' calculation of RI, rather than relying on either historical retention times for *n*-alkanes or using an alternative homologous series. Alternatively, Franke *et al.* (1993) have proposed the use of an RI reference mix (a selected group of compounds that structurally resemble those under investigation) rather than a homologous series. Retention indices for unknown compounds can then be normalised relative to the known RI values of the reference compounds. The main advantage of this approach is that both temperature-dependent and column-ageing effects on RI, which arise from polarity mismatch between the homologous series and the investigated solutes, are somewhat reduced and the data are therefore more reproducible over time.

As would be expected, there is better agreement for non-polar (most hydrocarbon-like) compounds than for polar compounds, and also for non-polar versus polar stationary phases where there is less difference in retention time between the *n*-alkane index markers. Using capillary columns, reproducibility can be as good as to within one RI unit for a non-polar phase, and to within a few units for a more polar one.

Good temperature, pressure and flow control, and precise measurement of the injection time and peak elution, are essential for accurate measurement of RIs. The carrier gas flow rate and the polarity of the stationary phase are temperature dependent. Thus, the partitioning of polar compounds into the stationary phase is affected by temperature to a greater extent than that for the *n*-alkanes. Differences between constant-flow and constant-pressure modes of operation are exaggerated when the column is ramped in several stages. It is common practice to have an initial fast rate of increase in temperature followed by one or more slower ramp rates, since the number of low-boiling compounds of interest is usually lower than the number of higher boiling compounds. Inaccuracies in RI calculation can also arise with high solute concentrations because of problems identifying the crest of the peak. This can also result in a trace component that elutes on the back of the concentrated one, as the major component begins to take part in the separation process by acting as a 'dynamic stationary phase'. This delays the elution of the trace component.

Column deterioration with use can lead to a preferential destruction or loss of the more labile component of a mixed phase. For example, in columns that contain mixed cyanopropyl- and methyl-PSX phases, the cyanopropyl group is preferentially lost, so that the column polarity is reduced. The elution of the index markers (*n*-alkanes) remains unchanged, but the progressive loss of cyanopropyl substituents results in a poorer interaction with polar compounds and an apparent *decrease* in their RI. With single-component stationary phases, the effect is still present, though less noticeable, as there is loss of retention of both RI markers and polar compounds.

Inlet systems

The inlet system provides the means of introducing the specimen into the GC. Obtaining a narrow sample band at the start of the chromatographic process is critical to achieve good resolution, since broad sample bands usually produce broad peaks, especially for analytes that elute early. The choice of injector depends on the characteristics of the specimen or residue, the quantity and characteristics of the analytes to be separated, and the temperature and nature of the stationary phase and the column. Solids may be dissolved in a suitable solvent and injected with a micro-syringe. It is best to keep the solution as concentrated as possible to reduce the size of the solvent peak. Liquids can be injected using a micro-syringe, but with sensitive detection systems the sample should be dissolved in a suitable solvent to reduce the sample size and avoid overloading the detector. Gases and vapours may be introduced by injection through the inlet port septum using a gas-tight syringe.

The three common types of GC injectors are split, splitless and cold on column. In reality, splitless injection is an extreme example of split injection and both are carried out using the same hardware. Conventional glass syringes of 1–10 µL volume with stainless-steel needles can be used on all but cold-on-column injectors, and the injection is made by piercing a silicone rubber septum. Care must be taken to select septa that have low bleed characteristics at the operating temperature, and those with Teflon backs are most reliable in this respect. Unstable materials can be decomposed by the high temperature of the injection system, particularly if the system is constructed of metal. For labile substances, cold-on-column injection is preferred, but clean extracts must be used to minimise column contamination.

Split and splitless injectors

Split injectors are used for more concentrated samples, since only a fraction of the sample actually enters the column. An inlet splitter allows a high flow of carrier gas through the injector while maintaining a low flow (1–4 mL/min) through the column: the excess gas and associated sample components are vented to the atmosphere through the split line. The ratio of these two flows (the split ratio) controls the proportion of the injected sample that reaches the column. The total flow through the injector may be from 10 mL/min to 100 mL/min, which gives split ratios of 10 : 1 to 100 : 1. A good splitter should be linear, i.e. it should split high- and low-boiling point compounds equally. The function of the splitter is not primarily to reduce sample volume, but rather to ensure that the sample enters the column as a compact plug. Split injections, therefore, produce some of the most efficient chromatographic separations, and allow the use of very narrow capillary columns. A lower split ratio channels a larger fraction of the injected sample down the column and may result in column overload. High split ratios waste large amounts of carrier gas and insufficient analyte may reach the column.

In splitless injection, all the carrier gas passes to the column. This is useful for very volatile compounds, for low sample concentrations or for trace analysis. The flow rate in the injector is the same as that in the column (1–4 mL/min), and the only path for the injection to take is into the column, since the split vent is closed. At a fixed time after injection (usually 15–60 s), the injector is purged by opening the split vent to introduce a much larger flow of carrier gas through the injector (typically 20–60 mL/min) and any remaining sample in the injector is discarded through the split vent. Since the rate of sample transfer onto the column is so slow (because of the low gas flow), peaks are usually somewhat broader than for split injections. Care should also be taken to ensure that the volume of the injector liner is not exceeded by the expanding solvent injected (Table 40.5), otherwise splitting of early peaks will be observed. Temperature conditions can be adjusted to narrow or focus the sample band at the top of the column. Splitless injections should therefore be made with the initial column temperature at least 10°C below the boiling point of the solvent (Table 40.5), and the initial temperature should be held at least until after the purge activation time. Solvent condenses on the front of the column and traps the solute molecules, which focuses the sample into a narrow band (known as the solvent effect). Individual solutes with a boiling point 150°C above the initial column temperature condense and focus at the top of the column in a process known as cold trapping. Either the solvent effect or cold trapping must occur before efficient chromatography can be obtained. Some newer chromatographs have the option of a pulsed splitless injection. In this mode, the column head pressure is increased immediately upon injection (typically to 174 kPa) and held there for 30–60 s, before returning to the normal operating pressure. This facilitates band sharpening and, while the process is not guaranteed to increase the fraction of the injection delivered onto the column, sensitivity is often improved because of improved chromatography.

Glass liners for split and splitless injectors come in a variety of shapes and volumes and it is prudent to start with a straight liner and to investigate some of those that cause turbulence (e.g. the inverted cup style) later if this is unsatisfactory. A plug of deactivated glass wool in the liner helps prevent the deposition of non-volatile or particulate material on the column, but may cause some peak deterioration, and for the best results needs to be placed at a consistent position in the liner. Packing of splitless injection liners with deactivated glass wool may decrease the chromatographic performance, but this must be weighed against the potential for damage to the stationary phase from the repeated injection of non-volatile or particulate material.

Table 40.5 Boiling points and expansion volumes for commonly used injection solvents

Solvent	*Boiling point (°C)*	*Expansion volume (μL) per L of solvent*[a]	*Suggested GC oven starting temperature (°C)*
Methylene chloride	40	330	15–30
Carbon disulfide	46	355	15–30
Acetone	56	290	30–45
Methanol	65	525	40–55
n-Hexane	69	165	40–60
Ethyl acetate	77	215	45–65
Acetonitrile	85	405	55–75
iso-Octane	99	130	70–90
Water	100	1180	70–90
Toluene	111	200	80–100

[a]Values are given at 250°C and 105 kPa head pressure.

Large-volume injectors

The analysis of trace amounts of components or contaminants in complex matrices such as foods, beverages and environmental samples is difficult. Adequate sensitivity to detect trace components is provided by specific detectors such as the NPD or ECD, but regulatory standards require positive identification of these compounds by mass spectroscopy (MS). To overcome the inferior sensitivity of MS, large-volume injectors have been developed. Examples include the Apex pre-column separating inlet (PSI), the temperature-programmed sample inlet (PTV) from Gerstel and time-coupled time-resolved chromatography (TCRC). The inlet typically consists of a length (10–50 cm) of standard (2 mm i.d.) glass chromatography column that can be deactivated or packed with traditional materials. The first two injectors are mounted directly in the GC injector port; the latter is a free-standing column coupled by a four-way valve into the GC inlet. Injection volumes range from 125 μL for the PSI, 1 mL for the PTV and up to 20 mL for the TCRC. Injection of larger volumes (up to 60 mL) is possible for some applications, but result in discrimination in favour of high-boiling components and loss of volatiles.

Large-volume injectors remove the solvent from the sample prior to its introduction onto the capillary column, typically by low-temperature evaporation through the split vent. As the sample is concentrated towards the bottom of the injector, the injector is heated, the split vent is closed and the analytes are introduced onto the GC column in splitless mode. Those injectors that can be heated selectively and cooled allow the precise introduction of selected components only from the sample, and thus reduce the quantity of non-volatile components (e.g. sugars) that might overload or destroy the analytical column. The TCRC has a small mobile oven (2–8 mm width) that can be scanned along the length of the column to produce band compression. Prior to the next injection, the injector columns are usually baked to vent high-boiling compounds to waste. Sensitivity can often be improved 50- to 100-fold and time is saved in sample preparation, since extensive clean-up or extraction procedures are no longer required.

Cold-on-column injection

Cold-on-column injection is most suited to compounds that are thermally labile. The injection needle must be fine enough to enter the column bore, usually fused-silica or stainless steel with a fused-silica insert. The top of the column is held at a temperature low enough for the solvent that contains the sample to condense, usually by an air- or carrier gas-cooled sleeve. The solvent temporarily swamps the stationary phase and ensures that the sample components concentrate in a narrow band. Any solvent or sample that remains in the injector is backflushed with carrier gas, often by automatic valves. The proximal end of the column is then brought rapidly to the operating temperature, when the solvent vaporises and chromatography begins. The potential for rapid column contamination or deterioration means that cold-on-column injection is usually restricted to those applications where its use is essential.

Volatiles interface

The volatiles interface allows automated analysis of gaseous samples. The interface is a low-volume highly inert switching block, and is ideally suited to trace-level detection. A portion of the carrier gas supply is diverted through the specimen sampler and released under controlled conditions onto the column. The remainder of the carrier gas goes to a flow sensor, which prevents fluctuations in column gas flow that would otherwise occur when the switching valves are opened and closed. The interface can be run in split, splitless or cold-on-column modes as described in the sections above. Samples may be introduced from external devices, such as air samplers or purge-and-trap devices (see section below), or from headspace analysis, which permits analysis of volatile substances in a liquid sample while minimising contamination of the column. This technique is used in the assay of ethanol and other solvents in blood and for complex household preparations, such as polishes, which contain volatile substances (see Chapter 14).

Thermal desorption and purge-and-trap injection

The analysis of samples that have been pre-concentrated onto solid adsorbents is common in the fields of industrial air monitoring, analysis of residues in food, soil and water, petrochemical analysis and environmental monitoring. The methods of preparing samples for analysis are described in the section on specimen preparation. These samples require special interfaces with GCs to ensure good chromatography. In some instances the sample preparation device and injector are manufactured as stand-alone pieces of equipment that require very little modification of conventional injectors, while others must be dedicated pieces of equipment. Once collected, the concentrated sample must be desorbed into the chromatograph using the heated injector port. The major problem here is the possible introduction of water into the chromatograph from moisture adsorbed during collection from high-humidity samples. Release of solutes from the adsorbent should be as rapid and complete as possible to allow for rapid and sensitive analysis and for a narrow sample band to be introduced into the chromatograph. This is achieved either by cooling the column oven cryogenically to refocus the sample in the injector prior to injection or by using a dry purge system coupled to the gas chromatograph via a volatile interface (see above) designed to operate above ambient temperature. Here, the specimen is thermally desorbed from the collection tube onto a narrower (1 mm i.d.) tube of the same adsorbent material. The concentrated solute is then released into the chromatograph, ensuring rapid and complete sample introduction. Adsorbents must be thermally stable to reduce interference from background contaminants.

With solid phase microextraction (SPME) the adsorbed sample is introduced into the heated injector port via a special sleeved needle (see under Specimen preparation). This technique requires the injector liner to be narrow (usually 0.75 mm as opposed to 2 or 4 mm) to increase the linear velocity of carrier gas through the liner and ensure that a narrow band of sample is introduced onto the column.

Solid injection

When solvent interference is serious the sample may be injected as a solid. The 'moving needle' injector has found application in steroid analysis and for the determination of anticonvulsant drugs. A solution of the material to be injected is placed on the tip of the glass needle with a syringe. A small flow of carrier gas sweeps the solvent out of the top of the device to waste. The dry residue is then introduced by moving the needle into the heated injection zone of the chromatograph with a magnet. This form of injection can be used only with compounds that do not volatilise with the solvent.

Backflush

Upon vaporisation, the injected sample undergoes considerable expansion, sometimes up to 100 to 1000 times its original volume, which creates a pulse of pressure that often exceeds the column carrier gas pressure. If the volume of the liner is smaller than the expanded solvent volume (see Table 40.5), some of the sample is propelled out of the injector in a process known as backflush. This can appear as a broad tailing solvent front, since it now takes longer to flush the expanded solvent out of the injector and carrier gas line. Backflush can also cause injector contamination, since the analytes condense in the cooler carrier gas line, from where they may bleed continuously into the injector and cause high background or spurious peaks. Carryover or peak ghosting can occur when the next injection backflushes and carries previously condensed compounds back into the vapour phase and onto the column. Backflush can usually be solved by using a smaller injection volume, a less expansive solvent, a lower injector temperature, a liner with an upper restrictor, or a faster carrier gas flow. The use of an adjustable septum purge gas (usually 0.5–1 mL/min) also decreases the potential for backflush, as components that would normally condense on the cooler septum and travel into the carrier gas lines are swept away by the septum purge. Too high a purge flow results in loss of highly volatile components.

Injector discrimination

Injector discrimination occurs because not all the compounds in the sample vaporise at the same rate. Since the sample remains in the liner for a limited time, this usually results in some loss of higher-boiling solutes. This can be alleviated by increasing the residence time of the sample within the injector, or by using a higher injector temperature or smaller injection volume. However, there is usually a compensatory loss in lower-boiling compounds. Discriminating behaviour can usually be managed by making reproducible injections.

Gas pressure and flow control

For accurate and reproducible GC, either a constant carrier gas flow or a constant carrier gas pressure must be maintained. Under isothermal conditions, simple pressure control is adequate for packed or capillary columns and back pressure can be monitored by a pressure gauge between the flow controller and the injector. A decrease indicates a leaking septum and an increase suggests contamination of the injector liner or the top of the column. This also ensures that the flow controller is performing correctly. Since the back pressure rises to equal the supply pressure, flow becomes pressure controlled. Flow control is highly desirable, if not essential, during temperature programming with packed columns and can be used to advantage with capillary columns. The added convenience of a digital (electronic) flow controller may be worthwhile.

Since the carrier gas becomes less viscous as the column oven temperature rises, the gas pressure must be increased as the run progresses to maintain constant velocity (or constant flow) throughout the analysis. Fig. 40.5 shows the effects of increasing the column temperature on the carrier gas flow and velocity if the head pressure is held constant during the run. As flow and velocity do not respond identically to increasing temperature (see Fig. 40.5D), late-eluting analytes are recovered more quickly using constant flow than under constant-pressure conditions. Furthermore, since column efficiency is a function of the carrier gas velocity (Fig. 40.6), resolution at the end of the chromatogram is improved under constant flow conditions. Switching between conditions of either constant flow or constant pressure can sometimes resolve otherwise co-eluting compounds. Table 40.6 shows the relationship between flow and pressure for various lengths and diameters of capillary columns. It shows the calculated head pressure (kPa) required to achieve the stated gas velocity or flow through a 25 m column operating at 150°C. Note that head pressure values above 280 kPa are not usually practicable using standard pressure regulators. Increasing the column length has a direct and proportional increase on head pressure for both velocity and flow calculations.

The way in which carrier gas velocity affects column efficiency is best demonstrated by reference to the van Deemter curves in Fig. 40.6. These demonstrate that the optimum column efficiency (minimum height equivalent of a theoretical plate, HETP) occurs at intermediate

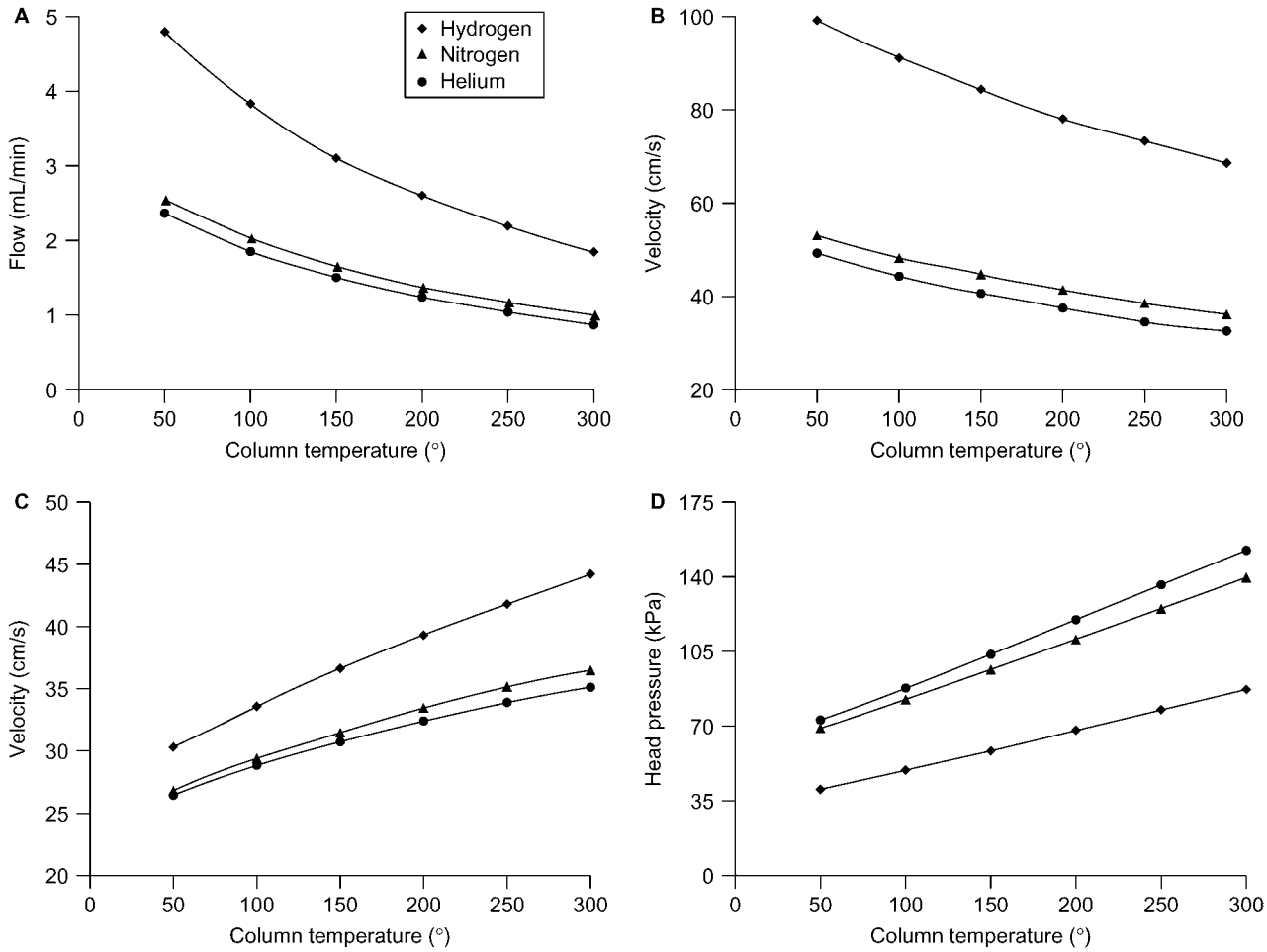

Figure 40.5 Effect of temperature on carrier gas flow and velocity. (A) and (B) are under conditions of constant carrier gas head pressure (140 kPa). (A) shows the change in column flow (mL/min) with change in temperature from 50°C to 300°C. (B) shows the change in velocity (cm/s) with change in temperature from 50°C to 300°C. (C) and (D) are under conditions of constant carrier gas flow (1 mL/min). (C) shows the change in carrier gas velocity (cm/s) with change in temperature from 50°C to 300°C. (D) shows the change in column head pressure (kPa) with change in temperature from 50°C to 300°C. All calculations are for a 25 m column of 0.25 mm i.d. operating at atmospheric pressure and 150°C.

velocity, and that column efficiency is compromised at both low and very high velocities. A small loss in efficiency for a shorter analysis time is usually tolerated. Curves are shown for the three most common carrier gases (helium, nitrogen and hydrogen), and it can be seen that the chromatography is much less tolerant to changes in nitrogen velocity than to helium. Helium is favoured by most users, as analysis times are half that with nitrogen, with only a slight loss in efficiency. While hydrogen gives the best dynamic range and shortest analysis times, there are safety issues relating to its use. While the gas used for the carrier gas should always be of the highest purity available, a lower-quality gas can sometimes be used for the makeup or detector, since these do not contribute to column deterioration by oxidation. Regardless of quality, it is advisable always to use a scrubber (to remove oxygen and hydrocarbons) followed by a dryer (to remove water vapour) between the supply and the instrument. Metal trap bodies are recommended, as plastics are permeable to impurities in laboratory air, especially when large amounts of organic solvents are used. Most traps have an indicator to show when they are saturated, and they can be changed without interruption to the gas flow. Stainless steel or copper tubing is recommended for plumbing of all gases, as plastics are permeable to moisture and oxygen, and Teflon, nylon, polyethylene, polypropylene and PVC contain contaminants that degrade gas purity.

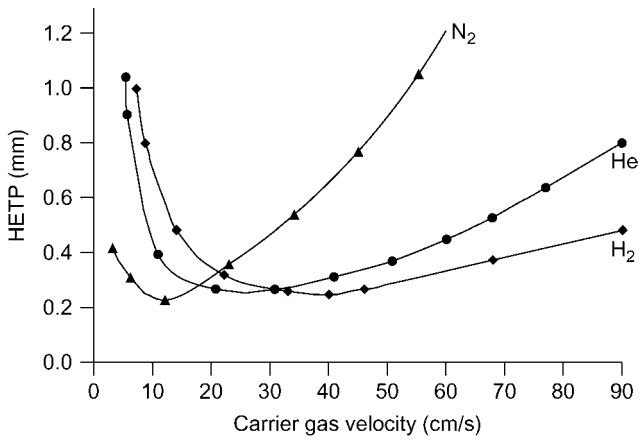

Figure 40.6 Van Deemter plots for a 25 m × 0.25 mm i.d. WCOT OV-101 column. HETP = height equivalent of a theoretical plate.

Detector systems

The choice of chromatography detector for an application depends on factors such as cost, ease of operation, consumables supply, sensitivity, selectivity and the linear working range.

Some detectors respond to almost all solutes, while others (selective detectors) respond only to solutes with specific functional groups,

Table 40.6 Relationship of gas chromatography column diameter to column flow, velocity and head pressure (kPa)

	Column internal diameter (mm)					
	0.10	***0.18***	***0.20***	***0.25***	***0.32***	***0.53***
Velocity 30 cm/s						
Hydrogen	329.1	93.2	10.8	74.5	28.7	10.4
Nitrogen	654.1	181.5	144.9	90.4	54.2	19.5
Helium	724.5	201.5	160.0	100.1	60.0	215.3
Flow 1 mL/min						
Hydrogen	675.5	157.3	115.9	58.2	24.8	3.66
Nitrogen	959.1	240.8	181.5	95.9	43.2	6.76
Helium	1007.4	254.6	193.2	102.8	46.9	7.38

Data for a 25 m column operating at 150°C.

atoms or structural configurations. Additional functional groups can often be added to solutes, generally after extraction (see below under Derivatisation), to achieve a response from a selective detector and gain additional sensitivity and selectivity. The use of detectors such as the ECD to identify amenable compounds, and the NPD to detect compounds that contain phosphorus and nitrogen, removes many of the extraneous peaks frequently observed when using non-selective detectors, such as the FID. However, these selective detectors have also led to the detection of substances such as plasticisers from blood-collection tubes or transfusion lines, which interfere in many toxicological analyses. Detectors that detect the presence of a solute and also give information about its structure are increasingly popular and MS, Fourier transform infrared spectroscopy and atomic emission spectrometry have been invoked to achieve this goal. Detector sensitivity is measured as signal-to-noise ratio, in which the signal corresponds to the height of the peak, and the noise to the height of the baseline variability. A signal-to-noise ratio of 8 to 10 is considered sufficient to confirm the presence of a peak. Each type of detector has a linear operating range in which the response obtained is directly proportional to the amount of solute that passes through, although this can be modified slightly by the nature of the solute and the chromatographic conditions (mobile phase type and flow, detector temperature). The linear operating range is considered to be exceeded when the incremental response obtained from the detector varies by more than 5% from that expected.

Most detectors (except MS) rely on gas other than the mobile phase (combustion, reagent or purge gas) for their operation. Usually, a total flow of at least 30 mL/min is necessary to sweep the solute molecules physically through the body of the detector at sufficient speed to prevent refluxing and produce narrow peaks. Thus, the addition of a 'makeup' gas is invariably required with capillary columns. Recommended gases and their flows for each detector are included in the manufacturer's instruction manuals, and it is important to follow these guidelines (and those on maintenance) to achieve the stated performance.

Here, only the most widely used detectors are considered in detail. Several other types of detectors are available; for a more detailed discussion, the reader is referred to the text by Scott (1996).

Flame ionisation detector

This is the most widely used of all detectors, since it responds to nearly all classes of compounds. The effluent from the column is mixed with hydrogen and the mixture is burnt at a small jet in a flow of air. A polarising current is applied between the jet and an electrode situated above it. When a component elutes from the column it burns in the flame to create ions that carry a current between the electrodes and provide the signal. The background current and noise are both low. Any of the usual carrier gases can be used and minor changes in gas flow are without effect. Sensitivity is moderate (0.1–10 ng), with linearity extending sometimes as high as six orders of magnitude. The response of the FID is dependent on the number of carbon atoms in the molecule, but the response is lowered if oxygen or nitrogen is also present in the molecule. It responds to all organic compounds that contain carbon–hydrogen bonds with the exception of formic acid. Both the sensor design and electronics are simple, and manufacturing cost is therefore low. The FID is easy to clean, and when operating with capillary columns it is virtually maintenance free. With packed columns, however, there is a tendency for a build-up of stationary phase bleeding from the column, which must be removed periodically. The insensitivity of the detector to water is a useful feature that allows aqueous solutions to be used.

Nitrogen–phosphorus detector or alkali flame ionisation detector

The introduction of alkali metal vapours (usually supplied by an electrically heated bead of rubidium chloride or caesium chloride) into the flame or 'plasma' of an FID confers an enhanced response to compounds containing phosphorus and nitrogen. By adjustment of the plasma gases the detector can be made virtually specific for phosphorus compounds (e.g. a phosphorus : carbon response ratio of 50 000 : 1 and a phosphorus : nitrogen response ratio of 100 : 1). Even when optimised for nitrogen compounds, it retains its response to phosphorus (e.g. a nitrogen : carbon response ratio of 5000 : 1 and a nitrogen : phosphorus response ratio of 10 : 1). This detector is particularly useful for drug analysis, since most drugs contain nitrogen, while the solvent and the bulk of the co-extracted material from a biological sample do not. The NPD is ideal for detecting pesticides that contain phosphorus, and therefore has wide application in environmental and regulatory analysis (air, soil, water and residues in food). The extreme sensitivity to compounds that contain phosphorus can be further exploited by the preparation of derivatives that contain this element. Sensitivity is excellent (1–10 pg), with a good linear range of up to four or six orders of magnitude. A disadvantage is the need for the supply of three gases and, unlike with the FID, their control is absolutely critical to selectivity. The detecting element (bead) lasts between 1 and 3 months depending on usage. Stationary-phase bleeding from packed columns coats the bead and collector assembly but can be rinsed off using methanol or dilute (0.1 mol/L) sulfuric acid. Most of the early problems that arose from poor reproducibility in bead coating have now been resolved, and the most stable detectors nowadays have a geometry that enables the bead to be located and fixed in its optimal position with relative ease.

Electron-capture detector

The early form of this detector consists of a small chamber with a pair of electrodes and a radioactive source, usually ^{63}Ni, placed close to the cathode to ionise the carrier gas. Potential applied to the electrodes produces a steady background current. Electron-capturing solutes arriving in the chamber remove some of the electrons and reduce the detector current. The response of the detector is therefore a loss of signal rather than an increase, as is given by most other detectors. Although the ECD can be polarised from a suitable low-voltage direct-current supply, it is more sensitive when a pulsed power supply is used, and in modern detectors the polarising pulses are modulated to maintain a constant current. A voltage that depends on the modulation frequency is generated as the output signal. Additional carrier gas is necessary, even with packed columns, to obtain a flow of at least 60 mL/min to purge the detector adequately and avoid peak broadening and distortion. Sensitivity can also be improved dramatically by raising the operating temperature of the detector, and decreasing the makeup gas flow.

The ECD is a selective detector with a very high sensitivity to compounds that have a high affinity for electrons; for many compounds, the sensitivity of the ECD often exceeds that of MS, and sometimes even that of the NPD. Compounds that contain a halogen, nitro group or carbonyl group are detected at 0.1–10 pg, 1–100 pg and 0.1–1 ng, respectively. This makes it very useful for compounds such as the benzodiazepines or halogenated pesticides and herbicides. Alternatively, the great sensitivity of the detector may be utilised by preparing derivatives with halogenated reagents, such as trifluoroacetic, heptafluorobutyric or pentafluoropropionic (PFP) anhydrides. Linearity (at best only two or three orders of magnitude) is a limiting factor for quantitative analysis.

In older models, the addition of a small amount of quench gas, such as methane, improves stability and linearity, and is essential if argon or helium carrier gas is used. Newer models can be operated successfully with helium as both carrier and detector gas. The ECD, because of its high sensitivity, can be contaminated easily: an impure cylinder of gas can damage a detector beyond repair in a matter of only a few hours. Cleaning is difficult, although some material can be removed by heating the detector to its maximum operating temperature overnight, and the injection of water in 100 µL aliquots through an empty glass column can also help. However, if contamination is avoided, it is virtually maintenance free. The radioactive source requires special handling procedures that may be subject to federal legislative regulations. More recently, it has been shown that this detector can work with greater sensitivity and operate over an increased linear range using a helium plasma in place of the radioactive source.

Fourier-transform infrared detector

In the Fourier-transform infrared detector (FTIRD), the column effluent is conducted through a light pipe and swept by a scavenging gas into the path of an infrared light beam that has been processed by an interferometer. The interferometer directs the entire source light to a beam splitter, which sends the light in two directions at right angles. One beam takes a fixed path length to a stationary mirror, while the other takes a variable path length to a computerised moving mirror. The two beams are recombined, and the difference in path lengths creates constructive and deconstructive interference, or an interferogram. The recombined beam is then passed through the sample. Analyte molecules absorb light energy of specific wavelengths from the interferogram, and the sensor reports variation in energy versus time for all wavelengths simultaneously. For molecules to be infrared active they must be able to undergo a change in dipole moment with the transition to their excited state. As a result, many compounds that are symmetrical do not respond.

Fourier transformation refers to the mathematical computation that converts the data from an intensity versus time plot into an intensity (percentage transmission) versus frequency spectrum. Each dip in the spectrum corresponds to light absorbed, and can be interpreted as characteristic of specific functional groups in the molecule. Computer libraries allow for easy and rigorous comparison of spectra. FTIR can be fully quantitative, but it is relatively insensitive (10 ng range). Its advantages are that it is non-destructive, and it can distinguish between isomers (MS cannot). Because of the logistical difficulties of combining FTIR with GC, this combination of techniques has started to emerge only recently.

Atomic emission detector

With the atomic emission detector (AED), carrier gas that elutes from the column delivers solutes into a high-temperature helium plasma, where heat energy is absorbed by the constituent elements. In returning to their ground state, they emit energy as light, the wavelength of which is characteristic for each element. Emitted light is focused by a quartz lens and spherical mirror onto a diffraction grating, and the dispersed light is focused onto a diode array that is continuously scanned (wavelength usually 170–800 nm). Typically, some 15 elements can be monitored simultaneously, and each is plotted against time. The composite chromatogram allows the percentage elemental composition of each peak to be determined. Sensitivity is very good, but the detector is complex and expensive to operate and is not widely used.

Mass spectrometer

A gas chromatograph is an almost ideal inlet device for quadruple MS. The detector is maintained under vacuum, and in the most common technique of electron impact (EI) the column effluent is bombarded with electrons. Compounds absorb energy, which causes them to ionise and fragment in a characteristic and reproducible fashion. The resultant ions are focused and accelerated into a mass filter that allows fragments of sequentially increasing mass to enter the detector stepwise. The mass filter scans through the designated range of masses (usually up to about 700 amu) several times per second. The abundance of each mass at a given scan time produces the mass spectrum, which can be summed and plotted versus time to obtain a total ion chromatogram. The MS detector can be operated either in full scan mode (collecting all the ions within a given mass range) or selected-ion monitoring (SIM) mode, which collects only pre-selected masses characteristic for the compound under study. Sensitivities for the two modes of operation are quite different: 1–10 ng for full scan, increasing to 1–10 pg in SIM because of the dramatic decrease in background noise. The linear range is excellent and often spans five or six orders of magnitude. Recent advances in computer technology, coupled with improved detector design, have revolutionised the use of the MS detector from a research tool to one of routine application. This technique is described in more detail in Chapter 37.

Ion-trap mass spectrometer

As with other forms of mass spectrometers, EI or chemical ionisation (CI) is used to produce an ion source, but this is focused into the ion-trap mass spectrometer in pulses rather than continuously. The fundamental difference is that all the solute ions generated over the entire pulse period are trapped in the detector and are then sequentially ejected in increasing mass number from the trap into the electron multiplier. The addition of helium into the trap (133 mPa) contracts the ion trajectory to the centre of the trap, where it is further focused by the ring electrode, to form dense ion packets that are expelled more efficiently than diffuse clouds, and thus greatly improve resolution. The spectral patterns can be quite different from those produced by mass filter spectrometers, and are often characteristic of the conditions under which the instrument is run, which makes comparison difficult between instruments. However, because the ion collection period is longer, the sensitivity of the ion trap in full scan mode is similar to that obtained in SIM on the average MS. Furthermore, an improved mass range (sometimes up to several thousand atomic mass units) gives this type of detector many applications, particularly for quantitative trace analysis, and for higher mass components. This technique is described in more detail in Chapter 37.

Dual detector systems

The simultaneous use of a combination of a universal detector (FID) with a specific detector to monitor the effluent of a column can provide useful information about the properties of functional groups and substituents in a molecule. The FID response is roughly dependent on the number of carbon atoms in a molecule and is quite predictable. However, the ECD response varies widely for different compounds is dependent on the electron-deficient part of the compound and is difficult to predict. The NPD response of a compound depends to some extent on the number of phosphorus or nitrogen atoms in a molecule, but it also depends on their environment. Thus, by using the FID as a reference, and measuring the ECD or NPD response relative to it, another characteristic for identification is obtained in addition to retention behaviour.

Dual detector systems can be used in several ways. The column can be split at the detector end and the effluent passed into two different detectors that operate in parallel. This approach allows the most flexibility, since the choice of detectors is wide, and the effluent can be split in proportion to the sensitivity required from each detector. For capillary columns this is accomplished easily with zero-dead-volume press-fit tee connectors, but it is a more complicated operation for packed columns. Additional makeup gas may be required to ensure a good flow through the detectors, and care should be taken to use tubing of a total area smaller than or equal to the analytical column to avoid loss of peak shape through refluxing at the detector. Alternatively, the GC oven houses two completely separate but identically matched columns, each connected to a single detector. This is not an ideal approach, as matching columns is difficult and has to be checked at frequent intervals. Another approach is to stack the detectors in series, and some manufacturers deliberately provide detectors in identical modules for this purpose. There are limitations to the choice of possible detector combinations, as the first

detector must always be a non-destructive detector, such as the ECD, AED or FTIRD.

Specimen preparation

Prior to chromatography, it is usually necessary to isolate the compound(s) of interest from either a biological matrix (plasma, urine, stomach contents, hair or tissue) or some other matrix, such as soil, air or water. Removal of extraneous material and concentration of the compounds of interest usually take place simultaneously. The high water solubility of some drug metabolites (e.g. glucuronide conjugates) requires chemical conversion to a less polar entity to permit isolation from water-based samples, and a hydrolysis procedure is often used for this purpose.

Isolation and concentration

Protein precipitation

If the analyte is present in blood in high concentration, a simple protein precipitation step often provides a suitable extract, although the possibility of losing significant amounts of analyte with the precipitate must be considered. Mixing with a solution of mercuric chloride or barium sulfate readily precipitates plasma proteins, and centrifugation provides a supernatant for direct injection onto the chromatography column. Use of perchloric or trichloroacetic acids (10%) is not advised, unless the resultant solution is neutralised prior to injection. Dimethylformamide is a good organic precipitation reagent that is well tolerated by most GC stationary phases. Other organic precipitating agents are methanol, acetone and acetonitrile, all of which should be added in the proportion of two volumes to each volume of blood. While the extract is still water based, most columns with a high stationary-phase loading (5 μm film thickness) can tolerate the injection of 1 μL of water. If the column is not water tolerant, it is possible to evaporate small volumes of the supernatant to dryness for reconstitution in a more suitable solvent.

Liquid–liquid extraction

Liquid–liquid extraction is the most frequently used method to isolate and concentrate solutes for GC. The pH of the specimen is adjusted to ensure that the compounds to be extracted are not ionised (basic for bases, acid for acidic compounds). Bearing in mind that some portion of the aqueous acid or base will dissolve in the solvent, the use of strong mineral acids or alkalis is not advised as this adversely affects column performance. Best results are obtained with acidic buffers (phosphate or acetate) and with ammonium hydroxide or basic buffers (borate), using a 5 : 1 ratio of solvent to specimen. The solvent chosen should be sufficiently polar to partition the compound of interest without co-extracting excessive amounts of polar contaminants. For more water-soluble drugs, such as beta-blockers, the addition of 2–10% of a polar solvent (e.g. isopropanol or butanol) is helpful, or solid sodium chloride can be added to 'salt out' the analyte. If a derivatisation step is to be carried out subsequently, the use of a solvent compatible with the derivatisation eliminates the need for an evaporation step. Use of solvents with a higher density than the sample (e.g. dichloromethane) can lead to difficulty in isolation of the organic phase. Purification of extracts by back extraction (re-extraction of the analytes from the organic solvent at the opposite pH followed by re-extraction into solvent at the original pH) may be helpful for trace analysis. The use of a small volume of solvent for the final extraction serves as a concentration step without the need for separation and evaporation of the organic phase.

Solid–liquid or solid-phase extraction

Solid–liquid extraction uses a polypropylene cartridge with a small amount (200 mg to 3 g) of high-capacity (1–20 mL) silica-based packing at the base of the reservoir. On introduction of the sample matrix, the compounds of interest are withheld by the packing. Impurities are then rinsed selectively from the column, and the final elution releases the compound of interest. Evaporation followed by reconstitution in a suitable solvent provides a clean, concentrated sample ready for analysis by GC. Bonded-phase packings that have been modified by the addition of various functional groups are available. The mechanisms of interaction for the matrix, analytes and packings are similar to those in LC (see Chapter 38). Polar stationary phases retain polar analytes (normal phase) and are eluted with organic solvents, while non-polar stationary phases retain non-polar analytes (reversed-phase) and are eluted with aqueous solvents. Ion-pair extraction uses a non-polar stationary phase and polar analyte, with a counter-ion added to the sample solution, and allows retention of the (now neutral) analyte by a reversed-phase mechanism. In ion-exchange extraction, the adsorbent surface is modified with ionisable functionalities. Analytes with ionic charges opposite to those on the packing are retained. Solvents that contain counter-ions of greater strength are used to elute the analytes of interest from the tube.

Solid-phase microextraction

Solid-phase microextraction (SPME) requires no solvents or complicated apparatus and can concentrate volatile and non-volatile compounds in both liquid and gas samples. The unit consists of a fused-silica fibre attached to a stainless-steel plunger coated with a stationary phase (mixed with solid adsorbents as required). The plunger is inserted through a septum into a vial that contains the sample, and the fibre is exposed by depressing the plunger either into the liquid or into the headspace for 20–30 min. The retracted fibre is inserted into the injection port of the GC, and is desorbed when the plunger is depressed. The unit may be reconditioned and used 50 to 100 times. For field analysis, adsorbed samples can be stored and transported in the needle sealed in a special container for subsequent analysis by GC (or LC). Pesticides recovered from water samples have been shown to be more stable when stored in this way than in water. The special small-volume injection liner fits any model of chromatograph, and produces sharper peaks because of the higher linear gas velocity, with little or no backflush. Suitable stationary phases are:

- 100 μm dimethyl-PSX film for low-molecular-weight compounds or volatiles, or a thinner film (7 μm) for higher-molecular-weight semi-volatile compounds
- 85 μm polyacrylate film for polar compounds
- 65 μm film of dimethyl-PSX-divinyl benzene for volatile alcohols and amines
- For surfactants, 50 μm Carbowax-templated resin
- For trace-level volatiles, a 75 μm Carbowax-carboxen phase is suitable.

An alternative approach uses a small magnetic stir bar encapsulated in glass and coated with a layer of dimethyl-PSX. The bar is left to stir in the sample for 30–120 minutes and then removed and placed in a thermal desorption tube. From there, it is introduced onto the GC as described in the section Thermal desorption. Both approaches give similar performance for higher-boiling compounds (>350°C), but SPME is inferior for lower-boiling compounds such as naphthalene and fluorene (b.p. 218°C and 298°C, respectively).

Supercritical fluid extraction

A supercritical fluid (SCF) is a substance that is maintained above its critical temperature and pressure, where it exhibits physicochemical properties intermediate between those of a liquid and those of a gas. Properties of gas-like diffusivity, gas-like viscosity and liquid-like density combined with a pressure-dependent solvating power provided the impetus to apply SCFs to analytical separation. The initial applications most often involved isolation of flavours and contaminant residues from food and soil. These have now been extended to the isolation of drugs from blood and other aqueous-based media by using adsorbents added in-line (such as molecular sieves, diatomaceous earth, silica gel, etc.) to filter proteinaceous material and adsorb water. It is possible, by adding small volumes of co-solvent to the SCF, to extract highly polar solutes with excellent efficiency. In contrast to the conventional extracting solvents, the fluid most often used in supercritical fluid extraction (SFE), supercritical CO_2, is non-polluting, non-toxic and relatively inexpensive. Additionally, extractions are carried out quickly at temperatures that avoid degradation of temperature-sensitive analytes and provide clean extracts with extremely high efficiency. Several dedicated SFE analysers are available; each consists of a gas supply, pump and controller used to pressurise the gas, temperature-controlled oven, extraction vessel, internal diameter regulator and collection device.

The CO_2 supply is compressed to a selected pressure (e.g. 28 000 kPa) and its is temperature adjusted (e.g. 50°C). As the supercritical CO_2 passes through the sample material, the solutes are extracted to an equilibrium solubility level, typically about 10% (w/w). The gaseous solution that leaves the extractor is passed through the pressure-reduction valve, where the pressure (and thus the dissolving power) of the CO_2 is reduced. The solutes precipitate in the separator, and the CO_2 is recycled through the system several times until the extraction is completed, when it is vented to waste.

Headspace analysis

This method of isolation is used for analytes with volatility higher than that of the common extraction solvents. A detailed description of the technique is given in Chapter 14.

Purge and trap

Purge and trap is a powerful procedure for extracting and concentrating volatile organic compounds from soil, sediment, water, food, beverages, etc. It is especially useful for poorly water-soluble compounds and those with boiling points above 200°C. The procedure involves bubbling an inert gas (nitrogen or helium) through an aqueous sample or suspension at ambient temperature, which causes volatile organic compounds to be transferred into the vapour phase. During the purge step, purge gas sweeps the vapour through a trap containing adsorbent materials that retain the volatilised compounds. Water vapour may be removed by dry purging. The trap is rapidly heated to 5–10°C below the desorption temperature. The valve is then switched to join the trap flow to the carrier gas flow, and the trap heated to its desorption temperature for a fixed time. Adsorbent tubes are usually packed with multiple beds of sorbent materials, each one more active than the preceding one, which allows compounds with a wide range of boiling points and polarities to be analysed simultaneously. During the purge, the smaller and more non-polar solutes are readily carried down the beds and, since the carrier gas passes in the opposite direction during the desorption phase, the larger and more polar compounds do not come into contact with the innermost active beds, from which their release may be difficult to effect.

Thermal desorption

This technique is used extensively for air monitoring in industrial hygiene, environmental air, indoor air or source-emission monitoring. The device may be portable or fixed and of varying size. Air is pumped continuously through the device at a fixed rate, during which time components are extracted gradually and concentrated onto the adsorbent beds; the arrangement of the beds is the same as described above for the purge and trap, and prevents potentially irreversible binding of large molecules. The direction of the flow is simply reversed during desorption. Analysis requires a special interface to the GC, which is described above in the section Thermal desorption and purge-and-trap injection. The adsorbents must have high capacity to remain active during the entire sampling period, and show an acceptable pressure drop during sampling. Ideally, a minimal amount of unwanted analytes should be absorbed, as these will contribute to the background noise.

Pyrolysis

Analytical pyrolysis can be a very useful tool for characterization of complex materials, including synthetic polymers (e.g. plastics) and natural organic polymers such as humic organic matter (HOM). Conventional pyrolysis with a unit connected to the exterior of the GC injection port is of only limited use for HOM, first because of the formation of much carbonaceous residue of virtually zero diagnostic value, and second because of loss of high-boiling pyrolysis products with great diagnostic value during the sample transfer from the pyrolysis unit to the GC column. Such products are long-chain alkanes, alkylbenzenes, fatty acids and dicarboxylic acids, as well as steranes and hopanes. These large compounds originate in the HOM as they are not formed during pyrolysis, and are diagnostically distinct from smaller products such as phenols that might originate either from the HOM itself or equally from the breakdown of lignin, carbohydrates and proteins, which are the starting material for HOM. To circumvent this deficiency, an in-column pyrolysis unit has been devised that sits in the GC oven (Gorecki, Poerschmann 2001). This is a silicosteel metal capillary (0.53 µm diameter) connected through butt connectors to a fused-silica restrictor inserted in the injection port which prevents backflush, and to the analytical column. The unit can be heated up to 750°C in 13 ms, and can be cooled again to ambient temperature in 4 s. The much reduced discrimination traditionally related to transfer of higher-boiling fractions is thus overcome, and this arrangement greatly extends the application of pyrolysis as a means of sample introduction into GC.

Tissues and hair

Tissues and hair require treatment prior to drug extraction to break down the biological matrix and enable a good recovery of the drug. For solid tissues, good results are obtained by incubation of a portion of the tissue with a mixture of a collagenase, a protease and a lipase in a buffer of suitable pH. For small amounts of tissues (100 mg), overnight treatment at room temperature suffices, although gentle agitation or occasional mixing speeds up the process. Larger amounts of tissue benefit from mechanical homogenisation prior to incubation. For the analysis of hair, an initial washing to remove residues from cosmetic products or environmental contaminants is recommended, followed by incubation with either caustic alkali (for basic drugs) or mineral acid (for acidic drugs). After adjustment of the pH, drug recovery can proceed by the usual procedures established for the specific compounds under investigation. For additional information see Chapter 10 and Chapter 19.

Hydrolysis

Recovery of conjugated drug metabolites from biological fluids can be increased by hydrolytic cleavage of the conjugate bond prior to extraction. This offers a vast improvement in sensitivity for qualitative analysis, particularly from urine, and is essential to identify drugs (e.g. laxatives) that are excreted almost exclusively as conjugated metabolites. However, reliable quantitative analysis of conjugated metabolites requires that the unconjugated metabolite must first be removed or quantified, and then the total (conjugated plus unconjugated) metabolite be measured after hydrolysis in a subsequent separate procedure. For quantitative work, appropriate standards that contain conjugated metabolites must be carried through the procedure to monitor the efficiency of the hydrolysis step.

Enzymatic hydrolysis

The use of a specific enzyme to cleave chemical bonds is the more specific of the two approaches but it incurs additional cost and time. It also provides cleaner extracts, and therefore prolongs the life of the chromatography column. There are a number of commercial preparations of purified glucurase and sulfatase harvested from different species. It is important to pay attention to the pH and temperature optima of the specific enzyme preparation. Temperature-tolerant preparations allow heating up to 60°C, which permits relatively short incubation times (2 h).

Chemical hydrolysis

This quicker and less expensive approach can provide suitable extracts for chromatography for some analytes, although they are generally more demanding in terms of clean-up procedures. Typically, strong mineral acids or alkalis are used, often with boiling or treatment in a microwave or pressure cooker. Extracts must be neutralised, otherwise the chromatography column deteriorates quickly. Care should be taken to ensure the stability of the analytes to the hydrolysis conditions. Vigorous hydrolysis conditions often yield undesirable by-products or, if several compounds can be hydrolysed to a single entity, preclude accurate identification of the original compound present. For example, both the acid and the enzymatic hydrolysis of benzodiazepines remove glucuronide conjugates, but acid hydrolysis also converts two or three drugs to the same benzophenone compound (diazepam, temazepam and ketazolam are all converted into 2-methylamino-5-chlorobenzophenone). While this compound has good chromatography characteristics, the approach is unsuitable for those applications (such as forensic analysis) that require absolute identification of the drug ingested.

Derivative formation

Derivatisation enables the analysis of compounds that otherwise could not be monitored readily by GC. To some extent the availability of stable polar stationary phases in capillary columns and the use of temperature programming has negated the requirement for derivatisation, although it is still widely used. Choice of reagent is based on the functional group that requires derivatisation, the presence of other functional groups in the molecule and the reason for performing the reaction. Although the retention characteristics are changed, the order of elution of a series of derivatives will be the same as that for the parent compounds. The preparation of derivatives modifies the functionality of the solute molecule to increase (or sometimes decrease) volatility, and thereby shortens or lengthens the retention time of a substance, or to speed up the analysis.

Another common reason for derivatisation is to improve resolution and reduce tailing of polar compounds (hydroxyl, carboxylic acids, hydrazines, primary amines and sulfhydryl groups). For instance, hydroxylated compounds often have long retention times and column adsorption causes tailing, which results in low sensitivity. However, they readily form silyl ethers and these derivatives show excellent chromatography, and sensitivity can often be improved by a factor of 10 or more. Derivatisation can also help to remove the substance peak away from interfering material. For example, the reaction of amfetamine with acetone enables successful differentiation from methyl ethyl ketone on most stationary phases. Derivatives may also be used to make the molecule amenable to detection by selective detectors, or can be used to improve the fragmentation pattern of the compound in the mass spectrometer.

The reaction may be carried out during extraction (e.g. extractive alkylation), on the dry residue after solvent extraction (e.g. silylation) or during injection (e.g. methylation). In choosing a suitable reagent, certain criteria must be used. A good reagent produces stable derivatives without harmful by-products that interact with the analytical column, in a reaction that is almost 100% complete. Poor reagents cause rearrangements or structural alterations during formation, and contribute to loss of sample during reaction. Most manufacturers of derivatising reagents provide information on the potential uses of each product, along with standard operating instructions. Entire texts, such as that by Blau and Halket (1993), are devoted to this topic.

Chiral separations

Chiral compounds can be derivatised to improve their chromatographic characteristics, and the enantiomers separated on a chiral stationary phase. Both enantiomers behave similarly, provided that steric hindrance does not preclude a reaction with one enantiomer. An alternative approach is to use a chiral derivatising reagent which, when reacted with enantiomers, produces diastereoisomers that can then be separated on a conventional stationary phase. As with enantiomers, diastereoisomers still produce similar mass spectra, but are resolved in time by the chromatography column. This approach is less expensive and also less restrictive, since a dedicated column is not required. Care should be taken to ensure the enantiomeric purity of the derivatising reagent, and to guard against racemisation during the reaction. *n*-Trifluoroacetyl-L-propyl chloride (TPC) in triethylamine and chloroform (or ethyl acetate) is a commonly used chiral reagent that couples with enantiomeric amines. Excess reagent is washed off with 6 mol/L HCl and the organic phase is dried over magnesium sulfate. For chiral alcohols, (1*R*,2*S*,5*R*)-(–)-menthylchloroformate (MCF) reacts well if pyridine is used as a catalyst.

Quantitative determinations

Quantitative work usually requires some form of sample preparation to isolate the drug from the bulk of the sample and some degree of concentration or, more rarely, dilution. These processes inevitably introduce a degree of analytical error. A further difficulty is caused by the non-reproducibility of injected volumes. To compensate for these errors, it is usual to compare the response of the unknown with the response of an added internal standard. The internal standard should be added as early as possible in the assay process and should have chromatographic properties matching the drug's as closely as possible, preferably with a longer retention time. It is often possible to obtain unmarketed analogues of drugs, or compounds specially synthesised for use as internal standards (e.g. a methyl addition or a halogen substitution). However, the internal standard usually does not behave exactly as the drug and careful control of variables, such as pH, is necessary. If a derivative is to be prepared, the internal standard should also be amenable to derivatisation. Use of an inappropriate internal standard can seriously affect precision (Dudley 1980). If a mass spectrometer is being used as the detector, then the ideal internal standard is a ^{3}H- or ^{13}C-substituted analogue of the drug, a number of which are readily available at reasonable cost. Calibration should include points of higher and lower concentrations than the sample, and quality assurance samples should be included at appropriate concentrations in frequently run assays. Peak measurement may be by peak height or by the peak area obtained by integration. If the peaks show even a modest degree of tailing, use of peak area usually provides a more accurate quantitative result. A plot of the ratio of peak height (or area) of the drug to internal standard versus concentration is a straight line with most detectors. Care should be taken in the preparation of standards to match the matrix to that of the specimens, and to allow for any associated salt or water of crystallisation in the calculation of the concentration. The best results are obtained when the amount of internal standard used produces a peak response ratio of 1 at the mid-point of the calibration range.

Optimising operation conditions to customise applications

Additional sensitivity can be achieved by increasing sample size, using a concentration step, derivatisation, injecting a larger sample volume, selecting a different stationary phase or using the detector at a higher sensitivity level.

When attempting a new analysis, it is advisable first to review published literature for a method that can be copied or for a method that involves a similar type of compound and can be adapted. Column manufacturers' catalogues are a useful source of information and invariably show examples of separations performed with their columns. Data on boiling points and RI (see monographs in Volume 2) are also useful indicators. If the review is not helpful, a start can be made with a standard column, such as a 100% methyl-PSX capillary column (25 m with a 0.5 µm film) and using standard flow conditions (1–2 mL/min helium). The oven temperature should be taken from 80°C to 300°C at 10°/min (or started at 200°C or 250°C if only an isothermal oven is available). A solution of the compounds of interest in ethanol or methanol should be injected with the injector temperature set at 250°C. If a peak tails, derivatisation or use of a more polar stationary phase should be considered. Fine-tuning is carried out once some peaks have been obtained. Having established the chromatography, the extraction and concentration steps can be determined. Manufacturers' catalogues are again a useful source for both derivatisation and solid-phase extraction procedures.

Good preventive maintenance is essential. The injector (or liner) should be cleaned periodically, and any glass wool changed regularly (approximately every 100 to 1000 injections, depending on the quality of the extracts). For capillary columns, the performance is improved by periodically removing the first 5–10 cm of capillary tubing, or a retention gap could be considered for dirty samples. It is advisable to monitor performance by selecting certain performance criteria (e.g. a certain response size or amount of acceptable separation between two closely eluting components) to indicate when maintenance is required. The manufacturers' instructions for cleaning detectors should be followed.

The presence of traces of contaminants in the carrier gas supply shortens the column life drastically, and also causes detector deterioration. In-line filters (to remove oxygen, hydrocarbons, etc.) and molecular sieves (to remove water vapour) are strongly recommended, and the use of stainless-steel gas tubing minimises further contamination.

Carrier gas flow should be optimised for a particular column and a particular carrier gas. This is most important for capillary columns. Fig. 40.6 shows the relationship between efficiency expressed as the HETP versus carrier gas velocity (van Deemter plot) for a 28 m × 0.25 mm i.d. WCOT OV-101 column. Modifying the mobile phase in GC has very little effect compared with that observed with HPLC or thin-layer chromatography (TLC) and, in general, affects efficiency rather than selectivity. Nitrogen gives higher efficiency but at the expense of longer analysis time, while the less dense, but more hazardous, hydrogen gives lower efficiency but faster analysis. In practice, nitrogen is usually used for packed columns and helium for capillary columns. Certain detectors impose restrictions on the choice of carrier gas, but an additional supply of gas can be added to the column effluent to purge the detector. Experimenting with higher flow and a lower operating temperature (or vice versa) can give rewarding results for the separation of compounds that elute closely. This effect is particularly noticeable for two compounds that have different polarities, as the retention of the more polar compound is influenced to a greater extent the longer it resides in the column (non-polar compounds elute in boiling point sequence). Conditions of constant flow improve the efficiency of late-eluting peaks and produce faster chromatography than do constant pressure conditions.

For a particular separation, the lowest temperature compatible with a reasonable analysis time should be used. In general, retention times double with each 20°C decrease in temperature. If the time is excessive, it is generally better to reduce the stationary phase loading or use a shorter column than to increase the column operating temperature. There is a maximum temperature at which a column can be operated and there is also a minimum temperature below which efficiency drops sharply. Manufacturers give the temperature operating ranges for each of their stationary phases (see Table 40.3). The stationary phase must be a liquid at the temperature of operation, and if a column is run at too low a temperature to obtain longer retention times the stationary phase may still be in the solid or semi-solid form. When using temperature programming, experimentation with a faster initial ramp followed by a slower subsequent ramp or an isothermal period can help resolve problematic separations.

Efficiency can also be improved by decreasing the column diameter or increasing the column length. The resultant increase in analysis time (particularly if the flow must be reduced to accommodate the increased pressure demand imposed by a narrower column) can usually be offset by using a slightly higher operating temperature (temperature increases affect retention time much more than do increases in gas flow). As shown in Table 40.7, reducing the diameter of a capillary column markedly increases efficiency, but the retention time remains constant only as long as the same phase ratio is maintained. Therefore, unless there is a simultaneous reduction in film thickness, retention increases in direct proportion to the phase ratio.

The solvent used for the sample can sometimes produce unexpected derivatives that give different retention times (traces of acetic anhydride that remain in butyl acetate avidly derivatise primary amines at room temperature). An inert non-polar solvent should be used if possible to minimise the co-extraction of unwanted contaminants. Acetone, other ketones, ethyl acetate and carbon disulfide readily form derivatives with primary amines and should be avoided.

The choice of injector type and injection solvent also play an important part in the chromatography. A solvent volume should be chosen that does not expand to exceed the capacity of the injector (see Table 40.5), otherwise backflush and irreproducible results are obtained. Split injection significantly reduces the amount of solvent and associated contaminants that enter the column and, although the analyte response is reduced, the improvement in the signal-to-noise ratio often results in enhanced sensitivity.

The use of a selective detector, such as an ECD (with the preparation of a strongly responsive derivative if appropriate), can improve sensitivity typically up to 100-fold. Similarly, switching from full scan to SIM in MS improves the sensitivity, usually by a factor of 10. However, selective detectors should not be used as a substitute for cleaning up of sample extracts, as loading contaminants onto the column affects the chromatography adversely, even if the selective detector does not respond to the compounds. Increasing the detector temperature may also improve sensitivity.

Fronting or splitting of peaks indicates column overload. If the detector sensitivity permits, the best option here is to inject a smaller sample volume (or a more dilute sample), rather than to increase the column loading or diameter, otherwise efficiency is also affected.

If trace impurities are sought in the presence of a preponderant component, a number of stationary phases of differing polarities should be tried. Trace impurities are seen easily if they emerge before the main component of a mixture, while they may be lost completely in the tail if they elute just after the large peak. Early peaks are also sharper and thus, for the same peak area, higher – an effect that can contribute enormously to the successful detection of trace substances.

Table 40.7 Relationship of film thickness, phase ratio (β)[(a)], efficiency (*N*)[(b)] and column diameter

Film thickness d (*μm*)	*Column internal diameter (mm)*						
	0.10	***0.18***	***0.20***	***0.25***	***0.32***	***0.45***	***0.53***
0.10	250[(a)]	450	500	625	800	1125	1325
0.18	139	250	278	347	444	625	736
0.25	100	180	200	313	400	450	663
0.40	63	113	125	156	200	282	331
0.42	—	107	119	149	190	265	315
0.50	—	90	100	125	160	225	265
0.83	—	—	60	75	96	136	160
0.85	—	—	59	74	94	133	156
1.00	—	—	50	63	80	113	133
1.27	—	—	—	49	63	88	104
1.50	—	—	—	42	53	75	88
2.55	—	—	—	25	31	44	52
3.00	—	—	—	21	27	38	44
5.00	—	—	—	13	16	23	27
Efficiency *N*[(b)]	12 500	6600	5940	4750	3710	2640	2240

[(a)]Phase ratio β = *r*/2*d*, where r = column radius (mm), *d* = film thickness (μm).
[(b)]*N*, theoretical plates per metre; maximum efficiency calculated for a solute with *k* = 5.

Two-dimensional GC

For most quantitative applications in drug analysis the chromatogram contains only two or three compounds, while some qualitative applications may contain 20 or more peaks of interest. Using an efficient column maximises the probability that a peak in a given time window consists of only one compound and that it is indeed the compound of interest. Other separations are far more complex, and the compound of interest may be present at minute concentrations relative to the background (e.g. flavours in foods or trace residue analysis in foods or groundwater). While these analyses can be fine-tuned to a limited extent by the use of element-specific detectors, the problem of obtaining a clean peak for positive identification and quantitation often remains. Consideration of equation (40.5c) shows column resolution to be related to two terms that can be varied by the analyst. The first of these is N, the number of theoretical plates, which in a typical capillary column is a few more than 100 000 plates. However, since R increases with the square root of N, a substantial increase in resolution can be obtained only by a very large increase in column length, and with a correspondingly large increase in analysis time. Some 500 million theoretical plates would be needed to separate 99 compounds out of a 100 component mixture. The other term is α, which describes the selectivity of a stationary phase for a particular pair of analytes. A modest increase in α can have a significant impact on resolution. This same philosophy was applied by analysts in the 1970s and 1980s who used two or more complementary stationary phases in parallel housed in different packed columns to make positive peak identifications (Moffat *et al.* 1974b). These columns were selected to have chemical properties as different as possible, but were limited by temperature compatibility since they were often placed in the same GC oven, and sometimes were split off from the same injector to allow reproducible temperature programming. However, recent advances in electronic pressure control and electronic proportional back-pressure regulators with pressure sensing, the manufacture of inert connector fittings and improvements in cryogenic focusing devices are enabling analysts to contemplate using two different analytical columns in series to achieve a satisfactory result.

Two main multidimensional approaches are receiving attention for routine use: two-dimensional gas chromatography (GC × GC) and 2D-GC with heartcutting. Instruments are now commercially available with two independently operated and controlled column ovens in a variety of injector and detector configurations. 2D-GC techniques began with heartcutting, in which only a timed portion of the chromatographed effluent from the first column was diverted from the detector or waste line into a second column of different polarity. A connector (or modulator) was employed to trap and focus the first eluent into narrow bands and transfer it to the second column at a rate that preserved the separation already achieved. In GC × GC the entire chromatography effluent from the first column is introduced to the second one. Difficulties arise when the retention of a solute on the second column exceeds the modulation cycle and 'wraparound peaks' appear with the solutes in subsequent cycles. A more recent modification, aimed at preventing this phenomenon, is called stopped-flow GC × GC. Here, the flow through the first column is stopped for a brief period, typically a few seconds, during each modulation cycle. This allows not only for better preservation of the separation of the first column, but also for longer separation times on the second column because subsequent bands are held up. This renders the secondary separation time independent of the modulation cycle, and increases the options for varying the chromatography conditions. A disadvantage is the longer run time and associated larger data file. Time-of-flight (TOF)-MS is almost mandatory to de-convolute the rapid analysis in the second column. These techniques have been demonstrated across many areas of industry, for example for identification of flavours in liquors, pesticide residues in foods and essential oils, and oxygenates in gasoline. The topic was recently reviewed by Pierce *et al.* (2008), and detailed examples showing hardware configurations can be found at www.chem.agilent.com/cag/prod/GC/Simplified_2DGC.

Specific applications

The systems given below are applicable to the routine screening, separation and identification of groups of drugs and chemicals. They are not exhaustive lists and references to specific systems for individual drugs and chemicals are given in the relevant monographs. Some of these systems use columns that are identical or very similar in terms of discriminating power (see Table 40.3), but are operated with different temperature programmes for specific groups of compounds. Moreover, some groups of substances are chromatographed as derivatives rather than as the parent compounds.

The most commonly used general screening system is a 100% dimethyl-PSX (methyl-PSX or X-1) capillary column (for packed columns, SE-30, OV-1 or OV-101 is equivalent). This should always be used for screening purposes, since it has the best chance of eluting any compound of interest. Analysts have collaborated to compile comprehensive lists of retention indices using this system (De Zeeuw 2002), some of which are included in the Index of Gas Chromatographic Data.

Most of the data are for the drugs themselves, but thermal decomposition may occur and the peak observed may be for the decomposition product (referred to as 'artefact') rather than the original drug. Where the drug is known to chromatograph badly, or to decompose, data are given for suitable derivatives (e.g. methyl or ethyl esters for the sulfonamides, and TMS derivatives for hydroxides). Wherever possible, the RI of the drug is given, since this is a more reproducible parameter than retention time or relative retention (see discussion above). However, if a laboratory prefers routinely to use the latter parameters, the RI data can be converted easily after chromatography of a few representative drugs and using a regression analysis of RI against either retention time or relative retention. RIs for some additional non-drug substances that might interfere with toxicological analyses, but are not included in the monographs. A nitrogen–phosphorus (alkali flame ionisation) detector is the best detector for nitrogenous drugs and phosphorus-containing pesticides, but an FID should also be used, since some drugs do not contain nitrogen (e.g. some anti-inflammatory agents). ECDs are excellent for benzodiazepines and halogen-containing compounds, such as some phenothiazines and herbicides. Extra selectivity can always be obtained by using element-specific detectors (e.g. those for phosphorus and sulfur for compounds that contain these elements). Additional specificity or confirmation of identity can be obtained by using a mass-selective detector, such as MS or an ion-trap detector. Where improved fragmentation can result from the use of derivatisation, data for suitable derivatives have been included. As mass spectrometry has matured as a technique, significant improvements in detection of higher-mass fragments have enabled the use of larger derivatising reagents such as heptafluorobutyrate (HFB). In any analysis for an unknown compound, the data obtained from complementary techniques, such as TLC, HPLC or colour tests, should always be assessed for compatibility with the GC result. (In the tables of retention indices given here, a dash indicates that no value is available for the compound, not that it does not elute.)

General screen, systems GA and GB

Both systems use standard columns that are able to chromatograph a wide variety of drugs and chemicals. System GB uses a slightly more polar column, which gives better peak shapes for hydroxylated compounds (many drug metabolites are hydroxylated), better resolution between structural isomers and improved peak shape for primary amines over the less polar GA. However, the retention indices are very similar for GA and GB, and can be interconverted using the equations:

$$\text{GB RI} = 1.079 \times (\text{GA RI}) - 66$$

or

$$\text{GA RI} = (\text{GB RI}+66)/1.079$$

As the stated values for drugs are retention indices, the operating conditions for the columns may be varied to suit particular laboratory situations.

System GA

Details are taken from the TIAFT book (De Zeeuw 2002) and the PMW Spectral Library (Pfleger *et al.* 2004). Chromatography details are given for both systems below.

- *Column:* 3% SE-30 or OV-1 on 80 to 100 mesh Chromosorb G HP (acid washed and dimethyldichlorosilane treated) glass (2 m × 2 mm i.d.); it is essential that the support be fully deactivated.
- *Temperature:* Normally between 100°C and 300°C; for isothermal conditions, an approximate guide to temperature is to use the RI divided by 10.
- *Carrier gas:* N_2, 45 mL/min.
- *Capillary column:* 100%-dimethyl-PSX (X-1) (10–15 m × 0.32 or 0.53 mm i.d., 1.5–3 µm).
- *Carrier gas:* He.
- *Temperature programme:* 135°C for 4 min to 200°C at 13°/min to 312° at 6°/min for 6 min.
- *Column:* HP1 (100%-dimethyl-PSX) fused-silica capillary (12 m × 0.2 mm i.d., 0.33 µm).
- *Injector:* 280°C splitless mode.
- *Temperature programme:* 100°C for 2 min to 310°C at 30°/min for 8 min.
- *Carrier gas:* He, 1 mL/min.

System GB

Data generated by the author.

- *Column:* 5% phenyl–95% dimethyl-PSX (X-5) capillary (20–30 m × 0.2 or 0.25 mm i.d., 0.5–1 µm).
- *Carrier gas:* He, 1 mL/min.
- *Temperature programme:* 90°C for 0.7 min to 240°C at 35°/min to 290°C at 8°/min to 325°C at 25°/min for 6 min.
- *Reference compounds: n*-Alkanes with an even number of carbon atoms, or a reference drug mix that contains amfetamine (1125), ephedrine (1365), benzocaine (1545), methylphenidate (1725), diphenhydramine (1870), tripelennamine (1976), methaqualone (2135), trimipramine (2215), codeine (2375), nordazepam (2490), prazepam (2648), papaverine (2825), haloperidol (2930) and strychnine (3116). (RI values for system GA are given in parentheses for the drug mix.)
- *Retention indices:* Values for drugs in these systems are found in drug monographs and in the Indexes of Analytical Data; they are also included in the systems for specific groups of drugs that follow. The search window should be ±50 RI units if hydrocarbons are used to calculate RI, or ±30 RI units if a reference drug mixture is used for the RI calculation.

Amfetamines and other stimulants

Amfetamines are basic drugs that require strongly alkaline conditions to be extracted from aqueous solution. The conditions are too basic to extract the phenolic metabolites, but these can be recovered at pH 8 or 9 and the extracts combined prior to chromatography. For high sensitivity, back extraction into dilute sulfuric acid (0.05 mol/L) is a useful clean-up procedure. When using packed columns, derivatives are almost always required for the primary and secondary amines, since the peaks tail badly. Suitable derivatives are acetyl, trifluoroacetyl, pentafluoropropionate or TMS (see Derivative formation). With capillary columns, derivatives are used most often to improve mass spectral patterns or to modify the separation of compounds that elute closely. For hydroxylated metabolites, derivatisation is invariably required to achieve acceptable chromatography. Data for the most commonly used derivatives are given in Table 40.8. Care must be taken to avoid drug loss during solvent evaporation, which can be obviated by adding a small amount of concentrated aqueous acid (20 µL 6 mol/L HCl) to the organic solvent. Unless otherwise stated, GC retention data and mass spectral data are identical for both D- and L- (+ and –) enantiomers. To differentiate enantiomers (such as D- and L-metamfetamine or amfetamine), a chiral column or chiral derivatising reagent is required (Cody, Schwarzhoff 1993). At present, all amfetamine- or metamfetamine-producing drugs (aminorex, amfetaminil, clobenorex, ethylamfetamine, fencamine, fenethylline, fenproporex, mefenorex, prenylamine, benzfetamine, dimethylamfetamine, famprofazone, furfenorex) are racemates (with the exception of L-selegiline, L-metamfetamine and dexamfetamine). Stereo-inversion does not occur in humans (Nagai, Kamiyama 1991). Drugs that are metabolised to amfetamines, but are not themselves classified as such, are also listed. System GA or GB, previously described, may be used as well as system GC.

Table 40.8 GC retention data and mass spectral data for the amfetamines and derivatives (reference compounds are *n*-alkanes with an even number of carbon atoms; AC, acetyl; ET, ethyl; HFB, heptafluorobutyrate; PFP, pentafluoropropionate; TFA, trifluoroacetyl; TMS, trimethylsilyl)

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
Amfetaminil (metabolised to amfetamine, see below)	1755	—	—	132	105	133	89	77	65
Amfetamine (D or L)	1125	1150	—	91	65	51	63	89	120
Amfetamine-TFA	1095	—	1536	140	118	91	69	65	117
Amfetamine-PFP	1330	—	—	118	190	91	119	65	117
Amfetamine-TMS	1190	—	—	116	73	100	91	117	192
Amfetamine-AC	1501	—	—	44	86	118	91	117	65
Art (formyl)	1100	1142	—	56	91	125	146	147	132
M (3OH-)-PFP_2	1520	—	—	190	280	119	253	69	—
M (3OH-)-TMS_2	1850	—	—	116	73	100	280	117	179
M (3OH-)-AC_2	1930	—	—	86	134	176	107	77	235
M (4OH-)	1480	—	—	56	107	77	108	91	151
M (4OH-)-AC	1890	—	—	134	107	86	77	133	193
M (4OH-)-AC_2	1900	—	—	134	86	176	107	77	133
M (3,4-di-OH-)-AC_3	2150	—	—	86	150	234	192	137	123
M (OH-methoxy-)	1465	—	—	138	137	122	123	94	181
M (OH-methoxy-)-AC_2	2065	—	—	164	86	206	137	165	265
M (desamino-oxo-OH-)-AC	1520	—	—	107	149	150	176	192	—
M (desamino-oxo-OH-methoxy-)	1510	—	—	137	180	94	122	138	107
M (desamino-oxo-OH-methoxy-)-AC	1600	—	—	137	180	138	109	122	222

table continued

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
M (desamino-oxo-di-OH-)-AC_2	1735	—	—	123	166	208	150	124	250
Aminorex (metabolised to amfetamine)	2065	—	—	56	118	162	91	119	145
Amiphenazole-AC_2	2575	—	—	191	233	121	275	149	257
3,4-Benzodioxazol butanamine (BDB)	1570	1622	—	58	136	77	135	164	193
BDB-TFA	1705	—	—	135	176	154	77	161	289
BDB-PFP	1700	—	—	135	176	119	204	126	339
BDB-AC	1950	—	—	58	176	162	100	135	235
Art (formyl)	1585	—	—	70	135	77	205	176	92
M (desmethylenyl-methyl-)-AC_2	2140	—	—	58	178	220	100	137	279
M (desmethylenyl-)-AC_3	2235	—	—	58	100	164	248	123	307
2,3-Benzodioxazol butanamine (2,3-BDB)	1550	1602	—	58	77	135	83	164	193
2,3-BDB-TFA	1705	—	—	176	154	135	77	136	289
2,3-BDB-PFP	1615	—	—	135	176	119	204	136	339
2,3-BDB-TMS	1670	—	—	130	73	135	236	250	77
2,3-BDB-AC	1895	—	—	58	176	100	135	235	131
Art (formyl)	1575	—	—	70	77	135	205	176	105
Bemegride	—	—	1253	—	—	—	—	—	—
Benzfetamine (metabolised to metamfetamine and amfetamine)	1855	1899	2172	91	148	65	149	92	56
N-Benzylpiperazine (BZP)	—	1530	—	91	134	176	56	120	146
BZP-AC	1920	—	—	91	146	85	134	132	218
BZP-HFB	1730	—	—	91	281	372	175	146	295
BZP-TFA	1665	—	—	91	181	272	195	146	132
BZP-TMS	1860	—	—	102	248	157	86	116	233
M (4-OH-) isomer 1-AC_2	2275	—	—	107	85	149	192	204	276
M (4-OH-) isomer 2-AC_2	2245	—	—	107	149	204	85	190	276
M (OH-methoxy-)-AC_2	2380	—	—	137	85	127	179	234	306
4-Bromo-2,5-dimethoxyamfetamine (DOB)	1804	1875	—	44	77	230	232	105	91
DOB-TFA	1935	—	—	229	231	256	258	69	369
DOB-PFP	1905	—	—	229	231	119	256	258	419
DOB-TMS	1920	—	—	116	73	117	229	272	201
DOB-AC	2150	—	—	86	256	258	162	315	317
Art (formyl)	1790	—	—	56	254	256	285	229	199
4-Bromo-2,5-dimethoxyphenethylamine (2C-B, BDMPEA)	1785	1867	—	230	232	215	217	259	261
2C-B-AC	2180	—	—	242	244	229	148	301	303
Art (formyl)	1840	1860	—	242	240	229	231	271	273
M (*O*-desmethyl-) isomer 1-AC_2	2410	—	—	228	230	287	289	215	329
M (*O*-desmethyl-) isomer 2-AC_2	2440	—	—	228	230	287	289	215	329
M (*O*-desmethyl deamino-OH-)-AC_2	2160	—	—	228	230	288	290	213	329
Cathine (see [D+]-norpseudoephedrine)									
Cathinone	—	—	—	—	—	—	—	—	—
Cathinone-TFA	1350	—	—	105	77	69	106	140	78
Cathinone-PFP	1335	—	—	190	119	105	280	253	225
Cathinone-TMS	1590	—	—	116	73	77	117	191	206
Cathinone-AC	1610	—	—	86	77	105	191	134	132
1-(3-Chlorophenyl)-piperazine (*m*CPP)	—	1806	—	154	196	138	111	156	75
*m*CPP-AC	2265	—	—	166	238	138	154	168	195
M (*p*OH-) isomer 1-AC_2	2515	—	—	182	254	169	184	211	296
M (*p*OH-) isomer 2-AC_2	2525	—	—	182	254	169	184	296	211
M (desethylene-)-AC_2	2080	—	—	140	195	153	142	111	169
M (chloroaniline)-AC	1580	—	—	127	129	169	171	99	100

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
M (OH-chloroaniline) isomer 1-AC_2	1980	—	—	143	145	167	185	227	169
M (OH-chloroaniline) isomer 2-AC_2	2020	—	—	143	185	145	187	78	114
Chlorphentermine	1355	1393	1725	58	107	108	125	168	89
Chlorphentermine-AC	1730	—	—	58	100	86	166	167	125
Chlorphentermine-HFB	1560	—	—	254	125	166	214	169	255
Chlorphentermine-PFP	1515	—	—	204	166	164	154	125	119
Chlorphentermine-TFA	1520	—	—	154	125	114	166	89	69
Chlorphentermine-TMS	1520	—	—	130	73	114	125	89	240
Clobenzorex (metabolised to amfetamine and norephedrine)	1940	—	—	168	125	170	127	89	244
Clobenzorex-AC	2290	—	—	168	125	210	170	91	266
Clobenzorex-PFP	2040	—	—	125	127	118	91	314	316
Clobenzorex-TFA	2075	—	—	125	127	91	118	264	266
M (chlorobenzyl-OH-)-AC_2	2565	—	—	226	141	183	200	268	324
M (OH-) isomer 1-AC_2	2585	—	—	168	125	210	107	272	364
M (OH-) isomer 2-AC_2	2630	—	—	168	125	210	170	134	176
M (OH-methoxy-)-AC_2	2690	—	—	168	125	164	137	206	210
M (OH-chlorobenzyl-OH-) isomer 1-AC_3	2705	—	—	226	141	183	161	215	268
M (OH-chlorobenzyl-OH-) isomer 2-AC_3	2725	—	—	141	226	268	183	125	150
M (OH-chlorobenzyl-OH-) isomer 3-AC_3	2775	—	—	226	141	183	161	215	268
M (OH-chlorobenzyl-OH-) isomer 4-AC_3	2795	—	—	141	226	183	134	107	268
M (OH-alkyl-OH-)-AC_3	2725	—	—	168	125	210	192	150	220
M (di-OH-)-AC_3	2765	—	—	168	125	210	192	150	234
Dexamfetamine (see Amfetamine)									
Diethylpropion (amfepramone)	1486	1532	1715	100	44	72	101	77	56
M (phenylpropanolamine)	1360	1352	—	44	77	79	51	45	42
M (diethylnorephedrine)	—	1599	—	—	—	—	—	—	—
M (ethylnorephedrine)	—	1457	—	—	—	—	—	—	—
M (*N*-desethyl-)	—	1423	—	—	—	—	—	—	—
M (*N*-didesethyl-)	—	1338	—	—	—	—	—	—	—
M (norephedrine)	—	—	1383	—	—	—	—	—	—
2,5-Dimethoxyamfetamine (DMA)	1546	1601	—	44	152	137	121	195	91
DMA-AC	1870	—	—	44	178	86	121	237	91
Art (formyl)	1550	—	—	56	176	151	121	207	91
2,5-Dimethoxy-4-ethyl-β-phenethylamine (2C-E)									
2C-E-AC	2000	—	—	192	177	149	179	91	251
2C-E-TFA	1770	—	—	179	192	305	177	149	193
M (*O*-desmethyl-) isomer 1-AC_2	2210	—	—	178	237	165	163	179	279
M (*O*-desmethyl-) isomer 2-AC_2	2240	—	—	178	237	165	163	179	279
M (OH-) isomer 1-AC_2	2340	—	—	250	191	207	309	175	237
M (OH-) isomer 2-AC_2	2420	—	—	190	191	250	309	164	295
M (OH-) isomer 3-AC_2	2500	—	—	196	195	250	208	309	212
M (desamino-OH-)-AC	1850	—	—	192	177	149	91	252	179
M (*O*-desmethyl-desamino-OH-) isomer 1-AC_2	1990	—	—	178	163	145	165	238	280
M (*O*-desmethyl-desamino-OH-) isomer 2-AC_2	2000	—	—	178	163	220	238	154	280
M (*O*-desmethyl-OH-) isomer 1-AC_3	2430	—	—	176	235	177	277	309	337
M (*O*-desmethyl-OH-) isomer 2-AC_3	2460	—	—	176	177	235	277	161	337
2,5-Dimethoxy-4-ethylthio-β-phenethylamine (2C-T-2)	1980	—	—	212	211	183	241	153	197
2C-T-2-AC	2310	—	—	224	211	283	209	153	181
2C-T-2-TFA	2210	—	—	211	337	224	181	151	222
M (*N*-acetyl-)	2310	—	—	224	211	283	209	153	181

table continued

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
M (*N*-acetyl-)-AC	2400	—	—	224	211	209	181	153	325
M (desamino-OH-)-AC	2050	—	—	224	284	209	167	225	100
M (*O*-desmethyl-)-AC_2 or (*O*-desmethyl-*N*-acetyl-)-AC	2120	—	—	269	210	197	252	311	297
M (OH-*N*-acetyl-)-TFA	2270	—	—	259	427	260	428	367	
M (*O*-desmethyl-)-TFA_2	1980	—	—	306	293	419	209	294	307
M (*O*-desmethyl-*N*-acetyl-)-TFA	2290	—	—	306	323	293	307	355	197
2,5-Dimethoxy-4-iodo-β-phenethylamine (2C-I)	2330	—	—	278	263	307	247	279	232
2C-I-AC	2260	—	—	290	349	275	277	148	247
2C-I-TFA	2100	—	—	290	277	247	275	231	403
M (*O*-desmethyl-) isomer 1-AC_2	2480	—	—	276	335	233	263	259	377
M (*O*-desmethyl-) isomer 2-AC_2	2500	—	—	276	335	263	261	377	358
M (desamino-OH-)-AC	2150	—	—	290	275	148	350	247	277
M (*O*-desmethyl-desamino-OH-) isomer 1-AC_2	2240	—	—	276	336	134	261	191	378
M (*O*-desmethyl-desamino-OH-) isomer 2-AC_2	2275	—	—	276	261	336	263	150	378
2,5-Dimethoxy-4-methyl-β-phenethylamine (2C-D)		—	—						
2C-D-AC	1940	—	—	178	135	163	165	179	237
M (*O*-desmethyl-) isomer 1-AC_2	2130	—	—	164	223	151	149	265	165
M (*O*-desmethyl-) isomer 2-AC_2	2200	—	—	164	223	151	149	265	206
M (OH-)-AC_2	2390	—	—	236	295	193	177	235	223
M (desamino-OH-)-AC	1740	—	—	178	163	135	238	79	104
M (*O*-desmethyl-desamino-OH-) isomer 1-AC_2	1880	—	—	164	149	72	224	182	266
M (*O*-desmethyl-desamino-OH-) isomer 2-AC_2	1890	—	—	164	149	121	224	266	206
2,5-Dimethoxy-4-propylthio β-phenethylamine (2C-T-7)	2470	—	—	226	183	225	153	169	255
2C-T-7-AC	2410	—	—	238	255	181	297	153	183
2C-T-7-TFA	2170	—	—	225	351	181	153	238	183
M (OH-*N*-acetyl-)-AC and (OH-)-AC_2	2590	—	—	296	236	101	355	356	283
M (desamino-OH-)-AC	2080	—	—	238	298	181	239	255	299
M (OH-)-TFA_2	2105	—	—	337	463	350	181		
M (OH-*N*-acetyl-)-TFA	2350	—	—	350	409	351	337	181	
2,5-Dimethoxy-4-methylamfetamine (STP or DOM)	1612	1652	—	44	166	151	135	91	209
DOM-PFP	1730	—	—	165	192	135	119	91	355
DOM-AC	2020	—	—	44	192	86	165	166	251
DOM-AC_2	2090	—	—	192	165	86	135	177	293
Art (formyl)	1565	—	—	56	190	165	135	221	91
M (*O*-desmethyl-)-PFP_2	1780	—	—	324	297	190	119	325	487
M (OH-)-PFP_2	1830	—	—	354	327	190	119	355	517
M (OH-)-AC_2	2260	—	—	250	309	86	164	191	91
M (desamino-oxo-OH-)-PFP_2	2045	—	—	353	326	516	233	206	396
M (desamino-oxo-OH-)-AC_2	2560	—	—	164	249	206	233	308	91
Dimethylamfetamine (trimethylbenzeneethenanamine; metabolised to metamfetamine)	1235	—	1429	72	91	73	44	42	56
Ephedrine (D or L)	1365	1410	—	58	77	105	146	131	—
Ephedrine-TFA_2	1345	—	—	154	110	69	115	244	338
Ephedrine-PFP_2	1370	—	—	204	119	95	160	294	338
Ephedrine-TMS_2	1620	—	—	130	73	147	149	163	294
Ephedrine-AC_2	1795	—	—	58	100	148	117	249	122
M (nor-)	1360	1356	—	77	79	91	107	118	132
M (nor-)-TFA_2	1355	—	—	140	69	230	203	105	175
M (nor-)-PFP_2	1380	—	—	190	119	105	117	280	253
M (nor-)-TMS_2	1555	—	—	116	73	117	147	280	163
M (nor-)-AC_2	1805	—	—	86	87	107	134	176	235
M (OH-)	1875	—	—	58	71	77	95	148	107

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
M (OH-)-AC_3	2145	—	—	58	100	205	247	123	307
Etafedrine	1519	1510	1737	86	58	87	42	56	77
Ethylamfetamine (etilamfetamine; also metabolised to amfetamine)	1230	—	—	72	91	117	148	162	—
Ethylamfetamine-AC	1675	—	—	72	114	91	205	148	119
Ethylamfetamine-HFB	1485	—	—	268	240	91	118	269	169
Ethylamfetamine-PFP	1450	—	—	218	190	118	91	119	117
Ethylamfetamine-TFA	1450	—	—	168	69	140	118	91	83
M (OH-methoxy-)	1640	—	—	72	94	137	122	77	209
M (4OH)-AC_2/(PHEA)-AC_2	1995	—	—	72	114	134	176	107	263
M (OH-methoxy-)-AC	2000	—	—	72	164	114	137	251	—
M (OH-methoxy-)-AC_2	2080	—	—	72	114	164	206	137	293
M (di-OH-)-AC_3	2200	—	—	72	114	150	234	192	321
Famprofazone (also metabolised to metamfetamine)	2965	—	—	286	229	91	135	377	—
M (3-OH-methylpropylphenazone)	2410	—	—	231	246	232	215	77	154
M (3-OH-methylpropylphenazone)-AC	2240	—	—	245	232	273	288	190	274
Fencamfamin (also metabolized to amfetamine)	1675	1723	—	98	58	84	215	71	186
Fencamfamin-AC	2085	—	—	170	142	58	97	91	84
Fencamfamin-HFB	1795	—	—	170	142	67	117	129	280
Fencamfamin-TFA	1970	—	2180	142	170	91	180	115	242
Fencamfamin-TMS	1780	—	—	170	258	287	272	259	130
Fencamfamin-PFP	1755	—	—	170	142	91	230	105	292
M (desethyl-)-AC	2005	—	—	170	142	91	171	115	229
M (desethyl-OH-)-AC_2	2305	—	—	142	168	228	91	119	287
Fenethylline (fenetylline; also metabolised to amfetamine)	2830	2900	—	250	70	207	91	119	148
Fenethylline-AC	3110	—	—	250	207	91	292	180	383
Fenethylline-HFB	2815	—	—	91	446	419	266	180	118
Fenethylline-PFP	2790	—	—	91	396	369	207	339	217
Fenethylline-TFA	2840	—	—	91	166	346	319	207	170
M (*N*-desalkyl)-AC	2480	—	—	206	180	193	265	122	86
M (etophylline)	2125	—	—	180	95	224	109	122	194
M (etophylline)-TMS	2160	—	—	180	73	281	296	252	123
M (etophylline)-AC	2200	—	—	87	266	206	180	122	223
Fenfluramine	1230	1252	—	72	44	159	73	58	42
Fenfluramine-TFA	1455	—	1621	168	140	159	169	186	308
Fenfluramine-PFP	1455	—	—	218	190	159	119	168	358
Fenfluramine-AC	1580	—	—	72	114	159	216	58	254
M (desethyl-, norfenfluramine)	1133	1157	—	44	42	159	43	45	184
M (desethyl-)-AC	1510	—	—	86	159	186	109	226	245
Fenproporex (metabolised to amfetamine)	1585	1648	—	97	56	91	68	132	173
Fenproporex-TFA	1705	—	—	193	118	140	91	56	152
Fenproporex-PFP	1685	—	—	243	118	190	56	91	202
Fenproporex-AC	1915	—	—	97	56	139	91	118	65
4-Hydroxyamfetamine (PHA)	1480	—	—	56	107	77	108	91	151
4-Hydroxyamfetamine-AC	1890	—	—	134	107	86	77	133	193
4-Hydroxyamfetamine-AC_2	1900	—	—	134	86	176	107	77	133
M (methoxy-)	1465	—	—	138	137	122	123	94	181
M (methoxy-)-AC_2	2065	—	—	164	86	206	137	165	265
M (1-OH-)/(4-OH-norephedrine)-AC_3	2150	—	—	86	150	234	192	137	123

table continued

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
Levamfetamine (see Amfetamine)									
Mebeverine (metabolised to PMEA)	3045	—	—	308	165	309	121	55	98
Meclofenoxate	1770	1804	2200	58	111	71	42	75	59
Mefenorex (also metabolised to Amfetamine)	1719	1602	—	120	122	91	56	65	121
Mefenorex-TFA	1715	—	—	216	218	140	118	91	154
Mefenorex-PFP	1710	—	—	266	190	91	118	268	119
Mefenorex-AC	1935	—	—	120	122	162	164	91	147
M (OH-dechloro-)-AC_2	2060	—	—	144	186	101	91	84	118
M (OH-) isomer 1-AC_2	2300	—	—	120	121	58	162	107	77
M (OH-) isomer 2-AC_2	2230	—	—	120	121	58	162	107	77
M (OH-methoxy-)	2145	—	—	120	84	122	137	107	256
M (OH-methoxy-)-AC	2360	—	—	120	122	162	164	137	257
M (OH-methoxy-)-AC_2	2410	—	—	120	164	162	206	137	341
M (dechloro-di-OH-)-AC_3	2400	—	—	144	84	101	107	134	186
M (dechloro-tri-OH-)-AC_4	2630	—	—	144	150	186	192	234	84
M (di-OH-)-AC_3	2510	—	—	120	122	162	150	192	234
M (dechloro-di-OH-methoxy-)-AC_3	2520	—	—	144	164	186	206	101	137
Mephentermine (metabolised to phentermine)	1240	1250	—	72	91	56	65	115	148
Mephentermine-TFA	1335	—	—	168	110	91	56	117	122
Mephentermine-AC	1501	—	—	72	114	91	132	148	117
Mescaline	1680	1737	—	182	167	211	151	148	—
Mescaline-TFA	1830	—	—	181	194	179	307	148	151
Mescaline-PFP	1835	—	—	181	194	357	179	119	151
Mescaline-TMS	1745	—	—	102	73	181	182	268	283
Mescaline-TMS_2	1990	—	—	174	73	175	86	340	100
Mescaline-AC	2160	—	—	194	179	181	253	151	148
Art (formyl)	1700	—	—	181	223	182	148	167	77
4-Methoxyamfetamine (PMA)	1412	1410	—	122	121	77	78	91	107
PMA-AC	1720	—	—	44	148	121	86	77	207
4-Methoxyethylamfetamine (PMEA; metabolised to PMA and 4OH-ethylamfetamine)	1660	1512	—	72	121	91	149	77	192
PMEA-TFA	1775	—	—	168	148	121	140	149	289
PMEA-PFP	1765	—	—	218	148	121	190	149	339
PMEA-TMS	2065	—	—	144	73	145	250	121	264
PMEA-AC	1855	—	—	72	148	141	121	77	235
Methoxyphenamine	1361	1416	—	58	91	59	56	42	121
Methylamfetamine (see Metamfetamine)									
Metamfetamine (also metabolised to amfetamine)	1175	1200	—	58	91	65	56	77	134
Metamfetamine-AC	1575	—	—	58	100	91	117	191	—
Metamfetamine-HFB	1460	—	—	254	210	118	91	169	69
Metamfetamine-PFP	1415	—	—	204	160	118	119	91	69
Metamfetamine-TFA	1300	—	1722	154	110	118	91	69	245
Metamfetamine-TMS	1325	—	—	130	73	59	91	131	206
M (4-OH-, pholedrine)	1885	—	—	58	77	107	135	150	—
M (4-OH-, pholedrine)-TFA_2	1585	—	—	154	110	69	230	155	357
M (4-OH-, pholedrine)-PFP_2	1605	—	—	204	160	119	280	154	253
M (4-OH-, pholedrine)-TMS_2	1620	—	—	179	206	73	154	110	309
M (4-OH-, pholedrine)-AC_2	1995	—	—	58	100	134	176	107	—
M (OH-methoxy-)	1810	—	—	58	137	94	122	65	195
M (OH-methoxy-)-AC_2	2115	—	—	58	100	164	206	136	279
M (di-OH-)-AC_3	2190	—	—	58	100	150	123	234	307

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
4-Methoxy-metamfetamine (PMMA) (also metabolized to PMA, MDMA and MDA)									
PMMA-AC	1820	—	—	58	100	148	121	77	221
M (*O*-desmethyl-, pholedrine)-AC_2	1995	—	—	58	100	134	176	249	107
M (1-OH-pholedrine)-AC_2	2095	—	—	58	100	164	206	137	279
5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT)	1965	—	—	114	72	160	174	144	274
Methyl-3,4-benzodioxazol butanamine (MBDB; also metabolised to BDB, see above)	1630	1690	—	72	57	135	77	178	207
MBDB-AC	1995	—	—	72	176	114	135	77	249
MBDB-PFP	1785	—	—	218	176	135	160	119	353
MBDB-TFA	1800	—	—	168	176	135	110	303	140
M (desmethylenylmethyl-)-AC_2	2170	—	—	72	114	178	220	137	293
M (desmethylenyl-)-AC_3	2295	—	—	72	114	164	248	123	321
Methyl-2,3-benzodioxazol butanamine (2,3-MBDB; metabolised to 2,3-BDB)	1610	1660	—	72	57	89	135	178	120
2,3-MBDB-PFP	1710	—	—	218	176	160	135	129	353
2,3-MBDB-TFA	1725	—	—	168	176	110	135	303	140
2,3-MBDB-AC	1965	—	—	72	114	176	135	249	77
2,3-MBDB-TMS	1730	—	—	144	73	135	145	250	264
2,3-Methylenedioxyamfetamine (2,3-MDA)	1470	—	—	44	77	51	135	179	161
2,3-MDA-AC	1770	—	—	162	77	135	105	86	51
2,3-MDA-HFB	1595	—	—	162	135	240	77	163	375
2,3-MDA-PFP	1545	—	—	162	135	119	190	77	325
2,3-MDA-TFA	1585	—	—	162	135	140	275	77	136
2,3-MDA-TMS	1655	—	—	116	73	77	135	236	251
Art (formyl)	1490	—	—	56	135	77	191	105	176
3,4-Methylenedioxyamfetamine (MDA; metabolised to amfetamine metabolites)	1480	1512	—	44	136	135	51	77	179
MDA-AC	1860	—	—	44	162	135	77	86	221
MDA-HFB	1650	—	—	135	162	169	77	240	375
MDA-PFP	1605	—	—	135	162	119	190	136	325
MDA-TFA	1615	—	—	135	162	77	105	136	275
Art (formyl)	1520	1689	—	56	77	135	191	136	105
3,4-Methylenedioxyethylamfetamine (MDEA; metabolised to MDA and ethylamfetamine metabolites)	1560	1630	—	72	77	135	105	163	207
MDEA-TFA	1770	—	—	168	162	140	135	125	303
MDEA-PFP	1755	—	—	218	190	162	135	119	353
MDEA-TMS	1825	—	—	144	73	135	264	100	77
MDEA-AC	1985	—	—	72	162	114	135	77	249
3,4-Methylenedioxymetamfetamine (MDMA; metabolised to MDA, and amfetamine metabolites)	1585	1572	—	58	135	77	177	105	193
MDMA-HFB	1770	—	—	254	162	135	210	77	389
MDMA-PFP	1830	—	—	204	162	160	135	119	339
MDMA-TFA	1720	—	—	154	162	135	110	77	289
MDMA-TMS	1710	—	—	58	100	162	77	135	235
Art (formyl)	—	1735	—	—	—	—	—	—	—
Methylephedrine (metabolised to ephedrine)	1405	1451	—	72	77	105	115	161	—
Methylephedrine-AC	1495	—	—	72	77	91	105	117	162
Methylephedrine-TFA	1185	—	—	72	134	91	162	115	117
Methylephedrine-TMS	1485	—	—	72	149	163	236	117	251
2,3-Dimethylbenzodioxazolbutanamine (MMBDB; also metabolised to MBDB)	1660	1700	—	86	71	96	135	192	105
M (desmethylenyl-methyl-)-AC	1890	—	—	86	87	123	180	222	264

table continued

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
3,4-Methylenedioxy-5-methoxyamfetamine (MMDA)	1690	1743	—	44	166	165	77	65	209
MMDA-AC	2050	—	—	44	192	165	86	166	77
Art (formyl)	1685	—	—	56	165	221	120	77	166
4-Methylthioamfetamine (MTA)	1300	1610	—	44	138	137	122	91	78
MTA-TFA	1750	—	—	137	164	122	69	277	140
MTA-PFP	1760	—	—	137	164	122	190	327	91
MTA-TMS	1750	—	—	116	73	117	100	137	238
MTA-AC	1760	—	—	164	86	137	122	117	265
Art (formyl)	1560	—	—	56	137	193	122	78	91
Methylphenidate	1725	1793	2200	84	56	91	115	77	85
Methylphenidate-AC	2085	—	—	84	126	112	56	275	—
Methylphenidate-TFA	1730	—	—	180	67	150	91	181	126
M (ritalinic acid)	—	—	—	84	91	56	55	136	175
Methylpiperidyl benzilate	2328	—	—	—	—	—	—	—	—
4-Methoxy-α-pyrrolidinopropiophenone (MOPPP)	1705	—	—	98	77	92	135	233	107
M (desmethyl-)-ET	1955	—	—	98	99	121	69	149	247
M (desmethyl-)-TMS	2005	—	—	98	73	56	135	193	276
M (desmethyl-3-OH-)-ET_2	2165	—	—	98	99	56	137	165	193
M (desmethyl-3-methoxy-)-ET	2135	—	—	98	99	56	151	123	179
M (desamino-oxo-)	1440	—	—	135	77	92	107	136	178
M (desmethyl-desamino-oxo-)-ET	1530	—	—	149	121	93	65	150	192
M (desmethyl-3-methyoxy-desamino-oxo-)-ET	1680	—	—	179	151	123	108	73	222
M (oxo-)	2120	—	—	112	121	135	164	150	246
M (dihydro-)-TMS	1880	—	—	98	121	135	209	218	292
4-Methyl-α-pyrrolidinopropiophenone (MPPP)	1725	—	—	98	56	65	91	119	216
M (carboxy-)-ET	2320	—	—	98	177	149	230	104	275
M (carboxy-)-TMS	2195	—	—	98	290	135	99	—	—
M (oxo-)	1920	—	—	112	69	119	84	113	231
M (OH-)-TMS	2095	—	—	98	290	135	90	99	—
M (dihydro-)-TMS	1730	—	—	98	73	163	193	276	202
M (desmethyl-3-methoxy-desamino-oxo)-ET	1680	—	—	179	151	123	108	73	222
M (desmethyl-3-methoxy-)-TMS	1960	—	—	98	223	306	321		
α-Pyrrolidinopropiophenone (PPP) (also metabolized to cathinone and norephedrine)	1595	—	—	98	56	77	69	105	202
M (oxo-)	1820	—	—	112	69	77	84	105	217
M (4-OH-)-ET	1955	—	—	98	99	121	69	149	247
M (4-OH-)-TMS	2005	—	—	98	73	56	135	193	276
M (dihydro-)-TMS	1665	—	—	98	73	105	56	188	262
3,4-Methylenedioxy-α-pyrrolidinopropiophenone (MDPPP)	1995	—	—	98	56	99	121	149	178
M (desmethylene-)-ET_2	2165	—	—	98	99	56	137	165	193
M (desmethylene-3-methyl-)-ET	2135	—	—	98	99	56	151	123	179
M (desmethylene-3-methyl-)-TMS	1960	—	—	98	223	306	321		
M (desamino-oxo-)	1525	—	—	149	121	192	65	91	150
M (desmethylene-desamino-oxo-)-ET_2	1720	—	—	193	165	137	109	194	136
M (desmethylene-3-methyl-desamino-oxo-)-ET	1680	—	—	179	151	123	108	73	222
M (desmethylene-3-methyl-oxo-)-ET	2290	—	—	112	179	151	208	123	290
M (oxo-)	2290	—	—	112	149	178	121	175	261
M (desmethylene-oxo-)-ET2	2325	—	—	112	151	69	193	222	305
M (dihydro-)-TMS	1965	—	—	98	121	149	232	306	223
4′-Methyl-α-pyrrolidinohexanophenone (MPHP)	1965	—	—	140	141	91	119	84	202
α-Methyltryptamine (AMT)	1740	—	—	44	131	77	103	174	—

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
Phendimetrazine (metabolised to phenmetrazine)	1334	1504	1735	57	85	191	77	91	105
Phenmetrazine	1432	1483	—	71	56	177	77	91	105
Phenmetrazine-TFA	1530	—	1873	70	167	98	105	134	273
Phenmetrazine-TMS	1620	—	—	100	73	114	115	143	249
Phenmetrazine-AC	1810	—	—	71	113	86	85	176	219
M (OH-) isomer 1	1830	—	—	71	56	193	121	107	105
M (OH-) isomer 2	1865	—	—	71	56	193	106	163	121
M (OH-methoxy-)	1900	—	—	71	56	223	151	107	137
M (OH-) isomer 1-AC_2	2150	—	—	71	70	113	85	234	277
M (OH-) isomer 2-AC_2	2200	—	—	71	70	113	85	234	277
M (OH-methoxy)-AC_2	2320	—	—	71	70	113	86	265	307
Phentermine	1155	1191	—	—	—	—	—	—	—
Phentermine-TFA	1100	—	1450	154	59	91	132	114	230
Phentermine-PFP	1305	—	—	204	91	132	164	129	280
Phentermine-TMS	1195	—	—	130	73	91	114	206	221
Phentermine-AC	1510	—	—	58	100	91	117	134	191
N-(1-Phenylcyclohexyl)-2-ethoxyethenamine (PCEEA)	1825	—	—	159	91	204	188	247	218
N-(1-Phenylcyclohexyl)-2-methoxyethenamine (PCMEA)	1770	—	—	91	190	159	283	188	218
N-(1-Phenylcyclohexyl)-3-ethoxypropanamine (PCEPA)	1915	—	—	218	91	117	261	232	174
N-(1-Phenylcyclohexyl)-propanamine (PCPR)	1630	—	—	174	91	58	217	159	188
Phenylephrine	1606	0000	—	—	—	—	—	—	—
Phenylephrine-TFA	1755	—	—	95	141	123	77	140	136
Phenylephrine-TFA_2	1755	—	—	140	69	232	121	219	359
Phenylephrine-TMS_3	2110	—	—	116	73	368	146	267	383
Phenylephrine-AC_3	2110	—	—	86	87	115	129	165	220
Phenyl-1-ethylamine	—	1078	—	—	—	—	—	—	—
Phenyl-2-ethylamine	1111	1122	—	—	—	—	—	—	—
Phenylpropanolamine (see Norephedrine)									
Prenylamine (also metabolised to amphetamine)	2555	—	—	58	238	91	45	239	167
Prenylamine-AC	2925	—	—	58	91	100	280	238	164
Prolintane	1634	1660	1849	—	—	—	—	—	—
M (oxo-)	1895	—	—	140	98	91	86	188	231
M (OH-phenyl-)	2135	—	—	126	127	96	107	190	232
M (OH-phenyl-)-AC	2110	—	—	126	127	107	190	232	274
M (OH-methoxy-phenyl-)-AC	2115	—	—	126	127	137	55	262	304
M (oxo-OH-alkyl-)	2200	—	—	86	71	156	91	188	—
M (oxo-OH-alkyl-)-AC	2255	—	—	138	86	198	156	91	71
M (oxo-OH-methoxy-phenyl-)	2240	—	—	140	98	192	86	163	277
M (oxo-OH-methoxy-phenyl-)-AC	2360	—	—	140	192	98	77	234	319
M (oxo-di-OH-)-AC_2	2485	—	—	198	156	128	162	279	107
M (oxo-di-OH-phenyl-)	2475	—	—	140	98	86	178	123	263
M (oxo-di-OH-phenyl)-AC_2	2450	—	—	140	98	77	141	178	220
M (oxo-OH-phenyl)-AC	2275	—	—	140	98	86	162	204	289
M (oxo-di-OH-methoxy-)AC_2	2560	—	—	198	192	156	234	128	377
M (di-OH-phenyl-)-AC_2	2295	—	—	126	123	248	150	290	232
M (tri-OH-)-AC_3	2630	—	—	198	156	128	178	151	123
Propylhexedrine	1175	1192	—	—	—	—	—	—	—
Propylhexedrine-TFA	1385	—	—	154	182	69	110	155	251
Propylhexedrine-PFP	1385	—	—	204	182	160	119	124	205
M (OH-)	1475	—	—	58	156	81	171	138	108
M (OH-)-AC_2	1915	—	—	58	100	74	240	195	255

table continued

Table 40.8 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GC*						
Propylhexedrine-AC	1570	—	—	100	58	182	140	101	114
Pseudoephedrine (see Ephedrine)									
Selegiline (also metabolised to L-metamfetamine and L-amfetamine)	1450	1453	—	—	—	—	—	—	—
M (OH-)	1580	—	—	96	56	97	107	76	171
M (OH-)-AC	1860	—	—	96	56	97	107	77	—
M (nor-)	1350	—	—	82	91	65	67	115	128
M (nor-)-AC	1735	—	—	82	124	91	65	118	214
M (nor-OH-)	1550	—	—	82	107	77	67	135	—
M (nor-OH-)-AC_2	2030	—	—	44	182	167	151	107	225
1,(3-Trifluoromethylphenyl)piperazine (TFMPP)									
TFMPP-AC	1890	—	—	200	56	174	188	172	272
M (OH-)-AC_2	2275	—	—	216	288	56	203	188	330
M (desethylene-)-AC_2	1865	—	—	174	187	229	73	175	145
M (OH-desethylene-)-AC_3	2275	—	—	190	203	245	287	191	232
M (trifluoroaniline)-AC	1400	—	—	161	203	184	142	114	111
M (OH-trifluoromethylaniline)-AC_2	1840	—	—	157	177	219	117	129	261
3,4,5-Trimethoxyamfetamine (3,4,5-TMA)		1740							
3,4,5-TMA-AC	2020	—	—	44	208	193	86	181	267
Art (formyl)	1680	1745	—	56	181	237	148	77	222
2,3,5-Trimethoxyamfetamine (2,3,5-TMA)	2040	—	—	182	167	181	151	107	225
2,3,5-TMA-AC	2285	—	—	208	181	193	86	267	167
2,3,5-Trimethoxymethamfetamine (TMMA)-AC	2310	—	—	58	208	100	224	281	177
Precursors and intermediates of synthesis of illicit amfetamines									
1-(1,3-Benzodioxol-5-yl)-butan-2-one	1525	—	—	135	57	77	192	136	105
1-(1,3-Benzodioxol-5-yl)-butan-1-ol	1560	—	—	151	93	65	123	194	77
Benzylmethylketone (BMK)	1110	1153	—	—	—	—	—	—	—
2,5-Dimethoxybenzaldehyde	1345	1381	—	166	63	95	120	151	123
2,5-Dimethoxyphenyl-2-nitroethene	1900	—	—	209	77	133	147	148	162
2,5-Dimethoxyphenylethylamine	1630	1689	—	152	44	137	121	162	181
2,5-Dimethoxytoluene	1020	—	—	137	152	77	109	65	94
Dimethylphenylethylamine	—	1954	—	—	—	—	—	—	—
Isosafrole	1215	—	—	162	104	103	131	77	65
3,4-Methylene dioxymethylbenzylamine	—	1423	—	—	—	—	—	—	—
Methylene dioxyphenylacetone	—	1530	—	—	—	—	—	—	—
Methylphenylethylamine acetate	—	1593	—	—	—	—	—	—	—
2-Methylhydroquinone	1210	—	—	124	123	67	95	77	107
Piperonal	1302	1373	—	149	150	63	121	91	—
Piperonylacetone	1315	1357	—	135	77	178	79	105	136
3,4,5-Trimethoxybenzaldehyde	1550	1630	—	196	181	125	118	93	95
3,4,5-Trimethoxyphenylacetonitrile	1610	—	—	192	207	164	78	149	124

System GC

DA Cowan, personal communication (2003).

In this system, the drugs are chromatographed as tertiary bases or trifluoroacetyl derivatives.

- *Column*: 3% OV-17 on 80–100 mesh Chromosorb W HP glass (2 m × 3 mm i.d).
- *Temperature programme*: 170°C for 2 min to 270°C at 16°/min for 8 min.
- *Carrier gas*: N_2, 30 mL/min.
- *Reference compounds*: *n*-Alkanes with an even number of carbon atoms.
- *Retention indices*: The retention indices given for system GC are those of the tertiary bases or of trifluoroacetyl derivatives.

Analgesics (non-narcotic) and non-steroidal anti-inflammatory drugs

Analgesics (non-narcotic) and non-steroidal anti-inflammatory drugs (NSAIDs) are acidic and/or neutral drugs and, although water soluble, are readily extracted at pH 5 (sodium acetate or phosphate buffer) into polar solvents, such as ethyl acetate or diethyl ether. Recovery can be improved by 'salting out' using excess solid sodium chloride. Many are arylacetic (indometacin), arylpropionic (ibuprofen), salicylic

(diflunisal) or fenamic (mefenamic acid) acid derivatives, and thus require the formation of suitable derivatives prior to GC (data for methyl derivatives are given in Table 40.9; the values given for system GD are retention times of methyl derivatives relative to *n*-$C_{16}H_{34}$). This is most pertinent at low concentrations or with packed columns, while at higher concentrations peaks may tail, and there is thus a tendency for retention time to increase with concentration. Capillary columns, especially those with higher phase ratios, give better peaks for underivatised phenols. The arylpropionic acid derivatives are chiral, with the non-steroidal anti-inflammatory (NSAI) activity usually residing in the *S*-enantiomer, but these are usually marketed as racemates and undergo enantiomeric inversion *in vivo*. Separation of NSAI enantiomers has been reviewed (Davies 1997).

Systems GA or GB, described above, may be used, or systems GD and GL.

System GD

In this system, the substances are chromatographed as their methyl derivatives.

- *Column*: 3% SE-30 on 80–100 mesh Chromosorb G (acid-washed and dimethyldichlorosilane-treated) glass (2 m × 3 mm i.d).
- *Temperature programme*: 120°C for 2 min to 260°C at 10°/min for 5 min.
- *Carrier gas*: N_2, 40 mL/min.
- *Reference compound*: Hexadecane (*n*-$C_{16}H_{34}$).
  Note: Free carboxylic acids and phenols will not generally give peaks, although large quantities may give tailing peaks.
- *Retention indices and relative retention times*: The values given for system GD are retention times of methyl derivatives relative to *n*-$C_{16}H_{34}$.

System GL

In this system (Maurer *et al.* 2001), the substances are chromatographed as their methyl derivatives after extractive methylation. Equal volumes of urine and phase-transfer reagent (0.02 mol/L tetrahexylammonium hydrogensulfate in 1 mol/L phosphate buffer, pH 12) are incubated with three volumes of 1 mol/L methyl iodide in toluene on a shaker at 50°C for 30 min. The organic phase is eluted on a diol solid-phase cartridge (conditioned with methanol, then toluene) using diethyl ether–ethyl acetate (95 : 5 v/v). After evaporation to dryness at 60°C, the residue is reconstituted in ethyl acetate for injection.

- *Column:* HP1 (methyl-PSX) fused-silica capillary (12 m × 0.2 mm i.d. 0.33 μm).
- *Injector:* 280°C splitless mode.
- *Temperature programme:* 100°C for 2 min to 310°C at 30°/min for 8 min.
- *Carrier gas:* He, 1 mL/min.
- *Retention indices* are given in Table 40.9.

Anticholinergics

The anticholinergics comprise a chemically diverse group of drugs, although most can be extracted successfully from biological specimens under mildly alkaline conditions. Some (atropine, hyoscine) are

Table 40.9 GC retention data and mass spectral data for analgesics (non-narcotic) and NSAIDs (ET, ethyl; Me, methyl)

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GD*	*GL*						
Acemetacin (metabolised to indometacin)	Not eluted	—	—	—						
Acemetacin-Me	3150	—	—	—	139	429	141	431	312	430
Acemetacin-ET	3220	—	—	—	139	443	141	312	445	442
Art-Me_2	2390	—	—	—	174	233	291	175	159	131
Acetanilide (also metabolised to paracetamol)	1368	1400	—	—	93	135	43	66	65	39
Alclofenac	—	—	—	—	41	226	77	143	181	141
Aletamine	1293	—	—	—	70	120	43	91	39	103
Amidopyrine/aminophenazoine	1895	1992	—	—	56	231	97	111	112	42
M (nor-)	1980	—	—	—	—	—	—	—	—	—
M (bis-nor-)	1955	—	—	—	—	—	—	—	—	—
M (desamino OH-)	1855	—	—	—	—	—	—	—	—	—
Aspirin (metabolised to salicylic acid and salicylamide)	1545	—	—	—	120	43	138	92	121	64
Aspirin-Me	1394	1430	—	—	135	194	179	136	91	76
Azapropazone (metabolised to paracetamol and aspirin)	2461	—	—	—	160	300	189	145	188	301
Art	1804	1779	—	—	—	—	—	—	—	—
Benorilate (metabolised to paracetamol and aspirin)	1840	—	—	—	121	163	151	109	43	122
Benoxaprofen	2550	Not eluted	—	—	256	301	91	258	119	65
Benoxaprofen-Me	2485	—	1.98	—	256	315	91	119	258	65
Benzydamine	2380	—	—	—	85	58	86	91	84	70
Bufexamac	—	—	—	—	—	—	—	—	—	—
Celecoxib	—	—	—	—	381	300	382	301	281	140
Clonixin-Me	—	—	1.61	—	—	—	—	—	—	—
Dexketoprofen (see Ketoprofen)	—	—	—	—	—	—	—	—	—	—
Diclofenac	2271	2231	—	—	214	216	242	295	215	297
Diclofenac-Me	2195	—	1.42	2200	214	242	309	216	311	179
Diclofenac-Me_2	2220	—	—	—	228	323	229	325	214	264
Art	2322	2418	—	—	320	355	357	322	228	292

table continued

Table 40.9 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GD*	*GL*						
Art (-H_2O)	2135	2231	—	—	214	277	242	279	179	216
Art (-H_2O)-Me	2300	2436	—	—	228	230	200	263	291	164
M (OH-)-Me_2	2460	—	—	2460	244	339	272	341	201	246
M	—	2592	—	—	214	216	242	277	179	294
M (OH-) isomer 1	—	2600	—	—	230	293	232	295	258	195
M (OH-) isomer 2	—	2941	—	—	230	293	232	271	158	310
Diflunisal	2095	—	—	—	232	250	175	204	176	233
Diflunisal-Me	2050	—	1.20	—	—	—	—	—	—	—
Diflunisal-Me_2	1990	—	—	1990	278	247	245	175	188	204
Dipyrone	—	2069	—	—	56	42	83	57	77	51
M (bisdesalkyl-)	1955	—	—	—	—	—	—	—	—	—
M (desalkyl-)-AC	2395	—	—	—	—	—	—	—	—	
Etenzamide (also metabolised to salicylamide)	—	—	—	—	—	—	—	—	—	—
M (desethyl-)-AC	1660				120	92	105	148	150	121
Etodolac	2333	—	—	—	228	258	287	198	57	229
Etodolac-Me	—	—	—	2225	228	272	301	198	229	115
Famprofazone (also metabolised to metamfetamine)	2965	—	—	—	286	229	91	81	287	77
M (OH-)	2410	2850	—	—	244	273	44	302	214	229
Fenbufen	3078	—	—	—	181	152	153	254	182	151
Fenbufen-Me	2315	—	1.79	1975	181	152	153	182	268	237
M (acetic acid OH-)-Me_2	—	—	—	2190	197	256	154	128	152	198
Fenclofenac-Me	—	—	1.55 and 1.26	—	—	—	—	—	—	—
Fenoprofen	2016	2040	—	—	197	241	198	77	242	104
Fenoprofen-Me	1906	—	1.31	1970	197	256	198	257	91	103
M (OH)-Me_2	—	—	—	2130	286	227	287	123	91	152
Feprazone	2380	—	—	—	183	77	252	320	184	41
Feprazone-Me	—	—	1.81							
Floctafenine	3132	—	—	—	—	—	—	—	—	—
Floctafenine-Me	2433	—	—	—	—	—	—	—	—	—
Flufenamic acid	1950	—	1.26	—	263	281	166	92	145	167
Flufenamic acid-Me	—	—	—	1875	263	295	235	166	264	92
M (OH-)-Me_2	—	—	—	2115	325	293	278	250	223	202
Flunixin-Me	—	—	1.39	—	—	—	—	—	—	—
Flupirtene	2603	—	—	—	109	304	231	124	258	110
Flurbiprofen	1900	—	—	—	199	244	200	178	179	184
Flurbiprofen-Me	1885	—	1.3	1880	199	178	183	258	170	200
M (OH)-Me_2	2180	—	—	2180	229	288	230	289	214	183
M (OH-methoxy-)-Me_2	2310	—	—	—	318	259	319	260	215	303
Glafenine-ME	2770	—	—	—	—	—	—	—	—	—
Ibuprofen	1615	1637	—	—	163	161	119	91	206	117
Ibuprofen-ME	1510	—	0.89	1505	161	177	220	119	91	117
M (2OH-)	—	2096	—	—	177	117	119	91	118	221
M (2OH-)-Me	1750	—	—	—	177	117	159	145	131	236
M (3OH-)-Me	1630	—	—	1680	119	118	91	59	178	162
M (HOOC-)-Me_2	1765	—	—	—	205	145	177	117	121	264
Indometacin	2550	—	—	—	139	141	357	111	359	140
Indometacin-Me	2770	—	1.55 and 0.49	2770	139	141	111	371	312	114
M (OH)-Me_2	—	—	—	2880	139	141	140	401	111	262
Indoprofen-Me	2708	—	2.27 and 2.07	—	236	295	237	296	218	206
Isopropylaminophenazone	2033	—	—	—	—	—	—	—	—	—

Table 40.9 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GD*	*GL*						
Kebuzone-Me	—	—	—	2510	77	336	105	183	266	117
M (OH)-Me_2	—	—	—	2690	77	107	366	367	213	296
Ketoprofen	2245	—	—	—	105	177	77	209	254	210
Ketoprofen-Me	2090	—	1.45	2090	209	105	77	268	191	210
M (OH)-Me_2	—	—	—	2250	239	298	135	191	107	103
Ketorolac	—	2686	—	—	105	210	77	44	132	254
Lonazolac-Me	—	—	—	2685	267	326	77	232	269	328
M (OH)-Me_2	—	—	—	2875	356	297	262	358	299	247
Meclofenamic acid	2420	—	—	—	—	—	—	—	—	—
Meclofenamic acid-Me	—	—	1.62	2240	242	244	309	311	277	214
Mefenamic acid	2201	2370	—	—	223	241	208	222	194	180
Mefenamic acid-Me	2069	—	1.45	2110	223	255	208	180	194	222
M (OH)-Me	—	—	—	2400	209	271	221	224	180	194
Methyl salicylate (metabolised to salicylic acid and salicylamide)	1195	1228	—	1210	92	120	152	65	121	93
Methylsalicylate-Me	1200	—	—	—	135	133	77	92	166	104
Mofebutazone-Me_2	—	—	—	1955	121	77	204	260	83	105
M (OH)-Me_2	—	—	—	2075	77	276	121	71	128	220
Morazone (metabolised to phenazone and phenmetrazine)	3130	—	—	—	201	56	176	202	70	258
Nabumetone	—	2084	—	—	171	228	172	185	128	115
M (6-methoxy-2-naphthyl acetic acid, 6-MNA)	—	2322	—	—	171	215	128	172	44	102
M (*O*-desmethyl-)	—	2385	—	—	157	201	128	158	127	44
Naproxen	2045	2337	—	—	185	230	141	186	184	115
Naproxen-Me	1980	—	1.37 and 1.18	2120	185	244	170	141	115	186
M (ET)	1830	2115	—	—	185	258	186	170	153	141
M (*O*-desmethyl-)	—	2396	—	—	171	215	115	141	153	130
M (*O*-desmethyl-)-Me_2	1980	—	—	2120	185	244	170	141	115	186
M (OH)-Me_2	1800	—	—	1800	274	215	259	171	184	275
Nifenazone	3080	—	—	—	—	—	—	—	—	—
M (desacyl-)	1955	—	—	—	—	—	—	—	—	—
Niflumic acid	2085	—	1.35	—	282	236	237	281	263	145
Niflumic acid-Me	1955	—	—	1960	236	295	296	263	237	145
M (OH-) Me_2	—	—	—	2140	326	325	251	293	294	266
Oxyphenbutazone	Not eluted	Not eluted	—	—	199	324	93	77	65	55
Art (phenyldiazophenol)	2070	—	—	—	93	77	65	198	121	51
Art (phenyldiazophenol)-Me	2020	—	—	—	77	107	212	135	64	51
Oxyphenbutazone-Me_2 (isomer 1)	2545	—	2.11	—	352	213	77	107	118	135
Oxyphenbutazone-Me_2 (isomer 2)	2720	—	—	—	352	77	160	190	309	278
Paracetamol (acetaminophen)	1665	1722	—	—	109	51	43	80	108	81
Art (*p*-aminophenol)	1253	1280	—	—	109	52	53	80	81	108
Paracetamol-Me	1512	—	—	1630	108	123	165	80	95	122
Art (*p*-aminophenol)-Me_2	1220	—	—	—	136	137	121	120	94	65
Phenacetin (also metabolised to paracetamol)	1675	1730	—	—	108	109	179	137	43	81
Phenacetin-Me	—	—	—	—	122	193	151	56	123	94
M (hydroquinone)	1240	—	—	—	110	81	55	53	82	39
M (*p*-phenetidine)	1275	—	—	—	108	137	109	80	53	65
Phenazone/antipyrine	1835	1951	—	—	188	96	77	56	105	189
M (4-OH)	1855	—	—	—	85	56	84	204	77	120
Phenazopyridine (also metabolised to paracetamol)	2245	2370	—	—	—	—	—	—	—	—
M (aniline)	1158	—	—	—	93	66	65	92	46	41

table continued

Table 40.9 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GD*	*GL*						
Phenylbutazone (also metabolised to oxyphenbutazone)	2367	2472	2.05 and 1.81	—	—	—	—	—	—	—
Art	2435	2550	—	—	183	77	184	324	325	119
Phenylbutazone-Me	2290	—	—	2290	183	77	322	266	118	323
M (OH-alkyl)-Me	—	—	—	2500	183	338	162	77	266	184
Phenyramidol	1960	—	—	—	—	—	—	—	—	—
Piroxicam	1413	—	—	—	173	117	145	78	104	94
Propyphenazone	1920	2030	—	—	215	230	56	77	216	96
M (nor-)	1772	—	—	—	174	216	77	173	129	145
M (OH-methyl-)	2410	—	—	—	231	246	232	77	215	154
M (OH-phenyl-)	2300	—	—	—	231	56	246	96	122	217
M (OH-propyl-)	2210	—	—	—	215	56	246	124	77	231
M (isopropanolyl-)	2020	—	—	—	231	246	213	56	232	61
M (isopropenyl-)	1970	—	—	—	136	228	95	77	108	106
M (nor-OH-)	1780	—	—	—	93	232	77	190	120	174
M (nor-OH-phenyl-)	2080	—	—	—	190	232	93	121	65	161
M (nor-di-OH-)	2090	—	—	—	248	109	136	206	121	232
M (nor)-Me	1735	—	—	—	215	77	230	51	200	185
M (OH-phenyl)-Me	2310	—	—	—	56	246	260	122	96	77
M (COOH)-Me	2160	—	—	—	215	274	56	77	105	165
M (nor-OH-phenyl isomer 1)-Me_2	2030	—	—	—	245	230	260	215	77	92
M (nor-OH-phenyl isomer 2)-Me_2	2060	—	—	—	245	230	260	77	92	215
M (nor-di-OH)-Me_3	2240	—	—	—	275	290	260	252	236	276
Rofecoxib	—	3119	—	—	257	314	178	131	176	165
Salicylamide	1414	1489	—	—	120	92	137	65	121	64
Salicylic acid	1307	1340	—	—	120	92	138	64	63	121
Salicylic acid-AC	1545	—	—	—	120	43	138	92	121	64
Salicylic acid-Me	1195	1228	—	1210	92	120	152	65	121	93
Salicylic acid-Me_2	1200	—	—	—	135	133	77	92	166	104
M (5-OH)-Me_3	—	—	—	1530	196	165	163	181	107	151
M (glycine conj)	1825	—	—	—	120	121	92	65	195	149
M (glycine conj)-Me	1810	—	—	—	121	120	209	119	65	92
M (glycine conj)-Me_2	1845	—	—	—	135	90	77	105	223	121
Salsalate (metabolised to salicylic acid)	—	Not eluted	—	—	121	120	92	65	138	258
Sulindac	2890	—	0.49	—	341	233	356	246	247	281
Sulindac-Me	3220	—	—	—	233	354	355	370	248	247
M (sulfide-)	2896	2959	—	—	328	233	234	313	159	247
M (sulfone)	—	3029	—	—	328	233	234	329	220	246
Sumatriptan	—	2715	—	—	58	143	142	115	156	295
Tenoxicam-Me_2	2690	—	—	—	—	—	—	—	—	—
Tiaprofenic acid	1976	—	—	—	216	139	201	77	173	105
Tiaprofenic acid-Me_2	2180	—	—	2175	229	288	230	77	105	201
Tolfenamic acid	—	—	—	—	—	—	—	—	—	—
Tolfenamic acid-Me	—	—	—	2255	208	243	275	180	89	245
Tolmetin	1890	—	—	—	212	213	122	198	44	91
Tolmetin-Me	2247	—	1.77 and 1.36	2235	212	271	256	119	270	91
M (COOH)	—	2615	—	—	212	91	256	119	44	65
M (COOH)-Me_2	—	—	—	2600	256	315	242	197	135	314
Zomepirac-Me	2343	—	—	—	246	305	248	304	139	111
M (-CO_2)	2025	—	—	—	246	247	248	211	230	136

relatively unstable and produce several artefacts, either from hydrolysis during extraction or from thermal degradation in the GC. The quaternary ammonium compounds (e.g. emepromium bromide) are not amenable to gas chromatography. Systems GA or GB, previously described, may be used. Retention indices and relative retention times are given in Table 40.10.

Anticonvulsants and barbiturates

Most barbiturates and anticonvulsants are acidic or mildly basic drugs and are extracted readily from aqueous medium into organic solvents. Although phenobarbital requires pH 7 or less for good recovery in liquid–liquid extractions, the other barbiturates and anticonvulsants (such as carbamazepine and phenytoin) may be extracted from aqueous

Table 40.10 GC retention data and mass spectral data for anticholinergics (AC, acetyl)

	System		*Principal ions* (m/z)					
	GA	*GB*						
Adiphenine	2200	—	86	167	99	87	58	165
Atropine	2190	2293	124	82	94	83	42	96
Art (-CH_2O)	1980	2051	124	259	140	94	221	178
Art (-H_2O)	2085	2250	124	271	96	82	140	94
Benzatropine (benztropine)	2302	2423	83	140	82	124	96	42
Biperiden	2276	—	98	218	99	55	41	77
M (OH-)	2645	—	98	218	114	327	284	85
Caramiphen	—	—	86	99	91	144	58	56
Chlorphenoxamine	2080	2190	58	59	179	42	178	72
M (OH-)	2470	—	58	152	165	181	195	231
M (nor-)	2094	2205	—	—	—	—	—	—
M (OH-methoxy-carbinol)-H_2O	2220	—	260	262	210	245	227	181
Cyclopentolate	2022	2092	58	71	72	207	42	91
Cyclopentolate-H_2O	2000	1551	58	71	91	115	129	273
Cycrimine	2114	—	98	41	42	55	99	77
Dicycloverine (dicyclomine)	2111	2175	86	71	99	58	55	56
Diethazine	2377	—	86	298	87	58	299	212
Eucatropine	2026	—	124	276	58	140	56	72
Homatropine	2072	2165	124	107	82	83	42	77
Hyoscine (scopolamine)	2300	2427	94	138	42	108	136	41
Art (H_2O)	2230	2255	94	103	138	154	108	285
M (desacyl-)	1210	—	96	94	155	126	110	70
Hyoscyamine (see Atropine)								
Metixine (methixine)	2480	2596	99	197	44	58	112	309
Orphenadrine	1935	2014	58	73	44	45	165	181
M (nor-, tofenacin)	1900	2007	180	179	86	255	165	240
M (methylbenzophenone)	1700	1827	195	196	77	105	119	165
M	1560	1630	167	182	107	108	165	119
Oxyphencyclimine	1661 and 2250	—	105	129	112	77	42	313
Piperidolate	2318	—	111	96	167	112	165	71
Procyclidine	2156	2261	84	204	205	85	42	55
M (OH-isomer 1)	—	2487	—	—	—	—	—	—
M (OH-Isomer 2)	—	2517	—	—	—	—	—	—
M	—	2548	—	—	—	—	—	—
M	—	2603	—	—	—	—	—	—
M (oxo-) art-H_2O	2490	2669	200	115	86	98	198	283
Scopolamine (see Hyoscine)								
Tigloidine	1687	—	124	82	83	94	55	42
Trihexyphenidyl (benzhexol)	2245	2354	98	105	55	99	77	218
M (OH-)	2500	2618	—	—	—	—	—	—
Tropicamide	2335	2442	92	91	65	103	93	163
Art (-CH_2O)	2230	—	92	91	163	65	254	107
Art (-H_2O)	2250	—	103	266	92	251	77	265
Tropicamide-AC	2410	—	92	104	266	65	163	326

solutions at pH values as high as 11. There is little to be gained in terms of sensitivity by using NPD over FID, especially for barbiturates, but the former is more specific and excludes fatty acids, which can be problematic in decomposing samples. For GC, some investigators prefer to methylate, either during the extraction (iodomethane–tetramethylammonium hydroxide in dimethyl sulfoxide) (Liu *et al.* 1994), or by flash methylation in the injection port (trimethylphenylammonium hydroxide in ethyl acetate) (Brugmann 1981). This method is sometimes considered unreliable for quantitative analysis, and is arguably only necessary when using packed chromatography columns. Data for the methyl derivatives are therefore given where they are available. When using packed columns for GC, the medium- and higher-polarity phases, such as *-1310, *-2100, DEGS and waxes, are useful, although methylation allows the use of some more non-polar phases such as *-1 (Stern, Caron 1977).

Systems GA and GB, previously described, may be used, or systems GE, GF and GAJ.

System GE

Quantitative analysis of underivatised antiepileptic drugs (Supelco 1979).

- *Column*: 2% SP-2110 and 1% SP-2510-DA on 100–120 mesh Supelcoport glass (1 m × 2 mm i.d.).
- *Temperature programme*: 120°C to 250°C at 16°/min.
- *Carrier gas*: N_2, 50 mL/min.
- *Reference compound*: Phenytoin. *Note:* This system separates cholesterol from all drugs in the group.
- *Retention indices and relative retention times*: The values given for system GE are retention times relative to phenytoin.

System GF

(Flanagan, Berry 1977).

- *Column*: 3% Poly A103 on 80–100 mesh Chromosorb W HP glass (1 m × 4 mm i.d.).
- *Temperature*: 200°C.
- *Carrier gas*: N_2, 60 mL/min.
- *Reference compounds*: *n*-Alkanes with an even number of carbon atoms.

System GAJ

Data generated by the author.

- *Column:* DB1301 capillary (25 m × 0.32 mm i.d., 0.25 µm).
- *Temperature programme:* 100°C to 235°C at 35°/min for 3.6 min to 290°C at 8°/min for 3.5 min.
- *Carrier gas:* He, 1.5 mL/min.
- *Reference compound:* Methylphenobarbital.
- *Detection* by FID and NPD split.
- *Retention indices and relative retention times* are given in Table 40.11. The values given for system GAJ are retention times relative to methylphenobarbital.

Antidepressants

Antidepressants (tricyclics, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs)) can be extracted readily under mildly basic conditions (pH 10) into many solvents, such as ethyl acetate, hexane, diethyl ether. Less polar solvents, such as hexane, limit the extraction of hydroxylated metabolites. An acidified (0.05 mol/L H_2SO_4) back extraction is a useful clean-up procedure

Table 40.11 GC retention data and mass spectral data for anticonvulsants and barbiturates (Me, methyl; ET, ethyl)

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GE*	*GF*	*GAJ*						
Allobarbital	1600	1636	—	2340	—	41	167	124	80	53	68
Me_2	1505	—	—	—	—	195	138	194	110	221	236
M	1785	—	—	—	—	—	—	—	—	—	—
Amobarbital (amylobarbital)	1710	1742	—	2430	0.794	156	141	157	41	55	142
Aobarbital-Me_2	1593	—	—	—	—	184	169	170	185	226	239
M (3OH-)	1915	2015	—	—	1.138	156	157	141	227	214	195
M (3OH)-Me_2	1750	—	—	—	—	137	184	169	185	255	270
M (COOH-)	1960	—	—	—	0.775	156	141	157	183	212	155
M (COOH)-Me_3	1850	—	—	—	—	169	184	137	185	240	211
Aprobarbital	1618	—	—	—	—	167	41	124	168	97	39
M (OH-)	1815	—	—	—	—	183	154	184	122	165	226
Aprobarbital-Me_2	1540	—	—	—	—	195	196	138	181	111	220
Barbital	1489	—	—	2230	0.612	156	141	55	155	98	82
Barbital-Me_2	1420	—	—	—	—	184	169	126	112	183	83
Barbituric acid	0000	—	—	—	—	42	128	85	44	70	69
Barbituric acid-Me_3	1645	—	—	—	—	170	55	82	98	113	155
Beclamide	1720	1778	—	—	—	91	106	197	162	107	148
Art	1680	1539	—	—	—	55	161	106	116	79	77
Brallobarbital	1853	—	—	2765	1.000	207	41	39	124	91	165
Brallobarbital-Me_2	1725	—	—	—	—	235	193	136	194	236	121
M (2OH-)	2040	—	—	—	—	223	165	180	136	152	122
M (desbromo-OH-)	1795	—	—	—	—	167	124	141	98	181	224
Butalbital	1665	1698	—	2395	0.778	41	167	168	124	97	141
Butalbital-Me_2	1655	—	—	—	—	196	195	138	209	169	237
M (OH-	1940	2016	—	—	—	168	167	153	141	222	240
Butobarbital (butobarbitone)	1660	—	—	2390	0.732	141	156	41	55	98	142

Table 40.11 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GE*	*GF*	*GAJ*						
Butobarbital-Me_2	1565	—	—	—	—	169	184	112	170	183	211
M (3′-OH-)	1920	—	—	—	1.053	156	141	157	199	181	213
M (3′-oxo-)	1880	—	—	—	—	156	141	198	157	199	211
Carbamazepine	2285	2435	0.83	—	1.716	193	192	236	191	194	165
Carbamazepine-Me	1905	—	—	—	—	—	—	—	—	—	—
M (10,11-epoxide)	2220	—	—	—	1.188	180	179	178	152	44	181
M (iminostilbene)	1998	2064	—	—	0.732	193	192	194	165	179	191
M (acridine)	1800	1880	—	—	0.591	179	178	151	152	180	177
M (formylacridine)	2025	2158	—	—	0.813	179	207	178	180	151	152
M (methylacridine)	—	2054	—	—	0.699	193	192	165	191	194	167
M	—	2316	—	—	—	180	209	181	210	152	190
M	—	2332	—	—	—	283	180	208	284	192	266
M	—	2387	—	—	—	209	180	208	210	152	167
M	—	2402	—	—	—	180	210	227	209	181	208
M (10,11-di-OH-)	—	2738	—	—	—	180	208	196	270	253	167
Clobazam (see Benzodiazepine section)											
Clonazepam (see Benzodiazepine section)											
Cyclobarbital	1955	—	—	2825	1.142	207	141	81	79	67	80
Cyclobarbital-Me_2	1845	—	—	—	—	235	169	236	178	121	264
M (oxo-)	2190	—	—	—	—	221	193	260	179	222	178
M (oxo)-Me_2	2050	—	—	—	—	249	221	250	164	192	278
Cyclopentobarbital	1865	—	—	—	—	67	193	66	41	169	39
Cyclopentobarbital-Me_2	1775	—	—	—	—	221	196	164	181	111	107
Dimethadione	1060	—	—	—	—	43	59	42	41	58	129
Enallylpropymal-Me_2	1520	—	—	—	—	181	41	182	124	53	138
Ethosuximide	1205	1258	0.18	—	0.453	113	70	55	42	41	39
Ethosuximide-Me	1130	—	—	—	—	55	127	70	112	140	155
M (OH-ET-)	1370	1436	—	—	—	113	85	98	69	71	142
M (3OH-)	1322	1395	—	—	—	71	86	129	139	142	157
M (oxo-)	1270	—	—	—	—	70	155	55	113	69	98
Ethotoin	1800	1751	0.57	—	0.940	104	105	204	77	78	133
Felbamate(H_2O)	1450	1475	—	—	—	104	103	91	77	121	134
Art (-$C_2H_3NO_2$)	1890	1854	—	—	—	104	103	91	77	121	134
Art (-CH_3NO_2)	2210	2212	—	—	—	134	104	103	91	77	177
Fosphenytoin (see Phenytoin)											
Gabapentin Art (-H_2O)	1750	1633	—	—	—	81	153	152	87	110	96
Art (-H_2O)-Me	1560	—	—	—	—	81	67	167	166	124	110
Heptabarb	2055	2110	—	2940	1.282	221	43	78	93	80	141
Heptabarb-Me_2	1915	—	—	—	—	249	169	250	133	183	192
M (OH-)	2275	—	—	—	—	219	93	141	115	237	157
M (3′-oxo-)	2320	—	—	—	—	—	—	—	—	—	—
Hexethal	1850	—	—	—	—	156	141	55	41	157	98
Hexethal-Me_2	1745	—	—	—	—	169	184	112	185	170	209
Hexobarbital	1855	—	—	2380	0.940	221	81	157	80	79	155
Hexobarbital-Me	1800	—	—	—	—	235	81	169	171	170	236
M (3′-oxo-)	2055	—	—	—	—	95	235	250	156	193	123
M (nor-)	1980	—	—	—	—	81	143	207	123	139	222
M (oxo)-ME	2020	—	—	—	—	249	264	95	221	207	170
M (3′-OH-)	1970	—	—	—	—	156	79	219	234	233	191

table continued

Table 40.11 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GE*	*GF*	*GAJ*						
Ibomal (propallylonal; also metabolised to aprobarbital)	1880	—	—	—	—	167	209	43	124	39	41
Ibomal-Me_2	1745	—	—	—	—	237	195	138	110	238	196
M (desbromo-OH-)	1770	—	—	—	—	169	141	142	98	184	226
M (desbromo-oxo)-Me_2	1720	—	—	—	—	169	197	112	212	140	170
M (desbromo-OH)-Me_2	1730	—	—	—	—	169	183	170	112	198	214
Idobutal	1700	—	—	—	—	167	41	168	124	97	141
Isobutal-Me_2	1610	—	—	—	—	195	196	138	181	169	223
Lamotrigine	2635	2562	—	—	1.941	185	187	255	257	123	124
Levetiracetam	—	1629	—	—	—	126	41	69	98	44	127
Metharbital (metabolised to barbital)	1470	—	—	—	—	155	170	112	169	55	82
Methohexital	1770	1827	—	—	0.798	41	81	53	221	79	39
Methohexital-Me	1735	1797	—	—	—	235	178	195	247	261	275
M (4′-OH-)	1880	—	—	—	—	219	124	181	245	261	278
Methoin (mephenytoin)	1785	—	0.55	—	0.918	189	104	190	77	44	105
M (*p*-OH-)	2400	—	—	—	—	205	120	109	152	176	234
M (OH-methoxy-)	2380	—	—	—	—	235	150	135	137	247	264
M (nor-)	1950	—	—	—	—	104	175	77	132	163	204
Mesuximide (methsuximide)	1705	—	0.35	—	0.689	181	117	203	103	77	78
M (nor-)	1750	—	—	—	0.779	118	117	103	77	189	155
M (OH-)	2220	—	—	—	—	134	219	119	107	91	204
M (nor-OH-)	2300	—	—	—	—	134	205	119	133	103	165
Methylphenobarbital	1890	2222	—	—	1.000	218	117	118	146	103	77
Methylphenobarbital-Me	1855	—	—	—	—	232	118	117	146	175	260
M (OH-, MHD)	2370	—	—	—	—	134	233	234	262	162	133
M (OH-methoxy-)	2310	—	—	—	—	231	292	263	164	188	174
Nealbarbital	1720	—	—	2460	0.789	57	41	141	167	39	83
Nealbarbital-Me_2	1620	—	—	—	—	169	195	209	112	138	250
Oxcarbazepine	—	2266	—	—	—	209	180	208	153	181	210
M (formylacridine)	2025	2158	—	—	—	179	207	178	180	151	152
M (methylacridine)	—	2054	—	—	—	193	192	165	191	194	167
M	—	2204	—	—	—	211	180	194	182	167	152
M	—	2296	—	—	—	193	192	180	165	191	237
M (10-OH-, MHD)	—	2580	—	—	—	193	180	194	210	254	167
M (carbamazepine)	2285	2435	—	—	1.716	193	192	236	191	194	165
Paramethadione	1115	—	0.06	—	—	43	129	57	56	41	72
Pentobarbital	1735	1776	—	2465	0.803	141	156	43	41	157	55
Pentobarbital-Me_2	1630	—	—	—	—	184	169	112	225	185	126
M (3′-OH-)	1955	2039	—	—	—	156	141	157	197	195	227
M (3′-OH)-Me_2	1820	—	—	—	—	169	184	185	223	225	241
Phenacemide	1473	—	—	—	—	91	92	118	44	43	135
Pheneturide	1465	—	—	—	—	91	146	44	119	206	41
Phenobarbital (phenobarbitone)	1953	2031	0.74	2960	1.150	204	117	146	161	77	103
Phenobarbital-Me_2	1855	—	—	—	—	232	118	117	146	175	260
M (4-OH-)	2295	2378	—	—	—	219	248	148	220	176	204
M (4-OH)-Me_3	2200	—	—	—	—	290	261	148	233	262	176
Phensuximide	1634	—	0.39	—	—	104	189	103	78	51	77
Phenylmethylbarbituric acid (heptobarbital)	1880	—	—	—	1.087	104	132	218	51	103	77
Phenylmethylbarbituric acid-Me_2	1790	—	—	—	—	132	104	246	103	79	189
Phenytoin	2320	2435	1.00	—	1.773	180	104	223	77	209	252

Table 40.11 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GE*	*GF*	*GAJ*						
M (OH-)	2795	2910	—	—	—	268	239	196	225	120	180
Phenytoin-Me	2245	—	—	—	—	180	266	237	209	189	165
M (*p*-OH)-Me_2	2720	—	—	—	—	296	267	219	210	180	134
M (*p*-OH-methoxy-)	2770	—	—	—	—	298	269	226	196	211	254
M (*p*-OH-methoxy)-Me_2	2740	—	—	—	—	326	397	249	282	210	196
Primidone (also metabolised to phenobarbital)	2250	2384	0.89	—	1.674	146	190	117	118	161	189
Primidone-Me_2	—	2161	—	—	—	146	218	117	118	217	246
M (phenylethylmalondiamide)	1884	1996	—	—	1.074	148	163	103	120	91	117
M (diamide)	1935	—	—	—	—	163	148	103	118	120	133
M (AC)	2115	2189	—	—	—	146	232	117	118	189	218
Secbutabarbital	1655	—	—	—	—	141	156	41	57	39	98
Secbutabarbital-Me_2	1565	—	—	—	—	—	—	—	—	—	—
M (2′-OH-)	1926	—	—	—	—	—	—	—	—	—	—
Secobarbital (quinalbarbitone)	1786	1827	—	—	0.865	167	168	41	43	97	124
Secobarbital-Me_2	1690	—	—	—	—	196	195	138	181	224	237
M (3′-OH-)	1865	2029	—	—	1.206	168	167	169	153	209	195
M (3′-keto)	—	—	—	—	—	43	168	69	85	167	124
M (desallyl-)	1665	—	—	—	—	129	128	85	86	154	169
M (2,3′-diOH-)	—	—	—	—	—	171	143	128	159	198	241
Succinimide	—	—	—	—	—	28	99	56	27	26	55
Sulthiame-Me	2880	—	—	—	—	304	274	226	198	210	211
Sulthiame-Me_2	2815	—	—	—	—	318	274	226	210	211	104
Sultiame	3000	—	—	—	—	290	184	185	104	77	168
Talbutal	1703	—	—	—	—	167	168	41	97	124	39
Talbutal-Me_2	1600	—	—	—	—	195	196	138	181	111	211
Thialbarbital	2116	—	—	—	—	81	223	79	80	157	185
Thiamylal	1899	—	—	—	—	43	41	184	168	167	97
Thiopental (thiopentone; also metabolised to pentobarbital)	1857	1923	—	2600	0.948	172	157	173	43	41	55
Thiopental-Me_2	1825	—	—	—	—	200	185	201	127	157	167
M (OH-)	—	2134	—	—	—	172	173	157	97	258	229
Taigabine-Me						156	157	113	111	358	96
Topiramate	—	2253	—	—	—	324	43	80	110	189	206
Art (fructopyranose)	—	1621	—	—	—	43	245	69	59	127	85
Troxidone (trimethadione; metabolised to dimethadione)	1090	—	0.04	—	—	43	58	143	42	41	128
Valproate (valproic acid)	1064	1098	0.09	—	0.350	73	102	41	57	43	55
M	—	1195	—	—	—	100	55	41	69	127	113
M	1200	—	—	—	—	100	55	41	69	127	113
M	—	1267	—	—	—	72	101	114	100	55	44
M	—	1312	—	—	—	100	55	41	113	99	69
Vigabatrin	—	Not eluted	—	—	Not eluted	56	84	111	69	82	54
Vinbarbital	1753	—	—	2495	—	195	41	141	69	152	135
Vinabarbital-Me_2	1670	—	—	—	—	223	224	166	169	138	135
M (OH-)	2070	—	—	—	—	167	169	85	211	193	155
Vinylbital	1729	—	—	—	0.798	154	83	71	55	155	67
Vinylbital-Me_2	1655	—	—	—	—	182	181	183	97	125	154
M (3′-OH-)	1995	—	—	—	—	154	155	83	112	139	195
M (desvinyl-)	1665	—	—	—	—	—	—	—	—	—	—
Zonisamide	—	2042	—	—	—	132	77	133	104	51	64

where sensitivity is important. Chromatography of primary and secondary amines is poor on packed columns, but is adequate on well-maintained capillary columns, particularly those of low–medium polarity such as PSX-5 (see Table 40.3). Some authors prefer to chromatograph the secondary amines and hydroxylated metabolites as acetylated derivatives, prepared by heating the dried residue with acetic anhydride and pyridine (3 : 2, v/v) (Maurer, Bickeboeller-Friedrich 2000). Others employ an enzymatic hydrolysis procedure to improve recovery of both parent drug and metabolites, although the additional sensitivity gained is often negated by the increased analytical time in the emergency setting. Acid hydrolysis is quicker, but some relevant compounds are destroyed under these conditions.

System GA, GF or GB, described above, may be used, or system GM.

System GM

System GM (Dawling *et al.* 1990) is ideal for plasma samples, since the isothermal conditions allow high throughput and the limited resolution of hydroxylated metabolites is not important as these do not constitute a significant fraction of the extract from plasma. Conditions are given for both packed and capillary column systems.

- *Column:* 3% SP2250 on Supelcoport 80–100 mesh glass (2.1 m × 2 mm i.d.).
- *Temperature:* 265°C.
- *Carrier gas:* He, 25 mL/min.
- *Column:* HP-50 + fused silica capillary (25 m × 0.53 mm i.d., 1 μm).
- *Temperature:* 250°C.
- *Carrier gas:* He, 7 mL/min.
- *Reference compound:* Iprindole.
- *Quantification:* NPD.
- *Retention indices*, relative retention to iprindole, are given in Table 40.12. Only the metabolites known to occur in urine and/or plasma specimens are included in this list; the list in De Zeeuw (2002) is more extensive.

Antihistamines

Antihistamines are a diverse group of drugs that includes the ethanolamines (diphenhydramine), ethylenediamines (pyrilamine), alkylamines (hydroxyzine), phenothiazines (promethazine) and piperidines (chlorphenamine). Many share common metabolites with other members of

Table 40.12 GC retention data and mass spectral data for antidepressants (AC, acetyl)

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GF*	*GM*						
Amitriptyline (also metabolised to nortriptyline)	2194	2284	2510	0.723	58	59	202	42	203	214
M (*cis*-10-OH-)	2348	2454	—	1.149	58	202	215	178	189	165
M (*cis*-10-OH-*N*-oxide)	—	2215	—	—	215	229	230	207	248	178
M (*trans*-10-OH-)	2348	2466	—	1.168	58	202	215	178	189	165
M (*trans*-10-OH-*N*-oxide)	—	2239	—	—	215	229	230	207	248	178
M (cyclobenzaprine)	2235	2330	—	0.850	58	215	202	189	176	163
Amitriptyline *N*-oxide	1975	2051	—	—	232	217	215	202	117	189
Amoxapine	2638	2746	—	2.831	245	257	247	193	56	246
M (7-OH-)	2951	3525	—	—	261	209	273	263	244	329
M (8-OH-)	2959	3546	—	—	261	209	273	263	244	329
Atomoxetine										
Bupropion	—	1645	—	—	44	100	111	139	224	57
M	—	1746	—	—	44	100	77	57	208	113
M	—	1764	—	—	44	100	77	57	208	113
M (OH-)	—	1898	—	—	44	100	116	139	224	110
M	—	1916	—	—	44	100	224	157	57	65
M	—	2107	—	—	44	100	57	84	260	—
Butriptyline	2181	2288	2465	0.683	58	293	45	59	193	100
M (nor-)	—	2330	—	0.761	—	—	—	—	—	—
Citalopram	2525	2499	—	1.121	58	238	208	42	324	190
M (nor-)	2500	2526	—	1.232	44	238	208	138	310	190
M	—	2846	—	—	238	81	136	192	265	221
M	—	2987	—	—	238	207	163	254	265	282
Clomipramine	2415	2511	2795	1.172	58	85	269	268	270	271
M (nor-)	2432	2540	—	1.374	268	269	229	227	242	300
M (*N*-oxide)	2146	2246	—	—	228	193	192	269	230	165
M (ring)	2230	2335	—	—	229	194	193	214	228	231
M (8-OH-)	2727	2843	—	—	58	285	243	209	284	330
M (8-OH-nor-)	2762	2880	—	—	44	245	243	284	258	316
M (2OH-)	2569	2735	—	—	—	—	—	—	—	—
M (10OH-)	2574	2698	—	—	58	85	86	268	329	313
Clorgiline	1883	—	—	—	—	—	—	—	—	—
Desipramine	2235	2338	—	0.896	235	195	208	44	234	193

Table 40.12 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GF*	*GM*						
M (2-OH-)	2553	2669	—	—	44	211	224	250	180	282
M (10-OH-)	—	2521	—	—	44	180	194	206	251	282
M (ring)	1930	2014	—	—	195	194	180	167	97	89
M (ring-OH-)	2240	2335	—	—	211	210	196	180	167	212
M (ring di-OH-)	2600	—	—	—	227	226	157	196	228	183
M (di-OH-)	—	2995	—	—	44	266	240	227	298	225
M (AC-)	2670	2811	—	—	208	114	308	193	194	222
M (OH-methoxy-)	—	2749	—	—	227	241	254	280	312	44
M (ring OH-methoxy-)	2390	—	—	—	241	240	226	180	210	198
Dibenzepin	2450	2566	2885	1.735	58	324	209	71	225	72
M (nor-)	2449	—	—	—	—	—	—	—	—	—
M (di-nor-)	2406	—	—	—	235	234	207	206	179	192
M (ter-nor-)	2680	—	—	—	235	234	206	207	179	192
M (N_5-desmethyl-)	2455	—	—	—	58	210	211	167	195	223
Dosulepin (dothiepin)	2380	2486	2770	1.259	—	—	—	—	—	—
M (nor-)	2421	2507	—	1.450	204	281	221	263	238	165
M (*N*-oxide)	2100	—	—	—	217	235	250	202	221	240
M (OH-*N*-oxide)	2130	—	—	—	266	165	251	233	237	215
M (sulfoxide)	2392	2533	—	—	—	—	—	—	—	—
M (norsulfoxide)	2421	2839	—	—	—	—	—	—	—	—
Doxepin *cis*-isomer	2220	2301	2570[a]	0.788	58	220	219	59	191	189
Doxepin *trans*-isomer	2220	2321	—	0.823	58	220	219	59	191	189
M (*cis*-*N*-oxide)	1970	2077	—	—	234	219	165	178	202	189
M (*trans*-*N*-oxide)	—	2081	—	—	234	219	165	178	202	189
M (*cis*-nor-)	2245	2333	—	0.830	44	165	178	189	202	219
M (*trans*-nor-)	2245	2339	—	0.933	44	165	178	189	202	219
M (*cis*-OH-)	2535	2528	—	—	58	165	295	152	178	220
M (*trans*-OH-)	2560	2544	—	—	58	165	295	152	178	220
M (*cis*-nor-OH-)	2540	2644	—	—	44	220	238	165	152	281
M (*trans*-nor-OH-)	—	2671	—	—	44	220	238	165	152	281
Duloxetine	—	2750	—	—	44	297	265	240	181	115
Escitalopram (see Citalopram)										
Fluoxetine	1859	1903	—	0.304	44	309	183	104	251	91
M (nor-)	1851	1888	—	0.284	104	134	103	77	162	191
M (AC-)	2250	2319	—	—	44	86	190	117	104	115
M (nor-AC-)	2190	2278	—	—	117	176	72	104	115	91
Fluvoxamine	1885	1911	—	0.295	187	71	45	276	172	145
Art (ketone)	1525	—	—	—	173	228	145	159	188	241
Art	1560	1602	—	—	187	172	200	228	244	259
M (AC-)	2240	2284	—	—	86	102	187	258	341	360
Art	1895	1921	—	—	258	71	226	242	311	329
M	—	2200	—	—	71	145	172	198	226	258
M	—	1791	—	—	241	172	212	145	144	198
M	–-	1687	—	—	86	257	198	145	281	341
Imipramine (also metabolised to desipramine)	2230	2314	2540	0.784	58	235	85	234	236	195
M (2-OH-)	2565	2636	—	—	58	250	251	211	296	224
M (10-OH-)	—	2494	—	—	58	193	180	232	251	296
M (di-OH-)	—	2962	—	—	58	266	267	227	312	252
M (OH-methoxy-)	—	2715	—	—	58	280	241	326	254	266
Iprindole	2335	2437	—	1.000	58	170	284	213	145	212
Iproniazid	1593	1609	—	—	123	58	106	79	43	78

table continued

Table 40.12 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GF*	*GM*						
Isocarboxazid	1949	0000	—	—	—	—	—	—	—	—
Lofepramine (metabolised to desipramine)	Not eluted	Not eluted	—	Not eluted	—	—	—	—	—	—
Maprotiline	2390	2440	—	1.086	44	70	59	277	71	191
M (nor-)	2293	2404	—	1.107	56	202	203	178	263	189
M (desamino-di-OH-)	2570	2620	—	—	252	207	280	253	219	195
M (OH-)		2622			44	70	203	189	187	293
Mebanazine	1240	—	—	—	—	—	—	—	—	—
Mianserin	2210	2302	2595	0.879	193	264	43	72	71	220
M (nor-)	2235	2348	—	1.105	193	208	250	165	178	220
M (8-OH-)	2495	2628	—	—	280	209	72	236	265	180
M (OH-methoxy-)	2530	—	—	—	310	239	266	295	224	72
Minaprine	2855	3023	—	—	100	113	186	56	198	77
M (CM30488)	3040	3222	—	—	—	—	—	—	—	—
Mirtazapine	2250	2361	—	—	195	194	208	180	167	265
M (nor-)	—	2414	—	—	195	194	209	180	167	251
M (oxo-)	—	2665	—	—	195	250	279	180	194	208
Moclobemide	—	2333	—	0.967	100	56	113	139	111	42
M	—	2578	—	2.191	—	—	—	—	—	—
Nefazodone	4510	Not eluted	—	Not eluted	303	274	260	304	454	317
M (*m*-chlorophenylpiperazine, *m*CPP)	—	1806	—	—	154	196	138	111	156	75
M (*m*CPP)-AC	2265	—	—	—	166	238	138	154	168	195
M (*N*-desalkyl-OH-) isomer 2-AC_2	2525	—	—	—	182	254	169	184	296	211
M (desamino-OH-)-	2340				120	198	291	127	171	140
M (desamino-OH-)-AC	2500	—	—	—	120	240	91	333	77	126
M (OH-ethyl-desamino-OH)-AC_2	2650	—	—	—	120	298	391	91	101	238
Nialamide	1500	—	—	—	91	177	44	106	45	78
Nomifensine	2130	2239	2670	0.850	194	195	238	193	72	178
M (4-OH-)	2450	—	—	—	86	210	211	194	254	228
M (OH,MeO-) isomer 1	2505	—	—	—	284	86	241	210	209	224
M (OH,MeO-) isomer 2	2590	—	—	—	284	86	241	210	209	224
Nortriptyline	2215	2304	—	0.816	44	202	45	220	218	215
M (*cis*-10-OH-)	2375	2480	—	1.261	44	218	203	202	178	165
M (*trans*-10-OH-)	2375	2494	—	1.323	44	218	203	202	178	165
M (norcyclobenzaprine)	—	2343	—	0.880	44	215	218	202	189	163
M (AC-)	2660	2774	—	—	44	232	202	217	86	203
M (norcyclobenzaprine-AC)	—	2949	—	—	44	230	215	202	86	178
Noxiptyline	2270	—	—	—	58	71	208	72	59	42
M (dibenzocycloheptanone)	1850	—	—	—	208	180	179	178	165	152
M (OH-dibenzocycloheptanone)-H_2O	2200	—	—	—	178	206	176	152	76	89
Opipramol	3050	3219	—	—	363	206	143	42	70	193
M (ring)	1985	—	—	—	—	—	—	—	—	—
Paroxetine	—	2691	—	2.047	44	329	192	70	138	109
M (desmethylenyl-3-methyl-)	—	2734	—	—	44	192	140	331	177	70
M	—	2687	—	—	105	210	77	254	132	44
Phenelzine	1335	1278	—	—	31	45	46	29	59	74
Protriptyline	2253	2329	2590	0.878	70	44	191	192	188	59
M (nor-)	—	2343	—	—	—	—	—	—	—	—
M (10-OH-)	—	2406	—	—	70	44	207	178	279	249
M (10,11-di-OH-)		2472	—	—	44	70	179	178	207	280
Sertraline	—	2481	—	1.166	274	276	159	262	239	304

Table 40.12 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GF*	*GM*						
M (nor-)	—	2468	—	1.218	119	145	274	246	130	290
M (ketone)	—	2496	—	—	227	290	292	199	163	248
M	—	2279	—	—	274	276	128	202	239	259
M	—	2333	—	—	202	272	274	200	236	100
M	—	2619	—	—	131	290	292	189	220	254
M	—	2786	—	—	287	289	217	251	189	108
M	—	2802	—	—	250	252	305	307	263	214
Tofenacin	1920	2013	—	0.420	44	59	165	166	181	179
Tranylcypromine	1220	1252	1455	—	133	132	56	115	30	117
Trazodone	3330	3564	—	Not eluted	205	70	231	78	135	166
M (*m*CPP)	—	1806	—	—	154	196	138	111	156	75
M (*m*CPP)-AC	2265	—	—	—	166	238	138	154	168	195
M (OH-AC-)	3380	3640	—	—	205	336	414	429	231	176
M (desalkyl AC-)	2265	2261	—	—	166	238	56	195	140	153
M (*N*-desalkyl-OH-) isomer 2-AC_2	2525	—	—	—	182	254	169	184	296	211
Trimipramine	2215	2302	2505	0.734	58	249	208	99	193	234
M (nor-)	—	2335	—	0.858	208	193	44	249	234	280
M (OH-)	2575	2631	—	—	58	265	224	250	209	310
M (nor-OH-)	—	2662	—	—	224	44	209	265	250	296
M (OH-methoxy-)	2590	2715	—	—	58	295	280	254	340	241
M (nor ring)	1930	2107	—	—	194	249	208	193	167	179
Venlafaxine	—	2163	—	0.544	58	134	179	119	91	277
M (*N*-desmethyl-)	—	2196	—	0.570	44	202	134	121	91	263
M (*O*-desmethyl-)	—	2230	—	0.625	58	120	165	107	91	263
M (*N*,*O*-didesmethyl-)	—	2264	—	0.687	44	188	120	107	145	249
M (nor-OH-)	—	2450	—	—	44	134	218	200	121	185
M (*O*-desmethyl-OH-) isomer 1	—	2373	—	—	58	134	91	179	121	77
M (*O*-desmethyl-OH-) isomer 2	—	2408	—	—	58	134	91	179	121	77
Viloxazine	1855	1923	—	—	56	100	138	110	57	237
M (di-oxo-)	2325	—	—	—	56	100	110	138	265	128
Zimeldine	2270	—	—	0.820	58	70	318	316	317	193
M (nor-)	2223	—	—	0.941	302	304	224	193	260	272

[a]Racemate.

their class, which may compromise the identification of the parent drug ingested. Some authors advocate the preparation of acetylated derivatives, particularly to analyse the hydroxylated metabolites, but to identify them in biological fluids this is an unnecessary additional step.

Systems GA, GB, GC or GF, described above, may be used. The retention indices and principal ions are given in Table 40.13.

Benzodiazepines

The analysis of benzodiazepines in biological specimens is hampered by their high potency and resultant low plasma concentrations, and by their inter-connected metabolic pathways. Several benzodiazepines appear in urine almost exclusively as glucuronide-conjugated metabolites, and these can be hydrolysed with glucuronidase (1000 U glucurase/mL of urine at 60°C for 1–2 h), although some can degrade with prolonged heating. Extraction can be performed at any pH between 3 and 12, but basic extracts (pH 9–11) give cleaner chromatograms. The extraction solvent should be moderately polar (ethyl acetate is appropriate), and TMS derivatives form easily in 20–30 min at 60°C using 50% bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) in acetonitrile. These derivatives markedly improve peak shape and sensitivity. All compounds except 7-aminonitrazepam show electron-capture responses with high sensitivity. However, quantitation by ECD is problematic as it has a narrow linear range, and a multiple point calibration is essential. Alternatively, for most compounds a nitrogen detector (NPD) gives adequate sensitivity with a much improved linear range, although it is not advisable to make TMS derivatives if using this detector. MS detection is required to confirm the identity. System GA or GB, described above, may be used or system GG. The retention indices and principal ions are given in Table 40.14.

System GG

M Möller, personal communication

- *Column*: 2.5% OV-17 on 80–100 mesh Chromosorb G, treatment and dimensions as for system GA.
- *Column temperature, carrier gas, reference compounds*: As for system GA.
- *Retention indices*: The retention indices of benzodiazepines have been shown to be dependent on column temperature (Schuetz, Westenberger 1978, 1979). The values given by these authors are about 50 RI units above those generally quoted. The values given below should therefore be checked before use by chromatographing a few sample compounds.

Table 40.13 GC retention data and mass spectral data for antihistamines (BP, benzophenone; CBPH, chlorobenzophenone)

Compound	*System*				*Principal ions (m/z)*					
	GA	*GB*	*GC*	*GF*						
Alimemazine (trimeprazine)	2315	2402	2646	2715	58	198	298	100	180	84
M (OH-)	2650	—	—	—	58	314	100	255	196	281
M (OH-)-AC	2600	—	—	—	58	100	356	269	214	196
M (bis-nor-)-AC	2765	—	—	—	212	312	114	198	199	180
M (nor-)	2335	2432	—	—	199	284	212	198	180	252
M (nor-OH-)-AC_2	2930	—	—	—	128	384	228	214	270	196
M (phenothiazine)	2120	2130	—	—	199	167	198	166	154	139
M (sulfoxide)	2665	2805	—	—	58	212	199	180	298	299
M (norsulfoxide)	—	2829	—	—	—	—	—	—	—	—
M (nor-OH-)	—	2845	—	—	—	—	—	—	—	—
Antazoline	2318	2421	2749	—	—	—	—	—	—	—
Astemizole	3900	Not eluted	—	—	96	337	109	338	294	458
M (*N*-desalkyl)	2470	—	—	—	109	241	132	83	242	111
Azatadine	2377	2499	—	—	246	280	232	247	291	217
M (OH-alkyl-)-H_2O	2410	—	—	—	244	255	288	230	216	229
M (nor-)-AC	2710	—	—	—	128	326	212	198	180	86
Bamipine	2250	—	—	—	97	91	96	70	77	182
M (OH-)	2580	—	—	—	97	91	296	198	98	96
M (OH-)-AC	2620				97	70	98	96	338	240
M (nor-)-AC	2675				91	182	77	217	308	183
M (nor-OH-)-AC_2	3020				91	366	240	56	199	275
Benzophenone (BPH)	1610	1673	—	—	105	77	51	182	106	183
M (OH-BPH) isomer 1	2065	—	—	—	198	121	77	199	192	151
M (OH-BPH) isomer 2	2080	—	—	—	121	198	77	105	199	122
M (OH-methoxy-BPH) isomer 1	2050	—	—	—	105	151	198	228	77	121
M (OH-methoxy-BPH) isomer 2	2070	—	—	—	105	151	198	228	77	121
M (carbinol)	1670	1722	—	—	79	219	77	218	78	108
Bromodiphenhydramine (Bromazine)	2155	2231	—	2480	58	73	45	165	59	166
Brompheniramine	2092	2184	2457	2470	247	249	58	72	167	168
M (nor-)	—	2219	—	—	247	249	167	44	246	168
M (bis-nor-)	—	2203	—	—	247	249	167	106	260	180
Buclizine (also metabolised to chlorobenzophenone)	3360	3461	—	—	231	147	285	201	132	165
M (desalkyl-) (norchlorcyclizine)	2520	2355	—	—	85	165	201	241	230	286
Carbinoxamine	2080	2147	2430	—	58	71	54	167	72	202
M (chlorobenzoylpyridine)	1645	—	—	—	139	189	111	216	217	191
M (nor-)	2150	—	—	—	203	167	202	220	205	204
Chlorcyclizine (also metabolised to chlorobenzophenone)	2220	2316	—	2560	99	56	72	165	300	228
M (nor-)	2520	2355	—	—	85	165	201	241	230	286
Chlorobenzophenone (CBPH)	1850	—	—	—	105	139	77	216	218	141
M (4OH-CBPH)	2300	—	—	—	121	232	111	139	234	197
M (4-chloromethylbiphenyl)	—	1688	—	—	167	165	202	152	82	166
M (4-chlorobiphenylmethanone)	—	1862	—	—	105	139	216	111	141	181
M (carbinol)	1750	—	—	—	105	77	139	218	165	111
Chloropyriline	2133	—	—	—	58	131	72	71	79	42
Chlorphenamine (see Chlorpheniramine)										
Chlorpheniramine	1996	2079	2586	2355	203	58	44	205	54	204
M (nor-)	2014	2115	—	—	203	167	44	205	202	204
M (bis-nor-)	—	2065	—	—	203	167	205	202	204	216
M (nor-AC-)	2530	2563	—	—	203	216	167	205	202	302
M (OH-)-AC	2405	—	—	—	—	—	—	—	—	—

Table 40.13 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*						
M (bis-nor-)-AC	2535	—	—	—	—	—	—	—	—	—
M (desamino-OH-)-AC	2530	—	—	—	—	—	—	—	—	—
Cinnarizine (also metabolised to benzophenone)	3050	3233	—	—	201	117	167	251	165	202
M (desalkyl-, norcyclizine)	2120	2128	—	—	167	165	207	85	152	252
Clemastine	2425	2521	—	2710	84	128	179	85	214	98
M (OH-methoxy-carbinol)-H_2O	2220	—	—	—	—	—	—	—	—	—
M (di-OH-)-H_2O	2440	—	—	—	246	248	288	247	330	152
Clemizole	2620	—	—	—	131	256	125	42	255	89
M (oxo-)	2965	—	—	—	255	256	214	339	131	186
Cyclizine (also metabolised to benzophenone)	2025	2104	2348	2320	99	56	167	207	194	266
M (nor-)	2120	2128	—	—	167	165	207	85	152	252
Cyproheptadine	2355	2460	2307	2710	287	96	286	215	70	58
M (OH-)	3060	—	—	—	303	203	202	217	304	205
M (nor-)	2400	—	—	—	273	215	216	231	229	272
M (oxo-)	2960	—	—	—	229	215	202	242	301	258
M (nor-OH)-H_2O	2450	2608	—	—	271	272	270	165	193	241
Deptropine	2615	—	—	—	83	140	82	124	96	193
Dimenhydrinate (see Diphenhydramine)										
Dimetindene	2275	2376	2669	—	58	59	72	45	292	218
Dimetotiazine	3060	3096	—	—	72	73	230	56	210	198
M (nor-)	3150	—	—	—	72	320	306	58	198	210
M (OH-)-AC	3200	—	—	—	72	245	59	198	398	263
M (bis-nor-)-AC	3380	—	—	—	319	405	346	211	210	320
M (nor-)-AC	3360	—	—	—	58	114	319	346	72	419
Diphenhydramine (also metabolised to benzophenone)	1873	1928	2387	2105	58	73	167	165	166	152
M (nor-)	1520	1922	—	—	44	165	59	167	152	166
M (nor-acetyl-)	2265	2360	—	—	44	101	167	86	165	152
M (di-nor-acetyl-)	2240	2318	—	—	167	165	87	183	72	152
M (desamino-)	—	1883	—	—	214	181	183	153	152	167
M (methoxy-)	2010	2239	—	—	58	73	165	181	152	153
M	—	2190	—	—	183	165	167	105	152	166
M	—	2631	—	—	167	165	152	168	253	115
M (diphenylmethane)	—	1465	—	—	167	168	165	152	153	91
M (diphenylmethanol)	1645	1644	—	—	167	165	152	162	168	115
M (4-phenylmethylphenol)	—	1780	—	—	184	183	165	107	152	115
Diphenylpyraline (metabolised to benzophenone)	2100	2128	2447	2405	99	114	98	167	70	165
Doxylamine	1910	1970	—	2170	58	71	167	182	180	72
M (nor-)	—	1974	—	—	182	183	167	200	149	44
M	1520	1623	—	—	182	173	167	168	94	106
M (carbinol)-H_2O	1560	1670	—	—	180	181	152	77	90	102
M (OH-)-AC	2300	—	—	—	58	71	183	198	72	182
M (OH-carbinol-)-AC	2980	—	—	—	137	257	78	79	106	200
M (OH-methoxy-)-AC	2320	—	—	—	58	71	72	183	198	196
M (bis-nor-)-AC	2280	—	—	—	182	86	167	183	181	198
M (desamino-OH-)-AC	1960	—	—	—	198	87	182	183	167	180
M (nor-)-AC	2340	—	—	—	182	183	167	100	181	58
Histapyrrodine	2240	—	—	—	84	91	196	280	197	65
M (OH-)	1650	—	—	—	84	91	212	296	213	297
M (oxo-)	2570	—	—	—	91	196	209	197	275	294

table continued

Table 40.13 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*						
M (*N*-desbenzyl-)	1800	—	—	—	84	190	106	111	122	77
M (*N*-desbenzyl-oxo-)	2120	—	—	—	106	119	118	98	77	204
M (*N*-desphenyl-oxo)-H_2O	2100	—	—	—	91	159	216	160	215	84
Hydroxyzine (also metabolised to chlorobenzophenone)	2880	3000	—	—	201	203	165	299	166	202
M (desalkyl-) (norchlorcyclizine)	2520	2355	—	—	85	165	201	241	230	286
Isothipendyl	2225	—	—	—	72	214	200	285	86	56
M (nor-)	2220	—	—	—	58	214	213	181	199	271
M (OH-)	2450	—	—	—	72	301	218	197	178	228
M (bis-nor-)	2230	—	—	—	214	257	213	58	181	215
Loratadine	—	3236	—	—	382	265	245	280	292	294
Mebhydrolin	2450	2575	2739	2920	91	233	232	276	275	65
Meclozine (also metabolised to chlorcyclizine)	3035	3193	—	—	189	105	201	285	165	190
M (*N*-desalkyl-)	2520	—	—	—	—	—	—	—	—	—
Mepyramine	2220	2328	—	2560	121	58	72	214	122	215
M (*N*-desalkyl-)	2120	—	—	—	121	214	78	165	136	154
M (*N*-desalkyl-)-AC	2150	—	—	—	107	214	78	256	163	197
M (*N*-desmethoxybenzyl-)	1580	—	—	—	58	78	107	60	95	119
Mequitazine	2765	2939	—	—	124	322	198	125	212	180
M (phenothiazine)	2120	2130	—	—	199	167	198	166	154	139
M (sulfoxide)	3120	—	—	—	124	198	338	321	180	—
M (sulfone)	3250	—	—	—	124	125	354	180	152	—
Methapyrilene	1981	—	—	2305	58	97	72	71	191	261
Methdilazine	2462	—	—	2920	97	98	296	199	55	212
Phenindamine	2165	2245	2926	2515	260	261	42	57	184	215
M (OH-)	2300	—	—	—	276	277	275	233	234	200
M (*N*-oxide)	2230	—	—	—	260	277	259	276	215	202
M (nor-)	2210	—	—	—	246	247	202	217	168	215
M (nor-OH-)	2590	—	—	—	262	263	261	184	233	228
Pheniramine	1805	1874	—	2100	169	58	168	170	72	167
M (nor-)	2080	1890	—	—	169	168	167	182	184	226
Phenyltoloxamine	1940	2030	—	—	58	255	42	71	59	44
M (*N*-oxide)-$(CH3)_2NOH$	1500	1580	—	—	165	210	181	167	195	152
M (nor-)	2140	2002	—	—	44	58	210	165	241	181
M (*O*-desalkyl-)	1680	1724	—	—	184	165	78	77	106	183
M (*O*-desalkyl-OH-)	2220	—	—	—	200	107	94	122	152	181
M (OH-) isomer 1	2280	—	—	—	58	72	271	152	226	197
M (OH-) isomer 2	2300	—	—	—	58	72	271	152	226	197
M (nor-OH-) isomer 1	2320	2398	—	—	58	257	226	107	152	197
M (nor-OH-) isomer 2	2340	2402	—	—	58	257	226	152	197	91
M (OH-methoxy-)	2320	—	—	—	58	72	301	271	152	107
M (desamino-OH-)	1830	1928	—	—	228	183	165	184	106	181
Promethazine	2339	2383	2546	2675	72	284	198	213	199	180
M (nor-)	2250	2333	—	—	58	213	180	198	152	270
M (phenothiazine)	2120	2130	—	—	199	167	198	166	154	139
M (sulfoxide)	2710	2797	—	—	72	198	180	152	213	229
M (norsulfoxide)	—	2732	—	—	58	212	180	198	229	152
M (nor-OH-)	2580	2717	—	—	212	58	180	229	198	286
M (OH-)	2590	—	—	—	72	196	300	229	214	288
Propiomazine	2738	—	—	3225	72	73	340	269	197	56

Table 40.13 continued

Compound	*System*				*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*						
Pyrrobutamine	2419	—	—	2815	205	240	91	84	125	242
M (oxo-)	2920	—	—	—	205	115	98	240	200	123
Thenalidine	2318	—	—	—	97	70	99	43	188	44
Thenyldiamine	1999	—	2300	2340	58	97	72	71	203	191
Thiazinamium methysulfate Art (promethazine)	2339	2383	2546	2675	72	284	198	213	199	180
Thonzylamine	2203	—	2576	—	58	121	72	71	216	215
Tolpropamine	1900	—	—	—	58	253	165	193	178	117
M (OH-)	2150	—	—	—	58	269	165	115	178	193
M (nor-)	2100	—	—	—	193	239	165	178	208	117
M (nor-OH-)	2200	—	—	—	255	193	167	165	178	115
M (*N*-oxide)	1750	—	—	—	115	193	208	178	165	116
Trimeprazine (see Alimemazine)										
Trimethobenzamide	3281	—	—	—	58	195	59	72	388	89
Tripelennamine	1976	—	—	—	58	91	72	71	197	185
M (nor-)	2420	—	—	—	91	129	197	58	147	241
M (OH-)	2400	—	—	—	58	91	72	213	200	271
M (benzylpyridylamine)	1650	—	—	—	184	106	79	183	78	107
Triprolidine	2253	2340	2954	2600	208	209	278	207	193	200

Table 40.14 GC retention data and mass spectral data for benzodiazepines (ET, ethyl; TMS, trimethylsilyl)

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GG*						
Adinazolam[(a)]	2955	—	—	308	307	309	310	58	280
Alprazolam[(a)]	3100	3108	—	308	279	204	273	77	307
M (αOH-)	3245	0000	—	287	322	321	323	288	324
M (αOH)-TMS	—	3183	—	381	396	382	383	398	397
M (4OH-)	3045	—	—	—	—	—	—	—	
Bromazepam	2665	2760	3280	236	317	318	288	316	286
Bromazepam-TMS	—	2702	—	388	386	387	389	372	374
M (3OH-)	2470	—	—	304	314	331	305	303	302
M (3OH)-TMS_2	—	2650	—	388	386	477	475	179	360
M (aminohydroxybromazepam)-TMS	—	2590	—	249	247	366	364	338	336
Brotizolam[(a)]	3070	—	—	394	245	316	210	291	176
M (OH-) Art (-CH_2O)	3050	—	—	380	378	379	299	301	245
Camazepam[(a)] (metabolised to oxazepam and temazepam)	2945	3162	—	58	72	43	78	271	44
Chlordiazepoxide (metabolised to nordazepam and oxazepam)	2795	2981 thermally unstable	3065	282	299	284	283	241	253
M (nor-)	2452	2679	—	268	269	270	233	271	205
M (demoxepam)	2529	2806	—	120	285	286	269	241	287
Clobazam[(a)]	2558	2683	3174	300	258	77	259	283	231
M (nor-)	2747	2759	—	286	218	215	217	216	244
M (OH-)	3000	—	—	316	318	274	271	299	247
M (OH-MeO-)	3255	—	—	346	316	301	348	274	271
Clonazepam	2823	3000	3600	280	314	315	285	234	288
Clonazepam-TMS	—	2781	—	387	352	306	372	386	388
M (7-amino-)	2890	2996	—	285	256	257	287	250	111
M (7-amino)-TMS_2	—	2742	—	429	394	414	430	431	314

table continued

Table 40.14 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GG*						
M (amino-OH-)	2935	—	—	283	220	225	285	254	284
Clorazepic acid (hydrolysed *in vivo* and absorbed as diazepam)	2457	2618	3125	242	43	270	269	241	103
Clotiazepam[a]	2532	—	—	289	318	291	320	275	290
M (OH-)	2705	—	—	287	316	318	289	288	317
M (OH-)-AC	2870	—	—	271	316	256	300	273	241
M (di-OH-)-AC_2	2995	—	—	332	374	319	291	303	434
Demoxepam	2529	2806	3043	285	286	269	287	241	242
Diazepam[a] (metabolised to nordazepam, oxazepam and temazepam)	2428	2556	2940	256	283	284	287	257	255
Estazolam[a]	3070	3050	—	259	294	293	205	239	101
Etizolam[a]	2980	—	—	342	266	313	224	239	45
Flunitrazepam[a]	2600	2744	3190	285	312	313	286	266	238
M (nor-)	2720	2816	—	224	299	298	372	271	252
M (nor-)-TMS	—	2622	—	371	370	352	356	324	372
M (7-amino-)	2723	2804	—	283	255	254	282	264	240
M (7-amino-)-TMS	—	2836	—	355	327	326	354	356	312
M (nor-amino-)	2825	—	—	269	240	241	268	270	213
Flurazepam[a]	2780	2896	3220	86	87	99	58	84	387
M (desalkyl-)	2470	2559	—	288	260	259	287	261	289
M (desalkyl-)-TMS	—	2350	—	359	360	341	361	345	362
M (2-OH-ET-)	2675	2805	—	288	273	287	332	331	304
M (2-OH-ET-)-TMS	—	2778	—	288	287	273	389	273	360
M (bis-desethyl-)	2694	2739	—	313	315	314	312	250	259
M (desalkyl-OH-)	2255	2373	—	223	286	258	75	257	251
Flutazolam	2460	—	—	289	245	246	210	259	211
Halazepam[a] (metabolised to nordazepam, oxazepam)	2285	—	—	324	352	323	325	351	353
Ketazolam (hydrolyses to diazepam)									
Loprazolam[a]	Not eluted	Not eluted	—	70	464	42	43	465	394
Lorazepam	2410	2528	2910	291	239	274	293	75	302
Lorazepam-TMS_2	—	2566	—	429	431	430	347	449	432
Lormetazepam	2660	2770	—	305	307	306	309	308	334
Lormetazepam-TMS (metabolised to lorazepam)	—	2799	—	377	379	391	291	317	406
Medazepam[a] (metabolised to diazepam, nordazepam, oxazepam and temazepam)	2235	2340	2620	242	207	244	270	243	271
M (nor-)	2280	—	—	228	193	256	257	165	110
Metaclazepam[a]	2640	—	—	349	347	321	351	350	394
M (*O*-desmethyl-)	2730	—	—	321	319	380	378	349	347
M (nor-)	2690	—	—	335	333	349	347	378	380
Mexazolam[a]	2600	—	—	251	70	253	41	42	139
Midazolam[a]	2575	2722	—	310	312	311	163	325	75
M (α-OH-)	2830	2901	—	310	311	312	341	283	313
M (α-OH-)-TMS	—	2866	—	310	398	413	312	400	415
M (4-OH-)-TMS	—	2775	—	268	269	297	412	298	397
Nitrazepam	2740	2915	3450	280	253	281	206	234	252
Nitrazepam-TMS	—	2642	—	352	353	306	338	354	307
M (7-amino-)[b]	2785	2878	—	251	223	222	250	252	235
M (7-amino-)-TMS_3	—	2634	—	394	395	396	380	280	322
M (7-acetamido-)	—	Not eluted	—	293	265	264	292	43	222
Nordazepam	2490	2625	3041	—	—	—	—	—	—
Nordazepam-TMS	—	2367	—	341	342	343	327	344	329
Oxazepam	2803	2325	2438	257	77	268	239	205	267

Table 40.14 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GG*						
Oxazepam-TMS_2	—	2468	—	429	430	431	313	415	401
Oxazolam[a] (metabolised to oxazepam)	2540	—	—	251	70	253	241	105	252
Prazepam[a] (metabolised to nordazepam and oxazepam)	2648	2783	3145	91	269	324	55	296	295
M (3-OH-)	2860	—	—	257	55	311	259	313	340
Quazepam[a] (also metabolised to desalkylflurazepam)	2440	2576	—	386	359	323	388	245	303
M (2-oxo-)	2255	—	—	342	341	343	370	369	259
Temazepam (metabolised to oxazepam)	2595	2727	3125	271	273	300	272	256	77
Temazepam-TMS	—	2713	—	343	344	257	283	357	372
Tetrazepam[a]	2430	—	—	253	288	287	289	225	259
M (nor-)	2530	—	—	239	274	273	275	245	240
M (OH-) isomer 1	2570	—	—	235	375	304	237	261	247
M (OH-) isomer 2	2580	—	—	235	375	304	237	261	247
M (oxo-)	2430	—	—	285	287	267	245	302	247
Triazolam[a]	3080	3219	—	313	238	342	315	75	344
M (α-OH-)	3000	0000	—	328	330	293	265	239	357
M (α-OH)-TMS	—	3308	—	415	417	430	432	416	380
Zolazepam[a]	—	2426	—	257	285	267	286	258	145

[a]Does not form a TMS derivative.
[b]No electron-capture response.

Hydrolysis of benzodiazepines (preparation of benzophenones)
Boil an aqueous solution (or urine) with concentrated hydrochloric acid (1 part to 10 parts urine or solution) for 30–60 min. Cool, and neutralise with solid $KHCO_3$ or adjust the pH to 8–9 with 10 mol/L KOH. Mix with an equal volume of petroleum ether for 10 min. Centrifuge and evaporate the upper organic phase to dryness at 60°C. The reconstituted extract can be used for GC or other analytical procedures such as TLC (Chapter 39). Data are also presented here for some acetylated hydrolysis products (treatment of the dried residue with acetic anhydride and pyridine (3 : 2) for 30 min at 60°C (Maurer, Pfleger 1987). Not all benzodiazepines make benzophenones when hydrolysed by acid, and a number of other degradation products are furnished. The α-OH-metabolites of alprazolam, brotizolam and triazolam are partly altered by the elimination of formaldehyde. Hydrolysis products of bis-desethylflurazepam and di-OH-tetrazepam are dehydrated; OH-bromazepam, lorazepam and oxazepam form artefacts by rearrangement; the nor-metabolites of clobazam are cleaved and rearranged to benzimidazole derivatives; tetrazepam, and its two hydroxylated metabolites, are transformed into a pair of *cis*- and *trans*-isomeric hexahydroacridone derivatives.

Since the metabolism of benzodiazepines is complex, assays that convert drugs and metabolites into hydrolysis products are not ideal, because they do not permit unequivocal identification of the parent compound. After acid hydrolysis, care must be taken to ensure that the acid is neutralised prior to extraction or before injecting the solvent onto the chromatograph, otherwise the column deteriorates rapidly. The retention indices and principal ions are given in Table 40.15.

Cardioactive drugs

Cardioactive drugs (beta-blockers, calcium channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, etc.) are a diverse group of

Table 40.15 GC retention data and mass spectral data for benzophenones, hydrolysis products (HY) and some acetylated derivatives (AC) of benzodiazepines and their metabolites

Abbreviation	*Structure*	*System GA*	*Parent compound (*in-vivo *by metabolism)*	*Principal ions* (m/z)					
ABP	2-(2-Amino-5-bromobenzoyl)pyridine	2245	3-OH-Bromazepam, bromazepam	247	249	276	278	246	248
ABP-AC		2490		121	247	249	318	320	289
	3OH-bromazepam HY Art 1	2255	(Bromazepam)	285	287	206	286	284	179
	3OH-bromazepam HY Art 2	2265	(Bromazepam)	299	301	220	300	298	179
ACB	2-Amino-5-chlorobenzophenone	2039	Nordazepam, oxazepam (camazepam, chlordiazepoxide, clorazepic acid, diazepam, halazepam, ketazolam, medazepam, oxazolam, prazepam)	230	77	231	232	233	195
ACB-AC		2245		230	231	232	273	77	105
ACB Art 1		2060		239	205	240	241	163	177
ACB Art 2		2070		253	219	254	255	110	238
ACDP	2-Amino-5-chlorodiphenylamine	2210	Norclobazam (clobazam)	242	241	77	166	206	243
	Nor-OH-MeO-clobazam HY	2405	(Clobazam)	288	290	289	272	245	281

table continued

Table 40.15 continued

Abbreviation	*Structure*	*System GA*	*Parent compound (in-vivo by metabolism)*	*Principal ions* (m/z)					
	Nor-OH-MeO-clobazam HY-AC	2615		288	290	330	332	290	235
	Nor-OH-clobazam HY	2650	(Clobazam)	258	257	259	260	224	246
	Nor-OH-clobazam HY-AC	3000		258	300	260	259	257	302
	OH-MeO-clobazam HY	2905	(Clobazam)	320	322	206	240	321	207
ACFB	2-Amino-5-chloro-2′-fluorobenzophenone	2030	Desalkylflurazepam, *N*-desalkylflutoprazepam, oxo-quazepam (flurazepam, flutoprazepam, quazepam)	249	248	250	251	123	124
ACFB-AC		2195		249	248	291	123	95	250
	Didesethylflurazepam Art (-H_2O)-AC	2460	Didesethylflurazepam (flurazepam)	246	316	245	211	273	275
ACNB	2-Amino-2′-chloro-5-nitrobenzophenone	2470	Clonazepam, loprazolam	241	276	139	111	195	165
ADCB	2-Amino-5,2′-dichlorobenzophenone	2120	Lorazepam, mexazolam (lormetazepam)	230	265	267	232	154	195
ADCB-AC		2300		239	265	307	287	232	309
	Lorazepam HY Art	2170	Lorazepam (lormetazepam)	239	241	274	273	275	276
AFMAB	5-Amino-2′-fluoro-2-methylaminobenzophenone	2753	7-Acetamidoflunitrazepam, 7-aminoflunitrazepam (flunitrazepam)	244	227	243	245	211	123
AFMAB-AC_2		2870		205	286	328	244	243	269
AFNB	2-Amino-2′-fluoro-5-nitrobenzophenone	2330	Desmethylflunitrazepam (flunitrazepam)	260	259	123	165	213	241
ANB	2-Amino-5-nitrobenzophenone	2388	Nitrazepam	242	241	77	105	195	165
CPMACB	2-Cyclopropylmethylamino-5-chlorobenzophenone	2385	3-Hydroxyprazepam, prazepam	56	77	105	285	270	165
CPMACB-AC		2595		257	256	259	241	283	343
CTFEAB	5-Chloro-2-(2,2,2-trifluoro)-ethylaminobenzophenone	2380	Halazepam	313	312	314	315	244	296
DAB	2,5-Diaminobenzophenone	2175	7-Acetamidonitrazepam, 7-aminonitrazepam (nitrazepam)	211	212	77	107	195	183
DAB-AC_2		2985		296	212	211	254	253	297
DACB	2,5-Diamino-2′-chlorobenzophenone	2305	7-Acetamidoclonazepam, 7-aminoclonazepam (clonazepam)	246	211	245	248	107	247
DACB-AC_2		2845		330	288	246	211	139	332
DAFB	2,5-Diamino-2′-fluorobenzophenone	2175	7-Aminodesmethyl flunitrazepam (flunitrazepam)	230	229	211	210	107	231
DAFB-AC_2		2715		230	314	272	229	123	201
DCMAB	2′,5-Dichloro-2-(methylamino) benzophenone	2220	Lormetazepam	244	279	229	281	111	75
DEACFB	2-Diethylaminoethylamino-5-chloro-2′-fluorobenzophenone	2505	Flurazepam	86	87	57	348	350	109
HEACFB	2-Hydroxyethylamino-5-chloro-2′-fluorobenzophenone	2400	Hydroxyethylflurazepam, flutazolam (flurazepam, quazepam)	262	109	166	264	293	275
HEACFB-AC		2470		262	109	166	275	335	264
MACB	2-Methylamino-5-chlorobenzophenone	2105	Diazepam, temazepam (camazepam, chlordiazepoxide, clorazepate, ketazolam, tetrazepam)	77	245	244	228	105	246
MACDP	2-Methylamino-5-chlorodiphenylamine	2220	Clobazam	257	259	77	274	215	231
MANFB	2-Methylamino-5-nitro-2′-fluorobenzophenone	2385	Flunitrazepam	274	273	257	211	123	275

Table 40.15 continued

Abbreviation	*Structure*	*System GA*	*Parent compound* (in-vivo *by metabolism)*	*Principal ions* (m/z)					
	α-OH-alprazolam HY Art (-CH_2O)	3070	(Alprazolam)	259	294	293	205	239	265
	α-OH-alprazolam-AC	3180	(Alprazolam)	323	324	325	366	271	295
	OH-brotizolam HY Art (-CH_2O-)	3050	(Brotizolam)	380	378	379	299	301	245
	OH-brotizolam-AC	3140	(Brotizolam)	409	407	410	450	452	408
	α-OH-midazolam-AC	2820	(Midazolam)	310	340	383	312	342	385
	α,4-Di-OH-midazolam-AC_2	3020	(Midazolam)	310	326	399	340	383	441
	Normedazepam-AC	2470	(Medazepam)	228	297	193	256	298	255
	Tetrazepam HY (isomer 1)	2220	Tetrazepam	207	249	220	206	209	234
	Tetrazepam HY (isomer 2)	2260	Tetrazepam	207	249	220	206	209	234
	Tetrazepam M (OH-) HY-AC (isomer 1)	2380	(Tetrazepam)	307	248	234	264	309	220
	Tetrazepam M (OH-) HY-AC (isomer 2)	2470	(Tetrazepam)	307	248	234	264	309	220
	Tetrazepam M (OH-) HY-AC (isomer 3)	2535	(Tetrazepam)	307	206	309	248	218	220
	Tetrazepam M (OH-) HY-AC (isomer 4)	2560	(Tetrazepam)	307	218	220	206	248	264
	Tetrazepam M (nor-OH-) HY-AC_2	2500	(Tetrazepam)	233	232	196	154	275	335
	α-OH-triazolam HY Art (-CH_2O)	3000	(Triazolam)	328	293	330	265	239	329
	α-OH-triazolam-AC	3200	(Triazolam)	357	359	358	400	402	329

chemicals that require different analytical strategies. The calcium channel antagonists of the phenylalkylamine class (e.g. verapamil) and the benzothiazines (e.g. diltiazem) chromatograph well on standard GC phases (X-1 or X-5) after solvent extraction under mildly basic conditions (pH 10–12). As a rule, the beta-blockers (those with names that end in '-olol') are more water soluble and often require 'salting out' of aqueous solution at mildly basic conditions (pH 10–12) with an excess of solid sodium chloride. When subjected to GC they have a tendency to produce artefacts by loss of water and/or their amino-alkyl side-chain. Although they chromatograph reasonably well on capillary columns such as X-1 or X-5, they often give tailing peaks on packed columns, and various derivatisation strategies have been employed to overcome this. Simultaneous preparation of *N*-TFA and *O*-TMS derivatives have been described (Leloux *et al.* 1989; Lho *et al.* 1990), and cyclic boronates may be formed from phenyl or *n*-butylboronic acids using either triethylamine or pyridine catalysts (Lee *et al.* 1998). Acetylation of acid-hydrolysed sample extracts is described using acetic anhydride with a pyridine catalyst; although this process results in the complete destruction of some of the parent compounds, data for many metabolites are given (Maurer, Pfleger 1986). The ACE inhibitors (those with names that end in '-pril') have a free carboxylic acid group, and acquire a second such group by enzymatic hydrolysis of their ethyl ester link ('prilates'). Neither of these groups of compounds, nor the dihydropyridine calcium channel antagonists (e.g. nifedipine), chromatographs in their native state, but GC has been applied successfully to their analysis following extractive methylation. Here, equal volumes of urine and phase-transfer reagent (0.02 mol/L tetrahexylammonium hydrogensulfate in 1 mol/L phosphate buffer pH 12) are incubated with three volumes of 1 mol/L methyliodide in toluene on a shaker at 50°C for 30 min. The organic phase is eluted on a diol solid-phase cartridge (conditioned with methanol, then toluene) using diethyl ether–ethyl acetate (92.5 : 7.5, v/v). After evaporation to dryness at 60°C, the residue is reconstituted in ethyl acetate for injection.

Systems GA and GB, described above, can be used, and system GP chromatographs many of the drug metabolites as their methyl derivatives (Maurer *et al.* 1998; Maurer, Arlt 1999).

System GP

- *Column:* HP1 (methyl-PSX) fused silica capillary (12 m × 0.2 mm i.d., 0.33 μm)
- *Injector:* 280° splitless mode.
- *Temperature programme:* 100°C for 3 min to 310°C at 30°/min for 8 min.
- *Carrier gas:* He, 1 mL/min.
- *Retention indices and principal ions* are given in Table 40.16.

Coumarins and other anticoagulants

Coumarins are extracted fairly readily from acidic solution, especially when 'salted out' with solid sodium chloride; the coumarin structure is

Table 40.16 GC retention data and mass spectral data for cardioactive drugs (Me, methyl)

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
Acebutolol	2811	2926	—	72	43	56	151	221	98
Art	2910	3014	—	151	221	333	98	86	348
Art	—	2761	—	72	193	43	151	136	122
Art (-H_2O)	2850	2569	—	303	98	140	318	82	233
M (phenol-)	2450	2056	—	151	221	136	108	43	132
Art (desacetyl-)	—	2089	—	194	209	264	279	166	234

table continued

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
Acecainide (NAPA)	2550	2724	—	86	99	58	162	120	205
Acetyldigitoxin	Not eluted	Not eluted	—	—	—	—	—	—	—
Ajmaline	2880	—	—	144	326	173	198	297	237
Alprenolol	1820	—	—	72	56	73	249	98	234
Amiodarone	3335	Not eluted	—	86	87	84	58	56	44
Art (*O*-desalkyl)	2800	—	—	142	121	294	265	251	237
Amlodipine	—	2982	—	297	208	44	254	298	347
Amlodipine-Me	2820	—	—	311	254	88	312	208	238
Amlodipine-Me_2	2815	—	—	72	58	325	165	208	347
M (dehydro-2-HOOC-)-Me	—	—	2430	356	296	224	268	357	391
M (dehydro-desamino-HOOC-)-Me	—	—	2635	260	347	318	316	349	400
Amyl nitrite (see Chapter 12)									
Aprindine	2462	—	—	113	84	116	98	117	115
M (*N*-desalkyl-)	1920	—	—	104	209	77	116	115	94
M (*p*-aminophenol)	1253	1280	—	109	52	53	80	81	108
Atenolol	2385	2469	—	72	56	98	43	107	41
Art (-H_2O)	2150	2090	—	98	56	107	248	72	190
Art	2400	2648	—	46	86	107	127	72	263
Art (HOOC)-Me	2140	—	—	72	107	116	237	56	266
Art (HOOC)-Me	2175	—	—	278	127	112	56	293	292
Benazepril	—	Not eluted	—	—	—	—	—	—	—
Benazepril-Me	—	—	3030	365	204	91	366	392	347
M (benazeprilate)-Me_3	—	—	2985	379	204	380	91	144	438
Benziodarone	—	Not eluted	—	518	173	264	519	373	376
Betaxolol	2370	2420	—	72	253	292	307	55	107
Art	2410	2508	—	304	319	318	290	127	55
Art (-H_2O)	2400	2519	—	72	98	53	56	107	158
Bethanidine	1925	—	—	71	91	106	177	57	72
Bisoprolol	2378	2427	—	72	116	107	100	281	73
Art	2595	2680	—	127	112	86	322	337	224
Art (-H_2O)	2400	2480	—	98	56	307	107	204	220
M (phenol)	1690	—	—	107	123	77	167	103	210
Bunazosin	3330	—	—	247	260	233	373	234	221
Bunitrolol	1960	—	—	86	233	70	71	58	204
Art	1980	—	—	245	70	56	119	158	260
Captopril	Not eluted	Not eluted	—	70	41	69	75	114	217
Captopril-Me	1730	—	—	70	128	172	199	231	198
Captopril-Me_2	—	—	—	70	128	89	245	203	130
Carazolol	2810	—	—	183	72	298	154	184	116
Art	2830	—	—	183	127	112	310	222	295
Carazolol-Me	2815	—	—	86	183	154	312	298	268
Carteolol	2588	—	—	86	57	277	70	292	87
Art	2690	—	—	289	141	126	202	304	290
Carvedilol Art 1	—	2056	—	222	223	11	151	98	77
Carvedilol Art 2	—	2224	—	183	154	155	127	77	184
Celiprolol	2610	—	—	86	58	250	44	291	307
Art 1	2350	—	—	333	86	56	96	112	216
Art 2	2650	—	—	86	151	291	277	114	265
Art 3	2740	—	—	86	209	323	294	114	56
Cilazapril	—	Not eluted	—	211	143	91	283	197	344
Cilazapril-Me	—	—	3010	157	225	297	344	91	358

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
M (cilazaprilate)-Me_3	—	—	2960	157	225	297	245	361	372
Clenbuterol	2100	—	—	86	57	127	190	243	90
Art (-H_2O)	1895	—	—	57	174	175	176	202	258
Art	2160	—	—	99	243	245	187	174	176
Clonidine	2090	2165	—	229	231	172	194	174	200
M	—	2046	—	192	157	227	194	229	193
M	—	2264	—	243	188	245	186	194	236
Art (dichlorophenylisocyanate)	1350	—	—	187	189	124	159	126	161
Art (dichlorophenylmethylcarbamate)	1500	—	—	184	186	174	133	160	219
Art 5	2110	—	—	248	194	250	229	243	283
Debrisoquine	0000	0000	—	132	104	44	175	103	117
Debrisoquine-acetylhydrazone deriv.	—	2100	—	—	—	—	—	—	—
M (4OH)-acetylhydrazone deriv.	—	2450	—	—	—	—	—	—	—
Deserpidine	—	—	—	578	195	577	367	351	579
Deslanoside (metabolised to digoxin)	Not eluted	Not eluted	—						
Diazoxide	Not eluted	Not eluted	—	125	189	230	127	191	63
Digitoxin	Not eluted	Not eluted	—						
Digoxin	Not eluted	Not eluted	—						
Diltiazem	2949	3076	—	58	71	72	121	150	136
M (desacetyl-)	2990	3092	—	58	71	121	136	150	109
M (*O*-desmethyl-)	3050	3147	—	58	71	159	283	207	253
M (*N*-desmethyl-)	—	3114	—	44	150	283	161	121	136
Disopyramide	2505	2608	—	195	212	114	167	72	194
M (*N*-desalkyl-)	—	2286	—	—	—	—	—	—	—
M	—	2264	—	194	196	167	180	280	252
Doxazosin	—	3054	—	—	—	—	—	—	—
Enalapril-H_2O	2770	2864	—	208	254	117	70	169	358
Enalapril-Me	2650	—	2675	234	91	70	130	160	317
M (enalaprilate)-Me_3	—	—	2680	234	130	174	235	331	91
M (enalaprilate-H_2O)-Me	2730	—	2735	208	240	91	70	117	344
Encainide	3016	—	—	98	135	70	99	77	352
Enoximone	—	Not eluted	—	248	247	151	201	249	200
Esmolol	—	2311	—	72	56	107	116	73	91
Art	—	2395	—	292	306	307	293	278	234
Felodipine	2670	2793	—	238	210	239	354	383	338
Felodipine-Me	2725	—	—	252	224	324	326	164	338
M (dehydro-desethyl)-Me	—	—	2235	322	324	323	300	258	173
M (dehydro-)	—	—	2280	346	318	348	320	286	173
Flecainide	2250	2351	—	84	56	97	301	219	209
Art (formyl)	2500	2240	—	301	125	42	97	218	343
Heptaminol	1120	—	—	59	56	69	113	95	127
Hexobendine	Not eluted	—	—	296	195	58	253	297	84
Hydralazine	1528	1914	—	—	—	—	—	—	—
Hydroquinidine	2810	—	—	—	—	—	—	—	—
Imidapril-Me	2700	—	—	234	91	346	159	160	117
M (desethyl-)-Me_2	2695	—	—	220	56	117	70	159	346
M (desethyl-)-Me_3	2710	—	—	234	360	130	235	56	117
Imolamine	2177	—	—	—	—	—	—	—	—
Indoramin	—	—	—	217	174	105	218	143	130
Isradipine	2680	—	—	210	252	77	150	178	284

table continued

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
Isradipine-Me	2670	—	—	298	224	268	56	385	284
M (dehydro-desiospropyl)-Me	2270	—	2270	309	264	341	310	294	279
M (dehydro-)	2360	—	—	295	327	265	251	369	310
Labetalol Art	1320	1270	—	44	91	132	117	65	78
Lacidipine	2955	—	—	252	196	326	169	119	382
Lanatoside c (hydrolyses to digoxin)	—	Not eluted	—	41	43	55	57	73	81
Lidocaine	1870	1947	—	86	87	58	44	72	42
M (MEGX)	1800	1870	—	58	121	120	163	91	77
M (GX)	—	1776	—	121	178	120	106	148	77
M (2,6-xylidine)	1180	1195	—	106	121	120	91	77	65
M (3OH-)	2350	—	—	86	58	250	194	110	120
Lidoflazine	3870	Not eluted	—	343	70	344	109	42	113
M (desaminocarboxy-)	2230	—	—	—	—	—	—	—	—
M (desaminocarboxy)-Me	2125	—	—	—	—	—	—	—	—
Lisinopril	Not eluted	Not eluted	—	70	91	84	113	245	224
Lorcainide	2810	2923	—	82	56	110	355	124	251
M (*N*-desmethyl-)	2660	2789	—	—	—	—	—	—	—
M (desacyl-)	2100	—	—	110	56	125	252	180	254
Losartan-Me_2	—	—	3555	192	201	249	165	450	435
Mecamylamine	—	—	—	98	84	71	56	99	124
Methoserpidine	—	—	—	608	195	607	397	609	395
Methyldopa	—	Not eluted	—	88	42	123	124	89	77
Metipranolol	2220	2320	—	72	152	56	116	102	194
Art	2240	—	—	127	114	112	86	152	306
Art	2190	—	—	72	152	116	56	137	223
Metoprolol	2035	2090	—	72	107	56	73	223	100
Art	2120	2176	—	56	127	112	114	264	279
M	2200	2284	—	72	107	116	251	280	145
M (OH-) Art	2240	2355	—	128	127	250	280	295	294
Mexiletine	1400	1431	—	58	44	83	77	69	85
M (desamino-oxo-)	1350	1395	—	105	178	135	134	133	121
M	—	1745		44	58	91	77	135	178
Minoxidil	—	—	—	84	209	67	43	110	192
Moexipril-Me	—	—	3575	234	305	190	250	91	439
M (moexiprilate)-Me_3	—	—	3580	234	305	190	91	220	453
M (moxeprilate-H_2O)-Me_3	—	—	3775	190	466	449	91	164	290
Moracizine	—	—	—	100	286	142	56	239	70
Nadolol	2540	2658	—	86	57	294	71	310	70
Art	2560	2670	—	306	70	86	141	201	307
Nicardipine	3900	Not eluted	—	91	134	147	146	148	165
M (dehydro-desbenzylMeNH_2)-Me	—	—	2300	327	297	313	312	344	252
M (dehydro-desamino-HOOC)-Me	—	—	2645	312	313	281	371	285	139
M (dehydro-desamino-OH-)	—	—	2665	312	313	299	281	252	374
M	2250	—	—	299	269	316	285	300	241
Nifedipine	2545	2708	—	239	284	224	268	330	285
M (dehydro-)	2250	2370	2255	298	299	252	267	313	344
M (dehydo-HOOC-)	2290	—	—	283	252	224	126	282	298
M (dehydro-2-HOOC)-Me	—	—	2695	342	343	139	195	357	388
Nilvaldipine	—	Not eluted	—	—	—	—	—	—	—
M (dehydro-desisopropyl)-Me	—	—	2565	340	324	355	308	164	341
M (dehydro-)	—	—	2565	324	341	310	383	294	164

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
Nimodipine	2929	3096	—	—	—	—	—	—	—
M (dehydro-desisopropyl-desmethoxyethyl)-Me_2	—	—	2300	327	297	313	312	344	252
M (dehydro-desmethoxyethyl)-Me	—	—	2390	313	298	252	330	283	372
M (dehydro-desisopropyl-*O*-desmethyl-HOOC)-Me	—	—	2645	312	313	281	371	285	139
M (dehydro-)	—	—	2655	298	299	340	341	342	357
M (dehydro-desisopropyl-*O*-desmethyl)-Me	—	—	2665	312	313	299	281	252	374
M (dehydro-*O*-desmethyl-HOOC)-Me	—	—	2740	298	340	281	299	341	371
Nisoldipine	2730	—	—	371	210	270	266	284	254
M (dehydro-desisobutyl)-Me	—	—	2255	298	299	252	267	313	344
M (dehydro-)	—	—	2450	284	340	57	285	236	303
M (dehydro-OH-)	—	—	2615	284	356	59	253	313	267
M (dehydro-desisobutyl-2-HOOC)-Me_2	—	—	2695	342	343	139	195	357	388
Nitrendipine	2635	—	—	238	210	239	287	360	150
M (dehydro-desethyl-)-Me	—	—	2300	327	297	313	312	344	252
M (dehydro-)	—	—	2370	341	313	358	312	281	252
M (dehydro-desethyl-)-CO_2	2275	—	—	269	329	255	286	139	180
M (dehydro-desmethyl-)-CO_2	2330	—	—	251	139	253	255	283	300
M (dehydro-desethyl-OH-)-H_2O	2650	—	—	311	281	328	297	312	250
M (dehydro-desethyl-OH-)-H_2O	2690	—	—	325	297	342	266	250	326
Oxprenolol	1870	1972	—	72	56	221	41	73	57
Art	1985	2062	—	56	262	248	148	277	235
M (desamino-OH-desalkyl-)	1700	—	—	—	—	—	—	—	—
Pargyline	1214	1257	—	82	68	91	159	42	158
Pempidine	—	—	—	140	84	51	41	72	69
Penbutolol	2139	2221	—	86	70	56	276	133	161
M (OH-)	2425	—	—	86	304	56	178	319	292
Art (formyl)	2150	—	—	288	289	303	141	91	159
Perhexiline	2245	—	—	84	194	55	85	56	99
M (OH-)	2485	—	—	84	56	210	192	97	110
M (di-OH-)	2660	—	—	84	56	98	210	70	249
M (di-OH-)-H_2O	2510	—	—	84	56	192	208	210	291
Perindopril	—	Not eluted	—	—	—	—	—	—	—
Perindopril-Me	—	—	2450	172	98	309	173	124	382
M (perindoprilate)-Me_3	—	—	2470	172	112	86	323	173	382
M (perindoprilate-H_2O)-Me_3	—	—	2560	222	249	277	336	133	294
Phenoxybenzamine	2235	2332	—	91	196	198	92	197	65
Phentolamine	Not eluted	Not eluted	—	281	120	91	122	280	160
Pindolol	2245	2335	—	72	133	116	248	134	56
Practolol	0000	2440	—	72	151	43	109	56	57
Prajamlium	2925	—	—	—	—	—	—	—	—
M (OH-) Art	3130	—	—	224	126	196	384	313	356
M (methoxy-) Art	2895	—	—	254	370	398	255	126	297
M (OH-methoxy-) Art	3200	—	—	224	196	206	414	343	399
Prazosin	Not eluted	Not eluted	—	233	383	259	245	95	56
Prenylamine (also metabolised to amphetamine)	2555	—	—	58	238	91	45	239	167
Prenylamine-AC	2925	—	—	58	91	100	280	238	164
M (*N*-desalkyl-)-AC	2320	—	—	73	165	167	193	253	152

table continued

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
M (*N*-desalkyl-OH-)-AC_2	2635	—	—	73	183	311	220	239	269
M (*N*-desalkyl-OH-methoxy-)-AC_2	2700	—	—	73	213	299	152	240	341
M (OH-methoxy-)-AC_2	3310	—	—	58	326	368	270	459	240
M (OH-)-AC_2	3200	—	—	58	296	338	297	100	429
M (desamino-OH-)-H_2O	1940	—	—	—	—	—	—	—	—
Procainamide	2255	2332	—	86	99	120	92	87	58
M (*N*-acetyl-)	2550	2724	—	86	99	58	162	120	205
M	—	2245	—	120	58	71	92	137	149
M	—	2292	—	86	146	99	117	120	189
M	—	2642	—	58	71	120	162	92	191
M	—	2783	—	86	99	120	58	176	219
Propafenone	2730	—	—	72	91	98	297	131	312
Art (-H_2O)	2300	—	—	91	98	105	294	323	230
M (OH-)-H_2O	2720	—	—	98	72	230	137	339	310
M (*O*-desalkyl-)	1830	—	—	—	—	—	—	—	—
M (*O*-desalkyl-OH-) isomer 1	2345	—	—	—	—	—	—	—	—
M (*O*-desalkyl-OH-) isomer 2	2355	—	—	—	—	—	—	—	—
M (*O*-desalkyl-OH-methoxy-)	2400	—	—	—	—	—	—	—	—
Propranolol	2147	2234	—	72	56	98	115	144	116
M (4OH-)	—	2546	—	72	116	160	131	275	199
M (1-naphthol)	1505	1534	—	144	115	116	72	89	63
M (desamino-OH-)	2065	—	—	144	115	218	131	116	101
Protoveratrine	2465	—	—	—	—	—	—	—	—
Quinapril	—	—	—	316	270	91	130	117	104
Art-H_2O	3380	3467	—	91	316	270	130	117	104
Quinapril-Me	—	—	3110	234	91	130	190	160	379
M (quinaprilate)-Me_3	—	—	3080	234	91	130	148	174	235
M (quinaprilate-H_2O)-Me_3	—	—	3310	91	130	270	302	103	132
Quinidine	2790	2979	—	136	81	322	188	55	172
M (*N*-oxide)	2950	3086	—	152	136	189	340	173	324
M	2940	3125	—	152	124	138	338	323	158
Ramipril	—	Not eluted	—	294	248	91	110	117	209
M (-H_2O)	2980	—	—	248	294	117	110	209	297
Ramipril-ME	—	—	2880	234	91	160	235	357	220
M (ramiprilate)-Me_3	—	—	2865	234	91	235	130	371	148
M (ramiprilate-H_2O)-Me_3	—	—	2925	280	248	91	110	193	284
Rescinnamine (Reserpinine)	2180	—	—	221	109	200	186	395	251
Reserpine	Not eluted	Not eluted	—	608	606	195	609	395	397
M (trimethoxybenzoic acid)	1780	—	—	212	197	141	154	169	111
M (trimethoxyhippuric acid)	2085	—	—	195	251	223	152	122	167
M (trimethoxybenzoic acid)-Me	1740	—	—	226	211	195	155	183	168
M (trimethyoxyhippuric acid)-Me	2350	—	—	283	195	284	152	268	252
Sotalol	2413	2520	—	72	43	122	73	106	121
Syrosingopine	—	Not eluted	—	181	395	198	251	397	396
Tertatolol	2310	—	—	86	166	251	280	57	151
Art	2400	—	—	292	293	307	141	166	151
Terazosin	—	Not eluted	—	233	71	316	245	43	387
Timolol	2266	2373	—	—	—	—	—	—	—
Art	2275	2380	—	86	96	72	142	154	313
Tocainide	1714	1769	—	44	121	77	120	42	106
Trandolapril-H_2O	3090	—	—	262	308	91	124	117	223

Table 40.16 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GB*	*GP*						
Trandolapril-Me	—	—	2970	234	91	235	160	371	130
Trandolapril-Me_2	2995			248	249	91	174	144	385
M (perindoprilate)-Me_3	—	—	3005	234	235	91	385	130	174
M (perindoprilate-H_2O)-Me_3	—	—	3070	294	262	91	398	117	223
Trimetazidine	—	—	—	181	85	56	166	266	182
Valsartan-Me_2	—	—	3420	264	378	192	320	249	164
Verapamil	3150	3305	—	303	58	43	304	151	44
M (nor-)	3180	3371	—	289	151	290	152	165	260
M (*N*-desalkyl-)	2100	2193	—	44	164	203	247	290	57
M (*O*-desmethyl-didesalkyl-)	—	2169	—	216	233	164	185	276	203
M (*O*-desmethyl-desalkyl-)	—	2246	—	44	257	212	171	247	290
M (didesalkyl-)	—	2300	—	247	248	275	233	216	290
M	—	2409	—	202	189	230	247	290	203
M (*N*-desalkyl-acetyl-)	2460	2546	—	247	289	332	216	248	290
M (*N*-didesalkyl-acetyl-)	2545	2579	—	275	318	233	276	234	170

not amenable to GC without prior derivatisation. Maurer and Arlt (1998) describe an extractive methylation procedure from aqueous alkali (5 mL) using iodomethane in toluene (5 mL of 0.5 mol/L) and 150 µL of a phase-transfer reagent (tetrahexylammonium hydrogensulfate (THA), 4.5 g in 50 mL of 0.5 mol/L NaOH). Removal of excess THA was achieved by passing the toluene layer through a solid-phase extraction (SPE) cartridge and eluting with diethyl ether–ethyl acetate (92.5 : 7.5, v/v), which enables GC with MS. System GA, described above, is used, and the retention indices and principal ions are given in Table 40.17. Anticoagulants of the heparin family (e.g. enoxaparin) are not included as these peptides cannot be chromatographed.

Diuretics

As a group, the diuretics chromatograph poorly on packed columns, and only marginally better on capillaries because of the presence of one or

Table 40.17 GC retention data and mass spectral data for coumarins and other anticoagulants (Me, methyl)

Compound	*System GA*	*Principal ions* (m/z)					
Acenocoumarol-Me	3035	324	325	367	189	121	278
M (amino)-Me_3	2985	308	365	292	309	293	249
M (acetamido)-Me_2	3265	350	351	393	278	56	394
M (OH-) isomer 1-Me_2	3350	354	355	397	151	308	219
M (OH-) isomer 2-Me_2	3500	354	355	397	151	308	219
Anisindione	2273	252	237	253	181	238	77
Coumachlor-Me	2770	313	315	356	128	189	201
M (OH-) isomer 1-Me_2	2990	343	345	386	125	151	231
M (OH-) isomer 2-Me_2	3035	343	345	386	125	151	231
M (OH-dihydro)-Me_2	3095	388	343	329	245	125	151
M (OH-methoxy)-Me_2	3195	373	375	416	372	359	125
M (di-OH)-Me_3	3195	373	375	416	372	359	125
Coumatetralyl	2635	292	188	130	128	293	187
Coumatetralyl isomer 1-Me	2655	306	175	291	115	121	189
Coumatetralyl isomer 2-Me	2690	306	291	175	115	202	91
M (OH-) isomer 1-Me	2910	203	303	304	187	121	322
M (OH-) isomer 2-Me_2	2925	336	205	217	232	302	321
M (OH-) isomer 3-Me_2	2935	321	320	336	175	319	305
M (OH-) isomer 4-Me_2	2990	336	205	232	217	321	337
Dicoumarol-Me	3235	—	—	—	—	—	—
Diphenadione	2934	173	167	340	165	89	152
Phenindione	2055	222	165	223	194	76	90
Phenprocoumon isomer 1-Me	2375	203	279	265	294	249	121
Phenprocoumon isomer 2-Me	2395	91	265	294	203	279	221

table continued

Table 40.17 continued

Compound	*System GA*	*Principal ions* (m/z)					
M (OH-) isomer 1-Me_2	2655	295	324	233	91	309	251
M (OH-) isomer 2-Me_2	2675	295	296	324	121	279	201
M (OH-) isomer 3-Me_2	2705	295	324	296	91	233	151
M (OH-methoxy)-Me_2	2770	325	354	326	151	279	201
M (di-OH)-Me_3	2770	325	354	326	151	279	201
Pyranocoumarin/cyclocumarol	2670	322	72	265	249	275	148
M (*O*-desmethyl-) Art-Me (warfarin-Me)	2580	279	280	322	91	121	189
M (*O*-desmethyl-OH-) isomer 1 Art-Me_2	2810	309	310	352	91	277	151
M (*O*-desmethyl-OH-) isomer 2 Art-Me_2	2830	309	121	201	295	352	310
M (*O*-desmethyl-OH-) isomer 3 Art-Me_2	2870	309	310	352	91	295	206
Warfarin-ME	2580	279	280	322	91	121	189
M (OH-) isomer 1-Me_2	2810	309	310	352	91	277	151
M (OH-) isomer 2-Me_2	2830	309	121	201	295	352	310
M (OH-) isomer 3-Me_2	2870	309	310	352	91	295	206

more sulfonamide (SO_2–NH_2) or carboxylic acid groups. However, they can be methylated easily, and then systems GA or GB, described above, may be used effectively. The diuretics can be extractively alkylated from aqueous alkali (5 mL) using iodomethane in toluene (5 mL of 0.5 mol/L) and 150 µL of a phase-transfer reagent. Tetrahexylammonium hydrogensulfate (4.5 g in 50 mL 0.5 mol/L NaOH) is far superior to tetrabutylammonium hydroxide (TBAH) and tetrapentylammonium hydroxide (TPAH) (Carreras *et al.* 1994). Removal of excess THA was achieved by passing the toluene layer through an SPE cartridge and eluting with diethyl ether–ethyl acetate (92.5 : 7.5, v/v). Alternatively, diuretics can be extracted from aqueous acidic solution into ethyl acetate, and the evaporated residue heated with 10% methyl iodide in acetone and 100 mg solid K_2CO_3 for 6 h at 60°C, and the resultant extract applied directly to the chromatograph. The former method tends to produce more completely substituted derivatives than the latter, which gives a more varied pattern of substituted derivatives. Although good sensitivity for plasma samples can be obtained with an NPD, the use of a mass spectrometer is required to confirm the identity (Lisi *et al.* 1991; Yoon *et al.* 1990).

System GA or GB, described above, can be used, as can systems GX and GY.

System GX

The details are taken from Carreras *et al.* (1994).

- *Column:* 5%-phenyl-PSX (X-5) (25 m × 0.2 mm i.d., 0.33 µm).
- *Temperature programme:* 230°C to 320°C at 35°/min (drugs elute isothermally).
- *Carrier gas:* He, 1 mL/min.
- *Retention indices and times* (min) are given in Table 40.18.

System GY

The details are taken from Lisi *et al.* (1991).

- *Column:* methyl-PSX (X-1) (25 m × 0.22 mm i.d., 0.1 µm).
- *Temperature programme:* 130°C to 320°C at 40°/min for 3 min.
- *Injection:* Split 10 : 1.
- *Carrier gas:* H_2, 1 mL/min.
- *Retention indices and times* (min) are given in Table 40.18.

Table 40.18 GC retention data and mass spectral data for diuretics (Me, methyl)

Compound	*System*			*Principal ions* (m/z)					
	GA	*GX*	*GY*						
Acetazolamide-*N*-Me_3	1827	3.62	2.90	249	83	108	43	264	265
Acetazolamide-*O*-Me	1930	—	2.69	70	44	129	236	237	—
Althiazide-Me_4	—	13.20	—	352	354	244	42	145	—
Art-Me	1840	—	—	202	144	171	116	204	101
Art-Me_2	1860	—	—	187	170	189	142	116	101
Bendrofluazide-Me_4	—	10.70	5.62	386	278	91	42	387	145
Bendroflumethiazide-Me_3	3344	12.00	—	386	278	42	387	388	—
Benzbromarone	2760	—	—	264	173	424	115	279	423
Benzbromarone-Me	—	2730	—	278	438	173	440	439	—
Benzthiazide	2680	—	—	309	91	311	123	176	121
Bumetanide-Me_3	2970	7.71	4.90	254	318	363	406	77	196
Chlorothiazide	1720	—	—	295	268	297	270	64	124
Chlorothiazide-Me_3	—	6.55	4.34	337	245	42	339	230	293
Chlortalidone	2145	—	—	148	130	76	321	299	300
Chlortalidone-Me_4	2630	7.67	4.81	176	287	363	365	364	289

Table 40.18 continued

Compound	*System*			*Principal ions* (m/z)					
	GA	*GX*	*GY*						
Clopamide-Me_2	2805	6.97	—	111	112	127	55	139	358
Clopamide-Me_3	2600	—	—	372	374	387	373	264	245
Art(-SO_2NH)	2195	—	—	111	127	139	83	96	251
Cyclopenthiazide-Me_4	—	—	6.33	352	354	233	42	145	435
Dichlorphenamide-Me_4	—	5.15	3.94	44	108	253	255	144	360
Ethacrynic acid-Me	2195	4.02	3.33	261	263	243	245	281	316
Furosemide (frusemide)	Not eluted	—	—	81	300	53	96	82	332
Furosemide-Me	2890	—	—	81	344	346	96	329	311
Furosemide-Me_2	2850	—	—	81	358	360	96	325	343
Furosemide-Me_3	2800	6.95	4.65	81	372	358	374	339	312
Art (-SO_2NH)	2040	—	—	81	53	251	96	253	233
Art (-SO_2NH)-Me	2020	—	—	81	265	53	96	232	250
Art (-SO_2NH)-Me_2	2050	—	—	81	232	279	250	234	204
M (*N*-desalkyl-)-Me	2750	—	—	264	232	266	234	248	200
M (*N*-desalkyl-)-Me_2	2450	—	—	278	200	280	248	185	169
Hydrochlorothiazide	Not eluted	—	—	269	297	271	221	268	188
Hydrochlorothiazide-Me_4	2966	9.01	4.99	310	353	218	288	355	202
Art (-SO_2NH)-Me	2170	—	—	139	232	127	63	167	189
Hydroflumethiazide	Not eluted	—	—	303	239	331	255	266	158
Hydroflumethiazide-Me_4	2653	6.30	4.38	387	236	215	344	252	322
Indapamide-Me_3	3035	9.01	—	161	132	131	407	409	130
Mefruside-Me_2	2860	7.43	4.74	85	43	86	110	325	367
M (5-oxo-)-Me_2	—	9.80	5.14	99	325	327	218	282	326
Methazolamide	2187	—	—	221	43	83	236	223	221
Methazolamide-Me_2	—	—	2.90	249	264	43	108	83	265
Methyclothiazide	Not eluted	—	—	310	312	42	311	230	359
Methyclothiazide-Me_3	—	9.90	—	352	354	244	246	—	—
Metolazone-Me_3	3910	—	6.23	392	394	393	284	118	407
Piretanide-Me_3	2965	8.40	—	295	296	404	266	297	—
Polythiazide	2380	—	—	310	312	42	129	311	230
Polythiazide-Me_3	2985	11.01	—	352	354	244	42	246	—
Probenecid	2336	—	—	256	185	121	224	257	65
Probenecid-Me	2205	3.90	3.23	270	135	199	271	228	299
Quinethazone	Not eluted	—	—	260	262	180	289	261	145
Quinethazone-Me_3	—	—	5.05	316	208	318	42	173	317
Spironolactone	3280	—	—	341	340	374	342	267	359
M (canrenone)	3250	—	5.57	340	267	107	91	341	325
M (canrenoic acid)	3100	—	—	358	84	85	329	359	274
M (canrenoic acid)-Me	3130	—	—	354	355	339	340	356	173
Triamterene	2010	—	—	253	252	43	104	254	235
Triamterene-Me_6	2875	9.15	—	336	338	322	308	293	309
Trichlormethiazide-Me_4	2810	10.72	—	352	354	244	42	—	—
Trometamol	1645	—	—	—	—	—	—	—	—
Xipamide-Me_2	3350	—	—	262	264	382	263	168	223
Xipamide-Me_3 isomer 1	2800	8.72	—	396	276	365	395	397	398
Xipamide-Me_3 isomer 2	3320	—	—	276	396	278	277	168	233
Xipamide-Me_4	2780	—	—	410	290	379	409	411	412
Art (-SO_2NH)	2385	—	—	121	155	275	157	106	99
Art (-SO_2NH)-Me	2480	—	—	169	170	289	126	290	291
Art (-SO_2NH)-Me_2	2115	—	—	183	303	272	257	302	304
M (OH-)-Me_4	3000	—	—	426	428	395	396	275	262

Essential oils, flavours and fragrances

Essential oils, flavours and fragrances are complex mixtures of many components, so Table 40.19 is representative of the most common ones only. For a more extensive list, the reader is referred to specialist texts (Adams 1995). In addition, many small esters and ketones are contributory and can be detected via the system described in the section below on volatile substances. Essential oils, once thought innocuous, are now recognised as a potential cause of serious poisonings, especially in children, and are encountered in highly concentrated forms with increasing frequency in everyday use as vehicles for medicines, aromatherapy and handicraft supplies. Since most natural flavours occur in predominantly one enantiomeric form, and the majority of synthetic flavours are racemates, chiral analysis of enantiomeric proportions is an effective way to determine the authenticity of flavours. The catalogues of chromatography column suppliers show many examples of chiral separations; some examples are found on-line at www.restek.com. Systems GA and GB, described above, can be used, as can systems GN and GO (Supelco 2000).

System GN

- *Column:* Supelcowax 10 (Wax10) (10 m × 0.25 mm i.d., 0.25 μm).
- *Temperature programme:* 50° for 2 min, to 280° at 2°/min
- *Carrier gas:* He, 25 cm/s.
- *Retention times* (min) are given in Table 40.19.

System GO

- *Column:* SPB-5 (5%-phenyl-PSX, X-5) (30 m × 0.25 mm i.d. 0.25 μm).
- *Temperature programme:* 75° for 8 min to 200° at 4°/min.
- *Carrier gas:* He, 25 cm/s.
- *Retention times* (min) are given in Table 40.19.

Narcotic analgesics, opiates and opioids

Many laboratories perform specific assays for opiates for federal or legal purposes; these are generally limited to codeine, morphine and more recently 6-monoacetyl morphine (MAM) (Paul *et al.* 1999).

Table 40.19 GC retention data for oils, flavours and fragrances

Compound	*System*			
	GA	*GB*	*GN (min)*	*GO (min)*
Anethole	1284	1316	—	—
Camphene	—	—	5.9	5.9
Camphor	1143	—	—	—
Carene	—	—	8.6	8.3
Carvone	—	1275	—	18.8
Cedrol	—	—	64.3	—
Cineole	—	1063	—	11.2
Citronellal	1265	—	—	14.4
Citronellol	—	—	45.8	—
Eugenol	1368	1380	—	—
Geranial	—	—	—	19.4
Geraniol	1192	—	51.7	18.8
Ionine	—	—	50.4	—
Jasmone	—	—	56	—
Lavandulol	—	—	40.5	—
Limonene	1053	1063	11.0	8.9
Linalool	1100	—	32.4	12.0
Menthol	1206	1194	—	—
Menthone	—	—	26.1	—
Methyl salicylate	1195	1228	—	—
Myrcene	—	—	9.6	7.1
Neral	—	—	—	18.1
Nerol	—	—	48.0	17.6
Nerolidol	—	—	59, 61	—
Patchouli	—	1691	—	—
α-Pinene	—	—	4.9	5.4
β-Pinene	—	—	7.1	6.7
Piperonal	—	1373	—	—
Sabinine	—	—	—	6.5
α-Terpinene	—	—	—	8.4
γ-Terpinene	—	—	18.4	10.2
Terpinen-4-ol	—	—	—	15.5
α-Terpineol	1126, 1176	—	41.5	16.1
Thymol	—	1316	—	—
Vanillin	1630	1632	86.5	—

However, for clinical purposes a wider range of analytes is desirable and can include codeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone and oxymorphone. All assays involve a hydrolysis step (acidic or enzymatic – see earlier discussion for an evaluation of these) to cleave the glucuronide conjugates, followed by a basic extraction (often using solid-phase or acidic back extraction for cleanliness). Derivatisation is possible with a number of reagents (PFP, TMS, TFA or AC derivatives are the most common) (Chen *et al.* 1990; Grinstead 1991; Maurer, Pfleger 1984), and retention data for some of these are included in Table 40.20. The derivatising reagent is selected on the basis of personal preference for a desired separation or the formation of unique ions on MS fragmentation. Analysis of hydromorphone, oxycodone and oxymorphone is complicated by the possibility that several structurally different

Table 40.20 GC retention data and mass spectral data for narcotic analgesics, opiates and opiods (AC, acetyl; HFB, heptafluorobutyrate; PFP, pentafluoropropionate; TFA, trifluoroacetyl; TMS, trimethylsilyl)

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
Acetorphine (hydrolyses to etorphine)											
Acetylcodeine	2503	2645	—	—	1.449	341	282	342	229	204	240
Alfentanil	2970	3108	—	—	Not eluted	289	268	290	140	222	170
Alphameprodine	1850	1927	—	—	—	—	—	—	—	—	—
Alphaprodine	1792	1862	—	—	—	172	187	84	57	42	188
Anileridine	2850	—	3469	—	—	246	247	218	120	277	106
Apomorphine	2715	0000	—	—	Not eluted	266	267	224	220	152	248
Apomorphine-AC_2	2830	—	—	—	—	351	350	266	308	309	292
Apomorphine-TMS_2	2715	—	—	—	—	410	411	322	73	368	412
Benzylmorphine	3015	—	—	—	—	284	91	81	375	285	175
Buprenorphine	3360	3610	—	—	Not eluted	378	410	379	435	434	449
Buprenorphine-HFB	2960	—	—	—	—	574	606	575	562	548	607
Buprenorphine-HFB_2	2820	—	—	—	—	55	562	83	630	646	604
Buprenorphine-PFP	3040	—	—	—	—	524	556	525	512	498	580
Buprenorphine-PFP_2	2775	—	—	—	—	55	512	580	554	513	595
Buprenorphine-TFA	2920	—	—	—	—	55	474	506	475	448	507
Buprenorphine-TFA_2	2800	—	—	—	—	55	462	530	463	504	546
Buprenorphine-TMS	3890	—	—	—	—	450	451	482	506	493	424
Art (-H_2O)	3240	—	—	—	—	449	434	408	419	435	450
Art (-H_2O)-AC	3320	—	—	—	—	491	476	450	434	477	492
Buprenorphine-AC	3410	—	—	—	—	420	452	421	408	394	509
Butorphanol	2761	2902	—	—	—	272	273	411	254	157	327
Butorphanol-TMS	—	2832	—	—	—	344	345	271	326	399	384
Butorphanol-TMS_2	—	2851	—	—	—	416	417	326	270	456	471
Cetobemidone	2045	—	—	—	—	70	71	190	247	119	57
Cetobemidone-AC	2095	—	—	—	—	70	71	232	289	190	247
Cetobemidone-HFB	1915	—	—	—	—	70	71	69	128	96	115
Cetobemidone-PFP	1865	—	—	—	—	70	57	128	336	393	129
Cetobemidone-TFA	1925	—	—	—	—	70	71	69	286	128	129
Cetobemidone-TMS	2070	—	—	—	—	70	71	262	319	191	304
M (nor-)-AC_2	2545	—	—	—	—	261	58	70	218	160	219
M (methoxy-)-AC	2265	—	—	—	—	70	71	188	319	220	262
Codeine (also metabolised to morphine, *O*-desmethylcodeine)	2375	2511	2681	2860	1.519	299	162	229	300	124	59
Codeine-AC	2503	2645	—	—	1.449	341	282	229	342	204	298
Codeine-HFB	2320	—	—	—	—	282	283	169	225	495	266
Codeine-PFP	2430	—	—	—	—	282	445	446	388	266	283
Codeine-TFA	2280	—	—	—	—	282	395	283	225	266	396
Codeine-TMS	2520	2592	—	—	—	371	178	196	234	343	229
M (nor-)	2388	2535	—	—	—	285	215	148	164	200	242

table continued

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
M (nor)-AC	2945	—	—	—	—	87	223	224	369	209	195
M (nor)-PFP_2	2440	—	—	—	—	563	355	209	400	327	387
M (nor)-TMS_2	—	2631	—	—	—	429	254	250	292	284	414
Cyclizine	2025	2104	—	—	0.514	194	98	165	167	207	208
M (nor-)	2120	2128	—	—	0.610	167	165	207	85	152	252
M (benzophenone) (BP)	1610	1673	—	—	—	105	77	51	182	106	183
M (carbinol)	1750	—	—	—	—	105	77	139	141	165	218
M (OH-BP) isomer 1	2065	—	—	—	—	198	121	77	199	192	151
M (OH-BP) isomer 2	2080	—	—	—	—	121	198	77	105	199	122
M (OH-methoxy-BP) isomer 1	2050	—	—	—	—	105	151	198	228	77	121
M (OH-methoxy-BP) isomer 2	2070	—	—	—	—	105	151	198	228	77	121
Dextromethorphan (see Methorphan)											
Dextromoramide	2940	3094	3625	—	—	100	128	265	56	165	266
M (OH)	3095	3310	—	—	—	100	128	281	165	194	322
M (OH)-AC	3210	—	—	—	—	100	128	194	323	325	365
M (methoxy-)	3269	—	—	—	—	100	128	194	323	422	423
Dextropropoxyphene	2188	2268	2173	2370	1.220	58	91	105	115	59	208
M (nor-)	2214	2487	—	—	1.248	44	220	100	205	129	307
M (nor-amide)	2526	2673	—	—	1.969	234	100	105	220	129	94
M (nor-*N*-proponyl-)	2400	2514	—	—	1.300	44	220	100	205	129	91
M	—	2520	—	—	1.250	205	220	91	126	115	160
M	—	2526	—	—	1.255	205	220	91	126	115	160
M	—	2624	—	—	—	220	91	147	105	135	115
M (nor-)-AC	2365	—	—	—	—	220	205	86	293	129	191
Art	1621	1659	—	—	—	115	208	91	193	130	117
Art	—	1756	—	—	—	115	91	208	193	130	117
Art	1890	1957	—	—	—	58	91	191	178	128	115
Art	—	1987	—	—	—	44	91	178	191	115	129
Art	—	2021	—	—	—	44	178	91	115	129	191
Dextrorphan	2230	2323	2230	—	—	257	59	150	256	200	157
Dextrorphan-AC	2280	—	—	—	—	59	150	299	198	231	256
Dextrorphan-PFP	2060	—	—	—	—	150	403	335	402	119	346
Dextrorphan-TFA	2015	—	—	—	—	150	285	353	352	128	296
Dextrorphan-TMS	2230	—	—	—	—	59	150	329	272	328	314
M (nor-)	2241	2328	—	—	—	243	157	136	198	200	242
M (nor)-AC_2	2710	—	—	—	—	87	72	198	211	327	285
M (OH)-AC_2	2555	—	—	—	—	357	231	356	355	298	315
Diamorphine (heroin; metabolised to MAM, morphine and codeine)	2615	2769	—	—	—	327	369	310	268	204	215
M (6-MAM)	2525	2646	—	—	—	327	268	215	328	285	310
M (3-MAM)	2495	2625	—	—	—	327	285	162	215	268	310
M (6-MAM)-PFP	2650	—	—	—	—	414	473	361	204	430	454
M (3-MAM)-PFP	2490	—	—	—	—	268	310	431	473	267	211
M (6-MAM)-HFB	2425	—	—	—	—	464	465	480	677	407	411
M (6-MAM)-TMS	2590	2688	—	—	—	399	340	287	204	282	266
M (3-MAM)-TMS	2570	2668	—	—	—	399	357	234	196	164	329
Diethylthiambutene	2008	—	—	—	—	276	111	219	42	277	97
Dihydrocodeine (metabolised to dihydromorphine, hydrocodone and hydromorphone)	2390	2511	2702	2840	1.493	301	164	59	300	301	115
Dihydrocodeine-AC	2445	—	—	—	—	343	300	284	344	226	328
Dihydrocodeine-HFB	2315	—	—	—	—	497	284	498	300	185	169

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
Dihydrocodeine-PFP	2360	—	—	—	—	447	448	284	300	392	432
Dihydrocodeine-TFA	2265	—	—	—	—	397	284	185	300	340	382
Dihydrocodeine-TMS	2480	2496	—	—	—	373	236	282	315	146	178
M (nor-)	—	2599	—	—	—	287	150	242	213	176	132
M (nor)-AC_2	2750	—	—	—	—	243	371	225	224	285	285
M (nor)-TMS_2	—	2559	—	—	—	431	316	226	294	416	340
Dihydromorphine	2400	2527	2504	—	—	287	70	164	286	230	288
Dihydromorphine-AC_2	2545	—	—	—	—	329	371	286	270	212	310
Dihydromorphine-PFP_2	2330	—	—	—	—	119	579	416	432	359	560
Dihydromorphine-TMS_2	2520	2518	—	—	—	431	236	146	416	373	326
M (nor)-AC_3	2790	—	—	—	—	357	399	229	211	272	315
Diphenoxylate	3514	3670	—	—	Not eluted	246	377	193	165	452	184
Dipipanone	2474	2586	2894	2710	1.309	112	113	91	165	334	223
Diprenorphine	—	3385	—	—	Not eluted	—	—	—	—	—	—
Ethoheptazine	1857	1923	1630	2110	—	—	—	—	—	—	—
Ethylmorphine (metabolised to morphine, see below)	2411	2530	—	—	—	—	—	—	—	—	—
Ethylmorphine-AC	2530	—	—	—	—	355	296	327	234	268	204
Ethylmorphine-PFP	2430	—	—	—	—	296	459	280	266	402	430
Ethylmorphine-TFA	2320	—	—	—	—	296	409	380	280	352	266
Ethylmorphine-TMS	2540	—	—	—	—	385	192	146	196	234	357
M (nor)-AC_2	2930	—	—	—	—	87	209	237	383	341	181
Etorphine	3033	3211	—	—	Not eluted	44	215	411	324	164	216
Etorphine-TMS		—	—	—	—	272	396	250	162	354	483
Fentanyl	2720	2833	—	—	—	146	245	189	105	202	158
M (nor-)	—	—	—	—	—	—	—	—	—	—	—
M (despropionyl)	—	—	—	—	—	146	189	44	118	132	280
Hydrocodone (also metabolised to hydromorphone, dihydromorphine and dihydrocodeine)	2440	2580	3028	2930	—	299	242	243	96	185	214
Hydrocodone-TMS	—	2674	—	—	—	297	386	371	329	298	387
M (nor)	—	2599	—	—	—	285	242	115	214	128	185
M (nor)-AC	2760	—	—	—	—	87	241	327	212	285	228
Hydromorphone (metabolised to dihydromorphine)	2445	2598	—	—	—	285	96	228	229	286	128
Hydromorphone-AC	2595	—	—	—	—	285	327	228	229	214	242
Hydromorphone-enol-AC_2	2625	—	—	—	—	327	284	162	228	369	270
Hydromorphone-HFB	2385	—	—	—	—	481	425	424	410	482	452
Hydromorphone-HFB_2	2325	—	—	—	—	481	425	424	410	482	452
Hydromorphone-PFP	2250	—	—	—	—	431	375	374	360	346	402
Hydromorphone-enol-PFP_2	2320	—	—	—	—	430	308	414	577	372	520
Hydromorphone-enol-TFA_2	2230	—	—	—	—	477	473	380	364	258	458
Hydromorphone-TMS	—	2621	—	—	—	357	300	243	342	314	286
Hydromorphone-enol-TMS_2	—	2595	—	—	—	429	414	234	184	357	324
Hydromorphone oxime TMS_2	—	2678	—	—	—	355	444	429	356	339	372
Ketamine	1840	1939	—	—	0.427	180	182	209	152	138	102
M (nor-)	1810	1907	—	—	0.423	166	168	195	131	138	223
M (nor-OH)-H_2O	1960	2058	—	—	—	166	221	168	193	131	138
M (nor-OH)-NH_3	1740	1840	—	—	—	187	222	117	159	131	224

table continued

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
M (nor-di-OH)-2H_2O	1920	2009	—	—	—	190	219	192	221	156	184
M (nor)-H_2O	—	1931	—	—	—	153	138	221	118	155	192
Ketobemidone	2040	—	—	—	—	70	71	42	44	57	190
Levallorphan (metabolised to nordextrorphan)	2355	2460	—	—	—	283	157	282	176	256	84
Levallorphan-AC	2390	—	—	—	—	85	325	298	176	157	257
Levomethadyl acetate (LAAM)	—	2267	—	—	—	72	43	73	91	255	165
M (nor-)	—	2262	—	—	—	58	101	100	208	281	165
M (di-nor-)	—	2255	—	—	—	44	120	193	165	178	208
Levopropoxyphene (see Dextropropoxyphene)											
Levorphanol (see Dextrorphan)											
Lofexidine	1910	—	—	—	—	243	67	95	223	245	97
Lofexidine-AC	2200	—	—	—	—	86	139	257	265	223	243
Meptazinol	1920	1980	—	—	0.429	58	84	98	71	85	233
M (nor-)	1995	2069	—	—	0.428	70	84	219	107	159	91
M (oxo-)	2410	2600	—	—	—	148	147	247	204	176	133
Methadone	2145	2228	2470	2370	0.606	72	73	294	57	223	91
Methadone-TMS	2260	—	—	—	—	72	73	296	85	165	178
M (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP)	2040	2120	—	—	0.520	277	276	262	220	165	115
M (dinor-;2-ethylidene-1-methyl-3,3-diphenylpyrrolidine, EMDP)	2021	2069	—	—	—	262	263	248	221	186	165
M (nor-)	2095	—	—	—	—	58	72	224	165	115	178
M (methadol)	2185	—	—	—	—	72	91	165	105	253	193
M (normethadol)	—	—	—	—	—	58	91	115	165	178	193
Methorphan (dextromethorphan/racemethorphan/levomethorphan; also metabolised to dextrorphan see above)	2138	2237	—	—	—	59	150	271	270	214	171
M (nor-methorphan/nor-racemethorphan)	2193	2244	—	—	—	257	212	171	136	213	214
M (OH-)	—	2420	—	—	—	287	59	230	150	187	228
M (OH)-AC_2	2555	—	—	—	—	357	231	356	355	298	315
Methylfentanyl (3- or α-) (china white)	2775	—	—	—	—	259	260	203	146	91	110
M (despropionyl-)	—	—	—	—	—	58	203	146	110	91	118
Morphine	2445	2564	2542	—	Not eluted	285	162	215	284	124	268
Morphine-AC_2 (diamorphine)	2615	2769	—	—	—	327	369	268	310	195	162
Morphine-PFP_2	2360	—	—	—	—	414	577	415	578	558	430
Morphine-TMS_2	2560	2602	—	—	—	429	236	357	414	401	196
Morphine-TFA_2	2250	—	—	—	—	364	477	478	365	380	458
M (nor-)	2459	—	—	—	—	271	150	201	148	162	81
M (nor)-AC_3	2955	—	—	—	—	87	209	210	355	397	181
M (nor)-PFP_2	2440	—	—	—	—	563	355	387	373	400	544
M (nor)-PFP_3	2405	—	—	—	—	355	709	367	382	533	546
M (nor)-TMS_3	2605	—	—	—	—	222	416	487	472	192	355
Nalbuphine	2960	Not eluted	—	—	—	302	303	357	284	272	254
Nalbuphene-AC_2	3110	—	—	—	—	386	387	441	344	296	326
Nalbuphene-AC_3	3080	—	—	—	—	428	429	368	483	326	440
Nalbuphene-AC	3030	—	—	—	—	344	345	399	326	302	—
Nalbuphene-TMS_3	—	3093	—	—	—	573	518	428	468	574	410
M (*N*-desalkyl-)	2930	—	—	—	—	289	272	271	202	115	242
M (*N*-desalkyl)-AC_2	2970	—	—	—	—	87	331	227	373	228	313
M (*N*-desalkyl)-AC_3	3020	—	—	—	—	87	373	227	228	296	415

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
Nalmefene	—	—	—	—	—	55	339	110	149	82	298
Nalorphine	2620	Not eluted	—	—	—	311	312	310	188	241	294
Nalorphine-AC_2	2820	—	—	—	—	353	395	226	294	354	396
Nalorphine-AC	2800	—	—	—	—	353	294	354	241	310	230
Nalorphine-TFA	2403	—	—	—	—	—	—	—	—	—	—
Nalorphine-TMS_2	—	2738	—	—	—	455	414	324	260	438	350
Naloxone	2715	Not eluted	—	—	—	327	328	242	96	286	229
Naloxone-AC	2840	—	—	—	—	327	369	328	286	244	310
Naloxone-AC_2	2750	—	—	—	—	369	411	285	310	326	352
Naloxone-enol-AC_2	2810	—	—	—	—	411	369	330	270	228	244
Naloxone-enol-AC_3	2770	—	—	—	—	327	369	328	411	286	453
Naloxone-enol-TMS_3	2645	2787	—	—	—	438	528	543	355	371	461
Naloxone-enol-TMS_2	2700	2843	—	—	—	471	456	366	390	229	398
Naloxone-TMS_2	2680	2881	—	—	—	399	471	456	314	358	384
Naloxone-oxime-TMS_3	—	2892	—	—	—	558	379	313	453	543	469
Naloxone-PFP	2530	—	—	—	—	473	388	432	375	348	446
Naloxone-enol-PFP_2	2360	—	—	—	—	619	472	456	428	620	592
Naloxone-enol-PFP_3	2270	—	—	—	—	765	602	618	738	519	454
Naloxone-PFP_2	2470	—	—	—	—	82	119	619	472	592	456
M (dihydro)-AC_2	2820	—	—	—	—	82	83	413	172	214	371
M (dihydro)-AC_3	2855	—	—	—	—	82	413	455	327	254	372
Naltrexol	—	3033	—	—	—	343	55	302	110	98	288
Naltrexone	2880	Not eluted	—	—	—	341	55	300	342	110	243
Naltrexone-AC	2980	—	—	—	—	341	383	342	243	300	286
Naltrexone-AC_2	2870	—	—	—	—	383	425	341	324	340	366
Naltrexone-enol-AC_2	3060	—	—	—	—	425	383	384	342	286	382
Naltrexone-enol-AC_3	2960	—	—	—	—	425	467	408	382	366	324
Naltrexone-TMS_2	—	3051	—	—	—	485	486	470	388	412	444
Naltrexone-TMS_3	—	2975	—	—	—	540	555	500	288	272	450
Naltrexone-enol-TMS_3	—	2945	—	—	—	557	542	355	242	484	452
Naltrexone-oxime-TMS_3	—	3071	—	—	—	572	573	475	327	557	499
M (methoxy-)	2920	—	—	—	—	371	286	274	330	356	316
M (methoxy)-AC	3150	—	—	—	—	413	274	372	371	328	358
M (methoxy)-AC_2	3130	—	—	—	—	455	412	396	456	413	273
M (methoxy)-enol-AC_2	3300	—	—	—	—	455	414	456	384	400	440
M (methoxy)-enol-AC_3	3180	—	—	—	—	497	454	498	396	440	412
M (dihydro)-AC_3	2990	—	—	—	—	413	427	469	384	370	426
Nefopam	2035	2106	—	—	0.586	58	179	180	225	178	165
M (nor-)	—	2116	—	—	—	—	—	—	—	—	—
M (nor)-AC	2080	—	—	—	—	208	87	194	179	165	281
M (*p*-OH-)	—	2266	—	—	—	58	195	165	178	210	241
M (OH)-AC isomer 1	2250	—	—	—	—	195	194	238	165	224	311
M (OH)-AC isomer 2	2285	—	—	—	—	178	195	208	179	268	311
M (nor di-OH-)	—	2649	—	—	—	—	—	—	—	—	—
M (nor di-OH)-AC_2 isomer 1	2610	—	—	—	—	87	266	337	295	224	252
M (nor di-OH)-AC_2 isomer 2	2640	—	—	—	—	87	337	195	295	209	178
Neopine	2395	2532	—	—	—	299	162	229	123	59	42
Normethadone	2095	—	—	—	—	58	72	59	224	178	165

table continued

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
Norpipanone	2488	—	—	—	—	98	111	99	112	165	178
Noscapine	3145	3358	—	—	—	220	221	205	147	42	118
Oxycodone (metabolised to oxymorphone)	2524	2671	—	—	—	315	230	258	70	201	316
Oxycodone-AC	2555	—	—	—	—	357	314	298	240	230	212
Oxycodone-enol-AC_2	2560	—	—	—	—	399	357	240	314	296	298
Oxycodone-TFA	2290	—	—	—	—	411	314	240	298	396	254
Oxycodone-TMS	—	2703	—	—	—	387	229	230	388	372	214
Oxycodone-enol-TMS_2	—	2602	—	—	—	459	460	444	312	297	242
Oxycodone-oxime-TMS_2	—	2740	—	—	—	474	229	214	459	295	385
M (nor-)	—	2703	—	—	—	313	187	314	115	214	229
M (nor)-enol-AC_3	2680	—	—	—	—	385	427	343	281	326	368
M (nor)-TMS_2	—	2763	—	—	—	373	445	288	258	226	240
M (nor)-enol-TMS_2	—	2621	—	—	—	445	446	312	430	354	288
M (nor)-enol-TMS_3	—	2746	—	—	—	517	518	502	312	342	428
M (dihydro-)	—	2666	—	—	—	317	230	115	242	260	216
M (dihydro)-AC_2	2570	—	—	—	—	359	401	242	230	224	282
M (nor-dihydro)-AC_3	2935	—	—	—	—	242	387	224	343	284	429
M (nor-dihydro)-AC_2	2900	—	—	—	—	343	258	201	239	242	387
Oxymorphone	2538	2723	—	—	—	301	216	44	42	70	302
Oxymorphone-TMS	—	2715	—	—	—	373	288	259	374	316	358
Oxymorphone-TMS_2	—	2728	—	—	—	445	446	430	287	331	372
Oxymorphone-TMS_3	—	2641	—	—	—	517	502	355	412	518	503
Oxymorphone-oxime-TMS_3	—	2748	—	—	—	532	533	517	287	443	459
M (nor-)	—	Not eluted	—	—	—	—	—	—	—	—	—
M (nor)-enol-TMS_2	—	2788	—	—	—	431	259	316	346	432	416
M (nor)-enol-TMS_3	—	2662	—	—	—	503	488	355	398	504	308
M (nor)-enol-TMS_4	—	2773	—	—	—	575	503	355	560	486	242
M (dihydro-)	—	2690	—	—	—	303	286	115	216	315	256
Papaverine	2825	2973	—	—	—	338	324	339	340	308	325
M (*O*-desmethyl-)	2805	—	—	—	—	324	310	325	294	266	309
Pentazocine	2280	2356	2225	3030	0.870	70	217	110	69	285	202
Pentazocine-AC	2330	—	—	—	—	259	110	327	312	244	217
Pentazocine-PFP	2120	—	—	—	—	363	348	110	416	431	430
Pentazocine-TFA	2075	—	—	—	—	69	313	110	298	366	381
Pentazocine-TMS	2320	—	—	—	—	289	244	245	274	342	357
M (desalkyl-)	—	2019	—	—	—	—	—	—	—	—	—
M (desalkyl)-AC_2	2380	—	—	—	—	87	88	72	73	301	172
M (OH-)	2545	2649	—	—	—	217	218	202	268	301	110
Pethidine (meperidine)	1754	2025	1995	1809	0.319	71	70	172	247	246	218
M (nor-)	1885	1842	—	—	0.357	57	233	158	103	131	117
M (nor)-AC	2240	2256	—	—	—	187	57	275	158	232	202
M (OH-)	2045	2145	—	—	—	71	140	263	262	189	234
M (OH)-AC	2205	—	—	—	—	71	305	188	230	261	276
M (nor-OH)-AC_2	2600	—	—	—	—	203	56	245	333	218	290
Phenazocine	2686	2833	—	—	—	230	231	58	105	158	173
Phenoperidine	2872	2983	—	—	—	246	247	367	91	158	172
Pholcodine	3070	3348	—	—	—	114	100	42	56	398	115
Pholcodine-AC	3260	—	—	—	—	114	100	56	70	115	440
Pholcodine-PFP	2980	—	—	—	—	114	100	277	354	380	544
Pholcodine-TFA	2800	—	—	—	—	114	100	277	354	380	494

Table 40.20 continued

Compound	*System*					*Principal ions* (m/z)					
	GA	*GB*	*GC*	*GF*	*GM*						
Pholcodine-TMS	3140	3410	—	—	—	114	100	115	470	196	356
M (nor)-PFP_2	3010	—	—	—	—	100	114	380	70	513	676
M (nor)-TMS_2	3260	—	—	—	—	114	100	73	468	528	456
M (nor)-PFP	3270	—	—	—	—	100	114	56	530	502	—
M (nor)-AC	3620	—	—	—	—	100	114	70	426	340	181
M (nor)-AC_2	3650	—	—	—	—	100	114	56	468	382	70
Piminodine	2884	—	—	—	—	246	366	106	234	247	260
Pipazethate (pipazetate)	2037	—	—	—	—	98	111	99	199	288	200
M (alcohol)	1830	—	—	—	—	98	112	99	156	103	84
M (ring sulfone)	2720	—	—	—	—	232	200	168	184	156	140
M (OH-ring)	2800	—	—	—	—	—	—	—	—	—	—
Piritramide	3560	—	—	—	—	386	138	387	84	110	42
Profadol	1748	—	—	—	—	—	—	—	—	—	—
Propoxyphene (see Dextropropoxyphene)											
Racemorphan (see Methorphan)											
Thebacon (metabolised to dihydrocodeine)	2533	2559	—	—	—	341	298	299	242	284	162
Thebacon-TMS	2475	—	—	—	—	371	234	356	184	370	313
Thebaine	2517	2672	—	—	—	311	296	312	297	242	139
Tilidine (tilidate)	1838	—	—	—	—	97	82	103	77	132	176
M (nor-)	1827	—	—	—	—	83	68	259	184	157	214
M (nor)-AC	2165	—	—	—	—	125	83	111	155	170	258
M (bis-nor-)	1830 —	—	—	—	—	69	83	119	135	170	245
M (bis-nor)-AC	2100	—	—	—	—	69	111	155	170	244	287
Tramadol	1943	2021	—	—	—	58	263	135	77	264	92
M (nor-)	—	2049	—	—	—	44	188	249	135	150	159
M (nor)-AC	2295	—	—	—	—	86	200	58	273	172	184
M (nor-) carbamate Art	—	2065	—	—	—	189	121	135	202	261	188
M (*O*-desmethyl-)	1995	2093	—	—	—	58	249	121	93	107	131
M (*O*-desmethyl)-AC	1998	—	—	—	—	58	121	248	163	291	128
M (*N*,*O*-didesmethyl-)	—	2122	—	—	—	44	174	235	121	145	159
M (*N*,*O*-didesmethyl)-AC_2	2464	—	—	—	—	86	186	301	228	107	113
M (didesmethyl-) carbamate Art	2148	—	—	—	—	73	173	174	188	145	247
M (OH-)	2200	2252	—	—	—	58	279	135	77	234	261
Trimeperidine	1808	1895	—	—	—	186	201	187	70	105	91

derivatives will form in non-reproducible proportions from the automerisation of the enol and keto forms. However, these compounds can be stabilised in their keto forms by incubating with hydroxylamine or methoxyamine–pyridine, and then yield only a single derivatised oxime product (Broussard *et al.* 1997; Meatherall 1999).

Systems GA, GB, GC or GF, described previously may be used. For plasma, system GM, described previously, is a rapid isothermal packed-column method with good sensitivity on NPD. Table 40.20 gives retention indices, or relative retention times to iprindole for GF.

Non-amfetamine stimulants and hallucinogens

Non-amfetamine stimulants and hallucinogens have a variety of clinical and toxic actions. Extraction of cocaine is straightforward under basic conditions, and most metabolites, except benzoylecgonine, can be detected in the clinical setting without derivatisation. For regulated testing, quantification of benzoylecgonine is required, and most laboratories use TMS as the derivatising reagent. As a result of the recent interest in other metabolites that may have clinical importance, data for these and their TMS derivatives are also included in Table 40.21.

For analysis of cannabis metabolites, hydrolysis of conjugates with 10 mol/L potassium hydroxide is usually performed on urine prior to weakly acidic extraction (pH 6.5); TMS is the derivative of choice. Phencyclidine (PCP) analysis is complicated by the low concentration present, although extraction is straightforward and derivatisation is required only for metabolite measurement (Nakahara *et al.* 1997). Chromatographic confirmation of lysergide (LSD) is hampered by the low concentrations and acidic nature of the metabolites, which necessitates both derivatisation (TMS) and tandem MS (Nelson, Foltz 1992). Systems GA or GB may be used. Table 40.21 gives the retention indices (reference compounds are the alkanes with an even number of carbon atoms).

Table 40.21 GC retention data and mass spectral data for non-amfetamine stimulants and hallucinogens (AC, acetyl; PFP, pentafluoropropionate; TFA, trifluoroacetyl; TMS, trimethylsilyl)

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Amiphenazole	2170	—	191	121	77	104	122	43
Bemegride	1367	—	55	83	82	113	70	69
Bufotenine	2057	—	58	204	146	59	160	42
Caffeine	1800	1904	94	109	55	67	82	195
M (1-nor-, theobromine)	1807	1920	180	55	67	109	82	137
M (7-nor-, theophylline)	1925	1990	180	95	68	53	181	96
Cannabidiol	2390	2480	231	232	245	174	314	187
Cannabidiol-TMS_2	2330	2510	390	337	301	351	319	324
Cannabigerol	2500	—	193	123	231	316	247	136
Cannabigerol-TMS_2	2440	2520	—	—	—	—	—	—
Cannabinol	2535	2644	295	296	310	238	251	223
Cannabinol-TMS	2485	2600	367	368	382	310	295	238
Δ^9-Tetrahydrocannabinol (Δ^9-THC)	2473	2578	299	314	231	271	243	258
Δ^9-THC-TMS	2405	2499	386	371	315	303	343	330
M (8α,11-di-OH-Δ^9-THC)-TMS	2710	—	—	—	—	—	—	—
M (8α,OH-Δ^9-THC)	2775	2975	271	295	297	311	312	214
M (8α,OH-Δ^9-THC)-TMS	2580	—	—	—	—	—	—	—
M (11-OH-Δ^9-THC)	2775	2975	299	300	330	217	231	193
M (11-OH-Δ^9-THC)-TMS_2	2630	2762	371	372	373	474	459	403
M (11-nor-Δ^9-THC-9-carboxylic acid)	—	—	325	268	326	340	281	253
M (11-nor-Δ^9-THC-9-carboxylic acid)-TMS_2	2660	2820	371	473	488	372	398	417
Cinnamoylcocaine isomer 1	2345	2489	82	182	96	131	238	329
Cinnamoylcocaine isomer 2	2450	2625	82	182	96	131	238	329
Cocaine	2187	2289	82	182	94	77	83	303
M (ecgonine)	—	0000	82	96	83	97	124	185
M (ecgonine)-TMS_2	1680	—	82	83	96	97	314	329
M (anhydroecgonine methyl ester)-methylecgonidine	1280	1430	152	181	82	122	166	138
M (anhydroecgonine methyl ester)-TMS	1345	1472	210	239	224	211	122	183
M (norecgonine)	—	1472	82	156	126	96	171	116
M (methylecgonine)	1472	1530	82	96	83	199	168	182
M (methylecgonine)-TMS	1580	1585	82	96	212	271	182	240
M (ethylecgonine)	—	1602	82	96	83	97	168	213
M (ethylecgonine)-TMS	1485	1651	82	96	83	240	285	196
M (nor-)	2162	2259	168	136	105	77	68	289
M (nor-)-TMS	—	2378	105	240	140	152	179	346
M (benzoylecgonine)	2570	2663	124	168	82	105	94	289
M (benzoylecgonine)-TMS	2285	2365	82	240	105	122	256	361
M (benzoylnorecgonine)-TMS_2	—	2400	404	140	298	—	—	—
M (*m*-OH-benzoylecgonine)-TMS_2	2505	2600	82	240	193	210	256	449
M (*p*-OH-benzoylecgonine)-TMS_2	—	2650	82	240	193	210	256	449
M (*m*-OH-)	2460	2608	82	182	94	121	198	319
M (*m*-OH-)-TMS	—	2550	182	82	94	193	391	198
M (*p*-OH-)	—	2650	82	182	94	121	198	319
M (*p*-OH-)-TMS	—	2610	182	82	94	193	391	198
M (OH-methoxy-)	2670	2729	82	182	151	349	18	168
M (OH-methoxy-)-TMS	2850	—	82	182	83	421	198	223
M (OH-dimethoxy-)-TMS	2970	—	82	182	83	94	451	240
M (cocaethylene)	2250	2345	196	82	317	272	94	105
M (norcocaethylene)	2115	2317	182	68	136	105	108	303
M (norcocaethylene)-TMS	—	2385	254	140	360	—	—	—

Table 40.21 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
M (OH-cocaethylene)	—	2709	82	196	94	121	333	288
M (OH-methoxy-cocaethylene)	—	2779	82	196	151	212	318	363
M (methoxy-cocaethylene)	—	2663	82	196	121	94	267	333
Cyclopentamine	1230	—	58	126	59	69	56	44
Cyclopentamine-AC	1680	—	58	100	168	126	104	183
Ethamivan	1900	—	151	72	223	123	222	152
Ethamivan-AC	1970	—	151	222	223	194	195	265
Harmaline (makes harmine on heating)	2430	—	213	214	170	198	169	115
Harmaline-TFA	2525	—	241	310	121	169	184	198
Harmaline-PFP	2540	—	241	360	242	121	198	184
Harmaline-AC	2670	—	213	256	214	170	186	115
Harmaline-AC_2	2800	—	255	298	256	241	212	141
Harmine	2291	2322	212	169	197	213	106	211
Harmine-AC	2545	—	212	254	197	169	213	140
M (*O*-desmethyl-)	2550	—	198	197	170	99	75	199
M (*O*-desmethyl)-AC	2600	—	198	240	169	197	199	115
Isometheptene	1052	—	58	55	128	44	59	56
Isoprenaline	1730	—	72	44	124	123	193	70
Isoprenaline-AC_4	2460	—	84	193	235	277	319	365
Lobeline	1820	—	96	105	77	97	216	218
Lysergamide	—	—	267	221	207	180	223	154
Lysergic acid	—	—	268	224	154	180	207	223
Lysergide (lysergic acid diethylamide, LSD)	3445	3332	323	221	207	181	196	280
LSD-TMS	3595	—	395	253	293	268	279	337
M (nor-)	—	—	207	309	182	280	128	100
M (nor-)-TMS_1	3705	—	381	279	254	100	265	205
M (nor-)-TMS_2	3515	—	453	253	351	279	326	337
M (2-oxo-)	—	—	—	—	—	—	—	—
M (2-oxo-3-OH-)-TMS_2	3430	—	309	499	235	325	397	409
Iso-LSD-TMS	3515	—	395	293	279	253	268	337
Mazindol	2325	2504	266	268	267	255	231	102
Mazindol-AC	2705	—	256	255	254	326	220	284
Meclofenoxate	1770	—	58	111	71	75	141	113
M (chlorophenoxyacetic acid)	1770	—	186	141	111	128	113	99
M (*N*-acetyl)	2160	—	194	179	181	253	151	148
Methoxamine	1726	1596	168	137	44	139	152	124
Myristic acid	1755	Not eluted	73	60	57	129	185	228
Myristic acid-Me	1715	—	74	87	143	129	199	242
Myristic acid-TMS	2280	—	73	117	285	149	101	300
Myristicin	1400	—	192	91	119	165	161	147
Naphazoline	2100	—	209	210	141	115	153	208
Nicotine	1350	1380	84	133	42	162	161	105
M (cotinine)	1715	1645	98	176	118	119	58	147
Nikethamide	1525	1569	106	78	177	51	178	107
M (*N*-ethylnicotinamide)	1605	—	106	150	78	149	51	135
M (nicotinamide)	1341	1418	122	78	106	51	50	52
Oxymetazoline	2170	2254	245	260	44	217	218	246
Oxymetazoline-AC_2	2760	—	302	344	287	320	203	245
Pemoline	1969	2081	107	176	90	77	70	105
Pemoline-Me_2	1590	—	118	204	90	105	77	70

table continued

Table 40.21 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
M (mandelic acid)			107	79	77	51	152	125
M (5-phenyloxazolidine-2,4-dione)			90	177	105	77	106	51
Pentetrazole	1550	1579	107	176	90	77	70	105
Phenbutrazate (fenbutrazate)	2675	—	69	56	71	91	261	84
Phencyclidine (PCP)	1900	1981	200	242	243	91	84	186
M (4-phenyl-4 piperidinocyclohexanol, PPC)	—	—	216	258	259	91	202	182
M (PPC)-TMS	—	—	200	254	331	—	—	—
M (1(1-phenylcyclohexyl)-4-OH-piperidine)-TMS	—	—	172	288	331	—	—	—
M (PCA) 5[*N*-(1′-phenylcyclohexyl)-amino]-pentanoic acid	—	—	—	—	—	—	—	—
Pipradrol	2145	2242	84	56	85	77	105	55
Pipradrol-AC	2478	—	249	248	165	229	291	206
Psilocin	1985	2080	58	204	150	146	205	155
Psilocin-AC	2270	—	58	246	146	130	202	117
Psilocin-AC_2	2340	—	58	288	80	202	122	246
Psilocybin	2046	—	—	—	—	—	—	—
Theobromine (see Caffeine)								
Theophylline (see Caffeine)								

Oral hypoglycaemics

Oral hypoglycaemics constitute three chemical classes: biguanides (metformin), sulfonylureas (chlorpropamide) and thiazolidenediones (rosiglitazone). The latter two classes contain sulfur, and the sulfonylureas also share structural similarities with the thiazide diuretics (see above) and the sulfonamide antibiotics (see later). Sulfonylureas and their metabolites are not amenable to GC because of their acidity and polarity. Extractive methylation has been employed, but the use of standard conditions leads to the formation of unstable compounds, and in addition there may be thermal decomposition during chromatography. Thus, the formation of common fragments (superscripts a to f in Table 40.22; HH Maurer, personal communication, 2003) complicates

Table 40.22 GC retention data and mass spectral data for oral hypoglycaemics (Me, methyl)

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Acetohexamide	1859	Not eluted	210	56	43	184	211	75
Acetohexamide-Me	2250	—	183	198	98	119	115	91
Buformin-nitrobenzoyltriazine	—	3200	—	—	—	—	—	—
Carbutamide-Me	2300	—	109	156	92	285	—	—
Chlorpropamide	1791	1887	111	175	75	85	276	127
Chlorpropamide-Me	2165	—	58	115	111	175	290	127
Chlorpropamide-Me_2	2250	—	109	156	92	304	—	—
Art 1-Me	1825	—	111	175	205	75	113	141
Art 1-Me_2	1690	—	111	219	75	175	113	221
Art (chlorosulfonamide)-Me	1740	—	111	75	141	175	205	—
Art (chlorosulfonamide)-Me_2	1655	—	111	75	219	175	155	—
Art (chloroamide)-Me	2135	—	111	75	125	248	175	—
Art (chloroamide)-Me_2	2150	—	87	111	125	175	262	—
Glibenclamide-Me	3800	—	169	82	97	171	198	381
Glibenclamide-Me_2	3840	—	169	289	171	291	353	126
Art 3-Me	3445	—	169	198	126	287	382	—
Art 3-Me_2	3355	—	169	289	198	353	396	—
Glibornuride Art[(d)] (methylsulfonamide)	1730	1660	91	65	171	155	107	—
Art[(e)] (methylsulfonamide)-Me	1740	—	91	65	185	155	121	108
Art[(f)] (amide)	1620	1695	91	155	197	65	106	—

Table 40.22 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Art 5	1845	1995	95	109	134	164	195	—
Art 5-Me	1715	—	95	209	109	139	150	—
M (OH)-Art	2305	—	95	109	125	181	211	—
M (OH)-Art (sulfonamide)-Me[(d)]	2265	—	107	89	201	172	141	—
M (OH)-Art (sulfonamide)-Me_2[(e)]	2030	—	107	89	215	171	151	—
M (COOH)-Art(sulfonamide)-Me_3[(f)]	1955	—	135	243	103	199	212	—
Gliclazide Art 1-Me	1545	—	110	181	125	184	151	—
Art[(c)] (amide)	1620	—	91	155	197	65	106	—
Art 3	1670	—	81	110	125	67	169	—
Art[(a)] (methylsulfonamide)	1730	1660	91	65	171	155	107	—
Art[(b)] (methylsulfonamide)-Me	1740	—	91	65	185	155	121	—
M (OH)-Art (sulfonamide)-Me[(d)]	2265	—	107	89	201	172	141	—
M (OH)-Art (sulfonamide)-Me_2[(e)]	2030	—	107	89	215	171	151	—
M (COOH)-Art (sulfonamide)-Me_3[(f)]	1955	—	135	243	103	199	212	—
Glipizide-Me	3420	—	150	111	93	459	98	—
Glipizide-Me_2	3455	—	150	121	93	334	392	197
Art 2-Me	3020	—	150	121	93	197	334	—
Art 2-Me_2	3005	—	150	241	121	93	348	—
Gliquidone	2024	Not eluted	—	—	—	—	—	—
Gliquidone-Me	3850	—	323	220	204	175	176	430
Art 4-Me	3460	—	204	219	176	321	416	—
Art 4-Me_2	3415	—	204	219	176	321	416	—
Glymidine	1632	2750	244	59	77	43	245	168
Glymidine-Me	—	—	—	—	—	—	—	—
Metformin-nitrobenzoyltriazine	—	3050	—	—	—	—	—	—
Tolazamide	1651	1720	91	155	114	65	197	42
Tolazamide-Me	2630	—	113	155	170	241	325	—
Tolazamide-Me_2	2540	—	91	155	339	184	229	114
Art 1-Me	1315	—	98	113	59	68	85	172
Art (methylsulfonamide)	1730	1660	91	65	171	155	107	—
Art (methylsulfonamide)-Me	1740	—	91	65	185	155	121	—
M (OH)-Art (sulfonamide)-Me[(d)]	2265	—	107	89	201	172	141	—
M (OH)-Art (sulfonamide)-Me_2[(e)]	2030	—	107	89	215	171	151	—
M (COOH)-Art(sulfonamide)-Me_3[(f)]	1955	—	135	243	103	199	212	—
Tolbutamide	1683	—	91	30	155	108	65	197
Art[(c)] (amide)	1620	—	91	155	197	65	106	—
Tolbutamide-Me	2320	—	91	129	155	284	269	87
Tolbutamide-Me_2	2170	—	91	155	113	121	220	184
Art (methylsulfonamide)	1730	1660	91	65	171	155	107	—
Art (methylsulfonamide)-Me	1740	—	91	65	185	155	121	—
M (OH)-Me	2645	—	129	171	300	285	200	—
M (OH)-Me_2	2740	—	134	215	107	197	314	—
M (COOH)-Me_3	2590	—	129	135	199	297	328	—
M (OH)-Art (sulfonamide)-Me[(b)]	2265	—	107	89	201	172	141	—
M (OH)-Art (sulfonamide)-Me_2[(a)]	2030	—	107	89	215	171	151	—
M (COOH)-Art(sulfonamide)-Me_3[(c)]	1955	—	135	243	103	199	212	—

[(a)–(f)]Hydrolysis artefacts common to several sulfonylureas.

identification of the parent compound and limits the application as a screening tool. For this purpose, HPLC may be more applicable (Maurer *et al.* 2002). However, stable *N*-methyl (sulfonamide nitrogen) derivatives can be formed by maintaining the extraction pH below 7, and by using TBAH as the ion-pairing reagent. With the alkylation pH above 10, and using TPAH as the counter-ion, there is almost complete hydrolysis to sulfonamide and amide artefacts (Hartvig *et al.* 1980). Thermal stability can also be achieved by the judicious choice of pairs of derivatising reagents: methyl iodide plus trifluoroacetic anhydride (TFAA) for parent compounds, and methyliodide plus heptafluorobutyric anhydride (HFBA) for the hydroxy and carboxy metabolites (Braselton *et al.* 1977). This strategy offers the additional advantage of an improved ECD response. The biguanides also present analytical difficulties, although successful chromatography is achieved by forming a triazine derivative by them reacting with *p*-nitrobenzoyl chloride, for which the retention data are given in Table 40.22 (Brohon, Nöel 1978; Paroni *et al.* 2000). Screening methods are not yet developed for the newer classes of drugs such as the alpha-glucosidase inhibitors, glinides, glitazones and gliptins, although LC-MS methods have been published for some of the individual compounds. Systems GA and GB may be used, and the reference compounds are *n*-alkanes with an even number of carbon atoms.

Pesticides

A comprehensive method for screening pesticides using systems GA and GK can be found in Chapter 16, Table 16.1. Systems GKA, GKB, GKC and GKD cater for a smaller set of compounds and these can be found in the Indexes of Analytical Data.

System GKA

(Osselton, Snelling 1986).

- *Column*: Chromosorb W HP 3% SE-30 on 80–100 mesh silanised glass (2 m × 4 mm i.d.).
- *Carrier gas*: O_2-free N_2, 50 mL/min.
- *Detector*: Flame ionisation and nitrogen–phosphorus.
- *Reference compound:* Straight-chain hydrocarbons

System GKB

(Osselton, Snelling 1986).

- *Column*: Chromosorb W HP 3% OV-7 on 80–100 mesh silanised glass (2 m × 4 mm i.d.).
- *Carrier gas*: O_2-free N_2, 50 mL/min.
- *Detector*: Flame ionisation and nitrogen–phosphorus.
- *Reference compound*: Straight-chain hydrocarbons.

System GKC

(Osselton, Snelling 1986).

- *Column*: Chromosorb W HP 3% OV-17 on 80–100 mesh silanised glass (2 m × 4 mm i.d.).
- *Carrier gas*: O_2-free N_2, 50 mL/min.
- *Detector*: Flame ionisation and nitrogen–phosphorus.
- *Reference compound*: Straight-chain hydrocarbons.

System GKD

(Junting, Chuichang 1991).

- *Column*: Fused silica HP-1, methyl silica gum (5 m × 0.53 mm i.d., 2.65 μm).
- *Temperature programme*: 190°C to 235°C at 10°/min.
- *Carrier gas*: N_2, 20 mL/min flow rate.
- *Detector*: Flame ionisation.

Phenothiazines and other tranquillisers

Phenothiazines and other tranquillisers can be extracted readily under mildly basic conditions (pH 10) into solvents such as ethyl acetate, hexane, butyl chloride and diethyl ether. An acidified (0.05 mol/L H_2SO_4) back extraction is a useful clean-up procedure where sensitivity is important. Chromatography of the primary and secondary amines is poor on packed columns, but is adequate on well-maintained capillary columns, particularly those of low-to-medium polarity, such as PSX-5 (see Table 40.3). Meprobamate is unstable in basic solution, and benefits from the use of mildly acidic (pH 5) extraction conditions. Some authors prefer to chromatograph the secondary amines and hydroxylated metabolites as acetylated derivatives, prepared by heating the dried residue with acetic anhydride and pyridine (3 : 2, v/v) (Maurer, Bickeboeller-Friedrich 2000). Others employ an enzymatic hydrolysis procedure to improve the recovery of both parent drug and metabolites, although the additional sensitivity gained is often negated by the increased analytical time in the emergency setting. Acid hydrolysis is quicker, but some relevant compounds are destroyed under these conditions. System GA or GB may be used (Table 40.23), and the reference compounds are *n*-alkanes with an even number of carbon atoms.

Laxatives

Table 40.24 lists the stimulant laxatives. Other types of laxatives, such as bulkers (bran), osmotic (PEG and lactulose), stool softeners and saline

Table 40.23 GC retention data and mass spectral data for phenothiazines and other tranquillisers

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Acepromazine	2735	2844	100	72	240	340	44	197
M (dihydro)-H_2O	2720	2824	58	86	310	225	224	251
M (7-OH-)	—	3160	58	86	342	326	296	257
Aceprometazine	2650	—	72	73	255	326	56	240
M (methoxy-dihydro)-H_2O	2920	—	72	56	84	238	270	340
Acetophenazine	—	Not eluted	254	143	42	70	411	113
Alimemazine (trimeprazine)	2305	2402	58	298	212	198	100	299
M (nor-)	2335	2432	199	284	212	198	180	252
M (sulfoxide)	2665	2805	58	212	199	180	298	297
M (norsulfoxide)	—	2817	—	—	—	—	—	—
M (OH-)	2650	2829	58	314	100	255	196	281
M (nor-OH-)	—	2845	—	—	—	—	—	—
Alpidem	—	3313	—	—	—	—	—	—
Amisulpride	3260	—	98	99	44	242	70	111
M (*O*-desmethyl-)	2960	—	98	135	182	228	99	107
Apronal	1331	—	55	44	142	141	61	81

Table 40.23 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Aripiprazole M	—	2108	174	176	218	220	75	44
M	—	2258	213	215	242	244	98	172
Azacyclonol	2243	2361	85	84	183	105	56	77
Azaperone	2705	—	107	165	123	95	121	—
Benactyzine	2255	—	86	105	77	87	182	99
Benperidol	3433	3667	230	109	82	187	243	363
M	1490	—	56	125	123	180	136	95
M (*N*-desalkyl-)	2415	—	134	79	51	106	217	161
Benzoctamine	2078	2172	218	44	191	221	219	178
Bromisoval	1540	—	55	70	163	165	83	222
M (Br-isovaleric acid)	1190	—	136	140	101	59	120	122
M (OH-isovaleric acid)	1140	—	76	73	55	58	57	74
M (iso-valeric acid carbide)	1850	—	102	59	85	57	70	61
Bromperidol	3037	—	42	268	270	281	283	123
Art (-H_2O)	3020	—	236	238	252	250	253	265
M	1890	—	233	235	56	94	127	154
M (*N*-desalkyl-oxo)-2H_2O	1850	—	233	235	127	154	63	101
Buspirone	3300	3468	177	277	265	122	148	108
M (1-pyrimidinyl piperazine)	—	1558	122	108	96	80	164	134
Butaperazine	3190	—	113	70	409	141	283	127
Captodiame	2774	—	58	165	255	359	166	73
Carbromal	1513	—	44	69	208	210	55	71
M (OH-carbromide)	1340	—	150	152	165	167	183	194
M (carbromide)	1215	—	69	165	167	114	71	150
M (desbromo-)	1380	—	87	113	71	130	86	115
Art	1450	—	69	58	70	105	179	97
Art	1470	—	69	70	140	151	193	191
Carphenazine	3590	—	268	143	245	70	269	394
Chlormezanone	2199	2346	98	152	154	42	69	174
Art	1235	1245	152	153	154	89	111	59
M (4-chlorobenzoic acid)	1400	—	139	156	111	75	141	113
M (*N*-methyl-4-chlorobenzamide)	1555	1596	139	111	75	169	141	168
Chlorpromazine	2495	2618	58	86	318	85	320	272
M (nor-)	2480	2656	44	232	233	196	214	304
M (didesmethyl-)	2480	2646	232	290	233	246	272	214
M (sulfoxide)	2809	3003	58	246	214	232	272	318
M (norsulfoxide)	2900	3046	44	246	232	302	214	196
M (7-OH-)	—	2939	58	86	334	248	288	262
M (*N*-oxide)	2100	2355	233	198	201	154	166	171
Chlorprothixene	2492	2608	58	59	221	42	222	255
M (OH-dihydro-) isomer 1	2750	—	58	333	335	247	334	215
M (OH-dihydro-) isomer 2	2790	—	58	333	247	335	249	334
M (OH-methoxy-dihydro-)	2810	—	58	363	277	173	262	249
M (*N*-oxide)-$(CH_3)_2$NOH	2410	—	234	235	270	269	202	255
M (*N*-oxide sulfoxide)- $(CH_3)_2$NOH	2560	—	203	234	202	251	286	269
M (sulfoxide)	2720	—	58	221	189	255	176	331
Art (dihydro-)	2490	—	58	317	231	73	152	195
Art (Cl-thioxanthenone)	2260	—	246	218	248	139	220	183
Clomethiazole (chlormethiazole)	—	1269	112	161	85	45	163	113
Clopenthixol (see Zuclopenthixol)								

table continued

Table 40.23 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Cloral betaine (see Chapter 14)								
Cloral hydrate (see Chapter 14)								
Clothiapine (clotiapine)	2712	2833	83	70	273	244	209	71
M (nor-)	—	2882	—	—	—	—	—	—
M (oxo-)	3030	—	357	209	244	285	273	291
Clozapine	2895	3024	—	—	—	—	—	—
M (nor-)	3105	3092	192	243	256	56	227	312
M (nor-acetyl-)	3490	3609	396	310	298	192	227	256
M	—	2833	58	300	256	243	299	160
M	—	2972	243	286	44	256	244	270
M	—	3150	225	238	294	209	250	264
M	—	3264	255	268	192	239	338	280
M	—	3320	255	192	268	324	239	280
M	—	3527	340	192	339	228	243	256
Dichloralphenazone	1855	—	188	47	82	96	77	84
M (phenazone)	1835	1951	188	96	77	56	105	189
M (4-OH-phenazone; metabolised to chloral hydrate)	1855	—	85	56	84	204	77	120
Diethazine	2377	—	86	298	87	58	299	212
Dimetotiazine	3060	3096	72	73	320	56	210	198
M (nor-)	3150	—	72	58	320	306	198	210
Dixyrazine	3220	—	212	42	187	70	180	56
M (phenothiazine)	2120	2130	199	167	198	166	154	139
Droperidol	3430	Not eluted	246	165	42	123	199	247
M (benzimidazolone)	1950	—	134	79	106	121	105	67
Emylcamate	1105	—	73	43	84	55	69	44
Ethchlorvynol	1015	1060	115	117	89	53	109	51
Ethinamate	1365	—	91	81	106	78	95	68
Ethomoxane	1975	—	86	44	265	180	87	—
Fluanisone	2785	—	205	218	123	356	219	162
M (*O*-desmethyl-)	2715	—	194	165	123	342	338	134
Fluopromazine (see Triflupromazine)								
Flupentixol (flupenthixol) *cis* isomer	3058	3199	143	70	100	144	98	58
Flupentixol *trans* isomer	—	3217	143	70	100	144	98	58
M (ring)	2190	—	267	235	247	222	198	216
M (*N*-oxide)	2120	—	304	303	234	235	289	283
M (desalkyl-) *cis*	—	2832	—	—	—	—	—	—
M (desalkyl-) *trans*	—	2855	—	—	—	—	—	—
Fluphenazine	3050	3194	280	143	70	437	406	113
M (ring)	2190	—	280	70	56	143	113	248
M (7-OH-)	—	3572	—	—	—	—	—	—
M (sulfoxide)	—	3752	—	—	—	—	—	—
Fluspirilene	1017	—	244	42	72	475	109	245
M (*N*-desalkyl-oxo-)	2405	—	57	56	245	68	228	206
M (desamino-OH-)	2120	—	203	201	262	183	216	244
M (desamino-carboxy-)	2230	—	203	201	183	216	276	167
Glutethimide	1830	1910	189	132	117	160	91	115
M (OH-ethyl-)	1865	1958	146	104	233	103	133	117
M (OH-phenyl-)	1875	2040	133	204	233	176	77	205
M (2-phenylglutarimide)	2235	—	104	189	103	117	78	91
M (desethylphenylgutarimide)	2370	—	—	—	—	—	—	—

Table 40.23 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Haloperidol	2930	3094	224	42	237	226	123	206
M (reduced)	3152	3152	224	206	226	193	377	139
M (*N*-desalkyl-oxo)-2H_2O	1650	1707	189	154	127	191	126	190
M	1750	1872	56	139	84	223	206	111
M (*N*-desalkyl)	1800	—	56	84	139	111	133	211
Hydroxyphenamate	1724	—	135	57	91	77	119	105
Hydroxyzine	2849	3000	201	203	165	45	299	166
M (norchlorcyclizine)	—	2355	165	201	166	85	241	230
M (4-chlorobenzophenone)	1850	1862	105	139	216	77	111	218
M (4-chloromethylbiphenyl)	1600	1688	167	165	202	152	82	204
M (OH-chlorobenzophenone)	2300	2230	121	139	95	234	111	152
M	—	2704	201	165	166	228	242	299
M	—	2847	85	165	166	201	242	256
Levomepromazine (methotrimeprazine)	2514	2641	58	328	100	228	185	329
M (norsulfoxide)	—	3088	—	—	—	—	—	—
M (sulfoxide)	—	3114	—	—	—	—	—	—
Loxapine	2555	2717	70	83	257	193	56	228
M (8-OH-)	2931	3077	70	83	273	209	260	343
M (7-OH-)	—	3068	70	273	260	209	244	343
M (amoxapine)	2638	2746	245	193	257	247	228	164
M (7-OH-amoxapine)	2951	3525	261	209	273	263	244	329
M (8-OH-amoxapine)	2959	3546	261	209	273	263	244	329
Mebutamate	1889	—	97	55	69	72	71	98
Mecloqualone	2255	—	235	111	75	76	236	50
Meprobamate (also carisoprodol metabolite)	1785	1854	83	84	55	56	43	71
Art	1535	1487	84	55	56	44	83	75
M	1720	1763	104	43	45	62	148	86
M	—	1932	104	43	45	71	119	204
M	—	2079	43	111	104	132	172	62
Mesoridazine	3380	3629	98	70	99	386	126	55
Methaqualone	2135	2256	235	250	91	233	236	65
M (2-formyl-)	2240	2370	235	132	264	206	248	192
M (2-OH-methyl-)	2360	2437	235	266	251	175	132	160
M (2-carboxy-)	2400	—	235	146	77	221	252	280
M (2′-OH-methyl-)	2410	2500	160	266	235	251	77	247
M (3′-OH-)	2490	—	251	266	249	77	148	252
M (4′-OH-)	2510	—	251	266	249	77	143	235
M (6-OH-)	2525	—	251	266	249	132	65	92
M (OH-methoxy-)	2560	2698	296	281	143	249	279	266
Methdilazine	2462	—	97	98	296	199	212	198
Methyprylon	1527	1581	155	140	83	98	55	41
M (OH)-H_2O	1540	1601	83	55	153	166	84	98
M (oxo-)	1870	1834	83	98	55	168	151	182
Molindone	2465	—	100	56	176	98	120	70
Olanzapine	—	2861	242	229	231	198	312	169
M (nor-)	—	2911	229	213	242	298	198	254
Oxypertine	2355	—	175	70	176	132	379	204
Pecazine	2540	2669	310	58	199	112	111	212
Penfluridol	3360	—	42	292	56	294	109	203

table continued

Table 40.23 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
M (*N*-desalkyl-oxo)-2H_2O	1920	—	257	259	258	222	167	202
M (*N*-desalkyl-)	2210	—	56	261	279	179	260	114
M (desamino-OH-)	2120	—	—	—	—	—	—	—
M (desamino-carboxy-)	2230	—	—	—	—	—	—	—
Perazine	2798	—	113	339	44	70	141	340
M (OH-)	3175	—	—	—	—	—	—	—
M (phenothiazine)	2120	2130	199	167	198	166	154	139
Pericyazine	3260	3486	114	44	142	365	223	115
M (ring)	2555	—	224	192	223	120	112	179
Perphenazine	3380	3594	246	143	403	70	404	248
M (ring)	2100	—	—	—	—	—	—	—
Phenothiazine	2120	2130	199	167	198	166	154	139
Phenprobamate	1520	—	118	117	91	92	119	65
Pimozide	3870	Not eluted	230	187	42	217	83	461
M (*N*-desalkyl-)	2415	—	—	—	—	—	—	—
M (benzimidazolone)	1950	—	—	—	—	—	—	—
M (desamino-OH-)	2120	—	—	—	—	—	—	—
M (desamino-carboxy-)	2130	—	—	—	—	—	—	—
Pipamperone	3040	—	165	138	331	123	110	194
M (OH-)	3250	—	165	154	347	123	292	194
Piperacetazine	0000	—	142	170	44	410	143	42
Pipotiazine (pipothiazine)	2932	Not eluted	142	44	140	198	170	96
Prochlorperazine	2954	3129	113	70	373	141	43	72
M (*N*-oxide)	2100	2356	233	198	235	218	272	201
M (norsulfoxide)	—	3571	70	113	373	246	319	232
M (sulfoxide)	—	3758	70	113	246	319	373	232
Promazine	2315	2425	58	284	86	238	198	199
M (nor-)	2405	2452	199	270	198	213	238	212
M (sulfoxide)	2705	2840	58	212	199	300	284	180
M (norsulfoxide)	—	2875	—	—	—	—	—	—
M (phenothiazine)	2120	2130	199	167	198	166	154	139
M (OH-)	2685	2781	58	86	300	215	254	228
M (nor-OH-)	—	2797	—	—	—	—	—	—
Promethazine	2339	2383	72	284	198	213	199	180
M (nor-)	2250	2333	58	213	180	198	152	270
M (phenothiazine)	2120	2130	199	167	198	166	154	139
M (sulfoxide)	2710	2797	72	198	180	152	213	229
M (norsulfoxide)	—	2732	58	212	180	198	229	152
M (nor-OH-)	2580	2717	212	58	180	229	198	286
M (OH-)	2590	—	72	196	300	229	214	288
Propiomazine	2738	—	72	340	269	197	73	71
Prothipendyl	2345	—	58	285	214	200	86	227
M (OH-)	2720	—	58	301	86	216	230	243
M (OH-ring)	2800	—	216	187	168	188	200	161
M (ring)	2045	—	200	168	199	156	201	155
M (sulfoxide)	2750	—	58	86	216	179	155	200
Quetiapine	—	3400	210	239	144	251	321	226
M	—	2745	227	210	239	251	265	295
M	—	2709	195	207	178	151	219	233
Remoxipride	2520	2588	98	99	70	228	230	243
M	—	2981	—	—	—	—	—	—

Table 40.23 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
M	—	3022	—	—	—	—	—	—
M	—	3313	—	—	—	—	—	—
Risperidone	—	1877	220	191	204	178	192	221
Art	2063	—	—	—	—	—	—	—
Sulforidazine (also thioridazine and mesoridazine metabolite)	3415	3690	98	402	70	197	198	290
M (ring)	3180	—	277	198	154	127	278	263
Sulpiride	3102	Not eluted	—	—	—	—	—	—
Art (-SO_2NH)	2295	—	98	70	77	135	99	111
Tetrabenazine	2490	2579	191	261	260	274	316	176
M (*O*-desmethyl-OH-)	2500	2638	205	191	274	318	319	232
Thalidomide	2440	Not eluted	173	104	76	111	148	170
Thiethylperazine	3226	—	70	113	141	399	72	259
M (ring)	2750	—	259	230	198	186	260	167
M (sulfone)	3400	—	70	113	127	305	431	212
Thiopropazate (metabolised to perphenazine)	3467	—	246	70	185	98	87	213
Thioproperazine	3552	—	70	113	127	212	320	141
M (ring)	3200	—	306	198	199	197	277	154
Thioridazine	3115	3292	98	370	126	99	371	250
M (nor-)	—	3275	84	56	112	356	245	185
M (sulfoxide) mesoridazine	3380	3629	98	70	99	386	126	55
M (ring)	2570	2639	245	198	186	230	154	166
M (oxo-)	3500	—	384	385	244	112	258	245
M (ring sulfone)	3420	3626	98	370	402	244	258	290
M (side chain sulfone)	3800	Not eluted	416	112	290	277	276	417
Thiothixene	3060	—	—	—	—	—	—	—
Trichloroethanol (see Chapter 14)								
Triclofos (metabolised to trichloroethanol)	—	1952	31	49	77	113	51	115
Trifluomeprazine	2250	—	58	366	100	266	248	84
Trifluoperazine	2683	2798	113	70	407	43	141	127
M (phenothiazine)	2120	2130	199	167	198	166	154	139
M (sulfoxide)	2990	3145	113	141	248	266	306	280
M (norsulfoxide)	—	3191	—	—	—	—	—	—
Trifluperidol	2675	—	42	271	258	123	83	240
M (*N*-desalkyl-oxo-)-$2H_2O$	1570	—	223	224	154	170	183	204
M (*N*-desalkyl-)	1970	—	56	227	226	245	223	198
Triflupromazine	2230	2318	58	352	86	353	306	266
M (phenothiazine)	2120	2130	199	167	198	166	154	139
M (OH-)	2700	—	58	368	86	322	282	323
M (OH-methoxy-)	2730	—	58	398	86	312	313	265
Trimetozine	2253	—	195	281	196	152	280	81
Tybamate	1725	—	55	72	97	158	118	56
Zolpidem	2715	2941	235	236	307	219	92	65
Zopiclone	2950	3263	143	245	112	99	139	217
Art (amino-chloropyridine)	1200	1261	128	101	130	73	98	93
Zotepine	2660	—	208	199	221	163	231	147
Zuclopenthixol (clopenthixol) *cis* isomer	3360	3557	143	70	144	100	42	56
Zuclopenthixol *trans* isomer	3400	3680	143	70	144	100	42	56
M (*N*-oxide)-$C_6H_{14}N_2O_2$	2410	—	—	—	—	—	—	—
Art (Cl-thioxanthenone)	2260	—	—	—	—	—	—	—

Table 40.24 GC retention data and mass spectral data for laxatives (AC, acetyl; Me, methyl; TMS, trimethylsilyl)

Compound	*System*		*Principal ions* (m/z)					
	GA	*GB*						
Aloe-emodin	2660	—	—	—	—	—	—	—
Aloe-emodin-AC	2735	—	270	241	312	271	242	225
Aloe-emodin-AC_2	3000	—	270	354	312	241	271	224
Aloe-emodin-Me	2900	—	284	266	238	225	209	237
Aloe-emodin-Me_2	2705	—	298	267	239	299	240	291
Aloe-emodin-TMS	2685	—	311	312	342	225	296	268
Aloe-emodin-TMS_2	2785	—	399	400	184	310	383	325
Aloe-emodin-TMS_3	2900	—	471	472	472	399	367	281
Aloin	0000	0000	—	—	—	—	—	—
Arecoline	1195	—	155	96	140	43	81	94
Bisacodyl	2818	2956	361	277	319	276	199	318
M (bismethoxybisdesacetyl)	2820	—	337	322	336	338	259	307
M (bismethoxydesacetyl-)	2890	—	379	322	364	336	378	380
M (desacetyl-)	2750	2876	319	276	277	199	318	246
M (bisdesacetyl-)	2655	2793	277	276	199	183	278	246
M (methoxybisdesacetyl-)	2680	—	307	306	229	292	275	198
M (methoxydesacetyl-)	2810	—	349	306	307	229	292	348
M (bismethoxybisdesacetyl)-AC_2	2950	—	379	421	364	322	336	378
M (methoxybisdesacetyl)-AC_2	2870	—	349	391	307	306	229	348
M (trimethoxybisdesacetyl)-AC_2	3060	—	409	367	451	329	352	203
M (desacetyl)-TMS	—	2830	391	348	349	271	390	392
M (bisdesacetyl)-TMS_2	—	2728	421	343	420	422	256	240
Dantron	2330	2450	240	212	241	184	138	92
Dantron-Me	2435	—	254	208	236	225	139	168
Dantron-Me_2	2475	—	253	268	139	152	209	180
Dantron-AC	2460	—	240	282	241	212	184	155
Dantron-AC_2	2595	—	240	282	241	212	184	155
Dantron-TMS	2465	2574	297	298	253	240	210	312
Dantron-TMS_2	2530	2611	369	370	297	371	268	210
Emetine	2505	Not eluted	192	206	272	480	288	246
Emetine-Me	4010	—	206	207	190	272	288	494
Frangula-emodin	2620	—	—	—	—	—	—	—
Frangula-emodin-AC	2740	—	270	312	271	242	213	241
Frangula-emodin-Me_2	2775	—	298	252	280	269	237	281
Frangula-emodin-Me_3	2845	—	297	312	295	283	266	251
Phenolphthalein	—	3292	274	225	318	273	275	226
Phenolphthalein-AC_2	3375	3351	360	318	274	225	257	402
Phenolphthalein-Me_2	3060	—	271	302	346	301	239	287
Phenolphthalein-TMS_2	—	3205	418	417	419	297	253	329
M (methoxy)-AC_2	3395	—	390	273	272	348	304	391
Physcion	2660	2732	284	285	255	128	283	241
Physcion-AC_2	2920	—	284	326	285	255	227	184
Physcion-Me_2	2845	—	—	—	—	—	—	—
Physcion-Me	2775	—	—	—	—	—	—	—
Physcion-TMS	2150	2247	341	117	129	132	145	356
Picosulfate (hydrolysed to bisacodyl metabolites, see above)								
Rhein	2675	—	284	285	255	128	241	139
Rhein-Me	2660	—	298	267	239	155	126	284
Rhein-Me_2	2740	—	312	266	294	251	235	126
Rhein-Me_3	2855	—	311	326	312	309	235	295

Table 40.25 GC retention data for steroids (AC, acetyl; TMS, trimethylsilyl)

	GA	*GAG*	*GAI*	*GAR*
Androstanolone	—	—	—	—
dihydrotestosterone	2510	—	—	—
dihydrotestosterone-AC	2630	—	—	—
dihydrotestosterone-TMS	2485	—	—	—
dihydrotestosterone enol-TMS_2	2450	—	—	—
5α-dihydrotestosterone	—	—	0.95	—
Androsterone	2475	—	—	11.9
-AC	2580	—	—	—
enol-TMS_2	2500	—	—	—
Boldenone	—	1.05	0.961	12.8
undecylenate	—	2.62	—	22.4
5β-androst-1-en-17β-ol-3-one	—	—	0.96	—
acetate	—	—	—	13.6
benzoate	—	—	—	18.7
undecylenate	—	—	—	22.4
DHEA	2530	—	—	11.8
DHEA-H_2O	2595	—	—	—
DHEA enol-TMS_2	2580	—	—	—
Drostanolone propionate	2985	—	0.974	—
drostanolone	2555	—	—	—
drostanolone-AC	2700	—	—	—
drostanolone-TMS	2575	—	—	—
drostanolone-enol-TMS_2	2625	—	—	—
drostanolone propionate	2985	—	0.974	—
Fluoxymesterone	2835	1.5	1.155	14.6
Methandienone	2672	1.2	—	13.2
17α-methyl-5β	—	—	0.925	—
17α-androstan-3α,17β-diol	—	—	0.925	—
17α-methyl-5β-androst-1-en-3α,17β-diol	—	—	0.921	—
17α-methyl-1,4-androstadien-6β,17β-diol-3-one	—	—	1.117	—
Methandriol	—	0.89	—	12.3
dipropionate	—	1.70	—	—
17α-methyl-5β-androstan-3α,17β-diol	—	—	0.925	—
Methyltestosterone	2645	1.05	—	13.1
-AC	2770	—	—	—
-TMS	2590	—	—	—
enol-TMS_2	2665	—	—	—
17α-methyl-5β	—	—	0.925	—
Nandrolone	2395	0.91	—	12.5
-TMS	2760	—	—	—
Oxandrolone	—	1.17	1.111	13.7
Oxymetholone	3005	1.28	—	13.7
enol-TMS_3	2870	—	—	—
17α-methyl-5α-androstan-3α,17β-diol	—	—	0.925	—
2-hydroxymethyl-17α-methyl-5-androstan-3,17-diol	—	—	1.106	—
2-hydroxymethyl-17α-methyl-5-androstan-3,6,17-triol	—	—	1.180	—
Stanozolol	3085	—	1.31	15.4
-TMS_2	3025	—	—	—
-AC	2120	—	—	—
3′-hydroxystanozolol	—	—	1.38	—
4β-hydroxystanozolol	—	—	1.393	—

table continued

Table 40.25 continued

	GA	GAG	GAI	GAR
Testosterone	2620	—	0.97	12.9
-AC	2750	—	—	—
enol-TMS_2	2690	—	—	—
propionate	2815	1.43	—	14.2
dipropionate	3350	—	—	—
methyltestosterone	—	1.05	—	13.1
acetate	—	1.21	—	13.5
isobutyrate	—	1.54	—	—
cipionate	—	2.19	—	18.7
enantate	—	1.92	—	16.7
undecylate	—	2.56	—	—
isocaproate	—	1.77	—	15.9
decanoate	—	2.36	—	19.8
benzoate	—	—	—	18.0
phenylpropionate	—	—	—	20.2

(magnesium and other salts), are detected by other means (Duncan 2000) and are not discussed here. Stools may be analysed after homogenisation, or alternatively purgatives may be detected in urine, both after enzymatic hydrolysis to release conjugated metabolites. Extraction of hydrolysis products with chloroform–isopropanol (9 : 1) or other moderately polar solvents at the hydrolysis pH yields good recovery, and derivatisation (e.g. silylation, methylation or acetylation) improves chromatography, particularly of the more polar compounds such as rhein. Rhein is a product of many vegetable glycoside laxatives, including sennosides, aloes and cascara (except frangula, which is metabolised to emodin), which are hydrolysed by colonic bacteria to active aglycones prior to absorption (this is important to remember when analysing pharmaceutical products). System GA or GB may be used, and the reference compounds are *n*-alkanes with an even number of carbon atoms.

Solvents and other volatile compounds

Methods for the analysis and analytical data for solvents and other volatile substances are described in detail in Chapter 16. An ECD run in parallel with an FID produces the optimal detection rates, since many of the compounds of interest are halogenated. The low boiling point of these compounds requires their careful isolation from biological samples by headspace analysis, and the GC may benefit from cryogenic cooling (Flanagan *et al.* 1997; Sharp 2001). Standard capillary columns can be used (e.g. dimethyl-PSX), but chromatography and durability benefit from the use of a column with a high-phase ratio (3–5 µm film thickness). Other columns, such as an X-wax phase (a 0.25 µm film thickness is adequate here), or those specifically designed for volatiles, such as X-624 (3 µm film thickness), are used commonly (see Table 40.3 for details of these stationary phases). System GA or GI may be used and a more comprehensive method for screening volatiles can be found in Chapter 16, Table 16.3.

System GA, previously described, may be used, or system GI, below.

System GI

(Ramsey, Flanagan 1982).

- *Column*: 0.3% Carbowax 20M on 80-100 mesh Carbopak C glass, (2 m × 2 mm i.d.).
- *Temperature programme*: 35°C for 2 min to 175°C at 5°/min for at least 8 min.
- *Carrier gas*: N_2, 30 mL/min.

Steroids (Table 40.25)

System GAR

(CND Analytical, 1989).

- *Column:* Fused silica capillary with methylsilicone (12 m × 0.25 mm i.d., 0.25 µm).
- *Temperature programme:* 70°C to 150°C at 15°/min to 250°C at 25°/min.

System GAG

(Lurie *et al.* 1994).

- *Column*: Bonded DB-1 fused silica capillary, cross-linked (30 m × 0.25 mm i.d., 0.25 µm). Split ratio of 30 : 1.
- *Temperature programme*: 180°C to 230°C at 10°/min to 245°C at 1°/min to 295°C at 30°/min for 15 min.
- *Carrier gas*: H_2.
- *Detector*: Flame ionisation.

System GAI

(Ayotte *et al.* 1996).

- *Column*: HP-5 5% phenyl polymethyl siloxane capillary (25 m × 0.25 mm i.d., 0.33 µm).
- *Temperature programme*: 100° for 1 min to 220° at 16°/min to 301° at 20°/min for 5.5 min.
- *Carrier gas*: He.
- *Detection*: Mass-selective detector.
- *Retention time*: Relative to 17α-methyl-5α-androstan-3β,17β-diol.

Sulfonamides

Gas chromatography of sulfonamides is only possible after extractive *N*-methylation of the secondary amino group (Gyllenhaal *et al.* 1978) and some authors additionally prepare HFB or PFP derivatives of any primary amino groups (Tarbin *et al.* 1999). These latter derivatives may require the use of positive chemical ionisation MS (Reeves 1999). As with the oral hypoglycaemics, differences in methylation conditions may lead to hydrolysis, and a number of sulfonamides may yield products or metabolites that correspond to sulfanilamide derivatives. The main metabolites are the N^4-acetylacted derivatives, which are hydrolysed relatively easily back to the parent compound under acidic conditions. System GA or GJ (Gyllenhaal *et al.* 1978) may be used.

System GJ

- *Column:* 5% OV-17 on 80 to 100 mesh Gas-Chrom Q glass (1.5 m × 2 mm i.d.).
- *Temperature:* 250°.
- *Carrier gas:* N_2, 30 mL/min.
- *Relative retention times* are given in Table 40.26 (retention times of methyl derivatives are relative to griseofulvin).

Table 40.26 GC retention data and mass spectral data for the sulfonamides (AC, acetyl; Me, methyl)

Compound	*System*		*Principal ions* (m/z)					
	GA	*GJ*						
Mafenide	2340	—	106	77	185	105	104	89
Mafenide-Me_2	1920	—	58	214	213	74	89	133
Mafenide-Me_3	1900	—	58	89	228	227	133	214
Mafenide-Me_4	1870	—	58	242	89	107	134	117
M (AC-)	2425	—	105	106	147	228	185	160
M (AC)-Me	2300	—	119	161	185	242	89	199
Phenylbenzenesulfonamide (see Sulfabenzamide)								
Phthalylsulfacetamide (metabolised to sulfacetamide)								
Phthalylsulfathiazole (metabolised to sulfathiazole)								
Succinylsulfathiazole (metabolised to sulfathiazole)								
Sulfabenzamide-Me_2	2770	0.09	118	105	77	304	170	240
Sulfabenzamide-Me (metabolised to sulfanilamide)	2700	—	118	105	77	92	226	290
Sulfacetamide	2132	—	92	109	156	180	65	214
Sulfacetamide-Me	—	0.16	—	—	—	—	—	—
Sulfadiazine	2502	—	185	186	92	65	108	170
Sulfadiazine-Me	2625	0.66	199	200	92	108	156	184
M (AC)-Me	3710	1.69	241	242	199	108	92	266
Sulfadimidine	2613	—	—	—	—	—	—	—
Sulfadimidine-Me	—	0.71	—	—	—	—	—	—
Sulfaethidole (also metabolised to sulfanilamide)	2620	—	92	284	65	108	156	220
Sulfadoxine	—	—	—	—	—	—	—	—
Sulfaethidole (also metabolised to sulfanilamide)	2620	—	—	—	—	—	—	—
Sulfaethidole-Me	3060	—	298	92	83	190	234	156
Sulfaethidole-Me_2	2840	—	106	92	65	161	156	234
M (AC-)	2490	—	213	108	80	326	136	283
M (AC)-Me_2	3410	—	148	106	203	276	302	354
Sulfafurazole (sulfisoxazole)	1212	—	156	92	108	140	65	267
Sulfafurazole-Me	—	0.42						
Sulfaguanidine	0000	—	108	214	92	65	148	156
Sulfaguanole-Me (metabolised to sulfanilamide)	2905	—	203	57	323	322	249	204
Sulfamerazine	2566	—	199	200	92	65	108	100
Sulfamerazine-Me	2625	0.69	199	200	65	92	108	140
Sulfamethizole-Me (metabolised to sulfanilamide)	2660	0.98	92	284	108	156	176	220
Sulfamethoxazole-Me (also metabolised to sulfanilamide)	2500	—	92	108	119	162	156	203
Sulfamethoxazole-Me_2	2460	—	92	108	119	62	156	188
Sulfamethoxazole-Me	2500	0.40	92	108	119	162	156	188
M (AC)-Me	3255	0.91	161	134	230	245	205	199
Sulfamethoxydiazine-Me_3 (also metabolised to sulfanilamide)	2925	1.38	229	230	92	108	138	156
M (AC)-Me	3620	—	271	272	229	65	92	139
Sulfamethoxypyridazine-Me	—	0.93	—	—	—	—	—	—
Sulfametopyrazine/sulfalene-Me	—	0.69	—	—	—	—	—	—
Sulfamoxole-Me	—	0.40	—	—	—	—	—	—
Sulfanilamide	2185	—	65	92	156	172	108	80
Sulfanilamide-Me	2135	—	92	186	56	65	108	122
Sulfanilamide-Me_3	—	—	122	214	170	106	77	79
Sulfanilamide-Me_4	2095	—	136	120	228	184	77	105
M (AC-)	2690	—	172	156	92	108	125	214
M (AC)-Me	2600	—	186	156	228	92	108	65
Sulfaperin-Me_3 (also metabolised to sulfanilamide)	1795	—	213	214	65	92	198	306
M (AC)-Me_2	3420	—	255	256	65	93	122	213
Sulfaphenazole-Me	—	1.71	—	—	—	—	—	—

table continued

Table 40.26 continued

Compound	*System*		*Principal ions* (m/z)					
	GA	*GJ*						
Sulfapyridine	2600	—	184	185	65	92	108	66
Sulfapyridine-Me	—	0.47	198	199	92	65	78	108
M (AC)-Me	—	1.16	—	—	—	—	—	—
Sulfasalazine (metabolised to sulfapyridine)								
Sulfasomidine/sulfaisomidine-Me	—	0.50	—	—	—	—	—	—
Sulfathiazole	Not eluted	—	—	—	—	—	—	—
Sulfathiazole-Me	—	0.49	—	—	—	—	—	—
Sulfaurea (metabolised to sulfanilamide)								

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CHAPTER

41 High Performance Liquid Chromatography

T Kupiec and P Kemp

Introduction

The ability to separate and analyse complex samples, both small and large molecules, is important to the biological and medical sciences. Classic column chromatography has evolved over the years, with chromatographic innovations introduced at roughly decade intervals. These techniques offered major improvements in speed, resolving power, detection, quantification, convenience and applicability to new sample types. The most notable of these modifications was high performance liquid chromatography (HPLC). Modern HPLC techniques became available in 1969; however, they were not widely accepted in the pharmaceutical industry until several years later. Once HPLC systems capable of quantitative analysis became commercially available, their usefulness in pharmaceutical analysis was fully appreciated.

By the 1990s, HPLC had begun an explosive growth that made it a popular analytical method judged by sales of instruments and also scientific importance. During the last decade developments in chromatographic supports and instrumentation for liquid chromatography have continued to evolve. The use of silica-based monolithic supports, elevated mobile phase temperatures and columns packed with particles $<2\,\mu m$ has resulted in better resolution and faster separations of complex mixtures. Ultra-fast HPLC is such an important topic that it has been given its own section in the chapter. This application has experienced phenomenal growth in the first decade of the twenty-first century.

Liquid chromatography's present popularity results from its convenient separation of a wide range of sample types, exceptional resolving power, speed and nanomolar detection levels. It is presently used in biological, forensic and pharmaceutical research and development:

- To purify synthetic or natural products
- To characterise metabolites
- To assay active ingredients, impurities, degradation products and in dissolution assays
- In pharmacodynamic and pharmacokinetic studies.

Improvements made in HPLC in recent years include:

- Changes in packing material, such as smaller particle size, new packing and column materials
- High-speed separation
- Micro-HPLC, automation and computer-assisted optimisation
- Improvements in detection methods, including the so-called hyphenated detection systems.

These innovations will be discussed in the appropriate sections of this chapter. Additionally, a few novel techniques are presented that have come to be used in the past few years. These techniques include denaturing high pressure liquid chromatography and ion chromatography.

Practical aspects of HPLC theory

The practical application of HPLC is aided by an awareness of the concepts of chromatographic theory, in particular the measurement of chromatographic retention and the factors that influence resolution.

Chromatographic principles

The retention of a drug with a given packing material and eluent can be expressed as a retention time or a retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (k), which is independent of these factors. The column capacity ratio of a compound (A) is defined by Equation (41.1):

$$k_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0} \qquad (41.1)$$

where V_A is the elution volume of A and V_0 is the elution volume of a non-retained compound (i.e. void volume). At constant flow rate, retention times (t_A and t_0) can be used instead of retention volumes. The injection of a solvent or salt solution can be used to measure V_0, but the solute used should always be recorded along with reported k data. The importance of selecting suitable solutes for the measurement of V_0 has been discussed (Wells, Clark 1981).

It is sometimes convenient to express retention data relative to a known internal standard (B). The ratio of retention times (t_A/t_B) can be used, but the ratio of adjusted retention times, $(t_A - t_0)/(t_B - t_0)$, is better when data needs to be transferred between different chromatographs (Ettre 1980).

Resolution is the parameter that describes the separation power of the complete chromatographic system relative to the particular components of the mixture. By convention, resolution (R) is expressed as the ratio of the distance between two peak maxima to the mean value of the peak width at the base line (Equation 41.2).

$$R = \frac{2(V_{R,2} - V_{R,1})}{W_1 + W_2} \qquad (41.2)$$

If we approximate peaks by symmetrical triangles, then if R is equal to or more than 1, the components are completely separated. If R is less than 1, the components overlap.

Sensitivity in chromatographic analysis is a measure of the smallest detectable level of a component in a chromatographic separation and is dependent on the signal-to-noise ratio in a given detector. Sensitivity can be increased by derivatisation of the compound of interest, optimisation of the chromatographic system or miniaturisation of the system. The limit of detection is normally taken as 3 times the signal-to-noise ratio and the limit of quantification as 10 times this ratio.

Chromatographic mechanisms

The systems used in chromatography are often described as belonging to one of four mechanistic types: adsorption, partition, ion exchange and size exclusion. *Adsorption chromatography* arises from interactions between solutes and the surface of the solid stationary phase. Generally, the eluents used for adsorption chromatography are less polar than the stationary phases and such systems are described as 'normal phase'. *Partition chromatography* involves a liquid stationary phase that is immiscible with the eluent and coated on an inert support. Partition systems can be normal phase (stationary phase more polar than eluent) or reversed-phase chromatography, referred to as RPC (stationary phase less polar than eluent). *Ion-exchange chromatography* involves a solid stationary phase with anionic or cationic groups on the surface to which solute molecules of opposite charge are attracted. *Size-exclusion chromatography* involves a solid stationary phase with controlled pore size.

Solutes are separated according to their molecular size, with the large molecules unable to enter the pores eluting first. However, this concept of four separation modes is an over-simplification. In reality, there are no distinct boundaries and several different mechanisms often operate simultaneously.

Other types of chromatographic separation have been described. *Ion-pair chromatography* is an alternative to ion-exchange chromatography. It involves the addition of an organic ionic substance to the mobile phase, which forms an ion pair with the sample component of opposite charge. This allows a reversed-phase system to be used to separate ionic compounds. *Chiral chromatography* is a method used to separate enantiomers, which can be achieved by various means. In one case, the mobile phase is chiral and the stationary phase is non-chiral. In another, the liquid stationary phase is chiral with the mobile phase non-chiral. Or, finally, the solid stationary phase may be chiral with a non-chiral mobile phase.

Hardware

HPLC instrumentation includes a pump, an injector, a column, a detector and a recorder or data system (Figure 41.1). The heart of the system is the column in which separation occurs. Since the stationary phase is composed of micrometer-sized porous particles, a high-pressure pump is required to move the mobile phase through the column. The chromatographic process begins by injecting the solute on to the top of the column. Separation of components occurs as the analytes and mobile phase are pumped through the column. Eventually, each component elutes from the column and is registered as a peak on the recorder. Detection of the eluting components is important; this can be either selective or universal, depending upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram. To collect, store and analyse the chromatographic data, computers, integrators and other data-processing equipment are used frequently.

Mobile phase reservoir

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with special caps, Teflon tubing and filters to connect to the pump inlet and to the sparge gas (helium) used to remove dissolved air. When the mobile phase contains excessive gas that remains dissolved at the pressure produced by the column, the gas may come out of the solution at the column exit or in the detector, which results in sharp spikes. Spikes are created by microscopic bubbles that change the nature of the flowing stream to make it heterogeneous, while drift may occur as these microscopic bubbles gradually collect and combine in the detector cell. The main culprit is oxygen (from the air) that dissolves in polar solvents, particularly water. Degassing may be accomplished by one or a combination of the following methods: applying a vacuum to the liquid; boiling the liquid; placing the liquid in an ultrasonic bath; bubbling a fine stream of helium through the liquid (sparging); or using commercial on-line degassing units.

Pumps

High-pressure pumps are needed to force solvents through packed stationary-phase beds. Smaller bed particles (e.g. 3 µm) require higher pressures. There are many advantages to using smaller particles, but they may not be essential for all separations. The most important advantages are higher resolution, faster analyses and increased sample load capacity. However, only the most demanding separations require these advances in significant degrees. Many separation problems can be resolved with larger particle packings (e.g. 5 µm) that require less pressure.

Flow-rate stability is another important pump feature that distinguishes pumps. Constant-flow systems are generally of two basic types: reciprocating piston and positive displacement (syringe) pumps. The basic advantage of both systems is their ability to repeat elution volume and peak area, regardless of viscosity changes or column blockage, up to the pressure limit of the pump. Although syringe-type pumps have a pressure capability of up to 540 000 kPa (78 000 psi), they have a limited ability to form gradients. Reciprocating piston pumps can maintain a liquid flow for an indefinite length of time, while a syringe pump needs to be refilled after the syringe volume has been displaced. Dual-headed reciprocating piston pumps provide more reproducible and pulse-free delivery of solvent, which reduces detector noise and enables more reliable integration of peak area. Reciprocating pumps now dominate the HPLC market and are even useful for micro-HPLC applications, as they can maintain a constant flow at flow rates in µL/min ranges.

An additional pump feature found on the more elaborate pumps is external electronic control. Although it adds to the expense of the pump, external electronic control is a very desirable feature when automation or electronically controlled gradients are to be run. Alternatively, this becomes unnecessary when using isocratic methods. The degree of flow control also varies with the expense of the pump. More expensive pumps include such state-of-the-art technology as electronic feedback and multiheaded configurations.

Modern pumps have the following parameters:

- Flow-rate range, 0.01–10 mL/min
- Flow-rate stability, not more than 1% (short term)

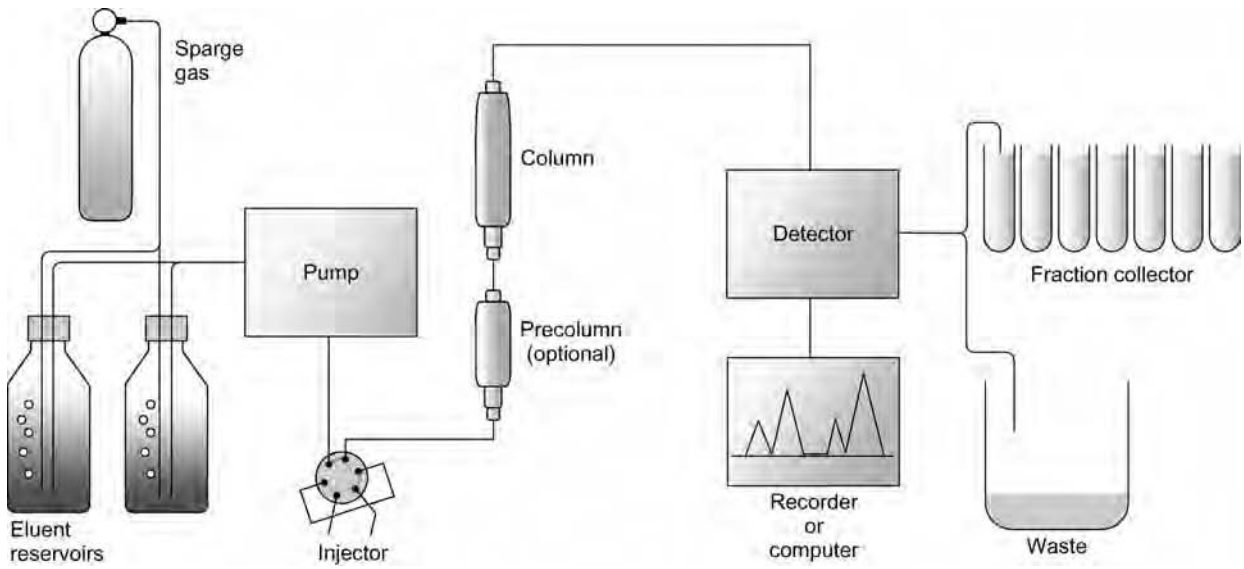

Figure 41.1 A typical HPLC system.

- For size exclusion chromatography (SEC), flow-rate stability should be <0.2%
- Maximum pressure, up to 34 500 kPa (5000 psi).

Injectors

An injector for an HPLC system should provide injection of the liquid sample within the range 0.1–100 mL of volume with high reproducibility and under high pressure (up to 27 600 kPa). The injector should also minimise disturbances to the flow of the mobile phase and produce minimum band broadening. Sample introduction can be accomplished in various ways. In most cases, the injection valve has replaced syringe injection. Valve injection offers rapid, reproducible and essentially operator-independent delivery of a wide range of sample volumes. The most common valve is a six-port Rheodyne valve in which the sample fills an external stainless-steel loop. A clockwise turn of the valve rotor places the sample-filled loop into the mobile-phase stream, which deposits the sample on to the top of the column. These valves can be operated manually or actuated via computer-automated systems. One minor disadvantage of valve injection is that the sample loop must be changed to obtain various sample volumes. However, this is a simple procedure that requires only a few minutes. Automatic sampling devices are incorporated in more sophisticated HPLC systems. These autosamplers have a piston-metering syringe-type pump to suck the preset sample volume into a line and transfer it to a sample loop of adequate size in a standard six-port valve. Most autosamplers are computer controlled and can serve as the master controller for the whole system.

In HPLC, liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent. The solvent need not be the mobile phase, but frequently it is wise to choose the mobile phase to avoid detector interference, column–component interference, loss in efficiency or all of these. It is always best to remove particles from the sample by filtration or centrifugation, since continuous injections of particulate material eventually cause blockage of injection devices or columns.

Sample sizes may vary widely. The availability of highly sensitive detectors frequently allows the use of small samples that yield the highest column performance.

Thermostats

It is often advantageous to run ion-exchange, size-exclusion and reversed-phase columns above room temperature and to control precisely the temperature of liquid–liquid columns. Accordingly, column thermostats are a desirable feature in modern HPLC instruments. Temperature variation within the HPLC column should generally be held within $\pm 0.2^{\circ}$C. It is especially important to maintain a constant temperature in quantitative analysis, since changes in temperature can seriously affect peak-size measurement. It is often important to be able to work at higher temperatures for size-exclusion chromatography of some synthetic polymers because of solubility problems. High-velocity circulating air baths, which usually consist of high-velocity air blowers plus electronically controlled thermostats, are the most convenient for HPLC. Alternatively, HPLC columns can be jacketed and the temperature controlled by contact heaters or by circulating fluid from a constant-temperature bath. This latter approach is practical for routine analyses but is less convenient when columns must be changed frequently.

Column switches

These valve devices are used to divert the flow from one column to another within a single HPLC system. Column-switching techniques can be used during method development when several columns are to be evaluated for their efficiency, retention, etc. More recently, the use of column switching has been employed in the on-line analysis of biological matrices. Raw plasma or other sample matrix is injected directly onto the first column. Chromatographic conditions are optimised such that interfering substances are eluted from the column while the analytes of interest are retained. The column switch then diverts the eluent that contains the analytes of interest from the 'clean-up column' onto the analytical column, which then separates the analytes of interest for quantification or characterisation. Another use of column switches is in gradient chromatography for which high throughput is essential. The first column is switched off-line to re-equilibrate to initial conditions, while the second column is brought on-line for the next injection. This conserves valuable analysis time that would otherwise be wasted waiting for the column to re-equilibrate. The most up-to-date information on the use of column switching can be found by searching the current literature.

Detectors

Today, optical detectors are used most frequently in HPLC systems. These detectors pass a beam of light through the flowing column effluent as it passes through a flow-cell. Flow-cells are available in preparative, analytical and micro-analytical sizes The variations in light intensity, caused by ultraviolet (UV) absorption, fluorescence emission or change in refractive index (depending on the type of detector used) from the sample components that pass through the cell, are monitored as changes in the output voltage. These voltage changes are recorded on a strip-chart recorder and are often fed into an integrator or computer to provide retention time and peak-area data.

Most applications in drug analysis use detectors that respond to the absorption of UV radiation (or visible light) by the solute as it passes through the flow-cell. Absorption changes are proportional to concentration, following the Beer–Lambert Law. Flow-cells generally have path-lengths of 5–10 mm with volumes between 5 and 10 µL. These detectors give good sensitivities with many compounds, are not affected by slight fluctuations in flow rate and temperature, and are non-destructive, which allows solutes to be collected and further analysed if desired.

The simplest detectors are of the fixed-wavelength type and usually contain low-pressure mercury lamps that have an intense emission line at 254 nm. Some instruments offer conversion kits that allow the energy at 254 nm to excite a suitable phosphor to give a new detection wavelength (e.g. 280 nm). Variable-wavelength detectors have a deuterium lamp with a continuous emission from 180 nm to 400 nm and use a manually operated diffraction grating to select the required wavelength. Tungsten lamps (400–700 nm) are used for the visible region.

Many organic compounds absorb at 254 nm and hence a fixed-wavelength detector has many uses. However, a variable-wavelength detector can be invaluable to increase the sensitivity of detection by using the wavelength of maximum absorption. This is particularly useful when analysing proteins that absorb at 280 nm, or peptides that are detected commonly at 215 nm. Using a variable-wavelength detector can also increase the selectivity of detection by enhancing the peak of interest relative to interfering peaks.

Eluents must have sufficient transparency at the selected detection wavelength. Buffer salts can also limit transparency. The spectra of some drugs change with pH and the sensitivity and selectivity of an assay can sometimes be controlled by changing the eluent pH. The influence of such changes on the chromatography must also be considered.

Other commonly used detectors include diode array, refractive index (RI), fluorescence (FL), electrochemical (EC) and mass spectrometry (MS) detectors. Infrared (IR) and nuclear magnetic resonance (NMR) spectrometers may also be used as detectors.

Photodiode array detectors

The photodiode array detector (DAD) is an advanced type of UV detector. Depending on the wavelength, a tungsten lamp and a deuterium lamp are used as light sources. The polychromatic light beam is focused on a flow-cell (volume 8–13 µL) and subsequently dispersed by a holographic grating or quartz prism. The spectral light then reaches a chip that contains 100–1000 light-sensitive diodes arranged side by side. Each diode registers only a well-defined fraction of the information and in this way all wavelengths are measured at the same time. Note that, although having more diodes in an array increases the resolution of UV spectra, it lowers the absolute sensitivity since less radiation is absorbed by each individual diode. The wavelength resolution of up-to-date detectors is of the order of 1 nm per diode, with a wavelength accuracy of better than ± 1 nm and a sensitivity below 10^{-4} absorbency

units. All operations of the detector are controlled by computer: correction of fluctuations of the lamp energy, collection of signals (I_λ) from all the diodes, storage of the data of the mobile phase ($I_{0\lambda}$, measured at the start of the chromatogram) and calculation of the absorbance according to the Beer–Lambert Law from I_λ to $I_{0\lambda}$. The number of spectra recorded per second can be chosen from between 0.1 and 10; usually one spectrum per second is optimum with respect to chromatographic resolution and noise. At the end of the run, a three-dimensional spectrochromatogram (absorbance as a function of wavelength and time) is stored on the computer and can be evaluated qualitatively and quantitatively. A detailed description of the DAD operation is given in Huber and George (1993).

Diode array detection offers several advantages. Knowledge of the spectra of compounds of interest enables interfering peaks to be eliminated so that an accurate quantification of peaks of interest can be achieved despite less than optimal resolution. Simultaneous detection at two wavelengths allows calculation of an absorbance ratio. If this ratio is not constant across a peak, the peak is not pure, regardless of its appearance. An additional advantage of the DAD is the subtraction of a reference wavelength. This reduces baseline drift during gradient elution. HPLC-DAD systems linked to libraries of UV spectra are particularly useful in clinical and forensic toxicology in screening for drugs in biological samples (Herzler *et al.* 2003; Pragst *et al.* 2004) and its use in this context is described in detail later.

Refractive index detector

The RI detector is a universal detector, in that changes in RI (either positive or negative) that arise from the presence of a compound in the eluent are recorded. However, it is also the least sensitive detector (as much as 100 times less sensitive than UV detection). RI detectors may be used for excipients such as sugars in pharmaceuticals. Many factors influence RI and must be controlled during separation, such as temperature, eluent composition and pressure. The chromatography is best facilitated using a thermostatically controlled cabinet and high-quality pump to minimise pressure fluctuations.

Fluorescence detector

In FL detectors, the solute is excited with UV radiation and emits radiation at a longer wavelength. Most detectors allow the selection of both excitation and emission wavelengths. There are only a few drugs and natural compounds that have strong natural fluorescence (e.g. ergot alkaloids); however, many drug derivatives are fluorescent compounds. FL detection can offer great selectivity, since excitation and emission wavelengths as well as retention time can be used to identify drugs. Eluents must be chosen carefully when using FL detection. The eluent must neither fluoresce nor absorb at the chosen wavelengths. It is also necessary to consider the pH of the system, in that some drugs show fluorescence only in certain ionic forms.

Electrochemical detectors

EC detectors measure the current that results from the electrolytic oxidation or reduction of analytes at the surface of an electrode. These detectors are quite sensitive (down to 10^{-15} mole) and also quite selective. Two types of detector are available. The coulometric detector has a large electrode surface at which the electrochemical reaction is taken to completion. The amperometric detector has a small electrode with a low degree of conversion. Despite the difference in conversion rate, in practice these two types have approximately the same sensitivity. Eluents for EC detection must be electrically conductive. This is accomplished by the addition of inert electrolytes. EC detection is most easily used in the oxidative mode, as use in the reductive mode requires the removal of dissolved oxygen from the eluent.

`Hyphenated' techniques

The recent development of the so-called hyphenated techniques has improved the ability to separate and identify multiple entities within a mixture. These techniques include HPLC-MS, LC-MS(-MS), HPLC-IR and HPLC-NMR. These techniques usually involve chromatographic separation followed by peak identification with a traditional detector such as UV, combined with further identification of the compound with the MS, IR or NMR spectrometer.

Mass spectrometry as a detector for an HPLC system has gained wide popularity over the past few years. Advances in data systems and the simplification of the user interface have facilitated the ease of use of a mass spectrometer as an HPLC detector. The most common types of mass spectrometers used in HPLC are quadrupoles and ion traps. Tandem mass spectrometers (also called triple–quadrupoles) are also commonly available and are widely used in the pharmaceutical industry for the quantitative analysis of trace concentrations of drug molecules.

The process of mass analysis is essentially the same as in any other mass-spectrometric analyses that utilise quadrupole or ion-trap technology. The unique challenge to interfacing an HPLC system to a mass spectrometer is the need to convert a liquid-phase eluent into a gas phase suitable for mass spectral analysis. Modern mass spectrometers commonly utilise a technique known as atmospheric pressure ionisation (API) to accomplish this. API can be subdivided into electrospray (ion spray) ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). Each technique has its own advantages. ESI is particularly useful for the analysis of a wide variety of compounds, especially proteins and peptides. APCI is also very well suited for the analysis of a large variety of compounds, particularly the less polar organic molecules. Both techniques are very rugged and well suited to pharmaceutical analysis.

An important consideration when using API is the need for volatile mobile phase modifiers in the chromatographic separation. Acetic acid, formic acid, etc., are commonly used as acidic modifiers. Ammonium formate and ammonium acetate salts can also be used when more pH control is required for the separation. Organic modifiers are most often methanol or acetonitrile. One very important issue that must be considered when developing a method using API (electrospray, in particular) is the phenomenon of ion suppression. Co-eluting contaminants compete with the analyte of interest for ionisation, which results in a loss of signal for the analyte of interest. This can be very problematic if extremely small quantities of analyte are to be measured (as is often the case when MS is being used). Additional sample clean-up or adjustment of the chromatography to prevent co-elution of the contaminant is often necessary to correct this problem.

LC-MS(-MS) is commonly used in the pharmaceutical industry and in forensic science to analyse trace concentrations of drug and/or metabolite. MS-MS offers the advantage of increased signal-to-noise ratio, which in turn lowers the limits of detection and quantification easily into the sub ng/mL range. MS-MS is also a very useful technique in the qualitative identification of previously unidentified metabolites of drugs, which thus makes MS-MS a very powerful technique in research laboratories. Several recently published studies have employed MS-MS as a high-throughput analytical technique in the pharmaceutical industry.

HPLC-IR has proved to be an effective method for detection of degradation products in pharmaceuticals. IR provides spectral information that can be used for compound identification or structural analysis. The IR spectra obtained after HPLC separation and IR analysis can be compared with the thousands of spectra available in spectral libraries to identify compounds, metabolites and degradation products. An advantage of IR spectroscopy is its ability to identify different isomeric forms of a compound based on the different spectra that result from alternative locations of a functional group on the compound. Unlike MS, IR is a non-destructive technique in which the original compound is deposited on a plate as pure, dry crystals and can be collected afterwards if desired.

HPLC-NMR is also growing in popularity for the identification of various components in natural products and other disciplines. Although a relatively new hyphenated system, HPLC-NMR has several applications on the horizon. The miniaturisation of the system and the possibility of measuring picomolar amounts of material are both areas currently attracting a large amount of attention. Also, in the future HPLC-NMR systems will be interfaced with other detectors, such as Fourier transform IR and mass spectrometers. This will provide a wide range of possibilities for further applications, which could include the analysis of mixtures of polymer additives and the ability to identify unknowns without first having to isolate them in a pure form.

Data systems

Since the detector signal is electronic, use of modern data-acquisition techniques can aid in the signal analysis. In addition, some systems can store data in a retrievable form for highly sophisticated computer analysis at a later time.

The main goal in using electronic data systems is to increase analysis accuracy and precision, while reducing operator attention. There are several types of data systems, which differ in terms of available features. In routine analysis, where no automation (in terms of data management or process control) is needed, a pre-programmed computing integrator may be sufficient. If higher control levels are desired, a more intelligent device is necessary, such as a data station or minicomputer. The advantages of intelligent processors in chromatographs are found in several areas. First, additional automation options become easier to implement. Second, complex data analysis becomes more feasible. These analysis options include such features as run-parameter optimisation and deconvolution (i.e. resolution) of overlapping peaks. Finally, software safeguards can be designed to reduce accidental misuse of the system. For example, the controller can be set to limit the rate of solvent switching. This acts to extend column life by reducing thermal and chemical shocks. In general, these stand-alone, user-programmable systems are becoming less expensive and increasingly practical.

Other more advanced features can also be applied to a chromatographic system. These include computer-controlled automatic injectors, multi-pump gradient controllers and sample fraction collectors. These added features are not found on many systems, but they do exist and can save much time and effort for the chromatographer.

Columns

Typical HPLC columns are 10, 15 and 25 cm in length and are fitted with extremely small-diameter (3, 5 or 10 μm) particles. The columns may be made of stainless steel, glass-lined stainless steel or polyetheretherketone (PEEK). The internal diameter of the columns is usually 4.0 or 4.6 mm for traditional detection systems (UV, FL, etc.); this is considered the best compromise of sample capacity, mobile phase consumption, speed and resolution. However, if pure substances are to be collected (preparative scale), larger-diameter columns may be needed. Smaller-diameter columns (2.1 mm or less) are often used when HPLC is coupled with MS. The smaller-diameter columns also have the advantage of consuming less solvent because of their lower optimal flow rates. HPLC systems sold today can often be plumbed with narrower tubing diameters to take advantage of the benefits of these smaller column diameters.

Packed capillary microcolumns are also gaining wider use when interfacing an HPLC system to a mass spectrometer and extremely low flow rates (nL/min) are needed to maximise sensitivity for the analysis of proteins and peptides.

Packing of the column tubing with small-diameter particles requires high skill and specialised equipment. For this reason, it is generally recommended that all but the most experienced chromatographers purchase pre-packed columns, since it is difficult to match the high performance of professionally packed HPLC columns without a large investment in time and equipment.

In general, HPLC columns are fairly durable and one can expect a long service life unless they are used in some manner that is intrinsically destructive, such as with highly acidic or basic eluents, or with continual injections of 'dirty' biological or crude samples. It is wise to inject some test mixture (under fixed conditions) into a column when it is new and to retain the chromatogram. If questionable results are obtained later, the test mixture can be injected again under specified conditions. The two chromatograms are compared to establish whether or not the column is still useful.

Column dimensions

The description of column dimensions and assignment of a category to a size vary greatly depending on the reference cited. The following categories were suggested by Rozing *et al.* (2001), and may be more stratified than other categories.

Preparative

Preparative columns generally are of larger bore than analytical columns. Some have inner diameters as large as 100 mm and may have lengths up to 600 mm. These columns are usually packed with packing materials of larger particle size that may range from 10 μm to 50 μm in particle size. The flow rate used with these columns normally exceeds 5 mL/min.

Normal bore

The normal bore for an analytical column can range from 3.9 mm to 5.0 mm inner diameter, but the most common is 4.6 mm. This diameter is the best compromise of sample capacity, mobile phase consumption, speed and resolution. The normal flow rate for this type of column is 1.5–5 mL/min.

Minibore

A minibore or narrow-bore column has an inner diameter of 2.1–3.9 mm. The flow rate for this column size ranges from 500 μL/min to 1500 μL/min.

Microbore

Microbore columns have a 1.0–2.1 mm inner diameter and have flow rates of 100–500 μL/min. These small columns save solvent, are popular when HPLC is interfaced with MS and provide increased sensitivity in situations of limited sample mass.

Capillary

Capillary columns have inner diameters of 50 μm to 1.0 mm and have a typical flow rate of 0.2–100 μL/min. So-called 'nanobore' columns usually fall into the lower end of this size range. The inner surface of these very narrow columns must be extremely smooth. Since this is difficult to obtain with stainless-steel columns, many of these columns are glass-lined stainless steel. Fused-silica columns also fall into this category.

Packing materials

Silica-based packing materials

Silica ($SiO_2 \cdot xH_2O$) is the most widely used substance for the manufacture of packing materials. It consists of a network of siloxane linkages (Si–O–Si) in a rigid three-dimensional structure that contains interconnecting pores. The size of the pores and the concentration of silanol groups (Si–OH) that line the pores can be controlled in the manufacturing process. Thus, a wide range of commercial products is available with surface areas that range from 100 m^2/g to 800 m^2/g and average pore sizes from 4 nm to 33 nm.

Spherical packing materials are now the only types being introduced for analytical HPLC. Irregularly shaped materials are still being used to pack preparative columns. The silanol groups on the surface of silica give it a polar character, which is exploited in adsorption chromatography using organic eluents. Silanol groups are also slightly acidic and hence basic compounds are adsorbed particularly strongly. Unmodified silicas can thus be used with aqueous eluents for the chromatography of basic drugs.

Silica can be altered drastically by reaction with organochlorosilanes or organoalkoxysilanes to give Si–O–Si–R linkages with the surface. The attachment of hydrocarbon chains to silica produces a non-polar surface suitable for RPC in which mixtures of water and organic solvents are used as eluents. The most popular material is octadecylsilica (ODS), which contains C_{18} chains, but materials with C_1, C_2, C_4, C_6, C_8 and C_{22} chains are also available. The latest silica-based bonded phase to be introduced is a long C_{30} phase, which has 24% carbon coverage to make it one of the most retentive phases available.

During manufacture, such materials may be reacted with a small monofunctional silane (e.g. trimethylchlorosilane) to further reduce the number of silanol groups that remain on the surface (end-capping). Recent advances in column technology include multiple reactant end-capping, use of Type B silica (high purity, low trace metals, low acidity) and encapsulating the surface with a polymeric phase. These silicas are often referred to as 'base-deactivated' and are especially useful in RPC in the pH range 4–8 when many basic compounds are partially ionised. Variations in elution order on different commercial packing materials of the same type (e.g. ODS) are often attributed to differences in surface coverage and the presence of residual silanol groups. For this reason it must not be assumed

that a method developed with one manufacturer's ODS column can be transferred easily to another manufacturer's ODS column.

Speciality silicas A vast range of materials have intermediate surface polarities that arise from the bonding to silica of organic compounds that contain groups such as phenyl, cyano, nitro, amino, fluoro, sulfono and diol. There are also miscellaneous chemical moieties bound to silica, as well as polymeric packings, designed to purify specific compounds.

Phenyl Propylphenylsilane ligands attached to the silica gel show weak dipole-induced dipole interactions with polar analytes. Usually this type of bonded phase is used for group separations of complex mixtures. Newer phases have phenyl backbones that allow π–π (stacking) interactions. These are recommended for peptide mapping applications. Amino compounds show some specific interactions with phenyl-modified adsorbents.

Cyano A cyano-modified surface is very slightly polar. Columns with this phase are useful for fast separations of mixtures that consist of very different components. These mixtures may show a very broad range of retention times on the usual columns.

Cyano-columns can be used in both normal-phase and reversed-phase modes of HPLC.

Amino Amino-phases are weak anion exchangers. This type of column is mainly used in normal-phase mode, especially for protein separation and also the selective retention of aromatic compounds.

Fluoro A newer type of silica packing has fluorinated surfaces. This phase is generally more hydrophilic than phases with hydrocarbons of similar chain length. It has increased retention and unique selectivity for halogenated organic compounds and lipophilic compounds.

Sulfono Sulfonic functional groups separate compounds on the basis of hydrophobic interactions. These packing materials allow the isocratic separation of mixtures that normally require gradient elution.

Diols Diols are slightly polar adsorbents for normal-phase separations. These are useful for separating complex mixtures of compounds with different polarities that usually have a strong retention on unmodified silica.

Miscellaneous Cyclodextrins, amylose, avidin, ristocetin, nitrophenylethyl, carbamate, ester, diphenylethyldiamine and Pirkle-type functional groups are all bound to silica packing material to enable enantiomeric separations. These columns are often referred to as chiral columns. Strong ion exchangers are also available, in which sulfonic acid groups or quaternary ammonium groups are bonded to silica. These packing materials are useful for separating proteins. There are also proprietary functional groups added to silica packing materials for a variety of uses. These include petrochemical analysis, environmental analysis, detection of DNA adducts, purification of double-stranded DNA, separation of cationic polymers and separation of nitro-aromatic explosives.

For size-exclusion chromatography, a special type of silica is available that has a narrow range of pore diameters. Size-exclusion chromatography can be complicated by adsorption, but this can be reduced by treating the surface with trimethylchlorosilane.

pH range The useful pH range for silica-based columns is 2–8, since siloxane linkages are cleaved below pH 2 while at pH values above 8 silica may dissolve. However, the pH range may be extended above 8 if a pre-column packed with microparticulate silica is included between the pump and injector to saturate the eluent before it enters the analytical column.

Zirconia packing materials

Zirconia is a metal oxide that is more chemically and thermally stable than silica. It can be used for separations conducted at temperatures as high as 200°C and is unaffected by changes in ionic strength or organic content of the mobile phase. Zirconia packings have a wider pH range and are especially useful for basic separations at pH 10 or higher, where silica gel starts to dissolve. Zirconia can be used for RPC and is extremely stable and efficient through surface modification with polymer or carbon coatings. Other chemical modifications of zirconia produce packing materials suitable for normal-phase or ion-exchange chromatography.

Polymer-based packing materials

Several packing materials based on organic polymers are available. For example, unmodified styrene–divinylbenzene co-polymers have a hydrophobic character and can be used for RPC. Although they traditionally give lower column efficiencies than ODS, this has improved greatly in the past few years. Polymeric materials are best when separation conditions require a mobile phase that can go beyond the upper pH limits of silica gel (usually pH 6.5–7), as they have the advantage of being stable over a wide pH range. Polymeric materials also provide selectivity and retention characteristics different from those of silica-based reversed-phase packings. They also avoid problems associated with residual silanol groups (e.g. peak tailing). Ion-exchange materials of the styrene–divinylbenzene type are also available in which sulfonic acids, carboxylic acids or quaternary ammonium groups are incorporated in the polymeric matrix.

Monolithic columns

Monoliths are chromatographic columns that are cast as continuous homogeneous phases rather than packed as individual particles, creating porous rods of polymerised silica that are mechanically stable. Monolithic phases have flow-through pores with macroporosity ($\sim$2 µm) and mesopores, which are diffusive pores with an average pore diameter that can be controlled. To create the column, a silica gel polymer is formed, which, after ageing, is dried into the form of a straight rod of highly porous silica with the bimodal pore structure. The rod is then encased (or clad) in a PEEK cover, ensuring that there is absolutely no void space between the silica and PEEK material. The pore structure yields a very large internal surface area and ensures high-quality separations. In addition, the high porosity of the column means that very high flow rates can be used with lower pressures. This enables separations in a fraction of the time needed when using a column with conventional packing materials.

Recently, a polymeric monolithic column was introduced. It contains a poly(glycidylmethacrylate–ethyleneglycol-dimethacrylate) co-polymer that has functional groups added to make various types of stationary phases.

Maintenance

An effective maintenance programme is essential to keep an HPLC system in proper working order. The maintenance programme should include preventive, periodic and necessary repairs of the HPLC system. This programme is essential to ensure that all the components of the system are in proper working condition. In this section, the general maintenance of columns, pumps, injection valves and detectors is discussed. For information on the functions and uses of these components, refer to the earlier sections of this chapter.

It is always recommended that the maintenance guidelines provided with the system should be consulted to ensure compliance with the manufacturers' suggestions. This guide should be used whenever maintenance is required.

Columns

The column is an essential key to good chromatography and its maintenance ensures proper functionality of the HPLC system. High back-pressures, poor resolution, non-uniform peak symmetry and decreasing retention times are several signs that may indicate that the column is in need of repair or is failing.

Column degradation is inevitable, but a column's life can be prolonged if it is maintained properly. Flushing of a column with a mobile phase of high elution strength after sample runs is essential. When a column is not in use, it should be capped to prevent it from drying out. Particulate samples should be filtered and, when possible, a guard column should be utilised. Column regeneration can instil some life into a column, but preventive maintenance is the vital key to prevent premature degradation.

Pumps

The pump forces the mobile phase through the HPLC system. A steady pump pressure is needed to ensure reproducibility and accuracy. Inability to build pressure, high pressures or leakage may indicate that the pump is not functioning correctly.

Pumps are typically known to be robust, but adequate maintenance must be performed to maintain that characteristic. Good maintenance practice includes replacing components, such as inlet check valves, outlet check valves, frits, pump seals and piston rods, on a routine schedule, based on the amount of usage. Proper maintenance of the pump system minimises downtime.

Injection valves

Injection valves play the role of directing injected volumes into the mobile phase, where they then travel on to the column. Proper valve function is a necessity to ensure reproducibility between injections. The symptoms of injection valve failure are low pump pressure, leakage or inadequate inert gas pressure to the switch valve.

The seals of the injection valve may eventually falter, after numerous injections. Replacement of these seals is necessary to maintain system reproducibility with respect to injections made.

Detectors

Detector maintenance is generally performed as needed. Baseline drift, erratic baseline and decreasing response may be indicators of a failing detector.

A malfunctioning or contaminated flow cell can also cause baseline drift. The cell should be flushed regularly with water to remove salts when using mobile phases of high salt concentration. An organic mobile phase of high elution strength should be used to remove any organic residue that may remain in the cell. An erratic baseline can occur because of an air bubble in the flow cell. Increasing the flow rate may push the bubble out of the cell. Decreasing responses can also result from a decrease in lamp intensity.

Eluent preparation

The quality of solvents and inorganic salts is an important consideration. Soluble impurities can give noisy baselines and spurious peaks or can build up on the surface of the packing material, eventually changing chromatographic retention. Furthermore, the eluate may need to be collected for further experimentation and all contamination must be avoided. In addition, particulate matter should be removed, otherwise pump filters, frits and tubing can become blocked.

There is now commercially available a wide range of HPLC-grade solvents that are free from particulate matter, have low residues on evaporation and have guaranteed upper limits of UV-absorbing and fluorescent impurities. However, if a detector is not to be operated at its maximum sensitivity, analytical grade solvents may be used. A general rule of thumb is to use the highest purity of solvent that is available and practical depending on the particular application.

Air dissolved in the mobile phase can lead to problems. The formation of a bubble in a pump head usually reduces or stops eluent flow, while bubbles formed in the detector can give spurious peaks. One commonly used remedy is to degas the eluent using an in-line vacuum chamber. HPLC solvents are pumped from the reservoirs into a vacuum chamber in line with the HPLC eluent flow. This method ensures continuous and efficient degassing of the mobile phase. Vacuum degassing can also be performed off-line by applying a weak vacuum to the mobile phase reservoir while sonicating. Off-line techniques do not offer the advantage of continuous degassing throughout the analysis. Eluents can also be degassed by purging with helium, which has a very low solubility and drives the air out. This technique can be performed on-line and be controlled by the HPLC system, or off-line. Care must always be taken when degassing eluents that contain volatile components to avoid changing the composition.

It is convenient to prepare eluents as volume-plus-volume mixtures of solvents (i.e. the volume of each solvent is measured separately and then they are mixed). Volume changes can occur when solvents are mixed (e.g. methanol and water show a contraction in volume), which must be remembered if the volume of only one solvent is measured and the second solvent is added to make up to volume.

True pH values can be measured only in aqueous solutions and any measurements made with a pH meter in aqueous–organic solvents should be described as 'apparent pH'. In general, the apparent pH of a buffer solution rises as the proportion of organic solvent in the aqueous mixture increases. When an eluent is prepared it is usually best to dissolve the required buffer salts in water at the appropriate concentrations, adjust the pH and then mix this solution (v/v) with the organic solvents.

Separation techniques

Isocratic

When the mobile-phase composition does not change throughout the course of the run, it is said to be isocratic. A mixed mobile phase can be delivered at a constant ratio by the pumps themselves or the solvent mixture can be prepared prior to analysis and pumped through a single reservoir. This is the simplest technique and should be the method of first choice when developing a separation.

Gradient elution

HPLC can be performed with changes in composition over time (gradient elution). The elution strength of the eluent is increased during the gradient run by changing polarity, pH or ionic strength. Gradient elution can be a powerful tool to separate mixtures of compounds with widely different retentions. A direct comparison can be drawn with temperature programming in gas chromatography (GC; see Chapter 40).

Eluent gradients are usually generated by combining the pressurised flows from two pumps and changing their individual flow rates with an electronic controller or data system, while maintaining the overall flow rate constant. Alternatively, a single pump with a low sweep volume can be used in combination with a proportioning valve, which controls the ratio of two liquids that enter the pump from two liquid reservoirs. Equipment and data systems that allow the gradient to take almost any conceivable form (e.g. step gradients, concave and convex gradient curves) are commonly available. The gradient can be programmed to return the system to the original eluent composition for the next analysis.

While most, if not all, commercially available pumps are capable of performing reliable gradient elutions, there are some potential difficulties. The technique can be very time-consuming, as the column must be reconditioned with the initial eluent between runs. This drawback can be overcome by utilising a column-switching apparatus (see earlier in this chapter). In addition, drifting of the detector response and the appearance of spurious peaks that arise from solvent impurities may occur. While isocratic elution is usually favoured over gradients for simplicity, gradient elution can be a very important and useful technique in the separation of complex mixtures.

Recently, the use of 'fast gradient' separation has enabled the implementation of high-throughput analysis in laboratories with a high sample load.

Derivatisation

Derivatisation involves a chemical reaction that alters the molecular structure of the analyte of interest to improve detection and/or chromatography. In HPLC, derivatisation of a drug is not usually necessary to achieve satisfactory chromatography. This applies to compounds of all polarities and molecular weights and is an important advantage of HPLC over GC. Derivatisation is used to enhance the sensitivity and selectivity of detection when available detectors are not satisfactory for the underivatised compounds. Both UV-absorbing and fluorescent derivatives have been used widely. UV derivatisation reagents include *N*-succinimidyl-*p*-nitrophenylacetate (SNPA), phenylhydrazine and 3,5-dinitrobenzoyl chloride (DNBC), while fluorescent derivatives can be formed with reagents such as dansyl chloride (DNS-Cl), 4-bromomethyl-7-methoxycoumarin (BMC) and fluorescamine. The characteristics of a good derivative in HPLC are similar to those in GC (stability, low background, convenience, and so on).

Derivative formation can be carried out before the sample is injected on to the column (pre-column) or by on-line chemical reactions

between the column outlet and the detector. Such post-column reactions generally involve the addition of reagents to the eluent. With pre-column derivatisation there are no restrictions on reaction conditions (e.g. solvent, temperature) and a large excess of reagent can be used, as this can be separated from the derivatives during the chromatography. The major drawback of pre-column reactions is the need to obtain reproducible yields for accurate quantification, which is best achieved when the reactions proceed to completion. Furthermore, it is important that the products of pre-column derivatisation reactions be characterised fully. With post-column derivatisation, the reaction is well controlled by the flow rates of eluate and reagents, temperature, etc. Hence, it is less necessary for the reaction to proceed to completion or even for the chemistry to be understood as the system is calibrated by the injection of known quantities of the reference standards. A much more detailed discussion can be found in Snyder *et al.* (1997).

Chiral separation

Separation of compounds by chiral chromatography began in the early 1980s. At that time, the separation of enantiomeric compounds was one of the most challenging problems in chromatography. However, in recent years more than 100 chiral columns have been made available. These columns are based on several different approaches to solving the many enantiomeric separation problems. Chiral columns are used in a variety of different applications that range from pharmacokinetic and pharmacodynamic studies to measuring enantiomeric impurity of amino acids.

Chiral stationary phases (CSPs) are designed to separate optical isomers. The use of these columns provides an efficient and economical way to separate optical isomers by HPLC. CSPs are used both for resolving optical isomers to determine enantiomeric purity and for isolating enantiomerically pure compounds.

Figure 41.2 shows the separation of enantiomers of flurbiprofen. Enantiomers were separated on a CHIRALPAKbAD-RHTM column using methanol–0.1% trifluoroacetic acid (TFA) as the mobile phase. This separation was performed at 15°C to improve selectivity.

The columns can be categorised according to two criteria: class and origin. The class category is based on the structural properties of the chiral selector. The category is made up of five different column types: macrocyclic, polymeric, π–π associations, ligand exchange, miscellaneous) plus hybrids. The macrocyclic chiral columns have had the largest impact on analytical enantiomeric separations. The origin category separates columns according to their source and classifies them into three types: naturally occurring, semisynthetic and synthetic chiral selectors.

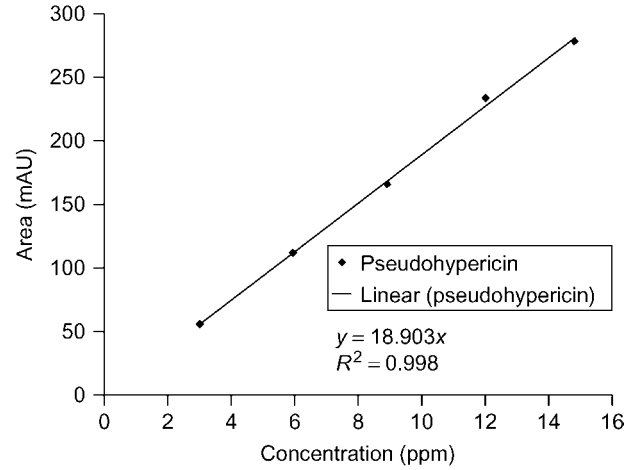

Figure 41.3 Example of a calibration curve for pseudohypericin.

High-speed/high-temperature HPLC

The speed of a chromatographic method directly affects the economy and operating cost of the separation. High-speed HPLC is accomplished by using short microbore columns packed with small particles (3 µm). In addition, the use of higher temperatures increases the speed of HPLC separations through the 5- to 10-fold decrease in eluent viscosity upon an increase of the eluent's temperature from 25°C to 200°C. High-temperature/high-speed HPLC is not universally useful because of several limitations. Silica-based stationary phases are unstable in aqueous media at temperatures above 50–60°C. Some detectors are also not able to tolerate high temperatures.

Quantitative analysis

The quantification methods incorporated in HPLC derive mostly from GC methods. The basic theory for quantification involves the measurement of peak height or peak area. To determine the concentration of a compound, the peak area or height is plotted versus the concentration of the substance (Figure. 41.3). For peaks that are well resolved, both peak height and peak area are proportional to the concentration. Three different calibration methods, each with its own benefits and

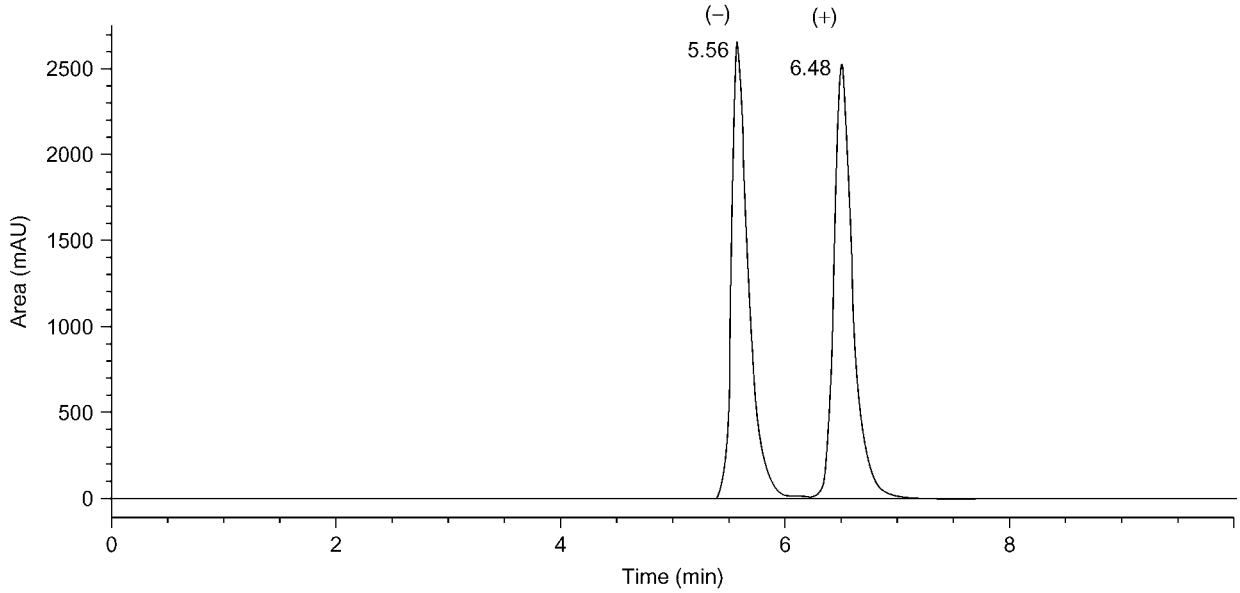

Figure 41.2 Chiral separation of the (+) and (−) enantiomers of flurbiprofen.

limitations, can be employed in quantitative analysis: external standard, internal standard and the standard addition method.

External standard

The external standard method is the simplest of the three methods. The accuracy of this method is dependent on the reproducibility of the injection of the sample volume. To perform this method, a standard solution of known concentration of the compound of interest is prepared. A fixed amount, which should be similar in concentration to the unknown, is injected. Peak height or area is plotted versus the concentration for each compound. The plot should be linear and go through the origin. The concentration of the unknown is then determined according to Equation (41.3).

$$\text{Concentration}_{\text{unknown}} = \left(\frac{\text{Area}_{\text{unknown}}}{\text{Area}_{\text{known}}}\right) \times \text{Concentration}_{\text{known}} \qquad (41.3)$$

The calibrator concentrations should cover the range of the likely concentration in the unknown sample. Only concentrations read within the highest and lowest calibration levels are acceptable. Concentrations read from an extrapolated regression line may not be accurate. This applies to all the quantification methods.

Internal standard

Although each method is effective, the internal standard method tends to yield the most accurate and precise results. In this method, an equal amount of an internal standard, a component that is not present in the sample, is added to both the sample and the standard solutions. The internal standard selected should be chemically similar to the analyte, have a retention time close to that of the analyte and derivatise in a similar way to the analyte. For biological samples, the internal standard should extract similarly to the analyte without significant bias towards the internal standard or the analyte. Additionally, it is important to ensure that the internal standard is stable and that it does not interfere with any of the sample components. The internal standard should be added before any preparation of the sample so that extraction efficiency can be evaluated. Quantification is achieved by using ratios of peak height or area of the component to the internal standard (Equation 41.4).

$$\text{Concentration}_{\text{unknown}} = \left(\frac{\text{Area}_{\text{internal standard in known}}}{\text{Area}_{\text{internal standard in unknown}}}\right) \times \left(\frac{\text{Area}_{\text{unknown}}}{\text{Area}_{\text{known}}}\right) \text{Concentration}_{\text{known}} \qquad (41.4)$$

Standard addition method

The third method for quantification is the standard addition approach. This is especially useful when there is a problem with interference from the sample matrix, since it cancels out these effects. To perform this quantification, the sample is divided into two portions, so that a known amount of the analyte (a spike) can be added to one portion. These two samples, the original and the original-plus-spike, are then analysed. The sample with the spike shows a larger analytical response than the original sample because of the additional amount of analyte added to it. The difference in analytical response between the spiked and unspiked samples results from the amount of analyte in the spike. This provides a calibration point to determine the analyte concentration in the original sample. The method has a drawback if only a small volume of sample is available. Equation (41.5) is used for this method.

$$\text{Amount}_{\text{unknown}} = \frac{\text{Area}_{\text{unspiked}}}{\text{Area}_{\text{spiked}}\text{Area}_{\text{unspiked}}} \times \text{Amount}_{\text{spiked}} \qquad (41.5)$$

Validation

It is important to use a validated HPLC method when carrying out analyses. Typical analytical characteristics evaluated in an HPLC validation may include precision, accuracy, specificity, limit of detection, limit of quantification, linearity and range. Some appropriate suggestions for LC validation for postmortem and body fluids samples are published in the *SOFT/AAFS Forensic Toxicology Laboratory Guidelines* (www.soft-tox.org). It is important to consider the guidelines of the US Food and Drug Administration (FDA; www.fda.gov/cder/guidance) and US Pharmacopoeia (USP; www.usp.org) when validating HPLC methods used for pharmaceutical samples. USP 24 section <1225> provides guidance on the validation of compendial methods including definitions and determination. International Conference on Harmonisation (ICH) guidelines (www.ich.org) provide suggestions concerning the validation of pharmaceuticals. Valuable sources of information providing regulatory guidance may be found on the FDA website at www.fda.gov/cder/guidance.

System suitability tests evaluate the function of the overall HPLC system. This includes all parts that make up a system, such as the instrument, reagents, packing material, details of the procedure and even the analyst. These tests imply that all the components of a system constitute a single system of which the overall function can be tested. These tests are very valuable and have been accepted in general application because reliable and reproducible chromatographic results are based on a wide range of specific parameters.

Most laboratories have a standard operating procedure that outlines the specifications of running a systems suitability test. For example, in pharmaceutical analysis at least five replicate injections should be made of a single solution that contains 100% of the expected active and excipient ingredient levels. The peak response is measured and the standard deviation of that response should not exceed the limit set by the testing monograph or 2%, whichever of the two is the lower. Using the USP method, the tailing factors of the analytes should be determined. The values should not exceed 2.0. Peak-to-peak resolutions are also determined by using the USP calculations, and the value should not be lower than 1.5. The system test should be used to ensure the quality of the data and of the analysis.

Ultra-high performance liquid chromatography

Analytical laboratories are consistently called upon to reduce turnaround times without affecting quality. In the forensic laboratory, decreased analytical time translates into a quicker dissemination of data to medical examiners and law enforcement agencies. For the pharmaceutical testing laboratory, demands for greater efficiency have spread industry wide, from drug discovery to clinical trials. There are many difficult applications dealing with the separation of a high number of analytes in complex sample matrices, such as proteomics, genomics and metabolomics. These difficult analyses require a high degree of efficiency and resolution within acceptable analysis times. A faster analysis potentially assists with moving a new drug out into the market in a more timely manner.

For decades, HPLC has been an integral component of analytical laboratories involved in comprehensive drug testing. Whether it is used to quantify active ingredients and/or excipients in the pharmaceutical laboratory, for pharmacokinetic studies or analysing for drugs in biological fluids in the forensic arena, HPLC continues to be a mainstay for the quantitative analysis of drugs. Newer techniques such as ultra-high performance liquid chromatography (UHPLC) have been developed to provide faster separation without compromising data quality. Improvements in detectors, column chemistries and hardware provide faster separations and data analysis without compromising data quality. This technique is known by many general names including ultra-high performance liquid chromatography or ultra-high pressure liquid chromatography (both of which are called UHPLC), high-speed liquid chromatography and fast liquid chromatography. Instrument manufacturers have come up with their own names for this technique. Examples include: Ultra Performance

Liquid Chromatography (UPLC; Waters); Rapid Resolution LC System (RRLC; Agilent); Xtreme Liquid Chromatography (X-LC; Jasco); Accela High Speed Chromatography System (Thermo). Whatever its name, this technology has advantages and disadvantages that should be considered by those laboratories thinking about adding it to their analytical capabilities.

In liquid chromatography, packing columns with small particles is a recognised method for simultaneous improvement of efficiency, optimum velocity and mass transfer. Martin and Synge (1941) proposed the use of smaller particles and high pressure differences across the entire column length as a means of achieving greater column efficiency. Halasz *et al.* (1975) demonstrated that the fastest HPLC separations could be obtained by using the smallest particle sizes. In recent years, particle sizes in columns have decreased from 10 µm to 2.5 µm. The advantages of 1–2 µm particles have been to increase sensitivity and column efficiency while reducing analysis time. This evolution of particle size has been governed by the van Deemter equation, which describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). The design and development of smaller particles presented a number of challenges including poor loading capacity and retention due to low surface area, limited pH ranges and poor mechanical strength.

The van Deemter equation (van Deemter 1956) indicates that the most efficient particle size is 1.7 µm at a high linear velocity. This relationship shows that, as particle size decreases, the flow rate (or linear velocity) required to attain maximum resolution increases. The increase in flow rate results in a proportional increase in back pressure, i.e. smaller particles require much higher operating pressures. The pressure required for percolating mobile phase through a column packed with small particles can be prohibitive for standard HPLC hardware. Halving the particle diameter results in a four fold increase in pressure. The significant increase in back pressure presented a challenge to conventional HPLC instrument systems. To address the increasing pressure requirements, MacNair *et al.* (1997) introduced UHPLC to overcome the pressure limitations that small particles impose on conventional pumping systems. This research showed that the pressures required to run at optimum flow rates are of the order of 20 000 psi, which translates into analysis times of less than 10 minutes.

The improved resolution with UHPLC may be optimised by reduction in particle size and bridging of the column packing. This characteristic is important in terms of sample throughput and sensitivity improvement. Resolution can be enhanced by the use of longer columns packed with small particles, although optimisation of mobile phase flow rate is not always possible. Relative to isocratic mode, UHPLC generates higher resolution separations in gradient mode. The maximum peak capacity for a 3-hour gradient is also substantially enhanced by a significant reduction of the column dead time in UHPLC compared with traditional approaches.

Some drawbacks of UHPLC include the need for a dedicated instrument, with optimised high-back-pressure pumps and an injector, acquisition rate of the detector, injection cycle time, dwell volume and system dead volume. At the high pressures necessary for UHPLC, columns may age faster if they are run routinely at greater than 12 000 psi. Intracolumn temperature gradients due to back pressure generated by frictional heat can also be problematic. This issue, especially critical for the 4.6 mm i.d. columns and/or when the pressure is close to or higher than 1000 bar, can usually be resolved by reducing the column inside diameter to 1.0–2.1 mm. The internal diameters of the connection tubing are very small (0.004–0.02 inch) and are less forgiving than those of traditional systems.

Applications

UHPLC is rapidly becoming an integral part of analytical laboratories from many disciplines. The benefits of decreased analysis time without sacrifice of quality have attracted the attention of contract pharmaceutical, forensic and research laboratories. Scientists have used this technology to improve the speed of conventional HPLC separations or to develop completely new UHPLC methods.

Method development in the pharmaceutical laboratory is often tedious and time-consuming. Optimisation of the various sample handling protocols and instrument parameters can be a daunting task. UHPLC technology has decreased method development time significantly. Many laboratories use UHPLC to rapidly develop an analytical method, then transfer, or scale, the analysis to conventional HPLC conditions for routine use. The theory behind scaling of chromatographic methods involves the use of mathematics to transform the UHPLC chromatographic conditions to conventional HPLC parameters.

The drug discovery process in the pharmaceutical industry continues to evolve, with complex drugs, metabolic profiles and extraction matrices requiring new technologies in the analytical laboratories to maintain progress. Toxic drug metabolites, for example, must be identified as early as possible in the discovery process so that poor compounds will not continue down the path to production. The rapid turnaround time, sensitivity and specificity of UHPLC coupled with mass spectrometry have provided researchers with a tool to identify potential problems quickly.

The use of UHPLC is not confined to the pharmaceutical industry. The forensic and environmental arenas have benefited from it as well. Recent research has shown UHPLC, coupled with mass spectrometry (UHPLC-MS), to be a rapid and sensitive method for the simultaneous detection of amfetamine and amfetamine-like stimulants, cocaine and metabolites, and the major metabolite of Δ^9-tetrahydocannabinol in surface water and urban wastewater (Bijlsma *et al.* 2009). Additional forensic research has also demonstrated the usefulness of UHPLC-MS for the detection of drugs of abuse in urine for doping control (Ventura *et al.* 2008; Badoud *et al.* 2010). Surface water and wastewater have been analysed for the presence of antibiotics using UHPLC and quadrupole time-of-flight mass spectrometry (Ibanez *et al.* 2009).

Saving time and money without sacrificing quality is a goal of any analytical laboratory. UHPLC continues to grow in popularity owing to the multiple analytical advantages described above. The expanding number of vendors of both instrumentation and column technology is evidence that UHPLC is becoming a valuable tool in a variety of analytical disciplines.

New emerging trends

Several new trends, including hyphenated systems and micro-HPLC, are discussed in other sections of this chapter. Two other trends that deserve mention, denaturing high performance liquid chromatography and ion chromatography, are described below.

Ion chromatography

Ion chromatography (IC) is a branch of liquid chromatography that has several unique characteristics to meet the needs of ion analysis. The eluents, or mobile phases, used are often acid, alkaline or high-saline solutions. The high-pressure pump used should consist of an entirely non-metallic flow path in order to deliver these eluents reliably without corrosion issues. The ion-exchange columns provide unique ion separation capabilities, and a conductivity detector is the primary detection device used for IC. Other detectors, such as ultraviolet/visible light absorbance (UV/VIS) and electrochemical detectors are often used as well. Like all forms of liquid chromatography, IC includes a pump, injection valve, separation column and detector. Figure 41.4 is a representation of a liquid chromatographic system used to determine ions. The hardware used to perform IC is similar to that used for HPLC, although it is typically made of inert polymer as corrosive acid and base solutions are frequently used in IC. A sample introduction device and the means to record the output signal are also required.

While a number of separation and detection set-ups can be used in IC, the most commonly used approach is the combination of an ion-exchange separation with suppressed conductivity detection, i.e. the concept first described by Small, Stevens and Bauman (Small *et al.* 1975). In an ion-exchange separation, ionic analytes are separated by virtue of their electrostatic attraction to a stationary phase that carries immobilised ion-exchange sites. The ion-exchange site carries a charge that is opposite to the charge on the analyte. For example, anions are

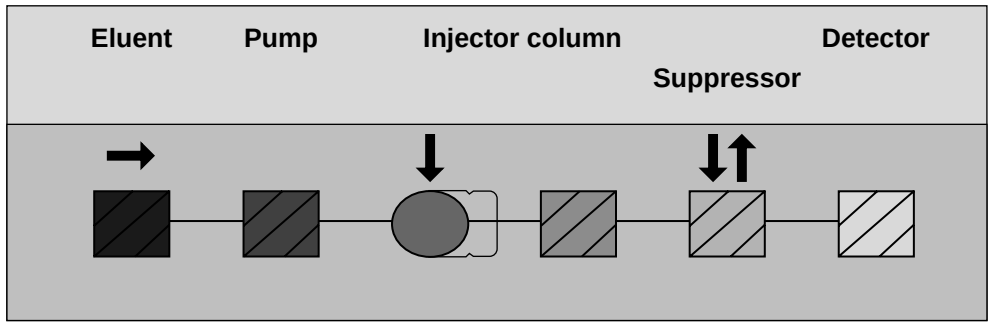

Figure 41.4 Elements of a liquid chromatography system used to determine ions.

separated using a column that carries positively charged ion-exchange sites. The more strongly an analyte interacts with the column, the longer the retention time. The relative affinity of the ion-exchange site for ions is dependent on the charge, charge density and hydrophobicity of the eluent and the analyte ion. Analytes are identified by their retention time and quantitatively determined (primarily) by peak area.

Components of an ion chromatograph

Eluent The function of the eluent (mobile phase) is primarily to provide counterions for the ion-exchange process. Secondary functions include providing a stable environment for the sample ions and providing kinetic flow of the sample ions through separation and detection. The most common eluents used in ion chromatography are sodium or potassium hydroxide or sodium or potassium carbonate/bicarbonate for anion determinations. For cations, methanesulfonic acid (MSA), sulfuric acid or nitric acid is most commonly used.

Pump The pump delivers liquid eluent to the resin-packed column. Modern pumps must deliver a minimum of detector noise while operating over a wide flow and pressure range. Because of the possibility of using strong acid and base eluents, the pump's wet components and system tubing are constructed from PEEK.

Injector A fixed length of tubing of known internal diameter constitutes the 'sample loop'. The sample loop can be filled either manually or by an autosampler. A high-pressure six-port or ten-port two-way valve is switched in-line into the flowing eluent stream and the loop contents are carried on to the ion-exchange guard and separator column.

Separation column (and guard column) The separation column provides stable support for stationary phase ions that act as active sites in the dynamic ion-exchange process. The active sites compete with the counterions in the mobile phase for retention of analytes. The choice of column is key to the separation of important analytes, capacity to retain any given sample component and analyte peak efficiency, i.e. minimal band broadening in the ion chromatographic separation process. The sample ions are separated into discrete bands as they move through the column. Different ions migrate at different rates, depending on their relative affinities for the resin. A guard column of shorter length serves to protect the separation column. It is typically packed with the same resin as the separation column or a neutral (non-functionalised) resin to remove possible hydrophobic contaminants.

Conductivity detector A conductivity detector measures the electrical conductance of analyte ions as they pass through a conductivity cell. Conductivity detection is the primary method of detection used in IC. The detector produces a signal depending on a chemical or physical property of an analyte. For optimal results, a detector must exhibit high sensitivity, low noise and a wide linear range. The conductivity detector is ideal for small inorganic and organic ions.

Chemical suppression technology is the breakthrough in IC that has allowed it to become a preferred technique for ions. The suppressor is a post-column reactor specific to IC, which allows more sensitive conductivity detection. Conductivity detection is a universal (bulk property) detector for IC as all ions are conductive. Chemical suppression provides a significant improvement in signal-to-noise ratio, making ion chromatography a truly practical tool.

Figure 41.5 illustrates the effect of chemical suppression in a typical ion-exchange separation of anions with conductivity detection. The effluent from the analytical column is monitored by conductivity as it passes immediately from the column; a chromatogram similar to that

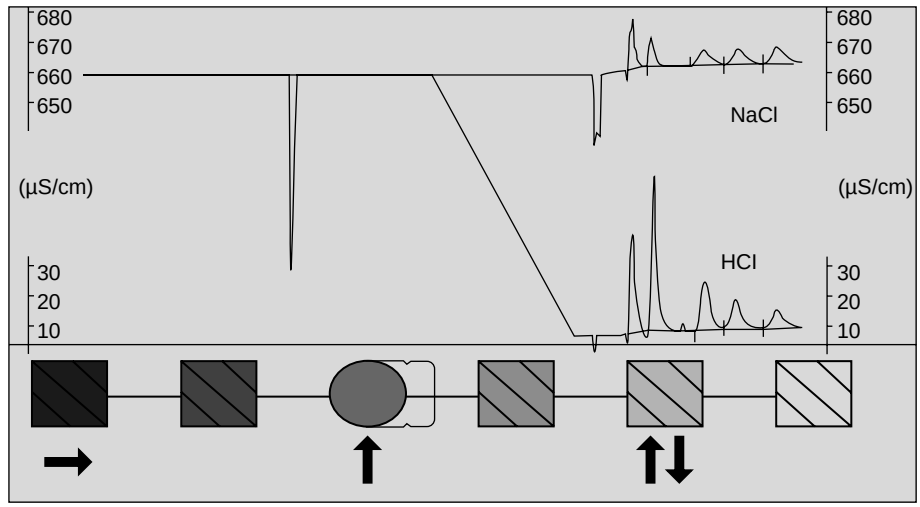

Figure 41.5 Effect of chemical suppression in ion-exchange chromatography of anions with conductivity suppression.

shown in the upper right area of the figure would be obtained. This chromatogram illustrates a very high background from the conductive eluent, a large counterion peak at the column void volume and analyte peaks with low response. In contrast, by passing the column effluent through a chemical suppressor, the chromatogram shown at the bottom is obtained. The chemical suppressor exchanges cations in the eluent for hydronium ions. The source of hydronium used in IC systems is dilute sulfuric acid. The hydronium ions then neutralise the carbonate ions in the eluent, resulting in fluoride, chloride and sulfate ions in carbonic acid, which is slightly dissociated resulting in typical background detector signals of 1–5 μS/cm. Counterions of the analytes, which are cationic and thus elute in the void, are also removed. This accounts for the absence of the large system peak after suppression. A third advantage is that the anions are now paired with hydronium counterions, resulting in a much larger response for these ions since they enter the conductivity detector cell in a more conductive form. The detector response for a peak using conductivity detection is proportional to the sum of the equivalent conductivity of the anion and its associated cation. The equivalent conductivities for anions and cations are typically between 35 and 80 S cm^2/mol. The hydronium ion, H_3O^+, with 350 S cm^2/mol and hydroxide ion (regenerants for cation separations) with 198 S cm^2/mol provide the only exceptions.

The use of suppression in IC results in an increase in detector response as much as 10-fold for anion methods. Suppressor design and performance have expanded the range of IC methods from packed-bed batch suppression to membrane-based continuous suppression. The first chemical suppressor was a batch suppressor and, while it delivered good signal-to-noise ratio, it had to be frequently removed from the chromatographic separation stream for regeneration. With modern technologies, automated micro-packed column suppressors automate the regeneration and rinsing of the suppressor channels. Figure 41.6 illustrates the analysis of anions using suppressed ion chromatography with conductivity detection.

Other detectors for ion chromatography

Amperometric detection Some inorganic and organic ion determinations are not well suited for conductivity detection. Amperometric detection is used to measure the current or charge resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. For analytes that can be oxidised or reduced, detection is sensitive and highly selective since many potentially interfering species cannot be oxidised or reduced and therefore are not detected. When a single potential is applied to the working electrode, the detection method is DC amperometry. Pulsed amperometry (PAD) employs a repeating sequence of potentials. Separation of carbohydrates by high-pH anion exchange followed by PAD detection is used for the detection of wood sugars, mannitol, fucose, arabinose, rhamnose, galactose, glucose, sucrose, xylose, mannose, fructose and ribose (Cheng, Kaplan 2003). This use of IC is for evaluating pulping and biomass conversion operations.

Ultraviolet/visible light absorbance Commonly used in HPLC, UV/VIS is also used in IC as a detection device complementary to conductivity detection for the identification and quantification of aromatic and heterocyclic molecules, transition metals and certain inorganic ions. UV/VIS detection used in tandem with conductivity detection quantifies trace level analytes in the presence of interfering high-concentration ions by measuring the amount of light that they absorb at a specific wavelength. An example is measurement of nitrite ions in the presence of high chloride ions (high-saline solution) (Miura, Hamada 1999). UV/VIS detection using post-column reaction chemistry permits selective measurement of transition metals (Atanassova *et al.* 2004).

Mass spectrometry Combining IC separations with mass spectrometry detection using standard IC conditions improves MS performance because the suppressor removes high-ionic-strength eluents. IC with MS detection offers high specificity of analytes by avoiding co-elution interferences and background interferences. It is also possible to quantify target analytes and identify unknowns.

Examples of ion chromatography applications in the pharmaceutical industry

- Sodium in buffered nasal drops
- Chloride in dextrin injection (USP)
- Cations in artificial saliva
- Neomycin by pulsed amperometric detection
- Triethylamine in cefixime (USP method)
- Betanacol chloride – Assay (BP method)
- Chloride and sulfate in gabapentin
- Sodium in basalazide
- Acetate in pantoprazole sodium
- 2-Aminoethanol in pantoprazole sodium
- Residual TFA in bulk drugs
- Molybdenum in tinidazole
- Tetrafluoroborate in antibiotics
- Ethyl sulfate in indinavir sulfate (IP method with Supp. 3)

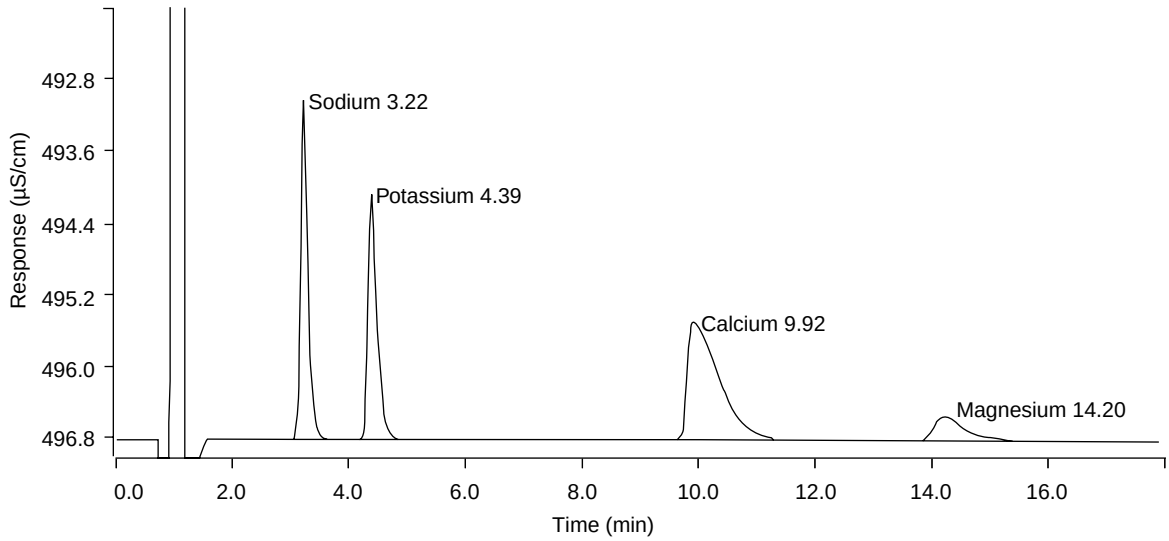

Figure 41.6 Anion analysis by suppressed ion chromatography.

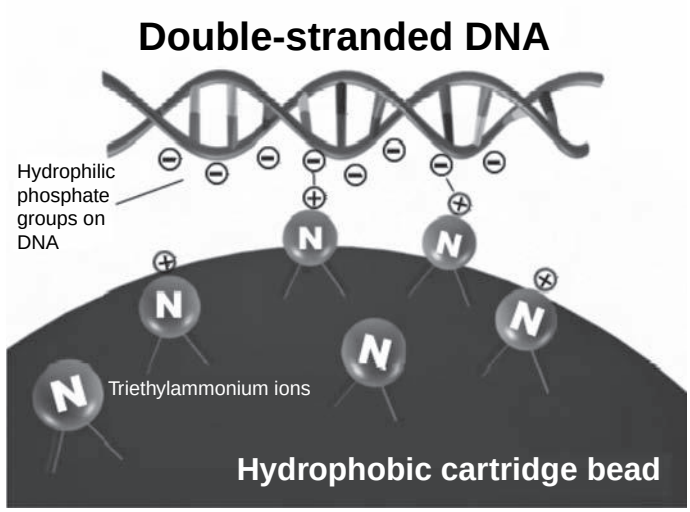

Figure 41.7 Interaction of DNA with the stationary phase in Denaturing HPLC.

- *N*-Methylpyrolidone in cefepime hydrochloride (USP Method)
- Sulfate and sulfamate in topiramate
- Pamidronic acid – assay
- Phosphate and phosphite as impurities in pamidronic acid
- Residual acetic acid and formic acid in ceftriaxone.

Denaturing high performance liquid chromatography

HPLC has also found applications in the field of molecular and clinical genetics in the form of denaturing HPLC (DHPLC). Some of the main applications of DHPLC include discovery of mutations and variations (Liu *et al.* 1998; Luquin *et al.* 2010), screening of genetic diseases (Kosaki *et al.* 2005; Costabile, Quach 2006) and purification and analysis of nucleic acid (Danielson *et al.* 2005). The technique is also finding important applications in forensic science, for example in pharmacogenomic investigations (Aquilante *et al.* 2006) and in DNA analyses (Budowle, van Daal 2009). A full account of the role of pharmacogenomics in forensic toxicology is given in Chapter 25.

For separations of DNA fragments, a specific combination of stationary and mobile phases is required. In general, the stationary phase is made of a C_{18} polystyrene–divinylbenzene bead polymer. Each of these beads is approximately 3 µm in size. The matrix is very hydrophobic. Binding of hydrophilic DNA can be highly efficient when the positive charges of the ion-pairing reagent TEAA (triethylammonium acetate) interact electrostatically with negatively charged DNA. The positively charged ammonium ion of TEAA interacts electrostatically with the phosphate groups on the DNA backbone. The triethyl groups are hydrophobic, and the result is that the hydrophilic DNA acquires a hydrophobic outer coating and can thus interact with the column matrix. Varying concentrations of acetonitrile in the mobile phase are used in order to elute the attached DNA fragments off the stationary phase. Figure 41.7 illustrates the interaction of DNA molecules with the stationary phase.

Detection of mutations in DNA fragments by DHPLC involves a process known as partial denaturation. In this process, heat is applied and the double-stranded DNA unwinds and separates into single strands by the breaking of hydrogen bonding between the cytosine/guanine and adenosine/thymine base-pairs. The sample is then cooled to allow the strands to re-hybridise (or anneal). If a mixture of two or more DNA fragments is heat denatured and allowed to cool, a combination of homoduplexes and heteroduplexes is formed. The homoduplexes represent the original components of the mixture, whereas the heteroduplexes are formed by cross-hybridisation of the different contributors of the mixture. Heteroduplexes derived from DNA fragments from a heterozygous individual have different denaturing (or melting) properties from those of perfectly matched homoduplexes formed from DNA fragments from a homozygous individual and it is this difference in temperature stability that is exploited by DHPLC. Thus, the annealed mixture is introduced onto a column heated to an optimum temperature (usually >50°C) to induce partial denaturation; with an increasing gradient of a non-polar solvent such as acetonitrile it is possible to separate the heteroduplexes and the homoduplexes. Heteroduplexes are generally retained less owing to their decreased interaction with the ion-pairing reagent and the fact that they are inherently unstable. An example of the use of DHPLC in investigating a genetic disorder is shown in Figure 41.8.

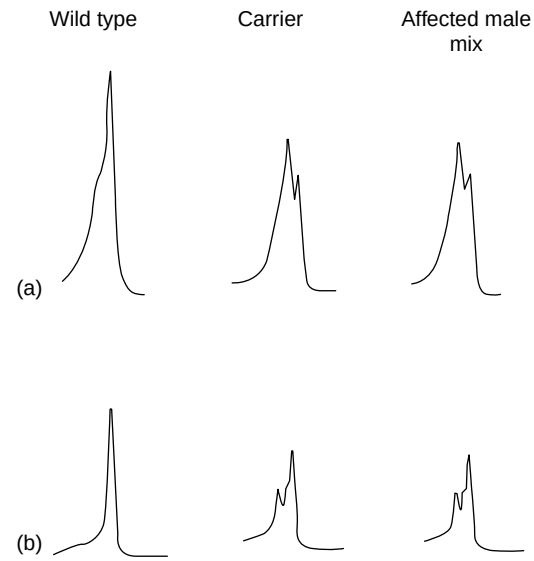

Figure 41.8 Partial denaturing HPLC chromatograms of DNA samples taken from two families (a and b) affected by the haemophilia A mutation. The wild-type DNA (predominant form) shows a homoduplex (a double-stranded molecule in which both strands are from the same source). The carrier DNA shows a heteroduplex (a double-stranded DNA molecule or a DNA–RNA hybrid where each strand is from a different source) as in the sample from an affected male derived after mixing with normal DNA. (From Lin *et al.* 2008.)

Most DHPLC systems have built-in features to perform analyses under non-denaturing, partially denaturing and fully denaturing conditions, depending upon the specific application. In order to effect each of the conditions listed, a column oven is employed that can vary column temperatures to within 0.1°C. Non-denaturing analysis allows for size-based separation and purification of double-stranded DNA and is usually carried out under a column temperature of around 50°C. Partially denaturing conditions (usually >50°C) are used for variation and single-nucleotide polymorphism (SNP) discovery and detection are based on DNA fragment sequence as described above. Fully denaturing temperatures (>70°C) can be used for the separation and analysis of oligonucleotides and nucleic acids as illustrated in Figure 41.9.

On-line sample preparation

The preparation of samples typically demands a large amount of time, work and cost in an analytical laboratory. The innovation of on-line sample preparation makes the process more efficient and reduces the cost. On-line sample preparation techniques usually involve direct elution of the extract from a solid-phase extraction cartridge into the system by the mobile phase. The on-line method gives superior analytical results and can be automated fully. Another benefit is that the sample preparation is reliable, reproducible and robust. This sample preparation method is also discussed in the column-switching section of this chapter.

Rapid screening

The need for high throughput in a laboratory environment is constantly increasing. The use of short, highly efficient, analytical columns, rapid gradients and column-switching apparatus in HPLC systems is helping to facilitate this. Sample turnaround time can often be reduced to a few minutes or less in highly automated and optimised systems. Other information on this topic is given earlier in this chapter in the sections on gradients and column switching.

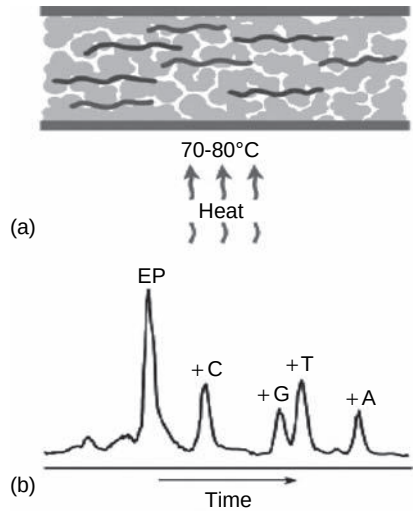

Figure 41.9 Principle of completely denaturing HPLC. (a) At column temperatures >70°C, double-stranded DNA fragments will denature completely. (b) The single-stranded components can then be resolved from each other even if they differ only in sequence and not in size. The chromatogram depicts the separation of an extension primer (EP) and the four possible isomeric products generated by single nucleotide extension sequencing. Resolution for any given pair of alleles can be optimised by varying column temperature. The order of elution of the alleles depends on the stationary phase used. On poly(styrene-divinylbenzene) monoliths, as depicted, extension products elute in the order C < G < T < A. On micropellicular alkylated poly(styrene-divinylbenzene) particles, in contrast, the elution order is G < C < A < T.

Systems for drug analysis

Eluent systems

A large number of eluent and/or packing material combinations have been used for drug analysis. However, currently most are performed on silica or one of the hydrocarbon-bonded silicas (usually ODS). Other types of packing are employed when these conventional materials fail. The majority of drug analyses can be carried out with the four types of system described next.

Silica with non-polar eluents

With silica normal-phase systems the principal mechanism is adsorption chromatography. Separation is controlled by the competition between solute molecules and molecules of the mobile phase for the adsorption sites on the silica surface. Polar groups are attracted most strongly to these sites and hence polar compounds are retained more strongly than non-polar ones. Retention can be decreased by increasing the polarity of the eluent.

Adsorption energies of numerous solvents on alumina (ε° values are given in Table 41.1) have been measured and this scale can be used as a good guide to the elution strengths of eluents on silica as well as alumina (Snyder 1968).

Mixtures of solvents can be employed to give elution strengths between those of the pure solvents. Furthermore, different solvent mixtures that have the same ε° value often give different separations of a group of compounds.

Water is strongly bound to silica and thus the water content of the eluent must be controlled strictly to maintain constant activity of the silica surface and hence reproducible retention times. This is most critical when the eluent is of very low polarity. However, because anhydrous systems are difficult to maintain, a low concentration of water can be used in the eluent, sufficient to deactivate the most active sites without deactivating the whole surface. Typical water concentrations range from 0.01% to 0.2% (v/v). The most satisfactory method used to prepare a solvent of known water content is to mix anhydrous and water-saturated solvents in known proportions. Anhydrous hydrocarbon or halo hydrocarbon solvents can be prepared by passing them through a bed of activated silica or alumina (200 μm) in a glass column. The problems associated with the control of water concentration mean

Table 41.1 ε° values for numerous solvents on alumina (Snyder 1968)

Solvent	ε°
Pentane	0.00
Hexane	0.01
Isooctane	0.01
Cyclohexane	0.04
Toluene	0.29
1-Chlorobutane	0.30
Ether	0.38
Chloroform	0.40
Methylene chloride	0.42
Tetrahydrofuran	0.45
Acetone	0.56
Ethyl acetate	0.58
Diethylamine	0.63
Acetonitrile	0.65
Isopropyl alcohol	0.82
Ethanol	0.88
Methanol	0.95
Acetic acid	Large
Water	Large

that commonly alcohols, such as methanol (0.01–0.5% v/v), are employed to moderate the silica surface (Engelhard 1977).

Silica with polar eluents

Several systems have been described that involve the use of silica with eluents of moderate-to-high polarity containing alcohols and/or water as major components. With such eluents, adsorption chromatography is most probably not the principal mechanism. The mechanisms are poorly understood, which makes the prediction of retention behaviour difficult; nevertheless, many of these systems are very useful for drug analysis.

An eluent that consists of methanol–ammonium nitrate buffer (90 : 10) is suitable for a wide range of basic drugs (e.g. amfetamines and opiates). Retention can be controlled by changes to the pH, ionic strength or methanol : water ratio, or by the addition of other organic solvents such as methylene chloride. With these alkaline eluents the silica surface must bear a negative charge and the principal mechanism is probably cation exchange.

Benzodiazepines can be chromatographed with methanol eluents that contain perchloric acid (typically 0.001 mol/L). Retention can be modified by the addition of other organic solvents (e.g. ether) or by changes to the acid concentration.

Both acidic and basic drugs can be chromatographed on silica using aqueous methanolic eluents that contain cetyltrimethylammonium bromide (Hansen 1981). Hydrophobic quaternary ammonium ions are strongly adsorbed on silica to give a dynamically coated stationary phase. Retention may be controlled by varying the concentration or nature of the quaternary ammonium ion, changing the ionic strength or pH of the buffer, or changing the concentration or nature of the organic component.

ODS with polar eluents

Eluents for RPC on ODS are usually mixtures of methanol or acetonitrile with an aqueous buffer solution. Retention is controlled mainly by the hydrophobic interactions between the drugs and the alkyl chains on the packing material. Retention increases as the analytes decrease in polarity (i.e. polar species are eluted first). Hence, the elution time is increased by increasing the polarity of the eluent (i.e. increasing the water content). The pH of the eluent and the pK_a of the drug are also important, since non-ionised species show greater retention. Thus, acids show an increase in retention as the pH is reduced, while bases show a decrease. It is important to use a buffer of sufficient capacity to cope with any injected sample size, otherwise tailing peaks can arise from changes in ionic form during chromatography. Phosphate buffers (0.05–0.2 mol/L) are widely used as they have a good pH range and low UV absorbance.

Drugs that contain basic nitrogen atoms sometimes show poor efficiencies and give tailing peaks caused by interactions with residual silanol groups on the packing material. This can often be improved by the addition of an amine or quaternary ammonium compound to the eluent, which competes with the analytes for adsorption sites on the silica. Amines of small molecular weight (e.g. diethylamine) can be used as part of the buffer system. Alternatively, low concentrations (0.001 mol/L) of long-chain hydrophobic modifiers (e.g. *N,N*-dimethyloctylamine) can be added to eluents together with conventional buffers.

Other hydrocarbon-bonded packing materials can be used in RPC. A decrease in retention is associated with a decrease in the alkyl chain length.

ODS with polar eluents that contain hydrophobic cations or anions

Drugs that bear positive or negative charges are retained poorly in reversed-phase systems. If the pH of the eluent cannot be changed to convert the drug into its non-ionised form, a hydrophobic ion of opposite charge can be added to form a neutral ion-pair and increase retention. Hence, for a basic drug an acidic eluent is chosen and a hydrophobic anion is added. This technique is referred to as reversed-phase ion-pair chromatography.

The sodium salts of alkylsulfonic acids (RSO_3^- Na^+, where R = pentyl, hexyl, heptyl or octyl) are used widely as ion-pair reagents for basic drugs, while quaternary ammonium compounds (e.g. tetrabutylammonium salts) are used for acidic drugs. Ion-pair reagents are generally added to eluents in the concentration range 0.001–0.005 mol/L, and within this range an increase in concentration leads to an increase in retention. When detergents such as sodium lauryl sulfate or cetyltrimethylammonium bromide are used as the ion-pair reagents, the method is sometimes referred to as 'soap chromatography'. With these salts, ions build up on the surface of the packing material and produce a stationary phase, which behaves like an ion exchanger. This type of mechanism has been described as 'dynamic ion exchange' and probably also occurs with less hydrophobic ion-pair reagents. It is virtually impossible to remove an ion-pair reagent completely from a hydrocarbon-bonded phase, and such columns should therefore not be re-used with other reversed-phase eluents.

Selection of chromatographic systems

Many different combinations of packing material and eluent may be suitable for the analysis of a particular compound or group of compounds and the final choice can be influenced by many factors. The time required to develop a new system can be shortened if it is possible to predict the way in which changes in eluent composition influence chromatographic retention. Systems that use hydrocarbon-bonded phases are particularly attractive from this viewpoint as a large range of parameters can be adjusted (pH, organic solvent, ionic strength, ion-pair reagents) with largely foreseeable consequences. Predictions for silica are generally less reliable. Silica is good for separating drugs that belong to different chemical classes, while hydrocarbon-bonded silicas are preferred for separations of drugs with closely related structures (e.g. barbiturates).

Most of the endogenous materials in biological extracts that can interfere with the analysis of a drug are fairly polar. In reversed-phase systems this material generally elutes before the drug and can obscure the drug peak. In these circumstances, reversed-phase ion-pair chromatography can be valuable to increase selectively the retention of the drug relative to the interfering peaks. Normal-phase systems that use silica do not generally suffer from this problem, as most of the endogenous material usually elutes after the drug. However, these slow-eluting compounds can lead to a noisy baseline or may remain adsorbed to the packing material and thus eventually lead to a loss in column performance.

The vast majority of compounds are separated using a silica-based column with C_{18}, and fine-tuning of the separation can be done by selecting a column with a shorter bonded phase, such as C_8 (see later).

Specially end-capped columns are available that are designed to minimise the tailing that is common with nitrogen-containing weak bases. These are often marketed as a 'basic' column (e.g. Metachem's MetaSil Basic). There are also specially end-capped columns designed to withstand extremely high concentrations of aqueous mobile phase (95–100%). These columns are end-capped with a hydrophilic moiety that ensures proper 'wetting' of the silica to prevent bonded-phase collapse. The columns are typically marketed as 'AQ' for aqueous (e.g. YMC's ODS-AQ).

Analysis of drugs in pharmaceutical preparations

HPLC has found widespread use for the quantitative analysis of drugs in preparations of pharmaceutical and illicit manufacture. Drug concentrations are generally high enough to allow dissolution of the sample (tablet, powder, ointment, etc.) in a suitable solvent followed by injection. UV, visible, FL, RI or mass spectrometric detection methods are used often. These techniques are well suited to provide specific data regarding the chemical composition of the sample in question (e.g. a UV spectrum, mass spectrum).

Within the pharmaceutical industry, HPLC is used at various stages of drug development, such as the optimisation of synthetic reactions and stability testing. Furthermore, it is used extensively for quality control during production to monitor the purity of drugs and excipients. HPLC systems can be automated easily (including injection and data handling), which allows large numbers of samples to be analysed rapidly and economically. HPLC is particularly valuable for the analysis of drugs that are polar (e.g. aspirin), thermally unstable (e.g. benzodiazepines) or present in oil-based formulations for which analysis by GC can be very difficult. Similarly, HPLC can be used for the forensic analysis of illicit preparations to aid the identification of an unknown drug by the measurement of retention times and UV spectra and

comparison to spectral libraries. Furthermore, as the technique can be non-destructive, depending on the detection system used, the eluted compounds can be collected for further analysis.

Example of a drug analysis system

Opiates have been separated by many methods in the past, and the system described here was developed for this purpose. The three opiates separated were morphine sulfate, hydrocodone bitartrate and oxycodone hydrochloride. The column used was a Phenomenex Luna C_{18} (2), $150 \times 4.60\,\text{mm} \times 5\,\mu\text{m}$. The mobile phase was 39 mmol/L dipotassium hydrogenphosphate (K_2HPO_4) and methanol in a 40 : 60 ratio. The final pH was 10 and the mobile phase flow rate was 1.0 mL/min. The retention times obtained (Figure 41.10) for morphine sulfate, hydrocodone bitartrate and oxycodone hydrochloride were 2.799, 4.696 and 6.143 min, respectively.

Analysis of drugs in biological fluids and tissues

Several factors determine the ability of HPLC to detect a drug among the endogenous compounds present in biological material. Clearly, selective detection of the drug relative to the endogenous material is advantageous. In addition, the stationary phase and/or mobile phase can be altered to separate the drug peak from interfering peaks (e.g. using ion-pair reagents). Finally, the sample may be extracted before HPLC to concentrate the drug relative to the endogenous material.

The chromatographic system and detector should always be chosen to minimise the time needed for sample preparation. The complexity of the sample preparation procedure is controlled by several factors, which include the nature of the sample (urine, blood, liver, etc.), the condition of the sample and the concentration of the drug. Interference from endogenous compounds is most acute when drug concentrations are low (e.g. in therapeutic drug monitoring), so more extensive sample preparation and more sensitive and specific detectors are often required. Such assays can be very susceptible to changes in the condition of the sample (e.g. a method developed for fresh blood may not be satisfactory for urine or hair samples), which can present severe difficulties in forensic toxicology. Thus, methods should be tested and validated with the most difficult samples that may be encountered. In contrast, the analysis by HPLC of biological samples that contain high drug concentrations (e.g. in fatal drug overdose) may require much less sample preparation and is less susceptible to changes in sample condition.

Sample preparation for HPLC is essentially the same as for other methods of drug analysis. A drug that is physically trapped within solid tissue (e.g. liver), or chemically bound to the surface of proteins, must be released; then the protein is precipitated to leave the drug in aqueous solution. The protein may be degraded by strong acids or enzymes, precipitated by various chemicals (e.g. tungstic acid, ammonium sulfate) or removed by ultrafiltration. Some drugs are destroyed by protein degradation methods, while ultrafiltration and precipitation can lead to drug losses through protein binding. No single procedure works well for all drugs and the method should be selected to give the maximum recovery of the drug being analysed.

When drug concentrations are high (typically μg/mL) and systems with polar mobile phases are used, the direct injection of deproteinised solutions may be acceptable. Proteins must be removed to protect the column from irreversible contamination. A rapid procedure is to: mix the biological fluid with at least two volumes of methanol or acetonitrile; centrifuge to remove the precipitated protein; evaporate the organic supernatant; and reconstitute the sample in a volume of mobile phase. Urine can be treated similarly to guard against the precipitation of salts on the column. Great care and consideration should be afforded when injecting minimally prepared biological samples on to a HPLC system. Particulates are more likely to become trapped in the system plumbing and a more rapid degradation of column performance may be observed from build-up of contaminants on the head of the column. To help maximise column performance and lifetime, it is good policy to use a guard column between the injector and analytical column. This is packed with the same material as the analytical column and replaced at frequent intervals. The configurations of guard columns range from easily replaceable and relatively inexpensive frit-like filters and/or cartridges to shorter versions of the analytical column itself. All are designed to protect the analytical column by acting as a trap for components that would otherwise irreversibly bind to the analytical column and thus decrease the usable life of the column.

Extraction of drugs and other analytes away from endogenous materials prior to analysis is a common procedure for all types of biological samples. This may also entail a concentration step, which increases the sensitivity of the method. Solvent extraction remains the most popular approach, as many factors can be modified to optimise the extraction. These modifications include changing the polarity of the organic solvent, the pH and ionic strength of the aqueous phase, and the use of ion-pairing agents. It is generally recommended that the collected organic phase be evaporated to dryness and the residue dissolved in a suitable solvent, typically something greater than or equal to the polarity and composition of the initial mobile phase before injection. Care must be taken that volatile drugs are not lost by evaporation and that lipid material in the residue does not prevent the drug from dissolving in the new solvent.

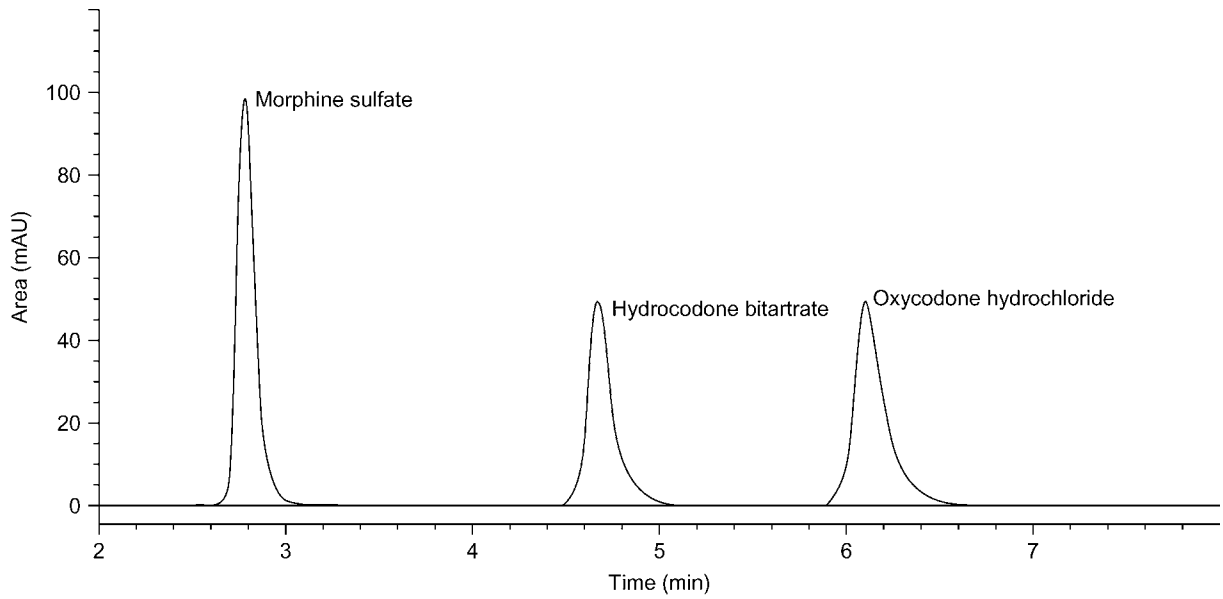

Figure 41.10 Separation of opiates by HPLC. Conditions for separation are described in the text.

Example protocol for the extraction of a wide variety of weak bases

- To 1 mL of plasma, urine or other homogenised matrix add 100 µL concentrated ammonium hydroxide.
- Extract the sample with 4 mL of a mixture of *n*-butyl chloride–acetonitrile (4 : 1) for 20 min.
- Centrifuge at high speed for 20–30 min to partition the phases.
- Carefully collect the organic phase into a clean tube.
- Evaporate the organic phase under a stream of air or nitrogen at 25–40°C, depending on the volatility of the analytes (a small volume of acidified methanol can be added to prevent the loss of amfetamine-type analytes).
- Reconstitute the residue in an HPLC mobile phase that is more polar than the LC mobile phase to be used for analysis (e.g. if the HPLC elution ratio is 60% aqueous, reconstitute the sample in a phase that is >60% aqueous). This ensures that, when injected, the sample is focused on the front end of the column and minimises band (peak) broadening.

An example of a chromatogram that utilises this extraction technique is shown in Figure 41.11. The urine was fortified with analytes and deuterated internal standards for amfetamine and methamfetamine (dashed chromatograms) and extracted as described above. The sample was eluted using a MetaSil Basic 3 × 100 mm × 3 µm column. The mobile phase was 85% (0.1% formic acid in water), 15% methanol, pumped isocratically at 0.2 mL/min. The instrument used was an Agilent 1100 LC/MSD with ESI.

SPE columns are also widely used to extract drugs from biological samples. The column is washed with suitable solvents to remove endogenous material before the drug is removed by passing through a solvent of higher elution strength. Such columns are usually attached to extraction manifolds utilising either positive or negative pressure to draw the liquids through the sorbent beds. Extraction selectivity can be controlled by adjustments to the biological fluid before extraction (e.g. pH, ionic strength) and the choice of washing solvents. Most, if not all, manufacturers of SPE columns offer methods and columns optimised for a particular drug class and/or matrix. As less traditional biological matrices are used for drug analysis (e.g. sweat, hair, oral fluids), some modifications of the sample preparation scheme are needed. Hair requires solubilisation prior to extraction; oral fluids and sweat may need to be isolated from their respective collection devices. Consideration of the pH and solubility may be needed prior to sample preparation, but in general the principles in place for the extraction of blood, urine, etc. apply to these alternative matrices. Some important issues unique to these matrices are:

- Sample volume is typically much less than blood or urine.
- The amount of drug extracted from a particular matrix may be much less than from traditional matrices, so that much more sensitive detectors (e.g. MS or MS–MS) are required.

Identification of drugs by HPLC with photodiode array detection and UV spectra library search

HPLC with DAD in combination with a UV spectra library has proved to be a very successful 'systematic toxicological analysis' (STA) technique for use in clinical and forensic toxicology (see Chapter 1). Any drugs or other poisons in the sample are identified by coincidence of the UV spectrum and of the retention time or another chromatographic retention parameter with the library data; one system and its use are described below (F Pragst and M Herzler, personal communication).

Chromatographic conditions

Since the method is used in combination with a database of UV spectra and retention parameters, the chromatographic conditions must be reproducible and the same as used to generate the database. The mobile phase must be suitable for the separation of a large variety of organic substances and must be transparent in the wavelength range used. These prerequisites are best met by reversed-phase columns (RP_8 or RP_{18}) and acidic acetonitrile–buffer mixtures as mobile phases. Systems described in the literature generally use either a gradient elution or two isocratic runs with different buffer : acetonitrile ratios.

Gradient elution has the advantages that strongly polar and non-polar substances can be analysed in one run, that peaks are not broadened with increasing retention time, and that the retention times of the toxicologically relevant compounds are distributed more evenly over the run time, but it has some disadvantages (see above). A system of HPLC retention indices was introduced by Bogusz *et al.* (1993) analogous to the Kovats indices used in GC and based on the retention times of the nitroalkanes.

Isocratic HPLC has the advantage of higher reproducibility of the retention times, greater ruggedness and a more economic use of the mobile phase by recycling. Disadvantages are an unfavourable distribution of the retention times of toxicologically relevant compounds with an increased number at the beginning of the chromatogram, and the need for a second mobile phase for non-polar compounds. Nevertheless, isocratic HPLC–DAD procedures are used successfully in many toxicological laboratories for screening purposes. Suitable experimental

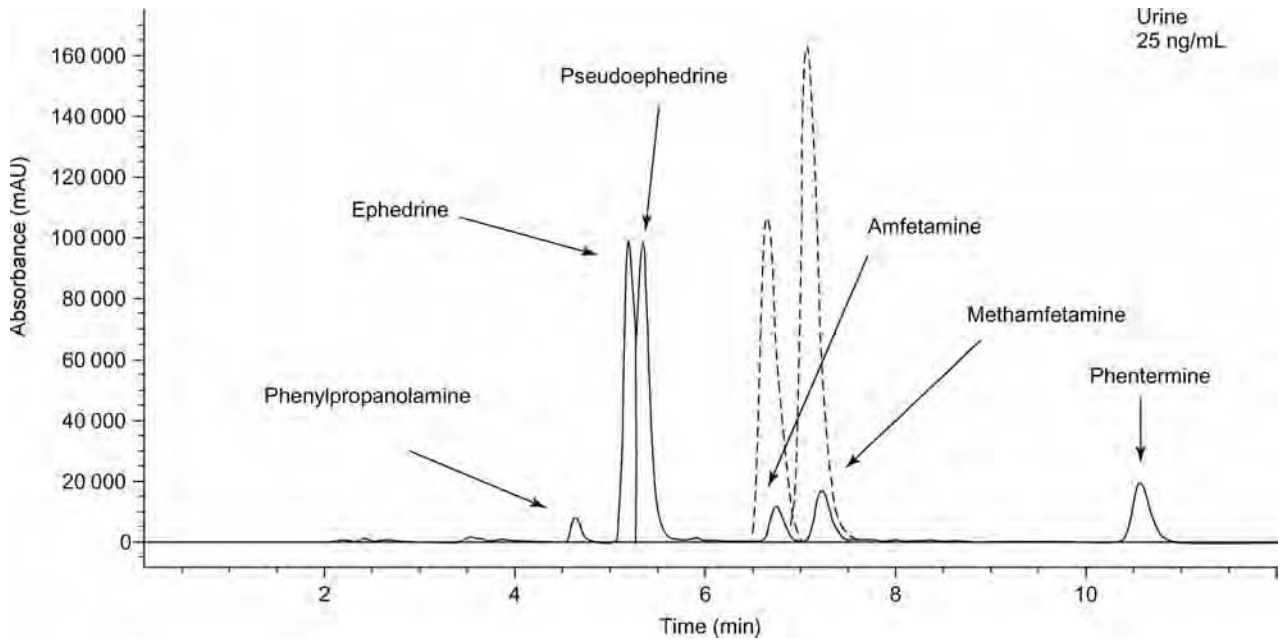

Figure 41.11 Separation of amfetamines by HPLC–MS. Conditions for separation are described in the text.

conditions, also used in the recording of an extensive UV spectra library, were as follows (Pragst *et al.* 2001):

- HPLC column: RP_8, end-capped, 5 µm, 250 × 4.0 mm
- Mobile phase A: 0.1 mol/L phosphate buffer pH 2.3–acetonitrile (67 : 33 v/v)
- Mobile phase B: 0.1 mol/L phosphate buffer pH 2.3–acetonitrile (33 : 67 v/v)
- Flow rate: 1 mL/min.

Standard compounds are histamine hydrochloride to measure the time of an unretained peak t_0 (dead time), 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) to calculate relative retention times (RRTs) in mobile phase A, and 4-phenylbenzophenone to calculate RRTs in mobile phase B.

The UV spectra of a large number of compounds listed in this book were measured under these conditions. An overview of HPLC–DAD conditions used for STA is given in Pragst *et al.* (2001).

Retention parameters

Absolute retention times are not suitable for peak identification purposes, since they depend strongly on the configuration and experimental conditions of the HPLC device. Moreover, the capacity ratio k_A (see above) is sensitive to small fluctuations of the experimental conditions and is not suitable for an identification system used in different laboratories. Therefore, for gradient elution, retention indices are preferred (Bogusz *et al.* 1993). Under isocratic conditions RRTs related to a standard compound are more reproducible (Equation 41.6).

$$\mathrm{RRT_x} = \frac{t_x - t_0}{t_s - t_0} \tag{41.6}$$

where RRT_x is the RRT of compound x, t_x is the absolute retention time of compound x, t_0 is the retention time of an unretained peak, and t_s is the retention time of the standard compound.

The relatively small peak resolution of HPLC and the differences between charges of the reversed-phase material mean that the value of retention indices or of RRTs in the identification of a compound from a large number of candidates is rather limited. However, it is very useful for distinguishing between compounds with very similar UV spectra. In this way an RRT window can be chosen as a pre-selection parameter for the spectra library search.

UV spectral library search and specificity of UV spectra

Before peak identification a 'peak purity check' should be carried out. A pure peak means that it originates only from one compound and that the UV spectrum does not change over the whole peak width.

A UV spectral library search is based on the comparison of the spectrum of the unknown peak with all spectra of the library. This comparison is not confined to UV maxima and minima, but can comprise all absorbance–wavelength pairs measured by DAD. Mathematical models to assess spectral similarity use the description of the spectrum as a vector in n-dimensional space, where n is the number of absorbance–wavelength pairs measured. For the complete identity of two spectra, both vectors point in exactly the same direction, that is the angle between them is $\theta = 0°$. Different concentrations have an effect on vector length, but not on its direction in space. The similarity index (SI) is defined as $\cos\theta$ and is calculated with Equation (29.7):

$$\mathrm{SI_x} = \cos\theta_{\bar{s}_1 \cdot \bar{s}_2} = \frac{\bar{s}_1 \cdot \bar{s}_2}{|\bar{s}_1| \times |\bar{s}_2|} \tag{41.7}$$

where $\bar{S}_i$ is the vectorised spectrum of compound i.

UV spectra can be measured with extremely high reproducibility. Therefore, small differences between spectra measured under identical conditions indicate that they originate from different compounds. SI is 1.000 for completely identical spectra. However, in practice two spectra with SI >0.9990 can be regarded as identical. At small concentrations, and in the case of partly overlapping peaks, SI >0.990 may be a sufficient criterion for identity.

It was shown in a systematic study on the selectivity of an HPLC–DAD method (Herzler *et al.* 2003) that, from 2888 toxicologically relevant compounds, 2682 (93%) exhibited UV absorption above 195 nm. Out of these, 1619 (60.4%) had a unique UV spectrum and could be identified unambiguously. By inclusion of the retention time this proportion was increased to 84.2%. Large UV spectra libraries can be divided into sub-libraries, according to the retention parameter or the effect or use of the substance, to facilitate a faster and more specific library search. The result can also be supported by the presence of metabolites, while in doubtful cases complementary methods may be used for confirmation (e.g. MS).

As an example, in Figure 41.12 the results of the library search for a peak with RRT = 0.0811 in an intoxication case are shown. In this case a sub-library of all compounds with RRT = 0.601–0.900 was used. Hit 1 was promethazine with SI = 0.9992; hit 2 (promazine, SI = 0.9964) and hit 3 (dixyrazine, SI = 0.9961) also originated from compounds of the phenothiazine type. The small difference between the spectra of hits 1 and 2 may arise because in these two compounds the amino group of the side chain is separated from the phenothiazine ring by two and three saturated carbon atoms, respectively. Dixyrazine could clearly be excluded by the much smaller retention time. However, promethazine and promazine could not be distinguished by the RRT values stored in the database. Therefore, to confirm the library search result, promazine and promethazine standards were measured immediately after the sample, which resulted in an exact agreement with promethazine.

As a prerequisite for the optimal use of a commercially available UV spectral library, the same mobile phase must be used and the technical parameters of the DAD (wavelength accuracy and resolution) need to be (and stay) sufficient. This can be controlled by daily measurement of a compound with a vibration fine structure of the UV spectrum, such as benzene.

UV spectra and retention times of metabolites

The use of HPLC–DAD has the advantage that in many cases, metabolites can be attributed easily to the parent drug by the UV spectrum. Depending on the site of metabolism, the UV spectrum may be altered significantly (change of the UV-absorbing unsaturated part of the molecule, the chromophore) or it may be the same as (or very similar to) that of the parent drug (reaction at the aliphatic part of the molecule). As an example, in Figure 41.13 the spectrum of flunitrazepam is compared with that of its metabolites, 7-aminoflunitrazepam (strong change of the chromophore by transformation of the aromatically bound nitro group into the amino group) and 3-hydroxyflunitrazepam (no essential change of the chromophore by hydroxylation at the aliphatic carbon atom 3).

The retention times of drugs on reversed-phase columns are shifted in a typical way by metabolism. Metabolism to more hydrophilic products (e.g. hydroxylation, reduction of the nitro to an amino group; Figure 41.13) leads to a decrease in retention time, whereas deamination strongly increases retention time, particularly in an acidic mobile phase (removal of the strongly hydrophilic, protonated amino group). For many drugs, the chromatograms obtained from blood or urine extracts have a typical metabolite pattern that supports identification in the context of STA.

Sample pretreatment

Tablets, powders or residues in syringes can simply be dissolved in the mobile phase and analysed by HPLC-DAD without further treatment. The investigation of biological samples, such as whole blood (serum, plasma), stomach contents, urine or tissue samples, is more complicated. In these cases the drug must be separated from the biological matrix.

Although SPE has been much improved in the past decade, liquid–liquid extraction (LLE) is still preferred if HPLC-DAD is used for toxicological screening, since it is less susceptible to interferences, more reproducible and easier to handle for single samples. An important advantage of UV detection is that cholesterol and fatty acids, co-extracted to a high extent from human samples by lipophilic solvents, show no UV absorption and therefore, in contrast to GC-MS, do not interfere with the analysis. Moreover, derivatisation is not necessary. A sample pretreatment method by extraction with n-butyl chloride–acetonitrile (4 : 1), which can be used for a wide variety of basic compounds,

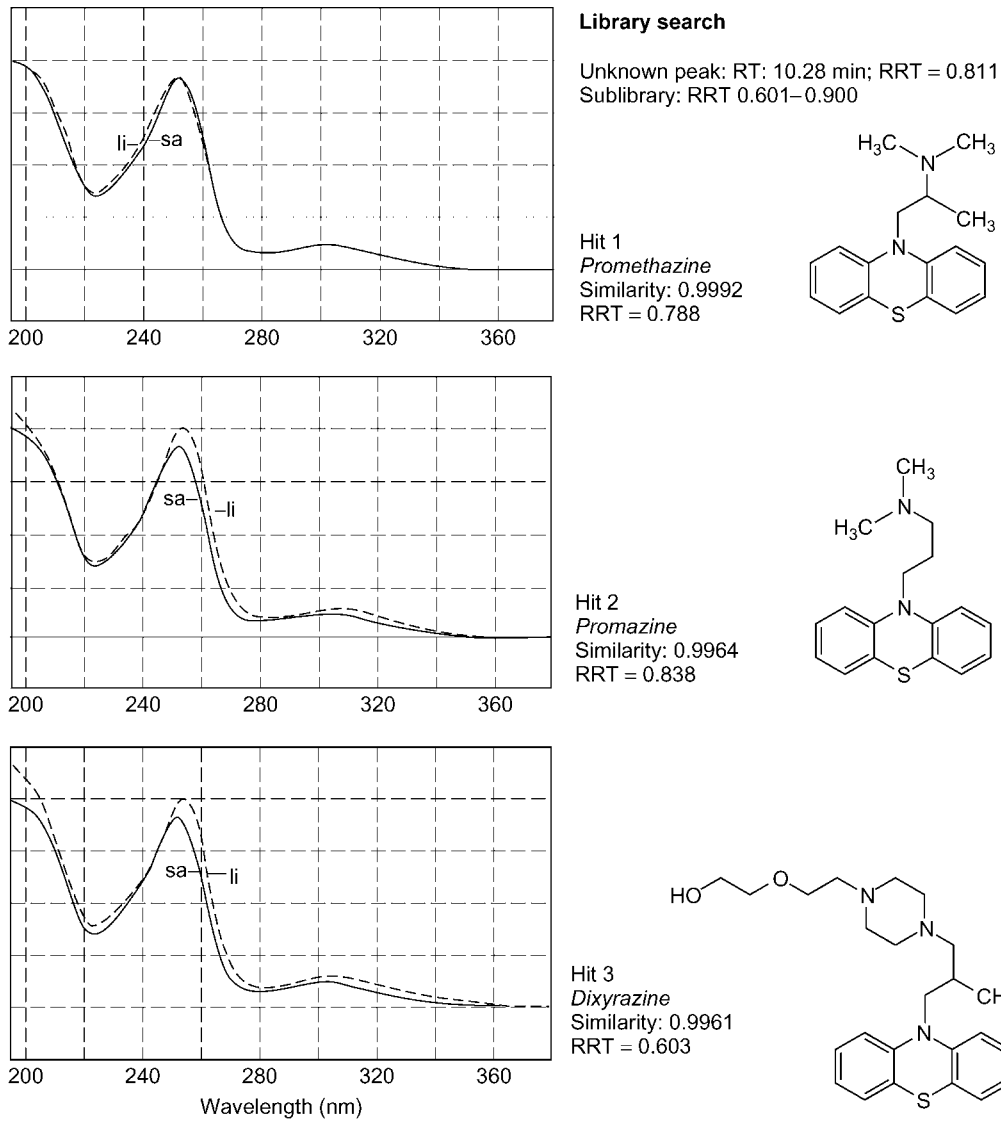

Figure 41.12 Results of the HPLC–DAD library search for a peak in the chromatogram of an alkaline extract of a lethal trimipramine–promethazine intoxication. Hit 1 (promethazine) was confirmed by exact agreement of the retention time with the reference compound measured immediately after the sample. sa, sample; li, library.

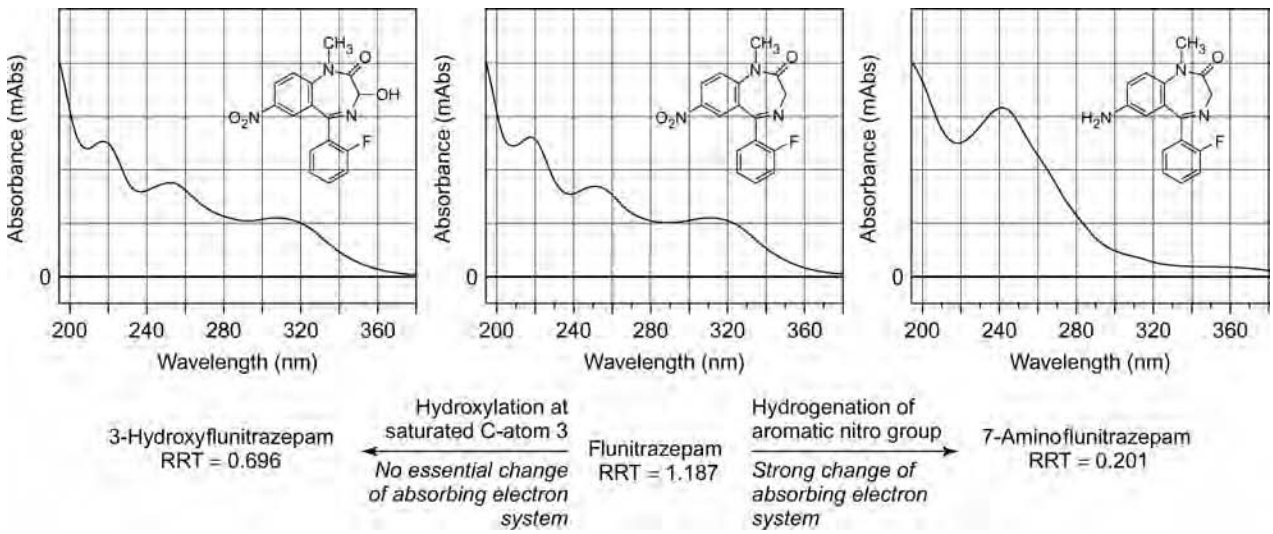

Figure 41.13 Change of the UV spectrum and the relative retention time (RRT) of flunitrazepam by metabolism.

is given above. For systematic toxicological screening of blood (serum, plasma) samples by HPLC-DAD, the measurement of two extracts obtained at pH 2 and pH 9 with dichloromethane and of the supernatant of a protein precipitation by acetonitrile has proved to be very useful (Pragst *et al.* 2002).

Preparation of a basic and an acidic methylene chloride extract

1. Dispense 500 µL of whole blood, serum or plasma into two 1.5-mL vials.
2. To vial 1 add 100 µL of a 0.2 mol/L solution of tri(hydroxymethyl) amine (basic extract).
3. To vial 2 add 100 µL of 0.1 mol/L hydrochloric acid (acidic extract).
4. To both vials add 400 µL of dichloromethane.
5. Vortex mix the vials for 1 min and centrifuge.
6. Withdraw 200 µL of the dichloromethane extract and evaporate the solvent at room temperature under a stream of nitrogen.
7. Dissolve the residue in 100 µL of mobile phase.
8. Analyse 50 µL of each extract (basic extract in mobile phase A and acidic extract in mobile phase B).

Protein precipitation by acetonitrile

1. To 500 µL of whole blood, serum or plasma add 500 µL of acetonitrile.
2. Vortex the mixture for 2 min and centrifuge.
3. Separate off the supernatant.
4. Analyse 50 µL in mobile phase A.

Protein precipitation is particularly useful for hydrophilic drugs, which are extracted poorly by the procedure mentioned above. These include paracetamol, salicylic acid and lamotrigine. The limits of detection are between 0.01 and 0.1 µg/mL for dichloromethane extraction (depending on the extinction coefficient and on the extraction yield) and between 0.1 and 1 µg/mL for protein precipitation.

Application example

In STA, the library search must be applied to all peaks of the HPLC-DAD chromatogram. As an example, the chromatogram at 225 nm of the basic extract from the blood sample of a lethal drug poisoning case and the UV spectra of the highest peaks are shown in Figure 41.14. To determine RRT, the standard compound (MPPH, peak no. 10, RRT = 1.000) was added. From the remaining 11 peaks of the chromatogram, 7 could be identified by both UV spectrum and RRT. As the result, a high overdose of trimipramine and promethazine was found to be the cause of death. The extensive metabolism indicated that there had been a long survival time after drug ingestion. The similarities between the UV spectra of the parent drugs (peaks 9 and 12) and some of their metabolites (peak 8, and peaks 6 and 11, respectively) are also demonstrated in this case. On the other hand, the sulfoxides of promethazine (peak 3) and desmethylpromethazine (peak 2) show completely changed spectra because of the transformation that takes place directly at the UV-absorbing phenothiazine ring. Caffeine (peak 1) is found in almost all samples. The poor separation of peaks 4, 5 and 7 meant that the UV spectra were not suitable for a library search.

Recommended HPLC systems

There are general screening methods based on gradient elution and retention indices that have proven value by many laboratories, and data from these are listed below (systems HA, HX, HZ, HY and HAA). Another (system HBK) is based on a combination of isocratic systems. The tabulated data are derived from systems in which groups of compounds have been chromatographed either as part of a general screening procedure or from systems that have been used specifically for that group of compounds. Other systems for the chromatography of individual compounds, especially those used for quantification, are given in the monographs.

Chromatographic retention data are presented as *k* values as well as retention times (RTs), retention indices (RIs) and relative retention times (RRTs).

Note In the tables, a dash indicates that no value is available for the compound, not that it does not elute.

General screens

System HA

Jane I *et al.* (1985). *J Chromatogr* 323: 191–225.

- *Column:* Silica Spherisorb S5W (125 × 4.9 mm i.d., 5 µm).
- *Mobile phase:* Solution containing 1.175 g (0.01 mol/L) ammonium perchlorate in 1 L methanol; adjust to pH 6.7 by the addition of 1 mL 0.1 mol/L sodium hydroxide in methanol.
- k *values:* Values for drugs in this system will be found in drug monographs and in the Indexes to Analytical Data; they are also included in the systems for specific groups of drugs that follow.

System HX

J Hartstra, JP Franke, RA de Zeeuw, personal communication.

- *Column:* Lichrospher 60 RP-Select B (125 × 4.0 mm i.d., 5 µm) with precolumn Lichrospher 60 RP-Select B (4 × 4.0 mm i.d., 5 µm).
- *Mobile phase:* (A:B) triethylammonium phosphate buffer (25 mmol/L, pH 3.0)–acetonitrile.
- *Elution programme:* (A:B) (100 : 0) to (30 : 70) in 30 min, hold 10 min, back to initial conditions in 3 min with equilibration for 10 min before next injection.
- *Flow rate:* 1 mL/min.
- *Detection:* DAD.
- *Standards:* Nitro-*n*-alkanes (C_1 to C_{11}) 10 µL in 10 mL acetonitrile.
- *RI values:* Values for drugs in this system will be found in the monographs and in the Indexes to Analytical Data; they are also included in the systems for specific groups of drugs that follow.

System HY

RK Watt, RA Waters, AC Moffat, unpublished information.

- *Column:* C_{18} symmetry (250 × 4.6 mm i.d., 5 µm).
- *Column temperature:* 40°C.
- *Mobile phase:* (A:B) sulfuric acid (0.5 mL 2.5 mol/L) in water (500 mL)–sulfuric acid (0.5 mL 2.5 mol/L) in acetonitrile (500 mL).
- *Elution programme:* (98 : 2) for 3 min to (2 : 98) over 23 min, hold for 10 min, back to initial conditions over 2 min with equilibration of 8 min before next injection.
- *Detection:* DAD.
- *Standards:* Nitro-*n*-alkanes (C_1 to C_{16}) 10 µL in 10 mL acetonitrile.
- *RI values:* Values for drugs in this system will be found in the monographs and in the Indexes to Analytical Data; they are also included in the systems for specific groups of drugs that follow.

System HZ

Conemans JMH *et al.* http://www.zanob.nl/pages/LSShowElementsPage_v2.asp?ListID=1650&elemid=29275&articleid=133751&token= (accessed 14 December 2010).

- *Column:* C_{18} end-capped LiChrospher 100 RP-18e (125 × 4.0 mm i.d., 5 µm), with precolumn LiChrocart 124-4.
- *Mobile phase:* Add 146 µL triethylamine and about 750 µL phosphoric acid to 530 mL water. Adjust pH to 3.3 using a 10% potassium hydroxide solution and finally add 470 mL acetonitrile.
- *Flow rate:* 0.6 mL/min.
- *Detection:* DAD.
- *Retention times:* Values for drugs in this system will be found in the monographs and in the Indexes to Analytical Data; they are also included in the systems for specific groups of drugs that follow.

System HAA

Gaillard Y, Pepin G. (1997). *J Chromatogr A* 763: 149–163.

- *Column:* C_8 Symmetry (250 × 4.6 mm i.d., 5 µm) with Symmetry C_{18} precolumn (20 mm).
- *Column temperature:* 30°C.
- *Mobile phase:* (A:B) phosphate buffer (pH 3.8)–acetonitrile.
- *Elution programme:* (85 : 15) for 6.5 min to (65 : 35) until 25 min to (20 : 80) for 3 min, and back to initial conditions for equilibration for 7 min.

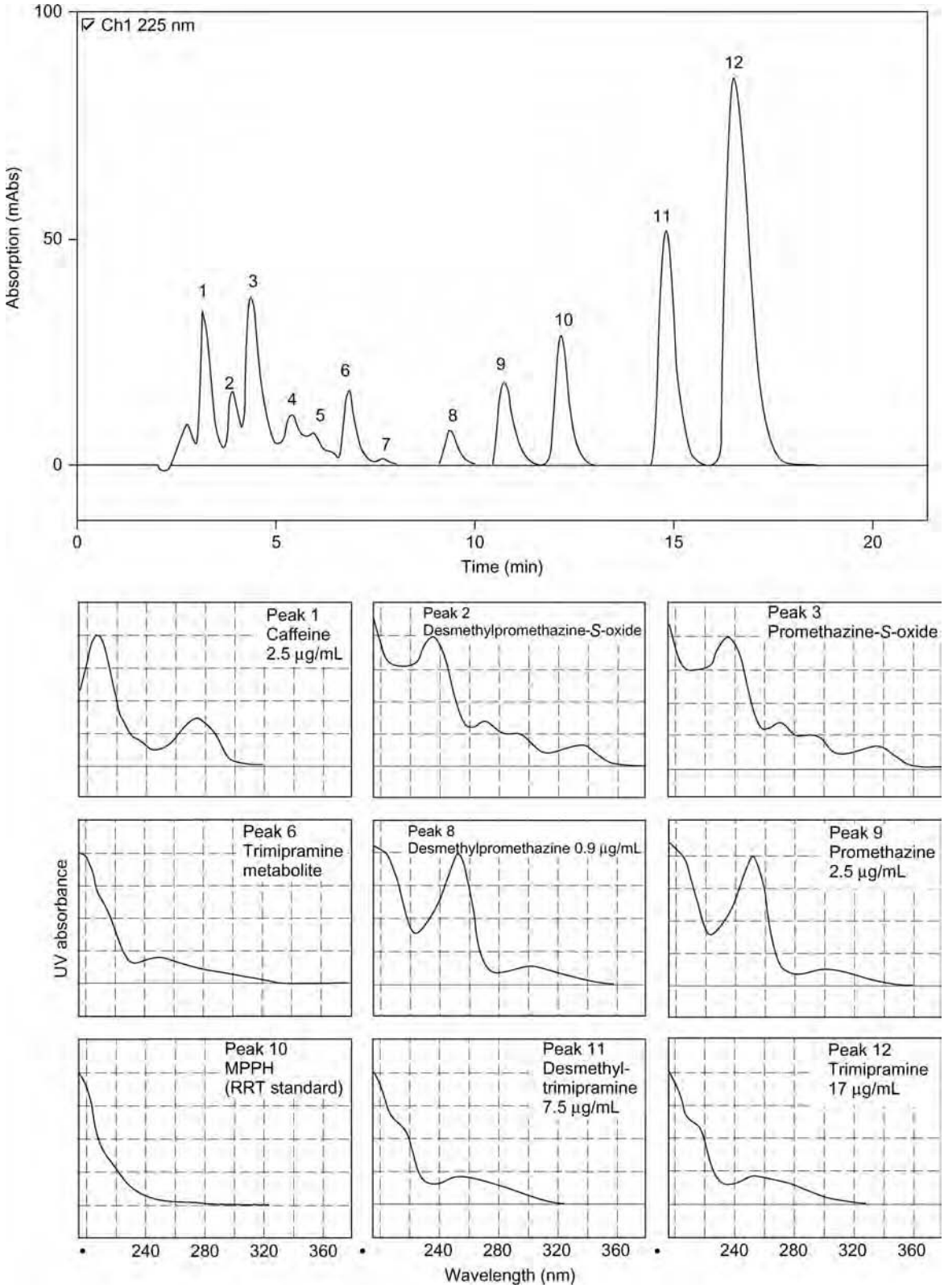

Figure 41.14 HPLC–DAD investigation of a combined trimipramine–promethazine poisoning. Chromatogram of a basic extract of a venous blood sample, UV spectra of the highest peaks, results of the library search and semiquantitatively determined concentrations.

- *Flow rate:* 1 mL/min for 6.5 min, then linear increase to 1.5 mL/min for 6.5–25 min and hold for 3 min (re-equilibration is made at 1.5 mL/min).
- *Detection:* DAD.
- *Retention times:* Values for drugs in this system will be found in the monographs and in the Indexes to Analytical Data; they are also included in the systems for specific groups of drugs that follow.

System HBK

Pragst F *et al.* (2001) *UV Spectra of Toxic Compounds*. Heppenheim: Verlag Dr Dieter Helm.

- *Column:* Lichrospher RP-8ec (250 × 4.0 mm i.d., 5 µm).
- *Mobile phase:* Three different composition are used: A: acetonitrile–phosphate buffer pH 2.3 (33 : 67). Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (for compounds eluting within 30 min). B: acetonitrile–phosphate buffer pH 2.3 (67 : 33). Internal standard: 4-phenylbenzophenone (for compounds eluting after 30 min). C: acetonitrile–phosphate buffer pH 2.3 (20 : 80). Internal standard: salicylamide (for compounds with RRTs below 0.2).
- *Flow rate:* 1 mL/min.
- *Detection:* DAD.
- *Note:* The phosphate buffer is prepared by dissolving 4.8 g phosphoric acid (85%) and 6.66 g potassium dihydrogenphosphate in 1 L water, adjust pH to 2.3. Values for drugs in this system will only be found in the Indexes to Analytical Data.

Amfetamines, other stimulants and anorectics

Systems HA, HX or HY previously described may be used, or Systems HB or HC below.

System HB

Gill R *et al.* (1981). *J Chromatogr* 218: 639–646.

- *Column:* ODS Hypersil (250 × 5 mm i.d., 5 µm).
- *Mobile phase:* Solution containing 19.60 g (0.2 mol/L) phosphoric acid and 7.314 g (0.1 mol/L) diethylamine in 1 L of a 10% v/v solution of methanol; adjust the pH to 3.15 by the addition of sodium hydroxide solution.

System HC

Law B *et al.* (1984). *J Chromatogr* 301: 165–172.

- *Column:* Silica Spherisorb (250 × 5 mm i.d., 5 µm).
- *Mobile phase:* Methanol–ammonium nitrate buffer solution (90 : 10). To prepare the buffer solution add 94 mL strong ammonia solution and 21.5 mL nitric acid to 884 mL water and adjust to pH 10 by the addition of strong ammonia solution.

Amfetamines, other stimulants and anorectics					
	HA	*HB*	*HC*	*HX*	*HY*
	k	k	k	*RI*	*RI*
Adrenaline	–	–	0.63	–	–
Amfetamine	0.9	8.48	0.98	244	–
Benzfetamine	1.2	–	0.15	–	–
Brucine	11.1	–	–	312	267
Caffeine	0.2	–	0.26	–	–
Cathine	1	4.39	0.83	–	–
Chlorphentermine	0.9	–	0.82	–	–
Diethylpropion	1.7	–	0.16	–	230
Dimethylamfetamine	–	11.08	1.89	–	–
DOM	–	–	1.13	–	–
Ephedrine	1.0	5.68	1.79	–	–
Fencamfamin	1.3	–	0.72	354	309
Fenethylline	–	–	0.27	–	–
Fenfluramine	1.3	–	0.88	371	315
Norfenfluramine	1	–	–	–	–
Fenproporex	–	–	–	–	226
Hordenine	–	2.00	–	–	–
Hydroxyamfetamine	–	2.24	1.11	–	–
Hydroxyephedrine	–	0.73	–	–	–
Mazindol	1.8	–	0.2	357	286
Mephentermine	1.5	–	2.48	–	–
Mescaline	1.3	16.82	2.17	–	–
Metamfetamine	2	10.52	2.07	262	216
Methoxyamfetamine	–	14.95	–	–	–
Methoxyphenamine	1.7	32.17	–	–	–
Methylamfetamine	2.0	10.52	2.07	–	–
Methylenedioxymethamfetamine	–	–	–	278	252
Methylephedrine	2.3	–	1.83	–	–
Methylphenidate	1.7	–	0.36	–	277
Noradrenaline	–	0.10	–	–	–
Normetanephrine	–	–	1.08	–	–
Oxedrine	–	0.27	–	–	–
Pemoline	0.2	–	0.1	307	271
Phendimetrazine	0.9	–	0.3	263	218
Phenelzine	1.0	5.91	0.37	–	–

continued

Amfetamines, other stimulants and anorectics, continued					
	HA	*HB*	*HC*	*HX*	*HY*
	k	k	k	*RI*	*RI*
Phenethylamine	1.2	3.64	1.31	–	–
Phenmetrazine	1.7	–	–	258	241
Phentermine	0.6	19.46	0.86	–	245
Phenylephrine	1.3	–	1.64	–	–
Phenylpropanolamine	0.9	3.87	0.70	–	–
Pipradrol	1.2	–	0.69	355	–
Prolintane	2	–	1.3	370	–
Pseudoephedrine	1.2	5.90	1.77	–	–
Tranylcypromine	1.0	–	0.26	–	–
Trimethoxyamfetamine	–	–	1.48	–	–
Tyramine	1.2	0.81	1.47	–	–

Analgesics, non-steroidal anti-inflammatory drugs

System HD

HM Stevens, R Gill, unpublished data.

- *Column:* ODS Hypersil (160 × 5 mm i.d., 5 µm).
- *Mobile phase:* Isopropyl alcohol–formic acid–0.1 mol/L potassium dihydrogenphosphate (13.61 g/L; 540 : 1 : 1000).

System HV

- *Column:* ODS Spherisorb (200 × 4.6 mm i.d., 5 µm).
- *Mobile phase:* Acetronitrile–acetic acid (45 : 55) for 2 min, to (75 : 25) at 3%/min, for 6 min.
- *Flow rate:* 1.7 mL/min.

System HW

HM Stevens, R Gill, unpublished data.

- *Column:* As for System HD, above.
- *Mobile phase:* Isopropyl alcohol–formic acid–0.1 mol/L potassium dihydrogenphosphate (13.61 g/L; 176 : 1 : 1000).

Antifungals

The general screening systems, previously described, may be used.

Antifungals				
	HX	*HY*	*HZ*	*HAA*
	RI	*RI*	*RT*	*RT*
Econazole	526	385	–	20.1
Fluconazole	340	289	–	11.4
Flucytosine	72	–	1.5	3.1
Griseofulvin	–	488	–	18.4
Ketoconazole	439	464	5.2	15.7

Analgesics, NSAIDs							
	HD	*HV*	*HW*	*HX*	*HY*	*HZ*	*HAA*
	k	*RRT*	k	*RI*	*RI*	*RT*	*RT*
Acetanilide	0.5	–	2.3	–	281	–	–
Paracetamol	0.1	–	0.32	–	–	–	–
Alclofenac	2.6	0.61	–	–	–	–	–
Aminophenazone	0.2	–	0.32	262	204	2.1	–
Aspirin	0.5	–	2.7	350	318	2.7	–
Salicylic acid	0.7	–	4.6	–	–	–	–
Benorilate	0.7	–	22.4	–	–	–	–
Aspirin	0.5	–	2.7	–	–	–	–
Paracetamol	0.1	–	0.32	–	–	–	–
Benoxaprofen	11.3	0.98	–	–	–	–	–
Clonixin	–	0.87	–	–	345	–	–
Diclofenac	11.5	0.85	–	616	592	14.8	22.1
Diflunisal	4.1	0.77	–	508	583	5.4	–
Dipyrone	0.1	–	0.45	316	194	1.4	–
Etenzamide	0.55	–	4.6	–	303	–	–
Fenbufen	4	0.81	–	520	461	–	19.3
Fenoprofen	7.9	–	–	574	524	10.9	21.2
Floctafenine	–	–	–	–	–	4.4	17.2
Flufenamic acid	19.7	1	–	671	667	–	–
Flunixin	–	0.99	–	–	414	–	–

Analgesics, NSAIDs, continued							
	HD	***HV***	***HW***	***HX***	***HY***	***HZ***	***HAA***
	k	***RRT***	**k**	***RI***	***RI***	***RT***	***RT***
Flurbiprofen	–	0.89	–	585	–	11.8	21.3
Glafenine	–	–	–	372	276	2.3	–
Ibuprofen	15.1	–	–	616	598	16.5	23.8
Indometacin	6.95	0.87	–	607	590	14.4	21.7
Indoprofen	1.2	0.52	–	–	406	–	–
Ketoprofen	2.4	0.66	–	495	–	6.4	19.6
Ketorolac	–	–	–	–	–	4.1	–
Meclofenamic acid	–	–	–	653	690	–	–
Mefenamic acid	21.1	0.95	–	661	686	–	–
Methyl salicylate	3.9	–	–	480	449	–	–
Salicylic acid	0.7	–	–	–	–	–	–
Morazone	0.4	–	2.05	–	294	–	–
Naproxen	3.3	–	–	501	468	6.8	–
Nefopam	–	–	–	–	313	–	12.7
Nifenazone	0.1	–	0.45	310	–	–	–
Niflumic acid	–	0.93	–	595	530	–	22
Oxyphenbutazone	1.95	0.69	–	501	459	6.7	–
Paracetamol	0.1	–	0.32	264	241	1.9	5.6
Phenacetin	0.6	–	4.4	377	335	3.0	–
Paracetamol	0.1	–	0.3	264	241	1.9	–
Phenazone	0.1	–	0.95	333	299	2.1	–
Phenylbutazone	6.5	0.95	–	672	643	19.5	24.1
Oxyphenbutazone	1.95	0.7	–	501	459	6.7	–
Piroxicam	0.6	–	7.7	431	382	4.9	16.6
M (5-hydroxy)	–	–	–	–	446	–	–
Propyphenazone	1.3	–	11	441	370	4.7	–
Salicylamide	0.4	–	2.5	327	289	–	–
Salsalate	3.6	0.69	–	–	–	–	–
Sulindac	1.25	0.78	–	488	462	3.9	16.6
Sulindac sulfoxide	–	–	–	–	–	7.2	–
Tenoxicam	–	–	–	366	–	–	12.7
Tiaprofenic acid	–	–	–	484	452	5.8	17.6
Tolfenamic acid	–	–	–	690	–	37.9	–
Tolmetin	2.05	0.60 and 0.99	–	470	434	5.4	–
Zomepirac	3.7	–	–	–	495	–	–

Antibacterials

The general screening systems, previously described, may be used.

Antibacterials			
	HX	***HY***	***HAA***
	RI	***RI***	***RT***
Amoxicillin	–	226	3.1
Ampicillin	–	250	3.8
Azithromycin	–	–	–
Ceftriaxone	239	–	5.3
Chloramphenicol	390	336	14.1
Ciprofloxacin	318	260	9.1
Clarithromycin	–	–	–
Clindamycin	354	291	12
Furazolidone	336	–	12.2

continued

Antibacterials, continued			
	HX	***HY***	***HAA***
	RI	***RI***	***RT***
Isoniazid	–	246	–
Metronidazole	257	226	6.8
Minocycline	–	240	22.6
Nalidixic acid	–	380	16
Nitrofurantoin	319	288	–
Ofloxacin	314	260	8.6
Oxytetracycline dehydrate	299	260	–
Rifampicin	–	417	16.2
Roxithromycin	–	–	15.8
Tetracycline	314	265	9.9
Trimethoprim	299	254	8.3

Anticholinergics

The general screening systems, previously described, may be used or systems HAX and HAY below.

System HAX

Koves EM (1995). *J Chromatogr A* 692: 103–119.

- *Column:* Supelcosil LC-DP (250 × 4.6 mm i.d., 5 µm).
- *Eluent:* (A : B : C) Acetonitrile–phosphoric acid (0.025% v/v)–triethylamine buffer.
- *Isocratic elution:* (25 : 10 : 5).
- *Flow rate:* 0.6 mL/min.
- *Detection:* DAD (λ × 229 nm).
- *Note:* The triethylamine (TEA) buffer is prepared by adding 9 mL concentrated phosphoric acid and 10 mL TEA to 900 mL water, adjusted to pH 3.4 with diluted phosphoric acid and made up to 1 L with water.

System HAY

Koves EM (1995). *J Chromatogr A* 692: 103–119.

- *Column:* LiChrospher 100 RP-8 (250 × 4.0 mm i.d., 5 µm).
- *Eluent:* (A : B : C) as for System HAX.
- *Isocratic elution:* (60 : 25 : 15).
- *Flow rate:* 0.6 mL/min.
- *Detection:* DAD (λ × 229 nm).

Anticonvulsants and barbiturates

System HE

Christofides JA, Fry DE (1980). *Clin Chem* 26: 499–501.

- *Column:* Alkyl-silica SAS-Hypersil (125 × 4.5 mm i.d., 5 µm).
- *Mobile phase:* Acetonitrile–tetrabutylammonium phosphate, 0.005 mol/L, pH 7.5 (20 : 80).

System HG

Gill R *et al.* (1981). *J Chromatogr* 204: 275–284.

- *Column:* ODS Hypersil (150 × 4.6 mm i.d., 5 µm).
- *Mobile phase:* Methanol–0.1 mol/L sodium dihydrogenphosphate (11.998 g/L) (40 : 60); adjust to pH 3.5 by the addition of phosphoric acid.

System HH

Gill R *et al.* (1981). *J Chromatogr* 226; *Biomed Appl* 15: 117–123.

- *Column:* As for System HG, above.
- *Mobile phase:* As for System HG except that the mixture is adjusted to pH 8.5 by the addition of sodium hydroxide solution.

Anticholinergics

	HA	*HX*	*HY*	*HZ*	*HAA*	*HAX*	*HAY*
	k	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***
Adiphenine	1.8	422	–	–	–	–	–
Atropine	3.9	306	251	2.2	10.4	7	3.8
Biperiden	–	–	–	6.4	14.8	–	–
Chlorphenoxamine	2.9	–	346	–	–	–	–
Clidinium	–	379	–	–	–	–	–
Clidinium bromide	–	–	–	–	13.3	–	–
Cyclopentolate	1.6	353	287	3.2	–	–	–
Dicycloverine	1.1	–	575	–	–	–	–
Diethazine	3.4	–	–	–	–	15.1	7.4
Emepronium bromide	5.2	420	–	–	–	–	–
Homatropine	4.2	272	223	–	–	6.8	3.6
Hyoscine	1.1	270	253	–	7.4	7	3.7
Hyoscyamine	3.7	–	–	–	9.7	–	–
Isopropamide Iodide	2.4	379	–	–	–	–	–
Metixene	3.6	451	–	–	–	–	–
Orphenadrine	3	418	323	6	–	–	–
N-Monodesmethylorphenadrine	1.7	–	–	–	–	–	–
N-Oxide	1.1	–	–	–	–	–	–
Oxyphencyclimine	2.8	424	–	–	–	–	–
Oxyphenonium bromide	2.6	424	–	–	–	–	–
Piperidolate	1.7	429	–	–	–	–	–
Procyclidine	2	406	–	6.2	–	>20	4.7
Profenamine	2.4	444	338	–	–	16.6	8.3
Propantheline bromide	4.4	454	–	–	–	–	–
Xanthanoic acid	–	499	–	–	–	–	–
Trihexyphenidyl	1.8	429	381	7.6	15.3	–	–

Anticonvulsants, barbiturates and antiepileptics					
	HG	***HH***	***HX***	***HY***	***HZ***
	k	**k**	***RI***	***RI***	***RT***
Allobarbital	2.46	1.33	346	–	2.7
Amobarbital	10.91	7.05	424	374	4
Aprobarbital	3.42	2.22	357	319	2.8
Barbital	1.11	0.63	308	258	2.2
Benactyzine	–	–	382	–	–
Brallobarbital	3.09	1.72	371	336	3
Butalbital	6.17	3.48	394	342	3.4
Butetamate	–	–	390	–	–
Butobarbital	5.43	3.42	384	355	3.2
Carbamazepine	–	–	418	368	–
Clonazepam	–	–	465	403	4.6
Cyclobarbital	5.25	2.61	384	352	3.2
Cyclopentobarbital	6	3.84	391	352	–
Enallylpropymal	8.65	6.96	–	394	–
Ethosuximide	–	–	301	276	2.3
Flavoxate	–	–	–	–	–
Heptabarb	9.9	4.93	416	377	3.9
Hexethal	34.28	20.39	–	451	–
Hexobarbital	7.37	5.67	419	242	4.3
Ibomal	4.01	2.58	379	352	–
Idobutal	8.12	4.77	–	357	–
Mebeverine	–	–	448	–	7.1
Mephenytoin	–	–	–	366	3.7
Mesuximide	–	–	–	387	4.8
Metharbital	2.69	1.99	435	324	–
Barbital	1.11	0.63	–	–	–
Methylphenobarbital	7.27	3.84	435	395	4.6
Nealbarbital	10.22	6.19	417	382	–
Papaverine	–	–	363	295	–
Pentobarbital	10.96	8.07	424	383	4.1
Phenacemide	–	–	339	266	–
Phenobarbital	3.09	1.23	379	335	3
Phenytoin	–	–	431	381	3.7
Primidone	–	–	322	288	2.1
Secbutabarbital	4.9	3.3	377	331	–
Secobarbital	16.28	11.47	437	407	4.7
Sultiame	–	–	344	275	–
Talbutal	7.2	4.7	403	370	–
Thiamylal	–	–	516	476	–
Thiopental	–	–	485	433	6.9
Vinbarbital	4.83	2.32	379	363	–
Vinylbital	–	–	424	–	4.1

Antidepressants

The general screening systems, previously described, may be used or systems HF and HAZ below.

System HF

R Gill, unpublished data, after Kabra PM *et al.* (1981). *Clin Chim Acta* 111: 123–132.

- *Column:* ODS Hypersil (160 × 5 mm i.d., 5 µm).
- *Mobile phase:* Acetonitrile–phosphate buffer (pH 3.0; 30 : 70). To prepare the phosphate buffer, add 0.6 mL nonylamine to 1 L 0.01 mol/L sodium dihydrogenphosphate (1.1998 g/L) and adjust the pH to 3.0 by the addition of phosphoric acid.

System HAZ

Chiba K *et al.* (1995). *J Chromatogr B* 668: 77–84.

- *Column:* C_{18} (250 × 4.0 mm i.d., 5 µm).
- *Mobile phase:* (A : B : C) Water–methanol–triethylamine adjusted to pH 5.5 with phosphoric acid.
- *Isocratic elution:* (70 : 30 : 0.1).
- *Flow rate:* 0.7 mL/min.
- *Detection:* UV (λ = 240 nm).

Antidepressants and antipsychotics								
	HA	***HF***	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAZ***
	k	**k**	***RI***	***RI***	***RT***	***RT***	***RT***	**k**
Amitriptyline	3.3	5.42	440	375	7.5	15.9	15.8	1.76
10-Hydroxyamitriptyline	2.9	–	–	–	–	–	–	–
10-Hydroxynortriptyline	1.8	–	–	–	–	–	–	–
Nortriptyline	2	4.58	–	–	–	–	–	1.71
Amoxapine	–	–	398	–	–	14.2	–	–
Benperidol	1.1	–	393	324	3.6	–	–	–
Butriptyline	2.7	7.33	–	369	–	–	–	–
Norbutriptyline	1.7	–	–	–	–	–	–	–
Citalopram	–	–	403	–	4.5	–	–	–
Desmethylcitalopram	–	–	–	–	3.7	–	–	–
Clomipramine	3.4	9.92	462	405	10.2	16.4	–	–
Monodesmethylclomipramine	2	–	–	–	–	–	–	–
Desipramine	2.1	3.6	424	361	5.9	14.9	13	1.52
Didesmethylimipramine	1.3	–	–	–	–	–	–	–
2-Hydroxydesipramine	1.2	–	–	–	–	–	–	–
M (2-OH-)	–	–	–	–	–	–	–	0.39
Dibenzepin	2.8	0.5	361	300	–	–	–	–
Dosulepin	3.2	3.6	428	367	5.7	–	–	–
M (sulfoxide)	4.6	–	–	–	–	–	–	–
M (nor-)	2.2	–	–	–	–	–	–	–
Doxepin	3.7	2.27	404	316	5	14.1	12.9	–
M (nor-)	2.2	–	–	–	4.6	–	–	–
Fluoxetine	–	–	–	400	7.6	16.2	12.2	–
Desmethylfluoxetine	–	–	–	–	6.7	–	–	–
Fluvoxamine	–	–	430	363	5.6	15.3	10	–
Imipramine	4.2	4.17	437	335	6.7	15.1	14.7	1.62
Desipramine	2.1	3.6	–	–	–	–	–	–
2-Hydroxydesipramine	1.2	–	–	–	–	–	–	–
2-Hydroxyimipramine	3.1	–	–	–	–	–	–	–
M (10-OH-)	–	–	–	–	–	–	–	0.39
M (2-OH-)	–	–	–	–	–	–	–	0.39
M (*N*-oxide)	–	–	–	–	–	–	–	1.85
Iprindole	4.1	10.83	–	–	–	–	–	–
Isocarboxazid	–	–	392	353	–	–	–	–
Maprotiline	2.2	4.92	438	389	6.6	15.5	–	1.44
Desmethylmaprotiline	1.1	–	–	–	–	–	–	–
Mianserin	1.8	–	391	342	4.6	13.8	–	1.18
M(nor-)	2.4	–	–	–	–	–	–	–
M (nor-)	–	–	–	–	–	–	–	0.88
M (*N*-oxide)	–	–	–	–	–	–	–	0.53
M (8-OH-)	–	–	–	–	–	–	–	0.19
Moclobemide	–	–	295	–	2.4	10.2	6.9	–
Nialamide	1.2	–	334	–	–	–	–	–
Nomifensine	0.9	0.42	349	296	–	–	–	–
Nortriptyline	2	4.58	–	338	6.6	15.6	13.7	1.71
10-Hydroxynortriptyline	1.8	–	–	–	–	–	–	–
Noxiptiline	–	1.63	–	330	–	–	–	–
Opipramol	2.2	1.63	377	340	3.9	14.2	–	–

Antidepressants and antipsychotics, continued								
	HA	***HF***	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAZ***
	k	**k**	***RI***	***RI***	***RT***	***RT***	***RT***	**k**
Paroxetine	–	–	426	337	5.6	15.3	11.1	–
Phenelzine	1	–	184	–	–	–	–	–
Protriptyline	2.1	3.6	418	362	–	–	–	–
Remoxipride	–	–	334	–	3	–	8.8	–
M (FLA-838)	–	–	316	–	–	–	–	–
M (NCM-001)	–	–	364	–	–	–	–	–
M (NCM-009)	–	–	341	–	–	–	–	–
Sertraline	–	–	460	–	8.2	–	14.5	–
(desmethylsertraline)	–	–	–	–	7.0	–	–	–
Tofenacin	1.7	–	–	–	5.3	–	–	–
Trazodone	0.6	–	378	305	3.3	12.7	–	–
Trimipramine	2.7	6.17	454	345	8.3	15.9	15.5	–
M (nor-)	1.8	–	–	–	–	–	–	–
Viloxazine	–	2.7	325	273	–	11	–	–
Zimeldine	3.2	0.67	–	270	–	–	–	–
M (nor-)	2.9	–	–	–	–	–	–	–

Antihistamines

The general screening systems, previously described, may be used.

Antimalarials

The general screening systems, previously described, may be used.

Antihistamines							
	HA	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAY***
	k	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***
Alimemazine	3.1	420	–	–	–	14.9	7.1
Antazoline	1.8	383	294	–	–	–	–
Astemizole	–	–	286	3.9	13.2	–	–
(astemizole)	–	383	–	–	–	–	–
(M-nor)	–	361	–	–	–	–	–
Bromazine	2.7	444	–	–	–	–	–
Brompheniramine	4.1	–	267	–	13.9	–	–
Buclizine	0.7	–	454	–	–	–	–
Carbinoxamine	4.7	359	–	–	12.8	–	–
Cetirizine	–	–	–	3.6	15.7	8.89	5.29
Chlorcyclizine	2.3	–	340	–	–	–	–
Chlorphenamine	3.9	356	264	3.5	12.9	10.8	5.3
Cinnarizine	0.8	560	–	22	19.3	–	–
Clemastine	3.7	501	–	14	–	–	–
Clemizole	4.8	420	–	–	–	–	–
Cyclizine	2.9	405	–	4.8	–	12.4	5.8
Norcyclizine	2.2	–	–	–	–	–	–
Cyproheptadine	3.2	–	354	6.5	15	–	–
Deptropine	5	471	–	10.3	–	–	–
Dimetindene	5.1	338	288	–	–	–	–
Diphenhydramine	3.3	393	336	–	–	12.2	6
Diphenylpyraline	3.3	401	–	–	–	–	–
Doxylamine	4.4	–	259	–	11.1	–	–
Hydroxyzine	1.4	437	326	5.7	15.3	11.4	6.3
Isothipendyl	3.8	390	–	–	13.5	–	–
Loratadine	–	523	362	14.6	22.9	10.9	13.3

continued

Antihistamines, continued							
	HA	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAY***
	k	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***
Mebhydrolin	3	411	–	5.3	–	–	–
Meclozine	0.7	587	398	–	20	–	–
Mepyramine	3.9	448	257	–	–	–	–
Methapyrilene	4.1	342	197	–	–	–	–
Methdilazine	6	–	–	–	–	15.2	6.7
Phenindamine	2.5	397	–	–	–	–	–
Pheniramine	4.1	283	206	–	–	9.5	4.5
Phenyltoloxamine	3.1	415	–	–	–	–	–
Pizotifen	3.4	435	–	6.6	15.2	–	–
Promethazine	5	409	324	5.7	14.5	13.2	6.4
Propiomazine	2.1	440	359	–	–	14.1	7.1
Pyrrobutamine	2.8	477	–	–	–	–	–
Thenyldiamine	4	317	–	–	–	–	–
Thiazinamium metilsulfate	–	–	–	6.4	–	–	–
Trimethobenzamide	4.7	347	–	–	–	–	–
Tripelennamine	3.6	336	265	–	–	–	–
Triprolidine	3.2	388	270	–	13.1	–	–

Antimalarials							
	HA	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAY***
	k	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***
Chloroquine	15.2	282	246	2.1	5.4	12.7	3.6
Cinchonidine	3.1	306	214	–	–	–	–
Cinchonine	–	304	209	–	10.2	–	–
Halofantrine	–	800	–	–	23	–	–
Hydroxychloroquine	–	280	–	1.9	–	9.6	3.2
Primaquine	1.4	–	276	–	–	–	–
Proguanil	–	379	–	3.8	13.6	–	–
Pyrimethamine	1	–	289	–	12.5	–	–
Quinine	2.4	327	246	2.6	11.3	8.3	4.5

Antineoplastics

The general screening systems, previously described, may be used.

Antineoplastics		
	HX	***HAA***
	RI	***RT***
Diethylstilbestrol	592	20.9
Doxorubicin	370	12.1
Fluorouracil	70	3.4
Methotrexate	292	–
Vinblastine	–	8.4

Antitussives

The general screening systems, previously described, may be used.

Antitussives				
	HA	***HX***	***HY***	***HAA***
	k	***RI***	***RI***	***RI***
Bromhexine	0.4	417	334	–
Dextromethorphan	5.6	377	298	13.3
Dextrorphan	4.7	–	–	–
Dextrorphan	–	325	–	–
Dropropizine	–	240	–	7.2
Guaifenesin	–	328	262	11.4
Noscapine	0.3	368	289	12.8
Pholcodine	6	65	92	2.7
Pipazetate	5.4	385	–	–

Antivirals

The general screening systems, previously described, may be used or systems HAB and HAC below.

System HAB
Sparidans RW *et al.* (2000). *J Chromatogr B Biomed Sci Appl* 742: 185–192.

- *Column:* C_{18} Symmetry (100 × 4.6 mm i.d., 3.5 µm) with Symmetry C_{18} precolumn (20 × 3.8 mm, 5 µm).
- *Mobile phase:* Acetonitrile–sodium phosphate buffer (25 mmol/L, pH 6.8) (40 : 60).
- *Flow rate:* 1.5 mL/min.
- *Detection:* Fluorescence ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 340$ nm).
- *Note:* 8 min after each injection, flush column for 5 min at 1.5 mL/min with acetonitrile–water (30 : 70). Equilibrate for about 8 min with the original eluent before injecting the next sample.

System HAC
Aymard G *et al.* (2000). *J Chromatogr B Biomed Sci Appl* 744: 227–240.

- *Column:* C_{18} Symmetry (250 × 4.6 mm i.d., 5 µm) with C_{18} precolumn (Guard-Pak, µBondapak).
- *Column temperature:* 37°C.
- *Mobile phase:* (A : B) Disodium hydrogenphosphate (0.04 mol/L) with 4% (v/v) octane sulfonic acid (0.25 mol/L)–acetonitrile.
- *Isocratic elution:* (50 : 50).
- *Flow rate:* 1.3 mL/min.
- *Detection:* DAD (λ = 261 nm between time 0 and 9 min; λ = 241 nm between time 9 and 20 min; λ = 254 nm between time 20 and end of the run (32 min).

Antivirals	*HAB*	*HAC*
	RT	k
Abacavir	1	–
Amprenavir	4	2.5
Efavirenz	–	8.5
Indinavir	4.2	2

Benzodiazepines

System HI
R Gill, unpublished data.

- *Column:* ODS Hypersil (200 × 5 mm i.d., 5 µm).
- *Mobile phase:* Methanol–water–phosphate buffer (55 : 25 : 20). To prepare the phosphate buffer, dissolve 11.038 g (0.092 mol/L) sodium dihydrogenphosphate and 1.136 g (0.008 mol/L) disodium hydrogenphosphate in sufficient water to produce 1 L.

System HJ
R Gill, unpublished data.

- *Column:* As for System HI, above.
- *Mobile phase:* Methanol–water–phosphate buffer (as in System HI) (70 : 10 : 20).

System HK
R Gill, unpublished data, after RJ Flanagan *et al.* (1980). *J Chromatogr* 187: 391–398.

- *Column:* Silica Spherisorb (250 × 5 mm i.d., 5 µm).
- *Mobile phase:* Methanol to which has been added 100 µL perchloric acid per litre.

Cannabinoids

System HL
Baker PB *et al.* (1980). *J Anal Toxicol* 4: 145–152.

- *Column:* ODS Spherisorb (250 × 4.6 mm i.d., 5 µm).
- *Mobile phase:* 0.01 mol/L sulfuric acid–methanol–acetonitrile (7 : 8 : 9).

Cannabinoids	*System HL*
	k
Cannabichromene	19.09
Cannabicyclol	14.78
Cannabidiol	7.47
Cannabidiolic acid	8.76
Cannabigerol	8.18
Cannabinol	11.77
Cannabivarin	7.47
Δ^8-Tetrahydrocannabinol	14.07
Δ^9-Tetrahydrocannabinol	13.35
Tetrahydrocannabinolic acid	25.83
Tetrahydrocannabivaric acid	14.64
Tetrahydrocannabivarin	8.18

Cardiac glycosides

System HM
Cobb PH (1976). *Analyst (Lond)* 101: 768–776.

- *Column:* Silica LiChrosorb SI60 (250 × 4 mm i.d., 10 µm).
- *Mobile phase:* Cyclohexane–ethanol–acetic acid (60 : 9 : 1).

Cardiac glycosides	*System HM*
	k
Digitoxigenin	2.0
Digitoxigenin bisdigitoxoside	3.9
Digitoxigenin monodigitoxoside	2.8
Digitoxin	5.4
Digoxigenin	4.5
Digoxigenin bisdigitoxoside	8.2
Digoxigenin monodigitoxoside	5.5
Digoxin	11.3
Gitaloxin	6.8
Gitoxigenin	3.7
Gitoxigenin bisdigitoxoside	6.5
Gitoxigenin monodigitoxoside	4.5
Gitoxin	8.6
Lanatoside A	17.9
Lanatoside B	31.8
Lanatoside C	39.5

Benzodiazepines	*HI*	*HJ*	*HK*	*HX*	*HY*	*HZ*	*HAA*	*HAX*	*HAY*
	k	k	k	*RI*	*RI*	*RT*	*RT*	*RT*	*RT*
Acecarbromal	–	–	–	429	374	–	–	–	–
Alprazolam	–	–	2.79	–	–	–	–	–	–
Bromazepam	–	–	2.99	–	–	–	–	–	–

continued

Benzodiazepines, continued									
	HI	*HJ*	*HK*	*HX*	*HY*	*HZ*	*HAA*	*HAX*	*HAY*
	k	k	k	*RI*	*RI*	*RT*	*RT*	*RT*	*RT*
Bromisoval	–	–	–	365	307	2.9	–	–	–
Brotizolam	–	–	–	484	–	4.6	–	7.4	7.9
Carbromal	–	–	–	410	377	3.9	–	–	–
Chlordiazepoxide	–	–	2.87	–	–	–	–	–	–
Clobazam	–	–	0.03	–	–	–	–	–	–
Clomethiazole	–	–	–	395	292	–	16	–	–
Clonazepam	–	–	0.35	–	–	–	–	–	–
Clorazepic acid	–	–	2.00	–	–	–	–	–	–
Demoxepam	–	–	0.03	–	–	–	–	–	–
Diazepam	–	–	2.49	–	–	–	–	–	–
Flumazenil	–	–	–	387	327	2.6	–	–	–
Flunitrazepam	3.15	–	0.47	483	305	5.6	18.6	–	–
Flurazepam	–	3.19	6.5	397	305	4.2	–	10.5	5.5
Glutethimide	–	–	–	436	401	4.8	–	6.6	6.2
Ketazolam	–	–	0.04	–	–	–	–	–	–
Loprazolam	–	–	–	388	–	–	13.4	–	–
Lorazepam	–	–	0.14	–	–	–	–	–	–
Lormetazepam	6.32	–	0.08	487	463	6.2	–	–	–
Medazepam	–	–	4.44	–	–	–	–	–	–
Methaqualone	–	–	–	459	400	5.4	–	6.8	7.4
Methyprylon	–	–	–	347	302	–	–	–	–
Midazolam	9.75	2.1	5.9	399	306	4.2	14.9	10.2	6.3
Nitrazepam	2.96	–	1.49	448	370	4.2	16.9	6.3	6
Nordazepam	–	–	1.99	–	–	–	–	–	–
Oxazepam	4.62	–	0.73	–	–	–	–	–	–
Prazepam	–	–	2.19	–	–	–	–	–	–
Quazepam	–	–	–	–	766	37.5	–	11.9	17.7
Temazepam	5.68	–	0.6	472	438	5.5	18.6	8.9	6.7
Oxazepam	–	–	0.73	–	–	–	–	–	–
Triazolam	4.38	–	1.83	476	390	4.2	17.4	6.4	6.7
Not detected	–	–	–	–	–	–	–	–	–
Zolpidem	–	–	–	–	291	3.2	11.9	–	–
Zopiclone	–	–	–	331	269	2.3	–	7.5	3.8

Cardioactive drugs

The general screening systems, previously described, may be used.

Cardioactive drugs					
	HA	*HX*	*HY*	*HZ*	*HAA*
	k	*RI*	*RI*	*RT*	*RT*
Ajmaline	2.8	–	277	–	–
Alfuzosin	–	–	–	2.4	10.4
Amiodarone	2.4	683	476	90.4	–
Monodesethyl-amiodarone	1.8	–	–	–	–
Aprindine	–	433	–	–	17
Bamethan	0.9	250	–	–	5.9
Benzthiazide	–	–	415	–	–
Betahistine	3.1	–	–	–	3.2
Bretylium tosilate	4.3	–	275	–	–

Cardioactive drugs, continued					
	HA	*HX*	*HY*	*HZ*	*HAA*
	k	*RI*	*RI*	*RT*	*RT*
Buphenine	0.9	370	–	–	–
Captopril	–	316	283	2.1	9.7
Cilazapril	–	420	–	4.5	14.4
Cilazaprilate	–	–	–	1.7	–
Clonidine	1.2	258	194	2.5	6.1
Clopamide	–	377	310	–	–
Debrisoquine	1.2	–	245	–	–
Diltiazem	–	–	361	4.5	14
Deacetyl-diltiazem	–	–	–	–	–
Desmethyl-diltiazem	–	–	–	–	–

Cardioactive drugs, continued					
	HA	***HX***	***HY***	***HZ***	***HAA***
	k	***RI***	***RI***	***RT***	***RT***
Desacetyldil-tiazem	–	–	–	–	–
Disopyramide	2.4	345	281	3	11.4
N-Monodesiso-propyldisopyramide	1.8	–	–	–	–
Enalapril	–	201	–	1.5	3.4
Encainide	–	363	–	–	–
Felodipine	–	690	–	25.8	24.4
Flecainide	–	419	355	5.2	–
Hydralazine	–	193	132	1.9	–
Isoxsuprine	0.8	353	301	–	–
Labetalol	1.7	365	290	3	–
Lidoflazine	0.6	530	–	–	–
Lisinopril	–	271	250	1.5	–
Lorcainide	1.8	425	–	6.6	–
Methyldopa	–	69	–	1.4	3
Mexiletine	1.2	329	278	–	11.5
Minoxidil	–	297	–	2.4	9.8
Naftidrofuryl oxalate	–	–	409	–	15.8
Nifedipine	0.2	527	464	7.2	19.5
Pargyline	0.2	–	203	–	–
Pentaerithrityl tetranitrate	–	663	–	–	–
(pentaerithrityl)	–	–	–	–	23.1
Pentoxifylline	–	355	274	2.1	11.5
Perindopril	–	–	–	1.6	13.7
(perindoprilat)	–	314	–	–	–
Phenoxybenzamine	0.1	396	–	–	–
Phentolamine	1.7	368	–	3	–
Prajmalium bitartrate	2.2	–	340	–	–
Prazosin	0.8	352	–	2.5	10.6
Procainamide	1.3	208	160	1.9	–
N-Acetylpro-cainamide	3	–	–	1.8	–
Quinapril	–	–	–	5.4	16.8
Quinidine	2.1	322	245	2.6	11
Ramipril	–	–	–	4.2	15.7
Rescinnamine	0.6	496	407	–	–
Reserpine	–	467	351	–	16.4
Sotalol	1.2	226	–	2	3.8
Tocainide	1.2	247	208	2.1	–
Tolazoline	2.1	225	179	–	–
Trandolapril	–	–	–	6.1	17
Trandolaprilat	–	–	–	2.1	–
Trimetazidine	3	–	–	–	6.1
Verapamil	2.6	447	386	7	15.4
M (nor-)	1.7	–	–	6.6	–

Diuretics

System HN

R Gill *et al.*, unpublished data, after Tisdall PA *et al.* (1980). *Clin Chem* 26: 702–706.

- *Column:* ODS Hypersil (160 × 5 mm i.d., 5 µm).
- *Mobile phase:* Acetonitrile–water containing 10 mL/L acetic acid (30 : 70).

Diuretics				
	HN	***HX***	***HY***	***HAA***
	k	***RI***	***RI***	***RT***
Acetazolamide	–	268	226	6.9
Amiloride	–	257	190	3.6
Bendroflumethiazide	15.35	508	–	18.6
Benzthiazide	9.32	–	415	–
Chlorothiazide	0.54	–	239	–
Chlortalidone	1.28	367	308	–
Clopamide	4.01	377	310	–
Clorexolone	7.26	–	391	–
Cyclopenthiazide	16.45	–	453	–
Cyclothiazide	10.78, 11.91, and 12.81	–	433	–
Etacrynic acid	–	521	497	–
Furosemide	–	435	380	15.2
Hydrochlorothiazide	0.7	294	255	–
Mefruside	8.67	–	417	–
Methyclothiazide	3.82	–	364	15.4
Metolazone	4.89	–	371	–
Spironolactone	–	592	539	20.7
Triamterene	–	298	263	8.7
Trichlormethiazide	3.1	–	341	14.9
Xipamide	–	488	–	18.8

Drugs of abuse

A comprehensive HPLC method for the screening of common drugs of abuse is described in Chapter 1, Table 1.24. Furthermore, an additional eight systems (HBC, HBD, HBE, HBF, HBG, HBI and HBJ) are provided in Chapter 11, Table 11.4.

Drugs of abuse						
	HA	***HC***	***HX***	***HY***	***HZ***	***HAA***
	k	**k**	***RI***	***RI***	***RI***	**k**
5-Methyltrypt-amine	–	–	–	–	–	–
Amfetamine	0.9	0.98	244	–	–	3.7
Benzfetamine	1.2	0.15	–	–	–	–
Benzoylecgonine	0.9	–	–	236	1.7	9.7
Bufotenine	3.1	–	–	181	–	–
Cannabidiol	–	–	990	902	–	–
Cannabinol	–	–	1080	1028	–	–
Cocaine	2.8	–	348	289	3.3	11.9
Δ^9-THC	–	–	–	–	–	–
Diamorphine	3	0.66	340	282	–	–
Diethyltrypt-amine	–	–	–	–	–	–
Dimethyltrypt-amine	–	–	–	228	–	–
DOM	–	1.13	340	–	–	–
Ketamine	–	–	311	262	2.4	9.6
Lysergic acid	0.8	–	–	236	–	–
Lysergide	0.7	–	362	–	–	12

continued

Drugs of abuse, continued						
	HA	***HC***	***HX***	***HY***	***HZ***	***HAA***
	k	**k**	***RI***	***RI***	***RI***	**k**
Mescaline	1.3	2.17	272	243	–	–
Metamfetamine	2	2.07	262	216	2.4	8.4
Methadone	2.2	1.03	440	343	8.5	15.8
Methylenedioxy-amfetamine	–	0.98	266	248	2.1	8.1
Methylenedioxy-methamfetamine	–	–	278	252	2.2	9.1
Monoacetyl-morphine	3.6	0.8	–	–	–	7.3
Morphine	3.8	1.3	200	182	1.8	3.3
N-Methyltrypt-amine	–	–	–	–	–	–
p-Methoxyam-fetamine	–	–	–	–	–	–
Psilocin	3.1	–	240	226	–	–
Psilocybine	–	–	–	185	–	–

Ergot alkaloids

System HA, previously described, may be used or System HP, below.

System HP

R Gill *et al*., unpublished data, after Twitchett PJ *et al*. (1978). *J Chromatogr* 150: 73–84.

- *Column:* ODS Hypersil (100 × 5 mm i.d., 5 µm).
- *Mobile phase:* Methanol–phosphate buffer (60 : 40). To prepare the phosphate buffer, dissolve 3.43 g (0.022 mol/L) sodium dihydrogen-phosphate and 10.03 g (0.028 mol/L) disodium hydrogenphosphate in sufficient water to produce 1 L.

Ergot alkaloids		
	HA	***HP***
	k	**k**
Bromocriptine	–	44.3
Dihydroergocristine	–	18.3
Dihydroergocryptine	–	15.9
Dihydroergotamine	0.6	11.4
Ergocornine	0.4	10.2
Ergocristine	0.3	17.3
Ergocryptine	0.4	15.2
Ergometrine	0.4	0.50
Ergosine	0.3	7.08
Ergosinine	0.3	17.7
Ergotamine	0.4	9.58
Iso-lysergic acid	–	0.83
Iso-lysergide	2.6	0.0
Lysergamide	0.5	0.33
Lysergic acid	0.8	0.0
Lysergic acid methylpropylamide	–	1.98
Lysergide	0.7	1.83
Lysergol	1.1	0.83
Methylergometrine	0.4	0.83
Methysergide	0.4	2.33
2-Oxylysergide	–	0.92

Local anaesthetics

The general screening systems, previously described may be used, as well as system HQ or HR, below.

System HQ

Gill R *et al*. (1984). *J Chromatogr* 301: 155–163.

- *Column:* ODS Hypersil (160 × 5 mm i.d., 5 µm).
- *Mobile phase:* Methanol–water–1% v/v solution of phosphoric acid–hexylamine (30 : 70 : 100 : 1.4).

System HR

Gill R *et al*. (1984). *J Chromatogr* 301: 155–163.

- *Column:* As for System HQ above.
- *Mobile phase:* Methanol–1% v/v solution of phosphoric acid–hexylamine (100 : 100 : 1.4).

Local anaesthetics						
	HA	***HQ***	***HR***	***HX***	***HY***	***HZ***
	k	**k**	**k**	***RI***	***RI***	***RT***
Benzocaine	0.1	20.06	1.61	404	358	4.3
Bupivacaine	0.9	7.19	0.86	366	310	4.1
Butacaine	1.2	8.97	–	392	331	–
Butanilicaine	–	4.42	–	–	280	–
Chloroprocaine	–	0.24	–	–	250	–
Cinchocaine	1.9	–	5.51	–	371	–
Cocaine	2.8	2.68	–	348	289	3.3
Benzoylecgonine	0.9	5.68	–	–	–	–
Ecgonine	1.1	–	–	–	–	–
Cyclomethycaine	–	–	10.31	–	413	–
Dyclonine	–	–	2.78	–	347	–
Etomidate	–	–	475	417	–	
Ketamine	–	–	–	311	262	2.4
Lidocaine	0.6	0.79	–	288	258	2.6
M (monoethyl-glycinexylidide)	1.2	–	–	–	–	–
Mepivacaine	0.9	1.09	–	296	260	2.6
Methohexital	–	–	–	503	484	–
Oxybuprocaine	–	16.25	0.86	405	–	–
Piperocaine	–	4.59	–	357	312	–
Pramocaine	0.6	–	2.48	415	–	6.5
Prilocaine	1	1.38	–	–	–	2.7
Procaine	1.9	–	–	264	225	–
Propofol	–	–	–	–	–	35
Proxymetacaine	2.1	1.38	–	–	269	–
Quinisocaine	2.2	–	11.24	–	–	–
Tetracaine	2	16.25	1.33	389	321	4.4

Narcotic analgesics

Systems HA or HC, previously described, may be used or System HS, below.

System HS

Baker PB, Gough TA (1981). *J Chromatogr Sci* 19: 483–489.

- *Column:* Aminopropyl-bonded silica Spherisorb S5NH_2 (250 × 4 mm i.d., 5 µm).
- *Mobile phase:* Acetonitrile–tetrabutylammonium phosphate, 0.005 mol/L, pH 7.5 (85 : 15).

Narcotic analgesics and narcotic antagonists									
	HA	***HC***	***HS***	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAY***
	k	**k**	**k**	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***
Alphaprodine	2.8	–	–	363	317	–	–	–	–
Bezitramide	0.2	–	–	564	–	22.5	–	–	–
Buprenorphine	0.4	0.05	–	397	339	5	14	–	–
Codeine	4.8	1.21	1.9	266	237	1.9	5	6.1	3.4
Morphine	3.8	1.3	5.16	–	–	–	–	–	–
M (nor-)	3.1	3.51	–	–	–	–	–	–	–
Cyclazocine	2.1	–	–	–	289	–	–	–	–
Dextromoramide	0.7	0.09	–	440	390	–	15.8	–	–
Dextropropoxyphene	1.9	0.19	–	–	374	7.6	15.8	–	–
Norpropoxyphene	1.3	–	–	–	–	–	–	–	–
Diamorphine	3	0.66	0.35	340	282	–	–	7.9	4.1
6-Monoacetylmorphine	3.6	0.8	1	–	–	–	–	–	–
Morphine	3.8	1.3	5.16	–	–	–	–	–	–
Dihydrocodeine	7.2	2.5	–	261	208	2	4.7	–	–
Dihydromorphine	5.7	2.75	–	237	156	–	–	–	–
Dipipanone	2.2	1.61	–	500	363	–	–	–	–
Ethoheptazine	3.3	1.55	–	359	–	–	–	–	–
Ethylmorphine	3.7	1.06	1.45	291	244	–	–	6.7	3.6
Fentanyl	0.8	1.11	–	373	299	–	14.2	11.4	6
Hydromorphone	7.9	–	–	240	187	–	–	5.8	3.4
Ketobemidone	2.8	–	–	294	245	–	–	–	–
Levallorphan	1.9	1.46	–	356	291	–	–	–	–
Levorphanol	4.4	3.2	–	–	265	–	–	–	–
Meptazinol	3.1	–	–	–	269	–	–	–	–
Methadone	2.2	1.03	–	440	343	8.5	15.8	16.5	8.4
M (EDDP)	2.8	–	–	–	–	–	–	–	–
M (EMDP)	0.2	–	–	–	–	–	–	–	–
Morphine	3.8	1.30	5.16	200	182	1.8	3.3	5.6	3.2
Morphine-3-glucuronide	–	1.56	–	–	–	–	–	–	–
N-oxide	3.2	–	–	–	–	–	–	–	–
Nalorphine	1	0.29	–	260	237	–	4.8	–	–
Naloxone	1.4	0.17	–	–	238	2	14	–	–
Norcodeine	3.1	3.51	–	–	235	–	–	–	–
Normethadone	–	0.53	–	–	366	–	–	–	–
Normorphine	2.9	3.92	–	–	133	–	–	–	–
Norpipanone	–	0.35	–	466	–	–	–	–	–
Oxycodone	6.9	0.85	–	277	246	–	–	6.5	5.8
Oxymorphone	6.7	–	–	–	–	–	–	–	–
Oxymorphone	6.7	–	–	217	184	–	–	–	–
Pentazocine	1.8	0.67	–	372	288	3.8	12.5	9.9	5.5
Pethidine	2.8	0.55	–	345	281	3.2	11.8	9.2	4.8
M (nor-)	1.7	2.04	–	–	–	–	–	–	–
Pethidinic acid	2.8	–	–	–	–	–	–	–	–
Phenazocine	1.3	0.3	–	409	299	–	–	–	–
Phenoperidine	0.8	0.1	–	434	–	–	–	–	–
Norpethidine	1.7	2.04	–	–	–	–	–	–	–
Pethidine	2.8	0.55	–	–	–	–	–	–	–
Piritramide	0.6	0.1	–	377	343	–	–	–	–
Thebacon	3.7	0.85	–	333	–	–	–	–	–
Tramadol	–	–	–	328	267	2.9	–	–	–

Oral hypoglyacemics and antidiabetics

The general screening systems, previously described, may be used.

Oral hypoglyacemics and antidiabetics				
	HX	*HY*	*HZ*	*HAA*
	RI	*RI*	*RT*	*RT*
Carbutamide	–	321	–	14.5
Chlorpropamide	450	411 and 413	5	17.7
Glibenclamide	637	571	14.4	22
Gliclazide	536	483	8.8	20.5
Glipizide	478	423	4.5	17.6
Metformin	60	–	1.7	2.8
Tolazamide	452	445	6.8	–
Tolbutamide	477	424	5.9	–

Pesticides

System HAO

Osselton MD, Snelling RD (1986). *J Chromatogr* 368: 265–271.

- *Column:* ODS Hypersil (160 × 5 mm i.d., 5 µm), stainless steel.
- *Mobile phase:* Acetonitrile–water (60 : 40).
- *Flow rate:* 2 mL/min.
- *Detection:* DAD (200–450 nm).

System HAP

Osselton MD, Snelling RD (1986). *J Chromatogr* 368: 265–271.

- *Column:* Silica Spherisorb S5W (250 × 5 mm i.d.).
- *Mobile phase:* Dichloromethane–isoctane (60 : 40).
- *Flow rate:* 2 mL/min.
- *Detection:* DAD (200–450 nm).

For more information on screening pesticides, see Chapter 16, Table 16.1.

Phenothiazines and other tranquillisers

The general screening systems, previously described, may be used.

Phenothiazines and other tranquillisers								
	HA	*HX*	*HY*	*HZ*	*HAA*	*HAX*	*HAY*	*HAZ*
	k	*RI*	*RI*	*RT*	*RT*	*RT*	*RT*	k
Acepromazine	4.1	–	350	–	10.8	–	–	–
Azacyclonol	1.2	–	–	–	–	8.7	4.5	–
Benzoctamine	1.7	380	322	–	–	–	–	–
Butaperazine	3.4	464	406	–	–	–	–	–
Captodiame	–	561	–	–	20.2	–	–	–
Chlordiazepoxide	–	363	285	3.2	15.2	6.9	5.3	1.68
Chlormezanone	–	–	334	–	15.5	6	5.3	–
Chlorpromazine	4.1	456	350	9.1	16	17	BASE	2.64
M (nor-)	2.2	–	–	–	–	–	–	–
M (sulfoxide)	–	–	–	–	–	8.4	4.3	0.62
Chlorprothixene	3	459	353	10.1	–	17.6	8.3	–
Clopenthixol	–	448	411	–	–	–	–	–
Clorazepic acid	–	475	388	5.6	–	–	–	–
Clorazepate	–	–	–	–	18.4	–	–	–
Fluanisone	–	423	349	–	–	–	–	–
Flupentixol	1.2	475	435	10.7	17.4	13.7	7.5	–
Sulfoxide	1.3	–	–	–	–	–	–	–
Fluphenazine	1.2	462	471	10.1	17.4	13.6	7.2	–
Fluspirilene	–	538	–	–	–	18.3	9.8	–
Haloperidol	1.2	421	316	5.8	14.4	11.1	6.2	0.72
Levomepromazine	3.2	435	381	7.5	–	15.2	7.2	1.82
Loxapine	1.1	407	336	–	14.6	–	–	–
Mesoridazine	5	–	337	3.4	–	10.1	5	–
Oxypertine	0.7	402	–	–	–	–	–	–
Pecazine	3.9	443	382	–	–	15.3	7	–
Penfluridol	–	659	656	43.4	20.2	–	–	–
Perazine	–	403	371	6.3	–	–	–	–
Pericyazine	1.3	410	356	4.4	–	10.2	5.1	–
Perphenazine	1.9	428	395	7.2	16	13.1	6.3	3.28
Pimozide	0.7	504	–	11.9	17.2	–	–	–
Pipamperone	–	299	241	2.7	10.9	–	–	–
Pipotiazine	–	431	–	–	14.7	–	–	–
Prochlorperazine	3.9	450	323	10.4	–	–	–	–
Promazine	5.9	407	326	5.9	–	–	–	–

Phenothiazines and other tranquillisers, continued								
	HA	***HX***	***HY***	***HZ***	***HAA***	***HAX***	***HAY***	***HAZ***
	k	***RI***	***RI***	***RT***	***RT***	***RT***	***RT***	**k**
Prothipendyl	4.4	388	–	–	–	–	–	–
Sulforidazine	–	421	–	4.8	–	–	–	–
Sulpiride	–	259	235	2	3.9	–	–	0.02
Thiopropazate	1	483	–	–	–	–	–	–
Thioproperazine	4.1	427	305	15.4	15.2	–	–	–
Thioridazine	5.2	490	427	13.5	17.2	–	9.8	3.88
Mesoridazine	5	–	–	–	–	–	–	–
Tiotixene	3.8	442	374	6.8	–	–	–	–
Triflupromazine	2.7	484	454	12.3	–	17.3	8.9	–

Steroids

System HATa

Walters MJ *et al.* (1990). *J Assoc Off Anal Chem* 73: 904–926.

- *Column:* ODS Zorbax (250 × 4.6 mm i.d., 5 µm), stainless steel.
- *Eluent:* (A) Methanol.
- *Isocratic elution:* (100).
- *Flow rate:* 1.5 mL/min.
- *Detection:* UV (λ = 240, 210 and 280 nm).

System HATb

Walters MJ *et al.* (1990). *J Assoc Off Anal Chem* 73: 904–926.

- *Column:* ODS Zorbax (250 × 4.6 mm i.d., 5 µm), stainless steel.
- *Eluent:* (A : B) Methanol–water.
- *Isocratic elution:* (75 : 25).
- *Flow rate:* 1.5 mL/min.
- *Detection:* UV (λ = 240, 210 and 280 nm).

System HAR

Lurie I *et al.* (1994). *J Forensic Sci* 39: 74–85.

- *Column:* ODS Zorbax (250 × 4.6 mm i.d., 5 µm).
- *Mobile phase:* (A : B) Water–methanol.
- *Gradient elution:* (30 : 70) to (0 : 100) over 15 min with 15 min hold.
- *Flow rate:* 1.0 mL/min.
- *Detection:* DAD.

System HT

Rose JQ, Jusko WJ (1979). *J Chromatogr Biomed Appl* 162: 273–280.

- *Column:* Silica Zorbax SIL (250 × 4.6 mm i.d., 5 µm).
- *Mobile phase:* Methylene chloride–methanol (97 : 3).

Steroids								
	HT	***HX***	***HY***	***HZ***	***HAA***	***HAR***	***HATa***	***HATb***
	k	***RI***	***RI***	***RT***	***RT***	***RRT***	***RRT***	***RRT***
Beclometasone	4.2	444	–	–	–	–	–	–
Dipropionate	–	–	711	–	–	–	–	–
Betamethasone	–	–	–	14.2	13.3	–	–	–
Betamethasone valerate	–	–	584	–	–	–	–	–
Boldenone	–	–	–	–	–	0.74	–	0.76
Undecylenate	–	–	–	–	–	–	1.94	–
Cortisone	2.4	–	372	–	–	–	–	–
Dexamethasone	4.8	–	381	3.4	13.1	–	–	–
Fluoxymesterone	–	–	427	–	–	0.78	–	0.7
Hydrocortisone	5.8	403	349	–	17.7	–	–	–
Hydroxyprogesterone	–	1054	–	–	–	–	–	–
Metenolone	–	–	–	–	–	–	–	–
Acetate	–	–	–	–	–	–	1.26	3.54
Enantate	–	–	–	–	–	–	1.87	–
Methandienone	–	–	–	–	–	0.86	–	0.87
Methandriol	–	–	–	–	–	1.25	–	1.29
Dipropionate	–	–	–	–	–	–	–	2.75
Methylprednisolone	7.5	426	390	–	18.9	–	–	–
Methyltestosterone	–	–	587	–	–	1.17	–	1.27
Nandrolone	–	–	–	–	–	0.84	–	0.92
Norethisterone	–	536	676	–	24	–	–	–
Prednisolone	8.4	401	361	2.5	14.1	–	–	–
Prednisone	3.4	250	340	2.6	14.2	–	–	–
Progesterone	–	672	698	–	23.8	–	–	–

continued

Steroids, continued								
	HT	*HX*	*HY*	*HZ*	*HAA*	*HAR*	*HATa*	*HATb*
	k	*RI*	*RI*	*RT*	*RT*	*RRT*	*RRT*	*RRT*
Testosterone	–	534	508	–	–	–	–	–
Acetate	–	–	894	–	–	1.76	–	2.59
Propionate	–	–	1003	–	–	2.01	1.31	4.06
Methyltestosterone	–	–	–	–	–	1.17	–	1.27
Isobutyrate	–	–	–	–	–	2.17	–	–
Cipionate	–	–	–	–	–	2.63	–	–
Enantate	–	–	–	–	–	2.6	1.8	–
Undecanoate	–	–	–	–	–	3.18	–	–
Phenylpropionate	–	–	–	–	–	–	1.48	–
Isocaproate	–	–	–	–	–	–	1.62	–
Cipionate	–	–	–	–	–	–	2.05	–
Undecenoate	–	–	–	–	–	–	2.53	–
Decanoate	–	–	–	–	–	–	2.78	–
Undecylate	–	–	–	–	–	–	3.27	–
Triamcinolone	–	438	312	–	–	–	–	–
Acetonide	2.5	–	–	–	–	–	–	–
Trenbelone	–	–	–	–	–	–	–	–
Hexahydrobenzylcarbonate	–	–	–	–	–	–	1.65	–
Acetate	–	–	–	–	–	–	–	1.71

Sulfonamides

System HU

Cobb PH, Hill GT (1976). *J Chromatogr* 123: 444–447.

- *Column:* Silica Spherisorb (250 × 4 mm i.d., 5 µm).
- *Mobile phase:* Cyclohexane–ethanol–acetic acid (85.7 : 11.4 : 2.9).

Sulfonamides	
	HU
	k
Phthalylsulfathiazole	14.0
Succinylsulfathiazole	16.8
Sulfadoxine	4.4
Sulfamerazine	8.1
Sulfaquinoxaline	4.8
Sulfacetamide	7.7
Sulfachlorpyridazine	3.3
Sulfadiazine	8.7
Sulfadimidine	7.1
Sulfafurazole	6.0
Sulfamethoxazole	4.8
Sulfamethoxydiazine	8.2
Sulfamethoxypyridazine	7.5
Sulfamoxole	12.6
Sulfanilamide	8.9
Sulfapyridine	3.8
Sulfathiazole	13.4

Xanthine stimulants

The general screening systems, previously described, may be used.

Xanthine stimulants					
	HA	*HX*	*HY*	*HZ*	*HAA*
	k	*RI*	*RI*	*RT*	*RT*
Caffeine	0.2	305	259	1.9	6.7
Diprophylline	–	275	227	–	3.6
Fenetylline	–	336	277	–	–
Proxyphylline	0.1	293	–	–	–
Theobromine	0.1	262	201	1.6	3.8
Theophylline	0.1	276	249	1.7	4.9

Additional systems

System HAD

Aymard *et al.* (2000). *J Chromatogr Biomed Sci Appl* 744: 227–240.

- *Column:* C_{18} Symmetry Shield (250 × 4.6 mm i.d., 5 µm) protected by 2 µm Upchurch filter.
- *Column temperature:* 30°C.
- *Mobile phase:* (A : B) M/15 potassium dihydrogenphosphate with 1% (v/v) octane sulfonic acid : acetonitrile. Mobile phase (MP) 1: (95 : 5) at flow rate 1 mL/min; MP 2: (80 : 20) at flow rate 1 mL/min; MP 3: (30 : 70) at flow rate 1.2 mL/min.
- *Eluent switching programme:* At injection, MP1 to the column. From time 12 min to 30 min, MP2 to the column. From time 30 min, MP3 to the column to rinse it. From time 35 min to 40 min, equilibration with MP1.
- *Detection:* DAD (λ = 260 nm).

k	*Compound*
2.7	Lamivudine
3.2	Didanosine
3.8	Stavudine
6.6	Zidovudine
8.1	Abacavir
11.1	Nevirapine

System HAF

Tanaka E *et al.* (1996). *J Chromatogr B Biomed Sci Appl* 682: 173–178.

- *Column:* ODS TSK-gel Super (100 × 4.6 mm i.d., 2 µm).
- *Mobile phase:* (A : B) Acetonitrile–5 mmol/L sodium dihydrogenphosphate (pH 6).
- *Isocratic elution:* (45 : 55).
- *Flow rate:* 0.65 mL/min.
- *Detection:* UV (λ = 254 nm).

Retention time (min)	*Compound*
5.3	Clonazepam
6.6	Bromazepam
9.1	Nitrazepam
13.7	Triazolam
15.0	Lorazepam
18.4	Etizolam
21.0	Chlordiazepoxide
29.8	Diazepam
32.2	Flutazolam

System HAV

Rutledge DR *et al.* (1994). *J Pharm Biomed Anal* 12: 135–140.

- *Column:* RP-short alkyl chain, silanol-deactivated (SCD 100; 250 × 4.6 mm i.d.), stainless steel.
- *Mobile phase:* (A : B) Methanol–0.04 mol/L dibasic potassium phosphate (pH 5.5).
- *Isocratic elution:* (50 : 50).
- *Flow rate:* 1 mL/min.
- *Detection:* UV (λ = 237 nm).

k	*Compound*
2.2	Celiprolol
2.3	Propranolol
3.6	Diltiazem deacetyldiltiazem
5.1	Diltiazem desmethyldiltiazem
6.1	Diltiazem
6.4	Imipramine
8.2	Verapamil

System HBA

Sastre-Toraño J, Guchelaar H-J (1998). *J Chromatogr B Biomed Sci Appl* 720: 89–97.

- *Column:* C_{18} base-deactivated silica (125 × 4.6 mm i.d., 5 µm) with base-deactivated C_{18} precolumn (20 × 4.6 mm i.d., 5 µm).
- *Eluent:* (A : B) Acetonitrile–50 mmol/L potassium dihydrogenphosphate (pH 7.5, containing 500 µL triethylamine).
- *Isocratic elution:* (60 : 40).
- *Flow rate:* 2 mL/min.
- *Detection:* Fluorescence (λ_{ex} = 255 nm, λ_{em} = 315 nm).

Retention time (min)	*Compound*
8.8	Erythromycin
15.7	Clarithromycin
17.1	Roxithromycin
20.7	Azithromycin

System HBB

Taninaka C *et al.* (2000). *J Chromatogr B Biomed Sci Appl* 738: 405–411.

- *Column:* C_{18} (250 × 6.0 mm i.d., 5 µm).
- *Eluent:* (A : B) Acetonitrile–50 mmol/L phosphate buffer (pH 7.2).
- *Isocratic elution:* (43 : 57).
- *Flow rate:* 1.7 mL/min.
- *Detection:* Electrochemical (working electrode: glassy carbon; reference electrode: Ag/AgCl).

Retention time (min)	*Compound*
6.8	Clarithromycin
6.8	Erythromycin
9.6	Azithromycin
16.3	Roxithromycin

System HAE

Proust V *et al.* (2000). *J Chromatogr B Biomed Sci Appl* 742: 453–458.

- *Column:* C_{18} (Lichrospher, 100 RP-18, 5 µm) with C_{18} precolumn (Lichrospher RP-18, 5 µm).
- *Mobile phase:* (A : B) Acetonitrile–25 mmol/L sodium phosphate modified with diethylamine (0.9%) and tetrahydrofuran (1%), pH 3.0.
- *Isocratic elution:* (44.8 : 55.2).
- *Flow rate:* 0.5 mL/min.
- *Detection:* UV (λ = 260 nm).

Retention time (min)	*Compound*
6.3	Delavirdine
7.0	Saquinavir
8.0	Nelfinavir
9.4	Amprenavir
22.2	Ritonavir
28.6	Efavirenz

System HAK

Le Guellec C *et al.* (1988). *J Chromatogr Sci Appl* 719: 227–233.

- *Column:* C_{18} Symmetry (250 × 4.6 mm i.d., 5 µm) with C_{18} precolumn Symmetry sentry.
- *Mobile phase:* (A : B) Acetonitrile–20 mmol/L potassium dihydrogenphosphate.
- *Elution programme:* (50 : 50) to (70 : 30) in 15 min.
- *Flow rate:* 1 mL/min.
- *Detection:* UV (λ = 313 nm).

Retention time (min)	*Compound*
4.7	Carbamazepine
6.2	Clonazepam
7.6	Nordazepam
9.3	Clobazamm
Not detected	Phenobarbital
Not detected	Phenytoin

System HAL

Boukhabza A *et al.* (1990). *J Chromatogr* 529: 210–216.

- *Column:* C_{18} Novapak (150 × 4.6 mm i.d., 5 µm).

- *Mobile phase:* (A : B : C) Acetonitrile–methanol–6 mmol/L phosphate buffer (pH 5.7).
- *Isocratic elution:* (30 : 10 : 60).
- *Flow rate:* 1.3 mL/min.
- *Detection:* DAD ($\lambda = 242$ nm).
- *Note:* The phosphate buffer stock solution is prepared using 94 mL 0.2 mol/L sodium dihydrogenphosphate added to 6 mL 0.2 mol/L disodium phosphate heptahydrate.

Retention time (min)	*Compound*
1.4	Barbital
1.45	Clonazepam 7-acetamidoclonazepam
1.55	Clonazepam 7-aminoclonazepam
2.0	Aprobarbital
2.4	Hexobarbital
3.7	Flunitrazepam M (nor)
4.4	Nordazepam oxazepam
4.4	Oxazepam
4.6	Nitrazepam
4.33	Clonazepam
5.1	Lorazepam
6.2	Flunitrazepam
6.3	Alprazolam
6.6	Triazolam
7.7	Chlordiazepoxide
7.8	Clobazam
7.9	Nordazepam
8.1	Bromazepam
8.2	Medazepam
13.2	Diazepam

System HAM

de Carvalho D, Lanchote VL (1991). *Ther Drug Monit* 13: 55–63.

- *Column:* C_{18} (150 × 4.0 mm i.d., 3 µm) with C_{18} precolumn (40 × 4.0 mm i.d., 3 µm).
- *Mobile phase:* (A : B) Water–acetonitrile.
- *Isocratic elution:* (50 : 50).
- *Flow rate:* 0.7 mL/min.
- *Detection:* UV ($\lambda = 313$ nm).

Retention time (min)	*Compound*
1.8	Theophylline
1.98	Caffeine
2.0	Paracetamol
2.2	Primidone
2.7	Sulfamethoxazole
2.8	Phenobarbital
3.1	Chlordiazepoxide
3.4	Diazepam
3.4, 4.4	Oxazepam
3.5	Phenytoin
4.2	Lorazepam
4.3	Clonazepam
4.5	Nitrazepam
9.0	Imipramine
9.1	Desipramine
10.3	Diazepam
Not detected	Alprazolam
Not detected	Bromazepam
Not detected	Clobazam
Not detected	Codeine
Not detected	Ephedrine
Not detected	Levomepromazine
Not detected	Lidocaine
Not detected	Medazepam
Not detected	Nortriptyline
Not detected	Propranolol
Not detected	Thioridazine
Not detected	Triazolam

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CHAPTER

42 Capillary Electrophoresis

F Tagliaro, A Fanigliulo, J Pascali and F Bortolotti

Introduction

Since its introduction in the early 1980s, and particularly in the first decade of this century, capillary electrophoresis (CE) has established a prominent role in many areas of applied analytical chemistry. This is demonstrated by an increasing number of applications and scientific papers in biomedical sciences, toxicology, biopharmaceutics, biotechnology, and environmental and forensic science. The spread of this application relies on its peculiar features:

- Wide analytical applications (from inorganic ions to large DNA fragments and even viruses and cells)
- Variety of separation modes (electrophoretic, electrokinetic, chromatographic and more)
- Variety of detection systems (from ultraviolet (UV) spectroscopy to mass spectrometry (MS))
- High separation efficiency (up to millions of theoretical plates) and mass sensitivity (from femtomoles (10^{-15} moles) down to yoctomoles (10^{-21} moles)); minimal consumption of samples (in the order of nanolitres) and solvents (a few millilitres per day)
- Simple and inexpensive operation coupled with instrumental ruggedness.

Moreover, being based on specific separation mechanisms, CE has established itself as an independent analytical technique complementary to chromatography.

Capillary electrophoresis originated from the optimisation of basic electrophoretic principles traditionally applied using slab gels. The range of applications of CE soon extended to include hybrid separation mechanisms, partially borrowed from chromatography, and it has become a highly versatile and flexible tool in the hands of separation scientists. CE has been recognised as potentially admissible in the US courts as a form of evidence in accordance with the Daubert Standard (Kuffner *et al.* 1996).

Although CE has received major attention in molecular biology, clinical chemistry, analytical toxicology and other areas of forensic analysis, it is still 'in its infancy'. Forensic science laboratories have to deal with a range of diverse analytical problems involving, for example, gunshot residues, explosives, inks, dusts, soils, illicit drugs, poisons, DNA fragments and proteins and others, and CE has particular characteristics that make it applicable to all these areas. Forensic samples are often limited in quantity and heavily contaminated and must be conserved as far as possible in order to allow for further investigations. CE has great potential as a practical and productive investigation tool for analytical toxicologists and other forensic scientists dealing with such samples. In addition, scientists working in the fields of clinical and biochemical analysis, where analytical versatility, minimum use of sample volume and low operative costs are extremely important, derive considerable benefits from the technique.

This chapter builds on that contributed to the third edition of this publication by Professor David Perrett in that it aims to illustrate the basic principles of CE by giving a description of its instrumentation and of the different modes of separation and detection. A major addition is the presentation of recent review papers that cover important topics of CE applications in analytical toxicology and to which the readers are referred for more detailed information. Further, a selected number of applications of particular interest are discussed.

Theoretical aspects

Capillary electrophoresis can be defined as high-voltage electrophoresis (10–30 kV) carried out in a capillary-shaped separation compartment (typical dimensions: internal diameter 20–100 μm, length 20–100 cm). The geometry of this set-up, with respect to traditional slab gel electrophoresis, offers the neat advantages of minimal band diffusion and improved joule heating control, thus permitting the application of high voltages. The reduced zone broadening results in excellent separation efficiency, reaching up to 10^6 theoretical plates.

The CE separation mechanism, as in any form of electrophoresis, is based on the principle that charged species subjected to an electric field tend to migrate, driven by electrostatic force, towards the electrode with the opposite charge. Their velocity depends on their electrophoretic mobility (μ), which is specific for each individual ionic species on the basis of the mass-to-charge ratio, as described by Equation (42.1):

$$\mu_i = \frac{q_i}{6\pi\eta r_i} \tag{42.1}$$

where μ_i = ion mobility, q_i = ion charge, η = electrolyte solution viscosity, and r_i = ion effective radius.

When an electric field of strength E is applied, the ion migration velocity (v_i) will equal the product $\mu_i \times E$, causing the physical separation of the components of a mixture of molecules with different μ_i (Equation 42.2):

$$v_i = \mu_i \times E \tag{42.2}$$

where the electric field strength E is given by Equation (42.3):

$$\text{Electric field } (E) = \frac{\text{Applied voltage}}{\text{Distance between electrodes}} = \frac{V}{d} \tag{42.3}$$

An additional phenomenon known as *electroosmosis* takes place inside the separation capillary and results from the double electrical layer that builds up at the solid–liquid interface whenever a solid surface is in contact with a solution of ions. This ionic double layer is described by the so-called zeta potential ζ. The inner wall of capillaries, silica being the most common material of which the CE capillaries are made, exhibits an excess of surface charge, since it contains a great number of silanol groups (SiOH), which at pH values higher than 2 are ionised as SiO^-. The resulting negative charge of the wall surface tends to be compensated for by cations attracted from the solution, thus building up a double electric layer. When an electric potential difference is established between the ends of the capillary (i.e. when electrophoresis starts), all the cations in the solution migrate towards the cathode (the negative electrode) and anions move in the opposite direction. The migration towards the cathode of excess cations close to the capillary wall (being not compensated by migration of the corresponding anions stationary in the wall) drags water in the same direction, thus producing a measurable flow of liquid inside the capillary (typically tens of nanolitres per minute), termed 'electroosmotic flow' (EOF). Capillaries made of materials other than silica (e.g. Teflon), or with the wall coated with neutral or charged coatings (negative or positive), will display the same phenomenon, depending on the degree of ionisation of the wall surface. It is

important to point out that even 'neutral' capillaries may display a charged surface because of the adsorption on the wall of molecules present in the solution.

Electrophoretic migration and electroosmotic flow may have either the same or the opposite direction. Thus, they sum as vectors and induce ionised species to migrate with an apparent velocity (v) resulting from the sum of their intrinsic mobility (μ_i) and the mobility of the EOF (μ_{EOF}) (Equation 42.4):

$$v = (\mu_i + \mu_{EOF}) \times E \tag{42.4}$$

The set-up of a capillary electropherograph is usually with the detector close to the end of the capillary towards which the EOF is directed, and liquid flow will drag towards the detector all the solutes contained in the injected sample, excluding those with an electrophoretic counter-migration velocity higher than the EOF itself (small ions with high charge-to-mass ratio). Inside this flow of solvent, the ionic species migrate according to the respective μ_i, whereas neutral solutes (with $\mu_i = 0$) migrate all together at the velocity of the EOF.

In short, with the usual instrumental arrangement, having the injector at the anode end and the detector close to the cathode end of an uncoated fused-silica capillary (negatively charged at the inner wall), the cations with the highest mobility will arrive at the detector first, followed by the cations with progressively lower mobility (lower charge-to-mass ratio); subsequently, the bulk of the neutrals will appear at the detector, followed by the anions in reversed order of mobility (slow anions first, fast anions last). Only the fastest anions with electrophoretic mobility higher than that of the EOF will not be detected, because they will escape from the opposite end of the separation capillary.

The EOF, being driven by a force generated close to the capillary wall, has a peculiar 'piston-like' flow profile, which is particularly beneficial for molecular separations. In fact, it minimises band broadening, which is a typical drawback of capillary liquid chromatography, where the pressure-driven flow of the mobile phase, hindered by shear forces at the wall, yields a parabolic flow profile.

The coexistence of both electrophoretic and electrokinetic phenomena, which can be tuned separately and in combination to achieve molecular separation, is a unique feature of CE that can be exploited to perform a great variety of separations modes. In addition, interactions taking place between analytes and other molecules (e.g. complex-forming molecules, organic solvents, micelles, polymer gels) present in the medium in which separation occurs further contribute to differentiation of the migration velocities of different chemical species according to non-electrophoretic principles.

For a detailed explanation of the theoretical principles of CE, readers are referred to publications listed in the 'Further reading' section of this chapter, and particularly to the book by Ahuja and Jimidar (2008).

Finally, although quantification methods used in CE are similar to those applied in other forms of chromatography, in that they mainly use internal standardisation, two important differences must be emphasised. First, in CE most separations have an 'on-column' detection step. In this process, the velocity of the peaks crossing the detection window differs according to the respective ion mobilities of the analytes. As a result, the 'apparent peak width' of zones moving at different velocities will differ, even if the 'real band width' is the same. Consequently, two equal bands differing only in mobility will show different 'peak areas', because of the difference in their residence times inside the detector cell. To overcome this problem, for quantitative computations the ratio of peak area to migration time can be used instead of peak areas. An alternative is to use peak heights.

Second, since only minute amounts of sample (of the order of a few tens of nanolitres) are injected onto the separation capillary, it is difficult to handle these volumes with precision using current technology. This seriously restricts the use of external standardisation for quantitative methods. Thus, as for example with gas chromatography (GC), internal standardisation is much preferred.

Instrumental hardware

The outstanding feature of CE instrumentation is its basic simplicity. A schematic representation of a capillary electropherograph is given in Figure 42.1.

In brief, as in other chromatographic techniques, a CE analysis is based on three major steps: sample injection, separation and detection. As a consequence, a capillary electropherograph can be described as an assembly of the following components:

- An injection system
- A high-voltage power supply
- A separation capillary (generally located in a thermostatted compartment)
- A detector.

In CE, only a minute amount of sample can be introduced into the capillary, in order to maintain the separation efficiency. Overloading of samples causes peak broadening and peak distortion. Sample loading is limited by capillary dimensions. Typically, the length of the sample plug should not exceed 1–2% of the total capillary length and this corresponds to volumes of between 10 and 100 nL, for typical CE capillaries, which have an inner volume ranging between 1 and 4 µL.

Sample injection is achieved by replacing the inlet buffer reservoir with the sample vial for a few seconds. The forces driving sample introduction into the separation capillary may be brought about by applying pressure, voltage or both. The most common modes are hydrodynamic and electrokinetic injection.

Hydrodynamic injection is the most widely used and is carried out by the application of a positive pressure at the inlet end of the capillary, by the application of a vacuum at the terminal end or by exploiting siphoning between the two capillary ends. In this way, the amount of sample loaded is almost independent of the sample composition and is representative of the whole sample solution. Hydrodynamic injection is described by Equation (42.5):

$$V = \frac{\Delta P d^4 \pi t}{128 \eta L} \quad \text{(Hagen–Poiseuille equation)} \tag{42.5}$$

where V = sample volume, ΔP = applied pressure, d = capillary internal diameter, t = injection time, L = capillary length and η = buffer viscosity.

The typical applied pressure range is between 5 and 30 psi (1 psi = 69 mbar = 6.9 kPa) and injection times are between 5 and 20 seconds, although these parameters depend strongly on method optimisation. The advantages of this injection mode are reproducibility and suitability for quantitative analysis. However, it is subject to limited sensitivity owing to the tiny volume of sample introduced into the capillary and hence the limited mass of analytes that can be introduced into the capillary.

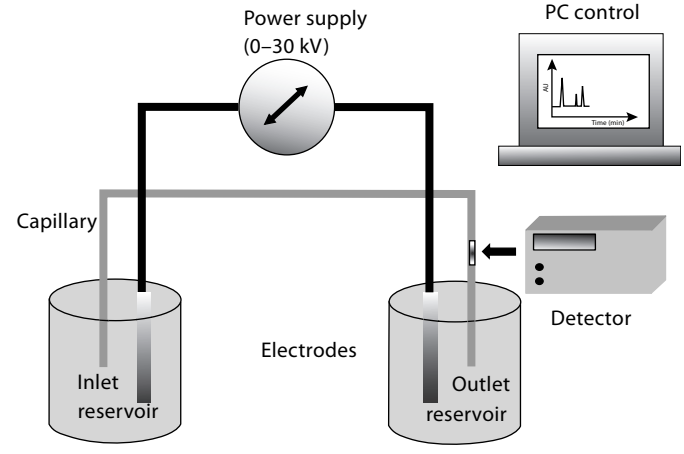

Figure 42.1 Schematic representation of a capillary electropherograph.

Electrokinetic injection is obtained by applying a voltage for a few seconds while the injection end of the capillary is dipped into the sample vial. In this way, analytes enter the separation capillary by both electrophoretic migration and the pumping effect of the EOF. The amount of sample introduced is given by Equation (42.6):

$$Q = \frac{(\mu_i + \mu_{EOF}) V \pi r^2 C t}{L} \quad (42.6)$$

where Q = grams or moles of injected amount, μ_i = analyte electrophoretic mobility, μ_{EOF} = EOF mobility, V = applied voltage, r = capillary radius, t = injection time, L = capillary length and C = analyte concentration.

It can be seen that, in electrokinetic injection, discrimination occurs on the basis of the electrophoretic mobility of the sample components, so that ionic species with mobilities towards the detector will be preferentially introduced with respect to neutral species and ions with the opposite charge. This results in selectivity and sensitivity enhancements for the analytes that are preferentially loaded, but also in delicate reproducibility, strongly affected by the sample composition. Electrokinetic injection is the injection mode exploited to realise 'sample stacking' procedures that allow large increases in analytical sensitivity, as discussed below with regard to sample pretreatment and sample enrichment.

Separation capillary

Fused silica is by far the most widely used material for CE capillaries, since it is chemically and electrically inert, physically resistant, UV transparent (for the needs of in-capillary UV detection) and displays good thermal conductivity (in order to ensure high dissipation of the joule heat). To achieve the necessary mechanical resistance, capillaries are externally coated with a protective polyimide layer, in a similar way to GC capillaries. This external layer is optically opaque and must be removed in the region where an optical detector is to be placed. This can easily be achieved by burning or scraping off the material in a capillary segment corresponding to the detection window. In-capillary optical detection has the advantage of avoiding any post-separation added volumes and consequent band broadening, but has disadvantages in terms of optical path length and cell geometry.

Typical ranges for inner and outer diameters of CE capillaries are 25–100 μm and 350–400 μm, respectively. Capillary length typically rages between 20 and 100 cm.

Fused-silica capillaries can be internally 'uncoated' (naked) or 'coated' with thin layers of polymers. The most common internal coatings include amines, polyacrylamide, cellulose, poly(vinyl alcohol)s, amino acids, surfactants, aryl pentafluoro compounds, poly (vinylpyrrolidinone) and poly(ethyleneamine). Also, liquid chromatography (C_2, C_8, C_{18}) or GC (poly(ethylene glycol) and phenyl methyl silicone) stationary phases can be used as capillary wall coatings.

An inherent problem in CE separations is reproducibility, since the EOF and consequently migration times of analytes are affected by the inner surface conditions. A common practice adopted to refresh the inner surface of uncoated silica capillaries and remove adsorbed materials is that of flushing with strong bases, which dissolve a thin layer of the silica surface. Usually, 1 mol/L sodium hydroxide solution is flushed through the capillary, followed by 0.1 mol/L sodium hydroxide solution and then by the separation buffer for final conditioning. The frequency of base conditioning during the experimental work depends on the nature of the samples, the buffer employed, the working pH, the background electrolyte (BGE) concentration and the nature of the capillary surface. Acid washing and flushing with solvents are less popular but effective practices, particularly when coated capillaries are used that would suffer from exposure to basic solutions.

Thermostatting is another key point to ensure reproducibility of capillary separations. Temperature variations due to joule heating should be minimised in CE analyses so as to minimise viscosity changes in both separation and injection. Thermostatting is better achieved by using a refrigerant fluid, but air streams are also effective when separations generate low currents.

Power supply

A high-voltage power supply is a fundamental part of a CE instrument. It should be capable of delivering up to 30 kV, with currents up to 200–300 μA. Because of the typical direction of the EOF (towards the cathode, in naked fused-silica capillaries), the standard polarity configuration has the anode (positive electrode) placed at the injection end of the capillary and the cathode (negative electrode) at the opposite end, close to the detector (a configuration known as 'normal polarity'). However, in many cases (e.g. when the EOF is directed towards the anode, because the capillary wall is coated with a positively charged wall modifier) the application of a 'reversed polarity' configuration is needed in order to allow the analytes to migrate to the detector. For this reason, dual-polarity power supplies that are capable of rapidly switching the electrode polarity are used in modern CE instrumentation.

CE separations are usually carried out at a constant potential, but separations at constant current can sometimes be needed. An advantage of constant-current mode is the possibility of compensating by automatic adjustments of the voltage for viscosity changes caused by inadequate temperature control.

Voltage programming, i.e. applying gradients of voltage or current during the analysis, has also been reported to improve separation efficiency in the case of complex samples, but the advantages of gradient application are not as important as in GC and high performance liquid chromatography (HPLC), where temperature or buffer composition gradients are the most popular mode for tuning separation.

Detectors

Although it might be expected that the detection of analytes directly inside the capillary through its transparent silica wall would be relatively simple, this is not the case. Inherently, the main problem related to detection is sensitivity, not in terms of detectable mass (picogram amounts of analytes are easily detectable inside the capillary with simple UV detectors), but in terms of concentration of analyte. Since only minute volumes (nanolitres) of samples can be introduced into the tiny separation compartment (i.e. the capillary, with a typical volume 1 μL), only very small amounts of analytes will reach the detector. On this basis, taking into account the high efficiency of CE (i.e. the ability to produce separations without generating dilution of the electrophoretic zones), it is mandatory to avoid zone broadening in order to achieve an acceptable sensitivity, either 'in-capillary' or 'off-capillary'. The way to achieve this is by limiting any 'dead volumes' in the system, particularly at the detection side, and increasing the response time of the detectors.

In the next subsections the most common CE detectors are described and their relative advantages and drawbacks discussed.

Optical methods

UV is the most commonly employed means of detection in CE instruments. Absorbance detection is usually performed 'in-capillary', with the optical beam focused directly into the capillary, crossing a transparent window made in it by removing the protective polyimide external coating. The limited optical path inside the capillary and the poor optical shape of the capillary section are the major factors negatively affecting sensitivity.

There are also other features peculiar to CE separation that have to be taken into account in quantitative analysis. As mentioned previously, detection in CE occurs when separation is still taking place, in contrast to chromatographic techniques where all analytes move at the same velocity in front of the detector, which is located after the separation column. In CE, the migration velocity differs from one analyte to another during detection such that their residence time in the detector is affected by their different velocities, and this affects the respective peak areas. To correct this artefact in quantitative analysis, peak areas in CE should be divided by their migration times.

Moreover, in CE the dynamic linear range of optical detection is less extended than in HPLC, particularly with UV detection, since deviations from the Beer–Lambert Law occur owing to the small size and curvature of the capillary cell.

Despite these limitations, UV detection is generally applicable, easy to perform and acceptably sensitive for most applications.

Capillary design and optical cell design may be optimised to improve sensitivity. Two examples are Z-shaped cells and bubble cells. In Z-cells, a double right angle, in the capillary region where detection takes place, results in an increased optical path length. The light beam runs axially for the length included between the two right angles. In bubble cells, solutes pass through an expanded region ('bubble') inside the capillary, where the analyte zone expands radially and, consequently, contracts longitudinally, increasing the path length without sacrificing separation.

The availability of multichannel, dispersive optical detectors (diode array detectors, DADs) for commercial CE instruments has greatly impacted CE instrumentation, allowing for enhanced information content at each analytical run and easier method development.

Fluorescence detection, mainly based on laser-induced fluorescence (LIF), has been proficiently applied both to fluorescent analytes and to molecules that can be made fluorescent after chemical derivatisation. In this case, sensitivity can be as high as 10^{-12} mol/L.

In the case of non-UV-absorbing ionised molecules, 'indirect detection' can be performed by introducing a detectable (UV-absorbing) additive, with the same charge as the analyte of interest and similar mobility to the running buffer. Displacement of the additive by the non-absorbing analyte will occur in the capillary in the zone of residence of the analyte and consequently a negative peak will be recorded by the detector (Beckers, Boček, 2003; Johns *et al.* 2003). Indirect detection, for instance, makes possible the determination of small organic and inorganic ions, most of which do not absorb UV light, using the UV detectors present in all commercial CE instruments.

Electrochemical detectors

Electrochemical (EC) detectors, particularly amperometric and conductimetric detectors, can be successfully coupled with CE. Electrochemical detection can be performed either 'in-capillary' or 'off-capillary'.

Amperometric detectors record the electronic current generated by an electrochemical reaction involving the analyte molecules, thus displaying, for oxidisable (or reducible) analytes, high sensitivity and good selectivity. This technique is mass sensitive and therefore independent of the cross-sectional pathlength of the capillary. Conductimetric detectors are simpler, although less selective, than amperometric detectors. Their application to CE allows direct detection of small ions, making possible ion analysis in a configuration resembling ion chromatography.

A general problem with EC detection in CE is the isolation of the separation circuit from the detection circuit to avoid interferences. A further problem of the EC detection mode is the necessity of using miniaturised electrodes, compatible with capillary dimensions. These devices are not commercially available so far, making EC detection (particularly amperometric) still reliant on home-made components.

Mass spectrometry

(See also Chapters 37 and 41.)

Hyphenation with mass spectrometry (CE-MS) is the latest major innovation in CE. In recent years the application of CE-MS has grown substantially, and now accounts for a large number of publications in the pharmaceutical, biotechnological, environmental and toxicological science literature (Schmitt-Kopplin, Frommberger 2003; Schmitt-Kopplin, Engelmann 2005).

The key issue when coupling CE with MS is the efficient transfer of ions from the CE capillary to the mass analyser without sacrifice of separation performance and sensitivity (Smith *et al.* 1988).

The electrospray ionisation (ESI) source has proved to be the most suitable for CE-MS coupling. This produces gas-phase ions from ions in solution, which are introduced into the mass spectrometer after being sprayed in a plume of droplets, as a result of a voltage applied on a holed metal tip. The ESI source is compatible with the CE-limited flows, since it is concentration sensitive and not mass sensitive. Other ion sources can be coupled with CE, for example atmospheric pressure chemical ionisation (APCI; Tanaka *et al.* 2003) and atmospheric pressure photoionisation (APPI; Mol *et al.* 2005), but the recent successful results of CE-MS interfacing are due to the development of the CE-ESI hyphenation.

Coupling CE with ESI involves joining a high-voltage-driven separation technique with a high-voltage-based molecule ionisation technique. Thus, it is necessary to ensure electrical continuity between the two systems while at the same time keeping the respective circuits independent. Second, a stable flow of ionised molecules must be constantly produced during analysis and therefore a BGE compatible with the ion source must be chosen.

CE can be interfaced with ESI with essentially two possible alternatives, as well described by Cai and Henion (1995): the 'sheath liquid' interface and the 'sheathless' interface. In the former case the electrical continuity is brought about by an additional aqueous/organic flow (the sheath liquid) of the order of magnitude of μL/min, which dilutes the liquid stream coming out of the separation capillary (nL/min). This accessory flow also ensures the onset of a stable ion spray. The diluting effect on the sample may be seen as a drawback in terms of sensitivity, but, on the other hand, it allows for the use of non-volatile BGEs or small amounts of additives or coating agents in the separation buffer (van Wijk *et al.* 2007).

In a sheathless interface, the electrical contact between the CE buffer and ion source is established by making the terminal end of the separation capillary conductive via several devices, such as stainless-steel connections, microelectrodes, conductive polymer coatings, and metal coatings of gold, silver, nickel or chromium. Unfortunately, the lack of reproducibility and robustness, due to delicate fabrication and manipulation of miniaturised components, limits its use on a large scale despite its advantages in terms of sensitivity (Smith 1990). Novel sheathless configurations have recently been designed, exploiting the results of the developments in nanospray and other miniaturised technology, with promising results (Janini *et al.* 2003; Kele *et al.* 2005).

Fast mass analysers with a high sampling rate along the electropherogram are required to follow the tiny, fast and closely moving CE peaks, which reflect the intrinsic high efficiency of this separation technique. CE has been hyphenated with almost all types of MS detector: magnetic sectors (Perkins, Tomer 1994), single and triple quadrupoles (Baidoo *et al.* 2003), ion traps (IT; McClean *et al.* 2000; Wey *et al.* 2000; Wey, Thormann 2001a; Iio *et al.* 2003), Fourier transform ion cyclotron resonance (FTICR; Hofstadler *et al.* 1994, 1996) and time-of-flight (TOF) mass spectrometers (Lazar *et al.* 1998a, 1998b; Ullsten 2004). At present, as in HPLC, ion traps remain the most widely employed detectors for CE because of their relative speed of scanning, relatively low cost and the possibility of performing multistep fragmentation experiments (MS^n).

The hyphenation of CE with TOF MS is seen as particularly promising, because of the high scan rate (10–200 μs per spectrum) and high sampling frequency of this detector (Lazar *et al.* 1998a, 1998b; 1999). TOF shows a particular advantage for toxicological analyses owing to the possibility of identifying molecules by determining their accurate mass and not requiring fragmentation databases.

Microchip CE

A specific advantage of CE is that instrumentation can easily be miniaturised in a microchip format, since a precise control of fluidics and other separation conditions can be obtained simply by changing the voltage and without the need of mechanical pumps, valves, etc. as in HPLC. CE microchips can also provide custom design, versatility, reduced consumption of reagents and sample, low waste generation, and increased analysis speed and portability. Because of the minimal amounts of sample injected, the most commonly used detection method is LIF, which offers the highest sensitivity. Microchip CE is mostly suitable for portable devices and fully automated analysers. Instrumentation based on this technique is now commercially available. Table 42.1, taken from the third edition of this work, gives an indication of the relative sensitivities of CE detectors towards various analytes in terms of mass and concentration limit of detection (LOD).

Modes of separation

A particular advantage of CE is the possibility of using the same hardware to perform different separation modes by simply adjusting a few

Table 42.1 Relative sensitivity of CE detection systems towards appropriate compounds

Detection mode	*LOD (moles injected)*	*LOD (mol/L)*	*Commercial availability*	*Comments*
UV/visible	10^{-13}–10^{-16}	10^{-3}–10^{-8}	Yes; DAD supplied with the major instruments	At <200 nm it is almost a universal detector; sensitivity is limited by capillary ID
Fluorescence	10^{-15}–10^{-17}	10^{-5}–10^{-9}	Yes – stand-alone add on	Limited to fluorescent compounds and derivatives; xenon lamp based
Laser-induced fluorescence	10^{-18}–10^{-22}	10^{-10}–10^{-16}	Yes	Very sensitive, but restricted to situations in which the wavelength of lasers matched the excitation wavelength of the analyte
Amperometric	10^{-18}–10^{-20}	10^{-5}–10^{-11}	No	Limited to electroactive species
Conductivity	10^{-15}–10^{-16}	10^{-4}–10^{-8}	Yes, but not currently manufactured	Universal towards ions, but lacks sensitivity
Mass spectrometry	10^{-15}–10^{-17}	10^{-8}–10^{-10}	Interfaces available from both CE and MS companies	Universal detection, but CE modes are limited
Indirect UV	10^{-10}–10^{-12}	10^{-2}–10^{-5}	Yes, with UV detector	Universal for ions, but lacks sensitivity
Indirect fluorescence	10^{-15}–10^{-17}	10^{-5}–10^{-6}	Yes, but requires fluorescent detector	Universal for ions, but lacks sensitivity

From Perrett D (2004). Capillary electrophoresis for drug analysis. In: Moffatt AC *et al.*, eds. *Clarke's Analysis of Drugs and Poisons*, 3rd edn. London: Pharmaceutical Press, 540.

analytical parameters, such as running buffer composition or capillary surface. This is illustrated in Figure 42.2, which summarises how a CE method is developed by evaluating the effects of changing these parameters. This unique characteristic of CE makes it a highly flexible tool with which to tackle the large and varied spectrum of compounds that are typically encountered in toxicological analysis, e.g. small ions (CN^-, K^+), drugs, drug enantiomers, drug metabolites, proteins, peptides and DNA. The most common separation modes are described below.

Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) is the basic mode of operation that can be applied in CE. Analytes migrate in discrete zones along the capillary under a high electric field (about 600–700 = V/cm), with different velocities, depending on their individual electrophoretic mobilities (μ).

The presence of the EOF produces an electrically driven flow of liquid, which, in fused-silica capillaries (exposing SiO^- groups), is directed from the anode to the cathode. The electroosmotic flow mobility (μ_{EOF}) is described by Equation (42.7):

$$\mu_{\mathrm{EOF}} = \frac{\varepsilon\zeta}{4\pi\eta} \tag{42.7}$$

where ε = dielectric constant, ζ = zeta potential and η = solution viscosity.

Because of the presence of the EOF, as already mentioned, both cationic and (most) anionic solutes will move towards the detector, which is typically located at the cathode end of the capillary. Neutral species will move, without separation, at the same velocity as the EOF.

The EOF can be modified, reduced almost to zero, or reversed by modifying buffer characteristics (pH, composition, ionic strength) and by using additives or capillary wall modifiers (coatings). Buffer ions should be chosen that are to be used within ±1 pH unit of their pK_a and carry low currents. All buffer systems absorb UV radiation, particularly in the 190–230 nm spectral region, i.e. the range of UV detection most commonly used in CE. Inorganic buffer systems such as phosphate and borate are preferable when low-wavelength UV detection is used. Table 42.2 gives physicochemical values for the buffers most commonly used in CE.

Capillary coating can be accomplished in a permanent way by chemical modifications of the capillary surface or, more commonly, by adding

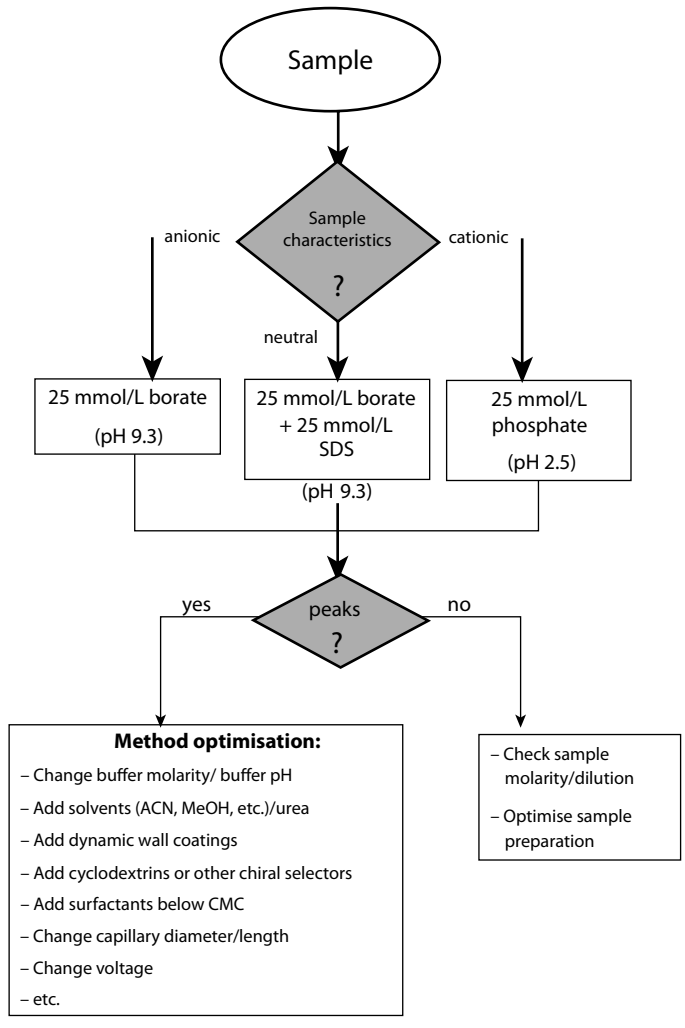

Figure 42.2 Simplified flow chart for developing a CE separation.

Table 42.2 Physicochemical values of the common buffers used in CE[a]

Buffer salt[b]	*pK_a*[c]	*Native pH*[d]	*pH/°C*	*Conductivity*	*Mobility $\times 10^5$ cm^2/V*	*Absorbance at 210 nm*[e]
Phosphate pK_{a1}	2.12	4.61	−0.024	3.43×10^{-3}	-7.76×10^{-3}	0.026
Glycine	2.35	6.25	−0.025	2.68×10^{-3}	-3.62×10^{-3}	0.839
Citrate pK_{a1}*	3.06	2.56	0.001	2.83×10^{-3}	-3.43×10^{-3}	1.103
Formate	3.75	6.77	0.002	-2.24×10^{-3}	-5.18×10^{-4}	1.201
Citrate pK_{a2}*	4.74	2.56	0.002	-1.64×10^{-3}	-7.77×10^{-3}	1.57
Acetate	4.76	7.16		-4.0×10^{-1}	−16.6	0.95
Citrate pK_{a3}	5.40	2.47	0.007	-2.09×10^{-3}	-3.62×10^{-3}	1.72
Bicarbonate pK_{a2}	6.15	8.58		1.25×10^{-3}		0.15
Phosphate pK_{a2}	7.21	4.52	0.003	-1.49×10^{-3}	-7.77×10^{-3}	0.176
HEPES*	7.55	5.06	0.024	-3.87×10^{-4}		2.10
TRIS	8.06	10.32	−0.028	-7.45×10^{-4}	-3.63×10^{-3}	0.647
Tetraborate	9.23	5.42	−0.008	-4.17×10^{-4}	-1.10×10^{-5}	0.085
Glycine pK_{a2}	9.78	5.85	−0.026	-4.02×10^{-4}	-3.62×10^{-3}	2.25
Carbonate pK_{a2}	10.25	10.7		-1.92×10^{-3}		2.56
Triethylamine	10.8					
Phosphate pK_{a3}	12.35	4.58	−0.025	-4.47×10^{-3}	-7.76×10^{-3}	2.44
Hydrochloric acid	NA					0.006
Trifluoroacetic acid	0.3					
Sodium hydroxide	NA					1.67
SDS (molecular biology grade)	NA					0.078

From Perrett D (2004). Capillary electrophoresis for drug analysis. In: Moffatt AC *et al.*, eds. *Clarke's Analysis of Drugs and Poisons*, 3rd edn. London: Pharmaceutical Press, 541.
HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); NA, not applicable; TRIS, tris(hydroxymethyl)aminomethane.
[a] All measurements obtained using AR-grade salts and usually the sodium salt.
[b] All determinations performed using 25 mmol/L solution at 25°C, except that the SDS solution was 75 mmol/L and those marked *for which 5 mmol/L was used.
[c] All values measured at the pK_a adjusted from the native pH with 25 mmol/L HCl or 25 mmol/L NaOH as appropriate.
[d] The native pH is the pH of the simple solution of the salt in water.
[e] Absorbance measured in a 1-cm silica cell against an empty silica cell.

modifiers to the running buffer (dynamic coating). A major advantage of dynamic coating is reproducibility, since it is continuously regenerated, the modifier always being present in the running buffer.

Although aqueous buffers are the standard separation electrolytes in CZE, the use of organic solvents as running media has been reported in several publications (Riekkola *et al.* 2000; Geiser, Veuthey 2009).

The advantages of non-aqueous capillary electrophoresis (NACE) are especially related to its suitability for molecules with poor solubility in water and to a change in selectivity with respect to aqueous buffers. Moreover, the high volatility of most organic solvents makes this technique particularly eligible for MS coupling. Several aspects of electrophoretic migration are changed in an organic medium. The most relevant are changes in the volume of solvated analyte ions and in the protonation equilibria of the analyte molecules owing to different dielectric constants, different acid–base properties and the different solvation power of organic solvents with respect to water. This results in a dramatic change in the pK_a of molecules that can be exploited to perform separations of molecules with similar pK_a values in aqueous buffers (Riekkola 2002). An additional advantage is the lower current generated on voltage application. The use of organic solvents, with small amounts of organic acids or buffer salts added, has proved to be effective in a wide range of applications (Porras *et al.* 2001; Scriba 2007).

Another special mode of CZE, known as complex-formation electrophoresis, can be used to achieve chiral separations. It is based on the addition of special modifiers to the running buffer, acting as chiral selectors. This technique is much less expensive and more efficient than chiral HPLC or GC, where expensive and delicate chiral stationary phases are needed. The chiral selectors most commonly used in CE are cyclodextrins (CDs) which are neutral cyclic oligosaccharides consisting of six, seven or eight glucose units, named α-, β- and γ-CD, respectively. Chiral selectivity is achieved by forming reversible inclusion complexes of different affinity constants with each of the two enantiomers of a chiral analyte. Since the mobility of the complex is different from that of the free drug, the chiral separation is achieved as a function of the affinity differences of the CD used for each of the two steric isomers (for a review see Scriba 2008). There is now increasing interest in the use of macrolytic antibiotics, which were first introduced as chiral selectors by Armstrong *et al.* in 1994. These exhibit a variety of interactions (inclusion, electrostatic, hydrogen bonding) and this enables high chiral resolution with a wider range of analytes (acidic, basic or neutral compounds). Six groups of antibiotics have been employed so far as chiral selectors in CE, namely glycopeptides, polypeptides, ansamycins, aminoglycosides and lincosamides. A comprehensive review of their application to the chiral analysis of pharmaceuticals such as NSAIDS, anticoagulants, beta-blockers, vasodilators and hormones has been given by Prokhorova *et al.* (2010).

Micellar electrokinetic chromatography

Micellar electrokinetic capillary chromatography (MEKC or MECC) was introduced by Terabe *et al.* (1984) to separate non-charged (neutral) analytes.

Micelles, made of anionic or cationic surfactants, have a net electrophoretic mobility because of their charged outer surface; in addition they can transport uncharged species along the capillary in their hydrophobic core. In MEKC, a micellar phase (formed by a surfactant at a concentration above its critical micelle concentration, CMC) with electrophoretic mobility opposite to the EOF is added to the running buffer. In this system, which resembles reversed-phase liquid chromatography, the separation occurs by the partitioning of neutral molecules between the BGE and the micelles, which act as a 'pseudo-stationary phase', moving slowly to the detector because of their electrophoretic mobility

opposite to the EOF. They therefore exert a retarding effect on the compounds that they interact with, while the EOF acts as a chromatographic mobile phase, driving the analytes towards the detector. In this system, the uncharged more polar analytes are excluded from the lipophilic core of the micelles and consequently migrate first, at a velocity matching that of the EOF. On the other hand, the most lipophilic compounds are highly retained by the micelles and move at a velocity matching the 'apparent' migration of the micelles towards the detector. The compounds with an intermediate polarity interact selectively with the micelles and are differentially retarded according to their individual degree of lipophilicity.

The most frequently used surfactant for MEKC is sodium dodecylsulfate (SDS), but cationic surfactants, such as cetyl trimethylammonium bromide (CTAB) or dodecyl trimethylammonium bromide (DTAB) are also used.

As in reversed-phase chromatography, the addition of organic modifiers (methanol, acetonitrile, etc.) to the running buffer can be used to modify selectively the 'elution' of the analytes.

MEKC can be seen as a very flexible mode of CE, capable of separating both neutral and charged species at the same time. Thus it proves to be a suitable tool for a wide range of applications and, particularly, for the analysis of drugs and other organic compounds.

Capillary electrochromatography

Capillary electrochromatography (CEC) is electrophoresis carried out inside a capillary containing a stationary phase, similar to a HPLC column. Thus, it can be seen as a kind of 'hybrid' technique between capillary liquid chromatography and capillary electrophoresis, exploiting different separation mechanisms, both chromatographic (partitioning of molecules between stationary and mobile phases) and electrophoretic (differential mobility of charged species in an electric field). As in MEKC, the driving force of the mobile phase is the EOF generated at the interface between the mobile phase and the stationary phase. The generation of the EOF inside the stationary phase achieves the advantage of minimised band spreading in comparison with capillary HPLC.

Although in theory CEC appears very efficient and selective, the limited availability and reproducibility of capillary columns has resulted in few applications being described.

Capillary isoelectric focusing

Capillary isoelectric focusing (CIEF) uses the basic principles of isoelectric focusing in a capillary format. In brief, under an electric field, a pH gradient is generated inside the capillary that is filled with a mixture of amphoteric species (known as ampholites). The separation in this case is not attained by migration differences but through the focusing of analytes in discrete zones of the capillary, on the basis of their isoelectric points. This technique is the gold standard for the separation of proteins (or other ampholites) which are differentiated with exceptional selectivity that is difficult to achieve with separation techniques simply based on charge-to-mass ratios or molecular weight differences.

In CIEF, proteins do not in fact migrate through the capillary, but settle (*focus*) inside the capillary where the local buffer pH equals their isoelectric points (pIs). In a subsequent step, an external force (e.g. pressure, electroosmosis) is applied to drive the discrete analyte zones out of the capillary towards the detector.

This mode of operation is particularly useful for the separation of isoforms of the same protein, which often differ only slightly in net charge or in mass-to-charge ratio, but exhibit different pIs.

Capillary isotachophoresis

Isotachophoresis means 'moving at the same velocity'. Capillary isotachophoresis (CITP) differs from CE in that the electric field is different in each section of the capillary. Ionic analytes are separated and migrate in discrete zones at the same speed between two ionic solutions, one with the highest mobility (the leading electrolyte) and the other with the lowest mobility (the terminating electrolyte) of all the analytes in the sample.

Because of its band-focusing ability, CITP can be used not only for direct separation but also for sample pretreatment with a concentration ability of up to 100–1000 times, followed by CZE analysis.

Capillary gel electrophoresis

Capillary gel electrophoresis (CGE) is electrophoresis performed in a capillary filled with a sieving medium, typically a linear or cross-linked polymer, where the separation mechanism is based on the different size of analytes. It is mainly applied for the separation of biopolymers in which, because of the similarity of the constituting monomeric units, values of charge-to-mass ratios are almost constant in spite of different molecular sizes. In these cases, the potential of plain electrophoretic separations is extremely poor and, consequently, an additional mechanism with different selectivity, i.e. molecular sieving, must be introduced into the separation process. CGE is typically used for DNA–RNA analysis and sequencing (e.g. in forensic DNA fingerprinting) and for the characterisation of proteins (resembling slab gel SDS–polyacrylamide electrophoresis, SDS-PAGE).

In CGE, entangled polymer solutions (linear polyacrylamide, hydroxyalkylcellulose, poly(vinyl alcohol), etc.) are often preferred to cross-linked gels.

Capillary immunoassay

Capillary immunoassay is a combination of immunochemistry and electrophoresis. In this analytical mode the selectivity relies on antigen–antibody recognition, whereas electrophoresis achieves the separation between the free and the bound antigen. The addition of excess antigen tagged with a label that is detectable by the detector of the CE instruments produces competition between labelled and native antigen for the binding sites on the antibody. Therefore, the amount of labelled antigen in the 'free fraction' will be inversely correlated with the concentration of the native antigen in the sample. Capillary immunoassay is rapid and versatile and appears to be particularly suitable for use as a screening method for drugs in biological fluids. Most publications on capillary immunoassay describe competitive immunometric methods to detect small concentrations of analytes in complex biological matrices. The introduction of labelled drugs as fluorescent tracers, which could be easily detected by LIF detectors, offers an opportunity to achieve high sensitivity. For example, Chen and Pentoney (1994) described an immunoassay technique coupled with CE separation for the analysis of digoxin in serum at clinically useful concentration levels of 10^{-10} mol/L. Thormann *et al.* (1998) proposed a screening method for the detection of urinary methadone by capillary electrophoretic immunoassay at a concentration of 10 ng/mL.

Sample pretreatment and sample injection

As previously discussed, the major drawback of CE is its moderate sensitivity in terms of analyte concentration because of the small sample volumes (typically ~10–50 nL) that can be injected into the separation capillary. In particular, for the most widely used detection method (UV absorbance) the sensitivity limit of CE is generally 10- to 100-fold higher than that of HPLC. Effective sample preconcentration techniques may overcome this limitation. Other problems in applying CE to biological samples are the high concentrations of inorganic constituents present in the samples, which cause electrodispersion and reduce the efficiency of separation, with resulting loss of resolution and sensitivity. Consequently, sample preparation, including clean-up and preconcentration, plays a key role in the application of CE to complex samples.

Basically, conventional sample clean-up and preconcentration methods, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), liquid–liquid extraction (LLE), protein precipitation and dialysis can be applied before CE analysis. The prepared sample (usually purified and concentrated) is injected into a capillary; CE separation and analyte detection follow.

Off-line preconcentration methods can lower the detection limit of CE by 10- to 30-fold or even more, mainly owing to the higher purity of the injected material. Their main disadvantage is sample loss, because no more than 1% of the extracted sample is injected into the capillary. Moreover, sample loss and sample contamination may occur during the preconcentration process, especially when a large volume of sample is concentrated in a tiny volume of extract. It is therefore desirable to have on-line sample clean-up and preconcentration methods (Veraart *et al.* 1999; Simpson *et al.* 2008). On-line sample clean-up and concentration can be achieved using selective chromatographic sorbents packed inside the capillary injection end. This type of on-line coupling is also called in-line SPE-CE coupling. The sample enrichment procedure includes washing, wetting, conditioning, sorption, washing, filling and desorption, before CE separation. This compact SPE technique lowers the detection limit of CE by factors of 700–1000.

In some cases only nanolitre or low microlitre volumes of sample are available and this places constraints on the sample preparation options. For such samples, direct sample injection may be necessary, possibly coupled with on-capillary concentration or derivatisation. Sample-stacking techniques are the most frequently used 'on-capillary' concentration methods, since they do not need any preconcentration device to be connected to the capillary. In sample stacking, the sample is prepared in a low-conductivity buffer, while the CE running buffer has a relatively high concentration of salts (high conductivity). It is well known that the electrophoretic velocity of an ion in a low-conductivity matrix is higher than that in a high-conductivity matrix, because of a higher electric field (in the low conductivity medium). Therefore, when the ions electromigrate across a boundary between low- and high-conductivity matrices, they are subjected to a sudden slow down, resulting in stacking (concentration) of the sample zone at the interface between injection plug and separation buffer. One merit of sample stacking is that it can inject a larger sample plug into the capillary. In some experiments, more than half the capillary can be filled with sample plug (partial-filling techniques). Sample stacking can also be accomplished during electrokinetic injection when the inlet capillary is immersed in a sample vial with a low-conductivity matrix. This stacking mode is also called field-amplification sample stacking (FASS). Injection of a short plug of water before the sample is reported to be able to enhance the electric field at the injection point and thus to increase the stacking. Other concentration methods, such as acetonitrile stacking, sweeping and the use of a pH junction, are also based on the velocity difference of ions moving across the boundary of the sample plug and the running buffer. An overview of the approaches to sample preparation for capillary electrophoresis has been presented by Wu (2003).

Applications of CE to forensic and clinical drug analyses

Drugs

Capillary electrophoresis was introduced in the early 1990s for the determination of potentially toxic, illicit and abused drugs in both clandestine preparations and biological fluids. CZE and MEKC are the most commonly employed electrophoretic techniques in this regard (Boone, Ensing 2003). Single-wavelength UV, DAD, fluorescence and MS detectors are reported as being employed in most literature applications. CE has been claimed to offer advantages over chromatography for the separation of chiral drug families, e.g. amfetamines and ring-substituted amfetamines.

Shihabi *et al.* (1998) reviewed the application of CE in the clinical field of therapeutic drug monitoring and cited references for the analysis of antiepileptics, antiarrhythmics, cardiovascular drugs, analgesics, antiasthmatics, immunosuppresants, antitumour agents and antibiotics in serum and urine. Examples of drugs being analysed in serum or urine by chiral separation are also listed. Although CE has not, as yet, found widespread routine use in this area, it remains a promising approach and its versatility has been demonstrated recently by the development of a rapid method for the measurement of serum lithium concentrations (Pascali *et al.* 2010). In an early review, Boone *et al.* (1999) listed details for the CE analysis of over 200 drugs and metabolites in biological samples including the sample pretreatment methods applied and the detection limits achieved.

A detailed review of the use of electrophoretic and electrokinetic separation techniques for profiling of drugs and related products was published by Hilhorst *et al.* (2001). Other more recent reviews on the application of capillary electrophoretic/electrokinetic techniques in forensic toxicology were published by Thormann (2002), Anastos *et al.* (2005) and Tagliaro *et al.* (2007).

The use of CE as an alternative to the more traditional GC and HPLC techniques is justified by the minimal amount of sample required, making this approach suitable for specific applications such as single-cell analysis (Yeung 1999; Phillips 2001; Woods *et al.* 2004; Arcibal *et al.* 2007) and determination of samples prepared by microdialysis (Denoroy *et al.* 1998; O'Brien 2003).

One of the most interesting applications of CZE showing the suitability of this technique for comprehensive toxicological drug screening in biological samples was reported by Hudson *et al.* (1995). Hudson analysed 326 basic and neutral drugs by CZE using two phosphate running buffers at pH 2.5 and pH 9.5, with UV detection and electrokinetic injection. LODs lower than 10 ng/mL for most of the tested drugs were obtained in spiked blood samples.

Alnajjar *et al.* (2004) used fluorescence detection for the CE analysis of multiple drugs of abuse in biological fluids. Morphine, normorphine, 6-acetylmorphine and codeine were separated and quantified using native fluorescence, although with moderate sensitivity. Derivatisation with fluorescein isothiocyanate coupled with LIF detection provided sensitivities higher than those for GC and HPLC.

CZE was also applied by Zhang *et al.* (2007) for the analysis of the toxic alkaloids lappaconitine, bullatine A, atropine sulfate, atropine methobromide, scopolamine hydrobromide, anisodamine hydrobromide, brucine, strychnine, quinine sulfate and chloroquine in human blood and urine, using procaine hydrochloride as an internal standard. The separation employed a fused-silica capillary of 75 μm i.d. × 60 cm length and a buffer containing 100 mmol/L phosphate and 5% acetonitrile (pH 4.0). The sample was injected in a pressure mode and the separation was performed at a voltage of 16 kV. The compounds were detected by UV absorbance at wavelengths of 195 and 235 nm. Electrophoretic peaks could be identified either by the relative migration time or by their UV spectrum.

In-house coated capillaries (with poly(ethylene oxide)) were used in a very simple CZE method (running buffer 100 mmol/L phosphate pH 6.4; UV detection at 200 nm wavelength) for the analysis of morphine, codeine, methadone and EDDP (a methadone metabolite) in human urine, after acid hydrolysis and LLE. Using electrokinetic injection, LODs ranging from 50 ng/mL to 100 ng/mL were achieved, thus making the method suitable for practical application in urine drug testing (Di Pietro *et al.* 2006).

Because of its ability to separate both neutral and charged compounds and its relatively high peak capacity, MEKC has traditionally been preferred to CZE for the analysis of illicit drug preparations.

In a paper by Ishii *et al.* (2001), the ability of MEKC to simultaneously separate and detect cocaine congeners and sugars added as excipients to clandestine preparations was exploited for analysing batches of seized drugs without any sample preparation. Under appropriate separation conditions (15% (v/v) acetonitrile in 8 mmol/L Na_2HPO_4–5 mmol/L phthalate–10 mmol/L CTAB, pH 12.5; capillary fused silica, 75 μm i.d. × 85 cm effective length; voltage −27 kV) and using indirect/UV absorption (310 nm) detection, sugars and polyhydric alcohols (ribose, glucose, lactose, sucrose, mannitol, inositol), dissociated as anions, were detected as positive peaks migrating faster than the EOF, while benzoylecgonine, cocaine, *cis*-cinnamoylcocaine and *trans*-cinnamoylcocaine (bearing a positive charge) were detected as 'negative peaks' after the peak corresponding to the EOF.

MEKC has also been applied to the determination of lysergide (LSD). The main problems in LSD analysis arise from the low dosage of drug taken, its extensive metabolism and its thermal instability, all of which yield extremely low concentrations to be determined in both biological fluids and clandestine preparations. On-line preconcentration of LSD from urine samples was exploited by Fang *et al.* (2002). To increase

sensitivity, the authors combined the use of two sample enrichment techniques, namely sweeping-MEKC and cation-selective exhaustive injection (CSEI-sweep-MEKC). The sweeping MEKC technique involved the concentration of analytes at the interface with the MEKC separation buffer, by introducing the sample hydrodynamically into the capillary in a solvent free of pseudostationary phase (Quirino, Terabe 1999). CSEI-sweep-MEKC caused sample stacking with electrokinetic injection and sweeping, thus providing further improvements in sensitivity (Quirino, Terabe 2000). Fang reported a LOD (S/N>3) of about 16 ng/mL in spiked urine using the sweeping-MEKC method with fluorescence detection ($\lambda_{ex} = 320$ nm; $\lambda_{em} = 390$ nm), and a LOD of about 1 ng/mL with CSEI-sweep-MEKC. The sweeping-MEKC was also used for the online concentration and analysis of LSD in clandestine tablets (Fang *et al.* 2003).

LIF detection, using a He–Cd laser at 325 nm wavelength, has also been applied to the determination of LSD in specimens of human blood (Frost, Koehler 1998). The CE method used a citrate–acetate system as running buffer.

The use of DAD as a CE detector in routine methods has attracted particular attention in drug analysis. As expected from a multichannel detector, recording an entire signal spectrum enhances the capability of correct identification and determination of analytes and the possibility of studying the peak purity. An example of a CE-DAD application can be found in Nieddu *et al.* (2005). This rapid method realises the screening of a class of amfetamine designer drugs, comprising 10 methylenedioxy derivatives of amfetamines and phenethylamine in human blood. The detection limits and recoveries from blood samples were between 10 and 30 ng/mL and 81 and 90%, respectively.

CSEI was used by Meng *et al.* (2006) as an on-line concentration method for the high-sensitivity analysis of illicit amfetamines. Using this approach (CSEI with micellar sweeping), a LOD lower than 50 pg/mL was achieved, whereas using normal MEKC it was about 10 ng/mL. The quantitative reproducibility of CSEI-micellar sweeping for the analysis of amfetamine, metamfetamine and MDMA using a benzylamine internal standard was satisfactory (standard deviation around 10%). The method was also tested on hair samples.

Among multichannel detectors, mass-selective detectors have rapidly become the gold standard in toxicological analysis for both generic and specific determinations. Recently CE-MS coupling has begun to find applications in the field of drug analysis (Smyth 2006).

Benzodiazepines are a challenging class of drugs to assay in biological fluids and represent a typical example of the proficient use of MS detection based on its outstanding identification power and selectivity. A double-dynamic coating CZE method with IT-MS detection was developed by Vanhoenacker *et al.* (2004a) for the separation of six benzodiazepines in spiked urine, and MS^2 experiments were performed for confirmation. McClean *et al.* (2000) optimised a CZE-ESI-IT-MS method for the determination of selected 1,4-benzodiazepines, identified by sequential product ion fragmentation.

MS^3 fragmentation was also exploited by Wey *et al.* (2000) to unambiguously identify codeine, morphine, dihydrocodeine and their glucuronides in urine samples previously screened using opiate immunoassay.

The simultaneous detection and quantification of a number of abused drugs in human hair (namely 6-monoacetylmorphine, morphine, amfetamine, metamfetamine (MA), methylenedioxyamfetamine (MDA), methylenedioxymetamfetamine (MDMA), benzoylecgonine, ephedrine and cocaine) was obtained by Gottardo *et al.* (2007a) with a rapid CZE-ESI-IT-MS method (Figure 42.3). Ammonium formate (25 mmol/L), pH 9.5 and 15 kV separation were employed for the separation using a bare fused-silica capillary. Under field-amplified sample stacking conditions, LODs were below 0.1 ng/mg for all drugs in hair matrix and good linearity was achieved in the concentration range 0.025–5 ng for each analyte per mg of sample. The same group investigated the application of these separation conditions when coupling CE-ESI with a TOF detector (Gottardo *et al.* 2007b), in order to exploit the advantages of high mass accuracy and fast scanning capability of this technique. Drugs and metabolites were identified in hair samples, after a single extraction procedure, by exact mass and isotopic pattern matching (Figure 42.4). Analytical precision in real matrices proved acceptable in both within-day and day-to-day tests.

MS detection can also be usefully employed for the detection of poorly UV absorbing molecules, such as γ-hydroxybutyric acid (GHB). Gottardo *et al.* (2004) described the use of IT-MS detection coupled to CZE as a confirmation method for a previously developed CZE screening assay using indirect UV detection (Bortolotti *et al.* 2004). A detection limit of 20 μg/mL was obtained in urine, which required no pretreatment other than dilution with water (1 : 4).

A triple quadrupole MS was used as detector by Baidoo *et al.* (2003) to detect and quantify nicotine and eight of its metabolites in smokers' urine. Detection limits of 0.55 and 11.25 ng/mL were reported for nicotine and cotinine, respectively when sample-stacking electrokinetic injection was used.

An interesting use of CE-MS was reported by Vanhoenacker *et al.* (2004b), who used a double dynamic coating, already reported by Lurie *et al.* (2001) for CE-UV, in a method with IT-MS detection. The double coating was prepared by subsequent flushing of the capillary with a polycation buffer solution ('initiator') and a polyanion solution ('accelerator'), the latter of which also acted as the running buffer. Thus, a double-layer coating was achieved, producing a high and stable EOF, with a significant reduction in analysis time and an increase in reproducibility. The separation of five basic drugs, including amfetamine, ephedrine, trazodone, codeine and salbutamol, was reported.

An important field of application of CE in toxicological analysis is the chiral determination of amfetamine and its congeners (Heo *et al.* 2000), either alone or in combination with other drugs (Ramseier *et al.* 1999). CE is a particularly easy and cost-effective means of developing chiral analytical methods. Additionally, mass spectrometry is often the detector of choice in these methods in order to ensure good specificity and discrimination power (Ramseier *et al.* 2000; Boatto *et al.* 2005).

Amfetamine designer drugs challenge both GC and HPLC techniques owing to their thermal instability, on the one hand, and their poor retention on reversed-phase columns, on the other. The advantage of CZE lies in the possibility of simply adding the chiral selector to the running buffer, with no need for especially manufactured and expensive chiral columns or a precolumn derivatisation procedure to form diasteroisomers (for a review see Zaugg and Thormann 2000).

An example of enantioseparation and discrimination of amfetamines and other pharmaceutical drugs was given by Cherkaoui *et al.* (2001). The authors used CE coupled with ESI-MS and adopted the partial filling technique. This involves the introduction of a small amount of chiral selector solution in the separation capillary prior to sample injection, while the remaining capillary is filled with BGE free of chiral selector. Usually, analytes of interest are basic compounds, positively charged at an acidic pH, whereas an anionic chiral selector is chosen as additive. Under these conditions, an opposite migration of analyte and chiral selector is obtained. As a result, the two species undergo complex formation inside

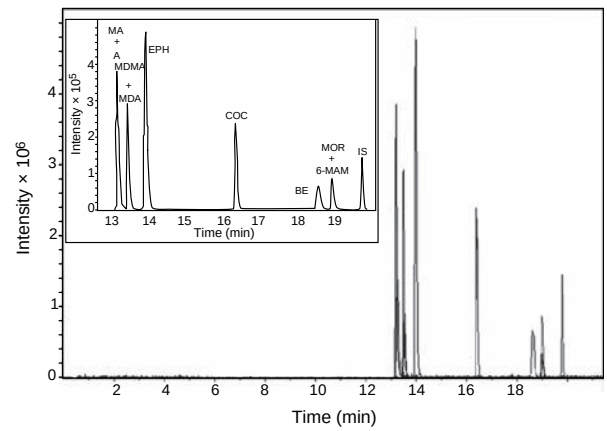

Figure 42.3 Toxicological analysis of drugs of abuse in hair using CZE-ion trap MS. Peak identification: A, amfetamine; MA, metamfetamine; MDA, methylenedioxyamfetamine; MDMA, methylenedioxymetamfetamine; EPH, ephedrine; COC, cocaine; BE, benzoylecgonine; MOR, morphine; 6-MAM, 6-acethylmorphine; IS, internal standard (folcodine). For analytical details, see text. (From *Gottardo et al.* 2007a, with permission.)

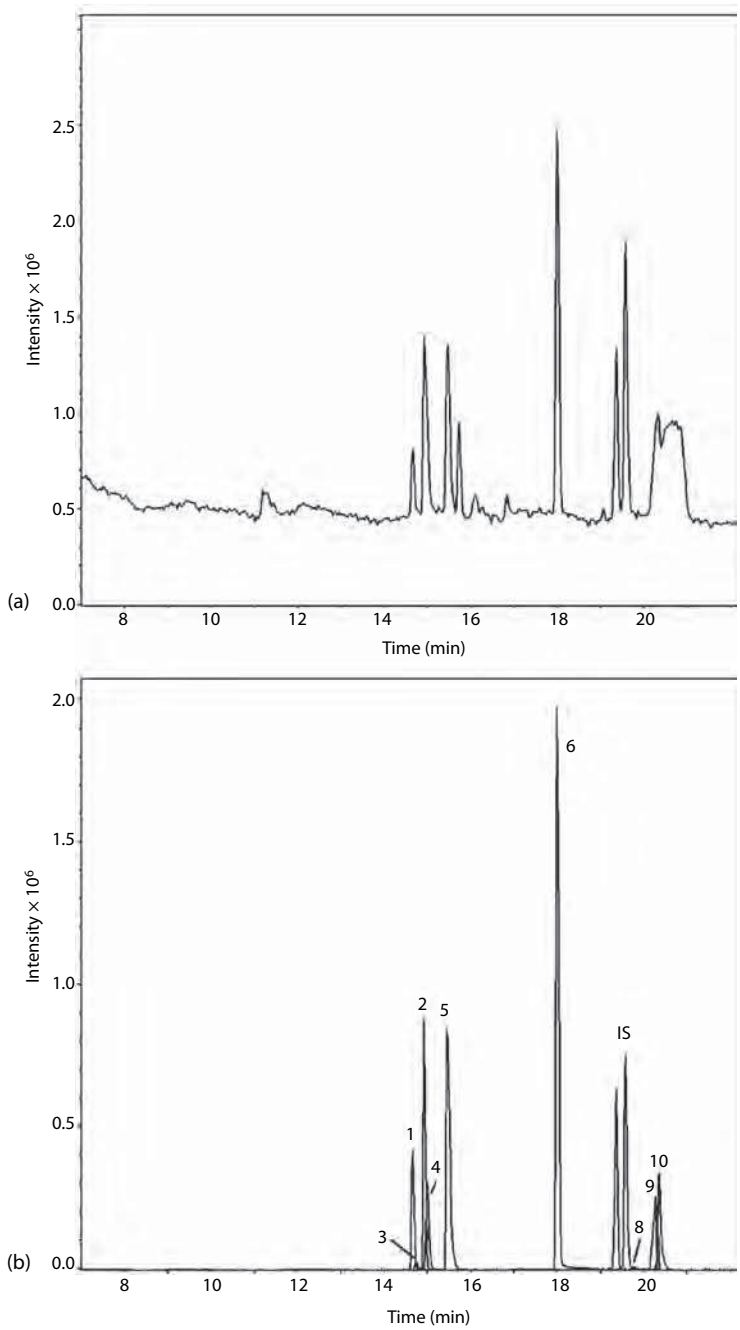

Figure 42.4 Toxicological analysis of drugs of abuse in hair using CZE-TOF MS. Peak identification: 1, MA, metamfetamine; 2, MDMA, methylenedioxymetamfetamine; 3, amfetamine; 4, MDA, methylenedioxyamfetamine; 5, ephedrine; 6, cocaine; 7, codeine; 8, benzoylecgonine; 9,6-acethylmorphine; 10, morphine, at a concentration of 0.2 ng/mg each drug; IS, internal standard (folcodine). For analytical details, see text. (From *Gottardo et al.* 2007b, with permission.)

the capillary, with resulting enantiomeric separation. When MS detection is used, the anionic chiral selector does not enter the MS ion source, because it is driven in the opposite direction by the applied voltage.

Iio *et al.* (2003) used the complete filling technique in which the chiral selector, a diluted mixture of 3 mmol/L β-cyclodextrin and 10 mmol/L heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, was instead added directly to the BGE. This method achieved the enantioseparation of metamfetamine and its metabolites in human urine samples with detection limits (with MS) in the range 0.03–0.05 μg/mL.

Amfetamine, metamfetamine, MDA, MDMA and MDEA were also used as test compounds in a study by Souverain *et al.* (2006) on the different strategies for rapid chiral analysis using CE. The adoption of hydroxypropyl-β-cyclodextrin as the chiral selector was investigated using different approaches (short-end injection, high electric fields, external pressure application, dynamic coating of the capillary) with a view to decreasing analysis time and increasing sensitivity.

A general paper on the optimisation of chiral separations using dual neutral cyclodextrins (β-CD and dimethyl-β-CD) was published by Nhujak *et al.* (2005), showing the potential advantages of adding two chiral selectors to the running buffer.

As previously discussed, non-aqueous capillary electrophoresis (NACE) is particularly eligible for CE-MS coupling, because of the high volatility of most organic solvents, which is beneficial for ionisation. Moreover, the observed change in selectivity in comparison with

separations in aqueous buffer may offer additional practical advantages in resolution. A complete review on NACE-MS has been published by Scriba (2007), collecting all the published NACE-MS applications up to November 2006. The low number of papers reviewed is probably due to the difficulties in adopting a separation technique whose physical–chemical mechanisms are still scarcely understood.

Peri-Okonny *et al.* (2003) exploited NACE-MS for the separation of nine basic drugs, including tricyclic antidepressant and bronchodilator drugs, with 80 mmol/L acetate buffer dissolved in methanol–acetonitrile (80 : 20 v/v). The results showed improved efficiency compared with HPLC separation.

In a paper by Steiner and Hassel (2005), a few organic solvents (namely methanol, acetonitrile, DMSO, formamide, *N*-methylformamide and *N,N*-dimethylformamide) were compared against water for the preparation of ammonium acetate separation buffer. Selectivity, peak efficiency and average plate counts were evaluated in the separation of basic drugs. In the seven different solvents (including water), the shortest run time was obtained with acetonitrile, the best peak resolution with the amphiprotic solvents (especially methanol), best peak efficiency with methanol and formamide, and the most sensitive ESI-MS detection with acetonitrile and methanol, but with only a slight advantage compared with water.

Anderson *et al.* (2004) developed a NACE-MS method for the determination of lidocaine and two of its metabolites in human plasma. The effects of sheath liquid composition, drying gas temperature and nebulising gas pressure on separation efficiency were evaluated.

Fluoxetine and related compounds were analysed with NACE-MS by Cherkaoui and Veuthey (2002), using 25 mmol/L ammonium acetate–1 mol/L acetic acid in acetonitrile as the running buffer and a 30 kV separation voltage. A significant increase in sensitivity was obtained compared with UV detection. NACE-MS was also reported by Geiser *et al.* (2000) for separation of amfetamine derivatives in spiked urine, after LLE.

Although proposed as an attractive alternative to chromatography and supported by positive results, CE traditionally lacks sensitivity compared with chromatographic methods. However, on-line and off-line sample enrichment and stacking injection techniques have been employed successfully to enhance sensitivity.

As previously mentioned, CSEI was used by Meng *et al.* (2006) for the high-sensitivity analysis of illicit amfetamines and achieved a LOD of $<$50 pg/mL.

The FASS approach was reported by Zhang and Thormann (1998) and Wey *et al.* (1999) for the determination of opioids in biological fluids and by Manetto *et al.* (2000) for the determination of opiate drugs in hair. This on-line sample preconcentration method has also proved useful for the CE-MS analysis of opioids in urine (Wey, Thormann 2001b).

FASS with electrochemiluminescence detection has been used for the determination of diamorphine and cocaine on banknotes contaminated with illicit drugs (Xu *et al.* 2006), offering a new tool with which to produce evidence of the diffusion of these illicit compounds in the population. Acetic acid (10 mmol/L) was used to elute the drugs from the banknotes. The detection limits obtained were 18.5 ng/mL for diamorphine and 18.1 ng/mL for cocaine. No interference from other compounds present on the banknote surfaces was observed.

A miniaturised method of SPE based on a novel monolith phase (methacrylic acid–ethyleneglycol dimethacrylate) coupled to CZE was reported by Wei *et al.* (2006) for the rapid determination of diamorphine, 6-monoacetylmorphine, morphine, codeine, papaverine and narcotine in human urine. The extraction device was contained in a capillary tube. By applying FASS injection, detection limits of 7–20 ng/mL were achieved.

Reviews of applications of capillary electrophoresis in the analysis of metal-based drugs and pharmaceutical analyses have been published by Timerbaev (2007) and by Suntornsuk (2007).

Small ions

Capillary electrophoresis, which is also known in this application as capillary ion analysis (CIA), and used most often with indirect UV detection, can offer a simple alternative to ion chromatography for the analysis of small ions. The advantages of using electrophoresis can be summarised as versatility (the same instrument can easily be switched without hardware modifications from other applications to ion analysis) and robustness, since all the separation occurs in solution without the need for any interaction with a stationary phase. This avoids problems of column contamination from injected materials. Last, but not least, because its separation mechanisms are substantially different from those of chromatography, CE can be used to confirm the results of an ion chromatographic analysis. Several applications of CIA in the determination of ions of toxicological interest have been reported, as reviewed by Timerbaev and Keppler (2007).

A method for ion screening without quantification was developed by Gillette *et al.* (2006) for the detection of 29 different anions (including thiosulfate, bromide, chromate, iodide, chloride, sulfate, sulfite, sulfide, nitrite, nitrate, oxalate, perchlorate, azide, thiocyanate, chlorate, fluoride, chlorite and phthalate) in human biological samples. This CE method was based on a background electrolyte composed of 2.25 mmol/L pyromellitic acid, 1.6 mmol/L triethanolamine, 0.75 mmol/L hexamethonium hydroxide and 6.5 mmol/L NaOH at pH 7.7; negative voltage was applied. Samples of human blood, plasma, urine and intestinal contents were analysed after protein precipitation and SPE. Using direct and indirect UV detection (at 200 and 250 nm, respectively), screening for all 29 compounds could be carried out in less than 20 minutes with a LOD of about 1 μg/mL for each compound in pure aqueous solution.

The quantitative determination of bromide (an old antiepileptic drug, recently reconsidered for treating refractory epilepsy) in human serum was performed by a very simple CZE method using 90 mmol/L sodium tetraborate and 10 mmol/L sodium chloride (pH 9.24) as the background electrolyte and reversed polarity (Pascali *et al.* 2006). Owing to the absorption of radiation in the low UV range by bromide ions, direct UV detection (200 nm) could be applied and achieved high selectivity, since most anions do not absorb UV radiation. The method gained further selectivity, because the separation of bromide ions was effected counter to the EOF. Thus, direct injection of serum samples, after 1 : 10 dilution with water, could be carried out and the reported LOD and limit of quantification (LOQ) were 0.05 and 0.1 mmol/L, respectively.

Eight arsenic compounds were recently analysed by CE with indirect UV detection. Two different buffers were optimised for the separation of the anionic and cationic species with good results in terms of sensitivity (LODs in the order of 7.8–250 μg/mL for the anionic species) (Kitagawa *et al.* 2006).

An optimised dynamic coating system, the CElixirOA (Analis, Namur, Belgium), was used in a method aimed at the characterisation of inorganic ions in metamfetamine tablets, produced in clandestine laboratories. The characterisation of phosphate, phosphite and hypophosphite ions helps to distinguish the various phosphorus–iodine methods used in these laboratories for producing metamfetamine by reduction of pseudoephedrine. The method separated acetate, azide, bromide, carbonate, chlorate, chloride, chromate, fluoride, hypophosphite, iodide, nitrate, nitrite, perchlorate, phosphate, phosphite, sulfate, sulfite and thiocyanate, with LODs in the order of μg/mL and percentage relative standard deviations of normalised migration times under 0.1% (Knops *et al.* 2006).

In recent years, a new and interesting application for CIA has emerged, on the boundary between ion analysis, toxicology and biochemistry. It concerns the determination of minor metabolites of ethanol formed by its enzymatic conversion into ethyl glucuronide (EtG) and ethyl sulfate (EtS). In comparison with ethanol and acetaldehyde, the major ethanol metabolites, EtG and EtS, are detectable for longer in body fluids and tissues and consequently can be used as markers of recent alcohol intake when ethanol and acetaldehyde are no longer detectable.

Two papers from the group of Thormann (Krivankova *et al.* 2005; Mrazkova *et al.* 2006) have recently described the optimisation and application of a CZE method based on separation in pH 4.4 nicotinic acid–ε-aminocaproic acid BGE in polyacrylamide-coated capillaries, reversed polarity and indirect detection. The method allowed the simultaneous determination of lactate and acetate, which are organic ions potentially affected by ethanol ingestion. Because of a transient isotachophoretic phenomenon, producing a sample self-stacking effect,

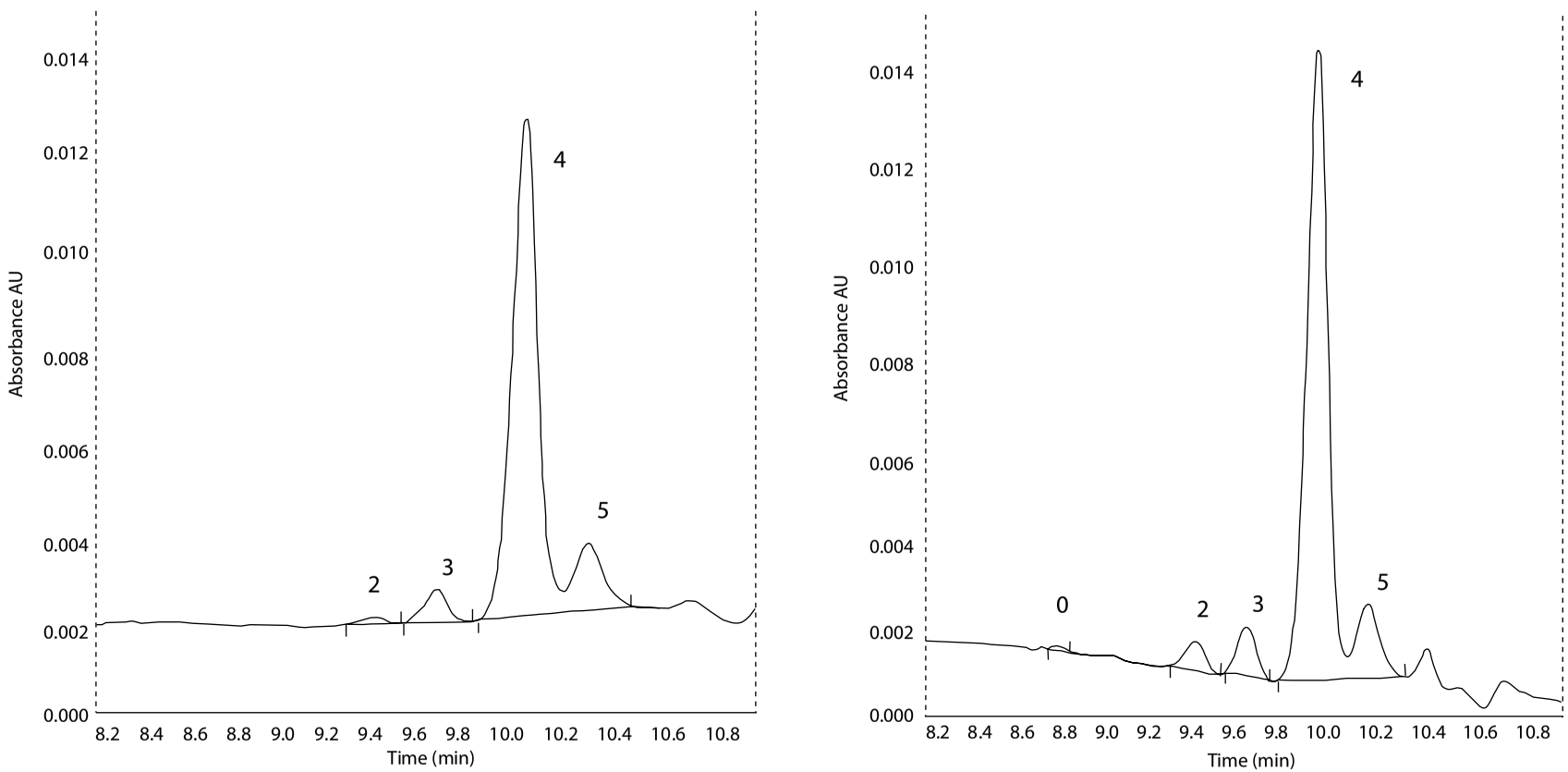

Figure 42.5 Transferrin (Tf) glycoform separation in serum with CE (for CDT analysis). On the left, analysis of a `normal' individual; on the right, analysis of an alcohol abuser. Peak identification: 0, asialo-Tf; 2, disialo-Tf; 3, trisialo-Tf; 4, tetrasialo-Tf; 5, pentasialo-Tf. Tf, human tranferrin.

a sensitivity of ~0.1 μg/mL for EtG could be achieved in untreated samples after only 1 : 1 dilution. This method was applied successfully to human serum, but no data were provided about its application to urine, which is the biological sample most often analysed for EtG.

Esteve-Turillas *et al.* (2006) and Fang *et al.* (2003) reported methods for EtS analysis in serum and urine. The BGE was composed of 15 mmol/L maleic acid, 1 mmol/L phthalic acid and 0.05 mmol/L CTAB. Again, negative polarity and indirect detection were applied and the selectivity of the method allowed for 1 : 5 dilution as the only sample pretreatment.

Proteins and peptides of toxicological interest

Since its introduction in 1930, protein analysis has always been a major field of application of electrophoresis and electrophoretic methods remain the basis of serum protein analysis. Although little attention has been paid to protein analysis in forensic toxicology, protein molecules have become attractive as biological markers of chronic alcohol abuse. Carbohydrate-deficient transferrin (CDT) has gained universal acceptance as one such marker and is assayed in many clinical and forensic laboratories world-wide (Arndt 2001).

CDT is the collective name of a group of minor isoforms of serum transferrin with a low degree of glycosylation, which include asialo-, monosialo- and disialo-transferrin (Tf). CDT concentrations increase after sustained alcohol intake (≥50–80 g/day), lasting for at least 7 days, and decrease, after cessation of drinking with a half-life of about 14 days.

First applied to CDT analysis in the second half of the 1990s, CE has rapidly gained acceptance and today is considered, along with HPLC, to be the most reliable method currently available (Bortolotti *et al.* 2006). An example of the separation of glycoforms of Tf by CE is shown in Figure 42.5.

Following the introduction of commercial reagents and multicapillary instrumentation, CE has become the most productive instrumental approach to routine protein and peptide analyses. Most CE methods are based on CZE separations using borate buffers with organic amines added (e.g. diaminobutane, spermine, diethylenetriamine) to hinder protein interactions with the capillary wall. Detection is always based on absorption of UV radiation at 200–214 nm wavelengths (Crivellente *et al.* 2000; Giordano *et al.* 2000; Lanz *et al.* 2002; Fermo *et al.* 2004). An interesting alternative was proposed by Wuyts *et al.* (2001), who described a CZE method using proprietary reagents containing polycations and polyanions (CEofix CDT buffer system, Analis, Namur, Belgium), which provided a dynamic double coating of the capillary. Further improvements of the results in terms of peak resolution were obtained using the same reagents by fine tuning of capillary length, temperature and voltage by the group of Thormann and his co-workers (Lanz and Thormann 2003; Lanz *et al.* 2004; Joneli *et al.* 2006).

Because of their high potential productivity, multicapillary systems, have rapidly replaced former methods (e.g. gel electrophoresis) in the routine analysis of DNA, RNA and serum proteins.

Toxins and venoms

Toxins and venoms are products of living organisms that may have a polypeptide nature, but which, in any case, are complex and very unstable molecules. For this reason they are often not amenable to chromatographic analysis. CE has been shown to offer interesting applications in this area (Weinberger 2001). Although no determinations in human fluids or tissues have so far been published, Bruggemann *et al.* (1996), described a method for the analysis of the principal toxins of *Amanita phalloides* (α-amanitin and β-amanitin) in body fluids. Although the method was successful when applied to the analysis of mushroom extracts, the detection limit did not offer sufficient sensitivity to cover the clinically relevant range of concentrations in biological fluids.

Conclusions

Claims that capillary electrophoresis has had limited success in terms of practical application ignore the fact that this technique has already gained a leading position in DNA analysis, where CE-based instruments in fully automated configurations are the standard tools for DNA fragment analysis and sequencing. CE is also rapidly gaining predominance in clinical protein analysis where multicapillary instrumentation is replacing cellulose acetate electrophoresis. In this area, the analysis of CDT is another example of the successful application of CE methods in routine laboratory practice. CE, particularly with multicapillary instruments, offers good separation performances, standardisation and simple documentation of results, together with full automation and unrivalled productivity.

In contrast to GC and HPLC, CE has not, as yet, achieved significant recognition as a valuable technique in drug analysis. In this area CE-MS is the configuration that most warrants attention since it fulfils the strictest forensic requirements of analytical toxicology, in terms of sensitivity, selectivity, identification power and reliability.

A major advantage of coupling CE technology to MS, which remains to be fully investigated, is associated with the miniaturisation of the separation capillary. This greatly reduces the number of ions that enter the ion source when compared with HPLC-MS and reduces to a minimum the risk of ion suppression, which is a major disadvantage associated with HPLC-MS. In addition, the speed of data production/acquisition of modern MS detectors (particularly TOF-MS) is fully compatible with the fast and narrow peaks typical of CE separations

In conclusion, CE technology shows considerable promise for future applications in analytical toxicology, particularly in combination with high-resolution MS techniques (Polettini 2008). The ease of use and robustness of CE-MS suggests a promising future for this fascinating analytical technique (Servais 2006).

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CHAPTER

43 Atomic Absorption Spectroscopy, Inductively Coupled Plasma-Mass Spectrometry and Other Techniques for Measuring the Concentrations of Metals

A Taylor

Introduction

The development of flame atomic absorption spectrometry (AAS) (Walsh 1955) followed by electrothermal AAS (ETAAS), which is also known as graphite furnace AAS (GFAAS) (Massman 1968), brought about a new era in the quantitative measurement of metals. These techniques provided for considerably lower detection limits than had previously been achievable and an increase in the range of elements that could be measured (Taylor 2006a). Although widely used, AAS is essentially a single-element technique and separate measurements must be made if more than one metal is to be determined. Multi-element analyses using atomic emission spectrometry (AES), neutron activation analysis (NAA) and X-ray fluorescence (XRF) have been available for some years, but these either lack the sensitivity of AAS or require very specialised instrumentation that is not readily accessible.

The field of trace element analysis advanced dramatically when inductively coupled plasma–mass spectrometry (ICP-MS) was developed (Gray, Date 1983). Not only are the detection limits and speed of analysis equal to or better than those seen with AAS, but it is also a powerful multi-element technique. Within a single analysis most elements within the periodic table, including their individual isotopes, may be measured. However, the costs associated with the purchase and operation of ICP-MS and, more importantly, the interferences that were evident in the analysis of biological samples, prevented the widespread application of the technique to clinical, toxicological and forensic situations.

The use of collision and dynamic reaction cells with ICP-MS has largely overcome most of these interferences, and equipment costs have reduced so that ICP-MS is now the primary technique for measurement of metals and other elements in biological specimens. Other important recent advances include the use of techniques that separate different molecular conformations of an element (speciation), *in vivo* analyses involving NAA or, more usually, XRF, and analysis of solid samples to provide quantitative measurements and/or mapping of elemental distribution across a structure using laser ablation (LA) ICP-MS or XRF.

Preparation of samples for analysis

Sample preparation

A range of biological samples including serum, blood, urine, tissues and hair may be used to measure concentrations of metals (see Chapter 17). While there are some exceptions, most analytical systems for measuring metals require samples to be in a liquid form. Processes for preparation, therefore, are designed to ensure that the specimen is in a suitable form for introduction to the analytical instrument and with the analytes at concentrations that will produce a measurable response. At the same time the method should also reduce or eliminate possible interferences.

With instrument sampling systems that involve narrow capillary tubing it is necessary that the transfer rates of samples and calibration solutions be equal. The presence of components such as proteins and cells in serum and blood gives rise to viscosity that is absent in aqueous calibration solutions. Accordingly, assays may be subject to matrix interferences unless the flow rates of sample and calibration solution are matched (Example 43.1). When the concentrations of analyte(s) are high, dilution with water may be all that is needed. Alternatively, protein precipitation can be performed by addition of, for example, 10% (w/v) trichloroacetic acid followed by centrifugation.

Liquid–liquid extraction is a particularly useful approach with addition of a chelating agent to the sample (either a body fluid or a solution post digestion) followed by solvent extraction of the resulting complex. Interfering material is retained in the aqueous phase and, if the volume of solvent is less than that of the sample, analyte enrichment also occurs. A common method uses ammonium pyrrolidine dithiocarbamate (APDC) and 4-methyl-2-pentanone (methyl isobutyl ketone, MIBK), but various chelating agents and solvents have been employed (Example 43.2).

As with sample collection (see Chapter 28), contamination must be avoided throughout the sample preparation procedure. Equipment and

Example 43.1. Sample preparation: dilution of serum for measurement of selenium by ICP-MS

Prepare a 1 : 14 dilution by mixing 200 µL serum with:

- 200 µL 1% Triton X-100
- 200 µL internal standard
- 200 µL modifier (0.14 mol/L NH_3, 3 mmol/L $(NH_4)_2H_2EDTA$, 29 mmol/L $NH_4H_2PO_4$)
- 500 µL 6% v/v butan-1-ol
- 1.7 mL water.

The modifier and Triton X-100 reduce viscosity and prevent blockage of the nebuliser, the butan-1-ol reduces the concentration of interfering Ar adducts such as $^{40}Ar^{38}Ar$, and the internal standard compensates for variation in nebuliser and plasma performance during a run.

Example 43.2. Sample preparation: extraction and concentration of vanadium from urine for measurement by ETAAS

- Adjust 5 mL of test urine to pH 1–2 and pour this solution into a glass-stoppered tube.
- Add 0.5 mL of 5% cupferron solution, stopper, vortex for 30 s and allow to stand for 10 min.
- Add 1 mL water-saturated MIBK, stopper and vortex for 30 s. Mix by repeated inversions for 10 min.
- Centrifuge at 3000 rpm for 5 min to clarify the two layers.
- Take the clear organic layer into sample cups for measurement of vanadium by ETAAS.

reagents should be of the highest quality available and must be tested for cleanliness. All re-usable materials should be cleaned before use. Glassware should be first soaked in aqueous acid, for example 2–10% v/v hydrochloric acid or nitric acid, and then rinsed with copious volumes of purified water. Pure water has an electrical resistivity of more than 18 MΩ and, ideally, should be used at all times. Reagent blank and internal quality control (IQC) samples must be included in the analysis procedure. In addition to guarding against false high results, it is necessary to check for losses of analyte due to volatilisation, adsorption onto container surfaces, or the precipitation of insoluble complexes.

Analysis of tissues

Solid specimens have to be digested to give an aqueous solution prior to analysis. Acid digestion is most commonly used. Methods with addition of nitric acid, perchloric acid and hydrogen peroxide in varying combinations are widely employed and a number of possible procedures are available (Bazzi *et al.* 2005). Sulfuric acid may be included to achieve higher temperatures (Welz *et al.* 1987). Care must be exercised if using the potentially explosive perchloric acid.

Heating may be performed in conical flasks or beakers placed on a hot plate, or in tubes that fit an aluminium heating block. Care is necessary to prevent more volatile elements, especially mercury, being lost. Digestion is normally complete within 2–6 h. Microwave heating in sealed digestion vessels is widely used (Grinberg *et al.* 2005). Specially designed ovens include features to prevent damage from acid fumes and excessive pressure within digestion vessels. As many as 40 or more samples can be digested at the same time, often in less than 30 min and, because each vessel is sealed, there should be no loss of volatile elements (Example 43.3).

Example 43.3. Sample preparation: digestion of hair for measurement of lead with microwave heating

- Wash and dry with acetone and water (Ryabukhin 1978)
- Accurately weigh the sample (the weight taken will depend on sensitivity of technique and amount available)
- Transfer to the digestion vessel
- Add 2.0 mL water and 3.0 mL nitric acid. Seal and load into the oven
- Set pre-determined heating parameters, e.g. power, temperature, time
- Initiate the heating cycle
- Allow to cool
- Quantitatively transfer contents to a tube and make up to required volume

An alternative to acid digestion involves dissolution of the sample in a concentrated alkaline solution. Tetramethylammonium hydroxide (TMAH) is preferred and samples are simply mixed with the reagent and heated for a few hours. Putting 50 mg of dried tissue and 2 mL 50 g/L TMAH into a screw capped vial, complete solubilisation is achieved by heating at 90°C for 2 h with occasional shaking of the vials A third approach to sample destruction is dry ashing, in which the sample is heated in a muffle furnace, typically to 400–450°C, followed by cooling and dissolution of the ash in dilute acid. Loss of volatile elements must be considered and this technique cannot be used if mercury is to be measured. These techniques for preparation of solid samples can be applied to liquid specimens for elimination of matrix interferences and to effect analyte enrichment.

Techniques to extract metals from tissues in a way that preserves their natural chemical state for subsequent speciation analysis have been developed recently. These methods involve use of ultrasonication (Zhou, Mo 2009) or extraction into a solvent at a very high pressure, e.g. 6 MPa, at 125°C (Sanz *et al.* 2007). Enzymes such as proteases and lipase have also been found useful for disrupting tissues to release elements without transformation of the species (Kurek, Ruszczynska 2009).

Analyte enrichment

Techniques for pre-concentration (analyte enrichment) are included in methods when low detection limits are required. Some examples have been mentioned above, but others have been developed for the specific purpose of concentrating the analyte. These involve trapping from a relatively large sample and subsequent elution into a smaller volume. The methods used include (1) adsorption onto materials such as charcoal or silica, (2) use of ion-exchange resins either with or without functionalised groups to trap specific elements, and (3) size-exclusion chromatography (SEC) (Loreti, Bettmer 2004). Enrichment in this way can be off-line, but many methods incorporate on-line sample processing.

Experience with ion-exchange chromatography and SEC has led to a further important development in sample preparation – speciation. In some cases measurement of the total concentration of an element is inadequate and can be misleading. In such circumstances it is necessary to measure particular species such as Cr(III) and Cr(VI), or As(III), monomethylarsonic acid and dimethylarsinic acid (Mandal *et al.* 2004). Methods for speciation involving chromatographic separation or differential solvent extraction are used routinely (Taylor 2006a).

Colorimetry and fluorimetry

A number of compounds react with metal ions to give coloured products (Table 43.1), forming the basis for quantitative assays, although selectivity and sensitivity are usually poor. Colorimetric measurements of calcium and magnesium in serum and urine, analytes that are present at concentrations of the order of mmoles per litre in these fluids, is routine in clinical laboratories. Measurement of zinc and copper in serum is also possible and commercial kits are available that can be used on modern clinical chemistry analysers (Makino 1999). However, there are interferences from the serum matrix, and in external quality assurance (EQA) schemes the performance of these methods is unsatisfactory (Taylor 2006b).

Some of the complexes formed between metals and dye compounds also fluoresce, but compounds have been specially developed to exploit the fluorimetric potential for greater sensitivity and selectivity (Table 43.2). Nevertheless, in practice, only the measurement of selenium using 2,3-diaminonaphthylamine is of interest. Methods can be as sensitive as those involving AAS, but a much more complex sample

Table 43.1 Some compounds that react with metals to give coloured products

Reagent	*Metal*
Catechol violet	Al
Eriochrome cyanide R	Al
o-Cresolphthalein complexone	Ca, Mg
Methylthymol blue	Ca
Sodium diethyldithiocarbamate	Cu
2,2′-Bipyridyl	Fe
Dithizone	Pb, Hg
3,3′-Diaminobenzidine	Se
2-Carboxy-2′-hydroxy-5′-sulfoformazylbenzene (Zincon)	Zn
1-(2-Pyridylazo)-2-naphthol (PAN)	Zn

Table 43.2 Some compounds that react with metal ions to produce fluorescent products

Reagent	*Metal*
3-(2,4-Dihydroxyphenylazo)-2-hydroxy-5-chlorobenzenesulfonic acid (Lumogallion)	Al
8-Hydroxyquinoline	Be
2′,3,4′,5,7-Pentahydroxyflavone (Morin)	Mg
2,3-Diaminonaphthylamine	Se
3,3′-Diaminobenzidine	Se

preparation procedure involving acid digestion is needed. Hence, it is generally used only when there is no alternative.

Electrochemical methods

Anodic stripping voltammetry

Anodic stripping voltammetry (ASV) is a technique in which there is preconcentration of metals on to an electrode followed by selective oxidation during an anodic potential sweep. It is ideally suited to the analysis of dilute solutions where the sample volume is not a limiting factor. The sensitivity can be similar to that associated with AAS for 12–15 elements. A reference electrode and a thin-film mercury graphite electrode are placed in the sample and a negative potential is applied to the mercury electrode, typically for periods of 2–30 min. Cations in the sample are thereby caused to concentrate ('plate-out') on the surface of the mercury electrode (the anode). The direction of the potential is then reversed to give an increasingly larger positive potential over 2–30 s. As the voltage reaches the half-wave potential of an element, lead (Pb^{2+}) for example, all such ions are oxidised and discharged (stripped) from the anode, producing a current that can be measured (Figure 43.1).

The current produced is proportional to the number of ions appearing at that voltage and is compared with those given by calibration solutions. In its simplest form, the potential is scanned linearly as a function of time (linear sweep voltammetry). In differential pulse anodic stripping voltammetry (DPASV), the current is measured in pulses by taking two readings and recording the difference as the potential is increased. This reduces the background current and therefore increases the sensitivity of measurement. While ASV is not widely used in the analysis of biological specimens, a niche application has developed, especially in the USA, for measuring lead in blood. A chelating agent is added to mobilise lead bound to red cells and protein, and the measurement is performed using an instrument specifically designed for this application (Figure 43.2). The equipment is compact and can be used in any location. A very simple hand-held instrument, suitable for concentrations up to about 60 μg/dL, is available in which the electrode and sample container are provided as precalibrated disposable units. A problem associated with the precalibration process was identified, but this has since been resolved (Stanton *et al.* 2006).

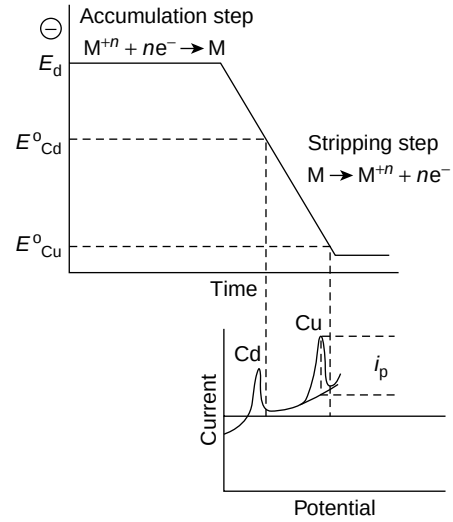

Figure 43.1 Principle of anodic stripping voltammetry illustrated by the potential–time waveform (top) for a solution containing cadmium and copper and the resulting voltammogram (bottom). E_d is the electrodeposition potential and E^o values are the respective oxidation potentials for the two elements. The current measured (i_p) is proportional to the concentration of copper in the sample. (Reproduced from http://www2.chemistry.msu.edu/courses/chem837/Anodic%20stripping%20Voltammetry.pdf.)

Ion-selective electrodes

Ion-selective electrodes (ISEs), of which the pH electrode is an example, are widely used to measure major cations (Na^+, K^+, Ca^{2+}) in biological specimens (Burnett *et al.* 2000). Electrodes for other metal ions and also for anions are available. In clinical laboratories, lithium (Li^+) is commonly measured in this way in plasma or serum (Greil, Steller 1992; Greffe, Gouget 1996). (Use of lithium-free blood collection tubes is essential.) Fluoride (F^-) may be measured in biological samples, not only to assess possible exposure to this poison or compounds giving rise to F^- by metabolism, but also in medicolegal work to establish that enough fluoride has been added to ensure inhibition of microbial growth (Shajani 1985; Kissa 1987).

ISEs are examples of membrane electrodes in which the membrane has physical or chemical properties that allow movement of only one kind of ion between the internal filling solution and any test solutions. If the activities of the ions in the two solutions are not equal, ions will move across the membrane towards the lower-activity solution, and the electric charge thus generated will oppose the migration of ions until equilibrium is established. The actual number of ions involved in these movements is small and does not involve a gradual interdiffusion between the two compartments. The potential established by the movement of ions is measured and is logarithmically related to the activity in the test solution. It is important to remember that it is *activity* and not concentration that is determined by an ISE.

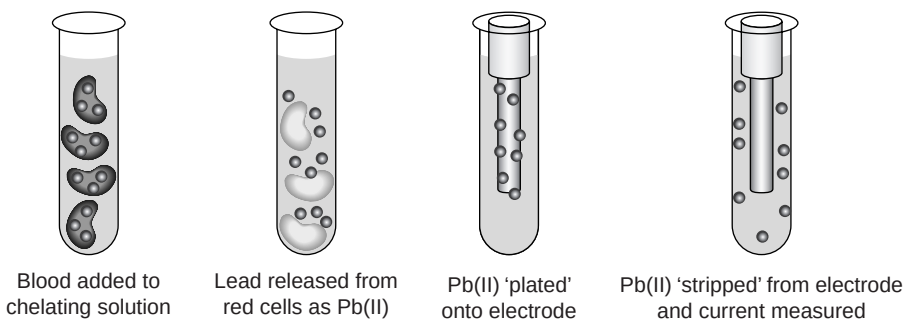

Figure 43.2 Measurement of lead in blood by anodic stripping voltammetry.

Membranes may be solid with a fixed ionic structure, e.g. glass that responds to Na^+, or a water-immiscible liquid containing dissolved material that will actively exchange the selected ions in solution. An effective calcium exchanger is a calcium salt of an alkyl phosphate dissolved in dioctylphenylphosphonate. The exchanger is prepared on a thin PVC layer to form the membrane. For a fluoride ISE, the membrane is a crystal of lanthanum fluoride (LaF_3) doped with europium fluoride (EuF_2) to provide holes in the crystal to allow the F^- ions to pass across.

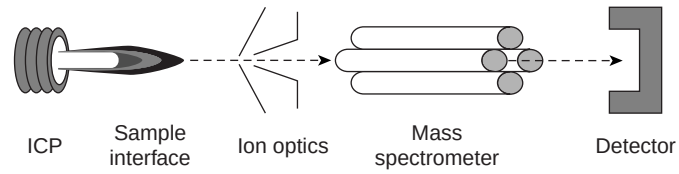

Figure 43.3 Components of an ICP-mass spectrometer.

Atomic spectrometry

Quantitative analytical atomic spectrometric techniques include inorganic MS, AAS, AES, atomic fluorescence spectrometry (AFS) and XRF. AAS, AES and AFS involve interactions between UV–visible light and the outer shell electrons of free, gaseous, uncharged atoms. In XRF, high-energy particles collide with inner shell electrons of atoms, initiating transitions that conclude with emission of X-ray photons. For inorganic MS, ionised analyte atoms are separated within electrical or magnetic fields according to their mass-to-charge (*m/z*) ratio.

Vapour generation procedures (see Vapour generation approaches below) were initially developed for AAS and later applied to other atomic spectrometric techniques.

Inductively coupled plasma–mass spectrometry

As a sample is taken to a high temperature, any organic component is destroyed and the thermal energy causes some or all of the inorganic elements to be ionised. When these ions are directed into a mass spectrometer they are separated by a mass filter according to the *m/z* ratio, and are then detected and counted using an electron multiplier. This process is generally described as *atomic* or *inorganic MS* (Hill 1999; Nelms 2005; Evans *et al.* 2009). Many elements can be measured virtually simultaneously and detection limits are in the range of a few µg/L or below. Elements that have hitherto been almost impossible to quantify can be measured reliably. The other major feature of mass spectrometry is the ability to measure isotopes of the same element (Al Saleh *et al.* 1993). The technique is now well established for analysis of biological specimens (Heitland, Köster 2004, 2006).

Various ion sources for inorganic MS have been employed, but most recent work makes use of a plasma, i.e. a volume of ionised gas, usually argon. The modules required for ICP-MS include sample introduction and ion generation, ion focusing, ion separation, ion detection, and data collection and display (Figure 43.3).

Plasma

Plasmas exist at temperatures of around 6000–10 000°C and in the instrument are constrained to the shape of a 'torch'. Figure 43.4 shows a radiofrequency induction coil around the tip of the torch. As power is supplied to the coil from an RF generator, oscillating electrical and magnetic fields are established at the end of the torch. The plasma is initiated by applying a high-voltage spark to the argon flowing through the ICP torch causing electrons to be stripped off the atoms, forming argon ions.

These ions are caught in the oscillating fields and collide with other argon atoms, forming an argon discharge or plasma that is sustained with the energy from the induction coil. This is known as an inductively coupled plasma (ICP) and samples are introduced via a nebuliser, from a vapour generation device (see Vapour generation approaches), by electrothermal vaporisation from a graphite atomiser, or by laser ablation of solid specimens (Figure 43.5).

Sample introduction

A range of sample introduction techniques have been developed (Figure 43.5) but the most widely used involve liquid samples converted to a fine aerosol using a nebuliser, followed by isolation of the fine droplets in a spray chamber. This provides for efficient ionisation in the plasma.

Samples are normally pumped at a rate of around 1 mL/min via a peristaltic pump into the nebuliser where the liquid is broken up into an aerosol by the pneumatic action of a flow of argon. As plasmas are inefficient at dissociating large droplets in the aerosol, the spray chamber – coupled to the nebuliser – functions to allow only the small droplets (~5–10 µm in diameter) to enter the plasma. Some spray chambers are externally cooled (typically to 2–5°C) to give thermal stability of the sample and to minimise the amount of solvent going into the plasma. This can have a number of beneficial effects such as a reduction of oxide species and the ability to aspirate volatile organic solvents.

The commonest spray chamber is the double-pass, in which the aerosol is directed into a central tube running the whole length of the chamber. Droplets greater than 10 µm in diameter fall out by gravity and exit through a drain tube at the end of the spray chamber. The fine droplets pass between the outer wall and the central tube and are then transported into the plasma torch. The cyclonic spray chamber operates

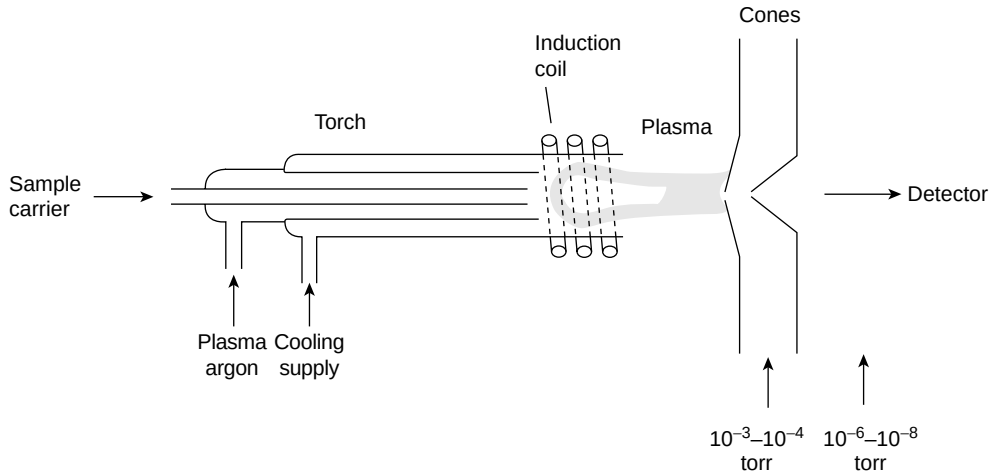

Figure 43.4 ICP torch.

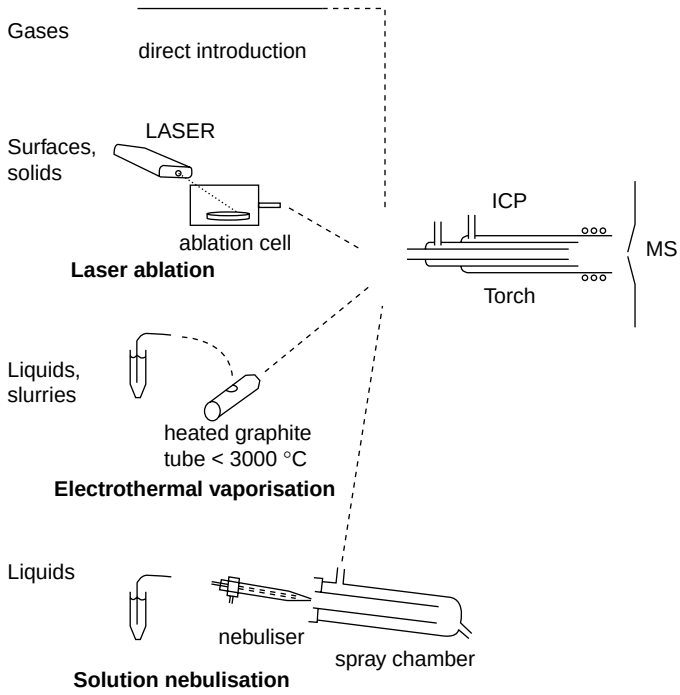

Figure 43.5 Sample introduction for ICP-MS.

by centrifugal force. Smaller droplets are carried with the gas stream into the ICP-MS set-up, while the larger droplets strike the walls and run to the drain. The types of nebulisers and spray chambers generally used for ICP-MS are listed in Table 43.3.

The plasma and ion generation

The plasma is responsible for the formation of analyte ions. This involves desolvation of the aerosol droplet to give the solid and then the gaseous form of the analyte. Any molecular forms are disintegrated/dissociated to leave the ground-state atom. The final step is removal of an outer shell electron, giving the ion. The ICP torch is interfaced to the mass analyser via two metallic cones (sampler and skimmer) with very small holes through which ions are extracted into the ion focusing unit. Because the orifice sizes of the sampler and skimmer cones used in ICP-MS are so small (~0.6–1.2 mm), they are susceptible to becoming blocked and the concentration of matrix components (dissolved solids) must generally be kept below 0.2%. The interface region accommodates the change from atmospheric pressure (101 kPa, 760 mmHg) in the plasma, to 2–3 mmHg (267–400 Pa) at the cones, down to 10^{-6} mmHg (0.000133 Pa) within the mass spectrometer.

Ion focusing and separation

Having passed through the sampler and skimmer cones into the spectrometer, the ion optics, a system of ion lenses, focus and direct ions to the entrance of the analyser for mass separation and measurement. Several analyser configurations are available commercially, although the most commonly used is the quadrupole mass filter.

The quadrupole design has four cylindrical metallic rods, typically 15–20 cm in length and about 1 cm in diameter. Figure 43.6 shows a cross-section of the quadrupole configuration. When a specific direct current (DC) field is applied to one pair of rods and a radiofrequency (RF) field to the opposite pair, the positive or negative bias on the rods will electrostatically steer the analyte ions of interest down the middle of the four rods to the end, where they emerge and produce an electrical pulse in the detector. Ions of other *m/z* ratios pass through the spaces between the rods and are ejected from the quadrupole. The settings are then changed so that ions at a different *m/z* ratio are measured and the process is repeated until all the analytes in a multi-element determination have been measured. While this represents a sequential analytical process, readings are made within milliseconds and a spectrum is presented as if measurements were simultaneous (Figure 43.7).

Limitations to the resolution achievable with quadrupole mass filters, 0.7–1.0 amu, require more powerful systems for certain applications. To exploit the full potential of the ICP-MS technique, a double-focusing sector field mass analyser is required (Figure 43.8). Such instruments provide up to 30 times greater ion resolution and offer higher sensitivity than a quadrupole ICP-MS. The units responsible for separation are an electrostatic analyser (ESA), followed by an electromagnet (in some instruments this order is reversed). Ions are sampled from the plasma and then accelerated in the ion optics region to a few kilovolts before entering the mass analyser. The ESA is dispersive with respect to ion energy while the magnetic field separates ions on the basis of ion energy and mass. By variation of the electric field and the magnetic field strength, ions are separated with much improved resolution. As with the quadrupole, readings are rapidly sequential.

Some commercial instruments employ time-of-flight (TOF) MS technology, which provides true simultaneous multi-element measurement. This is particularly useful for the analysis of rapid, transient multi-element signals, such as those generated by electrothermal vaporisation and laser ablation. Multi-collector instruments, with double-focusing sector field mass spectrometry, are used for applications requiring high-precision measurements, such as the determination of isotopic ratios.

Interferences

ICP-MS is subject to spectral interference caused by the presence of isotopes of either different elements (e.g. $^{64}Ni^{+}$ on $^{64}Zn^{+}$, $^{156}Gd^{2+}$ on $^{78}Se^{+}$) or ions formed from matrix components and the plasma gas (isobaric and polyatomic, respectively). Examples of polyatomic interferences that are important for analysis of biological samples include $^{40}Ar^{+}$ on ^{40}Ca, $^{31}P^{16}O_2{}^{+}$ on ^{63}Cu, $^{40}Ar^{35}Cl^{+}$ on ^{75}As and $^{40}Ar_2{}^{+}$ on ^{80}Se. These interferences can be avoided by counting a non-affected isotope if it is present with sufficient abundance, e.g. $^{82}Se^{+}$ in place of $^{80}Se^{+}$, $^{66}Zn^{+}$ in place of $^{64}Zn^{+}$. Sector field ICP-MS is not subject to most of these interferences, although there may be some loss of sensitivity at high resolution, which may lead to problems with

Table 43.3 Nebulisers and spray chambers used in ICP-MS

Unit/Design	*Advantages*	*Disadvantages*
Concentric nebuliser	Provides stable and sensitive readings	Unable to use samples with high dissolved solids, liable to blocking
Cross-flow nebuliser	Can use samples with higher dissolved solids. Rugged performance	Produces fewer small droplets and, therefore, less sensitive readings
Microflow nebuliser	Ideal for small samples, use flow rate of less than 0.1 mL/min	
	Low memory effects and low blanks	
Double-pass spray chamber	Rugged performance, suitable for most applications	
Cyclonic spray chamber	Good sampling efficiency with improved sensitivity and lower detection limits	Precision may be compromised

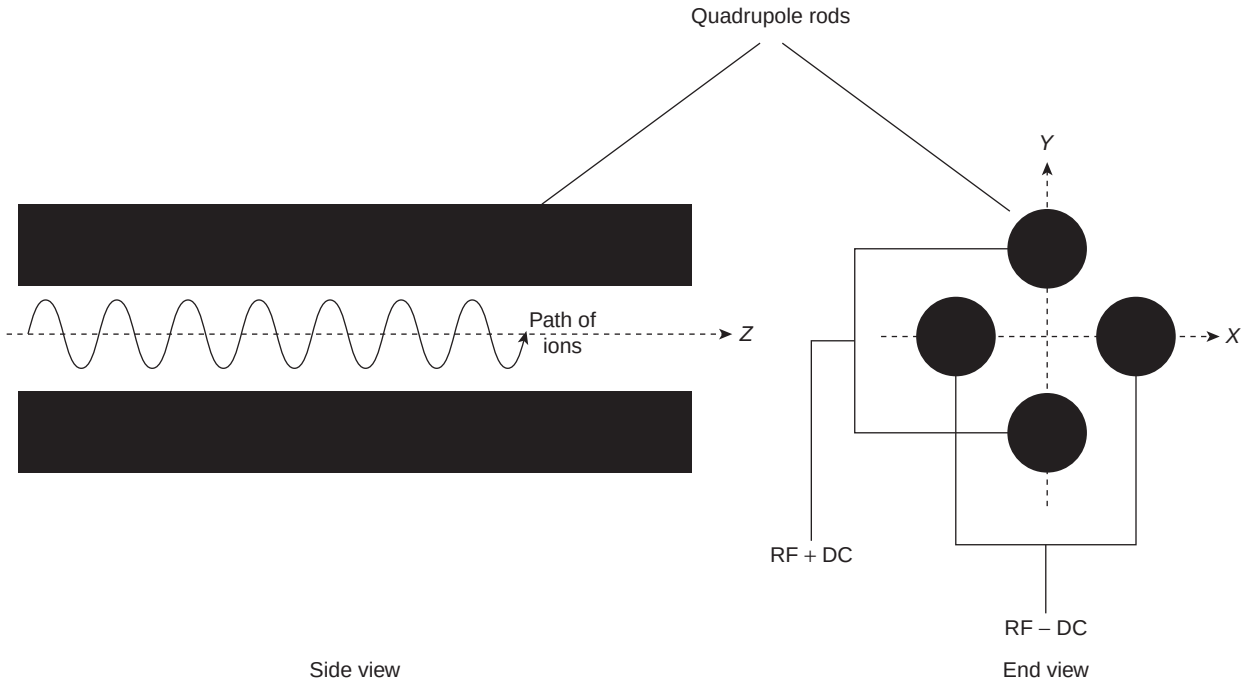

Figure 43.6 Cross-section of the quadrupole for ICP-MS.

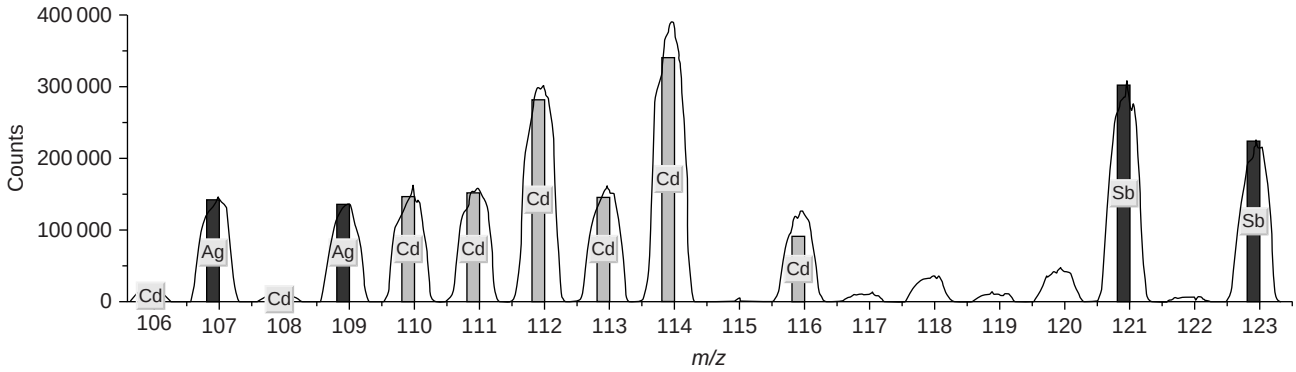

Figure 43.7 Example of an ICP-MS mass spectrum.

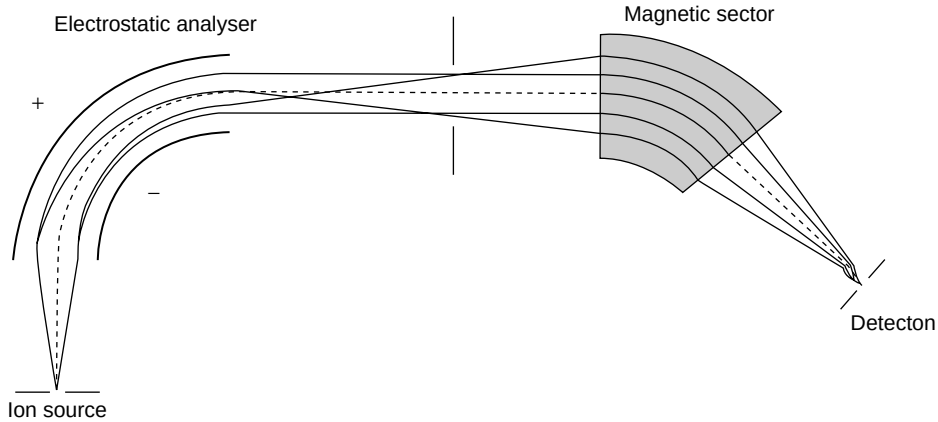

Figure 43.8 Sector field mass spectrometer.

some applications such as the measurement of blood selenium (Klaue, Blum 1999). The most usual procedure, however, is to use a collision, or dynamic reaction, cell.

This is a relatively new development for use in quadrupole MS instruments. The cell is a multipole – a quadrupole, a hexapole or an octapole – aligned between the ion optics and the quadrupole mass filter. Interactions between polyatomic ions and a gas fed into the cell greatly reduce spectral interferences. These interactions lead to the formation of secondary species, which are removed from the ion path on the basis of kinetic energy discrimination or mass discrimination so that only the analyte ion is transmitted to the detector.

The addition of nitrogen, helium, or methane to the carrier gas or an organic solvent to the diluent reduces some of the argon-based interferences (Branch *et al.* 1994). An alternative approach involves prior separation of analyte ions from those involved in the formation of polyatomic species. Separation may be achieved by vaporisation of the sample, for example by hydride generation or by electrothermal vaporisation, or by prior use of a chromatographic or other separation step such as liquid–liquid extraction.

Non-spectral interferences, associated with sample introduction and fluctuations in the inductively coupled plasma, are effectively eliminated by using an internal standard. This should be an element not present in the original sample, not subject to spectral interferences, and with mass and ionisation energy close to those of the analyte(s). Internal standards often used with biological specimens are scandium, indium and iridium for masses <80, 80–150 and >150 amu, respectively.

Applications

In most laboratories ICP-MS serves as a routine technique that affords high throughput, single- or multi-element analysis with very good sensitivity. It can, therefore, be applied to a wide range of investigations. Situations in which ICP-MS is particularly valuable are illustrated by the following studies.

Multi-element screening A welder developed neurological symptoms after working on a warship where the metal had been coated with non-standard paint. A urine sample was analysed with the ICP-MS instrument set in semi-quantitative mode, for a rapid scan to identify any elements present at abnormal levels. Metals identified as possibly of further interest were then quantified, but none was found at increased concentration (Taylor, 2009, unpublished observation).

Sensitivity Individuals from regions where there had been military action were concerned about possible exposure to uranium. With the exquisite sensitivity afforded by ICP-MS it was possible to demonstrate that concentrations of natural and depleted uranium in blood and urine samples were quite normal (Oeh *et al.* 2007).

Isotopic analysis Several children from one town had increased blood lead concentrations. Lead isotopes were measured in blood samples and a series of possible sources of exposure. The isotopic ratios of a 'market place' medicine with a high lead concentration matched the ratios determined in the blood samples. The public authorities arranged for the material to be removed from sale (Al Saleh *et al.* 1993).

Versatility Non-standard sample introduction and the excellent sensitivity of LA-ICP-MS were exploited to determine the profile of platinum along a single strand of hair from a patient treated with cisplatin. Each measurement corresponded to just a few days of growth and concentration peaks corresponded to discrete episodes of treatment (Stadlbauer *et al.* 2005).

General principles of AES, AAS and AFS

Atoms may exist at the most stable or *ground state* (E^0), having the lowest energy, or at any one of a series of *excited states* depending on how many electrons have been moved to higher energy levels, although it is usual to consider just the first transition (to E'). This may be visualised in an energy level diagram (Figure 43.9). It is important to understand that energy levels, and the differences (ΔE) between each energy level, are dependent on the composition of the atom and are unique to each element.

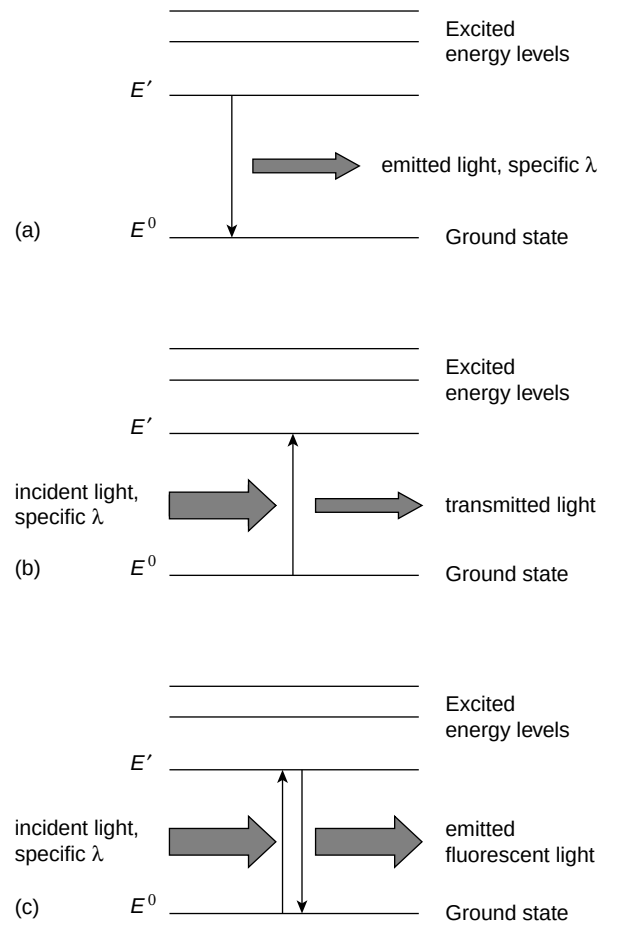

Figure 43.9 Energy-level diagrams to show transitions associated with (a) AES, (b) AAS and (c) AFS. The vertical arrows indicate absorption or emission of radiant energy (light).

The value of ΔE for movements of *outer shell electrons* in most elements corresponds to the energy equivalent to UV–visible radiation. The energy of a photon is represented as:

$$E = h\nu \tag{43.1}$$

where h is the Planck constant and ν is the frequency of the waveform corresponding to that photon. Furthermore, frequency and wavelength are related as:

$$\nu = \frac{c}{\lambda} \tag{43.2}$$

where c is the velocity of light and λ is the wavelength. Therefore:

$$E = \frac{hc}{\lambda} \tag{43.3}$$

and so the transition associated with each element, e.g. ΔE(Ca), is characterised by a unique wavelength.

Under appropriate conditions, outer shell electrons of vaporised, ground state atoms within the analytical system are excited by heating (thermal energy). As atoms return to the more stable ground state, this energy is lost. Some of this energy is in the form of emitted light that can be measured. The intensity of the light is proportional to the number of atoms present and in a quantitative system the process is *atomic emission spectrometry* (Figure 43.9a).

As light (i.e. radiant energy) of a specific wavelength enters an analytical system, outer shell electrons of corresponding vaporised ground

state atoms within the light path will be excited as the energy is absorbed. Consequently the amount of light transmitted through to the detector is reduced. The loss of light is proportional to the number of atoms present and the method is known as *atomic absorption spectrometry* (AAS) (Figure 43.9b).

Some of the radiant energy absorbed by ground-state atoms can be emitted as light as the atom returns to the ground state. This emission is known as resonance fluorescence and is again proportional to the number of atoms in the light path. The technique is known as *atomic fluorescence spectrometry* (AFS) (Figure 43.9c). This technique requires very bright light sources to generate useful amounts of fluorescence and in practice is limited to just a few elements.

It follows from equations 43.1–43.3 that the wavelengths of the absorbed and emitted light are the same, and are unique to any given element. This gives AAS, AES and AFS great specificity, so that one element can be measured accurately even in the presence of an enormous excess of a chemically similar element.

Atomic absorption spectrometry

Atomic absorption spectrometry can be used to measure more than 60 elements and is sufficiently sensitive to measure many of these elements at the concentrations present in biological specimens. The spectrometer consists of a light source, atomiser, monochromator, detector and readout/display. The essential requirement of a good light source for AAS is to provide a high-intensity, monochromatic output, which is achieved with hollow cathode or electrodeless discharge lamps. The monochromator, detector and display are similar to those of other spectrometers.

The *atomiser* is any device that will generate ground-state atoms as a vapour within the light path of the instrument. In the case of lead in blood, for example, the element is present bound to macromolecules within red cells and a small fraction in the blood plasma. Formation of the atomic vapour (atomisation) requires (1) removal of solvent (drying), (2) separation from anionic or other components of the matrix to give Pb^{2+} and (3) reduction ($Pb^{2+} + 2e^- \rightarrow Pb^0$). The necessary energy is supplied as heat, from either a flame or an electrically heated furnace. The uncharged lead atomic vapour, within the atomiser, then absorbs incident radiant energy coming from a lead-specific hollow cathode lamp.

Flame atomisation

The typical arrangement for flame AAS involves a *pneumatic nebuliser*, mixing chamber, and an air–acetylene laminar flame with a 10 cm path length (Figure 43.10). The sample uptake rate through the nebuliser is usually about 5 mL/min and aspiration for several seconds is necessary to achieve a steady signal. The sample emerges from the nebuliser as an aerosol with a wide range of droplet sizes, is mixed with the combustion gases and is transported to the flame for atomisation. As with the nebuliser used for ICP-MS, droplets of more than 10 µm diameter run to waste and only about 15% of the sample enters the flame. Hence, with the pneumatic nebuliser, the original sample undergoes dilution with the flame gases, a portion is lost in the mixing chamber and there is considerable thermal expansion (i.e. further dilution) within the flame. In addition to dispersion of sample through the flame, there are losses of atoms due to the formation of oxides or other species at the flame margins.

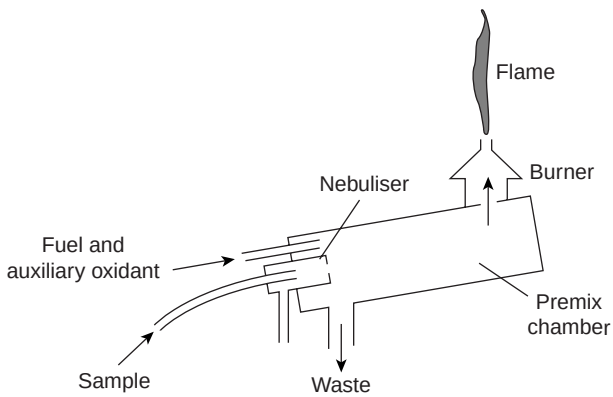

Figure 43.10 Pneumatic nebuliser for flame AAS.

Table 43.4 Advantages and disadvantages of pneumatic nebulisation

Advantages	*Disadvantages*
Rapid	Only about 15% of the sample enters the flame
Reproducible	Wide range of droplet sizes
Few interferences	Low atomic density of sample in the flame
Steady-state signal	Burner conditions impose limitations on nebuliser

The advantages and disadvantages of the pneumatic nebuliser–flame atomisation system are shown in Table 43.4. Because of its simplicity, speed and freedom from interferences, this approach is adopted whenever possible. The lowest analyte concentrations that can be measured are typically ~1 mg/L. Acetylene burns in air at ~2000°C. A nitrous oxide–acetylene flame, which reaches ~3000°C, is used for elements such as aluminium and chromium that form refractory oxides and do not atomise at the temperature of an air–acetylene flame.

Improved sensitivity is obtained when the limitations of pneumatic nebulisers listed above are overcome. This may be achieved with (1) use of atom traps that give a greater atom density within the light path, (2) by-passing the nebuliser so that all of the sample is atomised, and (3) introducing the sample as a single, rapid pulse rather than a continuous flow. Some accessories employ a combination of these features. Atom traps, e.g. the slotted quartz tube and the Delves cup system, are devices that effectively concentrate the analyte atoms in the flame by slowing down their dispersion so that more of the incident radiation is absorbed, increasing the sensitivity of the analysis. Traps are most effective with more volatile elements such as zinc, cadmium and lead (Delves 1970; Taylor, Brown 1983). These three approaches to improved sensitivity also feature in other atomisers used in AAS, AES and AFS.

Electrothermal atomisation

Various materials have been used to construct electrically heated furnaces to vaporise the analyte, but commercial instruments use a graphite tube and the technique is often called *graphite furnace atomisation*. Heating occurs when electrical contact is made to the ends of the tube and a voltage is applied; the resistance to the flow of current causes the furnace temperature to increase. A programme is used to increase the voltage and, consequently, the temperature, so that a solution placed inside the furnace is carefully dried, organic material is destroyed and the analyte ions are dissociated from anions. With a further rapid increase in temperature, analyte ions are vaporised and reduced to ground-state atoms for absorption of radiant energy and spectrophotometric measurement.

The atomisation temperatures achieved by these analysers can be up to 3000°C, allowing refractory elements such as aluminium and chromium to be measured. Typically only 10–50 µL of sample is needed for injection into the furnace and, because the entire sample is atomised within a small volume, a dense atom population is produced. The technique is very sensitive and analyte concentrations of a few µg/L can be measured. However, electrothermal atomisation AAS (ETAAS) is subject to greater potential interference than flame AAS (FAAS) and procedures to either eliminate or compensate for interferences are essential. Different forms of graphite (electrographite, pyrolytically coated graphite and total pyrolytic graphite) are used, and the design of the furnace and its mode of heating are optimised to promote atomisation and reduce interferences.

Interferences

Devices that involve flow of solutions, such as nebulisers or flow injection systems, will give inaccurate results if the samples and calibrators have different viscosities, as this causes rates of flow into the analyser to differ. If this occurs, internal standardisation, addition of standard or the addition of reagents to equalise analyte and calibrant flow rates can be used to improve accuracy. Thus, the viscosity of serum reduces the aspiration rates of samples compared with aqueous calibration solutions, giving low results if not adjusted. Sample viscosity can be reduced by dilution, but this may then give very low absorbance readings. Different strategies to overcome this problem in the measurement of serum copper and zinc are outlined in Example 43.4. Note that when the method includes several steps, the possibility for contamination to occur, especially with zinc, is increased. Whichever sample preparation method is used, it is important to measure the aspiration rates of samples and calibration solutions through the nebuliser pre-mix chamber to ensure that they are equivalent.

Table 43.5 Chemical modifiers used in ETAAS

Modifier	*Function*
Nickel Ammonium phosphate Palladium Ruthenium	Form a thermostable complex with the analyte ions to allow a higher ash temperature to be used and so remove interfering species
Gaseous oxygen	Assists the ashing of the organic matrix. Mediates formation of atoms via activation of the graphite surface
Nitric acid Ammonium nitrate	Promote low temperature volatilisation of halides to prevent analyte–chloride vapour phase interferences
Magnesium nitrate	Delays atomisation so that isothermal conditions can be established inside the furnace

Example 43.4. Techniques to match viscosities of sample and calibration solutions

- Prepare the calibration solution in the same matrix as the sample (matrix matching or standard additions).
- Prepare the calibration solutions in 2% v/v glycerol.
- Add 1 part 10% w/v trichloroacetic acid to 1 part serum or calibration solution. Mix well, centrifuge and remove the supernatant for analysis.
- Dilute the samples and calibrators with 5% v/v aqueous butan-1-ol.
- Dilute the mixture 5- to 10-fold in purified water.

Table 43.6 Approaches to eliminate non-atomic absorption in ETAAS

Technique	*Rationale*	*Examples*
Chemical modifiers	Promote the destruction of sample matrix. Delay atomisation of analyte	Oxygen, magnesium nitrate, diammonium hydrogen orthophosphate
Isothermal atomisation	Reduce vapour phase interactions by delaying atomisation until the furnace reaches constant temperature	L'Vov platform, graphite probe, novel furnaces
Background correction	Separately measure total and non-atomic absorption; difference = atomic absorption	Deuterium BC, Zeeman-effect BC, Smith-Heijfte BC

Chemical interactions may influence the rate of atomisation. During FAAS, calcium bound to phosphate in serum is not entirely separated at 2000°C and gives a lower result than an equivalent concentration in an aqueous calibrant. Addition of a releasing agent such as lanthanum (La^{3+}), which releases the calcium by binding preferentially to phosphate, avoids this interference.

Problems also occur in the graphite furnace. Components in the sample matrix can cause the analyte to become volatile and be lost during the ashing step, e.g. lead can be lost as lead chloride, $PbCl_2$. However, the most difficult problems are those that develop in the vapour phase as the samples are heated. Ground-state atoms are vaporised into a rapidly changing thermal environment where the gas-phase temperature is lower than that of the tube wall and the ends of the tube are at a lower temperature than the centre. Consequently, vaporised atoms may condense on the cooler parts of the tube and then revaporise as the gas-phase temperature increases to the analyte appearance temperature, giving double peaks. This is because the molecular species formed give non-atomic absorption. Compensation for non-atomic absorption is provided by using chemical modifiers to stabilise the atoms and/or to promote destruction of the matrix at an earlier stage of heating (Table 43.5), by using devices to establish isothermal atomisation conditions and by using background correction techniques (Table 43.6).

A typical method for the measurement of blood lead (Example 43.5) exploits each of these features. Matrix-matched calibration solutions are prepared in a sample of blood with a low concentration of lead. Samples are diluted 15-fold with a phosphate/Triton solution and introduced into the graphite tube furnace of the AAS set-up. Oxygen or air is bled into the furnace during the ash phase to facilitate the destruction of organic material. Background correction is essential to compensate for the non-atomic absorption.

Example 43.5. Measurement of lead in blood by electrothermal atomisation atomic absorption spectrometry

- Add whole blood (30 µL) to chemical modifier (0.05 % v/v Triton X-100 + 0.5 % (w/v) diammonium hydrogen orthophosphate (420 µL)).
- Mix well and introduce to furnace with deuterium or Zeeman-effect background correction.
- Heating programme (instrument: Thermo 939):

Phase	*Temperature (°C)*	*Hold time (s)*	*Ramp (°/s)*	*Gas*	*Gas setting*	*Remarks*
1	80	2	100	Ar	2	Drying
2	130	35	2	Ar	2	Drying
3	500	10	0	Air	2	Air ashing
4	600	15	10	Ar	3	Air/O_2 desorbed from furnace
5	1400	2	max.	Ar	0	Temperature control and read
6	2800	3	max.	Ar	3	Cool

Atomic emission and atomic fluorescence spectrometry

Atomic emission spectrometry

Flame atomic emission spectrometry (FAES; flame photometry) is convenient for the alkali metals at high concentrations, but AES is most useful with high-temperature energy sources when multi-element analyses can be undertaken. As indicated above, the heat source for atomisation and excitation to a higher energy level can be a flame. Historical alternatives include arcs and sparks, but modern instruments employ an argon plasma, as subsequently used for ICP-MS. Samples are normally introduced via a nebuliser but, as for ICP-MS and AAS, vapour generation (see below), electrothermal vaporisation from a graphite atomiser or laser ablation of solid specimens is also possible. Optical systems direct the emitted light either via a monochromator to a single detector or to an array of monochromators and detectors positioned around the plasma. With the first arrangement a sequential series of readings can be made with the monochromator driven to give each of the wavelengths of interest in turn. Simultaneous readings can be made with the second arrangement as each of the monochromators transmits light of different required wavelengths. A sequential reading instrument is less expensive than a simultaneous reading instrument, but more sample is required to take a series of readings. For most elements the analytical sensitivity for ICP-AES is similar or slightly superior to that obtained with FAAS.

At the high operating temperatures of the ICP, many energy transitions take place, giving rise to the potential for spectral interferences when emission of light from different elements occurs at wavelengths that are too close to be separated by the monochromator. Most of these spectral interferences are known so that, when interference is suspected, an alternative resonance line may be used for the measurement.

Atomic fluorescence spectrometry

Few commercial instruments for AFS are available and these are confined to the measurement of hydride-forming elements and mercury (see Vapour generation approaches). The components of the instrument are similar to those for AAS. Effective atomic fluorescence requires intense, stable light sources and these are difficult to construct reliably. Most success has been with electrodeless discharge lamps. The optical path of the emitted light is directed at 90° to that of the incident light so that only the emitted, fluorescent light reaches the detector. Very low detection limits can be achieved for the metalloid elements of groups 4–6 of the periodic table.

Vapour generation approaches

A family of chemical vapour generation techniques has been described (Tsalev 1999) where the analyte is separated from the matrix as an atomic or chemical vapour that is then transported to a suitable instrument for detection and measurement. Most practical applications involve either volatile hydride generation or the formation of mercury vapour, although some recent reports have demonstrated that it is possible to measure copper and zinc in this way (Matsumoto *et al.* 2007; He *et al.* 2008).

Hydride generation

Elements such as arsenic, selenium, antimony and bismuth form gaseous hydrides, e.g. arsine (AsH_3), and this can be exploited in an assay. Using simple instrumentation, a reducing agent, such as sodium borohydride, is added to a reaction flask containing an acidified sample. Hydrogen is formed, which reacts with the analyte and the gaseous hydride is evolved. The hydride is transferred by a flow of inert gas to an ICP for AES or MS, or to a heated silica tube positioned in the light path for AAS or AFS (Heilier *et al.* 2005). The tube is heated, by either an air–acetylene flame or an electric current, to a temperature that is sufficient to cause dissociation of the hydride and atomisation of the analyte (Figure 43.11). Of relevance to AAS, there is no loss of specimen in the nebuliser, all the atoms enter the light path within a few seconds and they are trapped within the silica tube, which retards their dispersion. Instrument development to give a continuous flow has simplified automation.

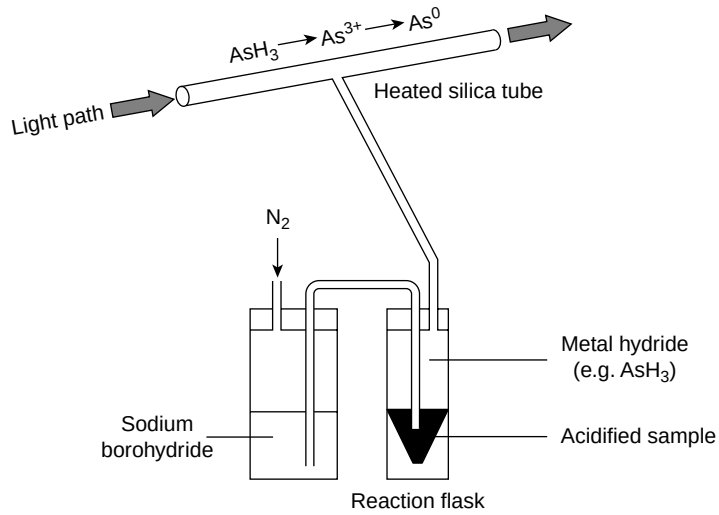

Figure 43.11 Hydride generation AAS.

Typically, the hydride-forming element will be present in a sample as a range of species (e.g. As(III), As(V), monomethylarsonic acid, dimethylarsinic acid, arsenobetaine). The rate of hydride formation is dependent on the species, so that to measure the total concentration of the element it is necessary to convert all of the different species present to the form that is the most reactive. This is usually achieved by heating with concentrated acids (Example 43.6) and then adding a reducing agent to give the reactive species (As(III), Se(IV), Sb(III), etc.).

Example 43.6. Preparation of sample for the measurement of total selenium concentrations (Welz *et al.* 1987)

- 1 g of blood, urine, tissue placed in a digestion tube. Add 5 mL HNO_3 and heat at 150°C for 30 min.
- Add 0.2 mL $HClO_4$ and 0.5 mL H_2SO_4. Heat at 150°C for 15 min, then at 200°C for 15 min, 250°C for 15 min.
- Heat for a further 20 min with the heater set at 320°C. Allow to cool to room temperature.
- To reduce Se(VI) to Se(IV), add 5 mL 50% v/v HCl. Heat at 90°C for 20 min.
- Cool, add 5 mL H_2O and take for the measurement.

Seafoods are a rich source of non-toxic organoarsenic species and urinary total arsenic concentrations will be high for several days following consumption of fish. It is possible to differentiate between dietary and non-dietary arsenic compounds by omitting the aggressive heating step and using a mild reducing agent to convert any As(III) metabolites to the hydride-forming species. The analysis will then reflect the exposure to toxic arsenic species (Cava-Montesinos *et al.* 2005; Leermakers *et al.* 2006).

Certain interferences are common to hydride generation whichever detector is used. If one hydride-forming element is present in the sample in large amounts it will consume the reducing agent so that other elements may not be detected (Welz, Stauss 1993). High concentrations of transition-element ions also inhibit hydride formation. If this is a problem, the use of masking agents, co-precipitation or chelation of the analyte followed by liquid–liquid extraction of the chelate can be used prior to the analysis (Welz, Melcher 1984).

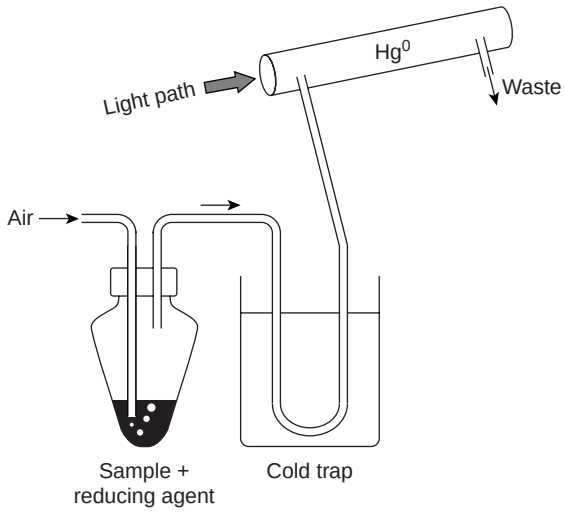

Figure 43.12 Cold vapour generation.

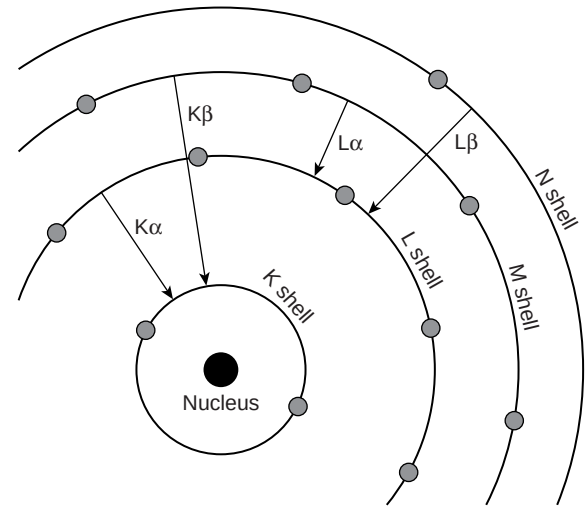

Figure 43.13 XRF electronic transitions in a calcium atom.

Mercury vapour generation

Mercury forms a vapour at ambient temperatures and this property is the basis for cold vapour generation. A reducing agent such as tin(II) chloride is added to the sample solution to convert Hg^{2+} to elemental mercury (Hg^0). Agitation or bubbling of gas through the solution causes rapid vaporisation of the atomic mercury, which is transferred to a flow-through cell placed in a light path (Figure 43.12). To break the carbon–mercury bond in any organomercury compounds present, potassium permanganate may be added to acidified urine and the sample incubated at room temperature overnight. Excess permanganate is destroyed by addition of hydroxylamine hydrochloride. The reducing agent is added to the sample immediately before connection to the vapour generation accessory (Example 43.7). As with hydride generation, the LOD is a few nanograms and instruments to perform both procedures have been developed.

Example 43.7. Measurement of mercury in urine by cold vapour generation AAS

- Place urine (1 mL) in a tube (4°C).
- Add 0.2 mL concentrated H_2SO_4 and 1.5 mL 6% w/v $KMnO_4$.
- Stand overnight and then add 0.3 mL 20% w/v hydroxylamine hydrochloride.
- Add 1 mL 20% w/v $SnCl_2$ in 50% v/v HCl to reduce Hg^{2+} to Hg^0.
- Connect to accessory and purge with air or inert gas to volatilise the analyte.

X-ray fluorescence

When solid samples are exposed to short-wavelength X-rays or to gamma rays (the primary radiation), an electron may be displaced, i.e. ionisation occurs. Energy associated with these rays is sufficient to displace electrons from the inner orbitals (K, L or M) of constituent atoms. The atomic structure is then unstable and the orbital vacancy is filled by an outer shell electron 'falling' into the hole. This event releases energy in the form of an X-ray photon. The energy (and hence wavelength) of the emitted photon is equal to the difference in the energy levels involved in the electron transition. This phenomenon is known as *X-ray fluorescence* (XRF).

The transitions are given names that describe the movement of electrons: L to K is Kα, M to K is Kβ, M to L is Lα (Figure 43.13).

The wavelength is characteristic of the atom (element) from which it originated, while the intensity of the emission is related to the concentration of the atoms in the sample (Janssens, Adams 1989; West *et al.* 2009). The spectrometer used to measure the emission may separate either the wavelengths of the radiation or the energies of the photons. Thus analytical XRF is described as wavelength-dispersive (WDXRF) or energy-dispersive (EDXRF). Total reflection XRF (TXRF) is usually described as a separate technique, although it may be considered as a variation of EDXRF (Klockenkämper, von Bohlen 1992). To perform an analysis, specimens are irradiated by high-energy photons, usually the polychromatic primary beam from an X-ray tube. To avoid having to use an elaborate power supply, radioactive isotopes such as ^{244}Cm, ^{241}Am, ^{55}Fe and ^{109}Cd may be used as sources. These have application to semi-portable instruments developed for *in vivo* XRF (Chettle 2006).

The signal may be complicated by contributions from the sample matrix, making calibration difficult. Techniques to overcome the interferences include the use of reference materials, matrix-matched standardisation and/or internal standardisation. In TXRF or when samples are prepared as very thin films, problems are reduced and simpler to resolve. In addition to the effect of the matrix, sensitivity is also influenced by wavelength, and lower-atomic-mass elements are more difficult to measure accurately.

With WDXRF the fluorescence energy is dispersed into individual spectral lines using an analyser crystal and the diffracted beams focused onto a photomultiplier tube. As with ICP-AES, readings may be taken sequentially, with a number of interchangeable crystals for measurement of the full range of elements, or simultaneously in a multichannel mode usually preset for specific analytes. Detection limits for light elements (silicon and below) are 10–100 times lower than with EDXRF. Resolution is good, although less so at shorter wavelengths. Sequential instruments require long analysis times to measure several elements compared with simultaneous instruments or EDXRF technology.

For EDXRF, X-ray emission is directed into a crystal detector. Pulses of current are generated with intensities that are proportional to the energy of the X-ray photons. The energies associated with the various elements in the sample are sorted electronically. Compared with WDXRF, lower-energy sources such as a low-power X-ray tube or radioactive element (^{244}Cm, ^{241}Am or ^{109}Cd) are employed. The detector has to be maintained in a vacuum at the temperature of liquid nitrogen (77 K). While analysis times are 10–30 times longer than with WDXRF, as a truly multi-element technique, the total time required for measurement is not necessarily increased.

If a collimated beam of X-rays is directed against an optically flat surface at a shallow angle (~5 minutes of arc), total reflection will occur.

This is the principle of TXRF in which the sample is exposed to both primary and total reflected beams, and is excited to fluoresce. Emitted radiation is detected and resolved as an energy-dispersive spectrum. As there is effectively no absorption by the matrix, measurement and calibration are much simpler and sensitivities are greater than with other X-ray techniques (Klockenkämper, von Bohlen 1992).

Applications of XRF include the analysis of solid samples such as artefacts, powders, dried tissues and fluids. Instruments to determine metals *in vivo* are being developed with particular interest in lead in bone. Elements such as silver and iron have been measured in skin. Reviews of recent developments and applications are published annually (West *et al.* 2009).

Catalytic methods

The concentration of an element can be measured from its catalytic role in a reaction, the rate of which is monitored using colorimetry or fluorimetry (Nakano *et al.* 1983). The decoloration of ceric sulfate by arsenious acid, which requires iodine as catalyst, was once used to measure iodine in serum when investigating thyroid function. Most other examples of this type of reaction involve the oxidation of a substrate by hydrogen peroxide:

- $H_2O_2 + KI \rightarrow I^{3-}$; catalysed by Mo
- *o*-Dianisidine oxidation by H_2O_2; catalysed by Cr(VI)
- Acid blue 45 oxidation by H_2O_2; catalysed by Mn.

Chemiluminescence for the measurement of metals is a special example of a catalytic technique. Oxidation of luminol by hydrogen peroxide at alkaline pH is accompanied by the emission of light. Chromium, cobalt, copper, iron and manganese have all been assayed in this way (Klopf, Nieman 1983). Such methods are very sensitive and can detect a few μg/L of analyte if interference from other metals can be excluded. However, these reactions have seldom been exploited in the analysis of biological samples. They are included here to illustrate the range of techniques available to the analyst.

Neutron activation analysis

Neutron activation analysis (NAA) can be used to measure several elements simultaneously. However, because access to an atomic reactor is required, the technique is generally used only for special projects. In summary, the sample is irradiated with neutrons which interact with the target nucleus via a non-elastic collision, causing neutron capture. This produces a compound nucleus with an unstable excited state which almost instantaneously reverts to a more stable configuration. In most cases, this more stable configuration is as a radioactive nucleus, which then decays by the emission of a range of radioisotopes that can be measured using conventional β- or γ-counters, dependent on the unique half-life of the radioactive nucleus. Although characteristic emissions from individual radioisotopes impart some selectivity, there may be interferences, especially from short-lived isotopes. Therefore, the sample is left for a specific decay period and/or post activation ion-exchange chromatography is often employed to isolate the element(s) of interest.

Up to 74 elements can be measured using NAA and it provides detection limits ranging from 0.1 ng/g to 1 mg/g. Heavier elements with larger nuclei have a larger neutron capture cross-section and are more likely to be activated. The analysis is non-destructive, requiring little or no sample preparation. However, it is an expensive technique requiring special equipment and highly trained operators. In order to attain good detection limits, irradiation times of several days may be needed. although NAA is essentially non-destructive, which means that the sample is available for further investigations, the irradiated samples remain radioactive for many years, requiring careful handling and disposal.

Separation science and metals

Chromatography

Chromatography has been used occasionally as the basic technique to determine metals, but conventional detectors lack the sensitivity for this to be a useful approach except where concentrations are unusually high. Ion chromatography is more usually associated with the measurement of anions, but it is possible to measure metal ions using anion exchange columns (Pohl *et al.* 1997). Methods usually involve derivatisation with a reagent such as 4-(2-pyridylazo)resorcinol and colorimetric detection, although other detection systems may be employed including AES and ICP-MS (Heitkemper *et al.* 2001). Detection limits of 0.02–0.5 ng using a 50 μL aqueous sample have been reported.

For measurements of metals, however, chromatography is important for two other applications. Sample preparation can include passage through a column prior to introduction to an ICP-mass spectrometer to eliminate spectral interferences. For example, Cl^- ions can be separated from the sample matrix to prevent formation of $^{40}Ar^{35}Cl^+$ in the plasma, which would otherwise interfere with the reading for ^{75}As (Sheppard *et al.* 1990). Chromatographic techniques are also widely used, together with atomic spectrometric methods, for metal speciation studies (see below).

Capillary electrophoresis

Capillary electrophoresis refers to a group of related separation techniques used for molecular separation within narrow-bore fused-silica capillaries. Capillary zone electrophoresis is the technique most widely used to separate metal-containing species and the two terms are generally used synonymously. The technique is applied almost exclusively to speciation studies.

The capillary, containing an electrolyte solution, has its ends placed in vials (inlet and outlet) also filled with electrolyte solution. The vials also contain electrodes connected to a high-voltage supply (Figure 43.14). The sample solution enters the capillary as a small plug

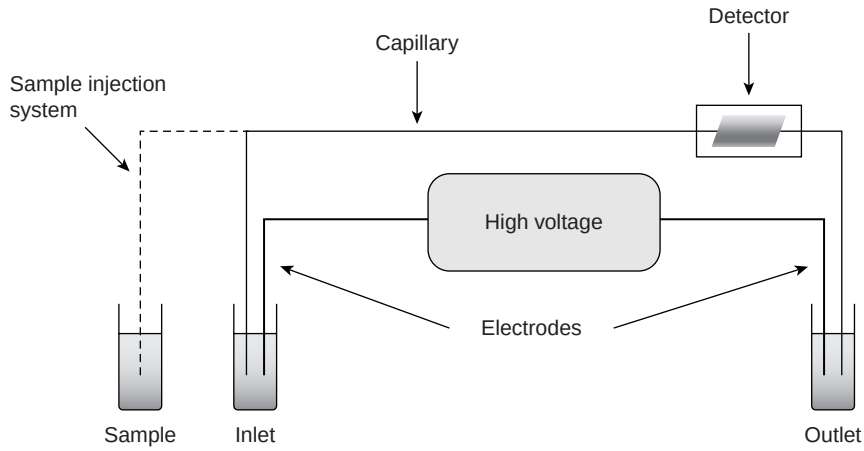

Figure 43.14 Capillary electrophoresis.

by capillary action or through application of pressure or voltage. As a high voltage (5–30 kV) is established across the capillary, zones of analyte form due to different electrophoretic mobilities (based on differences in charge, size and hydrophobicity) of ionic species and migrate toward the outlet side of the capillary. In fact, different ions can be separated when their charge/size ratio differs. For metal speciation investigations, the separated zones are fed into an appropriate detector, usually an ICP-mass spectrometer (Deng *et al.* 2008; Groessl *et al.* 2008; Nguyen, Moini 2008; Kuban *et al.* 2009)

Speciation

Knowing the total concentration of an element in a specimen is usually sufficient for most toxicological investigations, but sometimes this is inadequate or misleading. Non-toxic arsenic compounds derived from seafood may be present in blood and urine. Therefore, separation of arsenic species in a sample, to differentiate between dietary and other sources of exposure, may be vital in a poisoning incident. Similarly, separate determination of methylmercury and inorganic mercury in a sample, by selective measurement of the mercury species, will provide information about the source of exposure. Speciation analysis is also a feature of more fundamental work to investigate the metabolism of an element (Devos *et al.* 2002; Kremer *et al.* 2005) and is prominent in current literature (Taylor *et al.* 2009). Chromatography or electrophoresis is generally used for speciation prior to measurement. Detection is usually by AAS, AES, AFS or ICP-MS. Typically the separation and detection systems are linked in a tandem arrangement.

Apart from the examples for arsenic and mercury, other applications include separation of ionic species, e.g. Cr(III) and Cr(VI), investigation of metalloproteins in body fluid and cell fractions, studies of the metabolism of metal-containing drugs, and interactions between metals and nucleic acids. Identification of selenium species in yeast is a particularly topical challenge that is addressed by several research groups. All types of gas and liquid chromatography, capillary and polyacrylamide gel electrophoresis feature prominently among the techniques employed (Taylor *et al.* 2009).

Analyses involve any or all of the following steps: extraction (from tissue samples, for example), formation of a volatile derivative (for GC), separation and measurement. The extraction procedure should extract all the element in the sample and no species transformation should occur. Useful reviews of arsenic and selenium speciation are available (Francesconi, Kuehnelt 2004; Francesconi, Pannier 2004; Polatajko *et al.* 2006).

Summary

Analysts have an increasing range of techniques available for the quantitative measurement of metals in biological and other samples. Accurate measurements of a specific element, or a multi-element profile to indicate whether a metal is unexpectedly present or absent, can be provided in complex biological or other materials. For many years AAS and AES have been the most important techniques, but ICP-MS is increasingly the method of choice and it is likely that further developments will lead to greater sensitivity, analysis of smaller samples and more effective removal of interferences. XRF is proving a powerful technique, not only for powders or other solid materials; the number of applications is growing rapidly (West *et al.* 2009) and XRF could become equally in importance to ICP-MS in the future.

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CHAPTER

44 Emerging Techniques

D Rudd

Introduction

Throughout history, analytical technologies and the associated methodologies have evolved, and will continue to evolve, to fulfil unmet needs. As technological advances are made, and as user requirements become ever more demanding, so techniques and methods are refined and more widely applied to meet these expectations.

It is unusual, but not impossible, to find examples of completely new analytical technologies being discovered. The pioneering days of analytical science, in this respect at least, may be behind us. Nevertheless, these are exciting times for the pharmaceutical analyst and this chapter attempts to capture the flavour of some of the more interesting newer developments, application areas and ways of working.

At the forefront of these newer approaches lies the 'Quality by Design' philosophy that is revolutionising pharmaceutical product and process development and manufacture (FDA 2004; ICH Topic Q8 (R2) 2009) and which is discussed in some detail below. This philosophy has resulted in increasing demands and the need for innovative approaches in the field of pharmaceutical analysis. This is exemplified by, but not restricted to, movement away from laboratory-based measurements to more process-based analytical applications. The consequences of this shift in emphasis are significant and are dealt with in this chapter in a variety of ways.

The chapter has the following structure:

- The 'Quality by Design' philosophy, as applied to pharmaceutical product and process development and manufacture, is described and the analytical contribution is highlighted.
- The evolution of some existing laboratory-based analytical techniques is discussed, specifically of those (ultra-high performance liquid chromatography, ion-mobility spectrometry and solid-state nuclear magnetic resonance spectroscopy) that lend themselves to newer ways of working as required by the modern-day pharmaceutical analyst. Some detail is provided in terms of an overview of the less familiar techniques, but broadly the emphasis is placed on more recent developments and applications, and an indication of the direction in which the techniques are moving.
- Dose-form-specific developments are described using examples of tablet imaging and dissolution and the monitoring of powder and suspension inhalation products. Some recent advances in automation approaches are given to illustrate activity in this area
- A major part of this chapter is devoted to process-based measurement techniques as this is the area that is currently seeing the more significant advances and novel applications.

  Such developments generally involve much more than simple transition of the laboratory-based technique into a process environment and, as a result, a number of traditional analytical steps (sampling, sample treatment, data processing, etc.) need to be considered differently. To that end, a lengthy discourse is provided to help the practising laboratory-based analyst identify and address areas of analytical science with which they may be less familiar. This section forms the basis for a draft ASTM (American Society for Testing and Materials) standard undergoing approval by ballot at the time of writing (American Society for Testing and Materials 2010).

  Newer process-based techniques are described, and these include passive and active acoustics), a range of spectroscopic techniques and imaging approaches. This section includes a detailed discussion of process tomography techniques since they offer some interesting, and less appreciated, opportunities for the characterisation and control of dynamic fluid systems, particularly at the manufacturing scale.

  The section on process-based measurement techniques concludes with reference to light-scattering applications and thermal effusivity and an overview of data collection and interpretation techniques and how they are being developed for non-laboratory applications.
- Examples of some field-based measurement techniques are given. These systems can provide capability for rapid identification of pharmaceutical materials, and often with surprisingly good quantitative performance.
- The dramatic emergence of rapid microbiological techniques is discussed; these, possibly more than any other group of analytical technologies, reflect the way in which demands for greater speed of analysis, quality of data and capability, etc. may be met.
- Finally, the growing importance of biopharmaceuticals and the associated need for analytical methods for their characterisation and quantification are addressed. Many major pharmaceutical companies expect biopharmaceutical products to occupy a significant portion of the new product portfolio in the next few years (GlaxoSmithKline 2010). In that event, some of the technologies described are likely to become particularly important.

It is hoped that the format and scope of this chapter will offer a useful, if necessarily limited, insight into a number of interesting and emerging analytical areas. Not all of these, of course, necessarily offer the advantages that are claimed by the main protagonists, and it will be interesting to see exactly which techniques realise their potential and which fall by the wayside.

Inevitably in this chapter, reference is made to a number of existing commercial analytical systems and suppliers. Generally, this should be recognised as a means to illustrate a specific approach or new development rather than an endorsement of that particular supplier, although the examples are chosen, of course, because of their suitability.

'Quality by Design' in the pharmaceutical industry

Traditional pharmaceutical development and manufacture is capable of producing high-quality, safe and efficacious medicines for the patient based on well-defined and well-controlled manufacturing processes. The quality of the finished product is generally established using laboratory-based analytical testing that demonstrates compliance of the batch from which laboratory samples are taken and tested against a predetermined quality specification.

While it is fair to say that this approach has been used successfully for many years, there are a number of perceived shortcomings that can result in incorrect assessment of product quality, potential risk to the patient and/or waste of expensive commercial material. For example, issues with analytical methodology, non-representative sampling schemes, inhomogeneity of material, etc. may all result in an erroneous assessment of the overall batch quality. In addition, minor deviations in the manufacturing process (either deliberate or adventitious) may result in change to some finished-product quality attributes and these may go undetected during the laboratory analytical testing, or may be detected and assigned an inappropriate level of significance.

These potential scenarios result in greater dependence being placed on the outcome of the laboratory analytical testing to demonstrate the

overall quality of the product whereas, in an ideal world, such testing would simply be confirmatory rather than pivotal in terms of establishing batch quality.

In order to overcome some of the shortcomings that have been highlighted, the pharmaceutical industry is now becoming increasingly aware of the importance of incorporating 'Quality by Design' principles into its product and process development activities (FDA 2004; ICH Topic Q8 (R2) 2009). Broadly, this means that greater emphasis is placed on the incorporation of sound scientific and engineering principles into product and process development such that robust, fit-for-purpose and well-understood processes are established and operated throughout the life cycle of the product.

The 'Quality by Design' approach generally embraces four distinct stages, reflecting the sequence of events that needs to be followed in order to develop and maintain product and process quality. Various terms are used by different organisations for each of these stages but, in general, the following represents a summary of the scope and objectives of each stage:

Design intent

Design intent effectively defines the required performance of the product and process, including final commercial requirements, and will be based on input from various customer sources. The key areas of performance are patient safety and efficacy. However, the design intent will also address the need for appropriate formulation and manufacturing technologies to ensure that the product is chemically and physically stable, and that it is manufactured using a robust process.

Design selection

The design selection stage involves the selection of the formulation, manufacturing unit operations, device, packaging and analytical methods for the commercial product that meet the design intent. A key output of design selection is an understanding of how the input materials, manufacturing operations and packaging design impact the quality of the final product.

Control definition

Control definition identifies the ways in which the quality of the final product may be achieved. The attributes and parameters that contribute to variability in quality will be established and appropriate controls defined.

Control verification

Control verification is the final stage of a 'Quality by Design' approach and allows continued verification of the suitability of the defined control strategy throughout the life cycle of the product and process. This usually takes the form of data trending activities to ensure that process parameters, operating ranges, predictive models, etc. are all consistently providing material of suitable quality or, if this is not the case, to offer refinement of the control strategy such that quality requirements continue to be met.

This approach provides a number of key advantages to the patient and to the manufacturer of the product:

- Product quality and performance are ensured through the design of effective and efficient manufacturing processes.
- Product and process specifications are based on a mechanistic understanding of how formulation and process factors affect product quality and performance.
- Continuous real-time quality assurance can be achieved and demonstrated.
- Process control strategies may be applied to ensure that deliberate or adventitious excursions in process operating conditions do not necessarily result in batch failures or production of sub-optimal material.

It is evident from this brief summary of the key advantages of a 'Quality by Design' approach that the analytical component is an integral part of successful implementation. For example, during development, analytical technologies will be required to help identify the relationships between formulation and process factors (the so-called 'critical process parameters' (ICH Topic Q8 (R2) 2009)) and the key indicators of product quality and performance (the 'critical quality attributes' (ICH Topic Q8 (R2) 2009)). Such analytical technologies (or, more exactly, the methodologies associated with the techniques) may need to be relatively sophisticated and, as such, may not be ideally suited for routine application throughout the life cycle of the product. However, this is not too problematic, as such relatively complex analytical technologies may be used during product and process development to establish a suitable degree of process understanding (that is, the identification of the relationships between critical process parameters and critical quality attributes), with simpler methodology being employed for subsequent routine monitoring.

Continuous real-time quality assurance depends on a more targeted application of a number of measurement and control technologies, including the use of robust and reproducible analytical methodology. Such applications are likely to be in-process (at-line, on-line or in-line), although it is possible to provide continuous real-time quality assurance using laboratory-based (off-line) measurement technologies. The important factor is that the overall analytical cycle time (that is, the interval between taking a sample and being able to react to the measurement made on that sample) should be short relative to the time scale of the process in order to allow real-time quality assurance to be achieved.

As indicated earlier, analytical methodology to support routine monitoring throughout the life cycle of the product needs to be robust, reproducible and well established, and these requirements dictate strongly the suitability of some emerging technologies. This will be considered later in this chapter.

To summarise this section, it is apparent that the pharmaceutical industry is currently embracing the incorporation of 'Quality by Design' principles into its product and process development activities in order to ensure improved product quality and increased efficiency of manufacture. The application of analytical technologies is an integral part of this 'Quality by Design' philosophy, with different technical requirements being identified during product and process development compared with routine manufacture. As a result, some analytical technologies lend themselves more easily to these different application areas, and this distinction must be borne in mind at all times when emerging techniques are being considered.

Laboratory-based analytical techniques

Some of the newer developments in existing laboratory-based analytical techniques are discussed, including the ways in which some of those techniques are evolving and being applied. Adoption of 'Quality by Design' principles to the analytical method itself, rather than the use of the method to aid application of such principles to pharmaceutical product and process development and manufacture, is also introduced (Borman *et al.* 2007).

'Quality by Design' for analytical methods

As discussed above, the pharmaceutical industry is beginning to incorporate 'Quality by Design' principles into the development and routine manufacture of drug substances and drug products. This is based on the need to deliver more robust manufacturing processes and to facilitate continuous improvement through more flexible regulatory approaches. In a similar vein, the application of 'Quality by Design' concepts to analytical methods has recently gained impetus in order to improve their overall robustness, transferability and potential for continuous improvement.

As described previously, the application of 'Quality by Design' principles begins with a set of predefined objectives (design intent). For a pharmaceutical product and manufacturing process, this will be defined as a set of 'critical quality attributes' (ICH Topic Q8 (R2) 2009) that describe the characteristics and limits with which the product must comply in order to be safe and efficacious. For an analytical method, the equivalent may be considered as the 'analytical target profile', which is the combination of all the desired method performance characteristics (e.g. precision, sensitivity, accuracy) and associated criteria such that it is fit for the intended purpose.

The definition of the 'analytical target profile' is based on the critical quality attributes requiring measurement in support of the process control strategy. For a quantitative test, the key requirement is that the method has adequate accuracy and precision over the range of intended use in the presence of any potential interferences. Determination of targets for precision needs to be based on the specification limits associated with that test and to ensure that the method contribution to the overall process capability is minimised.

Once the method performance requirements are understood and documented, appropriate analytical technique and method conditions may be chosen to achieve the desired performance (design selection). During this phase, information such as prior experience with similar measurements or matrices, regional limitations, availability of reagents or specific technologies in laboratories conducting the testing, and/or cycle time requirements to support process operations can be used to ensure that the method will both meet the predefined 'analytical target profile' and be operationally efficient in routine use.

The next step in the 'Quality by Design' process is to develop an understanding of the method (where the term 'understanding' is used to refer to an appreciation of the relationship between method inputs and the desired performance characteristics). Here a systematic approach needs to be adopted in which the various steps involved in the method (sampling, sample preparation, measurement, data interpretation, etc.) are defined using process mapping tools and then method factors that may influence the overall performance are identified through use of tools such as Ishikawa (Fishbone) diagrams (Tague 2004).

The method design phase then continues by evaluation of the impact of each factor on the desired method performance. Initially this involves assessment of the factors that are considered most likely to be important and, once these are identified, experimentation to understand which of these factors significantly influence performance and, in the case of continuous variables, the associated ranges that continue to provide acceptable data. Typically, experimental design approaches are used for resource efficiency and to understand any multivariate relationships that exist. For non-continuous factors (operator, batch of column packing, equipment model, etc.), it is not possible to explore fully the range of variations that will produce acceptable results. However, if considered potentially important, such factors should also be examined to determine, from a limited sample, whether specific control is required. Techniques such as Measurement System Analysis (Automotive Industry Action Group 2010) can also be used to understand the influence of such variables.

This systematic approach will generally produce a set of method operating instructions that provides a high probability that the method will operate robustly in routine use (control definition). Moreover, evidence of compliance with these method operating instructions will prove highly useful in any trouble-shooting exercises in the event that unexpected results are obtained.

It is also important to capture any understanding generated during this systematic approach and to maintain and update this knowledge as further experience is gained during routine use. This may identify additional or modified control requirements, particularly where automated data capture systems allow identified variables to be monitored (control verification).

In summary, the 'Quality by Design' approach, as applied to analytical methods, starts with predefined method performance objectives, followed by experimentation that provides an understanding of the relationship between method inputs and performance characteristics. In turn, this defines the required control strategy, application of which ensures that the expected analytical method performance is achieved throughout the life cycle on the basis of continued monitoring and refinement during routine usage.

General developments in the key laboratory-based analytical techniques

Ultra-high performance liquid chromatography

High performance liquid chromatographic (HPLC) techniques have been the mainstay of the pharmaceutical analytical laboratory for several decades. This reflects the ability of the technique to resolve drug molecules and associated impurities from the complex excipient mixtures introduced during product formulation and from the biological matrices that are encountered in drug metabolism studies. It is a curiosity to many, however, that a technique that has such wide utilisation in routine laboratory analysis and screening finds little application in the process measurement environment. There are examples of process-based HPLC applications, of course, but, relative to the level of use as a laboratory technique, these are limited in number. This is primarily due to the lack of robustness of HPLC equipment when used in manufacturing environments, coupled with the relatively long analysis times and sample preparation required.

Progress in laboratory-based liquid chromatographic technology and capability has been steady rather than spectacular over the last 30 years, with significant advances being made in equipment design and reliability, but more particularly in terms of stationary-phase performance. In more recent years, the development of column packing materials of progressively smaller particle size (typically below 2.5 μm) has allowed high-efficiency separations to be achieved without an associated increase in analysis times. This new development in liquid chromatography (ultra-high performance liquid chromatography or UPLC) has now found wide application in many routine pharmaceutical analytical laboratories, especially where sample numbers are very high or where rapid throughput is required.

More recently, practitioners of UPLC have begun to identify other advantages that rapid, highly efficient separation capability might provide. On-line application is now regarded more favourably compared with traditional HPLC in that real-time information can easily be obtained, and this lends itself to applications such as chemical reaction monitoring (Cormier, Jenkins 2010), *in situ* cleaning verification (Fekete *et al.* 2009) and preparative scale-up monitoring (Boughtflower 2008).

Developments in equipment continue to be necessary in order to keep pace with the evolution of column packing materials and the requirements that their improved performance demands. Rapid analysis times are driving improvements in sample injection technology and liquid-phase delivery systems, while detector performance (especially in terms of sampling frequency and resolution) remains crucial if optimised applications are required. Against this backdrop of continuous improvement in performance, it is important to remember that equipment robustness is equally critical if successful process-based applications are to be achieved. It may be that the emergence of packing materials of smaller particle size, coupled with the associated improvements in equipment, finally allows liquid chromatographic techniques to bridge the gap between well-established routine laboratory usage and the more demanding (in terms of analysis time and equipment robustness) process-based applications.

Ion-mobility spectrometry

Ion-mobility spectrometry (IMS) is a type of separation technique, similar to time-of-flight mass spectrometry (TOF-MS), that distinguishes ions of a given compound on the basis of their velocities through a drift tube under the influence of a weak electric field. The technique characterises chemical substances on the basis of their gas-phase ion mobilities and provides detection and quantification of trace analytes.

IMS analysis is carried out by injecting a sample solution onto a substrate and then allowing it to dry. The substrate is then heated, and this results in desorption or vaporisation of the sample into an inlet tube. Primary ion formation occurs through atmospheric pressure chemical ionisation (APCI) and, following many collisions, product ions are formed and gated into the drift tube. These ions travel through the drift tube at different velocities based on their size, shape and charge. In contrast to mass spectrometry, separation of ions is based on a size/charge relationship rather than the mass/charge (m/z) ratio.

IMS is usually subdivided into three distinct techniques. Typically, IMS refers to low-field (e.g. 100 V/cm) linear measurements. The technique is linear in that the ions travel at a constant velocity through a linearly decreasing field. Differential mobility spectrometry (DMS) and field asymmetric waveform IMS (FAIMS) are both high-field (e.g. 10 kV/cm or greater), non-linear ion measurements and involve field gradients across the drift tube and/or different instrument configurations to provide increased speed and flexibility or higher resolution.

IMS has been used for direct formulation analysis (O'Donnell *et al.* 2008), particularly for product authentication purposes, but is more usefully applied for cleaning verification. In addition, portable IMS devices are finding wide application in the area of health and safety as well as from a security perspective (see below).

The use of IMS instruments for cleaning verification of manufacturing equipment offers several advantages over methods currently used by pharmaceutical manufacturers. The low levels of quantification for drug substances and contaminants using IMS are generally in the range of 0.4 mg to 10 ng, making the technique comparable to HPLC and TOC (total organic carbon) methods. However, the increased specificity associated with IMS helps to avoid some of the 'false positives' that TOC and HPLC can indicate. Cycle times are reduced as a consequence of the speed of analysis and instrument set-up compared with HPLC, while the elimination of solvents and of column packing materials are added advantages.

Solid-state and quantitative nuclear magnetic resonance spectroscopy

Solid-state NMR (SSNMR) has historically been used in the pharmaceutical industry as a 'fingerprint' technique, supporting vibrational spectroscopy and powder X-ray diffraction techniques. However, the role of SSNMR has grown beyond this to an information-rich technique that can address unique solid-state challenges and serve as a first-line technique in many roles (Vogt 2010b). These changes have been driven by developments in hardware and pulse sequences, which now allow access to a range of nuclei of interest in pharmaceuticals using commercial instrumentation. Some of these nuclei, such as ^{1}H and ^{19}F, allow SSNMR to obtain (simultaneously) high sensitivity and specificity, opening up a range of applications to low-dose products (e.g. low-level polymorph detection), which are not possible with other techniques (Katrincic *et al.* 2009). New multidimensional SSNMR methods allow the extraction of detailed information about molecular structure and association in both crystalline and amorphous materials, leading to applications to co-crystals (Vogt *et al.* 2009) and amorphous dispersions (Pham *et al.* 2010). SSNMR methods have recently been used in combination with X-ray diffraction to determine the crystal structure of a pharmaceutical from a powder sample (Vogt *et al.* 2008), while the ability to probe subtle phenomena, such as dynamic motion in solids, static disorder in crystal structures and crystal defects, is also established (Vogt 2010a).

Looking further ahead, it is likely that SSNMR will be applied more extensively to the characterisation of formulations containing low levels of drug and structural studies on drug delivery systems. Technology and performance improvements should also facilitate investigation of biopharmaceutical solids, for example, where biomolecule–excipient interactions need to be better understood to ensure product performance and efficacy. However, the role of SSNMR has grown beyond this to an information-rich technique that can address unique solid-state challenges and serve as a first-line technique in many roles (Vogt 2010b).

Dose-form-specific developments

Magnetic resonance imaging for solid dose forms

Three-dimensional imaging of solid dose forms, especially those where controlled or modified delivery of the drug from the matrix is intended, can provide a valuable insight for the formulation development scientist. An understanding of hydration mechanisms and the modes of moisture ingress, for example, may allow better formulation development and optimisation of drug release performance for complex solid dose forms.

Historically, tablet imaging has been achieved by mapping physical and/or chemical properties of the intact dose form, generally using spectroscopic techniques and, while such an approach can provide useful information concerning drug and excipient distribution within the tablet matrix, destructive sampling may be involved. In addition, such applications are usually limited to dose forms in their dry state, making it difficult to investigate dynamic hydration mechanisms, for example, under conditions similar to those experienced during use by patients.

Magnetic resonance imaging (MRI) has been used successfully for several years to study hydration mechanisms and drug release from pharmaceutical products (Richardson *et al.* 2005), but applications have been limited by high equipment running costs and long experimental run times. However, such disadvantages are now being overcome with the use of low-field MRI systems that incorporate compact permanent magnet technology (see www.oxford-instruments.com 2010), allowing non-invasive and non-destructive imaging of whole tablets during the dissolution process.

Broadly, the MRI technique provides information on water (and lipid, where present) proton density (M_0) as well as mobility (generally described by the spin–lattice relaxation time, T_1, and the spin–spin relaxation time, T_2). The fact that different materials in a given matrix exhibit different values of these density and mobility parameters allows discrimination during measurement such that mapping of key components becomes possible despite the presence of other, potentially interfering species. As an example, MRI has been used to compare the degree of hydration of a gel matrix formulation (chlorpheniramine maleate tablets) with the corresponding drug release profile under traditional pharmacopoeial testing conditions (Nott 2008). More recently, some immediate-release and modified-release formulations have been studied using the technique in order to understand more fully the mechanisms whereby drug is released (Butler, Nott 2010).

These examples indicate that the MRI approach could be used not only for formulation development and optimisation, but also as a potential real-time release technique for tablet dissolution studies. In addition, MRI could offer an insight into the extent of water binding during granulation and/or drying processes, for example, thus providing an improved level of understanding for these manufacturing steps.

Next-generation dissolution testing

Laboratory-based dissolution testing continues to be the main technique for both development and routine assessment of drug release from oral solid dose products. However, it is recognised that opportunities exist for improvements in reliability, capability and efficiency, even allowing for the advances made in the field of automation of dissolution testing. As a consequence, a number of major pharmaceutical companies are collaborating with key vendors in an attempt to influence the design and promote the development of a 'next-generation' automated dissolution system that will meet the identified current and future needs.

At present, two different approaches, each with their own advantages and disadvantages, are being pursued within this collaboration. The first is the RoboDis system developed by ERWEKA in collaboration with Bayer Schering Pharma (see www.erweka.com 2010) and is based on a centrally configured Stäubli industrial robot that sequentially services all dissolution vessels. Currently this system employs fibreoptic technology for in-situ ultra violet (UV) measurement with a Zeiss spectrometer and Helma probe. The RoboDis system is well engineered and provides operation for up to 12 dissolution vessels, with video monitoring integrated into the control software. However, it lacks some flexibility, in that no media addition or exchange is currently possible and cycle times for sample analysis are fairly lengthy. On-line HPLC analysis of samples is also yet to be addressed.

The second approach features the SOTAX AT-70 (see www.sotax.com 2010), which has dedicated pumps, valves and tubing to service each of the six dissolution vessels separately. On-line UV and HPLC measurement, as well as media addition and exchange, is supported. Sample analysis times are fairly rapid, but there are large dead volumes associated with the complex network of tubing and valves, etc.

It is interesting to note that these current developments largely centre on the automation and general improvement of existing dissolution technology. While it is recognised that dissolution testing in its current form is an important element in the quality assessment of oral solid dose products from a regulatory perspective, it is nevertheless surprising that radically different approaches are not being considered for drug release monitoring. Perhaps the task of demonstrating drug release from oral solid dose products based, for example, on tablet imaging technology and/or assessment of the mechanical properties of the tablet would

Figure 44.1 Xelair series automated inhalation product testing system. (Courtesy of Astech Projects Ltd; www.astechprojects.co.uk.)

prove too problematic – in which case developments in this area are likely to remain limited to those of the type described above.

Dose content uniformity of the emitted dose for inhalation products

Reliable assessment of the dose emitted by metered-dose inhalers (MDIs) and dry powder inhalation products (DPIs) has always been an important aspect of the performance and quality assurance of such dose forms. Regulatory emphasis has increased in this area in recent years, making the need for reproducible and accurate measurement of emitted doses particularly important as a means of ensuring that patients receive the correct dose on a consistent basis.

To this end, the more recent developments in analytical technologies used to determine emitted doses have concentrated on reducing the variability and increasing the volume of data produced. As the regulatory requirements for testing the dose content are well defined (FDA 1998, 2002), technology developments have centred on improved control of product preparation (including shaking regimes for MDIs), actuation, dose collection and waste firing. It is considered likely that this emphasis on the automation part of the testing will be maintained in the future, rather than the introduction of innovative approaches for the determination of the emitted dose itself.

Available commercial systems for emitted dose testing generally reflect the manual process – that is, the priming of the actuation system of the inhalation product, the collection of doses, the firing of doses to waste, the collection of subsequent doses, the analytical manipulation of the collected doses, washing, etc. – but using a heavily automated approach for some or all of these steps. Examples include the Xelair range (see www.astechprojects.co.uk 2010) and the Process Master range (see www.rtslifescience.com 2010), both of which address a number of issues encountered with manual testing of inhalation products – namely, variability in data, productivity and safety aspects – and which also offer a more integrated approach across dose collection, sample treatment and analytical assessment.

Interestingly, systems such as the Xelair (illustrated in Figure 44.1) and Process Master (Figure 44.2) ranges can provide different levels of capability and complexity, allowing applications that extend from simple automation of individual stages of emitted dose testing through to fully automated, completely unattended operation involving large numbers of samples within distinctly different batch sets.

Further refinements are likely to be made with these systems, particularly in terms of the shaking and firing regimes (which seem to be a significant source of variability during manual testing), although the challenge may be one of replicating the most representative manual approach so that any large historical body of data on a given product is not immediately lost on introduction of an automated alternative.

Developments in automation

Advances in automation of analytical technologies continue to be made, although still along fairly traditional lines. The perceived advantages of automation (ability to deal with large numbers of samples; improved quality of data generated; productivity and efficiency; environmental and

Figure 44.2 Process Master automated inhalation product testing system. (Courtesy of RTS Life Science; www.rtslifescience.com.)

safety considerations, etc.) mean that developments generally centre on improvements in these areas, and there are several examples of these types of advances, some of which have been discussed earlier under the dose-form-specific developments (see www.astechprojects.co.uk 2010; www.erweka.com 2010; www.rtslifescience.com 2010; www.sotax.com 2010).

More ambitious advances in automation are less commonplace, but some of the potentially more interesting are discussed here.

Development of an automated generic extraction technology

Traditionally, laboratory automation is generally developed and applied to late-stage, established pharmaceutical products – and, in particular, to routine, high-volume and/or high-throughput testing. This makes sense, in that large numbers of samples are generally produced only at later stages of product development and beyond, allowing the heavy validation burden of any automated technology to be justified.

However, there is an advantage in being able to apply automated approaches to earlier development samples, especially those where the final details of the product type (dose, formulation, pack, etc.) are not fully established. The development of a generic, automated sample treatment stage for application to such product variants therefore has merit as a precursor to a number of different analytical measurement steps.

Recent industry/vendor collaborations have seen the development of such a generic extraction technology based first on the use of acoustic energy to form a localised, high-velocity jet of solute to provide an effective extraction process (see www.covarisinc.com 2010), and second on the use of a commercialised lyophilisation system (Faulkes 2008). Results have shown improved extraction times and extraction efficiencies for early stage formulations and the potential of the systems to deal with diverse formulation types and sub-optimal products (unusually hard tablets or dose forms with high moisture content, and so on).

Automated method development

The incorporation of 'Quality by Design' principles for the automated development of liquid chromatography methods, in particular, has received some attention in recent years. This represents one of the more intriguing aspects of automation development and opens up the more ambitious idea of using automated approaches for the development of pharmaceutical products and manufacturing processes.

At this stage, however, the application of automated 'Quality by Design' is restricted to liquid chromatography methods, one example of which is based on the integration of commercial software from S-Matrix with UPLC technology from Waters (see www.waters.com 2010). It will be interesting to see whether this approach represents the first step towards intelligent, automated development of pharmaceutical products and manufacturing processes, or a short-lived marriage of related, but non-enhancing technologies.

Laboratory on a chip

This much-heralded field of automation and miniaturisation continues to offer advances and, with such a significant level of support and underpinning research effort, seems destined to occupy a key position for the foreseeable future. Environmental considerations have only served to underline the advantages that development in microfluidics, for example, can bring to laboratory on a chip applications and a recent point-of-care disease diagnostic application (Gervais, Delamarche 2009) illustrates how complex analyses may be conducted with these devices. The successful incorporation of pumps, valves and reaction chambers into a robust miniature device, which requires very small sample volumes and yet provides high sensitivity and versatility, demonstrates how this approach can provide point-of-care, field-based or in-process automated analytical capability.

Process-based measurement techniques

Process measurement technologies represent the heart of successful monitoring and control. This section provides an overview of the key measurement techniques, many of which are now being used on a fairly regular basis within the pharmaceutical sector. The areas of development and applicability are also described. In addition, the important aspects of sampling and data processing are discussed in view of the differences arising in comparison with laboratory-based techniques.

Sampling aspects are part of a range of analytical performance topics that need to be considered for process-based measurement techniques, and the following section provides guidance for addressing some of these issues. Note that the content of this section is based on a draft standard undergoing approval by ballot within the ASTM at the time of writing (American Society for Testing and Materials 2010).

Performance assessment for process-based measurement techniques, including sampling considerations

With any process-based measurement system, before any meaningful conclusions can be drawn from the data obtained, it is important to recognise that various factors may affect instrument performance and/or data quality and that these factors need to be well understood and controlled, where necessary.

Generally, such factors may be classified as follows:

- Instrument configuration and performance qualification (PQ)
- Factors affecting measurement
- Influence of effective sample size.

Within each of these categories, there are a number of technique-specific and application-specific factors that will contribute (often adversely) to the quality of the measurement data obtained. It is important, therefore, to identify the technique-specific and application-specific factors that need to be considered to ensure that high-quality data are obtained. In this way, the user can be assured that meaningful conclusions can be drawn about the process or system under investigation, rather than having such conclusions clouded by excessive variability or lack of sensitivity, for example in the measurement system being used.

Instrument configuration and performance qualification

It is important to establish the basic operating configuration of the instrument and expected performance standards. This is likely to have been carried out during method development and, to some extent, will be independent of the intended application (although some applications will necessitate specific instrumental operating configurations). For example, if a spectroscopic measurement technique allows the averaging of multiple spectral measurements to improve the signal-to-noise ratio (SNR), the user can decide how many spectra will be averaged. Too many and the overall measurement cycle time may become excessively long, while too few will result in minimal improvement in the SNR. The final decision on the number of spectra to be averaged may, therefore, be a compromise between maximum measurement cycle time and minimum acceptable SNR – and these become critical parameters to consider as aspects of PQ before the measurement system is used in the chosen application.

Generally, all aspects of instrument configuration that could potentially affect the instrument performance (and also affect the quality of the data produced) are considered and a default operating configuration is established based on an expected level of instrument performance (e.g. maximum relative standard deviation (RSD) of not greater than 2% on 10 replicate measurements of a standard system or sample, minimum SNR of 10, minimum response of measurement to change in amount of analyte of 0.2 mV/mg, and so on). Naturally, it becomes possible to move away from any default operating configuration if the specific application requires a modified approach (e.g. an improved SNR is required, whereas extended measurement cycle times are acceptable), but this would be reflected in similarly modified performance expectations.

Broadly, during the PQ exercise, the expected level of instrument performance is established (and documented) and a default set of instrumental operating parameters is identified which is capable of providing this level of performance. Idealised test systems or reference materials may be used for basic PQ work – that is, a system or process that itself can be considered consistent and/or well characterised. In this way, the user can be sure that any examples of failure to meet the predefined levels of instrument performance are truly attributable to the measurement system and not to the process or material under investigation.

Factors affecting measurement

Once satisfactory instrumental performance has been established, it becomes possible to evaluate systematically those factors (many of which are application specific) that might also affect the quality of the measurement.

Many process-based measurement techniques (spectroscopic, acoustic, thermal, etc.) are often configured to provide relatively simplistic data outputs (spectroscopic absorbance, average acoustic signal level, average thermal effusivity, etc.). While this approach has some advantages in terms of ease of comprehension of data, it must be recognised that meaningful interpretation of such data relies heavily on the validity of the underlying assumptions being made.

Univariate data output of this type (single measurement values being displayed against time, for example), based as it is on contributions from a number of key variables (analyte concentration and particle size, etc. in the case of spectroscopic absorbance; granule compressibility, granule size and flow properties in the case of acoustic signal level; average analyte effusivity, influence of air and water, etc. in the case of thermal effusivity), is useful only when the influence of particular variables can be isolated. The underlying assumption is that the influences of all variables other than the one of interest are consistent, so that variations in the univariate data output can be attributed (perhaps correlated) with the one key variable of interest.

In order to draw such conclusions, it becomes necessary to confirm the validity of these underlying assumptions – and, if this has not been done or if this cannot be done, the reliance on over-simplified univariate data output must be seriously questioned. It then becomes necessary to interrogate the unprocessed measurement data using more sophisticated data processing techniques in order to de-convolute the respective influences of multiple key variables. This is relatively easy to do for many process-based measurement techniques but may prove to be difficult for others, rendering the specificity (or discriminating ability) of such techniques questionable.

In short, where measurements are made that are known to be influenced by a number of key variables, it is important to understand the contribution to the measurement signal from each of these variables (especially in terms of consistency or otherwise), before conclusions can be drawn about the relationship between the measurement signal and the key variable of interest.

For any given measurement technique, the factors affecting measurement will be different, but there are a number of considerations that occur across the range of most common process-based measurement techniques.

Sensitivity or response of sensor Whether this takes the form of light throughput in a fibreoptic probe, frequency response of an acoustic sensor or speed of response of a thermocouple, for example, sensor performance, reliability and consistency are important. Failure to meet predetermined levels of performance may be indicative of poor fabrication, deterioration, poor installation and/or alignment, fouling or poisoning.

In addition, a poor choice of measurement technique or sensors could give rise to an inadequate level of specificity (discriminating ability) – and this is a common cause of poor SNRs.

Interfacing of the measurement system with the process under investigation During method development, basic application-specific measurement parameters such as number of sensors, position of sensors and interfacing of sensors are established. For example, several spectroscopic sensors may be needed if suspension homogeneity is being monitored, whereas a single (far-reaching) acoustic sensor may be sufficient to determine granulation end point.

The method of interfacing (mechanical fixing, insertion, chemical coupling, etc.) may also be critical, and this needs to be defined sufficiently well within the documented process analytical method that the interfacing can be reproduced – and demonstrated to be satisfactory – from one piece of equipment to another, and possibly across different geographical locations and over a wide time interval to allow comparison of data generated under different conditions.

Influence of system or process under investigation Most process-based measurement applications involve the monitoring of dynamic systems. This means that the system or process under investigation is itself changing during the measurement cycle. Of course, some of these changes are the very thing that the analyst is interested in monitoring (e.g. analyte homogeneity during a powder blending process). However, other aspects of the system or process may also be changing (degree of compaction of powder systems, level of moisture, particle size, etc.) and, if these factors contribute significantly to the overall measurement signal – and yet are not the primary factors that the user is interested in monitoring – once again, erroneous conclusions will be drawn from the data obtained if the variations in these 'secondary' factors are not considered. This is why many process-based measurement applications depend critically on the ability to monitor systems and processes simultaneously using a combination of diverse measurement techniques, so that the influence of changes in 'secondary' factors can be understood. There is probably no measurement technique that is universally sensitive to the influence of all potential factors, making it impossible to understand the contributions of each factor from one measurement technique alone.

Influence of external (environmental) factors Traditionally, the performance of laboratory-based measurement techniques is generally unaffected by environmental factors. While such instruments will usually have an environmental operating specification (such as 'do not operate at temperatures above 50°C'), such constraints are seldom a problem in a well-ventilated, air-conditioned modern laboratory. However, the process-based measurement is likely to be made in a relatively harsh process environment, where significant variations in environmental factors such as temperature, humidity, noise levels (audio and ultrasonic), etc., can confound the measurement. Data quality can be assured only when such influences are controlled, minimised and/or understood.

Effective sample size

On-line and in-line process measurement applications do not involve physical sampling of the system or process. Measurements are generally made using sensors or probes in contact with, or inserted into, the system or process – or, in many cases, using non-contact 'interrogation' of the system or process using beams of radiation focused though observation windows fabricated into the process equipment.

However, even though there may be no physical removal of samples from the system or process, all such process measurement techniques are effectively sampling from the bulk of the material under investigation. This is based on the fact that such techniques have a limited field of view or operation, will penetrate a system or process to a finite depth, and can only make measurements at a defined rate.

To illustrate this point, consider a spectroscopic technique that produces data based on an illumination area of about 4 mm diameter and that penetrates to a depth of about 1 mm. This equates to an effective sample volume (assuming a cylindrical shape) of about 13 mm^3 and, provided that the average density of the system under investigation is known (say, about 0.4 g/cm), allows calculation of the effective sample mass that is being monitored during each measurement (in this example, about 5 mg). Combining this with the frequency of measurement (say, a scan once a second, with about 30 scans being averaged) means that an effective sample size of about 150 mg is being investigated. Clearly this level of scrutiny would be acceptable for a powder blending application where blend homogeneity at a scale approximating to the nominal tablet unit dose is being pursued.

As a general principle, the effective sample size can be calculated (or, at least, estimated) on the basis of the contributions from the factors discussed, that is:

- volume of the system or process being monitored (this, in turn, may need to be estimated on the basis of the area of examination and depth of penetration)
- density of the system or process being monitored; this may change during the measurement cycle, so an average value or estimate may be needed
- frequency of measurement: where multiple measurements are averaged, or the output from several sensors is combined, these factors should also be included.

The required effective sample size is specific to the particular application. For example, a powder blending application involving blend homogeneity determination needs to be conducted at a scale that is comparable to the nominal tablet unit dose level (e.g. 150–750 mg typically), whereas monitoring a tablet granulation process using acoustic emission at this scale would be wholly inappropriate – a much greater effective sample size (perhaps the whole batch) being required (meaning that multiple or continuous measurement may be necessary).

Acoustic techniques

While much of analytical science is generally concerned with the study of optical spectroscopy, the equivalent use of sound has so far received less attention. In a similar vein to electromagnetic radiation, sound energy may be emitted by physical and chemical processes, so some similarities exist between the study of optics and acoustics.

The analytical aspects of sound can be divided in to two main areas – active acoustics and passive acoustics (acoustic emission). In active acoustics, an acoustic wave is launched into the sample (material) of interest and the physical properties of the sample affect the velocity and attenuation of this acoustic wave. The velocity is a function of the density and compressibility of the material, while the attenuation also depends on additional viscoelastic and thermal properties.

Acoustic emission monitoring refers to the technique of detecting and analysing sound produced by a process or system. Often these sounds are well above the frequencies that can be detected by the human ear (frequencies up to ~15 kHz are audible). Processes that give rise to acoustic emission include boiling, gas evolution, mixing, grinding and fluidisation. Acoustic emissions produced by chemical reactions can reach frequencies of 1 MHz or greater (Wentzell, Wade 1989) and such emissions are usually monitored using piezoelectric transducers (Figure 44.3) responding in the region between 50 and 450 kHz. At these frequencies, attenuation is high and sound propagates only relatively short distances. This has the advantage that processes can be monitored with little interference from extraneous noise.

Acoustic emission

Acoustic emission is well known in the study of fracture mechanics and is used extensively by materials scientists. A large amount of research has been conducted on the use of acoustic emission for the routine inspection of aircraft wings, pressure vessels, load-bearing structures and components. Acoustic emission is also used in the engineering industry for the monitoring of machine tool wear.

Several workers (Betteridge *et al.* 1981; Belchamber *et al.* 1986; Wentzell, Wade 1989) have reported the use of acoustic emission in the study of chemical reactions. This includes the acoustic emission produced by effervescent reactions and that generated by crystalline phase changes. In the area of pharmaceuticals, it has been shown that acoustic emission is capable of monitoring the blending of powder materials (Tily *et al.* 1987; Holroyd 1989), that it is useful for the monitoring and end-point determination of a high-shear granulation process (Whitaker *et al.* 2000) and that it can be used to monitor chemical systems that may be difficult to observe by other means (Betteridge *et al.* 1981).

In most process applications of acoustic emission, there are many sources of sound, each producing short bursts of energy. This means that the detected signal, for instance on the wall of a vessel, is a complex mixture of many overlapping waveforms resulting from many sources and many propagation modes.

At interfaces, depending on the relative acoustic impedance of the two materials, much of the energy is reflected back towards the source. In a fluidised bed, for instance, acoustic emissions will be detected only from particles directly impacting on the walls of the bed close to the transducer.

The simplest way of studying the acoustic data is to examine changes in the 'average signal level' (ASL), an example of which is presented in Figure 44.4.

However, other information can be derived from examining the power spectrum of the ASL and this is calculated by performing a fast Fourier transform (FFT) on the digitised raw data record. Power spectra may be averaged to produce a reliable estimate of power spectral density or to give a 'fingerprint' of a particular process regime. This type of averaging is not possible on the raw acoustic signals or ASL records as they are essentially random and average to zero. Interpretation of the power spectrum is complicated by the fact that the acoustic signal originating in the system is distorted by several factors, including transmission, reflection and signal transfer characteristics.

The shape of the power spectrum (Figure 44.5) of the ASL record is a function of the process dynamics. Periodic processes show high power at certain discrete frequencies (e.g. mechanical stirring, periodic bubbling of a fluidised bed). Random processes show either flicker-type properties, where power is inversely proportional to frequency, or white noise-type properties in which power is independent of frequency (van der Ziel 1976). The amplitude of the power spectrum is also affected by the energy of the acoustic emissions produced by the process. For instance, if hard material is being processed, the acoustic emission produced by particle impact will be greater than that produced by soft material.

Most recently, acoustic emission techniques have been applied to the on-line determination of the particle size, flow properties and compression properties of material produced during a high-shear granulation process (Whitaker *et al.* 2000). This application is based on the fact that particle size and granule density changes achieved during granulation affect the acoustic emission signals and, as these physical properties have a direct effect on powder flow and compression properties, the value in monitoring and controlling these parameters becomes apparent. As a non-invasive, sensitive and relatively inexpensive technique, acoustic emission lends itself well to both development applications and more routine usage. Key physical properties can be monitored in real time, providing indications of process end points and/or 'process signatures' (that is, the batch-specific information that characterises the actual

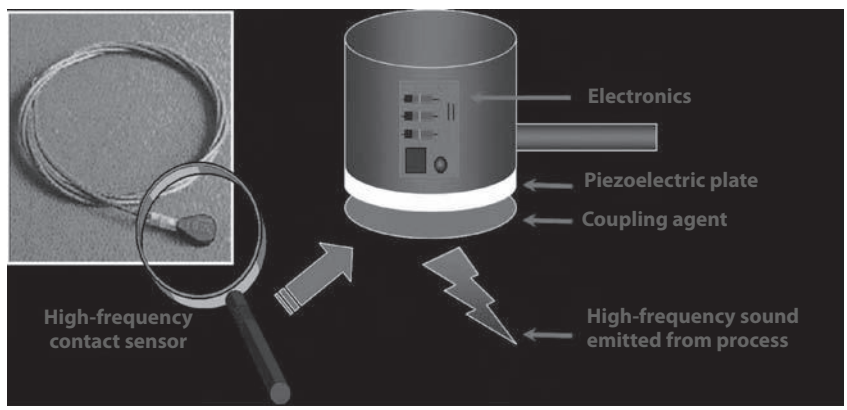

Figure 44.3 Piezoelectric transducer used in acoustic emission. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

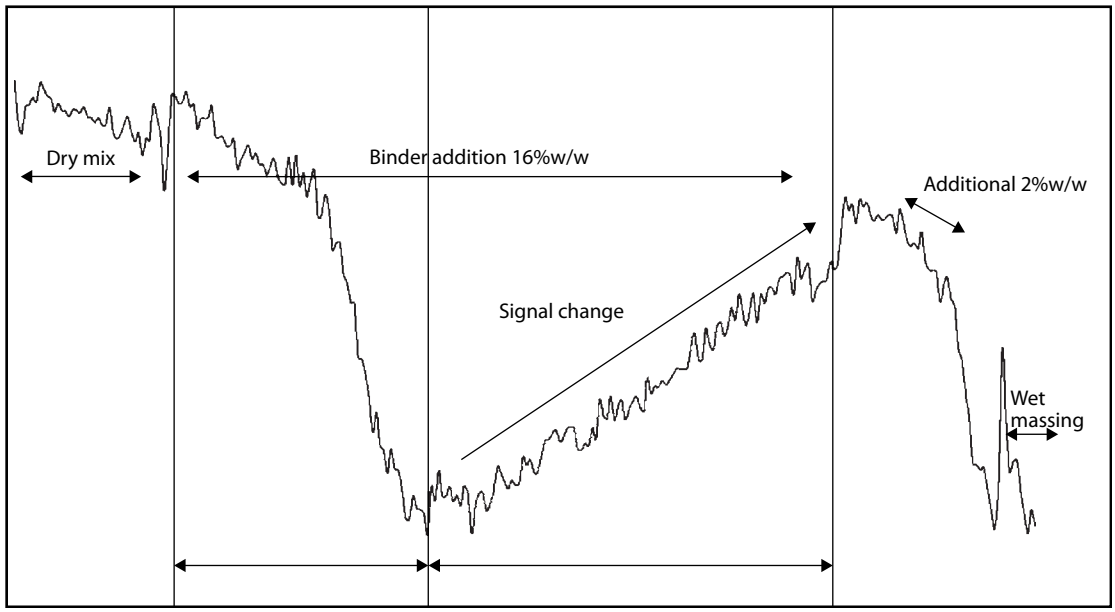

Figure 44.4 Average signal level monitored during a high-shear wet granulation process. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

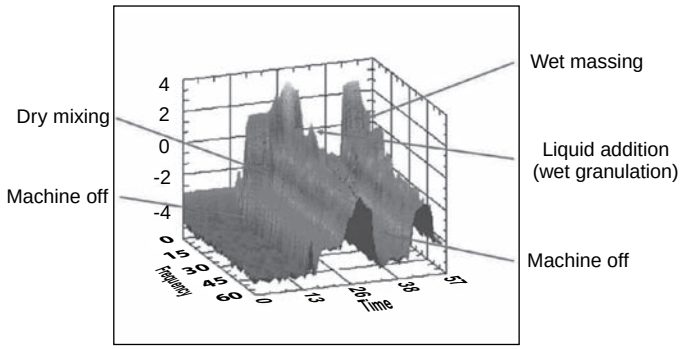

Figure 44.5 Power spectrum monitored during a high-shear wet granulation process. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

operating conditions and performance of a pharmaceutical process), which can help to guarantee the quality of intermediate pharmaceutical materials, such as granulation output. In turn, re-creation of the process signatures associated with successful process performance can provide a non-subjective basis for process optimisation, process scale-up and technology transfer.

Active acoustics

Active ultrasound spectroscopy, based on its capability of establishing material characteristics that are not easily assessed using existing measurement approaches, may be considered as a powerful addition to the current range of analytical monitoring and control techniques. It is particularly useful when applied to fluid, rather than solid, systems.

Active ultrasound spectroscopy differs from passive acoustic emission in that an ultrasound signal is applied to the material under test. Ultrasound, transmitted by a transducer responding to electrical input, travels over a defined pathlength through the test material (as depicted schematically in Figure 44.6) to a receiving transducer, at which an electrical response is generated and digitised for processing into information about the test material. Generally, measurements of attenuation (loss in amplitude of the primary, longitudinal mode of ultrasound propagation in fluids, shown as the ratio of I to I_0 in Figure 44.6) and velocity (shown as V in Figure 44.6) are made and these can provide useful insight into viscoelastic properties, for example, of the test material. The practical implementation of this approach is shown in

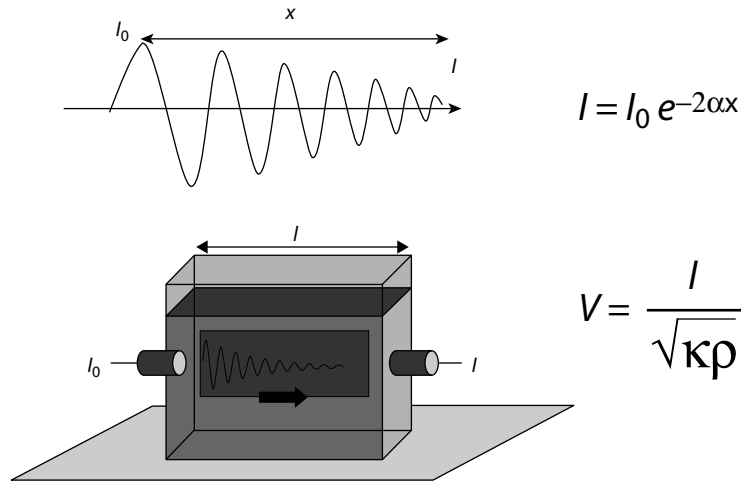

Figure 44.6 Schematic describing attenuation and velocity measurements in active ultrasound spectroscopy. α, absorbance coefficient; κ, compressibility; ρ, density. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

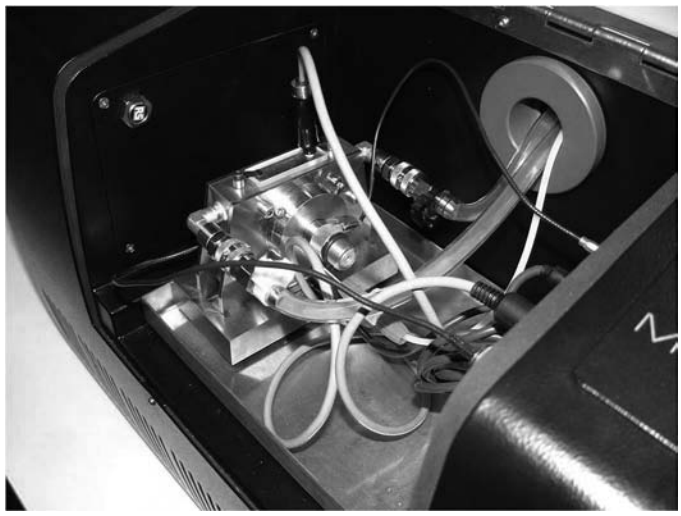

Figure 44.7 Sample cell from the ICHOS active ultrasound spectrometer. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

Figure 44.7, which depicts the active ultrasound sample cell from the commercially available ICHOS system (see www.paa.co.uk 2010).

It may be seen that active ultrasound spectroscopy offers the user flexibility of approach because, given an instrument of appropriate capability, there is the opportunity to define frequencies and other characteristics of the ultrasound waves used to probe the test material.

High-frequency ultrasound enables non-invasive analysis, with negligible transfer of energy to the sample. This can be important if, for example, fragile test materials are being investigated, e.g. during protein conformation studies or investigations into chemical and biochemical equilibria. An example of this is provided in Figure 44.8, which shows how changes in acid–base equilibria during a titration manifest themselves in the ultrasound domain. Information of this type may be used to determine reaction rate constants, equilibrium shifts and pK values, for example.

The active ultrasound process is also non-invasive in the sense that optically opaque samples, and those enclosed by vessels or pipework, can be tested *in situ*. The ultrasound source may be used in continuous signal mode or with a series of discrete, regular tone bursts. This latter approach allows precise scanning across a range of ultrasound frequencies, and can deliver more instantaneous sonic power into the medium as the system has time to recover between bursts.

Spectroscopic techniques

Terahertz spectroscopy

Terahertz radiation corresponds to the far infrared region of the electromagnetic spectrum (3–333 cm^{-1} or 100 GHz–10 THz), or parts thereof, residing between microwave and mid-infrared radiation. Rather than representing information originating from intramolecular vibrations in the mid-infrared region of the spectrum, terahertz radiation induces intermolecular non-covalent bond vibrations and translations in solids. As terahertz radiation directly probes interactions between molecules, it is intrinsically sensitive to changes in crystalline structure.

A photoconductive semiconductor antenna that generates broadband pulses of terahertz radiation by injecting charge carriers into the substrate using a femtosecond laser is used as the radiation source. After transmission through the sample material, the radiation is detected as a time-domain waveform.

After performing a Fourier transformation of both sample and reference waveforms into the frequency domain, the terahertz absorbance spectrum is calculated. For the structural image generation in terahertz pulsed imaging (TPI), the measured time delay originating from reflections at interfaces within the sample in the time-domain waveform is used to resolve the depth dimension. Spatial information (in horizontal and vertical dimensions) is obtained by point mapping of the sample. These TPI waveform data for each pixel can also be Fourier transformed to yield a fourth dimension that contains spectral information relating to the sample. Using this approach, the interrogated sample can be described in terms of structural information and chemical composition.

Applications to date fall into two main areas (Strachan *et al.* 2006): polymorph detection and quantification (usually carried out using terahertz pulsed spectroscopy) and tablet imaging (using TPI as described above). Demonstration of stability of polymorphic form in drug substances and pharmaceutical formulations is important from a quality perspective in terms of product efficacy and patient safety, and terahertz spectroscopy may have an important part to play in this respect. Similarly, the tablet imaging applications allow improved

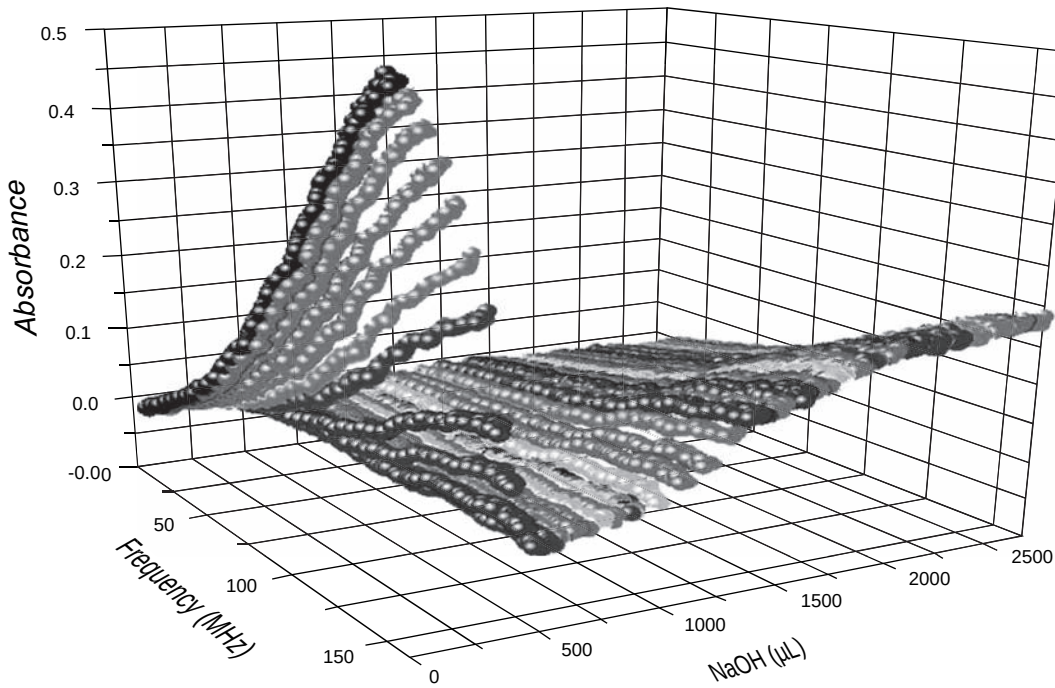

Figure 44.8 Acid–base titration monitored using active ultrasound spectroscopy. (Courtesy of Process Analysis & Automation; www.paa.co.uk.)

understanding and control of film-coating processes and/or some insight into the mechanical and physical characteristics of solid dose forms, which, in turn, may dictate the way in which the contained drug substance is released during treatment of the patient.

Despite the apparent attractions of terahertz spectroscopy, there is a feeling that the technique may not offer significantly unique advantages over other forms of infrared spectroscopy. While there are examples of undoubtedly successful applications of terahertz spectroscopy, it seems generally true that these may be equally well achieved using infrared or near-infrared spectroscopy, meaning that the technique is still looking for its unique place in the analytical toolkit. Couple this with the relative lack of maturity of the technique in terms of pharmaceutical applications, high cost of equipment and questions about instrument robustness (compared with near-infrared systems, for example) and it becomes clear that terahertz spectroscopy still has to win over a number of sceptics. Nevertheless, developments in instrument capability continue, meaning that the potential of the technique remains high.

Light-induced fluorescence

Light-induced fluorescence (LIF) is a well-established technique that can provide a number of advantages, especially in terms of selectivity and sensitivity, compared with other forms of spectroscopy (Lai *et al.* 2004). Specific excitation and emission wavelengths are associated with those individual molecules that exhibit fluorescence properties and, while these wavelength regions are often quite broad, the combination for a particular molecule may result in greatly improved selectivity. Moreover, the fluorescence process can result in very large gains in sensitivity compared with absorption spectroscopy, making the fluorescence technique highly advantageous in suitable applications. Some of these advantages can be offset, however, if scattered radiation (i.e. radiation from the excitation source) is not distinguished from the emitted fluorescence signal during detection. Failure to do this will result in significant loss of accuracy owing to non-specific signals being included in any analyte measurements.

Recent developments have seen the emergence of LED (light-emitting diode) array sensors as high-intensity, robust and versatile light sources for LIF applications. These small, self-contained, solid-state devices lend themselves well to process-based applications, especially manufacturing operations such as powder blending where interfacing of sensor-based LIF systems into a variety of large-scale blenders has been achieved successfully (Dickens 2010). The selectivity of the LIF sensor allows the monitoring system to be customised by the user to monitor the analyte of choice on the basis of its fluorescent spectral properties (using the excitation and emission wavelengths). This makes the technique ideal for monitoring relatively minor components (e.g. drug substances) in complex powder matrices (such as pharmaceutical formulations).

Extending this idea, an array of several sensors, each tailored to a specific analyte, allows multicomponent systems to be monitored simultaneously, provided that each of the analytes exhibits suitable fluorescence spectral properties. Portable devices are also available (see www.customsensors.com 2010), making the LIF technique potentially suitable for raw material identification and analysis as well as cleaning verification applications.

Transmittance Raman spectroscopy and deep Raman spectroscopy

Compared with other optical techniques, Raman spectra are relatively rich in features and can offer high chemical specificity. Physical changes, such as modifications to crystal structure or polymorphic form, are also detectable and, unlike infrared and terahertz spectroscopy, the technique is able to deal with systems containing high amounts of water.

Unlike conventional Raman spectroscopy (in which the signal is collected after back-scattering from the sample illumination area), the transmission Raman spectroscopy signal is collected from the opposite side of the sample (i.e. after transmission through the sample). This allows bulk assessment of the sample to be carried out, rather than the surface or subsurface interrogation that conventional Raman spectroscopy provides. Transmission Raman spectroscopy provides an additional benefit in that fluorescence interference and Raman signals from surface or near-surface layers are suppressed, meaning that analysis of drug content in fluorescing capsules, for example, becomes relatively easy compared with conventional Raman spectroscopy. Transmission Raman spectroscopy provides an additional benefit in that fluorescence interference and Raman signals from surface or near-surface layers are suppressed, meaning that analysis of drug content in fluorescing capsules, for example, becomes relatively easy compared with conventional Raman spectroscopy (see www.cobaltlight.com/sites/cobaltlight.com/files/CTN003%20-%20Transmission%20Raman%20Spectroscopy.pdf).

Current technical developments are exemplified by the TRS100 system produced by Cobalt Light Systems (see www.cobaltlight.com 2010). This instrument capitalises on the light-scattering properties of common pharmaceutical materials and offers a number of important application areas ranging from the characterisation of crystalline and amorphous forms of drug substance (including some quantitative aspects) through to complete and rapid non-destructive assay of intact solid dose products such as capsules and tablets, with no sample preparation required. Claims of high accuracy, good repeatability and rapid throughput of samples (typically 100 dose units in 5 minutes) for capsule and tablet assay applications make the system highly attractive for process control as well as offering a strong statistically based foundation for batch sanctioning.

A variation on conventional Raman spectroscopy that lends itself to some pharmaceutical applications is the spatially offset Raman spectroscopy (SORS) technique. This is based on the collection of the back-scattered Raman signal at spatial offsets from the incident radiation. Broadly, a laser excites the sample and the signal is collected at both the excitation point and an offset distance. The surface signal is then subtracted to reveal the spectrum of the contents. Greater spatial offsets result in Raman signals that are more representative of deeper sub-layers within the sample structure, making it possible to interrogate the contents of a diffusely scattering container, for example. Applications to date include the non-invasive detection of counterfeit drugs in plastic bottles and identification of tablets within an unopened blister pack. Greater spatial offsets result in Raman signals that are more representative of deeper sub-layers within the sample structure, making it possible to interrogate the contents of a diffusely scattering container, for example. Applications to date include the non-invasive detection of counterfeit drugs in plastic bottles and identification of tablets within an unopened blister pack (see www.cobaltlight.com/sites/cobaltlight.com/files/CAN002%20%20Counterfeit%20Drugs%20Detection%20Using%20SORS.pdf).

Process NMR spectroscopy

Chemical reaction monitoring using process-based NMR spectroscopy is commonplace and these applications are well reviewed elsewhere (Wawer *et al.* 2008; Edwards, Giammatteo 2010). More interestingly, the technique seems to have potential as part of a 'Quality by Design' strategy for the optimisation and control of drug substance manufacturing processes, where establishment of process parameter ranges can provide flexibility within the registered manufacturing process without compromising product quality. This approach, whereby small but controlled variations in the manufacturing process may be shown to produce material of acceptable quality – especially when supported by real-time analytical data that confirm the acceptability of these changes – represents the way in which process control and on-going verification need to be performed in the future.

While this philosophy is true for all real-time measurement techniques, process NMR is felt to occupy a key position for chemical reaction monitoring and control in view of the information that it can provide for important chemical and physical quality attributes.

Imaging techniques

Vibrational imaging

The term 'vibrational imaging' is generally applied to the use of near-infrared, mid-infrared and/or Raman spectroscopy to establish the presence and distribution of chemical species within a sample. Effectively, chemical imaging of this nature provides the user with an additional level

of information by combining material identification with spatial visualisation of its distribution. As such, there are a number of pharmaceutical applications ranging over blend homogeneity monitoring (El Hagrasy *et al.* 2001), tablet content uniformity determination (Gendrin *et al.* 2007), detection of polymorphism (Clark *et al.* 2007) and product authentication (Dubois *et al.* 2007). Different forms of spectroscopy are used depending on the nature of the chemical species that need to be mapped and there is generally a balance to be considered in terms of the level of scrutiny applied to any given sample. For example, highly detailed information may be obtained using microspectroscopy (i.e. the use of near-infrared, mid-infrared or Raman microscopes) and this can be useful for establishing chemical distributions within a tablet, although data acquisition times can be lengthy. In contrast, a manufacturing-scale powder blend monitoring application relies more on spatial mapping at the product target dose level (perhaps several tens of milligrams), so microscopy would be inappropriate. In terms of resolution, less detailed information is obtained in these circumstances, but data acquisition times are considerably shorter, allowing real-time process control as appropriate.

Different mapping strategies may be employed depending on the nature of the application and these can help to establish the distribution of minor components in a complex matrix or when dealing with rough-surfaced samples.

Current developments in vibrational chemical imaging technologies reflect the improvements in camera speed and capability, with wavelength ranges covering the visible to short-wave infrared (700–2500 nm) and acquisition rates of several hundred frames per second being used. Couple this with spatial resolutions of 1 mm or better and you have a powerful technology, not only for the types of application mentioned above (tablet content uniformity, powder blend monitoring, etc.), but also for more challenging examples such as film-coating quality assessment and quantification and the monitoring of outputs from processes such as continuous granulation and roller compaction.

Thermal imaging

Thermal imaging is a visualisation technique based on the principle that infrared radiation is emitted by all objects, with more radiation being emitted as the temperature of the object increases. When a system is viewed using a thermographic camera, differences in the thermal properties of materials associated with that system may allow visual distinction, in much the same way that objects appear differently coloured in the optical domain.

The principle of operation of thermal imaging devices is comparable to the way in which optical cameras work, with the obvious difference that the detection system within thermal cameras responds to the infrared region of the electromagnetic spectrum rather than the visible. High-resolution thermal cameras are widely available, with the capabilities and overall performance constantly being improved.

As with other imaging techniques, application to pharmaceutical manufacturing processes can be achieved by monitoring bulk systems in order to establish the degree of homogeneity (for example, during a powder blending operation) or to confirm that a reaction end point has been reached. Such monitoring techniques allow hot spots in vessels or regions of poor flow and inadequate mixing to be rapidly identified, allowing corrective action to be taken in real time.

At present, the overall resolution of commercially available thermal cameras is insufficient to provide the detailed image maps required for the monitoring and control of bulk pharmaceutical processes. The problem of how to achieve a detailed level of scrutiny across a large-scale manufacturing process, and with rapid data processing, is common to most imaging techniques, but it is likely that developments with thermal cameras will allow this issue to be addressed in the near future.

Process tomography

Tomography is the general name given to the imaging by sections of three-dimensional systems using sensors arranged around the periphery. Process tomography, where sectional images of systems such as pipes and vessels are obtained, is generally based on the electrical properties of the materials involved, although ultrasound is often used (particularly in the medical diagnostic field). In principle, any useful property may be exploited to obtain suitable images, but positron emission, electrical resistance, electrical impedance, electrical capacitance and magnetic induction are the most widely used at present. Figure 44.9 shows the type of information that can be obtained – in this case an electrical resistance image of a human hand, in which the wrist and five fingers can be seen clearly. This provides an idea of the level of resolution achievable and its suitability when applied to large, manufacturing-scale processes and chemical reactors.

To illustrate the basic principles and utility of tomographic techniques, electrical capacitance tomography (ECT) is chosen as an example for further description in this chapter. However, the general points discussed are applicable to other forms of process tomography.

A basic ECT system will consist of a capacitance sensor (an example of which is shown in Figure 44.10), a capacitance measuring unit and a computerised control system.

ECT is most successful when applied to materials such as oils, plastics, dry powders and, under favourable circumstances, aqueous systems

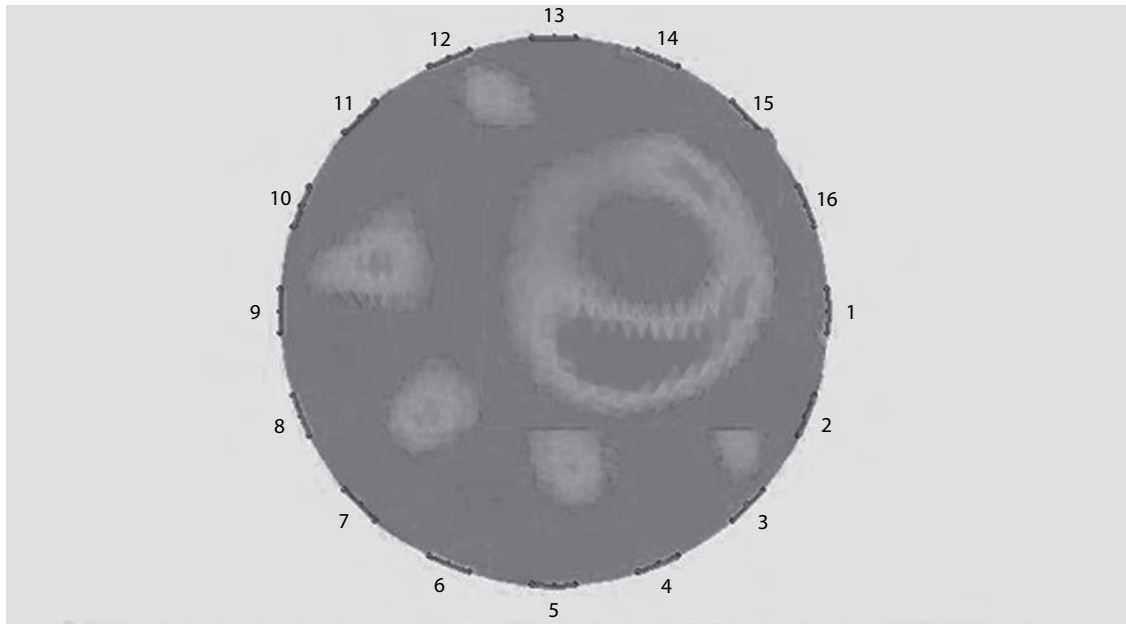

Figure 44.9 Electrical resistance tomography image of a wrist and five fingers. (Courtesy of Industrial Tomography Systems plc; www.itoms.com.)

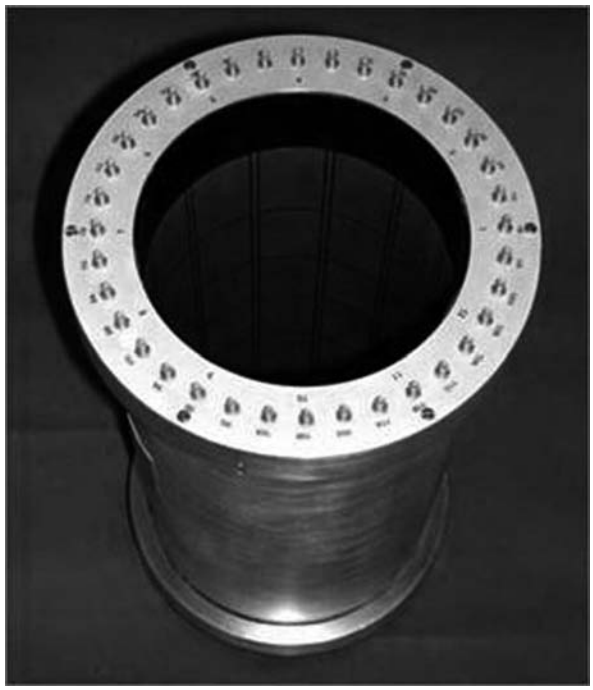

Figure 44.10 Electrical capacitance tomography sensor system. (Courtesy of Process Tomography Ltd; www.tomography.com.)

(all of which have low electrical conductivity). ECT allows information about the distribution of the contents of closed pipes or vessels to be obtained by measuring variations in the dielectric properties of the material inside the vessel. Typical information obtainable includes cross-sectional images of the vessel contents.

The cross-section to be imaged is surrounded by one or more circumferential sets of capacitance electrodes and the electrical capacitances between all combinations of the electrodes within each set are measured. This information is then used to construct an image of the contents of the cross-section of the vessel enclosed by the sensor, based on variations in the permittivity of the material inside the vessel. An example of the kind of image obtained is shown in Figure 44.11, which depicts a plastic tube inside a pipe.

In practice, the capacitance electrodes can be mounted either inside or outside the vessel. If the vessel wall is an electrical insulator such as plastic, then the electrodes are normally mounted on the outside surface of the pipe or vessel. In this case, the measurement is non-invasive and can be made intrinsically safe. The sensor is surrounded by an earthed shield that minimises the influence of external fields or objects.

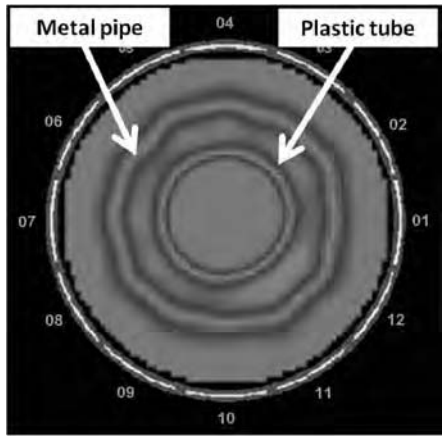

Figure 44.11 Process tomography image of a plastic tube inside a pipe. (Courtesy of Process Tomography Ltd; www.tomography.com.)

The number and size of the capacitance electrodes used depends on the application. A larger number of electrodes will give a higher-resolution image, but the measurement sensitivity will be low, although this can be increased by using longer electrodes, although at the expense of axial resolution. If high axial resolution is required, a small number of short electrodes can be used, together with separately excited axial guard electrodes, and these prevent the electric field from spreading excessively at each end of the sensor electrodes.

ECT images are normally of relatively low resolution (at present a 32×32 pixel grid is typical), but they can be captured at high speed. With existing technology, image data can be captured at 100 frames per second for a 12-electrode sensor and displayed on-line. Higher image capture rates (up to 300 frames per second) can be achieved for sensors with fewer electrodes.

The spatial resolution achievable depends on the size and radial position of the target object, together with its permittivity difference relative to that of the other material in the pipe. Typically, target objects (or local changes in permittivity) with a diameter of around 5% of that of the pipe or vessel can be detected provided that there is sufficient contrast between the permittivity of the target and the surrounding media. The accuracy of the ECT image depends on the method used for its reconstruction from the interelectrode capacitance measurements. At present, the only image-construction algorithm that is fast enough to be used for on-line image display is the linear back-projection method. This produces approximate images that are of acceptable quality for many applications. Other methods can be used to produce improved images off-line from captured capacitance measurements and these can often involve the use of iterative computational methods or the application of neural network techniques.

Pharmaceutical applications of process tomography to date include chemical reaction monitoring (Ricard *et al.* 2005), imaging of powders in a fluidised bed, allowing confirmation of satisfactory and uniform operation during the drying process (Yang 2010) and non-invasive check-weighing of bulk materials during pharmaceutical manufacture (see www.tomography.com 2010).

Light scattering

Developments in light-scattering techniques for pharmaceutical analysis seem relatively limited at present. The use of dynamic light scattering (DLS) to monitor protein aggregation is well established (see www.horiba.com 2010), and multi-angle light scattering (MLS) detector systems feature routinely where measurements of absolute molecular sizes are required (Johann 2005). However, significant advances in light-scattering instrumentation and application areas are considered unlikely at present, although any technology that provides insight into particle size aspects and conformation of large molecules and their potential for aggregation will always be considered useful.

Turbidity for metered-dose inhaler suspension monitoring

Many pharmaceutical products incorporate drug particles in suspension, using both aqueous-based and non-aqueous suspension matrices. Such products include MDIs, aqueous nasal sprays and some oral dose forms (e.g. syrups or reconstituted powders). For products of this type, drug concentration monitoring and particle sizing can be hindered by the presence of the suspension matrix, and elaborate sample manipulation is often required in order to eliminate measurement interference.

Direct measurement of drug content in suspension matrices depends heavily on the use of analytical measurement technologies that are not responsive to the components of the suspension. In this respect, it would be natural to assume that a high degree of discrimination or selectivity is required, and this is undoubtedly true in terms of achieving a distinction between the drug substance being analysed and the matrix in which it is suspended. However, beyond this distinction and, somewhat curiously, a high level of selectivity between different analyte molecules is much less important for pharmaceutical systems where a single drug substance is suspended, even in a complex matrix. In such cases, as long as the measurement technique provides sufficient discrimination for the drug substance under investigation, relatively poor selectivity can be tolerated, as no other drug molecules are present in the system. For this

reason, light-scattering techniques based on suspension turbidity have found favour recently when applied to MDI concentration monitoring (Marsh *et al.* 2010).

Most MDI products consist of one or, sometimes, two drugs suspended in non-aqueous media (usually volatile propellant systems). The relative simplicity of such formulations (where a single drug substance is present in a single-component suspension medium) means that properties such as suspension turbidity (the extent of which is directly attributable to the amount of drug present in the formulation matrix) can be monitored to establish overall drug concentration in bulk systems. Back-scattered light from simple near-infrared sources is generally used for systems that have relatively high levels of turbidity, whereas forward scattering is more frequently used for applications where the degree of turbidity is less. Good linearity of response may be obtained over a relatively large concentration range, making the technique ideal for real-time monitoring during filling operations, for example. In addition, flexible laboratory-based equipment and robust process-based versions are available commercially (see http://us.mt.com 2010), allowing development of suitable methodology and transfer to systems that are fully integrated into manufacturing production lines. Application to multicomponent MDIs (i.e. products that contain two different drugs) is also possible using dual-source turbidity systems with the irradiation wavelength of each source chosen specifically to match the spectral absorption characteristics of the two drugs.

Turbidity measurements also allow for some particle size trending in view of the fact that the degree and nature of the light scattering have some dependency on particle size.

Thermal effusivity

Thermal effusivity is a material property that combines the thermal conductivity, density and heat capacity of the material. The measurement of thermal effusivity values of materials is based on the principle that the material will exchange heat energy with its surroundings in a manner characterised by the relevant material properties. As a result, the technique can be used to differentiate between various components of a system on the basis of their heat transfer properties and this means that the composition of pharmaceutical formulations (for example) could be monitored during a blending and/or granulation process. Determination of the extent of mixing or blending using thermal effusivity is based on the uniformity of measurement across the bulk material. As with many other techniques, well-mixed materials will show a relatively low degree of variability when replicate measurements are made, whereas poor mixing will reveal itself in the form of high variation from sample to sample.

The thermal effusivity value of a pure material is characteristic of that material and, since measurements are essentially based on an averaging principle, it becomes possible to calculate the theoretical thermal effusivity values of multicomponent systems such as pharmaceutical formulations. Thus, mixing and blending processes may be monitored not only in terms of variability across the bulk material, but also by assessing how the average thermal effusivity value compares with the calculated end point. In principle, this provides a highly advantageous method for monitoring dynamic systems, allowing process control to be achieved on the basis of meeting defined requirements of homogeneity and/or overall composition.

Instrumentation is relatively simple (see www.ctherm.com 2010) and involves the application of a small amount of heat to the system under investigation, followed by measurement of the amount of heat reflected back to the sensor. A number of sensors may be used simultaneously (up to eight, typically), in order to allow assessment of the distribution or homogeneity of materials across bulk containers or vessels. This lends itself to process-based applications where monitoring equipment may be fixed directly onto powder blenders, for example Figure 44.12.

A number of practical issues seem inherent in this technique, however, and this has led to a general curtailing of the initial enthusiasm that prevailed in the pharmaceutical industry at one stage. First, the reliability of current instrumentation is questionable. Sensors need to be calibrated using reference materials (substances such as high-density polyethylene may be used) and differences in individual sensor performance (sensitivity, reproducibility, etc.) may adversely affect the conclusions when systems are monitored using multiple sensors. Second, the reliability of an individual thermal effusivity measurement depends on reproducible and adequate contact between the sensor and the bulk material. Variations in the degree of contact will result in variable and inaccurate data. This is regarded as a significant problem

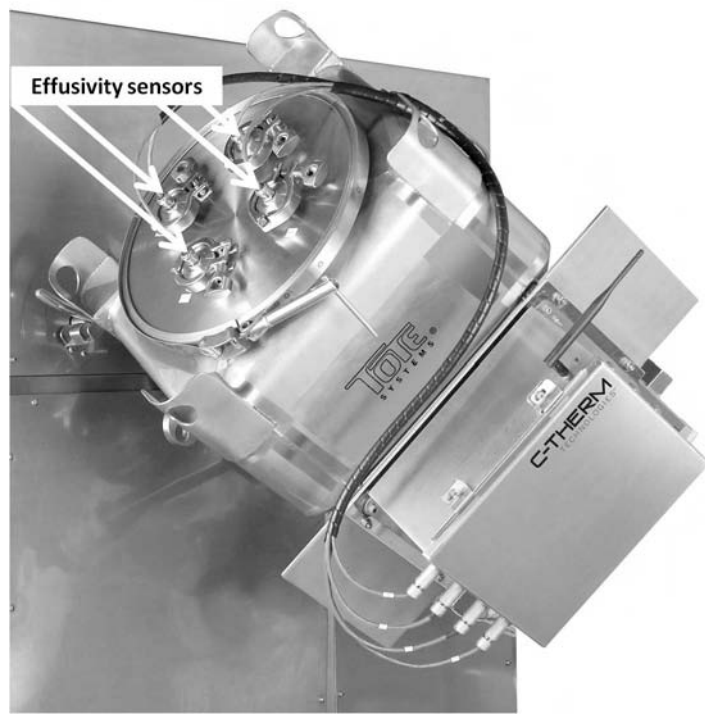

Figure 44.12 Thermal effusivity equipment for powder blend homogeneity monitoring. (Courtesy of C-Therm Technologies; www.ctherm.com.)

during mixing and blending applications, for example, where the extent of sensor contact is based entirely on the natural pressure that the bulk material exerts on the sensor when the blender is inverted. To allow a potentially critical variable to have such an uncontrolled influence on the reliability of the measurement would seem to require some considerable attention.

Finally, and perhaps most significantly of all, it is noted that the thermal effusivity values of common pharmaceutical materials are relatively similar to one another. Typically a range of 200–500 $W\,s^{1/2}/m^2\,K$ encompasses most common excipients, and it is likely that many drug substances exhibit similar thermal characteristics. Contrast this with the nominal values for water (about 1600 $W\,s^{1/2}/m^2\,K$) and air (about 5 $W\,s^{1/2}/m^2\,K$) and it becomes apparent that the presence of even relatively small amounts of these materials can be highly influential in terms of the overall average thermal effusivity value of a multicomponent system. As a result, questions may be raised about the effective 'selectivity' of the technique and its ability to distinguish between the apparent mixing of multicomponent systems as opposed to simple consolidation of particles (with attendant loss of interstitial air) or general drying, leading to an apparent increase in homogeneity according to the thermal effusivity data.

Until such fundamental questions are resolved, and despite the attractiveness of the general principle upon which the technique is based, thermal effusivity is likely to remain in the unproven category, with applications limited by the apparent lack of selectivity. It may find application, of course, where monitoring of water and/or air removal from pharmaceutical systems is important and this may explain the partial successes in monitoring of drying processes, lubrication of granules and particle consolidation during granulation and tablet compression. However, if such success is based simply on water and/or air removal, then it must be recognised that the capability of the technique is limited at best, and that it provides a relatively crude way of monitoring pharmaceutical processes almost independently of the nature and composition of the bulk materials.

Data collection and interpretation

As analytical technologies continue to evolve, it becomes progressively more important to ensure that advances are made in data collection and interpretation techniques. Currently, there are some interesting developments in two key areas associated with data generation and assessment:

- Synchronisation and integration of product and process data
- Data mining and data trending.

This section reviews some recent progress in these areas and attempts to indicate some possible future developments.

Synchronisation and integration of product and process data

The emergence of 'Quality by Design' principles in pharmaceutical development and manufacture has emphasised the importance of process understanding – that is, understanding the relationship between the critical quality attributes of the product and the process parameters (and input raw material attributes) of the manufacturing process. Development of process understanding is dependent, therefore, on collection of data associated with both the product and the manufacturing process. In this way, correlations and cause-and-effect relationships may be established, enabling suitable process control strategies to be developed and implemented to ensure good and consistent quality of product.

Generally, data collection systems associated with analytical instrumentation (laboratory-based information management systems or stand-alone measurement equipment) have been viewed separately from those that record the electromechanical measurements made on the manufacturing process (temperatures, flow rates, mixing times, etc.). The principle of 'Quality by Design' and the importance of developing process understanding highlight the need to synchronise and integrate these diverse measurements, thus allowing correlations between product and process data to be made with increased confidence.

Extensive industry/vendor collaboration in recent years has seen the development of a number of commercial products that address this need (as well as other requirements associated with the quality assurance of pharmaceutical products) and that may be exemplified by the SIPAT software produced by Siemens (see www.siemens.com 2010) or synTQ from Optimal (see www.optimal-ltd.co.uk 2010).

Software of this nature can provide the user with a common interface for all of the analytical systems (product and process analysers), process control systems, data analysis and reporting tools, etc. In this way, a fully integrated approach towards quality assurance and (potentially) real-time release of pharmaceutical products becomes possible, and this is particularly important as continuous processing begins to feature more prominently in pharmaceutical manufacture.

Data mining and data trending

Data mining involves the extraction of information from data-sets and usually results in features and relationships being identified within those data-sets. Groups, clusters, associations and patterns may be established, and significant features or characteristics identified, thus allowing predictions or subsequent additions, interpolations and extrapolations to be made.

Traditional approaches involving projection techniques such as PCA (principal components analysis) and PLSR (partial least squares regression or projection to latent structures regression, depending on your point of view) are now well established in the pharmaceutical industry, but recent developments include the application of genetic algorithms for variable selection in pharmaceutical development (Tabora *et al.* 2010) and locally weighted PLSR for powder blend uniformity monitoring (Kim *et al.* 2010). Generally, approaches of this type provide improved model accuracy and/or predictive capability and offer an indication of the application areas in which multivariate data interpretation techniques appear to be most useful.

Elsewhere, some of the machine learning approaches (e.g. artificial neural networks and support vector machines) are now being utilised extensively for establishing structure–activity relationships, but do not yet seem to be finding application to pharmaceutical model development and historical product and process characterisation. Several vendors, for example StatSoft (see www.statsoft.com 2010), offer products with this type of capability, and an increase in usage designed to establish trends and relationships in historical data-sets, stability studies and continuous process manufacturing data is considered likely.

Field-based measurement techniques

Near-infrared systems

Portable analytical systems are playing an increasingly important role in a number of different application areas. Raw material identification and analysis at the point of receipt is an important and long-established application and hand-held near-infrared (NIR) devices continue to evolve to support this type of usage.

Optoelectronic developments have allowed vendors to produce rugged, portable 'point and shoot' devices that now offer performance comparable to that of laboratory-based FT-NIR systems, and this is providing the user with high-resolution NIR capability and rapid spectral acquisition even under harsh field-based conditions. Examples include the microPHAZIR Rx system from Thermo Scientific (see www.ahurascientific.com 2010) and Axsun's Anavo device (see www.axsun.com 2010), both of which are similar in capability, but which differ in terms of the light source used. The advantage of Axsun's tunable diode laser over the tungsten light bulb favoured by Polychromix lies in the simplicity of design and elimination of power and cooling requirements, but perhaps at the expense of light throughput and battery and source life. Either way, the use of hand-held NIR devices for raw material identification and analysis, as well as some process monitoring applications, is now quite commonplace.

Other portable NIR systems include the briefcase-sized RxSpec 700Z from ASD (see www.asdi.com 2010), which is marketed as an anti-counterfeiting drug analyser and allows field agents to test drug samples on the spot to investigate authenticity.

Raman systems

Although NIR finds wide applicability in field-based raw material identification and analysis, Raman spectroscopy can offer some significant advantages in certain circumstances. The high specificity of the technique can provide increased confidence when attempting to identify or discriminate between chemically similar materials, but it is the ability to obtain high-quality spectra directly through a range of packaging materials that distinguishes Raman systems from alternative approaches (Perkin-Elmer 2010), although the quantitative aspects may be less impressive.

Ion-mobility spectrometry

Ion-mobility spectrometry (IMS) instruments are easy to use, as demonstrated by their widespread employment in military and aviation security applications.

For IMS, volatile samples are introduced by sampling the headspace of open containers or vapour streams from processing lines. Less volatile particulates are collected by wiping surfaces with an inert material followed by their thermal desorption directly into the instrument and, because sampling is accomplished without the use of solvents, manual processing or derivatisation, non-scientists can perform the measurements. This ease of use and portability of equipment, coupled with the sensitivity of detection and broad selectivity, has resulted in IMS becoming the current technique of choice for field-based security applications (Ewing *et al.* 2001).

Similarly, the technique lends itself to environmental and personal monitoring, especially in terms of exposure to drug substances or hazardous materials during pharmaceutical manufacture (Eiceman *et al.* 1995). A number of commercial units are currently available, allowing individual personal monitoring, as well as static field-based systems that provide more of an overview of general, rather than localised, exposure.

Field-based systems for fault detection and trace analysis of solvents and chemicals are also available, in particular using IMS to provide the sensitivity and quantitative elements that such applications require. Progress in this area involves the use of programmable systems that can be 'trained' to create a chemical fingerprint characteristic of the process or system under investigation, and that can then be used to detect anomalous events or out-of-range situations.

Such systems (see www.owlstonenanotech.com 2010) can provide point-of-use capability for reaction monitoring, as well as solvent estimation in drug substances and measurement of contaminants at trace levels and in real time.

Rapid microbiological techniques

Clinical and food industry microbiological laboratories have used rapid microbiological methods for many years because of the need for quick diagnosis of infectious diseases and the assessment of quality of perishable goods. In contrast, the pharmaceutical industry has seemed reluctant to adopt rapid microbiological methods, largely owing to the potential lack of acceptance by regulatory agencies, but these concerns have been alleviated in recent years as the 'Quality by Design' initiatives gather pace. As a result, many rapid microbiological methods have now become firmly established within the pharmaceutical sector, particularly for screening and other specialised applications, but the traditional culture-plate methods are generally still preferred for routine use. This is partly historical and reflects the fact that rapid microbiological methods do not necessarily give equivalent information to that obtained from the more traditional methods. As an example, some rapid microbiological methods (particularly those that do not rely on microbial growth) can detect a significantly wider range of organisms than existing more traditional methods, and this can cause obvious problems in terms of demonstrating equivalence of data during method validation programmes.

Unlike traditional culture-plate methods (which involve the growth of microorganisms in media), rapid microbiological methods rely on the instrumental detection of suitable markers. Broadly, rapid microbiological methods may be classified under three main headings: identification (where the types of microbial contaminants are specified), qualitative (where the presence or absence of microbial contamination is indicated), and quantitative (where the total level of contamination is estimated). Different technologies are employed in each of these application areas, with the most common involving nucleic acid-based detection (using DNA or RNA targets), antibody-based detection, biochemical, enzymatic detection, impedance methods and solid-state and flow cytometry.

Some current developments are now discussed.

Nucleic acid-based methods

Molecular-based methods are now being used to classify strains at the genetic level and as easy-to-use assays for the rapid detection of objectionable organisms. For example, molecular strain typing by sequencing or enzyme digestion patterns can allow comparison of strain against previously observed contaminants.

Nucleic acid amplification techniques (e.g. based on polymerase chain reaction) are also finding application and operate by amplifying and detecting the nucleic acid within the microbial cell rather than detecting the cell itself. Amplification tests may be tailored to detect only a single species or strain and therefore provide high sensitivity and selectivity. Equally, the use of commonly shared genetic sequences as amplification targets allows detection of much broader categories of organisms, thus providing a more universal identification system where necessary (Rodger *et al.* 2010).

Fluorescence-based methods

These methods utilise either the natural autofluorescence of a bacterial cell or the fluorescence resulting from the absorption or activation of a fluorescent dye. Sometimes a short pre-enrichment growth phase is required with this type of method in order to obtain adequate sensitivity but, in general, high selectivity can be achieved.

Oligonucleotide probes can be designed that offer differing levels of selectivity depending on the intended application. When such probes are labelled with fluorescent dyes or the enzyme horseradish peroxidase, for example, identification of single microbial cells is possible using fluorescence *in-situ* hybridisation (FISH). Development of group-specific probes continues (Amann, Fuchs 2008), allowing more reliable quantification of microbial populations *in situ* and in increasingly complex sample types.

Commercially, the Biovigilant system (see www.biovigilant.com 2010) offers a detection approach based on Mie scattering size information coupled with UV laser-induced fluorescence of certain metabolites inside microbial cells and spores. This combination of measurements greatly facilitates classification of microbes.

Bioluminescence methods

Adenosine triphosphate (ATP) bioluminescence methods are well accepted for rapid microbial screening, but can have limited effectiveness for biopharmaceuticals owing to the presence of non-microbial ATP in the sample matrix. Pre-treatment of the sample with proprietary reagents can help to overcome this problem, however, allowing more rapid detection of microbial contamination during manufacture (see www.celsis.com 2010a).

More recently, adenylate kinase (AK) has been used to achieve amplification of ATP bioluminescence, thereby providing confirmation of the absence of microorganisms, including slow-growing moulds, in pharmaceutical products within an overall cycle time of 18–24 h (see www.celsis.com 2010b) for microbial limit testing. Such technology can also be used for sterility testing (see www.celsis.com 2010c), with improvements in sample applicability and handling, reproducibility of detection and overall reduction in analytical cycle times compared with the traditional pharmacopoeial approach.

MALDI-TOF methods

Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry can be used for the accurate identification of selected bacteria and fungi. Application of the technique to clinical samples has demonstrated excellent performance (Seng *et al.* 2009) and provides a

good indication that MALDI-TOF mass spectrometry could replace Gram staining and biochemical identification and become a reliable standard in medical microbiological laboratories in the next few years.

The emergence of biopharmaceuticals

The pharmaceutical industry has placed, and continues to place, great emphasis on the development of biopharmaceutical products in recent years in order to provide new forms of therapy or to address disease areas that are not adequately treated using conventional 'small' molecules.

This upsurge has presented a number of analytical challenges in terms of the physical and chemical characterisation of biopharmaceuticals and the control of the associated manufacturing processes in view of the relative complexity involved. Conventional 'small' molecules generally have well-defined structures, and the quality (potency, morphology, impurity levels, etc.) may be established with confidence. Biopharmaceuticals are less easy to characterise, however, and those that are chemically identical (some proteins, for example) may have different biological effects resulting from their structural folding patterns. In addition, the biopharmaceutical manufacturing process (involving host cell development, cell production, purification, etc.) is often difficult to operate reproducibly and the characteristics of the finished product (and hence efficacy, and safety) are likely to be influenced by small variations during production.

In view of this high level of complexity, a number of established analytical techniques have been and are being applied, with further development as necessary, for the characterisation of biopharmaceuticals and the associated manufacturing processes. Some of the major applications are now described.

Surface plasmon resonance spectroscopy

Traditionally surface plasmon resonance spectroscopy (SPR spectroscopy) has been used for measuring adsorption of materials onto planar (usually metal) surfaces and has found application in ligand binding studies, for example. For biopharmaceutical characterisation, biosensor-based SPR spectroscopy is being used as a replacement for traditional ELISA (enzyme-linked immunosorbent assay) technology for antibody detection as it offers the advantages of speed and simplicity, ease of automation and provision of label-free immunoassays (Gong 2009). More ambitiously, SPR spectroscopy provides the capability of therapeutic antibody screening based on mechanism of action, and can include a multiplexed approach to allow analysis of several multiple interactions in parallel (Corbin 2009).

The real-time capability of SPR spectroscopy lends itself to process-based application and allows product yield and integrity to be monitored and optimised (Hoffman 2010), as well as providing the basis for process development and control (Thillaivinayagalingam *et al.* 2010).

Quantitative polymerase chain reaction

The polymerase chain reaction (PCR) is widely used in a number of different application areas to produce multiple copies of a DNA sequence. After this amplification, end-point detection usually involves gel electrophoresis followed by staining, but this approach is fairly time-consuming, prone to interference and provides, at best, only qualitative information.

Quantitative PCR (qPCR) overcomes many of these shortcomings by performing the amplification and detection simultaneously, and allows instrumental measurement while the reaction is progressing. Quantitative PCR has been applied to address contamination of host cell DNA and viruses, as this is a critical step in ensuring biological purity of the organisms being used to express the required product (see www.home.agilent.com 2010).

In addition, a multiplex real-time qPCR assay method has been proposed recently that allows the viral clearance capacity of biopharmaceutical purification processes to be established and that may prove useful as a validation technique during process development and implementation (Lute *et al.* 2009).

Capillary electrophoresis and isoelectric focusing

Capillary electrophoresis techniques now play an important role in many biopharmaceutical development and quality control laboratories, with modern instrumentation offering considerable advantages over traditional slab gel methods.

General application areas include the determination of molecular purity, usually involving separations based on hydrodynamic size under denaturing conditions (Guo *et al.* 2008) or determination of the isoelectric point of proteins and establishing the charge heterogeneity due to post-translational modifications (see www.m-scan.co.uk 2010).

The modes of separation used in capillary electrophoresis techniques offer complementary information to that obtained using HPLC, for example, and this can provide a more complete approach to the characterisation of complex biopharmaceutical products (Girard 2009).

Cation-exchange chromatofocusing

Classically, cation-exchange chromatography is used to characterise charge heterogeneity in some classes of biopharmaceuticals (monoclonal antibodies, for example). The inherent heterogeneity of these species demands thorough characterisation, but traditional cation-exchange chromatography methods employing salt gradients can suffer from a lack of robustness and stability of performance owing to fluctuations in eluent composition.

Chromatofocusing is performed on an ion-exchange column and uses a pH gradient to achieve elution of the analytes of interest. Buffer composition and the shape of the pH gradient dictate the order and point of elution of the components of the sample. However, there are limitations in terms of robustness of performance, particularly owing to the chemical composition of elution buffers and when shallow pH gradients are used.

Chromatofocusing using gradual pH gradients (produced by the external mixing of two different elution buffers) can provide improved method performance and flexibility compared with existing approaches and this technique can be used for separation of monoclonal antibody charge species and determination of acidic species and isoform content (Rozhkova 2009).

Conclusion

It may be apparent from the brief review of developments, newer application areas and improved ways of working presented in this chapter that the field of pharmaceutical analysis is in the midst of quite a revolution. There may be few truly 'new' analytical technologies emerging, perhaps because the analytical toolbox is already quite well furnished with techniques suitable for most existing applications, but the change in emphasis that is being felt within the analytical community is providing the impetus for a great deal of innovative thinking.

No longer is the pharmaceutical analyst content simply to review the results of product and process development, or to establish product quality using traditional tests and methods. Now the emphasis is on gaining an improved understanding of pharmaceutical products and processes such that quality is ensured 'by design', and the on-going analytical assessment, mainly during this design and development phase, plays an integral part in obtaining this understanding. Control of drug substance manufacturing processes to ensure *and demonstrate* correct morphology, crystal habit, physical properties, etc. is as important now as establishing purity, for these are the aspects that will govern subsequent processability during product manufacture and final quality of the pharmaceutical product. Tablet hydration studies during formulation development will provide an insight into the mechanisms whereby the tablet releases the active ingredient and it is this, rather than the traditional laboratory dissolution test, that will aid design of a product of suitable quality.

Analytical techniques have evolved to meet these needs and will continue to do so. More established techniques are finding newer application areas. Different approaches are being adopted to establish aspects

of product and process understanding and final product quality. In all of this, the challenge for the pharmaceutical analyst is to keep sight of the real objectives and to choose the most appropriate techniques and methods, and the required analytical performance, that will achieve them. May the revolution continue.

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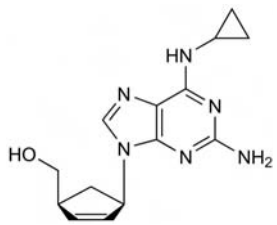
PART TWO

Monographs

Abacavir

Antiretroviral, Nucleoside Reverse Transcriptase Inhibitor
$C_{14}H_{18}N_6O$ = 286.3
CAS—136470-78-5

IUPAC Name [(1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)purin-9-yl]cyclopent-2-en-1-yl]methanol
Synonyms (1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol; (–)-*cis*-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol; 1592U89.
Proprietary Names *Epzicom*; *Kivexa*.



Chemical Properties White to off-white solid. White solid foam from acetonitrile. Mp 165°. Soluble in water (0.077 mg/L at 25°). pK_a 5.01 [Kumar *et al.* 1999]. Log *P* (octanol/sodium phosphate, pH 7.4) 1.22 [Kumar *et al.* 1999].

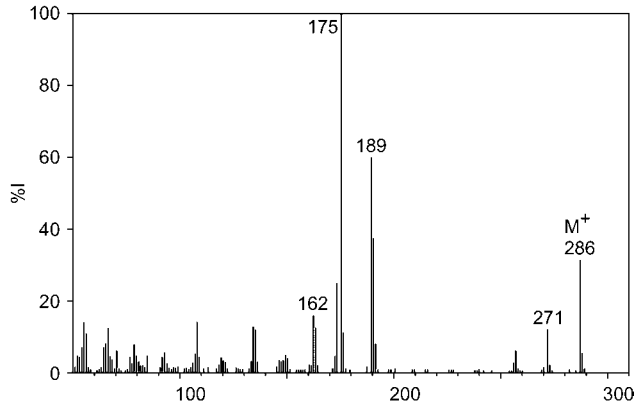
Abacavir Succinate

$C_{18}H_{24}N_6O_5$ = 404.4
CAS—168146-84-7
IUPAC Name [(1*R*)-4-[2-Amino-6-(cyclopropylamino)purin-9-yl]cyclopent-2-en-1-yl]methanol butanedioic acid

Abacavir Sulfate

$(C_{14}H_{18}N_6O)_2,H_2SO_4$ = 670.8
CAS—188062-50-2
IUPAC Name [(1*R*)-4-[2-Amino-6-(cyclopropylamino)purin-9-yl]cyclopent-2-en-1-yl]methanol sulfuric acid
Proprietary Names *Kivexa*; *Trizivir*; *Ziagen*.
Chemical Properties Log *P* (octanol/water, pH 7.1–7.3), 1.20 (25°).

High Performance Liquid Chromatography System HAB—RT 1.0 min; system HAD—*k* 8.10.
Ultraviolet Spectrum Aqueous alkali—217, 260, 285 nm.
Mass Spectrum Principal ions at *m/z* 175, 189, 190, 286, 173, 162, 271, 163.



Quantification

Plasma **HPLC** Column: Waters Symmetry C_{18} (100 × 4.6 mm i.d., 3.5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 7.8, 85 : 15), flow rate 1.5 mL/min. UV detection (λ = 285 nm). Limit of quantification, 0.08 mg/L [Sparidans *et al.* 2001]. Column: Symmetry Shield C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer + ion pair (50 : 50). UV detection (λ = 260 nm) [Aymard *et al.* 2000]. Column: Symmetry C_{18} (100 × 4.6 mm i.d., 3.5 µm). Temperature: 41°. Mobile phase: 25 mmol/L phosphate buffer (pH 7.0) : acetonitrile (85 : 15), flow rate 1.0 mL/min. UV detection (λ = 285 nm). Retention time: 4.8 min. Limit of quantification, 0.02 mg/L [Veldkamp *et al.* 1999]. Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm) Mobile phase: acetonitrile: water (83 : 17), flow rate 1.0 mL/min. UV detection (λ = 285 nm). Limit of quantification, 0.05 mg/L. [Ferrer *et al.* 2004].

LC-MS Column: Machery-Nagel Nucleosil C_{18}-100 (125 × 3 mm i.d., 3 µm). Mobile phase: 20 mmol/L ammonium acetate-0.1% aqueous acetic acid : acetonitrile, flow rate 0.4 mL/min. ESI, positive ion mode [Gehrig *et al.* 2007]. Column: Zorbax Eclipse XDB C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 6.3) : acetonitrile (30 : 70 for 1.5 min to 85 : 15 at 2 min), flow rate 0.25 mL/min. ESI, positive ion mode. Limit of detection not reported [Clark *et al.* 2004]. Column: Phenomenex Luna $C_8(2)$ (50 × 2.0 mm i.d., 5 µm). Mobile phases: 20 mmol/L DMHA (pH 7) and methanol : water (80 : 20), flow rate 0.2 mL/min. Limit of detection, <25 nmol/L [Fung *et al.* 2001].

CE Capillary: uncoated fused silica (50 µm i.d., 70 µm o.d.). Buffer: 40 µmol/L ammonium acetate (pH 9.2). SIM acquisition mode, *m/z* 288. Limit of detection, 1 µmol/L [Cai *et al.* 2003].

Urine **HPLC** Column: Phenomenex Kromasil octadecyl (150 × 3.2 mm i.d., 5 µm). Mobile phase: 25 mmol/L ammonium acetate buffer (pH 4.0) : methanol (95 : 5), flow rate 0.7 mL/min. UV detection (λ = 295 nm). Limit of quantification, 56 mg/L [Ravitch, Moseley 2001].

Amniotic Fluid **LC-MS** See Plasma [Clark *et al.* 2004].

CSF **HPLC** See Urine [Ravitch, Moseley 2001].

Placenta **LC-MS** See Plasma [Clark *et al.* 2004].

Foetus **LC-MS** See Plasma [Clark *et al.* 2004].

Disposition in the Body Abacavir is extensively and rapidly absorbed after oral administration with a bioavailability of ~83%, depending on formulation [Chittick *et al.* 1999]. It is metabolised by alcohol dehydrogenase to 5′-carboxylic acid and by glucuronyl transferase to the 5′-glucuronide. It is mainly excreted in urine: 1.2% of a dose is excreted unchanged in urine, 30% as the 2 metabolites and 15% as an unidentified metabolite; 16% of the dose can also be detected in faeces [Yuen *et al.* 2008].

Therapeutic Concentration

Twelve HIV-infected adult patients were administered with single oral doses of 100, 300, 600, 900 and 1200 mg abacavir. Peak plasma concentrations of 0.6, 2.9, 4.7, 8.1 and 9.6 mg/L were reached for these doses, respectively, attained within 1.0–1.7 h [Kumar *et al.* 1999].

Abacavir was administered to 41 HIV-infected adult patients as an oral dose of 100, 300 or 600 mg twice daily for 12 weeks. Average peak plasma concentrations of 0.6, 2.2 and 4.4 mg/L were reached for these doses, respectively, attained within 1.0 h [Weller *et al.* 2000].

Abacavir was administered to 22 HIV-infected children, aged between 3 months and 13 years, either as a 4 or a 8 mg/kg dose. Peak plasma concentrations of 1.69 and 3.94 mg/L, respectively, were reached within 1.5 h [Hughes *et al.* 1999].

Toxicity Life-threatening hypersensitivity reactions have been reported, as well as life-threatening lactic acidosis and severe hepatomegaly with steatosis.

Half-life Adults, elimination half-life 0.9–1.7 h [Kumar *et al.* 1999], 0.8–1.0 h [Weller *et al.* 2000]. Children, 1.0 h [Hughes *et al.* 1999]. The half-life of the active intracellular anabolite, carbovir triphosphate, is >20 h [Yuen *et al.* 2008].

Volume of Distribution Apparent volume of distribution: adults 0.65–0.95 L/kg (57.6–77.6 L) [Weller *et al.* 2000]; children 1.82 L/kg (45.3 L) [Jullien *et al.* 2005].

Clearance Mean apparent clearance: adults 0.56 L/h/kg (single 1200 mg dose)–1.94 L/h/kg (single 100 mg dose) [Kumar *et al.* 1999]; children 1.6 L/h/kg (8 mg/kg dose)–1.1 L/h/kg (4 mg/kg dose) [Hughes *et al.* 1999], 0.9 L/h/kg (8 mg/kg dose) [Jullien *et al.* 2005].

Protein Binding ≈50% [Yuen *et al.* 2008].

Dose Adults: 300 mg (base) twice daily or 600 mg once daily. Children aged between 3 months and 16 years: 8 mg/kg body weight (maximum 300 mg) twice daily.

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Yuen GJ *et al.* (2008). A review of the pharmacokinetics of abacavir. *Clin Pharmacokinet* 47: 351–371.

Acamprosate Calcium

Alcohol Deterrent

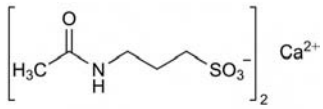
$Ca(C_5H_{10}NO_4S)_2 = 400.5$

CAS—77337-76-9 (acamprosate); 77337-73-6 (acamprosate calcium)

IUPAC Name Calcium 3-acetamidopropane-1–sulfonate

Synonyms Ca-AOTA; calcium 3-acetamido-1-propanesulfonate; calcium acetylhomotaurinate; calcium bisacetyl homotaurine.

Proprietary Names *Aotal*; *Campral.*



Chemical Properties A colourless crystalline powder. Mp 270°.

High Performance Liquid Chromatography System HAA—retention time 3.0 min.

Ultraviolet Spectrum Water—192 nm.

Quantification

Plasma **GC-MS** Column: fused-silica capillary coated with OV-1701 (25 m × 0.35 mm i.d., 0.2 µm). Temperature programme: 240° to 310° at 10°/min for 2 min. NICI, SIM acquisition mode at *m/z* 424. Retention time: 5.5 min [Girault *et al.* 1990]. Limit of detection, 0.001 mg/L [Girault *et al.* 1990].

HPLC Fluorescence detection. Limit of detection, 0.001 mg/L [Chabenat *et al.* 1987].

Disposition in the Body Absorption is slow after oral administration but sustained and shows large intersubject variability. Steady state concentrations are observed 7 days after administration. The drug crosses the blood–brain barrier. It is not significantly metabolised and is excreted unchanged in urine.

Therapeutic Concentration

Following single oral doses of 666 mg to 24 healthy subjects, aged 19 to 35 years, the mean maximum plasma concentration was 0.180 mg/L. Most of the dose was absorbed within 4 h [Girault *et al.* 1990].

Six healthy volunteers and 12 patients with moderate or severe chronic renal impairment were administered a single dose of 666 mg. Maximum peak plasma concentrations were 0.198 mg/L for the healthy individuals and 0.813 mg/L for those with severe impairment. These were observed at 6 and 23 h, respectively [Sennesael 1994].

Half-life Plasma 13 to 18 h (healthy individuals); 47 h (patients with severe renal impairment).

Clearance Plasma 184 L/h (healthy volunteers); 16 L/h (patients with moderate renal impairment); 66.5 L/h (severe impairment).

Protein Binding Not bound.

Note For a review of acamprosate calcium, see Wilde and Wagstaff [1997].

Dose Patients with a body weight <60 kg: 1.3 g daily. Patients with a body weight >60 kg: 2 g daily.

Chabenat C *et al.* (1987). Determination of calcium acetylhomotaurinate by liquid chromatography with fluorimetric and electrochemical detection. *J Chromatogr* 414: 417–422.
Girault J *et al.* (1990). Determination of calcium acetylhomotaurinate in human plasma and urine by combined gas chromatography-negative-ion chemical ionization mass spectrometry. *J Chromatogr* 530: 295–305.
Sennesael J (1994). Data on file. France: Lipha.
Wilde MI, Wagstaff AJ (1997). Acamprosate. A review of its pharmacology and clinical potential in the management of alcohol dependence after detoxification. *Drugs* 53: 1038–1053.

Acarbose

Antidiabetic

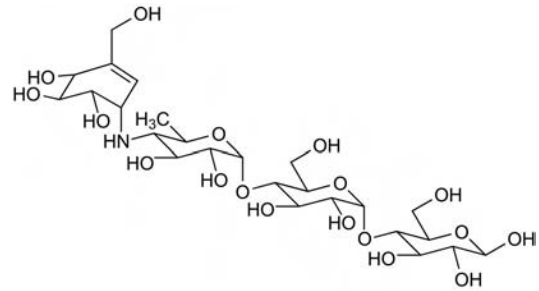
$C_{25}H_{43}NO_{18} = 645.6$

CAS—56180-94-0

IUPAC Name (2*R*,3*R*,4*R*,5*S*,6*R*)-5-[(2*R*,3*R*,4*R*,5*S*,6*R*)-5-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3, 4-Dihydroxy-6-methyl-5-[[(1*S*,4*S*,5*S*,6*S*)-4,5, 6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino]oxan-2-yl]oxy-3, 4-dihydroxy-6-(hydroxymethyl)oxan-2-yl] oxy-6-(hydroxymethyl)oxane-2,3, 4-triol

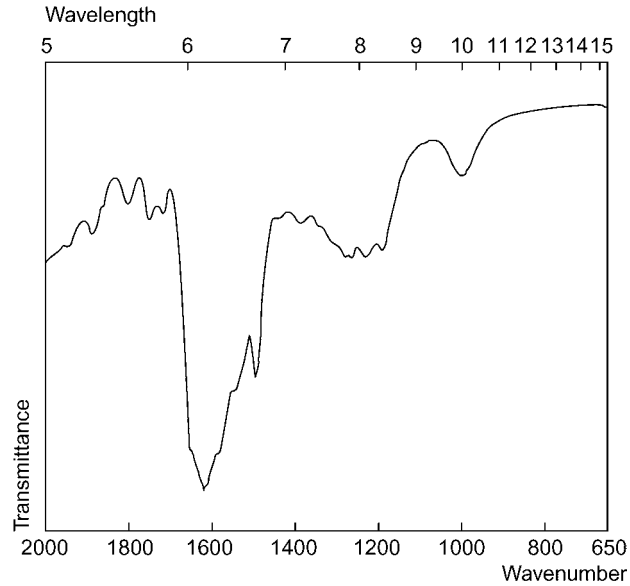
Synonyms Bay-g-5421; *O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl] amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose.

Proprietary Names *Glicobase*; *Glucobay*; *Glucor*; *Glumide*; *Prandase*; *Precose*.



Chemical Properties A white to off-white amorphous powder, which is soluble in water. pK_a 5.1. Log *P* (octanol/water), −8.08.

Infrared Spectrum Principal peaks at wavenumbers 1033, 1153, 1458 cm^{-1} (KBr disk).



Quantification

Plasma **Bioassay** Limit of detection, 5 µg/L [Ahr *et al.* 1989].

Urine **Bioassay** Limit of detection, 150 µg/L [Ahr *et al.* 1989].

Other **HPLC** In Pharmaceuticals. ELS detection. Limit of quantification, >15 mg/L, limit of detection, <5 mg/L [Cherkaoui *et al.* 1998]. Column: Nucleosil aminopropyl-bonded (250 × 4.6 mm, i.d. 5 µm). Mobile phase: methanol: dichloromethane (65:35). Detection: ELS. Retention time: 4.3 min [Cherkaoui *et al.* 1998].

Disposition in the Body Acarbose is metabolised by intestinal digestive enzymes and by the microbial flora of the gastrointestinal tract, where the majority of the active unchanged drug remains. The major metabolites are the 4-methylpyrogallol derivatives (sulfate, methyl and glucuronide conjugates) produced by biotransformation (mainly reduction and conjugation). 35% of an oral dose is absorbed as the metabolites and only 1 to 2% absorbed as the active drug. After intravenous administration, approximately 90% of a dose is excreted in urine within 48 h as the active drug (parent drug or active metabolite). After oral administration, <2% of the dose is recovered in urine as the active drug and 34% as metabolites, and approximately 50% as the unabsorbed drug in faeces (within 96 h post ingestion).

Therapeutic Concentration Patients with severe renal impairment, creatinine clearance <25 mL/min/1.73 m^2, have a peak plasma concentration of acarbose 5 times greater than those with normal renal function.

Six healthy volunteers were administered with a single oral 200 mg dose of acarbose. Peak plasma concentrations of 49.5 ± 27.0 µg/L were reached approx. 2 h after administration [Ahr *et al.* 1989].

Toxicity Acarbose is associated with hepatotoxicity and has low toxicity by ingestion, subcutaneous and intravenous routes. A 360 mg/kg body weight dose in women over a 60-day period and given intermittently in a number of separate and discrete doses can cause non-fatal toxic effects. Acarbose competitively and reversibly inhibits the α-glucosidase enzymes, glucoamylase, sucrase, maltase and isomaltase, found in the small intestine, and this delays hydrolysis of complex carbohydrates. It is unlikely to produce hypoglycaemia in overdose, but abdominal discomfort and diarrhoea may occur.

Bioavailability Systemic bioavailability is 0.5 to 1.7%.

Half-life Elimination half-life is 3.2 ± 0.9 h.

Volume of Distribution Steady state volume of distribution is 0.32 L/kg.

Clearance Total body clearance, 600 L/h.

Protein Binding Minimal protein binding with plasma concentrations of more than 1 µg/L and up to 98% with 0.008 µg/L.

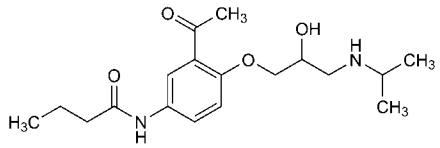
Dose An initial dose of 25 mg three times a day is administered and can be increased to a maintenance dose of 50 to 100 mg three times daily. Doses up to 200 mg three times daily have been reported. Patients with a body weight ≤60 kg have a maximum dose of 50 mg three times a day.

Ahr HJ *et al.* (1989). Pharmacokinetics of acarbose. Part I: Absorption, concentration in plasma, metabolism and excretion after single administration of [14C]acarbose to rats, dogs and man. *Arzneimittelforschung* 39: 1254–1260.
Cherkaoui S *et al.* (1998). Development and validation of liquid chromatography and capillary electrophoresis methods for acarbose determination in pharmaceutical tablets. *J Pharm Biomed Anal* 18: 729–735.

Acebutolol

β-*Adrenoceptor Antagonist*
$C_{18}H_{28}N_2O_4$ = 336.4
CAS—3751-730-9

IUPAC Name *N*-[3-Acetyl-4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]butanamide
Synonyms (±)-3′-Acetyl-4′-(2-hydroxy-3-isopropylaminopropoxy)butyranilide; *N*-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]butanamide.



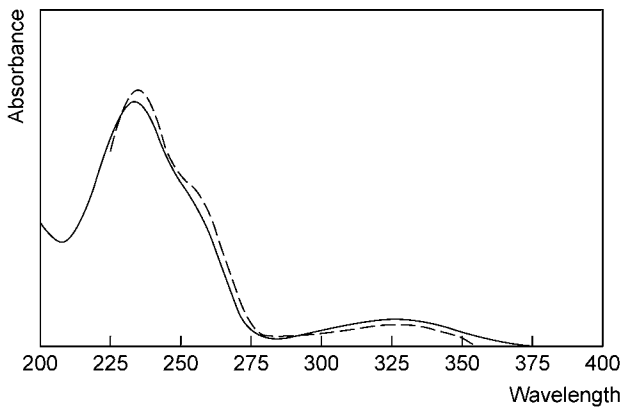
Chemical Properties Crystals. Mp 119° to 123°. pK_a 9.4. Log *P* (octanol/water), 0.1 [Dohta *et al.* 2007], −0.40 [Krzek *et al.* 2006]. Extraction yield (chlorobutane), 0.05 [Demme *et al.* 2005]. Acebutolol is stable in plasma for more than 24 h at room temperature, after 3 freeze–thaw cycles and after storage at –20° for 1 month [Maurer *et al.* 2004]. Acebutolol is stable in plasma for at least 48 h at 4° and for at least 8 weeks at –80°. Stock standard solutions were stable for at least 3 months when kept at 4° [Umezawa *et al.* 2008].
Note For a study on the degradation of acebutolol in various concentrations of sodium hydroxide, see Kwiecień *et al.* [2008] and in hydrochloric acid, see Krzek *et al.* [2006].

Acebutolol Hydrochloride

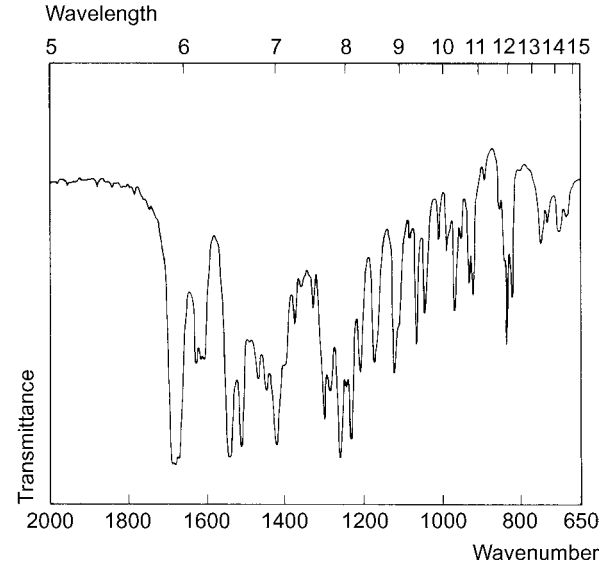
$C_{18}H_{28}N_2O_4$,HCl = 372.9
CAS—34381-68-5
Proprietary Names *Acecor*; *Acetanol*; *Neptal*; *Prent*; *Sectral*. It is an ingredient of *Secadrex*.
Chemical Properties A white or slightly cream-coloured powder. Mp 141° to 143°.

Colour Tests Methanolic potassium hydroxide—yellow; Nessler's reagent—orange (slow); sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.00; system TC—R_f 0.03; system TE—R_f 0.33; system TAE—R_f 0.13; system TAF—R_f 0.71; system TAG—R_f 0.06.

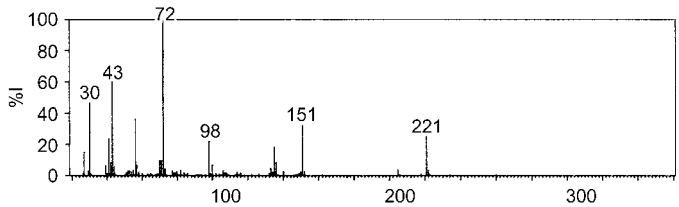
Plates: F_{254}. Solvent system: chloroform : methanol : ammonia (15 : 7 : 0.2). UV detection (λ = 240 nm). R_f 0.53 [Krzek *et al.* 2006;Kwiecień *et al.* 2008].
Gas Chromatography System GA—RI 2811, RI 2850 Art (-H_2O), RI 2450 M (phenol-); system GB—RI 2926, RI 2569 Art (-H_2O), RI 2056 M (phenol-), RI 2089 Art (desacetyl-).
High Performance Liquid Chromatography System HA—*k* 1.4; system HX—RI 325; system HX—RI 264; system HZ—RT 2.25 min; system HAA—RT 10.2 min.
Ultra Performance Liquid Chromatography Column: Acquity C_{18} BEH (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile (80 : 20 to 50 : 50 in 10 min), flow rate 0.5 mL/min. UV detection (λ = 270 nm). Retention time: 1.41 min. Limit of detection, not reported [Wren, Tchelitcheff 2006].
Ultraviolet Spectrum Aqueous acid—234 (A^1_1=655b), 320 nm (A^1_1=75b); methanol—235 (A^1_1=866a), 328 nm.



Infrared Spectrum Principal peaks at wavenumbers 1665, 1245, 1525, 1495, 1217, 1285 cm^{-1} (hydrochloride), KBr disk.



Mass Spectrum Principal ions at *m/z* 72, 43, 30, 56, 151, 221, 41, 98.



Quantification

Plasma **HPLC** Column: HyPurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 3.8) : acetonitrile (90 : 10 to 65 : 35 at 25 min for 1 min), flow rate 1.0 mL/min. DAD (λ = 220 nm). Retention time: 14.5 min. Limit of quantification, 25 µg/L; limit of detection, 6 µg/L [Delamoye *et al.* 2004]. Column: Spherisorb ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L phosphate buffer (pH 4.0) : acetonitrile (6 : 55), flow rate 1.0 mL/min. UV detection (λ = 243 nm). Limit of quantification, 20 µg/L for acebutolol and its metabolites [Buskin *et al.* 1982].

LC-MS Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium formate : acetonitrile (100 : 0 for 3 min to 0 : 100 at 4 min for 5.5 min), flow rate 0.55 mL/min. ESI, positive ion mode. Retention time: 7.2 min. Limit of detection, 1 µg/L [Umezawa *et al.* 2008]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (80 : 20 for 2.2 min to 60 : 40 at 5.5 min to 10 : 90 at 8.0 min to 80 : 20 at 9.5 min for 0.5 min), flow rate 0.4 mL/min to 0.7 mL/min at 8 min to 0.65 mL/min at 9.5 min to 0.4 mL/min at 10.0 min. APCI, SIM acquisition mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.01 mg/L [Maurer *et al.* 2004].

Urine **GC-MS** Column: Supelco Equity 1701 (10 m × 100 mm i.d., 0.1 µm). Temperature programme: 100° for 0.1 min to 300° at 2.1 min at 55°/min. Carrier gas: He, 0.6 mL/min. EI ionisation at 70 eV, full scan or SIM acquisition mode. Retention time: ~5.8 min. Limit of detection, 10 µg/L [Brunelli *et al.* 2006].

HPLC See Plasma [Buskin *et al.* 1982].

Other **LC-MS** Sewage. Column: Zorbax SB-C_8 (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: water : acetonitrile : formic acid (94.5 : 5.0 : 0.5) : acetonitrile : formic acid (99.5 : 0.5; 100 : 0 to 75 : 25 in 13 min for 13 min), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Retention time: 17.4 min. Limit of detection, 9 ng/L [Lee *et al.* 2007].

Disposition in the Body Absorbed after oral administration; bioavailability approx. 40%. The major active metabolite is the acetyl derivative diacetalol. After an oral dose, approximately 20 to 40% of the dose is excreted in the urine in 24 h, approximately 9 to 12% of the dose as unchanged drug and approximately 12 to 24% as diacetalol. Up to 60% of the dose is eliminated in the faeces as unchanged drug and diacetalol. After an IV dose, relatively more is excreted in the urine than in the faeces, and the proportion of unchanged drug in the urine is greater than after an oral dose.

Therapeutic Concentration

Ten healthy subjects ingested 200 mL grapefruit juice or water 3 times a day for 3 days and twice on day 4. On day 3, each subject ingested 400 mg acebutolol with the grapefruit juice or water. The grapefruit juice decreased the mean peak plasma concentration from 872 ± 207 to 706 ± 140 µg/L [Lilja *et al.* 2005].

After a single oral dose of 400 mg, administered to 8 subjects, a mean peak plasma concentration of 700 µg/L was attained in 2 h; a mean peak concentration of diacetalol of 800 µg/L was attained in 4 h [Gulaid *et al.* 1981].

Steady-state concentrations of 0.51 to 1.23 mg/L (mean, 0.7 mg/L) were reported during chronic oral administration of 300 mg every 6 to 8 h to 6 subjects; the concentration of diacetalol was 0.63 to 4.43 mg/L (mean, 2.2 mg/L) [Winkle *et al.* 1977].

Toxicity A fatal case of self-poisoning with acebutolol was reported with a post-mortem blood concentration of 34.7 µg/mL [Tracqui *et al.* 1992].
Bioavailability ~40%.

A

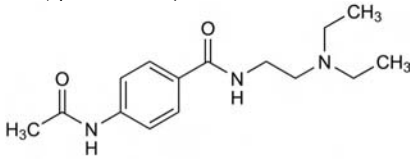
Half-life Plasma half-life acebutolol ~7 to 11 h, diacetalol ~12 h.
Volume of Distribution Approximately 1 to 3 L/kg.
Distribution in Blood Plasma : whole blood ratio, ~0.8.
Protein Binding Approximately 20%.
Dose The equivalent of 0.2 to 1.2 g of acebutolol daily.

Brunelli C *et al.* (2006). High-speed gas chromatography in doping control: fast-GC and fast-GC/MS determination of beta-adrenoceptor ligands and diuretics. *J. Sep Sci* 29: 2765–2771.
Buskin JN *et al.* (1982). High-performance liquid chromatography assay of acebutolol and two of its metabolites in plasma and urine. *J Chromatogr* 230: 438–442.
Delamoye M *et al.* (2004). Simultaneous determination of thirteen beta-blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection. *Forensic Sci Int* 141: 23–31.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT,* Seoul: 481–486.
Dohta Y *et al.* (2007). A system for Log D screening of 96-well plates using a water-plug aspiration/injection method combined with high-performance liquid chromatography-mass spectrometry. *Anal Chem* 79: 8312–8315.
Gulaid AA *et al.* (1981). The pharmacokinetics of acebutolol in man, following the oral administration of acebutolol HCl as a single dose (400 mg), and during and after repeated oral dosing (400 mg, b.d). *Biopharm Drug Dispos* 2: 103–114.
Krzek J *et al.* (2006). Stability of atenolol, acebutolol and propranolol in acidic environment depending on its diversified polarity. *Pharm Dev Technol* 11: 409–416.
Kwiecień A *et al.* (2008). Stability of chosen beta-adrenolytic drugs of different polarity in basic environment. *J AOAC Int* 91: 322–331.
Lee HB *et al.* (2007). Determination of beta-blockers and beta2-agonists in sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1148: 158–167.
Lilja JJ *et al.* (2005). Effects of grapefruit juice on the pharmacokinetics of acebutolol. *Br J Clin Pharmacol* 60: 659–663.
Maurer HH *et al.* (2004). Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization. *J Chromatogr A* 1058: 169–181.
Tracqui A *et al.* (1992). Toxicological findings in a fatal case of acebutolol self-poisoning. *J Anal Toxicol* 16: 398–400.
Umezawa H *et al.* (2008). Simultaneous determination of beta-blockers in human plasma using liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 22: 702–711.
Winkle RA *et al.* (1977). Acebutolol metabolite plasma concentration during chronic oral therapy. *Br J Clin Pharmacol* 4: 519–522.
Wren SA, Tchelitcheff P (2006). UPLC/MS for the identification of beta-blockers. *J Pharm Biomed Anal* 40: 571–580.

Acecainide

Antiarrhythmic
$C_{15}H_{23}N_3O_2$ = 277.4
CAS—32795-44-1

IUPAC Name 4-Acetamido-*N*-(2-diethylaminoethyl)benzamide
Synonyms *N*-Acetylprocainamide; NAPA.

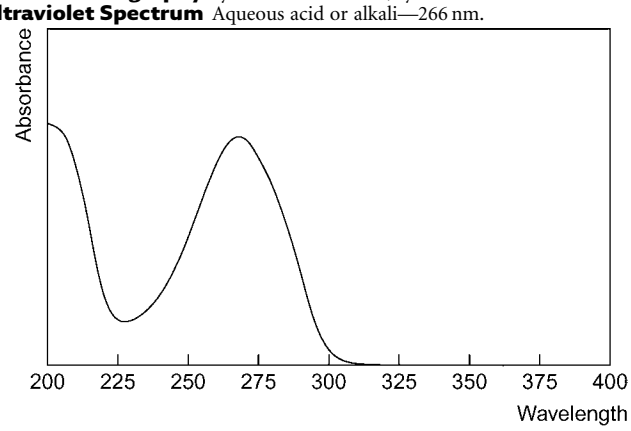


Chemical Properties Log *P* 1.99 [Hollosy *et al.* 2006].

Acecainide Hydrochloride

$C_{15}H_{23}N_3O_2$,HCl = 313.8
CAS—34118-92-8
Chemical Properties Crystals. Mp 190° to 193°.

Gas Chromatography System GA—RI 2550; system GB—RI 2724.
Ultraviolet Spectrum Aqueous acid or alkali—266 nm.



Infrared Spectrum Principal peaks at wavenumbers 1639, 1509, 1527, 1696, 1600, 1259 cm^{-1} (hydrochloride), KBr disk.
Mass Spectrum Principal ions at *m/z* 86, 58, 99, 56, 162, 132, 149, 205.
Quantification See also under Procainamide.
Plasma **GC** Column: Chromosorb W-HP 3% OV-17 (1.5 m × 1 mm i.d.). Temperature: 255°. Carrier gas: He, 5 mL/min. EI ionisation at 70 eV. Limit of detection, 500 µg/L [Strong *et al.* 1975].

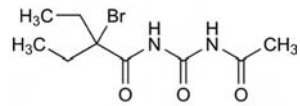
HPLC Column: Ultrasphere octyl C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : acetic acid : tetraethylammonium (78 : 22 : 1 : 0.01), flow rate 1.3 mL/min. UV detection (λ = 280 nm). Retention time: 6.7 min. Limit of quantification, 4 µg/L; limit of detection, 2 µg/L [Lessard *et al.* 1998].
Disposition in the Body Readily absorbed after oral administration; bioavailability approx. 85%. Up to 85% of a dose is excreted in the urine as unchanged drug in 48 h, with only 2 to 3% excreted as procainamide and <1% as the monodesethyl metabolite. Acecainide is the principal active metabolite of procainamide.
Therapeutic Concentration In plasma, usually in the range 10 to 30 mg/L. After a single oral dose of 1.5 g to nine subjects, peak plasma concentrations of 7.4 to 17.2 mg/L (mean, 12 mg/L), were attained in 1.5 to 4 h [Lee *et al.* 1976]. Following oral administration of 1 g four times a day to 8 subjects, steady-state plasma concentrations of 9.3 to 25.5 mg/L (mean, 18 mg/L) were reported [Atkinson *et al.* 1977].
Toxicity Toxic effects may occur at plasma concentrations within the therapeutic range.
Bioavailability Approximately 85%
Half-life Plasma half-life, approximately 6 to 9 h in normal subjects; increased in subjects with heart disease or renal impairment.
Volume of Distribution Approximately 1.5 L/kg.
Clearance Plasma clearance, approx. 3 mL/min/kg.
Protein Binding Approximately 10%.
Note For a review of the pharmacokinetics of acecainide, see Connolly and Kates [1982].
Dose Acecainide has been given in doses of 2 to 6 g daily.

Atkinson AJ Jr *et al.* (1977). Dose-ranging trial of *N*-acetylprocainamide in patients with premature ventricular contractions. *Clin Pharmacol Ther* 21: 575–587.
Connolly S, Kates JRE (1982). Clinical pharmacokinetics of *N*-acetylprocainamide. *Clin Pharmacokinet* 7: 206–220.
Hollosy F *et al.* (2006). Estimation of volume of distribution in humans from high throughput HPLC-based measurements of human serum albumin binding and immobilized artificial membrane partitioning. *J Med Chem* 49: 6958–6971.
Lee WK *et al.* (1976). Antiarrhythmic efficacy of *N*-acetylprocainamide in patients with premature ventricular contractions. *Clin Pharmacol Ther* 19: 508–514.
Lessard E *et al.* (1998). Improved high-performance liquid chromatographic assay for the determination of procainamide and its *N*-acetylated metabolite in plasma: application to a single-dose pharmacokinetic study. *J Chromatogr Sci* 36: 49–54.
Strong JM *et al.* (1975). Pharmacokinetics in man of the *N*-acetylated metabolite of procainamide. *J Pharmacokinet Biopharm* 3: 223–235.

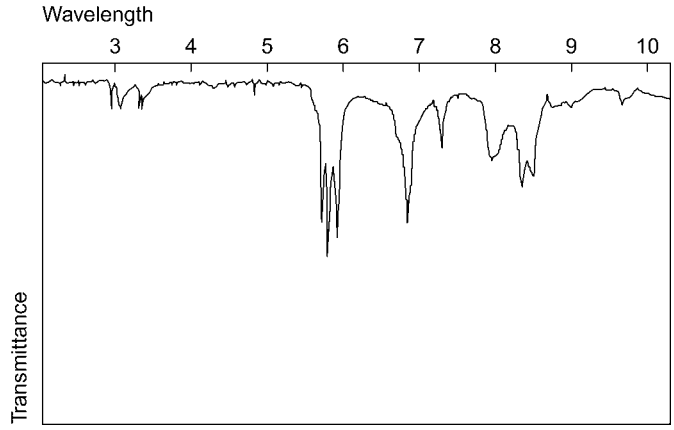
Acecarbromal

Sedative, Hypnotic
$C_9H_{15}BrN_2O_3$ = 279.1
CAS—77-66-7

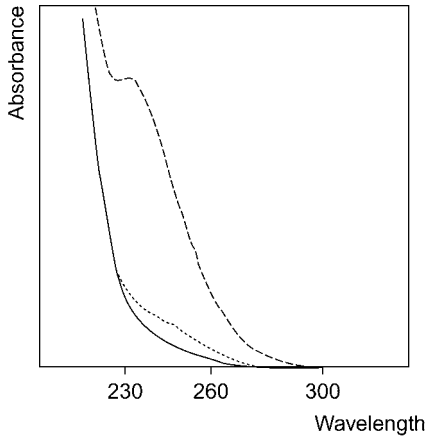
IUPAC Name *N*-(Acetylcarbamoyl]-2-bromo-2-ethylbutanamide
Synonyms *N*-[(Acetylamino)carbonyl]-2-bromo-2-ethylbutanamide; acetylbromodiethylacetylcarbamide; *N*-acetyl-*N*-bromodiethylacetylurea; *N*-acetyl-*N*′-α-bromo-α-ethylbutyrylcarbamide; acetylcarbromal.
Proprietary Names *Abasin*; *Sedamyl.*



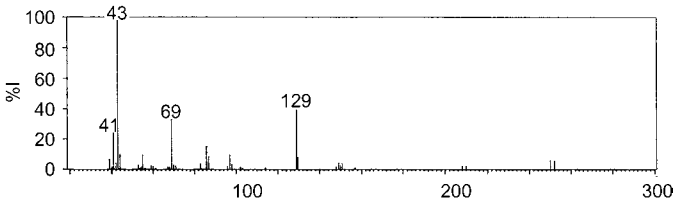
Chemical Properties Colourless crystals or white crystalline powder. Mp 109°. Soluble 1 in 1000 of water; soluble in ethanol, chloroform and ether.
Colour Test Nessler's reagent—brown-orange (slow).
Thin-layer Chromatography System TD—R_f 0.49; system TE—R_f 0.57; system TF—R_f 0.48; system TAD—R_f 0.60; system TAE—R_f 0.84; system TAF—R_f 0.84; system TAJ—R_f 0.61; system TAK—R_f 0.58; system TAL—R_f 0.90.
Gas Chromatography System GA—acecarbromal RI 1720, M (carbromal) RI 1513, M (desbromocarbromal) RI 1380.
High Performance Liquid Chromatography System HX—RI 429; system HY—RI 374.
Infrared Spectrum Principal peaks at wavenumbers 1776, 1730, 1183, 1267, 1149, 1236 cm^{-1} (Nujol mull).



Ultraviolet Spectrum Aqueous alkali—230 nm.



Mass Spectrum Principal ions at *m/z* 43, 129, 69, 41, 86, 97, 55, 44.



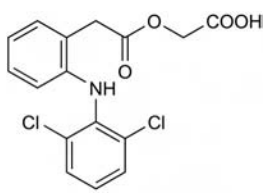
Quantification
Plasma **HPLC** Column: LiChrosorb Si 60 (30 cm × 3 mm i.d., 5 µm). Mobile phase: *n*-hexane : methylene chloride : ethanol (235 : 10.5 : 7.5), flow rate 1.2 mL/min. Retention time: 4.5 min. Limit of detection not reported [Hobel, Bender 1977].
Urine **GC-MS** Column: HP capillary (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 100° to 310° at 30°/min. Carrier gas: He, 1 mL/min. EI ionisation at 70 eV, scan mode. Limit of detection, 10–20 µg/L [Maurer 1990].
HPLC See Plasma [Hobel, Bender 1977].
Dose 0.75 to 1.5 g daily.

Hobel M, Bender G (1977). Separation and quantitative determination of acecarbromal, carbromal, and bromisoval as well as their main metabolites by means of high-pressure liquid chromatographic analysis. *Arch Toxicol* 37: 307–312.
Maurer HH (1990). Identification and differentiation of barbiturates, other sedative-hypnotics and their metabolites in urine integrated in a general screening procedure using computerized gas chromatography-mass spectrometry. *J Chromatogr* 530: 307–326.

Aceclofenac

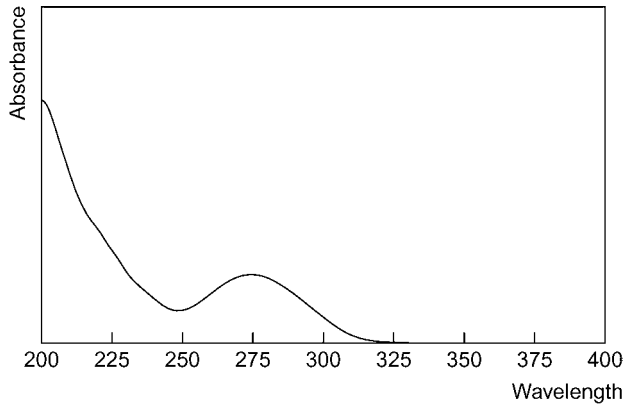
Antiinflammatory
$C_{16}H_{13}Cl_2NO_4 = 354.2$
CAS—89796-99-6

IUPAC Name 2-[2-[2-(2,6-Dichloroanilino)phenyl]acetyl]oxyacetic acid
Synonyms Aceclofenacum; 2-[(2,6-dichlorophenylamino)phenyl]acetoxyacetic acid; PR-82/3.
Proprietary Names *Airtal*; *Barcan*; *Biofenac*; *Difucrem*; *Falcol*; *Gerbin*; *Preservex*; *Sanein*; *Tresquim*.



Chemical Properties A white to almost white crystalline powder. Mp 149° to 150°. It is practically insoluble in water; soluble in alcohol and methyl alcohol; freely soluble in acetone and dimethylformamide.
Thin-layer Chromatography Plate: silica gel. Mobile phase: chloroform: methanol:acetic acid (90:10:1). Aceclofenac R_f 0.44; 4′-OH aceclofenac R_f 0.25 [Bort *et al.* 1996].
High Performance Liquid Chromatography System HZ—retention time 10.7 min.

Ultraviolet Spectrum Ethanol—275 nm.



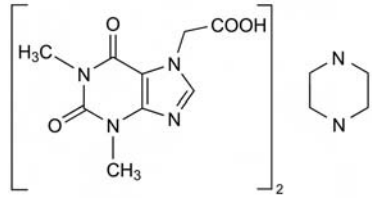
Quantification
Plasma **HPLC** UV detection (λ=278 nm). Limit of detection, 0.010 mg/L [Lee *et al.* 2000]. Column: Spherisorb ODS–2 C_{18} (200 × 4.6 mm, 5 µm). Mobile phase: 0.02% triethanolamine in 100 mmol/L phosphate buffer, pH 7.4:acetonitrile (75:25), flow rate 1.0 mL/min. UV detection (λ=282 nm). Retention time: aceclofenac, 23.4 min; 4′-hydroxyaceclofenac, 7.6 min [Bort *et al.* 1996]. Limit of detection, 6.5 mg/L [Bort *et al.* 1996].
Urine **HPLC** See Plasma [Bort *et al.* 1996].
Disposition in the Body Aceclofenac is well absorbed after oral administration and circulates mainly as the unchanged drug. It is metabolised in hepatocytes and microsomes to 4′-hydroxyaceclofenac, which can undergo further conjugation. Other metabolites also identified include diclofenac and 4-hydroxydiclofenac, and their hydroxylated derivatives. Aceclofenac penetrates into synovial fluid and concentrations here reach approximately 57% of those in plasma. 70% of an administered dose is excreted in urine as glucuronides of aceclofenac and diclofenac.
Therapeutic Concentration
Eight healthy, male, Caucasian volunteers, aged between 26 and 68 years, were orally administered a 100 mg dose of aceclofenac. Peak plasma concentrations of 7.6 ± 1.3 mg/L were reached within 2.6 ± 1.8 h [Bort *et al.* 1996].
Toxicity Type III hypersensitivity reactions have been reported.
Half-life Elimination half-life is approximately 4 h.
Volume of Distribution Apparent volume of distribution is ~25 L.
Protein Binding >99% protein bound.
Dose A usual dose of 100 mg twice daily is administered with a reduction to 100 mg daily for patients with hepatic impairment.

Bort R *et al.* (1996). Metabolism of aceclofenac in humans. *Drug Metab Dispos* 24(8): 834–841.
Lee HS *et al.* (2000). Simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using column-switching. *J Pharm Biomed Anal* 23: 775–781.

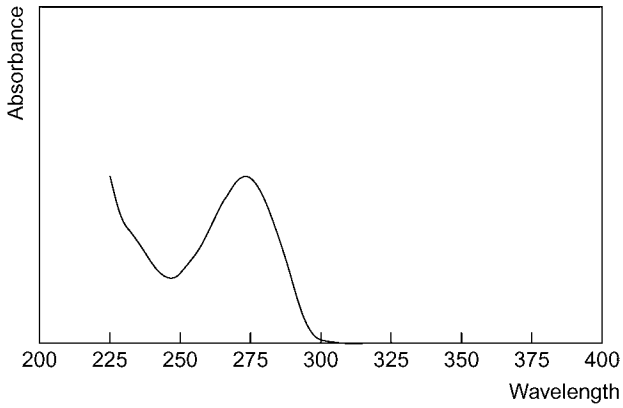
Acefylline Piperazine

Xanthine Bronchodilator
$(C_9H_{10}N_4O_4)_2,C_4H_{10}N_2 = 562.5$
CAS—18833-13-1

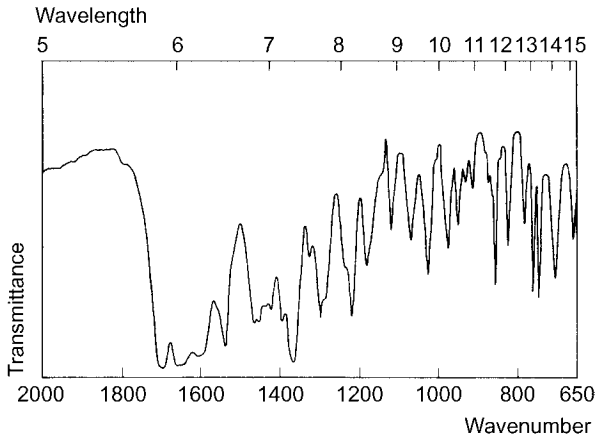
IUPAC Name 2-(1,3-Dimethyl-2,6-dioxopurin-7-yl)acetic acid; piperazine
Synonyms Acepifylline; Piperazine bis(theophyllin-7-ylacetate); piperazine theophylline ethanoate.
Proprietary Names *Dynaphylline*; *Etaphylline*; *Etophylate*.



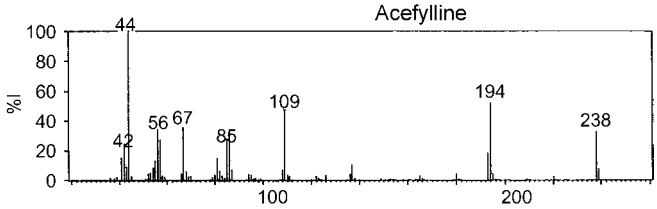
Chemical Properties A white crystalline powder. Mp 260°. Freely soluble in water; slightly soluble in ethanol.
Colour Test Amalic acid test—orange/violet.
Thin-layer Chromatography System TA—R_f 0.04; system TB—R_f 0.01; system TC—R_f 0.01; system TL—R_f 0.01 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1000.
Ultraviolet Spectrum Aqueous acid—274 nm (A^1_1=317a).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1660, 1612, 1538, 1298, 1219 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 194, 109, 67, 86, 238, 56, 85.

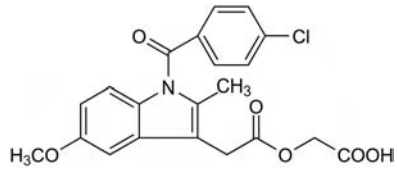


Quantification
Plasma **HPLC** UV detection. Limit of detection, 25 µg/L for theophylline-7-acetic acid [Sved *et al.* 1981].
Urine **GC** FID [Zuidema, Hilbers 1980].
Disposition in the Body Very poorly absorbed after oral administration. It is metabolised to theophylline-7-acetic acid but does not appear to be converted to theophylline.
Therapeutic Concentration Following a single oral dose of 1 g to 2 subjects, peak plasma concentrations of 0.28 and 0.14 µg/mL of theophylline-7-acetic acid were attained in 1.5 and 2 h, respectively [Sved *et al.* 1981].
Half-life Plasma half-life, theophylline-7-acetic acid about 1 to 2 h.
Volume of Distribution Theophylline-7-acetic acid 0.5 L/kg (single subject).
Clearance Plasma clearance, theophylline-7-acetic acid 7 mL/min/kg (single subject).
Dose 1.5 to 3 g daily.

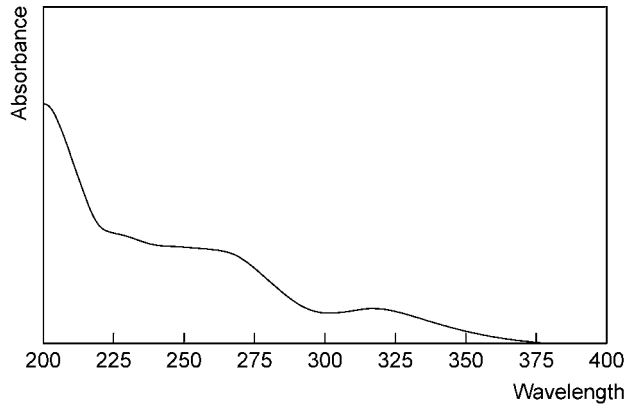
Sved S *et al.* (1981). The assay and absorption kinetics of oral theophylline-7–acetic acid in the human. *Biopharm Drug Disp* 2: 177–184.
Zuidema J, Hilbers H (1980). Gas-liquid chromatographic determination of acephylline in urine. *J Chromatogr* 182 (8): 445–447.

Acemetacin

NSAID
$C_{21}H_{18}ClNO_6$ = 415.8
CAS—53164-05-9
IUPAC Name 2-[2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetyl]oxyacetic acid
Synonyms 1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid carboxymethyl ester; TV-1322, TVX-1322.
Proprietary Names *Acemix*; *Altren*; *Emflex*; *Espledol*; *Oldan*; *Peran*; *Rantudil*; *Rheumibis*; *Rheutrop*; *Solart*; *Tilur*.



Chemical Properties Very fine, pale yellow crystals from petroleum ether. Mp 150° to 153°. pK_a 2.9. Log *P* (octanol/water), 4.13.
Gas Chromatography System GA—acemetacin RI 0, acemetacin-ME RI 3150, acemetacin-ET RI 3220, acemetacin art-ME_2 RI 2390.
Ultraviolet Spectrum



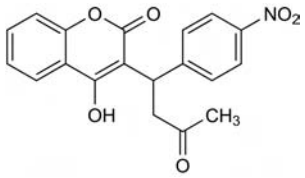
Mass Spectrum Principal ions at *m/z* 355, 139, 113, 371, 341, 415.
Quantification
Blood **HPLC** Column: Hypersil ODS (150 × 4.5 mm i.d., 5 µm). Mobile phase: methanol:0.02 mol/L potassium dihydrogen phosphate (57:43), pH 4.5, flow rate 1.25 mL/min. UV detection (λ = 245 nm). Retention time: acemetacin, 5.58 min; indometacin, 9.03 min [Notarianni, Collins 1987]. Limit of detection, 10 µg/L for acemetacin and indometacin, 20 µg/L for *p*-chlorobenzoic acid [Notarianni, Collins 1987]. UV detection. Limit of detection, 25 µg/L for acemetacin and indometacin [Schöllnhammer *et al.* 1986].
Plasma **HPLC** UV detection. Limit of detection, 25 µg/L for acemetacin and indometacin [Schöllnhammer *et al.* 1986].
Disposition in the Body Acemetacin is rapidly and well absorbed after oral administration. It is metabolised to indometacin (major metabolite) and *p*-chlorobenzoic acid (PCBA, minor metabolite and degradation product) by hydrolysis in the liver. The drug is eliminated by both hepatic and renal routes; the pharmacokinetics are unaffected by moderate renal or hepatic impairment.
Therapeutic Concentration
Following administration of 60 mg orally twice a day for 8 days to 10 young (mean age of 30.9 years, between 22 and 44) and 10 elderly subjects (mean 75.9 years, ranging between 68 and 80 years), mean peak plasma concentrations of 187 and 277 µg/L were reached after 2.6 and 2.5 h in the 2 groups, respectively, on day 8 of the study. The peak concentrations for the metabolite, indometacin, were 705 µg/L for the young and 804 µg/L for the elderly. These were observed at 3.1 and 2.9 h, respectively. Each individual suffered with osteoarthritis and was fasted overnight before administration of the drug with a standard breakfast [Jones *et al.* 1991].
Half-life Plasma, 1 h.
Dose 120 to 180 mg daily.

Jones RW *et al.* (1991). The comparative pharmacokinetics of acemetacin in young subjects and elderly patients. *Br J Clin Pharmacol* 31: 543–545.
Notarianni LJ, Collins AJ (1987). Method for the determination of acemetacin, a non-steroidal anti-inflammatory drug, in plasma by high-performance liquid chromatography. *J Chromatogr* 413: 305–308.
Schöllnhammer G *et al.* (1986). Quantitative determination of acemetacin and its metabolite indometacin in blood and plasma by column liquid chromatography. *J Chromatogr* 375: 331–338.

Acenocoumarol

Anticoagulant
$C_{19}H_{15}NO_6$ = 353.3
CAS—152-72-7
IUPAC Name 2-Hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]chromen-4-one
Synonyms Acenocumarin; 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2*H*-1-benzopyran-2-one; nicoumalone; G-23350.

Proprietary Names *Sinthrome*; *Sintrom*.



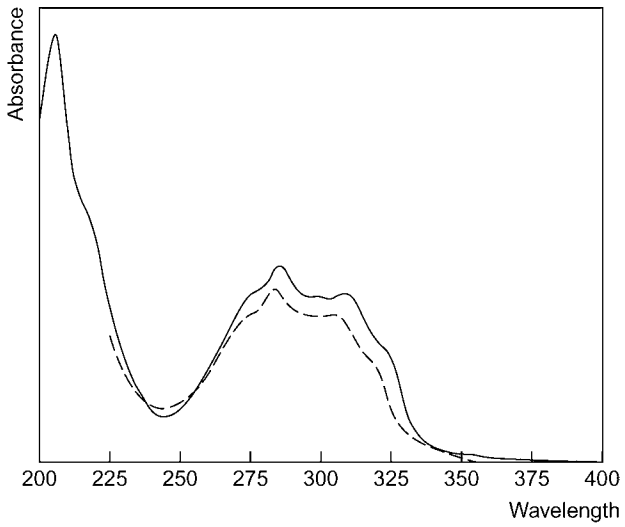
Chemical Properties An almost white to buff-coloured powder. Mp 196° to 199°. Sparingly soluble in water; soluble 1 in 400 of ethanol and 1 in 200 of chloroform; soluble in solutions of alkali hydroxides. pK_a 4.7. Log *P* (octanol/water), 2.0.

Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.52; system TE—R_f 0.16; system TF—R_f 0.48; system TAD—R_f 0.60; system TAE—R_f 0.92; system TAF—R_f 0.92; system TAJ—R_f 0.68; system TAK—R_f 0.51; system TAL—R_f 0.92 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

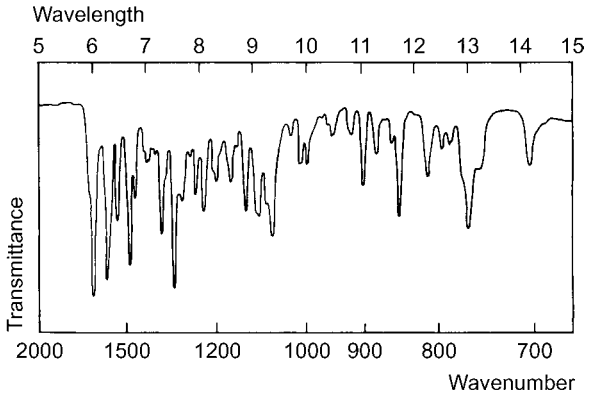
Gas Chromatography System GA—acenocoumarol-Me RI 3035; M (amino-)-Me_3 RI 2985; M (acetamido-)-Me_2 RI 3265; M (OH-)isomer 1-Me_2 RI 3350; M (OH-)isomer 2-Me_2 RI 3500.

High Performance Liquid Chromatography System HX—RI 563; system HZ—retention time 9.1 min; system HAA—retention time 20.1 min.

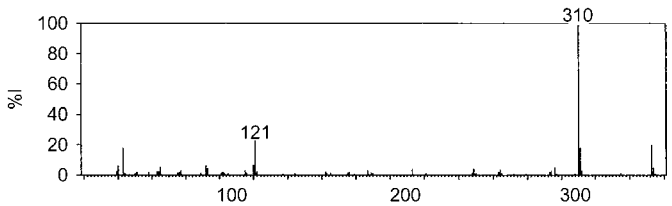
Ultraviolet Spectrum Methanol—283 (A^1_1=640a), 306 nm.



Infrared Spectrum Principal peaks at wavenumbers 1686, 1616, 1508, 1070, 762, 1570 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 310, 121, 353, 311, 43, 120, 92, 296.



Quantification

Plasma **TLC** Fluorescence detection. Limit of detection, 10 µg/L [van Kempen *et al.* 1978].

GC ECD detection. Limit of detection, 500 pg [Bianchetti *et al.* 1976].

GC-MS Limit of detection, 0.8 µg/L [Pommier *et al.* 1994].

HPLC Acenocoumarol enantiomers [Gill *et al.* 1988].

LC-MS Limit of detection, 10 µg/L [Kollroser, Schober 2002].

Serum **HPLC** UV detection. Limit of detection, 15 µg/L [de Wolff *et al.* 1980].

Disposition in the Body Acenocoumarol is readily absorbed after oral administration. It is extensively metabolised by reduction to a number of metabolites including the amino derivative, and the two diastereoisomers of 4-hydroxy-3-[1-(4-nitrophenyl)-3-hydroxybutyl]coumarin. Two further inactive metabolites, the 6-hydroxy- and 7-hydroxycoumarin derivatives have been identified in urine. About 50 to 60% of a dose is excreted in the urine in 48 h, mostly as metabolites, with less than 1% as unchanged drug; about 30% of a dose is eliminated in the faeces.

Therapeutic Concentration

Following a single oral dose of 10 mg to 3 subjects, peak plasma concentrations of about 0.3 mg/L were attained in 2 to 3 h; the amino metabolite was not detected in plasma [Thijssen, Baars 1983].

After a single oral dose of 12 mg to 2 subjects, peak plasma acenocoumarol concentrations of 0.17 and 0.41 mg/L were attained in 3 h; peak concentrations of the amino metabolite of 0.28 and 0.16 mg/L were reported after 6 to 10 h [Dieterle *et al.* 1977].

Following oral administration of 2.5 mg twice daily to 2 subjects, steady-state plasma concentrations of 0.02 to 0.07 mg/L were reported [Bianchetti *et al.* 1976].

Half-life Plasma half-life, about 8 h.

Volume of Distribution About 0.3 L/kg.

Clearance Plasma clearance, about 0.5 mL/min/kg.

Protein Binding About 98%.

Dose Maintenance, 1 to 8 mg daily.

Bianchetti G *et al.* (1976). Gas chromatographic determination of acenocoumarin in human plasma. *J Chromatogr* 124: 331–335.

de Wolff FA *et al.* (1980). Determination of nanogram levels of the anticoagulant acenocoumarin in serum by high-performance liquid chromatography. *J Anal Toxicol* 4: 156–159.

Dieterle W *et al.* (1977). Biotransformation and pharmacokinetics of acenocoumarol (Sintrom) in man. *Eur J Clin Pharmacol* 11: 367–375.

Gill TS *et al.* (1988). Stereospecific assay of nicoumalone: application to pharmacokinetic studies in man. *Br J Clin Pharmacol* 25: 591–598.

Kollroser M, Schober C (2002). Determination of coumarin-type anticoagulants in human plasma by HPLC-electrospray ionization tandem mass spectrometry with an ion trap detector. *Clin Chem* 48: 84–91 and 1372.

Pommier F *et al.* (1994). Determination of acenocoumarol in human plasma by capillary gas chromatography with mass-selective detection. *J Chromatogr* 654: 35–41.

Thijssen HH, Baars LG (1983). Active metabolites of acenocoumarol: do they contribute to the therapeutic effect? *Br J Clin Pharmacol* 16: 491–496.

van Kempen GM *et al.* (1978). Quantitative and qualitative analysis of the anticoagulant acenocoumarol in human plasma. *J Chromatogr* 145: 332–335.

Acepromazine

Phenothiazine, Tranquilliser

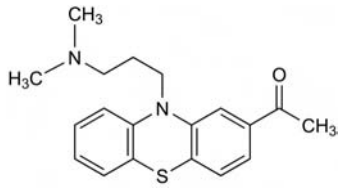
$C_{19}H_{22}N_2OS$ = 326.5

CAS—61-00-7

IUPAC Name 1-[10-[3-(Dimethylamino)propyl]phenothiazin-2-yl]ethanone

Synonyms Acetazine; acetylpromazine; 1522-CB; 1-[10-[3-(dimethylamino) propyl]-10*H*-phenothiazin-2-yl]ethanone.

Proprietary Name *Plégicil*



Chemical Properties An orange-coloured oil. Acepromazine is converted to 2-(1-hydroxyethyl)promazine in whole blood [Elliott, Hale 1999].

Acepromazine Maleate

$C_{19}H_{22}N_2OS,C_4H_4O_4$ = 442.5

CAS—3598-37-6

Proprietary Names *Atravet*; *Calmivet*; *Plégicil*; *PromAce*; *Sedalin*; *Vetranquil*. It is an ingredient of *Immobilon* (for large animals).

Chemical Properties A yellow crystalline powder. Mp 135° to 136°. Soluble 1 in 27 of water, 1 in 13 of ethanol and 1 in 3 of chloroform; slightly soluble in ether and light petroleum. pK_a 9.3. Log *P* (octanol/pH 7.4), 2.3.

Colour Tests Formaldehyde-sulfuric acid—blue-violet; Forrest reagent—red; FPN reagent—brown-orange; Liebermann's reagent—brown; Mandelin's test—green→red; Marquis test—yellow-green-red.

Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.26; system TC—R_f 0.24; system TE—R_f 0.63; system TAE—R_f 0.28; system TAG—R_f 0.12 (acidified iodoplatinate solution, positive).

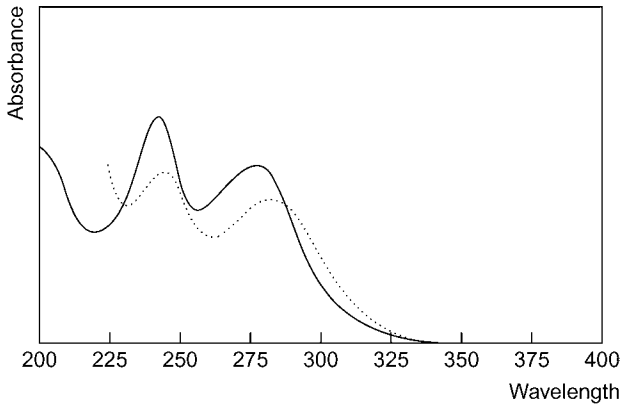
Gas Chromatography System GA—RI 2735 acepromazine, RI 2720 M (dihydro-)-H_2O; system GB—RI 2844 acepromazine, RI 2824 M (dihydro-)-H_2O, RI 3160 M (7-OH-); system GF—RI 3230.

Column: Fused silica capillary (1) J & W DB-1 (15 m × 0.32 mm, 0.25 μm), (2) J & W DB-1301 (15 m × 0.25 mm, 0.25 μm), (3) J & W DB-1 capillary (10 m × 0.18 mm, 0.4 μm). Temperature programme: 120° to 280° at 20°/min (1 and 2), 80° to 300° at 20°/min (3). Carrier gas: He. Detection: NPD (1 and 2); MS (*m/z* 41 to 550) (3). Retention time: 8.1 min (1); 9.4 min (2); 12.2 min (3) [Stowell 1998].

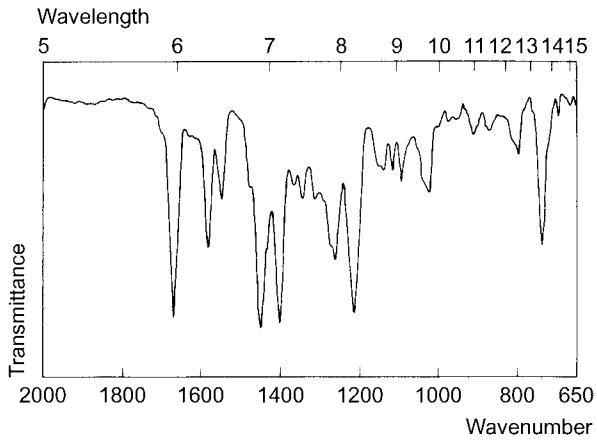
High Performance Liquid Chromatography System HA—*k* 4.1; system HY—RI 350; system HAA—RT 10.8 min.

Column: Spherisorb S5OD/CN (150 × 4.6 mm). Mobile phase: 0 to 70% acetonitrile in 15 min for 3 min, flow rate 2 mL/min. UV detection (λ = 200 to 595 nm). Retention time: 10.5 min [Elliott, Hale 1999].

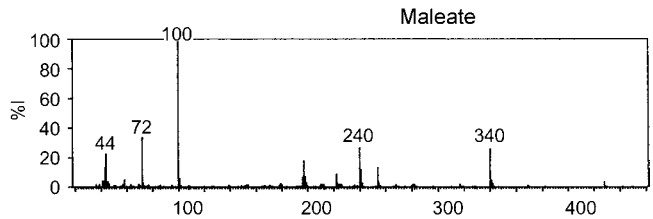
Ultraviolet Spectrum Aqueous acid—243 (A_1^1 = 765a), 279 nm; aqueous alkali—245, 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 1675, 1220, 1265, 1587, 746, 1562 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 100, 72, 240, 340, 44, 197, 254, 43 (maleate).



Quantification

Blood **GC-MS** Column: J & W DB-1 (15 × 0.32 mm, 0.25 μm). Temperature programme: 120° to 280° at 20°/min. Carrier gas: He, 9 psi. NPD. Retention time: 8.1 min. Limit of detection not reported [Stowell 1998].

HPLC Column: Spherisorb S5OD/CN (150 × 4.6 mm i.d.). Mobile phase: 0 to 70% acetonitrile in 15 min, for 3 min, flow rate 2 mL/min. UV detection. Limit of detection, 5 μg/L [Elliott, Hale 1999]. Column: NovaPak C_{18} (300 × 3.9 mm i.d., 4 μm). Mobile phase: methanol : tetrahydrofuran : 10 mmol/L potassium dihydrogen phosphate (pH 2.6; 65 : 5 : 30), flow rate 0.8 mL/min. DAD. Limit of detection not reported [Tracqui *et al.* 1993].

Serum **LC-MS** Column: Betasil C_{18} reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1% trifluoroacetic acid (50 : 50 for 2.5 min to 65 : 35 in 1 min for 2 min to 50 : 50 for 0.5 min), flow rate 0.6 mL/min. APCI, positive ion mode, SIM acquisition mode. Retention time, 4.5 min. Limit of quantification, 5.0 μg/L; limit of detection, 2.5 μg/L [Miksa *et al.* 2005].

Stomach Contents **GC-MS** See Blood [Stowell 1998].

Bile **GC-MS** See Blood [Stowell 1998].

Hair **LC-MS** Column: Xterra MS C_{18} reversed phase (50 × 2.1 mm i.d., 5 μm). Mobile phase: 2 mmol/L ammonium formate (pH 3.0) : 2 mmol/L ammonium formate (pH 3.0)-acetonitrile (10 : 90; 85 : 15 at 1 min to 70 : 30 at 6 min to 10 : 90 at 7 min to 85 : 15 at 8 min). ESI, positive ion mode. Limit of quantification, 10 ng/g, limit of detection, 5 ng/g [Gaulier *et al.* 2008].

Liver **GC-MS** See Blood [Stowell 1998].

Disposition in the Body Acepromazine is converted to 2-(1-hydroxyethyl)promazine by red blood cells, and 2-(1-hydroxyethyl)promazine, which may be the major unconjugated metabolite of acepromazine in humans.

Toxicity

A 43-year-old woman was found dead. Acepromazine was found in chest-cavity blood, the liver, brain and bile at concentrations of 0.6, 3.0, 0.4 and 6.5 mg/L, respectively. The stomach contents contained a total of 2.5 mg acepromazine [Stowell 1998].

A 28-year-old woman was found dead and toxicological analysis showed a blood concentration of 2.4 mg/L acepromazine and 3.29 mg/L zolpidem, urine concentration of 0.37 and 2.54 mg/L, respectively, and gastric contents concentration of 20.1 and 34.3 mg/L, respectively [Tracqui *et al.* 1993].

Note For a report of an overdose with Large Animal Immobilon (2.45 g/L etorphine hydrochloride and 10 g/L acepromazine maleate), see Sterken *et al.* [2004].

Dose Acepromazine has been given in doses of 6 to 60 mg daily.

Elliott SP, Hale KA (1999). A previously unidentified acepromazine metabolite in humans: implications for the measurement of acepromazine in blood. *J Anal Toxicol* 23: 367–371.

Gaulier JM *et al.* (2008). Identification of acepromazine in hair: an illustration of the difficulties encountered in investigating drug-facilitated crimes. *J Forensic Sci* 53: 755–759.

Miksa IR *et al.* (2005). Determination of acepromazine, ketamine, medetomidine, and xylazine in serum: multi-residue screening by liquid chromatography-mass spectrometry. *J Anal Toxicol* 29: 544–551.

Sterken J *et al.* (2004). Intentional overdose of Large Animal Immobilon. *Eur J Emerg Med* 11: 298–301.

Stowell LI (1998). Suicide with the veterinary drug acepromazine. *J Anal Toxicol* 22: 166–168.

Tracqui A *et al.* (1993). A fatality involving two unusual compounds: zolpidem and acepromazine. *Am J Forensic Med Pathol* 14: 309–312.

Acetaldehyde

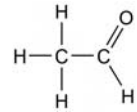
Aldehyde

C_2H_4O = 44.1

CAS—75-07-0

IUPAC Name Acetaldehyde

Synonyms Acetic aldehyde; aldehyde; ethanal; ethyl aldehyde.



Chemical Properties A colourless inflammable liquid. Mp −123.5°. Bp 21.0°. Refractive index, at 20°, 1.3316. Miscible with water, ethanol, and ether.

Colour Tests Potassium dichromate (Method 2)—green; Schiff's reagent—violet; sodium nitroprusside (method 2)—red.

Gas Chromatography System GA—RI 372; system GI—retention time 0.7 min.

Column: Alltech Carbowax megabore capillary (30 m × 0.54 mm, 1.2 μm). Carrier gas: He, 7.9 mL/min. Internal standard: 1-propanol. FID. Retention time: 1.44 min [McCarver-May, Durisin 1997].

Mass Spectrum Principal ions at *m/z* 29, 44, 43, 42, 26, 41, 28, 27.

Quantification

Blood **GC** FID head-space analysis. Limit of detection, 18 μg/L [Christensen *et al.* 1981].

Disposition in the Body Acetaldehyde is a major intermediate metabolite of ethanol and is also a metabolite of metaldehyde, paraldehyde and phenacetin. It undergoes further metabolism by oxidation to acetic acid and, eventually, to carbon dioxide and water. A minor pathway involves condensation with pyruvic acid to form acetoin.

Toxicity Acetaldehyde is more toxic than ethanol or acetic acid. The maximum permissible atmospheric concentration is 100 ppm.

Christensen JM *et al.* (1981). Determination of acetaldehyde in human blood by a gas chromatographic method with negligible artefactual acetaldehyde formation. *Clinica Chim Acta* 116: 389–395.

McCarver-May DG, Durisin L (1997). An accurate, automated, simultaneous gas chromatographic headspace measurement of whole blood ethanol and acetaldehyde for human in vivo studies. *J Anal Toxicol* 21: 134–141.

Acetanilide

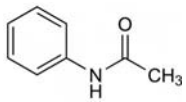
Analgesic

C_8H_9NO = 135.2

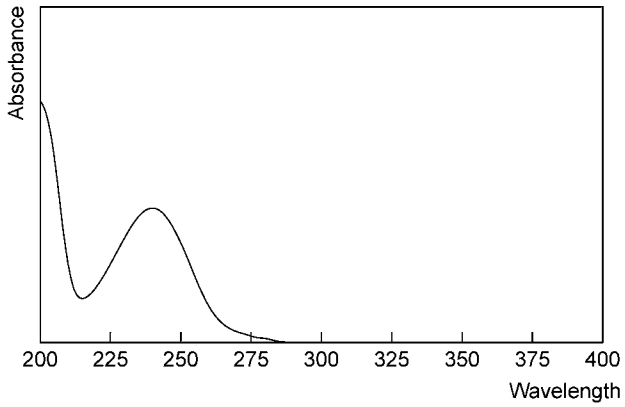
CAS—103-84-4

IUPAC Name *N*-Phenylacetamide

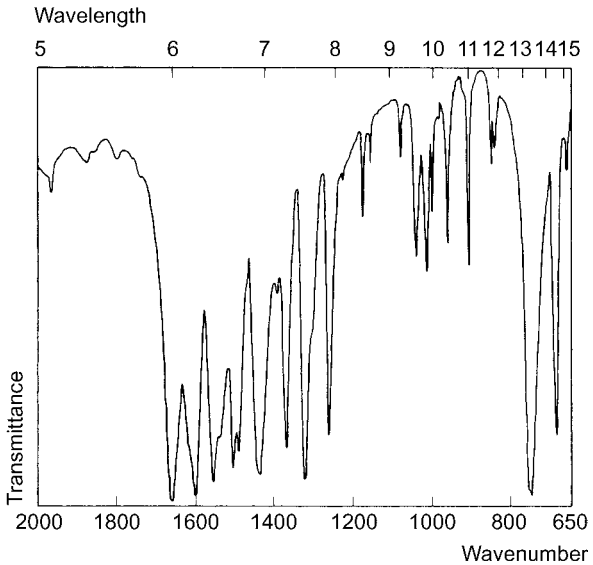
Synonyms Acetylaniline; acetylaminobenzene; antifebrin.



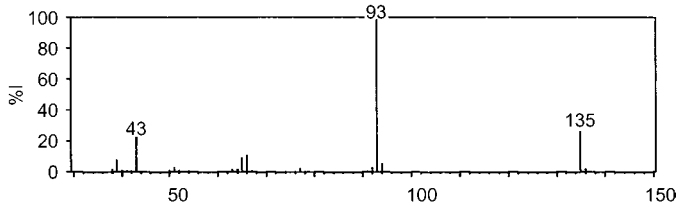
Chemical Properties Colourless shining lamellar crystals or white crystalline powder. Mp 113° to 115°. Soluble 1 in 200 of water, 1 in 20 of boiling water, 1 in 3.5 of ethanol, 1 in 8 of chloroform and 1 in 50 of ether; soluble in acetone. pK_a 0.6 (25°). Log *P* (octanol/water), 1.2.
Colour Test Liebermann's reagent—red→orange
Thin-layer Chromatography System TD—R_f 0.45; system TE—R_f 0.70; system TF—R_f 0.45; system TAD—R_f 0.52; system TAE—R_f 0.80; system TAF—R_f 0.84 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—acetanilide RI 1368, paracetamol RI 1687; system GB—RI 1400.
High Performance Liquid Chromatography System HA—*k* 0.1; system HD—acetanilide *k* 0.5, paracetamol *k* 0.1; system HW—acetanilide *k* 2.30, paracetamol *k* 0.32; system HY—RI 281.
Ultraviolet Spectrum Aqueous acid—239 nm ($A_1^1 = 815$a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1598, 752, 1538, 1315, 1500 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 93, 135, 43, 66, 65, 39, 94, 92; paracetamol 109, 151, 43, 80, 108, 81, 53, 52.



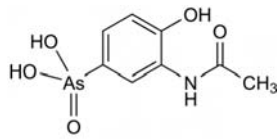
Quantification
Plasma **GC** Column: 3% OV-17 Gas Chrom Q 100-120. Temperature: 195°. Carrier gas: N_2, 50 mL/min. FID. Retention time: 2.5 min. Limit of detection, not reported [Buchanan *et al.* 1980].
Disposition in the Body Rapidly and completely absorbed after oral administration, with maximum plasma concentrations achieved after 1 to 2 h. It is distributed throughout the body. The main metabolic reaction is oxidation to paracetamol through which the analgesic and antipyretic effects of the drug are chiefly exerted; paracetamol is then conjugated with glucuronic acid or sulfate. A minor reaction is deacetylation of acetanilide to aniline. Approximately 0.1% of an oral dose is excreted unchanged in the urine in 24 h, approx. 4% as free paracetamol, 80% as paracetamol conjugates, and 0.05% as aniline. Approximately 0.1% of an oral dose may be eliminated in the faeces.
Toxicity The main toxic effect is the formation of methaemoglobin via production of aniline or other toxic metabolites.
Half-life Plasma half-life, approx. 1.5 h.
Volume of Distribution Approximately 0.7 L/kg.
Clearance Plasma clearance, approx. 6 mL/min/kg.
Protein Binding Not significantly bound.
Dose Acetanilide was formerly given in doses of 0.36 to 1.2 g daily.

Buchanan N *et al.* (1980). Acetanilide pharmacokinetics in kwashiorkor. *Br J Clin Pharmacol* 9: 525–526.

Acetarsone

Antiprotozoal
$C_8H_{10}AsNO_5 = 275.1$
CAS—97-44-9

IUPAC Name (3-Acetamido-4-hydroxyphenyl)arsonic acid
Synonyms Acetarsol; acetphenarsine; acetphenarsinum; [3-(Acetylamino)-4-hydroxyphenyl]arsonic acid; osarsolum.
Proprietary Names *Ehrlich 594*; *Fourneau 190*; *F—190*; *Gynoplix*; *Orarsan*; *Spirocid*; *Stovarsol*; *SVC*.

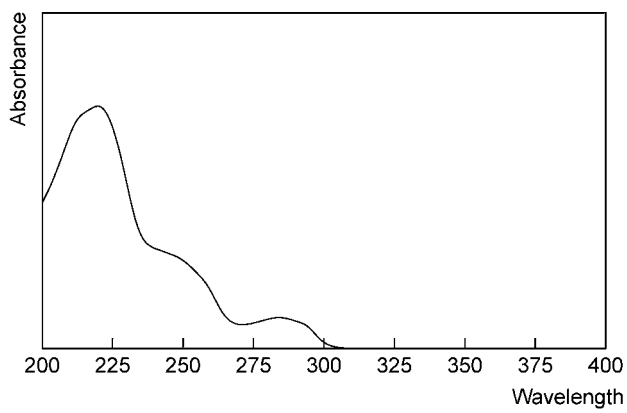


Chemical Properties A white crystalline powder. Mp about 240°, with decomposition. Practically insoluble in cold water, moderately soluble in boiling water; practically insoluble in ethanol; soluble in dilute alkalis. pK_a 3.7, 7.9, 9.3.

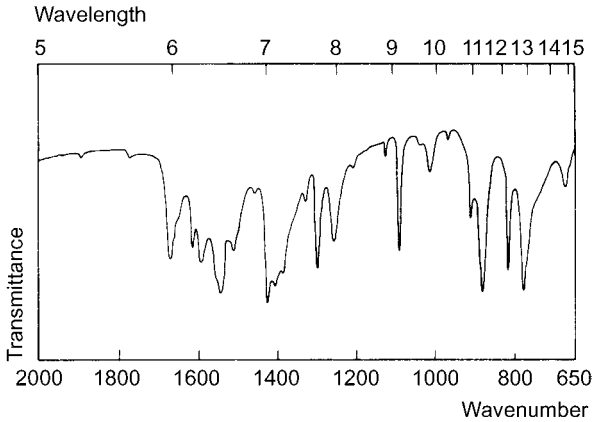
Acetarsone Sodium

$C_8H_9AsNNaO_5,5H_2O = 387.2$
CAS—5892-48-8 (anhydrous)
Chemical Properties White crystals or crystalline powder. Soluble 1 in 7 of water; practically insoluble in ethanol, chloroform and ether.

Ultraviolet Spectrum Aqueous acid—281 nm (A_1^1=94b);aqueous alkali—250 nm (A_1^1=567b), 299 nm (A_1^1=177b).



Infrared Spectrum Principal peaks at wavenumbers 887, 1538, 784, 823, 1297, 1584 cm^{-1} (KBr disk).

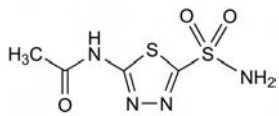


Dose Up to 500 mg daily for 10 days.

Acetazolamide

Antiglaucoma, Diuretic, Carbonic Anhydrase Inhibitor
$C_4H_6N_4O_3S_2$ = 222.3
CAS—59-66-5

IUPAC Name *N*-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide
Synonyms 6063; acetazolam; acetazoleamide; *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide.
Proprietary Names *Acetamox*; *Atenezol*; *Défiltran*; *Diamox*; *Didoc*; *Diuriwas*; *Donmox*; *Edemox*; *Fonurit*; *Glaucomide*; *Glaupax*; *Inidrase*.

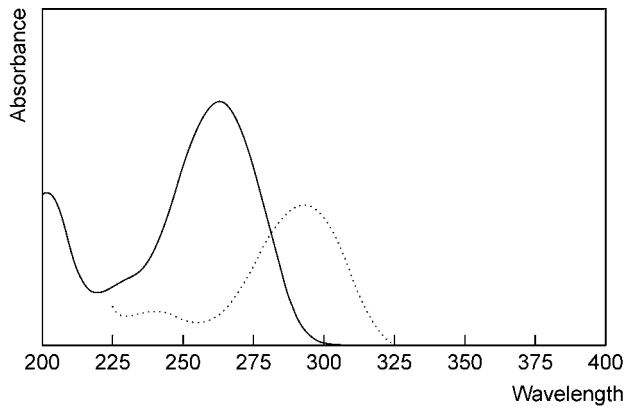


Chemical Properties A fine, white to yellowish-white crystalline powder. Mp about 260°, with decomposition. Soluble 1 in 1400 of water, 1 in 400 of ethanol, and 1 in 100 of acetone; practically insoluble in carbon tetrachloride, chloroform and ether. pK_a 7.2, 9.0 (25°).

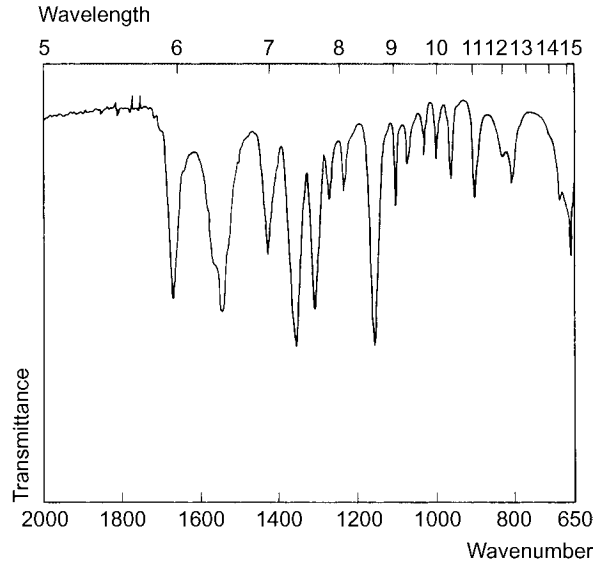
Acetazolamide Sodium

$C_4H_5N_4NaO_3S_2$ = 244.2
CAS—1424-27-7
Proprietary Names *Diamox Sodium*; *Vetamox*.
Chemical Properties Soluble in water.

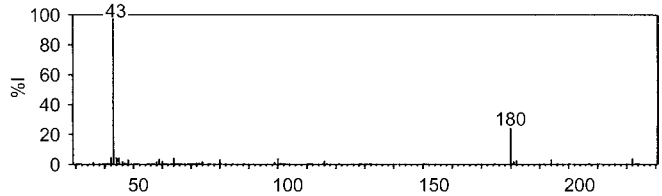
Colour Test Koppanyi-Zwikker test—red-violet (transient).
Thin-layer Chromatography System TA—R_f 0.85; system TD—R_f 0.04; system TE—R_f 0.03; system TF—R_f 0.31; system TAD—R_f 0.18; system TAE—R_f 0.84; system TAJ—R_f 0.18; system TAK—R_f 0.01; system TAL—R_f 0.60.
Gas Chromatography-Mass Spectrometry Column: fused silica capillary cross-linked 5% phenylmethylsilicone (25 m × 0.2 mm, 0.33 µm). Temperature programme of 230° to 320° at 35°/min. Injector: 280°. Detector: 300°. Carrier gas: He, 1 mL/min flow rate. Internal standard (IS): mefruside. (SIM acquisition mode at *m/z* 249 and *m/z* 85 for IS). Retention time: acetazolamide, 3.62 min (*k* 0.684); IS, 7.43 min (*k* 2.46) [Carreras *et al.* 1994].
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 268; system HY—RI 226; system HZ—retention time 1.9 min; system HAA—retention time 6.9 min.
Ultraviolet Spectrum Aqueous acid—265 nm (A^1_1=475a); aqueous alkali—240, 291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1167, 1548, 1316, 1665, 671, 1115 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 180, 42, 45, 44, 64, 100, 222.



Quantification
Blood **GC** ECD detection. Limit of detection, 10 pg [Wallace *et al.* 1977].
GC-MS Limit of detection, 25 pg [Kishida *et al.* 1981].
HPLC UV detection. Limit of detection, 50 µg/L [Chapron, White 1984].
Plasma **GC** ECD. Limit of detection, 10 pg [Wallace *et al.* 1977].
HPLC UV detection (λ=254 nm). Limit of detection, 5 µg/L [Zarghi, Shafaati 2002]. UV detection. Limit of detection, 50 µg/L [Chapron, White 1984].
Saliva **GC** ECD. Limit of detection, 10 pg [Wallace *et al.* 1977].
GC-MS Limit of detection, 25 pg [Kishida *et al.* 1981].
Urine **HPLC** UV detection. Limit of detection, 50 µg/L [Chapron, White 1984].
Disposition in the Body Acetazolamide is readily absorbed after oral administration. It binds tightly to carbonic anhydrase and will accumulate in tissues in which this enzyme is present, particularly in red blood cells and the renal cortex. About 70 to 90% of a dose is excreted in the urine as unchanged drug in 24 h but the renal clearance is increased if the urine is alkaline; small amounts of unchanged drug are excreted in the bile. The plasma:saliva ratio is about 100.
Therapeutic Concentration In plasma, usually in the range 10 to 15 mg/L.
Following a single oral dose of 250 mg to 5 subjects, peak plasma concentrations of 10 to 18 (mean 14) mg/L were attained in 1 to 3 h; peak erythrocyte concentrations of 13 to 29 mg/L were reached about 1 h after the peak plasma concentrations [Wallace *et al.* 1977].
A patient with type I diabetes and end-stage renal disease (ESRD) undergoing continuous ambulatory peritoneal dialysis (CAPD) was administered a 250 mg oral dose of acetazolamide after surgery for a detached retina. The concentration of acetazolamide in serum was measured prior to the dose and 12 additional times over a 24 hrs dosing period. The serum concentration at the beginning of the dosing period was 18 µg/mL and at the end 17 µg/mL with a maximum concentration of 27 µg/mL at 6.5 h. The therapeutic range was 5 to 10 µg/mL [Schwenk *et al.* 1994].
Toxicity Fatal cases of agranulocytosis, aplastic anaemia and thrombocytopenia have been reported.
Half-life Plasma half-life, about 13 h; blood and erythrocyte concentrations decrease more slowly.
Volume of Distribution About 0.2 L/kg.
Protein Binding About 90 to 95%.
Dose In the treatment of glaucoma 0.25 to 1 g daily.

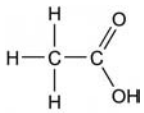
Carreras D *et al.* (1994). Comparison of derivatization procedures for the determination of diuretics in urine by gas chromatography-mass spectrometry. *J Chromatogr A* 683: 195–202.
Chapron DJ, White LB (1984). Determination of acetazolamide in biological fluids by reverse-phase high-performance liquid chromatography. *J Pharm Sci* 73: 985–989.
Kishida K *et al.* (1981). *Anal Lett (Part B)* 14: 335–347.
Schwenk MH *et al.* (1994). The pharmacokinetics of acetazolamide during CAPD. *Adv Perit Dial* 10: 44–46.
Wallace SM *et al.* (1977). GLC analysis of acetazolamide in blood, plasma, and saliva following oral administration to normal subjects. *J Pharm Sci* 66: 527–530.

Zarghi A, Shafaati A (2002). Rapid determination of acetazolamide in human plasma. *J Pharm Biomed Anal* 28: 169–172.

Acetic Acid Glacial

Acid
$CH_3{\cdot}COOH = 60.1$
CAS—64-19-7

IUPAC Name Acetic acid
Synonyms Acetic acid; acide acetique cristallisable; concentrated acetic acid



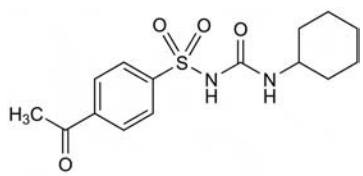
Chemical Properties A translucent crystalline mass or a clear colourless liquid. Mp 15°. Bp about 117°. Refractive index, at 20°, 1.3718. Miscible with water, ethanol, chloroform and ether. pK_a 4.8 (25°).
Colour Test Ferric chloride—red.
Mass Spectrum Principal ions at *m/z* 45, 29, 43, 60, 42, 28, 44, 41.
Quantification
Blood **GC** FID. Limit of detection, 1 µg/mL [Lester 1964].
Disposition in the Body Acetic acid occurs as a metabolite of ethanol and also of paraldehyde after its depolymerisation to acetaldehyde. It is further oxidised to carbon dioxide and water.
Toxicity The estimated minimum lethal dose is 5 mL and the maximum permissible atmospheric concentration is 10 ppm.

Lester D (1964). *Anal Chem* 36: 1810–1812.

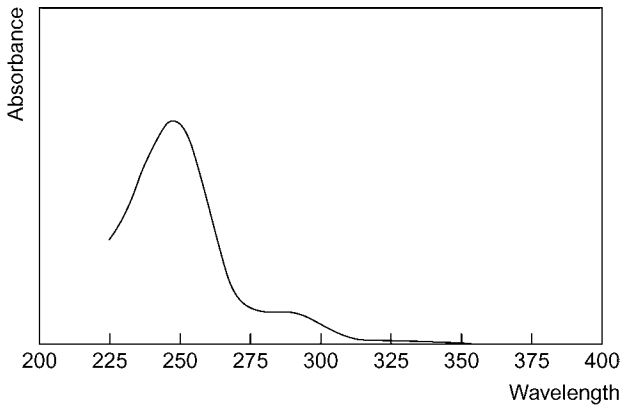
Acetohexamide

Antidiabetic
$C_{15}H_{20}N_2O_4S = 324.4$
CAS—968-81-0

IUPAC Name 1-(4-Acetylphenyl)sulfonyl-3-cyclohexylurea
Synonyms 4-Acetyl-*N*-[(cyclohexylamino)carbonyl]benzenesulfonamide; cyclamide; tsiklamid.
Proprietary Names *Dimelor*; *Dimeline*; *Dymelor*; *Ordimel*.

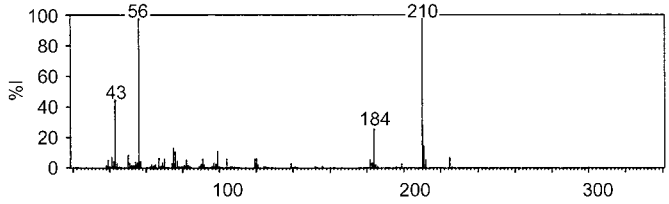


Chemical Properties A white crystalline powder. Mp 182° to 187°. Practically insoluble in water and ether; soluble 1 in 230 of ethanol and 1 in 210 of chloroform; soluble in pyridine and in dilute solutions of alkali hydroxides.
Colour Test Koppanyi-Zwikker test—violet.
Thin-layer Chromatography System TD—R_f 0.39; system TE—R_f 0.12; system TF—R_f 0.43; system TAD—R_f 0.53; system TAJ—R_f 0.60; system TAK—R_f 0.66; system TAL—R_f 0.91.
High Performance Liquid Chromatography System HY—RI 438.
Ultraviolet Spectrum Aqueous acid—247 nm (A_1^1=508b);aqueous alkali—249 nm (A_1^1=427a).



Infrared Spectrum Principal peaks at wavenumbers 1165, 1031, 1681, 1531, 905, 1264 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 210, 56, 43, 184, 211, 75, 99, 76.



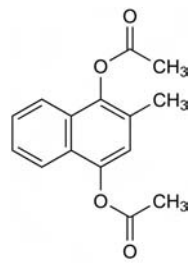
Quantification
Plasma **Spectrofluorimetry** Limit of detection, 200 ng [Girgis-Takla, Chroneos 1979].
Biological Fluids **GC** FID [Kleber *et al.* 1977].
Disposition in the Body Readily absorbed after oral administration and rapidly metabolised to (−)-1-hydroxyhexamide which has about 2.5 times the hypoglycaemic activity of the unchanged drug. Minor metabolites include 4′-*trans*-hydroxyacetohexamide, 4′-*trans*-hydroxy-1-hydroxyhexamide and, to a lesser extent, the 4′-*cis*-, 3′-*cis*- and 3′-*trans*-isomers. About 80% of a dose is excreted in the urine in 24 h, mainly as metabolites.
Therapeutic Concentration In plasma, usually in the range 20 to 60 µg/mL. Following oral administration of 750 mg of 3 different tablet formulations to 8 subjects, mean peak plasma-acetohexamide concentrations of about 28, 40, and 44 mg/L were attained 2 h after a dose; mean peak plasma concentrations of (−)-1-hydroxyhexamide averaged about 30 mg/L [Kleber *et al.* 1977].
After daily oral doses of 0.5 g to 18 subjects, an average serum concentration of 42 mg/L was reported 3 to 5 h after the dose [Sheldon *et al.* 1965].
Toxicity Prolonged hypoglycaemia has been reported.
Half-life Plasma half-life, acetohexamide about 1.3 h, (−)-1-hydroxyhexamide about 5 h.
Protein Binding About 96%.
Note For a review of the pharmacokinetics of oral hypoglycaemic agents, see Balant [1981].
For a review of the relationship between the pharmacokinetics and pharmacodynamic effects of oral hypoglycaemic drugs, see Ferner and Chaplin [1987].
Dose 0.25 to 1.5 g daily.

Balant L (1981). Clinical pharmacokinetics of sulphonylurea hypoglycaemic drugs. *Clin Pharmacokinet* 6: 215–241.
Ferner RE, Chaplin S (1987). The relationship between the pharmacokinetics and pharmacodynamic effects of oral hypoglycaemic drugs. *Clin Pharmacokinet* 12: 379–401.
Girgis-Takla P, Chroneos I (1979). Fluorimetric determination of acetohexamide in plasma and tablet formulations using 1-methylnicotinamide. *Analyst* 104: 117–123.
Kleber JW *et al.* (1977). GLC determination of acetohexamide and hydroxyhexamide in biological fluids. *J Pharm Sci* 66: 635–638.
Sheldon J *et al.* (1965). Serum concentration and urinary excretion of oral sulfonylurea compounds: relation to diabetic control. *Diabetes* 14: 362–367.

Acetomenaphthone

Vitamin K Activity
$C_{15}H_{14}O_4 = 258.3$
CAS—573-20-6

IUPAC Name (4-Acetyloxy-2-methylnaphthalen-1-yl) acetate
Synonyms Acetomenadione; 1,4-diacetoxy-2-methylnaphthalene; dihydrovitamin K_3; menadiol diacetate; vitamin-K_4.
Proprietary Names *Kapilin*; *Prokayvit Oral*; *Vitavel K*. It is an ingredient of *Pernivit*.



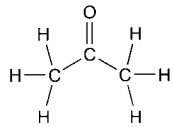
Chemical Properties A white crystalline powder. Mp 112° to 115°. Practically insoluble in water; slightly soluble in cold ethanol; soluble 1 in 3.3 of boiling ethanol; soluble in acetic acid.
Colour Test Liebermann's reagent—black.
Ultraviolet Spectrum Dehydrated alcohol—285 nm (A_1^1=245b), 322 nm (A_1^1=37b).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1760, 1163, 1070, 775, 1030 cm^{-1} (KBr disk).
Dose 5 to 20 mg daily.

A

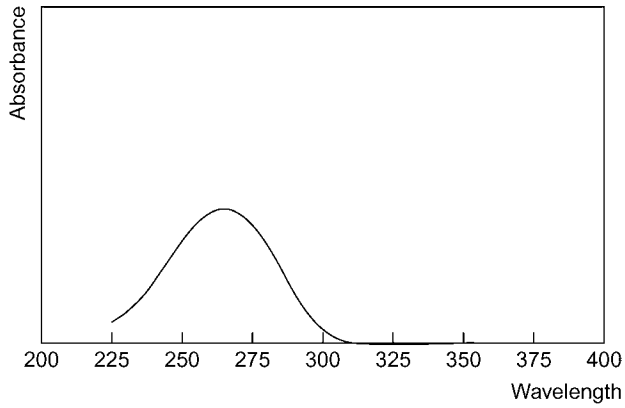
Acetone

Solvent
C_3H_6O = 58.08
CAS—67-64-1

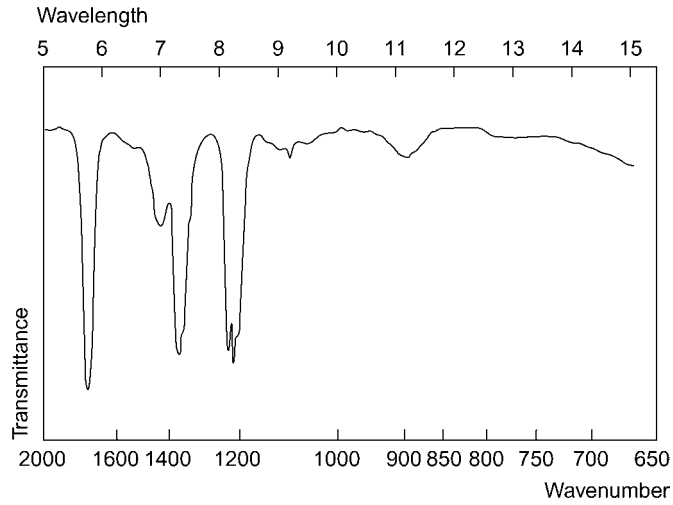
IUPAC Name Propan-2-one
Synonyms Cetona; dimethylformaldehyde; dimethyl ketone; β-ketopropane; 2-propanone; pyroacetic ether.



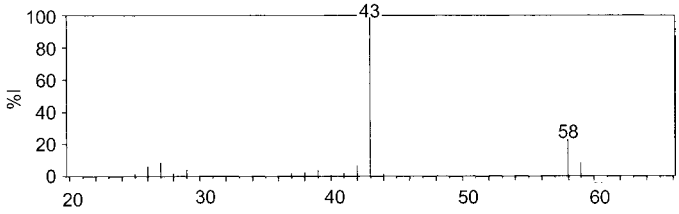
Chemical Properties A clear, colourless, volatile, mobile, inflammable liquid. Mp −94°. Bp approx. 56°. Refractive index 1.3591. Miscible with water, ethanol, chloroform and ether. Log *P* (octanol/water), −0.2.
Colour Test Sodium nitroprusside (method 1)—red.
Gas Chromatography System GA—RI 469; system GI—RT 2.5 min.
Ultraviolet Spectrum Aqueous acid—265 nm.



Infrared Spectrum Principal peaks at wavenumbers 1750, 1330 and 1200 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 43, 58, 59, 27, 42, 26, 39, 29.



Quantification
Plasma **GC** Column: Chromosorb 102 (1.5 m × 4 mm i.d.). Temperature: 115°. Carrier gas: N_2, 50 mL/min. FID. Retention time: 7.3 min. Limit of detection, 0.1 mmol/L [Trotter *et al.* 1971].

HPLC Column: Pecosphere 3 × 3 C_{18} (33 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (45 : 55), flow rate 1.0 mL/min. UV detection. Retention time: 3 min. Limit of detection, 0.034 mmol/L [Brega *et al.* 1991].

Serum **GC** Column: Restek Rtx-200 (30 m × 0.53 mm i.d., 3 µm). Temperature programme: 40° for 1 min to 260° at 70°/min. Carrier gas: He, 80.1 mL/min. IS: *n*-propanol. FID. Retention time: acetone, 1.76 min; IS, 1.54 min Limit of quantification, 2.5 mg/L, limit of detection, 1.0 mg/L [Williams *et al.* 2000].
Urine **GC** Column: 10% SBS-100 (4.1 m × 3.2 mm i.d.). Temperature: 60°. Carrier gas: N_2. FID. Limit of detection not reported [Fujino *et al.* 1992].

HPLC Column: Pecosphere 3 × 3 C_{18} (33 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (45 : 55), flow rate 1.0 mL/min. UV detection. Limit of detection, 0.034 mmol/L [Brega *et al.* 1991].
Alveolar Air **GC** Column: BX100 (1 m × 3 mm i.d.). Temperature: 90°. FID. Limit of detection not reported [Fujino *et al.* 1992]. Column: Chromosorb 102 (1.5 m × 4 mm i.d.). Temperature: 115°. Carrier gas: N_2, 50 mL/min. FID. Retention time: 7.3 min. Limit of detection, 0.1 mmol/L [Trotter *et al.* 1971].
Disposition in the Body Acetone is absorbed through the lungs and skin. It is excreted in the urine and through the lungs; large amounts are mainly excreted unchanged but small doses may be oxidised to carbon dioxide or utilised in the body as acetate or formate. Acetone is the main metabolite of isopropyl alcohol; it occurs naturally in the blood and urine of diabetics.
Therapeutic Concentration

Observations were made for 89 non-occupationally exposed subjects and 3 groups of workers exposed to acetone or isopropanol on a daily basis. Acetone was detected in all samples from the non-exposed subjects at mean concentrations of 840 µg/L in blood, 842 µg/L in urine, 715 mg/L in alveolar air and 154 ng/L in environmental air. The morning after a shift, workers had concentrations of acetone in their blood and urine of 3.5 mg/L and 13 mg/L, respectively. This was after mean environmental air acetone exposure of 336 µg/L [Wang *et al.* 1994].

One-hundred and ten male workers exposed to acetone or acetate fibres had urine acetone concentrations of 2.4 ± 5.6 mg/L compared with 1.3 ± 2.4 mg/L in non-exposed workers [Satoh *et al.* 1995].

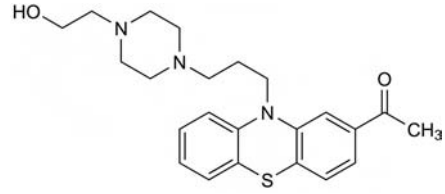
Toxicity Acetone is one of the solvents abused in 'glue-sniffing'. Severe toxic effects have been associated with blood concentrations of 200 to 300 mg/L; a blood concentration of 550 mg/L has been reported in a fatality. The maximum permissible atmospheric concentration is 1000 ppm. Exposure to 1600 ppm for about 15 min causes irritation to the eyes and nose. Up to 20 mL has been ingested without ill-effect.

Brega A *et al.* (1991). High-performance liquid chromatographic determination of acetone in blood and urine in the clinical diagnostic laboratory. *J Chromatogr* 553: 249–254.
Fujino A *et al.* (1992). Biological monitoring of workers exposed to acetone in acetate fibre plants. *Br J Ind Med* 49: 654–657.
Satoh T *et al.* (1995). Acetone excretion into urine of workers exposed to acetone in acetate fiber plants. *Int Arch Occup Environ Health* 67: 131–134.
Trotter MD *et al.* (1971). The rapid determination of acetone in breath and plasma. *Clin Chim Acta* 35: 137–143.
Wang G *et al.* (1994). Blood acetone concentration in "normal people" and in exposed workers 16 h after the end of the workshift. *Int Arch Occup Environ Health* 65: 285–289.
Williams RH *et al.* (2000). Simultaneous detection and quantitation of diethylene glycol, ethylene glycol, and the toxic alcohols in serum using capillary column gas chromatography. *J Anal Toxicol* 24: 621–626.

Acetophenazine

Antipsychotic, Tranquilliser
$C_{23}H_{29}N_3O_2S$ = 411.6
CAS—2751-68-0

IUPAC Name 1-[10-[3-[4-(2-Hydroxyethyl)piperazin-1-yl]propyl]phenothiazin-2-yl] ethanone
Synonyms Acephenazine; 1-[10-[3-[4-(2-hydroxyethyl)-1-piperazinyl]propyl]-10*H*-phenothiazin-2-yl]ethanone.

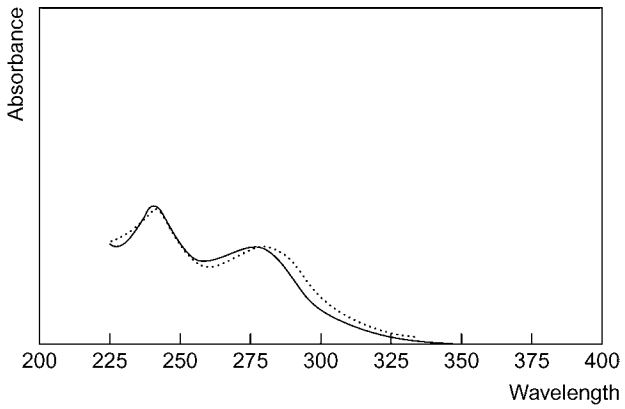


Acetophenazine Dimaleate

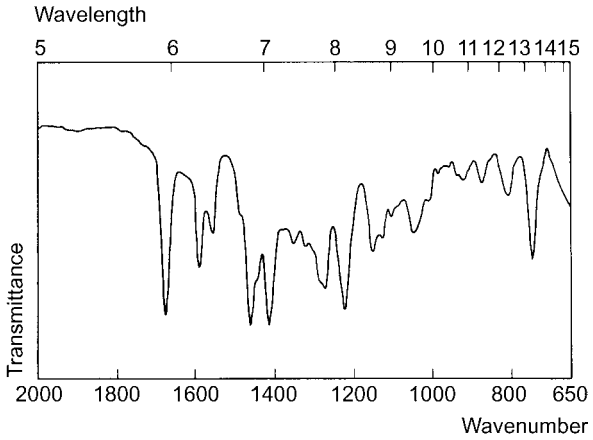
$C_{23}H_{29}N_3O_2S,2C_4H_4O_4$ = 643.7
CAS—5714-00-1
Proprietary Name *Tindal*
Chemical Properties A fine yellow powder. Mp about 165°, with decomposition. Soluble 1 in 10 of water, 1 in 260 of ethanol, 1 in 370 of acetone, 1 in 2850 of chloroform, 1 in 6000 of ether, and 1 in 11 of propylene glycol.

Colour Tests Formaldehyde–sulfuric acid—blue-violet; Forrest reagent—pink-orange; FPN reagent—orange; Liebermann's reagent—brown; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.03; system TC—R_f 0.25; system TE—R_f 0.38; system TL—R_f 0.03; system TAE—R_f 0.34; system TAF—R_f 0.32; system TAJ—R_f 0.05; system TAK—R_f 0.00; system TAL—R_f 0.33(Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, orange).
Gas Chromatography System GA—not eluted.

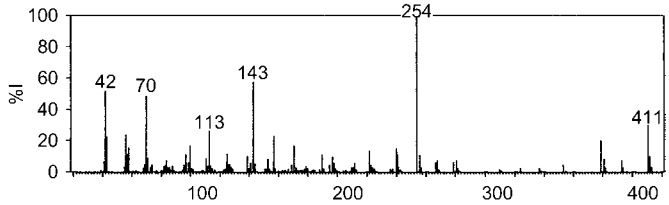
High Performance Liquid Chromatography System HA—*k* 1.9.
Ultraviolet Spectrum Aqueous acid—243 nm (A^1_1=500a), 278 nm.



Infrared Spectrum Principal peaks at wavenumbers 1670, 1220, 1265, 1587, 746, 1149 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 254, 143, 42, 70, 411, 113, 56, 157.

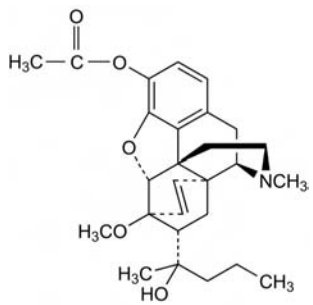


Dose 40 to 120 mg of acetophenazine maleate daily; up to 600 mg daily has been given.

Acetorphine

Narcotic Analgesic (Veterinary)
$C_{27}H_{35}NO_5$ = 453.6
CAS—25333-77-1

Synonym O^3-Acetyl-7,8-dihydro-7α-[1(*R*)-hydroxy-1-methylbutyl]-O^6-methyl-6,14-*endo*-ethenomorphine

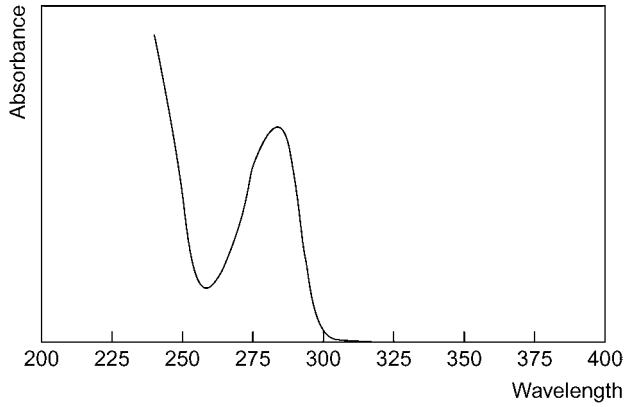


Chemical Properties Mp 193°. Soluble 1 in 4000 of water; freely soluble in ethanol, chloroform and ether.

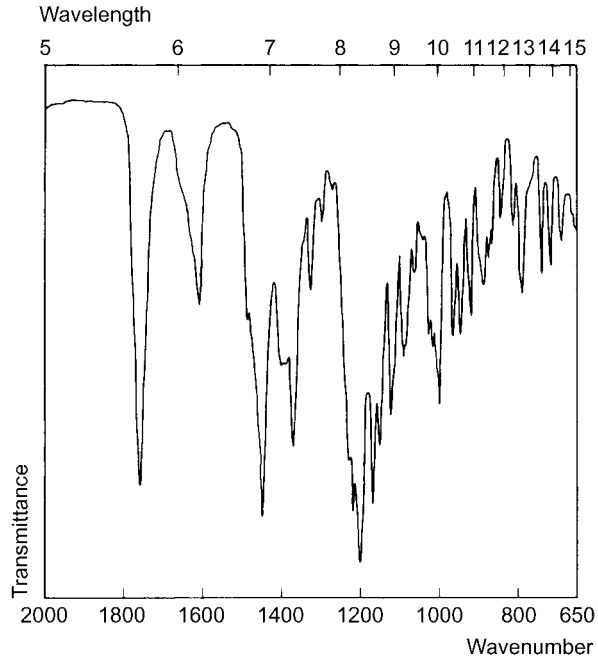
Caution It is dangerous to smell or taste this substance.

Acetorphine Hydrochloride
$C_{27}H_{35}NO_5$,HCl = 490.0
CAS—25333-78-2
Synonym M183
Chemical Properties A white, crystalline, hygroscopic powder. Mp 204°. It is hydrolysed in solution to etorphine and acetic acid.
Soluble 1 in 50 of water and 1 in 10 of ethanol.

Colour Test Marquis test—blue-grey→yellow-brown.
Thin-layer Chromatography System TA—R_f 0.72 (acidified iodoplatinate solution, positive; Marquis reagent, grey; acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HA—*k* 0.4.
Ultraviolet Spectrum Aqueous acid—284 nm (A^1_1=45b).



Infrared Spectrum Principal peaks at wavenumbers 1200, 1218, 1168, 1760, 1150, 1122 cm^{-1} (acetorphine hydrochloride, KBr disk).



Acetylcholine Chloride

Parasympathomimetic
$C_7H_{16}ClNO_2$ = 181.7
CAS—60-31-1

IUPAC Name 2-Acetyloxyethyl(trimethyl)azanium chloride
Synonym 2-(Acetyloxy)-*N*,*N*,*N*-trimethylethanaminium chloride
Proprietary Names *Acecoline*; *Arterocoline*; *Miochol*; *Ovisot*.
Chemical Properties A white, very hygroscopic, crystalline powder. Mp about 150°. Very soluble in water yielding unstable solutions; very soluble in ethanol and propylene glycol; freely soluble in chloroform; practically insoluble in ether.

A

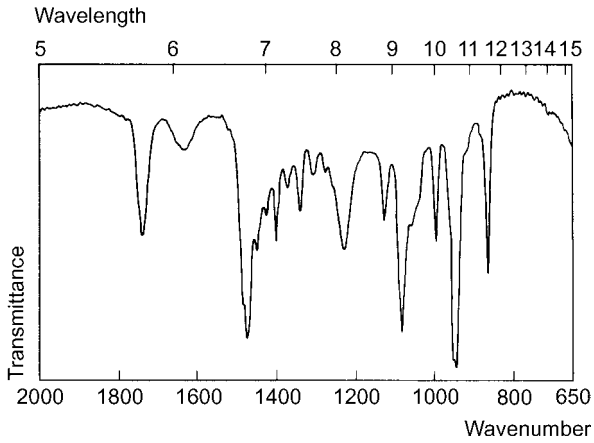
Acetylcholine Bromide

$C_7H_{16}BrNO_2$ = 226.1
CAS—66-23-9

Chemical Properties Deliquescent colourless crystals or white crystalline powder. Hydrolysed by hot water and alkalis. Mp 143°. Freely soluble in water; soluble in ethanol; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.02; system TN—R_f 0.70; system TO—R_f 0.60 (acidified iodoplatinate solution, positive).

Infrared Spectrum Principal peaks at wavenumbers 949, 1089, 869, 1231, 1000, 1740 cm^{-1} (KBr disk).



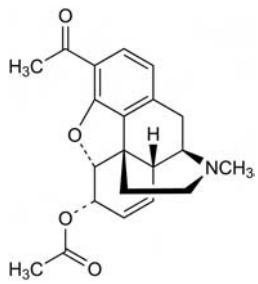
Mass Spectrum Principal ions at *m/z* 58, 43, 57, 149, 71, 42, 41, 55.

Dose Acetylcholine chloride is used as a 1% ophthalmic solution; it was formerly given parenterally in doses of 20 to 200 mg.

Acetylcodeine

Morphine Impurity
$C_{20}H_{23}NO_4$ = 341.4
CAS—6703-27-1

Synonym 3-*O*-Methyl-6-*O*-acetylmorphine



Chemical Properties Mp 134° to 135°. It is a contaminant of codeine and up to 12% may be found in illicit heroin. Stable for 48 h at 4°, 16 h at room temperature and after 3 freeze-thaw cycles [Brunet *et al.* 2008]. Stable in urine for 23 weeks [O'Neal, Poklis 1997].

Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.23; system TC—R_f 0.43; system TE—R_f 0.54; system TL—R_f 0.12; system TAE—R_f 0.26; system TAF—R_f 0.25; system TAJ—R_f 0.32; system TAK—R_f 0.05; system TAL—R_f 0.61.

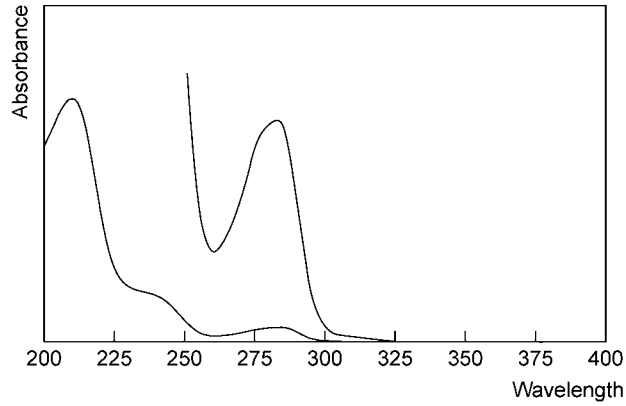
Gas Chromatography System GA—RI 2503; system GB—RI 2645; system GM—RRT 1.449 (relative to iprindole).

High Performance Liquid Chromatography System HC—*k* 0.78; system HS—*k* 0.50; system HX—RI 336.

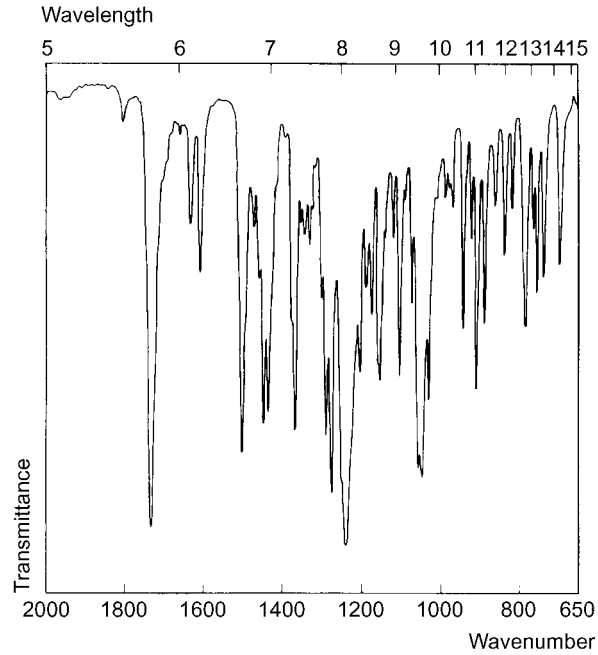
Column: aminopropyl-bonded silica (250 × 4.0 mm). Mobile phase: acetonitrile : 0.005 mol/L tetrabutylammonium phosphate (85 : 15), flow rate 1.0 mL/min. UV detection (λ = 284 nm). Relative retention time: 1.13. Limit of detection, 2.0 µg [Baker, Gough 1981].

Liquid Chromatography-Gas Chromatography Column: Zorbax Extend C_{18} (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: 5 mmol/L ammonium formate-0.1% formic acid : acetonitrile (95 : 5 to 85 : 15 in 18 min, to 80 : 20 in 5 min, to 95 : 5 in 0.1 min), flow rate 0.4 mL/min. ESI, positive ion mode [Zhang *et al.* 2008].

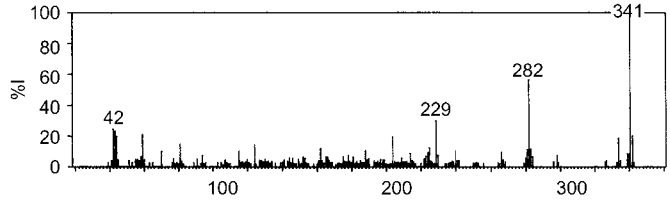
Ultraviolet Spectrum Aqueous acid—284 nm (A_1^1 = 37b)



Infrared Spectrum Principal peaks at wavenumbers 1233, 1731, 1272, 1042, 1055, 1501 cm^{-1}.



Mass Spectrum Principal peaks at *m/z* 341, 282, 229, 42, 43, 59, 342, 204 [Paul *et al.* 1985].



Quantification

Plasma **LC-MS** Column: Zorbax Bonus (150 × 4.6 mm i.d., 5 µm). Mobile phase: ammonium formate buffer (pH 4.0) : acetonitrile (97 : 3 for 2 min to 87 : 13 at 2.6 min to 84.5 : 15.5 at 8.0 min to 20 : 80 at 8.1 min until 11 min, to 97 : 3 at 11.1 min until 15 min). ESI, MRM acquisition mode. Retention time: 7.8 min. Limit of quantification, 5 µg/L [Rook *et al.* 2005; Rook *et al.* 2006].

Urine **GC-MS** Column: DB-5MS (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 20.4 cm/s. Temperature programme: 180° for 1 min to 280° at 10°/min for 14 min. MSD, SIM acquisition mode. Limit of quantification, 0.2 µg/L, limit of detection, 0.1 µg/L [Brenneisen *et al.* 2002]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 170° for 1 min to 240° at 20°/min to 256° at 2°/min to 270° at 10°/min for 0.6 min. EI ionisation. Limit of quantification, 1 µg/L [Staub *et al.* 2001]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 170° for 1 min to 280° at 10°/min. MSD, SIM acquisition mode. Limit of detection, 2 µg/L [O'Neal, Poklis 1998]. Limit of quantification, 1.0 µg/L, limit of detection, 0.5 µg/L [O'Neal, Poklis 1997]. Column: DB-5 (15 m × 0.25 mm i.d.). Carrier gas: He, 1.6 mL/min. EI ionisation at 70 eV. Retention time: 3.26 min [Paul *et al.* 1985].

LC-MS Column: Phenomenex C_{18} AQUA (150 × 2 mm i.d., 4 µm). Mobile phase: water-acetonitrile (98 : 2) and 5 mmol/L ammonium acetate : water-acetonitrile (10 : 90) and 5 mmol/L ammonium acetate (95 : 5 to 0 : 100 in 6 min for 4 min to 95 : 5 at 13 min for 6 min), flow rate 200 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.16 µg/L, limit of detection, 0.35 µg/L [Musshoff *et al.* 2004]. Column: RP18 (125 × 3 mm i.d.). Mobile phase: acetonitrile: 0.05 mmol/L ammonium formate buffer (pH 3.0, 30 : 70), flow rate 0.4 mL/min. SIM acquisition mode, positive ion mode. Limit of detection, 2.0 µg/L [Bogusz *et al.* 2001].

Oral Fluid **GC-MS** Column: capillary (15 m × 0.25 mm i.d.). EI ionisation. Limit of quantification, 2.0 µg/L [Cone *et al.* 2007].

LC-MS Column: EXSIL BDS C_8 (50 × 3.2 mm i.d.). Mobile phase: 5% methanol in 4 mmol/L ammonium formate: 1% propan-2-ol and 0.05% formic acid in methanol (100 : 0 for 1 min to 5 : 95 over 3 min for 2 min to 100 : 0 over 0.1 min for 1 min. TIS, ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 µg/L, limit of detection, 1 µg/L [Phillips, Allen 2006].

Sweat **GC-MS** Column: HP-5MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 100° for 0.5 min to 245° at 25°/min to 255° at 2°/min to 300° at 30°/min for 0.7 min. EI ionisation. Retention time: 11.1 min. Limit of detection, 5 ng/patch [Brunet *et al.* 2008].

Hair **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 180° for 1 min to 190° at 15°/min for 10 min to 250° at 5°/min to 290° at 30°/min for 2 min. Limit of quantification, 0.13 ng/mg, limit of detection, 0.02 g/mg [Musshoff *et al.* 2005; Musshoff *et al.* 2009]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 170° for 1 min to 240° at 20°/min to 256° at 2°/min to 270° at 10°/min for 0.6 min. SIM acquisition mode. Limit of quantification, 0.09 ng/mg [Girod, Staub 2001]. Column: HP5-MS 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 295° at 30°/min for 6 min. EI ionisation. Retention time: 10.1 min. Limit of quantification, 10 pg/mg [Kintz *et al.* 1998; Polettini *et al.* 1997].

Baker PB, Gough TA (1981). The separation and quantitation of the narcotic components of illicit heroin using reversed-phase high performance liquid chromatography. *J Chromatogr Sci* 19: 483–489.

Bogusz MJ *et al.* (2001). Detection of non-prescription heroin markers in urine with liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J Anal Toxicol* 25: 431–438.

Brenneisen R *et al.* (2002). Acetylcodeine as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin. *J Anal Toxicol* 26: 561–566.

Brunet BR *et al.* (2008). Development and validation of a solid-phase extraction gas chromatography–mass spectrometry method for the simultaneous quantification of methadone, heroin, cocaine and metabolites in sweat. *Anal Bioanal Chem* 392: 115–127.

Cone EJ *et al.* (2007). Prevalence and disposition of drugs of abuse and opioid treatment drugs in oral fluid. *J Anal Toxicol* 31: 424–433.

Girod C, Staub C (2001). Acetylcodeine as a marker of illicit heroin in human hair: method validation and results of a pilot study. *J Anal Toxicol* 25: 106–111.

Kintz P *et al.* (1998). Evaluation of acetylcodeine as a specific marker of illicit heroin in human hair. *J Anal Toxicol* 22: 425–429.

Musshoff F *et al.* (2004). Validated assay for the determination of markers of illicit heroin in urine samples for the control of patients in a heroin prescription program. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 47–52.

Musshoff F *et al.* (2005). Opiate concentrations in hair from subjects in a controlled heroin-maintenance program and from opiate-associated fatalities. *J Anal Toxicol* 29: 345–352.

Musshoff F *et al.* (2009). Cocaine and opiate concentrations in hair from subjects in a heroin maintenance program in comparison to a methadone substituted group. *Int J Legal Med* 123: 363–369.

O'Neal CL, Poklis A (1997). Simultaneous determination of acetylcodeine, monoacetylmorphine, and other opiates in urine by GC–MS. *J Anal Toxicol* 21: 427–432.

O'Neal CL, Poklis A (1998). The detection of acetylcodeine and 6-acetylmorphine in opiate positive urines. *Forensic Sci Int* 95: 1–10.

Paul BD *et al.* (1985). Simultaneous identification and quantitation of codeine and morphine in urine by capillary gas chromatography and mass spectroscopy. *J Anal Toxicol* 9: 222–226.

Phillips SG, Allen KR (2006). Acetylcodeine as a marker of illicit heroin abuse in oral fluid samples. *J Anal Toxicol* 30: 370–374.

Polettini A *et al.* (1997). Determination of opiates in hair. Effects of extraction methods on recovery and on stability of analytes. *Forensic Sci Int* 84: 259–269.

Rook EJ *et al.* (2005). The quantitative analysis of heroin, methadone and their metabolites and the simultaneous detection of cocaine, acetylcodeine and their metabolites in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 824: 213–221.

Rook EJ *et al.* (2006). Screening for illicit heroin use in patients in a heroin-assisted treatment program. *J Anal Toxicol* 30: 390–394.

Staub C *et al.* (2001). Detection of acetylcodeine in urine as an indicator of illicit heroin use: method validation and results of a pilot study. *Clin Chem* 47: 301–307.

Zhang Z *et al.* (2008). Fragmentation pathways of heroin-related alkaloids revealed by ion trap and quadrupole time-of-flight tandem mass spectrometry. *Rapid Commun Mass Spectrom* 22: 2851–2862.

Acetylcysteine

Mucolytic, Antidote (Parametamol)

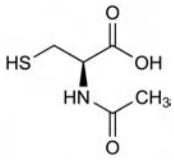
$C_5H_9NO_3S$ = 163.2

CAS—616-91-1

IUPAC Name (2*R*)-2-Acetamido-3-sulfanylpropanoate

Synonym *N*-Acetylcysteine

Proprietary Names *Airbron*; *Brunac*; *Fabrol*; *Fluimucil*; *Fluprowit*; *Mucocedyl*; *Mucolyticum*; *Mucolator*; *Mucomyst*; *Muco sanigen*; *Mucosil*; *Mucret*; *Nac*; *Neo-Flumucil*; *Parvolex*; *Tixair*.



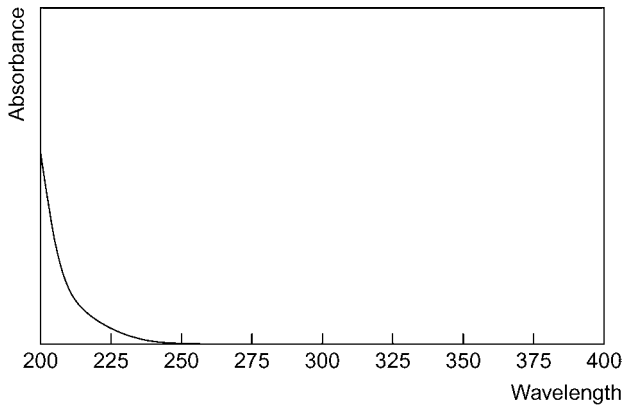
Chemical Properties A white, crystalline, deliquescent powder. Mp 104° to 110°. Soluble 1 in 8 of water and 1 in 2 of ethanol; practically insoluble in chloroform and ether. pK_a 9.5 (30°).

Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

Gas Chromatography System GA—RI 1547.

High Performance Liquid Chromatography System HX—RI 149; system HY—RI 176; system HZ—RT 1.57 min.

Ultraviolet Spectrum No significant absorption, 230–360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1530, 1300, 1230, 1275, 1580, 1715 cm^{-1} (Nujol mull).

Quantification

Plasma **HPLC** Column: Kromasil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : tetrahydrofuran : triprotic citric acid–sodium hydroxide buffer (pH 6.6, 75 : 18 : 4 : 3), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 500 nm, λ_{em} = 510 nm). Limit of detection, 0.3 nmol/L [Guo *et al.* 2009]. Column: Nucleosil (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium dihydrogen phosphate-10 mmol/L 1-octanesulfonic acid in acetonitrile : water (9 : 91), flow rate 1.5 mL/min. UV detection (λ = 333 nm). Limit of detection, 170 nmol/L [Tsikas *et al.* 1998]. Column: C_{18} bonded reversed phase (250 × 0.46 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.05 mol/L trisodium citrate-0.001 mol/L EDTA buffer (pH 7.0, 30 : 70), flow rate 1.0 mL/min. UV detection (λ = 360 nm). Limit of detection, 60 µg/L [Lewis *et al.* 1984].

Urine **HPLC** See Plasma. Limit of detection, 200 mg/L [Lewis *et al.* 1984].

Disposition in the Body *N*-Acetylcysteine is readily absorbed after oral administration, peak plasma concentrations being attained in 2–3 h; concentrations in the lung are similar to those in plasma. Approximately 20% of a dose is excreted in the urine in 24 h. *N*-Acetylcysteine is primarily metabolised to cysteine.

Therapeutic Concentration

Five healthy male and female subjects with a mean age of 28.8 years were fasted overnight for 12 h and administered 600 mg *N*-acetylcysteine orally. *N*-Acetylcysteine was observed in plasma at a mean concentration of 4.6 µg/L after 60 min and 2.5 µg/L after 90 min. Cysteine was observed at a mean maximum concentration of 18.6 µg/L [Tsikas *et al.* 1998].

Nine male and female patients with cirrhosis, mean age 51.1 years (range, 40–74), and 6 healthy subjects with mean age 39.8 years (range, 28–61) were administered 600 mg *N*-acetylcysteine IV over 3 min. After 30 min, the peak plasma concentration was 60 mg/L for both groups; after 10 h, the plasma concentration was 4.5 mg/L for the patients and 1.5 mg/L for the healthy subjects [Jones *et al.* 1997].

Half-life Healthy subjects 2.6 h; patients with cirrhosis 4.9 h.

Volume of Distribution Healthy subjects 25.5 L; patients with cirrhosis 17.4 L.

Clearance Body clearance: healthy subjects, 6.5 L/h; patients with cirrhosis 4.5 L/h.

Protein Binding ≈78%.

Dose As a mucolytic, 600 mg daily by mouth. In the treatment of paracetamol overdosage, initially 150 mg/kg by rapid IV infusion.

Guo XF *et al.* (2009). Simultaneous analysis of plasma thiols by high-performance liquid chromatography with fluorescence detection using a new probe, 1,3,5,7-tetramethyl-8-phenyl-(4-iodoacetamido)difluoroboradiaza-s-indacene. *J Chromatogr A* 1216: 3874–3880.

Jones AL *et al.* (1997). Pharmacokinetics of *N*-acetylcysteine are altered in patients with chronic liver disease. *Aliment Pharmacol Ther* 11: 787–791.

Lewis PA *et al.* (1984). High-performance liquid chromatographic assay for *N*-acetylcysteine in plasma and urine. *J Pharm Sci* 73: 996–998.

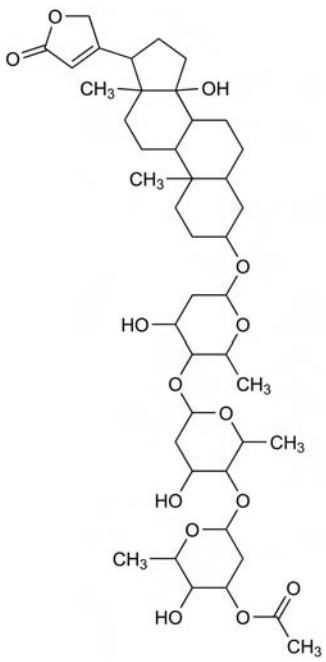
Tsikas D *et al.* (1998). Analysis of cysteine and *N*-acetylcysteine in human plasma by high-performance liquid chromatography at the basal state and after oral administration of *N*-acetylcysteine. *J Chromatogr B Biomed Sci Appl* 708: 55–60.

A

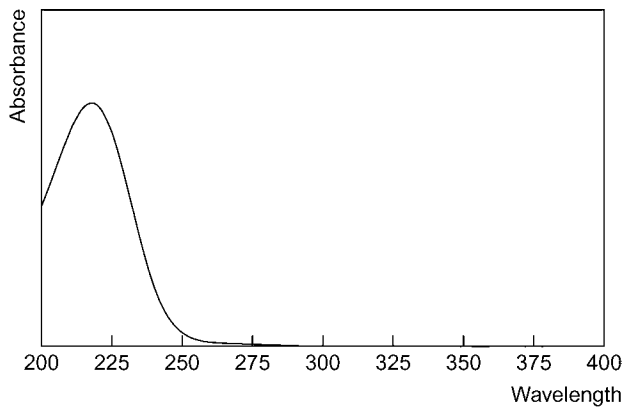
Acetyldigitoxin

Cardiac Glycoside
$C_{43}H_{66}O_{14}$ = 807.0
CAS—1111-39-3

IUPAC Name [(2*R*,3*R*,4*S*,6*S*)-3-Hydroxy-6-[(2*R*,3*S*,4*S*,6*S*)-4-hydroxy-6-[(2*R*,3*S*,4*S*,6*R*)-4-hydroxy-6-[[(3*S*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-14-hydroxy-10,13-dimethyl-17-(5-oxo-2H-furan-3-yl)-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-3-yl]oxy]-2-methyloxan-3-yl] oxy-2-methyloxan-3-yl]oxy-2-methyloxan-4-yl] acetate
Synonyms 3β-[(*O*-3-*O*-Acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl) oxy]-14-hydroxy-5β,14β-card-20(22)-enolide; acetyldigitoxoside; β-digitoxin monoacetate.
Proprietary Name *Acylanid(e)*



Chemical Properties A white hygroscopic crystalline powder. Mp 217° to 221°. Soluble 1 in 6100 of water, 1 in 63 of ethanol and 1 in 12 of chloroform; practically insoluble in ether and light petroleum; soluble in methanol.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Quantification

Plasma **LC-MS**. Limit of detection, 0.15 µg/L [Tracqui *et al.* 1997].
Disposition in the Body About 65% of a dose is absorbed after oral administration. It is slowly excreted in the urine, about 20% of a dose being excreted in 6 days; digitoxin accounts for about 60% of the urinary material. About 15 to 20% of a dose is eliminated in the faeces over a period of 18 days.
Half-life Plasma half-life, about 8 to 9 days.
Protein Binding In plasma, about 80%.
Dose Maintenance, 100 to 200 µg daily.

Tracqui A *et al.* (1997). High-performance liquid chromatography-ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. *J Chromatogr B Biomed Sci Appl* 692: 101–109.

Acetyldihydrocodeine

Cough Suppressant, Opioid Derivative
$C_{20}H_{25}NO_4$ = 343.4
CAS—3861-72-1

Synonym 4,5-Epoxy-3-methoxy-9a-methylmorphinan-6-yl acetate
Chemical Properties Soluble in water (510 mg/L). Log *P* (octanol/water), 2.50 [Meylan, Howard 1995].

Acetyldihydrocodeine Hydrochloride

$C_{20}H_{25}NO_4$,HCl = 379.9
Proprietary Name *Acetylcodone*

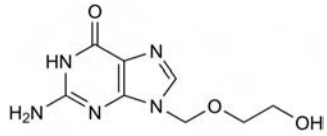
Colour Tests Ammonium molybdate test—green→blue (limit of detection, 0.1 µg); ammonium vandate test—faint grey-green (limit of detection, 1.0 µg); sulfuric acid-formaldehyde test—purple (limit of detection, 0.1 µg); Vitali's test—faint yellow/faint yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System TA—R_f 0.26 (location reagent iodoplatinate spray positive reaction).
Gas Chromatography System G2/225—retention time 1.27 min (relative to codeine); system G4—retention time 0.95 min (relative to codeine).
Ultraviolet Spectrum Ethanol—284.5 nm; aqueous acid (0.05 N sulfuric acid)—283 nm; aqueous alkali (0.5 N sodium hydroxide)—283 nm.
Dose Given by mouth in a usual daily dose of 20 to 50 mg; no more than 20 mg should be taken as a single dose.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Aciclovir

Antiviral
$C_8H_{11}N_5O_3$ = 225.2
CAS—59277-89-3

IUPAC Name 2-Amino-9-(2-hydroxyethoxymethyl)-3H-purin-6-one
Synonyms Acycloguanosine; acyclovir; 2-amino-1,9-dihydro-9-(2-hydroxyethoxymethyl)-6*H*-purin-6-one; BW-248U.
Proprietary Names *Aciviran*; *Activir*; *Acyvir*; *Alovir*; *Avirase*; *Avyclor*; *Clovix*; *Cusiviral*; *Cycloviran*; *Dravyr*; *Efriviral*; *Esavir*; *Geavir*; *Maynar*; *Milavir*; *Neviran*; *Rexan*; *Sifiviral*; *Viclovir*; *Vipral*; *Virherpes*; *Virmen*; *Zovir*; *Zovirax*; *Zyclir.*

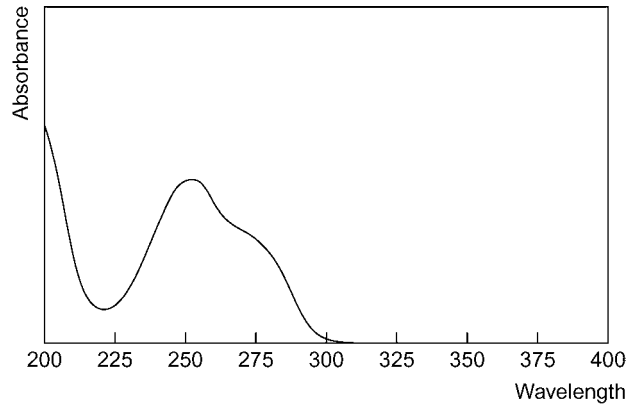


Chemical Properties A white to almost white crystalline powder. Mp 255°, with decomposition. It is sparingly to slightly soluble in water; practically insoluble or very slightly soluble in ethanol; dissolves in dilute solutions of mineral acids and alkali hydroxides; and is freely soluble in dimethyl sulfoxide. pK_a 2.3. Log *P* (octanol/water), −1.56.

Aciclovir Sodium

$C_8H_{10}N_5NaO_3$ = 247.2
CAS—69657-51-8
Proprietary Names *Cusiviral*; *Geavir*; *Herpotern*; *Maynar*; *Viclovir*; *Virherpes*; *Virmen*; *Zovirax*; *Zyclir.*
Chemical Properties A white crystalline powder. It is soluble 1 in 10 of water.

High Performance Liquid Chromatography System HAA—retention time 3.1 min.
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—255 nm;basic—261 nm.



Infrared Spectrum Principal peaks at wavenumbers 1717, 1632, 1485, 1104 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 151, 164, 45, 43, 165, 109, 110, 108.

Quantification

Plasma **HPLC** Column: C_8 Symmetry (150 × 4.6 mm, 5 µm). Mobile phase: acetate citrate buffer (0.1 mol/L) containing 3.7 mmol/L octane sulfonic acid:methanol (92:8 pH 3.0), flow rate 1 mL/min. UV detection (λ=250 nm). Retention time: 5.3 min [Poirier *et al.* 1999]. Limit of detection, 62.5 µg/L [Poirier *et al.* 1999]. Column: LiChrosorb RP-8 (250 × 4 mm i.d., 7 µm). Mobile phase: 1% acetonitrile in 0.02 mol/L disodium hydrogen orthophosphate, (pH 2.5) flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}=270 nm, λ_{em}=380 nm). Retention time: 9.6 min [Peh, Yuen 1997].Limit of detection, 30 µg/L [Peh, Yuen 1997]. Column: ODS R-P Ultrasphere (75 × 4.6 mm, 3 µm). Mobile phase: 30 mmol/L phosphate buffer, pH 2.1 containing 5 mmol/L dodecyl sulfate and 18% acetonitrile, flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}=285 nm, λ_{em}=380 nm). Retention time: aciclovir, 1.7 min; 9-carboxy-methoxymethylguanine, 1.5 min [Svensson *et al.* 1997]. Limit of detection, 0.12 µg/L [Svensson *et al.* 1997]. UV detection. Limit of detection, 10 µg/L [Swart *et al.* 1994].

Serum **HPLC** Column: RP-8 SymmetryShield (6 mm i.d., 5 µm). Mobile phase: acetonitrile: monoammonium phosphate buffer 0.025 mol/L (pH 4.0) (2:98), flow rate 1.0 mL/min. UV detection. Retention time: 9.7 min [Pham-Huy *et al.* 1999]. Limit of quantification, 200 µg/L, limit of detection, 50 g/L [Pham-Huy *et al.* 1999]. UV detection. Limit of detection, 50 µg/L [Nebinger, Koel 1993].

Urine **HPLC** Fluorescence detection (λ_{ex}=285 nm, λ_{em}=380 nm). Limit of detection, 0.60 µg/L [Svensson *et al.* 1997].

Note For an ELISA for the quantification of aciclovir see Tadepalli *et al.* [1986].

Disposition in the Body Aciclovir is poorly absorbed after oral administration. Peak plasma concentrations occur about 1.5 to 2.5 h after administration. It is excreted mostly unchanged through the kidney by both glomerular filtration and tubular secretion. 30 to 70% of an administered dose can be detected in urine. About 14% is excreted as the inactive metabolite 9-carboxymethoxymethylguanine (the main metabolite), and also 8-hydroxy-9-(2-hydroxyethoxymethyl)guanine has been detected (<0.2% of the dose). Faecal excretion accounts for about 2% of a dose. It is widely distributed into various tissues, including CSF where concentrations reach about 50% of those of plasma. It crosses the placenta and is distributed into breast milk. Aciclovir is removed by haemodialysis but not by peritoneal dialysis.

Therapeutic Concentration The trough serum therapeutic concentration range is 0.5 to 1.5 mg/L while the peak is 5 to 15 mg/L.

Single dose and multiple dosing studies were carried out on adult volunteers aged 45 to 60 years. Single dose studies involved administering intravenous doses of 0.5, 1.0, 2.5, 5.0, 10.0 and 15.0 mg/kg over a 1 h infusion period resulting in mean peak plasma concentrations of 1.0, 2.1, 4.2, 8.8, 14.6 and 22.7 mg/L, respectively. All concentrations were observed by the end of infusion. The mean peak and trough plasma concentrations measured after administration of 2.5 mg/kg intravenously over 1 h every 8 h were 5.1 and 0.5 mg/L, respectively. Following doses of 5.0, 10.0, and 15.0 mg/kg administered similarly, the mean peak and trough concentrations were: 9.8 and 0.7, 20.7 and 2.3, 23.6 and 2.0 mg/L, respectively. A study was also carried out on children, from birth up to 17 years old, who were administered a 250 or 500 mg/m^2 dose. The mean peak concentrations were 4 to 18 mg/L and 14 to 30 mg/L, respectively [Blum *et al.* 1982].

Bioavailability Oral, 20%.

Toxicity Nephrotoxicity may occur occasionally. The risk may be increased if large doses are given or if it is administered very rapidly or as a bolus. Neurotoxicity may also occur. Patients have ingested up to 20 g with no unexpected effects. If the solubility of aciclovir in intratubular fluid is exceeded (2.5 g/L), aciclovir may precipitate in the renal tubules and urine flow must therefore be maintained in cases of overdosage.

Severe neurological impairment and nonoliguric renal failure developed in a 68-year-old man a week after starting treatment with aciclovir 600 mg four times daily by mouth. On presentation the blood-aciclovir concentration was 18 mg/L. Intravascular volume depletion secondary to profuse diarrhoea and a history of chronic renal failure were contributory factors. Symptoms resolved on discontinuation of aciclovir and haemodialysis [Bradley *et al.* 1997].

Seven of 8 subjects treated with high-dose aciclovir (400 to 1200 mg/m^2/day) by i.v. infusion for cytomegalovirus pneumonia following bone-marrow transplant died. Plasma aciclovir concentrations at postmortem for 4 of the subjects were 0.018 to 0.2 mg/L. The following corresponding concentrations in various organs were reported: lung 0.023 to 0.28 mg/L, heart 0.027 to 0.25 mg/L, liver 0.022 to 0.31 mg/L, renal medulla 0.16 to 2.68 mg/L, renal cortex 0.13 to 2.62 mg/L, brain 0.142 mg/L, spinal cord 0.0063 to 0.118 mg/L [Wade *et al.* 1982].

Half-life Plasma, 2 to 3 h.

Volume of Distribution 4 to 55 L/1.73 m^2.

Clearance Plasma, 327 mL/min/1.73 m^2.

Protein Binding 9 to 33%.

Dose Up to 30 mg/kg body weight daily intravenously; up to 4 g daily by mouth.

Blum MR *et al.* (1982). Overview of acyclovir pharmacokinetic disposition in adults and children. *Am J Med* 73: 186–192.

Bradley J *et al.* (1997). Progressive somnolence leading to coma in a 68-year-old man. *Chest* 112: 538–540.

Nebinger P, Koel M (1993). Determination of acyclovir by ultrafiltration and high-performance liquid chromatography. *J Chromatogr* 619: 342–344.

Peh KK, Yuen KH (1997). Simple high-performance liquid chromatographic method for the determination of acyclovir in human plasma using fluorescence detection. *J Chromatogr Biomed Sci Appl* 693: 241–244.

Pham-Huy C *et al.* (1999). Rapid determination of valaciclovir and acyclovir in human biological fluids by high-performance liquid chromatography using isocratic elution. *J Chromatogr Biomed Sci Appl* 732: 47–3.

Poirier JM *et al.* (1999). Determination of acyclovir in plasma by solid-phase extraction and column liquid chromatography. *Ther Drug Monit* 21(1): 129–133.

Svensson JO *et al.* (1997). Determination of acyclovir and its metabolite 9-carboxymethoxymethylguanine in serum and urine using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 690: 363–366.

Swart KJ *et al.* (1994). Automated high-performance liquid chromatographic method for the determination of acyclovir in plasma. *J Chromatogr A* 663: 65–69.

Tadepalli SM *et al.* (1986). A competitive enzyme-linked immunosorbent assay to quantitate acyclovir and BW B759U in human plasma and urine. *Antimicrob Agents Chemother* 29: 93–98.

Wade JC *et al.* (1982). Treatment of cytomegalovirus pneumonia with high-dose acyclovir. *Am J Med* 73: 249–256.

Acipimox

Hyperlipidaemic, Nicotinic Acid Analogue

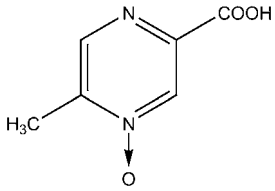
$C_6H_6N_2O_3$ = 154.1

CAS—51037-30-0

IUPAC Name 5-Methyl-4-oxidopyrazin-4-ium-2-carboxylic acid

Synonyms 2-Carboxy-5-methylpyrazine-4-oxide; K-9321; 6-methyl-1-oxidopyrazin-1-ium-2-carboxylic acid.

Proprietary Names *Olbemox*; *Olbetan*.



Chemical Properties Crystals. Mp 177° to 180°. Freely soluble in water, insoluble in ether. pK_a 3.25 [Ghabrial *et al.* 1991]. Log *P* (octanol/water), −0.52 [Meylan, Howard 1995].

Quantification

Urine **HPLC** Column: Whatman Partisil PAC 10 (250 × 3 mm i.d.). Mobile phase: citrate phosphate buffer (pH 2.5) : acetonitrile (80 : 20), flow rate 1.4 mL/min. UV detection (λ = 269 nm). Limit of detection not reported [Musatti *et al.* 1981].

Placenta **HPLC** Column: Novapak phenyl (4 µm). Mobile phase: 25 mmol/L dipotassium hydrogen phosphate buffer containing 40 mL/L methanol, 5 mmol/L tetrabutyl ammonium and 5 mmol/L triethylamine (pH 6.8), flow rate 3 mL/min. UV detection (λ = 264 nm). Limit of detection not reported [Ghabrial *et al.* 1991].

Disposition in the Body Acipimox is rapidly and completely absorbed from the gastrointestinal tract, reaching peak plasma concentrations in approx. 2 h. The drug is not significantly metabolised [Musatti *et al.* 1981] but the main metabolite is 5-methylpyrazine-2-carboxylic acid [Efthymiopoulos *et al.* 1993]. Approximately 90% of an administered dose is excreted unchanged in the urine [Musatti *et al.* 1981], although only 6% is excreted via this route in patients with severe renal failure [Bonadonna *et al.* 1985].

Therapeutic Concentration

Ten healthy volunteers were administered a single 250 mg acipimox dose followed by a dose 3 times daily for 6 days. After the single administration, the mean peak plasma concentration was 5.74 mg/L (2.56 to 8.38 mg/L) at 1.7 h (1 to 3 h). The elimination half-life was 1.15 h (0.79 to 1.48 h). These values were not significantly different after repeated doses [Efthymiopoulos *et al.* 1993].

Six uraemic patients on dialysis were given single oral doses of 50 mg acipimox. The mean peak plasma concentration was 1.6 mg/L at 2 h. During dialysis, the mean plasma levels declined rapidly [Bonadonna *et al.* 1985].

Four healthy male volunteers (aged 22 to 28 years; weight 66 to 75 kg) were administered 150, 250 or 400 mg acipimox at 1 week intervals according to a randomised sequence. Mean peak plasma concentrations were 1.75 ± 0.22, 3.57 ± 0.22, and 6.03 ± 0.63 mg/L, respectively, attained at 1.75 ± 0.25, 2 ± 0, and 1.25 ± 0.24 h, respectively. Six healthy male volunteers (aged 19 to 37 years; weight 60 to 83 kg) were administered one 250 mg capsule either on an empty stomach or after a 750 calorie breakfast. After a 1 week washout period, the same 6 men started oral treatment of 250 mg three times daily for 6 days with one capsule taken on the morning of the 7th day. Mean peak plasma levels were as follows:

	C_{max} (mg/L)	t_{max} (h)	$t_{1/2}$ (4-10) (h)
Fasting	4.24 ± 0.34	1.33 ± 0.21	1.95 ± 0.10
After food	3.74 ± 0.18	3.16 ± 0.16	1.90 ± 0.12
19th dose	6.01 ± 0.18	1.50 ± 0.22	1.92 ± 0.08

[Musatti *et al.* 1981]

A

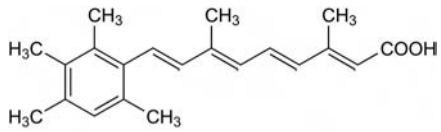
Toxicity Diffusion through the human placenta is slow, which should afford the fetus some protection if acipimox is administered to the mother [Ghabrial *et al.* 1991].
Half-Life Healthy patients, 1.15 h; patients in renal failure and on haemodialysis, 2.6 h.
Clearance Approximately 220 mL/min.
Dose 500 to 750 mg daily in divided doses.

Bonadonna A *et al.* (1985). A pilot study of the pharmacokinetics and triglyceride lowering activity of acipimox in dialyzed uremic patients. *Int J Clin Pharmacol Ther Toxicol* 23: 112–114.
Efthymiopoulos C *et al.* (1993). Pharmacokinetics of acipimox and of its *N*-deoxy metabolite following single and repeated oral administration to healthy volunteers. *Therapie* 48: 23–26.
Ghabrial H *et al.* (1991). Transfer of acipimox across the isolated perfused human placenta. *Placenta* 12: 653–661.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Musatti L *et al.* (1981). Bioavailability and pharmacokinetics in man of acipimox, a new antilipolytic and hypolipemic agent. *J Int Med Res* 9: 381–386.

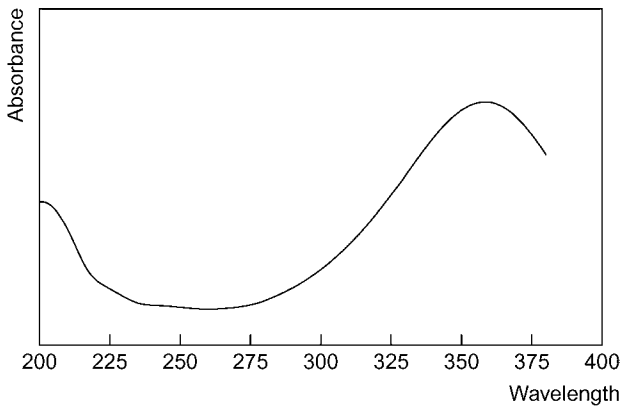
Acitretin

Dermatological Agent, Retinoid
$C_{21}H_{26}O_3$ = 326.4
CAS—55079-83-9

IUPAC Name (2*E*,4*E*,6*E*,8*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid
Synonyms Etretin; Ro-10-1670.
Proprietary Names *Neotigason*; *Soriatane*.



Chemical Properties A yellow to greenish-yellow powder. Mp 228° to 230°. Protect from light. pK_a 3.7. Log *P* (octanol/water), 6.4. The retinoids easily undergo photoisomerisation and oxidation, making it necessary to handle and store all substances and solutions in yellow dark-room light [Palmskog 1980]. Plasma samples spiked with 50 and 100 µg/L were stable for 2, 6, 10 and 24 h at ambient temperature and for 7, 21 and 90 days at –20° [Al Mallah *et al.* 1987]. Plasma samples stored at 10° were stable for at least 24 h [Wyss, Bucheli 1992].
Thin-layer Chromatography System TF—R_f 0.43.
High Performance Liquid Chromatography Column: Nucleosil RP C_{18} (250 × 4.2 mm i.d., 5 µm). Mobile phase: methanol-acetonitrile (7 : 3) : 1.5% acetic acid (85 : 15), flow rate 1.2 mL/min. UV detection (λ = 350 nm). Retention time: 13-*cis*-acitretin, 6.0 min; all-*trans*-acitretin, 6.9 min. Limit of quantification, 1 µg/kg [Laugier *et al.* 1994].
Ultraviolet Spectrum



Mass Spectrum Principal peaks at *m/z* 150, 326, 163, 203, 251, 281, 41, 311.
Quantification
Blood **HPLC** Column: Nucleosil C_{18} (250 × 4 mm i.d., 5 µm). Mobile phase: methanol : 1% aqueous acetic acid (85 : 15), flow rate 1.5 mL/min. UV detection (λ = 350 nm). Retention time: 8.9 min. Limit of detection not reported [Al Mallah *et al.* 1987].
Plasma **HPLC** Column: Nucleosil C_{18} reversed phase (250 × 4.2 mm i.d., 5 µm). Mobile phase: methanol-acetonitrile (7 : 3) : 1.5% acetic acid (85 : 15), flow rate 1.2 mL/min. UV detection (λ = 350 nm). Retention time: 6 and 6.9 min for 13-*cis*-acitretin and all-*trans*-acitretin, respectively. Limit of quantification, 1 µg/L [Laugier *et al.* 1994]. Column: Spherisorb ODS (1.5 µm). Mobile phase: 1% ammonium acetate-acetonitrile-acetic acid (30 : 70 : 3) : 1.7% ammonium acetate-acetonitrile-acetic acid (6 : 95 : 3) : acetonitrile-tetrahydrofuran-water (80 : 20 : 2; 100 : 0 : 0 through to 0 : 0 : 100). UV detection (λ = 230 nm). Limit of detection, 0.1 µg/L [Sturkenboom *et al.* 1994]. Column: Spherisorb ODS1 (250 × 4 mm i.d., 5 µm). Mobile phase: 10% ammonium acetate-glacial acetic acid-water-acetonitrile (4 : 3 : 300 : 700) : 10% ammonium acetate-glacial acetic acid-water-acetonitrile (4 : 1 : 146 : 850; 100 : 0 to 0 : 100 at 8 min for 3 min to 100 : 0 at 11.1 min), flow rate 1.0 mL/min. UV detection (λ = 360 nm). Limit of detection, 2 µg/L [Klein *et al.* 1992]. Column: Spherisorb ODS 1 (125 × 4 mm, 5 µm). UV detection (λ = 360 nm). Limit of quantification, 0.3 µg/L, limit of detection, 0.1 µg/L for acitretin and metabolites [Wyss, Bucheli 1992]. Column: Chromspher silica (150 × 4.6 mm i.d., 5 µm). Mobile phase: *n*-hexane: methylsalicylate : acetic acid (200 : 18 : 0.6). UV detection (λ = 360 nm). Limit of quantification, 3 to 4 µg/L [Meyer *et al.* 1991].
See also De Leenheer *et al.* [1990], Wyss and Bucheli [1988], McNamara *et al.* [1988], Larsen *et al.* [1987, 1988], Al Mallah *et al.* [1987], Jakobsen *et al.* [1987] and Palmskog [1980].
Urine **HPLC** Column: Nucleosil C_{18} (250 × 4 mm i.d., 5 µm). Mobile phase: methanol : 1% aqueous acetic acid (85 : 15), flow rate 1.5 mL/min. UV detection (λ = 350 nm). Retention time: 8.9 min. Limit of detection not reported [Al Mallah *et al.* 1987].
Skin **HPLC** See Plasma [Laugier *et al.* 1994].
Subcutaneous Tissue **HPLC** See Plasma. Limit of quantification, 13 ng/g (50 mg tissue sample) [Sturkenboom *et al.* 1994].
Suction Blister **HPLC** See Plasma [Laugier *et al.* 1994]
Disposition in the Body Acitretin is absorbed after oral administration and peak plasma concentrations occur after approximately 1 to 6 h. Administration with food increases bioavailability. The drug is widely distributed in the body. It undergoes extensive metabolism and interconversion by simple isomerisation to 13-*cis*-acitretin. Both acitretin and 13-*cis*-acitretin may be conjugated. An additional three metabolites have been identified. Etretinate has been detected following administration of acitretin; this may be a route of metabolism if there is concomitant alcohol consumption. It is excreted in urine and bile. Neither acitretin nor its 13-*cis* metabolite are removed by dialysis.
Therapeutic Concentration
Following administration of a single dose of acitretin (40 mg) to 6 subjects with psoriasis, aged between 23 and 76 years (mean, 52 years), peak plasma concentrations of 98 to 526 µg/L (mean, 241 µg/L) occurred after 0.6 to 3.0 h. The dose was administered after an overnight fast followed by an additional 3 h fast post-administration [Larsen *et al.* 1987].
Following administration of 50 mg acitretin to 18 healthy male subjects (18 to 40 years old) with or without food, peak plasma concentrations of 87 to 480 µg/L and 196 to 728 µg/L were measured, respectively. A mean peak plasma concentration of 245 and 416 µg/L was observed for the doses administered with food and without, respectively. Peak concentrations were reached between 2 and 5 h [McNamara *et al.* 1988].
Peak plasma concentrations of 229 to 408 µg/L occurred after 1.0 to 2.8 h in four subjects who were administered 40 mg acitretin daily for 3 months. This dose was ingested after an overnight fast followed by a 3 h additional fast post-administration. The individuals used in the study were aged 23 to 59 years (mean, 55 years) and suffered from severe psoriasis (healthy otherwise) [Larsen *et al.* 1988].
Toxicity Acitretin is teratogenic. Although acitretin has a relatively short half-life, etretinate has also been detected in the plasma of some patients taking acitretin. Since etretinate is also teratogenic and has a much longer half-life than acitretin, pregnancy must be avoided for at least 3 years after stopping acitretin therapy [Sturkenboom *et al.* 1994]. Plasma concentrations may be a poor guide to presence of etretinate in the body since etretinate concentrates in fatty tissues. The minimum plasma concentrations associated with teratogenicity are unknown.
A woman had taken a cumulative dose of 9.9 g acitretin but had stopped taking it 52 months previously. Etretinate concentrations of of 0.30 µg/L and 128.5 µg/kg were found in plasma and SC fat, respectively. Another woman who was still taking acitretin (cumulative dose, 1.075 g) had plasma and fat concentrations of 0.48 µg/L and 18.7 µg/kg etretinate, respectively [Maier, Hönigsmann 1996].
Bioavailability Approximately 60%.
Half-life Plasma, acitretin, 50 h; 13-*cis*-acitretin, 63 h.
Volume of Distribution Mean, 3.47 L/kg; range, 2.3 to 4.5 L/kg.
Clearance Plasma, 175.9 ± 81.4 mL/kg/h.
Protein Binding Greater than 99.9%.
Note For general data and references, see Brindley [1989] and Pilkington and Brogden [1992].
Dose Usually 25 to 50 mg daily.

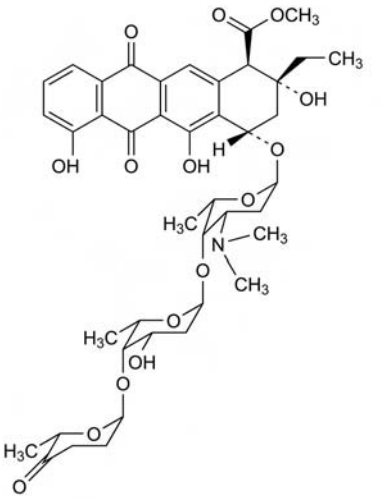
Al Mallah NR *et al.* (1987). Determination of the aromatic retinoids (etretin and isoetretin) in biological fluids by high-performance liquid chromatography. *J Chromatogr* 421: 177–186.
Brindley CJ (1989). Overview of recent clinical pharmacokinetic studies with acitretin (Ro 10-1670, etretin). *Dermatologica* 178: 79–87.
De Leenheer AP *et al.* (1990). High-performance liquid chromatographic determination of etretinate and all-*trans*- and 13-*cis*-acitretin in human plasma. *J Chromatogr* 500: 637–642.
Jakobsen P *et al.* (1987). Simultaneous determination of the aromatic retinoids etretin and etretinate and their main metabolites by reversed-phase liquid chromatography. *J Chromatogr* 415: 413–418.
Klein J *et al.* (1992). Therapeutic drug monitoring of retinoids. *Ther Drug Monit* 14: 197–202.
Larsen FG *et al.* (1987). Single dose pharmacokinetics of etretin and etretinate in psoriatic patients. *Pharmacol Toxicol* 61: 85–88.
Larsen FG *et al.* (1988). Pharmacokinetics of etretin and etretinate during long-term treatment of psoriasis patients. *Pharmacol Toxicol* 62: 159–165.
Laugier JP *et al.* (1994). Determination of acitretin in the skin, in the suction blister, and in plasma of human volunteers after multiple oral dosing. *J Pharm Sci* 83: 623–628.
Maier H, Hönigsmann H (1996). Concentration of etretinate in plasma and subcutaneous fat after long-term acitretin. *Lancet* 348: 1107.

McNamara PJ *et al.* (1988). Food increases the bioavailability of acitretin. *J Clin Pharmacol* 28: 1051–1055.
Meyer E *et al.* (1991). Improved quantitation of 13-*cis*- and all-*trans*-acitretin in human plasma by normal-phase high-performance liquid chromatography. *J Chromatogr* 570: 149–156.
Palmskog G (1980). Determination of plasma levels of two aromatic retinoic acid analogues with antipsoriatic activity by high-performance liquid chromatography. *J Chromatogr* 221: 345–351.
Pilkington T, Brogden RN (1992). Acitretin. A review of its pharmacology and therapeutic use. *Drugs* 43: 597–627.
Sturkenboom MC *et al.* (1994). Inability to detect plasma etretinate and acitretin is a poor predictor of the absence of these teratogens in tissue after stopping acitretin treatment. *Br J Clin Pharmacol* 38: 229–235.
Wyss R, Bucheli F (1988). Quantitative analysis of retinoids in biological fluids by high-performance liquid chromatography using column switching. II. Simultaneous determination of etretinate, acitretin and 13-*cis*-acitretin in plasma. *J Chromatogr* 431: 297–307.
Wyss R, Bucheli F (1992). Use of direct injection precolumn techniques for the high-performance liquid chromatographic determination of the retinoids acitretin and 13-*cis*-acitretin in plasma. *J Chromatogr* 593: 55–62.

Aclarubicin

Antineoplastic
$C_{42}H_{53}NO_{15}$ = 811.8
CAS—57576-44-0

IUPAC Name Methyl (1*R*,2*R*,4*S*)-4-[(2*R*,4*S*,5*S*,6*S*)-4-(dimethylamino)-5-[(2*S*,4*S*,5*S*, 6*S*)-4-hydroxy-6-methyl-5-[(2*R*, 6*S*)-6-methyl-5-oxooxan-2-yl]oxy-oxan-2-yl]oxy-6-methyloxan-2-yl]oxy-2- ethyl-2,5,7-trihydroxy-6,11-dioxo-3,4-dihydro-1H-tetracene-1-carboxylate
Synonym Aclacinomycin A; [1*R*-(1α,2β,4β)]-2-ethyl-1,2,3,4,6,11-hexahydro-2,5,7-trihydroxy-6,11-di-oxo-4-[[2,3,6-trideoxy-4-*O*-[2,6-dideoxy-4-*O*-[(2*R*-*trans*)-tetrahydro-6-methyl-5-oxo-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyl]-3-(dimethyl-amino)-α-L-*lyxo*-hexopyranosyl]oxy]-1-naphthacenecarboxylic acid methyl ester.

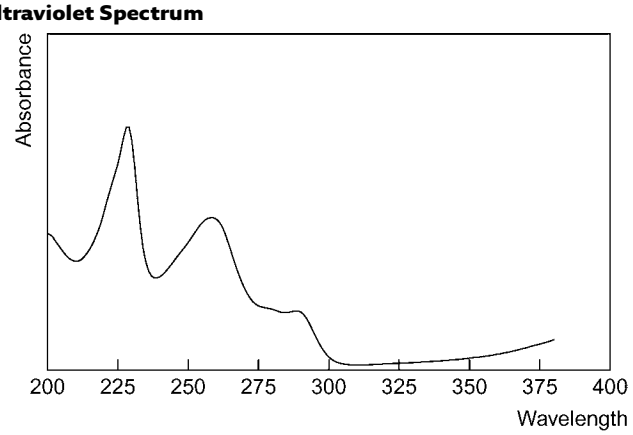


Chemical Properties It is soluble in chloroform and in ethyl acetate; insoluble in ether, in *n*-hexane and in light petroleum.

Aclarubicin Hydrochloride

$C_{42}H_{53}NO_{15}$,HCl = 848.3
Proprietary Names *Aclacin*; *Aclacinomycine*; *Aclaplastin*.

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 113, 100, 71, 43, 73, 81, 96, 376.
Quantification
Serum **HPLC** Column: C_{18} μBondapak (300 × 4 mm i.d., 10 μm). Mobile phase: acetonitrile: methanol: water (40:90:70) acidified with orthophosphoric acid 85% (1.5 mL per 100 mL), flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: 5.9 min [Erttmann 1983]. Limit of detection, 10.8 μg/L [Erttmann 1983].

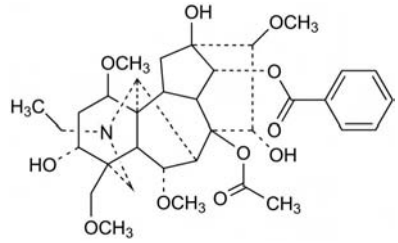
Disposition in the Body Aclarubicin is rapidly distributed following intravenous administration. It is rapidly and highly metabolised to biologically active glycosides and inactive aglycones. The metabolites are excreted mainly in urine, and small amounts may be excreted in faeces.
Therapeutic Concentration The mean peak concentration of the principal active metabolite is 130 μg/L and occurs at a mean time of 191 min after dosing.
Half-life Plasma, aclarubicin, 3 h; principal metabolite, 13 h.
Clearance Plasma, 1.6 to 8.8 L/min/m^2.
Volume of Distribution Apparent, 600 to 1900 L/m^2.
Dose 25 to 100 mg/m^2 as a single infusion every 3 to 4 weeks (maintenance therapy). Usual maximum total dose is 400 mg/m^2.

Erttmann R (1983). Determination of aclacinomycin A by reversed-phase high-performance liquid chromatography. *J Chromatogr* 277: 433–435.

Aconitine

Alkaloid, Diterpine
$C_{34}H_{47}NO_{11}$ = 645.7
CAS—302-27-2

Synonyms Acetylbenzoylaconine; (1α,3α,6α,14α,15α,16β)-20-ethyl-1,6,16-trimethoxy-4-(methoxymethyl)aconitane-3,8,13,14,15-pentol 8-acetate 14-benzoate.

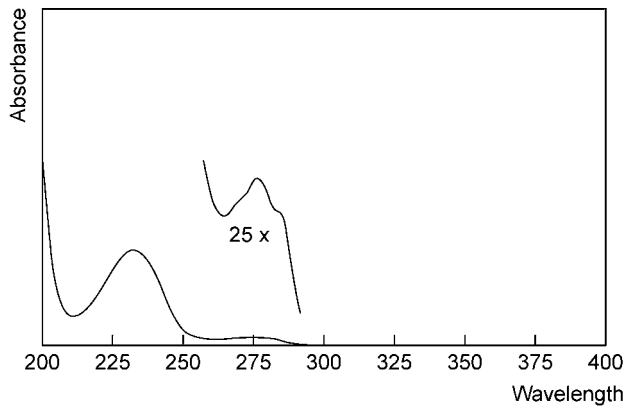


Chemical Properties An alkaloid present in species of *Aconitum*, including *A. napellus* agg. (Ranunculaceae). Colourless crystals or white crystalline powder. Mp 204°, with decomposition. Solubility (g/mL): 1 in 3300 water, 1 in 28 abs. alcohol, 1 in 2 chloroform, 1 in 50 ether, 1 in 7 benzene and slightly soluble in petroleum ether. In phosphate buffer (pH 7.4) at 37°, aconitine is converted *in vitro* to benzoylaconine and aconine over time [Mizugaki *et al.* 1998]. In extracts, aconitine was stable at high and low concentrations for more than 24 h, before and after 3 freeze–thaw cycles and after storage at –20° for 1 month [Beyer *et al.* 2007].

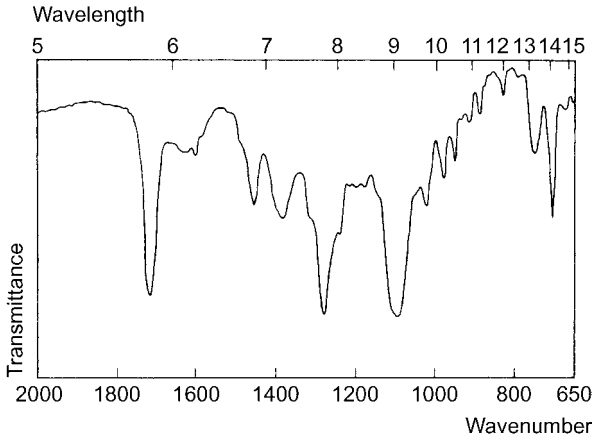
Aconitine Nitrate

$C_{34}H_{47}NO_{11}$,HNO_3 = 708.8
CAS—6509-18-8
Proprietary Names *Aconitysat*; *Bromofotyl*; *Herbadon*.
Chemical Properties Colourless crystals or white crystalline powder. Mp approx. 200°, with decomposition. Soluble 1 in 10 in boiling water; less soluble in cold water; soluble in alcohol. pK_a 8.1 (25°). Log *P* (octanol/water), 0.13.

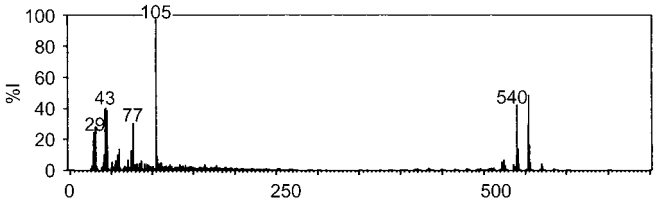
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.11; system TC—R_f 0.39; system TE—R_f 0.75; system TL—R_f 0.58; system TAE—R_f 0.42; system TAF—R_f 0.68; system TAJ—R_f 0.06; system TAK—R_f 0.00; system TAL—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Gas Chromatography-Mass Spectrometry Column: Fused silica cross-linked with 5% phenylsilicone (15 m × 0.25 mm i.d.). Temperature programme: 250° for 1 min to 320° at 16°/min. Carrier gas: He, 25 cm/s. SIM acquisition mode [Ito *et al.* 1997].
High Performance Liquid Chomatography System HX—RI 432.
Liquid Chomatography-Mass Spectrometry Column: Inertsil ODS-2 (150 × 0.32 mm i.d.). Mobile phase: acetonitrile : 0.3% trifluoroacetic acid solution (0 : 100 to 80 : 20 over 40 min) containing 0.8% glycerin, flow rate 5 μL/min. FAB. Retention time: 28.5 min. Limit of detection, ~1 pg [Ohta *et al.* 1998].
Ultraviolet Spectrum Aqueous acid—234 (A_1^1 = 233a), 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1092, 1273, 1713, 1235, 710, 1020 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 105, 554, 540, 43, 45, 77, 31, 29.



Quantification

Blood **GC-MS** Column: DB-5 fused silica capillary cross-linked with methyl 5% phenylsilicone. Temperature programme: 250° for 1° /min to 320° at 16°/min. Carrier gas: He, 25 cm/s. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 pg for aconitine and mesaconitine [Ito *et al.* 1998].

HPLC Column: XTerra RP18150 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium hydrogen carbonate (pH 10.05) : acetonitrile (100 : 0 to 50 : 50 at 10 min for 15 min), flow rate 1 mL/min. UV detection (λ = 237 nm). Limit of detection, 0.1 ng [Wang *et al.* 2004]. Column: Spherisorb S5OD/CN (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (0 : 100 to 70 : 30 at 15 min for 3 min), flow rate 2 mL/min. DAD (λ = 200–595 nm). Retention time: 3.78 min. Limit of detection, ~2 μg/L [Elliott 2002]. Column: L-column ODS (150 × 4.6 mm i.d.). Mobile phase: organic solvent mixed with a dilute acidic solution, flow rate 1 mL/min. UV detection (λ = 235 nm). Limit of detection, 1 ng for aconitine and other aconite alkaloids, [Ohta *et al.* 1997].

LC-MS Column: XTerra MS C_{18} (50 × 2.1 mm, 3.5 μm). Mobile phase: 2 mmol/L ammonium acetate : water : acetonitrile (10 : 80 : 10 for 0.5 min to 10 : 40 : 50 from 0.5 to 1.5 min to 10 : 50 : 40 for 1 min to 10 : 80 : 10 over 1 min), flow rate, 0.4 mL/min. ESI, positive ion mode. Retention time: 2.9 min. Limit of detection, 0.4 μg/L [Pullela *et al.* 2008]. Column: XTerra RP_8 (150 × 2.1 mm i.d., 3.5 μm). ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 ng/g, limit of detection, 0.1 ng/g [Beike *et al.* 2004]. Column: Inertsil ODS-2 (150 × 0.32 mm i.d.). Mobile phase: acetonitrile : 0.3% trifluoroacetic acid solution (0 : 100 to 80 : 20 over 40 min) containing 0.8% glycerin, flow rate 5 μL/min. FAB. Retention time: 28.5 min. Limit of detection, ~1 μg/L [Ohta *et al.* 1998].

Plasma **LC-MS** Column: LiChroCART (125 × 2 mm i.d.). Mobile phase: 50 mmol/L aqueous ammonium formate (pH 3.5) : acetonitrile (90 : 10 for 2 min to 20 : 80 at 5 min for 2 min to 90 : 10 at 10 min), flow rate 0.4 mL/min for 2 min to 0.6 mL/min for 5 min to 0.4 mL/min for 3 min. APCI, positive ion mode, SIM acquisition mode or tandem MS, ESI, MRM acquisition mode. Limit of quantification, 50 and 0.1 μg/L for single-stage MS and tandem MS, respectively [Beyer *et al.* 2007]. Column: Shiseido UG80 C_{18}. Mobile phase: 20 mmol/L ammonium acetate : acetonitrile (70 : 30), flow rate 200 μL/min. ESI, positive ion mode. Limit of detection, 0.2 μg/L [Kaneko *et al.* 2006]. Column: Altima C_{18} (150 × 3 mm i.d., 3 μm). Mobile phase: 2 mmol/L formate buffer (pH 3.0) : acetonitrile (20 : 80), flow rate 400 μL/min. ESI, MRM acquisition mode. Limit of quantification, 0.02 μg/L, limit of detection, 0.01 μg/L [Moritz *et al.* 2005].

Serum **GC-MS** Column: DB-5 fused silica capillary cross-linked with methyl 5% phenylsilicone (15 m × 0.25 mm i.d.). Temperature programme: 250° for 1 min to 320° at 16°/min. Carrier gas: He, 25 cm/s. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 pg [Mizugaki *et al.* 1998]. Column: DB-5 cross-linked with methyl 5% phenylsilicone (15 m × 0.25 mm i.d.). Carrier gas: He, 25 cm/s. Temperature programme: 250° for 1 min to 320° at 16°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 pg [Ito *et al.* 1997].

LC-MS Column: Xterra RP18 (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.1% formic acid in acetonitrile : 0.1% formic acid in aqueous solution (24 : 76), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection not reported [Fujita *et al.* 2007]. Column: Eclipse XDB-C_{18}(150 × 2.1 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium acetate (pH 7.0): acetonitrile containing 0.5% acetate (80 : 20 to 30 : 70 at 7 min to 20 : 80 at 14 min), flow rate 0.25 mL/min. ESI, positive ion mode. Limit of detection, 0.2–1 μg/L [Hayashida *et al.* 2003].

Urine **GC-MS** See Blood [Ito *et al.* 1998]. See Serum [Mizugaki *et al.* 1998].

HPLC See Blood [Elliott 2002; Wang *et al.* 2004]. Column: L-column ODS (150 × 4.6 mm i.d.). Mobile phase: organic solvent mixed with a dilute acidic solution, flow rate 1 mL/min. UV detection (λ = 235 nm). Limit of detection, 1 ng for aconitine and other aconite alkaloids [Ohta *et al.* 1997].

LC-MS See Blood [Pullela *et al.* 2008]. Column: XTerra (150 × 2.1 mm i.d.). Mobile phase: acetonitrile : water both containing 0.3% formic acid (25 : 75 for 5 min to 60 : 40 at 6.5 min for 3.5 min to 25 : 75 at 11 min), flow rate 300 μL/min. ESI, positive ion mode. Limit of detection not reported [Van Landeghem *et al.* 2007]. Column: Agilent Zorbax Eclipse XDB C_8 (150 × 4.5 mm i.d., 5 μm). Mobile phase: 0.2% formic acid in water : 0.2% formic acid in acetonitrile (70 : 30 to 50 : 50 in 20 min to 70 : 30 in 3 min), flow rate 0.5 mL/min. ESI, positive ion mode. Limit of detection, 2.5 μg/L [Lai *et al.* 2006]. Column: Hypersil BDS-C_{18}(150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : formic acid (50 : 50 : 1), flow rate 0.5 mL/min. ESI, SRM acquisition mode. Limit of detection not reported [Zhang *et al.* 2005]. See Blood [Beike *et al.* 2004]. See Blood. Limit of detection, ~0.5 μg/L [Ohta *et al.* 1998].

Stomach Contents **LC-MS** See Blood [Beike *et al.* 2004].

Kidney **LC-MS** See Urine [Van Landeghem *et al.* 2007].

Liver **LC-MS** See Urine [Van Landeghem *et al.* 2007].

Other **LC-MS** Tuber Samples. See Plasma [Kaneko *et al.* 2006].

Disposition in the Body

Toxicity Aconitine affects both the heart and the CNS and is one of the most potent and quick-acting poisons. It is well absorbed from the gastrointestinal tract and death may occur within a few minutes. The estimated minimum lethal dose is 2 mg of aconitine, 5 mL of aconite tincture (25% v/v) or 1 g of aconite root, although recovery after ingestion of 10 mg of aconitine has been reported.

A 25-year-old individual ingested some pink flowers and some blackberries. The pink flowers were later identified as *A. napellus*. Postmortem femoral blood and urine had aconitine concentrations of 3.6 and 149 μg/L, respectively [Pullela *et al.* 2008].

A man in his fifties was poisoned with *A. napellus*. On re-analysis of samples 5 years later, his aconitine concentrations were 810 μg/L in urine and 6.5 and 1.3 μg/kg in his liver and kidneys, respectively [Van Landeghem *et al.* 2007].

A 21-year-old man ingested 3 homemade capsules containing 237 mg of root and 19 μg of aconitine. At 7, 9, 14 and 19 h post-ingestion, his plasma aconitine concentrations were 1.75, 0.75, 0.35, and 0.02 μg/L, respectively [Moritz *et al.* 2005].

Two cases of aconitine poisoning gave the following postmortem concentrations:

	Blood (μg/kg)	*Stomach contents (μg/kg)*	*Urine (μg/L)*
Case 1	10.0	3	NA
Case 2	12.1	NA	180

NA, not available [Beike *et al.* 2004].

In a fatality resulting from the deliberate ingestion of *A. napellus* extract, the following postmortem tissue concentrations of aconitine were reported: femoral blood 10.8 μg/L, urine 264 μg/L. The antemortem concentrations were 6 μg/L in the serum (estimate) and 334 μg/L in the urine [Elliott 2002].

A 61-year-old man developed nausea and diarrhoea 2 h after ingesting aconite, mistaken for edible grass. Resuscitation and antiarrhythmic drugs did not work. Ventricular tachycardia and fibrillation developed and lasted for 6 h. The man fell into a coma and died of brain oedema on the 6th day. No aconitine was detected in serum after 24 h. Urine was tested after 9, 13 and 18 h and aconitine levels were 14.9, 20.2 and 2.8 μg/L, respectively [Yoshioka *et al.* 1996].

Note But *et al.* [1994] describe 3 fatal cases of aconite poisoning and Dickens *et al.* [1994] describe a further 2. For a review of aconitine poisonings since the 1950s see Chan [1994], for a global perspective see Chan [2002] and see Chan *et al.* [1994] for cases specifically regarding Chinese herbal medicines.

Beike J *et al.* (2004). Determination of aconitine in body fluids by LC-MS-MS. *Int J Legal Med* 118: 289–293.
Beyer J *et al.* (2007). Detection and validated quantification of toxic alkaloids in human blood plasma: comparison of LC-APCI-MS with LC-ESI-MS/MS. *J Mass Spectrom* 42: 621–633.
But PP *et al.* (1994). Three fatal cases of herbal aconite poisoning. *Vet Hum Toxicol* 36: 212–215.
Chan TY (1994). Aconitine poisoning: a global perspective. *Vet Hum Toxicol* 36: 326–328.
Chan TY (2002). Incidence of herb-induced aconitine poisoning in Hong Kong: impact of publicity measures to promote awareness among the herbalists and the public. *Drug Saf* 25: 823–828.
Chan TY *et al.* (1994). Aconitine poisoning due to Chinese herbal medicines: a review. *Vet Hum Toxicol* 36: 452–455.
Dickens P *et al.* (1994). Fatal accidental aconitine poisoning following ingestion of Chinese herbal medicine: a report of two cases. *Forensic Sci Int* 67: 55–58.
Elliott SP (2002). A case of fatal poisoning with the aconite plant: quantitative analysis in biological fluid. *Sci Justice* 42: 111–115.
Fujita Y *et al.* (2007). Five cases of aconite poisoning: toxicokinetics of aconitines. *J Anal Toxicol* 31: 132–137.
Hayashida M *et al.* (2003). A column-switching LC/MS/ESI method for detecting tetrodotoxin and *Aconitum* alkaloids in serum. *Leg Med (Tokyo)* 5(Suppl1): S101–S104.

Ito K *et al.* (1997). Method for the simultaneous determination of *Aconitum* alkaloids and their hydrolysis products by gas chromatography–mass spectrometry in human serum. *Planta Med* 63: 75–79.
Ito K *et al.* (1998). Report on the preparation of deuterium-labelled aconitine and mesaconitine and their application to the analysis of these alkaloids from body fluids as internal standard. *J Chromatogr B Biomed Sci Appl* 714: 197–203.
Kaneko R *et al.* (2006). Sensitive analysis of aconitine, hypaconitine, mesaconitine and jesaconitine in human body fluids and *Aconitum* tubers by LC/ESI-TOF-MS. *J Mass Spectrom* 41: 810–814.
Lai CK *et al.* (2006). Hidden aconite poisoning: identification of yunaconitine and related aconitum alkaloids in urine by liquid chromatography-tandem mass spectrometry. *J Anal Toxicol* 30: 426–433.
Mizugaki M *et al.* (1998). Quantitative analysis of *Aconitum* alkaloids in the urine and serum of a male attempting suicide by oral intake of aconite extract. *J Anal Toxicol* 22: 336–340.
Moritz F *et al.* (2005). Severe acute poisoning with homemade *Aconitum napellus* capsules: toxicokinetic and clinical data. *Clin Toxicol (Phila)* 43: 873–876.
Ohta H *et al.* (1997). Determination of *Aconitum* alkaloids in blood and urine samples. I. High-performance liquid chromatographic separation, solid-phase extraction and mass spectrometric confirmation. *J Chromatogr B Biomed Sci Appl* 691: 351–356.
Ohta H *et al.* (1998). Determination of *Aconitum* alkaloids in blood and urine samples. II. Capillary liquid chromatographic-frit fast atom bombardment mass spectrometric analysis. *J Chromatogr B Biomed Sci Appl* 714: 215–221.
Pullela R *et al.* (2008). A case of fatal aconitine poisoning by Monkshood ingestion. *J Forensic Sci* 53: 491–494.
VanLandeghem AA *et al.* (2007). Aconitine involvement in an unusual homicide case. *Int J Legal Med* 121: 214–219.
Wang ZH *et al.* (2004). Quantitative determination of *Aconitum* alkaloids in blood and urine samples by high-performance liquid chromatography. *Phytochem Anal* 15: 16–20.
YoshiokaN *et al.* (1996). A case of aconitine poisoning with analysis of aconitine alkaloids by GC/SIM. *Forensic Sci Int* 81: 117–123.
Zhang HG *et al.* (2005). Separation and identification of *Aconitum* alkaloids and their metabolites in human urine. *Toxicon* 46: 500–506.

Acriflavinium Chloride

Antiseptic

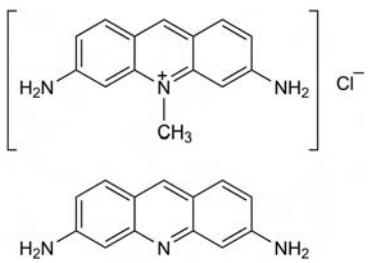
$C_{27}H_{25}Cl_2N_6 = 505.4$

CAS—8063-24-9

IUPAC Name Acridine-3,6-diamine; 10-methylacridin-10-ium-3,6-diamine; chloride; hydrochloride

Synonyms Acid trypaflavine; acriflavinium chloride hydrochloride.

Proprietary Names *Diacrid*; *Panflavin*.



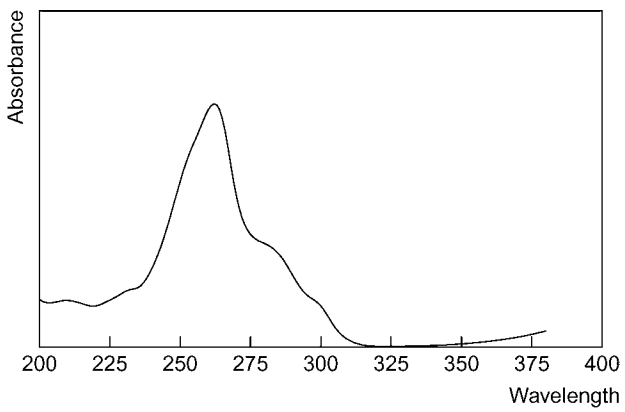
Chemical Properties An orange-red to red crystalline powder. A precipitate may form in aqueous solutions on dilution or on standing. Soluble 1 in about 3 of water and 1 in 40 of ethanol; practically insoluble in chloroform and ether. pK_a 9.1 (25°).

Note A mixture of 3,6-diamino-10-methylacridinium chloride hydrochloride and 3,6-diaminoacridine dihydrochloride, the latter being present to the extent of approximately one-third.

Colour Test Marquis test—yellow→red.

Thin-layer Chromatography System TA—four spots at R_f 0.07, R_f 0.22, R_f 0.28, R_f 0.62 (location under ultraviolet light, yellow-green fluorescence).

Ultraviolet Spectrum Aqueous acid—262 nm (A^1_1=1515a). No alkaline shift



Infrared Spectrum Principal peaks at wavenumbers 1631, 1590, 1171, 1181, 1316, 1242 cm^{-1}.

Acrivastine

Antihistamine

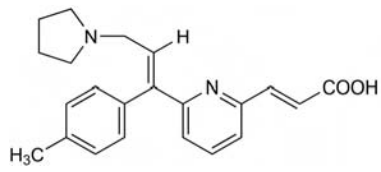
$C_{22}H_{24}N_2O_2 = 348.4$

CAS—87848-99-5

IUPAC Name (*E*)-3-[6-[(*E*)-1-(4-Methylphenyl)-3-pyrrolidin-1-ylprop-1-enyl] pyridin-2-yl]prop-2-enoic acid

Synonyms BW-270C; BW-825C; BW-A825C; (*E*)-3-{6-[(*E*)-3-Pyrrolidin-1-yl-1-*p*-tolylprop-1-enyl]-2-pyridyl}acrylic acid.

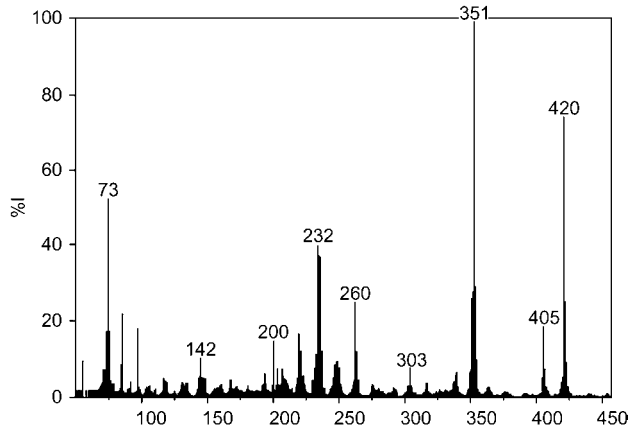
Proprietary Names *Benadryl*; *Semprex*.



Chemical Properties An odourless, white to pale cream crystalline powder. Mp 222°. It is slightly soluble in water; soluble in chloroform and ethanol. Log *P* (octanol/water), 2.83.

High Performance Liquid Chromatography Column: RP-C_{18} LiChrosorb (200 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile : methanol : perchloric acid : *n*-octylamine (500:130:25:13:0.3), flow rate 3 mL/min. UV detection (λ=260 nm). Retention time: 6.6 min [Altuntas *et al.* 1998].

Mass Spectrum Principal ions at *m/z* 351, 420, 73, 232, 233, 260, 405, 200 (TMS derivative).



Quantification

Plasma **GC-MS** Limit of detection, about 2 µg/L for acrivastine and propionic acid metabolite [Chang *et al.* 1989].

Disposition in the Body Acrivastine is well absorbed after oral administration. Peak plasma concentrations are reached in about 1.5 h. It is does not cross the blood–brain barrier to a significant extent. The drug undergoes some metabolism to form a propionic acid analogue that has pharmacological activity and makes up approximately 10% of the total plasma drug concentration. About 60% of an administered dose is excreted unchanged in urine and 15 to 17% of this is detected as the metabolite.

Therapeutic Concentration

A mean peak plasma concentration of 73.1 (range, 48.1 to 101.0) µg/L was obtained 1.42 h (range, 0.75 to 2 h) after oral administration of a single dose of 4 mg to 12 healthy male subjects, aged between 20 and 42 years [Cohen *et al.* 1985].

Bioavailability 18%.

Half-life Plasma, 1.4 to 2.1 h (acrivastine); 2.3 h (metabolite).

Volume of Distribution 0.64 L/kg (single dose); 0.75 L/kg (multiple).

Clearance Total body, 4.41 ± 0.63 mL/min/kg.

Protein Binding 50%.

Note For a review of acrivastine see Brogden and McTavish [1991].

Dose 8 mg 3 times daily (in Europe and UK) or 4 times daily (USA).

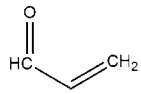
Altuntas TG *et al.* (1998). Quantitative determination of acrivastine and pseudoephedrine hydrochloride in pharmaceutical formulation by high performance liquid chromatography and derivative spectrophotometry. *J Pharm Biomed Anal* 17: 103–109.
Brogden RN, McTavish D (1991). Acrivastine. A review of its pharmacological properties and therapeutic efficacy in allergic rhinitis, urticaria and related disorders. *Drugs* 41: 927–940 ; erratum. 42: 639.
Chang SY *et al.* (1989). Quantitative gas chromatographic-mass spectrometric analysis of acrivastine and a metabolite in human plasma. *J Chromatogr* 497: 288–295.
Cohen AF *et al.* (1985). Pharmacodynamic and pharmacokinetics of BW 825C: a new antihistamine. *Eur J Clin Pharmacol* 28: 197–204.

A

Acrolein

Aldehyde, Herbicide, Lachrymator
$C_3H_4O = 56.1$
CAS—107-02-8

IUPAC Name Prop-2-enal
Synonyms Acraldehyde; acrylaldehyde; acrylic aldehyde; aqualin (shell); 2-propenal.



Chemical Properties Colourless to slightly yellow liquid with a pungent odour. Mp −88°. Soluble in 2 to 3 parts water, in alcohol, in ether. Log *P* (octanol/water), −0.01 [Hansch *et al.* 1995]. Unstable, polymerises (especially under light or in the presence of alkali or strong acid) forming disacryl, a plastic solid. Stable in PBS for at least 2 h at 4° but in urine stable for 30 min at 4° [Sakura *et al.* 1998].

For the absorption spectrum, see O'Neil *et al.* [2006].

Quantification

Plasma **HPLC** Column: C_{18} RP ODS Uptisphere (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : imidazole buffer (pH 7.5, 30 : 70), flow rate 1.8 mL/min. Fluorescence detection (λ_{ex}=407 nm, λ_{em}=507 nm). Limit of quantification, 16.8 µg/L, limit of detection, 5.6 µg/L [Paci *et al.* 2000].

Urine **GC-MS** Column: DB-WAX capillary (30 m × 0.32 mm i.d., 0.5 µm). Carrier gas: He, 2.0 mL/min. EI ionisation, SIM acquisition mode. Retention time: 1.4 min. Limit of detection not reported [Takamoto *et al.* 2004]. Column: DB-WAX capillary (30 m × 0.32 mm i.d., 0.5 µm). Carrier gas: He, 2.0 mL/min. Temperature: 70°. EI ionisation, SIM acquisition mode. Limit of detection, 1.0 nmol/L [Takamoto *et al.* 2001]. Column: DB-1 capillary (60 m × 0.5 mm i.d., 0.25 µm). Carrier gas: He, 1.7 mL/min. Temperaature: 70°. SIM acquisition mode. Limit of detection, 1–5 nmol/L [Sakura *et al.* 1998].

HPLC Column: C_{18} (10 cm × 8 mm i.d., 10 µm). Mobile phase: 0.05 mol/L dibasic ammonium phosphate solution (pH 2.5) : acetonitrile : methanol (92 : 6 : 2), flow rate 3 mL/min. Fluorescence detection (λ_{ex}= 360 nm, λ_{em}= 495 nm). Retention time: 4.3 min. Limit of detection, 1.0 mg/L [Al Rawithi *et al.* 1993].

LC-MS Column: Luna C_8 (150 × 4.6 mm i.d., 3 µm). Mobile phase: 0.1% formic acid (pH 2.5) : acetonitrile (75 : 25 for 2 min to 50 : 50 within 6 min for 4 min to 0 : 100 in 2 min for 4 min to 75 : 25 in 4 min for 4 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Retention time: 10.7 min. Limit of detection, 5 µg/L for 3-HPMA [Schettgen *et al.* 2008].

Saliva **HPLC** Column: C_{18} Synergi Phenomenex (50 × 4.6 mm i.d., 4 µm). Mobile phase: water-tetrahydrofuran (80 : 20) : acetonitrile- tetrahydrofuran (80 : 20, 100 : 0 to 20 : 80 in 20 min), flow rate 1.2 mL/min. UV detection (λ= 365 nm). Limit of detection, 10 pmol [Annovazzi *et al.* 2004].

CE Capillary: fused silica (57/50 cm total/effective length × 50 µm i.d.). Running buffer: 20 mmol/L sodium tetraborate (pH 9.0) containing 60 mmol/L SDS. UV detection (λ= 365 nm). Limit of detection, 0.6 pmol [Annovazzi *et al.* 2004].

Brain **LC-MS** Column: C_{18} reversed phase. Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (95 : 5 for 2 min to 80 : 20 in 8 min to 30 : 70 in 20 min to 10 : 90 in 20 min to 5 : 95 in 5 min to 95 : 5), flow rate 4 µL/min. ESI. Limit of detection, 6.5 pg [Williams *et al.* 2005].

Expired Air Condensate **LC-MS** Column: Supelcosil LC-18-DB (75 × 4.6 mm i.d., 3 µm). Mobile phase: 20 mmol/L aqueous acetic acid : methanol (57 : 43 to 2 : 98 in 3.2 min for 2.5 min. ESI or APCI, negative ion mode, SRM acquisition mode. Limit of detection, 1.0 nmol/L [Andreoli *et al.* 2003].

Other **HPLC** Human Liver Microsomes. Column: LiChrosorb 100 RP-8 (125 × 3 mm i.d., 5 µm). Mobile phase: 0.5% phosphoric acid : acetonitrile (96 : 4), flow rate 0.4 mL/min. Fluorescence detection (λ_{ex}= 358 nm, λ_{em}= 505 nm). Retention time: 8 min. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Bohnenstengel *et al.* 1997].

Disposition in the Body When glycerol is heated to 280° it decomposes to acrolein. It is one of the main components of mainstream cigarette smoke, and its local noxious and carcinogenic effects in the oral cavity and upper gastrointestinal tract are well known [Annovazzi *et al.* 2004]. It is also the metabolite of cyclophosphamide and ifosphamide [Kaijser *et al.* 1993], irritates mucous membranes, and is considered pathogenetically important in hemorrhagiccystitis [Takamoto *et al.* 2004].

Toxicity Potential symptoms of overexposure are irritation of eyes, skin and mucous membranes; decreased pulmonary function; delayed pulmonary edema; chronic respiratory disease [NIOSH, 1997).

LD_{50} (oral) in rats: 0.046 g/kg [O'Neil *et al.* 2006].

Uses Manufacture of colloidal forms of metals, making plastics, perfumes, warning agent in methyl chloride refrigerant. Has been used in military poison gas mixtures. Used in organic syntheses [O'Neil *et al.* 2006].

Al Rawithi S *et al.* (1993). Determination of acrolein in urine by liquid chromatography and fluorescence detection of its quinoline derivative. *Pharm Res* 10: 1587–1590.

Andreoli R *et al.* (2003). Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* 17: 637–645.

Annovazzi L *et al.* (2004). High-performance liquid chromotography and capillary electrophoresis: methodological changes for the determination of biologically relevant low-aliphatic aldehydes in human saliva. *Electrophoresis* 25: 1255–1263.

Bohnenstengel F *et al.* (1997). High-performance liquid chromatographic determination of acrolein as a marker for cyclophosphamide bioactivation in human liver microsomes. *J Chromatogr B Biomed Sci Appl* 692: 163–168.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants.* Washington DC: American Chemical Society.

Kaijser GP *et al.* (1993). The analysis of ifosfamide and its metabolites (review). *Anticancer Res* 13: 1311–1324.

NIOSH (1997). *Pocket Guide to Chemical Hazards.* London: DHHS/NIOSH.

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn, Whitehouse Station NJ: Merck Research Laboratories.

Paci A *et al.* (2000). Quantitative high-performance liquid chromatographic determination of acrolein in plasma after derivatization with Luminarin 3. *J Chromatogr B Biomed Sci Appl* 739: 239–246.

Sakura N *et al.* (1998). Determination of acrolein in human urine by headspace gas chromatography and mass spectrometry. *J Chromatogr B Biomed Sci Appl* 719: 209–212.

Schettgen T *et al.* (2008). Simultaneous determination of mercapturic acids derived from ethylene oxide (HEMA), propylene oxide (2-HPMA), acrolein (3-HPMA), acrylamide (AAMA) and *N*,*N*-dimethylformamide (AMCC) in human urine using liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 22: 2629–2638.

Takamoto S *et al.* (2001). Determination of acrolein by headspace solid-phase microextraction gas chromatography and mass spectrometry. *J Chromatogr B Biomed Sci Appl* 758: 123–128.

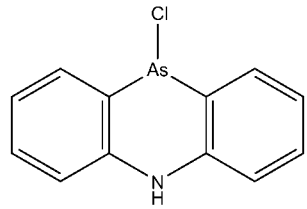
Takamoto S *et al.* (2004). Monitoring of urinary acrolein concentration in patients receiving cyclophosphamide and ifosphamide. *J Chromatogr B Analyt Technol Biomed Life Sci* 806: 59–63.

Williams TI *et al.* (2005). Analysis of derivatized biogenic aldehydes by LC tandem mass spectrometry. *Analytical Chemistry* 77: 3383–3389.

Adamsite

Chemical Warfare Agent, Organic Arsenical, Riot Control Agent
$C_{12}H_9AsClN = 277.6$
CAS—578-94-9

IUPAC Name 10-Chloro-5*H*-phenarsazinine
Synonyms 5-Aza-10-arsenaanthracene chloride; 10-chloro-5,10-dihydroarsacridine; 10-chloro-5,10-dihydrophenarsazine; diphenylaminechlorarsine; DM; phenazarsine chloride.



Chemical Properties Canary-yellow crystals from carbon tetrachloride; also reported as yellow to green solid. Dimorphous. The stable form occurs as orthorhombic crystals. Mp 195°. Bp 410° with decomposition. Sublimes readily. Practically insoluble in water (0.4 mg/L; also reported as 0.64 mg/L). Slightly soluble in benzene, xylene, carbon tetrachloride. Soluble in acetone (13 g/100 g at 15°). Corrodes iron, bronze, brass (The metastable form melts at 186° if monoclinic, and at 182° if triclinic) [Ludemann *et al.* 1969; Olajos, Salem 2001; O'Neil MJ *et al.* 2006]. Log *P* (octanol/water), 4.05 [Sanderson *et al.* 2008]. As a solid, the rate of hydrolysis is not significant owing to the formation of an oxide coating; however, the rate of hydrolysis is rapid when it is in the form of an aerosol. Hydrolysis products are hydrochloric acid and diphenylarsenious acid [Olajos, Salem 2001]. Fungal manganese peroxidase has been shown to be a useful biocatalyst to convert hazardous organopollutants that include adamsite [Haas *et al.* 2004].

Thin-layer Chromatography Plates: basic silica gel-G (250 µm). Solvent systems: (1) acetone : chloroform (20 : 80); (2) ethyl acetate : methanol (10 : 90). Locating reagent *o*-dianisidine and sulfuric acid. R_f values reported as follows:

Compound	*R_f value*		*Spot colour*
	System 1	**System 2**	
Adamsite	0.35	0.23	Orange
Diphenylcyanoarsine	0.65	—	Rose
Diphenylamine	0.08	0.15	Green
Adamsite oxide	—	0.38	Orange

Limit of quantification not reported [Ludemann *et al.* 1969].

High Performance Liquid Chromatography Column: C_{18} (250 × 3.0 mm i.d., 5 µm). Mobile phase: methanol : water (20 : 80 to 80 : 20 over 45 min to 100 : 0 over 1 min), flow rate 0.5 mL/min. UV detection (λ = 210 and 230 nm). Retention time: ~36 min. Limit of quantification not reported [Haas *et al.* 2004].

Disposition in the Body

Toxicity Adamsite is one of several compounds classified as vomiting agents, including diphenylchloroarsine (DA), diphenylcyanoarsine (DC) and chloropicrin. It is more toxic than other riot control compounds and is considered a potentially dangerous agent. The effects from adamsite begin approx. 30 min after the onset of exposure and may last an hour or two. In addition to other standard signs and symptoms of exposure to lachrymatory agents (eye and upper respiratory tract irritation, coughing, etc.), exposure to adamsite can cause unsteady gait, weakness in the limbs, and trembling, and even mental depression. Exposure to high concentrations may result in serious illness and even death [Olajos, Salem 2001]. The maximum 'safe' inhaled dose of adamsite for humans was reported as 100 mg•min/m^3; however, many of the experiments were terminated so as to not exceed the 'safe' dosage [Punte *et al.* 1962]. Human ocular irritancy thresholds and toxicity estimates are reported as follows: irritancy threshold 1 mg/m^3, intolerable concentration 5 mg/m^3, lethal concentration (10 min exposure) 650 mg/m^3. The inhalation toxicity of chemical warfare agents, military chemicals, and riot control agents is, by convention, expressed by the notation Ct. It is defined as the product of the concentration in mg/m^3 multiplied by the exposure time (t) in minutes (mg•min/m^3). The terms LCt_{50} and ICt_{50} describe the airborne dosages that are lethal (L) or incapacitating (I) to 50% of the exposed population. The estimated LCt_{50} for adamsite in humans is between 11 000 and 35 000 mg•min/m^3, whereas the ICt_{50} is reported as between 20 and 150 mg•min/m^3. Animal LCt_{50} estimates (mg•min/m^3) are reported as rat 3700, mouse 22400, and guinea pig 7900. Computed inhaled LD_{50} values (mg/kg) are reported as: rat 14.1, mouse 17.9, and guinea pig 2.4 [Olajos, Salem 2001]. LCt_{50} in fish (mg/L) is 0.44 [Sanderson *et al.* 2007].

Note For reviews of chemical warfare and riot control agents, see Olajos and Stopford [2004] and Olajos and Salem [2001].

Haas R *et al.* (2004). Conversion of adamsite (phenarsarzin chloride) by fungal manganese peroxidase. *Appl MicroBiol Biotechnol* 63: 564–566.

Ludemann WD *et al.* (1969). Qualitative thin-layer chromatography of some irritants. *Anal Chem* 41: 679–681.

Olajos E, Salem JH (2001). Riot control agents: pharmacology, toxicology, biochemistry and chemistry. *J Appl Toxicol* 21: 355–391.

Olajos EJ, Stopford W (2004). *Riot Control Agents: Issues in Toxicology, Safety and Health Care*. Boca Raton, FL: CRC Press.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories

Punte CL *et al.* (1962). Inhalation studies with chloracetophenone, diphenylamino-chloroarsine, and pelargonic morpholide. II. Human exposures. *Am Ind Hyg Assoc J* 23: 199–202.

Sanderson H *et al.* (2007). PBT screening profile of chemical warfare agents (CWAs). *J Hazard Mater* 148: 210–215.

Sanderson H *et al.* (2008). Screening level fish community risk assessment of chemical warfare agents in the Baltic Sea. *J Hazard Mater* 154: 846–857.

Adenosine

Antiarrhythmic

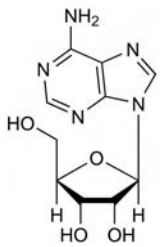
$C_{10}H_{13}N_5O_4 = 267.2$

CAS—58-61-7

IUPAC Name (2*R*,3*R*,4*S*,5*R*)-2-(6-Aminopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol

Synonyms Adenine nucleoside; adenine riboside; 6-amino-9-β-D-ribofuranosyl-9*H*-purine.

Proprietary Names *Adenocard*; *Adenocor*; *Adenoscan*; *Adenosin*; *Adrekar*; *Krenosin*.



Chemical Properties Adenosine is a white crystalline powder. Mp 234°, with decomposition >300°. Adenosine is soluble in water (approx. 8 g/L) and practically insoluble in alcohol. pK_a 3.5. Log *P* (octanol/water), −1.05. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Adenosine Triphosphate

$C_{10}H_{16}N_5O_{13}P_3 = 507.2$

CAS—56-65-5

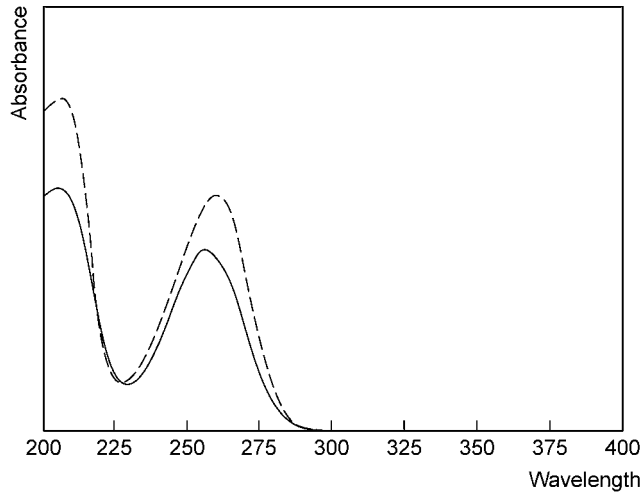
Synonyms Adenosine 5′-triphosphate; 5′-adenyldiphosphoric acid; adenylpyrophosphoric acid; ATP; adenosine 5′-(tetrahydrogen triphosphate).

Proprietary Names *Atepadene*; *Atepodin*; *Bio-Regenerat*; *Striadyne*.

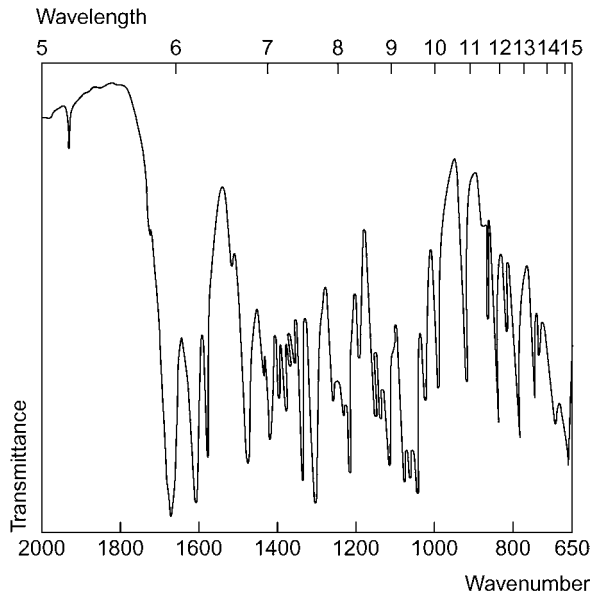
Thin-layer Chromatography Plate: octadecyl-modified silica treated with copper acetate and (2*S*,4*R*,2′*RS*)-4-hydroxyl-1-(2-hydroxydodecyl)proline (Chiral). Mobile phase: methanol : water : acetonitrile (50:50:50). R_f 0.8 [Feldburg, Reppuci 1987]. Plate: Whatman LK5DF. Mobile phase: IPA : water : ammonia (7:2:1). R_f 0.6. Plate: Whatman LHPKDF. Mobile phase: IPA : water : ammonia (7:2:1). R_f 0.7. Plate: Whatman LKC18DF. Mobile phase: IPA : water : DMSO (16:4:1). R_f 0.6. Plate: Whatman C2. Mobile phase: IPA : water : DMSO (16:4:1). R_f 0.8. Plate: Whatman SCX. Mobile phase: 0.1 mol/L ammonium hydrogen phosphate and 1 mol/L sodium chloride. R_f 0.7 [Sherma 1982]. Plate: silanised aminopropyl. Mobile phase: methanol : water (46:60) and sodium chloride (0.18 mol/L). R_f 0.8 [Hauck, Jost 1983].

High Performance Liquid Chromatography System HAA—retention time 2.7 min.

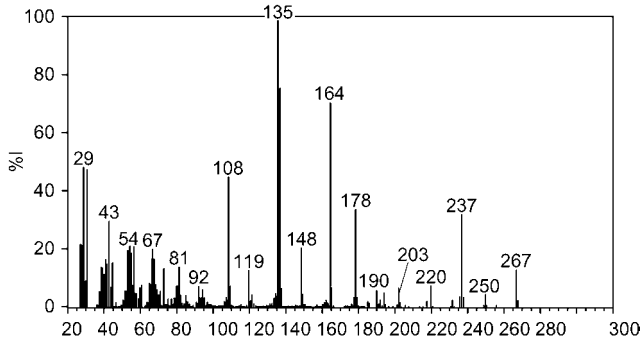
Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid), alkali (0.1 mol/L sodium hydroxide) and water—209, 259 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 135, 136, 164, 29, 31, 108, 178, 237.



Disposition in the Body Extremely efficient re-uptake of adenosine from plasma by red blood cells and blood vessel endothelial cells. Once inside the blood cells, adenosine is phosphorylated, by adenosine kinase, to adenosine monophosphate (AMP). Alternatively, it is deaminated, by adenosine deaminase, to inactive inosine, which can be further degraded to hypoxanthine and then uric acid. Adenosine is almost completely eliminated after a single pass through the coronary circulation. It is naturally found in various forms in all cells of the body.

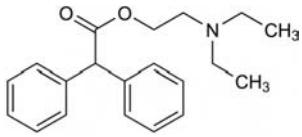
Therapeutic Concentration Administered dose is minute in comparison with adenosine concentrations already in the body and due to the efficient re-uptake, classic ADME studies are not possible.
Half-life 0.6 to 10 s.
Distribution in Blood Erythrocytes transport adenosine from the liver to tissues which cannot synthesise it.
Dose Initial dose is 6 mg (rapid IV bolus injection, administered over 1 to 2 s), followed by a saline flush. Up to 2 additional 12 mg boluses, within 1 to 2 min after initial dose, if necessary. A dose of 0.05 to 0.1 mg/kg body weight is effective in children with a maximum of 0.3 mg/kg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Feldburg RS, Reppuci LM (1987). Rapid separation of anomeric purine nucleosides by thin-layer chromatography on a chiral stationary phase. *J Chromatogr* 410: 226–229.
Hauck HE, Jost W (1983). *J Chromatogr* 261235.
Sherma BP*et al.* 1982. *Techniques and Application of Thin Layer Chromatography.* New York: John Wiley & Sons

Adiphenine

Anticholinergic
$C_{20}H_{25}NO_2 = 311.4$
CAS—64-95-9

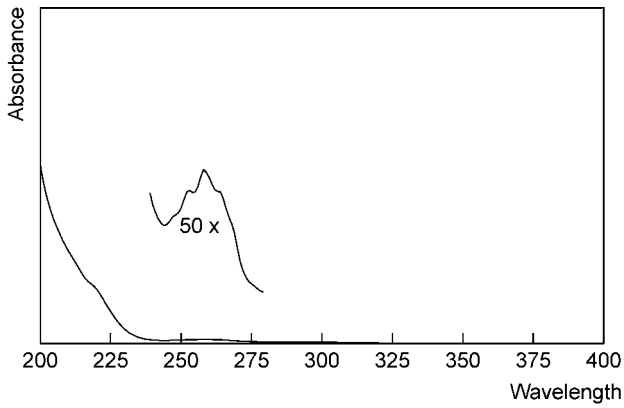
IUPAC Name 2-Diethylaminoethyl 2,2-diphenylacetate
Synonyms Diphacil; diphenylacetyldiethylaminoethanol; α-phenylbenzeneacetic acid 2-(diethylamino)ethyl ester.



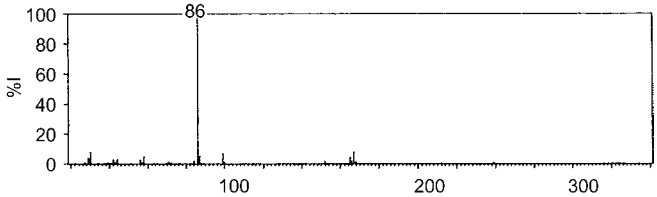
Adiphenine Hydrochloride

$C_{20}H_{25}NO_2,HCl = 347.9$
CAS—50-42-0
Synonym Spasmolytine
Proprietary Name *Trasentine*
Chemical Properties A white crystalline powder. Mp 112° to 115°. Soluble in water, ethanol and chloroform; practically insoluble in ether.

Colour Tests Liebermann's reagent—brown; Mandelin's test—green→blue.
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.56; system TC—R_f 0.60; system TE—R_f 0.83; system TAE—R_f 0.49; system TL—R_f 0.51 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2186.
High Performance Liquid Chromatography System HA—*k* 1.8; system HX—RI 422.
Ultraviolet Spectrum Aqueous acid—253, 258 (A^1_1=14.5a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1736, 1145, 700, 1190, 1500, 746 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 30, 167, 99, 87, 58, 165, 29.

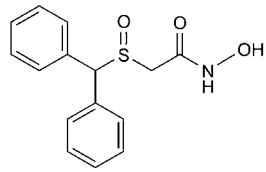


Dose Adiphenine hydrochloride has been given in doses of 225 to 450 mg daily.

Adrafinil

α-Adrenoceptor Agonist
$C_{15}H_{15}NO_3S = 289.4$
CAS—63547-13-7

IUPAC Name 2-Benzhydrylsulfinyl-*N*-hydroxyacetamide
Synonyms Adrafinilum; 2-(benzhydrylsulfinyl)acetohydroxamic acid; 2-[(diphenylmethyl)sulfinyl]-acetohydroxamic acid; 2-[(Diphenylmethyl)sulfinyl]-N-hydroxyacetamide.
Proprietary Name *Olmifon*



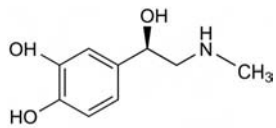
Chemical Properties White to off-white crystalline powder. Mp 154° to 160°. Slightly soluble in water (<1 g/L), more soluble in ethanol and soluble in methanol.
High Performance Liquid Chromatography System HAA—retention time 13.8 min.
Quantification
Other **LC-MS** Rat Serum. Column: Kromasil C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water: acetic acid (35 : 65 : 0.1), flow rate 1.0 mL/min. ESI, positive ion mode. Limit of quantification, 11.6 μg/L, limit of detection, 3.5 μg/L [Rao *et al.* 2008].
Disposition in the Body After oral administration, adrafinil is partly metabolised to its amide, 2-(benzhydrylsulfinyl)acetamide (2-[(diphenylmethyl)sulfinyl]acetamide; modafinil), which is also metabolised into its inactive acid and sulfone forms.
Toxicity Low toxicity.
Half-life Elimination half-life, adrafinil 1 h; modafinil 3 h.
Protein Binding To albumin, 80%.
Dose Between 600 mg and 1.2 g daily by mouth (for the elderly).

Rao RN *et al.* (2008). LC-ESI-MS determination and pharmacokinetics of adrafinil in rats. *J Chromatogr B Analyt Technol Biomed Life Sci* 873: 119–123.

Adrenaline

Sympathomimetic
$C_9H_{13}NO_3 = 183.2$
CAS—51-43-4

IUPAC Name 4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol
Synonyms Epinephrine; epirenamine; levorenin; suprarenin.
Proprietary Names *Bronkaid Mistometer*; *Dyspné-Inhal*; *Epi-glaufrin*; *Epinal*; *Eppy*; *Glauposine*; *Primatene Mist*; *Simplene*; *Suprarenaline*; *Sus-Phrine*; *Vaponefrin*. It is an ingredient of *Asma-Vydrin*; *Brovon*; *Ganda*; *Isopto*; *Epinal*; *Riddovydrin Inhalant*; *Rybarvin*.



Chemical Properties A white or creamy-white crystalline powder or granules. It darkens in colour on exposure to air and light. It is unstable in neutral or alkaline solution. Mp ≈212°, with decomposition. Sparingly soluble in water; practically insoluble in ethanol, chloroform, ether, and light petroleum; freely soluble in solutions of mineral acids and boric acid solution, and in solutions of sodium or potassium hydroxide. pK_{a1} 8.7, pK_{a2} 10.2, pK_{a3} 12.0 (20°).

Adrenaline Acid Tartrate

$C_9H_{13}NO_3,C_4H_6O_6 = 333.3$
CAS—51-42-3
Synonyms Adrenaline bitartrate; adrenaline tartrate; epinephrine bitartrate.
Proprietary Names *E1*; *E2*; *Epitrate*; *Liadren*; *Lyophrin*; *Medihaler Epi*. It is an ingredient of *E-Pilo*; *Mydricaine*; *PE*; *Welder's Flash Drops*.
Chemical Properties A white to greyish-white or light brownish-grey, crystalline powder. It slowly darkens on exposure to air and light. Incompatible with alkalis. Mp 147° to 152°, with decomposition. Soluble 1 in 3 of water and 1 in 520 of ethanol; practically insoluble in chloroform and ether.

Adrenaline Hydrochloride

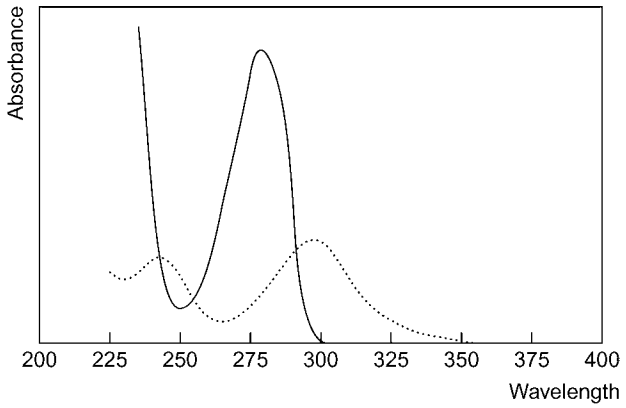
$C_9H_{13}NO_3,HCl = 219.7$
CAS—55-312

Proprietary Names *Epifrin*; *Glaucon*; *Glycirenan*. It is an ingredient of *Riddobron Inhalant*; *Riddofan*.
Colour Tests Ammoniacal silver nitrate—red-brown/red-brown; ferric chloride—green; folin–Ciocalteu reagent—blue; Marquis test—orange→violet; methanolic potassium hydroxide—pink-orange→brown; Nessler's reagent—black; palladium chloride—orange→brown; potassium dichromate (method 1)—green→brown.

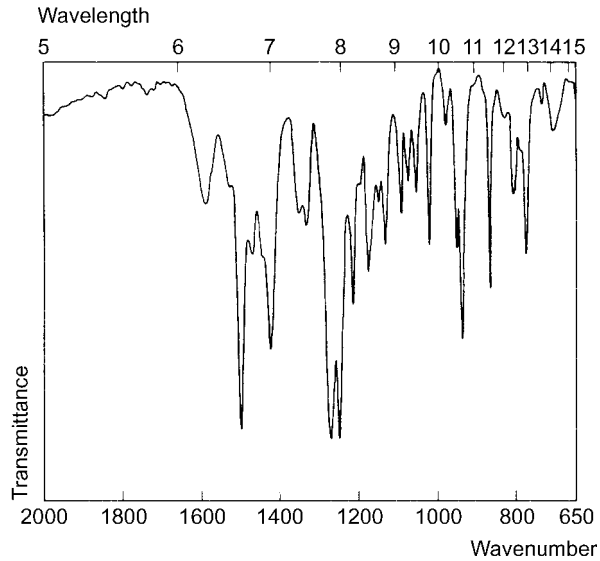
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.13; system TAE—R_f 0.30; system TAG—R_f 0.00 (acidified potassium permanganate solution—positive).

High Performance Liquid Chromatography System HC—*k* 0.63; system HX—RI 30.

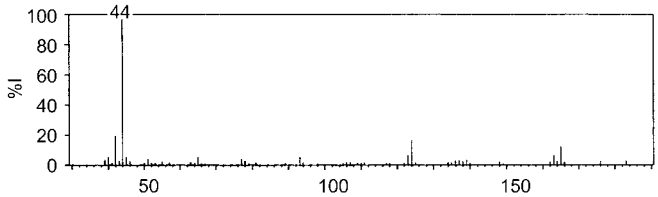
Ultraviolet Spectrum Aqueous acid—280 (A^1_1 = 155a); aqueous alkali—242, 297 nm.



Infrared Spectrum Principal peaks at wavenumbers 1253, 1274, 1500, 945, 1224, 871 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 42, 124, 165, 163, 123, 93, 65.



Quantification

Plasma **GC** Column: 7% DC-11 on 80/100 mesh Gas Chrom P (1.8 m × 3.1 mm [6 ft × 0.125 in] o.d.). Carrier gas: He, 20 mL/min. Temperature: 115°. FID. Limit of detection, 0.1 pg [Lovelady, Foster 1975].

HPLC Column: PLRP-S 100 Å (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.025 mol/L sodium phosphate-0.025 mol/L citric acid-0.001 mol/L heptane and sulfonic acid (pH 2.85), flow rate 0.75 mL/min. Fibre optic detection. Retention time: 210 s. Limit of detection, 3.5 ng/L [Ferreira *et al.* 2009]. Column: reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.025 mol/L sodium phosphate-0.025 mol/L citric acid-0.001 mol/L heptane (pH 2.8, 55 : 95), flow rate 0.75 mL/min. Electrochemical detection. Limit of detection, 3.4 ng/L [Silva *et al.* 2009]. Column: Deverosil RP Aqueous-AR-5 triacontylsilyl silica C_{30} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10.5 g/L citric acid and 20 mg/L EDTA (pH 2.8, 2 : 98), flow rate 0.3 mL/min. Electrochemical detection. Retention time: 9.2 min. Limit of quantification, 20 ng/L, limit of detection, 10 ng/L [Machida *et al.* 2006]. Column: RP C_{18} Ultrasphere-ODS (250 × 4.6 mm i.d., 5 mm). Mobile phase: 0.1 mol/L citrate buffer, 0.3 mol/L disodium EDTA and 0.5 µmol/L sodium 1-octanesulfonic acid in methanol : water (pH 2.5, 7 : 93), flow rate 1.2 mL/min. Electrochemical detection. IS: isoproterenol. Retention time: noradrenaline 3.5 min, adrenaline 4.1 min, IS 9.6 min. Limit of detection, <10 pg [Wang *et al.* 1999].

Serum **GC** See Plasma [Lovelady, Foster 1975].

Urine **GC** See Plasma [Lovelady, Foster 1975].

HPLC See Plasma [Ferreira *et al.* 2009; Silva *et al.* 2009].

Note For spectrofluorimetry for the quantification of adrenaline, see O'Hanlon *et al.* [1970].

Disposition in the Body Adrenaline is rapidly destroyed in the gastrointestinal tract after oral administration; the effects of adrenaline after SC injection are produced within 5 min and appear more slowly than after IM injection. It is rapidly taken up by the heart, spleen, several glandular tissues and adrenergic nerves; only metabolites are detectable in CSF. The major metabolic reactions are oxidative deamination and *O*-methylation followed by reduction or by glucuronic acid or sulfate conjugation. Approximately 70–95% of an IV dose is excreted in the urine; of the excreted material, ~80% is *O*-methyl metabolites, 2% is catechol metabolites and only 1% is unchanged drug. The major urinary metabolite is 4-hydroxy-3-methoxymandelic acid (HMMA); other metabolites include 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), conjugated metanephrine and 4-hydroxy-3-methoxyphenylglycol, together with minor amounts of 3,4-dihydroxymandelic acid in free or conjugated form and *N*-methyladrenaline.

Endogenous plasma concentrations of adrenaline in normal subjects are in the range 30–160 ng/L.

Toxicity The minimum SC lethal dose is ~4 mg, but recoveries have occurred after accidental overdosage with 16 mg SC and 30 mg IV, followed by immediate supportive treatment.

Protein Binding ~50%.

Dose 200 to 500 µg, SC or IM, as a single dose which may be repeated.

Ferreira FD *et al.* (2009). High performance liquid chromatography coupled to an optical fiber detector coated with laccase for screening catecholamines in plasma and urine. *J Chromatogr A* 1216: 7049–7054.

Lovelady HG, Foster LL (1975). Quantitative determination of epinephrine and norepinephrine in the picogram range by flame ionization gas–liquid chromatography. *J Chromatogr* 108: 43–52.

Machida M *et al.* (2006). Simultaneous analysis of human plasma catecholamines by high-performance liquid chromatography with a reversed-phase triacontylsilyl silica column. *J Chromatogr B Analyt Technol Biomed Life Sci* 830: 249–254.

O'Hanlon JFJr *et al.* (1970). A fluorometric assay for subnanogram concentrations of adrenaline and noradrenaline in plasma. *Anal Biochem* 34: 568–581.

Silva LI *et al.* (2009). Optical fiber biosensor coupled to chromatographic separation for screening of dopamine, norepinephrine and epinephrine in human urine and plasma. *Talanta* 80: 853–857.

Wang Y *et al.* (1999). A simple high-performance liquid chromatography assay for simultaneous determination of plasma norepinephrine, epinephrine, dopamine and 3,4-dihydroxyphenyl acetic acid. *J Pharm Biomed Anal* 21: 519–525.

Adrenalone

Haemostatic, Sympathomimetic, Vasoconstrictor

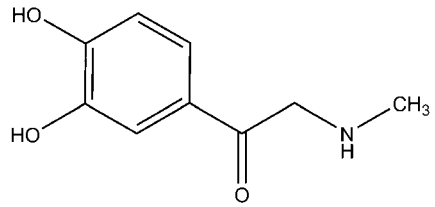
$C_9H_{11}NO_3$ = 181.2

CAS—99-45-6

IUPAC Name 1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanone

Synonyms Adrenone; 3,4-dihydroxy-α-methylaminoacetophenone; 3′,4′-dihydroxy-2-(methylamino)-acetophenone; 4-methylaminoacetopyrocatechol.

Proprietary Name *Stryphon*



Chemical Properties Mp 235.5°. Sparingly soluble in water. Log *P* (octanol/water), 0.47 [Meylan, Howard 1995].

Adrenalone Hydrochloride

$C_9H_{11}NO_3$,HCl = 217.7

CAS—62-13-5

Proprietary Names *Haemodan*; *Kephrine*; *Levorene*; *Stryphnnasal*.

Chemical Properties Crystals. Mp 243°. Freely soluble in water, soluble in alcohol, insoluble in ether.

Ultraviolet Spectrum Peaks at 205, 232, 280 and 312 nm.

Quantification

Other **HPLC** USP Adrenaline Injections. Column: Zorbax CN (250 × 4.6 mm i.d., 0.45 µm). Mobile phase: 5 mmol/L octane sulfonic acid sodium salt, 0.1 mmol/L sodium EDTA in 20 mL acetonitrile, and 10 mL methanol per litre of water (pH 3.5), flow rate 1.5 mL/min. Electrochemical detection followed by UV detection (λ = 254 nm). Retention time: 10.5 min. Limit of detection not reported [Kirchhoefer *et al.* 1985].

Dose In adults, IM or SC, 500 µg to 2 mg daily; orally, 2.5 mg to 10 mg daily.

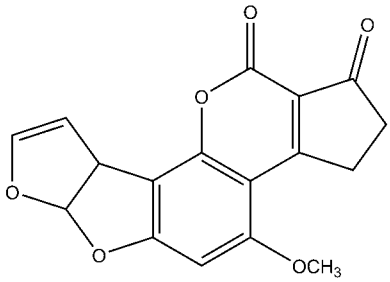
Kirchhoefer RD *et al.* (1985). Analysis of USP epinephrine injections for potency, impurities, degradation products, and *d*-enantiomer by liquid chromatography, using ultraviolet and electrochemical detectors. *J Assoc Off Anal Chem* 68: 163–165.

Meylan W, Howard MPH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

A

Aflatoxins B

Difuranocoumarin, Mycotoxin



Chemical Properties Aflatoxins are a closely related group of secondary fungal metabolites produced by *Aspergillus flavus, A. bombycis*, *A. ochraceoroseus*, *A. parasiticus, A. pseudotamari*, *A. nomius* and *Penicillium puberulum*, but not all strains of *A. flavus* and *A. parasiticus* produce aflatoxins. The four naturally occurring aflatoxins are aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), although AFB_1 is the most commonly occurring compound. Aflatoxins M_1 (AFM_1) and M_2 (AFM_2) are the 4-hydroxylated aflatoxin B derivatives found in the milk of cows fed contaminated meal. Further aflatoxins have been described, especially as mammalian biotransformation products of the major metabolites. Conditions for the growth and production of the naturally occurring aflatoxins in foodstuffs include a moisture content of 18 to 19.5% and a temperature of 25° to 40°. The next most important factor is the presence of zinc as a trace element (e.g. in groundnuts and corn). The B toxins are so named because they exhibit blue fluorescence under UV light [Bennett, Klich 2003; Denning 1987; Wild, Hall 2000]. Aflatoxins are freely soluble in moderately polar solvents (e.g. chloroform and methanol), especially in dimethyl sulfoxide, and also have some water solubility. They are very stable at high temperatures, with little or no destruction occurring under ordinary cooking conditions or during pasteurisation. The presence of the lactone ring in their structure makes the aflatoxins susceptible to alkaline hydrolysis. Acid treatments are also used frequently for their detoxification [McLean, Dutton 1995].

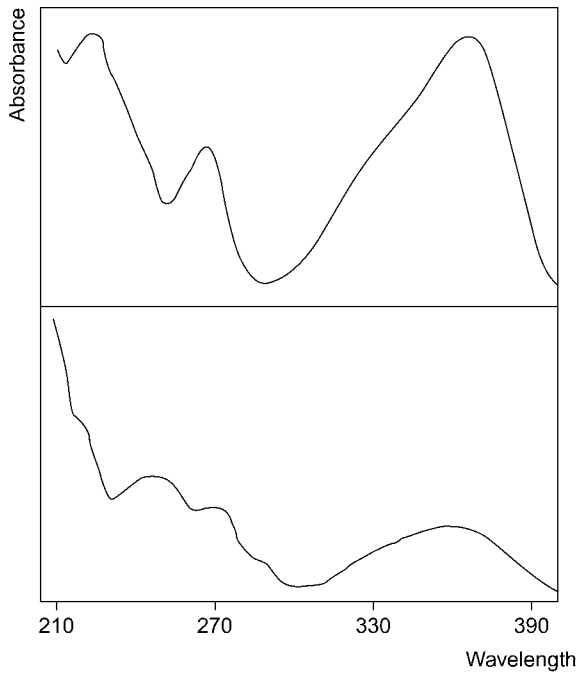
Aflatoxin B_1

$C_{17}H_{12}O_6$ = 312.3
CAS—1162-65-8
IUPAC Name (6a*R-cis*)-2,3,6a,9a-Tetrahydro-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione
Synonym AFB_1
Chemical Properties Crystals. Mp 268° to 269° [O'Neil *et al.* 2006].

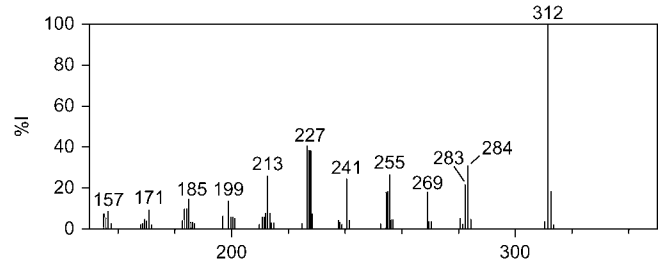
Aflatoxin B_2

$C_{17}H_{14}O_6$ = 314.3
CAS—7220-81-7
IUPAC Name (6a*R-cis*)-2,3,6a,8,9,9a-Hexahydro-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione
Synonym AFB_2
Chemical Properties The 8,9-dihydro derivative of AFB_1. Crystals. Mp 268° to 269° [O'Neil *et al.* 2006].

Ultraviolet Spectrum Ethanol—223, 362, 265 nm (AFB_1); 363,265 nm (AFB_2) [Dragsted *et al.* 1988].



Mass Spectrum Tsuboi *et al.* [1984].



Quantification

Blood **HPLC** Column: C_{18} (250 × 5.0 mm i.d., 5 µm). Mobile phase: water : acetonitrile (75 : 25), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 418 nm). Limit of detection, AFB_1 250 ng/L, AFM_1 100 ng/L, AFM_2 30 ng/L [Abdulrazzaq *et al.* 2002]. Column: C_{18} (200 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (32 : 68), flow rate 0.2 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 432 nm). Relative retention times: AFB_1 1.60, AFB_2 1.29, AFG_1 1.22, AFG_2 1.00, AFM_1 0.776, AFB_{2a} 0.568, AFG_{2a} 0.493, AFM_{2a} 0.353 (relative to AFG_2, retention time: 19.1 min). Limit of detection, AFB_1 3 ng/L, AFB_2 10 ng/L, AFG_1 3 ng/L, AFG_2 10 ng/L [Chao *et al.* 1994].

Serum **HPLC** Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: methanol : water : acetonitrile (25 : 25 : 50), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 366 nm, λ_{em} = 418 nm). Retention time: AFB_1 2.5 min. Limit of quantification not reported [Lopez *et al.* 2002]. Column: C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: 0.02 mol/L sodium phosphate (pH 7.2) : methanol (100 : 0 for 2 min to 40 : 60 over 20 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 405 nm, λ_{em} = 470 nm). Retention time: aflatoxin–lysine adduct 18.2 min. Limit of quantification, 9.1 pg on-column [Sabbioni 1990]. Column: C_{18} (250 × 5.0 mm i.d., 5 µm). Mobile phase: water : methanol (50 : 50), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 418 nm). Retention times: AFB_1 ~7.5 min, AFB_2~ 6.0 min, AFG_1 ~5.0 min, AFG_2 ~4.5 min, AFM_1 ~4.0 min, AFM_2 ~3.0 min, aflatoxicol ~11.0 min. Limit of detection, AFB_1 250 ng/L; AFG_1 500 ng/L; AFM_1 and aflatoxicol 100 ng/L; AFB_2, AFG_2, and AFM_2 25 ng/L [Lamplugh 1983].

Urine **TLC** Plates: silica gel G-HR (20 × 20 cm, 0.5 mm). Solvent system: acetone : chloroform : propan-2-ol (10 : 85 : 5). UV detection. R_f values: AFB_1 0.73, AFG_1 0.59, AFM_1 0.44, AFB_{2a} 0.39, AFG_{2a} 0.29, aflatoxicol-1 0.69, aflatoxicol-2 0.62, tetrahydrodeoxy-AFB_1 0.79. Limit of detection, AFB_1 90 ng/L, AFG_1 13 ng/L, AFM_1 27 ng/L, AFB_{2a} 125 ng/L, AFG_{2a} 73 ng/L, aflatoxicol-1 57 ng/L, aflatoxicol-2 42 ng/L, tetrahydrodeoxy-AFB_1 33 ng/L [Lovelace *et al.* 1982].

HPLC Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-methanol (1 : 1) : 20 mmol/L ammonium acetate buffer (pH 3.9; 30 : 70), flow rate 1.2 mL/min. Fluorescence detection. Limit of detection, AFB_1 25 ng/L, AFB_2 0.35 ng/L, AFG_1 50 ng/L, AFG_2 0.8 ng/L, AFM_1 5 ng/L, AFQ_1 100 ng/L [Polychronaki *et al.* 2008]. Column: phenyl (100 × 8.0 mm i.d., 4 µm). Mobile phase: water containing 1 mmol/L potassium bromide and 1 mmol/L nitric acid : acetonitrile (70 : 30), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 440 nm). Limit of quantification, AFB_1, AFB_2, AFG_1 and AFG_1 6.8 µg/L; AFM_1 and AFQ_1 18 µg/L; limit of detection, 1 ng/L [Kussak *et al.* 1995a; Kussak *et al.* 1998]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: water containing 1 mmol/L potassium bromide and 1 mmol/L nitric acid : methanol : acetonitrile (60 : 20 : 20), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 440 nm). Limit of detection, AFB_1, AFB_2, AFG_1 and AFG_2 50 ng/L [Kussak *et al.* 1993].

LC-MS Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (20 : 20 : 60), flow rate 1.0 mL/min. ESI, positive ion mode, SIM and MRM acquisition modes. Retention times: AFB_1 11.7 min, AFB_2 9.7 min, AFG_1 8.7 min, AFG_2 7.5 min. Limit of detection, AFB_1 and AFB_2 2 pg, AFG_1 3 pg, AFG_2 8 pg on column [Kussak *et al.* 1995b].

Liver **TLC** Plates: silica kieselgel 60G (10 × 10 cm, 0.2 mm). Solvent systems: first direction, methanol : water : ether (94 : 4.5 : 1.5), second direction chloroform : acetone (97 : 3). R_f values not reported. Limit of quantification not reported [Apeagyei *et al.* 1986].

Other **TLC** Food Samples. Plates: precoated silica gel 60 (10 × 10 cm, 0.2 mm). Solvent systems: first direction, chloroform : acetone (9 : 1), second direction toluene : ethyl acetate : formic acid (5 : 4 : 1). UV detection. R_f values: first direction, AFB_1 0.62, AFB_2 0.56, AFG_1 0.48, AFG_2 0.40; second direction, AFB_1 0.22, AFB_2 0.20, AFG_1 0.15, AFG_2 0.12. Limit of detection, 1.2 µg/kg [Fernandez Pinto *et al.* 2001; Tapia 1985].

HPLC Rat Liver. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium phosphate (pH 3.0)-methanol (75 : 25) : methanol (90 : 10 to 65 : 35 over 15 min, to 49 : 51 over 5 min), flow rate 1.0 mL/min. UV detection (λ = 365 nm). Retention times: AFB_1 21.6 min, AFG_1 19.9 min, AFM_1 16.3 min, AFQ_1 14.1 min, AFB_1-8,9-epoxide-glutathione *S*-transferase (AFBO-GSH) conjugate 9.2 min. Limit of quantification, AFB_1 25 pmol, AFBO-GSH 50 pmol [Kamdem *et al.* 2006]. Food Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (1 : 3 : 6), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 450 nm). Limit of quantification not reported [Sugita-Konishi *et al.* 2006]. Traditional Herbal Remedies Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile (70 : 20 : 10), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 450 nm). Limit of detection, AFB_1, AFG_1 and AFG_2 (trifluoroacetic acid [TFA] derivatives) 0.01 µg/kg [Ali *et al.* 2005]. Rice Samples (polished and cooked). Column: C_{18} (150 × 3.9 mm i.d.). Mobile phase:

methanol : acetonitrile : water (17 : 17 : 70), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 440 nm). Limit of detection, AFB_1 (TFA derivative) 0.80 μg/kg [Park *et al.* 2005]. Spice Samples. Column: C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: water : methanol : acetonitrile (65 : 20 : 15). Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 430 nm). Limit of detection, AFB_1 0.1 μg/kg; AFB_2, AFG_1, and AFG_2 (TFA derivatives) 0.2 μg/kg [Fazekas *et al.* 2005]. Food Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile (25 : 75), flow rate 0.7 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 435 nm). Limit of quantification, AFB_1 and AFB_2 0.13 μg/kg, AFG_1 0.5 μg/kg, AFG_2 2.5 μg/kg, limit of detection, AFB_1 and AFB_2 0.04 μg/kg, AFG_1 0.15 μg/kg, AFG_2 0.75 μg/kg [Blesa *et al.* 2004]. Beer Samples. Column: Lichrospher C_{18}. Mobile phase: water : acetonitrile : methanol (75 : 15 : 10), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 425 nm). Limit of quantification, AFB_1 5 μg/L; AFB_2, AFG_1 and AFG_2 (TFA derivatives) 8 μg/L [Odhav and Naicker 2002]. Baby Food Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile : methanol (6 : 2 : 3) containing 120 mg/L potassium bromide and 350 μL 4 mol/L nitric acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 420 nm). Post-column bromination. Retention times: AFB_1, AFB_2, AFG_1, and AFG_2 11, 9, 8, and 6 min, respectively. Limit of quantification, 0.05 μg/kg [Stroka *et al.* 2001; Tam *et al.* 2006]. Airborne Dust Samples. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: water : methanol : acetonitrile (60 : 20 : 20), flow rate 1.1 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 450 nm). Retention time: AFB_1 9.0 min. Limit of detection, 33 μg/L [Selim *et al.* 1998].

LC-MS Corn Silage Samples. Column: C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: acetonitrile : water with 0.5% acetic acid (pH 3, 5 : 95 to 50 : 50 over 15 min to 80 : 20 over 10 min), flow rate 0.3 mL/min. ESI, positive and negative ion modes, SIM acquisition mode. Retention times: AFB_1 15.4 min, citrinin 16.7 min, deoxynivalenol 6.9 min, fumonisin B_1 15.1 min, gliotoxin 14.2 min, ochratoxin A 20.3 min, zearalenone 20.4 min. Limit of quantification, AFB_1 5 ppb; limit of detection, AFB_1 1.5 ppb [Richard *et al.* 2007]. Food Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol (55 : 45), flow rate 0.7 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, AFB_1 and AFG_1 0.07 μg/kg, AFB_2 and AFG_2 0.2 μg/kg [Blesa *et al.* 2004]. Cell Culture Samples. Column: LichroCart 250-3 Purospher C_{18}. Mobile phase: methanol : 10 mmol/L ammonium acetate containing 20 μmol/L sodium acetate (20 : 80 for 4 min, to 70 : 30 over 4 min for 18 min to 90 : 10 in 1 min for 4 min, flow rate 0.4 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times: AFG_2, AFG_1, AFB_2 and AFB_1 13.5 min, 13.8 min, 14.2 min and 14.5 min, respectively; citrinine, ochratoxin A, and sterigmatocystin also detected. Limit of quantification, 200 ng [Tuomi *et al.* 2001].

Note For a review of LC-MS methods for the analysis of aflatoxins in different media, see Zollner and Mayer-Helm [2006]. For the analysis of AFB_1–lysine adduct in serum using LC-MS, see McCoy *et al.* [2005]; using HPLC-fluorescence, see Sheabar *et al.* [1993]. For methods for the analysis of aflatoxin nucleic acid adducts and metabolites in urine using HPLC, see Groopman *et al.* [1985] and Autrup *et al.* [1985]. For methods for the analysis of aflatoxins in human tissues using normal phase HPLC, see Anukarahanonta and Chudhabuddhi [1983] and Siraj *et al.* [1981]. For a radioimmunoassay of AFB and AFG, see Tsuboi *et al.* [1984]. For ELISA methods for the detection of aflatoxins, see Blesa *et al.* [2004], Dragsted *et al.* [1988], Kim *et al.* [2001] and Lipigorngoson *et al.* [2003].

Disposition in the Body Following ingestion, AFB_1 is metabolised mainly by CYP1A2, CYP3A4 and CYP3A5 in the liver, generating several hydroxylated derivatives and a highly reactive epoxide (AFB_1-8,9-*exo*-epoxide) that binds to DNA to form the predominant 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB_1 adduct, that damages DNA. Other metabolites are formed from AFB_1, including aflatoxicol (reductive metabolite; interconversion occurs in red blood cells), AFQ_1 (hydroxylated at position 3), AFM_1 (hydroxylated at position 4), and AFP_1 (position 4 methoxy group replaced by hydroxyl group). These metabolites and other naturally occurring aflatoxins (AFG_1, AFB_2, and AFG_2) are poorer substrates for epoxidation and, consequently, are less mutagenic, carcinogenic, and toxic than AFB_1. Urinary aflatoxin-N^7-guanine adducts and AFM_1, AFQ_1, AFP_1 metabolites together with serum aflatoxin–albumin adducts (aflatoxin–lysine) have been found to be useful biomarkers of aflatoxin exposure. Typically, urinary metabolites reflect recent exposure (2 to 3 days), whereas blood or serum aflatoxin–albumin adducts reflect exposure over a longer period (2 to 3 months). The metabolism of AFB_1 to the epoxide and to AFM_1 can be blocked by treatment with oltipraz, an antischistosomal drug, which induces the major aflatoxin detoxification enzyme glutathione-*S*-transferase. Aflatoxins are known to cross the placenta. The role of extrahepatic metabolism of AFB_1, particularly in the small intestine, may be important in modulating the toxic and carcinogenic effects *in vivo*. Lipoxygenase and prostaglandin H synthase may also make a significant contribution to AFB_1 metabolism in some extrahepatic organs.

Toxicity The disorders caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other pathological conditions. AFB_1 is the most potent heptatocarcinogen known in mammals [Creppy 2002], included in category 1A by the International Agency for Research on Cancer [IARC 1993]. Exposure to aflatoxins is associated with increased risk of hepatocellular carcinoma, particularly in combination with hepatitis B virus [IARC 1993, 2002]. The maximum limit for AFB_1 and AFB_2 in foods is 4 μg/kg (or 4 μg/L) in the UK and 20 μg/kg (or 20 μg/L) in the USA [Creppy 2002]. The LD_{50} values for AFB_1 are 18.2 μg/50 g bodyweight oral dose in 1-day-old duckling and 9.50 mg/kg bodyweight IP dose in newborn mice; for AFB_2, the oral dose is 84.8 μg/50 g bodyweight in 1-day-old duckling [Buchi *et al.* 1973; Carnaghan *et al.* 1963]. The order of acute and chronic toxicity is $AFB_1 > AFG_1 > AFB_2 > AFG_2$, reflecting the role played by epoxidation of the 8,9-double bond and also the greater potency associated with the cyclopentenone ring of the B series compared with the 6-membered lactone ring of the G series [McLean, Dutton 1995; Wogan 1966]. Kwashiorkor, a form of protein energy malnutrition disease, may be a form of paediatric aflatoxicosis [de Vries *et al.* 1990; Hendrickse 1997; Lamplugh, Hendrickse 1982; Maxwell 1998; Peraica *et al.* 1999].

A group of 50 lactating mothers and their infants were screened for aflatoxin exposure. AFB_1 was detected in the serum and milk of 24 mothers at mean concentrations of 8.9 μg/L and 1.9 μg/L, respectively. The corresponding mean serum concentration in infants was 1.8 μg/L [Hassan *et al.* 2006].

An outbreak of hepatitis that affected 400 people, of whom 100 died, almost certainly resulted from aflatoxins. The outbreak was traced to maize that was heavily contaminated with *A. flavus*, and containing up to 15 mg/kg aflatoxins. Consumption of the affected adults was calculated to be 2 to 6 mg in a single day. From this value, a dose of the order of 10 to 20 mg was estimated as lethal for adults [Krishnamachari *et al.* 1975].

In an attempted suicide with purified AFB_1, a young woman ingested 5.5 mg AFB_1 over 2 days and, 6 months later, a total of 35 mg over 2 weeks. Following admission to hospital after each attempt, physical, radiological, and laboratory examinations were normal and liver biopsies appeared normal. A follow-up examination 14 years later did not reveal any signs or symptoms of disease. These findings suggest that the hepatotoxicity of AFB_1 may be lower in well-nourished persons or that the latent period for tumour formation exceeds 14 years [Peraica *et al.* 1999; Willis *et al.* 1980].

The possibility that IV heroin users might be exposed to aflatoxins was investigated. Four heroin samples submitted for analysis yielded AFB_1 in concentrations of 1.63, 4.31, 5.23 and 30.82 nmol/kg. Random urine samples obtained from 132 heroin addicts and from 99 normal adult volunteers were screened for AFB_1. Approximately 20% of the heroin users showed AFB_1, AFB_2, AFM_1, AFM_2 and aflatoxicol in their urine, in quantities that ranged from 0.73 to 29.09 nmol/L, whereas only 2 of the control urines showed any aflatoxins (AFB_2 at 0.13 and 0.24 nmol/L) [Hendrickse, Maxwell 1988; Hendrickse *et al.* 1989].

Postmortem concentrations of aflatoxins were reported following an outbreak in Malaysia where 17 people were taken ill following ingestion of contaminated noodles. Of these victims, 13 died; all were children (aged between 2.5 and 11 years old). The duration of survival ranged from 2 to 6 days, with a median of 4 days 4 h. Aflatoxin concentrations in tissues were reported as follows:

Victim	*Tissue sample (μg/kg)*					
	Liver	*Kidney*	*Spleen*	*Lung*	*Heart*	*Brain*
1	14.5 (AFM_1)	–	4.8 (AFM_1)	–	2.4 (AFB_1)	–
2	1.6 (AFB_1)	–	3.5 (AFB_1)	1.4 (AFB_1)	–	–
3	0.7 (AFB_1)			0.4 (AFB_1)		13.3 (AFM_1); 5.2 (AFM_2)
4	1.2 (AFB_1)			3.5 (AFB_1); 0.03 (AFL)		0.4 (AFM_2)
5	0.5 (AFB_1); 0.04 (AFB_2); 1.1 (AFM_1)			0.04 (AFB_1)		
6	0.8 (AFB_1); 0.1 (AFB_2); 9.1 (AFG_1); 7.6 (AFM_1)	0.2 (AFB_1); 18.5 (AFM_1)	2.3 (AFM_1); 1.5 (AFM_2)	0.13 (AFB_1)	1.2 (AFB_1); 0.02 (AFB_2)	
7	0.04 (AFB_2); 1.0 (AFM_1)				0.8 (AFB_1)	
8		0.34 (AFB_1)		1.3 (AFM_1); 1.6 (AFM_2)		1.2 (AFM_1); 1.4 (AFM_2)

Table continued

Table continued

Victim	Tissue sample (μg/kg)					
	Liver	Kidney	Spleen	Lung	Heart	Brain
9	3.2 (AFB_1)	0.9 (AFM_1); 0.5 (AFM_2)	0.02 (AFM_1); 0.6 (AFB_2)	0.05 (AFB_2)		
10	0.3 (AFM_1)	1.3 (AFM_1)				

[Cheng 1992].

Note For postmortem reports giving lung and liver aflatoxin concentrations in kwashiorkor deaths, see Oyelami *et al.* [1997] and Oyelami *et al.* [1998].

Protein Binding AFB_1 is mainly bound to γ-globulin.

Note For a review of human aflatoxicosis in developing countries, see Williams *et al.* [2004]. For a study on the inhibitory effect of oltipraz on the activation of aflatoxins in humans, see Wang *et al.* [1999]. For a review on the interactions and metabolism of aflatoxins, see Forrester *et al.* [1990], McLean and Dutton [1995] and Wild and Turner [2002]. For studies on urinary and serum biomarkers of aflatoxins, see Gan *et al.* [1988]; Groopman *et al.* [1992a,1992b], Sabbioni [1990] and Wild *et al.* [1990].

Use It has been reported that aflatoxins have been developed in some countries as biological weapons.

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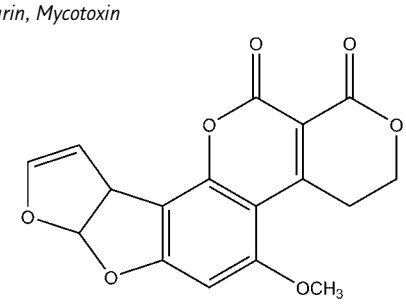
Aflatoxins G

Difuranocoumarin, Mycotoxin



Chemical Properties Aflatoxins G are a closely related group of secondary fungal metabolites produced by *Aspergillus bombycis*, *A. ochraceoroseus*, *A. parasiticus*,

A. pseudotamari, *A. nomius* and *Penicillium puberulum*. The 4 naturally occurring aflatoxins are aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2). The G toxins are so named because they exhibit green fluorescence under UV light [Bennett, Klich 2003; Denning 1987; Wild, Hall 2000]. See Aflatoxins B for further properties.

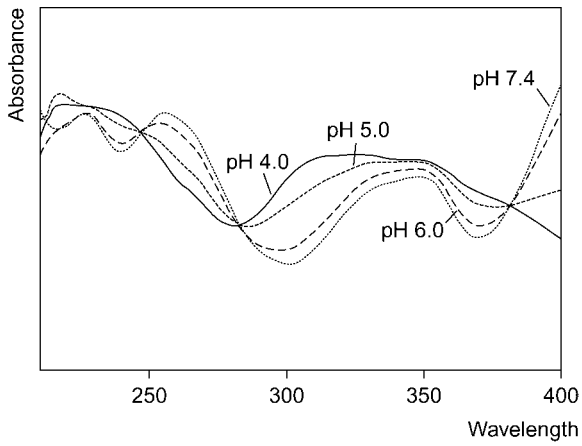
Aflatoxin G_1

$C_{17}H_{12}O_7 = 328.3$
CAS—1165-39-5
IUPAC Name (7a*R*,10a*S*)-3,4,7a,10a-Tetrahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][1]benzopyran-1,12-dione
Synonym AFG_1
Chemical Properties Crystals. Mp 244° to 246° [O'Neil *et al.* 2006].

Aflatoxin G_2

$C_{17}H_{14}O_7 = 330.3$
CAS—7241-98-7
IUPAC Name (7a*R*,10a*S*)-3,4,7a,9,10,10a-*H*exahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][1]benzopyran-1,12-dione
Synonym AFG_2
Chemical Properties The 9,10-dihydro derivative of AFG_1. Crystals. Mp 237° to 240° [O'Neil *et al.* 2006].

Ultraviolet Spectrum Ethanol—362, 243, 264, 257 nm (AFG_1); 363, 265 nm (AFG_2); 0.1 mol/L sodium phosphate buffer—(pH 7.4) 413, 255, 225, 346 nm; (pH 6.0) 413, 225, 255, 346 nm; (pH 5.0) 218, 345 nm; (pH 4.0) 326, 216 nm (*N*-α-acetyllysine–AFG_1 adduct) [Sabbioni and Wild 1991].



Quantification

See Aflatoxins B.
Note For a review of LC-MS methods for the analysis of aflatoxins in different media, see Zollner and Mayer-Helm [2006].

Disposition in the Body

Toxicity The LD_{50} values orally in 1-day-old duckling are 39.2 μg/50 g bodyweight for AFG_1 and 172.5 μg/50 g bodyweight for AFG_2 [Carnaghan *et al.* 1963]. See also Aflatoxins B. For epidemiological studies of AFG, AFB and AFM exposure to mothers and their lactating infants, see Polychronaki *et al.* [2008]. The maximum limit for AFG_1 and AFG_2 in foods is 4 μg/kg (or 4 μg/L) in the UK and 20 μg/kg (or 20 μg/L) in the US [Creppy 2002].

Postmortem AFG_1 concentrations in lung tissue of children who died from kwashiorkor were between 3.4 and 52.1 ng/g. AFG_2, AFM_1 and AFM_2 were also detected but at much lower concentrations. In another similar study, AFG_1 concentrations in kidney ranged between 7.9 and 42.5 ng/g. AFG_2 was detected more often but at much lower concentrations (between 0.006 and 0.9 ng/g) [Oyelami *et al.* 1997, 1998].
Note For a study investigating the use of an AFG_1–serum albumin adduct as a measure of human exposure to aflatoxins, see Sabbioni and Wild [1991].
Protein Binding AFG_1 is mainly bound to albumin.

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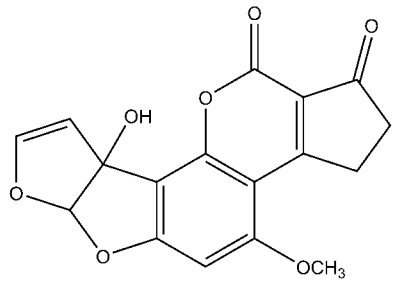
Aflatoxins M

Haemostatic, Sympathomimetic, Vasoconstrictor, Difuranocoumarin, Mycotoxin



Chemical Properties Aflatoxins M_1 (AFM_1) and M_2 (AFM_2) are the highly toxic 4-hydroxylated aflatoxin B derivatives found in the milk of cows fed contaminated meal. Aflatoxins are freely soluble in moderately polar solvents (e.g. chloroform and methanol), especially in dimethyl sulfoxide, and also have some water solubility. They are very stable at high temperatures, with little or no destruction occurring under ordinary cooking conditions or during pasteurisation. The presence of the lactone ring in their structure makes the aflatoxins susceptible to alkaline hydrolysis. Acid treatments are also used frequently for their detoxification [McLean, Dutton 1995]. See Aflatoxins B for further properties.

Aflatoxin M_1

$C_{17}H_{12}O_7 = 328.3$
CAS—6795-23-9
IUPAC Name (6a*R*,9a*R*)-2,3,6a,9a-Tetrahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione
Synonyms AFM_1; 4-hydroaflatoxin B_1.
Chemical Properties Crystals from methanol. Mp 299° with decomposition. Exhibits blue–violet fluorescence [O'Neil *et al.* 2006].

Aflatoxin M_2

$C_{17}H_{14}O_7 = 330.3$
CAS—6885-57-0
IUPAC Name 2,3,6a,8,9,9a-Hexahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione
Synonyms AFM_2; 4-hydroaflatoxin B_2.

Chemical Properties The 8,9-dihydro derivative of AFM_1. Crystals from methanol/chloroform. Mp 293° with decomposition. Exhibits violet fluorescence [O'Neil *et al.* 2006].
Ultraviolet Spectrum Ethanol—226, 357, 265 nm (AFM_1); 357, 221, 264 nm (AFM_2).

Quantification

Serum **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : methanol (33 : 62 : 5), flow rate 0.7 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 425 nm). Limit of detection, 1 μg/L [Okumura *et al.* 1993]. Column: C_{18} (250 × 5.0 mm i.d., 5 μm). Mobile phase: water : acetonitrile (75 : 25), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 418 nm). Limit of detection AFM_1, 0.1 μg/L, AFM_2 0.03 μg/L, AFB_1 0.25 μg/L [Abdulrazzaq *et al.* 2002].
Urine **TLC** Plates: silica gel G-HR (20 × 20 cm, 0.5 mm). Solvent system: acetone : chloroform : propan-2-ol (10 : 85 : 5). UV detection. R_f values: AFB_1 0.73, AFG_1 0.59, AFM_1 0.44, AFB_{2a} 0.39, AFG_{2a} 0.29, aflatoxicol-1 0.69, aflatoxicol-2 0.62, tetrahydrodeoxy-AFB_1 0.79. Limit of detection, AFB_1 90 ng/L, AFG_1 13 ng/L, AFM_1 27 ng/L, AFB_{2a} 125 ng/L, AFG_{2a} 73 ng/L, aflatoxicol-1 57 ng/L, aflatoxicol-2 42 ng/L, tetrahydrodeoxy-AFB_1 33 ng/L [Lovelace *et al.* 1982].

HPLC Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile-methanol (1 : 1) : 20 mmol/L ammonium acetate buffer (pH 3.9; 30 : 70), flow rate 1.2 mL/min. Fluorescence detection. Limit of detection, AFB_1 25 ng/L, AFB_2 0.35 ng/L, AFG_1 50 ng/L, AFG_2 0.8 ng/L, AFM_1 5 ng/L, AFQ_1 100 ng/L [Polychronaki *et al.* 2008]. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 13% ethanol and 3% methanol in tetraethylammonium fluoride, flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 365 nm). Retention time: 12 min. Limit of detection, 0.5 ng/L [Cheng *et al.* 1997]. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol : 0.01 mol/L potassium dihydrogen phosphate (1 : 1), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 435 nm). Retention time: 4.0 min (trifluoroacetic acid derivative). Limit of detection, 0.01 ppb [Liu *et al.* 1990].
Faeces **HPLC** Column: C_{18} (220 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile-methanol (1 : 1) : 20 mmol/L ammonium acetate buffer (pH 3.9, 30 : 70), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 366 nm, λ_{em} = 440 nm). Retention times: AFM_1 8.6 min, AFQ_1 7.8 min, AFB-N^7-guanine 4.6 min. Limit of detection, AFM_1 and AFB-N^7-guanine 5 ng/kg; AFQ_1 0.5 μg/kg [Mykkanen *et al.* 2005].
Other **TLC** Egg Samples. Plates: precoated silica gel 60 (20 × 20 cm). Solvent system: hexane : tetrahydrofuran : ethanol (95 : 4 : 1). UV detection. Limit of quantification, 0.04 μg/kg [Trucksess and Stoloff 1984]. Milk Samples (powdered and liquid). Plates: precoated silica G25HR. Solvent systems: toluene : ethyl acetate : ethyl ether : formic acid (25 : 35 : 40 : 5); plates are heated at 110° for 10 min and developed in hexane : acetone : chloroform. Fluorescence detection (*p*-anisaldehyde derivative). Limit of detection, 0.3 ng [Serralheiro, Quinta 1986]. Dog Liver Samples. Plates: precoated silica gel washed (developed) twice in chloroform : acetone (9 : 1) and once

A

in diethyl ether to remove impurities, activated at 105° for 1 h. Solvent systems: (1) chloroform: acetone: propan-2-ol (82.5 : 15 : 2.5); (2) chloroform : acetone (8 : 2); (3) benzene: ethyl acetate: acetone (50 : 4 : 6). R_f values: (1) AFM_1 0.48, aflatoxicol M_1 0.37, AFB_{2a} 0.43, aflatoxicol 0.78, AFB_1 0.83; (2) AFM_1 0.37, aflatoxicol M_1 0.28, AFB_{2a} 0.33, aflatoxicol 0.71, AFB_1 0.77; (3) AFM_1 0.06, aflatoxicol M_1 0.09, aflatoxicol 0.37, AFB_1 0.24. Limit of quantification not reported [Salhab *et al.* 1977].

HPLC Human Breast Milk. Column: C_{18} (220 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol : acetonitrile (66 : 17 : 17), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 440 nm). Limit of detection, 4.2 ng/L [Polychronaki *et al.* 2006]. Column: C_{18} (250 × 4.0 mm i.d., 10 μm). Mobile phase: 2% acetic acid : acetonitrile : methanol (40 : 35 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 430 nm). Retention times: AFM_1 3.1 min, ochratoxin A 7.3 min. Limit of quantification, 0.01 μg/L [Navas *et al.* 2005]. Column: C_{18} (220 × 4.6 mm i.d., 5 μm). Mobile phase: water-methanol-acetonitrile-acetic acid (77 : 15 : 8 : 0.05): water-methanol-acetonitrile-acetic acid (52 : 40 : 8 : 0.05; 100 : 0 for 12 min to 0 : 100 over 8 min for 8 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 365 nm, λ_{em}= 418 nm). Retention time: 23.3 min. Limit of detection, 10 ng/L [el Nezami *et al.* 1995]. Cheese Samples. Column: C_{18} (125 × 4.0 mm i.d., 4 μm). Mobile phase: water: acetonitrile (75 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 365 nm, λ_{em}= 440 nm). Retention time: 5.2 min. Limit of detection, 5 ng/kg [Peitri *et al.* 1997]. Milk Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water: acetonitrile: methanol (55 : 24 : 21 for 6 min, to 15 : 40 : 45 over 1 min for 3 min), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex}= 360 nm, λ_{em}= 440 nm). Retention time: 7 min. Limit of quantification, 0.3 μg/L, limit of detection, 0.12 μg/L [Muscarella *et al.* 2007]. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: water : acetonitrile : methanol (50 : 30 : 20), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 430 nm). Retention time: 7.9 min. Limit of quantification, 10 ng/L, limit of detection, 3 ng/L [Diaz, Espitia 2006]. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (25 : 75), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 435 nm). Retention times: AFM_1 13.5 min, AFM_2 10.2 min, AFM_{2a} 5.1 min. Limit of detection, AFM_1 1 ng/kg, AFM_2 0.3 ng/kg, AFM_{2a} 0.2 ng/kg [Nakajima *et al.* 2004]. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile : methanol (60 : 22 : 18), flow rate 1.0 mL/min. Fluorescence detection. Limit of detection, 0.05 μg/L [Carvajal *et al.* 2003]. Column: C_{18} (150 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (25 : 75), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 435 nm). Retention time: 7 min. Limit of detection, 5 μg/L [Roussi *et al.* 2002; Van Egmond and Dragacci 2001]. Milk, Dried Milk, and Yoghurt Samples. Column: C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (28 : 72), flow rate 1.5 mL/min. Retention time: 5.5 min. Limit of detection, 1 ng/L [Galvano *et al.* 1998]. Pig Liver Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (25 : 75), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 435 nm). Retention time: 8.3 min. Limit of detection, 1 μg/kg [Chiavaro *et al.* 2005]. Column: C_{18} (250 × 4.6 mm i.d., 10 μm). Mobile phase: acetonitrile : water (30 : 70), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 366 nm, λ_{em} = 430 nm). Limit of detection, 10 ng/kg [Tyczkowska *et al.* 1987]. Milk and Cheese samples. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetic acid : acetonitrile : propan-2-ol : water (2 : 10 : 10 : 78), flow rate 1.2 mL/min. Post-column derivatisation with *p*-bromophenacyl bromide. Fluorescence detection (λ_{ex} = 353 nm, λ_{em} = 423 nm). Retention time: 6 min. Limit of detection, 1 and 5 ng/kg for milk and cheese, respectively [Manetta *et al.* 2005].

LC-MS Rat Urine. Column: phenyl-hexyl (150 × 2.0 mm i.d., 3 μm). Mobile phase: water : acetonitrile both containing 0.1% formic acid (70 : 30 for 10 min to 5 : 95 over 0.1 min for 1.4 min), flow rate 0.325 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: AFM_1 4 min, AFP_1 3.8 min, AFG_2 5.2 min, AFB_2 6.6 min, AFG_1 7 min, AFB_1 9 min. Limit of quantification, 392 fg on-column [Everley *et al.* 2007]. Cow Milk Samples. Column: C_{18} (250 × 4.6 mm i. d., 5 μm). Mobile phase: water-acetone (87 : 13) : methanol-acetone (87 : 13; 85 : 15 for 1 min to 15 : 85 over 10 min to 5 : 95 for 2 min), flow rate 1.0 mL/min. APPI, MRM acquisition mode. Retention time: 8.0 min. Limit of quantification, 6 ng/L [Cavaliere *et al.* 2006a]. Column: PRP-1 poly(styrene-divinylbenzene) (100 × 2.1 mm i.d., 3 μm). Mobile phase: 10 mmol/L 4-methylmorpholine aqueous solution (pH 9.7) : acetonitrile (80 : 20 to 0 : 100 over 6 min for 6.5 min), flow rate 0.2 mL/min. ESI, negative ion mode, SRM acquisition mode. Retention time: 9.3 min. Limit of detection, 0.59 ng/L with immunoaffinity column clean-up [Chen *et al.* 2005]. Cheese Samples. Column: C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: water : acetonitrile both containing 0.1% acetic acid (75 : 25 for 16 min to 10 : 90 for 24 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: AFM_1 4.7 min, AFG_2 6.4 min, AFB_2 8.2 min, AFG_1 8.6 min, AFB_1 11.3 min, penicillic acid 3.5 min, roquefortine C 4.2 min, mycophenolic acid 27.0 min, ochratoxin A 31.9 min. Limit of quantification, AFM_1, mycophenolic acid and ochratoxin A 0.3 μg/kg; roquefortine C 0.4 μg/kg; AFB_1, AFB_2, AFG_1 and AFG_2 0.8 μg/kg; penicillic acid 2.0 μg/kg. Limits of detection, 0.6, 0.8, 1.6 and 4.0 μg/kg, respectively [Kokkonen *et al.* 2005].

Note See Aflatoxins B for further methods. For a review of LC-MS methods for the analysis of aflatoxins in different media, see Cavaliere *et al.* [2006b] and Zollner and Mayer-Helm [2006]. For a comparison of methods using different extraction methods for AFM_1 in cheese, see Cavaliere *et al.* [2006b], and using different ionisation sources for LC-MS analysis, see Cavaliere *et al.* [2006a]. For a two-dimensional TLC method for the quantification of AFM_1 in milk, see Grosso *et al.* [2004], in cheese, see Bijl *et al.* [1987]. For a comparison of methods for the extraction and clean-up of AFM_1, see Shepherd *et al.* [1986].

Disposition in the Body AFM_1 is one of the major urinary metabolites found in subjects exposed to AFB_1 and its level correlates with the presence of AFB_1–serum albumin adducts. AFM_1 can be further activated to form AFM_1-8,9-epoxide, which binds to DNA and is excreted into urine in the form of AFM_1-N^7-guanine. Quantitative data on AFM_1 excretion into faeces are limited.

Toxicity The carcinogenic potency of AFM_1 is approx. 10-fold lower than that of AFB_1 [Creppy 2002]. It is classified as a probable human carcinogen in group 2B by the International Agency for Research on Cancer [IARC 1993]. The European regulatory limits for AFM_1 in milk are 0.05 μg/L for liquid milk and 0.5 μg/kg for milk powder. This was recently amended to include maximum permissible levels of 0.025 μg/kg AFM_1 for infant formulae, follow-on formulae, and dietary foods. The Codex Alimentarius Committee for Food Additives and Contaminants (CCFAC) recommend a limit of 0.5 μg/L for liquid milk and 5.0 μg/kg for milk powder. Exposure to AFM_1 accounts for a substantial part of the risk of hepatocellular carcinoma in men with chronic hepatitis B virus hepatitis [Sun *et al.* 1999]. For a study on AFM_1 absorption and cytotoxicity in a human intestinal *in vitro* model, see Caloni *et al.* [2006]. Kwashiorkor, a form of protein energy malnutrition disease, may be a form of paediatric aflatoxicosis, and exposure of neonates to AFM_1 present in breast milk should be monitored [Coulter *et al.* 1984, 1986; de Vries *et al.* 1990; Hendrickse 1997; Lamplugh, Hendrickse 1982; Maxwell 1998; Peraica *et al.* 1999]. The oral LD_{50} in 1-day-old ducklings is 16.6 μg/duckling for AFM_1 and 62.0 μg/duckling for AFM_2 [Holzapfel *et al.* 1966].

In a study investigating morbidity in neonates of 166 mothers exposed to aflatoxins, AFM_1 was detected in 113 (68%) of maternal blood samples (0.03 to 8.49 μg/L) and in 111 (67%) of cord blood samples (0.05 to 10.44 μg/L). No correlation was found between AFM_1 concentration and rates of jaundice or infection in neonates [Abdulrazzaq *et al.* 2004].

During a survey of the occurrence of aflatoxins in breast milk, a group of 445 women had AFM_1 concentrations ranging from 2 to 3 ng/L. Consequently, an average 3-month-old baby (bodyweight 4.5 kg) with a daily milk intake of approximately 500 to 700 mL, might receive a daily AFM_1 dose between 7.5 and 10.5 ng/kg bodyweight [Saad *et al.* 1995].

Note See Aflatoxins B for further case reports of exposure.

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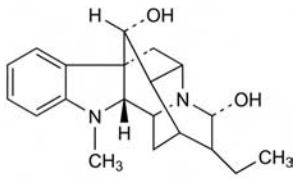
Serralheiro ML, Quinta ML (1986). Thin layer chromatographic confirmation of aflatoxin M_1 extracted from milk. *J Assoc Off Anal Chem* 69: 886–888.
Shepherd MJ *et al.* (1986). Comparison and critical evaluation of six published extraction and clean-up procedures for aflatoxin M_1 in liquid milk. *J Chromatogr* 354: 305–315.
Sun, Z *et al.* (1999). Increased risk of hepatocellular carcinoma in male hepatitis B surface antigen carriers with chronic hepatitis who have detectable urinary aflatoxin metabolite M_1. *Hepatology* 30: 379–383.
Trucksess MW, Stoloff L (1984). Determination of aflatoxicol and aflatoxins B_1 and M_1 in eggs. *J Assoc Off Anal Chem* 67: 317–320.
Tyczkowska, K *et al.* (1987). Liquid chromatographic determination of aflatoxicol in porcine liver. *J Assoc Off Anal Chem* 70: 475–478.
VanEgmond HP, Dragacci S (2001). Liquid chromatographic method for aflatoxin M_1 in milk. *Methods Mol Biol* 157: 59–69.
Zollner P, Mayer-Helm B (2006). Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionisation mass spectrometry. *J Chromatogr A* 1136: 123–169.

Ajmaline

Antiarrhythmic
$C_{20}H_{26}N_2O_2 = 326.4$
CAS—4360-12-7

Synonyms Ajmalan-17,21-diol; rauwolfine.
Proprietary Names *Aritmina*; *Cardiorythmine*; *Gilurytmal*; *Ritmos*; *Tachmalin*.



Chemical Properties An alkaloid obtained from the root of *Rauwolfia serpentina* (Apocynaceae). A white or slightly yellowish crystalline powder. Mp ~195°, with decomposition. Practically insoluble in water; freely soluble in ethanol, chloroform and glacial acetic acid; sparingly soluble in ether and methanol; soluble in dilute hydrochloric acid. pK_a 8.2. Extraction yield (chlorobutane), 0.5 [Demme *et al.* 2005]. Ajmaline is stable under acidic and basic conditions. There is no conversion of ajmaline into its more thermodynamically stable isomer isoajmaline or into sandwicine [Köppel *et al.* 1992].

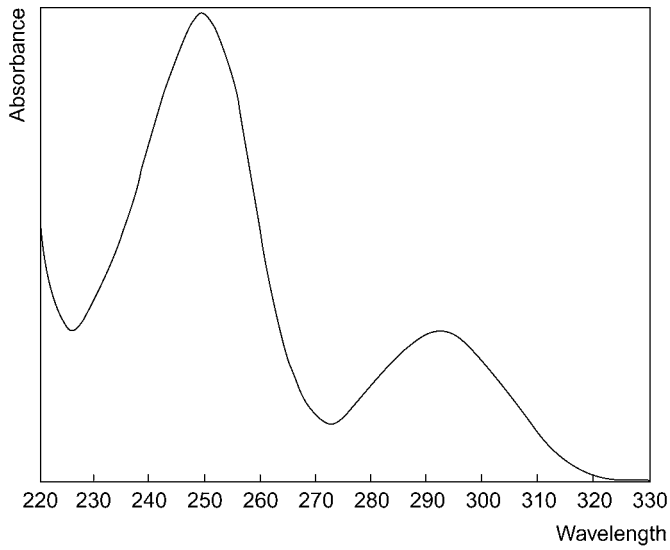
Ajmaline Hydrochloride
$C_{20}H_{26}N_2O_2,2HCl,2H_2O = 435.4$
Chemical Properties Crystals. Mp 140°. Soluble 1 in 40 of water.
Proprietary Name *Cardiorythmine*

Ajmaline Monoethanolate
$C_{20}H_{26}N_2O_2,C_2H_6O = 372.5$
CAS—60991-48-2
Chemical Properties A white or slightly yellowish crystalline powder. Practically insoluble in water; freely soluble in ethanol, chloroform and glacial acetic acid; sparingly soluble in ether and methanol.

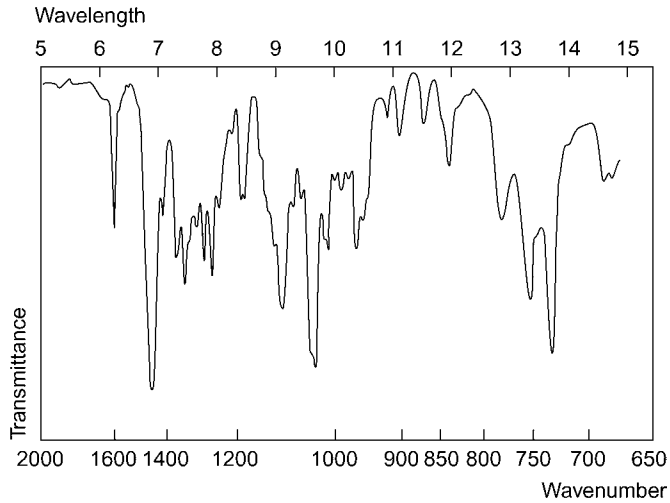
Colour Tests Liebermann's reagent—red; Mandelin's test—red; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.07; system TE—R_f 0.56; system TAE—R_f 0.22 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2880.
High Performance Liquid Chromatography System HA—*k* 2.8 (tailing peak)); system HY—RI 277.

Column: RP-18e (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer containing 0.5% glacial acetic acid (pH 3.0, 15 : 85 for 9 min to 25 : 75 for 1 min to 30 : 70 for 2 min to 35 : 65 for 18 min to 15 : 85 for 20 min), flow rate 1.0 mL/min. DAD. Retention time: 6.05 min. Limit of quantification, 19 mg/L; limit of detection, 6 mg/L [Srivastava *et al.* 2006]. Column: RP-18 (125 × 4 mm i.d., 5 µm). Column temperature: 40°. Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 3.5; 26 : 74), flow rate 1 mL/min. IS: detajmium. Detection: fluorescence (λ_{ex} = 247 nm, λ_{em} = 353 nm). Retention time: ajmaline 3.5 min, IS, 7.0 min [Oertel *et al.* 1998]. Column: Nucleosil C_{18} (120 × 4 mm i.d., 5 µm) Mobile phase: acetontrile : 0.07% orthophosphoric acid (22 : 78), flow rate 1.5 mL/min or aqueous 0.2 mol/L trifluoroacetic acid: acetonitrile (80 : 20), flow rate 1.1 mL/min. UV detection (λ = 254 nm). Retention time: ajmaline 5.88 min, isoajmaline 5.1 min, ajmaline *N*-oxide 4.35 min, dihydroajmaline 7.05 min [Köppel *et al.* 1992].
Ultraviolet Spectrum Aqueous acid—245 (A^1_1 = 223a), 289 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 756, 741, 1103, 1092, 1046, 1280 cm^{-1}.



Quantification
Blood **GC-MS** Column: 3% OV-1 on Gas Chrom Q, 100/120 mesh (6 ft (180 cm) × 2 mm i.d.). Temperature: 250°. Carrier gas: He, 40 mL/min. EI ionisation. Limit of detection, 0.4 mg/L [Clemans *et al.* 1977].

HPLC Column: Zorbax CN (250 × 4.6 mm i.d.). Mobile phase: hexane : ethanol : ethanolamine (83.18 : 16.79 : 0.03), flow rate 1.5 mL/min. UV detection (λ = 235 nm). Retention time: 8.15 min. Limit of detection not reported [Ikeda *et al.* 1988].

Plasma **HPLC** Column: Nucleosil C_{18} (120 × 0.4 cm i.d., 5 µm). Mobile phase: acetonitrile : 0.07% orthophosphoric acid (22 : 78), flow rate 1.5 mL/min.

A

Fluorescence detection ($\lambda_{ex} = 296$ nm, $\lambda_{em} = 358$ nm). Retention time: ajmaline 5.88 min, isoajmaline and sandwicine 5.10 min, ajmaline *N*-oxide 4.35 min, and hydroajmaline 7.05 min. Limit of detection, 1 µg/L with extraction and 50 µg/L without [Köppel *et al.* 1992].

Gastric Contents **HPLC** See Blood [Ikeda *et al.* 1988].

Other **TLC** Dog Plasma. Plates: precoated (20 × 20 cm). Solvent system: benzene : acetic acid : methanol (86 : 7 : 7). R_f 0.20. Limit of detection, 0.1 µg [Dombrowski *et al.* 1975].

Note For a spectrofluorometric method for the determination of ajmaline in plasma, see Welman *et al.* [1977].

Disposition in the Body Variably absorbed after oral administration and mainly metabolised by the liver. The main metabolite is hydroxyajmaline *N*-oxide [Köppel *et al.* 1990].

Therapeutic Concentration

Six patients with acute myocardial infarction and ventricular arrhythmia were administered 20 mg/h IV ajmaline for 24 h. After 24 h, the plasma concentrations ranged from 0.25 to 0.95 mg/L [Köppel *et al.* 1992].

After IV injection of 50 mg to 1 subject, a plasma concentration of approx. 1.7 mg/L was reported at 1 min [Welman *et al.* 1977].

Toxicity

A 4-year-old girl died 3 h after ingestion of her mother's ajmaline tablets: seventeen 50 mg tablets were found missing. The ajmaline concentrations were determined to be 5.5 mg/L in her blood and 178.2 µg/g in her stomach contents (46.3 mg in total was found in the stomach). An estimated lethal dose of 100 to 500 mg/kg was reported [Ikeda *et al.* 1988].

A 57-year-old man ingested 1.0 g of ajmaline with suicidal intent. His blood concentrations and urinary excretion of ajmaline during the first 3 days post-ingestion were as follows:

Day	*Blood concentration (mg/L)*	*Urine*	
		mg/L	*mg/24 h*
1	3	1.26	35.3
2	0	0.609	6.09
3	0	0	0

[Almog *et al.* 1979].

In a fatality resulting from the ingestion of 2.5 g ajmaline and 300 mg diazepam, the following postmortem tissue concentrations of ajmaline were reported: blood 10 mg/L, liver 50 µg/g, urine 44 mg/L. A blood-alcohol concentration of 1590 mg/L was also reported [AR Alha 1966, personal communication].

Note For an account of a suicide attempt with ajmaline, see Jornod and Barrelet [1965].

Bioavailability Oral, 5%.

Half-life 1.5 h.

Volume of Distribution 2 to 3 L/kg.

Protein Binding 76%.

Dose Maintenance, 200 to 300 mg daily; therapeutic 150 to 400 mg daily.

Almog C *et al.* (1979). Acute intoxication with ajmaline. *Isr J Med Sci* 15: 570–572.

Clemans SD *et al.* (1977). A gas chromatography–mass fragmentographic method for the assay of ajmaline and its monochloroacetyl ester. *Arzneimittelforschung* 27: 1128–1130.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481– 486.

Dombrowski LJ *et al.* (1975). Determination of 17-monochloroacetylajmaline and its metabolite in plasma by TLC fluorescence detection. *J Pharm Sci* 64: 643–645.

Ikeda, N *et al.* (1988). An infant fatality involving ajmaline. *J Forensic Sci* 33: 558–561.

Jornod J, Barrelet C, JA (1965). Suicidal attempt by overdosage of ajmaline. *Am Heart J* 70: 719–720.

Köppel, C *et al.* (1990). Clinical course and outcome in class IC antiarrhythmic overdose. *J Toxicol Clin Toxicol* 28: 433–444.

Köppel, C *et al.* (1992). Monitoring of ajmaline in plasma with high-performance liquid chromatography. *J Chromatogr* 575: 87–91.

Oertel, R *et al.* (1998). Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing. *J Chromatogr A* 797: 203–209.

Srivastava, A *et al.* (2006). Quantitative determination of reserpine, ajmaline, and ajmalicine in *Rauvolfia serpentina* by reversed-phase high-performance liquid chromatography. *J Chromatogr Sci* 44: 557–560.

Welman, E *et al.* (1977). A spectrofluorometric method for the determination of ajmaline in plasma. *Br J Clin Pharmacol* 4: 549–551.

Alacepril

ACE Inhibitor, Antihypertensive

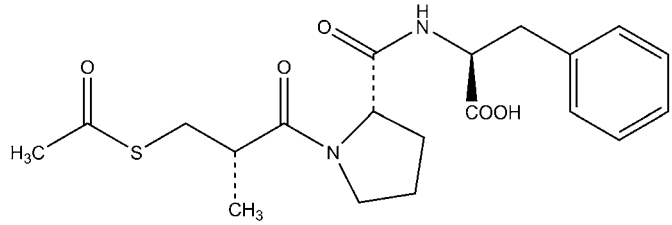
$C_{20}H_{26}N_2O_5S = 406.5$

CAS—74258-86-9

IUPAC Name (2*S*)-2-[[(2*S*)-1-[(2*S*)-3-Acetylsulfanyl-2-methylpropanoyl]pyrrolidine-2-carbonyl]amino]-3-phenylpropanoic acid

Synonyms 1-[(2*S*)-3-(Acetylthio)-2-methyl-1-oxopropyl]-l-prolyl-l-phenylalanine; 1-(*D*-3-acetylthio-2-methylpropanoyl)-l-prolyl-l-phenylalanine; DU-1219; *N*-[1-[(*S*)-3-mercapto-2-methylpropionyl]-l-prolyl]-3-phenyl-l-alanine acetate (ester).

Proprietary Name *Cetapril*



Chemical Properties Crystals. Mp 155° to 156° [O'Neil *et al.* 2006]. Captopril is stable in plasma and urine at approx. −20° for 30 days [Hayashi *et al.* 1985].

Quantification

Plasma **HPLC** Column: µBondapak C_{18} (300 × 4.0 mm i.d., 10 µm). Mobile phase: acetonitrile : ethanol : 1% acetic acid (45 : 11 : 75), flow rate 1.0 mL/min. UV detection ($\lambda = 254$ nm). Limit of detection, 10 µg/L for captopril [Hayashi *et al.* 1985].

Urine **HPLC** See Plasma. Limit of detection, 50 µg/L for captopril [Hayashi *et al.* 1985].

Disposition in the Body Alacepril thiolesterase catalyses the hydrolysis of alacepril to desacetylalacepril (DU-1227). Alacepril is also converted to captopril after absorption.

Therapeutic Concentration

Forty-seven patients with chronic renal failure or normal renal function were administered 6.25, 12.5, 25, or 50 mg alacepril. At a dosage of 25 mg/day, the plasma concentration of captopril ranged from 123 to 3010 mg/L, while serum creatinine levels ranged from 0.4 to 11.3 mg/L. There was a linear relationship between plasma concentrations of captopril and serum creatinine levels at all doses given. In patients with creatinine clearance (CL_{CR}) <10 mL/min who were administered 6.25, 12.5, or 25 mg/day alacepril, the plasma concentrations were 621 ± 99, 1055 ± 190, and 1679 ± 275 µg/L, respectively. A dosage of 25 mg/day resulted in a mean plasma concentration of 1993 ± 725 µg/L in patients on chronic haemodialysis, with a decrease to 1115 ± 415 µg/L after 4 h on haemodialysis [Nonoguchi *et al.* 2008].

Nine patients with chronic renal failure were administered 50 mg alacepril orally. Mean maximum plasma concentrations were 239, 1090, and 1433 µg/L for free captopril, protein-conjugated captopril, and total captopril attained at 1.1, 3.6, and 2.8 h, respectively. The mean peak plasma concentration, the time to peak plasma concentration, the elimination rate constant and the area under curve of free captopril did not differ from those in healthy subjects, although cumulative urinary excretion was significantly diminished. The same parameters of plasma protein-conjugated captopril and total captopril in the uraemic patient were 2 to 3 times greater than those in healthy subjects, while the elimination of total captopril in the urine was significantly decreased [Onoyama *et al.* 1986; Singlas, Fillastre 1991].

Seven healthy male subjects (aged 33 to 50 years) were given 50 mg alacepril orally in the fasting or fed state. Mean maximum plasma concentrations were 266, 513 and 764 µg/L for free captopril, protein-conjugated captopril and total captopril, respectively, attained at 1, 1.7 and 1.6 h, respectively, in the fasting state. The elimination half lives for free, protein-bound and total captopril were 1.9, 4.2 and 5 h, respectively. In the fed state, the half lives remained the same but the t_{max} for free captopril was prolonged to 1.9 h [Kelly, O'Malley 1990; Onoyama *et al.* 1985].

Note For a study investigating the efficacy of alacepril in combination with candesartan silextil in ovarian hyperstimulation syndrome, see Ando *et al.* [2003]. For a study of alacepril in decreasing atrial and brain natriuretic peptides in congestive heart failure, see Yoshimura *et al.* [1994]. For a study comparing the withdrawal of diuretics or ACE inhibitors as antihypertensive therapy in subjects with essential hypertension, see Takata *et al.* [1992].

Half-life In the fasted state: 1.9, 4.2 and 5 h for free, protein-bound and total captopril, respectively.

Dose Daily oral dose 25 to 75 mg, as a single dose or in two divided doses.

Ando H *et al.* (2003). Dual renin–angiotensin blockade therapy in patients at high risk of early ovarian hyperstimulation syndrome receiving IVF and elective embryo cryopreservation: a case series. *Hum Reprod* 18: 1219–1222.

Hayashi K *et al.* (1985). Determination of captopril and its mixed disulphides in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 338: 161–169.

Kelly JG, O'Malley K (1990). Clinical pharmacokinetics of the newer ACE inhibitors. A review. *Clin Pharmacokinet* 19: 177–196.

Nonoguchi H *et al.* (2008). Long-term plasma levels and dose modulation of alacepril in patients with chronic renal failure. *Hypertens Res* 31: 29–36.

O'Neil MJ *et al.* (2006) *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Onoyama K *et al.* (1985). Pharmacokinetics of a new angiotensin I converting enzyme inhibitor (alacepril) after oral dosing in fasting or fed states. *Clin Pharmacol Ther* 38: 462–468.

Onoyama K *et al.* (1986). Pharmacokinetic properties of a new angiotensin I-converting enzyme inhibitor in patients with chronic renal failure. *Curr Ther Res* 39: 671–680.

Singlas E, Fillastre JP (1991). Pharmacokinetics of newer drugs in patients with renal impairment (Part II). *Clin Pharmacokinet* 20: 389–410.

Takata Y *et al.* (1992). Comparison of withdrawing antihypertensive therapy between diuretics and angiotensin converting enzyme inhibitors in essential hypertensives. *Am Heart J* 124: 1574–1580.

Yoshimura M *et al.* (1994). Responses of plasma concentrations of A type natriuretic peptide and B type natriuretic peptide to alacepril, an angiotensin-converting enzyme inhibitor, in patients with congestive heart failure. *Br Heart J* 72: 528–533.

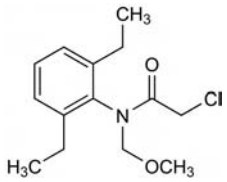
Alachlor

Herbicide

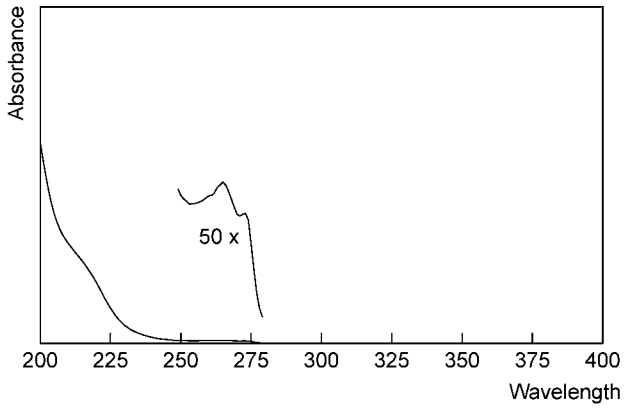
$C_{14}H_{20}ClNO_2$ = 269.8

CAS—15972-60-8

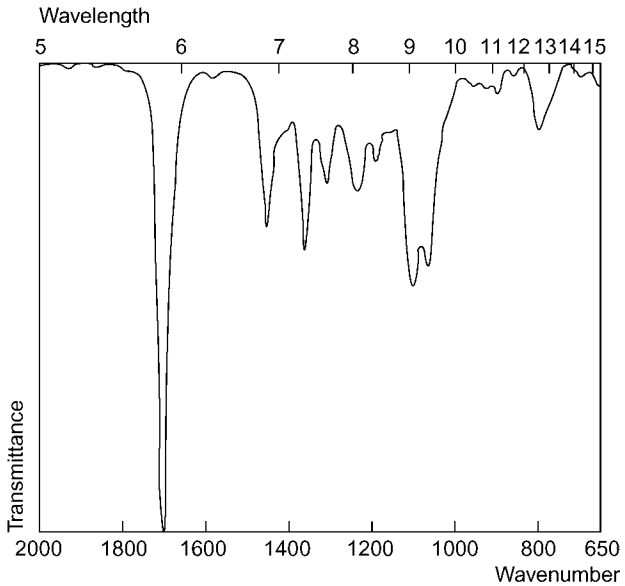
IUPAC Name 2-Chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide
Synonyms Alachlore; CP-50144; metachlor.
Proprietary Names *Alanex*; *Lasso*.



Chemical Properties A colourless to yellow crystalline solid. Mp 40° to 41°. It is soluble in water (240 mg/L at 25°), acetone, benzene, ethanol, ethyl acetate, diethyl ether and chloroform; sparingly soluble in heptane. Log *P* (octanol/water), 2.64, 2.92 and 3.52.
Thin-layer Chromatography System TX—R_f 0.40; system TY—R_f 0.45.
Gas Chromatography System GA—RI 1876.
Ultraviolet Spectrum Aqueous acid—265, 273 nm.



Infrared Spectrum Principal peaks at 1106, 1702, 1365, 1458 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 45, 160, 188, 146, 237, 132, 117, 77.
Quantification
Serum **GC** Column: DB-1 (2 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 0.3 min to 180° at 15°/min. Carrier gas: 1.9 mL/min. FID. Reference compounds: pentachlorophenol, heptadecanoic acid. Retention time: 2.75 min [Liu *et al.* 1994]. Limit of detection, 11.5 µg/L [Liu *et al.* 1994].

HPLC Limit of detection, <50 mg/L [Galati *et al.* 1998].

Note For an ELISA for the quantification of alachlor see Biagini *et al.* [1995].
Disposition in the Body Alachlor is absorbed through the skin and can be metabolised to 2,6-diethylbenzoquinoneimine (DEBQI) which is a carcinogenic metabolite and 2-chloro-*N*-(2,6-diethylphenyl)acetamide (CDEPA) which can subsequently be converted into DEBQI. Additionally, 2,6-diethylaniline (DEA), alachlor mercapturate and conjugated metabolites have been detected in urine. No free alachlor has been detected in urine.
Toxicity Alachlor may cause mild eye and skin irritations after exposure.

Biagini RE *et al.* (1995). Urinary biomonitoring for alachlor exposure in commercial pesticide applicators by immunoassay. *Bull Envir Contam Toxicol* 54(2): 245–250.
Galati R *et al.* (1998). Determination of serum levels of 2,6 diethylaniline in laboratory animal treated with Alachlor. *Anticancer Res* 18: 979–982.
Liu Z *et al.* (1994). Comprehensive two-dimensional gas chromatography for the fast separation and determination of pesticides extracted from human serum. *Anal Chem* 66: 3086–3092.

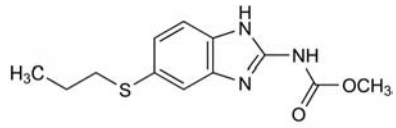
Albendazole

Anthelmintic (Veterinary)

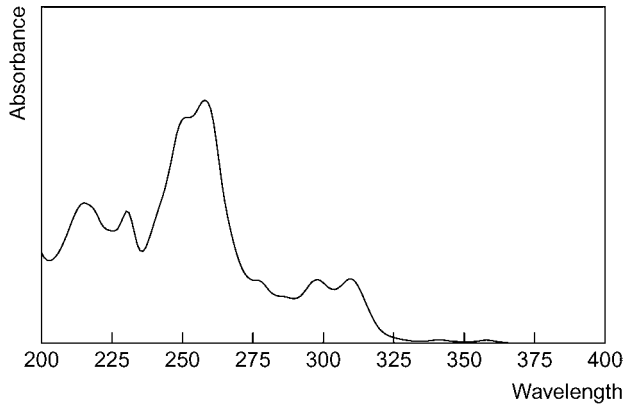
$C_{12}H_{15}N_3O_2S$ = 265.3

CAS—54965-21-8

IUPAC Name Methyl *N*-(6-propylsulfanyl-1H-benzimidazol-2-yl)carbamate
Synonym [5-(Propylthio)-1*H*-benzimidazol-2-yl]carbamic acid methyl ester.
Proprietary Names *Albenza*; *Eskazole*; *Proftril*; *Valbazen*; *Zental*.



Chemical Properties Colourless crystals. Mp 208° to 210°.
Ultraviolet Spectrum Aqueous acid—292 nm (A^1_1=370b); aqueous alkali—309 nm (A^1_1=742b).



High Performance Liquid Chromatography Column: amylose tris(3-dimethylphenyl carbamate) coated on silica gel substrate, Chiralpak AD (250 × 4.6 mm, 10 µm). Mobile phase: hexane: isopropanol: ethanol (81:14.25:4.74), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}=280 nm; λ_{em}=320 nm). Retention time: 8.5 min for albendazole; 13.7 (*trans*) 21.6 (*cis*) min for albendazole sulfoxide; 25.7 min for albendazole sulfone [Lanchote *et al.* 1998].
Quantification
Plasma **HPLC** Limit of quantification 10 µg/L and 7.5 µg/L for albendazole and metabolites, respectively [Chiap *et al.* 2000]. Column: RP C_{18} LiChroCART (125 × 4 mm, 5 µm). Mobile phase: acetonitrile: 0.25 N sodium acetate buffer (3:7), flow rate 1.5 mL/min. Internal standard (IS): mebendazole. UV detection (λ=290 nm). Retention time: 1.2 min for albendazole sulfoxide; 1.9 min for albendazole sulfone; 5.6 min for IS; 11.7 min for albendazole [Valois *et al.* 1994]. Limit of quantification, 40 µg/L for albendazole sulfoxide, and 10 µg/L for sulfone [Valois *et al.* 1994]. UV detection (λ=290 nm). Limit of detection, 14 µg/L for albendazole sulfoxide, and 30 µg/L for albendazole sulfone [Zeugin *et al.* 1990].
Disposition in the Body
Therapeutic Concentration

Thirteen subjects, male and female, with a mean age of 38.9 (15 to 64) years were administered a mean daily dose of 18.3 mg/kg albendazole (range 16.0 to 22.2 mg/kg). The mean peak plasma concentration of albendazole sulfoxide was 0.71 mg/L (range 0.36 to 1.52 mg/L) and albendazole sulfone was 0.06 (range 0.02 to 0.14) mg/L [Valois *et al.* 1994].

A patient with echinococcosis was administered 200 mg albendazole orally and a peak plasma concentration of 1.57 µmol/L was observed at 0.9 h [Zeugin *et al.* 1990].

Chiap P *et al.* (2000). Determination of albendazole and its main metabolites in ovine plasma by liquid chromatography with dialysis as an integrated sample preparation technique. *J Chromatogr A* 870(1–2): 121–134.
Lanchote VL *et al.* (1998). Simultaneous determination of albendazole sulfoxide enantiomers and albendazole sulfone in plasma. *J Chromatogr B, Biomed Sci Appl* 709: 273–279.

Valois MEC *et al.* (1994). Determination of albendazole metabolites in plasma by HPLC. *J Anal Toxicol* 18: 86–89.
Zeugin T *et al.* (1990). Therapeutic monitoring of albendazole: a high-performance liquid chromatography method for determination of its active metabolite albendazole sulfoxide. *Ther Drug Monit* 12: 187–190.

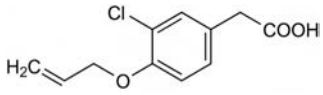
Alclofenac

Analgesic

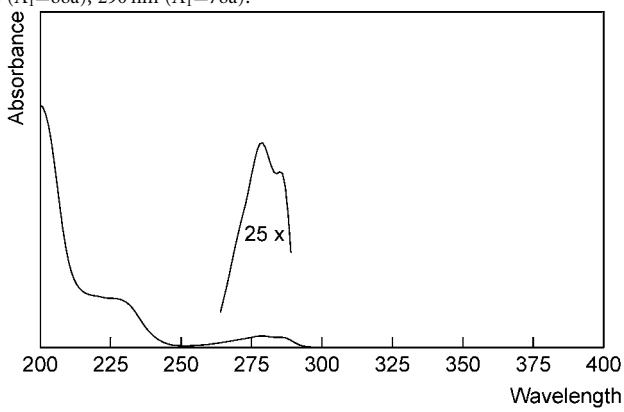
$C_{11}H_{11}ClO_3 = 226.7$

CAS—22131-79-9

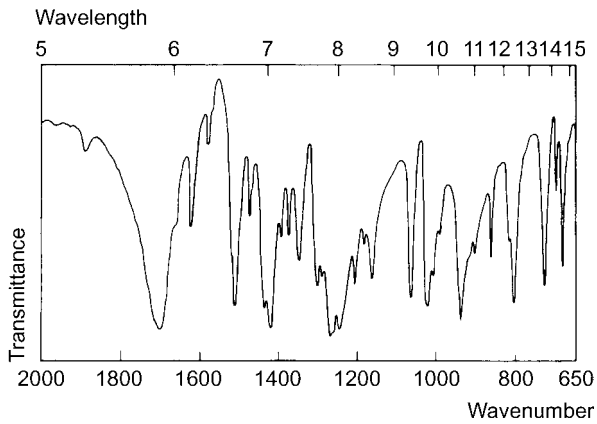
IUPAC Name 2-(3-Chloro-4-prop-2-enoxyphenyl)acetic acid
Synonym 3-Chloro-4-(2-propenyloxy)benzeneacetic acid.
Proprietary Names *Allopydin*; *Epinal*; *Mervan*; *Reufenac*; *Zumaril*.



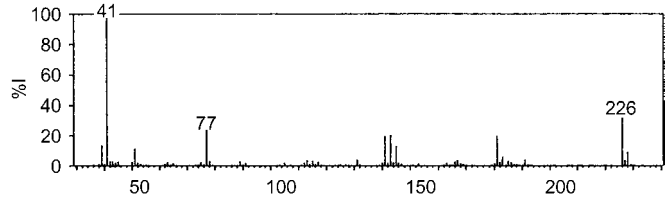
Chemical Properties A white or slightly yellowish-white crystalline powder. Mp about 91°. Slightly soluble in water; soluble 1 in 3 of ethanol, 1 in 4 of chloroform, and 1 in 6 of ether. pK_a 4.6.
Thin-layer Chromatography System TD—R_f 0.18; system TE—R_f 0.04; system TF—R_f 0.28; system TG—R_f 0.12; system TAD—R_f 0.33; system TAJ—R_f 0.17; system TAK—R_f 0.70; system TAL—R_f 0.90 (Lucy Tenger reagent, orange).
Gas Chromatography System GD—(methyl derivative)RRT 1.13.
High Performance Liquid Chromatography System HD—*k* 2.6; system HV—retention time 0.61 relative to meclofenamic acid.
Ultraviolet Spectrum Aqueous acid—277 (A^1_1=77b); dehydrated alcohol—282 (A^1_1=88a), 290 nm (A^1_1=78a).



Infrared Spectrum Principal peaks at wavenumbers 1258, 1689, 1235, 933, 1016, 1500 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 41, 226, 77, 143, 181, 141, 39, 145.



Quantification
Plasma **GC** FID. Alclofenac and 2 metabolites [Roncucci *et al.* 1971].
Urine **GC** See Plasma [Roncucci *et al.* 1971].
HPLC Limit of quantification, 1 mg/L [Delbeke *et al.* 1993].
Disposition in the Body Alcofenac is variably absorbed after oral or rectal administration and distributed into the synovial fluid. It is metabolised by glucuronic acid conjugation; there is some conjugation with glycine, deallylation to form 3-chloro-4-hydroxyphenylacetic acid, and hydroxylation to form 3-chloro-4-(2,3-dihydroxypropyloxy)phenylacetic acid which may be methylated; an epoxide metabolite has also been identified. Up to about 90% of an oral dose is excreted in the urine in 24 h, mostly as unchanged drug and the glucuronide conjugate.
Therapeutic Concentration
After an oral dose of 1 g to 10 subjects, a mean peak plasma concentration of 136 mg/L was attained in about 1 h; concentrations in the synovial fluid reached a mean peak of 32 mg/L in 2 h [Thomas *et al.* 1975].
Half-life Plasma half-life, 1.5 to 5.5 h (mean 2.5 h).
Volume of Distribution About 0.1 L/kg.
Protein Binding About 99%.
Note For a review of the pharmacokinetics of alclofenac, see Brogden *et al.* [1977].
Dose Alclofenac has been given in doses of 1.5 to 3 g daily.

Brogden RN *et al.* (1977). Alclofenac: a review of its pharmacological properties and therapeutic efficacy in rheumatoid arthritis and allied rheumatic disorders. *Drugs* 14: 241–259.
Delbeke FT *et al.* (1993). Determination of alclofenac in equine plasma and urine by high-performance liquid chromatography. *J Chromatogr* 621(2): 209–214.
Roncucci R *et al.* (1971). Gas chromatographic determination of 4-allyloxy-3-chlorophenylacetic acid (alclofenac) and its metabolites. *J Chromatogr* 62: 135–137.
Thomas GM *et al.* (1975). Simultaneous pharmacokinetics of alclofenace in plasma and synovial fluid in patients with rheumatoid arthritis. *Curr Med Res Opinion* 3(5): 264–267.

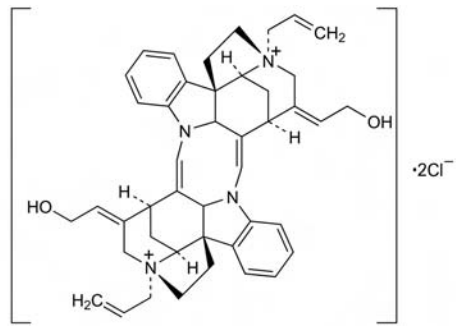
Alcuronium Chloride

Muscle Relaxant

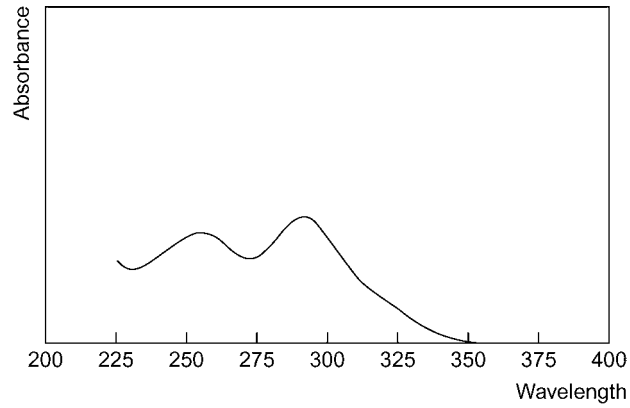
$C_{44}H_{50}Cl_2N_4O_2,5H_2O = 827.9$

CAS—23214-96-2 (alcuronium); 15180-03-7 (chloride)

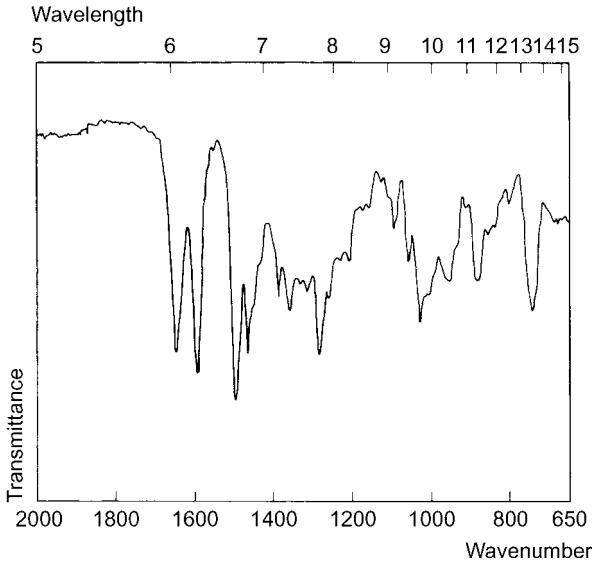
Synonyms Allnortoxiferin chloride; diallylnortoxiferine dichloride; diallyltoxiferine chloride; 4,4′-didemethyl-4,4′-di-2-propenyltoxiferine dichloride.
Proprietary Name *Alloferin*



Chemical Properties A colourless crystalline powder. Mp 220°. Soluble in water and ethanol; soluble 1 in 5 of methanol.
Colour Tests Mandelin's test—blue-violet; Marquis test—green-brown.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00 (alcuronium); system TE—R_f 0.00; system TAE—R_f 0.00 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HX—RI 354.
Ultraviolet Spectrum Aqueous acid—255, 291 nm (A^1_1=185b).



Infrared Spectrum Principal peaks at wavenumbers 1495, 1592, 1283, 1650, 1030, 757 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** Column: Spherisorb 5-CN (250 × 4 mm). Mobile phase: acetonitrile: aqueous solution of 60 mmol/L Na_2SO_4 and 5 mmol/L H_2SO_4 (46:54), flow rate 1 mL/min. Internal standard (IS): laudanosine. UV detection (λ=294 nm). Retention time: 3.2 min for IS; 5.4 min for alcuronium. Limit of detection, 25 µg/L [Kunzer *et al.* 1994]. Electrochemical detection. Limit of detection, 100 µg/L [Tovey *et al.* 1983].

Spectrofluorimetry [Walker *et al.* 1980].

Urine **HPLC** See Plasma [Kunzer *et al.* 1994].

Disposition in the Body After i.v. administration it is widely distributed throughout the tissues. About 80 to 85% of a dose is excreted in the urine as unchanged drug and about 10 to 15% is secreted in the bile and eliminated in the faeces.

Therapeutic Concentration

Following i.v. administration of 0.25 mg/kg to 17 subjects, plasma concentrations of 0.74 to 2.25 mg/L (mean 1.4 mg/L) were reported after 3 to 30 min [Walker *et al.* 1980].

Half-life Plasma half-life, about 3 h; increased in subjects with renal failure.

Volume of Distribution About 0.4 L/kg.

Clearance Plasma clearance, about 1.3 mL/min/kg.

Protein Binding About 40%.

Note For a review of the pharmacokinetics of alcuronium chloride and other muscle relaxants, see Ramzan *et al.* [1981].

Dose Initially 200 to 300 µg/kg intravenously.

Kunzer T *et al.* (1994). Simple and rapid high-performance liquid chromatography method for the determination of alcuronium in human plasma and urine. *J Chromatogr B, Biomed Appl* 653(1): 63–68.

Ramzan MI *et al.* (1981). Clinical pharmacokinetics of the non-depolarising muscle relaxants. *Clin Pharmacokinet* 6: 25–60.

Tovey C *et al.* (1983). Determination of alcuronium dichloride in plasma by high-performance liquid chromatography without solvent extraction. *J Chromatogr* 278: 216–219.

Walker J *et al.* (1980). Clinical pharmacokinetics of alcuronium chloride in man. *Eur J Clin Pharmacol* 17: 449–457.

Aldesleukin

Antineoplastic, Immunomodulator, Biological Response Modifier

$C_{690}H_{1115}N_{177}O_{203}S_6$ = 15330.7

CAS—110942-02-4

Synonyms DRG-0021; recombinant human interleukin-2; rIL-2; r-serHuIL-2; serine-125 human interleukin-2.

Proprietary Name *Proleukin*

Chemical Properties A human recombinant interleukin-2 product, approximate molecular weight 15 300 daltons.

Quantification

Serum **Immunobioassay** Limit of quantification, 0.1 µg/L [Nadeau *et al.* 1989].

Disposition in the Body The pharmacokinetics of aldesleukin show considerable inter- and intra-subject variation. Following IV administration it undergoes rapid distribution into the extravascular space. Distribution may be related to the method of administration and may therefore affect its efficacy. It is eliminated by the kidneys by both glomerular filtration and peritubular secretion.

Therapeutic Concentration

Twelve patients with either metastatic renal cell carcinoma or melanoma were administered with aldesleukin at a dose of 1.1 mg/m^2 daily (18×10^6 IU/m^2 daily) as a continuous IV infusion for 5 days. The maximum serum concentration was 2.2 ± 1.1 µg/L (40 IU/L) after 24 or 48 h. After that time, the serum concentrations decreased to 0.59 ± 0.43 µg/L (10.6 IU/L) by the end of the 5-day treatment [Fish *et al.* 1991].

Toxicity Capillary leak syndrome leading to pulmonary oedema and hypertension is a major adverse effect.

Half-life Plasma, 85 min to 4 h (IV bolus); 12 h (1-h infusion).

Volume of Distribution 6.3 to 7.9 L (following IV bolus or 2-h infusion) which increases with repeated doses.

Clearance Plasma, 140 to 300 mL/min (mean clearance 268 mL/min); 8.4 to 18 L/h.

Note For a review of aldesleukin, see Whittington and Faulds [1993].

Dose 600 000 units/kg every 8 h by IV infusion over 15 min for a maximum of 14 doses repeated after 9 days. Alternatively, a dose of up to 18 million international units may be given subcutaneously.

Fish *et al.* (1991). *Br J Cancer* 64 (Suppl.15)4.

Nadeau RW *et al.* (1989). Quantification of recombinant interleukin-2 in human serum by a specific immunobioassay. *Anal Chem* 61: 1732–1736.

Whittington R, Faulds D (1993). Interleukin-2. A review of its pharmacological properties and therapeutic use in patients with cancer. *Drugs* 46: 446–514.

Aldicarb

Acaricide, Carbamate, Insecticide, Nematocide

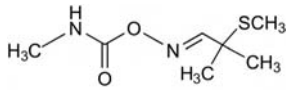
$C_7H_{14}N_2O_2S$ = 190.3

CAS—116-06-3

IUPAC Name [(*E*)-(2-Methyl-2-methylsulfanylpropylidene)amino] *N*-methyl carbamate

Synonyms Aldicarbe; carbanolate; ENT 27093; 2-methyl-2-(methylthio)propanal-*O*-[methylamino(carbonyl)]oxime; NCI-CO8640; OMS 771; UC 21149.

Proprietary Names *Sentry*; *Temic*; *Temik*.



Chemical Properties A white crystalline powder. Mp 90° to 100°. It is soluble in water (0.1–1.0 mg/mL at 22°), DMSO (>100 mg/mL at 21°), 95% ethanol (>100 mg/mL at 21°), acetone (>100 mg/mL at 21°), chloroform, isopropane, toluene and most organic solvents; insoluble in heptane and mineral oils. Log *P* (octanol/water), 1.13 (25°). Aldicarb and its metabolites are not stable in beef or chicken liver when stored at −4° with 100% depletion of fortified samples occurring at 3–6 months [Ali *et al.* 1993].

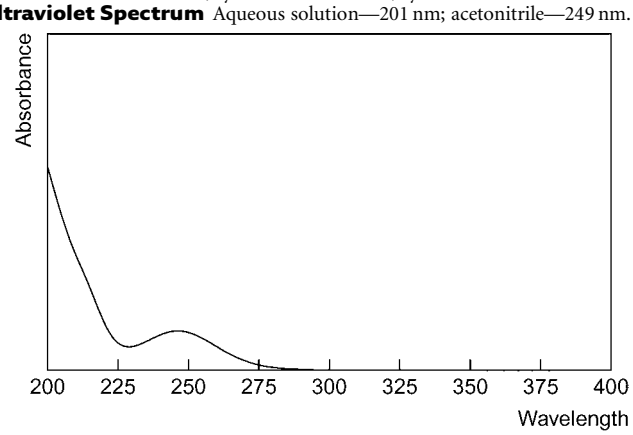
Thin-layer Chromatography System TX—R_f 0.18; system TY—R_f 0.12; system TZ—R_f 0.65; system TAA—R_f 0.12; system TAB—R_f 0.04; system TAC—R_f 0.03.

Plate: silica gel G. Mobile phase: *n*-hexane : acetone (4 : 1). Developed with zinc chloride–diphenylamine spray reagent (110° for 10 min). R_f 0.4 [Sevalkar *et al.* 1991].

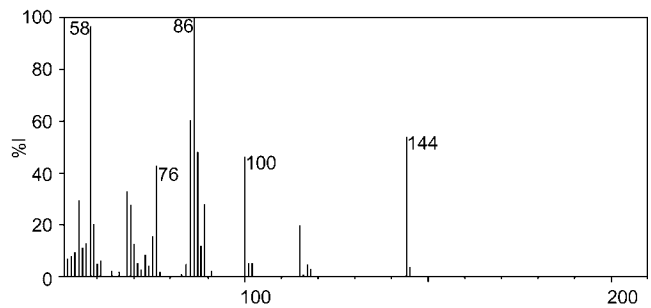
Gas Chromatography System GA—RI 1320; system GKA—RI 866; system GKB—RI 966; system GKC—RI 1088.

High Performance Liquid Chromatography System HZ—RT 3.6 min; system HAA—RT 15.1 min; system HAO—*k* 7.63; system HAP—*k* 0.69.

Ultraviolet Spectrum Aqueous solution—201 nm; acetonitrile—249 nm.



Mass Spectrum Principal peaks at *m/z* 86, 58, 144, 84, 100, 76.



A

Quantification

Blood **HPLC** Column: Waters C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: water : methanol : acetonitrile, flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 339 nm, λ_{em} = 445 nm). Limit of detection, 1 µg/L [Proença *et al.* 2004].

Serum **HPLC** Column: Alltima RP-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (35 : 65), flow rate 1.5 mL/min. DAD (λ = 210 nm). Retention time: 7.2 min. Limit of detection, 0.05 mg/L [Covaci *et al.* 1999].

Urine **GC** Column: 3% polyethylene glycol 20M–GasChrom Q (2.1 m × 2.7 mm i.d.). Temperature: 160°. FPD. Limit of detection, 2.4 µg/L [Lian *et al.* 1991].

HPLC Column: Hypersil C_{18} (100 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (15 : 85 to 100 : 0 at 6 min for 3 min to 15 : 85 at 14 min), flow rate 1 mL/min. UV detection (λ = 210 nm). Limit of quantification, 1 µg/L for aldicarb, 3 µg/L for the metabolites, limit of detection, 0.3 µg/L for aldicarb and 1 µg/L for the metabolites [Parilla Vázquez *et al.* 2000]. Column: Hypersil C_{18} (100 × 3 mm i.d., 5 µm). Mobile phase: water : acetonitrile : methanol (90 : 10 : 0 for 4 min to 90 : 5 : 5 at 4.01 min for 2 min to 0 : 100 : 0 in 4 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 4 µg/L for aldicarb and 10 µg/L for metabolites [Martínez Fernández *et al.* 2000]. See Serum [Covaci *et al.* 1999].

LC-MS Column: Hypersil C_{18} (100 × 3 mm i.d., 5 µm). Mobile phase: water : acetonitrile (80 : 20 for 3 min to 0 : 100 at 7 min for 2 min), flow rate 0.8 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 0.1 µg/L for aldicarb and aldicarb sulfoxide, 0.5 µg/L for aldicarb sulfone [Martínez Fernández *et al.* 2000].

Other **HPLC** Food. Column: Waters (150 × 3.9 mm i.d., 4 µm). Mobile phase: water : methanol : acetonitrile. Fluorescence detection (λ_{ex} = 339 nm, λ_{em} = 445 nm). Limit of quantification, 0.7, 0.9 and 0.07 µg/kg for aldicarb, aldicarbsulfoxide and aldicarbsulfone, respectively [Borkovcová *et al.* 2004]. Environmental Water. Column: Varian octadecylsilica (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (18 : 82 to 19 : 81 at 11 min to 20 : 80 at 13 min to 27 : 73 at 15 min to 49 : 51 at 25 min to 70 : 30 at 35 min to 80 : 20 at 45 min to 100 : 0 at 46 min), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 465 nm). Limit of detection, 3–4 µg/L [García de Llasera, Bernal-González 2001]. Drinking Water. Column: Phenomenex ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (70 : 30), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 465 nm). Retention time: 11.9 min. Limit of detection, <0.1 µg/L [Morrica *et al.* 2005]. Air. Column: Nova-Pak C_{18} (300 × 3.9 mm i.d.). Mobile phase: propan-1-ol–TEA phosphate buffer 0.02 mol/L (pH 7.0; 2 : 98): propan-1-ol-acetonitrile (2 : 98; 97 : 3 to 5 : 95 at 30 min for 5 min), flow rate 1.0 mL/min. DAD (λ = 200 nm). Limit of quantification, 2.42 µg/sample [Kennedy *et al.* 1997]. Tobacco. Column: Hypersil C_{18} (200 × 4.6 mm i.d.). Mobile phase: 0.1% triethanolamine in acetonitrile-water (20 : 80) : 0.1% triethanolamine in water (10 : 90 for 4 min to 30 : 70 in 7 min to 90 : 10 over 5 min for 10 min), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 465 nm). Limit of detection not reported [Yang, Smetena 1994]. Drinking Water. Column: Nucleosil 100 C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L phosphate buffer (pH 3.0) : acetonitrile-water (90 : 10; 95 : 5 to 0 : 100 at 25 min for 5 min), flow rate 1 mL/min. DAD (λ = 215 nm) or fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 460 nm). Limit of quantification, 0.1 µg/L for DAD, 0.01 µg/L for fluorescence; limit of detection, 0.1 µg/L for DAD, 0.005 µg/L for fluorescence [Patsias, Papadopoulou-Mourkidou 1999]. Column: Supersphere 60 RP-8 (250 × 4.6 mm i.d., 4 µm). Mobile phase: acetonitrile : methanol : water (40 : 40 : 20) : acetonitrile : water (10 : 90; 5 : 95 to 20 : 80 at 15 min to 30 : 70 in 20 min to 65 : 35 in 20 min to 100 : 0 in 7 min), flow rate 0.8 mL/min. UV detection (λ = 220 nm). Limit of detection, 0.03 µg/L for aldicarb, 5 µg/L for aldicarb sulfone and aldicarb sulfoxide (lower limits with post-column fluorescence derivatisation) [Chiron, Barcelo 1993]. Column: Beckman Ultrasphere ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (15 : 85 to 100 : 0 in 32 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 418 nm). Limit of detection, 1.0 µg/L for aldicarb, 2.0 µg/L for aldicarb sulfone and aldicarb sulfoxide [Edgell *et al.* 1991].

LC-MS Apples. Column: Atlantis dC_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile: 0.1 mol/L ammonium acetate in acetonitrile : water (20 : 80): water (8 : 10 : 82 to 90 : 10 : 0 at 7 min to 90 : 10 : 0 at 25 min to 100 : 0 : 0 at 28 min to 8 : 10 : 82 at 34 min), flow rate 0.2 mL/min for 25 min to 0.3 mL/min until 33 min to 0.2 mL/min for 1 min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.6 µg/kg [Wang, Wotherspoon 2007]. Soy-based Infant Formulas. Column: YMC ODS-AQ S-3 (50 × 2 mm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate with 20% acetonitrile in water : water (80 : 20 : 0 to 90 : 10 : 0 over 9 min for 4 min to 100 : 0 : 0 for 2 min to 8 : 10 : 82 for 5 min), flow rate 0.2 mL/min for 13 min to 0.3 mL/min for 6 min to 0.2 mL/min for 1 min. ESI, positive ion mode, MRM acquisition mode. Retention time: 8.46 min. Limit of detection, 0.6 µg/kg [Wang, Cheung 2006]. Apple-based Infant Food. Column: YMC ODS-AQ S-3 (50 × 2 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate in 20% acetonitrile : water (8 : 10 : 82 to 90 : 10 : 0 at 9 min for 4 min to 100 : 0 : 0 for 2 min to 8 : 10 : 82 for 5 min), flow rate 0.2 mL/min for 13 min to 0.3 mL/min for 6 min to 0.2 mL/min for 1 min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.2, 0.1 and 0.09 µg/kg for aldicarb, aldicarb sulfoxide and aldicarb sylfone, respectively [Wang *et al.* 2005]. Fruit and Vegetables. Column: Zorbax Eclipse XDB C_{18} (150 × 3 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (30 : 70 to 100 : 0 at 15 min), flow rate 500 µL/min. APCI, positive ion mode, SIM acquisition mode. Limit of detection, 0.5–5.0 µg/L [Takino *et al.* 2004]. Natural Waters. Column: Hypersil reversed phase C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (20 : 80 to 100 : 0 in 35 min), flow rate 1.0 mL/min. ESI, positive and negative ion modes. Limit of detection, 0.6 ng/L [Di Corcia *et al.* 2000]. Fruit and Vegetables. Column: Zorbax C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L ammonium formate (5 : 95 to 30 : 70 at 10 min to 5 : 95 for 20 min), flow rate 1.0 mL/min. APCI, SIM acquisition mode. Limit of detection, 0.5 ng [Nunes *et al.* 2000].

CE Groundwater. Capillary: fused silica (57/50 cm total/effective length, 75 µm i.d.). Buffer: sodium borate : 20 mmol/L hydrochloric acid (pH 8). DAD (λ = 210 nm). Limit of quantification, 24.9 µg/L, limit of detection, 7.47 µg/L [Arráez-Román *et al.* 2004]. Drinking Water. Capillary: untreated fused silica (52/40 cm total/effective length, 50 µm i.d.). Buffer: 50 mmol/L SDS-10 mmol/L borate buffer-15 mmol/L β-cyclodextrin : acetonitrile (78 : 22). UV detection (λ = 202 nm). Limit of detection, 0.46 µg/L [Fung, Mak 2001].

CE-MS Spiked Tap Water. Capillary: bare fused silica (60/67 cm total/effective length, 50 µm i.d.). Buffer: 5 mmol/L ammonium acetate in water : 5 mmol/L ammonium acetate in methanol (50 : 50), flow rate 4 µL/min. ESI, SIM acquisition mode. Limit of detection, 0.01 mg/L [Van Biesen, Bottaro 2006].

Disposition in the Body Aldicarb is efficiently absorbed from the gastrointestinal tract and to a lesser extent through the skin. It is distributed to all tissues, but it does not significantly cross the blood–brain barrier. It is metabolised to aldicarb sulfoxide and aldicarb sulfone, both of which are toxic. Both metabolites can be detoxified by hydrolysis to oximes and nitriles. The drug and metabolites are excreted primarily in urine but a small amount is also excreted through biliary elimination. Aldicarb does not accumulate in the body during long-term exposure.

Toxicity Aldicarb features prominently in accidental pesticide poisonings because of the way it is misused. Aldicarb exposure may be fatal and may occur by ingestion, inhalation or when absorbed through the skin. Very high doses may result in paralysis of the respiratory system and the nervous system. Toxicity can occur between 15 min and 3 h postexposure but effects can disappear quite quickly within 4–12 h. The allowed daily intake is 0.005 mg/kg body weight.

A 24-year-old man was found unconscious, frothing at the mouth. He was dead on arrival at hospital. Postmortem aldicarb concentrations were 6.2 mg/L (blood), 48.9 mg/kg (stomach), 0.80 mg/kg (liver), 8.10 mg/kg (kidney), 6.70 mg/kg (heart), and 17.5 mg/L (urine) [Proença *et al.* 2004].

A 65-year-old man and a 63-year-old woman were admitted to hospital with signs of cholinergic crisis. Their serum and urine aldicarb concentrations were as follows:

Concentration (mg/L)	*Male*	*Female*
Serum		
Day 1 (19 : 30)	0.90	0.85
Urine		
Day 1 (20 : 00)	1.00	0.61
Day 1 (24 : 00)	0.28	0.25
Day 2	—	—

[Covaci *et al.* 1999].

A 40-year-old woman was found unconscious and later pronounced dead. It was believed that she had ingested a black granular substance known as Temic. Postmortem analysis revealed the following aldicarb levels: blood concentration 11 mg/L, total stomach contents 213 mg and liver >1 mg/kg [Hoai Ngo 1991].

Ali MS *et al.* (1993). Analyte stability study of *N*-methylcarbamate pesticides in beef and poultry liver tissues by liquid chromatography. *J AOAC Int* 76: 1309–1316.

Arráez-Román D *et al.* (2004). Determination of aldicarb, carbofuran and some of their main metabolites in groundwater by application of micellar electrokinetic capillary chromatography with diode-array detection and solid-phase extraction. *Pest Manag Sci* 60: 675–679.

Borkovcová I *et al.* (2004). Determination of *n*-methylcarbamates in foods. *Cent Eur J Public Health* 12: 220–223.

Chiron S, Barcelo D (1993). Determination of pesticides in drinking water by on-line solid-phase disk extraction followed by various liquid chromatographic systems. *J Chromatogr* 645: 125–134.

Covaci A *et al.* (1999). A case of aldicarb poisoning: a possible murder attempt. *J Anal Toxicol* 23: 290–293.

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Edgell KW *et al.* (1991). Direct aqueous injection-liquid chromatography with post-column derivatization for determination of *N*-methylcarbamoyloximes and *N*-methylcarbamates in finished drinking water: collaborative study. *J Assoc Off Anal Chem* 74: 309–317.

Fung YS, Mak JL (2001). Determination of pesticides in drinking water by micellar electrokinetic capillary chromatography. *Electrophoresis* 22: 2260–2269.

García de Llasera MP, Bernal-González M (2001). Presence of carbamate pesticides in environmental waters from the northwest of Mexico: determination by liquid chromatography. *Water Res* 35: 1933–1940.

Hoai Ngo S (1991). A fatal case involving aldicarbe. *TIAFT Bull Case Notes*,21.

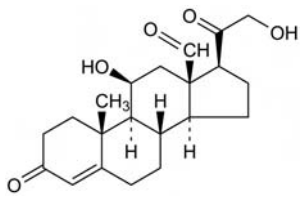
Kennedy ER *et al.* (1997). A sampling and analytical method for the simultaneous determination of multiple organonitrogen pesticides in air. *Am Ind Hyg Assoc J* 58: 720–725.

Lian DX *et al.* (1991). Gas chromatographic determination of aldicarb and its metabolites in urine. *J Chromatogr* 542: 526–530.
Martínez Fernández J *et al.* (2000). Analysis of *N*-methylcarbamate insecticides and some of their main metabolites in urine with liquid chromatography using diode array detection and electrospray mass spectrometry. *Anal Chim Acta* 412: 131–139.
Morrica P *et al.* (2005). Liquid chromatographic determination of nine *N*-methylcarbamates in drinking water. *Biomed Chromatogr* 19: 107–110.
Nunes GS *et al.* (2000). Determination of aldicarb, aldicarb sulfoxide and aldicarb sulfone in some fruits and vegetables using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr A* 888: 113–120.
Parilla Vázquez P *et al.* (2000). Reversed-phase liquid chromatographic column switching for the determination of *N*-methylcarbamates and some of their main metabolites in urine. *J Chromatogr B Biomed Sci Appl* 738: 387–394.
Patsias J, Papadopoulou-Mourkidou E (1999). A fully automated system for analysis of pesticides in water: on-line extraction followed by liquid chromatography–tandem photodiode array/post-column derivatization/fluorescence detection. *J AOAC Int* 82: 968–981.
Proença P *et al.* (2004). Aldicarb poisoning: one case report. *Forensic Sci Int* 146: S79–S81.
Sevalkar MT *et al.* (1991). Zinc chloride–diphenylamine reagent for thin layer chromatographic detection of some organophosphorus and carbamate insecticides. *J Assoc Off Anal Chem* 74: 545–546.
Takino M *et al.* (2004). Determination of carbamate pesticide residues in vegetables and fruits by liquid chromatography–atmospheric pressure photoionization-mass spectrometry and atmospheric pressure chemical ionization-mass spectrometry. *J Agric Food Chem* 52: 727–735.
VanBiesen G, Bottaro CS (2006). Ammonium perfluorooctanoate as a volatile surfactant for the analysis of *N*-methylcarbamates by MEKC-ESI-MS. *Electrophoresis* 27: 4456–4468.
Wang J, Cheung W (2006). Determination of pesticides in soy-based infant formula using liquid chromatography with electrospray ionization tandem mass spectrometry. *J AOAC Int* 89: 214–224.
Wang J, Wotherspoon D (2007). Determination of pesticides in apples by liquid chromatography with electrospray ionization tandem mass spectrometry and estimation of measurement uncertainty. *J AOAC Int* 90: 550–567.
Wang J *et al.* (2005). Determination of pesticides in apple-based infant foods using liquid chromatography electrospray ionization tandem mass spectrometry. *J Agric Food Chem* 53: 528–537.
Yang SS, Smetena I (1994). Determination of aldicarb, aldicarb sulfoxide and aldicarb sulfone in tobacco using high-performance liquid chromatography with dual post-column reaction and fluorescence detection. *J Chromatogr A* 664: 289–294.

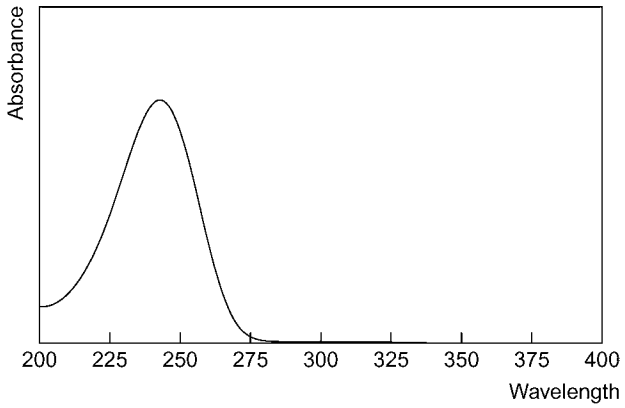
Aldosterone

Corticosteroid
$C_{21}H_{28}O_5$ = 360.4
CAS—52-39-1

IUPAC Name (8*S*,9*S*,10*R*,11*S*,13*R*,14*S*, 17*S*)-11-Hydroxy-17-(2-hydroxyacetyl)-10-methyl-3-oxo-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthrene-13-carbaldehyde
Synonyms (11β)-11,21-Dihydroxy-3,20-dioxopregn-4-en-18-al; electrocortin.
Proprietary Name *Aldocorten*



Chemical Properties Mp 164°.
Ultraviolet Spectrum Methanol—241 nm (A^{1}_{1}=437b).



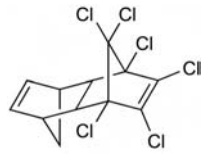
Infrared Spectrum Principal peaks at wavenumbers 1650, 986, 1020, 999, 1062, 1075 cm^{-1} (KBr disk).
Quantification
Plasma **LC-MS** (*m/z* 359.2 to 331.2). APCI Limit of quantification, 15 ng/L, limit of detection, 10 ng/L [Fredline *et al.* 1997].
Serum **HPLC** MS detection (*m/z* 359.2 to 331.2). See Plasma [Fredline *et al.* 1997].
Dose Aldosterone has been given in doses of 500 µg intravenously, repeated several times a day.

Fredline VF *et al.* (1997). A reference method for the analysis of aldosterone in blood by high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. *Anal Biochem* 252: 308–313.

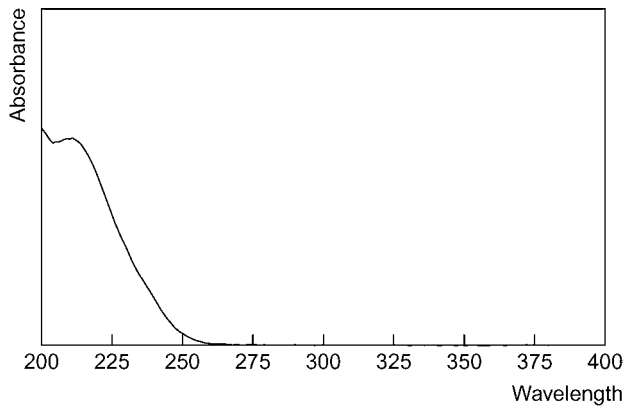
Aldrin

Insecticide
$C_{12}H_8Cl_6$ = 364.9
CAS—309-00-2

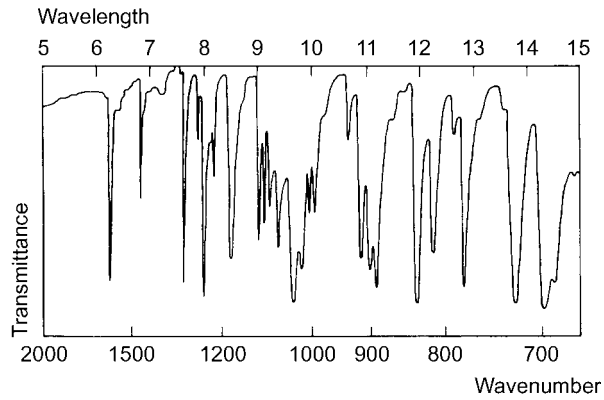
Synonyms Compd 118; HHDN.
Proprietary Names *Alderstan*; *Aldrex*; *Octalene*.



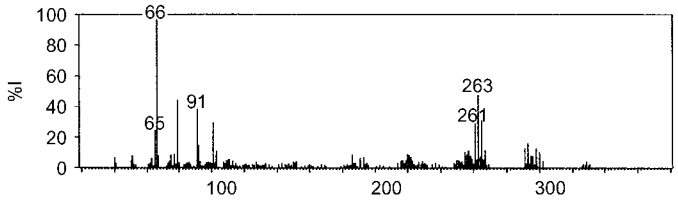
Note Aldrin usually contains 95% w/w of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene (HHDN) together with 5% w/w of active related compounds.
Chemical Properties White crystals. When heated to decomposition it evolves highly toxic fumes of phosgene and hydrogen chloride. The technical grade is a tan to dark brown solid containing 85% or more of HHDN. Mp 104° (pure grade), 49° to 60° (technical grade). Practically insoluble in water; soluble 1 in 20 of ethanol, 1 in 1 of acetone, 1 in 0.55 of benzene, and 1 in 0.33 of carbon tetrachloride; soluble in ether. Log *P* (octanol), 3.0.
Colour Tests Nitric–sulfuric acid (Erdman's reagent)—pink; sulfuric acid–fuming sulfuric acid—red (slow).
Thin-layer Chromatography System TX—aldrin R_f 0.89, dieldrin R_f 0.65; system TY—aldrin R_f 0.98, dieldrin R_f 0.87; system TAB—aldrin R_f 0.67, dieldrin R_f 0.51; system TAC—aldrin R_f 0.52, dieldrin R_f 0.27.
Gas Chromatography System GA—aldrin RI 1943, dieldrin RI 2110; system GK—aldrin retention time 0.88, dieldrin retention time 1.13 (both relative to caffeine); system GKA—aldrin RI 2008, dieldrin RI 2170; system GKB—aldrin RI 2096, dieldrin RI 2304; system GKC—aldrin RI 2226, dieldrin RI 2528.
High Performance Liquid Chromatography System HAO—*k* 0.00; system HAP—*k* 33.86.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 696, 1036, 835, 723, 1255, 893 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 66, 91, 79, 263, 65, 101, 261, 265; dieldrin 79, 82, 81, 263, 77, 108, 265, 80.



Quantification

Blood **GC** ECD. Limit of detection, 0.1 µg/L [Rosell *et al.* 1993].

GC-MS Column: fused silica capillary (30 m x 0.25 mm i.d., 0.25 µm). Temperature: 250°. Carrier gas: He. IS: endosulfan-I-d_4. Retention time: aldrin, 21.9 min; dieldrin, 31.4 min [Liu, Pleil 2002]. Limit of quantification, 25–50 pg (*m/z* 263, 378) [Liu, Pleil 2002].

Disposition in the Body Poorly absorbed after oral ingestion but when dissolved in oils or other lipids it is readily absorbed by the skin and gastrointestinal tract. It is rapidly metabolised in the body by epoxidation to dieldrin; dieldrin is stored in the body fat and persists for several weeks after the cessation of exposure. It is slowly eliminated in the faeces, mainly as unknown hydrophilic metabolites; a small amount is excreted in the urine as metabolites.

Blood Concentration Blood concentrations of dieldrin averaging 1 µg/L have been reported in 10 subjects with no occupational exposure to insecticides. Serum concentrations in subjects with low to high occupational exposure averaged 1 to 2 µg/L for aldrin and 9 to 27 µg/L for dieldrin.

Toxicity Severe symptoms may follow absorption of 1 to 3 g; the estimated minimum lethal dose is 5 g. The maximum permissible atmospheric concentration is 0.25 mg/m^3 and the maximum permissible concentration in food is 0.1 ppm.

The body fat of a 23-year-old man poisoned by aldrin, while working in a formulating plant, contained 60 µg/g of dieldrin 15 days after the last exposure; a blood concentration of 0.1 mg/L was reported 4 months later. The blood concentrations of two men exposed to aldrin in the same plant and showing symptoms of poisoning were 0.28 and 0.13 mg/L of dieldrin 1 month after the last exposure. In 2 further cases of poisoning, the concentrations of dieldrin in the body fat 3 and 5 weeks after exposure were 149 and 44 µg/g respectively; the corresponding blood concentrations were 0.53 and 0.13 mg/L [Kazantzis *et al.* 1964].

Half-life Blood half-life, dieldrin 50 to 170 days (mean 97).

Distribution in Blood Plasma : whole blood ratio, dieldrin about 1.5.

Kazantzis G *et al.* (1964). Poisoning in industrial workers by the insecticide aldrin. *Br J Ind Med* 21: 46–51.

Liu S, Pleil JD (2002). Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography-mass spectrometry analysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 769: 155–167.

Rosell MG *et al.* (1993). Determination of chlorinated insecticides in blood samples of agricultural workers. *J Chromatogr* 655(1): 151–154.

Alefacept

Antipsoriatic, Immunosuppressant, Protein, T-Cell Activation Inhibitor

CAS—222535-22-0

IUPAC Name Dimer of 1-92 antigen LFA-3 (human) fusion protein with human immunoglobulin G1 (hinge-C_H2-C_H3 γ1-chain)

Synonyms BG-9273; BG9712; LFA3TIP; recombinant human LFA-3-IgG1 fusion protein.

Proprietary Name *Amevive*

Disposition in the Body Following IM injection, serum concentrations of alefacept are detectable ≈6 h later, with peak concentrations achieved between 24 and 192 h.

Therapeutic Concentration

Twelve young healthy male volunteers were administered 0.15 mg/kg of alefacept by IV bolus. The mean peak serum concentration was 3.1 mg/L at 0.4 h. In another study, two groups of eight young healthy volunteers were administered 0.04 mg/kg alefacept either as an IV infusion or IM injection. Peak serum concentrations for the IV and IM groups were 0.96 mg/L and 0.36 mg/L, respectively, and attained at 2.8 h and 86 h, respectively [Vaishnaw, TenHoor 2002].

Bioavailability Approximately 70% following IM injection.

Half-life Serum, ≈12 days.

Volume of Distribution 0.09 to 0.13 L/kg.

Clearance 0.18 to 0.34 mL/h/kg.

Dose Given in a dose of 7.5 mg once weekly by IV injection, or 15 mg once weekly by IM injection, for 12 weeks.

Vaishnaw AK, TenHoor CN (2002). Pharmacokinetics, biologic activity, and tolerability of alefacept by intravenous and intramuscular administration. *J Pharmacokinet Pharmacodyn* 29: 415–426.

Alendronic Acid

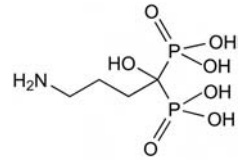
Bone Modulator

$C_4H_{13}NO_7P_2$ = 249.1

CAS—66376-36-1

IUPAC Name (4-Amino-1-hydroxy-1-phosphonobutyl)phosphonic acid

Synonyms Alendronate; (4-amino-1-hydroxybutylidene)bisphosphonic acid; aminohydroxybutylidene diphosphonic acid.



Chemical Properties A fine white powder. Mp 233° to 235°, with decomposition. pK_a (25°) 2.7, 8.7, 10.5, 11.6. Log *P* (octanol/water), −1.30.

Alendronate Sodium

$C_4H_{12}NNaO_7P_2,3H_2O$ = 325.1

CAS—121268-17-5

Proprietary Names *Adronat*; *Alendros*; *Dronal*; *Fosamax*.

Chemical Properties A white crystalline non-hygroscopic powder. It is soluble in water; very slightly soluble in ethanol; practically insoluble in chloroform.

Quantification

Plasma **HPLC** Electrochemical detection. Limit of quantification, 5 µg/L [Kline, Matuszewski 1992].

Urine **HPLC** Electrochemical detection. Limit of quantification, 1 µg/L. Fluorescence detection, 2.5 µg/L [Kline, Matuszewski 1992]. Fluorescence detection. Limit of quantification, 5 µg/L [Kline *et al.* 1990].

Disposition in the Body Alendronate is poorly absorbed after oral administration and absorption is decreased by food, especially products containing calcium or other polyvalent cations. The drug is rapidly cleared from plasma and about half of the absorbed dose is excreted in urine with the remainder being sequestered to bone for a prolonged period. It does not appear to be metabolised and is slowly released from skeletal deposits. Approximately 45% is excreted in urine in the first 8 h after an IV dose and then 5% is excreted much more slowly between 8 and 72 h. The drug is probably bound to the mineral phase of the skeleton and of that retained, approximately one-third is excreted over the first 6 months.

Half-life 10.5 years.

Volume of Distribution Steady state (estimated), greater than 28 L.

Clearance Systemic clearance (estimated), no more than 11.94 L/h.

Protein Binding 78%.

Dose For Paget's disease of bone the equivalent of 40 mg of alendronic acid daily; for treatment of postmenopausal osteoporosis 10 mg daily.

Kline WF *et al.* (1990). Determination of 4-amino-1-hydroxybutane-1,1-bisphosphonic acid in urine by automated pre-column derivatization with 2,3-naphthalene dicarboxyaldehyde and high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 534: 139–149.

Kline WF, Matuszewski BK (1992). Improved determination of the bisphosphonate alendronate in human plasma and urine by automated precolumn derivatization and high-performance liquid chromatography with fluorescence and electrochemical detection. *J Chromatogr* 583: 183–193.

Aletamine

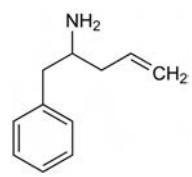
Analgesic

$C_{11}H_{15}N$ = 161.2

CAS—4255-23-6

IUPAC Name 1-Phenylpent-4-en-2-amine

Synonyms Alfetamine; α-allylphenethylamine.



Aletamine Hydrochloride

$C_{11}H_{15}N,HCl$ = 197.7

CAS—4255-24-7

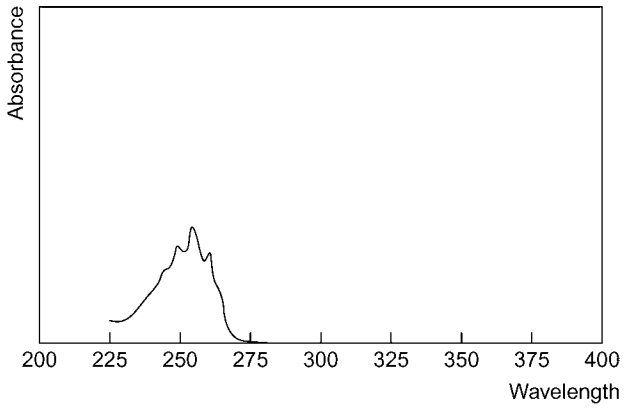
Chemical Properties White crystals. Soluble in water.

Colour Tests Liebermann's reagent—orange; Marquis test—orange.

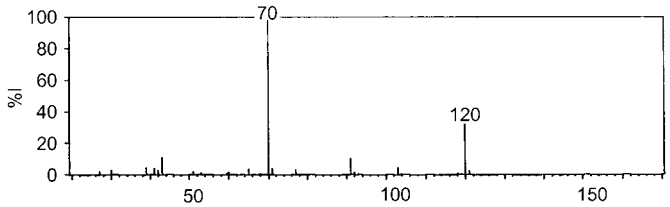
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.37; system TC—R_f 0.40; system TL—R_f 0.42 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 1293.

Ultraviolet Spectrum Aqueous acid—251, 257 (A^1_1=10b), 263 nm.



Mass Spectrum Principal ions at *m/z* 70, 120, 43, 91, 39, 103, 71, 65.



Dose Aletamine hydrochloride has been given in doses of 375 to 750 mg daily.

Alfacalcidol

Vitamin

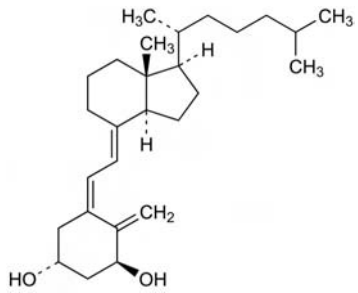
$C_{27}H_{44}O_2 = 400.6$

CAS—41294-56-8

IUPAC Name (1*R*,3*S*,5*Z*)-5-[(2E)-2-[(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(2*R*)-6-methylheptan-2-yl]-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1, 3-diol

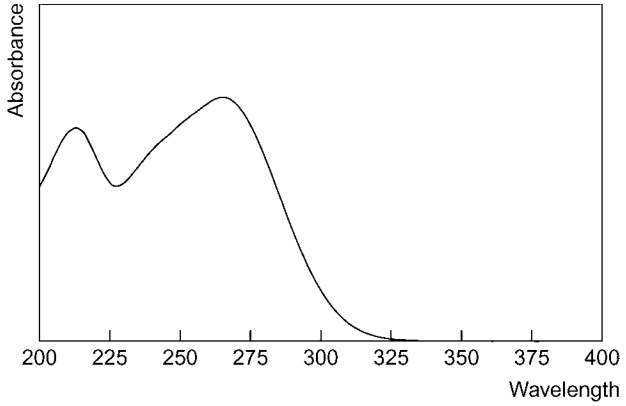
Synonyms 1α-Hydroxycholecalciferol; 1α-hydroxyvitamin D_3; (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β-diol.

Proprietary Names *Alfa D*; *AlfaD*; *Alfadelta*; *Alfarol*; *Bondiol*; *Dediol*; *Diseon*; *Doss*; *EinsAlpha*; *Etalpha*; *One-Alpha*; *Un-Alfa*.



Chemical Properties White or almost white crystals that are sensitive to air, heat and light. Mp 134° to 136°. It is practically insoluble in water; freely soluble in ethanol; soluble in fatty oils. Reversible isomerisation to pre-alfacalcidol may take place in solution. Log *P* (octanol/water), 9.11.

Ultraviolet Spectrum Principal peaks at 214, 267 nm.



Disposition in the Body Alfacalcidol is well absorbed after oral administration. Bile is essential for intestinal absorption. It is converted rapidly in the liver to calcitriol and eliminated via bile and kidneys.

Therapeutic Concentration Peak serum concentrations occur about 12 h after a single dose.

Half-life Plasma, calcitriol 3 to 6 h.

Protein Binding Bound to specific α-globulins for transport.

Dose Initially 1 µg daily, usual maintenance 0.25 to 1 µg daily.

Alfadolone

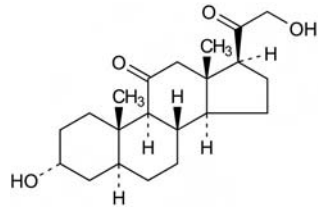
Anaesthetic (General)

$C_{21}H_{32}O_4 = 348.5$

CAS—14107-37-0

IUPAC Name (3*R*,5*S*,9*S*,14*S*)-3-Hydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-1,2,3,4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-11-one

Synonyms Alphadolone; 3α,21-dihydroxy-5α-pregnane-11,20-dione.



Chemical Properties Mp 167° to 170°.

Alfadolone Acetate

$C_{23}H_{34}O_5 = 390.5$

CAS—23930-37-2

Synonym It is an ingredient of alphadione (with alfaxalone)

Proprietary Names It is an ingredient of *Alfatesin(e)*, *Alfathesin*, *Althesin* and *Saffan* (vet.).

Chemical Properties A white to creamy-white powder. Mp 175° to 177°. Practically insoluble in water; soluble 1 in 15 of ethanol and 1 in 2 of chloroform. Log *P* (octanol/water), 2.67.

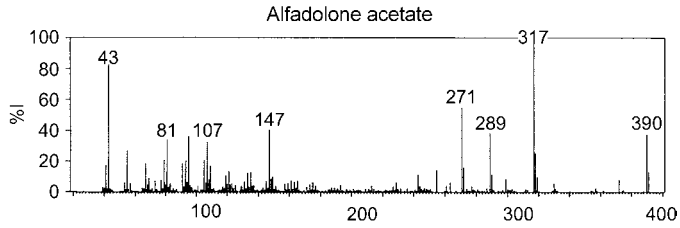
Colour Tests Antimony pentachloride—yellow; naphthol–sulfuric acid—orange-brown/orange; sulfuric acid—yellow.

Thin-layer Chromatography Alfadolone acetate: system TD—R_f 0.35; system TE—R_f 0.71; system TF—R_f 0.40; system TP—R_f 0.59; system TQ—R_f 0.22; system TR—R_f 0.80; system TS—R_f 0.45, streaking may occur; system TAD—R_f 0.62 (*p*-toluenesulfonic acid solution, positive).

Ultraviolet Spectrum Alfadolone acetate: methanol—290 nm (A^1_1=about 2).

Infrared Spectrum Principal peaks at wavenumbers 1228, 1758, 1710, 1278, 1010, 1043 cm^{-1} (alfadolone acetate, KBr disk).

Mass Spectrum Principal ions at *m/z* 317, 43, 271, 147, 289, 390, 95, 81 (alfadolone acetate).



Quantification

Plasma **GC-MS** ECD. Limit of detection, 10 µg/L [Pateman 1981].

Biological material **GC** [Heusler 1985].

Disposition in the Body Rapidly and widely distributed after injection. Alfadolone acetate is metabolised in the liver to alfadolone and alfadolone glucuronide which are excreted in the urine. Alfadolone is considered to be about 50% as potent an anaesthetic as alfaxalone.

Protein Binding 20 to 40%.

Dose See under Alfaxalone.

Heusler H (1985). Quantitative analysis of common anaesthetic agents. *J Chromatogr* 340: 273–319.

Pateman AJ (1981). Sensitive gas chromatographic method for the determination of alphadolone in plasma. *J Chromatogr* 226(15): 213–218.

Alfaxalone

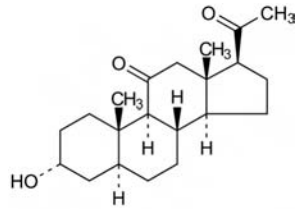
Anaesthetic (General)

$C_{21}H_{32}O_3 = 332.5$

CAS—23930-19-0

A

IUPAC Name (3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Acetyl-3-hydroxy-10,13-dimethyl-1,2,3, 4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-11-one
Synonyms Alphaxalone; (3α,5α)-3-hydroxypregnane-11,20-dione.
Proprietary Names It is an ingredient of *Alfatesin(e)*, *Alfathesin*, *Althesin* and *Saffan* (vet.).

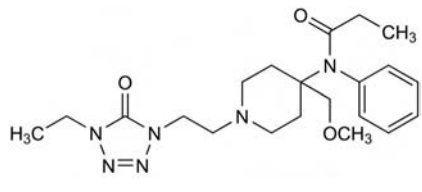


Chemical Properties A white to creamy-white powder. Mp 165° to 171°. Practically insoluble in water; soluble 1 in 10 of ethanol and 1 in 2 of chloroform.
Thin-layer Chromatography System TP—R_f 0.60; system TQ—R_f 0.22; system TR—R_f 0.90; system TS—R_f 0.72, streaking may occur (*p*-toluenesulfonic acid solution, positive).
Ultraviolet Spectrum Methanol—290 nm (A^1_1=about 2).
Infrared Spectrum Principal peaks at wavenumbers 1695, 1148, 1220, 1267, 1000, 1252 cm^{-1} (KBr disk).
Quantification
***Plasma* GC** AFID. Limit of detection, 10 µg/L [Sear *et al.* 1980].
Disposition in the Body Alfaxalone is rapidly and widely distributed following injection. It undergoes almost 100% first-pass metabolism by reduction of the 20-oxo group and conjugation with glucuronic acid, and is excreted in the urine. About 60% of a dose is excreted in the urine in 24 h and 80% in 5 days.
Therapeutic Concentration
During constant IV infusion of 18, 52 and 90 µg/min/kg to 22 subjects, mean steady-state plasma concentrations of 1.9, 2.9 and 3.9 mg/L, respectively, were reported. [Sear, Prys-Roberts 1979].
Half-life Plasma half-life, about 0.5 h.
Volume of Distribution About 0.8 L/kg.
Clearance Plasma clearance, about 20 mL/min/kg.
Protein Binding 20 to 50%.
Note For a review of the pharmacokinetics of IV anaesthetic agents, see Duvaldestin [1981].
Dose A solution containing alfaxalone 9 mg and alfadolone acetate 3 mg/mL has been administered intravenously; doses of 0.05 to 0.075 mL/kg and 10 to 20 mL/h have been given to induce and maintain anaesthesia, respectively.

Duvaldestin P (1981). Pharmacokinetics in intravenous anaesthetic practice. *Clin Pharmacokinet* 6: 61–82.
Sear JW, Prys-Roberts C (1979). Plasma concentrations of alphaxalone during continuous infusion of Althesin. *Br J Anaesth* 51: 861–865.
Sear JW *et al.* (1980). Plasma concentrations of alphaxalone by gas chromatography: comparison with other gas chromatographic methods and gas chromatography-mass spectrometry. *J Pharm Pharmacol* 32: 349–352.

Alfentanil

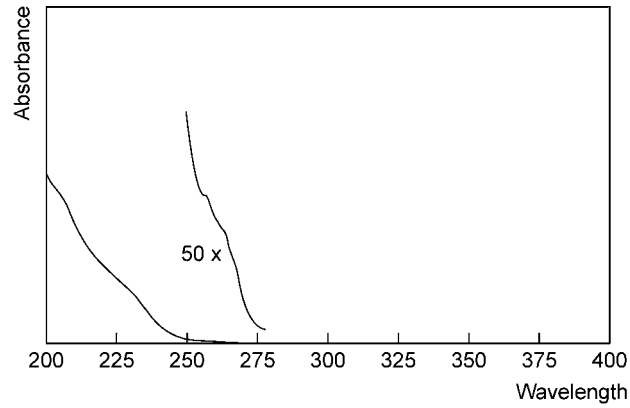
Narcotic Analgesic
$C_{21}H_{32}N_6O_3$ = 416.5
CAS—71195-58-9
IUPAC Name *N*-[1-[2-(4-Ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-{methoxymethyl)-4-piperidinyl]-*N*-phenylpropanamide



Chemical Properties pK_a 6.5. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Alfentanil Hydrochloride

$C_{21}H_{32}N_6O_3$,HCl, H_2O = 471.0
CAS—69049-06-5 (anhydrous); 70879-28-6 (monohydrate)
Proprietary Names *Alfenta*; *R-39209*; *Rapifen*.
Chemical Properties A white powder. Mp 135° to 140°. Readily soluble in water; soluble 1 in 5 of ethanol, 1 in <2 of chloroform, and 1 in <2 of methanol; practically insoluble in ether.
Thin-layer Chromatography System TB—R_f 0.16; system TE—R_f 0.72; system TF—R_f 0.08; system TAE—R_f 0.78.
Gas Chromatography System GA—RI 2970; system GB—RI 3108; system GM—not eluted.
High Performance Liquid Chromatography System HX—RI 378.
Ultraviolet Spectrum Isopropyl alcohol—258 (A^1_1=6.3b), 264 (A^1_1=4.5b), 268 nm (A^1_1=3.1b).



Infrared Spectrum Principal peaks at wavenumbers 1722, 1654, 1252, 1109, 715, 967 cm^{-1} (alfentanil hydrochloride, KBr disk).
Quantification
***Plasma* GC** Column: 3% OV-17 on 80/100 mesh Supelcoport (1 m × 3 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 290°. AFID. Retention time: 2.7 min. Limit of detection, 1 µg/L [Woestenborghs *et al.* 1981].
GC-MS Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 14 kPa. Temperature programme: 40° for 1 min to 120° at 40°/min to 290° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 µg/L [Mautz *et al.* 1994].
HPLC Column: Econosphere CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate (pH 2.8) : acetonitrile (65 : 35), flow rate 1.4 mL/min. IS: papaverine. UV detection (λ= 195 nm). Retention time: IS, 4.8 min; alfentanil, 5.3 min. Limit of quantification 2 µg/L, limit of detection, 0.25 µg/L [Kumar *et al.* 1996].
***Urine* GC-MS** Column: DB5-MS (30 m × 0.25 mm i.d., 0.15 µm). Carrier gas: He, 2.5 mL/min. Temperature programme: 70° to 280° at 60°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 7.5 ng/L [Van Nimmen *et al.* 2004]. Column: 5% cross-linked phenylmethylsiloxane HP-5 (15 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 85° for 0.75 min to 315° at 22.5°/min for 0.75 min. EI ionisation at 70 eV. Limit of detection, 300 ng/L for norsufentanil [Valaer *et al.* 1997].
LC-MS Column: Macherey-Nagel Pyramid C_{18} (70 × 4 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate with 0.1% acetic acid : acetonitrile (100 : 0 for 1 min to 0 : 100 within 7 min for 1 min to 100 : 0 for 2.7 min), flow rate 800 µL/min. CID, MRM acquisition mode. Limit of detection, 0.5 µg/L [Thevis *et al.* 2005].
Disposition in the Body
Therapeutic Concentration
Eight patients undergoing lower abdominal surgery were anaesthetised and 1 mg alfentanil was administered epidurally before induction of the general anaesthesia. The mean peak plasma concentration was 9.7 µg/L, observed between 30 and 120 min [Haak-van der Lely *et al.* 1994].
Following an IV injection of 50 µg/kg to 2 subjects, a mean plasma concentration of 0.54 mg/L was reported at 1 min, decreasing to 0.038 mg/L at 1 h [Michiels *et al.* 1983].
Half-life Plasma half-life, ~1.5 h.
Volume of Distribution ~0.5–1.0 L/kg.
Clearance Plasma clearance, ~3–8 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.6.
Protein Binding Approximately 90% (concentration dependent; decreases at plasma concentrations >0.1 µg/mL).
Note For a review of alfentanil see Mather [1983].
Dose Initially the equivalent of up to 500 µg alfentanil IV, followed by supplementary doses of 250 µg. With assisted ventilation, an initial dose of 30 to 50 µg/kg IV may be given.

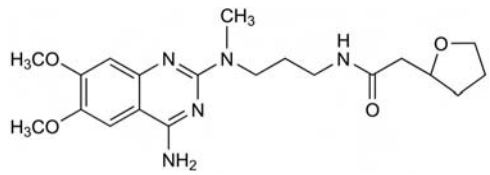
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Haak-van der Lely F *et al.* (1994). Plasma concentrations of alfentanil following epidural administration. *Anaesthesia* 49: 850–852.
Kumar K *et al.* (1996). A sensitive assay for the simultaneous measurement of alfentanil and fentanyl in plasma. *J Pharm Biomed Anal* 14: 667–673.

Mather LE (1983). Clinical pharmacokinetics of fentanyl and its newer derivatives. *Clin Pharmacokinet* 8: 422–446.
Mautz DS *et al.* (1994). Determination of alfentanil and noralfentanil in human plasma by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 658: 149–153.
Michiels M *et al.* (1983). Radioimmunoassay of the new opiate analgesics alfentanil and sufentanil. Preliminary pharmacokinetic profile in man. *J Pharm Pharmacol* 35: 86–93.
Thevis M *et al.* (2005). Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. *Eur J Mass Spectrom* 11: 419–427.
Valaer AK *et al.* (1997). Development of a gas chromatographic-mass spectrometric drug screening method for the *N*-dealkylated metabolites of fentanyl, sufentanil, and alfentanil. *J Chromatogr Sci* 35: 461–466.
VanNimmen NF *et al.* (2004). Highly sensitive gas chromatographic-mass spectrometric screening method for the determination of picogram levels of fentanyl, sufentanil and alfentanil and their major metabolites in urine of opioid exposed workers. *J Chromatogr B Analyt Technol Biomed Life Sci* 804: 375–387.
Woestenborghs R *et al.* (1981). Rapid and sensitive gas chromatographic method for the determination of alfentanil and sufentanil in biological samples. *J Chromatogr* 224: 122–127.

Alfuzosin

α_1-Adrenoceptor Antagonist
$C_{19}H_{27}N_5O_4 = 389.5$
CAS—81403-80-7

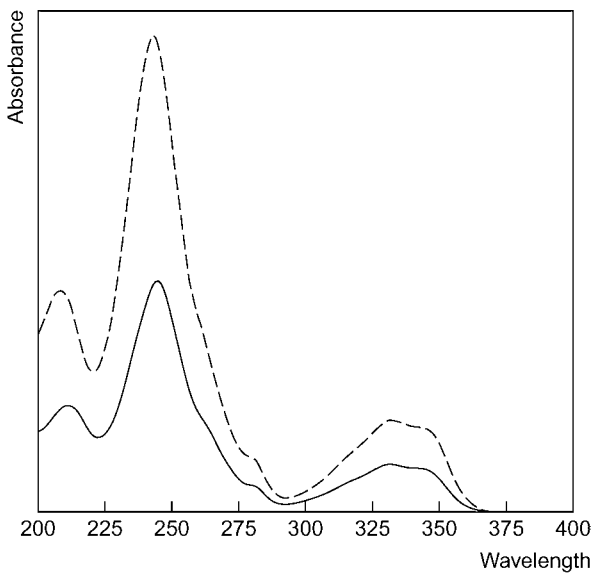
IUPAC Name *N*-[3-[(4-Amino-6, 7-dimethoxyquinazolin-2-yl)-methylamino] propyl]oxolane-2-carboxamide
Synonyms *N*-[(3-[(4-Amino-6,7-dimethoxy-2-quinazolinyl)(methylamino] propyl]tetrahydro-2-furancarboxamide; SL-77.499.



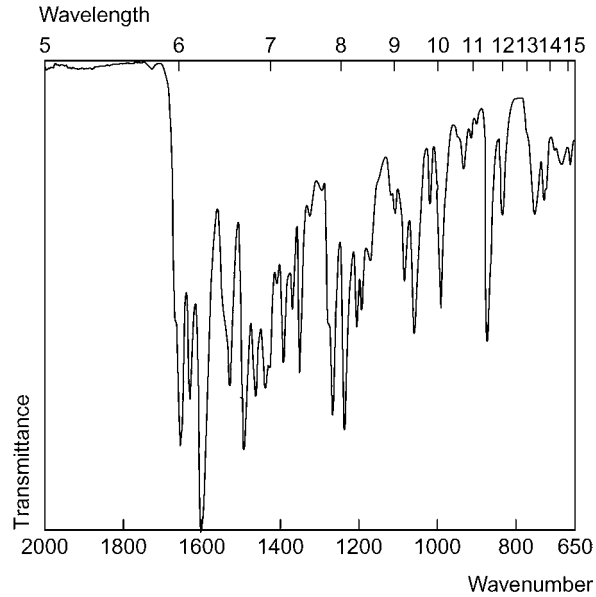
Chemical Properties pK_a 8.1.

Alfuzosin Hydrochloride
$C_{19}H_{27}N_5O_4,HCl = 425.9$
CAS—81403-68-1
Synonym SL-77.499-10
Proprietary Names *Alfoten*; *Benestan*; *Dalfaz*; *Mittoval*; *Urion*; *UroXatral*; *Xatral.*
Chemical Properties A white or almost white slightly hygroscopic crystalline powder. Mp 225°, also reported as 235°. It is freely soluble in water; sparingly soluble in ethanol; practically insoluble in dichloromethane.

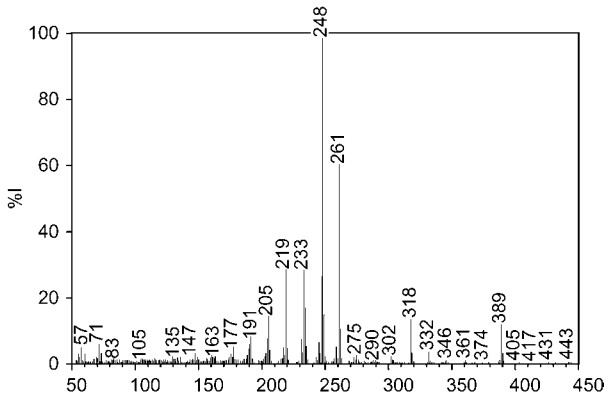
High Performance Liquid Chromatography System HZ—retention time 2.4 min; system HAA—retention time 10.4 min.
Ultraviolet Spectrum Neutral (water)—245, 210, 331 nm (hydrochloride).



Infrared Spectrum Principal peaks at wavenumber 1598, 1492, 1238, 3366, 873 cm^{-1} (hydrochloride).



Mass Spectrum Principal ions at *m/z* 248, 261, 219, 233, 247, 205, 318, 389.



Quantification
Blood **HPLC** Fluorescence detection [Guinebault *et al.* 1986].
Plasma **HPLC** Limit of detection, 1 µg/L for each enantiomer [Rouchouse *et al.* 1990]. See Blood. Limit of detection, 0.5 to 1.0 µg/L [Guinebault *et al.* 1986].
Urine **HPLC** See Blood [Guinebault *et al.* 1986].
Disposition in the Body Alfuzosin is readily absorbed after oral administration. Peak plasma concentrations occur about 0.5 to 3 h after a dose. It is extensively metabolised in the liver to inactive metabolites and excreted primarily in faeces via bile. About 11% of a dose is excreted unchanged in urine.
Therapeutic Concentration
Following oral administration of 1, 2.5, 5 and 10 mg twice daily for 3 days to 6 subjects and 10 mg twice daily to 3 subjects, peak plasma concentrations measured after a dose on the fourth day were 4.1, 11.8, 20.8 and 46.0 µg/L, respectively. These concentrations were observed after 1.8, 1.3, 1.0 and 2.0 h for the doses 1, 2.5, 5 and 10 mg, respectively. All subjects were healthy males, aged between 21 and 32 years with a mean of 23.8 years [Scott *et al.* 1989].
Bioavailability About 64%.
Half-life Plasma, 3 to 5 h.
Protein Binding 90%.
Dose Up to 10 mg daily.

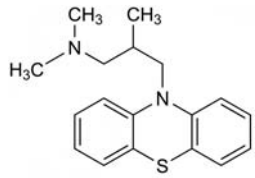
Guinebault P *et al.* (1986). High-performance liquid chromatographic determination of alfuzosin in biological fluids with fluorimetric detection and large-volume injection. *J Chromatogr* 353: 361–369.
Rouchouse A *et al.* (1990). Direct high-performance liquid chromatographic determination of the enantiomers of alfuzosin in plasma on a second-generation alpha 1-acid glycoprotein chiral stationary phase. *J Chromatogr* 506: 601–610.
Scott MG *et al.* (1989). Haemodynamic and pharmacokinetic evaluation of alfuzosin in man. A dose ranging study and comparison with prazosin. *Eur J Clin Pharmacol* 37: 53–58.

Alimemazine

Antihistamine, Phenothiazine, Sedative
$C_{18}H_{22}N_2S = 298.4$
CAS—84-96-8

A

IUPAC Name *N,N*,β-Trimethyl-10*H*-phenothiazine-10-propanamine
Synonym Trimeprazine



Chemical Properties Crystals. Mp 68°. Solubility in water is 0.9415 mg/L at 25°. Log *P* (buffer/hexane), 1.5 to 129.4 (see table). Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Dissolved in mobile phase, the drug was stable for 6 months when stored at –20° and 3–5 months at 60°. In whole blood, the drug was stable for at least 24 h at 4° and for at least 1 h at 25° and at 37° [Hu *et al.* 1986].

pH	*Log P*
4.82	1.5
6.00	4.53
7.00	7.53
8.2	8.58
9.3	16.28
10.54	129.4

[Hu *et al.* 1986].

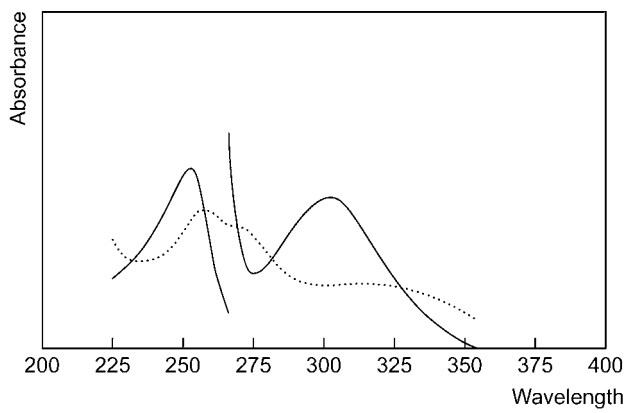
Standard solutions were stable for at least 2 months when stored at 4° [Shinmen *et al.* 2008].

Alimemazine Tartrate

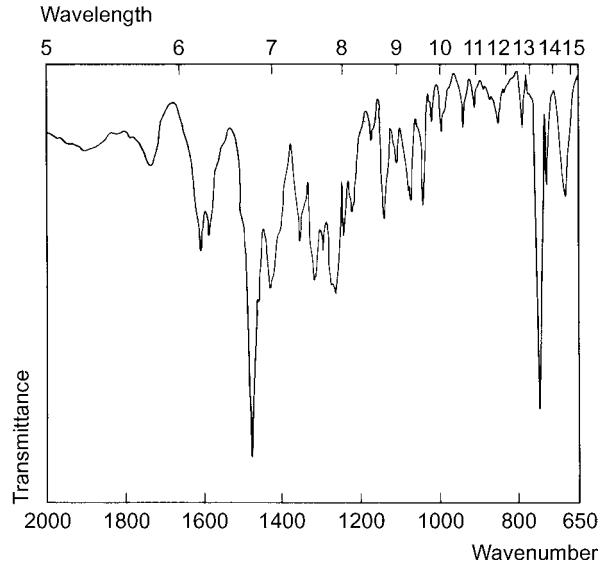
$(C_{18}H_{22}N_2S)_2,C_4H_6O_6 = 747.0$
CAS—4330-99-8
Proprietary Names *Nedeltran*; *Panectyl*; *Repeltin*; *Theralen(e)*; *Vallergan*; *Variargil*.
Chemical Properties White or slightly cream-coloured crystalline powder that darkens in colour on exposure to light. Mp 159° to 163°. Soluble 1 in 4 of water, 1 in 30 of ethanol, and 1 in 5 of chloroform; very slightly soluble in ether. Log *P* (octanol/pH 7.4), 2.9; log *P* (octanol/water), 4.71.

Colour Tests Formaldehyde–sulfuric acid—red-violet; Forrest reagent—brown-red; FPN reagent—brown-red; Mandelin's test—violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.54; system TC—R_f 0.39; system TE—R_f 0.77; system TL—R_f 0.31; system TAE—R_f 0.32; system TAF—R_f 0.46 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, pink; ninhydrin spray, positive).
Gas Chromatography System GA—alimemazine RI 2305; M (OH-) RI 2650; M (OH-)-AC RI 2600; M (bis-nor-)-AC RI 2765; M (nor-) RI 2335; M (nor-OH-)-AC_2 RI 2930; M (ring, phenothiazine) RI 2020; M (sulfoxide) RI 2665; system GB—alimemazine RI 2402; M (nor-) RI 2432; M (sulfoxide) RI 2805; M (OH-) RI 2829; M (norsulfoxide) RI 2817; M (nor-OH-) RI 2845; system GC—RI 2646; system GF—RI 2715; system GW—alimemazine RT 20.3 min.
High Performance Liquid Chromatography System HA—*k* 3.1; system HX—RI 420; system HAX—RT 14.9 min; system HAY—RT 7.1 min.

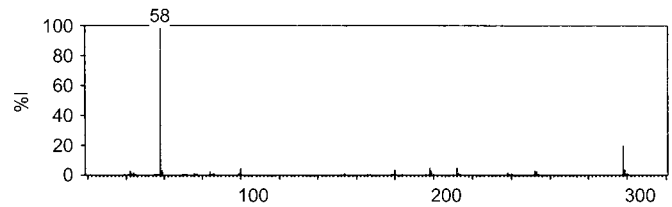
Column: Discovery C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 575 mg ammonium formate with 1.3 mL formic acid in 500 mL water: acetonitrile (60 : 40 for 3 min to 20 : 80 at 11 min to 0 : 100 at 12 min to 60 : 40 at 13 min for 2 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 378$ $\lambda_{em} = 502$ at 0.01 min to $\lambda_{ex} = 344$ nm $\lambda_{em} = 380$ nm at 4.75 min to $\lambda_{ex} = 351$ nm, $\lambda_{em} = 436$ nm at 8.35 min to $\lambda_{ex} = 344$ nm, $\lambda_{em} = 389$ nm at 10 min; $\lambda_{ex} = 344$ nm, $\lambda_{em} = 379$ nm). Limit of detection, 4 nmol/L [Diehl, Karst 2000].
Ultraviolet Spectrum Aqueous acid—251 nm ($A_1^1 = 926$a), 300 nm; aqueous alkali—256, 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 748, 1248, 1260, 1305, 1590, 1275 cm^{-1} (alimemazine tartrate, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 298, 212, 198, 100, 299, 252, 199.



Quantification

Blood **GC** Column: Equity-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 140° for 1 min to 300° at 15°/min. NPD. Limit of detection, 0.6 µg/L [Shinmen *et al.* 2008]. Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 120° to 280° at 6°/min. SID. Retention time: 19.3 min. Limit of detection, 250–500 ng/L [Hattori *et al.* 1992].

HPLC Column: Spherisorb CN (250 × 3.2 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.015 mol/L sodium acetate-acetic acid (pH 6.5, 95 : 5), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 20 µg/L [Kintz *et al.* 1995]. Column: Micropak CN (10 µm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (90 : 10), flow rate 2.0 mL/min. Electrochemical detection. Retention time: 4.4 min. Limit of detection not reported [Hu *et al.* 1986].

Plasma **GC** See Blood. Limit of detection, 0.8 µg/L [Shinmen *et al.* 2008].

HPLC Column: Zorbax CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium acetate buffer in acetonitrile (10 : 90), flow rate 4.0 mL/min. Electrochemical detection. Retention time: 2.74 min. Limit of quantification, 0.25 µg/L, limit of detection, 0.125 µg/L [McKay *et al.* 1982]. Column: Spherisorb (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L aqueous dipotassium hydrogen phosphate (pH 6.5) : methanol : acetonitrile (11 : 4 : 6). Electrochemical detection. Limit of detection, 0.1 µg/L [Holt *et al.* 1983].

HPLC Fluorescence detection. Limit of detection, 0.004 µg/L [Diehl, Karst 2000].

Urine **GC** See Blood [Kintz *et al.* 1995]. See Blood [Hattori *et al.* 1992].

Bile **GC** See Blood [Kintz *et al.* 1995].

Gastric Contents **GC** See Blood [Kintz *et al.* 1995].

Brain **GC** See Blood [Kintz *et al.* 1995].

Hair **LC-MS** Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : formate buffer (pH 3.0, 5 : 95 to 80 : 20 at 10 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 2 pg/mg [Kintz *et al.* 2006].

Heart **GC** See Blood [Kintz *et al.* 1995].

Kidney **GC** See Blood [Kintz *et al.* 1995].

Liver **GC** See Blood [Kintz *et al.* 1995].

Muscle **GC** See Blood [Kintz *et al.* 1995].

Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the body. About 70% of an oral dose is excreted in the urine in 48 h, mostly as sulfoxides and glucuronides.

Therapeutic Concentration

Following single oral doses of 5 mg to 2 subjects, peak plasma concentrations of 0.8 and 1.8 µg/L were reported at 4 h [McKay *et al.* 1982].

Six healthy males, aged 20–40 years, were administered 5 mg alimemazine as a syrup and in tablet form after an overnight fast. The mean peak blood concentrations after the syrup were 2.34 µg/L and 0.95 µg/L with the tablet. These concentrations were observed at 3.5 and 4.5 h, respectively [Hu *et al.* 1986].

Six children aged 1.5–9.5 years were orally administered 3 mg/kg body weight for 30–85 min (median, 65 min). The mean peak blood alimemazine concentration was 0.357 µmol/L (range, 0.27–0.51) observed at 55–105 min [Sponheim *et al.* 1990].

Toxicity

A 50-year-old male was found dead at his home with a suicide note. Toxicological analysis showed that he had an alimemazine concentration of 1.91 mg/L in his blood and 25.05 mg/L in his urine. Noralimemazine was detected in his urine at 13.59 mg/L and alimemazine sulfoxide at 7.84 mg/L. In this case, the blood alimemazine concentration was 1000-times greater than reported therapeutic concentrations [Kintz, Mangin 1995] (last accessed on 18 October 2010, http://www.tiaft.org/tmembers/cnrarchive/25_3_5.php

A 58-year-old male with a history of clinical depression was found dead at his home. Several empty containers of alimemazine were found near his bed. Alimemazine was detected in his femoral blood at a concentration of 6.52 mg/L; in urine at 6.22 mg/L; bile, 4.44 mg/L; gastric contents, 80.19 mg/L; brain, 19.9 µg/g (major site of drug disposition); liver, 18.69 µg/g; kidney, 19.13 µg/g; heart, 16.62 µg/g and in muscle at a concentration of 1.43 µg/g. The postmortem blood concentration was 10,000-times that observed at therapeutic levels [Kintz *et al.* 1995].

Half-life Mean, 6.8 h (children).

Clearance Blood, 3.7 L/kg/h (children).

Blood Distribution Plasma : red blood cell ratio, 1.17.

Dose Usually 10 to 40 mg of alimemazine tartrate daily; up to 100 mg daily has been given. Elderly, 10 to 20 mg daily. Children >2 years' old, 2.5 to 5 mg three or four times daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Diehl G, Karst U (2000). Post-column oxidative derivatization for the liquid chromatographic determination of phenothiazines. *J Chromatogr A* 890: 281–287.

Hattori H *et al.* (1992). Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 579: 247–252.

Holt, JE *et al.*1983 "Sensitive high performance liquid chromatographic assay method for monitoring trimeprazine levels in plasma", pp. 604P-605P.

Hu OY *et al.* (1986). Relative bioavailability of trimeprazine tablets investigated in man using HPLC with electrochemical detection. *J Pharm Pharmacol* 38: 172–176.

Kintz P *et al.* (1995). A fatal case of alimemazine poisoning. *J Anal Toxicol* 19: 591–594.

Kintz P, Mangin P (1995). A Rare Lethal Case of Alimemazine Poisoning. *TIAFT Bull Case Notes* 25 (3).

Kintz P *et al.* (2006). Determination of trimeprazine-facilitated sedation in children by hair analysis. *J Anal Toxicol* 30: 400–402.

McKay G *et al.* (1982). Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses. *J Chromatogr* 233: 417–422.

Shinmen N *et al.* (2008). Simultaneous determination of some phenothiazine derivatives in human blood by headspace solid-phase microextraction and gas chromatography with nitrogen-phosphorus detection. *J AOAC Int* 91: 1354–1362.

Sponheim S *et al.* (1990). Pharmacokinetics of trimeprazine in children. *Pharmacol Toxicol* 67: 243–245.

Allantoin

Urea Derivative, Vulnerary

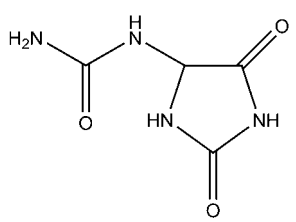
$C_4H_6N_4O_3$ = 158.1

CAS—97-59-6

IUPAC Name (2,5-Dioxo-4-imidazolidinyl)urea

Synonyms Alyoxyldiureide; cordianine; 2,5-dioxoimidazolidin-4-ylurea; glyoxyldiureide; 5-ureidohydantoin; 5-ureidoimidazolidine-2,4-dione.

Proprietary Names *Alantan*; *Masse*. It is also an ingredient in *Actinac*; *Alasulf*; *Alphosyl HC*; *Anbesol Cold-Sore Therapy*; *Anodesyn*; *Atopiclair*; *Blistex*; *Blistex Lip Balm*; *Cicatryl*; *Contractubex*; *Deltavac*; *DIT1-2*; *Dr Dermi-Heal*; *Lonax Astringent*; *Orabase Lip*; *Tanac*; *Tanac Dual Core*; *Vesagex Heelbalm*. For further information see Sweetman [2009].



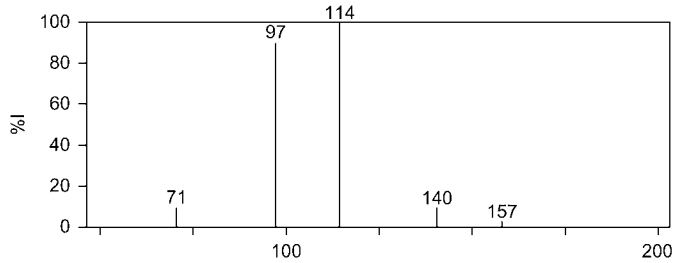
Chemical Properties Monoclinic plates or prisms from water. Mp 238°. Slightly soluble in water (5.26 g/L), very slightly soluble in alcohol (2 g/L), more soluble in hot water and hot ethanol; almost insoluble in ether [O'Neil *et al.* 2006]. Log *P* (octanol/water) −3.14 [Meylan, Howard 1995]. Urine samples spiked with allantoin were stable for at least 24 h, and also after 3 freeze–thaw cycles; post-process samples stored in injection vials at room temperature were stable for up to 72 h [Berthemy *et al.* 1999].

Colour Test Treat a solution with concentrated furfuraldehyde solution to which a little hydrochloric acid is added—violet.

Ultraviolet Spectrum Aqueous alkali (pH 9.4)—224 nm.

Infrared Spectrum Principal peaks at wavenumbers 1710, 1652, 1530 cm^{-1} (KBr disc).

Mass Spectrum Principal ions at *m/z* 114, 97, 71, 140, 157.



Quantification

Plasma **HPLC** Column: C_{18} (150 × 4.0 mm i.d., 5 µm). Mobile phase: 5% acetonitrile in 8.3 mmol/L phosphate buffer (pH 6.1): 50% acetonitrile in 8.3 mmol/L phosphate buffer (pH 6.1; 95 : 5 to 40 : 60 over 20 min to 0 : 100 over 2 min for 13 min), flow rate 0.5 mL/min. UV detection (λ = 360 nm). Retention times: *syn*-isomer 14.8 min, *anti*-isomer 20.8 min (glyoxylate-2,4-dinitrophenylhydrazine derivatives). Limit of quantification not reported [Kand'ar *et al.* 2006]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium phosphate (pH 5.5) : methanol (100 : 0 to 90 : 10 over 10 min to 80 : 20 over 10 min), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 3.3 min. Limit of quantification, 0.2 µmol/L [Terzuoli *et al.* 1999]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate with 5 mmol/L 1-heptanesulfonic acid (pH 3.1), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Retention time: 3.0 min. Limit of detection, 5 µmol/L [Benzie *et al.* 1999].

CE Column: uncoated silica capillary (total/effective length: 57/50 cm, 75 µm i.d.). Buffer: 20 mmol/L borate buffer (pH 10.1). UV detection (λ = 214 nm). Migration time: 13.7 min. Limit of quantification not reported [Terzuoli *et al.* 1999].

Serum **GC-MS** Column: HP-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° to 270° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.111 µmol/L for methyl-(tert-butyldimethylsilyl)trifluoroacetamide derivative [Pavitt *et al.* 2002].

Urine **HPLC** Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 4.7), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 4.4 min. Limit of quantification not reported [George *et al.* 2006]. Column: C_2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate with 0.005 mol/L sodium 1-pentane sulfonate monohydrate, flow rate 1.0 mL/min. UV detection (λ = 225 and 286 nm). Retention time: 3.4 min. Limit of quantification, 34.5 µg/L, limit of detection, 10.4 µg/L [Carlson, Thompson 2001].

LC-MS Column: DVB polyamine (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (0 : 100 to 8 : 92 over 5 min to 10 : 90 over 2 min for 8 min), flow rate 1.5 mL/min for 5 min to 1.0 mL/min over 0.1 min for 10 min. ESI, negative ion mode, SRM acquisition mode. Retention time: ≈10 min. Limit of quantification, 14.6 mg/L [Berthemy *et al.* 1999].

CE Column: fused silica capillary (total/effective length: 44/37 cm, 75 µm i.d.). Buffer: 30 mmol/L sodium tetraborate (pH 10) for CZE, with the addition of 75 mmol/L sodium dodecyl sulfate for MECC. UV detection (λ = 195 nm). Migration time: 5.4 min. Limit of detection, 5 µmol/L [Alfazema *et al.* 1998].

Note For a TLC determination of allantoin in urine, see Thrasher and Hansen [1980].

Other **HPLC** Microdialysate Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ortophosphoric acid (pH 2.4) with 1.5 mmol/L tetrabutylammonium hydrogen sulfate, flow rate 0.8 mL/min. UV detection (λ = 205 nm). Limit of quantification, 0.25 mmol/L [Marklund *et al.* 2000].

LC-MS Herbal Tablets (Liuwei Dihuang). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : formic acid in water (pH 3.3; 2 : 0 : 98 for 3 min to 0 : 2 : 98 over 1 min to 0 : 6 : 94 over 8 min to 0 : 20 : 80 over 14 min for 4 min to 0 : 30 : 70 over 10 min), flow rate 1.0 mL/min. ESI, positive ion mode. Retention time: 6 min. Limit of quantification not reported [Zhao *et al.* 2007]. Carotid Artery Plaque. Column: Supelcosil LC-ABZ (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate solution (pH 4.8) : methanol (100 : 0 for 10 min to 25 : 75 over 20 min to 0 : 100 over 20 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.8 min. Limit of quantification not reported [Catinella *et al.* 2001].

CE Carotid Artery Plaque. Column: uncoated silica capillary (total/effective length: 57/50 cm, 75 µm i.d.). Buffer: 20 mmol/L borate buffer (pH 10.1). UV detection (λ = 214 nm). Migration time: 13.7 min. Limit of quantification not reported [Terzuoli *et al.* 1999].

Dose It is present in multi-ingredient preparations intended for various skin disorders. It is also used for its astringent properties in preparations for the treatment of haemorrhoids and other anorectal disorders.

Alfazema LN *et al.* (1998). Determination of allantoin in biofluids using micellar electrokinetic capillary chromatography. *J Chromatogr A* 817: 345–352.

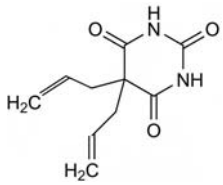
Benzie IF *et al.* (1999). Simultaneous measurement of allantoin and urate in plasma: analytical evaluation and potential clinical application in oxidant:antioxidant balance studies. *Clin Chem* 45: 901–904.

Berthemy A *et al.* (1999). Quantitative determination of an extremely polar compound allantoin in human urine by LC-MS/MS based on the separation on a polymeric amino column. *J Pharm Biomed Anal* 19: 429–434.
Carlson M, Thompson RD (2001). Identification of allantoin, uric acid, and indoxyl sulfate as biochemical indicators of filth in food packaging by LC. *J AOAC Int* 84: 782–788.
Catinella S *et al.* (2001). Determination of purine compounds in carotid artery plaque by liquid chromatography electrospray ionization tandem mass spectrometry. *J Mass Spectrom* 36: 441–442.
George SK *et al.* (2006). Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 832: 134–137.
Kand'ar R *et al.* (2006). Monitoring of antioxidant properties of uric acid in humans for a consideration measuring of levels of allantoin in plasma by liquid chromatography. *Clin Chim Acta* 365: 249–256.
Marklund N *et al.* (2000). Hypoxanthine, uric acid and allantoin as indicators of in vivo free radical reactions, Description of a HPLC method and human brain microdialysis data. *Acta Neurochir (Wien)* 142: 1135–1141.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
O'Neil MJ *et al.* (2006). *The Merck Index: An encyclopedia of chemicals, drugs and biologicals,* 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.
Pavitt DV *et al.* (2002). Assay of serum allantoin in humans by gas chromatography-mass spectrometry. *Clin Chim Acta* 318: 63–70.
Sweetman SC, ed. (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.
Terzuoli L *et al.* (1999). Comparative determination of purine compounds in carotid plaque by capillary zone electrophoresis and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 728: 185–192.
Thrasher JJ, Hansen MA (1980). Extraction and thin layer chromatographic confirmation of urine residues: new plate development. *J Assoc Off Anal Chem* 63: 189–193.
Zhao X *et al.* (2007). Quantitative and qualitative determination of Liuwei Dihuang tablets by HPLC-UV-MS-MS. *J Chromatogr Sci* 45: 549–552.

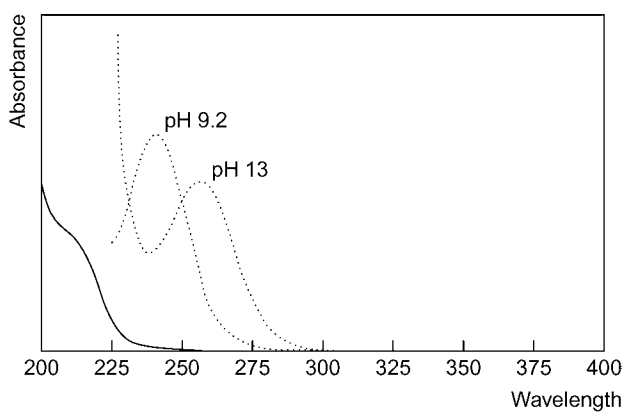
Allobarbital

Sedative, Barbiturate
$C_{10}H_{12}N_2O_3 = 208.2$
CAS—52-43-7

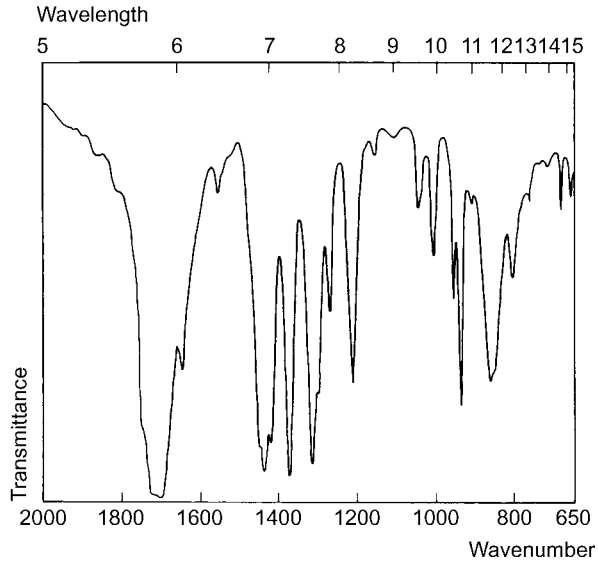
IUPAC Name 5,5-Bis(prop-2-enyl)-1,3-diazinane-2,4,6-trione
Synonyms Allobarbitone; diallylbarbitone; diallylmalonylurea; diallymalum; 5,5-di-2-propenyl-2,4,6(1*H*,3*H*,5*H*)pyrimidinetrione.
Proprietary Name It is an ingredient of *Dialog*.



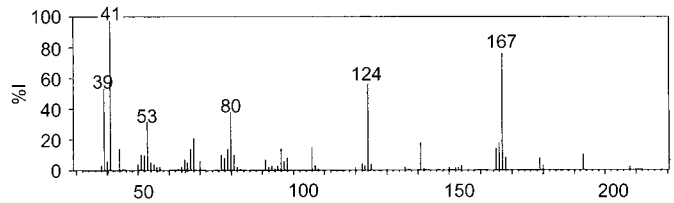
Chemical Properties A white crystalline powder. Mp about 173°. Soluble 1 in 700 of water, 1 in 15 of ethanol and 1 in 20 of ether; soluble in solutions of alkalis. pK_a 7.8 (25°).
Colour Tests Koppanyi-Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown-orange/violet (transient).
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.34; system TF—R_f 0.66; system TH—R_f 0.53; system TAD—R_f 0.56; system TAE—R_f 0.87 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown).
Gas Chromatography System GA—allobarbital RI 1600, allobarbital-Me_2 RI 1636; system GB—allobarbital-Me_2 RI 1505; system GF—allobarbital RI 2340.
High Performance Liquid Chromatography System HG—*k* 2.46; system HH—*k* 1.33; system HX—RI 346; system HZ—retention time 2.7 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—241 (A_1^1=460a); 1 mol/L sodium hydroxide (pH 13)—256 nm (A_1^1=356b).



Infrared Spectrum Principal peaks at wavenumbers 1687, 1315, 925, 1219, 847, 1640 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 41, 167, 124, 39, 80, 53, 68, 141.



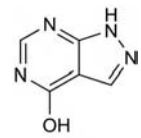
Quantification
Blood **HPLC** Column: (1) C_{18} RP TSK gel Super-ODS (100 × 4.6 mm i.d., 2 µm); (2) C_{18} RP Hypersil ODS-C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 8 mmol/L KH_2PO_4 (30:70), flow rate 0.4 mL/min. Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin. UV detection (λ=215 nm). Retention time: (1) 5.1 min; (2) 5.7 min [Tanaka *et al.* 1997]. Limit of quantification, 50 µg/L [Tanaka *et al.* 1997].
Urine **HPLC** See Blood [Tanaka *et al.* 1997].
Brain **HPLC** See Blood [Tanaka *et al.* 1997].
Liver **HPLC** See Blood [Tanaka *et al.* 1997].
See under Amobarbital.
Disposition in the Body Allobarbital is absorbed after oral administration. It may persist unchanged in the body for up to one week; about 10 to 35% of a dose is slowly excreted unchanged in the urine.
Therapeutic Concentration In plasma, usually in the range 15 to 40 mg/L.
Toxicity The estimated minimum lethal dose is 2 g. Plasma concentrations of 50 mg/L or more are usually toxic.
Dose Allobarbital has been given in doses of 90 to 200 mg daily.

Tanaka E *et al.* (1997). Forensic analysis of 10 barbiturates in human biological samples using a new reversed-phase chromatographic column packed with 2-micrometre porous microspherical silica-gel. *Forens Sci Int* 85: 73–82.

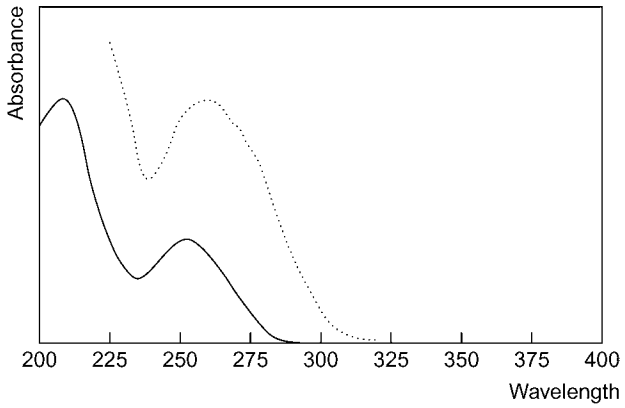
Allopurinol

Xanthine Oxidase Inhibitor
$C_5H_4N_4O = 136.1$
CAS—315-30-0

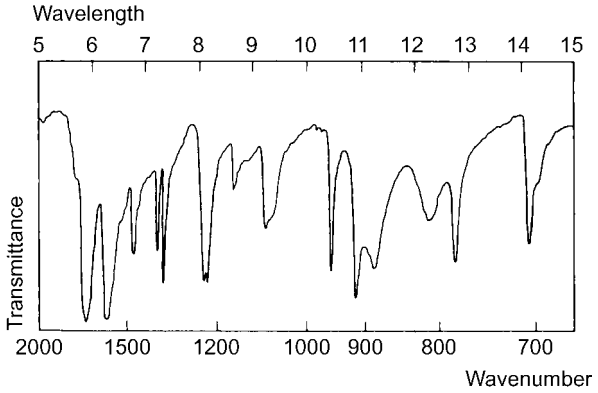
IUPAC Name 1,2-Dihydropyrazolo[3,4-d]pyrimidin-4-one
Synonyms 1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one; HPP; isopurinol; BW-56158.
Proprietary Names *Adenock*; *Alloprin*; *Allopur*; *Aloral*; *Alositol*; *Aluline*; *Anoprolin*; *Blemimol*; *Bloxanth*; *Caplenal*; *Cosuric*; *Dabroson*; *Embarin*; *Epidropal*; *Foligan*; *Gichtex*; *Hexanurat*; *Ledopor*; *Lysuron*; *Miniplanor*; *Monarch*; *Progout*; *Remin*; *Sigapural*; *Takanarumin*; *Uriscel*; *Urobenyl*; *Urtias*; *Xanturat*; *Zyloprim*; *Zyloric*.



Chemical Properties A white microcrystalline powder. No characteristic melting point; it is unchanged up to 300°, after which it begins to darken and at an indefinite high temperature it chars and decomposes. Very slightly soluble in water and ethanol; practically insoluble in chloroform and ether; soluble in dimethylformamide and in dilute solutions of alkali hydroxides. pK_a 9.4.
Colour Test Folin-Ciocalteu reagent—grey-blue.
Thin-layer Chromatography System TE—R_f 0.21; system TAE—R_f 0.75.
Gas Chromatography System GA—RI 882.
High Performance Liquid Chromatography System HY—RI 128; system HZ—retention time 1.55 min.
Ultraviolet Spectrum Aqueous acid (prepared by dissolving in alkali and diluting with acid)—250 (A^1_1=563a); aqueous alkali—257 nm (A^1_1=523a).



Infrared Spectrum Principal peaks at wavenumbers 1692, 1587, 916, 1224, 1235, 956 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 136, 135, 52, 28, 137, 109, 29, 18.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 0.1 mg/L [de Vries *et al.* 1993]. UV detection. Limit of detection, 100 µg/L for allopurinol and oxipurinol [Breithaupt, Goebel 1981].
Serum **GC-MS** Limit of detection, 25 µg/L [Lartigue-Mattei *et al.* 1982].
Urine **GC-MS** See Serum [Lartigue-Mattei *et al.* 1982].
HPLC See Plasma. Limit of detection, 0.2 mg/L [de Vries *et al.* 1993]. See Plasma [Breithaupt, Goebel 1981].
Disposition in the Body Allopurinol is rapidly absorbed after oral administration. The major metabolite, oxipurinol (alloxanthine), is active but less potent than allopurinol. Subjects with a genetic deficiency of xanthine oxidase are unable to metabolise allopurinol to oxipurinol. The excretion of unchanged drug appears to vary with acute or chronic administration, <10% of a single dose being excreted unchanged in the urine whereas about 30% is excreted in the urine as unchanged drug after chronic administration. Most of the remainder of a dose is slowly excreted in the urine as oxipurinol; about 20% of a dose is eliminated in the faeces in 72 h.
Therapeutic Concentration Oxipurinol accumulates during chronic administration and may contribute significantly to the therapeutic effect.

Ten elderly patients with bronchitis, angina and degenerative bone/joint disease (mean age 76.5 years; range 71 to 93 years) and 9 young volunteers (30.3; 24 to 35 years) were administered 200 mg allopurinol orally and 200 mg intravenously after a standard breakfast. The peak allopurinol concentrations were 1.24 mg/L at 0.8 h and 0.64 mg/L at 1.2 h for the young and elderly subjects, respectively. The peak oxipurinol concentrations were 3.75 mg/L at 4.2 h and 5.63 mg/L at 4.5 h, respectively [de Vries *et al.* 1993].

Eighteen healthy volunteers, aged 22 to 27 years, were administered a single dose of 2 different tablet formulations of allopurinol with an interval of 2 weeks. The mean peak concentrations were 1.91 and 1.93 mg/L for allopurinol at 1.17 and 1.53 h, respectively, and for the metabolite, oxipurinol, 4.59 and 4.78 mg/L at 5.22 and 4.94 h, respectively [Barthel *et al.* 1999].

Following a single oral dose of 300 mg given to 6 subjects, peak plasma concentrations of 1.4 to 2.6 mg/L (mean 2) of allopurinol and 4.4 to 7.8 mg/L (mean 6.3) of oxipurinol were attained in about 0.5 to 2 h and 2 to 5 h, respectively [Breithaupt, Tittel 1982].

Following daily oral administration of 300 mg to 7 subjects, steady-state serum concentrations of oxipurinol, determined immediately prior to a dose, were reported to range from 3.4 to 19.4 mg/L (mean 9.7) [Rodnan *et al.* 1975].

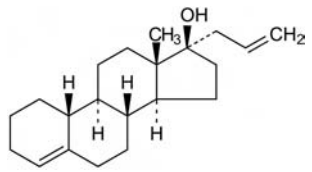
Bioavailability About 90%.
Half-life Plasma half-life, allopurinol about 0.5 to 2 h, oxipurinol 12 to 40 h.
Volume of Distribution About 0.6 L/kg.
Clearance Plasma clearance, about 11 mL/min/kg.
Protein Binding Allopurinol < 5%, oxipurinol about 17%.
Dose 100 to 600 mg daily.

Barthel W *et al.* (1999). Bioequivalence of allopurinol-containing tablet preparations. *Int J Clin Pharmacol Ther* 37(3): 148–152.
Breithaupt B, Tittel M (1982). Kinetics of allopurinol after single intravenous and oral doses. Noninteraction with benzbromarone and hydrochlorothiazide. *Eur J Clin Pharmacol* 22: 77–84.
Breithaupt H, Goebel G (1981). Determination of allopurinol and oxipurinol in biological fluids by high-performance liquid chromatography. *J Chromatogr* 226: 237–242.
de Vries JX *et al.* (1993). Simultaneous determination of allopurinol and oxipurinol in human plasma and urine by high-performance liquid chromatography. *Arzneimittelforschung* 43(1): 1072–1075.
Lartigue-Mattei C *et al.* (1982). Simultaneous determination of allopurinol and oxipurinol in biological fluids by mass fragmentography. *J Chromatogr* 229: 211–216.
Rodnan GP *et al.* (1975). Allopurinol and gouty hyperuricemia. Efficacy of a single daily dose. *J Am Med Assoc* 231(11): 1143–1147.

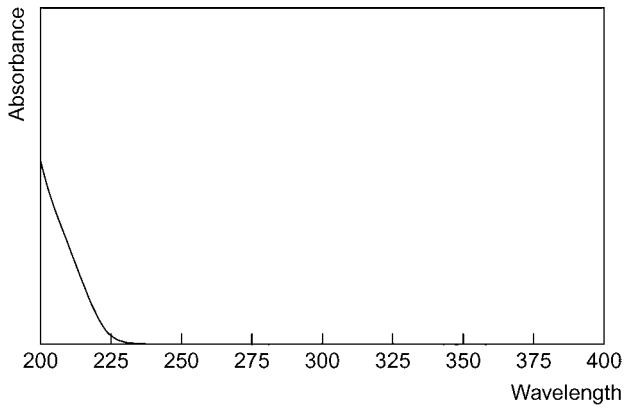
Allylestrenol

Progestogen
$C_{21}H_{32}O$ = 300.5
CAS—432-60-0

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-13-Methyl-17-prop-2-enyl-2,3,6,7,8,9,10,11,12,14,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-17-ol
Synonym Allyloestrenol; (17β)-17-(2-propenyl)-estr-4-en-17-ol.
Proprietary Names *Gestanin*; *Gestanon*; *Gestanyn*; *Orageston*; *Turinal*.



Chemical Properties Crystals. Mp 80°. Practically insoluble in water; soluble in ethanol, chloroform and ether.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

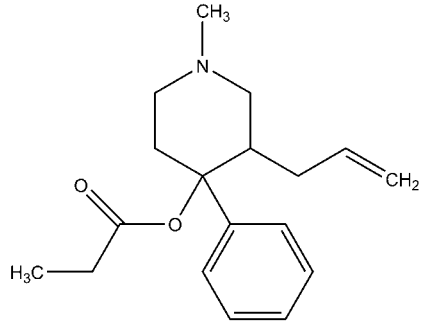


Infrared Spectrum Principal peaks at wavenumbers 905, 1008, 999, 1020, 1101, 985 cm^{-1} (Nujol mull).
Dose Allylestrenol has been given in doses of 5 to 20 mg daily.

Allylprodine

Narcotic Analgesic
$C_{18}H_{25}NO_2$ = 287.4
CAS—25384-17-2

IUPAC Name 1-Methyl-4-phenyl-3-(2-propenyl)-4-piperidinol propanoate
Synonyms NIH-7440; Ro-2-7113.



Chemical Properties Very slightly soluble in water (430 mg/L). Log *P* (octanol/water) 2.97 [Biobyte].

Allylprodine Hydrochloride

$C_{18}H_{25}NO_2$,HCl = 323.9

Chemical Properties Crystals from acetone + methanol. Mp 186° to 187° [O'Neil *et al.* 2006].

Colour Tests Ammonium molybdate test—grey→green; ammonium vanadate test—grey-olive; sulfuric acid-formaldehyde test—blue-black (limit of detection 0.1 µg); Vitali's test—grey-purple→brown.

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—252, 258, 264 nm.

Biobyte, Biobyte Inc. *Allylprodine.* www.biobyte.com/blqsar/index.html. (accessed 5 February 2008).
O'Neil MJ *et al.* (2006). *The Merck Index: An encyclopedia of chemicals, drugs and biologicals,* 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Alminoprofen

Antiinflammatory, Antirheumatic, COX-2 Inhibitor, NSAID, Phenylpropionic Acid

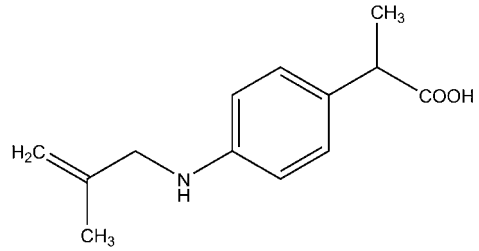
$C_{13}H_{17}NO_2$ = 219.3

CAS—39718-89-3

IUPAC Name 2-[4-(2-Methylprop-2-enylamino)phenyl]propanoic acid

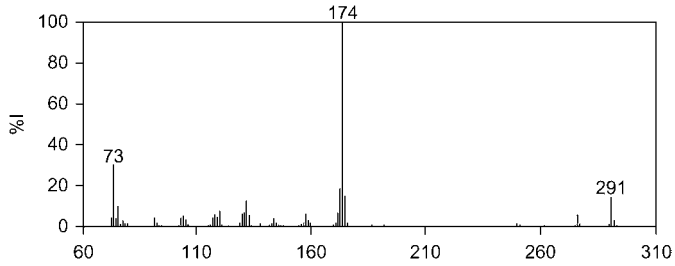
Synonyms EB-382; 4-[(2-methylallyl)amino]hydratropic acid; α-methyl-4-[(2-methyl-2-propenyl)amino]benzeneacetic acid.

Proprietary Name *Minalfene*



Chemical Properties Mp 107°. Soluble in water (307 mg/L). Log *P* (octanol/water), 2.87 [Meylan, Howard 1995].

Mass Spectrum Principal ions at *m/z* 174, 73, 291(TMS TFA derivative) [Premel-Cabic *et al.* 1980].



Quantification

Plasma **GC** Column: 3% OV1 on Gaschrom Q 80-100 mesh (2 m × 3.0 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 200°. FID and EI ionisation at 70 eV. Retention time: ~5 min. Limit of detection, 1 mg/L [Premel-Cabic *et al.* 1980].

HPLC Column: µBondapak C_{18} (300 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : water (50 : 50) with 1% glacial acetic acid, flow rate 0.85 mL/min. UV detection (λ = 235 nm). Retention time: approx. 8 min. Limit of quantification, 4 mg/L, limit of detection, 1 mg/L [Paillet *et al.* 1985].

Urine **HPLC** Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : acetic acid : dimethyl sulfoxide (50 : 46 : 0.8 : 3), flow rate 1 mL/min. UV detection (λ = 251 nm). Retention time: 8.1 min. Limit of quantification, 2 mg/L [Tod *et al.* 1995].

GC Column: 3% OV1 Gas-chrom Q 80-100 mesh (2 m × 3.0 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 200°. FID. Retention time: ~5 min. Limit of detection, 0.5 mg/L [Premel-Cabic *et al.* 1980].

Synovial Fluid **HPLC** Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : acetic acid : dimethyl sulfoxide (50 : 46 : 0.8 : 3), flow rate 1 mL/min. UV detection (λ = 251 nm). Retention time: 8.1 min. Limit of quantification, 2 mg/L [Tod *et al.* 1995].

Disposition in the Body Readily absorbed following ingestion. Eliminated mainly by the renal route, conjugated as glucuronide.

Therapeutic Concentration

Four patients were administered 300 mg alminoprofen orally ≈0.5 h before breakfast. Peak plasma concentrations were 36.2 to 41.5 mg/L, reached between 0.5 and 1.5 h. In another study, 7 patients were administered 300 mg alminoprofen orally 3 times daily during meals for a period of 5 days. On day 5, the mean morning plasma alminoprofen concentration was 3.1 mg/L and in the evening it was 28.5 mg/L [Premel-Cabic *et al.* 1980].

A group of healthy subjects was administered a single 300 mg oral dose of alminoprofen at either 8 am (group A) or 6 pm (group B). In Group A, a mean peak plasma concentration of 34.4 g/L was reached after 1.5 h. Bioavailability seemed slightly decreased when the drug was given in the evening. The pharmacokinetics were not significantly different in group B, although bioavailability was slightly decreased [Paillet *et al.* 1985].

A group of 45 patients with knee effusion was administered 300 mg alminoprofen orally 3 times daily for 4 days. Plasma and synovial fluid concentrations were measured over 12 h post-ingestion of the final dose and were reported as:

Time (h)	*Plasma (mg/L)*	*Synovial fluid (mg/L)*
1	24.2	4.7
2	19.0	9.3
4	15.8	7.2
6	8.6	5.6
8	4.0	4.7
12	2.1	2.3

[Tod *et al.* 1995].

Half-life Approximately 3 h.

Volume of Distribution Apparent, 7.8 L.

Clearance Approximately 43 mL/min.

Dose Up to 900 mg daily by mouth in inflammatory and rheumatic disorders.

Meylan WM, Howard P (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Paillet M *et al.* (1985). Rapid determination of alminoprofen in plasma by high-performance liquid chromatography. *J Chromatogr* 343: 455–459.
Premel-Cabic A *et al.* (1980). Pharmacokinetics of 2-(*p*-methylallylaminophenyl) propionic acid, alminoprofene, in man after single and multiple oral doses. *Eur J Clin Pharmacol* 18: 419–422.
Tod M *et al.* (1995). A population pharmacokinetic study of alminoprofen penetration into synovial fluid. *Biopharm Drug Dispos* 16: 627–634.

Almotriptan

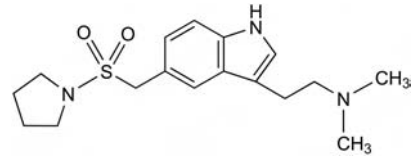
Antimigraine

$C_{17}H_{25}N_3O_2S$ = 335.5

CAS—154323-57-6

IUPAC Name *N*, *N*-Dimethyl-2-[5-(pyrrolidin-1-ylsulfonylmethyl)-1H-indol-3-yl]ethanamine

Synonyms 1-[[[3-[2-(Dimethylamino)ethyl-1H-indol-5-yl]methyl]sulfonyl] pyrrolidine; LAS-31416.



Almotriptan Maleate

$C_{19}H_{25}N_3O_2S,C_4H_6O_5$ = 469.6

CAS—181183-52-8

Synonym PNU-180638E

Proprietary Names *Almogran*; *Axert*.

Quantification

Plasma **LC-MS** Limit of quantification, 0.5 µg/L [Fleishaker *et al.* 2001a,b].

Urine **LC-MS** Limit of quantification, 50 µg/L [Fleishaker *et al.* 2001a,b].

Disposition in the Body Almotriptan is well absorbed after oral administration and metabolised in the liver by monoamine oxidase and cytochrome P450 isoenzymes (CYP3A4 and CYP2D6). Metabolites are inactive. It is eliminated by a number of pathways and 40 to 50% of a dose is recovered unchanged in urine.

Therapeutic Concentration

Twelve healthy male and female volunteers aged 18 to 51 years (mean, 31 years) were treated with 12.5 mg almotriptan with 1 week wash-out periods in-between. The peak plasma concentration was 39.6 µg/L at 2.67 h [Fleishaker *et al.* 2000].

Eleven healthy women and 3 men, aged 18 to 55 years (mean, 35.3 years), were administered with a single 12.5 mg dose of almotriptan after a 10 h fast. The same dose was also administered on day 8 of treatment after 20 mg 3 times daily of fluoxetine for days 1 to 8. The peak plasma concentration of almotriptan was 44.3 µg/L for the single dose and 52.5 µg/L for the dose administered with fluoxetine. The time to reach these concentrations was 2.46 and 1.86 h, respectively [Fleishaker *et al.* 2001b].

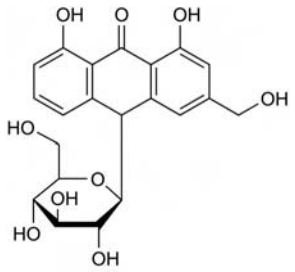
Bioavailability 69 to 80%.
Half-life 3.1 to 3.6 h.
Volume of Distribution 249 L.
Clearance 26.6 L/h.
Dose 12.5 mg repeated after 2 h if migraine recurs with a maximum of 25 mg in 24 h.

Fleishaker JC *et al.* (2000). Pharmacokinetic interaction between verapamil and almotriptan in healthy volunteers. *Clin Pharmacol Ther* 67(5): 498–503.
Fleishaker JC *et al.* (2001a). Effect of MAO-A inhibition on the pharmacokinetics of almotriptan, an antimigraine agent in humans. *Br J Clin Pharmacol* 51: 437–441.
Fleishaker JC *et al.* (2001b). Evaluation of the potential pharmacokinetic interaction between almotriptan and fluoxetine in healthy volunteers. *J Clin Pharmacol* 41(2): 217–223.

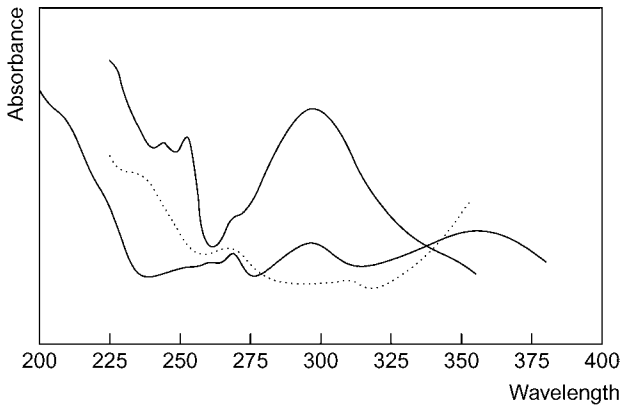
Aloin

Purgative
$C_{21}H_{22}O_9$ = 418.4
CAS—8015-61-0 (aloin); 1415-73-2 (barbaloin)

IUPAC Name (10R)-1,8-Dihydroxy-3-(hydroxymethyl)-10-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-10*H*-anthracen-9-one
Synonyms Barbaloin; 10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9 (10*H*)-anthracenone.



Chemical Properties A crystalline substance extracted from aloes and consisting almost entirely of barbaloin. A pale or dull yellow crystalline powder. It darkens on exposure to light. Mp 148° to 80°. Almost completely soluble 1 in 130 of water; soluble 1 in 20 of ethanol; soluble in acetone; very slightly soluble in chloroform and ether.
Colour Tests Ferric chloride—brown; Folin-Ciocalteu reagent—blue; Liebermann's reagent—black; Millon's reagent—pink→red.
Ultraviolet Spectrum Aqueous acid—245, 253, 300 nm; aqueous alkali—268 nm.



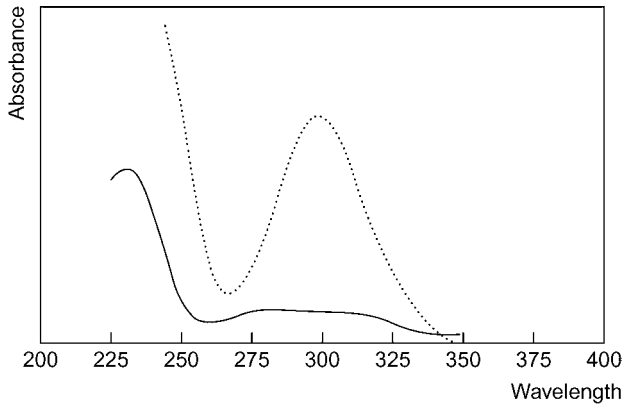
Thin-layer Chromatography Plate: silica gel (Merck). Mobile phase: methylisobutylketone : m-xylene : hexane : glacial acetic acid (60:20:12:0.5). Detection: sprayed with 3 mol/L potassium hydroxide and examined at 254 nm (yellow/red) and 366 nm (orange/red). R_f 0.75 [Perkins, Livesey 1993].
Dose Aloin has been given in doses of 13 to 60 mg.

Perkins SL, Livesey JF (1993). A rapid high-performance thin-layer chromatographic urine screen for laxative abuse. *Clin Biochem* 26(3): 179–181.

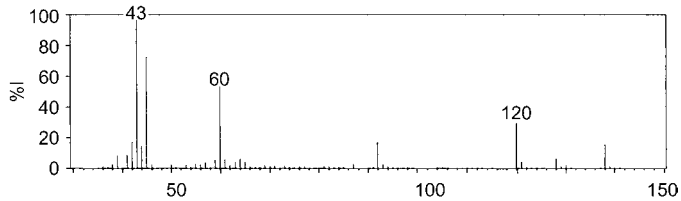
Aloxiprin

Analgesic
CAS—9014-67-9

Note A polymeric condensation product of aluminium oxide and aspirin which contains about 83% of total salicylates. Approx. formula: $Al_3O_2[C_6H_4(OOCCH_3)COO]_5$
Proprietary Names *Lyman*; *Palaprin Forte*; *Rumatral*.
Chemical Properties A fine white or slightly pink powder. Practically insoluble in water, ethanol and ether; soluble 1 in 200 of chloroform.
Thin-layer Chromatography System TD—R_f 0.04; system TE—R_f 0.09; system TF—R_f 0.10; system TAD—R_f 0.22.
Ultraviolet Spectrum Aqueous acid—231 nm; aqueous alkali—298 nm.



Infrared Spectrum Principal peaks at wavenumbers 1597, 1198, 1562, 1220, 1745, 760 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 45, 60, 120, 42, 92, 138, 44.

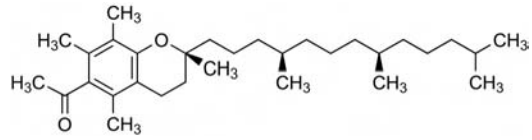


Disposition in the Body Aloxiprin is hydrolysed to aspirin in the gastrointestinal tract.
Dose Up to 100 mg/kg daily, orally.

Alpha Tocoferil Acetate

Vitamin E Activity
$C_{31}H_{52}O_3$ = 472.8
CAS—52225-20-4

IUPAC Name [(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3, 4-dihydrochromen-6-yl] acetate
Synonyms α-Tocopherol acetate; (±)-α-tocopheryl acetate; *dl*-α-tocopherol acetate; α-tocoferil acetate; vitamin E acetate.
Proprietary Names *Aquasol E*; *Ephynal*; *Evion*; *Tocomine*; *Tokols (d=+)*; *Vita-E (d=+)*; *Detulin*; *Eusovit*.



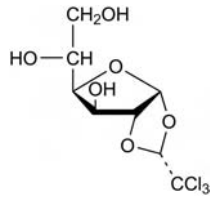
Chemical Properties A clear, slightly greenish-yellow, viscous, oily liquid. Relative density 0.952 to 0.966. Practically insoluble in water; soluble in ethanol; freely soluble in dehydrated alcohol, chloroform, and ether.
Ultraviolet Spectrum Methanol—284 nm (A_1^1=43a).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1755, 1075, 1105, 1160, 1250 cm^{-1} (thin film).

A

Alphachloralose

Rodenticide
$C_8H_{11}Cl_3O_6$ = 309.5
CAS—15879-93-3

IUPAC Name (1*R*)-1-[(2*R*,3a*R*,5*R*,6*S*,6a*R*)-6-Hydroxy-2-(trichloromethyl)-3a,5,6, 6a-tetrahydrofuro[2,3-d][1,3]dioxol-5-yl]ethane-1,2-diol
Synonyms α-Chloralosane; chloralose; glucochloral; glucochloralose; (*R*)-1,2-*O*-(2,2,2-trichloroethylidene)-α-D-glucofuranose.
Proprietary Names *Alfamat*; *Alphabird*; *Alphakil*; *Alphamouse*; *Aphosal*; *Dulcidor*; *Murex*; *Somi*.



Chemical Properties Mp 182° to 184°. It is soluble in water 4.44 g/L at 15°. Log *P* (octanol/water), 1.02.
Quantification
Blood **GC-MS** Limit of detection, 0.5 mg/L (mg/kg) [Kintz *et al.* 1999].
Bile **GC-MS** See Blood [Kintz *et al.* 1999].
Tissue **GC-MS** See Blood [Kintz *et al.* 1999].
Disposition in the Body Alphachloralose is detected in urine as the parent compound and also as the β-isomer.
Toxicity The toxic dose of alphachloralose is approximately 1 g in adults and 20 mg/kg in infants.

An 18-year-old man was found dead at home with 3 bags, which had contained 5 g 100% alphachloralose. Postmortem toxicological analysis showed that the man had a peripheral blood alphachloralose concentration of 175.7 mg/L, cardiac blood concentration of 282.2 mg/L, and bile concentration of 279.6 mg/L. No other drugs or ethanol was detected [Kintz *et al.* 1999].

Kintz P *et al.* (1999). Testing for alpha-chloralose by headspace-GC/MS. A case report. *Forens Sci Int* 104: 59–63.

Alphameprodine

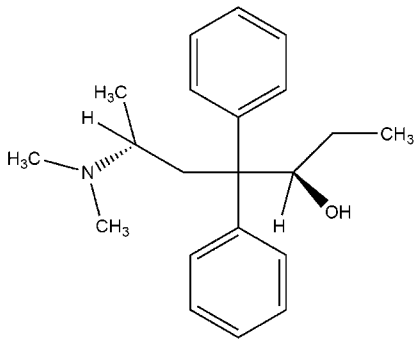
Narcotic Analgesic
$C_{17}H_{25}NO_2$ = 275.4
IUPAC Name [(3*S*,4*R*)-3-Ethyl-1-methyl-4-phenylpiperidin-4-yl] propanoate

Alphameprodine Hydrochloride
Chemical Properties White crystals. Soluble in water.

Colour Tests Ammonium molybdate test—blue-grey→green with blue rim (limit of detection 0.5 μg); ammonium vanadate test—grey (limit of detection 0.5 μg); sulfuric acid-formaldehyde test—brownish-red (limit of detection 0.25 μg).
Thin-Layer Chromatography System TA—R_f 50, streaking (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—RRT 1.00 (relative to diphenhydramine); system G4—RRT 0.48 (relative to diphenhydramine).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—247, 252, 263 nm.

Alphamethadol

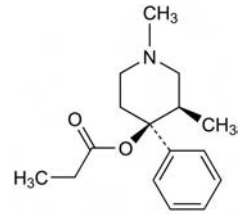
Narcotic Analgesic
$C_{21}H_{29}NO$ = 311.5
IUPAC Name 6-Dimethylamino-4,4-di(phenyl)heptan-3-ol
Synonym α-6-Dimethylamino-4,4-diphenylheptan-3-ol



Chemical Properties A white crystalline solid.
Colour Test Sulfuric acid-formaldehyde test—purple-brown→grey-green (limit of detection, 0.25 μg); ammonium molybdate test—brown-purple→green (limit of detection, 0.25 μg); ammonium vandate test—grey-green (limit of detection, 0.25 μg).
Thin-layer Chromatography System T1—R_f 57 (acidified iodoplatinate-spray—positive reaction).
Gas Chromatography System G2/225—RRT 2.55 (relative to diphenhydramine); system G4—RRT 2.44 (relative to diphenhydramine); system G5—RRT 0.69 (relative to barbital).
UV Spectrum Aqueous acid (0.05 mol/L sulfuric acid)—maximum at 253.5 nm (A^{1}_{1} 17.5) and 259 nm (A^{1}_{1} 20.2).

Alphaprodine

Narcotic Analgesic
$C_{16}H_{23}NO_2$ = 261.4
CAS—77-20-3; 15867-21-7 (± alphaprodine)
IUPAC Name [(3*S*,4*R*)-1,3-Dimethyl-4-phenylpiperidin-4-yl] propanoate
Synonyms (*cis*)-1,3-Dimethyl-4-phenyl-4-piperidinol propanoate; prisilidene; (±)-α-prodine.

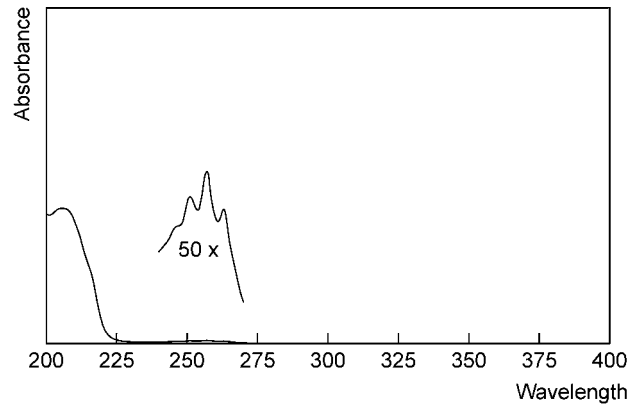


(+)-form

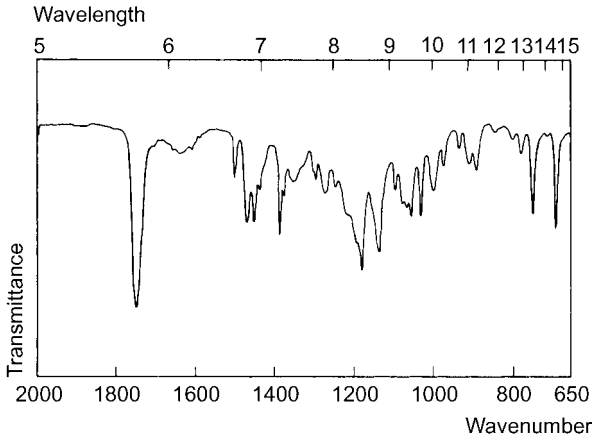
Chemical Properties Practically insoluble in water; soluble in chloroform. pK_a 8.7 (20°).

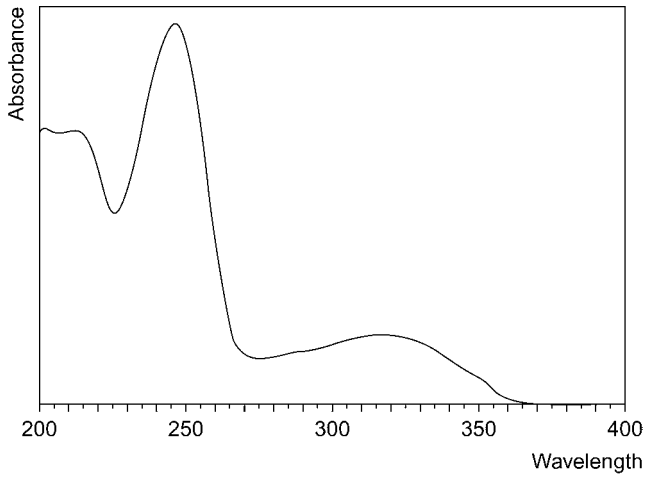
Alphaprodine Hydrochloride
$C_{16}H_{23}NO_2$,HCl = 297.8
CAS—561-78-4 (± alphaprodine hydrochloride)
Proprietary Name *Nisentil*
Chemical Properties A white crystalline powder. Mp 218° to 220°. Soluble 1 in 2 of water, 1 in 7 of ethanol, 1 in 47 of acetone and 1 in 3 of chloroform; very slightly soluble in ether.

Colour Tests Mandelin's test—blue-grey; Marquis test—brown-red.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.30; system TC—R_f 0.35; system TE—R_f 0.62; system TF—R_f 0.01; system TL—R_f 0.11; system TAE—R_f 0.28; system TAJ—R_f 0.23; system TAK—R_f 0.05; system TAL—R_f 0.60 (dragendorff spray, positive; Acidified iodoplatinate solution, positive; Marquis reagent, red-brown).
Gas Chromatography System GA—RI 1792; system GB—RI 1862.
High Performance Liquid Chromatography System HA—*k* 2.8 (tailing peak); system HX—RI 363; system HY—RI 317.
Ultraviolet Spectrum Aqueous acid—251, 257 (A^{1}_{1}=9a), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1740, 1178, 1134, 694, 1030, 1055 cm^{-1} (KBr disk).





Mass Spectrum Principal ions at *m/z* 172, 187, 84, 57, 42, 188, 44, 43.

Quantification

Plasma **GC** FID. Limit of detection, 30 µg/L [Fung *et al.* 1980].

Disposition in the Body Alphaprodine is a homologue of pethidine but its action is more rapid in onset and of shorter duration.

Therapeutic Concentration

After a rapid IV injection of 0.5 mg/kg to 6 subjects, plasma concentrations of about 0.8 mg/L were measured after 5 min, declining to 0.07 mg/L at 5 h [Fung *et al.* 1980].

Toxicity The estimated minimum lethal dose is 0.1 g. Prolonged use of alphaprodine is liable to produce dependence of the morphine type.

The following postmortem tissue concentrations were reported in an accidental fatality caused by the IV administration of 40 mg of alphaprodine: blood 0.62 mg/L, liver 0.97 µg/g, urine 0.30 mg/L; a blood-alcohol concentration of 1.7 mg/L was also reported [Griesemer, Nakamura 1973].

Half-life Plasma half-life, about 2 h.

Volume of Distribution About 2 L/kg.

Clearance Plasma clearance, about 10 mL/min/kg.

Dose 20 to 60 mg of alphaprodine hydrochloride by subcutaneous injection, or 10 to 30 mg intravenously.

Fung DL *et al.* (1980). A comparison of alphaprodine and meperidine pharmacokinetics. *J Clin Pharmacol* 20: 37–41.

Griesemer E, Nakamura GR (1973). *TIAFT Bull* 9(1–2)5.

Alpidem

Anxiolytic, Imidazopyridine

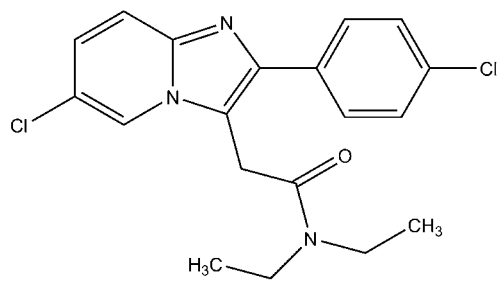
$C_{21}H_{23}Cl_2N_3O = 404.3$

CAS—82626-01-5

IUPAC Name 2-[6-Chloro-2-(4-chlorophenyl)imidazo[3,2-a]pyridin-3-yl]-*N*,*N*-dipropylacetamide

Synonyms 6-Chloro-2-(4-chlorophenyl)-*N*,*N*-dipropylimidazo[1,2-a]pyridine-3-acetamide; SL-80.0342-00.

Proprietary Name *Ananxyl*



Chemical Properties White, fluffy, microcrystalline powder. Mp 140° to 141°. Sparingly soluble in water (20 mg/L). pK_a 4.25, Log *P* (octanol/water), 4.4. [Durand *et al.* 1992].

Ultraviolet Spectrum

Principal peak at 249 nm.

Quantification

Plasma **GC** Column: 5% phenylmethylsilicone (25 m × 0.32 mm i.d., 0.52 µm). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD. Retention time: 1.77 (relative to prazepam). Limit of quantification not reported [Gaillard *et al.* 1993].

HPLC Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.025 mol/L phosphate buffer (pH 4.5, 40 : 15 : 45), flow rate 1.5 mL/min. Fluorescence detection ($\lambda_{ex} = 255$ nm, $\lambda_{em} = 423$ nm). Retention time: 14 min. Limit of quantification, 2.5 µg/L for alpidem; 1.5 µg/L for metabolites [Flaminio *et al.* 1994]. Column: C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : phosphate buffer (pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. DAD. Retention time: 11 min. Limit of quantification, not reported [Tracqui *et al.* 1993]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.025 mol/L potassium dihydrogen phosphate (pH 4.8; 45 : 10 : 45), flow rate 1.5 mL/min. Fluorescence detection ($\lambda_{ex} = 255$ nm, $\lambda_{em} = 423$ nm). Retention time: 24 min. Limit of detection, 1 µg/L [Ascalone *et al.* 1987].

Disposition in the Body Alpidem undergoes first-pass metabolism, being metabolised to several active products via *N*-alkylation or oxidation (or a combination of both processes) of the propyl side chain and aromatic oxidation reactions. Elimination is mainly via the faeces and less than 0.1% of a dose is found in urine.

Therapeutic Concentration

A group of 21 healthy man volunteers was administered single oral doses of 25, 50, 100 or 200 mg alpidem. Mean peak plasma concentrations for the four doses were 39, 128, 237 and 270 µg/L, respectively, attained at 8, 3, 4, and 3 h, respectively [Jonkman *et al.* 1991].

A group of 10 healthy volunteers was administered single oral doses of 25, 50, or 100 mg alpidem. Mean peak plasma concentrations for the three doses were 24.7, 78.1 and 119.3 µg/L, respectively, reached after 2.2, 2.6 and 1.9 h [Saletu *et al.* 1986].

Toxicity Alpidem was withdrawn from the market following reports of hepatic dysfunction.

Bioavailability Approximately 32%.

Half-life Approximately 18 h (range 7 to 44 h); increases to 23 h in elderly subjects.

Volume of Distribution 8.7 L/kg.

Clearance 0.86 L/h/kg.

Protein Binding 99.1%.

Ascalone V *et al.* (1987). Determination of alpidem and its metabolites in human plasma by high-performance liquid chromatography and fluorimetric detection. *J Chromatogr* 414: 101–108.

Durand A *et al.* (1992). Comparative pharmacokinetic profile of two imidazopyridine drugs: zolpidem and alpidem. *Drug Metab Rev* 24: 239–266.

Flaminio L *et al.* (1994). Determination of alpidem, an imidazopyridine anxiolytic, and its metabolites by column-switching high-performance liquid chromatography with fluorescence detection. *J Chromatogr A* 668: 403–411.

Gaillard Y *et al.* (1993). Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction. *J Chromatogr* 622: 197–208.

Jonkman JH *et al.* (1991). Clinical pharmacokinetics and tolerability of alpidem in healthy subjects given increasing single doses. *Eur J Clin Pharmacol* 41: 369–374.

Saletu B *et al.* (1986). Pharmacokinetic and dynamic studies with a new anxiolytic imidazo-pyridine alpidem utilizing pharmaco-EEG and psychometry. *Int Clin Psychopharmacol* 1: 145–164.

Tracqui A *et al.* (1993). High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma. *J Chromatogr* 616: 95–103.

Alprazolam

Anxiolytic, Benzodiazepine

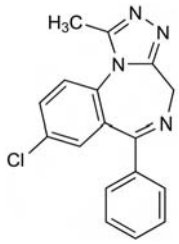
$C_{17}H_{13}ClN_4 = 308.8$

CAS—28981-97-7

IUPAC Name 8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]-triazolo[4,3-*a*][1,4]-benzodiazepine

A

Proprietary Names *Alpralid*; *Alpraz*; *Alprox*; *Alzam*; *Anxirid*; *Apo-Alpraz*; *Apox*; *Azor*; *Calmax*; *Cassadan*; *Drimpam*; *Esparon*; *Gerax*; *Kalmor*; *Mialin*; *Novo-Alprazol*; *Nu-Alpraz*; *Panix*; *Ralozam*; *Valeans*; *Xanagis*; *Xanax*; *Xanolam*; *Xanor*; *Zopax*.



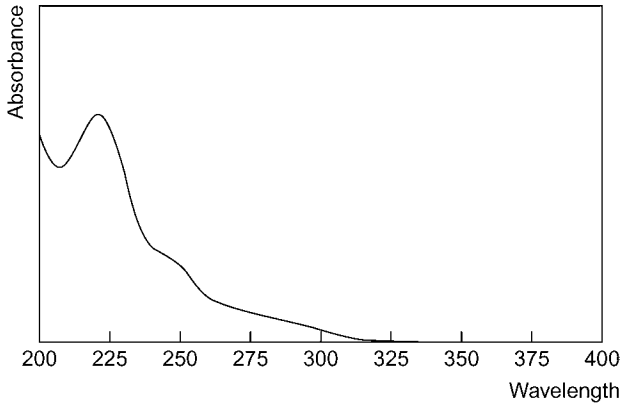
Chemical Properties A white crystalline powder. Mp 228° to 228.5°. Practically insoluble in water; sparingly soluble in alcohol and in acetone; freely soluble in chloroform and dichloromethane. pK_a 2.4. Log *P* 2.12. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.01; system TC—R_f 0.57; system TD—R_f 0.07; system TE—R_f 0.47; system TF—R_f 0.02; system TL—R_f 0.14; system TAD—R_f 0.40; system TAE—R_f 0.67; system TAF—R_f 0.66.

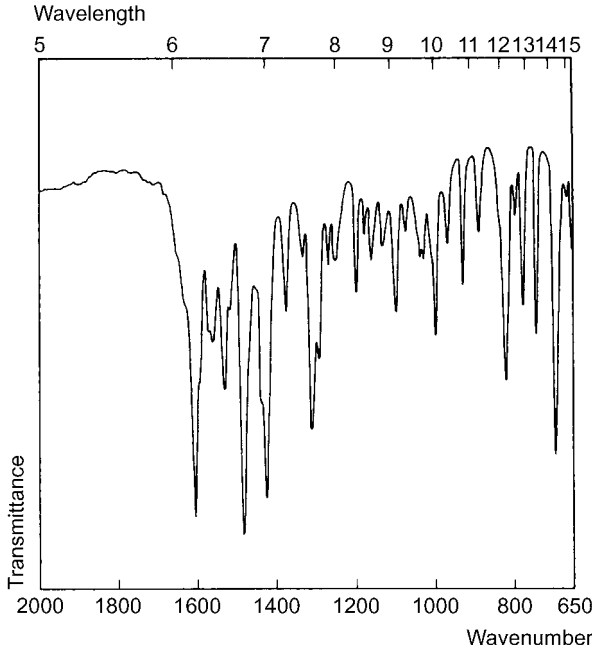
Gas Chromatography System GA—alprazolam RI 3100, M (α-OH-) RI 3045, M (OH-) RI 3245, M (4-OH-) not eluted; system GB—alprazolam RI 3108, M (OH-) not eluted; .

High Performance Liquid Chromatography System HI—*k* 4.70; system HK—*k* 2.79; system HX—RI 470; system HY—RI 379; system HZ—RT 4.0 min; system HAA—RT 17.0 min; system HAI—(relative to temazepam) RRT 1.47; system HAL—RT 6.3 min; system HAM— not detected; system HAX—RT 6.4 min; system HAY—RT 6.4 min; system HBH—*k* 3.35; system HBI—*k* 1.28.

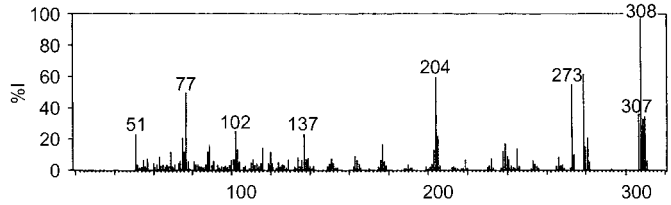
Ultraviolet Spectrum Aqueous acid—260 nm.



Infrared Spectrum Principal peaks at wavenumbers 1490, 1610, 697, 1316, 1540, 827 cm^{-1} (KBr disk). Three polymorphic forms may occur.



Mass Spectrum Principal ions at *m/z* 308, 279, 204, 273, 77, 307, 310, 309.



Quantification

Blood **GC** Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 μm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: HP-1 methylsilicone (12.5 m × 0.2 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° to 280° at 20°/min. Retention time: 6.6 min. Limit of quantification, 18 μg/L [Hall *et al.* 1995]. Column: Ultra-2 (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 40 cm/s. Temperature programme: 150° for 1 min to 280° at 30°/min for 12 min. NICI. Limit of quantification, 4 μg/L [Cairns *et al.* 1994].

HPLC Column: Acquity BEH Phenyl (100 × 2.1 mm i.d., 1.7 μm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (80 : 20 for 0.25 min to 65 : 35 over 2.25 min for 2.5 min to 20 : 80 over 1 min to 80 : 20 over 0.01 min for 1.4 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of detection not reported [Gunn *et al.* 2010]. Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5), flow rate 0.45 mL/min. DAD. Limit of quantification, 2 μg/L [Dussy *et al.* 2006].

Plasma **GC** Column: CPSIL 8 CB (25 × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 150° to 230° at 40°/min for 2 min to 250° at 5°/min for 1 min to 300° at 15°/min for 3 min. NPD. Limit of detection, 0.01–0.48 μmol/L [Reubsaet *et al.* 1998]. Column: C_8 Ultra-2 (25 m × 0.32 mm i.d., 0.52 m). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD and ECD. Relative retention time: 1.48. Limit of quantification, 2 μg/L [Gaillard *et al.* 1993]. See Blood [Lillsunde, Seppälä 1990].

GC-MS Column: Restek-200 capillary (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 7 psi. Temperature programme: 190° for 1 min to 320° at 20°/min for 2 min. NCI. Limit of quantification, 1 μg/L [Höld *et al.* 1996].

HPLC Column: C_{18} (250 × 4.6 mm). Mobile phase: methanol : water (65 : 35), flow rate 1.0 mL/min. UV detection (λ = 230 nm) [Liang *et al.* 2009]. Column: Kromasil C_8 (250 × 5 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : 0.05 mol/L ammonium acetate. DAD (λ = 240 nm). Limit of quantification, 0.07–1.57 mg/L, limit of detection, 0.02–0.47 mg/L [Uddin *et al.* 2008]. Column: Inertsil C_8 (250 × 4 mm i.d., 5 μm). Mobile phase: ammonium acetate : 0.05 mol/L methanol : acetonitrile (33 : 57 : 10). Limit of detection, 3.3–10.2 ng [Samanidou *et al.* 2007]. Column: reversed phase C_{18}. Mobile phase: methanol : water (60 : 40) containing 0.1% formic acid, flow rate 250 μL/min. Limit of detection, 0.05 μg/L [Crouch *et al.* 1999]. Column: C_{18} ODS (150 × 4.6 mm i.d., 5 μm) at 30°. Mobile phase: methanol : water with 1% diethylamine (pH 6, 60 : 40), flow rate, 1.5 mL/min. UV detection (λ = 240 nm). Limit of detection, 200 μg/L [Jenkins *et al.* 1997].

See also Akerman *et al.* [1996], and Rieck, Platt [1992].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Retention time: 4.93 min. Limit of quantification, 20 μg/L, limit of detection, 10 μg/L [Marin *et al.* 2008; Marin, McMillin 2010].

Serum **HPLC** See Blood [Dussy *et al.* 2006; Gunn *et al.* 2010]. See Plasma [Akerman *et al.* 1996]. Column: LiChrosorb RP 18 (100 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water (65 : 35), flow rate 1.2 mL/min. UV detection (λ = 230 nm). Retention time: 3.1 min. Limit of detection, 10 mg/L [Goldnik *et al.* 1994]. Column: Supelco C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 0.001 mol/L phosphate buffer with 0.003 mol/L heptyltriethylammonium phosphate in water (pH 7.4, 40 : 60), flow rate 2 mL/min. Limit of detection, 1 μg/L [Schmith *et al.* 1991].

LC-MS See Plasma [Marin, McMillin 2010]. Unison UK-C_{18} RP ODS (150 × 2 mm i.d., 3 μm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 over 20 min to 20 : 80 over 5 min), flow rate 0.25 mL/min. MRM acquisition mode. Limit of detection, 0.3–11.4 μg/L [Nakamura *et al.* 2009]. See Plasma [Marin *et al.* 2008].

Urine **GC** See Plasma [Reubsaet *et al.* 1998].

GC-MS Column: Column: BP1 polydimethylsiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° for 1 min to 250° at 15°/min, for 8 min, to 300° at 10°/min for 2 min. SIM acquisition mode. Retention time: 21.49 min. Limit of detection, 1.0–1.7 μg/L (SIM) and 13–30 μg/L (scan mode) for alprazolam and other benzodiazepines [Borrey *et al.* 2001a; Borrey *et al.* 2001b]. Column: 5% phenylmethylsiloxane (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 0.5 mL/min. Temperature programme: 240° to 260° at 25°/min to 300° at 30°/min. SIM acquisition mode. Limit of detection, 0.1 mg/L [Black *et al.* 1994]. Column: DB-5 bonded-phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 0.9 mL/min. Temperature programme: 130° for 1 min to 270° at 30°/min for 5 min to 300° at 40°/min for 6 min. EI ionisation, SIM acquisition mode. Limit of quantification, 16.5 μg/L, limit of detection, 6.8 μg/L [Joern 1992].

HPLC See Plasma [Uddin *et al.* 2008]. Column: Inertsil C_8 (250 × 4 mm i.d., 5 µm). Mobile phase: ammonium acetate : 0.05 mol/L methanol : acetonitrile (33 : 57 : 10). Limit of detection, 2.6–12.6 ng [Samanidou *et al.* 2007]. Column: Hypersil C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.04 mol/L phosphate buffer (pH 4.5; 35 : 65), flow rate, 1 mL/min. UV detection: DAD at 240 nm. Retention time: 3.48 min for α-hydroxyalprazolam and 4.98 min for alprazolam. Limit of detection, 9.2 µg/L [Segura *et al.* 2001].

LC-MS See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Symmetry C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 5.0, 10 : 90 to 30 : 70 at 5 min to 80 : 20 at 9.0 min to 10 : 90 at 9.1 min), flow rate 0.3 mL/min. Limit of quantification, 0.002 µmol/L [Hegstad *et al.* 2006].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Oral Fluid **HPLC** See Plasma [Uddin *et al.* 2008].

LC-MS Column: Atlantis dC_{18} (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 5 mmol/L aqueous ammonium acetate (pH 5, 10 : 90 to 40 : 60 at 4 min to 90 : 10 at 4.1 min until 8 min to 10 : 90 at 8.1 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.093 µg/L [øiestad *et al.* 2007].

Liver **GC-MS** See Blood [Cairns *et al.* 1994].

Disposition in the Body Alprazolam is readily absorbed after oral administration and undergoes extensive metabolism. The major metabolites are α-hydroxyalprazolam, 4-hydroxyalprazolam (both active) and a benzophenone derivative of alprazolam. Approximately 80% of a dose is excreted in the urine in 72 h, of which 11% is unchanged drug, 15% is α-hydroxyalprazolam and 9% is the benzophenone metabolite. Small amounts of desmethylalprazolam and 4-hydroxyalprazolam are also excreted in the urine. Approximately 7% of a dose is eliminated in the faeces.

Therapeutic Concentration The serum therapeutic concentration range is 0.005–0.05 mg/L; it has also been reported as 0.025–0.102 mg/L.

After a single oral dose of 1 mg alprazolam given to 10 subjects, peak plasma concentrations of 0.011–0.020 mg/L (mean, 0.015) were attained in ~1.75 h. Following daily oral doses of 0.5 mg 3 times a day to the same subjects, minimum steady-state plasma concentrations of 0.006–0.017 mg/L (mean, 0.011) and a mean maximum steady-state plasma concentration of 0.018 mg/L were reported [Smith *et al.* 1983].

In 227 patients treated for panic disorder with alprazolam (dosage titrated up to 6 mg daily), after 3 weeks of treatment, alprazolam plasma concentrations were significantly correlated with daily dosage: 70% of those with levels greater than 0.02 mg/L achieved complete remission of attacks compared with 30% of those with levels below 0.02 mg/L. At week 8, plasma alprazolam levels no longer correlated with efficacy [Greenblatt *et al.* 1993a].

Toxicity The serum toxic concentration range is 0.1–0.4 mg/L.

In a fatality attributed to acute alprazolam toxicity, the following postmortem tissue concentrations were reported for alprazolam and α-hydroxyalprazolam, respectively: heart blood 2.1 mg/L and not detected, subclavian blood 2.3 and 0.12 mg/L, urine 1.0 and 1.4 mg/L, bile 2.8 and 1.3 mg/L, vitreous humour 0.58 mg/L and not detected, liver 9.2 and 0.83 µg/g, kidney 3.8 µg/g and not detected, stomach contents 13 mg in 110 mL and not detected; the alprazolam concentration in the heart blood (2.1 mg/L) was the highest reported so far in the literature [Jenkins *et al.* 1997].

The following postmortem concentrations were reported in a 30-year-old female who ingested an unknown quantity of alprazolam and tramadol: alprazolam, α-hydroxyalprazolam and tramadol, respectively, in peripheral blood 0.21, not detected and 38.3 mg/L; bile 0.27, 0.12 and 44.0 mg/L, liver 0.23, <0.005 and 27.6 µg/g, and gastric contents (100 mL) 2.73, not detected and 130 mg [Michaud *et al.* 1999].

Half-life Plasma half-life, 11–15 h (mean, 12).

Volume of Distribution ~0.7 L/kg.

Distribution in Blood Plasma : whole blood ratio, 1.35

Clearance Plasma clearance, ~0.7 mL/min/kg.

Protein Binding ~70–80%.

Note For a review of alprazolam, see Dawson *et al.* [1984]; for its pharmacokinetics, see Greenblatt, Wright [1993] and Greenblatt *et al.* [1993b].

Dose 1 to 3 mg daily; up to 10 mg in the treatment of panic attacks.

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Borrey D *et al.* (2001b). Simultaneous determination of fifteen low-dosed benzodiazepines in human urine by solid-phase extraction and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765: 187–197.

Cairns ER *et al.* (1994). Quantitative analysis of alprazolam and triazolam in hemolysed whole blood and liver digest by GC/MS/NICI with deuterated internal standards. *J Anal Toxicol* 18: 1–6.

Crouch DJ *et al.* (1999). Quantitation of alprazolam and alpha-hydroxyalprazolam in human plasma using liquid chromatography electrospray ionization MS–MS. *J Anal Toxicol* 23: 479–485.

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Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

Dussy FE *et al.* (2006). Quantification of benzodiazepines in whole blood and serum. *Int J Legal Med* 120: 323–330.

Gaillard Y *et al.* (1993). Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen–phosphorus detection after solid-phase extraction. *J Chromatogr* 622: 197–208.

Goldnik A *et al.* (1994). Determination of estazolam and alprazolam in serum by HPLC. *Acta Pol Pharm* 51: 311–312.

Greenblatt DJ, Wright CE (1993). Clinical pharmacokinetics of alprazolam: therapeutic implications. *Clin Pharmacokinet* 24: 453–471.

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Greenblatt DJ *et al.* (1993b). Alprazolam pharmacokinetics, metabolism, and plasma levels: clinical implications. *J Clin Psychiatry* 54(Suppl): 4–11.

Gunn J *et al.* (2010). Simultaneous determination and quantification of 12 benzodiazepines in serum or whole blood using UPLC/MS/MS. *Meth Mol Biol* 603: 107–119.

Hall MA *et al.* (1995). High-performance liquid chromatography of alprazolam in postmortem blood using solid-phase extraction. *J Anal Toxicol* 19: 511–513.

Hegstad S *et al.* (2006). Determination of benzodiazepines in human urine using solid-phase extraction and high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. *J Anal Toxicol* 30: 31–37.

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Jenkins AJ *et al.* (1997). A fatality due to alprazolam intoxication. *J Anal Toxicol* 21: 218–220.

Joern WA (1992). Confirmation of low concentrations of urinary benzodiazepines, including alprazolam and triazolam, by GC/MS: an extractive alkylation procedure. *J Anal Toxicol* 16: 363–367.

Liang X *et al.* (2009). [Simultaneous determination of 5 sedative hypnotics in human plasma by reversed phase high-performance liquid chromatography]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 34: 689–692.

Lillsunde P, Seppälä T (1990). Simultaneous screening and quantitative analysis of benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen–phosphorus detection. *J Chromatogr* 533: 97–110.

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øiestad EL *et al.* (2007). Drug screening of preserved oral fluid by liquid chromatography–tandem mass spectrometry. *Clin Chem* 53: 300–309.

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Rieck W, Platt D (1992). High-performance liquid chromatographic method for the determination of alprazolam in plasma using the column-switching technique. *J Chromatogr* 578: 259–263.

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Schmith VD *et al.* (1991). New high-performance liquid chromatographic method for the determination of alprazolam and its metabolites in serum: instability of 4-hydroxyalprazolam. *J Chromatogr* 568: 253–260.

Segura M *et al.* (2001). Analytical methodology for the detection of benzodiazepine consumption in opioid-dependent subjects. *J Anal Toxicol* 25: 130–136.

Smith RB *et al.* (1983). Single- and multiple-dose pharmacokinetics of oral alprazolam in healthy smoking and nonsmoking men. *Clin Pharm* 2: 139–143.

Uddin MN *et al.* (2008). Validation of SPE-HPLC determination of 1,4-benzodiazepines and metabolites in blood plasma, urine, and saliva. *J Sep Sci* 31: 3704–3717.

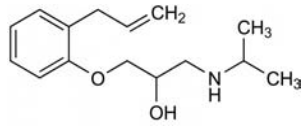
Alprenolol

β-*Adrenoceptor Antagonist*

$C_{15}H_{23}NO_2$ = 249.4

CAS—13655-52-2; 23846-70-0 (±)

IUPAC Name 1-(Propan-2-ylamino)-3-(2-prop-2-enylphenoxy)propan-2-ol



Chemical Properties Mp 57° to 59.° pK_a 9.5 (20°). Log *P* (octanol/water pH 7.0), 0.5. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Alprenolol Hydrochloride

$C_{15}H_{23}NO_2$,HCl = 285.8

CAS—13707-88-5; 13678-97-2 (±)

Proprietary Names *Apllobal*; *Aptin(e)*; *Betacard*; *Duriles*; *Gubernal*; *Regletin*; *Yobir*.

Chemical Properties Colourless crystals or a white crystalline powder. Mp 108° to 111°. Soluble 1 in <1 of water, 1 in 2 of ethanol, and 1 in 3 of chloroform; soluble in acetone and dilute acetic acid; practically insoluble in ether.

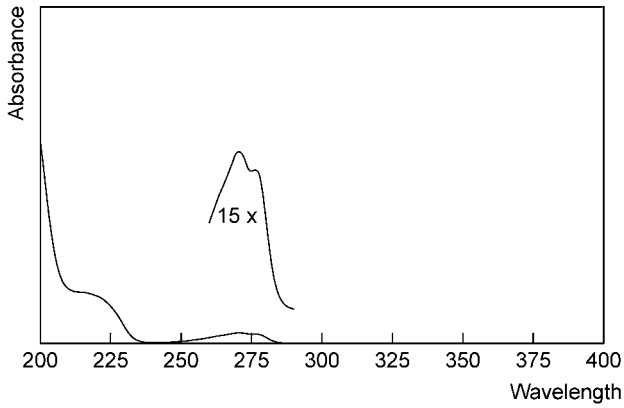
Colour Tests Liebermann's reagent—red; Mandelin's test—brown-violet; Marquis test—red; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.11; system TC—R_f 0.12; system TE—R_f 0.49; system TAE—R_f 0.22; system TAG—R_f 0.11 (acidified iodoplatinate solution—positive).

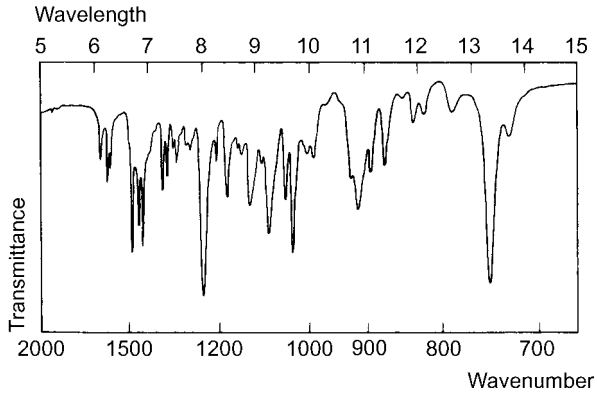
Gas Chromatography System GA—RI 1820.

High Performance Liquid Chromatography System HA—*k* 1.2; system HY—RI 323; system HZ—RT 3.87 min.

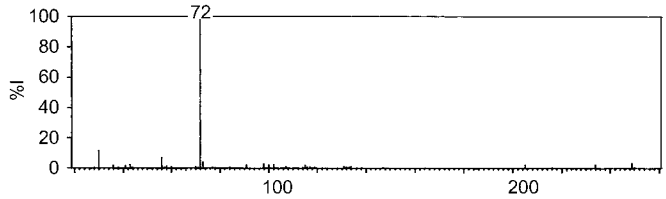
Ultraviolet Spectrum Aqueous acid—270 (A_1^1 = 69b), 276 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1242, 746, 1031, 1492, 1079, 916 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 30, 56, 73, 249, 98, 234, 102.



Quantification

***Plasma* GC** Column: 3% OV-17 on Gas-Chrom Q 100/120 mesh (1.5 m × 0.2 cm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 235°. FID. Limit of detection, 2.5 µg/L [Poole *et al.* 1980].

LC-MS Column: Chiral-CBH (100 × 3.0 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate and 0.02% acetic acid (pH 5.0) : acetonitrile (65 : 35), flow rate 0.9 mL/min. TIS, MRM acquisition mode. Limit of quantification, 0.5 µg/L [Jiang *et al.* 2008]. Column: LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (80 : 20 for 2.2 min to 60 : 40 at 2.21 min until 5.5 min to 10 : 90 at 5.51 min until 8 min to 80 : 20 at 8.01 min until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min at 5.51 mL/min to 6.5 mL/min at 8.01 min to 0.4 mL/min at 9.51 min. APCI, positive ion mode, SIM acquisition mode. Limit of detection, 0.01 mg/L [Maurer *et al.* 2004].

***Serum* GC** Column: OV-101 (25 m × 0.25 mm i.d., 0.3 µm). Carrier gas: N_2, 16 cm/s. Temperature: 205°. ECD. Relative retention time: 6 min. Limit of detection, 2 pg [Debruyne *et al.* 1979].

***Urine* HPLC** Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm); Mobile phase: acetonitrile : water (40 : 60) containing 5 µmol/L potassium dihydrogen phosphate/dipotassium hydrogen phosphate (pH 6.5), flow rate 1.3 mL/min. Electrochemical detection. Retention time: 11.42 min (k 4.71). Limit of quantification, 500 µg/L [Maguregui *et al.* 1995].

Disposition in the Body Alprenolol is rapidly and almost completely absorbed after oral administration but undergoes extensive first-pass metabolism; bioavailability ~5–10%. Approximately 90% of an oral dose is excreted in the urine in 24 h, with ~40% as the glucuronide conjugate of the active metabolite 4-hydroxyalprenolol, 5% as free 4-hydroxyalprenolol, 20–30% as alprenolol glucuronide, 1% as desisopropylalprenolol, and <1% as unchanged drug; unknown metabolites account for the remainder of the dose.

Therapeutic Concentration There is considerable intersubject variation in plasma concentrations and in the ratio of 4-hydroxyalprenolol to alprenolol.

After oral administration of 200 mg to 2 subjects, peak plasma alprenolol concentrations of 0.07 and 0.22 mg/L were reported; peak plasma concentrations of the 4-hydroxy metabolite were 0.04 and 0.06 mg/L [Alvan *et al.* 1977].

Following oral administration of 200 mg three times a day to 16 subjects, average steady-state plasma alprenolol concentrations of 0.01–0.14 mg/L (mean, 0.04) were reported [Collste *et al.* 1976].

Toxicity

In 2 fatalities, caused by the ingestion of ~4 g and 20 g, respectively, the following postmortem tissue alprenolol concentrations were reported: blood 40 and 43 mg/L, liver 72 and 91 µg/g [Dickson *et al.* 1978].

Half-life Plasma half-life, alprenolol and 4-hydroxyalprenolol ~2–3 h.

Volume of Distribution ~3 L/kg.

Clearance Plasma clearance, ~15 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.6.

Protein Binding 80–90%.

Dose 200 to 800 mg of alprenolol hydrochloride daily.

Alvan G *et al.* (1977). Importance of 'first-pass elimination' for inter-individual differences in steady-state concentrations of the adrenergic beta-receptor antagonist alprenolol. *J Pharmacokinet Biopharm* 5: 193–205.

Collste P *et al.* (1976). Pharmacokinetics and pharmacodynamics of alprenolol in the treatment of hypertension. I. Relationship between plasma concentration and adrenergic beta-receptor blockade. *Eur J Clin Pharmacol* 10: 85–88.

Debruyne D *et al.* (1979). Improved electron-capture GLC determination of alprenolol and oxprenolol in serum using a wall-coated open tubular column. *J Pharm Sci* 68: 511–512.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dickson SJ *et al.* (1978). The gas chromatographic determination of alprenolol in human postmortem liver and blood samples. *J Anal Toxicol* 2: 242–244.

Jiang H *et al.* (2008). Enantioselective determination of alprenolol in human plasma by liquid chromatography with tandem mass spectrometry using cellobiohydrolase chiral stationary phases. *J Chromatogr B Analyt Technol Biomed Life Sci* 872: 121–127.

Maguregui MI *et al.* (1995). High-performance liquid chromatography with amperometric detection applied to the screening of beta-blockers in human urine. *J Chromatogr B Biomed Appl* 674: 85–91.

Maurer HH *et al.* (2004). Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization. *J Chromatogr A* 1058: 169–181.

Poole CF *et al.* (1980). Formation of electron-capturing derivatives of alprenolol by *trans*boronation: application to the determination of alprenolol in plasma. *J Chromatogr* 194: 365–377.

Alprostadil

Prostaglandin, Vasodilator

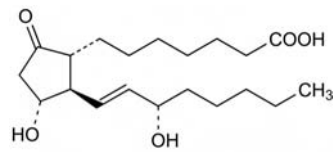
$C_{20}H_{34}O_5$ = 354.5

CAS—745-65-3

IUPAC Name 7-[(1*R*,3*R*)-3-Hydroxy-2-[(E, 3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoic acid

Synonyms (*E*)-(8*R*,11*R*,12*R*,15*S*)-11,15-Dihydroxy-9-oxoprost-13-en-1-oic acid; prostaglandin E_1; PGE_1.

Proprietary Names *Alprostar (alprostadil alfadex)*; *Caverject*; *Edex*; *Liple*; *Minprog*; *Muse*; *Prostandin (alprostadil alfadex)*; *Prostavasin (alprostadil alfadex)*; *Prostin VR*; *Prostine VR*; *Prostivas*; *Sugiran (alprostadil alfadex)*; *Viridal (alprostadil alfadex)*.



Chemical Properties A white to off-white crystalline powder. Mp about 110°. It is soluble in water and in acetone; freely soluble in ethanol; very slightly soluble in chloroform and in ether; slightly soluble in ethyl acetate. pK_a 4.85. Log *P* (octanol/water), 3.20.

Thin-layer Chromatography Plate: silica LK6D (5 × 20 cm). Mobile phase: ethyl acetate: acetic acid (99.5:0.5). PGE_0, R_f 0.50; PGE_2 R_f 0.31 [Schweer *et al.* 1994].

Quantification

***Plasma* GC-MS** Limit of quantification, 1 ng/L for alprostadil and 5 ng/L for its metabolite [Schweer *et al.* 1994].

Disposition in the Body Alprostadil is rapidly distributed throughout the body (except in the CNS) following IV administration. Between 60 and 90% (depending on dose) is rapidly metabolised by oxidation during passage through pulmonary circulation. It is also metabolised by the liver and kidneys to prostaglandin E_0 (PGE_0) and 15-hydroxy-PGE_0. The drug is excreted principally in urine (88% of a dose) as metabolites and a small amount is excreted in faeces via bile (12%).

Therapeutic Concentration

Following IV infusion of 20 µg over 30 min in 24 subjects, mean peak plasma concentrations of 8.2 ng/L were observed. Following intracavernous injection of 20 µg, mean peak plasma concentrations of 17.6 ng/L were reached after a mean of 4.8 min [Cawello *et al.* 1997].

Mean peak plasma concentrations of 4.8 (range, 3.4 to 6.3), 7.7 (6.7 to 10.8) and 12.9 (10.2 to 14.6) ng/L were measured in 12 healthy male subjects (aged between 20 and 35 years) after IV infusion over 2 h of 30, 60 and 120 µg, respectively. These levels were observed by the end of the infusion. The mean

peak plasma PGE_0 concentrations were 8.3, 15.1 and 29.2 ng/L for the 30, 60 and 120 µg doses and the 15-hydroxy-PGE_0 metabolite, 108.6, 203.6 and 395.6 ng/L, respectively [Cawello *et al.* 1995].

Bioavailability 15%.

Half-life Plasma, 0.2 to 8 min (after single dose).

Volume of Distribution 0.8 L/kg.

Clearance Plasma, 115 L/min; also reported as 20 mL/min/kg.

Protein Binding 81 to 93% (to albumin).

Note For a review of the pharmacokinetics of alprostadil see Kirsten *et al.* [1998].

Dose Usually, 5 to 20 µg by intracavernous injection; 250 µg by transurethral delivery (initial dose); 50 to 100 ng/kg/min by IV infusion (reduced to lowest dose).

Cawello W *et al.* (1995). Dose proportional pharmacokinetics of alprostadil (prostaglandin E1) in healthy volunteers following intravenous infusion. *Br J Clin Pharmacol* 40: 273–276.

Cawello W *et al.* (1997). Pharmacokinetics of prostaglandin E1 and its main metabolites after intracavernous injection and short-term infusion of prostaglandin E1 in patients with erectile dysfunction. *J Urol* 158: 1403–1407.

Kirsten R *et al.* (1998). Clinical pharmacokinetics of vasodilators. Part II. *Clin Pharmacokinet* 35: 9–36.

Schweer H *et al.* (1994). Determination of prostaglandin E1 and its main plasma metabolites 15-keto-prostaglandin E0 and prostaglandin E0 by gas chromatography/negative ion chemical ionization triple-stage quadrupole mass spectrometry. *Biol Mass Spectrom* 23: 165–170.

Alteplase

Antithrombotic

$C_{2569}H_{3894}N_{746}O_{781}S_{40}$ = 59007.6

CAS—105857-23-6

Synonyms G-11044; G-11021; recombinant tissue-type plasminogen activator; rt-PA.

Proprietary Names *Actilyse*; *Actiplas*; *Activase*; *Besospartin*; *Lysatec-rt-PA*.

Chemical Properties A white to off-white powder.

Quantification

Note For an ELISA for the quantification of alteplase see Matsuo *et al.* [1983].

Disposition in the Body Alteplase is rapidly cleared from plasma and metabolised mainly in the liver to low molecular weight compounds and amino acids. After IV infusion, 50% of the dose is cleared from plasma within 5 min and 80% within 10 min. 80% of a dose of alteplase is excreted within 18 h, via the kidneys.

Therapeutic Concentration

Six healthy volunteers were administered with a 0.25 mg/kg dose of alteplase and 6 with 0.5 mg/kg. Peak plasma concentrations of 0.96 mg/L and 1.83 mg/L, respectively, were reached by the end of infusion [Tanswell *et al.* 1989]. Patients with acute myocardial infarction show increased peak plasma concentrations with similar doses.

Toxicity Acute overdosing (over 150 mg) can lead to internal bleeding, including intracranial, gastrointestinal and peripheral and ocular haemorrhaging.

Half-life Plasma half-life is 4 to 5 min (α-phase), ~40 min (β-phase).

Volume of Distribution Initial volume of distribution approximates plasma volume, 2–5 L.

Clearance Plasma clearance, 550 to 650 L/min.

Dose Adult: dose ranges between 0.02 to 0.04 mg/kg body weight and 1.5 mg/kg body weight, depending on symptoms. Maximum dose of 100 mg. Children: 0.01 to 0.05 mg/kg body weight.

Matsuo O *et al.* (1983). Determination of tissue plasminogen activator by an enzyme-immunoassay method. *Anal Biochem* 135: 58–63.

Tanswell P *et al.* (1989). *Clin Pharmacol Ther* 46: 1310–1319.

Altretamine

Antineoplastic

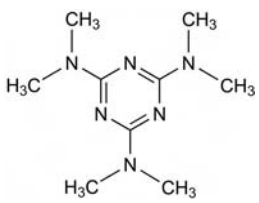
$C_9H_{18}N_6$ = 210.3

CAS—645-05-6

IUPAC Name 2-*N*,2-*N*,4-*N*,4-*N*,6-*N*,6-*N*-Hexamethyl-1,3,5-triazine-2,4,6-triamine

Synonyms ENT-50852; hexamethylmelamine; HMM; NSC-13875; 2,4,6-tris (dimethylamino)-1,3,5-triazine.

Proprietary Names *Hexalen*; *Hexastat*; *Hexinawas*.



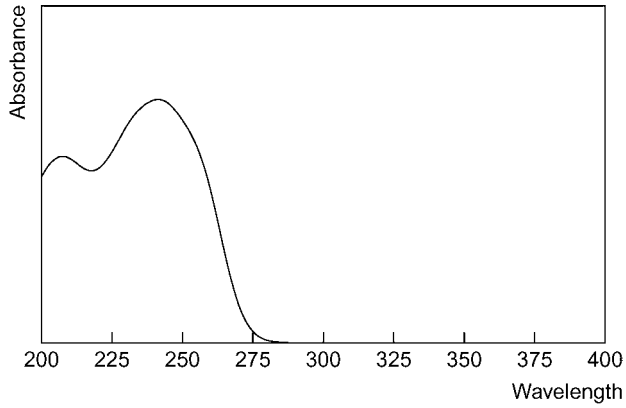
Chemical Properties A white crystalline powder. Mp about 172°. It is practically insoluble in water and physiological solvents; soluble in chloroform, DMSO, ethanol and acetone. Log *P* (octanol/water), 2.73.

Note Altretamine is an irritant; avoid contact with skin and mucous membranes.

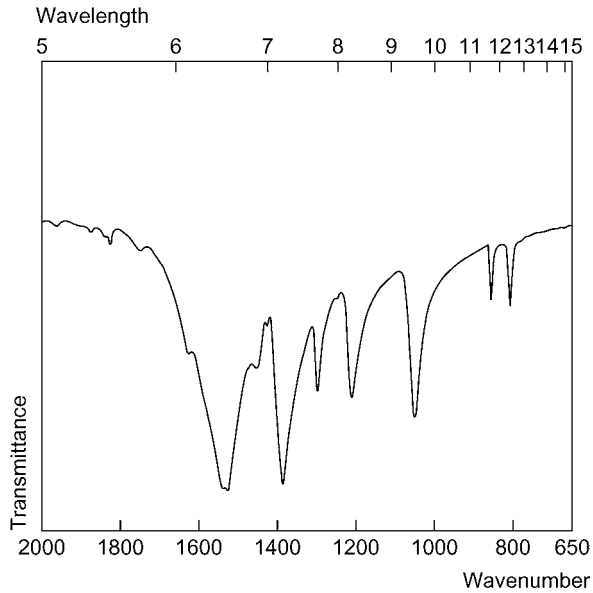
High Performance Liquid Chromatography System HAA—retention time 17.8 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. DAD. Retention time: 3.2 min [Mills, Roberson 1993].

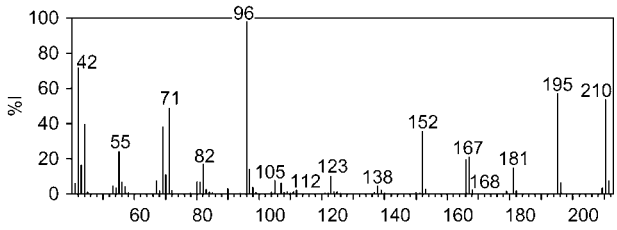
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—240 nm; Ethanol—226 nm; basic—241 nm.



Infrared Spectrum Principal peaks at wavenumber 1527, 1387, 1051 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 96, 42, 195, 210, 71, 69, 44, 152.



Quantification

Plasma **GC** AFID. Limit of detection, 0.005 mg/L [Hulshoff *et al.* 1980]. AFID. Limit of detection, 0.2 mg/L for altretamine and 0.025 mg/L for pentamethylmelamine [Ames, Powis 1979].

HPLC UV detection. Limit of quantification, 0.15 mg/L [Barker *et al.* 1994].

Serum **GC** See Plasma [Hulshoff *et al.* 1980].

Urine **GC** See Plasma [Ames, Powis 1979].

Disposition in the Body Altretamine is rapidly and well absorbed after oral administration and quickly distributed to tissues with a high lipid component. It is rapidly demethylated in the liver to pentamethylmelamine and other metabolites that are excreted in urine. Formaldehyde is also produced which is subsequently oxidised to carbon dioxide and expired. Other metabolites include dimethylmelamine, monomethylmelamine, trimethylmelamine and melamine, and these have been detected in urine. No parent drug has been detected in urine. Metabolism is

A

catalysed by cytochrome P450 enzymes. Accumulation in plasma has not been observed with multiple dosing.

Therapeutic Concentration

Twenty patients with gynaecological malignancies were administered with 200 to 300 mg/m^2 doses and peak plasma concentrations of 0.32 to 4.28 mg/L were observed after 0.5 to 3 h. 12 patients administered with a 200 mg dose reached peak concentrations of 0.3 to 0.9 mg/L at ~1 h. Peak plasma concentrations vary widely among patients because of the rapid hepatic metabolism [Damia, D'Incalci 1995].

Half-life Plasma, 4 to 13 h.

Protein Binding Altretamine 94%; pentamethylmelamine 75%.

Dose 260 mg/m^2 daily for 14 consecutive days in a 28-day cycle.

Ames MM, Powis G (1979). Determination of pentamethylmelamine and hexamethylmelamine in plasma and urine by nitrogen-phosphorus gas-liquid chromatography. *J Chromatogr* 174: 245–249.

Barker IK *et al.* (1994). Determination of altretamine in human plasma with high-performance liquid chromotography. *J Chromatogr B Biomed Appl* 660: 121–126.

Damia G, D'Incalci M (1995). Clinical pharmacokinetics of altretamine. *Clin Pharmacokinet* 28: 439–448.

Hulshoff A *et al.* (1980). Determination of hexamethylmelamine and metabolites in plasma or serum by gas-liquid chromatography with a nitrogen-sensitive detector. *J Chromatogr* 181: 363–371.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. 4–5: Boca Raton, FL: CRC Press.

Aluminium

Metal

Al = 26.98

CAS—7429-90-5

Synonyms Aluminum; aluminium bronze; aluminium dehydrated; alumina fibre; aluminium flake; aluminium powder; metana.

Proprietary Names *Aluminum-27*; *JISC 3108/3110*; *Metana*; *Noral Aluminum*; *Pap-1*.

Chemical Properties Malleable, ductile tin-white with bluish tint metal, also exists as a crystalline solid. Mp 660°. Bp 2467°. Insoluble in water, soluble in alkalis and acids. Flammable solid if finely divided and is easily ignited. Valency Al (0) and Al (+3). Occurrence: owing to its high reactivity, it is never found as the free metal in nature. It is found combined with other elements (oxygen, silicone, and fluorine). The metal is usually obtained from the mineral bauxite. Uses include as an alloy for food and drink packaging, construction materials, bodies and mechanical parts of transportation vehicles, electrical applications, and cooking utensils. It is used for the absorption of occluded gases in the manufacture of steel; testing for gold, arsenic, and mercury; precipitating copper; in explosives; and in flashes for photography. Aluminium powder is used in paints, protective coatings, and fireworks. Naturally occurring aluminium-containing minerals such as bentonite and zeolite are used in water purification, sugar refining and in the brewing and paper industries.

Aluminium Chloride

$AlCl_3$ = 133.3

CAS—7446-70-0

Synonyms Aluminum chloride; aluminium chloride (1 : 3); aluminium trichloride; trichloroaluminium.

Proprietary Name *Pearsall*

Chemical Properties White or yellowish crystals with a strong odour of hydrochloric acid. Mp 190° at 2.5 atm, also reported to volatilise without melting. Bp 182.7°. Freely soluble in benzophenone, benzene, nitrobenzene, carbon tetrachloride, trichloromethane; soluble in alcohol and ether; reacts explosively with water, evolving HCl gas. Used as an acid catalyst in Friedel–Crafts-type reactions, in the cracking of petroleum in the manufacture of rubbers and lubricants. In cosmetics, it is used as a topical astringent; it is also used in refining crude oil, dyeing fabrics, and manufacturing parchment paper. The hexahydrate form is used in preserving wood, as a disinfectant, and in deodorants and antiperspirants.

Aluminium Chlorhydrate

$AlClH_5O_5$ = 147.5 (other formulae have been suggested, including $Al_2(OH)_5Cl{,}2H_2O$ or $Al_6(OH)_{15}C_{13}$ or $[Al_2(OH)_5Cl]_x$)

CAS—1327-41-9; 11097-68-0; 84861-98-3

Synonyms Aluminium chloride, basic; aluminium chloride hydroxide; aluminium chlorohydroxide; aluminium hydroxychloride; polyaluminium chloride.

Proprietary Names *Astringen*; *Chlorhydrol*; *Locron*.

Chemical Properties Glassy solid. Soluble in water, forming turbid colloidal suspensions. Used as an active ingredient in many antiperspirants and deodorants.

Aluminium Hydroxide

$Al(OH)_3$ = 78.0

CAS—21645-51-2

Synonyms α-Alumina trihydrate; aluminium (III) hydroxide; aluminium oxide hydrate; hydrated alumina.

Proprietary Names *Alcoa331*; *Alugel*; *Alumigel*; *British Aluminum AF260*; *Calmogastrin*; *Higilite H 31S*; *Hychol 705*; *Hydrafil*; *Hydral 705*; *Martinal A*; *Reheis F1000*.

Chemical Properties White, bulky amorphous powder. Mp 300°. Practically insoluble in water, forming gels on prolonged contact; soluble in alkaline aqueous solutions or in hydrochloric and sulfuric acids. Used in stomach antacids, as a desiccant powder, in antiperspirants and dentifrices, in packaging materials, as a soft abrasive for brass and plastics, as a glass additive, in ceramics, and as a filler in plastics, rubber, cosmetics, and paper. It is also used to lower plasma phosphorus levels in patients with renal failure.

Aluminium Nitrate

AlN_3O_9 = 213.0 (also as hydrated form, $AlN_3O_9{,}9H_2O$ = 375.1)

CAS—13473-90-0

Synonyms Aluminium (III) nitrate (1 : 3); aluminium trinitrate; nitric acid, aluminium salt.

Chemical Properties Odourless white nonahydrate deliquescent crystals. Mp 73°, decomposes at 135°. Very soluble in water; very slightly soluble in acetone; almost insoluble in ethyl acetate and pyridine. Used in antiperspirants, for tanning leather, as a corrosion inhibitor, in the preparation of insulating papers, in transformer core laminates, in incandescent filaments, and in cathode ray tube heating elements.

Aluminium Oxide

Al_2O_3 = 101.9

CAS—1344-28-1

Synonyms Activated aluminium oxide; alumina; β-aluminium oxide; aluminium sesquioxide; aluminium trioxide.

Proprietary Names *Almite*; *Alon*; *Aloxite*; *Alumite*; *Alundum*; *Campalox*; *Dispol Alumina*; *Exolon XW 60*; *Faserton*; *Hypalox II*; *Ludox CL*; *Martoxin*; *Microgrit WCA*; *Poraminar*.

Chemical Properties White crystalline powder. Mp 2000°. Bp 3000°. Practically insoluble in water; slowly soluble in aqueous alkaline solutions; practically insoluble in non-polar organic solvents. Used in the production of aluminium; manufacture of abrasives, ceramics, refractories, electrical insulators, catalyst and catalyst supports, paper, spark plugs, crucibles, and laboratory works; adsorbent for gases and water vapours; in chromatographic analysis, fluxes, light bulbs, artificial gems; heat-resistant fibres; food additive; in fibre membrane units in water desalination, industrial ultrafiltration, haemodialysis; as dosimeter for measuring personnel radiation exposure.

Aluminium Phosphate

$AlPO_4$ = 122.0

CAS—7784-30-7

Synonyms Aluminium orthophosphate; aluminium phosphate tribasic; phosphoric acid, aluminium salt (1 : 1).

Proprietary Names It is an ingredient in *Alaphos*; *Phosphaljel*; *Phosphalugel*; *Phosphalutab*; *Ukocid*.

Chemical Properties White crystals. Mp>1460°. Insoluble in water; very slightly soluble in concentrated hydrochloric acid and nitric acid. Used in OTC stomach antacids.

Aluminium Phosphide

AlP = 58.0

CAS—20859-73-8

Synonym Aluminium monophosphide

Proprietary Names *Celphos*; *Delicia*; *Delicia Gastoxin*; *Detia GAS EX-B/EX-T*; *Detia phospine pellets*; *Phostoxin*; *Quick-Phos*; *Quick-Fume*.

Chemical Properties Dark grey or dark yellow crystals with a garlic odour. Does not melt or decompose thermally at temperatures up to 1000°. Decomposes in water to give phosphine gas. Used as insecticidal grain fumigant.

Aluminium Sulfate

$Al_2(SO_4)_3$ = 342.1

CAS—10043-01-3

Synonyms Alum; aluminium sulphate (2 : 3); cake alum; dialuminium sulphate; filter alum; papermakers' alum; patent alum; peral alum; pickle alum.

Proprietary Names *Cake Alum*; *Patent Alum*.

Chemical Properties Odourless white lustrous crystals (also pieces, granules or powder). Decomposes at 770°. Soluble in water and dilute acids; practically insoluble in alcohol. Used primarily for water purification systems and sewage treatment systems as a flocculent; in the paper and pulp industry; in fireproofing and waterproofing cloth; in clarifying oils and fats; in waterproofing concrete; in antiperspirants; in tanning leather; as a mordant in dyeing; in agricultural pesticides; as a soil conditioner to increase acidity for plants; and in cosmetics and soaps. Solutions containing 5–10% have been used as local applications to ulcers and to stop discharges from mucous surfaces.

Colour Test Addition of sodium hydroxide to aluminium ions—forms a white precipitate that dissolves in excess sodium hydroxide.

Quantification

Specimen Collection Blood/serum/plasma—5 mL, plain or lithium heparin tube; urine—20 mL plastic universal container; dialysis fluid—20 mL plastic universal container.

Note Aluminium is the most abundant element on earth and contamination from needles, syringes, collection tubes, glassware, analytical reagents, and equipments is a major problem. It is always advisable to analyse a sample of aluminium-free water alongside the test samples.

Blood **DPV** Buffer: 0.5 mol/L ammonium acetate plus 0.5 mol/L ammonia with 13.3% 2 mol/L sodium chloride and dopamine. Scan rate: 20 mV/s. Pulse

amplitude: 50 mV. Pulse width: 60 ms. Limit of detection, 19 nmol/L [Zhang *et al.* 2002]. Buffer: 0.08 mol/L ammonium acetate : ammonia (pH 8.5). Limit of detection, 76 nmol/L [Zhang *et al.* 2000].

ETAAS Dry cycle: 110° at 10 s for 5 s to 150° in 60 s for 15 s (to 400° at 1 s for 15 s in blood with a haematocrit of >0.5 L/L). Char cycle: 1400° at 40 s for 20 s (ramp time extended to 100 s in blood with a haematocrit of >0.5 L/L. Atomisation cycle: 260° for 8 s. Gas: N_2, 100 mL/min. Limit of detection, 2.3 µg/L [van der Voet *et al.* 1985]. Dry cycle: 120° at 15 s for 15 s, 300 mL/min. Char cycle: 300° in 5 s for 5 s to 1530° in 25 s for 10 s, 300 mL/min. Atomisation cycle: 2700° in 1 s for 13 s, 20 mL/min for first 6 s to 300 mL/min for last 7 s. Limit of detection, 20 pg [D'Haese *et al.* 1985].

ICP-AES Limit of detection, 0.1 mg/kg [Dinya *et al.* 2005]. Plasma gas: Ar, 12 L/min. Nebuliser gas: Ar, 0.5 L/min. Nebuliser pressure: 2.3 bar (λ = 396.2 nm). Limit of detection, 0.12 µmol/L [Chappuis *et al.* 1992].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 1.13 L/min. Nebuliser gas: 1.0 mL/min. Limit of detection not reported [Rainska *et al.* 2007]. Plasma gas: Ar, 14.8 L/min. Auxiliary gas: Ar, 0.9 L/min. Carrier gas: 1.1 L/min. Babington nebuliser (*m/z* 27). Limit of detection not reported [Botta *et al.* 2006]. Limit of detection, 0.1 µg/L [Nagaoka, Maitani 2005]. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 40 µg/L [De Boer *et al.* 2004]. Limit of detection, 171 ng/L [Liao *et al.* 2004]. Outer gas: Ar, 15 L/min. Intermediate gas: 0.85 L/min. Carrier gas: 0.91 L/min. Limit of detection not reported [Nagaoka, Maitani 2001].

Plasma **ETAAS** Dry cycle: 120° at 1 s for 20 s to 160° in 10 s for 10 s. Char cycle: 1000° in 10 s for 20 s. Atomisation cycle: 2600° for 5 s to 2650° in 1 s for 7 s. Gas: Ar (λ = 309 nm). Limit of detection not reported [Progar *et al.* 1996]. Dry cycle: 110° at 10 s for 5 s to 150° in 60 s for 15 s. Char cycle: 1400° in 40 s for 20 s. Atomisation cycle: 260° for 8 s. Gas: N_2, 100 mL/min. Limit of detection, 1.8 µg/L [van der Voet *et al.* 1985]. See Blood. Dry cycle: 100° at 15 s for 15 s to 120° in 10 s for 10 s, 300 mL/min. Char cycle: 700° in 2 s for 1 s to 1580° in 120 s for 10 s, 300 mL/min [D'Haese *et al.* 1985].

ICP-AES Plasma gas: Ar, 0.01 L/min. Auxiliary gas: Ar, 10 L/min. Nebuliser gas: Ar, 0.8 L/min. McPherson 216 or Minuteman 310-SMP spectrometer (λ = 308 nm). Limit of detection not reported [Progar *et al.* 1996].

Serum **ETAAS** See Blood. Limit of detection, 1.9 µg/L [van der Voet *et al.* 1985]. Dry cycle: 350° for 60 s. Char cycle: 1500° for 60 s. Atomisation cycle: 2600° for 12 s. Aluminium hollow cathode lamp (λ = 309 nm). Limit of detection not reported [Gorsky, Dietz 1978].

ICP-AES Plasma gas: Ar, 12 L/min. Meinhard nebuliser (λ = 167 nm). Limit of quantification, 0.97 µg/L; limit of detection, 0.5 µg/L [Bianchi *et al.* 2007]. External gas: 14.0 L/min. Indirect gas: 0.5 L/min. Bearing gas: 1.0 L/min. Meinhard nebuliser (λ = 165–460 nm). Limit of detection not reported [Olszewski *et al.* 2006]. See Blood [Chappuis *et al.* 1992].

ICP-MS Outer gas: 15.0 L/min. Carrier gas: 0.8 L/min. Make-up gas: 0.17 L/min. Limit of detection, 5 µg/L [Murko *et al.* 2007].

FPLC-ICP-MS Column: Mono-Q HR 5/5 FPLC (50 × 5 mm i.d., 10 µm). Mobile phase: 0.05 mol/L TRIS hydrochloride : 0.05 mol/L TRIS hydrochloride with 0.25 mol/L ammonium acetate (100 : 0 to 0 : 100 at 15 min), flow rate 1 mL/min. UV-vis detection (proteins, λ = 295 nm). Carrier gas: 1.1 or 1.15 L/min. Intermediate gas: 1.0 or 0.9 L/min. Outer gas: 15.0 or 14.5 L/min for quadrupole and double-focusing ICP-MS, respectively. Limit of detection, <5 µg/L [Soldado Cabezuelo *et al.* 1997, 1998].

CSF **DPV** See Blood [Zhang *et al.* 2002].

ETAAS Palladium-triton modifier (λ = 396.2 nm). Limit of detection, 2 ppb [Gane *et al.* 1996].

Urine **CE** Capillary: Uncoated. Limit of detection, 2 ppb [Blanco-Gonzalez *et al.* 1998].

DPV See Blood [Zhang *et al.* 2000, 2002].

ETAAS Dry cycle: 110° at 1 s for 20 s to 130° in 15 s for 20 s. Char cycle: 1250° in 30 s for 15 s, change gas from Ar to 5% H_2 in Ar for 5 s. Atomisation cycle: 2300° for 5 s. Normal gas: Ar, 250 mL/min. Purge gas: 5% H_2 in Ar, 250 mL/min. Hollow cathode lamp (λ = 309.3 nm). Limit of detection, 0.06 µg/L [Lin, Huang 2001]. Perkin-Elmer 5100. Limit of detection, 0.25 µg/L [White 1999]. See Plasma. Limit of detection, 26 pg [D'Haese *et al.* 1985]. Dry cycle: 100 for 60 s. See Serum [Gorsky, Dietz 1978].

ICP-MS See Blood [Botta *et al.* 2006; Rainska *et al.* 2007]. Limit of detection, 15 µg/L [De Boer *et al.* 2004], 4.92 µg/L [Liao *et al.* 2004]. Perkin-Elmer Sciex Elan 5000. Nebuliser gas: 1.0 mL/min. Limit of detection, 35 ng/L [White 1999].

Milk **ICP-MS** Plasma gas: 12–13 L/min. Auxiliary gas: 0.9 to 1.0 L/min. Sample gas: 1.0 –1.2 L/min. Limit of detection, 39 µg/L [Krachler *et al.* 2000].

Ascitic Fluid **DPV** See Blood [Zhang *et al.* 2002].

ETAAS Dry cycle: 60° to 90° in 15 s for 5 s to 100° in 10 s for 5 s to 110° in 15 s for 5 s to 140° for 5 s, 200 mL/min. Char cycle: 140° to 1000° in 10 s for 20 s, 100 mL/min. Atomisation cycle: 2700° for 4 s. Lamp current: 10.0 mA (λ = 309.3 nm). Limit of detection, 1.0 µg/L [Milacic, Benedik 1999; Scancar *et al.* 1999].

Brain **ICP-AES** Sample flow rate: 2.0 mL/min. Plasma gas: 15 L/min. Auxiliary gas: 0.5 L/min. Aerosol gas: 0.8 L/min. UV detection (λ = 167 to 395 nm). Limit of quantification, 1 mg/kg [Andrasi *et al.* 2005]. Plasma gas: Ar, 16 L/min. Auxiliary gas: 0 L/min. Nebuliser gas: 0.8 L/min. Limit of detection not reported [Carpenter 1985].

ETAAS Hollow cathode lamp (λ = 309.3 nm). Limit of detection, 5.0 µg/kg [van Ginkel *et al.* 1990]. Dry cycle: 110° for 20 s to 250° for 5 s. Char cycle: 1100° for 20 s. Atomisation cycle: 2600° for 13 s. Aluminium hollow cathode lamp (λ = 309.3 nm). Gasflow: Ar. Limit of detection, 1.9 µg/L [Bouman *et al.* 1986].

Hair **DPV** See Blood [Zhang *et al.* 2000].

Note For a study following the trace element hair analysis of 1 man over 2 decades, see Klevay *et al.* [2004].

Heart **ETAAS** See Brain [Bouman *et al.* 1986].

ICP-AES Plasma gas: Ar, 1.2 L/min. Cooling gas: 14 L/min. Shimadzu ICP-1000III (λ = 396 nm). Limit of detection, 50 µg/L [Minami *et al.* 1996].

Iliac Crest **ETAAS** Dry cycle: 60° to 90° in 10 s for 5 s to 100° in 10 s for 5 s to 150° in 10 s, 200 mL/min. Char cycle: 150° to 1000° in 10 s for 20 s, 100 mL/min. Atomisation cycle: 2700° for 4 s. Lamp current: 10.0 mA (λ = 309.3 nm). Limit of detection, 1.0 µg/L [Scancar *et al.* 2000].

Kidney **ETAAS** See Brain [Bouman *et al.* 1986].

Liver **ICP-AES** See Brain [Carpenter 1985].

ETAAS See CSF [Gane *et al.* 1996]. See Blood. Dry cycle: 100° at 10 s for 10 s, 300 mL/min. Char cycle: 700° in 10 s for 10 s to 1500° in 30 s for 10 s, 300 mL/min. Limit of detection, 50 µg/kg [D'Haese *et al.* 1985].

Rib Bone **ICP-AES** See Brain [Carpenter 1985].

Skeletal Muscle **ICP-AES** See Brain [Carpenter 1985].

Spinal Cord **ETAAS** See Brain [Bouman *et al.* 1986].

Other **HPLC** Environmental and Biological Samples. Column: Spherisorb ODS 2 (150 × 4.6 mm i.d., 5 mm). Mobile phase: methanol : water (pH 1.0, 30 : 70), flow rate 1.0 mL/min. Diode array and fluorometric detection (λ_{ex} = 418 nm, λ_{em} = 490 nm). Limit of detection, 2 nmol/L for Al–morin complex [Lian *et al.* 2003].

DPV Drinking Water, Hydrothorax, Sodium Chloride Injections, Sucrafate, and Synthetic Renal Dialysate. See Blood [Zhang *et al.* 2000].

ETAAS Cocaine Samples. Char cycle: 1400°. Atomisation cycle: 2300° (λ = 309.3 nm). Limit of detection not reported [Bermejo-Barrera *et al.* 1999]. Infusion Solutions and Solutions for Nutritional Support. Char cycle: 1400°. Atomisation cycle: 2500°. Graphite tube pyrolytically coated with L'vov platform (λ = 396 nm). Limit of detection, 0.6 µg/L [Recknagel *et al.* 1994]. Aqueous Solutions. See Blood. Limit of detection, 1.3 µg/L [van der Voet *et al.* 1985].

ICP-AES Argentine Wine. Outer gas: 8.5 L/min. Auxiliary gas: 1.0 L/min. Nebuliser gas: 1.0 L/min (λ = 308.1 nm). Limit of detection, 0.3 µg/L [Lara *et al.* 2005]. Infusion solutions and solutions for nutritional support. Outer gas: Ar, 12 L/min. Auxiliary gas: Ar, 0.3 L/min. Nebuliser gas, 0.8 L/min. Meinhard nebuliser (λ = 167 nm). Limit of detection, 2.5 µg/L [Recknagel *et al.* 1994]. Infant Formulae. Plasma gas: Ar, 18 L/min. Coating gas: Ar, 0.9 L/min. Carrier gas: Ar, 0.1 L/min. Meinhard nebuliser (λ= 237 nm). Limit of quantification, 15 µg/L, limit of detection, 4.5 µg/L [Coni *et al.* 1993]. Infant Milk Formulae. Nebuliser gas: Ar, 0.8 L/min (λ = 308 nm). Limit of detection, 50 µg/L [Bloodworth *et al.* 1991].

ICP-MS Meals from Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 0.12 mg/kg [Noel *et al.* 2003]. Infant Formulae. See Milk [Krachler *et al.* 2000].

Note For a review of detection methods to evaluate the speciation of metals for toxicity assessment and the difficulties in measuring aluminium, see Caruso *et al.* [2006]. For a comparison of 6 catechols as electroactive ligands in the speciation analysis of aluminium, see Liu *et al.* [2002]. For the detection of aluminium fluoride in natural waters by ICP-MS, see Bayon *et al.* [1999]. For a review of the measurement of aluminium in clinical samples, see Taylor and Walker [1992]. For a DC plasma emission spectrometry method for the measurement of aluminium in serum, see Roberts and Williams [1988].

Disposition in the Body In living organisms, aluminium exists as the free ion, low-molecular-weight complexes, physically bound macromolar complexes (e.g. transferrin), and as covalently bound macromolecular complexes. The complexes are active metabolites, particularly the non-polar ones.

Inhalation exposure Occupational exposure to fumes, dust and flakes results in increased serum, tissue and urinary concentrations of aluminium. It has been suggested that the fractional absorption of aluminium from lung to blood is higher in individuals exposed to fumes as opposed to dust. Studies have also shown that aluminium may directly enter the brain via the olfactory tract. However, it has also been demonstrated that exposure to aluminium oxide or chlorhydrate does not necessarily result in significant increases in tissue or serum levels which would indicate lung retention rather than absorption is taking place. This is supported further by the fact that lungs have the highest aluminium concentration when compared with other organs and that the pulmonary concentration of aluminium increases with age.

Oral exposure As little as 0.1 to 0.3% of aluminium ingested in the diet and drinking water is absorbed. More bioavailable forms, particularly complexes with carboxylic acids (e.g. citrate), can reach 1%. In antacid therapy, where patients may consume several grams of aluminium per day, approx. 99% of the ingested aluminium is recovered in the faeces, with blood concentrations rarely rising more than 50% above the pre-antacid level. Aluminium bioavailability is mainly related to the form in which it is ingested and the presence of dietary constituents with which the metal cation can complex. For example, presence of citric acid greatly increases the bioavailability of the cation.

Dermal exposure Aluminium cholrohydrate salts are commonly used in underarm antiperspirants, which are thought to act as an obstructive plug of aluminium hydroxide within the sweat duct. Studies in animals have demonstrated that aluminium can be directly absorbed through the skin.

Excretion Aluminium is excreted by the kidneys and no accumulation occurs in subjects with normal renal function. Uraemic patients are susceptible to aluminium

A

toxicity owing to accumulation in the bone and tissues either from dosage with phosphate binders or exposure to parenteral intake from dialysis fluid.

Normal Concentrations Serum—1–4 μg/L (0.04–0.16 μmol/L); urine—<15 μg/L (0.5 μmol/L). Guideline concentration for potable water—50 μg/L (1.9 μmol/L). Recommended upper limit for dialysis fluid—30 μg/L (1.1 μmol/L). Minimum risk level: 2 mg/kg/day oral.

A study of aluminium in tobacco and cannabis using ETAAS found the following concentrations [Exley *et al.* 2006]:

Brand/type of tobacco/cannabis	*Al content (mg/g product)*
Bangladeshi tobacco	2.3–3.7
Rothmans	0.6–1.5
Natural tobacco	1.2–2.0
Medium tobacco	0.8–1.4
Cannabis (2 types)	0.1–0.4
Cannabinoid extract (THC)	0.1–0.4

Toxicity Uraemic patients undergoing dialysis with fluid prepared from aluminium-rich tap water may develop an encephalopathy, dialysis dementia, which can be fatal. The same illness may ensue in uraemic patients receiving large quantities of phosphate-binding aluminium salts. Aluminum phosphide, which releases phosphine when ingested, is highly toxic, with a fatal dose as low as 1.5 g.

Twenty-seven patients with end-stage renal disease received chronic intermittent haemodialysis with dialysate contaminated with both calcium and aluminium. Ten of the patients died following convulsions, sepsis, and coma. Antemortem serum concentrations of aluminium were 808 μg/L, with concentrations of 255 μg/L found in the serum of the survivors. Postmortem toxicological analysis was performed on four of the patients and the following concentrations were found [De Wolff *et al.* 2002]:

Patient	*Serum (μg/L)*	*Liver (μg/g)*	*Bone (μg/g)*[a]	*Cerebral cortex (μg/g)*[b]
1	517	43.0	21.9	1.09
2	696	32.7	88.7	1.40
3	1275	51.7	7.54	1.12
4	894	4.7	77.0	1.78
Reference values	<10	<2	<2	0.14–0.22

[a]Collected from the femoral shaft.
[b]Collected from the gyrus temporalis inferior and superior.

Serum aluminium concentrations were measured in patients undergoing haemodialysis. Concentrations were 44.5 ± 29.0 μg/L compared with 10.8 ± 2.5 μg/L in controls [Neiva *et al.* 2002].

One hundred patients undergoing chronic haemodialysis had blood and serum concentrations, respectively, of 79 and 77 μg/L, compared with 12.1 and 2.0 μg/L in controls [D'Haese *et al.* 1985].

Serum aluminium levels have also been measured in blood donors, exposed (directly and indirectly) and non-exposed workers. Concentrations were as follows [Ruangyuttikarn *et al.* 2002]:

	N	*Al (μg/L)*
Blood donors	500	8.8 ± 5.7
Directly exposed	62	13.6 ± 4.5
Indirectly exposed	130	9.4 ± 5.3
Not exposed	207	7.9 ± 6.5

Aluminium has been implicated in Alzheimer's disease (AD). Brain samples from 3 control patients (aged 55 to 71 years) and 3 patients with AD (aged 72 to 80 years) were tested for their aluminium, magnesium, and phosphorus content. The concentration of aluminium was consistently higher in AD specimens than in controls. The mean aluminium concentration in brain regions ranged from 1.4 to 2.5 mg/kg in control samples and from 3.5 to 10.2 mg/kg in AD samples [Andrasi *et al.* 2005]:

Brain region	*Control patients*			*AD patients*		
	Al	*Mg*	*P*	*Al*	*Mg*	*P*
Ammon's horn	1.4 ± 0.6	680 ± 100	13190 ± 960	4.9 ± 3.0	557 ± 82	11000 ± 480
Cortex entorhinalis	1.5 ± 0.9	666 ± 106	12560 ± 900	10.2 ± 9.0	540 ± 55	10700 ± 500
Cortex frontalis parasagittalis	1.8 ± 0.6	606 ± 89	12040 ± 850	6.8 ± 4.3	625 ± 35	10860 ± 550
Cortex frontalis basalis	2.5 ± 0.7	673 ± 48	12500 ± 940	6.4 ± 2.9	623 ± 53	10650 ± 730
Globus pallidus	1.8 ± 0.7	628 ± 80	13000 ± 1000	3.5 ± 0.4	552 ± 48	10850 ± 860

In another study of postmortem hippocampal (dentate gyrus) tissue from 12 patients with AD (aged 65 to 93 years), the concentration of aluminium was 0.3 ± 0.08 μg/g in AD compared with 0.12 ± 0.07 μg/g in controls (aged 63 to 89 years) [Corrigan *et al.* 1993].

The parietal cortex of patients with AD has also been found to have higher concentrations of aluminium when compared with controls [Srivastava and Jain 2002].

Aluminium concentrations in 4 brain regions from 6 patients with pathologically verified amyloid lateral sclerosis (ALS) were significantly higher than in controls [Yasui *et al.* 1991]:

Motor area	*ALS*			*Controls (5)*
	Case 1	*Case 2*	*Other 4 cases*	
Precentral gyrus	213.0	169.2	33.8 ± 8.0	22.3 ± 1.5
Internal capsule	69.5	40.5	18.6 ± 2.7	18.0 ± 1.5
Crus cerebri	102.4	–	26.1 ± 1.8	19.4 ± 2.8
Spinal cord	517.8	88.2	33.5 ± 4.2	22.1 ± 1.2

A 39-year-old man committed suicide by ingesting aluminium phosphide. He was discovered 10 days after the event. Postmortem concentrations were reported as follows [Anger *et al.* 2000]:

Biological media	*Phosphorus found (mg/L or mg/g)*	*Usual values*	*Aluminium found (μg/L or μg/g)*	*Usual values*
Blood	76.3	25–45	1537.5	9–15
Urine	564	681–1300	<0.1	<0.1
Brain	4.3	2.36–0.5	36	2 ± 1
Heart	1.37	1.27–0.3	4.6	<1
Surrenal	4.52	—	44	—
Liver	8.22	200–0.05	75	3 ± 1
Kidney	2.05	1.75–0.05	3	3 ± 1

A 24-year-old pregnant woman accidentally exposed to aluminium phosphide visited her physician with tachycardia (218 bpm). She vomited but remained lucid. Clear frothy sputum began emanating from her mouth and nostrils. She was transferred to hospital but shortly after arrival suffered a cardiac arrest and died. Her blood aluminium concentration was 713 μg/L (normal range, 2 to 42 μg/L) [Garry *et al.* 1993].

Note For a review of aluminium phosphide poisoning cases, see Gupta and Ahlawat [1995]; for an investigation of toxic trace elements in the hair of children with autism, see Fido and Al Saad [2005]. For an evaluation of metal levels in welders, see Iarmarcovai *et al.* [2005]. For a comparison of trace metal profiles in hair samples from children in urban or rural areas, see Hasan *et al.* [2004] and for a study on the environmental influences on the trace element content of teeth, see Brown *et al.* [2004]. Rahil-Khazen *et al.* [2002] have studied trace element levels in the autopsy tissue of 30 Norwegians. For a review of aluminium and neurotoxicity, see Yokel [2000]. For a study of serum aluminium concentrations in dialysis patients, see van Landeghem *et al.* [1998]; for concentrations in the urine of glass-manufacturing workers, see Apostoli *et al.* [1998].

Bioavailability Dependent on the route of exposure, see Yokel and McNamara [2001].
Half-life From blood or urine, very short. Because of this, the use of human biomonitoring to estimate aluminium exposure in environmental medicine is not recommended [Wilhelm *et al.* 2004].
Distribution in Blood Blood : plasma ratio approx. 0.95. For a study on the distribution of aluminium between plasma and erythrocytes, see van der Voet and De Wolff [1985].
Protein Binding Approximately 90%, mainly to transferrin and influenced by the concentration of iron [Nagaoka, Maitani 2001; van Landeghem *et al.* 1997].

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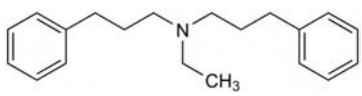
Alverine

Antispasmodic
$C_{20}H_{27}N = 281.4$
CAS—150-59-4

Synonyms Dipropyline; *N*-ethyl-*N*-(3-phenylpropyl)-benzenepropanamine; phenpropamine.



Alverine Citrate

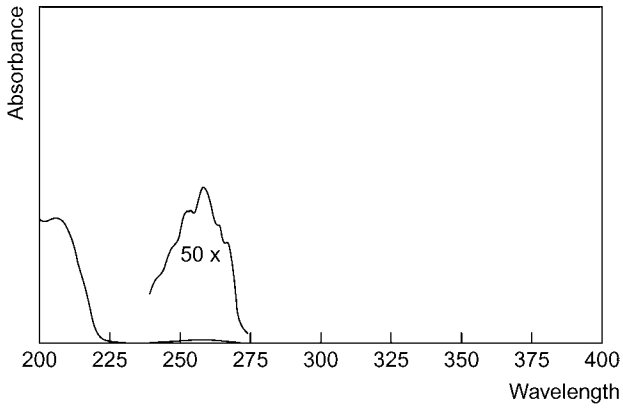
$C_{20}H_{27}N,C_6H_8O_7 = 473.6$
CAS—5560-59-8

Proprietary Names *Profenil*; *Spasmavérine*; *Spasmonal*.
Chemical Properties A white powder. Mp 100° to 103°. Slightly soluble in water and chloroform; sparingly soluble in ethanol; very slightly soluble in ether.

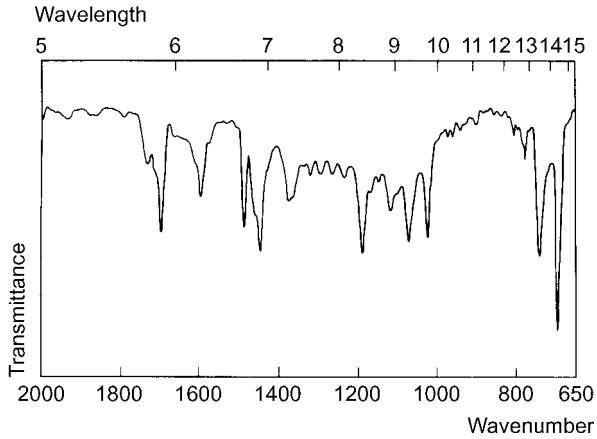
Colour Tests Liebermann's reagent—orange; Mandelin's test—grey–green; Marquis test—orange–red.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.65; system TC—R_f 0.39; system TL—R_f 0.38 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2142.
High Performance Liquid Chromatography System HA—*k* 1.8.

A

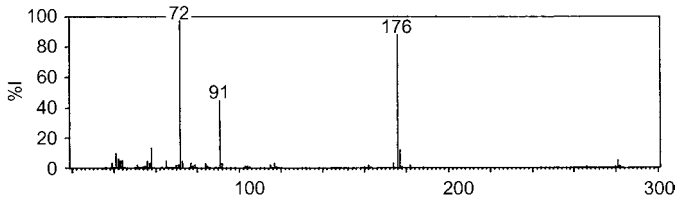
Ultraviolet Spectrum Aqueous acid—253, 259 (A_1^1=14.4 b), 264, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 694, 1190, 740, 1075, 1020, 1694 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 176, 91, 58, 177, 41, 42, 30.



Dose The equivalent of 40 to 240 mg of alverine daily.

Amantadine

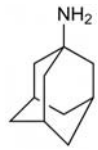
Antiviral, Antiparkinsonian

$C_{10}H_{17}N$ = 151.3

CAS—768-94-5

IUPAC Name Adamantan-1-amine

Synonym 1-Adamantanamine



Chemical Properties Crystals. Mp 160° to 190°, also reported as 180 to 192°. Sparingly soluble in water; soluble in chloroform. pK_a 10.1. Extraction yield (chlorobutane), 0.5 [Demme *et al.* 2005].

Amantadine Hydrochloride

$C_{10}H_{17}N,HCl$ = 187.7

CAS—665-66-7

Synonyms EXP-105-1; NSC-83653.

Proprietary Names *Amazolon*; *Antadine*; *Mantadine*; *Mantadix*; *Mantandan*; *Symmetrel*; *Virofral*.

Chemical Properties A white crystalline powder. Mp 360° with decomposition. Soluble 1 in 2.5 of water, 1 in ~5 of ethanol and 1 in 18 of chloroform; practically insoluble in ether.

Amantadine Sulfate

$C_{10}H_{17}N,^1/_2H_2SO_4$ = 200.3

CAS—31377-23-8

Proprietary Names *Contenton*; *PK-Merz*; *Trivaline*.

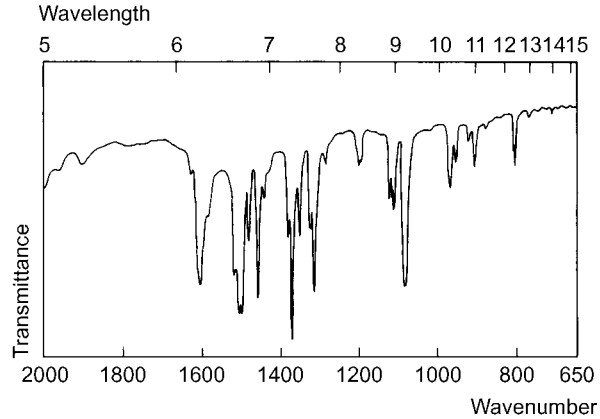
Colour Test Ninhydrin—pink-violet

Thin-layer Chromatography System TA—R_f 0.23; system TB—R_f 0.19; system TC—R_f 0.07; system TE—R_f 0.35; system TL—R_f 0.04; system TAE—R_f 0.07; system TAF—R_f 0.77 (acidified iodoplatinate solution—positive).

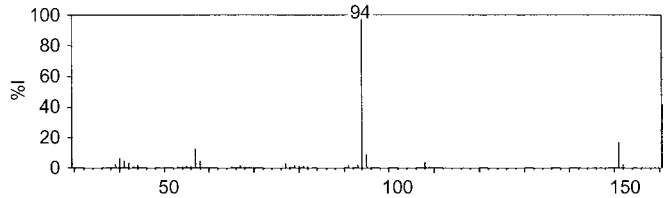
Gas Chromatography System GA—amantadine RI 1240, amantadine-AC RI 1640.

Ultraviolet Spectrum No significant absorption, 230–360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1503, 1497, 1316, 1603, 1089, 1517 cm^{-1} (amantadine hydrochloride, KBr disk).



Mass Spectrum Principal peaks at *m/z* 94, 151, 57, 95, 40, 41, 58, 108.



Quantification

Plasma **GC** Column: HP-1 capillary (10 m × 0.53 mm i.d., 2.65 µm). Carrier gas: He, 20 mL/min. Temperature: 180°. ECD. Limit of quantification, 2.3 µg/L [Rakestraw 1993]. FID. Limit of detection, 100 µg/L [Bélanger, Grech-Belanger 1982]. Column: 5% SE-30 on Chromosorb W HP 80/100 mesh (2 m × 3 mm i.d.). Carrier gas: 40 mL/min. Temperature: 200°. ECD. Limit of detection, 10 µg/L [Sioufi, Pommier 1980].

HPLC Column: stainless steel (27 × 2.1 mm i.d.). Mobile phase: acetonitrile : water (55 : 45 for 1.5 min to 85 : 15 at 5.5 min for 5 min to 55 : 45 at 11.5 min until 13.47 min), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}= 254 nm, λ_{em}= 305–395 nm). Limit of detection, 0.79 ng [Zhou *et al.* 1993].

LC-MS Column: Agilent Zorbax SB-CN (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : 0.5% formic acid (20 : 80), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of quantification, 20 µg/L [Feng *et al.* 2009]. Column: Hypersil-HypurityC_{18} reversed-phase (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 20 µmol/L ammonium acetate (pH 4.0, 45 : 10 : 45) containing 1% acetic acid. Limit of quantification, 3.9 µg/L [Wang *et al.* 2007].

Serum **HPLC** Column: Phenomenex Luna C_8(2) (100 × 2.0 mm i.d., 3 µm). Mobile phase: water : acetonitrile (60 : 40) with 5 g/L formic acid, flow rate 0.2 mL/min. Limit of detection, 20 mg/L [Arndt *et al.* 2005].

Urine **GC** See Plasma. Limit of detection, 4 µg/L [Bélanger, Grech-Belanger 1982]. See Plasma [Sioufi, Pommier 1980].

HPLC Column: TSK gel ODS-80TM (250 × 4.6 mm i.d.). Mobile phase: methanol : water (10 : 1). Fluorescence detection (λ_{ex}= 355 nm, λ_{em}= 405 nm). Limit of detection, 2 mg/L [Fujino *et al.* 1993]. See Plasma [Zhou *et al.* 1993]. Column: Hypersil ODS (150 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L citrate buffer (pH 2.5, 75 : 25), containing 20 mmol/L tetramethylammonium. UV detection (λ= 350 nm). Limit of detection, 75 µg/L [van der Horst *et al.* 1990].

Disposition in the Body Amantadine is slowly but almost completely absorbed after oral administration. It is mainly excreted unchanged in the urine (~56% of a dose being excreted unchanged in 24 h and ~86% in 4 days) although small amounts of metabolites have also been detected. The major metabolic pathway is *N*-acetylation but several unusual routes (*N*-methylation, formation of Schiff bases and *N*-formiates) have also been observed. Amantadine crosses the placenta and the blood–brain barrier. It is also distributed into breast milk.

Therapeutic Concentration

Following a single oral dose of 150 mg given to 6 subjects, peak plasma concentrations of 0.32–0.56 µg/mL (mean, 0.4) were attained in ~3–4 h [Aoki *et al.* 1979].

After oral administration of 100 mg twice daily, steady-state plasma concentrations of 0.1–1.1 μg/mL (mean, 0.5) were observed in 22 patients [Greenblatt *et al.* 1977].

Toxicity

An adolescent who ingested 1.3 g amantadine developed complex ventricular arrhythmias and altered mental status. The arrhythmias were completely suppressed with IV lidocaine [Pimentel, Hughes 1991].

In a fatality involving the ingestion of amantadine, the following postmortem tissue concentrations were reported: blood 21 μg/mL, bile 418.6 μg/mL, liver 135.4 μg/g, urine 1330 μg/mL [Reynolds, van Meter 1984].

Half-life Plasma half-life, 10 to 15 h in patients with normal renal function but significantly prolonged in the elderly and in patients with renal impairment.

Protein Binding ~67%.

Note For studies on the use of amantadine as a medication for cocaine dependence, see Shoptaw *et al.* [2002].

Dose Usually 100 to 200 mg of amantadine hydrochloride daily. Doses up to 400 mg daily have occasionally been used, but this dose should not be exceeded.

Aoki FY *et al.* (1979). Amantadine kinetics in healthy young subjects after long-term dosing. *Clin Pharmacol Ther* 26: 729–736.

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Wang P *et al.* (2007). Quantitative determination of amantadine in human plasma by liquid chromatography–mass spectrometry and the application in a bioequivalence study. *J Pharm Biomed Anal* 43: 1519–1525.

Zhou FX *et al.* (1993). Direct determination of adamantanamine in plasma and urine with automated solid phase derivatization. *J Chromatogr* 619: 93–101.

Amatoxins

Protein Synthesis Inhibitor, Toxin

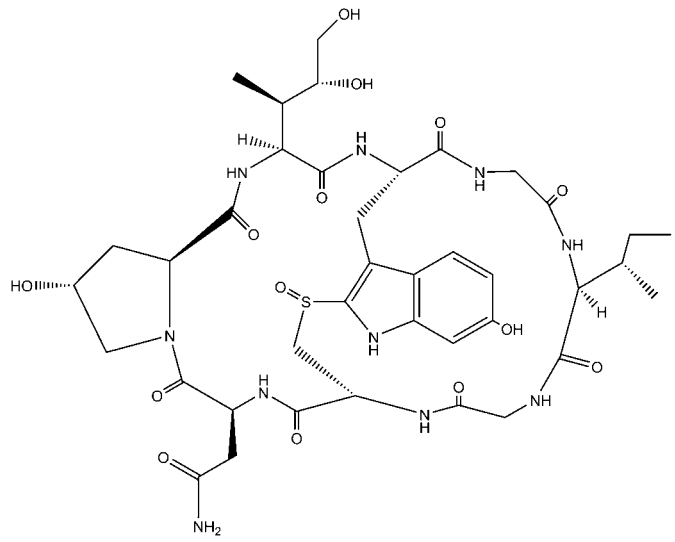
CAS—11030-71-0

Amatoxins are group 1 mushroom toxins from *Amanita, Galerina* and *Lepita* species of fungi, comprising α- β- and γ-amanitin and amanin. α-Amanitin is 10 to 20 times more toxic than phalloidin. Amatoxins are bicyclic octapeptides.

α-Amanitin

$C_{39}H_{54}N_{10}O_{14}S = 919$

CAS—23109-05-9



Chemical Properties Needles from methanol. Mp 254° to 255°. pK_a 9.72 [Morris *et al.* 1978]. Log *P* (octanol/water), −9.28 [Meylan, Howard 1995].

β-Amanitin

$C_{39}H_{53}N_9O_{15}S = 920$

CAS—21150-22-1

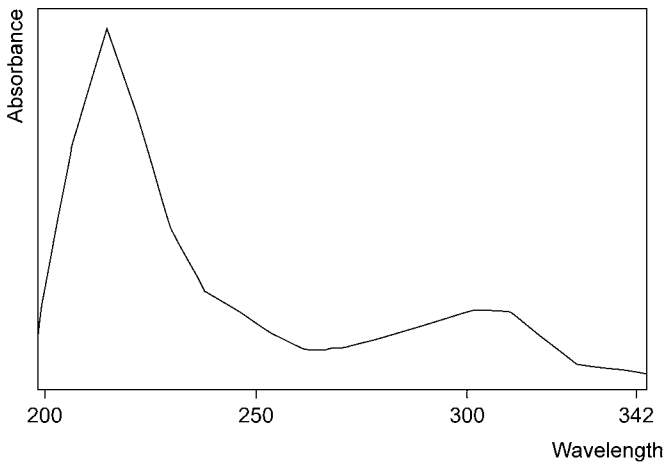
Chemical Properties Needles from methanol. Mp 300°. Soluble in water, methanol, ethanol and aqueous butanol.

Thin Layer Chromatography Plates: silica gel G. Solvent system: methyl ethyl ketone : methanol (1 : 1). Cinnamaldehyde spray (1% in methanol) followed by exposure to hydrochloric acid vapours. R_f 0.20, 0.38 and 0.51 for β- α- and γ-amanitin, respectively. Limit of detection not reported [Benedict *et al.* 1966].

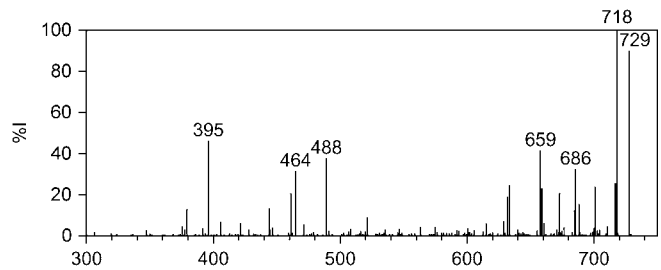
High Performance Liquid Chromatography Column: Spherisorb (25 cm × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water 915 : 85), flow rate 1 mL/min. UV detection (λ= 302 nm). Limit of detection, 0.5 mg/L [Pastorello *et al.* 1982].

Capillary Electrophoresis Capillary: fused silica (84 cm length). Running buffer: aqueous solution of 20 mmol/L ammonium formate (pH 10.8). ESI, negative ion mode, SIM acquisition mode. Limit of detection, 27, 42 and 87 nmol/L for α-, β- and γ-amanitin, respectively [Rittgen *et al.* 2008].

Ultraviolet Spectrum Principal peak at 302 nm.



Mass Spectrum



Quantification

Plasma **HPLC** Column: PLRP-S 100 Å (150 × 4.6 mm i.d.). Mobile phase: 0.05 mol/L phosphate buffer : acetonitrile (pH 9.5; 91 : 9), flow rate 0.5 mL/min. Electrochemical detection. Limit of detection, 2 μg/L [Tagliaro *et al.* 1991].

Serum **HPLC** Column: Develosil RP AQUEOUS. Mobile phase: acetonitrile containing 0.01 mol/L ammonium acetate (pH 5.0). UV detection (λ= 295, 302 and 230 nm). Limit of detection, 0.2 mg/L [Nishizawa, Yamaura 2003]. Column: Ultrasphere ODS C_{18} silica (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02 mol/L aqueous ammonium acetate : acetonitrile (88 : 12), flow rate 1 mL/min. UV detection (λ= 280 nm). Retention time 12.1 and 7.4 min for α- and β-amanitin, respectively. Limit of detection, 10 μg/L for α- and β-amanitin [Jehl *et al.* 1985]. Column: Lichrosorb RP-18 (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.01 mol/L acetic acid–ammonium acetate buffer (pH 5.0; 7 : 93 for 7 min to 25 : 75 at 30 min), flow rate 1 mL/min. UV detection (λ= 302 nm). Retention time: β-amanitin 9.1 min; α-amanitin 14.9 min. Limit of detection, 10 ng on column [Caccialanza *et al.* 1985].

LC-MS Column: Synergi RP-Polar (100 × 4.6 mm i.d.). Mobile phase: 0.01 mol/L ammonium acetate in 0.1% aqueous formic acid : 0.01 mol/L ammonium acetate in 0.1% aqueous methanolic formic acid (40 : 60 for 5 min to 10 : 90 for 2 min to 40 : 60 for 8 min), flow rate 0.5 mL/min. ESI, full scan mode. Retention time: α-amanitin 4.5 min. Limit of detection, 0.26 μg/kg [Filigenzi *et al.* 2007].

Urine **HPLC** Column: Inertsil ODS-3 (150 × 4.6 mm i.d.). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 5.0) : chloroform (84 : 16), flow rate 1.0 mL/min. DAD. Limit of detection not reported [Gonmori, Yoshioka 2003]. Column: HP ODS Hypersil RP_{18} (100 × 2.1 mm i.d., 3 μm). Mobile phase: methanol-ammonium acetate (pH 5.0; 10 : 90) : methanol-ammonium acetate (pH 5.0; 70 : 30; 0 : 100 for 6 min to 100 : 0 for 5.5 min), flow rate 350 μL/min for 3 min to 50 μL/min for 2 min to 150 μL/min for 1 min to 460 μL/min for 3.5 min to 350 μL/min. UV detection (λ= 302 nm). Limit of quantification, 5 μg/L, limit of detection,

A

2.5 μg/L [Maurer *et al.* 2000]. Column: Supelcosil LC_{18} (250 × 4.6 mm i.d.). Mobile phase: 5 mmol/L bisodic phosphate aqueous solution (pH 7.2) : acetonitrile (90 : 10), flow rate 1 mL/min. Electrochemical detection. Limit of quantification, 10 μg/L [Defendenti *et al.* 1998]. Column: Ultrasphere ODS C_{18} silica (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02 mol/L aqueous ammonium acetate : acetonitrile (88 : 12), flow rate 1 mL/min. UV detection (λ = 280 nm). Retention time: 12.1 and 7.4 min for α- and β-amanitin, respectively. Limit of detection, 10 μg/L for α- and β-amanitin [Jehl *et al.* 1985]. Column: Lichrosorb RP-18 (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile: 0.01 mol/L acetic acid-ammonium acetate buffer (pH 5.0; 7 : 93 for 7 min to 25 : 75 at 30 min), flow rate 1 mL/min. UV detection (λ = 302 nm). Retention time: β-amanitin 9.1 min, α-amanitin 14.9 min. Limit of detection, 10 ng on column [Caccialanza *et al.* 1985].

LC-MS Column: Chromcart CC Kromasil RP_{18} (125 × 2 m i.d., 5 μm). Mobile phase: methanol : 0.02 mol/L ammonium acetate (pH 5; 22 : 78), flow rate 75 μL/min. API, ESI, SIM acquisition mode. Limit of detection, 10 μg/L for α- and β-amanitin [Maurer *et al.* 1997a, b].

Gastric Contents **HPLC** Column: Ultrasphere ODS C_{18} silica (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02 mol/L aqueous ammonium acetate : acetonitrile (88 : 12), flow rate 1 mL/min. UV detection (λ = 280 nm). Retention times 12.1 and 7.4 min for α- and β-amanitin, respectively. Limit of detection, 10 μg/L for α- and β-amanitin [Jehl *et al.* 1985].

Liver **LC-MS** Column: Synergi RP-Polar (100 × 4.6 mm i.d.). Mobile phase: 0.01 mol/L ammonium acetate in 0.1% aqueous formic acid : 0.01 mol/L ammonium acetate in 0.1% aqueous methanolic formic acid (40 : 60 for 5 min to 10 : 90 for 2 min to 40 : 60 for 8 min), flow rate 0.5 mL/min. ESI, full scan mode. Retention time: α-amanitin 4.5 min. Limit of detection, 0.50 μg/kg [Filigenzi *et al.* 2007].

Other **TLC** *Amanita verna* Bull. Plates: Silica gel 60 (10 × 10 cm). Solvent system: chloroform : methanol : acetic acid : water (75 : 33 : 5 : 7.5). Densitometric detection. Limit of detection not reported [Seeger, Stijve 1979]. *Amanita phalloides* and *Galerina marginata.* Plates: Silica gel G (8 × 8 in [20 × 20 cm]). Solvent system: methanol : methyl ethyl ketone (1 : 1). Visualised using 1% cinnamaldehyde in methanol and exposure to hydrochloric acid vapour. R_f 0.46 and 0.23 for α- and β-amanitin, respectively. Limit of detection not reported [Sullivan *et al.* 1965].

HPTLC *Amanita phalloides*. Plates: Silicagel 60 for nano-TLC (10 × 10 cm). Solvent system: chloroform : methanol : acetic acid : water (75 : 33 : 5 : 7.5) or 2-butanol : ethylacetate : water (56 : 48 : 20). Visualisation with 1 mL cinnamaldehyde in 100 mL methanol in a tank of fuming hydrochloric acid. Limit of detection, 50 ng [Stijve, Seeger 1979].

HPLC *Amanita exitialis*. Column: YWG C_{18} reversed phase (300 × 4 mm i.d., 10 μm). Mobile phase: 0.02 mol/L aqueous ammonium acetate-acetonitrile (90 : 10) : 0.02 mol/L aqueous ammonium acetate-acetonitrile (76 : 24; 100 : 0 to 95 : 5 at 15 min to 20 : 40 at 40 min to 0 : 100 at 50 min to 100 : 0 at 60 min), flow rate 1.0 mL/min. UV detection (λ = 295 nm). Retention times: 13.86 and 8.41 min for α- and β-amanitin, respectively. Limit of detection not reported [Zhang *et al.* 2005]. Rat Urine. Column: NH2 Dynamax (250 × 4.6 mm i.d., 8 μm). Mobile phase: water : acetonitrile (90 : 10 for 10 min to 70 : 30 at 11 min for 19 min), flow rate 1.0 mL/min. UV detection (λ = 305 nm). Limit of detection, 5 μg/L for α-amanitin [Lee *et al.* 2001]. *Galerina fasciculate* and *Galerina helvoliceps*. Column: μBondasphere C_{18} 100 Å (5 μm). Mobile phase: 20 mmol/L ammonium acetate containing 5% methanol (pH 5.0) : 20 mmol/L ammonium acetate containing 80% methanol (pH 5.85; 80 : 20 for 15 min to 1 : 99 at 30 min for 10 min), flow rate 10 mL/min. DAD (λ = 210–550 nm). Retention times 16.5, 18.5 and 25.2 min for β-, α- and γ-amanitin, respectively. Limit of detection not reported [Muraoka *et al.* 1999]. *A. phalloides.* Column: Ultrasphere ODS reversed phase (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02 mol/L aqueous ammonium acetate-acetonitrile (pH 5.0, 90 : 10) : 0.02 mol/L aqueous ammonium acetate-acetonitrile (pH 5.0, 76 : 24; 100 : 0 for 4 min to 43 : 57 for 16 min to 0 : 100 for 10 min to 100 : 0), flow rate 1 mL/min. UV detection (λ = 214 and 295 nm). Limit of detection, 10 μg/L [Enjalbert *et al.* 1992].

LC-MS Dog Liver. Column: Polar-RP (150 × 4.6 mm i.d.). Ion trap MS. Limit of detection, 0.5 μg/L [Puschner *et al.* 2007].

Note For a study of the distribution of amatoxins and phallotoxins in *A. phalloides,* see Enjalbert *et al.* [1999]; in various other mushrooms, see Faulstich *et al.* [1974]. For a fluorescence method for the detection of amanitins, see Vlaskin *et al.* [2006].

Disposition in the Body Kinetics studies of amanitins in human poisoning have shown that they are present in plasma at low concentrations 24–48 h after ingestion; they are present in urine at high concentrations, and large amounts may be eliminated 3–4 days following ingestion. High concentrations are found in the gastrointestinal aspiration fluid from 48–110 h and large amounts may be eliminated in the faeces [Jaeger *et al.* 1993].

Toxicity People eating *A. phalloides* present with gastrointestinal problems such as vomiting and diarrhoea within 8 to 14 h of ingestion. Amatoxins are taken up into hepatocytes where they inhibit RNA polymerase II, ultimately resulting in a severe acute hepatitis that is indistinguishable from acute viral hepatitis [Lampe, McCann 1987]. If untreated, mortality is as high as 80%. Treatment includes supportive measures, inactivation of the toxin, and, in cases of liver failure, liver transplantation [Yildiz *et al.* 2008]. The lethal dose in humans is 0.1 mg/kg and the toxicity of α-amanitin is increased by lysine-orotate [Halacheva *et al.* 1988].

A 56-year-old man was admitted to hospital 42 h after mushroom ingestion. His main complaints of nausea and vomiting had begun 12 h after the ingestion. His laboratory findings were normal apart from the renal, liver and cardiac function markers, which were all elevated. The patient was treated with activated charcoal, high-dose IV penicillin G, and IV silibinin (5 mg/kg bolus and 20 mg/kg/24 h continuous infusion for 3 days). Because of the elevated cardiac enzymes, he was also given acetylsalicylic acid and metoprolol. After 480 h, the majority of the patient's biochemical parameters had returned to normal. The patient was discharged but with a haemodialysis schedule owing to chronic renal failure [Unverir *et al.* 2007].

Forty-five patients were admitted to hospital in France after accidental intoxication with *A. phalloides*. Amanitin analysis was performed on plasma (43 cases), urine (35 cases), gastroduodenal fluid (12 cases), faeces (12 cases), and tissues (4 cases). The concentration ranges were as follows:

	α-Amanitin	*β-Amanitin*
Plasma (μg/L)	8–190	15.9–162
Urine (mg)	0.02–3.29	0.04–5.21
Gastric fluid	ND	ND
Faeces (μg/L)	28–6986	42–14 900
Liver (μg/kg)	0–19	0–3298
Kidney (μg/kg)	122–1719	1017–1391

ND, not detectable [Jaeger *et al.* 1993].

A middle aged Finnish-born engineer was admitted to hospital in upstate New York 30 h after ingesting ~30 tiny fruiting bodies of *Lepiota josserandii* Bon and Boif, which he had mistakenly identified as *Lepiota excoriate*. His respiratory rate was 40 breaths/min; his systolic blood pressure was 60 mmHg, his abdomen was soft, and his liver was markedly enlarged and diffusely tender. His distal extremities were cold and cyanotic. Initial laboratory findings were as follows:

	Result (normal range)
Haematocrit (%)	60 (40–49)
Sodium (mEq/L)	120 (135–145)
Potassium (mEq/L)	6.7 (3.5–5.2)
Bicarbonate (mEq/L)	7.5 (21–30)
Glucose (mg/L)	390 (650–1150)
Urea nitrogen (mg/L)	820 (100–200)
Arterial pH	7.16 (7.37–7.45)
Platelet count (μL)	30 000 (130 000–350 000)

The prothrombin time and activated partial thromboplastin time were elevated beyond routine measurement capabilities. These findings suggested severe metabolic acidosis, haemoconcentration, coagulopathy, and renal and hepatic failure. Despite invasive haemodynamic monitoring, ventilatory support, and large amounts of IV fluids, the patient became comatose 100 h after ingestion and died 10 h later. Postmortem examination revealed hepatic necrosis consistent with amatoxin mushroom poisoning [Haines *et al.* 1986]

Note For a report on acetylcysteine as a life-saving antidote in *A. phalloides* poisoning, see Montanini *et al.* [1999], although there is some evidence that it is ineffective against α-amanitin [Schneider *et al.* 1992]. For a report of amanita toxicosis in a dog, see Puschner *et al.* [2007]. For a review of the clinical characteristics of amanita and non-amanita mushroom poisoning in Turkish children, see Erguven *et al.* [2007]; in Turkish adults, see Yilmaz *et al.* [2006]. For a review investigating the geographical distribution of mushroom poisoning in Japan, see Gonmori and Yoshioka [2003].

Half-life 32.2 ± 51.9 and 80.2 ± 94.6 μg/h for α- and β-amanitin, respectively.

Protein Binding Amanitin does not bind to plasma proteins.

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Ambazone

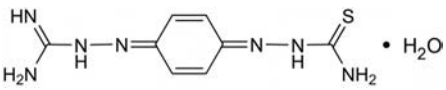
Bacteriostat

$C_8H_{11}N_7S,H_2O = 255.3$

CAS—6011-12-7 (monohydrate); 539-21-9 (anhydrous)

Synonym 2-[4-[(Aminoiminomethyl)hydrazono]-2,5-cyclohexadien-1-ylidene]-hydrazinecarbothioamide monohydrate

Proprietary Names *Bridal*; *Iversal*; *Primal(s)*.

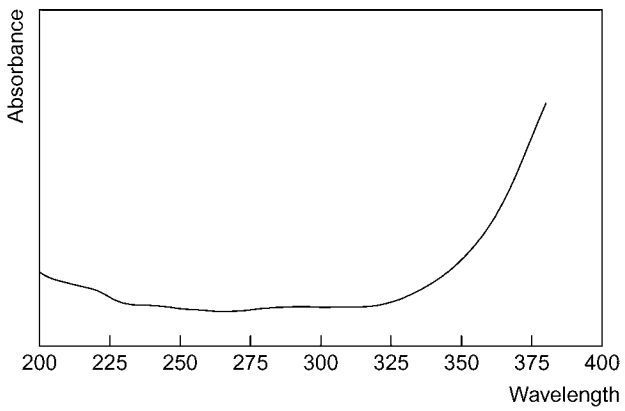


Chemical Properties A brown microcrystalline powder. Mp 192° to 194°, with decomposition. Practically insoluble in water; very slightly soluble in ethanol; soluble in DMF and in solutions of acids and of alkali hydroxides.

Colour Test Palladium chloride—brown.

Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.00; system TC—R_f 0.04; system TE—R_f 0.38; system TL—R_f 0.08; system TAD—R_f 0.00; system TAE—R_f 0.70 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1130, 1515, 1587, 1612, 1639, 1315 cm^{-1}.

Note For the chemical and analytical characterisation of ambazone, see Miosga *et al.* [1988].

Dose Ambazone is given as 10-mg lozenges in doses of 30 to 50 mg daily.

Miosga N *et al.* (1988). Chemical-analytical characterization of dihydroambazone. *Pharmazie* 43: 541–543.

Ambenonium Chloride

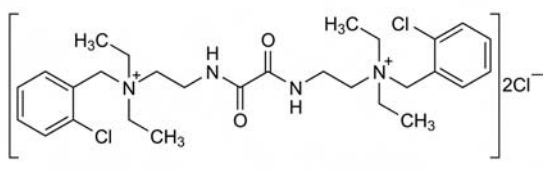
Anticholinesterase

$C_{28}H_{42}Cl_4N_4O_2 = 608.5$

CAS—7648-98-8 (ambenonium); 115-79-7 (chloride, anhydrous); 52022-31-8 (chloride, tetrahydrate)

Synonyms Ambestigmini chloridum; *N,N′*-[(1,2-dioxo-1,2-ethanediyl)bis(imino-2,1-ethanediyl)]bis[2-chloro-*N,N*-diethylbezenemethanaminium] dichloride; oxazyl.

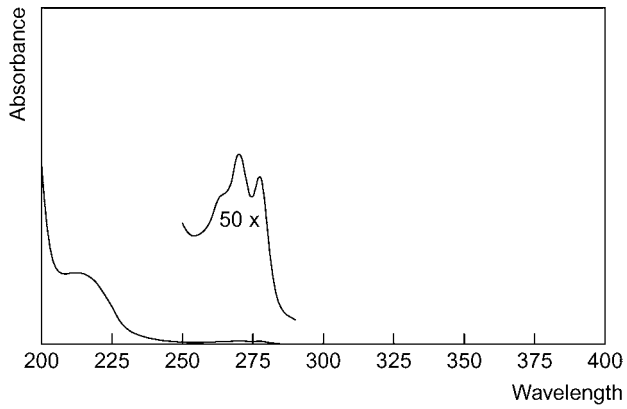
Proprietary Names *Mysuran*; *Mytelase*.



Chemical Properties A white powder. Mp about 200°. Soluble 1 in 5 of water and 1 in 20 of ethanol; slightly soluble in chloroform; practically insoluble in acetone and ether.

Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—271 (A^1_1=30c), 277 nm (A^1_1=29c). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1675, 1505, 767, 806, 1136, 1190 cm^{-1} (KBr disk).

Therapeutic Concentration

Six patients with myasthenia gravis with mean age 47.8 years (34 to 56 years) were administered 10 mg ambenonium chloride (group A) and 7 patients with mean age 48.1 years (25 to 68 years) administered 5 mg (group B) after an overnight fast of 12 h or 30 min after a standard meal. The mean peak concentrations were 9.12 µg/L observed at 85 min for group A when fasting and 2.50 µg/L at 120 min after a meal. For group B, mean concentrations were 5.61 µg/L at 55.7 min when fasting and 1.29 µg/L at 72.9 min after a meal [Ohtsubo *et al.* 1992].

Dose 15 to 100 mg daily; doses of over 200 mg daily have been given.

Ohtsubo K *et al.* (1992). Influence of food on serum ambenonium concentration in patients with myasthenia gravis. *Eur J Clin Pharmacol* 42(4): 371–374.

Ambucetamide

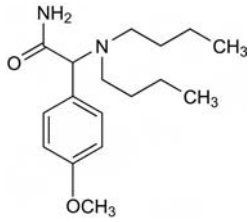
Antispasmodic

$C_{17}H_{28}N_2O_2 = 292.4$

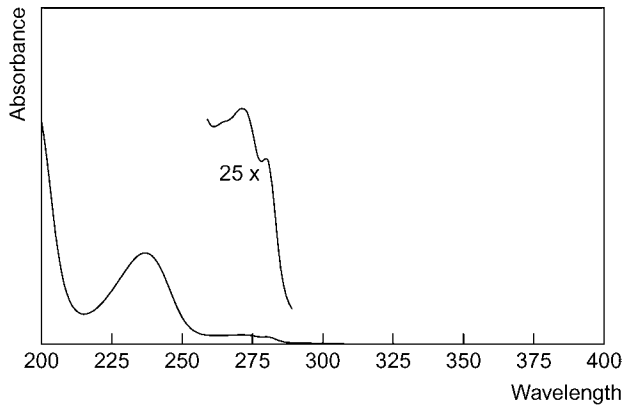
CAS—519-88-0

Synonym 2-Dibutylamino-2-(4-methoxyphenyl)acetamide

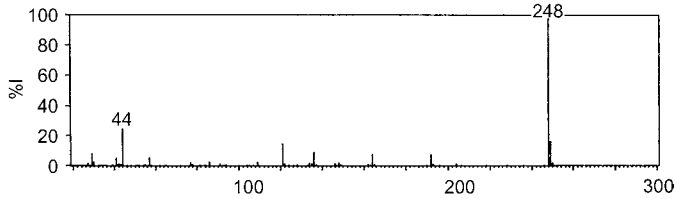
Proprietary Name It is an ingredient of *Femerital*.



Chemical Properties A white crystalline powder. Mp 134°. Practically insoluble in water; soluble in ethanol, chloroform, glacial acetic acid and isopropyl alcohol.
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.05; system TC—R_f 0.68; system TE—R_f 0.76; system TL—R_f 0.61; system TAE—R_f 0.76; system TAF—R_f 0.88 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2330.
Ultraviolet Spectrum Aqueous acid—235 nm; aqueous alkali—279, 286 nm.



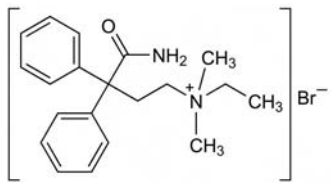
Infrared Spectrum Principal peaks at wavenumbers 1656, 1513, 1176, 1250, 1026, 820 cm^{-1} (KBr disk).
Mass Spectrum Principal peaks at *m/z* 248, 44, 249, 121, 136, 29, 164, 192.



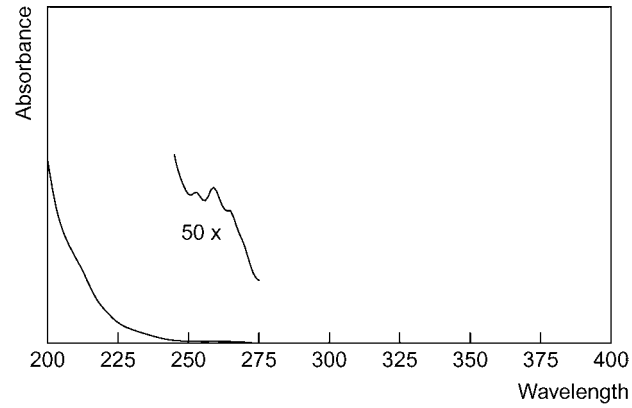
Dose 300 to 600 mg daily.

Ambutonium Bromide

Anticholinergic
$C_{20}H_{27}BrN_2O$ = 391.4
CAS—14007-49-9 (ambutonium); 115-51-5 (bromide)
Synonym γ-(Aminocarbonyl)-*N*-ethyl-*N*,*N*-dimethyl-γ-phenylbenzenepropanaminium bromide
Proprietary Name It is an ingredient of *Aludrox SA* and *Praxiten SP*.



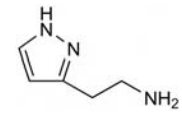
Chemical Properties White crystals. Mp 228° to 229°, with decomposition. Soluble 1 in 1.4 of water, 1 in 27 of ethanol, 1 in 500 of acetone and 1 in 3.5 of chloroform.
Colour Test Liebermann's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.03 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—253, 259 (A^1_1=11.5b), 265 nm.



Infrared Spectrum Principal peaks at wavenumbers 700, 1664, 764, 1590, 1315, 979 cm^{-1}.
Dose 7.5 to 20 mg daily.

Ametazole

Diagnostic Agent (Gastric Secretion)
$C_5H_9N_3$ = 111.1
CAS—105-20-4
IUPAC Name 2-(1*H*-Pyrazol-5-yl)ethanamine
Synonyms Betazole; gastramine; 1*H*-pyrazole-3-ethanamine.

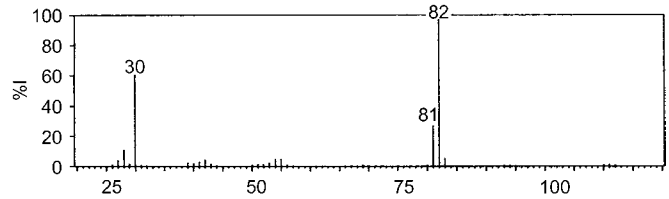


Chemical Properties A viscous liquid. Bp 118° to 123°. pK_a 2.2, 9.6 (20°).

Ametazole Hydrochloride

$C_5H_9N_3$,2HCl = 184.1
CAS—138-92-1
Proprietary Names *Betazol*; *Histalog*.
Chemical Properties A white, hygroscopic, crystalline powder. Mp about 240°. Soluble 1 in 3 of water and 1 in 50 of ethanol; practically insoluble in chloroform; very slightly soluble in ether.

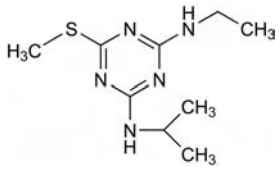
Thin-layer Chromatography System TA—R_f 0.26; system TB—R_f 0.11; system TC—R_f 0.00 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown; ninhydrin spray, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1390.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1563, 1316, 1647, 775, 928, 1044 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 82, 30, 81, 83, 55, 54, 42, 27.



Dose 500 µg/kg of ametazole hydrochloride by subcutaneous or intramuscular injection.

Ametryne

Herbicide
$C_9H_{17}N_5S$ = 227.3
CAS—834-12-8
IUPAC Name 4-*N*-Ethyl-6-methylsulfanyl-2-*N*-propan-2-yl-1,3,5-triazine-2,4-diamine
Synonyms Ametryn; *N*-ethyl-*N*′-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine.
Proprietary Names *Ametrex*; *Evik*; *Gesapax*; *G-34162*.

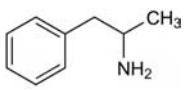


Chemical Properties Colourless crystals. Mp 84° to 86°. Very slightly soluble in water; soluble in chloroform and methanol.
Thin-layer Chromatography System TA—R_f 0.76; system TX—R_f 0.26; system TY—R_f 0.23 (dragendorff spray, positive).
Gas Chromatography System GA—RI 1879.
Infrared Spectrum Principal peaks at wavenumbers 1515, 1595, 1294, 1166, 806, 1140 cm^{-1} (KBr disk).

Amfetamine

Phenethylamine, Stimulant (Central), Sympathomimetic
$C_9H_{13}N = 135.2$
CAS—300-62-9

IUPAC Name 1-Phenylpropan-2-amine
Synonyms Amphetamine; desoxynorephedrine.
Proprietary Names *Elastonon*; *Fenopromin*; *Mydrial*; *Phenamine*. It is an ingredient of *Biphetmaine* and *Durophet*.



Chemical Properties A colourless, mobile, slowly volatile liquid. It absorbs carbon dioxide from the air forming a violent carbonate. Bp 200° to 203°. Soluble 1 in 50 of water; soluble in ethanol and ether; readily soluble in acids. pK_a 9.9 [Baselt 2008]. Log *P* (octanol/water), 1.8 [Baselt 2008]. Extraction yield (chlorobutane), 0.5 [Demme *et al.* 2005].

Amfetamine Hydrochloride

$C_9H_{13}N,HCl = 141.7$
IUPAC Name 1-Phenylpropan-2-amine hydrochloride

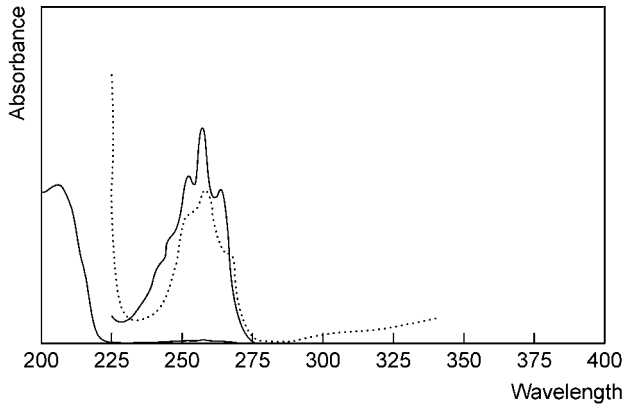
Amfetamine Phosphate

$C_9H_{13}N,H_3PO_4 = 233.2$
CAS—139-10-6
IUPAC Name 1-Phenylpropan-2-amine; phosphoric acid
Synonyms Amphetamine phosphate; monobasic racemic amphetamine phosphate.
Proprietary Names *Dynaphenil*; *Monophos*; *Profetamine*.
Chemical Properties A white crystalline powder with no characteristic melting point; it sinters at ~150° and decomposes at ~300°. Freely soluble in water; slightly soluble in ethanol; practically insoluble in benzene, chloroform and ether.

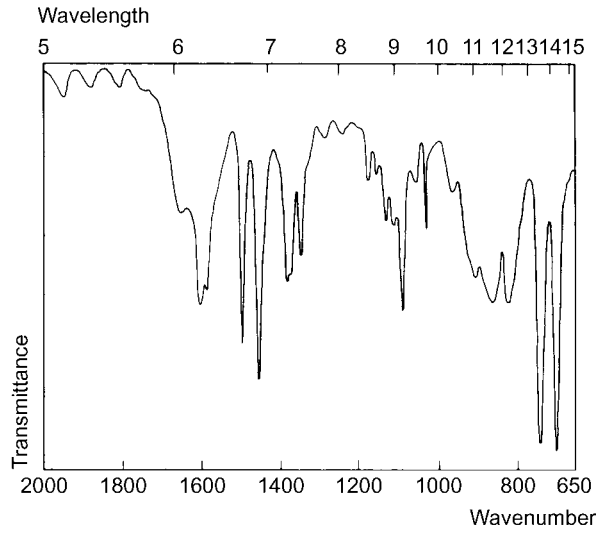
Amfetamine Sulfate

$(C_9H_{13}N)_2,H_2SO_4 = 368.5$
CAS—60-13-9
IUPAC Name 1-Phenylpropan-2-amine sulfuric acid
Synonym Amphetamine sulfate
Proprietary Names *Benzedrine*; *Benzpropamine*; *Centramina*; *Fabedrine*; *Fenamin*; *Phenaminum*; *Phenopromin*; *Sympamine*. It is an ingredient of *Adderall*, *Epipropane* and *Ortenal*.
Chemical Properties A white crystalline powder. Mp above 300°, with decomposition. Soluble 1 in 9 of water and 1 in 515 of ethanol; practically insoluble in chloroform and ether.

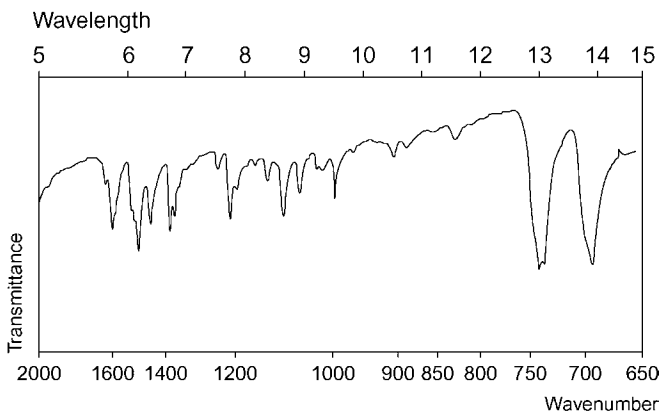
Colour Test Liebermann's reagent—red-orange; Marquis test—orange→brown; ninhydrin—pink-orange.
Thin-layer Chromatography System TA—R_f 0.43; system TB—R_f 0.20; system TC—R_f 0.09; system TE—R_f 0.43; system TL—R_f 0.18; system TAE—R_f 0.12; system TAF—R_f 0.75 (acidified iodoplatinate solution—positive; acidified potassium permanganate solution—positive; Dragendorff spray—positive; FPN reagent— pink; Marquis test—brown; ninhydrin—positive).
Gas Chromatography System GA—RI 1125, amfetamine-trifluroacetic acid RI 1095, amfetamine-PFP RI 1330, amfetamine-TMS RI 1190, amfetamine-AC RI 1501, art (formyl) RI 1100, M (3-OH-)-PFP_2 RI 1520, M (3-OH-)-TMS_2 RI 1850, M (3-OH-)-AC_2 RI 1930, M (4-OH-) RI 1480, M (4-OH-)-AC_2 RI 1900, M (3,4-di-OH-)-AC_3 RI 2150, M (OH-methoxy-) RI 1465, M (OH-methoxy-)-AC_2 RI 2065, M (desamino-oxo-OH-)-AC RI 1520, M (desamino-oxo-OH-methoxy-) RI 1510, M (desamino-oxo-OH-methoxy-)-AC RI 1600, M (desamino-oxo-di-OH-)-AC_2 RI 1735; system GB—RI 1150art (formyl) RI 1142; system GC—RI 1536; system GF—RI 1315; system GAD—RT 7.5 min; system GAK—RT 4.9 min.
High Performance Liquid Chromatography System HA—*k* 0.9; system HB—*k* 8.48; system HC—*k* 0.98; system HX—RI 244; system HAA—RT 3.7 min; system HBC—RT 2.1 min; system HBD—RT 3.7 min.
Ultraviolet Spectrum Aqueous acid—251 nm, ($A\frac{1}{1}$ = 14a), 257 nm, 263 nm.



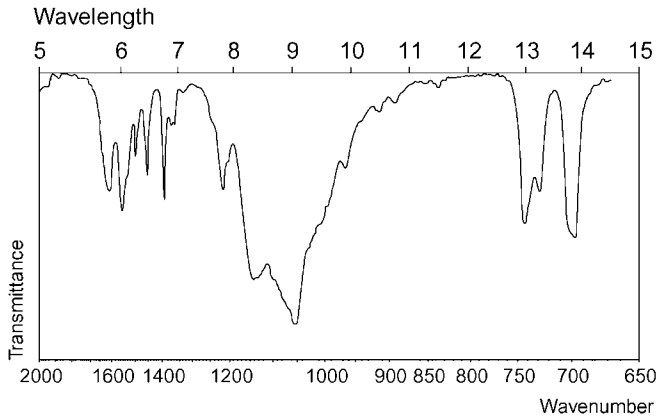
Infrared Spectrum Principal peaks at wave numbers 700, 740, 1495, 1090, 1605, 825 cm^{-1} (thin film, see below).



D-Amfetamine Hydrochloride

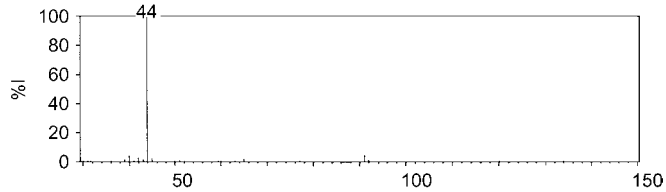


DL-Amfetamine Sulfate



A

Mass Spectrum Principal ions at *m/z* (amfetamine) 44, 91, 40, 42, 65, 45, 39, 43; (phenylacetone) 43, 91, 134, 92, 65, 39, 63, 135.



Quantification

Blood **GC-MS** Column: HP-5 MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 60° for 1 min, to 220° at 20°/min. EI ionisation, SIM acquisition mode. Retention time: 4.66 min for amfetamine, 5.3 min for metamfetamine, 7.14 min for 3,4-methylenedioxymethamfetamine (MDMA) [Gunn *et al.* 2010]. Column: Supelco, PTE-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.4 mL/min. Temperature programme: 60° for 3 min to 280° at 20°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.5 ng/g for amfetamines and metamfetamine [Okajima *et al.* 2001]. Column: Supelco PTE-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 kPa. Temperature programme: 60° for 3 min, to 280° at 20°/min. EI ionisation, SIM acquisition mode. Limit of detection, 10 ng/g [Namera *et al.* 2000]. Column: HP-5 MS capillary (30 m × 0.32 mm i.d.). El ionisation, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection 0.5–8 µg/L [Marquet *et al.* 1997]. Column: fused silica (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 8 psi. Temperature programme: 45° for 3.5 min to 270° at 25°/min. EI ionisation, SIM acquisition mode. Limit of detection, 0.01 µg/g for amfetamine and metamfetamine [Nagasawa *et al.* 1996].

LC-MS Column: Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: 2 mmol/L ammonium formate buffer: acetonitrile (pH 5.3, 92 : 8): methanol (97 : 3 for 3 min, to 80 : 20 over 4 min for 4 min to 70 : 30 over 6 min, to 10 : 90 over 13 min), flow rate 0.3 mL/min. MRM acquisition mode. Limit of quantification, 0.010 mg/g, limit of detection, 0.0008 mg/g [Bjork *et al.* 2010]. Column: Sphinx RP. Mobile phase: 10 mmol/L ammonium formate buffer : acetonitrile. Limit of quantification, 2.5 µg/L, limit of detection, 0.05–0.5 µg/L [Fernandez *et al.* 2009]. Column: Uptisphere ODB C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 2 mmol/L formate buffer (pH 3.0, 20 : 80 for 8 min to 90 : 10 over 5 min to 20 : 80 over 7 min), flow rate 250 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 µg/L [Chèze *et al.* 2007]. Column: BEH C_{18} (50 × 2.1 mm). Mobile phase: aqueous pyrrolidine : methanol (52 : 48), flow rate 0.4 mL/min. Retention time: 1.32 min [Apollonio *et al.* 2006]. Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.001% formic acid (6 : 94 for 5 min to 50 : 50 over 10 min to 6 : 94 over 5 min for 15 min), flow rate 0.3 mL/min. Sonic spray ionisation. Limit of detection, 5 µg/L [Mortier *et al.* 2002].

Plasma **GC** Column: HP (25 m × 0.3 mm i.d., 1.05 µm). Carrier gas: He, 2 mL/min. Temperature programme: 105° for 0.50 min to 300° at 5°/min for 15 min. ECD. Limit of detection, <1 µg/L [Asghar *et al.* 2001]. Column: HP-5 (25 m × 0.32 mm i.d., 0.52 µm). NPD. Limit of quantification, 1 µg/L, limit of detection, 1 µg/L [Cheung *et al.* 1997].

GC-MS See Blood [Gunn *et al.* 2010]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° to 280° at 20°/min for 1.5 min. EI ionisation, SIM acquisition mode. Limit of detection not reported [Kronstrand *et al.* 2003]. Column: SGE-BPX5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 100° for 1 min to 180° at 40°/min to 195° at 5°/min to 310° at 40°/min for 2 min. NICI, SIM acquisition mode. Limit of quantification, 0.049 µg/L for (*R*)-(−)-amfetamine and 0.195 µg/L for (*S*)-(+)-amfetamine [Leis *et al.* 2003]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, 5 µg/L [Peters *et al.* 2003a]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 180° at 30°/min to 230° at 5°/min to 310° at 30°/min. NICI, SIM acquisition mode. Limit of detection not reported [Peters *et al.* 2003b].

HPLC Fluorescence detection. Limit of detection, 0.87 µg/L for amfetamine and metamfetamine [Nakashima *et al.* 2003]. Column: MetaSil Basic (100 × 2 mm i.d., 3 µm). Mobile phase: 0.1% formic acid: methanol (73 : 27), flow rate 0.25 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.2 µg/L [Slawson *et al.* 2002]. Column: ODS. Mobile phase: acetonitrile : citrate buffer (55 : 45). UV detection. Limit of detection, 20 µg/L [Farrell, Jefferies 1983].

LC-MS Column: Alltima RP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L formic acid (10 : 90 for 2 min to 25 : 75 in 16 min to 30 : 70 in 0.1 min to 100 : 0 over 12 min), flow rate 1.0 mL/min. Positive ion mode, MRM acquisition mode. Limit of quantification, 1.0 µg/L, limit of detection, 0.6 µg/L [Sergi *et al.* 2009]. Column: Atlantis dC_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : ammonium formate buffer (5 : 95 for 1 min, to 50 : 50 in 10 min for 1 min, to 5 : 95 in 1 min for 4 min), flow rate 0.2 mL/min. ESI, SIM acquisition mode. Limit of quantification, 2 µg/L, limit of detection, 0.5–1 µg/L [Concheiro *et al.* 2006]. Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.5 µg/L [Wood *et al.* 2003].

Serum **GC-MS** See Blood [Gunn *et al.* 2010]. Column: DB-5 MS (30 m × 0.32 mm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 130° for 2 min, to 320° at 15°/min for 3 min. SIM acquisition mode. Limit of detection not reported [Kankaanpää *et al.* 2004]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° to 120° at 30°/min to 180° at 20°/min to 250° at 30°/min for 0.5 min. SIM acquisition mode. Limit of detection, 0.6 µg/L [Lee *et al.* 2000]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 200° at 20°/min to 240° at 5°/min to 310° at 30°/min for 6 min. SIM acquisition mode. Limit of quantification, 4.9 µg/L, limit of detection, 1.5 µg/L [Weinmann *et al.* 2000].

HPLC Superspher 100 RP C_{18} (125 × 3 mm i.d., 4 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium formate buffer (25 : 75), flow rate 0.3 mL/min. APCI, SIM acquisition mode. Limit of detection, 2 µg/L [Bogusz *et al.* 2000]. Column: Superspher Select B (125 × 3 mm i.d.). Mobile phase: 50 mmol/L ammonium formate buffer (pH 3.0) : acetonitrile (60 : 40), flow rate 0.8 mL/min. UV detection (λ= 250 nm). Limit of detection, 30 µg/L [Bogusz *et al.* 1997]. Column: Superspher Select B (125 × 3 mm i.d.). Mobile phase: 50 mmol/L ammonium formate buffer (pH 3.0) : acetonitrile (60 : 40), flow rate 0.8 mL/min. DAD. Limit of detection, 100 µg/L [Bogusz *et al.* 1997].

Urine **GC** Column: HP-5 (300 × 32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 140° to 300° at 20°/min. PDHID. Limit of detection, 0.5 mg/L [Casari, Andrews 2001]. See Plasma. Limit of quantification, 20 µg/L, limit of detection, 1 µg/L [Cheung *et al.* 1997]. NPD. Limit of quantification, 0.05 mg/L [Jonsson *et al.* 1996]. Column: DB-5 MS (150 × 2.5 mm i.d.). Carrier gas: He, 1 mL/min. Temperature programme: 70° to 200° at 15°/min. FTIR. Limit of quantification, 20 µg/L, limit of detection, 10 µg/L [Kalasinsky *et al.* 1993]. ECD. Limit of detection, 10 µg/L [Terada *et al.* 1982]. See also Ugland *et al.* [1999], Ugland *et al.* [1997] and Van Bocxlaer *et al.* [1997].

GC-MS Column: DB-5 MS (5 m × 100 µm i.d., 0.1 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 85° to 115° at 62°/min for 0.8 min to 300° at 65°/min for 0.52 min. SIM acquisition mode. Limit of detection, 6.25 µg/L [Marais, Laurens 2009]. Column: HP-5 MS (150 mm). Temperature programme: 100° for 1.8 min to 200° at 15 to 30°/min. SIM acquisition mode. Limit of detection, 62.5 µg/L [Stout *et al.* 2002]. See Serum. Limit of quantification, 200 µg/L [Kankaanpää *et al.* 2004]. Column: HP-5 MS (300 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 1 min to 300° at 10°/min for 3 min. EI ionisation, SIM acquisition mode. Limit of detection, 5 µg/L [Nishida *et al.* 2003]. Column: HP-5 MS (300 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 3 min to 280° at 20°/min for 3 min. SIM acquisition mode. Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Huang *et al.* 2002]. See also Centini *et al.* [1996], Dallakian *et al.* [1996], Dasgupta, Spies [1998], Hensley, Cody [1999], Jurado *et al.* [2000], Karacic, Skender [2000], McCambly *et al.* [1997], Myung *et al.* [1998], Namera *et al.* [2002], Pellegrini *et al.* [2002], Valentine *et al.* [1995], Wang *et al.* [2001] and Yashiki *et al.* [1995].

HPLC Column: Supelcosil LC-CN (33 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium acetate (15 : 85), flow rate 0.4 mL/min. ESI, SIM acquisition mode. Limit of quantification, 13 µg/L [Wu *et al.* 2001]. Column: RP C_{18} ODS (250 × 4 mm i.d.). Mobile phase: tetrahydrofuran : acetonitrile : 0.1% TEA (15 : 15 : 70). UV detection (λ= 260 nm). Limit of detection, 10 µg [Veress 2000]. Column: ODS. Mobile phase: 0.1 mol/L Tris-hydrochloride buffer (pH 7.0) : acetonitrile (45 : 55), flow rate 0.2 mL/min. Fluorescence detection [[Al-Dirbashi *et al.* 2000a]. UV detection. Limit of quantification, 47 µg/L, limit of detection, 14 µg/L [Fisher, Bourque 1993]. See Plasma. Limit of detection, 4 µg/L [Farrell, Jefferies 1983]. See also Campins-Falcó [1995], Campins-Falcó *et al.* [1996], Al-Dirbashi *et al.* [1997], Al-Dirbashi *et al.* [1998], Katagi *et al.* [1996] and Molins Legua *et al.* [1995].

LC-MS See Blood. Limit of quantification, 25 µg/L, limit of detection, 0.25–2.5 µg/L [Fernandez *et al.* 2009]. Column: CAPCELL PAK C_{18} MG-II (150 × 2.0 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (95 : 5 to 20 : 80 in 8 min for 1 min), flow rate 0.23 mL/min. ESI, MRM acquisition mode. Limit of quantification, 5.0 µg/L, limit of detection, 0.87 µg/L [Kim *et al.* 2008]. See Blood [Chèze *et al.* 2007]. Column: Symmetry Shield RP C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetronitrile : 0.05% trifluoroacetic acid (10 : 90), flow rate 0.2 mL/min. MRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 1 µg/L [Fuh *et al.* 2006]. Column: Symmetry Shield RP C_{18} (50 × 2.1 mm i.d., 3.5 µm). MRM acquisition mode. Limit of quantification, 9.1 µg/L, limit of detection, 2.8 µg/L [Wu, Fuh 2005].

Bile **GC-MS** See Vitreous Humour [Kiely *et al.* 2009].

LC-MS Column: Shiseido CAPCELL PAK SCX80 (250 × 1.5 mm i.d.). Mobile phase: 25 mmol/L ammonium acetate (pH 4.0) : acetonitrile (3 : 7), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection not reported [Kuwayama *et al.* 2008].

CSF **GC-MS** SIM acquisition mode [Narasimhachari *et al.* 1979].

Meconium **GC-MS** Column: HP-5 MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 5 min, to 260° at 35°/min for 0.5 min. EI ionisation, SIM acquisition mode. Limit of quantification, 75 ng/g, limit of detection, 30 ng/g [Gunn *et al.* 2008]. DB-1 (10 m × 0.18 mm i.d., 0.4 µm). Temperature programme: 120° for 1 min to 150° at 5°/min. Limit of quantification, 100 ng/g, limit of detection, 50 ng/g [Elsohly *et al.* 1999].

LC-MS Column: Synergi Hydro RP (150 × 2 mm i.d., 4 µm). Mobile phase: 1 mmol/L ammonium formate, (pH 3.4) : acetonitrile and 5% 1 mmol/L ammonium formate (100 : 0 to 70 : 30 over 8 min, to 65 : 35 over 2 min for 1 min, to

100 : 0 over 2.5 min), flow rate 0.250 mL/min. MRM acquisition mode. Limit of quantification, 5 ng/g, limit of detection, 1 ng/g [Gray *et al.* 2009]. Column: Phenomenex Synergi Hydro RP (50 × 2.0 mm i.d., 2.0 µm). Mobile phase: 10 mmol/L ammonium acetate with 0.1% formic acid : acetonitrile with 0.1% formic acid (88 : 12), flow rate 0.6 mL/min. Limit of quantification, 0.6 ng/g, limit of detection, 0.2 ng/g [Jones *et al.* 2009]. Column: Synergi Polar RP (150 × 2.0 mm i.d., 4 µm). Mobile phase: 10 mmol/L ammonium acetate containing 0.01% formic acid : acetonitrile (90 : 10 for 1 min to 50 : 50 over 13 min for 2 min to 10 : 90 over 2 min for 2 min to 90 : 10 over 2 min), flow rate 200 µL/min. MRM acquisition mode. Limit of quantification, 12.5 ng/g, limit of detection, 4 ng/g [Kelly *et al.* 2008]. Column: C_{18}. Mobile phase: 10 mmol/L ammonium bicarbonate : methanol. SIM acquisition mode. Limit of quantification, 0.005 µg/g [Pichini *et al.* 2004].

HPLC Column: Supelcosil LC-18 DB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: potassium dihyrogen phosphate : methanol : acetonitrile : tetrahydrofuran : TEA (600 : 100 : 25 : 7 : 1 : 5), flow rate 1 mL/min. DAD (λ = 204 nm). Limit of quantification, 0.5 µg/g [Franssen *et al.* 1994]

Milk **HPLC** Phenomenex Aqua C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 45 mmol/L phosphate buffer (12 : 88), flow rate 1.2 mL/min. UV detection (λ = 200 nm). Limit of quantification, 3 mg/L [Bartu *et al.* 2009].

Oral Fluid **GC-MS** Column: DB-5 (30 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 230° at 10°/min for 10 min. SIM acquisition mode. Limit of detection, 100 µg/L [Meng, Wang 2010]. Column: Varian (15 m × 0.25 mm i.d.). Limit of quantification, 3 µg/L [Cone *et al.* 2007]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 190° at 30°/min, to 230° at 5°/min to 245° at 30°/min to 260° at 5°/min to 310° at 30°/min. NICI mode, SIM acquisition mode. Limit of detection not reported [Peters *et al.* 2007]. Column: HP-5 MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 70° to 170° at 25°/min for 2 min to 195° at 5°/min for 0.5 min to 300° at 30°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, 5.0 µg/L, limit of detection, 2.5 µg/L [Scheidweiler, Huestis 2006]. See Serum. Limit of quantification, 5 µg/L [Kankaanpää *et al.* 2004].

LC-MS ESI. Limit of quantification, 0.4–5 µg/L [Fritch *et al.* 2009]. See Plasma. Limit of quantification, 1.5 µg/L, limit of detection, 0.5 µg/L [Sergi *et al.* 2009]. Column: Pinnacle II C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : methanol containing 0.1% formic acid, flow rate 1.2 mL/min. MRM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 10 µg/L [Kala *et al.* 2008]. Column: Atlantis dC_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : ammonium formate buffer (pH 3.0, 5 : 95 for 1 min to 50 : 50 in 10 min for 1 min to 5 : 95 in 1 min), flow rate 0.2 mL/min. ESI, SIM acquisition mode. Limit of quantification, 1 µg/L, limit of detection, 0.5 µg/L [Concheiro *et al.* 2007]. See Plasma [Wood *et al.* 2003].

Stomach Contents **GC-MS** See Vitreous Humour [Kiely *et al.* 2009]. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.9 mL/min. Limit of detection not reported [Takekawa *et al.* 2007]. Column: DB-5 fused silica (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 1 min, to 100° at 30°/min to 270° at 10°/min. Limit of detection not reported [Meyer *et al.* 1997].

Vitreous Humour **GC-MS** Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° to 120° at 40°/min to 240° at 10°/min for 5 min. Limit of detection not reported [Kiely *et al.* 2009].

HPLC Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3 µm). Fluorescence detection (λ_{ex} = 288 nm, λ_{em} = 324 nm). Limit of quantification, 2.0 µg/L, limit of detection, 0.8 µg/L [Clauwaert *et al.* 2000].

LC-MS Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3 µm). Limit of quantification, 2.0 µg/L, limit of detection, 0.8 µg/L [Clauwaert *et al.* 2000].

Bone and Bone Marrow **GC-MS** [Sato *et al.* 2000].

Brain **GC-MS** See Vitreous Humour [Kiely *et al.* 2009]. Column: HP-1 MS (3 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 70° for 1 min to 175° at 30°/min to 250° at 23°/min to 310° at 18°/min for 5 min. SIM acquisition mode. Limit of quantification, 50 ng/g, limit of detection, 50 ng/g [Lowe *et al.* 2006]. See Stomach Contents [Meyer *et al.* 1997].

Hair **GC** Column: CBJ-17. NPD. Limit of detection, 0.1 µg/g for amfetamine and and 0.4 µg/g for metamfetamine [Koide *et al.* 1998].

GC-MS Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 90° for 0.5 min to 150° at 25°/min for 1.5 min to 230° at 15°/min for 1 min to 300° at 40°/min for 1.3 min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.1 µg/g, limit of detection, 0.027 µg/g [Kim *et al.* 2010a]. G&W Scientific Agilent Technologies 5% phenyl-MS (17 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 20 psi. Temperature programme: 100° for 1 min to 140° at 8°/min to 310° at 30°/min for 2 min. Limit of quantification, 0.2 µg/g for (*R*)-(−) and (*S*)-(+)-enantiomers, limit of detection, 0.1 µg/g for (*R*)-(−) and (*S*)-(+)-enantiomers [Strano-Rossi *et al.* 2009]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 300° at 10°/min for 3 min. Limit of detection, 1.6 µg/g [Nishida *et al.* 2006a]. See Plasma [Kronstrand *et al.* 2003]. See Urine. Limit of detection, 0.25 µg/g [Nishida *et al.* 2003]. See also Cirimele *et al.* [1995], Kintz *et al.* [1995], Liu *et al.* [2001], Musshoff *et al.* [2002], Rohrich, Kauert [1997] and Skender *et al.* [2002].

HPLC Column: SP-120-5-ODS-BP (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (30 : 40 : 30), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 325 nm, λ_{em} = 430 nm). Limit of detection, 11 ng/g [Kaddoumi *et al.* 2004]. See Plasma. Mobile phase: acetonitrile : methanol : citrate buffer (45 : 20 : 37.5). Limit of detection, 0.12 ng/g for amfetamine and metamfetamine [Nakashima *et al.* 2003]. See Urine. Limit of quantification, 0.06 µg/g [Wu *et al.* 2001]. Column: Chiralcel OD-RH (150 × 2 mm i.d.). Mobile phase: phosphate-citrate buffer (pH 4.0) containing sodium hexafluorophosphate : acetonitrile (43 : 57), flow rate 0.1 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 440 nm). Limit of detection, 54.0 ng/g [Al-Dirbashi *et al.* 2000b].

LC-MS See Blood. Limit of quantification, 14.7 ng/g [Chèze *et al.* 2007]. Column: Chiral DRUG (150 × 2 mm i.d.). Mobile phase: 2 mmol/L ammonium formate buffer (pH 5) in methanol : 2 mmol/L ammonium formate buffer (pH 5.0, 40 : 60 for 20 min to 95 : 5 over 55 min), flow rate 0.1 mL/min. ESI, SIM acquisition mode. Limit of detection, 0.05 µg/g for (*R*)-(−) and (*S*)-(+)-enantiomers [Nishida *et al.* 2006b]. Column: Betasil C_8. Mobile phase: water : acetonitrile containing 0.1% formic acid (80 : 20). MRM acquisition mode. Limit of quantification, 25 ng/g, limit of detection, 25 ng/g [Cairns *et al.* 2004]. Column: LiChroCART Purospher 60 RP C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (100 : 0 to 60 : 40 at 15 min to 100 : 0 at 15.2 min for 5 min), flow rate 1 mL/min. APCI, SIM acquisition mode. Limit of detection, 0.10 µg/g [Stanaszek, Piekoszewski 2004]. Column: CAPCELL PAK SCX UG 80 (150 × 1.5 mm i.d., 5 µm). Mobile phase: 12.5 mmol/L ammonium acetate containing formic acid : acetonitrile (40 : 60), flow rate 0.15 mL/min. Positive ion mode, SIM acquisition mode. Limit of detection, 0.02 µg/g [Miki *et al.* 2003].

Kidney **GC-MS** See Stomach Contents [Meyer *et al.* 1997].

Liver **GC-MS** See Vitreous Humour [Kiely *et al.* 2009]. See StomachContents [Meyer *et al.* 1997].

Lung **GC-MS** [Sato *et al.* 2000]

Muscle **GC-MS** [Sato *et al.* 2000]. XTI-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.7 mL/min. Temperature programme: 60° for 1 min to 290° at 20°/min. SIM acquisition mode. Limit of detection not reported [Hara *et al.* 1997].

Nail Clippings **GC-MS** Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 3 min to 170° at 15°/min for 2 min to 210° at 25°/min for 1.5 min to 230° at 20°/min for 0.5 min, to 300° at 40°/min for 0.3 min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.063 µg/g, limit of detection, 0.019 µg/g [Kim *et al.* 2010b]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.1–1.4 mL/min. Temperature programme: 100° for 1 min to 150° at 20°/min for 1.5 min to 285° at 30°/min for 0.5 min to 300° at 30°/min for 2.5 min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.1 µg/g, limit of detection, 0.016 µg/g [Kim *et al.* 2008]. HP-1 MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 60° for 1 min to 300° at 20°/min for 1 min. Limit of detection, 0.2 µg/g [Lin *et al.* 2004]. See Hair. Limit of detection, 12.0 µg/g [Cirimele *et al.* 1995].

Placenta **GC-MS** Column: ZB-5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 2 min, to 160° at 30°/min to 170° at 5°/min to 200° at 20°/min to 210° at 10°/min to 300° at 30°/min for 3 min. EI ionisation. Limit of quantification, 3.4 µg/L, limit of detection, 1.1 µg/L [Joya *et al.* 2010].

Disposition in the Body

Amfetamine is readily absorbed after oral or rectal administration. It is rapidly distributed extravascularly and taken up, to some extent, by red blood cells. The main metabolic reaction is oxidative deamination to form phenylacetone, which is then oxidised to benzoic acid and conjugated with glycine to form hippuric acid; minor reactions include aromatic hydroxylation to form 4-hydroxyamfetamine (an active metabolite), β-hydroxylation, which is stereoselective for the (+)-isomer of amfetamine, to form norephedrine (phenylpropanolamine), and *N*-oxidation to form a hydroxylamine derivative. The products of aromatic hydroxylation and *N*-oxidation may be conjugated with sulfate or glucuronic acid.

Excretion of amfetamine is markedly dependent on urinary pH, being greatly increased in acid urine. After large doses, amfetamine may be detected in urine for several days. Under uncontrolled urinary pH conditions, ~30% of the dose is excreted unchanged in the urine in 24 h and a total of ~90% of the dose is excreted in 3–4 days. The amount excreted unchanged in 24 h may increase to 74% of the dose in acid urine and decrease to 1 to 4% in alkaline urine; under alkaline conditions, hippuric acid and benzoic acid account for ~50% of the urinary material. Under normal conditions, 16–28% is excreted as hippuric acid, ~4% as benzoylglucuronide, 2–4% as 4-hydroxyamfetamine and ~2% as norephedrine in 24 h; small amounts of conjugated 4-hydroxynorephedrine and phenylacetone are also excreted. No elimination in the faeces has been detected.

Amfetamine is a metabolite of benzfetamine, metamfetamine and selegiline.

Therapeutic Concentration After normal therapeutic doses the plasma concentration is usually <0.1 mg/L. However, continued use of amfetamine may cause addiction, and ingestion of 10 times the usual therapeutic dose is common among addicts; in such cases the plasma concentration may be up to 3 mg/L.

The IV administration of 160 mg amfetamine to a regular user resulted in a plasma concentration of 0.59 mg/L after 1 h [Anggård *et al.* 1970].

After a single oral dose containing 10 mg amfetamine to 4 subjects, peak plasma concentrations of ~0.02 mg/L were attained [Wan *et al.* 1978].

Toxicity The estimated minimum lethal dose in non-addicted adults is 200 mg. Toxic effects may be produced with blood concentrations of 0.2–3 mg/L, and fatalities with concentrations greater than 0.5 mg/L. Death from overdosage are comparatively rare.

In a fatality caused by IV administration of amfetamine, the following postmortem tissue concentrations were reported: blood 41 mg/L, liver 23 µg/g and urine 39 mg/L [Adjutantis *et al.* 1975].

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Urine concentrations of >0.5–320 mg/L (mean, 76) were reported in 11 fatalities caused by amfetamine [Holmgren, Lindquist 1975].

In a 22-year-old man who died of cardiorespiratory arrest following amfetamine ingestion, the following postmortem tissue concentrations were reported for racemic, (*R*)- and (*S*)-amfetamine, respectively: blood 2.44, 1.26 and 1.18 mg/L; urine 33.4, 16.7 and 16.7 mg/L; liver 11.7, 6.07 and 5.64 µg/g; kidney 3.85, 2.00 and 1.85 µg/g; and brain 5.50, 2.95 and 2.55 µg/g [Meyer *et al.* 1997].

Half-life Plasma half-life, 4 to 8 h when the urine is acidic and approx. 12 h in subjects whose urinary pH values are uncontrolled.

Volume of Distribution ≈3–4 L/kg.

Distribution in Blood Plasma : whole blood, 1.0

Protein Binding 15–40%.

Saliva Plasma : saliva ratio, ~0.35

Dose 20 to 100 mg of amfetamine sulfate daily has been used in the treatment of narcolepsy.

Note For a review of the toxicokinetics of amfetamines, see Kraemer, Maurer [2002]; for a study of the pharmacokinetic and pharmacodynamic drug interactions in the treatment of attention-deficit hyperactivity disorder, see Markowitz, Patrick [2001].

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Slawson MH *et al.* (2002). Quantitative analysis of selegiline and three metabolites (*N*-desmethylselegiline, methamphetamine, and amphetamine) in human plasma by high-performance liquid chromatography–atmospheric pressure chemical ionization-tandem mass spectrometry. *J Anal Toxicol* 26: 430–437.
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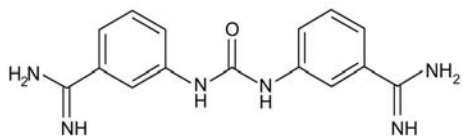
Stout PR *et al.* (2002). Rapid simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine in urine by solid-phase extraction and GC-MS: a method optimized for high-volume laboratories. *J Anal Toxicol* 26: 253–261.
Strano-Rossi S *et al.* (2009). A rapid method for the extraction, enantiomeric separation and quantification of amphetamines in hair. *Forensic Sci Int* 193: 95–100.
Takekawa K *et al.* (2007). Methamphetamine body packer: acute poisoning death due to massive leaking of methamphetamine. *J Forensic Sci* 52: 1219–1222.
Terada M *et al.* (1982). Rapid and highly sensitive method for determination of methamphetamine and amphetamine in urine by electron-capture gas chromatography. *J Chromatogr* 237: 285–292.
Ugland HG *et al.* (1997). Aqueous alkylchloroformate derivatisation and solid-phase microextraction: determination of amphetamines in urine by capillary gas chromatography. *J Chromatogr B Biomed Sci Appl* 701: 29–38.
Ugland HG *et al.* (1999). Automated determination of 'Ecstasy' and amphetamines in urine by SPME and capillary gas chromatography after propylchloroformate derivatisation. *J Pharm Biomed Anal* 19: 463–475.
Valentine JL *et al.* (1995). GC-MS determination of amphetamine and methamphetamine in human urine for 12 hours following oral administration of dextro-methamphetamine: lack of evidence supporting the established forensic guidelines for methamphetamine confirmation. *J Anal Toxicol* 19: 581–590.
VanBocxlaer JF *et al.* (1997). Quantitative determination of amphetamine and alpha-phenylethylamine enantiomers in judicial samples using capillary gas chromatography. *J Anal Toxicol* 21: 5–11.
Veress T (2000). Determination of amphetamine by HPLC after acetylation. *J Forensic Sci* 45: 161–166.
Wan SH *et al.* (1978). Kinetics, salivary excretion of amphetamine isomers, and effect of urinary pH. *Clin Pharmacol Ther* 23: 585–590.
Wang SM *et al.* (2001). Simultaneous supercritical fluid extraction and chemical derivatization for the gas chromatographic-isotope dilution mass spectrometric determination of amphetamine and methamphetamine in urine. *J Chromatogr B Biomed Sci Appl* 759: 17–26.
Weinmann W *et al.* (2000). Automated solid-phase extraction and two-step derivatisation for simultaneous analysis of basic illicit drugs in serum by GC/MS. *Int J Legal Med* 113: 229–235.
Wood M *et al.* (2003). Development of a rapid and sensitive method for the quantitation of amphetamines in human plasma and oral fluid by LC-MS-MS. *J Anal Toxicol* 27: 78–87.
Wu J *et al.* (2001). Determination of stimulants in human urine and hair samples by polypyrrole coated capillary in-tube solid phase microextraction coupled with liquid chromatography–electrospray mass spectrometry. *Talanta* 54: 655–672.
Wu TY, Fuh MR (2005). Determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxymethamphetamine in urine by online solid-phase extraction and ion-pairing liquid chromatography with detection by electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 19: 775–780.
Yashiki M *et al.* (1995). Detection of amphetamines in urine using head space-solid phase microextraction and chemical ionization selected ion monitoring. *Forensic Sci Int* 76: 169–177.

Amicarbalide

Antiprotozoal (Veterinary)
$C_{15}H_{16}N_6O$ = 296.3
CAS—3459-96-9

IUPAC Name 1,3-Bis(3-carbamimidoylphenyl)urea
3,3′-(Carbonyldiimino)bisbenzenecarboximidamide



Amicarbalide Isetionate

$C_{19}H_{28}N_6O_9S_2$ = 548.6
CAS—3671-72-5
Synonym M&B 5062A
Proprietary Name *Diampron*
Chemical Properties A white or slightly cream-coloured powder. Mp 200° to 204°. Soluble 1 in less than 1 of water and 1 in 250 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Aromaticity (method 2)—yellow/orange; Liebermann's test (100°)—yellow.
Thin-layer Chromatography System TA—R_f 0.05, streaking (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—231 nm (A^1_1=1236a), inflexion at 256 nm.
Infrared Spectrum Principal peaks at wavenumbers 1195, 1590, 1672, 1250, 1562, 1052 cm^{-1} (KBr disk).

A

Amidefrine

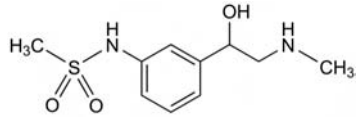
Sympathomimetic

$C_{10}H_{16}N_2O_3S = 244.3$

CAS—3354-67-4

IUPAC Name *N*-[3-[1-Hydroxy-2-(methylamino)ethyl]phenyl]methanesulfonamide

Synonyms Amidephrine; MJ-1996.



Chemical Properties Crystals. Mp 159° to 161°. pK_a 9.1.

Amidefrine Mesilate

$C_{10}H_{16}N_2O_3S,CH_3SO_3H = 340.4$

CAS—1421-68-7

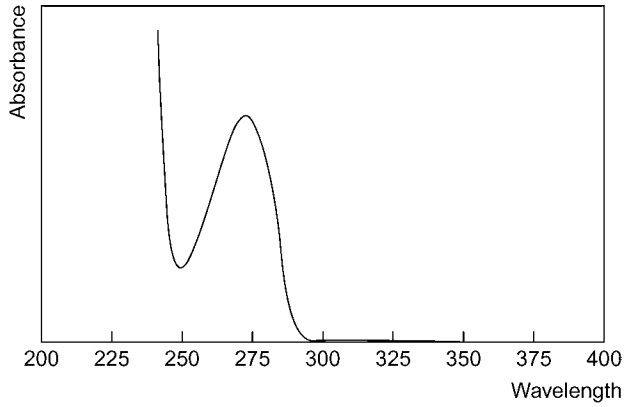
Chemical Properties A white crystalline solid. Mp 207° to 209°. Soluble 1 in about 5 of water; very slightly soluble in chloroform.

Colour Test Mandelin's test—violet.

Thin-layer Chromatography System TA—R_f 0.15; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.00; system TL—R_f 0.02; system TAE—R_f 0.01 (acidified potassium permanganate solution, positive).

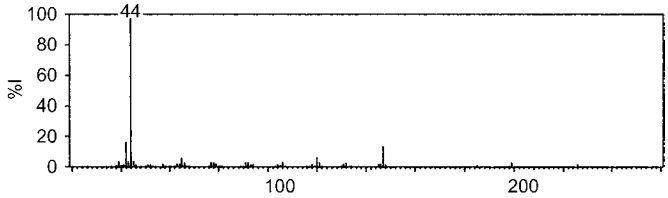
Gas Chromatography System GA—not eluted.

Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=22a).



Infrared Spectrum Principal peaks at wavenumbers 1101, 1153, 1270, 799, 1207, 1316 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 44, 42, 147, 120, 65, 43, 45, 39.



Use A 0.1% solution of amidefrine mesilate has been used as a nasal decongestant.

Amifostine

Cytoprotective

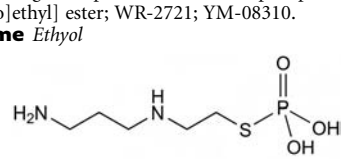
$C_5H_{15}N_2O_3PS = 214.2$

CAS—20537-88-6

IUPAC Name 2-(3-Aminopropylamino)ethylsulfanylphosphonic acid

Synonyms Ethiofos; gammaphos; NSC-296961; phosphorothioic acid *S*-[2-[(3-aminopropyl)amino]ethyl] ester; WR-2721; YM-08310.

Proprietary Name *Ethyol*



Chemical Properties A white to off-white crystalline powder. Mp 160°. It is freely soluble in water, >9 g/100 mL. pK_a(water), 10.14, 7.26. Log *P* (octanol/water), −1.07.

Thin-layer Chromatography (All systems are for the *S*-2-(3-aminopropylamino)-ethanethiol, WR-1065, metabolite.)

Plate 1: silica 60 (0.25 mm). Mobile phase: 0.50 mol/L sodium acetate buffer (pH 5.5): methanol (50 to 90%). R_f 0.03. Plate 2: alumina 60 (0.25 mm). Mobile phase 1: 0.50 mol/L sodium acetate buffer (pH 5.5): methanol (50 to 90%). R_f 0.70 (50% methanol), R_f 0.45 (60%) and R_f 0.03 (70 to 90%). Mobile phase 2: 0.1 mol/L phosphate buffer (pH 5.5): methanol (50 to 90%). R_f 0.03 [Mank *et al.* 1995].

Quantification

Blood **HPLC** Column: LC-ABZ Supelcosil (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol: 10 mmol/L ethylamine in 0.1 mol/L monochloroacetic acid (pH 2.8) (10:90), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=385 nm, λ_{em}=515 nm). Retention time: 4.0 min for amifostine metabolite, WR-1065. Limit of detection, 0.25 mg/L [Bonner, Shaw 2000].

Disposition in the Body Amifostine is rapidly cleared from plasma with <10% remaining in plasma 6 min after administration. It is metabolised in the liver by alkaline phosphatase to the active free thiol metabolite, *S*-2-(3-aminopropylamino)-ethanethiol, WR-1065, and the inactive WR-33278 metabolite. <4% of the parent drug and metabolites are excreted in urine, WR-1065 is found in bone marrow.

Therapeutic Concentration

Five cancer patients were administered a 740 mg/m^2 dose of amifostine and 7 patients with 910 mg/m^2. Peak plasma levels of 0.10 and 0.24 nmol/L were reached [Shaw *et al.* 1994].

Toxicity Hypotension is the main toxic effect.

Half-life Elimination half-life is ~9 min.

Volume of Distribution Steady state between 3.5 and 7 L.

Clearance Plasma clearance 2.17 L/min.

Protein Binding Approximately 4%

Dose Usual dose given is 910 mg/m^2 body surface but can be reduced to 740 mg/m^2, if necessary.

Bonner HS, Shaw LM (2000). Measurement of both protein-bound and total S-2-(3-aminopropylamino)ethanethiol (WR-1065) in blood by high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 739: 357–362.

Mank AJ *et al.* (1995). Determination of the anticancer drug metabolite WR1065 using pre-column derivatization and diode laser induced fluorescence detection. *J Pharm Biomed Anal* 13(3): 255–263.

Shaw L *et al.* (1994). *Proc Am Soc Cancer Res* 13144.

Amikacin

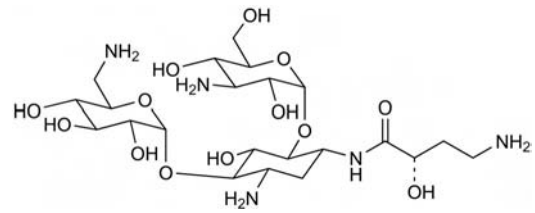
Antibacterial

$C_{22}H_{43}N_5O_{13} = 585.6$

CAS—37517-28-5

IUPAC Name (2S)-4-Amino-N-[(1R,2S,3S,4R,5S)-5-amino-2-[(2S,3R,4S,5S,6R)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-[(2R,3R,4S,5S,6R)-6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy-3-hydroxycyclohexyl]-2-hydroxybutanamide

Synonym *O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-N^1-[(2*S*)-4-amino-2-hydroxy-1-oxobutyl]-2-deoxy-D-streptamine



Chemical Properties A white crystalline powder. Mp 201° to 204°, with decomposition. Sparingly soluble in water.

Amikacin Sulfate

$C_{22}H_{43}N_5O_{13},2H_2SO_4 = 781.8$

CAS—39831-55-5

Proprietary Names *Amiglyde-V*; *Amikin*; *BB-K8*; *Biklin*; *Lukadin*; *Mikavir*; *Novamin*; *Pierami*.

Chemical Properties Freely soluble in water.

Thin-layer Chromatography System TAE—R_f 0.00.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1050, 1630, 1120, 1145, 1545, 940 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** Column: C_{18} µBondapak (300 × 3.9 mm, 10 µm). Mobile phase: acetonitrile: 2-methoxyethanol: tetrahydrofuran: glacial acetic acid: tris(hydroxymethyl): aminoethane (1% aqueous solution) (41:4:52:4:0.21:50). Flow rate 1.0 mL/min for 8.5 min; 0.5 mL/min to 19 min; 3.0 mL/min to 25 min; 1.0 mL/min for 5 min. Internal standard (IS): Cefepine. UV detection. Retention time(s): 16.5 min. Limit of quantification, 0.5 mg/L [Papp *et al.* 1992]. Fluorescence detection (λ_{ex}=340 nm; λ_{em}=418 nm). Limit of quantification, 25 µg/L [Wichert *et al.* 1991].

Serum **GC-MS** ECD. Limit of detection, amikacin, gentamicin and tobramycin, 600 µg/L [Mayhew, Gorbach 1978].

HPLC Fluorescence detection. Limit of detection 1 mg/L for amikacin, gentamicin and tobramycin [Anhalt, Brown 1978].

Urine **HPLC** See Plasma. Limit of quantification, 0.5 mg/L [Papp *et al.* 1992].
Note For a fluoroimmunoassay for amikacin, see Thompson and Burd [1980]. For a bioassay for aminoglycoside antibiotics, see Broughall [1978]. For a review of methods for the determination of aminoglycoside antibiotics, see Maitra *et al.* [1979].
Disposition in the Body Poorly absorbed after oral administration but rapidly absorbed after i.m. injection. About 90% of a dose is excreted in the urine unchanged in 24 h.
Therapeutic Concentration During treatment, the serum concentration should be in the range 15 to 25 mg/L and should be monitored regularly, especially in patients who have renal insufficiency. During multiple dosing, the trough concentration immediately preceding a dose should not exceed 10 mg/L.

A male patient aged 36 years with multi-drug resistant tuberculosis was administered 750 mg amikacin intravenously for 5 days. The mean peak concentration was 39.3 mg/L observed between 0.5 and 1.5 h. [Yew *et al.* 1999].

Eighteen patients with cystic fibrosis, aged 1.7 to 22.2 years, were administered a daily IV dose of 35 mg/kg amikacin over 30 min. The mean peak serum concentration was 121.4 mg/L (range 42.7 to 176 mg/L) after the 30 min and the trough concentration was 0.88 (0.2 to 1.9) mg/L. The peak concentration in sputum was 10.95 mg/L observed at 2 h which decreased slowly to a mean concentration of 2.14 mg/L just before the next infusion [Canis *et al.* 1997].

A patient received 300 mg of amikacin intravenously on its own over a 30-min infusion or in combination with 2000 mg cefepime. The mean plasma amikacin concentration was 27.4 mg/L when the drug was administered alone and 24.6 mg/L when in combination with cefepime. The peak concentrations were observed at the end of the infusion [Papp *et al.* 1992].

A single IM dose of 500 mg given to 6 subjects, produced a mean peak serum concentration of 20 mg/L in 1.5 h [Yates *et al.* 1978].

Toxicity Toxic effects may be produced at serum concentrations of 30 mg/L or more or, during chronic treatment, if the trough serum concentration exceeds 10 mg/L.

The following postmortem tissue concentrations were reported in a patient who died 5 days after discontinuation of amikacin treatment: serum 2.2 mg/L, heart 20 µg/g, kidney 794 µg/g, liver 30 µg/g, lungs 48 µg/g, muscle 11 µg/g [French *et al.* 1981].

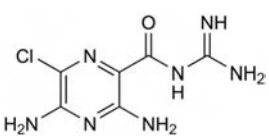
Half-life Plasma half-life, 2 to 3 h.
Volume of Distribution About 0.2 L/kg.
Clearance Plasma clearance, about 1 mL/min/kg.
Protein Binding <10%.
Note For a review of the pharmacokinetics of amikacin, see Andrews [1977].
Dose The equivalent of 15 mg of amikacin/kg daily, given parenterally; maximum of 1.5 g daily. The total dose should not exceed 15 g.

Andrews D (1977). *Can J Hosp Pharm* 30: 146–148.
Anhalt JP, Brown SD (1978). High-performance liquid-chromatographic assay of aminoglycoside antibiotics in serum. *Clin Chem* 24: 1940–1947.
Broughall M (1978). Aminoglycosides. In: Reeves DS *et al*, eds. *Laboratory Methods in Antimicrobial Chemotherapy* Edinburgh: Churchill Livingstone, 194–207.
Canis F *et al.* (1997). Pharmacokinetics and bronchial diffusion of single daily dose amikacin in cystic fibrosis patients. *J Antimicrob Chemother* 39(3): 431–433.
French MA *et al.* (1981). Amikacin and gentamicin accumulation pharmacokinetics and nephrotoxicity in critically ill patients. *Antimicrob Agents Chemother* 19: 147–152.
Maitra SK *et al.* (1979). Determination of aminoglycoside antibiotics in biological fluids: a review. *Clin Chem* 25: 1361–1367.
Mayhew JW, Gorbach SL (1978). Gas-liquid chromatographic method for the assay of aminoglycoside antibiotics in serum. *J Chromatogr* 151: 133–146.
Papp EA *et al.* (1992). High-performance liquid chromatographic assays for the quantification of amikacin in human plasma and urine. *J Chromatogr* 574: 93–99.
Thompson SG, Burd JF (1980). Substrate-labeled fluorescent immunoassay for amikacin in human serum. *Antimicrob Agents Chemother* 18: 264–268.
Wichert B *et al.* (1991). Sensitive liquid chromatography assay for the determination of amikacin in human plasma. *J Pharm Biomed Anal* 9(3): 251–254.
Yates RA *et al.* (1978). Disposition studies with amikacin after rapid intravenous and intramuscular administration to human volunteers. *J Antimicrob Chemother* 4: 335–341.
Yew WW *et al.* (1999). Serum pharmacokinetics of antimycobacterial drugs in patients with multidrug-resistant tuberculosis during therapy. *Int J Clin Pharmacol Res* 19(3): 65–71.

Amiloride

Diuretic
$C_6H_8ClN_7O$ = 229.6
CAS—2609-46-3

IUPAC Name 3,5-Diamino-6-chloro-*N*-(diaminomethylidene)pyrazine-2-carboxamide
Synonyms Amipramidin; amipramizide; 3,5-diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamide; guanamprazine.

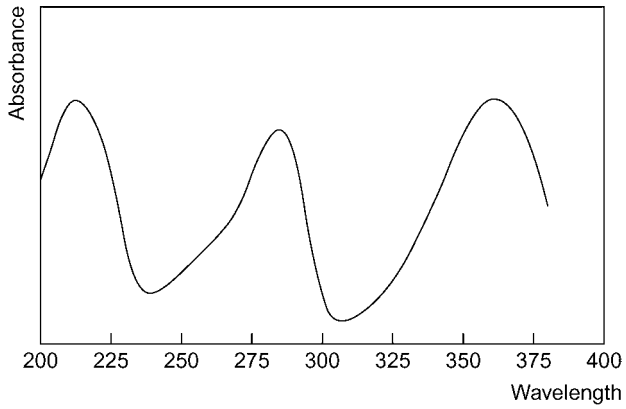


Chemical Properties Mp 240° to 242°. pK_a 8.7.

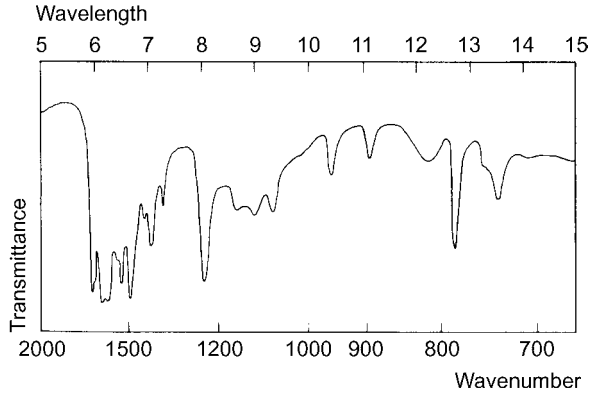
Amiloride Hydrochloride

$C_6H_8ClN_7O,HCl,\ 2H_2O$ = 302.1
CAS—2016-88-8 (anhydrous)
Synonym Amipramizide
Proprietary Names *Arumil*; *Midamor*; *Modamide*. It is an ingredient of *Amilco*, *Frumil*, *Moducren* and *Moduretic*.
Chemical Properties A pale yellow to greenish-yellow powder. Mp 285° to 288°, with decomposition. Soluble in water and ethyl acetate; soluble 1 in 350 of ethanol; practically insoluble in chloroform and ether.

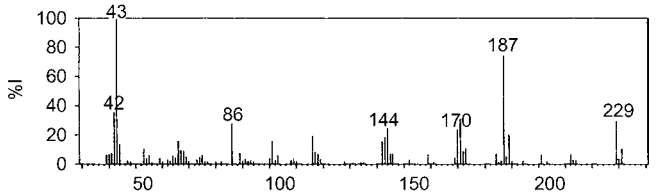
Colour Tests Marquis test—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.24; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.24; system TL—R_f 0.02; system TAD—R_f 0.00; system TAE—R_f 0.06; system TAF—R_f 0.74; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.29 (acidified iodoplatinate solution, strong reaction).
High Performance Liquid Chromatography System HY—RI 190; system HX—RI 257; system HAA—retention time 3.6 min.
Ultraviolet Spectrum Aqueous acid—285 (A^1_1=731a), 361 nm (A^1_1=802a).



Infrared Spectrum Principal peaks at wavenumbers 1634, 1602, 1504, 1686, 1238, 1538 cm^{-1} (amiloride hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 187, 42, 171, 229, 86, 144, 170.



Quantification
Plasma **TLC** See Reuter *et al.* [1982].

HPLC Fluorescence detection (λ_{ex}=360 nm; λ_{em}=413 nm). Limit of detection, 0.03 µg/L [Reeuwijk *et al.* 1992]. Fluorescence detection (λ_{ex}=286 nm; λ_{em}=418 nm). Limit of detection, 0.5 µg/L [Xu *et al.* 1991].

Spectrofluorimetry See Baer *et al.* [1967].
Serum **Spectrofluorimetry** See Plasma [Baer *et al.* 1967].
Urine **GC-MS** Limit of detection, 4 mg/L [Bi *et al.* 1992].

HPLC UV detection (λ=363, 213 and 285 nm). Limit of detection, 0.12 mg/L [Bi *et al.* 1992]. Fluorescence detection (λ_{ex}=286 nm; λ_{em}=418 nm). Limit of detection, 0.5 µg/L [Xu *et al.* 1991]. DAD. Limit of detection, 1.0 mg/L [Park *et al.* 1990].

Spectrofluorimetry See Plasma Baer *et al.* [1967].
Disposition in the Body Incompletely absorbed after oral administration; it does not appear to be metabolised. About 50% of an oral dose is excreted unchanged in the urine and 40% is eliminated in the faeces in 72 h.

A

Therapeutic Concentration

Eight healthy subjects aged 22 to 33 years (mean 25 years) inhaled 1 mg/mL (a volume of 4.5 mL in container) amiloride for 12 min. The mean volume aerolised was 3.5 mL in the 12 min. The peak plasma concentrations of amiloride were 3.36 µg/L after 30 min [Noone *et al.* 1997].

Six adult subjects were administered 10 mg amiloride as a Chinese formulation and an imported formulation. After 4 h, the peak plasma amiloride concentration was 27.76 µg/L for the Chinese and 23.17 µg/L for the imported formulations, respectively. After 24 h, the plasma concentrations had decreased to 5.76 µg/L and 3.57 µg/L, respectively [Xu *et al.* 1991].

After a single oral dose of 20 mg to 6 subjects, peak serum concentrations of about 0.05 mg/L were attained in 4 h [Smith, Smith 1973].

Half-life Plasma half-life, 6 to 10 h.

Volume of Distribution About 5 L/kg.

Protein Binding Not significantly bound.

Dose 5 to 20 mg of amiloride hydrochloride daily.

Baer JE *et al.* (1967). The potassium-sparing and natriuretic activity of N-amidino-3,5-diamino-6-chloropyrazinecarboxamide hydrochloride dihydrate (amiloride hydrochloride). *J Pharmacol Exp Ther* 157: 472–485.

Bi H *et al.* (1992). Determination and identification of amiloride in human urine by high-performance liquid chromatography and gas chromatography-mass spectrometry. *J Chromatogr* 582 (1–2): 93–101.

Noone PG *et al.* (1997). Airway deposition and clearance and systemic pharmacokinetics of amiloride following aerosolization with an ultrasonic nebulizer to normal airways. *Chest* 112(5): 1283–1290.

Park SJ *et al.* (1990). Systematic analysis of diuretic doping agents by HPLC screening and GC/MS confirmation. *J Anal Toxicol* 14(2): 84–90.

Reeuwijk HJ *et al.* (1992). Simultaneous determination of furosemide and amiloride in plasma using high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 575(2): 269–274.

Reuter K *et al.* (1982). [Fluorimetric determination of amiloride in human plasma using thin-layer chromatography]. *J Chromatogr* 233: 432–436.

Smith AJ, Smith RN (1973). Kinetics and bioavailability of two formulations of amiloride in man. *Br J Pharmacol* 48: 646–649.

Xu DK *et al.* (1991). High-performance liquid chromatographic assay for amiloride in plasma and urine. *J Chromatogr* 567: 451–458.

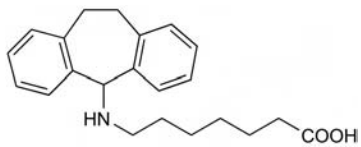
Amineptine

CNS Stimulant, Dopamine Reuptake Inhibitor, Tricyclic Antidepressant

$C_{22}H_{27}NO_2 = 337.5$

CAS—57574-09-1

Synonym 7-[10,11-Dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ylamino]heptanoic acid



Chemical Properties Log P (octanol/water), 2.66 [Meylan, Howard 1995].

Amineptine Hydrochloride

$C_{22}H_{28}ClNO_2 = 373.9$

CAS—30272-08-3

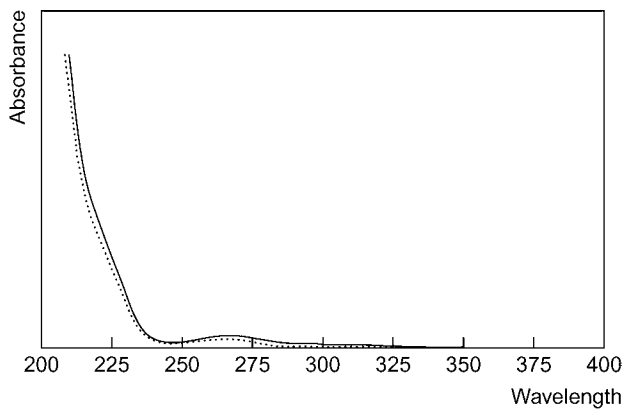
Synonyms S-1694

Proprietary Names *Maneon*; *Survector*.

Chemical Properties Crystals from distilled water. Mp 226° to 230°.

High Performance Liquid Chromatography System HAA—RT 14.0 min.

Ultraviolet Spectrum Aqueous acid—266 nm.



Mass Spectrum Principal peaks at *m/z* 191, 192, 207, 208, 209.

Quantification

Blood **HPLC** Column: Polygosil C_{18} (250 × 4.6 mm i.d., 10 µm). Mobile phase: M/400 TEA–trifluoroacetic acid (pH 2.0) : acetonitrile (100 : 0 to 20 : 80), flow rate 1 mL/min. DAD. Limit of detection not reported [Grislain *et al.* 1990].

Plasma **GC-MS** Column: fused silica capillary OV-1 (25 m × 0.35 mm i.d., 0.20 µm). Carrier gas: N_2, 20 mL/min. EI ionisation at 35 eV. Limit of detection not reported [Tsaconas *et al.* 1989].

HPLC Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.025 mol/L potassium dihydrogen phosphate (45 : 55), flow rate 0.8 mL/min UV detection (λ = 210 nm). *k*: amineptine 1.47, metabolite 0.93. Limit of detection, <0.05 mg/L [Rop *et al.* 1990]. Column: Nucleosil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (38 : 62). UV detection (λ = 220 nm). Limit of detection, ≥0.01 mg/L [Lachatre *et al.* 1989]. Column: Nucleosil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (38 : 62). UV detection (λ = 220 nm). Limit of detection, 10 µg/L [Nicot *et al.* 1984].

Disposition in the Body Amineptine is rapidly absorbed and metabolised to produce 7 metabolites. Metabolism is mainly via β-oxidation of the heptanoic side-chain, resulting in pentanoic and propanoic side-chain metabolites, and hydroxylation of the dibenzocycloheptyl ring to produce 2 diestereoisomers. *N*-Dealkylation of the lateral alkyl chain can also occur to produce dibenzosuberamine (DBZ), which is only found as a trace. Elimination is mainly (70–75%) via urine and complete within ~12 h [Grislain *et al.* 1990].

Therapeutic Concentration

Six healthy males and 6 females, aged between 23 and 46 years, were administered a single dose of 100 mg amineptine hydrochloride. Mean peak plasma concentrations of 0.28–2.22 mg/L amineptine were reached within 0.36–1.40 h. Peak plasma concentrations of the metabolites reached 0.14–1.07 mg/L within 0.44–2.32 h. No significant differences were observed between males and females [Lachatre *et al.* 1989].

Ten healthy volunteers were administered a single oral dose of 200 mg amineptine. Peak plasma concentrations were reached within 1 h, and blood concentrations had fallen below the limit of quantification after 4 h. The amineptine metabolite appeared ~30 min after administration of amineptine and the peak concentration occurred after ~1 h. The concentrations of the metabolite decreased more slowly than the parent compound and could still be found in the blood 10 h after administration [Riché *et al.* 1989].

Six healthy volunteers (5 men, 1 woman) were administered 200 mg amineptine in capsule form. Peak blood concentrations were reached within an hour and decreased and were no longer detectable 240 min after administration. Three of the same volunteers took 300 mg as 3 × 100–mg tablets three times daily for 10 days. Elimination half-lives were 106 ± 10 min for the capsule and 90 ± 7 min for the tablet [Sbarra *et al.* 1981].

Toxicity Severe acne-type lesions are associated with chronic self-administration of high doses (200–1000 mg) [Grimalt *et al.* 1999; Vexiau *et al.* 1988; Vexiau *et al.* 1990]. Amineptine-induced acne, like other iatrogenic acne, is dose dependent and usually appears in adulthood [Bettoli *et al.* 1998]. Lesions occur mainly on the face, back and thorax but also on the extremities and in the perineal region [Farella *et al.* 1996]. Inflammatory lesions are usually absent or scarce [De Gálvez Aranda *et al.* 2001]. Amineptine produces toxic hepatitis after therapeutic doses [Lazaros *et al.* 1996; Ricca Rosellini *et al.* 1990; Sebastián Domingo *et al.* 1994], which can be linked to genetic polymorphism [Larrey *et al.* 1989].

Note For 2 cases of amineptine addiction, see Biondi *et al.* [1990]; for a review of the pharmacology of amineptine, see Garattini [1997].

Half-life 0.5–1.2 h.

Volume of Distribution Apparent, 0.8–4.0 L/kg.

Clearance Plasma clearance, 124.8 L/h.

Dose A usual dose of 100 to 200 mg is administered daily.

Bettoli V *et al.* (1998). Acne due to amineptine abuse. *J Eur Acad Dermatol Venereol* 10: 281–283.

Biondi F *et al.* (1990). Chronic amineptine abuse. *Biol Psychiatry* 28: 1004–1006.

DeGálvez Aranda MV *et al.* (2001). Acneiform eruption caused by amineptine: a case report and review of the literature. *J Eur Acad Dermatol Venereol* 15: 337–339.

Farella V *et al.* (1996). Acne-like eruption caused by amineptine. *Int J Dermatol* 35: 892–893.

Garattini S (1997). Pharmacology of amineptine, an antidepressant agent acting on the dopaminergic system: a review. *Int Clin Psychopharmacol* 12(Suppl3): S15–S19.

Grimalt R *et al.* (1999). Guess what? Macronodular iatrogenic acne due to amineptine *Eur J Dermatol* 9: 491–492.

Grislain L *et al.* (1990). Metabolism of amineptine in rat, dog and man. *Eur J Drug Metab Pharmacokinet* 15: 339–345.

Lachatre G *et al.* (1989). Single-dose pharmacokinetics of amineptine and of its main metabolite in healthy young adults. *Fundam Clin Pharmacol* 3: 19–26.

Larrey D *et al.* (1989). Genetic predisposition to drug hepatotoxicity: role in hepatitis caused by amineptine, a tricyclic antidepressant. *Hepatology* 10: 168–173.

Lazaros GA *et al.* (1996). Amineptine induced liver injury: report of two cases and brief review of the literature. *Hepatogastroenterology* 43: 1015–1019.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Nicot G *et al.* (1984). High-performance liquid chromatographic method for the determination of amineptine and its main metabolite in human plasma. *J Chromatogr* 306: 279–290.

Ricca Rosellini S *et al.* (1990). Hepatic injury associated with amineptine therapy. *Ital J Gastroenterol* 22: 40–43.

Riché C *et al.* (1989). Pharmacokinetics of amineptine after single-dose, repeated treatment and study of the at-risk populations. *Clin Neuropharmacol* 12(Suppl2): S32–S40.

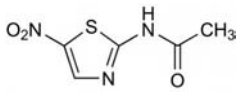
Rop PP *et al.* (1990). Determination of amineptine and its main metabolite in plasma by high-performance liquid chromatography after solid-phase extraction. *J Chromatogr* 532: 351–361.

Sbarra C *et al.* (1981). Pharmacokinetics of amineptine in man. *EurJ Drug Metab Pharmacokinet* 6: 123–126.
Sebastián Domingo JJ *et al.* (1994). Hepatic and pancreatic injury associated with amineptine therapy. *J Clin Gastroenterol* 18: 168–169.
Tsaconas C *et al.* (1989). Gas chromatographic–mass spectrometric assessment of the pharmacokinetics of amineptine and its main metabolite in volunteers with liver impairment. *J Chromatogr* 487: 313–329.
Vexiau P *et al.* (1988). Severe acne-like lesions caused by amineptine overdose. *Lancet* i: 585.
Vexiau P *et al.* (1990). Severe acne due to chronic amineptine overdose. *Arch Dermatol Res* 282: 103–107.

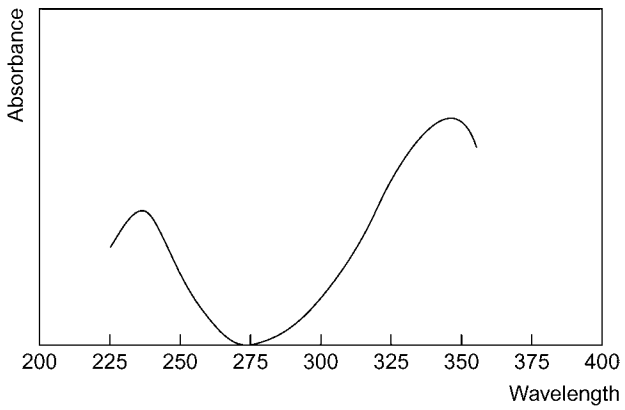
Aminitrozole

Antiprotozoal
$C_5H_5N_3O_3S$ = 187.2
CAS—140-40-9

IUPAC Name N-(5-Nitro-1,3-thiazol-2-yl)acetamide
Synonyms Acinitrazole; nithiamide; *N*-(5-nitro-2-thiazolyl)acetamide.
Proprietary Names *Enheptin-A*; *Trichorad*; *Tricosil*; *Trigamma*; *Tritheon*.



Chemical Properties A buff or yellowish-buff powder. Mp 264° to 265°; slight decomposition may occur. Very slightly soluble in water; soluble 1 in 300 of ethanol and 1 in 900 of chloroform; slightly soluble in ether; soluble in aqueous solutions of sodium hydroxide and ammonia.
Colour Test Methanolic potassium hydroxide—orange.
Thin-layer Chromatography System TA—R_f 0.80 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—237, 345 nm (A^1_1=435b).

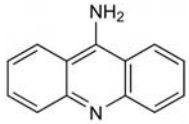


Infrared Spectrum Principal peaks at wavenumbers 1314, 1176, 1709, 1215, 818, 740 cm^{-1}.
Dose Aminitrozole has been given in doses of 200 to 400 mg daily.

Aminoacridine

Antiseptic
$C_{13}H_{10}N_2$ = 194.2
CAS—90-45-9

IUPAC Name Acridin-9-amine
Synonym 9-Acridinamine; aminacrine.



Chemical Properties Yellow needles. Mp 241°. Freely soluble in ethanol; soluble in acetone; slightly soluble in chloroform. pK_a 9.5 (25°).

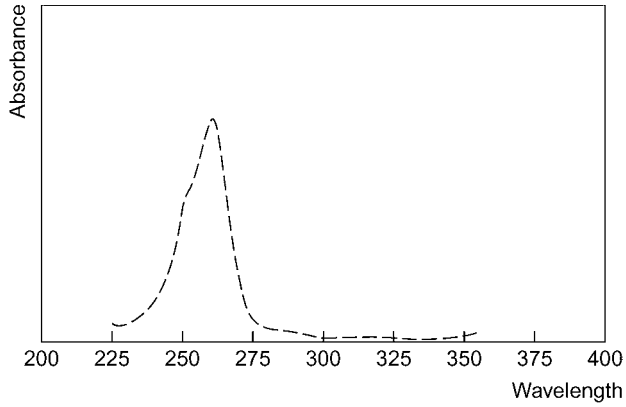
Aminoacridine Hexylresorcinate

$C_{13}H_{10}N_2,C_{12}H_{18}O_2$ = 388.5
CAS—7527-91-5
Synonyms Acrisorcin; aminoacridine 4-hexylresorcinate.
Chemical Properties A yellow crystalline powder. Slightly soluble in water and ethanol.

Aminoacridine Hydrochloride

$C_{13}H_{10}N_2$,HCl, H_2O = 248.7
CAS—134-50-9 (anhydrous)
Synonym Acramine Yellow
Proprietary Names *Aminopt*; *Monacrin*.
Chemical Properties A yellow crystalline powder. Mp about 235°. Soluble 1 in 300 of water; a saturated solution in water is pale yellow with a greenish-blue fluorescence, becoming blue when freely diluted. Soluble 1 in 150 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Aromaticity (method 2)—yellow/red; Liebermann's reagent (100°)—red. Cold nitric acid gives a red colour in 15 s.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.00; system TE—R_f 0.57; system TAE—R_f 0.09 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2240.
High Performance Liquid Chromatography System HX—RI 320.
Ultraviolet Spectrum Aqueous acid—260 (A^1_1=4442a), 313 (A^1_1=74b), 326 nm (A^1_1=81b); methanol—260 (A^1_1=4480b), 311, 325 nm.

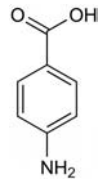


Infrared Spectrum Principal peaks at wavenumbers 760, 1556, 1645, 1655, 750, 1612 cm^{-1} (KBr disk).

Aminobenzoic Acid

Sunscreen Agent
$C_7H_7NO_2$ = 137.1
CAS—150-13-0

IUPAC Name 4-Aminobenzoic acid
Synonyms Amben; PAB; PABA; pabacidum; para-aminobenzoic acid; vitamin H′.
Proprietary Names *Hill-Shade*; *Pabagel*; *Pabanol*; *Paraminan*; *Presun 8*; *RVPaba*.

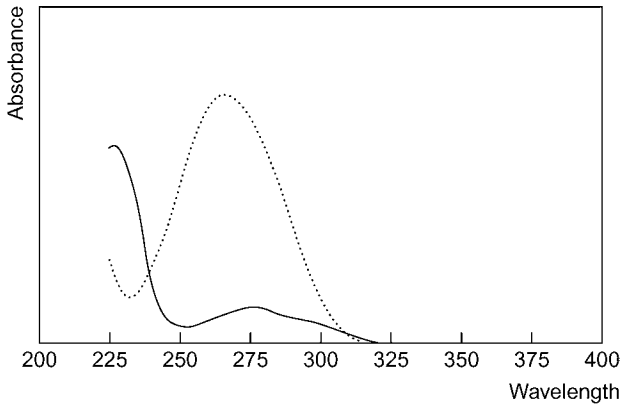


Chemical Properties White or slightly yellow crystals or crystalline powder. It gradually darkens on exposure to air and light. Mp 186° to 189°. Soluble 1 in 200 of water, 1 in 10 of boiling water, 1 in 8 of ethanol, and 1 in 50 of ether; slightly soluble in chloroform; freely soluble in solutions of alkali hydroxides and carbonates. pK_a 2.4, 4.9 (25°).

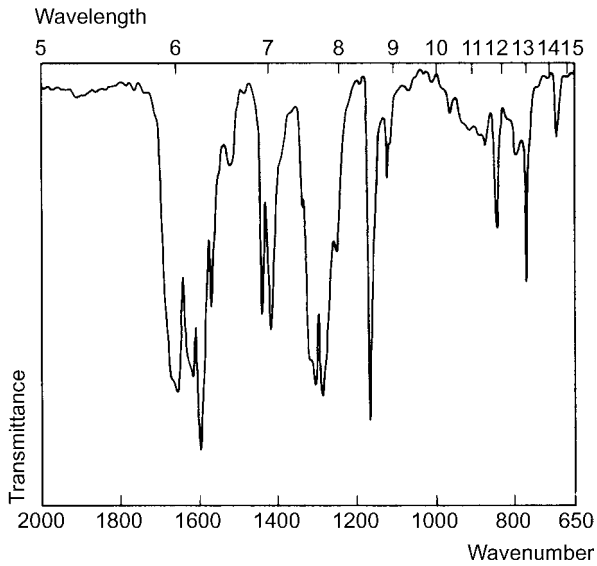
Potassium Aminobenzoate

$C_7H_6KNO_2$ = 175.2
CAS—138-84-1
Proprietary Name *Potaba*
Chemical Properties A crystalline powder. Very soluble in water; less soluble in ethanol; practically insoluble in ether.

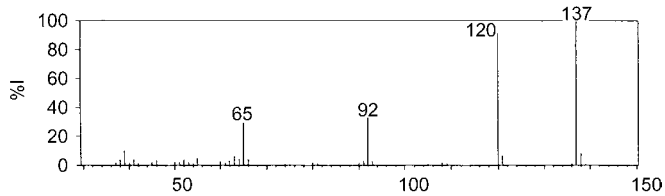
Colour Tests Coniferyl alcohol—orange; diazotisation—red.
Thin-layer Chromatography System TA—R_f 0.58; system TD—R_f 0.19; system TE—R_f 0.01; system TF—R_f 0.43; system TAD—R_f 0.31; system TAJ—R_f 0.44; system TAK—R_f 0.44; system TAL—R_f 0.83 (*p*-aminobenzoic acid) (acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—RI 1547.
High Performance Liquid Chromatography System HY—RI 66 (*m*-aminobenzoic acid), RI 235 (*o*-aminobenzoic acid), RI 212 (*p*-aminobenzoic acid).
Ultraviolet Spectrum Aqueous acid—270 nm (A^1_1=95b); aqueous alkali—265 nm (A^1_1=1063a).



Infrared Spectrum Principal peaks at wavenumbers 1597, 1168, 1290, 1665, 1305, 1625 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 137, 120, 92, 65, 39, 138, 121, 63.



Quantification
Serum **HPLC** UV detection. Aminobenzoic acid and metabolites [Brown, Michalski 1976].
Urine **HPLC** Column: C_{18} Separon-6 RP (150 × 3.2 mm i.d., 5 µm). Mobile phase: methanol: 0.02 mol/L ammonium acetate (20:80 pH 4), flow rate 0.5 mL/min. UV detection (λ=280 nm). *k* value: *p*-aminobenzoic acid, 1.86; *m*-hydroxybenzoic acid, 2.74; *N*-acetyl-*p*-aminobenzoic acid, 3.89. Limit of detection, 12.5 mg/L [Kastel *et al.* 1994]. See Serum [Brown, Michalski 1976].
Disposition in the Body Aminobenzoic acid is readily absorbed after oral administration. It is conjugated with aminoacetic acid to form *p*-aminohippuric acid which is excreted in the urine together with a small amount of *p*-aminobenzoyl glucuronide, *p*-acetamidobenzoyl glucuronide, and traces of *p*-acetamidohippuric acid, *p*-acetamidobenzoic acid and unchanged aminobenzoic acid.

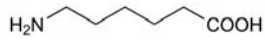
Aminobenzoic acid may be detected in the urine as a metabolite of tetracaine, benzocaine and procaine.
Toxicity Toxic effects are infrequent and are usually associated with plasma concentrations greater than about 600 mg/L.
Uses Aminobenzoic acid is used topically as a 5% solution; potassium aminobenzoate has been given orally, in doses of 12 g daily.

Brown ND, Michalski EJ (1976). An improved high-performance liquid chromatographic method for quantifying p-aminobenzoic acid and some of its metabolites. *J Chromatogr* 121: 76–78.
Kastel R *et al.* (1994). Simultaneous determination of p-aminobenzoic acid and its metabolites in urine by high performance liquid chromatography. *Biomed Chromatogr* 8: 294–296.

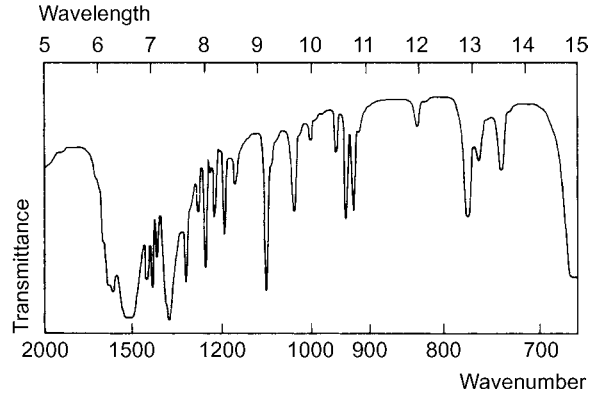
Aminocaproic Acid

Haemostatic
$C_6H_{13}NO_2$ = 131.2
CAS—60-32-2

IUPAC Name 6-Aminohexanoic acid
Synonyms CY-116; EACA; epsilcapramin; epsilon aminocaproic acid.
Proprietary Names *Amicar*; *Capracid*; *Capralense*; *Capramol*; *Ekaprol*; *Epsikapron*; *Hemocaprol*; *Ipsilon*.



Chemical Properties Colourless crystals or white crystalline powder. Mp about 204°, with decomposition. Soluble 1 in 1.5 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether; freely soluble in solutions of acids and alkalis. pK_a 4.4, 10.8 (25°).
Thin-layer Chromatography System TAJ—R_f 0.01; system TAL—R_f 0.45.
Infrared Spectrum Principal peaks at wavenumbers 1538, 1613, 1098, 1319, 1258, 1203 cm^{-1} (KBr disk).



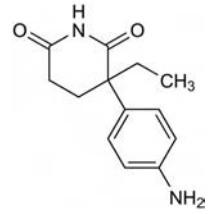
Quantification
Plasma **HPLC** Fluorescence detection. Limit of detection, 50 µg/L [Lam 1990].
Serum **HPLC** Fluorescence detection. Limit of detection, 0.01 ng [Farid 1979]. UV detection. Limit of detection 3 ng [Farid 1979].
Urine **HPLC** See Plasma [Lam 1990].
Disposition in the Body Aminocaproic acid is readily absorbed after oral administration and widely distributed throughout the body fluids. About 70 to 80% of a dose is excreted in the urine unchanged in 12 h.
Therapeutic Concentration In plasma, usually in the range 100 to 400 mg/L.
Half-life Plasma half-life, about 2 to 5 h.
Volume of Distribution About 0.4 L/kg.
Note For a review of aminocaproic acid, see Anon [1981].
Dose 3 to 6 g may be given 4 to 6 times daily.

(1981). Profile on Epsilon aminocaproic acid. *Aust J Pharm* 62: 403–407.
Farid NA (1979). Fluorescamine use in high-performance liquid chromatographic determination of aminocaproic acid in serum. *J Pharm Sci* 68: 249–252.
Lam S (1990). High performance liquid chromatographic assay of Amicar, epsilon-aminocaproic acid, in plasma and urine after pre-column derivatization with o-phthalaldehyde for fluorescence detection. *Biomed Chromatogr* 4(4): 175–177.

Aminoglutethimide

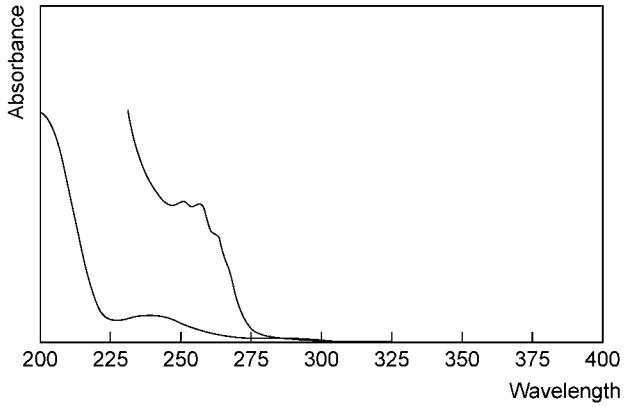
Antineoplastic
$C_{13}H_{16}N_2O_2$ = 232.3
CAS—125-84-8

IUPAC Name 3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione
Synonym 3-(4-Aminophenyl)-3-ethyl-2,6-piperidinedione
Proprietary Names *Cytadren*; *Elipten*; *Orimeten*.

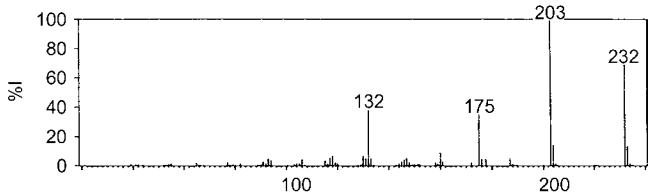


Chemical Properties A white crystalline powder. Mp about 151°. Very slightly soluble in water; freely soluble in organic solvents.
Colour Tests Coniferyl alcohol—yellow; Koppanyi-Zwikker test—violet; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.32; system TE—R_f 0.65; system TF—R_f 0.47; system TAD—R_f 0.53.
Gas Chromatography System GA—RI 2227.
High Performance Liquid Chromatography System HZ—retention time 2.5 min.
Ultraviolet Spectrum Aqueous acid—251 (A^1_1=18b), 257 nm (A^1_1=18b).



Infrared Spectrum Principal peaks at wavenumbers 1695, 1185, 1200, 1515, 1623, 1267 cm^{-1} (KCl disk).
Mass Spectrum Principal ions at *m/z* 203, 232, 132, 175, 204, 233, 160, 118.



Quantification
Plasma **HPLC** Column: Chiralcel OD cellulose tris(3,5-dimethylphenyl carbamate). Mobile phase: hexane: methanol: isopropyl alcohol (65:17.5:17.5), flow rate 0.7 mL/min. UV detection. Retention time: *R*-*N*-acetylaminoglutethimide 17.7 min (*k* 1.2); *S*-*N*-acetylaminoglutethimide 21.5 min (*k* 1.5); (*R*-aminoglutethimide) 32.7 min (*k* 2.6); (*S*-aminoglutethimide) 37.7 min (*k* 3.3). Limit of detection, 0.24 mg/L for *N*-acetylaminoglutethimide and 0.32 mg/L for aminoglutethimide [Alshowaier *et al.* 1995]. UV detection. Limit of detection, <230 µg/L [Robinson, Cornell 1983].
Urine **HPLC** See Plasma [Alshowaier *et al.* 1995].
Saliva **HPLC** See Plasma [Alshowaier *et al.* 1995].
Disposition in the Body Aminoglutethimide is readily absorbed after oral administration. About 10% of an oral dose is excreted in the urine as unchanged drug in 48 h; acetamidoglutethimide has been identified as a metabolite. Aminoglutethimide appears to induce its own metabolism.
Therapeutic Concentration

Postmenopausal women with advanced metastatic breast carcinoma, mean age 53 years, were administered 500 mg aminoglutethimide after an overnight fast. The mean peak concentration of *R*-aminoglutethimide was 3.8 (range 2.5 to 4.3) g/L observed at 1.4 (0.5 to 2.0) h and for *S*-aminoglutethimide 3.3 (2.4 to 4.3) g/L at 1.3 (1 to 2) h [Alshowaier *et al.* 1999].

After a single oral dose of 500 mg given to 6 subjects, peak plasma concentrations of 5.8 to 6.3 (mean 5.9) mg/L were attained in 1.3 h [Thompson *et al.* 1981].

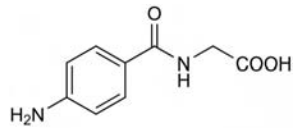
Steady-state serum concentrations of 4.7 to 32.4 (mean 11.5) mg/L were reported in 7 subjects receiving chronic treatment with oral doses of 1 g daily [Murray *et al.* 1979].

Toxicity The estimated minimum lethal dose is 5 g.
Half-life Plasma half-life about 13 h following a single dose but appears to decrease during chronic treatment.
Volume of Distribution About 1.4 L/kg.
Clearance Plasma clearance about 1.3 mL/min/kg.
Distribution in Blood Plasma:whole blood ratio, about 0.7.
Protein Binding About 25%.
Dose 0.5 to 1 g daily.

Alshowaier IA *et al.* (1995). Liquid chromatographic separation and measurement of optical isomers of aminoglutethimide and its acetyl metabolite in plasma, saliva, and urine. *Ther Drug Monit* 17: 538–543.
Alshowaier IA *et al.* (1999). Pharmacokinetics of *S*- and *R*- entantiomers of aminoglutethimide following oral administration of racemic drug in breast cancer patients. *J Clin Pharmacol* 39(11): 1136–1142.
Murray FT *et al.* (1979). Serum aminoglutethimide levels: studies of serum half-life, clearance, and patient compliance. *J Clin Pharmacol* 19: 704–711.
Robinson BA, Cornell FN (1983). Liquid-chromatographic determination of aminoglutethimide in plasma. *Clin Chem* 29: 1104–1105.
Thompson TA *et al.* (1981). Aminoglutethimide bioavailability, pharmacokinetics, and binding to blood constituents. *J Pharm Sci* 70: 1040–1043.

Aminohippuric Acid

Diagnostic Agent (Renal Function)
$C_9H_{10}N_2O_3$ = 194.2
CAS—61-78-9
IUPAC Name 2-[(4-Aminobenzoyl)amino]acetic acid
Synonyms *N*-(4-Aminobenzoyl)glycine; *p*-aminobenzoylglycine; PAHA; para-aminohippuric acid.



Chemical Properties A white crystalline powder, which discolours on exposure to light. Mp about 195°, with decomposition. Soluble 1 in 45 of water, 1 in 50 of ethanol and 1 in 5 of dilute hydrochloric acid; very slightly soluble in carbon tetrachloride, chloroform and ether; freely soluble, with decomposition, in solutions of alkali hydroxides and carbonates. pK_a 3.6.

Sodium Aminohippurate

$C_9H_9N_2NaO_3$ = 216.2
CAS—94-16-6
Proprietary Name *Nephrotest*
Chemical Properties Soluble in water.

Colour Test Diazotisation—red.
Thin-layer Chromatography System TA—R_f 0.55 (Van Urk reagent, yellow).
Ultraviolet Spectrum Methanol—280 nm (A^1_1=880b).
Quantification
Plasma **HPLC** Column: Ultracarb ODS 30 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 7 mmol/L 1-decanesulfonic acid (pH 3.7) : acetonitrile (82:18), flow rate 1.0 mL/min. Internal standard (IS): *p*-aminobenzoic acid. UV detection (λ=280 nm). Retention time: 2.4 min. Limit of detection, 1 mg/L [Pastore *et al.* 2001]. UV detection (λ=285 nm). Limit of quantification, 6.25 mg/L [Dowling *et al.* 1999]. UV detection. Limit of detection, 1 mg/L [Prueksaritanont *et al.* 1984].
Serum **HPLC** UV detection (λ=275 nm). Limit of detection, 0.18 mg/L [Marsilio *et al.* 1997].
Urine **HPLC** See Plasma [Pastore *et al.* 2001]. See Plasma [Dowling *et al.* 1999]. See Serum [Marsilio *et al.* 1997]. See Plasma [Prueksaritanont *et al.* 1984].
Disposition in the Body Aminohippuric acid is a major metabolite of aminobenzoic acid. After IV administration it is rapidly excreted in the urine in subjects with normal renal function.
Half-life Biological half-life, about 0.2 h.
Dose 2 to 30 g of sodium aminohippurate intravenously, to measure renal function.

Dowling TC *et al.* (1999). *J Chromatogr B Biomed Sci Appl* 124(6): 833–836.
Pastore A *et al.* (2001). Simultaneous determination of inulin and p-aminohippuric acid in plasma and urine by reversed-phase high-performance liquid chromatography. *J Chromatogr B, Biomed Sci Appl* 751: 187–191.
Prueksaritanont T *et al.* (1984). Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids. *J Chromatogr* 306: 89–97.
Marsilio R *et al.* (1997). Rapid determination of p-aminohippuric acid in serum and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 704: 359–364.

Aminometradine

Pyrimidinedione, Diuretic
$C_9H_{13}N_3O_2$ = 195.2
CAS—642-44-4
IUPAC Name 6-Amino-3-ethyl-1-(2-propenyl)-2,4(1*H*,3*H*)-pyrimidinedione
Synonyms Aminometramide; SC 3497.
Proprietary Names *Mictine*; *Mincard*.
Chemical Properties White crystals from water. Mp 75° to 115° (monohydrate), Mp 143° to 144° (anhydrous) [O'Neil *et al.* 2006]. Soluble 1 in 50 of water, 1 in 2.5 of ethanol and 1 in 8 of chloroform. Log *P* (octanol/water), 0.08 [Meylan, Howard 1995].
Ultraviolet Spectrum Ethanol—267 nm.
Infrared Spectrum Principal peaks at wavenumbers 1608, 1642, 1508 cm^{-1} (KBr disc).
Dose Up to 800 mg daily.

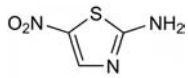
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
O'Neil MJ *et al.* (2006). *The Merck Index: An encyclopedia of chemicals, drugs and biologicals,* 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Aminonitrothiazole

Antiprotozoal (Veterinary)
$C_3H_3N_3O_2S$ = 145.1
CAS—1320-42-9
IUPAC Name 4-Nitro-1,3-thiazol-2-amine

A

Synonym 5-Nitro-2-thiazolamine

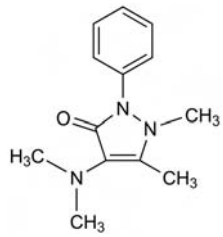


Chemical Properties A greenish-yellow to orange-yellow light powder. Mp 200°, with decomposition. Slightly soluble in water; soluble 1 in 250 of ethanol and ether; practically insoluble in chloroform; soluble in dilute mineral acids and propylene glycol.
Colour Test Diazotisation—violet.
Thin-layer Chromatography System TA—R_f 0.75 (visible yellow spot; acidified iodoplatinate solution, strong reaction).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Water—386 nm (A_1^1=1060a).
Infrared Spectrum Principal peaks at wavenumbers 1200, 1623, 1176, 1504, 1515, 1252 cm^{-1} (KBr disk).

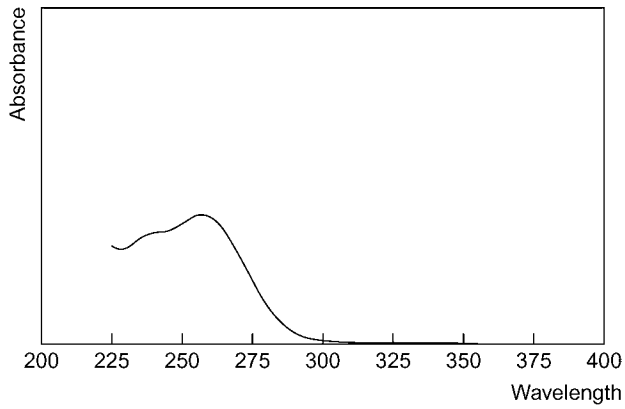
Aminophenazone

Analgesic
$C_{13}H_{17}N_3O$ = 231.3
CAS—58-15-1

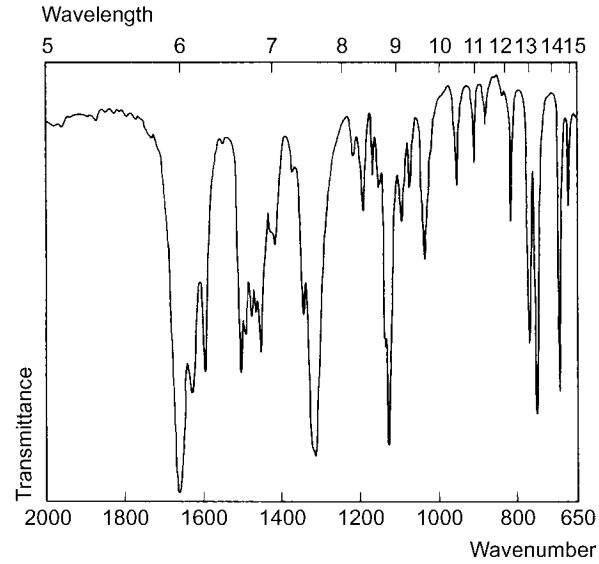
IUPAC Name (1*R*)-1-[(2*R*,3a*R*,5*R*,6*S*,6a*R*)-6-Hydroxy-2-(trichloromethyl)-3a,5,6,6a-tetrahydrofuro[2,3-d][1,3]dioxol-5-yl]ethane-1,2-diol
Synonyms Amidazofen; amidopyrine; amidopyrine-pyramidon; aminopyrine; dimethylaminoantipyrine; 4-(dimethylamino)-1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one; dimethylaminophenazone.
Proprietary Names It is an ingredient of *Gineburno*; *Thermocutan*; *Virdex*.



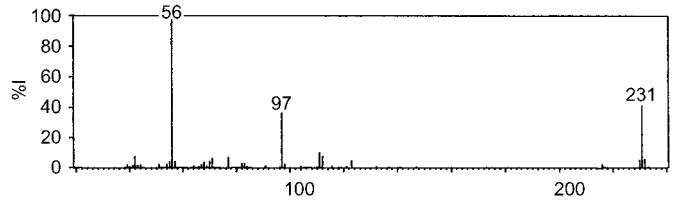
Chemical Properties Small colourless crystals or white crystalline powder. Mp 107° to 109°. Soluble 1 in 18 of water, 1 in 12 of benzene, 1 in 1.5 of ethanol, 1 in 1 of chloroform, and 1 in 13 of ether. pK_a 5.0 (20°). Log *P* (octanol/water), 1.0. A number of salts of aminophenazone have been used, including the ascorbate, gentisate, hydroxyisophthalate and salicylate.
Colour Tests Ferric chloride—blue-violet; Liebermann's reagent (100°)—blue; nitrous acid—violet (transient).
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.21; system TD—R_f 0.25; system TE—R_f 0.62; system TF—R_f 0.10; system TAD—R_f 0.58; system TAE—R_f 0.70; system TAF—R_f 0.68; system TAJ—R_f 0.53; system TAK—R_f 0.00; system TAL—R_f 0.76 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—aminophenazone RI 1895, M(nor-) RI 1980, M(bis-nor-) RI 1955, M(desamino-OH-) RI 1855; system GB—RI 1992; system GC—RI 2370; system GF—RI 2265.
High Performance Liquid Chromatography System HA—*k* 0.3; system HD—*k* 0.2; system HW—*k* 0.32; system HX—RI 262; system HY—RI 204; system HZ—retention time 2.1 min.
Ultraviolet Spectrum Aqueous acid—257 nm (A_1^1=443c); aqueous alkali—264 nm (A_1^1=353b).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1315, 1126, 750, 700, 1620 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 56, 231, 97, 111, 112, 42, 77, 71.



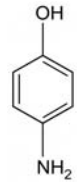
Quantification
Plasma **GC** FID. Limit of detection, 100 µg/L [Sioufi, Colussi 1978].
Urine **HPLC** Column: Spherisorb ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : triethylamine : acetic acid (70.9:27.7:0.9:0.5), flow rate 1 mL/min. UV detection (λ=254 nm). Retention time 29 min [Agundez *et al.* 1994]. UV detection. Limit of quantification, 1 mg/L for aminophenazone. Limit of detection, 0.10 mg/L for aminophenazone, and dipyrone and metabolites [Agundez *et al.* 1994]. UV detection [Shimada, Nagase 1980].
Disposition in the Body Absorbed after oral administration. It is rapidly and extensively metabolised. About 30 to 50% of a dose is excreted in the urine in 3 days as 4-aminophenazone and its acetyl derivative 4-acetamidophenazone which is the major urinary metabolite; other metabolites found in the urine include two red pigments, rubazonic acid and methylrubazonic acid; dimethylnitrosamine may be formed in the stomach. <5% of a dose is excreted in the urine unchanged.
Toxicity The use of aminophenazone is discouraged due to the risk of fatal agranulocytosis. The estimated minimum lethal dose is 5 g.
Half-life Plasma half-life about 2 to 3 h.
Protein Binding In plasma 25 to 30%.
Dose Aminophenazone was formerly given in doses of up to 1.5 g in 24 h.

Agundez JA *et al.* (1994). Determination of aminopyrine, dipyrone and its metabolites in urine by high-performance liquid chromatography. *Ther Drug Monit* 16: 316–322.
Shimada K, Nagase Y (1980). Quantitative high-performance liquid chromatographic determinations of aminopyrine and its metabolites in man. *J Chromatogr* 181(7): 51–57.
Sioufi A, Colussi D (1978). Gas chromatographic determination of phenazone derivatives in human plasma. I. Aminophenazone. *J Chromatogr* 146(3): 503–507.

4-Aminophenol

Phenol
C_6H_7NO = 109.1
CAS—123-30-8

IUPAC Name 4–Aminophenol
Synonyms *p*-Aminophenol; *p*-hydroxyaniline.



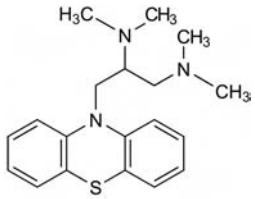
Chemical Properties Crystals. Mp about 190°. The commercial product is usually pink; Mp 186°. Slightly soluble in water; soluble in dehydrated alcohol; practically insoluble in chloroform.
Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TB—R_f 0.03; system TD—R_f 0.21; system TE—R_f 0.59; system TF—R_f 0.40; system TAD—R_f 0.30; system TAE—R_f 0.75 (ferric chloride solution, violet; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—4-aminophenol RI 1253, 4-aminophenol-AC_2 RI 1765, 4-aminophenol-AC_3 RI 2085, 4-aminophenol-Me RI 1100; system GB—4-aminophenol RI 1280.
Ultraviolet Spectrum Aqueous acid—271 nm (A^1_1=133b); aqueous alkali—266 nm (broad) (A^1_1=731b); methanol—233 nm (A^1_1=587b), 303 nm (A^1_1=177b).
Mass Spectrum Principal ions at *m/z* 109, 80, 53, 81, 108, 52, 54, 110.
Quantification
Urine **HPLC** UV detection (λ=215 nm). Limit of detection, 1 mg/L [Brega *et al.* 1990].
Disposition in the Body 4-Aminophenol is a metabolite of aniline, nitrobenzene, paracetamol and phenazopyridine.
Toxicity

Following paracetamol overdose, 3 patients were observed to have urine discoloration. Investigation by chromatographic and colorimetric methods revealed the presence of 4-aminophenol, responsible for the dark brown discoloration [Clark *et al.* 1986].

Brega A *et al.* (1990). Determination of phenol, m-, o- and p-cresol, p-aminophenol and p-nitrophenol in urine by high-performance liquid chromatography. *J Chromatogr* 535: 311–316.
Clark PM *et al.* (1986). Urine discoloration after acetaminophen overdose. *Clin Chem* 32: 1777–1778.

Aminopromazine

Antispasmodic (Veterinary)
$C_{19}H_{25}N_3S$ = 327.5
CAS—58-37-7
IUPAC Name 1-*N*,1-*N*,2-*N*,2-*N*-Tetramethyl-3-phenothiazin-10-ylpropane-1,2-diamine
Synonyms Proquamezine; tetrameprozine; *N*,*N*,*N*′,*N*′-tetramethyl-3-(10*H*-phenothiazin-10-yl)-1,2-propanediamine.



Chemical Properties pK_a 9.8. Log *P* (octanol/water), 4.8.

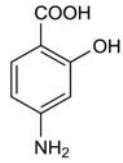
Aminopromazine Fumarate

$(C_{19}H_{25}N_3S)_2,C_4H_4O_4$ = 771.1
CAS—3688-62-8
Synonym Proquamezine fumarate
Proprietary Names *Lispamol*; *Sedofarmolo*.
Chemical Properties White powder. Mp about 166° to 170°, with decomposition. Soluble 1 in 11 of water, 1 in 200 of ethanol, and 1 in 20 of methanol; practically insoluble in ether.

Colour Tests Forrest reagent—violet→red→orange; FPN reagent—violet-red→red→orange; Mandelin's test—orange→violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.50; system TAE—R_f 0.12; system TAG—R_f 0.08; system TB—R_f 0.42; system TC—R_f 0.19; system TE—R_f 0.60 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2434.
High Performance Liquid Chromatography System HX—RI 427.
Ultraviolet Spectrum Aqueous acid—248 (A^1_1 = 965a), 297 nm (broad) (A^1_1 = 106a)
Infrared Spectrum Principal peaks at wavenumbers 1584, 745, 660, 1278, 1190, 765 cm^{-1} (aminopromazine fumarate, KBr disk).
Mass Spectrum Principal ions at *m/z* 198, 58, 115, 70, 269, 199, 72, 71.

Aminosalicylic Acid

Antituberculosis
$C_7H_7NO_3$ = 153.1
CAS—65-49-6
IUPAC Name 4-Amino-2-hydroxybenzoic acid
Synonyms Aminosalylum; para-aminosalicylic acid; PAS; pasalicylum.
Proprietary Names *Nemasol*; *Parasal*; *Rezipas*; *Teebacin Acid*.



Chemical Properties A white bulky powder, which darkens on exposure to air and light. Aqueous solutions are unstable. Mp 150° to 151°, with effervescence. Soluble 1 in about 600 of water, 1 in about 20 of ethanol, 1 in 6 of acetone, 1 in 4000 of chloroform, and 1 in 50 of ether. pK_a 1.8 (-NH_2), 3.6 (-COOH). Log *P* (octanol/water), 0.87.

Calcium Aminosalicylate

$(C_7H_6NO_3)_2Ca,3H_2O$ = 398.4
CAS—133-15-3 (anhydrous)
Synonym Aminosalicylate calcium
Chemical Properties A white or slightly yellow, hygroscopic, crystalline powder. Aqueous solutions are unstable and darken in colour. Soluble 1 in 7 to 1 in 10 of water; slightly soluble in ethanol.

Phenyl Aminosalicylate

$C_{13}H_{11}NO_3$ = 229.2
CAS—133-11-9
Synonym Fenamisal
Chemical Properties A white crystalline solid. Mp 153°. Practically insoluble in water.

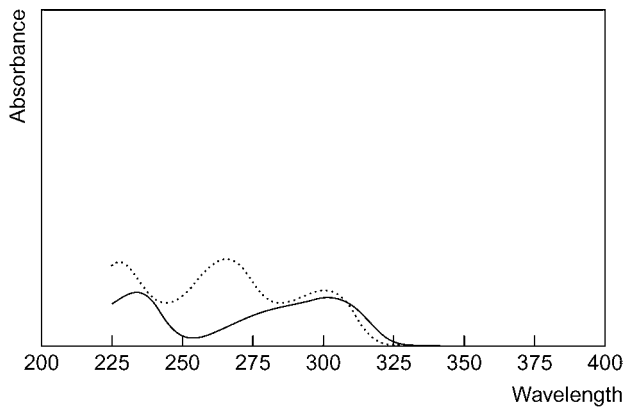
Potassium Aminosalicylate

$C_7H_6KNO_3$ = 191.2
CAS—133-09-5
Synonym Aminosalicylate potassium
Proprietary Name *Teebacin Kalium*
Chemical Properties A white to cream-coloured crystalline powder. Aqueous solutions are unstable. Freely soluble in water; sparingly soluble in ethanol; very slightly soluble in chloroform and ether.

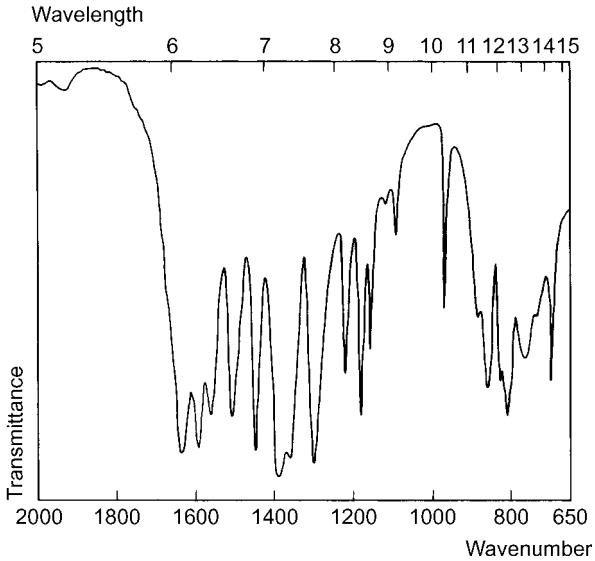
Sodium Aminosalicylate

$C_7H_6NNaO_3,2H_2O$ = 211.1
CAS—133-10-8 (anhydrous); 6018-19-5 (dihydrate)
Synonyms Aminosalicylate sodium; pasalicylum solubile.
Proprietary Names *Eupasal Sodico*; *Italpas Sodico*; *Parasal Sodium*; *Pasalba*; *Teebacin*.
Chemical Properties White or cream-coloured crystals or crystalline powder. Aqueous solutions are unstable. Soluble 1 in 2 of water; sparingly soluble in ethanol; practically insoluble in chloroform and ether.

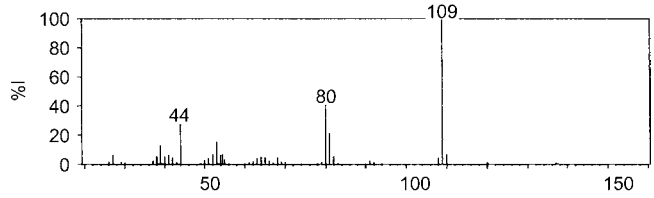
Colour Tests Coniferyl alcohol—orange; Ferric chloride—violet; Folin-Ciocalteu reagent—blue; McNally's test—brown precipitate.
Thin-layer Chromatography System TA—R_f 0.70; system TD—R_f 0.05; system TE—R_f 0.07; system TF—R_f 0.24; system TAD—R_f 0.15 (location under UV light, blue fluorescence; ferric chloride solution, violet; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—RI 1309.
High Performance Liquid Chromatography System HZ—retention time 1.5 min.
Ultraviolet Spectrum Aqueous acid—234 (A^1_1=496a), 300 nm (A^1_1=330a); aqueous alkali—265 (A^1_1=846a), 300 nm (A^1_1=532a).



Infrared Spectrum Principal peaks at wavenumbers 1301, 1638, 1595, 1510, 805, 1180 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 109, 80, 44, 81, 53, 39, 52, 54.



Quantification

Plasma **HPLC** Column: LiChrospher 60 RP-select B (250 × 4.6 mm i.d., 5 µm). Mobile phase: water (pH 3): methanol: acetonitrile (60:20:20), flow rate 1.2 mL/min. Internal standard (IS): *N*-acetyl-anthranilic acid. Fluorescence detection (λ_{ex}=311 nm; λ_{em}=449 nm). Retention time: 4 min for acetyl-5-aminosalicylic acid. Limit of quantification, 100 µg/L and limit of detection, 20 µg/L [Bystrowska *et al.* 2000]. Fluorescence detection. Limit of detection, 0.5 ng [Honigberg *et al.* 1980]. Fluorescence detection. Limit of detection, 500 µg/L for aminosalicylic acid and *p*-acetamidosalicylic acid [Stoll *et al.* 1972].

Urine **HPLC** See Plasma [Bystrowska *et al.* 2000]. See Plasma [Stoll *et al.* 1972].

Intestinal Biopsy Samples **HPLC** Column: C_{18} Erbasil S (250 × 4.6 mm i.d., 10 µm). Mobile phase: 0.01 mol/L Na_2HPO_4 containing 0.1 mmol/L EDTA, 0.1 mol/L citric acid and 0.1 mmol/L heptanesulfonic acid: methanol (85:15), pH 3, flow rate 1.0 mL/min. IS: 3,4-dihydroxybenzylamine. Electrochemical detection. Retention time: 3.2 min for 5-aminosalicylic acid; 8.3 min for acetyl-5-aminosalicylic acid; 6.5 min for 2,5-dihydroxybenzoic acid [Palumbo *et al.* 1995].

Disposition in the Body Aminosalicylic acid is readily absorbed after oral administration and widely distributed throughout the body, with high concentrations in the kidneys, lungs and liver. It is metabolised by acetylation to *p*-acetamidosalicylic acid and by conjugation with glycine, producing *p*-aminosalicyluric acid; both metabolites are inactive. More than 80% of a dose is excreted in the urine in 24 h with about 50% of the dose consisting of *p*-acetamidosalicylic acid; up to 25% of the dose may be excreted as free and acetylated *p*-aminosalicyluric acid, and the remainder is mostly unchanged drug with small amounts of 2,4-dihydroxybenzoic acid.

Therapeutic Concentration

Following a single oral dose of 4 g of aminosalicylic acid to 12 subjects, a mean peak plasma concentration of 50 mg/L was attained in 3.5 h; after oral administration of 4 g of sodium aminosalicylate to the same subjects, a mean peak plasma concentration of 155 mg/L was attained in about 0.8 h [Wan *et al.* 1974].

Toxicity Prolonged administration may give rise to toxic symptoms characteristic of the salicylates.

Half-life Plasma half-life, aminosalicylic acid about 0.5 to 1 h, *p*-acetamidosalicylic acid about 1.5 h.

Protein Binding 60 to 70%.

Dose 12 g of sodium aminosalicylate daily; up to 20 g daily has been given.

Bystrowska B *et al.* (2000). Validation of a LC method for the determination of 5-aminosalicylic acid and its metabolite in plasma and urine. *J Pharm Biomed Anal* 22: 341–347.

Honigberg IL *et al.* (1980). Non-extractive fluorometric measurement of p-aminosalicylic acid in plasma by ion-pairing techniques and high-performance liquid chromatography. *J Chromatogr* 181: 266–271.

Palumbo G *et al.* (1995). Simultaneous determination of 5-aminosalicylic acid, acetyl-5-aminosalicylic acid and 2,5-dihydroxybenzoic acid in endoscopic intestinal biopsy samples in humans by high-performance liquid chromatography with electrochemical detection. *J Pharm Biomed Anal* 14: 175–180.

Stoll RG *et al.* (1972). A sensitive and specific method for the analysis of para-aminosalicyclic acid and its N-acetyl metabolite in human plasma. *Res Commun Chem Path Pharmacol* 4: 327–338.

Wan SH *et al.* (1974). Bioavailability of aminosalicylic acid and its various salts in humans. 3. Absorption from tablets. *J Pharm Sci* 63: 708–711.

Aminotriazole

Herbicide

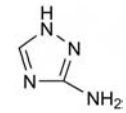
$C_2H_4N_4$ = 84.1

CAS—61-82-5

IUPAC Name 1*H*-1,2,4-Triazol-5-amine

Synonyms Amitrole; ATA; ENT-25445.

Proprietary Names *Amizol*; *Cytrol*; *Weedazol*. It is an ingredient of a number of weed-killers.



Chemical Properties A white crystalline powder. Mp 159°. Soluble 1 in 3.5 of water and 1 in 4 of ethanol; practically insoluble in acetone, ether, and non-polar solvents; sparingly soluble in ethyl acetate.

Colour Test Add sodium hypobromite solution to the sample—orange-red.

Thin-layer Chromatography System TA—R_f 0.68 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—aminotriazole RI 1312, aminotriazole-Me_2 RI 1050, aminotriazole-AC RI 1010; system GK—not eluted.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1639, 1047, 1534, 1595, 1211, 971 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 84, 58, 43, 44, 42, 53, 55, 86.

Toxicity

A 54-year-old man was hospitalised following poisoning with *Radoxone TL*, a weed-killer containing ammonium thiocyanate and aminotriazole. The patient was in a coma with myoclonic jerks and vascular collapse. Thiocyanate and aminotriazole blood levels were 750 mg/L and 138 mg/L, respectively, >12 h after ingestion. The patient died 48 h later of potanoxic coma [Legras *et al.* 1996].

Legras A *et al.* (1996). Herbicide: fatal ammonium thiocyanate and aminotriazole poisoning. *J Toxicol Clin Toxicol* 34: 441–446.

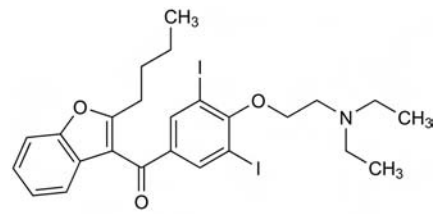
Amiodarone

Antiarrhythmic

$C_{25}H_{29}I_2NO_3$ = 645.3

CAS—1951-25-3

IUPAC Name (2-Butyl-1-benzofuran-3-yl)[4-(2-diethylaminoethoxy)-3,5-diiodophenyl]methanone



Chemical Properties pK_a 5.6. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Amiodarone Hydrochloride

$C_{25}H_{29}I_2NO_3$,HCl = 681.8

CAS—19774-82-4

Proprietary Names *Amiodar*; *Ancaron*; *Cordarex*; *Cordarone*; *Cordarone X*; *Ortacrone*; *Tachydaron*; *Trangorex*.

Chemical Properties A white crystalline powder. Mp about 161°. Very slightly soluble in water; soluble in ethanol; freely soluble in chloroform.

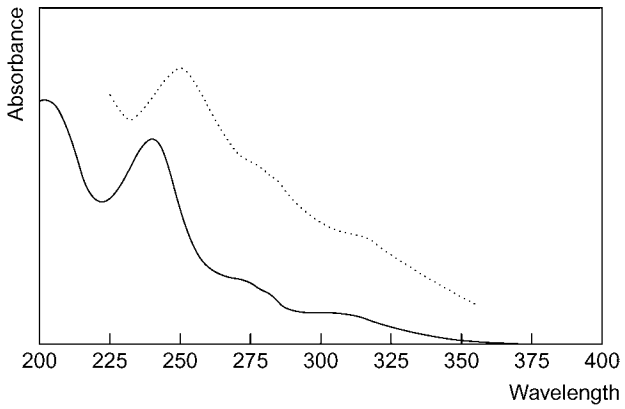
Colour Tests Iodine test (omitting MnO_2)—positive; Liebermann's reagent—brown-yellow; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.62; system TC—R_f 0.68; system TE—R_f 0.82; system TL—R_f 0.55; system TAE—R_f 0.54; system TAF—R_f 0.64 (dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, yellow).

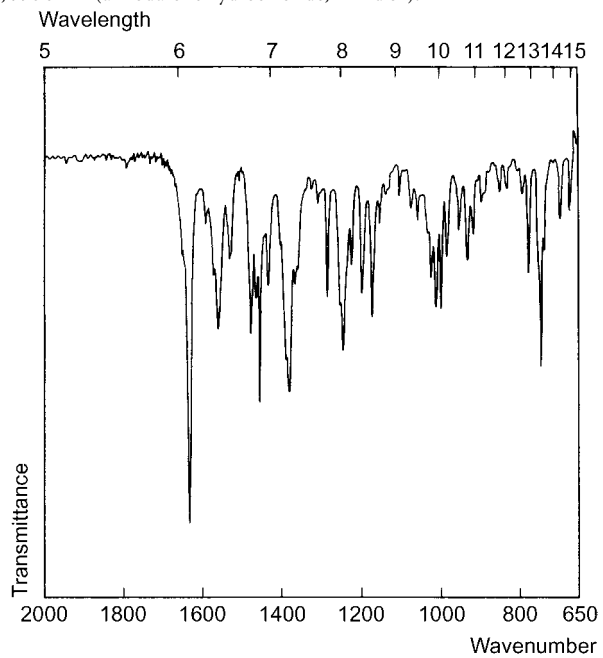
Gas Chromatography System GA—RI 3335amiodarone; RI 2800O-desalkylamiodarone (art).

High Performance Liquid Chromatography System HA—amiodarone *k* 2.4, monodesethylamiodarone *k* 1.8; system HX—RI 683; system HY—RI 476; system HZ—retention time 90.4 min.

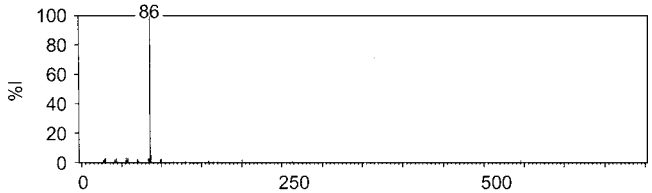
Ultraviolet Spectrum Aqueous acid—241 nm; aqueous alkali—251 nm.



Infrared Spectrum Principal peaks at wavenumbers 1630, 748, 1245, 1558, 1170, 998 cm^{-1} (amiodarone hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 36, 87, 84, 58, 56, 44, 38.



Quantification

Plasma **HPLC** Limit of quantification, 50 µg/L for drug and metabolite. UV detection. Limit of detection, 1 µg/L for amiodarone and 0.5 µg/L for desethylamiodarone [Kollroser, Schober 2002]. UV detection. Limit of detection, 25 µg/L for amiodarone and monodesethylamiodarone [Plomp *et al.* 1983]. UV detection. Limit of detection, 100 µg/L for amiodarone and monodesethylamiodarone [Storey *et al.* 1982].

Urine **HPLC** See Plasma [Plomp *et al.* 1983].

Tissues **HPLC** See Plasma [Plomp *et al.* 1983].

Disposition in the Body Amiodarone is slowly and incompletely absorbed after oral administration; it is distributed to the tissues where it is strongly bound. It is metabolised by *N*-dealkylation to monodesethylamiodarone which is the major plasma metabolite during chronic dosing. High concentrations of amiodarone and monodesethylamiodarone are found in the liver, lungs and adipose tissue. Enterohepatic circulation may occur. Only a small amount is excreted in the urine as unchanged drug.

Therapeutic Concentration Accumulates on chronic administration; steady state plasma concentrations are attained in about 1 month.

Fifteen patients were administered 400 mg amiodarone 3 times daily and peak plasma concentrations of the drug were 1.478 to 1.983 mg/L. Peak plasma concentrations of the metabolite, desethylamiodarone, were 0.522 to 1.008 mg/L [Kollroser, Schober 2002].

Following oral administration of 400 mg to 7 subjects, peak plasma concentrations of about 0.5 to 1 mg/L were attained in about 7 h. After administration of 200 mg 8-hourly to 6 subjects for 1 month, plasma concentrations determined immediately before the morning dose ranged from 0.75 to 2.8 mg/L (mean 1.5 mg/L) [Andreasen *et al.* 1981].

During chronic treatment with 400 mg daily to 33 subjects, mean steady-state plasma concentrations of 2.2 mg/L of amiodarone and 2.0 mg/L of monodesethylamiodarone were reported. [Flanagan *et al.* 1982].

Half-life Plasma half-life during chronic dosing, 14 to 107 days (mean 50).

Distribution in Blood Plasma:whole blood ratio, 1.3.

Protein Binding Extensively bound.

Note For a review of the pharmacokinetics of amiodarone, see Latini *et al.* [1984].

Dose Initially 600 mg of amiodarone hydrochloride daily; maintenance, 200 to 400 mg daily.

Andreasen F *et al.* (1981). Pharmacokinetics of amiodarone after intravenous and oral administration. *Eur J Clin Pharmacol* 19: 293–299.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Flanagan RJ *et al.* (1982). Identification and measurement of desethylamiodarone in blood plasma specimens from amiodarone-treated patients. *J Pharm Pharmacol* 34: 638–643.

Kollroser M, Schober C (2002). Determination of amiodarone and desethylamiodarone in human plasma by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry with ion trap detector. *J Chromatogr B Biomed Sci Appl* 766: 219–226.

Latini R *et al.* (1984). Clinical pharmacokinetics of amiodarone. *Clin Pharmacokinet* 9: 136–156.

Plomp TA *et al.* (1983). Simultaneous determination of amiodarone and its major metabolite desethylamiodarone in plasma, urine and tissues by high-performance liquid chromatography. *J Chromatogr* 273: 379–392.

Storey GCA *et al.* (1982). High-performance liquid chromatographic measurement of amiodarone and its desethyl metabolite: methodology and preliminary observations. *Ther Drug Monit* 4: 385–388.

Amiphenazole

Narcotic Antagonist, Respiratory Stimulant

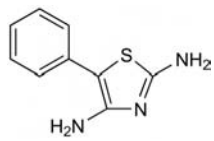
$C_9H_9N_3S$ = 191.3

CAS—490-55-1

IUPAC Name 5-Phenyl-1,3-thiazole-2,4-diamine

Synonyms DAPT; phenamizole; 5-phenyl-2,4-thiazolediamine.

Proprietary Names *Daptazile*; *Daptazole*; *Dizol*; *Fenamizol*.



Chemical Properties Flakes which turn brown on exposure to light and air. Mp 163° to 164°, with decomposition.

Amiphenazole Hydrochloride

$C_9H_9N_3S,HCl$ = 227.7

CAS—942-31-4

Synonym Amiphenazole chloride

Proprietary Names *Daptazile*; *Daptazole*.

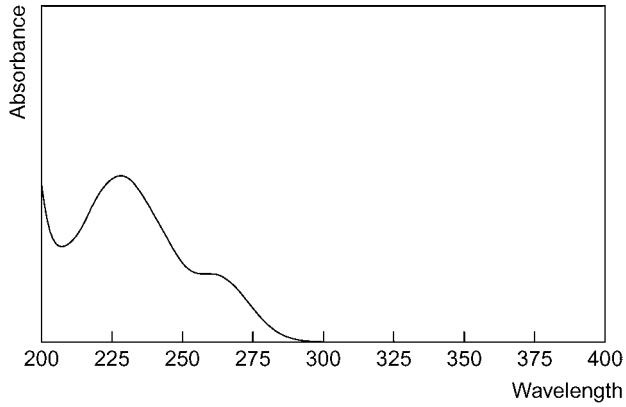
Chemical Properties A white, fine crystalline or granular, mobile powder. Aqueous solutions hydrolyse slowly. Mp 236°. Soluble 1 in 16 of water and 1 in 50 of ethanol; slightly soluble in acetone, chloroform and ether.

Colour Test Liebermann's reagent (100°)—green-blue.

Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.02; system TC—R_f 0.33; system TE—R_f 0.62; system TL—R_f 0.57 (acidified iodoplatinate solution, positive).

Gas Chromatography System GB—RI 1150; system GC—RI 2563.

Ultraviolet Spectrum Aqueous acid—230 (A^1_1=998a), 261 nm.



Infrared Spectrum Principal peaks at wavenumbers 1495, 1637, 688, 750, 1665, 1050 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 191, 121, 77, 104, 122, 43, 51, 192.

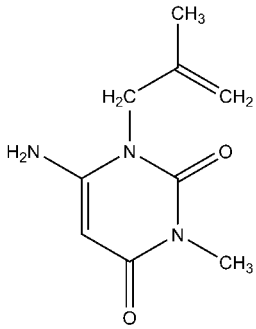
Dose Amiphenazole hydrochloride has been given parenterally in doses of 100 to 150 mg.

A

Amisometradine

Pyrimidine, Diuretic
$C_9H_{13}N_3O_2$ = 195.2
CAS—550-28-7

IUPAC Name 6-Amino-1,2,3,4-tetrahydro-3-methyl-1-methyl-allyl-2,4-dioxo-pyrimidine
Synonym Aminoisometradine
Proprietary Name *Rolicton*



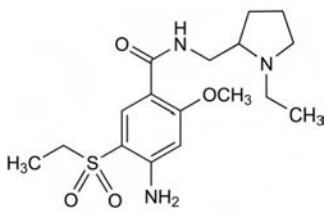
Chemical Properties White crystalline powder. Mp 175°. Slightly soluble in cold water, the solubility increasing rapidly on heating; freely soluble in ethanol and acetone; insoluble in ether. It is extracted by organic solvents from aqueous acid or alkaline solutions. Amisometradine is an isomer of aminometradine. Log *P* (octanol/water) 0.13 [Meylan, Howard 1995].
Thin-Layer Chromatography System T1—R_f 0.68 (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—268.5 nm; methanol—267 nm.
Infrared Spectrum Principal peaks at wavenumbers 1639, 1492 or 1604, 1680 cm^{-1} (KBr disk).
Disposition in the Body
Toxicity LD_{50} (oral) in mice: 610 mg/kg.
Dose Up to 1.6 g daily.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Amisulpride

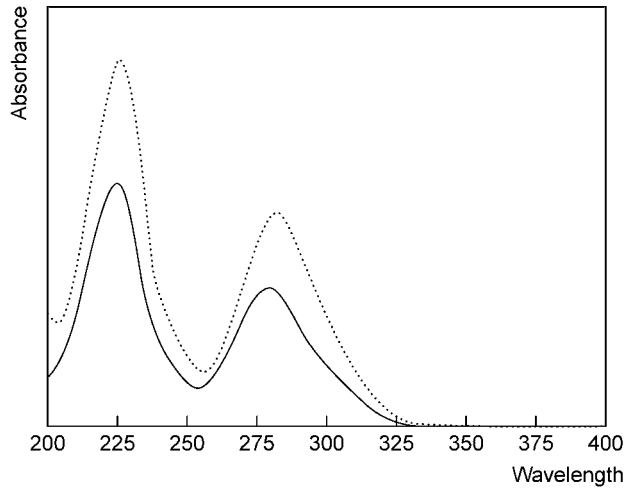
Antipsychotic
$C_{17}H_{27}N_3O_4S$ = 369.5
CAS—71675-85-9

IUPAC Name 4-Amino-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide
Synonyms Aminosultopride; 4-amino-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide; DAN-2163.
Proprietary Names *Deniban*; *Solian*; *Sulamid*.

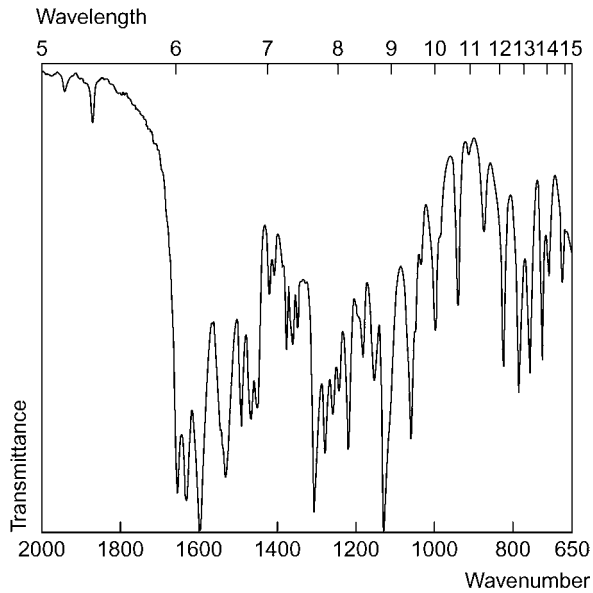


Chemical Properties Crystals from acetone. Mp 126° to 127°. pK_a 9.37. Log *P* (octanol/water), 1.10. Extraction yield (chlorobutane), 0.6 [Demme *et al.* 2005]. Stock solutions were stable for at least 1 month stored at 0–5°. Amisulpride is stable in human plasma for at least 24 h either at room temperature or at 37° [Malavasi *et al.* 1996]. Stability of amisulpride in methanol and human plasma was satisfactory at room temperature and at 37°. It was also satisfactory in human plasma following 2 freeze-thaw cycles and in human plasma diluted with borate buffer (pH 9.0) [Ascalone *et al.* 1996].
High Performance Liquid Chromatography System HAA—amisulpride RT 8.9 min, M1 RT 7.1 min, M2 RT 10.9 min.

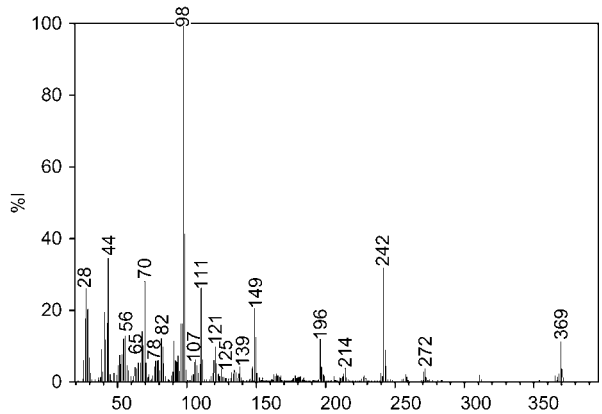
Column: Hypersil C_{18} BDS (150 × 4.6 mm i.d., 5 µm). Mobile phase: water-TEA-1 mol/L potassium dihydrogen phosphate (pH 3.0, 974 : 1 : 25) : acetonitrile (850 : 150), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 370 nm). Retention time: 4 min [Malavasi *et al.* 1996].
Ultraviolet Spectrum Aqueous acid—230, 285 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 98, 44, 242, 70, 28, 111, 149, 369.



Quantification
Blood **HPLC** Column: Nova-Pak C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : potassium dihydrogen phosphate (pH 2.6, 65 : 5 : 30). DAD. Retention time: 5.67 min. Limit of detection, 49 µg/L [Tracqui *et al.* 1995].
Plasma **HPLC** Column: Chiralpak AS (250 × 4.6 mm i.d.). Mobile phase: *n*-hexane: ethanol (67 : 33) containing 0.2% diethylamine, flow rate 0.5 mL/min. UV (λ = 280 nm) or fluorescence (λ_{ex} = 280 nm, λ_{em} = 370 nm) detection. Limit of quantification, 2.5 µg/L for both enantiomers [Ascalone *et al.* 1996]. Column: Hypersil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 850 mL 1 mol/L potassium dihydrogen phosphate (25 mL in 950 mL water with 1 mL TEA, pH 3.0 to 1 L with

water) to 1 L with acetonitrile, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 370 nm). Limit of quantification, 0.5 µg/L [Malavasi *et al.* 1996]. Column: RP-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : diethylamine (532 : 468 : 0.8), flow rate 1 mL/min. UV detection (λ = 226 nm). Retention time: 10.3 min. Limit of detection, 5 µg/L [Bohbot *et al.* 1987].

Serum **LC-MS** Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.5 min. Limit of quantification, 28.3 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **HPLC** See Plasma. Limit of quantification, 50 µg/L for both enantiomers [Ascalone *et al.* 1996]. See Plasma. Limit of quantification, 100 µg/L [Malavasi *et al.* 1996].

Disposition in the Body Following oral administration, amisulpride is readily absorbed, with 2 absorption peaks observed, the first after ~1 h and the second between 3 and 4 h after administration. It is only weakly metabolised; metabolites account for only ~4% of the dose and are inactive. It is excreted largely unchanged in urine (70%) and a small amount in faeces as the unchanged drug. Amisulpride is widely distributed throughout the body and is only very weakly dialysed.

Therapeutic Concentration

Following administration of a single oral dose of 50 mg amisulpride to 18 healthy subjects the mean plasma concentrations were 18.8, 17.9 and 17.2 µg/L at 2, 4 and 6.5 h, respectively. Following administration of a single 200 mg dose, the plasma concentrations were 76.2, 83.0, and 92.1 µg/L at 2, 4 and 6.5 h, respectively [Mattila *et al.* 1996].

Toxicity

In a non-fatal overdose, a 30-year-old Caucasian woman who had taken ~3 g amisulpride and an unknown amount of dosulepin had a blood amisulpride concentration of 9.63 mg/L. Her gastric fluid concentration was 14.3 mg/L. The patient experienced generalised convulsions, which resolved spontaneously, followed by coma, motor restlessness, tachycardia and slight prolongation of the QT interval. The subject was treated with gastric lavage and recovered within 48 h. The drug blood concentration was 10–50 times higher than that observed at therapeutic levels. A second subject who had been found dead had a blood amisulpride concentration of 41.7 mg/L. This is 40–200 times the observed therapeutic concentration [Tracqui *et al.* 1995].

Half-life Plasma, 12 h.

Volume of Distribution 5.8 L/kg.

Clearance 20 L/h

Protein Binding 16%.

Dose Up to 1200 mg daily orally; up to 400 mg daily has been given IM.

Ascalone V *et al.* (1996). Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. Application to pharmacokinetics. *J Chromatogr B Biomed Appl* 676: 95–105.

Bohbot M *et al.* (1987). Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography. *J Chromatogr* 416: 414–419.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Malavasi B *et al.* (1996). Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid–liquid extraction or solid-phase extraction in combination with high-performance liquid chromatography and fluorescence detection. application to pharmacokinetics. *J Chromatogr B Biomed Appl* 676: 107–115.

Mattila MJ *et al.* (1996). Single oral doses of amisulpride do not enhance the effects of alcohol on the performance and memory of healthy subjects. *Eur J Clin Pharmacol* 51: 161–166.

Tracqui A *et al.* (1995). Amisulpride poisoning: a report on two cases. *Hum Exp Toxicol* 14: 294–298.

Amitriptyline

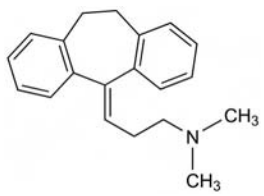
Tricyclic Antidepressant

$C_{20}H_{23}N$ = 277.4

CAS—50-48-6

IUPAC Name 3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine

Synonym 10,11-Dihydro-*N,N*-dimethyl-5*H*-dibenzo[*a,d*]cycloheptene-Δ5,γ-propylamine



Chemical Properties A colourless oil, which becomes yellow on standing through oxidation to a ketonic product. pK_a 9.4 [Sangster 1997]. Log *P* (octanol/water), 4.92 [Hansch *et al.* 1995], 4.94. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Amitriptyline Embonate

$(C_{20}H_{23}N)_2,C_{23}H_{16}O_6$ = 943.2

CAS—17086-03-2

Proprietary Name *Tryptizol* (syrup).

Chemical Properties A pale yellow to brownish-yellow powder. Mp ~140°. Practically insoluble in water; soluble 1 in 120 of ethanol, 1 in 6 of acetone, and 1 in 8 of chloroform.

Amitriptyline Hydrochloride

$C_{20}H_{23}N,HCl$ = 313.9

CAS—549-18-8

Proprietary Names *Adepril*; *Amavil*; *Amiline*; *Amineurin*; *Amioxid*; *Amitid*; *Amitril*; *Amitrip*; *Amitrol*; *Domical*; *Elatrol*; *Elavil*; *Endep*; *Equilibrin*; *Klotriptyl*; *Laroxyl*; *Lentizol*; *Levate*; *Meravil*; *Novoprotect*; *Novotriptyn*; *Redomex*; *Saroten*; *Sarotex*; *Syneudon*; *Triptyl*; *Tryptanol*; *Tryptil*; *Tryptizol*. It is an ingredient of *Etrafon*; *Limbatril*; *Limbitrol*; *Triavil*; *Triptafen*.

Chemical Properties Colourless crystals or white powder. Mp 196° to 197°. Soluble 1 in 1 of water, 1 in 1.5 of ethanol, 1 in 56 of acetone, 1 in 1.2 of chloroform, and 1 in 1 of methanol; practically insoluble in ether. pK_a 9.4 (25°). Log *P* (octanol/water), 2.18 [Meylan, Howard 1995].

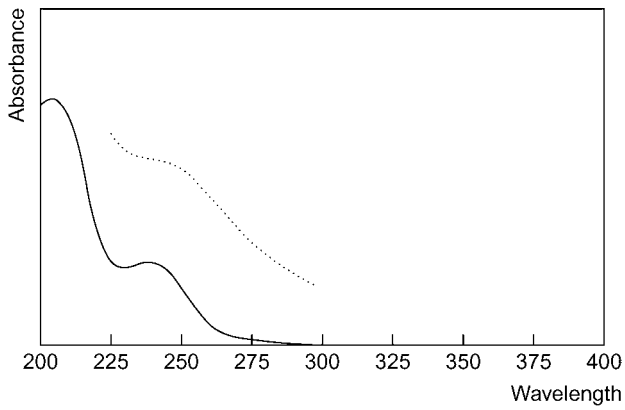
Colour Tests Mandelin's test—brown→green; Marquis test—brown-orange; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.50; system TC—R_f 0.32; system TE—R_f 0.69; system TL—R_f 0.15; system TAE—R_f 0.27; system TAF—R_f 0.51; system TAJ—R_f 0.13; system TAK—R_f 0.05; system TAL—R_f 0.56 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis test, brown).

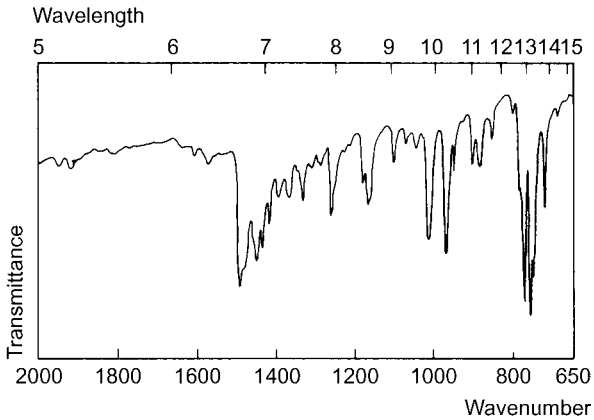
Gas Chromatography System GA—amitriptyline RI 2194, M (*cis*-10-OH-) RI 2348, M (*trans*-10-OH-) RI 2348, M (*N*-oxide) RI 1975, M (nortriptyline) RI 2215, M (cyclobenzaprine) RI 2235, M (OH-) RI 2380, M (nor-OH-) RI 2390; system GB—amitriptyline RI 2284, M (*cis*-10-OH-) RI 2454, M (*cis*-10-OH-*N*-oxide) RI 2215, M (*trans*-10-OH-) RI 2466, M (*trans*-10-OH-*N*-oxide) RI 2239, M (cyclobenzaprine) RI 2330, M (*N*-oxide) RI 2051; system GF—amitriptyline RI 2510, M (10-OH-) RI 2830 or 2880 (stereoisomers); system GM—amitriptyline RRT 0.723, M (*cis*-10-OH-) RRT 1.149, M (*trans*-10-OH-) RRT 1.168, M (cyclobenzaprine) RRT 0.850 (all relative to iprindole); system GS—RT 16.1 min.

High Performance Liquid Chromatography System HA—amitriptyline *k* 3.3, M (10-OH-) *k* 2.9, M (nor-10-OH-) *k* 1.8, M (nortriptyline) *k* 2.0; system HF—amitriptyline *k* 5.42, M (nortriptyline) *k* 4.58; system HX—RI 440; system HY—RI 375; system HZ—RT 7.5 min; system HAA—RT 15.9 min; system HAX—RT 15.8 min; system HAY—RT 7.3 min; system HAZ—amitriptyline *k* 1.76, M (nortriptyline) k 1.71 [Aymard *et al.* 1997].

Ultraviolet Spectrum Aqueous acid—239 nm (A^1_1 = 504a).

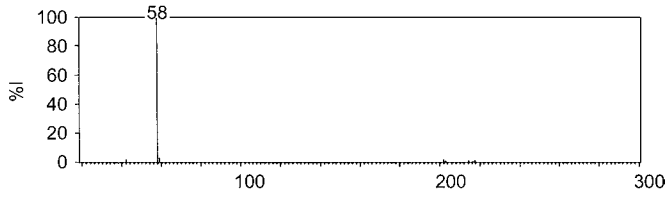


Infrared Spectrum Principal peaks at wavenumbers 756, 770, 746, 969, 1014, 1258 cm^{-1} (amitriptyline hydrochloride, KBr disk).



A

Mass Spectrum Principal ions at *m/z* 58, 59, 202, 42, 203, 214, 217; 10-OH-amitriptyline 58, 42, 69, 41, 30, 215, 202, 59; 10-OH-nortriptyline 44, 45, 26, 218, 215, 203, 202, 42; nortriptyline 44, 202, 45, 220, 218, 215, 91.



Quantification

Blood **GC** Carrier gas: He, 4 mL/min. Temperature programme: 100° for 1 min to 300° at 15°/min. FID. Limit of detection, 32 µg/L [Lee *et al.* 1997].

GC-MS Column: AT-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 20 cm/s. Temperature programme: 70° for 5 min to 310° at 20°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 18.4 min. Limit of detection, 0.12 mg/L [Stiakakis *et al.* 2009]. Ultra 2 capillary 5% phenylmethylsiloxane (12 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 150° for 2 min to 200° at 15°/min for 2 min to 270° at 30°/min for 7 min. EI ionisation, SIM acquisition mode. Limit of quantification, 100 µg/L, limit of detection, 9 µg/L [Margalho *et al.* 2007]. Column: DB-5 cross-linked 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 50° for 2 min to 180° at 30°/min to 280° at 5°/min for 19 min. Full scan mode. Retention time: 17.8 min. Limit of quantification, 0.05 mg/L [Paterson *et al.* 2004].

HPLC Column: Spheri-5 RP-18 (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L sodium dihydrogen phosphate : diethylamine (40 : 57.5 : 2.5), flow rate 2.0 mL/min. UV detection (λ = 220 and 254 nm). Limit of detection, 0.05 mg/L [McIntyre *et al.* 1993].

LC-MS Column: Xterra RP-18. Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2). ESI, MRM acquisition mode. Limit of quantification, 2 µg/L [Titier *et al.* 2007].

Plasma **GC** Column: 5% phenylmethylsilicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 150° to 300° at 10°/min. NPD. Limit of quantification, 19.3 µg/L, limit of detection, 5.8 µg/L [de la Torre *et al.* 1998]. NPD. Limit of quantification, 125 µg/L [Ulrich, Martens 1997; Vandel *et al.* 1992]. Column: 3% SP-2250 on Supelcoport 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: Ar, 3×10^5 N/m^2. Temperature: 250°. AFID. Retention time: 3.5 min. Limit of detection, 10 µg/L [Dawling, Braithwaite 1978].

GC-MS Column: DB-5MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min. EI ionisation, positive ion mode, SIM acquisition mode. Limit of quantification, 2 µg/L, limit of detection, 0.5 µg/L [Lee *et al.* 2008]. PBMS. Limit of detection, 2 ng/g for amitriptyline and 5 ng/g for nortriptyline [Kudo *et al.* 1997].

HPLC Column: µBondapak C_{18} (250 × 3.9 mm i.d.). Mobile phase: phosphate buffer : acetonitrile : TEA (65 : 35 : 0.1, pH 5.1). UV detection (λ = 239 nm). Limit of detection, 5 µg/L [Zarghi *et al.* 2001]. Column: Supelcosil LC-PCN cyanopropyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.005 mol/L ammonium phosphate buffer (pH 7.0, 70 : 15 : 15), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection, 29 nmol/L [Johansen, Rasmussen 1998]. Column: Nova-Pak RP C_{18} (150 × 4.6 mm i.d., 4 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate : acetonitrile : diethylamine (pH 8, 500 : 500 : 2), flow rate, 0.9 mL/min. UV detection (λ = 242 nm). Retention time: 17 min. Limit of detection, 60 nmol/L [Theurillat, Thormann 1998]. Column: Symmetry RP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.067 mol/L potassium dihydrogen phosphate (pH 3, 65 : 35); flow rate, 1.2 mL/min. DAD (λ = 200 nm or 450 nm). Retention time: 11.3 min. Limit of detection, 5 µg/L [Aymard *et al.* 1997]. Column: silica. Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (94 : 6). UV detection. Limit of detection, 5 ng/g for amitriptyline and 10 ng/g for nortriptyline [Kudo *et al.* 1997]. Column: Nova-Pak C_{18} (5 µm). Mobile phase: water : acetonitrile (70 : 30) containing 1% TEA (pH 3.0), flow rate 2.0 mL/min. UV detection (λ = 240 nm). Retention time: 10.5 min. Limit of detection, 2 µg/L [Ghahramani, Lennard 1996].

See also Härtter, Hiemke [1992] and Queiroz *et al.* [1995].

LC-MS Column: Symmetry C_{18} (150 × 3.0 mm i.d., 5 µm). Mobile phase : acetonitrile: 0.1% formic acid (28 : 72 for 4 min to 70 : 30 in 1 min for 3 min to 28 : 72 in 0.7 min). APCI, positive ion mode, full scan mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Kollroser, Schober 2002]. Column: C_{18} (15 × 2.1 mm i.d.). Mobile phase: 3 mmol/L ammonium acetate (pH 3.3) : acetonitrile (66 : 34), flow rate 1.4 mL/min. API, TIS. Limit of quantification, 1–2 µg/L, limit of detection, 5 µg/L [Zhang *et al.* 2000].

Note For a fluorescence polarisation immunoassay for amitriptyline and other tricyclic antidepressants and its comparison with HPLC see Hackett *et al.* [1998].

Serum **GC** Column: HP-5 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 120° to 240° at 30°/min for 20 min. NPD. Limit of detection, 1.5 ng/mL [Ulrich *et al.* 1996].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 280° at 10°/min for 20 min. EI ionisation at 70 eV. Retention time: 22.5 min. Limit of quantification, 0.025 mg/L, limit of detection, <0.025 mg/L [Maresova *et al.* 2008].

HPLC See Plasma [Theurillat, Thormann 1998]. Column: Separon SGX CN (150 × 3 mm i.d., 7 µm). Mobile phase: 0.05 mol/L phosphoric acid with 0.05 mol/L ammonium phosphate containing 28 mmol/L diethylamine (pH 2.55) : acetonitrile (74 : 26), UV detection (λ = 210 nm). Limit of detection, 20–25 µg/L [Dolezalova 1992]. Column: Nucleosil 100 CN cyanopropyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol: 0.01 mol/L phosphate buffer (pH 6.8, 578 : 188 : 235), flow rate 1.5 mL/min. UV detection (λ = 214 nm). Retention time: 8.54 min. Limit of quantification, ~5 µg/L, limit of detection, 5–10 µg/L [Härtter, Hiemke 1992]. Column: reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.0, 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 254 nm). *k'*: 3.333. Limit of detection, 10 µg/L [Segatti *et al.* 1991].

LC-MS Column: Xterra MS C_{18} (50 × 2.1 mm i.d., 5 µm). ESI, positive ion mode, MRM acquisition mode. Limit of detection, 10 µg/L [Sauvage *et al.* 2006].

Note For an immunoassay for the detection of amitriptyline see Rao *et al.* [1994].

Urine **HPLC** DAD [Li *et al.* 1994]. Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L perchloric acid (pH 2.5) : acetonitrile (60 : 40), flow rate 1.2 mL/min. UV detection (λ = 254 nm). Retention time: 13.0 min [Fischer, Breyer-Pfaff 1995].

Bile **GC-MS** See Blood [Stiakakis *et al.* 2009].

Ocular Fluid **GC-MS** See Blood [Stiakakis *et al.* 2009].

Brain **GC-MS** See Blood [Stiakakis *et al.* 2009].

Hair **GC-MS** Column: BP-5 (12.5 m × 0.22 mm i.d.). Carrier gas: He, 3.2 mL/min. Temperature programme: 60° for 1 min to 280° at 30°/min for 5 min. EI ionisation at 70 eV. Limit of detection not reported [Couper *et al.* 1995; Tracqui *et al.* 1992].

Kidney **GC-MS** See Blood [Stiakakis *et al.* 2009].

Liver **GC-MS** See Blood [Stiakakis *et al.* 2009].

HPLC See Blood [McIntyre *et al.* 1993].

Liver Microsomes **HPLC** Column: RP C_{18} ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 12 mmol/L tetramethylethylene diamine (pH 5.5, 40 : 60), flow rate, 1.5 mL/min. UV detection (λ = 242 nm). Retention time: 8.9 min. Limit of detection, 10 µg/L [Shu *et al.* 1998]. See Plasma [Ghahramani, Lennard 1996].

Other **HPLC** Biological Samples. UV detection [Tanaka *et al.* 1997].

Note For a review of analytical methods see Scoggins *et al.* [1980].

Disposition in the Body Amitriptyline is readily absorbed after oral administration and rapidly taken up by the tissues. The main metabolic reaction, demethylation to form the major active metabolite nortriptyline, is catalysed by CYP2C19 and CYP3A4 isoforms. Hydroxylation is catalysed by CYP2D6; *N*-oxidation and conjugation also occur; other metabolites include didesmethylamitriptyline, 10-hydroxy-derivatives, conjugates and amitriptyline *N*-oxide; the 10-hydroxy metabolites may also have some activity. It is mainly excreted in the urine as free and conjugated metabolites, up to ~35% of a single dose being excreted in 24 h. Approximately 50% of the excreted material is 10-hydroxynortriptyline and its glucuronide conjugate; up to 27% is 10-hydroxyamitriptyline (mainly conjugated); unchanged drug and nortriptyline each account for <5% of the excreted material. Approximately 8% of a dose may be eliminated in the faeces as unchanged drug. There appears to be polymorphic variation in the metabolite pattern. Amitriptyline and nortriptyline are widely distributed; they cross the placenta and are excreted in breast milk

Therapeutic Concentration In plasma, usually in the range 0.1–0.2 mg/L (combined amitriptyline and nortriptyline).

After a single oral dose of 50 mg to 3 subjects, peak plasma concentrations for amitriptyline of 0.02–0.04 mg/L (mean 0.025) and for nortriptyline of ~0.01 mg/L were attained in 2–4 h [Garland 1977].

Following daily oral doses of 150 mg to 14 subjects for 3 weeks, steady-state serum concentrations of 0.05–0.24 mg/L (mean 0.13) amitriptyline, 0.04–0.26 mg/L (mean 0.11) nortriptyline, 0.05–0.25 mg/L (mean 0.13) (*E*)-10-hydroxynortriptyline, and 0.005–0.04 mg/L (mean 0.02) 10-hydroxyamitriptyline were reported [Edelbroek *et al.* 1984].

Toxicity Moderate intoxication is associated with plasma concentrations of 0.05–0.43 mg/L (mean: 0.17) and severe toxic symptoms with concentrations >0.3 mg/L. The fatal blood concentration range is 0.55–16.1 mg/L (mean 3.3). Corresponding toxic doses are >1 g (moderate intoxication) and >2 g (severe or fatal).

In 3 deaths attributed to amitriptyline overdose, postmortem tissue concentrations were:

	Amitriptyline	*Nortriptyline*
Blood (mg/L)	6, 18, 3	5, –, 2
Liver (µg/g)	72, 66, 58	98, 24, 60
Urine (mg/L)	6, 28, 7	10, 12, 7

[Munksgaard 1969]

In 7 deaths attributed to amitriptyline overdose, postmortem concentrations (mean), were:

	Amitriptyline	*Nortriptyline*
Blood (mg/L)	0.43–8.30 (3.4)	0.29–6.50 (1.6)
Liver (μg/g)	10.4–243 (92)	4.2–456 (94)

[Bailey, Shaw 1980]

In a fatality involving the suicidal ingestion of amitriptyline, 10 blood samples taken 21 h after discovery of the body and ~28.5 h after the ingestion revealed concentrations of 2.5–12 mg/L amitriptyline, 0.7–3.1 mg/L nortriptyline, and 81–244 salicylate; a further 10 h later, the concentrations were 1–39 mg/L, 0.6–7.0 mg/L, and 86–310 mg/L, respectively. Of tissue samples, drug concentrations were highest in the liver (amitriptyline 301 μg/g, salicylates 670 μg/g) [Pounder *et al.* 1994].

A 44-year-old female was found dead in bed. Postmortem blood amitriptyline concentration was 85.9 mg/L [Margalho *et al.* 2007].

A 37-year-old female was found dead in her apartment. Amitriptyline and nortriptyline were quantified in her blood at concentrations of 7.0 and 7.4 mg/L, respectively [Stiakakis *et al.* 2009].

Bioavailability 30–60%.
Half-life Plasma half-life, 9–36 h, increased in overdosage.
Volume of Distribution ≈15 L/kg.
Clearance Plasma clearance, 11.5 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.2.
Saliva Saliva : plasma ratio, 3.
Protein Binding 91–97%.
Note For a review of the pharmacokinetics of tricyclic antidepressants see Molnar, Gupta [1980].
Dose For depression, 50 to 150 mg amitriptyline hydrochloride daily; up to 300 mg daily has been given.

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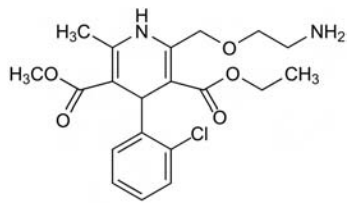
Amlodipine

Calcium Channel Blocker
$C_{20}H_{25}ClN_2O_5$ = 408.9
CAS—88150-42-9

IUPAC Name 3-*O*-Ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3,5-dicarboxylate
Synonyms 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester; UK-48340.



Chemical Properties pK_a 8.6. Log *P* (octanol/water), 3.00. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Amlodipine Maleate
$C_{20}H_{25}ClN_2O_5,C_4H_4O_4$ = 525.0
CAS—88150-47-4
Synonym UK-48340-11
Chemical Properties A white crystalline powder. Mp 178° to 179°.

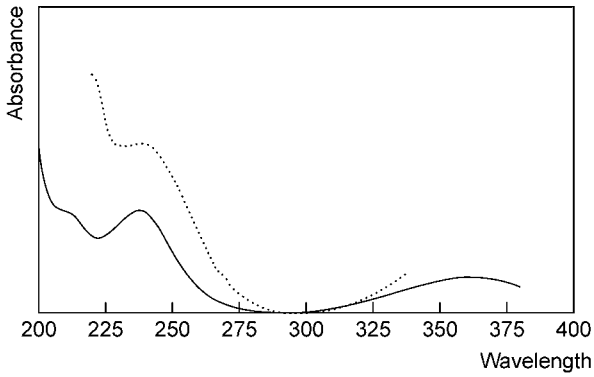
Amlodipine Besilate
$C_{20}H_{25}ClN_2O_5,C_6H_5SO_3H$ = 567.1
CAS—111470-99-6
Synonyms Amlodipine besylate; amlodipine monobenzenesulfonate.
Proprietary Names *Amlodin*; *Amlor*; *Antacal*; *Astudal*; *Istin*; *Monopina*; *Norvas*; *Norvasc*.
Chemical Properties A white crystalline powder. It is slightly soluble in water; sparingly soluble in ethanol.

A

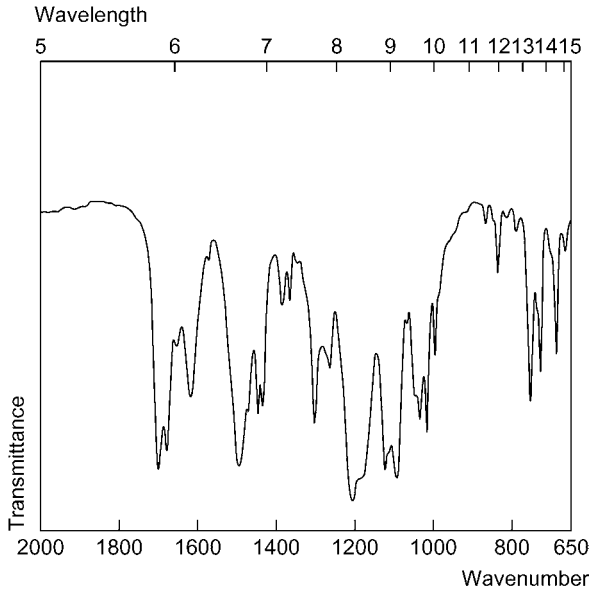
Thin-layer Chromatography System TE—R_f 0.36; system TB—R_f 0.02; system TAE—R_f 0.11.
Gas Chromatography System GB—RI 2982; system GP—M (dehydro-2-$HOOC^-$)-ME-, RI 2430, M (dehydro-desamino-$HOOC^-$)-ME-, RI 2635.
High Performance Liquid Chromatography System HX—RI 428; system HZ—retention time 4.9 min; system HAA—retention time 15.1 min.

Column: ODS Cosmosil 5 C_{18}-P (150 × 4.6 mm, 5 µm). Mobile phase: 0.05 mol/L phosphate buffer solution (pH 3.1) : acetonitrile (65:35) containing 0.005 mol/L sodium octane sulfonate and 5 mg/L EDTA, flow rate 1 mL/min. Electrochemical detection. Retention time: 10.1 min [Shimooka *et al.* 1989].

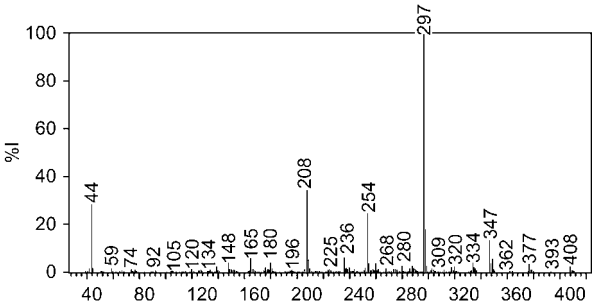
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—239 nm; basic—238 nm (besilate).



Infrared Spectrum Principal peaks at wavenumber 1208, 1182, 1094 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 297, 208, 44, 254, 298, 347, 236, 165.



Quantification

Plasma **GC** Column: DB1 (40 m × 0.25 mm i.d. × 0.25 mm); Temperature: 250°. Carrier gas: H_2, 32 cm/s. Detection: NPD. Retention time: 9.9 min [Monkman *et al.* 1996]. Limit of detection, 0.5 µg/L [Monkman *et al.* 1996]. ECD. Limit of detection, 0.02 µg/L for each enantiomer [Scharpf *et al.* 1994]. ECD. Limit of detection, 0.2 µg/L [Beresford *et al.* 1987]. Column: fused silica coated with cross-linked 5% phenylmethyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Temperature programme: 280° to 320° at 20°/min, for 9 min. Carrier gas: N_2, flow rate 1 mL/min. ECD. Retention time: 4.4 min [Faulkner *et al.* 1986]. Limit of detection, 0.2 µg/L [Faulkner *et al.* 1986].

HPLC Electrochemical detection. Limit of detection, about 0.2 µg/L for each enantiomer [Josefsson, Norlander 1996].

Serum **HPLC** Electrochemical detection. Limit of detection, 0.1 µg/L [Shimooka *et al.* 1989].

Gingival Crevicular Fluid **GC-MS** ECD. Limit of detection, 0.5 µg/L [Monkman *et al.* 1996].

Tissue **GC-MS** ECD. Limit of detection, 0.5 ng/g for each enantiomer [Scharpf *et al.* 1994].

Note For GC-MS method see Beresford *et al.* [1988].

Disposition in the Body Amlodipine is slowly and almost completely absorbed after oral administration; peak plasma concentrations occur after 6 to 12 h. Absorption is not affected by food. It undergoes minimal presystemic metabolism; bioavailability is about 60 to 65% and it undergoes extensive but slow metabolism in the liver. Metabolites lack significant pharmacological activity and are excreted predominantly in urine (about 60% of a dose); <10% of a dose is excreted as the unchanged drug. About 20 to 25% of a dose is eliminated via bile. It is not removed by haemodialysis.

Therapeutic Concentration

The mean peak plasma concentration 7.6 h after administration of a 10 mg single oral dose in 12 healthy male subjects (with a mean age of 25.8 years) was 5.9 µg/L. The drug was administered after an overnight fast. Following oral administration of 15 mg daily for 14 days in 56 subjects mean peak plasma concentration was 18.1 µg/L after 8.7 h [Faulkner *et al.* 1986].

Toxicity

A 42-year-old female ingested 50 to 100 mg of amlodipine besylate together with alcohol in a suicide attempt. Symptoms included hypotension and sinus tachycardia. Serum-amlodipine concentration measured 2.5 h after ingestion was 88 µg/L; this had reduced to 79 µg/L 37.5 h after ingestion. Treatment included activated charcoal, whole bowel irrigation and intravenous sodium chloride 0.9%; the woman was discharged on day 2 [Stanek *et al.* 1997].

Bioavailability 60 to 65%.

Half-life Plasma, 35 to 50 h; 65 h in elderly subjects; 56 h in patients with hepatic impairment.

Volume of Distribution 21 L/kg.

Clearance Plasma, 18.5 L/h (elderly); 24.6 L/h (young); also, stated as 7 mL/min/kg.

Protein Binding 95 to 98%.

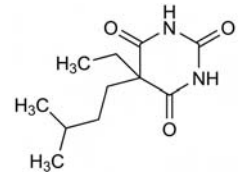
Dose Up to 10 mg (of the base) daily.

Beresford AP *et al.* (1987). Analysis of amlodipine in human plasma by gas chromatography. *J Chromatogr* 420: 178–183.
Beresford AP *et al.* (1988). Metabolism and kinetics of amlodipine in man. *Xenobiotica* 18: 245–254.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Faulkner JK *et al.* (1986). The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily. *Br J Clin Pharmacol* 22: 21–25.
Josefsson M, Norlander B (1996). Coupled-column chromatography on a Chiral-AGP phase for determination of amlodipine enantiomers in human plasma: an HPLC assay with electrochemical detection. *J Pharm Biomed Anal* 15: 267–277.
Monkman SC *et al.* (1996). Automated gas chromatographic assay for amlodipine in plasma and gingival crevicular fluid. *J Chromatogr Biomed Sci Appl* 678: 360–364.
Scharpf F *et al.* (1994). Enantioselective gas chromatographic assay with electron-capture detection for amlodipine in biological samples. *J Chromatogr Biomed Sci Appl* 655: 225–233.
Shimooka K *et al.* (1989). Analysis of amlodipine in serum by a sensitive high-performance liquid chromatographic method with amperometric detection. *J Pharm Biomed Anal* 7: 1267–1272.
Stanek EJ *et al.* (1997). Amlodipine overdose. *Ann Pharmacother* 31: 853–856.

Amobarbital

Barbiturate
$C_{11}H_{18}N_2O_3$ = 226.3
CAS—57-43-2

IUPAC Name 5-Ethyl-5-(3-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Amylobarbital; amylobarbitone; pentymalum.
Proprietary Names *Amal*; *Amytal*; *Eunoctal*; *Neur-Amyl*; *Stadadorm*.



Chemical Properties A white crystalline powder. Mp 156° to 158°. Soluble 1 in 1300 of water, 1 in 5 of ethanol, 1 in 17 of chloroform and 1 in 6 of ether; freely soluble in benzene; soluble in aqueous solutions of alkali hydroxides and carbonates; insoluble in petroleum ether and aliphatic hydrocarbons. pK_a 7.9 (25°). Log *P* (octanol/pH 7.4), 1.6.

Amobarbital Sodium

$C_{11}H_{17}N_2NaO_3$ = 248.3
CAS—64-43-7
Synonyms Amylobarbitone sodium; barbamylum; soluble amylobarbital.

Proprietary Names *Amylbarb Sodium*; *Amylobeta*; *Neur-Amyl Sodium*; *Sodium Amytal*. It is an ingredient of *Tuinal*.

Chemical Properties A white, hygroscopic, granular powder. Mp about 156°. Soluble 1 in <1 of water and 1 in 1 of ethanol; practically insoluble in chloroform and ether. Solutions in water decompose on standing.

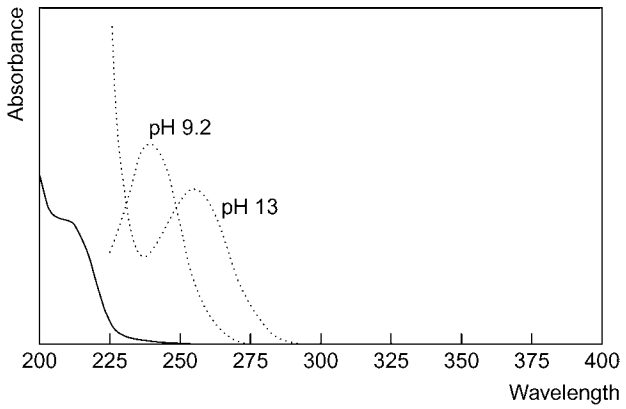
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.52; system TE—R_f 0.44; system TF—R_f 0.66; system TH—R_f 0.74; system TAD—R_f 0.58; system TAE—R_f 0.88. (Mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; Zwikker's reagent, pink.)

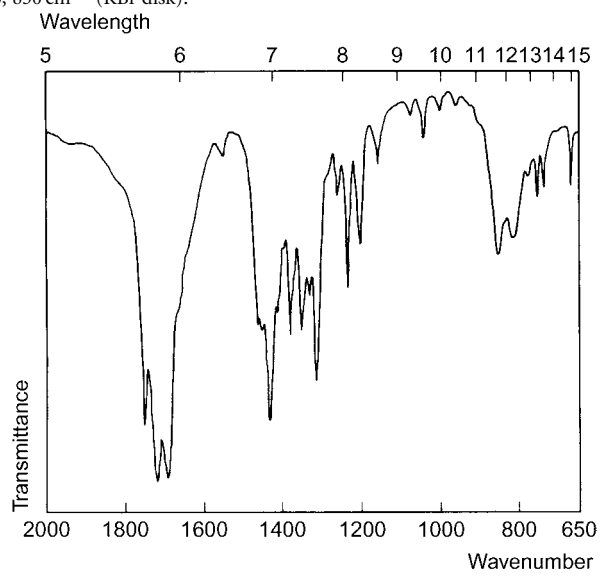
Gas Chromatography System GA—amobarbital RI 1710; amobarbital-Me_2 RI 1593, M (3-OH-) RI 1915, M (3-OH-)-Me_2- RI 1750, M (COOH-) RI 1960; system GB—amobarbital RI 1742; M (3-OH-) RI 2015; system GF—RI 2430; system GAJ—amobarbital RRT 0.794, M (3-OH-) RRT 1.138, M (COOH-) RRT 0.775 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HG—*k* 10.91; system HH—*k* 7.05; system HX—RI 424; system HY—RI 374; system HZ—retention time 4.0 min; system HAA—retention time 16.6 min.

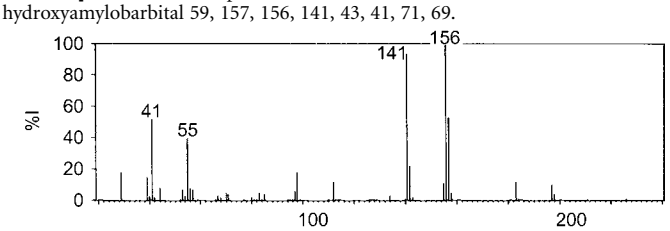
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—240 nm (A^1_1=445a); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=364b).



Infrared Spectrum Principal peaks at wavenumbers 1725, 1696, 1758, 1317, 1240, 850 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 156, 141, 157, 41, 55, 142, 98, 39; 3′-hydroxyamylobarbital 59, 157, 156, 141, 43, 41, 71, 69.



Quantification The following are general methods for most barbiturates.

Blood **HPLC** UV detection. Limit of detection, <1 mg/L [Gill *et al.* 1981].

Plasma **GC** AFID. Limit of detection, <500 ng/L [Villén, Petters 1983]. AFID. Limit of detection, <500 µg/L [Turcant *et al.*].

Immunoassay FPIA on COBAS INTEGRA. For comparison with GC–MS, see Cannon *et al.* [1999].

Serum **GC** NPD. Limit of detection, 500 µg/L [Soo *et al.* 1986].

Immunoassay FPIA on COBAS INTEGRA. For comparison with EMIT and GC–MS, see Schwenzer *et al.* [2000]. FPIA on COBAS INTEGRA. For comparison with GC–MS, see Cannon *et al.* [1999]].

Urine **GC** AFID. Limit of detection, <500 ng/L [Villén, Petters 1983].

GC–MS Limit of detection, about 20 µg/L [Liu *et al.* 1994].

HPLC UV detection. For amobarbital *N*-glycosides, see Nandi, Soine [1997].

Immunoassay For comparison with EMIT and GC–MS, see Schwenzer *et al.* [2000]. EMIT. Limit of detection, 2 mg/L for quinalbarbital [Law, Moffat 1981]. RIA. Limit of detection, 10 µg/L for quinalbarbital [Law, Moffat 1981].

Saliva **HPLC** UV detection. Limits of detection, 0.5 to 2.5 ng [Haginaka, Wakai 1987].

Note For a review of gas chromatographic methods, see Pillai, Dilli [1981].

Disposition in the Body Readily and almost completely absorbed after oral administration. It is metabolised by hydroxylation to give the major metabolite 3′-hydroxyamobarbital, which has about one-third of the activity of the parent substance. About 80 to 90% of a dose is excreted in the urine in 6 days, of which 30 to 50% is 3′-hydroxyamobarbital, up to 30% is *N*-β-D-glucopyranosylamobarbital and about 1% is unchanged drug. The metabolite pattern appears to be genetically determined. 5-(3′-Carboxybutyl)-5-ethylbarbituric acid has also been identified as a metabolite in urine. About 5% of a dose is eliminated in the faeces in 6 days.

Therapeutic Concentration In plasma, usually in the range 2 to 12 mg/L.

Toxicity The estimated minimum lethal dose is 1.5 g. Toxic effects are associated with plasma concentrations greater than 9 mg/L and fatalities with postmortem blood concentrations of 9 to 26 to 72 mg/L.

In a review of 55 cases of fatal overdosage, postmortem blood concentrations were in the range 13 to 96 mg/L [Gupta, Kofed 1966].

In 3 fatalities attributed to amobarbital overdose, the following postmortem tissue concentrations, mg/L or µg/g, were reported:

	Amobarbital	*3′-Hydroxyamobarbital*
Blood	36, 35, 25	2, –, 2
Bile	198, 178, –	6, –, –
Kidney	32, 59, 87	–, 2, 4
Liver	71, 79, 224	1, 2, 2
Lung	43, 40, 84	–, 2, 4
Spleen	77, 63, 104	–, 1, –
Urine	12, 12, –	9, –, –

[Robinson, McDowall 1979].

Bioavailability About 95%

Half-life Plasma half-life, 8 to 40 h (mean 24).

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, about 0.5 mL/min/kg.

Saliva Plasma : saliva ratio, about 2.8.

Protein Binding 40 to 60%.

Note For a review of the clinical pharmacokinetics of barbiturates, see Breimer [1977].

Dose 30 to 240 mg daily; up to 600 mg daily has been given.

Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.

Cannon RD *et al.* (1999). Comparison of the serum barbiturate fluorescence polarization immunoassay by the COBAS INTEGRA to a GC/MS method. *Ther Drug Monit* 21: 553–558.

Gill R *et al.* (1981). Analysis of barbiturates in blood by high-performance liquid chromatography. *J Chromatogr* 226: 117–123.

Gupta RC, Kofed J (1966). Toxicological statistics for barbiturates, other sedatives, and tranquillizers in Ontario: a 10-year survey. *Can Med Assoc J* 94: 863–865.

Haginaka J, Wakai J (1987). Liquid chromatographic determination of barbiturates using a hollow-fibre membrane for postcolumn pH modification. *J Chromatogr* 390: 421–428.

Law B, Moffat AC (1981). The evaluation of an homogeneous enzyme immunoassay (Emit) and radioimmunoassay for barbiturates. *J Forensic Sci Soc* 21: 55–66.

Liu RH *et al.* (1994). Improved gas chromatography/mass spectrometry analysis of barbiturates in urine using centrifuge-based solid-phase extraction, methylation, with d5-pentobarbital as internal standard. *J Forensic Sci* 39: 1504–1514.

Nandi V, Soine WH (1997). HPLC analysis for amobarbital N-glycosides in urine. *J Pharm Biomed Anal* 15: 1187–1195.

Pillai DN, Dilli S (1981). Analysis of barbiturates by gas chromatography. *J Chromatogr* 220: 253–274.

Robinson AE, McDowall RD (1979). The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man. *J Pharm Pharmacol* 31: 357–365.

Schwenzer KS *et al.* (2000). New fluorescence polarization immunoassays for analysis of barbiturates and benzodiazepines in serum and urine: performance characteristics. *J Anal Toxicol* 24: 726–732.

Soo VA *et al.* (1986). Screening and quantification of hypnotic sedatives in serum by capillary gas chromatography with a nitrogen-phosphorus detector, and confirmation by capillary gas chromatography-mass spectrometry. *Clin Chem* 32: 325–328.

Turcant A *et al.* (1982). Micromethod for automated identification and quantitation of fifteen barbiturates in plasma by gas-liquid chromatography. *J Chromatogr* 229: 222–226.

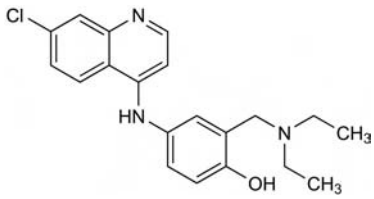
Villén T, Petters I (1983). Analysis of barbiturates in plasma and urine using gas chromatography without prior derivatization. *J Chromatogr* 258: 267–270.

A

Amodiaquine

Antimalarial
$C_{20}H_{22}ClN_3O = 355.9$
CAS—86-42-0

IUPAC Name 4-[(7-Chloroquinolin-4-yl)amino]-2-(diethylaminomethyl) phenol
Synonym Amodiachin
Proprietary Name *Basoquin*

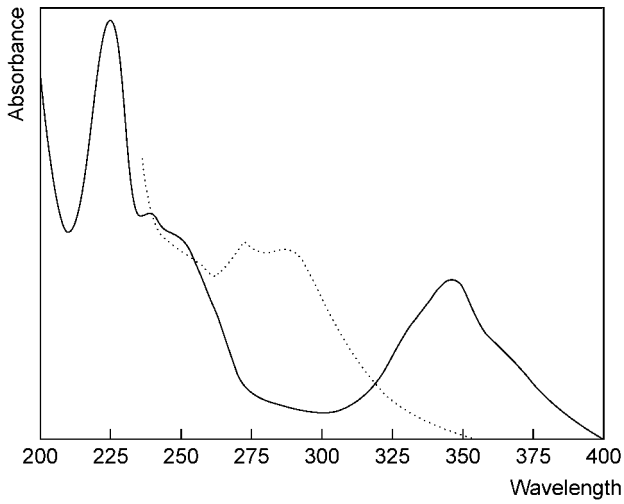


Chemical Properties White crystals. Mp 208°.

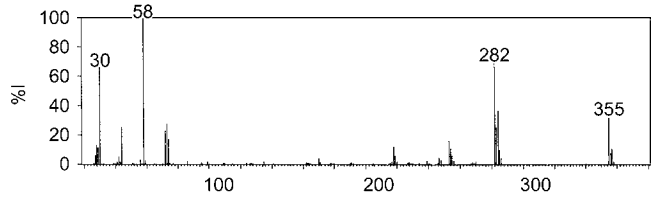
Amodiaquine Hydrochloride

$C_{20}H_{22}ClN_3O,2HCl, 2H_2O = 464.8$
CAS—69-44-3 (anhydrous); 6398-98-7 (dihydrate)
Proprietary Names *Camoquin*; *Flavoquine*.
Chemical Properties A yellow crystalline powder. Mp about 158°. Soluble 1 in about 22 of water and 1 in about 70 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Folin-Ciocalteu reagent—blue; Liebermann's reagent—black; Millon's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.08; system TC—R_f 0.40; system TE—R_f 0.74; system TL—R_f 0.37; system TAE—R_f 0.38; system TAJ—R_f 0.15; system TAK—R_f 0.00; system TAL—R_f 0.09 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—237 (A^{1}_{1}=600b), 343 nm; aqueous alkali—273, 287 nm.



Infrared Spectrum Principal peaks at wavenumbers 1565, 815, 1535, 1255, 869, 847 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 282, 30, 284, 355, 73, 283, 44.



Quantification

Note For a spectrofluorimetry method for the measurement of amodiaquine see Trenholme *et al* [1974].
Disposition in the Body Amodiaquine is readily absorbed after oral administration and widely distributed throughout the tissues. After absorption it is slowly released into the blood and excreted in the urine for at least 7 days after a single dose; the rate of excretion is increased in acid urine.

Therapeutic Concentration

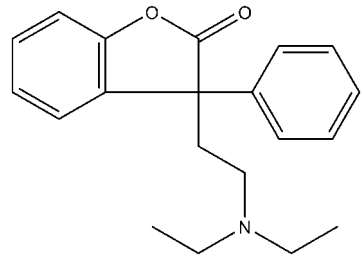
Following a single oral dose of 10 mg/kg to 5 subjects, serum concentrations of 0.30 to 0.68 (mean 0.5) mg/L were reported after 4 h; the ratio of erythrocyte to serum concentration varied with time and between individuals, but erythrocyte concentrations were generally higher than serum concentrations after 48 h [Trenholme *et al.* 1974].

Dose For an acute attack, the equivalent of 1.5 g of amodiaquine in 48 h (1.2 g in the first 24 h); for suppression, the equivalent of 300 to 600 mg every 7 days.

Trenholme GM *et al.* (1974). A method for the determination of amodiaquine. *Bull WHO* 51: 431–434.

Amolanone

Butyrolactone, Anaesthetic (Local)
$C_{20}H_{23}NO_2 = 309.4$
IUPAC Name 3-(2-Diethylaminoethyl)-3-phenyl-1-benzofuran-2-one
Synonyms AP 43; γ-diethylamino-α-o-hydroxyphenyl-α-phenylbutyrolactone.



Chemical Properties White crystals. Mp 43° to 44°. Bp 192° to 194°. Extracted by organic solvents from aqueous alkaline solutions.

Amolanone Hydrochloride

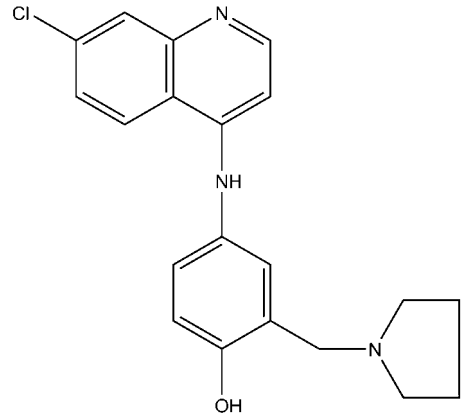
$C_{20}H_{23}NO_2,HCl = 345.9$
Proprietary Name *Amethone Hydrochloride*
Chemical Properties White crystals. Mp 152° to 153°. Soluble in water.

Colour Test Vitali's test—pale-brown/yellow (limit of detection, 0.25 μg).
Thin-layer Chromatography System T1—R_f 0.71 (location reagent acidified iodoplatinate spray positive reaction).
Gas Chromatography System G2/225—retention time 0.66 relative to codeine; system G4—retention time 0.43 relative to codeine.
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—265, 269, 276.5 nm.
Infrared Spectrum Principal peaks at wavenumbers 1795, 1025, 750, 1455 cm^{-1} (KBr disk).
Dose Used for local anaesthesia of the lower urinary tract; a 0.33% solution is instilled intra-urethrally. The maximum safe amount for topical use is 30 mL of a 0.33% solution.

Amopyroquine

Aminoquinoline, Antimalarial
$C_{20}H_{20}ClN_3O = 353.9$
CAS—550-81-2

IUPAC Name 4-(7-Chloro-4-quinolylamino)-2-(pyrrolidin-1-ylmethyl)phenol
Synonyms CI-356; PAM-780; WR-4835.



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

Amopyroquine Hydrochloride

$C_{20}H_{20}ClN_3O,2HCl = 426.8$
CAS—10350-81-9
Proprietary Name *Propoquin*
Chemical Properties Yellow crystalline powder.

Colour Tests Ammonium molybdate—(yellow) blue-black→green (limit of detection, 0.1 µg); Vitali's test—(yellow) orange/yellow/brown (limit of detection, 0.1 µg).
Thin-Layer Chromatography System T1—R_f 0.45 (location reagent acidified iodoplatinate spray, strong reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—224, 238, 342 nm.
Infrared Spectrum Principal peaks at wavenumbers 1563, 1490, 1252 cm^{-1} (KBr disk).
Quantification
***Other* HPLC** Rabbit plasma and red blood cells. Column: C_{18} Nucleosil (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 45 mmol/L potassium dihydrogenphosphate (pH 3.0; 15 : 85), flow rate 1.0 mL/min. UV detection (λ = 340 nm). Retention time: 3.5 min. Limit of detection, 7.1 µg/L in both media [Pussard *et al.* 1987]. Rat Plasma and Urine. Column: phenyl (100 × 8.0 mm i.d., 10 µm). Mobile phase: water : acetonitrile : methanol (90 : 8 : 2) containing triethylamine to pH 2.55 with orthophosphoric acid, flow rate 4.0 mL/min. UV detection (λ = 254 nm). Retention time: 4.0 min. Limit of detection, 5 to 10 µg/L in plasma [Coleman *et al.* 1987].
Disposition in the Body Rapidly absorbed following IM administration with peak plasma concentrations reached within 0.25 h. A primary amine metabolite is present in blood and plasma but in very low concentrations, with unchanged amopyroquine being the major circulating compound. The rapid absorption phase is followed by a tri-phasic decline with plasma concentrations representing only 9.5, 4.5 and 2.5% of the peak concentration after 3, 24 and 48 h, respectively. Unchanged amopyroquine and its amine metabolite are detectable in urine 48 h post-administration (1.2 and 0.2% of the administered dose, respectively).
Therapeutic Concentration

Ten healthy volunteers (mean age 27 years) were administered a single IM dose of amopyroquine base (2 mg/kg). The mean peak plasma concentration was 536 nmol/L after 0.25 h (range 296 to 883 nmol/L). In another study, 3 groups of patients diagnosed with *Plasmodium falciparum* malaria were administered different doses of IM amopyroquine. Patients in Group A were given a single 3 mg/kg injection; Group B 6 mg/kg; and Group C 6 mg/kg followed by 3 mg/kg after a 24-h interval. Whole blood concentrations in Group A ranged from 62 to 123 nmol/L at 48 h; Group B from 131 to 237 nmol/L at 24 h; and Group C from 106 to 236 nmol/L at 48 h [Verdier *et al.* 1989].

Twelve healthy male Caucasians (mean age 24 years) were administered 2 IM doses of amopyroquine base (6 mg/kg) with a 24-h interval. Peak blood and plasma concentrations for amopyroquine and its amine metabolite were reported as follows:

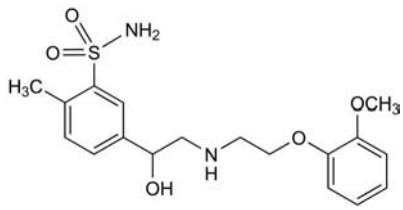
	Amopyroquine		*Amine metabolite*	
	C_{max} *(nmol/L)*	*Time (h)*	C_{max} *(nmol/L)*	*Time (h)*
Blood	2100	0.8	35	3.3
Plasma	800	0.6	10	2.7

An unidentified metabolite was also detected in blood and plasma at 3.3 h and 2.7 h, respectively [Pussard *et al.* 1994].
Volume of Distribution Apparent, 238 L/kg.
Clearance Systemic, approximately 2.06 L/min; renal 119 mL/min.
Distribution in Blood Blood : plasma ratio, 4.8.
Note For a review of the pharmacokinetics of amopyroquine and other 4-aminoquinoline antimalarials, see Pussard and Verdier [1994].
Dose Up to 9 mg/kg of the hydrochloride IM.

Coleman MD *et al.* (1987). High-performance liquid chromatographic method for the determination of amopyroquine in biological fluids. *J Chromatogr* 414: 242–247.
Pussard E *et al.* (1994). Pharmacokinetics and metabolism of amopyroquin after administration of two doses of 6 mg/kg im 24 h apart to healthy volunteers. *J Antimicrob Chemother* 34: 803–808.
Pussard E *et al.* (1987). Liquid chromatographic determination of amopyroquine in rabbit plasma and red blood cells. *J Chromatogr* 421: 192–197.
Pussard E, Verdier F (1994). Antimalarial 4-aminoquinolines: mode of action and pharmacokinetics. *Fundam Clin Pharmacol* 8: 1–17.
Verdier F *et al.* (1989). Pharmacokinetics of intramuscular amopyroquin in healthy subjects and determination of a therapeutic regimen for *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother* 33: 316–321.

Amosulalol

Antihypertensive
$C_{18}H_{24}N_2O_5S = 380.5$
CAS—85320-68-9
IUPAC Name 5-[1-Hydroxy-2-[2-(2-methoxyphenoxy)ethylamino]ethyl]-2-methylbenzenesulfonamide



Chemical Properties pK_a 7.72; 10.04. Log *D* (octanol/pH 7.5), 0.82.

Amosulalol Monohydrochloride

$C_{18}H_{25}ClN_2O_5S = 416.9$
CAS—70958-86-0; 93633-92-2
Synonym YM-09538
Proprietary Name *Lowgan*
Chemical Properties Colourless crystals. Mp 158° to 160°.

Ultraviolet Spectrum Aqueous acid—272 nm; aqueous alkali—272 nm.
Infrared Spectrum Principal peaks at wavenumber 1330, 1260, 1165, 755 cm^{-1}.
Mass Spectrum Principal ions at *m/z* (5-hydroxyl-) 196, 239, 208, 200, 225, 397, 396, 257.
Quantification
***Plasma* HPLC** Column: LiChrosorb RP-18 (15 cm × 4 mm i.d., 5 µm), stainless steel. Mobile phase: acetonitrile: methanol: 0.6 mol/L acetic acid : 0.02 mol/L ammonium acetate (20:5:30:50). UV detection (λ=271 nm). Retention time: amosulalol, 7.2 min; 5-hydroxylated amosulalol, 3.3 min [Kamimura *et al.* 1985]. Limit of detection, 0.02 mg/L [Kamimura *et al.* 1981].
***Urine* GC** Limit of detection, 0.2 mg/L [Kamimura *et al.* 1983].
Disposition in the Body Amosulalol is metabolised to at least 6 metabolites (free and conjugated forms), the main metabolite in humans being the sulfate conjugate of 5-hydroxyl amosulalol. 25 to 35% of an administered dose is excreted in urine unchanged within the first 6 h.
Therapeutic Concentration

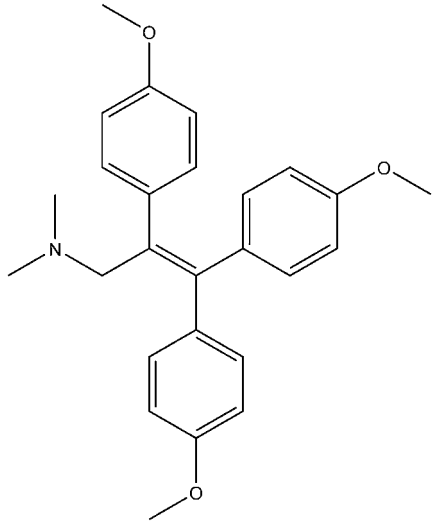
Twenty five healthy men, aged between 24 and 45 years, were administered with single oral doses of 12.5, 25, 50, 100 and 150 mg amosulalol after a fast. Mean peak plasma concentrations of 0.2, 0.49, 1.11, 1.87 and 4.09 mg/L were reached within 2.5 to 4 h respectively [Nakashima *et al.* 1984].

Toxicity Toxic by subcutaneous and IV routes.
Bioavailability Approximately 100%.
Half-life Approximately, 2.8 h (IV administration) or 5.7 h (oral).
Volume of Distribution 0.75 L/kg.
Clearance Approximately 8.09 L/h (IV); 7.28 L/h (oral).
Protein Binding 97 to 98%.
Dose Starting oral dose of 10 mg twice daily, up to a maximum daily dose of 60 mg.

Kamimura H *et al.* (1983). Determination of the alpha, beta-adrenoceptor blocker YM-09538 in urine by gas chromatography with a nitrogen-sensitive detector. *J Chromatogr Biomed Sci Appl* 275: 81–87.
Kamimura H *et al.* (1981). Determination of the alpha,beta-adrenoceptor blocker YM-09538 in plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr Biomed Sci Appl* 255: 115–121.
Kamimura H *et al.* (1985). Metabolism of amosulalol hydrochloride in man: quantitative comparison with laboratory animals. *Xenobiotica* 15(5): 413–420.
Nakashima M *et al.* (1984). Amosulalol, a combined alpha and beta adrenoceptor antagonist: kinetics after intravenous and oral doses. *Clin Pharmacol Ther* 36: 436–443.

Amotriphene

Vasodilator, Antiarrhythmic (Coronary)
$C_{26}H_{29}NO_3 = 403.5$
CAS—5585-64-8
IUPAC Name 2,3,3-Tris(4-methoxyphenyl)-*N*,*N*-dimethyl-prop-2-en-1-amine
Synonyms Aminoxitrifeno; aminoxytriphen; aminoxytriphenum; 2,3,3-tris(*p*-methoxyphenyl)-*N*,*N*-dimethylallylamin; 2,3,3-tris(*p*-methoxyphenyl)-*N*,*N*-dimethylallylamine.



Amotriphene Hydrochloride

$C_{26}H_{29}NO_3$,HCl = 439.9

CAS—568-69-4

IUPAC Name 2,3,3-Tris(4-methoxyphenyl)-*N*,*N*-dimethyl-prop-2-en-1-amine hydrochloride

Synonyms Aminooxytriphene hydrochloride; 3-dimethylamino-1,1,2-tris(4-methoxyphenyl)-1-propene hydrochloride; Win 5494.

Proprietary Name *Myordil*

Disposition in the Body

Therapeutic Concentration

Amotriphene hydrochloride (100 mg) was administered to 40 angina patients. This dose caused gastric irritation in a moderate number of patients, so the dose was reduced to 50 mg for the last 8 months of the study. Two patients stopped drug therapy due to nausea, 1 patient was classified as a failure, and 2 died. Of the remaining 35 patients, 50% remained free of anginal pain whereas the remaining 17 were put onto their original therapy with concomitant therapy with amotriphene [Day 1961].

In another study, a dose of 25 to 50 mg was orally administered 3 times daily to 75 patients: 36 had coronary artery disease, 6 had acute myocardial infarction, and 33 had various cardiac arrhythmias. The drug improved the status and wellbeing of patients, and reduced the incidence of ventricular premature contractions [Harris 1961].

Day HW (1961). Angina pectoris: a clinical note in the use of Myordil (Win 5494). *J Kans Med Soc* 62: 143.

Harris R (1961). Clinical observations of amotriphene hydrochloride. *NY State J Med* 61: 4009–4014.

Amoxapine

Dibenzoxazepine, Tricyclic Antidepressant

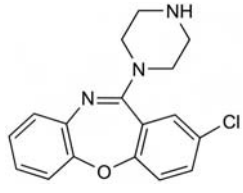
$C_{17}H_{16}ClN_3O$ = 313.8

CAS—14028-44-5

IUPAC Name 8-Chloro-6-piperazin-1-ylbenzo[*b*][1,5]benzoxazepine

Synonyms 2-Chloro-11-(1-piperazinyl)dibenz[b,f]oxazepine; CL-67772.

Proprietary Names *Asendin*; *Asendis*; *Defanyl*; *Demolox*; *Moxadil*.

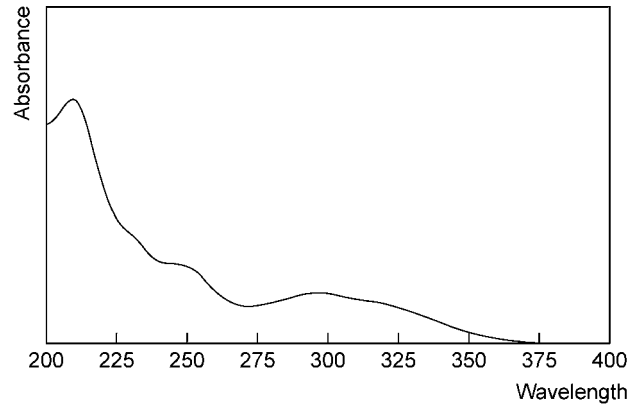


Chemical Properties Crystals. Mp 175° to 176°. Log *P* (octanol/water) 3.38 [Meylan, Howard 1995].

Gas Chromatography System GA—amoxapine RI 2638, M (7-OH-) RI 2951, M (8-OH-) RI 2959; system GB—amoxapine RI 2746, M (7-OH-) RI 3525, M (8-OH-) RI 3546, system GM—amoxapine RRT 2.831 (relative to iprindole).

High Performance Liquid Chromatography System HX—RI 398; system HAA—RT 14.19 min.

Ultraviolet Spectrum Aqueous acid—252 (A_1^1=362 a), 293 (A_1^1=315 a); aqueous alkali—250, 299 nm.



Infrared Spectrum Principal peaks at wavenumbers 1604, 1592, 752, 1181, 1246, 1107 cm^{-1}.

Mass Spectrum Principal ions at *m*/*z* 245, 257, 247, 193, 56, 246, 228, 259.

Quantification

Blood **GC** Column: Cross-linked methyl silicone fused silica capillary (25 m × 0.20 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 140–204 µg/L; limit of detection, 42–62 µg/L depending on the Bond-Elut column used for extraction [Martinez *et al.* 2002]. Column; Rtx-50 (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 130° for 3 min to 270° at 10°/min for 10 min to 295° at 10°/min for 5 min. Retention time: 1.22 min. Limit of quantification, 0.05 mg/L [Mazzola *et al.* 2000]. Column: 3% GasChrom Q 100/120 mesh (90 cm [3 ft]). Temperature programme: 200° for 8 min to 260° at 8°/min for 16 min. FID. Limit of detection not reported [Rohrig, Backer 1986]. Column: 3% OV-17 on Chromosorb WHP 100/120 mesh (1.83 m × 6.3 mm o.d.). Carrier gas: He, 32 mL/min. Temperature programme: 190° for 1 min to 300° at 16°/min for 19 min. Retention time: 10.0 min. Limit of detection, 0.5 mg/L [Winek *et al.* 1984]. Column: 3% OV-17 Gas-Chrom Q, 100/120 mesh (1.2 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. FID. Limit of detection, 0.25 mg/L [Taylor *et al.* 1982].

GC-MS Column: 5% OV-225 Chromosorb-WHP, 80/100 mesh (1.2 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 240°. EI ionisation at 70 eV. Limit of detection, 0.5 mg/L [Wu Chen *et al.* 1983].

HPLC Column: (1) C_8 RP TSK gel Super-Octyl (100 × 4.6 mm i.d., 2 µm); (2) Hypersil MOS-C_8 (100 × 4.6 mm, 5 µm). Mobile phase: methanol : 20 mmol/L potassium dihydrogen phosphate (pH 7, 60 : 40), flow rate 0.6 mL/min. Retention time: 5.6 min for column 1, 7.8 min for column 2. Column: C_8 reversed phase (100 × 4.6 mm i.d., 2 µm). Mobile phase: methanol : 20 mmol/L potassium dihydrogen phosphate (pH 7.0, 60 : 40), flow rate 0.6 mL/min. UV detection (λ = 254 nm). Retention time: 5.6 min. Limit of quantification, 0.5 mg/L [Tanaka *et al.* 1997].

LC-MS Column: Xterra RP_{18} (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2, 5 : 95 for 2 min to 20 : 80 over 2 min to 30 : 70 over 12 min for 2 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 µg/L [Titier *et al.* 2007].

Plasma **GC-MS** Column: DB-5MS fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 1.0 µg/L [Lee *et al.* 2008].

HPLC Column: Reversed phase CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.5 N acetic acid (30 : 70) containing 0.05% hexylamine, flow rate 0.8 mL/min. UV detection (λ = 310 nm). Limit of detection, 5.9 µg/L [Hüe *et al.* 1998]. Column: Spherisorb C_6 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate containing 14 mmol/L orthophosphoric acid : acetonitrile containing 105 µmol/L nonylamine (77 : 23), flow rate 2.2 mL/min. UV detection (λ = 210 nm). Limit of detection, 2 µg/L [Cheung *et al.* 1991]. Column: ODS-2 Spherisorb (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.15% TEA (pH 3.0) : acetonitrile (1 : 1), flow rate 1.5 mL/min. UV detection (λ = 212 nm). Limit of quantification, 2.0 µg/L [Selinger *et al.* 1989]. Column: Supelco LC-1 TMS-bonded silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate : acetonitrile (75 : 25) containing 1.2 mL/L *n*-butylamine, 1.0 mL/L orthophosphoric acid and 0.005 mol/L heptanesulfonic acid, flow rate 1.8 mL/min. Electrochemical detection. Limit of detection, 5 µg/L [Suckow, Cooper 1985]. Column: Cyanopropylsilane (250 × 4.6 mm i.d.). Mobile phase: 0.03 mol/L sodium acetate (pH 6.0) : acetonitrile (35 : 65), flow rate 3.0 mL/min. UV detection (λ = 250 nm). Limit of detection, 25 µg/L for amoxapine and metabolites [Johnson *et al.* 1984]. See also Yufu *et al.* [1984] and Kimball, Lampert [1982].

LC-MS Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 1.0 mL/min. Positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.2 mg/L, limit of detection, 0.16 mg/L [Shinozuka *et al.* 2006].

Serum **GC** Column: 3% SP 2100 on 100/120 mesh (1.8 or 2 m × 4 mm i.d.). Temperature: 255°. Carrier gas: Ar : CH_4. ECD. Limit of detection not reported [Cooper, Kelly 1979].

HPLC See Blood. Limit of quantification, 0.05 mg/L [Tanaka *et al.* 1997]. Column: TSkgel ODS-80TM (150 × 4.0 mm i.d.). Mobile phase: acetonitrile : 100 mmol/L potassium phosphate buffer (pH 2.7) containing sodium 1-heptanesulfonate (22.5 : 77.5 to 30 : 70 at 10 min). UV detection (λ = 210 nm). Limit of detection, 10 µg/L [Matsumoto *et al.* 1989]. Column: Cosmosil C_{18} reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: 1% TEA (pH 3.0) : methanol : tetrahydrofuran (70 : 20 : 10), flow rate 1 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 µg/L [Kobayashi *et al.* 1985]. Column: µBondapak C_{18}. Mobile phase: acetonitrile-water (85 : 15) with 5 mL 10 mol/L Tris buffer (pH 9), flow rate 4.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 50 µg/L for amoxapine and 8-hydroxyamoxapine [Tasset, Pesce 1984]. Column: Zorbax cyanopropyl (250 × 4.6 mm i.d., 5–6 µm). Mobile phase: 0.5 mol/L acetic acid: acetonitrile : *n*-butylamine (59 : 41 : 0.024), flow rate 1.5 mL/min). UV detection (λ = 254 nm). Limit of detection, 8–10 µg/L [Lensmeyer, Evenson 1984]. See also Beierle, Hubbard [1983], Ketchum *et al.* [1983], and Tasset, Hassan [1982].

LC-MS Column: Xterra MS C_{18} (50 × 2.1 mm i.d., 5 µm). ESI, positive ion mode, MRM acquisition mode. Retention time: 4.9 min. Limit of detection, 10 µg/L [Sauvage *et al.* 2006].

Urine **GC** See Blood [Winek *et al.* 1984]. Column: 3% OV-17 Gas-Chrom Q 100/120 mesh (1.2 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. FID. Limit of detection, 0.25 mg/L [Taylor *et al.* 1982]. Column: 3% SP 2100 on 100/120 mesh (1.8 or 2 m × 4 mm i.d.). Temperature: 255°. Carrier gas: He. FID. Limit of detection not reported [Cooper, Kelly 1979].

HPLC See Serum [Tanaka *et al.* 1997].

Bile **GC** See Blood [Winek *et al.* 1984]. See Urine [Taylor *et al.* 1982].

CSF **GC** See Blood [Winek *et al.* 1984].

Gastric Contents **GC** See Blood [Winek *et al.* 1984]]. See Urine [Taylor *et al.* 1982].

Ocular Fluid **GC** See Blood [Winek *et al.* 1984].

Bone Marrow **GC** See Blood [Winek *et al.* 1984].

Brain **GC** See Blood [Winek *et al.* 1984].

HPLC See Blood [Tanaka *et al.* 1997].

Liver **GC** See Blood [Winek *et al.* 1984]. See Urine [Taylor *et al.* 1982].

HPLC See Blood [Tanaka *et al.* 1997].

Disposition in the Body Amoxapine is rapidly and almost completely absorbed following oral administration, reaching a peak after ~90 min [Kimball, Lampert 1982]. It is metabolised by hydroxylation to 7-hydroxyamoxapine and 8-hydroxyamoxapine, which are both active. The hydroxylated metabolites are excreted in the urine as glucuronide conjugates and are also eliminated in the faeces in the unconjugated form. <5% of a dose is excreted in the urine as unchanged drug. Amoxapine is the *N*-demethylated metabolite of the antipsychotic agent loxapine.

Therapeutic Concentration

After a single oral dose of 50 mg to 26 subjects, a mean peak serum amoxapine concentration of 0.03 mg/L was attained in 1–2 h; the peak concentration of 8-hydroxyamoxapine was of a similar order [Cooper, Kelly 1979].

Following oral administration of 300 mg amoxapine daily in divided doses to 10 subjects, steady-state serum concentrations of 0.02–0.09 mg/L of amoxapine and 0.16–0.51 mg/L of 8-hydroxyamoxapine were reported [Boutelle 1980].

Six healthy male volunteers were administered a single dose of 150 mg amoxapine. A mean peak plasma concentration of 78 µg/L was reached at 1.64 h [Selinger *et al.* 1989].

Toxicity

A 69-year-old woman was found dead with a green, powdery substance on her lips. She was in possession of a prescription for loxapine. The concentration of amoxapine in her heart blood and bile at postmortem were 0.6 and 4.7 mg/L, respectively [Mazzola *et al.* 2000].

A 46-year-old female committed suicide with aspirin, diphenhydramine and amoxapine. Amoxapine and its metabolite concentrations at postmortem were as follows:

	Amoxapine	*N-acetylated amoxapine*
Premortem		
Blood (mg/L)	0.4	0.7
Postmortem		
Blood (mg/L)		
16 : 10	0.3	0.4
22 : 00	0.8	0.6
Liver (mg/kg)	27.6	3.7
Brain (mg/kg)	12.9	2.4
Stomach (mg)	123	1978
Small bowel (mg/kg)	2.1	16.8

[Osiewicz, Middleberg 1989].

Two fatal cases of amoxapine overdose (a 48-year-old white female and a 41-year-old white male) had blood amoxapine concentrations of 5.7 and 3.2 mg/L, respectively. The metabolites were not detected [Rohrig, Backer 1986].

The following postmortem concentrations were reported in 3 fatalities caused by amoxapine overdose: blood 11.5, 2.8 and 0.89 mg/L; bile 1264.5, 69.1 and 14.3 mg/L; and liver 112.2, 40.1 and 16.8 µg/g; in the first case brain and urine concentrations of 2.5 µg/g and 28.3 mg/L, respectively, were also reported [Winek *et al.* 1984].

Five patients with amoxapine overdose achieved maximum serum concentrations ranging from 308–2900 and 405–1480 µg/L for amoxapine and 8-hydroxyamoxapine, respectively [Tasset, Pesce 1984].

In a review of 33 cases of amoxapine overdose, 3 subjects had no toxic symptoms, 10 suffered mild toxic reactions, 15 developed severe toxicity and 5 died. Postmortem blood concentrations of 0.26–6.70 mg/L (mean, 2.8) of amoxapine plus 8-hydroxyamoxapine were reported in 4 of the 5 fatalities [Litovitz, Troutman 1983].

The following postmortem tissue concentrations were reported in death following ingestion of 2 g amoxapine: blood 7.2 mg/L, bile 823 mg/L, liver 36 µg/g; a blood-alcohol concentration of 280 mg/L was also reported. In a further 2 cases where amoxapine was a contributory cause of death, postmortem concentrations were blood 1.66 and 2.95 mg/L, bile 75 mg/L, liver 23.2 µg/g and urine 22 mg/L [Wu Chen *et al.* 1983].

In 5 fatal overdoses in which the subjects had ingested between 2000 and 8500 mg amoxapine, postmortem blood concentratins ranged from 261–6700 µg/L [Litovitz, Troutman 1983].

A 53-year-old male (A) and a 21-year-old female (B) died following the ingestion of amoxapine and the following concentrations were measured at postmortem:

Sample	*Case A*	*Case B*
Liver (mg/kg)	150 ± 13	ND
Gastric contents (mg/L)	110 ± 12	ND
Bile (mg/L)	61 ± 11	ND
Blood (mg/L)	18 ± 2.2	6.7 ± 0.9
Urine (mg/L)	13 ± 2.3	11 ± 0.8

ND, not determined
[Taylor *et al.* 1982].

In a non-fatal case of overdose in a 36-year-old woman, the following serum amoxapine and metabolite concentrations (µg/L) were measured at various time points after her admission to hospital:

Time after admission (h)	*Amoxapine*	*7-OH-Amoxapine*	*8-OH-Amoxapine*
0	2300	110	820
3	1150	60	860
8	700	<50	630

[Bock *et al.* 1982].
For a case of recovery from amoxepine overdose, see Miller *et al.* [1990].

Half-life Plasma half-life: amoxapine ~8 h, 7-hydroxyamoxapine ~6 h, 8-hydroxyamoxapine ~33 h.

Protein Binding ~90%.

Note For a review of the pharmacokinetics of amoxapine, see Jue *et al.* [1982] or Calvo *et al.* [1985].

Dose 150 to 600 mg daily.

Beierle FA, Hubbard RW (1983). Liquid chromatographic separation of antidepressant drugs: II. Amoxapine and maprotiline. *Ther Drug Monit* 5: 293–301.

Bock JL *et al.* (1982). Amoxapine overdose: a case report. *Am J Psychiatry* 139: 1619–1620.

Boutelle WE (1980). Clinical response and blood levels in the treatment of depression with a new antidepressant drug, amoxapine. *Neuropharmacology* 19: 1229–1231.

Calvo B *et al.* (1985). Pharmacokinetics of amoxapine and its active metabolites. *Int J Clin Pharmacol Ther Toxicol* 23: 180–185.

Cheung SW *et al.* (1991). Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high-performance liquid chromatography. *J Chromatogr* 564: 213–221.

Cooper TB, Kelly RG (1979). GLC analysis of loxapine, amoxapine, and their metabolites in serum and urine. *J Pharm Sci* 68: 216–219.

Hüe B *et al.* (1998). Concurrent high-performance liquid chromatographic measurement of loxapine and amoxapine and of their hydroxylated metabolites in plasma. *Ther Drug Monit* 20: 335–339.

Johnson SM *et al.* (1984). Isocratic liquid chromatographic method for the determination of amoxapine and its metabolites. *J Pharm Sci* 73: 696–699.

Jue SG *et al.* (1982). Amoxapine: a review of its pharmacology and efficacy in depressed states. *Drugs* 24: 1–23.
Ketchum C *et al.* (1983). Analysis of amoxapine, 8-hydroxyamoxapine, and maprotiline by high-pressure liquid chromatography. *Ther Drug Monit* 5: 309–312.
Kimball DF, Lampert AA (1982). Analysis of amoxapine and its metabolites in human plasma by high pressure liquid chromatography. *SDJ Med* 35: 31–33.
Kobayashi A *et al.* (1985). Determination of amoxapine and its metabolites in human serum by high-performance liquid chromatography. *Neuropharmacology* 24: 1253–1256.
Lee XP *et al.* (2008). Determination of tricyclic antidepressants in human plasma using pipette tip solid-phase extraction and gas chromatography–mass spectrometry. *J Sep Sci* 31: 2265–2271.
Lensmeyer GL, Evenson MA (1984). Stabilized analysis of antidepressant drugs by solvent-recycled liquid chromatography: procedure and proposed resolution mechanisms for chromatography. *Clin Chem* 30: 1774–1779.
Litovitz TL, Troutman WG (1983). Amoxapine overdose. Seizures and fatalities. *JAMA* 250: 1069–1071.
Martinez MA *et al.* (2002). Simultaneous determination of viloxazine, venlafaxine, imipramine, desipramine, sertraline, and amoxapine in whole blood: comparison of two extraction/cleanup procedures for capillary gas chromatography with nitrogen-phosphorus detection. *J Anal Toxicol* 26: 296–302.
Matsumoto K *et al.* (1989). Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites. *Clin Chem* 35: 453–456.
Mazzola CD *et al.* (2000). Loxapine intoxication: case report and literature review. *J Anal Toxicol* 24: 638–641.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Miller MT *et al.* (1990). Amoxapine overdose: recovery after severe metabolic acidosis (pH 6.69) and status epilepticus. *Anaesth Intens Care* 18: 246–248.
Osiewicz RJ, Middleberg R (1989). Detection of a novel compound after overdoses of aspirin and amoxapine. *J Anal Toxicol* 13: 97–99.
Rohrig TP, Backer RC (1986). Amoxapine overdose: report of two cases. *Anal Toxicol* 10: 211–212.
Sauvage FL *et al.* (2006). A fully automated turbulent-flow liquid chromatography–tandem mass spectrometry technique for monitoring antidepressants in human serum. *Ther Drug Monit* 28: 123–130.
Selinger K *et al.* (1989). A high-performance liquid chromatographic method for the analysis of amoxapine in human plasma. *J Pharm Biomed Anal* 7: 1001–1007.
Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.
Suckow RF, Cooper TB (1985). Determination of amoxapine and metabolites in plasma by liquid chromatography with electrochemical detection. *J Chromatogr* 338: 225–229.
Tanaka E *et al.* (1997). Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 microm porous microspherical silica gel. *J Chromatogr B Biomed Sci Appl* 692: 405–412.
Tasset JJ, Hassan FM (1982). Liquid-chromatographic determination of amoxapine and 8-hydroxyamoxapine in human serum. *Clin Chem* 28: 2154–2157.
Tasset JJ, Pesce AJ (1984). Amoxapine in human overdose. *J Anal Toxicol* 8: 124–128.
Taylor RL *et al.* (1982). The determination of amoxapine in human fatal overdoses. *J Anal Toxicol* 6: 309–311.
Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.
Winek CL *et al.* (1984). Amoxapine fatalities: three case studies. *Forensic Sci Int* 26: 33–38.
Wu Chen NB *et al.* (1983). Analysis of blood and tissue for amoxapine and trimipramine. *J Forensic Sci* 28: 116–121.
Yufu N *et al.* (1984). Simultaneous measurement of various antidepressants in the plasma of depressed patients by high performance liquid chromatography. *Folia Psychiatr Neurol Jpn* 38: 57–64.

Amoxicillin

Antibacterial

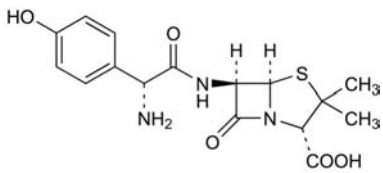
$C_{16}H_{19}N_3O_5S = 365.4$

CAS—26787-78-0

IUPAC Name (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Synonyms D(−)-α-Amino-*p*-hydroxybenzylpenicillin; Amoxycillin; Amoxicilline.

Proprietary Names *AMPC*; *Amolin*; *Amopenixin*; *Helvamox*; *Moxal*; *Pasetocin*; *Penimox*; *Zamocilline*.



Chemical Properties pK_a 2.4, 7.4, 9.6. Log *P* (octanol/water), 0.87.

Amoxicillin Sodium

$C_{16}H_{18}N_3NaO_5S = 387.4$

CAS—34642-77-8

Proprietary Names *Amoxil (injection)*; *Clamoxyl (injection)*.

Chemical Properties A white powder. Soluble 1 in less than 1 of water.

Amoxicillin Trihydrate

$C_{16}H_{19}N_3O_5S,3H_2O = 419.4$

CAS—61336-70-7

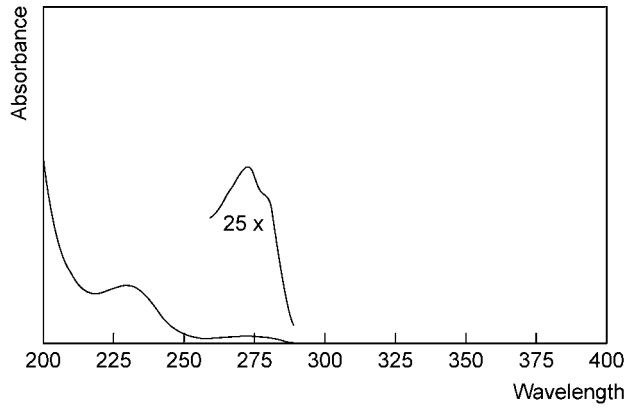
Synonym Amoxicillin

Proprietary Names *Amoxican*; *Amoxil*; *Amoxypen*; *Clamoxyl*; *Imacillin*; *Larotid*; *Moxacin*; *Moxilean*; *Novamoxin*; *Penamox*; *Polymox*; *Robamox*; *Trimox*; *Utimox*; *Wymox*. It is an ingredient of *Augmentin*.

Chemical Properties A white crystalline powder. Soluble 1 in 400 of water, 1 in 1000 of ethanol, and 1 in 200 of methanol; practically insoluble in chloroform and ether.

High Performance Liquid Chromatography System HY—RI 226; system HAA—retention time 3.1 min.

Ultraviolet Spectrum Amoxicillin trihydrate, aqueous acid—230 (A_1^1=225a), 272 (A_1^1=26a); aqueous alkali—247 nm (A_1^1=286b), 291 nm (A_1^1=62a).



Infrared Spectrum Principal peaks at wavenumbers 1775, 1583, 1684, 1248, 1613, 1313 cm^{-1} (amoxicillin trihydrate, KBr disk).

Quantification

Blood **HPLC** UV detection (λ=210 nm). Limit of quantification, 1.0 mg/L [Yuan *et al.* 1997].

Plasma **HPLC** DAD. Limit of quantification, 1.0 mg/L [Menelaou *et al.* 1999]. Fluorescence detection (λ_{ex}=395 nm; λ_{em}=485 nm). Limit of quantification, 0.1 mg/L [Mascher, Kikuta 1998]. UV detection (λ=210 nm). Limit of quantification, 0.25 mg/L [Yuan *et al.* 1997]. UV detection. Limit of detection, 500 µg/L [Lee, Brooks 1984].

Serum **HPLC** See Plasma [Mascher, Kikuta 1998].

Urine **HPLC** Fluorescence detection. Limit of detection, 2.5 mg/L for amoxicillin and penicilloic acid [Lee 1979].

Ear Fluid **HPLC** See Plasma [Yuan *et al.* 1997].

Disposition in the Body Amoxicillin is rapidly absorbed after oral administration. About 60% of an oral dose is excreted in the urine as unchanged drug in 6 h and 20% as the inactive metabolite, penicilloic acid, in the same period. After parenteral administration, up to 75% of a dose is excreted in the urine unchanged in 6 h.

Therapeutic Concentration

Twelve healthy male and female subjects with a mean age of 33.7 years (range 18 to 47 years) were fasted overnight and after administration of 1 g standard reference formulation amoxicillin (A), dispersible tablet (B) and 1 g dispersible tablet in suspension (C) the mean peak serum concentrations observed were 14.1 mg/L at 2.3 h for A, 15.1 mg/L at 2.0 h for B and 15.1 mg/L at 1.5 h for C [Prevot *et al.* 1997].

Following an oral dose of 500 mg to 8 subjects, peak plasma concentrations of 6.0 to 15.3 (mean 10) mg/L were attained in 1 to 2 h [Arancibia *et al.* 1980].

Half-life Plasma half-life, about 1 h, increased in renal failure.

Volume of Distribution About 0.2 to 0.4 L/kg.

Clearance Plasma clearance, 3 to 5 mL/min/kg.

Protein Binding About 20%.

Note For reviews of the pharmacokinetics of amoxicillin, see Brogden *et al.* [1975, 1979].

Dose The equivalent of 0.75 to 1.5 g of amoxicillin daily. One-day courses of 3 to 6 g are also given.

Arancibia A *et al.* (1980). Absorption and disposition kinetics of amoxicillin in normal human subjects. *Antimicrob Agents Chemother* 17: 199–202.
Brogden RN *et al.* (1975). Amoxycillin: A review of its antibacterial and pharmacokinetic properties and therapeutic use. *Drugs* 9: 88–140.
Brogden RN *et al.* (1979). Amoxycillin injectable: a review of its antibacterial spectrum, pharmacokinetics and therapeutic use. *Drugs* 18: 169–184.
Lee TL (1979). High-pressure liquid chromatographic determination of amoxicillin in urine. *J Pharm Sci* 68: 454–458.
Lee TL, Brooks MA (1984). High-performance liquid chromatographic determination of amoxicillin in human plasma using a bonded-phase extraction. *J Chromatogr* 306: 429–435.
Mascher HJ, Kikuta C (1998). Determination of amoxicillin in human serum and plasma by high-performance liquid chromatography and on-line postcolumn derivatisation. *J Chromatogr A* 812 (1–2): 221–226.
Menelaou A *et al.* (1999). Simultaneous quantification of amoxycillin and metronidazole in plasma using high-performance liquid chromatography with photodiode array detection. *J Chromatogr B, Biomed Sci Appl* 731: 261–266.
Prevot MH *et al.* (1997). Pharmacokinetics of a new oral formulation of amoxicillin. *Eur J Drug Metab Pharmacokinet* 22(1): 47–52.
Yuan Z *et al.* (1997). High-performance liquid chromatographic analysis of amoxicillin in human and chinchilla plasma, middle ear fluid and whole blood. *J Chromatogr B Biomed Sci Appl* 692(2): 361–366.

Amphotericin B

Antifungal

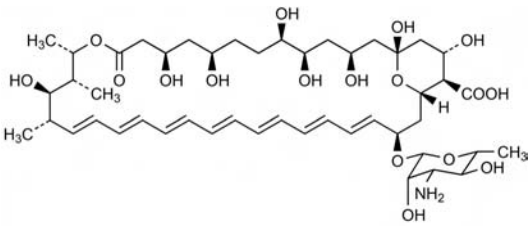
$C_{47}H_{73}NO_{17}$ = 924.1

CAS—1397-89-3

IUPAC Name (1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*, 36*R*,37*S*)-33-[(2*R*,3*S*,4*S*,5*S*,6*R*)-4-Amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid.

Synonyms Amphotericin; anfotericina B.

Proprietary Names *Ampho-Moronal*; *Fungilin*; *Fungizone*. It is an ingredient of *Mysteclin (syrup)*.



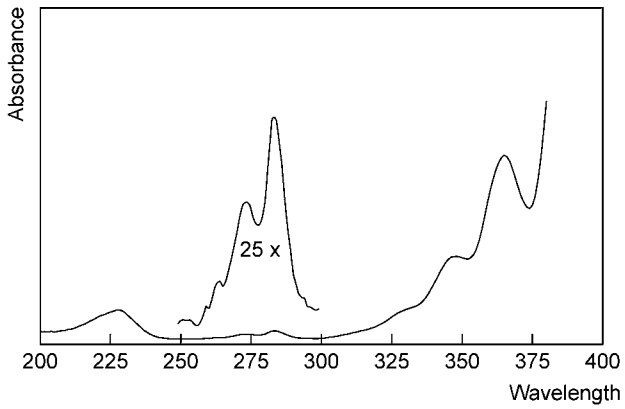
Chemical Properties A yellow to orange powder. It gradually decomposes above 170°. Practically insoluble in water, ethanol, chloroform and ether; soluble 1 in 200 of dimethylformamide and 1 in 20 of dimethylsulfoxide; soluble in propylene glycol. pK_a 5.5, 10.0.

Note A mixture of antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means.

High Performance Liquid Chromatography System HAA—retention time 15.7 min.

Column: RP-C_{18} μBondapak (300 × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile: 10 mmol/L acetate buffer , pH 4 (37:63 v/v). Flow rate: 1 mL/min for first 6 min and 2 mL/min until completion. Internal standard (IS): natamycin. UV detection (λ=383 nm). Retention time: 15 min [Lambros *et al.* 1996].

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1010, 1065, 1038, 1103, 1183, 1126 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** Column: RP-C_{18} μBondapak (300 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile: 20 mmol/L disodium edetate , pH 5 (45:55), flow rate 1 mL/min. IS: 1-amino-4-nitronaphthalene. UV detection (λ=407 nm). Retention time: 6.3 min [Eldem, Arica-Cellat 2001]. Limit of detection, 5 μg/L [Eldem, Arica-Cellat 2001].

LC-MS detection. Limit of quantification, 2 mg/L [Lee *et al.* 2001].

Serum **HPLC** UV detection. Limit of quantification, 0.05 mg/L [Alak *et al.* 1996]. UV detection (λ=383 nm). Limit of detection, 0.1 mg/L [Lambros *et al.* 1996]. UV detection. Limit of detection, 20 μg/L [Nilsson-Ehle *et al.* 1977].

Cerebrospinal Fluid **HPLC** See Serum [Nilsson-Ehle *et al.* 1977].

Urine **LC-MS** See Plasma. Limit of quantification, 0.05 mg/L [Lee *et al.* 2001].

Liver **HPLC** See Serum [Lambros*et al.* 1996].

Lung **HPLC** See Plasma [Lambros*et al.* 1996].

Faeces **LC-MS** See Plasma. Limit of quantification, 0.4 mg/L [Lee *et al.* 2001].

Note For an immunoassay for the quantification of amphotericin see Machard *et al.* [2000].

Disposition in the Body Amphotericin B is poorly absorbed after oral, i.m. or s.c. administration. It is slowly excreted in the urine, less than 10% of a dose being excreted unchanged in 24 h; up to 40% of a dose is excreted in the urine in 7 days and traces are still detectable in urine 2 months after cessation of treatment.

Therapeutic Concentration

Ten critically ill patients, aged 20 to 67 years, were administered liposomal amphotericin B IV at a dose of 1.2 to 4.2 mg/kg over 1 h, and 6 male and female patients, aged 49 to 76 years, were administered amphotericin B deoxycholate at a standard dose of 1.0 mg/kg over 1 h. The median peak concentration of amphotericin B was 14.4 (range 6.4 to 89.0) mg/L after the infusion and for amphotericin B deoxycholate, 1.7 (1.5 to 2.1) mg/L [Heinemann *et al.* 1997].

Adult patients, children (aged 1 to 12 years) and neonates (aged 14 to 30 days) all with invasive fungal infection were administered 1.0 mg/kg liposomal amphotericin B as a 60-min infusion. Mean peak plasma concentrations of 1.02 mg/L were detected at 0.83 to 1.22 h on day 1 which increased to 1.66 mg/L on day 28 for the adults. In the children, the peak concentrations were 0.63 mg/L at 0.36 to 0.95 h on day 1 and 1.1 mg/L on day 28. For the neonates, the peak plasma concentration was 0.54 mg/L at 0.34 to 1.0 h on day 1 and 0.73 mg/L on day 28 [Kotwani *et al.* 2002].

A mean serum concentration of 1.2 mg/L was reported in 20 subjects 1 h after an intravenous infusion of 50 mg [Fields *et al.* 1970].

Half-life Plasma half-life, about 24 to 48 h; a longer terminal elimination phase of about 15 days has also been reported.

Volume of Distribution About 4 L/kg.

Clearance Plasma clearance, about 0.4 mL/min/kg.

Protein Binding 90 to 97%.

Note For a review of amphotericin B, see Daneshead and Warnock [1983].

Dose Up to 800 mg daily by mouth. Doses of 0.25 to 1 mg/kg daily are given intravenously.

Alak A *et al.* (1996). A high-performance liquid chromatographic assay for the determination of amphotericin B serum concentrations after the administration of AmBisome, a liposomal amphotericin B formulation. *Ther Drug Monit* 18: 604–609.

Daneshead TK, Warnock DW (1983). *Clin Pharmacokinet* 8: 19–23.

Eldem T, Arica-Cellat N (2001). Determination of amphotericin B in human plasma using solid-phase extraction and high-performance liquid chromatography. *J Pharm Biomed Anal* 25: 53–64.

Fields BT *et al.* (1970). Amphotericin B serum concentrations during therapy. *Appl Microbiol* 19: 955–959.

Heinemann V *et al.* (1997). Pharmacokinetics of liposomal amphotericin B (Ambisome) in critically ill patients. *Antimicrob Agents Chemother* 41(6): 1275–1280.

Kotwani RN *et al.* (2002). A comparative study of plasma concentrations of liposomal amphotericin B (L-AMP-LRC-1) in adults, children and neonates. *Int J Pharm* 238(1–2): 11–15.

Lambros MP *et al.* (1996). New high-performance liquid chromatographic method for amphotericin B analysis using an internal standard. *J Chromatogr B Biomed. Appl* 685: 135–140.

Lee JW *et al.* (2001). Quantitation of free and total amphotericin B in human biologic matrices by a liquid chromatography tandem mass spectrometric method. *Ther Drug Monit* 23(3): 268–276.

Machard S *et al.* (2000). A sensitive amphotericin B immunoassay for pharmacokinetic and distribution studies. *Antimicrob Agents Chemother* 44(3): 546–550.

Nilsson-Ehle I *et al.* (1977). Quantitation of amphotericin B with use of high-pressure liquid chromatography. *J Infect Dis* 135: 414–422.

Ampicillin

Antibiotic

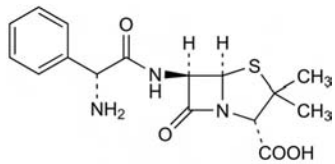
$C_{16}H_{19}N_3O_4S$ = 349.4

CAS—69-53-4

IUPAC Name (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Synonyms AY-6108; BRL-1341; P-50; aminobenzylpenicillin; anhydrous ampicillin.

Proprietary Names *A-Cillin*; *Albipen*; *Alpen*; *Amblosin*; *Amcill*; *Amfipen*; *Amipenix*; *Ampipenin*; *Amperil*; *Ampilar*; *Ampilean*; *Ampitab*; *Binotal*; *Biosan*; *Bonapicillin*; *Britacil*; *Britcin*; *D-Amp*; *Doktacillin*; *Domicillin*; *Dumopen*; *Grampenil*; *Nuvapen*; *Omnipen*; *Pen A*; *Penbritin(e)*; *Penicicline*; *Pensyn*; *Pentrexyl*; *Polycillin*; *Principen*; *Robamox*; *Supen*; *Tokiocillin*; *Totacillin*; *Vidopen*.



Chemical Properties A white crystalline powder. Mp about 200°. Soluble 1 in 170 of water; practically insoluble in ethanol, acetone, carbon tetrachloride, chloroform and ether. pK_a 2.5 (-COOH), 7.3 (-NH_2) (25°).

Ampicillin Sodium

$C_{16}H_{18}N_3NaO_4S$ = 371.4

CAS—69-52-3

Chemical Properties A white, hygroscopic, crystalline or amorphous powder. Aqueous solutions containing 10% or more deteriorate rapidly on storage. Mp about 205°, with decomposition. Soluble 1 in 2 of water and 1 in 50 of acetone; slightly soluble in chloroform; practically insoluble in ether. With ethanol, ampicillin sodium forms a colloidal dispersion which gels on standing.

Ampicillin Trihydrate

$C_{16}H_{19}N_3O_4S,3H_2O$ = 403.4

CAS—7177-48-2

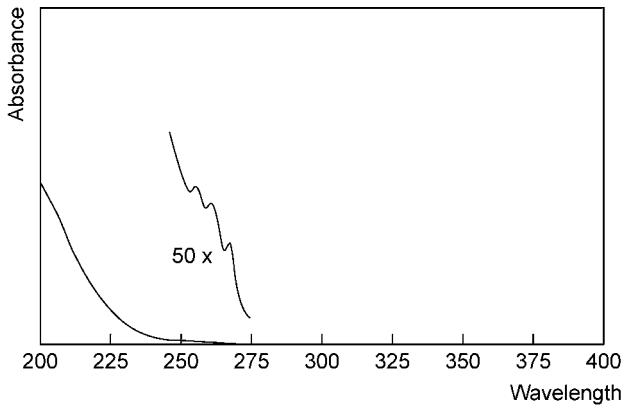
Chemical Properties A white crystalline powder. Soluble 1 in 150 of water; practically insoluble in ethanol, acetone, carbon tetrachloride, chloroform and ether.

A

Colour Tests Liebermann's reagent—orange. Suspend 10 mg in 1 mL of water and add 2 mL of a mixture of 2 mL of potassium cupritartrate solution and 6 mL of water—red-violet.

High Performance Liquid Chromatography System HY—RI 250; system HAA—retention time 3.8 min.

Ultraviolet Spectrum Aqueous acid—257 (A_1^1=9.2a), 262, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1775, 1693, 1526, 1308, 1497, 1583 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection. Limit of detection, 500 µg/L [Vree *et al.* 1978].

Serum **HPLC** Fluorescence detection (λ_{ex}=364 nm; λ_{em}=422 nm). Limit of detection, 2 µg/L [Lal *et al.* 1994].

Spectrofluorimetry Limit of detection, 50 µg/L [Keshavan *et al.* 1979].

Oral fluid **HPLC** See Plasma [Vree *et al.* 1978].

Urine **HPLC** See Plasma [Vree *et al.* 1978].

Note For a Polarography method for the detection of ampicillin, see Vree *et al.* [1978].

Disposition in the Body Ampicillin is readily but incompletely absorbed after oral administration. About 30% of an oral dose is excreted in the urine in 6 h as unchanged drug and about 10% as penicilloic acid; after parenteral administration about 75% is excreted unchanged in 6 h. High concentrations of ampicillin are attained in the bile.

Therapeutic Concentration

After an oral dose of 500 mg given to 6 young subjects, peak plasma concentrations of 2.65 to 4.0 (mean 3.4) mg/L were attained in about 2 h; higher concentrations were reported when a similar dose was given to 6 elderly subjects [Triggs *et al.* 1980].

Half-life Plasma half-life, about 1 to 2 h.

Volume of Distribution About 0.2 to 0.5 L/kg.

Clearance Plasma clearance, about 3 to 4 mL/min/kg.

Distribution in Blood Plasma:whole blood ratio, 1.8.

Protein Binding About 20%.

Note For a review of the pharmacokinetics of penicillins see Barza, Weinstein [1976].

Dose 1 to 8 g daily.

Barza M, Weinstein L (1976). Pharmacokinetics of the penicillins in man. *Clin Pharmacokinet* 1: 297–308.

Keshavan HJH *et al.* (1979). A modified quantitative determination of ampicillin in biological fluids. *Clin Chem* 25: 1674–1675.

Lal J *et al.* (1994). Determination of ampicillin in serum by high-performance liquid chromatography with precolumn derivatization. *J Chromatogr* 655: 142–146.

Schroeder S *et al.* (1978). [Polarographic determination of ampicillin in biological fluids]. *Pharmazie* 33: 432–434.

Triggs EJ *et al.* (1980). Absorption and disposition of ampicillin in the elderly. *Eur J Clin Pharmacol* 18: 195–198.

Vree TB *et al.* (1978). Rapid determination of amoxycillin (clamoxyl) and ampicillin (penbritin) in body fluids of many by means of high-performance liquid chromatography. *J Chromatogr* 145: 496–501.

Amprenavir

Antiviral

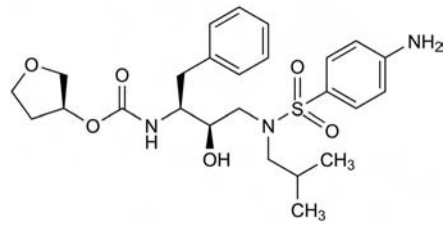
$C_{25}H_{35}N_3O_6S$ = 505.6

CAS—161814-49-9

IUPAC Name [(3*S*)-Oxolan-3-yl] *N*-[(2*S*, 3*R*)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2-yl]carbamate

Synonyms [(1*S*,2*R*)-3-[[(4-Aminophenyl)sulfonyl]-(2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid (3*S*)-tetrahydro-3-furanyl ester; KVX-478; VX-478; 141-W94.

Proprietary Names *Agenerase*; *Vertex*.



Chemical Properties A white to cream coloured solid with a solubility of 0.04 g/L in water, 86 g/L in alcohol (25°). pK_a 1.97 (25°).

High Performance Liquid Chromatography System HAB—Retention time 4.0 min; system HAC—*k* 2.5; system HAE—retention time 9.40 min.

Column: C_{18} AB Nucleosil 100 (5 µm). Mobile phase: acetonitrile : phosphate buffer, (pH 5.15) containing 0.02% sodium heptanesulfonate 15:85 to 30:70 at 2 min, to 32:68 at 8 min, to 42:58 at 18 min, to 46:54 at 34 min. UV detection (λ=201 nm). Retention time: 16.3 min [Marzolini *et al.* 2000].

Ultraviolet Spectrum Aqueous alkali—265 nm.

Quantification

Plasma **HPLC** UV detection (λ=261 nm). Limit of quantification, 25 µg/L [Aymard *et al.* 2000]. Column: C_{18} Novapak (150 × 2.1 mm i.d., 4 µm). Mobile phase: 0.5%, 5.8 mol/L orthophosphoric acid, 0.02% triethylamine (pH 5.0): acetonitrile: methanol. (40:35 for 10 min, to 25:75 for 15 min), flow rate 0.4 mL/min . UV detection (λ=265 nm). Retention time: 3.1 min [Poirier *et al.* 2000]. Limit of quantification, 5 µg/L [Poirier *et al.* 2000]. UV detection (λ=260 nm). Limit of quantification, 50 µg/L [Proust *et al.* 2000]. Limit of quantification, 1 µg/L [Sparidans *et al.* 2000]. Limit of quantification, 10 µg/L [Veronese *et al.* 2000].

Semen **HPLC** Limit of quantification, 1 µg/L [Sparidans *et al.* 2000].

Disposition in the Body Amprenavir is rapidly absorbed and metabolised in the liver to 2 major metabolites by oxidation, and minor metabolites which are glucuronide conjugates of these oxidised metabolites. In total there are 24 detected metabolites. <2% of the drug is recovered unchanged with the majority as metabolites in urine (14%) and faeces (75%).

Therapeutic Concentration

Ten healthy volunteers, 10 patients with moderate cirrhosis and 10 with severe cirrhosis, male and female, aged between 18 and 65 years, were administered with a 600 mg dose of amprenavir. Peak plasma concentrations of 4.9, 6.5 and 9.4 mg/L were observed for 3 groups, respectively. These levels were reached within 1 h [Veronese *et al.* 2000].

Twelve HIV infected patients, 18 to 55 years old, were administered with single oral doses of 150, 300, 600, 900 and 1200 mg. Peak plasma concentrations reached 2.00, 3.51, 6.27, 7.76 and 9.11 mg/L, respectively, within 1 to 2 h [Sadler *et al.* 1999].

Toxicity A severe or life-threatening rash including Stevens–Johnson syndrome has been observed.

Half-life Approximately, 8 h (range, 5.6 to 10.6 h).

Volume of Distribution 430 L.

Clearance 946 mL/min (healthy volunteers); 564 mL/min (patients with moderate cirrhosis); 295 mL/min (severe cirrhosis).

Distribution in Blood Partitioning into erythrocytes is low.

Protein Binding 90%.

Note For a review of the pharmacokinetics of amprenavir see Veronese *et al.* [2000] and Sadler *et al.* [1999].

Dose The usual dose administered is 1200 mg twice daily. Children aged between 4 and 12 years or those weighing below 50 kg: 40 to 45 mg/kg body weight is administered. The maximum dose is 2400 mg daily. The dose is reduced in patients with hepatic impairment.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 744: 227–240.

Marzolini C *et al.* (2000). Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction. *J Chromatogr Biomed Sci Appl* 740(1): 43–58.

Poirier JM *et al.* (2000). Simultaneous determination of the five HIV-protease inhibitors: amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir in human plasma by solid-phase extraction and column liquid chromatography. *Ther Drug Monit* 22: 465–473.

Proust V *et al.* (2000). Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma. *J Chromatogr Biomed Sci Appl* 742: 453–458.

Sadler BM *et al.* (1999). Safety and pharmacokinetics of amprenavir (141W94), a human immunodeficiency virus (HIV) type 1 protease inhibitor, following oral administration of single doses to HIV-infected adults. *Antimicrob Agents Chemother* 43(7): 1686–1692.

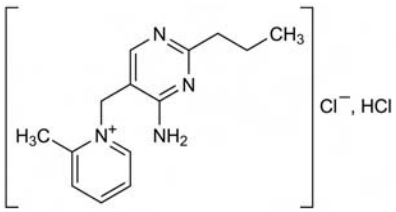
Sparidans RW *et al.* (2000). Sensitive liquid chromatographic assay for amprenavir, a human immunodeficiency virus protease inhibitor, in human plasma, cerebrospinal fluid and semen. *J Chromatogr Biomed Sci Appl* 742: 185–192.

Veronese L *et al.* (2000). Single-dose pharmacokinetics of amprenavir, a human immunodeficiency virus type 1 protease inhibitor, in subjects with normal or impaired hepatic function. *Antimicrob Agents Chemother* 44: 821–826.

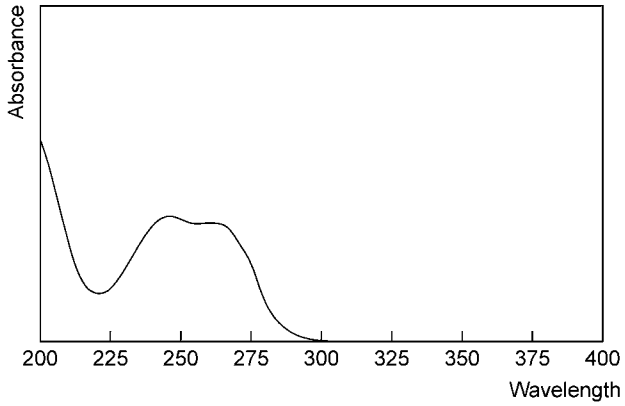
Amprolium Hydrochloride

Coccidiostat (Veterinary)
$C_{14}H_{19}ClN_4,HCl = 315.2$
CAS—121-25-5 (amprolium); 137-88-2 (hydrochloride)

IUPAC Name 5-[(2-Methylpyridin-1-ium-1-yl)methyl]-2-propylpyrimidin-4-amine chloride hydrochloride
Synonym 1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride hydrochloride
Proprietary Names It is an ingredient of *Amprolmix-UK*, *Amprol-Plus*, *Pancoxin* and *Supacox*.



Chemical Properties A white powder. Mp 247°, with decomposition. Soluble 1 in 2 of water and 1 in 170 of ethanol; practically insoluble in chloroform; very slightly soluble in ether.
Colour Test Aromaticity (method 2)—colourless/yellow.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—246 (A^1_1=420b), 262 nm.

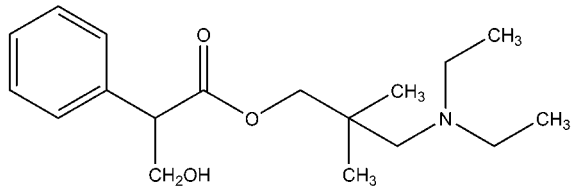


Infrared Spectrum Principal peaks at wavenumbers 1638, 1522, 1593, 787, 1562, 1149 cm^{-1}, (Amprolium, KBr disk).

Amprotropine

Atropine Derivative, Parasympatholytic
$C_{18}H_{29}NO_3 = 307.4$
CAS—148-32-3

IUPAC Name 3-Diethylamino-2,2-dimethylpropyl(±)tropate



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

Amprotropine Phosphate

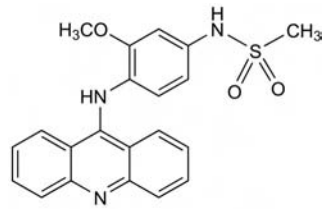
CAS—134-53-2
Proprietary Name *Syntropan*
Chemical Properties White crystalline powder. Mp 142° to 145°. Soluble in water; slightly soluble in ethanol; insoluble in ether and chloroform.

Colour Test Ammonium molybdate—blue (limit of detection 0.1 µg).
Thin-Layer Chromatography System T1—R_f 0.71 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.70 relative to diphenhydramine; system G4—retention time 2.40 relative to diphenhydramine.
Infrared Spectrum Principal peaks at wavenumbers 1730, 1162, 1060, 1195, 1378 cm^{-1} (KBr disk).
Dose Up to 400 mg daily.

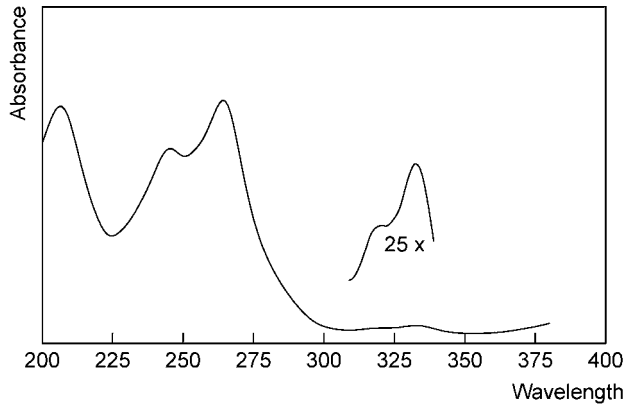
Amsacrine

Antineoplastic
$C_{21}H_{19}N_3O_3S = 393.5$
CAS—51264-14-3

IUPAC Name *N*-[4-(9-Acridinylamino)-3-methoxyphenyl]methanesulfonamide
Synonyms Acridinyl anisidide; *m*-AMSA; CI-880; NSC-249992; SN-11841
Proprietary Names *Amekrin*; *Amsa P-D*; *Amsidine*; *Amsidyl*.



Chemical Properties Log *P* (octanol/water), 3.89.
Ultraviolet Spectrum



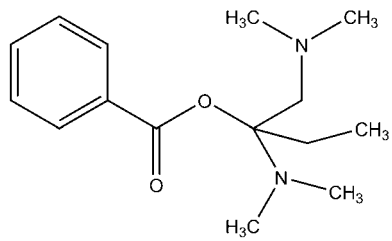
Mass Spectrum Principal ions at *m/z* 314, 315, 393, 270, 283, 79, 271, 394.
Quantification
Plasma **GC** FID. Limit of detection, 0.125 mg/L [Eksborg, Ehrsson 1985]. NPD. Limit of detection, 0.05 mg/L [Eksborg, Ehrsson 1985].
HPLC UV detection (λ=254 nm). Limit of detection, 50 nmol/L [Jurlina, Paxton 1983].
Disposition in the Body Amsacrine is poorly absorbed after oral administration. Following intravenous administration it is well distributed, except to the brain and CSF (cerebrospinal fluid). It is metabolised in the liver mostly as amasacrine–glutathione metabolites. It is excreted primarily in the bile by an active transport mechanism. >20% of the drug is recovered unchanged in urine.
Therapeutic Concentration The trough serum therapeutic concentration is 0.03 mg/L and the peak, 0.15 to 5.5 mg/L.
Half-life Plasma, 7 h.
Volume of Distribution 1.67 L/kg; 87.1 L/m^2.
Clearance 150 $mL/min/m^2$.
Protein Binding 98%.
Dose For induction therapy up to 120 mg/m^2 intravenously daily for 5 to 8 days.

Eksborg S, Ehrsson H (1985). Drug level monitoring: cytostatics. *J Chromatogr* 340: 31–72.
Jurlina JL, Paxton JW (1983). High-performance liquid-chromatographic method for the determination of 4′-(9-acridinylamino)methanesulfon-m-anisidide in plasma. *J Chromatogr* 276: 367–374.

Amydricaine

Benzoic Acid Ester, Anaesthetic (Local)
$C_{16}H_{26}N_2O_2 = 278.4$

IUPAC Name 1,1-Di(dimethylaminomethyl)propyl benzoate



A

Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

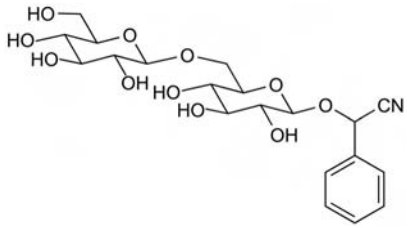
Amydricaine Hydrochloride

$C_{16}H_{26}N_2O_2$,HCl = 314.9
Proprietary Name *Alypin Hydrochloride*
Chemical Properties White hygroscopic crystalline powder. Mp ≈169°. Soluble 1 in 1 of water and 1 in 4 of ethanol; soluble in chloroform; insoluble in ether.

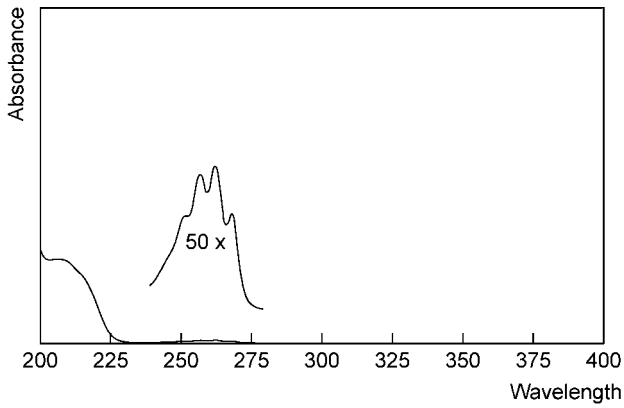
Thin-Layer Chromatography System T1—R_f 0.41 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—237, 277 nm (base); water—234 nm (hydrochloride).
Infrared Spectrum Principal peaks at wavenumbers 1282, 1715, 710 cm^{-1} (KBr disk).
Toxicity Amydricaine is as toxic as cocaine. The minimum SC lethal dose is 70 mg/kg in dogs and 200 to 430 mg/kg in rats.

Amygdalin

Cyanogenic Glycoside
$C_{20}H_{27}NO_{11}$ = 457.4
CAS—29883-15-6
IUPAC Name [(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]benzeneacetonitrile
Synonym Amygdaloside



Note The name 'laetrile' is frequently used to describe amygdalin but it has also been used to describe L-mandelonitrile-β-glucuronide, a substance that may be derived from amygdalin.
Chemical Properties A colourless, crystalline, cyanogenic glycoside present in bitter almond seeds (*Prunus amygdalus* var. *amara*) apricot kernels and other seeds of the Rosaceae. Mp about 220°; after solidifying the substance 125° to 130°. Soluble 1 in 12 of water and 1 in 900 of ethanol; almost insoluble in ether. Log *P* (octanol/water), −4.3.
Thin-layer Chromatography System TA—R_f 0.87; system TE—R_f 0.56; system TAJ—R_f 0.54; system TAK—R_f 0.62; system TAL—R_f 0.92.
Infrared Spectrum Principal peaks at wavenumbers 1065, 1025, 695, 758, 1272, 890 cm^{-1} (KCl disk).



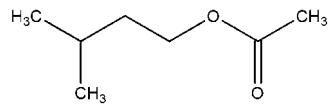
Mass Spectrum Principal ions at *m/z* 43, 31, 57, 73, 60, 29, 55, 44.
Quantification
Plasma **GC** FID. Limit of detection, 2 mg/L [Stobaugh 1978].
HPLC UV detection. Limit of detection, 1 mg/L for amygdalin and prunasin [Rauws *et al.* 1982].
Urine **HPLC** UV detection. Limit of detection, 1 mg/L for amygdalin and prunasin [Rauws *et al.* 1982].
Note For review of methods in body fluids or tissues, including GC–MS, HPLC or TLC, see Balkon [1982].
Disposition in the Body Poorly absorbed after ingestion and slowly hydrolysed in the gastro-intestinal tract to mandelonitrile-β-glucoside (prunasin), mandelonitrile and glucose, and finally to benzaldehyde and hydrogen cyanide. After ingestion, about 8 to 32% of a dose is excreted unchanged in the urine; after parenteral administration, only small amounts are hydrolysed and most of a dose is excreted unchanged in the urine. Urinary cyanide and thiocyanate concentrations are not significantly elevated after oral or intravenous administration.

Blood Concentration
In 6 subjects ingesting 500 mg of amygdalin three times a day, peak plasma concentrations of <1 mg/L of amygdalin were attained in 30 to 60 min; peak plasma-cyanide concentrations of 0.4 to 2.1 mg/L (mean 1.0) were reported 1.5 to 2 h after a dose on the second day; plasma-thiocyanate concentrations accumulated on repeated administration of amygdalin to about 25 mg/L on the last day of ingestion [Moertel *et al.* 1981].
Following an intramuscular injection of 6 g to 1 subject, a peak plasma concentration of 180 mg/L was attained in 1.25 h [Ames *et al.* 1978].
Toxicity Several reports of cyanide poisoning following administration of amygdalin have been recorded, mostly involving the ingestion of apricot or other fruit kernels. Cyanide release from amygdalin is known to occur in the presence of β-glucosidase enzymes that are present in some raw fruits and vegetables. Toxic effects or fatalities have been associated with blood-cyanide concentrations greater than 2 mg/L.
A blood-cyanide concentration of 10 mg/L was reported in one subject with toxic effects following daily ingestion of 1500 mg of amygdalin [Smith *et al.* 1977].
In a fatality involving amygdalin ingestion, a postmortem blood-cyanide concentration of 2.18 mg/L was reported [Vogel *et al.* 1981].
Note For a review of the literature on amygdalin, see Chandler *et al.* [1984].

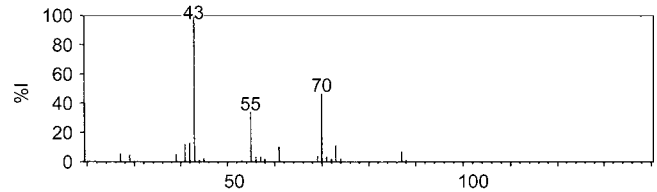
Ames MM *et al.* (1978). Initial pharmacologic studies of amygdalin (laetrile) in man. *Res Commun Chem Pathol Pharmacol* 22: 175–185.
Balkon J (1982). Methodology for the detection and measurement of amygdalin in tissues and fluids. *J Anal Toxicol* 6: 244–246.
Chandler RF *et al.* (1984). *Pharm J* 232: 330–332.
Moertel CG *et al.* (1981). A pharmacologic and toxicological study of amygdalin. *JAMA* 245: 591–594.
Rauws AG *et al.* (1982). Determination of amygdalin and its major metabolite prunasin in plasma and urine by high pressure liquid chromatography. *Pharm Weekbl (Sci.)* 4: 172–175.
Smith FP *et al.* (1977). Laetrile toxicity: a report of two cases. *JAMA* 238: 1361.
Stobaugh JF (1978). *Anal Lett (Part B)* 11: 753–764.
Vogel SN *et al.* (1981). Cyanide poisoning. *Clin Toxicol* 18: 367–383.

Amyl Acetate

Solvent
$C_7H_{14}O_2$ = 130.2
CAS—123-92-2 (iso); 53496-15-4 (sec); 628-63-7 (*n*)
IUPAC Name 3-Methylbutyl acetate
Synonyms Amyl acetic ester; banana oil; pear oil; pentyl acetate.



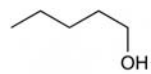
Chemical Properties A colourless, mobile, inflammable liquid. Bp ~140°. Refractive index 1.1400. Slightly soluble in water; miscible with ethanol and ether. Log *P* (octanol/water), 2.3. A mixture of isomers, principally isoamyl acetate (3-methylbutyl acetate), *sec*-amyl acetate (2-methylbutyl acetate) and *n*-amyl acetate.
Mass Spectrum Principal ions at *m/z* 43, 70, 55, 42, 41, 73, 61, 7.



Toxicity The estimated minimum lethal dose is 50 g and the maximum permissible atmospheric concentration is 100 ppm for isoamyl acetate or *n*-amyl acetate and 125 ppm for *sec*-amyl acetate.

Amyl Alcohol

Solvent
$C_5H_{12}O$ = 88.15
CAS—123-51-3 (3-methyl-1-butanol); 137-32-6 (2-methyl-1-butanol).
IUPAC Name Pentan-1-ol
Synonyms Butylcarbinol; 1-pentanol; pentyl alcohol.

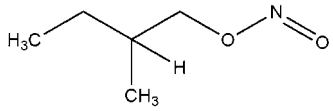


Chemical Properties A colourless liquid obtained by purifying fusel oil. Bp 128° to 132°. Refractive index 1.4075. Slightly soluble in water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 1.2 (3-methyl-1-butanol), 1.3 (2-methyl-1-butanol). A mixture of mainly 3-methyl-1-butanol (primary isoamyl alcohol; $(CH_3)_2CHCH_2CH_2OH$) with some 2-methyl-1-butanol (primary amyl alcohol; $CH_3CH_2CH(CH_3)CH_2OH$).

Mass Spectrum Principal ions at *m/z* 55, 41, 42, 43, 70, 57, 29, 1.
Disposition in the Body
Toxicity Amyl alcohol is toxic after ingestion. The estimated minimum lethal dose is 30 g and the maximum permissible atmospheric concentration is 100 ppm.

Amyl Nitrite

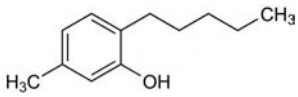
Antianginal Vasodilator
$C_5H_{11}NO_2$ = 117.1
CAS—8017-89-8; 110-46-3
IUPAC Name 3-Methylbutyl nitrite
Synonyms Amilnitrite; isoamyl nitrite; isopentyl nitrite.



Chemical Properties A clear, yellow, volatile, inflammable liquid with a fragrant odour. Incompatible with ethanol. Store in a cool place in airtight containers; protect from light. Bp 96°. Refractive index 1.3871. Practically insoluble in water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 2.8. It consists of the nitrites of 3-methylbutan-1-ol [$(CH_3)_2CHCH_2CH_2OH$] and 2-methylbutan-1-ol [$CH_3CH_2CH(CH_3)CH_2OH$], with other nitrites of the homologous series.
Colour Tests To 0.2 mL add 2 mL of a 2% ferrous sulfate solution and 5 mL of dilute hydrochloric acid—green-brown; to 0.2 mL add 0.5 mL of aniline and 5 mL of acetic acid—orange-red.
Gas Chromatography System GA—RI >1000; system GI—RT 20.3 min.
Mass Spectrum Principal ions at *m/z* 70, 43, 55, 41, 57, 42, 71, –.
Disposition in the Body Absorbed from the mucous membranes after inhalation but rapidly inactivated by hydrolysis. Inactive after ingestion owing to hydrolysis in the gastrointestinal tract.
Toxicity Recovery has occurred after the ingestion of 12 mL.
Dose 0.12 to 0.3 mL, by inhalation.
Caution Amyl nitrite forms an explosive mixture with air or oxygen. It is very inflammable and must not be used where it may be ignited.

Amylmetacresol

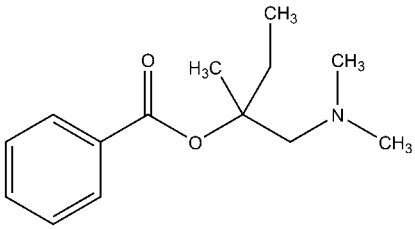
Antiseptic
$C_{12}H_{18}O$ = 178.3
CAS—1300-94-3; 53043-14-4
IUPAC Name 5-Methyl-2-pentylphenol
Synonym 6-Pentyl-*m*-cresol
Proprietary Name It is an ingredient of *Strepsils*.



Chemical Properties A colourless or slightly yellow, clear liquid or solid crystalline mass, which darkens on keeping. Mp 24°. Practically insoluble in water; soluble in ethanol and ether. Log *P* (octanol/water), 4.6.
Ultraviolet Spectrum Acidified dehydrated alcohol—278 nm (A^1_1=132a), 286 nm.
Infrared Spectrum Principal peaks at wavenumbers 1120, 1225, 1272, 805, 1584, 938 cm^{-1} (thin film).

Amylocaine

Anaesthetic (Local)
$C_{14}H_{21}NO_2$ = 235.3
CAS—644-26-8
IUPAC Name [1-(Dimethylamino)-2-methylbutan-2-yl] benzoate
Synonym 1-Dimethylaminomethyl-1-methylpropyl benzoate
Chemical Properties Log *P* (octanol/water), 2.89 [Meylan, Howard 1995].



Amylocaine Hydrochloride

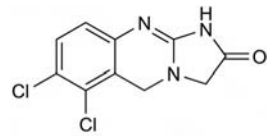
$C_{14}H_{21}NO_2$,HCl = 271.8
CAS—532-59-2
IUPAC Name Methylethyldimethylamino-methylcarbinol benzoyl ester hydrochloride
Synonyms Amyleine hydrochloride; 1-(dimethyylamino)-2-methyl-2-butanol benzoate hydrochloride.
Proprietary Name *Stovaine*

Chemical Properties Crystals. Freely soluble in water and ethanol, practically insoluble in ether. pK_a 7.96 [Polasek *et al.* 1992]. Log *P* (octanol/water), 0.12 [Meylan, Howard 1995].
Thin-layer Chromatography System T1—R_f 0.66; system TA—R_f 0.73; System TB—R_f 0.60; System TC—R_f 0.67; System TL—R_f 0.63.
Atomic Absorption Spectrometry Coil: (700 × 0.5 mm i.d.). Carrier: 0.1% Reinecke's salt (ammonium tetrathiocyanoammonochromate) solution (pH 5.0). Limit of detection, 2.1 mg/L [Eisman *et al.* 1993].
Ultraviolet Spectrum Peaks at 234 and 275 nm.
Infrared Spectrum Principal peaks at 1275, 1710, 710 and 1110 cm^{-1} (KBr disk).
Disposition in the Body
Toxicity When investigated in cultured human conjunctival and other cells, the order of toxicity was procaine < lidocaine < piperocaine < amylocaine < amethocaine < cinchocaine [Dawson, Mustafa 1985]. A temperature of 45° was optimum for the sporicidal activity of amylocaine versus *Aspergillus niger*, while a temperature of 84° was optimum against *Bacillus subtilis* [Abdelaziz, el Nakeeb 1988].

Abdelaziz AA, el Nakeeb MA (1988). Sporicidal activity of local anaesthetics and their binary combinations with preservatives. *J Clin Pharm Ther* 13: 249–256.
Dawson M, Mustafa AF (1985). Use of cultured human conjunctival and other cells to assess the relative toxicity of six local anaesthetics. *Food Chem Toxicol* 23: 305–308.
Eisman M *et al.* (1993). Automatic determination of amylocaine and bromhexine by atomic absorption spectrometry. *J Pharm Biomed Anal* 11: 301–305.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Polasek M *et al.* (1992). Determination of limiting ionic mobilities and dissociation constants of some local anaesthetics. *J Chromatogr* 596: 265–270.

Anagrelide

Antithrombotic
$C_{10}H_7Cl_2N_3O$ = 256.1
CAS—68475-42-3
IUPAC Name 6,7-Dichloro-1,5-dihydroimidazo-[2,1-*b*]quinazolin-2-(3*H*)-one



Anagrelide Hydrochloride

$C_{10}H_8Cl_3N_3O$ = 292.5
CAS—58579-51-4
Synonyms BL-4162A; BMY-26538-01.
Proprietary Names *Agrelin*; *Agrylin*.
Chemical Properties It is an off-white powder. Mp >280°. It is very slightly soluble in water; sparingly soluble in dimethylsulfoxide and dimethylformamide.

High Performance Liquid Chromatography Column: phenyl μBondapak (300 × 3.9 mm i.d.). Mobile phases: (A) acetonitrile : 0.01 mol/L sodium acetate (pH 4) (25:75), flow rate 2.5 mL/min for first 10 min; (B) dimethylsulfoxide, flow rate 2.5 mL/min, reduced to 1 mL/min at 13 min. Mobile phase A reused at minute 18, flow rate 2.5 mL/min. UV detection (λ=254 nm). Retention time: 6 to 8 min [Gaver *et al.* 1981].
Quantification
Plasma **HPLC** UV detection (λ=254 nm). Limit of detection, 5 μg/L [Gaver *et al.* 1981].
Disposition in the Body Anagrelide is rapidly absorbed after oral administration (minimum 75%) and is extensively metabolised. A minimum of four metabolites have been identified, none of which are glucuronide or sulfate conjugates. It is excreted mainly in urine (70 to 75% over 5 days) and in faeces (10%). <1% of the dose is excreted unchanged. It is extensively distributed.
Therapeutic Concentration
Five healthy men, aged 19 to 26 years, were administered with a 1.2 mg anagrelide hydrochloride (1 mg free base) dose after an overnight fast. Mean peak plasma concentrations were 4.5 ± 0.9 μg/L and reached within 0.9 ± 0.4 h, rapidly declining in the first 6 to 8 h [Gaver *et al.* 1981].
Toxicity Cardiac and CNS toxicity can be expected with overdose. It may also cause fetotoxicity and renal failure. Doses greater than 5 mg daily can lead to hypotension.
Bioavailability 13.8% after administration with food.
Half-life 1.3 h (fasting state); 1.8 h (dose administered with food).
Volume of Distribution 12 L/kg.
Clearance 9 L/h.

Dose An initial dose of 2 mg daily is administered which can be increased to a maximum of 10 mg daily.

Gaver RC *et al.* (1981). Disposition of anagrelide, an inhibitor of platelet aggregation. *Clin Pharmacol Ther* 29(3): 381–386.

Anastrozole

Antineoplastic

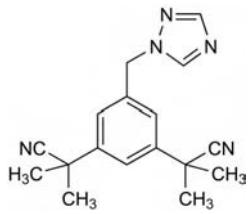
$C_{17}H_{19}N_5 = 293.4$

CAS—120511-73-1

IUPAC Name α,α,α′,α′-Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-benzenediacetonitrile

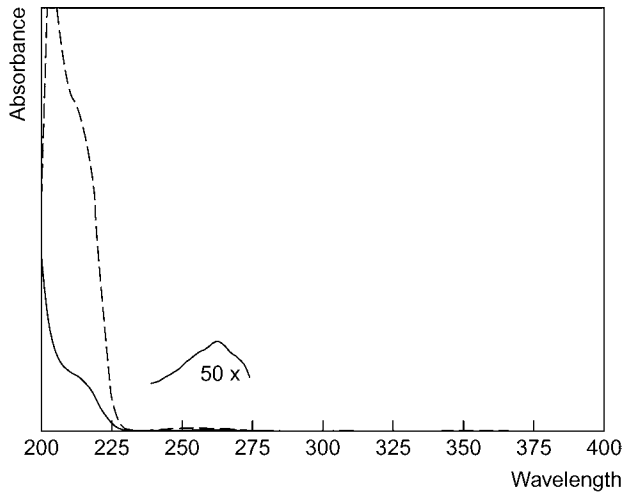
Synonyms ZD1033; ICI-D-1033.

Proprietary Name *Arimidex*

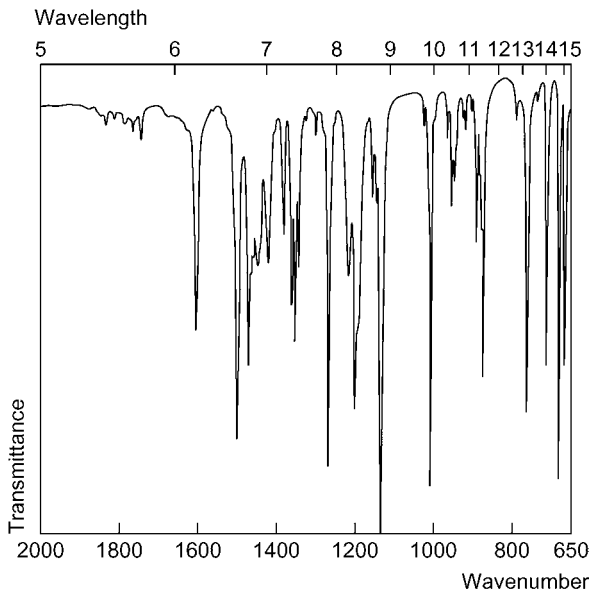


Chemical Properties An off-white powder. Mp 81° to 82°. It is soluble in water 0.5 mg/mL at 25°; freely soluble in acetone, ethanol, methanol and tetrahydrofuran; very soluble in acetonitrile.

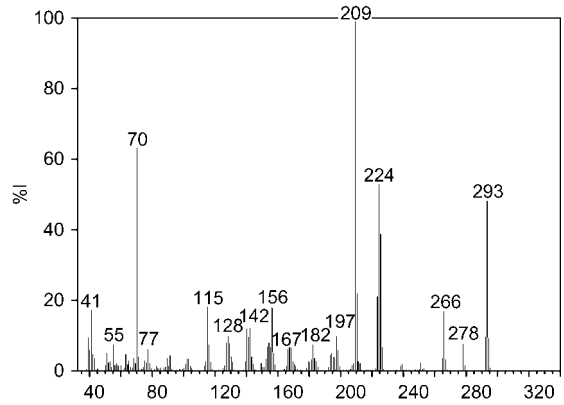
Ultraviolet Spectrum



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 209, 70, 224, 293, 115, 41, 156, 266.



Quantification

Plasma **GC** ECD. Limit of quantification, 3 µg/L, limit of detection, 0.5 µg/L [Bock *et al.* 1997].

Disposition in the Body Anastrozole is rapidly and almost completely absorbed after oral administration; peak plasma concentrations occur within about 2 h. The drug undergoes metabolism in the liver by *N*-dealkylation, hydroxylation and glucuronidation. The main inactive metabolite is triazole. The metabolites are excreted mainly in urine; less than 11% is excreted unchanged. It is removed by dialysis.

Half-life Plasma, 50 h.

Protein Binding 40%.

Dose 1 mg daily.

Bock MJ *et al.* (1997). Validated assay for the quantification of anastrozole in human plasma by capillary gas chromatography-63Ni electron capture detection. *J Chromatogr B Biomed Sci Appl* 700: 131–138.

Androstanediol

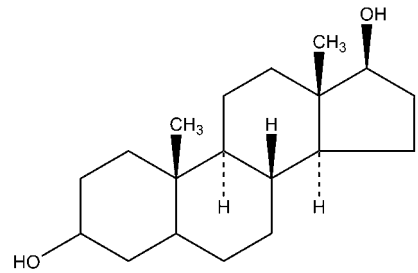
Anabolic Steroid

$C_{19}H_{32}O_2 = 292.5$

CAS—25126-76-5

IUPAC Name 10,13-Dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,17-diol

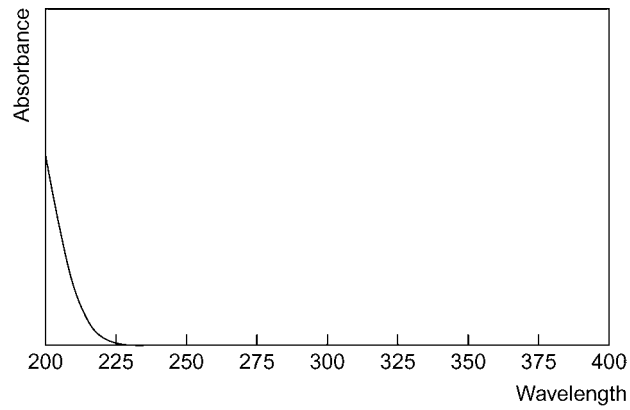
Synonym Androstane-3,17-diol



Chemical Properties Yellow-white crystalline powder. Mp 227° to 228°.

Thin-layer Chromatography Plates: silica gel 60F 254 (20 × 20 cm) with concentrating zone (4 × 20 cm) precoated (2.0 mm). System I: dichloromethane : diethyl ether (90 : 10). System IIa: toluene : ethyl acetate (50 : 50). System IIb: toluene : ethyl acetate (70 : 30). Biological samples: (1) Double run, *y* direction, system I at 4° (up to 10 spots per plate); (2) double run, *y* direction, system I at 4° followed by double run, *x* direction system IIa (single spot) or system IIb (two tracks or more). Limit of detection, 100 dpm [Vingler *et al.* 1991].

Ultraviolet Spectrum



Mass Spectrum Principal ion at *m/z* 470.

Quantification

Plasma **HPLC** Column: Rainin Hibar RT Lichrosorb (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: 12.5% ethanol in heptane: heptane (5 : 95 to 95 : 5 over 180 min), flow rate 1.5 mL/min. UV detection (λ= 220 nm). Limit of detection not reported [Rao *et al.* 1987].

Urine **GC-MS** Column: Fused silica CP Sil 5CB (25 m × 0.2 mm i.d.). Carrier gas: He, 0.8 mL/min. Temperature programme: 204° for 20 min to 240° at 4°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Vierhapper *et al.* 1988].

GC-MS Column: HP cross-linked 50% phenylmethysiloxane fused silica capillary (30 m × 0.25 mm i.d., 0.15 µm). Temperature programme: 80° for 1 min to 270° at 15°/min to 300° at 35°/min for 3 min. Limit of quantification not reported, limit of detection not reported [Maitre *et al.* 2004].

LC-MS Column: Spherisorb ODS 2 (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (10 : 90 to 100 : 0 at 25 min), flow rate 1 mL/min. Column: BPX35 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 100° to 260° at 2.4°/min. FID. Limit of detection not reported [Minut *et al.* 1999].

Amniotic Fluid **GC-MS** Column: Macherey-Nagel OV-1 fused silica (25 m × 0.2 mm, 0.1 µm). SIM acquisition mode. Limit of detection not reported although highly sensitive for androstanediol [Wudy *et al.* 1999].

Other **LC-MS** Rat Plasma. Column: Synergy Max-RP (50 × 2 mm i.d., 5.0 µm). Mobile phase: 0.05% acetic acid in water : 0.05% acetic acid in methanol (50 : 50 for 0.3 min to 0 : 100 at 5.7 min to 50 : 50 at 6 min), flow rate 150 µL/min. APCI, positive ion mode. Limit of quantification, 10 µg/L, limit of detection, 2 µg/L [Reddy *et al.* 2005].

Disposition in the Body

Therapeutic Concentration

Six healthy men (20 to 27 years) and 5 healthy women (24 to 30 years, in the follicular phase of the menstrual cycle) were given infusions of [^{13}C] testosterone. The doses administered were 20 mg in 500 mL for men and 5 mg in 500 mL for women, at a rate of 125 mL/h over 4 h. Urine was collected before (−10 to 0 h), during (0 to 4 h) and after (4 to 6 h, 8 to 10 h, and 10 to 24 h) the infusion and analysed by GC-MS. Cumulative excretion rates were expressed in µg/24 h and as the percentage of the infused amount of [^{13}C]testosterone [Vierhapper 1990].

	Testosterone	*3α-Androstanediol*
Men	15.6 ± 9.60 µg/24 h 0.08 ± 0.05%	67.7 ± 19.9 µg/24 h 0.30 ± 0.10%
Women	1.14 ± 1.55 µg/24 h 0.02 ± 0.03%	9.98 ± 5.95 µg/24 h 0.20 ± 0.12%

The mean urinary excretion rates for the male subjects were as follows [Vierhapper *et al.* 1988].

Time (h)	*^{12}C (µg/h)*	*^{13}C (µg/h)*
−10 to 0	2.40 ± 0.82	—
0–4	1.70 ± 1.35	4.70 ± 3.82
4–6	1.07 ± 0.59	5.36 ± 2.78
6–8	1.89 ± 1.05	6.23 ± 4.28
8–10	1.86 ± 1.54	3.98 ± 3.92
10- 24	1.81 ± 1.63	1.77 ± 1.61

Young (21 to 49 years) and elderly (62 to 77 years) men undergoing cardiac catheterisation were given infusions of [^{14}C]testosterone and [^{3}H] androstanediol. The plasma concentration of androstanediol was 180 ± 20 and 150 ± 40 ng/L in the young and elderly groups, respectively [Morimoto *et al.* 1981].

Maitre A *et al.* (2004). Urinary analysis of four testosterone metabolites and pregnanediol by gas chromatography–combustion-isotope ratio mass spectrometry after oral administrations of testosterone. *J Anal Toxicol* 28: 426–431.

Minut GJ *et al.* (1999). Urinary 5alpha-androstanediol and 5beta-androstanediol measurement by gas chromatography after solid-phase extraction and high-performance liquid chromatography. *Int J Biol Markers* 14: 154–159.

Morimoto I *et al.* (1981). Studies on the origin of androstanediol and androstanediol glucuronide in young and elderly men. *J Clin Endocrinol Metab* 52: 772–778.

Rao PN *et al.* (1987). Isolation and identification of androstanediol glucuronide from human plasma. *J Steroid Biochem* 28: 565–569.

Reddy DS *et al.* (2005). A high-performance liquid chromatography–tandem mass spectrometry assay of the androgenic neurosteroid 3alpha-androstanediol (5alpha-androstane-3alpha,17beta-diol) in plasma. *Steroids* 70: 879–885.

Vierhapper H (1990). Formation of androstanediol from ^{13}C-labeled testosterone in humans. *Steroids* 55: 177–180.

Vierhapper H *et al.* (1988). Estimation by gas chromatography-mass spectrometry with selected ion monitoring of urinary excretion rates of 3alpha-androstanediol during/after IV administration of ^{13}C-labelled testosterone in man. *J Steroid Biochem* 29: 105–109.

Vingler P *et al.* (1991). Direct quantitative digital autoradiography-thin-layer chromatography of 3alpha, 3beta- and 5alpha-reduced and 17beta-dehydrogenated androgens derived from testosterone metabolism. *J Chromatogr* 571: 73–86.

Wudy SA *et al.* (1999). Profiling steroid hormones in amniotic fluid of midpregnancy by routine stable isotope dilution/gas chromatography-mass spectrometry: reference values and concentrations in fetuses at risk for 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 84: 2724–2728.

Androstanolone

Anabolic Steroid

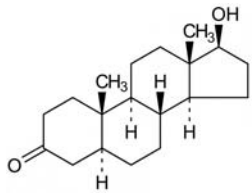
$C_{19}H_{30}O_2$ = 290.4

CAS—521-18-6

IUPAC Name (5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13-dimethyl-1,2,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[*a*]phenanthren-3-one

Synonyms Dihydrotestosterone; (5α,17β)-17-hydroxyandrostan-3-one; stanolone.

Proprietary Names *Anabolex*; *Andractim*; *Androlone*.



Chemical Properties White crystalline powder. Mp 181°. Practically insoluble in water; soluble 1 in 20 of ethanol and 1 in 70 of ether; soluble in acetone. Log *P* (octanol/water), 3.6.

Thin-layer Chromatography System TP—R_f 0.78; system TQ—R_f 0.11; system TR—R_f 0.90; system TS—R_f 0.72.

Gas Chromatography System GA—dihydrotestosterone RI 2510; dihydrotestosterone-AC RI 2630; dihydrotestosterone-TMS RI 2485; dihydrotestosterone enol-TMS_2 RI 2450; system GAI—urinary metabolite:5α-dihydrotestosterone RRT 0.95 (relative to 17α-methyl-5α-androstan-3β,17β-diol.

Infrared Spectrum Principal peaks at wavenumbers 1698, 1046, 1277, 1027, 1064, 1079 cm^{-1} (KBr disk).

Quantification

Hair **GC-MS** Column: CP-Sil 5 CB (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.3 min. Limit of quantification, 0.05 ng/mg, limit of detection, 0.01 ng/mg [Deng *et al.* 1999].

Dose Androstanolone has been given sublingually or sublabially in doses of 50 to 75 mg daily.

Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.

Androstenedione

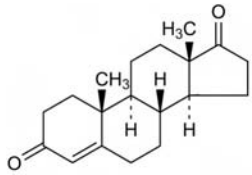
Anabolic Steroid

$C_{19}H_{26}O_2$ = 286.4

CAS—63-05-8

IUPAC Name Androst-4-ene-3,17-dione

Proprietary Name *Androtex*

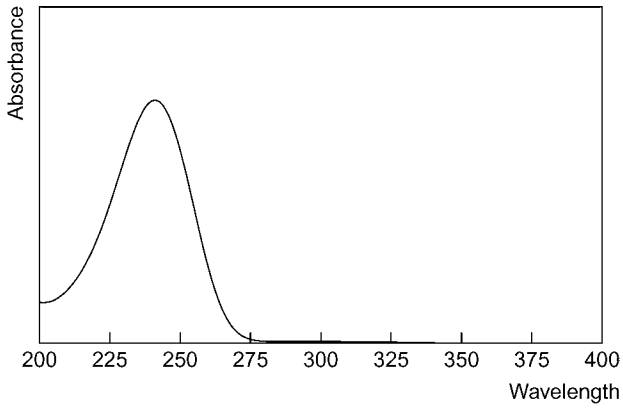


Chemical Properties Appears as needles or crystals. Mp 158°. Soluble in water (57.82 mg/L at 25°). Log *P* (octanol/water), 2.75.

Gas Chromatography System GA—RI 2600.

Column: 5% phenyl, methyl silicone HP Ultra-2 (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 100° for 1 min, 16°/min to 220°, 3.8°/min to 300°, for 10 min. Carrier gas: He, flow rate 0.6 to 0.8 mL/min. IS: 5α-androstane-17-one. MS detection (EI, SIM). Retention time: androstenediol 24 min; androstenedione 24.5 min [Masse *et al.* 1989].

Ultraviolet Spectrum Water—235 nm.

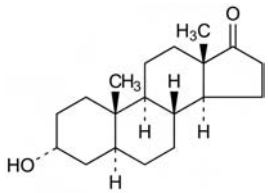


Mass Spectrum Principal ions at *m/z* 286, 124, 244, 148, 109, 107, 287, 147.
Quantification
Plasma **HPLC** Limit of detection, 5 pg [Walker *et al.* 1996].
Serum **HPLC** Limit of detection, 5 pg [Walker *et al.* 1996].
Disposition in the Body After oral administration, androstenedione is metabolised in the liver to testosterone. Blood testosterone levels increase ~15 min after oral administration and remain elevated for up to 3 h. Blood testosterone peaks at ~1 to 1.5 h after ingestion.
Therapeutic Concentration In young males, a single oral dose of 100 mg can increase endogenous androstenedione serum concentrations by 175% within the first 60 min after administration and 325 to 350% between 90 and 270 min after administration.
Dose The recommended dosage is between 50 and 300 mg daily.

Masse R *et al.* (1989). Studies on anabolic steroids. I. Integrated methodological approach to the gas chromatographic-mass spectrometric analysis of anabolic steroid metabolites in urine. *J Chromatogr B Biomed Sci Appl* 489: 23–50.
Walker VR *et al.* (1996). Semiautomated method for the quantitation of plasma or sera androstenedione, testosterone, and dihydrotestosterone in population studies. *Anal Biochem* 234: 194–203.

Androsterone

Androgen
$C_{19}H_{30}O_2$ = 290.4
CAS—53-41-8
IUPAC Name (3α,5α)-3-Hydroxyandrostan-17-one

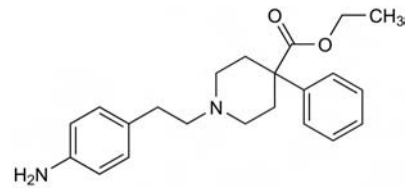


Chemical Properties A white crystalline powder. Mp about 185°. Practically insoluble in water; soluble in ethanol, ether and most organic solvents. Log *P* (octanol/water), 3.7.
Colour Tests Antimony pentachloride—yellow→brown; naphthol-sulfuric acid—orange-brown/orange; sulfuric acid—yellow.
Thin-layer Chromatography System TB—R_f 0.16; system TE—R_f 0.72; system TF—R_f 0.52; system TAE—R_f 0.90.
Gas Chromatography System GA—androsterone RI 2475, androsterone-AC RI 2580, androsterone enol-TMS_2 RI 2500; system GAR—retention time 11.9 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1724, 1000, 1031, 1062, 1242, 1282 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 290, 67, 108, 107, 79, 55, 41, 93.
Quantification
Urine **GC–MS** Limits of detection, 15 to 20 pg for androsterone glucuronide and other androgen glucuronides [Choi *et al.* 2000].
Disposition in the Body Androsterone is a naturally occurring androgen which may be isolated from male urine. It is a major metabolite of testosterone.

Choi MH *et al.* (2000). Simultaneous determination of urinary androgen glucuronides by high temperature gas chromatography-mass spectrometry with selected ion monitoring. *Steroids* 65: 54–59.

Anileridine

Narcotic Analgesic
$C_{22}H_{28}N_2O_2$ = 352.5
CAS—144-14-9
IUPAC Name Ethyl-1-[2-(4-aminophenyl)ethyl]-4-phenyl-4-piperidinecarboxylate



Chemical Properties A white to yellowish-white crystalline powder. When exposed to light and air it oxidises and darkens in colour. There are 2 crystalline forms which melt at about 80° and about 89°. Very slightly soluble in water; soluble 1 in 2 of ethanol and 1 in 1 of chloroform; soluble in ether but solutions may be turbid. pK_a 3.7, 7.5. Log *P* (octanol/water), 4.1.

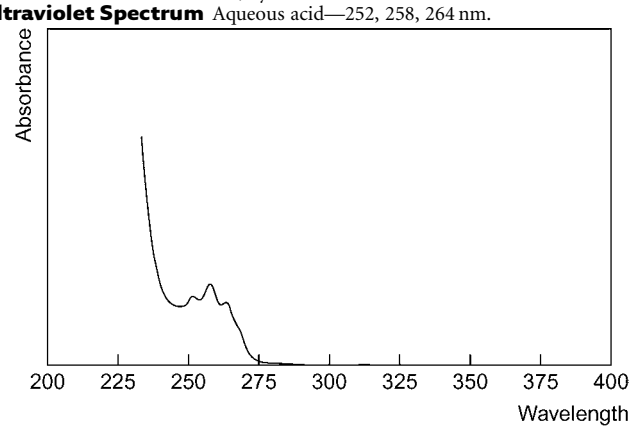
Anileridine Hydrochloride

$C_{22}H_{28}N_2O_2,2HCl$ = 425.4
CAS—126-12-5
Proprietary Name *Leritine* (tablets)
Chemical Properties A white crystalline powder. Mp about 270°, with decomposition. Soluble 1 in 5 of water and 1 in 80 of ethanol; practically insoluble in chloroform and ether.

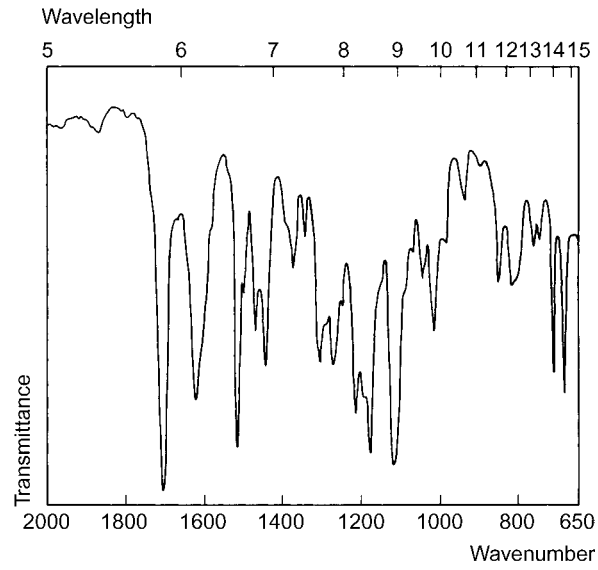
Anileridine Phosphate

$C_{22}H_{28}N_2O_2,H_3PO_4$ = 450.5
CAS—4268-37-5
Proprietary Name *Leritine* (injection)
Chemical Properties A white crystalline powder. Very soluble in water. Solutions are unstable above pH 4.

Colour Tests Diazotisation—red; Marquis test—orange (slow).
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.12; system TC—R_f 0.56; system TE—R_f 0.79; system TL—R_f 0.51; system TAE—R_f 0.60; system TAF—R_f 0.66; system TAJ—R_f 0.20; system TAK—R_f 0.00; system TAL—R_f 0.68 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2850; system GC—RI 3469.
High Performance Liquid Chromatography System HA—*k* 1.1; System HAX—retention time 11.7 min; System HAY—retention time 5.9 min.
Ultraviolet Spectrum Aqueous acid—252, 258, 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1700, 1120, 1179, 1512, 1219, 1620 cm^{-1} (KCl disk).



Mass Spectrum Principal ions at *m/z* 246, 247, 42, 120, 218, 172, 106, 91.

Disposition in the Body Readily absorbed after oral administration. Metabolised by hydrolysis to anileridinic acid followed by acetylation to acetylanileridinic acid; conjugation with acetic acid followed by partial de-esterification to acetylanileridinic acid also occurs. After oral administration, 5% of the dose is excreted in the urine as unchanged drug, with 7 to 14% as free anileridinic acid, 1 to 2% as acetylanileridinic acid, 0.5 to 2% as acetylanileridine, and 15 to 35% as diazotisable metabolites, tentatively identified as *p*-acetylaminophenylacetic acid; norpethidine has also been detected in urine.

Toxicity The estimated minimum lethal dose is 0.5 g but it may be up to 5 g in addicts.

The following postmortem concentrations were reported in a fatality attributed to oral overdosage of anileridine: blood 0.9 mg/L, bile 2.4 mg/L, urine 11.4 mg/L, vitreous humour 0.07 mg/L; diazepam was also detected [Peat, Kopjak 1979].

Dose Up to the equivalent of 200 mg of anileridine daily.

Peat MA, Kopjak L (1979). *Bull Int Assoc Forensic Toxicol* 14(3): 19.

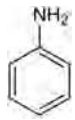
Aniline

Dye

$C_6H_5NH_2 = 93.1$

CAS—62-53-3

Synonym Phenylamine



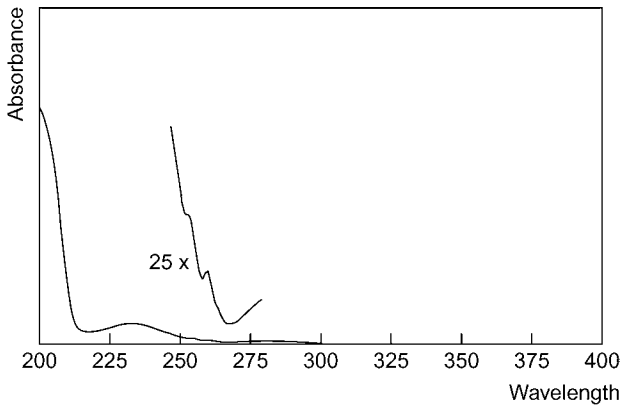
Chemical Properties A colourless or pale yellow oily liquid, with a characteristic odour. It readily darkens to brown on exposure to air and light. Fp −6°. Bp about 183°. Refractive index 1.5863. Soluble 1 in about 29 of water; miscible with ethanol, benzene, chloroform and ether. pK_a 4.6 (25°). Log *P* (octanol/water), 0.9.

Colour Tests Coniferyl Alcohol—orange; Diazotisation—red; Liebermann's Reagent—red-orange; Potassium Dichromate—blue-green (2 min).

Thin-layer Chromatography System TA—R_f 0.72; system TE—R_f 0.00; system TAJ—R_f 0.64; system TAK—R_f 0.00; system TAL—R_f 0.96 (acidified potassium permanganate solution, positive; van Urk reagent, bright yellow).

Gas Chromatography System GA—aniline RI 1158, 4-aminophenol RI 1253.

Ultraviolet Spectrum Aqueous acid—229 nm (A^1_1=13b), 255 nm (A^1_1=16b), 261 nm (A^1_1=12.5b); ethanol—235 nm (A^1_1=1007b), 286 nm (A^1_1=179b).



Mass Spectrum Principal ions at *m/z* 4-aminophenol 109, 80, 53, 81, 108, 52, 54, 110.

Quantification

Serum **GC–MS** Limit of detection, 0.1 mg/L [Dasgupta 1998; Dasgupta, Jagannath 1999].

Urine **GC–MS** Limits of detection, 0.05 to 2 µg/L for aniline and other aromatic amines and aromatic nitro metabolites [Weiss, Angerer 2002].

Tissues **GC** AFID. See Oliver and Williams [1974].

Disposition in the Body Readily absorbed from the skin and mucous membranes. Metabolised to 4-aminophenol and 4-acetamidophenol which are excreted in the urine as glucuronide and sulfate conjugates. *N*-Phenylsulfamic acid (aniline *N*-sulfate) is also a metabolite. The main toxic effect of aniline, the formation of methaemoglobin, is thought to be due to an oxidation product of aniline, phenylhydroxylamine.

Aniline is a metabolite of phenazopyridine.

Toxicity The minimum lethal dose may be as low as 1 g although recovery has followed the ingestion of 30 g. Serious toxic effects may occur after exposure for about 1 h to atmospheric concentrations of 100 to 160 ppm. The maximum permissible atmospheric concentration is 2 ppm. Urinary concentrations of >10 mg/L of 4-aminophenol may indicate toxic exposure to aniline.

The following postmortem tissue concentrations were reported in a fatality attributed to ingestion of an aniline-based ink: blood 6.3 mg/L, liver 20 µg/g, urine 40 mg/L [Oliver, Williams 1974].

Dasgupta A (1998). Gas chromatographic-mass spectrometric identification and quantification of aniline after extraction from serum and derivatization with 2,2,2-trichloroethyl chloroformate, a novel derivative. *J Chromatogr B Biomed Sci Appl* 716: 354–358.

Dasgupta A, Jagannath C (1999). Gas chromatographic/mass spectrometric identification and quantification of aniline after extraction from serum and derivatization with 4-carbethoxyhexafluorobutyryl chloride, a new derivative. *Ther Drug Monit* 21: 238–242.

Oliver JS, Williams DJ (1974). *Bull Int Assoc Forensic Toxicol* 10: 6–7.

Weiss T, Angerer J (2002). Simultaneous determination of various aromatic amines and metabolites of aromatic nitro compounds in urine for low level exposure using gas chromatography-mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 778: 179–192.

Aniracetam

Cognition Enhancer, Nootropic

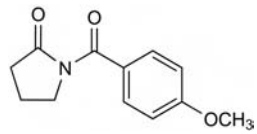
$C_{12}H_{13}NO_3 = 219.2$

CAS—72432-10-1

IUPAC Name 1-(4-Methoxybenzoyl)-2-pyrrolidinone; 1-*p*-Anisoyl-2-pyrrolidinone

Synonym Aniracetamum

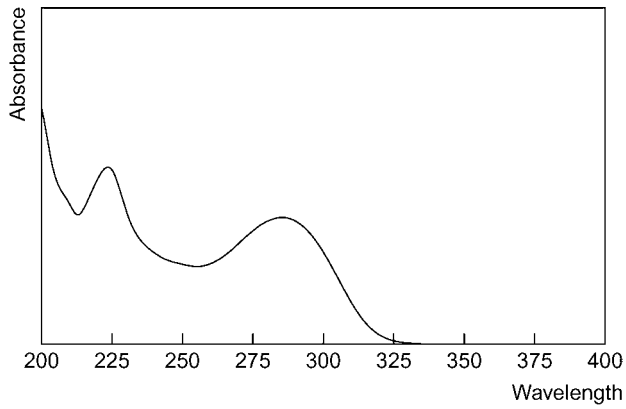
Proprietary Names *Ampamet*; *Draganon*; *Reset*; *Sarpul*.



Chemical Properties Odourless and colourless crystals. Mp 121° to 122°. It has limited aqueous solubility (approximately, 1.5 g/L) which is not affected by pH (1.47 g/L at pH 1.2 and 1.38 g/L at pH 7.5). Log *P* (octanol/buffer pH 7.4), 10 (22°). It is essentially a neutral molecule with moderate lipophilicity.

High Performance Liquid Chromatography Column: ODS-silica Hypersil ODS (60 × 4.0 mm i.d., 3 µm). Mobile phase : water : methanol : acetonitrile (70:30:10), flow rate 1.0 mL/min. UV detection (λ=282 nm). Retention time: 4.3 min.

Ultraviolet Spectrum Principal peaks at 224, 288 nm.



Quantification

Plasma **HPLC** Column: C_{18} µBondapak (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : triethylamine : 0.1 mol/L phosphate buffer (pH 3.0; 530 : 0.05 : 470), flow rate 1.0 mL/min. DAD. Retention time: *N*-anisoyl-GABA, 5.3 min [Guenzi, Zanetti 1990]. Limit of quantification, 5 µg/L and 50 µg/L for aniracetam and *N*-anisoyl-GABA, respectively, limit of detection, 2 µg/L and 5 µg/L, respectively [Guenzi, Zanetti 1990].

Disposition in the Body Aniracetam is rapidly absorbed from the gastrointestinal tract and essentially absorption is complete. Aniracetam undergoes extensive biodegradation and therefore bioavailability is very low at ~0.2% (only 0.2% reaches the systemic circulation as the parent drug). Aniracetam is metabolised in the body and the main metabolite produced is *N*-anisoyl-GABA (~70%) but 2-pyrrolidinone, 5-hydroxy-2-pyrrolidinone and anisic acid are also produced. Rapid and complete elimination occurs via urine (84%), faeces (0.8%) and expired air (11%) as CO_2. Elimination is biphasic. *N*-Anisoyl-GABA and anisic acid are secreted in breast milk. All can traverse the placenta from blood and/or metabolites formed before reaching fetus.

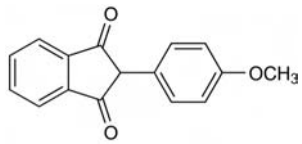
A

Therapeutic Concentration
After oral administration, 1000 mg, peak plasma concentrations varied greatly between 42 and 246 μg/L for 6 subjects and were detected at 5 min. Peak plasma concentrations for a 2 g dose varied between 55 and 116 μg/L, and between 42 and 1087 μg/L for a 4 g dose Roncari *et al* 1984.
Toxicity Toxicity testing in several animal species, including mouse, rat, rabbit and dog shows that aniracetam has extremely low toxicity.
Half-life For the parent drug, aniracetam, the half-life is 30 min and for its metabolites, 12.8 to 15.7 h.
Volume of Distribution Steady state, 2.5 L/kg (ranging between 1.9 and 4.1 L/kg).
Clearance Total clearance, ~9.9 L/min (range between 6.4 and 14.9 L/min).
Distribution in Blood Aniracetam is equally distributed between red blood cells and plasma (blood : plasma concentration ratio range 0.92 to 0.95). Distribution outside the vascular space and probable binding with tissue components also occurs.
Protein Binding 66%.
Dose Recommended dose of 1500 mg daily orally, either as a single dose (as sugar-free sachets) or as 750 mg doses in the form of a tablet.

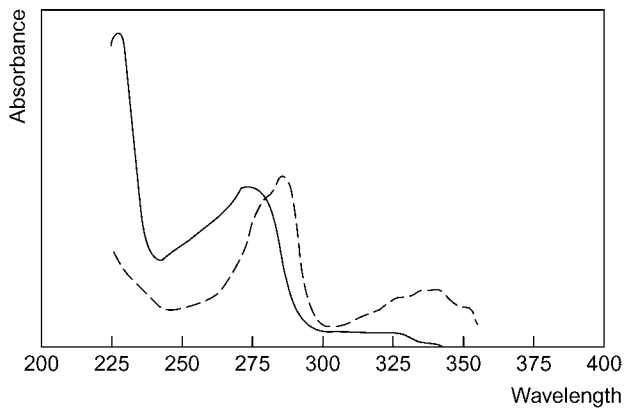
Guenzi A, Zanetti M (1990). Determination of aniracetam and its main metabolite, N-anisoyl-GABA, in human plasma by high-performance liquid chromatography. *J Chromatogr* 530: 397–406.
Roncari *et al.*, 1984, F. Hoffman-La Roche data on file B-104′488

Anisindione

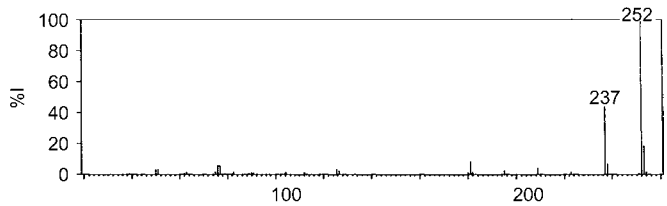
Anticoagulant
$C_{16}H_{12}O_3$ = 252.3
CAS—117-37-3
IUPAC Name 2-(4-Methoxyphenyl)-1*H*-indene-1,3(2*H*)-dione
Proprietary Names *Miradon*; *Unidone*.



Chemical Properties A white or creamy-white powder. Mp 152° to 158°. Practically insoluble in water; soluble in ether and methanol. pK_a 4.1. Log *P* (octanol/water), 2.9.
Thin-layer Chromatography System TA—R_f 0.98; system TE—R_f 0.15; system TAJ—R_f 0.82; system TAK—R_f 0.70; system TAL—R_f 0.95.
Gas Chromatography System GA—RI 2273.
Ultraviolet Spectrum Aqueous acid—273 nm; ethanol—286 nm (A^{1}_{1}=1400b), 340 nm.



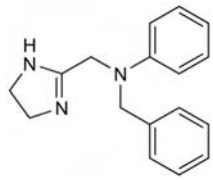
Mass Spectrum Principal ions at *m/z* 252, 237, 253, 181, 238, 77, 76, 209.



Dose Maintenance, 25 to 250 mg daily.

Antazoline

Antihistamine
$C_{17}H_{19}N_3$ = 265.4
CAS—91-75-8
IUPAC Name 4,5-Dihydro-*N*-phenyl-*N*-(phenylmethyl)-1*H*-imidazole-2-methanamine
Synonyms Imidamine; phenazoline.



Chemical Properties Crystals. Mp 120° to 122°. pK_a 2.5, 10.1 (25°). Log *P* (octanol/water), 3.4.

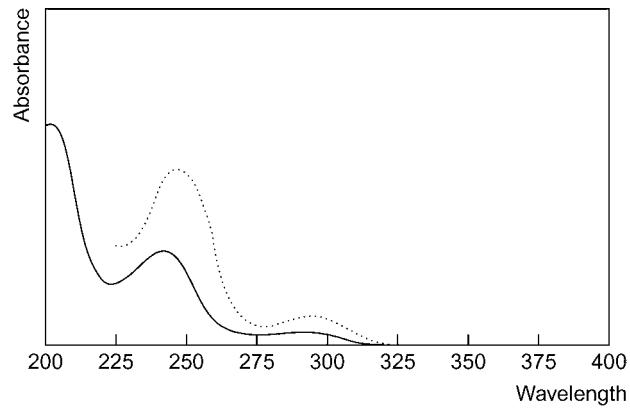
Antazoline Hydrochloride
$C_{17}H_{19}N_3$,HCl = 301.8
CAS—2508-72-7
Synonyms Imidamine hydrochloride; phenazolinum.
Proprietary Names *Wasp-Eze* (ointment). It is an ingredient of *Modantis* and *RBC*.
Chemical Properties A white crystalline powder. Mp 237° to 241°. Soluble 1 in 40 of water and 1 in 25 of ethanol; practically insoluble in ether, benzene and chloroform.

Antazoline Mesilate
$C_{17}H_{19}N_3$,CH_3SO_3H = 361.5
CAS—3131-32-6
Synonyms Antazoline mesylate; antazoline methanesulphonate; imidamine mesilate.
Chemical Properties A white, slightly hygroscopic powder. Mp 165° to 168°. Soluble 1 in 6 of water, 1 in 7 of ethanol and 1 in 12 of chloroform; practically insoluble in ether.

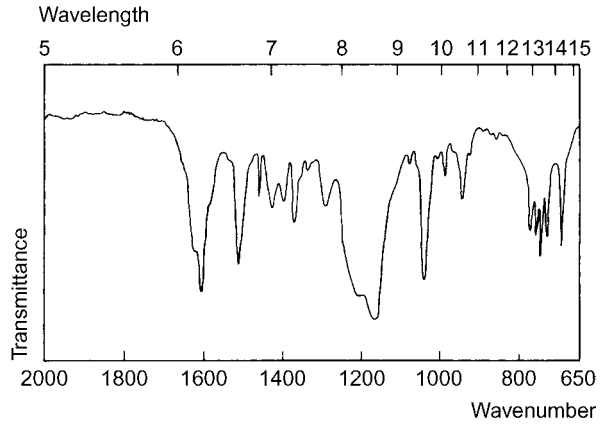
Antazoline Phosphate
$C_{17}H_{19}N_3$,H_3PO_4 = 363.4
CAS—154-68-7
Synonym Imidamine phosphate
Proprietary Names It is an ingredient of *Albalon-A*, *Antazoline-V*and *Vasocon A*.
Chemical Properties A white crystalline powder. Mp 194° to 198°. Soluble in water; sparingly soluble in methanol; practically insoluble in benzene and ether.

Antazoline Sulfate
$(C_{17}H_{19}N_3)_2$,H_2SO_4, $2H_2O$ = 664.8
CAS—24359-81-7 (anhydrous)
Synonym Imidamine sulphate
Proprietary Names It is an ingredient of *Antistin(e)-Privin(e)* and *Otrivine-Antistin*.

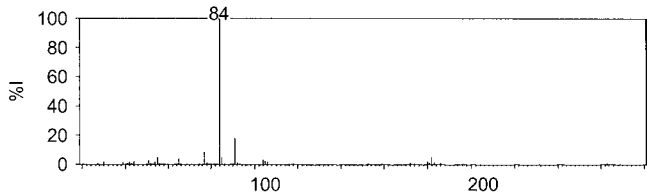
Colour Tests Liebermann's reagent—red; Mandelin's test—red-violet.
Thin-layer Chromatography System TA—R_f 0.31; system TB—R_f 0.06; system TC—R_f 0.07; system TE—R_f 0.47; system TL—R_f 0.03; system TAE—R_f 0.05; system TAF—R_f 0.66; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.15 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2318; system GB—RI 2421; system GC—RI 2749.
High Performance Liquid Chromatography System HA—*k* 1.8; system HX—RI 383; system HY—RI 294.
Ultraviolet Spectrum Aqueous acid—241 nm (A^{1}_{1}=578a), 291 nm; aqueous alkali—247, 295 nm.



Infrared Spectrum Principal peaks at wavenumbers 1164, 1599, 1042, 1508, 750, 700 cm^{-1} (antazoline mesilate, KBr disk).



Mass Spectrum Principal ions at *m/z* 84, 91, 77, 55, 182, 85, 65, 104.



Quantification

Urine **Colorimetry** See Wahba *et al.* [1974].

Disposition in the Body Readily absorbed after oral or parenteral administration. About 2% of an oral dose is excreted unchanged in the urine in 24 h.

Dose Antazoline hydrochloride has been given in doses of 100 to 600 mg daily.

Wahba N *et al.* (1974). Colorimetric determination of antazoline excreted in urine. *Pharmazie* 29: 790–791.

Antimony

Antiprotozoal, Metal

Sb = 121.8

CAS—7440-36-0

Synonyms Antimony black; antimony regulus; stibium.

Chemical Properties Silvery white solid. Mp 630.5°. Bp 1750°. Insoluble in water. Valencies: Sb (−3), Sb(0), Sb (+3) and Sb (+5). Metallic antimony is the only allotropic form of antimony that is stable under normal conditions and is occasionally found uncombined in nature. Antimony has two stable isotopes, ^{121}Sb and ^{123}Sb. One radioactive isotope, ^{125}Sb, is a fission product released in nuclear explosions or nuclear fuel reprocessing plants. Used in alloys, solder, sheet and pipe, bearings, castings and type metal, ammunition, and cable sheathing. High-purity antimony is used as a dopant in semi-conductors and in devices such as infrared detectors and diodes.

Antimony Pentasulfide

Sb_2S_5 = 403.8

CAS—1315-04-4

Synonyms Antimony persulfide; antimony red; antimonial saffron; antimonic sulfide; golden antimony sulfide.

Chemical Properties Odourless yellow solid. Mp 75° with decomposition. Insoluble in water and alcohol.

Antimony Pentoxide

Sb_2O_5 = 323.5

CAS—1314-60-9

Synonyms Antimonic acid; antimonic anhydride; antimonic oxide; antimony pentaoxide; diantimony pentoxide; stibic anhydride.

Chemical Properties Yellow solid. Mp 380° with decomposition. Very slightly soluble in water.

Antimony Potassium Tartrate

$Sb_2C_8H_4K_2O_{12},3H_2O$ = 333.9

CAS—28300-74-5

Synonyms Antimonyl potassium tartrate; tartar emetic; tartox; tartrated antimony.

Chemical Properties Odourless, colourless solid. Mp 100°. Soluble in water and glycerine. Insoluble in alcohol. Used in the treatement of schistosomiasis.

Antimony Trichloride

$SbCl_3$ = 228.1

CAS—10025-91-9

Synonyms Antimonous chloride; antimony butter; antimony (III) chloride; chloride antimony; trichlorostibine.

Chemical Properties Colourless solid with a sharp, unpleasant odour. Mp 73.4°. Bp 283°. Very soluble in water; soluble in alcohol, tartaric acid, methylene chloride, benzene and acetone.

Antimony Trioxide

Sb_2O_3 = 291.5

CAS—1309-64-4

Synonyms Antimonious oxide; antimony peroxide; antimony sesquioxide; antimony white; diantimony trioxide; exitelite; flowers of antimony; senarmontite; timothox; valentinite.

Proprietary Names *Fire Shield H*; *H Grade*; *HP*; *KR-LTS*; *LP*; *KR*; *Montana Brand*; *Thermoguard S*; *White Star*.

Chemical Properties Senarmontite is a white solid; valentinite is a colourless solid. Mp 656°. Bp 1550°. Very slightly soluble in water; soluble in tartaric acid, acetic acid and hydrochloric acid. Used as a fire retardant.

Antimony Trisulfide

Sb_2S_3 = 339.7

CAS—1345-04-6

Synonyms Antimonous sulphide; antimony crimson; antimony glance; antimony needles; antimony orange; antimony sesquisulfide; antimony sulphide; antimony vermilion; stibite.

Chemical Properties Black solid. Mp 550°. Bp 1150°. Slightly soluble in water; insoluble in acetic acid.

Stibine

SbH_3 = 124.8

CAS—7803-52-3

Synonyms Antimony hydride; antimony trihydride; hydrogen antimonide.

Chemical Properties Colourless gas with hydrogen sulfide-type odour. Mp −88°. Bp −17°. Soluble in carbon disulfide and ethanol.

Colour Tests

Reinsch test. Applicable to urine, gastric contents, scene residues. Clean a 5 × 10 mm square of copper foil (2–3 cm copper wire) with aqueous nitric acid (500 mL/L) until shiny. Rinse the copper with purified water and add 10 mL of concentrated hydrochloric acid and 20 mL of test sample in a 100 mL conical flask. Heat in a boiling water bath in a fume cupboard for 1 h. Cool and gently wash the copper with purified water.—Antimony imparts a black stain to the copper (arsenic, bismuth, selenium and tellurium also give black deposits).

Confirmatory test. Place the stained copper in 100 g/L potassium cyanide solution and leave for 10 min. Wash any undissolved stain with purified water and add 1 mL of freshly prepared sodium sulfite solution (50 g/L) and 1 mL of 3 mol/L nitric acid. Agitate frequently for 5 min and add 1 mL of purified water and 1 mL of a mixture of quinine/potassium iodide reagent (prepared by dissolving 1 g of quinine sulfate in 100 mL of purified water containing 0.5 mL of concentrated nitric acid and adding 2 g of potassium iodide when the quinine has dissolved).—Stains caused by arsenic dissolve in potassium cyanide solution; stains caused by antimony and bismuth do not. Bismuth slowly forms an orange/brown suspension with quinine/potassium iodide reagent.

Quantification

Specimen Collection Blood—10 mL K-EDTA tube; urine—20 mL plastic universal container.

Blood **FAAS** Flame: air–acetylene. Carrier gas: N_2, 105 mL/min. Hollow cathode lamp (λ = 217.6 nm). Limit of detection not reported [Le *et al.* 1992].

ETAAS Limit of detection, 0.5 µg/L [Gebel *et al.* 1998a]. Dry cycle: 120° in 10 s for 20 s, Ar, 300 mL/min. Char cycle: 1000° in 10 s for 20 s; Ar, 300 mL/min. Atomisation cycle: 2050° for 3 s (gas stop). Electrodeless discharge lamp (λ = 217 nm). Limit of detection, 2.6 µg/L [Subramanian *et al.* 1997]. Dry cycle: 90° at 15 s for 4 s to 130° in 30 s for 10 s. Char cycle: 700° in 60 s for 30 s to 1100° in 30 s for 16 s. Atomisation cycle: 2500° in 1 s for 4 s. Limit of detection, 25 µg/L following digestion [Costantini *et al.* 1985a].

ICP-MS Plasma gas: 15 L/min. Auxillary gas: 0 L/min. Carrier gas: 0.86 L/min. Glass expansion micromist nebuliser. Limit of detection, 0.03 µg/L [Bazzi *et al.* 2005]. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 0.2 µg/L [De Boer *et al.* 2004]. ELAN 5000A. Limit of detection, 8 ng/L [Miekeley *et al.* 2002]. Limit of detection, 142 ng/L [Liao *et al.* 2004]. Nebuliser gas: Ar, 1.0 L/min. Auxillary gas: Ar, 0.95 L/min. Coolant gas: Ar, 15.0 L/min. Limit of quantification, 0.01 µg/L [Delves *et al.* 1997].

Plasma **ICP-MS** See Blood [Miekeley *et al.* 2002].

Serum **ETAAS** See Blood [Subramanian *et al.* 1997].

ICP-MS See Blood [Delves *et al.* 1997].

Urine **FAAS** See Blood [Le *et al.* 1992].

ETAAS Limit of detection, 0.5 µg/L [Gebel *et al.* 1998a, 1998b]. Dry cycle: 140° at 30 s for 20 s. Char cycle: 800° in 30 s for 45 s; Ar 300 mL/min. Atomisation cycle: 2000° for 4 s (gas stop). Limit of detection, 0.69 µg/L [Smith *et al.* 1995]. See Blood [Costantini *et al.* 1985b]. Dry cycle: 110° for 20 s. Char cycle: 700° for 20 s. Atomisation cycle: 2200° for 2 s. Carbon furnace mode (λ = 217.6 nm). Limit of detection, not reported [Smith, Griffiths 1982].

ICP-AES Plasma gas: 0.8 L/min. Coolant gas: 16 L/min. Carrier gas: 0.4 L/min. Limit of detection, 0.09 µg/L for trivalent antimony [Li *et al.* 2006].

GC-ICP-MS Limit of detection, 2 to 12 pg/L [Kresimon *et al.* 2001].

ICP-MS Plasma gas: Ar, 15 L/min. Auxillary gas: Ar, 1.3 L/min. Sample gas: 1.0 L/min. Meinhard nebuliser. Limit of detection, 0.43 ng/L [Alimonti *et al.* 2005]. See Blood. Limit of detection, 0.15 µg/L [De Boer *et al.* 2004]. Limit of detection, 3 ng/L [Liao *et al.* 2004]. See Blood [Delves *et al.* 1997; Miekeley *et al.* 2002]. Plasma gas:

15 L/min. Nebulizer gas: 0.825 L/min. Auxillary gas: 0.8 L/min. Limit of detection, 0.03 μg/L [Schramel *et al.* 1997]. See also Dezateux *et al.* [1997].

Note For a candoluminescence spectrometry method for the determination of antimony in urine, see Clark and Patel [1986]. For a colorimetric method for the determination of antimony in urine (limit of quantification, 20 μg/L), see Kneip *et al.* [1976].

Hair **ETAAS** Limit of detection, 5 μg/kg [Gebel *et al.* 1998a; Gebel *et al.* 1998b].

Note For an instrumental neutron activation analysis of antimony in hair, see Dorea *et al.* [1987].

Liver **ICP-MS** Nebuliser gas: Ar, 1.04 L/min. Limit of detection, 23 ng/L [Patriarca *et al.* 1999]. Nebuliser gas: Ar, 1.0 L/min. Auxillary gas: Ar, 0.95 L/min. Coolant gas: Ar, 15.0 L/min. Limit of quantification, 0.7 μg/kg [Delves *et al.* 1997].

Lung **ICP-MS** See Blood. Limit of quantification, 0.8 μg/kg [Delves *et al.* 1997].

Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000].

Other **AAS** Fresh and Freeze-dried Plant Samples. Carrier gas: Ar, 50 mL/min. Atomisation cycle: 770°. Hollow cathode lamp ($\lambda = 217.6$ nm). Limit of detection not reported [Krachler, Emons 2000]. Bovine liver and porcine kidney. Carrier gas: Ar, 50 mL/min. Atomisation cycle: 770° ($\lambda = 217$ nm). Limit of quantification, 23 ng/kg, limit of detection, 7 ng/kg [Krachler *et al.* 1999].

ETAAS River Water and Seawater. Dry cycle: 100° at 10 s for 10 s. Char cycle: 1000° in 5 s for 10 s, Ar 250 mL/min. Atomisation cycle: 2200° for 3 s (gas stop). Antimony hollow cathode lamp ($\lambda = 217$ nm). Limit of detection, 8.0 ng/L for Sb (III) and 9.2 ng/L for Sb [Fan 2007]. Waste. Dry cycle: 120° in 1 s for 20 s to 130° in 3 s for 35 s. Char cycle: 1300° at 8 s for 25 s; Ar, 250 mL/min. Atomisation cycle: 1900° for 5 s (gas stop). Antimony hollow cathode lamp ($\lambda = 217$ nm). Limit of detection, 0.3 μg/L [Bosch Ojeda *et al.* 2005]. Naphtha. Dry cycle: 50° for 10 s to 90° in 25 s to 120° in 10 s; Ar, 200 mL/min. Atomisation cycle: 2500° for 8 s (gas stop; $\lambda = 217.6$ nm). Limit of detection, 2.5 μg/L [Cassella *et al.* 2004].

ICP-AES Pharmaceutical Formulations. Plasma gas: Ar, 15 L/min. Nebuliser gas: Ar, 0.48 L/min. Meinhard nebuliser. Limit of quantification, 21.7 ng/L [Cabral *et al.* 2008]. Bullet wounds. Plasma gas: Ar, 1.2 L/min. Cooling gas: 14 L/min. Carrier gas: 1.0 L/min. ICPS-7510 ($\lambda = 207$ nm). Limit of detection not reported [Wunnapuk *et al.* 2007].

ICP-MS Lymph Node Biopsy Microwave Digests. Plasma gas: 15 L/min. Carrier gas: 1.05 L/min. Limit of detection, 0.048 μg/L [Beavis *et al.* 2008]. Solid and liquid food samples. Limit of quantification, 3 μg/kg and 0.3 μg/L for solid and liquid samples, respectively; limit of detection, 1 μg/kg and 0.1 μg/L for solid and liquid samples, respectively [Cheung Chung *et al.* 2008]. Well Water. Method 200.8 of the EPA. Limit of detection, 1 μg/L [McCarty *et al.* 2004]. Plasma gas: 15 L/min. Nebuliser gas: 0.95 L/min. Auxillary gas: 0.8 L/min. Limit of detection, 0.044 μg/L [Dawson *et al.* 2003]. Meals from Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 2 μg/kg [Noel *et al.* 2003]. GSR. Coolant gas: 15 L/min. Auxiliary gas: 1.1 L/min. Sample gas: 0.97 L/min. Limit of detection, 0.045 μg/L [Reis *et al.* 2003].

Note For a review of analytical methods for the detection of antimony in waters, see Shotyk *et al.* [2005]. For a study of antimony in airborne particulate matter collected in Tokyo between 1995 and 2004, see Furuta *et al.* [2005]. For a study of antimony in soils, plants, water, and sediments in Korea, see Jung *et al.* [2002]. For the polarographic analysis of antimony compounds in antibilharzial preparations, see Gawargious *et al.* [1987].

Disposition in the Body Soluble forms of trivalent and pentavalent antimony administered by inhalation or parenteral injection are rapidly absorbed and eliminated, mainly via the kidneys, with greater than 50% in the urine 6 h following injection. Orally ingested soluble forms are slowly absorbed and urinary elimination is prolonged. Highest concentrations of antimony are found in the liver after therapeutic administration of antimonials, and the liver can reduce the pentavalent form to the trivalent form. Trivalent antimony is predominantly excreted in the faeces and not as rapidly excreted in the urine as the pentavalent form (25% excreted in the urine 24 h post injection).

Normal Concentrations Blood—<1 μg/L (8 nmol/L); urine—<1 μg/L (8 nmol/L); kidney—0.01 mg/kg; liver—0.02 mg/kg; lung—0.3 mg/kg.

Note For studies on antimony concentrations in infants, see Cullen *et al.* [1998] and Dezateux *et al.* [1997].

Therapeutic Concentration

Twenty-nine male patients with cutaneous leishmaniasis were administered sodium stibogluconate IM equivalent to 600 mg antimony per day for 10 days. The mean peak plasma concentration was 8.77 mg/L at 1.34 h [Jaser *et al.* 1995].

Five patients with visceral leishmaniasis were treated with sodium stibogluconate (2 patients) or meglumine antimonite (3 patients) given IM equivalent to antimony 10 mg/kg bodyweight. Treatment lasted 30 days. Mean peak blood concentrations of 9 to 12 mg/L occurred at 2 h [Chulay *et al.* 1988].

Toxicity Occupational inhalation of antimony compounds such as the oxide or trichloride from dust or fumes damages the upper respiratory tract, and prolonged exposure leads to gastrointestinal symptoms similar to those seen in arsenic poisoning. Symptoms of acute antimony poisoning resemble those of acute arsenic poisoning.

Urine antimony concentrations up to 150 μg/L (1.2 μmol/L) developed in a group of occupationally exposed workers [Smith *et al.* 1995].

A 32-year-old man suffering from AIDS was admitted to hospital for treatment of visceral leishmaniasis. He was also inadvertently treated with 10 times the prescribed dose of sodium stibogluconate (6500 mg instead of 650 mg). The concentration of antimony was ≈11 μmol/L at 20 h with biexponential elimination [Reymond, Desmeules 1998].

A 27-year-old woman acquired cutaneous leishmaniasis in Central America. She was inadvertently treated with 10 times the intended daily dose of the antimonial compound sodium stibogluconate (Pentostam), 8500 mg (143 mg/kg) instead of 850 mg. Concentrations of antimony in her serum and urine were as follows:

	Time	*Sb (mg/L)*
Serum		
Day 1	11.00 pm	210
Day 2	2.30 am	27.5
Day 2	7.15 am	4.6
Day 3	7.00 am	<2
Urine		
Day 1–2	24 h	579
Day 4	10.45 am	4.6

[Herwaldt *et al.* 1992].

Four adults were admitted to the emergency department with severe abdominal cramps, nausea, continuous vomiting and watery diarrhoea. Three of the patients survived with no sequelae but one, a 93-year-old man, had marked cyanosis and haematemesis. He subsequently died. The cause of the poisoning was the use of 'tartar emetic' in the place of 'cream of tartar' in baking. Antimony concentrations in the deceased were as follows:

	Antimony (μg/L)
Bile	471
Colon	53
Gallbladder	459
Heart	6
Kidney	103
Liver	491
Lung	32
Muscle (upper arm)	3
Nails	176
Skin/hair	6
Small bowel	224
Stomach	114
Vertebra	10

[Lauwers *et al.* 1990].

A 44-year-old white male doctor dosed himself with wine made of *Cephaelis ipecacuanha* and requested his apothecary to administer James' powder, a mixture of antimony oxide and potassium tartrate. A severe bout of vomiting and diarrhoea lasting more than 18 h followed and despite physician intervention the patient died [Miller 1982].

A woman ingested antimony trichloride solution and died 48 h later. She had tissue concentrations of 4.6 mg/L (blood), 6 mg/kg (brain), 6 mg/kg (lung), 45 mg/L (liver), 404 mg/L (bile) and 32 mg/kg (kidney) [Baselt 2005].

Note For an investigation of toxic trace elements in the hair of children with autism, see Fido and Al Saad [2005]. For a study of postmortem concentrations in deceased smelter workers, see Gerhardsson *et al.* [1982]. For concentrations in the urine of glass-manufacturing workers, see Apostoli *et al.* [1998], Arai *et al.* [1994] and Ludersdorf *et al.* [1987].

Volume of Distribution Apparent, ~0.22 L/kg.

Half-life Elimination half-life 38 days after IV injection. Following IM injection, biphasic, 2.02 and 76 h.

Distribution in Blood Trivalent antimony is bound predominantly to erythrocytes. Pentavalent antimony is predominantly found in serum.

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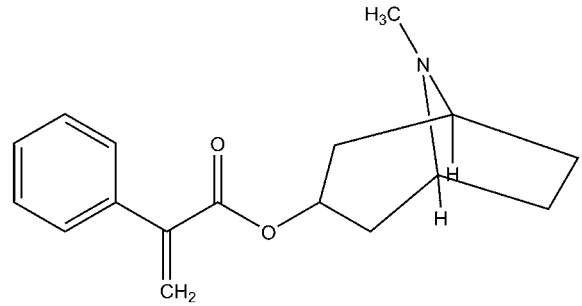
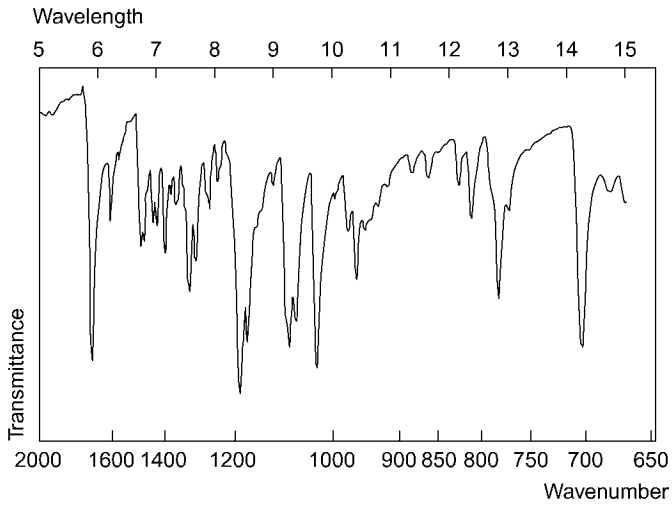
Apoatropine

Tropane, Alkaloid
$C_{17}H_{21}NO_2 = 271.4$
CAS—500-55-0

IUPAC Name Tropyl atropate
Synonyms Atropamine; atropoyltropeine.



Chemical Properties Obtained from the root of *Atropa belladonna*, *Latua pubiflora* (Solanaceae) and other plants of the genera *Datura* and *Scopolia*. Apoatropine is formed during the racemisation of hyoscyamine to atropine. Prisms from chloroform. Mp 60° to 62°. Almost insoluble in water; freely soluble in ethanol, ether, and chloroform; soluble in dilute acetic acid. Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 3.37 [Meylan, Howard 1995].
Colour Test Vitali's test—purple (limit of detection, 0.1 μg).
Thin-layer Chromatography System T1—R_f 0.18 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—RRT 1.75 (relative to diphenhydramine); system G4—RRT 0.43 (relative to diphenhydramine).
High Performance Liquid Chromatography Column: Lichrosorb Diol (250 × 4.0 mm i.d., 7 μm). Mobile phase: 0.0125 mol/L sodium phosphate buffer (pH 7.2) : acetonitrile (80 : 20), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: apoatropine 6.3 min, atropine 4.7 min, scopolamine 3.6 min. Limit of quantification not reported [Leroy, Nicolas 1987].
Infrared Spectrum Principal peaks at wavenumbers 1190, 1710, 1025 cm^{-1} (KBr disk).



Mass Spectrum See Wada *et al* [1994].
Quantification
***Other* TLC** Plant Material (*Scopolia tangutica*). Plates: silica gel G (10 × 15 cm). Solvent system: chloroform : methanol : 25% ammonium hydroxide (85 : 15 : 0.7). Location reagent: modified Dragendorff reagent. R_f values: apoatropine 0.77, tropine 0.04, cuscohygrine 0.12, (–)–6β-hydroxyhyoscyamine 0.23, hyoscyamine 0.34, daturamine 0.85, scopolamine 0.92. Limit of quantification not reported [Peigen, Liyi 1982].

GC-MS Plant Material (*Latua pubiflora*). Column: DB-1 fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 34.0 cm/s. Temperature

A

programme: 90° for 1 min to 300° at 6°/min for 4 min. EI ionisation at 70 eV (SIR). Retention time: apoatropine 21.2 min, 3α-cinnamoyloxytropane, hyoscyamine 24.8 min, scopolamine 26.3 min. Limit of quantification not reported [Munoz, Casale 2003]. Guinea Pig Tissue. Column: 2% OV-17 on Chromosorb W (AW-DMCS) (1.1 m × 3.2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 220°. EI ionisation at 70 eV. Retention time: 2.5 min. Limit of quantification not reported [Wada *et al.* 1994].

HPLC Plant Material (*Datura innoxia* and *Atropa belladonna*). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 30 mmol/L potassium dihydrogen phosphate-dipotassium hydrogen phosphate (815 : 185 g/g, pH 6.0) : methanol : acetonitrile (80.1 : 7.9 : 12), flow rate 1.0 mL/min. DAD. Retention times: apoatropine 9.2 min, (–)–6β-hydroxyhyoscyamine 10.6 min, scopolamine 15.7 min, hyoscyamine 18.6 min. Limit of quantification, 4 mg/L [Kursinszki *et al.* 2005]. Belladonna Preparations (tincture and powder). Column: Lichrosorb Diol (250 × 4.0 mm i.d., 7 µm). Mobile phase: 0.0125 mol/L sodium phosphate buffer (pH 7.2) : acetonitrile (80 : 20), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Retention times: apoatropine 6.3 min, atropine 4.7 min, scopolamine 3.6 min. Limit of quantification not reported [Leroy, Nicolas 1987].

Note For a study on the characterisation of alkaloids in hairy root cultures of *Datura stramonium*, see Berkov *et al.* [2003].

Toxicity Similar to atropine. LD_{50} (oral) in mice: 160 mg/kg.

Berkov S *et al.* (2003). Alkaloid spectrum in diploid and tetraploid hairy root cultures of Datura stramonium. *Z Naturforsch [C]* 58: 42–46.

Kursinszki L *et al.* (2005). Simultaneous analysis of hyoscyamine, scopolamine, 6beta-hydroxyhyoscyamine and apoatropine in Solanaceous hairy roots by reversed-phase high-performance liquid chromatography. *J Chromatogr A* 1091: 32–39.

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Munoz O, Casale JF (2003). Tropane alkaloids from *Latua pubiflora*. *Z Naturforsch [C]* 58: 626–628.

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Wada S *et al.* (1994). Sulphotransferase-dependent dehydration of atropine and scopolamine in guinea pig. *Xenobiotica* 24: 853–861.

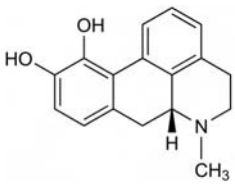
Apomorphine

Antiparkinsonian, Emetic

$C_{17}H_{17}NO_2$ = 267.3

CAS—58-00-4

IUPAC Name (6a*R*)-5,6,6a,7-Tetrahydro-6-methyl-4*H*-dibenzo[*de,g*]quinoline-10,11-diol



Chemical Properties Crystals. Mp about 195°, with decomposition. The crystals and aqueous solutions oxidise rapidly in light and air, turning green. Slightly soluble in water, benzene, ether and petroleum ether; soluble in ethanol and chloroform. pK_a 7.2, 8.9 (15°). Log *P* (octanol/water), 2.3. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005].

Apomorphine Hydrochloride

$C_{17}H_{17}NO_2$,HCl, $^1/_2H_2O$ = 312.8

CAS—314-19-2 (anhydrous); 41372-20-7 (hemihydrate)

Proprietary Names *Apofin*; *APO-go*; *Apokinon*; *Apomine*; *Britaject*; *Uprima*.

Chemical Properties White or greyish-white glistening crystals or microcrystalline powder. Decomposes between 225° and 236°. Soluble 1 in 50 of water and 1 in 17 of water at 80°; soluble 1 in 50 of ethanol; very slightly soluble in chloroform and ether.

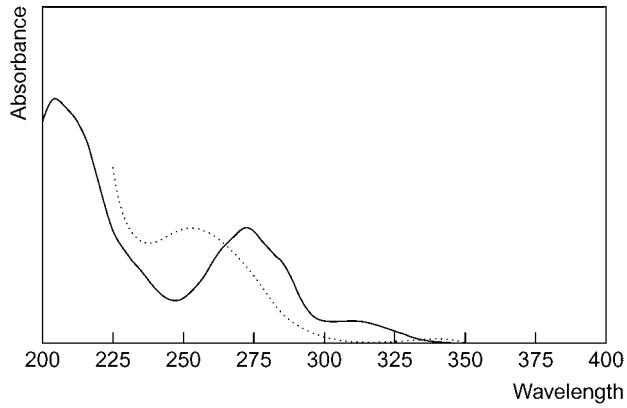
Colour Tests Ferric chloride—blue; Liebermann's reagent—black; Marquis test—violet→black; methanolic potassium hydroxide—green→red; Nessler's reagent—black.

Thin-layer Chromatography System TA—R_f 0.83; system TB—R_f 0.00; system TC—R_f 0.21; system TE—R_f 0.58; system TL—R_f 0.27; system TAE—R_f 0.54; system TAJ—R_f 0.06; system TAK—R_f 0.00; system TAL—R_f 0.22 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—apomorphine RI 2530; apomorphine-AC_2 RI 2830.

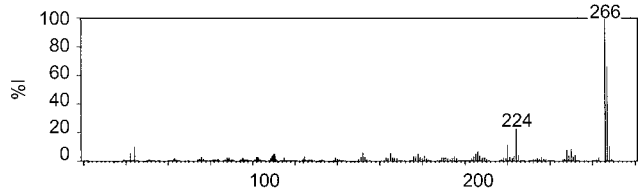
High Performance Liquid Chromatography System HA—*k* 3.7 (tailing peak); system HX—RI 348; system HY—RI 256; system HAA—retention time 9.0 min.

Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=626a); aqueous alkali—253 nm.



Infrared Spectrum Principal peaks at wavenumbers 1265, 1298, 1204, 752, 985, 790 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 266, 267, 224, 220, 268, 44, 250, 248.



Quantification

Plasma **GC** FID. Limit of detection, 1 mg/L [Baaske *et al.* 1977].

HPLC Electrochemical detection. Limit of detection, 20 pg [Bolner *et al.* 1997]. Electrochemical detection. For *R*- and *S*-apomorphine, apocodeine, isoapocodeine and conjugated metabolites. Limits of detection, 0.3 µg/L and 0.6 µg/L for *R*- and *S*-apomorphine, respectively [van der Geest *et al.* 1997]. Fluorescence detection, see Priston, Sewell [1996]. Electrochemical detection, see Sam *et al.* [1994]. Electrochemical detection, limit of detection, 0.5 µg/L [Essink *et al.* 1991]. Fluorescence detection, limit of detection, 100 µg/L [Smith, De Moreno 1983].

Urine **HPLC** See Plasma [van der Geest *et al.* 1997].

Disposition in the Body

Therapeutic Concentration

Apomorphine was administered by IV infusion (the rate was increased by 10 µg/kg every 20 min up to 100 µg/kg/h or until adverse effects occurred) to 10 subjects with parkinsonism, 8 of whom responded. Clinical efficacy occurred at a minimum concentration of 1.4 to 10.7 µg/L (mean 4.7) and dyskinesia occurred at 2.7 to 20 µg/L (mean 8.5); the minimal toxic concentration was 6.5 to24.5 µg/L (mean 16.7) [van Laar *et al.* 1998].

After a single sublingual dose of apomorphine 0.21 to 0.91 mg/kg to 5 patients (2 received 18 mg, 3 received 39 mg), peak plasma concentrations of 11.3 to 144.0 µg/L were attained at 45 to 90 min [Durif *et al.* 1991].

Dose 100 µg/kg of apomorphine hydrochloride subcutaneously, as a single dose.

Baaske DM *et al.* (1977). Gas chromatographic determination of apomorphine in plasma. *J Chromatogr* 140: 57–64.

Bolner A *et al.* (1997). Determination of apomorphine in human plasma by alumina extraction and high-performance liquid chromatography with electrochemical detection. *Forensic Sci Int* 89: 81–91.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Durif F *et al.* (1991). Relation between plasma concentration and clinical efficacy after sublingual single dose apomorphine in Parkinson's disease. *Eur J Clin Pharmacol* 41: 493–494.

Essink AW *et al.* (1991). Selective and quantitative isolation and determination of apomorphine in human plasma. *J Chromatogr* 570: 419–424.

Priston MJ, Sewell GJ (1996). Novel liquid chromatographic assay for the low-level determination of apomorphine in plasma. *J Chromatogr B Biomed Appl* 681: 161–167.

Sam E *et al.* (1994). Stability of apomorphine in plasma and its determination by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* 658: 311–317.

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van der Geest R *et al.* (1997). Assay of R-apomorphine, S-apomorphine, apocodeine, isoapocodeine and their glucuronide and sulfate conjugates in plasma and urine of patients with Parkinson's disease. *J Chromatogr B Biomed Sci Appl* 702: 131–141.

van Laar T *et al.* (1998). Stepwise intravenous infusion of apomorphine to determine the therapeutic window in patients with Parkinson's disease. *Clin Neuropharmacol* 21: 152–158.

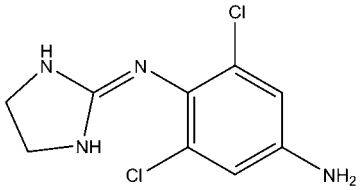
Apraclonidine

α_2-Adrenoceptor Agonist, Antiglaucoma

$C_9H_{10}Cl_2N_4$ = 245.1

CAS—66711-21-5

IUPAC Name 2,6-Dichloro-N^1-(4,5-dihydro-1*H*-imidazol-2-yl) benzene-1,4-diamine
Synonyms *p*-Aminoclonidine; aplonidine.



Chemical Properties Solid. Mp >230°. Log *P* (octanol/water), 1.53 [Wishart 2006].

Apraclonidine Hydrochloride

$C_9H_{10}C_{l2}N_4$,HCl = 281.6
CAS—73218-79-8
Synonyms ALO-2145; p-aminoclonidine Hydrochloride; aplonidine hydrochloride; NC-14.
Proprietary Names *Iopidine*; *Alcon*.
Chemical Properties A white to off-white, odourless to practically odourless powder. Soluble in methyl alcohol, sparingly soluble in water and alcohol, and insoluble in chloroform, ethyl acetate and hexanes.

Apraclonidine Dihydrochloride

$C_9H_{10}C_{l2}N_4$,2HCl = 318.1
CAS—73217-88-6

Ultraviolet Spectrum Peaks at 215, 252 and 300 nm.
Quantification
Plasma **GC-MS** Column: capillary. ECD. Limit of detection, 0.2 µg/L [Coleman *et al.* 1990; Robin, Coleman 1990].
Disposition in the Body
Therapeutic Concentration A 0.5% apraclonidine solution has been shown to be effective in reducing intra-ocular pressure in short and longer-term situations [Gross *et al.* 1997]. A 0.5% solution is as effective as a 1% solution in preventing intra-ocular pressure elevation after trabeculoplasty, iridotomy or capsulotomy [Rosenberg *et al.* 1995]. Plasma levels were not reported in either study.

Twenty healthy female volunteers (aged 21 to 47 years) were administered 2 drops of either 0.25% or 0.5% apraclonidine hydrochloride into both eyes or 0.5% apraclonidine into one eye and placebo into the other, with a 1 h break between each drop. Median plasma concentrations were as follows:

	Concentration (µg/L)		
Time (h)	Apraclonidine HCl 0.25%	Apraclonidine 0.5%	Unilateral 0.5% apraclonidine
2	ND	0.45	0.60
5	0.30	0.55	0.65
8	0.20	0.60	0.20

ND: not detectable (<0.2 µg/L) [Coleman *et al.* 1990; Robin, Coleman 1990].

Toxicity Systemic absorption of apraclonidine can occur following use of apraclonidine eye drops and cardiovascular effects have been reported. The drops should not be used concomitantly with monoamine oxidase inhibitors, tricyclic antidepressants, or systemic sympathomimetics. Ocular intolerance can occur with regular use.

A 56-year-old woman who underwent laser capsulotomy for cataract removal was prescribed apraclonidine hydrochloride ophthalmic solution. She was admitted to the emergency department with hypotension (BP 80/60 mmHg), drowsiness (Glasgow Coma Score 13) and bradycardia (heart rate 40 bpm). After a bolus of normal saline and IV atropine (1 mg), her blood pressure and heart rate returned to normal. A detailed history revealed that she had used the entire 5 mL apraclonidine solution within 12 h of the operation [Pekdemir *et al.* 2005]

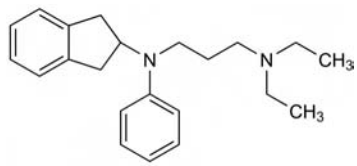
Half-life Approximately 8 h.
Protein Binding Approximately 99%.
Dose To control postoperative increase in intra-ocular pressure, one drop of a 1% solution is instilled before and following laser eye surgery. One or two drops of a 0.5% solution is instilled three times a day for control of raised intra-ocular pressure (short-term use only).

Coleman AL *et al.* (1990). Cardiovascular and intraocular pressure effects and plasma concentrations of apraclonidine. *Arch Ophthalmol* 108: 1264–1267.
Gross RL *et al.* (1997). Clinical experience with apraclonidine 0.5%. *J Glaucoma* 6: 298–302.
Pekdemir M *et al.* (2005). More than just an ocular solution. *Emerg Med J* 22: 753–754.
Robin AL, Coleman AL (1990). Apraclonidine hydrochloride: an evaluation of plasma concentrations, and a comparison of its intraocular pressure lowering and cardiovascular effects to timolol maleate. *Trans Am Ophthalmol Soc* 88: 149–159.
Rosenberg LF *et al.* (1995). Apraclonidine and anterior segment laser surgery. Comparison of 0.5% versus 1.0% apraclonidine for prevention of postoperative intraocular pressure rise. *Ophthalmology* 102: 1312–1318.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

A

Aprindine

Antiarrhythmic
$C_{22}H_{30}N_2$ = 322.5
CAS—37640-71-4
IUPAC Name *N*-(2,3-Dihydro-1*H*-inden-2-yl)-*N*′,*N*′-diethyl-*N*-phenyl-1,3-propanediamine

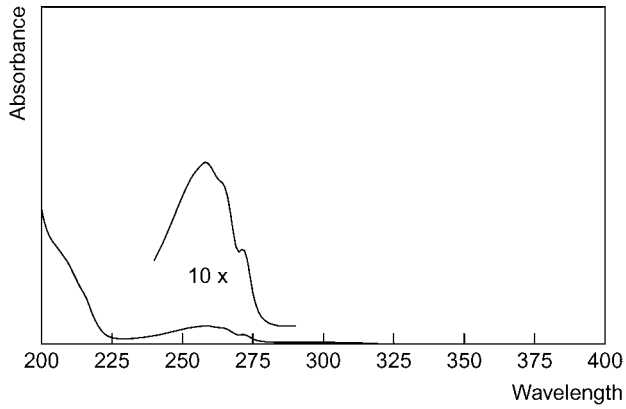


Chemical Properties pK_a 10.1. Log *P* (octanol/water), 4.9. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

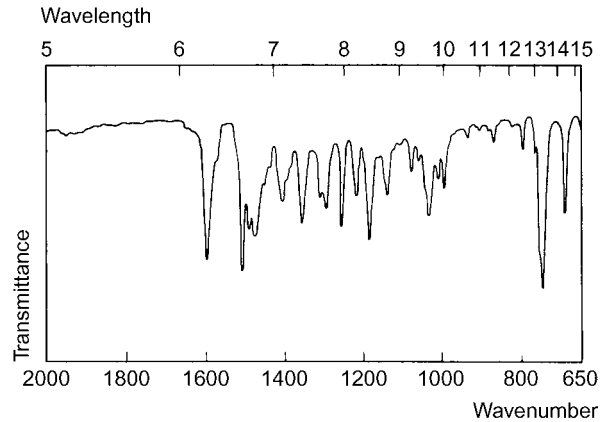
Aprindine Hydrochloride

$C_{22}H_{30}N_2$,HCl = 359.0
CAS—33237-74-0
Proprietary Names *Amidonal*; *Fiboran*.
Chemical Properties A white to yellowish-white microcrystalline powder. Mp 127° to 130°. Very soluble in water; freely soluble in ethanol and chloroform; practically insoluble in ether.

Thin-layer Chromatography System TB—R_f 0.63; system TE—R_f 0.76; system TAE—R_f 0.20.
Gas Chromatography System GA—aprindine RI 2462; M (*N*-desalkyl-) RI 1920, M (*p*-aminophenol-) RI 1253, M (OH-)-AC- RI 2850, M (OH-methoxy-)-AC- RI 2995, M (desindane)-AC- RI 1880, M (desphenyl-)-AC- RI 2300.
High Performance Liquid Chromatography System HX—RI 433; system HAA—retention time 17.0 min.
Ultraviolet Spectrum Aqueous acid—260, 266 nm (A_1^1=38a), 272 nm; ethanol—258 nm (A_1^1=389a), 296 nm (A_1^1=47a).



Infrared Spectrum Principal peaks at wavenumbers 745, 1510, 1602, 1187, 1258, 1035 cm^{-1} (aprindine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 113, 86, 116, 98, 117, 115, 58, 84; M (*N*-desalkyl-)aprindine *m/z* 104, 209, 77, 116, 115, 94.
Quantification
Plasma **GC** AFID. Limit of detection, 20 µg/L [Nash, Carmichael 1980].

A

HPLC UV detection, see Misztal and Przyborowski [1995]. UV or fluorescence detection. For aprindine and other antiarrhythmic drugs, see Verbesselt *et al.* [1991]. UV detection. Limits of detection, 20 µg/L and 50 µg/L for aprindine and monodesethylaprindine, respectively [Kobari *et al.* 1983].

***Serum* GC** Surface ionization detection. Limit of detection, 16 pg [Kawano *et al.* 1989].

Disposition in the Body Almost completely absorbed after oral administration. Extensively metabolised by aromatic hydroxylation and *N*-dealkylation followed by glucuronic acid conjugation; the monodesethyl metabolite, which can be detected in plasma after chronic administration, is active. <1% of a dose is excreted in the urine as unchanged drug and about 40 to 65% as conjugated metabolites; 35 to 60% of the dose is eliminated in the faeces over a period of 5 days.

Therapeutic Concentration In plasma, usually in the range 0.7 to 2 mg/L.

After a single oral dose of 200 mg to 12 subjects, peak plasma concentrations of 0.6 to 2 mg/L (mean 0.9) were attained in 2 h [Fasola, Carmichael [1974]].

Following daily oral doses of 100 to 150 mg, plasma concentrations in 28 patients ranged from 0.73 to 2.55 mg/L [Van Durme *et al.* 1974].

In 38 patients given a single oral dose of 100 to 150 mg aprindine hydrochloride, mean peak plasma concentrations of 0.33 to 1.40 mg/L (mean 0.77) were achieved in 1.5 to 4.0 h (mean 2.9). With repeated administration of 10 or 20 mg doses every 8 h, steady-state plasma concentrations of 0.155 to 0.455 mg/L and 0.39 to 1.53 mg/L, respectively, were attained in 7 to 19 days [Yokota *et al.* 1987].

Toxicity Toxic effects are usually associated with plasma concentrations greater than 2 mg/L.

Toxic effects were found in 9 patients whose plasma concentrations ranged from 1.2 to 4.9 mg/L (mean 2.6) [Van Durme *et al.* 1974].

Bioavailability About 75%.

Half-life Plasma half-life, 12 to 66 h (mean 30) in normal subjects but the half-life is apparently longer in patients with chronic ventricular arrhythmias and myocardial infarction (average, about 50 h).

Volume of Distribution About 4 L/kg.

Clearance Plasma clearance, about 2.5 mL/min/kg in normal subjects; decreased to about 1 mL/min/kg in patients with cardiac disease.

Protein Binding >95% at therapeutic concentrations, decreasing at higher concentrations.

Note For a review of the pharmacokinetics of antiarrhythmic agents, see Ronfeld [1980].

Dose 50 to 100 mg of aprindine hydrochloride daily; initial doses of up to 200 mg daily may be given.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings at the 12th TIAFT*, Seoul: 481–486.

Fasola AF, Carmichael R (1974). The pharmacology and clinical evaluation of aprindine a new antiarrhythmic agent. *Acta Cardiol Suppl* 18: 317–333.

Kawano H *et al.* (1989). Rapid and sensitive determination of aprindine in serum by gas chromatography using a surface ionization detector. *J Chromatogr* 493: 71–78.

Kobari T *et al.* (1983). High-performance liquid chromatographic determination of aprindine and its active desethyl metabolite in plasma. *J Chromatogr* 278: 220–224.

Misztal G, Przyborowski L (1995). Determination of aprindine in human plasma using reversed phase HPLC. *Pharmazie* 50: 187–188.

Nash JF, Carmichael RH (1980). GLC determination of aprindine in human plasma using a nitrogen–phosphorus flame-ionization detector. *J Pharm Sci* 69: 1094–1096.

Ronfeld RA (1980). Comparative pharmacokinetics of new antiarrhythmic drugs. *Am Heart J* 100: 978–983.

Van Durme JP *et al.* (1974). Therapeutic effectiveness and plasma levels of aprindine, a new antidysrhythmic drug. *Eur J Clin Pharmacol* 7: 343–346.

Verbesselt R *et al.* (1991). High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Ther Drug Monit* 13: 157–165.

Yokota M *et al.* (1987). Non-linear pharmacokinetics of aprindine hydrochloride in oral administration. *Arzneimittelforschung* 37: 184–188.

Aprobarbital

Sedative, Barbiturate

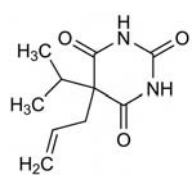
$C_{10}H_{14}N_2O_3$ = 210.2

CAS—77-02-1

IUPAC Name 5-(1-Methylethyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione

Synonyms Allylisopropylmalonylurea; allypropymal; aprobarbitone.

Proprietary Name *Alurate*



Chemical Properties A white crystalline powder. Mp 140° to 141.5°. Soluble 1 in 350 of water, 1 in 30 of boiling water, 1 in 2.5 of ethanol, 1 in 40 of chloroform, and 1 in 5 of ether; soluble in acetone, glacial acetic acid and aqueous solutions of alkali hydroxides and carbonates; almost insoluble in petroleum ether and aliphatic hydrocarbons. pK_a 8.0 (25°). Log *P* (octanol/water), 1.2.

Aprobarbital Sodium

$C_{10}H_{13}N_2NaO_3$ = 232.2

CAS—125-88-2

Synonym Aprobarbitone sodium

Chemical Properties A white, hygroscopic, microcrystalline powder. Very soluble in water; slightly soluble in ethanol; practically insoluble in ether.

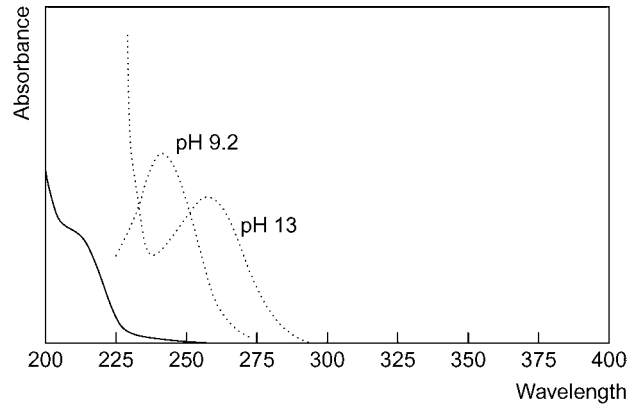
Colour Test Koppanyi-Zwikker test—violet.

Thin-layer Chromatography System TD—R_f 0.48; system TE—R_f 0.40; system TF—R_f 0.65; system TH—R_f 0.66; system TAD—R_f 0.57; system TAE—R_f 0.86 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).

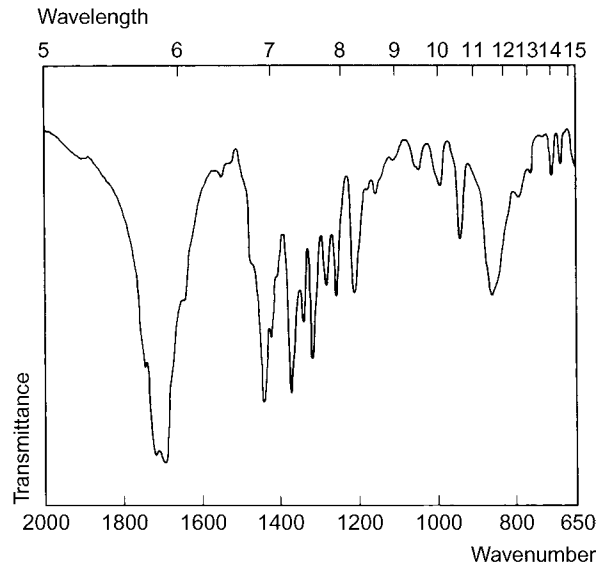
Gas Chromatography System GA—aprobarbital RI 1618, M (OH-) RI 1815, aprobarbital-Me_2 RI 1540.

High Performance Liquid Chromatography System HG—*k* 3.42; system HH—*k* 2.22; system HX—RI 357; system HY—RI 319; system HZ—retention time 2.8 min; system HAL—retention time 2.0 min.

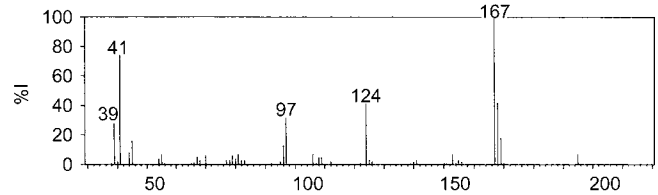
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—241 nm (A^1_1=451a); 1 mol/L sodium hydroxide (pH 13)—257 nm (A^1_1=331b).



Infrared Spectrum Principal peaks at wavenumbers 1693, 1720, 1745, 1316, 1255, 860 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 167, 41, 124, 168, 97, 39, 169, 45; *N*-hydroxyaprobarbital 41, 43, 183, 167, 140, 184, 124, 109.



Quantification See also under Amobarbital.

***Urine* GC–MS** For aprobarbital, *N*-hydroxyaprobarbital and aprobarbital diol, see Gilbert *et al.* [1978].

Disposition in the Body Absorbed after oral administration and slowly excreted in the urine, mainly as metabolites, <3% of a dose being excreted as

unchanged drug in 24 h. About 12% of a dose is excreted in the urine as the diol derivative and 4% as *N*-hydroxyaprobarbital in 3 days, together with 9% as unchanged drug.

Therapeutic Concentration In plasma, usually in the range 10 to 40 mg/L.

Toxicity The estimated minimum lethal dose is 2 g. Plasma concentrations greater than 40 mg/L are usually associated with toxic effects; concentrations greater than 50 mg/L may be fatal.

In 4 cases of death attributed to overdoses ranging from 2.5 to 7.7 g, antemortem serum concentrations ranged from 120 to 150 mg/L. In 44 cases of overdose in which the patients subsequently recovered, the serum concentrations 24 h after ingestion were in the range 40 to 130 mg/L [Lous 1954].

In a number of fatalities attributed to aprobarbital, a mean postmortem blood concentration of 50 mg/L in 9 cases and a mean postmortem liver concentration of 83 μg/g in 12 cases were reported [Bonnichsen *et al.* 1961].

Half-life Plasma half-life, 0.5 to 1.5 days.

Protein Binding 55 to 70%.

Dose 40 to 160 mg daily.

Bonnichsen R *et al.* (1961). Barbiturate analysis: method and statistical survey.. *J Forensic Sci* 6: 411–443.

Gilbert JN *et al.* (1978). The synthesis and urinary estimation of N-hydroxyaprobarbitone. *J Pharm Pharmacol* 30: 173–175.

Lous P (1954). Barbituric acid concentration in serum from patients with severe acute poisoning.. *Acta Pharmacol Toxicol* 10: 261–280.

Apronal

Hypnotic, Sedative

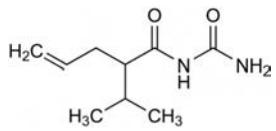
$C_9H_{16}N_2O_2$ = 184.2

CAS—528-92-7

IUPAC Name *N*-(Aminocarbonyl)-2-(1-methylethyl)-4-pentenamide

Synonyms Allylisopropylacetylurea; apronalide.

Proprietary Name *Sedormid*



Chemical Properties Colourless crystals or white crystalline powder. Mp 194°. Soluble 1 in 3000 of cold water, 1 in 210 of boiling water, 1 in 10 of ethanol, 1 in 45 of chloroform, and 1 in 75 of ether. Log *P* (octanol/water), 1.7.

Thin-layer Chromatography System TD—R_f 0.33; system TE—R_f 0.67; system TF—R_f 0.52 (acidified potassium permanganate solution, positive).

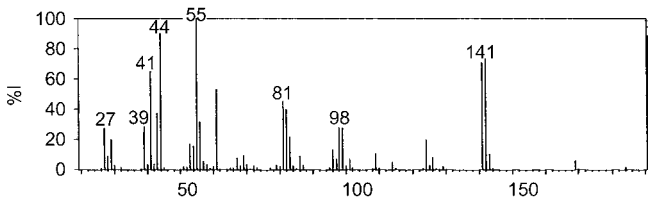
Gas Chromatography System GA—RI 1331.

High Performance Liquid Chromatography System HX—RI 396.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1676, 1704, 1092, 1620, 1185, 750 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 55, 44, 142, 141, 41, 61, 81, 82.



Dose Apronal was formerly given in doses of 250 to 750 mg daily.

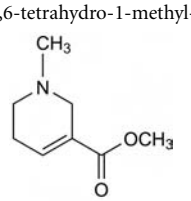
Arecoline

Purgative

$C_8H_{13}NO_2$ = 155.2

CAS—63-75-2

IUPAC Name Methyl-1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxylate



Chemical Properties Arecoline is an alkaloid which is obtained from the seeds of *Areca catechu* (Palmae). An oily liquid which is a strong base. Bp 209°. Miscible with water, ethanol and ether; soluble in chloroform. pK_a 7.4 (20°). Log *P* (octanol/water), 0.4.

Arecoline Hydrobromide

$C_8H_{13}NO_2$,HBr = 236.1

CAS—300-08-3

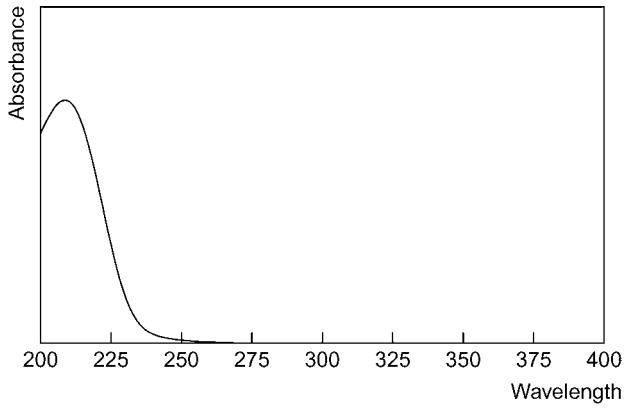
Chemical Properties A white crystalline powder. Unstable in light. Mp 169° to 171°. Soluble 1 in 1 of water and 1 in 10 of ethanol; slightly soluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.39; system TE—R_f 0.59; system TAE—R_f 0.41; system TAJ—R_f 0.28; system TAK—R_f 0.00; system TAL—R_f 0.28 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 1195.

High Performance Liquid Chromatography System HX—RI 79; system HAA—retention time 3.1 min.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1712, 1262, 1135, 1282, 1020, 1650 cm^{-1} (KCl disk).

Mass Spectrum Principal ions at *m/z* 155, 96, 140, 43, 42, 81, 94, 53.

Quantification

Plasma **GC–MS** Limit of detection, <1 μg/L [Hayes *et al.* 1989].

Biological Tissue **GC–MS** Limit of detection, <25 pmol for arecoline, acetylcholine and choline [Patterson, Kosh 1992].

Hayes MJ *et al.* (1989). Quantitative determination of arecoline in plasma by gas chromatography chemical ionization mass spectrometry. *Biomed Environ Mass Spectrom* 18: 1005–1009.

Patterson TA, Kosh JW (1992). Simultaneous quantitation of arecoline, acetylcholine, and choline in tissue using gas chromatography/electron impact mass spectrometry. *Biol Mass Spectrom* 21: 299–304.

Argatroban

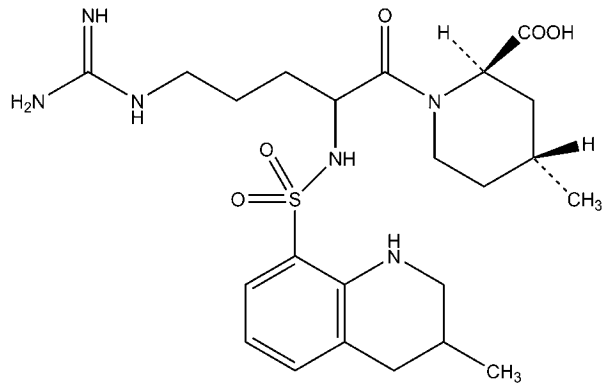
Antithrombotic

$C_{23}H_{36}N_6O_5S$ = 508.6

CAS—74863-84-6 (anhydrous)

IUPAC Name (2*R*,4*R*)-1-[(2*S*)-5-(Diaminomethylideneamino)-2-[(3-methyl-1,2,3,4-tetrahydroquinolin-8-yl)sulfonylamino]pentanoyl]-4-methylpiperidine-2-carboxylic acid hydrate

Synonyms (2*R*,4*R*)-1-[(2*S*)-5-[(Aminoiminomethyl)amino]-1-oxo-2-[[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]pentyl]-4-methyl-2-piperidinecarboxylic acid; argipidine; 1-[5-guanidino-2-[(3-methyl-1,2,3,4-tetrahydroquinolin-8-yl)sulfonylamino]pentanoyl]-4-methyl-piperidine-2-carboxylic acid; MQPA.



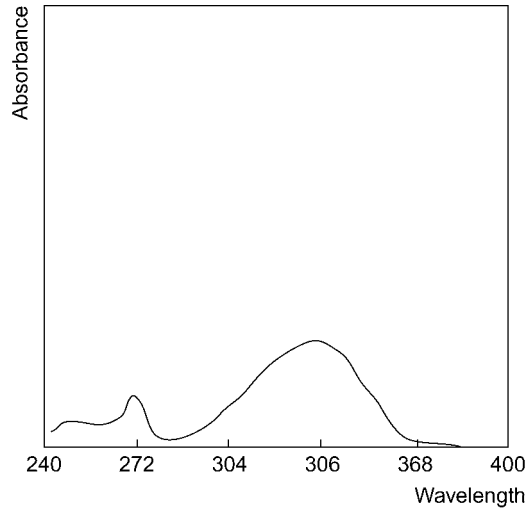
Chemical Properties Crystals. Mp 188° to 191°. Log *P* (octanol/water), 0.749 [Wishart 2006].

A

Argatroban Monohydrate

$C_{23}H_{36}N_6O_5S,H_2O = 526.7$
CAS—141396-28-3
Synonyms DK-7419; GN-1600; MCI-9038; MD-805; OM-805.
Proprietary Names *Novastan*; *Slonnon*.
Chemical Properties Crystals. Mp 176° to 180°.

Ultraviolet Spectrum Normal saline—333 nm.



Quantification

Plasma **HPLC** Column: Reversed phase C_{18}. Mobile phase: 1% TEA in water (pH 7.5 with 85% phosphoric acid) : acetonitrile (70 : 30), flow rate 1.0 mL/min. UV detection ($\lambda = 320$ nm). Limit of quantification, 0.4 mg/L [Ahmad *et al.* 1999].

Note For the enantiomeric separation of argatroban, see Rawson *et al.* [1993].

Disposition in the Body Extensively metabolised in the liver, mainly by hydroxylation and aromatisation of the 3-methyltetrahydroquinolone ring, to at least 4 metabolites. It appears to be metabolised by CYP3A4/5. The antithrombin potency of the major metabolite is about 30% that of the parent compound. It is excreted primarily in the faeces, via the bile as metabolites and as unchanged drug. Approximately 16% of a dose is excreted unchanged in the urine and 14% unchanged in faeces.

Therapeutic Concentration

A bolus dose of 125 µg/kg argatroban over 1 min followed by a 4 h continuous infusion of 2.5 µg/kg/min was administered to 20 young and 20 elderly volunteers. Mean steady-state argatroban plasma concentrations were as follows:

	Young		*Elderly*	
	Men	**Women**	**Men**	**Women**
Steady state concentration (µg/L)	541	473	657	511

In a separate study, 12 healthy adults were administered 2.5 µg/kg/min argatroban over 4 h and 12 patients with moderate hepatic impairment were administered 1.25 µg/kg/min over the same period. Mean steady-state plasma concentrations were 0.539 mg/L for the first group and 1.05 mg/L for the second [Swan, Hursting 2000].

Half-life In the range 40 to 50 min.
Volume of Distribution Steady state, approx. 0.2 L/kg.
Clearance Approximately 0.3 L/h/kg.
Protein Binding Approximately 54%.

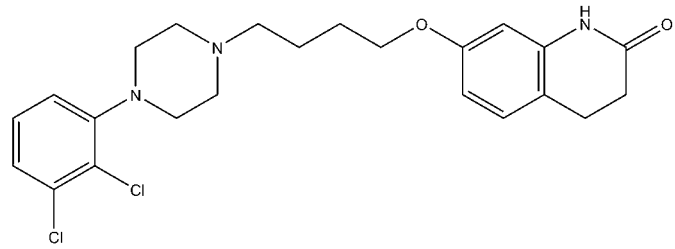
Dose In the management of heparin-induced thrombocytopenia, 2 to 10 µg/kg/min argatroban hydrochloride solution IV. In percutaneous coronary interventions in patients at risk of heparin-induced thrombocytopenia, 25 to 350 µg/kg/min IV over 3 to 5 min.

Ahmad S *et al.* (1999). Simultaneous monitoring of argatroban and its major metabolite using an HPLC method: potential clinical applications. *Clin Appl Thromb Hemost* 5: 252–258.
Rawson TE *et al.* (1993). Separation of 21-(*R*)- and 21-(*S*)-argatroban: solubility and activity of the individual diastereoisomers. *J Pharm Sci* 82: 672–673.
Swan SK, Hursting MJ (2000). The pharmacokinetics and pharmacodynamics of argatroban: effects of age, gender, and hepatic or renal dysfunction. *Pharmacotherapy* 20: 318–329.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

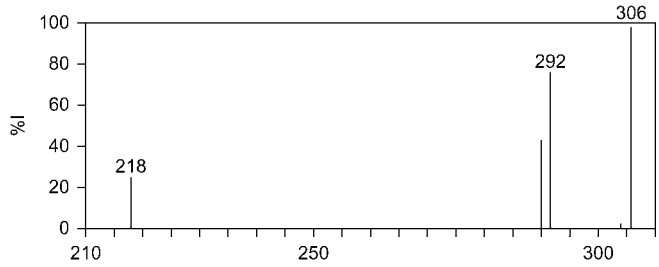
Aripiprazole

5-HT_{2A} Receptor Antagonist, 5-HT_1 Receptor Agonist, Dopamine D_2 Agonist, Antipsychotic, Dehydroquinoline
$C_{23}H_{27}Cl_2N_3O_2 = 448.4$
CAS—129722-12-9

IUPAC Name 7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]-3,4-dihydro-1*H*-quinolin-2-one
Synonyms Aripiprazol; 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril; 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro-2(1*H*)-quinolinone; OPC-14597; OPC-31.
Proprietary Names *Abilify*; *Arlemide*; *Azymol*; *Groven*; *Irazem*; *Real One*; *Siblix*; *Viza*.



Chemical Properties Colourless flake crystals. Mp 139° to 139.5° [O'Neil *et al.* 2006]. Insoluble in water.
Mass Spectrum Principal ions at *m/z* 306, 292, 280, 218 (TMS derivative).



Quantification

Plasma **GC-MS** Column: 5% phenyl polysilphenylene-siloxane (25 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 90° to 240° at 15°/min to 300° at 10°/min for 15 min. EI ionisation at 70 eV, scan acquisition mode. Retention time: aripiprazole 9.7 min, dehydroaripiprazole 9.8 min (both as TMS derivatives). Limit of quantification, 14.4 µg/L for aripiprazole, 6.9 µg/L for dehydroaripiprazole; limit of detection, 4.8 µg/L for aripiprazole, 2.3 µg/L for dehydroaripiprazole [Huang *et al.* 2007].

LC-MS Column: ODS-W (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% acetic acid in water : acetonitrile (65 : 35), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.1 µg/L [Kubo *et al.* 2005].

Serum **HPLC** Column: LiChrospher CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1.825 g/L dipotassium phosphate trihydrate (pH 6.4; 50 : 50), flow rate 1.2 mL/min. UV detection ($\lambda = 210$ nm). Retention time: approx. 17.8 min. Limit of quantification, 50 µg/L [Kirschbaum *et al.* 2005].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.0 min. Limit of quantification, 0.8 µg/L [Kirchherr, Kuhn-Velten 2006].

Other **HPLC** Rat Brain. Column: NovaPak phenyl (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : methanol : 20 mmol/L sodium sulfate : acetic acid (27 : 25 : 48 : 1), flow rate 1.0 mL/min. UV detection ($\lambda = 254$ nm). Limit of quantification, 30 ng/g [Shimokawa *et al.* 2005]. Rat plasma. Column: NovaPak phenyl (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : methanol : 20 mmol/L sodium sulfate : acetic acid (27 : 25 : 48 : 1), flow rate 1.0 mL/min. UV detection ($\lambda = 254$ nm). Limit of quantification, 10 µg/L [Shimokawa *et al.* 2005].

LC-MS *In vitro* Samples. Column: C_{18} (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: acetonitrile : 30 mmol/L ammonium acetate (62 : 38), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 50 ng/L; limit of detection, 5 ng/L [Li *et al.* 2007].

Disposition in the Body Aripiprazole is rapidly absorbed following oral administration, with peak plasma concentrations reached within 3 to 5 h. Steady-state plasma concentrations are achieved by day 14, with evidence of accumulation (mean AUC values of 10 or 15 mg/day are 4-fold greater on day 14 than on day 1). Aripiprazole has extensive extravascular distribution. It is extensively metabolised by CYP3A4 and CYP2D6 via *N*-dealkylation, hydroxylation or dehydrogenation pathways, with the formation of dehydroaripiprazole as the main systemic metabolite. At steady state, ≈40% of the plasma aripiprazole concentration is represented by the major metabolite, although it is unclear if dehydroaripiprazole is pharmacologically active. Aripiprazole is excreted via the kidney and liver, with 25% of the dose being recovered in urine (<1% unchanged) and 55% in the faeces (18% unchanged). Aripiprazole is distributed into breast milk.

Therapeutic Concentration

In a dose-ranging study of aripiprazole, groups of healthy volunteers were administered daily oral doses of either 5, 10, 15 or 20 mg in tablet form. Peak plasma concentrations were reported as follows:

Day	Parameter	Aripiprazole dose (mg/day)			
		5	10	15	20
1	C_{max} (μg/L)	27.3	38.9	70.8	65.6
	Time (h)	3.4	5.0	3.5	6.8
8	C_{max} (μg/L)	76.7	149	218	302
	Time (h)	3.5	3.5	4.7	4.6
14	C_{max} (μg/L)	98.2	163	242	393
	Time (h)	3.3	2.8	3.0	3.8

[Mallikaarjun *et al.* 2004].

In a study investigating the effects of concomitant administration of carbamazepine to an aripiprazole regimen, a group of chronically ill patients with schizophrenia or schizoaffective disorder were given 30 mg/day aripiprazole therapy for 14 days. Co-administration with carbamazepine was then started at 200 mg once daily for 3 days and then increased to 400 mg daily for 1 week. Plasma concentrations for both aripiprazole and its metabolite were reported as follows:

Parameter	Aripiprazole		Dehydroaripiprazole	
	Monotherapy	With carbamazepine	Monotherapy	With carbamazepine
C_{max} (μg/L)	519	176	118	39
Time (h)	3	3	3.5	5

[Citrome *et al.* 2007].

A 30-year-old lactating woman was administered 10 mg aripiprazole daily, which was increased to 15 mg on day 4. Aripiprazole concentrations in plasma and milk on day 15 were 71 and 13 μg/L, respectively [Schlotterbeck *et al.* 2007].

Note For a study on the effects of several types of co-medication on the serum levels of aripiprazole, see Castberg and Spigset [2007].

Toxicity As aripiprazole is a substrate for CYP3A4 and CYP2D6, it has potential for drug interactions, for example with fluoxetine, paroxetine and carbamazepine. It has also been reported that poor metabolisers of CYP2D6 substrates undergo an ~80% increase in aripiprazole plasma concentrations and a 30% decrease in dehydroaripiprazole plasma concentrations [Bristol-Myers Squibb, Otsuka America Pharmaceuticals 2007].

A 2.5-year-old girl ingested 13 tablets of aripiprazole 15 mg (17 mg/kg). Ten hours post-ingestion, the serum concentration of aripiprazole was 1.42 mg/L and of dehydroaripiprazole 0.45 mg/L. At 57.25 h post-ingestion, the concentrations were 0.34 and 0.30 mg/L, respectively. The child was discharged 7 days later with no apparent sequelae [Seifert *et al.* 2005].

A 3-year-old boy ingested one half of a 15 mg tablet of aripiprazole. The serum aripiprazole concentration 87 h post-ingestion was 63 μg/L. The peak serum concentration was estimated at 136 μg/L [Schonberger *et al.* 2004].

A 27-year-old suicidal woman ingested 22 tablets of aripiprazole 15 mg, one tablet of cyclobenzaprine 10 mg, and one tablet of quetiapine 25 mg. Approximately 3.5 h post-ingestion, the serum concentrations of aripiprazole and dehydroaripiprazole were 0.60 and 0.12 mg/L, respectively [Carstairs, Williams 2005].

Bioavailability Approximately 87%.

Half-life Reported as between 47 to 68 h and also 75 to 146 h (depending on CYP2D6 phenotype).

Volume of Distribution Approximately 4.9 L/kg.

Clearance Oral, 3.3 to 4.0 L/h.

Protein Binding 88 to 99%, primarily to albumin.

Note For more information on the pharmacokinetic variability of aripiprazole, see Molden *et al.* [2006].

Dose For the treatment of schizophrenia, given by mouth in an initial dose of 10 or 15 mg once daily. The usual maintenance dose is 15 mg once daily although the dose may be adjusted at intervals of not less than 2 weeks up to a maximum of 30 mg daily. In the USA, aripiprazole is also used for the treatment of mania associated with bipolar disorder; it is given orally in an initial dose of 30 mg once daily, this may subsequently be decreased to 15 mg once daily according to tolerance.

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Arsenic

Metal

As = 74.9

CAS—7440-38-2

Synonyms Arsenic black; colloidal arsenic; grey arsenic.

Chemical Properties Odourless grey solid. Mp 817° (at 28 atm). Bp 613°. Insoluble in water, soluble in nitric acid. Urine samples stored at room temperature had low (58 to 75%) recoveries of As^3, dimethylarsinic acid (DMA), and As^5. When stored at 4° or -20° the species were better recovered (87 to 100%) with the urine samples stored at -20° having a better recovery of As^3 (98.9%) compared with those stored at 4° (87%) [Morton, Mason 2006]. Valency: As (−3), As (0), As (+3) and As (+5). As(0) occurs as two allotropic forms, yellow and metallic grey. The metallic grey form is the stable form under ordinary conditions. It occurs most often as the sulfide in a variety of complex minerals containing copper, lead iron, nickel, cobalt, and other metals. It is widely distributed in the biosphere and is present in soil, water, and food. Non-toxic organic arsenic compounds are present in fish and shellfish. Human poisoning from contaminated well-water supplies is widespread in parts of India, Bangladesh and Vietnam.

Uses include the production of non-ferrous alloys, principally lead alloys used in lead–acid batteries. It may be added to alloys for bearing, type metal, lead ammunition, and automotive body solder. It can be added to brasses to improve corrosion resistance. High-purity arsenic is used in the manufacture of gallium arsenide and other intermetallic compounds used in semiconductors.

Arsenic Acid

AsH_3O_4 = 151

CAS—7778-39-4

Synonym Orthoarsenic acid

Proprietary Names *Desican L-10*; *Scorch*.

Chemical Properties White solid. Mp 35.5°, loses water at 160°. Soluble in water and alcohol. Used in herbicides, as a defoliant in cotton plants.

Arsenic Pentoxide

As_2O_5 = 229.8

CAS—1303-28-2

Synonyms Arsenic acid anhydride; arsenic (V) oxide; diarsenic pentoxide.

Chemical Properties White solid. Mp 315° with decomposition. Soluble in water, alcohol and acid. Used as a wood preservative.

Arsenic Trioxide

As_2O_3 = 197.8

CAS—1327-53-3

Synonyms Arsenic oxide; arsenious acid; arsenious oxide; white arsenic.

Proprietary Names *Arsenolite*; *Claudelite*; *Trisenox*.

Chemical Properties Odourless white solid. Mp 312.3°. Bp 465°. Soluble in water, hydrogen chloride, slightly soluble in alcohol. Used as a antineoplastic; wood preservative.

Calcium Arsenate

$Ca_3(AsO_4)_2$ = 398.1

CAS—7778-44-1

Synonyms Arsenic acid calcium salt; calcium orthoarsenate.

Proprietary Names *Pencal*; *Spra-cal*.

A

Chemical Properties Odourless, colourless solid. Slightly soluble in water, soluble in acids, insoluble in organic solvents. Formerly used in pesticides and herbicides.

Gallium Arsenide
GaAs = 144.6
CAS—1303-00-0
Synonym Gallium monoarsenide
Chemical Properties Dark grey solid. Mp 1238°. Used in semiconductor applications including solar cells; light-emitting diodes; lasers and integrated circuits.

Sodium Arsenate
Na_2HAsO_4 = 185.9
CAS—7778-43-0
Synonyms Disodium arsenate; disodium hydrogen arsenate.
Chemical Properties Odourless solid. Soluble in water and glycerol; slightly soluble in alcohol. Used in pesticides.

Sodium Arsenite
$NaAsO_2$ = 129.9
CAS—7784-46-5
Synonyms Arsenious acid, sodium salt; sodium metaarsenite.
Proprietary Names *Atlas A*; *Chem Sen*; *Kill-All*.
Chemical Properties Grey-white solid. Very soluble in water; slightly soluble in alcohol. Formerly used in herbicides and to debark trees.

Arsanilic Acid
$(C_6H_4NH_2)H_2AsO_3$ = 217.1
CAS—98-50-0
Synonyms 4-(Aminophenyl)arsenic acid; atoxylic acid.
Proprietary Names *Premix*; *Pro Gen*.
Chemical Properties Practically ordourless white solid. Mp 232°. Very soluble in hot water, soluble in alcohol, insoluble in ether, slightly soluble in acetic acid. Used in the manufacture of medicinal arsenicals; veterinary medicine: as a growth promoter, to improve feed efficiency, and to control swine dysentery.

Arsenobetaine
$(CH_3)_3As^+CH_2CO_2^-$ = 178.1
CAS—64436-13-1
Synonym Fish arsenic
Chemical Properties Solid. Mp 204 to 210°. Soluble in alcohol.

Dimethylarsinic Acid
$(CH_3)_2As(O)OH$ = 138.0
CAS—75-60-5
Synonyms Cacodylic acid; DMA; DMAA; hydroxydimethylarsine oxide.
Proprietary Names *Ansar*; *Arsan*; *Silvisar*; *Phytar*.
Chemical Properties Odourless. Colourless solid. Mp 195° to 196°. Very soluble in water, alcohol and acetic acid. pK_{a1} 1.3, pK_{a2} 6.2 [Sur, Dunemann 2004]. Used as a dermatologic agent; tonic (vet.).

Disodium Methanearsonate
$CH_3Na_2AsO_3$ = 183.9
CAS—144-21-8
Synonyms Disodium monomethane arsonate; DSMA.
Proprietary Names *Ansar 8100*; *Arrhenal*; *Arsinyl*; *Clout*; *Crab-E-Rad*; *Dal-E-Rad*; *Metar*; *Sodar*.
Chemical Properties Colourless solid. Mp >355°. Very soluble in water, slightly soluble in alcohol. Used as a herbicide.

Methanearsonic Acid
$CH_3H_2AsO_3$ = 134.0
CAS—124-58-3
Synonyms Arsonic acid, methyl-; MA; MMA; monomethylarsonic acid.
Chemical Properties White solid. Pleasant acid taste. Mp 161°. Soluble in water and alcohol. pK_{a1} 3.6, pK_{a2} 8.2 [Sur, Dunemann 2004]. Used as a herbicide.

Roxarsone
$C_6H_6AsNO_6$ = 263.1
CAS—121-19-7
Synonyms 4-Hydroxy-3-nitrophenylarsonic acid; NSC-2101; nitrophenolarsonic acid.
Proprietary Name *Ren-o-sal*
Chemical Properties Pale yellow solid. Puffs up and deflagrates on heating. Slightly soluble in cold water; soluble in approx. 30 parts boiling water; freely soluble in methanol, ethanol, acetic acid, acetone and alkalis; sparingly soluble in dilute mineral acids. Insoluble in ether, ethyl acetate. Used as an antibacterial.

Sodium Arsanilate
$C_6H_7AsNNaO_3$ = 239.1
CAS—127-85-5
Synonyms Arsanilic acid sodium salt; sodium aminarsonate; sodium anilarsonate.
Proprietary Names *(4-Aminophenyl)-arsonic acid sodium salt*; *Arsamin*; *Atoxyl*; *Nuarsol*; *Protoxyl*; *Soamin*; *Sonate*; *Piglet Pro-Gen V*; *Trypoxyl*.
Chemical Properties The tetrahydrate is a white odourless crystalline powder. Soluble in water and alcohol. Formerly used as an antisyphilitic.

Sodium Cacodylate
$C_2H_6AsNaO_2$ = 160.0
CAS—124-65-2
Synonyms [(Dimethylarsino)oxy]sodium As-oxide; sodium dimethylarsonate.
Proprietary Names *Arsecodile*; *Arsicodile*; *Arsycodile*; *Rad-e-cate*; *Silvisar*.
Chemical Properties The trihydrate is a granular solid with a slight odour. Liquifies at approx. 60° becoming anhydrous at 120°. Burns with a bluish flame, emitting an odour similar to garlic. Soluble in water and alcohol. Used as a herbicide; in treatment of chronic anaemia, eczema, and as a tonic (vet.).

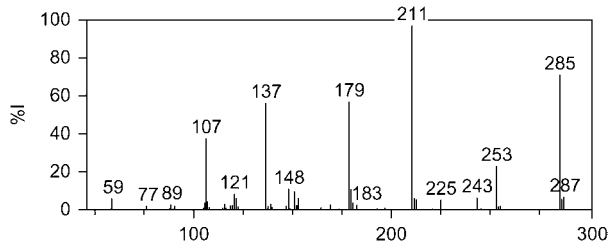
Sodium Methanearsonate
$CH_3NaHAsO_3$ = 162.0
CAS—2163-80-6
Synonyms Monosodium methanearsenate; MSMA.
Proprietary Names *Bueno*; *Daconate*; *Phybane*.
Chemical Properties Solid. Mp 115° to 119°. Used as a herbicide.

Colour Test Reinsch test (applicable to urine, gastric contents, scene residues)—arsenic imparts black stain to copper (antimony, bismuth, selenium and tellurium also give black deposits).

Clean a 5 × 10 mm square of copper foil (2–3 cm copper wire) with aqueous nitric acid (500 mL/L) until shiny. Rinse the copper with purified water, add 10 mL of concentrated hydrochloric acid and 20 mL of test sample in a 100 mL conical flask. Heat on a boiling water bath in a fume cupboard for 1 h. Cool and gently wash the copper with purified water. Confirmatory Test: place the stained copper in 100 g/L potassium cyanide solution and leave for 10 min. Arsenic stains dissolve in the cyanide solution (stains due to antimony or bismuth remain). Limit of detection, 5 mg/L.

Note For reference values for arsenic in the German population, see Wilhelm *et al.* [2004].

Mass Spectrum Principal ions at *m/z* 75; 211, 285, 179, 137, 107, 253 (thioglycolic acid methyl ester derivative of As^3).



Quantification Specimen collection: Blood—10 mL, K-EDTA tube; urine—20 mL plastic universal container.

Blood **AAS** Perkin-Elmer 3300. Arsenic hollow cathode lamp (λ = 193.7 nm). Limit of detection, 1.0–2.0 µg/L [Alauddin *et al.* 2003]. Limit of detection, 1.95 ng [Concha *et al.* 1998]. GBC hollow cathode lamp (λ = 193.7 nm). Carrier gas: N_2, 200 mL/min. Limit of detection, 0.02 µg/L [Tripathi *et al.* 1997]. Perkin-Elmer 5000 (λ = 193.7). Arsenic hollow cathode lamp. Limit of detection, 0.5 µg/L [Foà *et al.* 1984].

FAAS Flame: air-acetylene. Carrier gas: N_2, 90 mL/min. Hollow cathode lamp (λ = 193.7 nm). Limit of detection not reported [Le *et al.* 1992].

ETAAS Carrier gas: Ar. Dry cycle: 200° at 35 s for 30 s. Char cycle: 1600° at 20 s for 15 s. Atomisation cycle: 2500° for 4 s, gas stop. Limit of detection, 2 µg/L [Campillo *et al.* 2000].

HPLC-AAS Column: Phenomenex Bondclone (300 × 3.9 mm i.d., 10 µm). Arsenic hollow cathode lamp (λ = 193.7 nm). Limit of detection, 10 µg/L for arsenobetaine, DMA, and arsenite; 15 µg/L for MMA; and 20 µg/L for arsenate [Le *et al.* 1994].

HPLC-ICP-MS Column: Inertsil AS (150 × 2.1 mm i.d., 3.0 mm). Mobile phase: 10 mmol/L sodium butanesulfate : 4 mmol/L tetramethyl ammonium hydroxide : 4 mmol/L malonic acid : 0.05% methanol (pH 3.0), flow rate 0.2 mL/min. Limit of quantification, 0.1 µg/L for As^3, As^5 and the methylated metabolites [Fujisawa *et al.* 2007]. Column: Phenomenex Bondclone (300 × 3.9 mm i.d., 10 µm). Mobile phase: 50 mmol/L phosphate or carbonate buffer (pH 7.5, 9.0, or 10.3), flow rate 1.0 mL/min. Outer gas: 13.8 L/min. Auxiliary gas: 0.70 L/min. Nebuliser gas: 0.96 L/min. Meinhard nebuliser. Limit of detection not reported [Le *et al.* 1994].

ICP-MS Limit of detection, 0.97 µg/L [Liao *et al.* 2004]. Coolant gas: 16 L/min. Auxiliary gas: 1.4 L/min. Nebuliser gas: 0.7 L/min. Limit of quantification, 5 µg/L for DMA, arsenocholine, and As^5; 4 µg/L for arsenobetaine; 0.8 µg/L for As^3; and 0.6 µg/L for MMA. Limit of detection, 2 µg/L for DMA and As^5, 1 µg/L for arsenobetaine and arsenocholine, and 0.2 µg/L for As^3 and MMA [Milstein *et al.* 2003]. Plasma gas: 15 L/min. Auxiliary gas: 1.0 L/min. Method of detection, 30, 45, 27, and 61 ng/L for AsB, DMA, MMA, and As^5, respectively [Wei *et al.* 2000]. Plasma gas: Ar, 16 L/min. Auxiliary gas: 1.4 L/min. Carrier gas: 0.5 L/min. Limit of detection not reported [Tanaka *et al.* 1996].

Note For spectrophotometric methods for the detection of arsenic in blood, see Pillai *et al.* [2000] and Lakso *et al.* [1979].

Serum **ETAAS** See Blood [Campillo *et al.* 2000].

ICP-MS Column: Inertsil AS (150 × 2.1 mm i.d., 3 µm). Mobile phase: 10 mmol/L butane sulfonic sodium : 4 mmol/L malonic acid: 4 mmol/L tetramethylammonium hydroxide (pH 3.0), flow rate 0.2 mL/min. Plasma gas: Ar, 18 L/min. Limit of detection,not reported [Fukai *et al.* 2006].

Urine **LC-MS** Column: weak anion exchange. Mobile phase: methanol ammonium dihydrogen phosphate : ammonium acetate : glacial acetic acid. Limit of

detection, 0.5 µg/L for As^3 and As^5, 2.0 µg/L for MMA, 1.5 µg/L for DMA, and 1.0 µg/L for arsenobetaine [Apostoli *et al.* 1999].

AAS Perkin-Elmer MHS-20. Varian AA 1275. Limit of detection, 1 µg/L (approx. 1 ng) [Heilier *et al.* 2005]. Phillips AAS-PU-9100 ($\lambda = 193.7$ nm). Arsenic hollow cathode lamp. Limit of detection, 0.5 µg/L [Dang *et al.* 1999]. Limit of detection, 1.95 ng [Concha *et al.* 1998]. Carrier gas: Ar, 200 mL/min. Dry cycle: 50° to 80° for 10 s to 120° for 10 s. Char cycle: 120° to 400° for 20 s. Atomisation cycle: 2600° for 5 s, gas stop. Limit of detection not reported [Das *et al.* 1995]. See Blood [Foà *et al.* 1984].

HPLC-AFS Column: Hamilton PRP-X100 anion exchange (250 × 4.1 mm i.d., 10 µm). Mobile phase: 10 mmol/L ammonium dihydrogen phosphate (pH 5.8) : 60 mmol/L ammonium dihydrogen phosphate (pH 5.8, 100 : 0 for 2 min to 0 : 100 at 2 min for 4 min to 100 : 0 for 6 min), flow rate 1.0 mL/min. Retention time: 2.6, 3.6, 6.4 and 8.8 min for As^3, DMA, MA, and As^5, respectively. Arsenic-boosted discharge hollow cathode super lamp ($\lambda = 197.3$, 193.7 and 189.0 nm). Carrier gas: Ar, 0.25 L/min. Drying gas: N_2, 2.5 L/min. Limit of quantification, 10, 11, 6, and 6 µg/L for As^3, DMA, MMA, and As^5, respectively. Limit of detection, 3, 4, 2, and 2 µg/L for As^3, DMA, MMA, and As^5, respectively [Lindberg *et al.* 2007].

FAAS See Blood [Le *et al.* 1992].

ETAAS Dry cycle: 130° at 20 s for 10 s. Char cycle: 1400° in 5 s for 30 s. Atomisation cycle: 2500° for 5 s. Limit of detection, 0.03 µg/L [Horng *et al.* 2002]. See Blood [Campillo *et al.* 2000]. Limit of detection, 0.5 µg/L [Gebel *et al.* 1998].

ICP-MS See Serum [Fukai *et al.* 2006]. Limit of quantification, 0.18 µg/L; limit of detection, 0.05 µg/L [Brima *et al.* 2006], 9.98 µg/L [Liao *et al.* 2004]. Coolant gas: 16 L/min. Auxiliary gas: 1.4 L/min. Nebuliser gas: 0.7 L/min. Limit of quantification, 5 µg/L for DMA, arsenocholine and As^5; 4 µg/L for arsenobetaine; 0.8 µg/L for As^3; 0.6 µg/L for MMA. Limit of detection, 2 µg/L for DMA and As^5; 1 µg/L for arsenobetaine and arsenocholine; 0.2 µg/L for As^3 and MMA [Milstein *et al.* 2003]. Plasma gas: 15 L/min. Auxiliary gas: 1.0 L/min. Limit of detection, 30, 45, 27 and 61 ppt for AsB, DMA, MMA, and As^5, respectively [Wei *et al.* 2000]. See also Apostoli *et al.* [1999], Amarasiriwardena *et al.* [1998] and Alves *et al.* [1993].

GC-ICP-MS Limit of detection, 2–12 pg/L [Kresimon *et al.* 2001].

HPLC-ICP-MS Column: Inertsil AS (150 × 2.1 mm i.d., 3.0 mm). Mobile phase: 10 mmol/L sodium butanesulfate : 4 mmol/L tetramethyl ammonium hydroxide : 4 mmol/L malonic acid : 0.05% methanol (pH 3.0), flow rate 0.2 mL/min. Limit of quantification, 0.1 ppb for As^3, As^5, and the methylated metabolites [Fujisawa *et al.* 2007]. Column: Hamilton PRP-X100 (250 × 4.1 mm i.d., 10 µm). Mobile phase: 10 mmol/L ammonium carbonate in 2% methanol (pH 9.0) : 50 mmol/L ammonium carbonate in 2% methanol (pH 9.0; 100 : 0 for 0.5 min to 0 : 100 at 0.5 min for 12.5 min), flow rate 1.0 mL/min. Plasma gas: 15 L/min. Auxiliary gas: 1.1 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, 0.003, 0.01, 0.004, 0.003 and 0.002 µg/L for arsenobetaine, As^3, DMA, MMA and As^5, respectively [Wang *et al.* 2007]. Column: Shodex RSpak NN-614 cation exchange (150 × 4.6 mm i.d.) Mobile phase: 5 mmol/L nitric acid : 6 mmol/L ammonium nitrate : 1.5 mmol/L 2, 6-pyridinedicarboxylic acid, flow rate 1.0 mL/min. Limit of detection, 0.1 µg/L [Hata *et al.* 2007]. Column: Hamilton PRPX100 spherical poly(styrene-divinyl-benzene) trimethylammonium exchanger (250 × 4.1 mm i.d.). Mobile phase: 10 mmol/L ammonium carbonate : 20 mmol/L ammonium carbonate (100 : 0 for 5 min to 0 : 100 at 6 min for 16 min to 100 : 0 at 23 min for 4 min), flow rate 1 mL/min. Nebuliser gas: 0.85 L/min. Limit of detection, 0.8 µg/L [Morton, Mason 2006]. Limit of detection, 0.3 µg/L for As^3, MMA, and DMA; 0.4 µg/L for As^5 [Christian *et al.* 2006]. See also Chowdhury *et al.* [2003]; Kavanagh *et al.* [1998]; Lai *et al.* [2004]; Le *et al.* [1994]; Mandal *et al.* [2003]; Meza *et al.* [2004]; Sur, Dunemann [2004]; Zheng *et al.* [1999].

Note For spectrophotometric methods for the detection of arsenic in urine, see Lakso *et al.* [1979] and Kneip *et al.* [1977].

Hair **AAS** See Urine [Das *et al.* 1995]. Perkin-Elmer 403 ($\lambda = 193.7$ nm). Carrier gas: Ar. Fuel: H_2. Oxidant: Air. Limit of detection, 0.02 µg [Curatola *et al.* 1978].

ETAAS Dry cycle: 80° to 120° in 10 s. Char cycle: 300° to 400° at 10 s. Atomisation cycle: 2700° to 2800° in 5 s. Carrier gas: 200 mL/min. Hitachi model 180-50, S.N.5721-2 ($\lambda = 193.8$ nm). Limit of detection not reported [Kazi *et al.* 2006]. See Urine. Limit of detection, 5 µg/kg [Gebel *et al.* 1998]. Dry cycle: 140° at 5 s for 15 s. Char cycle: 1300° in 15 s for 30 s. Atomisation cycle: 2700° for 5 s. Perkin-Elmer 5000 ($\lambda = 193.7$ nm). Limit of detection, 10 pg [Koons and Peters 1994].

ICP-MS Plasma gas: Ar, 13 L/min. Auxiliary gas: Ar, 0.9 L/min. Nebuliser gas: Ar, 0.95 L/min. Limit of quantification, 0.036 µg/kg [Kintz *et al.* 2007]. Limit of detection, 0.13 mg/kg [Nadal *et al.* 2005]. Plasma gas: 15 L/min. Auxiliary gas: 0.8 L/min. Nebuliser gas: 0.8 L/min. Limit of detection not reported [Samanta *et al.* 2004].

HPLC-ICP-MS Column: RP-C_{18} (100 × 4.6 mm i.d.). Mobile phase: 0.35 mmol/L tetrabutylammonium hydrogen sulfate (pH 5.75), flow rate 1 mL/min. Carrier gas: Ar, 0.75 L/min. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Limit of detection not reported [Yáñez *et al.* 2005]. Column: Shodex Asahipak ES-502N 7C (100 × 7.6 mm i.d.). Mobile phase: 15 mmol/L citric acid monohydrate (pH 2.0), flow rate 1.0 mL/min. Plasma gas: 15 L/min. Auxiliary gas: 1.2 L/min. Limit of detection not reported [Mandal *et al.* 2003].

Liver **AAS** See Urine [Das *et al.* 1995].

Nail **AAS** See Urine [Das *et al.* 1995].

ICP-MS See Hair [Samanta *et al.* 2004]. Perkin-Elmer Sciex ELAN 5000. Plasma gas: Ar. Limit of detection, 0.07 µg/L [Chen *et al.* 1999].

HPLC-ICP-MS Column: Shodex Asahipak ES-502N 7C (100 × 7.6 mm i.d.). Mobile phase: 15 mmol/L citric acid monohydrate (pH 2.0), flow rate 1.0 mL/min. Plasma gas: 15 L/min. Auxiliary gas: 1.2 L/min. Limit of detection not reported [Mandal *et al.* 2003].

Milk **AAS** See Urine [Concha *et al.* 1998].

ICP-MS Plasma gas: 12 to 13 L/min. Auxiliary gas: 0.9 to 1.0 L/min. Sample gas: 1.0 to 1.2 L/min. Limit of detection, 0.2 µg/L [Krachler *et al.* 2000].

Other **GC-MS** Water. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 75° for 0.5 min to 280° at 30°/min for 1 min. Carrier gas: He, 0.75 mL/min. EI ionisation at 70 eV. Limit of detection, 0.1 µg/L for DMA and MMA, 3 µg/L for total inorganic arsenic [Claussen 1997].

AAS Fresh and Freeze-dried Plant Ssamples. Carrier gas: Ar, 50 mL/min. Atomisation cycle: 770°. Hollow cathode lamp ($\lambda = 193.7$ nm). Limit of detection not reported [Krachler, Emons 2000]. Seafood Products. Perkin-Elmer 5000. Limit of detection, 0.7 µg/kg [Muñoz *et al.* 1999]. Water. See Urine [Das *et al.* 1995].

ETAAS Naphtha. Dry cycle: 50° for 10 s to 90° in 25 s to 120° in 10 s, Ar, 200 mL/min. Atomisation cycle: 2500° for 8 s (gas stop; $\lambda = 193.7$ nm). Limit of detection, 2.7 µg/L [Cassella *et al.* 2004].

ICP-MS Seafood. Perkin-Elmer Elan 6000. Limit of detection, 0.05 mg/kg [Falcó *et al.* 2006]. Infant Food Products. Coolant gas: Ar, 12 L/min. Auxiliary gas: Ar, 1.0 L/min. Nebuliser gas: 0.8 L/min. Limit of detection, 5 µg/kg for As^3, MMA and DMA; 10 µg/kg for As^5 [Vela, Heitkemper 2004]. Food. Varian-Vista with an ultrasonic nebuliser. Limit of detection, 0.06 mg/kg [Llobet *et al.* 2003]. Meals from a Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 16 µg/kg [Noel *et al.* 2003]. Vegetables. Limit of detection, 0.04 µg/L [Alam *et al.* 2003]. Well Water. Limit of detection, 3 µg/L [Rahman *et al.* 2001]. Beverages. Limit of detection, 0.3 µg/kg [MacIntosh *et al.* 2000]. Method 200.8 of the EPA. Limit of detection, 1 µg/L [McCarty *et al.* 2004].

HPLC-ICP-MS Rice. Column: CAPCELL PAK C_{18}MG (250 × 4.6 mm i.d.). Mobile phase: 10 mmol/L sodium 1-butanesulfonate : 4 mmol/L tetramethylammonium hydroxide : 4 mmol/L malonic acid : 0.05% methanol (pH 3.0), flow rate 0.75 mL/min. Plasma gas: 15 L/min. Carrier gas: 0.70 L/min. Auxiliary gas: 0.9 L/min. Makeup gas: 0.43 L/min. Limit of quantification, 7.9 µg/kg; limit of detection, 2.4 µg/kg [Hamano-Nagaoka *et al.* 2008]. Drinking Water. Column: Agilent (250 × 4.6 mm i.d., 5 µm). Mobile phase: 2.0 mmol/L sodium dihydrogen phosphate : 0.2 mmol/L EDTA (pH 6.0), flow rate 1.0 mL/min. Plasma gas: Ar, 15 L/min. Auxiliary gas: 1.0 L/min. Carrier gas: 1.0 L/min. Limit of detection, 67, 74, 35 and 89 ng/L for As^3, DMA, MMA and As^5, respectively [Day *et al.* 2002].

Note For colourimetric assays for arsenic, see George *et al.* [1973] and Crawford and Tavares [1974]. For a study of arsenic in soils, plants, water and sediments in Korea, see [Jung *et al.* [2002].

Disposition in the Body

Absorption **Inhalation exposure** Arsenic absorption via the lungs is a two-step process: deposition of the particles on to the lung surface, and absorption of arsenic from the deposited material. In patients with lung cancer exposed to arsenic in cigarette smoke, deposition was estimated to be approx. 40% and absorption 75 to 85%. Thus, overall absorption was approx. 30 to 34%. In workers exposed to arsenic trioxide dusts in smelters, the amount excreted in the urine was approx. 40 to 60% of the inhaled dose.

Oral exposure Arsenates and arsenites are well absorbed from the gastrointestinal tract. In one study where faecal excretion was measured in humans given oral doses of arsenite, less than 5% was recovered. This indicates absorption was at least 95%. This is supported in studies where urinary excretion in humans was found to account for 55 to 80% of daily oral intakes of arsenate or arsenite. However, gastrointestinal absorption may be lower if highly insoluble forms or arsenic are ingested.

Dermal exposure Studies investigating percutaneous absoption of ^{73}As as arsenic acid alone and mixed with soil has been measured in skin from cadavers. Labelled arsenic was applied to skin in diffusion cells and transit through the skin into receptor fluid measured. After 24 h, 0.93% of the dose passed through the skin and 0.98% remained in the skin after washing. Absorption was lower with ^{73}As mixed with soil.

Arsenates and arsenites are well absorbed following both oral and inhalation exposure. Data on distribution are limited but it appears arsenic is transported to nearly all tissues with the highest concentrations found in muscle. Metabolism involves mainly reduction-oxidation reactions that interconvert $As^{+5}$and As^{+3}; and methylation of As^{+3} to form MA and DMA. Most arsenic is rapidly excreted in urine as a mixture of inorganic arsenics, MA and DMA, although some may remain bound in tissues (especially skin, hair and fingernails). The capacity of the body to methylate inorganic arsenic is very important in terms of detoxification. Limited data suggest that the methylation system might begin to become saturated at intakes of approx. 0.2 to 1 mg per day. Urinary excretion is complete within 6 days and accounts for approx. 90% of the dose. Trivalent arsenic salts are excreted as DMA (50%), MA (14%), As(+5) (8%), and As(+3) (8%). Organo-arsenic compounds (e.g. arsenobetaine, arsenochlorine), which are present in various types of seafood, are excreted unchanged.

Normal Concentrations Blood—<10 µg/L (0.1 µmol/L); urine—<10 µg/L (0.1 µmol/L); hair—<1 µg/g (0.01 µmol/L).

Therapeutic Concentration

A group of 12 Japanese patients with relapsed or refractory acute promyelocytic leukaemia (APL) were treated with arsenic trioxide at a daily dose of 15 mg/kg. As^3 and As^5 reached mean peak plasma concentrations of

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12.4 ± 8.4 and 10.2 ± 3.9 µg/L, respectively, attained at 1.9 ± 0.7 and 1.8 ± 0.9 h, respectively [Fujisawa *et al.* 2007].

A 72-year-old Japanese male with APL was treated with arsenic trioxide IV at a daily dose of 0.08 mg/kg. Concentrations of As^3, MMA and DMA in serum ranged from 18 to 41 µg/L during 24 h on day 4. The concentration of total arsenic in urine on day 4 was 4.46 mg [Fukai *et al.* 2006].

Toxicity Chronic arsenic poisoning is linked to lung and skin cancers, neurological disturbances, and cardiovascular abnormalities. Urine arsenic concentrations of 78 to 220 µg/L have been associated with chronic toxicity (equivalent to exposure to 50 µg/m^3) in workers [Apostoli *et al.* 1999]. Hair arsenic concentrations in chronically poisoned subjects range from 1 to 5 µg/g and can be as high as 47 µg/g [Hindmarsh *et al.* 1977], but there are difficulties in distinguishing between endogenous arsenic and external contamination [Hindmarsh 2002]. Trivalent salts of arsenic, such as sodium arsenate ($NaAsO_2$), cause serious toxicity and death after ingestion of as little as 200 mg. Lethal acute doses lead to convulsions, coma and death from respiratory failure. More moderate acute doses cause symptoms of gastro-enteritis with vomiting and bloody diarrhoea. Exposure to arsine gas (AsH_3) can result in massive haemolysis and renal failure.

A 22-month-old boy ingested an insecticide ant bait gel containing arsenic trioxide. His serum arsenic concentration was 1.7 mg/L 5 h post ingestion. Urinary concentrations at a number of times post ingestion were as follows:

Time post-ingestion (h)	*Urinary As (µg/L)*	*Cumulative As excreted (µg)*	*Urine volume (L)*
6.7	2085	876	0.42
12	583	2028	1.98
36	308	2762	2.38
60	229	3495	3.2
88	372	4027	1.43
110	201	4397	1.84
131	83	4499	1.23
162	46	ND	ND

ND, not determined [Stephanopoulos *et al.* 2002].

A 26-year-old man was admitted to hospital with multiple organ dysfunction. A screen for heavy metals on day 4 revealed a urine arsenic concentration of 867 µg/L. It was estimated that the patient's wife had administered a dose of 10 g As_2O_3 over the course of the 2 weeks prior to admission [Hantson *et al.* 2003].

A 41-year-old woman ingested 5 g of trivalent arsenic. Upon admission to hospital (approx. 4 h post-ingestion) the concentration of arsenic in her urine was 3.7 mg/L. The following day the urine arsenic concentration was 11.3 mg/24 h. The concentration of arsenic was measured in each 2 L of gastric effluent. Two days following intoxication, the arsenic concentration was 2.51 mg/L in the first 2 L collection of gastric effluent. Thereafter, there was an exponential decrease in the arsenic content [Michaux *et al.* 2000].

A 28-year-old man died following the ingestion of approx. 8 g of arsenic trioxide. Concentrations of total arsenic and arsenic species in his organs and blood were as follows:

Organ	*Total arsenic and arsenic species (mg/kg dry weight)*				
	As^3	*DMA*	*MMA*	*As^5*	*Total*
Brain	4.41	1.10	2.65	<LoQ	8.33
Cerebellum	5.15	1.60	3.76	<LoQ	11.0
Haemolysed blood	0.224	0.0539	0.135	0.009	0.422
Heart	9.05	0.64	1.61	<LoQ	11.8
Kidneys	20.0	1.60	4.52	0.53	26.6
Liver	122.0	5.91	14.7	2.94	147
Lung	9.46	0.45	1.11	<LoQ	11.1
Muscle	9.17	0.73	1.96	<LoQ	12.3
Pancreas	9.18	0.45	1.34	<LoQ	11.2
Skin	1.62	0.34	0.91	<LoQ	2.9
Spleen	9.50	0.598	1.65	<LoQ	11.7

[Benramdane *et al.* 1999].

A man who survived swallowing approx. 1 g of arsenic trioxide had arsenic concentrations of 0.415 µg/L (blood) and 51.4 mg/L (urine) 20 h after ingestion [Kamijo *et al.* 1998].

A 22-month-old girl ingested approx. 1 oz (27 g) of 2.27% sodium arsenate contained in an ant killer. Her 24 h urinary arsenic levels were as follows:

Day	*Concentration (µg/L)*
1	4880
3	1355
4	682
5	650
8	—
9	139
12	96

[Cullen *et al.* 1995]

A 35-year-old man experienced bouts of sudden extreme malaise after the introduction of a new detergent cleanser in the workplace. At first, his serum arsenic concentration was normal (10 ng/L), but 2 months later a 24 h urine collection revealed arsenic excretion of 11.3 mg/24 h, 450 times the normal level. A repeat urine sample showed arsenic excretion of 0.95 mg/24 h. His hair arsenic concentration was 0.9 g/kg, 1000 times the normal level, attesting to protracted exposure [Risk, Fuortes 1991].

A 29-year-old man injected unknown amounts of potassium cyanide and sodium arsenite IV. The initial urine sample was negative for arsenic but the 12 h collection started on admission contained 10 065 µg. On his third hospital day his blood concentration was 70 µg/L and the 24 h urine contained 19500 µg. [DiNapoli *et al.* 1989].

A 51-year-old man injected barbital IV and ingested arsenic orally. Antemortem blood and postmortem concentrations of arsenic were as follows:

Specimen	*Concentration (mg/L or mg/kg)*
Antemortem blood (serum)	0.3
Postmortem blood	1.3
Injection site	Trace
Urine	Present
Kidney	35
Liver	14
Spleen	11
Syringe	Absent

[Graham *et al.* 1983]

A person ingested an unknown quantity of wood-preserving solution containing large amounts of copper sulfate and sodium dichromate with a smaller but still substantial arsenic compound. Concentrations of arsenic in various tissues were reported as follows:

Tissue	*Concentration (µg/kg)*
Blood	2.0
Brain	2.7
Heart	7.7
Kidney	17.5
Liver	13.9
Lung	7.7
Spleen	7.4
Stomach	21.1

[Cross *et al.* 1979]

In 2 men who died after being exposed to arsine gas, arsenic concentrations were as follows:

Tissue	*Case 1*	*Case 2*
Bile (mg/kg)	139	104
Blood (mg/L)	58	11
Brain (mg/kg)	76	49
Kidney (mg/kg)	282	18
Liver (mg/kg)	401	136
Lungs (mg/kg)	35	—
Spleen (mg/kg)	342	66
Urine (mg/L)	12	35

[Pothel, Brosseau 1976]

Note For an investigation of toxic trace elements in the hair of children with autism, see Fido and Al Saad [2005]. For 5 cases of acute oral intoxication by trivalent arsenic, see Mahieu *et al.* [1981]. For concentrations in the urine of glass-manufacturing workers, see Apostoli *et al.* [1998].
Half-life In blood approximately 7 h.
Volume of Distribution 0.2 L/kg.
Distribution in Blood Blood : plasma ratio 1.

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Arsenic Trioxide

Antineoplastic
As_2O_3 = 197.8
CAS—1327-53-3

IUPAC Name Arsenic (3+); oxygen(2−)
Synonyms Arsenic oxide; arsenic (III) oxide; arsenious acid; arsenious acid anhydride; arsenious oxide; arsenite; arsenous acid; arsenous acid anhydride; arsenous oxide; arsenic sesquioxide; white arsenic.
Proprietary Name *Trisenox*
Chemical Properties White or transparent, glassy, amorphous lumps or crystalline powder. Two crystalline modifications: claudetite, mp 313°, and arsenolite, mp 275°. Bp 465°. It is intensely poisonous. It is sparingly and extremely slowly soluble in cold water; soluble in boiling water, dilute hydrochloric acid, alkali hydroxide and carbonate solutions; practically insoluble in alcohol, chloroform and ether.
Quantification
Blood **LC-ICP-MS** Column: PRP X100 (100 × 4.1 mm i.d.). Mobile phase: 20 mmol/L ammonium dihydrogen phosphate (pH 5.0) in methanol, flow rate 1.5 mL/min. Limit of detection, ng/g range [Slejkovec *et al.* 2008].
Plasma **GC** Column: 3% OV-17 (2 m). Carrier gas: N_2, 45 mL/min. Limit of detection, 0.15 nmol [Ni *et al.* 1998].
Urine **GC** See Plasma [Ni *et al.* 1998].
AAS λ= 193.7 nm. Limit of detection, 1 μg/L [Heinrich-Ramm *et al.* 2003].
LC-ICP-MS See Blood. Limit of detection, mg/L range [Slejkovec *et al.* 2008]. Column: C_{18} ODS-3 (150 × 4/6 mm i.d., 3 μm). Mobile phase: 5 mmol/L tetrabutylammonium hydroxide : 3 mmol/L malonic acid : 5% methanol, flow rate 1.2 mL/min. Plasma gas: Ar, 15 L/min, auxiliary gas, Ar, 1.2 L/min, nebuliser gas, Ar, 0.9 L/min [Wang *et al.* 2004]. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 1 mL/min. Plasma gas: Ar, 13 L/min, auxiliary gas, Ar, 0 L/min, nebuliser gas, Ar, 0.4 L/min. Retention time: 3.1 min. Limit of quantification, 36 μg/L [Do *et al.* 2000].
Hair **GC** See Plasma [Ni *et al.* 1998].
Nail **GC** See Plasma [Ni *et al.* 1998].
Disposition in the Body The extent of arsenic trioxide absorption depends on the physical form of the compound; for example, a coarse powder may be eliminated in faeces before significant dissolution and absorption can occur. Arsenic trioxide is partly methylated (detoxification) to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the liver. Both of these metabolites are relatively non-toxic and are excreted mostly in urine along with other inorganic arsenic compounds. During treatment with arsenic trioxide, the arsenic content of urine over a 24 h period accounted for 1–8% of the administered daily dose. Smaller amounts of arsenic are also excreted in faeces and sweat and via the lungs and skin. It has also been detected in breast milk and is known to cross the placenta. After ingestion of large doses, arsenic is widely distributed throughout the body to all organs, with the highest concentrations in liver and kidneys, with 10-fold and 3-fold higher concentrations, respectively.
Therapeutic Concentration
Arsenic trioxide was administered at a dose of 10 mg daily to 4 male patients with acute promyelocytic leukaemia. The relative proportions of arsenic (III), arsenic (V), MMA and DMA in 24 h urine samples were 27.6, 2.8, 22.8, and 43.7%, respectively [Wang *et al.* 2004].
Arsenic trioxide was administered at a dose of 10 mg daily to 8 patients with relapsed acute promyelocytic leukaemia. The peak plasma concentration reached 0.94 mg/L at 4 h after dosing. [Ni *et al.* 1998]
Toxicity The major toxic effect in severe arsenic poisoning is haemorrhagic gastroenteritis, which may lead to severe dehydration, collapse, shock and even death. Cardiac arrhythmias and convulsions have also been reported. A dose of 70–300 mg arsenic trioxide has been associated with fatalities depending on the physical form of the compound and the extent of its absorption. In the absence of adequate treatment, death may occur within 1 h but can be prolonged to 12–48 h.
A 21-year-old man ingested approximately 0.6 g arsenic trioxide. After 11 h, his arsenic trioxide concentration was 95096 μg/L [Heinrich-Ramm *et al.* 2003].
A 28-year-old man committed suicide by ingesting ~8 g of arsenic trioxide; he died 3 days after ingestion. Major intoxication symptoms observed were hepatic and renal failure and cardiovascular and neurological effects. After toxicological analysis of his organs, the highest arsenic concentrations were found in the liver (147 μg/g) and kidneys (26.6 μg/g). Other organs contained lower but very similar concentrations: muscle, 12.3 μg/g; heart, 11.75 μg/g; spleen, 11.72 μg/g; pancreas, 11.2 μg/g; lungs, 11.13 μg/g; cerebellum, 10.95 μg/g; and brain, 8.33 μg/g. Skin had the lowest concentration, at 2.9 μg/g. These concentrations were made up of arsenic (III) and the 2 metabolites MMA and DMA [Benramdane *et al.* 1999].
Half-life Plasma half-life, 12 h.
Clearance Systemic, 1.43 L/h.

Benramdane L *et al.* (1999). Arsenic speciation in human organs following fatal arsenic trioxide poisoning: a case report. *Clin Chem* 45: 301–306.
Do B *et al.* (2000). On-line reversed-phase liquid chromatography hydride generation emission spectrometry: speciation of arsenic in urine of patients intravenously treated with As_2O_3. *J Chromatogr B Biomed Sci Appl* 740: 179–186.
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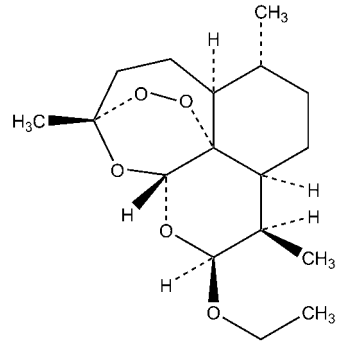
Ni J *et al.* (1998). Pharmacokinetics of intravenous arsenic trioxide in the treatment of acute promyelocytic leukemia.. *Chin Med J* 111: 1107–1110.
Slejkovec Z *et al.* (2008). Analytical artefacts in the speciation of arsenic in clinical samples. *Anal Chim Acta* 607: 83–91.
Wang Z *et al.* (2004). Arsenic speciation in urine from acute promyelocytic leukemia patients undergoing arsenic trioxide treatment. *Chem Res Toxicol* 17: 95–103.

Arteether

Antimalarial
$C_{17}H_{28}O_5$ = 312.4
CAS—75887-54-6

Synonyms α/β-Arteether; artemotil; dihydroartemisinin ethyl ether; (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-10-ethoxydecahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin; SM-227.
Proprietary Names *Rapither AB*; *EMAL*.



Chemical Properties White crystalline solid. Mp 80° to 82°. Log *P* (octanol/water), 3.89 [Ramu, Baker 1995]. Biosamples were stable at –50° for over 60 days. Analytes were stable after 3 freeze–thaw cycles [Sabarinath *et al.* 2003, 2005, 2006]. Analytes were stable after reconstitution for at least 24 h at 4° [Rajanikanth *et al.* 2003]. Storage in plasma in plastic containers results in a considerable loss of drug, even over a 24 h period [Idowu *et al.* 1989].
Thin-layer Chromatography Plate: Reversed phase C_{18}. Solvent system: acetonitrile : water (50 : 25). Derivatised with 4-methoxybenzaldehyde in methanol : water. Limit of quantification, 0.5 μg [Gabriels, Plaizier-Vercammen 2004]. Plate: Silica gel. Solvent system: chloroform. Visualisation in 4-methoxybenzaldehyde reagent of 1% acidic solution of sulfuric acid (98%) and acetic acid (96 to 98%, 2% and 10% in alcohol : water 60 : 30). Limit of quantification, 0.5 μg [Gabriels, Plaizier-Vercammen 2003].
Mass Spectrum Principal ions at *m/z* 267, 284, 330, 221, 253 and 239.
Quantification
Blood **LC-MS** Column: Spheri-5 C_{18} reversed phase (100 × 4.6 mm i.d.). Mobile phase: methyl alcohol : 10 mmol/L ammonium acetate buffer (pH 4.0, 93 : 7), flow rate 0.65 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Sabarinath *et al.* 2005].
Plasma **LC-MS** See Blood. Limit of quantification, 0.39 μg/L [Sabarinath *et al.* 2005].
Urine **LC-MS** See Plasma [Sabarinath *et al.* 2005].
Other **HPLC** Tablets. Column: Macherey-Nagel Nucleosil 120-4 C_{18} (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : water : 0.05 mol/L potassium hydrogen phosphate (pH 5; 500 : 100 : 320), flow rate 1.0 mL/min. UV detection (λ = 215 nm). Limit of detection not reported [Atemnkeng *et al.* 2007]. Rat Plasma. Column: Beckman Ultrasphere-ODS C_{18} reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (60 : 40), flow rate 0.75 mL/min. UV detection (λ = 253 nm). Limit of detection not reported [Idowu *et al.* 1989].
LC-MS Rat Plasma. Column: Ultracarb Phenomenex C_{18} (30 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 4; 90 : 10), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.78 μg/L [Sabarinath *et al.* 2006]. Monkey Plasma. Column: Spheri-10 RP_{18} (100 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : ammonium acetate buffer (pH 4, 92 : 8), flow rate 0.65 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.78 μg/L; limit of detection, 0.39 μg/L [Sabarinath *et al.* 2003]. Rat Plasma. Column: Spheri-10 RP_{18} (100 × 4.6 mm i.d.,

5 μm). Mobile phase: methanol : potassium phosphate buffer (pH 4; 73 : 27 to 100 : 0 at 3 min for 5 min), flow rate 1.0 mL/min. ESI, positive ion mode. Limit of quantification, 4.38 and 10 μg/L for the α- and β-isomers, respectively; limit of detection, 2.5 μg/L [Rajanikanth *et al.* 2003]. Rat Plasma. Column: Whatman Partisil P/5 ODS 3 C_{18} reversed phase (235 × 4.7 mm i.d., 5 μm). Mobile phase: methanol : water (60;40 for 5 min to 82 : 18 over 10 min, maintaining 0.1 mol/L ammonium acetate throughout), flow rate 1.0 mL/min. Positive ion mode, SIM acquisition mode. Limit of detection, 2 μg/L [Chi *et al.* 1991].

Disposition in the Body Arteether is converted to artenimol, primarily by CYP3A4 with a secondary contribution by CYP2B6, which is extensively eliminated via biliary excretion.

Therapeutic Concentration

Thirteen healthy male volunteers (aged 25 to 50 years) were administered a single IM injection of 150 mg arteether containing both α- and β-isomers in the ratio 30 : 70. Maximum plasma concentrations of 60.7 and 49.8 μg/L were reached at 4.77 ± 1.21 h and 6.96 ± 1.62 h for the α- and β-isomers, respectively [Sabarinath *et al.* 2005].

Fifty-six Thai patients with *Plasmodium falciparum* malaria were administered β-arteether according to one of the following regimens. A dose of 3.2 mg/kg on day 0 followed by 1.6 mg/kg on days 1 to 4 or a dose of 4.8 mg/kg followed by 1.6 mg/kg 6 h later and 1.6 mg/kg on days 1 to 4. Mean peak plasma concentrations of 63.7 μg/L at 6.1 h and 140.8 μg/L at 5.7 h were reached in the low- and high-dose groups, respectively [Li *et al.* 2004].

Toxicity In rats, dogs and primates, IM injections of arteether have produced selective damage to brain stem centres involved in auditory processing and vestibular reflexes [Nontprasert *et al.* 1998].

Bioavailability 34% following IM injection [Navaratnam *et al.* 2000].

Half-life 13.2 ± 1.08 and 30.2 ± 2.44 h for the α- and β-isomers, respectively.

Volume of Distribution 12 ± 1.37 and 36.2 ± 4.78 for the α- and β-isomers, respectively.

Clearance 1.41 ± 0.49 and 1.36 ± 0.23 mL/h for the α- and β-isomers, respectively.

Distribution in Blood Partition ratios between whole blood and plasma: 1.8 and 1.91 for α- and β-arteether, respectively.

Protein Binding 78.7 ± 2.1%, binding to α_1-acid glycoprotein is 20-fold greater than to albumin [Wanwimolruk *et al.* 1992].

Note For a study investigating the interaction of arteether with blood, see Edwards *et al.* [1992].

Atemnkeng MA *et al.* (2007). Quality control of active ingredients in artemisinin-derivative antimalarials within Kenya and DR Congo. *Trop Med Int Health* 12: 68–74.

Chi HT *et al.* (1991). Identification of the in vivo metabolites of the antimalarial arteether by thermospray high-performance liquid chromatography/mass spectrometry. *Biol Mass Spectrom* 20: 609–628.

Edwards G *et al.* (1992). Interaction of arteether with the red blood cell in-vitro and its possible importance in the interpretation of plasma concentrations in-vivo. *J Pharm Pharmacol* 44: 280–281.

Gabriels M, Plaizier-Vercammen JA (2003). Densitometric thin-layer chromatographic determination of artemisinin and its lipophilic derivatives, artemether and arteether. *J Chromatogr Sci* 41: 359–366.

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Idowu OR *et al.* (1989). Determination of arteether in blood plasma by high-performance liquid chromatography with ultraviolet detection after hydrolysis acid. *J Chromatogr* 493: 125–136.

Li Q *et al.* (2004). Pharmacokinetic investigation on the therapeutic potential of artemotil (beta-arteether) in Thai patients with severe *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 71: 723–731.

Navaratnam V *et al.* (2000). Pharmacokinetics of artemisinin-type compounds. *Clin Pharmacokinet* 39: 255–270.

Nontprasert A *et al.* (1998). Assessment of the neurotoxicity of parenteral artemisinin derivatives in mice. *Am J Trop Med Hyg* 59: 519–522.

Rajanikanth M *et al.* (2003). An HPLC-MS method for simultaneous estimation of alpha,beta-arteether and its metabolite dihydroartemisinin, in rat plasma for application to pharmacokinetic study. *Biomed Chromatogr* 17: 440–446.

Ramu K, Baker JK (1995). Synthesis, characterization, and antimalarial activity of the glucuronides of the hydroxylated metabolites of arteether. *J Med Chem* 38: 1911–1921.

Sabarinath S *et al.* (2003). A sensitive and selective liquid chromatographic/electrospray ionization tandem mass spectrometric assay for the simultaneous quantification of alpha-, beta-arteether and its metabolite dihydroartemisinin in plasma, useful for pharmacokinetic studies. *J Mass Spectrom* 38: 732–742.

Sabarinath S *et al.* (2005). Clinical pharmacokinetics of the diastereomers of arteether in healthy volunteers. *Clin Pharmacokinet* 44: 1191–1203.

Sabarinath S *et al.* (2006). Simultaneous quantification of alpha-/beta-diastereomers of arteether, sulphadoxine and pyrimethamine: a promising anti-relapse antimalarial therapeutic combination, by liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 842: 36–42.

Wanwimolruk S *et al.* (1992). The binding of the antimalarial arteether to human plasma proteins in-vitro. *J Pharm Pharmacol* 44: 940–942.

Artemether

Antimalarial

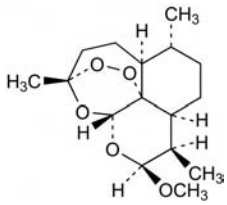
$C_{16}H_{26}O_5$ = 298.4

CAS—71963-77-4

IUPAC Name (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin

Synonyms Dihydroartemisinin methyl ether; dihydroqinghaosu methyl ether; *o*-methyl-dihydroartemisinin; SM-224.

Proprietary Names *Artemos*; *Qing Hao*.



Chemical Properties White crystalline powder. Mp 86° to 88°.

Quantification

Plasma **GC–MS** Limit of quantification, 5 μg/L [Mohamed *et al.* 1999].

HPLC Electrochemical detection. Limit of quantification, 26 μg/L for artemether and metabolite, dihydroartemisinin (DHA) [Teja-Isavadharm *et al.* 1996].

Note For a bioassay, limit of quantification 60 μg/L for DHA, see Teja-Isavadharm *et al.* [1996].

Disposition in the Body Artemether is rapidly but incompletely absorbed after oral administration and is metabolised in the liver to the demethylated derivative DHA. It is rapidly eliminated. Oral bioavailability, after IM injection, is ~43%.

Therapeutic Concentration

Six healthy Malaysian males were administered with a single oral dose of 200 mg artemether. Peak plasma concentrations of 310 ± 153 μg/L were reached within 1.88 ± 0.21 h. The peak concentration of the metabolite, DHA, was 273 ± 64 μg/L which was reached within 1.92 ± 0.13 h [Mordi *et al.* 1997].

Six patients with severe falciparum malaria and acute renal failure and 11 patients without renal failure were intramuscularly administered with a total dose of 640 mg (over 7 days) artemether. Patients with renal failure had a slightly higher peak plasma concentration compared with those without failure (1.89 to 3.95 μg/L/mg dose versus 1.05 to 3.38 μg/L/mg dose) [Karbwang *et al.* 1998].

Toxicity Neurotoxicity.

Half-life Elimination half-life for artemether is 1.5 to 3.5 h and for DHA is 1.4 to 2.4 h.

Volume of Distribution Apparent volume of distribution is 3.2 to 6.9 L/kg for patients with acute renal failure and 4.2 to 12.3 L/kg for those without.

Clearance Plasma clearance is 5.4 to 13.8 mL/min/kg for patients with acute renal failure and 8.5 to 25.1 mL/min/kg for those without.

Protein Binding About 50%.

Dose An IM dose of 3.2 mg/kg is administered initially followed by 1.6 mg/kg daily for a maximum of 7 days, then changed to oral administration. Capsule formulation: 160 mg on the first day followed by 80 mg daily for the next 4 days.

Karbwang J *et al.* (1998). Pharmacokinetics of intramuscular artemether in patients with severe falciparum malaria with or without acute renal failure. *Br J Clin Pharmacol* 45: 597–600.

Mohamed SS *et al.* (1999). Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography-mass spectrometry-selected ion monitoring. *J Chromatogr Biomed Sci Appl* 731: 251–260.

Mordi MN *et al.* (1997). Single dose pharmacokinetics of oral artemether in healthy Malaysian volunteers. *Br J Clin Pharmacol* 43: 363–365.

Teja-Isavadharm P *et al.* (1996). Comparative bioavailability of oral, rectal, and intramuscular artemether in healthy subjects: use of simultaneous measurement by high performance liquid chromatography and bioassay. *Br J Clin Pharmacol* 42: 599–604.

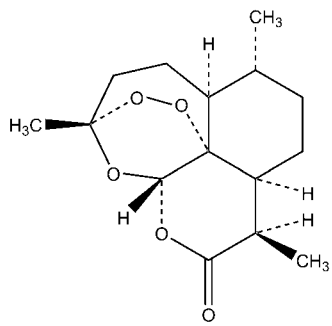
Artemisinin

Antimalarial

$C_{15}H_{22}O_5$ = 282.3

CAS—63968-64-9

Synonyms Arteannuin; artemisine; huanghuahaosu; (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano-[4,3-j]-1,2-benzodioxepin-10(3*H*)-one; QHS; qinghaosu; qing hau sau.



Chemical Properties Isolated from the traditional Chinese medicinal herb *Artemisia annua* L., Compositae. Needles. Mp 156° to 157°. Soluble in methanol,

A

100% ethanol, dimethyl formamide, DMSO, chloroform and acetone. Almost insoluble in water. Log *P* (octanol/water), 2.9 [Avery *et al.* 1995]. Stability established after storage in plasma at room temperature for 4 h. Processed samples were stable for up to 24 h in the autosampler, at –20° for at least 4 weeks and over at least 3 freeze–thaw cycles [Xing *et al.* 2006].

Dihydroartemisinin

$C_{15}H_{24}O_5$ = 284.3
CAS—71939-50-9
Synonym Dihydroqinghaosu

Thin-layer Chromatography Plate: Reversed phase C_{18}. Solvent system: acetonitrile : water (50 : 25). Derivatised with 4-methoxybenzaldehyde in methanol : water. Limit of quantification, 0.5 µg [Gabriels, Plaizier-Vercammen 2004].

Quantification

Blood **SCFC** Column: Deltabond cyano (200 × 1 mm i.d., 5 µm). ECD. Limit of detection, 20 µg/L [Mount *et al.* 1995].

Plasma **HPLC** Column: ADS-C_{18} reversed phase (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (2 : 98), flow rate 1.2 to 3.0 mL/min. UV detection (λ = 289 nm). Limit of quantification, 10 µg/L [Gordi *et al.* 2000]. Column: Versapak cyanopropyl (300 × 4.6 mm, 10 µm). Mobile phase: 100 mmol/L acetate buffer : acetonitrile (pH 5.0; 60 : 40), flow rate 1.5 mL/min. Electrochemical detection. Limit of quantification, 5 µg/L [van Agtmael *et al.* 1998].

Serum **HPLC** Column: Supelco discovery HS C_{18} (250 × 4.6 mm i.d.). Mobile phase: 20 mmol/L imidazole–nitric acid buffer (pH 8.5) : acetonitrile (30 : 70), flow rate 0.5 mL/min. Chemiluminescence detection. Limit of detection, 17.5 µg/L [Amponsaa-Karikari *et al.* 2006]. Column: Ultrasphere ODS bonded silica (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (50 : 50), flow rate 0.3 mL/min. Chemiluminescence detection. Limit of quantification, 25 µg/L [Green *et al.* 1995].

Oral Fluid **HPLC** See Plasma. Limit of quantification, 2 µg/L [Gordi *et al.* 2000].

Other **LC-MS** Rat Urine. Column: Luna ODS C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate containing 0.1% formic acid (85 : 15), flow rate 0.8 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.0 µg/L [Xing *et al.* 2006].

Disposition in the Body The metabolism of artemisinin is primarily mediated by CYP2B6, with a secondary contribution of CYP3A4. Urinary metabolites include deoxyarteminisin, deoxydihydroarteminisin, and 9,10-dihydrodeoxyarteminisin. The amount of unchanged drug excreted in human urine or faeces is negligible.

Therapeutic Concentration

Eight male volunteers given 10 mg/kg of artemisinin orally as an aqueous suspension had a mean peak plasma concentration of 0.58 mg/L at 2.5 h after dosing [Chan *et al.* 1997].

After administration of 400 mg artemisinin as an aqueous oral suspension to healthy subjects, a mean peak plasma concentration of 0.26 mg/L was attained after 1 h. The elimination half-life of 1.9 h was prolonged to 7.4 h by IM administration of the same dose [Titulaer *et al.* 1990].

Fourteen healthy male volunteers received 1 L of tea prepared from 9 g of *A. annua* L. leaves. This corresponded to a dose of 94.5 mg artemisinin. The mean peak plasma concentration was 0.24 mg/L after 0.6 h [Rath *et al.* 2004].

Bioavailability Between 19 and 35%.

Half-life Between 0.7 and 2.4 h for oral administration.

Volume of Distribution 16 L/kg.

Protein Binding Approximately 64%.

Note For a study investigating protein binding of dihydroartemisinin *in vivo*, see Batty *et al.* [2004] and for pharmacokinetic values, see Teja-Isavadharm *et al.* [2001].

Dose For the treatment of malaria in areas of multidrug resistance, artemisinin may be given orally as a 3-day course: 25 mg/kg bodyweight on the first day with 12.5 mg/kg on the second and third days.

Amponsaa-Karikari A *et al.* (2006). Determination of artemisinin in human serum by high-performance liquid chromatography with on-line UV irradiation and peroxyoxalate chemiluminescence detection. *Biomed Chromatogr* 20: 1157–1162.

Avery MA *et al.* (1995). Structure-activity relationships of the antimalarial agent artemisinin, 2. Effect of heteroatom substitution at O-11: synthesis and bioassay of *N*-alkyl-11-aza-9-desmethylartemisinins. *J Med Chem* 38: 5038–5044.

Batty KT *et al.* (2004). Protein binding and alpha : beta anomer ratio of dihydroartemisinin in vivo. *Br J Clin.Pharmacol* 57: 529–533.

Chan KL *et al.* (1997). A high-performance liquid chromatography analysis of plasma artemisinin using a glassy carbon electrode for reductive electrochemical detection. *Planta Med* 63: 66–69.

Gabriels M, Plaizier-Vercammen J (2004). Development of a reversed-phase thin-layer chromatographic method for artemisinin and its derivatives. *J Chromatogr Sci* 42: 341–347.

Gordi T *et al.* (2000). Direct analysis of artemisinin in plasma and saliva using coupled-column high-performance liquid chromatography with a restricted-access material pre-column. *J Chromatogr B Biomed Sci Appl* 742: 155–162.

Green MD *et al.* (1995). Chemiluminescent detection of artemisinin. Novel endoperoxide analysis using luminol without hydrogen peroxide. *J Chromatogr A* 695: 237–242.

Mount DL *et al.* (1995). Packed-column supercritical fluid chromatography of artemisinin (qinghaosu) with electron-capture detection. *J Chromatogr B Biomed Appl* 666: 183–187.

Rath K *et al.* (2004). Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of *Artemisia annua* L (annual wormwood). *Am J Trop Med Hyg* 70: 128–132.

Teja-Isavadharm P *et al.* (2001). Comparative pharmacokinetics and effect kinetics of orally administered artesunate in healthy volunteers and patients with uncomplicated falciparum malaria. *Am J Trop Med Hyg* 65: 717–721.

Titulaer HA *et al.* (1990). The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. *J Pharm Pharmacol* 42: 810–813.

van Agtmael MA *et al.* (1998). Validation of an improved reversed-phase high-performance liquid chromatography assay with reductive electrochemical detection for the determination of artemisinin derivatives in man. *Ther Drug Monit* 20: 109–116.

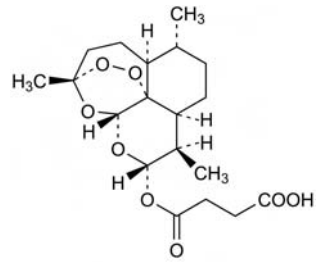
Xing J *et al.* (2006). A high-performance liquid chromatography/tandem mass spectrometry method for the determination of artemisinin in rat plasma. *Rapid Commun Mass Spectrom* 20: 1463–1468.

Artesunate

Antimalarial
$C_{19}H_{28}O_8$ = 384.4
CAS—88495-63-0

IUPAC Name Butanedioic acid mono[(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-yl] ester

Synonyms Artesunic acid; dihydroartemisinin hemisuccinate; dihydroqinghaosu hemisuccinate.



Chemical Properties A fine white crystalline powder.

Artesunate Sodium

$C_{19}H_{27}NaO_8$ = 406.4
Synonyms Dihydroartemisinin hemisuccinate sodium; dihydroqinghaosu hemisuccinate sodium; SM-804.
Proprietary Name *Arsumex*
Chemical Properties Poor stability in aqueous solutions.

Mass Spectrum Principal ions at *m/z* (cyclohexane pyrolysis product of DQHS) 43, 55, 69, 81, 152, 210, 95, 180.

Quantification

Blood **GC–MS** Column: 3% OV-3 on 100-120 Supelcocort (silanised glass, 2 m × 2 mm i.d.). Temperature programme: 160° to 24° at 10°/min. Carrier gas: methane, flow rate 20 mL/min. MS detection (EI, SIM at *m/z* 221). Internal standard: cedrol. Retention time: dihydroqinghaosu (DQHS) (cyclohexane pyrolysis product) 5.5 min. Limit of detection, 2 µg/L for DQHS [Theoharides *et al.* 1988].

Plasma **HPLC** Column: C_{18} Nova-Pak (150 × 3.9 mm i.d., 5 µm), column and detector oven set at 20°. Mobile phase (kept at 35°): acetonitrile : 0.1 mol/L dilute acetic acid (pH 4.80) (45:55). Flow rate 1.5 mL/min. Electrochemical detection. Retention time: α-dihydroartemisinin (DHA), 2.9 min; β-DHA, 4.2 min; artesunate, 4.5 min. Limit of quantification, 5 µg/L for artesunate, 3 µg/L for DHA [Na-Bangchang *et al.* 1998]. Column: C_8 Econosil (250 × 4.6 mm, 10 µm). Mobile phase: acetonitrile : 0.05 mol/L acetic acid (42:58), (pH 5.0), flow rate 1.5 mL/min . Electrochemical detection. Retention time: artesunate, 6.6 min; DHA, 8.7 min. Limit of detection, 4.0 µg/L for artesunate and the metabolite, DHA [Navaratnam *et al.* 1997].

Disposition in the Body Artesunate is rapidly metabolised to its active metabolite dihydroartemisinin (DHA) (dihydroqinghaosu, DQHS). After oral administration, bioavailability of DHA is 82%.

Therapeutic Concentration

Twenty six Vietnamese patients with falciparum malaria were administered with either a 120 mg IV dose or 100 mg oral dose of artesunate. With the IV dose, peak plasma concentrations of 11 mg/L artesunate and 2.64 mg/L DHA were reached. With the oral dose of artesunate, peak concentrations of 0.74 mg/L DHA were observed [Batty *et al.* 1998].

Eleven Thai patients, aged between 15 and 37 years, with acute uncomplicated falciparum malaria were administered with an initial, oral dose of 200 mg, followed by 100 mg daily (total of 700 mg). After the initial dose, peak plasma concentrations of artesunate at 0.5 to 1.9 µg/L were reached within 0.5 to 1 h and 1.2 to 4.1 µg/L for the metabolite, DHA (1.5 to 2.5 h). After the first 100 mg dose, peak concentrations reached 0.62 to 2.9 µg/L and 1.3 to 4.2 µg/L, respectively, within 2 h [Karbwang *et al.* 1998].

Bioavailability Bioavailability of DHA is 82%.

Half-life Elimination half-life of artesunate is 2.7 min and 40 min for DHA (IV administration) and 21.6 and 38.4 min, respectively, for oral administration.

Volume of Distribution Artesunate, 0.14 L/kg; DHA, 0.76 L/kg (IV administration). Oral administration, 6.8 L/kg for artesunate and DHA.

Clearance Clearance of artesunate is 2.33 L/h/kg and of DHA is 0.75 L/kg/h (intravenous administration). Oral administration, 0.005 and 0.003 L/kg/h for artesunate and DHA, respectively.

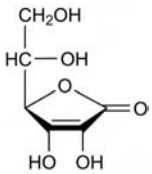
Distribution in Blood Blood cells : plasma concentration ratio 5:5.
Note Refer to Karbwang *et al.* [1998] and Batty *et al.* [1998] for a review of the pharmacokinetics.
Dose A dose of 10 mg/kg body weight daily (intravenously) is administered for a minimum of 5 days (3 days if in combination). Lowest dose of 2 mg/kg body weight daily. Total oral dose of 600 mg over 5 days.

Batty KT *et al.* (1998). A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. *Br J Clin Pharmacol* 45: 123–129.
Karbwang J *et al.* (1998). Pharmacokinetics of oral artesunate in thai patients with uncomplicated falciparum malaria.. *Clin Drug Invest* 15: 37–43.
Na-Bangchang K *et al.* (1998). Simple high-performance liquid chromatographic method with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in biological fluids. *J Chromatogr B Biomed Sci Appl* 708: 201–207.
Navaratnam V *et al.* (1997). Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J Chromatogr B Biomed Sci Appl* 692: 157–162.
Theoharides AD *et al.* (1988). Determination of dihydroqinghaosu in blood by pyrolysis gas chromatography/mass spectrometry. *Anal Chem* 60: 115–120.

Ascorbic Acid

Vitamin
$C_6H_8O_6 = 176.1$
CAS—50-81-7

IUPAC Name (2R)-2-[(1S)-1,2-Dihydroxyethyl]-4,5-dihydroxyfuran-3-one
Synonyms L-Ascorbic acid; cevitamic acid; 3-oxo-L-gulofuranolactone (enolic form); vitamin C.
Proprietary Names *Alba CE*; *Ascorbicap*; *Ascorbicin*; *Ascorvit*; *Buffered C*; *Cantan*; *Cebid*; *Cebion*; *Cecon*; *Celaskon*; *Celin*; *Cenolate*; *Cetebe*; *Cevi-Bid*; *Cevilan*; *Ce-Vi-Sol*; *Cevitan*; *Cewin*; *Dull-C*; *Favorcee*; *Haliborange Halibonbons*; *Hybrin*; *N'ice Vitamin C*; *RedoxonVitacin*; *Vitascorbol*.

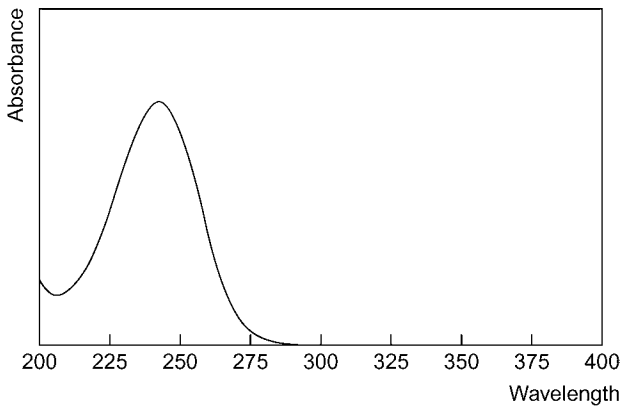


Chemical Properties Ascorbic acid and ascorbates are ingredients of many proprietary preparations. Colourless crystals or white or very pale yellow crystalline powder. Solutions, especially when made alkaline, deteriorate rapidly in air. Mp 190° to 192°, with some decomposition. Soluble 1 in about 3 of water, 1 in 30 of ethanol, 1 in 10 of methanol and 1 in 20 of propylene glycol; soluble in acetone; insoluble in benzene, chloroform, ether, petroleum ether, oils, fats and fat solvents. pK_a 4.2, 11.6 (25°). Log *P* (octanol/water), −1.8.

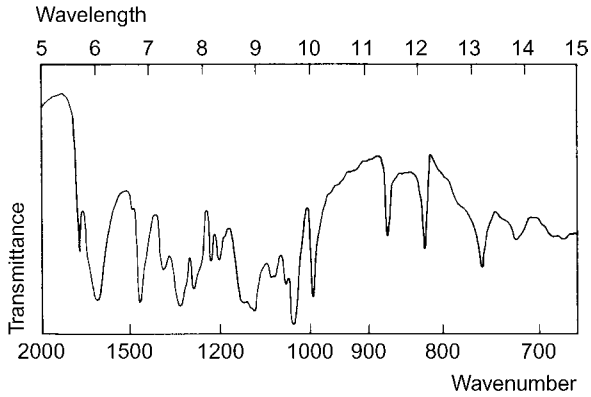
Sodium Ascorbate

$C_6H_7NaO_6 = 198.1$
CAS—134-03-2
Chemical Properties White or very faintly yellow crystals or crystalline powder. It gradually darkens on exposure to light. Mp 218° with decomposition. Soluble 1 in 1.3 of water; very slightly soluble in ethanol; practically insoluble in chloroform and ether.

Colour Tests Ammoniacal silver nitrate—black; Benedict's reagent—red; Nessler's reagent—black; palladium chloride—black.
Gas Chromatography System GA—RI 2120, ascorbic acid-AC RI 2065, ascorbic acid (methylated) RI 1700.
High Performance Liquid Chromatography System HY—RI 52; system HZ—retention time 1.5 min (ascorbate).
Ultraviolet Spectrum Aqueous acid—243 nm (A_1^1=556a).



Infrared Spectrum Principal peaks at wavenumbers 1026, 1111, 1312, 1136, 1653, 990 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 29, 41, 39, 42, 69, 116, 167, 168.
Quantification
Plasma **GC–MS** For ascorbic and dehydroascorbic acids, see Deutsch and Kolhouse [1993].

HPLC Fluorescence detection. For ascorbic and dehydroascorbic acids, see Kall and Andersen [1999]. UV detection. See Rumelin *et al.* [1999]. UV detection. For ascorbic and dehydroascorbic acids, see Esteve *et al.* [1997]. For ascorbate and dehydroascorbate, see Koshiishi and Imanari [1997]. Fluorescence detection (comparison with coulometric detection) ascorbic acid and dehydroascorbic acid. See Tessier *et al.* [1996]. Coulometric detection. For ascorbic acid and dehydroascorbic acid, see Lykkesfeldt *et al.* [1995]. Electrochemical detection. Limit of detection, 240 µg/L [Iwase, Ono 1994]. UV detection. Limit of detection, 0.4 mg/L [Manoharan, Schwille 1994]. UV detection. For ascorbic acid and uric acid, see Ross [1994]. Electrochemical detection. See Umegaki *et al.* [1994]. UV detection (comparison with electrochemical detection). Limit of detection, 120 µg/L [Liau *et al.* 1993]. UV detection. See Tanishima and Kita [1993]. Electrochemical detection. See Watson *et al.* [1993]. Electrochemical detection. For ascorbic acid and dehydroascorbic acid, see Dhariwal *et al.* [1991]. See Kutnink *et al.* [1987]. For ascorbate-2-phosphate, see Moore and Fishman [1987].

Colorimetry For comparison with HPLC, see Chung *et al.* [2001].
Serum **HPLC** See Plasma [Esteve *et al.* 1997]. Electrochemical detection. See Pachla and Kissinger [1976].
Urine **HPLC** See Plasma [Rumelin *et al.* 1999]. See Plasma [Koshiishi and Imanari 1997]. UV detection. Limit of detection 0.1 mg/L [Manoharan, Schwille 1994]. Electrochemical detection. See Pachla and Kissinger [1976].
Aqueous Humour **HPLC** See Plasma Watson *et al.* [1993].
Vitreous Humour **HPLC** UV detection. See Takano *et al.* [1997].
Blood Cells **HPLC** See Kutnink *et al.* [1987]. See Plasma [Moore and Fishman 1987]. See Omaye *et al.* [1987].
Postmortem Tissues **HPLC** UV detection. See Allender [1982].
Note For review of analysis methods in biological samples, pharmaceuticals and food, see Pachla *et al.* [1985].
Disposition in the Body Readily absorbed after oral administration; the proportion of a dose absorbed tends to decrease with increasing dose; it is widely distributed in the body tissues. The concentration of ascorbic acid is higher in leucocytes and platelets than in erythrocytes and plasma. Ascorbic acid is metabolised to dehydroascorbic acid, 2,3-diketogulonic acid, oxalate and carbon dioxide; some conjugation with sulfate occurs to form ascorbate-3-sulfate. Ascorbic acid in excess of the body's requirements is rapidly eliminated in the urine. About 85% of an IV dose, given to subjects previously saturated with the vitamin, is excreted in the urine in 24 h, with about 70% of the dose excreted unchanged and 15% as dehydroascorbic acid and diketogulonic acid. The amount normally present in the body is in excess of 1.5 g.
Blood Concentration Concentrations in plasma and in leucocytes are normally about 5 to 12 mg/L and 25 to 30 µg/10^8 cells respectively; these concentrations exhibit circadian and seasonal rhythms.
Protein Binding About 25%.
Dose 0.2 to 3 g daily.

Allender WJ (1982). Post mortem tissue levels of ascorbic acid in a scurvy case. *J Anal Toxicol* 6: 202–204.
Chung WY *et al.* (2001). Plasma ascorbic acid: measurement, stability and clinical utility revisited. *Clin Biochem* 34: 623–627.
Deutsch JC, Kolhouse JF (1993). Ascorbate and dehydroascorbate measurements in aqueous solutions and plasma determined by gas chromatography-mass spectrometry. *Anal Chem* 65: 321–326.
Dhariwal KR *et al.* (1991). Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. *Am J Clin Nutr* 54: 712–716.
Esteve MJ *et al.* (1997). Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. *J Chromatogr B Biomed Sci Appl* 688: 345–349.
Iwase H, Ono I (1994). Determination of ascorbic acid in human plasma by high-performance liquid chromatography with electrochemical detection using a hydroxyapatite cartridge for precolumn deproteinization. *J ChromatogrB Biomed Appl* 655: 195–200.
Kall MA, Andersen C (1999). Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and biological samples. *J Chromatogr B Biomed Sci Appl* 730: 101–111.

Koshiishi I, Imanari T (1997). Measurement of ascorbate and dehydroascorbate contents in biological fluids. *Anal Chem* 69: 216–220.
Kutnink MA *et al.* (1987). An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components. *Anal Biochem* 166: 424–430.
Liau LS *et al.* (1993). Determination of plasma ascorbic acid by high-performance liquid chromatography with ultraviolet and electrochemical detection. *J Chromatogr* 612: 63–70.
Lykkesfeldt J *et al.* (1995). Determination of ascorbic acid and dehydroascorbic acid in plasma by high-performance liquid chromatography with coulometric detection–are they reliable biomarkers of oxidative stress? *Anal Biochem* 229: 329–335.
Manoharan M, Schwille PO (1994). Measurement of ascorbic acid in human plasma and urine by high-performance liquid chromatography. Results in healthy subjects and patients with idiopathic calcium urolithiasis. *J Chromatogr B Biomed App* 654: 134–139.
Moore GL, Fishman RM (1987). High-performance liquid chromatographic analysis of ascorbate-2-phosphate, adenine and hypoxanthine in stored human blood. *J Chromatogr* 419: 95–102.
Omaye ST *et al.* (1987). Measurement of vitamin C in blood components by high-performance liquid chromatography. Implication in assessing vitamin C status. *Ann N Y Acad Sci* 498: 389–401.
Pachla LA, Kissinger PT (1976). Determination of ascorbic acid in foodstuffs, pharmaceuticals, and body fluids by liquid chromatography with electrochemical detection. *Anal Chem* 48: 364–367.
Pachla LA *et al.* (1985). Analytical methods for determining ascorbic acid in biological samples, food products, and pharmaceuticals. *J AOAC* 68: 1–12.
Ross MA (1994). Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 657: 197–200.
Rumelin A *et al.* (1999). Determination of ascorbic acid in plasma and urine by high performance liquid chromatography with ultraviolet detection. *Clin Chem Lab Med* 37: 533–536.
Seki T *et al.* (1987). Determination of ascorbic acid in human urine by high-performance liquid chromatography coupled with fluorimetry after post-column derivatization with benzamidine. *J Chromatogr* 385: 287–291.
Takano S *et al.* (1997). Determination of ascorbic acid in human vitreous humor by high-performance liquid chromatography with UV detection. *Curr Eye Res* 16: 589–594.
Tanishima K, Kita M (1993). High-performance liquid chromatographic determination of plasma ascorbic acid in relationship to health care. *J Chromatogr* 613: 275–280.
Tessier F *et al.* (1996). Validation of a micromethod for determining oxidized and reduced vitamin C in plasma by HPLC-fluorescence. *Int J Vitam Nutr Res* 66: 166–170.
Umegaki K *et al.* (1994). Improved method for the analysis of ascorbic acid in plasma by high-performance liquid chromatography with electrochemical detection. *J Nutr Sci Vitaminol (Tokyo)* 40: 73–79.
Wagner ES *et al.* (1979). High-performance liquid chromatographic determination of ascorbic acid in urine: effect on urinary excretion profiles after oral and intravenous administration of vitamin C. *J Chromatogr* 163: 225–229.
Watson DG *et al.* (1993). Measurement of ascorbic acid in human aqueous humour and plasma and bovine aqueous humour by high-performance liquid chromatography with electrochemical detection. *J Pharm Biomed Anal* 11: 389–392.

Asparaginase

Antineoplastic
CAS—9015-68-3

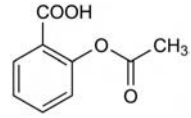
Synonyms L-Asnase; L-asparaginase; L-asparagine amidohydrolase; colaspase; E. C.3.5.1.1; Mk-965; NSC-109229.
Proprietary Names *Crasnitin*; *Crasnitine*; *Elspar*; *Erwinase (Crisantaspase-recombinant)*; *Kidrolase*; *Laspar*; *Leucogen*; *Leunase*; *Oncaspar (Pegaspargase-recombinant)*; *Paronal*.
Chemical Properties Commonly obtained from *E. coli* and *Erwinia* sp. Asparaginase from *E.coli* has a molecular weight of about 136000. A white crystalline powder. It is freely soluble in water where it appears to be global in shape; practically insoluble in methanol, in acetone and in chloroform.
Quantification
***Serum* TLC** Reaction with ^{14}C-asparagine and detection with scintillation counter. Limit of detection, 0.05 U/mL [Clausen, Christensen 1986].
Disposition in the Body It is sequestered slowly by the reticulo-endothelial system. It is found in the lymph at about 20% of the concentration in plasma. There is virtually no diffusion into the CSF. Little is excreted in the urine. Preparations derived from *E. coli* and *Erwinia* differ in their pharmacokinetic and pharmacodynamic properties.
Toxicity Allergic reactions are frequent and can lead to death.
Half-life Plasma, 8 to 30 h (IV administration); up to 49 h (intramuscular administration).
Protein Binding 30%.
Dose Up to 1000 units/kg intravenously daily for 10 days.

Clausen N, Christensen E (1986). Determination of L-asparaginase activity in serum by thin-layer chromatography: application to the treatment of acute lymphoblastic leukemia. *Clin Chim Acta* 161: 111–116.

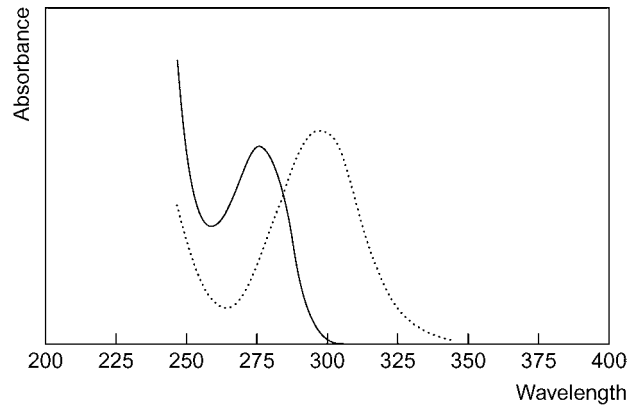
Aspirin

Analgesic, Antiplatelet, Antipyretic, NSAID
$C_9H_8O_4$ = 180.2
CAS—50-78-2

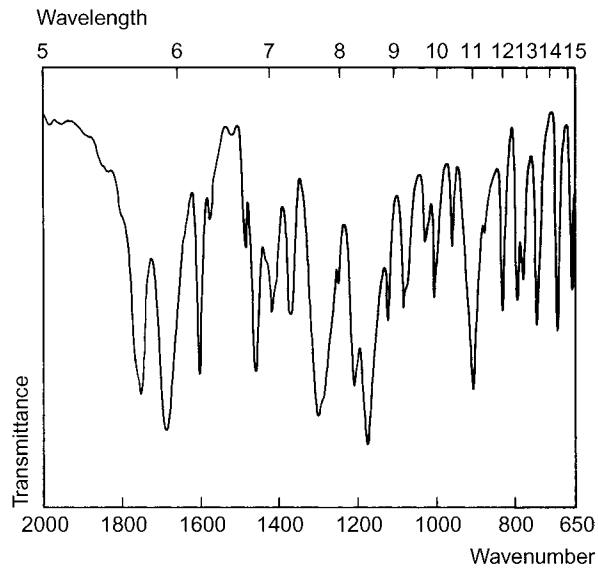
IUPAC Name 2-Acetyloxybenzoic acid
Synonyms 2-Acetoxybenzoic acid; acetylsalicylic acid; salicylic acid acetate.
Proprietary Names *Adprin-B*; *Angettes*; *Ascriptin*; *Aspergum*; *Asprimox*; *Aspro*; *Asatard*; *Beechams Lemon Tablets*; *Bufferin*; *Buffex*; *Caprin*; *Cardiprin*; *Cemerit*; *Claragine*; *Disprin*; *Easprin*; *Ecotrin*; *Empirin*; *Encaprin*; *Enprin*; *Gencardia*; *Genprin*; *Halfprin*; *Micropirin*; *Magnaprin*; *Nu-Seals*; *Platet*; *PostMI*; *Rhonal*; *Solprin*; *ZORprin*. Aspirin is an ingredient of many proprietary preparations [Sweetman 2007].



Chemical Properties Colourless or white crystals or white crystalline powder or granules. It is stable in dry air but gradually hydrolyses in contact with moisture to acetic and salicylic acids. Mp 135°. Soluble 1 in 300 of water, 1 in 5 of ethanol, 1 in 17 of chloroform, and 1 in 10 to 15 of ether; soluble in solutions of acetates and citrates and, with decomposition, in solutions of alkali hydroxides and carbonates. pK_a 3.5 (25°). Log *P* (octanol/pH 7.4), −1.1.
Colour Test McNally's test—red (after hydrolysis).
Thin-layer Chromatography System TA—R_f 0.90; system TD—aspirin R_f 0.18, salicylic acid R_f 0.07, salicyluric acid R_f 0.00; system TE—aspirin R_f 0.09, salicylic acid R_f 0.10, salicyluric acid R_f 0.00; system TF—aspirinR_f 0.30, salicylic acid R_f 0.01, salicyluric acid R_f 0.00; system TAD—aspirin R_f 0.31, salicylic acid R_f 0.24; system TAE—aspirin R_f 0.78, salicylic acid R_f 0.86; system TAJ—R_f 0.40; system TAK—R_f 0.65; system TAL—R_f 0.90.
Gas Chromatography System GA—aspirin RI 1545, M (aspirin-Me) RI 1394, salicylic acid RI 1307, M (salicylic acid-Me) RI 1195; system GB—salicylic acid RI 1340, M (salicylic acid-Me) RI 1228.
High Performance Liquid Chromatography System HD—aspirin *k* 0.5, salicylic acid *k* 0.7; system HW—aspirin *k* 2.70, salicylic acid *k* 4.60; system HX—aspirin RI 350; system HY—aspirin RI 318; system HZ—aspirin RT 2.7 min; system HAX—salicylic acid RT 5.2 min; system HAY—salicylic acid RT 4.4 min.
Ultraviolet Spectrum Aqueous acid—230 (A^1_1 = 466a), 278 nm (A^1_1 = 68a); aqueous alkali—231 (A^1_1 = 409b), 298 nm (A^1_1 = 190b).



Infrared Spectrum Principal peaks at wavenumbers 1183, 1688, 1305, 1755, 925, 1219 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 120, 43, 138, 92, 121, 39, 64, 63 (aspirin); 120, 92, 138, 64, 39, 63, 121, 65 (salicylic acid); 121, 120, 69, 92, 195, 39, 93, 45 (salicyluric acid).

Quantification

Plasma **GC** Column: 5% OV-17 on CQ 80/100 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature programme: 160° to 200° at 2°/min. FID. Limit of detection, 200–500 μg/L for aspirin, salicylic acid and salicylamide [Rance *et al.* 1975].

GC-MS Column: reversed phase Luna C_{18} (50 × 2.0 mm i.d., 3 μm). Mobile phase: acetonitrile and water containing 0.1% formic acid (8 : 2), flow rate 0.2 mL/min. MRM acquisition mode, negative ion mode. Limit of quantification, 5 μg/L for aspirin and 50 μg/L for salicylic acid [Bae *et al.* 2008]. Column: Column: DB-1701 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 25 kPa. Temperature programme: 100° for 2 min to 300° at 25°/min. NICI or EI ionisation. Limit of quantification, 200 ng/L [Tsikas *et al.* 1998].

HPLC Column: MC-SAX (10 × 4 mm i.d., 50 μm). Mobile phase: water-acetonitrile (1000 : 10) containing 0.2% trifluroacetic acid : water-acetonitrile (100 : 900) containing 0.1% trifluroacetic acid (100 : 0 to 70 : 30 for 18 min for 2 min), flow rate 1.0 mL/min. UV detection (λ = 235 nm). Limit of quantification, 60 μg/L for aspirin and salicylic acid [Yamamoto *et al.* 2007]. Column: C_8 Nucleosil (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol : acetonitrile : orthophosphoric acid (650 : 200 : 150 : 1), flow rate 1 mL/min. UV detection (λ = 225 nm). Limit of quantification, 0.1 mg/L, limit of detection, 0.04 mg/L for aspirin and salicylic acid [McMahon, Kelly 1998]. Column: LiChrospher 100 RP-18. Mobile phase: water : phosphate buffer (pH 2.5) : acetonitrile (35 : 40 : 25), flow rate 1 mL/min. Limit of detection, 0.1 mg/L for aspirin and salicylic acid [Pirola *et al.* 1998]. Column: Alltech ODS (150 × 4.6 mm i.d., 5 μm). Mobile phase: 30 mmol/L sodium citrate : 30 mmol/L sodium acetate (pH 4.8), flow rate, 1.0 mL/min. UV detection (λ = 305 nm). Limit of detection, <5 μmol/L for salicylic acid [Coudray *et al.* 1996]. Column: Nova-Pak C_{18} (150 × 4 mm i.d., 4 μm). Mobile phase: water : 85% orthophosphoric acid : acetonitrile (740 : 0.9 : 180). UV detection (λ = 237 nm). Limit of quantification, 100 μg/L for aspirin and salicylic acid [Kees *et al.* 1996]. See Urine [Krivosiková *et al.* 1996]. See Oral Fluid [Legaz *et al.* 1992]. See also Buskin *et al.* [1982], Gaspari, Locatelli [1987], Owen *et al.* [1987] and Rumble *et al.* [1981].

Serum **HPLC** Column: ACE C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1% aqueous orthophosphoric acid (40 : 60 to 45 : 55 at 3.5 min to 70 : 30 at 5.5 min until 8 min to 40 : 60 at 9 min for 1 min), flow rate 1 mL/min for 3.5 min to 2 mL/min at 5.5 min to 3 mL/min at 7 min. Fluorescence detection (λ_{ex} = 290 nm, λ_{em} = 445 nm). Limit of detection, <0.05 pmol for aspirin, salicylic acid, piroxicam and mefanamic acid [Ibrahim *et al.* 2007]. Column: ODS. Mobile phase: methanol : 0.7 mmol/L phosphoric acid (pH 2.5, 50 : 50). UV detection. Limit of detection, 114 μg/L for aspirin and 38 μg/L for salicylic acid [Ohwaki *et al.* 2007].

Urine **HPLC** Column: LiChroCART (250 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : 0.05% trifluoroacetic acid in water (96 : 3 : 1 to 70 : 29 : 1 at 30 min to 50 : 49 : 1 at 60 min), flow rate 1.0 mL/min to 1.2 mL/min at 30 min to 1.4 mL/min at 60 min. DAD (λ = 200–450 nm). Limit of quantification, 0.46 mg/L, limit of detection, 0.14 mg/L [Baranowska *et al.* 2009]. Column: Separon SGX C_{18} (150 × 3.3 mm i.d.). Mobile phase: water : 85% phosphoric acid : butanol : tetrabutylammonium hydroxide : methanol (134 : 1 : 1 : 1 : 63), flow rate, 0.9 mL/min. UV detection (λ = 305 nm). Limit of detection, 0.2 μmol/L salicylic acid and salicyluric acid [Krivosiková *et al.* 1996]. Column: μBondapak Phenyl (300 × 4.6 mm i.d., 10 μm). Mobile phase: methanol : acetonitrile : TEA : 10 mmol/L potassium dihydrogen phosphate (18 : 110 : 1 : 871, pH 2.8), flow rate 1.0 mL/min. UV detection (λ = 237 nm). Limit of detection, 2 mg/L for salicyl acyl glucuronide, salicyl phenolic acid glucuronide and salicyluric phenolic glucuronide; 1 mg/L for gentisic acid; and 0.5 mg/L for salicylic acid and salicyluric acid [Shen *et al.* 1991].

Oral Fluid **HPLC** Column: Spherisorb ODS-2 (200 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water : acetic acid (400 : 98 : 2), flow rate 1.0 mL/min. UV detection (λ = 237 nm). Limit of detection, 100 g/L [Legaz *et al.* 1992].

Skin **HPLC** See Plasma. Limit of detection, 0.1 μg/cm^2 for aspirin and salicylic acid [Pirola *et al.* 1998].

Note For a review of the analysis of salicylates, see Hori *et al.* [2003].

Disposition in the Body

Aspirin is readily absorbed after oral administration and rapidly hydrolysed to salicylic acid, which is the active agent. Salicylic acid is conjugated with glucuronic acid and glycine to form acyl and ether glucuronides and salicyluric acid; some hydroxylation also occurs to give dihydroxy and trihydroxy derivatives of salicylic acid. Aspirin is excreted almost entirely in the urine with ~50–80% of a dose as salicyluric acid, 10–30% as salicyl *O*-glucuronide, 5% as salicyl ester glucuronide, and 5–10% as free salicylic acid, together with small amounts of gentisic acid, gentisuric acid, and unchanged drug; salicylates are reabsorbed by the renal tubules from acid urine and thus alkaline diuresis increases the rate of salicylate elimination; ~85% of a dose is excreted as free salicylic acid if the urine is made alkaline.

Aspirin is a metabolite of aloxiprin and benorilate.

Therapeutic Concentration In plasma, salicylic acid is usually in the range 20–100 mg/L for analgesia and 150–300 mg/L for anti-inflammatory effect.

After a single oral dose of 900 mg given to 5 subjects, peak plasma aspirin concentrations of 16–50 mg/L (mean, 37) were attained in ~14 min, and peak plasma salicylic acid concentrations of 47–113 mg/L (mean, 78) were reported at 0.5–1 h [Cham *et al.* 1980].

Following daily oral doses of 3.9 g to 8 subjects for 8 days, steady-state plasma salicylic acid concentrations of 105–227 mg/L (mean, 173) were reported; by the 36th day of treatment, the steady-state plasma concentrations had declined to 45–208 mg/L (mean, 129) [Rumble *et al.* 1980].

Toxicity The estimated minimum lethal dose is 15 g. Plasma concentrations of salicylic acid greater than 300 mg/L are likely to produce toxic reactions and concentrations greater than 500 mg/L are associated with moderate to severe intoxication. The maximum permissible atmospheric concentration is 5 mg/m^3.

In a review of 62 fatalities attributed to salicylate poisoning, the following postmortem tissue concentrations were reported: blood 61–7320 mg/L (mean, 661; 52 cases), brain 22–700 μg/g (mean, 218; 20 cases), kidney 19–1200 μg/g (mean, 408; 21 cases), liver 13–1000 μg/g (mean, 437; 24 cases), spleen 103–1230 μg/g (mean, 421; 16 cases), urine 180–1350 mg/L (mean, 593; 15 cases) [Rehling 1967].

A 43-year-old female attempted suicide by self-administration, in the form of an enema, of ~700 aspirin tablets dissolved in water. The initial salicylate concentration in the serum was 590 mg/L and rose to 900 mg/L 12 h later; after haemodialysis, serum salicylate concentration fell to 160 mg/L but the patient remained in a coma for more than a year. The patient's poor outcome was attributed to retention of aspirin products in the rectal vault plus poor recognition of the delayed absorption properties of rectally administered aspirin [Watson, Tagupa 1994].

In 3 cases of severe salicylate poisoning, haemofiltration treatment reduced serum salicylate from 8.5–3.5 mmol/L after 3 h in 1 patient and in another patient from 6.2–4 mmol/L after 4 h and to 1.4 mmol/L after a further 7 h. The third patient who had persistent salicylism (serum level of 3 mmol/L), serum salicylate levels were decreased with haemofiltration [Wrathall *et al.* 2001]

An 86-year-old woman who took an overdose of tablets each containing 300 mg aspirin had respiratory alkalosis and a blood salicylate concentration of 850 mg/L. As the patient was elderly and frail, she was treated with urinary alkalinisation and she showed rapid clinical improvement [Basavarajaiah *et al.* 2004].

A 14-year-old girl who had ingested 120 tablets each containing 81 mg aspirin had a salicylate level of 10 mg/L 4 h after ingestion and she denied having any adverse symptoms. She was given one dose of activated charcoal. At 35 h she had dizziness, tinnitus and epigastric discomfort and a salicylate level of 460 mg/L. The delayed salicylate toxicity was treated with a second dose of activated charcoal and IV bicarbonate and potassium [Rivera *et al.* 2004].

A 52-year-old woman who died after ingesting ~300 tablets each containing 325 mg aspirin had the following post-mortem salicylate levels: 1.1 g/L (heart blood), 1.3 g/L (femoral blood), 0.3–0.4 mg/g (brain), 0.9–1.4 mg/g (lung), 0.6–0.8 mg/g (liver) and 0.9 g/L (kidney) [Ihama *et al.* 2007].

A 41-year-old man died after ingesting ~200 aspirin tablets (65 g asprin). Blood levels of salicylate ~12 h after ingestion were 475 to 557 mg/L. Postmorten levels were 762 mg/L (heart blood) and 215 mg/L (femoral blood). Lethal levels were also found in all tested organs (e.g. 503 mg/L (liver) and 251 mg/L (brain) [Wollersen *et al.* 2007].

A 74-year-old patient admitted to intensive care because of impaired consciousness developed irreversible asystole ~6 h later and was found to have a plasma salicylate concentration of 876 mg/L [Galbois *et al.* 2009].

A 35-year-old man admitted to hospital 7.5 h after ingesting 400 tablets each containing 325 mg aspirin was treated with activated charcoal and IV hydration with sodium bicarbonate. 2 h after admission his salicylate concentration was 916 mg/L. He was treated with haemodialysis and 22 h after presentation, the salicylate level was 884 mg/L and he died 40 h after the overdose [Minns *et al.* 2010].

Half-life Plasma half-life, aspirin ~17 min, salicylic acid dose dependent (2–4 h after doses of less than 3 g, increasing to ~19 h after large doses).

Volume of Distribution Aspirin ~0.15 L/kg, salicylic acid ~0.1–0.2 L/kg (dose dependent).

Protein Binding Salicylic acid ~90% at concentrations below 100 μg/mL, decreasing to 50% at concentrations above 400 μg/mL.

Note For reviews of the pharmacokinetics of salicylates, see Levy [1978] and Mandelli, Tognoni [1980]. For reviews of salicylate poisoning, see Pearlman, Gambhir [2009] and Reingardiene, Lazauskas [2006].

Dose

Usually 1.2 to 4 g daily; doses of up to 8 g daily are given in acute rheumatic disorders. Low-dose aspirin for antiplatelet effects; initially 300 mg then 75 to 150 mg daily.

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Astemizole

Antihistamine

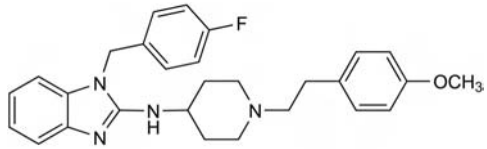
$C_{28}H_{31}FN_4O$ = 458.6

CAS—68844-77-9

IUPAC Name 1-[(4-Fluorophenyl)methyl]-*N*-[1-[2-(4-methoxyphenyl)ethyl] piperidin-4-yl]-benzimidazol-2-amine

Synonym R-43512

Proprietary Names *Alermizol*; *Esmacen*; *Hismacap*; *Hismanal*; *Histamen*; *Histaminos*; *Hubermizol*; *Laridal*; *Paralergin*; *Pollon-eze*; *Pollonis*; *Retolen*; *Rifedot*; *Rimbol*; *Romadin*; *Simprox*; *Urdrim*.



Chemical Properties A white or almost white powder. Mp 175° to 178°. Also described as white, odourless crystals. Mp 149.1°. Practically insoluble in water; soluble in ethanol; freely soluble in dichloromethane, methanol and other organic solvents. Log *P* (octanol/water), 6.43.

Thin-layer Chromatography System TAE—astemizoleR_f 0.56, M (nor) R_f 0.55; system TB—astemizole R_f 0.01, M (nor) R_f 0.00; system TE—astemizole R_f 0.64, M (nor) R_f 0.51.

Plates: silica gel 60 F_{254}. Solvent system: *n*-butanol : acetic acid : water (12 : 5 : 2). UV detection (λ= 280 nm). R_f 0.45 [Mangalan *et al.* 1991].

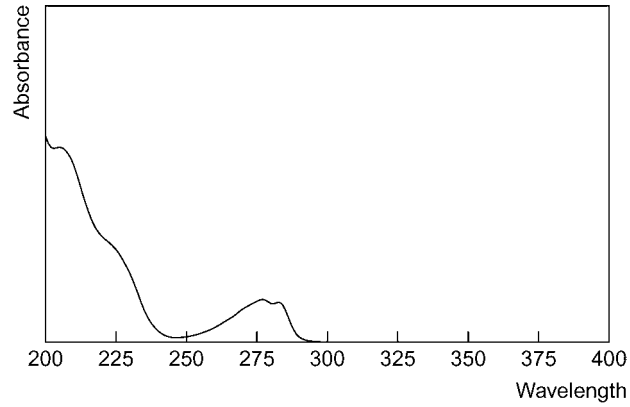
Plates: silica gel 60 F_{254} (20 × 20 cm, 0.25 mm). Solvent system: chloroform : methanol (85 : 15). UV detection (λ= 254 nm). R_f 0.67 (astemizole); 0.36 (demethylated derivative). R_f 1.86 relative to its metabolite [al Deeb *et al.* 1992].

Gas Chromatography System GA—astemizole RI 3150, M (*N*-desalkyl) RI 2470.

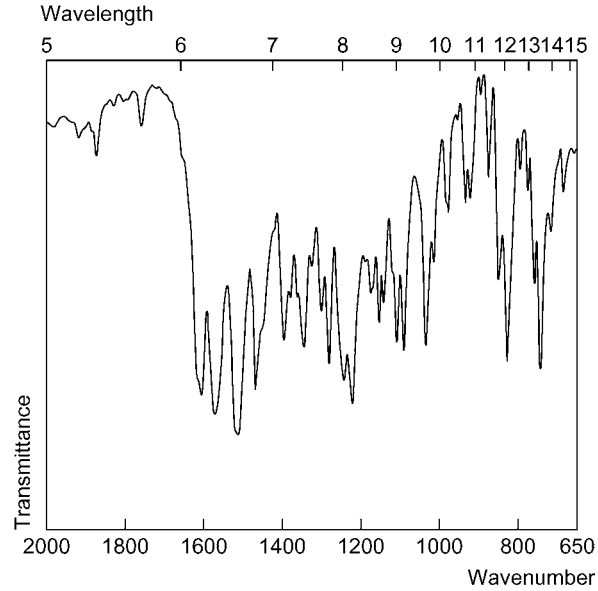
High Performance Liquid Chromatography System HX—astemizole RI 383, M (nor) RI 361; system HY—RI 286; system HZ—RT 3.9 min; system HAA—RT 13.2 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer (pH 3.5, 70 : 30), flow rate 1 mL/min. DAD. Retention time: 3.2 min [Mills, Roberson 1993].

Ultraviolet Spectrum Aqueous acid (ethanol)—219, 249, 286 (A^1_1= 306.5) nm; 0.1 mol/L hydrochloric acid—209, 277 nm.



Infrared Spectrum Principal peaks at wavenumber 1506, 1217, 738 cm^{-1} (KBr pellets).



Mass Spectrum Principal peaks at *m/z* 96, 109, 97, 294, 95, 135, 121, 242 (astemizole); 109, 241, 132, 83, 242, 111 M (*N*-desalkyl).

Quantification

***Other* HPLC** Animal Plasma and Tissue. Column: RSIL C_{18} HL reversed phase (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (50 : 50), flow rate 0.6 mL/min. UV detection (λ= 254 nm). Limit of detection, 1 µg/L in plasma, 5 ng/g in tissue [Woestenborghs *et al.* 1983].

Note For a spectrophotometric method for the determination of astemizole in pharmaceutical preparations, see Güngör, Onur [2001].

Disposition in the Body Astemizole is rapidly absorbed after oral administration. It is widely distributed although does not cross the blood–brain barrier to a significant extent. It is distributed into breast milk. The drug undergoes extensive first-pass metabolism mediated by the cytochrome P450 enzyme system (mainly by isoenzyme CYP3A4 and to a lesser extent by CYP2D6 and CYP2A6) [Matsumoto *et al.* 2002]. Desmethylastemizole is the major metabolite and has histamine H_1-blocking activity. Other metabolites include norastemizole and 6-hydroxydesmethylastemizole. The metabolites are slowly excreted, primarily in faeces via bile and undergo enterohepatic recycling. No unchanged drug has been detected and it is not known to accumulate once steady state levels have been reached.

Therapeutic Concentration Peak plasma concentrations are reached within 1–2 h. At steady state, the average peak plasma concentration of astemizole plus desmethylastemizole is 3–5 µg/L.

Toxicity Elevated plasma astemizole concentrations are associated with QT interval prolongation and serious cardiotoxicity. Arrhythmias, including torsade de pointes, have occurred rarely at doses as low as 20–30 mg daily.

A 4-year-old boy who ingested 1.7 mg/kg astemizole became lethargic 4 h later. The serum astemizole concentration measured 4.5 h after ingestion was 14 μg/L. Four other children aged 1.5–11 years who had ingested 2.2–12.2 mg/kg all had prolonged QT intervals on ECG recordings, which took 1–3 days to resolve [Wiley *et al.* 1992].

Plasma astemizole concentrations following accidental overdose in a 28-month-old child who had taken 16.7 mg/kg and in a 24-month old child who had taken 2.5 to 3.3 mg/kg were 250 and 15.9 μg/L, respectively (time after ingestion not stated). The child who had ingested 16.7 mg/kg developed ventricular fibrillation requiring DC cardioversion 46 h after ingestion. Both children made a full recovery [Hoppu *et al.* 1991].

A 14-year-old girl took an overdose of 200 mg astemizole. The peak plasma concentration was ~10 times greater than that seen after a single 20 mg dose. The apparent plasma half-life was 31 h [Kingswood *et al.* 1986].

A 16-year-old girl who had taken an overdose of 200 mg astemizole became unconscious with no cardiac output 7 h later. After another hour she developed ventricular tachyarrhythmia requiring DC cardioversion [Craft 1986].

Half-life Plasma half-life: astemizole 1–2 days, desmethylastemizole 9–13 days. The half-life of the drug and metabolites may increase to 18–20 days after long-term administration.

Volume of Distribution 250 L/kg.

Clearance Plasma, 11 mL/min/kg.

Protein Binding 96%.

Note For reviews of astemizole, see Richards *et al.* [1984] and Paton, Webster [1985].

Dose 10 mg daily (maximum).

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Atenolol

β-*Adrenoceptor Antagonist*

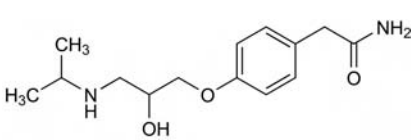
$C_{14}H_{22}N_2O_3 = 266.3$

CAS—29122-68-7

IUPAC Name 2-[4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide

Synonyms 2-{*p*-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}acetamide; 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide; ICI-66082.

Proprietary Names *Anselol*; *Antipressan*; *Ate*; *Atebeta*; *Atehexal*; *Atendol*; *Atenix*; *Ateno*; *Atenol*; *Betatop*; *Blocotenol*; *Cuxanorm*; *Duratenol*; *Evitocor*; *Falitonsin*; *Jenatenol*; *Juvental*; *Myocord*; *Noten*; *Prenormine*; *Tenoblock*; *Tenlol*; *Teno*; *Tenormin(e)*; *Tensig*; *Tonoprotect*; *Totamol*; *Uniloc*; *Xaten*. It is an ingredient of *AtenixCo*; *Beta-Adalat*; *Kalten*; *Tenben*; *Tenchlor*; *Tenif*; *Tenoret(ic)*; *Totaretic*.



Chemical Properties Crystals. Mp 146° to 148°. Slightly soluble in water and isopropanol; freely soluble in methanol; soluble in acetic acid and dimethylsulfoxide; very slightly soluble in acetone and dioxane; practically insoluble in acetonitrile, ethylacetate and chloroform. pK_a 9.6 (24°). Log *P* (octanol), 0.23. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

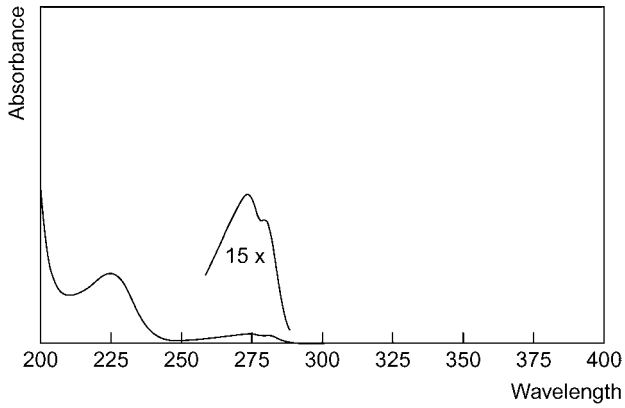
Colour Tests Liebermann's reagent—black; Nessler's reagent—yellow-brown (slow).

Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.00; system TC—R_f 0.02; system TE—R_f 0.22; system TL—R_f 0.02; system TAE—R_f 0.14.

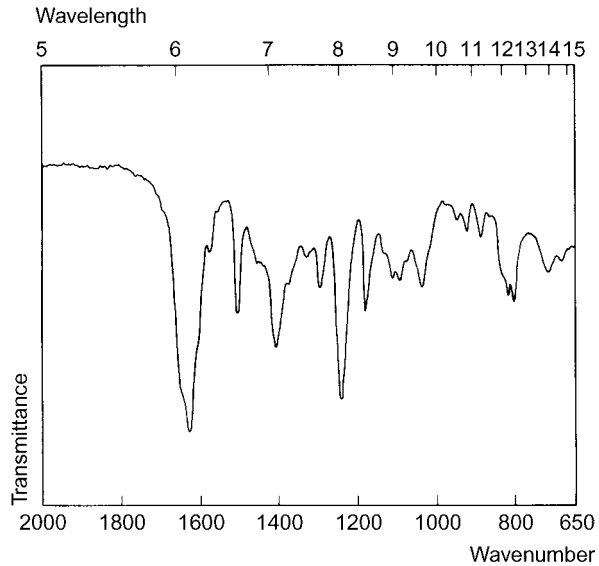
Gas Chromatography System GA—atenolol RI 2385, M (-H_2O) RI 2150, M (HOOC-)-Me RI 2140 and RI 2175; system GB—atenolol RI 2469, M (-H_2O) RI 2090.

High Performance Liquid Chromatography System HA—*k* 1.3; system HX—RI 243; system HY—RI 194; system HZ—RT 1.7 min; system HAA—RT 3.6 min.

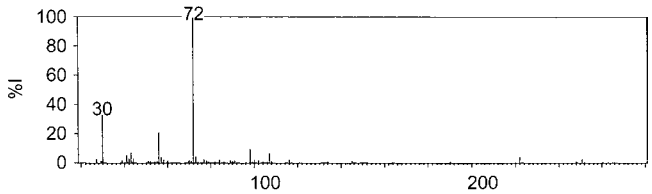
Ultraviolet Spectrum Aqueous acid—274 nm ($A_1^1 = 48$b), 280 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1633, 1245, 1510, 1184, 805, 820 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 72, 30, 56, 98, 43, 107, 41, 73.



Quantification

Blood **GC-MS** Column: HP-ULTRA-1 cross-linked 100% methylsiloxane capillary (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° to 290° at 30°/min for 2.67 min. SIM acquisition mode, full scan mode. Retention time: 5.74 min. Limit of detection not reported [Angier *et al.* 2005].

HPLC Column: Nucleosil C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : 0.05 mol/L phosphate triethylamine buffer (pH 3.3, 2 : 8 : 90), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 300 nm). Retention time: 4.4 min. Limit of quantification, 20 μg/L, limit of detection, 10 μg/L [Miller 1991]. Column: Varian Micro-Pak MCH-10 C_{18} (250 × 2.0 mm i.d.). Mobile phase: 0.01 mol/L heptanesulfonic acid in water : 0.01 mol/L heptanesulfonic acid in methanol (28 : 72), flow rate 40 mL/h. Fluorescence detection

A

(λ_{ex}= 212 nm, λ_{em}= 326.5 nm). Retention time: 10.8 min. Limit of detection, 1 μg/L [Yee *et al.* 1979].

LC-MS Column: Atlantis dC_{18} (150 × 2.1 mm i.d., 3.0 μm). Mobile phase: 10 mmol/L ammonium formate (pH 3.1) : acetonitrile (90 : 10 to 10 : 90 over 10 min for 3 min to 90 : 10 over 5 min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 0.03 μmol/L [Kristoffersen *et al.* 2007].

Plasma **TLC** Fluorescence detection. Limit of detection, 5 μg/L [Schäfer, Mutschler 1979].

GC Column: 3% OV-1 on Gas-Chrom Q 100/120 mesh (2 m × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 30 mL/min. Temperature: 300°. ECD. Limit of detection, 5 μg/L [Ervik *et al.* 1980].

HPLC Column: C_{18} Shim Pack (150 × 4.6 mm i.d., 4 μm). Mobile phase: 0.05 mol/L phosphate buffer (pH 5.5) : methanol (80 : 20), flow rate 0.7 mL/min. Fluorescence detection (λ_{ex}= 235 nm, λ_{em}= 290 nm). Retention time: 12.7 min. Limit of quantification, 8 μg/L, limit of detection, 4 μg/L [Leite Fda *et al.* 2006]. Column: ThermoHypersil Hypurity C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Limit of detection, 5–10 μg/L for atenolol and other β-blockers [Delamoye *et al.* 2004]. Fluorescence detection (λ_{ex}= 258 nm; λ_{em}= 300 nm) [de Abreu *et al.* 2003]. Column: Chiracel OD-H. Mobile phase: hexane : ethanol (85 : 15) plus 0.1% diethylamine. Limit of quantification, 10 μg/L for atenolol enantiomers [Iha *et al.* 2002]. Column ODS. Mobile phase: 0.05 mol/L SDS in phosphate buffer (pH 5.8) : propan-1-ol (95 : 5), flow rate 1.3 mL/min. Fluorimetric detection. Limit of detection, 10 μg/L [Giachetti *et al.* 1997]. Column: Alltech Spherisorb ODS-2 (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 0.5% TEA (pH 3.5, 18 : 72), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 229 nm, λ_{em}= 298 nm). Retention time: 4.5 min. Limit of detection, 1 μg/L [Chiu *et al.* 1997]. See also Egginger *et al.* [1993a] and He *et al.* [1993]. See Urine [Bühring, Garbe 1986]; Enquist, Hermansson 1989; [Mehvar 1989; Morris *et al.* 1991]. See Milk [Bhamra *et al.* 1983]. See Blood [Yee *et al.* 1979].

LC-MS Column: Capcell Pak C_{18} (50 × 2 mm i.d., 5 μm). Mobile phase: acetonitrile and water, both containing 0.02% formic acid, flow rate 0.3 mL/min. ESI, MRM acquisition mode. Atenolol and other antiarrhythmic drugs [Li *et al.* 2007]. Column: Chromsep SS (30 × 3.0 mm i.d.). Mobile phase: 5% methanol in 10 mmol/L formic acid and 10 mmol/L heptanfluorobutyric acid : 50% methanol in 10 mmol/L formic acid and 10 mmol/L heptanfluorobutyric acid (95 : 5 to 15 : 85 at 8 min for 1 min to 95 : 5 at 10 min for 6 min). ESI, SIM acquisitin mode. Limit of quantification, 25 μg/L [Ho *et al.* 2005]. Column: Hypersil (50 × 4.6 mm). Mobile phase: acetonitrile : water : acetic acid : trifluoroacetic acid (85 : 15 : 0.5 : 0.04), flow rate 2.0 mL/min. ESI mode [Li *et al.* 2005]. APCI, SIM acquisition mode. Atenolol and other β-blockers [Maurer *et al.* 2004].

Serum **HPLC** Fluorescence detection [Alebic-Kolbah *et al.* 1989].

Urine **TLC** See Plasma. Limit of detection, 500 μg/L [Schäfer, Mutschler 1979].

GC See Plasma [Ervik *et al.* 1980].

GC-MS See Blood [Angier *et al.* 2005].

HPLC Column: ADS-LiChrospher RP-18 (25 × 4 mm i.d., 25 μm). Mobile phase: acetonitrile : methanol : acetic acid : triethylamine (55 : 45 : 0.3 : 0.3). Fluorescence detection (λ_{ex}= 230 nm, λ_{em}= 310 nm). Limit of detection, 15 μg/L [Lamprecht *et al.* 2000]. Column: RP-18 (3.00 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : glacial acetic acid : water (30 : 1 : 69), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}= 222 nm, λ_{em}= 300 nm). Limit of detection, 0.5 μg/L [Morris *et al.* 1991]. Column: Chiral α1-AGP (100 × 4.0 mm i.d., 5 μm). Mobile phase: 0.01 mol/L phosphate buffer (pH 7.1) containing 0.25% acetonitrile, flow rate 0.9 mL/min. Limit of detection, <6 μg/L for atenolol enantiomers [Enquist, Hermansson 1989]. Column: C_{18} (100 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile : methanol (43 : 35 : 22), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}= 195 nm, no emission filter). Limit of detection not reported [Mehvar 1989]. Column: LiChrosorb Si-60 (125 × 4 mm i.d., 7 μm). Mobile phase: 1 mol/L ammonium phosphate buffer (pH 4) : water : acetonitrile (5 : 92 : 3), flow rate 1.8 mL/min. Fluorimetric detection (λ_{ex}= 225 nm, λ_{em}= 300 nm). Limit of detection, 50 μg/L (5 μg/L in plasma) [Bühring, Garbe 1986]. See Plasma [Chiu *et al.* 1997; Iha *et al.* 2002]. See Milk [Bhamra *et al.* 1983].

LC-MS Column: Uptisphere HDD C_{18} (100 × 2 mm i.d., 3 μm). Mobile phase: 10 mmol/L ammonium formate buffer (pH 3.9) : methanol (83 : 17 to 60 : 40 in 22 min to 0 : 100 in 8 min to 83 : 17 for 15 min), flow rate 0.3 mL/min. ESI. Limit of quantification, 0.69 μg/L [Pujos *et al.* 2009]. Column: fused silica capillary (75 μm; Nano-LC), packed with chiral modified silica particles (5 μm). Mobile phase: 500 mmol/L ammonium acetate (pH 4.5) : methanol: acetonitrile (1 : 60 : 39), flow rate 900 nL/min. ESI. Limit of quantification, 400 μg/L, limit of detection, 50 μg/L, for atenolol enantiomers [D'Orazio, Fanali 2006].

Biological Samples **HPLC** Enantiomers of atenolol and other β-blockers [Egginger *et al.* 1993b].

LC-MS Column: Supelcosil LC_{18} (150 × 4.6 mm i.d., 3 μm). Mobile phase: aqueous mixture : methanol (90 : 10 to 10 : 90 at 5 min to 90 : 10 at 7 min for 3 min), flow rate 1.0 mL/min. APCI, PCI. Limit of quantification, 1.6 μg/L, limit of detection, 0.78 μg/L [Johnson, Lewis 2006].

Milk **HPLC** Column: Microparticulate silica (5 μm). Mobile phase: methanol containing 1 mmol/L *d*-10-camphorsulfonic acid monohydrate. Fluorescence detection (λ_{ex}= 195 nm). Limit of quantification, 20 μg/L [Bhamra *et al.* 1983].

Heart **GC-MS** See Blood [Angier *et al.* 2005].

Kidney **GC-MS** See Blood [Angier *et al.* 2005].

Liver **GC-MS** See Blood [Angier *et al.* 2005].

Disposition in the Body Atenolol is rapidly but incompletely absorbed after oral administration; bioavailability is ~50%. It is excreted almost entirely as unchanged drug, 35–50% of an oral dose being excreted in the urine and 30–50% in the faeces in 24 h; small amounts of 2-hydroxyatenolol and atenolol glucuronide are excreted in the urine.

Therapeutic Concentration

After a single oral dose of 100 mg given to 12 subjects, peak plasma concentrations of 0.41–0.87 mg/L (mean, 0.6) were attained in ~3 h [Mcainsh *et al.* 1980].

Following daily oral doses of 25, 50 and 100 mg to 7, 7 and 6 subjects, respectively, mean maximum steady-state plasma concentrations of 0.12, 0.22 and 0.39 mg/L, respectively, were reported 4 h after a dose; the corresponding mean trough concentrations were 0.02, 0.04 and 0.07 mg/L [Ishizaki *et al.* 1983].

Toxicity Atenolol is relatively non-toxic; there is one case report of ingestion of 1.2 g with subsequent recovery.

A 44-year-old obese female survived after ingesting 200 mg diazepam and 1000 mg atenolol in a suicide attempt. Upon admission to hospital 24 h later, she was in deep coma with a blood atenolol level of 250 mg/L [Hagemann 1986].

A man survived after ingesting a massive overdose of atenolol and diltiazem despite having an atenolol level of 35 mg/L [Snook *et al.* 2000].

A 28-year-old patient with end-stage renal failure suffered cardiac arrest and cardiogenic shock after receiving an overdose of atenolol. During haemodialysis with charcoal haemoperfusion (four dialyses carried out over 72 h), plasma atenolol levels declined from 7.4–2.1 mg/L [Salhanick, Wax 2000].

A woman in her twenties had fatal cardiac arrhythmias after an overdose of atenolol and pilsicainide. Blood levels were 4.94 mg/L and 7.83 mg/L, respectively [Hikiji *et al.* 2008].

A 45-year-old woman with liver cirrhosis who took a massive overdose of atenolol and nifedipine (blood levels of 69.6 mg/L and 63 μg/L, respectively), survived after treatment with continuous veno-venous haemo-diafiltration [Pfaender *et al.* 2008].

Half-life Plasma half-life, 4–14 h (mean, 7).

Volume of Distribution ~0.5–1.5 L/kg.

Clearance Plasma clearance, ~2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~0.8–0.9.

Protein Binding <5%.

Note For reviews of the pharmacokinetics of atenolol, see Heel *et al.* [1979] and Kirch, Gorg [1982]. For a discussion of the pharmacokinetics, toxicology and management of β-blocker overdose, see O'Grady *et al.* [2001].

Dose 50 to 100 mg daily.

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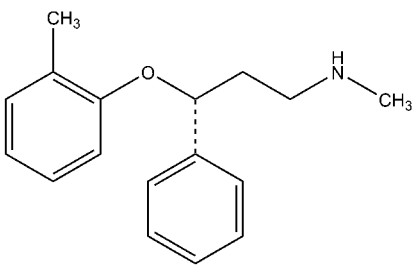
Atomoxetine

SSNRI, Treatment of ADHD
$C_{17}H_{21}NO$ = 255.4
CAS—83015-26-3

IUPAC Name (3*R*)-*N*-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine
Synonyms LY-135252; LY-139602; LY-139603; (γ*R*)-*N*-methyl-γ-(2-methylphenoxy)-benzenepropanamine; (—)-*N*-methyl-3-(*o*-tolyloxy)-3-phenylpropylamine; tomoxetine.



Chemical Properties Log *P* (octanol/water), 4.66 [Wishart 2006].

Atomoxetine Hydrochloride

$C_{17}H_{21}NO,HCl$ = 291.8
CAS—82248-59-7
IUPAC Name (3*R*)-*N*-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine hydrochloride
Proprietary Name *Strattera*
Chemical Properties Crystals or white solid. Mp 166° to 168° or 162° to 164°. Soluble in water. Provided in the pure *R*(−)-isomer form. pK_a 10.1. Stability was demonstrated for stock solutions as well as in plasma and urine [Mullen *et al.* 2005].

Mass Spectrum Principal ions at *m/z* 44, 148, 255 [Garside *et al.* 2006].

Quantification

Blood **GC** Column: DB-5 capillary (15 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 6.7 mL/min. Temperature programme: 120° to 300° at 15°/min for 3 min. NPD. Limit of quantification, 0.1 mg/L; limit of detection, 0.05 mg/L [Garside *et al.* 2006].

Plasma **HPLC** Column: Intersil ODS-3 C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 6.6; 39 : 61), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 2.5 µg/L [Guo *et al.* 2007]. Column: Agilent SB-C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L heptane sulfonic acid buffer with 1% triethylamine (pH 4.8; 40 : 60), flow rate 1 mL/min. UV detection (λ = 272 nm). Limit of quantification, 50 µg/L [Patel *et al.* 2007]. Column: Luna C_{18} (2) reversed phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (75 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 318 nm, λ_{em} = 448 nm). Limit of detection, 0.3 µg/L [Zhu *et al.* 2007].

LC-MS Column: Brownlee Spheri-5 C_{18} polyfunctional (100 × 4.6 mm i.d., 5 µm). Mobile phase: water : 5 mmol/L ammonium acetate-47.2 mmol/L formic acid-4 mmol/L trifluoroacetic acid in acetonitrile-water (85 : 15; 25 : 75 until 0.8 min to 0 : 100 at 1 min until 2.5 min to 25 : 70 at 3 min), flow rate 1.0 mL/min. APCI , positive ion mode. SRM acquisition mode. Limit of detection not reported [Mullen *et al.* 2005].

Urine **GC** See Blood [Garside *et al.* 2006].

LC-MS Column: Spheri-5 C_{18} polyfunctional (100 × 4.6 mm i.d., 5 µm). Mobile phase: water : 5 mmol/L ammonium acetate-47.2 mmol/L formic acid-4 mmol/L trifluoroacetic acid in acetonitrile-water (85 : 15; 25 : 75 until 0.8 min to 0 : 100 at 1 min until 2.5 min to 25 : 70 at 3 min), flow rate 1.0 mL/min. APCI , positive ion modeSRM acquisition mode. Limit of detection not reported [Mullen *et al.* 2005].

Vitreous Humour **GC** See Blood [Garside *et al.* 2006].

Bile **GC** See Blood [Garside *et al.* 2006].

Liver **GC** See Blood [Garside *et al.* 2006].

Disposition in the Body Rapidly absorbed after oral administration and eliminated primarily through oxidative metabolism by CYP2D6 and subsequent glucuronidation. The major oxidative metabolite is 4-hydroxyatomoxetine-*O*-glucuronide, which is mainly excreted in urine (80% of the administered dose) and <17% in faeces. A second metabolite, *N*-desmethylatomoxetine, is formed by the action of CYP2C19. Less than 3% is excreted as the unchanged drug. Peak plasma concentrations occur 1 to 2 h after dosing. Administration of the drug with a high fat meal decreases the rate of absorption and peak plasma concentrations. The drug is primarily distributed into total body water.

Therapeutic Concentration Therapeutic plasma concentrations after 40 mg a day for 5 days are 0.036 to 0.116 mg/L for extensive metabolisers and 0.500 to 0.915 mg/L for poor metabolisers [Garside *et al.* 2006].

A single-dose, open label dose titration study with 21 children who were CYP2D6 extensive metabolisers showed that atomoxetine was rapidly absorbed, with peak plasma concentrations occurring 1 to 2 h after dosing. Half-life averaged 3.12 and 3.28 h after a single dose and at steady state, respectively [Witcher *et al.* 2003].

Toxicity CYP2D6 inhibitors, such as paroxetine, fluoxetine and quinidine, may increase plasma concentrations of atomoxetine in extensive, but not poor, metabolisers. For a case report of acute atomoxetine and oxcarbazepine overdose with quetiapine, see Barker *et al.* [2004][No levels reported]. Garside *et al.* [2006] have provided 3 case reports:

Case 1. An 11-year-old Caucasian female collapsed during a ball game at school. She had been diagnosed with attention-deficit hyperactivity disorder (ADHD) for which she was originally prescribed dextroamfetamine. Several weeks prior to her death, she was started on atomoxetine while continuing treatment with dextroamfetamine. The death was ruled to be unrelated to atomoxetine [Garside *et al.* 2006].

Case 2. A 19-year-old Caucasian female with a 3-year history of depression and previous suicide attempts was found at home with poor respiration. A suicide note was found at the scene with several empty pill bottles. She was pronounced dead 2 h later. The deceased had been prescribed venlafaxine, ziprasidone, clonazepam, and atomoxetine [Garside *et al.* 2006].

Case 3. A 24-year-old Hispanic male with a history of ADHD was found dead on the floor of his home. He had been prescribed atomoxetine 20 mg twice a day. The death was ruled to be unrelated to atomoxetine [Garside *et al.* 2006].

Postmortem fluid and tissue distribution of atomoxetine were measured by GC-MS for all three cases:

	Atomoxetine (mg/L or mg/kg)						
	Aorta blood	*Femoral blood*	*Vitreous*	*Bile*	*Urine*	*Liver*	*Gastric (mg total)*
Case 1	0.65	0.33	0.1	1	NA	3.9	0.0097
Case 2	8.3	5.4	0.96	33	NA	29	16.8
Case 3	<0.1	<0.1	N/A	NA	<0.1	<0.44	NA

NA, not available [Garside *et al.* 2006].

Bioavailability Approximately 63% for extensive metabolisers and 94% for poor metabolisers.

Half-life Approximately 5.2 h for adult extensive metabolisers and 21.6 h for poor metabolisers.

Volume of Distribution Steady state, 0.85 L/kg.

Clearance Plasma, 0.35 L/h/kg for extensive metabolisers and 0.03 L/h/kg for poor metabolisers

Protein Binding Approximately 98%.

A

Dose Children and adults <70 kg bodyweight: 0.5 mg/kg atomoxetine hydrochloride daily, increased to 1.2 mg/kg bodyweight daily after 3 days. Patients >70 kg bodyweight: 40 mg daily increased to 80 mg after 3 days and up to 100 mg after 2 to 4 weeks.

Barker MJ *et al.* (2004). Acute oxcarbazepine and atomoxetine overdose with quetiapine. *Vet Hum Toxicol* 46: 130–132.

Garside D *et al.* (2006). Postmortem tissue distribution of atomoxetine following fatal and nonfatal doses–three case reports. *J Forensic Sci* 51: 179–182.

Guo W *et al.* (2007). Determination of atomoxetine in human plasma by a high performance liquid chromatographic method with ultraviolet detection using liquid-liquid extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 128–134.

Mullen JH *et al.* (2005). Simultaneous quantification of atomoxetine as well as its primary oxidative and *O*-glucuronide metabolites in human plasma and urine using liquid chromatography tandem mass spectrometry (LC/MS/MS). *J Pharm Biomed Anal* 38: 720–733.

Patel C *et al.* (2007). A new high performance liquid chromatographic method for quantification of atomoxetine in human plasma and its application for pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 356–360.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Witcher JW *et al.* (2003). Atomoxetine pharmacokinetics in children and adolescents with attention deficit hyperactivity disorder. *J Child Adolesc Psychopharmacol* 13: 53–63.

Zhu HJ *et al.* (2007). Sensitive quantification of atomoxetine in human plasma by HPLC with fluorescence detection using 4-(4,5-diphenyl-1H-imidazole-2-yl) benzoyl chloride derivatization. *J Chromatogr B Analyt Technol Biomed Life Sci* 846: 351–354.

Atorvastatin

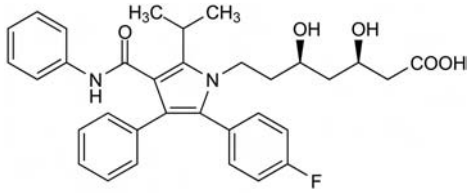
HMG-CoA Reductase Inhibitor

$C_{33}H_{35}FN_2O_5$ = 558.7

CAS—134523-00-5

IUPAC Name [*R*-(*R**,*R**)]-2-(4-Fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid

Synonym CI-981



Chemical Properties pK_a 4.46. Log *P* (octanol/water), 6.36.

Atorvastatin Calcium

$C_{66}H_{68}CaF_2N_4O_{10},3H_2O$ = 1209.4

CAS—134523-03-8

Proprietary Names *Lipitor*; *Sortis*; *Torvast*; *Totalip*; *Xarator*.

Chemical Properties A white to off-white crystalline powder, which is insoluble in aqueous solutions with pH≤4.0. It is very slightly soluble in distilled water, phosphate buffer (pH 7.4) and acetonitrile; slightly soluble in ethanol; freely soluble in methanol.

Atorvastatin Lactone

$C_{33}H_{33}FN_2O_4$ = 540.6

Chemical Properties Mp 159.2° to 160.7°. Solubility, 1.34 ± 0.53 mg/L (pH 2.3 to 7.7 at 30°).

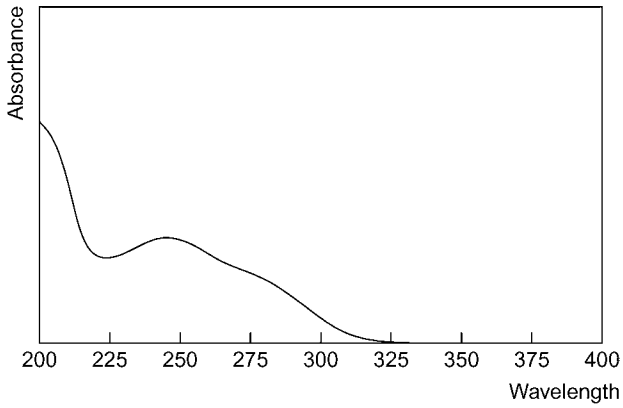
Atorvastatin Sodium

$C_{33}H_{34}FN_2NaO_5$ = 580.6

Chemical Properties Soluble in water at 30°, with a solubility of 20.4 mg/L at pH 2.1 and 1.23 g/L at pH 6.0.

High Performance Liquid Chromatography Column: ODS Ultrasphere (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : sodium acetate in water (pH 4.0), flow rate 1.0 mL/min. UV detection (λ=246 nm). Retention time: atorvastatin sodium, 4 min; atorvastatin lactone, 7 min [Kearney *et al.* 1993].

Ultraviolet Spectrum



Quantification

***Plasma* HPLC** Column: C_{18} YMC J'Sphere H80 (150 × 2 mm i.d., 4 µm). Mobile phase: acetonitrile : acetic acid (0.1%) (70:30). Flow rate 0.2 mL/min. MS–MS detection. Retention time: para-hydroxyatorvastatin, 2.27 min; ortho-hydroxyatorvastatin, 3.36 min; atorvastatin, 4.12 min. Limit of quantification, 0.25 µg/L [Bullen *et al.* 1999].

Bioassay Limit of quantification, 0.36 µg/L [Shum *et al.* 1998]. Limit of quantification, 0.3 µg/L [Gibson *et al.* 1996].

***Serum* HPLC** Column: YMC basic (2 × 50 mm, 5 µm) (at 40°). Mobile phases: (gradient elution) (A) water: methanol : formic acid (88%) (950 mL : 50 mL : 43 µL), (B) acetonitrile : methanol: formic acid (950 mL : 50 mL : 43 µL). 70% A and 30% B to 45% A and 55% B, 0 to 1 min; hold 0.5 min; to 70% A and 30% B, 0.1 min; hold, flow rate 0.3 mL/min. MS–MS detection. Retention time: atorvastatin, 2.78 min; 2-hydroxyatorvastatin, 2.69 min; 4-hydroxyatorvastatin, 2.31 min. Limit of quantification, 0.5 µg/L [Jemal *et al.* 1999].

Disposition in the Body Atorvastatin is rapidly absorbed after oral administration, which increases in proportion to the dose administered. Absolute bioavailability is ~12% due to presystemic clearance in the gastrointestinal mucosa and metabolism by cytochrome P450 3A4 in the liver. A number of metabolites have been identified, all with inhibitory activity, including 2- and 4-hydroxyatorvastatin, *ortho*- and *para*-hydroxylated derivatives and various β-oxidation products. Atorvastatin is excreted as metabolites, primarily in bile, with <2% of the dose present in urine.

Therapeutic Concentration

Sixteen young, healthy men and women, aged between 19 and 35 years, and 16 healthy, elderly men and women, 66 to 92 years old, were administered with a 20-mg dose of atorvastatin, after fasting for 8 h. Maximum plasma concentrations for the young volunteers reached 12.7 µg/L within 1 to 2 h and 18.1 µg/L for the elderly. Differences were also observed between the men and women with 14.2 µg/L (within 2.3 h) and 16.7 µg/L (1.4 h), respectively [Gibson *et al.* 1996].

Toxicity

A 73-year-old, moderately obese woman with type II diabetes and hypertension was admitted to hospital. She had been receiving a number of medications with no other problems and was prescribed a 10 mg daily dose of atorvastatin. 4 days later, she developed a red, itchy, painful rash (potentially life-threatening dermatosis). Treatment with atorvastatin was stopped but she still developed severe stomatitis, diffuse erythema, oedema of face, trunk and extremities, and other symptoms. Supportive care including intravenous fluids, morphine, corticosteroids and a liquid diet were required. She eventually recovered after being seriously ill for 4 months [Pfeiffer *et al.* 1998].

Half-life Elimination half-life, approximately 14 h.

Volume of Distribution Mean, approximately 565 L.

Distribution in Blood Blood cells : plasma, 0.25.

Protein Binding 98%

Dose An initial dose of 10 mg daily is administered with a maximum of 80 mg daily.

Bullen WW *et al.* (1999). Development and validation of a high-performance liquid chromatography tandem mass spectrometry assay for atorvastatin, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin in human, dog, and rat plasma. *J Am Soc Mass Spectrom* 10: 55–66.

Gibson DM *et al.* (1996). Effect of age and gender on pharmacokinetics of atorvastatin in humans. *J Clin Pharmacol* 36: 242–246.

Jemal M *et al.* (1999). Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 13: 1003–1015.

Kearney AS *et al.* (1993). The interconversion kinetics, equilibrium, and solubilities of the lactone and hydroxyacid forms of the HMG-CoA reductase inhibitor, CI-981. *Pharm Res* 10: 1461–1465.

Pfeiffer CM *et al.* (1998). Toxic epidermal necrolysis from atorvastatin. *JAMA* 279: 1613–1614.

Shum YY *et al.* (1998). Development, validation, and interlaboratory comparison of an HMG-CoA reductase inhibition assay for quantitation of atorvastatin in plasma matrices. *Ther Drug Monit* 20: 41–49.

Atovaquone

Antiprotozoal

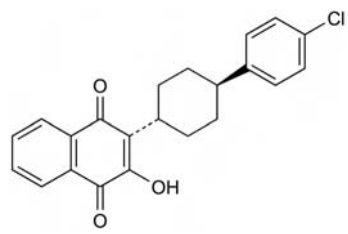
$C_{22}H_{19}O_3Cl$ = 366.8

CAS—95233-18-4

IUPAC Name *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthalenedione

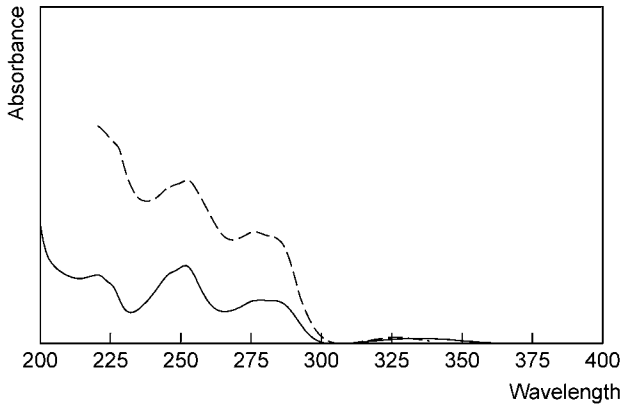
Synonyms 566C80; BW-566C; BW-566C-80.

Proprietary Names *Acuvel*; *Mepron*; *Wellvone*.

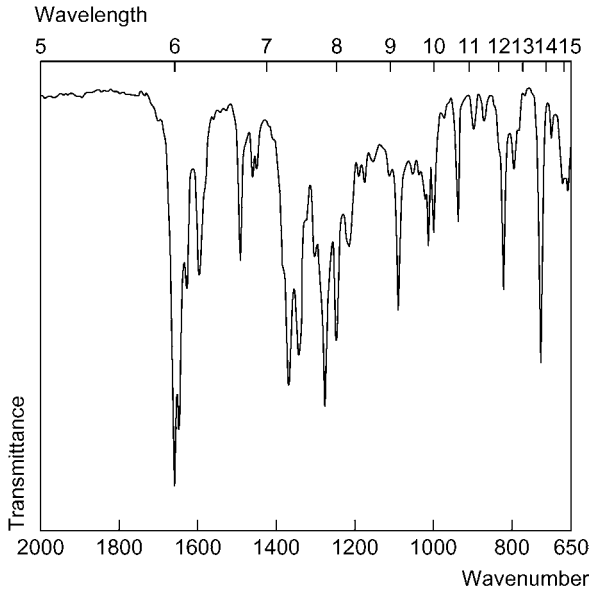


Chemical Properties A yellow crystalline solid. Also, reported as crystals from acetonitrile. Mp 216° to 219°. It is practically insoluble in water (>0.2 mg/L); poorly soluble in a variety of organic solvents.

Ultraviolet Spectrum Basic—252, 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1659, 1278, 1370 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 202, 366, 115, 77, 368, 125, 105, 213.

Quantification

Plasma **GC** ECD. Sensitivity, 0.01 mg/L [Rolan *et al.* 1994].

HPLC UV detection (λ=254 nm). Limit of quantification, 0.1 mg/L [Hannan *et al.* 1996]. Column: C_6 Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L triethylamine in 0.2% aqueous trifluoroacetic acid, pH 2 (76:24), flow rate 1 mL/min. Retention time: 8.8 min. UV detection. Limit of detection, 0.5 mg/L [Hansson *et al.* 1996]. UV detection. Limit of detection, 0.25 mg/L [Studenberg *et al.* 1995]. UV detection (λ=254 nm). Limit of quantification, 0.25 mg/L [DeAngelis *et al.* 1994]. UV detection. Limit of detection, 0.05 mg/L [Rolan *et al.* 1994].

Disposition in the Body Atovaquone is rapidly but poorly absorbed after oral administration; bioavailability shows considerable inter-individual variation and is increased by concomitant administration with food, especially food with high fat content. The drug is excreted almost entirely in faeces (94% of an administered dose over 21 days) as the unchanged drug, with little present in urine. There is no evidence that atovaquone is metabolised in the body. It may undergo enterohepatic recycling. Plasma concentrations do not increase proportionally with dose.

Therapeutic Concentration

Six male individuals, HIV-sero-positive, with CD4 counts >200 cells/mm^3 were administered with single 500, 1000 and 1500 mg doses after an overnight fast. Peak plasma concentrations reached 2.7, 5.6 and 4.3 mg/L, respectively, between 1.5 and 2.5 h. Following administration of 1000 mg daily with a high-fat meal and 1000 mg twice daily with or without a high-fat meal, for 14 days, the mean concentrations were 24.2, 41.3 and 34.4 mg/L, respectively. These levels were detected at 9.1, 3.7 and 2.1 h [Dixon *et al.* 1996].

The mean peak plasma concentration occurring 6 h after administration of 750 mg three times a day for 5 days followed by 750 mg twice a day for 16 days, with a standard breakfast, was 51 mg/L in 4 male subjects, aged over 18 years, with CD4 counts ≥250 cells/mm^3. Single doses of 100, 250, 750, 1500 and 3000 mg administered to the same group produced peak concentrations of 4.5, 14.3, 37.9, 33.4 and 39.0 mg/L, respectively, observed between 4 and 8 h [Hughes *et al.* 1991].

Toxicity It is safe to give doses of up to 3000 mg daily.

Half-life Plasma, between 60 and 70 h.

Volume of Distribution 0.6 L/kg.

Clearance Plasma, 0.15 mL/min/kg; also, reported as 1.56 L/h.

Protein Binding >99%.

Note For reviews of atovaquone see Haile, Flaherty [1993] and Spencer, Goa [1995].

Dose 750 mg as an oral suspension twice a day or 750 mg as tablets three times a day.

DeAngelis DV *et al.* (1994). High-performance liquid chromatographic assay for the measurement of atovaquone in plasma. *J Chromatogr* 652: 211–219.

Dixon R *et al.* (1996). Single-dose and steady-state pharmacokinetics of a novel microfluidized suspension of atovaquone in human immunodeficiency virus-seropositive patients. *Antimicrob Agents Chemother* 40: 556–560.

Haile LG, Flaherty JF (1993). Atovaquone: a review. *Ann Pharmacother* 27: 1488–1494.

Hannan SL *et al.* (1996). Determination of the potent antiprotozoal compound atovaquone in plasma using liquid-liquid extraction followed by reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 678: 297–302.

Hansson AG *et al.* (1996). Rapid high-performance liquid chromatographic assay for atovaquone. *J Chromatogr B Biomed Sci Appl* 675: 180–182.

Hughes WT *et al.* (1991). Safety and pharmacokinetics of 566C80, a hydroxynaphthoquinone with anti-Pneumocystis carinii activity: a phase I study in human immunodeficiency virus (HIV)-infected men. *J Infect Dis* 163: 843–848.

Rolan PE *et al.* (1994). Examination of some factors responsible for a food-induced increase in absorption of atovaquone. *Br J Clin Pharmacol* 37: 13–20.

Spencer CM, Goa KL (1995). Atovaquone. A review of its pharmacological properties and therapeutic efficacy in opportunistic infections. *Drugs* 50: 176–196.

Studenberg SD *et al.* (1995). A robotics-based liquid chromatographic assay for the measurement of atovaquone in plasma. *J Pharm Biomed Anal* 13: 1383–1393.

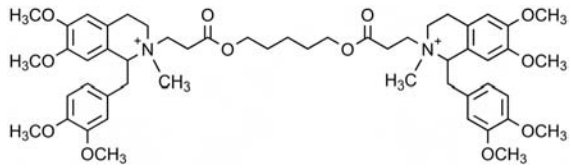
Atracurium

Skeletal Muscle Relaxant

$C_{53}H_{72}N_2O_{12}$ = 929.2

CAS—64228-79-1

IUPAC Name 2,2′-[1,5-Pentanediylbis[oxy(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)-methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolinium]



Atracurium Besilate

$C_{53}H_{72}N_2O_{12}.2C_6H_5O_3S$ = 1243.5

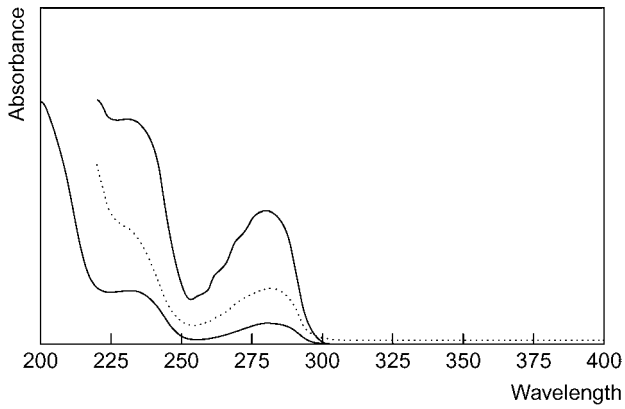
CAS—64228-81-5

Synonyms Atracurium besylate; BW-33A.

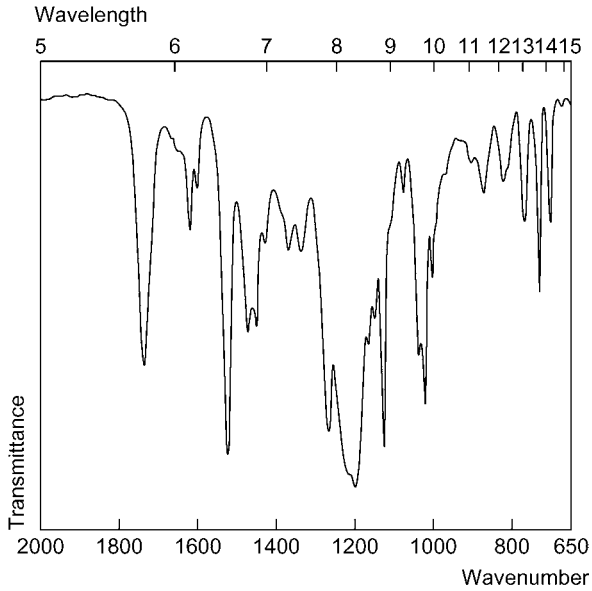
Proprietary Name *Tracrium*

Chemical Properties An off-white powder. Mp 85° to 90°. Softens at 60°.

Ultraviolet Spectrum Ethanol—231, 280 nm; basic—281 nm.



Infrared Spectrum Principal peaks at wavenumber 1725, 1513, 1189 cm^{-1} (KBr pellets).



Quantification

Plasma **HPLC** Column: μPorasil (150 × 3.9 mm i.d.). Mobile phase: acetonitrile : 0.002 mol/L sulfuric acid (50:50), flow rate 2 mL/min. UV detection (λ=210 nm). Retention time: 3.5 min. Limit of detection, 0.025 mg/L for atracurium and laudanosine [Bjorksten *et al.* 1990]. Column: Spherisorb C_8 (100 × 4.9 mm, 5 μm). Mobile phase: (A) acetonitrile : methanol : 0.03 mol/L dibasic potassium phosphate buffer (37.5:5:57.5); (B) acetonitrile : methanol : 0.1 mol/L dibasic potassium phosphate buffer (37.5:15:47.5), final pH 5. Elution programme: 0 to 100% (B) over 8 min, flow rate 1.7 mL/min. Fluorescence detection (λ_{ex}=240 nm, λ_{em}=320 nm). Retention time: 6.2 min. Limit of detection, 0.02 mg/L for atracurium, 0.01 mg/L for laudanosine [Varin *et al.* 1990]. Fluorescence detection. Limit of detection, about 0.01 mg/L for atracurium, 0.005 mg/L for laudanosine [Simmonds 1985].

Disposition in the Body After IV administration, it is inactivated in plasma by Hofmann elimination, a non-enzymatic breakdown process occurring at physiological pH and temperature, to produce laudanosine and other metabolites. It also undergoes ester hydrolysis by non-specific plasma esterases. The metabolites have no neuromuscular blocking activity although laudanosine may have some stimulatory action on the CNS. Excreted in urine and bile, mostly as metabolites. It crosses the placenta.

Therapeutic Concentration The serum therapeutic concentration range is 0.1 to 1.0 mg/L.

A study in 20 male and female subjects undergoing major surgery, with a mean age of 59.4 years, found that 90% paralysis was maintained by a mean infusion rate of 4.25 μg/kg/min of atracurium (mean plasma concentration 1.13 mg/L) [Beemer *et al.* 1990].

A study in 20 subjects on mechanical ventilation receiving atracurium infusion found that stable neuromuscular blockade was associated with a plasma-atracurium concentration of 1.5 mg/L, but this had increased to 5.3 mg/L at 38 h. Plasma-laudanosine concentrations were measured in 6 of the subjects and the maximum concentrations measured ranged from 1.9 to 5.1 mg/L [Yate *et al.* 1987].

An atracurium infusion was given for 71 days to a 35-year-old IV drug abuser who contracted tetanus. The mean rate of infusion over the period was 1.3 mg/kg/h. There was no evidence of accumulation of laudanosine as plasma concentration at the end of the infusion was 0.985 mg/L. The plasma-atracurium concentration was 1.5 mg/L [Peat *et al.* 1988].

Toxicity

A 59-day-old infant weighing 2.75 kg received 37 mg of atracurium over 75 min when the syringe driver was set up incorrectly. The infant made a full recovery by 135 min after the infusion was stopped. It was estimated that the plasma-laudanosine concentration at the time neuromuscular function recovered was about 19 mg/L. No atracurium was detectable in the plasma sample taken [Charlton *et al.* 1989].

A 3-week-old ventilated neonate weighing 3.9 kg received a bolus overdose of atracurium of 5.1 mg/kg in error. Intense bronchospasm occurred, systolic blood pressure fell to 28 mmHg and pulse rate increased from 140/min to 180/min. The neonate recovered within 3 h following treatment with adrenaline and ventilatory support [Durcan, Carter 1986].

Half-life Plasma, atracurium 20 min; laudanosine 3 h.

Volume of Distribution 0.16 L/kg.

Clearance Plasma, 4.4 to 6.5 mL/min/kg.

Protein Binding 80%

Dose Initially 300 to 600 μg/kg of atracurium besilate intravenously with subsequent doses of 100 to 200 μg/kg as necessary; 5 to 10 μg/kg/min has been given as a continuous IV infusion.

Beemer GH *et al.* (1990). Pharmacokinetics of atracurium during continuous infusion. *Br J Anaesth* 65: 668–674.
Bjorksten AR *et al.* (1990). Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia. *J Chromatogr* 533: 241–247.
Charlton AJ *et al.* (1989). Atracurium overdose in a small infant. *Anaesthesia* 44: 485–486.
Durcan J, Carter JA (1986). Overdose of atracurium. *Anaesthesia* 41: 767.
Peat SJ *et al.* (1988). The prolonged use of atracurium in a patient with tetanus. *Anaesthesia* 43: 962–963.
Simmonds RJ (1985). Determination of atracurium, laudanosine and related compounds in plasma by high-performance liquid chromatography. *J Chromatogr* 343: 431–436.
Varin F *et al.* (1990). Determination of atracurium and laudanosine in human plasma by high-performance liquid chromatography. *J Chromatogr* 529: 319–327.
Yate PM *et al.* (1987). Clinical experience and plasma laudanosine concentrations during the infusion of atracurium in the intensive therapy unit. *Br J Anaesth* 59: 211–217.

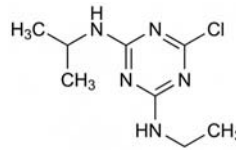
Atrazine

Herbicide

$C_8H_{14}ClN_5$ = 215.7

CAS—1912-24-9

IUPAC Name 6-Chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine



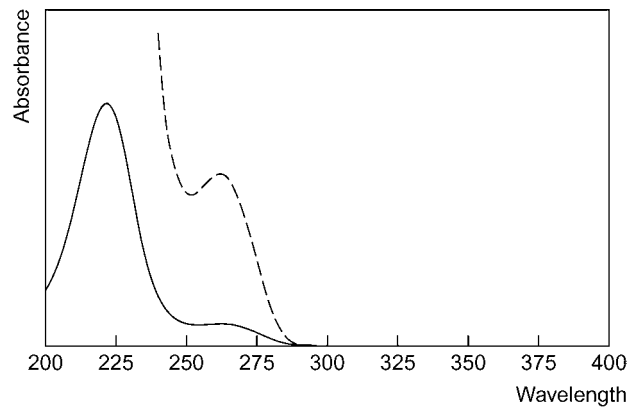
Chemical Properties Colourless crystals. Mp 171° to 174°. Practically insoluble in water; soluble 1 in 20 of chloroform, 1 in 80 of ether and 1 in 55 of methanol. pK_a 1.7. Log *P* (octanol/water), 2.6.

Thin-layer Chromatography System TA—R_f 0.77; system TAB—R_f 0.04; system TAC—R_f 0.08.

Gas Chromatography System GA—atrazine RI 1714, M (desethyl-) RI 1680, M (desethyl-deschloro-methoxy-) RI 1670; system GK—RRT 0.79(relative to caffeine).

High Performance Liquid Chromatography System HY—RI 401; system HAA—retention time 18.2 min; system HAO—*k* 10.75; system HAP—*k* 1.24.

Ultraviolet Spectrum Ethanol—263 nm (A^1_1=195b).



Infrared Spectrum Principal peaks at wavenumbers 1539, 1612, 1295, 804, 1161, 1121 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 43, 58, 44, 200, 68, 215, 41, 42.

Quantification

Urine **HPLC** Limits of detection, 0.2 to 0.5 μg/L for atrazine and other pesticide metabolites [Baker *et al.* 2000]. Limit of detection, 0.5 μg/L for atrazine metabolites [Beeson *et al.* 1999]. For atrazine metabolites, see Buchholz *et al.* [1999].

Note For an ELISA for the quantification of atrazine and its major metabolites, see Lucas *et al.* [1993].

Disposition in the Body

Toxicity

In a fatality involving the ingestion of 500 mL of a herbicide mix containing atrazine (100 g), aminotriazole (25 g), ethylene glycol (25 g), and formaldehyde (0.15 g), the postmortem tissue concentrations of atrazine were reported to be: liver 32.04 μg/g, pancreas 30.99 μg/g, small intestine μg/g, kidney 97.62 μg/g, lung 79.53 μg/g, heart 15.27 μg/g, muscle 19.93 μg/g. At the time of death, about 3 days after ingestion, the plasma atrazine concentration was 1.49 mg/L [Pommery *et al.* 1993].

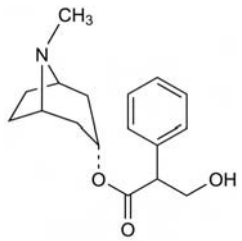
Baker SE *et al.* (2000). Quantification of selected pesticide metabolites in human urine using isotope dilution high-performance liquid chromatography/tandem mass spectrometry. *J Expo Anal Environ Epidemiol* 10: 789–798.

Beeson MD *et al.* (1999). Isotope dilution high-performance liquid chromatography/tandem mass spectrometry method for quantifying urinary metabolites of atrazine, malathion, and 2,4-dichlorophenoxyacetic acid. *Anal Chem* 71: 3526–3530.
Buchholz BA *et al.* (1999). HPLC-accelerator MS measurement of atrazine metabolites in human urine after dermal exposure. *Anal Chem* 71: 3519–3525.
Lucas AD *et al.* (1993). Determination of atrazine metabolites in human urine: development of a biomarker of exposure. *Chem Res Toxicol* 6: 107–116.
Pommery J *et al.* (1993). Atrazine in plasma and tissue following atrazine-aminotriazole-ethylene glycol-formaldehyde poisoning. *J Toxicol Clin Toxicol* 31: 323–331.

Atropine

Anticholinergic
$C_{17}H_{23}NO_3$ = 289.4
CAS—51-55-8

IUPAC Name [(1*R*,5*S*)8-Methyl-8-azabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate
Synonyms (±)-Hyoscyamine; (3-endo)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-α-(hydroxymethyl)benzene acetate; (1*R*,3*R*,5*S*,8*R*)-tropan-3-yl (*RS*)-tropate.
Proprietary Name *Atropinol*. Atropine and its salts are ingredients in and also used as adjuncts in many proprietary preparations [Sweetman 2007].

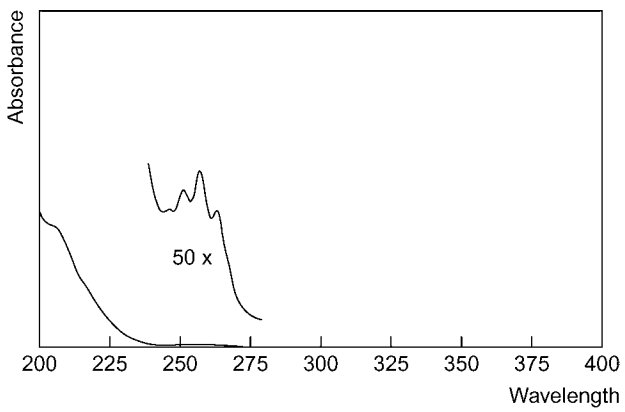


Chemical Properties An alkaloid obtained from *Duboisia* spp. and other solanaceous plants, or prepared by synthesis. Colourless crystals or white crystalline powder. Mp 114° to 118°. Soluble 1 in 455 of water, 1 in 50 of boiling water, 1 in 2 of ethanol, 1 in 1 of chloroform, 1 in 25 of ether and 1 in 27 of glycerol. pK_a 9.9 (20°). Log *P* (octanol), 1.8. Extraction yield (chlorobutane), 0.6 [Demme *et al.* 2005].

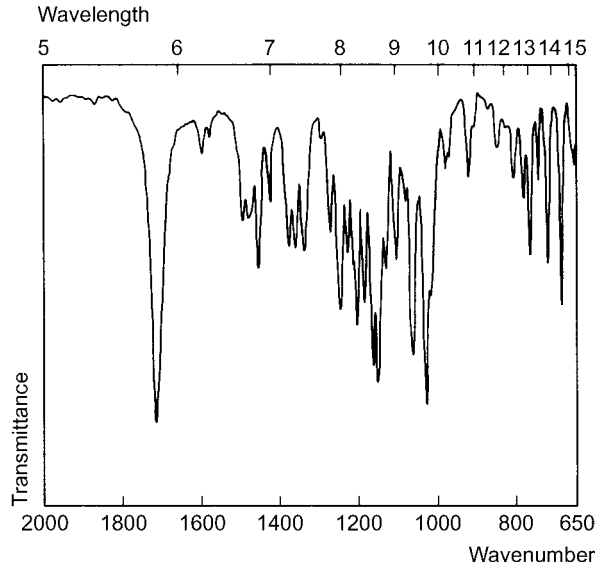
Atropine Sulfate

$(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ = 694.8
CAS—55-48-1 (anhydrous); CAS—5908-99-6 (monohydrate).
Proprietary Names *AtroPen*; *Atropisol*; *Atropocil*; *Atropt*; *Atrospan*; *Liotropina*; *Ocu-Tropine*; *Sal-Tropine*.
Chemical Properties Colourless crystals or white crystalline powder. It effloresces in dry air. Mp ≈190°, with decomposition, after drying at 135° for 15 min. Soluble 1 in 0.4 of water and 1 in 5 of ethanol; practically insoluble in chloroform and ether.

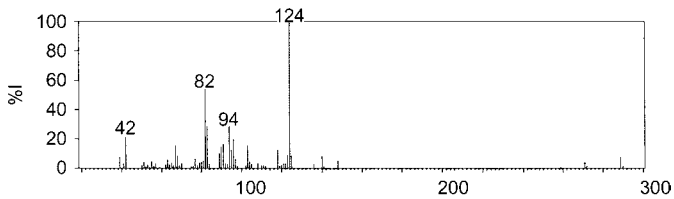
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.18; system TB—R_f 0.05; system TC—R_f 0.03; system TE—R_f 0.24; system TL—R_f 0.01; system TAE—R_f 0.05; system TAF—R_f 0.28 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—pink).
Gas Chromatography System GA—atropine RI 2190, M (-CH_2O) RI 1980, M (-H_2O) RI 2085, M (-AC) RI 2275; system GB—atropine RI 2293, M (-CH_2O) RI 2051, M (-H_2O) RI 2250; system GF—RI 2660.
High Performance Liquid Chromatography System HA—*k* 3.9 (tailing peak); system HX—RI 306; system HY—RI 251; system HZ—RT 2.2 min; system HAA—RT 10.4 min.
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1= 6.3 c), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1720, 1035, 1153, 1163, 1063, 1204 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 124, 82, 94, 83, 42, 96, 103, 67.



Quantification

Blood **GC-MS** Column: cross-linked methyl silicone capillary (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 100° to 240° at 20°/min. EI ionisation, SIM acquisition mode. Retention time: 1.7 min. Limit of quantification, 10 µg/L [Saady, Poklis 1989].

LC-MS Column: LiChroCART LiChrospher 60 RP-select B. Mobile phase: 0.1% formic acid in water and in acetonitrile. APCI. Atropine, fentanyl and scopolamine. Limit of quantification, 0.9 µg/L, limit of detection, 0.6–0.7 µg/L [Skulska *et al.* 2007].

CE Capillary: 50 cm. Buffer: borate-phosphate (pH 9.2) with 50 mol/L SDS. Limit of quantification, 0.2 µg/L, limit of detection, 0.06 µg/L for atropine (in presence of strychnine and tetracaine [Plaut, Staub 1998].

Plasma **HPLC** Column: ODS. Mobile phase: acetonitrile : 50 mmol/L potassium phosphate buffer (pH 3.0) containing 2 mmol/L sodium heptane sulfonate (16 : 84). UV detection (λ= 220 nm). Limit of quantification, 1 mg/L [Rbeida *et al.* 2005].

LC-MS Column: Atlantis T3 C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% formic acid : acetonitrile-water and 0.1% formic acid (80 : 20, 77 : 23 to 62 : 38 at 5 min to 20 : 80 at 6 min for 1 min to 77 : 23 at 7.5 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.05 µg/L, limit of detection, 0.01 µg/L [John *et al.* 2010]. Column: XTerraMS C_8 (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 2 mmol/L formate buffer (pH 3) : acetonitrile (99 : 1 for 2 min to 98 : 2 at 3 min to 5 : 95 at 3.1 min for 7.5 min to 99 : 1 at 8 min until 11 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.25 µg/L, limit of detection, 0.075 µg/L [Abbara *et al.* 2008]. Column: C_8 Superspher 60 RP select B (125 × 2 mm i.d.). Mobile phase: 50 mmol/L ammonium formate (pH 3.5) : acetonitrile (90 : 10 for 2 min to 20 : 80 at 5 min for 2 min to 90 : 10 at 7.01 min until 10 min), flow rate 0.4 mL/min for 2 min to 0.6 mL/min at 2.01 min to 0.4 mL/min at 7.01 min. APCI or ESI. Limit of quantification, 5 µg/L (APCI) and 0.1 µg/L (ESI) [Beyer *et al.* 2007].

Radioimmunoassay Limit of detection, 2.5 µg/L for atropine and hyoscyamine [Virtanen *et al.* 1980].

Serum **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 50° for 1 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 µg/L for tropane alkaloids [Namera *et al.* 2002].

LC-MS Column: Hypurity C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: aqueous buffer (10 g/L ammonium acetate, 15 mL/L acetic acid and 2 mL/L trifluoroacetic anhydride) : water : acetonitrile (5 : 95 : 0 for 1 min to 5 : 0 : 95 at 5 min to 5 : 95 : 0 at 5.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 5 µg/L [Boermans *et al.* 2006].

Radioimmunoassay See Plasma [Virtanen *et al.* 1980].

Urine **GC-MS** See Serum [Namera *et al.* 2002].

LC-MS See Blood [Skulska *et al.* 2007]. See Serum [Boermans *et al.* 2006].

Stomach Contents **CE** See Blood [Plaut, Staub 1998].

A

Biological Specimens **HPLC** Column: TSK gel ODS-120A (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (2 : 8) containing 6 mmol/L phosphoric acid (pH 2.7), flow rate 1.0 mL/min. UV detection (λ = 215 nm). Limit of detection, 8.5 μg/L [Okuda *et al.* 1991].

Disposition in the Body Atropine is readily absorbed from mucous membranes, skin and the gastrointestinal tract but not from the stomach. Approximately 80–90% of a dose is excreted in the urine in 24 h, 50% of the dose as unchanged drug, <2% as tropic acid and tropine, and ~30% as unknown metabolites. Traces of the dose are eliminated in the faeces.

Therapeutic Concentration

Following an IV dose of 1 mg atropine to 6 subjects, plasma concentrations of ~0.25 mg/L were observed at 2 min, decreasing to 0.005 mg/L at 20 min; following an IM dose of 1 mg to 4 subjects, peak plasma concentrations of ~0.003 mg/L were attained in 30 min [Berghem *et al.* 1980].

Toxicity The amount of a toxic dose appears to vary considerably with individuals. Fatalities are reported to have occurred with doses of 50–100 mg, but recovery has occurred after administration of 1 g; doses of 10 mg or less may be lethal in children or in susceptible individuals.

A man was found dead after having ingested an unknown amount of atropine sulfate tablets. Blood contained 0.2 mg/L atropine and urine 1.5 mg/L [Corbett *et al.* 1978].

Of 240 children accidentally injected with automatic atropine injectors, which had been distributed in Israel during the Persian Gulf Crisis as an antidote to chemical warfare agents, 20 (8%) showed signs of severe atropinization; no fatalities occurred. High serum atropine levels of 0.0062 to 0.061 mg/L were noted approximately 1 h after injection in six of the children [Amitai *et al.* 1992].

A 58-year-old man who ingested 500 mg atropine sulfate became unconscious within 1 h and was admitted to hospital. Although he was only prescribed 0.5 mg atropine sulfate orally he was inadvertently given 500 mg dispensed by the pharmacy. At 4 h after ingestion, his serum atropine concentration was 244 μg/L. He recovered after treatment with IV fluids, sedation and ventilator control [Otani *et al.* 2004].

A patient who became restless and excitable after eating boiled jimson weed (*Datura stramonium*) had serum levels of 31.3 μg/L atropine and 30.6 μg/L scopolamine, which decreased to 6.7 and 8.5 μg/L, respectively, after 2 h. The patient was treated with gastric lavage, activated charcoal and neostigmine [Matsuda *et al.* 2006].

A 17-year-old man who ingested *D. stramonium* had a blood concentration of 1.7 μg/L atropine at 12 h; a 17-year-old woman who had also ingested *D. stramonium* had urine levels of 114 μg/L atropine at day 1 [Marc *et al.* 2007].

A 46-year-old man who had ingested three handfuls of *Atropa belladonna* showed symptoms of toxicity (disorientation, aggressiveness, tachycardia) within 6 h; he was treated with diazepam, physostigmine and activated charcoal. After an initial improvement, his condition deteriorated, requiring sedation and continuous physostigmine, before complete recovery within 2 days. Muscarinic receptor total binding equivalent to binding of 130 μg/L atropine was found in the early phase of hospitalisation [Bogan *et al.* 2009].

Seven cases of the anticholinergic syndrome associated with ingestion of cooked vegetables containing *Datura innoxia* have been reported. Atropine and scopolamine were detected in urine and vegetable samples but not in plasma (possibly because of delay in sample collection). Atropine concentrations in urine ranged from 67.1–691.7 μg/L and the cooked vegetables contained 0.8 μg/g [Papoutsis *et al.* 2010].

Half-life Plasma half-life, ~2–4 h; a longer terminal phase of 13–38 h has also been reported.

Volume of Distribution ~2–3 L/kg.

Clearance Plasma clearance, ~8 mL/min/kg.

Protein Binding ~50%.

Note For the symptoms of atropine and other toxic alkaloid poisoning and a review of methods of their detection in biological fluids, see Beyer *et al.* [2009].

Dose Usually 0.25 to 2 mg atropine sulfate daily.

Abbara C *et al.* (2008). High-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of diazepam, atropine and pralidoxime in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 874: 42–50.

Amitai Y *et al.* (1992). Atropine poisoning in children during the Persian Gulf crisis: a national survey in Israel. *JAMA* 268: 630–632.

Berghem L *et al.* (1980). Plasma atropine concentrations determined by radioimmunoassay after single-dose i.v. and i.m. administration. *Br J Anaesth* 52: 597–601.

Beyer J *et al.* (2007). Detection and validated quantification of toxic alkaloids in human blood plasma: comparison of LC-APCI-MS with LC-ESI-MS/MS. *J Mass Spectrom* 42: 621–633.

Beyer J *et al.* (2009). Analysis of toxic alkaloids in body samples. *Forensic Sci Int* 185: 1–9.

Boermans PA *et al.* (2006). Quantification by HPLC-MS/MS of atropine in human serum and clinical presentation of six mild-to-moderate intoxicated atropine-adulterated-cocaine users. *Ther Drug Monit* 28: 295–298.

Bogan R *et al.* (2009). Plasma level of atropine after accidental ingestion of *Atropa belladonna*. *Clin Toxicol (Phila)* 47: 602–604.

Corbett *et al.* (1978). Atopine poisoning. *Bull TIAFT* 14: 37–38.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

John H *et al.* (2010). LC-ESI MS/MS quantification of atropine and six other antimuscarinic tropane alkaloids in plasma. *Anal Bioanal Chem* 396: 751–763.

Marc B *et al.* (2007). [Acute *Datura stramonium* poisoning in an emergency department]. *Presse Med* 36: 1399–1403.

Matsuda K *et al.* (2006). [Toxicological analysis of a case of *Datura stramonium* poisoning]. *Rinsho Byori* 54: 1003–1007.

Namera A *et al.* (2002). Quantitative analysis of tropane alkaloids in biological materials by gas chromatography–mass spectrometry. *Forensic Sci Int* 130: 34–43.

Okuda T *et al.* (1991). Determination of atropine in biological specimens by high-performance liquid chromatography. *J Chromatogr* 567: 141–149.

Otani N *et al.* (2004). [Case report of atropine poisoning, after accidental ingestion of 500 mg atropine sulfate]. *Chudoku Kenkyu* 17: 55–59.

Papoutsis I *et al.* (2010). Mass intoxication with *Datura innoxia*: case series and confirmation by analytical toxicology. *Clin Toxicol (Phila)* 48: 143–145.

Plaut O, Staub C (1998). Determination of atropine in biological fluids by micellar electrokinetic capillary chromatography in the presence of strychnine and tetracaine. *Electrophoresis* 19: 3003–3007.

Rbeida O *et al.* (2005). Integrated on-line sample clean-up using cation exchange restricted access sorbent for the LC determination of atropine in human plasma coupled to UV detection. *J Pharm Biomed Anal* 36: 947–954.

Saady JJ, Poklis A (1989). Determination of atropine in blood by gas chromatography/mass spectrometry. *J Anal Toxicol* 13: 296–299.

Skulska A *et al.* (2007). [Determination of fentanyl, atropine and scopolamine in biological material using LC-MS/APCI methods]. *Przegl Lek* 64: 263–267.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35 edn. London: Pharmaceutical Press.

Virtanen R *et al.* (1980). Radioimmunoassay for atropine and *l*-hyoscyamine. *Acta Pharmacol Toxicol (Copenh)* 47: 208–212.

Atropine Methobromide

Anticholinergic

$C_{18}H_{26}BrNO_3$ = 384.3

CAS—2870-71-5

Synonyms Atropine methylbromide; methylatropine bromide; mydriasine.

Chemical Properties Colourless crystals or a white crystalline powder. Mp 222° to 223°. Soluble 1 in 1 of water and 1 in 20 of ethanol; very slightly soluble in dehydrated alcohol; almost insoluble in chloroform and ether. Log *P* (octanol/water), −1.6.

Colour Tests Aromaticity (method 2)—colourless/yellow; Liebermann's reagent—orange.

Atropine Methonitrate

Anticholinergic

$C_{18}H_{26}N_2O_6$ = 366.4

CAS—52-88-0

Synonym Methylatropine nitrate

Chemical Properties Colourless crystals or a white crystalline powder. Mp 163°. Soluble 1 in less than 1 of water and 1 in 13 of ethanol; very slightly soluble in chloroform and ether. Log *P* (octanol/water), −2.2. Aqueous solutions are unstable.

Colour Tests Aromaticity (method 2)—colourless/violet (transient); Liebermann's reagent—orange.

Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.00; system TE—R_f 0.00; system TN—R_f 0.95; system TO—R_f 0.35; system TAE—R_f 0.00 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 299.

Ultraviolet Spectrum Aqueous acid—252 nm (A^1_1=about 5), 258 nm (A^1_1=about 5), 264 nm (A^1_1=about 4).

Infrared Spectrum Principal peaks at wavenumbers 1159, 1045, 1718, 1176, 700, 1212 cm^{-1} (Nujol mull).

Atropine Oxide

Anticholinergic

$C_{17}H_{23}NO_4$ = 305.4

CAS—4438-22-6

IUPAC Name (3-*endo*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl-α-(hydroxymethyl)-benzeneacetate *N*-oxide

Synonyms Aminoxytropine tropate; atropine aminoxide; atropine *N*-oxide.

Chemical Properties A very hygroscopic, crystalline powder. Mp 127° to 128°; it decomposes at 135°. Soluble in ethanol and chloroform; practically insoluble in ether.

Atropine Oxide Hydrochloride

$C_{17}H_{23}NO_4$,HCl = 341.8

CAS—4574-60-1

Proprietary Name *Génatropine*

Chemical Properties Crystals. Mp 192° to 193°.

Thin-layer Chromatography System TA—R_f 0.21 (acidified iodoplatinate solution, positive).

Infrared Spectrum Principal peaks at wavenumbers 1164, 1730, 1118, 1026, 1056, 1195 cm^{-1}.

Aviptadil

Hormone, Muscle Relaxant
$C_{147}H_{238}N_{44}O_{42}S$ = 3325.8
CAS—40077-57-4

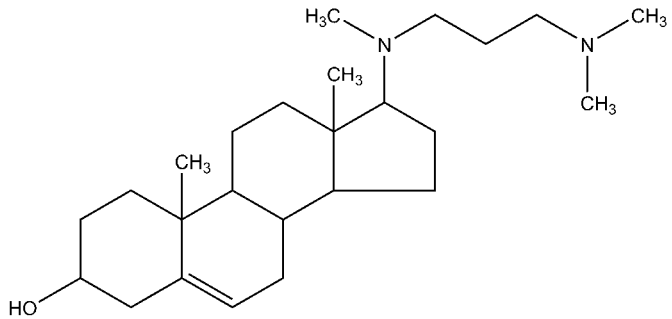
Synonym Vasoactive intestinal octacosapeptide (swine)
Proprietary Name *Invicorp*
Disposition in the Body Vasoactive intestinal peptide acts as a hormone and neurotransmitter in various parts of the body; it is a potent relaxant of smooth muscle and has vasodilator and bronchodilator properties as well as stimulating the gastrointestinal tract to increased secretion.
Dose Has been used in different doses in the management of acute oesophageal food impaction, and for the treatment of acute respiratory distress syndrome, pulmonary arterial hypertension, acute lung injury, and chronic thromboembolic pulmonary hypertension. It has also been used as a combination product with phentolamine for erectile dysfunction.
Note For a review of the clinical trials of aviptadil, see Keijzers [2001].

Keijzers GB (2001). Aviptadil (Senatek). *Curr Opin Investig Drugs* 2: 545–549.

Azacosterol

Anticholesteraemic, Contraceptive (Veterinary)
$C_{25}H_{44}N_2O$ = 388.6
CAS—1249-84-9

IUPAC Name 17β-{[3-(Dimethylamino)propyl]methylamino}androst-5-en-3β-ol
Synonyms 20,25-Diazacholesterol; SC-12937.



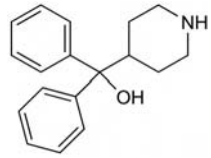
Chemical Properties Slightly soluble in water (7.05 mg/L). Log *P* (octanol/water), 4.35 [Meylan, Howard 1995]. Extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—dull orange→green (limit of detection 0.1 µg); Ammonium vanadate test—orange→blue (limit of detection 0.1 µg); sulfuric acid-formaldehyde test—brown-orange (limit of detection 0.1 µg).
Infrared Spectrum Principal peaks at wavenumbers 1065, 1455, 1345 cm^{-1} (KBr disk).
Quantification
Note For a HPLC method for the analysis of azacosterol in quail feed or serum, see Johnston *et al.* [2001].

Johnston JJ *et al.* (2001). Determination of DiazaCon in quail feed and quail serum by ion pair reversed-phase chromatography. *J AOAC Int* 84: 634–639.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Azacyclonol

Tranquilliser
$C_{18}H_{21}NO$ = 267.4
CAS—115-46-8

IUPAC Name α,α-Diphenyl-4-piperidinemethanol



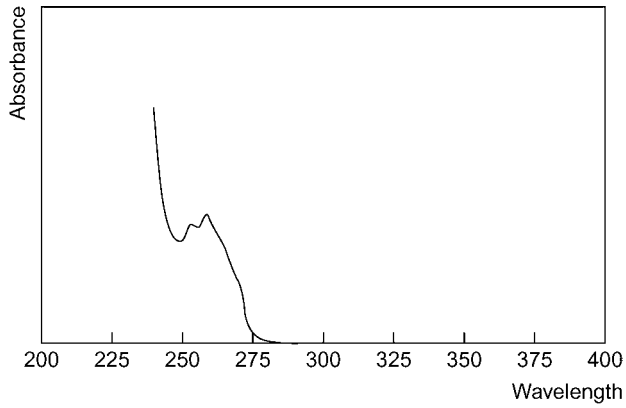
Chemical Properties Crystals. Mp 160° to 161°. Log *P* (octanol/water), 3.4.

Azacyclonol Hydrochloride

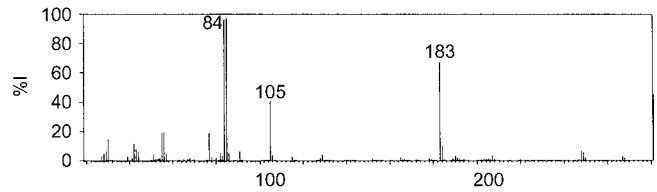
$C_{18}H_{21}NO,HCl$ = 303.8
CAS—1798-50-1
Synonym Azacyclonolium chloride
Proprietary Names *Frenoton*; *Frenquel.*

Chemical Properties Small white crystals or crystalline powder. Mp 270° to 281°. Soluble 1 in 200 of water and 1 in 1000 of ethanol; practically insoluble in acetone, chloroform, ether and light petroleum.

Colour Tests Liebermann's reagent—brown; Mandelin's test—red; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.10; system TB—R_f 0.00; system TC—R_f 0.03; system TE—R_f 0.14; system TL—R_f 0.01; system TAE—R_f 0.03; system TAL—R_f 0.40 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2243; system GB—RI 2361.
High Performance Liquid Chromatography System HA—*k* 1.2; system HAX—retention time 8.7 min; system HAY—retention time 4.5 min.
Ultraviolet Spectrum Aqueous acid—253, 259 nm (A^1_1=17.4a).



Infrared Spectrum Principal peaks at wavenumbers 702, 745, 1316, 1063, 971, 1155 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 85, 84, 183, 105, 56, 77, 55, 30.

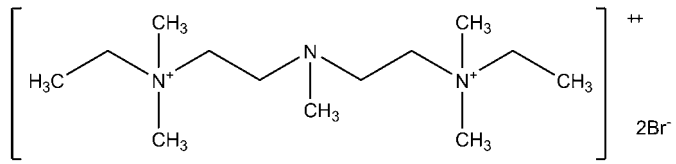


Dose Azacyclonol hydrochloride has been given in doses of 100 to 800 mg daily.

Azamethonium Bromide

Quaternary Ammonium, Ganglion Blocker, Antihypertensive
$C_{13}H_{33}Br_2N_3$ = 391.3
CAS—306-53-6; 60-30-0 (azamethonium)

IUPAC Name 2,2′-Methyliminobis(diethyldimethylammonium) dibromide
Synonyms Ciba 9295; pentamethazene bromide; pentamin.
Proprietary Name *Pendiomide*



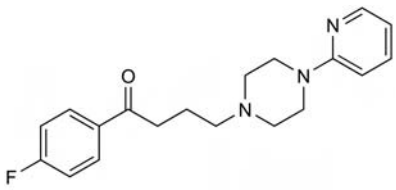
Chemical Properties White hygroscopic crystalline powder. Mp 212° to 215°. Soluble in water and ethanol; almost insoluble in ether.
Colour Test Vitali's test—pale brown/yellow (limit of detection 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.00 (location reagent acidified iodoplatinate spray, positive reaction).
Infrared Spectrum Principal peaks at wavenumbers 1477, 1014, 968 or 1620 cm^{-1} (KBr disk).
Disposition in the Body
Toxicity LD_{50} (oral) in mice: 2.5 g/kg.

Azaperone

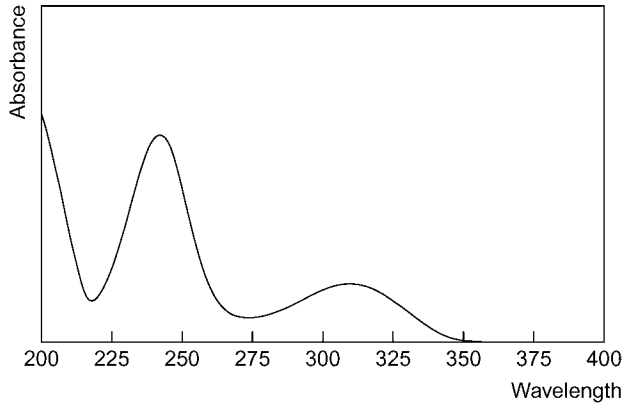
Tranquilliser (Veterinary)
$C_{19}H_{22}FN_3O$ = 327.4
CAS—1649-18-9

A

IUPAC Name 1-(4-Fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanone
Proprietary Names *Stresnil*; *Suicalm*. It is an ingredient of *Fentazin*. (All veterinary.)



Chemical Properties A white to yellowish-white microcrystalline powder. Mp 73° to 75°. Practically insoluble in water; soluble 1 in 29 of ethanol, 1 in 4 of chloroform and 1 in 31 of ether; soluble in dilute organic acids. Log *P* (octanol/water), 3.3.
Colour Tests Cyanogen bromide—orange; Mandelin's test—yellow.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.30; system TE—R_f 0.72; system TL—R_f 0.09; system TAE—R_f 0.65; system TAJ—R_f 0.54; system TAK—R_f 0.00; system TAL—R_f 0.67 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2705; system GV—retention time 19.0 min.
High Performance Liquid Chromatography System HX—RI 319.
Ultraviolet Spectrum Aqueous acid—242 nm (A^1_1=800a), 312 nm; methanol—247 nm (A^1_1=1260b), 300 nm (A^1_1=200b).

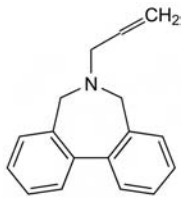


Infrared Spectrum Principal peaks at wavenumbers 1587, 1669, 775, 1236, 1222, 1152 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 107, 165, 123, 95, 121.
Quantification
Urine **GC–MS** Animal urine (azaperone and other tranquillisers). Limit of detection, 5 µg/L for azaperone [Olmos-Carmona, Hernandez-Carrasquilla 1999].
Kidney **GC–MS** For azaperone, azaperol, and carazolol in swine kidney, see Fluchard *et al.* [2000].
Muscle **GC–MS** See Kidney Fluchard *et al.* [2000].

Fluchard D *et al.* (2000). Determination of a method for detecting and quantifying azaperone, azaperol and carazolol in pig tissues by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 744: 139–147.
Olmos-Carmona ML, Hernandez-Carrasquilla M (1999). Gas chromatographic-mass spectrometric analysis of veterinary tranquillizers in urine: evaluation of method performance. *J Chromatogr B Biomed Sci Appl* 734: 113–120.

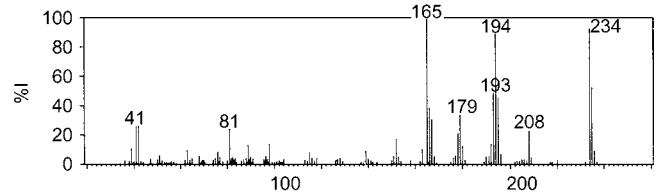
Azapetine

Vasodilator
$C_{17}H_{17}N$ = 235.3
CAS—146-36-1
IUPAC Name 6-Allyl-6,7-dihydro-5*H*-dibenz[*c*,*E*]azepine
Synonym Azepine



Azapetine Phosphate

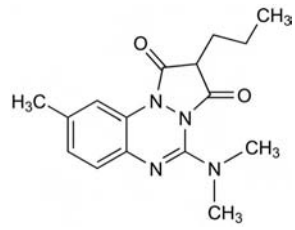
$C_{17}H_{17}N,H_3PO_4$ = 333.3
CAS—130-83-6
Chemical Properties Crystals. Mp 211° to 215°, with decomposition. Soluble at least 1 in 100 of water.
Colour Test Mandelin's test—brown.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.57; system TC—R_f 0.67; system TE—R_f 0.78; system TL—R_f 0.56; system TAE—R_f 0.14; system TAJ—R_f 0.59; system TAK—R_f 0.26; system TAL—R_f 0.90 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1939.
Ultraviolet Spectrum Aqueous acid—248 nm.
Infrared Spectrum Principal peaks at wavenumbers 748, 918, 1063, 990, 1111, 1639 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 165, 234, 194, 235, 193, 196, 166, 179.



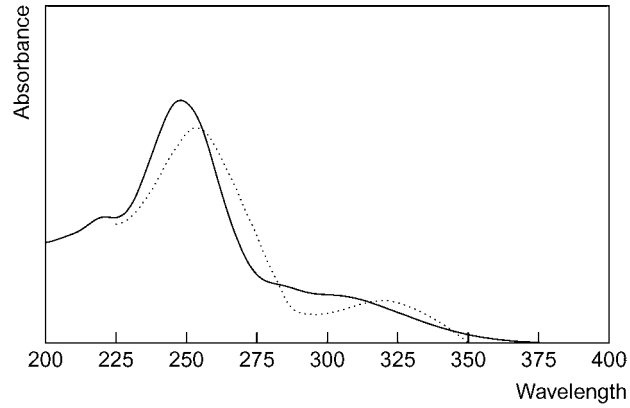
Dose The equivalent of 75 to 225 mg of azapetine has been given daily.

Azapropazone

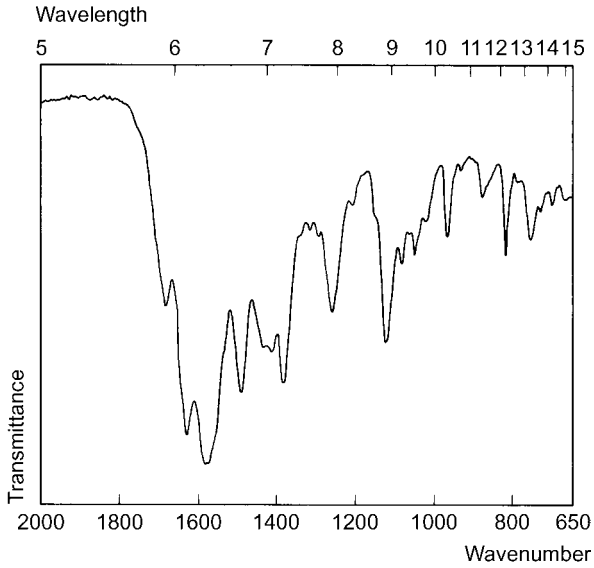
Analgesic
$C_{16}H_{20}N_4O_2,2H_2O$ = 336.4
CAS—13539-59-8 (anhydrous)
IUPAC Name 5-(Dimethylamino)-9-methyl-2-propyl-1*H*-pyrazolo[1,2-*a*][1,2,4]-benzotriazine-1,3(2*H*)-dione dihydrate
Synonym Apazone
Proprietary Names *Prolixan(a)*; *Rheumox*; *Tolyprin*.



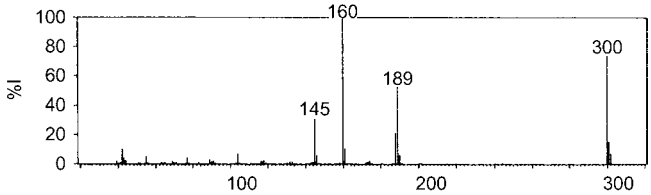
Chemical Properties Almost colourless crystals. Mp 228°; 247° to 248° (dihydrate). Log *P* (octanol/pH 7.4), 1.0.
Colour Tests Liebermann's reagent—grey-green; Mandelin's test—grey-green.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.53; system TC—R_f 0.05; system TE—R_f 0.08; system TL—R_f 0.67; system TAE—R_f 0.88; system TAJ—R_f 0.11; system TAK—R_f 0.06; system TAL—R_f 0.61.
Gas Chromatography System GA—RI 2461; system GF—RI 3010.
High Performance Liquid Chromatography System HZ—retention time 2.0 min.
Ultraviolet Spectrum Azapropazone dihydrate: aqueous acid—250 nm (A^1_1=1040b); aqueous alkali—255 nm (A^1_1=1080b), 325 nm.



Infrared Spectrum Principal peaks at wavenumbers 1590, 1640, 1500, 1133, 1270, 1695 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 160, 300, 189, 145, 188, 301, 161, 42.



Quantification
Plasma **HPLC** UV detection. Limit of detection, 400 µg/L for azapropazone, 1.5 to 3 mg/L for 6-hydroxyazapropazone [Kline *et al.* 1983].

Ultraviolet spectrophotometry For comparison with gas chromatographic and colorimetric methods, see Leach [1976].
Serum **Colorimetry** Limit of detection, 10 mg/L [Farrier 1974].
Urine **HPLC** See Plasma [Kline *et al.* 1983].
Disposition in the Body Readily absorbed after oral administration. About 95% of a dose is excreted in the urine, of which about 60% is unchanged drug and about 20% is the inactive metabolite, 6-hydroxyazapropazone.
Therapeutic Concentration

After a single oral dose of 600 mg, administered to 6 subjects, plasma concentrations of 34 to 54 mg/L (mean 43) were attained in 4 h [Ritch *et al.* 1982].

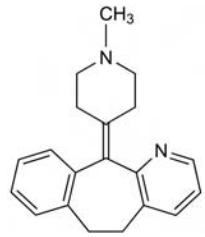
Steady-state plasma concentrations of 32 to 89 mg/L (mean 57) were reported during the daily administration of 800 mg to 10 subjects [Thune 1976].

Half-life Plasma half-life, 8 to 24 h (mean 15), increased in elderly subjects and in subjects with impaired renal or liver function.
Volume of Distribution About 0.2 L/kg.
Clearance Plasma clearance, 0.1 to 0.2 mL/min/kg.
Protein Binding About 99%.
Dose 0.6 to 1.2 g daily.

Farrier DS (1974). Spectroscopic methods for the determination of azapropazone in serum developed and evaluated using radio-isotopic techniques. *Arzneimittelforschung* 24: 747–751.
Kline BJ *et al.* (1983). The determination of azapropazone and its 6-hydroxy metabolite in plasma and urine by HPLC. *Arzneimittelforschung* 33: 504–506.
Leach H (1976). The determination of azapropazone in blood plasma. *Curr Med Res Opin* 4: 35–43.
Ritch AE *et al.* (1982). Pharmacokinetics of azapropazone in the elderly. *Br J Clin Pharmacol* 14: 116–119.
Thune S (1976). A comparative study of azapropazone and indomethacin in the treatment of rheumatoid arthritis. *Curr Med Res Opin* 4: 70–75.

Azatadine

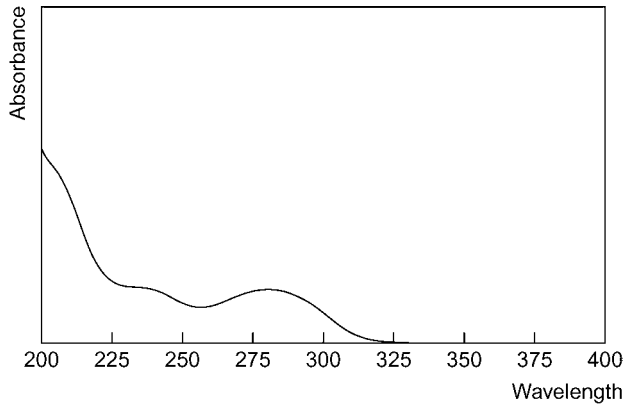
Antihistamine
$C_{20}H_{22}N_2 = 290.4$
CAS—3964-81-6
IUPAC Name 6,11-Dihydro-11-(1-methyl-4-piperidinylidene)-5*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridine



Chemical Properties Log *P* (octanol/water), 3.6.

Azatadine Maleate
$C_{20}H_{22}N_2,2C_4H_4O_4 = 522.6$
CAS—3978-86-7
Proprietary Names *Idulamine*; *Idulian*; *Lergocil*; *Optimine*; *Zadine*.
Chemical Properties A white crystalline powder. Mp 145° to 154°. Soluble in water, ethanol, chloroform, dimethylformamide and methanol; practically insoluble in ether.

Colour Tests Cyanogen bromide—pink; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.39 (acidified potassium permanganate solution, strong reaction).
Gas Chromatography System GA—azatadine RI 2377, M (OH-alkyl-)-H_2O- RI 2410, M (OH-alkyl-)-AC- RI 2520, M (OH-aryl-)-AC- RI 2540, M (nor-)-AC- RI 2720; system GB—azatadine RI 2499.
High Performance Liquid Chromatography System HAX—retention time 10.7 min; System HAY—retention time 4.6 min.
Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1=330b).

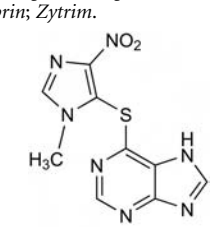


Infrared Spectrum Principal peaks at wavenumbers 752, 1271, 1124, 803, 984, 795 cm^{-1} (KBr disk).
Quantification
Urine **HPLC** For UV detection, see Alton *et al.* [1987].
Dose 2 to 4 mg of azatadine maleate daily.

Alton KB *et al.* (1987). High-performance liquid chromatographic assay for azatadine in human urine. *J Chromatogr* 385: 249–259.

Azathioprine

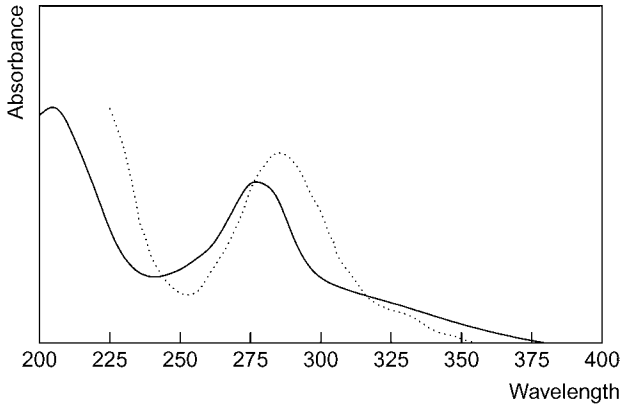
Immunosuppressant
$C_9H_7N_7O_2S = 277.3$
CAS—446-86-6
IUPAC Name 6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine
Proprietary Names *Aseroprim*; *Azahexal*; *Azamun(e)*; *Azatrilem*; *Azopi*; *Azopine*; *Berkaprine*; *Immunoprin*; *Imuprin*; *Imuran*; *Imurek*; *Imurel*; *Oprisine*; *Satedon*; *Thioprine*; *Tiosalprin*; *Zytrim*.



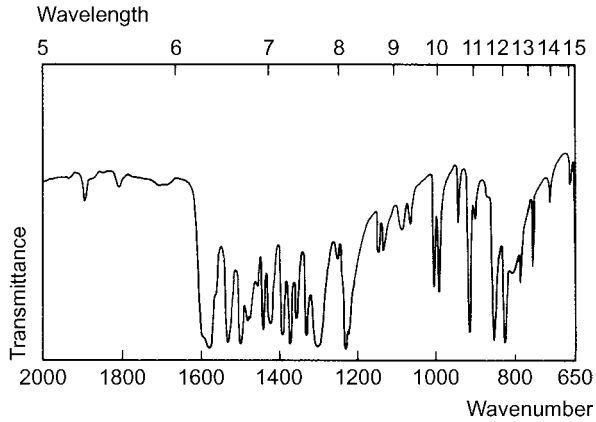
Chemical Properties A pale yellow powder. Mp about 238°, with decomposition. Practically insoluble in water; very slightly soluble in ethanol and chloroform; sparingly soluble in dilute mineral acids; soluble in dilute solutions of alkali hydroxides but decomposes in stronger solutions. pK_a 8.2 (25°). Log *P* (octanol/water), 0.1. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

A

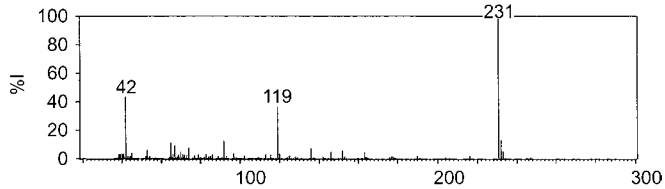
Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.03; system TC—R_f 0.08; system TE—R_f 0.06; system TL—R_f 0.04; system TAD—R_f 0.71; system TAE—R_f 0.80.
High Performance Liquid Chromatography System HX—RI 323.
Ultraviolet Spectrum Aqueous acid—280 nm (A^{1}_{1}=600a); aqueous alkali—285 nm.



Infrared Spectrum Principal peaks at wavenumbers 1237, 1306, 1580, 832, 1500, 1531 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 231, 42, 119, 232, 92, 65, 67, 74.



Quantification
Blood **HPLC** For azathioprine metabolites, see Dervieux and Boulieu [1998]. For azathioprine metabolites, see Weller *et al.* [1995].
Plasma **HPLC** Azathioprine and 6-mercaptopurine. Limit of detection, <5 µg/L for azathioprine [Van Os *et al.* 1996]. Azathioprine metabolites. Limit of detection, 5 µg/L [Weller *et al.* 1995]. UV detection. Limit of detection, 40 µg/L for azathioprine, 5 µg/L for mercaptopurine [Ding, Benet 1979].

Spectrofluorimetry Azathioprine and mercaptopurine. Limit of detection, 10 µg/L [Maddocks 1979].
Serum **HPLC** UV detection. Limit of detection, <2.5 ng [Binscheck *et al.* 1996].
Urine **HPLC** For azathioprine metabolites, see Weller *et al.* [1995].
Biological Fluids **HPLC** For thiopurine metabolites of azathioprine, see Boulieu *et al.* [1993].
Disposition in the Body Absorbed after oral administration and distributed throughout the body. It is readily metabolised to mercaptopurine, which is the major active metabolite, and to 1-methyl-4-nitro-5-(*S*-glutathionyl)-imidazole; other metabolites include 1-methyl-4-nitroimidazole, 1-methyl-4-nitro-5-thioimidazole and 6-thiouric acid; mercaptopurine is further metabolised to its ribonucleotide, thioinosinic acid, which is the active moiety. About 50% of a dose is excreted in the urine in 24 h, mainly as thiouric acid and other metabolites, with about 10% consisting of unchanged drug; about 12% of a dose is eliminated in the faeces in 48 h.
Therapeutic Concentration
After daily oral doses of 50 to 100 mg to 3 renal transplant patients, peak steady-state plasma concentrations of 0.05 to 0.08 mg/L of mercaptopurine were reported 1 h after a dose [Ding, Benet 1979].

Half-life Plasma half-life, azathioprine about 3 h, mercaptopurine about 0.5 to 1.5 h.
Protein Binding About 30%.
Dose 1 to 5 mg/kg daily, orally.

Binscheck T *et al.* (1996). High-performance liquid chromatographic assay for the measurement of azathioprine in human serum samples. *J Chromatogr* 675 B Biomed. Appl.: 287–294.
Boulieu R *et al.* (1993). High-performance liquid chromatographic determination of thiopurine metabolites of azathioprine in biological fluids. *J Chromatogr* 615: 352–356.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Dervieux T, Boulieu R (1998). Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 44: 551–55.
Ding TL, Benet LZ (1979). Determination of 6-mercaptopurine and azathioprine in plasma by high-performance liquid chromatography. *J Chromatogr* 163: 281–288.
Maddocks JL (1979). Assay of azathioprine, 6-mercaptopurine and a novel thiopurine metabolite in human plasma. *Br J Clin Pharmacol* 8: 273–278.
Van Os EC *et al.* (1996). Simultaneous determination of azathioprine and 6-mercaptopurine by high-performance liquid chromatography. *J Chromatogr* 679 B Biomed. Appl.: 147–154.
Weller S *et al.* (1995). HPLC analysis of azathioprine metabolites in red blood cells, plasma and urine in renal transplant recipients. *Int J Clin Pharmacol Ther* 33: 639–645.

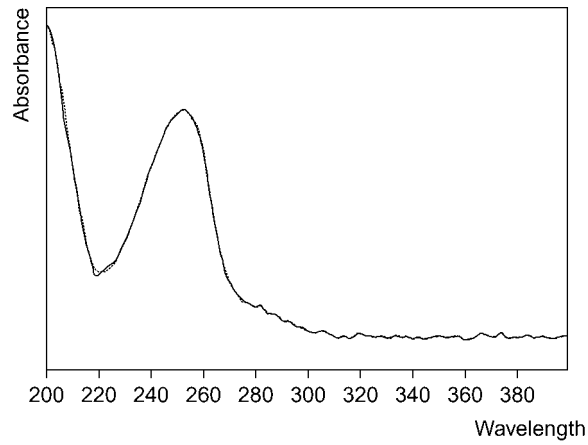
Azide

Anion
HN_3 = 22

Synonym Hydrazoic acid
Chemical Properties Characteristic pungent odour. Bp 37°. pK_a 4.7 [Baselt 2008].

Sodium Azide
N_3Na = 65.01
CAS—26628-22-8
Synonym Smite
Chemical Properties Colourless hexagonal crystals. Very soluble in water; soluble in liquid ammonia; slightly soluble in alcohol; insoluble in ether. Used in the preparation of hydrazoic acid, lead azide, pure sodium; as preservative for laboratory reagents and urine specimens; as a propellant for inflating automotive safety bags; as an agricultural nematocide, herbicide; in fruit rot control.

Colour Test Add 400 µL of test solution to 400 µL of $CuCl_2$ (0.4 g of hydrated cupric chloride in 20 mL dH_2O). Centrifuge. Add 500 µL 0.5 mol/L NaOH. Centrifuge—Sodium azide initially results in a bright green solution with a dark brown precipitate (if present). Following NaOH, there is a green flash followed by a milky blue solution. After centrifugation, the pellet is grey-blue with a clear supernatant [Fleming, Nixon 1986]. Limit of detection, 19 mmol/L.
Ultraviolet Spectrum Principal peaks at 252 nm (benzoyl derivative) [Marquet *et al.* 1996].



Quantification
Blood **GC** Column: HP-5 fused silica capillary (15 m × 0.53 mm i.d., 1.5 µm). Temperature programme: 80° to 230° at 10°/min. Carrier gas: He, 6.0 mL/min. NPD. Limit of detection, 5 µg/L [Kikuchi *et al.* 2001].

GC-MS Column: HP-5 fused silica capillary (20 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 40° for 1 min to 90° at 30°/min to 130° at 10°/min to 300° at 30°/min. Carrier gas: He, 1 mL/min. EI ionisation at 70 eV, full scan mode. Limit of detection, 100 µg/L [Kikuchi *et al.* 2001]. Column: DB-225 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 50° for 3 min to 220° at 10°/min. Carrier gas: He, 2 mL/min. EI ionisation at 70 eV, positive and negative ion mode, full scan mode. Limit of detection, approx. 0.5 µmol/L [Kage *et al.* 2000].

HPLC Column: Ultrasphere ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (1 : 1), flow rate 1 mL/min. DAD (λ= 240–254 nm). Limit of detection, 80 µg/L [Lambert *et al.* 1995a]. Column: Novapak C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate Tris buffer

(pH 4.6) : acetonitrile (55 : 45), flow rate 1.1 mL/min. DAD (λ = 200–400 nm). Limit of detection, 200 µg/L [Marquet *et al.* 1996].

Note For an ion chromatography method for the determination of sodium azide in blood, see Kruszyna *et al.* [1998].

Urine **GC** Column: HP-5 fused silica capillary (15 m × 0.53 mm i.d., 1.5 µm). Temperature programme: 80° to 230° at 10°/min. Carrier gas: He, 6.0 mL/min. NPD. Limit of detection, 0.5 µg/L [Kikuchi *et al.* 2001].

GC-MS See Blood. Limit of detection, 10 µg/L [Kikuchi *et al.* 2001]. See Blood [Kage *et al.* 2000].

Disposition in the Body Little is known of the fate of azide in the body. It is converted to nitric oxide by erythrocytes; cyanide, which is produced *in vitro* by incubation in whole blood, is thought to be a metabolite. For a review of azide pharmacokinetics in animals, see Reinhardt and Brittelli [1981].

Toxicity Azide is similar to cyanide in that it exerts the majority of its toxic effects by inhibiting cytochrome oxidase. Acute poisoning following inhalation of the vapour or ingestion of the salts is characterised by tachycardia, metabolic acidosis, severe hypotension, respiratory depression, and convulsions. There is some evidence from mouse experiments that the formation of an azide–methaemoglobin complex may afford some protection against toxicity [Abbanat, Smith 1964].

A 47-year-old male ingested approx. 9 g of sodium azide. Postmortem sodium azide concentrations in his gastric contents, bile, brain and lung were 1036, 21.4, 2.7 and 17.6 mg/kg, respectively. Concentrations were 7.4 and 8.3 mg/L in his thoracic and suprahepatic blood, respectively [Marquet *et al.* 1996].

A 25-year-old female was found with no signs of life. She had a lethal concentration of cyanide in her blood (9 mg/L) together with a high azide concentration (262 mg/L). Concentrations of azide in her stomach contents, liver, kidney and bile were 754, 14, 205 and 1283 mg/L, respectively [Lambert *et al.* 1995a, b].

A woman who was administered 800 mg of sodium azide accidentally by mouth died 3.5 days later. She had a urine azide concentration of 0.14 mg/L [Howard *et al.* 1990].

A 52-year-old man ingested between 1.2 and 2 g of sodium azide. On arrival at hospital (approx. 3 h after ingestion) he had a plasma cyanide concentration of 1.6 mg/L. At 12, 18 and 24 h later his cyanide concentrations were 1.7, 0.6 and 0.1 mg/L [Klein-Schwartz *et al.* 1989].

A 20-year-old man ingested a white unknown powder and died 40 min later. The amount of sodium azide found by volumetric analysis was 86.7 mg% in the stomach wall and 42.9 mg% in the small intestine. There was no sodium azide found in the liver [Kozlicka-Gajdzinska, Brzyski 1966].

Note For cases of fatal sodium azide ingestion, see Abrams *et al.* [1987]; Albertson *et al.* [1986]; Emmett and Ricking [1975]; Judge and Ward [1989].

Abbanat R, Smith RP (1964). The influence of methaemoglobinaemia on the lethality of some toxic anions. I. Azide. *Toxicol Appl Pharmacol* 6: 576–583.

Abrams J *et al.* (1987). Suicidal sodium azide ingestion. *Ann Emerg Med* 16: 1378–1380.

Albertson TE *et al.* (1986). A case of fatal sodium azide ingestion. *J Toxicol Clin Toxicol* 24: 339–351.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, CA: Biomedical Publications.

Emmett EA, Ricking JA (1975). Fatal self-administration of sodium azide. *Ann Intern Med* 83: 224–226.

Fleming N, Nixon RA (1986). A versatile transition metal salt reaction for a wide range of common biochemical reagents: an instantaneous and quantifiable color test. *Anal Biochem* 154: 691–701.

Howard JD *et al.* (1990). Death following accidental sodium azide ingestion. *J Forensic Sci* 35: 193–196.

Judge K, Ward WNE (1989). Fatal azide-induced cardiomyopathy presenting as acute myocardial infarction. *Am J Cardiol* 64: 830–831.

Kage S *et al.* (2000). Determination of azide in blood and urine by gas chromatography–mass spectrometry. *J Anal Toxicol* 24: 429–432.

Kikuchi M *et al.* (2001). Application of a new analytical method using gas chromatography and gas chromatography-mass spectrometry for the azide ion to human blood and urine samples of an actual case. *J Chromatogr B Biomed Sci Appl* 752: 149–157.

Klein-Schwartz W *et al.* (1989). Three fatal sodium azide poisonings. *Med Toxicol Adverse Drug Exp* 4: 219–227.

Kozlicka-Gajdzinska H, Brzyski J (1966). A case of fatal intoxication with sodium azide. *Arch Toxikol* 22: 160–163.

Kruszyna R *et al.* (1998). Determining sodium azide concentration in blood by ion chromatography. *J Forensic Sci* 43: 200–202.

Lambert WE *et al.* (1995). Application of high-performance liquid chromatography to a fatality involving azide. *J Anal Toxicol* 19: 261–264.

id="c-d1e113789">Lambert WE *et al.* (1995). Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions. *J Anal Toxicol* 19: 73–78.

Marquet P *et al.* (1996). Anall findings in a suicide involving sodium azide. *J Anal Toxicol* 20: 134–138.

Reinhardt CF, Brittelli MR (1981). Azides. In: Clayton GD, Clayton FE, eds. *Patty's Industrial Hygiene and Toxicology,* Vol IIA. New York: Wiley, 2778-2784.

Azinphos-(Et)

Acaricide, Insecticide

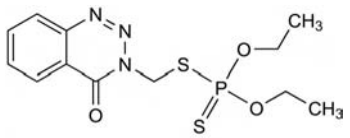
$C_{12}H_{16}N_3O_3PS_2$ = 345.4

CAS—2642-71-9

IUPAC Name Phosphorodithioic acid *O,O*-diethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] ester

Synonyms Bayer 16259; ENT-22014; ethyl guthion; gusathion A; R-1513.

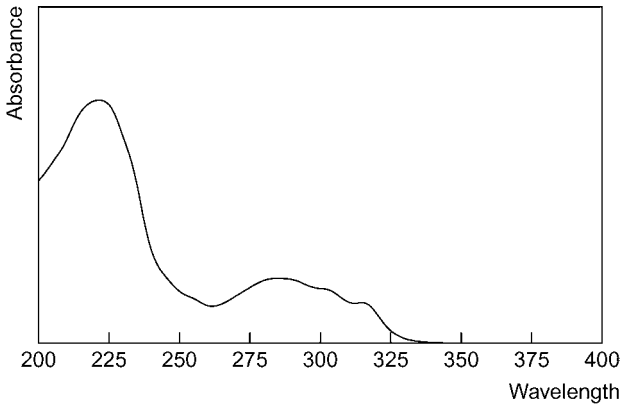
Proprietary Names *Azinos*; *Azinugec E*; *Bionex*; *Contrion-Ethyl*; *Gutex*; *Sepizin L*; *Triazotion*.



Chemical Properties Colourless crystals. Mp 53°. Bp 111°. It is soluble in water (4 to 5 mg/L at 20°); readily soluble in most organic solvents except aliphatic hydrocarbons. Log *P* (octanol/water), 3.40. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Thin-layer Chromatography System TX—R_f 0.24; system TY—R_f 0.48; system TAE—R_f 0.81.

Gas Chromatography System GA—RI 2560.



Mass Spectrum Principal ions at *m/z* 132, 77, 160, 105, 29, 65, 76, 97.

Quantification

Blood **GC** FPD. Limit of detection, 1 µg/L [Marques 1990].

Plasma **GC** Column: 3% OV-1 on 100 to 120 mesh Gas-Chrom Q (1.8 m × 0.25 mm i.d.). Temperature: 245°. Detector temperature: 300°. Carrier gas: 5% methane in argon, flow rate 50 mL/min. Detection: electron capture ^{63}Ni. Retention time: azinphos-ethyl, 1.5 min. Limit of detection, 1 µg/L [Stein, Pittman 1976].

Disposition in the Body Azinphos is rapidly absorbed from the gastro-intestinal tract after ingestion. It is excreted in urine.

Toxicity

In a series of azinphos-ethyl poisoning cases, fatal cases were found to have blood levels of 1.112 mg/L and above, serious cases of intoxication with levels of 494 µg/L and above, and less serious cases with levels above 57 µg/L. Serious intoxication was always observed with concentrations above 370 µg/L [Marques 1990].

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Marques EG (1990). Acute intoxication by azinphos-ethyl. *J Anal Toxicol* 14(4): 243–246.

Stein VB, Pittman KA (1976). Gas-liquid chromatographic determination of azinphos ethyl in human plasma and in mouse plasma, tissue, and fat. *J Assoc Off Anal Chem* 59(5): 1094–1096.

Azinphos-(Me)

Acaricide, Insecticide

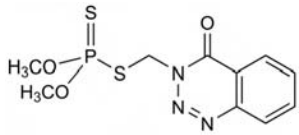
$C_{10}H_{12}N_3O_3PS_2$ = 317.3

CAS—86-50-0

IUPAC Name Phosphorodithioic acid *O,O*-dimethyl-*S*-[(4-Oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] ester

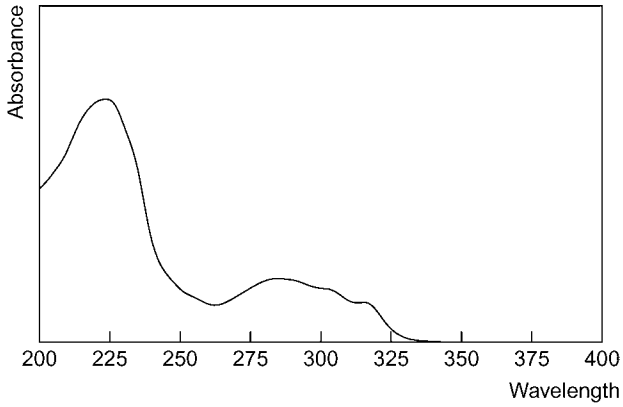
Synonym DBD

Proprietary Names *Gusathion M*; *Guthion*.



A

Chemical Properties Colourless crystals. It is rapidly hydrolysed in alkaline or acid media. Mp 73° to 74°. Soluble 1 in 30 000 of water; soluble in ethanol, methanol and propylene glycol. Log *P* (octanol/water), 2.8. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Palladium chloride—brown; phosphorus test—yellow.
Thin-layer Chromatography System TA—R_f 0.84; system TB—R_f 0.40; system TC—R_f 0.99; system TD—R_f 0.91; system TE—R_f 0.99; system TF—R_f 0.93; system TL—R_f 0.99; system TW—R_f 0.68; system TX—R_f 0.20; system TY—R_f 0.42; system TAD—R_f 0.94; system TAE—R_f 0.81; system TAF—R_f 0.87.
Gas Chromatography System GA—RI 2473; system GK—RRT 1.60 (relative to caffeine).
High Performance Liquid Chromatography System HY—RI 513.
Ultraviolet Spectrum Hexane—285 nm ($A_1^{\downarrow}$=280b), 302, 315 nm.



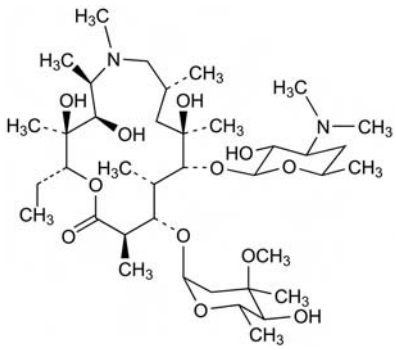
Infrared Spectrum Principal peaks at wavenumbers 1000, 1666, 833, 781, 1041, 775 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 77, 160, 132, 44, 105, 104, 93, 76.
Disposition in the Body
Toxicity Azinphos-methyl is less toxic than mevinphos or parathion. The maximum permissible atmospheric concentration is 0.2 mg/m^3.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

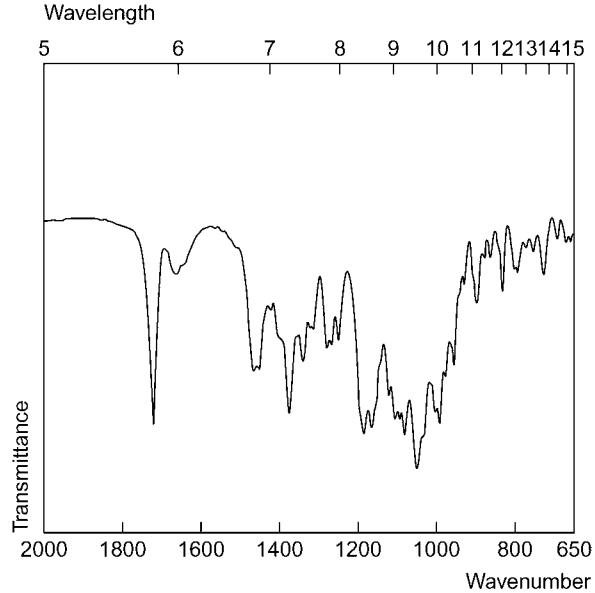
Azithromycin

Antibacterial
$C_{38}H_{72}N_2O_{12}$ = 749.0
CAS—83905-01-5 (anhydrous); 117772-70-0 (dihydrate)

IUPAC Name [2*R*-(2*R**,3*S**,4*R**,5*R**,8*R**,10*R**,11*R**,12*S**,13*S**,14*R**)]-13-[2,6-Dideoxy-3-*C*-methyl-3*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one
Synonyms 9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A; CP-62993; XZ-450.
Proprietary Names *Azitrocin*; *Azitromax*; *Goxil*; *Ribotrex*; *Sumamed*; *Toraseptol*; *Trozocina*; *Vinzam*; *Zentavion*; *Zithromax*; *Zitromax*.



Chemical Properties Mp 113° to 115°. pK_a 8.1 and 8.8. Log *P* (octanol/water), 4.02.
High Performance Liquid Chromatography System HBA—retention time 20.7 min; system HBB—retention time 9.6 min.
Infrared Spectrum Principal peaks at wavenumbers 1721, 1188, 1052 cm^{-1} (KBr pellet).



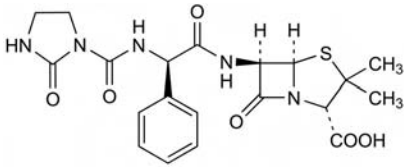
Quantification
Plasma **HPLC** Electrochemical detection. Limit of quantification, 0.03 mg/L [Taninaka *et al.* 2000].
Serum **HPLC** Fluorescence detection (λ_{ex}=255 nm, λ_{em}=315 nm). Limit of detection, 0.09 mg/L [Sastre Toraño, Guchelaar 1998]. Electrochemical detection. Limit of detection, 0.01 mg/L [Shepard *et al.* 1991].
Tissue **HPLC** Electrochemical detection. Limit of detection, 100 mg/g [Shepard *et al.* 1991].
Note For a comparison of microbiological assay and high performance liquid chromatography methods for the determination of azithromycin, see Riedel *et al.* [1992].
Disposition in the Body Azithromycin is rapidly absorbed after oral administration with a bioavailability of about 40%. Absorption from the capsule formulation, but not the tablet formulation, is reduced by food. It is extensively distributed to the tissues; tissue concentrations are much higher than those in blood and high concentrations are taken up into white blood cells. There is little diffusion into CSF unless meninges are inflamed. Some demethylation of the drug occurs in the liver and it is excreted in bile as the unchanged drug and its metabolites (~10 with no microbial activity); more than 50% of a dose is excreted in faeces as the unchanged drug. About 12% of an oral dose is excreted in urine over the course of about a week.
Therapeutic Concentration
Peak plasma concentrations of 0.165 to 0.623 mg/L (mean, 0.383 mg/L) were reported following oral administration of 10 mg/kg on day 1 followed by 5 mg/kg on days 2 to 5 in 14 children aged 6 to 15 years, both boys and girls. Peak concentrations were reached after 1 to 4 h (mean 2.4 h) [Nahata *et al.* 1993].
Following administration of a 1-g oral dose to 14 male subjects, mean peak serum concentrations at 1.7 h were 0.82 mg/L and mean peak lymph concentrations after 3.1 h were 0.22 mg/L [Bergan *et al*].
Bioavailability 37%.
Half-life Plasma half-life, 14 to >40 h.
Volume of Distribution 23 to 31 L/kg.
Clearance Plasma clearance, 630 mL/min.
Protein Binding Decreasing from 51% at 0.02 to 0.05 mg/L to 7% at 1 mg/L.
Note For reviews of azithromycin, see Hopkins [1991], Peters *et al.* [1992] and Lalak, Morris [1993].
Dose Usually 500 mg daily.

Bergan T *et al.* (1992). Azithromycin pharmacokinetics and penetration to lymph. *Scand J Infect Dis Suppl* 83: 15–21.
Hopkins S (1991). Clinical toleration and safety of azithromycin. *Am J Med* 91: 40S–45S.
Lalak NJ, Morris DL (1993). Azithromycin clinical pharmacokinetics. *Clin Pharmacokinet* 25: 370–374.
Nahata MC *et al.* (1993). Pharmacokinetics of azithromycin in pediatric patients after oral administration of multiple doses of suspension. *Antimicrob Agents Chemother* 37: 314–316.
Peters DH *et al.* (1992). Azithromycin. A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. *Drugs* 44: 750–799.
Riedel KD *et al.* (1992). Equivalence of a high-performance liquid chromatographic assay and a bioassay of azithromycin in human serum samples. *J Chromatogr* 576: 358–362.
Sastre Toraño J, Guchelaar HJ (1998). Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 720: 89–97.
Shepard RM *et al.* (1991). High-performance liquid chromatographic assay with electrochemical detection for azithromycin in serum and tissues. *J Chromatogr* 565: 321–337.
Taninaka C *et al.* (2000). Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *J Chromatogr B Biomed Sci Appl* 738: 405–411.

Azlocillin

Antibacterial
$C_{20}H_{23}N_5O_6S = 461.5$
CAS—37091-66-0

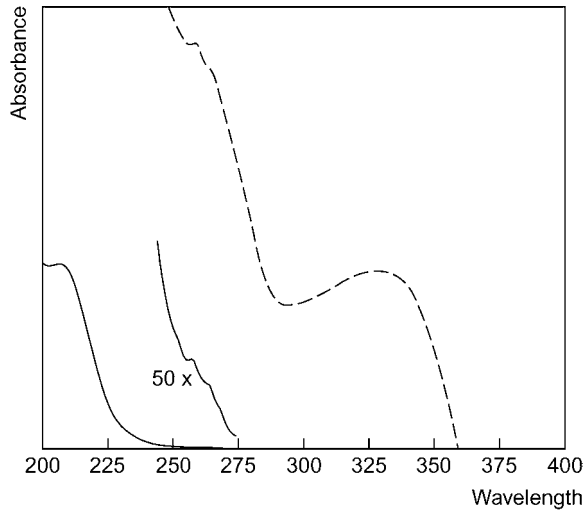
IUPAC Name (6*R*)-6-[D-2-(2-Oxoimidazolidine-1-carboxamido)-2-phenylacetamido]penicillin
Synonym Bay e 6905



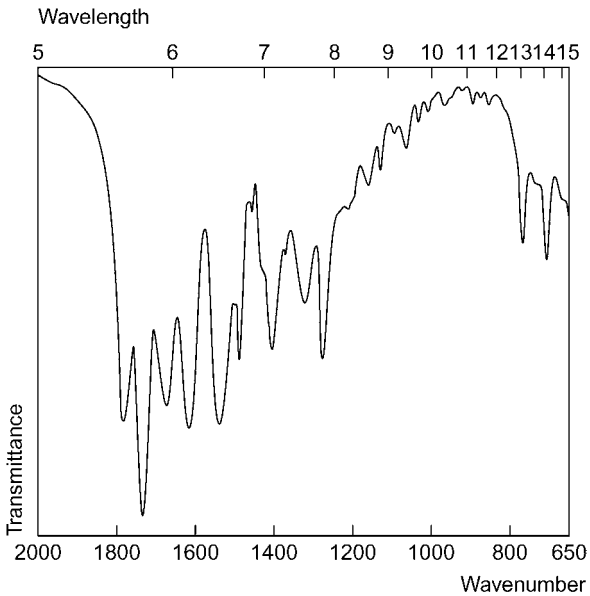
Azlocillin Sodium

$C_{20}H_{22}N_5NaO_6S = 483.5$
CAS—37091-65-9
Proprietary Names *Azlin*; *Securopen*.
Chemical Properties A white to pale yellow hygroscopic powder. It is freely soluble in water; soluble in dimethyl formamide and methanol; slightly soluble in ethanol and isopropanol; practically insoluble in acetone, chloroform and ether.

Ultraviolet Spectrum Water—257, 320 nm (sodium salt).



Infrared Spectrum Principal peaks at wavenumber 1722, 1606, 1532, 1772, 1272, 3295 cm^{-1} (sodium salt) (KBr disk).



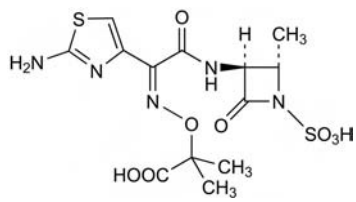
Quantification
Plasma **HPLC** UV detection (λ=220 nm). Limit of detection, 0.1 mg/L [Knöller *et al.* 1988]. UV detection (λ=220 nm). Limit of quantification, 5 µg/mL, limit of detection, 1.3 µg/mL [Hildebrandt, Gundert-Remy 1982]. UV detection. Limit of detection, 0.7 mg/L [Gundert-Remy and De Vries 1979].
Serum **HPLC** See Plasma [Knöller *et al.* 1988]. UV detection (λ=214 nm). Limit of detection, 0.1 mg/L [Jehl *et al.* 1987]. UV detection. Limit of detection, 0.4 mg/L [Weber *et al.* 1983].
Urine **HPLC** See Plasma [Knöller *et al.* 1988]. See Serum [Jehl *et al.* 1987]. UV detection. Limit of detection, 1 to 2 mg/L, azlocillin, 5 to 7 mg/L penicilloic acid metabolite [Gau, Horster 1979].
Bile **HPLC** See Serum [Jehl *et al.* 1987].
Tissues **HPLC** See Plasma [Knöller *et al.* 1988].
Disposition in the Body Azlocillin is poorly absorbed from the gastro-intestinal tract but is widely distributed following IV administration in body tissues and fluids. It crosses the placenta and small amounts are distributed into breast milk. There is little diffusion into CSF unless meninges are inflamed. It is metabolised to a limited extent and about 50 to 70% of a dose is excreted unchanged in the urine by glomerular filtration and tubular secretion. This occurs within 24 h of administration, therefore, producing high urinary concentrations. It is partly excreted in bile. Concomitant administration of probenecid increases plasma concentrations. It can be removed by haemodialysis.
Therapeutic Concentration
Following administration of 4 g as an IV infusion over 20 min to 12 healthy male and female subjects, the mean peak plasma concentration was 451 mg/L. This decreased to 223 mg/L after 30 min and 33 mg/L 4 h from the beginning of infusion [Colaizzi *et al.* 1986].
Half-life Plasma half-life, 1 h; 2 to 6 h in renal impairment.
Volume of Distribution 0.22 L/kg.
Clearance Serum clearance, 153 mL/min/1.73 m^2.
Protein Binding Between 20 and 46%.
Note For a review of the pharmacokinetics of azlocillin, see Bergan [1983].
Dose Up to 5 g (of the base) intravenously every 8 h; reduced in patients with renal dysfunction.

Bergan T (1983). Review of the pharmacokinetics and dose dependency of azlocillin in normal subjects and patients with renal insufficiency. *J Antimicrob Chemother* 11: 101–114.
Colaizzi PA *et al.* (1986). Comparative pharmacokinetics of azlocillin and piperacillin in normal adults. *Antimicrob Agents Chemother* 29: 938–940.
Gau W, Horster FA (1979). [High pressure liquid chromatographic analysis of azlocillin and its penicilloate in urine]. *Arzneimittelforschung* 29: 1941–1943.
Gundert-Remy U, De Vries JX (1979). Determination of the ureidopenicillins azlocillin, mezlocillin and bay K 4999 in plasma by high performance liquid chromatography. *Br J Clin Pharmacol* 8: 589–592.
Hildebrandt R, Gundert-Remy U (1982). Improved procedure for the determination of the ureidopenicillins azlocillin and mezlocillin plasma by high-performance liquid chromatography. *J Chromatogr* 228: 409–412.
Jehl F *et al.* (1987). Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. *J Chromatogr* 413: 109–119.
Knöller J *et al.* (1988). Application of high-performance liquid chromatography of some antibiotics in clinical microbiology. *J Chromatogr* 427: 257–267.
Weber A *et al.* (1983). High-pressure liquid chromatographic quantitation of azlocillin. *Antimicrob Agents Chemother* 24: 750–753.

Aztreonam

Antibacterial
$C_{13}H_{17}N_5O_8S_2 = 435.4$
CAS—78110-38-0

IUPAC Name [2*S*-[2α,3β(*Z*)]]-2-[[[1-(2-Amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methylpropanoic acid
Synonyms Azthreonam; SQ-26776.
Proprietary Names *Azactam*; *Azonam*; *Aztreon*; *Nebactam*; *Primbactam*; *Urobactam*.



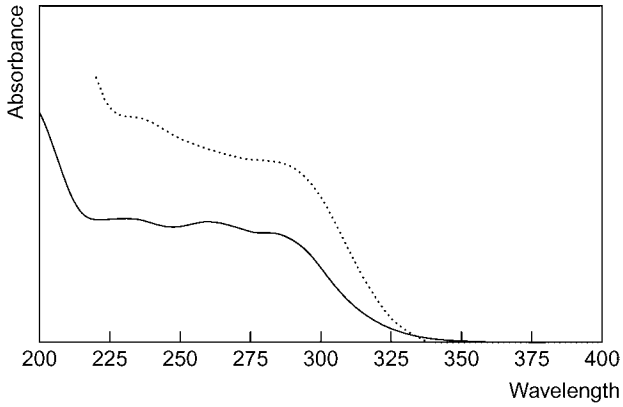
Chemical Properties A white crystalline odourless powder. Decomposes at 227°. It is very slightly soluble in dehydrated alcohol; slightly soluble in methanol; soluble in dimethylformamide and dimethylsulfoxide; practically insoluble in chloroform, ethyl acetate and toluene. pK_a, less than 2.0.

Aztreonam Disodium

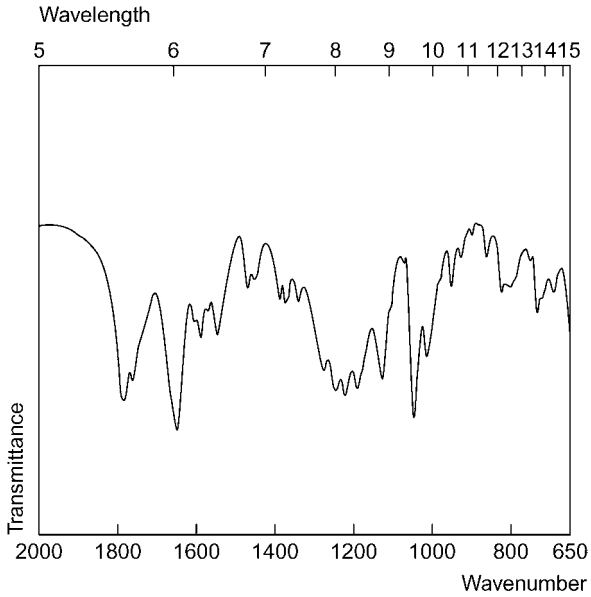
$C_{13}H_{15}N_5Na_2O_8S_2 = 479.4$

A

Ultraviolet Spectrum Aqueous acid (ethanol)—268, 281 nm; basic—289 nm.



Infrared Spectrum Principal peaks at wavenumber 1640, 1041, 633 cm^{-1} (KBr disk).



Quantification

Serum **HPLC** UV detection. Limit of detection, 0.5 mg/L [Jehl *et al.* 1987]. UV detection. Limit of detection, 1 mg/L [Mihindu *et al.* 1983]. UV detection (λ=293 nm). Limit of detection, 1 mg/L [Pilkiewicz *et al.* 1983]. UV detection. Limit of detection, 1 mg/L [Swabb *et al.* 1981].

Urine **HPLC** See Serum [Jehl *et al.* 1987]. See Serum [Mihindu *et al.* 1983]. UV detection (λ=293 nm). Limit of detection, 5 mg/L [Pilkiewicz *et al.* 1983]. UV detection. Limit of detection, 5 mg/L [Swabb *et al.* 1981].

Bile **HPLC** See Serum [Jehl *et al.* 1987].

Faeces **HPLC** UV detection. Limit of detection, about 2 μg/g [Ehret *et al.* 1987].

Bioassay Limit of detection, 0.03 mg/L [Ehret *et al.* 1987].

Disposition in the Body Aztreonam is completely absorbed after IM administration, with peak concentrations reached within 1 h. It takes 1 to 3 h for equilibrium to be reached between tissues and plasma. It is widely distributed in body tissues and fluids, including bile, but diffusion into CSF is poor unless meninges are inflamed. It crosses the placenta and small amounts are distributed into breast milk. Only about 6 to 16% is metabolised to inactive metabolites by hydrolysis. The drug is excreted predominantly in urine by renal tubular secretion and glomerular filtration; about 60 to 75% of a dose appears within 8 h as the unchanged drug with only small quantities of metabolites. Only small amounts of the unchanged drug and metabolites are excreted in faeces. It is removed by haemodialysis and to a lesser extent by peritoneal dialysis.

Therapeutic Concentration The trough serum therapeutic concentration range is 1 to 10 mg/L and the peak, 50 to 250 mg/L.

Twenty-four male volunteers were divided into 4 groups according to the degree of creatinine clearance. Group 1 (mean age, 27 years): creatinine clearance, ≥80 mL/min, group 2 (mean age, 43): 30 to 79 mL/min, group 3 (mean age, 44): 10 to 29 mL/min, group 4 (mean, 40 years old): <10 mL/min. Each group was administered with an IV dose of 1 g aztreonam over 2 min, after an overnight fast followed by a 2-h fast after administration. The mean peak serum concentrations for the groups were 78, 98, 98 and 145 mg/L, respectively, observed after 10 min [Mihindu *et al.* 1983].

Half-life Plasma half-life, 1.4 to 2.2 h; prolonged to 4.7 to 6 h in renal impairment and to some extent in hepatic impairment.

Volume of Distribution 0.11 to 0.21 L/kg; increased in neonates and paediatric patients (from 1 week to 12 years) to 0.3 to 1 L/kg.

Clearance Plasma clearance, 93 mL/min; reduced in neonates.

Protein Binding About 56%.

Note For reviews of aztreonam, see Brogden, Heel [1986] and Neu [1990].

For reviews of the pharmacokinetics of aztreonam, see Mattie [1988] and Mattie [1994].

Dose Up to 8 g daily by intravenous or intramuscular injection. Dose may need to be reduced in patients with severe renal impairment.

Brogden RN, Heel RC (1986). Aztreonam. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 31: 96–130.

Ehret W *et al.* (1987). Determination of aztreonam in faeces of human volunteers: a comparison of reversed-phase high pressure liquid chromatography and bioassay. *J Antimicrob Chemother* 19: 541–549.

Jehl F *et al.* (1987). Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. *J Chromatogr* 413: 109–119.

Mattie H (1988). Clinical pharmacokinetics of aztreonam. *Clin Pharmacokinet* 14: 148–155.

Mattie H (1994). Clinical pharmacokinetics of aztreonam. An update. *Clin Pharmacokinet* 26: 99–106.

Mihindu JC *et al.* (1983). Pharmacokinetics of aztreonam in patients with various degrees of renal dysfunction. *Antimicrob Agents Chemother* 24: 252–261.

Neu HC (1990). Aztreonam activity, pharmacology, and clinical uses. *Am J Med* 88: 2S–6S.

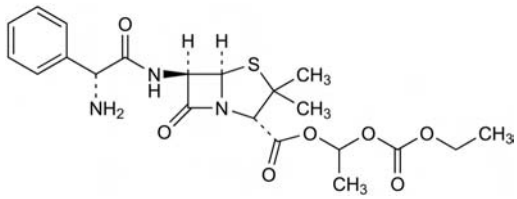
Pilkiewicz FG *et al.* (1983). High-pressure liquid chromatographic analysis of aztreonam in sera and urine. *Antimicrob Agents Chemother* 23: 852–856.

Swabb EA *et al.* (1981). Pharmacokinetics of the monobactam SQ 26,776 after single intravenous doses in healthy subjects. *J Antimicrob Chemother* 8: 131–140.

Bacampicillin

Antibacterial
$C_{21}H_{27}N_3O_7S = 465.5$
CAS—50972-17-3

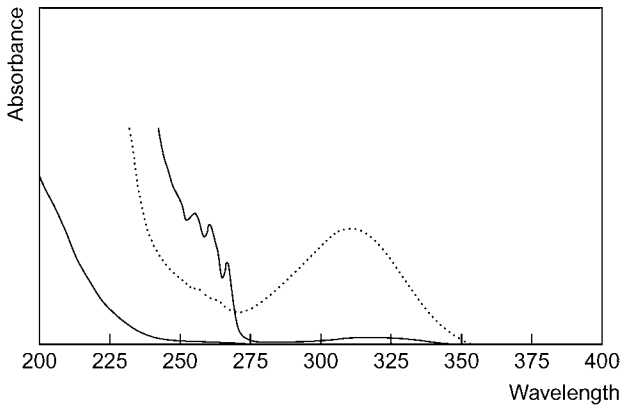
IUPAC Name (2*S*,5*R*,6*R*)-6-[[(2*R*)-Aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 1-[(ethoxycarbonyl) oxy]ethyl ester
Synonym Carampicillin



Bacampicillin Hydrochloride

$C_{21}H_{27}N_3O_7S,HCl = 502.0$
CAS—37661-08-8
Proprietary Names *Ambaxin*; *Penglobe*; *Spectrobid*.
Chemical Properties A white crystalline powder. Mp 171°. Soluble 1 in 15 of water, 1 in 7 of ethanol, and 1 in 10 of chloroform; practically insoluble in ether.

High Performance Liquid Chromatography System HAA—retention time 14.7 min.
Ultraviolet Spectrum Water—258, 263 (A_1^1=13b), 269 nm; aqueous alkali—310 nm.

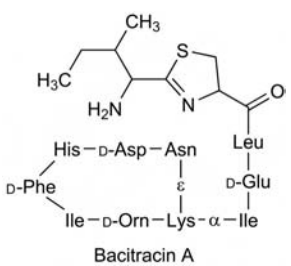


Infrared Spectrum Principal peaks at wavenumbers 1770, 1075, 1788, 1263, 1687, 1744 cm^{-1} (bacampicillin hydrochloride, KBr disk).
Dose 0.8 to 2.4 g of bacampicillin hydrochloride daily.

Bacitracin

Antibacterial
CAS—92528-87-5

IUPAC Name 4-[[2-[[2-(1-Amino-2-methylbutyl)-4,5-dihydro-1, 3-thiazole-4-carbonyl]amino]-4-methylpentanoyl]amino]-5-[[1-[[19-(2- amino-2-oxoethyl)-4-(3-aminopropyl)-10-benzyl-7-butan-2-yl-16- (carboxymethyl)-13-(4H-imidazol-4-ylmethyl)-2,5,8,11,14,17, 20-heptaoxo-3,6,9,12,15,18, 21-heptazacyclopentacos-1-yl]amino]-3-methyl-1-oxopentan-2-yl]amino]-5- oxopentanoic acid; zinc
Synonym Bacitracin is a polypeptide produced by the growth of an organism of the licheniformis group of *Bacillus subtilis*.
Proprietary Names *Baciguent*; *Bacitin*. It is an ingredient of *Ototrips* and *Polybactrin Soluble GU*.



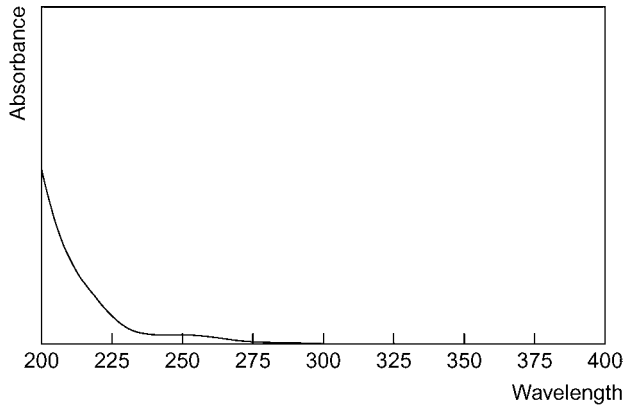
Bacitracin A

Chemical Properties A white to pale buff hygroscopic powder. Freely soluble in water; soluble in ethanol; practically insoluble in acetone, chloroform and ether.

Bacitracin Zinc

CAS—1405-89-6
Synonyms Bacitracins zinc complex; zinc bacitracin.
Proprietary Names It is an ingredient of *Cicatrene*, *Cicatrex*, *Cicatrin*, *Dispray Antibiotic*, *Polyfax*, and *Tribiotic*.
Chemical Properties A white, pale buff or tan, hygroscopic powder. Soluble 1 in 900 of water and 1 in 500 of ethanol; very slightly soluble in ether; practically insoluble in chloroform.

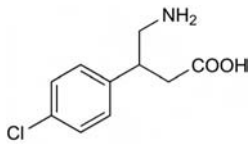
Ultraviolet Spectrum Bacitracin zinc: aqueous acid—252 nm (A_1^1=18b).



Baclofen

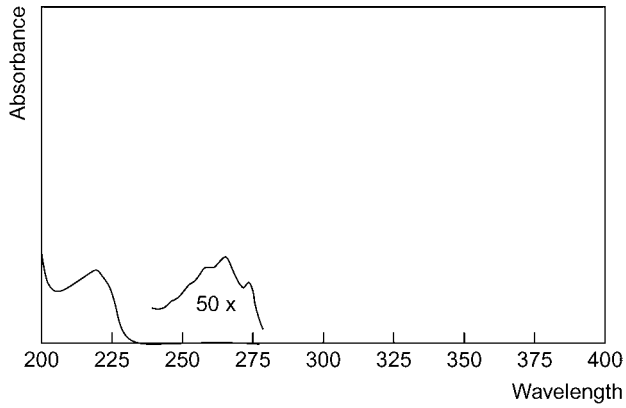
Muscle Relaxant
$C_{10}H_{12}ClNO_2 = 213.7$
CAS—1134-47-0

IUPAC Name β-(Aminomethyl)-4-chlorobenzenepropanoic acid
Synonym Aminomethyl chlorohydrocinnamic acid
Proprietary Name *Lioresal*

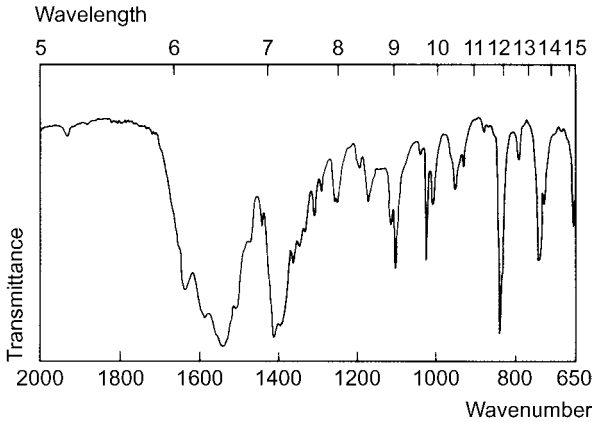


Chemical Properties A white crystalline solid. Mp about 207°. Slightly soluble in water; poorly soluble in organic solvents. pK_a 3.9, 9.6. Log *P* (octanol/water), −1.0.
Colour Test Liebermann's reagent—orange.
Thin-layer Chromatography System TD—R_f 0.01; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.00.
Gas Chromatography System GA—RI 2010.
High Performance Liquid Chromatography System HZ—retention time 2.0 min.

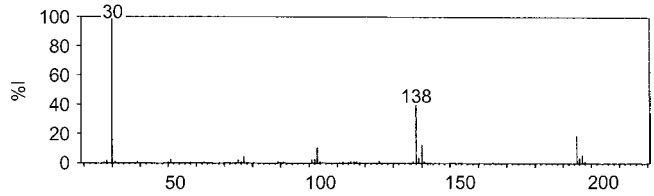
Column: C_{18} Bondapak (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : water (45:55), flow rate 0.4 mL/min. Fluorescence detection (λ_{ex}=463 nm; λ_{em}=524 nm). Retention time: 4.0 min [Tosunoglu, Ersoy 1995].
Ultraviolet Spectrum Aqueous acid—259, 266 (A_1^1=11.3a), 274 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1527, 835, 1574, 1495, 1624, 1095 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 30, 138, 195, 140, 103, 197, 77, 196.



Quantification

Plasma **GC** ECD. Limit of detection, 50 µg/L [Degen, Riess 1976].

HPLC Electrochemical detection. Limit of quantification, 10 µg/L, limit of detection, 2.5 µg/L [Millerioux *et al.* 1996].

Serum **GC–MS** Limit of detection, 5 µg/L [Swahn *et al.* 1979].

Urine **GC** See Plasma [Degen, Riess 1976].

Cerebrospinal Fluid **GC–MS** See Serum [Swahn *et al.* 1979].

Disposition in the Body Baclofen is rapidly absorbed after oral administration. About 80% of a dose is excreted in the urine in 24 h, mostly as unchanged drug. About 15% of a dose is metabolised, mainly to the deaminated derivative; about 20% of a dose may be eliminated in the faeces as unchanged drug and metabolites.

Therapeutic Concentration

A 45-year-old, healthy, woman administered 20 mg baclofen orally produced a peak plasma concentration of 270 µg/L after 2.5 h [Tosunoglu, Ersoy 1995].

After an oral dose of 40 mg to 1 subject, a peak plasma concentration of unchanged drug of about 0.6 mg/L was attained in 2 h; a peak concentration of metabolites of about 0.1 mg/L was attained in about 4 h [Faigle, Keberle 1972].

Toxicity Recovery has occurred after the ingestion of 1.5 g.

A fatality occurred after ingestion of baclofen. Baclofen was detected in serum at a concentration of 17 mg/L and in urine at 760 mg/L which was collected 12 h after ingestion of the drug [Fraser *et al.* 1991].

Coma, respiratory failure and severe seizures occurred in a 39-year-old female subject after ingestion of 450 mg of baclofen; following treatment, the patient regained consciousness within 36 h, at which time a plasma concentration of 0.2 mg/L was reported; the plasma half-life was found to be 35 h [Ghose *et al.* 1980].

Half-life Plasma half-life, about 2 to 4 h.

Protein Binding About 30%.

Dose 15 to 60 mg daily; maximum of 100 mg daily.

Degen PH, Riess W (1976). The determination of gamma-amino-beta-(p-chlorophenyl)butyric acid (baclofen) in biological material by gas-liquid chromatography. *J Chromatogr* 117: 399–405.
Faigle JW, Keberle H (1972). The chemistry and kinetics of Lioresal. *Postgrad Med J* 48: 9–13.
Fraser AD *et al.* (1991). Toxicological analysis of a fatal baclofen (Lioresal) ingestion. *J Forensic Sci* 36 (5): 1596–1602.
Ghose K *et al.* (1980). Complications of baclofen overdosage. *Postgrad Med J* 56: 865–867.
Millerioux L *et al.* (1996). High-performance liquid chromatographic determination of baclofen in human plasma. *J Chromatogr A* 729: 309–314.
Swahn CG *et al.* (1979). Mass fragmentographic determination of 4-amino-3-p-chlorophenylbutyric acid (baclofen) in cerebrospinal fluid and serum. *J Chromatogr* 162: 433–438.
Tosunoglu S, Ersoy L (1995). Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection. *Analyst* 120: 373–375.

Balsalazide

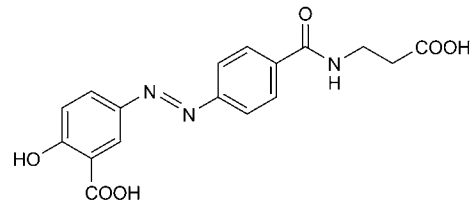
Mesalamine, Antiinflammatory

$C_{17}H_{15}N_3O_6 = 357.3$

CAS—80573-04-2

IUPAC Name (3*Z*)-3-[[4-(2-Carboxyethylcarbamoyl)phenyl]hydrazinylidene]-6-oxocyclohexa-1,4-diene-1-carboxylic acid

Synonyms 5-[(1*E*)-[4-[[2-Carboxyethyl)amino]carbonyl]phenyl]azo]-2-hydroxybenzoic acid; 5-[4-(2-carboxyethylcarbamoyl)phenylazo]salicylic acid; (*E*)-5-[[*p*-[2-carboxyethyl)carbamoyl]phenyl]azo]-2-salicylic acid.



Chemical Properties Crystals from hot ethanol. Mp 254° to 255°.

Balsalazide Disodium Dihydrate

$C_{17}H_{17}N_3Na_2O_8,2H_2O = 437.3$

CAS—150399-21-6; 82101-18-6 (anhydrous)

Synonyms Balsalazide sodium; BX-661A.

Proprietary Names *Colazal*; *Colazide*.

Chemical Properties Orange-to-yellow microcrystalline powder. Mp >350°. Non-hygroscopic. Freely soluble in water and isotonic saline; sparingly soluble in methanol and ethanol; practically insoluble in organic solvents.

Disposition in the Body Balsalazide is metabolised in the colon by bacterial azoreductases to 5-aminosalicyclic acid (5-ASA). 5-ASA is responsible for the antiinflammatory action, and 4-aminobenzoyl-β-alanine (4-ABA) is an inert carrier. Twenty-five percent of the released 5-ASA is inactivated in the colonic mucosa and liver to the *N*-acetylated metabolite NASA. 4-ABA is also converted to its *N*-acetylated metabolite NABA. Only NASA and NABA are detected in urine; the parent drug is excreted mainly in the faeces.

Therapeutic Concentration

Twenty healthy male and female volunteers were administered 2.25 g balsalazide (equivalent to 800 mg 5-ASA). A mean peak plasma 5-ASA concentration of 348 µg/L was reached at 9.1 h [Sandborn *et al.* 2004].

Fifty-four adult patients with ulcerative colitis (in remission) were administered with 3 to 6 g daily for at least 1 year. Peak plasma concentrations of 0.324 mg/L were reached within 2 h. [Green *et al.* 1998].

Peak plasma concentrations of the metabolite 4-ABA were <0.096 mg/L within 9 to 10 h after administration of a 2.25-g dose of balsalazide [Riddell *et al.* 1998].

Toxicity

Co-administration of balsalazide with azathioprine or 6-mercaptopurine in patients with Crohn's disease may result in leucopaenia [Lowry *et al.* 2001].

Hypersensitivity reaction: a 59-year-old woman was administered 2.25 g balsalazide 3 times a day. After 8 days, she was admitted into hospital with central chest pain, shortness of breath, and back pain which worsened over the next 3 days. Upon examination, she was apyrexial, with splinter haemorrhages in her fingernails, raised jugular venous pressure, a loud pericardial rub, and a soft, tender liver. Balsalazide treatment was stopped and all symptoms resolved within 1 month. [Adhiyaman *et al.* 2000].

Half-life Elimination half-life of NASA is 6 to 9 h, and for 5-ASA is ≈1 h.

Clearance 4.5 L/h.

Protein Binding 5-ASA ≈40% and NASA 80%.

Dose An initial dose of 2.25 g three times a day is administered for up to 12 weeks. Maintenance dose of 1.5 g twice daily with a maximum of 6 g daily.

Adhiyaman V *et al.* (2000). Hypersensitivity reaction to balsalazide. *BMJ* 320: 613.
Green JRB *et al.* (1998). Pharmacokinetic study of balsalazide disodium (Colazide®) in patients with ulcerative colitis receiving long term maintenance therapy. *Glycyx Pharmaceuticals Limited. GLY 01/93.*
Lowry PW *et al.* (2001). Leucopenia resulting from a drug interaction between azathioprine or 6-mercaptopurine and mesalamine, sulphasalazine, or balsalazide. *Gut* 49: 656–664.
Riddell JG *et al.* (1998). A comparative tolerability and pharmacokinetic study of balsalazide disodium (Colazide®), sulphasalazine (Salazopyrin®) and mesalazine (Asacol®) following a single oral dose. *Glycyx Pharmaceuticals Limited. Harris Project No. 20060.*
Sandborn WJ *et al.* (2004). Comparative pharmacokinetics of equimolar doses of 5-aminosalicylate administered as oral mesalamine (Asacol) and balsalazide: a randomized, single-dose, crossover study in healthy volunteers. *Aliment Pharmacol Ther* 19: 1089–1098.

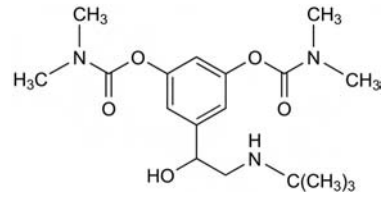
Bambuterol

Bronchodilator, β_2-Adrenoceptor Agonist

$C_{18}H_{29}N_3O_5 = 367.5$

CAS—81732-65-2

IUPAC Name Dimethylcarbamic acid 5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-1,3-phenylene ester

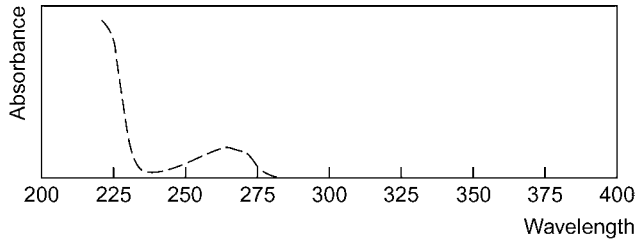


Bambuterol Hydrochloride

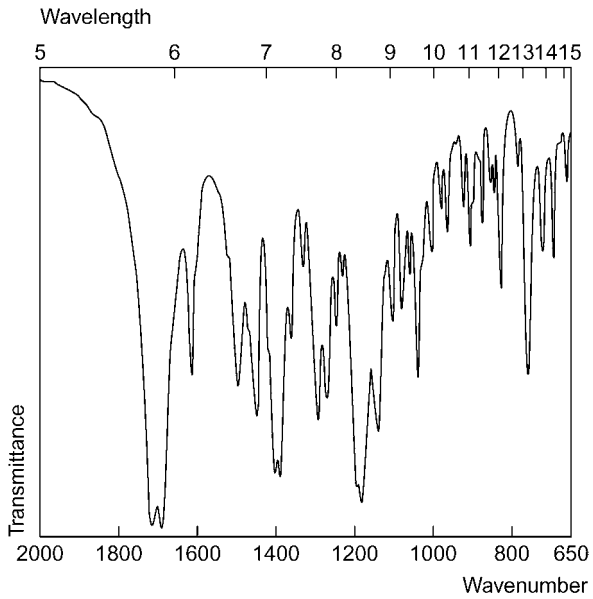
$C_{18}H_{29}N_3O_5$,HCl = 403.9
CAS—81732-46-9
Synonym KWD-2183
Proprietary Name *Bambec*
Chemical Properties A white or almost white crystalline powder. Exhibits polymorphism. It is freely soluble in water; soluble in ethanol.

Thin-layer Chromatography System TB—R_f 0.02 (bambuterol), R_f 00 (bambuterol monocarbamate); system TE—R_f 0.37 (bambuterol), R_f 0.21 (bambuterol monocarbamate); system TAE—R_f 0.18 (bambuterol); R_f 0.19 (bambuterol monocarbamate).
High Performance Liquid Chromatography System HX—RI 353 (bambuterol), RI 304 (bambuterol monocarbamate).

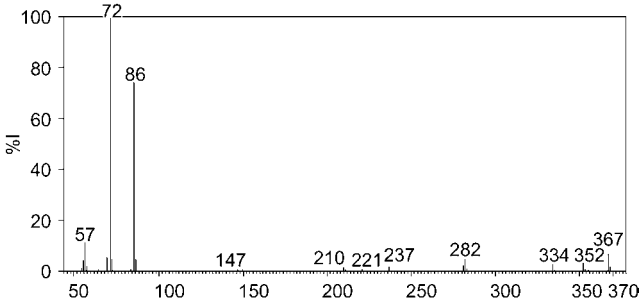
Column: LC-18-DB Supelcosil (150 × 4.6 mm i.d., 5 µm). Mobile phase: 6.0 mmol/L sodium octane sulfonate and methanol : acetonitrile : 50 mmol/L phosphate buffer pH 3.0 (34:11:55), flow rate 1.0 mL/min. UV detection (λ=214 nm). Retention time: 10 min [Wannerberg, Persson 1988].
Ultraviolet Spectrum Water—264 nm.



Infrared Spectrum Principal peaks at wavenumber 1695, 1720, 1190, 1200, 1390, 1350 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 72, 86, 57, 70, 367, 282, 352, 334.



Quantification
Plasma **GC–MS** Limit of quantification, 0.2 µg/L [Lindberg *et al.* 1990].
Urine **GC–MS** Limit of quantification, 1.5 µg/L [Lindberg *et al.* 1990].
Disposition in the Body About 20% of a dose is absorbed after oral administration. It concentrates in lung tissue where it is hydrolysed primarily by a plasma cholinesterase to its active metabolite terbutaline (β_2 adrenergic receptor agonist). It is also metabolised by oxidative enzymes in the liver. Approximately one-third of an IV dose is converted to terbutaline. It also undergoes oxidative metabolism to products that are then metabolised to terbutaline. Very little bambuterol is excreted unchanged in urine.

Therapeutic Concentration

Twelve healthy volunteers, male and female, aged between 23 and 62 years, were administered with IV doses of 30 g/kg over 5 min and oral doses of 7 g/kg as an aqueous solution. Peak plasma concentrations after the IV dose ranged between 72.5 and 184 nmol/L with a mean of 129 nmol/L. The mean peak plasma concentration, after a single oral dose, was 6.9 nmol/L (2.3 to 24.3 nmol/L) and was reached within 2.3 h (ranging between 1 and 4 h). [Nyberg *et al.* 1998].

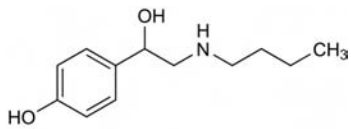
Twenty-three healthy Caucasian volunteers (12 women), aged between 20 and 56 years, were administered with oral tablets: 10, 20 and 30 mg, and a 20 mg solution as an open, crossover, random study. After the first dose, the mean peak plasma concentration was 3.69 nmol/L (1.0 to 8.8 nmol/L) for the 10 mg tablet, 8.56 nmol/L (ranging between 3.5 and 16.9 nmol/L) for the 20 mg tablet, 14.23 nmol/L (range, 5.3 to 30.4 nmol/L) for 30 mg and 6.13 nmol/L (1.7 to 13.6 nmol/L) for the 20 mg solution. The mean time to the peak concentration was 1.4 to 1.8 h [Rosenborg *et al.* 2000].

Bioavailability <15%.
Half-life Plasma, 12 h (oral administration); 2.6 h (IV administration).
Volume of Distribution Women, between 1.6 and 2.1 L/kg; men, 1.2 to 1.4 L/kg.
Clearance Plasma, 1250 mL/min.
Protein Binding 40 to 50%.
Distribution in Blood Distributes extensively outside the plasma component.
Note For a review of the pharmacokinetics of bambuterol, see Sitar [1996].
Dose Usually 20 mg daily.

Lindberg C *et al.* (1990). Determination of bambuterol, a prodrug of terbutaline, in plasma and urine by gas chromatography/mass spectrometry. *Biomed Env Mass Spectrom* 19: 218–224.
Nyberg L *et al.* (1998). Pharmacokinetics of bambuterol in healthy subjects. *Br J Clin Pharmacol* 45 (5): 471–478.
Rosenborg J *et al.* (2000). Pharmacokinetics of bambuterol during oral administration of plain tablets and solution to healthy adults. *Br J Clin Pharmacol* 49: 199–206.
Sitar DS (1996). Clinical pharmacokinetics of bambuterol. *Clin Pharmacokinet* 31: 246–256.
Wannerberg O, Persson B (1988). Liquid chromatographic method for the determination of bambuterol hydrochloride and related compounds. *J Chromatogr* 435: 199–203.

Bamethan

Vasodilator
$C_{12}H_{19}NO_2$ = 209.3
CAS—3703-79-5
IUPAC Name α-[(Butylamino)methyl]-4-hydroxybenzenemethanol

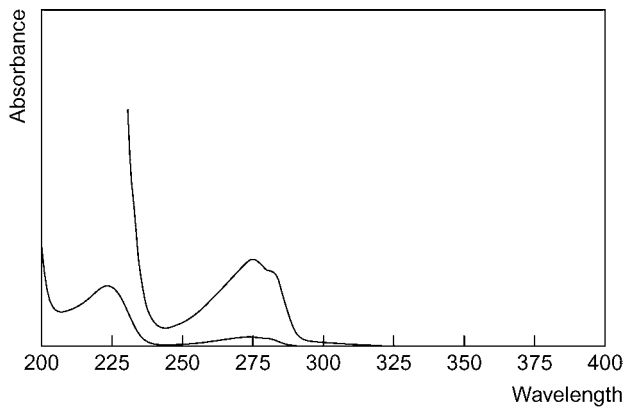


Chemical Properties Crystals. Mp 123.5° to 125°. pK_a 9.0, 10.2 (25°). Log *P* (octanol/water), 1.3.

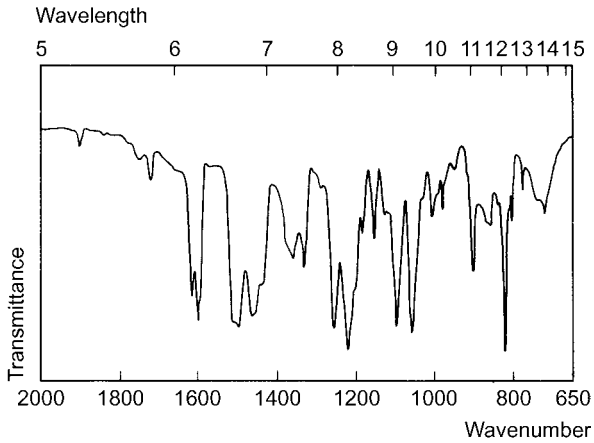
Bamethan Sulfate

$(C_{12}H_{19}NO_2)_2,H_2SO_4$ = 516.7
CAS—5716-20-1
Proprietary Names *Vasculat*; *Vasculit*.
Chemical Properties Soluble in water.

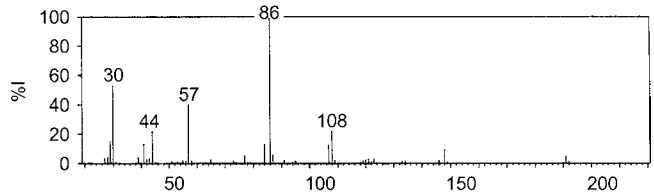
Colour Tests Folin-Ciocalteu reagent—blue; Liebermann's reagent—violet-brown; Mandelin's test—blue→green.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.04; system TC—R_f 0.06; system TL—R_f 0.00; system TAE—R_f 0.23 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1920.
High Performance Liquid Chromatography System HA—*k* 0.9; system HX—RI 250; system HAA—retention time 5.9 min.
Ultraviolet Spectrum Aqueous acid—275 nm (A^{1}_{1}=64a); aqueous alkali—242, 290 nm.



Infrared Spectrum Principal peaks at wavenumbers 832, 1228, 1066, 1264, 1494, 1108 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 30, 57, 108, 44, 29, 84, 41.



Disposition in the Body Bamethan is rapidly absorbed after oral administration. About 80% of a dose is excreted in the urine in 24 h, with about 30% of the dose as unchanged drug.

Therapeutic Concentration

After a single oral dose of 25 mg given to 5 subjects, peak serum concentrations of 0.04 to 0.11 mg/L (mean 0.07) were attained in 0.5 to 1 h [Hengstmann, Steinkamp 1981].

Half-life Plasma half-life, about 2.5 h.

Volume of Distribution 2.5 to 5 L/kg (mean 3.7).

Clearance Plasma clearance, 12 to 20 (mean 16) mL/min/kg.

Dose 100 mg of bamethan sulfate daily.

Hengstmann JH, Steinkamp B (1981). [Pharmacokinetics of 3H-bamethan in humans]. *Arzneimittelforschung* 31: 843–848.

Bamifylline

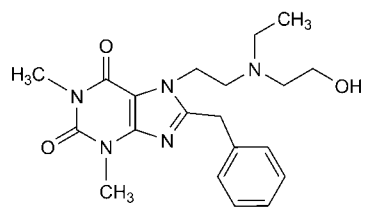
Adenosine A1 Antagonist, Bronchodilator

$C_{20}H_{27}N_5O_3 = 385.5$

CAS—2016-63-9

IUPAC Name 7-[2-(Ethyl-(2-hydroxyethyl)amino)ethyl]-1,3-dimethyl-8-(phenylmethyl)purine-2,6-dione

Synonyms Bamifyllin; benzetamophylline; 7-[2-[Ethyl(2-hydroxyethyl)amino] ethyl]-3,7-dihydro-1,3-dimethyl-8-(phenylmethyl)-1*H*-purine-2,6-dione.



Chemical Properties Crystals. Mp 80° to 80.5°.

Bamifylline Hydrochloride

$C_{20}H_{27}N_5O_3,HCl = 421.9$

CAS—20684-06-4

IUPAC Name 8-Benzyl-7-[2-(ethyl-(2-hydroxyethyl)amino)ethyl]-1,3-dimethylpurine-2,6-dione hydrochloride

Proprietary Names *Pulmac*; *Trentadil*.

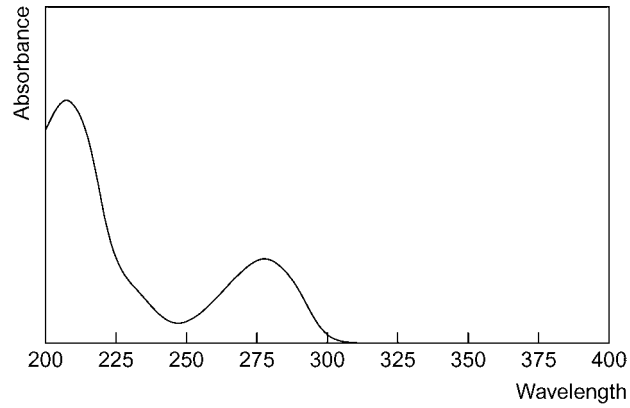
Chemical Properties Crystalline solid. Mp 185° to 186°. Soluble in water. Log *P* (octanol/water) 1.6 [Meylan, Howard 1995].

Colour Test Amalic acid test—orange/violet.

Thin-layer Chromatography System TA—R_f 0.65; system TAE—R_f 0.71; system TAG—R_f 0.34; system TB—R_f 0.00; system TC—R_f 0.54 (acidified iodoplatinate solution, positive).

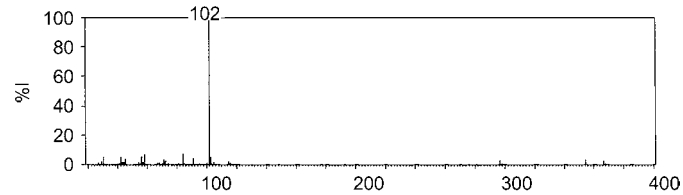
High Performance Liquid Chromatography System HAA—RT 10.3 min.

Ultraviolet Spectrum Aqueous acid—278 nm ($A^1_1 = 310$a); methanol—277 nm ($A^1_1 = 280$b).



Infrared Spectrum Principal peaks at wavenumbers 1650, 1698, 1538, 729, 1041, 1205 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 102, 84, 58, 56, 42, 30, 103, 91.



Quantification

Blood **GC-MS** Column: OV 101 fused silica capillary (15 m, 0.1 μm). Temperature programme: 120° for 1 min to 280° at 10°/min for 1 min. Limit of detection not reported [Offidani *et al.* 1993].

Plasma **HPLC** UV detection (λ = 280 nm). Limit of detection, 20 μg/L [Segre *et al.* 1990]. Column: Ultrasphere IP (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen phosphate containing 10 g tetrabutylammonium hydrogen sulphate/L (5 : 95). UV detection (λ = 276 nm). Limit of detection, 0.1 mg/L [Gerlo, Maes 1988]. Column: Hypersil ODS (5 μm). Mobile phase: 1.5 g heptanesulfonate and 3 g potassium chloride/L (pH 3.0) : acetonitrile (78 : 22), flow rate 1.3 mL/min. UV detection (λ = 275 nm). Limit of detection, 10 μg/L for bamifylline and desalkyl metabolites [Nicot *et al.* 1983].

Other **HPLC** Bulk Materials and Pharmaceutical Forms. Column: Column: Erbasil ODS (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : tetrahydrofuran : 0.01 mol/L potassium dihydrogen phosphate (pH 7.5; 60 : 4 : 40), flow rate 1.0 mL/min. UV detection (λ = 278 nm). Limit of detection, 10 μg/L [Carlucci *et al.* 1990].

Disposition in the Body

Therapeutic Concentration

Six male and female healthy subjects, mean age 33.3 years, were administered 600 mg bamifylline every 12 h for 7 days. The mean peak plasma concentration after the first dose was 1165 μg/L (range, 463 to 1708 μg/L) observed 1 h after dosing and 1038 (777 to 1238) μg/L for the last dose administered [Segre *et al.* 1990].

Toxicity

A 21-year-old woman who suffered from asthma was found dead. She had a blood bamifylline concentration of 205 mg/L [Offidani *et al.* 1993].

Dose Bamifylline hydrochloride has been given in doses of 0.9 to 1.8 g daily.

Carlucci G *et al.* (1990). Determination of bamifylline hydrochloride impurities in bulk material and pharmaceutical forms using liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* 8: 1067–1069.

Gerlo E, Maes V (1988). HPLC method for assay of bamifylline in plasma from neonates. *Clin Chem* 34: 1657.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

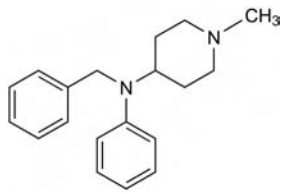
Nicot G *et al.* (1983). High-performance liquid chromatographic method for the determination of bamifylline and its three metabolites in human plasma. *J Chromatogr* 277: 239–249.

Offidani C *et al.* (1993). Fatal case of bamifylline intoxication. *Am J Forensic Med Pathol* 14: 244–245.

Segre G *et al.* (1990). Pharmacokinetics of bamifylline during chronic therapy. *Arzneimittelforschung* 40: 450–452.

Bamipine

Antihistamine
$C_{19}H_{24}N_2$ = 280.4
CAS—4945-47-5
IUPAC Name 1-Methyl-*N*-phenyl-*N*-(phenylmethyl)-4-piperidinamine

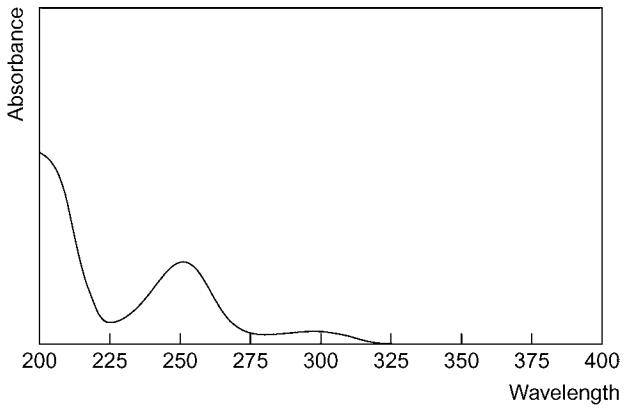


Chemical Properties Crystals. Mp about 115°. Soluble 1 in 75 of water. Log *P* (octanol), 4.1.

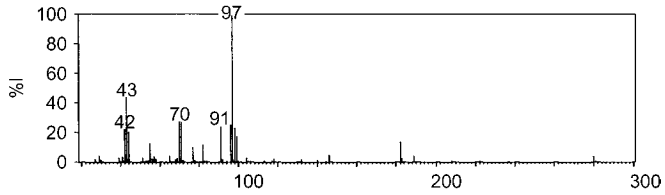
Bamipine Hydrochloride

$C_{19}H_{24}N_2$,HCl = 316.9
CAS—1229-69-2
Proprietary Names *Soventol*; *Taumidrine*.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—brown.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.40; system TC—R_f 0.43; system TL—R_f 0.13; system TAE—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2250, RI 2580 M (OH^-)-.
High Performance Liquid Chromatography System HX—RI 468.
Ultraviolet Spectrum Aqueous acid—251, 298 nm; methanol—251 (A_1^1=439b), 298 nm (A_1^1=62b).



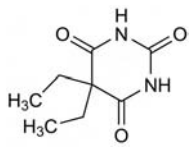
Infrared Spectrum Principal peaks at wavenumbers 745, 1593, 725, 1495, 690, 1275 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 97, 43, 71, 70, 96, 91, 98, 42.



Dose Bamipine hydrochloride has been given in doses of 75 to 400 mg daily.

Barbital

Sedative, Barbiturate
$C_8H_{12}N_2O_3$ = 184.2
CAS—57-44-3
IUPAC Name 5,5-Diethyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Barb; barbitalum; barbitone; diemalum; diethylmalonylurea; malonal.

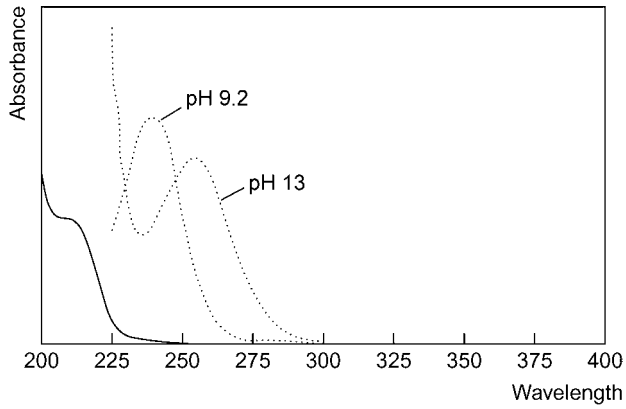


Chemical Properties Colourless crystals or white crystalline powder. Mp 188° to 192°. Soluble 1 in about 130 of water, 1 in 13 of boiling water, 1 in 14 of ethanol, 1 in 75 of chloroform and 1 in 35 of ether; soluble in acetone, ethyl acetate, petroleum ether, acetic acid, amyl alcohol, pyridine, aniline and nitrobenzene, and in solutions of alkalis. pK_a 8.0 (25°). Log *P* (octanol/water), 0.7.

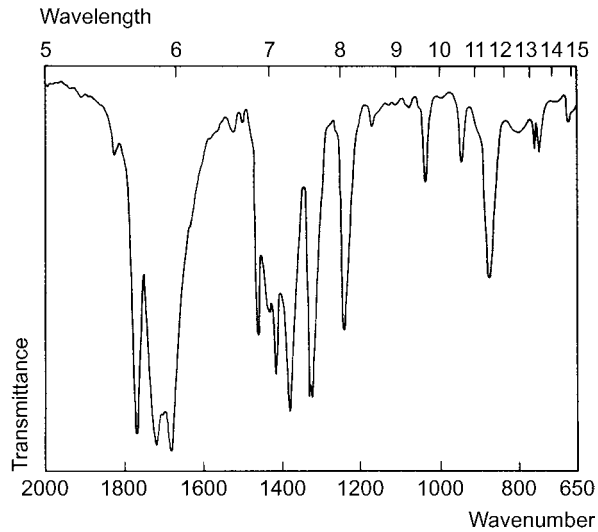
Barbital Sodium

$C_8H_{11}N_2NaO_3$ = 206.2
CAS—144-02-5
Synonyms Barbitalum natricum; barbitone sodium; diemalnatrium; soluble barbitone.
Proprietary Name *Neurinase*
Chemical Properties A white crystalline powder. A solution in water slowly decomposes. Mp about 190°. Soluble 1 in 5 of water (1 in 2.5 of boiling water) and 1 in 400 of ethanol; practically insoluble in chloroform and ether.

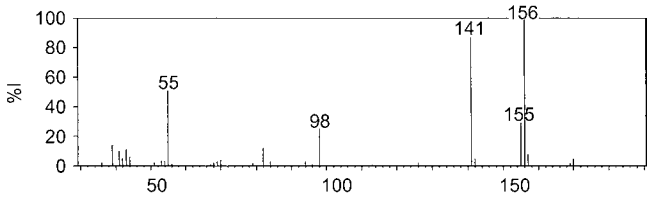
Colour Tests Koppanyi-Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.41; system TE—R_f 0.32; system TF—R_f 0.61; system TH—R_f 0.51; system TAD—R_f 0.57; system TAE—R_f 0.84 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; Zwikker's reagent, pink).
Gas Chromatography System GA—barbital RI 1489, barbital-Me_2 RI 1420, barbital-Me (metharbital) RI 1470, barbituric acid-Me_3 RI 1645; system GF—RI 2230; system GAJ—RRT 0.612 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 1.11; system HH—*k* 0.63; system HX—RI 308; system HY—RI 258; system HZ—retention time 2.2 min; system HAA—retention time 10.4 min; system HAL—retention time 1.4 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A_1^1=549a); 1 mol/L sodium hydroxide (pH 13)—254 nm (A_1^1=427b).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1720, 1767, 1320, 1245, 875 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 156, 141, 55, 155, 98, 39, 82, 43.



Quantification See also under Amobarbital.
Plasma **GC–MS** Limit of detection, 0.5 mg/L [Varin *et al.* 1980].
Disposition in the Body Readily absorbed after oral administration. It is excreted slowly in the urine almost entirely as unchanged drug; about 2% of a dose is excreted in 8 h, about 16% in 32 h, and detectable amounts may still be present in the urine after 16 days. Barbital is a metabolite of metharbital.
Therapeutic Concentration In plasma, usually in the range 5 to 30 mg/L.
Toxicity The estimated minimum lethal dose is 2 g. Toxic effects may be produced with blood concentrations of about 20 mg/L or more, and concentrations greater than 90 mg/L may be lethal.

In 6 cases of acute poisoning, the amounts ingested ranged from 5 to about 25 g. In 2 cases, death ensued after 150 and 160 h when the serum concentrations were about 100 and 170 mg/L respectively. In the other 4 cases, the serum concentrations on awakening from coma were 70 to 150 µg/L. [Lous 1954a, 1954b].

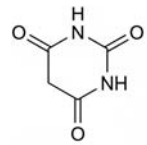
Half-life Plasma half-life, about 2 days.
Volume of Distribution About 0.5 L/kg.
Saliva Plasma : saliva ratio, about 1.
Protein Binding <20%.
Dose Barbital has been given in a dose of 300 to 600 mg.

Lous P (1954a). Plasma levels and urinary excretion of three barbituric acids after oral administration to man. *Acta Pharmacol Toxicol (Copenh)* 10: 147–165.
Lous P (1954b). Barbituric acid concentration in serum from patients with severe acute poisoning. *Acta Pharmacol Toxicol (Copenh)* 10: 261–280.
Varin F *et al.* (1980). GLC-mass spectrometric procedure with selected-ion monitoring for determination of plasma concentrations of unlabeled and labeled barbital following simultaneous oral and intravenous administration. *J Pharm Sci* 69(6): 640–643.

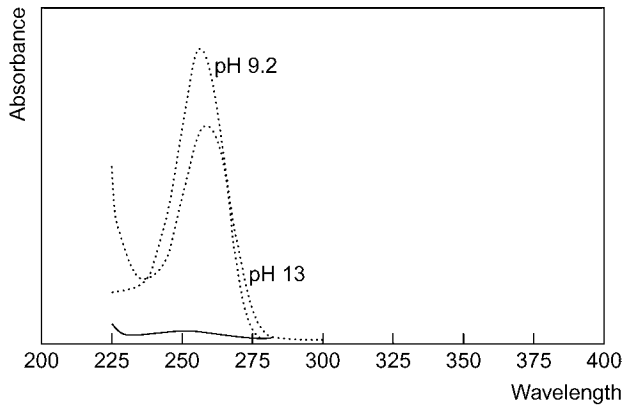
Barbituric Acid

Barbiturate
$C_4H_4N_2O_3$ = 128.1
CAS—67-52-7

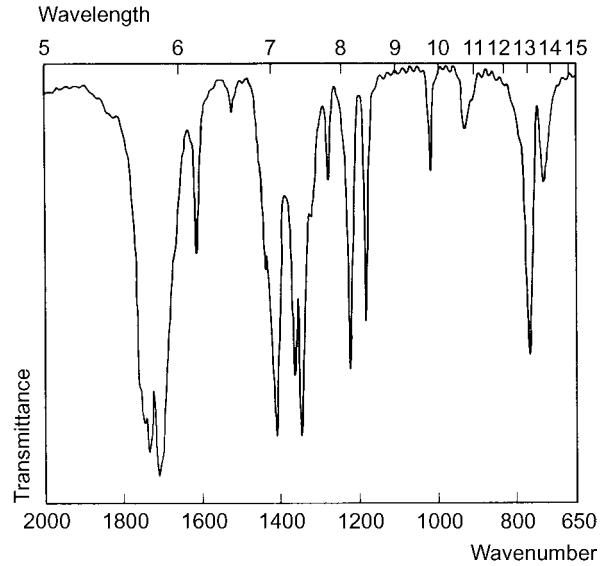
IUPAC Name 2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonym Malonylurea



Chemical Properties Crystals. Mp about 248°, with some decomposition. pK_a 4.0 (25°). Log *P* (octanol/water), −1.5.
Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TH—R_f 0.03; system TAD—R_f 0.00; system TAE—R_f 0.84.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—257 nm; 1 mol/L sodium hydroxide (pH 13)—259 nm.



Infrared Spectrum Principal peaks at wavenumbers 1710, 1734, 1750, 1225, 775, 1190 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 42, 128, 85, 43, 44, 41, 70, 69.

Barium

Potassium Channel Antagonist, Diagnostic Aid (Radiopaque Medium)
Ba^{2+} = 137.3
CAS—7440-39-3

Synonyms Barium, alloys non-pyrophoric; barium, alloys, pyrophoric; barium ion; barium, metal, non-pyrophoric; elemental barium.
Chemical Properties Silver-white malleable metal. Mp 710°. Bp 1600°. Soluble in benzene. Used as a gas absorbent.

Barium Acetate

$Ba(CH_3CO_2)_2$ = 255.5
CAS—543-80-6
Synonyms Acetic acid, barium salt; barium acetate monohydrate; barium diacetate.
Chemical Properties White crystals. Mp 41° (monohydrous). Very soluble in water, soluble in alcohol. Used as a mordant for printing fabrics; in lubricants; as catalyst in organic reactions.

Barium Carbonate

$BaCO_3$ = 197.3
CAS—513-77-9
Synonyms Carbonic acid, barium salt; witherite.
Proprietary Names *C. I. Pigment White 10*; *C. I. 77099*.
Chemical Properties White heavy powder. Decomposes at 1300° into BaO and CO_2. Almost insoluble in water; slightly soluble in CO_2–water; soluble in dilute hydrochloric acid, nitric acid or acetic acid; soluble in ammonium chloride and ammonium nitrate. Used as rat poison; in ceramics, paints, enamels; as a marble substitute; in the manufacture of paper, barium salts, electrodes, optical glass; as an analytical agent.

Barium Chloride

$BaCl_2$ = 208.2
CAS—10361-37-2
Synonyms Barium chloride dihydrate; barium dichloride.
Proprietary Name *SBA-0108E*
Chemical Properties The dihydrate occurs as crystals, granules or powder with a bitter salty taste. Mp 963°. Very soluble in water; soluble in methanol; practically insoluble in ethanol, acetone and ethyl acetate. Used in the manufacture of pigments, glass; in aluminium refining; as a pesticide; in tanning and finishing leather; as a boiler compound for softening water; formerly used as purgative in horses and as ruminatoric in cattle.

Barium Cyanide

$Ba(CN)_2$ = 189.4
CAS—542-62-1
Synonym Barium dicyanide
Chemical Properties Crystals; slowly decomposes in air. Very soluble in water, soluble in alcohol. Used in electroplating processes, metallurgy.

Barium Hydroxide

$Ba(OH)_2$ = 171.3
CAS—17194-00-2
Synonyms Barium dihydroxide; barium hydrate; barium hydroxide lime; barium hydroxide monohydrate; barium hydroxide octahydrate.
Proprietary Name *Caustic baryta*

Chemical Properties The monohydrate is a white powder. Slightly soluble in water; soluble in dilute acids. The octahydrate occurs as transparent crystals or white masses. Freely soluble in water, methanol; slightly soluble in ethanol; practically insoluble in acetone. Used in the manufacture of alkali, glass; in synthetic rubber vulcanisation; in corrosion inhibitors, drilling fluids, lubricants, pesticides, sugar industry, boiler scale remedy; refining animal and vegetable oils; softening water; fresco painting.

Barium Oxide

BaO = 153.3

CAS—1304-28-5

Synonyms Barium monoxide; barium protoxide.

Chemical Properties White to yellowish-white powder or lumps. Mp 1200°. Soluble in water, dilute acids, methanol and ethanol. Used for drying gases and solvents; in the manufacture of lubricating oil detergents.

Barium Sulfate

$BaSO_4$ = 233.4

Synonyms Artificial heavy spar; artificial barite; barites; blanc fixe.

Proprietary Names *Baridol*; *C.I. 77120*; *C.I. Pigment White 21*; *Citobaryum*; *Enamel White*; *E-Z-Paque*; *Solbar*; *Steripaque*.

Chemical Properties Fine heavy, odourless powder or polymorphous crystals. Mp 1600° with decomposition. Practically insoluble in water, dilute acids, alcohol. Soluble in hot concentrated sulfuric acid. Used in the manufacture of photographic papers, artificial ivory, cellophane; as a filler for rubber, linoleum, oil cloth, polymeric fibres and resins, paper, lithographic inks; as a water-colour pigment for coloured paper; in wallpaper; in heavy concrete for radiation shield.

Colour Tests

Qualitative Test (Applicable to gastric contents and scene residues.) Dip one end of a platinum wire into concentrated hydrochloric acid. Dip the moistened end into the test material. Insert the material into the hot region of a microburner and observe the colour of the flame—Barium imparts a yellow green colour to the flame (copper and thallium salts also produce a green flame). Limit of detection, 50 mg/L.

Confirmatory Test Mix 2 mL of lead acetate solution (100 g/L) with 2 mL of 1 mol/L sulfuric acid and add sufficient aqueous acetic acid (50 mL/L) to dissolve the lead sulfate precipitate. Add 0.1 mL of dilute acetic acid to 1 mL of sample, add 1 mL of the lead sulfoacetate solution (from step 1) and vortex mix for 5 s. Centrifuge for 2 min and examine the tube against a black background—The presence of barium is indicated by a white turbidity or a white precipitate (calcium and strontium interfere). Limit of detection, 100 mg/L.

Quantification

Specimen Collection Blood—5 mL K-EDTA tube; urine—20 mL plastic universal container.

Blood **ICP-AES** Limit of detection, 2 µg/kg [Dinya *et al.* 2005].

ICP-MS Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 10 µg/L [De Boer *et al.* 2004].

Serum **ICP-AES** Outer gas: 16.0 L/min. Nebulizer gas: 0.9 L/min. RCA 1P28 photomultiplier ($\lambda = 455$ nm). Limit of quantification, 11 µg/L, limit of detection, 0.22 µg/L [Mianzhi, Barnes 1985].

Urine **CE** Capillary: Polyimide-coated fused silica (total/effective length 81/60 cm, 100 µm). UV detection ($\lambda = 255$ nm). Limit of detection not reported [Xu *et al.* 1994]. Capillary: AccuSep fused silica (52 cm effective length, 75 µm). UV detection ($\lambda = 214$ nm). Limit of detection not reported [Buchberger *et al.* 1994].

ICP-MS See Blood. Limit of detection, 5 µg/L [De Boer *et al.* 2004].

Oral Fluid **ICP-MS** Plasma gas: 13 L/min. Auxillary gas: 0.55 L/min. Nebulizer gas: 0.1 L/min. Limit of detection, 0.11 µg/L [Menegario *et al.* 2001].

Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, a few nanograms per litre [Krachler *et al.* 2000].

Other **ICP-MS**. GSR. Coolant gas: 15 L/min. Auxiliary gas: 1.1 L/min. Sample gas: 0.97 L/min. Limit of detection, 0.507 µg/L [Reis *et al.* 2003].

Disposition in the Body

Approximately 90% of the total body burden of barium is found in the bone and teeth, with trace amounts present in all other human tissues. It is estimated that the gastrointestinal absorption of barium is <5%. Barium is not metabolised in the body but may be metabolically transported or incorporated into complexes or tissues. Only a fraction of an ingested dose is excreted in the urine (less than 3%), with the main route of elimination being via the faeces.

Note For a pharmacokinetic model of the transfer of alkaline earth elements to the fetus, see Fell *et al.* [2001] and for data on whole-body retention of injected ^{133}Ba, see Newton *et al.* [2001].

Normal Concentrations Plasma—<1 µg/L (<7 nmol/L); urine—< 20 µg/24h (<0.15 nmol/24 h); bone—2000 µg/kg; brain—4 µg/kg; heart—9 µg/kg; kidney—16 µg/kg; liver—3 µg/kg; lung—160 µg/kg.

Toxicity Insoluble barium salts (e.g. barium sulfate) are relatively innocuous by oral ingestion, although inhalation of barium dusts can produce a benign pneumoconiosis (baritosis) in mine workers. Soluble barium salts (e.g. the carbonate and chloride) are very toxic after oral or pulmonary ingestion and induce abdominal pain, diarrhoea, vomiting and muscular paralysis. This can be accompanied by a severe hypokalaemia, which causes disturbances in the cardiac rhythm. Doses as low as 0.5 g of a soluble barium salt can be lethal in an adult.

A 42-year-old man presented with marked lethargy and fever. He had ingested approx. 50 mL of an industrial chemical solution approx. 1 h prior to admission. His serum barium concentration was 34.1 µg/L [Hung, Chung 2004].

A 52-year-old woman attempted suicide by ingesting 70 mg amlodipine, 280 mg fluoxetine, and an unknown amount of an unidentified white powder. Barium carbonate intoxication was suspected so continuous venovenous haemodiafiltration (CVVHDF) was initiated. A total of 125 mg of barium was eliminated 14 h after initiation of CVVHDF and 152 mg after 42 h, at discontinuation of CVVHDF. Average barium concentrations in the 24 h urine collections on days 2 and 3 were 18.7 and 69.7 mg/L, respectively. Urinary barium excretion on days 2 and 3 was 69.7 and 101 mg, respectively [Koch *et al.* 2003].

A 61-year-old woman was hospitalised for the treatment of suspected acute pancreatitis. Following the administration of barium contrast material on day 27, the patient presented with severe abdominal bloating and prostration. The patient's condition continued to deteriorate and she died on day 41. The serum barium concentration was 370 µg/L on day 39 and 150 µg/L on day 41. The concentration of barium in the CSF was 440 µg/L [Pelissier-Alicot *et al.* 1999].

A study of welders using consumable electrodes with high barium content for 4 h each day showed high concentrations of barium in the urine, but they suffered no discernable health effects [Zschiesche *et al.* 1992].

A 22-year-old man swallowed approximately 5 to 10 g barium carbonate in an attempt to commit suicide. On admission, his plasma barium concentration was approx. 250 µg/L. Following haemodialysis, his plasma barium concentration had decreased to approx. 18 µg/L, with a half-life of 1.9 h. After haemodialysis, the plasma barium concentration increased transiently (redistribution) and then steadily decreased with a half-life of 18 h [Schorn *et al.* 1991].

An adult who died several hours after ingesting a depilatory containing barium sulfide had barium concentrations of 1.9 mg/L (blood), 0.4 mg/kg (brain), 1.6 mg/kg (liver), 6.1 mg/kg (bile), 5.9 mg/kg (spleen), 7.5 mg/kg (kidney), and 0.5 mg/L (vitreous) [Baselt 2005].

In 2 cases of non-fatal barium poisoning, serum barium concentrations of 3.4 and 7.8 mg/L were measured [Boehnert *et al.* 1985; Phelan *et al.* 1984].

A 62-year-old man sustained a barium burn when his jackhammer penetrated a pocket of molten barium chloride. Approximately 2 h after the burn, the plasma barium concentration was 12.4 mg/L. At 4, 8, 12 and 18 h postburn the barium concentrations were 10.3, 8.2, 8.9 and 4.0 mg/L, respectively. After several successful skin grafts, the patient was discharged [Stewart, Hummel 1984].

Two individuals who died 14 and 31 h after ingesting barium sulfide were found to have barium concentrations of 132 and 14 mg/kg in liver and 160 and 162 mg/kg in kidney, respectively [Baisane *et al.* 1978].

Note For a report of 2 cases of acute dyspnoea following aspiration of large amounts of barium sulfate, see Tamm and Kortsik [1999]; for a study on the environmental influences on the trace element content of teeth, see Brown *et al.* [2004]. Rahil-Khazen *et al.* [2002] have studied trace element levels in the autopsy tissue of 30 Norwegians.

Half-life 10–18 h [Baselt 2008].

Baisane SO *et al.* (1978). Spectrographic determination of barium in biological material. *Forensic Sci Int* 12: 127–129.

Baselt RC (2005). *Disposition of Toxic Drugs and Chemicals in Man*, 7th edn. Chemical Toxicology Institute.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, CA: Biomedical Publications.

Boehnert M *et al.* (1985). Measurement of serum levels in acute barium chloride overdose. *Vet Hum Toxicol* 27291.

Brown CJ *et al.* (2004). Environmental influences on the trace element content of teeth: implications for disease and nutritional status. *Arch Oral Biol* 49: 705–717.

Buchberger W *et al.* (1994). Applications of capillary zone electrophoresis in clinical chemistry. Determination of low-molecular-mass ions in body fluids. *J Chromatogr A* 671: 375–382.

DeBoer JL *et al.* (2004). Practical and quality-control aspects of multi-element analysis with quadrupole ICP-MS with special attention to urine and whole blood. *Anal Bioanal Chem* 379: 872–880.

Dinya M *et al.* (2005). Major and trace elements in whole blood of phlebotomized patients with porphyria cutanea tarda. *J Trace Elem Med Biol* 19: 217–220.

Fell TP *et al.* (2001). A model for the transfer of alkaline earth elements to the fetus. *Radiat Prot Dosimetry* 95: 309–321.

Hung Y, Chung M, H.M. (2004). Acute self-poisoning by ingestion of cadmium and barium. *Nephrol Dial Transplant* 19: 1308–1309.

Koch M *et al.* (2003). Acute barium intoxication and hemodiafiltration. *J Toxicol Clin Toxicol* 41: 363–367.

Krachler M *et al.* (2000). Concentrations of trace elements in osteoarthritic knee-joint effusions. *Biol Trace Elem Res* 75: 253–263.

Menegario AA *et al.* (2001). Determination of Ba, Cd, Cu, Pb and Zn in saliva by isotope dilution direct injection inductively coupled plasma mass spectrometry. *Analyst* 126: 1363–1366.

Mianzhi Z, Barnes RM (1985). Determination of major, minor, and trace elements in human serum by using inductively coupled plasma-atomic emission spectroscopy. *Appl Spectroscopy* 39: 793–796.

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Pelissier-Alicot AL *et al.* (1999). Fatal poisoning due to intravasation after oral administration of barium sulfate for contrast radiography. *Forensic Sci Int* 106: 109–113.

Phelan DM *et al.* (1984). Is hypokalaemia the cause of paralysis in barium poisoning? *Br Med J (Clin Res Ed)* 289: 882.

Rahil-Khazen R *et al.* (2002). Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). *J Trace Elem Med Biol* 16: 15–25.

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Schorn TF *et al.* (1991). Barium carbonate intoxication. *Intensive Care Med* 17: 60–62.

Stewart D, Hummel W, R.P. (1984). Acute poisoning by a barium chloride burn. *J Trauma* 24: 768–770.

Tamm I, Kortsik C (1999). Severe barium sulfate aspiration into the lung: clinical presentation, prognosis and therapy. *Respiration* 66: 81–84.

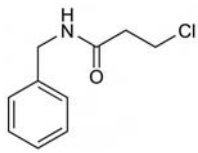
Xu X *et al.* (1994). Simultaneous determination of urinary creatinine, calcium and other inorganic cations by capillary zone electrophoresis with indirect ultraviolet detection. *J Chromatogr B Biomed Appl* 661: 35–45.
Zschiesche W *et al.* (1992). Exposure to soluble barium compounds: an interventional study in arc welders. *Int Arch Occup Environ Health* 64: 13–23.

B

Beclamide

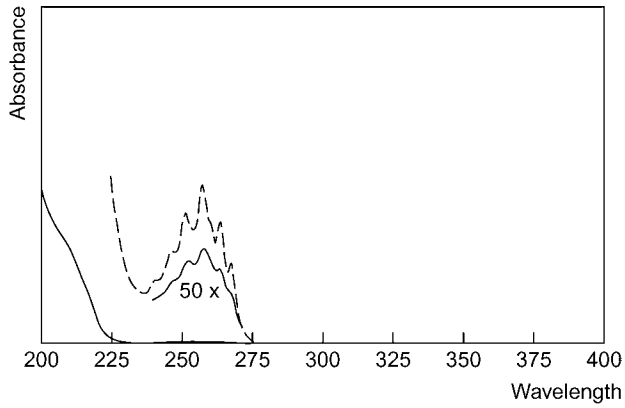
Anticonvulsant
$C_{10}H_{12}ClNO$ = 197.7
CAS—501-68-8

IUPAC Name 3-Chloro-*N*-(phenylmethyl)propanamide
Synonyms Benzchlorpropamide; chloroethylphenamide.
Proprietary Names *Neuracen*; *Nydrane*; *Posedrine*.

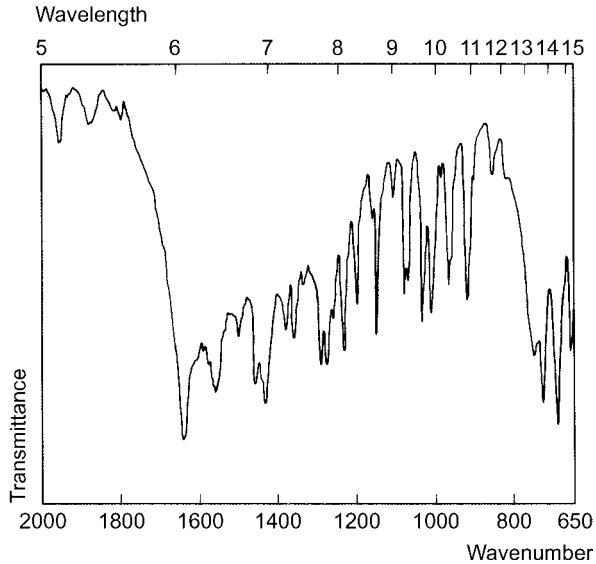


Chemical Properties Colourless crystals. Mp 91° to 94°. Slightly soluble in water; soluble 1 in 14 of ethanol, 1 in 5 of chloroform, and 1 in less than 100 of ether. Log *P* (octanol/water), 1.8.

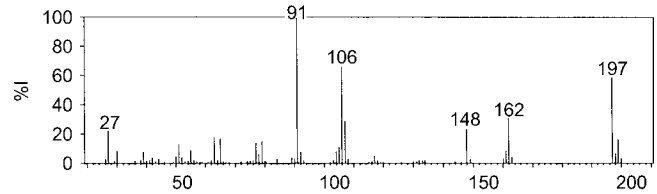
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.08; system TC—R_f 0.65; system TL—R_f 0.64; system TAE—R_f 0.90 (acidified potassium permanganate solution, positive, developing slowly).
Gas Chromatography System GA—beclamide RI 1720, beclamide-Art RI 1680; system GB—beclamide RI 1778, beclamide-Art RI 1539.
Ultraviolet Spectrum Aqueous acid—252, 258, 264 nm; methanol—252, 258 nm (A^1_1=9.6b), 264, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1640, 696, 736, 1558, 1275, 1290 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 106, 197, 162, 107, 148, 27, 63.



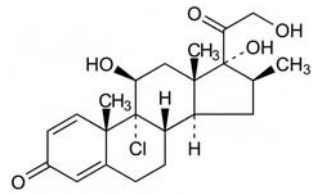
Quantification
***Plasma* GC** FID. Limit of detection, 200 µg/L [Jones *et al.* 1975].
***Urine* HPLC** Column: Apex ODS (250 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : water (22.5:77.5): potassium dihydrogen phosphate (1.4 g/L), flow rate 2 mL/min. Internal standard: hexobarbital. UV detection (λ=215 nm). Retention time: beclamide 8.8 min; 4-hydroxybeclamide 3.0 min; 3-hydroxybeclamide 3.5 min. Limit of detection, 0.2 mg/L for beclamide and metabolites [Ahmadi *et al.* 1995].
Disposition in the Body Beclamide is absorbed after oral administration. <0.5% of a dose is excreted in the urine as unchanged drug in 24 h; a metabolite, tentatively identified as an *N*-hydroxybenzyl-3-chloropropionamide, has been detected in the urine as a glucuronide conjugate. Its 3-hydroxyphenyl (7% excreted over 24 h) and 4-hydroxyphenyl (24% over 24 h) metabolites are detected as both glucuronide (majority) and sulfate conjugates in urine. Approximately 22% of an administered dose is excreted as hippuric acid.
Therapeutic Concentration
Following a single oral dose of 1 g to 6 subjects, peak plasma concentrations of 8.7 to 18.9 (mean 13) mg/L were attained in 1 to 4 h [Leach *et al.* 1975].
Dose 1.5 to 4 g daily.

Ahmadi M *et al.* (1995). Metabolism of beclamide after a single oral dose in man: quantitative studies. *J Pharm Pharmacol* 47: 876–878.
Jones OP *et al.* (1975). A simple method for the determination of beclamide in plasma by gas chromatography. *Br J Clin Pharmacol* 2: 364–365.
Leach H *et al.* (1975). Studies of plasma levels and the excretion of beclamide in normal human subjects [proceedings]. *Br J Clin Pharmacol* 2: 377P–378P.

Beclometasone

Corticosteroid
$C_{22}H_{29}ClO_5$ = 408.9
CAS—4419-39-0

IUPAC Name (11β,16β)-9-Chloro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione
Synonyms Beclomethasone; 9α-chloro-16β-methylprednisolone.



Chemical Properties Log *P* (octanol/water), 2.0.

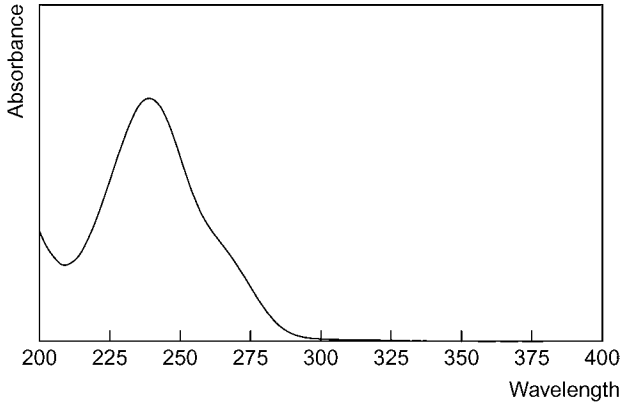
Beclometasone Dipropionate

$C_{28}H_{37}ClO_7$ = 521.0
CAS—5534-09-8
Proprietary Names *Aldecin*; *Becloforte*; *Beclovent*; *Beconase*; *Becotide*; *Clenil*; *Propaderm*; *Sanasthmyl*; *Turbinal*; *Vancenase*; *Vanceril*; *Viarox*. It is an ingredient of *Ventide*.
Chemical Properties A white to creamy-white powder. Mp about 212°, with decomposition. Practically insoluble in water; soluble 1 in 60 of ethanol and 1 in 8 of chloroform; freely soluble in acetone.

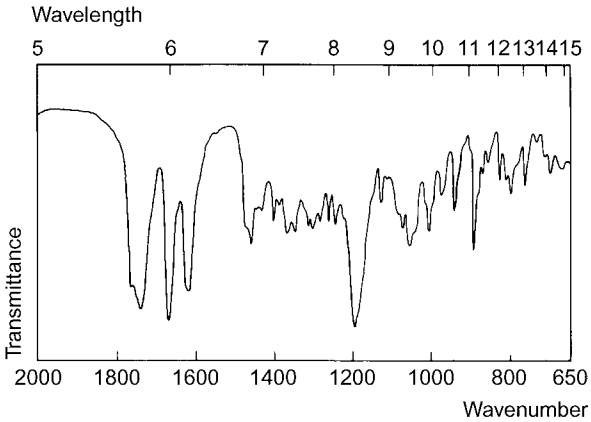
Colour Tests Naphthol–sulfuric acid—green/brown-yellow; sulfuric acid—orange (slow).
Thin-layer Chromatography Beclometasone dipropionate: system TP—R_f 0.75; system TQ—R_f 0.38; system TR—R_f 0.89; system TS—R_f 0.42, streaking may occur (*p*-Toluenesulfonic acid solution, positive).
High Performance Liquid Chromatography System HT—*k* 4.2; system HX—RI 444; system HY—RI 711 (dipropionate).
Column: C_{18} Alltima (250 × 4.6 mm i.d.). Mobile phase: methanol : water : acetonitrile : glacial acetic acid (650:262:88:1.75), flow rate 1.3 mL/min. Internal standard (IS): dexamethasone-21-acetate. UV detection (λ=242 nm). Retention time: beclometasone, 3.9 min; beclometasone propionate, 15.4 min [Foe *et al.* 1998a].
Column: (1) C_{18} Alltima 16% carbon load (250 × 4.6 mm i.d., 5 µm); (2) C_{18} Econosphere 10% carbon load (250 × 4.6 mm i.d., 5 µm); (3) C_8 Ultrasphere (150 × 4.6 mm i.d., 5 µm). Mobile phase: (1) methanol : water : acetonitrile : acetic acid (309:146:44:1), flow rate 1.3 mL/min; (2) (250:205:44:1), 1.2 mL/min; (3) (275:180:44:1), 1.3 mL/min. UV detection (λ=242 nm). Retention time:

beclometasone, (1) 4.9 min, (2) 5.5 min, (3) 3.3 min; beclometasone propionate, (1) 23.0 min, (2) 25.9 min, (3) 17.6 min [Foe *et al.* 1998b].

Ultraviolet Spectrum Beclometasone dipropionate: dehydrated alcohol—239 nm (A^{1}_{1}=292a).



Infrared Spectrum Principal peaks at wavenumbers 1190, 1658, 1730, 1608, 890, 1053 cm^{-1} (beclometasone dipropionate, KBr disk).



Quantification

Hair **HPLC–MS** MS detection (*m/z* 411). Limit of detection, 0.17 ng/g [Cirimele *et al.* 2000].

Disposition in the Body Beclometasone dipropionate is rapidly converted to the metabolite beclometasone-17-monopropionate.

Therapeutic Concentration

Twelve healthy males, aged 22 to 39 years, were administered a single IV dose of 1.0 mg over a 10-min infusion, a single intranasal dose of 1.344 mg, a single inhaled dose of 1.0 mg and a single oral dose of 4.0 mg as an aqueous solution. Subjects were fasted 12 h before dosing and for 2 h afterwards. The median peak plasma beclometasone dipropionate concentrations for the above doses were 35.356 µg/L (range 20.2 to 61.7 µg/L) observed at 0.2 to 0.3 h, 0.31 (0.256 to 0.375) µg/L at 4 to 8 h, 0.319 (0.184 to 0.553) µg/L at 0.2 to 0.5 h and 0.703 (0.563 to 0.879) µg/L at 1.5 to 6.0 h, respectively [Daley-Yates *et al.* 2001].

Half-life Beclometasone dipropionate, 0.3 to 0.7 h; beclometasone 17-monopropionate, 1.3 to 5.3 h.

Volume of Distribution Beclometasone dipropionate, median, 20 L (range 10.6 to 39.4); beclometasone 17-monopropionate, 424 (362 to 496) L.

Clearance Plasma, beclometasone dipropionate, median, 150 (98.8 to 228) L/h; beclometasone 17-monopropionate, 120 (104 to 139) L/h.

Dose Usually 300 to 400 µg of beclometasone dipropionate daily, by aerosol inhalation; maximum of 2 mg daily.

Cirimele V *et al.* (2000). Identification of ten corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry. *Forensic Sci Int* 107(1–3): 381–383.

Daley-Yates PT *et al.* (2001). Beclomethasone dipropionate: absolute bioavailability, pharmacokinetics and metabolism following intravenous, oral, intranasal and inhaled administration in man. *Br J Clin Pharmacol* 51(5): 400–409.

Foe K *et al.* (1998a). Decomposition of beclomethasone propionate esters in human plasma. *Biopharm Drug Dispos* 19: 1–8.

Foe K *et al.* (1998b). Degradation products of beclomethasone dipropionate in human plasma. *Drug Metab Dispos* 26(2): 132–136.

Bemegride

CNS Stimulant, Antidote (Barbiturate Poisoning)

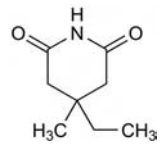
$C_8H_{13}NO_2$ = 155.2

CAS—64-65-3

IUPAC Name 4-Ethyl-4-methyl-2,6-piperidinedione

Synonym Methetharimide

Proprietary Names *Eukraton*; *Megimide*.



Chemical Properties White flakes or crystalline powder. Mp 126° to 128°. Soluble 1 in 170 of water, 1 in 30 of ethanol, 1 in 12 of acetone, 1 in 4 of chloroform and 1 in 100 of ether; soluble in aqueous solutions of alkali hydroxides. pK_a 11.6. Log *P* (octanol/water), 0.5.

Bemegride Sodium

$C_8H_{12}NNaO_2$ = 177.2

CAS—25334-00-3

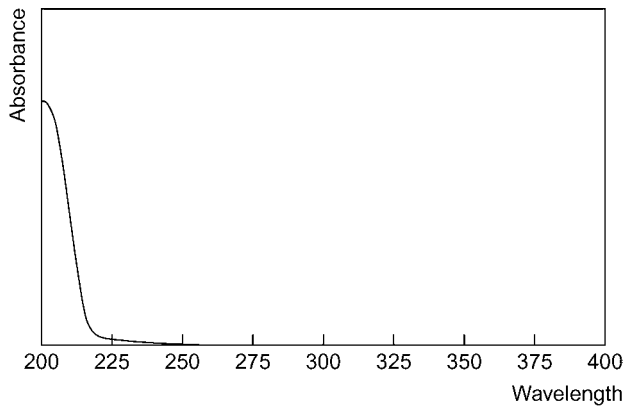
Chemical Properties A fine white powder. Soluble 1 in 11 of water and 1 in 20 of ethanol; practically insoluble in acetone and ether.

Colour Tests Koppanyi-Zwikker test—violet; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.52; system TE—R_f 0.68; system TF—R_f 0.53; system TAD—R_f 0.64; system TAF—R_f 0.88 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black).

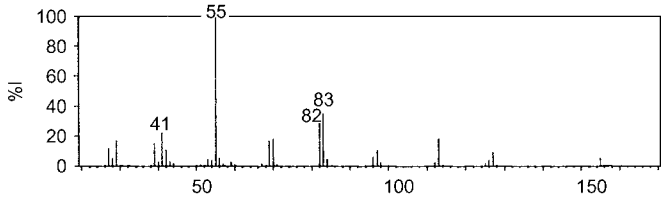
Gas Chromatography System GA—RI 1373; system GC—RI 1253.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1680, 1277, 1730, 860, 1160, 1145 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 55, 83, 82, 41, 113, 70, 29, 69.



Disposition in the Body Metabolised by hydroxylation of the ethyl side-chain and excreted in the urine as unchanged drug and as the hydroxy metabolite.

Dose Bemegride has been given in doses of 25 to 50 mg intravenously, repeated as necessary.

Bemiparin

Anticoagulant, Heparin, Anti-Factor Xa Activity

CAS—9041-08-1

Synonyms Bemiparin sodium; RO-11.

Proprietary Names *Hibor*; *Ivor*; *Zibor*.

Chemical Properties Prepared by alkaline degradation of heparin obtained from the intestinal mucosa of pigs. The majority of the components have a 2-*O*-sulfo-4-enepyranosuronic acid structure at the non-reducing end and a 2-*N*,6-*O*-disulfo-D-glucosamine structure at the reducing end of their chain. The average molecular weight is approx. 3600 (3000 to 4200). The degree of sulfation is approx. 2 per disaccharide unit.

Disposition in the Body Rapidly absorbed after SC injection, with peak plasma activity reached in about 2 to 4 h, depending on the dose. Peak plasma

activity following IV administration is approx. 3 times that following SC administration of the same dose. Data concerning the metabolism of bemiparin are not available, although it is suggested that elimination occurs via the renal route.

Therapeutic Concentration

Twelve healthy young male volunteers were administered three SC injections of bemiparin: 7500 IU anti-FXa (90 mg), 9000 IU anti-FXa (120 mg), and 12 500 IU anti-FXa (150 mg), leaving a washout period of 1 week between treatments. Tissue factor pathway inhibitor (TFPI, total and free) plasma levels were reported as follows:

Bemiparin anti-FXa (IU)	*Plasma TFPI (μg/L)*
Total TFPI	
7 500	112.8
9 000	133.0
12 500	139.2
Free TFPI	
7 500	43.5
9 000	53.2
12 500	61.7

These were reached within 2 to 6 h but returned to baseline levels 10 to 12 h after injection [Falkon *et al.* 1998].

In 2 dose-ranging studies, healthy volunteers were administered SC injections of bemiparin: 2500, 5000, 7500, 9000, and 12 500 IU anti-FXa. Maximum anti-Xa activity (E_{Max}) was reported as follows:

Regimen (anti-Xa IU)	*E_{max} (IU/mL)*	*t_{max} (h)*
2 500	0.34	2 to 3
5 000	0.54	3 to 4
7 500	1.22	3 to 6
9 000	1.42	3 to 6
12 500	2.03	2 to 6

Another set of subjects was administered an IV injection of 5000 IU anti-Fxa; the E_{max} was reported as 1.30 IU/mL [Falkon *et al.* 1995, 1997].

Twelve healthy volunteers were administered SC injections of bemiparin 3500 IU anti-XFa. The maximum anti-Xa activity was reported as 0.34 IU/mL after 2.5 h [Depasse *et al.* 2003].

Bioavailability Approximately 96%.

Half-life Approximately 5 to 6 h.

Volume of Distribution Approximately 5.1 L.

Clearance 0.90 L/h.

Dose In the prophylaxis of venous thromboembolism during general surgery with moderate risk, bemiparin sodium is given SC in a dose of 2500 IU once daily, with the first dose given 2 h before or 6 h after surgery; in patients undergoing orthopaedic surgery with high risk of thromboembolism, the dose should be 3500 IU initially and then once daily. Treatment should be continued for at least 7 to 10 days and until the patient is fully ambulant. For treatment of thromboembolism, a dose of 115 IU/kg is given SC once daily. For the prevention of clotting in the extracorporeal circulation during haemodialysis, bemiparin sodium is administered into the arterial side of the dialyser in a single dose of 2500 IU for patients weighing less than 60 kg and 3500 IU for patients weighing more than 60 kg.

Depasse F *et al.* (2003). Comparative study of the pharmacokinetic profiles of two LMWHs – bemiparin (3500 IU, anti-Xa) and tinzaparin (4500 IU, anti-Xa) – administered subcutaneously to healthy male volunteers. *Thromb Res* 109: 109–117.

Falkon L *et al.* (1995). Bioavailability and pharmacokinetics of a new low-molecular-weight heparin (RO-11): a three way cross-over study in healthy volunteers. *Thromb Res* 78: 77–86.

Falkon L *et al.* (1997). Pharmacokinetics and tolerability of a new low molecular mass heparin (RO-11) in healthy volunteers: a dose-finding study within the therapeutical range. *Thromb Haemost* 77: 133–136.

Falkon L *et al.* (1998). Tissue factor pathway inhibitor and anti-FXa kinetic profiles of a new low-molecular-mass heparin, bemiparin, at therapeutic subcutaneous doses. *Blood Coagul Fibrinolysis* 9: 137–141.

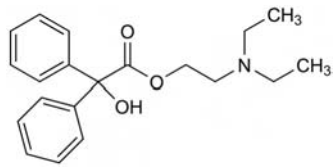
Benactyzine

Antispasmodic

$C_{20}H_{25}NO_3$ = 327.4

CAS—302-40-9

IUPAC Name α-Hydroxy-α-phenylbenzeneacetic acid 2-(diethylamino)ethyl ester



Chemical Properties Crystals. Mp 51°. pK_a 6.6. Log *P* (octanol/water), 2.9.

Benactyzine Hydrochloride

$C_{20}H_{25}NO_3$,HCl = 363.9

CAS—57-37-4

Proprietary Name It is an ingredient of *Deprol*.

Chemical Properties A white crystalline powder. Mp 177° to 181°. Soluble 1 in 14 of water and 1 in 22 of ethanol; very slightly soluble in ether.

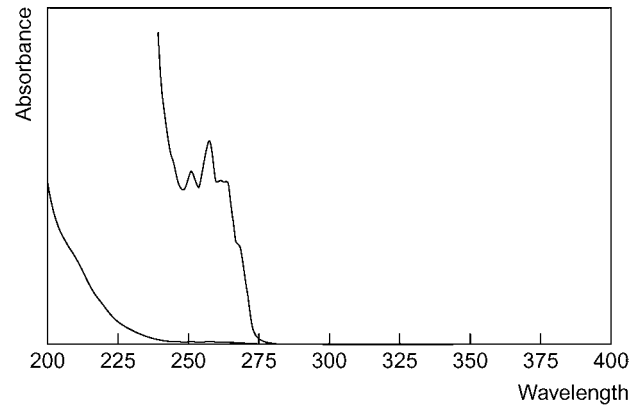
Colour Tests Liebermann's reagent—orange→brown; Marquis test—orange→green→blue; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.40; system TC—R_f 0.53; system TL—R_f 0.53; system TAE—R_f 0.52; system TAF—R_f 0.57; system TAJ—R_f 0.34; system TAK—R_f 0.03; system TAL—R_f 0.48 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2255.

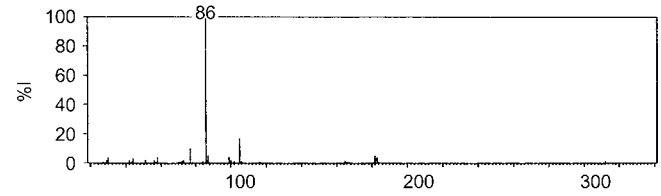
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 382.

Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=14a), 262, 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 695, 1723, 1231, 1054, 1162, 751 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 86, 105, 77, 87, 182, 99, 183, 58.



Disposition in the Body Rapidly metabolised and excreted.

Dose 3 to 9 mg of benactyzine hydrochloride daily.

Benaprizine

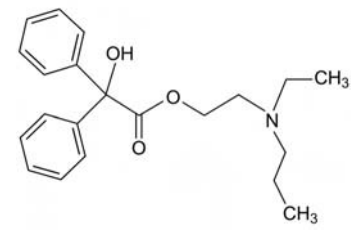
Anticholinergic

$C_{21}H_{27}NO_3$ = 341.4

CAS—22487-42-9

IUPAC Name 2-(*N*-Ethylpropylamino)ethyl benzilate

Synonym Benapryzine



Benaprizine Hydrochloride

$C_{21}H_{27}NO_3$,HCl = 377.9

CAS—32702-55-9

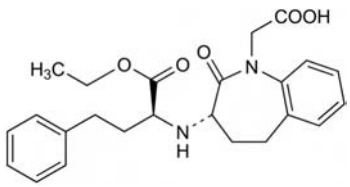
Chemical Properties A white crystalline powder. Mp about 166°. Soluble 1 in 35 of water, 1 in 56 of dehydrated alcohol, 1 in 120 of acetone and 1 in 8 of chloroform.

Thin-layer Chromatography System TAJ—R_f 0.48; system TAK—R_f 0.05; system TAL—R_f 0.58.
Ultraviolet Spectrum Aqueous acid—251, 257, 261, 264 nm.
Infrared Spectrum Principal peaks at wavenumbers 1753, 1215, 700, 690, 1180, 1020 cm^{-1} (benaprizine hydrochloride, KCl disk).
Dose 150 to 200 mg of benaprizine hydrochloride daily.

Benazepril

ACE Inhibitor, Antihypertensive
$C_{24}H_{28}N_2O_5$ = 424.5
CAS—86541-75-5

IUPAC Name 2-[(3*S*)-3-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-2-oxo-4,5-dihydro-3*H*-1-benzazepin-1-yl]acetic acid
Synonym [*S*-(*R**, *R**)]-3-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-oxo-1*H*-1-benzazepine-1-acetic acid



Chemical Properties Mp 148.5°. Log *P* (octanol/water), 3.50.

Benazepril Hydrochloride

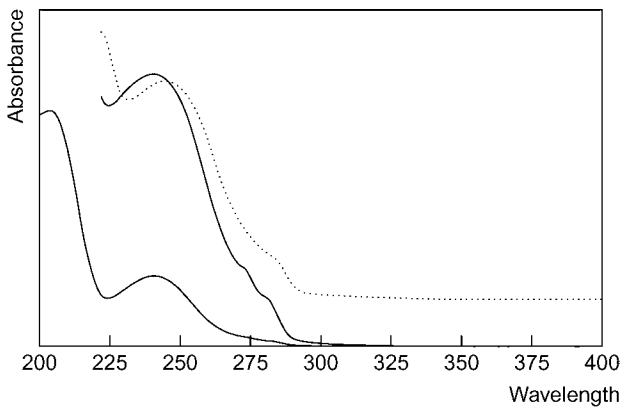
$C_{24}H_{28}N_2O_5$,HCl = 461.0
CAS—86541-74-4
Synonym CGS 14824A
Proprietary Names *Briem*; *Cibace*; *Cibacen*; *Cibacene*; *Fortekor*; *Labopal*; *Lotensin*; *Tensanil*; *Zinandril*.
Chemical Properties A white to off-white crystalline powder. It is soluble in water, ethanol and methanol.

Gas Chromatography System GP—methyl ester (-ME) derivative RI 3030, M (-ME_3) RI 2985.
High Performance Liquid Chromatography System HAA—RT 17.0 min.

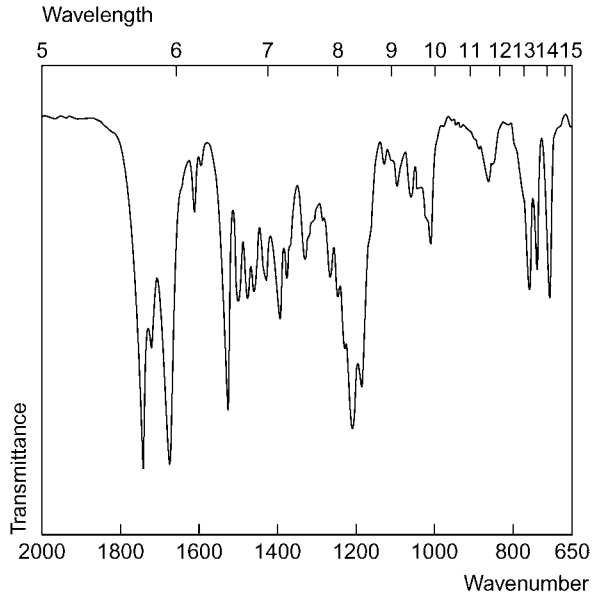
Column: RP-BDS C_{18} (250 × 3 mm i.d., 5 µm). Mobile phase: 0.025 mol/L sodium dihydrogen phosphate (pH 4.8) : acetonitrile (55 : 45), flow rate 0.4 mL/min. UV detection (λ = 250 nm). Retention time: benazepril hydrochloride, 4.95 min. [Panderi, Parissi-Poulou 1999].

Column: Hypersil ODS (250 × 4.5 mm i.d., 5 µm). Mobile phase: 20 mmol/L sodium heptane sulfonate (pH 2.5) : acetonitrile (5% THF; 52 : 48), flow rate 1.0 mL/min. UV detection (λ = 215 nm). Retention time: 15 min [Bonazzi *et al.* 1997].

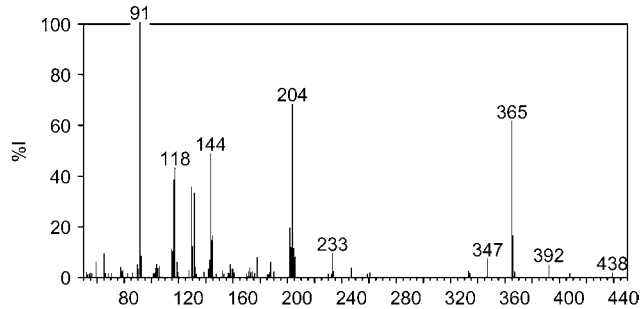
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid)—237 nm; basic—241 nm; aqueous acid (0.1 mol/L hydrochloric acid)—237.2 nm (hydrochloride salt).



Infrared Spectrum Principal peaks at wavenumber 1739, 1674, 1211 cm^{-1} (KBr pellet).



Mass Spectrum Principal peaks at *m/z* 365, 204, 91, 366, 392, 347 (benazepril methyl ester derivative); 379, 204, 380, 91, 144, 438 (benzaprilat).



Quantification

Plasma **GC-MS** Column: Resteck Rtx-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 50 mL/min. Temperature programme: 190° for 0.5 min to 290° at 30°/min. MSD. Limit of quantification, 2.5 µg/L [Pommier *et al.* 2003]; Column: cross-linked methyl silicone (12.5 m × 0.2 mm i.d.). Carrier gas: He, 55 kPa. Temperature programme: 210° for 0.5 min to 290° at 50°/min. MSD. Limit of quantification, 5.4 nmol/L [Sioufi *et al.* 1988]. Column: 3% OV-101 on Gaschrom Q, 80/100 mesh (1.5 m × 2 mm i.d.). Temperature: 275°. Carrier gas: He, 30 mL/min. EI ionisation at 70 eV, SIM acquisition mode. Retention times: benazepril (ME derivative) 2.55 min, benazeprilat 2.3 min. Limit of quantification, 26 µg/L for benazepril and 23 µg/L for benazeprilat, limit of detection, 3.4 µg/L for benazepril and 2.3 µg/L for benazeprilat [Kaiser *et al.* 1987].

HPLC Column: Hypersil BDS-C_{18} (300 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L phosphate buffer (pH 2.6) : acetonitrile 70 : 30 for 6 min to 50 : 50 at 15 min for 1 min to 70 : 30 at 20 min for 3 min), flow rate 1.0 mL/min. UV detection (λ = 237 nm). Limit of quantification, 20 µg/L, limit of detection, 10 µg/L for benazepril and benazeprilat [Wang *et al.* 2007].

LC-MS Column: reversed-phase porous graphitised carbon (125 × 2.1 mm i.d., 5 µm). Mobile phase: 55% acetonitrile : 0.3% formic acid (55 : 45), flow rate 0.15 mL/min. SIM acquisition mode. Limit of quantification, 5 µg/L for benazepril, benazeprilat and hydrochlorothiazide [Vonaparti *et al.* 2006]. Column: Spherigel C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% aqueous formic acid (35 : 65 for 5 min to 65 : 35 at 5.5 min until 12 min to 35 : 65 at 12.5 min until 15 min), flow rate 0.8 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 6.67 µg/L, limit of detection, 2.5 µg/L [Xiao *et al.* 2005].

Bioassay Limit of detection, 8.5 µg/L [Graf *et al.* 1988].

Urine **GC-MS** See Plasma. Limit of quantification, 11 nmol/L [Sioufi *et al.* 1988]. SIM acquisition mode. Limit of quantification, 34 µg/L for benazepril and 64.5 µg/L for benazeprilat, limit of detection, 6.4 µg/L for benazepril and 7.6 µg/L for benazeprilat [Kaiser *et al.* 1987].

Bioassay Limit of detection, 4.25 µg/L [Graf *et al.* 1988].

Other **HPLC** Tablets. Column: BDS C_{18} (250 × 3.0 mm i.d., 5 µm). Mobile phase: 0.025 mol/L sodium dihydrogen phosphate (pH 4.8) : acetonitrile (55 : 45), flow rate 0.4 mL/min. UV detection (λ = 250 nm). Limit of quantification, 2.92 mg/L, limit of detection, 0.88 mg/L [Panderi, Parissi-Poulou 1999].

Disposition in the Body A minimum of 37% of an oral dose of benazepril is absorbed (fasting) and is rapidly converted to its active carboxylic metabolite benazeprilat by enzymatic hydrolysis in the liver. Benazepril is cleared mainly by

metabolic transformation, with <1% being excreted unchanged in urine along with its acyl glucuronide metabolite. Approximately 18% of an oral dose is recovered in urine as the diacid benazeprilat. Peak plasma concentrations of benazeprilat occur 1–2 h after administration if fasting, and 2–4 h in the non-fasting state. The majority of benazeprilat is excreted in urine, with 11–12% excreted in bile. Elimination is slow in renal impairment. Very small amounts of benazepril and benazeprilat are secreted into breast milk.

Therapeutic Concentration

A daily (oral) dose of 10 mg benazepril was administered to 9 young subjects (male; 21–39 years) and 9 elderly subjects (6 male, 3 female; between 66 and 76 years), over 8 consecutive days. After repeated administration, benazeprilat was detected in plasma immediately before the last dose with mean values of 0.015 µg/L for the young subjects and 0.037 µg/L for the elderly. These values, and those obtained 24 h later, are consistent with steady-state disposition of benazeprilat. Time to peak concentrations was 1.5 h for both groups, after acute and chronic administration [Macdonald *et al.* 1993].

Toxicity Severe hypotension with acute overdose.

Half-life Apparent elimination half-life for benazepril is 0.6 h and it is eliminated from plasma completely within 4 h. Benazeprilat is eliminated in a biphasic manner with an initial half-life of 3 h and a terminal half-life of 17 h.

Volume of Distribution Steady state, ~0.7 L/kg (metabolite).

Distribution in Blood Benazeprilat is not extensively distributed into extravascular sites with minimum passage across the blood–brain barrier.

Protein Binding Benazepril and benazeprilat both bind serum proteins, ~94% and 93%, respectively.

Note For a study on the pharmacokinetics and pharmacodynamics of benazepril, see Shionoiri *et al.* [1992].

Dose For hypertension, an initial dose of 10 mg once daily is recommended in patients with creatinine clearance of 30 mL/min or greater and in those not receiving diuretics. For those with a creatinine clearance <30 mL/min or receiving diuretics, an initial daily dose of 5 mg is recommended. For heart failure, an initial daily dose of 2.5 mg is recommended. A usual daily dose is between 20 and 40 mg with a maximum of 80 mg (lower in heart failure and renal impairment).

Bonazzi D *et al.* (1997). Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). *J Pharm Biomed Anal* 16: 431–438.

Graf P *et al.* (1988). Determination of the angiotensin converting enzyme inhibitor benazeprilat in plasma and urine by an enzymic method. *J Chromatogr* 425: 353–361.

Kaiser G *et al.* (1987). Determination of a new angiotensin converting enzyme inhibitor and its active metabolite in plasma and urine by gas chromatography–mass spectrometry. *J Chromatogr* 419: 123–133.

Macdonald NJ *et al.* (1993). A comparison in young and elderly subjects of the pharmacokinetics and pharmacodynamics of single and multiple doses of benazepril. *Br J Clin Pharmacol* 36: 201–204.

Panderi IE, Parissi-Poulou M (1999). Simultaneous determination of benazepril hydrochloride and hydrochlorothiazide by micro-bore liquid chromatography. *J Pharm Biomed Anal* 21: 1017–1024.

Pommier F *et al.* (2003). Quantitative determination of benazepril and benazeprilat in human plasma by gas chromatography–mass spectrometry using automated 96-well disk plate solid-phase extraction for sample preparation. *J Chromatogr B Analyt Technol Biomed Life Sci* 783: 199–205.

Shionoiri H *et al.* (1992). Pharmacokinetics and pharmacodynamics of benazepril in hypertensive patients with normal and impaired renal function. *J Cardiovasc Pharmacol* 20: 348–357.

Sioufi A *et al.* (1988). Determination of benazepril, a new angiotensin-converting enzyme inhibitor, and its active metabolite, benazeprilat, in plasma and urine by capillary gas chromatography–mass-selective detection. *J Chromatogr* 434: 239–246.

Vonaparti A *et al.* (2006). Development and validation of a liquid chromatographic/electrospray ionization mass spectrometric method for the determination of benazepril, benazeprilat and hydrochlorothiazide in human plasma. *J Mass Spectrom* 41: 593–605.

Wang XD *et al.* (2007). Simultaneous and rapid quantitation of benazepril and benazeprilat in human plasma by high performance liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* 44: 224–230.

Xiao W *et al.* (2005). Simultaneous determination of benazepril hydrochloride and benazeprilat in plasma by high-performance liquid chromatography/electrospray-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 814: 303–308.

Bendroflumethiazide

Diuretic

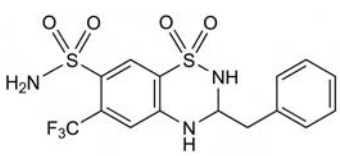
$C_{15}H_{14}F_3N_3O_4S_2 = 421.4$

CAS—73-48-3

IUPAC Name 3,4-Dihydro-3-(phenylmethyl)-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Synonyms Bendrofluazide; benzydroflumethiazide.

Proprietary Names *Aprinox*; *Berkozide*; *Centyl*; *Naturetin*; *Neo-NaClex*; *Pluryl*; *Polidiuril*; *Salural*; *Sinesalin*; *Uriside*. It is an ingredient of *Abicol*, *Corgaretic*, *Inderetic*, *Inderex*, *Prestim*, *Rauzide* and *Tenavoid*.



Chemical Properties A white or cream-coloured crystalline powder. Mp about 220°, with decomposition. Practically insoluble in water and chloroform; soluble 1 in 17 of ethanol, 1 in 1.5 of acetone and 1 in 500 of ether. pK_a 8.5 (25°). Log *P* (octanol/water), 1.9.

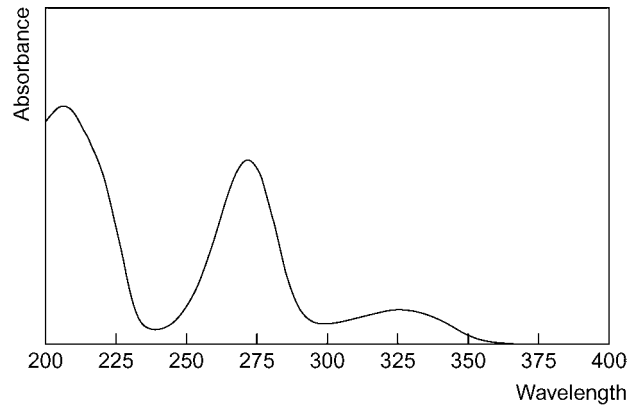
Colour Tests Koppanyi-Zwikker test—violet; Liebermann's reagent—blue; sulfuric acid—violet.

Thin-layer Chromatography System TD—R_f 0.25; system TE—R_f 0.52; system TF—R_f 0.71; system TAD—R_f 0.30; system TAJ—R_f 0.38; system TAK—R_f 0.11; system TAL—R_f 0.72 (location under UV light, violet fluorescence).

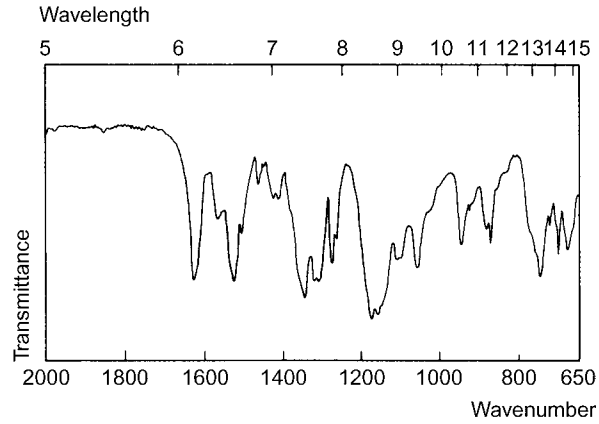
Gas Chromatography System GX—retention time 10.7 min, bendrofluazide-ME4; system GY—retention time 5.6 min, bendrofluazide-ME4.

High Performance Liquid Chromatography System HN—*k* 15.35; system HX—RI 508; system HAA—retention time 18.6 min.

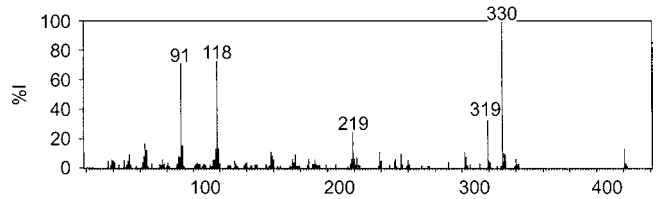
Ultraviolet Spectrum Aqueous acid—207, 273 (A^1_1=585a), 325 nm; aqueous alkali—273 (A^1_1=410a), 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1170, 1155, 1306, 1518, 1621, 750 cm^{-1} (KBr disk). There are 2 polymorphic forms.



Mass Spectrum Principal ions at *m/z* 330, 118, 91, 319, 219, 64, 92, 421.



Quantification

Plasma **GC** ECD. Limit of detection, 5 µg/L [Beermann *et al.* 1976].

Disposition in the Body Readily and almost completely absorbed after oral administration. Up to about 30% of a dose is excreted unchanged in the urine in 48 h; the remainder is excreted as unidentified metabolites.

Therapeutic Concentration

A single oral dose of 10 mg given to 9 subjects, produced peak plasma concentrations of 0.07 to 0.10 mg/L in 1.6 to 2.4 h [Beermann *et al.* 1977].

Peak plasma concentrations of 0.04 to 0.06 (mean 0.05) mg/L were reported 2 h after a dose, following administration of 5 mg daily to 8 hypertensive patients [Beermann *et al.* 1978].

Half-life Plasma half-life, about 9 h.

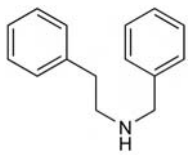
Volume of Distribution About 1.5 L/kg.

Protein Binding About 94%.
Note For a review of the clinical pharmacokinetics of diuretics, see Beermann, Groschinsky-Grind [1980].
Dose 2.5 to 10 mg daily.

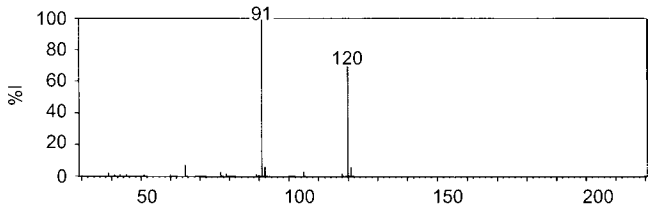
Beermann B *et al.* (1976). A GLC assay for bendroflumethiazide. Preliminary data about its plasma level in man. *Eur J Clin Pharmacol* 10: 293–295.
Beermann B *et al.* (1977). Pharmacokinetics of bendroflumethiazide. *Clin Pharmacol Ther* 22: 385–388.
Beermann B *et al.* (1978). Pharmacokinetics of bendroflumenthiazide in hypertensive patients. *Eur J Clin Pharmacol* 13: 119–124.
Beermann B, Groschinsky-Grind M (1980). Clinical pharmacokinetics of diuretics. *Clin Pharmacokinet* 5: 221–245.

Benethamine

Pharmaceutical Adjuvant
$C_{15}H_{17}N = 211.3$
IUPAC Name *N*-Benzylphenethylamine

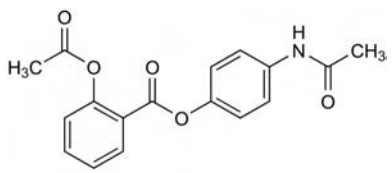


Chemical Properties Soluble in chloroform.
Colour Tests Liebermann's reagent—red-orange; Marquis test—orange-brown→brown.
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.48; system TC—R_f 0.59; system TL—R_f 0.43 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1798.
Ultraviolet Spectrum Water—258 nm (A_1^1=12b).
Infrared Spectrum Principal peaks at wavenumbers 696, 745, 1111, 1022, 1592, 1067 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 91, 120, 65, 121, 92, 105, 77, 118.

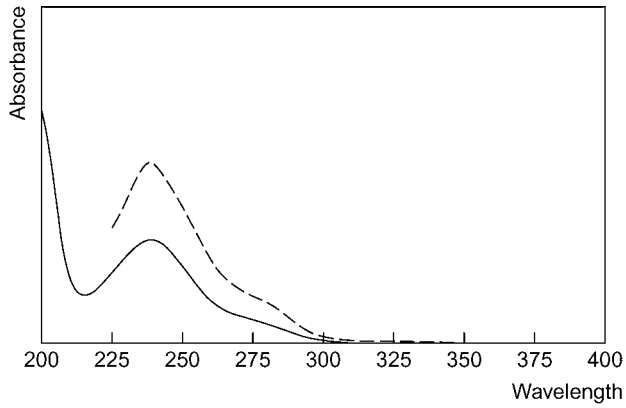


Benorilate

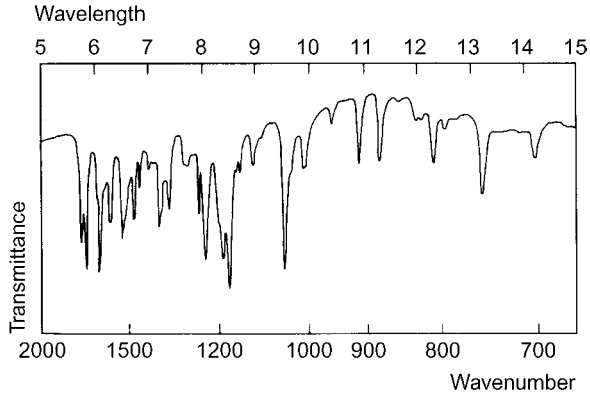
Analgesic
$C_{17}H_{15}NO_5 = 313.3$
CAS—5003-48-5
IUPAC Name 2-(Acetyloxy)benzoic acid 4-(acetylamino)phenyl ester
Synonyms Benorylate; fenasprate.
Proprietary Names *Benoral*; *Benortan*; *Salipran*.



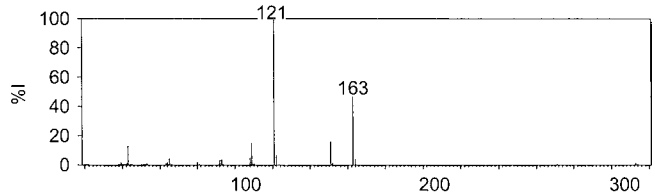
Chemical Properties A white crystalline powder. Mp 178° to 181°. Practically insoluble in water; soluble 1 in 88 of ethanol, 1 in 22 of acetone, 1 in 18 of chloroform and 1 in 900 of ether. Log *P* (octanol/water), 2.2.
Colour Tests Liebermann's reagent—black; Mandelin's test—green; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.00; system TC—R_f 0.51; system TL—R_f 0.62; system TAE—R_f 0.86 (marquis reagent, positive).
Gas Chromatography System GA—benorilate RI 1840, aspirin RI 1309, paracetamol RI 1687.
High Performance Liquid Chromatography System HD—benorilate *k* 0.7, aspirin *k* 0.5, paracetamol *k* 0.1; system HW—benorilate *k* 22.4, aspirin *k* 2.7, paracetamol *k* 0.32.
Ultraviolet Spectrum Dehydrated alcohol—240 nm (A_1^1=740a).



Infrared Spectrum Principal peaks at wavenumbers 1182, 1050, 1661, 1730, 1248, 1194 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 121, 163, 151, 109, 43, 122, 108, 65; aspirin 120, 43, 138, 92, 121, 39, 64, 63; paracetamol 109, 151, 43, 80, 108, 81, 53, 52.

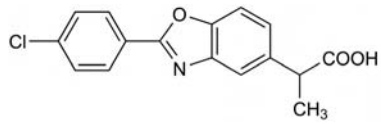


Quantification See also under Aspirin and Paracetamol.
Plasma **GC** FID. Aspirin and salicylic acid. Limit of detection, 2 mg/L for aspirin [Cailleux *et al.* 1979].
HPLC UV detection. Limit of detection, 200 µg/L for benorilate, 400 µg/L for paracetamol [Cailleux *et al.* 1979].
Disposition in the Body Benorilate is readily absorbed after oral administration. It is hydrolysed in the blood to aspirin and paracetamol, and about 85% of a dose is excreted in the urine in 72 h by the normal metabolic routes for these substances. About 15% of a dose is eliminated in the faeces.
Therapeutic Concentration
Following oral administration of 4 g as a suspension to 20 subjects, mean peak plasma concentrations of 2.2 mg/L of benorilate and about 120 mg/L of salicylate were attained in 0.5 and 3 h, respectively [Aylward *et al.* 1975].
Half-life Plasma half-life, about 1 h.
Dose 4.5 to 8 g daily.

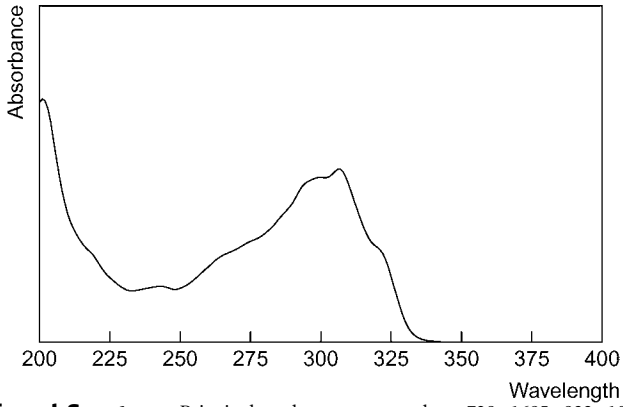
Aylward M *et al.* (1975). Simultaneous pharmacokinetics of benorylate in plasma and synovial fluid of patients with rheumatoid arthritis. *Scand J Rheumatol* Suppl. 13: 9–12.
Cailleux A *et al.* (1979). [Analysis of benorylate and its metabolites in blood by high performance liquid chromatography and gas chromatography]. *Therapie* 34: 73–79.

Benoxaprofen

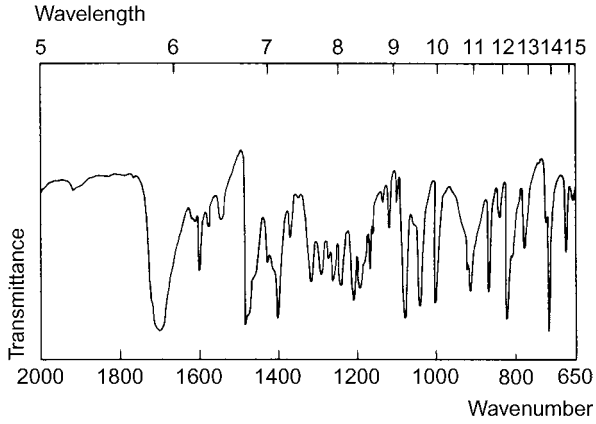
Analgesic
$C_{16}H_{12}ClNO_3 = 301.7$
CAS—67434-14-4
IUPAC Name 2-(4-Chlorophenyl)-α-methyl-5-benzoxazoleacetic acid
Proprietary Names *Opren*; *Oraflex*.



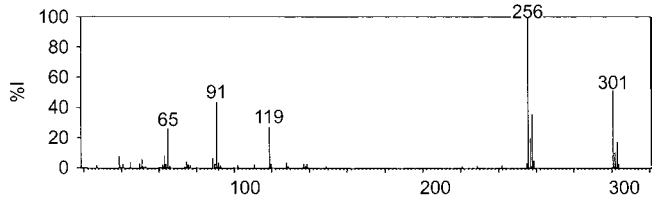
Chemical Properties A cream-coloured solid. Mp 189° to 190°. pK_a 3.5. Log *P* (octanol/water), 3.2.
Thin-layer Chromatography System TG—R_f 0.14; system TAJ—R_f 0.56; system TAK—R_f 0.80; system TAL—R_f 0.99 (Ludy Tenger reagent, orange).
Gas Chromatography System GA—RI 2550, RI 2485benoxaprofen-ME; system GB—not eluted; system GD—RRT 1.98 methyl derivative to *n*-$C_{16}H_{34}$.
High Performance Liquid Chromatography System HD—*k* 11.3; system HV—RRT 0.98 to meclofenamic acid.
Ultraviolet Spectrum Aqueous alkali—244 (A_1^1=295b), 309 nm (A_1^1=900b).



Infrared Spectrum Principal peaks at wavenumbers 728, 1695, 833, 1086, 1050, 1010 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 256, 301, 91, 258, 119, 65, 257, 303.



Quantification
Plasma **GC** ECD. Limit of detection, 10 µg/L [Chatfield, Woodage 1978].
HPLC UV detection. Limit of detection, 25 µg/L [Ekman *et al.* 1980].
Urine **GC** See Plasma [Chatfield, Woodage 1978].
HPLC See Plasma [Ekman *et al.* 1980].
Disposition in the Body Benoxaprofen is readily absorbed after oral administration. About 15% of a dose is excreted in the urine in 24 h, mainly as the glucuronic acid conjugate, together with small amounts of unchanged drug. In 5 days about 60% of a dose is excreted in the urine and 40% is eliminated in the faeces.
Therapeutic Concentration
After a single oral dose of 400 mg, administered to 4 subjects, a mean peak plasma concentration of 45 mg/L was attained in 2 to 4 h [Smith *et al.* 1977].
Toxicity Because of reports of adverse reactions and fatalities, benoxaprofen was withdrawn worldwide in 1982.
Half-life Plasma half-life, about 30 to 35 h; increased in renal impairment.
Protein Binding >99%.

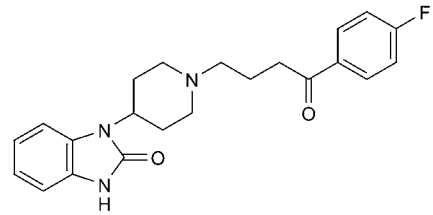
Dose Benoxaprofen has been given in doses of 600 mg daily.

Chatfield DH, Woodage TJ (1978). Determination of benoxaprofen [2-(4-chlorophenyl)-alpha-methyl-5-benzoxazoleacetic acid, LRCL 3794] in biological fluids. *J Chromatogr* 153: 101–106.
Ekman L *et al.* (1980). Determination of benoxaprofen in plasma and urine by liquid chromatography. *J Chromatogr* 182: 478–481.
Smith GL *et al.* (1977). Preliminary studies of absorption and excretion of benoxaprofen in man. *Br J Clin Pharmacol* 4: 585–590.

Benperidol

Butyrophenone, Antipsychotic
$C_{22}H_{24}FN_3O_2$ = 381.4
CAS—2062-84-2

IUPAC Name 3-[1-[4-(4-Fluorophenyl)-4-oxobutyl]piperidin-4-yl]-1*H*-benzimidazol-2-one
Synonyms Benzperidol; 1-[1-[3-(*p*-fluorobenzoyl)propyl]-4-piperidyl]-2-benzimidazolinone; 1-[1-[4-(*p*-fluorophenyl)-4-oxybutyl]piperidin-4-yl]-2-benzimidazolinone; 1-[1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one; McN-JR-4854; R-4584.
Proprietary Names *Anquil*; *Frénactil*; *Glianimon*.

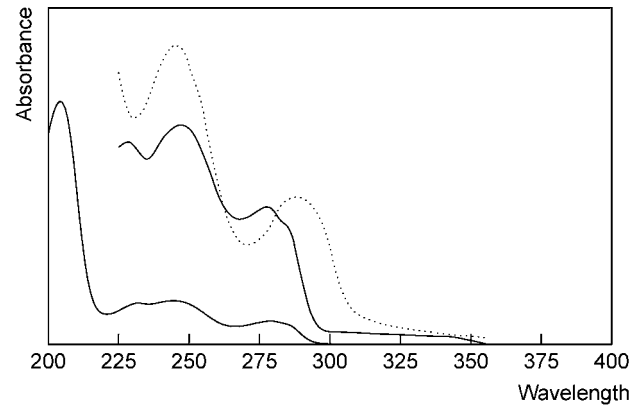


Chemical Properties An off-white amorphous powder or crystals. It darkens slowly on exposure to light. Mp 170° to 171.8°. Practically insoluble in water; soluble 1 in 1000 of ethanol, 1 in 4 of chloroform, and 1 in 625 of ether; sparingly soluble in dilute mineral acids. pK_a 7.9 [Seiler *et al.* 1994]. Log *P* (octanol/water) 3.9 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

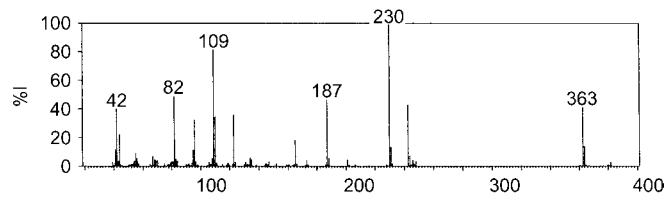
Benperidol Hydrochloride

$C_{22}H_{24}FN_3O_2$,HCl = 417.9
Chemical Properties Solid. Mp 134° to 142°.

Colour Tests Koppanyi–Zwikker test—violet; Mandelin's test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TAE—R_f 0.62; system TAF—R_f 0.69; system TAG—R_f 0.32; system TF—R_f 0.03.
Gas Chromatography System GA—RI 3433, RI 2415 M (*N*-desalkyl-); system GB—RI 3667.
High Performance Liquid Chromatography System HA—*k* 1.1; system HX—RI 393; system HY—RI 324; system HZ—RT 3.6 min.
Ultraviolet Spectrum Aqueous acid—231, 249 (A_1^1 = 348b), 279 nm; aqueous alkali—248 (A_1^1 = 445b), 288 nm.



Infrared Spectrum Principal peaks at wavenumbers 1694, 757, 1587, 1162, 1204, 1219 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 230, 109, 82, 187, 243, 363, 42, 123.



Quantification

Plasma **GC** Column: 2% OV3 or 3% Dexil 300 on HP Chrom W AW DMCS 80/100 mesh (1 m × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature programme: 230° to 285° at 2°/min to 290° at 3°/min. FID. Limit of detection, 30 µg/L [Quaglio *et al.* 1982].

HPLC Column: CPS (3 µm). Mobile phase: 0.15 mol/L acetate buffer (pH 4.7) : acetonitrile (75 : 25). Electrochemical detection. Limit of quantification, 2.4 µg/L; limit of detection, 0.8 µg/L [Seiler *et al.* 1994]. Column: Hypersil-CPS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.15 mol/L acetate buffer (pH 4.7) : acetonitrile (75 : 25), flow rate 1.5 mL/min. Electrochemical detection. Limit of detection 0.5 µg/L [Suss *et al.* 1991]. Column: C_{18} Nucleosil (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L potassium dihydrogen phosphate (pH 2.8; 32 : 68), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 5.9 min. Limit of detection, 0.5–1 µg/L [Kruger *et al.* 1984].

Serum **HPLC** Column: C_8 (250 cm × 2.6 mm i.d., 10 µm). Mobile phase: 0.12 mol/L acetate buffer (pH 4.2) : acetonitrile (60 : 40), flow rate 1 mL/min. Electrochemical detection. Limit of detection, 0.2 µg/L [Furlanut *et al.* 1987].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.17 µg/L [Kirchherr, Kühn-Velten 2006].

Disposition in the Body

Therapeutic Concentration

Thirteen schizophrenic patients were orally administered 6 mg benperidol as liquid and 6 mg as tablets. The mean peak plasma concentration was 10.2 µg/L at 1.0 h for the liquid and 7.3 µg/L at 2.7 h for the tablet formulation [Seiler *et al.* 1994].

Dose 0.25 to 1.5 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Furlanut M *et al.* (1987). Electrochemical detection of benperidol in serum for drug monitoring in humans. *Ther Drug Monit* 9: 343–346.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Kruger R (1984). Determination of benperidol in human plasma by high-performance liquid chromatography. *J Chromatogr* 311: 109–116.

Quaglio MP *et al.* (1982). Determination of benperidol, droperidol and pimozide in human plasma by GLC. *Boll Chim Farm* 121: 276–284.

Seiler W *et al.* (1994). Pharmacokinetics and bioavailability of benperidol in schizophrenic patients after intravenous and two different kinds of oral application. *Psychopharmacology (Berl)* 116: 457–463.

Suss S *et al.* (1991). Determination of benperidol and its reduced metabolite in human plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr* 565: 363–373.

Benserazide

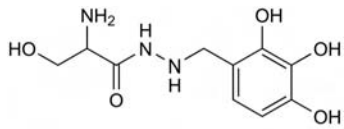
Dopa-Decarboxylase Inhibitor, Antiparkinsonian

$C_{10}H_{15}N_3O_5$ = 257.2

CAS—322-35-0

IUPAC Name DL-Serine 2-[(2,3,4-trihydroxyphenyl)methyl] hydrazide

Synonym Serazide



Chemical Properties Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Benserazide Hydrochloride

$C_{10}H_{15}N_3O_5$,HCl = 293.7

CAS—14919-77-8; 14046-64-1

Proprietary Names It is an ingredient of *Madopar* and *Prolopa*.

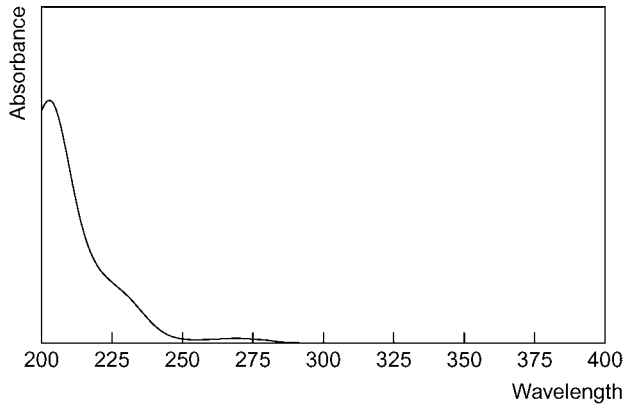
Chemical Properties An off-white crystalline powder. Mp 146° to 148°. Soluble 1 in 3 of water, 1 in 118 of ethanol, 1 in 66 of acetone, 1 in 180 of chloroform and 1 in 455 of ether.

Colour Tests Ammoniacal silver nitrate—black; *p*-dimethyl-aminobenzaldehyde—red/-; ferric chloride—green-brown; Folin-Ciocalteu reagent—blue; methanolic potassium hydroxide—red; Millon's reagent—red-orange; Nessler's reagent—black; palladium chloride—orange→brown; potassium dichromate—brown.

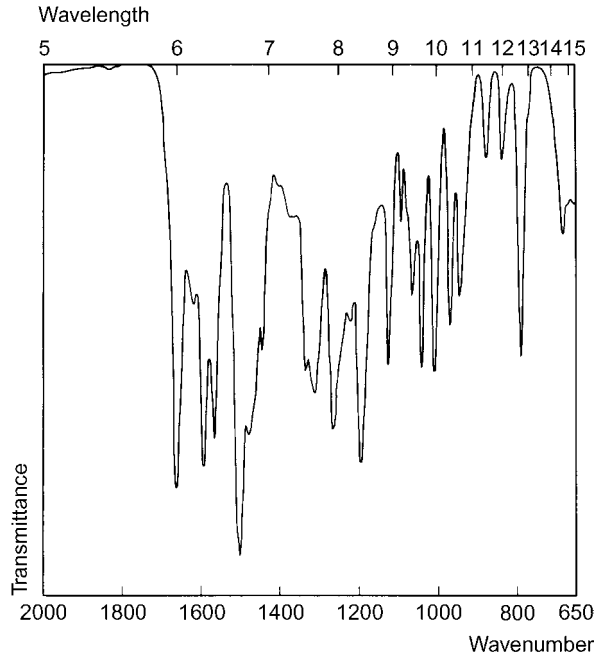
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.03; system TAE—R_f 0.07.

High Performance Liquid Chromatography System HX—RI 35.

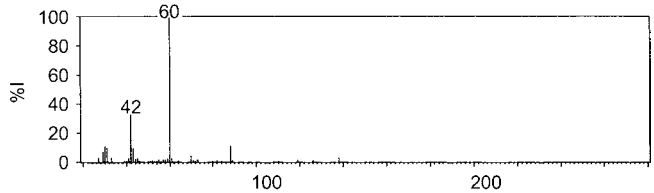
Ultraviolet Spectrum Aqueous acid—203, 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1500, 1665, 1593, 1195, 1570, 1265 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 60, 42, 88, 30, 31, 43, 29, 70.



Disposition in the Body Rapidly absorbed after oral administration and appears to be rapidly metabolised. About 50 to 60% of an oral dose and 80% of an IV dose is excreted in the urine in 24 h, and about 30% and 10% of the respective doses are eliminated in the faeces in 7 days.

Therapeutic Concentration

After an oral dose of 50 mg of ^{14}C-benserazide hydrochloride, peak plasma concentrations of about 1 mg/L were attained in 1 h [Schwartz *et al.* 1974].

Dose Usually the equivalent of 50 mg of benserazide daily, increasing to 100 to 250 mg daily (given in combination with levodopa in the treatment of parkinsonism).

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Schwartz DE *et al.* (1974). Pharmacokinetics of the decarboxylase inhibitor benserazide in man; its tissue distribution in the rat. *Eur J Clin Pharmacol* 7: 39–45.

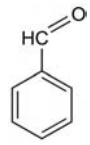
Benzaldehyde

Flavouring Agent

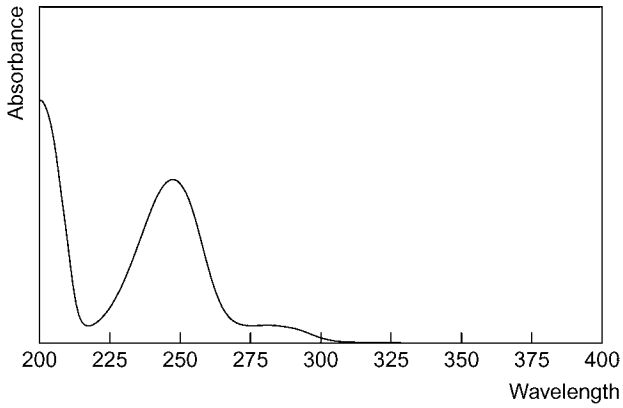
C_6H_5CHO = 106.1

CAS—100-52-7

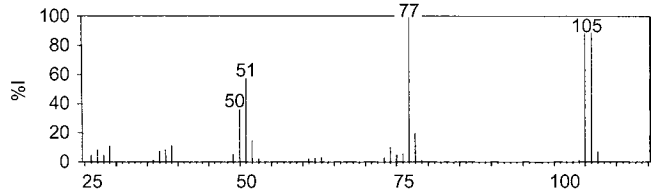
Synonym Artificial essential oil of almond



Chemical Properties A clear, colourless, strongly refractive liquid, with a characteristic odour of bitter almonds. It becomes yellowish on storage and oxidises in air to benzoic acid. Bp about 179°. Refractive index, at 20°, 1.544 to 1.5465. Soluble 1 in 350 of water; miscible with ethanol and ether. Log *P* (octanol/water), 1.5.
Gas Chromatography System GA—RI 947; system GI—retention time 34.2 min.
Ultraviolet Spectrum Ethanol—245 (A^1_1=1294b), 279 (A^1_1=126b), 289 nm (A^1_1=105b).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1210, 1601, 1175, 1318, 753 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 77, 106, 105, 51, 50, 78, 52, 39.



Quantification
Blood **GC** For detection and identification of volatile compounds using head-space analysis and FID, see Ramsey and Flanagan [1982].
Serum **GC–MS** For head-space analysis, see Zlatkis *et al.* [1974].

Ramsey JD, Flanagan RJ (1982). Detection and identification of volatile organic compounds in blood by headspace gas chromatography as an aid to the diagnosis of solvent abuse. *J Chromatogr* 240: 423–444.
Zlatkis A *et al.* (1974). Analysis of trace volatile metabolites in serum and plasma. *J Chromatogr* 91: 379–383.

Benzalkonium Bromide

Quaternary Ammonium, Antiseptic
CAS—8001-54-5

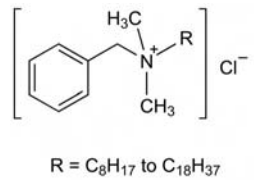
Proprietary Name *Morpan BB*
Chemical Properties A mixture of alkylbenzyldimethylammonium bromides of the general formula $[C_6H_5CH_2N(CH_3)_2R]Br$ in which R represents a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$. An aqueous solution containing the equivalent of 50% of $[C_6H_5CH_2N(CH_3)_2C_{13}H_{27}]Br$ is a colourless or pale-yellow syrupy liquid, which is miscible with water, ethanol, and acetone.
Note See Benzalkonium Chloride.

Benzalkonium Chloride

Cationic Disinfectant, Antiseptic
CAS—8001-54-5

Note A mixture of alkylbenzyldimethylammonium chlorides of the general formula $[C_6H_5{\cdot}CH_2{\cdot}N(CH_3)_2{\cdot}R]Cl$, in which R represents a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$.

Proprietary Names *Benzalchlor-50*; *Cetal Conc. A and B*; *Empigen BAC*; *Hyamine 3500*; *Laudamonium*; *Morpan BC*; *Ovules Pharmatex*; *Quartamon*; *Roccal*; *Sabol*; *Silquat B10*; *Silquat B50*; *Vantoc CL*; *Zephiran*. It is an ingredient of *Polycide*, *Stomosol* and *Timodine*.



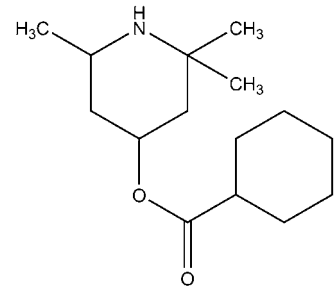
R = C_8H_{17} to $C_{18}H_{37}$

Chemical Properties A white or yellowish-white amorphous powder, thick gel or gelatinous pieces. A solution in water gives a clear, colourless or pale yellow, syrupy liquid, which foams strongly when shaken. Very soluble in water, ethanol and acetone; practically insoluble in ether.
Thin-layer Chromatography System TL—R_f 0.00; system TAE—R_f 0.04; system TAF—R_f 0.66.
Ultraviolet Spectrum Water—256 (A^1_1=4.9b), 262 (A^1_1=5.8b), 268 nm (A^1_1=4.6b).
Infrared Spectrum Principal peaks at wavenumbers 702, 726, 780, 875, 1618, 1211 cm^{-1} (thin film).

Benzamine

Piperidine, Anaesthetic (Local)
$C_{15}H_{21}NO_2$ = 247.3
CAS—500-34-5

IUPAC Name 4-Benzoyloxy-2,2,6-trimethylpiperidine
Synonyms Betacaine; racemic benzamine.



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 2.95 [Meylan, Howard 1995].

Benzamine Hydrochloride

Synonyms Beta-eucaine hydrochloride; eucaine hydrochloride.
Chemical Properties White crystalline powder. Mp 274°, with decomposition. Soluble 1 in 30 of water, 1 in 35 of ethanol and 1 in 30 of chloroform.

Benzamine Lactate

Chemical Properties White crystalline powder. Mp 152°. Soluble 1 in 5 of water and 1 in 8 of ethanol.

Thin-layer Chromatography System T1—R_f 0.57 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—233, 274 nm.
Infrared Spectrum Principal peaks at wavenumbers 1714, 1276, 711 or 1108 cm^{-1} (KBr disk).
Toxicity The minimum IV lethal dose in rats is 15 to 25 mg/kg.
Dose Up to 30 mg.

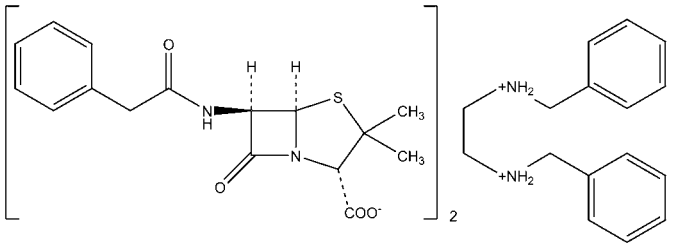
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Benzathine Benzylpenicillin

Natural Penicillin, Antibiotic
$C_{16}H_{20}N_2(C_{16}H_{18}N_2O_4S)_2$ = 909.1
CAS—1538-09-6 (anhydrous benzathine benzylpenicillin); 5928-83-6 (benzathine benzylpenicillin monohydrate); 41372-02-5 (benzathine benzylpenicillin tetrahydrate)

IUPAC Name *N*, *N*′-Dibenzylethylenediammonium bis[(6*R*)-6-(2-phenylacetamido)penicillanate]

Synonyms Benzathine benzylpenicillin; benzathine penicillin G; benzethacil; dibenzylamine penicillin G; penzaethinum G.
Proprietary Names *Benzatron*; *Benzetacil*; *Bepeben*; *Bicillin*; *Dibencil*; *Extencilline*; *Galtamicina*; *Longacilin*; *Neolin*; *Penadur*; *Pen di Ben*; *Penditan*; *Pendysin*; *Penadur*; *Penidural*; *Permapen*; *Retarpen*. It is also an ingredient in *Bicillin C-R*; *Pecivax*; *Tardocillin*.



Chemical Properties White powder. Mp 129° to 133° with decomposition. Soluble 1 in 6000 of water and 1 in 1000 of ethanol; freely soluble in dimethylformamide and in formamide; almost insoluble in ether and chloroform. The tetrahydrate is a white, odourless, crystalline powder. Soluble 1 in 5000 of water and 1 in 65 of alcohol. Dissolve 50 mg of dehydrate alcohol in 50 mL of water, the pH will be between 4.0 and 6.5.
High Performance Liquid Chromatography Column: Hypersil C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (4.32 g/L potassium hydrogen phosphate; 6.30 g/L sodium heptane-1-sulfonate) : acetonitrile (pH 3.17; 71.5 : 28.5), flow rate 1.0 mL/min. UV detection (λ = 258 nm). Retention times: penicillin G ≈4 min, benzathine ≈8 min. Limit of quantification not reported [Irwin *et al.* 1984].
Ultraviolet Spectrum Methanol—251, 257, 263, 266, 320 nm.
Disposition in the Body Relatively stable in the presence of gastric juice or serum, and penicillin is slowly released after PO or IM administration. When benzathine benzylpenicillin is given by mouth, the maximum concentration of penicillin in the blood is obtained less rapidly than after a comparable dose of a soluble salt of penicillin by injection, but the level is maintained for a longer period; doses of 225 mg usually give effective blood levels for 6 h. When given by IM injection, benzathine benzylpenicillin forms a depot from which penicillin is released over several days. After a single dose of 450 mg, an effective concentration in the blood may be maintained for ≥1 week. Distribution into the CSF is reported to be poor. Due to the slow absorption from the injection site, benzylpenicillin has been detected in the urine for up to 12 weeks after a single dose.

Therapeutic Concentration

In a group of 193 patients receiving a 4-weekly dose of IM benzathine benzylpenicillin (1 200 000 units) for the prevention of secondary rheumatic fever, mean serum concentrations at days 1, 3, 10, 21 and 28 were reported as being ≈0.17, 0.11, 0.04, 0.03 and 0.02 mg/L, respectively [Kaplan *et al.* 1989].

Twenty-five healthy pregnant volunteers at 38–39 weeks' gestation scheduled for elective caesarean delivery were administered benzathine benzylpenicillin (2400000 units) IM preoperatively. Ten women delivered 1 day after injection, 5 delivered 2–3 days after and 10 delivered 7 days after. Penicillin concentrations (mg/L) in compartments of the maternal-placental-fetal unit were reported as follows:

Sample	*Day 1*	*Days 2–3*	*Day 7*
Maternal serum	0.14	0.14	0.08
Maternal CSF	0.005	0.005	0.005
Cord serum	0.12	0.04	0.04
Amniotic fluid	0.16	0.04	0.03
Maternal/cord serum	4.03	19.2	5.8

[Nathan *et al.* 1993]

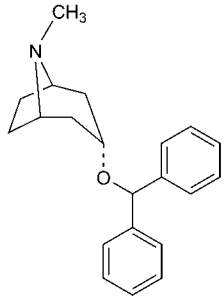
Toxicity Allergic reactions may occur. It should not be injected IV because ischaemic reactions may occur.
Dose Benzathine benzylpenicillin has the same antimicrobial action as benzylpenicillin, to which it is hydrolysed gradually after deep IM injection. This results in a prolonged effect but, because of the relatively low blood concentrations of benzylpenicillin produced, its use should be restricted to micro-organisms that are highly susceptible to benzylpenicillin. In acute infections (and if bacteraemia is present), the initial treatment should be with benzylpenicillin by injection. Usually up to 900 mg daily by mouth or up to 1.8 g IM as a single dose.

Irwin WJ *et al.* (1984). Controlled-release penicillin complexes. High-performance liquid chromatography and assay. *J Chromatogr* 287: 85–96.
Kaplan EL *et al.* (1989). Pharmacokinetics of benzathine penicillin G: serum levels during the 28 days after intramuscular injection of 1,200,000 units. *J Pediatr* 115: 146–150.
Nathan L *et al.* (1993). Penicillin levels following the administration of benzathine penicillin G in pregnancy. *Obstet Gynecol* 82: 338–342.

B

Benzatropine

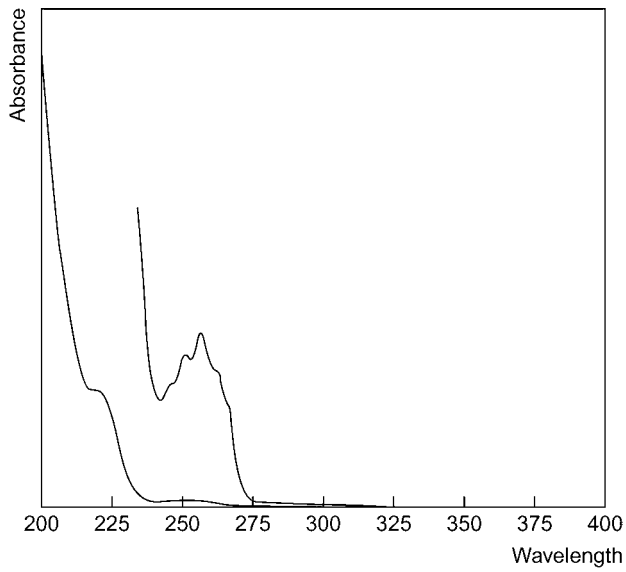
Dopamine Uptake Inhibitor, Anticholinergic
$C_{21}H_{25}NO$ = 307.4
CAS—86-13-5
IUPAC Name (1*R*,5*R*)-3-[Di(phenyl)methoxy]-8-methyl-8-azabicyclo[3.2.1] octane
Synonyms Benztropine; 3-(diphenylmethoxy)-8-methyl-8-azabicyclo[3.2.1] octane.



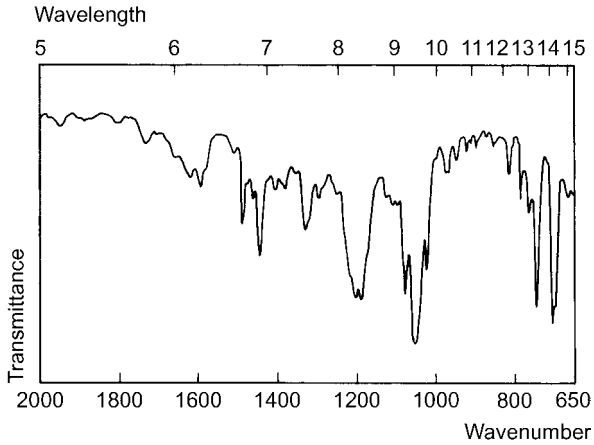
Chemical Properties Soluble in chloroform. Log *P* (octanol/water) 4.28 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Benzatropine Mesilate

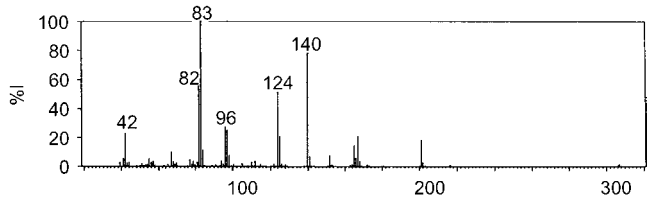
$C_{21}H_{25}NO,CH_4O_3S$ = 403.5
CAS—132-17-2
IUPAC Name (1*R*,5*R*)-3-Benzhydryloxy-8-methyl-8-azabicyclo[3.2.1]octane methanesulfonic acid
Synonyms Benzatropine methanesulfonate; tropine diphenylmethyl ether.
Proprietary Names *Bensylate*; *Cogentin*.
Chemical Properties A white, slightly hygroscopic, crystalline powder. Mp 141° to 145°. Soluble 1 in 0.7 of water, 1 in 1.5 of ethanol, and 1 in 2 of chloroform; practically insoluble in ether. pK_a 10.0 (20°). Log *P* (octanol/water) 3.91 [Meylan, Howard 1995], (heptane) 0.4.
Colour Tests Mandelin's test—yellow; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.13; system TAE—R_f 0.06; system TAG—R_f 0.02; system TB—R_f 0.26; system TC—R_f 0.06 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2302; system GB—RI 2423.
High Performance Liquid Chromatography System HA—*k* 3.7 (tailing peak).
Ultraviolet Spectrum Aqueous acid—253, 259 nm (A_1^1 = 14.5a).



Infrared Spectrum Principal peaks at wavenumbers 1054, 700, 742, 1191, 1204, 1075 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 83, 140, 82, 124, 96, 97, 42, 125.



Quantification

Blood **GC-MS** Column: DB-5MS 5% phenyl-methylpolysiloxane capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 125° to 290° at 20°/min for 5 min. EI ionisation. Limit of detection not reported [Rosano *et al.* 1994].

Plasma **GC-MS** Column: Silanised glass with 5% dimethyldichlorosilane in toluene packed with 1.5% OV-17 on 100/200 mesh gas chrom Q (2 mm i.d.). Carrier gas: He, 20.0 mL/min. Temperature: 220°. EI ionisation at 70 eV, full scan mode. Limit of detection, 2 µg/L [Jindal *et al.* 1981].

HPLC Column: C_8 Spherisorb (150 × 3.9 mm i.d., 5 µm). Mobile phase: 0.15% triethylamine (pH 3) : acetonitrile (40 : 60), flow rate 1.5 mL/min. UV detection (λ = 199 nm). Retention time: 7.0 min. Limit of quantification, 0.25 µg/L [Selinger *et al.* 1989].

Serum **GC-MS** See Blood [Rosano *et al.* 1994].

Urine **GC-MS** See Blood [Rosano *et al.* 1994]. See Plasma [Jindal *et al.* 1981].

Disposition in the Body Absorbed after oral administration.

Toxicity

In a fatality attributed to the ingestion of an unknown quantity of benzatropine tablets, the following postmortem concentrations were reported: blood 0.7 mg/L, liver 1.6 µg/g, and urine 0.8 mg/L. In a second case, a liver concentration of 2.3 µg/g was found [del Villar, Liddy 1976].

A 41-year-old male with a history of schizophrenia and drug abuse was found dead. Benzatropine at a concentration of 0.183 mg/L in his blood and 7.12 mg/L in his urine was found [Rosano *et al.* 1994].

Dose 0.5 to 6 mg of benzatropine mesilate daily.

del Villar G, Liddy M (1976). *TIAFT Bull* 12: 11–12.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Jindal SP *et al.* (1981). A stable isotope dilution assay for the antiparkinsonian drug benztropine in biological fluids. *Clin Chim Acta* 112: 267–273.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Rosano TG *et al.* (1994). Benztropine identification and quantitation in a suicidal overdose. *J Anal Toxicol* 18: 348–353.
Selinger K *et al.* (1989). High-performance liquid chromatographic method for the analysis of benztropine in human plasma. *J Chromatogr* 491: 248–252.

Benzbromarone

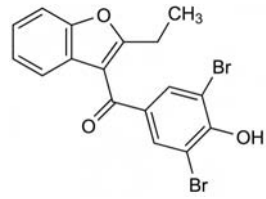
Uricosuric

$C_{17}H_{12}Br_2O_3$ = 424.1

CAS—3562-84-3

IUPAC Name (3,5-Dibromo-4-hydroxyphenyl)(2-ethyl-3-benzofuranyl) methanone

Proprietary Names *Desuric*; *Narcaricin*; *Normurat*; *Uricovac M*.

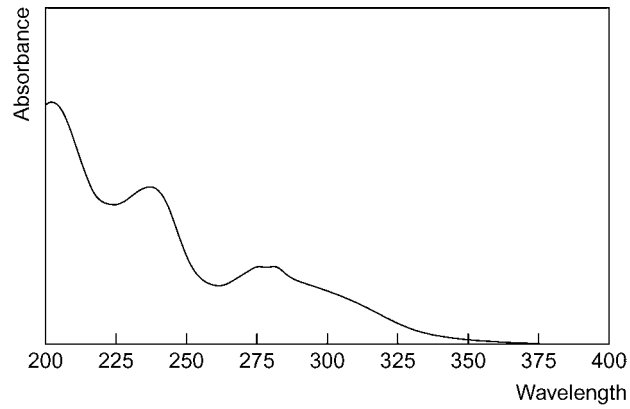


Chemical Properties Yellowish crystals. Mp 151°. Log *P* (octanol/water), 6.0.

Thin-layer Chromatography System TF—R_f 0.49; system TAE—R_f 0.94.

High Performance Liquid Chromatography System HX—RI 860; system HAA—retention time 26.1 min.

Ultraviolet Spectrum Methanolic acid—237 (A^1_1=666b), 274 (inflexion), 281 (A^1_1=314b); aqueous alkali—240 nm (A^1_1=440b), 281 (inflexion), 355 nm (A^1_1=513b).



Quantification

Plasma **HPLC** UV detection. Limit of detection, 0.01 mg/L for benzarone [de Vries *et al.* 1986].

Serum **HPLC** UV detection. Limit of detection, 140 µg/L for benzbromarone, 90 µg/L for benzarone [Vergin, Bishop 1980].

Urine **HPLC** See Plasma [de Vries *et al.* 1986].

Disposition in the Body Benzbromarone is incompletely absorbed after oral administration. It is metabolised by debromination to bromobenzarone and benzarone which are both active metabolites. About 50% of a dose is excreted in the bile as free and conjugated (glucuronide) metabolites and less than 10% of a dose is excreted in the urine.

Therapeutic Concentration

Following a single oral dose of 100 mg to 7 subjects, peak plasma concentrations of 1.4 to 2.9 (mean 2.2) mg/L of benzbromarone and 0.6 to 1.2 (mean 0.8) mg/L of benzarone were attained in about 3 and 6 h, respectively [Ferber *et al.* 1981].

Half-life Plasma half-life, benzbromarone about 3 h, benzarone about 13 h.

Note For a review of benzbromarone, see Heel *et al.* [1977].

Dose Usually 100 mg daily; up to 300 mg daily has been given.

de Vries JX *et al.* (1986). Determination of benzarone in human plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry. Identification of the conjugates. *J Chromatogr* 382: 167–174.
Ferber H *et al.* (1981). Pharmacokinetics and biotransformation of benzbromarone in man. *Eur J Clin Pharmacol* 19: 431–435.
Heel RC *et al.* (1977). Benzbromarone: a review of its pharmacological properties and therapeutic use in gout and hyperuricaemia. *Drugs* 14: 349–366.
Vergin H, Bishop G (1980). High-performance liquid chromatographic determination of benzbromarone and the main metabolite benzarone in serum. *J Chromatogr* 183: 383–386.

Benzene

Solvent

C_6H_6 = 78.11

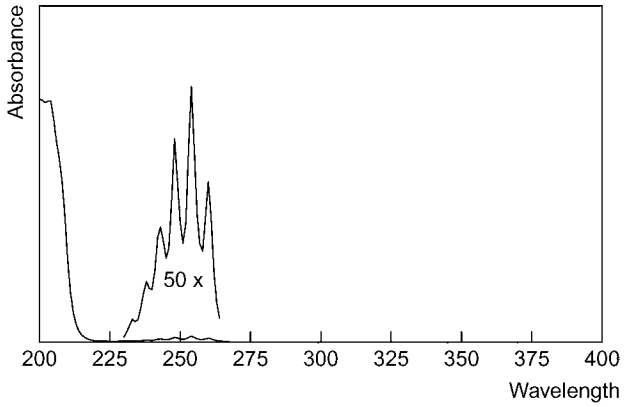
CAS—71-43-2

Synonyms Cyclohexatriene; phenyl hydride.

Chemical Properties A clear, colourless, mobile, inflammable liquid, with a characteristic aromatic odour, which burns with a smoky flame. Bp 80.1°. It solidifies when cooled to 0°. Practically immiscible with water; miscible with dehydrated alcohol, acetone, chloroform, ether and glacial acetic acid. Log *P* (octanol/water), 2.1.

Gas Chromatography System GA—RI 1000; system GI—retention time 14.8 min.

High Performance Liquid Chromatography System HAA—retention time 19.5 min.
Ultraviolet Spectrum Ethanol—255 nm (A_1^1=28b).



Mass Spectrum Principal ions at *m/z* 78, 77, 52, 51, 50, 39, 79, 74.
Quantification
Blood **GC** FID. For head-space analysis, see Collom, Winek [1970].
Urine **GC** FID. Limit of quantification, 23 µg/L, limit of detection, 7 µg/L [Ljungkvist *et al.* 2001]. FID. Limit of detection, 10 µg/L for phenol [Rick *et al.* 1982].

HPLC Fluorescence detection (λ_{ex}=395 nm; λ_{em}=470 nm). Limit of detection, 1 µg/L [Einig, Dehnen 1995]. SIM acquisition mode. Limit of detection, 0.01 mg/L for *trans, trans*-muconic acid (metabolite) [Ruppert *et al.* 1995]. UV detection (λ = 265 nm). Limit of detection, 125 ng [Lee *et al.* 1993].
Tissue **GC** See Snyder *et al.* [1977]. See Blood Collom, Winek [1970].
Disposition in the Body Benzene is absorbed from the gastrointestinal tract, through the skin and from the lungs. About 50% of inhaled benzene is retained in the body; eventually up to 50% of the retained benzene may be eliminated via the lungs, only very small amounts (~0.1%) appearing unchanged in the urine. The remainder of the retained benzene is excreted in the urine, mainly as phenol together with small amounts of catechol and quinol. The excretion of phenol is highest in the first 24 h and is complete within 48 h of exposure. The phenolic metabolites are excreted mainly in the conjugated form as ethereal sulfates and glucuronides.
Toxicity The maximum permissible atmospheric concentration is 10 ppm or 30 mg/m^3. The maximum exposure limit is 1 ppm. Chronic poisoning may result from exposure to low concentrations over a period of time; 1500 ppm for an hour causes marked depression of the CNS and 7500 ppm for half an hour or 20 000 ppm for a few minutes may cause death; chronic inhalation of as little as 100 ppm is likely to cause severe bone marrow depression. The ingestion of 10 mL has caused death.

In a death attributed to inhalation of benzene vapours (glue sniffing), the postmortem concentration of benzene in the blood was 0.94 mg/L and in the kidney 5.5 µg/g [Collom, Winek 1970].

The following postmortem tissue concentrations were reported in a fatality due to the ingestion of benzene: blood 38 mg/L, brain 253 µg/g, kidney 21 µg/g, liver 105 µg/g, urine 20 mg/L [Alha *et al.* 1975].

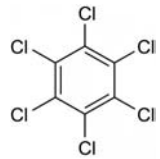
A 3-year-old child drank some benzene and died 2 h later; postmortem concentrations were: brain 250 µg/g, kidney 20 µg/g, liver 800 µg/g [Heyndrickx *et al.* 1966].

Half-life Plasma half-life, 9 to 24 h.
Note For a review of the effects on health of benzene inhalation, see Haley [1977].

Alha A *et al.* (1975). *Bull Int Assoc Forensic Toxicol* 11(3): 9.
Collom WD, Winek CL (1970). Detection of glue constitutents in fatalities due to "glue sniffing". *Clin Toxicol* 3: 125–130.
Einig T, Dehnen W (1995). Sensitive determination of the benzene metabolite S-phenylmercapturic acid in urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr A* 697(1–2): 371–375.
Haley TJ (1977). Evaluation of the health effects of benzene inhalation. *Clin Toxicol* 11: 531–548.
Heyndrickx A *et al.* (1966). Distribution of benzene in a fatal child poisoning. *J Pharm Belg* 21: 406–408.
Lee BL *et al.* (1993). Urinary trans,trans-muconic acid determined by liquid chromatography: application in biological monitoring of benzene exposure. *Clin Chem* 39(9): 1788–1792.
Ljungkvist G *et al.* (2001). Determination of low concentrations of benzene in urine using multi-dimensional gas chromatography. *Analyst* 126(1): 41–45.
Rick DL *et al.* (1982). Determination of phenol and pentachlorophenol in plasma and urine samples by gas liquid chromatography. *J Anal Toxicol* 6: 297–300.
Ruppert T *et al.* (1995). Determination of urinary trans,trans-muconic acid by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 666(1): 71–76.
Snyder CA *et al.* (1977). An extraction method for determination of benzene in tissue by gas chromatography. *Am Ind Hyg Assoc J* 38: 272–276.

Benzene Hexachloride

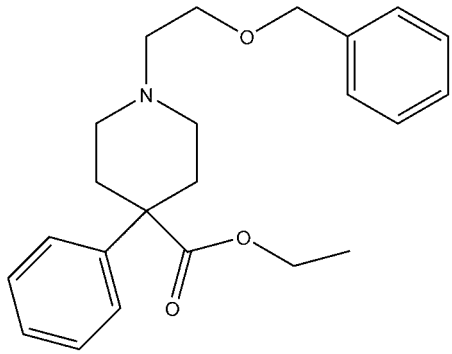
Insecticide
$C_6H_6Cl_6$ = 290.8
CAS—319-84-6 (α-isomer); 319-85-7 (β-isomer); 58-89-9 (γ-isomer); 319-86-8 (δ-isomer); 6108-10-7 (ε-isomer)
Synonyms BHC; HCH; hexachlorocyclohexane; technical benzene hexachloride.



Chemical Properties A mixture of the several isomers of 1,2,3,4,5,6-hexachlorocyclohexane; it contains not less than 12% of the gamma isomer. Benzene hexachloride has 5 known isomers designated alpha, beta, gamma, delta and epsilon; of these only the gamma isomer (see Lindane) is outstandingly active as an insecticide. White to light brown granules, flakes or powder, with a characteristic musty odour. Mp 159°. Practically insoluble in water; its solubility in organic solvents depends on the proportions of the various isomers present.
Gas Chromatography System GA—α-isomer RI 1690, β-isomer RI 1710, δ-isomer RI 1755, γ-isomer RI 1745.
Infrared Spectrum Principal peaks at wavenumbers 688, 855, 704, 781, 917, 957 cm^{-1} (Nujol mull).
Quantification See under Lindane.
Disposition in the Body Absorbed after ingestion, inhalation or through the skin. It is stored in the body fat and adrenal glands. The β-isomer accumulates on chronic exposure (see under Lindane).
Toxicity Benzene hexachloride has a greater chronic toxicity than lindane. Ingestion of 20 to 30 g may produce serious toxic effects but death is unlikely unless it is dissolved in an organic solvent.

Benzethidine

Narcotic Analgesic
$C_{23}H_{29}NO_3$ = 367.5
CAS—3691-78-9
IUPAC Name Ethyl-1-(2-benzyloxyethyl)-4-phenylpiperidine-4-carboxylate
Synonyms Benzyloxyethylnorpethidine; TA-28.

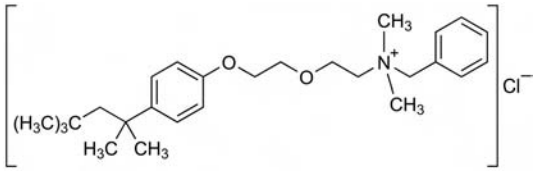


Chemical Properties Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 4.46 [Meylan, Howard 1995].
Colour Tests Ammonium molybdate test—orange-brown (limit of detection 0.5 µg); sulfuric acid-formaldehyde test—dull orange (limit of detection 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.74 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 2.53 relative to codeine; system G4—retention time 1.70 relative to codeine.
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—252, 258, 264 nm and an inflexion at 267 nm.

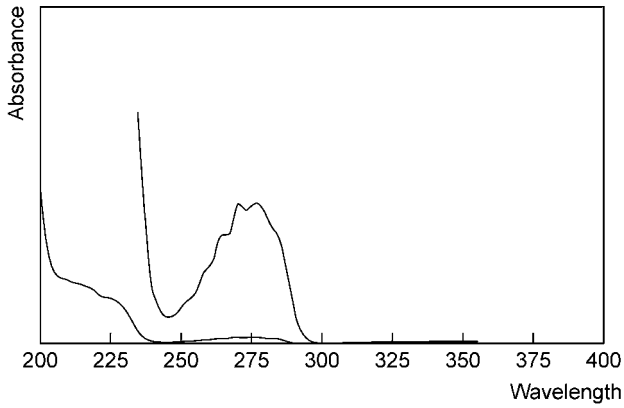
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Benzethonium Chloride

Cationic Disinfectant, Antiseptic
$C_{27}H_{42}ClNO_2$ = 448.1
CAS—121-54-0
IUPAC Name *N,N*-Dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy] ethoxy]-ethyl]-benzenemethanaminium chloride
Proprietary Names *Hyamine 1622*; *Phemerol Chloride*.



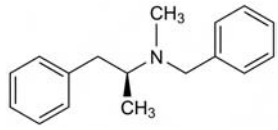
Chemical Properties White crystals. A solution in water foams strongly when shaken. Mp 160° to 165°. Soluble 1 in 0.6 of water, 1 in 0.6 of ethanol and 1 in 1 of chloroform; slightly soluble in ether; practically insoluble in light petroleum. Log *P* (octanol/water), 4.0.
Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.03 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—263, 269 (A^1_1=29c), 274 nm (A^1_1=28c). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1240, 1505, 1120, 826, 1063, 769 cm^{-1} (KBr disk).
Use Used as 0.1 to 0.2% solutions.

Benzfetamine

Anorectic
$C_{17}H_{21}N$ = 239.4
CAS—156-08-1
IUPAC Name (α*S*)-*N*,α-Dimethyl-*N*-(phenylmethyl)benzeneethanamine
Synonym Benzphetamine
Proprietary Name *Didrex*



Chemical Properties A liquid. Bp 127°. Practically insoluble in water; soluble in methanol, ethanol, chloroform, acetone, benzene and ether. pK_a 6.6. Log *P* (octanol/water), 4.1.

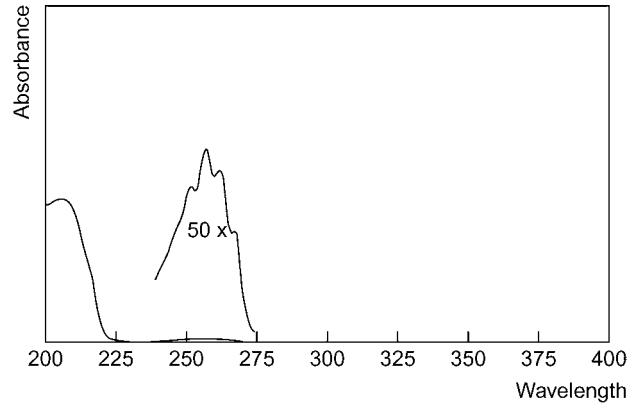
Benzfetamine Hydrochloride

$C_{17}H_{21}N,HCl$ = 275.8
CAS—5411-22-3
Proprietary Names *Didrex*; *Inapetyl*.
Chemical Properties A white crystalline powder. Mp about 131°. Freely soluble in water, ethanol and chloroform; slightly soluble in ether.

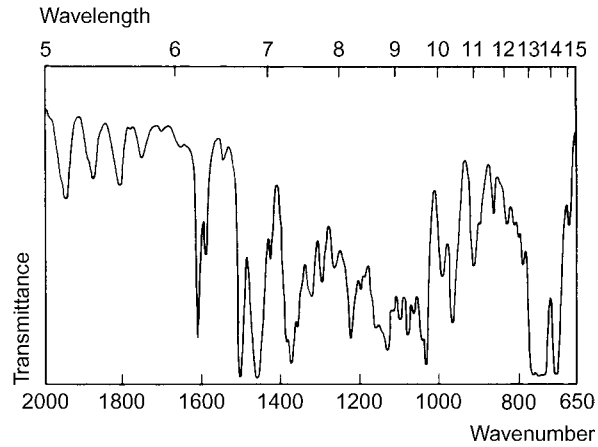
Colour Test Marquis test—orange
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.67; system TC—R_f 0.70; system TE—R_f 0.87; system TL—R_f 0.70; system TAE—R_f 0.60 (dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown).
Gas Chromatography System GA—benzfetamine RI 1855, amfetamine RI 1125, metamfetamine RI 1175; system GB—benzfetamine RI 1899, amfetamine RI 1150, metamfetamine RI 1200; system GC—benzfetamine RI 2172, amfetamine RI 1536, metamfetamine RI 1722; system GF—benzfetamine RI 2050, amfetamine RI 1315, metamfetamine RI 1335.

Column: TC-1 cross-linked methylsilicone (20 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 60° for 0.5 min, to 280° at 20°/min. Carrier gas: He, flow rate 1 mL/min. SIM acquisition mode. Retention time: amfetamine, 4.6 min; metamfetamine, 5.4 min; benzfetamine, 8.2 min [Kikura, Nakahara 1995].
High Performance Liquid Chromatography System HA—benzfetamine *k* 1.2, amfetamine *k* 0.9, metamfetamine *k* 2.0; system HC—benzfetamine *k* 0.15, amfetamine *k* 0.98, metamfetamine *k* 2.07.

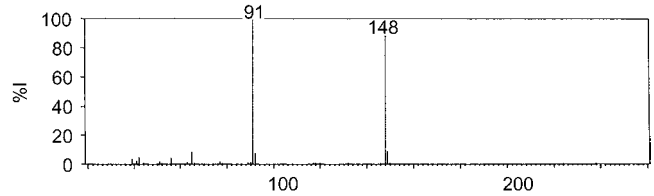
Ultraviolet Spectrum Aqueous acid—252, 258 nm (A^1_1=19c), 262, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1497, 740, 698, 1028, 1125, 1220 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 148, 149, 65, 92, 42, 56, 39.



Quantification

***Urine* GC-MS** Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 80° for 1 min, to 210° at 20°/min for 2 min. Carrier gas: He, flow rate 1 mL/min. Retention time: amfetamine, 4.2 min; metamfetamine, 5.1 min; benzfetamine, 8.2 min. Limit of detection, 5 µg/L for amfetamine and metamfetamine, 2 µg/L for benzfetamine [Cody, Valtier 1998].

HPLC Benzfetamine metabolites Fujinami *et al.* [1998].

***Hair* GC-MS**. Limit of detection, 0.5 ng/mg [Nakahara 1995].

Disposition in the Body Readily absorbed after oral administration and mainly excreted in the urine as amfetamine and metamfetamine; the metabolism of benzfetamine to desmethylbenzfetamine may be a major pathway in its metabolism. It is also excreted in the urine as conjugated metabolites, *p*-hydroxy-*N*-benzylamfetamine glucuronide and *p*-hydroxybenzfetamine glucuronide; very little is excreted as unchanged drug.

Therapeutic Concentration

Urinary concentrations of *p*-hydroxy-*N*-benzylamfetamine glucuronide (pHBAG) and *p*-hydroxybenzfetamine glucuronide (pHBZG) were determined in 2 healthy volunteers after the administration of 10 mg benzfetamine hydrochloride. After 6 h, pHBAG concentrations were 450 nM (subject 1) and 800 nM (subject 2), and pHBZG 800 nM (subject 1) and 1900 nM (subject 2). After 24 h, pHBAG levels dropped to 50 nM for both subjects and pHBZG concentrations were negligible [Fujinami *et al.* 1998].

Toxicity

A 16-year-old male was found dead with several medicine bottles in the room. Postmortem examination and toxicological analysis revealed the following benzfetamine concentrations: blood 13.9 mg/L, urine 8.0 mg/L, liver 106.3 mg/kg, brain 30.5 mg/kg, and gastric contents (total) 53.1 mg [Brooks *et al.* 1982].

Dose 25 to 150 mg of benzfetamine hydrochloride daily.

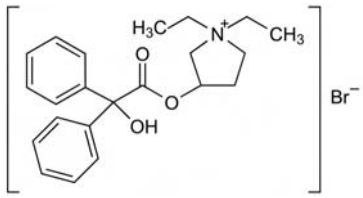
Brooks JP *et al.* (1982). A case of benzphetamine poisoning. *Am J Forensic Med Pathol* 3: 245–247.
Cody JT, Valtier S (1998). Detection of amphetamine and methamphetamine following administration of benzphetamine. *J Anal Toxicol* 22: 299–309.
Fujinami A *et al.* (1998). Development of a method for the quantitation of benzphetamine metabolites in human urine by high-performance liquid chromatography. *Ann Clin Biochem* 35: 775–779.
Kikura R, Nakahara Y (1995). Hair analysis for drugs of abuse. XI. Disposition of benzphetamine and its metabolites into hair and comparison of benzphetamine use and methamphetamine use by hair analysis. *Biol Pharm Bull* 18: 1694–1699.
Nakahara Y (1995). Detection and diagnostic interpretation of amphetamines in hair. *Forensic Sci Int* 70: 135–153.
Cody JT, Valtier S (1998). Detection of amphetamine and methamphetamine following administration of benzphetamine. *J Anal Toxicol* 22: 299–309.

Benzilonium Bromide

Anticholinergic
$C_{22}H_{28}BrNO_3 = 434.4$
CAS—1050-48-2

IUPAC Name 1,1-Diethyl-3-[(hydroxydiphenylacetyl)oxy]pyrrolidinium bromide

Proprietary Names *Portyn*; *Ulcoban*.

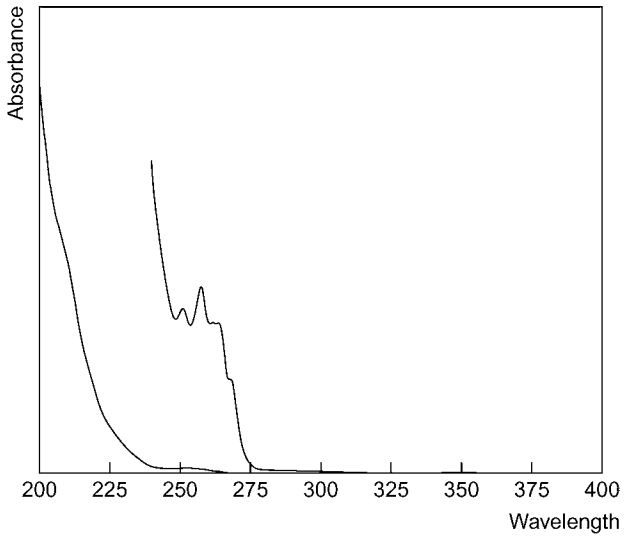


Chemical Properties A white crystalline powder. Mp 203° to 204°. Soluble in water. Log *P* (octanol/water), −0.3.

Colour Tests The following tests are performed on benzilonium nitrate: Liebermann's reagent—brown; Marquis test—orange→green→blue; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.03; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.05 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—252, 258 (A^{1}_{1}=11a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1728, 1220, 1190, 1162, 690, 1052 cm^{-1} (KBr disk).

Quantification
Plasma **GC–MS** Limit of detection, 5 µg/L [Dahlström *et al.* 1980].

Dose 30 to 70 mg daily.

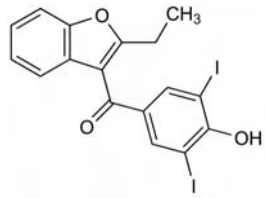
Dahlström H *et al.* (1980). Quantitative determination of benzilonium bromide in plasma by gas chromatography-mass spectrometry after oxidation to benzophenone. *J Chromatogr* 183: 511–513.

Benziodarone

Uricosuric, Vasodilator
$C_{17}H_{12}I_2O_3 = 518.1$
CAS—68-90-6

IUPAC Name 2-Ethyl-3-benzofuranyl 4-hydroxy-3,5-diiodophenyl methanone

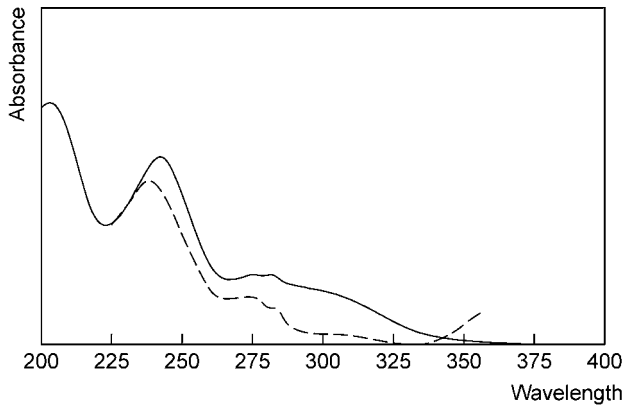
Proprietary Name *Amplivix*



Chemical Properties A yellowish powder. Mp 167°. Soluble 1 in about 500 of water at 25° and 1 in about 100 of water at 45°; soluble in acetone and chloroform. Log *P* (octanol/water) 6.6.

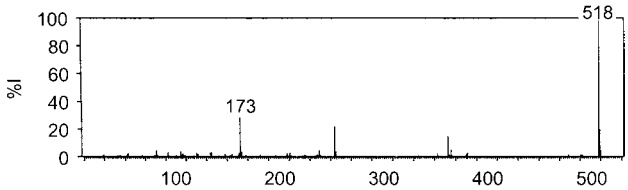
Thin-layer Chromatography System TD—R_f 0.62; system TE—R_f 0.23; system TF—R_f 0.58; system TAD—R_f 0.65.

Ultraviolet Spectrum Methanol—240 nm (A^{1}_{1}=1280b), 275, 357 nm.



Infrared Spectrum Principal peaks at wavenumbers 1618, 756, 1577, 1135, 1293, 1176 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 518, 173, 264, 519, 373, 376, 520, 249.



Dose Benziodarone has been given in initial doses of 600 mg daily and maintenance doses of 300 to 400 mg daily.

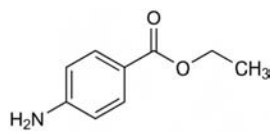
Benzocaine

Anaesthetic (Local)
$C_9H_{11}NO_2 = 165.2$
CAS—94-09-7

IUPAC Name 4-Aminobenzoic acid ethyl ester

Synonyms Anaesthesinum; anesthamine; éthoforme; ethyl aminobenzoate.

Proprietary Names *Americaine*; *Anaesthesin*; *Flavamed*; *Subcutin*. It is an ingredient of *AAA*, *Audicort*, *Auralgan*, *Auralgicin*, *Auraltone*, *Merocaine*, *Transvasin*, *Tyrosolven* and *Tyrozets*.



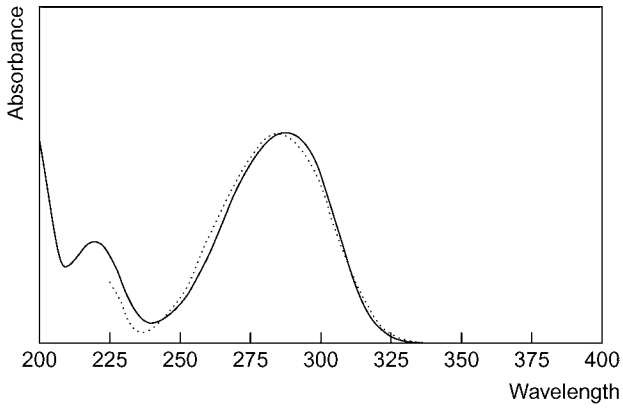
Chemical Properties Colourless crystals or white crystalline powder. Mp 88° to 92°. Soluble 1 in 2500 of water, 1 in 8 of ethanol, 1 in 2 of chloroform and 1 in 4 of ether; soluble in dilute acids. pK_a 2.5 (25°). Log *P* (octanol/water), 1.9.

Colour Test Diazotisation—red.

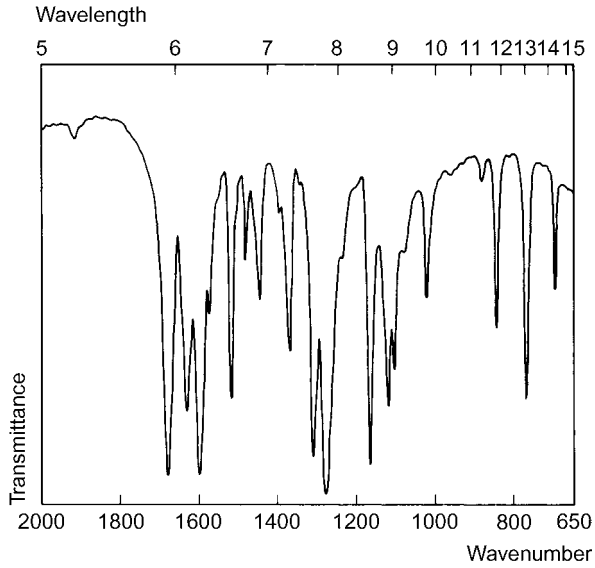
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.06; system TC—R_f 0.57; system TD—R_f 0.56; system TE—R_f 0.77; system TF—R_f 0.62; system TL—R_f 0.66; system TAD—R_f 0.63; system TAE—R_f 0.84; system TAF—R_f 0.87 (ninhydrin spray, positive; acidified potassium permanganate solution, positive; Van Urk reagent, bright yellow).

Gas Chromatography System GA—benzocaine RI 1555, 4-aminobenzoic acid RI 1547; system GF—RI 2100.

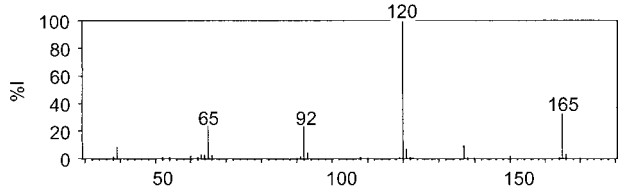
High Performance Liquid Chromatography System HA—*k* 0.1; system HQ—*k* 20.06; system HR—*k* 1.61; system HX—RI 404; system HY—RI 358; system HZ—retention time 4.3 min.
Ultraviolet Spectrum Aqueous acid—272 (A^1_1=90c), 278 nm; aqueous alkali—285 nm (A^1_1=930a); ethanol—293 nm (A^1_1=1238a).



Infrared Spectrum Principal peaks at wavenumbers 1280, 1680, 1598, 1170, 1315, 1634 cm^{-1} (KBr disk).



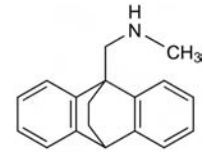
Mass Spectrum Principal ions at *m/z* 120, 165, 92, 65, 137, 39, 121, 93; 4-aminobenzoic acid 137, 120, 92, 65, 39, 138, 121, 63.



Disposition in the Body Benzocaine is metabolised by hydrolysis to 4-aminobenzoic acid. At the concentrations normally used (2 to 10%) it is comparatively non-irritant and non-toxic, having only about one-tenth the toxicity of cocaine. The maximum safe amount for topical use is 5000 mg (25 mL of a 20% solution).

Benzoctamine

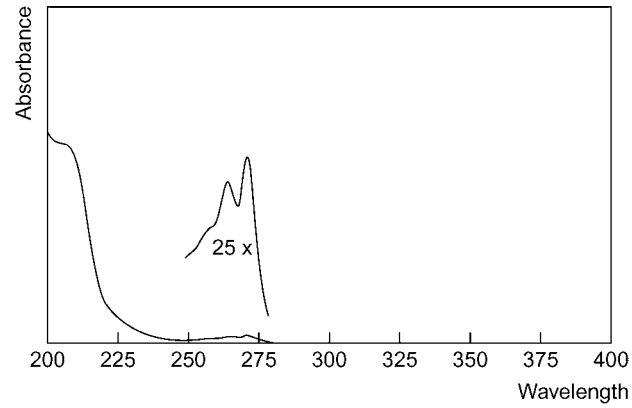
Tranquilliser
$C_{18}H_{19}N$ = 249.4
CAS—17243-39-9
IUPAC Name *N*-Methyl-9,10-ethanoanthracene-9(10*H*)-methenamine



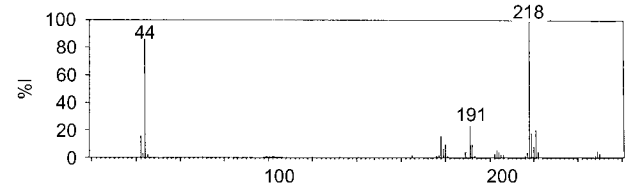
Chemical Properties pK_a 7.6. Log *P* (octanol/water), 3.5.

Benzoctamine Hydrochloride
$C_{18}H_{19}N,HCl$ = 285.8
CAS—10085-81-1
Synonym Ba-30803
Proprietary Name *Tacitin(e)*
Chemical Properties A white crystalline powder. Mp 320° to 322°. Soluble in water, ethanol and chloroform; sparingly soluble in acetone and ether.

Colour Tests Mandelin's test—blue-green; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.57; system TC—R_f 0.52; system TL—R_f 0.43; system TAE—R_f 0.38; system TAJ—R_f 0.31; system TAK—R_f 0.14; system TAL—R_f 0.65 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2078; system GB—RI 2172; system GF—RI 2445.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 380; system HY—RI 322.
Ultraviolet Spectrum Aqueous acid—264, 271 nm (A^1_1=57a).



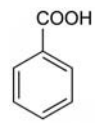
Infrared Spectrum Principal peaks at wavenumbers 757, 743, 732, 1134, 1060, 1020 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 218, 44, 191, 221, 219, 178, 42, 180.



Dose 30 to 60 mg of benzoctamine hydrochloride daily.

Benzoic Acid

Preservative
$C_7H_6O_2$ = 122.1
CAS—65-85-0
Synonyms Benzenecarboxylic acid; phenylformic acid.
Proprietary Names It is an ingredient of *Aserbine* and *Malatex*.

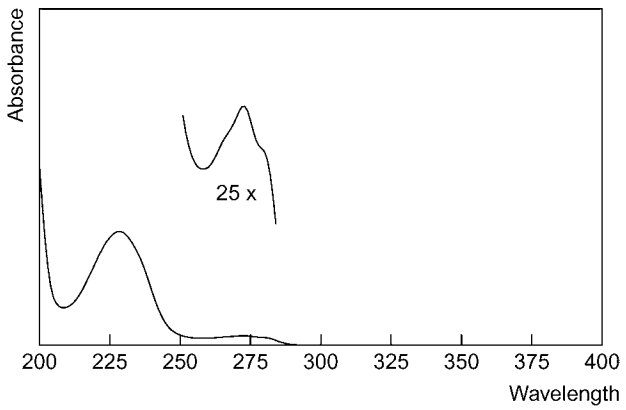


Chemical Properties Colourless, light feathery crystals or white scales or powder. Mp 122.4°. It sublimes on heating. Soluble 1 in about 350 of water, 1 in 20 of boiling water, 1 in 3 of ethanol, 1 in 5 of chloroform and 1 in 3 of ether; freely soluble in acetone. pK_a 4.2 (25°). Log *P* (octanol/water), 1.9.

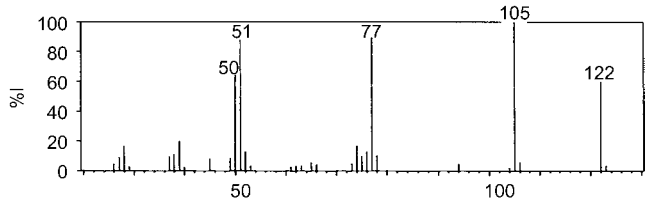
Sodium Benzoate
$C_7H_5O_2Na$ = 144.1
CAS—532-32-1

Chemical Properties A white, amorphous, granular, flaky, or crystalline powder. Soluble 1 in 2 of water and 1 in 90 of ethanol.

Thin-layer Chromatography System TD—R_f 0.26; system TE—R_f 0.07; system TF—R_f 0.28; system TAD—R_f 0.35; system TAJ—R_f 0.55; system TAK—R_f 0.81; system TAL—R_f 0.92.
Gas Chromatography System GA—RI 1180.
High Performance Liquid Chromatography System HX—RI 360; system HY—RI 327; system HZ—retention time 3.0 min (benzoate).
Ultraviolet Spectrum Aqueous acid—230 (A^1_1=923a), 273 nm (A^1_1=85a); methanol—227 (A^1_1=895a), 272 (A^1_1=73a), 280 nm (A^1_1=61b).



Infrared Spectrum Principal peaks at wavenumbers 709, 1689, 1296, 667, 935, 685 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 105, 77, 51, 122, 50, 39, 74, 76; hippuric acid 105, 135, 51, 134, 77, 106, 50, 78.



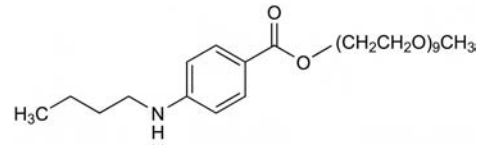
Quantification
Plasma **GC** FID. Limit of detection, 0.1 mg/L [Li, Zhao 2001]. ECD. Limit of detection, 10 µg/L [Sioufi, Pommier 1980].
HPLC UV detection (λ=283 nm). Limit of quantification, 0.05 mg/L [Nomeir *et al.* 1994].
Urine **GC** FID. Limit of detection, 0.2 mg/L [Li, Zhao 2001]. ECD. Limit of detection, 0.5 µg/L [Aprea *et al.* 1997]. See Plasma [Sioufi, Pommier 1980].
Disposition in the Body Benzoic acid is metabolised in the liver by conjugation with glycine and is rapidly and completely excreted in the urine as hippuric acid. Normal urinary excretion of hippuric acid is 1 to 2.5 g daily, equivalent to 0.7 to 1.7 g of benzoic acid. When taken in large doses, a proportion may be excreted as benzoylglucuronic acid. Benzoic acid may be found in the urine as a metabolite of benzaldehyde, and is also a metabolite of numerous other compounds.
Uses Benzoic acid is used as a preservative in a concentration of 0.1%. Sodium benzoate is given by mouth in a dose of 6 g to test liver function.

Aprea C *et al.* (1997). Analytical method for the determination of urinary 3-phenoxybenzoic acid in subjects occupationally exposed to pyrethroid insecticides. *J Chromatogr B Biomed Sci Appl* 695 (2): 227–236.
Li AQ, Zhao XL (2001). Determination and pharmacokinetic study of 1-p-(3.3-dimethyl-1-triazeno) benzoic acid in cancer patients by capillary gas chromatography. *Biomed Chromatogr* 15 (2): 75–78.
Nomeir AA *et al.* (1994). Liquid chromatographic analysis, stability and protein binding studies of the anti-HIV agent benzoic acid, 2-chloro-5[[(1-methylethoxy)thioxomethyl]amino]-,1-methylethyl ester. *J Pharm Biomed Anal* 12(5): 693–698.
Sioufi A, Pommier F (1980). Gas chromatographic determination of low concentrations of benzoic acid in human plasma and urine. *J Chromatogr* 181(7); B Biomed. Appl.: 161–168.

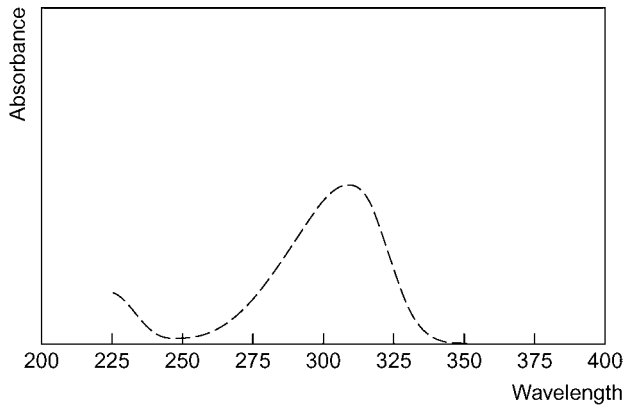
Benzonatate

Cough Suppressant
$C_{30}H_{53}NO_{17}$ = 603.7
CAS—104-31-4
IUPAC Name 4-(Butylamino)benzoic acid 3,6,9,12,15,18,21,24,27-nonaoxaoctacos-1-yl ester
Synonyms Benzonatine; benzononatine.

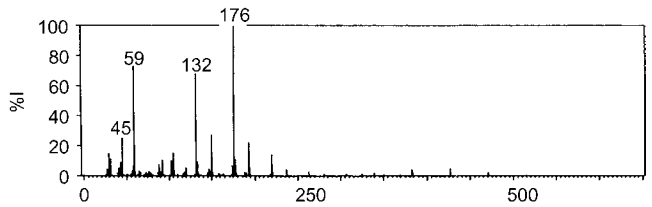
Proprietary Name *Tessalon*



Chemical Properties A clear, pale yellow, viscous liquid. Miscible with water, ethanol, chloroform and ether. Log *P* (octanol/water), 2.4.
Colour Tests Aromaticity (method 2)—yellow/red; Liebermann's test (at 100°)—blue.
Thin-layer Chromatography System TA—R_f 0.61; system TAJ—R_f 0.56; system TAK—R_f 0.23; system TAL—R_f 0.90 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Ethanol—308 nm (A^1_1=473c).



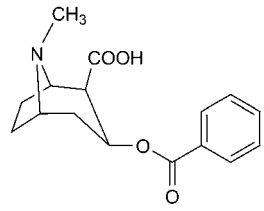
Infrared Spectrum Principal peaks at wavenumbers 1095, 1605, 1266, 1172, 1698, 1527 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 176, 59, 132, 150, 45, 193, 105, 29.



Dose 300 to 600 mg daily.

Benzoylecgonine

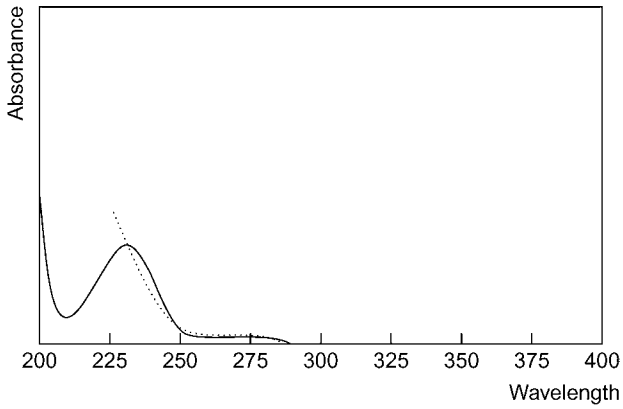
Alkaloid
$C_{16}H_{19}NO_4$ = 289.3
CAS—519-09-5
IUPAC Name 3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid
Synonym Ecgonine benzoate



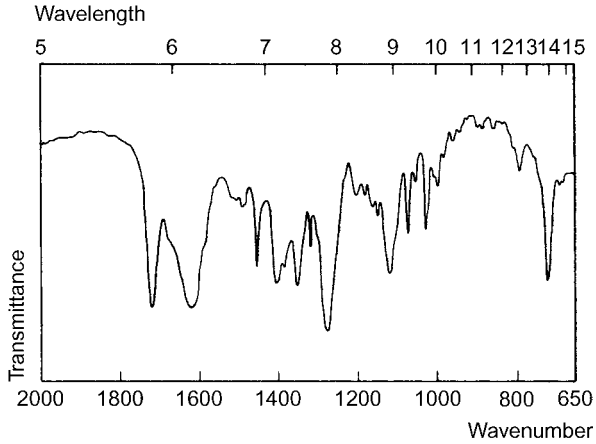
Chemical Properties An alkaloid obtained from coca leaves, *Erythroxylum coca* (Erythroxylaceae) and its varieties. The hydrated form occurs as crystals. Mp 86° to 92° (anhydrous) 195° (with decomposition). Very soluble in hot water; soluble in ethanol; practically insoluble in ether; soluble in dilute acids and alkalis. Log *P* (octanol/water) −1.3. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Thin-layer Chromatography System TA—R_f 0.21; system TB—R_f 0.00; system TC—R_f 0.01 (acidified iodoplatinate solution, positive).
Plate: silica 60 F_{254} (10 × 10 cm). Mobile phase: hexane : toluene : diethylamine (65 : 20 : 5). UV detection. R_f 0.02 [Antonilli *et al.* 2001].
Gas Chromatography System GA—benzoylecgonine RI 2570; cocaine RI 2187; system GB—benzoylecgonine RI 2663, cocaine RI 2289.

High Performance Liquid Chromatography System HA—*k* 0.9 (tailing peak); system HQ—*k* 5.68; system HY—RI 236; system HZ—RT 1.7 min; system HAA—RT 9.7 min.

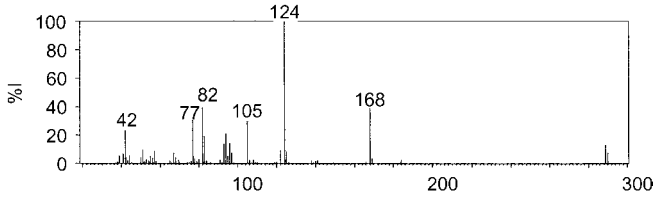
Ultraviolet Spectrum Aqueous acid—234 ($A_1^1 = 376a$), 274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1275, 1720, 1618, 717, 1116, 1316 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 124, 82, 168, 77, 105, 42, 94, 83.



Quantification See also under Cocaine.

Blood **LC-MS** CI. Limit of detection, 2 µg/L [Sosnoff *et al.* 1996].

Plasma **GC-MS** SIM acquisition mode (*m/z* 404, 140 and 298). Limit of detection, 5 µg/L [Wang *et al.* 1994].

Urine **GC-MS** SIM acquisition mode (*m/z* 318, 439). Limit of detection, 1 µg/L [de la Torre *et al.* 1995]. See Plasma [Wang *et al.* 1994].

HPLC Column: LiChrocart-LiCrospher 100 RP-18 (250 × 4 mm i.d., 5 µm). Mobile phase: 0.045 mol/L ammonium acetate-methanol-acetonitrile (80 : 10 : 10) : 0.045 mol/L ammonium acetate-methanol-acetonitrile (20 : 40 : 40; 100 : 0 to 47.2 : 52.8 in 20 min), flow rate 1 mL/min. UV detection (λ = 235 nm). Retention time: 8.48 min. Limit of quantification, 200 µg/L, limit of detection, 25 µg/L [Antonilli *et al.* 2001

LC-MS Column: Allure Basix (30 × 2.1 mm, 5 µm). Mobile phase: 50 mmol/L formic acid-100 mmol/L ammonium formate-acetonitrile : acetone (60 : 40). Tandem MS detection (*m/z* 290 to 168 transition). Retention time: 1.5 min. Limit of detection, 0.5 µg/L [Jeanville *et al.* 2001].

Oral Fluid See Plasma [Wang *et al.* 1994].

Hair **GC-MS** SIM acquisition mode (*m/z* 240 and 361). Limit of detection, 0.2 µg/g using a 30 mg sample [Kintz, Mangin 1995]. See Plasma. Limit of detection, 0.5 µg/g [Wang *et al.* 1994].

Disposition in the Body Benzoylecgonine is the first hydrolysis product formed in the metabolism of cocaine; it is then further hydrolysed to ecgonine.

Antonilli L *et al.* (2001). Analysis of cocaethylene, benzoylecgonine and cocaine in human urine by high-performance thin-layer chromatography with ultraviolet detection: a comparison with high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 751: 19–27.

de la Torre R *et al.* (1995). Determination of cocaine and its metabolites in human urine by gas chromatography/mass spectrometry after simultaneous use of cocaine and ethanol. *J Pharm Biomed Anal* 13: 305–312.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Jeanville PM *et al.* (2001). Rapid confirmation/quantitation of ecgonine methyl ester, benzoylecgonine, and cocaine in urine using on-line extraction coupled with fast HPLC and tandem mass spectrometry. *J Anal Toxicol* 25: 69–75.

Kintz P, Mangin P (1995). Simultaneous determination of opiates, cocaine and major metabolites of cocaine in human hair by gas chromotography/mass spectrometry (GC/MS). *Forensic Sci Int* 73: 93–100.

Sosnoff CS *et al.* (1996). Analysis of benzoylecgonine in dried blood spots by liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. *J Anal Toxicol* 20: 179–184.

Wang WL *et al.* (1994). Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Appl* 660: 279–290.

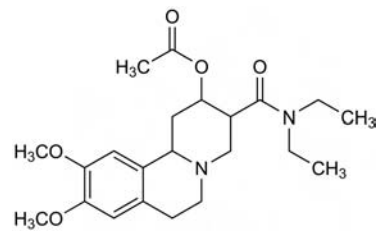
Benzquinamide

Antiemetic

$C_{22}H_{32}N_2O_5 = 404.5$

CAS—63-12-7

IUPAC Name 2-(Acetyloxy)-*N*,*N*-diethyl-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-2*H*-benzo[*a*]-quinolizine-3-carboxamide



Chemical Properties A yellowish crystalline powder. Mp 130° to 131.5°. Soluble in dilute acetic acid. pK_a 5.9. Log *P* (octanol/water), 1.9.

Benzquinamide Hydrochloride

$C_{22}H_{32}N_2O_5,HCl = 441.0$

CAS—113-69-9

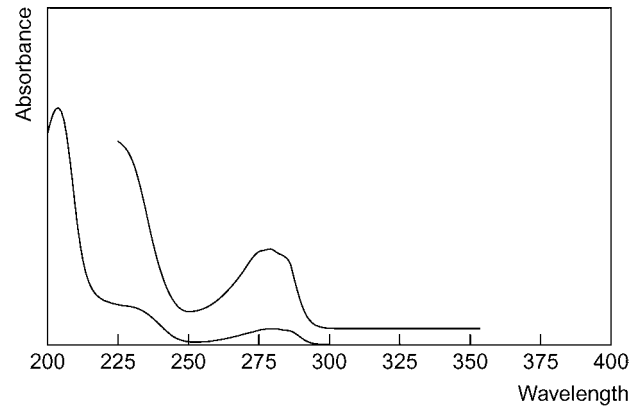
Proprietary Name *Emete-con*

Colour Tests Liebermann's reagent—black; Marquis test—yellow; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.07; system TC—R_f 0.69; system TL—R_f 0.36; system TAJ—R_f 0.53; system TAK—R_f 0.06; system TAL—R_f 0.91 (acidified iodoplatinate solution, positive).

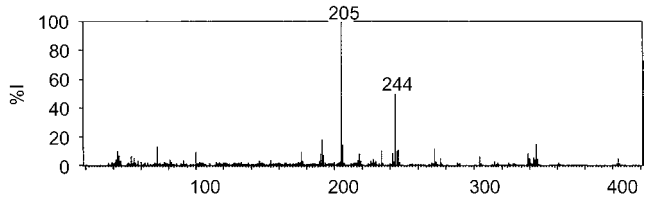
High Performance Liquid Chromatography System HA—*k* 0.3.

Ultraviolet Spectrum Aqueous acid—282 nm (A_1^1=95b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1244, 1733, 1633, 1204, 1515, 1123 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 205, 244, 191, 345, 206, 72, 272, 246.



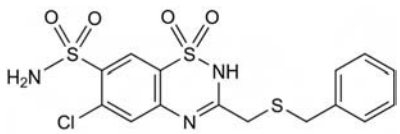
Quantification
Plasma **GC–MS** Limit of detection, 10 µg/L [Hobbs, Connolly 1978].
Disposition in the Body Readily absorbed after oral administration; bioavailability about 35%. Metabolised by *O*-demethylation, deacetylation and *N*-dealkylation. After an oral dose, up to about 7% is excreted unchanged in the urine in 24 h.
Therapeutic Concentration
Following oral administration of 200 mg to 20 subjects, a mean peak plasma concentration of 0.6 mg/L was attained in 1.2 h. Following intramuscular injection of 50 mg to 20 subjects, a mean peak plasma concentration of 0.7 mg/L was attained in 0.26 h [Hobbs, Connolly 1978].
Half-life Plasma half-life, about 1 h.
Volume of Distribution About 1 L/kg.
Dose The equivalent of 50 mg of benzquinamide by intramuscular injection, repeated as necessary.

Hobbs DC, Connolly AG (1978). Pharmacokinetics of benzquinamide in man. *J Pharmacokinet Biopharm* 6: 477–485.

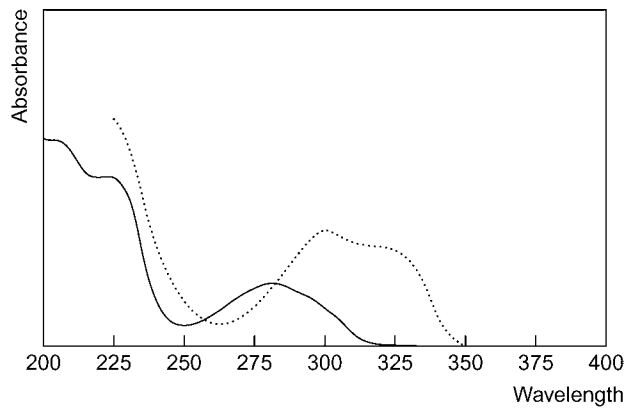
Benzthiazide

Diuretic, Antihypertensive
$C_{15}H_{14}ClN_3O_4S_3 = 431.9$
CAS—91-33-8

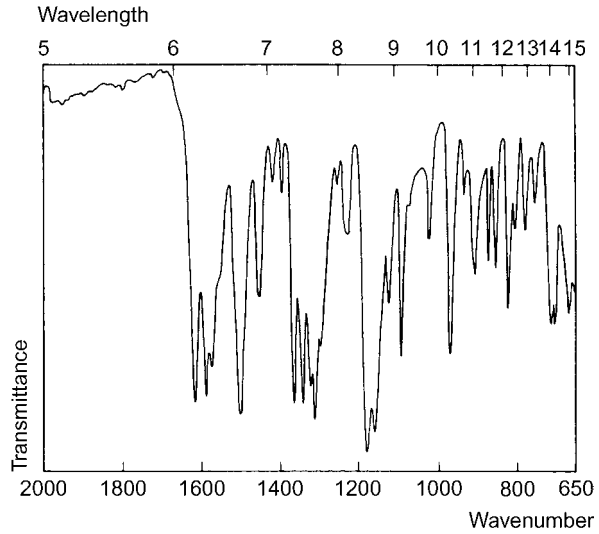
IUPAC Name 6-Chloro-3-[[(phenylmethyl)thio]methyl]-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
Proprietary Names *Aquatag*; *Exna*; *Hydrex*. It is an ingredient of *Decaserpyl Plus* and *Dytide*.



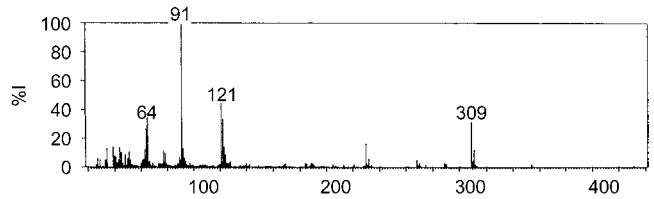
Chemical Properties A white crystalline powder. Mp 231° to 232°. Practically insoluble in water, chloroform and ether; slightly soluble in ethanol; soluble 1 in 100 of acetone; freely soluble in dimethylformamide and in solutions of alkalis. pK_a 6.0. Log *P* (octanol/water), 1.7.
Colour Tests Koppanyi-Zwikker test—violet; Liebermann's reagent—red-brown; sulfuric acid—yellow.
Thin-layer Chromatography System TD—R_f 0.14; system TE—R_f 0.09; system TF—R_f 0.51; system TAD—R_f 0.30; system TAJ—R_f 0.31; system TAK—R_f 0.06; system TAL—R_f 0.71.
Gas Chromatography System GA—RI 2680.
High Performance Liquid Chromatography System HN—*k* 9.32; system HY—RI 415.
Ultraviolet Spectrum Aqueous acid—283 nm (A^1_1=292a); aqueous alkali—297 nm (A^1_1=310b).



Infrared Spectrum Principal peaks at wavenumbers 1180, 1160, 1310, 1502, 1620, 1590 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 121, 65, 122, 309, 64, 230, 123.



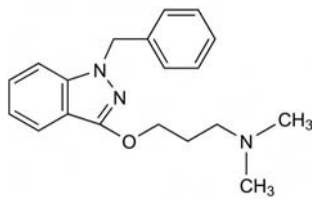
Quantification
Plasma **HPLC** Column: reversed phase. UV detection (λ=280 nm). Limit of detection, 10 µg/L [Meyer *et al.* 1982].
Urine **HPLC** See Plasma [Meyer *et al.* 1982].
Faeces **HPLC** See Plasma [Meyer *et al.* 1982].
Disposition in the Body Poorly absorbed after oral administration. Less than 10% of a dose is excreted in the urine as unchanged drug in 48 h, and about 80% of a dose is eliminated in the faeces.
Therapeutic Concentration
Following an oral dose of 50 mg to 4 subjects, peak plasma concentrations of about 0.008 and 0.02 mg/L were attained in 3 h in 2 subjects; in the remaining 2 subjects benzthiazide was not detectable in the plasma [Meyer *et al.* 1982].
Half-life Derived from urinary excretion data, about 10 to 15 h.
Dose 50 to 200 mg daily.

Meyer MC *et al.* (1982). HPLC determination of benzthiazide in biologic material. *Biopharm Drug Dispos* 3: 1–9.

Benzydamine

Analgesic
$C_{19}H_{23}N_3O = 309.4$
CAS—642-72-8

IUPAC Name *N*,*N*-Dimethyl-3-[[1-(phenylmethyl)-1*H*-indazol-3-yl]oxy]-1-propanamine
Synonym Benzindamine

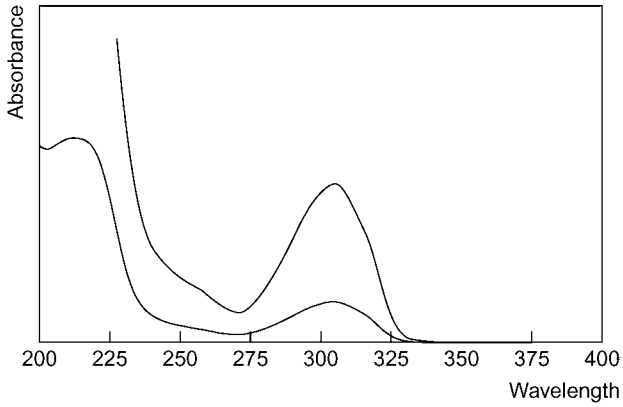


Chemical Properties Bp 160°. Log *P* (octanol/water), 4.2.

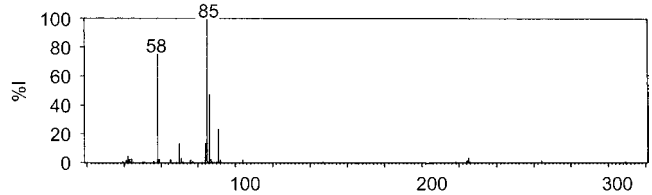
Benzydamine Hydrochloride
$C_{19}H_{23}N_3O,HCl = 345.9$
CAS—132-69-4
Proprietary Names *Afloben*; *A-Termadol*; *Difflam*; *Imotryl*; *Multum*; *Tantum*; *Verax*.

Chemical Properties A white crystalline powder. Mp 160°. Soluble 1 in 1 of water, 1 in 8 of ethanol and 1 in 4 of chloroform; practically insoluble in ether.

Colour Test Mandelin's test—brown-green.
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.36; system TC—R_f 0.22; system TL—R_f 0.09; system TAE—R_f 0.16 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2380.
High Performance Liquid Chromatography System HAA—retention time 15.0 min.
Ultraviolet Spectrum Aqueous acid—307 nm (A^1_1=144c). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1529, 740, 1491, 1613, 1182, 1141 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 85, 58, 86, 91, 84, 70, 42, 225.



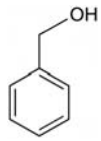
Quantification
Plasma **HPLC** Limit of quantification, 0.5 µg/L [Baldock *et al.* 1990].
Urine **HPLC** Limit of quantification, 1.0 µg/L [Baldock *et al.* 1990].
Disposition in the Body Absorbed after oral administration. About 50% of a dose is excreted unchanged in the urine.
Therapeutic Concentration
An oral dose of 1 mg/kg produced a blood concentration of about 0.8 mg/L within 2 h and a significant concentration was maintained for several hours [Catanese *et al.* 1966].
Protein Binding A fraction of benzydamine is bound to proteins in the blood.
Dose 150 to 200 mg of benzydamine hydrochloride daily.

Baldock GA *et al.* (1990). Determination of benzydamine and its N-oxide in biological fluids by high-performance liquid chromatography. *J Chromatogr* 529(1): 113–123.
Catanese B *et al.* (1966). Studies on the absorption and elimination of benzydamine in the mouse, rat, dog, and man. *Arzneimittelforschung* 16: 1354–1357.

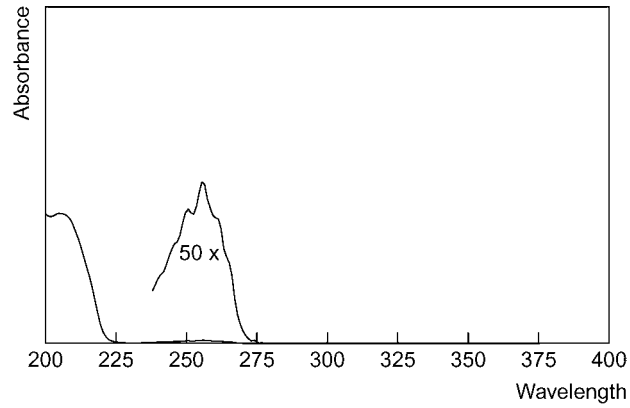
Benzyl Alcohol

Anaesthetic (Local), Disinfectant
C_7H_8O = 108.1
CAS—100-51-6

Synonyms Phenylcarbinol; phenylmethanol.



Chemical Properties A colourless liquid with a faint aromatic odour. Mass per mL 1.043 to 1.046 g. Bp 203° to 208°. Soluble 1 in 25 of water; miscible with ethanol, chloroform and ether. pK_a 15.4. Log *P* (octanol/water), 1.1.
Thin-layer Chromatography System TAE—R_f 0.86.
Gas Chromatography System GA—RI 1040.
Ultraviolet Spectrum Methanol—252 (A^1_1=14b), 258 (A^1_1=17b), 264 nm (A^1_1=13b).



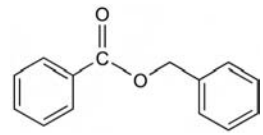
Mass Spectrum Principal ions at *m/z* 79, 77, 108, 101, 51, 50, 39, 40.
Quantification
Blood **GC–MS** Limit of detection, 1 mg/L [Dasgupta, Steinagel 1997].
Plasma **HPLC** Column: C_{18}. Mobile phase: water : acetonitrile : glacial acetic acid. UV detection (λ=254 nm). Limit of detection, 10 ng [Tan *et al.* 1991].
Serum **GC–MS** SIM (*m/z* 69, 77, 91, 105, 121, 504 and 518). Limit of detection, 0.1 mg/L [Dasgupta, Humphrey 1998]. See Blood [Dasgupta, Steinagel 1997].
Use Topically in concentrations of up to 10%.

Dasgupta A, Steinagel G (1997). Gas chromatographic-mass spectrometric identification and quantitation of benzyl alcohol from human serum and postmortem blood after derivatization with 4-carbethoxy hexafluorobutyryl chloride: a novel derivative. *J Forensic Sci* 42(4): 697–700.
Dasgupta A, Humphrey PE (1998). Gas chromatographic-mass spectrometric identification and quantitation of benzyl alcohol in serum after derivatization with perfluorooctanoyl chloride: a new derivative. *J Chromatogr B Biomed Sci Appl* 708(1–2): 299–303.
Tan HS *et al.* (1991). Determination of benzyl alcohol and its metabolite in plasma by reversed-phase high-performance liquid chromatography. *J Chromatogr* 568(1): 145–155.

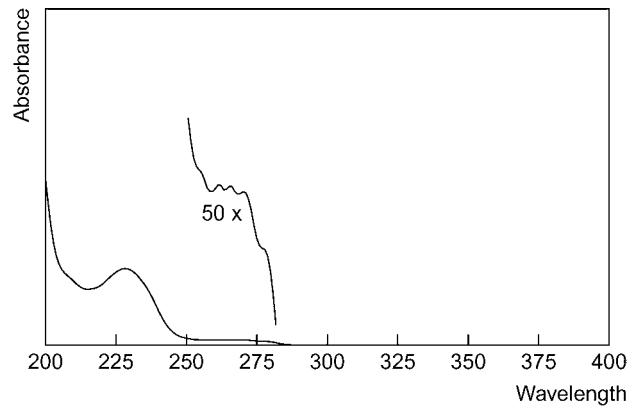
Benzyl Benzoate

Acaricide
$C_{14}H_{12}O_2$ = 212.2
CAS—120-51-4

Proprietary Names *Antiscabiosum Mago*; *Ascabiol*; *Benzemul*; *Scabanca*.



Chemical Properties Colourless crystals or a clear, colourless, oily liquid. Bp 323° to 324°. Practically insoluble in water; miscible with ethanol, acetone, carbon disulfide, chloroform and ether. Log *P* (octanol/water), 4.0.
Thin-layer Chromatography System TF—R_f 0.73; system TAD—R_f 0.09.
Gas Chromatography System GA—RI 1738.
Ultraviolet Spectrum Ethanol—230 nm (A^1_1=843b).

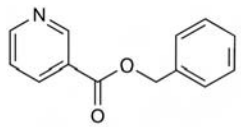


Infrared Spectrum Principal peaks at wavenumbers 1263, 1706, 1106, 713, 698, 1067 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 77, 105, 91, 51, 65, 212, 50, 78.
Disposition in the Body Rapidly hydrolysed to benzoic acid and benzyl alcohol; benzyl alcohol then undergoes further oxidation to benzoic acid followed by conjugation with glycine to form hippuric acid. Excreted in the urine mainly as hippuric acid.
Uses Topically as a 25% application; a 5% solution is used as an insect repellent.

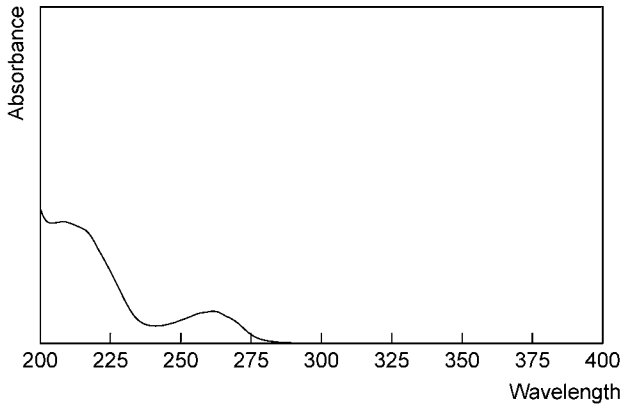
Benzyl Nicotinate

Rubefacient (Topical)
$C_{13}H_{11}NO_2$ = 213.2
CAS—94-44-0

Proprietary Names *Rubriment*. It is an ingredient of *Bayolin*.



Chemical Properties A liquid. Log *P* (octanol/water), 2.4.
Colour Tests Cyanogen bromide—red-orange; Liebermann's reagent—brown; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.42; system TC—R_f 0.71; system TL—R_f 0.60 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Methanol—262 nm (A^1_1=152b).

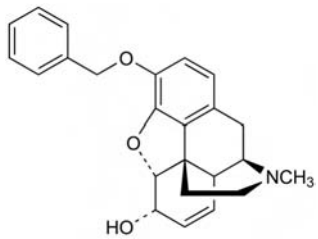


Infrared Spectrum Principal peaks at wavenumbers 1278, 1724, 1108, 740, 699, 1587 cm^{-1} (KCl disk).
Use Topically in a concentration of 2.5%.

Benzylmorphine

Narcotic Analgesic
$C_{24}H_{25}NO_3$ = 375.5
CAS—14297-87-1

IUPAC Name 7,8-Didehydro-4,5-epoxy-17-methyl-3-(phenylmethoxy)morphinan-6-ol



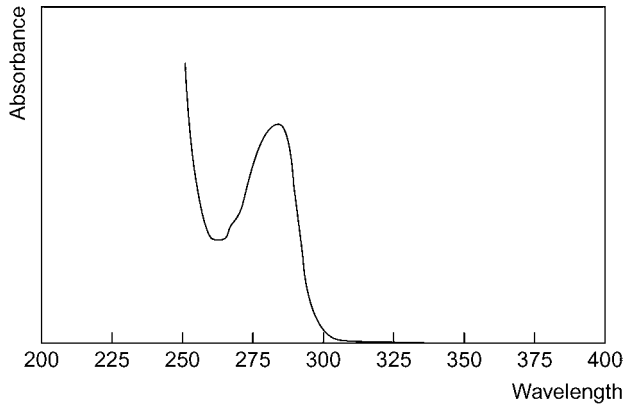
Chemical Properties A colourless crystalline powder. Mp 132°. Soluble 1 in 2500 of cold water; 1.6 parts dissolves in 100 parts ether; readily soluble in 50% ethanol and in benzene. pK_a 8.1 (20°). Log *P* (octanol/water), 3.0.

Benzylmorphine Hydrochloride

$C_{24}H_{25}NO_3$,HCl = 411.9
CAS—630-86-4

Proprietary Name *Peronine*
Chemical Properties A colourless microcrystalline powder. Soluble 1 in 200 of water and 1 in 160 of ethanol; practically insoluble in chloroform and ether.

Colour Test Marquis test—red→violet.
Thin-layer Chromatography System TA—R_f 0.41; system TB—R_f 0.06; system TC—R_f 0.23; system TL—R_f 0.08; system TAE—R_f 0.20; system TAF—R_f 0.23 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3015.
High Performance Liquid Chromatography System HA—*k* 4.4 (tailing peak); system HC—*k* 1.03.
Ultraviolet Spectrum Aqueous acid—284 nm (A^1_1=48b).

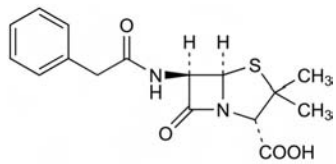


Infrared Spectrum Principal peaks at wavenumbers 1274, 1040, 1502, 1017, 763, 1056 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 284, 91, 375, 81, 42, 36, 285, 175.
Dose Benzylmorphine hydrochloride was formerly given in doses of 8 to 30 mg.

Benzylpenicillin

Antibiotic
$C_{16}H_{18}N_2O_4S$ = 334.4
CAS—61-33-6

IUPAC Name (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Synonyms Crystalline penicillin G; penicillin; penicillin G; penicillin II.



Note The name 'benzylpenicillin' and its synonyms are commonly used to describe either benzylpenicillin potassium or benzylpenicillin sodium.
Chemical Properties An antimicrobial acid produced by the growth of certain strains of *Penicillium notatum*. pK_a 2.8 (25°). Log *P* (octanol/water), 1.8.

Benzylpenicillin Potassium

$C_{16}H_{17}KN_2O_4S$ = 372.5
CAS—113-98-4

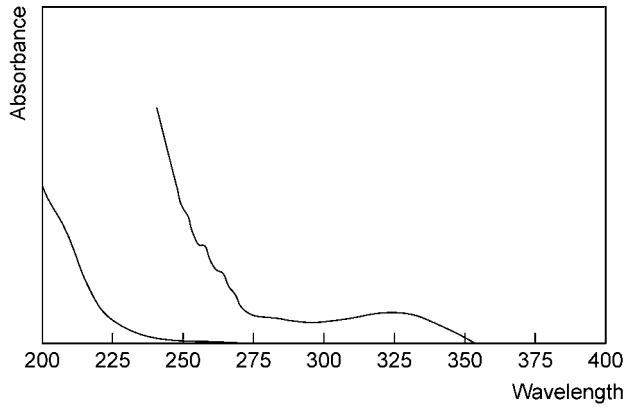
Proprietary Names *Abbocillin-G*; *Crystapen G*; *Falapen*; *Hyasorb*; *M-Cillin B*; *Megacillin*; *Novopen*; *P-50*; *Paclin G*; *Pentids*; *Pfizerpen*; *Sugracillin*.
Note *Megacillin* is also used as a proprietary name for clemizole penicillin, phenoxymethylpenicillin and procaine penicillin (procaine benzylpenicillin).
Chemical Properties A white, finely crystalline powder. Mp 214° to 217°, with decomposition. Very soluble in water; soluble in ethanol; practically insoluble in chloroform and ether.

Benzylpenicillin Sodium

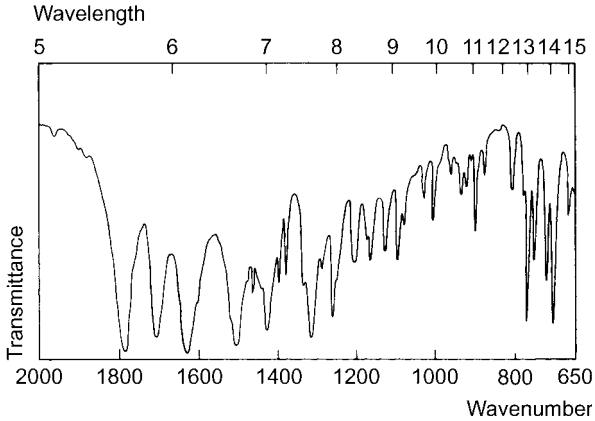
$C_{16}H_{17}N_2NaO_4S$ = 356.4
CAS—69-57-8

Proprietary Names *Crystapen*; *Gonopen*; *Spécilline G*. It is an ingredient of *Bicillin* and *Triplopen*.
Note *Bicillin* is also used as a proprietary name for benzathine benzylpenicillin.
Chemical Properties A white to slightly yellow, finely crystalline powder. Very soluble in water; soluble in ethanol; practically insoluble in chloroform and ether.

High Performance Liquid Chromatography System HY—RI 376.
Ultraviolet Spectrum Benzylpenicillin sodium: water—257 (A^1_1=7b), 264, 325 nm.



Infrared Spectrum Principal peaks at wavenumbers 1620, 1777, 1500, 1310, 1700, 703 cm^{-1} (benzylpenicillin sodium, KBr disk).



Quantification

Serum **HPLC** UV detection (λ=208 nm). Limit of quantification, 0.5 to 50 mg/L [Mendez-Alvarez *et al.* 1991].

Disposition in the Body About 30% of an oral dose is absorbed, the remainder being inactivated by gastric acid; maximum concentrations are attained about 1 h after oral administration. After IM injection it is rapidly absorbed, peak concentrations being attained in 15 to 30 min. About 60 to 90% of an IM dose is excreted in the urine, mainly in the first few hours; the urinary material consists of unchanged drug and penicilloic acid (about 20% of the dose). Biliary excretion also occurs.

Therapeutic Concentration In plasma, minimum inhibitory concentration 0.006 to 2 mg/L.

Half-life Plasma half-life, about 0.5 to 1 h; increased in infants and elderly subjects, and in renal impairment.

Distribution in Blood Plasma : whole blood ratio, 1.6.

Protein Binding 45 to 65%.

Note For a review of the pharmacokinetics of penicillin antibiotics, see Barza, Weinstein [1976]. For a review of clinical pharmacology and therapeutic use of penicillins, see Nathwani, Wood [1993].

Dose 0.6 to 2.4 g daily, given parenterally; up to 24 g daily in severe infections.

Barza M, Weinstein L (1976). Pharmacokinetics of the penicillins in man. *Clin Pharmacokinet* 1: 297–308.

Mendez-Alvarez E *et al.* (1991). A reversed phase liquid chromatographic method for the simultaneous determination of several common penicillins in human serum. *Biomed Chromatogr* 5(2): 78–82.

Nathwani D, Wood MJ (1993). Penicillins. A current review of their clinical pharmacology and therapeutic use. *Drugs* 45: 866–894.

N-Benzylpiperazine

Stimulant

$C_{11}H_{16}N_2$ = 176.3

CAS—2759-28-6

IUPAC Name 1-(Phenylmethyl)piperazine

Synonyms 1-Benzyl-1,4-diazacyclohexane; 1-benzylpiperazine; BZP.

Street Names A2; Bliss; Charge; Frenzy; Herbal ecstasy; Legal E; Legal X.

Chemical Properties Pale yellow viscous liquid. Mp 17° to 20°. pK_a 9.59 [Bishop *et al.* 2005]. Log *P* (octanol/water), 1.36 [Meylan, Howard 1995]. Human urine samples were stable for up to 3 months at –20° [Nordgren, Beck 2004]. There was no indication of instability of processed samples over a time period of 30 h. Freeze–thaw stability was established [Peters *et al.* 2003].

N-Benzylpiperazine Hydrochloride

$C_{11}H_{16}N_2$,HCl = 212.8

CAS—72878-35-4

Chemical Properties White solid.

N-Benzylpiperazine Dihydrochloride

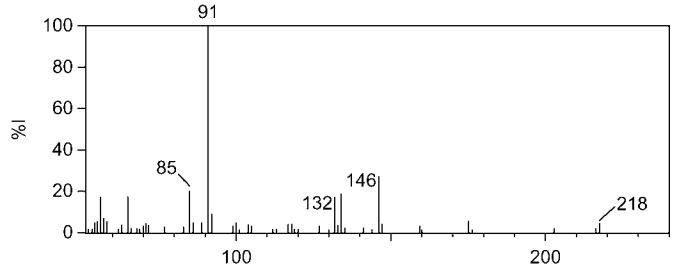
$C_{11}H_{16}N_2$,2HCl = 249.3

CAS—5321-63-1

Chemical Properties White solid. Mp 287° to 292°.

Ultraviolet Spectrum Neutral—211 nm.

Mass Spectrum Principal ions at *m/z* 91, 134, 56, 176, 120, 118, 119, 77, 91, 146, 134, 132, 85, 56, 218, 175 (AC derivative), Staack *et al.* [2002]; 91, 272, 181, 56, 69, 175, 146, 196 (TFAA derivative), Wikstrom *et al.* [2004]; 91, 281, 372, 175, 295 (HFBA derivative).



Quantification

Blood **GC-MS** Column: HP-5MS 5% phenylmethylsilicone (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 0.9 mL/min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.02 μg/g [Wikstrom *et al.* 2004].

Plasma **GC-MS** Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 μg/L; limit of detection, approx. 1 μg/L [Peters *et al.* 2003]. Column: HP-5MS (19 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 1 min to 300° at 15°/min for 5 min. EI ionisation at 70 eV. Limit of quantification not reported [de Boer *et al.* 2001].

Urine **GC-MS** Column: Fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 80° for 1 min to 250° at 10°/min. EI ionisation at 70 eV. Limit of detection, 20 μg/L for BZP and 500 μg/L for 3-hydroxy-BZP and 4-hydroxy-BZP [Tsutsumi *et al.* 2005].

LC-MS Direct injection, positive ion mode, MRM acquisition mode. Limit of detection, 8.8 μg/L (screening), 0.9 μg/L (confirmation) [Nordgren *et al.* 2005]. Column: SCX (150 × 0.2 mm i.d.). Mobile phase: acetonitrile : 40 mmol/L ammonium acetate (pH 4; 75 : 25), flow rate 0.15 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 0.2 μg/L for BZP and 0.4 μg/L for 3-hydroxy-BZP and 4-hydroxy-BZP [Tsutsumi *et al.* 2005]. Column: HyPURITY Advance (30 × 2.1 mm i.d., 3 μm). Mobile phase: 10 mmol/L ammonium acetate : methanol (95 : 5 at 0.1 min to 20 : 80 at 1 min to 95 : 5 for 5 min), flow rate 400 μL/min. APCI, positive ion mode. Retention time: 1.44 min. Limit of detection, 0.9 μg/L [Nordgren, Beck 2004].

Other **GC-MS** Rat Urine. Column: Fused silica capillary DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 80° for 1 min to 250° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Tsutsumi *et al.* 2006].

LC-MS Rat Urine Column: SCX (150 × 0.2 mm i.d.). Mobile phase: acetonitrile : 40 mmol/L ammonium acetate (pH 4; 75 : 25), flow rate 0.15 mL/min. ESI, positive ion mode. Limit of detection not reported [Tsutsumi *et al.* 2006].

Disposition in the Body Metabolised by CYP2D6 to form 4-hydroxy-BZP, 3-hydroxy-BZP, 4-hydroxy-3-methoxy-BZP, piperazine, benzylamine and *N*-benzylethylenediamine. The 4-hydroxy-BZP and 4-hydroxy-methoxy-BZP metabolites are also excreted as glucuronic and/or sulfuric acid conjugates in urine.

BZP has been measured in 13 non-fatal (0.02 to 1.2 μg/g blood) and 1 fatal instance of ingestion (1.7 μg/g blood) [Wikstrom *et al.* 2004].

Note For further reading on the metabolism of BZP, see Staack *et al.* [2002]. BZP is a metabolite of the antidepressant drug piberaline [Olajos, Sztaniszlav 1986].

Toxicity There have been few published cases of fatal BZP toxicity. It stimulates the release of dopamine and noradrenaline and also inhibits dopamine, noradrenaline and serotonin uptake. It acts as a CNS stimulant, producing effects comparable to amfetamine [Maurer *et al.* 2004; Wikstrom *et al.* 2004].

Bishop S *et al.* (2005). Simultaneous separation of different types of amphetamine and piperazine designer drugs by capillary electrophoresis with a chiral selector. *J Forensic Sci* 50: 326–335.

deBoer D *et al.* (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Sci Int* 121: 47–56.

Maurer HH *et al.* (2004). Chemistry, pharmacology, toxicology, and hepatic metabolism of designer drugs of the amphetamine (ecstasy), piperazine, and pyrrolidinophenone types: a synopsis. *Ther Drug Monit* 26: 127–131.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Nordgren HK, Beck O (2004). Multicomponent screening for drugs of abuse: direct analysis of urine by LC-MS-MS. *Ther Drug Monit* 26: 90–97.

Nordgren H K *et al.* (2005). Application of direct urine LC-MS-MS analysis for screening of novel substances in drug abusers. *J Anal Toxicol* 29: 234–239.

Olajos S, Sztaniszlav D (1986). Gas chromatographic method for determination of a piperazine derivative (Trelibet) and its metabolites in human plasma and urine. *J Chromatogr* 378: 155–162.

Peters FT *et al.* (2003). Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom* 38: 659–676.

Staack RF *et al.* (2002). Studies on the metabolism and toxicological detection of the new designer drug N-benzylpiperazine in urine using gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 773: 35–46.

Tsutsumi H *et al.* (2005). Development of simultaneous gas chromatography-mass spectrometric and liquid chromatography–electrospray ionization mass spectrometric determination method for the new designer drugs, *N*-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and their main metabolites in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 315–322.

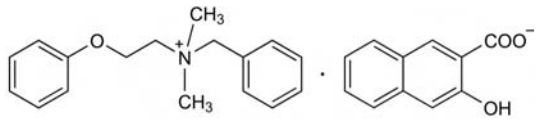
Tsutsumi H *et al.* (2006). Metabolism and the urinary excretion profile of the recently scheduled designer drug N-benzylpiperazine (BZP) in the rat. *J Anal Toxicol* 30: 38–43.

Wikstrom M *et al.* (2004). A2 (*N*-benzylpiperazine) a new drug of abuse in Sweden. *J Anal Toxicol* 28: 67–70.

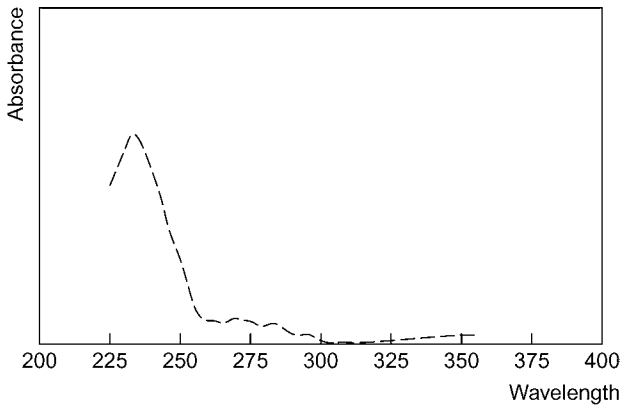
Bephenium Hydroxynaphthoate

Anthelmintic
$C_{28}H_{29}NO_4$ = 443.5
CAS—7181-73-9 (bephenium); 3818-50-6 (hydroxynaphthoate)

IUPAC Name *N*,*N*-Dimethyl-*N*-(2-phenoxyethyl)benzenemethanaminium 3-hydroxy-2-naphthoate
Synonym Naphthammonum
Proprietary Name *Alcopar(a)*



Chemical Properties A yellow to greenish-yellow crystalline powder, which gives a green fluorescence when examined under ultraviolet light. Mp 170° to 171°. Practically insoluble in water; soluble 1 in 50 of ethanol. Log *P* (octanol/water), 0.5.
Colour Tests Mandelin's test—green; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.77 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Methanol—234 (A^{1}_{1}=1300b), 263, 270, 283, 295, 352 nm.



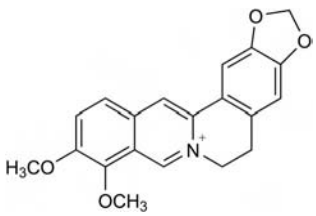
Infrared Spectrum Principal peaks at wavenumbers 1233, 768, 1653, 1590, 858, 726 cm^{-1} (KBr disk).
Quantification
Urine **Colorimetry** See Rogers [1958].
Disposition in the Body Bephenium hydroxynaphthoate is poorly absorbed after oral administration; less than 1% of a dose is excreted in the urine in 24 h.
Dose The equivalent of 2.5 g of bephenium, as a single dose.

Rogers EW (1958). Excretion of bephenium salts in urine of human volunteers. *BMJ* 2: 1576–1577.

Berberine

Alkaloid
$[C_{20}H_{18}NO_4]^+$ = 336.4
CAS—2086-83-1 ($C_{20}H_{18}NO^+_4$); 117-74-8 ($C_{20}H_{18}NO_4$·OH)

IUPAC Name 5,6-Dihydro-9,10-dimethoxybenzo[*g*]-1,3-benzodioxolo[5,6-*a*] quinolizinium



Chemical Properties A quaternary alkaloid present in *Hydrastis*, in various species of *Berberis*, and in many other plants. Yellow crystals. Mp 145°. Soluble 1 in 4.5 of water and 1 in 100 of ethanol; very slightly soluble in ether. Log *P* (octanol/water), 2.1.

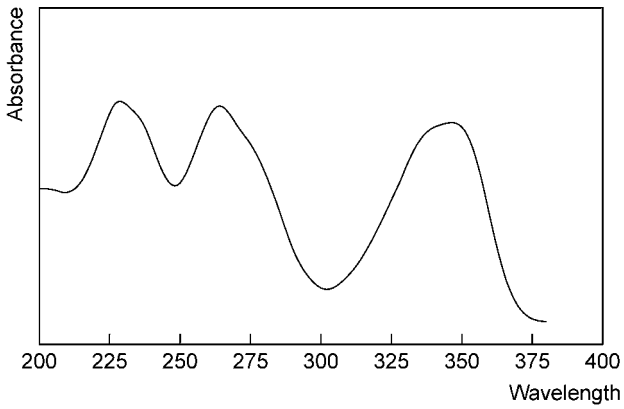
Berberine Hydrochloride

$C_{20}H_{18}ClNO_4,2H_2O$ = 407.8
CAS—633-65-8 (anhydrous); 5956-60-5 (dihydrate)
Synonym Berberine chloride
Chemical Properties Bright yellow acicular crystals or powder. Soluble 1 in 400 of water; freely soluble in boiling water; soluble in ethanol; practically insoluble in chloroform and ether.

Berberine Sulfate

$C_{20}H_{18}NO_4,HSO_4$ = 433.4
CAS—633-66-9
Synonyms Berberine acid sulfate; berberine bisulfate.
Chemical Properties Bright yellow acicular crystals or dark yellow powder. Soluble 1 in about 100 of water; slightly soluble in ethanol.

Colour Tests Mandelin's test—blue-green→brown; Marquis test—green.
Thin-layer Chromatography System TA—R_f 0.07; system TL—R_f 0.00; system TAE—R_f 0.04; system TAF—R_f 0.56; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.20 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2070.
High Performance Liquid Chromatography System HY—RI 327.
Ultraviolet Spectrum Aqueous acid—264 (A^{1}_{1}=554b), 345 nm.



Infrared Spectrum Principal peaks at wavenumbers 1505, 1271, 1234, 1030, 1587, 1098 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 321, 278, 320, 292, 306, 191, 322, 304.
Quantification
Plasma **HPLC** UV detection (λ=346 nm). Limit of detection, 0.4 ng [Zeng 1999]. Limit of detection, 18.1 µg/L [Chen, Chang 1995].
Urine **GC–MS** Limit of detection, 1 µg/L [Miyazaki *et al.* 1978].
HPLC Limit of detection, 2.3 µg/L [Chen, Chang 1995].
Bile **HPLC** Limit of detection, 90.4 µg/L [Chen, Chang 1995].
Disposition in the Body After oral administration, <0.1% of a dose is excreted in the urine unchanged in 24 h.
Dose Berberine sulfate has been given in doses of 150 mg daily.

Chen CM, Chang HC (1995). Determination of berberine in plasma, urine and bile by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 665: 117–123.
Miyazaki H *et al.* (1978). Quantitative analysis of berberine in urine samples by chemical ionization mass fragmentography. *J Chromatogr* 152: 79–86.
Zeng X (1999). Relationship between the clinical effects of berberine on severe congestive heart failure and its concentration in plasma studied by HPLC. *Biomed Chromatogr* 13(7): 442–444.

Beryllium

Metal
Be = 9.01
CAS—7440-41-7

Synonyms Beryllium-9; beryllium metallic; glucinium; glucinum.
Chemical Properties Grey solid. Mp 1287° to 1292°. Bp 2970°. Insoluble in water; soluble in dilute acid and alkali. Valencies: Be(0) and Be(+2). Naturally occurring element found in surface rocks at levels of <1 to 15 mg/kg. It is not found as the free metal because of its high reactivity. There are approx. 45 mineralised forms of beryllium, but the most important are beryl and bertrandite. Beryl is known as the gemstones: emerald, aquamarine and beryl. Used in aircraft disc brakes, X-ray transmission windows, space vehicle optics, aircraft/satellite structures, missile guidance systems, nuclear reactor neutron reflectors, nuclear warhead triggering devices, fuel containers, precision instruments, rocket propellants, navigational systems, heat shields, mirrors, computers.

Beryllium Chloride

$BeCl_2$ = 79.92
CAS—7787-47-5
Synonym Beryllium dichloride
Chemical Properties Colourless needles. Mp 405°. Bp 520°. Very soluble in water, alcohol, ether, pyridine; slightly soluble in benzene and chloroform. Used in the manufacture of beryllium and as an acid catalyst in organic reactions.

Beryllium Fluoride

BeF_2 = 47.01
CAS—7787-49-7
Synonym Beryllium difluoride

Chemical Properties Colourless glassy hygroscopic mass. Mp 555°. Bp 1175°. Very soluble in water; slightly soluble in alcohol. Used in the manufacture of beryllium, in beryllium alloys and glass, in nuclear reactors.

Beryllium Hydroxide

$Be(OH)_2 = 43.03$
CAS—13327-32-7
Synonyms Beryllium dihydroxide; beryllium hydrate.
Chemical Properties White amorphous powder or crystalline solid. Decomposes in water. Very slightly soluble in water; soluble in hot concentrated acid and alkali. Used in the manufacture of beryllium and beryllium oxide.

Beryllium Oxide

BeO = 25.01
CAS—1304-56-9
Synonyms Beryllia; beryllium monoxide.
Chemical Properties White light amorphous powder. Mp 2508° to 2547°. Bp 3787°. Very sparingly soluble in water; soluble in concentrated acids. Used in the manufacture of beryllium oxide ceramics and glass, in nuclear reactor fuels and moderators, as a catalyst for organic reactions.

Beryllium Nitrate

$Be(NO_3)_2 = 133.0$
CAS—13597-99-4 (anhydrous); 13510-48-0 (tetrahydrate)
Synonym Nitric acid, beryllium salt
Chemical Properties White crystals. Mp 60.5°. Bp 142° with decomposition. Very soluble in water, ethanol. Used in stiffening mantles in gas and acetylene lamps.

Quantification

Specimen collection Blood—10 mL, K-EDTA tube; urine—20 mL, plastic universal container; lung—for post-mortem examination of beryllium-related deaths.
Blood **ICP-MS** Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 0.3 µg/L [De Boer *et al.* 2004].
Urine **ETAAS** Dry cycle: 130° at 20 s for 10 s. Char cycle: 1400° in 5 s for 30 s. Atomisation cycle: 2500° for 5 s. Limit of detection, 0.02 µg/L [Horng *et al.* 2002]. Dry cycle: 80° to 120° for 30 s. Char cycle: 600° to 1200° for 45 s. Atomisation cycle: 288° for 4 s. Limit of detection, 0.37 µg/L [Wang *et al.* 2001].

FAAS Dry cycle: 180° at 5 s for 30 s. Char cycle: 1400° in 5 s for 5 s. Atomisation cycle: 2400° for 4 s. Limit of detection, 1.7 pg [Paschal, Bailey 1986]. Dry cycle: 100° for 50 s. Char cycle: 1000° for 60 s. Atomisation cycle: 2600° for 10 s. Ar, 50 mL/min ($\lambda = 235$ nm). Limit of quantification, 10 ng/L [Hurlbut 1978].

ICP-MS See Blood. Limit of detection, 0.2 µg/L [De Boer *et al.* 2004]. Limit of quantification, 0.03 µg/L [Apostoli, Schaller 2001].

ICP-MS-ETV Outer gas: 15 L/min. Intermediate gas: 0.9 L/min. Nebulizer gas: 0.7 L/min. Carrier gas: 0.4 L/min. Dry cycle: 60° in 4 s for 4 s. Vaporisation cycle: 900° for 4 s. Limit of detection, 0.072 ng/L [Xia *et al.* 2004].
Hair **ETAAS** Dry cycle: 80° to 120° for 30 s; Ar, 200 mL/min. Char cycle: 600° to 1400° for 30 s; Ar, 200 mL/min. Atomisation cycle: 2900° for 5 s (gas stop). Limit of detection, 2 µg/kg [Li, Kuo 2002].

FAAS Dry cycle: 100° for 50 s. Char cycle: 1000° for 60 s. Atomisation cycle: 2600° for 10 s. Ar, 50 mL/min ($\lambda = 235$ nm). Limit of quantification, <1 µg/kg [Hurlbut 1978].

ICP-MS Limit of detection, 0.13 mg/kg [Nadal *et al.* 2005].

ICP-MS-ETV Outer gas: 15 L/min. Intermediate gas: 0.9 L/min. Nebuliser gas: 0.7 L/min. Carrier gas: 0.4 L/min. Dry cycle: 60° in 4 s for 4 s. Vaporisation cycle: 900° for 4 s. Limit of detection, 0.072 ng/L [Xia *et al.* 2004].
Note For a spectroflurometric method for the determination of beryllium in mineral water and hair, see Jiang and He [2003].
Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000].
Faeces **FAAS** See Hair. Limit of quantification, 1 µg/L [Hurlbut 1978].
Other **FAAS** Industrial Dust. Limit of detection, 30 µmol/L [Rouleau *et al.* 2005].

ICP-MS Industrial Dust. Limit of detection, 5 nmol/L [Rouleau *et al.* 2005].
Note For an overview of the beryllium content of food and water, see Vaessen and Szteke [2000].
Disposition in the Body Approximately 90% of an ingested dose of beryllium is excreted via the kidneys over a period of 3 to 5 days and the remainder is deposited, mainly in the liver and bones. The metal or its insoluble salts, when inhaled, are deposited in the lungs and are only slowly absorbed and excreted. Workers exposed in this way may continue to excrete beryllium in the urine 10 years later. The biological half-life of beryllium is inversely proportional to the dose.
Normal Concentrations Blood—<1 µg/L (0.1 µmol/L); urine—<1 µg/L (0.1 µmol/L).
Toxicity Contact dermatitis has been reported after beryllium exposure. Inhalation can cause bronchitis and severe pneumonitis. Chronic exposure by inhalation leads to pulmonary granulomatous (berylliosis) with fatigue, weight loss, and increased risk of lung cancer [Aller 1990]. Beryllium levels >20 µg/kg in dried lung tissue indicate significant exposure [Sprince *et al.* 1976]. Beryllium concentrations in urine samples from 65 exposed workers ranged from 0.12 to 0.15 µg/L (13 to 17 nmol/L) compared with <0.063 µg/L (7 nmol/L) in control subjects [Apostoli, Schaller 2001].

A 27-year-old white woman worked as a dental technician for 3 years. She was referred to hospital with shortness of breath, weakness, nausea, vomiting, diarrhoea and weight loss. She was referred and sent home but 6 months later she was referred back. This time her chest radiograph showed an increased interstitial pattern with hilar lymphadenopathy. Her beryllium lymphocyte transformation test revealed values of 5.90 and 2.54 on the stimulation index at 10^{-4} mol/L (1 mmol/L) and 10^{-5} mol/L (0.1 mmol/L) of $BeSO_4$, respectively (normal values SI <1.7) [Fireman *et al.* 2001].

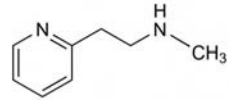
Concentrations of 0.9 ± 0.5 µg/kg were found in 20 people with no professional contact with beryllium. High concentrations of 2 µg/kg were measured in the urine of smokers of filterless cigarettes, the cigarettes themselves containing 120 ng [Stiefel *et al.* 1980].

Note For an investigation of toxic trace elements in the hair of children with autism, see Fido and Al Saad [2005]. For a thermodynamic model predicting the chemistry of beryllium in simulated biological fluids, see Sutton and Burastero [2003]. For biological monitoring of beryllium in workers in hazardous-waste incineration, see Schuhmacher *et al.* [2002] and Domingo *et al.* [2001]; in steel production workers, see Horng *et al.* [2002]. For a study on the suitability of urinary beryllium for assessing occupational exposure, see Apostoli and Schaller [2001]. For a mortality study of workers at a beryllium plant, see Ward *et al.* [1992].
Distribution in Blood 60–70% bound to serum proteins prealbumins and gammaglobulins.

Aller AJ (1990). The clinical significance of beryllium. *J Trace Elem Electrolytes Health Dis* 4: 1–6.
Apostoli P, Schaller KH (2001). Urinary beryllium: a suitable tool for assessing occupational and environmental beryllium exposure? *Int Arch Occup Environ Health* 74: 162–166.
De Boer JL *et al.* (2004). Practical and quality-control aspects of multi-element analysis with quadrupole ICP-MS with special attention to urine and whole blood. *Anal Bioanal Chem* 379: 872–880.
Domingo JL *et al.* (2001). Levels of metals and organic substances in blood and urine of workers at a new hazardous waste incinerator. *Int Arch Occup Environ Health* 74: 263–269.
Fido A, Al Saad S (2005). Toxic trace elements in the hair of children with autism. *Autism* 9: 290–298.
Fireman E *et al.* (2001). Beryllium disease: first case reported in Israel. *Isr Med Assoc J* 3: 224–225.
Horng CJ *et al.* (2002). Determination of urinary beryllium, arsenic, and selenium in steel production workers. *Biol Trace Elem Res* 88: 235–246.
Hurlbut JA (1978). Determination of beryllium in biological tissues and fluids by flameless atomic absorption spectroscopy. *Atomic Absorption Newslett* 17: 121–124.
Jiang C, He F (2003). Spectrofluorimetric determination of trace amounts of beryllium in mineral water and human's hair. *Spectrochim Acta A Mol Biomol Spectrosc* 59: 1321–1328.
Krachler M *et al.* (2000). Concentrations of trace elements in osteoarthritic knee-joint effusions. *Biol Trace Elem Res* 75: 253–263.
Li CC, Kuo MS (2002). Application of the acetylacetone chelation solid-phase extraction method to measurements of trace amounts of beryllium in human hair by GFAAS. *Anal Sci* 18: 607–609.
Nadal M *et al.* (2005). Monitoring metals in the population living in the vicinity of a hazardous waste incinerator: levels in hair of school children. *Biol Trace Elem Res* 104: 203–213.
Paschal DC, Bailey GG (1986). Determination of bryllium in urine with electrothermal atomic absorption using the L'vov platform and matrix modification. *Atomic Spectroscopy* 7: 1–3.
Rouleau M *et al.* (2005). Physical and chemical characterization of beryllium particles from several workplaces in Quebec, Canada. Part B: Time-of-flight secondary-ion mass spectroscopy. *J Toxicol Environ Health A* 68: 1907–1916.
Schuhmacher M *et al.* (2002). Biological monitoring of metals and organic substances in hazardous-waste incineration workers. *Int Arch Occup Environ Health* 75: 500–506.
Sprince NL *et al.* (1976). Current (1975) problem of differentiating between beryllium disease and sarcoidosis. *Ann N Y Acad Sci* 278: 654–664.
Stiefel T *et al.* (1980). Toxicokinetic and toxicodynamic studies of beryllium. *Arch Toxicol* 45: 81–92.
Sutton M, Burastero SR (2003). Beryllium chemical speciation in elemental human biological fluids. *Chem Res Toxicol* 16: 1145–1154.
Vaessen HA, Szteke B (2000). Beryllium in food and drinking water: a summary of available knowledge. *Food Addit Contam* 17: 149–159.
Wang HC *et al.* (2001). Determination of beryllium and selenium in human urine and of selenium in human serum by graphite-furnace atomic absorption spectrophotometry. *Anal Sci* 17: 527–532.
Ward E *et al.* (1992). A mortality study of workers at seven beryllium processing plants. *Am J Ind Med* 22: 885–904.
Xia L *et al.* (2004). Single-drop microextraction combined with low-temperature electrothermal vaporization ICPMS for the determination of trace Be, Co, Pd, and Cd in biological samples. *Anal Chem* 76: 2910–2915.

Betahistine

Vasodilator
$C_8H_{12}N_2 = 136.2$
CAS—5638-76-6
IUPAC Name *N*-Methyl-2-pyridine-ethanamine



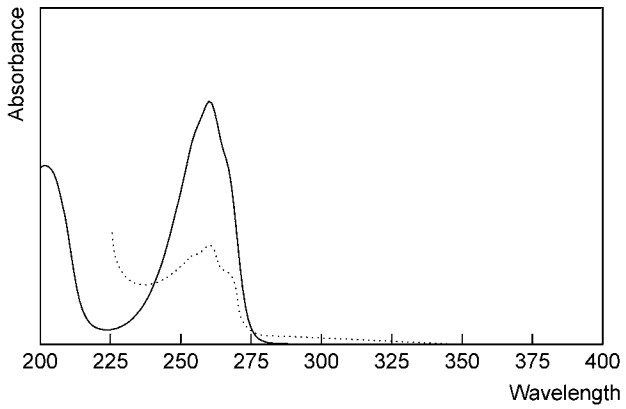
Chemical Properties A liquid. Bp 113° to 114°. Miscible with water, ethanol, chloroform and ether. pK_a 3.5, 9.7. Log *P* (octanol/water), 0.7.

Betahistine Hydrochloride

$C_8H_{12}N_2,2HCl = 209.1$
CAS—5579-84-0
Proprietary Names *Betaserc*; *Microser*; *Serc*; *Vasomotal*.
Chemical Properties A white or creamy-white, hygroscopic, crystalline powder. Mp about 152°. Freely soluble in water; soluble in ethanol and methanol; practically insoluble in carbon tetrachloride, chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.10 (acidified iodoplatinate solution, strong reaction).

Gas Chromatography System GA—RI 1235.
High Performance Liquid Chromatography System HA—*k* 3.1; system HAA—retention time 3.2 min.
Ultraviolet Spectrum Aqueous acid—261 nm (A_1^1=358b).

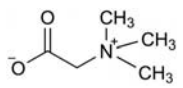


Infrared Spectrum Principal peaks at wavenumbers 1585, 1560, 1290, 762, 1238, 1145 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 79, 105, 104, 51, 78, 52, 50, 77.
Quantification
Serum **GC** FID. Limit of detection, 600 µg/L [Douglas, Hohing 1978].
Disposition in the Body Betahistine is readily absorbed after oral administration; peak plasma concentrations of the metabolites are attained in 3 to 5 h. Most of a dose is excreted in the urine as metabolites.
Dose 24 to 48 mg of betahistine hydrochloride daily.

Douglas JF, Hohing TL (1978). GLC determination of betahistine in serum. *Experientia* 34: 499–500.

Betaine

Treatment of Hypochlorhydria
$C_5H_{11}NO_2$ = 117.2
CAS—107-43-7
IUPAC Name 1-Carboxy-*N*,*N*,*N*-trimethylmethanaminium inner salt
Synonyms Glycine betaine; glycocoll betaine; lycine; oxyneurine; trimethylglycine.



Chemical Properties Deliquescent crystals. Mp about 310°, with decomposition. Soluble 1 in 0.6 of water and 1 in 11 of ethanol; sparingly soluble in ether; soluble 1 in 2 of methanol. pK_a 1.8 (25°). Log *P* (octanol/water), −4.9.

Betaine Hydrochloride

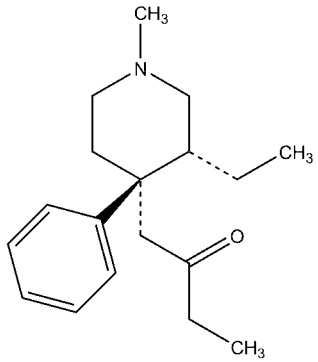
$C_5H_{11}NO_2$,HCl = 153.6
CAS—590-46-5
Proprietary Names It is an ingredient of *Acidol-Pepsin* and *Kloref*.
Chemical Properties Colourless crystals or white crystalline powder. When dissolved in water betaine hydrochloride hydrolyses and almost 25% of its weight of hydrochloric acid is formed. Mp about 230°, with decomposition. Soluble 1 in 2 of water and 1 in 20 of ethanol (90%); practically insoluble in chloroform and ether.

Colour Test To an aqueous solution add iodine solution—red precipitate.
Thin-layer Chromatography System TA—R_f 0.23; system TAE—R_f 0.16; system TAF—R_f 0.11 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1731, 1206, 885, 994, 902, 1250 cm^{-1} (KBr disk).
Quantification
Blood **HPLC** UV detection (λ=254 nm). Limit of detection, 5 µmol/L (=0.6 mg/L) [Laryea *et al.* 1998].
Urine **HPLC** See Blood [Laryea *et al.* 1998]. UV detection. Limit of detection, 0.9 µg [Laryea *et al.* 1994].
Dose 0.18 to 3.5 g of betaine hydrochloride daily.

Laryea MD *et al.* (1994). Simultaneous determination of betaine and N,N-dimethylglycine in urine. *Clin Chim Acta* 230(2): 169–175.
Laryea MD *et al.* (1998). Simple method for the routine determination of betaine and N,N-dimethylglycine in blood and urine. *Clin Chem* 44(9): 1937–1941.

Betameprodine

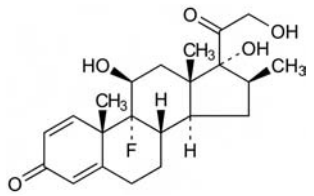
Narcotic
$C_{17}H_{25}NO_2$ = 275.4
CAS—468-51-9
IUPAC Name β-3-Ethyl-1-methyl-4-phenyl-4-propionyloxypiperidine
Synonym β-Meprodine



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—blue-grey→green (limit of detection 0.5 µg); ammonium vanadate test—blue-grey (limit of detection 0.5 µg); sulphuric acid-formaldehyde test—reddish-purple (limit of detection 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.64 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 0.92 relative to diphenhydramine; system G4—retention time 0.40 relative to diphenhydramine.

Betamethasone

Corticosteroid
$C_{22}H_{29}FO_5$ = 392.5
CAS—378-44-9
IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-3-one
Synonyms Flubenisolonum; 9α-fluoro-16β-methylprednis-olone; (11β,16β)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione; NSC-39470; Sch-4831.
Proprietary Names *Betnelan*; *Betnesol*; *Celeston(e)*; *Desacort-Beta*; *Minisone*; *No-Reumar*; *Pertene*.



Chemical Properties Betamethasone is a synthetic glucocorticoid and an isomer of dexamethasone. A white to creamy-white crystalline powder. Mp ≈240°, with decomposition. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 65 of ethanol, 1 in 15 of warm ethanol, and 1 in 325 of chloroform; very slightly soluble in ether; sparingly soluble in acetone and methanol. Log *P* (octanol), 1.9. Stable at ambient temperature for 5 h, in the autosampler for at least 24 h, after 3 freeze-thaw cycles and plasma samples were stable at −70° for 2 weeks [Zou *et al.* 2008].

Betamethasone Acetate

$C_{24}H_{31}FO_6$ = 434.5
CAS—987-24-6
Proprietary Name It is an ingredient of *Celestone Suluspan*.
Chemical Properties A white to creamy-white powder. Mp ≈165° and, with decomposition, 200° to 205–8°. Practically insoluble in water; soluble 1 in 9 of ethanol and 1 in 16 of chloroform; freely soluble in acetone.

Betamethasone Benzoate

$C_{29}H_{33}FO_6$ = 496.6
CAS—22298-29-9
Proprietary Names *Beben*; *Benisone*; *Uticort*.
Chemical Properties A white powder. Mp ≈225° to 228°, with decomposition. Practically insoluble in water; soluble in ethanol, chloroform, and methanol.

Betamethasone Dipropionate

$C_{28}H_{37}FO_7$ = 504.6
CAS—5593-20-4

Proprietary Names *Alphatrex*; *Diprolene*; *Diprosone*; *Maxivate*; *Psorion*; *Teladar.*
Chemical Properties A white or creamy-white powder. Mp 170° to: 179°, with decomposition. Practically insoluble in water; sparingly soluble in ethanol; freely soluble in acetone and chloroform.

B

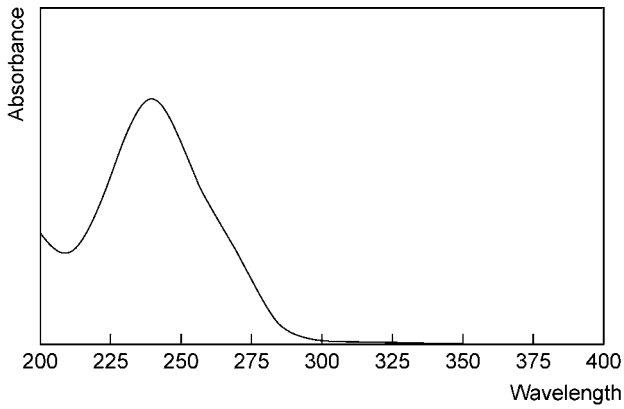
Betamethasone Sodium Phosphate

$C_{22}H_{28}FNa_2O_8P = 516.4$
CAS—151-73-5
Synonym Betamethasone disodium phosphate.
Proprietary Names *Bentelan*; *Betameson*; *Betnesol*; *B-S-P*; *Celeston(e)*; *Cel-U-Jec*; *Emilan*; *Paucisone*; *Selestoject*; *Vista-Methasone.*
Chemical Properties A white hygroscopic powder. Soluble 1 in 2 of water and 1 in 470 of dehydrated alcohol; freely soluble in methanol; practically insoluble in acetone, chloroform and ether.

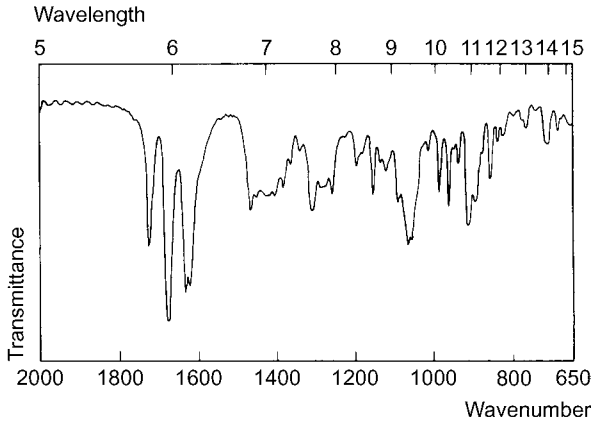
Betamethasone Valerate

$C_{27}H_{37}FO_6 = 476.6$
CAS—2152-44-5
Synonym 9α-Fluoro-16β-methylprednisolone 17-valerate.
Proprietary Names *Bedermin*; *Betacap*; *Betacort*; *Betaderm*; *Betatrex*; *Beta-Val*; *Betnelan-V*; *Betnesol-V*; *Betneval*; *Betnovat(e)*; *Bettamousse*; *Bextasol*; *Celestoderm (-V)*; *Celestone-V*; *Dermovaleas*; *Ecoval*; *Luxiq*; *Valisone.*
Chemical Properties A white to creamy-white powder. Mp ≈183° to 184°, with decomposition. Practically insoluble in water; soluble 1 in 12 to 1 in 16 of ethanol, 1 in <10 of chloroform, and 1 in 50 of isopropyl alcohol; freely soluble in acetone; slightly soluble in ether.
Note In alkaline media, betamethasone 17-valerate undergoes a rearrangement to betamethasone 21-valerate.

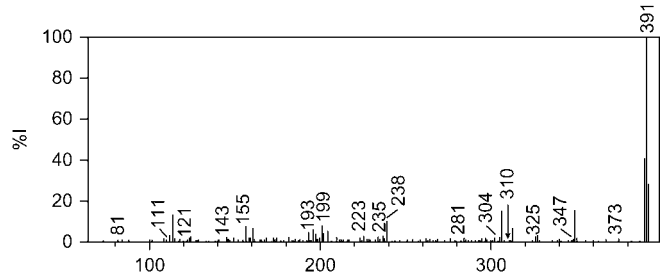
Colour Tests Antimony pentachloride—green→brown; naphthol–sulfuric acid—green-brown/orange-brown; sulfuric acid—pink-orange.
Thin-layer Chromatography System TP—betamethasone R_f 0.30, betamethasone valerate R_f 0.58; system TQ—betamethasone R_f 0.00, betamethasone valerate R_f 0.27; system TR—betamethasone R_f 0.00, betamethasone valerate R_f 0.20; system TS—betamethasone R_f 0.00, betamethasone valerate R_f 0.02, streaking may occur (DPST solution). Betamethasone sodium phosphate remains on the baseline in all systems.
High Performance Liquid Chromatography System HAA—RT 13.3 min; system HY—betamethasone valerate RI 584; system HZ—RT 14.2 min.
Ultraviolet Spectrum Betamethasone: ethanol—240 nm ($A^1_1 = 390a$). Betamethasone sodium phosphate: water—241 nm ($A^1_1 = 296a$).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1617, 1606, 1710, 1056, 907 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 121, 43, 122, 223, 147, 91, 41, 135.



Quantification

Plasma **LC-MS** Column: Venusil XBP-C_8 (200 × 3.9 mm i.d., 5 µm). Mobile phase: methanol : ammonium formate (80 : 20), flow rate 0.4 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 µg/L [Qu *et al.* 2008]. Column: Hanbon Lichrospher C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase methanol : water (85 : 15), flow rate 0.7 mL/min. ESI. Limit of quantification, 0.1 µg/L for betamethasone and 0.05 µg/L for betamethasone 17-monopropionate [Zou *et al.* 2008]. Column: C_{18} Genesis (100 × 2.1 mm i.d., 4 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (80 : 20), flow rate 350 µL/min. APPI, negative ion mode, MRM acquisition mode. Limit of quantification, 50 ng/L [Pereira Ados *et al.* 2005].
Serum **HPLC** Column: Kromasil C_{18} (150 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : water (70 : 30) with 0.1% hydrochloric acid, flow rate 0.9 mL/min. UV-vis detection (λ = 240 nm). Limit of detection, 20–50 µg/L [Quintana *et al.* 2004].
LC-MS Column: SYNERGI MAX-RP (50 × 4.6 mm i.d., 4 µm). Mobile phase: acetonitrile : 0.1 mmol/L ammonium acetate (29 : 71 for 9.5 min to 65 : 35 for 4 min to 29 : 71 for 0.5 min), flow rate 750 µL/min. TIS, positive ion mode, MRM acquisition mode. Limit of detection, 0.6–1.6 nmol/L for betamethasone and other corticosteroids [Taylor *et al.* 2004].
Urine **LC-MS** See Serum [Taylor *et al.* 2004]. Column: Chrompack microspher C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : water (50 : 50) containing 0.1 mol/L ammonium acetate or acetonitrile : water (37 : 63) containing 0.15 mol/L ammonium acetate or 0.05 mol/L ammonium acetate, flow rate 1.3 mL/min. SIM acquisition mode. Limit of quantification, 1 µg/L LC-LC-MS(-MS), limit of detection, 10 µg/L LC-MS(-MS) or LC-LC(-MS) , 0.2 µg/L LC-LC-MS(-MS) [Polettini *et al.* 1998].
Note For a comparison of ELISA, GC-MS and LC-MS for the analysis of corticosteroids, see Pujos *et al.* [2005].

Disposition in the Body

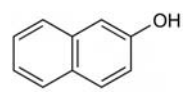
Therapeutic Concentration

Five healthy volunteers were administered 0.6 mg betamethasone orally and betamethasone 17-valerate as a topical application prepared as a suspension and applied to a 100 cm^2 area on the back for 28 h. The mean peak plasma concentrations were 5.0 and 0.24 mg/L, respectively [Kubota *et al.* 1994].
Dose 0.5 to 5 mg daily.

Kubota K *et al.* (1994). Plasma concentrations of betamethasone after topical application of betamethasone 17-valerate: comparison with oral administration. *Br J Clin Pharmacol* 37: 86–88.
Pereira Ados S *et al.* (2005). Quantification of betamethasone in human plasma by liquid chromatography–tandem mass spectrometry using atmospheric pressure photoionization in negative mode. *J Chromatogr B Analyt Technol Biomed Life Sci* 828: 27–32.
Polettini A *et al.* (1998). Development of a coupled-column liquid chromatographic-tandem mass spectrometric method for the direct determination of betamethasone in urine. *J Chromatogr B Biomed Sci Appl* 713: 339–352.
Pujos E *et al.* (2005). Comparison of the analysis of corticosteroids using different techniques. *Anal Bioanal Chem* 381: 244–254.
Qu TT *et al.* (2008). Determination of betamethasone in human plasma by liquid chromatography with tandem mass. *Yao Xue Xue Bao* 43: 402–407.
Quintana MC *et al.* (2004). Development of a solid phase extraction method for simultaneous determination of corticoids and tranquilizers in serum samples. *J Sep Sci* 27: 53–58.
Taylor RL *et al.* (2004). Quantitative, highly sensitive liquid chromatography–tandem mass spectrometry method for detection of synthetic corticosteroids. *Clin Chem* 50: 2345–2352.
Zou JJ *et al.* (2008). Determination of betamethasone and betamethasone 17-monopropionate in human plasma by liquid chromatography–positive/negative electrospray ionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 873: 159–164.

Betanaphthol

Parasiticide
$C_{10}H_8O = 144.2$
CAS—135-19-3
IUPAC Name 2-Naphthalenol
Synonyms β-Naftol; naphthol.



Chemical Properties White crystalline leaflets or powder; stable in air but darkens on exposure to light. Mp 121° to 123°. Bp 285° to 286°. Soluble 1 in 1000 of

cold water, 1 in 80 of boiling water, 1 in 0.8 of ethanol and 1 in 1.3 of ether; soluble in chloroform and in solutions of alkali hydroxides. pK_a 9.5 (25°). Log *P* (octanol/water), 2.7.

Colour Tests Ferric chloride—green; Liebermann's reagent—green. To 200 mg add 2 mL of an 8% sodium hydroxide solution and 1 drop of chloroform, then warm—blue.

Ultraviolet Spectrum Ethanol—274 (A_1^1=326b), 285 (A_1^1=230b), 322 (A_1^1=120b), 330 nm (A_1^1=140b).

Disposition in the Body Rapidly absorbed from the gastrointestinal tract and may be absorbed through intact skin. It is excreted mainly as the glucuronide and gives a reddish tint to the urine.

Toxicity The estimated minimum lethal dose is 2 g. Severe nephritis and fatalities have occurred following its absorption through intact skin.

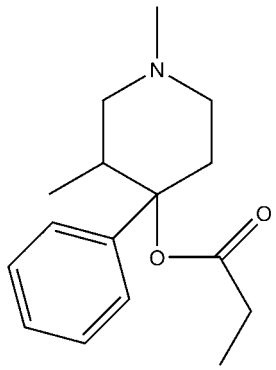
Betaprodine

Narcotic, Piperidine

$C_{16}H_{23}NO_2$ = 261.4

CAS—468-59-7

IUPAC Name β-1,3-Dimethyl-4-phenyl-4-propionyloxypiperidine



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 3.45 [Meylan, Howard 1995].

Betaprodine Hydrochloride

Synonym NU-1779

Chemical Properties Crystals from acetone : methanol. Mp 190° to 192°.

Colour Tests Ammonium molybdate test—blue-green→green with blue rim (limit of detection 0.5 µg); ammonium vanadate test—blue-grey (limit of detection 0.5 µg); sulphuric acid-formaldehyde test—reddish-purple (limit of detection 0.25 µg).

Thin-layer Chromatography System T1—R_f 0.61 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—retention time 0.84 relative to diphenhydramine; system G4—retention time 0.41 relative to diphenhydramine.

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—248, 253, 258, 264, 268 nm.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

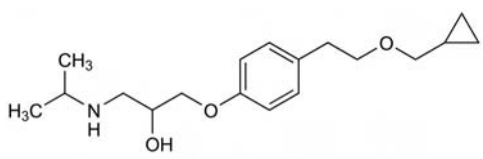
Betaxolol

β-*Blocker*

$C_{18}H_{29}NO_3$ = 307.4

CAS—63659-18-7

IUPAC Name 1-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl) amino]-2-propanol



Chemical Properties Crystals from petroleum ether. Mp 70° to 72°. pK_a 9.4. Log *P* (octanol/water), 2.81. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Betaxolol Hydrochloride

$C_{18}H_{29}NO_3$,HCl = 343.9

CAS—63659-19-8

Synonyms SLD-212; SL-75.212.

Proprietary Names *Betoptic*; *Betoptima*; *Betoquin*; *Kerlon*; *Kerlone*; *Oxodal*.

Chemical Properties A white or almost white crystalline powder. Mp 113° to 117°. It is very soluble to freely soluble in water; freely soluble in chloroform, ethanol and methanol; soluble in dichloromethane; practically insoluble in ether.

Thin-layer Chromatography System TB—R_f 0.11; system TE—R_f 0.51; system TF—R_f 0.00; system TAE—R_f 0.22.

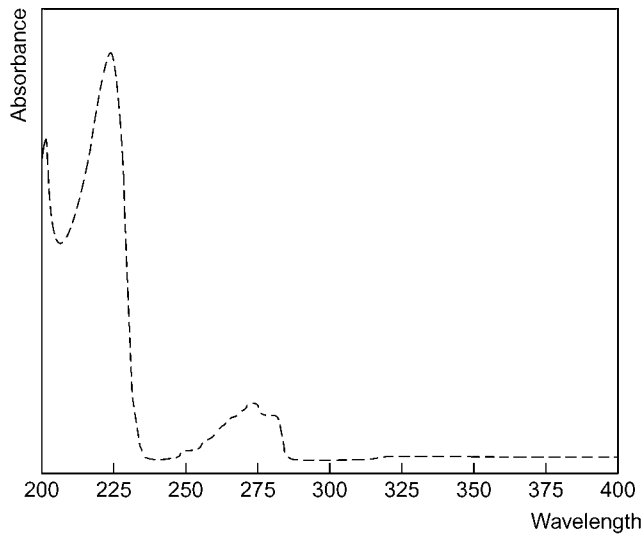
Gas Chromatography System GA—RI 2370 (betaxolol), RI 2410 (Art), RI 2400 (Art-H_2O); system GB—RI 2420 (betaxolol), RI 2508 (Art), RI 2519 (Art-H_2O).

Column: 5% phenyl-methyl silicone RTx-5 fused-silica capillary (30 m × 0.25 mm i.d.). Temperature programme: 110°, held for 1 min; increased to 170° at 20°/min; increased to 225° at 7°/min; 290° at 24°/min, held 10 min. Carrier gas: He. MS detection. Retention time (relative to isoproteronol, internal standard): 1:32. [Branum *et al.* 1998].

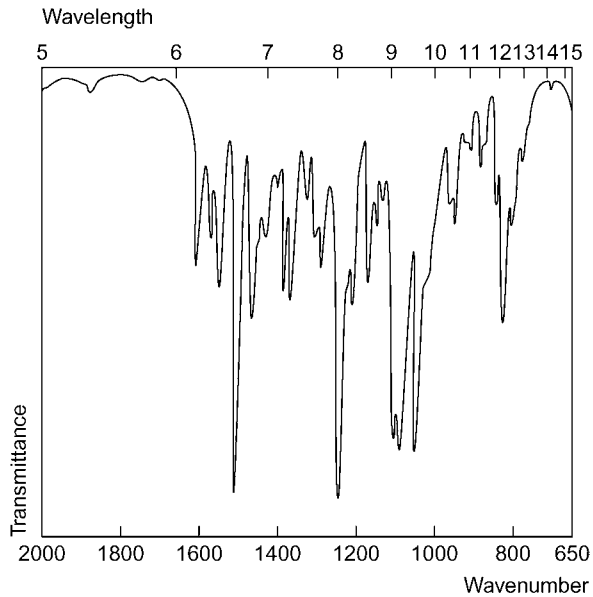
High Performance Liquid Chromatography System HX—RI 386; system HY—RI 301; system HZ—retention time 3.8 min; system HAA—retention time 13.4 min.

Column: C_{18} Ultrasphere (75 × 4.5 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.05 mol/L DMOA (pH 3) : water (8:10:82), 1 mL/min. Fluorescence detection (λ_{ex}=200 nm). Retention time: betaxolol, 9.9 min; α-hydroxybetaxolol, 2.3 min.

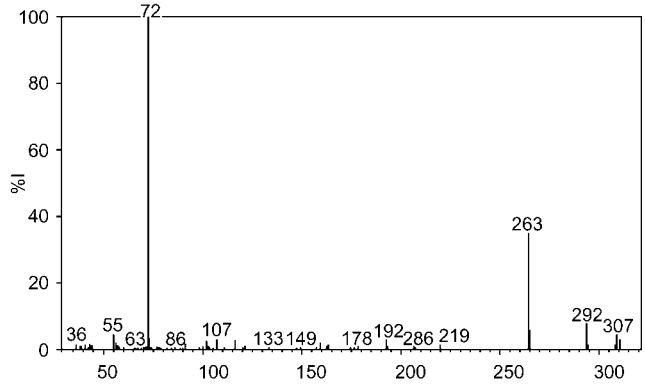
Ultraviolet Spectrum Ethanol—224, 280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1515, 1249, 1091, 1053, 3250, 830 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 72, 263, 292, 307, 55, 107, 192, 219 (betaxolol); *m/z* 55, 91, 316, 140, 125, 98, 43, 331 (betaxolol methaneboronate).



Quantification

Blood **GC** Limit of detection, 0.5 µg/L [Ganansia *et al.* 1983].

HPLC Column: C_{18} Nova-Pak (150 × 3.9 mm, 5 µm). Mobile phase: acetonitrile : glacial acetic acid : distilled water (29:1:70), 0.9 mL/min flow rate. Fluorescence detection (λ_{ex}=200 nm). Retention time: acid metabolites, 7.4 min. Limit of detection, 1 to 5 µg/L betaxolol, 5 to 20 µg/L betaxolol metabolites [Wong, Ludden 1990]. Fluorescence detection. Limit of detection 0.5 µg/L (separate enantiomers) [Darmon, Thenot 1986]. Fluorescence detection (λ_{ex}=275 nm, λ_{em}=305 nm). Limit of detection, 5 µg/L [Caqueret, Bianchetti 1984].

Plasma **HPLC** Fluorescence detection. Limit of detection, 5 µg/L [Bhamra *et al.* 1987]. Fluorescence detection (λ_{ex}=270 nm, λ_{em}=320 nm). Limit of detection, 4 µg/L [Canal, Flouvat 1985].

Serum **HPLC** See Plasma [Bhamra *et al.* 1987].

Urine **GC** See Blood [Ganansia *et al.* 1983].

HPLC See Blood [Wong, Ludden 1990]. See Blood [Caqueret, Bianchetti 1984].

Tissues **GC** See Blood [Ganansia *et al.* 1983].

Disposition in the Body Betaxolol is completely absorbed after oral administration. Administration with food does not affect the rate or extent of absorption. The primary route of elimination is hepatic metabolism and urinary excretion. About 15% of a dose is excreted in urine as the unchanged drug but the majority is oxidatively metabolised to inactive and weakly active metabolites, including α-hydroxybetaxolol. There are no active metabolites of clinical importance. It has high lipid solubility and crosses the placenta. It is distributed into breast milk where higher concentrations have been achieved than in maternal blood. It is not removed by dialysis.

Therapeutic Concentration The serum therapeutic concentration range is 5 to 50 µg/L.

Six healthy male and female volunteers, aged between 20 and 25 years, were administered with a 20-mg oral dose of betaxolol after a light breakfast and moderate exercise. The peak plasma concentrations were between 48 and 75 µg/L 2 to 4 h after administration. The drug could be measured up to 25 h after administration in all the subjects [Giudicelli *et al.* 1980].

Bioavailability Oral, 80 to 90%.

Half-life Plasma, 14 to 22 h.

Volume of Distribution 8 L/kg; reported as 4.9, 6.0 and 9.8 L/kg for single doses and 7.7 to 8.8 L/kg for repeated doses.

Clearance Plasma, 0.28 L/h/kg.

Protein Binding 55%.

Note For a review of betaxolol, see Beresford and Heel [1986].

Dose Usually 10 to 20 mg daily.

Beresford R, Heel RC (1986). Betaxolol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension. *Drugs* 31: 6–28.

Bhamra RK *et al.* (1987). Column liquid chromatographic measurement of betaxolol in plasma or serum. *J Chromatogr* 417: 229–232.

Branum GD *et al.* (1998). The feasibility of the detection and quantitation of beta-adrenergic blockers by solid-phase extraction and subsequent derivatization with methaneboronic acid. *J Anal Toxicol* 22: 135–141.

Canal M, Flouvat B (1985). Determination of betaxolol in plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 342: 212–215.

Caqueret H, Bianchetti. G (1984). Simple method for routine determination of betaxolol in blood and urine by automated high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 311: 199–205.

Darmon A, Thenot JP (1986). Determination of betaxolol enantiomers by high-performance liquid chromatography. Application to pharmacokinetic studies. *J Chromatogr* 374: 321–328.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Ganansia J *et al.* (1983). Improved determination of betaxolol in biological samples by capillary column gas chromatography. *J Chromatogr* 275: 183–188.

Giudicelli JF *et al.* (1980). Beta-adrenoceptor blocking effects and pharmacokinetics of betaxolol (SL 75212) in man. *Br J Clin Pharmacol* 10: 41–49.

Wong YW, Ludden TM (1990). Determination of betaxolol and its metabolites in blood and urine by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 534: 161–172.

Bethanechol Chloride

Parasympathomimetic

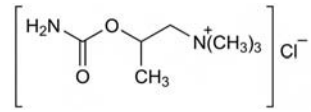
$C_7H_{17}ClN_2O_2$ = 196.7

CAS—674-38-4 (bethanechol); 590-63-6 (chloride)

IUPAC Name 2-[(Aminocarbonyl)oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium chloride

Synonym Carbamylmethylcholine chloride

Proprietary Names *Duvoid*; *Mechothane*; *Myotonachol*; *Myotonine Chloride*; *Urecholine*; *Urocarb*; *Vesicholine*.

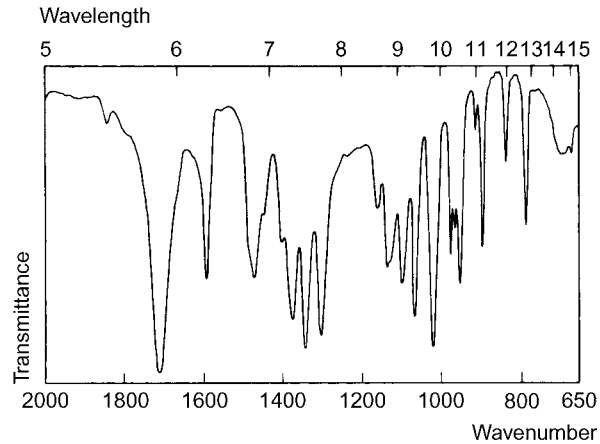


Chemical Properties Colourless or white hygroscopic crystals, or white crystalline powder. Mp 211°. Soluble 1 in 0.6 of water and 1 in 10 of ethanol, less soluble in dehydrated alcohol; practically insoluble in chloroform and ether. Log *P* (octanol/water), −3.4.

Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1712, 1014, 1300, 1062, 1094, 946 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 58, 42, 143, 85, 171, 157, 102.

Dose 15 to 200 mg daily.

Bethanidine

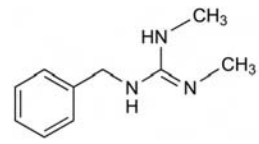
Antihypertensive

$C_{10}H_{15}N_3$ = 177.2

CAS—55-73-2

IUPAC Name *N*,*N*′-Dimethyl-*N*″-(phenylmethyl)guanidine

Synonym Betanidine



Chemical Properties Crystals. Mp 195° to 197°. pK_a 12 (20°). Log *P* (octanol/water), 1.6.

Bethanidine Sulfate

$(C_{10}H_{15}N_3)_2,H_2SO_4$ = 452.6

CAS—114-85-2

Proprietary Names *Esbaloid*; *Esbatal*; *Regulin*.

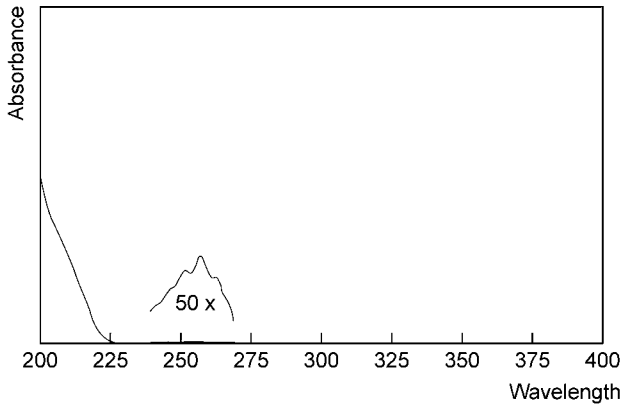
Chemical Properties A white crystalline powder. Mp about 280°. Soluble 1 in 1 of water and 1 in 30 of ethanol; practically insoluble in ether.

Colour Tests Liebermann's reagent—orange→brown; Marquis test—orange-red.

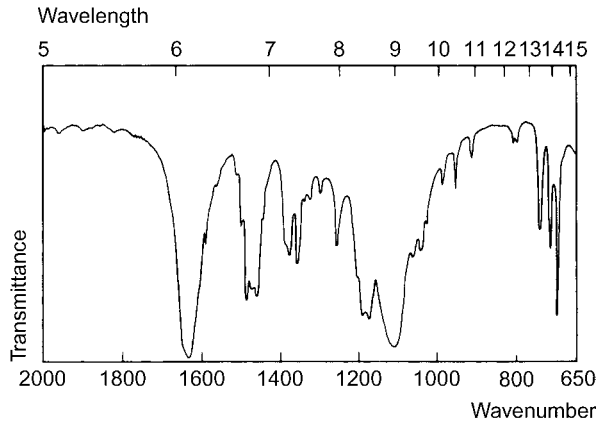
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00 (Dragendorff spray, positive).

Gas Chromatography System GA—RI 1925.

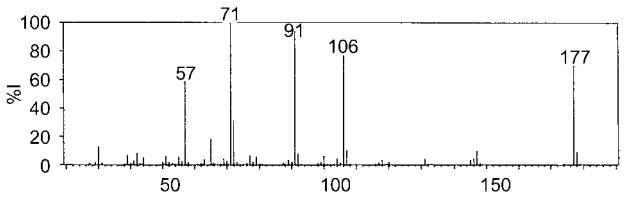
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=11a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1630, 1111, 1174, 701, 1192, 1066 cm^{-1} (bethanidine sulfate, KBr disk).



Mass Spectrum Principal ions at *m/z* 71, 91, 106, 177, 57, 72, 65, 30.



Quantification

Plasma **GC–MS** Limit of detection, 1 µg/L for guanethidine and other guanido-containing drugs [Hengstmann *et al.* 1974].

HPLC UV detection. Limit of detection, 20 µg/L [Shipe *et al.* 1983].

Spectrofluorimetry Limit of detection, 4 µg/L [Corder *et al.* 1975].

Urine **GC–MS** Limit of detection, 1 µg/L for guanethidine and other guanido-containing drugs [Hengstmann *et al.* 1974].

Disposition in the Body Bethanidine is readily but incompletely absorbed after oral administration. It does not appear to be metabolised. After an IV dose, about 90% is excreted unchanged in the urine in 3 to 4 days; after an oral dose, 50 to 85% is excreted in the urine and up to about 50% is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 0.02 to 0.5 mg/L. After a single oral dose of 10 mg to 3 subjects, peak plasma concentrations of about 0.02 to 0.15 mg/L were attained in 2 h [Corder *et al.* 1975].

After daily oral administration of 30 to 150 mg in divided doses to 12 subjects, steady-state plasma concentrations of 0.02 to 0.5 (mean 0.12) mg/L were reported [Corder *et al.* 1978].

Half-life The plasma half-life is multiphasic and there is considerable intersubject variation; after IV administration, plasma half-lives of about 2 to 6 h and terminal half-lives of several days have been reported.

Protein Binding Not significantly bound.

Dose 20 to 200 mg of bethanidine sulfate daily.

Corder CN *et al.* (1975). Fluorometric assay of bethanidine in plasma. *J Pharm Sci* 64: 785–788.

Corder CN *et al.* (1978). Bethanidine dose, plasma levels, and antihypertensive effects. *J Clin Pharmacol* 18: 249–258.

Hengstmann JH *et al.* (1974). Quantitative determination of guanethidine and other guanido-containing drugs in biological fluids by gas chromatography with flame ionization detection and multiple ion detection. *Anal Chem* 46: 34–39.

Shipe JR *et al.* (1983). Determination of bethanidine in plasma by liquid-chromatography with a microbore reversed-phase column. *Clin Chem* 29: 1793–1795.

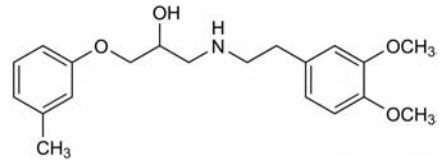
Bevantolol

β-Blocker

$C_{20}H_{27}NO_4$ = 345.4

CAS—59170-23-9

IUPAC Name 1-[[2-(3,4-Dimethoxyphenyl)ethyl]-amino]-3-(3-methylphenoxy)-2-propanol



Chemical Properties pK_a 8.1. Log *P* (octanol/water), 3.0; (pH 7), 13.0.

Bevantolol Hydrochloride

$C_{20}H_{28}ClNO_4$ = 381.9

CAS—42864-78-8

Synonyms CI-775; NC-1400.

Proprietary Names *Ranestol*; *Sentiloc*; *Vantol*.

Chemical Properties Mp 137° to 138°. Water solubility is 184.3 mg/L (25°).

High Performance Liquid Chromatography System HZ—retention time 4.2 min.

Column: Partisil RAC II (100 × 4.6 mm, 5 µm). Mobile phase: 75 mmol/L dibasic ammonium phosphate buffer (pH 3.5) : acetonitrile (50:50), flow rate 2.0 mL/min. UV detection (λ=220 nm). Retention time: (+)-bevantolol, 7.4 min; (−)-bevantolol, 6.4 min [Rose, Randinitis 1991].

Quantification

Plasma **HPLC** Column: ODS-2 Spherisorb (5 µm). Mobile phase: 0.5 mol/L phosphate buffer (pH 3.5) : acetonitrile : methanol (50:30:20). Fluorescence detection. Retention time: 3.0 min [Nattel *et al.* 1987]. Column: LC-CN Supelcosil (250 × 4.6 mm i.d. 5 µm). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 3.5) : acetonitrile : methanol (70:20:10), flow rate 1.0 mL/min. UV detection (λ=205 nm). Retention time: 9.6 min. Limit of detection, 0.05 mg/L. Limit of detection, 0.026 mg/L [Selen *et al.* 1986].

Disposition in the Body Bevantolol is virtually completely absorbed (>85%) and undergoes moderate first pass metabolism. It is extensively metabolised to 4 metabolites; the principal metabolite makes up 16% of the dose and 3 minor metabolites in trace amounts. It is excreted in urine (72%) mostly as metabolites and small amounts of the unchanged drug (<10%). It is also excreted in faeces (~12%).

Therapeutic Concentration

Twelve healthy volunteers (11 women and 1 man) with a mean age of 37 years, were administered with a single 100-mg dose of bevantolol in a fasting state, 15 min before a meal or after a meal. The mean peak plasma concentrations observed were 1.05, 1.06 and 0.92 mg/L, respectively, and these levels were reached at ~1.0, 0.9 and 1.8 h [Toothaker *et al.* 1987].

Bioavailability 60%.

Half-life 1.5 to 2 h.

Volume of Distribution Approximately, 1.5 L/kg.

Protein Binding 95 to 98% bound especially to plasma glycoproteins.

Dose The usual dose of 150 to 400 mg is administered daily.

Nattel S *et al.* (1987). Bevantolol disposition in patients with hepatic cirrhosis. *J Clin Pharmacol* 27: 962–966.

Rose SE, Randinitis EJ (1991). A high-performance liquid chromatographic assay for the enantiomers of bevantolol in human plasma. *Pharm Res* 8(6): 758–762.

Selen A *et al.* (1986). Comparative single dose and steady-state pharmacokinetics of bevantolol in young and elderly subjects. *Eur J Clin Pharmacol* 30: 699–704.

Toothaker RD *et al.* (1987). The influence of food on the oral absorption of bevantolol. *J Clin Pharmacol* 27: 297–299.

Bezafibrate

Lipid-Regulating Agent

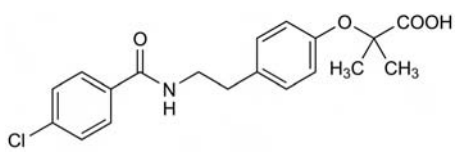
$C_{19}H_{20}ClNO_4$ = 361.8

CAS—41859-67-0

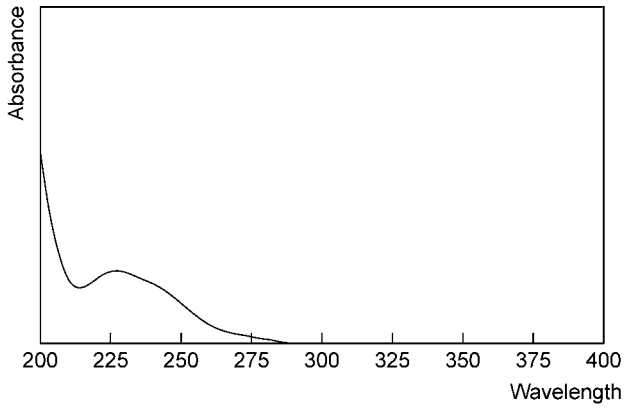
IUPAC Name 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid

Synonym BM-15075

Proprietary Names *Bezalip*; *Cedur*.



Chemical Properties An off-white crystalline powder. Mp 183° to 186°. Practically insoluble in water; slightly soluble in ethanol, acetone and methanol; soluble in dimethylformamide. Log *P* (octanol/water), 4.2.
Thin-layer Chromatography System TF—R_f 0.00; system TAE—R_f 0.89.
Gas Chromatography System GA—RI 3100.
High Performance Liquid Chromatography System HX—RI 513; system HAA—retention time 18.3 min.
Ultraviolet Spectrum Methanol—230 nm (A^1_1=644b).



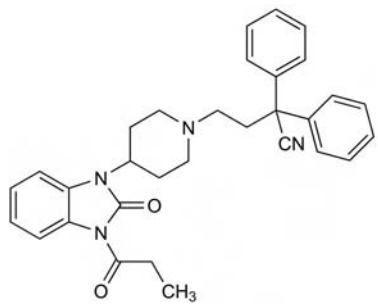
Infrared Spectrum Principal peaks at wavenumbers 1715, 1540, 1610, 1140, 1500, 1220 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** UV detection (λ=232 nm). Limit of quantification, 0.25 mg/L [Masnatta *et al.* 1996].
Dose 400 to 600 mg daily.

Masnatta LD *et al.* (1996). Determination of bezafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 687(2): 437–442.

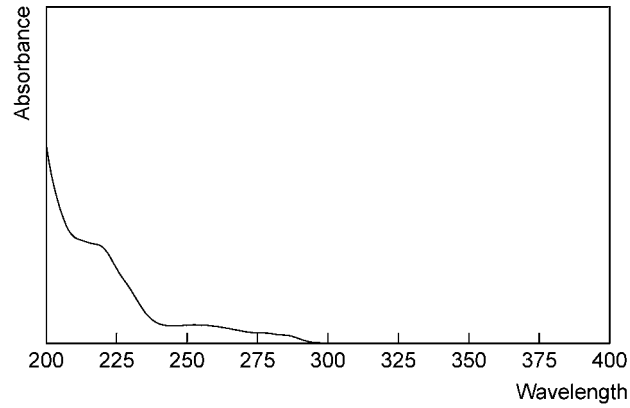
Bezitramide

Narcotic Analgesic
$C_{31}H_{32}N_4O_2$ = 492.6
CAS—15301-48-1

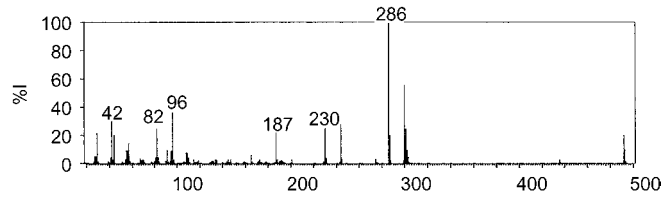
IUPAC Name 1-[1-(3-Cyano-3,3-diphenylpropyl)-4-piperidinyl]-1,3-dihydro-3-(1-oxopropyl)-2*H*-benzimidazol-2-one
Synonyms Benzitramide; R-4845.
Proprietary Name *Burgodin*



Chemical Properties A white, amorphous or crystalline powder. Mp 145° to 149°. Practically insoluble in water and ethanol; soluble 1 in 45 of acetone, 1 in 2 of chloroform, and 1 in 400 of ether. Log *P* (octanol/water), 4.8.
Colour Test Mandelin's test—violet→orange.
Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.41; system TC—R_f 0.79; system TL—R_f 0.70; system TAE—R_f 0.92; system TAF—R_f 0.96 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 0.2; system HX—RI 564; system HZ—retention time 22.5 min.
Ultraviolet Spectrum After solution in chloroform and dilution with acid isopropyl alcohol—251 nm (A^1_1=118b), inflexion at 274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1733, 755, 1708, 700, 1286, 1054 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 286, 300, 96, 42, 244, 230, 301, 82.



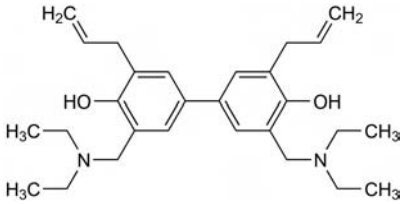
Quantification
Blood **LC-MS**. Column: Hypersil ODS (5 microm). Mobile phase: 1.0 mmol/L ammonium acetate: methanol- acetonitrile (50:50): 1.0 mmol/L ammonium acetate (80:20). ESI, SRM acquisition mode. Limit of detection, 1 microg/L [De Baere *et al.* 1999].
HPLC Fluorescence detection. Limit of detection, 1 µg/L [De Baere *et al.* 1997].
Urine **GC** NPD. Limit of quantification, 10 µg/L [De Baere *et al.* 1998]. NPD. Limit of quantification, 10 µg/L for acidic metabolite [De Baere *et al.* 1996]. AFID. Limit of detection, 600 µg/L for 3-cyano-3,3-diphenylpropionic acid [van Rooy *et al.* 1978].
LC-MS See Blood [De Baere *et al.* 1999].
HPLC Fluorescence detection. Limit of detection, 1 µg/L [De Baere *et al.* 1997]. UV detection. Limit of detection, 150 µg/L for 1,3-dihydro-1-(piperidin-4-yl)benzimidazol-2-one [van Rooy, Soe-Agnie 1978].
Bile **LC-MS** See Blood [De Baere *et al.* 1999].
Kidneys **LC-MS** See Blood [De Baere *et al.* 1999].
Liver **LC-MS** See Blood [De Baere *et al.* 1999].
Stomach contents **LC-MS** See Blood [De Baere *et al.* 1999].
Disposition in the Body Absorbed after oral administration. It is metabolised by hydrolysis to 3-cyano-3,3-diphenylpropionic acid and 1,3-dihydro-1-(piperidin-4-yl)benzimidazol-2-one which are excreted in the urine.
Therapeutic Concentration
Following a single oral dose of 5 mg to 7 subjects, peak plasma concentrations (bezitramide plus major metabolite) of about 5 µg/L were attained in about 3 h; in 3 of the subjects, plasma concentrations remained fairly constant over a period of 7 h; in a further 2 subjects, distinct secondary peak plasma concentrations were observed [Meijer *et al.* 1984].
Dose Bezitramide has been given in doses of 5 mg; maximum of 30 mg in 24 h.

De Baere SM *et al.* (1996). Quantitative gas chromatographic analysis of 3-cyano-3,3-diphenylpropionic acid, the acidic metabolite of bezitramide (Burgodin), in urine. *J Anal Toxicol* 20(3): 159–164.
De Baere SM *et al.* (1997). Quantitative analysis of despropionyl-bezitramide, the active metabolite of bezitramide (Burgodin), in biological samples by high-performance liquid chromatography with fluorescence detection. *Anal Chem* 69(24): 5186–5192.
De Baere SM *et al.* (1998). Quantitative gas chromatographic analysis of [1-(4-piperidinyl)-1,3-dihydro-2H-benzimidazole-2-one], the basic metabolite of bezitramide (Burgodin), in human urine. *J Anal Toxicol* 22(1): 18–26.
De Baere SM *et al.* (1999). Identification and quantitation of despropionyl-bezitramide in post-mortem samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. *Anal Chem* 71(14): 2908–2914.
Meijer DK *et al.* (1984). Pharmacokinetics of the oral narcotic analgesic bezitramide and preliminary observations on its effect on experimentally induced pain. *Eur J Clin Pharmacol* 27: 615–618.
van Rooy HH, Soe-Agnie C (1978a). Determination of the metabolites of bezitramide in urine. II. The basic metabolite. *J Chromatogr* 156: 189–195.
van Rooy HH *et al.* (1978b). Determination of the metabolites of bezitramide in urine. I. Acidic metabolite. *J Chromatogr* 148: 447–452.

Bialamicol

Antiamoebic
$C_{28}H_{40}N_2O_2$ = 436.6
CAS—493-75-4

IUPAC Name 3,3′-Bis[(diethylamino)methyl]-5,5′-di-2-propenyl-[1,1′-biphenyl]-4,4′-diol
Synonyms Biallylamicol; biethylamicol.

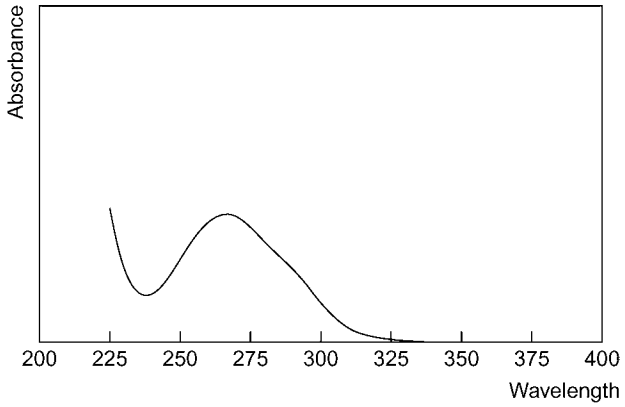


Chemical Properties Log *P* (octanol/water), 7.0.

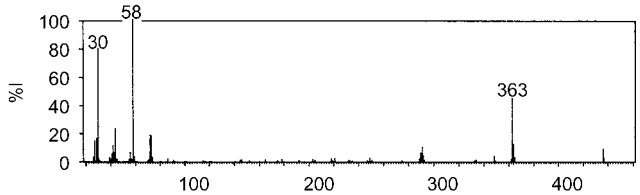
Bialamicol Hydrochloride

$C_{28}H_{40}N_2O_2$,2HCl = 509.6
CAS—3624-96-2
Proprietary Name *Camoform*
Chemical Properties A white crystalline powder. Mp 209° to 210°. Soluble 1 in 5 of water, 1 in 40 of ethanol and 1 in 150 of chloroform; slightly soluble in ether.

Colour Tests Folin-Ciocalteu reagent—blue; Liebermann's reagent—green. Dissolve 100 mg in 2 mL of water and add 2 mL of nitric acid—intense orange-red.
Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.62; system TC—R_f 0.80; system TL—R_f 0.73.
Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1=440a).



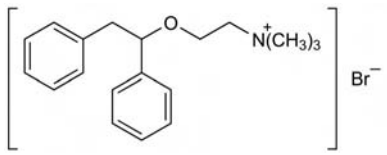
Mass Spectrum Principal ions at *m/z* 58, 30, 363, 44, 72, 29, 27, 364.



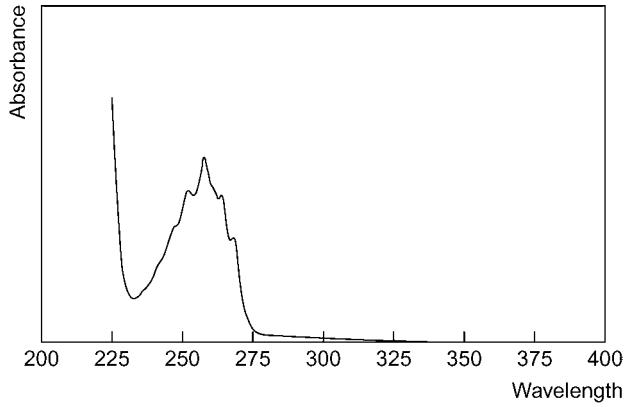
Disposition in the Body Bialamicol is rapidly absorbed from the gastrointestinal tract and stored in high concentrations in the liver, lungs and tissues. Slowly excreted in the bile and eliminated in the faeces.
Dose Bialamicol hydrochloride has been given in doses of 0.75 to 1.5 g daily.

Bibenzonium Bromide

Cough Suppressant
$C_{19}H_{26}BrNO$ = 364.3
CAS—59866-76-1 (bibenzonium); 15585-70-3 (bromide)
IUPAC Name 2-(1,2-Diphenylethoxy)-*N*,*N*,*N*-trimethylethanaminium bromide
Synonym Diphenetholine bromide
Proprietary Name *Lysobex*, *Medipectol*, *Thoragol*.



Chemical Properties Crystals. Mp 142° to 144°. Very soluble in water and ethanol; practically insoluble in benzene and in ether. Log *P* (octanol/water), 0.6.
Colour Tests The following tests are performed on bibenzonium nitrate: Mandelin's test—green; Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1923.
Ultraviolet Spectrum Aqueous acid—253, 258 (A^1_1=11.4b), 264, 268 nm.

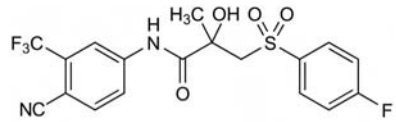


Infrared Spectrum Principal peaks at wavenumbers 705, 1095, 749, 759, 1020, 952 cm^{-1} (KBr disk).
Dose 40 to 120 mg daily.

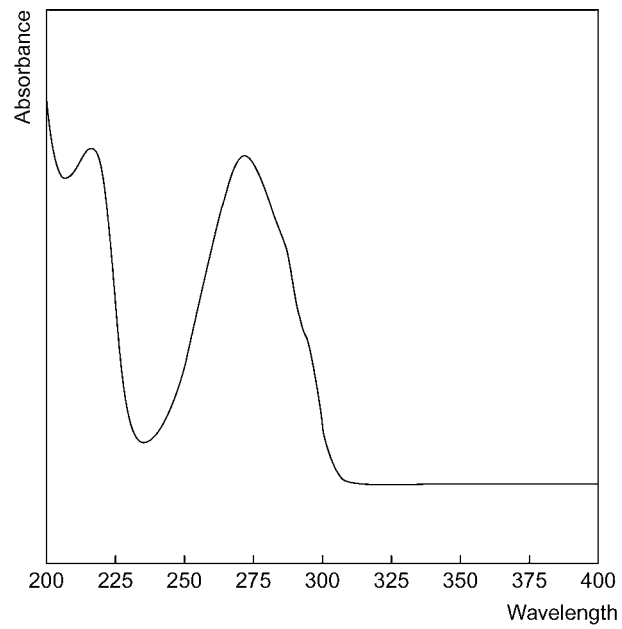
Bicalutamide

Antiandrogen, Antineoplastic
$C_{18}H_{14}F_4N_2O_4S$ = 430.4
CAS—90357-06-5

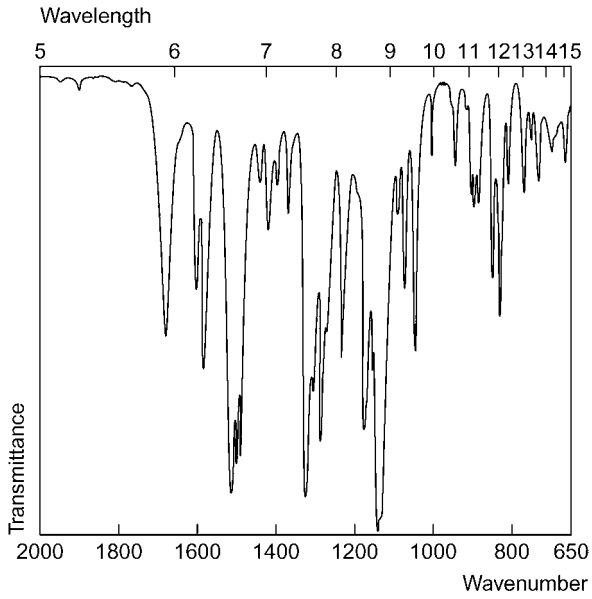
IUPAC Name (*RS*)-*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide
Synonym ICI-176334
Proprietary Names *Casodex*; *Cosudex*.



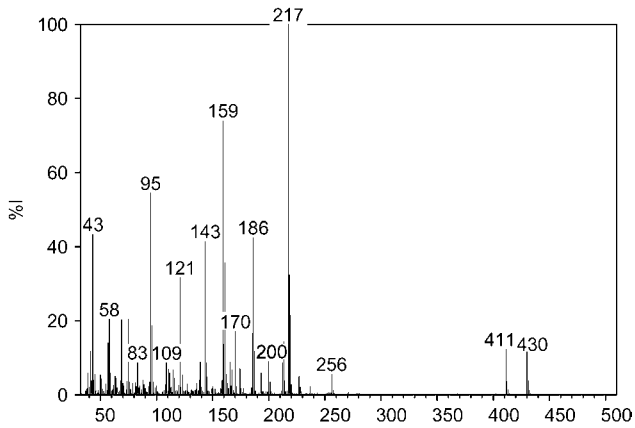
Chemical Properties A fine white to off-white powder. Mp 191° to 193°. It is practically insoluble in water (solubility in aqueous solution is 5 mg/L); slightly soluble in chloroform and anhydrous ethanol; sparingly soluble in methanol; soluble in acetone and in tetrahydrofuran. pK_a 12.
Ultraviolet Spectrum Aqueous acid (pH 4.0)—217, 272 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 217, 159, 95, 43, 186, 143, 160, 218.



Quantification

Plasma **HPLC** Limit of detection, 5 µg/L for (*R*)-bicalutamide, 3.8 µg/L for (*S*)-bicalutamide [Cockshott *et al.* 1997]. UV detection (λ=270 nm). Limit of quantification, 7.9 µg/L for (*R*)-bicalutamide, 7.6 µg/L for (*S*)-bicalutamide [McKillop *et al.* 1993].

Disposition in the Body Bicalutamide is extensively absorbed after oral administration and undergoes extensive metabolism in the liver; the active *R*-enantiomer is metabolised predominantly by oxidation, the inactive *S*-enantiomer primarily by glucuronidation. The drug is rapidly cleared and the metabolites are excreted in approximately equal amounts in urine and faeces. Approximately half of an administered dose is excreted in urine as the glucuronide conjugates of the drug and its metabolite, hydroxy-bicalutamide. The remainder is detected in faeces as the drug and metabolite. Bicalutamide is not removed in significant amounts by dialysis.

Therapeutic Concentration

Fifteen healthy males, aged 36 to 54 years (mean, 44 years), were administered with a 50 mg single dose after an overnight fast and a standard high-fat cooked breakfast. The mean peak plasma (*R*)-enantiomer concentrations were 836 µg/L for those administered the drug with food and 734 µg/L for those in the fasting state. These concentrations were observed at 15.8 and 23.4 h, respectively. For the (*S*)-enantiomer, the peak concentrations were 100.2 µg/L at 20.8 h for the fed individuals and 84 µg/L for those who were being fasted, observed at 20.7 h [Cockshott *et al.* 1997].

Toxicity Hepatic failure has occurred rarely and consideration should be given to periodic liver function monitoring.

Half-life Plasma, *R*-enantiomer, 5.8 days to 1 week; the *S*-enantiomer is cleared more rapidly.

Clearance Plasma, 0.32 L/h (*R*-enantiomer).

Protein Binding Racemate, 96%; *R*-enantiomer, >99%.

Note For a review of the pharmacokinetics of bicalutamide, see Mahler *et al.* [1998].

Dose Up to 150 mg daily.

Cockshott ID *et al.* (1997). The effect of food on the pharmacokinetics of the bicalutamide ('Casodex') enantiomers. *Biopharm Drug Dispos* 18(6): 499–507.

Mahler C *et al.* (1998). Clinical pharmacokinetics of the antiandrogens and their efficacy in prostate cancer. *Clin Pharmacokinet* 34: 405–417.

McKillop D *et al.* (1993). Metabolism and enantioselective pharmacokinetics of Casodex in man. *Xenobiotica* 23(11): 1241–1253.

Biperiden

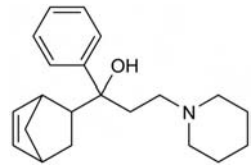
Anticholinergic

$C_{21}H_{29}NO$ = 311.5

CAS—514-65-8

IUPAC Name α-Bicyclo[2.2.1]hept-5-en-2-yl-α-phenyl-1-piperidinepropanol

Proprietary Name *Roloken*



Chemical Properties A white crystalline powder. Mp 101°. Practically insoluble in water; slightly soluble in ethanol; readily soluble in methanol. Log *P* (octanol/water), 4.25. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Biperiden Hydrochloride

$C_{21}H_{29}NO,HCl$ = 347.9

CAS—1235-82-1

Proprietary Names *Akineton (tablets)*; *Akinophyl*; *Dekinet*; *Ipsatol*; *Norakin N*.

Chemical Properties A white crystalline powder. Mp 238°. Slightly soluble in water, ethanol, chloroform, and ether; sparingly soluble in methanol.

Biperiden Lactate

$C_{21}H_{29}NO,C_3H_6O_3$ = 401.5

CAS—7085-45-2

Proprietary Names *Akineton (injection)*; *Dekinet*; *Ipsatol*.

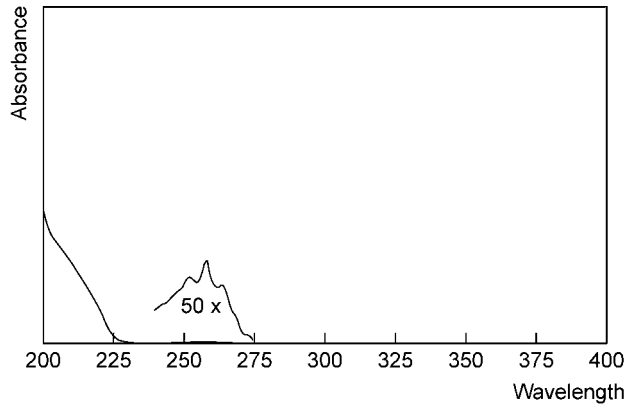
Colour Tests Liebermann's reagent—brown; Marquis test—red-brown; sulfuric acid—orange-brown.

Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.68; system TC—R_f 0.64; system TE—R_f 0.83; system TL—R_f 0.64; system TAE—R_f 0.45; system TAJ—R_f 0.37; system TAK—R_f 0.12; system TAL—R_f 0.73 (acidified iodoplatinate solution, positive).

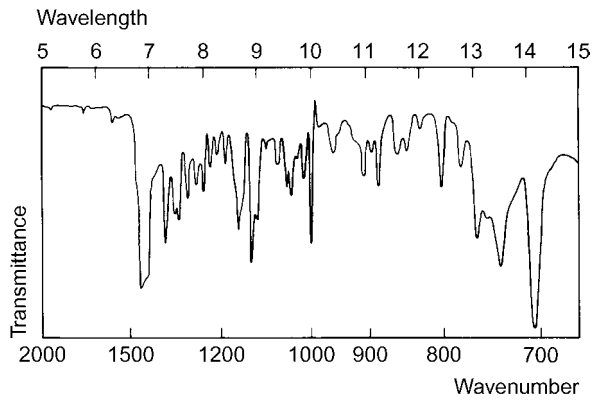
Gas Chromatography System GA—biperiden RI 2276, M (OH-) RI 2645.

High Performance Liquid Chromatography System HZ—retention time 6.4 min; system HAA—retention time 14.8 min.

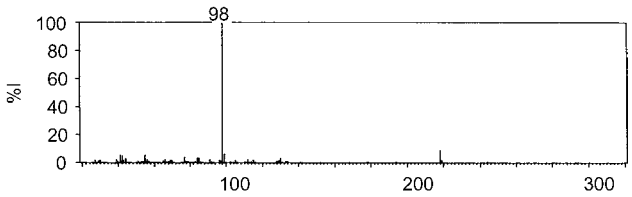
Ultraviolet Spectrum Aqueous acid—252, 259 (A^1_1=5.6b), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 702, 735, 1121, 997, 760, 1153 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 98, 218, 99, 55, 41, 42, 77, 84.

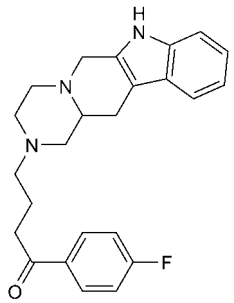


Quantification
Plasma **GC** AFID. Limit of quantification, 250 ng/L [Le Bris, Brode 1985].
Disposition in the Body Rapidly absorbed after oral administration.
Therapeutic Concentration
Following a single oral dose of 4 mg to 6 subjects, peak plasma concentrations of 4 to 6 µg/L were attained in 1.5 h [Hollman *et al.* 1984].
Toxicity For a summary of fatalities caused by anticholinergics in Norway, see Gjerden *et al.* [1998].
Bioavailability About 30%.
Half-life Plasma half-life, about 18 h.
Clearance 146 L/h (oral).
Dose 2 to 12 mg of biperiden hydrochloride daily, by mouth. Biperiden lactate is given parenterally in doses of 5 to 20 mg daily.

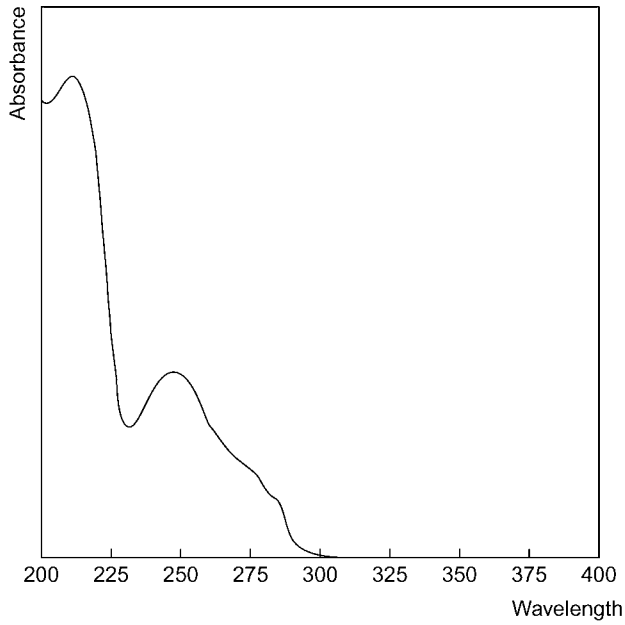
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Gjerden P *et al.* (1998). [Fatalities caused by anticholinergic antiparkinsonian drugs. Analysis of findings in a 11-year national material]. *Tidsskr Nor Laegeforen* 118: 42–44.
Hollmann M *et al.* (1984). Biperiden effects and plasma levels in volunteers. *Eur J Clin Pharmacol* 27: 619–621.
Le Bris T, Brode E (1985). Capillary gas chromatographic determination of biperiden in human plasma. *Arzneimittelforschung* 35: 149–151.

Biriperone

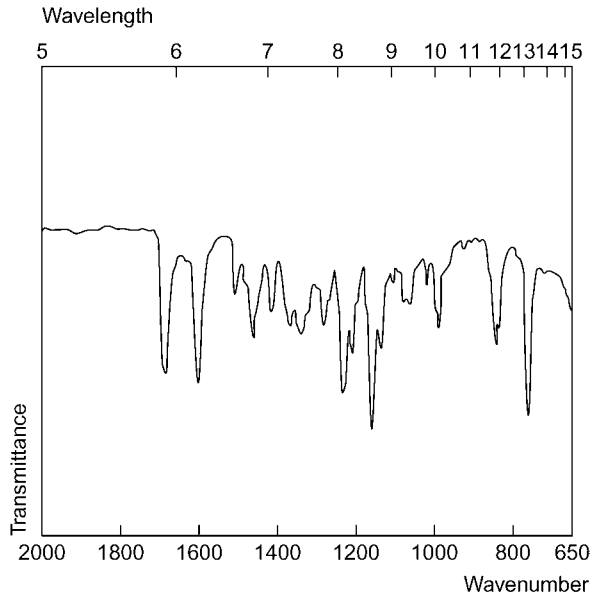
Antipsychotic
$C_{24}H_{26}FN_3O = 391.5$
CAS—41510-23-0
Synonyms Centbutindole; 2-γ-[(*p*-fluorobenzoyl)propyl]-1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrido[3,4-b]indole.
Proprietary Names *Biriperona*; *Biriperonum*.



Chemical Properties Faint-yellow, odourless powder. Mp 184° to 186°. Soluble in methanol, ethanol, isopropanol, *n*-butanol, acetone and dilute acids; practically insoluble in *n*-hexane, cyclohexane, propylene glycol and glycerol.
Thin-layer Chromatography Plate: neutral alumina (20% Gypsum) (6 g) (5 × 20 cm). Mobile phase: chloroform : methanol (99 : 1). Detection by exposure to iodine vapour or by spraying with Dragendorff reagent. R_f 0.48.
Plates: silica gel GF_{254} (4G) (10 × 20 cm). Mobile phase: chloroform : methanol (19 : 1). Detection as above. R_f 0.25 [Seth, Sarin 1989].
High Performance Liquid Chromatography Column: Nucleosil Cyano (200 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate : 0.3 mmol/L orthophosphoric acid (pH 2.3; 20 : 70 : 10), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 6.6 min. Limit of detection, 0.4 mg/L [Paliwal *et al.* 1990].
Ultraviolet Spectrum Aqueous acid (0.1 N HCl)—215, 250, 286 nm.



Infrared Spectrum Principal peaks at wavenumber 1170, 770, 1290, 1620, 1700, 2850 cm^{-1}.



Quantification
Serum **HPLC** Column: Cyano (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogen phosphate (60 : 40). Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 350 nm). Retention time: ≈10 min. Limit of detection, 0.25 µg/L [Paliwal *et al.* 1992]. Column: Cyano (10 cm × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogenphosphate (pH 3.0; 60 : 40) flow rate 1.0 ml/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 350 nm). Retention time: biriperone, 10 min and hydroxy-metabolite, 8.6 min. Limit of quantification, 0.25 and 0.05 µg/L for biriperone and its hydroxy-metabolite, respectively, limit of detection, 0.1 and 0.02 µg/L for biriperone and its hydroxy-metabolite, respectively [Paliwal *et al.* 1991].
Other **HPLC** Rat Serum. Column: Spheri-5 C_{18} reversed phase (220 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 50 mmol/L potassium dihydrogen orthosphosphate (pH 3.5; 35 : 25 : 40), flow rate 1 mL/min. Limit of quantification, 2.5 and 1.25 µg/L for biriperone and its hydroxy-metabolite, respectively [Issar *et al.* 2002].
Disposition in the Body Large inter-subject variation for absorption and bioavailability of centbutindole has been observed and extensive tissue distribution has been shown. Biriperone is metabolised to its hydroxy-metabolite, 2-γ-hydroxy-δ-(4-fluorophenyl)butyl]-1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrid-o [3,4-b]indole.

Therapeutic Concentration
A single 3 mg oral dose of biriperone was administered to 5 healthy males aged between 24 and 36 years old. The mean peak serum level was 3.48 mg/L (range, 2.24 to 6.13 mg/L), which was achieved within 4.0 h (range, 1.41 to 5.87 h) [Paliwal *et al.* 1992].

Half-life The absorption half-life for centbutindole varies between 0.23 and 2 h and the terminal phase elimination half-life is approximately 12.45 h.

Volume of Distribution 765 L.

Clearance Systemic clearance is approximately 44.2 h (range, 23.4 to 64.1 h).

Issar M *et al.* (2002). A sensitive LC assay for the simultaneous determination of centbutindole and its metabolite in rat serum using fluorescence detection. *J Pharm Biomed Anal* 27: 347–353.

Paliwal JK *et al.* (1990). A simple high performance liquid chromatographic method for the estimation of centbutindole. *Indian J Pharm Sci* 52: 22–25.

Paliwal JK *et al.* (1991). Simultaneous determination of centbutindole and its hydroxy metabolite in serum by high-performance liquid chromatography. *J Chromatogr* 572: 219–225.

Paliwal JK *et al.* (1992). Single oral dose pharmacokinetics of cenbutindole, a new neuroleptic agent, in healthy human volunteers. *Drug Invest* 4: 246–251.

Seth RK, Sarin JPS (1989). Analytical and shelf-life studies on centbutindole, a new neuroleptic compound. *Indian J Pharm Sci* 51: 244–247.

Bisacodyl

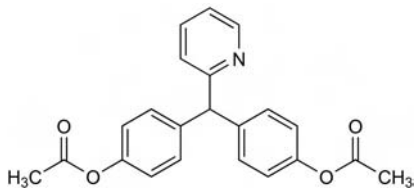
Purgative

$C_{22}H_{19}NO_4$ = 361.4

CAS—603-50-9

IUPAC Name 4,4′-(2-Pyridylmethylene)bisphenol diacetate

Proprietary Names *Bisacolax*; *Biscolax*; *Contlax*; *Deficol*; *Delco-Lax*; *Dulcolax*; *Laco*; *Laxbene*; *Perilax*; *Theralax*; *Toilax*. It is an ingredient of *Dulcodos*.



Chemical Properties A white crystalline powder. Mp 138°. Practically insoluble in water and in alkaline solutions; soluble 1 in 100 of ethanol, 1 in 35 of chloroform and 1 in 170 of ether; sparingly soluble in methanol; soluble in dilute acids, in acetone, and in propylene glycol. Log *P* (octanol/water), 3.4. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].

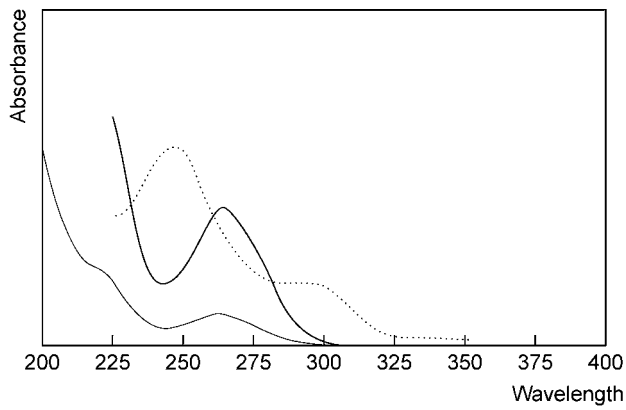
Colour Tests Liebermann's reagent—red-brown; Mandelin's test—violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.15; system TC—R_f 0.76; system TL—R_f 0.66; system TAE—R_f 0.82; system TAF—R_f 0.87 (acidified iodoplatinate solution, positive).

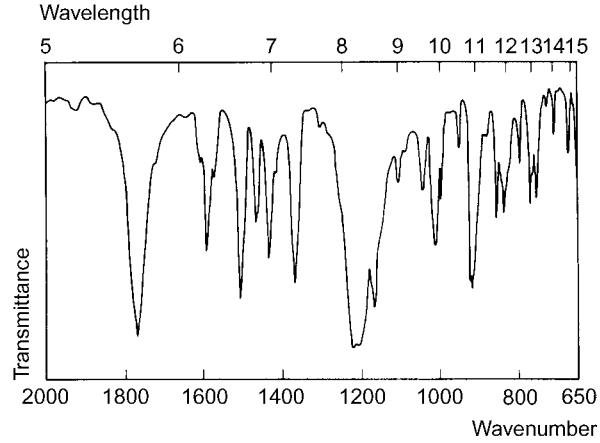
Gas Chromatography System GA—bisacodyl RI 2818, M (bismethoxybisdesacetyl) RI 2820, M (bismethoxydesacetyl) RI 2890, M (desacetyl) RI 2750, M (bisdesacetyl) RI 2655, M (methoxybisdesacetyl) RI 2680, M (methoxydesacetyl) RI 2810; system GB—bisacodyl RI 2956, M (desacetyl) RI 2876, M (bisdesacetyl) RI 2793.

High Performance Liquid Chromatography System HX—RI 531; system HY—RI 431; system HZ—retention time 9.6 min.

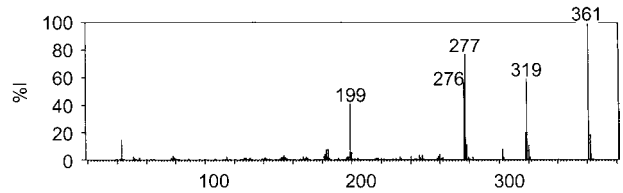
Ultraviolet Spectrum Aqueous acid—264 nm (A^1_1=270a); methanolic potassium hydroxide—248 nm (A^1_1=650a).



Infrared Spectrum Principal peaks at wavenumbers 1212, 1198, 1754, 1162, 1500, 909 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 361, 277, 319, 276, 199, 318, 362, 43.



Quantification

Serum **GC–MS** SIM *m/z* 305. Limit of detection, 10 µg/L [Kudo *et al.* 1998].

Urine **TLC–colorimetry** For bisacodyl diphenol, see Jauch *et al.* [1975].

GC–MS SIM *m/z* 305. Limit of detection, 5 µg/L [Kudo *et al.* 1998].

HPLC See Perkins and Livesey [1993]. UV detection. Limit of detection, 1 mg/L for bisacodyl diphenol [Loof *et al.* 1980].

Disposition in the Body Variably absorbed after oral administration and metabolised by deacetylation to the active metabolite, bis(4-hydroxyphenyl)-2-pyridylmethane (bisacodyl diphenol). Up to about 30% of a dose is excreted in the urine in 48 h as bisacodyl diphenol glucuronide; about 50% of a dose is eliminated in the faeces as unconjugated bisacodyl diphenol.

Dose 5 to 10 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Jauch R *et al.* (1975). Bis-(p-hydroxyphenyl)-pyridyl-2-methane: The common laxative principle of Bisacodyl and sodium picosulfate. *Arzneimittelforschung* 25: 1796–1800.

Kudo K *et al.* (1998). Laxative poisoning: toxicological analysis of bisacodyl and its metabolite in urine, serum, and stool. *J Anal Toxicol* 22(4): 274–278.

Loof L *et al.* (1980). Quantitation of a bisacodyl metabolite in urine for the diagnosis of laxative abuse. *Ther Drug Monit* 2: 345–349.

Perkins SL, Livesey JF (1993). A rapid high-performance thin-layer chromatographic urine screen for laxative abuse. *Clin Biochem* 26(3): 179–181.

Bismuth

Metal

Bi = 208.9

Chemical Properties Used in alloys, pigments; in various compounds (alluminate, carbonate, gallate, nitrate, subsalicylate, subcitrate) as antacids, astringents, and for treatment of peptic ulcers, diarrhoea, warts.

Colour Tests Reinsch test. Applicable to urine, gastric contents, scene residues. Clean a 5 × 10 mm square of copper foil (2–3 cm copper wire) with aqueous nitric acid (500 mL/L) until shiny. Rinse the copper with purified water and add 10 mL of concentrated hydrochloric acid and 20 mL of test sample in a 100 mL conical flask. Heat on a boiling water bath in a fume cupboard for 1 h. Cool and gently wash the copper with purified water.—Bismuth imparts a shiny black stain to the copper (antimony, arsenic, selenium, and tellurium also give black deposits).

Confirmation test. Place the stained copper in 100 g/L potassium cyanide solution and leave for 10 min. Wash any undissolved stain with purified water and add 1 mL of freshly prepared sodium sulfite solution (50 g/L). Shake frequently for 5 min and add 1 mL of purified water followed by 1 mL of a quinine/potassium iodide reagent (prepared by dissolving 1 g of quinine sulfate in 100 mL of purified water containing 0.5 mL of concentrated nitric acid. Add 2 g of potassium iodide when the quinine has dissolved completely).—Stains from arsenic dissolve in the potassium cyanide solution, whereas stains from antimony and bismuth do not. An orange/brown suspension is slowly formed with the quinine/iodide reagent if bismuth is present. Limit of detection, 2 mg/L.

Quantification

Specimen collection Blood—10 mL, K-EDTA; urine—20 mL universal plastic container.

Plasma **FAAS** Carrier gas: N_2. Hollow cathode lamp ($\lambda = 223.1$ nm). Flame: air–acetylene. Limit of detection, 0.1 μg/L [Froomes *et al.* 1988].

ICP-MS Outer gas: Ar, 12 L/min. Nebuliser gas: Ar, 0.75 L/min. Limit of quantification, 1.2 nmol/L, limit of detection, 0.07 nmol/L [Mauras *et al.* 1993].

Serum **ETAAS** Dry cycle: 100° at 10 s for 20 s. Char cycle: 330° in 10 s for 20 s. Atomisation cycle: 2000° for 5 s, gas stop. Perkin Elmer 5000 ($\lambda = 223.1$ nm). Limit of detection, 1 μg/L [Hundal *et al.* 1999]. Dry cycle: 95° in 5 s to 100° in 40 s to 200° in 15 s for 10 s. Char cycle: 1030° in 10 s for 30 s. Atomisation cycle: 2300° for 3 s. Varian hollow cathode lamp ($\lambda = 223.1$ nm). Limit of detection, approx. 0.9 nmol/L [Dean *et al.* 1992].

ICP-MS Plasma gas: 13 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 0.7 L/min. Limit of detection, 0.007 to 0.027 μg/L [Vanhoe *et al.* 1993].

CSF **ETAAS** Palladium–triton modifier ($\lambda = 223.1$ nm). Limit of detection, 2 ppb [Gane *et al.* 1996].

Urine **AAS** Purge gas: N_2, 120 mL/min. Bismuth hollow cathode lamp ($\lambda = 223.1$ nm). Limit of detection, 320 ng/L [Cadore *et al.* 1998].

FAAS See Plasma [Froomes *et al.* 1988].

TAAS See Serum [Dean *et al.* 1992].

ICP-MS Plasma gas: 15 L/min. Nebuliser gas: 0.825 L/min. Auxillary gas: 0.8 L/min. Limit of detection, 5 ng/L [Schramel *et al.* 1997]. See Plasma [Mauras *et al.* 1993].

Bone **ETAAS** Dry cycle: 90° at 20 s for 10 s, gas flow 100 mL/min, to 110° in 30 s for 10 s, gas flow 30 mL/min. Char cycle: 800° in 30 s for 15 s, gas flow 30 mL/min for the first 15 s to 10 mL/min for 5 s to 20° in 1 s for 15 s. Atomisation cycle: 2100° for 5 s, gas stop, to 2300° in 1 s for 1 s, gas flow 300 mL/min. Limit of detection, 1 μg/L [Slikkerveer *et al.* 1993].

Brain **ETAAS** See Bone [Slikkerveer *et al.* 1993].

Kidney **ETAAS** See Bone [Slikkerveer *et al.* 1993].

Liver **ETAAS** Palladium–triton modifier ($\lambda = 223.1$ nm). Limit of detection, 2 ppb [Gane *et al.* 1996]. See Bone [Slikkerveer *et al.* 1993].

Spleen **ETAAS** See Bone [Slikkerveer *et al.* 1993].

Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000].

Other **ETAAS** Rat Bone, Brain, Kidney, Liver and Spleen. See Bone [Slikkerveer *et al.* 1993].

Note For an autometallographic technique for tracing bismuth in postmortem brain samples, see Stoltenberg *et al.* [2001]. For a study of bismuth in soils, plants, water and sediments in Korea, see Jung *et al.* [2002].

Disposition in the Body Bismuth salts with low solubility are eliminated almost entirely unabsorbed in the faeces. Water-soluble salts (e.g. the subsalicylate) given parenterally are excreted mainly in the urine over several weeks.

Normal Concentrations Blood—<1 μg/L (<5 nmol/L); urine—<1 μg/L (<5 nmol/L).

Therapeutic Concentration Blood—up to 50 μg/L (239 nmol/L). The presence of an active ulcer does not significantly influence the absorption of colloidal bismuth citrate or bismuth subnitrate [Hundal *et al.* 1999].

Eighteen healthy male volunteers were assigned to receive a capsule containing 140 mg bismuth biskalcitrate, 125 mg metronidazole, and 125 mg tetracycline four times daily for 6 days, alone or in combination with 20 mg omeprazole twice daily. Mean peak plasma concentrations of bismuth were 8.06 ± 6.79 μg/L at 0.67 ± 0.2 h in the former group compared with 25.5 ± 17.7 μg/L at 0.84 ± 0.35 h in the group that also received omeprazole [Spénard *et al.* 2004].

Toxicity Bismuth toxicity resembles that of lead and mercury. The insoluble salts are relatively non-toxic, whereas the soluble compounds, particularly the organic lipid-soluble forms, can accumulate in the body after excessive dosage and lead to neurotoxicity. Water-soluble compounds usually cause renal damage, including acute renal failure. Many cases of severe poisoning, including deaths, have been recorded after chronic and acute overdose with therapeutic bismuth agents. Generally toxicity is associated with blood bismuth concentrations in excess of 100 μg/L (480 nmol/L) [Serfontein, Mekel 1979; Serfontein *et al.* 1979].

A 16-year-old girl ingested 60 tablets (18 g) of colloidal bismuth citrate. Twelve days later her serum bismuth concentration was 495 μg/L [Cengiz *et al.* 2005].

A 66-year-old man worked for 20 years as a repairer in a glassworks. The hard tissue bismuth content of one of his teeth was 50.1 ppm [Bachanek *et al.* 2000].

Patients undergoing elective myelography after the administration of therapeutic doses of tripotassium dicitrato bismuthate for 1 month had significantly higher bismuth concentrations in their liver and CSF than patients receiving sucralfate or aluminium hydroxide [Gane *et al.* 1996].

A 60-year-old man developed an encephalopathy and had a blood bismuth level of 72 μg/L after chronic usage of bismuth subsalicylate [Hasking, Duggan 1982].

Half-life In blood, ≈5 days

Protein Binding Preferably to transferrin instead of albumin in aqueous solutions and plasma [Sun *et al.* 2001].

Bachanek T *et al.* (2000). Heavy metal poisoning in glass worker characterised by severe dental changes. *Ann Agric Environ Med* 7: 51–53.

Cadore S *et al.* (1998). Determination of bismuth in urine and prescription medicines using atomic absorption with an on-line hydride generation system. *Analyst* 123: 1717–1719.

Cengiz N *et al.* (2005). Acute renal failure after overdose of colloidal bismuth subcitrate. *Pediatr Nephrol* 20: 1355–1358.

Dean S *et al.* (1992). Elimination of matrix effects in electrothermal atomic absorption spectrophotometric determinations of bismuth in serum and urine. *Clin Chem* 38: 119–122.

Froomes PR *et al.* (1988). Improved assay for bismuth in biological samples by atomic absorption spectrophotometry with hydride generation. *Clin Chem* 34: 382–384.

Gane E *et al.* (1996). Hepatic and cerebrospinal fluid accumulation of aluminium and bismuth in volunteers taking short course anti-ulcer therapy. *J Gastroenterol Hepatol* 11: 911–915.

Hasking GJ, Duggan JM (1982). Encephalopathy from bismuth subsalicylate. *Med J Aust* 2: 167.

Hundal O *et al.* (1999). Absorption of bismuth from two bismuth compounds before and after healing of peptic ulcers. *Hepatogastroenterology* 46: 2882–2886.

Jung MC (2002). Arsenic, Sb and Bi contamination of soils, plants, waters and sediments in the vicinity of the Dalsung Cu-W mine in Korea. *The Science of The Total Environment* 295: 81–89.

Krachler M *et al.* (2000). Concentrations of trace elements in osteoarthritic knee-joint effusions. *Biol Trace Elem Res* 75: 253–263.

Mauras Y *et al.* (1993). Simultaneous determination of lead, bismuth and thallium in plasma and urine by inductively coupled plasma mass spectrometry. *Clin Chim Acta* 218: 201–205.

Schramel P *et al.* (1997). The determination of metals (antimony, bismuth, lead, cadmium, mercury, palladium, platinum, tellurium, thallium, tin and tungsten) in urine samples by inductively coupled plasma-mass spectrometry. *Int Arch Occup Environ Health* 69: 219–223.

Serfontein WJ, Mekel R (1979). Bismuth toxicity in man II. Review of bismuth blood and urine levels in patients after administration of therapeutic bismuth formulations in relation to the problem of bismuth toxicity in man. *Res Commun Chem Pathol Pharmacol* 26: 391–411.

Serfontein WJ *et al.* (1979). Bismuth toxicity in man: I. Bismuth blood and urine levels in patients after administration of a bismuth protein complex (Bicitropeptide). *Res Commun Chem Pathol Pharmacol* 26: 383–389.

Slikkerveer A *et al.* (1993). Analysis for bismuth in tissue by electrothermal atomic absorption spectrometry. *Clin Chem* 39: 800–803.

Spénard J *et al.* (2004). Influence of omeprazole on bioavailability of bismuth following administration of a triple capsule of bismuth biskalcitrate, metronidazole, and tetracycline. *J Clin Pharmacol* 44: 640–645.

Stoltenberg M *et al.* (2001). Autometallographic tracing of bismuth in human brain autopsies. *J NeuroPathol Exp Neurol* 60: 705–710.

Sun H *et al.* (2001). Competitive binding of bismuth to transferrin and albumin in aqueous solution and in blood plasma. *J Biol Chem* 276: 8829–8835.

Vanhoe H *et al.* (1993). Bismuth in human serum: reference interval and concentrations after intake of a therapeutic dose of colloidal bismuth subcitrate. *Clin Chim Acta* 219: 79–91.

Bisoprolol

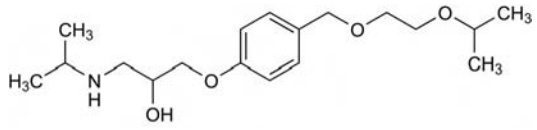
β-*Blocker*

$C_{18}H_{31}NO_4 = 325.5$

CAS—66722-44-9

IUPAC Name 1-[4-[[2-(1-Methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol

Synonym EMD-33512



Chemical Properties pK_a 9.57. Log *P* (octanol/water), 2.15; (dodecane/water), −0.24. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Bisoprolol Fumarate (hemifumarate)

$(C_{18}H_{31}NO_4)_2,C_4H_4O_4 = 767.0$

CAS—104344-23-2; 66722-45-0

Proprietary Names *Bisobloc*; *Bisomerck*; *Concor*; *Cordalin*; *Detensiel*; *Emconcor*; *Emcor*; *Euradal*; *Fondril*; *Godal*; *Isoten*; *Monocor*; *Soprol*; *Zebeta*.

Chemical Properties A white crystalline powder. Mp 100°. It is readily soluble in water, chloroform, ethanol and methanol.

Thin-layer Chromatography System TB—R_f 0.08; system TE—R_f 0.48; system TF—R_f 0.00; system TAE—R_f 0.22.

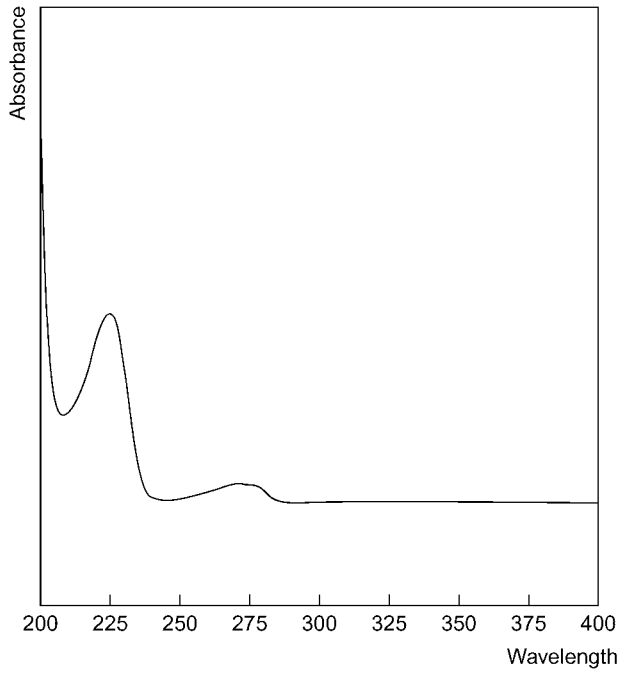
Gas Chromatography System GA—RI 2378 (bisoprolol), RI 2595 (Art), RI 2400 (Art(-H_2O)), RI 1690 (M (phenol)); system GB—RI 2427 (bisoprolol), RI 2680 (Art), RI 2480 (Art(-H_2O)).

Gas Chromatography-Mass Spectrometry

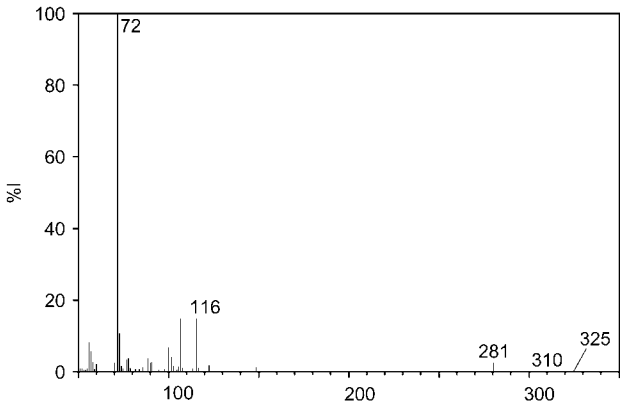
Column: 5% phenylmethyl silicone RTx-5 fused-silica capillary (30 m × 0.25 mm i.d.). Injector port: 250°, splitless conditions. Temperature programme: 110° for 1 min, to 170° at 20°/min to 225° at 7°/min to 290° at 24°/min, for 10 min. Carrier gas: He. Retention time (relative to isoproteronol, internal standard): 1.31 [Branum *et al.* 1998].

High Performance Liquid Chromatography System HX—RI 358; system HZ—retention time 3.1 min; system HAA—retention time 12.3 min.

Ultraviolet Spectrum Aqueous acid (pH 4)—225, 273 nm.



Mass Spectrum Principal ions at *m/z* 72, 116, 107, 100, 281, 73, 43, 325.



Quantification

Plasma **HPLC** Column: Chiralcel OD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : 2-propanol (10:0.9) containing 0.01% diethylamine, flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}=228 nm, λ_{em}=298 nm). Retention time: *R*(+)-bisoprolol, 20 min; *S*(−)-bisoprolol, 30 min. Limit of detection, 2 µg/L for each enantiomer [Suzuki *et al.* 1993]. Fluorescence detection. Limit of detection, 1 µg/L [Poirier *et al.* 1988]. Fluorescence detection. Limit of detection; 1 to 2 µg/L[Bühring, Garbe 1986].

Urine **HPLC** See Plasma Suzuki *et al.* [1993]. See Plasma. Limit of detection, 10 µg/L [Poirier *et al.* 1988]. See Plamsa. Limit of detection, 10 µg/L [Bühring, Garbe 1986].

Disposition in the Body Bisoprolol is almost completely absorbed after oral administration and undergoes only minimal first-pass metabolism. Peak plasma concentrations are reached 2 to 4 h after oral administration. It is moderately lipid-soluble and rapidly and widely distributed in the body. Approximately 50% of a dose is metabolised in the liver to pharmacologically inactive polar metabolites that are then excreted by the kidneys; the remaining 50% is excreted unchanged by the kidneys. <2% of a dose is excreted in faeces.

Therapeutic Concentration The serum therapeutic concentration range is 0.01 to 0.06 mg/L.

Group 1: 8 healthy subjects (with a mean age of 23 years), group 2: 11 patients with impaired renal function (mean age, 59 years), group 3: 5 patients with acute hepatitis (mean age, 38 years) and group 4: 13 patients with liver cirrhosis (mean age, 52 years). Each group was administered with 10 mg bisoprolol daily for 7 days. The mean peak plasma concentrations were 52, 74, 54 and 62 µg/L for the groups, respectively, and the mean trough concentrations 11, 32, 19 and 22 µg/L. All peak concentrations were observed 1.7 to 3 h after administration [Kirch *et al.* 1987].

Bioavailability 90%.

Half-life Plasma, 10 to 12 h; increased in patients with renal or liver impairment.

Volume of Distribution 2.9 L/kg for healthy individuals; 3.1 L/kg for patients with impaired renal function; 3.9 L/kg for patients with acute hepatitis; 2.9 L/kg for those with liver cirrhosis.

Clearance Total, 14.2 L/h for healthy individuals; 7.8 L/h for patients with impaired renal function; 11.9 L/h for patients with acute hepatitis; 10.8 L/h for those with liver cirrhosis.

Protein Binding 30 to 35%.

Note For reviews of bisoprolol, see Lancaster, Sorkin [1988] and Johns, Lopez [1995].

Dose Up to 20 mg of bisoprolol fumarate daily.

Branum GD *et al.* (1998). The feasibility of the detection and quantitation of beta-adrenergic blockers by solid-phase extraction and subsequent derivatization with methaneboronic acid. *J Anal Toxicol* 22: 135–141.

Bühring KU, Garbe A (1986). Determination of the new beta-blocker bisoprolol and of metoprolol, atenolol and propranolol in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 382: 215–224.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Johns TE, Lopez LM (1995). Bisoprolol: is this just another beta-blocker for hypertension or angina? *Ann Pharmacother* 29: 403–414.

Kirch W *et al.* (1987). Pharmacokinetics of bisoprolol during repeated oral administration to healthy volunteers and patients with kidney or liver disease. *Clin Pharmacokinet* 13: 110–117.

Lancaster SG, Sorkin EM (1988). Bisoprolol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension and angina pectoris. *Drugs* 36: 256–285.

Poirier JM *et al.* (1988). Rapid and sensitive high-performance liquid chromatographic determination of bisoprolol in plasma and urine. *J.Chromatogr* 426: 431–437.

Suzuki T *et al.* (1993). Sensitive determination of bisoprolol enantiomers in plasma and urine by high-performance liquid chromatography using fluorescence detection, and application to preliminary study in humans. *J Chromatogr* 619: 267–273.

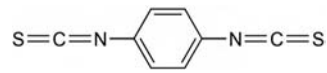
Bitoscanate

Anthelmintic

$C_8H_4N_2S_2$ = 192.3

CAS—4044-65-9

IUPAC Name 1,4-Di-isothiocyanatobenzene

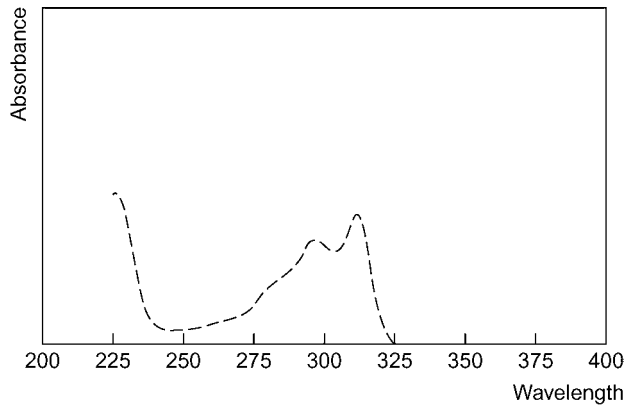


Chemical Properties A yellowish-white crystalline powder. Mp 130° to 132°. Practically insoluble in water; soluble in ethanol, chloroform and ether. Log *P* (octanol/water), 4.7.

Colour Tests Mandelin's test—brown-violet; palladium chloride—orange.

Thin-layer Chromatography System TA—R_f 0.65 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Ethanol—296, 311 nm (A^1_1=1795a).



Disposition in the Body Partly absorbed after oral administration. About 28% of a dose is excreted in the urine in 30 days and 55% is eliminated in the faeces, mainly in the first week.

Therapeutic Concentration

Following a single oral dose of 100 mg of ^{14}C-labelled bitoscanate to 6 subjects, peak blood concentrations of total radioactivity equivalent to 0.58 to 2.1 mg/L (mean 1.4) of bitoscanate were reported at about 24 h [Christ *et al.* 1970].

Half-life Plasma half-life (total radioactivity), about 26 h.

Dose 100 mg at 12-hourly intervals for 3 doses.

Christ OE *et al.* (1970). *Arzneimittelforschung* 20: 756–762.

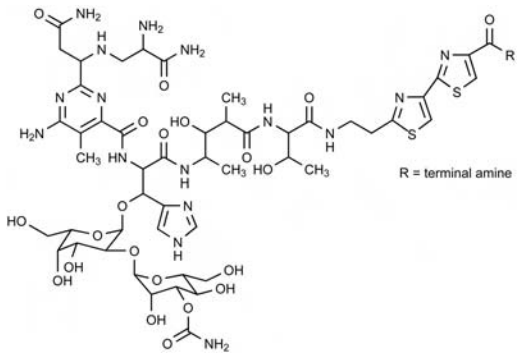
Bleomycin

Antineoplastic

$C_{55}H_{84}N_{17}O_{21}S_3$ = 1415.6

CAS—11056-06-7

Note Bleomycins are a group of related glycopeptide antineoplastic antibiotics isolated from *Streptomyces verticillus*. The main component of bleomycin used clinically is bleomycin A_2(N^1-[3-(dimethylsulfonio)propyl]bleomycinamide).
Synonyms NSC-125066; bleo.

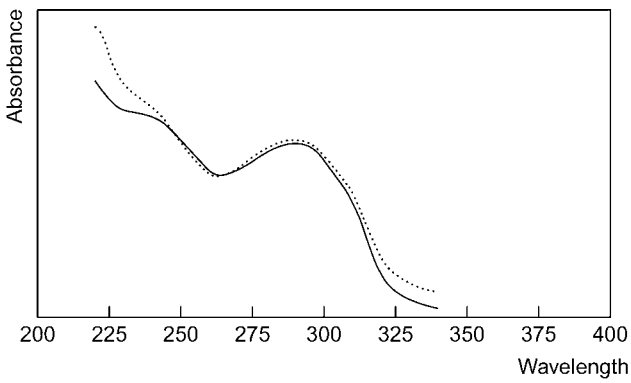


Chemical Properties A colourless or yellowish powder which becomes blueish depending on copper content. It is very soluble in water and in methanol; slightly soluble in ethanol; practically insoluble in acetone, in ethyl acetate, in butyl acetate and in ether.

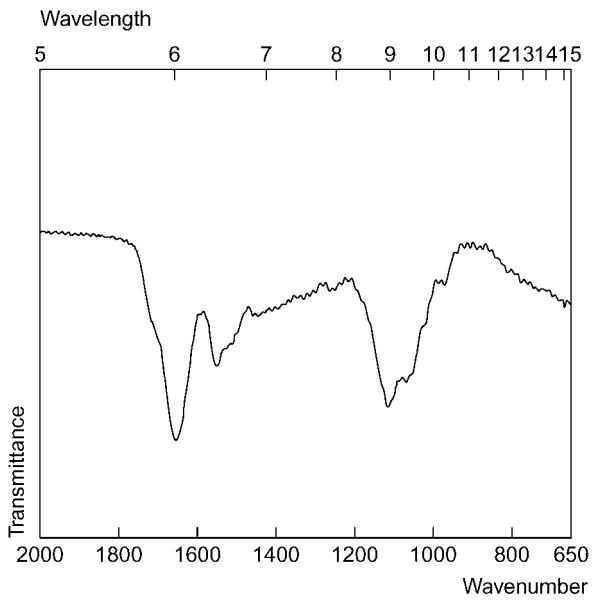
Bleomycin Sulfate

CAS—9041-93-4
Proprietary Names *Blenoxane*; *Bleo-S*; *Oil Bleo*.
Chemical Properties A white or yellowish white or cream-coloured amorphous very hygroscopic powder. It is very soluble in water; slightly soluble in ethanol; practically insoluble in acetone and in ether.

Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—290 nm: basic—290 nm.



Infrared Spectrum Principal peaks at wavenumbers 1653, 1548, 1118, 618 cm^{-1} (sulfate salt).



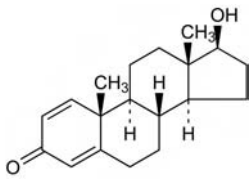
Quantification

Plasma **HPLC** UV detection. Limit of detection, 0.5 mg/L [Shiu *et al.* 1979].
Disposition in the Body It is poorly absorbed after oral administration. After parenteral administration 60 to 70% is excreted in urine as the active drug. Most tissues, except skin and lung, can degrade bleomycin enzymically. About 45% of a dose is absorbed into the systemic circulation following intrapleural or intraperitoneal administration. It does not cross the blood–brain barrier.
Therapeutic Concentration
Following intraperitoneal administration of 150 units of bleomycin in 2 L of peritoneal dialysis fluid to 3 subjects, mean peak peritoneal concentration at 15 min was 38 milli-units/L. The corresponding mean plasma concentration was 0.075 milli-units/L [Bitran 1985].
Toxicity Pulmonary toxicity is the most serious adverse effect and is more likely in those given total cumulative doses >400 units.
Half-life Plasma, 2 to 4 h.
Volume of Distribution 0.35 to 0.45 L/kg.
Clearance Plasma, 119 to 128 mL/min/m^2.
Protein Binding 1%.
Note For review of bleomycin, see Dorr [1992].
Dose Usually 15 to 60 units weekly intravenously. The maximum total cumulative dose is 400 or 500 units.

Bitran JD (1985). Intraperitoneal bleomycin. Pharmacokinetics and results of a phase II trial. *Cancer* 56: 2420–2423.
Dorr RT (1992). Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. *Semin Oncol* 19: 3–8.
Shiu GK *et al.* (1979). Rapid high-performance liquid chromatographic determination of bleomycin A2 in plasma. *J Pharm Sci* 68: 232–234.

Boldenone

Anabolic Steroid
$C_{19}H_{26}O_2$ = 286.4
CAS—846-48-0
IUPAC Name (17β)-17-Hydroxyandrosta-1,4-dien-3-one
Synonym Dehydrotestosterone



Chemical Properties Crystals. Mp 164° to 166°. Log *P* (octanol/water), 3.05.

Boldenone Acetate

$C_{21}H_{28}O_3$ = 328.5
Chemical Properties Crystals. Mp 151° to 153°.

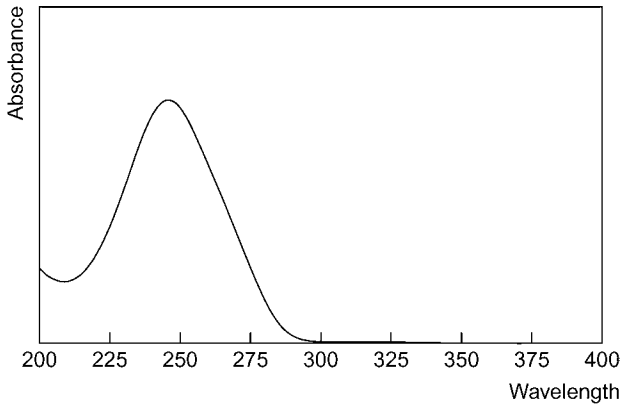
Boldenone Benzoate

$C_{26}H_{30}O_5$ = 422.5

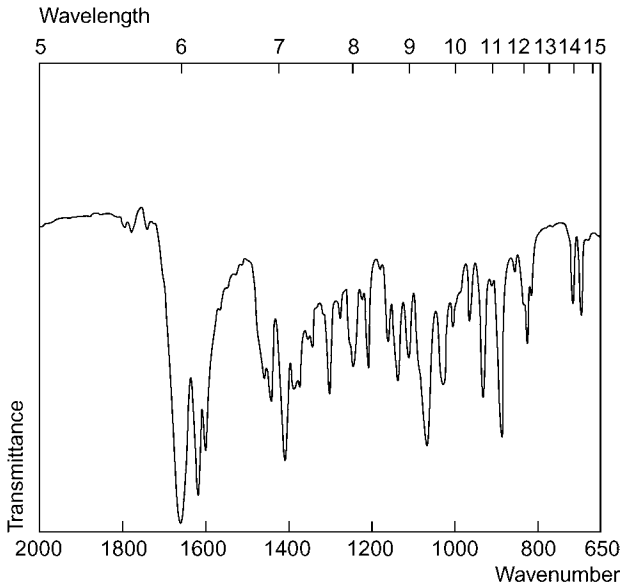
Boldenone Undecylenate

$C_{30}H_{44}O_3$ = 452.7
CAS—13103-34-9
Synonyms Ba-29038; 10-undecenoate.
Proprietary Names *Parenabol*; *Vebonol*.

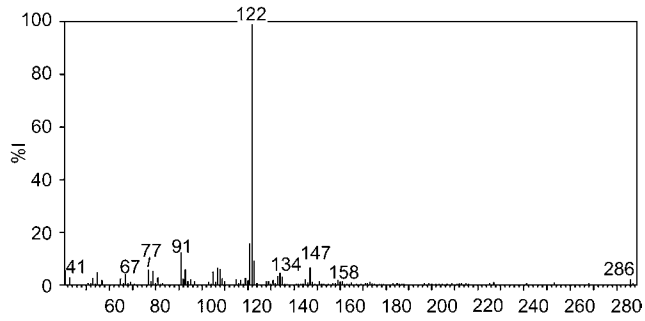
Thin-layer Chromatography System TA—R_f 0.98; system TE—R_f 0.98; system TAJ—R_f 0.90; system TAK—R_f 0.88; system TAL—R_f 0.98; system TAM—R_f 0.99.
Gas Chromatography System GAG—boldenone RRT 1.05, boldenone undecylenate RRT 2.62 (both relative to testosterone); system GAI—boldenone RRT 0.961, 5β-androst-1-en-17β-ol-3-one metabolite RRT 0.96 (both relative to 17α-methyl-5α-androstan-3β, 17β-diol); system GAR—boldenone retention time 12.8 min, boldenone acetate retention time 13.6 min, boldenone benzoate retention time 18.7 min, boldenone undecylenate retention time 22.4 min.
Column: silica SE54, cross-linked 5% phenyl, 1% vinyl methyl silicone (17 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, flow rate 1 mL/min. Split 1:10. MS detection (EI), SIM. Internal standard: 5α-androstane-3,17-dione,bis-TMS. RI: boldenone (TMS derivative) 2685.
Column: silica OV1, cross-linked methyl silicone (20 m × 0.25 mm i.d., 0.33 μm). Carrier gas: He, flow rate 1 mL/min. MS detection (EI), SIM. Internal standard: 5α-androstane-3,17-dione,bis-TMS. RI: boldenone (TMS derivative) 2672 [Schanzer, Donike 1992].
High Performance Liquid Chromatography System HAR—RRT 0.74 (relative to testosterone); system HATa—boldenone undecylenate RRT 1.94(relative to testosterone); system HATb—boldenone RRT 0.76(relative to testosterone).
Column: C_{18} Nucleosil (250 × 10 mm i.d., 7 μm). Mobile phase: (A) water, (B) acetonitrile : water (90:10). Elution programme: (A:B) (70:30) to (20:80) over 25 min, flow rate 6 mL/min. Retention time: 13.1 min [Schanzer, Donike 1992].
Ultraviolet Spectrum Aqueous acid—248 nm; aqueous base—250 nm; ethanol—243 nm.



Infrared Spectrum Principal peaks at wavenumbers 1654, 1400, 1055 cm^{-1}; (acetate)1725, 1231, 1034 cm^{-1}; (benzoate)1658, 1276, 716 cm^{-1}; (undecylenate) 1725, 1654, 1175 cm^{-1} (KBr disks).



Mass Spectrum Principal ions at *m/z* 122, 121, 91, 123, 147, 77, 107, 93.



Quantification

Urine **GC–MS** Column: 5% phenylmethylpolysiloxane Ultra-2 (30 m × 0.2 mm i.d., 0.33 µm), cross-linked capillary. Temperature programme: 150° held for 2 min, step rate of 20°/min to 300°, held for 2 min. MS detection negative chemical ionisation (NCI): Internal standard: 1,2-d2-testosterone. Retention time: boldenone metabolite, 9.17 min. Limit of detection, 2 ppb [Choi *et al.* 1998].

Disposition in the Body Boldenone is metabolised to 5β-androst-1-en-17β-ol-3-one, 5β-androst-1-ene-3α,17β-diol, 5β-androst-1-en-3α-ol-17-one, 5β-androst-1-ene-3,17-dione, androsta-1,4-diene-6β,17β-diol-3-one, androsta-1,4-dien-6β-ol-3,17β-dione and 5β-androstan-3α-ol-17-one, by 5β-reduction. Excreted, via the kidneys, to a large extent as a conjugate.

Choi MH *et al.* (1998). Determination of four anabolic steroid metabolites by gas chromatography/mass spectrometry with negative ion chemical ionization and tandem mass spectrometry. *Rapid Commun Mass Spectrom* 12: 1749–1755.

Schanzer W, Donike M (1992). Metabolism of boldenone in man: gas chromatographic/mass spectrometric identification of urinary excreted metabolites and determination of excretion rates. *Biol Mass Spectrom* 21: 3–16.

Boldione

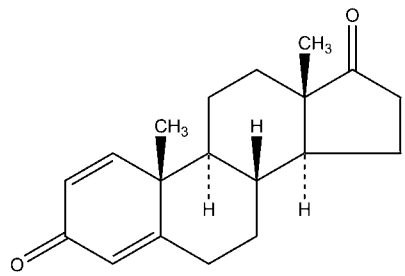
Anabolic Steroid

$C_{19}H_{24}O_2$ = 284.4

CAS—897-06-3

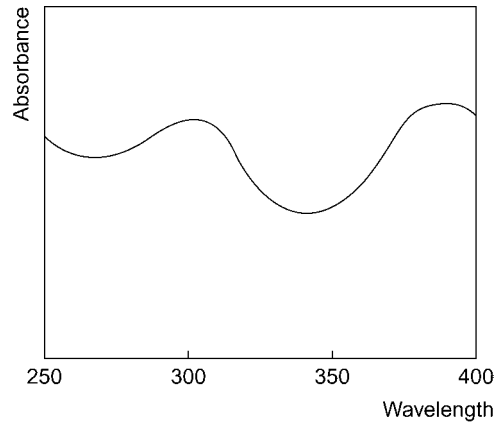
IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-Dimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthrene-3,17-dione

Synonyms Androstadienedione; 1,4-androstadiene-3,17-dione; 1-dehydroandrostenedione.



Chemical Properties Prohormone of the anabolic steroid boldenone. Log *P* (octanol/water), 2.92 [Meylan, Howard 1995]. Stability of boldione in bovine urine was verified for 4 weeks in the dark at –20° [Draisci *et al.* 2003].

Ultraviolet Spectrum



Quantification

Urine **GC-MS** Column: Ultra-2 cross linked phenylmethylsiloxane (17 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 180° for 2 min to 260° at 5°/min to 310° at 6°/min for 2 min. EI ionisation, scan mode. Limit of detection, 4.6 and 4.2 µg/L for boldione and boldenone, respectively [Kim *et al.* 2006].

LC-MS Column: Agilent Hypersil ODS C_{18} (150 × 2.0 mm i.d., 5.0 µm). Mobile phase: acetonitrile : water (20 : 80 to 45 : 55 at 5 min). ESI, positive ion mode. Limit of detection not reported [Kim *et al.* 2006].

Other **GC-MS** Bovine Urine. Column: 30 m × 0.25 mm i.d., 0.25 µm. Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 2 min to 250° at 15°/min to 300° at 5°/min for 10 min. EI ionisation, negative ion mode. Limit of detection, 0.05 µg/L [Le Bizec *et al.* 2006].

HPLC Bovine Liver and Kidney. Column: SYNERGY fusion RP 80 (150 × 4.6 mm i.d., 4.0 µm). Mobile phase: acetonitrile : water (20 : 80 for 9 min to 40 : 60 at 42 min to 20 : 80 at 47 min). Retention time: approx. 41.9 min. Limit of detection, 7.11 mg/L [Merlanti *et al.* 2007].

LC-MS Bovine Urine. Column: Uptisphere C_{18} TF (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: methanol : 0.5% acetic acid (10 : 90 to 0 : 100 at 9 min for 6 min). ESI, positive ion mode, SRM acquisition mode. Limit of detection not reported [Le Bizec *et al.* 2006]. Equine Urine. Column: Supelcosil LC-8-DB reversed phase (100 × 2.1 mm i.d., 3.0 µm). Mobile phase: 0.1% acetic acid : methanol (60 : 40 to 0 : 100 at 5 min for 5 min), flow rate 0.2 mL/min. API, ESI, positive ion mode, MRM acquisition mode. Retention time: 4.88 min. Limit of detection, 5 µg/L [Yu *et al.* 2005]. Bovine Urine. Column: Allure C_{18} reversed phase. Mobile phase: acetonitrile : 5 mol/L ammonium acetate (60 : 40), flow rate 130 µL/min. APCI, positive ion mode. Limit of quantification, 0.2 µg/L [Draisci *et al.* 2003].

Note Neoformation of 17α-boldenone in bovine faeces requires that only uncontaminated urine be analysed, see Pompa *et al.* [2006]. There is a natural occurrence of various boldenone metabolites in the urine of non-treated animals; for more information, see Le Bizec *et al.* [2006].

Disposition in the Body Rapidly metabolised to boldenone and 2 minor metabolites, M2 and M3. M2 appears to be a hydroxylated version of boldione and M3 the reduced form of M2.

A healthy male volunteer (age 26 years, weight 71 kg) was administered one 100 mg boldione tablet orally. Urine samples were collected over 48 h and analysed by GC-MS. The maximum amount of boldione excreted occurred after 1.8 h and was 16.1 mg (in 750 mL urine), whereas figures for boldenone (the main metabolite) were 6.8 mg (in

700 mL urine) after 3.6 h. The total amount excreted after the 48 h period was 34.5 mg for boldione and 16.0 mg for boldenone (in 4.16 L urine) [Kim *et al.* 2006].

Draisci R *et al.* (2003). Confirmatory analysis of 17beta-boldenone, 17alpha-boldenone and androsta-1,4-diene-3,17-dione in bovine urine by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 789: 219–226.
Kim Y *et al.* (2006). Characterization of boldione and its metabolites in human urine by liquid chromatography/electrospray ionization mass spectrometry and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 20: 9–20.
Le Bizec B *et al.* (2006). Criteria to distinguish between natural situations and illegal use of boldenone, boldenone esters and boldione in cattle 1. Metabolite profiles of boldenone, boldenone esters and boldione in cattle urine. *Steroids* 71: 1078–1087.
Merlanti R *et al.* (2007). An in vitro study on metabolism of 17beta-boldenone and boldione using cattle liver and kidney subcellular fractions. *Anal Chim Acta* 586: 177–183.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Pompa G *et al.* (2006). Neoformation of boldenone and related steroids in faeces of veal calves. *Food Addit Contam* 23: 126–132.
Yu NH *et al.* (2005). Screening of anabolic steroids in horse urine by liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 37: 1031–1038.

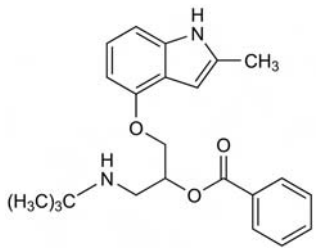
Bopindolol

Antihypertensive

$C_{23}H_{28}N_2O_3 = 380.5$

CAS—62658-63-3

IUPAC Name 1-[(1,1-Dimethylethyl)amino]-3-[(2-methyl-1*H*-indol-4-yl)oxy]-2-propanol benzoate ester



Chemical Properties It is soluble in ether and methylene chloride.

Bopindolol Maleate

$C_{27}H_{32}N_2O_7 = 496.6$

Proprietary Name *Sandonorm*

Bopindolol Malonate

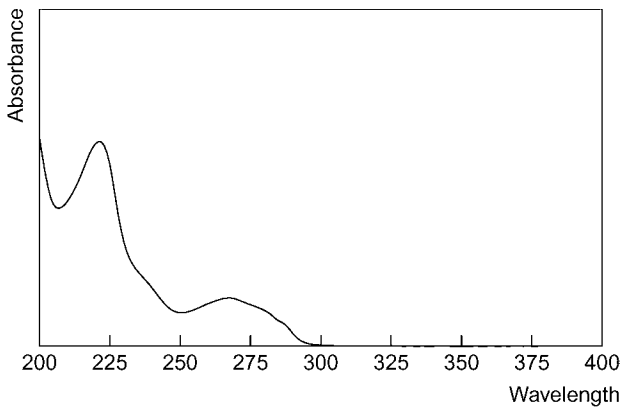
$C_{26}H_{32}N_2O_7 = 484.5$

CAS—82857-38-3

Synonyms Bopindolol hydrogen malonate; LT-31-200.

Proprietary Name *Wandonorm*

Ultraviolet Spectrum Principal peaks at 221, 267 nm.



Quantification

Plasma **HPLC** Electrochemical detection. Limit of detection, 0.025 µg/L [Humbert *et al.* 1987].

Disposition in the Body Bopindolol is rapidly hydrolysed in plasma and no measurable amounts of the parent drug can be detected after oral administration. The two main metabolites identified are benzoic acid and an active hydrolysed metabolite.

Therapeutic Concentration

Twenty young male volunteers, aged between 18 and 42 years, and 20 elderly males, 62 to 83 years old, were administered with a single 1-mg dose of bopindolol once daily for 7 days. Mean peak plasma concentrations of 4.5 and 5.2 µg/L were reached for the young and elderly volunteers, respectively. All peak concentrations were observed approximately 1.5 h after administration. No significant differences were observed between the young and elderly [Holmes *et al.* 1991].

Fourteen patients with cirrhosis (male and female, aged between 40 and 66 years) and 15 healthy volunteers (21 to 28 years) were administered with a 1.2-g dose antipyrine on day 1 and 2-mg bopindolol on day 3. The patients with cirrhosis reached a mean peak plasma concentration of 5.8 µg/L at 1.6 h and the healthy volunteers 6.9 µg/L within 1.5 h. No significant difference was observed [Wensing *et al.* 1990].

Half-life Mean, 4.4 h (range 3.1 to 6.0 h).

Volume of Distribution Mean, 202 L (range, 102 to 504 L) after oral administration; 148 L intravenously.

Clearance After single dose administration, plasma clearance is 30.9 L/h and for multiple dosing, 38.0 L/h.

Dose Between 0.5 and 4 mg.

Holmes D *et al.* (1991). Steady state pharmacokinetics of hydrolysed bopindolol in young and elderly men. *Eur J Clin Pharmacol* 41: 175–178.
Humbert H *et al.* (1987). Column liquid chromatographic determination of hydrolysed bopindolol, in the picogram per millilitre range in plasma, using cartridge extraction and dual electrochemical detection. *J Chromatogr* 422: 205–215.
Wensing G *et al.* (1990). Pharmacokinetics after a single oral dose of bopindolol in patients with cirrhosis. *Eur J Clin Pharmacol* 39: 569–572.

Borates

Anion

Boric Acid

$H_3BO_3 = 61.8$

CAS—10043-35-3

Synonyms Boracic acid; orthoboric acid; occurs in nature as the mineral sassolite.

Proprietary Name *Borofax*

Chemical Properties Colourless, odourless, transparent crystals, or white granules or powder. Mp 171°. Very soluble in water, alcohol, glycerol. Used for weatherproofing wood and fireproofing fabrics; as a preservative; in manufacture of cements, crockery, porcelain, enamels, leather, soaps, artificial gems; in cosmetics; photography; hardening steel; as an insecticide.

Sodium Borate Decahydrate

$Na_2B_4O_7{,}10H_2O = 381.2$

CAS—1330-43-4

Synonyms Sodium biborate; sodium pyroborate; sodium tetraborate; fused sodium borate, borax glass, or fused borax (anhydrous form).

Proprietary Names *Borax*; *Jaikin* (decahydrate).

Chemical Properties Hard odourless crystals, granules or crystal powder. Mp 75°; loses $5H_2O$ at 100° and $9H_2O$ at 150°; becomes anhydrous at 320°. Soluble in water; very soluble in glycerol; insoluble in alcohol. Used for soldering metals; in manufacture of glazes and enamels; in tanning; in cleaning compounds; artificially ageing wood; as a preservative; fireproofing fabrics and wood; in cockroach control; pharmaceutical aid.

Colour Test Test applicable to gastric contents and scene residues. A portion of the sample is acidified with 1 mol/L hydrochloric acid and applied to turmeric paper (prepared by soaking strips of filter paper in turmeric spice dissolved in methanol (10 g/L) and allowing them to dry at room temperature)—A brown-red colour, which intensifies when the paper dries, indicates the presence of borate; moisten the filter paper with 4 mol/L ammonium hydroxide solution—A colour change to green-black suggests the presence of borates (oxidising agents such as bromates, chlorates, iodates and nitrates interfere by bleaching the turmeric). Limit of detection, 20 mg/L.

Confirmation Test Stomach contents, 5 mL, is filtered into a 10 mL glass tube and 0.5 mL of the filtrate is added to a clean glass tube before slowly adding 0.5 mL of carminic acid solution (0.5 g/L in concentrated sulfuric acid) down the side of the tube so that it forms a layer underneath the sample—A blue-violet ring at the interface of the two layers suggests the presence of borate. Strong oxidising agents (e.g. bromates, chlorates, iodates and nitrates) also give a positive result with this test.

Quantification Specimen collection: Blood—10 mL EDTA tube; urine—20 mL plastic universal container; gastric contents are useful in postmortem examinations.

Blood **ICP-AES** Nebuliser gas: 0.70 L/min ($\lambda = 249.7$ nm). Limit of detection not reported [Svantesson *et al.* 2002].

ICP-MS Plasma gas: Ar, 15 L/min. Auxiliary gas: Ar, 0.8 L/min. Nebuliser gas: Ar, 0.9 L/min. Limit of detection, 5 µg/L [Moreton, Delves 1999]. Jobin-Yvon JY 48 spectrometer. Plasma-Therm source. Limit of detection, 0.06 µmol/L [Mauras *et al.* 1986].

Note For a DEAE cellulose method for the analysis of boron-10, see Schremmer and Noonan [1987]. For a colorimetric method for the detection of boron in blood, see Dill *et al.* [1977]. For a spectrophotometric method for the detection of boron in blood, see Edwall *et al.* [1979].

Plasma **ICP-MS** Plasma gas: Ar, 15 L/min. Auxiliary gas: Ar, 0.8 L/min. Nebuliser gas: Ar, 0.9 L/min. Limit of detection, 5 µg/L [Moreton, Delves 1999].

Serum **FAAS** Gas: nitrous oxide–acetylene. Perkin-Elmer ($\lambda = 249.7$ nm). Limit of detection, 15 mg/L [Bader, Brandenberger 1968]. Gas: air–acetylene. Limit of quantification, 0.2 µg [Siemer 1982].

Urine **FAAS** See Serum [Bader, Brandenberger 1968; Siemer 1982].

ICP-AES See Blood [Svantesson *et al.* 2002].

CSF **FAAS** See Serum [Bader, Brandenberger 1968].

Dialysis Fluid **ICP-MS** See Blood [Mauras *et al.* 1986].

Brain **ICP-MS** See Plasma [Moreton, Delves 1999].

Note For a review of the considerations for measuring boron at low concentrations, see Downing *et al.* [1998]. For a spectrometric method for the determination of boron, see Aznárez and Mir [1984].

Disposition in the Body Boric acid is well absorbed through mucous membranes and damaged skin, but not when the skin is intact. Intestinal absorption of

boron is rapid and almost complete following ingestion of boric acid or borates, with peak blood concentrations being reached within 2 h [Locatelli *et al.* 1987]. The main route of elimination of borates is via the kidneys and they are excreted substantially unchanged, regardless of the route of administration. Urinary excretion accounts for 85 to 100% of a dose within 5 to 7 days. Smaller amounts are excreted in the faeces and in sweat. Boric acid is not metabolised in humans or animals [Murray 1998].

Note For a possible role of dietary boron, see Hunt [1998].

Normal Concentrations Blood—<2 mg/L (as borate). Normal boron concentrations from 50 humans have been reported as follows:

Sample	*Median (ppm)*	*Range (ppm)*
Whole blood	0.057	0.008–0.17
Blood serum	0.022	0.008–0.048
Urine	0.75	0.16–2.9
Scalp hair	4.3	0.83–10.2
Fingernails	15.2	7.4–82.7
Toenails	17.9	7.6–57.4

[Moseman 1994].

Therapeutic Concentration Infusion of sodium borocaptate (mercaptoundecahydrododecaborate [BSH]) is used in boron neutron capture therapy of brain tumours (glioblastomas and astrocytomas). It is a system that tries to combine chemotherapy with radiotherapy with a greater selectivity, efficacy and safety than chemotherapy or radiotherapy alone. An IV infusion of 25 mg/kg is given over the course of 1 h [Gibson *et al.* 2003; Horn *et al.* 1998; Shibata *et al.* 2003].

Toxicity The minimal lethal dose of boric acid for humans is estimated as ~2–3 g in infants, 5–6 g in children and 15–20 g in adults. The serum toxic concentration is cited as 4 mg/L and the lethal concentration is reported to be 50–100 mg/L [Ishii *et al.* 1993].

A 77-year-old man mistakenly ingested approximately 30 g boric acid to stop hiccups. At 55 h after ingestion, his serum boric acid was 25.3 mg/L and his plasma boric acid concentration was 25.5 mg/L. The patient died 63 h post-ingestion; a postmortem was not performed [Ishii *et al.* 1993].

A 45-year-old ingested 2 cups of boric acid crystals in a suicide attempt. He died 63 h later. Whole-blood and urine concentrations of boric acid at 52 h post-ingestion were 42 and 160 mg/L, equivalent to 7 and 28 mg boron/L, respectively. At postmortem, his urinary boron concentration was 29.4 mg/L [Restuccio *et al.* 1992].

In a fatal case of a man who ingested a large quantity of boric acid, a serum borate concentration of 440 mg/L was reported [Litovitz *et al.* 1991].

A review of 784 exposures to boric acid between 1981 and 1985 showed that the median age was 2 years and that children under 6 years made up 80.2% of cases. All but 2 cases were acute ingestions:

A 14-month-old boy ingested 20 g of 50% boric acid. His serum boric acid concentration was 8 mg/L. A 2-year-old ingested 10 g of boric acid. His serum boric acid concentrations were 53 and 58 mg/L, attained at 1 and 7 h, respectively [Litovitz *et al.* 1988].

After an attempted suicide by ingesting 80 g of boric acid, a 35-year-old woman had serum borate concentrations at 1 and 13 h of 2320 mg/L and 1360 mg/L, respectively. Similarly, a 28-year-old woman ingested an estimated 297 g of a 99% boric acid solution. Her serum boric acid concentrations was 4.9 mg/L 2 h later [Linden *et al.* 1986].

The mean blood plasma boron concentrations was 2.6 ± 0.9 µmol/L in control subjects and 16.1 ± 5.6 µmol/L in haemodialysed patients before dialysis. After dialysis, the plasma concentration of boron was 9.5 ± 3.2 [Mauras *et al.* 1986].

Chronic ingestion has more severe consequences than acute ingestion: acute ingestion of up to 20 g of boric acid by infants induced only diarrhoea and sickness, with serum borate concentrations of 80–580 mg/L [Linden *et al.* 1986]. In contrast, seizures were observed in infants who ingested between 4 and 30 g over a period of 4–10 weeks and had blood borate levels of 2.6–8.5 mg/L. Control values in 15 children who had received no boron in their diet were 0.0–0.63 mg/L [O'Sullivan, Taylor 1983].

Application of boric acid for nappy rash has caused neurological disorders, severe erythema of the skin, and gastrointestinal problems, as well as deaths in infants.

In a child who survived application of boric acid, a serum borate concentration of over 300 mg/L was recorded, which reduced to 32 mg/L after 54 h of peritoneal dialysis [Baliah *et al.* 1969].

Eleven infants who were accidentally administered boric acid in their food developed the same symptoms. The 6 who survived (doses 2–4.5 g) had serum borate levels of 20–150 mg/L; the remaining 5 (doses 4.5–14 g) died 3 days later, with serum borate levels of 200–1600 mg/L [Wong *et al.* 1964].

Note For a case of poisoning with boron trifluoride, see Stewart and Waisberg [1998]. For a study of the relationship between blood and urine concentrations of boron and boron exposure in borax workers, see Culver *et al.* [1994].

Volume of Distribution 0.17–0.50 L/kg.

Clearance 0.107–0.294 mL/min/kg. For a study on the effect of boron supplementation on its urinary excretion, see Naghii and Samman [1997].

Distribution in Blood Plasma : blood, 1.3 ± 0.2.

Half-life 12–27 h.

Aznárez J, Mir JM (1984). Spectrophotometric determination of boron with curcumin after extraction with 2-methylpentane-24-diol-chloroform. *Analyst* 109: 183–184.

Bader H, Brandenberger H (1968). Boron determination in biological materials by atomic absorption spectrophotometry. *Atomic Absorption Newslett* 7: 1–3.

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Culver BD *et al.* (1994). The relationship of blood- and urine-boron to boron exposure in borax-workers and usefulness of urine-boron as an exposure marker. *Environ Health Perspect* 102: 7133–137.

Dill H *et al.* (1977). Blood boron levels in patients using buffered sodium peroxyborate monohydrate mouthwash three times daily for four weeks. *Int J Clin Pharmacol Biopharm* 15: 16–18.

Downing RG *et al.* (1998). Considerations in the determination of boron at low concentrations. *Biol Trace Elem Res* 66: 3–21.

Edwall L *et al.* (1979). Absorption of boron after mouthwash treatment with Bocosept. *Eur J Clin Pharmacol* 15: 417–420.

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Horn V *et al.* (1998). Disposition and tissue distribution of boron after infusion of borocaptate sodium in patients with malignant brain tumors. *Int J Radiat Oncol Biol Phys* 41: 631–638.

Hunt CD (1998). Regulation of enzymatic activity: one possible role of dietary boron in higher animals and humans. *Biol Trace Elem Res* 66: 205–225.

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Linden CH *et al.* (1986). Acute ingestions of boric acid. *J Toxicol Clin Toxicol* 24: 269–279.

Litovitz TL *et al.* (1988). Clinical manifestations of toxicity in a series of 784 boric acid ingestions. *Am J Emerg Med* 6: 209–213.

Litovitz TL *et al.* (1991). 1990 annual report of the American Association of Poison Control Centers National Data Collection System. *Am J Emerg Med* 9: 461–509.

Locatelli C *et al.* (1987). Human toxicology of boron with special reference to boric acid poisoning. *G Ital Med Lav* 9: 141–146.

Mauras Y *et al.* (1986). Increase in blood plasma levels of boron and strontium in hemodialyzed patients. *Clin Chim Acta* 156: 315–320.

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Moseman RF (1994). Chemical disposition of boron in animals and humans. *Environ Health Perspect* 102(Suppl7): 113–117.

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Naghii M, Samman RS (1997). The effect of boron supplementation on its urinary excretion and selected cardiovascular risk factors in healthy male subjects. *Biol Trace Elem Res* 56: 273–286.

O'Sullivan K, Taylor M (1983). Chronic boric acid poisoning in infants. *Arch Dis.Child* 58: 737–739.

Restuccio A *et al.* (1992). Fatal ingestion of boric acid in an adult. *Am J Emerg Med* 10: 545–547.

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Botulinum Toxin

Neurotoxin, Skeletal Muscle Relaxant

Botulinum toxins are potent neurotoxins produced by *Clostridium botulinum*. There are 7 serologically distinct types designated A to G. The proteins targeted by each serotype are:

Toxin	*SNARE target*
A, E	SNAP-25
B, D, F, G	Synaptobrevin (VAMP)
C	Syntaxin

Only types A and B are approved by the FDA for use in humans. The active components of types A and B consist of an amino acid complex composed of a heavy and a light chain linked by disulfide bonds. The heavy chain is thought to be responsible for binding to target cells and the translocation of the toxin across the synaptic membrane, while the light chain is responsible for the toxicity. For a review, see Thakker and Rubin [2004].

Synonym BoNT

Botulinum Toxin A

Treatment of Blepharospasm/Treatment of Cervical Dystonia/Treatment of Severe Primary Axillary Hyperhidrosis/Treatment of Strabisimus

149.3 kDa

CAS—93384-43-1

Synonyms AGN-191622; BoNT-A; botulin A; oculinum.

Proprietary Names *BoTox*; *Dysport*.

Chemical Properties The fluorescence emission of the CBQCA-labelled products is stable at 4° for up to 14 days [Laing *et al.* 2006]. Most stable in the pH range 5–7 [Callaway 2004]. The toxin must be kept frozen at –5° or colder until reconstituted, when it should be refrigerated and administered within 4 h [Thakker, Rubin 2004].

Botulinum Toxin B

Treatment of Spasmodic Torticollis

150.6 kDa

CAS—93384-44-2

Synonyms BoNT-B; botulin B.
Proprietary Names *Myobloc*; *NeuroBloc*.
Chemical Properties Colourless to light yellow solution. Most stable in the pH range 5–7. Purified toxin stable for 9 months when stored at room temperature and for >36 months when refrigerated (2° to 8°) [Callaway 2004].

Note The mouse bioassay is the currently accepted method for detecting BoNT. Drawbacks of this method include the need to euthanise a number of animals and that the results are not available for several hours. ELISAs, which recognise protein antigenic sites, are less sensitive than the mouse bioassay [Karner, Allerberger 2006]. For an overview of immunoassays and polymerase chain reaction techniques, see Shone *et al.* [2006].
High Performance Liquid Chromatography Column: BioRad HiPore C_{18} reversed phase 304 (250 × 4.6 mm). Mobile phase: 0.1% trifluroacetic acid in water : 0.1% trifluroacetic acid in 70% acetonitrile (100 : 0 for 2.5 min to 55 : 45 for 28.5 min to 0 : 100 for 6 min), flow rate 1.0 mL/min. UV detection ($\lambda = 210$ nm). Total time for separation, ~60 min. Limit of detection not reported [Laing *et al.* 2006].
Liquid Chromatography-Mass Spectrometry Column: Phenomenex Luna C_{18} (150 × 1 mm i.d., 5 µm). Mobile phase: 1% formic acid in water : acetonitrile-1% formic acid in water (80 : 20; 100 : 0 to 20 : 80 at 25 min), flow rate 50 µL/min. ESI, MRM acquisition mode. Limit of detection, 0.62 LD_{50}/mL in mouse for serotypes A, B and F, and <0.31 LD_{50}/mL in mouse for serotype E [Barr *et al.* 2005]. Column: C_{18} (1 mm). MALDI-TOF or ESI, positive ion mode. Limits of detection for serotypes A, B, E and F were as follows:

Detection method	*Reaction time (h)*	*Limit of detection (U/mL)*			
		BoNT-A	*BoNT-B*	*BoNT-E*	*BoNT-F*
MALDI-TOF-MS	4	1.250	1.250	1.250	6.250
MALDI-TOF-MS	10 to 17	0.625	0.625	0.313	6.250
ESI-MS-(MS)	4	1.250	0.156	0.078	0.781
ESI-MS-(MS)	10 to 17	1.000	0.039	0.039	0.781

[Boyer *et al.* 2005].

Capillary Electrophoresis Capillary: Bare fused silica (37 cm × 50 µm i.d., 30 cm effective length). Buffer: 50 mmol/L HEPES (pH 8.4), 20 mol/L sodium chloride, 0.005% Tween-20 and 1 mmol/L EDTA. Fluorescence detection ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 520$ nm). Total time for separation, 8 min. Limit of detection not reported [Laing *et al.* 2006].
Note For the AOAC official ELISA method for the detection of serotypes A, B, E and F, see Ferreira *et al.* [2003]; for a characterisation of serotypes C, D, E and F by MALDI and ESI mass spectrometry, see van Baar *et al.* [2004]. For a review of the laboratory diagnostics of botulism, see Lindstrom and Korkeala [2006]. The avian eyelid assay may represent a new diagnostic method for the detection of serotypes A, B and E, see Wang *et al.* [2007].
Disposition in the Body Once internalised in the presynaptic terminal, botulinum toxin causes an irreversible block of acetyl choline release. Functional recovery occurs after sprouting of new motor nerve terminals, which reaches its peak after 5–10 weeks.
Therapeutic Concentration Botulinum toxin A is currently in clinical trials for benign prostatic hyperplasia.
Toxicity BoNT-A is defined in terms of its biological activity; 1 unit is equal to the LD_{50} for IP injections in mice. The LD_{50} in monkeys is 39 U/kg, which implies LD_{50} in humans of 3000 units. The Centers for Disease Control and Prevention (CDC) report that there are 110 cases of botulism each year, 72% of which are infantile. For a case report of botulism caused by BoNT-B in a 4-week-old white boy, see Lavoie *et al.* [2006].

Most foodborne botulism results from the consumption of improperly prepared home-canned foods, although fermented foods, such as fish, seal and whale, have also been associated. Foodborne botulism has been reported from home-prepared fermented tofu [Centers for Disease Control and Prevention 2007], barbequed pork [Meusburger *et al.* 2006] and commercial carrot juice [Centers for Disease Control and Prevention 2006].

No human deaths have been associated with cosmetic or therapeutic botulinum toxin injections.

In late 2004, four cases of botulism following injections of a highly concentrated, unlicensed preparation of BoNT-A were reported. Serum toxin levels showed that three of the four patients had 23 to 41 times the estimated human lethal dose. A 100 µg vial of toxin from the same batch was found to contain sufficient toxin to cause 14 286 deaths by injection if disseminated evenly [Chertow *et al.* 2006].

A 34-year-old woman developed shortness of breath, difficulty swallowing, double vision, and generalised weakness 2 days after receiving what she had thought to be cosmetic BoNT. It was, in fact, research-grade toxin purchased from the Internet. She had plasma toxin concentrations that were 20 times the mouse LD_{50}. The patient showed signs of improvement 4 weeks after admission and had achieved a remarkable recovery after 14 weeks [Souayah *et al.* 2006].

A 3-year-old patient with cerebral palsy received a total dose of 40 U/kg BoNT-A as injections into both legs and her right arm. She presented with dysphagia, excessive drooling, and she had begun to aspirate on liquids. Her mother reported that the child suffered from sleep apnoea and within a month of the injections she had developed generalised weakness and a decline in her functional abilities. The severe weakness lasted 6 weeks but she was unable to hold her head up for 3 months. Since the initial evaluation, the child had received additional doses of BoNT-A in doses ranging from 17.7 to 20.0 U/kg with no adverse events [Crowner *et al.* 2007].
Note For a case of botulinophilia, see Kreyden *et al.* [2002].
Dose For cervical dystonia, 236 U IM divided between the affected muscles. For bletharospasm 1.25 to 2.5 U IM at each site. For strabismus 1.25 to 2.5 IM in vertical muscles and horizontal strabismus of less than 20 prism dioptres, 2.5 to 5.0 U in horizontal strabismus of 20 to 50 prism dioptres, and 1.25 to 2.5 U IM for persistant cranial nerve VI palsy ≥1 month in duration. For severe primary axillary hyperhydrosis, 50 U per axilla divided in multiple intradermal injections [Anderson 2004].

Anderson ERJr (2004). Proper dose, preparation, and storage of botulinum neurotoxin serotype A. *Am J Health Syst.Pharm* 61: S24–S29.
Barr JR *et al.* (2005). Botulinum neurotoxin detection and differentiation by mass spectrometry. *Emerg Infect Dis* 11: 1578–1583.
Boyer AE *et al.* (2005). From the mouse to the mass spectrometer: detection and differentiation of the endoproteinase activities of botulinum neurotoxins A–G by mass spectrometry. *Anal Chem* 77: 3916–3924.
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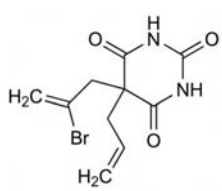
Centers for Disease Control and Prevention (2006) Botulism associated with commercial carrot juice: Georgia and Florida, September 2006. *MMWR Morb Mortal Wkly Rep* 55: 1098-1099.
Centers for Disease Control and Prevention (2007) Foodborne botulism from home-prepared fermented tofu: California, 2006. MMWR Morb Mortal Wkly Rep 56: 96-97.
Chertow DS *et al.* (2006). Botulism in 4 adults following cosmetic injections with an unlicensed, highly concentrated botulinum preparation. *JAMA* 296: 2476–2479.
Crowner BE *et al.* (2007). Iatrogenic botulism due to therapeutic botulinum toxin A injection in a pediatric patient. *Clin Neuropharmacol* 30: 310–313.
Ferreira JL *et al.* (2003). Detection of botulinal neurotoxins A, B, E, and F by amplified enzyme-linked immunosorbent assay: collaborative study. *J AOAC Int* 86: 314–331.
Karner J, Allerberger F (2006). Detecting *Clostridium botulinum*. *Emerg Infect Dis* 12: 1292.
Kreyden OP *et al.* (2002). Delusional hyperhidrosis as a risk for medical overtreatment: a case of botulinophilia. *Arch Dermatol* 138: 538–539.
Laing TD *et al.* (2006). Capillary electrophoresis laser-induced fluorescence for screening combinatorial peptide libraries in assays of botulinum neurotoxin A. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 240–246.
Lavoie D *et al.* (2006). Infantile botulism: a case report. *W V Med J* 102: 19–20.
Lindstrom M, Korkeala H (2006). Laboratory diagnostics of botulism. *Clin MicroBiol Rev* 19: 298–314.
Meusburger S *et al.* (2006). Outbreak of foodborne botulism linked to barbecue. *Austria 2006 Euro Surveill* 11: E061214.
Shone C *et al.* (2006). Workshop review: assays and detection. [In: The 5th International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Neurotoxins.]. *Neurotox Res* 9: 205–216.
Souayah N *et al.* (2006). Severe botulism after focal injection of botulinum toxin. *Neurology* 67: 1855–1856.
Thakker MM, Rubin PA (2004). Pharmacology and clinical applications of botulinum toxins A and B. *Int Ophthalmol Clin* 44: 147–163.
vanBaar BL *et al.* (2004). Characterisation of botulinum toxins type C, D, E, and F by matrix-assisted laser desorption ionisation and electrospray mass spectrometry. *J Chromatogr A* 1035: 97–114.
Wang J *et al.* (2007). Avian eyelid assay, a new diagnostic method for detecting botulinum neurotoxin serotypes A, B and E. *Toxicon* 49: 1019–1025.

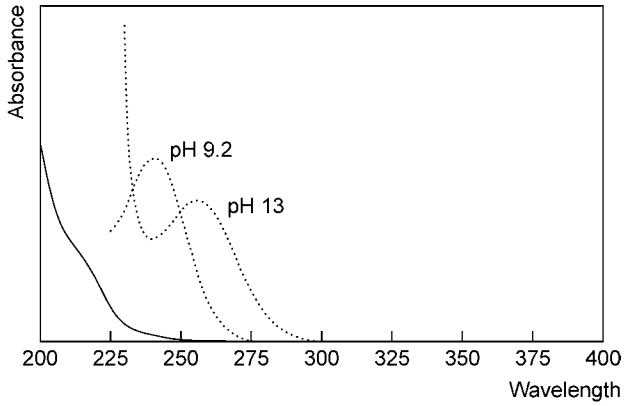
Brallobarbital

Sedative, Barbiturate
$C_{10}H_{11}BrN_2O_3 = 287.1$
CAS—561-86-4
IUPAC Name 5-(2-Bromoprop-2-enyl)-5-prop-2-enyl-1,3-diazinane-2,4,6-trione
Synonyms Brallobarbitone; 5-(2-bromo-2-propenyl)-5-(2-propenyl)-2,4,6 (1*H*,3*H*,5*H*)-pyrimidinetrione.
Proprietary Name It is an ingredient of *Vesparax*.

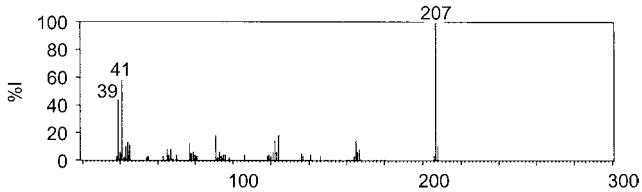


Chemical Properties Mp 168° to 169°. Log *P* (octanol/water), 1.4.
Colour Test Vanillin reagent—brown-orange.
Thin-layer Chromatography System TD—R_f 0.52; system TE—R_f 0.30; system TF—R_f 0.68; system TH—R_f 0.47; system TAD—R_f 0.57; system TAE—R_f 0.87.
Gas Chromatography System GA—brallobarbital RI 1853, brallobarbital-Me_2 RI 1725, M (2-OH-) RI 2040, M (desbromo-OH-) RI 1795; system GF—brallobarbital RI 2765; system GM—brallobarbital RRT 1.000 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 3.09; system HH—*k* 1.72; system HX—RI 371; system HY—RI 336; system HZ—retention time 3.0 min.

Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—240 (A^{1}_{1}=304b); 1 mol/L sodium hydroxide (pH 13) —256 nm (A^{1}_{1}=236b).



Mass Spectrum Principal ions at *m/z* 207, 41, 39, 124, 91, 165, 122, 44.



Quantification See under Amobarbital.

Plasma **GC** NPD. Limit of detection, 0.5 mg/L [Coudore *et al.* 1993].

Disposition in the Body Brallobarbital is absorbed after oral administration. About 5% of a dose is excreted in the urine as unchanged drug, 9% as 5-acetonyl-5-allylbarbituric acid and <1% each as 5-acetonyl-5-(2-bromoallyl)barbituric acid and 5-allyl-5-(2-hydroxypropyl)barbituric acid.

Toxicity

In 10 fatalities involving the ingestion of a preparation containing brallobarbital, quinalbarbital and hydroxyzine, the following postmortem concentrations, µg/g (mean, *N*) were reported:

	Brallobarbital	*Quinalbarbital*
Blood	9.8–25 (16.3, 9)	10–33.4 (17.7, 9)
Brain	1.9–24.1 (12.7, 10)	8.6–50 (29.3, 10)
Kidney	8.9–28.1 (20.4, 10)	14.5–83.4 (30.6, 10)
Liver	14.9–241 (50.2, 10)	23.1–231 (95.7, 10)
Urine	1.8–201 (40.8, 6)	1.0–7.1 (4.0, 6)

[Sticht, Kaferstein 1980].

In an overdose involving the ingestion of >3 g of brallobarbital (in combination with quinalbarbital), plasma concentrations of 106 mg/L of brallobarbital and 57 mg/L of quinalbarbital were reported 6 h and 4 h, respectively, after ingestion; the subject slowly recovered after treatment by haemoperfusion [de Groot *et al.* 1979].

Coudore F *et al.* (1993). Rapid toxicological screening of barbiturates in plasma by wide-bore capillary gas chromatography and nitrogen-phosphorus detection. *J Anal Toxicol* 17: 109–113.

de Groot G *et al.* (1979). An evaluation of the use of hemoperfusion in acute poisoning. *Vet Hum Toxicol* 21: 8–11.

Sticht G, Kaferstein H (1980). [Results of toxicological investigations on vesparax-poisonings (author's transl)]. *Z Rechtsmed* 85: 169–175.

Bretylium Tosilate

Antiarrhythmic, Antihypertensive

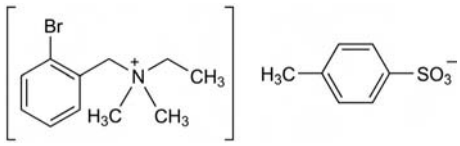
$C_{11}H_{17}BrN,C_7H_7O_3S = 414.4$

CAS—59-41-6 (bretylium); 61-75-6 (tosilate)

IUPAC Name (2-Bromophenyl)methyl-ethyl-dimethylazanium; 4-methylbenzenesulfonate

Synonyms Bretylium tosylate; 2-bromo-*N*-ethyl-*N*,*N*-dimethylbenzenemethanaminium 4-methylbenzenesulfonate.

Proprietary Names *Bretylate*; *Bretylol*.

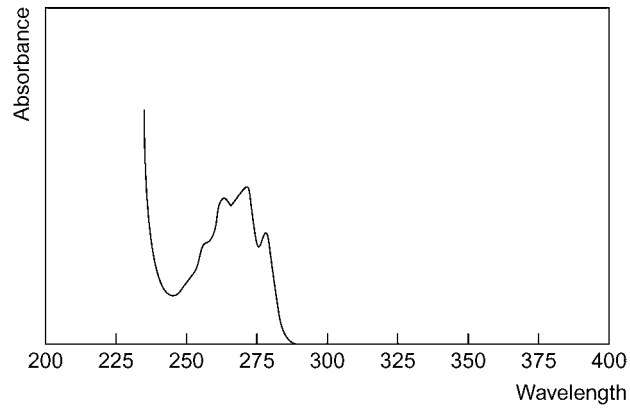


Chemical Properties A white crystalline powder. Mp 97° to 99°. Soluble 1 in 1 of water and 1 in 0.4 of ethanol; practically insoluble in ether, ethyl acetate and hexane.

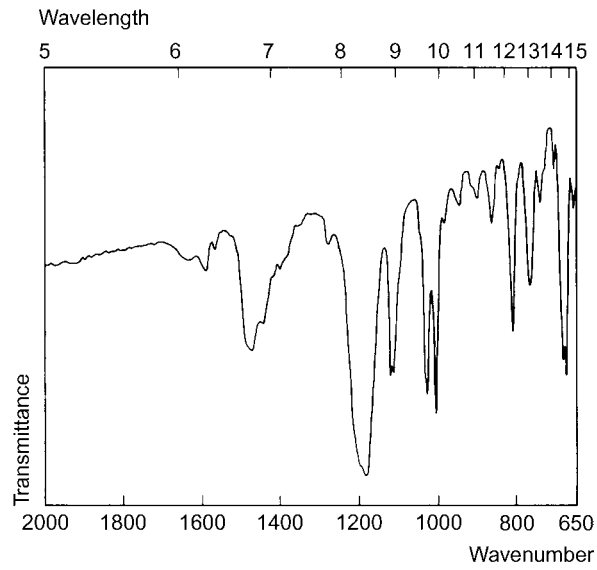
Thin-layer Chromatography System TA—R_f 0.01; system TN—R_f 0.94; system TO—R_f 0.40 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HA—*k* 4.3 (tailing peak); system HY—RI 275.

Ultraviolet Spectrum Aqueous acid—263, 272 (A^{1}_{1}=22a), 278 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1188, 1012, 1032, 1120, 680, 815 cm^{-1} (KBr disk).



Quantification

Plasma **GC** ECD. Limit of detection, 5 µg/L [Lai *et al.* 1980]. ECD. Limit of detection, 1 µg/L [Patterson *et al.* 1980].

Urine **GC** See Plasma [Lai *et al.* 1980]. See Plasma [Patterson *et al.* 1980].

Myocardial Tissue **GC** See Plasma [Patterson *et al.* 1980].

Disposition in the Body Bretylium tosilate is poorly absorbed after oral administration. Excreted mainly in the urine as unchanged drug but a high proportion of bretylium is also excreted into the bile. After IV infusion about 70% of the dose is excreted in the urine in 24 h and 90% in 48 h. After chronic oral dosing about 20% of a dose is excreted in the 24-hour urine.

Therapeutic Concentration

After a single oral dose of 5 mg/kg to 10 subjects, peak serum concentrations of 0.04 to 0.14 (mean 0.08) mg/L were attained in 1 to 8 h; following IV infusion of 5 mg/kg over 30 min to the same subjects, serum concentrations at the end of the infusion were reported to be in the range 1.1 to 2.4 (mean 1.9) mg/L [Anderson *et al.* 1980].

Bioavailability About 25%.

Half-life Plasma half-life, about 7 to 10 h, increased in renal impairment.

Volume of Distribution About 8 L/kg.

Clearance Plasma clearance, about 11 mL/min/kg.

Protein Binding Not significantly bound.

Note For further information on bretylium tosilate, see Garrett *et al.* [1982].

Dose 5 to 10 mg/kg by IM injection. Bretylium tosilate has been given orally in doses of 0.3 to 1.2 g daily.

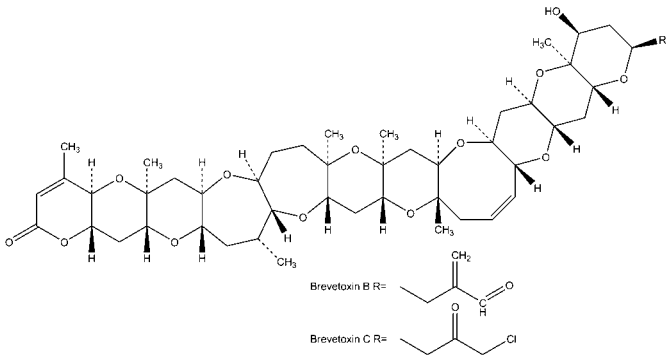
Anderson JL *et al.* (1980). Oral and intravenous bretylium disposition. *Clin Pharmacol Ther* 28: 468–478.

Garrett ER *et al.* (1982). Bretylium pharmacokinetics and bioavailabilities in man with various doses and modes of administration. *Biopharm Drug Disp* 3: 129–164.
Lai CM *et al.* (1980). GLC determination of bretylium in biological fluids. *J Pharm Sci* 69: 681–683.
Patterson E *et al.* (1980). Sensitive gas chromatographic assay for the quantitation of bretylium in plasma, urine and myocardial tissue. *J Chromatogr* 181: 33–39.

Brevetoxin

Sodium Channel Activator, Neurotoxin

Synonym BTX



Chemical Properties Brevetoxin is produced by the 'red-tide' dinoflagellate *Ptychodiscus brevis* Davis (*Karenia brevis,* formerly known as *Gymnodinium breve* Davis) , prevalent in the Gulf of Mexico and along the Florida coast [O'Neil *et al.* 2006]. One generic chemical structure of brevetoxins consists of an 11-member heterocyclic oxygen-containing fused ring system (or backbone) culminating in an unsaturated lactone at one end and at the other an unsaturated aldehyde (brevetoxin B), a halogen-containing alkyl group (brevetoxin C), an unsaturated alcohol (brevetoxin D), or an ether-ketone group (brevetoxin E). Another ring system includes the compound brevetoxin A and consists of a 10-member heterocyclic oxygen-containing ring backbone with an unsaturated lactone at one end and a saturated aldehyde at the other [Viviani 1992]. Several more compounds exist for both ring systems although they are not as commonly encountered. Decomposition of brevetoxins is accelerated in aqueous solution below pH 2 and above pH 10. Aqueous 0.1 mol/L sodium hydroxide degrades brevetoxins in minutes by saponification of the lactone in ring A. Ozonolysis and aqueous chlorine degrade brevetoxins by addition and cleavage at double bonds and by oxidation, respectively [Poli *et al.* 1990a].

Brevetoxin A

$C_{49}H_{70}O_{13}$ = 867.1

Synonym GB-1

Chemical Properties Fine prisms from acetonitrile. Mp 197° to 199°, 228° to 220° (double melting point) [O'Neil *et al.* 2006].

Brevetoxin B

$C_{50}H_{70}O_{14}$ = 895.1

Synonyms BTX-B; GB-2; T-34; T-47.

Chemical Properties Needles from acetonitrile. Mp 270° with decomposition [O'Neil *et al.* 2006].

Brevetoxin C

$C_{49}H_{69}ClO_{14}$ = 917.5

Synonym BTX-C

Ultraviolet Spectrum Methanol—208 nm (brevetoxins B and C).

Quantification

Urine **LC-MS** Column: C_{18} (250 × 2.0 mm i.d.). Mobile phase: acetonitrile : water with 0.1% formic acid (20 : 80 to 65 : 35 in 45 min), flow rate 0.2 mL/min. ESI or APCI, positive ion mode. Limit of quantification, 25 ng on-column [Poli *et al.* 2000].

Other **TLC** Rat Faeces. Plates: silica (10 × 20 cm, 0.2 mm thickness). Solvent system: chloroform : ethyl acetate : ethanol (50 : 25 : 25), drying stage and redeveloped in chloroform : ethyl acetate : ethanol (80 : 10 : 10). Radioactivity detection. R_f 0.74 (brevetoxin C). Limit of quantification not reported [Poli *et al.* 1990b].

HPLC Rat Skeletal Muscle. Column: C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : water (40 : 60 for 5 min to 85 : 15 over 10 min for 15 min), flow rate 0.8 mL/min. Radioactivity detection. Retention time: 22.5 min. Limit of quantification not reported [Poli *et al.* 1990b]. Monkey Buccal Mucosa and Skin. Column: C_{18} (360 × 2.0 mm i.d., 10 µm). Mobile phase: methanol : water (85 : 15), flow rate 0.5 mL/min. UV detection (λ = 215 nm). Retention time: 4.1 min (brevetoxin C). Limit of quantification not reported [Mehta *et al.* 1991]. Air, Seawater and Seafoam. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (85 : 15), flow rate 1 mL/min. UV detection (λ = 215 nm). Limit of quantification not reported [Pierce *et al.* 2003].

LC-MS *K. brevis* Cultures, Oysters. Column: C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: water : acetonitrile with 0.1% acetic acid (50 : 50 for 2 min to 20 : 80 over 20 min, to 5 : 95 over 1 min for 6 min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification not reported [Twiner *et al.* 2007]. Air and Seawater. Column: C_{18} (75 × 2.0 mm i.d., 3 µm). Mobile phase: water : 1 mmol/L ammonium acetate in methanol (80 : 20 for 1 min to 10 : 90 over 2 min for 7 min), flow rate 0.15 to 0.20 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification not reported [Cheng *et al.* 2005]. *K. brevis* Cultures, Oysters. Column: C_{18} (150 × 2.0 mm i.d.). Mobile phase: water : acetonitrile with 0.1% acetic acid (65 : 35 for 2 min to 20 : 80 over 28 min to 5 : 95 over 5 min for 15 min), flow rate 0.2 mL/min. Limit of quantification, 0.15 ng on-column (brevetoxin C) [Abraham *et al.* 2006; Plakas *et al.* 2004; Wang *et al.* 2004]. Rat Liver Microsomes. Column: C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : water (80 : 20 for 2.5 min, to 90 : 10 over 1 min), flow rate 0.2 mL/min. ESI, positive ion mode, CID. Retention time: brevetoxin B, 8.5 min; brevetoxin D, 7.8 min. Limit of quantification not reported [Wang *et al.* 2005]. Rat Blood and Urine. Column: C_{18} (150 × 2.0 mm i.d.). Mobile phase: water : acetonitrile with 0.1% acetic acid (65 : 35 for 2 min to 20 : 80 over 28 min to 5 : 95 over 5 min for 15 min), flow rate 0.2 mL/min. Limit of quantification, 0.15 ng on-column [Radwan *et al.* 2005].

CE Fish Samples. Column: Bare silica capillary (50 cm × 75 µm i.d.). Buffer: 10 mmol/L disodium borate : 30 mmol/L SDS : 10% methanol (pH 9.3). Fluorescence or UV detection (λ = 214 nm). Limit of quantification not reported [Shea 1997].

Note For the detection of brevetoxins using immunoassay methodology, see Poli *et al.* [2007] and Woofter *et al.* [2003].

Toxicity Brevetoxins, which are lipid soluble, act as depolarising substances in the membrane of the excitable cells, opening membrane channels permeable to sodium, leading to a sodium influx and causing neurotoxic poisoning.

One man, aged 49, and his 2 young children (3 and 4 years old) ate boiled whelks contaminated with brevetoxin. Urine samples were collected from the children ~3 h post-ingestion and the presence of brevetoxin was confirmed, with concentrations reported as 42 µg/L and 117 µg/L. No brevetoxin was detected 4 days later at follow-up examination. Total brevetoxin content in the whelks was measured and reported as 24.5 mg/kg [Poli *et al.* 2000].

Protein Binding Brevetoxin has a 50 to 100% preference to bind to high density lipoprotein over other lipoprotein classes, which is consistent with the difference in surface lipid to core lipid ratio of high density lipoprotein and high density lipoprotein particles. The binding of brevetoxin to the plasma remnants, of which albumin and α-glycoprotein represent the predominant components, is a third less than any of the lipoproteins. Brevetoxin in plasma can, therefore, be distributed by lipoproteins either to tissues for storage and/or biological action or to the liver for metabolism [Woofter, Ramsdell 2007].

Note For a study of the distribution of brevetoxin to lipoproteins in human plasma, see Woofter and Ramsdell [2007]. For a distribution and elimination study of brevetoxins in rats, see Poli *et al.* [1990b]. For a study on the metabolism of brevetoxin in rats, see Wang *et al.* [2005] and Radwan and Ramsdell [2006].

Dose Used as tools in neurochemical research.

Abraham A *et al.* (2006). Characterization of polar brevetoxin derivatives isolated from *Karenia brevis* cultures and natural blooms. *Toxicon* 48: 104–115.
Cheng YS *et al.* (2005). Concentration and particle size of airborne toxic algae (brevetoxin) derived from ocean red tide events. *Environ Sci Technol* 39: 3443–3449.
Mehta M *et al.* (1991). In vitro penetration of tritium-labelled water (THO) and [^{3}H]PbTx-3 (a red tide toxin) through monkey buccal mucosa and skin. *Toxicol Lett* 55: 185–194.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.
Pierce RH *et al.* (2003). Brevetoxin concentrations in marine aerosol: human exposure levels during a *Karenia brevis* harmful algal bloom. *Bull Environ Contam Toxicol* 70: 161–165.
Plakas SM *et al.* (2004). Brevetoxin metabolism and elimination in the Eastern oyster (*Crassostrea virginica*) after controlled exposures to *Karenia brevis*. *Toxicon* 44: 677–685.
Poli MA *et al.* (1990a). Detection, metabolism, and pathophysiology of brevetoxins. In: Hall S, Strichartz G, eds. *Marine Toxins: Origin, Structure, and Molecular Pharmacology.* Washington DC: American Chemical Society, pp. 176–191.
Poli MA *et al.* (1990). Distribution and elimination of brevetoxin PbTx-3 in rats. *Toxicon* 28: 903–910.
Poli MA *et al.* (2000). Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. *Toxicon* 38: 981–993.
Poli MA *et al.* (2007). An electrochemiluminescence-based competitive displacement immunoassay for the type-2 brevetoxins in oyster extracts. *J AOAC Int* 90: 173–178.
Radwan FF, Ramsdell JS (2006). Characterization of in vitro oxidative and conjugative metabolic pathways for brevetoxin (PbTx-2). *Toxicol Sci* 89: 57–65.
Radwan FF *et al.* (2005). Identification of a rapid detoxification mechanism for brevetoxin in rats. *Toxicol Sci* 85: 839–846.
Shea D (1997). Analysis of brevetoxins by micellar electrokinetic capillary chromatography and laser-induced fluorescence detection. *Electrophoresis* 18: 277–283.
Twiner MJ *et al.* (2007). Extraction and analysis of lipophilic brevetoxins from the red tide dinoflagellate *Karenia brevis*. *Anal Biochem* 369: 128–135.
Viviani R (1992). Eutrophication, marine biotoxins, human health. *Sci Total Environ* ,631–662.
Wang Z *et al.* (2004). LC/MS analysis of brevetoxin metabolites in the Eastern oyster (*Crassostrea virginica*). *Toxicon* 43: 455–465.
Wang W *et al.* (2005). Characterization of rat liver microsomal and hepatocytal metabolites of brevetoxins by liquid chromatography–electrospray tandem mass spectrometry. *Anal Bioanal Chem* 383: 67–75.
Woofter RT, Ramsdell JS (2007). Distribution of brevetoxin to lipoproteins in human plasma. *Toxicon* 49: 1010–1018.
Woofter R *et al.* (2003). Measurement of brevetoxin levels by radioimmunoassay of blood collection cards after acute, long-term, and low-dose exposure in mice. *Environ Health Perspect* 111: 1595–1600.

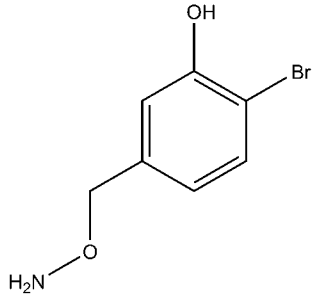
Brocresine

Histidine Decarboxylase Inhibitor

$C_7H_8BrNO_2$ = 218.0

CAS—555-65-7

IUPAC Name 5-(Aminooxymethyl)-2-bromophenol
Synonyms 5-Amino-oxymethyl-2-bromophenol; CL 54998; NSD 1055.
Proprietary Name *Contramine* is a phosphate.

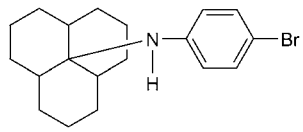


Chemical Properties White crystalline powder. Soluble in dilute acetic acid and chloroform. Brocresine is extracted by chloroform from aqueous alkaline solutions.
Colour Tests Ammonium vanadate test—green (limit of detection, 1.0 µg); Vitali's test—pale yellow/pale yellow/bright yellow (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.75 (location reagent potassium permanganate spray, positive reaction).
Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.
Ultraviolet Spectrum Brocresine in 0.1 N sulfuric acid, maximum at 280 nm (E1%, 1 cm 86); inflexion at 225 nm (E1%, 1 cm 248); minimum at 250 nm.
Infrared Spectrum Principal peaks at wavenumbers A 1412, B 1121, C 1028 or 1285 cm^{-1} (KBr disk).
Disposition in the Body Brocresine is rapidly cleared from the plasma and/or metabolised. The inhibitory activity of the blood plasma disappears 2 h after administration [Ellenbogen *et al.* 1971].

Ellenbogen L *et al.* (1971). Assay and rapid disappearance of brocresine activity in blood. *Biochem Pharmacol* 20: 1501–1506.

Bromantane

Psychostimulant
$C_{16}H_{20}BrN$ = 306.2
CAS—87913-26-6
Synonyms Bromantan; *N*-(4-bromophenyl)tricyclo[3.3.1.13,7]decan-2-amine



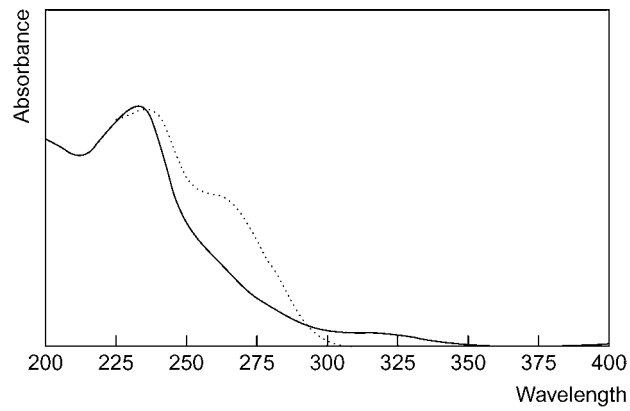
Disposition in the Body
Toxicity LD_{50} (oral) in rats >10000 mg/kg [Iezhitsa *et al.* 2002].
Use Bromantane is used as a performance-enhancing agent for muscles in athletes and as an immunostimulant [Burnat *et al.* 1997].

Burnat P *et al.* (1997). Bromontan, a new doping agent. *Lancet* 350: 963–964.
Iezhitsa IN *et al.* (2002). Toxic effect of single treatment with bromantane on neurological status of experimental animals. *Bull Exp Biol Med* 133: 380–383.

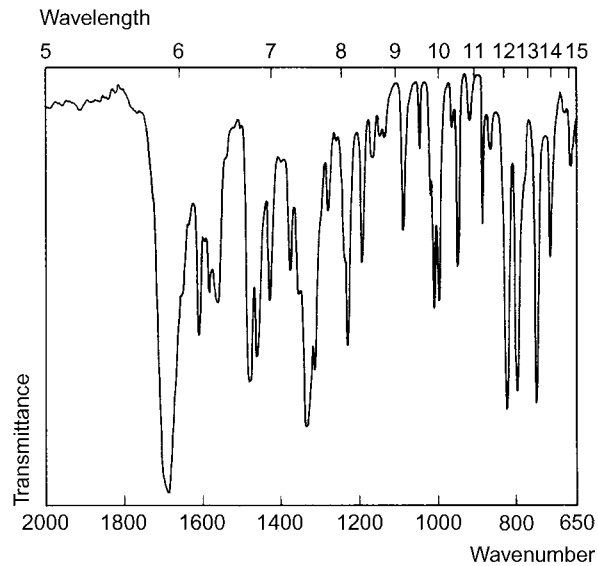
Bromazepam

Anxiolytic, Benzodiazepine, Tranquilliser
$C_{14}H_{10}BrN_3O$ = 316.2
CAS—1812-30-2
IUPAC Name 7-Bromo-5-pyridin-2-yl-1,3-dihydro-1,4-benzodiazepin-2-one
Synonyms Bromazepamas; bromazepamum; 7-bromo-1,3-dihydro-5-(2-pyridinyl)-2*H*-1,4-benzodiazepin-2-one; Ro-5-3350.
Proprietary Names *Compendium*; *Creosedin*; *Durazanil*; *Lectopam*; *Lexomil*; *Lexotan*; *Lexotanil*; *Normoc.*

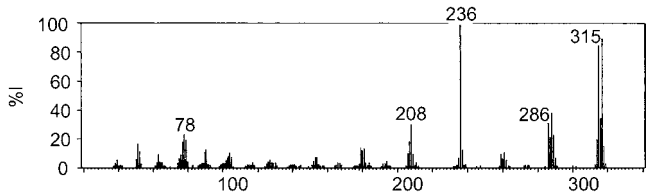
Chemical Properties A white or yellowish crystalline powder. Mp 237° to 238.5° with decomposition. Practically insoluble in water, sparingly soluble in alcohol and in dichloromethane. pK_a 2.9, 11.0. Log *P* (octanol/water), 2.05. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].
Colour Test Formaldehyde–sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.06; system TC—R_f 0.41; system TD—R_f 0.13; system TE—R_f 0.63; system TF—R_f 0.18; system TL—R_f 0.53; system TAD—R_f 0.47; system TAE—R_f 0.73; system TAF—R_f 0.69; system TAJ—R_f 0.34; system TAK—R_f 0.04; system TAL—R_f 0.63.
Gas Chromatography System GA—bromazepam RI 2665; M (3-OH-) RI2470; system GB—bromazepam RI 2760; bromazepam-TMS RI 2702; M (3-OH-)-TMS_2 RI 2650; system GG—RI 3280.
Column: DB-17 (30 m × 0.32 mm, 0.25 µm). Temperature programme: 150° for 1 min to 230° for 5 min to 300° at 10°/min for 9 min. Injector and detector temperatures 270° and 300°, respectively. Carrier gas: He (pre-column pressure, 80 kPa). ECD. Retention time: 18 min [Guan *et al.* 1999].
Gas Chromatography-Mass Spectrometry Column: HP5-MS 5% phenyl 95% siloxane (30 m × 0.25 mm, 0.25 µm). Temperature programme: 60° for 1 min to 295° at 30°/min for 6 min. Injector temperature: 250°. Carrier gas: He, 1 mL/min. NCI. Retention time: 9.65 min [Cirimele *et al.* 1997].
High Performance Liquid Chromatography System HI—*k* 2.32; system HK—*k* 2.99; system HX—RI 397; system HY—RI 331; system HZ—RT 3.0 min; system HAA—RT 14.7 min; system HAF—RT 6.6 (tailing peak) min; system HAG—RT 25.5 (tailing peak) min; system HAX—RT 5.8 min; system HAY—RT 5.1 min; system HBH—*k* 1.63; system HBI—*k* 0.80; system HAL—RT 8.1 min; system HAM—not detected.
Column: RP C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: water : acetonitrile : TEA (700 : 300 : 4, pH 7.4), flow rate 2 mL/min . UV detection (λ = 240 nm). Retention time: bromazepam, 2.05 min, α-hydroxytriazolam (I.S.), 3.16 min [Le Solleu *et al.* 1993].
Ultraviolet Spectrum Aqueous acid—239, 345 nm; aqueous alkali—237 nm (A_1^1 = 920b), 348 nm; methanol—233 nm (A_1^1 = 1050b), 320 nm (A_1^1 = 61b)



Infrared Spectrum Principal peaks at wavenumbers 1685, 825, 750, 802, 1315, 1230 cm^{-1}.



Mass Spectrum Principal ions at m/z 236, 317, 315, 288, 316, 286, 208, 78 (bromazepam); 79, 78, 52, 105, 304, 314, 316, 51 (3-hydroxybromazepam).



Quantification

Blood **GC** Column: SE-54 fused silica 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 μm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD. Retention time: 6.99 min. [Lillsunde, Seppälä 1990].

GC-MS Column: DB5-MS (20 m × 0.18 mm i.d., 0.18 μm). Temperature programme: 70° for 0.5 min to 320° at 40°/min for 1 min. TOF-MS. Limit of detection, 0.63 ng [Aebi *et al.* 2002]. Column: HP-1 (13 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 60 kPa. Temperature programme: 100° to 250° at 30°/min to 300° at 20°/min for 5 min. SIM acquisition mode (*m/z* 317). Limit of detection, 2–5 ng/g [Zhang *et al.* 1996].

LC-MS Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium acetate (pH 4.75) : acetonitrile : methanol (90 : 5 : 5 to 50 : 25 : 25 at 7 min to 10 : 45 : 45 at 27 min for 3 min to 90 : 5 : 5 at 31 min), flow rate 0.45 mL/min. DAD-ESI detection. Limit of detection, 6 μg/L [Dussy *et al.* 2006]. Column: Uptisphere ODB 18. Mobile phase: 2 mmol/L formate buffer and acetonitrile. Limit of detection, <2 pg/mg [Chèze *et al.* 2005]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: methanol : 0.006 mol/L formic acid (pH 3.0, 30 : 70 to 40 : 60 at 5 min to 50 : 50 at 25 min to 40 : 60 at 30 min to 60 : 40 at 35 min to 30 : 70 at 36 min for 9 min), flow rate 0.2 mL/min. Limit of quantification, 4.3 μg/L, limit of detection, 1.3 μg/L [Smink *et al.* 2004].

Plasma **GC** See Blood [Lillsunde, Seppälä 1990]. ECD. Limit of detection, 5 ng/mL [Klotz 1981].

HPLC Column: Kromasil C(8) (250 × 5 mm, 5 μm). Mobile phase: methanol, acetonitrile and 0.05 mol/L ammonium acetate. Limit of quantification, 0.3–20 mg/L [Uddin *et al.* 2008]. Column: Inertsil C_8 (250 × 4 mm, 5 μm). Mobile phase: 0.05 mol/L ammonium acetate, acetonitrile and methanol. UV detection (λ = 240 nm). Limit of detection, 3.3–10.2 ng/20 μL injection volume [Samanidou *et al.* 2007]. MRM acquisition mode. Limit of detection, 1 ng/mL [Goncalves *et al.* 2005]. SIM acquisition mode. Run time, 2.5 min. Limit of detection, 1 ng/mL [Andraus *et al.* 2004]. Column: Genesis C_{18} (100 × 2.1 mm, 4 μm). ESI. Run time 5 min. Limit of quantification, 5 ng/mL [Laurito *et al.* 2004]. See also Le Solleu *et al.* [1993], Boukhabza *et al.* [1989] and Hirayama *et al.* [1983].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.04 mg/L (SIM), limit of detection, 0.01 mg/L (scan) [Kratzsch *et al.* 2004].

Serum **HPLC** Column: C_{18} reversed phase (100 × 4.6 mm, particle size 2 μm) or TSK gel Super-ODS (100 × 4.6 mm, particle size 5 μm). Mobile phase: methanol : 5 mmol/L monobasic sodium phosphate pH 6 (45 : 55), flow rate 0.65 mL/min. UV detection (λ = 254 nm) [Tanaka *et al.* 1996]. See Blood [Dussy *et al.* 2006].

LC-MS Column: Unison UK-C_{18} (150 × 2 mm i.d., 3 μm) or Cadenza CD-C_{18} (150 × 2 mm i.d., 3 μm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 to 20 : 80 at 20 min for 5 min), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 12.6 min. Limit of quantification, 5.2 μg/L, limit of detection, 17.5 μg/L [Nakamura *et al.* 2009].

Urine **GC** Column: DB-17 (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 80 kPa. Temperature programme: 150° for 1 min to 230° at 10°/min for 5 min to 300° at 10°/min for 9 min. ECD. Limit of detection, 160 ng/mL [Guan *et al.* 1999]

HPLC See Plasma [Uddin *et al.* 2008]. Limit of detection, 2.6–12.6/20 μL injection sample [Samanidou *et al.* 2007].

LC-MS See Blood [Chèze *et al.* 2005]. Column: Uptisphere ODB (100 × 2 mm i.d., 5 μm). Mobile phase: acetonitrile : 2 mmol/L formate buffer pH 3 (15 : 85 for 0.5 min to 10 : 90 for 10 min to 85 : 15 for 5 min), flow rate 200 μL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 1 μg/L, limit of detection, 0.1 μg/L [Chèze *et al.* 2004].

Note For a fluorometric method for the detection of bromazepam, see Salem *et al.* [2004].

Adipose Tissue **GC-MS** See Blood [Zhang *et al.* 1996].

Brain **GC-MS** See Blood [Zhang *et al.* 1996].

Hair **LC-MS** See Blood [Chèze *et al.* 2005]. See Urine. Limit of quantification, 5 pg/mg, limit of detection, 1–2 pg/mg [Chèze *et al.* 2004].

Kidney **GC-MS** See Blood [Zhang *et al.* 1996].

Liver **GC-MS** See Blood [Zhang *et al.* 1996].

Lung **GC-MS** See Blood [Zhang *et al.* 1996].

Saliva **HPLC** See Plasma [Uddin *et al.* 2008].

Skeletal Muscle **GC-MS** See Blood [Zhang *et al.* 1996].

Spleen **GC-MS** See Blood [Zhang *et al.* 1996].

Disposition in the Body Bromazepam is well absorbed after oral administration, and peak plasma concentrations are usually achieved within 2 h. Approximately 70% of a dose is excreted in the urine in 72 h, including ~2% of the dose as unchanged bromazepam, ~27% as the glucuronide of 3-hydroxybromazepam, ~40% as the glucuronide of 2-amino-5-bromo-3-hydroxybenzoylpyridine and <1% as 2-(2-amino-5-bromobenz-oyl)pyridine.

Therapeutic Concentration

After a single oral dose of 12 mg, administered to 10 subjects, peak plasma concentrations of 0.11–0.17 mg/L (mean 0.13) were attained in 1–4 h. Steady-state concentrations of 0.08–0.15 mg/L (mean 0.12) were measured during dosing of 6 subjects with 9 mg daily [Kaplan *et al.* 1976].

Administration of bromazepam as a slow-release formulation to 24 healthy subjects resulted in maximum plasma concentrations of 11.05 μg/L at 8 h (dose 3 mg); the corresponding values for a conventional-release preparation were 10.21 μg/L, also at 8 h (2 doses of 1.5 mg) [Lerner *et al.* 2001].

After administration of a single oral dose of 6 mg to 32 healthy subjects, peak serum levels of 132 ng/mL were attained in subjects aged 60–81 years and lower levels (82 ng/mL) in those aged 21–29 years. Compared with the younger age group, the older age group had a smaller volume of distribution (1.44 and 0.88 L/kg, respectively), lower oral clearance (0.76 and 0.41 mL/min/kg, respectively) and increased serum free fraction (28.8% and 34.8%, respectively, unbound) [Ochs *et al.* 1987].

Toxicity

In a 68-year-old woman who was found unconscious and barely breathing, bromazepam intoxication was discovered to be the cause (a serum level of 6 mg/L was detected); normal functions were restored 12 days after the ingestion [Rudolf *et al.* 1998].

A 42-year-old woman ingested 420 mg bromazepam in a suicide attempt and survived despite being found unconscious outdoors in a state of semi-undress and suffering from hypothermia. Approximately 12 h after the ingestion, the blood concentration of bromazepam was 7.7 mg/L [Michaud *et al.* 2001].

In 57 patients who were admitted to hospital ~7.3 h after ingesting ~88 mg bromazepam, mostly with other drugs, average serum levels of bromazepam were 1.87 mg/L and the elimination half-life was ~29 h. Signs of toxicity were not seen at serum levels below 2.3 mg/L [Koyama *et al.* 2003]

Half-life Plasma half-life, 8–19 h (mean 12).

Volume of Distribution Approximately 0.9 L/kg.

Protein Binding 70%.

Dose Usually 3 to 18 mg daily in divided doses; up to a maximum of 60 mg daily in divided doses has been given to hospitalised patients.

Aebi B *et al.* (2002). Quantitation using GC-TOF-MS: example of bromazepam. *Forensic Sci Int* 128: 84–89.

Andraus MH *et al.* (2004). Determination of bromazepam in human plasma by high-performance liquid chromatography with electrospray ionization tandem mass spectrometric detection: application to a bioequivalence study. *J Mass Spectrom* 39: 1348–1355.

Boukhabza A *et al.* (1989). High-performance liquid chromatographic determination of bromazepam in human plasma. *Analyst* 114: 639–641.

Chèze M *et al.* (2004). Determination of bromazepam, clonazepam and metabolites after a single intake in urine and hair by LC–MS/MS. Application to forensic cases of drug facilitated crimes. *Forensic Sci Int* 145: 123–130.

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Cirimele V *et al.* (1997). Screening for forensically relevant benzodiazepines in human hair by gas chromatography-negative ion chemical ionization–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 700: 119–129.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dussy FE *et al.* (2006). Quantification of benzodiazepines in whole blood and serum. *Int J Legal Med* 120: 323–330.

Goncalves JC *et al.* (2005). On-line solid-phase extraction coupled with high-performance liquid chromatography and tandem mass spectrometry (SPE-HPLC-MS-MS) for quantification of bromazepam in human plasma: an automated method for bioequivalence studies. *Ther Drug Monit* 27: 601–607.

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Klotz U (1981). Determination of bromazepam by gas–liquid chromatography and its application for pharmacokinetic studies in man. *J Chromatogr* 222: 501–506.

Koyama K *et al.* (2003). [Pharmacokinetics of bromazepam in 57 patients with acute drug intoxication.]. *Chudoku Kenkyu* 16: 51–56.

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Laurito TL *et al.* (2004). Bromazepam determination in human plasma by high-performance liquid chromatography coupled to tandem mass spectrometry: a highly sensitive and specific tool for bioequivalence studies. *J Mass Spectrom* 39: 168–176.

LeSolleu H *et al.* (1993). The determination of bromazepam in plasma by reversed-phase high-performance liquid chromatography. *J Pharm Biomed Anal* 11: 771–775.

Lerner FE *et al.* (2001). Comparative bioavailability of two oral formulations of bromazepam in healthy volunteers. *Arzneimittelforschung* 51: 955–958.

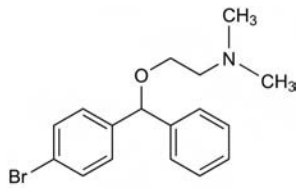
Lillsunde P, Seppälä T (1990). Simultaneous screening and quantitative analysis of benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection. *J Chromatogr* 533: 97–110.

Michaud K *et al.* (2001). Hypothermia and undressing associated with non-fatal bromazepam intoxication. *Forensic Sci Int* 124: 112–114.
Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography-tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.
Ochs HR *et al.* (1987). Bromazepam pharmacokinetics: influence of age, gender, oral contraceptives, cimetidine, and propranolol. *Clin Pharmacol Ther* 41: 562–570.
Rudolf J *et al.* (). [Protracted course of bromazepam poisoning in advanced age.]. *Dtsch Med Wochenschr* 123: 832–834.
Salem AA *et al.* (2004). Spectrophotometric and fluorimetric determination of diazepam, bromazepam and clonazepam in pharmaceutical and urine samples. *Spectrochim Acta A Mol Biomol Spectrosc* 60: 771–780.
Samanidou VF *et al.* (2007). Development of a validated HPLC method for the determination of four 1,4-benzodiazepines in human biological fluids. *J Sep Sci* 30: 679–687.
Smink BE *et al.* (2004). Quantitative analysis of 33 benzodiazepines, metabolites and benzodiazepine-like substances in whole blood by liquid chromatography–(tandem) mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 13–20.
Tanaka E *et al.* (1996). Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-microns porous microspherical silica gel. *J Chromatogr B Biomed Appl* 682: 173–178.
Uddin MN *et al.* (2008). Validation of SPE-HPLC determination of 1,4-benzodiazepines and metabolites in blood plasma, urine, and saliva. *J Sep Sci* 31: 3704–3717.
Zhang XX *et al.* (1996). Sensitive determination of bromazepam in human tissues using capillary gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 677: 111–116.

Bromazine

Antihistamine
$C_{17}H_{20}BrNO$ = 334.3
CAS—118-23-0

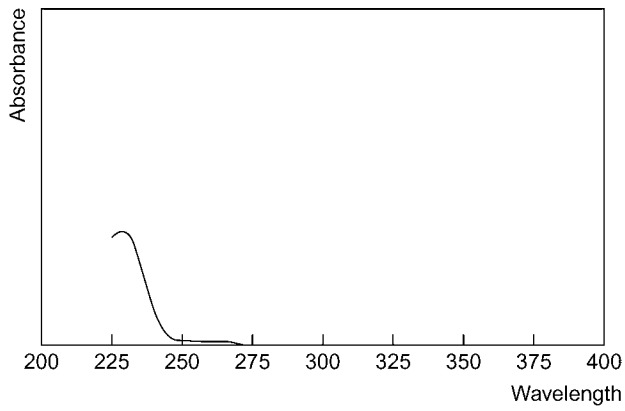
IUPAC Name 2-[(4-Bromophenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine
Synonyms Bromodiphenhydramine; bromdiphenhydramine; histabromamine.



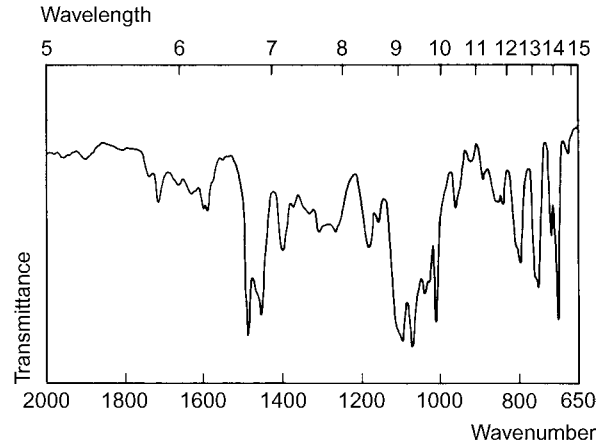
Chemical Properties pK_a 8.6 (25°).

Bromazine Hydrochloride
$C_{17}H_{20}BrNO,HCl$ = 370.7
CAS—1808-12-4
Proprietary Name *Ambodryl*
Chemical Properties A white to pale buff-coloured crystalline powder. Mp 148° to 152°. Soluble 1 in less than 1 of water, 1 in 2 of ethanol, 1 in 2 of chloroform and 1 in 31 of isopropyl alcohol; practically insoluble in ether.

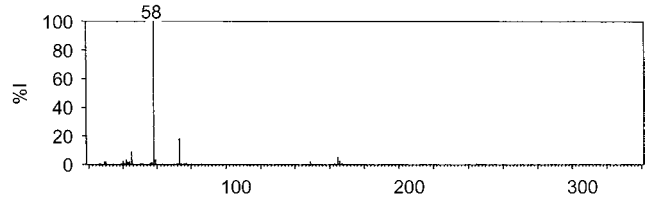
Colour Test Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.44; system TC—R_f 0.43; system TL—R_f 0.13; system TAE—R_f 0.27; system TAF—R_f 0.48 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, yellow).
Gas Chromatography System GA—RI 2155; system GF—RI 2480.
High Performance Liquid Chromatography System HA—*k* 2.7; system HX—RI 444.
Ultraviolet Spectrum Aqueous acid—230 nm (A_1^1=465a).



Infrared Spectrum Principal peaks at wavenumbers 1067, 1094, 1007, 696, 1041, 746 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 73, 45, 165, 59, 42, 166, 149.

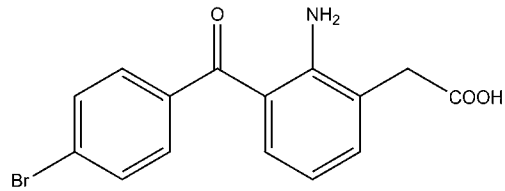


Disposition in the Body Absorbed after oral administration. A number of metabolites have been identified in the urine including the *N*-oxide, monodesmethyl and didesmethyl derivatives, 4-bromobenzhydrol, 4-bromobenzophenone and 4-hydroxybenzophenone; glucuronide conjugates of some of these metabolites have also been reported. Unchanged drug is also excreted in the urine.
Dose 75 to 100 mg of bromazine hydrochloride daily.

Bromfenac

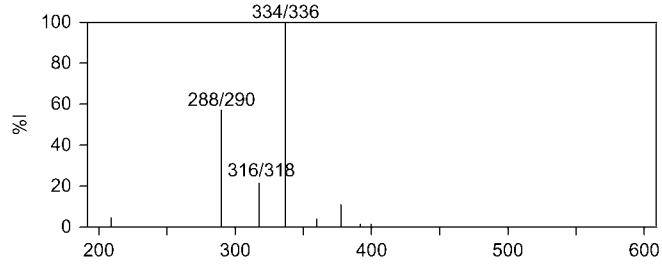
NSAID, Phenylacetic Acid Derivative
$C_{15}H_{12}BrNO_3$ = 334.2
CAS—91714-94-2

IUPAC Name 2-[2-Amino-3-(4-bromobenzoyl)phenyl]acetic acid
Synonyms AHR-10282; 2-amino-3-(4-bromobenzoyl)benzeneacetic acid.



Bromfenac Sodium
$C_{15}H_{11}BrNNaO_3,1^1/_2H_2O$ = 383.2
CAS—120638-55-3 (sesquihydrate); 91714-93-1
Synonym AHR-10282B
Proprietary Names *Bronuck*; *Duract*; *Xibrom*.
Chemical Properties Bright orange-yellow crystalline powder. Mp 284° to 286°, with decomposition. Soluble in water, methanol and in dilute base; insoluble in chloroform and in dilute acid. pK_a 4.29 [O'Neil *et al.* 2006].

Thin-layer Chromatography Solvent system: chloroform : acetone (80 : 20). Detection: Bratton-Marshall reagent. R_f 70 (acid hydrolysis product) [Holland, Schutz 1989].
Mass Spectrum



Quantification

Plasma **HPLC** Column: Hichrom (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (pH 4.9; 10 : 90 to 45 : 55 over 50 min for 10 min), flow rate 1.0 mL/min. UV detection (λ = 270 nm). Retention time: ~42 min. Limit of quantification not reported [Osman *et al.* 1998]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.05 mol/L sodium acetate buffer (pH 6.5) : acetonitrile : tetrahydrofuran (55 : 39 : 6), flow rate 1.5 mL/min. UV detection (λ = 270 nm). Retention time: ~9.5 min. Limit of quantification, 30 µg/L [Osman *et al.* 1989].

LC-MS Column: C_{18} DB (250 × 2.0 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (pH 4.9; 10 : 90 to 45 : 55 over 50 min for 10 min), flow rate 0.2 mL/min. ESI, negative ion mode. Limit of quantification not reported [Osman *et al.* 1998].

Urine **HPLC** Column: Hichrom (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (pH 4.9; 10 : 90 to 45 : 55 over 50 min for 10 min)), flow rate 1.0 mL/min. UV detection (λ = 270 nm). Retention time: ~42 min. Limit of quantification not reported [Osman *et al.* 1998].

LC-MS Column: C_{18} DB (250 × 2.0 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (pH 4.9; 10 : 90 to 45 : 55 over 50 min for 10 min), flow rate 0.2 mL/min. ESI, negative ion mode. Limit of quantification not reported [Osman *et al.* 1998].

Disposition in the Body Rapidly absorbed after oral administration in the fasted state. When administered with food, less drug is absorbed and the peak plasma concentration decreases by ≈25%. The major metabolic pathway involves hepatic biotransformation via oxidative and conjugative mechanisms. One third of an orally administered dose is subject to first-pass metabolism in the liver and gut wall. Approximately 80% of a dose is excreted in the urine within 24 h, with the majority eliminated in the first 8 h. Urinary metabolites include a cyclic amide and 2 pairs of diastereomeric glucuronides of the hydroxylated cyclic amide. No metabolites have been observed in plasma.

Therapeutic Concentration

Sixteen healthy volunteers were administered 50 mg of bromfenac orally every 8 h for 3 days. Doses were given 30 min before or 2 h after food. The mean peak plasma concentration was 4.8 mg/L after 0.7 h. The concomitant administration of 300 mg phenytoin for 7 days resulted in a 40% fall in the bromfenac peak plasma concentration [Gumbhir-Shah *et al.* 1997].

A single drop of bromfenac ophthalmic solution (0.09%) was administered to 54 patients before cataract surgery. The mean peak aqueous humour concentration was 78.7 µg/L at between 2.5 and 3 h after dosing [Donnenfeld, Donnenfeld 2006].

A single oral dose of 50 mg bromfenac A was administered to 40 patients in the fasted state who had varying degrees of impaired kidney function. Pharmacokinetic parameters were reported as follows:

Treatment group	*Plasma bromfenac (mg/L)*	*Time (h)*	*Half-life (h)*	*Clearance (L/h/kg)*	*Volume of distribution (L/kg)*
Normal	3.4	0.8	0.9	0.13	0.15
Renal impairment	3.9	0.7	1.8	0.12	0.22
Dialysis	3.3	0.6	1.7	0.17	0.24

Total urinary excretion of unchanged bromfenac was less than 2% of the administered dose [Ermer *et al.* 1997].

Toxicity The oral formulation has been withdrawn from the market following reports of severe and sometimes fatal hepatic failure. For a case study of severe hepatotoxicity associated with bromfenac use, see Moses *et al.* [1999].

Bioavailability Approximately 67%.

Half-life 30 min to 4 h (with most studies reporting <1 h).

Volume of Distribution Apparent, 0.15 to 0.36 L/kg.

Clearance 0.12 to 9.6 L/h.

Distribution in Blood Blood : plasma ratio 0.2 to 0.3.

Protein Binding Extensively (>99%) bound to albumin.

Dose Used as 0.1% eye drops for ocular pain and inflammation, including postoperative inflammation in patients who have undergone cataract extraction.

Donnenfeld ED, Donnenfeld A (2006). Global experience with Xibrom (bromfenac ophthalmic solution) 0.09%: the first twice-daily ophthalmic nonsteroidal anti-inflammatory drug. *Int Ophthalmol Clin* 46: 21–40.

Ermer JC *et al.* (1997). Bromfenac disposition in patients with impaired kidney function. *Clin Pharmacol Ther* 61: 312–318.

Gumbhir-Shah K *et al.* (1997). Evaluation of pharmacokinetic interaction between bromfenac and phenytoin in healthy males. *J Clin Pharmacol* 37: 160–168.

Holland EM, Schutz H (1989). Screening des neuen analgetikums bromfenac. *Arzneimittelforschung* 39: 831–832.

Moses PL *et al.* (1999). Severe hepatotoxicity associated with bromfenac sodium. *Am J Gastroenterol* 94: 1393–1396.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Osman MA *et al.* (1989). Determination of bromfenac in plasma by high-performance liquid chromatography. *J Chromatogr* 489: 452–458.

Osman M *et al.* (1998). Metabolic disposition of ^{14}C-bromfenac in healthy male volunteers. *J Clin Pharmacol* 38: 744–752.

Bromhexine

Mucolytic Expectorant

$C_{14}H_{20}Br_2N_2$ = 376.1

CAS—3572-43-8

IUPAC Name 2,4-Dibromo-6-[[cyclohexyl(methyl)amino]methyl]aniline

Synonym 2-Amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzenemethanamine

Chemical Properties Log *P* (octanol/water), 4.9.

Bromhexine Hydrochloride

$C_{14}H_{20}Br_2N_2$,HCl = 412.6

CAS—611-75-6

Synonym NA-274

Proprietary Names *Auxit*; *Bisolvon*; *Broncokin*; *Dakryo Biciron*; *Ophtosol*; *Quentan*. It is an ingredient of *Alupent Expectorant* and *Bisolvomycin*.

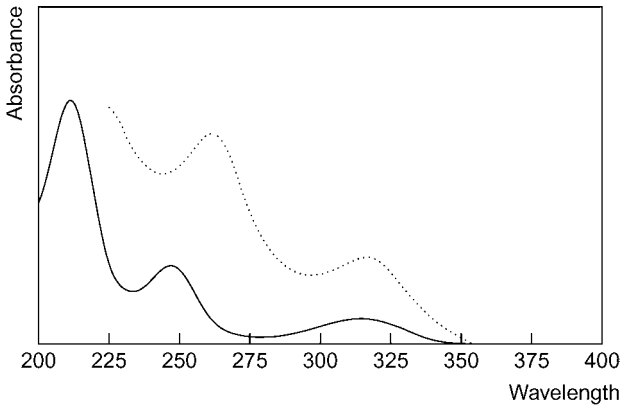
Chemical Properties A white crystalline powder. Mp about 235°. Soluble 1 in 250 of water, 1 in 100 of ethanol, 1 in 300 of chloroform and 1 in 50 of methanol; practically insoluble in acetone; soluble in glacial acetic acid.

Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.67; system TC—R_f 0.79; system TL—R_f 0.71; system TAE—R_f 0.84; system TAJ—R_f 0.98; system TAK—R_f 0.28; system TAL—R_f 0.78 (acidified iodoplatinate solution, positive).

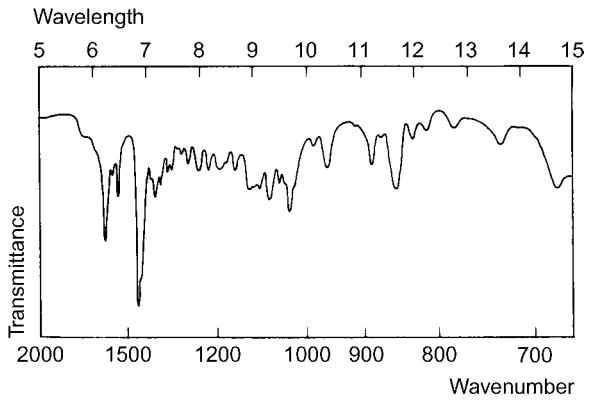
Gas Chromatography System GA—RI 2337.

High Performance Liquid Chromatography System HA—*k* 0.4; system HX—RI 417; system HY—RI 334; system HZ—retention time 7.5 min.

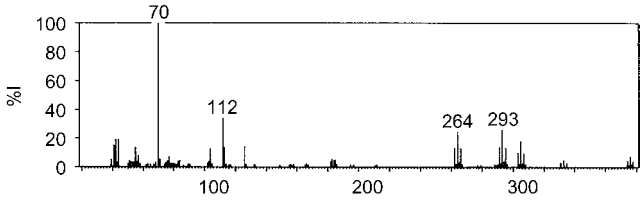
Ultraviolet Spectrum Aqueous acid—245 (A^{1}_{1}=259a), 310; aqueous alkali—262, 317 nm.



Infrared Spectrum Principal peaks at wavenumbers 1602, 1031, 1074, 1548, 1122, 857 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 70, 112, 293, 264, 44, 42, 305, 41.



Quantification
Plasma **GC** ECD. Limit of detection, 1 µg/L [DeLeenheer, Vandecasteele-Thienpont 1980].

HPLC UV detection. Limit of detection, 4 µg/L [Bechgaard, Nielsen 1982].
Urine **HPLC** UV detection. Limit of detection, 2 µg/L [Bechgaard, Nielsen 1982].
Disposition in the Body Bromhexine is well absorbed after oral administration but undergoes considerable first-pass metabolism including conjugation with glucuronic acid or sulfate; ambroxol, *trans*-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol, is an active metabolite of bromhexine. About 70% of an oral dose is excreted in the urine in 24 h as metabolites with <1% as unchanged drug.
Therapeutic Concentration
Following single oral doses of 32 mg to 10 subjects, peak plasma concentrations of 0.01 to 0.14 (mean 0.04) mg/L were attained in 0.5 to 2.5 h [Bechgaard, Nielsen 1982].
Half-life Derived from plasma and urinary data, about 6 h.
Dose 24 to 64 mg of bromhexine hydrochloride daily.

Bechgaard E, Nielsen A (1982). Determination of bromhexine in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 228: 392–397.
Bechgaard E, Nielsen A (1982). Bioavailability of bromhexine tablets and preliminary pharmacokinetics in humans. *Biopharm Drug Disp* 3: 337–344.
De Leenheer AP, Vandecasteele-Thienpont LM (1980). Electron-capture GLC determination of bromhexine in human plasma. *J Pharm Sci* 69: 99–100.

Bromides

Anion

Organic bromide compounds such as the fumigant methyl bromide and the sedative agent carbromal, which give rise to inorganic bromide following ingestion, are dealt with in separate monographs.

Ammonium Bromide

BrH_4N = 97.9
CAS—12124-97-9
Chemical Properties White, odourless, slightly hygroscopic crystals or granules, slowly becomes yellowish in air. Sublimes at high temperature without melting. Freely soluble in water, methanol, ethanol and acetone; slightly soluble in ether; practically insoluble in ethyl acetate. Used in the manufacture of photographic film, plates and papers; in process engraving and lithography; for fireproofing of wood; in corrosion inhibitors; as a sedative.

Calcium Bromide

Br_2Ca = 199.9
CAS—7789-41-5
Chemical Properties Odourless, deliquescent granules or rhombic crystals; becomes yellow on long exposure to air. Mp 730° (anhydrous). Very soluble in water, methanol and ethanol; soluble in acetone; practically insoluble in dioxane, chloroform and ether. Used in photography, manufacture of mineral waters, fire extinguishing compositions, as a sedative, anticonvulsant.

Potassium Bromide

BrK = 119.0
CAS—7758-02-3
Chemical Properties Colourless crystals or white granules or powder. Mp 730°. Very soluble in water and glycerol. Used in the manufacture of photographic papers and plates, in process engraving, as a sedative, anticonvulsant.

Sodium Bromide

BrNa = 102.9
CAS—7647-15-6
Proprietary Name *Sedoneural*
Chemical Properties White crystals, granules or powder. Mp 755°. Very soluble in water and methanol; soluble in alcohol. Used in photography; as a sedative, hypnotic, anticonvulsant.

Colour Tests Applicable to urine, gastric contents and scene residues. To 1 mL of a clear test solution, add 0.1 mL of 2 mol/L nitric acid. Mix for 5 s and add 0.1 mL of 10 g/L aqueous silver nitrate solution. Centrifuge and add 0.1 mL of concentrated ammonium hydroxide—A white precipitate that dissolves in ammonium hydroxide indicates chloride; an off-white precipitate sparingly soluble in ammonium hydroxide indicates bromide, and a creamy-yellow insoluble precipitate indicates iodide. Limit of detection, 50 mg/L.

Confirmatory test. To a 1 mL portion of the clear test sample add 5 mL of chlorine solution and 3 mL of chloroform.—A yellow colour, which is extracted into the chloroform layer, indicates the presence of bromide; iodides give a violet colour and chlorides do not react. Limit of detection, 50 mg/L

Quantification
Specimen Collection Blood—10 mL, heparinised or EDTA tube; urine—20 mL plastic universal container; gastric contents (useful for postmortem examinations).
Note For a method for the detection of bromide in biological fluids using ion-sensitive electrodes, see Poser *et al.* [1974].
Blood **GC** Column: 60/80 Carbopak B/1% SP-1000. Carrier gas: He, 38 mL/min. Temperature programme: 45° for 3 min to 200° at 8°/min for 5 min. Retention time: 2.6 min. Limit of detection not reported [Michalodimitrakis *et al.* 1997].
Plasma **HPLC** Column: Hypersil APS (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.03 mol/L phosphate buffer (pH 2.8). UV detection (λ = 214 nm). Limit of detection, 1 ng [Goewie, Hogendoorn 1985].
Serum **GC** Column: Chromosorb G HP (2 m × 3 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 140°. Limit of detection, 1.2 nmol/L [Maros *et al.* 1989]. Column: 3% OV-17 Diatomite CQ (2.1 m × 2 mm i.d.). Carrier gas: N_2, 15 mL/min. Temperature programme: 145°. FID. Retention time: 2.1 min for bromo-2,4-dimethylphenol, 5.0 min for 2,3-dibromocyclohexanol. Limit of detection, 10 mg/L [Corina *et al.* 1979].

HPLC Column: Partisil-10 SAX (250 × 4.6 mm i.d.). Mobile phase: 30 mmol/L potassium dihydrogen phosphate, flow rate 0.7 mL/min. UV detection (λ = 195 nm). Limit of detection, 60 µg/L [Miller, Cappon 1984].

CE Capillary: Fused silica (total/effective length 100/87.7 cm × 75 µm i.d.). Mobile phase: 250 mmol/L sodium chloride and 7.5 mmol/L cetyltrimethylammonium chloride (pH 2.2). UV detection (λ = 226 nm). Limit of detection, 0.54 mg/L [Hirokawa *et al.* 2004].
Urine **GC** See Serum. Limit of detection, 10 mg/L [Corina *et al.* 1979].

CE Capillary: Fused silica (100/87.7 cm (total/effective length) × 75 µm i.d.). Mobile phase: 250 mmol/L sodium chloride and 7.5 mmol/L cetyltrimethylammonium chloride (pH 2.2). UV detection (λ= 226 nm). Limit of detection, 0.44 mg/L [Hirokawa *et al.* 2004].
Bile **GC** See Blood [Michalodimitrakis *et al.* 1997].
Brain **GC** See Blood [Michalodimitrakis *et al.* 1997].
Kidney **GC** See Blood [Michalodimitrakis *et al.* 1997].
Liver **GC** See Blood [Michalodimitrakis *et al.* 1997].
Lung **GC** See Blood [Michalodimitrakis *et al.* 1997].
Skin **GC** See Blood [Michalodimitrakis *et al.* 1997].
Spleen **GC** See Blood [Michalodimitrakis *et al.* 1997].
Testis **GC** See Blood [Michalodimitrakis *et al.* 1997].
Adrenal Gland **GC** See Blood [Michalodimitrakis *et al.* 1997].
Milk **HPLC** See Plasma [Goewie, Hogendoorn 1985].
Disposition in the Body Bromide ion is poorly absorbed after oral ingestion. Approximately 4.3% of a dose is excreted daily in the urine. Smaller amounts are excreted in sweat and hair. Bromide ion is not bound to plasma proteins and is not sequestered in cells.
Normal Concentration Blood—<5 mg/L.
Toxicity Acute poisoning with inorganic bromide medicines is rare and has fewer severe consequences than those of chronic poisoning (bromism), which include fatigue, abdominal pain, hallucinations, delirium and coma. Therapeutic concentrations are 750 to 1000 mg/L. Following poisoning with these agents and organic forms that metabolise to inorganic bromide, signs of intoxication develop when serum bromide concentrations exceed 1000 mg/L and in severe cases these may exceed 3000 mg/L [Maes *et al.* 1985; Vaiseman *et al.* 1986].

A 30-year-old woman was admitted to hospital after ingesting 20 calcium bromogalactogluconate effervescent tablets daily for the prior 1.5 months. Her serum bromide concentration was 1717 mg/L (21.5 Eq/L) [Frances *et al.* 2003].

A 30-year-old woman presented with lethargy and fever. Her serum bromide concentration was 19.7 Eq/L following ingestion of 20 mg tablets of dextromethorphan hydrobromide [Hung 2003].

A 12-year-old girl was admitted to hospital with dizziness, nausea, vomiting and uncoordinated movements. Her blood bromide concentration was 202 mg/L. In a barn near her house were approximately forty 50-year-old fire extinguishers containing methyl bromide [Hoizey *et al.* 2002].

A 39-year-old man, a 34-year-old woman, and a 5-year-old girl were found in a state of akinetic mutism. Twenty seven cans of methyl bromide had been damaged in their building a few days before the onset of symptoms. On day 1, the man had a plasma bromide concentration of 72.9 mg/L, which decreased to 6.6 mg/L after dialysis on day 9. The woman had a plasma bromide concentration of 67.8 mg/L on day 1 and also had dialysis on day 9, which decreased her plasma bromide concentration to 7.3 mg/L. The 5-year-old girl had a plasma bromide concentration of 91.5 mg/L on day 1 that decreased to 7.5 mg/L following peritoneal lavage [Yamano *et al.* 2001].

A 36-year-old woman ingested 15 calcium bromogalactogluconate tablets daily prior to being admitted to hospital (recommended daily dose is one to two tablets). Her plasma bromide concentration was 37.5 mEq/L [Danel *et al.* 2001].

A 36-year-old woman was exposed to methyl bromide gas with 0.5% chloropicrin. At postmortem, methyl bromide was detected in the blood (2.9 mg/L), bile (1.7 mg/L), liver (24 mg/kg), and adipose tissue (28 mg/kg) [Horowitz *et al.* 1998].

A 40-year-old man complained of headache, fatigue and loss of concentration. His serum bromide concentration was 3180 mg/L (39.8 mmol/L). Following haemodialysis, his bromide concentration fell to 377 mg/L (4.7 mmol/L). The

patient had drunk a case or 2 of a cola beverage containing brominated vegetable oil daily [Horowitz 1997].

A 43-year-old man inhaled an unknown quantity of methylbromide solution. Postmortem concentrations (mg/kg or mg/L) of methyl bromide and inorganic bromide were as follows:

Specimen	*Methyl bromide*	*Inorganic bromide*
Adrenal gland	3.4	130
Bile	1.2	ND
Brain	3.5	30
Epididymis	1.2	ND
Kidney	2.6	310
Liver	1.9	290
Lung	2.9	410
Peripheral blood	3.3	480
Spleen	ND	460
Subclavian blood	3.8	530
Testis	2.8	120

ND, not determined. [Michalodimitrakis *et al.* 1997].

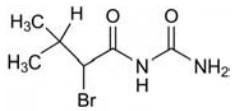
Note For a non-fatal ingestion of ethylene dibromide, see Prakash *et al.* [1999]; for an accidental exposure to methyl bromide, see Lifshitz and Gavrilov [2000].
Half-life 9–15 days.
Volume of Distribution 0.35–0.48 L/kg.

Corina DL *et al.* (1979). Bromide measurement in serum and urine by an improved gas chromatographic method. *J Chromatogr* 162: 382–387.
Danel V *et al.* (2001). Bromide intoxication and pseudohyperchloremia. *Ann Pharmacother* 35: 386–387.
Frances C *et al.* (2003). Bromism from daily over intake of bromide salt. *J Toxicol Clin Toxicol* 41: 181–183.
Goewie CE, Hogendoorn EA (1985). Liquid chromatographic determination of bromide in human milk and plasma. *J Chromatogr* 344: 157–165.
Hirokawa T *et al.* (2004). High-sensitivity capillary electrophoresis determination of inorganic anions in serum and urine using on-line preconcentration by transient isotachophoresis. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 165–170.
Hoizey G *et al.* (2002). An unusual case of methyl bromide poisoning. *J Toxicol Clin Toxicol* 40: 817–821.
Horowitz BZ (1997). Bromism from excessive cola consumption. *J Toxicol Clin Toxicol* 35: 315–320.
Horowitz BZ *et al.* (1998). An unusual exposure to methyl bromide leading to fatality. *J Toxicol Clin Toxicol* 36: 353–357.
Hung YM (2003). Bromide intoxication by the combination of bromide-containing over-the-counter drug and dextromethorphan hydrobromide. *Hum Exp Toxicol* 22: 459–461.
Lifshitz M, Gavrilov V (2000). Central nervous system toxicity and early peripheral neuropathy following dermal exposure to methyl bromide. *J Toxicol Clin Toxicol* 38: 799–801.
Maes V *et al.* (1985). Acute and chronic intoxication with carbromal preparations. *J Toxicol Clin Toxicol* 23: 341–346.
Maros L *et al.* (1989). Simultaneous determination of bromide and iodide as acetone derivatives by gas chromatography and electron capture detection in natural waters and biological fluids. *Anal Chem* 61: 733–735.
Michalodimitrakis MN *et al.* (1997). Death following intentional methyl bromide poisoning: toxicological data and literature review. *Vet Hum Toxicol* 39: 30–34.
Miller ME, Cappon CJ (1984). Anion-exchange chromatographic determination of bromide in serum. *Clin Chem* 30: 781–783.
Poser S *et al.* (1974). Use of bromide electrodes for rapid screening of elevated bromide concentrations in biological fluids. *Z.Klin.Chem Klin.Biochem* 12: 350–351.
Prakash MS *et al.* (1999). Ethylene dibromide poisoning with acute renal failure: first reported case with non-fatal outcome. *Ren Fail* 21: 219–222.
Vaiseman N *et al.* (1986). Pharmacokinetics of oral and intravenous bromide in normal volunteers. *J Toxicol Clin Toxicol* 24: 403–413.
Yamano Y *et al.* (2001). Three cases of acute methyl bromide poisoning in a seedling farm family. *Ind Health* 39: 353–358.

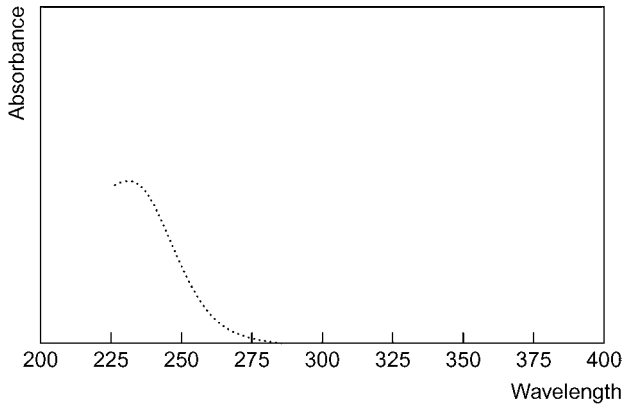
Bromisoval

Hypnotic, Sedative
$C_6H_{11}BrN_2O_2 = 223.1$
CAS—496-67-3

IUPAC Name 2-Bromo-*N*-carbamoyl-3-methylbutanamide
Synonyms *N*-(Aminocarbonyl)-2-bromo-3-methylbutanamide; bromvaletone; bromisovalerylurea; bromisovalum; bromvalerylurea; bromylum.
Proprietary Names *Bromural*. Bromisoval is an ingredient of *Seduan*, *Sekundal* and *Steno-Valocordin*.



Chemical Properties Small, white, acicular or scale-like crystals, which sublime on heating. Mp 147° to 149°. Soluble 1 in 500 of water, 1 in 15 of ethanol, 1 in 6 of chloroform, and 1 in 25 of ether; soluble in solutions of alkali hydroxides. pK_a 10.8. Log *P* (octanol/water), 1.14.
Colour Test Nessler's reagent—brown-orange.
Thin-layer Liquid Chromatography System TB—R_f 0.06; system TD—R_f 0.35; system TE—R_f 0.68; system TF—R_f 0.51; system TAD—R_f 0.52; system TAE—bromisoval R_f 0.84, α-(N-acetylcysteine-S-yl)-isovalerylurea R_f 0.87, dl-isovalthine R_f 0.77.
Gas Chromatography System GA—bromisoval RI 1540, M (Br-isovalerianic acid) RI 1190, M (OH-isovalerianic acid) RI 1140, M (isovalerianic acid carbamide) RI 1850.
High Performance Liquid Chromatography System HX—bromisoval RI 365, α-(N-acetylcysteine-S-yl)-isovalerylurea RI 312, dl-isovalthine RI 211, α-(cystein-S-yl)isovalerylurea RI 237; system HY—RI 307; system HZ—retention time 2.9 min.
Ultraviolet Spectrum Aqueous alkali—233 nm (A^1_1=160b).



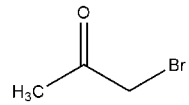
Infrared Spectrum Principal peaks at wavenumbers 1720, 1700, 1581, 1210, 1170, 1300 cm^{-1} (KBr disk). Polymorphism may occur.
Mass Spectrum Principal ions at *m/z* 44, 83, 180, 182, 137, 143, 139, 41.
Quantification
Biological Fluids **GC** FID. Limit of detection, 200 mg/L [Goldbaum, Domanski 1966].
Tissues **GC** See Biological fluids [Goldbaum, Domanski 1966].
Dose Bromisoval has been given in doses of 300 to 900 mg.

Goldbaum LR, Domanski TJ (1966). Detection and identification of micrograms of neutral drugs in biological samples. *J Forensic Sci* 11: 233–242.

Bromoacetone

Lachrymator
$C_3H_5BrO = 136.9$
CAS—598-31-2

IUPAC Name 1-Bromopropan-2-one
Synonyms Acetonyl bromide; 1-bromo-2-propanone; α-bromoacetone; martonite.



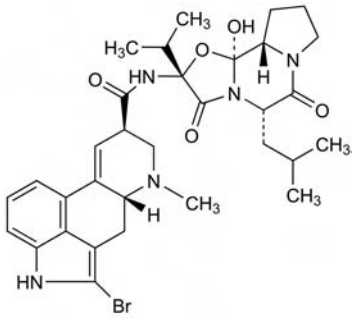
Chemical Properties Liquid, Mp −36.5°. Bp 137°. Turns violet rapidly even in the absence of air. Sparingly soluble in water, soluble in alcohol and acetone. Log *P* (octanol/water), 0.11 [Meylan, Howard 1995].
Quantification
Plasma **GC** Column: Heliflex AT 225 (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 105° for 9 min to 185° at 40°/min for 5 min. ECD. Retention time 5.99 min [van Kreel 1994].
Urine See Plasma [van Kreel 1994].
Faeces See Plasma [van Kreel 1994].
Use Chemical war gas.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
van Kreel BK (1994). An improved bromide assay for the estimation of extracellular water volume by capillary gas chromatography. *Clin Chim Acta* 231: 117–128.

Bromocriptine

Dopaminergic Agent
$C_{32}H_{40}BrN_5O_5 = 654.6$
CAS—25614-03-3

Synonyms 2-Bromo-α-ergocryptine; bromocryptine; 2-bromoergocryptine; (5′α)-2-bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)-ergotaman-3′,6′,18-trione.

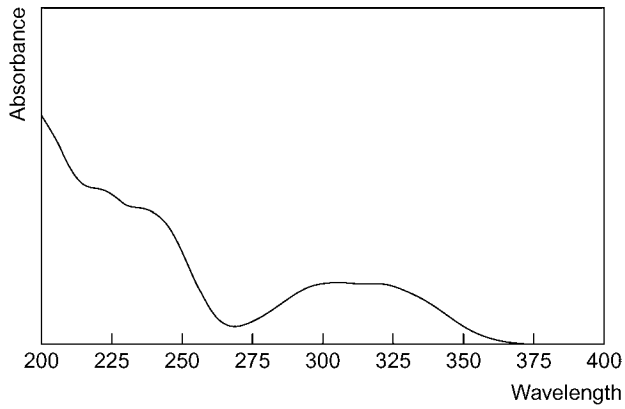


Chemical Properties Crystals. Mp 215° to 218°, with decomposition. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

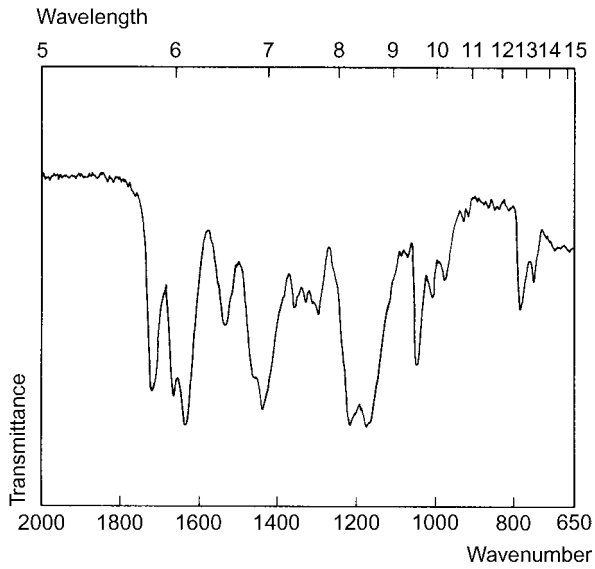
Bromocriptine Mesilate

$C_{32}H_{40}BrN_5O_5,CH_4O_3S = 750.7$
CAS—22260-51-1
Synonym Bromocriptine methanesulfonate
Proprietary Names *Parlodel*; *Pravidel*.
Chemical Properties A yellowish-white crystalline powder. Mp 192° to 196°, with decomposition.

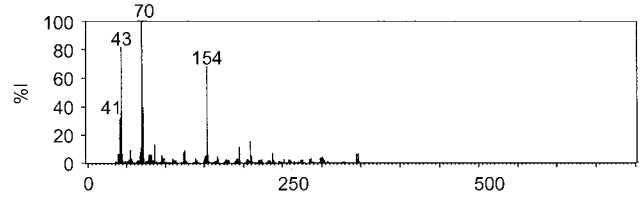
Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.00; system TC—R_f 0.69; system TL—R_f 0.61; system TAE—R_f 0.84; system TAF—R_f 0.88.
Gas Chromatography System GA—RI 2156.
High Performance Liquid Chromatography System HP—*k* 44.3; system HX—RI 456; system HZ—retention time 5.7 min; system HAA—retention time 16.7 min.
Ultraviolet Spectrum Aqueous acid—306 nm (A^1_1=138b); aqueous alkali—239 (A^1_1=373b), 300 nm (A^1_1=144b).



Infrared Spectrum Principal peaks at wavenumbers 1170, 1633, 1217, 1660, 1715, 1045 cm^{-1} (bromocriptine mesilate).



Mass Spectrum Principal ions at *m/z* 70, 43, 154, 71, 41, 209, 86, 195.



Quantification
Plasma **GC** Limit of detection, 0.5 µg/L [Larsen *et al.* 1979].
GC-MS Limit of detection, 1 µg/L [Larsen *et al.* 1979].
HPLC Limit of detection, 1 µg/L [Larsen *et al.* 1979].
Disposition in the Body Rapidly absorbed after oral administration. It undergoes extensive first-pass metabolism by hydrolysis and isomerisation to 2-bromolysergic acid and 2-bromoisolysergic acid, and, by hydroxylation, further oxidation and conjugation to produce a large number of metabolites. About 7% of a dose is excreted in the urine as metabolites together with a small fraction as unchanged drug. About 70% of a dose is eliminated in the faeces via the bile within 5 days of a single oral dose.
Therapeutic Concentration
Following a single oral dose of 25 mg to 9 subjects, peak plasma concentrations of 1 to 4 µg/L were attained in about 90 min; after single doses of 50 and 100 mg to 7 and 5 subjects, respectively, peak plasma concentrations of 3 to 20 µg/L (mean 10) and 6 to 25 µg/L (mean 10) were attained in about 90 to 120 min [Price *et al.* 1978.]

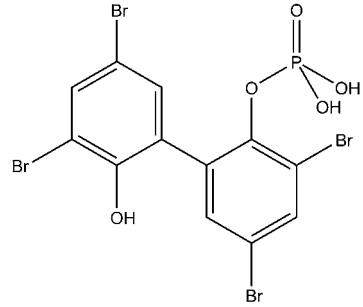
Following daily oral administration of 60 to 150 mg in divided doses to 4 subjects, minimum steady-state plasma concentrations of 9 to 54 µg/L (mean 30) and saliva concentrations of 0.1 to 0.4 µg/L were reported [Friis *et al.* 1980.]

Half-life Plasma half-life, about 3 h.
Volume of Distribution About 3 L/kg.
Protein Binding 90 to 96%.
Dose The equivalent of 1.25 to 30 mg of bromocriptine daily. In Parkinsonism, the usual maintenance dose is the equivalent of 40 to 100 mg daily, but up to 300 mg daily has been given.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Friis ML *et al.* (1980). Bromocriptine concentration in saliva plasma after long-term treatment of patients with Parkinson's disease. *Eur J Clin Pharmacol* 18: 171–174.
Larsen NE *et al.* (1979). Determination of bromocriptine in plasma: comparison of gas chromatography, mass fragmentography and liquid chromatography. *J Chromatogr* 174: 341–349.
Price P *et al.* (1978). Plasma bromocriptine levels, clinical and growth hormone responses in Parkinsonism. *Br J Clin Pharmacol* 6: 303–309.

Bromofenofos

Anthelmintic, Organophosphate
$C_{12}H_7Br_4O_5P = 581.8$
CAS—21466-07-9
IUPAC Name [2,4-Dibromo-6-(3,5-dibromo-2-hydroxyphenyl)phenyl] dihydrogen phosphate
Synonyms BFF; bromophenophos; bromphenphos; PH-1882; 3,3′,5,5′-tetrabromo-(1,1′-biphenyl)-2,2′-diol mono(dihydrogen phosphate); 3,3′,5,5′-tetrabromo-2,2′-biphenyldiol mono(dihydrogen phosphate); 4,4′,6,6′-tetrabromobiphenyl-2,2′-diol mono(dihydrogen phosphate).
Proprietary Name *Acedist*



Chemical Properties Crystals with no mp; decompostion >350°. Very slightly soluble in water (0.0074 mg/L). Log *P* (octanol/water), 4.28 [Meylan, Howard 1995].
Quantification
Other **HPLC** Cow Milk. Column: Kaseisorb ODS-300-5 (250 × 4.6 mm i.d., 5.0 µm). Mobile phase: acetonitrile : 0.05 mol/L potassium dihydrogen phosphate

(pH 3; 55 : 45), flow rate 1 mL/min. Electrochemical detection. Limit of detection, 0.2 µg/L for dephosphate bromofenofos [Takeba *et al.* 1994]. Rat Plasma. Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5.0 µm). Mobile phase: acetonitrile : methanol : water : phosphoric acid (35 : 35 : 30 : 0.1), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Retention times: 8.8 min for bromofenofos; 21.1 min for dephosphate metabolite. Limit of detection, 1.0 mg/L for both compounds [Endoh *et al.* 1988].

Disposition in the Body In animal toxicity studies, bromofenofos is hydrolysed mostly in the gastrointestinal tract to the dephosphate bromofenofos metabolite, which is the only compound detected in animal tissues.

Toxicity In animal studies, placental transfer occurs, resulting in teratogenic and embryolethal effects.

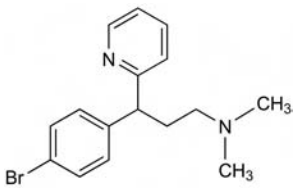
Endoh YS *et al.* (1988). High-performance liquid chromatographic determination of bromofenofos and its metabolite in rat plasma. *J Chromatogr* 426: 202–206.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Takeba K *et al.* (1994). Determination of dephosphate bromofenofos in milk by liquid chromatography with electrochemical detection. *J AOAC Int* 77: 904–908.

Brompheniramine

Antihistamine
$C_{16}H_{19}BrN_2$ = 319.2
CAS—86-22-6

IUPAC Name 3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-pyridin-2-ylpropan-1-amine

Synonym γ-(4-Bromophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine; parabromdylamine.



Chemical Properties A slightly yellow, oily liquid. Miscible with dilute acids. pK_a 3.9, 9.2. Log *P* (octanol/water), 4.1.

Brompheniramine Maleate

$C_{16}H_{19}BrN_2,C_4H_4O_4$ = 435.3
CAS—980-71-2; 32865-01-3 (±)

Proprietary Names *Antial*; *Dimegan*; *Dimetane*; *Dimotane*; *Drauxin*; *Ebalin*; *Gammistin*; *Ilvin*; *Veltane*. It is an ingredient of *Dimotapp* and *Exyphen*.

Chemical Properties A white crystalline powder. Mp 132° to 134°. Soluble 1 in 5 of water, 1 in 15 of ethanol, and 1 in 15 of chloroform; slightly soluble in ether.

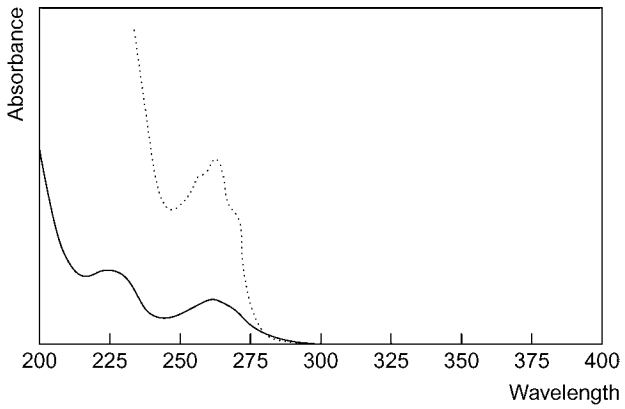
Colour Test Cyanogen bromide—orange.

Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.33; system TC—R_f 0.16; system TL—R_f 0.06; system TAE—R_f 0.12; system TAJ—R_f 0.01; system TAK—R_f 0.00; system TAL—R_f 0.28 (acidified iodoplatinate solution, positive).

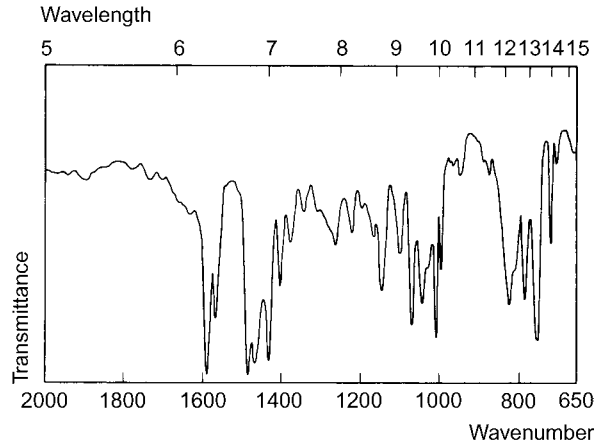
Gas Chromatography System GA—RI 2092; system GB—brompheniramine RI 2184, M (nor-) RI 2219, M (bis-nor-) RI 2203; system GC—RI 2457; system GF—RI 2470.

High Performance Liquid Chromatography System HA—*k* 4.1; system HY—RI 267; system HAA—retention time 13.9 min.

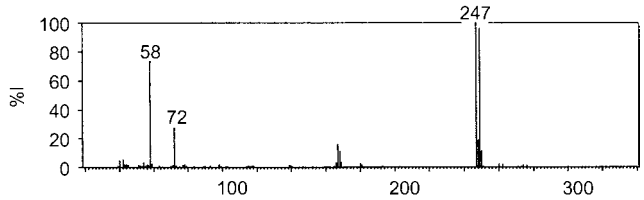
Ultraviolet Spectrum Aqueous acid—265 nm (A_1^1=272a); aqueous alkali—262 (A_1^1=177a), 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1585, 750, 1003, 1067, 1565, 1040 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 247, 249, 58, 72, 248, 167, 250, 168.



Quantification

Blood **GC** ECD. Limit of detection, 10 µg/L [Bruce *et al.* 1968].

Urine **GC** ECD. See Blood Bruce *et al.* [1968].

Disposition in the Body Absorbed after oral administration; accumulates in the body during chronic daily dosing. The main metabolic reactions are *N*-demethylation and deamination. About 50% of a ^{14}C-labelled dose is excreted in the urine in 5 days, with 10% of the dose as unchanged brompheniramine, 11% as monodesmethylbrompheniramine, 10% as didesmethylbrompheniramine, 4% as 3-(4-bromophenyl)-3-(2-pyridyl)-propionic acid, and 2% as its glycine conjugate; other unidentified polar metabolites are also present. <3% of the dose is eliminated in the faeces. Under steady-state conditions the daily excretion rate appears to be dependent on urinary pH and volume.

Therapeutic Concentration

After a single oral dose of 8 mg administered to 2 subjects, peak blood concentrations of 12 and 17 µg/L were attained in 3 h [Bruce *et al.* 1968.]

Fourteen children with a mean age of 9.5 years were administered 4 mg brompheniramine. Mean peak drug concentrations of 7.7 µg/L at 3.2 h [Simons *et al.* 1999.]

Half-life Plasma half-life, about 15 h.

Dose 12 to 32 mg of brompheniramine maleate daily.

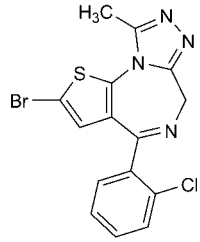
Bruce RB *et al.* (1968). Determination of brompheniramine in blood and urine by gas-liquid chromatography. *Anal Chem* 40: 1246–1250.
Simons FE *et al.* (1999). The clinical pharmacology of brompheniramine in children. *J Allerg Clin Immunol* 103(2): 223–226.

Brotizolam

Thienotriazolodiazepine, Sedative, Hypnotic
$C_{15}H_{10}BrClN_4S$ = 393.7
CAS—57801-81-7

Synonyms 2-Bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine; WE-941-BS.

Proprietary Names *Dormex*; *Landormin*; *Lendorm*; *Lendormin*; *Lindormin*; *Mederantil*; *Nimbisan*; *Noctilen*; *Sintonal*.



Chemical Properties Colourless crystalline powder. Mp 212° to 214°. Water solubility of 1.078 mg/L at 25°. Log *P* (octanol/water) 2.79. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable in serum after freeze–thaw cycles, in the

autosampler for at least 6 h and for 62 days at –30° [Nakamura *et al.* 2009]. Brotizolam was found to be stable in extracts for > 24 h at room temperature [Kratzsch *et al.* 2004].

Thin-layer Chromatography System TA—R_f 0.72 (brotizolam), R_f 0.68 (M-6-hydroxy), R_f 0.72 (M-α-hydroxy); system TAD—R_f 0.53 (brotizolam), R_f 0.37 (M-6-hydroxy), R_f 0.41 (M-α-hydroxy); system TAE—R_f 0.72 (brotizolam), R_f 0.76 (M-6-hydroxy), R_f 0.78 (M-α-hydroxy); system TAF—R_f 0.71 (brotizolam), R_f 0.76 (M-6-hydroxy), R_f 0.78 (M-α-hydroxy); system TAG—R_f 0.27 (brotizolam), R_f 0.13 (M-6-hydroxy), R_f 0.31 (M-α-hydroxy); system TB—R_f 0.05 (brotizolam), R_f 0.01 (M-6-hydroxy), R_f 0.02 (M-α-hydroxy); system TC—R_f 0.52 (brotizolam), R_f 0.35 (M-6-hydroxy), R_f 0.46 (M-α-hydroxy); system TD—R_f 0.15 (brotizolam), R_f 0.05 (M-6-hydroxy), R_f 0.07 (M-α-hydroxy); system TE—R_f 0.52 (brotizolam), R_f 0.28 (M-6-hydroxy), R_f 0.45 (M-α-hydroxy); system TF—R_f 0.05 (brotizolam), R_f 0.04 (M-6-hydroxy), R_f 0.06 (M-α-hydroxy).

Plates: silica gel 60 F_{254}. Mobile phase: methylene chloride : methanol (9 : 1) followed by toluene : isopropanol: 25% ammonia (5 : 4 : 1). UV detection (λ= 254 nm). We 964 R_f 0.59–0.62; We 1061 R_f 0.37–0.40 [Bechtel *et al.* 1986a].

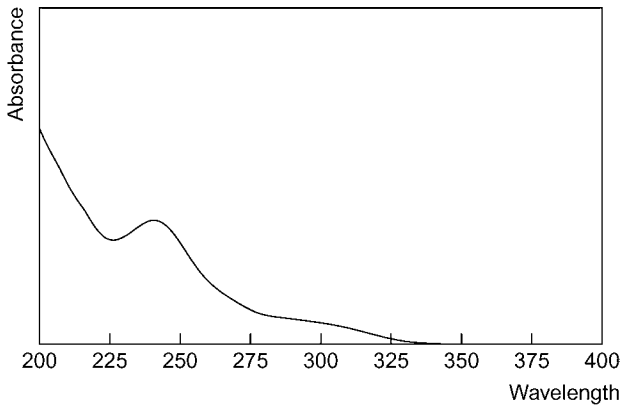
Plate: silica gel 60 F_{254} (10 × 10 cm). Mobile phase 1: cyclohexane : toluene : diethylamine (75 : 15 : 10); mobile phase 2: chloroform : methanol (90 : 10); mobile phase 3: chloroform : acetone (80 : 20). Dragendorff reagent for visualision. R_f 0.06 (mobile phase 1); R_f 0.52 (mobile phase 2); R_f 0.13 (mobile phase 3) [Otsubo *et al.* 1995].

Gas Chromatography System GA—RI 3070 (brotizolam), RI 3050 (M (OH-) Art (-CH_2O)).

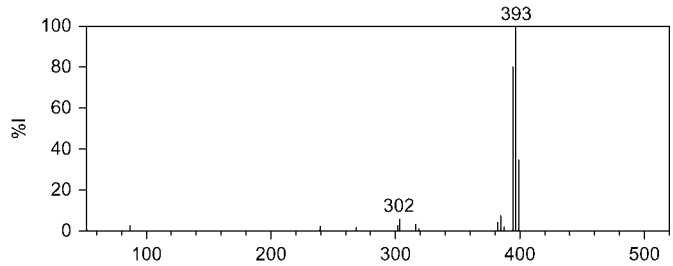
High Performance Liquid Chromatography System HAX—RT 7.4 min; system HAY—RT 7.9 min; system HX—RI 484; system HZ—RT 4.6 min.

Column: Nucleosil C_{18} (200 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L NH_4CO_3 : diethylamine (35 : 65 : 0.1), flow rate 1 mL/min. UV detection (λ=254 nm). Retention time: (metabolites) We 964, 10 to 11 min; We 1061, 12 to 13 min [Bechtel *et al.* 1986a].

Ultraviolet Spectrum Principal peak at 240 nm



Mass Spectrum Principal peaks at *m/z* 394, 245, 316, 210, 291, 176, 365, 313; (Hydroxybrotizolam) 55, 69, 83, 149, 95, 410, 245, 111.



Quantification

Blood **LC-MS** Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : 0.006 mol/L formic acid (pH 3.0; 30 : 70 to 40 : 60 at 5 min to 50 : 50 at 25 min to 60 : 40 at 30 min for 5 min to 30 : 70 at 36 min for 9 min. APCI. Retention time: 29.8 min. Limit of quantification, 0.9 µg/L, limit of detection, 0.3 µg/L [Smink *et al.* 2004].

Plasma **GC** Column: 0.5% PPE and 3% OV-17 SCOT. Carrier gas: He, 10 mL/min. Temperature: 230°. Limit of detection, 2.0 µg/L [Jochemsen *et al.* 1983c]. Column: 1% OV-17 80-100 Chromosorb WHP (1.83 m × 2 mm i.d.). Carrier gas: He, 50 mL/min. Temperature: 290°. ECD. Limit of detection, 0.25 µg/L [Greenblatt *et al.* 1983].

HPLC C_{18} ultrabase (35 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (60 : 40), flow rate 0.9 mL/min. UV detection (λ= 240 nm). Limit of detection, 76 µg/L [Casas *et al.* 1993].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.5 µg/L (SIM); limit of detection, 0.5 µg/L (scan) [Kratzsch *et al.* 2004].

Serum **HPLC** Column: LichroCART RP-18 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water: 0.1 mol/L phosphate buffer (pH 5.7; 60 : 20 : 20 or 65 : 15 : 20), flow rate 0.75 mL/min. Limit of detection, 0.1 mg/L [Otsubo *et al.* 1995]. Limit of detection, 2 µg/L [Musshoff, Daldrup 1992].

LC-MS Column: Unison UK-C_{18} ODS (150 × 2 mm i.d., 3 µm) or Cadanza CD-C_{18} ODS (150 × 2 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 to 20 : 80 at 20 min for 5 min), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 3.5 µg/L; limit of detection, 1.0 µg/L [Nakamura *et al.* 2009].

Urine **HPLC** See Plasma [Casas *et al.* 1993].

Disposition in the Body Brotizolam is rapidly absorbed after oral administration and is completely metabolised to 2 major hydroxylated metabolites, including 6-hydroxylated brotizolam. Evidence from human liver microsomes and trials in healthy volunteers suggests that CYP3A4 is the isoenzyme predominantly responsible for the α- and 6-hydroxylation of brotizolam [Osanai *et al.* 2004; Senda *et al.* 1997; Tokairin *et al.* 2005]. The drug is more slowly absorbed in the elderly and if metabolic clearance is impaired. Metabolites are excreted in urine as glucuronide and sulfate conjugates (65%) and in the faeces (22%) with only 2 to 3% of the dose excreted unchanged [Bechtel *et al.* 1986b]. Brotizolam is rapidly distributed throughout the body.

Therapeutic Concentration The serum therapeutic concentration range is 1–20 µg/L.

Six healthy male volunteers were administered single 0.25 and 0.5 mg doses of brotizolam on 2 occasions in random sequence separated by at least 1 week. They fasted 8 h before and 3 h after each dose. Mean peak plasma brotizolam concentrations were 9.2 and 5.5 µg/L after the 0.5 and 0.25 mg dose, respectively. Peak concentrations were attained within 1.5 h of dosing [Greenblatt *et al.* 1983].

Twenty elderly patients; 10 males and 10 females aged between 71 and 93 years, and 8 young, healthy volunteers; 5 males and 3 females, 21 to 26 years old, were administered a 0.25 mg dose of brotizolam in the fasting state. Peak plasma concentrations ranged between 3.8 and 13.6 µg/L for the elderly patients and 5.0–6.6 µg/L for the young. These were reached within 1.1–1.7 h, respectively [Jochemsen *et al.* 1983d; Jochemsen *et al.* 1983b].

Eight healthy volunteers; 5 males and 3 females, 21–26 years old, were administered an oral dose of 0.5 mg brotizolam. Mean peak plasma concentrations were 7.3 ± 3.1 µg/L at 1.1 ± 1.0 h [Jochemsen *et al.* 1983e].

Eighteen patients with mild (CL_{CR} 45 to 80 mL/min), moderate (CL_{CR} 15–45 mL/min) or severe (CL_{CR} <15 mL/min) renal insufficiency were administered 0.25 mg brotizolam twice daily for 6 days and once on the seventh day. There was no delay in elimination in severe renal failure and there was no drug accumulation [Evers *et al.* 1983].

Eight male patients with liver cirrhosis aged between 34 and 64 years were administered 0.5 mg brotizolam orally. Peak plasma concentrations were 7.1 (3.2–10.7) µg/L in patients compared with 9.4 (2.9–19.0) µg/L in controls. Elimination half-lives were 12.8 (9.4–25) h in patients compared with 6.9 (4.4–8.4) h in controls [Jochemsen *et al.* 1983a].

Toxicity

A 42-year-old male was found dead; toxicological screening was carried out on his blood, urine and stomach contents. In his blood, 10.4 µg/L brotizolam, 3000 µg/L ibuprofen and 130 µg/L dihydrocodeine were found. In his urine, the brotizolam metabolite We 964, α-OH-triazolam, ibuprofen, dihydrocodeine and phenylpropanolamine were found at varying concentrations. Brotizolam and ibuprofen were detected in the stomach contents. It was determined that death occurred due to a combination of these drugs and alcohol poisoning [Saito *et al.* 1997].

Note For a review of the toxicology of brotizolam in animals, see Hewett *et al.* [1983].

Half-life The mean elimination half-life is 4.4 h for the unchanged drug and 9.5 h for the metabolites. In the elderly, the half-life increases to ~9.8 h for the parent drug.

Bioavailability 70%.

Volume of Distribution 0.45–0.72 L/kg in the elderly and 0.40–0.77 L/kg in the young.

Clearance For the elderly, 109 mL/min and the young, 40 mL/min.

Protein Binding ≈86–91% with serum albumin and 89–95% other plasma proteins [Bechtel 1983].

Dose The usual dose of 250 µg daily is reduced to 125 µg in the elderly and debilitated patients [Nicholson 1983].

Bechtel WD (1983). Pharmacokinetics and metabolism of brotizolam in humans. *Br J Clin Pharmacol* 16(suppl2): 279S–283S.

Bechtel WD *et al.* (1986a). Metabolic fate of [^{14}C]-brotizolam in the rat, dog, monkey and man. *Arzneimittelforschung* 36: 578–586.

Bechtel WD *et al.* (1986b). Blood level, excretion, and metabolite pattern of [^{14}C]-brotizolam in humans. *Arzneimittelforschung* 36: 575–578.

Casas M *et al.* (1993). Solid-phase extraction of 1,4-benzodiazepines from biological fluids. *J Pharm Biomed Anal* 11: 277–284.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

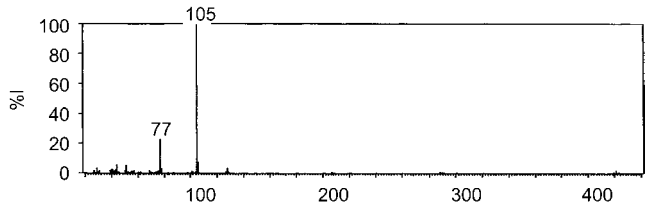
Evers J *et al.* (1983). Pharmacokinetics of brotizolam in renal failure. *Br J Clin Pharmacol* 16(suppl2): 309S–313S.

Greenblatt DJ *et al.* (1983). Pilot pharmacokinetic study of brotizolam, a thienodiazepine hypnotic, using electron-capture gas-liquid chromatography. *Sleep* 6: 72–76.
Hewett C *et al.* (1983). The toxicology of brotizolam. *Br J Clin Pharmacol* 16(suppl2): 267S–274S.
Jochemsen R *et al.* (1983a). Pharmacokinetics of oral brotizolam in patients with liver cirrhosis. *Br J Clin Pharmacol* 16(suppl2): 315S–322S.
Jochemsen R *et al.* (1983b). Pharmacokinetics of brotizolam in the elderly. *Br J Clin Pharmacol* 16 (suppl2): 299S–307S.
Jochemsen R *et al.* (1983c). Assay of midazolam and brotizolam in plasma by a gas chromatographic and a radioreceptor technique. *Pharm Weekbl Sci* 5: 308–312.
Jochemsen R *et al.* (1983d). Pharmacokinetics of brotizolam in healthy subjects following intravenous and oral administration. *Br J Clin Pharmacol* 16(suppl2): 285S–290S.
Jochemsen R *et al.* (1983e). Comparative pharmacokinetics of brotizolam and triazolam in healthy subjects. *Br J Clin Pharmacol* 16(suppl2): 291S–297S.
Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.
Musshoff F, Daldrup T (1992). A rapid solid-phase extraction and HPLC/DAD procedure for the simultaneous determination and quantification of different benzodiazepines in serum, blood and post-mortem blood. *Int J Legal Med* 105: 105–109.
Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography-tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.
Nicholson AN (1983). Brotizolam: review of clinical studies. *Br J Clin Pharmacol* 16(suppl2): 433S–440S.
Osanai T *et al.* (2004). Effect of itraconazole on the pharmacokinetics and pharmacodynamics of a single oral dose of brotizolam. *Br J Clin Pharmacol* 58: 476–481.
Otsubo K *et al.* (1995). Rapid and sensitive detection of benzodiazepines and zopiclone in serum using high-performance thin-layer chromatography. *J Chromatogr B Biomed Appl* 669: 408–412.
Saito T *et al.* (1997). A case of homicidal poisoning involving several drugs. *J Anal Toxicol* 21: 584–586.
Senda C *et al.* (1997). Identification of human cytochrome P450 isoforms involved in the metabolism of brotizolam. *Xenobiotica* 27: 913–922.
Smink BE *et al.* (2004). Quantitative analysis of 33 benzodiazepines, metabolites and benzodiazepine-like substances in whole blood by liquid chromatography-(tandem) mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 13–20.
Tokairin T *et al.* (2005). Inhibition of the metabolism of brotizolam by erythromycin in humans: in vivo evidence for the involvement of CYP3A4 in brotizolam metabolism. *Br J Clin Pharmacol* 60: 172–175.

Broxaldine

Antiprotozoal
$C_{17}H_{11}Br_2NO_2$ = 421.1
CAS—3684-46-6
IUPAC Name 5,7-Dibromo-2-methyl-8-quinolyl benzoate
Synonym Brobenzoxaldine
Proprietary Name It is an ingredient of *Intestopan*.

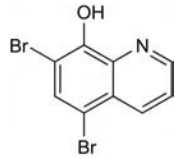
Chemical Properties An off-white powder. Practically insoluble in water; soluble in ether.
Colour Tests Mandelin's test—yellow; Marquis test—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.52; system TC—R_f 0.79; system TL—R_f 0.71; system TAE—R_f 0.78 (acidified iodoplatinate solution, strong reaction).
Gas Chromatography System GA—RI 2686.
Mass Spectrum Principal ions at *m/z* 105, 77, 106, 44, 51, 128, 78, 40.



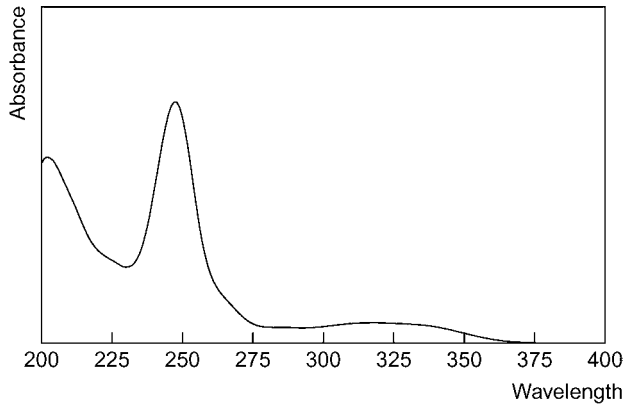
Dose 120 to 300 mg daily, usually in combination with broxyquinoline.

Broxyquinoline

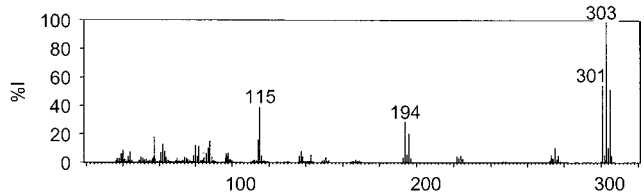
Antiprotozoal
$C_9H_5Br_2NO$ = 303.0
CAS—521-74-4
IUPAC Name 5,7-Dibromo-8-quinolinol
Synonym Broxichinolinum
Proprietary Name It is an ingredient of *Intestopan*.



Chemical Properties A cream-coloured powder. Mp 196°. Practically insoluble in water; freely soluble in acetic acid, benzene, chloroform and ethanol; slightly soluble in ether. Log *P* (octanol/water) 3.4.
Colour Tests Liebermann's reagent—orange; Marquis test—yellow; Millon's reagent—red; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.51; system TL—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.06 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—263 nm (A^1_1=800b); ethanol—249 (A^1_1=1525b), 325 nm (A^1_1=125b).



Infrared Spectrum Principal peaks at wavenumbers 1316, 1190, 930, 806, 1258, 782 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 303, 301, 305, 115, 194, 196, 114, 87.

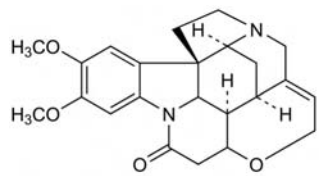


Quantification
Urine **Colorimetry** See Berggren, Hansson [1968] and Rodriguez, Close [1968].
Disposition in the Body Absorbed after oral administration. About 36% of a dose is excreted in the urine in 24 h, mainly as the glucuronide conjugate; very little unchanged drug is excreted and 8-hydroxyquinoline accounts for <2% of the dose.
Dose 0.6 to 1.5 g daily, usually in combination with broxaldine.

Berggren L, Hansson O (1968). Absorption of intestinal antiseptics derived from 8-hydroxyquinolines. *Clin Pharmacol Ther* 9: 67–70.
Rodriguez LA, Close JA (1968). The metabolism of the 5-7-dibromo-8-hydroxyquinoline (broxyquinoline) in man. *Biochem Pharmacol* 17: 1647–1653.

Brucine

CNS Stimulant
$C_{23}H_{26}N_2O_4$ = 394.5
CAS—357-57-3 (anhydrous)
Synonyms 2,3-Dimethoxystrychnidin-10-one; dimethoxystrychnine.

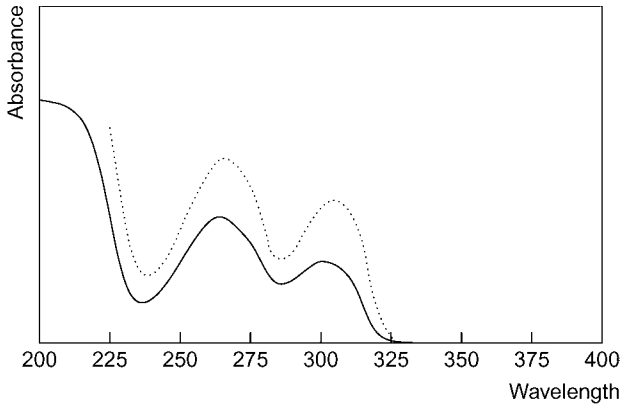


Chemical Properties An alkaloid present in the seeds of *Strychnos nux-vomica* and other species of *Strychnos (Loganiaceae)*. Small white crystals. Mp 178°. pK_a 2.3, 8.3 (25°). Log *P* (octanol/water), 1.0.

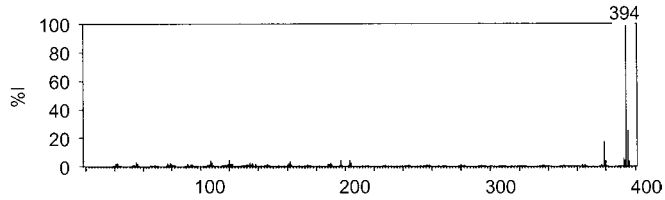
Brucine Sulfate
$(C_{23}H_{26}N_2O_4)_2,H_2SO_4,\ 7H_2O = 1013$
CAS—4845-99-2 (anhydrous); 60583-39-3 (heptahydrate)
Chemical Properties Small white crystals or powder. Soluble 1 in 75 of cold water, 1 in about 10 of boiling water, 1 in 105 of ethanol and 1 in 170 of chloroform.

Brucine tetrahydrate
$C_{23}H_{26}N_2O_4,4H_2O = 466.5$
Chemical Properties Becomes anhydrous at 100°. Soluble 1 in 1320 of water, 1 in 100 of benzene, 1 in 1.3 of ethanol, 1 in 5 of chloroform, 1 in 187 of ether, 1 in 25 of ethyl acetate and 1 in 36 of glycerol.

Colour Test Liebermann's reagent—red.
Thin-layer Chromatography System TA—R_f 0.16; system TB—R_f 0.00; system TC—R_f 0.17; system TL—R_f 0.01; system TAE—R_f 0.05; system TAF—R_f 0.07; system TAJ—R_f 0.04; system TAK—R_f 0.00; system TAL—R_f 0.64 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3280.
High Performance Liquid Chromatography System HA—*k* 11.1 (tailing peak); system HX—RI 312; system HY—RI 267.
Ultraviolet Spectrum Aqueous acid—265 (A_1^1=330a), 300 nm; aqueous alkali—266 (A_1^1=320a), 304 nm.



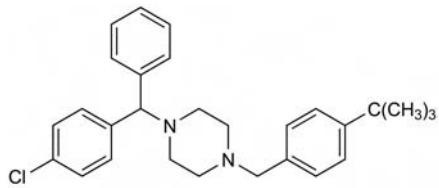
Infrared Spectrum Principal peaks at wavenumbers 1500, 1660, 1280, 1195, 1120, 1212 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 394, 395, 379, 392, 120, 197, 203, 393.



Buclizine

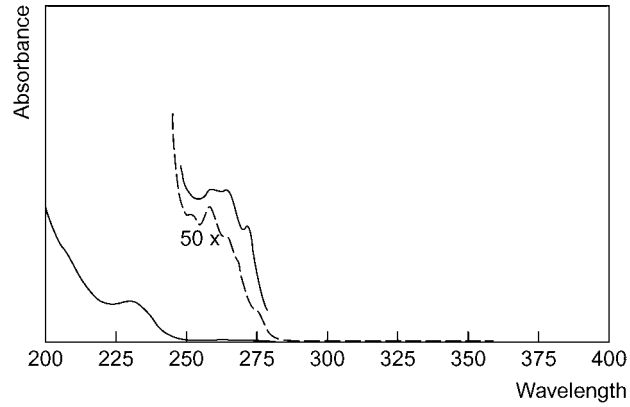
Antihistamine
$C_{28}H_{33}ClN_2 = 433.0$
CAS—82-95-1

IUPAC Name 1-[(4-Tert-butylphenyl)methyl]-4-[(4-chlorophenyl)-phenylmethyl] piperazine
Synonym 1-[(4-Chlorophenyl)phenylmethyl]-4-[[4-(1,1-dimethylethyl)phenyl]-methyl]piperazine

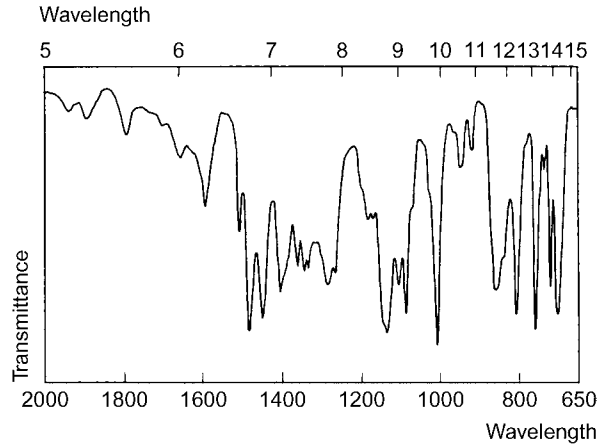


Buclizine Hydrochloride
$C_{28}H_{33}ClN_2,2HCl = 506.0$
CAS—129-74-8
Proprietary Names *Aphilan R*; *Bucladin-S*; *Longifene*. It is an ingredient of *Equivert* and *Migraleve* (pink tablets).
Chemical Properties A white crystalline powder. Mp 230° to 240°. Slightly soluble in water.

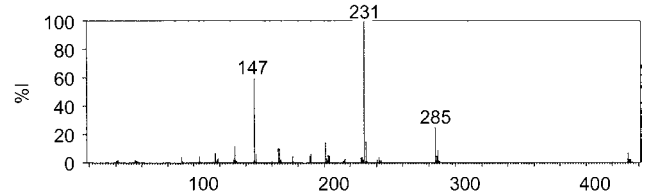
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.61; system TC—R_f 0.83; system TL—R_f 0.72 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—buclizine RI 3360; M (desalkyl-) (norchlorcyclizine)-RI 2520; M (chlorobenzophenone (ClBP)-) RI 1850; M (OH-ClBP-) RI 2300; M (carbinol) RI 1750; system GB—buclizine RI 3461; M (4-chloromethylbiphenyl) RI 1688; M (4-chlorobiphenylmethanone) RI 1862.
High Performance Liquid Chromatography System HA—*k* 0.7; system HY—RI 454.
Ultraviolet Spectrum Methanol—255, 260 nm (A_1^1=19b).



Infrared Spectrum Principal peaks at wavenumbers 1002, 1131, 754, 800, 1075, 694 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 231, 147, 285, 232, 201, 132, 165, 166.

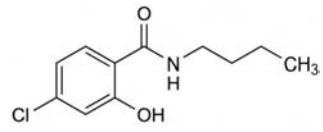


Dose 25 to 150 mg of buclizine hydrochloride daily.

Buclosamide

Antifungal
$C_{11}H_{14}ClNO_2 = 227.7$
CAS—575-74-6

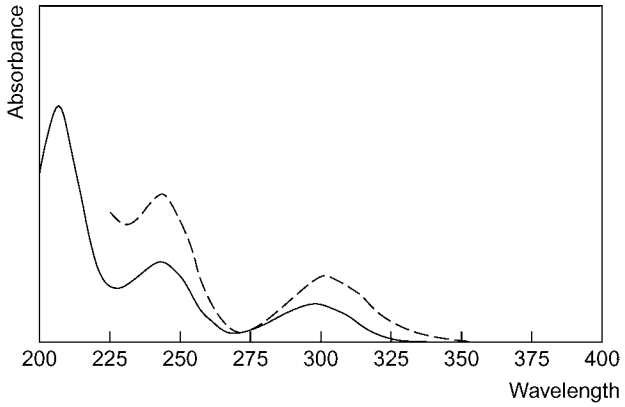
IUPAC Name *N*-Butyl-4-chloro-2-hydroxybenzamide



Chemical Properties A white crystalline powder. Mp about 91°. Practically insoluble in water; soluble 1 in 3 of ethanol, and 1 in 3 of ether. Log *P* (octanol/water) 3.6.

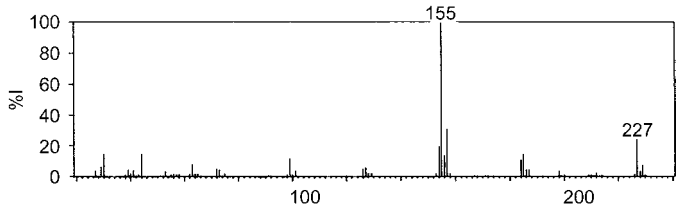
Colour Tests Folin-Ciocalteu reagent—blue; Mandelin's test—green→blue rim; Millon's reagent—red.
Thin-layer Chromatography System TA—R_f 0.90; system TB—R_f 0.02; system TC—R_f 0.67; system TL—R_f 0.70; system TAE—R_f 0.90 (location under ultraviolet light, blue fluorescence; acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—245 (A_1^1=532b), 297 nm (A_1^1=204b); aqueous alkali—247 (A_1^1=391b), 325 nm (A_1^1=323a); ethanol—243 (A_1^1=497a), 301 nm.



Infrared Spectrum Principal peaks at wavenumbers 1543, 898, 1190, 1590, 1201, 1506 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 155, 157, 227, 154, 185, 44, 30, 156.



Use Buclosamide has been applied topically at a concentration of 10%.

Budipine

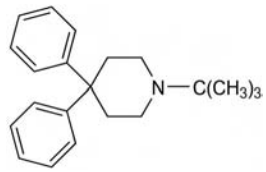
Dopaminergic Agent

$C_{21}H_{27}N$ = 293.4

CAS—57982-78-2

IUPAC Name 1-Tert-butyl-4,4-diphenylpiperidine

Synonyms Budipina; BY 701; 1-(1,1-dimethylethyl)-4,4-diphenylpiperidine.



Chemical Properties Crystals. Mp 73° to 75°. Bp 160° to 165°. pK_a 10.55. Log *P* (octanol/water), 5.10; −0.55 (pH 4.5); 1.49 (pH 7.4). Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Budipine Hydrochloride

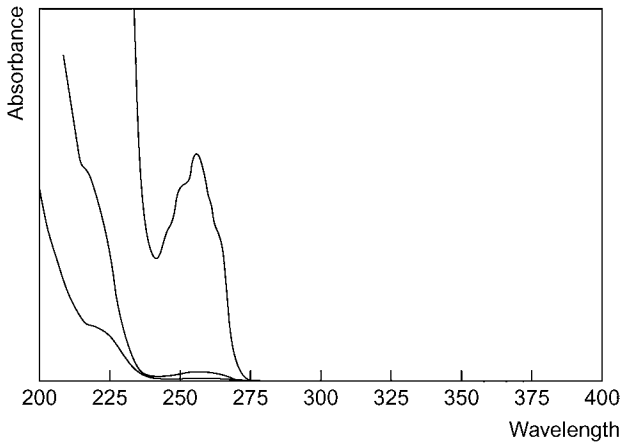
$C_{21}H_{28}ClN$ = 329.9

CAS—63661-61-0

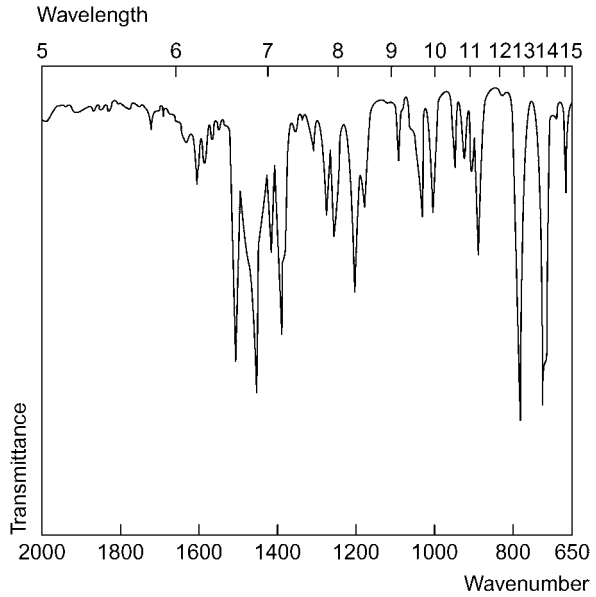
Proprietary Name *Parkinsan*

Chemical Properties Crystals.

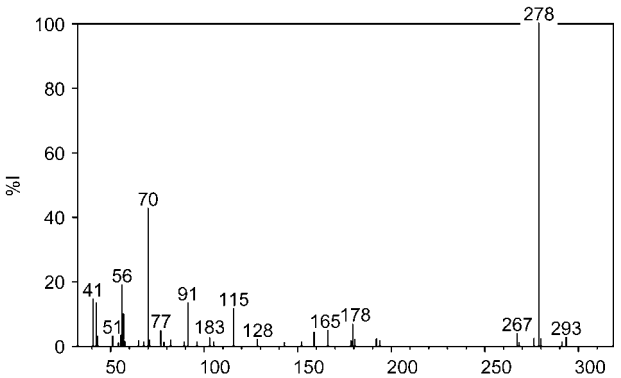
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 767, 698, 1446, 1496, 1383, 1242 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 278, 70, 56, 41, 42, 91, 115, 178.



Disposition in the Body It is well absorbed after oral administration (about 80%). It mainly metabolises to mono-*p*-hydroxybudipine, which is significantly less active than budipine. About 80% of a dose is excreted via the kidneys, partly as unchanged budipine and, to a greater extent, as the hydroxymetabolite.

Toxicity Can be poisonous intravenously.

Bioavailability Approximately 47%.

Half-life About 30 h.

Volume of Distribution 2.7 L/kg.

Protein Binding About 96%.

Dose Initially, 10 mg three times daily. Maximum dose of 60 mg. In patients with severe impairment of renal function or liver damage, the dose should not exceed 30 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Bufexamac

Analgesic

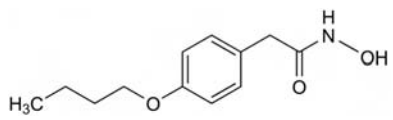
$C_{12}H_{17}NO_3$ = 223.3

CAS—2438-72-4

IUPAC Name 4-Butoxy-*N*-hydroxybenzeneacetamide

Synonym CP-1044-J3

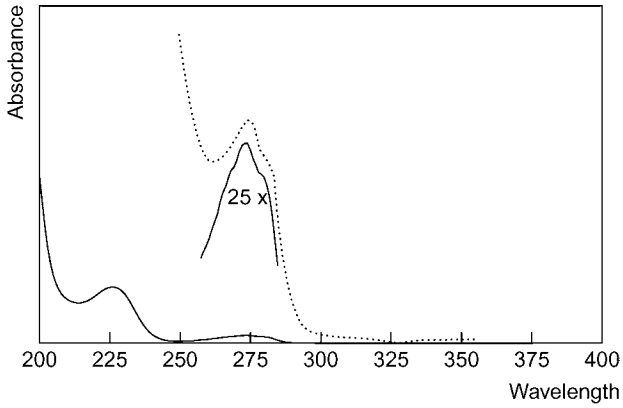
Proprietary Names *Droxaryl*; *Feximac*; *Malipuran*; *Mofenar*; *Norfemac*; *Paraderm*; *Parfenac*; *Parfenal*.



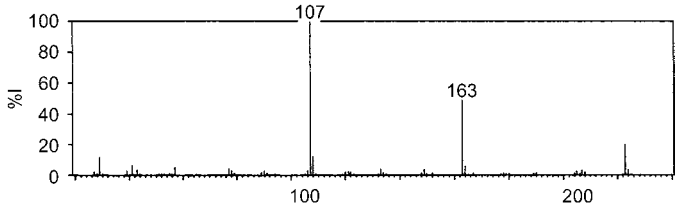
Chemical Properties Almost colourless pearly flakes. Mp 153° to 155°, with decomposition. Practically insoluble in water. Log *P* (octanol/water), 2.1.

Thin-layer Chromatography System TD—R_f 0.11; system TE—R_f 0.18; system TF—R_f 0.19; system TG—R_f 0.36; system TAD—R_f 0.31.

Gas Chromatography System GD—RRT 1.12 of methyl derivative, relative to n-$C_{16}H_{34}$.
High Performance Liquid Chromatography System HD—*k* 1.95.
Ultraviolet Spectrum Aqueous alkali—275 nm (A^1_1=72b).



Infrared Spectrum Principal peaks at wavenumbers 1612, 1234, 1515, 980, 1052, 1176 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 107, 163, 223, 108, 29, 164, 41, 57.

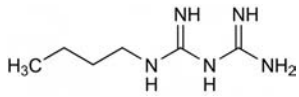


Dose 0.75 to 1.5 g daily.

Buformin

Antidiabetic
$C_6H_{15}N_5$ = 157.2
CAS—692-13-7

IUPAC Name 2-Butyl-1-(diaminomethylidene)guanidine
Synonym *N*-Butylimidodicarbonimidic diamide

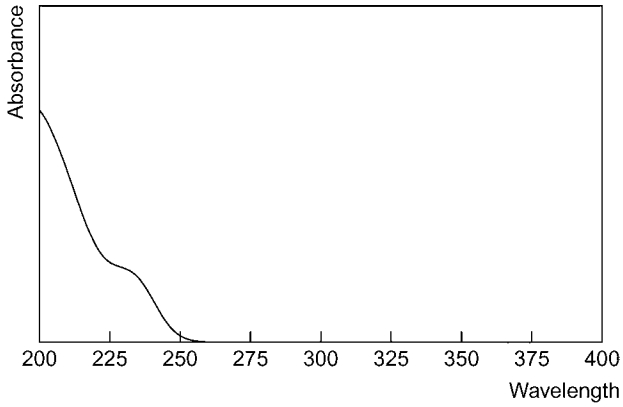


Chemical Properties Log *P* (octanol), −1.2.

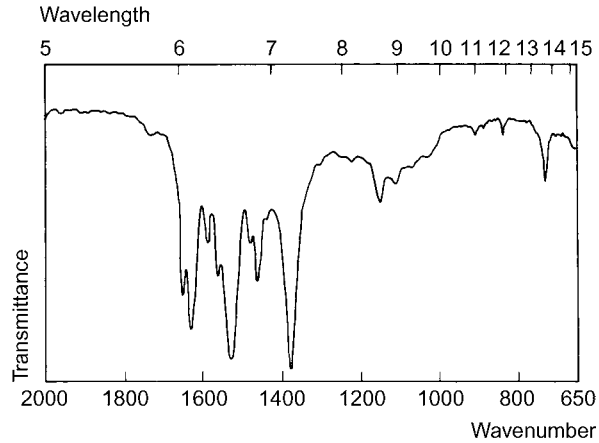
Buformin Hydrochloride

$C_6H_{15}N_5$,HCl = 193.7
CAS—1190-53-0
Proprietary Names *Silubin*; *Sindiatil*.
Chemical Properties A fine white crystalline powder. Mp 174° to 177°. Freely soluble in water and ethanol.

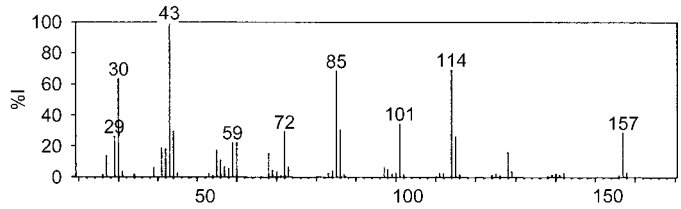
Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00.
Gas Chromatography System GB—not eluted.
Ultraviolet Spectrum Aqueous alkali—230 nm (A^1_1=919b).



Infrared Spectrum Principal peaks at wavenumbers 1530, 1634, 1666, 1562, 1587, 1149 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 114, 85, 30, 101, 86, 44, 72.



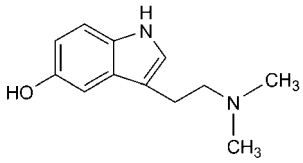
Quantification
Blood **UV Spectrophotometry** See Garrett *et al.* [1972].
Plasma **GC** ECD. Limit of detection, 1 µg/L [Matin *et al.* 1975].
UV Spectrophotometry See Garrett *et al.* [1972].
Urine **GC** See Plasma [Matin *et al.* 1975].
UV Spectrophotometry See Garrett, Tsau [1972].
Biological Fluids **GC** AFID. See de Groot *et al.* [1980].
Tissues **GC** AFID. See de Groot *et al.* [1980].
Disposition in the Body Absorbed after oral administration. About 35% of an oral dose is excreted in the urine as unchanged drug in 24 h and up to 30% is eliminated in the faeces; after IV administration about 90% is excreted unchanged in the urine in 12 h.
Therapeutic Concentration In plasma, usually in the range 0.2 to 0.6 mg/L. Following a single oral dose of 100 mg to 6 subjects, a mean peak plasma concentration of 0.4 mg/L was reported after 2.5 h; plasma concentrations >0.2 mg/L were maintained up to 8 h after the dose [Gutsche *et al.* 1976].
Toxicity Lactic acidosis is associated with plasma concentrations greater than 1 mg/L.
An 84-year-old diabetic died 6 h after admission to hospital with severe lactic acidosis caused by buformin. The plasma concentration on admission was 5.5 mg/L. Postmortem tissue concentrations were: plasma 3.2 mg/L, bile 6.3 mg/L, heart 3.0 µg/g, kidney 98 µg/g, liver 5.2 µg/g, lung 2.8 µg/g [de Groot *et al.* 1980].
Half-life Plasma half-life, about 2 to 6 h.
Volume of Distribution About 2 L/kg.
Protein Binding About 10%.
Dose 100 to 300 mg of buformin hydrochloride daily.

de Groot G *et al.* (1980). Gas chromatographic determination of buformin in body fluids and tissues, using a nitrogen phosphorus detector: application to a postmortem case. *J Anal Toxicol* 4: 281–285.
Garrett ER, Tsau J (1972). Application of ion-pair methods to drug extraction from biological fluids. I. Quantitative determination of biguanides in urine. *J Pharm Sci* 61: 1404–1410.
Garrett ER *et al.* (1972). Application of ion-pair methods to drug extraction from biological fluids. II. Quantitative determination of biguanides in biological fluids and comparison of protein binding estimates. *J Pharm Sci* 61: 1411–1418.
Gutsche H *et al.* (1976). [Concentration of 14C-1-butylbiguanide in plasma of diabetic patients and its elimination after administration of a new Galenical formulation (author's transl)]. *Arzneimittelforschung* 26: 1227–1229.
Matin SB *et al.* (1975). Simple elcetron capture gas chromatographic method for the determination of oral hypoglycemic biguanidis in biological fluids. *Anal Chem* 47: 545–548.

Bufotenine

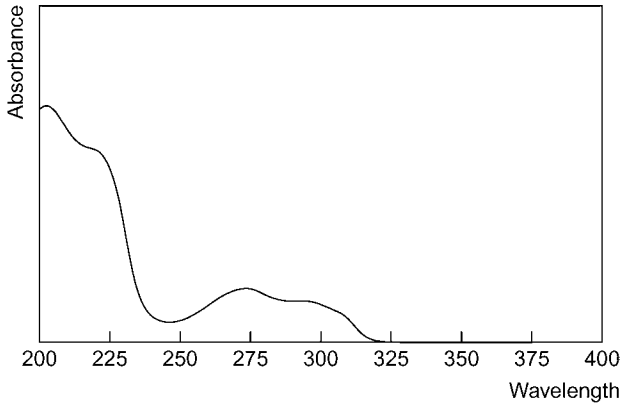
Hallucinogen
$C_{12}H_{16}N_2O$ = 204.3
CAS—487-93-4

IUPAC Name 3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-ol
Synonyms *N,N*-Dimethylserotonin; 5-hydroxy-*N,N*-dimethyltryptamine; mappine.

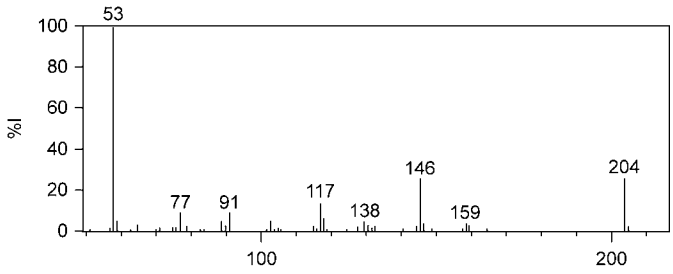


Chemical Properties An indole alkaloid obtained from the seeds and leaves of *Piptadenia peregrina* and *P. macrocarpa* (Mimosaceae). It has also been isolated from species of *Amanita* (Agaricaceae) and from the skin glands of toads (Bufo spp.). A white crystalline powder. Mp 138° to 140°. Almost insoluble in water; freely soluble in ethanol; slightly soluble in ether; soluble in dilute acids and alkalis. Log *P* (octanol) 0.06 [McBride 2000], 1.5.
Colour Test Marquis test—green-brown.
Thin-layer Chromatography System TA—R_f 0.35; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.33; system TL—R_f 0.01; system TAE—R_f 0.10; system TAF—R_f 0.34 (Van Urk reagent, violet).
Gas Chromatography System GA—RI 2057.

Column: 2% SE-30 on 80/100 mesh Gas-Chrom Q or 1% OV-225 on 100/200 mesh Gas-Chrom Q. Temperature: 190°. EI ionisation at 70 eV. SIM acquisition mode. Retention time: 0.55 and 0.62 min on the SE-30 and OV-225 columns, respectively (di-TMS derivative). Limit of detection, <5 pmol [Räisänen, Kärkkäinen 1978].
High Performance Liquid Chromatography System HA—*k* 3.1; system HY—RI 181.
Ultraviolet Spectrum Aqueous acid—278 (A_1^1 = 269b), 297 nm.



Infrared Spectrum Principal peaks at wavenumbers 1232, 824, 1493, 1615, 792, 1052 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 53, 204, 146, 117, 91, 77, 138, 159.



Quantification
Blood **HPLC** Column: Bondapak μC_{18} (1 ft × 0.25 in o.d.). Mobile phase: 0.01 mol/L sodium phosphate buffer (pH 7.6) : acetonitrile (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 5 pmol/L [Riceberg, Vunakis 1978].
Plasma **LC-MS** Column: Xterra MS C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: water : methanol (70 : 30) containing 1% acetic acid, flow rate 60 µL/min to 120 µL/min at 3 min for 2 min to 60 µL/min at 8 min for 2 min. ESI, SIR acquisition mode. Limit of detection, 300 ng/L [Kärkkäinen *et al.* 2005].
Serum **LC-MS** See Plasma [Kärkkäinen *et al.* 2005].
Urine **GC** Column: 1%-OV-101 on 80/100 mesh Gas-Chrom Q. Temperature: 210°. EI ionisation at 70 eV, MID. Limit of detection, 300 ng/L [Räisänen, Kärkkäinen 1979].

GC-MS Column: 1% OV-101 on 80/100 mesh Gas-Chrom Q. Temperature: 210°. EI ionisation. Limit of detection not reported [Räisänen 1984].

HPLC See Blood [Riceberg, Vunakis 1978].

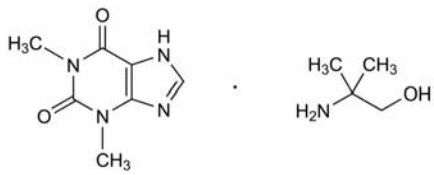
LC-MS Column: C_8 Sunfire (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate buffer (pH 3.5) : acetonitrile-methanol (2 : 1, 98 : 2 at 3.5 min to 80 : 20 at 4 min for 4 min to 2 : 98 at 11 min for 4 min to 98 : 2 at 11.5 min for 6.5 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 0.0125 µg/L [del Mar Ramirez Fernandez *et al.* 2007]. See Plasma [Kärkkäinen *et al.* 2005]. Column: Spheri-5 RP-18 (100 × 1.0 mm i.d., 5 µm). Mobile phase: methanol : water with 0.2% formic acid (50 : 50), flow rate 40 µL/min. ESI, MRM acquisition mode. Limit of detection, 0.35 µg/L [Forsström *et al.* 2001].
Kidney **LC-MS** See Plasma [Kärkkäinen *et al.* 2005].
Lung **LC-MS** See Plasma [Kärkkäinen *et al.* 2005].
Other **GC-MS** Aphrodisiac Preparations. Column: DB-17 (30 m × 0.32 mm, 0.25 µm). Carrier gas: He, 12 psi. Temperature programme: 50° for 3 min to 320° at 20°/min. EI ionisation at 70 eV. Limit of detection not reported [Barry *et al.* 1996].
Disposition in the Body After the IV infusion of 0.2 and 1 mg bufotenine-s′-^{14}C to 2 healthy volunteers, >90% was excreted within 12 h, mainly as 5-hydroxyindoleacetic acid (68 and 74%). Only small amounts were excreted as the unchanged drug (1 and 6%) [Sanders-Bush *et al.* 1976].
Note For reviews of bufotenine, see Lyttle *et al.* [1996] and McBride [2000]. Bufotenine and other bufadienolides derived from toad venom or secretions have been used for their aphrodisiac properties, see Barry *et al.* [1996].

Barry TL *et al.* (1996). GC/MS comparison of the West Indian aphrodisiac "Love Stone" to the Chinese medication "chan su": bufotenine and related bufadienolides. *J Forensic Sci* 41: 1068–1073.
del Mar Ramirez Fernandez M *et al.* (2007). Liquid chromatography-tandem mass spectrometry method for the simultaneous analysis of multiple hallucinogens, chlorpheniramine, ketamine, ritalinic acid, and metabolites, in urine. *J Anal Toxicol* 31: 497–504.
Forsström T *et al.* (2001). Determination of potentially hallucinogenic *N*-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 61: 547–556.
Kärkkäinen J *et al.* (2005). Potentially hallucinogenic 5-hydroxytryptamine receptor ligands bufotenine and dimethyltryptamine in blood and tissues. *Scand J Clin Lab Invest* 65: 189–199.
Lyttle T *et al.* (1996). Bufo toads and bufotenine: fact and fiction surrounding an alleged psychedelic. *J Psychoactive Drugs* 28: 267–290.
McBride MC (2000). Bufotenine: toward an understanding of possible psychoactive mechanisms. *J Psychoactive Drugs* 32: 321–331.
Räisänen M (1984). The presence of free and conjugated bufotenin in normal human urine. *Life Sci* 34: 2041–2045.
Räisänen M, Kärkkäinen J (1978). Quantitative assay of the *N*-methylated metabolites of tryptamine and serotonin by gas chromatography mass spectrometry as applied to the determination of lung indoleethylamine N-methyltransferase activity. *Biomed Mass Spectrom* 5: 596–600.
Räisänen M, Kärkkäinen J (1979). Mass fragmentographic quantification of urinary *N*,*N*-dimethyltryptamine and bufotenine. *J Chromatogr* 162: 579–584.
Riceberg LJ, Vunakis HV (1978). Determination of *N*,*N*-dimethylindolealkylamines in plasma, blood and urine extracts by radioimmunoassay and high pressure liquid chromatography. *J Pharmacol Exp Ther* 206: 158–166.
Sanders-Bush E *et al.* (1976). Metabolism of bufotenine-2′-14C in human volunteers. *Life Sci* 19: 1407–1411.

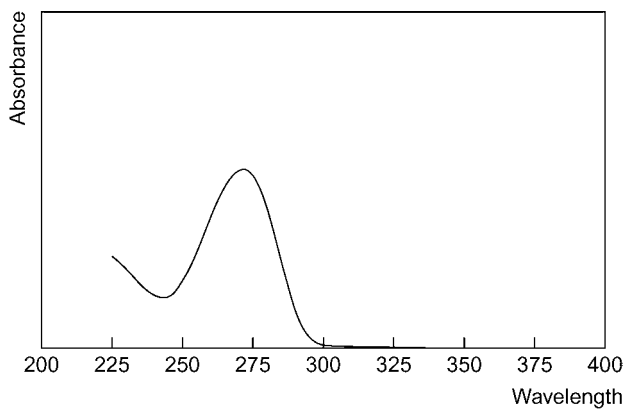
Bufylline

Xanthine Bronchodilator
$C_{11}H_{19}N_5O_3$ = 269.3
CAS—5634-34-4

IUPAC Name 2-Amino-2-methylpropan-1-ol; 1,3-dimethyl-7*H*-purine-2,6-dione
Synonyms Ambuphylline; 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione compound with 2-amino-2-methyl-1-propanol (1:1); theophylline-aminoisobutanol.
Proprietary Names *Butaphyllamine*; *Buthoid*. It is an ingredient of *Nethaprin* and *Nethaprin Dospan*.

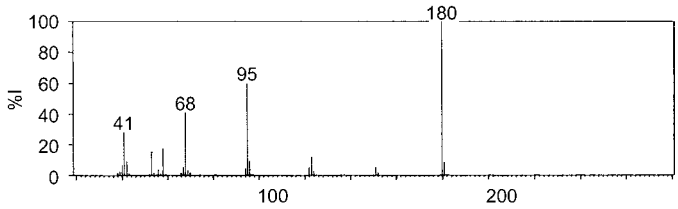


Chemical Properties A white crystalline powder. Mp 254° to 256°. Slightly soluble in water.
Colour Test Amalic acid test—red/violet.
Gas Chromatography System GA—RI 2301.
Ultraviolet Spectrum Aqueous acid—271 nm (A_1^1=350b).



Infrared Spectrum Principal peaks at wavenumbers 1665, 1712, 1636, 1562, 740, 1180 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 180, 95, 68, 41, 58, 53, 123, 96.



Dose 60 to 120 mg every 3 to 4 h.

Bumetanide

Diuretic

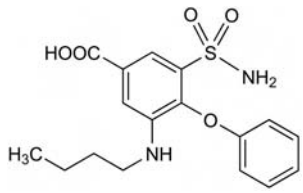
$C_{17}H_{20}N_2O_5S = 364.4$

CAS—28395-03-1

IUPAC Name 3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid

Synonyms 3-(Aminosulfonyl)-5-(butylamino)-4-phenoxybenzoic acid; PF-1593; Ro-10-6338.

Proprietary Names *Burinex*; *Fordiuran*.



Chemical Properties A white crystalline powder. Mp about 230°. Soluble 0.1 mg/mL water; 30.6 mg/mL ethanol; 18.7 mg/mL propylene glycol; >500 mg/mL dimethylacetamide; 76.5 mg/mL methanol; 0.4 mg/mL benzene; 21.6 mg/mL benzyl alcohol; 50.2 mg/mL acetone. Soluble in alkaline solutions. Log *P* (octanol/water), 2.6.

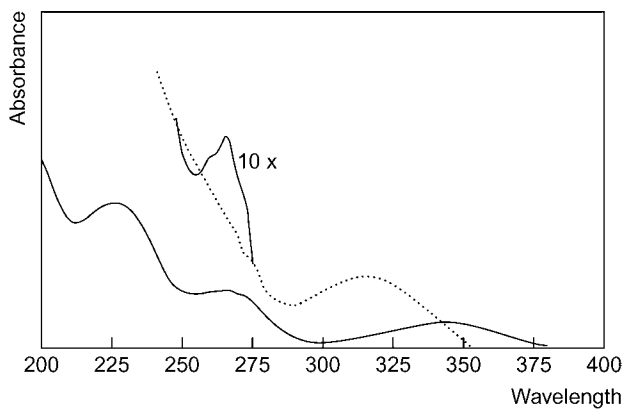
Colour Tests Koppanyi-Zwikker test—violet; Liebermann's reagent—brown-orange; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.01; system TE—R_f 0.04; system TF—R_f 0.10; system TAD—R_f 0.06; system TAE—R_f 0.87; system TAJ—R_f 0.18; system TAK—R_f 0.42; system TAL—R_f 0.80.

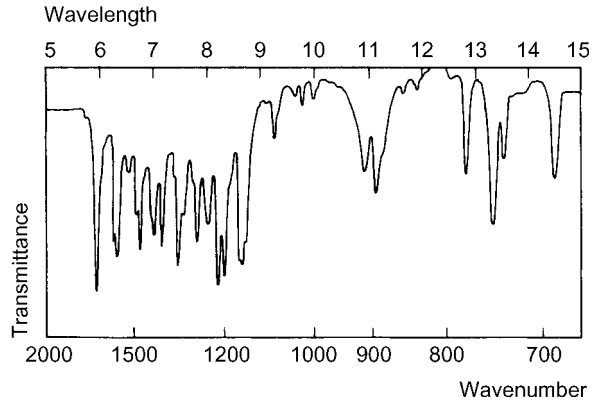
Gas Chromatography System GA—bumetanide-Me_3 RI 2970; system GX—bumetanide-Me_3 retention time 7.7 min; system GY—bumetanide-Me_3 retention time 4.9 min.

High Performance Liquid Chromatography System HAA—retention time 19.1 min.

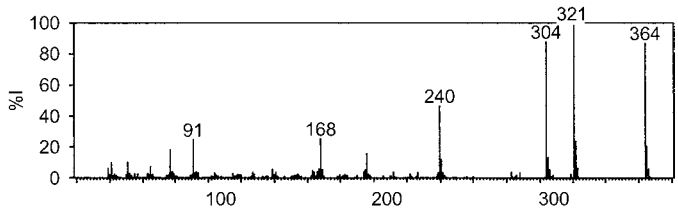
Ultraviolet Spectrum Aqueous acid—340 nm (A_1^1=80b); aqueous alkali—317 nm (A_1^1=87b).



Infrared Spectrum Principal peaks at wavenumbers 1695, 1215, 1199, 1153, 1587, 1280 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 321, 364, 304, 240, 168, 91, 322, 365.



Quantification

Plasma **HPLC** Fluorescence detection (λ_{em}=418 nm; λ_{ex}=228 nm). Limit of detection, 5 µg/L [Wells *et al.* 1991]. Fluorescence detection. Limit of detection, 5 µg/L [Smith 1982].

Serum **GC** FID. Limit of detection, 1 µg/L [Davies *et al.* 1974].

Urine **GC** See Serum [Davies *et al.* 1974].

GC–MS Limit of detection, 10 to 50 µg/L [Lisi *et al.* 1992].

HPLC Amperometric detection. Limit of detection, 0.25 µg/L [Legoburu *et al.* 2001]. See Plasma [Wells *et al.* 1991]. Fluorescence detection. Limit of detection, <10 µg/L [Graaden *et al.* 1990].

Disposition in the Body Rapidly and completely absorbed after oral administration. Metabolised to some extent by hydroxylation of the butyl side-chain to give the 2′-, 3′-, and 4′-alcohols; the 3′-carboxylic acid and desbutyl metabolites have also been reported. About 50% of a dose is excreted in the urine as unchanged drug in 24 h together with 4 to 6% as the 3′-alcohol. About 80% of a dose is excreted in the urine and 10% to 15% is eliminated in the faeces in 48 h.

Therapeutic Concentration

After a single dose of 1 mg given to 12 subjects, a mean peak plasma concentration of 0.03 mg/L was attained in 1 to 8 h [Holazo *et al.* 1984].

Half-life Plasma half-life, about 1 h.

Volume of Distribution About 0.2 L/kg.

Clearance Plasma clearance about 3 mL/min/kg.

Protein Binding About 96%.

Note For a review of bumetanide, see Ward, Heel [1984].

Dose Usually 1 to 2 mg daily; doses of 20 mg or more daily have been given.

Davies DL *et al.* (1974). Renal action, therapeutic use, and pharmacokinetics of the diuretic bumetanide. *Clin Pharmacol Ther* 15: 141–155.

Gradeen CY *et al.* (1990). Analysis of bumetanide in human urine by high-performance liquid chromatography with fluorescence detection and gas chromatography/mass spectrometry. *J Anal Toxicol* 14(2): 123–126.

Holazo AA *et al.* (1984). Pharmacokinetics of bumetanide following intravenous, intramuscular, and oral administrations to normal subjects. *J Pharm Sci* 73: 1108–1113.

Legorburu MJ *et al.* (2001). Quantitative determination of the loop diuretic bumetanide in urine and pharmaceuticals by high-performance liquid chromatography with amperometric detection. *J Chromatogr Sci* 39(10): 425–430.

Lisi AM *et al.* (1992). Diuretic screening in human urine by gas chromatography-mass spectrometry: use of a macroreticular acrylic copolymer for the efficient removal of the coextracted phase-transfer reagent after derivatization by direct extractive alkylation. *J Chromatogr* 581(1): 57–63.

Smith DE (1982). High-performance liquid chromatographic assay for bumetanide in plasma and urine. *J Pharm Sci* 71: 520–523.

Ward A, Heel RC (1984). Bumetanide. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs* 28: 426–464.

Wells TG *et al.* (1991). Measurement of bumetanide in plasma and urine by high-performance liquid chromatography and application to bumetanide disposition. *J Chromatogr* 570(1): 235–242.

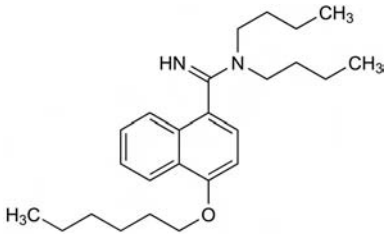
Bunamidine

Anthelmintic (Veterinary)

$C_{25}H_{38}N_2O = 382.6$

CAS—3748-77-4

IUPAC Name *N*,*N*-Dibutyl-4-(hexyloxy)-1-naphthalenecarboximidamide

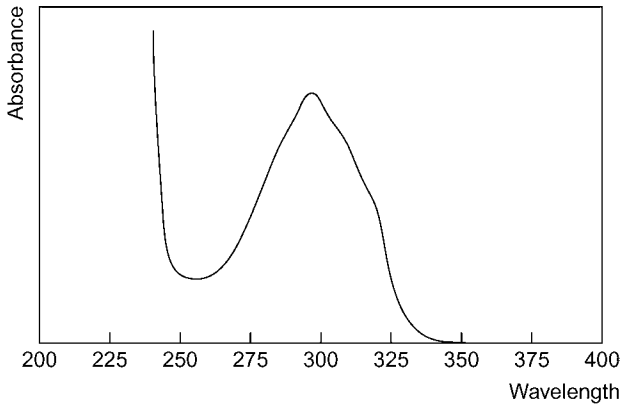


Bunamidine Hydrochloride
$C_{25}H_{38}N_2O,HCl = 419.0$
CAS—1055-55-6
Proprietary Name *Scolaban*
Chemical Properties A white crystalline powder. Mp about 210°. Soluble 1 in 200 of water, 1 in 2 of ethanol and 1 in 2 of chloroform; practically insoluble in ether.

Caution Bunamidine hydrochloride is irritant, especially to the eyes, and should be handled with care.

Bunamidine Hydroxynaphthoate
$C_{36}H_{46}N_2O_4 = 570.8$
CAS—13501-04-7
Proprietary Name *Buban*
Chemical Properties A pale yellow crystalline powder. Mp 169° to 170°. Practically insoluble in water; soluble 1 in 35 of ethanol, 1 in 3 of chloroform and 1 in 300 of ether.

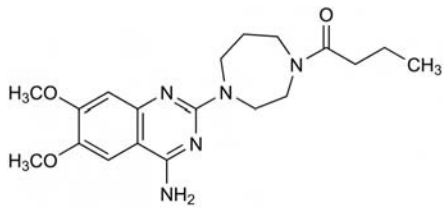
Colour Tests Mandelin's test—green; Marquis test—orange→red.
Thin-layer Chromatography System TA—R_f 0.64 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—298 nm (A^1_1=240a).



Infrared Spectrum Principal peaks at wavenumbers 1572, 1079, 1263, 1234, 763, 1511 cm^{-1} (KBr disk).

Bunazosin

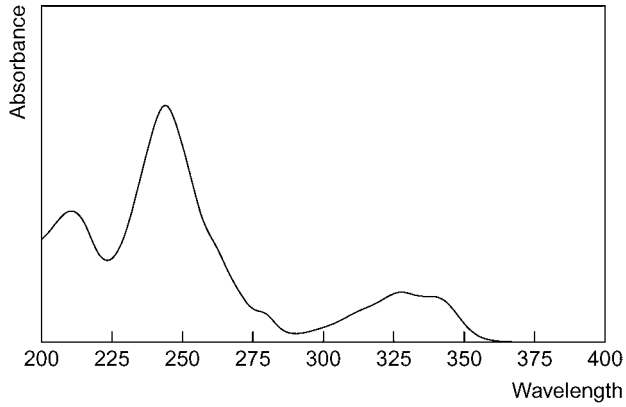
Antihypertensive
$C_{19}H_{27}N_5O_3 = 373.5$
CAS—80755-51-7
IUPAC Name 1-[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-1, 4-diazepan-1-yl]butan-1-one
Synonyms 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-hexahydro-4-(1-oxobutyl)-1*H*-1,4-diazepine; bunazocine; bunazosine; DDQ.



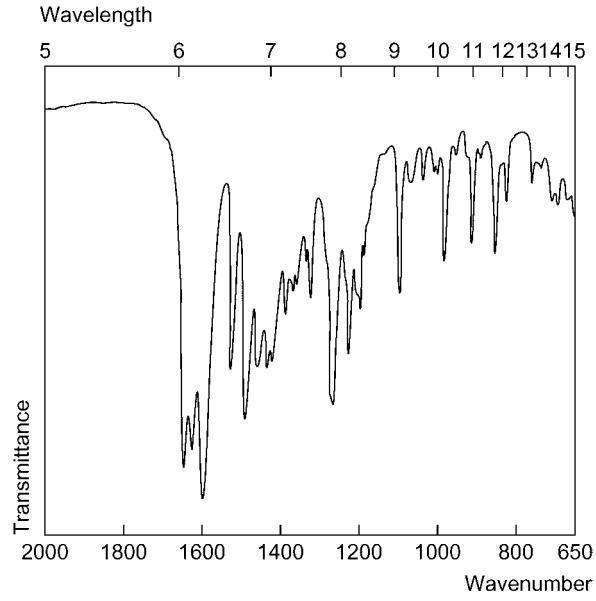
Chemical Properties Log *P* (octanol/water), 0.215.

Bunazosin Hydrochloride
$C_{19}H_{28}ClN_5O_3 = 409.9$
CAS—52712-76-2
Synonym E643
Proprietary Names *Andante*; *Bunazosin Retard*; *Detantol*.
Chemical Properties A white crystalline powder. Mp 273°. It is very soluble in formic acid; slightly soluble in water and methanol; very slightly soluble in dehydrated ethanol; practically insoluble in ether.

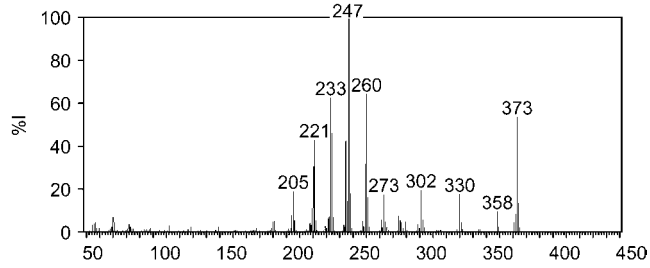
Ultraviolet Spectrum Principal peaks at 211, 244 nm.



Infrared Spectrum Principal peaks at wavenumbers 1598, 1648, 1628, 2953, 3144, 864 cm^{-1} (hydrochloride salt).



Mass Spectrum Principal ions at *m/z* 247, 260, 233, 373, 234, 221, 245, 259.



Quantification
Plasma **HPLC** Fluorescence detection (λ_{ex}=254 nm; λ_{em}=370 nm). Limit of detection, 0.1 µg/L [Nobumichi *et al.* 1993].
Urine **HPLC** Fluorescence detection (λ_{ex}=254 nm; λ_{em}=370 nm). Limit of detection, 0.5 µg/L [Nobumichi *et al.* 1993].
Disposition in the Body Bunazosin is rapidly absorbed after oral administration and undergoes metabolism in the liver. It is eliminated mainly in faeces (60%) and the rest in urine as the unchanged drug (0.7%) and its metabolites. Bunazosin can pass through the placenta to the fetus and is secreted in milk.
Therapeutic Concentration
Twelve healthy, adult males were administered with a 2 mg dose of bunazosin hydrochloride after a meal. The peak plasma concentration of the unchanged drug was 22.5 µg/L which was observed 1 h after administration [Eisai Product Information Sheets].

Twelve healthy volunteers, aged between 65 and 75 years (2 males and 10 females), were administered with a single dose of 6 mg bunazosin (sustained release formulation). Peak plasma concentrations of 9.0 ± 4.0 µg/L were reached within 5 to 7 h. 12 healthy young volunteers, 22 to 39 years old

(6 males and 6 females), were administered with the same dose. Peak plasma concentrations were lower, reaching 6.1 ± 2.6 µg/L for the males and 5.4 ± 1.5 µg/L for females within 3.5 to 6 h [Nobumichi *et al.* 1993].

Toxicity Acute overdose, over 12 mg/day, can result in tachycardia, hypotension, miosis and oliguria. Poison by SC, IM, IV and IP routes. Moderate toxicity by ingestion.

Bioavailability Approximately 45%.

Half-life Elimination half-life is approximately 2 h.

Volume of Distribution Apparent volume of distribution is ~3 to 6 L/kg.

Clearance Body clearance, ~0.25 to 0.35 L/h/kg.

Protein Binding Up to 97%.

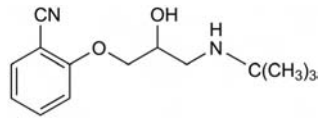
Dose An initial dose of 1.5 mg bunazosin (as the hydrochloride) is administered, which may be increased to 3 to 6 mg daily (sustained-release formulation). The maximum dose is 12 mg/day. The usual adult daily dose for the sustained release preparation is 3 to 9 mg.

Nobumichi NM *et al.* (1993). *Drug Invest* 6: 5–9.
Nobumichi NM *et al.* (1993). *Drug Invest* 6: 62–69.

Bunitrolol

β-*Blocker*
$C_{14}H_{20}N_2O_2 = 248.3$
CAS—34915-68-9

IUPAC Name 2-[3-(Tert-butylamino)-2-hydroxypropoxy]benzonitrile
Synonyms 2-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]benzonitrile; Ko-1366.

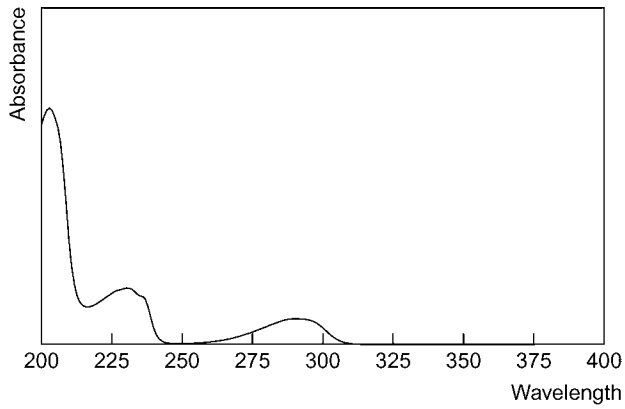


Chemical Properties Log *P* (octanol/water), 1.6.

Bunitrolol Hydrochloride

$C_{14}H_{20}N_2O_2,HCl = 284.8$
CAS—23093-74-5
Proprietary Name *Stresson*
Chemical Properties A crystalline solid. Mp about 164°.

Thin-layer Chromatography System TE—R_f 0.40; system TAE—R_f 0.16.
Gas Chromatography System GA—bunitrolol RI 1948, bunitrolol-Art RI 1980.
Ultraviolet Spectrum Aqueous acid—232 (A^1_1=332b), 292 nm (A^1_1=142b).



Quantification

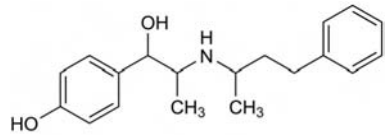
Plasma **HPLC** Fluorescence detection. Limit of detection, 2.5 µg/L for bunitrolol and 4-hydroxybunitrolol [Nagakura, Kohei 1982].
Urine **HPLC** See Plasma [Nagakura, Kohei 1982].
Disposition in the Body Bunitrolol is absorbed after oral administration and metabolised by *p*-hydroxylation followed by conjugation with glucuronic acid. About 1% of a dose is excreted in the urine as unchanged drug and 5% as the *p*-hydroxy metabolite, in 6 h.
Dose Bunitrolol hydrochloride has been given in doses of 20 to 40 mg daily.

Nagakura A, Kohei H (1982). Simultaneous determination of bunitrolol and its metabolite in biological fluids, plasma and urine. *J Chromatogr* 232: 137–143.

Buphenine

Vasodilator
$C_{19}H_{25}NO_2 = 299.4$
CAS—447-41-6

IUPAC Name 4-[1-Hydroxy-2-(4-phenylbutan-2-ylamino)propyl]phenol
Synonyms 4-Hydroxy-α-[1-[(1-methyl-3-phenylpropyl)amino]ethyl]benzenemethanol; nylidrin.

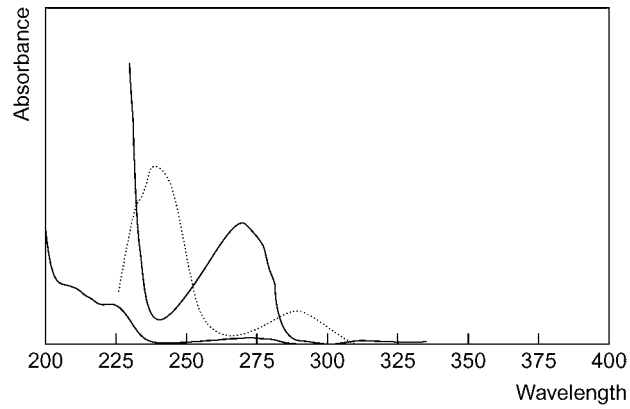


Chemical Properties Mp 111° to 112°. Log *P* (octanol/water), 3.3.

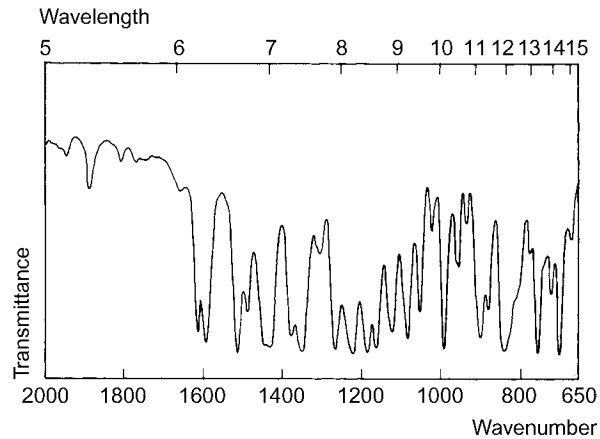
Buphenine Hydrochloride

$C_{19}H_{25}NO_2,HCl = 335.9$
CAS—849-55-8
Proprietary Names *Arlidin*; *Dilatol*; *Opino*; *Penitardon*; *Pervadil*.
Chemical Properties A white crystalline powder. Mp 223° to 226°. Soluble 1 in 65 of water and 1 in 40 of ethanol; practically insoluble in benzene, chloroform and ether.

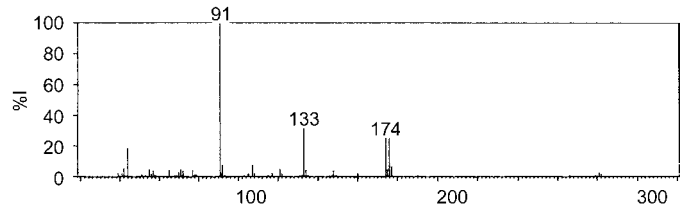
Colour Test Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.03; system TC—R_f 0.14; system TE—R_f 0.62; system TL—R_f 0.50; system TAE—R_f 0.33; system TAF—R_f 0.83 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2314.
High Performance Liquid Chromatography System HA—*k* 0.9; system HX—RI 370.
Ultraviolet Spectrum Aqueous acid—273 nm (A^1_1=40a); aqueous alkali—242 (A^1_1=390b), 291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1512, 1220, 750, 695, 1182, 837 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 133, 176, 174, 44, 107, 92, 177.



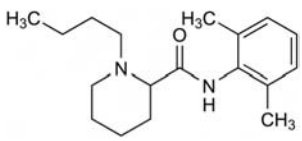
Quantification
Urine **GC** FID. See Li, Cervoni [1976].
Disposition in the Body Buphenine is readily absorbed after oral administration. About 5% of a single dose is excreted in the urine as unchanged drug in 24 h.
Toxicity The estimated minimum lethal dose in adults is 2 g and in children 200 mg.
Dose 18 to 48 mg of buphenine hydrochloride daily.

Li H, Cervoni P (1976). GLC determination of nylidrin in human urine samples. *J Pharm Sci* 65: 1352–1356.

Bupivacaine

Anaesthetic (Local)
$C_{18}H_{28}N_2O$ = 288.4
CAS—2180-92-9

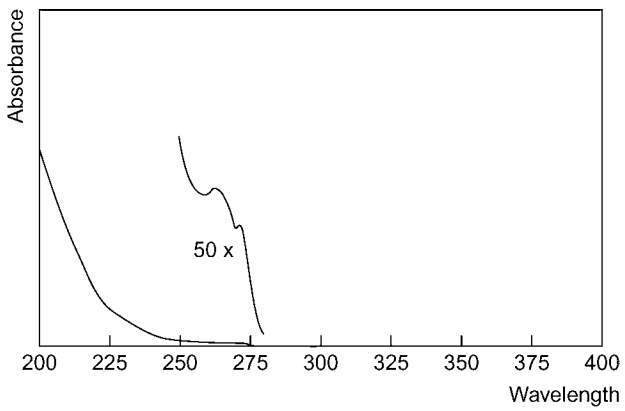
IUPAC Name 1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide
Synonym 1-Butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide



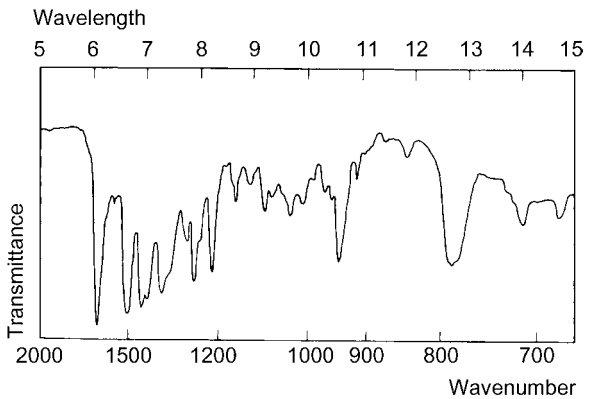
Chemical Properties Mp 107.5° to 108°. pK_a 8.1. Log *P* (octanol), 3.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Bupivacaine Hydrochloride
$C_{18}H_{28}N_2O,HCl,H_2O$ = 342.9
CAS—18010-40-7 (anhydrous); CAS—14252-80-3 (monohydrate).
Proprietary Names *Carbostesin*; *Marcain(e)*; *Sensorcaine*.
Chemical Properties A white crystalline powder. Mp 258.5°. Soluble 1 in 25 of water and 1 in 8 of ethanol; slightly soluble in acetone, chloroform, and ether.

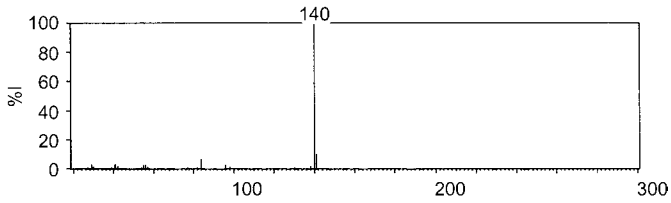
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.42; system TC—R_f 0.73; system TE—R_f 0.80; system TL—R_f 0.65; system TAE—R_f 0.69; system TAF—R_f 0.79; system TAG—R_f 0.65 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—RI 2269.
High Performance Liquid Chromatography System HA—*k* 0.9; system HQ—*k* 7.19; system HR—*k* 0.86; system HX—RI 366; system HY—RI 310; system HZ—RT 4.1 min.
Ultraviolet Spectrum Aqueous acid—263 (A_1^1 = 16a), 271 nm.



Infrared Spectrum Principal peaks at wavenumbers 1667, 1522, 1279, 1222, 787, 944 cm^{-1} (bupivacaine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 140, 141, 84, 41, 29, 96, 56, 55.



Quantification
Blood **GC** FID. Limit of detection, 15 µg/L [Berlin *et al.* 1973].
Plasma **HPLC** Column: C_8 Phenomenex (150 × 4.6 mm i.d., 5 µm). Mobile phase: 70 mmol/L sodium sulfate (pH 3.1) : acetonitrile (70 : 30), flow rate 1.5 mL/min. UV detection (λ = 205 nm). Limit of quantification, 8 µg/L [Gaudreault *et al.* 2009]. Column: LC_8 DB (50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 50 mmol/L phosphate buffer (pH 6.0, 20 : 20 : 60), flow rate 1.2 mL/min. UV detection (λ = 210 nm) Retention time: 3.0 min. Limit of detection, 5 µg/L for bupivacaine and ropivacaine [Rifai *et al.* 2001]. Column: Symmetry C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: 10 mmol/L octanesulfonic acid in acetonitrile-0.05 mol/L phosphate buffer (3 : 97) : 10 mmol/L octanesulfonic acid in acetonitrile-0.05 mol/L phosphate buffer (pH 2, 50 : 50, 70 : 30 for 10 min to 10 : 90 at 45 min to 70 : 30 at 48 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of quantification, 0.1 µmol/L for bupivacaine, ropivacaine and their major metabolites [Arvidsson *et al.* 1999]. Column: Cyano (CN) (250 × 4.6 mm). Mobile phase: methanol : acetonitrile : orthophosphoric acid : 0.01 mol/L sodium dihydrogen phosphate (200 : 80 : 2 : 718). UV detection (λ = 215 nm). Limit of quantification, 125 µg/L [Gross *et al.* 1999]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : acetonitrile : 0.02 mol/L sodium phosphate buffer (pH 6.0, 15 : 40 : 45), flow rate 1.2 mL/min. UV detection (λ = 210 nm). Limit of detection, 10 µg/L for bupivacaine and metabolites [Kastrissios *et al.* 1992]. See also Abraham *et al.* [1997].

LC-MS Column: Mercury MS Luna C_{18} (10 × 4.0 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (95 : 5 for 0.5 min to 10 : 90 at 3 min for 1 min to 95 : 5 at 4.01 min until 5 min), flow rate 3.0 mL/min. ESI, SIM acquisition mode. Limit of quantification, 2.5 µg/L [Kawano *et al.* 2003]. Column: Phenomenex Luna C_{18} (75 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium acetate buffer (pH 7.0, 60 : 40), flow rate 0.8 mL/min. ESI, SIM acquisition mode. Limit of quantification, 2.5 µg/L, limit of detection, 0.5 µg/L [Stumpe *et al.* 2000].
Serum **GC** Column: HP-Innowax (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2, 35 mL/min. Temperature programme: 50° for 1 min to 150° at 25°/min to 240° at 5°/min. FID. Limit of quantification, ~50 µg/L [Baniceru *et al.* 2004]. Column: 3% OV-17 on Chromosorb W HP 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 16 mL/min. Temperature programme: 180° to 240° at 20°/min for 8 min. AFID. Limit of detection, 50 µg/L for bupivacaine and 2′,6′-pipecoloxylidide [Lesko *et al.* 1980].

HPLC Column: reversed phase C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : sodium dihydrogen phosphate (pH 5.6, 100 : 100 : 300), flow rate 1 mL/min. UV detection (λ = 210 nm). Limit of quantification, 5 µg/L for bupivacaine and ropivacaine and 2 µg/L for mepivacaine [Tanaka *et al.* 2006].

LC-MS Column: Synergy Polar-RP 80A (150 × 2 mm i.d., 4 µm). Mobile phase: acetonitrile-2 mmol/L ammonium acetate–formic acid (5 : 95 : 0.2) : acetonitrile-2 mmol/L ammonium acetate-formic acid (95 : 5 : 0.2, 100 : 0 for 0.1 min to 20 : 80 at 2.5 min for 0.5 min to 100 : 0 at 3.2 min until 7 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1 µg/L for bupivacaine and other local anaesthetics [Koehler *et al.* 2005].
Urine **GC-MS** Column: HP5MS cross-linked 5% phenyl methyl silicone (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 2 min to 270° at 20°/min for 6 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 14.07 min. Limit of detection, 5 µg/L for phenolic bupivacaine metabolites [Zhang *et al.* 1998].

HPLC Column: Johnssen Spherigel C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile-11 mmol/L triethylamine solution with 0.1% phosphoric acid (10 : 90) : acetonitrile-20 mmol/L triethylamine with 0.1% phosphoric acid (50 : 50; 100 : 0 to 30 : 70 over 15 min to 0 : 100 at 30 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 0.03 mg/L [Ma *et al.* 2006]. See Plasma. Limit of quantification, 1 µmol/L for bupivacaine, ropivacaine and their major metabolites [Arvidsson *et al.* 1999]. See Plasma [Kastrissios *et al.* 1992].
Disposition in the Body Bupivacaine is metabolised in the liver by oxidative dealkylation to 2′,6′-pipecoloxylidide; 3′- and 4′-hydroxylation also occur. <10% of a dose is excreted in the urine as unchanged drug in 24 h.
Therapeutic Concentration
After epidural administration of 150 mg with adrenaline (epinephrine) to 12 subjects, mean peak plasma concentrations of 1.1 mg/L were reported at 0.3 h and peak CSF concentrations averaged 30 mg/L at 0.5 h [Wilkinson, Lund 1970].

After slow IV infusion of 1.35 mg/kg to three subjects, peak venous plasma concentrations of 2.6–4.5 mg/L were reported 3–17 min after completion of the infusion [Mather *et al.* 1971].

A healthy female patient undergoing tubal ligation was induced with anaesthesia and maintained with propofol, droperidol and fentanyl. Lidocaine (4 mL at 1%) was also administered prior to incision followed by 2 mL (*R,S*)-bupivacaine (0.5%). Peak plasma concentrations of 144 µg/L (*R*)-bupivacaine and 212 µg/L (*S*)-bupivacaine were reached 5.4 min after infiltration with the drug. [Abraham *et al.* 1997].

Fifteen infants, aged <5 months, undergoing minor lower abdominal procedures under a standard general anaesthesia, were administered 0.25%

bupivacaine at a dose of 2.5 mg/kg or 0.25% bupivacaine with adrenaline. The total plasma drug concentration was 114–446 μg/L (median, 400.5) for those receiving the drug only compared with 372–1423 μg/L (median, 742) for those receiving the drug with adrenaline [Hansen *et al.* 2001].

Toxicity Bupivacaine is several times more toxic than lidocaine.

Muscular rigidity has been reported in 2 subjects having blood concentrations of 9 and 12 mg/L following the administration of approximately 210 mg, and convulsions have been reported at plasma concentrations greater than 4 mg/L.

A case report describes the accidental high thoracic epidural infusion of 337.5 mg of 0.75% bupivacaine and 180 μg sufentanil over 30 min. The patient experienced severe hypotension, mild respiratory depression and an extensive sensory and motor block developed, but recovered after treatment [Wolff *et al.* 1992].

A 54-year-old man died after self-injecting bupivacaine into external genitalia. Bupivacaine concentrations of 3.8 mg/L (femoral blood), 2.8 mg/L (heart blood), 1.3 mg/L (vitreous humour) and 11.4 mg/L (urine) were found at postmortem [Yazzie *et al.* 2004].

Half-life Plasma half-life, ~1–3 h, increased in neonates.

Volume of Distribution ~1 L/kg.

Clearance Plasma clearance, ~8 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.4.

Protein Binding ~90%.

Note For a review of the clinical pharmacokinetics of local anaesthetics, see Tucker, Mather [1979].

Dose Bupivacaine hydrochloride is given by injection as a 0.25 or 0.5% solution (calculated as the anhydrous hydrochloride); maximum recommended dose is 2 mg/kg in any 4 h period.

Abraham I *et al.* (1997). Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 703: 203–208.

Arvidsson T *et al.* (1999). Liquid chromatographic bioanalytical determination of ropivacaine, bupivacaine and major metabolites. *Biomed Chromatogr* 13: 286–292.

Baniceru M *et al.* (2004). Determination of some local anesthetics in human serum by gas chromatography with solid-phase extraction. *J Pharm Biomed Anal* 35: 593–598.

Berlin A *et al.* (1973). Micromethod for the determination of bupivacaine in maternal and foetal blood during obstetric analgesia. *J Pharm Pharmacol* 25: 466–469.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Gaudreault F *et al.* (2009). High-performance liquid chromatography using UV detection for the simultaneous quantification of ropivacaine and bupivacaine in human plasma. *Ther Drug Monit* 31: 753–757.

Gross AS *et al.* (1999). Simultaneous analysis of ketamine and bupivacaine in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 728: 107–115.

Hansen TG *et al.* (2001). Plasma concentrations and pharmacokinetics of bupivacaine with and without adrenaline following caudal anaesthesia in infants. *Acta Anaesthesiol Scand* 45: 42–47.

Kastrissios H *et al.* (1992). High-performance liquid chromatographic method for the quantitation of bupivacaine, 2,6-pipecoloxylidide and 4′-hydroxybupivacaine in plasma and urine. *J Chromatogr* 577: 103–107.

Kawano S *et al.* (2003). Direct analysis of drugs in plasma by column-switching liquid chromatography–mass spectrometry using a methylcellulose-immobilized reversed-phase pretreatment column. *J Chromatogr B Analyt Technol Biomed Life Sci* 792: 49–54.

Koehler A *et al.* (2005). Simultaneous determination of bupivacaine, mepivacain, prilocaine and ropivacain in human serum by liquid chromatography–tandem mass spectrometry. *J Chromatogr A* 1088: 126–130.

Lesko LJ *et al.* (1980). Simultaneous determination of bupivacaine and 2,6-pipecoloxylidide in serum by gas–liquid chromatography. *J Chromatogr* 182: 226–231.

Ma M *et al.* (2006). Liquid-phase microextraction combined with high-performance liquid chromatography for the determination of local anaesthetics in human urine. *J Pharm Biomed Anal* 40: 128–135.

Mather LE *et al.* (1971). The intravenous toxicity and clearance of bupivacaine in man. *Clin Pharmacol Ther* 12: 935–943.

Rifai N *et al.* (2001). Simultaneous measurement of plasma ropivacaine and bupivacaine concentrations by HPLC with UV detection. *Ther Drug Monit* 23: 182–186.

Stumpe M *et al.* (2000). Determination of free concentrations of ropivacaine and bupivacaine in plasma from neonates using small-scale equilibrium-dialysis followed by liquid chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 748: 321–330.

Tanaka E *et al.* (2006). Simultaneous determination of three local anesthetic drugs from the pipecoloxylidide group in human serum by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 834: 213–216.

Tucker GT, Mather LE (1979). Clinical pharmacokinetics of local anaesthetics. *Clin Pharmacokinet* 4: 241–278.

Wilkinson GR, Lund PC (1970). Bupivacaine levels in plasma and cerebrospinal fluid following peridural administration. *Anesthesiology* 33: 482–486.

Wolff AP *et al.* (1992). Accidental overdose of epidural bupivacaine and sufentanil. *Reg Anesth* 17: 237–238.

Yazzie J *et al.* (2004). Fatal bupivacaine intoxication following unusual erotic practices. *J Forensic Sci* 49: 351–353.

Zhang AQ *et al.* (1998). The application of capillary gas chromatography–selective ion mass spectrometry for the separation, identification and quantification of phenolic bupivacaine metabolites from human urine. *J Pharm Biomed Anal* 17: 1139–1142.

Buprenorphine

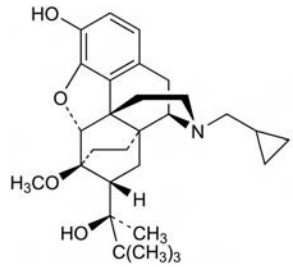
Narcotic Analgesic

$C_{29}H_{41}NO_4$ = 467.6

CAS—52485-79-7

Synonyms Buprenorphinum; [5α,7α(*S*)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol; (2*S*)-2-[(-)-(5*R*,6*R*,7*R*,14*S*)-9α-cyclopropylmethyl-4,5-epoxy-6,14-ethano-3-hydroxy-6-methoxymorphinan-7-yl]-3,3-dimethylbutan-2-ol; RX-6029-M.

Proprietary Names *BuTrans*; *Nopan*; *Transtec*.



Chemical Properties A white or almost white crystalline powder. Very slightly soluble in water; freely soluble in acetone; soluble in methanol; slightly soluble in cyclohexane. Also dissolves in dilute solutions of acids. Mp 209°. pK_{a1}, 8.5, pK_{a2}, 10.0. Log *P* (octanol/water), 4.98. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Buprenorphine Hydrochloride

$C_{29}H_{41}NO_4$,HCl = 504.1

CAS—53152-21-9

Synonyms CL-112302, NIH-8805, UM-952.

Proprietary Names *Anorfin*; *Buprenex*; *Buprex*; *Buprine*; *Finibron*; *Lepetan*; *Nopan*; *Prefin*; *Suboxone*; *Subutex*; *Temgesic*.

Chemical Properties A white or almost white crystalline powder. Sparingly soluble in water; soluble in alcohol; freely soluble in methanol; practically insoluble in cyclohexane.

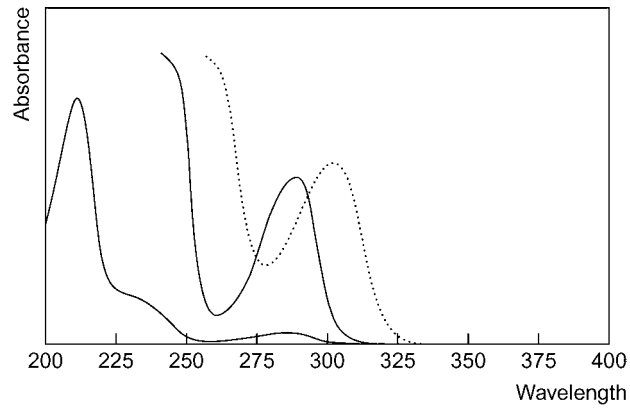
Colour Tests Liebermann's reagent—black; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.09; system TC—R_f 0.68; system TE—R_f 0.80; system TL—R_f 0.69; system TAE—R_f 0.80; system TAJ—R_f 0.62; system TAK—R_f 0.04; system TAL—R_f 0.77.

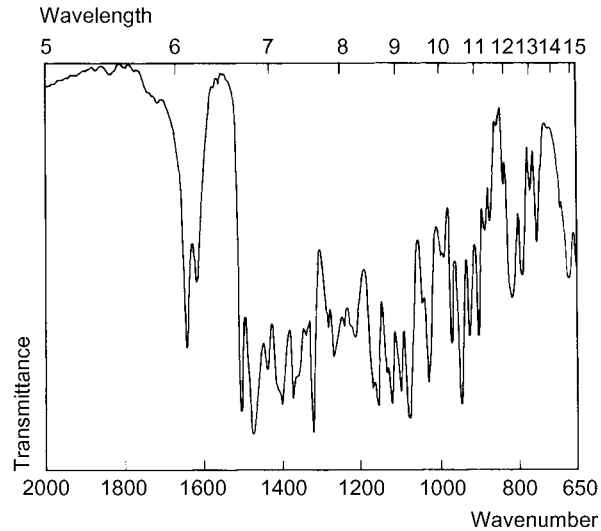
Gas Chromatography System GA—RI 3360, M (-AC) 3410, art (-H_2O) RI 3240, art (-H_2O)-AC RI 3320; system GB—RI 3610; system GM—not eluted.

High Performance Liquid Chromatography System HA—*k* 0.4; system HC—*k* 0.05; system HX—RI 397; system HY—RI 339; system HZ—RT 5.0 min; system HAA—RT 14.0 min.

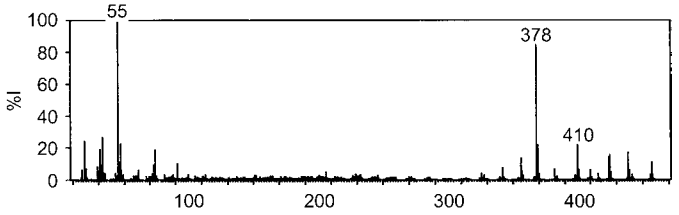
Ultraviolet Spectrum Aqueous acid—286 nm (A_1^1 = 33b); aqueous alkali—300 nm.



Infrared Spectrum Principal peaks at wave numbers 1320, 1077, 1503, 1155, 1120, 947 cm^{-1} (buprenorphine hydrochloride, KCl disk).



Mass Spectrum Principal ions at *m/z* 55, 378, 43, 29, 57, 410, 379, 84.



Quantification

Blood **LC-MS** Column: Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 μm). Mobile phase: methanol : ammonium formate. MRM acquisition mode, positive ion mode. Limit of quantification, 0.0005–0.01 mg/kg for buprenorphine and other drugs of abuse [Bjork *et al.* 2010]. Column: Genesis C_{18} reversed phase. Mobile phase: acetonitrile ammonium acetate (pH 3.2). MRM acquisition mode, positive ion mode [Gergov *et al.* 2009] Column: Phenomenex Synergi reverse phase. Mobile phase: 10 mmol/L ammonium formate (pH 3) : acetonitrile. Limit of quantification, 0.5–4.09 μg/L, limit of detection, 0.16–1.2 μg/L for buprenorphine and other opioids [Al Asmari, Anderson 2007]. Column: cyanopropyl (150 × 2.1 mm). ESI, positive ion mode. Limit of detection, 0.05 μg/L for buprenorphine and norbuprenorphine [Favretto *et al.* 2006]. APCI, SIM acquisition mode [Bogusz *et al.* 1998]. Column: Nova-Pak C_{18} (150 × 2 mm i.d., 4 μm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate buffer (pH 3.0, 80 : 20), flow rate 200 μL/min. Limit of detection, 0.1 μg/L for buprenorphine and 0.05 μg/L for norbuprenorphine [Tracqui *et al.* 1997]. See also [Hoja *et al.* 1997].

Plasma **GC** ECD. Limit of quantification, 0.1 μg/L [Everhart *et al.* 1997].

GC-MS Column: HP-1 fused silica. Carrier gas: He. EI ionisation, SIM acquisition mode. Limit of quantification, 0.05 μg/L for buprenorphine and 0.1 μg/L for norbuprenorphine [Gopal *et al.* 2001]. PICI. Limit of quantification, 0.5 μg/L [Moody *et al.* 1997].

HPLC Column: reversed-phase C_8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: phosphate buffer containing TEA : methanol : acetonitrile (pH 6.0, 40 : 50 : 10). Limit of quantification, 1.0 μg/L, limit of detection, 0.4 μg for buprenorphine and norbuprenorphine [Mercolini *et al.* 2007]. Column: reversed phase Spherisorb C_8. Mobile phase: 0.06 mol/L potassium dihydrogen phosphate-disodium hydrogen phosphate (pH 6.4) : acetonitrile : TEA : Pic B5 (520 : 480 : 0.5 : 15). UV detection (λ = 214 nm) [Lagrange *et al.* 1998]. ECD. Limit of detection, 40 ng/L for buprenorphine and norbuprenorphine [Schleyer *et al.* 1993].

LC-MS Buprenorphine, norbuprenorphine and glucuronide metabolites [Concheiro *et al.* 2010]. Limit of quantification, 0.25 μg/L for buprenorphine and norbuprenorphine [DiFrancesco *et al.* 2007]. Column: chiral α_1-acid glycoprotein. Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 7.0, 18 : 82), flow rate 0.9 mL/min. Buprenorphine, norbuprenorphine and methadone enantiomers and metabolite [Rodriguez-Rosas *et al.* 2007]. Limit of quantification, 0.1 μg/L for buprenorphine, norbuprenorphine and glucuronides [Huang *et al.* 2006]. Column: Phenomenex C_{12} MX-RP (150 × 2 mm i.d., 4 μm). Mobile phase: ammonium formate buffer (pH 3.5) : acetonitrile. Buprenorphine and other opioids [Musshoff *et al.* 2006]. See also Ceccato *et al.* [2003], Moody *et al.* [1997], Moody *et al.* [2002], Murphy, Huestis [2005] and Polettini, Huestis [2001].

Serum **LC-MS** ESI. Limit of detection, 0.2 μg/L for buprenorphine and its major metabolites [Oechsler, Skopp 2010]. Column: LiChroCART with a Purospher RP-18e cartridge. SIM acquisition mode. Limit of quantification, 0.2 μg/L for buprenorphine and norbuprenorphine [Scislowski *et al.* 2005]. See Blood [Bogusz *et al.* 1998].

Urine **TLC** Fluorescence densiometric detection (λ = 340 nm) [Alemany *et al.* 1996].

GC-MS EI ionisation. Limit of quantification, 1 μg/L for buprenorphine and norbuprenorphine [Fuller 2008]. Limit of detection, 1.0 μg/L for buprenorphine and norbuprenorphine [George *et al.* 2004]. EI ionisation, SIM acquisition mode. Limit of quantification, 0.25 μg/L for buprenorphine and 0.20 μg/L for norbuprenorphine [Vincent *et al.* 1999]. Limit of quantification, 1 μg/L, limit of detection, 0.2 μg/L for buprenorphine and norbuprenorphine [Lisi *et al.* 1997].

HPLC Electrochemical detection. Limit of detection, 0.2 μg/L for buprenorphine and 0.15 μg/L for norbuprenorphine [Debrabandere *et al.* 1992]. Electrochemical detection. Limit of detection, 250 ng/L for buprenorphine and 100 ng/L for norbuprenorphine [Debrabandere *et al.* 1991]. See Plasma [Mercolini *et al.* 2007; Schleyer *et al.* 1993].

LC-MS Column: Synergy Polar RP. Mobile phase: 10 mmol/L ammonium formate (pH 3) : acetonitrile. Limit of quantification, 0.7–1.2 μg/L, limit of detection, 0.2–0.4 μg/L for buprenorphine and its metabolites [Al Asmari, Anderson 2008]. Buprenorphine and its metabolites [Kacinko *et al.* 2008]. Column: C_{18}. ESI [Liu *et al.* 2008]. MRM acquisition mode. Limit of quantification, 4.6 μg/L for buprenorphine glucuronide and 11.8 μg/L for norbuprenorphine glucuronide [Hegstad *et al.* 2007]. Limit of quantification, 0.5 μg/L for buprenorphine, buprenorphine and norbuprenorphine glucuronides and 2.5 μg/L for norbuprenorphine [Huang *et al.* 2006]. See Blood [Bogusz *et al.* 1998; Favretto *et al.* 2006; Gergov *et al.* 2009]. See also Fox *et al.* [2006] and Kronstrand *et al.* [2003].

Immunoassay Immunoassay versus LC-MS [Twigger *et al.* 2008]. Buprenorphine and norbuprenorphine, validation with GC-MS [Wang *et al.* 2007]. Limit of detection, 20 μg/L [Debrabandere *et al.* 1995].

Milk **LC-MS** Limit of quantification, 0.18 μg/L for buprenorphine and 0.20 μg/L for norbuprenorphine, limit of detection, 0.05 μg/L for buprenorphine and norbuprenorphine [Grimm *et al.* 2005].

Hair **GC-MS** Limit of quantification, 0.005 μg/g for buprenorphine and norbuprenorphine [Vincent *et al.* 1999].

HPLC Column: Lichrosorb CN Mobile phase: phosphate buffer (pH 4.0) : acetonitrile : 1-heptanosulfonic acid : butylamine (85 : 17 : 2 : 0.01). Electrochemical detection. Limit of detection, 0.02 μg/g for buprenorphine and 0.01 μg/g for norbuprenorphine [Kintz *et al.* 1994].

LC-MS Column: cyanopropyl (150 × 2.1 mm i.d.). ESI, positive ion mode. Limit of detection, 4 ng/g for buprenorphine and norbuprenorphine [Favretto *et al.* 2006]. Column: Nova-Pak C_{18} (150 × 2 mm i.d., 4 μm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate buffer (pH 3.0, 80 : 20), flow rate 200 μL/min. Limit of detection, 4 ng/g for buprenorphine and 2 ng/g for norbuprenorphine [Tracqui *et al.* 1997].

Disposition in the Body Following IM administration, buprenorphine rapidly reaches peak plasma concentration. Absorption also takes place through the buccal mucosa following sublingual administration. It is metabolised mainly by *N*-dealkylation via cytochrome P450 CYP3A4 to *N*-dealkylbuprenorphine (norbuprenorphine) and by conjugation. It is eliminated mainly in the faeces, with a small proportion excreted in the urine, as metabolites. Buprenorphine is subject to considerable first-pass metabolism following oral administration. Small amounts of buprenorphine are distributed into breast milk.

Therapeutic Concentration

In 11 patients receiving maintenance doses of 8 mg buprenorphine sublingually, those receiving the dose daily had a mean steady-state plasma concentration (at 24 h) of 0.00080 mg/L; those receiving the dose on alternate days had a level of 0.00077 mg/L at 24 h. Daily and alternate-day steady-state norbuprenorphine plasma concentrations were 0.0010 and 0.0009 ng/L, respectively [Kuhlman, Jr *et al.* 1998].

After sublingual administration of buprenorphine solution at a dose of 16 mg/70 kg body weight daily to 10 subjects, mean plasma concentrations of 0.00067 mg/L were attained 24 h after each dose. Different thrice-weekly dosing schedules with doses of 16–44 mg/70 kg resulted in plasma concentrations varying from 0.0009–0.00169 mg/L at 24 h and 0.00041–0.00096 mg/L at 48 h [Chawarski *et al.* 1999].

In 6 subjects each receiving a 7.7 mg dose of buprenorphine in liquid form (sublingually) and 8 mg in tablet form, 1 week apart, peak plasma levels were 0.0071 mg/L attained at 0.9 h and 0.0029 mg/L attained at 1.2 h for the liquid and tablet dosages, respectively [Nath *et al.* 1999].

Toxicity

A 25-year-old man who apparently had committed suicide (drug packages including high-dose sublingual tablets of buprenorphine were found next to his body) had the following postmortem tissue concentrations of buprenorphine and norbuprenorphine, respectively: blood 3.3 and 0.4 mg/L, urine 3.4 and 0.6 mg/L, bile 2035 and 536 mg/L and brain 6.4 and 3.9 μg/g. High concentrations of amino-7-flunitrazepam, the main metabolite of flunitrazepam, were also detected in blood, urine and gastric fluid [Gaulier *et al.* 2000].

A 14-year-old boy died of asphyxia caused by a combination of buprenorphine and benzodiazepines in drug-facilitated sexual abuse. Postmortem buprenorphine concentrations were 1.1 μg/L in blood and 23 ng/g in hair [Kintz *et al.* 2003].

In a survey of 21 cases in which buprenorphine was detected in postmortem blood and/or urine, 18 were sudden deaths. Of these, 6 deaths were attributed to natural causes and blood buprenorphine levels ranged from undetected (detected in urine) to 3.2 μg/L. The other 12 cases were attributed directly or indirectly to mixed drug poisoning, and blood buprenorphine levels ranged from undetected (detected in urine) to 17 μg/L [Lai *et al.* 2006].

In an analysis of 86 cases of buprenorphine overdoses in children under 6 years of age, 54 children developed signs of toxicity. The mean time to onset of effects was 64.2 min (range, 20–180). No child ingesting <4 mg experienced a severe effect [Hayes *et al.* 2008].

In 2 series of 39 and 78 fatalities involving buprenorphine, postmortem blood levels were 0.0005–0.0510 mg/L (mean, 0.0102) and 0.0001–0.0760 mg/L (mean, 0.0126); norbuprenorphine levels were 0.0002–0.0471 mg/L (mean, 0.0082) and <0.0001–0.0650 mg/L (mean, 0.0106), respectively. Two of the deaths were suicides, with blood buprenorphine concentrations of 0.1440 and 3.276 mg/L [Kintz 2001].

For an earlier report of 20 fatalities involving buprenorphine, see Tracqui *et al.* [1998] and for a later report of 13 buprenorphine-related deaths, see Kintz [2002].

Half-life Plasma half-life, 1.2–7.2 h.

Volume of Distribution ~1.4 L/kg.

Clearance Plasma, ~13 mL/min/kg.

Protein Binding ~96%.

Dose In the treatment of pain, equivalent of 300 to 600 μg buprenorphine given parenterally, or 200 to 400 μg sublingually, every 6 to 8 h. In the treatment of opioid dependence, initially 0.8 to 4 mg sublingually once daily; maintenance, up to maximum of 32 mg daily.

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Bupropion

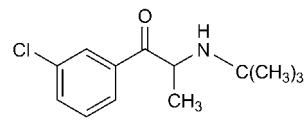
Aminoketone, Antidepressant

$C_{13}H_{18}ClNO = 239.7$

CAS—34911-55-2

IUPAC Name 2-(*tert*-Butylamino)-1-(3-chlorophenyl)propan-1-one

Synonyms Amfebutamone; 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)-amino]-1-propanone.



Chemical Properties A pale-yellow oil. Bp 52°. Soluble in methanol, ethanol, acetone, ether and benzene. Bupropion is unstable in plasma at 22° and 37°, but samples frozen at –20° were stable for at least 6 months [Laizure, DeVane 1985]. When stored at −20°, plasma and urine samples were within 15% of the original concentration for both bupropion and hydroxybupropion enantiomers. After 3 freeze–thaw cycles, analyte concentrations in plasma and urine were within 15% of the initial value. When extracted samples were stored in an autosampler at 10°, bupropion and hydroxybupropion began to racemise within 24 h. When the autosampler temperature was lowered to 4°, the enantiomeric ratio remained stable for 24 h but began to racemise by 4 days. When extracted samples were stored at −20° before analysis, the enantiomeric ratio remained constant for at least 7 days [Coles, Kharasch 2007].

Bupropion Hydrochloride

$C_{13}H_{19}Cl_2NO = 276.2$

CAS—31677-93-7

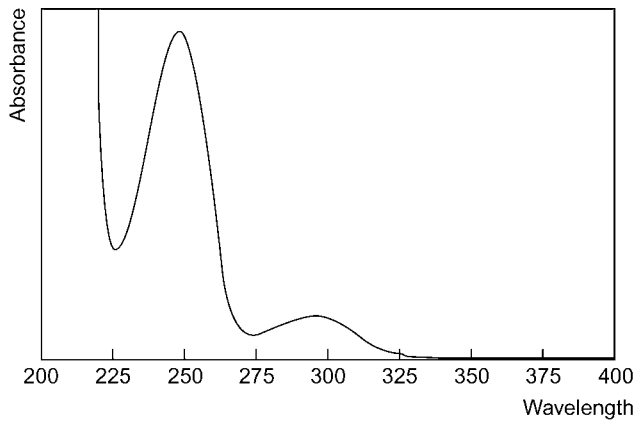
Synonyms Amfebutamone hydrochloride; BW-323.

Proprietary Names *Wellbutrin*; *Zyban*.

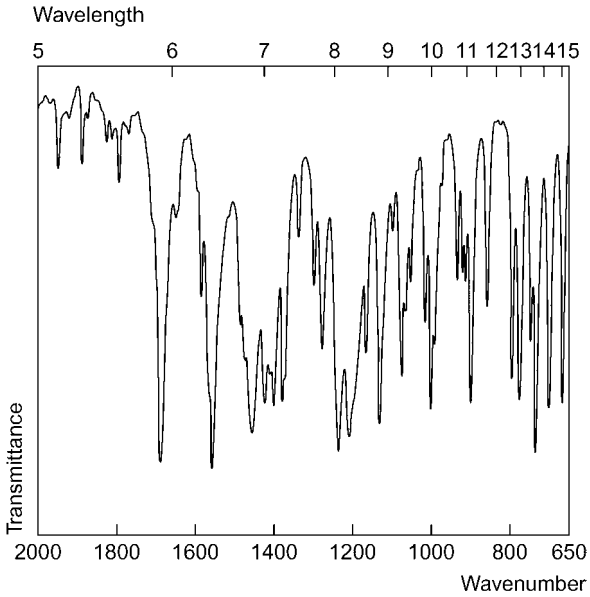
Chemical Properties White crystalline powder. Mp 233° to 234°. Soluble in water, alcohol and hydrochloric acid.

Gas Chromatography System GB—RI 1645 (bupropion), RI 1898 (M (OH-)).

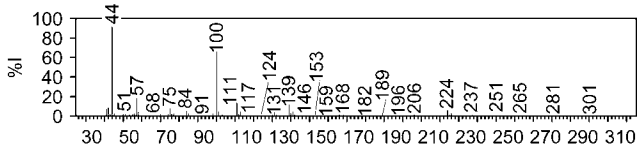
Ultraviolet Spectrum Aqueous acid (methanol)—249, 296 nm.



Infrared Spectrum Principal peaks at wavenumbers 1560, 1691, 2676, 2982, 1458, 739 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 44, 100, 57, 111, 139, 75, 84, 224.



Quantification

Blood **GC** Column: DB-1 (15 m × 0.54 mm i.d., 1.5 µm) or DB-17 (15 m × 0.55 mm i.d., 1.0 µm). Temperature programme: 120° for 1 min to 220° at 20°/min for 5 min. NPD. Limit of detection not reported [Meeker *et al.* 1992]. Column: DB-5 (12 m) and HP-1 (10 m). Temperature programme: 100° to 300° at 10°/min for 10 min. NPD. Reference compound: *n*-propylamphetamine. Retention time: 8.62 min. Limit of detection not reported [Rohrig, Ray 1992].

GC-MS Column: BP-1 capillary (15 m). Temperature programme: 80° for 1 min to 295° at 15°/min for 5 min. R_f 0.75. Limit of detection not reported [Friel *et al.* 1993]. Column: HP-5 cross-linked 5% phenyl methyl silicone fused silica capillary (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 30°/min to 260° at 10°/min to 300° at 20°/min for 8 min. Limit of detection not reported [Ramcharitar *et al.* 1992]. Column: DB-5 capillary (30 m × 0.25 mm i.d.). Temperature programme: 150° for 5 min to 280° at 10°/min for 5 min. Ion trap detection. Retention time: 8.14 min. Limit of detection, 0.1 mg/L [Wohlenberg *et al.* 1992].

LC-MS Column: SymmetryShield RP 18 (150 × 1.0 mm i.d., 3.5 µm). Mobile phase: 2 mmol/L ammonium formate (pH 4.0) : acetonitrile 2 mmol/L ammonium formate buffer (pH 4.0; 90 : 10; 95 : 5 for 1 min to 65 : 35 at 1.5 min to 60 : 40 at 5.5 min to 5 : 95 at 7.5 min to 95 : 5 at 8.5 min for 3.5 min), flow rate 50 µL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L; limit of detection, 5 µg/L [Mercerolle *et al.* 2008].

Plasma **HPLC** Column: Phenomenex Aqua C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.05 mol/L phosphate buffer (pH 5.5; 45 : 55), flow rate 1.0 mL/min. UV detection (λ = 254 nm for bupropion, 214 nm for hydroxybupropion). Limit of quantification, 2.5, 10, 5 and 10 µg/L for bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion, respectively, limit of detection, 2.0, 7.5, 2.5 and 7.5 µg/L for bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion, respectively [Loboz *et al.* 2005]. Column: TMS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L monobasic potassium phosphate buffer : acetonitrile (80 : 20) with 0.007 mol/L sodium heptane sulfonate and 0.01 mol/L triethylamine (pH 3.0), flow rate 2.3 mL/min. UV detection (λ = 254 nm). Retention time: 11.5 min. Limit of detection, 5.0 µg/L for bupropion and 0.1 mg/L for its metabolites [Cooper *et al.* 1984]. Column: silica (100 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.05 mol/L ammonium acid phosphate (pH 3.2), flow rate 0.9 mL/min. UV detection (λ = 248 nm). Retention time: 4.29 min. Limit of detection, 5.0 µg/L [Jennison *et al.* 1995].

LC-MS Column: Chiral α_1-acid glycoprotein (AGP; 100 × 2 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium formate (pH 5.7) : methanol (90 : 10 for 0.5 min to 70 : 30 at 1.0 min for 4.0 min to 50 : 50 at 8 min for 7 min), flow rate 0.22 mL/min. Retention time: 8.4 and 10.1 min for (*R*)- and (*S*)-bupropion and 9.3 and 13.5 min for (*S,S*)- and (*R,R*)-hydroxybupropion, respectively. Limit of detection, 0.5 and 2.5 µg/L for the bupropion and hydroxybupropion enantiomers, respectively [Coles, Kharasch 2007]. Column: Chromolith SpeedROD RP-18 (50 × 4.6 mm i.d.). Mobile phase: 8 mmol/L ammonium acetate : acetonitrile (55 : 45), flow rate 5.0 mL/min. TIS, positive ion mode, SRM acquisition mode. Retention time: 0.38, 0.25 and 0.23 min for bupropion, threohydrobupropion and hydroxybupropion, respectively. Limit of quantification, 0.25 µg/L for bupropion and threohydrobupropion, 1.25 µg/L for hydroxybupropion [Borges *et al.* 2004].

Serum **HPLC** Column: Silica (100 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.05 mol/L ammonium acid phosphate (pH 3.2), flow rate 0.9 mL/min. UV detection (λ = 248 nm). Retention time: 4.29 min. Limit of detection, 5.0 µg/L [Jennison *et al.* 1995].

Urine **GC-MS** See Blood [Ramcharitar *et al.* 1992].

LC-MS See Blood [Mercerolle *et al.* 2008]. See Plasma. Limit of detection, 5 and 25 µg/L for the bupropion and hydroxybupropion enantiomers, respectively [Coles, Kharasch 2007]. Column: Gemini C_{18} (150 × 2.0 mm i.d., 5.0 µm). Mobile phase: 0.1% acetic acid : methanol (98 : 2 to 10 : 90 in 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Retention time: 10.4 min. Limit of detection not reported [Petsalo *et al.* 2007].

Bile **GC-MS** See Blood [Ramcharitar *et al.* 1992].

Stomach **GC-MS** See Blood [Ramcharitar *et al.* 1992].

Vitreous Humour **GC** See Blood [Meeker *et al.* 1992].

Kidney **GC-MS** See Blood [Ramcharitar *et al.* 1992].

Liver **GC** See Blood [Meeker *et al.* 1992]. See Blood [Rohrig, Ray 1992].

GC-MS See Blood [Ramcharitar *et al.* 1992].

Note For an enantiomeric separation of the phenylmorphinol metabolite of bupropion, see Suckow *et al.* [1997].

Disposition in the Body Bupropion is well absorbed and undergoes hepatic metabolism via oxidation, hydroxylation and reduction. Peak plasma concentrations reached within 3 h for sustained-release preparations and 2 h for immediate release. Several metabolites are produced, which are pharmacologically active. The 3 active metabolites are hydroxybupropion (the major metabolite), threohydrobupropion and erythrohydrobupropion. The enzyme CYP2B6 is involved in the hydroxylation of bupropion [Turpeinen *et al.* 2004]. Greater than 60% of a dose is excreted in urine within 24 h, 87% over 96 h and 10% in the faeces, with <1% being the parent drug [Lai, Schroeder 1983; Schroeder 1983]. Bupropion crosses the placenta and is secreted in milk.

Therapeutic Concentration The serum therapeutic concentration range is 0.025 to 0.1 mg/L.

Six healthy male volunteers were administered a single oral dose of 200 mg bupropion hydrochloride. Mean peak plasma concentrations ranged from 126 to 388 µg/L and were reached within 100 min [Laizure *et al.* 1985].

Eight healthy male volunteers were administered bupropion in the following doses: a single oral dose of 100 mg, 100 mg 8-hourly oral dose for 14 days, and a single 100 mg oral dose 14 days later. Mean peak plasma concentrations were 0.31 ± 0.08, 0.31 ± 0.07 and 0.26 ± 0.07 µmol/L at 1.72 ± 0.6, 1.73 ± 0.38, and 1.25 ± 0.30 h, respectively [Posner *et al.* 1985].

Toxicity Blood levels >0.17 mg/L are associated with seizures, and those >0.45 mg/L can be fatal. Other toxic effects include tachycardia, hallucinations and loss of consciousness.

A 35-year-old male was found dead next to an empty box of thirty 150 mg slow-release bupropion tablets. Blood and urine concentrations (mg/L) of bupropion and its metabolites were as shown below:

Sample	*Bupropion*	*Hydroxybupropion*	*Threohydrobupropion*
Femoral blood	N.D.	5.8	30.4
Urine	42.9	100	617

N.D. Not detected [Mercerolle *et al.* 2008].

In 3 fatal overdose cases involving bupropion, the victims had blood concentrations of 4.0, 0.16 and 4.2 mg/L bupropion. In the 2 cases where bupropion was highest, the lethal dose was estimated to be <10 g [Friel *et al.* 1993].

A 45-year-old female with a history of psychological problems took 2 bottles of bupropion (100 to 150 tablets, up to 22.5 g). She was admitted to hospital semi-conscious and died after failing to respond to resuscitation. At postmortem her bupropion concentrations in subclavian blood, vitreous humour and liver were 13.0, 11.0 and 8.7 mg/L, respectively [Meeker *et al.* 1992].

A 62-year-old female was found to be unresponsive. Her heart and femoral blood bupropion concentrations were 4.62 and 0.12 mg/L, respectively. A 34-year-old female attempted suicide by stabbing herself in the abdomen. She was treated with bupropion in hospital but died 6 days later. Her heart and femoral blood bupropion concentrations were 1.02 and 1.05 mg/L, respectively [Wohlenberg *et al.* 1992].

A 40-year-old female was found dead in bed. Her heart and femoral blood bupropion concentrations were 20.8 and 11.0 mg/L, respectively with 13.8 mg/kg bupropion in her liver. There was 1.58 g bupropion in her stomach [Rohrig, Ray 1992].

A 38-year-old female with a history of depression was found unresponsive in the street. The bupropion and metabolite concentrations described below were found at postmortem.

Specimen	*Bupropion*	*Hydroxybupropion*	*Erythroamino Alcohol*	*Threoamino Alcohol*
Heart blood (mg/L)	4.2	5.0	0.6	4.6
Subclavian blood (mg/L)	6.2	5.8	1.0	5.1
Bile (mg/L)	1.4	5.0	1.0	5.1
Kidney (mg/kg)	2.4	14	3.5	21
Liver (mg/kg)	1.0	13	6.3	35
Stomach contents (mg)	16	9	0.7	3.4
Urine (mg/L)	37	27	14	54

[Ramcharitar *et al.* 1992]

Half-life Terminal half-life is approximately 14 h (regular release formulation) and 21 h (sustained release).
Volume of Distribution Apparent, 27 to 63 L/kg [Lai, Schroeder 1983].
Clearance ≈2 L/h/kg [Lai, Schroeder 1983].
Protein Binding ≈84% [Findlay *et al.* 1981].
Dose Initial dose of 100 mg twice daily, after 3 days increased to 100 mg three times a day. Maximum dose 450 mg/day. With modified-release preparations, initial dose is 150 mg daily, increasing to 150 mg twice daily. Maximum dose is 200 mg twice daily. The dose is reduced for patients with renal or hepatic failure.

Borges V *et al.* (2004). High-throughput liquid chromatography-tandem mass spectrometry determination of bupropion and its metabolites in human, mouse and rat plasma using a monolithic column. *J Chromatogr B Analyt Technol Biomed Life Sci* 804: 277–287.
Coles R, Kharasch ED (2007). Stereoselective analysis of bupropion and hydroxybupropion in human plasma and urine by LC/MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 67–75.
Cooper TB *et al.* (1984). Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection. *J Pharm Sci* 73: 1104–1107.
Findlay JW *et al.* (1981). Pharmacokinetics of bupropion, a novel antidepressant agent, following oral administration to healthy subjects. *Eur J Clin Pharmacol* 21: 127–135.
Friel PN *et al.* (1993). Three fatal drug overdoses involving bupropion. *J Anal Toxicol* 17: 436–438.
Jennison TA *et al.* (1995). A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum. *J Anal Toxicol* 19: 69–72.
Lai AA, Schroeder DH (1983). Clinical pharmacokinetics of bupropion: a review. *J Clin Psychiatry* 44: 82–84.
Laizure SC, DeVane CL (1985). Stability of bupropion and its major metabolites in human plasma. *Ther Drug Monit* 7: 447–450.
Laizure SC *et al.* (1985). Pharmacokinetics of bupropion and its major basic metabolites in normal subjects after a single dose. *Clin Pharmacol Ther* 38: 586–589.
Loboz KK *et al.* (2005). HPLC assay for bupropion and its major metabolites in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 823: 115–121.
Meeker JE *et al.* (1992). A suicidal overdose of bupropion (Wellbutrin). *TIAFT Bulletin Case Notes* 22.
Mercerolle M *et al.* (2008). A fatal case of bupropion (Zyban) overdose. *J Anal Toxicol* 32: 192–196.
Petsalo A *et al.* (2007). Identification of bupropion urinary metabolites by liquid chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 21: 2547–2554.
Posner J *et al.* (1985). The disposition of bupropion and its metabolites in healthy male volunteers after single and multiple doses. *Eur J Clin Pharmacol* 29: 97–103.
Ramcharitar V *et al.* (1992). Bupropion and alcohol fatal intoxication: case report. *Forensic Sci Int* 56: 151–156.
Rohrig TP, Ray NG (1992). Tissue distribution of bupropion in a fatal overdose. *J Anal Toxicol* 16: 343–345.
Schroeder DH (1983). Metabolism and kinetics of bupropion. *J Clin Psychiatry* 44: 79–81.
Suckow RF *et al.* (1997). Enantiomeric determination of the phenylmorpholinol metabolite of bupropion in human plasma using coupled achiral-chiral liquid chromatography. *Biomed Chromatogr* 11: 174–179.
Turpeinen M *et al.* (2004). Selective inhibition of CYP2B6-catalyzed bupropion hydroxylation in human liver microsomes in vitro. *Drug Metab Dispos* 32: 626–631.
Wohlenberg N *et al.* (1992). Postmortem quantitation of bupropion (Wellbutrin) in blood using the Finnigan ion trap detector. *TIAFT Bulletin Case Notes* 22.

Buserelin

Antineoplastic, Gonadorelin Analogue
$C_{60}H_{86}N_{16}O_{13}$ = 1239.4
CAS—57982-77-1
IUPAC Name (2*S*)-*N*-[(2*S*)-1-[[(2*S*)-1-[[(2*S*)-1-[[(2*S*)-1-[[(2*R*)-1-[[(2*S*)-1-[[(2*S*)-5-(Diaminomethylideneamino)-1-[(2*S*)-2-(ethylcarbamoyl)pyrrolidin-1-yl]-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-[(2-methylpropan-2-yl)oxy]-1-oxopropan-2-yl]amino]-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino]-3-hydroxy-1-oxopropan-2-yl]amino]-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-3-(1*H*-imidazol-5-yl)-1-oxopropan-2-yl]-5-oxopyrrolidine-2-carboxamide
Synonym 6-[*O*-(1,1-Dimethylethyl)-D-serine]-9-(*N*-ethyl-L-prolinamide)-10-deglycinamide-luteinising hormone-releasing factor (pig)

5-oxoPro—His—Trp—Ser—Tyr—D-Ser(t-Bu)—Leu—Arg—ProNHCH$_2$CH$_3$

Chemical Properties A white or slightly yellowish hygroscopic powder. It is sparingly soluble in water and in dilute mineral acids.

Buserelin Acetate

$C_{60}H_{86}N_{16}O_{13},C_2H_4O_2$ = 1299.5
CAS—68630-75-1
Synonym HOE-766
Proprietary Names *Bigonist*; *Profact*; *Receptal*; *Suprecur*; *Supreface*.

Disposition in the Body Buserelin is completely absorbed after SC injection, with peak plasma concentrations occurring after about 1 h. It accumulates in the liver and kidneys as well as in the anterior pituitary gland. A high degree of inactivation of the drug occurs by gastrointestinal enzymes (peptidases), and the proportion of active compound reaching systemic circulation is very low. The main serum metabolite is inactive buserelin (5–9) pentapeptide. Buserelin is excreted in urine and bile as the unchanged drug (66% dose) and its metabolites (28% in 24 h). 17 to 32% of a dose is detected in urine after IV or SC administration. Very small amounts are distributed into breast milk.

Therapeutic Concentration

A mean peak plasma concentration of 54.2 µg/L was reached 30 min after administration of 30 µg/kg SC to 11 children being treated for central precocious puberty. Following administration of 200 µg intranasally to 10 children, a mean peak plasma concentration of 0.65 µg/L was obtained after 30 min [Holland *et al.* 1986].

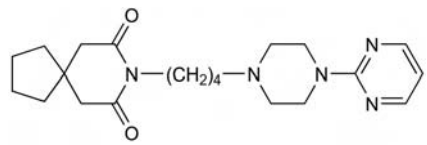
Following administration of 1000 µg SC every 12 h for 7 days to 3 women (aged between 18 and 30 years, with laparoscopically diagnosed endometriosis), the mean plasma concentration was 41.7 µg/L at 15 min. Concentrations were also 23.7, 7.3 and 0.97 µg/L after 2, 4 and 8 h, respectively. Mean plasma concentrations of 1.39 µg/L were measured 15 min after the third dose of 300 µg intranasally every 8 h to 5 women [Kiesel *et al.* 1989].

Half-life Plasma, 80 min.
Protein Binding 15%.
Note For a review of buserelin, see Brogden *et al.* [1990].
Dose In prostatic carcinoma, 500 µg (of the base) SC every 8 h for 7 days, then 200 µg into each nostril every 8 h.

Brogden RN *et al.* (1990). Buserelin. A review of its pharmacodynamic and pharmacokinetic properties, and clinical profile. *Drugs* 39: 399–437.
Holland FJ *et al.* (1986). Pharmacokinetic characteristics of the gonadotropin-releasing hormone analog D-Ser(TBU)-6EA-10luteinizing hormone-releasing hormone (buserelin) after subcutaneous and intranasal administration in children with central precocious puberty. *J Clin Endocrinol Metab* 63: 1065–1070.
Kiesel L *et al.* (1989). Serum concentration and urinary excretion of the luteinizing hormone-releasing hormone agonist buserelin in patients with endometriosis. *J Clin Endocrinol Metab* 68: 1167–1173.

Buspirone

Anxiolytic
$C_{21}H_{31}N_5O_2$ = 385.5
CAS—36505-84-7
IUPAC Name 8-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione



Chemical Properties Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Buspirone Hydrochloride

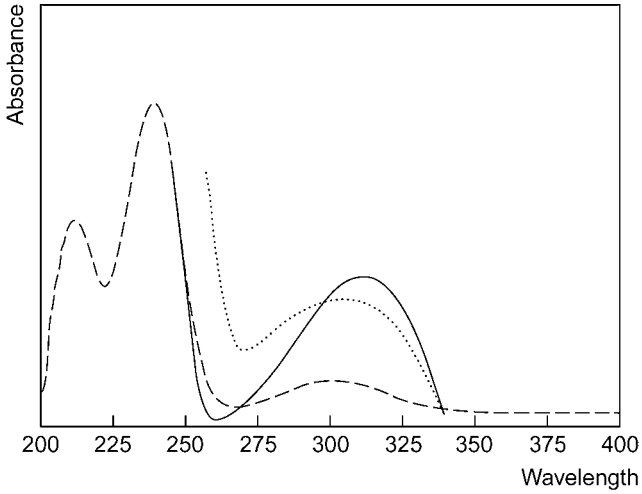
$C_{21}H_{31}N_5O_2$,HCl = 422.0
CAS—33386-08-2
Proprietary Names *Ansial*; *Ansiced*; *Axoren*; *Bespar*; *Buspar*; *Buspimen*; *Buspinol*; *Buspisal*; *Censpar*; *Lucelan*; *Narol*; *Travin*.
Chemical Properties A white crystalline powder. Mp 201.5° to 202.5°. It is very soluble in water; sparingly soluble in ethanol and in acetonitrile; very slightly soluble in ethyl acetate; freely soluble in methanol and in methylene chloride; practically insoluble in hexanes.

Thin-Layer Chromatography System TB—R_f 0.30; system TE—R_f 0.71; system TF—R_f 0.05; system TAE—R_f 0.64.
Gas Chromatography Column: methyl silicone (HP1, 0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. MS detection. Retention index: 3299 [Mills, Roberson 1993].
High Performance Liquid Chromatography System HX—RI 369; system HY—RI 310; system HZ—retention time 3.5 min; system HAA—retention time 12.5 min; system HAX—retention time 9.1 min; system HAY—retention time 5.0 min.

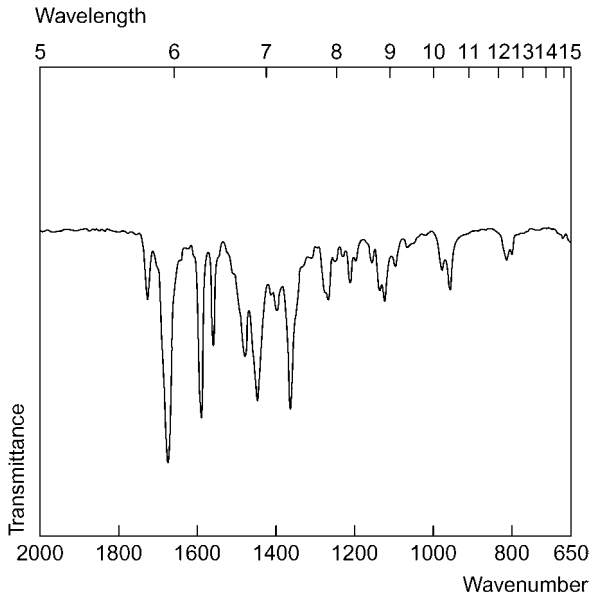
Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer, (pH 3.5) (70:30), flow

rate 1 mL/min. UV diode array detection. Retention time: 2.1 min [Mills, Roberson 1993].

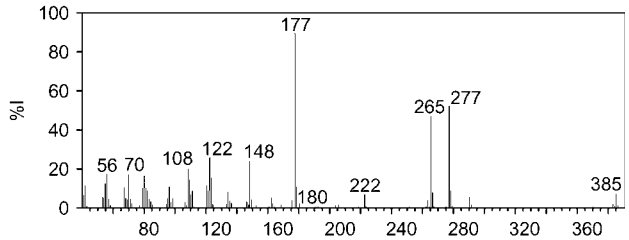
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—312 nm; basic—307 nm.



Infrared Spectrum Principal peaks at wavenumbers 1668, 1436, 1351 cm^{-1} (KBr pellets).



Mass Spectrum Principal peaks at *m/z* 177, 277, 265, 122, 148, 108, 56, 70.



Quantification

Plasma **GC** Column: fused-silica capillary coated with Ultra 2.5% phenyl methyl silicone (25 m × 0.32 mm i.d., 0.52 µm). Temperature programme: 210° to 285° at 2.5°/min. Carrier gas: He, flow rate 2.1 mL/min. Detection: electron-capture/nitrogen–phosphorous. Relative retention time (to prazepam, internal standard): 2.03. ECD and NPD. Limit of detection, 5 µg/L [Gaillard *et al.* 1993].

GC–MS Limit of detection, 0.1 µg/L [Sciacca *et al.* 1988]. Limit of detection, 0.05 µg/L [Gammans 1985].

HPLC Electrochemical detection. Limit of detection, 0.1 µg/L [Ary *et al.* 1998].

Serum **HPLC** UV detection. Limit of detection, 0.2 µg/L [Kristjánsson 1991].

Urine **GC–MS** Limit of detection, 0.2 µg/L [Sciacca *et al.* 1988].

Disposition in the Body It is rapidly absorbed after oral administration; peak plasma concentrations occur after about 40 to 90 min. It undergoes extensive first-pass metabolism primarily by oxidation and bioavailability is therefore low. Metabolism is probably mediated by CYP3A4. Most metabolites are inactive, although oxidative dealkylation produces 1-(2-pyrimidinyl)-piperazine which is about 20 to 25% as potent as the parent drug. The major metabolite is 5-hydroxybuspirone which may be oxidised further to many more metabolites and its glucuronide conjugate. The metabolites are excreted mainly in urine (65%); some are also excreted in faeces (35%). <1% of a dose is excreted as the unchanged drug. It is not removed by haemodialysis.

Therapeutic Concentration The serum therapeutic concentration range is 0.9 to 5 mg/L.

Plasma concentrations of buspirone are usually very low and variable between subjects. Studies suggest that buspirone has nonlinear pharmacokinetics.

Bioavailability 4%.

Half-life Plasma, 2 to 11 h; mean, 2.4 h.

Volume of Distribution 5.3 L/kg after a 20 mg IV dose.

Clearance Plasma, 2.0 to 3.5 L/h/kg, also reported as 1.70 L/h/kg.

Protein Binding 95%.

Note For a review of buspirone, see Gammans *et al.* [1986].

Dose Usually 15 to 30 mg of buspirone hydrochloride daily.

Ary K *et al.* (1998). High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique. *J Chromatogr A* 797: 221–226.

Betto P *et al.* (1992). Simultaneous high-performance liquid chromatographic analysis of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in plasma using electrochemical detection. *J Chromatogr* 575: 117–121.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Franklin M (1990). Determination of plasma buspirone by high-performance liquid chromatography with coulometric detection. *J Chromatogr* 526: 590–596.

Gaillard Y *et al.* (1993). Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction. *J Chromatogr* 622: 197–208.

Gammans RE (1985). Capillary gas chromatographic-mass spectrometric determination of buspirone in plasma. *J Chromatogr* 345: 285–297.

Gammans RE *et al.* (1986). Metabolism and disposition of buspirone. *Am J Med* 80: 41–51.

Kristjánsson F (1991). Sensitive determination of buspirone in serum by solid-phase extraction and two-dimensional high-performance liquid chromatography. *J Chromatogr* 566: 250–256.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton. CRC Press, 5: 76–77.

Sciacca MA *et al.* (1988). Simultaneous quantitation of buspirone and 1-(2-pyrimidinyl)piperazine in human plasma and urine by capillary gas chromatography-mass spectrometry. *J Chromatogr* 428: 265–274.

Busulfan

Antineoplastic

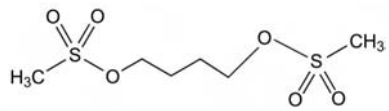
$C_6H_{14}O_6S_2$ = 246.3

CAS—55-98-1

IUPAC Name 4-Methylsulfonyloxybutyl methanesulfonate

Synonyms Busulphan; 1,4-butanediol dimethanesulfonate esters; CB-2041; GT-41.

Proprietary Names *Misulban*; *Myleran*.



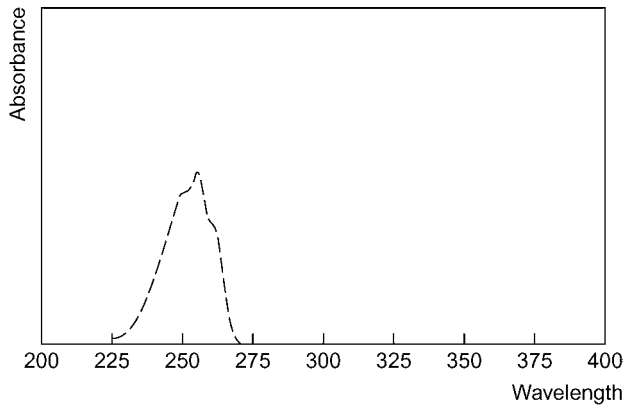
Chemical Properties A white crystalline powder. Mp 114° to 118°. Soluble 1 in 750 of water and 1 in 25 of acetone; slightly soluble in ethanol. Log *P* (octanol/water), −0.5.

Caution Busulfan is irritant; avoid contact with skin and mucous membranes.

Thin-layer Chromatography System TAE—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 368.

Ultraviolet Spectrum Methanol—256 nm (A^1_1=20b).



Infrared Spectrum Principal peaks at wavenumbers 1178, 934, 861, 980, 962, 773 cm^{-1} (KBr disk).

Quantification

***Plasma* GC** ECD. Limit of quantification, 0.01 mg/L [Burns *et al.* 1995]. ECD. Limit of quantification, 0.1 mg/L, limit of detection, 0.06 mg/L [Embree *et al.* 1993].

GC–MS SIM (*m/z* 183 and 197). Limit of quantification, 0.04 mg/L [Lai *et al.* 1998]. Limit of detection, 10 mg/L [Ehrsson, Hassan 1983].

HPLC Fluorescence detection (λ_{ex}=360 nm; λ_{em}=425 nm). Limit of quantification, 20 µg/L, limit of detection, 9 µg/L [Peris *et al.* 1999]. UV detection (λ=251 nm). Limit of quantification, 0.06 mg/L, limit of detection, 0.02 mg/L [Heggie *et al.* 1997].

***Serum* HPLC** UV detection (λ=278 nm). Limit of quantification, 10 mg/L [Funakoshi *et al.* 1994].

Disposition in the Body Readily absorbed after oral administration. It is mainly excreted in the urine as metabolites; about 1% of a dose is excreted in the urine unchanged in 24 h.

Therapeutic Concentration

Following single oral doses of 6 mg to 5 subjects, peak plasma concentrations of 0.05 to 0.13 mg/L (mean 0.08) were reported [Ehrsson *et al.* 1983].

A patient was administered a 1 mg/kg dose of busulfan on two consecutive days. The maximum plasma concentrations reached were 1.2 mg/L 20 min after dosing on the first day and 1.6 mg/L after approx. 60 min on the following day [Peris *et al.* 1999].

Six adult patients, aged 31.8 to 56.9 years, were administered a single low dose of busulfan of 4.87 to 8.2 mg, and six children, aged 0.9 to 15.8 years were administered 2.29 to 7.72 mg. A mean maximum concentration of 0.504 mg/L was reached by the children and 0.352 mg/L by the adults less than 2 h after dosing [Hassan *et al.* 2001].

Half-life Plasma half-life, about 2 to 3 h.

Dose Usually 2 to 4 mg daily; maintenance, 0.5 to 2 mg daily.

Burns RB *et al.* (1995). A gas-chromatographic assay method for busulfan with sensitivity for test dose therapeutic monitoring. *J Pharm Biomed Anal* 13(9): 1073–1078.

Ehrsson H, Hassan M (1983). Determination of busulfan in plasma by GC-MS with selected-ion monitoring. *J Pharm Sci* 72: 1203–1205.

Ehrsson H *et al.* (1983). Busulfan kinetics. *Clin Pharmacol Ther* 34: 86–89.

Embree L *et al.* (1993). Gas-chromatographic analysis of busulfan for therapeutic drug monitoring. *Cancer Chemother Pharmacol* 32(2): 137–142.

Funakoshi K *et al.* (1994). High-performance liquid chromatographic determination of busulfan in human serum with on-line derivatization, column switching and ultraviolet absorbance detection. *J Chromatogr B Biomed Sci Appl* 660(1): 200–204.

Hassan Z *et al.* (2001). Pharmacokinetics of liposomal busulphan in man. *Bone Marrow Transplant* 27(5): 479–485.

Heggie JR *et al.* (1997). Validation of a high-performance liquid chromatographic assay method for pharmacokinetic evaluation of busulfan. *J Chromatogr B Biomed Sci Appl* 692 (2): 437–444.

Lai WK *et al.* (1998). Routine analysis of plasma busulfan by gas chromatography-mass fragmentography. *Clin Chem* 44(12): 2506–2510.

Peris JE *et al.* (1999). Determination of busulfan in human plasma using high-performance liquid chromatography with pre-column derivatization and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 730(1): 33–40.

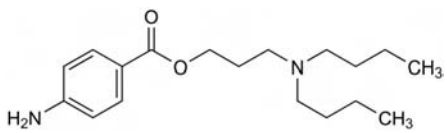
Butacaine

Anaesthetic (Local)

$C_{18}H_{30}N_2O_2$ = 306.4

CAS—149-16-6

IUPAC Name 3-(Dibutylamino)-1-propanol 4-aminobenzoate



Chemical Properties A liquid. pK_a 9.0. Log *P* (octanol/water), 4.4.

Butacaine Sulfate

$(C_{18}H_{30}N_2O_2)_2,H_2SO_4$ = 711.0

CAS—149-15-5

Proprietary Name It is an ingredient of *Rhinamid*.

Chemical Properties A white, very hygroscopic, crystalline powder. Unstable to light and moisture. Mp 100° to 103°. Soluble 1 in 1.5 of water, 1 in 2 of ethanol, 1 in 2.5 of chloroform and 1 in 2000 of ether; quite soluble in acetone.

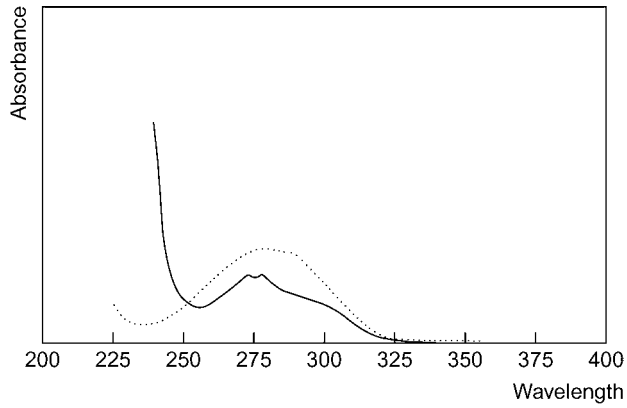
Colour Test Diazotisation—red.

Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.07; system TC—R_f 0.30; system TE—R_f 0.83; system TL—R_f 0.64; system TAE—R_f 0.44; system TAF—R_f 0.76; system TAJ—R_f 0.27; system TAK—R_f 0.05; system TAL—R_f 0.20 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Ninhydrin spray, positive; Van Urk reagent, bright yellow).

Gas Chromatography System GA—RI 2457.

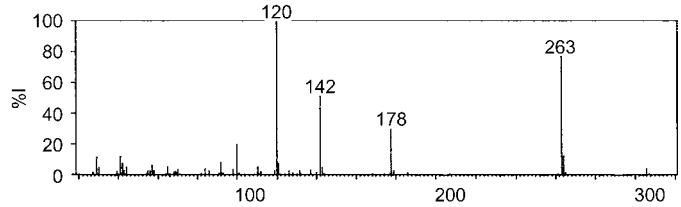
High Performance Liquid Chromatography System HA—*k* 1.2; system HQ—*k* 8.97; system HX—RI 392; system HY—RI 331.

Ultraviolet Spectrum Aqueous acid—272, 278 nm (A_1^1=72b); aqueous alkali—282 nm.



Infrared Spectrum Principal peaks at wavenumbers 1275, 1165, 1598, 1694, 1639, 1111 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 120, 263, 142, 178, 100, 264, 41, 29.



Use A 2% solution of butacaine sulfate has been used for surface anaesthesia.

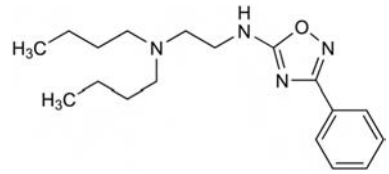
Butalamine

Vasodilator

$C_{18}H_{28}N_4O$ = 316.4

CAS—22131-35-7

IUPAC Name *N*,*N*-Dibutyl-*N′*-(3-phenyl-1,2,4-oxadiazol-5-yl)-1,2-ethanediamine



Chemical Properties Log *P* (octanol/water), 4.4.

Butalamine Hydrochloride

$C_{18}H_{28}N_4O,HCl$ = 352.9

CAS—56974-46-0

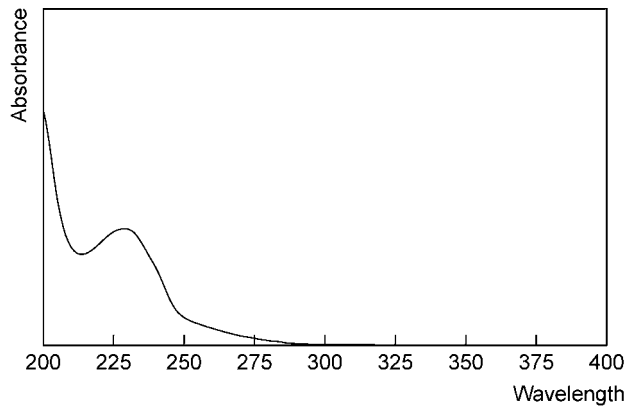
Proprietary Names *Adrevil*; *Surheme*.

Chemical Properties A white crystalline powder. Mp 145°. Soluble 1 in 7 of water, 1 in 10 of ethanol and 1 in 2.5 of chloroform.

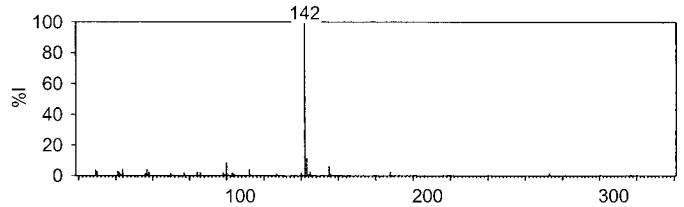
Thin-layer Chromatography System TA—R_f 0.68; system TD—R_f 0.11; system TE—R_f 0.86; system TF—R_f 0.29; system TAD—R_f 0.46 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2490.

Ultraviolet Spectrum Aqueous acid—228 nm (A_1^1=812a).



Infrared Spectrum Principal peaks at wavenumbers 1639, 751, 1603, 694, 1492, 1170 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 142, 143, 100, 155, 44, 112, 57, 29.

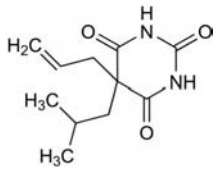


Dose Butalamine hydrochloride has been given in doses of 160 to 320 mg daily.

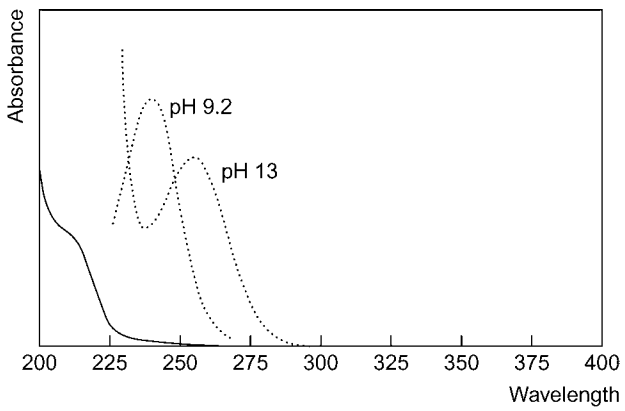
Butalbital

Sedative, Barbiturate
$C_{11}H_{16}N_2O_3 = 224.3$
CAS—77-26-9

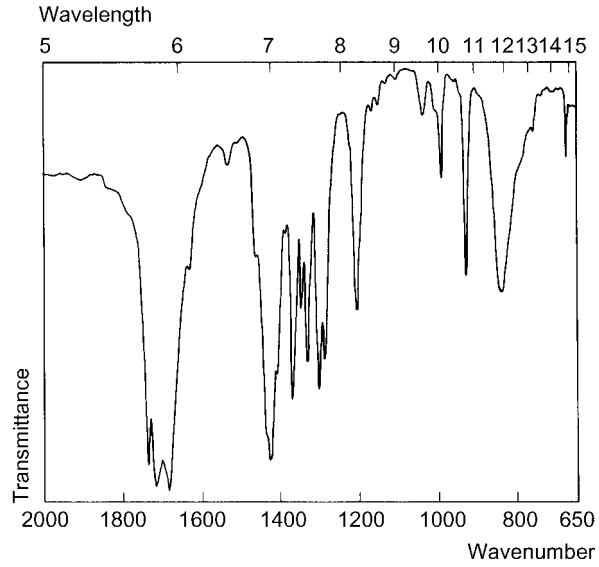
IUPAC Name 5-(2-Methylpropyl)-5-prop-2-enyl-1,3-diazinane-2,4,6-trione
Synonyms Alisobumalum; allylbarbituric acid; itobarbital; 5-(2-methylpropyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; tetrallobarbital.
Proprietary Names *Sandoptal*. It is an ingredient of *Fiorinal*.
Note The name Butalbital has also been applied to talbutal, the *sec*-butyl analogue.



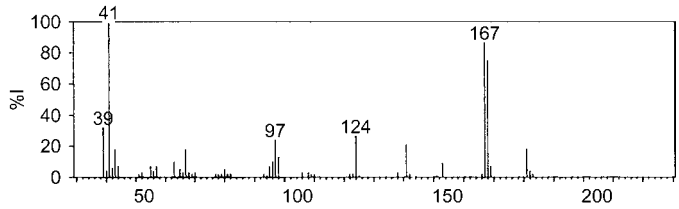
Chemical Properties pK_a 7.6 (20°). Log *P* (octanol/water), 1.9. A white crystalline powder. Mp 138° to 139°. Slightly soluble in cold water; soluble in boiling water; soluble in acetone, ethanol, chloroform, ether, and glacial acetic acid; soluble in aqueous solutions of alkali hydroxides and carbonates.
Colour Tests Koppanyi-Zwikker test—violet; vanillin reagent—orange/colourless.
Thin-layer Chromatography System TB—R_f 0.01; system TD—R_f 0.54; system TE—R_f 0.44; system TF—R_f 0.67; system TH—R_f 0.67; system TAD—R_f 0.57; system TAE—R_f 0.87 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown).
Gas Chromatography System GA—butalbital RI 1665, butalbital-Me_2 RI 1655, M (OH-) RI 1940; system GB—butalbital RI 1698, M (OH-) RI 2016; system GF—RI 2395; system GAJ—butalbital RRT 0.778 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 6.17; system HH—*k* 3.48; system HX—RI 394; system HY—RI 342; system HZ—retention time 3.4 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—240 nm (A^1_1=439a); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=329b).



Infrared Spectrum Principal peaks at wavenumbers 1690, 1720, 1740, 1310, 1290, 1200 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 41, 167, 168, 39, 124, 97, 141, 181.



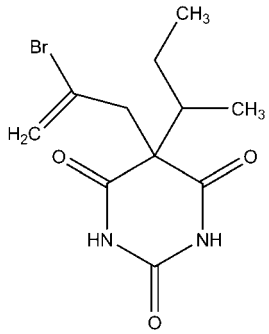
Quantification See under Amobarbital.
Blood **GC–MS** Limit of quantification, 50 mg/L, limit of detection, 5 mg/L [Meatherall 1997].
Urine **GC–MS** Limit of quantification, 20 mg/L, limit of detection, 5 mg/L [Meatherall 1997].
Disposition in the Body Absorbed after oral administration. About 5% of a dose is excreted in the urine as unchanged drug in 96 h and 20 to 60% as 5-(2,3-dihydroxypropyl)-5-isobutylbarbituric acid.
Therapeutic Concentration Usually in the range 1 to 10 mg/L.
A small group of healthy volunteers was administered 100 mg butalbital orally. Peak blood concentrations reached 2.1 mg/L and fell to 1.5 mg/L after 24 h [Drost, Walter 1988].
Toxicity Plasma concentrations of 10 to 25 mg/L are usually associated with toxic effects.
The following concentrations were reported in one fatality attributed to butalbital overdose: blood 26 mg/L, liver 50 µg/g [Baselt, Cravey 1977].
Half-life Derived from urinary excretion data, about 30 to 40 h.
Dose 200 mg as a hypnotic; 150 to 400 mg daily as a sedative.

Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–103.
Drost ML, Walter L (1988). Blood and plasma concentrations of butalbital following single oral doses in man. *J Anal Toxicol* 12(6): 322–324.
Meatherall R (1997). GC/MS confirmation of barbiturates in blood and urine. *J Forensic Sci* 42(6): 1160–1170.

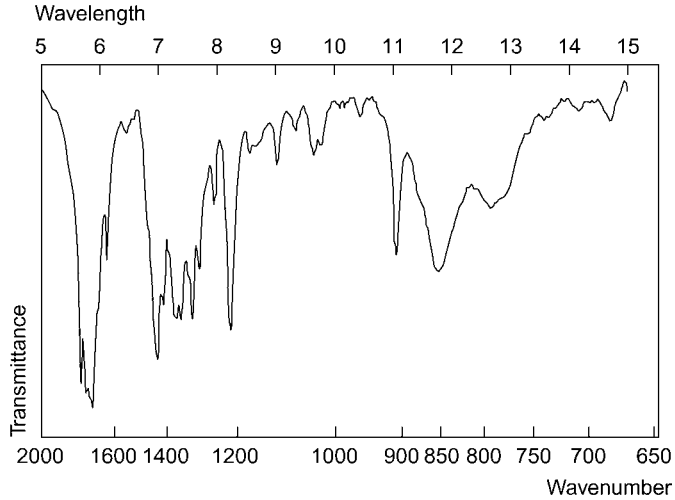
Butallylonal

Barbiturate
$C_{11}H_{15}BrN_2O_3 = 303.2$
CAS—1142-70-7

IUPAC Name 5-(2-Bromoallyl)-5-*s*-butylbarbituric acid
Synonym Butylbromallylbarbituric acid
Proprietary Name *Pernocton*



Chemical Properties Fine white crystalline powder. Mp 130° to 133°. Very slightly soluble in water; soluble in ethanol, ether, and aqueous solutions of alkalis. May be isolated in the A2 fraction. Log *P* (octanol/water) 2.20 [Meylan, Howard 1995].
Colour Test Zwikker's reagent—violet-blue
Thin-layer Chromatography System T10—R_f 0.28 (location reagents: fluorescein spray, pink; mercurous nitrate spray, black; potassium permanganate spray, yellow-brown).
Infrared Spectrum

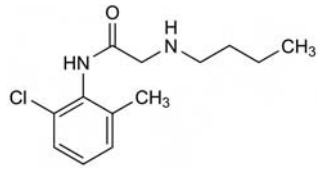


Disposition in the Body Intermediate-acting barbiturate. Metabolised by side-chain oxidation to 5-(2-acetonyl)-5-(1-methylpropyl)barbituric acid with 5 to 17% of the dose appearing in the urine in this form with only traces of unchanged drug.
Toxicity The minimum oral lethal dose in rabbits is 400 mg/kg.
Dose Usually 200 mg.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Butanilicaine

Anaesthetic (Local)
$C_{13}H_{19}ClN_2O$ = 254.8
CAS—3785-21-5
IUPAC Name 2-(Butylamino)-*N*-(2-chloro-6-methylphenyl)acetamide

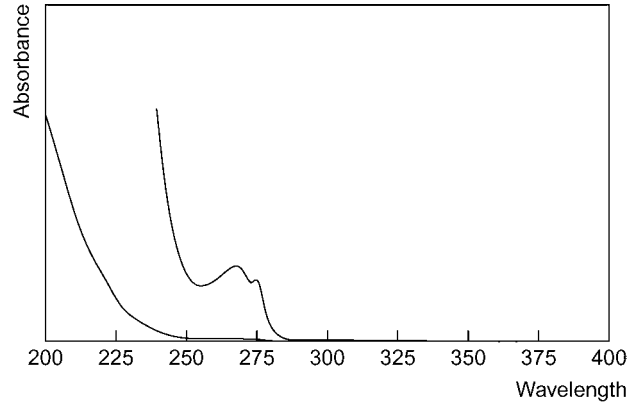


Chemical Properties Crystals. Mp 45° to 46°. Log *P* (octanol/water), 2.0.

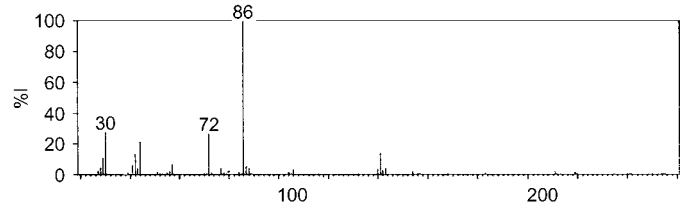
Butanilicaine Phosphate
$C_{13}H_{19}ClN_2O,H_3PO_4$ = 352.8
CAS—2081-65-4
Proprietary Name *Hostacain* (also as hydrochloride).
Chemical Properties Crystals. Mp 126° to 127°.

Colour Tests Aromaticity (method 2)—colourless/orange; Koppanyi–Zwikker test—violet; Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.14; system TC—R_f 0.54; system TE—R_f 0.75; system TL—R_f 0.61; system TAE—R_f 0.65 (Acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2025.
High Performance Liquid Chromatography System HQ—*k* 4.42; system HY—RI 280.
Ultraviolet Spectrum Aqueous acid—267 (A_1^1=15b), 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1495, 1694, 770, 1136, 1562, 1587 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 86, 30, 72, 44, 141, 42, 29, 57.

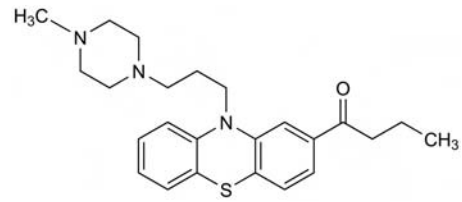


Quantification
Plasma (horse) **GC** AFID. Limit of detection, 5 µg/L [Delbeke, Debackere 1982].
Urine (horse) **GC** See Plasma [Delbeke, Debackere 1982].
Dose Butanilicaine phosphate has been administered by injection, as a 0.5 to 3% solution.

Delbeke FT, Debackere M (1982). Determination of butanilicaine in horse plasma and urine by extractive benzoylation and gas chromatography with a nitrogen-phosphorus detector. *J Chromatogr* 237: 344–349.

Butaperazine

Tranquilliser
$C_{24}H_{31}N_3OS$ = 409.6
CAS—653-03-2
IUPAC Name 1-[10-[3-(4-Methyl-1-piperazinyl)propyl]-10*H*-phenothiazin-2-yl]-1-butanone
Synonym Butyrylperazine



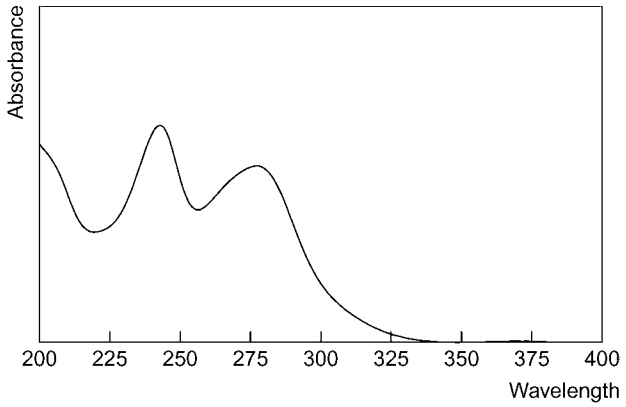
Chemical Properties A yellow oil. Bp 195° to 210°. Log *P* (octanol/water), 4.8.

Butaperazine Maleate
$C_{24}H_{31}N_3OS,2C_4H_4O_4$ = 641.7
CAS—1063-55-4
Synonym Butaperazine dimaleate
Proprietary Name *Repoise*
Chemical Properties A yellow crystalline powder. Mp about 195°. Soluble in water; practically insoluble in chloroform and ether.

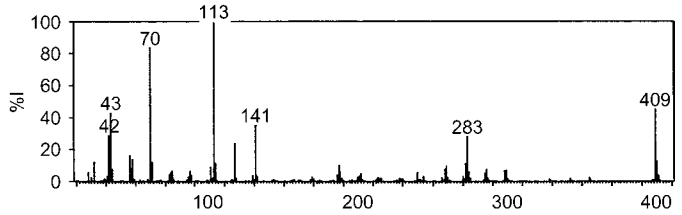
Butaperazine Phosphate
$C_{24}H_{31}N_3OS,2H_3PO_4$ = 605.6
CAS—7389-45-9
Synonym Butaperazine diphosphate
Chemical Properties A yellow crystalline powder. Mp 161° to 162°. Soluble in water; practically insoluble in chloroform and ether.

Colour Tests Mandelin's test—brown-violet; Marquis test—brown-violet.
Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.28; system TC—R_f 0.37; system TE—R_f 0.52; system TL—R_f 0.05; system TAE—R_f 0.26;

system TAJ—R_f 0.10; system TAK—R_f 0.00; system TAL—R_f 0.42 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 3190.
High Performance Liquid Chromatography System HA—*k* 3.4; system HX—RI 464; system HY—RI 406.
Ultraviolet Spectrum Aqueous acid—242 (A^{1}_{1}=609a), 277 nm.



Infrared Spectrum Principal peaks at wavenumbers 1675, 749, 1279, 1193, 1163, 1143 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 113, 70, 409, 43, 141, 283, 42, 127.



Quantification
Plasma **Spectrofluorimetry** For method, see Manier *et al.* [1974].
Biological Fluids **GC** AFID. Limit of detection, 5 µg/L [Javaid *et al.* 1979].
Disposition in the Body Butaperazine is readily absorbed after oral administration. It is metabolised to the sulfoxide and sulfone; other unidentified metabolites have been detected in plasma.
Therapeutic Concentration
Following oral administration of 40 mg as a single dose to 13 subjects, peak plasma concentrations of 0.07 to 0.69 (mean 0.28) mg/L were attained in 2 to 4 h; peak erythrocyte concentrations of 0.01 to 0.52 (mean 0.11) mg/L were also reported [Garver *et al.* 1976].

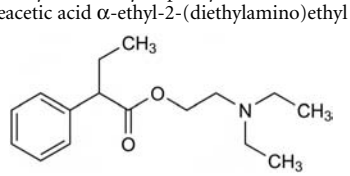
Following oral administration of 20 mg twice a day to 9 subjects, minimum steady-state plasma concentrations of 0.02 to 0.20 (mean 0.09) mg/L, and erythrocyte concentrations of 0.01 to 0.06 (mean 0.03) mg/L were reported; therapeutic effect appeared to correlate with erythrocyte concentrations in the range 0.03 to 0.06 mg/L [Casper *et al.* 1980].

Half-life Plasma half-life, 5 to 30 (mean 12) h.
Dose Usually the equivalent of 15 to 30 mg of butaperazine daily; maximum of 100 mg daily.

Casper R *et al.* (1980). Phenothiazine levels in plasma and red blood cells. Their relationship to clinical improvement in schizophrenia. *Arch Gen Psychiatry* 37(3): 301–305.
Garver DL *et al.* (1976). Pharmacokinetics of red blood cell phenothiazine and clinical effects. Acute dystonic reactions. *Arch Gen Psychiatry* 33(7): 862–866.
Javaid JI *et al.* (1979). Determination of butaperazine in biological fluids by gas chromatography using nitrogen specific detection system. *J Chromatogr Sci* 17: 666–670.
Manier DH *et al.* (1974). A fluorometric method for the measurement of butaperazine in human plasma. *Clin Chim Acta* 57: 225–230.

Butetamate

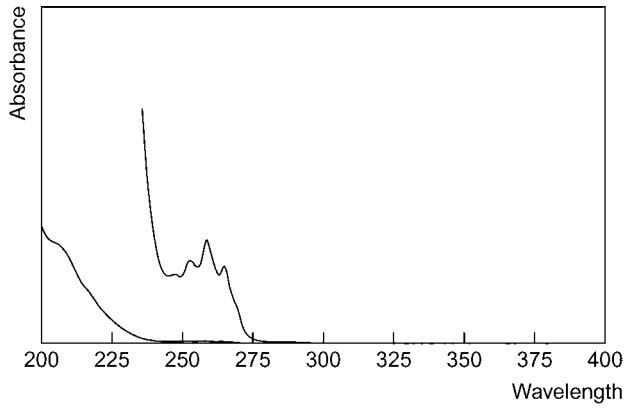
Antispasmodic, Bronchodilator
$C_{16}H_{25}NO_2$ = 263.4
CAS—14007-64-8
IUPAC Name 2-Diethylaminoethyl 2-phenylbutanoate
Synonym Benzeneacetic acid α-ethyl-2-(diethylamino)ethyl ester; butethamate.



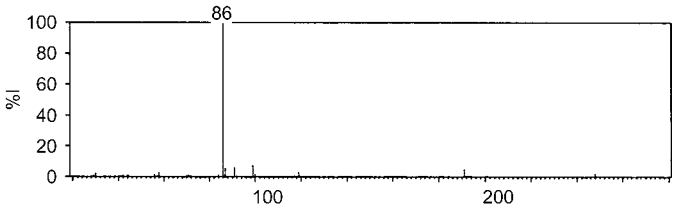
Chemical Properties Log *P* (octanol/water), 3.7.

Butetamate Citrate
$C_{16}H_{25}NO_2$,$C_6H_8O_7$ = 455.5
CAS—3639-12-1
Proprietary Name It is an ingredient of *CAM*.
Chemical Properties Colourless crystals. Mp 107° to 110°. Soluble 1 in 10 of water and 1 in 40 of ethanol.

Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.59; system TC—R_f 0.57; system TE—R_f 0.81; system TL—R_f 0.47; system TAE—R_f 0.48; system TAF—R_f 0.56 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1754.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 390.
Ultraviolet Spectrum Aqueous acid—253, 258 (A^{1}_{1}=7.6b), 264 nm.



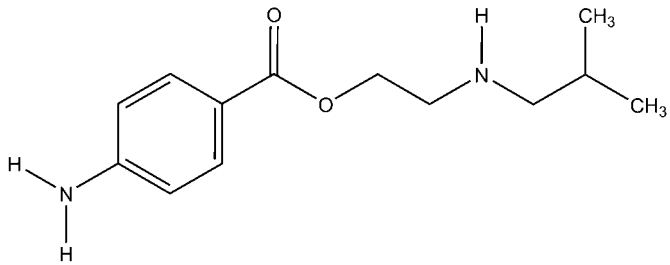
Infrared Spectrum Principal peaks at wavenumbers 1730, 1158, 1194, 694, 1265, 1219 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 99, 91, 191, 87, 119, 58, 248.



Dose Butetamate citrate has been given in doses of 18 to 90 mg daily.

Butethamine

Anaesthetic (Local)
$C_{13}H_{20}N_2O_2$ = 236.3
CAS—2090-89-3
IUPAC Name 2-Isobutylaminotheyl *p*-aminobenzoate



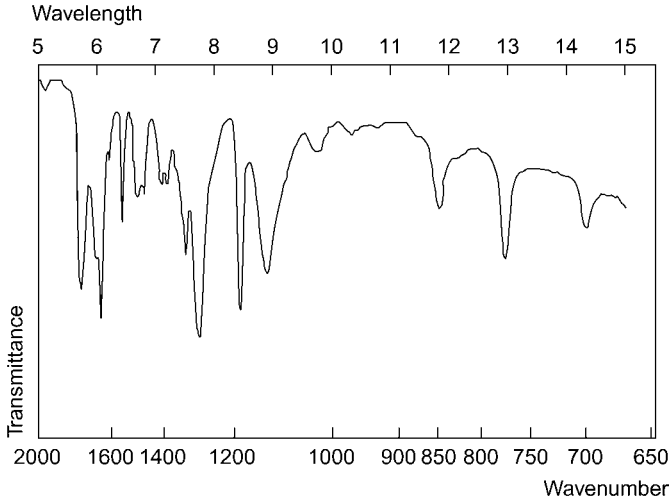
Chemical Properties Butethamine and metabutethamine are isomers. Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 2.19 [Meylan, Howard 1995].

Butethamine Formate
Proprietary Name *Monocaine Formate*
Chemical Properties White crystals or crystalline powder. Mp 136° to 139°. Readily soluble in water and ethanol; slightly soluble in ether and chloroform.

Butethamine Hydrochloride
Proprietary Name *Monocaine Hydrochloride*
Chemical Properties White crystalline powder. Mp 192° to 196°. Sparingly soluble in water; slightly soluble in ethanol and chloroform; practically insoluble in ether.

Colour Tests Diazotisation test—red; Vitali's test—yellow/yellow (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.60 (location reagents: acidified iodoplatinate spray, positive reaction; *p*-dimethylaminobenzaldehyde spray, yellow).
Gas Chromatography System G2/225—retention time 1.80 relative to diphenhydramine.
Ultraviolet Spectrum Water—290 nm; aqueous acid (0.1 N sulfuric acid)—228, 280 nm and inflexions at 275, 290 nm.
Infrared Spectrum Principal peaks at wavenumbers 1272, 1600, 1702 cm^{-1} (KBr disk).



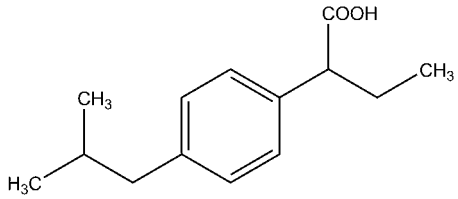
Disposition in the Body Metabolised by hydrolysis to *p*-aminobenzoic acid.
Toxicity It is one-third more potent and one-third more toxic than procaine hydrochloride; it is not recommended as a surface anaesthetic for mucous membranes. The maximum safe amount by injection or topical application is 750 mg, or 75 mL of a 1% solution. LD_{50} (IP) in rats: 183 mg/kg.
Dose Used by injection as a 1 to 1.5% solution with adrenaline.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Butibufen

Butyric Acid, NSAID
$C_{14}H_{20}O_2$ = 220.3
CAS—55837-18-8

IUPAC Name 2-[4-(2-Methylpropyl)phenyl]butanoic acid
Synonyms α-Ethyl-4-(2-methylpropyl)benzeneacetic acid; FF-106; 2-(4-isobutylphenyl)butyric acid.



Chemical Properties Solid. Mp 51° to 53°. Soluble in water (43 mg/L). pK_a 6.04 [Barrigon *et al.* 1985]. Log *P* (octanol/water), 4.28 [Meylan, Howard 1995].

Butibufen Sodium

$C_{14}H_{19}NaO_2$ = 242.3
CAS—60682-24-8
Proprietary Names *Butilopan*; *Mijal.*

Quantification

Plasma **GC** Column: 3% D-C 550 on 80-100 Chromosorb (1.82 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 170° isothermal. FID. Retention time: 5.5 min. Limit of quantification, 0.5 mg/L [Barrigon *et al.* 1985].
Other **HPLC** Pharmaceutical Dosage Forms. Column: C_{18} Novapak (150 × 3.9 mm i.d., 4 µm). Mobile phase: water : acetonitrile : orthophosphoric acid (528 : 472 : 0.4), flow rate 1 mL/min. UV detection (λ = 264 nm). Retention time: 10.1 min. Limit of quantification not reported [Gonzalez Tavares *et al.* 1992].
Note For a chiroptical characterisation method, see Hoult *et al.* [1999].

Disposition in the Body

Therapeutic Concentration

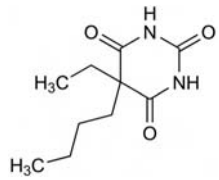
Four healthy young volunteers (aged 18 to 21 years) were administered a single dose of 0.5 g butibufen as either enteric-coated microcapsules (ECM) or non-enteric-coated (gastric release) microcapsules (GRM), with a washout period of a week between doses. The mean peak plasma concentration in the ECM study was 22.2 mg/L after 1.73 h and in the GRM study, 23.7 mg/L after 1.26 h. There was a significant difference in half-life absorption values, with 39.3 min for the ECM study and 23.0 min in the GRM study [Barrigon *et al.* 1985].
Dose Given orally in inflammatory and rheumatic disorders.

Barrigon S *et al.* (1985). Butibufen enteric-coated vs non-enteric-coated microcapsules: pharmacokinetics and gastrointestinal blood loss effect relationships in healthy volunteers. *Arch Farmacol Toxicol* 11: 189–197.
Gonzalez Tavares L *et al.* (1992). High pressure liquid chromatographic determination of the new non-steroidal anti-inflammatory agent butibufen. *Arzneimittelforschung* 42: 818–820.
Hoult JR *et al.* (1999). Chromatographic resolution, chiroptical characterization and preliminary pharmacological evaluation of the enantiomers of butibufen: a comparison with ibuprofen. *J Pharm Pharmacol* 51: 1201–1205.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

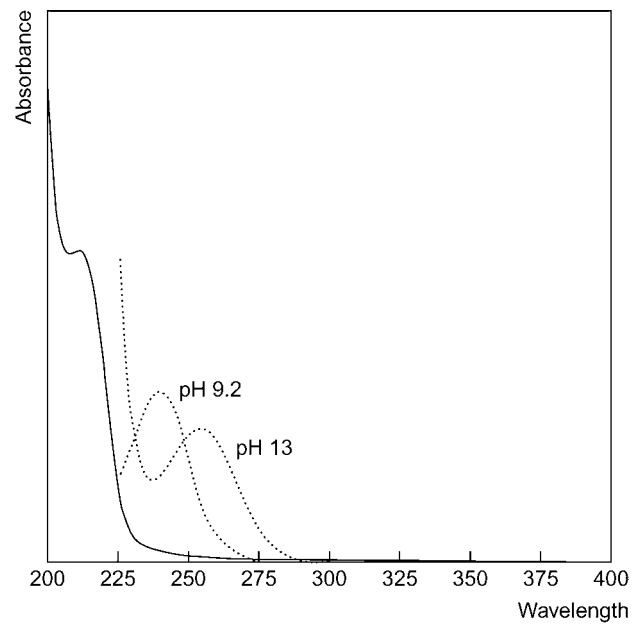
Butobarbital

Hypnotic, Barbiturate
$C_{10}H_{16}N_2O_3$ = 212.3
CAS—77-28-1

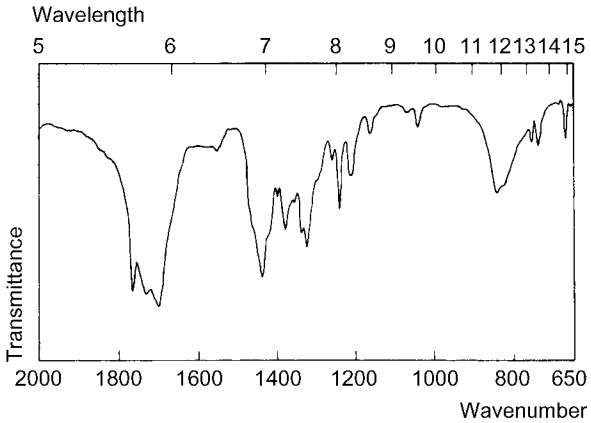
IUPAC Name 5-Butyl-5-ethyl-1,3-diazinane-2,4,6-trione
Synonyms Butethal; butobarbitalum; butobarbitone; 5-butyl-5-ethyl-2,4,6 (1*H*,3*H*,5*H*)-pyrimidinetrione.
Proprietary Names *Sonabarb*; *Soneryl.* It is an ingredient of *Belloid* and *Hypnasmine.*



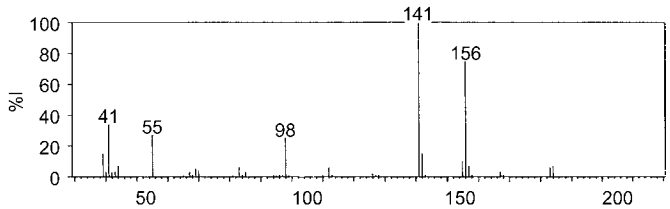
Chemical Properties Colourless crystals or white crystalline powder. Mp 124° to 127°. Soluble 1 in 5 alcohol and 1 in 10 ether; practically insoluble in water, insoluble in petroleum ether and aliphatic hydrocarbons. pK_a 8.0 (25°). Log *P* (octanol/water), 1.73.
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.41; system TF—R_f 0.65; system TH—R_f 0.68; system TAD—R_f 0.58; system TAE—R_f 0.86 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; Zwikker's reagent, pink).
Gas Chromatography System GA—butobarbital RI 1660, butobarbital-Me_2 RI 1565, M (3′-OH-) RI 1920, M (3′-OH-)-AC RI 1940, M (3′-oxo-) RI 1880; system GF—RI 2390; system GAJ—butobarbital RRT 0.732, M (3′-OH-) RRT 1.053 (both relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 5.43; system HH—*k* 3.42; system HX—RI 384; system HY—RI 355; system HZ—retention time 3.2 min; system HAA—retention time 14.9 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=477a); M sodium hydroxide (pH 13)—254 nm (A^1_1=388b).



Infrared Spectrum Principal peaks at wavenumbers 1696, 1727, 1760, 1242, 850, 1215 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 141, 156, 41, 55, 98, 39, 142, 155; 3′-hydroxybutobarbitone 156, 141, 45, 157, 41, 29, 55, 27.



Quantification See also under Amobarbital.
Urine **GC–MS** For butobarbital and metabolites see Gilbert and Powell [1976].
Disposition in the Body Readily absorbed after oral administration. Metabolic reactions include side-chain oxidation to form the 3′-hydroxy, 3′-oxo, and 3′-carboxypropyl metabolites. About 5 to 9% of an oral dose is excreted in the urine unchanged, 22 to 28% as 3′-hydroxybutobarbital, 14 to 18% as 3′-oxobutobarbital, and 4 to 8% as 5-(3′-carboxypropyl)-5-ethylbarbituric acid.
Therapeutic Concentration In plasma, usually in the range 2 to 15 mg/L.
A single oral dose of 200 mg, given to 5 subjects, produced peak plasma concentrations of 2.9 to 4.1 mg/L (mean 3.6) in 0.6 to 2 h; following oral doses of 200 mg daily for 3 days to 2 subjects, plasma concentrations of 6.5 and 6.4 mg/L were observed 9 h after the last dose [Breimer 1976].
Toxicity The estimated minimum lethal dose is 2 g. Toxic effects are associated with plasma concentrations of 14 to 32 to 98 mg/L and fatalities with blood concentrations of 11 to 30 to 75 mg/L.
In 3 fatalities attributed to butobarbital overdose, the following postmortem tissue concentrations, mg/L or μg/g, were reported:

	Butobarbital	*3′-Hydroxybutobarbital*
Blood	18, 22, 49	—, 3, —
Bile	49, 27, 131	204, 4, 312
Kidney	31, 23, 72	—, 8, 12
Liver	47, 58, 83	2, 10, 17
Lung	26, 19, 52	—, 8, 19
Urine	4, 26, 38	259, —, 5

[Robinson, McDowall 1979].

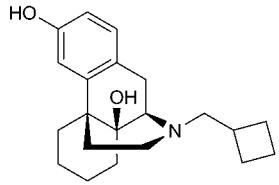
Half-life Plasma half-life, about 40 h following single doses, but may be reduced to about 30 h after multiple dosing.
Volume of Distribution About 0.8 L/kg.
Protein Binding About 26%.
Dose 100 to 200 mg, as a hypnotic.

Breimer DD (1976). Pharmacokinetics of butobarbital after single and multiple oral doses in man. *Eur J Clin Pharmacol* 10: 263–271.
Gilbert JNT, Powell JW (1976). *Eur J Drug Metab Pharmacokinet* 1: 188–193.
Robinson AE, McDowall RD (1979). The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man. *J Pharm Pharmacol* 31: 357–365.

Butorphanol

Narcotic Analgesic
$C_{21}H_{29}NO_2 = 327.5$
CAS—42408-82-2

Synonyms 17-(Cyclobutylmethyl)morphinan-3,14-diol; Levo-BC-2627.

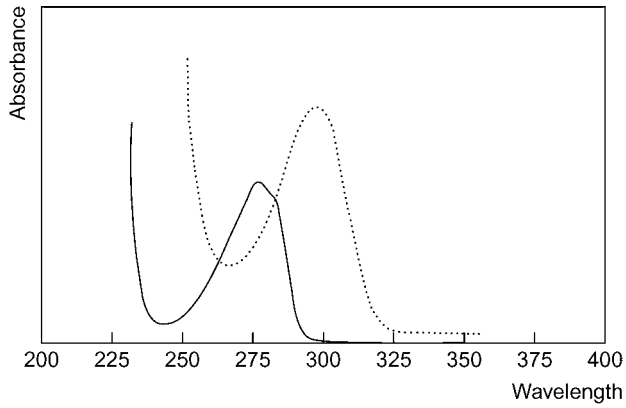


Chemical Properties Solid. Mp 215° to 217°. Butorphanol and its metabolites were stable in plasma for 123 and 87 days, respectively, when stored at −20°. Reconstituted PFB-derivatives of butorphanol were stable for at least 42 h, and the TFA-derivatives were stable for at least 24 h [Mulvana *et al.* 1996]. Butorphanol and its metabolites were stable in frozen human urine for 1 month. This was independent of concentration. Reconstituted samples were stable in the autosampler for at least 68 h [Willey *et al.* 1994].

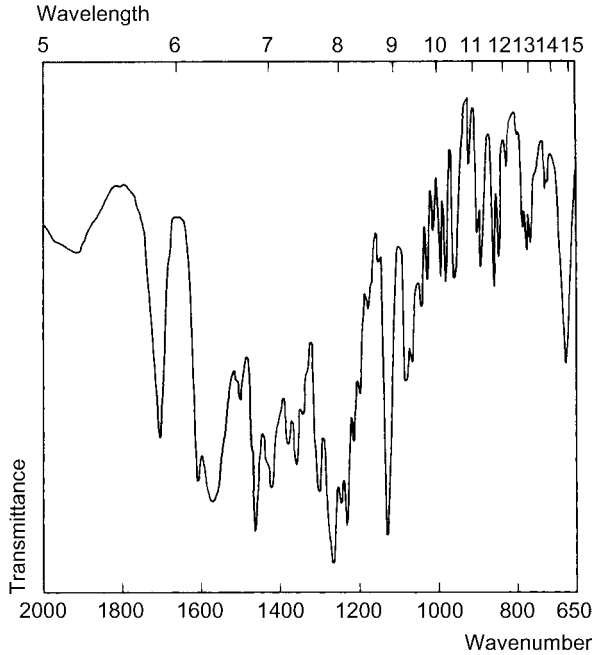
Butorphanol Tartrate

$C_{21}H_{29}NO_2,C_4H_6O_6 = 477.6$
CAS—58786-99-5
Proprietary Name *Stadol*
Chemical Properties White crystalline powder. Mp 217° to 219°, with decomposition. Sparingly soluble in water; slightly soluble in methanol; practically insoluble in ethanol, chloroform, and ether; soluble in dilute acids. Log *P* (octanol) 3.7.

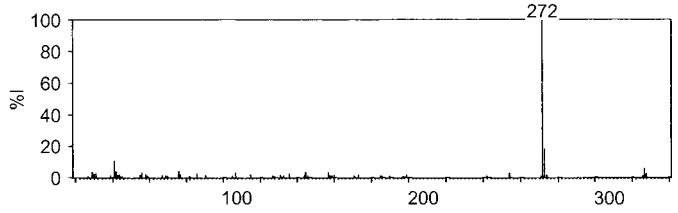
Colour Tests Liebermann's reagent—black; Marquis test—grey.
Gas Chromatography System GA—RI 2761; system GB—RI 2902.
High Performance Liquid Chromatography System HY—RI 300.
Ultraviolet Spectrum Aqueous acid—278 nm. ($A^{1}_{1} = 62$b); aqueous alkali—299 nm.



Infrared Spectrum Principal peaks at wavenumbers 1269, 1130, 1233, 1575, 1249, 1303 cm^{-1} (butorphanol tartrate), (KBr disk).



Mass Spectrum Principal ions at *m/z* 272, 273, 41, 327, 145, 76, 42, 29.



Quantification

Plasma **GC-MS** Column: DB-1 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 50 cm/s. Temperature programme: 200° for 1 min to 310° at 25°/min for 2.1 min. NCI, SIM acquisition mode. Limit of detection, 20 ng/L [Mulvana *et al.* 1996].

HPLC Column: Bondapak-NH_2 (30 cm × 4 mm i.d.). Mobile phase: ethanol : benzene (10 : 90 for 12 min to 100 : 0), flow rate 1 mL/min for 12 min to 6 mL/min. UV detection (λ = 280 nm). Retention time: butorphanol 3–6 min, hydroxybutorphanol 6–12 min, norbutorphanol 19–24 min. Limit of detection, 0.1 ng butorphanol [Gaver *et al.* 1980].

LC-MS Column: Partisil C_8 (100 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water : formic acid (90 : 10 : 0.1), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 13.7 ng/L, [Boulton *et al.* 2002]. Column: Capcell Pak-ODS.UG120 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L potassium phosphate buffer (3 : 7), flow rate 1.0 mL/min. UV detection (λ = 220 nm) followed by APCI, SIM acquisition mode. Retention time: 14.0 min. Limit of quantification, 10.7 ng [Kanazawa *et al.* 1998].

Serum **GC** Column: 3% SE-30 on 100/200 mesh GasChrom Q (30.5 cm × 4 mm i.d.). ECD. Limit of quantification, 0.9 μg/L; limit of detection, 2 μg/L [Pfeffer *et al.* 1980].

Urine **HPLC** Column: Octyl (250 × 4.6 mm i.d., 5 μm). Mobile phase: 1.0 mol/L tetramethyl ammonium hydroxide 1.0 mol/L ammonium acetate (pH 6.0) : water : methanol : acetonitrile (20 : 1380 : 200 : 400), flow rate, 1.0 mL/min. Fluorescence detection (λ_{ex} = 200 nm, λ_{em} = 325 nm). Limit of quantification, 1 mg/L for butorphanol and hydroxybutorphanol, 2 mg/L for norbutorphanol [Willey *et al.* 1994].

Disposition in the Body Well absorbed after oral or IM administration. Undergoes extensive first-pass metabolism, mainly by hydroxylation, dealkylation and conjugation. The major metabolite is 3′-hydroxybutorphanol. Approximately 70% of a dose is excreted in the urine and 13% is eliminated in the faeces in 5 days. In 24 h, 5 to 10% of a dose is excreted in the urine as unchanged drug, 5 to 10% as free and conjugated norbutorphanol, and approximately 50% as 3′-hydroxybutorphanol.

Therapeutic Concentration

Two healthy male volunteers were administered 1 mg butorphanol tartrate as an intranasal dose for 24 h. The maximum plasma concentrations were 1.47 μg/L 0.25 h after the dose and 1.33 μg/L 0.5 h after [Boulton *et al.* 2002].

Nine healthy male volunteers received a 1 mg nasal dose of butorphanol on days 1 and 6 and a 1 mg transnasal dose every 6 h on days 2–5. After the first dose, the mean peak plasma butorphanol concentration was 1.54 μg/L and was reached at 0.25 h. The steady-state plasma butorphanol concentration was 1.38 μg/L at 0.5 h. After the first dose, the mean peak plasma hydroxybutorphanol concentration was 0.376 μg/L and was reached at 6.0 h. The steady-state plasma hydroxybutorphanol concentration was 1.81 μg/L at 4.0 h [Vachharajani *et al.* 1997].

A single IM dose of 2 mg butorphanol administered to 6 subjects produced a mean peak plasma concentration of 2 μg/L butorphanol in 0.5 to 1 h, and a mean peak plasma concentration of 1 μg/L of 3′-hydroxybutorphanol after 4 to 6 h [Gaver *et al.* 1980].

After oral administration of 16 mg of butorphanol tartrate to 4 subjects, peak serum concentrations of 0.3 to 3.0 μg/L (mean 1.0) were attained in 2 to 4 h [Pittman *et al.* 1980].

Bioavailability ≈17%.

Half-life Plasma half-life, 2 to 4 h.

Volume of Distribution ≈5 L/kg.

Protein Binding ≈80%.

Note For a review of butorphanol, see Gillis *et al.* [1995].

Dose 1 to 4 mg of butorphanol tartrate every 4 h by IM injection; doses of 4 to 16 mg have been given by mouth.

Boulton DW *et al.* (2002). Validation and application of a sensitive assay for butorphanol in human plasma by high-performance liquid chromatography with tandem mass spectrometry detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 775: 57–62.

Gaver RC *et al.* (1980). Disposition of parenteral butorphanol in man. *Drug Metab Dispos* 8: 230–235.

Gillis JC *et al.* (1995). Transnasal butorphanol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute pain management. *Drugs* 50: 157–175.

Kanazawa H *et al.* (1998). Determination of sedatives and anesthetics in plasma by liquid chromatography-mass spectrometry with a desalting system. *J Chromatogr A* 797: 227–236.

Mulvana DE *et al.* (1996). Quantitative determination of butorphanol and its metabolites in human plasma by gas chromatography-electron capture negative-ion chemical ionization mass spectrometry. *J Chromatogr B Biomed Appl* 682: 289–300.

Pfeffer M *et al.* (1980). Pharmacokinetics of subcutaneous and intramuscular butorphanol in dogs. *J Pharm Sci* 69: 801–803.

Pittman KA *et al.* (1980). Serum levels of butorphanol by radioimmunoassay. *J Pharm Sci* 69: 160–163.

Vachharajani NN *et al.* (1997). The pharmacokinetics of butorphanol and its metabolites at steady state following nasal administration in humans. *Biopharm Drug Dispos* 18: 191–202.

Willey TA *et al.* (1994). High-performance liquid chromatographic method for the quantitative determination of butorphanol, hydroxybutorphanol, and norbutorphanol in human urine using fluorescence detection. *J Chromatogr* 652: 171–178.

Butoxamine

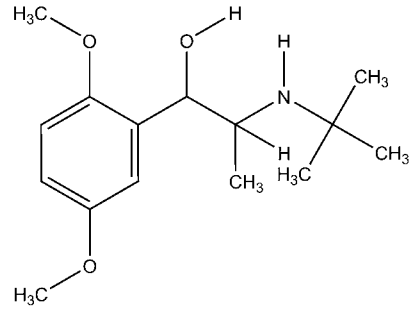
β_2-Adrenoceptor Antagonist, Antidiabetic

$C_{15}H_{25}NO_3$ = 267.4

CAS—2922-20-5

IUPAC Name (±)–Erythro-1-(2,5-dimethoxyphenyl)-2-*t*-butylaminopropan-1-ol

Synonym BW64-9



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

Colour Tests Ammonium molybdate test—green (limit of detection 0.1 μg); sulfuric acid-formaldehyde test—green (limit of detection 0.1 μg); Vitali's test—yellow/yellow/yellow (limit of detection 0.5 μg).

Thin-layer Chromatography System T1—R_f 0.60 (location reagent potassium permanganate spray, positive reaction).

Infrared Spectrum Principal peaks at wavenumbers 1485, 1206, 1263 cm^{-1} (KCl disk).

Butoxyethyl Nicotinate

Vasodilator (Topical)

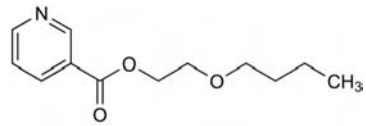
$C_{12}H_{17}NO_3$ = 223.3

CAS—13912-80-6

IUPAC Name 2-Butoxyethyl pyridine-3-carboxylate

Synonym 2-Butoxyethyl nicotinate

Proprietary Names It is an ingredient of *Actinac* and *Finalgon*.

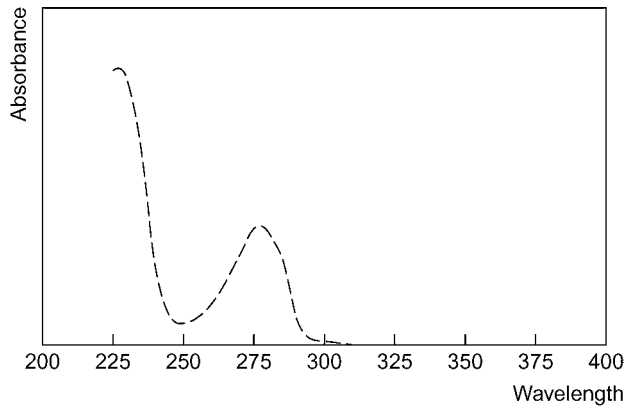


Chemical Properties Soluble in ether; slightly soluble in dilute acetic acid.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.45; system TC—R_f 0.69; system TL—R_f 0.62 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1640.

Ultraviolet Spectrum Methanol—279 nm (A^1_1=152b).



Infrared Spectrum Principal peaks at wavenumbers 1282, 1724, 1111, 740, 1020, 1587 cm^{-1}.

Use Topically in a concentration of 2.5%.

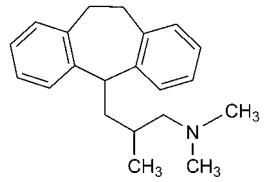
Butriptyline

Tricyclic Antidepressant

$C_{21}H_{27}N$ = 293.5

CAS—35941-65-2

Synonym 10,11-Dihydro-*N*,*N*,β-trimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-5-propanamine



Chemical Properties Oil. Butriptyline is stable in human serum at room temperature for 8 days [Bruderlein *et al.* 1977].

Butriptyline Hydrochloride

$C_{21}H_{27}N,HCl = 329.9$
CAS—5585-73-9

Proprietary Name *Evadyne*

Chemical Properties White crystalline powder. Mp ≈186°. Freely soluble in water; moderately soluble in aliphatic alcohols and chloroform; insoluble in ether.

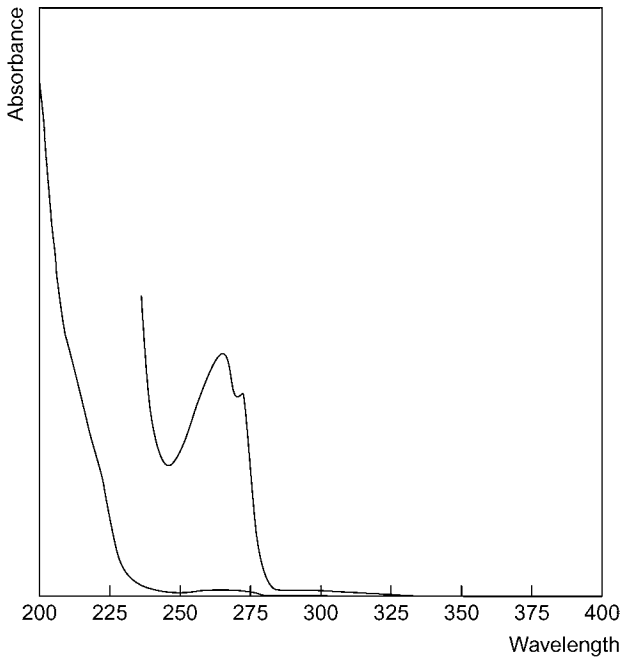
Colour Test Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.59; system TAG—R_f 0.38; system TB—R_f 0.61; system TC—R_f 0.48 (acidified iodoplatinate solution, positive).

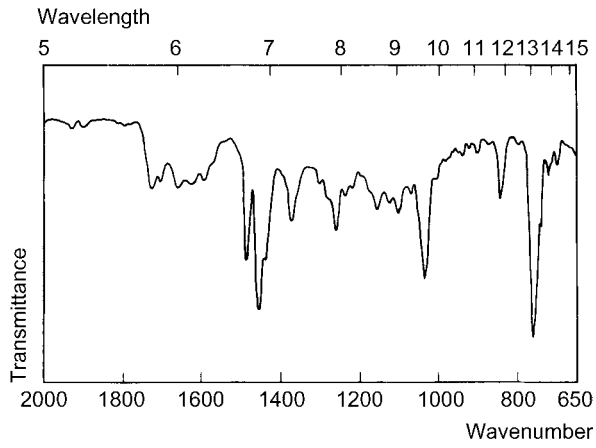
Gas Chromatography System GA—RI 2181; system GB—butriptyline RI 2288, M (nor-) RI 2330; system GF—RI 2465; system GM—butriptyline RRT 0.683,M (nor-) RRT 0.761.

High Performance Liquid Chromatography System HA—butriptyline *k* 2.7, norbutriptyline *k* 1.7; system HF—*k* 7.33; system HY—RI 369.

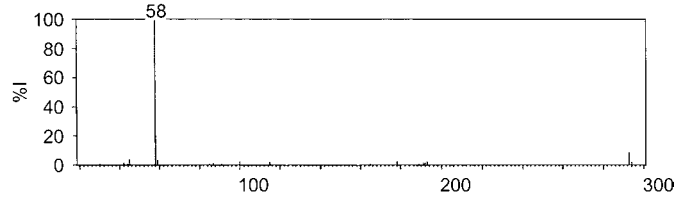
Ultraviolet Spectrum Aqueous acid—265 nm.



Infrared Spectrum Principal peaks at wavenumbers 757, 1033, 1265, 740, 1098, 1149 cm^{-1} (KCl disk).



Mass Spectrum Principal ions at *m/z* 58, 293, 45, 59, 193, 100, 178, 294.



Quantification

Blood **GC-MS** DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 2 min to 195° at 25°/min to 300° at 5°/min for 2 min. NPD. Limit of detection, 250 µg/L [Hutchison *et al.* 1994].

Plasma **GC** Column: 3% OV-17 on Gas-Chom Q (80/100 mesh; 6′ × 2 mm i.d.). Carrier gas: He, 60 mL/min. Temperature: 208°. Limit of detection not reported [Bourgouin *et al.* 1981]. Column: Coiled glass (1.8 m × 4 mm i.d.). Carrier gas: N_2, 21 mL/min. Temperature: 215°. FID. Retention time: 9.3 min. Limit of detection, 10 µg/L [Norman *et al.* 1977].

Serum **GC** Column: U-shaped glass (6′ × 2 mm i.d.). Carrier gas: He, 15 mL/min. FID. Limit of detection, 10 µg/L [Bruderlein *et al.* 1977].

Disposition in the Body Readily absorbed after oral administration. It is rapidly metabolised, and the metabolites are thought to undergo enterohepatic circulation; the major metabolite is norbutriptyline. Butriptyline is slowly excreted in the urine mainly as metabolites; up to 25% of a dose is excreted in the urine in 24 h with <2% of the dose as unchanged drug, and about 4% as glucuronide conjugates; approximately 1 to 2% of a dose is eliminated in the faeces in 24 h [Cameron *et al.* 1974].

Therapeutic Concentration

After a single oral dose of 75 mg to 14 subjects, peak plasma concentrations of 0.024 to 0.11 mg/L (mean 0.05) were attained in ~ 3 h [Bourgouin *et al.* 1981].

After oral administration of 50 mg three times a day to 9 subjects, steady-state plasma butriptyline concentrations of 0.06 to 0.28 mg/L (mean 0.15) were reported 6 h after the final dose; plasma concentrations of norbutriptyline were of a similar order [Burrows *et al.* 1977].

Toxicity

A 16-year-old female was found dead with several medication bottles nearby. It was estimated that she had taken approximately 137 butriptyline tablets (25 mg each). The drug was detected in her blood at a concentration of 14.9 mg/L and in her urine at 2.96 mg/L. Salicylate and acetaminophen were also detected in her blood [Hutchison *et al.* 1994].

Half-life Plasma half-life, ≈20 h.

Protein Binding >90%.

Dose The equivalent of 75 to 150 mg of butriptyline daily.

Bourgouin J *et al.* (1981). Butriptyline: human pharmacokinetics and comparative bioavailability of conventional and sustained release formulations. *Biopharm Drug Dispos* 2: 123–130.

Bruderlein H *et al.* (1977). A gas-liquid chromatographic method for the determination of butriptyline in serum. *Clin Biochem* 10: 3–7.

Burrows GD *et al.* (1977). A new antidepressant butriptyline: plasma levels and clinical response. *Med J Aust* 2: 604–606.

Cameron BD *et al.* (1974). The disposition of butriptyline in rats, dogs and man. *Arzneimittelforschung* 24: 93–96.

Hutchison JDJr *et al.* (1994). Concentrations of butriptyline in biological fluids following a fatal overdose. *J Anal Toxicol* 18: 220–224.

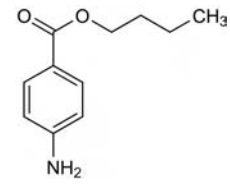
Norman TR *et al.* (1977). Determination of therapeutic levels of butriptyline in plasma by gas-liquid chromatography. *J Chromatogr* 134: 524–528.

Butyl Aminobenzoate

Anaesthetic (Local)
$C_{11}H_{15}NO_2 = 193.2$
CAS—94-25-7

IUPAC Name Butyl 4-aminobenzoate

Synonyms Butamben; butoforme; scuroforme.



Chemical Properties A white crystalline powder. It slowly hydrolyses when boiled with water. Mp 57° to 59°. Very slightly soluble in water; soluble 1 in 3 of ethanol, and 1 in 2 of ether; soluble in chloroform and in dilute mineral acids. pK_a 2.5 (25°). Log *P* (octanol/water), 2.9.

Butyl Aminobenzoate Picrate

$(C_{11}H_{15}NO_2)_2,C_6H_3N_3O_7 = 615.6$
CAS—577-48-0

Proprietary Name *Butesin Picrate*
Chemical Properties A yellow powder. Mp 109° to 110°. Very slightly soluble in water; soluble in ethanol, chloroform, and ether.

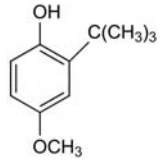
Colour Test Diazotisation—red.
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.06; system TC—R_f 0.63; system TL—R_f 0.70; system TAE—R_f 0.83; system TAF—R_f 0.90.
Gas Chromatography System GA—RI 1742.
High Performance Liquid Chromatography System HY—RI 483.
Ultraviolet Spectrum Aqueous acid—279 nm; aqueous alkali—285 nm; ethanol—294 nm (A^1_1=1065a).
Infrared Spectrum Principal peaks at wavenumbers 1273, 1595, 1681, 1630, 1111, 1163 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 120, 137, 193, 92, 65, 121, 138, 41.
Quantification
Plasma **GC** Nitrogen selective detection. Limit of quantification, 6 mg/L, limit of detection, 3 mg/L [Grouls *et al.* 1995].
Note For a review of the toxicology of topical local anaesthetics, see Bangha *et al.* [1996].
Dose The maximum safe dose for topical use is estimated to be 5 g.

Bangha E *et al.* (1996). Toxicology of topical local anesthetics. *Skin Pharmacol* 9: 376–380.
Grouls RJ *et al.* (1995). Capillary gas chromatographic method for the determination of n-butyl-p-aminobenzoate and lidocaine in plasma samples. *J Chromatogr B Biomed Sci Appl* 673(1): 51–57.

Butylated Hydroxyanisole

Antioxidant
$C_{11}H_{16}O_2$ = 180.2
CAS—25013-16-5

IUPAC Name 2-Tert-butyl-4-methoxyphenol
Synonym BHA; (1,1-dimethylethyl)-4-methoxyphenol.
Proprietary Names *Embanox BHA*; *Nipantiox 1-F*; *Tenox BHA*.



Chemical Properties A white crystalline powder or a yellowish-white waxy solid. Mp 48° to 55°. It contains a variable proportion of 3-*tert*-butyl-4-methoxyphenol. Practically insoluble in water; soluble 1 in 4 of ethanol, 1 in 2 of chloroform, 1 in 1.2 of ether and 1 in 2 of propylene glycol; soluble in solutions of alkali hydroxides. Log *P* (octanol/water), 3.5.
Colour Tests Dissolve 0.1 g in 10 mL of ethanol, add 2 mL of a 2% solution of borax and a few crystals of 2,6-dichloroquinone-chloroimide—blue (compare blue colour produced by butylated hydroxytoluene).

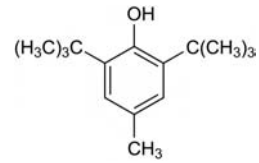
Dissolve a few crystals in 10 mL of ethanol and add 0.5 mL of a 0.2% solution of ferric ammonium sulfate in 0.5 mol/L sulfuric acid—green-blue.
Gas Chromatography System GA—RI 1462.
Ultraviolet Spectrum Acid ethanol—228 (A^1_1=340a), 292 nm (A^1_1=205a).
Infrared Spectrum Principal peaks at wavenumbers 1202, 1220, 1050, 805, 1185, 1294 cm^{-1} (KBr disk).
Quantification
Plasma **GC** FID. Limit of detection, <100 µg/L [El-Rashidy, Niazi 1979].
HPLC For method, see Verhagen *et al.* [1987].
Urine **GC** See Plasma [El-Rashidy, Niazi 1979].
Disposition in the Body Butylated hydroxyanisole is absorbed after oral administration and may be stored in body fat after large doses. It is metabolised by *O*-demethylation and conjugation with glucuronic acid and sulfate. Less than 1% of a dose is excreted in the urine unchanged in 24 h together with 44% as the glucuronide, 26% as the sulfate and about 22% each as the glucuronide and sulfate conjugates of the desmethyl metabolite.
Protein Binding Highly bound.

El-Rashidy R, Niazi S (1979). GLC determination of butylated hydroxyanisole in human plasma and urine. *J Pharm Sci* 68: 103–104.
Verhagen H *et al.* (1987). Sensitive high-performance liquid chromatographic method for the routine determination of butylated hydroxyanisole in plasma. *J Chromatogr* 413: 282–286.

Butylated Hydroxytoluene

Antioxidant
$C_{15}H_{24}O$ = 220.4
CAS—128-37-0

IUPAC Name 2,6-Ditert-butyl-4-methylphenol
Synonyms BHT; 2,6-bis-(1,1-dimethylethyl)-4-methylphenol; DBPC.
Proprietary Names *Anullex BHT*; *Embanox BHT.*



Chemical Properties Colourless crystals or white crystalline powder. Mp 70°. Insoluble in water; freely soluble in toluene; soluble in acetone; soluble 1 in 4 of ethanol, 1 in 1.1 of chloroform, and 1 in 0.5 of ether; practically insoluble in propylene glycol and in solutions of alkali hydroxides. pK_a 12.2. Log *P* (octanol/water), 5.1.

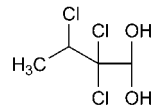
Colour Test Dissolve 0.1 g in 10 mL of ethanol, add 2 mL of a 2% solution of borax and a few crystals of 2,6-dichloroquinonechloroimide—faint blue (compare blue colour produced by butylated hydroxyanisole).
Gas Chromatography System GA—RI 1490.
Ultraviolet Spectrum Dehydrated alcohol—278 nm (A^1_1=85a).
Quantification
Plasma **HPLC** Electrochemical detection. Limit of detection, 0.4 mg/L [Bianchi *et al.* 1997].
Tissue **HPLC** See Plasma [Bianchi *et al.* 1997].
Disposition in the Body Readily absorbed after oral administration. About 50% of a dose is excreted in the urine in 24 h, mainly as glucuronide conjugates of oxidation products.

Bianchi L *et al.* (1997). Measurement of synthetic phenolic antioxidants in human tissues by high-performance liquid chromatography with coulometric electrochemical detection. *J Chromatogr B Biomed Sci Appl* 694(2): 359–365.

Butylchloral Hydrate

Hypnotic
$C_4H_7Cl_3O_2$ = 193.5
CAS—76-40-4

IUPAC Name 2,2,3-Trichlorobutane-1,1-diol
Synonyms Croton-cloral hydrate; trichlorobutylidene glycol.

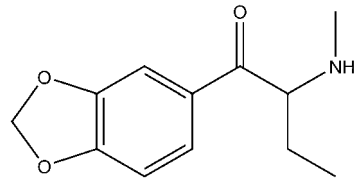


Chemical Properties Pearly-white crystalline scales. Mp ≈78°. Soluble 1 in 40 of water, 1 in 0.6 of ethanol (forming an ethanolate), 1 in 20 of chloroform and 1 in 2 of ether. Log *P* (octanol) 1.9.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 830, 1090, 1051, 1020, 690, 1300 cm^{-1} (KBr disk).
Dose Butylchloral hydrate has been given at a dose of 0.3 to 1.2 g.

Butylone

Cathinone Derivative, Phenethylamine, Stimulant
$C_{12}H_{15}NO_3$ = 221.2
CAS—17762-90-2

IUPAC Name 2-Methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one
Synonym bk-MBDB

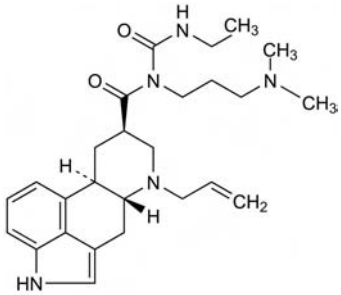


Chemical Properties Cathinone analogue of MDBD.
Note The name Butylone is also a trademarked brand name for pentobarbital.
Dose Reported as 160 mg orally.

Cabergoline

Dopamine D_2 Agonist
$C_{26}H_{37}N_5O_2 = 451.6$
CAS—81409-90-7

IUPAC Name 2,6-Ditert-butyl-4-methylphenol
Synonym (8β)-*N*-[3-(Dimethylamino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)ergoline-8-carboxamide; FCE-21336.
Proprietary Names *Cabaser*; *Dostinex*.

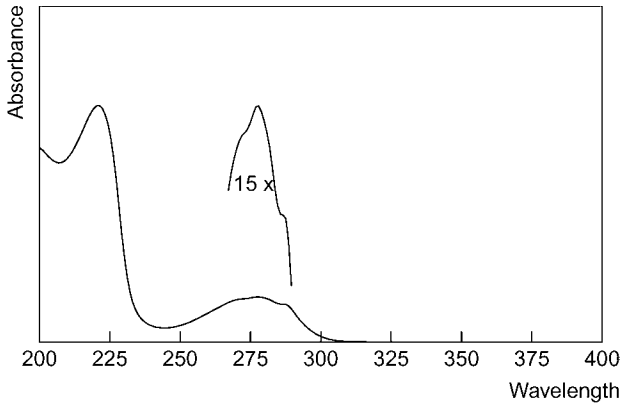


Chemical Properties A white powder. It is insoluble in water; soluble in chloroform, ethanol, and dimethylformamide; slightly soluble in 0.1 mol/L hydrochloric acid; very slightly soluble in hexane.

Cabergoline Diphosphate

$C_{26}H_{37}N_5O_2, 2H_3PO_4 = 647.6$
CAS—85329-89-1
Chemical Properties Mp 153° to 155°.

Ultraviolet Spectrum



Quantification

Plasma **HPLC** Column: C_{18} (Nucleosil, 250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 75 mM phosphate buffer pH 3 (20:80), 0.6 mL/min. Electrochemical detection. Retention time: 14 min. Limit of detection 0.25 µg/L [Pianezzola *et al.* 1992].
Urine **HPLC** Electrochemical detection. Limit of detection 0.30 µg/L [Pianezzola *et al.* 1992].
Disposition in the Body Cabergoline is absorbed after oral administration and undergoes significant first-pass metabolism. It is widely distributed and extensively metabolised, mainly by hydrolysis, to several inactive metabolites. The drug is mainly eliminated via faeces with a small proportion excreted in urine. <4% is excreted unchanged; 1% as the parent drug.
Therapeutic Concentration
Twelve healthy males, aged 21 to 31 years (mean 26 years), were administered with single doses of 0.5, 1.0 and 1.5 mg cabergoline. Mean maximum plasma concentrations were 33.3, 40.3 and 67.0 ng/L, respectively, for the three doses and all were observed at ~2 h [Andreotti *et al.* 1995].
Half-life Plasma half-life, 63 to 68 h (in healthy subjects); 79 to 115 h (in hyperprolactinaemic subjects).
Protein Binding 40%.
Note For reviews of cabergoline, see Rains *et al.* [1995] and Fariello [1998].
Dose Usually 1 mg weekly for hyperprolactinaemia, up to 6 mg daily for Parkinson's disease.

Andreotti AC *et al.* (1995). Pharmacokinetics, pharmacodynamics, and tolerability of cabergoline, a prolactin-lowering drug, after administration of increasing oral doses (0.5, 1.0, and 1.5 milligrams) in healthy male volunteers. *J Clin Endocrinol Metab* 80(3): 841–845.
Fariello RG (1998). Pharmacodynamic and pharmacokinetic features of cabergoline. Rationale for use in Parkinson's disease. *Drugs,* 55: 10–16.
Pianezzola E *et al.* (1992). Determination of cabergoline in plasma and urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 574: 170–174.
Rains CP *et al.* (1995). Cabergoline. A review of its pharmacological properties and therapeutic potential in the treatment of hyperprolactinaemia and inhibition of lactation. *Drugs* 49: 255–279.

Cadaverine

Putrefactive Base
$C_5H_{14}N_2 = 102.2$
CAS—462-94-2

IUPAC Name 1,5-Pentanediamine
Synonym Pentamethylenediamine

Chemical Properties A colourless syrupy basic liquid, which fumes and absorbs carbon dioxide on exposure to air. Fp 9°. Bp 178° to 180°. Soluble in water and ethanol; slightly soluble in ether. Log *P* (octanol/water), −0.2.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1035.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1565, 1508, 1600, 1120, 1173, 923 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 56, 55, 41, 43, 45, 85, 42, 84.
Quantification
Serum **GC** FID. Limit of detection, 0.5 mg/L [Khuhawar *et al.* 1999].
Hair **GC–MS** For method, see Choi *et al.* [2000].
Disposition in the Body Cadaverine is formed during putrefaction by bacterial decarboxylation of lysine in the gastrointestinal tract. It may be deaminated to yield ammonia and an aldehyde, and it may undergo cyclisation to piperidine. The piperidine normally excreted in the urine is from cadaverine.

Choi MH *et al.* (2000). Determination of hair polyamines as N-ethoxycarbonyl-N-pentafluoropropionyl derivatives by gas chromatography-mass spectrometry. *J Chromatogr A* 897(1–2): 295–305.
Khuhawar MY *et al.* (1999). Capillary gas chromatographic determination of putrescine and cadaverine in serum of cancer patients using trifluoroacetylacetone as derivatizing reagent. *J Chromatogr B Biomed Sci Appl* 723(1–2): 17–24.

Cadmium

Dermatological Agent, Nematocide
Cd = 112.4
CAS—7440-43-9

Synonym Colloidal cadmium
Chemical Properties Odourless silver-white lustrous solid. Mp 321°. Bp 765°. Insoluble in water, soluble in acids, ammonium nitrate, hot sulfuric acid. Valency Cd(+2). Widely but sparsely distributed element found in the earth's crust at concentrations ranging from 0.1 to 1 ppm, primarily in association with zinc ores. Used in alloys, nickel–cadmium batteries.

Cadmium Carbonate

$CdCO_3 = 172.4$
CAS—513-78-0
Synonyms Cadmium monocarbonate; carbonic acid, cadmium salt; otavite.
Chemical Properties White powder or rhombohedral leaflets. Mp <500° with decomposition. Insoluble in water; soluble in dilute acid and concentrated ammonia solution. Formerly used as a fungicide.

Cadmium Chloride

$CdCl_2 = 183.3$
CAS—10108-64-2
Synonyms Cadmium dichloride; dichlorocadmium.
Proprietary Names *Caddy*; *Vi-Cad*.
Chemical Properties Odourless, colourless rhombohedral crystals. Mp 568°. Bp 960°. Soluble in water and acetone; slightly soluble in methanol and ethanol. Used in the preparation of cadmium sulfide; in the manufacture of special mirrors; in dyeing and calico printing. Formerly used as a fungicide.

Cadmium Oxide

CdO = 128.4
CAS—1306-19-0
Synonyms Cadmium fume; Cadmium monoxide.

Chemical Properties Dark brown infusible powder or cubic crystals. Sublimes at 1559°. Insoluble in water; soluble in dilute acids; slowly soluble in ammonium salts. Used in semiconductors; manufacture of silver alloys; in battery electrodes; as nematocide; in ceramic glazes.

Cadmium Sulfate

$CdSO_4 = 208.5$

CAS—10124-36-4

Synonyms Sulfuric acid, cadmium (2+) salt

Chemical Properties Odourless, colourless monoclinic crystals. Mp 1000°. Soluble in water; insoluble in alcohol, acetone and ammonia. Used in electrodeposition of cadmium, copper and nickel; as nematocide, fungicide, bactericide, lubricant.

Cadmium Sulfide

CdS = 144.5

CAS—1306-23-6

Synonyms Cadmium monosulfide; cadmium orange; cadmium yellow; cadmopur yellow; capsebon; greenockite.

Chemical Properties Light yellow or orange cubic or hexagonal crystals. Mp 1750°. Slightly soluble in water; soluble in concentrated or warm dilute mineral acids. Used as a dermatologic agent; as a pigment; colour for soaps; colouring glass yellow; colouring textiles, paper, rubber; in printing inks; ceramic glazes; fireworks; in phosphors and fluorescent screens; in scintillation counters; semiconductors; photoconductors.

Colour Test There are no simple colour tests applicable to biological samples.

Note For reference values for cadmium in the German population, see Wilhelm *et al.* [2004].

Quantification

Specimen collection Blood—10 mL K-EDTA; urine—20 mL plastic universal container.

Blood **DPASV** Limit of detection, 0.1 µg/L [Moreno *et al.* 1999].

FAAS Lead and hydrogen hollow cathode lamps ($\lambda = 228.8$ nm). Limit of quantification, 30 µg/L [Delves 1977].

ETAAS Perkin-Elmer 5100 PC/HGA with Zeeman background collection. Limit of detection, 0.06 µg/L [Palminger Hallén *et al.* 1995].

Dry cycle: 120° in 1 s for 20 s. Char cycle: 1200° in 10 s for 20 s to 90° in 1 s for 10 s to 120° in 1 s for 20 s to 550° in 20 s for 20 s to 700° in 10 s for 20 s to 20° in 1 s for 10 s; Ar, 300 mL/min. Atomisation cycle: 1700° for 4 s (gas stop). Cadmium discharge lamp. Limit of detection, 0.22 µg/L [Moreira *et al.* 1995]. Dry cycle: 110° to 120°. Char cycle: 350°. Atomisation cycle: 2000° ($\lambda = 228.8$ nm). Limit of detection not reported [Baranowska 1995].

ICP-MS Plasma gas: Ar, 14.8 L/min. Auxiliary gas: Ar, 0.9 L/min. Carrier gas: 1.1 L/min. Babington nebuliser (*m/z* 27). Limit of detection, not reported [Botta *et al.* 2006]. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 0.3 µg/L [De Boer *et al.* 2004]. Plasma gas: 14.8 L/min. Auxiliary gas: 0.85 L/min. Nebulizer gas: 0.93 L/min. Limit of detection, 5 ng/kg [Diemer *et al.* 2001]. Perkin-Elmer Sciex Elan 5000. Nebuliser gas: 1.0 mL/min. Limit of detection, 20 ng/L [White 1999].

Note For an immunoassay to determine concentrations of cadmium in serum, see Darwish and Blake [2002].

Urine **ETAAS** Perkin-Elmer 5100. Limit of detection, 0.05 µg/L [White 1999]. See Blood [Moreira *et al.* 1995].

ETV-ID-ICP-MS Outer gas: 15.0 L/min. Intermediate gas: 0.74 L/min. Carrier gas: 1.08 L/min. Dry cycle: 90° at 30 s for 10 s to 130° in 10 s for 10 s. Char cycle: 300° in 10 s for 20 s. Atomisation cycle: 1000° in 1 s for 20 s. Limit of detection, 0.02 µg/L [Lee *et al.* 1998].

ICP-MS Plasma gas: Ar, 14.8 L/min. Auxiliary gas: Ar, 0.9 L/min. Carrier gas: 1.1 L/min. Babington nebuliser (*m/z* 27). Limit of detection not reported [Botta *et al.* 2006]. See Blood. Limit of detection, 0.15 µg/L [De Boer *et al.* 2004]. See Blood. Limit of detection, 0.1 µg/L [White 1999]. Plasma gas: 15 L/min. Nebuliser gas: 0.825 L/min. Auxiliary gas: 0.8 L/min. Limit of detection, 0.02 µg/L [Schramel *et al.* 1997].

Oral Fluid ICP-MS Plasma gas: 13 L/min. Auxiliary gas: 0.55 L/min. Nebulizer gas: 0.1 L/min. Limit of detection, 0.03 µg/L [Menegario *et al.* 2001].

Ocular Fluid **ICP-MS** Perkin-Elmer Sciex Elan 6100. Limit of detection, 0.2 µg/L [Erie *et al.* 2005].

Bone **ETAAS** Dry cycle: 110° to 120° in 30 s. Char cycle: 300° to 350° in 30 s. Atomisation cycle: 1200° for 20 s. Carrier gas: Ar, 200 mL/min ($\lambda = 228.8$ nm). Limit of detection, 1 µg/L [Baranowska *et al.* 1995].

Hair **ETAAS** Dry cycle: 80° to 120° in 10 s. Char cycle: 300° to 400° at 10 s. Atomisation cycle: 2700° to 2800° in 5 s. Carrier gas: 200 mL/min. Hitachi model 180-50, S.N.5721-2 ($\lambda = 193.8$ nm). Limit of detection not reported [Kazi *et al.* 2006]. Dry cycle: 100° for 15 s to 140° for 20 s. Char cycle: 850° for 20 s. Atomisation cycle: 1650° for 5 s. Perkin-Elmer 700 spectrometer ($\lambda = 228.8$ nm). Limit of detection, 4.6 µg/L [Sweileh 2003].

ICP-MS Limit of detection, 0.03 mg/kg [Nadal *et al.* 2005]. Plasma gas: 15 L/min. Auxiliary gas: 0.8 L/min. Nebuliser gas: 0.8 L/min. Limit of detection, not reported [Samanta *et al.* 2004].

Note For a study following the trace element hair analysis of 1 man over 2 decades, see [Klevay *et al.* 2004].

Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000].

Liver **ICP-MS** Perkin-Elmer Elan 6000. Nebuliser gas: Ar, 1.04 L/min. Limit of detection, 26 ng/L [Patriarca *et al.* 1999].

Note For a portable system for detecting cadmium in human liver, see Al Hiti *et al.* [1979].

Nail **ICP-MS** See Hair [Samanta *et al.* 2004].

Colostrum **ETAAS** Dry cycle: 50° to 120° at 20 s for 20 s. Char cycle: 120° to 900° in 30 s for 30 s. Atomisation cycle: 1600° for 7 s. Limit of detection, 0.62 µg/L [Turan *et al.* 2001].

Milk **ETAAS** Perkin-Elmer 5100 PC/HGA 600 with Zeeman background correction. Limit of detection, 0.06 µg/L [Palminger Hallén *et al.* 1995].

Placenta **ETAAS** Dry cycle: 110° to 120°. Char cycle: 350°. Atomisation cycle: 2000° ($\lambda = 228.8$ nm). Limit of detection not reported [Baranowska 1995]. Atomisation cycle: 1400° ($\lambda = 228.8$ nm). Limit of detection, <4 µg/kg [Fiala *et al.* 1998].

Teeth **DPV** Limit of quantification, 0.394 µg/L, limit of detection, 0.078 µg/L [Bayo *et al.* 2001].

Other **DPASV** Yemeni Khat. Limit of detection, 0.15 µg/kg [Matloob 2003].

ETAAS Eggs and Chicken Feed. Dry cycle: 120° at 1 s for 50 s. Char cycle: 1400° in 1 s for 30 s to 20° in 1 s for 5 s; Ar, 300 mL/min. Atomisation cycle: 2300° for 5 s (gas stop). Limit of detection, 0.07 mg/kg [Fakayode, Olu-Owolabi 2003]. Cocaine Samples. Char cycle: 800°. Atomisation cycle: 1400° ($\lambda = 228.8$ nm). Limit of detection not reported [Bermejo-Barrera *et al.* 1999].

ICP-AES Argentine Wine. Outer gas: 8.5 L/min. Auxiliary gas: 1.0 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, 8 ng/L [Lara *et al.* 2005].

ICP-MS Seafood. Perkin-Elmer Elan 6000. Limit of detection, 0.02 mg/kg [Falcó *et al.* 2006]. Food. Varian-Vista with an ultrasonic nebuliser. Limit of detection, 0.02 mg/kg [Llobet *et al.* 2003]. Meals from Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 0.6 µg/kg [Noel *et al.* 2003]. Beverages. Limit of detection, 0.2 µg/kg [MacIntosh *et al.* 2000].

Disposition in the Body Cadmium can be absorbed by the inhalation, oral and dermal routes of exposure in any of its chemical forms. Dermal absorption is relatively insignificant, although small amounts are absorbed percutaneoulsy over time. Gastrointestinal absorption of cadmium in any chemical form is relatively low when compared with the inhalation route and is in the range of 3 to 8%. Several blood and dietary factors can influence gastrointestinal absorption, such as deficiencies in calcium or iron, or blood ferritin content in women. Zinc decreases the dietary absorption of cadmium. A higher fraction of cadmium is absorbed by inhalation and depends very much on the particle size of the cadmium salt and its solubility.

Cadmium accumulates in the lungs, liver and kidney after chronic exposure and is excreted very slowly in the urine. Cadmium may bind to plasma proteins, plasma metallothionein, or directly to the erythrocytes, and it tends to accumulate in the liver and kidney. Spleen, pancreas and testes also have relatively high concentrations of cadmium. It is not known to undergo any direct metabolic processes, but binds as the bivalent cation ion to anionic groups in proteins and other molecules. The majority of an oral dose is excreted in faeces; following inhalation and cadmium distribution throughout the body, the amounts of faecal and urinary excretion are approximately equal. Excessive occupational exposure results in a high body burden, which leads to elevated blood and urine cadmium concentrations that persist into old age.

Normal Concentrations Blood (non-smokers)—<2 µg/L (18 nmol/L); blood (smokers)—<6 µg/L (54 nmol/L); urine (smokers and non-smokers)—<1 µg/L (9 nmol/L); kidney—2.1 to 22.0 mg/kg; liver—1.2 to 3.7 mg/kg. Occupational Guidance Values: blood and urine—5 µg/L (45 nmol/L).

Toxicity Chronic cadmium poisoning causes proximal renal tubular damage, with disturbances in calcium and phosphate metabolism, and it is associated with urine concentrations >15 µg/L. Prolonged excessive exposure by the oral route leads to skeletal deformities, gastrointestinal disorders, and kidney, lung and heart defects. Inhalation of approx. 4 mg of cadmium can be fatal; the lethal dose by the oral route is of the order of several hundred milligrams of a soluble salt. Cadmium exposure from industrial contamination of water supplies led to an epidemic of cadmium poisoning in the Jinzu river basin of Japan during the 1940s and the name 'itai-itai' (meaning 'ouch-ouch') disease was coined to describe the painful symptoms associated with the condition. This multiple-system disorder primarily affects postmenopausal women and is characterised by renal osteomalacia, proteinuria and enteropathy [Nogawa *et al.* 2004]. Blood cadmium concentrations are higher in exposed workers than in non-exposed workers: 9.3 versus 3.8 µg/L [Baker *et al.* 1979].

In 5 men who survived exposure to cadmium fumes over a long period and developed fatigue, chest pains and coughing, urine cadmium levels ranged from 10 to 50 µg/L [Hardy, Skinner 1947].

In a further 5 cases of non-fatal acute exposure to cadmium, blood and urine concentrations of 1.2 to 3.0 mg/L and 0.10 to 0.36 mg/L, respectively, were reported [Cotter, Cotter 1951].

A man who ingested 5 g of cadmium iodide had a urine cadmium concentration of 5.6 mg/L after 7 days. The postmortem blood concentration was 1.1 mg/L; the liver contained 80 mg/kg and the renal cortex 80 mg/kg [Wisniewska-Knypl *et al.* 1971].

A 43-year-old man who worked in copper alloy manufacture was admitted to hospital with breathlessness having been exposed to smolten copper scrap. On his 11th day in hospital, 12 days after exposure, he died. The cadmium concentration in the right upper lobe of the lung was 1.06 mg/kg wet weight [Yamamoto *et al.* 1983].

An analysis of 41 cadmium workers predicted that there is a 50% probability of having kidney dysfunction when there are concentrations of 38.4 and 42.3 mg/kg cadmium in the kidney and liver, respectively [Ellis *et al.* 1984].

A study of 29 patients whose clinical findings motivated a kidney biopsy and/or had a history of possible exposure to cadmium found a mean cadmium concentration of 12.9 µg/g kidney tissue. The highest concentrations (30 to 45 µg/g) were found in 3 patients with tubulo-interstitial damage. In a control group of 22 postmortem samples, the mean cadmium concentration was 8.7 µg/g [Lindqvist *et al.* 1989].

A 68-year-old man developed diffuse abdominal pain within days of beginning to fashion boxes from sheet metal. On the third day he developed a fever, cough, increased abdominal pain, and ileus. He was hospitalised and treatment for occult sepsis was begun. He developed peritoneal signs but an exploratory laparotomy was inconclusive. His hypoxia worsened and there were progressive alveolar infiltrates. On his fifth day in hospital he died. Postmortem examination revealed congestive cardiomegaly with pulmonary congestion and oedema and small bilateral pulmonary effusions. A heavy metal screen revealed dramatically elevated cadmium in the cardiac blood of 280 µg/L and slightly elevated kidney concentrations of 24 mg/kg [Fuortes *et al.* 1991].

A 43-year-old man sought medical attention for acute respiratory insufficiency. He had been suffering from cough, chest tightness, abdominal pain and diarrhoea. He worked in a crematorium and smoked 20 cigarettes a day. Screening for heavy metals showed an initial urinary cadmium concentration of 438 mmol/L. Analysis of dialysate fluid showed a cadmium concentration of 130 mmol/L. A renal biopsy 3 weeks later showed a cadmium concentration of 0.015 mg/kg. The patient was discharged after 10 weeks [Nicholson *et al.* 1997].

A 66-year-old man worked for 20 years as a repairer in a glassworks. The hard tissue cadmium content of one of his teeth was 1.4 mg/kg [Bachanek *et al.* 2000].

A 35-year-old woman was found dead in bed. She had last eaten beetroot. Because of the purple discoloration it was deemed that an investigation for manganese and other elements was appropriate. Manganese and cadmium concentrations were as follows:

Medium	*Mn (µg/L or µg/g)*	*Cd (µg/L or µg/g)*
Serum (heart blood)	127	ND
Heart blood	899	238
Stomach contents	0.1	0.4
Brain	0.2	3.6
Kidney	3.6	34.3
Liver	0.8	20.2
Small bowel	1266	<0.1

[Dressler *et al.* 2002].

A 42-year-old man presented with marked lethargy and fever. He had ingested approx. 50 mL of an industrial chemical solution approx. 1 h prior to admission. His serum cadmium concentration was 24.9 µg/L (normal range 0.2 to 6.0 µg/L) [Hung, Chung 2004].

Note For a review of cadmium in the environment and human health, see Yost [1986] and for an investigation of toxic trace elements in the hair of children with autism, see Fido and Al Saad [2005]. For an evaluation of metal levels in welders, see Iarmarcovai *et al.* [2005]. For a comparison of trace metal profiles in hair samples from children in urban or rural areas, see Hasan *et al.* [2004]. For a study of cadmium in the blood, serum and red blood cells of patients with motor neuron disease, see Pamphlett *et al.* [2001]. Rahil-Khazen *et al.* [2002] have studied trace element levels in the postmortem tissue of 30 Norwegians. For biological monitoring of cadmium in workers in hazardous-waste incineration, see Schuhmacher *et al.* [2002]; for concentrations in the urine of glass-manufacturing workers, see Apostoli *et al.* [1998] and Arai *et al.* [1994]. For a study of cadmium concentrations in the Greenlandic population, see Hansen *et al.* [1985].

Half-life Total body: 16 years.

Distribution Blood : serum ratio approx. 2

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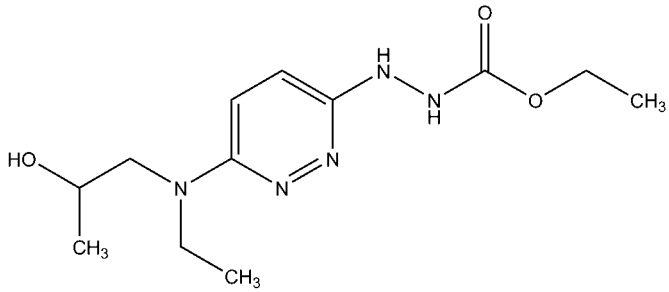
Cadralazine

Antihypertensive
$C_{12}H_{21}N_5O_3$ = 283.3
CAS—64241-34-5

IUPAC Name Ethyl *N*-[[6-(ethyl-(2-hydroxypropyl)amino)pyridazin-3-yl]amino]carbamate
Synonyms 3-(2-Carbethoxyhydrazino)-6-[*N*-(2-hydroxypropyl)ethylamino]-pyridazine; DC-826; 2-[6-[ethyl(2-hydroxy-propyl)amino]-3-pyridazinyl]hydrazinecarboxylic acid ethyl ester; ISF-2469.
Proprietary Names *Cadral*; *Cadraten*; *Cadrilan*; *Presmode*.

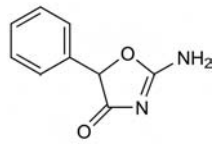


Chemical Properties Crystals. Mp 160° to 162°. pK_a 6.0.
Dose 10 mg orally once a day.

Caffeine

Xanthine Stimulant
$C_8H_{10}N_4O_2$ = 194.2
CAS—58-08-2

IUPAC Name 1,3,7-Trimethylpurine-2,6-dione
Synonyms 1,3,7-Trimethyl-2,6-dioxopurine; 1,3,7-trimethylxanthine; 3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione; anhydrous caffeine; coffeine; guaranine; methyltheobromine; methylxanthine theophylline.
Proprietary Names *Caffedrine*; *Enerjets*; *Keep Alert*; *NoDoz*; *Pro-Plus*; *Quick Pep*; *Spray-n-Wake*; *Stay Alert*; *Tirend*; *Vivarin*; *Wake-Up Tablets*. Caffeine is an ingredient of many proprietary preparations [Sweetman 2007].



Chemical Properties Silky white crystals, usually matted together, or a white crystalline powder. It sublimes at 178°. Mp 238°. When crystallised from water, caffeine contains 1 molecule of water of crystallisation, but it is anhydrous when crystallised from ethanol, chloroform or ether. It is decomposed by strong solutions of caustic alkalis. Freely soluble in pyrrole, in tetrahydrofuran containing approx. 4% water; soluble in ethyl acetate; soluble 1 in 46 of water, 1 in 5.5 of water at 80°, 1 in 1.5 of boiling water, 1 in 66 of alcohol, 1 in 22 of alcohol at 60°, 1 in 50 of acetone, 1 in 5.5 of chloroform; 1 in 530 of ether, 1 in 100 of benzene, 1 in 22 of boiling benzene; slightly soluble in petroleum ether. Solubility in water is increased by alkali benzoates, cinnamates, citrates or salicylates. pK_a 10.4 (40°) [Dean *et al.* 1985], 14.0 (25°). Log *P* (octanol/water), −0.07 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 0.3 [Demme *et al.* 2005].

Caffeine Citrate

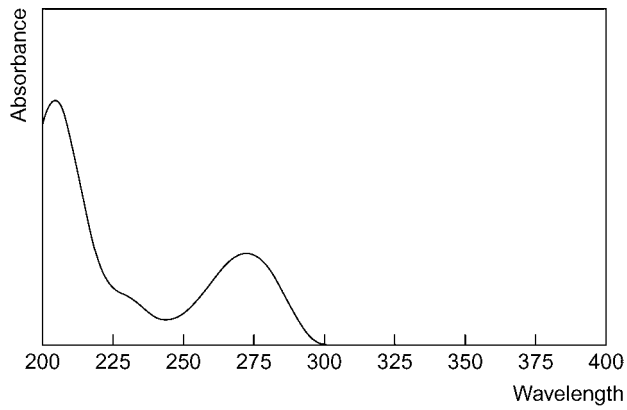
$C_8H_{10}N_4O_2,C_6H_8O_7$ = 386.3
CAS—69-22-7
Synonyms Caffeina citrate; citrated caffeine; coffeinum citricum; cofeincitrat.
Proprietary Names *Cafcit*. An ingredient of *Askit*.

Chemical Properties A mixture of caffeine and citric acid containing 47–50% of anhydrous caffeine. A white, crystalline powder that is decomposed by water. Soluble 1 in 4 of hot water, dissociating on further dilution with the separation of caffeine on cooling, which redissolves in ~1 in 32 of water; soluble 1 in 25 of ethanol. Log *P* (octanol/water), 0.16.

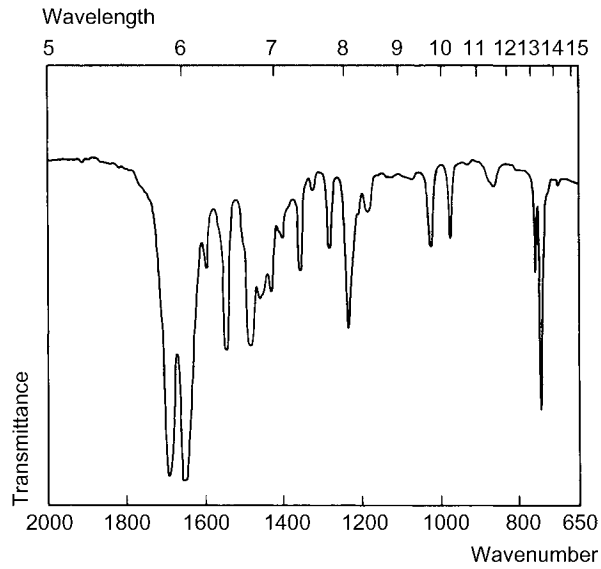
Caffeine Hydrate

$C_8H_{10}N_4O_2,H_2O$ = 212.2
CAS—5743-12-4
Synonyms Caféine monohydratée; caffeine monohydrate; coffein monohyrat; coffeinum monohydricum.
Proprietary Names An ingredient of *Migril* and *Pardale*.
Chemical Properties Silky white crystals, usually matted together, or a white crystalline powder containing 8.5% water. It effloresces in dry air and loses its water of crystallisation when heated, becoming anhydrous at 80°. It sublimes at ~180°. Mp 234° to 239°. Soluble 1 in 60 of water and 1 in 110 of ethanol; soluble in chloroform with the separation of water; slightly soluble in ether.

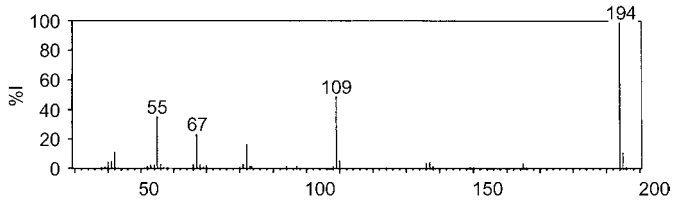
Colour Test Amalic acid test—orange/violet.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.03; system TC—R_f 0.58; system TD—R_f 0.15; system TE—R_f 0.52; system TF—R_f 0.10; system TL—R_f 0.25; system TAD—R_f 0.55; system TAE—R_f 0.59; system TAF—R_f 0.55; system TAJ—R_f 0.54; system TAK—R_f 0.18; system TAL—R_f 0.81 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).
Gas Chromatography System GA—RI 1800, M (theobromine) RI 1807, M (theophylline) RI 2005; system GB—RI 1904, M (theobromine) RI 1920, M (theophylline) RI 1990; system GC—RI 2376; system GF—RI 2340.
High Performance Liquid Chromatography System HA—*k* 0.2; system HC—*k* 0.26; system HS—*k* 0.21; system HX—RI 305; system HY—RI 259; system HZ—RT 1.9 min; system HAA—RT 6.7 min; system HAM—RT 1.98 min; system HAX—RT 4.8 min; system HAY—RT 3.8 min; system HAZ—*k* 0.18.
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1 = 504a). No alkaline shift.



Infrared Spectrum Principal peaks at wave numbers 1658, 1698, 747, 1548, 1242, 760 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 194, 109, 55, 67, 82, 195, 42, 110.



Quantification

Plasma **GC** Column: DB-17 (15 m × 530 µm i.d., 1 µm). Carrier gas: He, 21 mL/min. Temperature programme: 70° to 280°. NPD. Limit of detection, 50 µg/L [Carregaro *et al.* 2001]. Column: OV-17. NSD. Limit of detection, 1 µg/L [Teeuwen *et al.* 1991]. AFID. Limit of detection, 50 µg/L [Bradbrook *et al.* 1979].

HPLC Column: Partisil-10 SCX. Mobile phase: 0.1 mol/L sodium phosphate buffer (pH 4.9) : methanol (92 : 8), flow rate.1.5 mL/min. UV detection (λ = 254 nm). Retention time: 5.1 min. Limit of detection, 10 µg/L [Ghosheh *et al.* 2000]. Column: reversed phase. UV detection. Limit of detection, 0.1 mg/L for caffeine and theophylline [Schreiber-Deturmeny, Bruguerolle 1996]. Caffeine and its metabolites [Dobrocky *et al.* 1994]. Column: TSK gel ODS-80TM (150 × 4.6 mm i.d.). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (30 : 70), flow rate 0.8 mL/min. UV detection (λ = 274 nm). Limit of detection, 5 µg/L for caffeine [Tanaka 1992]. UV detection. Limit of detection, 100 µg/L [Haughey *et al.* 1982]. UV detection (λ = 273 nm). Limit of detection, 100 µg/L [O'Connell, Zurzola 1984].

LC-MS FAB. Limit of detection, ~5 µg/L [Hieda *et al.* 1995].

Serum **HPLC** Caffeine and its metabolites. Column: MZ Kromasil C4 (250 × 4 mm i.d., 5 µm). Mobile phase: acetate buffer (pH 3.5) : methanol (97: 3 to 80 : 20 in 20 min). UV detection (λ = 275 nm). Limit of detection, 20 mg/L for caffeine [Georgia *et al.* 2001]. Caffeine and paraxanthine. Column: reversed phase. UV detection. Limit of detection, 0.3 mg/L for caffeine [Koch *et al.* 1999]. Caffeine and paraxanthine. UV detection. Limit of quantification, 50 µg/L [Holland *et al.* 1998]. Caffeine and theophylline. Column: ODS reversed phase. Mobile phase: 0.2 mmol/L sodium hydrogen phosphate solution (pH 7.4). UV detection (λ = 273 nm). Limit of detection, 0.5 mg/L for caffeine [Umemura *et al.* 1998]. See Plasma [Haughey *et al.* 1982].

Urine **HPLC** Caffeine, theobromine and theophylline. UV detection. Limit of detection, <1 ng/L [Bispo *et al.* 2002]. Caffeine metabolites. Column: Eclipse XDB-C_{18}. DAD [Caubet *et al.* 2002]. See Serum [Georgia *et al.* 2001]. Column: YMC-Pack Polyamine-II (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.75% formic acid (91 : 9), flow rate 1.0 mL/min. UV detection (λ = 260 and 280 nm) [Nyeki *et al.* 2001]. Caffeine and its metabolites. Column: ODS. Mobile phase: 0.05% acetic acid: methanol (92.5 : 7.5). UV detection (λ = 280 nm) [Bendriss *et al.* 2000]. Caffeine metabolites. UV detection. Limit of detection, 1–2 µmol [Rasmussen, Brosen 1996]. See Plasma [Dobrocky *et al.* 1994].

LC-MS See Plasma [Hieda *et al.* 1995].

CE Caffeine and its metabolites. Limit of detection, 2–5 µmol/L [Rodopoulos, Norman 1994].

Oral Fluid **GC** See Blood [Teeuwen *et al.* 1991]. See Plasma [Haughey *et al.* 1982].

Sweat **GC-MS** [Tsuda *et al.* 2000].

Disposition in the Body Caffeine is rapidly absorbed after oral administration; bioavailability is almost 100%. It is widely distributed, crossing the placenta, entering the CNS and saliva; small amounts are excreted in breast milk. Metabolic reactions include *N*-demethylation, acetylation and oxidation to uric acid derivatives. Approximately 85% of a dose is excreted in the urine in 48 h with up to 40% of the dose as 1-methyluric acid, 10–15% as 1-methylxanthine and up to 35% as 5-acetylamino-6-formylamino-3-methyluracil and 5-acetylamino-6-amino-3-methyluracil; other metabolites excreted in the urine include theophylline, 1,7-dimethylxanthine (paraxanthine), 7-methylxanthine, and 1,3-dimethyluric acid. Approximately 1% is excreted in the urine as unchanged drug. The extent of *N*-acetylation is genetically determined. In neonates, caffeine is largely excreted unchanged in the urine, because the capacity to metabolise the drug is reduced until approx. 6 months of age. Caffeine, theophylline, theobromine and paraxanthine are found in plasma from dietary sources, especially coffee, tea and cocoa. An average cup of coffee or tea contains approximately 100 mg caffeine.

Therapeutic Concentration

Following a single dose of 130 mg caffeine to 36 subjects, peak plasma concentrations of 2.5–6.8 mg/L (mean, 4.0) were attained in 20–40 min [O'Connell, Zurzola 1984].

Following a single oral dose of 5 mg/kg to 10 subjects, peak plasma concentrations of 6.9–16.1 mg/L (mean, 10) were attained in ~0.5 h [Blanchard, Sawers 1983].

Toxicity Fatalities have occurred after the ingestion of 5–50 g caffeine, but recovery following the ingestion of 30 g has been reported. Toxic effects are associated with blood concentrations greater than 15 mg/L and fatalities with blood concentrations greater than 80 mg/L.

In a fatality resulting from ingestion of mail order diet pills containing caffeine as their only active ingredient, a 1560 mg/L postmortem serum concentration was reported in a 22-year-old woman [Mrvos *et al.* 1989].

The following postmortem tissue concentrations were reported in a fatality resulting from ingestion of 5.3 g caffeine by a 5-year-old child: blood 159 mg/L, kidney 230 µg/g, liver 198 µg/g and urine 114 mg/L [DiMaio, Garriott 1974].

In a death caused by the ingestion of 50 g caffeine, the following postmortem tissue concentrations were reported: blood 79 mg/L, brain 105 µg/g, kidney 145 µg/g, liver 214 µg/g and urine 280 mg/L [Grusz-Harday 1973].

A 1-year-old girl survived after ingesting 2–3 g caffeine (200–300 mg/kg) despite having a maximum serum concentration of 385 mg/L 4 h after ingestion. Symptoms of toxicity included arrhythmias, seizures, metabolic disturbances and severe pulmonary oedema [Dietrich, Mortensen 1990].

Note For a review of caffeine toxicology, see Abbott [1986]. For a review of deaths involving caffeine, see Shum *et al.* [1997].

Half-life Plasma half-life, 2–10 h (mean, 4).

Volume of Distribution ~0.5 L/kg.

Clearance Plasma clearance, 1–2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.93;

Saliva Plasma : saliva ratio, ~1.3.

Protein Binding ~35%.

Dose As a mild stimulant 50 to 200 mg; maximum 1 g daily.

Abbott PJ (1986). Caffeine: a toxicological overview. *Med J Aust* 145: 518–521.

Bendriss EK *et al.* (2000). Liquid chromatographic method for the simultaneous determination of caffeine and fourteen caffeine metabolites in urine. *J Chromatogr B Biomed Sci Appl* 746: 331–338.

Bispo MS *et al.* (2002). Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography. *J Chromatogr Sci* 40: 45–48.

Blanchard J, Sawers SJ (1983). The absolute bioavailability of caffeine in man. *Eur J Clin Pharmacol* 24: 93–98.

Bradbrook ID *et al.* (1979). Comparison of thin-layer and gas chromatographic assays for caffeine in plasma. *J Chromatogr* 163: 118–122.

Carregaro AB *et al.* (2001). Comparison of the quantification of caffeine in human plasma by gas chromatography and ELISA. *Braz J Med Biol Res* 34: 821–824.

Caubet MS *et al.* (2002). Analysis of urinary caffeine metabolites by HPLC-DAD: the use of metabolic ratios to assess CYP1A2 enzyme activity. *J Pharm Biomed Anal* 27: 261–270.

Dean JA *et al.* (1985). A new approach to the analysis of complex dose–response relationships: a multivariate study of octopamine action on the leech Leydig neurone. *Comp Biochem Physiol C* 82: 165–170.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dietrich AM, Mortensen ME (1990). Presentation and management of an acute caffeine overdose. *Pediatr Emerg Care* 6: 296–298.

DiMaio VJ, Garriott JC (1974). Lethal caffeine poisoning in a child. *Forensic Sci* 3: 275–278.

Dobrocky P *et al.* (1994). Rapid method for the routine determination of caffeine and its metabolites by high-performance liquid chromatography. *J Chromatogr* 652: 104–108.

Georgia KA *et al.* (2001). Use of novel solid-phase extraction sorbent materials for high-performance liquid chromatography quantitation of caffeine metabolism products methylxanthines and methyluric acids in samples of biological origin. *J Chromatogr B Biomed Sci Appl* 759: 209–218.

Ghosheh OA *et al.* (2000). A simple high performance liquid chromatographic method for the quantification of total cotinine, total 3′-hydroxycotinine and caffeine in the plasma of smokers. *J Pharm Biomed Anal* 23: 543–549.

Grusz-Harday E (1973). Lethal caffeine poisoning. *TIAFT Bull* 9: 6–7.

Hansch C *et al.* (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society

Haughey DB *et al.* (1982). Liquid chromatographic determination of caffeine in biologic fluids. *J Chromatogr* 229: 387–395.

Hieda Y *et al.* (1995). Highly sensitive and rapid determination of theophylline, theobromine and caffeine in human plasma and urine by gradient capillary high-performance liquid chromatography–FRIT-fast atom bombardment mass spectrometry. *J Chromatogr B Biomed Appl* 667: 241–246.

Holland DT *et al.* (1998). Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation. *J Chromatogr B: Biomed Sci Appl* 707: 105–110.

Koch JP *et al.* (1999). Validation of a high-performance liquid chromatography assay for quantification of caffeine and paraxanthine in human serum in the context of CYP1A2 phenotyping. *Biomed Chromatogr* 13: 309–314.

Mrvos RM *et al.* (1989). Massive caffeine ingestion resulting in death. *Vet Hum Toxicol* 31: 571–572.

Nyeki A *et al.* (2001). Extractionless method for the simultaneous high-performance liquid chromatographic determination of urinary caffeine metabolites for *N*-acetyltransferase 2, cytochrome P450 1A2 and xanthine oxidase activity assessment. *J Chromatogr B Biomed Sci Appl* 755: 73–84.

O'Connell SE, Zurzola FJ (1984). Rapid quantitative liquid chromatographic determination of caffeine levels in plasma after oral dosing. *J Pharm Sci* 73: 1009–1011.

Rasmussen BB, Brosen K (1996). Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and *N*-acetyltransferase activity in humans. *Ther Drug Monit* 18: 254–262.

Rodopoulos N, Norman A (1994). Determination of caffeine and its metabolites in urine by high-performance liquid chromatography and capillary electrophoresis. *Scand J Clin Lab Invest* 54: 305–315.

Schreiber-Deturmeny E, Bruguerolle B (1996). Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. *J Chromatogr B Biomed Appl* 677: 305–312.

Shum S *et al.* (1997). Acute caffeine ingestion fatalities: management issues. *Vet Hum Toxicol* 39: 228–230.

Sweetman S, ed. (2007). *Martindale, The Complete Drug Reference*, 35 edn. London: Pharmaceutical Press.

Tanaka E (1992). Simultaneous determination of caffeine and its primary demethylated metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr* 575: 311–314.

Teeuwen HW *et al.* (1991). Rapid and sensitive gas-chromatographic determination of caffeine in blood plasma, saliva, and xanthine beverages. *Mol Biol Rep* 15: 1–7.

Tsuda T *et al.* (2000). Proposal of sampling process for collecting human sweat and determination of caffeine concentration in it by using GC/MS. *Biomed Chromatogr* 14: 505–510.

Umemura T *et al.* (1998). Direct injection determination of theophylline and caffeine in blood serum by high-performance liquid chromatography using an ODS column coated with a zwitterionic bile acid derivative. *Analyst* 123: 1767–1770.

Calfactant

Pulmonary Surfactant
CAS—183325-78-2

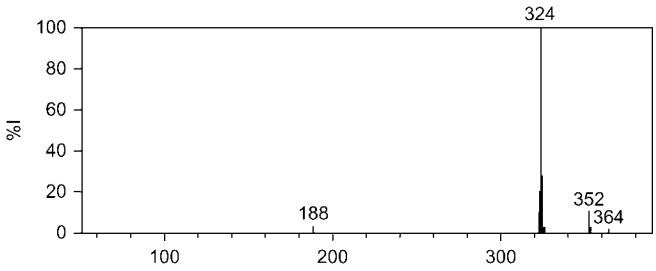
Synonyms CLSE; CLL.
Proprietary Name *Infasurf*
Disposition in the Body Chloroform/methanol extract of calf lung lavage fluid consisting of 35 mg/mL phospholipid (of which 55 to 70% is dipalmitoylphosphatidylcholine), 0.9 mg/mL fatty acids, 1.8 mg/mL neutral lipids, and low-molecular-weight surfactant proteins (SP-B and SP-C).
Protein binding Approximately 50%.
Note For the separation of subfractions of the hydrophobic components of calfactant, see Hall *et al.* [1994]; for a review of calfactant, see Willson [2001].
Dose In prophylaxis of respiratory distress syndrome at birth, 3 mL/kg of birth weight in two doses. In treatment of respiratory distress syndrome within 72 h of birth, 3 mL/kg of birth weight, in two doses. Repeat doses of 3 mL/kg of birth weight to a maximum of 3 doses 12 h apart.

Hall SB *et al.* (1994). Separation of subfractions of the hydrophobic components of calf lung surfactant. *J Lipid Res* 35: 1386–1394.
Willson D (2001). Calfactant. *Expert Opin Pharmacother* 2: 1479–1493.

Californine

Anxiolytic, Pavine Alkaloid
$C_{19}H_{18}NO_4 = 324.4$

IUPAC Name (5*S*,12*S*)-5,6,12,13-Tetrahydro-15-methyl-cycloocta[1,2-*f*:5,6-*f*′]bis[1,3]benzodioxol-5,12-imine
Synonym Eschscholtzine
Proprietary Name It is an ingredient of the phytopharmaceutical *Sympathyl* as a component of *Eschscholtzia californica*.
Chemical Properties Alkaloid isolated from *Eschscholtzia californica* (Papaveraceae, 'California poppy' or 'Yellow poppy').
Mass Spectrum



Quantification

Other **GC-MS** Rat Urine. Column: cross-linked methylsilicone capillary (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, flow rate 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV and PICI. RI 2615 (acetyl derivative). Limit of quantification not reported [Paul, Maurer 2003].

HPLC Rat Liver Microsome Samples. Column: Supersher 60 RP-select B (125 × 2.0 mm i.d.). Mobile phase: 20 mmol/L ammonium formate buffer (pH 3.0) : acetonitrile (83 : 17), flow rate 0.4 mL/min. Fluorescence detection ($\lambda_{ex} = 230$ nm, $\lambda_{em} = 334$ nm). Retention time: approx. 16.5 min. Limit of quantification not reported [Paul *et al.* 2004].

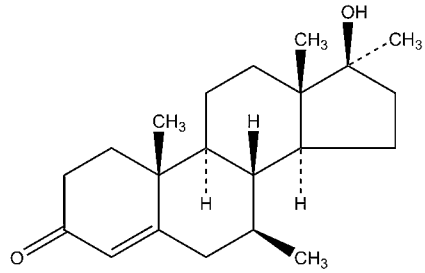
Disposition in the Body *In vitro* studies have shown that californine is extensively metabolised by *N*-demethylation and/or single or double demethylation, with consecutive catechol *O*-methylation of 1 of the hydroxyl groups. This is mainly mediated by CYP3A2 and to a minor extent by CYP1A2 and CYP2D1.
Toxicity *Eschscholtzia* has been described as a substitute drug for marijuana, with similar mild euphoria after ingestion or smoking, lasting for 20–30 min. Gradual increase of the dose is recommended until the desired effect occurs. Regular and prolonged use is not thought to be habit forming.
Note For a study on the metabolism of californine in rats, see Paul and Maurer [2003].
Dose Plant sources of californine are used in phytotherapy preparations for the treatment of anxiety disorders. They are also misused as herbal drugs of abuse (marijuana substitutes).

Paul LD, Maurer HH (2003). Studies on the metabolism and toxicological detection of the *Eschscholtzia californica* alkaloids californine and protopine in urine using gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 789: 43–57.
Paul LD *et al.* (2004). Cytochrome P450 isoenzymes involved in rat liver microsomal metabolism of californine and protopine. *Eur J Pharmacol* 485: 69–79.

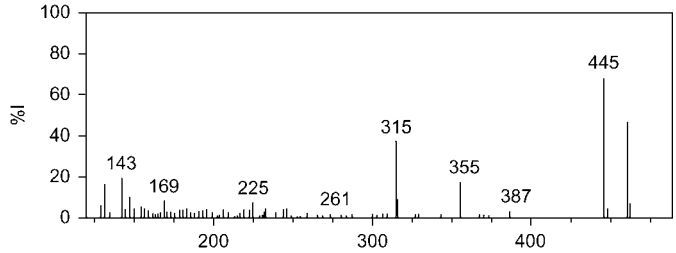
Calusterone

Antineoplastic, Anabolic Steroid
$C_{21}H_{32}O_2 = 316.5$
CAS—17021-26-0

IUPAC Name (7*S*,8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-7,10,13,17-tetramethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-3-one
Synonyms 7β,17α-Dimethyltestosterone; (7β,17β)-17-hydroxy-7,17-dimethyl-androst-4-en-3-one; U-22550.
Proprietary Name *Methosarb*



Chemical Properties Crystals. Mp 127° to 129°. Log *P* (octanol/water), 4.14 [Meylan, Howard 1995].
Mass Spectrum Principal ions at *m/z* 445, 460, 315, 143, 355, 169, 225 (MSTFA/TMSI derivative) Zhang *et al.* [1993].



Disposition in the Body Metabolised to dihydrocalusterone by 5-reductase and undergoes further hydrogenation through 3-hydroxysteroid-dehydrogenase to tetrahydrocalusterone. Both dihydrocalusterone and tetrahydrocalusterone form hydroxy analogs via hydroxidase.
Therapeutic Concentration

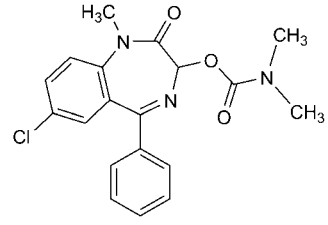
Forty-five postmenopausal women with advanced metastatic breast cancer were given 200 mg calusterone daily. Of the 40 evaluable patients, 11 were unable to tolerate its toxicity. Disease regresson was seen in 4 patients for an average of 15.2 weeks. Lesions continued to grow in 12 patients. Thirty three patients experienced side effects, including nausea, vomiting, fluid retention, and androgenic side effects, and 11 discontinued the drug. Plasma concentrations of calusterone or its metabolites were not reported [Aslam, Maxwell 1977].

Aslam J, Maxwell I (1977). Calusterone therapy for advanced breast cancer. *Cancer Treat Rep* 61: 371–373.
Meylan W, Howard M, P.H (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Zhang YZ *et al.* (1993). Studies on urinary metabolites of calusterone in man. *Yao Xue Xue Bao* 28: 918–923.

Camazepam

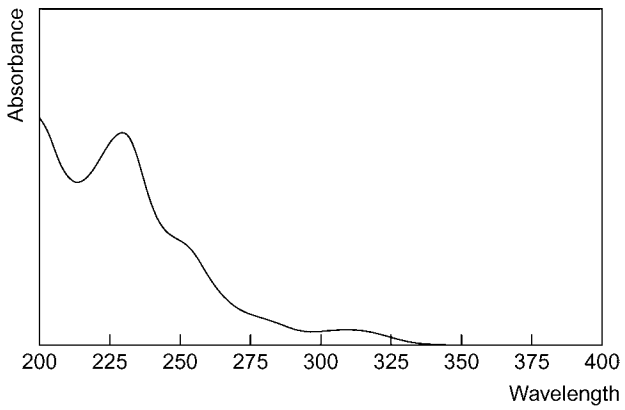
Benzodiazepine, Tranquilliser
$C_{19}H_{18}ClN_3O_3 = 371.8$
CAS—36104-80-0

IUPAC Name (7-Chloro-1-methyl-2-oxo-5-phenyl-3*H*-1,4-benzodiazepin-3-yl) *N*,*N*-dimethylcarbamate
Synonyms 7-Chloro-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl dimethylcarbamate; 3-*N*,*N*-dimethylcarbamoyloxy-7-chloro-5-phenyl-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one; SB 5833.
Proprietary Names *Albego*; *Limpidon*.

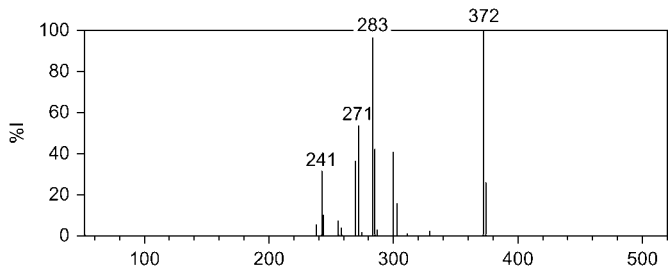


Chemical Properties White crystalline powder. Mp 173° to 174°. Moderately soluble in water; soluble in ethanol. Log *P* (octanol) 2.7. Stock solutions prepared in acetone were stable for 6 months at 4° [Cuisinaud *et al.* 1985]. In extracts, camazepam was found to be stable for >24 h at room temperature [Kratzsch *et al.* 2004].

Thin-layer Chromatography System TA—R_f 0.76; system TAD—R_f 0.69; system TAE—R_f 0.82; system TAF—R_f 0.83; system TAG—R_f 0.65; system TB—R_f 0.12; system TC—R_f 0.73; system TD—R_f 0.55; system TE—R_f 0.75; system TF—R_f 0.32.
Gas Chromatography System GA—RI 2945; system GB—RI 3162.
High Performance Liquid Chromatography System HY—RI 506; system HZ—RT 9.6 min.
Ultraviolet Spectrum Methanol—231 ($A^1_1 = 937b$), 315 nm ($A^1_1 = 67b$).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1718, 1193, 1318, 1110, 703 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 58, 72, 43, 78, 271, 57, 44, 77.



Quantification

Plasma **GC-MS** Column: 3% OV 17 dimethylchlorosilane GasChrom Q (100/120 mesh; 1 m × 3 mm i.d.). Carrier gas: N_2, 50 mL/min. ECD. Limit of detection, 1 µg/L [Riva *et al.* 1982]. Column: 3% OV-17 on Chromosorb W HP 80/100 mesh (1 m × 3 mm i.d.). Carrier gas: Ar: CH_4 (9 : 1), 80 mL/min. Temperature: 260°. ECD at 70 eV. Limit of detection, 10 µg/L [Cuisinaud *et al.* 1979].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.05 mg/L (SIM), limit of detection, 0.01 mg/L (scan) [Kratzsch *et al.* 2004].

Urine **LC-MS** Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Other **GC** Rat and Mouse Blood and Brain. Column: 3% OV-17 on 100/120 mesh GasChrom Q (1 m × 3 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 275°. ECD. Limit of detection, 15 µg/L of blood or 15 µg/kg brain [Marcucci *et al.* 1978].

HPLC Rat Plasma and Brain. Column: Nucleosil 7C 18 (250 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : water (10 : 1 : 7), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Morino, Sugiyama 1985].

Note For a comparison of achiral and chiral analysis of camazepam by supercritical fluid chromatography, see Wang *et al.* [1995].

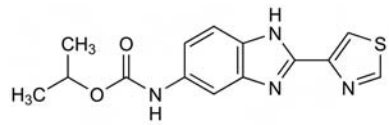
Disposition in the Body Metabolises to oxazepam and temazepam.
Toxicity LD_{50} (mice) is 970 and 800 mg/kg oral and IP, respectively [Deberdt 1975].
Protein Binding ≈91% [Morino *et al.* 1985].
Dose 20 to 60 mg daily.

Cuisinaud G *et al.* (1979). Simple and sensitive gas chromatographic method for the determination of camazepam in human plasma. *J Chromatogr* 178: 314–319.
Cuisinaud G *et al.* (1985). High-performance liquid chromatographic determination of cicletanide, a new diuretic, in plasma, red blood cells, urine and saliva. *J Chromatogr* 341: 97–104.
Deberdt R (1975). Camazepam versus diazepam: a double-blind trial on psychoneurotic patients. *Curr Ther Res Clin Exp* 17: 32–39.
Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.
Marcucci F *et al.* (1978). Distribution of camazepam in rats and mice. *J Pharm Sci* 67: 1470–1471.
Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography-mass spectrometry. *J Chromatogr* 422: 85–101.
Morino A, Sugiyama M (1985). Relation between time courses of pharmacological effects and of plasma levels of camazepam and its active metabolites in rats. *J Pharmacobiodyn* 8: 597–606.
Morino A *et al.* (1985). Species differences in the disposition and metabolism of camazepam. *Xenobiotica* 15: 1033–1043.
Riva R *et al.* (1982). Quantitative determination of camazepam and its metabolite temazepam in man by gas-liquid chromatography with electron-capture detection. *Farmaco [Prat]* 37: 15–19.
Wang MZ *et al.* (1995). Achiral and chiral analysis of camazepam and metabolites by packed-column supercritical fluid chromatography. *J Chromatogr B Biomed Appl* 665: 139–146.

Cambendazole

Anthelmintic
$C_{14}H_{14}N_4O_2S = 302.4$
CAS—26097-80-3

IUPAC Name Propan-2-yl N-[2-(1,3-thiazol-4-yl)-3H-benzimidazol-5-yl] carbamate
Synonym MK-905; [2-(4-thiazolyl)-1*H*-benzimidazol-5-yl]carbamic acid 1-methylethyl ester.
Proprietary Names *Bovicam (vet.)*. It is an ingredient of *Porcam (vet.)*.

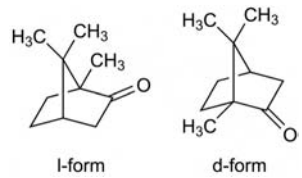


Chemical Properties A white crystalline powder. Mp 238° to 240°, with decomposition. Practically insoluble in water; soluble in ethanol and dimethylformamide; sparingly soluble in acetone; slightly soluble in benzene; very slightly soluble in 0.1 M HCl. Log *P* (octanol/water), 2.7.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1=620b), 319 nm (A^1_1=689b); aqueous alkali—237 (A^1_1=936b), 314 nm (A^1_1=649b).
Mass Spectrum Principal ions at *m/z* 260, 302, 216, 215, 243, 189, 242, 188.

Camphor

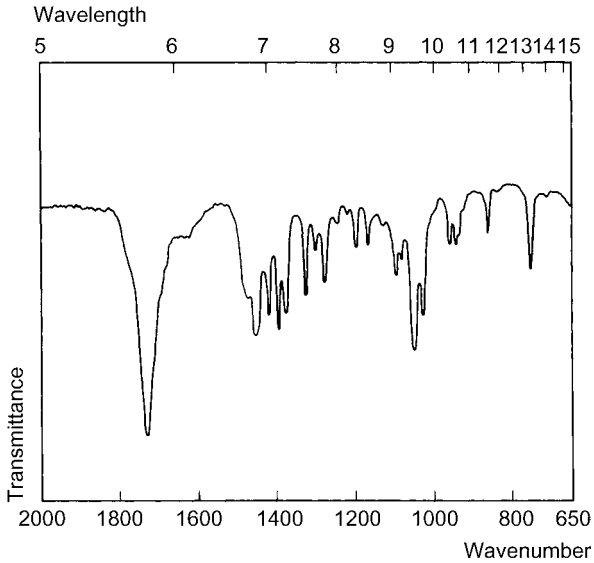
Rubefacient
$C_{10}H_{16}O = 152.2$
CAS—76-22-2; 464-49-3 (+)

IUPAC Name 1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
Synonyms Alcanfor; 2-camphanone; camphre droit (natural); camphre du Japon (natural); cânfora.
Proprietary Name It is an ingredient of *Pernomol*.



l-form d-form

Chemical Properties Camphor is obtained by distillation from the wood of *Cinnamomum camphora* (Lauraceae) and purified by sublimation, or it may be prepared synthetically. Natural camphor is dextrorotatory; the synthetic product is optically inactive. Colourless transparent or white crystals, crystalline masses, blocks, or powdery masses known as 'flowers of camphor'. Mp 179°. Soluble 1 in 700 to 1 in 800 of water, 1 in 1 of ethanol, 1 in 0.5 of chloroform, and 1 in 1 of ether. Log *P* (octanol/water), 2.4.
Thin-layer Chromatography System TAF—R_f 0.93; system TAJ—R_f 0.98; system TAK—R_f 0.95; system TAL—R_f 0.98.
Gas Chromatography System GA—RI 1143; system GI—retention time 38.2 min.
Ultraviolet Spectrum Methanol—289 nm (A^1_1=about 2b).
Infrared Spectrum Principal peaks at wavenumbers 1730, 1047, 1025, 1277, 1095, 755 cm^{-1} (KCl disk).



Mass Spectrum Principal ions at *m/z* 95, 81, 41, 69, 55, 83, 67, 137.

Quantification

Plasma **GC** FID. Limit of quantification, 5 mg/L, limit of detection, 1 mg/L [Valdez *et al.* 1999].

Disposition in the Body Absorbed after ingestion through the mucous membranes and through the skin. Metabolised by hydroxylation and excreted in the urine mainly as the glucuronides of 2-hydroxy- and 3-hydroxycamphor. Oxidation also occurs at the 7-position yielding a small amount of a carboxylic acid.

Toxicity Poisoning by camphor has usually been due to ingestion of camphorated oil. The fatal dose in a one-year-old child is stated to be about 1 g of camphor, although some children have survived the ingestion of 5 g. In adults, doses in the region of 2 g are likely to produce toxic symptoms and a dose of 4 g or more may be lethal, although one man survived after ingestion of as much as 30 g in the form of 150 mL of camphorated oil. The maximum permissible atmospheric concentration is 2 ppm.

A plasma concentration of 1.7 mg/L was reported in a severely intoxicated subject, 12 h after the ingestion of about 18 g of camphor; the patient recovered after treatment by resin haemoperfusion [Kopelman *et al.* 1979].

A serum concentration of 19.5 mg/L was observed 7 h after the ingestion of 0.7 g of camphor in a moderately intoxicated 3-year-old child [Phelan 1976].

Dose Camphor was formerly given orally in doses of 120 to 300 mg.

Kelly RC *et al.* (1979). *J Anal Toxicol* 3: 76–77.

Kopelman R *et al.* (1979). Camphor intoxication treated by resin hemoperfusion. *JAMA* 241: 727–728.

Phelan WJ (1976). Camphor poisoning: over-the-counter dangers. *Pediatrics* 57: 428–431.

Valdez JS *et al.* (1999). Sensitive and selective gas chromatographic methods for the quantitation of camphor, menthol and methyl salicylate from human plasma. *J Chromatogr B Biomed Sci Appl* 729(1–2): 163–171.

Candesartan

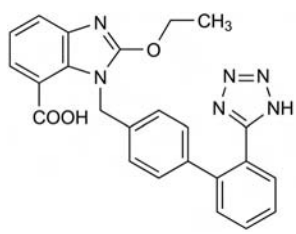
Angiotensin II Receptor Antagonist

$C_{24}H_{20}N_6O_3 = 440.5$

CAS—139481-59-7

IUPAC Name 2-Ethoxy-3-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl] benzimidazole- 4-carboxylic acid

Synonym CV-11974; 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl] methyl]-1*H*-benzimidazole-7-carboxylic acid.



Chemical Properties Colourless crystals. Mp 183° to 185°.

Candesartan Cilexetil

$C_{33}H_{34}N_6O_6 = 610.7$

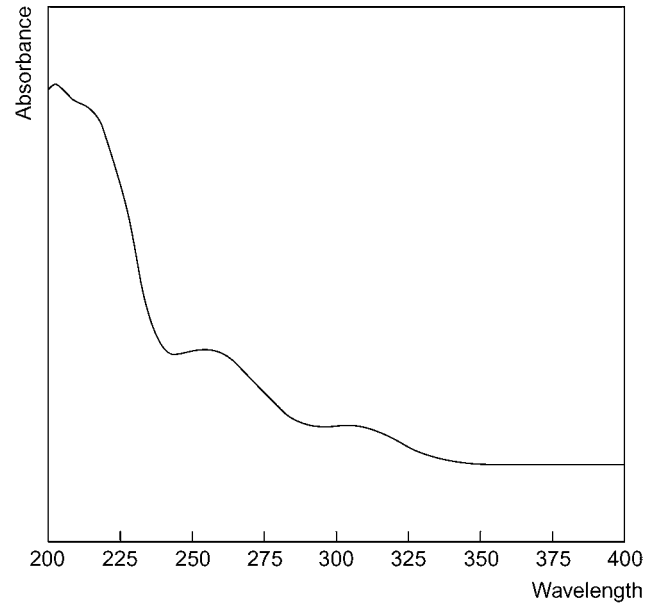
CAS—145040-37-5

Synonym TCV-116

Proprietary Names *Amias*; *Atacand*.

Chemical Properties A white to off-white powder. Mp 163°. It is practically insoluble in water; sparingly soluble in methanol.

Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—246, 281 nm; (ethanol)—253 nm; aqueous base (0.5 mol/L NaOH)—251 nm.



Quantification

Serum **HPLC** Limit of detection, 0.8 µg/L [Delacrétaz *et al.* 1995]. UV detection. Limit of detection, 2 µg/L for candesartan and candesartan cilexetil [Lee *et al.* 1995].

Urine **HPLC** UV detection. Limit of detection, 10 µg/L for candesartan cilexetil [Lee *et al.* 1995].

Disposition in the Body Candesartan cilexetil is absorbed after oral administration with an absolute bioavailability for candesartan of about 40% following administration of a solution or 14% following administration of tablets. Candesartan is the active metabolite. Peak plasma concentrations occur after about 3 to 4 h. It is excreted in urine and bile mainly as unchanged drug and a small amount of inactive metabolites. Hydrolysed during absorption to form candesartan. Not removed by haemodialysis.

Therapeutic Concentration

Twenty-seven male, healthy volunteers, aged 20 to 36 years (mean age, 26 years), were administered with doses of 1, 2, 4 or 8 g candesartan cilexetil on 8 consecutive days, after an overnight fast. Plasma concentrations of the parent drug were not detected but the active metabolite was detected at peak concentrations between 3.5 and 6.0 h. The mean concentrations on day 1 were 5.5, 12.7, 34.3 and 47.7 µg/L for the 1, 2, 4 and 8 mg doses, respectively. On day 8, the concentrations were 8.8, 17.6, 42.8 and 48.2 µg/L, respectively [Delacrétaz *et al.* 1995].

Half-life Plasma half-life, 9 h.

Protein Binding >99%.

Dose Usually 8 mg of candesartan cilexetil daily, with a maximum dose of 16 mg.

Delacrétaz E *et al.* (1995). Characterization of the angiotensin II receptor antagonist TCV-116 in healthy volunteers. *Hypertension* 25: 14–21.

Lee JW *et al.* (1995). Development and validation of column-switching high-performance liquid chromatographic methods for the determination of a potent AII receptor antagonist, TCV-116, and its metabolites in human serum and urine. *J Chromatogr* 670: 287–298.

Cannabis

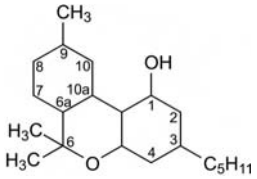
Psychomimetic

CAS—8063-14-7

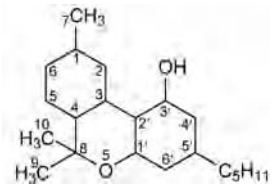
Synonyms Bhang; cannabis indica; chanvre; charas; dagga; ganja; guaza; hashish; hanfkraut; Indian Hemp; kif; maconha; marijuana; marihuana.

Note Many other synonyms, approximate synonyms and street names for cannabis and cannabis resin have been used [Sweetman 2007]. Marijuana usually refers to a mixture of the leaves and flowering tops. Bhang, dagga, ganja, kif and maconha are commonly used in various countries to describe similar preparations. Hashish and charas are names often applied to the resin, although in some countries hashish is applied to any cannabis preparation.

Dibenzofuran numbering



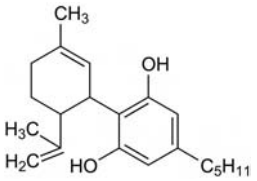
Monoterpenoid numbering



The most important cannabinoids are cannabidiol (CBD), cannabinol (CBN), (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC [Δ^1-THC]), (−)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC [Δ^{1-6}-THC]) and Δ^9-tetrahydrocannabinolic acid.

Cannabidiol

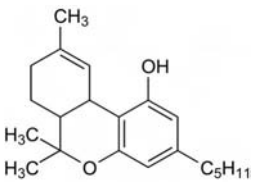
$C_{21}H_{30}O_2 = 314.5$
CAS—13956-29-1
IUPAC Name 2-[3-Methyl-6-(-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol
Synonym CBN



Chemical Properties Pale yellow resin or crystal. Mp 66° to 67°. Practically insoluble in water or 10% sodium hydroxide; readily soluble in ethanol, methanol, ether, benzene, chloroform and light petroleum.

Δ^9-Tetrahydrocannabinol

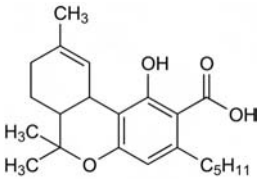
$C_{21}H_{30}O_2 = 314.5$
CAS—1972-08-3
Synonyms (−)-*trans*-Δ^9-Tetrahydrocannabinol; Δ^1-THC; Δ^9-THC.



Chemical Properties A viscous oil. Practically insoluble in water; soluble 1 in 1 of ethanol and 1 in 1 of acetone; readily soluble in chloroform and light petroleum.

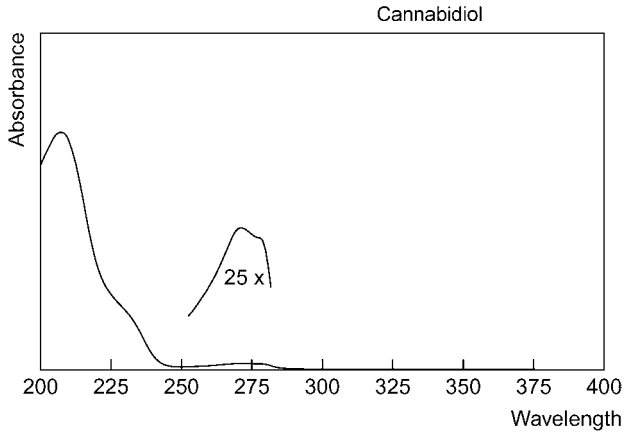
Δ^9-Tetrahydrocannabinolic Acid

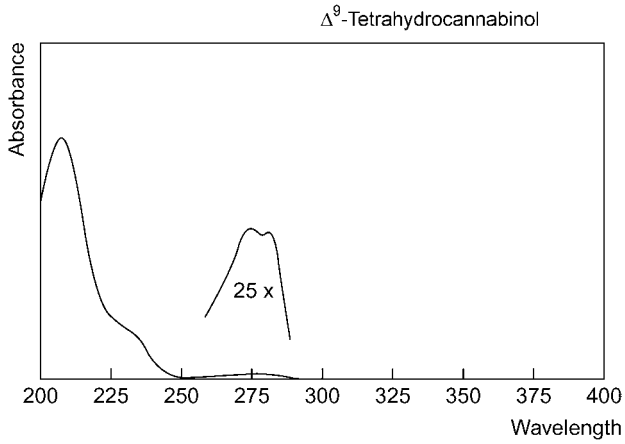
$C_{22}H_{30}O_4 = 358.5$
CAS—23978-85-0



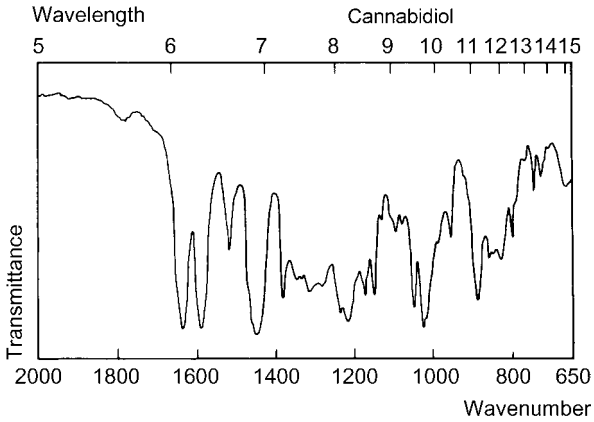
The amount of the major constituents in various samples of *Cannabis* spp. varies between ~5% and zero, depending on climatic and genetic factors. Almost all the psychomimetic activity of the plant is associated with the Δ^9-THC content, which is usually 20 times as great as that of Δ^8-THC. The Δ^9-THC content may average 1, 3 and 5% in marihuana, ganja and hashish, respectively. However, newer plant strains ('skunk weed' and derivatives) can contain much larger Δ^9-THC percentages. The potency of confiscated marijuana samples rose from <1.5% Δ^9-THC in 1980 to 4.2% Δ^9-THC in 1997. Δ^9-Tetrahydrocannabinolic acid, which is present in abundance in some cannabis samples, is itself inactive but it is converted by smoking into active Δ^9-THC. Cannabinol and CBD may be present in large amounts but have little activity.
Note Δ^9-THC binds readily to glass and plastic, reducing recoveries during analytical procedures. Δ^9-THC adsorption can be minimised with storage of solutions in amber silylated glassware and by maintenance of the compound in a basic solution or organic solvent.

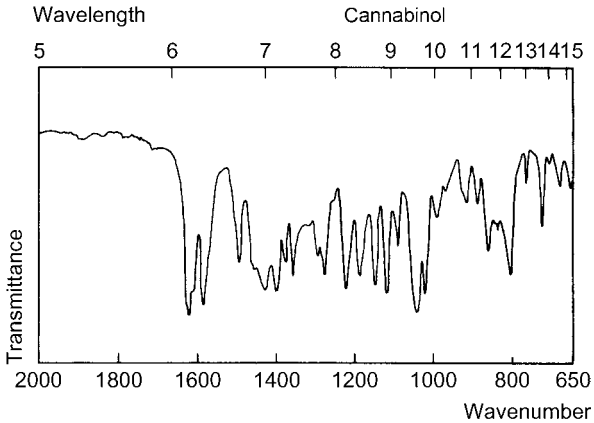
Colour Tests Marquis test—slow/weak 'salmon orange' (pink, orange red); Mecke's reagent—dark orange→dark purple-ish orange; sodium nitroprusside—cherry red colour [Zimmerman 2001].
Thin-layer Chromatography System TA—Δ^9-THC R_f 0.11, CBN R_f 0.94, CBD R_f 0.94; system TE—Δ^9-THC R_f 0.31, CBN R_f 0.95, CBD R_f 0.95; system TAJ—Δ^9-THC R_f 0.00, CBN R_f 0.90, CBD R_f 0.88; system TAK—Δ^9-THC R_f 0.01, CBN R_f 0.77, CBD R_f 0.76; system TAL—Δ^9-THC R_f 0.31, CBN R_f 0.97, CBD R_f 0.97; system TI—Δ^9-THC R_f 0.30, CBN R_f 0.52, CBD R_f 0.05; system TJ—Δ^9-THC R_f 0.29, CBN R_f 0.20, CBD R_f 0.36; system TAH—Δ^9-THC R_f 0.50, CBN R_f 0.45, CBDR_f 0.60 (fast blue B solution—Δ^9-THC red colour, CBD orange colour and CBN purple colour; Duquenois reagent—cannabinoids give blue to violet colours after spraying reagent and overspray with hydrochloric acid).
Gas Chromatography System GA—Δ^9-THC RI 2473, CBD RI 2390, CBN RI 2535, cannabigerol RI 2500, M (11-hydroxy-Δ^9-THC) RI 2775; system GB—Δ^9-THC RI 2578, CBNRI 2644, CBD RI 2480, M (11-hydroxy-Δ^9-THC) RI 2975; system GH—Δ^9-THC RI 2350, CBD RI 2270, CBN RI 2430, cannabicyclol RI 2280, cannabigerol RI 2440, propyl-CBDRI 2110, propyl-Δ^9-THC RI 2170, M (8α,11-dihydroxy-Δ^9-THC) RI 2710, M (8α-hydroxy-Δ^9-THC) RI 2580, M (11-hydroxy-Δ^9-THC) RI 2620, M (11-nor-Δ^9-THC-^{9}-carboxylic acid) RI 2756; system GAK—CBN RT 13.9 min, CBDRT 13.3 min.
High Performance Liquid Chromatography System HL—Δ^9-THC *k* 13.35, Δ^8-THC *k* 14.07, CBN *k* 11.77, CBD*k* 7.47, cannabicyclol *k* 14.78, cannabichromene *k* 19.09, cannabidiolic acid *k* 8.76, cannabigerol *k* 8.18, cannabivarin*k* 7.47, tetrahydrocannabinolic acid *k* 25.83, tetrahydrocannabivaric acid *k* 14.64, tetrahydrocannabivarin *k* 8.18; system HX—CBD RI 990, CBD RI 1080; system HY—CBD RI 902, CBN RI 1028, M(9-carboxy-11-nor-9-tetrahydrocannabinol) RI 680.
Ultraviolet Spectrum Ethanol—CBD278, CBD 285, Δ^9-THC 278, Δ^9-THC acid 278 and 283 nm.

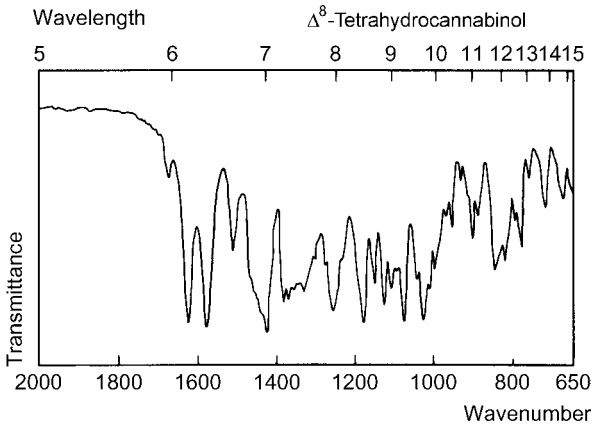


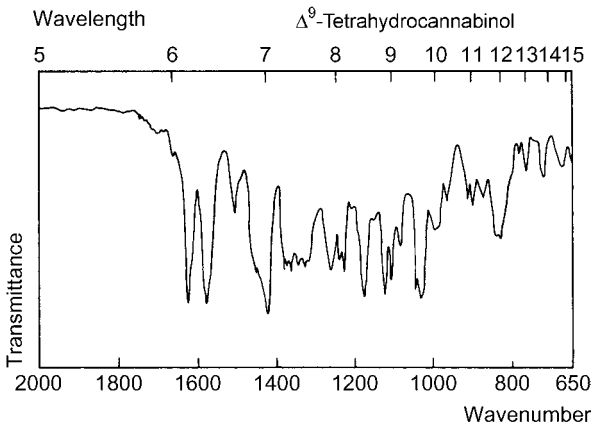


Infrared Spectrum Main components have principal peaks at the following wavenumbers (all thin films): 1585, 1630, 1020, 1210, 1240, 1050 cm^{-1} (CBD); 1620, 1050, 1580, 1030, 1120, 1228 cm^{-1} (CBN); 1580, 1030, 1620, 1180, 1080, 1260 cm^{-1} (Δ^8-THC); 1580, 1040, 1620, 1180, 1130, 1050 cm^{-1} (Δ^9-THC).

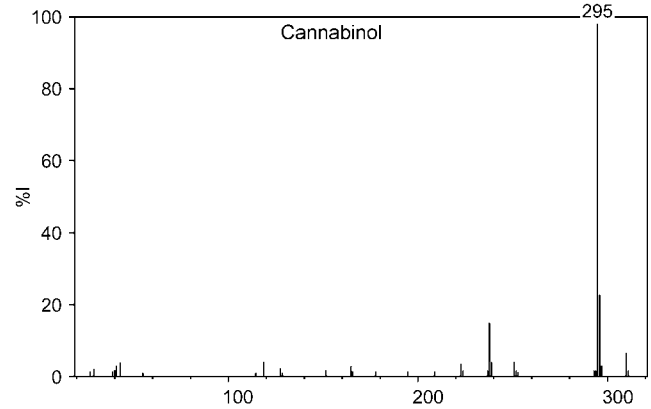








Mass Spectrum Main components and metabolites have principal peaks at the following *m/z*: 231, 246, 314, 232, 121, 193, 74, 174 (CBD); 295, 296, 238, 310, 119, 43, 251, 239 (CBN); 221, 314, 248, 261, 193, 236, 222, 315 (Δ^8-THC); 299, 231, 314, 43, 41, 295, 55, 271 (Δ^9-THC); 271, 43, 41, 311, 295, 312, 297, 91 (8α-hydroxy-Δ^9-THC); 271, 43, 295, 41, 297, 29, 330, 272 (8β-hydroxy-Δ^9-THC); 299, 43, 41, 67, 300, 69, 231, 29 (11-hydroxy-Δ^9-THC); 41, 229, 43, 329, 69, 344, 29, 283 (Δ^9-THC-11-oic acid).



Quantification

Blood **GC** Column: 6% OV-1 or Hi-Eff 9BP. TID. Limit of detection, 0.2 μg/L [McCallum, Shaw 1981].

GC-MS Limit of detection, 1 μg/L [Kintz, Cirimele 1997]. Column: 2% OV-17 Chromosorb W (HP). Temperature programme: 190° to 275° at 10°/min. Limit of detection, 1 μg/L [Bergman *et al.* 1981].

Plasma **GC-MS** Column: ZB-50 (30 m × 0.25 mm i.d., 0.25 μm) and DB-1MS (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 185° for 0.5 min to 225° at 45°/min for 3 min to 275° at 15°/min for 1.58 min to 195° at 80°/min for 2.3 min to 230° at 10°/min to 275° at 25°/min. FID, SIM acquisition mode. Limit of quantification, 0.25 μg/L for CBD, THC, and THC-COOH, 0.125 μg/L for H-OH-THC, limit of detection, 0.25 μg/L for CBD and THC, 0.125 μg/L for H-OH-THC and THC-COOH [Karschner *et al.* 2010]. See Brenneisen *et al.* [2010]. Column: HP 5 MS 95% dimethylsiloxane 5% diphenylsiloxane (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 130° at 2 min to 300° at 20° for 5 min. Limit of quantification, 0.80, 0.51, 0.88, 0.95 and 3.9 μg/L for THC, 11-THC-OH, THC-COOH, CBD and CBN, respectively, limit of detection, 0.24, 0.15, 0.26, 0.29 and 1.1 μg/L for THC, 11-THC-OH, THC-COOH, CBD and CBN, respectively [Nadulski *et al.* 2005]. Limit of quantification, 0.5 μg/L [Huang *et al.* 2001].

HPLC Column: C_8 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 50 mmol/L phosphate buffer (pH 2.7) : acetonitrile (35 : 65), flow rate 0.3 mL/min to 2.5 mL/min at 8 min for 4 min to 0.3 mL/min between 12 and 14 min. UV detection (λ = 220 nm). Limit of quantification, 2 μg/L, limit of detection, 0.8 μg/L [Mercolini *et al.* 2008]. Column: Synergi MAX-RP 80A C_{12} (75 × 2 mm i.d., 4 μm). Mobile phase: 10 mmol/L ammonium formate (pH 3.0) : acetonitrile–10 mmol/L ammonium formate (pH 3.0, 90 : 10; 50 : 50 to 21 : 79 at 12 min to 5 : 95 at 12.5 min for 2.5 min to 50 : 50 at 15.01 min until 25 min), flow rate 400 μL/min. APCI, SIM acquisition mode. Limit of quantification, 0.2 μg/L, limit of detection, 0.1 μg/L [Grauwiler *et al.* 2007]. Electrochemical detection. Limit of detection, 0.5 μg/L [Nakahara *et al.* 1989]. Limit of detection, 6.5 μg/L [Law *et al.* 1984a].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 1 mmol/L ammonium formate : methanol (10 : 90), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 μg/L [Laloup *et al.* 2006].

Serum **HPLC** Limit of detection, 3.3 μg/L [Law *et al.* 1984a].

Urine **GC-MS** See Kintz *et al.* [1995].

HPLC See Plasma [Grauwiler *et al.* 2007; Mercolini *et al.* 2008]. Column: reversed phase C_8-selent B (150 × 2 mm i.d., 5 μm). Mobile phase: 0.1% formic acid-1 mmol/L ammonium formate : acetonitrile-0.1% formic acid (90 : 10 to 70 : 30 to 30 : 30 to 10 : 90 for 4 min). TIS. Retention time, 26.8 min. Limit of detection, <3–10 μg/L [Weinmann *et al.* 2000]. Column: C_8 RP. Mobile phase: acetonitrile : acid phosphate buffer (55 : 45). UV detection. Limit of quantification, 10 μg/L [Bianchi, Donzelli 1996].

Oral Fluid **HPLC** Column: Lichrospher RP-Select B C_8 (125 × 4 mm i.d., 5 μm). Mobile phase: methanol-0.15 mol/L aqueous NaCl solution (20 : 80) : methanol-0.15 mol/L aqueous NaCl solution (82 : 18, 100 : 0 for 5 min to 0 : 100 at 5.01 min until 25 min to 100 : 0 at 25.1 min until 35 min), flow rate 0.8 mL/min. UV detection (λ = 220 nm). Limit of detection, 2, 1, and 8 mg/L for Δ^9-THC, CBD and CBN, respectively [Kircher, Parlar 1996].

GC-MS Column: ZB-50 (30 m × 0.25 mm i.d., 0.25 μm) followed by DB-1MS (15 m × 0.25 mm i.d., 0.25 μm). EI ionisation, SIM acquisition mode. Limit of quantification, 0.5 μg/L for THC, 11-OH-THC and CBD; 1 μg/L for CBN; 7.5 ng/L for THC-COOH (NCI) [Milman *et al.* 2010]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 290° at 10°/min for 10 min. SIM acquisition mode. Limit of quantification, 2 μg/L for THC-COOH and THC, limit of detection, 1 μg/L for THC-COOH and THC [Choi *et al.* 2009].

LC-MS See Plasma. Limit of quantification, 1.2 μg/L [Laloup *et al.* 2006]. Column: XTerra MS C_{18} (50 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.05% ammonia (70 : 30), flow rate 0.3 mL/min. SIR acquisition mode, positive ion mode. Limit of quantification, 2 μg/L, limit of detection, 1 μg/L [Teixeira *et al.* 2004].

Brain **GC** See Blood [McCallum, Shaw 1981].

Hair **GC-MS** Column: cross-linked 5% phenylmethysiloxane (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: 1.0 mL/min. Temperature programme: 150° for 1 min to 270 at 20° at 5 min. EI ionisation. HP Ultra 2 cross-linked 5% phenylmethylsiloxane (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 2 min to 230° at 20 min to 270° at 10°/min for 1 min. SIM acquisition mode. Limit of quantification, 10 ng/g for Δ^9-THC-COOH (GC-MS-NCI); 0.1 μg/mg for Δ^9-THC, CBD and CBN; and 1.00 μg/g for Δ^9-THC-COOH (GC-MS-EI) [Baptista *et al.* 2002]. Column: HP-5 cross-linked 5% phenyl methyl silicone (12 m × 0.32 mm i.d., 0.33 μm). Temperature programme: 100° for 2 min to 200° at 50°/min to 270° at 15°/min. SIM acquisition mode. Limit of detection, 0.1 μg/L for CBN and HBC, 0.2 ng/kg for CBD [Strano-Rossi, Chiarotti 1999].

HPLC Limit of quantification, 1.1 ng/g. Limit of detection, 0.3 ng/g [Sachs, Dressler 2000].

Nail Clippings **GC-MS** Column: HP-1 cross-linked dimethyl dimethyl silicone (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Column: 150° to 300° at 10°/min for 10 min. EI ionisation, SIM acquisition mode. Limit of detection, <0.1 ng/mg [Lemos *et al.* 1999].

Note For a study of the detection of cannabis in oral fluid and forehead wipes from impaired drivers, see Kintz *et al.* [2000]. For a review of radioimmunoassay for the quantification of cannabinoid metabolites, see Law *et al.* [1984a].

Disposition in the Body Δ^9-THC is absorbed from the gastrointestinal tract but absorption is slow and irregular. However, Δ^9-THC can be measured in plasma within seconds after inhalation of the first puff of marijuana smoke. Δ^9-THC is lipophilic and is widely distributed in the body. It is oxidised to the active metabolites 11-hydroxy-Δ^9-THC and 8β-hydroxy-Δ^9-THC by an hepatic cytochrome P450 enzyme. The inactive substances 8α-hydroxy-Δ^9-THC and 8α,11-dihydroxy-Δ^9-THC are also formed. 11-Hydroxy-Δ^9-THC is further oxidised to 11-nor-Δ^9-THC-9-carboxylic acid (Δ^9-THC-11-oic acid), and many other polar mono- and dicarboxylic acids are formed; these are conjugated with glucuronic acid to a variable extent. Enterohepatic circulation of metabolites may occur. Up to ~25% of a dose is excreted in the urine in 3 days, mainly as 11-nor-Δ^9-THC-9-carboxylic acid glucuronide, together with the other carboxylic acids in free and conjugated form. Δ^9-THC-*O*-glucuronide has also been detected in urine. The major excretion route is via the faeces, up to around 65% of a dose being excreted in 5 days, mainly as 11-hydroxy-Δ^9-THC and the carboxylic acids in conjugated form. Δ^9-THC metabolites have been detected in urine for up to 12 days following a single oral dose. It crosses the placenta and is distributed into breast milk.

Therapeutic Concentration

Six healthy males with histories of marijuana use smoked a single marijuana cigarette (placebo or 1.75% or 3.55% Δ^9-THC). The smoking protocol consisted of a 2-s inhalation, a 10-s hold period and a 72-s exhalation and rest period. A total of eight puffs were inhaled in 11.2 min. Mean plasma levels of 7.0 and 18.1 μg/L Δ^9-THC, respectively, were observed after the first inhalation of the 1.75% and 3.55% Δ^9-THC cigarettes. Levels continued to increase rapidly to mean peak plasma concentrations of 79.0 and 152.0 μg/L for the 1.75% and 3.55% Δ^9-THC cigarettes, respectively, and were reached after 9 min (before initiation of the last puff sequence at 9.8 min) [Huestis *et al.* 1992a].

After 11 subjects had smoked 11.6 to 15.6 mg (mean, 13.0) of Δ^9-THC from a cigarette over a period of 5 to 7 min, peak plasma concentrations of 0.03 to 0.12 mg/L (mean, 0.08) of Δ^9-THC were observed within 3 min after termination of smoking; the concentration fell rapidly to 0.003–0.01 mg/L (mean, 0.007) at 1 h and to 0.0006–0.003 mg/L (mean, 0.0016) at 4 h, after smoking. When 20 mg Δ^9-THC was given orally to the same subjects, peak plasma concentrations of 0.004–0.01 mg/L Δ^9-THC were produced between 60 and 300 min after ingestion [Ohlsson *et al.* 1980].

Toxicity Cannabis or Δ^9-THC intoxication may result in loss of consciousness or even death, but reports of fatalities are rare.

In 6 deaths from cardiovascular effects folllowing cannabis use, the following Δ^9-THC levels were found in the blood: 22 μg/L, 4 μg/L, 2 μg/L, 5 μg/L, 3 μg/L and 7 μg/L; no other drugs were detected except for 400 mg/L ethanol in the 4th case [Bachs, Morland 2001].

In a fatal poisoning by Δ^9-THC, the following postmortem concentrations of Δ^9-THC in tissues were reported: liver 37.5 μg/g, kidney 42 μg/g and spleen 12 μg/g [Tewari, Sharma 1980].

Bioavailability After ingestion, ~6–20%. During smoking, ~18–50%.

Half-life Plasma half-life, Δ^9-THC ~20–36 h.

Volume of Distribution Δ^9-THC ~10 L/kg.

Distribution in Blood Plasma : whole blood ratio, Δ^9-THC 1.8.

Protein Binding Δ^9-THC and 11-hydroxy-Δ^9-THC: 94–99%.

Note For a general review of cannabis and its effects on human behaviour and performance, see Huestis [2002]; for a review of the therapeutic aspects of cannabis and cannabinoids, see Robson [2001]; for a review of Δ^9-THC metabolism and disposition, see Wall, Perez-Reyes [1981]. See also Law *et al.* [1984b] andWall *et al.* [1983]. For a review of the clinical pharmacokinetics of non-opiate abused drugs, see Busto *et al.* [1989]. For relating blood concentrations of tetrahydrocannabinol and metabolites to pharmacological effects and time of marijuana use, see Cone, Huestis [1993] and Huestis *et al.* [1992b]. For detection and persistence of cannabis DNA on skin, see Wilkinson, Linacre [2000] and for swabbing the hands for traces of marihuana, see Thibault *et al.* [1983]. For the determination of the distribution of cannabinoids in cannabis resin, see Baker *et al.* [1980]; for the Δ^9-THC and Δ^9-tetrahydrocannabinolic acid content of cannabis products, see Baker *et al.* [1981]. For the role of cannabis in motor vehicle crashes, see Bates, Blakely [1999].

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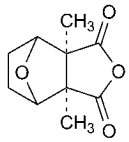
C

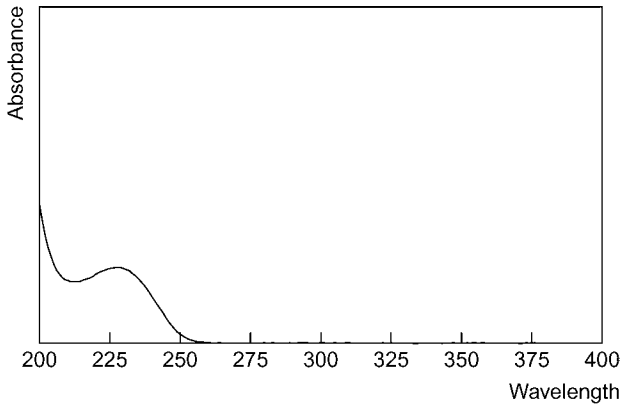
Cantharidin

Protein Phosphatase 1 and 2A Inhibitor, Vesicant
$C_{10}H_{12}O_4 = 196.2$
CAS—56-25-7

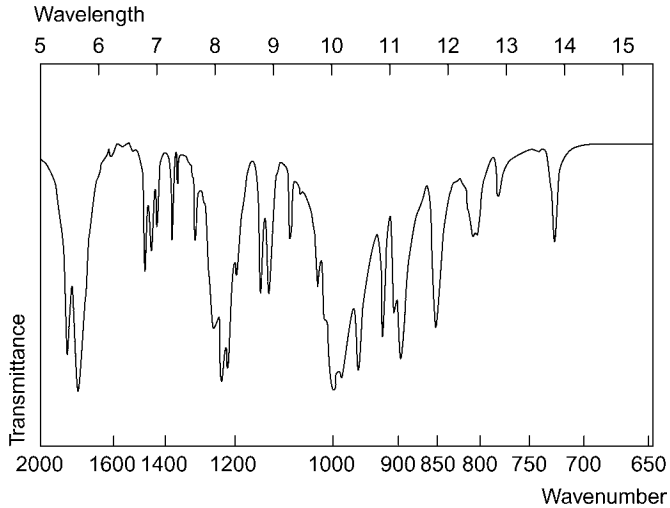
Synonyms Hexahydro-3a,7a-dimethyl-4,7-epoxyisobenzofuran-1,3-dione; Spanish fly.
Proprietary Name *Cantharone*



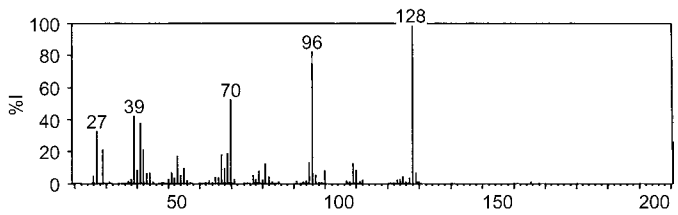
Chemical Properties Cantharidin is obtained from cantharides, the dried beetle *Cantharis vesicatoria* (*Lytta vesicatoria*) (Meloidae) or other spp., containing not less than 0.6%, or from mylabris, the dried beetles *Mylabris sidae* (*M. phalerata*), *M. cichorii* and *M. pustulata* (Meloidae), containing not less than 1%. Colourless, glistening crystals which sublime at ≈120°. Mp 218°. Insoluble in cold water, somewhat soluble in hot water; soluble 1 in about 1100 of ethanol, 1 in 40 of acetone, 1 in 65 of chloroform, 1 in 560 of ether and 1 in 150 of ethyl acetate. Soluble in oils. Log *P* (octanol/water) 1.2.
Gas Chromatography System GA—RI 1492.
Ultraviolet Spectrum Principal peak at 230 nm



Infrared Spectrum Principal peaks at wavenumbers 1242, 962, 900, 1786, 1852, 1002 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 128, 96, 70, 39, 41, 27, 42, 29.



Quantification

Blood **GC** Column: OV-1 fused silica (15 m × 0.53 mm i.d.). Carrier gas: He, 5.0 mL/min. Temperature: 150°. FID. Limit of detection, 0.24 mg/L [Cheng *et al.* 1990].

GC-MS HP Ultra fused silica capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 1.5 min to 290° at 20°/min for 3.5 min. EI ionisation. Limit of detection not reported [Polettini *et al.* 1992].

Serum **GC-MS** Column: CPSIL-8-CB 5% phenyl 95% methyl silicone (25 m × 0.22 mm i.d., 0.11 µm). Carrier gas: He, 40 cm/s. Temperature programme: 80° for 3 min to 280° at 12 /min for 10 min. EI ionisation at 70 eV. SIM acquisition mode. Retention time: 11 min. Limit of detection, 5 µg/L [Hundt *et al.* 1990; Steyn, Hundt 1988].

Urine **GC** See Blood. Limit of detection, 0.9 mg/L [Cheng *et al.* 1990].

GC-MS HP Ultra fused silica capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 1.5 min to 290° at 20°/min for 3.5 min. EI ionisation. Limit of detection not reported [Polettini *et al.* 1992].

Liver **GC** See Blood [Cheng *et al.* 1990].

Other **GC** Insect Tissue. Column: DB-5 fused silica capillary (27 m × 0.329 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 270° at 20°/min. FID. Limit of detection, 30 pg [Carrel *et al.* 1985]. Rat Liver. Column: 3% SE-30 silicon (8′). Temperature: 200°. Limit of detection, 0.12 µg [Bagatell *et al.* 1966].

GC-MS Horse Urine and Stomach. Column: 3% OV-101 on Chromosorb W-HP 100/120 mesh (2′ or 6′ × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 140° to 220° at 4°/min. Retention time: 1.9 and 5.8 min for the 2 and 6′ columns, respectively. Limit of detection, 10 ng [Ray *et al.* 1980].

HPLC Animal Liver, Kidney, and Blood. Column: µPorasil (30 cm × 4 mm i.d., 10 µm). Mobile phase: 0.5% acetonitrile in chloroform, flow rate 2 mL/min. UV detection (λ = 254 nm). Retention time: 6.6 min. Limit of detection, 0.2 µg [Ray *et al.* 1979].

Disposition in the Body Readily absorbed from the skin and mucous membranes. It does not appear to be metabolised.

Toxicity When taken orally it causes great pain as well as blistering and gross inflammation of the gastrointestinal tract, the kidneys and the bladder. The lethal dose is between 10 and 60 mg although a crystal weighing not more than 0.5 mg lodged in the mucosa may produce a blister that could be fatal [Till, Majmudar 1981].

A 27-year-old female died approximately 17 h after she had eaten coconut ice into which a small quantity of cantharidin had been introduced by a fellow employee. Postmortem findings revealed that between 65 and 130 mg of cantharidin was circulating in the organs. At the same time as the above incident, a 19-year-old girl also ate a piece of the cantharidin-impregnated coconut ice. She died 26 h later. The postmortem level of cantharidin in the organs was somewhat less than in the former case [Nickolls, Teare 1954].

A 43-year-old male ingested between 32 and 65 mg cantharidin when he spilt some on his thumb while fishing. He died 2 days later. Pathological symptoms were those of cantharidin, but none was detected at postmortem [Nickolls, Teare 1954].

Cantharidin was detected at a concentration of 0.11 mg/L in postmortem blood after ingestion of a crude extract of cantharides (over 200 dried beetles). The liver concentration was 1.24 mg/kg [Cheng *et al.* 1990].

In another case, cantharidin was detected at a concentration of 72.3 mg/L in postmortem serum after ingestion of cantharide powder containing 0.87% cantharidin [Hundt *et al.* 1990].

A 30-year-old male ingested ~26 to 45 mg cantharidin. He died 30 h post-ingestion. Blood and urine concentrations (mg/L) were as shown below.

Time (h)	*Blood*	*Urine*
6	–	2.35
10	0.29	1.12
20	0.18	0.59
30	0.15	0.38

[Polettini *et al.* 1992].

Note For an account of the findings from 4 cases of poisoning from cantharidin, see Karras *et al.* [1996] and for cases of poisoning after the ingestion of a beetle, see Mallari *et al.* [1996], Wertelecki *et al.* [1967] and Tagwireyi *et al.* [2000]. For a case of poisoning after the ingestion of liquor epispasticus (≈20 mg cantharidin), see Rosin [1967] and after the ingestion of wart remover (0.7% cantharidin), see Ewart *et al.* [1978]. For a review of cantharidin, see Moed *et al.* [2001].

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Capecitabine

Antineoplastic

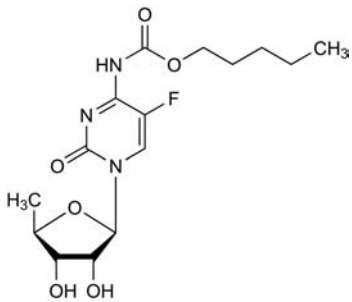
$C_{15}H_{22}FN_3O_6 = 359.4$

CAS—154361-50-9

IUPAC Name Pentyl *N*-[1-[(2*R*,3*R*,4*S*,5*R*)-3, 4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxopyrimidin-4-yl]carbamate

Synonym [1-(5-Deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]carbamic acid pentyl ester; Ro-09-1978/000

Proprietary Name *Xeloda*



Chemical Properties A white to off-white crystalline powder. Aqueous solubility approx. 26 g/L at 20°.

Disposition in the Body Capecitabine is extensively absorbed after oral administration and hydrolysed first to 5′-deoxy-5-fluorocytidine (5′-DFCR) by a carboxylesterase. 5′-DFCR is then converted to 5′-deoxy-5-fluorouridine (5′-DFUR) by cytadine deaminase to 5′-fluorouracil by thymidine phosphorylase to 5-fluoro-5,6-dihydrofluorouracil (FUH_2) to 5-fluoro-ureidopropionic acid (FUPA) and to α-fluoro-β-alanine (FBAL) by β-ureidopropionase. >95% of an administered dose is excreted in urine, with ~3% unchanged and 5% as FBAL, the major metabolite. 2.5% of the dose is recovered in faeces.

Therapeutic Concentration

A single oral dose of 892 to 2510 mg/m^2 was administered and taken within 30 min of the end of a meal. Mean peak plasma concentrations of the drug ranged from 2.4 to 3.9 mg/L and were reached within ~1.5 h [Roche Laboratories Inc. 1998].

Toxicity In patients treated with doses of up to 3514 mg/m^2 daily (i.e. cases of acute overdose), nausea, vomiting, diarrhoea and bleeding were observed.

Half-life 0.5 to 1 h.

Protein Binding <60% for the drug and metabolites.

Dose A usual dose of 2.5 g/m^2 body surface is administered daily.

Roche Laboratories Inc., (1998) Xeloda (Capecitabine) prescribing information, USA

Capreomycin

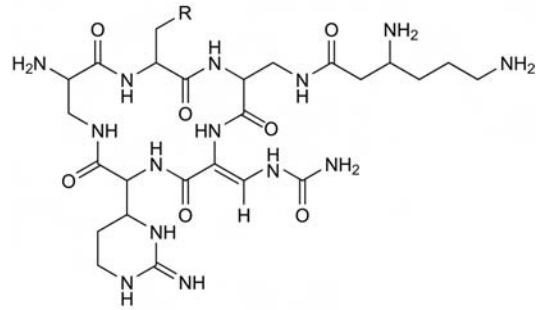
Antibacterial

$C_{25}H_{44}N_{14}O_8 = 668.7$

CAS—11003-38-6

IUPAC Name (3*S*)-3,6-Diamino-N-[[(2*S*,5*S*,8*Z*,11*S*,15*S*)-15-amino-11-[(6*S*)-2-amino-1,4,5,6-tetrahydropyrimidin-6-yl]-8-[(carbamoylamino)methylidene]-2-(hydroxymethyl)-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide

Synonyms Capromycin; caprolin; capostatin.



Capreomycin IA R = OH
Capreomycin IB R = H

Chemical Properties A mixture of the polypeptide antimicrobial substances produced by certain strains of *Streptomyces capreolus*. Capreomycin I consists of capreomycin IA ($C_{25}H_{44}N_{14}O_8 = 668.7$), Mp 246° to 248°; and capreomycin IB ($C_{25}H_{44}N_{14}O_7 = 652.7$), Mp 253° to 255°, which predominates. Capreomycin II that makes up about 10% of the mixture consists of capreomycin IIA and capreomycin IIB.

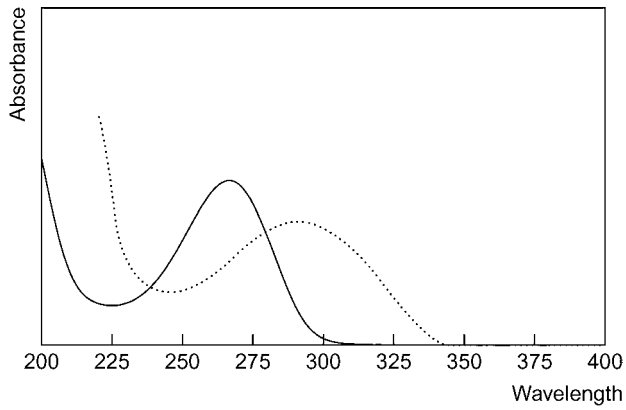
Capreomycin Sulfate

CAS—1405-37-4

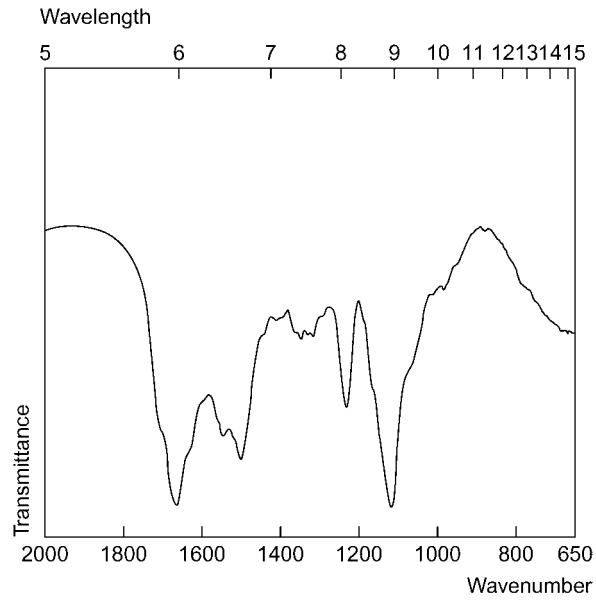
Proprietary Names *Capastat*; *Caprocin*; *Ogostal*.

Chemical Properties A white or almost white solid or amorphous powder. It is very soluble in water: practically insoluble in chloroform, ethanol, ether, and most other organic solvents.

Ultraviolet Spectrum (Capreomycin sulfate) Aqueous acid (0.2 mol/L H_2SO_4)—268 nm; basic—289 nm.



Infrared Spectrum Principal peaks at wavenumbers 1668, 1498, 1231, 1118 cm^{-1} (sulfate salt).



Quantification

Urine **Electrophoresis** UV detection. Limit of detection, 0.15 mg/L [Zhang *et al.* 1998].

Disposition in the Body Capreomycin is poorly absorbed after oral administration (<1%). It is not distributed into cerebrospinal fluid (CSF), but it does cross the

placenta. About 50% of a dose is excreted unchanged in urine by glomerular filtration within 12 h. Small amounts are excreted in bile. It is removed by haemodialysis.

Therapeutic Concentration A 1 g IM dose produces peak serum concentrations of about 30 mg/L (range 20 to 47 mg/L) after 1 to 2 h.

Toxicity

Nephrotoxicity, including acute tubular necrosis, and ototoxicity, including dizziness and vertigo, have been reported. Neuromuscular blockage or respiratory paralysis may also occur after rapid IV administration [Eli Lilly & Co.].

Half-life Plasma half-life, 4 to 6 h.

Clearance Plasma clearance, 57 mL/h/kg.

Dose The equivalent of 1 g of capreomycin daily is administered IM for 60 to 120 days, followed by 1 g two or three times a week. 20 mg/kg daily should not be exceeded. The dose should be reduced in patients with reduced renal function.

Data as supplied by Eli Lilly & Company Ltd.

Zhang SS *et al.* (1998). A reproducible, simple, and sensitive high-performance capillary electrophoresis method for simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine. *J Pharm Biomed Anal* 17: 617–622.

Captodiame

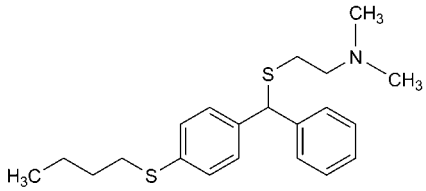
Tranquilliser

$C_{21}H_{29}NS_2$ = 359.6

CAS—486-17-9

IUPAC Name 2-[(4-Butylsulfanylphenyl)-phenylmethyl]sulfanyl-*N*,*N*-dimethylethanamine

Synonyms 2-[[[4-(Butylthio)phenyl]phenylmethyl]thio]-*N*,*N*-dimethylethanamine; captodiamine.



Captodiame Hydrochloride

$C_{21}H_{29}NS_2$, HCl = 396.1

CAS—904-04-1

Proprietary Name *Covatine*

Chemical Properties Crystals. Mp 131° to 132°.

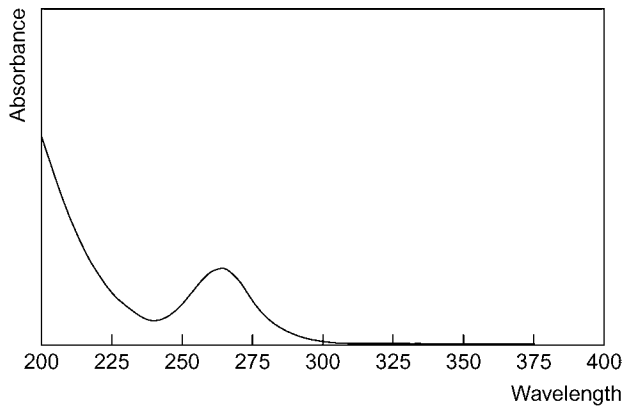
Colour Tests Mandelin's test—violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.66; system TAE—R_f 0.47; system TB—R_f 0.49; system TE—R_f 0.77 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2774.

High Performance Liquid Chromatography System HAA—RT 20.2 min; system HX—RI 561.

Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1 = 370b); aqueous alkali—273 nm.



Infrared Spectrum Principal peaks at wavenumbers 694, 751, 1086, 1041, 1010, 714 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 165, 255, 359, 166, 73, 199, 45.

Dose Captodiame hydrochloride has been given in doses of 150 mg daily.

Captopril

Antihypertensive

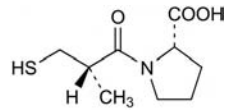
$C_9H_{15}NO_3S$ = 217.3

CAS—62571-86-2

IUPAC Name 1-[(2*S*)3-Mercapto-2-methyl-1-oxopropyl]-l-proline

Synonyms SQ-14225; captoprilum.

Proprietary Names *Acediur*; *Aceomel*; *Acepril*; *Aceplus*; *Alopresin*; *Acepress*; *Capoten*; *Captolane*; *Captoril*; *Cesplon*; *Dilabar*; *Ecopace*; *Garranil*; *Hipertil*; *Kaplon*; *Lopirin*; *Lopril*; *Tensiomin-Cor*; *Tensobon*; *Tensoprel*; *Tensopril*.



Chemical Properties Polymorphic white to off-white crystals. Mp ~106°. Freely soluble in water, ethanol, chloroform, methylene chloride and methanol. It dissolves in dilute solution of alkali hydroxides. pK_{a1} 3.7, pK_{a2} 9.8. Log *P* (octanol/water), 0.34.

Colour Tests Palladium chloride—orange; sodium nitroprusside (method 2)—violet.

Thin-layer Chromatography System TD—R_f 0.01; system TE—R_f 0.00; system TF—R_f 0.01; system TAD—R_f 0.06 (acidified potassium permanganate solution—yellow; ferric chloride—yellow; mercuric chloride–diphenylcarbazone reagent—violet (turns pink on heating); Van Urk reagent–yellow (fades on heating).

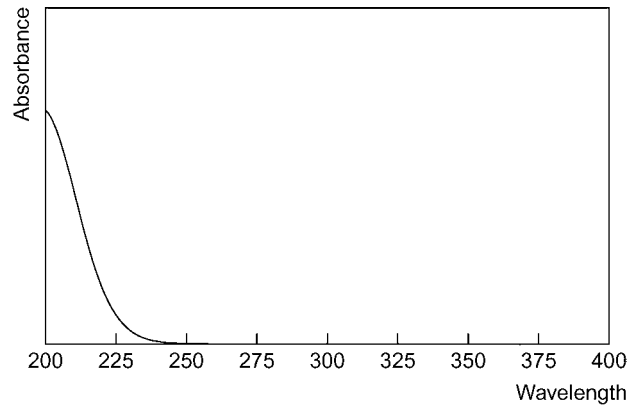
Gas Chromatography System GA—captopril, not eluted; methylcaptopril RI 1730; system GB—captopril, not eluted.

High Performance Liquid Chromatography System HZ—RT 2.1 min; system HY—RI 283; system HX—RI 316; system HAA—RT 9.7 min.

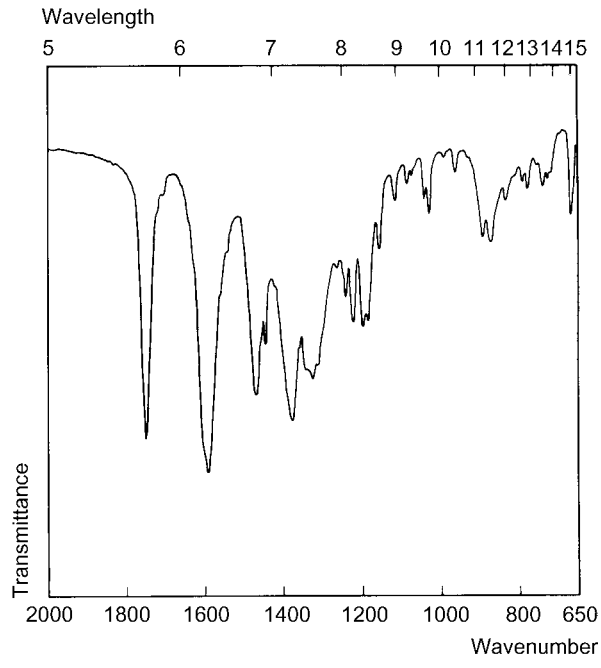
Column: Nova Pak RP C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 1% acetic acid pH 2.8 (42 : 58), flow rate 1.5 mL/min. I.S.: *N*-acetyl-L-cysteine. Fluorescence detection (λ_{ex} = 340 nm, λ_{em} = 389 nm). Retention time: captopril, 3.7 min; I.S. 2.2 min [Arroyo *et al.* 1997].

Column: RP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1% acetic acid (60 : 40), flow rate 1.3 mL/min. I.S.: nitrazepam. UV detection (λ = 260 nm). Retention time: captopril, 4.1 min; I.S., 4.6 min [Jankowski *et al.* 1995].

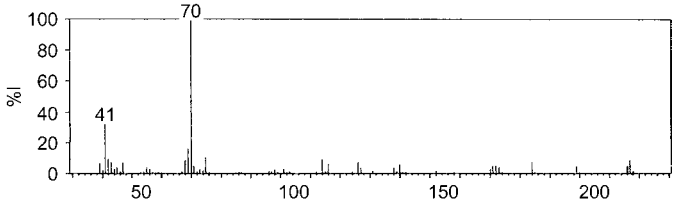
Ultraviolet Spectrum Aqueous acid—no significant absorption, 230–360 nm; aqueous alkali—238 nm (A^1_1 = 235c) (See below).



Infrared Spectrum Principal peaks at wavenumbers 1589, 1742, 1202, 1192, 1229, 1245 cm^{-1} (KBr disk). Polymorphism may occur.



Mass Spectrum Principal ions at *m/z* 70, 41, 69, 75, 114, 42, 217, 68.



Quantification

Blood **GC** Column: 3% OV-101 on Chromosorb W-HP. ECD. Limit of detection, 20 µg/L [Bathala *et al.* 1984].

HPLC Column: Separon SGX C_{18} (150 × 3.3 mm i.d., 5 µm). Mobile phase: 20 mmol/L sodium octanesulfonate (pH 2.5) with 0.1 mol/L citric buffer : acetonitrile (3 : 1), flow rate 0.5 mL/min. UV detection (λ = 314 nm). Retention time: 23 min. Limit of detection, 0.3 µg/L [Sypniewski, Bald 1996].

Plasma **GC** See Blood. Limit of detection, 50 µg/L [Bathala *et al.* 1984].

GC-MS Column: HP-5 capillary column (25 m × 0.2 mm i.d., 33 µm). Carrier gas: He, 0.8 mL/min. Temperature: 310°. Limit of quantification, 10 ng/mL [Franklin *et al.* 1998]. Column: 3% OV-101 on 100/120 Chromosorb WAW DMCS (2 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 150° for 1 min to 290° at 10°/min. EI ionisation at 70 eV, positive ion mode. Limit of detection, 1 µg/L for captopril, 25 µg/L for captopril disulfide [Drummer *et al.* 1984]. Limit of detection, 20 µg/L [Ivashkiv *et al.* 1984].

HPLC Column: DIAMONSIL (150 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : trifluoroacetic acid : water, flow rate 1.2 mL/min. UV detection (λ = 263 nm). Limit of quantification, 7 µg/L [Huang *et al.* 2006]. Column: Inertsil 5 ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% aqueous trifluoacetic acid : acetonitrile (85 : 15), flow rate 1.5 mL/min. Fluoresence detection. Limit of detection, 4 ppb [Tache *et al.* 2002]. Column: µbondapak NH_2 (150 × 3.9 mm i.d., 10 µm). Mobile phase: *n*-hexane : propan-2-ol : methanol : acetic acid (68 : 15 : 15 : 2), flow rate 2 mL/min. UV detection (λ = 246 nm). Limit of quantification, 10 mg/L [Amini *et al.* 1999]. Column: C_{18} reversed phase. Mobile phase: methanol: acetonitrile: phosphate buffer (pH 6.4, 30 : 30 : 135), flow rate 1 mL/min. Fluorescence detection. Limit of quantification, 5 µg/L [Zhong *et al.* 1998]. Column: Nova-Pak C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 100% acetic acid (pH 2.30, 42 : 58). Fluoresence detection (λ_{ex} = 340 nm and λ_{em} = 389 nm). Limit of quantification, 25 µg/L [Arroyo *et al.* 1997]. Mobile phase *o*-phthalaldehyde : acetonitrile : water : trifluoroacetic acid (15 : 85 : 0.1) with glycine 100 mg/L, flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 345 nm and λ_{em} = 455 nm). Limit of quantification, 12.5–25 µg/L [Kok *et al.* 1997]. Column: Spherisorb C_{18}. Mobile phase: water : acetonitrile : acetic acid (44 : 55 : 0.2). UV detection (λ = 258 nm). Limit of detection, 2 ng/L [Li *et al.* 1996]. Column: Kontron Analytical S5 ODS-2 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 1% acetic acid (60 : 40), flow rate 1.3 mL/min. UV detection (λ = 260 nm). Limit of detection, 15 µg/L [Jankowski *et al.* 1995]. Column: µBondapak C_{18} (37–50 µm) and YWG-C_{18} (10 µm). Mobile phase: acetonitrile : water : acetic acid (35 : 65 : 0.4). UV detection (λ = 260 nm). Limit of quantification, 20 and 10 µg/L, for columns respectively [Gao *et al.* 1992]. Column: Nova-Pak C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: 2-methyl-1,5-benzoylbenzimidazole : acetonitrile : water : trifluoroacetic acid (20 : 80 : 0.1), flow rate 1.0 mL/min. Fluoresence detection (λ_{ex} = 400 nm and λ_{em} = 280 nm) [Perrot *et al.* 1988].

LC-MS Column: Chromolith C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (65 : 35, pH 3.1). ESI, positive ion mode, MRM acquisition mode. Retention time: 1.45 min. Limit of quantification, 10 µg/L [Rezende *et al.* 2007].

Note For a radioimmunoassay for the detection of captopril see Duncan *et al.* [1983].

Serum **HPLC** Column: ODS reversed phase column. Mobile phase: acetonitrile : water : acetic acid (225 : 270 : 5), flow rate 1 mL/min. UV detection (λ = 263 nm). Limit of quantification, 50 ng/mL [Bahmaei *et al.* 1997].

Urine **GC-MS** See Plasma [Drummer *et al.* 1984].

HPLC See Plasma. Limit of detection, 250 µg/L [Kok *et al.* 1997]. See Plasma [Gao *et al.* 1992]. See Blood [Sypniewski, Bald 1996]. See Plasma [Perrot *et al.* 1988].

Disposition in the Body Captopril is readily absorbed after oral administration; ~60–75% of the dose is absorbed and peak plasma concentrations are achieved within ~1 h. It crosses the placenta and is found in breast milk at ~1% of maternal blood concentrations. Urinary excretion of an oral dose is 40–50% as unchanged drug, ~3% as captopril disulfide and ~30% as polar metabolites. Excretion in the urine is rapid, about half the dose being excreted within the first 4 h. An *S*-methyl metabolite has been identified in plasma and urine. Captopril is removed by haemodialysis.

Therapeutic Concentration

Analysis of captopril concentrations in plasma samples from 20 volunteers following oral administration of 100 mg captopril resulted in a mean peak concentration of 1.47 mg/L after 0.75 h [Franklin *et al.* 1998].

After a single oral dose of 100 mg of ^{35}S-labelled captopril to 10 subjects, peak blood concentrations of 0.51–1.31 mg/L (mean 0.8 mg/L) captopril and 0.11–0.49 mg/L (mean 0.23 mg/L) captopril disulfide were attained in 0.5–1.5 h [Kripalani *et al.* 1980].

Following oral administration of 25 mg three times a day to 12 subjects, a mean maximum steady-state blood concentration of 0.14 mg/L captopril was reported 0.9 h after a dose [Cody *et al.* 1982].

Toxicity

A 75-year-old man committed suicide by taking an overdose of captopril. He took ~90 tablets each containing 12.5 mg. The postmortem plasma concentration of captopril was 60.4 mg/L. This was the first case report of fatal captopril overdose with the measurement of plasma concentration of the drug [Park *et al.* 1990].

Serum levels of captopril reached 20 mg/L in a 43-year-old individual who had attempted suicide by ingesting 5–7.5 g captopril 7 h earlier. The patient survived and displayed minimal symptoms [Lechleitner *et al.* 1990].

Half-life ≈1–2 h in blood.

Volume of Distribution ≈0.7 L/kg in blood.

Clearance ≈13 mL/min/kg from blood.

Protein Binding ≈30% in plasma.

Note For a review of captopril, see Romankiewicz *et al.* [1983].

Dose Daily dose 75 to 150 mg; maximum of 450 mg daily in divided doses.

Amini M *et al.* (1999). Sensitive high-performance liquid chromatographic method for determination of captopril in plasma. *Pharm Acta Helv* 73: 303–306.

Arroyo C *et al.* (1997). Determination of captopril in plasma by high-performance liquid chromatography for pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 688: 339–344.

Bahmaei M *et al.* (1997). Determination of captopril in human serum by high performance liquid chromatography using solid-phase extraction. *J Pharm Biomed Anal* 15: 1181–1186.

Bathala MS *et al.* (1984). Quantitative determination of captopril in blood and captopril and its disulfide metabolites in plasma by gas chromatography. *J Pharm Sci* 73: 340–344.

Cody RJ *et al.* (1982). Captopril kinetics in chronic congestive heart failure. *Clin Pharmacol Ther* 32: 721–726.

Drummer OH *et al.* (1984). Combined gas chromatographic–mass spectrometric procedure for the measurement of captopril and sulfur-conjugated metabolites of captopril in plasma and urine. *J Chromatogr* 305: 83–93.

Duncan FM *et al.* (1983). Development and optimisation of a radioimmunoassay for plasma captopril. *Clin Chim Acta* 131: 295–303.

Franklin ME *et al.* (1998). Improved analytical procedure for the measurement of captopril in human plasma by gas chromatography–mass spectrometry and its application to pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 705: 47–54.

Gao S *et al.* (1992). Simple high-performance liquid chromatographic method for the determination of captopril in biological fluids. *J Chromatogr* 582: 258–262.

Huang T *et al.* (2006). Simultaneous determination of captopril and hydrochlorothiazide in human plasma by reverse-phase HPLC from linear gradient elution. *J Pharm Biomed Anal* 41: 644–648.

Ivashkiv E *et al.* (1984). Determination of total captopril in human plasma by gas chromatography–mass spectrometry with selected-ion monitoring after reduction of disulfides. *J Pharm Sci* 73: 1113–1117.

Jankowski A *et al.* (1995). Captopril: determination in blood and pharmacokinetics after single oral dose. *J Pharm Biomed Anal* 13: 655–660.

Kok RJ *et al.* (1997). Bioanalysis of captopril: two sensitive high-performance liquid chromatographic methods with pre- or postcolumn fluorescent labeling. *J Chromatogr B Biomed Sci Appl* 693: 181–189.

Kripalani KJ *et al.* (1980). Disposition of captopril in normal subjects. *Clin Pharmacol Ther* 27: 636–641.

Lechleitner P *et al.* (1990). Uneventful self poisoning with a very high dose of captopril. *Toxicology* 64: 325–329.

Li K *et al.* (1996). HPLC determination of captopril in human plasma and its pharmacokinetic study. *Biomed Chromatogr* 10: 237–239.

Park H *et al.* (1990). Suicide by captopril overdose. *J Toxicol Clin Toxicol* 28: 379–382.

Perrot D *et al.* (1988). A case of sotalol poisoning with fatal outcome. *J Toxicol Clin Toxicol* 26: 389–396.

Rezende KR *et al.* (2007). Determination of captopril in human plasma, using solid phase extraction and high-performance liquid chromatography, coupled to mass spectrometry: application to bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 59–67.

Romankiewicz JA *et al.* (1983). Captopril: an update review of its pharmacological properties and therapeutic efficacy in congestive heart failure. *Drugs* 25: 6–40.

Sypniewski S, Bald E (1996). Determination of captopril and its disulphides in whole human blood and urine by high-performance liquid chromatography with ultraviolet detection and precolumn derivatization. *J Chromatogr A* 729: 335–340.

Tache F *et al.* (2002). Validation of a LC-fluorescence method for determination of free captopril in human plasma, using a pre-column derivatization reaction with monobromobimane. *J Pharm Biomed Anal* 28: 549–557.

Zhong D *et al.* (1998). [Determination of captopril plus its disulfide metabolites in human plasma.]. *Yao Xue Xue Bao* 33: 605–609.

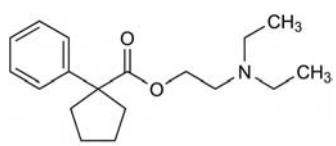
Caramiphen

Anticholinergic

$C_{18}H_{27}NO_2$ = 289.4

CAS—77-22-5

IUPAC Name 2-(Diethylamino)ethyl 1-phenylcyclopentanecarboxylate



Chemical Properties A liquid. Log *P* (octanol/water), 4.5.

Caramiphen Edisilate

$C_{18}H_{27}NO_2$, $^1/_2C_2H_6O_6S_2$ = 384.5

CAS—125-86-0

Synonym Caramiphen ethanedisulfonate
Chemical Properties Crystals. Mp 115° to 116°. Soluble in water and ethanol.

Caramiphen Hydrochloride

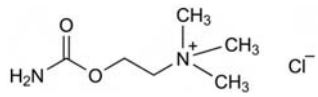
$C_{18}H_{27}NO_2$, HCl = 325.9
CAS—125-85-9
Chemical Properties White crystals or crystalline powder. Mp 145° to 146°. Soluble 1 in 4 of water and 1 in 8 of ethanol; practically insoluble in ether.

Colour Tests Liebermann's reagent—red-orange; Marquis test—yellow; sulfuric acid (when warmed)—red.
Thin-layer Chromatography System TA—R_f 0.66 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1971.
Ultraviolet Spectrum Aqueous acid—252 (A^1_1=7.9b), 259 (A^1_1=7.9b), 265 nm.
Infrared Spectrum Principal peaks at wavenumbers 1718, 1154, 694, 1173, 1220, 1060 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 99, 91, 144, 58, 56, 41, 87.
Quantification
Blood **GC** AFID. Limit of detection, <2.5 µg/L [Levandoski, Flanagan 1980].
Disposition in the Body
Therapeutic Concentration
Following daily oral administration of 20 mg of caramiphen edisilate at 0, 4, and 8 h to 11 subjects for 4 days, peak blood concentrations of 0.018 to 0.078 (mean 0.046) mg/L were reported 1 to 2 h after a dose [Levandoski, Flanagan 1980].
Dose Caramiphen hydrochloride has been given in doses of 50 to 600 mg daily.

Levandoski P, Flanagan T (1980). Use of nitrogen-specific detector for GLC determination of caramiphen in whole blood. *J Pharm Sci* 69: 1353–1354.

Carbachol

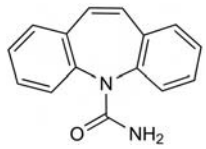
Parasympathomimetic
$C_6H_{15}ClN_2O_2$ = 182.6
CAS—51-83-2
IUPAC Name 2-Carbamoyloxyethyl(trimethyl)azanium chloride
Synonyms 2-[(Aminocarbonyl)oxy]-*N*,*N*,*N*-trimethylethanaminium chloride; carbacholine; carbamoylcholine chloride; carbamylcholine chloride; choline chloride carbamate.
Proprietary Names *Carbyl*; *Doryl*; *Miostat*.



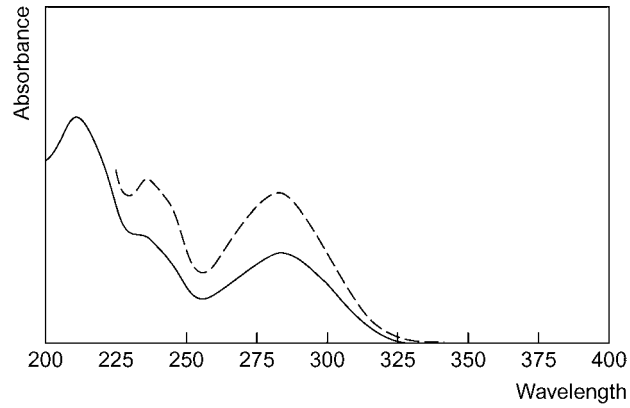
Chemical Properties White or faintly yellow, hygroscopic, crystals or crystalline powder. Mp 200° to 204°, with decomposition. Soluble 1 in 1 of water and 1 in 50 of ethanol; very slightly soluble in dehydrated alcohol, more readily soluble on boiling; practically insoluble in acetone, chloroform and ether. pK_a 4.8. Log *P* (octanol/water), −3.8.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.04; system TAF—R_f 0.23 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1730, 1083, 1056, 930, 1102, 1200 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 58, 42, 44, 30, 129, 36, 143.
Dose 6 mg daily orally. For acute symptoms, 250 µg by SC injection, repeated if necessary.

Carbamazepine

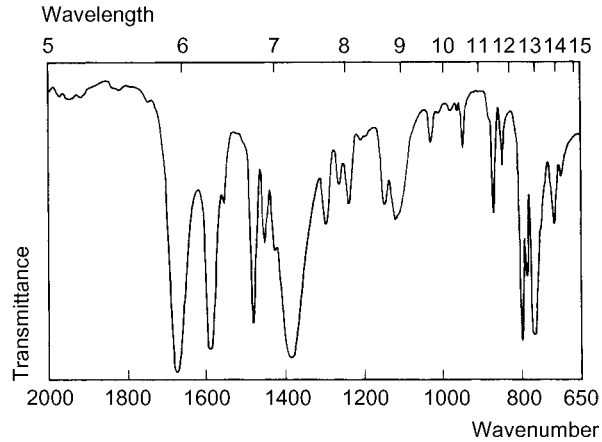
Anticonvulsant
$C_{15}H_{12}N_2O$ = 236.3
CAS—298-46-4
IUPAC Name Benzo[*b*][1]benzazepine-11-carboxamide
Synonyms Carbamazepinum; *5H*-Dibenz[*b*,*f*]azepine-5-carboxamide; G-32883.
Proprietary Names *Atretol*; *Biston*; *Calepsin*; *Carbagen*; *Carbatrol*; *Carbium*; *Carpaz*; *Convuline*; *Epimaz*; *Epitol*; *Finlepsin*; *Fokalepsin*; *Gericarb*; *Hermolepsin*; *Neurotop*; *Prozine*; *Sirtal*; *Stazepin*; *Tegretol*; *Telesmin*; *Teril*; *Timonil*.



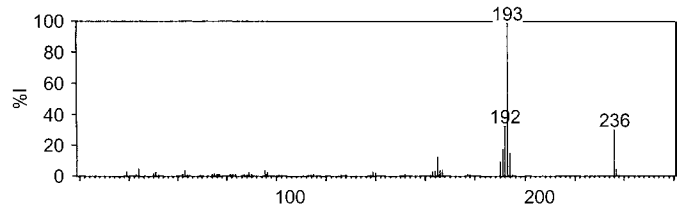
Chemical Properties A white or yellowish-white crystalline powder. Mp 189° to 193°. Practically insoluble in water and ether; soluble in alcohol, acetone, chloroform and propylene glycol, pK_a 7.0 [Martinavarro-Dominguez *et al.* 2002]. Log *P* (octanol/water), 2.45 [Martinavarro-Dominguez *et al.* 2002]. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].
Colour Test Dissolve the sample in 1 mL of chloroform, add 0.2 mL of sodium hypobromite solution and mix for 1 min—blue-violet (limit of detection, 250 mg/L).
Thin-layer Chromatography System TA—R_f 0.6; system TB—R_f 0.02; system TC—R_f 0.56; system TE—carbamazepine R_f 0.56, M (OH-) R_f 0.36; system TL—R_f 0.47; system TAE—carbamazepine R_f 0.79, M (OH-) R_f 0.79; system TAF—R_f 0.75; system TAJ—R_f 0.44; system TAK—R_f 0.64; system TAL—R_f 0.94 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—carbamazepine RI 2285, M (-Me) RI 1905, M (OH-)(methoxy-ring) RI 2340, M (OH-ring) RI 2240, M (epoxide) RI 2220, M (iminostilbene) RI 1998, M (acridine) RI 1800, M (formylacridine) RI 2025; system GB—carbamazepine RI 2435, M (iminostilbene) RI 2064, M (acridine) RI 1880, M (formylacridine) RI 2158, M (methylacridine) RI 2054, M (10,11-di-OH-) RI 2738; system GE—carbamazepine RRT 0.83 (relative to phenytoin); system GF—carbamazepine RI 2610, M (epoxide) RI 2560, M (iminostilbene) RI 2620; system GAJ—carbamazepine RRT 1.716, M (epoxide) RRT 1.188, M (iminostilbene) RRT 1.065 (all relative to methylphenobarbital).
High Performance Liquid Chromatography System HE—*k* 8.30; system HX—carbamazepine RI 418, M (10,11-di-OH-) RI 355; system HY—carbamazepine RI 368, M (epoxide) RI 336; system HZ—carbamazepine RT 3.5 min, M (epoxide) RT 1.9 min, M (10,11-di-OH-) RT 2.6 min; system HAA—carbamazepine RT 15.8 min; system HAX—carbamazepine RT 6.2 min; system HAY—carbamazepine RT 5.5 min; system HAZ—carbamazepine *k* 0.82, M (epoxide) *k* 0.38; system HAK—carbamazepine RT 4.7 min.
Ultraviolet Spectrum Methanol—237, 285 nm (A^1_1 = 490a).



Infrared Spectrum Principal peaks at wavenumbers 1678, 1594, 800, 769, 787, 1298 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 193, 192, 236, 191, 194, 165, 190, 237; carbamazepine-10,11-epoxide 180, 179, 178, 152, 44, 181, 223, 51; *trans*-10,11-dihydro-10,11-dihydroxycarbamazepine 180, 77, 181, 44, 179, 51, 209, 167.



Quantification
Blood **GC-MS** Column: Bond Elut Certify [Speed *et al.* 2000].

Plasma **GC** Thermionic detection. Limit of quantification, 50–200 μg/L [Queiroz *et al.* 2002]. Column: GP-2% SP-2510-DA on 100/120 mesh Supelcoport. Limit of detection, 1 mg/L [Riva *et al.* 1980].

HPLC Column: reversed phase monolithic. Mobile phase: 0.1 mol/L phosphate buffer (pH 6.5) : methanol : acetonitrile (77 : 20 : 3). UV detection (λ = 210 nm) [Heideloff *et al.* 2010]. Column: reversed phase C_{18}. Mobile phase: water : acetonitrile (78 : 22). UV detection (λ = 210 nm). Limit of quantification, 80 μg/L [Queiroz *et al.* 2008]. Column: Hisep (150 × 0.46 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (12 : 88), flow rate 1.5 mL/min. UV detection (λ = 280 nm). Limit of detection, 0.5 mg/L [Ma *et al.* 2002], Column: C_8 reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : phosphate buffer (pH 1.9). UV detection [Mandrioli *et al.* 2001]. Column: reversed phase C_8. Mobile phase: potassium dihydrogen phosphate (pH 2.5) : acetonitrile (67 : 33). UV detection (λ = 254 nm). Limit of quantification, 20 μg/L [Shimoyama *et al.* 2000].

See also Bhatti *et al.* [1998], Bonato *et al.* [1992], Chollet *et al.* [1996], Christofides, Fry [1980], Johansen *et al.* [1995], MacKichan [1980], Martens, Banditt [1993], Matar *et al.* [1999], Romanyshyn *et al.* [1994] and Rukhadze *et al.* [2000].

Note For a comparison of HPLC with immunoassays see Hermida *et al.* [2002].

LC-MS Column: Shimdazu Shimpack XR-ODS (50 × 4.6 mm i.d., 2.2 μm). Mobile phase: acetate buffer : methanol : acetonitrile : tetrahydrofuran. APCI, SIM acquisition mode [Subramanian *et al.* 2008].

Serum **TLC-spectofluorimetry** Limit of detection, 100 μg/L for carbamazepine, 50 μg/L for its metabolites [Hundt, Clark 1975].

GC-MS Column: cross-linked 5% phenyl methyl silicone capillary HP-5 (25 m × 0.2 mm i.d., 0.33 μm). Limit of detection, 10 μg/L [Minkova, Getova 2001].

HPLC Column: C_{18}. Mobile phase: methanol : water : acetic acid (65 : 34 : 1), flow rate, 1.0 mL/min. UV detection (λ = 285 nm). Limit of quantification, 240 μg/L, limit of detection, 160 μg/L [Dordevic *et al.* 2009]. Column: μBondapak C_{18}. Mobile phase: 30 mmol/L aqueous potassium phosphate buffer (pH 3.7) : acetonitrile (65 : 35), flow rate, 1.2 mL/min. UV detection (λ = 270 nm). Retention time: 7.3 min. Limit of detection, 250 μg/L [Greiner-Sosanko *et al.* 2007]. Column: Merck C_{18} (250 × 4 mm i.d., 5 μm). Mobile phase: water : acetonitrile (70 : 30, pH 3.0), flow rate 1 mL/min. UV detection (λ = 210 nm). Retention time, 13.9 min. Limit of detection, 1300 pg [Gupta *et al.* 2006]. Column: Cadenza CD-C_{18} (150 × 4.6 mm, 3 μm). Mobile phase: 25 mmol/L phosphate buffer (pH 4.6) : methanol : acetonitrile (65 : 20 : 15), flow rate, 1.0 mL/min. UV detection (λ = 215 nm). Limit of quantification, 500 μg/L [Yoshida *et al.* 2006]. Column: Kromasil C_{18} (250 × 4 mm i.d., 5 μm). Mobile phase: 0.05 mol/L SDS plus 70 mL/L butan-1-ol, flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of detection, 10 μg/L [Martinavarro-Dominguez *et al.* 2002].

See also Alexishvili *et al.* [1997], Kouno *et al.* [1993], Kouno *et al.* [1997], Lensmeyer *et al.* [1997], Levert *et al.* [2002], Liu *et al.* [1993a], [Liu *et al.* [1993b], Pienimäki *et al.* [1995] and Rambeck *et al.* [1994].

Note For a fluorescence polarisation immunoassay for the quantification of carbamazepine, see Steijns *et al.* [2002].

Urine **GC** See Plasma [Riva *et al.* 1980].

HPLC See Serum [Liu *et al.* 1993a].

LC-MS See Maggs *et al.* [1997].

Breast Milk **HPLC** See Plasma [Shimoyama *et al.* 2000].

Oral Fluid **HPLC** See Serum [Alexishvili *et al.* 1997; Dordevic *et al.* 2009; Liu *et al.* 1993a]. See Plasma [Rukhadze *et al.* 2000].

Hair **HPLC** Column: Reversed phase. Mobile phase: acetonitrile : methanol : water (9 : 37 : 54). UV detection (λ = 214 nm). Limit of detection, 1.33 ng/mg [Mei, Williams 1997]. Column: Reversed phase. UV detection [Saris *et al.* 1997].

Liver **GC-MS** See Blood [Speed *et al.* 2000].

Disposition in the Body Carbamazepine is slowly but almost completely absorbed after oral administration; bioavailability >70%. Metabolic reactions, catalysed by P450 isoforms CYP3A4 and CYP2C8, include epoxidation to form the 10,11-epoxide, which is active, followed by hydroxylation to *trans*-10,11-dihydro-10,11-dihydroxycarbamazepine; glucuronic acid conjugation also occurs. The rate of metabolism is higher in children than in adults and levels of the epoxide may be higher in children than in adults. Approximately 25% of a dose is excreted in the urine as the dihydroxy metabolite, together with 2% as the 10,11-epoxide and <10% as unchanged drug; other metabolites identified in the urine include carbamazepine-*N*-glucuronide, iminostilbene and several monohydroxy- and trihydroxycarbamazepine isomers. Approximately 30% of a dose is eliminated in the faeces. Carbamazepine crosses the placental barrier and is distributed into breast milk.

Therapeutic Concentration In plasma, usually in the range 4–12 mg/L.

Following single oral doses of 200 mg given to 9 subjects, peak plasma concentrations of 7.8–14 mg/L (mean 10) were attained in ~9 h [Anttila *et al.* 1979].

Steady-state plasma concentrations of 3–13 mg/L (mean 8) for carbamazepine and 0.6–5.7 mg/L (mean 3) for the epoxide metabolite were reported in 24 subjects receiving daily oral doses of 8–30 mg/kg [MacKichan *et al.* 1981].

Twenty-two children receiving long-term therapy with slow-release carbamazepine tablets (300 to 800 mg daily in 2 divided doses) had their treatment replaced with carbamazepine suppositories for 7 days (at an equivalent dosage; 375 to 1000 mg daily in 2 to 4 divided doses). Minimum to maximum plasma concentrations over 24 h were reported as follows: carbamazepine 20.4–33.1 μmol/L after slow-release tablets and 18.9–31.2 μmol/L after suppositories; 10,11-epoxy-carbamazepine 3.33–5.49 μmol/L after slow-release tablets and 3.11–4.90 μmol/L after suppositories [Arvidsson *et al.* 1995].

In a study involving 435 blood samples taken from 248 patients receiving carbamazepine therapy (dosage 2.86 to 37.50 mg/kg daily), steady-state plasma concentrations of 2.5–82.9 μmol/L (mean 22.3) carbamazepine, 0.85–16.9 μmol/L (mean 5.17) carbamzepine epoxide, and 0.77–36.4 μmol/L (mean 11.3) carbamazepine-diol were found [Svinarov, Pippenger 1996].

Toxicity The estimated minimum lethal dose is 5 g. Plasma concentrations of 2.4–10.5 mg/L (mean 6.0) have been associated with slight toxic effects and concentrations of 3.2–21 mg/L (mean 10) with severe toxicity.

A 15-year-old girl was admitted having taken at least 23 tablets of Tegretol (4.6 g) 6 h earlier. Her serum carbamazepine level was 190 μmol/L. Orogastric lavage was followed by activated charcoal. Within 20 h of admission, there was no improvement in her neurological status, so it was decided to perform plasmapheresis. The carbamazepine level immediately after the procedure was 101 μmol/L, and at the hours 36, 60 and 84 was 72, 33 and 20 μmol/L, respectively. The patient was discharged on the fourth day [Duzova *et al.* 2001].

In a massive overdose with controlled-release carbamazepine by a 31-year-old female, serum levels were 75 μmol/L on admission, fell to 66 μmol/L 22 h later (39 h post-ingestion), and rose to 196 μmol/L on day 4 despite treatment with activated charcoal and whole bowel irrigation. Benzodiazepines were also detected. At this time, coma and intermittent general seizures developed, so charcoal haemoperfusion was started and serum carbamazepine levels fell from 176 to 106 μmol/L after 1 h. Upon recovery the patient admitted ingesting 300 controlled-release carbamazepine (200 mg) tablets as well as diazepam, nitrazepam and alcohol [Graudins *et al.* 2002].

In a fatality involving overdose with carbamazepine, blood concentrations were reported as 47.7 mg/L 2 h before death and 53 mg/L 9 h after death [Spiller, Carlisle 2001].

In 28 cases of massive carbamazepine overdose, peak concentrations ranged from 78–285 μmol/L (18.4–67.4 mg/L). Serum levels of at least 170 μmol/L (40 mg/L) were associated with serious complications such as coma, seizures, respiratory failure and cardiac conduction defects. Two of the cases were fatalities [Hojer *et al.* 1993].

In a fatality attributed to carbamazepine and alcohol, postmortem kidney and liver concentrations of 306 μg/g and 173 μg/g, respectively, of carbamazepine were reported: the blood alcohol concentration was 1600 mg/L [Borkowski, Janowska 1983].

Half-life Plasma half-life, 18–65 h (mean 35) after single doses; reduced during chronic treatment to ~10–30 h for adults and 8–19 h for children.

Volume of Distribution ~1.4 L/kg.

Clearance Plasma clearance, ~1.3 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.6.

Saliva Plasma : saliva ratio, ~3–5.

Protein Binding Carbamazepine, ~75% and 10,11-epoxycarbamazepine, ~50%.

Note For reviews of the pharmacokinetics of carbamazepine see Bertilsson [1978] and Bertilsson, Tomson [1986].

Dose Initially 100 to 400 mg daily, increasing to 0.4 to 1.2 g daily; occasionally up to 2 g daily in epilepsy; in trigeminal neuralgia and bipolar disorder, up to 1.6 g daily.

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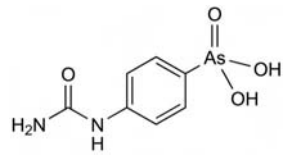
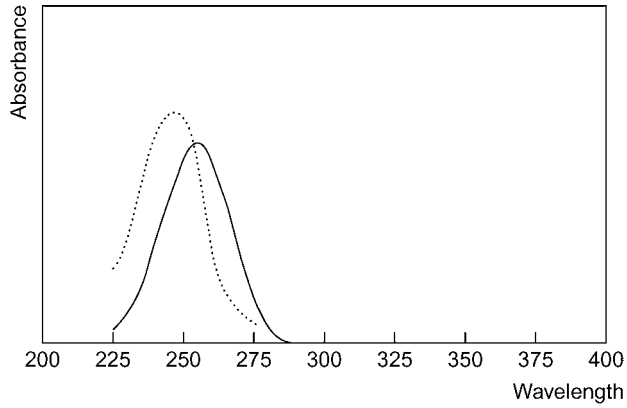
Carbarsone

Antiamoebic
$C_7H_9AsN_2O_4 = 260.1$
CAS—121-59-5

IUPAC Name [4-[(Aminocarbonyl)amino]phenyl]arsonic acid
Synonym Aminarsonum
Proprietary Names *Amabevan*; *Ameban*; *Carb-O-Sep*; *Fenarsone*; *Histocarb*; *Leucarsone*.



Chemical Properties A white powder. Mp 174°. Soluble 1 in 330 of water and 1 in 400 of ethanol; nearly insoluble in chloroform and ether; soluble in solutions of alkali hydroxides and carbonates. Log *P* (octanol/water), −1.2.
Ultraviolet Spectrum Aqueous acid—257 nm; aqueous alkali—248 nm.

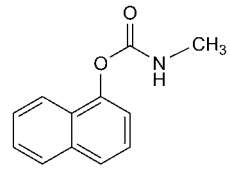


Infrared Spectrum Principal peaks at wavenumbers 1508, 1580, 1101, 903, 1681, 1248 cm^{-1}.
Dose 500 to 750 mg daily, for 10 days.

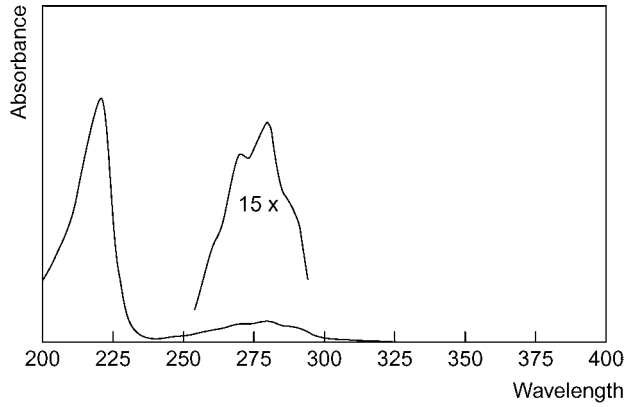
Carbaryl

Carbamate, Anticholinesterase, Insecticide, Parasiticide
$C_{12}H_{11}NO_2 = 201.2$
CAS—63-25-2

IUPAC Name Naphthalen-1-yl *N*-methylcarbamate
Synonyms Carbaril; 1-naphthalenol methylcarbamate.
Proprietary Names *Carylderm*; *Derbac* (shampoo); *Murvin*; *Sevin*; *Suleo-C*.



Chemical Properties A white crystalline solid. Mp 142°. Practically insoluble in water; soluble in most polar organic solvents. Log *P* (octanol/water) 2.4. Stock solutions in tetrohydrofuran were stable for >6 months when stored at –30° and for >3 months when stored at 4° in dark-coloured vials [Petropoulou *et al.* 2006].
Colour Tests Liebermann's reagent—black→green; Marquis test—green.
Thin-layer Chromatography System TAA—R_f 0.17; system TAB—R_f 0.12; system TAC—R_f 0.04; system TX—R_f 0.18; system TY—R_f 0.25; system TZ—R_f 0.68.
Gas Chromatography System GA—carbaryl RI 1865, carbaryl-TFA RI 1785, M (1-naphthol) RI 1490, M (1-naphthol)-AC RI 1555; system GK—M (1-naphthol) RT 0.47 relative to caffeine.
High Performance Liquid Chromatography System HAA—RT 18.0 min; system HY—RI 402.
Ultraviolet Spectrum Ethanol—279 nm ($A_1^1 = 310$b).



Infrared Spectrum Principal peaks at wavenumbers 1724, 1219, 1250, 1111, 781, 1265 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 144, 115, 116, 57, 58, 63, 145, 89.

Quantification

Blood **GC-MS** Column: 5% diphenyl-dimethyl polysiloxane capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 75° for 1.5 min to 135° at 15°/min to 138° at 2°/min to 175° at 10°/min for 1 min to 191° at 20°/min to 300° at 25°/min for 4.0 min. EI ionisation at 70 eV, positive ion mode, full scan mode. Retention time: 16.1 min. Limit of quantification, 0.5 µg/L; limit of detection, 0.1 µg/L [Petropoulou *et al.* 2006].

HPLC Column: Zorbax Cyano (250 × 4.6 mm i.d., 5 µm). Mobile phase: ethyl acetate : *iso*-octane (20 : 80), flow rate 1.0 mL/min. Refractive index detection. Relative retention time: 1.72 min. Limit of detection, 100 ng [Sharma *et al.* 1990]. Column: C_{18} reversed phase (5 mm i.d., 10 µm). Mobile phase: acetonitrile : water (37.5 : 62.5) containing 0.1% diethylamine (pH 7.0), flow rate 1.8 mL/min. UV detection (λ = 214 nm). Retention time: 2.85 min. Limit of detection, 1mg/L [Duck, Woolias 1985]. Column: Alltech C_8 (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.13 mol/L phosphate buffer (pH 6.2; 2 : 3), flow rate 1.42 mL/min. Fluorescence detection (λ_{ex} = 285 nm, λ_{em} = 340 nm). Retention time: carbaryl 10.6 min, 1-naphthol 12.5 min, napropamide 30.0 min. Limit of detection, 40 µg/L [DeBerardinis, Wargin 1982].

Serum **GC** Column: DB-1701 intermediately fused silica capillary (15 m × 0.53 mm i.d., 1.5 µm). Temperature programme: 100° for 3 min to 200° at 8°/min. FID. Limit of detection not reported [Yamazaki *et al.* 2001].

GC-MS Column: DB-5 slightly polar fused silica capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° to 250° at 20°/min for 20 min. EI ionisation. Limit of detection not reported [Yamazaki *et al.* 2001].

Urine **GC-MS** Column: AT-MS 5 5% diphenyl 95% dimethyl polysiloxane capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 75° for 1.5 min to 135° at 15°/min for 2.0 min to 138° at 2°/min to 175° at 10°/min for 1.0 min to 191° at 20°/min to 300° at 25°/min for 4 min. EI ionisation. Limit of quantification, 1 µg/L, limit of detection, 0.2 µg/L [Petropoulou *et al.* 2006].

HPLC See Blood [Duck, Woolias 1985]. Column: C_{18} µBondapak reversed phase. Mobile phase: acetonitrile : water (30 : 40), flow rate 1 mL/min. UV detection (λ = 254 nm). *k*′: 10.5 min for 1-naphthol. Limit of detection not reported [Keiser *et al.* 1983].

Stomach **HPLC** See Blood [Duck, Woolias 1985].

Liver **HPLC** See Blood [Sharma *et al.* 1990]. See Blood [Duck, Woolias 1985].

Lung **HPLC** See Blood [Sharma *et al.* 1990].

Other **GC** Animal Blood, Brain and Liver. Column: 3% OV-17 on 80/100 mesh Chromosorb W-HP (180 cm × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 180°. ECD. Limit of detection, 0.02 mg/L for blood, 0.1 mg/L for tissues [Mount, Oehme 1980].

HPLC Fruit. Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: water : tetrahydrofuran (100 : 0 to 30 : 70 in 20 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 450 nm). Limit of detection not reported [Stuart *et al.* 1999]. Drinking Water. Column: Supelcosil LC_8 (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (30 : 70 for 5 min to 60 : 40 at 15 min for 10 min to 30 : 70 at 30 min for 5 min), flow rate, 1.5 mL/min. UV detection, 220 nm. Retention time: 17.35 min. Limit of detection, 10 ng/L [Marvin *et al.* 1990].

Disposition in the Body It is rapidly metabolised after acute exposure (although metabolism in humans is yet to be investigated). Hydrolysis would yield 1-naphthol whereas the low pH of gastric juice may lead to the production of *N*-nitrosocarbaryl [Kubacki, Kupryszewski 1980]. Metabolism is slower in women and older adults [Wiener, Young 1995].

Toxicity Repeated exposure may have effects similar to those of 'irreversible' organophosphate cholinesterase inhibitors. Neuropsychiatric sequelae may include aggression, memory loss, progressive muscle weakness and peripheral neuropathy. One case of depression after carbaryl exposure has been reported [Wiener, Young 1995].

A 47-year-old male died due to possible pesticide poisoning. Carbaryl was found in gastric contents at a concentration of 1.27 g/L and in serum at 8.1 mg/L. Propanil, ethylbenzene and xylene were also detected [Yamazaki *et al.* 2001].

A 17-year-old female accidentally drank a glass of carbaryl liquid. She presented with abdominal pain, constipation, pain, and weakness in her limbs. Two hours later she had nausea, vomiting and cramp-like abdominal pain. By the fourth day her limbs had become weak. She was diagnosed with porphyria and, despite supportive therapy, died of respiratory and circulatory failure [Sargin *et al.* 1992].

A 48-year-old female ingested an unknown quantity of carbaryl garden spray. Post-mortem concentrations of carbaryl were 6 mg/L and 12 and 2400 mg/kg in the blood, liver, and stomach, respectively. There were trace amounts in her urine [Duck, Woolias 1985].

Note For a review of the toxicology of carbaryl, see Cranmer [1986] and for a case of pontine myelinosis in a 4-year-old boy with carbamate poisoning, see Santinelli *et al.* [2006].

Cranmer MF (1986). Carbaryl. A toxicological review and risk analysis. *Neurotoxicology* 7: 247–328.

DeBerardinis M,Jr Wargin WA (1982). High-performance liquid chromatographic determination of carbaryl and 1-naphthol in biological fluids. *J Chromatogr* 246: 89–94.

Duck BJ, Woolias M (1985). Reversed-phase high performance liquid chromatographic determination of carbaryl in postmortem specimens. *J Anal Toxicol* 9: 177–179.

Keiser JE *et al.* (1983). Reversed-phase high-performance liquid chromatography separation of human phenolic metabolites of propoxur (Baygon), carbofuran and carbaryl. *J Chromatogr* 259: 186–188.

Kubacki SJ, Kupryszewski G (1980). The formation, chemistry and stability of N-nitrosocarbaryl under simulated stomach conditions. *IARC Sci Publ*,245–257.

Marvin CH *et al.* (1990). Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water. *J Chromatogr* 503: 167–176.

Mount ME, Oehme FW (1980). Microprocedure for determination of carbaryl in blood and tissues. *J Anal Toxicol* 4: 286–292.

Petropoulou SS *et al.* (2006). Gas chromatographic-tandem mass spectrometric method for the quantitation of carbofuran, carbaryl and their main metabolites in applicators' urine. *J Chromatogr A* 1108: 99–110.

Santinelli R *et al.* (2006). Pontine myelinolysis in a child with carbamate poisoning. *Clin Toxicol (Phila)* 44: 327–328.

Sargin H *et al.* (1992). A case of porphyria due to carbaryl intoxication. *Hum Exp Toxicol* 11: 373.

Sharma VK *et al.* (1990). High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides. *Forensic Sci Int* 48: 21–25.

Stuart IA *et al.* (1999). Surface partitioning studies of N-methylcarbamate-treated post-harvest crops using SFE-HPLC-postcolumn reaction-fluorescence. *Analyst* 124: 275–280.

Wiener PK, Young RC (1995). Late-onset psychotic depression associated with carbaryl exposure. *Am J Psychiatry* 152: 646–647.

Yamazaki M *et al.* (2001). Pesticide poisoning initially suspected as a natural death. *J Forensic Sci* 46: 165–170.

Carbazochrome

Haemostatic

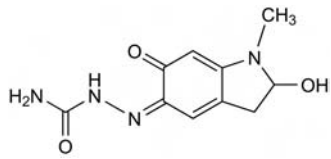
$C_{10}H_{12}N_4O_3$ = 236.2

CAS—69-81-8

IUPAC Name [(*Z*)-(3-Hydroxy-1-methyl-6-oxo-2,3-dihydroindol-5-ylidene) amino]urea

Synonym Adrenochrome monosemicarbazone; 5,6-dihydro-3-hydroxy-1-methylindoline-5,6-dione 5-semicarbazone.

Proprietary Names *Adrenoxyl*; *Cromosil*.



Chemical Properties An oxidation product of adrenaline. Yellowish-red or red crystals or crystalline powder. Mp about 222°, with decomposition. Very slightly soluble in water and ethanol; practically insoluble in ether.

Carbazochrome Salicylate

CAS—13051-01-9

IUPAC Name A complex of carbazochrome with sodium salicylate.

Proprietary Name *Adrenosem Salicylate*

Chemical Properties A fine, orange-red, crystalline powder. Mp 196° to 197.5°, with decomposition. Soluble in water and ethanol; practically insoluble in ether and chloroform.

Carbazochrome Sodium Sulfonate

$C_{10}H_{11}N_4NaO_5S$ = 322.3

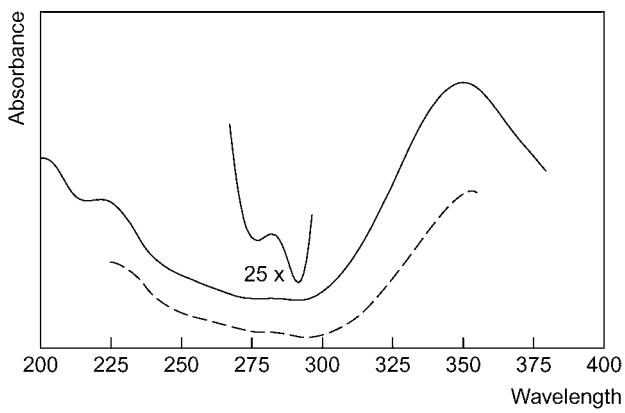
CAS—51460-26-5

Proprietary Names *Adona*; *Emex*.

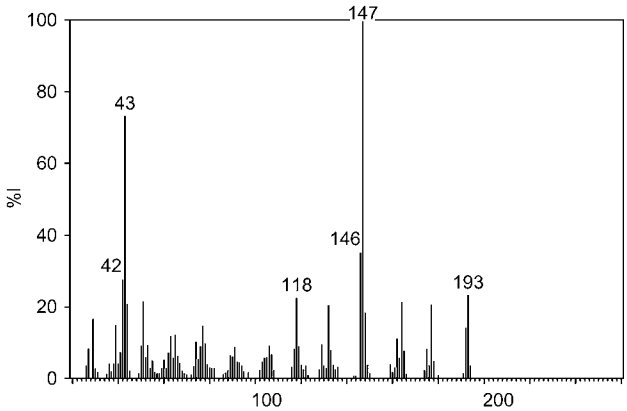
Chemical Properties Orange to yellow, fine, needle-like crystals. Mp 227° to 228°, with decomposition. Soluble 1 in 67 of cold water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.16; system TF—R_f 0.00; system TAD—R_f 0.10.

Ultraviolet Spectrum Methanol—356 nm (A_1^1=960b).



Mass Spectrum Principal ions at *m/z* 147, 43, 146, 42, 193, 118, 164, 44.

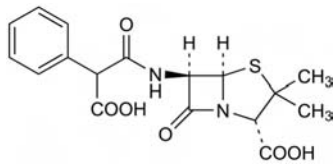


Dose The equivalent of 5 to 10 mg of carbazochrome has been given IM or orally.

Carbenicillin

Antibiotic
$C_{17}H_{18}N_2O_6S = 378.4$
CAS—4697-36-3

IUPAC Name (2*S*,5*R*,6*R*)-6-[(Carboxyphenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Synonym α-Carboxybenzylpenicillin

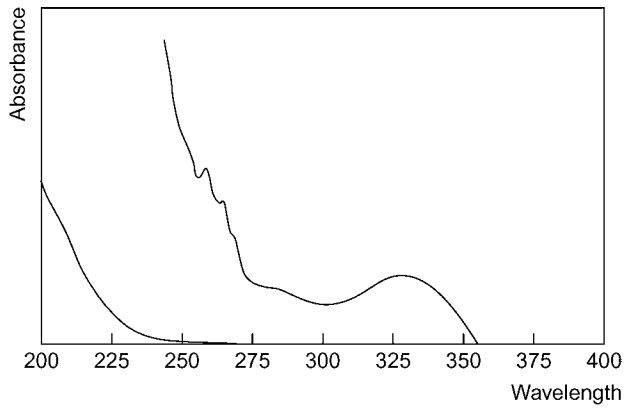


Chemical Properties pK_a 2.6, 2.7. Log *P* (octanol/water), 1.1.

Carbenicillin Sodium

$C_{17}H_{16}N_2Na_2O_6S = 422.4$
CAS—4800-94-6
Proprietary Names *Anabactyl*; *Carbapen*; *Fugacillin*; *Geopen*; *Microcillin*; *Pyopen*.
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 1.2 of water and 1 in 25 of ethanol; practically insoluble in chloroform and ether.

Ultraviolet Spectrum Aqueous acid—259, 265, 327 nm.



Infrared Spectrum Principal peaks at wavenumbers 1612, 1754, 1315, 1123, 1030, 1000 cm^{-1}.

Quantification
Plasma **HPLC** Limit of detection, 10 mg/L [Ishida *et al.* 1994].
Serum **HPLC** UV detection (λ=208 nm). Limit of quantification, 0.25 mg/L [Naidong *et al.* 1994].
Urine **HPLC** See Plasma [Ishida *et al.* 1994].

Disposition in the Body
Therapeutic Concentration
Four healthy male volunteers aged 20 to 23 years were fasted overnight and administered 2 g of carbenicillin IV. The peak plasma concentrations of the drug were 88 mg/L for *R*-carbenicillin and 83 mg/L for *S*-carbenicillin ~30 min after dosing [Ishida *et al.* 1994].

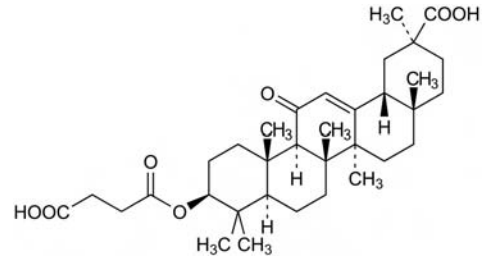
Dose The equivalent of 20 to 30 g of carbenicillin daily, IV.

Ishida M *et al.* (1994). Determination of carbenicillin epimers in plasma and urine with high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 652(1): 43–49.
Naidong W *et al.* (1994). Development and validation of an LC method for the quantitation of carbenicillin in human serum. *J Pharm Biomed Anal* 12: 845–850.

Carbenoxolone

Treatment of Peptic Ulcer
$C_{34}H_{50}O_7 = 570.8$
CAS—5697-56-3

IUPAC Name (2*S*,4*aS*,6*aR*,6*aS*,6*bR*,8*aR*,10*S*,12*aS*, 14*bR*)-10-(4-Hydroxy-4-oxobutanoyl)oxy-2,4*a*,6*a*,6*b*,9,9, 12*a*-heptamethyl-13-oxo-3,4,5,6,6*a*,7,8,8*a*,10,11,12, 14*b*-dodecahydro-1*H*-picene-2-carboxylic acid
Synonyms (3β,20β)-3-(3-Carboxy-1-oxopropoxy)-11-oxoolean-12-en-29-oic acid; glycerrhetic acid hydrogen succinate; glycyrrhetinic acid hydrogen succinate.

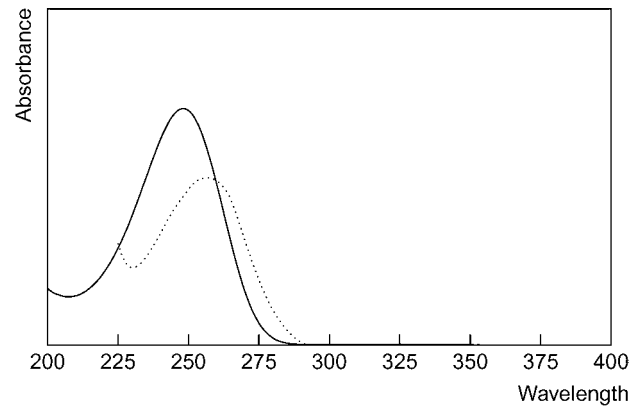


Chemical Properties Cream-coloured crystals. Mp 291° to 294°. pK_a 6.7, 7.1. Log *P* (octanol/water), 7.1.

Carbenoxolone Sodium

$C_{34}H_{48}Na_2O_7 = 614.7$
CAS—7421-40-1
Synonym Disodium enoxolone succinate
Proprietary Names *Biogastrone*; *Duogastrone*; *Gastrausil*; *Neogel*; *Ulcus-Tablinen*. It is an ingredient of *Pyrogastrone*.
Chemical Properties A white or pale cream-coloured hygroscopic powder. Soluble 1 in 6 of water and 1 in 30 of ethanol; insoluble in chloroform and ether.

Caution Carbenoxolone sodium powder is irritating to nasal membranes.
Colour Tests Antimony pentachloride—brown; naphthol–sulfuric acid—yellow-brown/orange; sulfuric acid—yellow; mix 5 mg with 50 mg of resorcinol and 2 mL of 80% v/v sulfuric acid, heat at 200° for 10 min, cool, and add to 200 mL of water; make just alkaline with sodium hydroxide solution—an intense green fluorescence is produced.
Thin-layer Chromatography System TD—R_f 0.07; system TE—R_f 0.00; system TF—R_f 0.17; system TAD—R_f 0.28 (acidified potassium permanganate solution, positive, faint).
High Performance Liquid Chromatography System HY—RI 824.
Ultraviolet Spectrum Carbenoxolone sodium: aqueous acid—248 nm; aqueous alkali—257 nm (A^1_1=172b).



Infrared Spectrum Principal peaks at wavenumbers 1562, 1642, 1715, 1289, 1261, 1210 cm^{-1} (carbenoxolone sodium, KBr disk).

Quantification
Serum **GC** FID. Limit of detection, 5 mg/L [Rhodes, Wright 1974].
Radioimmunoassay Limit of detection, 1 µg/L [Peskar *et al.* 1976].

Disposition in the Body Carbenoxolone is rapidly and almost completely absorbed after oral administration; absorption is reduced at gastric pH levels above 2 and delayed after food. It is almost entirely eliminated in the faeces via the bile, <5% being excreted in the urine. Carbenoxolone is excreted mainly as the glucuronide conjugate together with small amounts as the sulfate conjugate of β-glycyrrhetic acid (enoxolone).

Therapeutic Concentration

After a single oral dose of 200 mg to 10 subjects, peak blood concentrations of 13 to 35 (mean 24) mg/L were attained in 1 to 2 h, followed by a second peak of 3 to 32 (mean 20) mg/L at 3 to 6 h; in some subjects, the second peak was higher than the first [Downer *et al.* 1970].

Following oral administration of 100 mg to 3 fasting subjects, peak serum concentrations of 15 to 20 mg/L were attained in 1 to 2 h; following administration of 100 mg to 2 subjects after meals, peak serum concentrations of 8 to 9 mg/L were reported at 5 to 6 h.

After oral doses of 100 mg three times a day to 9 subjects, serum concentrations of 20 to 98 (mean 49) mg/L were reported on the eighth day [Baron *et al.* 1975].

Half-life Plasma half-life 8 to 20 (mean 13) h, increased in elderly subjects.
Volume of Distribution About 0.1 L/kg.
Protein Binding >99%.
Note For a review of carbenoxolone, see Pinder *et al.* [1976].
Dose 150 to 300 mg of carbenoxolone sodium daily.

Baron JH *et al.* (1975). Factors affecting the Absorption of Carbenoxolone in Patients with Peptic Ulcer. In: Jones FA, Parke DV, eds. *4th Symposium on Carbenoxolone.* London: Butterworths, 115–124

Downer HD *et al.* (1970). The absorption and excretion of carbenoxolone in man. *J Pharm Pharmacol* 22: 479–487.

Peskar BM *et al.* (1976). Radioimmunoassay for carbenoxolone. *J Pharm Pharmacol* 28: 720–721.

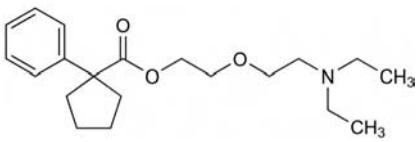
Pinder RM *et al.* (1976). Carbenoxolone: a review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease. *Drugs* 11: 245–307.

Rhodes C, Wright PA (1974). A gas chromatographic determination of carbenoxolone in human serum. *J Pharm Pharmacol* 26: 894–898.

Carbetapentane

Cough Suppressant
$C_{20}H_{31}NO_3$ = 333.5
CAS—77-23-6

IUPAC Name 2-(Diethylaminoethoxy)ethyl 1-phenylcyclopentanecarboxylate
Synonym Pentoxyverine

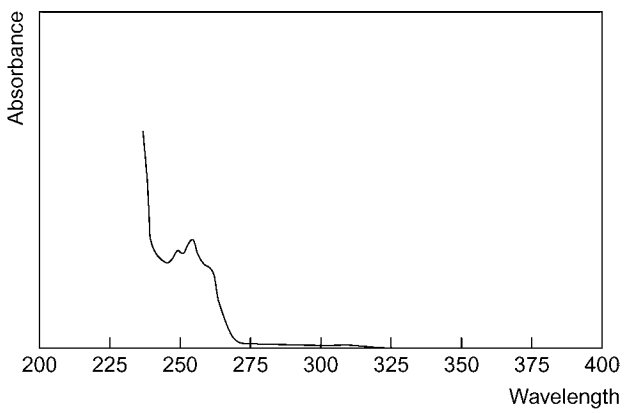


Chemical Properties Log *P* (octanol/water), 4.2.

Carbetapentane Citrate

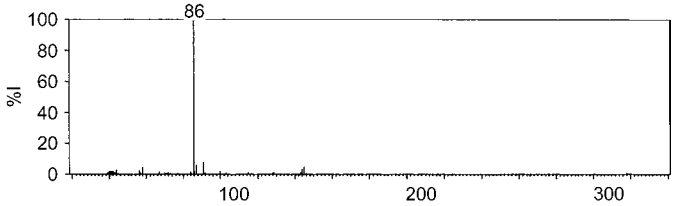
$C_{20}H_{31}NO_3$, $C_6H_8O_7$ = 525.6
CAS—23142-01-0
Proprietary Names *Atussil*; *Germapect*; *Sedotussin*; *Toclase*; *Tuclase*; *Tussa-Tablinen*.
Chemical Properties A white crystalline powder. Mp 93°. Freely soluble in water and chloroform; soluble in ethanol, acetone and ethyl acetate; practically insoluble in ether and in benzene.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—brown (slow); Marquis test—orange (slow).
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.48; system T—R_f 0.22 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown).
Gas Chromatography System GA—RI 2232; system GF—RI 2455.
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=6.1a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1725, 1120, 1158, 1176, 694, 1234 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 86, 91, 87, 145, 58, 144, 30, 44.

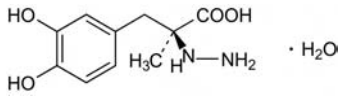


Dose 25 to 150 mg of carbetapentane citrate daily.

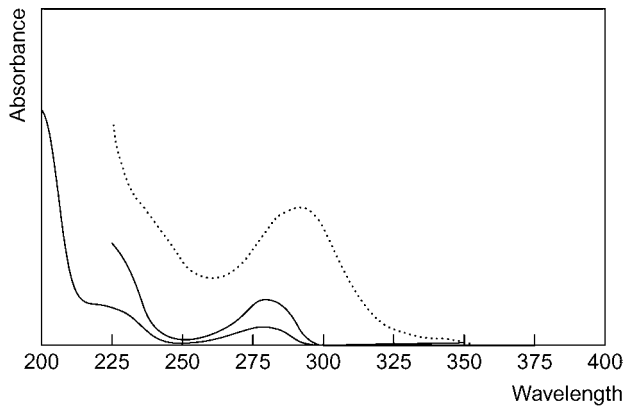
Carbidopa

Dopa-Decarboxylase Inhibitor
$C_{10}H_{14}N_2O_4$, H_2O = 244.2
CAS—28860-95-9 (anhydrous); 38821-49-7 (monohydrate)

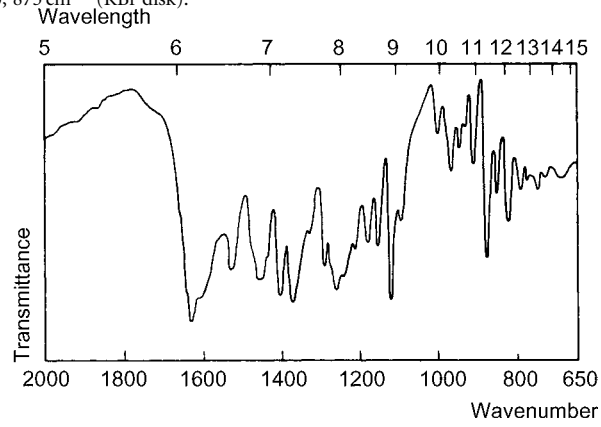
IUPAC Name (2*S*)-3-(3,4-Dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid hydrate
Synonyms HMD; (α*S*)-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid monohydrate; (−)-L-α-Methyldopa hydrazine; Mk-486.
Proprietary Name It is an ingredient of *Sinemet*.



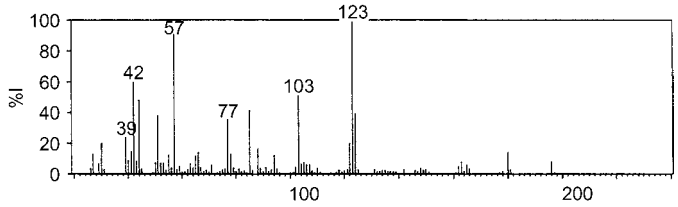
Chemical Properties A white or creamy-white powder. Mp 203° to 205°, with decomposition. Soluble 1 in 500 of water; practically insoluble in ethanol, chloroform, and ether; freely soluble in 3 mol/L hydrochloric acid. Log *P* (octanol/water), −0.1. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Tests Ammoniacal silver nitrate (on warming) —silver mirror; ferric chloride—brown; Folin-Ciocalteu reagent—blue; Liebermann's reagent—black; Marquis test—brown; methanolic potassium hydroxide—orange; Millon's reagent (cold)—orange; Nessler's reagent—orange→black; palladium chloride—orange→brown; potassium dichromate (method 1)—red.
Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.02; system TAD—R_f 0.04.
Gas Chromatography System GA—carbidopa, not eluted; carbidopa-Me_2 RI 1660.
High Performance Liquid Chromatography System HY—RI 190.
Ultraviolet Spectrum Methanolic acid—282 nm (A^1_1=130a); aqueous alkali—291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1625, 1121, 1260, 1525, 1290, 875 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 123, 57, 42, 103, 44, 85, 124, 51.



Quantification

Plasma **HPLC** Electrochemical detection. Limit of detection, 8 µg/L [Sagar, Smyth 2000]. Electrochemical detection. See Lucarelli *et al.* [1990]. Electrochemical detection. See Titus *et al.* [1990]. Electrochemical detection. Limit of detection, 15 µg/L [Nissinen, Taskinen 1982].

Urine **HPLC** See Plasma Titus *et al.* [1990].

Disposition in the Body Readily but incompletely absorbed after oral administration. About 50% of an oral dose is excreted in the urine in 48 h and 47% is eliminated in the faeces. Of the urinary material, about 30% is unchanged drug, 10 to 14% is 2-(4-hydroxy-3-methoxybenzyl)propionic acid, 10% is 2-(3,4-dihydroxybenzyl)propionic acid, 5% is 3,4-dihydroxyphenylacetone and 10% is 2-(3-hydroxybenzyl)propionic acid. The metabolites are excreted mainly as glucuronide conjugates.

Therapeutic Concentration

Following an oral dose of 50 mg to 10 subjects, a mean peak plasma concentration of 0.2 mg/L was attained in 2 to 4 h [Vickers *et al.* 1974].

Half-life Plasma half-life, about 2 h.

Protein Binding About 36%.

Note For a review of the pharmacokinetics of carbidopa, see Pinder *et al.* [1976].

Dose Usually the equivalent of 75 to 150 mg of anhydrous carbidopa daily, given in combination with levodopa.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Lucarelli C *et al.* (1990). Simultaneous measurement of L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients by improved sample pretreatment and high-performance liquid chromatographic determination. *J Chromatogr* 511: 167–176.

Nissinen E, Taskinen J (1982). Simultaneous determination of carbidopa, levodopa and 3,4-dihydroxyphenyl-acetic acid using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* 231: 459–462.

Pinder RM *et al.* (1976). Levodopa and decarboxylase inhibitors: a review of their clinical pharmacology and use in the treatment of parkinsonism. *Drugs* 11: 329–377.

Sagar KA, Smyth MR (2000). Simultaneous determination of levodopa, carbidopa and their metabolites in human plasma and urine samples using LC-EC. *J Pharm Biomed Anal* 22: 613–624.

Titus DC *et al.* (1990). Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection. *J Chromatogr* 534: 87–100.

Vickers S *et al.* (1974). Metabolism of carbidopa (1-(-)-alpha-hydrazino-3,4-dihydroxy-alpha-methylhydrocinnamic acid monohydrate), an aromatic amino acid decarboxylase inhibitor, in the rat, rhesus monkey, and man. *Drug Met Disp* 2: 9–22.

Carbimazole

Antithyroid Agent

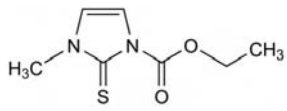
$C_7H_{10}N_2O_2S$ = 186.2

CAS—22232-54-8

IUPAC Name Ethyl 3-methyl-2-sulfanylideneimidazole-1-carboxylate

Synonym 2,3-Dihydro-3-methyl-2-thioxo-1*H*-imidazole-1-carboxylic acid ethyl ester

Proprietary Names *Carbazole*; *Carbotiroid*; *Neo-Mercazole*; *Neo-Morphazole*; *Neo-Thyreostat*; *Neo-Tireol.*



Chemical Properties A white or creamy-white crystalline powder. Mp 122° to 125°. Soluble 1 in 500 of water, 1 in 50 of ethanol, 1 in 17 of acetone, 1 in 3 of chloroform and 1 in 330 of ether. Log *P* (octanol/water), 0.5.

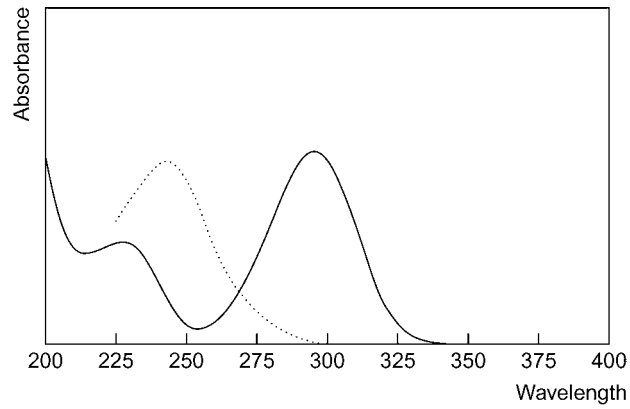
Colour Tests Palladium chloride—orange; to a small quantity add 1 drop of iodobismuthous acid solution—red.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.01; system TD—R_f 0.63; system TE—R_f 0.42; system TF—R_f 0.47; system TAD—R_f 0.68; system TAE—R_f 0.75; system TAF—R_f 0.75 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

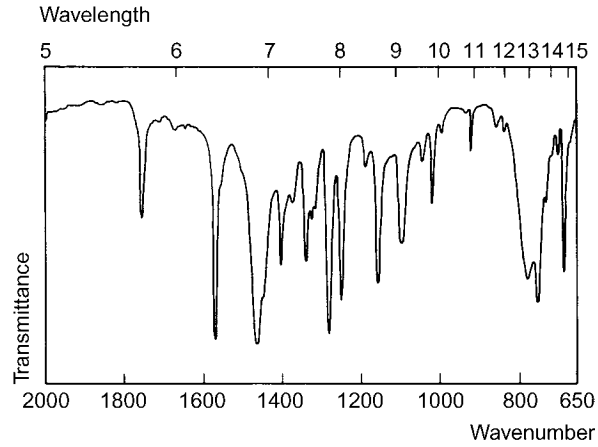
Gas Chromatography System GA—carbimazole RI 1678, thiamazole RI 1550.

High Performance Liquid Chromatography System HX—RI 318; system HAA—retention time 11.1 min.

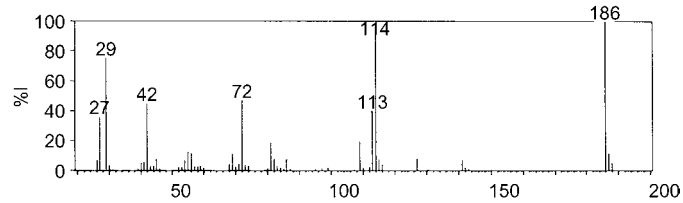
Ultraviolet Spectrum Aqueous acid—291 nm (A_1^1=557a); aqueous alkali—244 nm.



Infrared Spectrum Principal peaks at wavenumbers 1574, 1275, 740, 1246, 1150, 767 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 186, 114, 29, 72, 42, 113, 27, 109; thiamazole 114, 42, 72, 113, 69, 81, 54, 115.



Quantification

Plasma **GC** AFID. Limit of detection, 30 µg/L for thiamazole [Bending, Stevenson 1978].

GC–MS Limit of detection, 2 µg/L for thiamazole [Floberg *et al.* 1980].

HPLC Absorbance detection at 405 nm. Limit of detection, 5 µg/L for thiamazole [Meulemans *et al.* 1980].

Disposition in the Body Carbimazole is rapidly and almost completely absorbed after oral administration and converted to the active metabolite thiamazole. It is almost completely excreted in the urine in 24 h as metabolites; 3-methyl-2-thiohydantoin has been identified as a minor metabolite in urine and plasma. About 3% of a dose is eliminated in the faeces.

Therapeutic Concentration

After an oral dose of 60 mg, given to 11 subjects, peak serum concentrations of thiamazole of 0.5 to 3.4 (mean 0.9) mg/L were attained in about 0.7 to 3 h [Melander *et al.* 1980].

Half-life Plasma half-life, thiamazole about 3 to 5 h.

Volume of Distribution Thiamazole, about 0.5 L/kg.

Protein Binding Thiamazole, not significantly bound.

Note For a review of the pharmacokinetics of antithyroid drugs, see Kampmann and Hansen [1981].

Dose Initially 30 to 60 mg daily; maintenance, 5 to 20 mg daily.

Bending MR, Stevenson D (1978). Measurement of methimazole in human plasma using gas-liquid chromatography. *J Chromatogr* 154: 267–271.

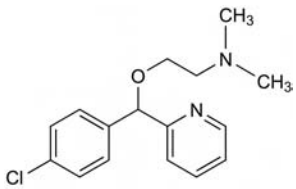
Floberg S *et al.* (1980). Determination of methimazole in plasma using gas chromatography–mass spectrometry after extractive alkylation. *J Chromatogr* 182: 63–70.

Kampmann JP, Hansen JM (1981). Clinical pharmacokinetics of antithyroid drugs. *Clin Pharmacokinet* 6: 401–428.
Melander A *et al.* (1980). Comparative in vitro effects and in vivo kinetics of antithyroid drugs. *Eur J Clin Pharmacol* 17: 295–299.
Meulemans A *et al.* (1980). Determination of methimazole in plasma by high performance liquid chromatography. *J Liq Chromatogr* 3(2): 287–298.

Carbinoxamine

Antihistamine
$C_{16}H_{19}ClN_2O = 290.8$
CAS—486-16-8

IUPAC Name 2-[(4-Chlorophenyl)-2-pyridinylmethoxy]-*N*,*N*-dimethylethanamine
Synonym Paracarbinoxamine

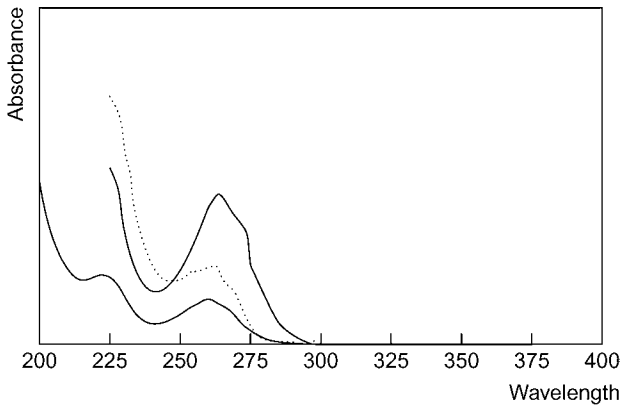


Chemical Properties A liquid. pK_a 8.1 (25°). Log *P* (octanol/water), 2.6.

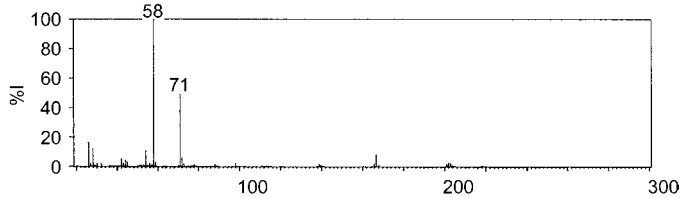
Carbinoxamine Maleate

$C_{16}H_{19}ClN_2O$, $C_4H_4O_4 = 406.9$
CAS—3505-38-2
Proprietary Names *Allergefon*; *Clistin*; *Histex*; *Ziriton*. It is an ingredient of *Davenol*, *Extil*, and *Rondec*.
Chemical Properties A white crystalline powder. Mp 117° to 119°. Soluble 1 in less than 1 of water, 1 in 1.5 of ethanol, and 1 in 1.5 of chloroform; very slightly soluble in ether.

Colour Test Cyanogen bromide—orange-pink.
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.26; system TC—R_f 0.19; system TE—R_f 0.50; system TL—R_f 0.04; system TAE—R_f 0.13; system TAF—R_f 0.16; system TAJ—R_f 0.04; system TAK—R_f 0.00; system TAL—R_f 0.27 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, pink).
Gas Chromatography System GA—carbinoxamine RI 2080, M (chlorobenzoylpyridine) RI 1645, M (carbinol) RI 1670, M (nor-) RI 2150; system GB—RI 2147; system GC—RI 2430.
High Performance Liquid Chromatography System HA—*k* 4.7 (tailing peak); system HX—RI 359; system HAA—retention time 12.8 min.
Ultraviolet Spectrum Aqueous acid—263 nm (A^1_1=323a); aqueous alkali—261 nm (A^1_1=181a).



Infrared Spectrum Principal peaks at wavenumbers 1084, 1110, 1584, 1041, 763, 1010 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 71, 26, 54, 167, 72, 42, 44.



Quantification
Plasma **GC** AFID. Limit of detection, 200 ng/L [Hoffman *et al.* 1983].

HPLC Coulometric detection. Limit of quantification, 2 µg/L, limit of detection, 0.5 µg/L [Stockis *et al.* 1995]. Coulometric detection. For method see Stockis *et al.* [1992].

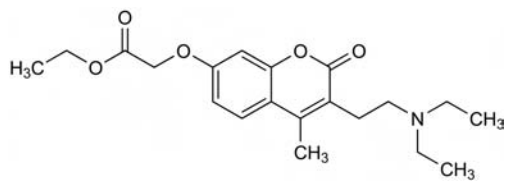
Disposition in the Body
Therapeutic Concentration Twenty healthy males and females (20 to 42 years) were administered a single medication containing 20 mg phenylephrine and 4 mg carbinoxamine maleate. The peak plasma carbinoxamine concentration was 6.5 µg/L, 2 to 6 h after dosing for a single dose and a steady state concentration of 13.5 µg/L, approximately 4.8 h after dosing was observed over a 4-day dosing period [Stockis *et al.* 1995].
Dose 12 to 32 mg of carbinoxamine maleate daily.

Hoffman DJ *et al.* (1983). Capillary GLC assay for carbinoxamine and hydrocodone in human serum using nitrogen-sensitive detection. *J Pharm Sci* 72: 1342–1344.
Stockis A *et al.* (1995). Relative bioavailability of carbinoxamine and phenylephrine from a retard capsule after single and repeated dose administration in healthy subjects. *Arzneimittelforschung* 45: 1009–1112.
Stockis A *et al.* (1992). Relative bioavailability of carbinoxamine and phenylpropanolamine from a retard suspension after single dose administration in healthy subjects. *Arzneimittelforschung* 42 (12): 1478–1481.

Carbocromen

Antianginal Vasodilator
$C_{20}H_{27}NO_5 = 361.4$
CAS—804-10-4

IUPAC Name Ethyl 2-[3-(2-diethylaminoethyl)-4-methyl-2-oxochromen-7-yl] oxyacetate
Synonyms Chromonar; [[3-[2-(diethylamino)ethyl]-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl]oxy]-acetic acid ethyl ester.

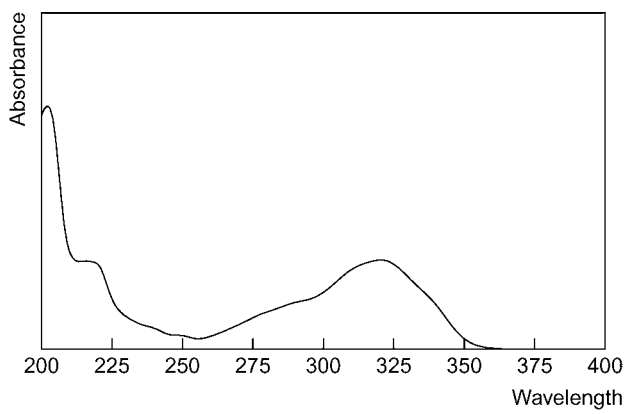


Chemical Properties Practically insoluble in water; soluble in chloroform and ether. Log *P* (octanol/water), 3.4.

Carbocromen Hydrochloride

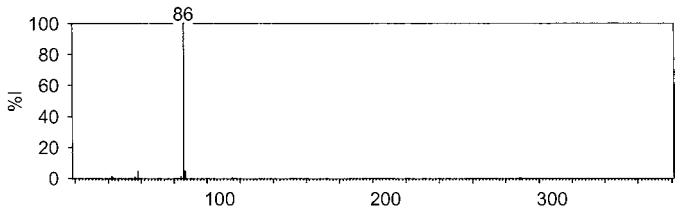
$C_{20}H_{27}NO_5$, HCl = 397.9
CAS—655-35-6
Synonym Cassella 4489
Proprietary Name *Intensain*
Chemical Properties A white crystalline powder. Mp 159° to 160°. Freely soluble in water, ethanol, methylene chloride, and chloroform; sparingly soluble in acetone, benzene, and ether.

Colour Tests Aromaticity (method 2)—yellow/orange; Liebermann's reagent (100°)—blue (3 min); sulfuric acid—violet (blue fluorescence at 350 nm).
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.17; system TC—R_f 0.24; system TE—R_f 0.62; system TL—R_f 0.12; system TAE—R_f 0.18 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2835.
Ultraviolet Spectrum Aqueous acid—202, 216, 321 nm (A^1_1=527a).



Infrared Spectrum Principal peaks at wavenumbers 1708, 1608, 1210, 1755, 1178, 1085 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 86, 87, 58, 30, 29, 84, 56, 42.

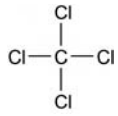


Quantification
Plasma **Spectrofluorimetry** Limit of detection, 40 µg/L [Martin, Wiegand 1970].
Urine **Spectrofluorimetry** See Plasma [Martin, Wiegand 1970].
Disposition in the Body Incompletely absorbed after oral administration (about 35%); rapidly hydrolysed to the corresponding carboxylic acid. About 70% of an IV dose and 20% of an oral dose are excreted in the 24-h urine as the carboxylic acid metabolite.
Therapeutic Concentration
Following oral administration of 150 mg three times a day to 6 subjects, a peak plasma concentration of 0.9 mg/L of the carboxylic acid metabolite was reported, declining to 0.06 mg/L, 10 h after the last dose [Martin, Wiegand 1970].
Half-life Plasma half-life, carboxylic acid metabolite about 0.8 h.
Volume of Distribution Carboxylic acid metabolite, about 0.4 L/kg.
Dose Carbocromen hydrochloride has been given in doses of 225 to 675 mg daily.

Martin YC, Wiegand RG (1970). Metabolism and excretion of chromonar and its metabolite in dog and man. *J Pharm Sci* 59: 1313–1318.

Carbon Tetrachloride

Anthelmintic (Veterinary), Solvent
CCl_4 = 153.8
CAS—56-23-5
IUPAC Name Tetrachloromethane
Synonyms Carboneum tetrachloratum medicinale; methane tetrachloride; perchloromethane; tetrachlorocarbon; tetracloruro de carbono.

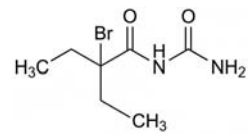


Chemical Properties A heavy, clear, colourless, volatile liquid, with a chloroform-like odour. It is non-inflammable, but in contact with a flame it decomposes and gives rise to toxic products (phosgene, carbon dioxide and hydrochloric acid), which have an acrid odour. Weight per millilitre, 1.592–1.595 g. Bp 76° to 78°. Refractive index 1.4607. Soluble 1 in 2000 water; miscible with dehydrated alcohol, benzene, chloroform and ether. Log *P* (octanol), 2.8.
Colour Test Fujiwara test—red.
Gas Chromatography System GA—RI 1000; system GI—RT 8.6 min; system GAA—RI 661.
Mass Spectrum Principal ions at *m/z* 117, 119, 47, 35, 121, 82, 84, 49.
Quantification
Blood **GC** Column: Carbowax 20 M on Carbopak P 60/80 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 90°. FID. Limit of detection, 50 mg/L [Tombolini, Cingolani 1996]. Column: 2.5% OV 17 silicone on Chromosorb G 80/100 mesh (6 ft × 1/8 in i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 150°. FID. Limit of detectin, 2 µg/L [Goldermann *et al.* 1983]. Column: 20% Supelco SP-2100 plus 0.1% Carbowax 1500 on Supelcoport 80/100 mesh (3.0 m × 2.0 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 95°. FID. Limit of detection, 5 µg [Reddrop *et al.* 1980].
GC-MS See GC. Carrier gas: 45 mL/min. Temperature: 75°. ECD. Limit of detection, 15 ng [Reddrop *et al.* 1980].
Urine **GC** See Blood [Tombolini, Cingolani 1996].
Bile **GC** See Blood [Tombolini, Cingolani 1996].
Vitreous Humour **GC** See Blood [Tombolini, Cingolani 1996].
Brain **GC** See Blood [Tombolini, Cingolani 1996].
Kidney **GC** See Blood [Tombolini, Cingolani 1996].
Liver **GC** See Blood [Tombolini, Cingolani 1996]. See Blood [Reddrop *et al.* 1980].
GC-MS See Blood [Reddrop *et al.* 1980].
Lung **GC** See Blood [Tombolini, Cingolani 1996].
Expired Air **GC** See Blood [Reddrop *et al.* 1980].
GC-MS See Blood [Reddrop *et al.* 1980].
Muscle **GC** See Blood [Tombolini, Cingolani 1996].
Myocardium **GC** See Blood [Tombolini, Cingolani 1996].
Spleen **GC** See Blood [Tombolini, Cingolani 1996].
Disposition in the Body Carbon tetrachloride is readily absorbed after inhalation and also absorbed after ingestion or through the skin; the rate of absorption is increased by the concomitant ingestion of alcohol. It is excreted mainly from the lungs as carbon tetrachloride and carbon dioxide; it is excreted in urine as urea and an unidentified metabolite, and is eliminated in faeces.
Toxicity The minimum lethal dose is 3–5 mL, but recoveries have occurred following ingestion of 30–40 mL. Carbon tetrachloride injures almost all cells of the body including those of the blood, CNS, liver and kidney; the kidneys and liver of those who have died often show marked fatty degeneration. The maximum permissible atmospheric concentration is 10 ppm. Inhalation of concentrations of 1000 ppm, even for short periods, may cause acute toxic reactions. Continued exposure to concentrations of approx. 100 ppm may give rise to chronic poisoning. When carbon tetrachloride is ingested together or immediately after alcohol, its toxicity, particularly nephrotoxicity, is greatly increased. Toxic effects are associated with blood concentrations of 20–50 mg/L and a postmortem blood concentration of 260 mg/L has been reported in one fatality.
A 75-year-old man died in hospital after ingesting an unknown amount of carbon tetrachloride. Concentrations were 328.5 mg/L in urine, 169.8 mg/L in bile, 143.4 mg/L in systemic venous blood, 57.5 mg/L in arterial blood and 170.5 mg/L in vitreous humour. In tissues, the concentrations were 657.9 mg/kg in pancreas, 243 mg/kg in brain, 237.3 mg/kg in testis, 127.3 mg/kg in lungs, 150.5 mg/kg in kidneys, 71.1 mg/kg in muscle, 78.5 mg/kg in myocardium, 68.3 mg/kg in spleen and 58.6 mg/kg in liver [Tombolini, Cingolani 1996].
The following postmortem tissue concentrations were reported in a fatality caused by inhalation of carbon tetrachloride: blood 18 mg/L, brain 175 µg/g, lung 12.5 µg/g [Franc 1983].
A 36-year-old man drank 50 mL of carbon tetrachloride. At the beginning of hyperventilation therapy blood concentrations were 120 µg/L. These levels fell to 30 µg/L within 6 days but the patient died from pneumonia 39 days after admission [Goldermann *et al.* 1983].
A 61-year-old man ingested a large amount of carbon tetrachloride, well in excess of the adult lethal dose. He was treated and survived with relatively mild clinical and biochemical evidence of toxicity [Mathieson *et al.* 1985].
A middle-aged alcoholic ingested ~30 mL carbon tetrachloride under the impression that it was alcohol. He was seriously ill but recovered. On his admission to hospital, his serum concentration of carbon tetrachloride was 20 mg/L. The first 24-h urine collection contained 8 mg/L and the first peritoneal dialysate contained 1 mg/L of carbon tetrachloride [SL Tompsett, personal communication 1967].
The following postmortem tissue concentrations were reported in a fatality caused by inhalation of carbon tetrachloride: kidney 32 µg/g, liver 142 µg/g, lung 39 µg/g, muscle 46 µg/g [Korenke, Pribilla 1969].

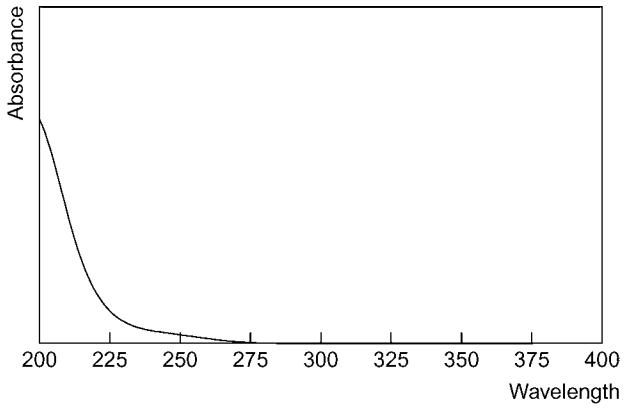
Franc A (1983). *TIAFT Bull* 17: 22–25.
Goldermann L *et al.* (1983). Quantitative assessment of carbon tetrachloride levels in human blood by head-space gas chromatography: application in a case of suicidal carbon tetrachloride intoxication. *Intensive Care Med* 9: 131–135.
Korenke HD, Pribilla O (1969). [Suicide by single inhalation of carbon tetrachloride (CCl_4), with resulting leukoencephalopathy.]. *Arch Toxikol* 25: 109–126.
Mathieson PW *et al.* (1985). Survival after massive ingestion of carbon tetrachloride treated by intravenous infusion of acetylcysteine. *Hum Toxicol* 4: 627–631.
Reddrop CJ *et al.* (1980). Two rapid methods for the simultaneous gas–liquid chromatographic determination of carbon tetrachloride and chloroform in biological material and expired air. *J Chromatogr* 193: 71–82.
Tombolini A, Cingolani M (1996). Fatal accidental ingestion of carbon tetrachloride: a postmortem distribution study. *J Forensic Sci* 41: 166–168.

Carbromal

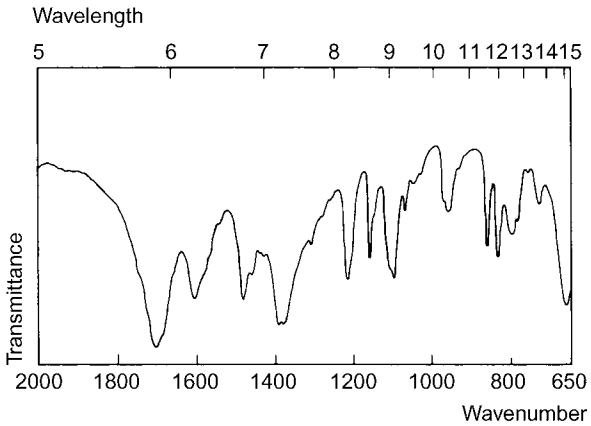
Hypnotic
$C_7H_{13}BrN_2O_2$ = 237.1
CAS—77-65-6
IUPAC Name 2-Bromo-N-carbamoyl-2-ethylbutanamide
Synonyms *N*-(Aminocarbonyl)-2-bromo-2-ethylbutanamide; bromadal; bromodiethylacetylurea; karbromal; uradal.
Proprietary Names *Adalin*; *Mirfudorm*.



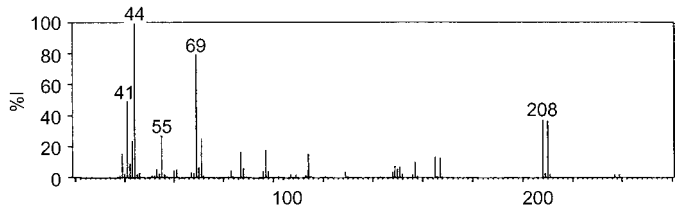
Chemical Properties A white crystalline powder. Mp 116° to 119°. Soluble 1 in 3000 of water, 1 in 18 of ethanol, 1 in 3 of chloroform and 1 in 14 of ether. Log *P* (octanol/water), 1.5.
Colour Test Nessler's reagent—brown-orange.
Thin-layer Chromatography System TB—R_f 0.12; system TD—R_f 0.53; system TE—R_f 0.75; system TF—R_f 0.55; system TAD—R_f 0.64; system TAE—R_f 0.85; system TAF—R_f 0.87 (fluorescein solution, pink).
Gas Chromatography System GA—carbromal RI 1513, M (carbromide) RI 1215, M (OH-carbromide) RI 1340, M (desbromo-) RI 1380, carbromal-Art RI 1450.
High Performance Liquid Chromatography System HX—RI 410; system HY—RI 377; system HZ—retention time 3.9 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1694, 660, 1600, 1094, 1212, 834 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 69, 41, 208, 210, 55, 71, 43; 2-bromo-2-ethylbutyramide 69, 43, 41, 44, 71, 167, 165, 55; 2-bromo-2-ethyl-3-hydroxybutyramide 150, 152, 165, 41, 167, 43, 44, 130; 2-ethylbutyrylurea 45, 130, 44, 71, 42, 61, 115, 55.



Quantification

Blood **GC** FID. For determination of inorganic bromide, see Wells, Cimbura [1973].

Plasma **HPLC** UV detection. Carbromal, 2-bromo-2-ethylbutyramide and 2-ethylbutyrylurea. Limit of detection, 200 µg/L for carbromal [Eichelbaum *et al.* 1978].

Disposition in the Body Carbromal is readily absorbed after oral administration. The major metabolite is free bromide ion; hydrolysis to an active metabolite, 2-bromo-2-ethylbutyramide, also occurs followed by oxidation to 2-bromo-2-ethyl-3-hydroxybutyramide; other metabolites include 2-ethylbutyrylurea and 2-ethyl-2-hydroxybutyric acid. Carbromal is excreted in the urine mainly as bromide ion and partly as 2-ethyl-2-hydroxybutyric acid, with very little as unchanged drug. Peak bromide excretion is attained after about 48 h.

Therapeutic Concentration

Following a single oral dose of 1 g given to 4 subjects, mean peak serum concentrations of about 6 mg/L of carbromal and 3 mg/L of 2-bromo-2-ethylbutyramide were attained in 0.5 and 4 h respectively; the total bromide concentration reached about 12 mg/L in 9 h and was still increasing [Vohland *et al.* 1976].

Toxicity Fatalities have occurred in adults following the ingestion of 10 to 25 g but they are rare. Long-term use of carbromal may give rise to symptoms of chronic toxicity resembling bromism. Toxic effects are associated with serum-bromide concentrations of 300 to **1200** to 3200 mg/L.

In 11 fatalities attributed to carbromal overdose, the following post-mortem tissue concentrations, mg/L or µg/g (mean, *N*), were reported

	Carbromal	*Bromoethylbutyramide*
Blood	19.9–26.1 (23, 3)	0.2–27.2 (15, 10)
Brain	0.6–120.9 (45, 8)	23.3–118.4 (66, 11)
Kidney	0.4–13 (6, 4)	16.1–79.6 (45, 9)
Liver	1.1–2.8 (2.3, 4)	1.75–50.5 (14, 9)
Urine	0.85–47.4 (24, 5)	2.5–36.9 (23, 8)

Total bromide concentrations in the blood ranged from 145 to 1898 (mean 536) mg/L [Kaferstein, Sticht 1978].

Post-mortem serum-carbromal concentrations of 158 and 71 mg/L were found in 2 fatalities resulting from acute intoxication [Gruska *et al.* 1970 and [Gruska *et al.* 1971].

Half-life Plasma half-life, carbromal 7 to 15 h, bromide about 15 days.

Dose 0.3 to 1 g, as a hypnotic.

Eichelbaum M *et al.* (1978). Determination of monoureides in biological fluids by high-pressure-liquid-chromatography. *Arch Toxicol* 41(3): 187–193.

Gruska H *et al.* (1970). Klinik, toxikologie und therapie einer schweren carbromalvergiftung mit letalem ausgang. *Arch Toxicol* 26(2): 149–160.

Gruska H *et al.* (1971). Klinik, toxikologie und therapie einer schweren carbamazepin-vergiftung. *Arch Toxicol* 27: 193–203.

Kaferstein H, Sticht G (1978). [Post mortem determination of bromureides (author's transl)]. *Z Rechtsmed* 81: 269–283.

Vohland HW *et al.* (1976). [On the toxicology of carbromal. I. Estimation of carbromal and its hypnotically active metabolites in rats and humans (author's transl)]. *Arch Toxicol* 36: 31–42.

Wells J, Cimbura G (1973). The determination of elevated bromide levels in blood by gas chromatography. *J Forensic Sci* 18(4): 437–440.

Carbutamide

Antidiabetic

$C_{11}H_{17}N_3O_3S = 271.3$

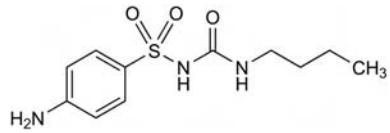
CAS—339-43-5

IUPAC Name 1-(4-Aminophenyl)sulfonyl-3-butylurea

Synonyms 4-Amino-*N*-[(butylamino)carbonyl]benzenesulfonamide; BZ-55; glybutamide; U-6987.

Proprietary Names *Diabetoplex*; *Dia-Tablinen*; *Glucidoral*; *Insoral*; *Invenol*; *Nadisan*.

Note *Insoral* is also used as a proprietary name for phenformin hydrochloride.

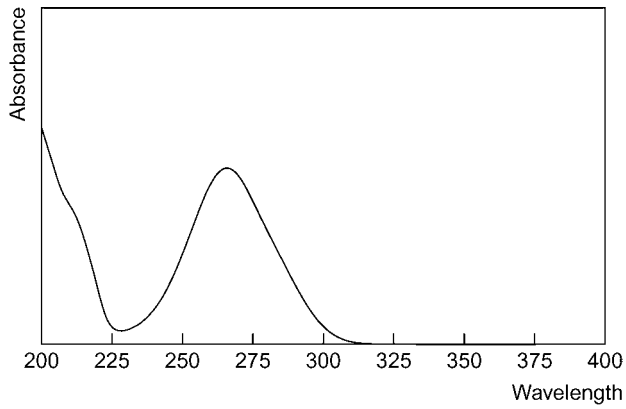


Chemical Properties A white finely crystalline powder. M.p. 144° to 145°. Soluble in water at pH 5–8. Practically insoluble in chloroform, and ether; soluble in ethanol and acetone. pK_a 6.0 (20°). Log *P* (octanol/water), 1.0.

Thin-layer Chromatography System TA—R_f 0.78; system TT—R_f 0.90; system TU—R_f 0.27; system TV—R_f 0.07; system TAE—R_f 0.87.

High Performance Liquid Chromatography System HY—RI 321; system HAA—retention time 14.5 min.

Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1=149a); aqueous alkali—255 nm (A^1_1=616a).



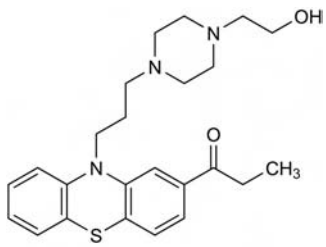
Infrared Spectrum Principal peaks at wavenumbers 1661, 1147, 1599, 1089, 1635, 1310 cm^{-1} (KBr disk).

Quantification
Serum **HPLC** UV detection. See Saffar *et al.* [1982].
Disposition in the Body Absorbed after oral administration. It is excreted in the urine mainly as the acetyl derivative.
Therapeutic Concentration
Following an oral dose of 250 mg to 5 subjects, a mean peak serum concentration of 48.8 mg/L was reported at 2.6 h [Saffar *et al.* 1982].
Half-life Plasma half-life, about 24 h.
Dose Carbutamide has been given in doses of 0.5 to 1 g daily.

Saffar F *et al.* (1982). Biopharmaceutical studies on the clinical inequivalence of two carbutamide tablets. *Chem Pharm Bull* 30: 679–683.

Carfenazine

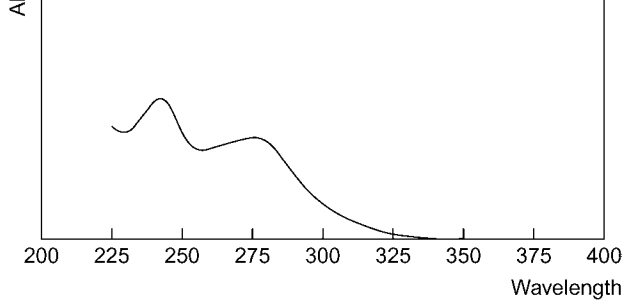
Antipsychotic, Tranquilliser
$C_{24}H_{31}N_3O_2S = 425.6$
CAS—2622-30-2
IUPAC Name 1-(10-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]-10*H*-phenothiazin-2-yl)-1-propanone
Synonym Carphenazine



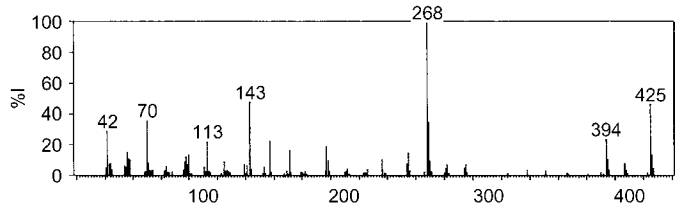
Chemical Properties Log *P* (octanol/water), 3.3.

Carfenazine Maleate
$C_{24}H_{31}N_3O_2S, 2C_4H_4O_4 = 657.7$
CAS—2975-34-0
Proprietary Name *Proketazine*
Chemical Properties A fine yellow powder. Mp 176° to 185°, with decomposition. Soluble 1 in 600 of water and 1 in 400 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Formaldehyde–sulfuric acid—blue; Forrest reagent—red; FPN reagent—red-orange; Liebermann's reagent—red-brown; Mandelin's test—red-violet; Marquis test—orange→red-violet.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.05; system TC—R_f 0.27; system TE—R_f 0.39; system TL—R_f 0.07; system TAE—R_f 0.39; system TAJ—R_f 0.08; system TAK—R_f 0.00; system TAL—R_f 0.51 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3590.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 419.
Ultraviolet Spectrum Aqueous acid—243 (A^{1}_{1}=606a), 277 nm. No alkaline shift.



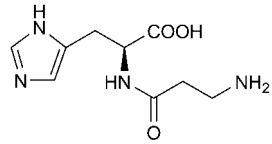
Infrared Spectrum Principal peaks at wavenumbers 1585, 1557, 1666, 1204, 751, 1298 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 268, 143, 425, 70, 269, 42, 394, 157.



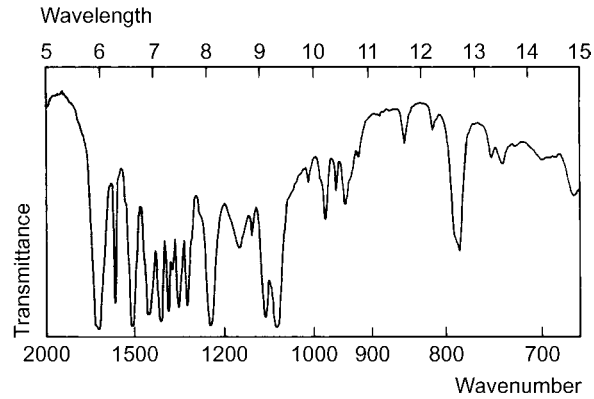
Dose 75 to 400 mg of carfenazine maleate daily.

Carisoprodol

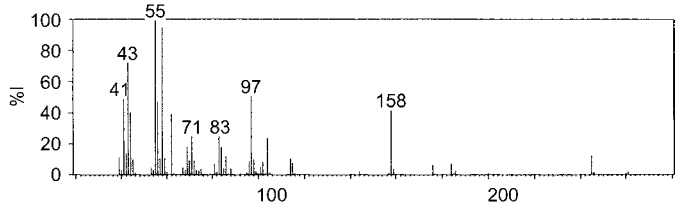
Skeletal Muscle Relaxant
$C_{12}H_{24}N_2O_4 = 260.3$
CAS—78-44-4
IUPAC Name [2-(Carbamoyloxymethyl)-2-methylpentyl] *N*-propan-2-ylcarbamate
Synonyms 2-[[(Aminocarbonyl)oxy]methyl]-2-methylpentyl (1-methylethyl) carbamate; carisoprodate; carisoprodolum; isobamate; *N*-isopropylmeprobamate.
Proprietary Names *Artifar*; *Caridolin*; *Carisoma*; *Chinchen*; *Dolaren*; *Flexartal*; *Mio Relax*; *Mioxom*; *Muslax*; *Myolax*; *Neotica*; *Rela*; *Rotalin*; *Sanoma*; *Scutamil-C*; *Soma*; *Somadril*; *Somalgit*. It is also an ingredient of *Flectomas*; *Flexidone*; *Lagaflex*; *Relaxibys*; *Sodol Compound*; *Soma Compound*; *Teknadone*.
Note The name Soma has also been applied to a hallucinogenic fungus.



Chemical Properties White crystalline powder. Mp 92° to 93°. Soluble in water: 30 mg/100 mL at 25°, 140 mg/100 mL at 50°; soluble in most common organic solvents; practically insoluble in vegetable oils. Log *P* (octanol/water) 2.36. Stable in dilute acids and alkalis. Carisoprodol in plasma was stable at −20° for up to 37 days [Kucharczyk *et al.* 1986].
Thin-layer Chromatography System TB—R_f 0.04; system TD—R_f 0.36; system TE—R_f 0.75; system TF—R_f 0.53; system TAD—R_f 0.59; system TAE—R_f 0.85; system TAF—R_f 0.79 (furfuraldehyde reagent, positive).
Gas Chromatography System GA—RI 1830; RI 1785 meprobamate.
Column: 2% GE-SE 30 on Aeropak 30* 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature programme: 100° to 210° at 4.2°/min. FID. Retention time: ≈16 min [Cardini *et al.* 1968].
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1695, 1527, 1075, 1246, 1101, 1319 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 55, 57, 43, 97, 41, 56, 158, 44; meprobamate 83, 84, 55, 56, 43, 71, 41, 62 (no peaks above 160).



Quantification
Blood **GC** Column: 5% phenyl methyl silicone (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 175° for 30 s to 220° at 10 /min to 260° at

20°/min for 6 min. FID. Limit of detection, 0.5 mg/L [Logan *et al.* 2000]. Column: 3% SE-30 on 80/100 mesh Chromosorb W AW-DMDS (4′ × 0.25" o.d.). Carrier gas: He, 60 mL/min. Temperature: 190°. FID. Retention time: 2.25 min. Limit of detection not reported [Maes *et al.* 1969].

GC-MS Column: DB-35 ms cross-linked silica capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 mg/L; limit of detection, 0.1 mg/L [Gunnar *et al.* 2004]. Column: DB-5 capillary (30 m × 0.25 mm i.d.). Temperature programme: 100° to 280° at 10/min for 11 min. Retention time: 7.53 and 8.44 for carisoprodol and meprobamate, respectively. Limit of detection not reported [Backer *et al.* 1990].

Plasma **GC** Column: 3% OV-17 on 100/120 mesh Chromosorb WHP (6′ × 2 mm i.d.). Carrier gas: N_2, 14 psi. Temperature: 180°. FID. Retention time: 5.25 and 4.81 min for carisoprodol and meprobamate, respectively. Limit of detection, 0.5 and 1 mg/L for carisoprodol and meprobamate, respectively [Kintz *et al.* 1988]. Column: 3% GP SP2100 DB on 100/120 mesh Supelcoport (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 180°. Retention time: ≈5.1 min. Limit of detection, 230 μg/L [Kucharczyk *et al.* 1986].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 190° for 5 min to 245° at 30°/min to 255° at 2°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1.93 mg/L, limit of detection, 0.58 mg/L, both for meprobamate [Daval *et al.* 2006].

LC-MS Column: Symmetry C_{18} reversed phase ODS (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (90 : 10 for 5 min to 20 : 80 at 15 min for 10 min), flow rate 150 μL/min. ESI, full scan and SIM acquisition modes. Limit of quantification, 0.5 μg/L [Matsumoto *et al.* 2003].

Serum **GC** Column: GP 2% SP-2110/1% SP-2510 DA on 100/120 Supelcoport (1.2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 180° for 2 min to 240° at 8°/min for 2 min. FID. Limit of detection not reported [Olsen *et al.* 1994]. Column: 3% OV-1 or 3% OV-17 (6′ × 0.25" o.d.). Carrier gas: N_2. Temperature programme: 140° for 1 min to 275° at 24°/min for 12 min. FID. Limit of detection not reported [Adams *et al.* 1975].

GC-MS See Blood [Gunnar *et al.* 2004].

Oral Fluid **LC-MS** Column: Atlantis dC_{18}(50 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 5 mmol/L aqueous ammonium acetate (pH 5.0; 10 : 90 to 40 : 60 at 4.0 min to 90 : 10 at 4.1 min until 8.0 min to 10 : 90 at 8.1 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, <0.02 μmol/L, limit of detection, <5.7 μg/L [øiestad *et al.* 2007].

Urine **GC** Column: 3% OV-17 on 100/120 mesh Chromosorb WHP (6′ × 2 mm i.d.). Carrier gas: N_2, 14 psi. Temperature: 180°. FID. Retention time: 5.25 and 4.81 min for carisoprodol and meprobamate, respectively. Limit of detection not reported [Kintz *et al.* 1988]. Column: 3% OV-1 or 3% OV-17 (6′ × 0.25" o.d.). Carrier gas: N_2. Temperature programme: 140° for 1 min to 275° at 24°/min for 12 min. FID. Limit of detection not reported [Adams *et al.* 1975]. See Blood [Maes *et al.* 1969].

GC-MS See Blood [Backer *et al.* 1990].

LC-MS Column: Symmetry C_{18} reversed phase ODS (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (90 : 10 for 5 min to 20 : 80 at 15 min for 10 min), flow rate 150 μL/min. ESI, full scan and SIM acquisition modes. Limit of quantification, 0.5 μg/L [Matsumoto *et al.* 2003].

Bile **GC** See Blood [Maes *et al.* 1969].

Stomach **GC** See Blood [Maes *et al.* 1969].

Vitreous Humour **GC-MS** See Blood [Backer *et al.* 1990].

Hair **GC-MS** Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° to 240° at 25°/min 300° at 10/min for 0.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.43 μg/g, limit of detection, 0.13 μg/g [Kim *et al.* 2005].

LC-MS Column: Zorbax SB-Phenyl (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: 25 mmol/L formic acid : acetonitrile (98 : 2 to 80 : 20 for 3 min to 10 : 90 in 4 min for 2 min), flow rate 0.25 mL/min. ESI, MRM acquisition mode. Limit of quantification, 12.5 ng/g [Hegstad *et al.* 2008].

Kidney **GC** See Blood [Maes *et al.* 1969].

Liver **GC** See Blood [Maes *et al.* 1969].

Spleen **GC** See Blood [Maes *et al.* 1969].

Disposition in the Body Rapidly absorbed after oral administration. It is metabolised principally to meprobamate by *N*-dealkylation, which is active, and to hydroxymeprobamate and their glucuronide conjugates. The enzyme responsible for the formation of meprobamate is CYP2C19 [Dalén *et al.* 1996], with heterozygous individuals with the CYP2C19*1/*2 genotype having a reduced capacity to metabolise carisoprodol. Such individuals are termed 'intermediate metabolisers' [Bramness *et al.* 2003]. The use of oral contraceptives has been shown to inhibit the metabolism of carisoprodol in intermediate and extensive metabolisers [Bramness *et al.* 2005]. Less than 1% of a dose is excreted in the urine as unchanged drug in 24 h, and approximately 5% is excreted as meprobamate in the same period. It is distributed in substantial amounts into breast milk [Nordeng *et al.* 2001].

Therapeutic Concentration

After administration of a 700 mg oral dose of carisoprodol to 9 healthy adults, the mean peak plasma carisoprodol concentration was 3.5 mg/L at 0.8 h and the mean peak meprobamate concentration was 4.0 mg/L at 3.7 h [Olsen *et al.* 1994].

Toxicity CNS toxicity of short duration is the chief manifestation of acute carisoprodol poisoning. Symptoms start at serum concentrations >33 mg/L and last <16 h, although very small amounts of drug may still be detectable 10 days post-ingestion [Goldberg 1969].

A 37-year-old female attempted suicide by ingesting carisoprodol/acetaminophen tablets. On admission to hospital her carisoprodol plasma concentration was 29.5 mg/L. This was reduced 40 h later to 0.07 mg/L [Matsumoto *et al.* 2003].

In 21 cases of driving impairment where carisoprodol was the sole drug involved, most overt symptoms of intoxication were noted when the combined blood concentration of carisoprodol and meprobamate was >10 mg/L [Logan *et al.* 2000].

A 39-year-old male ingested 35 g carisoprodol. He became agitated, developed tachycardia and myoclonus, and slipped into a coma. The blood carisoprodol concentration was 71 mg/L with a meprobamate concentration of 26 mg/L. Carisoprodol overdose is thought to induce simple CNS depression [Roth *et al.* 1998].

In 3 cases involving overdose with carisoprodol, the following tissue concentrations were reported in the respective cases: heart blood 39.4, 14.0 and 8.1 mg/L, femoral blood 39.3, 6.5 and 7.9 mg/L, urine 12.6, – and –, vitreous humour 18.9, 8.7 and 8.1 mg/L. The concentration of meprobamate in the respective cases was: 40.1, 18.9 and 20.9 mg/L, femoral blood 51.9, 20.5 and 34.3 mg/L, urine 61.0, – and –, vitreous humour 45.9, 50.2 and 102.0 mg/L. Verapamil was also detected in case 2 and propoxyphene was detected in case 3 in addition to carisoprodol [Backer *et al.* 1990].

The following concentrations were reported 4.5 h after the ingestion of 3.5 g by a young child: blood, carisoprodol 36.4 mg/L, meprobamate 15 mg/L; urine, carisoprodol 24.4 mg/L, meprobamate 166.4 mg/L; the child's condition deteriorated and death occurred within 36 h [Adams *et al.* 1975].

After the ingestion of some pills, a female had postmortem concentrations of carisoprodol of 110, 165, 64 and 350 mg/L in her blood, urine, bile and stomach, respectively, and 127, 50 and 110 mg/kg in her liver, spleen, and kidney, respectively [Maes *et al.* 1969].

An 18-year-old white male took approximately 27 tablets of 350 mg carisoprodol. He was admitted to hospital and he had the serum and urine concentrations (mg/L) shown below.

Serum		*Urine*	
Time (h)	*Carisoprodol*	*Time*	*Carisoprodol*
4.5	38	3–8	2775
8.5	36	8–16	2400
16	22	16–25	2640
25	11	—	—

The patient was given supportive treatment and suffered no sequelae [Goldberg 1969].

Note For a review of acute intoxications with carisoprodol, see Høiseth *et al.* [2007] or Høiseth *et al.* [2008]. For a review of deaths involving carisoprodol, see Davis and Alexander [1998].

Half-life 0.9 to 2.4 h.

Protein Binding Approximately 58%.

Dose Usual dose is 350 mg given three to four times daily by mouth. Half the usual dose or less is recommended in elderly patients.

Adams HR *et al.* (1975). Carisoprodol-related death in a child. *J Forensic Sci* 20: 200–202.

Backer RC *et al.* (1990). Carisoprodol concentrations from different anatomical sites: three overdose cases. *J Anal Toxicol* 14: 332–334.

Bramness JG *et al.* (2003). Association between blood carisoprodol : meprobamate concentration ratios and CYP2C19 genotype in carisoprodol-drugged drivers: decreased metabolic capacity in heterozygous CYP2C19*1/CYP2C19*2 subjects? *Pharmacogenetics* 13: 383–388.

Bramness JG *et al.* (2005). The CYP2C19 genotype and the use of oral contraceptives influence the pharmacokinetics of carisoprodol in healthy human subjects. *Eur J Clin Pharmacol* 61: 499–506.

Cardini C *et al.* (1968). Systematic application of gas chromatography to the analysis of pharmaceutical preparations. *J Chromatogr* 37: 190–193.

Dalén P *et al.* (1996). Formation of meprobamate from carisoprodol is catalysed by CYP2C19. *Pharmacogenetics* 6: 387–394.

Daval S *et al.* (2006). A one-step and sensitive GC-MS assay for meprobamate determination in emergency situations. *J Anal Toxicol* 30: 302–305.

Davis GG, Alexander CB (1998). A review of carisoprodol deaths in Jefferson County, Alabama. *South Med J* 91: 726–730.

Goldberg D (1969). Carisoprodol toxicity. *Mil Med* 134: 597–601.

Gunnar T *et al.* (2004). Validated semiquantitative/quantitative screening of 51 drugs in whole blood as silylated derivatives by gas chromatography-selected ion monitoring mass spectrometry and gas chromatography electron capture detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 806: 205–219.

Hegstad S *et al.* (2008). Drug screening of hair by liquid chromatography-tandem mass spectrometry. *J Anal Toxicol* 32: 364–372.

Høiseth G *et al.* (2007). Carisoprodol intoxications: a retrospective study of forensic autopsy material from 1992-2003. *Int J Legal Med* 121: 403–409.

Høiseth G *et al.* (2008). Acute intoxications with carisoprodol. *Clin Toxicol (Phila)* 46: 307–309.

Kim JY *et al.* (2005). Simultaneous determination of carisoprodol and meprobamate in human hair using solid-phase extraction and gas chromatography/mass spectrometry of the trimethylsilyl derivatives. *Rapid Commun Mass Spectrom* 19: 3056–3062.

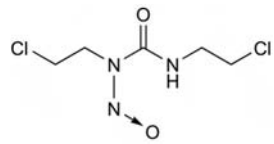
Kintz P *et al.* (1988). A rapid and sensitive gas chromatographic analysis of meprobamate or carisoprodol in urine and plasma. *J Anal Toxicol* 12: 73–74.

Kucharczyk N *et al.* (1986). Gas chromatographic determination of carisoprodol in human plasma. *J Chromatogr* 377: 384–390.
Logan BK *et al.* (2000). Carisoprodol, meprobamate, and driving impairment. *J Forensic Sci* 45: 619–623.
Maes R *et al.* (1969). The gas chromatographic determination of selected sedatives (ethchlorvynol, paraldehyde, meprobamate and carisoprodol) in biological material. *J Forensic Sci* 14: 235–254.
Matsumoto T *et al.* (2003). Simultaneous determination of carisoprodol and acetaminophen in an attempted suicide by liquid chromatography-mass spectrometry with positive electrospray ionization. *J Anal Toxicol* 27: 118–122.
Nordeng H *et al.* (2001). Transfer of carisoprodol to breast milk. *Ther Drug Monit* 23: 298–300.
øiestad EL *et al.* (2007). Drug screening of preserved oral fluid by liquid chromatography-tandem mass spectrometry. *Clin Chem* 53: 300–309.
Olsen H *et al.* (1994). Carisoprodol elimination in humans. *Ther Drug Monit* 16: 337–340.
Roth BA *et al.* (1998). Carisoprodol-induced myoclonic encephalopathy. *J Toxicol Clin Toxicol* 36: 609–612.

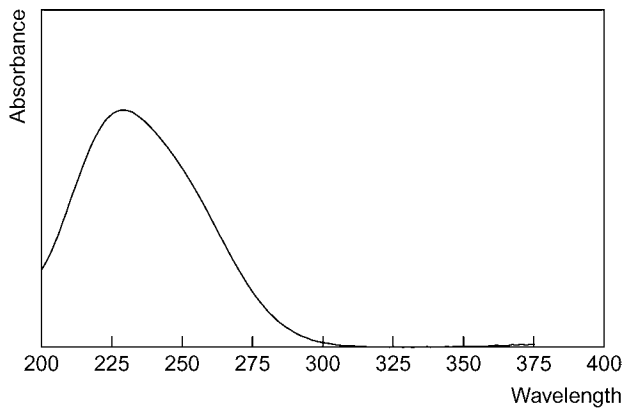
Carmustine

Antineoplastic
$C_5H_9Cl_2N_3O_2$ = 214.0
CAS—154-93-8

IUPAC Name 1,3-Bis(2-chloroethyl)-1-nitrosourea
Synonyms *N*,*N*′-Bis(2-chloroethyl)-*N*-nitrosourea; BCNU.
Proprietary Names *Becenun*; *BiCNU*; *Carmubris*; *Gliadel*; *Nitrourean*; *Nitrumon*.



Chemical Properties A yellowish granular powder that melts to an oily liquid. Mp 31°, with decomposition. It is slightly soluble in water; freely soluble in ethanol; very soluble in dichloromethane and ether. Log *P* (octanol/water), 1.53.
Ultraviolet Spectrum Principal peak at 229 nm.



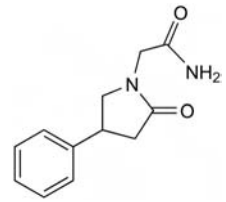
Mass Spectrum Principal ions at *m/z* 56, 62, 42, 77, 69, 49, 105, 64.
Quantification
Plasma **HPLC** Column: ODS RP (Ultrasphere, 250 × 4.6 mm i.d., 5 µm). Mobile phase: 35% acetonitrile : 0.1% glacial acetic acid in water, flow rate 1.2 mL/min. Retention time: 13.8 min. UV detection (λ=230 nm). Limit of detection, 50 µg/L [Yeager *et al.* 1984].
Brain Tissue **GC** Column: SPB1 fused-silica capillary (60 m × 0.25 mm i.d., 0.25 µm coating). Temperature programme: held at 50° for 5 min; increased to 100° at 10°/min; increased to 105.5° at 0.1°/min. Detection: ^{63}Ni electron capture. Retention time: 30 min. Limit of detection, 0.02 µg [Hassenbusch *et al.* 1995].
Disposition in the Body Rapidly metabolised after IV administration. Readily crosses the blood–brain barrier. Alkylates DNA/RNA and has also been shown to inhibit several enzymes by carbamoylation of amino acids in proteins. Metabolites have a longer half-life and are presumed to be responsible for its activity. Some enterohepatic circulation is believed to occur. Excreted primarily in the urine; about 10% is excreted as carbon dioxide via the lungs. Very small amounts (1%) are detected in the faeces.
Therapeutic Concentration
Ten patients with advanced neoplasms were being treated with a high-dose combination of chemotherapy, cyclophosphamide, cisplatin and carmustine. Each patient received 300 to 750 mg/m^2 carmustine over a 2 h infusion period, but this was increased to 1 g/m^2 for maximum results. Peak plasma concentrations reached 7.8 µg/L at the end of the infusion for the corrected dose [Henner *et al.* 1986].
Toxicity Hypotension was the most prominent feature with high-dose carmustine administration.
Half-life Plasma half-life, 22 min.
Volume of Distribution 3.25 L/kg; 5.1 L/kg (patients with advanced neoplasms).
Clearance Plasma clearance, 56 mL/min/kg; 77.6 mL/min/kg (patients with advanced neoplasms).
Protein Binding 77%.
Dose 150 to 200 mg/m^2 IV as a single dose or divided into two doses given on successive days. Implants are also available (containing 7.7 mg/implant).

Hassenbusch SJ *et al.* (1995). Determination of nitrosourea compounds in brain tissue by gas chromatography and electron capture detection. *J Pharm Sci* 84(7): 840–842.
Henner WD *et al.* (1986). Pharmacokinetics and immediate effects of high-dose carmustine in man. *Cancer Treat Rep* 70(7): 877–880.
Yeager RL *et al.* (1984). Quantitation of 1,3-bis(2-chloroethyl)-1-nitrosourea in plasma using high-performance liquid chromatography. *J Chromatogr* 305: 496–501.
Yeager RL *et al.* (1984). Quantitation of 1,3-bis(2-chloroethyl)-1-nitrosourea in plasma using high-performance liquid chromatography. *J Chromatogr* 305: 496–501.

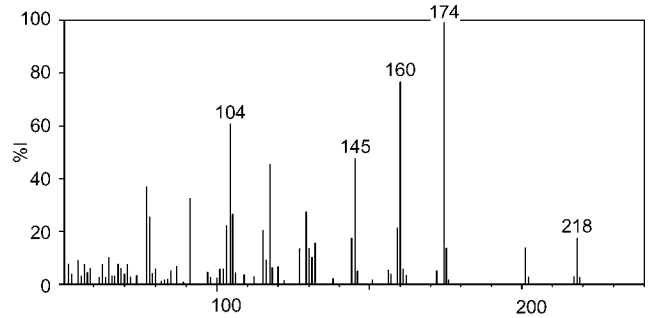
Carphedon

Psychostimulant, Treatment of Amnesia
$C_{12}H_{14}N_2O_2$ = 218.3
CAS—77472-70-9

IUPAC Name (2-Oxo-4-phenylpyrrolidine)acetamide
Synonyms BRN-5030440; 4-phenylpiracetam.



Mass Spectrum Principal ions at *m/z* 174, 160, 104, 145, 117, 77, 91, 129.



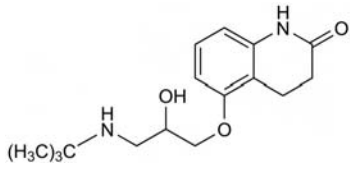
Quantification
Urine **GC** Column: DB-17 (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° to 280° at 25°/min, held at 280° for 8 min. Carrier gas: He, flow rate 0.9 mL/min. Detection: nitrogen–phosphorous. Retention time: 11.5 min. Limit of detection, 0.01 mg/L [Kim *et al.* 1999].
Disposition in the Body
Therapeutic Concentration
A healthy man was administered with a 0.38 mg/kg dose of carphedon. A concentration of 6.34 mg/L was detected in urine after 18 h [Kim *et al.* 1999].

Kim S *et al.* (1999). Determination of carphedon in human urine by solid-phase microextraction using capillary gas chromatography with nitrogen-phosphorus detection. *Analyst* 124: 1559–1562.

Carteolol

β-*Blocker*
$C_{16}H_{24}N_2O_3$ = 292.4
CAS—51781-06-7

IUPAC Name 5-[3-(Tert-butylamino)-2-hydroxypropoxy]-3,4-dihydro-1H-quinolin-2-one
Synonym 5-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-2(1*H*)-quinolinone



Chemical Properties pK_a 9.74. Log *P* (octanol/water), 1.42. Extraction yield (chlorobutane), 0.06 [Demme *et al.* 2005].

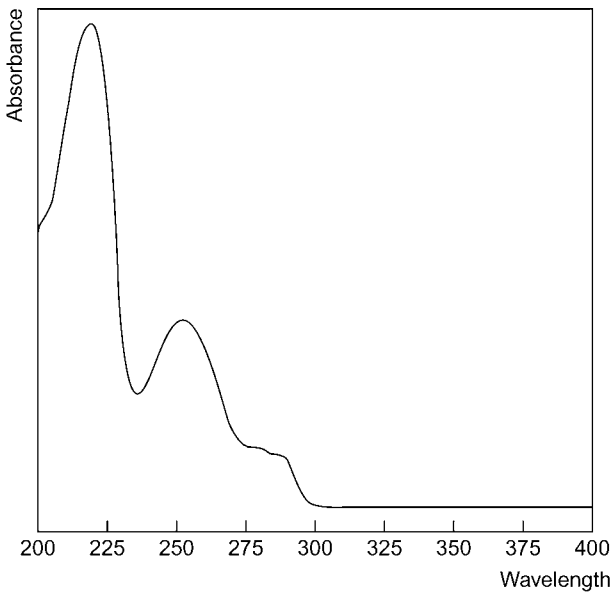
Carteolol Hydrochloride

$C_{16}H_{24}N_2O_3$, HCl = 328.8
CAS—51781-21-6
Synonyms Abbott-43326;OPC-1085.
Proprietary Names *Arteolol*; *Arteoptic*; *Carteol*; *Cartrol*; *Dispersa*; *Elebloc*; *Endak*; *Mikelan*; *Ocupress*; *Teoptic*.
Chemical Properties White crystals or a white crystalline powder., Mp 278°. It is soluble in water; very slightly soluble in ethanol and glacial acetic acid; sparingly soluble in methanol; practically insoluble in ether.

Thin-layer Chromatography System TE—R_f 0.33; system TAE—R_f 0.14.
Gas Chromatography System GA—RI 2588 carteolol); RI 2690 Art).

Column: 5% phenyl methyl silicone RTx−5 fused-silica capillary (30 m × 0.25 mm). Temperature programme: held at 110° for 1 min; increased to 170° at 20°/min; increased to 225° at 7°/min; 290° at 24°/min, held for 10 min. Carrier gas: He. MS detection. Retention time (relative to isoproteronol, internal standard): 1.20 [Branum *et al.* 1998].

High Performance Liquid Chromatography System HAA—retention time 5.9 min.
Ultraviolet Spectrum Aqueous acid (pH 4)—219, 252, 288 nm.



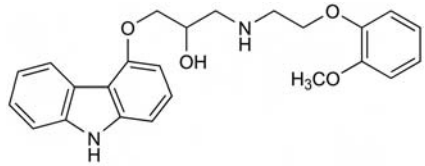
Mass Spectrum Principal ions at *m/z* 86, 57, 277, 70, 292, 87, 114, 60 (carteolol); *m/z* 301, 138, 41, 70, 96, 84, 316, 153 (carteolol methaneboronate).
Quantification
Plasma **GC** Column: fused-silica capillary coated with dimethylpolysiloxane (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: held at 50°, 0.7 min after injection, 55°/min to 320°, held for 3 min. Carrier gas: He. MS detection, SIM. Retention time: PFB amide−DMES ether derivative, 6.7 min. Limit of quantification, <0.03 µg/L [Nagasawa *et al.* 1995].

HPLC UV detection. Limit of detection, 5 µg/L [Ishizaki *et al.* 1983]. Electrochemical detection. Limit of detection, 5 µg/L for 8-hydroxycarteolol [Chu 1978].
Urine **HPLC** UV detection. Limit of detection, 100 µg/L [Ishizaki *et al.* 1983]. Electrochemical detection. Limit of detection, 25 µg/L for 8-hydroxycarteolol [Chu 1978].
Disposition in the Body Carteolol is well absorbed after oral administration with peak plasma concentrations being reached within 1 to 3 h. It has low lipid solubility. It is distributed into breast milk. Carteolol undergoes minimal hepatic metabolism to form the active metabolite, 8-hydroxycarteolol and glucuronic acid conjugates of carteolol and 8-hydroxycarteolol. The major route of elimination is renal with 50 to 70% being excreted unchanged in urine.
Bioavailability Absolute bioavailability, 85%.
Half-life Plasma, carteolol, 5 to 6 h; 8-hydroxycarteolol, 8 to 12 h.
Volume of Distribution 4 L/kg.
Clearance Plasma clearance, 10 mL/min/kg.
Protein Binding 20 to 30%.
Dose Usually 2.5 or 5 mg of carteolol hydrochloride daily.

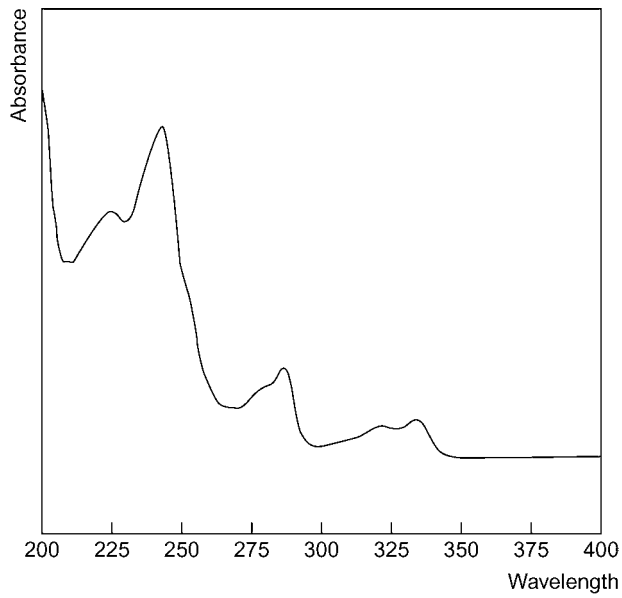
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Branum GD *et al.* (1998). The feasibility of the detection and quantitation of beta-adrenergic blockers by solid-phase extraction and subsequent derivatization with methaneboronic acid. *J Anal Toxicol* 22: 135–141.
Chu SY (1978). High-pressure liquid chromatographic determination of 8-hydroxycarteolol in plasma and urine using electrochemical detection. *J Pharm Sci* 67: 1623–1625.
Ishizaki T *et al.* (1983). Pharmacokinetics and absolute bioavailability of carteolol, a new beta-adrenergic receptor blocking agent. *Eur J Clin Pharmacol* 25: 95–101.
Nagasawa M *et al.* (1995). Determination of the beta-blocker carteolol in human plasma by a sensitive gas chromatographic-negative-ion chemical ionization high-resolution mass spectrometric method. *J Chromatogr* 673(2); Biomed. Appl.: 294–298.

Carvedilol

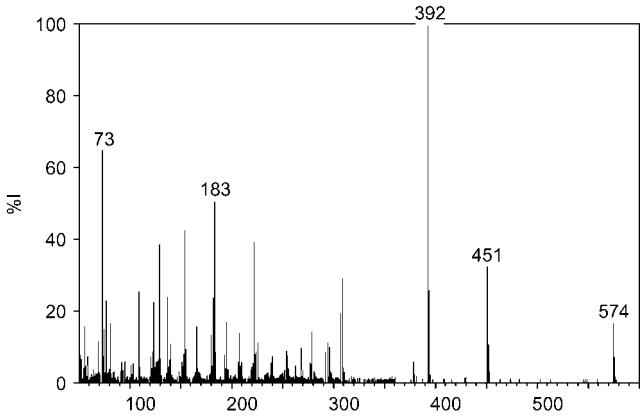
β-*Blocker*
$C_{24}H_{26}N_2O_4$ = 406.5
CAS—72956-09-3
IUPAC Name 1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol
Synonyms BM-14190; DQ-2466.
Proprietary Names *Carvipress*; *Dilatrend*; *Dimitone*; *Eucardic*; *Kredex*; *Querto*.



Chemical Properties A white to off-white powder. Mp 114° to 115°. It is practically insoluble in water; freely soluble in dimethyl sulfoxide; soluble in methylene chloride and in methanol; sparingly soluble in ethanol and in isopropyl alcohol; slightly soluble in ethyl ether. Log *P* (octanol/water), 4.19. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
High Performance Liquid Chromatography System HZ—carvedilol retention time 5.2 min, desmethylcarvedilol retention time 4.4 min.
Ultraviolet Spectrum Aqueous acid (2.0 mol/L HCl)—241, 285, 320, 332 nm; (methanol)—224, 243, 286, 319, 332 nm.



Mass Spectrum Principal ions at *m/z* 392, 73, 183, 154, 129, 222, 451, 309 (TMSTFA derivative).



Quantification

Plasma **HPLC** Fluorescence detection. Limit of detection, 1.55 µg/L [Fujimaki *et al.* 1990]. Limit of quantification, 1 µg/L *S*(−)-enantiomer, 2 µg/L *R*(+)-enantiomer [Neugebauer *et al.* 1990]. Fluorescence detection (λ_{ex}=285 nm, λ_{em}=360 nm). Limit of detection, 1.1 µg/L (*S*(−)-enantiomer), 0.6 µg/L (*R*(+)-enantiomer), [Eisenberg *et al.* 1989]. Fluorescence detection (λ_{ex}=285 nm, λ_{em}=340 nm). Limit of detection, 0.38 µg/L [Reiff 1987].

Serum **HPLC** Column: Spherisorb octyl (125 × 5 mm i.d., 5 µm). Mobile phase: acetonitrile : water (45:55) containing 5 mM dibutylamine, flow rate 2 mL/min. Retention time: 3.8 min Fluorescence detection (λ_{ex}=240 nm, λ_{em}=340 nm). Limit of detection, 0.1 µg/L [Varin *et al.* 1986].

Urine **HPLC** Fluorescence detection (λ_{ex}=285 nm, λ_{em}=340 nm). Limit of detection, 0.82 µg/L [Reiff 1987].

Disposition in the Body Carvedilol is rapidly and well absorbed after oral administration, but is subject to considerable first-pass metabolism in the liver. The rate of absorption is impaired by the co-administration with food but the bioavailability is not affected. The drug is widely distributed and extensively metabolised, primarily by aromatic ring oxidation and glucuronidation. The oxidative metabolites undergo further metabolism by glucuronidation and sulfation. The metabolites are excreted mainly via bile into faeces. Approximately 16% of a dose is detected in urine with <2% as the unchanged drug. Some of the metabolites have beta-blocking and vasodilating activity; one metabolite has greater beta-blocking activity than carvedilol but all have weaker vasodilating effects than carvedilol. The *o*-demethyl-*p*-hydroxyl and *m*-hydroxyl metabolites possess beta-blocking activity. Its metabolism is stereoselective and plasma concentrations of *R*(+)-carvedilol are about 2–3-times higher than *S*(−)-carvedilol. The principal enzymes involved are CYP2D6 and CYP2C9. The metabolism of carvedilol is subject to genetic polymorphism with poor metabolisers of debrisoquine having plasma-*R*(+)-carvedilol concentrations 2–3-fold higher than extensive metabolisers. It is not significantly cleared by haemodialysis. No accumulation of the drug has been observed.

Therapeutic Concentration In plasma, concentrations are linearly related to the administered dose.

The mean maximum plasma concentration after a 20 mg dose, administered orally after an overnight fast, to 5 healthy Japanese males was 0.025 (range, 0.0065 to 0.044) mg/L for the (*R*)-enantiomer and 0.0098 (0.0023 to 0.0187) mg/L, (*S*)-enantiomer. These concentrations were observed 0.5 to 1.0 h after administration [Fujimaki *et al.* 1990].

Following oral administration of a 50 mg tablet to 20 healthy males, aged between 19 and 45 years, maximum plasma concentrations ranged from 34 to 130 mg/L (mean 66 mg/L) after 0.65 to 2.25 h (mean 1.20 h). Peak concentrations of 128 mg/L (range, 55 to 237 mg/L), 0.66 (range, 0.24 to 1.29 h after administration were observed, however, for a 50 mg suspension. Following administration of a 25 mg tablet, maximum plasma concentrations were 5 to 99 mg/L (mean 21 mg/L) seen after 1.46 h (range, 0.68 to 3.10 h). After IV administration of 12.5 mg over a 1-h period, the peak concentration was 173 mg/L (range, 109 to 234) by the end of infusion [von Möllendorff *et al.* 1987].

Bioavailability Absolute bioavailability, 25 to 35%; *S*(−)-enantiomer, 15%; *R*(+)-enatiomer, 31%.

Half-life Plasma, 4 to 7 h (values outside this range have also been noted).

Volume of Distribution 1.5 to 2.0 L/kg.

Clearance Plasma clearance, 0.52 L/h/kg.

Protein Binding >98% with *R*(+)-enantiomer being more tightly bound.

Note For reviews of carvedilol, see McTavish *et al.* [1993] and Morgan [1994].

Dose It is recommended that all receive 12.5 mg for the first 2 days and then increase if necessary up to 50 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Eisenberg EJ *et al.* (1989). High-performance liquid chromatographic method for the simultaneous determination of the enantiomers of carvedilol and its O-desmethyl metabolite in human plasma after chiral derivatization. *J Chromatogr* 493: 105–115.

Fujimaki M *et al.* (1990). Assay and disposition of carvedilol enantiomers in humans and monkeys: evidence of stereoselective presystemic metabolism. *J Pharm Sci* 79: 568–572.

McTavish D *et al.* (1993). Carvedilol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* 45: 232–258.

Morgan T (1994). Clinical pharmacokinetics and pharmacodynamics of carvedilol. *Clin Pharmacokinet* 26: 335–346.

Neugebauer G *et al.* (1990). Stereoselective disposition of carvedilol in man after intravenous and oral administration of the racemic compound. *Eur J Clin Pharmacol* 38: S108–S111.

Reiff K (1987). High-performance liquid chromatographic method for the determination of carvedilol and its desmethyl metabolite in body fluids. *J Chromatogr* 413: 355–362.

Varin F *et al.* (1986). Liquid chromatographic assay and disposition of carvedilol in healthy volunteers. *J Pharm Sci* 75(12): 1195–1197.

von Möllendorff E *et al.* (1987). Pharmacokinetics and bioavailability of carvedilol, a vasodilating beta-blocker. *Eur J Clin Pharmacol* 33: 511–513.

Cathine

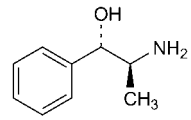
Amine, Anorectic, Sympathomimetic

$C_9H_{13}NO$ = 151.2

CAS—492-39-7; 36393-56-3

IUPAC Name (1*S*,2*S*)-2-Amino-1-phenylpropan-1-ol

Synonyms (α*S*)-α-[(1*S*)-1-Aminoethyl] benzenemethanol; katine; (+)-norpseudoephedrine; pseudonorephedrine.



Chemical Properties An alkaloid obtained from catha, the fresh or dried leaves of *Catha edulis* (Celastraceae). Catha (also known as Abyssinian, African, or Arabian Tea; Kat; Kath; Khat; Miraa) is used in northern and eastern Africa as a stimulant, the leaves being chewed or used as an infusion. Crystals. Mp 77° to 78°. Soluble in ethanol, chloroform, ether, and dilute acids. Extraction yield (chlorobutane), 0.07 [Demme *et al.* 2005]. Cathine is stable in urine if stored at –20°, 4°, and 22° for up to 9 months [Van Eenoo *et al.* 2007].

Cathine Hydrochloride

$C_9H_{13}NO$, HCl = 187.7

CAS—2153-98-2

Proprietary Names *Adiposetten N*; *Amorphan Depot*.

Chemical Properties Crystals. Mp 180° to 181°. Soluble in water. pK_a 9.4 (20°), 8.92 for *D*-cathine [Frosch 1977]. Log *P* (octanol/water) 0.8.

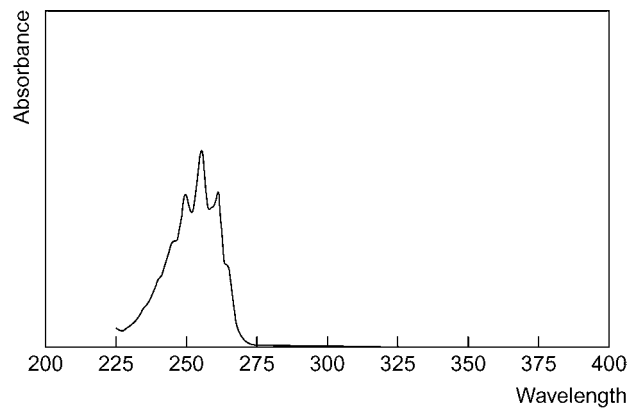
Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.25; system TC—R_f 0.05 (acidified potassium permanganate solution, positive).

The 'Toxilab' TLC system cannot distinguish phenylpropanolamine (*d,l*-norephedrine) from *d*-norpseudoephedrine [Gal, Lichtenstein 1987].

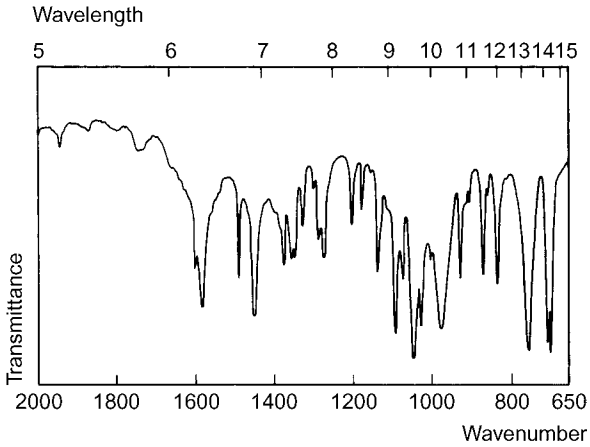
Gas Chromatography System GA—RI 1360; system GB—RI 1352; system GC—RI 1383; system GAL—RI 1362.

High Performance Liquid Chromatography System HA—*k* 1.0; system HB—*k* 4.39; system HC—*k* 0.83.

Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1 = 10a), 263 nm No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1045, 695, 754, 704, 1091, 976 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 57, 43, 40, 55, 41, 79, 77.

Quantification

Plasma **GC** Column: GP 2% SP-2510-DA on 100/120 mesh Suppelcoport (1.8 m × 4 mm i.d.). Carrier gas: CH_3 : Ar (5 : 95), 90 mL/min. Temperature: 280°. ECD. Limit of detection, 50 µg/L [Lo *et al.* 1981]. Column: 3% OV-17 on 100/120 mesh Gas-Chrom Q (6′ × 0.125" o.d.). Carrier gas: N_2, 6 mL/min. Temperature: 150°. ECD. Limit of detection, 0.5 mg/L [Lin *et al.* 1977].

GC-MS Column: HP-1 MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 0.5 min to 170° at 10°/min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 1 µg/L [Toennes *et al.* 2003].

HPLC Column: LiChrosorb SI 60 (5 µm). Mobile phase: diisopropylether : isopropanol : 25% ammonium hydroxide (480 : 25 : 3), flow rate, 0.9 mL/min. Fluorescence detection (λ_{ex} = 354 nm, λ_{em} = 450–520 nm). Limit of detection, 25 µg/L [Meyer, Portmann 1982].

LC-MS Column: Agilent Zrbax (150 × 2.1 mm i.d., 5 µm). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (95 : 5 to 70 : 30 at 10.0 min for 1 min to 95 : 5), flow rate 1.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 10 µg/L [Beyer *et al.* 2007]. Column: Hypersil Phenyl BDS (50 × 4.6 mm i.d.). APCI. Limit of quantification, 1 µg//L [Jacob, III *et al.* 2004]. Column: Hypersil Phenyl BDS (50 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium formate : 0.1% formic acid : methanol (100 : 0 to 20 : 80 in 7 min to 100 : 0 over 0.5 min), flow rate 0.7 mL/min. APCI, positive ion mode, SRM acquisition mode. Limit of detection, 0.5 µg/L [Haller *et al.* 2002].

Urine **TLC** Plates: Silica. Solvent system: ethyl acetate : methanol : ammonia (A, 17 : 2 : 1) or cyclohexane : chloroform : diethylamine (B, 5 : 4 : 1). UV and 0.3% ninhydrin at 105° for 10 min. R_f value: 0.42 and 0.47 for solvent systems A and B, respectively [Guantai, Maitai 1983]. Plates: Silica. Solvent system: butanol : acetic acid : water (A, 60 : 15 : 25); butanol saturated with water (upper phase, B); methanol : ammonia (C, 100 : 1.5); or isopropanol : water : ammonia (80 : 15 : 5). R_f value: 0.61, 0.31, 0.47 and 0.80 for solvent systems A, B, C and D, respectively [Maitai, Mugera 1975].

GC Column: HP-5MS cross-linked 5% diphenyl 95% dimethylpolysiloxane capillary (25 m × 0.25 mm i.d., 0.33 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 100° for 1 min to 200° at 10°/min to 300° at 20°/min for 4 min. EI ionisation at 70 eV. NPD. Limit of detection, 0.25 mg/L [Tseng *et al.* 2006]. Column: Rtx-5 Amine cross-linked 5% diphenyl 95% dimethylpolysiloxane (15 m × 0.25 mm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 100° to 105° at 0.5°/min to 118° at 2°/min to 280° at 50°/min for 2 min. NPD. Retention time: 12.1 min. Limit of detection, 2 mg/L [Van Eenoo *et al.* 2001]. Column: Chromosorb W 100/200 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 80° to 200° at 5°/min for 10 min. FID. Retention time: 7.5 min. Limit of detection not reported [Guantai, Maitai 1983]. Column: GP 2% SP-2510-DA on 100/120 mesh Suppelcoport (1.8 m × 4 mm i.d.). Carrier gas: CH_3: Ar (5 : 95), 90 mL/min. Temperature: 280°. ECD. Limit of detection, 50 µg/L [Lo *et al.* 1981]. Column: 3% OV-17 on 100/120 mesh Gas-Chrom Q (6 ft × 0.125 in o.d.). Carrier gas: N_2, 6 mL/min. Temperature: 150°. ECD. Limit of detection, 0.5 mg/L [Lin *et al.* 1977]. Column: Celite CQ 100/120 mesh with 3% methyl silicone (1.5 m × 0.3 cm o.d.). Carrier gas: N_2, 50 mL/min. Temperature: 150°. FID. Limit of detection not reported [Maitai, Mugera 1975].

GC-MS Column: Ultra-2 fused silica capillary (12 m × 0.20 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 95° to 270° at 10°/min for 2.5 min. EI ionisaton at 70 eV, full scan mode. Retention time: 6.42 min. Limit of detection not reported [de Oca Porto *et al.* 2007]. Column: HP fused silica capillary (17 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 320° at 20°/min for 2 min. Limit of detection, 0.1 µg/L [Shin *et al.* 1998]. Column: OV-1701 fused silica capillary (25 m). Carrier gas: He, 3 mL/min. Temperature programme: 84° for 1.5 min to 170° at 3°/min. EI ionisation at 70 eV. Retention time: 25.1 min [Brenneisen *et al.* 1986].

HPLC Column: Spherisorb ODS 1 C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: tetraethylammonium phosphate : methanol (985 : 15), flow rate 1.0 mL/min. DAD (λ = 206 nm). Limit of quantification, 0.3 mg/L, limit of detection, 0.1 mg/L [Chan *et al.* 2005]. Column: HP-1 MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 0.5 min to 170° at 10°/min to 310° at 30°/min for 5 min. EI ionisation at 70 eV. SIM acquisition mode. Limit of detection, <0.01 mg/L [Toennes, Kauert 2002]. Column: Phase Sep Spherisorb ODS 1 (150 × 4.6 mm i.d., 5 µm). Mobile phase: tetraethylammoniumphosphate : methanol (98 : 2), flow rate 1.0 mL/min. Limit of detection, 0.5 mg/L [van der Merwe *et al.* 1994]. Column: LiChrospher 60 RP Select B (125 × 4 mm i.d., 5 µm). Mobile phase: 200 mmol/L phosphate buffer : 150 mmol/L tetraethylammonium (pH 5.5), flow rate 1.3 mL/min. DAD (λ = 215 nm). Limit of detection, 0.2 mg/L [Imaz *et al.* 1993]. Column: Spherisorb ODS-1 (150 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (8.5 : 91.5) containing 8.5 g/L ortho-phosphoric acid and 200 µL/L hexylamine, flow rate 1.0 mL/min. DAD (λ = 192 nm). Limit of detection, 25 µg/L [Mathys, Brenneisen 1992].

LC-MS Column: Hypersil Phenyl BDS (50 × 4.6 mm i.d.). APCI. Limit of quantification, 1 µg//L [Jacob, III *et al.* 2004]. Column: Hypersil Phenyl BDS (50 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium formate : 0.1% formic acid : methanol (100 : 0 to 20 : 80 in 7 min to 100 : 0 over 0.5 min), flow rate 0.7 mL/min. APCI, positive ion mode, SRM acquisition mode. Limit of detection, 0.5 µg/L [Haller *et al.* 2002].

Milk **GC** Column: Fused silica cross-linked methylsilicone capillary (12 m × 0.2 mm i.d.). NPD. Retention time: 1.19 min. Limit of detection, 10 µg/L [Kristiansson *et al.* 1987].

Hair **GC-MS** Column: HP5-MS capillary (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 80° for 3 min to 150° at 10°/min to 300° at 20°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.1 µg/g, limit of detection, 0.03 µg/g [Sporkert *et al.* 2003].

Disposition in the Body Approximately 40% of an ingested dose is excreted unchanged in the urine in 6 h. Cathine is excreted in the breast-milk of lactating mothers 2–4 h after starting to chew khat. [Kristiansson *et al.* 1987]. Cathine is a metabolite of pseudoephedrine, famprofazone [Shin *et al.* 1998] and cathinone [Brenneisen *et al.* 1986; Guantai, Maitai 1983].

Therapeutic Concentration

Following a single oral dose of 60 mg to 6 subjects, a mean peak plasma concentration of 0.2 mg/L was attained in 1.3 h; cathine could not be detected in the plasma after 24 h [Frosch 1977].

Six khat-naïve subjects were administered 0.8 mg/kg cathinone (~54–71 g fresh khat leaves). The mean peak plasma concentration was 89 ± 49 µg/L at 183 ± 73 min [Widler *et al.* 1994].

Toxicity Two previously healthy female patients (43 and 55-years-old) took norpseudoephedrine to lose weight. Both patients developed dyskinesias which resolved only after botulinum toxin therapy [Thiel, Dressler 1994]. Addiction to catha has been reported.

Note For a review of the pharmacology of khat consumption, see Nencini and Ahmed [1989], Kalix [1984] or Kalix and Braenden [1985]. For a review of psychoactive alkaloids from *Ephedra* and *Catha*, see Kalix [1991].

Half-life Plasma half-life, ≈3 h.

Bioavailability ≈94% [Meyer, Portmann 1982].

Dose Cathine hydrochloride has been given in doses of 20 to 60 mg daily.

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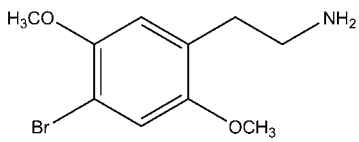
2C-B

5-HT$_{2A}$ Receptor Antagonist, Entactogen, Hallucinogen, Phenethylamine
$C_{10}H_{14}BrNO_2$=260.1
CAS—66142-81-2

IUPAC Name 2-(4-Bromo-2,5-dimethoxy-phenyl)ethanamine
Synonyms BDMPEA; 4-bromo-2,5-dimethoxybenzeneethanamine; 4-bromo-2,5-dimethoxy-β-phenethylamine; 2-(4-bromo-2,5-dimethoxyphenyl)-1-aminoethane; α-desmethyl DOB; 2,5-dimethoxy-4-bromophenethylamine; MFT.
Street Names Bees; Bromo; Erox; Nexus; Performax; Venus; XTC.



Chemical Properties Log *P* (octanol/water), 2.03 [ACD 2007]. No indication of instability of processed samples over a time period of 32 h. When the samples were freeze-thawed, the ratio of the means was within 90 and 110% for all analytes at high concentrations [Habrdova *et al.* 2005].

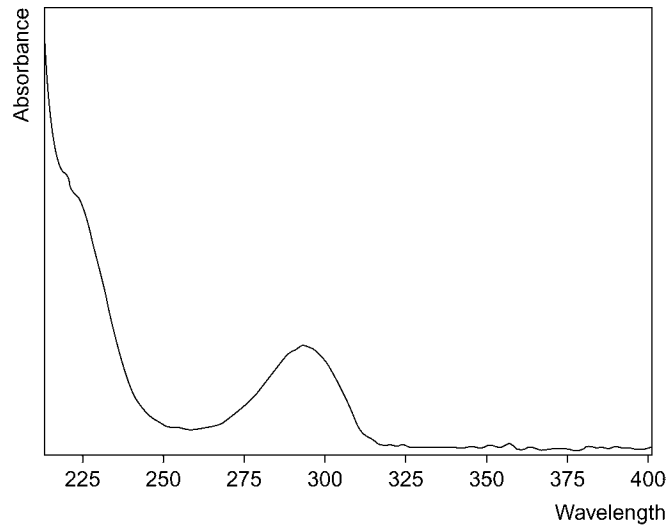
2C-B Hydrochloride

$C_{10}H_{14}BrNO_2$, HCl = 296.6
CAS—56281-37-9

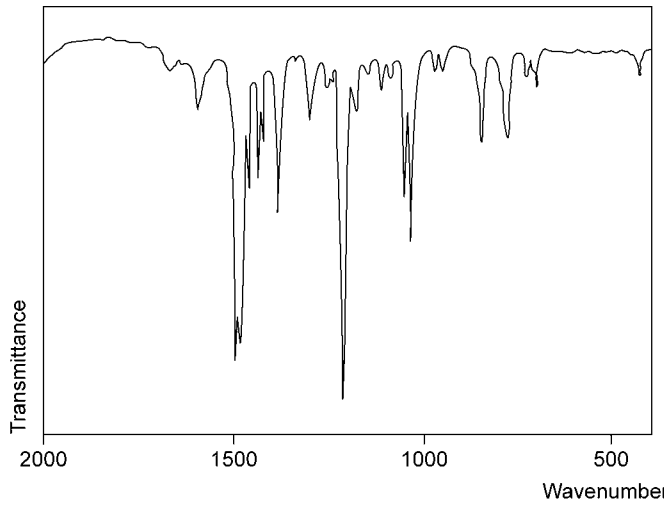
Synonyms BDMPEA hydrochloride; 4-bromo-2,5-dimethoxybenzeneethanamine hydrochloride; 4-bromo-2,5-dimethoxy-β-phenethylamine hydrochloride; 2-(4-bromo-2,5-dimethoxyphenyl)-1-aminoethane hydrochloride; α-desmethyl DOB hydrochloride; 2,5-dimethoxy-4-bromophenethylamine hydrochloride; MFT hydrochloride.
Chemical Properties Pale pink crystals, fine white crystals when dry. Mp 237° to 239°. Soluble in water.

Colour Test Marquis test—yellow→green [Cole *et al.* 2002].
Gas Chromatography-Mass Spectrometry Column: HP-5MS cross-linked phenylmethylysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 1 min to 200° at 20°/min for 8.84 min. EI ionisation at 70 eV. Limit of detection, 4.94 mg/L [Tsai *et al.* 2006]. Column: HP Ultra-2 fused silica capillary (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He. Temperature programme: 55° for 1 min, to 190° at 20°/min to 305° at 10°/min for 11 min. EI ionisation at 70 eV. Retention time: 7.4 min [Giroud *et al.* 1998].
High Performance Liquid Chromatography Column: Spherisorb C_{18} ODS2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate (pH 3.9) buffer (0.1% triethylamine) : methanol (77 : 23 for 19 min to 65 : 35 for 16 min), flow rate 0.7 mL/min for 9 min, 1.2 mL/min for 12 min, and 1.4 mL/min for 14 min. DAD (λ= 210 nm). Retention time: 27.6 min. Limit of detection, 105 mg/L [Soares *et al.* 2004]. Column: Lichrospher RP-8 Select B (250 × 4 mm i. d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 3.2; 5 : 95 to 60 : 40 after 20 min), flow rate 0.5 mL/min. DAD (λ= 210 nm). Limit of detection, 1 mg/L [Giroud *et al.*, 1998].

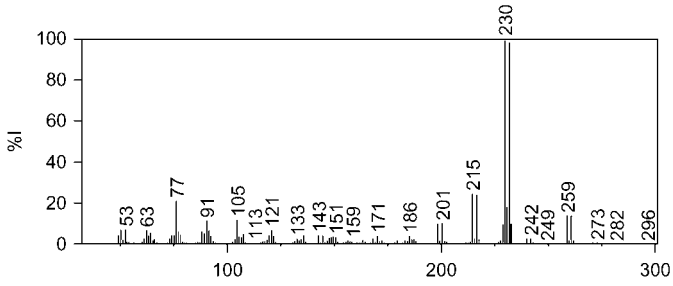
Capillary Electrophoresis Capillary: fused silica (total/effective length: 96/90 cm for sweeping MEKC and 100/94 cm for stacking MEKC, 50 µm i.d.). Lamp: Xe. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 340 nm). Limit of detection, 36.4 and 4.94 µg/L for sweeping and stacking MEKC, respectively [Tsai *et al.* 2006].
Ultraviolet Spectrum Methanol—203 nm, 296 nm.



Infrared Spectrum Principal peaks at wavenumbers 1250, 1500, 825, 740, 750 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 230, 232, 215, 217, 77, 259, 261, 91; 242, 244, 229, 148, 301, 303, 77, 201 (AC); 244, 242, 229, 231, 148, 455, 457, 199 (HFB derivative) [Giroud *et al.* 1998].



Quantification

Blood **GC-MS** Column: Phenomex ZBI (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 10°/min to 300° at 60°/min for 14 min. EI ionisation, SIM acquisition mode. Limit of detection, 5 to 10 µg/L [Vorce, Sklerov 2004].
Plasma **HPLC** Column: Daisopak SP-120-5-ODS-BP (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol-water-ethyl acetate (69 : 30 : 1) : acetonitrile-water (70 : 30; 60 : 40 for 20 min, 5 : 95 at 26 min until 49 min to 60 : 40). Fluorescence detection (λ_{ex}=325 nm, λ_{em}= 430 nm). Limit of detection, 312 ng/L [Kaddoumi *et al.* 2001].

GC-MS Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 1.1 mL/min. Temperature programme: 80° for 0.5 min to 310° at 30°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 µg/L [Habrdova *et al.* 2005].

Urine **GC-MS** See Blood [Vorce, Sklerov 2004]. Column: Supelco fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 80° for 3 min to 220° at 40°/min to 280°at 8°/min for 3 min. SIM acquisition mode. Limit of detection, 10 µg/L [Namera *et al.* 2002].

HPLC Column: Waters Spherisorb C_{18} ODS2 (250 × 4.6 mm, 5 µm). Mobile phase: methanol: 0.05 mol/L ammonium acetate buffer containing 0.1% triethylamine (pH 3.9, 23 : 77 for 19 min to 35 : 65 for 16 min), flow rate 0.7 mL/min to 8 min to 1.2 mL/min to 19 min to 1.4 mL/min to 35 min. UV detection (λ= 210 nm). Limit of detection, 105 µg/mL [Soares *et al.* 2004].

LC-MS Direct injection, positive ion mode, MRM acquisition mode. Limit of detection, 50 µg/L [Nordgren *et al.* 2005]. Column: HyPURITY Advance (30 × 2.1 mm, 3 µm). Mobile phase: 10 mmol/L ammonium acetate : methanol (95 : 5 at 0.1 min to 20 : 80 at 1 min to 95 : 5 for 5 min), flow rate 400 µL/min. APCI, positive ion mode. Retention time: 1.44 min. Limit of detection, 0.5 µg/L [Nordgren, Beck 2004].

Hair **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 90° for 3 min to 170° at 15°/min for 3 min to 210° at 25°/min for 1.5 min to 230° at 20°/min for 0.5 min to 300° at 35°/min for 0.5 min. EI ionisation mode at 70 eV, SIM acquisition mode. Limit of quantification, 20 pg/mg, limit of detection, 4 pg/mg [Kim *et al.* 2007].

Other **HPLC** Tablets. Column: Lichrospher RP-8 Select B (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 3.2; 5 : 95 to 60 : 40 at 20 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Quantification, down to 1 ng/L [Giroud *et al.* 1998].

GC-MS Rat Urine. Column: HP-1 capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Theobald *et al.* 2007]. Tablets. Column: HP Ultra-2 silica phenylmethylsilicone (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He. Temperature programme: 55° to 190° at 20°/min to 305° at 10°/min for 11 min. Limit of detection, 1 mg/L [Giroud *et al.* 1998].

Disposition in the Body Metabolised by oxidative deamination and demethylation to form 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol, 4-bromo-2,5-dimethoxyphenylacetic acid, 4-bromo-2,5-dimethoxybenzoic acid, and 2-(4-bromo-2-hydroxy-5-methoxyphenyl)ethanol [Carmo *et al.* 2005].

Toxicity There have been no published cases of fatal 2C-B toxicity. It is a partial agonist of serotonergic receptors producing euphoric and hallucinogenic effects (auditory and visual), which increase with dosage.

Note For further information, see Giroud *et al.* [1998] and Shulgin, Shulgin [1991].

Dose Light dose 5 to 15 mg; strong dose 20 to 50 mg [Cole *et al.* 2002].

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Cefaclor

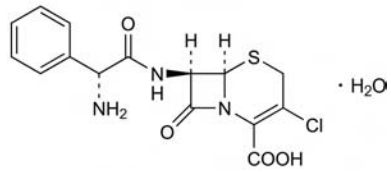
Antibacterial

$C_{15}H_{14}ClN_3O_4S$, H_2O = 385.8

CAS—53994-73-3 (anhydrous); 70356-03-5 (monohydrate)

IUPAC Name (6*R*,7*R*)-7-[[(2*R*)-Aminophenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate

Proprietary Names *Alfatil*; *Cec*; *Ceclor*; *Ceclorbeta*; *Cefabiocin*; *Cefallone*; *Cefa-Wolff*; *Cef-diolan*; *Distaclor*; *Infectocef*; *Keflor*; *Kefolor*; *Keftid*; *Kloclor*; *Panacef*; *Panoral*; *Sigacefal*; *Vercef*.

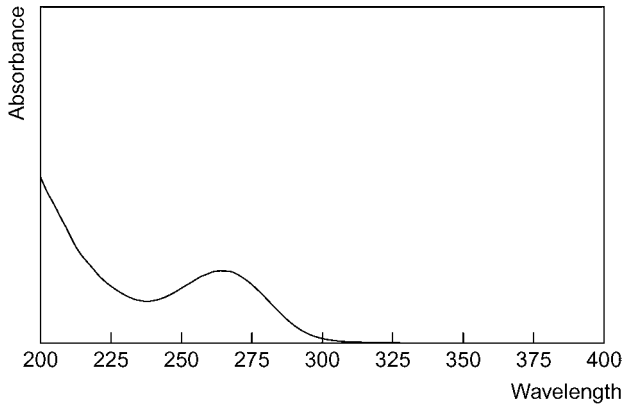


Chemical Properties A white to off-white, or slightly yellow, crystalline powder. It is soluble to slightly soluble in water (10 g/L); practically insoluble in benzene, chloroform, dichloromethane, and methanol (all <0.5 g/L). pK_a 1.5, 7.2 (water); 4.33, 7.34 (66% DMF). Log *P* (octanol/water), 0.35.

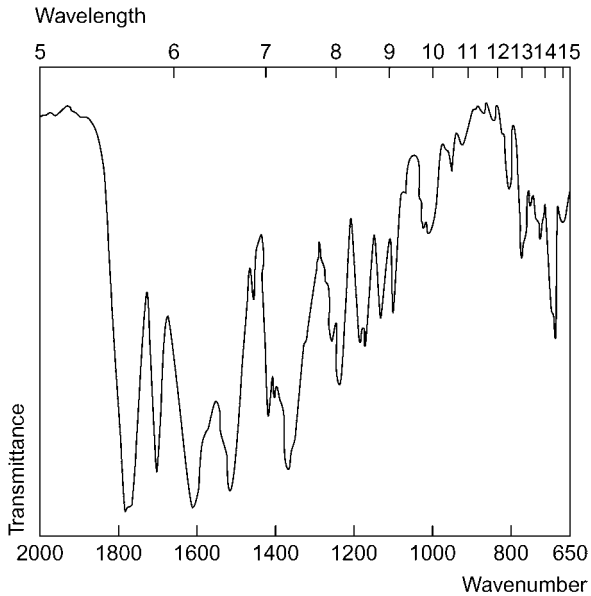
Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 268.

Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—265 nm.



Infrared Spectrum Principal peaks at wavenumbers 1775, 1693, 1600, 1365, 1500, 1560 cm^{-1}.



Quantification

Plasma **HPLC** UV detection (λ=265 nm). Limit of detection, 0.5 mg/L [Kovach, Lantz 1991]. UV detection. UV detection (λ=254 nm). Limit of detection, 0.5 mg/L [Signs *et al.* 1984].

Serum **HPLC** See Plasma [Kovach, Lantz 1991]. UV detection. Limit of detection, about 1 mg/L [Lindgren 1987]. UV detection (λ=240 nm). Limit of detection, 1 mg/L [McAteer *et al.* 1987].

Urine **HPLC** See Plasma [Kovach, Lantz 1991].

Disposition in the Body Cefaclor is well absorbed after oral administration; food may delay absorption but the total amount absorbed is unchanged. The peak concentration, however, with food is 50 to 75% of that observed for the drug when administered in the fasting state. It is widely distributed in the body and crosses the placenta and low concentrations appear in breast milk. Cefaclor is rapidly excreted by the kidneys; up to 85%

of a dose appears unchanged in urine within 8 h, with the greater proportion appearing in the first 2 h. Probenecid delays excretion. Some cefaclor is removed by haemodialysis.

Therapeutic Concentration The serum therapeutic concentration range is 13 to 35 mg/L for an oral dose and up to 900 mg/L for an IV dose.

Oral doses of 250, 500 and 1000 mg administered to fasting individuals, produce peak plasma concentrations of about 7, 13 and 23 mg/L, respectively at 0.5 to 1 h [Eli Lilly & Co. Ltd].

Half-life Plasma half-life, 0.5 to 1 h; 2.3 to 2.8 h in patients with the complete absence of renal function. Serum, 0.6 to 0.9 h; slightly prolonged in patients with renal impairment.

Protein Binding 25%.

Dose Adults: usually 250 to 500 mg of anhydrous cefaclor every 8 h. Total daily dose should not exceed 4 g. Children: usual recommended daily dose is 20 mg/kg every 8 h.

Data as supplied by Eli Lilly & Company Ltd.

Kovach PM, Lantz RJ (1991). High-performance liquid chromatographic determination of loracarbef, a potential metabolite, cefaclor and cephalexin in human plasma, serum and urine. *J Chromatogr* 567: 129–139.

Lindgren K (1987). Determination of cefaclor and cephradine in serum by ion-pair reversed-phase chromatography. *J Chromatogr* 413: 351–354.

McAteer JA *et al.* (1987). Liquid-chromatographic determination of five orally active cephalosporins–cefixime, cefaclor, cefadroxil, cephalexin, and cephradine–in human serum. *Clin Chem* 33 (10): 1788–1790.

Signs SA *et al.* (1984). High-pressure liquid chromatographic method for analysis of cephalosporins. *Antimicrob Agents Chemother* 26(5): 652–655.

Cefalexin

Antibiotic

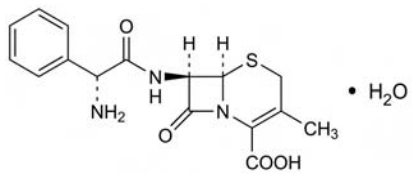
$C_{16}H_{17}N_3O_4S, H_2O = 365.4$

CAS—15686-71-2 (anhydrous); 23325-78-2 (monohydrate)

IUPAC Name (6*R*, 7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate

Synonym (6*R*,7*R*)-7-[[(2*R*)-Aminophenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate; cephalexin.

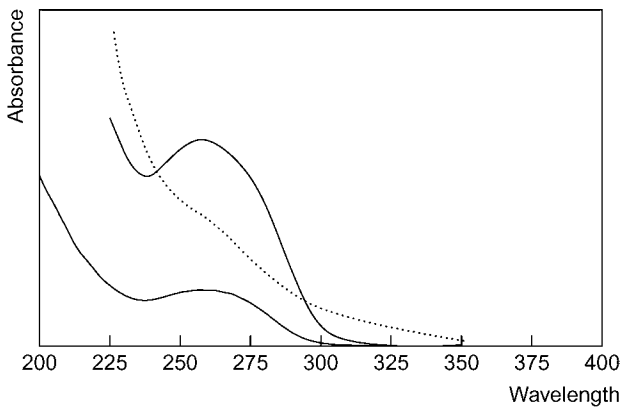
Proprietary Names *Ceporex*; *Ceporexin(e)*; *Keflex*; *Keforal*; *Oracef*.



Chemical Properties A white to cream-coloured, slightly hygroscopic, crystalline powder. Soluble 1 in 100 of water and 1 in 30 of 0.2% hydrochloric acid; practically insoluble in ethanol, chloroform, and ether; soluble in solutions of dilute alkalis. pK_a 2.5, 5.2, 7.3. Log *P* (octanol/water), 0.6.

High Performance Liquid Chromatography System HAA—Retention time 3.9 min (peak 1).

Ultraviolet Spectrum Aqueous acid—258 nm; water—260 nm (A^1_1=232a).



Infrared Spectrum Principal peaks at wavenumbers 1754, 1582, 1681, 1271, 695, 1186 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection (λ=265 nm). Limit of quantification, 0.5 mg/L [Kovach *et al.* 1991].

Serum **HPLC** See Serum [Kovach *et al.* 1991].

Urine **HPLC** See Serum [Kovach *et al.* 1991].

Biological Fluids **HPLC** For a review of methods for the analysis of antibiotics, see Nilsson-Ehle [1983].

Disposition in the Body

Therapeutic Concentration

Non-fasting and fasting patients were administered a single oral dose of 250 mg or 500 mg cephalexin. The highest concentration of the drug in radicular granuloma was observed 2 h after dosing at a concentration of 1.65 mg/g after the 250 mg dose in the non-fasting patients. The highest concentration for the 500 mg dose was observed to be 3.35 mg/g after 2 h for the non-fasting and 3.42 mg/g after 1.5 h for the fasting patients [Akimoto *et al.* 1994].

Dose 1 to 6 g daily.

Kovach PM *et al.* (1991). High-performance liquid chromatographic determination of loracarbef, a potential metabolite, cefaclor and cephalexin in human plasma, serum and urine. *J Chromatogr* 567(1): 129–139.

Akimoto Y *et al.* (1994). Cephalexin concentrations in radicular granuloma following a single oral administration of 250- or 500-mg cephalexin. *Gen Pharmacol* 25(8): 1563–1566.

Nilsson-Ehle I (1983). High-performance liquid chromatography for analyses of antibiotics in biological fluids. *J Liq Chromatogr* 6(9): 2251–293.

Cefaloridine

Antibiotic

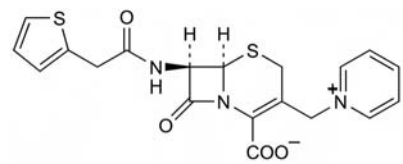
$C_{19}H_{17}N_3O_4S_2 = 415.5$

CAS—50-59-9

IUPAC Name (6*R*, 7*R*)-8-Oxo-3-(pyridin-1-ium-1-ylmethyl)-7-[(2-thiophen-2-ylacetyl)amino]- 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Synonym 1-[[(6*R*,7*R*)-2-Carboxy-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]pyridinium inner salt; cephaloridine.

Proprietary Names *Cepaloridin*; *Cepalorin*; *Ceporan*; *Ceporin(e)*; *Keflodin*; *Loridine*.



Chemical Properties A white crystalline powder, which discolours on exposure to light. Soluble 1 in 5 of water and 1 in 1000 of ethanol; practically insoluble in chloroform and ether. pK_a 3.2. Log *P* (octanol/water), −1.6.

Colour Tests Mandelin's test—violet; Marquis test—red-violet.

Thin-layer Chromatography System TA—R_f 0.73 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—241 (A^1_1=350a), 254 nm (A^1_1=341a); aqueous alkali—261 nm (A^1_1=88b); methanol—234 nm (A^1_1=372a).

Infrared Spectrum Principal peaks at wavenumbers 1600, 1764, 1656, 1538, 715, 1143 cm^{-1} (KBr disk).

Dose 1 to 3 g daily, IM; maximum of 6 g daily.

Cefalothin

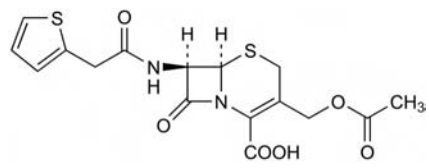
Antibiotic

$C_{16}H_{16}N_2O_6S_2 = 396.4$

CAS—153-61-7

IUPAC Name (6*R*,7*R*)-3-[(Acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl) amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Synonyms Cefalotin; cephalothin.



Chemical Properties Mp 160°. pK_a 2.2 (35°). Log *P* (octanol/water), 0.0.

Cefalothin Sodium

$C_{16}H_{15}N_2NaO_6S_2 = 418.4$

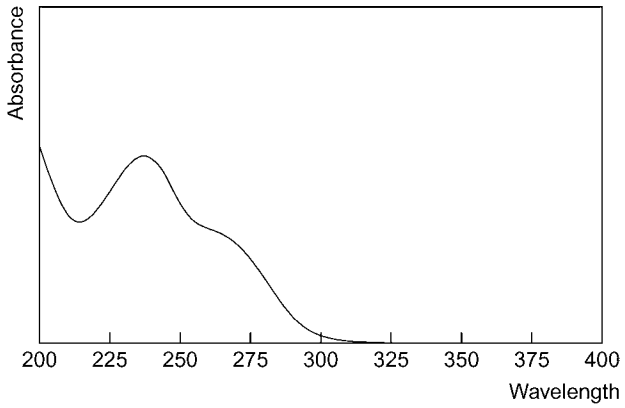
CAS—58-71-9

Proprietary Names *Ceporacin*; *Cepovenin*; *Keflin*.

Chemical Properties A white crystalline powder. Mp 204° to 205°. Soluble 1 in 3.5 of water and 1 in 700 of ethanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TAJ—R_f 0.00; system TAK—R_f 0.06; system TAL—R_f 0.41.

Ultraviolet Spectrum Cefalothin sodium: water—237 (A^1_1=335a), 265 nm (A^1_1=204b).

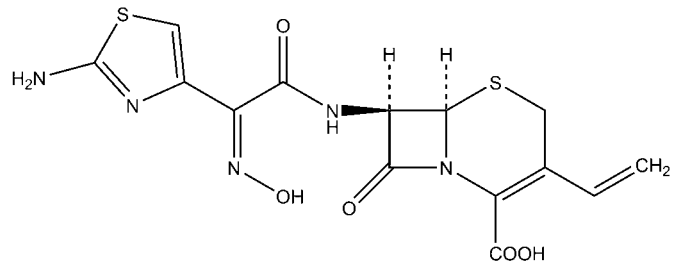


Infrared Spectrum Principal peaks at wavenumbers 1704, 1724, 1637, 1600, 1227, 1510 cm^{-1} (cefalothin sodium, KBr disk).
Dose The equivalent of 4 to 12 g of cefalothin daily, IV.

Cefdinir

Antibacterial, Cephalosporin
$C_{14}H_{13}N_5O_5S_2 = 395.4$
CAS—91832-40-5

IUPAC Name (−)-(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 72-(*Z*)-oxime
Synonyms 7-{(2-Amino-1,3-thiazol-4-yl)-2-[(*Z*)-hydroxyimino]acetamido}-3-vinylcephem-4-carboxylic acid; BMY-28488; FK-482.
Proprietary Names *Cefzon*; *Kefdir*; *Omnicef*; *Sefdin*.



Chemical Properties White to slightly brownish-yellow solid. Mp 170° (with decomposition). Slightly soluble in dilute hydrochloric acid; sparingly soluble in 0.1 mol/L phosphate buffer (pH 7.0). pK_a 9.70 [O'Neil *et al.* 2006]. Cefdinir degrades via 2 major routes in aqueous solution, β-lactam ring opening and pH-dependent isomerisation: lactonisation and epimerisation at C-6 under acidic to neutral conditions, isomerisation of the *N*-oxime function under neutral to basic conditions, and epimerisation at C-7 under basic conditions [Okamoto *et al.* 1996a].
High Performance Liquid Chromatography Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate : methanol : TEA (75 : 25 : 0.2; pH 2.2 with perchloric acid), flow rate 1.0 mL/min. UV detection (λ = 287 nm). Retention time: ~10 min. Limit of quantification not reported [Mehta *et al.* 2005]. Column: TSK-gel ODS-80T × M (75 × 4.6 mm i.d., 5 µm). Mobile phase: dioxane : methanol : 33 mmol/L citric acid buffer (pH 2.0; 1 : 4 : 36), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: ~4.0 min. Limit of quantification, 0.5 mg/L [Okamoto *et al.* 1996a, b].
Quantification
***Plasma* HPLC** Column: TSK-gel ODS-80TM (75 × 4.6 mm i.d., 5 µm). Mobile phase: dioxane : methanol : 33 mmol/L citric acid buffer (pH 2.0; 1 : 4 : 36), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention time: 12.0 min. Limit of quantification not reported [Fujita *et al.* 2007]. Column: TSK-gel ODS-80TM (250 × 4.6 mm i.d.). Mobile phase: citrate buffer (pH 2.5) : acetonitrile, flow rate 1.2 mL/min. UV detection (λ = 280 nm). Limit of detection, 0.05 mg/L [Hishida *et al.* 1998]. Column: NovaPak C_{18} (4 µm). Mobile phase: acetonitrile: 0.015 mol/L dibasic potassium phosphate (pH 3.1; 12 : 88), flow rate 1.0 mL/min. UV detection (λ = 287 nm). Retention time: 7.6 min. Limit of detection, 0.015 mg/L [Richer *et al.* 1995].
LC-MS Column: C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (25 : 75 : 0.075), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 2.4 min. Limit of quantification, 5 µg/L [Chen *et al.* 2006].
***Urine* HPLC** Column: TSK-gel ODS-80TM (75 × 4.6 mm i.d., 5 µm). Mobile phase: dioxane : methanol : 33 mmol/L citric acid buffer (pH 2.0; 1 : 4 : 36), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention time: 12.0 min. Limit of quantification not reported [Fujita *et al.* 2007].
***Other* HPLC** Blister Fluid. Column: NovaPak C_{18} (4 µm). Mobile phase: acetonitrile : 0.015 mol/L dibasic potassium phosphate (pH 3.3, 11 : 89), flow rate 1.0 mL/min. UV detection (λ = 287 nm). Retention time: 8.2 min. Limit of detection, 0.05 mg/L [Richer *et al.* 1995]. Pharmaceutical Suspension. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate : methanol : TEA (pH 2.2; 75 : 25 : 0.2), flow rate 1.0 mL/min. UV detection (λ = 287 nm). Retention time: approx. 10 min. Limit of quantification, 0.03 mg/L, limit of detection, 0.01 mg/L [Mehta *et al.* 2005].
Disposition in the Body Readily absorbed from the gastrointestinal tract after oral doses, with peak plasma concentrations occurring 2 to 4 h after a dose. It is widely distributed and shows good penetration into bronchial mucosa and epithelial lining fluid, tonsillar tissue, sinus tissue, skin blister fluid, and middle ear fluid. Cefdinir does not penetrate into alveolar macrophages. Distribution into skin blisters is reported to average 48% of plasma concentrations. It is not appreciably metabolised in adults and is predominantly eliminated via the kidneys as unchanged drug (12 to 18% of the total dose). Cefdinir is removed by dialysis.
Therapeutic Concentration

Twenty-nine young patients (aged 6 months to 10 years) were administered a single 14 mg/kg dose of cefdinir on day 1 followed by another 25 mg/kg dose on day 2, under fasted or non-fasted conditions. Mean peak plasma cefdinir concentrations were reported as follows:

	14 mg/kg		*25 mg/kg*	
	Non-fasting	*Fasting*	*Non-fasting*	*Fasting*
C_{max} (mg/L)	2.07	1.80	3.70	4.42
Time (h)	3.40	2.90	2.90	2.70

[Bowlware *et al.* 2006].

Sixteen healthy male volunteers (age 19 to 30 years) were administered a single dose of 200, 300, 400 or 600 mg cefdinir on each of study days 1, 8, 15 and 22. Mean peak plasma and blister fluid cefdinir concentrations were reported as follows:

Dose (mg)	*Plasma*		*Blister fluid*	
	C_{max} (mg/L)	*Time (h)*	*C_{max} (mg/L)*	*Time (h)*
200	1.00	3.3	0.54	4.9
300	1.55	3.2	0.67	4.9
400	2.15	3.0	0.89	4.8
600	2.35	3.2	1.09	4.8

[Richer *et al.* 1995].

Sixteen elderly patients (mean age 62 years) requiring bronchoscopy were administered a single oral dose of either 300 or 600 mg cefdinir. Concentrations of cefdinir in serum, bronchial mucosa and epithelial lining fluid were 2.00, 0.78 and 0.29 mg/L, respectively, for the 300 mg dose and 4.20, 1.14 and 0.49 mg/L, respectively, following the 600 mg dose. The extent of penetration of cefdinir into bronchial mucosa after both doses was 31 to 41% [Cook *et al.* 1996].
Note For a study of cefdinir pharmacokinetics in haemodialysis patients, see Hishida *et al.* [1998].
Toxicity Absorption of cefdinir is decreased by antacids or iron supplements, and doses should be separated by an interval of at least 2 h. Probenecid reduces the renal excretion of cefdinir. The pharmacokinetics of cefdinir are altered in patients with renal impairment.
Bioavailability Between 16 and 25%.
Half-life 1.7 h.
Volume of Distribution Approximately 1.6 to 2.1 L/kg.
Protein Binding Approximately 60 to 70%.
Dose The usual adult dose is 600 mg daily as a single dose or in two divided doses given orally. Children may be given 14 mg/kg daily up to a maximum of 600 mg daily. Doses may need to be reduced in patients with renal impairment.

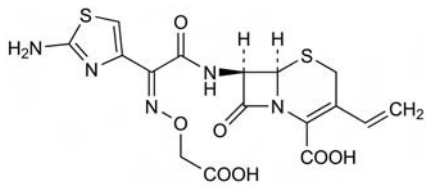
Bowlware KL *et al.* (2006). Cefdinir pharmacokinetics and tolerability in children receiving 25 mg/kg once daily. *Pediatr Infect Dis J* 25: 208–210.
Chen ZJ *et al.* (2006). Selective method for the determination of cefdinir in human plasma using liquid chromatography electrospray ionization tandam mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 834: 163–169.
Cook PJ *et al.* (1996). Distribution of cefdinir, a third generation cephalosporin antibiotic, in serum and pulmonary compartments. *J Antimicrob Chemother* 37: 331–339.
Fujita T *et al.* (2007). Effect of L-phenylalanine supplementation and a high-protein diet on pharmacokinetics of cefdinir in healthy volunteers: an exploratory study. *J Clin Pharm Ther* 32: 277–285.
Hishida A *et al.* (1998). Pharmacokinetic study of an oral cephalosporin, cefdinir, in hemodialysis patients. *Antimicrob Agents Chemother* 42: 1718–1721.
Mehta TN *et al.* (2005). Determination of cefdinir by a stability-indicating liquid chromatographic method. *J AOAC Int* 88: 1661–1665.
Okamoto Y *et al.* (1996a). Method development for the determination of cefdinir and its related substances by high-performance liquid chromatography. *J Pharm Biomed Anal* 14: 739–748.

Okamoto Y *et al.* (1996b). Degradation kinetics and isomerization of cefdinir, a new oral cephalosporin, in aqueous solution 1. *J Pharm Sci* 85: 976–983.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.
Richer M *et al.* (1995). Suction-induced blister fluid penetration of cefdinir in healthy volunteers following ascending oral doses. *Antimicrob Agents Chemother* 39: 1082–1086.

Cefixime

Antibacterial
$C_{16}H_{15}N_5O_7S_2 = 453.5$
CAS—79350-37-1

IUPAC Name (6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Synonyms Cefiximum; CL-284635; FK-027; FR-17027.
Proprietary Names *Aerocef*; *Cefixoral*; *Cefspan*; *Cephoral*; *Denvar*; *Fixim(e)*; *Oroken*; *Necopen*; *Suprax*; *Tricef*; *Unixine*.



Chemical Properties A white/almost white to light-yellow crystalline powder. It is slightly soluble in water and alcohol; sparingly soluble in dehydrated alcohol and acetone; freely soluble in methyl alcohol, glycerol and propylene glycol; very slightly soluble in 70% sorbitol and octanol; practically insoluble in ether, ethyl acetate and hexane. pK_a, 3.73.

Cefixime Trihydrate

$C_{16}H_{21}N_5O_{10}S_2 = 507.5$
Chemical Properties Pale-yellow solid. Mp 218° to 225°.

Cefixime Disodium Salt

$C_{16}H_{13}N_5Na_2O_7S = 465.4$
Chemical Properties Mp >250°.

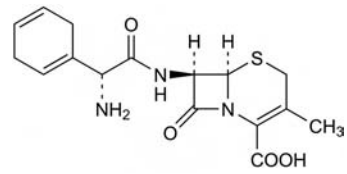
Thin-layer Chromatography Plate: silica gel with (2.5 × 10 cm). Mobile phase: ethylacetate: acetone: methanol: water (5:2.5:2.5:1.5). R_f 0.67 [Eric-Jovanovic *et al.* 1998].
High Performance Liquid Chromatography System HAA—retention time 4.8 min.
Quantification
Serum **HPLC**. Column: reversed phase Altex Ultrasphere Octyl C_8 (15 cm x 4.6 mm i.d., 5 µm). Mobile phase: methanol: 12.5 mmol/L monobasic sodium potassium buffer (pH 2.6, 20 : 80), flow rate 2 mL/min. UV detection (λ=240 nm). Limit of detection, 0.1 mg/L [McAteer *et al.* 1987].
Bioassay Limit of detection 0.1 mg/L [Brittain *et al.* 1985].
Urine **Bioassay** Limit of detection, 2.0 mg/L [Brittain *et al.* 1985].
Disposition in the Body 40 to 50% of an orally administered dose of cefixime (tablet form or as a suspension) is slowly absorbed. 20% of a dose (or 50% of the absorbed dose) is excreted unchanged in the urine within 24 h. Possibly up to 60% is eliminated by non-renal pathways, with some excretion in faeces as bile. There is no evidence of metabolism. Cefixime crosses the placenta.
Therapeutic Concentration An oral suspension dose provides on average a peak concentration 25 to 50% higher than that for the tablet form.
Twelve healthy subjects, aged between 24 and 31 years, were administered with a 50, 100, 200 and 400 mg dose of cefixime, after fasting. Peak serum concentrations of 1.02 ± 0.12, 1.46 ± 0.09, 2.63 ± 0.2 and 3.85 ± 0.23 mg/L were reached, respectively, within 2.5 to 4.5 h [Brittain *et al.* 1985].
Toxicity Diarrhoea and pseudomembranous colitis can be mild to life-threatening. Doses up to 2 g have been administered and no other serious toxic effects observed.
Bioavailability 22 to 54% (oral).
Half-life Elimination half-life is between 3 and 4 h.
Volume of Distribution 0.1 L/kg.
Clearance Serum, 0.4 mL/min/kg.
Protein Binding Approximately 65%.
Dose Adults: 200 to 400 mg daily. Children with a body weight <50 kg: 8 mg/kg body weight daily. Patients with a creatinine clearance <20 mL/min: maximum 200 mg daily.

Brittain DC *et al.* (1985). The pharmacokinetic and bactericidal characteristics of oral cefixime. *Clin Pharmacol Ther* 38(5): 590–594.
Eric-Jovanovic S *et al.* (1998). HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms. *J Pharm Biomed Anal* 18(4–5): 893–898.
McAteer JA *et al.* (1987). Liquid-chromatographic determination of five orally active cephalosporins–cefixime, cefaclor, cefadroxil, cephalexin, and cephradine–in human serum. *Clin Chem* 33 (10): 1788–1790.

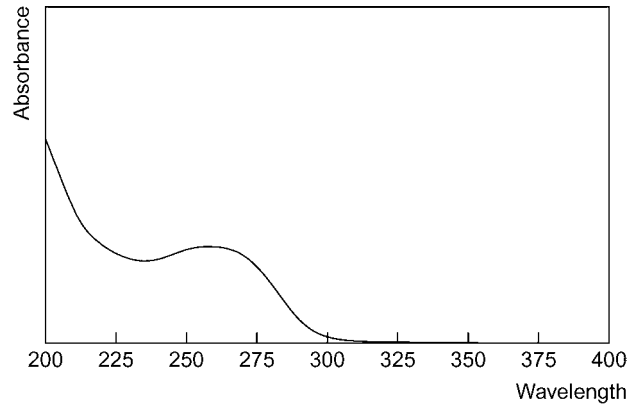
Cefradine

Antibiotic
$C_{16}H_{19}N_3O_4S = 349.4$
CAS—38821-53-3 (anhydrous); 75975-70-1 (monohydrate); 31828-50-9 (dihydrate)

IUPAC Name (6*R*,7*R*)-7-[[(2*R*)-Amino-1,4-cyclohexadien-1-ylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Synonyms Cephradine; SQ-11436.
Proprietary Names *Anspor*; *Eskacef*; *Maxisporin*; *Megacef*; *Sefril*; *Velosef*.



Chemical Properties A white to cream-coloured crystalline powder. Soluble 1 in 100 of water and 1 in 70 of methanol; practically insoluble in ethanol, chloroform, and ether. pK_a 2.5, 7.3 (35°). Log *P* (octanol/water), 0.4.
Ultraviolet Spectrum Aqueous acid—257 nm.



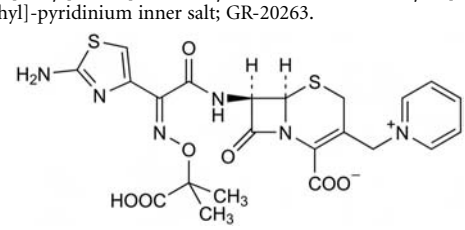
Infrared Spectrum Principal peaks at wavenumbers 1754, 1582, 1678, 1271, 1235, 1190 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** UV detection (λ=260 nm). Limit of quantification, 0.2 mg/L [Johnson *et al.* 2000].
Disposition in the Body
Therapeutic Concentration
Ten healthy, young male and female volunteers (aged 19 to 25 years) and nine elderly volunteers (aged 65 to 81 years) were administered 1 g cephradine as a 5-min IV infusion followed by 1 g oral dose the next day. The mean serum drug concentration was 1.52 mg/L 6 h after dosing and 0.73 mg/L after 8 h for the elderly volunteers compared with 0.43 mg/L at 6 h for the younger volunteers [Schwinghammer *et al.* 1990].
Dose 1 to 4 g daily.

Johnson VM *et al.* (2000). Determination of the cephalosporin antibiotic cephradine in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 740(1): 71–80.
Schwinghammer TL *et al.* (1990). Pharmacokinetics of cephradine administered intravenously and orally to young and elderly subjects. *J Clin Pharmacol* 30(10): 893–899.

Ceftazidime

Antibacterial
$C_{22}H_{22}N_6O_7S_2 = 546.6$
CAS—72558-82-8

IUPAC Name 6*R*,7*R*)-7-[[(2*Z*)-2-(2-Amino-1, 3-thiazol-4-yl)-2-(1-hydroxy-2-methyl-1-oxopropan-2-yl)oxyiminoacetyl] amino]-8-oxo-3-(pyridin-1-ium-1-ylmethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
Synonym 1-[[(6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-pyridinium inner salt; GR-20263.



Chemical Properties pK_a 1.9, 2.7, 4.1.

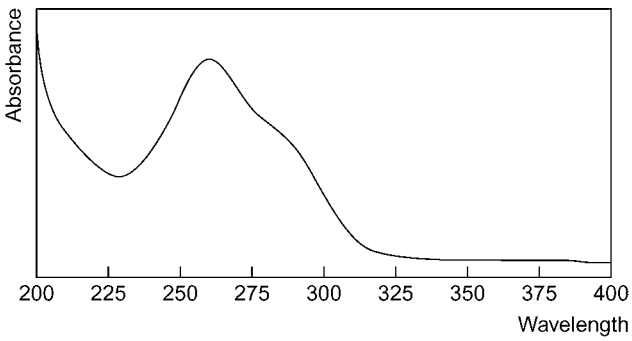
Ceftazidime Pentahydrate

$C_{22}H_{22}N_6O_7S_2$, $5H_2O$ = 636.7
CAS—78439-06-2

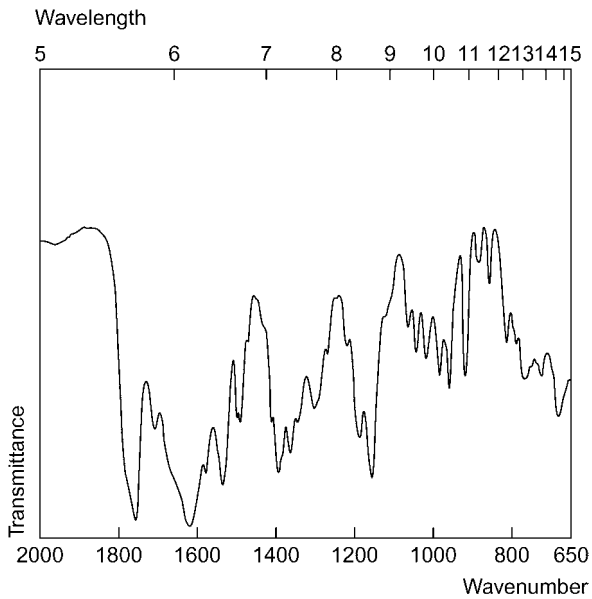
Proprietary Names *Ceftim*; *Ceptaz*; *Fortam*; *Fortaz*; *Fortum*; *Glazidim*; *Kefadim*; *Kefamin*; *Kefazim*; *Kefzim*; *Panzid*; *Potendal*; *Spectrum*; *Starcef*; *Tazicef*; *Tazidime*.

Chemical Properties A white to cream-coloured crystalline powder. Mp 135° to 137°, with decomposition (dark brown). It is slightly soluble in water (5 g/L); soluble in dimethylsulfoxide and in alkali; slightly soluble in dimethyl formamide and in methanol; practically insoluble in acetone, in chloroform, in dioxan, in ethanol (<1 g/L), in ether, in ethyl acetate, and in toluene; soluble in alkali.

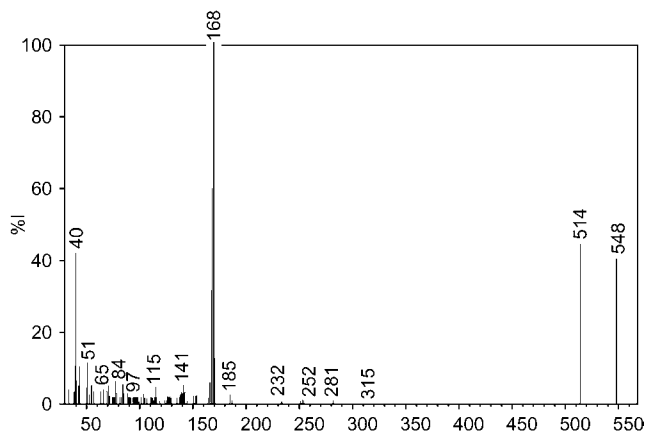
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—259 nm; (methanol)—257 nm; (0.1 mol/L sulfuric acid)—260 nm (A=2.514); basic—257 nm.



Infrared Spectrum Principal peaks at wavenumbers 1621, 1757, 1537, 1157, 3000–3600 cm^{-1} (pentahydrate salt) (KBr pellet).



Mass Spectrum Principal ions at *m/z* 168, 167, 514, 548, 40, 166, 51.



Quantification

Serum **HPLC** Column: C_{18} LiChrosorb (250 × 4.6 mm, 10 µm). Mobile phase: methanol (6%) : 100 mM sodium phosphate buffer (pH 6.0), flow rate 2.5 mL/min. Retention time: 9.6 min. UV detection (λ=254 nm). Limit of detection, 1 mg/L [Holt *et al.* 1990]. UV detection. Limit of detection, 0.2 mg/L (conventional HPLC), 0.05 mg/L (high-speed HPLC) [Jehl *et al.* 1987]. Column: C_{18} µBondapak (300 × 3.9 mm i.d.). Mobile phase for serum analysis: 150 mmol/L potassium dihydrogen phosphate buffer (pH 6.5) : methanol (82:18), flow rate 1.2 mL/min. For urine analysis: 50 mM potassium dihydrogen phosphate buffer (pH 6.5) : methanol (88:12). Retention time in serum and urine: 5.0 min. UV detection (λ=255 nm). Limit of detection, 1 mg/L [Leeder *et al.* 1983]. Column: RP MCH 10 Micropak (300 × 4.0 mm). Temperature: 50°. Mobile phase: (method A) 20% methanol : 50 mmol/L 80% aqueous ammonium dihydrogen phosphate and 117 µM perchloric acid, flow rate 1.0 mL/min. (Method B) 9% acetonitrile : 91% phosphate–perchloric acid solution (as method A), flow rate 0.5 mL/min, increased to 1.5 mL/min over first 4 min. *k* value: 0.3. UV detection (λ=257 nm). Limit of detection, 0.3 mg/L [Myers, Blumer 1983].

Urine **HPLC** See Serum [Holt *et al.* 1990]. See Serum [Jehl *et al.* 1987]. See Serum [Leeder *et al.* 1983]. See Serum [Myers, Blumer 1983].

Bile **HPLC** See Serum [Jehl *et al.* 1987].

Cerebrospinal Fluid **HPLC** See Serum [Holt *et al.* 1990].

Disposition in the Body Ceftazidime is not absorbed by oral administration but is given by injection as its sodium salt or in solution with arginine. It is widely distributed in body tissues and fluids including bone, synovial fluid, heart and bile; therapeutic concentrations are achieved in the CSF when the meninges are inflamed. The drug crosses the placenta and is distributed into breast milk. It is passively excreted in bile, although only a small proportion is eliminated by this route. The drug is mainly excreted by the kidneys, almost exclusively by glomerular filtration. About 80 to 90% of a dose is recovered unchanged in urine in 24 h as it does not undergo metabolism. It is removed by haemodialysis and peritoneal dialysis. There is no evidence of accumulation of the drug in serum after multiple dosing.

Therapeutic Concentration The trough serum therapeutic concentration range is 20 to 40 mg/L and peak, 50 to 200 mg/L.

In healthy adults, mean peak serum concentrations of 45 and 90 mg/L have been reported after IV administration of 500 mg and 1 g, respectively, over 5 min. Mean peak serum concentrations of 42, 69 and 170 mg/L have been reported after IV infusion of 500 mg, 1 g and 2 g, respectively, over 20 to 30 min. These concentrations were observed up to 30 min after the end of infusion. After IM injection of 500 mg and 1 g ceftazidine, mean peak serum concentrations were 17 and 39 mg/L, respectively, at approximately, 1 h. Serum concentrations stayed above 4 mg/L for 6 to 8 h after these doses [Eli Lilly & Co.].

Toxicity

Ceftazidime overdosage may lead to neurological effects, including encephalopathy, neuromuscular excitability, convulsions and coma. Seizures may occur in patients with renal impairment [Eli Lilly & Co.].

Half-life Plasma half-life, ~2 h.

Volume of Distribution Steady state, 0.2 to 0.3 L/kg.

Clearance Plasma clearance, 115 mL/min; serum, 98 to 122 mL/min (healthy individuals); 142 to 376 mL/min/1.73m^2 for patients suffering with cystic fibrosis; 79 mL/min for geriatric patients aged between 63 and 83 years.

Protein Binding <10%.

Note For a review of ceftazidime, see Rains *et al.* [1995].

Dose Adults: usual dose is 500 mg to 2 g every 8 to 12 h. In patients >80 years, daily dose should not exceed 2 g. Infants and children over 2 months: between 50 and 150 mg/kg daily IV. Maximum is 6 g daily. Neonates and children up to 2 months: 25 to 60 mg/kg daily. Dose should be reduced in patients with impaired renal function.

Data as supplied by Eli Lilly & Company Ltd.

Holt DE *et al.* (1990). A high performance liquid chromatography system for the simultaneous assay of some antibiotics commonly found in combination in clinical samples. *J Antimicrob Chemother* 26: 107–115.

Jehl F *et al.* (1987). Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. *J Chromatogr* 413: 109–119.

Leeder JS *et al.* (1983). High-pressure liquid chromatographic analysis of ceftazidime in serum and urine. *Antimicrob Agents Chemother* 24: 720–724.

Myers CM, Blumer JL (1983). Determination of ceftazidime in biological fluids by using high-pressure liquid chromatography. *Antimicrob Agents Chemother* 24: 343–346.

Rains CP *et al.* (1995). Ceftazidime. An update of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 49: 577–617.

Ceftriaxone

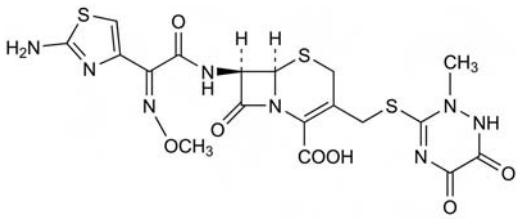
Antibacterial

$C_{18}H_{18}N_8O_7S_3$ = 554.6
CAS—73384-59-5

IUPAC Name (6*R*,7*R*)-7-[[(2*Z*)-2-(2-Amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-5,6-dioxo-1H-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Synonyms (6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)

thio]-methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid; cefatriaxone, Ro-13-9904/000.

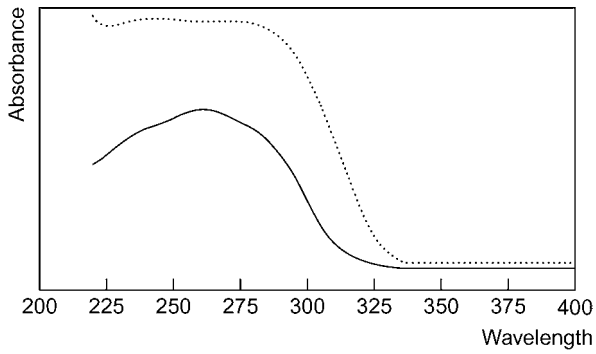


Chemical Properties pK_a 3, 3.2, 4.1. (disodium salt, hemiheptahydrate).

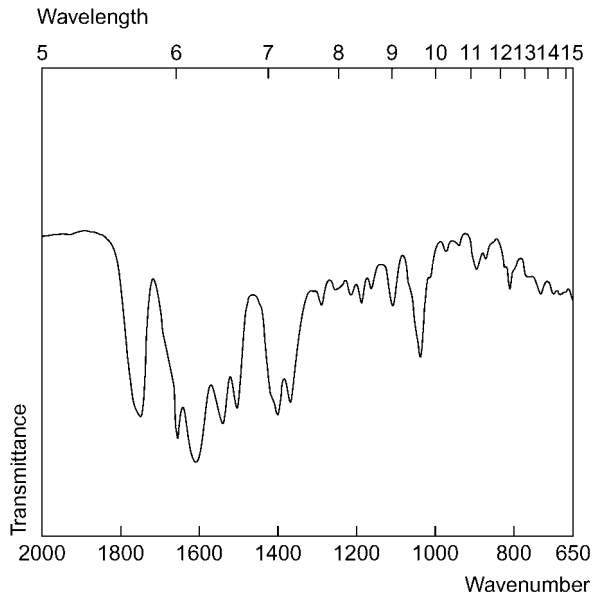
Ceftriaxone Sodium

$C_{18}H_{16}N_8Na_2O_7S_3$, $3^1/_2H_2O$ = 661.6
CAS—74578-69-1 (anhydrous); 104376-79-6 (hemiheptahydrate)
Synonym Cefatriaxone sodium
Proprietary Names *Hosbocin*; *Rocefalin*; *Rocefin*; *Rocephalin*; *Rocephin*; *Rocephine*.
Chemical Properties An almost white to yellowish-orange, slightly hygroscopic, crystalline powder. Mp >155°, with decomposition. It is freely or very soluble in water (40 g/100 mL at 25°); very slightly soluble in ethanol; sparingly soluble in methanol.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.88.
High Performance Liquid Chromatography System HX—RI 239; system HAA—retention time 5.3 min.
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—261 nm; basic—242, 269 nm.



Infrared Spectrum Principal peaks at wavenumber 1739, 1598, 1527, 1393 cm^{-1} (KBr pellets).



Quantification
Plasma **HPLC** UV detection. Limit of detection, 0.4 mg/L [Ascalone, Dal Bò 1983].
Serum **HPLC** Column: C_{18} RP μBondapak (300 × 4.0 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 9) containing 10 mmol/L hexadecyltrimethylammonium bromide (46:54), flow rate 1.5 mL/min. UV detection (λ=274 nm). Retention time: 4.6 min. Limit of detection, 1 mg/L [Granich, Krogstad 1987]. UV detection. Limits of detection, 0.2 mg/L (conventional HPLC), 0.05 mg/L (high-speed HPLC) [Jehl *et al.* 1987].
Urine **HPLC** See Serum [Granich, Krogstad 1987]. See Serum [Jehl *et al.* 1987]. UV detection. Limit of detection, 3.0 mg/L [Ascalone, Dal Bò 1983].
Bile **HPLC** See Serum [Jehl *et al.* 1987].
Cerebrospinal Fluid **HPLC** See Serum [Granich, Krogstad 1987].
Saliva **HPLC** UV detection. Limit of detection, 0.03 mg/L [Ascalone, Dal Bò 1983].
Disposition in the Body Following IM administration of ceftriaxone, peak plasma concentrations occur after about 2 to 3 h. It is widely distributed in body tissues and fluids; therapeutic concentrations may occur in the CSF when the meninges are inflamed. It crosses the placenta and is distributed into breast milk. High concentrations are found in bile. About 40 to 65% of a dose is excreted unchanged in urine, mainly by glomerular filtration; the remainder is excreted in bile and is found in faeces as the unchanged drug and inactive metabolites.
Therapeutic Concentration The serum therapeutic concentration range is 15 to 75 mg/L.

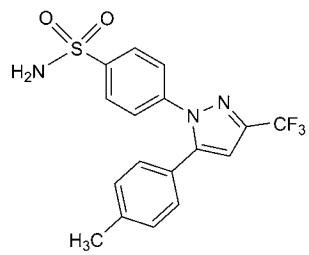
Peak plasma concentrations of 46.2 to 66.5 mg/L (mean, 54.9 mg/L) occurred 1 to 2 h after an IM injection of 500 mg administered to 6 subjects. Those participating in the study were males, mean age 25.7 years (range 19 to 43 years) with maturity-induced diabetes controlled by diet alone. Doses were repeated every 12h for a total of 5 doses. At steady state, the plasma concentrations ranged from 64.8 to 86.5 mg/L (mean 74.1 mg/L) [Pickup *et al.* 1981].

Half-life Plasma, 6 to 9 h.
Volume of Distribution 5.8 to 13.5 L.
Clearance Plasma, 0.58 to 1.45 L/h.
Protein Binding 85 to 95% depending on plasma concentration of ceftriaxone.
Note For reviews of the pharmacokinetics of ceftriaxone, see Yuk *et al.* [1989], Patel, Kaplan [1984] and Brogden, Ward [1988].
Dose 1 g daily or for severe infections, the equivalent of up to 4 g of ceftriaxone intravenously or intramuscularly daily.

Ascalone V, Dal Bò L (1983). Determination of ceftriaxone, a novel cephalosporin, in plasma, urine and saliva by high-performance liquid chromatography on an NH2 bonded-phase column. *J Chromatogr* 273: 357–366.
Brogden RN, Ward A (1988). Ceftriaxone. A reappraisal of its antibacterial activity and pharmacokinetic properties, and an update on its therapeutic use with particular reference to once-daily administration. *Drugs* 35(6): 604–645.
Granich GG, Krogstad DJ (1987). Ion pair high-performance liquid chromatographic assay for ceftriaxone. *Antimicrob Agents Chemother* 31: 385–388.
Jehl F *et al.* (1987). Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. *J Chromatogr* 413: 109–119.
Patel IH, Kaplan SA (1984). Pharmacokinetic profile of ceftriaxone in man. *Am J Med* 77: 17–25.
Pickup ME *et al.* (1981). A pharmacokinetic and tolerance study of Ro13-9904, a new cephalosporin antibiotic. *Br J Clin Pharmacol* 12: 111–115.
Yuk JH *et al.* (1989). Clinical pharmacokinetics of ceftriaxone. *Clin Pharmacokinet* 17: 223–235.

Celecoxib

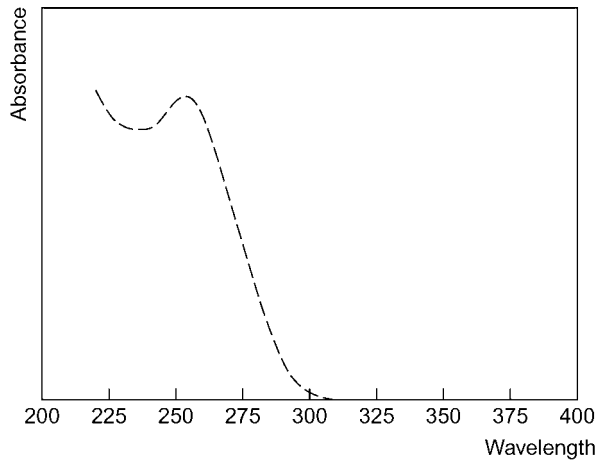
COX-2 Inhibitor, NSAID
$C_{17}H_{14}F_3N_3O_2S$ = 381.4
CAS—169590-42-5
IUPAC Name 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide
Synonyms C-58635; *p*-[5-*p*-methylphenyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide; YM-177.
Proprietary Name *Celebrex*



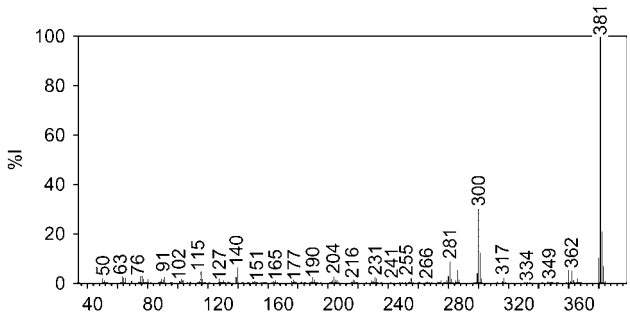
Chemical Properties Pale-yellow solid. Mp 157° to 159°. pK_a 11.1. Spiked plasma samples were stable at –20° for up to 2 months, 4° for at least 1 month, and at room temperature for 12 h [Zarghi *et al.* 2006]. Stable in plasma and breast milk for at least 4 freeze-thaw cycles if stored at –30°. The standard stock solution was stable for at least 4 months at 4° [Zhang *et al.* 2006]. The stability of analytes was established for 21 days at −20°, in frozen plasma at –20° for at least 3 freeze-thaw cycles, over 24 h at 5° and on the benchtop for 8 h [Pavan Kumar *et al.* 2006]. Standards and plasma samples were stable at −20° for 2 months and at room temperature for 24 h [Jalalizadeh *et al.* 2004]. Stable in plasma after 3 freeze-thaw cycles and at −70° for up to 9 months [Chow *et al.* 2004]. Serum samples were stable for at least 1 year when stored at −20°, after 3 freeze-thaw cycles, at room temperature for at least 3 days, and at 4° for at least 2 weeks [Schönberger *et al.* 2002]. Standard solutions were found to be stable at −80° for at least 2 months and for a

minimum of 2 weeks when stored protected from light at room temperature [Werner *et al.* 2002].

Ultraviolet Spectrum Aqueous acid (ethanol)—254 nm.



Mass Spectrum Principal ions at *m/z* 381, 300, 281, 140, 115, 362, 360, 204.



Quantification

Plasma **HPLC** Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : distilled water (45 : 10 : 45) containing 0.2% acetic acid (pH 3.5), flow rate 2 mL/min. UV detection (λ = 254 nm). Retention time: 3.6 min. Limit of quantification, 10 µg/L [Zarghi *et al.* 2006]. Column: Aqua C_{18} (75 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 3.5; 50 : 50) containing 0.1% triethylamine, flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 8.2 min. Limit of quantification, 10 µg/L [Zhang *et al.* 2006]. Column: Kromasil KR 100-5 C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water-acetonitrile (5 : 95) : methanol-water (90 : 10; 100 : 0 : 0 at 0 min, 90 : 0 : 10 at 2 min, 50 : 20 : 30 at 9 min, 30 : 50 : 20 at 25 min, 10 : 85 : 5 at 35 min, 100 : 0 : 0 at 36 min until 45 min), flow rate 1.0 mL/min. UV detection (λ = 235 nm). Retention time: 32.2 min. Limit of quantification, 0.1 mg/L [Pavan Kumar *et al.* 2006]. Column: Zorbax SB-CN (5 µm). Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen orthophosphate buffer (pH 2.4; 42 : 58), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 14.6 min. Limit of quantification, 10 µg/L [Hamama *et al.* 2005]. Column: Inertsil C_{18} ODS (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.05% glacial acetic acid (68 : 32), flow rate 1.0 mL/min. DAD (λ = 230 nm). Retention time: 19 min. Limit of quantification, 3.2 mg/L; limit of detection, 1.0 mg/L [Rao *et al.* 2005]. Column: Nucleosil 100-5 CN (250 × 4.6 mm i.d.). Mobile phase: water : acetonitrile (60 : 40), flow rate 0.9 mL/min. UV detection (λ = 260 nm). Retention time: 7.02 min. Limit of quantification, 10 µg/L, limit of detection, 4 µg/L [Jalalizadeh *et al.* 2004]. Column: Nova Pak C_8 (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : tetrahydrofuran: 0.02 mol/L sodium acetate buffer (30 : 8 : 62), flow rate 1.5 mL/min. UV detection (λ = 215 nm). Retention time: 18 min. Limit of quantification, 40 µg/L [Chow *et al.* 2004]. Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-0.01 mol/L sodium hydrogen phosphate buffer (pH 5.4; 10 : 90): acetonitrile-0.01 mol/L sodium hydrogen phosphate buffer (pH 5.4; 80 : 20, 90 : 10 for 10 min to 62 : 38 at 47 min to 27 : 73 at 63 min for 5 min to 90 : 10 at 68.1 min), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of quantification, 10 µg/L, limit of detection, 0.5 ng [Störmer *et al.* 2003]. Column: Nucleosil-NO_2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : methylene chloride : isopropyl alcohol (70 : 25 : 5), flow rate 1.4 mL/min. UV detection (λ = 260 nm). Limit of quantification, 25 µg/L [Rose *et al.* 2000].

LC-MS Column: Nucleosil C_8 120-5 (11 × 2.0 mm i.d.). Mobile phase: methanol : water (50 : 50) with 1% acetic acid, flow rate 200 µL/min. APCI, SRM acquisition mode. Limit of quantification, 5 mg/L [Werner *et al.* 2002]. Column: Nucleosil C_{18} (30 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 25% ammonium hydroxide solution (85 : 15 : 0.1), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention time: 0.8 min. Limit of quantification, 0.25 µg/L [Brautigam *et al.* 2001].

Serum **HPLC** Column: SGE C_{18} (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium dihydrogen orthophosphate (pH 3.2) : acetonitrile (50 : 50), flow rate 1.0 mL/min. UV detection (λ = 250 nm). Retention time, 9.6 min. Limit of quantification, 10 µg/L [Jayasagar *et al.* 2002]. Column: Prontosil C_{18} AQ (150 × 3.0 mm i.d., 3 µm). Mobile phase: water : acetonitrile (40 : 60), flow rate 0.35 mL/min. Fluorescence detection (λ_{ex} = 240 nm, λ_{em} = 380 nm). Retention time: 11.7 min. Limit of quantification, 12.5 µg/L [Schönberger *et al.* 2002].

Milk **HPLC** See plasma [Zhang *et al.* 2006].

Liver Microsomes **HPLC** Column: Betasil reversed phase C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05% phosphoric acid : acetonitrile-water (90 : 10) in 0.05% phosphoric acid (70 : 30 to 20 : 80 over 13 min. UV detection (λ = 254 nm). Retention time: 14.8 min. Limit of quantification, 25 nmol/L [Tang *et al.* 2000].

Skin **HPLC** Hypersil ODS (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (55 : 45), flow rate, 1.0 mL/min. UV detection (λ = 252 nm). Limit of detection not reported [Ventura *et al.* 2006].

Disposition in the Body Celecoxib is well and rapidly absorbed and metabolised in the liver to primary alcohol, the corresponding carboxylic acid (the primary metabolite) and its glucuronide conjugate. The drug is widely distributed throughout the body. It is eliminated predominantly by hepatic metabolism (>97%), with approximately 57% of the dose excreted in the faeces and 27% in urine [Paulson *et al.* 2000]. CYP2C9 is the major isoform responsible for the metabolism of celecoxib [Davies *et al.* 2000], although CYP3A4 also plays a part [Tang *et al.* 2000].

Note For studies on the influence of CYP2C9 genetic polymorphisms, see Kirchheiner *et al.* [2003] or Brenner *et al.* [2003].

Therapeutic Concentration

Twelve healthy volunteers were administered a single 200 mg oral dose of celecoxib. The mean peak plasma concentration was 700 µg/L at 18 h [Zarghi *et al.* 2006].

After an overnight fast, 12 healthy male volunteers received 200 mg celecoxib. After 2 days, a once-daily dose of 600 mg rifampicin was given for 5 consecutive days, and on day 9 another 200 mg dose of celecoxib was administered. Before rifampicin, the mean peak plasma celecoxib concentration was 545 ± 274 µg/L reached at 4 ± 0.9 h, and after rifampicin the mean peak plasma celecoxib concentration was 239 ± 146 µg/L at 4 ± 0.8 h. Rifampicin pretreatment decreased the AUC of celecoxib by 64% and increased clearance by 185% [Jayasagar *et al.* 2003].

Twelve healthy volunteers were administered a single oral dose of 200 mg celecoxib. The mean maximum plasma concentration was 840 ± 280 µg/L reached at 2.9 ± 1.2 h [Werner *et al.* 2002].

Four healthy young volunteers were administered a single 200 mg dose of celecoxib. Peak plasma concentrations of 0.797 mg/L were reached within 1.8 h for those who had celecoxib administered without food, and 0.877 mg/L within 6.3 h for the volunteers who had the drug administered with a high-fat breakfast [Davies *et al.* 2000].

Toxicity Acute overdose can result in coma, hypertension, acute renal failure and respiratory depression. Several cases of cholestatic hepatitis have been reported after celecoxib therapy [Alegria *et al.* 2002; Galan *et al.* 2001; Nachimuthu *et al.* 2001; O'Beirne, Cairns 2001], one case also involved pancreatitis [Carrillo-Jimenez, Nurnberger 2000]. Celecoxib has been associated with toxic epidermal necrolysis [Friedman *et al.* 2002; Giglio 2003] and allergic vasculitis [Drago *et al.* 2004; Jordan *et al.* 2002], in one case fatally [Schneider *et al.* 2002]. Celecoxib has also been associated with acute febrile neutrophilic dermatoses [Fye *et al.* 2001], acute generalised exanthematous pustulosis [Yang *et al.* 2004] as well as 'drug rash with eosinophilia and systemic symptoms (DRESS)' syndrome [Lee *et al.* 2008].

Half-life 11.2 to 15.6 h.

Volume of Distribution 5.7 to 7.1 L/kg (455 ± 166 L) [Davies *et al.* 2000].

Clearance 27.7 L/h.

Distribution in Blood Red blood cells : plasma ratio is 0.89.

Protein binding 97% to albumin.

Dose 200 mg daily and increased (if necessary) to 400 mg.

Alegria P *et al.* (2002). Celecoxib-induced cholestatic hepatotoxicity in a patient with cirrhosis. *Ann Intern Med* 137: 75.

Brautigam L *et al.* (2001). Determination of celecoxib in human plasma and rat microdialysis samples by liquid chromatography tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 761: 203–212.

Brenner S *et al.* (2003). Influence of age and cytochrome P450 2C9 genotype on the steady-state disposition of diclofenac and celecoxib. *Clin Pharmacokinet* 42: 283–292.

Carrillo-Jimenez R, Nurnberger M (2000). Celecoxib-induced acute pancreatitis and hepatitis: a case report. *Arch Intern Med* 160: 553–554.

Chow HH *et al.* (2004). Determination of celecoxib in human plasma using solid-phase extraction and high-performance liquid chromatography. *J Pharm Biomed Anal* 34: 167–174.

Davies NM *et al.* (2000). Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. *Clin Pharmacokinet* 38: 225–242.

Drago F *et al.* (2004). Cutaneous vasculitis induced by cyclo-oxygenase-2 selective inhibitors. *J Am Acad Dermatol* 51: 1029–1030.

Friedman B *et al.* (2002). Toxic epidermal necrolysis due to administration of celecoxib (Celebrex). *South Med J* 95: 1213–1214.

Fye KH *et al.* (2001). Celecoxib-induced Sweet's syndrome. *J Am Acad Dermatol* 45: 300–302.

Galan MV *et al.* (2001). Celecoxib-induced cholestatic hepatitis. *Ann Intern Med* 134: 254.

Giglio P (2003). Toxic epidermal necrolysis due to administration of celecoxib (Celebrex). *South Med J* 96: 320–321.

Hamama AK *et al.* (2005). Simultaneous determination of rofecoxib and celecoxib in human plasma by high-performance liquid chromatography. *J Chromatogr Sci* 43: 351–354.

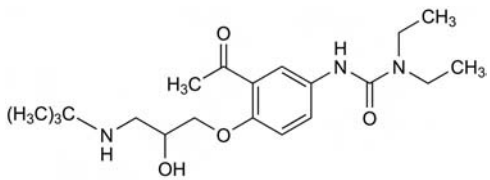
Jalalizadeh H *et al.* (2004). Determination of celecoxib in human plasma by high-performance liquid chromatography. *J Pharm Biomed Anal* 35: 665–670.

Jayasagar G *et al.* (2002). Validated HPLC method for the determination of celecoxib in human serum and its application in a clinical pharmacokinetic study. *Pharmazie* 57: 619–621.
Jayasagar G *et al.* (2003). Influence of rifampicin pretreatment on the pharmacokinetics of celecoxib in healthy male volunteers. *Drug Metabol Drug Interact* 19: 287–295.
Jordan KM *et al.* (2002). Allergic vasculitis associated with celecoxib. *Rheumatology (Oxford)* 41: 1453–1455.
Kirchheiner J *et al.* (2003). Influence of CYP2C9 genetic polymorphisms on pharmacokinetics of celecoxib and its metabolites. *Pharmacogenetics* 13: 473–480.
Lee JH *et al.* (2008). Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) syndrome induced by celecoxib and anti-tuberculosis drugs. *J Korean Med Sci* 23: 521–525.
Nachimuthu S *et al.* (2001). Acute hepatocellular and cholestatic injury in a patient taking celecoxib. *Postgrad Med J* 77: 548–550.
O'Beirne JP, Cairns SR (2001). Drug points: cholestatic hepatitis in association with celecoxib. *Brit Med J* 323: 23.
Paulson SK *et al.* (2000). Metabolism and excretion of [(14)C]celecoxib in healthy male volunteers. *Drug Metab Dispos* 28: 308–314.
Pavan Kumar VV *et al.* (2006). Simultaneous quantitation of etoricoxib, salicylic acid, valdecoxib, ketoprofen, nimesulide and celecoxib in plasma by high-performance liquid chromatography with UV detection. *Biomed Chromatogr* 20: 125–132.
Rao RN *et al.* (2005). Development and validation of a reversed-phase liquid chromatographic method for separation and simultaneous determination of COX-2 inhibitors in pharmaceuticals and its application to biological fluids. *Biomed Chromatogr* 19: 362–368.
Rose MJ *et al.* (2000). Determination of celecoxib in human plasma by normal-phase high-performance liquid chromatography with column switching and ultraviolet absorbance detection. *J Chromatogr B Biomed Sci Appl* 738: 377–385.
Schneider F *et al.* (2002). Fatal allergic vasculitis associated with celecoxib. *Lancet* 359: 852–853.
Schönberger F *et al.* (2002). Simple and sensitive method for the determination of celecoxib in human serum by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 768: 255–260.
Störmer E *et al.* (2003). Simultaneous determination of celecoxib, hydroxycelecoxib, and carboxycelecoxib in human plasma using gradient reversed-phase liquid chromatography with ultraviolet absorbance detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 783: 207–212.
Tang C *et al.* (2000). Major role of human liver microsomal cytochrome P450 2C9 (CYP2C9) in the oxidative metabolism of celecoxib, a novel cyclooxygenase-II inhibitor. *J Pharmacol Exp Ther* 293: 453–459.
Ventura CA *et al.* (2006). Influence of modified cyclodextrins on solubility and percutaneous absorption of celecoxib through human skin. *Int J Pharm* 314: 37–45.
Werner U *et al.* (2002). Investigation of the pharmacokinetics of celecoxib by liquid chromatography-mass spectrometry. *Biomed Chromatogr* 16: 56–60.
Yang CC *et al.* (2004). Acute generalized exanthematous pustulosis caused by celecoxib. *J Formos Med Assoc* 103: 555–557.
Zarghi A *et al.* (2006). Simple and rapid high-performance liquid chromatographic method for determination of celecoxib in plasma using UV detection: application in pharmacokinetic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 835: 100–104.
Zhang M *et al.* (2006). Determination of celecoxib in human plasma and breast milk by high-performance liquid chromatographic assay. *J Chromatogr B Analyt Technol Biomed Life Sci* 830: 245–248.

Celiprolol

β-*Blocker*
$C_{20}H_{33}N_3O_4$ = 379.5
CAS—56980-93-9

IUPAC Name 3-[3-Acetyl-4-[3-(tert-butylamino)-2-hydroxypropoxy]phenyl]-1,1-diethylurea
Synonyms *N′*-[3-Acetyl-4-[3-[(1,1-dimethylethyl)-amino]-2-hydroxypropoxy]phenyl]-*N*,*N*-diethylurea; ST-1396.

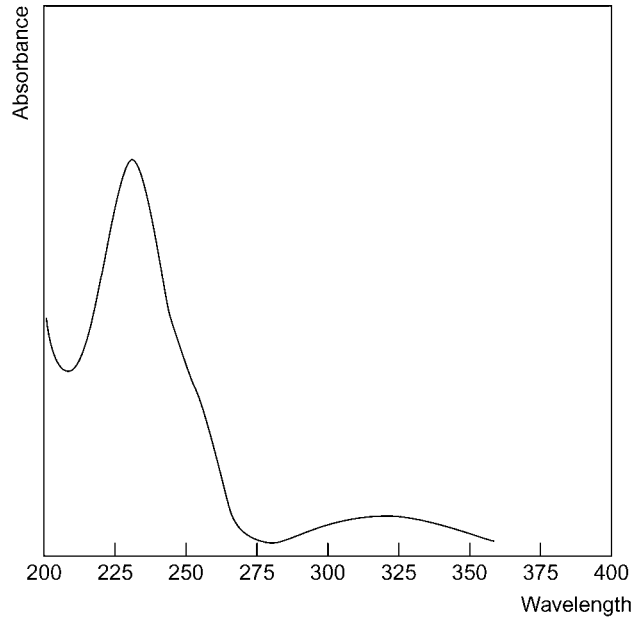


Chemical Properties Crystals. Mp 110° to 112°. pK_a 9.68 (25°). Log *P* (octanol/aqueous phosphate buffer, pH 7.4), 0.8 (37°); log *P* (chloroform/water), 0.14; log *P* (chloroform/pH 7.1 aqueous phosphate buffer), 2.42; log *P* (octanol/water), 1.92. Extraction yield (chlorobutane), 0.1 [Demme *et al.* 2005].

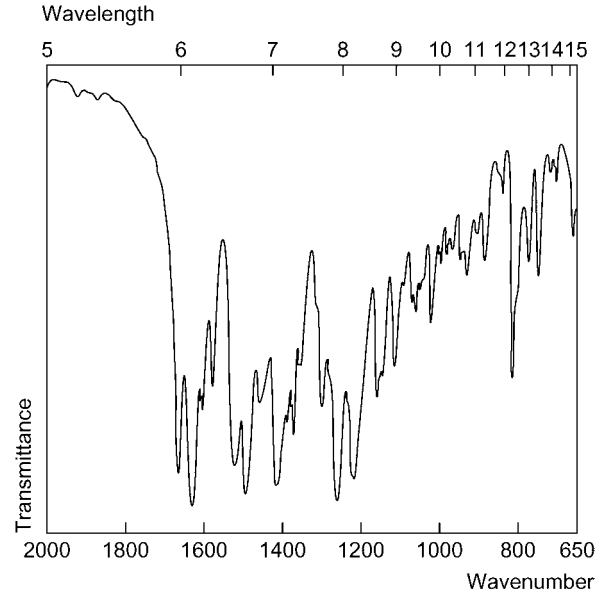
Celiprolol Hydrochloride

$C_{20}H_{33}N_3O_4$, HCl = 416.0
CAS—57470-78-7
Synonyms REV-5320A; RG-5320A; RHC-5320A; ST-1236.
Proprietary Names *Cardem*; *Celectol*; *Celipro*; *Corliprol*; *Dilanorm*; *Jofurol*; *Moderator*; *Selectol*.
Chemical Properties White odourless crystals. Mp 197° to 200°, with decomposition. Solubility at 25° in water, 15.1 g/100 mL; methanol, 18.2 g/100 mL; ethanol, 1.61 g/100 mL; chloroform, 0.42 g/100 mL.

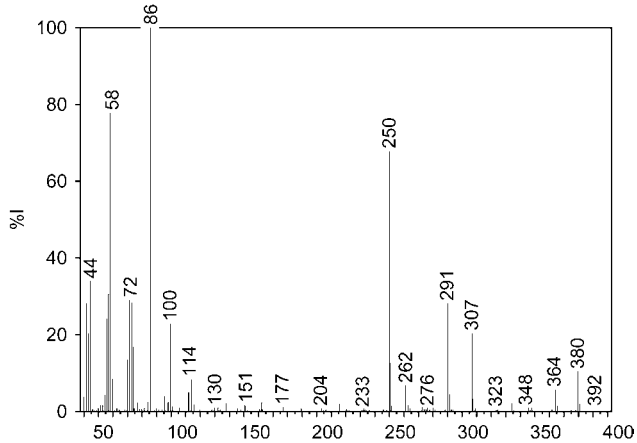
Thin-layer Chromatography System TAE—R_f 0.15; system TE—R_f 0.33.
Gas Chromatography System GA— RI 2610.
High Performance Liquid Chromatography System HAV—*k* 2.2; system HAA—retention time 11.5 min; system HZ—RI 2.5 min.
Ultraviolet Spectrum Aqueous acid (0.01 mol/L hydrochloric acid)—231 (A_1^1=660) and 324 (A_1^1=60) nm; aqueous alkali (0.01 mol/L sodium hydroxide)—231 (A_1^1=640) and 324 (A_1^1=60) nm; methanol—232 (A_1^1=775) and 329 (A_1^1=58) nm.



Infrared Spectrum Principal peaks at wavenumbers 1674, 1636, 823, 591 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 86, 58, 250, 44, 57, 72, 291, 71.



Quantification
Blood **HPLC** For fluorescence detection and UV detection methods, see Buskin *et al.* [1982].

Plasma **HPLC** UV detection. Limit of detection, about 16 µg/L [Braza *et al.* 1998]. Fluorescence detection. Limit of detection, 5 µg/L [Chiu, Raymond 1996]. UV and fluorescence detection. Limit of detection, 5 µg/L (total celiprolol), 2.5 µg/L (each enantiomer) [Verbesselt *et al.* 1996]. UV detection. Limit of detection, 4 µg/L [Rutledge *et al.* 1994a]. UV detection. Limit of detection, 25.7 µg/L [Rutledge *et al.* 1994b]. Fluorescence detection. Limit of detection, 1.5 µg/L (each enantiomer) [Hartmann *et al.* 1989]. Fluorescence detection. Limit of detection, 10 µg/L [Hippmann, Takacs 1983]. UV detection. Limit of detection, 10 µg/L. Fluorescence detection. Limit of detection, 5 µg/L [Buskin *et al.* 1982].
Urine **HPLC** Fluorescence detection. Limit of detection, 2.5 µg/L (each enantiomer) [Hartmann *et al.* 1989]. See Plasma [Hippmann, Takacs 1983]. See Blood Buskin *et al.* [1982].
Disposition in the Body Absorbed after oral administration in a non-linear fashion; the percentage of dose absorbed increases with increasing dose. The extent of absorption is reduced in the presence of food. Peak plasma concentrations are reached after 2 to 3 h. Crosses the placenta. Low lipid solubility. Undergoes only minimal metabolism and is excreted unchanged in the urine (~11%) and faeces (84% in 24 h after an oral dose). After an IV dose, ~50% is excreted in urine and 31% in faeces. A very low percentage of the drug is excreted as three metabolites.
Bioavailability 30 to 70%, which is reduced in the presence of food.
Therapeutic Concentration Considerable inter-individual variation in peak plasma concentrations.

Mean peak plasma concentration measured after administration of a 200 mg single oral dose to 12 subjects was 687 µg/L after 3.71 h. Following administration of 200 mg and 400 mg daily for 7 days to 12 subjects, the mean peak plasma concentration was 597 µg/L and 1676 µg/L, respectively [Norris *et al.* 1986].

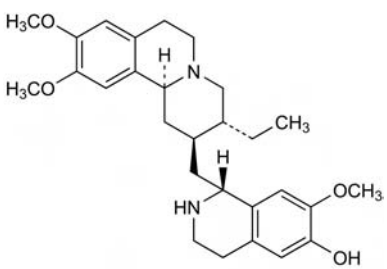
Half-life Plasma, 5 to 6 h.
Protein Binding 25%.
Note For reviews of celiprolol, see Riddell *et al.* [1987] and Milne, Buckley [1991].
Dose 200 mg of the hydrochloride daily, increased to 400 mg daily if necessary.

Braza AJ *et al.* (1998). Determination of celiprolol and oxprenolol in human plasma by high-performance liquid chromatography and the analytical error function. *J Chromatogr B Biomed Sci Appl* 718: 267–272.
Buskin JN *et al.* (1982). Specific and sensitive assay of celiprolol in blood, plasma and urine using high-performance liquid chromatography. *J Chromatogr* 230: 454–460.
Chiu FC, Raymond K (1996). Validated assay for the determination of celiprolol in plasma using high-performance liquid chromatography and a silanol deactivated reversed-phase support. *J Chromatogr B Biomed Appl* 687(2): 462–465.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings at the 12th TIAFT*, Seoul: 481–486.
Hartmann C *et al.* (1989). Simultaneous determination of (R)- and (S)-celiprolol in human plasma and urine: high-performance liquid chromatographic assay on a chiral stationary phase with fluorimetric detection. *J Chromatogr* 496: 387–396.
Hippmann D, Takacs F (1983). *Arzneimittelforschung* 33: 8–12.
Milne RJ, Buckley MM (1991). Celiprolol. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in cardiovascular disease. *Drugs* 41: 941–969.
Norris RJ *et al.* (1986). A pharmacokinetic evaluation of celiprolol in healthy elderly volunteers. *J Cardiovasc Pharmacol* 8: S91–S92.
Riddell JG *et al.* (1987). Celiprolol. A preliminary review of its pharmacodynamic and pharmacokinetic properties and its therapeutic use in hypertension and angina pectoris. *Drugs* 34: 438–458.
Rutledge DR *et al.* (1994a). Simultaneous determination of verapamil and celiprolol in human plasma. *J Chromatogr Sci* 32: 153–156.
Rutledge DR *et al.* (1994b). Liquid chromatographic determination of celiprolol, diltiazem, desmethyldiltiazem and deacetyldiltiazem in plasma using a short alkyl chain silanol deactivated column. *J Pharm Biomed Anal* 12: 135–140.
Verbesselt R *et al.* (1996). Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma. *J Chromatogr B Biomed Sci Appl* 683: 231–236.

Cephaëline

Emetic, Expectorant
$C_{28}H_{38}N_2O_4$ = 466.6
CAS—483-17-0

IUPAC Name (1*R*)-1-[[(3*R*,11*bS*)-3-ethyl-9,10-dimethoxy-2,3,4,6,7,11*b*-hexahydro-1*H*-pyrido[2,1-*a*]isoquinolin-2-yl]methyl]-7-methoxy-1,2,3,4-tetrahydroisoquinolin-6-ol
Synonyms Desmethylemetine; dihydropsychotrine; 7′,10,11-trimethoxyemetan-6′-ol.

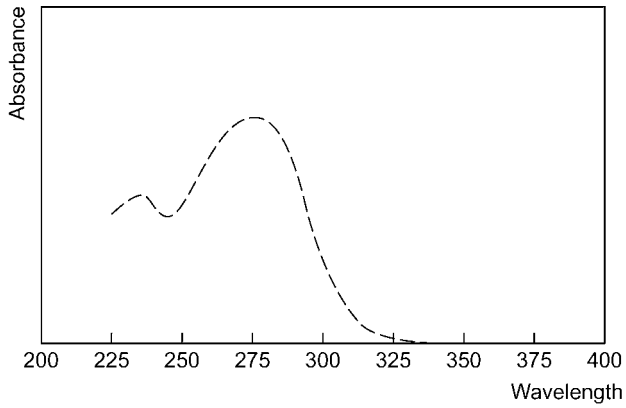


Chemical Properties An alkaloid present in ipecacuanha, the dried root, or rhizome and root, of *Cephaëlis ipecacuanha* (=*Uragoga ipecacuanha*) (Rubiaceae) or *C. acuminata*. Needles. Mp 115° to 116°. Practically insoluble in water; freely soluble in acetic acid, acetone, ethanol, and chloroform; slightly soluble in ether. Log *P* (octanol/water), 4.9.

Cephaëline Hydrochloride

$C_{28}H_{38}N_2O_4$,2HCl = 539.5
CAS—5853-29-2
Chemical Properties White crystals or crystalline powder. Solutions turn yellow. Soluble in water; less soluble in alcohol, acetone and chloroform. Practically insoluble in benzene.

Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TA—Rf 53; system TB—Rf 01; system TC—Rf 19; system TL—Rf 08 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HA—*k* 7.7 (tailing peak).
Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1=127b); ethanol—235, 276 nm (A_1^1=144b).



Infrared Spectrum Principal peaks at wavenumbers 1264, 1515, 1116, 1229, 1213, 1616 cm^{-1} (cephaëline hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 178, 192, 272, 466, 244, 288, 191, 273.
Quantification See under Emetine.
Plasma **HPLC** Fluorescence detection. Limit of quantification, 1.0 mg/L [Asano *et al.* 2001].
Urine **HPLC** See Plasma. Limit of quantification, 5 mg/L [Asano *et al.* 2001].
Disposition in the Body
Therapeutic Concentration

Six healthy male volunteers were administered Ipecac syrup containing 0.843 g/L cephaëline and 0.503 g/L emetine at doses of 5, 10, 15, 20, 25, and 30 mL. Cephaëline was found in plasma at a concentration of 1.7, 3.0, 3.1, 3.6, 5.6, and 3.1 mg/L, respective, to the dosings at times of 2.1, 1.7, 0.8, 1.1, 2.4, and 1.5 h, respectively [Asano *et al.* 2001].

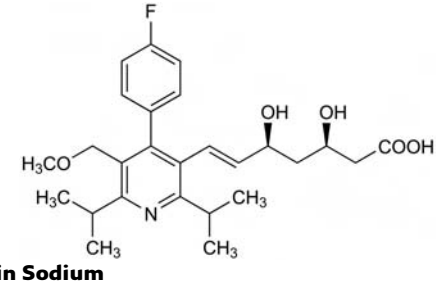
Dose Cephaëline hydrochloride has been given in doses of 5 to 10 mg.

Asano T *et al.* (2001). High-performance liquid chromatographic assay with fluorescence detection for the determination of cephaeline and emetine in human plasma and urine. *J Chromatogr B Biomed Sci Appl* 757: 197–206.

Cerivastatin

Lipid-Regulating Agent
$C_{26}H_{34}FNO_5$ = 459.6
CAS—145599-86-6

IUPAC Name (*E*,3*R*,5*S*)-7-[4-(4-Fluorophenyl)-5-(methoxymethyl)-2,6-di(propan-2-yl)pyridin-3-yl]-3,5-dihydroxyhept-6-enoic acid
Synonym (3*R*,5*S*,6*E*)-7-[4-(4-Fluorophenyl)-5-(methoxymethyl)-2,6-bis(1-methylethyl)-3-pyridinyl]-3,5-dihydroxy-6-heptenoic acid

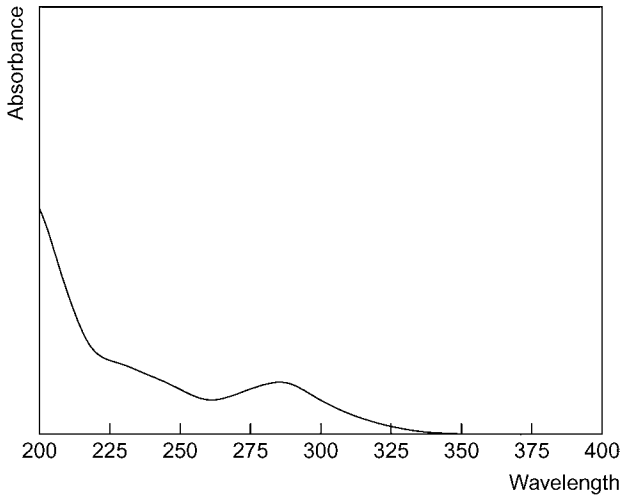


Cerivastatin Sodium

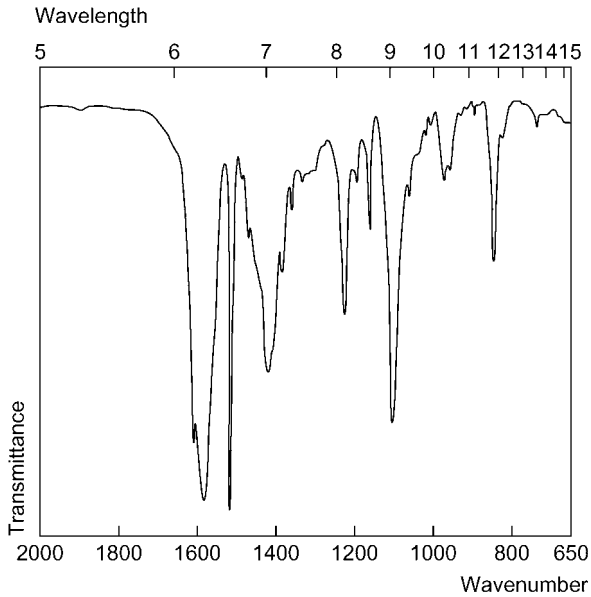
$C_{26}H_{33}FNNaO_5$ = 481.5
CAS—143201-11-0

Synonym Bay-W-6228
Proprietary Names *Baycol*; *Lipobay*.
Chemical Properties A white to off-white hygroscopic amorphous powder. It is soluble in water, ethanol, and methanol; very slightly soluble in acetone.

Ultraviolet Spectrum Methanol—228, 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1603, 1098, 1381, 1222 cm^{-1} (KBr disc).



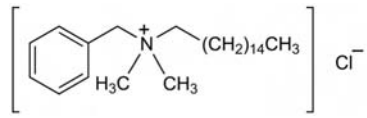
Quantification
Plasma **HPLC** Fluorescence detection. Limit of quantification, 0.025 µg/L [Krol *et al.* 1993].
Serum **HPLC** MS detection. Limit of quantification, 0.01 µg/L for cerivastatin and the lactone form, 0.05 to 0.5 µg/L for its biotransformation products [Jemal *et al.* 1999].
Disposition in the Body Cerivastatin is readily and almost completely absorbed after oral administration. It undergoes metabolism in the liver and is catalysed by at least two cytochrome P450 isoenzymes, CYP2C8 and CYP3A4. Three active metabolites are formed. The demethylated metabolite (about 50% as potent as the parent drug) and the hydroxylated metabolite (about 80% as potent) are the major metabolites formed; the product of both demethylation and hydroxylation also has activity, but it is only a minor metabolite. The metabolites are excreted in urine (about 30%) and in faeces (about 70%). There is no unchanged drug found in urine. There is no significant clearance by haemodialysis.
Therapeutic Concentration
Twenty-four healthy, Caucasian males, aged between 21 and 44 years, were administered with a single dose of 0.8 mg after an overnight fast (group 1), with breakfast (group 2), in the evening with dinner (group 3) or 4 h after dinner (group 4). The peak plasma concentrations were 7.7, 8.7, 7.1 and 7.1 µg/L for the four groups, respectively. These concentrations were reached within 1.5, 2.3 and 2.5 h, respectively. The mean concentration detected for the demethylated metabolite, M1, was 0.6 µg/L for the four groups and this was observed approx. 4 h after administration. The hydroxylated metabolite, M23, showed a concentration of 1.6 µg/L at approx. 4 to 6 h [Muck *et al.* 2000].
Bioavailability Absolute bioavailability, 60%.
Half-life Plasma, 2 to 3 h.
Volume of Distribution 0.3 L/kg.
Clearance Plasma, 0.2 L/h/kg.
Protein Binding 99%; 80% to albumin.
Dose Initially 0.1 mg of cerivastatin sodium daily, increasing to 0.3 mg daily.

Jemal M *et al.* (1999). Quantitation of cerivastatin and its seven acid and lactone biotransformation products in human serum by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B, Biomed Sci Appl* 736(1–2): 19–41.
Krol GJ *et al.* (1993). LC separation and induced fluorometric detection of rivastatin in blood plasma. *J Pharm Biomed Anal* 11: 1269–1275.
Muck W *et al.* (2000). Pharmacokinetics of cerivastatin when administered under fasted and fed conditions in the morning or evening. *Int J Clin Pharmacol Ther* 38(6): 298–303.

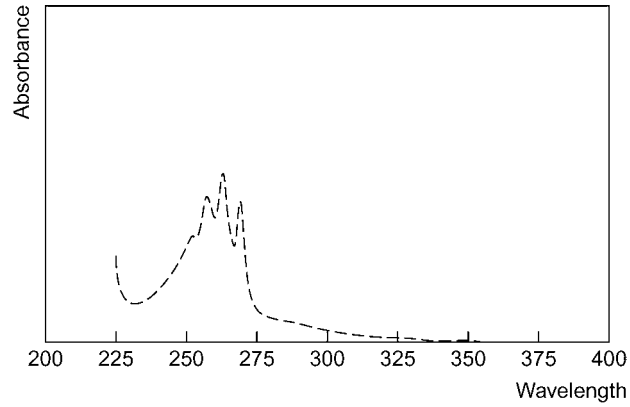
Cetalkonium Chloride

Cationic Disinfectant
$C_{25}H_{46}ClN$ = 396.1
CAS—122-18-9

IUPAC Name Benzyl-hexadecyl-dimethylazanium chloride
Synonym *N*-Hexadecyl-*N*,*N*-dimethylbenzenemethanaminium chloride
Proprietary Names *Baktonium*. It is an ingredient of *AAA*, *Bonjela*, and *Teejel*.



Chemical Properties A white crystalline powder. Mp 58° to 60°. Sparingly soluble in cold water, soluble in hot water; soluble in acetone, ethanol, chloroform, ether, ethyl acetate, propylene glycol, and glycerol. Log *P* (octanol/water), 4.9.
Thin-layer Chromatography System TA—R_f 0.12, streaking (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—258 (A^1_1=8b), 264 (A^1_1=10b), 270 (A^1_1=8b) nm; methanol—253, 258, 263 (A^1_1=11b), 270 nm.

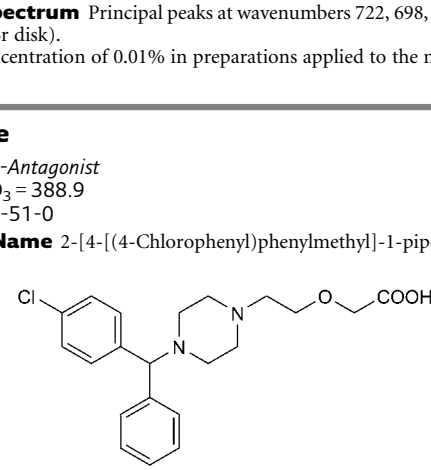


Infrared Spectrum Principal peaks at wavenumbers 722, 698, 1612, 1204, 875, 790 cm^{-1} (KBr disk).
Use In a concentration of 0.01% in preparations applied to the mouth or throat.

Cetirizine

Histamine H_1-Antagonist
$C_{21}H_{25}ClN_2O_3$ = 388.9
CAS—83881-51-0

IUPAC Name 2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy] acetic acid

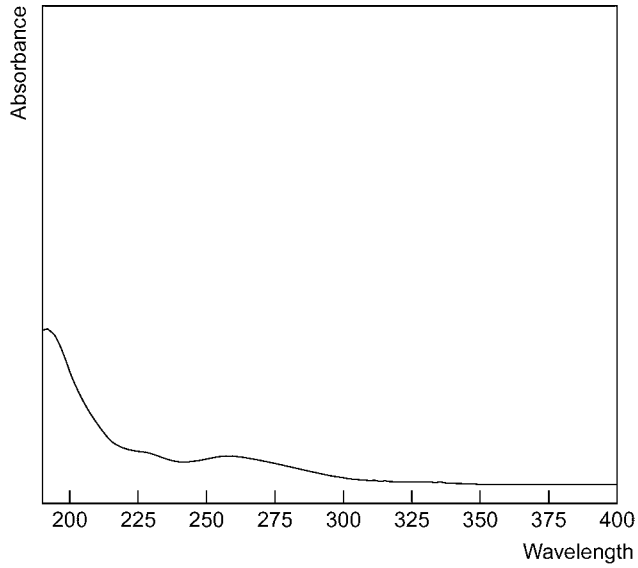


Chemical Properties Crystals from ethanol. Mp 110° to 115°.

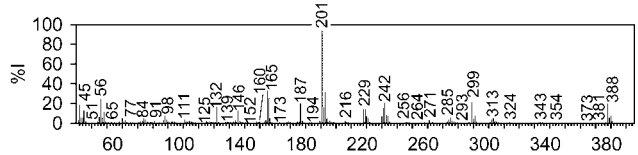
Cetirizine Dihydrochloride
$C_{21}H_{27}Cl_3N_2O_3$ = 461.8
CAS—83881-52-1

Synonyms P-071; UCB-P071.
Proprietary Names *Alerlisin*; *Formistin*; *Reactine*; *Virlix*; *Voltric*; *Zirtek*; *Zyrlex*; *Zyrtec*.
Chemical Properties White-to-almost-white crystalline powder. Mp 225°. Freely soluble in water; practically insoluble in acetone and dichloromethane. Log *P* octanol/water) −0.61.

Thin-layer Chromatography Plate: silica 60 F_{254}. Solvent system: chloroform followed by chloroform : methanol (85 : 15). R_f 0.31 [Pandya *et al.* 1996].
High Performance Liquid Chromatography. System HAA—RT 15.7 min; system HAX—RT 8.89 min; system HAY—RT 5.29 min; system HZ—RT 3.6 min.
Ultraviolet Spectrum Aqueous acid (pH 2.38)—192, 258 nm.



Mass Spectrum Principal ions at *m/z* 201, 165, 203, 56, 187, 242, 299, 388.



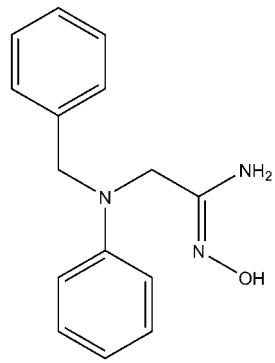
Quantification
Plasma **GC** Column: fused silica methylsilicone (25 m × 0.31 mm i.d., 0.17 µm). Temperature: 260°. Carrier gas: He, 35 cm/s. NPD or FID. Retention time: 8.5 min. Limit of detection, 0.02 mg/L [Baltes *et al.* 1988].

HPLC Column: C_{18} Nucleosil 100-3 (150 × 3.2 mm, 3 µm). Mobile phase: 30 mmol/L potassium dihydrogen phosphate (pH 6.8). UV detection (λ= 232 nm). Retention time: 8.25 min. Limit of quantification, 0.01 mg/L. [Macek *et al.* 1999]. Column: Beckman 5 gm octyl reversed phase (25 cm × 2 mm). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogen phosphate-0.02 mol/L sodium octane sulfonic acid (pH 3.0; 40 : 60), flow rate 0.25 mL/min. UV detection (λ = 230 nm). Limit of detection, 10 µg/L [Pariente-Khayat *et al.* 1995]. Column: reversed phase C_{18} RP-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate-0.02 mol/L sodium dodecyl sulfate (pH 2.9) : acetonitrile (55 : 45), flow rate 2.5 mL/min. UV detection (λ= 230 nm). Retention time: 15 min. Limit of detection, 5 µg/L [Muscará, De Nucci 1995].
Serum **HPLC** Column: C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : water (39 : 61) containing 13 mmol/L phosphate buffer (pH 2.8). UV detection (λ= 230 nm). Retention time: 1.69 min. Limit of detection, 0.005 mg/L [Zaater *et al.* 2000]. Column: Spherisorb S5 ODS2 reversed phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol: 0.05 mol/L ammonium phosphate (pH 2.5; 33 : 9 : 58), flow rate 2 mL/min. UV detection (λ= 211 nm). Retention time: 6.4 min. Limit of detection, 0.02 mg/L [Moncrieff 1992]. Column: Spherisorb C_8 (5 µm). Mobile phase: methanol : acetonitrile : 0.2 mol/L sodium hydrogen phosphate (30 : 20 : 50). UV detection. Limit of detection, 5 µg/L [Awni *et al.* 1990].
Urine **HPLC** See Plasma [Pariente-Khayat *et al.* 1995]. Column: Spherisorb 5ODS-2 (25 cm × 4.6 mm i.d.). Mobile phase: Pic A: methanol : tetrahydrofuran (33 : 65 : 2), flow rate 1.0 mL/min. DAD (λ = 230 nm). Retention time: 6.17 min. Limit of detection, 20 µg/L [Rosseel, Lefebvre 1991].
Renal Dialysate **HPLC** See Serum. Limit of detection, 2.1 µg/L [Awni *et al.* 1990].
Disposition in the Body Cetirizine is rapidly absorbed after oral administration and undergoes metabolism in the liver, to a very limited extent by *O*-dealkylation. The metabolite produced has negligible antihistaminic activity. Cetirizine is primarily excreted in urine (70%) and 10% in the faeces, as the unchanged drug [Wood *et al.* 1987]. It is secreted in breast milk.
Therapeutic Concentration
Fifteen infants and toddlers, with a mean age of 12.3 months, were admitted to hospital for reoccurring respiratory infections or other hypersensitivity-related diseases and treated with a single dose of 0.25 mg/kg cetirizine solution. A mean peak plasma concentration of 0.39 mg/L was observed at 2.0 h [Spicák *et al.* 1997].
Eight children (2–6-years-old) were administered a single 5-mg oral dose of 10 mg/mL cetirizine 1.5 h before anaesthesia. The mean maximum plasma concentration of 607 ± 231 µg/L was reached at 1.93 ± 1.39 h [Pariente-Khayat *et al.* 1995].
Fourteen healthy male volunteers (age: 21 to 46 years) received a single oral dose of 10 mg cetirizine hydrochloride as Zyrtek or Zetir. Mean peak plasma concentrations were 307 and 302 µg/L for Zetir and Zyrtek, respectively, both reached at 0.5 h.
Ten elderly volunteers (aged between 60 and 90 years) and 10 healthy, young volunteers (21–29-years-old) were administered with a single oral dose of 10 mg. Mean plasma concentrations were 0.362 mg/L for the elderly individuals and 0.337 mg/L for the young. These concentrations were observed at 1.30 and 1.12 h, respectively [Lefebvre *et al.* 1988].
Five patients with end-stage renal disease ingested 10-mg cetirizine dihydrochloride. The mean maximum plasma concentration was 285 µg/L reached at 2 h [Awni *et al.* 1990].
Toxicity Two cases of overdose have been reported: an adult took a 150-mg dose of cetirizine and was admitted to hospital with somnolence, but no other clinical signs or blood chemistry/haematology. An 18-month-old child overdosed on 180 mg of cetirizine and became restless, irritable and drowsy. Multiple-dose toxicity targets the liver and a single dose the central nervous system.
Note For a review of the comparative safety of histamine H_1 antagonists, see Simons [1994].
Half-life Elimination half-life is 7–11 h in adults; 6–7 h in children; 3.1 h in infants.
Volume of Distribution In adults, volume of distribution is 0.6–0.8 l/kg; children, 0.44 L/kg.
Clearance Body clearance, 0.76 mL/min; 2.13 mL/min/kg (infants). 1.27 ± 0.80 mL/min/kg in children [Pariente-Khayat *et al.* 1995].
Protein Binding ≈93% bound.
Dose Adults and children >6 years: a usual dose of 10 mg is administered daily. Children (2 to 6 years) and patients with renal impairment: usual dose 5 mg daily.

Awni WM *et al.* (1990). Effect of haemodialysis on the pharmacokinetics of cetirizine. *Eur J Clin Pharmacol* 38: 67–69.
Baltes E *et al.* (1988). Gas chromatographic method for the determination of cetirizine in plasma. *J Chromatogr* 430: 149–155.
Lefebvre RA *et al.* (1988). Single dose pharmacokinetics of cetirizine in young and elderly volunteers. *Int J Clin Pharmacol Res* 8: 463–470.
Macek J *et al.* (1999). Determination of cetirizine in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 736: 231–235.
Moncrieff J (1992). Determination of cetirizine in serum using reversed-phase high-performance liquid chromatography with ultraviolet spectrophotometric detection. *J Chromatogr* 583: 128–130.
Muscará MN, DeNucci G (1995). Comparative bioavailability of single doses of tablet formulations of cetirizine dihydrochloride in healthy male volunteers. *Int J Clin Pharmacol Ther* 33: 27–31.
Pandya KK *et al.* (1996). High-performance thin-layer chromatography for the determination of cetirizine in human plasma and its use in pharmacokinetic studies. *J Pharm Pharmacol* 48: 510–513.
Pariente-Khayat A *et al.* (1995). Pharmacokinetics of cetirizine in 2- to 6-year-old children. *Int J Clin Pharmacol Ther* 33: 340–344.
Rosseel MT, Lefebvre RA (1991). Determination of cetirizine in human urine by high-performance liquid chromatography. *J Chromatogr* 565: 504–510.
Simons FE (1994). H1-receptor antagonists. Comparative tolerability and safety. *Drug Safety* 10: 350–380.
Spicák V *et al.* (1997). Pharmacokinetics and pharmacodynamics of cetirizine in infants and toddlers. *Clin Pharmacol Ther* 61: 325–330.
Wood SG *et al.* (1987). The metabolism and pharmacokinetics of 14C-cetirizine in humans. *Ann Allergy* 59: 31–34.
Zaater MF *et al.* (2000). RP-LC method for the determination of cetirizine in serum. *J Pharm Biomed Anal* 22: 739–744.

Cetoxime

Antihistamine
$C_{15}H_{17}N_3O$ = 255.3
IUPAC Name *N*-Benzylanilinoacetamidoxime



Cetoxime Hydrochloride
CAS—22204-29-1
Proprietary Name *Febramine*
Chemical Properties Mp 160°. Soluble in water.

Colour Tests Ammonium vanadate test—red (limit of detection, 0.1 μg); Vitali's test—red/yellow/red-brown (limit of detection, 0.1 μg).
Thin-layer Chromatography System T1—R_f 0.75 (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—242, 290 nm; aqueous alkali (0.1 N sodium hydroxide)—249, 300 nm.
Disposition in the Body
Toxicity LD_{50} (oral): in mice 300 mg/kg.
Dose Up to 800 mg daily has been given.

Cetrimide

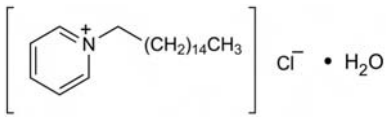
Cationic Disinfectant
$C_{17}H_{38}BrN = 336.4$
CAS—8044-71-1

IUPAC Name Trimethyl(tetradecyl)azanium bromide
Note Consists chiefly of tetradecyltrimethylammonium bromide together with smaller amounts of dodecyl- and hexadecyltrimethylammonium bromides.
Proprietary Names *Cetavlon*; *Morpan CHSA*; *Silquat C100*. It is an ingredient of *Ceanel*, *Cetal (liquid)*, *Savloclens*, *Savlodil*, *Savlon (liquid)*, and *Travasept*.
Chemical Properties A white to creamy-white, voluminous, free-flowing, hygroscopic powder. A solution in water foams on shaking. Mp 232° to 247°. Soluble 1 in 2 of water; freely soluble in ethanol; soluble in ether.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.00; system TL—R_f 0.00; system TN—R_f 0.100; system TO—R_f 0.50; system TAE—R_f 0.00; system TAF—R_f 0.29 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1699.
High Performance Liquid Chromatography System HX—RI 56.
Infrared Spectrum Principal peaks at wavenumbers 961, 909, 952, 719, 970, 724 cm^{-1} (KC1 disk).

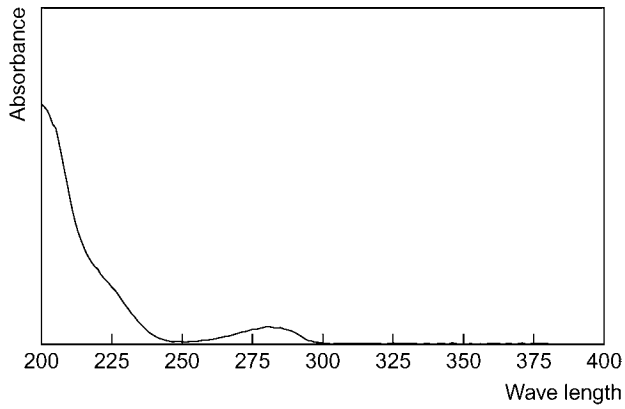
Cetylpyridinium Chloride

Cationic Disinfectant
$C_{21}H_{38}ClN, H_2O = 358.0$
CAS—7773-52-6 (cetylpyridinium); 123-03-5 (cetylpyridinium chloride, anhydrous); 6004-24-6 (cetylpyridinium chloride, monohydrate)

IUPAC Name 1-Hexadecylpyridin-1-ium chloride hydrate
Synonym 1-Hexadecylpyridinium chloride monohydrate
Proprietary Names *Ceepryn*; *Cepacol*; *Dobendan*; *Merocet(s)*. It is an ingredient of *Merocaine* and *Tyrosolven*.



Chemical Properties A white powder. A solution in water foams strongly when shaken. Mp 77° to 83°. Soluble 1 in 20 of water; freely soluble in ethanol and chloroform; very slightly soluble in ether and in benzene. Log *P* (octanol), 1.7.
Thin-layer Chromatography System TA—R_f 0.20; system TL—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.29 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—280 nm. No alkaline shift.

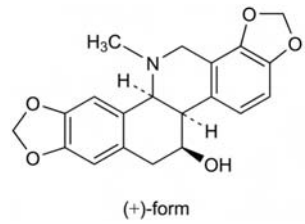


Infrared Spectrum Principal peaks at wavenumbers 685, 1629, 776, 1174, 715, 1205 cm^{-1}.
Use In concentrations of 0.01 to 1%.

Chelidonine

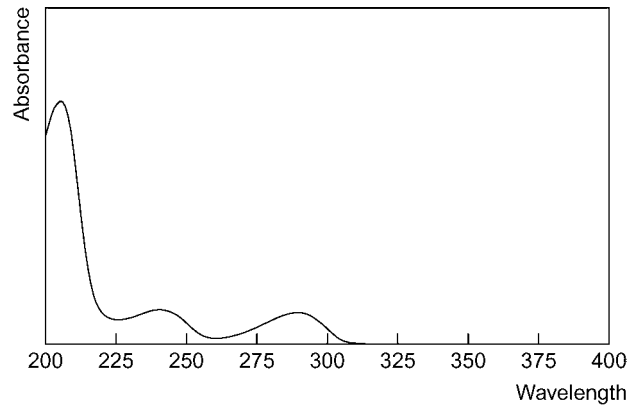
Alkaloid
$C_{20}H_{19}NO_5 = 353.4$
CAS—476-32-4

Synonyms [5b*R*-(5bα,6β,12bα)]-5b,6,7,12b,13,14-Hexahydro-13-methyl[1,3]-benzodioxolo[5,6-*c*]-1,3-dioxolo[4,5-*i*]phenanthridin-6-ol; stylophorin.



(+)-form

Chemical Properties An alkaloid obtained from the greater celandine, *Chelidonium majus* (Papaveraceae). A white crystalline powder. Mp 135° to 136°. Practically insoluble in water; soluble in ethanol, chloroform, and ether. Log *P* (octanol/water), 2.9.
Colour Tests Mandelin's test—yellow→green; Marquis test—green; sulfuric acid—brown.
Thin-layer Chromatography System TA—R_f 0.72 (acidified iodoplatinate solution, positive).



Infrared Spectrum Principal peaks at wavenumbers 1035, 1257, 1222, 1497, 748, 1070 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 332, 333, 304, 335, 176, 303, 334, 162.

Chloral Betaine

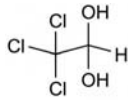
Hypnotic, Sedative
$C_7H_{12}Cl_3NO_3, H_2O = 282.6$
CAS—2218-68-0

IUPAC Name 2,2,2-Trichloroethane-1,1-diol; 2-(trimethylazaniumyl)acetate
Chemical Properties 1-Carboxy-*N*,*N*,*N*-trimethanaminium inner salt compound with 2,2,2-trichloro-1,1-ethanediol (1 : 1). The adduct formed by chloral hydrate, $CCl_3{\cdot}CH(OH)_2$, and betaine, $C_5H_{11}NO_2$, containing about 58% of chloral hydrate. A white crystalline powder. Mp about 124°, with decomposition. Soluble 1 in 1 of water and 1 in 4 of ethanol; practically insoluble in chloroform and ether.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 840, 1630, 1085, 1130, 730, 1500 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 81, 83, 46, 110, 82, 112, 84, 117.
Dose Usually 0.87 to 2.61 g daily, but doses of up to 3.5 g may be necessary.

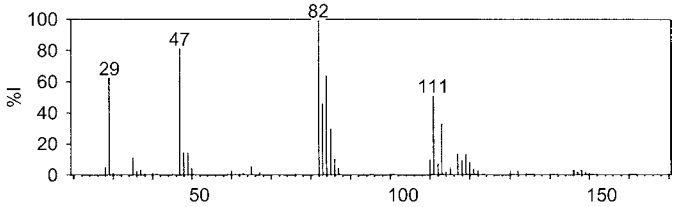
Chloral Hydrate

Hypnotic, Sedative
$C_2H_3Cl_3O_2 = 165.4$
CAS—302-17-0

IUPAC Name 2,2,2-Trichloro-1,1-ethanediol
Synonyms Cloral; cloral hydrate.
Proprietary Names *Aquachloral*; *Chloradorm*; *Chloraldurat*; *Chloralex*; *Chloralvan*; *Dormel*; *Elix-Nocte*; *Escre*; *Medianox*; *Noctec*; *Novo-chlorhydrate*; *Nycton*; *Rectules*; *Somnos*; *Welldorm*. Also an ingredient of *Babygencal*; *Dermophil Indien*; *Synthol*.



Chemical Properties Colourless or white crystals which volatilise slowly on exposure to air and are decomposed by caustic alkalis, liberating chloroform. Mp 50°. Bp 98°. 1 mL of water dissolves the following amounts of cloral hydrate: 2.4 g at 0°, 8.3 g at 25°, 14.3 g at 140°. Soluble: 1 in 0.2 of ethanol, 1 in 1.3 of alcohol, 1 in 2 of chloroform, 1 in 1.4 olive oil, 1 in 0.5 glycerol, 1 in 68 g carbon disulfide and 1 in 1.5 of ether. Freely soluble in acetone and methylethyl ketone. Moderately or sparingly soluble in turpentine, petroleum ether, carbon tetrachloride, benzene, and toluene. Ethanolic solutions may deposit crystals of cloral ethanolate. pK_a 10.0. Log *P* (octanol/water), 0.99.
Colour Tests Fujiwara test—red; palladium chloride—black.
Gas Chromatography System GA—RI 695 (tailing peak); system GI—retention time 12.5 min (tailing peak).
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 835, 1083, 1300, 970, 1620 cm^{-1}.
Mass Spectrum Principal peaks at *m/z* 82, 47, 84, 29, 111, 83, 113, 85; trichloroacetic acid 44, 83, 85, 36, 28; trichloroethanol 31, 49, 77, 113, 115, 82, 51, 117.



Quantification
Blood **UV-Vis** Trichloroethanol (modified Fujiwara reaction) [McBay *et al.* 1980].
GC ECD [Meyer *et al.* 1995] ECD. Chloral hydrate, trichloroethanol, and trichloroacetic acid, head-space analysis. Limit of detection, 500 µg/L for chloral hydrate and trichloroethanol [Breimer *et al.* 1974].
Plasma **GC** ECD. Trichloroethanol [Berry 1975].
GC-MS Chloral hydrate and metabolites, see [Yan *et al.* 1999].
Urine **UV-Vis** See Blood [McBay *et al.* 1980].
GC See Blood [Breimer *et al.* 1974]. See Plasma [Berry 1975].
Disposition in the Body Readily absorbed following oral administration. It is rapidly metabolised by reduction to trichloroethanol, the major active metabolite, which is further metabolised by conjugation with glucuronic acid to give urochloralic acid and by oxidation to trichloroacetic acid, the major urinary metabolite. Trichloroethanol passes into the CSF, into breast milk, and across the placenta. About 10–30% of a dose is excreted in the urine as urochloralic acid and up to 5% as trichloroethanol in 24 h. Trichloroacetic acid is slowly excreted in urine over several days; a small amount of urochloralic acid may be excreted in the bile.
Therapeutic Concentration Chloral hydrate is difficult to detect in body fluids after normal doses. The plasma concentration of trichloroethanol is usually in the range 1.5 to 15 mg/L. Trichloroacetic acid and urochloralic acid are present in plasma at concentrations similar to, or greater than, those of trichloroethanol. When alcohol has been taken, peak plasma concentrations of trichloroethanol are increased and remain elevated for about 6 h after ingestion, and those of trichloroacetic acid are decreased. Trichloroacetic acid accumulates in the plasma during chronic administration of cloral hydrate.

Following a single oral dose of 825 mg to 5 subjects, peak plasma trichloroethanol concentrations of 7.6 to 12.2 mg/L (mean 9.7) were attained in 0.5 to 1 h [Berry 1975].

Toxicity Fatalities have occurred following the ingestion of 1.25 and 3 g but recovery has occurred after ingestion of 30 g. Plasma concentrations greater than 40 mg/L of trichloroethanol are likely to produce toxic effects; fatalities have been reported at blood concentrations of 20–495 mg/L (mean 155) of trichloroethanol.

In a fatality caused by the admistration of chloral hydrate, lidocaine, and nitrous oxide, postmortem trichloroethanol concentrations were: plasma, 79.0 mg/L; urine, 31.0 mg/L; gastric contents, 454.0 mg/L; bile, 111.0 mg/L; vitreous humour, 40.2 mg/L; CSF, 68.3 mg/L; and liver, 164 mg/kg. Lidocaine concentrations ranged from 3.7 mg/L in urine to 19.0 mg /L in bile. Nitrous oxide was measured at 4.4 mL/L in blood [Engelhart *et al.* 1998].

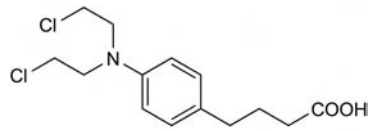
In 4 fatalities known to involve the acute ingestion of 15 to 30 g of chloral hydrate, postmortem blood trichloroethanol concentrations ranged from 100 to 640 mg/L (mean 265) [Baselt, Cravey 1977].

In a fatality due to the ingestion of chloral hydrate, the following postmortem tissue concentrations of trichloroethanol were reported: blood 55 mg/L, brain 91 mg/kg, liver 200 mg/kg, urine 30 mg/L [Poklis *et al.* 1973].
Half-life Plasma half-life, chloral hydrate about 4 min, trichloroethanol about 7 to 11 h, urochloralic acid about 7 h, trichloroacetic acid about 4 days.
Volume of Distribution Trichloroethanol, about 0.6 L/kg.
Distribution in Blood Plasma : whole blood ratio, trichloroethanol, about 0.9.
Protein Binding Trichloroethanol 35% and trichloroacetic acid 94%.
Note For a review of the pharmacokinetics of sedatives in neonates, see Jacqz-Aigrain, Burtin [1996]; for a review of the pharmacokinetics of hypnotic drugs, see Breimer [1977].
Dose 0.5 to 2 g daily. Children may be given 30 to 50 mg/kg body-weight with a maximum single dose of 1 g.

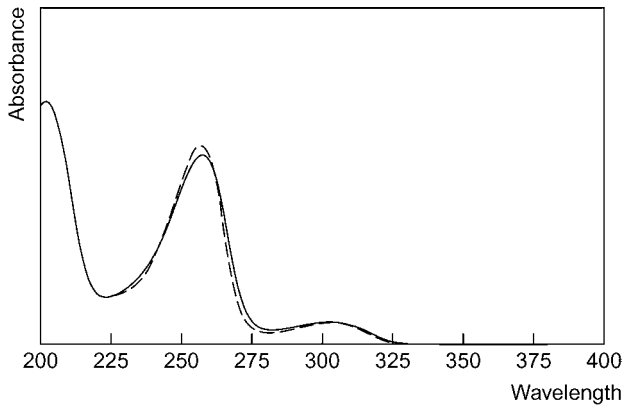
Baselt RC, Cravey RH (1977). *J Anal Toxicol* 1: 81–103.
Berry DJ (1975). Determination of trichloroethanol at therapeutic and overdose levels in blood and urine by electron capture gas chromatography. *J Chromatogr* 107: 107–114.
Breimer DD *et al.* (1974). Gas chromatographic determination of chloral hydrate, trichloroethanol and trichloroacetic acid in blood and in urine employing head-space analysis. *J Chromatogr A* 88 (1): 55–63.
Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.
Engelhart DA *et al.* (1998). Unusual death attributed to the combined effects of chloral hydrate, lidocaine, and nitrous oxide. *J Anal Toxicol* 22: 246–247.
Jacqz-Aigrain E, Burtin P (1996). Clinical pharmacokinetics of sedatives in neonates. *Clin Pharmacokinet* 31: 423–443.
McBay AJ *et al.* (1980). Spectrophotometric determination of trichloroethanol in chloral hydrate poisoning. *J Anal Toxicol* 4: 99–101.
Meyer E *et al.* (1995). Determination of chloral hydrate and metabolites in a fatal intoxication. *J Anal Toxicol* 19: 124–126.
Poklis A *et al.* (1973). *Bull Int Assoc Forensic Toxicol* 9(3–4): 8–9.
Yan Z *et al.* (1999). Determination of chloral hydrate metabolites in human plasma by gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 19: 309–318.

Chlorambucil

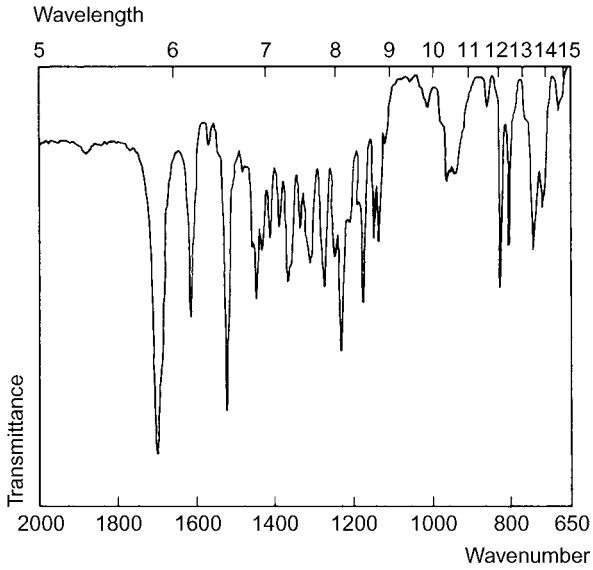
Antineoplastic
$C_{14}H_{19}Cl_2NO_2$ = 304.2
CAS—305-03-3
IUPAC Name 4-[4-[Bis(2-chloroethyl)amino]phenyl]butanoic acid
Synonyms 4-[Bis(2-chloroethyl)amino]benzenebutanoic acid; CB-1348; chlorbutinum.
Proprietary Names *Chloraminophène*; *Leukeran*.



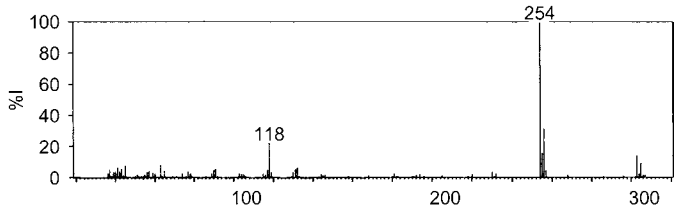
Chemical Properties A white crystalline or granular powder. Mp 66°. Practically insoluble in water; soluble 1 in 1.5 of ethanol, 1 in 2 of acetone, and 1 in 2.5 of chloroform; soluble in ether. pK_a 5.8. Log *P* (octanol/pH 7.4), 1.7.
Caution Chlorambucil is irritant; avoid contact with skin and mucous membranes.
Thin-layer Chromatography system TD—R_f 0.33; system TE—R_f 0.06; system TF—R_f 0.40; System TAD—R_f 0.50; system TAE—R_f 0.84.
Gas Chromatography System GA—chlorambucil RI 2420, chlorambucil-Me RI 2340.
High Performance Liquid Chromatography System HAA—Retention time 22.4 min.
Ultraviolet Spectrum Methanol—258 (A_1^1=642a), 303 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1695, 1520, 1229, 1610, 1175, 1270 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 254, 256, 118, 255, 303, 305, 63, 45.



Quantification

Plasma **GC–MS** Limit of detection, <10 mg/L [Ehrsson *et al.* 1980].

HPLC MS/MS. Limit of detection, 4 to 800 mg/L for drug and metabolites [Davies *et al.* 1999]. UV detection. Limit of detection, 100 mg/L [Ahmed *et al.* 1982].

Serum **HPLC** See Plasma [Davies *et al.* 1999].

Disposition in the Body Rapidly absorbed after oral administration with peak plasma concentrations being attained in 0.5 to 2 h. It is extensively metabolised; <1% of a dose is excreted in the urine unchanged. Phenylacetic acid mustard is a major metabolite.

Therapeutic Concentration

A single oral dose of 0.2 mg/kg (three different formulations tested: UK, US, and worldwide) was administered to healthy volunteers. Mean peak plasma drug concentrations of 475, 575, and 534 mg/L were observed for the three different formulations, respectively, and 340, 381, and 338 mg/L for the major metabolite, respectively [Davies *et al.* 1999].

Half-life Plasma half-life, about 1 to 2 h.

Dose Initially 200 µg/kg daily, orally; maintenance, 30 to 100 µg/kg daily.

Ahmed AE *et al.* (1982). Studies on the quantitation of chlorambucil in plasma by reversed-phase high-performance liquid chromatography. *J Chromatogr* 233(22): 392–397.

Davies ID *et al.* (1999). Rapid determination of the anti-cancer drug chlorambucil (Leukeran) and its phenyl acetic acid mustard metabolite in human serum and plasma by automated solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 732(1): 173–184.

Ehrsson H *et al.* (1980). Determination of chlorambucil in plasma by GLC with selected-ion monitoring. *J Pharm Sci* 69: 710–712.

Chloramphenicol

Antibiotic

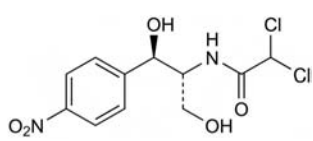
$C_{11}H_{12}Cl_2N_2O_5 = 323.1$

CAS—56-75-7

IUPAC Name 2,2-Dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide

Synonyms Chloranfenicol; cloranfenicol; 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide; laevomycetinum.

Proprietary Names *Amphicol*; *Chloromycetin*; *Chloroptic*; *Econochlor*; *Fenicol*; *Kemicetine*; *Novochlorocap*; *Sno Phenicol*.



Chemical Properties Fine, white to greyish-white or yellowish-white crystals. Mp 149° to 153°. A solution in dehydrated alcohol is dextrorotatory and a solution in ethyl acetate is laevorotatory. Soluble 1 in 400 of water and 1 in 2.5 of ethanol; very soluble in acetone and ethyl acetate; slightly soluble in chloroform and ether. pK_a 5.5. Log *P* (octanol/water), 1.1.

Chloramphenicol Cinnamate

$C_{20}H_{18}Cl_2N_2O_6 = 453.3$

CAS—14399-14-5

Chemical Properties A white or yellowish-white crystalline powder. Mp about 119°. Very slightly soluble in water; soluble 1 in 25 of ethanol, 1 in 50 of chloroform, and 1 in 500 of ether.

Chloramphenicol Palmitate

$C_{27}H_{42}Cl_2N_2O_6 = 561.5$

CAS—530-43-8

Synonyms Chloramphenicol α-palmitate; palmitylchloramphenicol.

Proprietary Names *Chloromycetin Palmitate Suspension*; *Globenicol*.

Chemical Properties A fine, white, unctuous, crystalline powder. Mp 87° to 95°. Very slightly soluble in water; soluble 1 in 45 of ethanol, 1 in 6 of chloroform, and 1 in 14 of ether; freely soluble in acetone; soluble in ethyl acetate.

Chloramphenicol Sodium Succinate

$C_{15}H_{15}Cl_2N_2NaO_8 = 445.2$

CAS—982-57-0

Synonym Chloramphenicol α-sodium succinate

Proprietary Names *Chloromycetin Succinate*; *Globenicol*; *Kemicetine Succinate*; *Mychel-S*.

Chemical Properties A white or yellowish-white hygroscopic powder.

Soluble 1 in less than 1 of water and 1 in 1 of ethanol; practically insoluble in chloroform and ether.

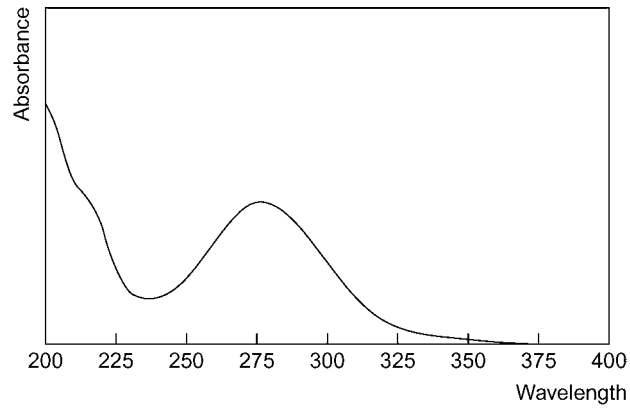
Colour Tests Fujiwara test—red; Nessler's reagent—brown-orange.

Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.00; system TC—R_f 0.31; system TD—R_f 0.11; system TE—R_f 0.36; system TF—R_f 0.31; system TL—R_f 0.48; system TAD—R_f 0.34; system TAE—R_f 0.86; system TAF—R_f 0.90; system TAJ—R_f 0.32; system TAK—R_f 0.08; system TAL—R_f 0.71.

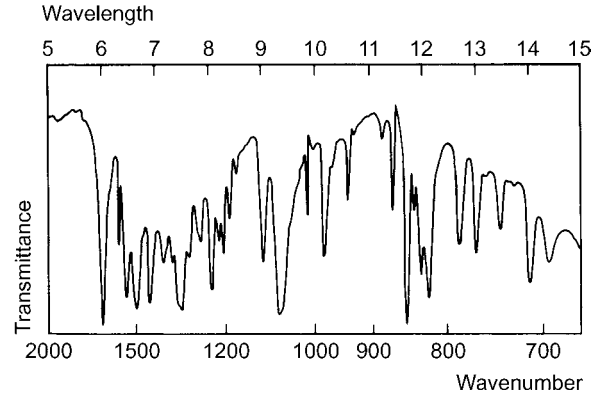
Gas Chromatography System GA—RI 2310.

High Performance Liquid Chromatography System HX—RI 390; system HY—RI 336; system HAA—retention time 14.1 min.

Ultraviolet Spectrum Water—278 nm (A^1_1=298a). Chloramphenicol palmitate: dehydrated alcohol—271 nm (A^1_1=178a).



Infrared Spectrum Principal peaks at wavenumbers 1681, 847, 1072, 1515, 816, 1562 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 70, 150, 151, 153, 77, 51, 118, 60.

Quantification

Plasma **GC** ECD. See Pickering *et al.* [1979].

HPLC UV detection. Limit of detection, 500 µg/L for chloramphenicol and 1 mg/L for chloramphenicol succinate [Velagapudi *et al.* 1982]. UV detection. Limit of detection, 100–200 µg/L [Sample *et al.* 1979].

Serum **HPLC** See Plasma [Sample *et al.* 1979].

Urine **GC** See Plasma Pickering *et al.* [1979]. FID. For quantification of chloramphenicol or thiamphenicol and their metabolites see Nakagawa *et al.* [1975].

Cerebrospinal Fluid **GC** See Plasma Pickering *et al.* [1979].

HPLC See Plasma [Sample *et al.* 1979].

Disposition in the Body Readily absorbed after oral administration. The inactive palmitate ester is hydrolysed to free drug in the gastro-intestinal tract before absorption and the inactive sodium succinate ester, given parenterally, is similarly hydrolysed *in vivo*. It is widely distributed in the body, giving a concentration in the CSF about 50% of that in the blood. The main metabolic pathway is conjugation with glucuronic acid; chloramphenicol is also hydrolysed to 1-(4-nitrophenyl)-2-aminopropane-1,3-diol. Both metabolites are inactive. Up to about 90% of a dose is excreted in the urine in 24 h, mainly as the glucuronide, with about 5 to 10% of the dose in unchanged form. About 3% of the dose is excreted in the bile but this is mainly reabsorbed to give about 1% eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 10 to 20 mg/L.

Following oral administration of chloramphenicol palmitate, equivalent to 15 to 27 mg/kg of chloramphenicol, four times a day to 18 children, plasma concentrations of 15.2 to 38.9 mg/L (mean 27.5) were reported 1.5 h after a dose [Kauffman *et al.* 1981].

Twenty-nine adults with uncomplicated enteric fever were administered 30 mg/kg chloramphenicol succinate ester IV or IM. The mean peak plasma concentrations of chloramphenicol were 16.2 and 7.8 mg/L, respectively [Acharya *et al.* 1997].

Toxicity There is an increased incidence of reversible bone-marrow depression when plasma concentrations exceed 25 mg/L. The 'grey' syndrome (cardiovascular collapse, respiratory depression, and coma) has been reported in patients with plasma concentrations in the range 40 to 200 mg/L.

A 26-year-old woman received an initial 1-g dose followed in 7 and 12 h by two 10-g doses. A plasma concentration of 201 mg/L was measured $5^1/_2$ h after the last dose while she was in severe shock, but she recovered [Thompson *et al.* 1975].

In a fatality involving ingestion of an unknown quantity of chloramphenicol (probably 2.5 to 4 g) by a 5-year-old boy, post-mortem liver and urine concentrations of 10.7 µg/g and 2.09 g/L, respectively, were reported; death occurred about 18 h after ingestion [Grusz-Harday 1973].

Half-life Plasma half-life, about 2 to 5 h.

Volume of Distribution About 0.5 to 1 L/kg.

Clearance Plasma clearance, about 4 mL/min/kg.

Protein Binding About 40 to 60%.

Note For a review of the pharmacokinetics of chloramphenicol, see Ambrose [1984].

Dose Usually 2 g daily.

Acharya GP *et al.* (1997). Factors affecting the pharmacokinetics of parenteral chloramphenicol in enteric fever. *J Antimicrob Chemother* 40(1): 91–98.

Ambrose PJ (1984). Clinical pharmacokinetics of chloramphenicol and chloramphenicol succinate. *Clin Pharmacokinet* 9: 222–238.

Grusz-Harday E (1973). *Bull Int Assoc Forensic Toxicol* 9((3 and 4)): 10–11.

Kauffman RE *et al.* (1981). Relative bioavailability of intravenous chloramphenicol succinate and oral chloramphenicol palmitate in infants and children. *J Pediatr* 99: 963–967.

Nakagawa T *et al.* (1975). Gas chromatographic determination and gas chromatographic-mass sepectrometric analysis of chloramphenicol, thiamphenicol and their metabolites. *J Chromatogr* 111: 355–364.

Pickering LK *et al.* (1979). Assays for chloramphenicol compared: radioenzymatic, gas chromatographic with electron capture, and gas chromatographic-mass spectrometric. *Clin Chem* 25: 300–305.

Sample RH *et al.* (1979). High-pressure liquid chromatographic assay of chloramphenicol in biological fluids. *Antimicrob Agents Chemother* 15(3): 491–493.

Thompson WL *et al.* (1975). Letter: Overdoses of chloramphenicol. *JAMA* 234: 149–150.

Velagapudi R *et al.* (1982). Simultaneous determination of chloramphenicol and chloramphenicol succinate in plasma using high-performance liquid chromatography. *J Chromatogr* 228(17): 423–428.

Chlorcyclizine

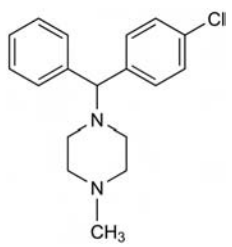
Antihistamine

$C_{18}H_{21}ClN_2 = 300.8$

CAS—82-93-9

IUPAC Name 1-[(4-Chlorophenyl)phenylmethyl]-4-methylpiperazine

Synonym Histachlorazine



Chemical Properties A liquid. pK_a 2.4, 7.8 (25°).

Chlorcyclizine Hydrochloride

$C_{18}H_{21}ClN_2$, HCl = 337.3

CAS—1620-21-9;14362-31-3

Synonym Chlorcyclizinium chloride

Proprietary Names *Di-Paralene*; *Trihistan*. It is an ingredient of *Fedrazil*.

Chemical Properties A white crystalline powder. Mp 226° to 227°. Soluble 1 in 2 of water, 1 in 11 of ethanol, and 1 in 4 of chloroform; practically insoluble in ether and benzene.

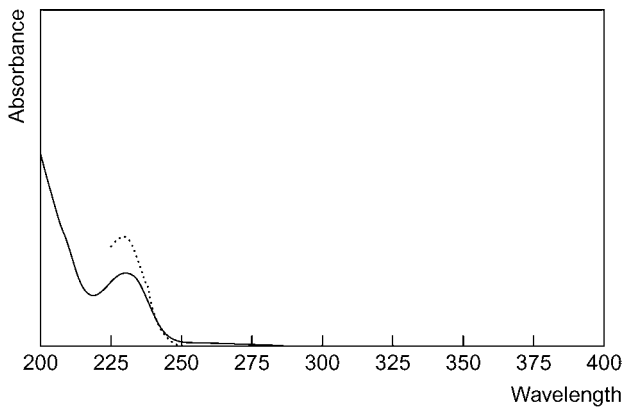
Colour Test Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.42; system TC—R_f 0.46; system TE—R_f 0.67; system TL—R_f 0.14; system TAE—R_f 0.35; system TAF—R_f 0.52; system TAJ—R_f 0.21; system TAK—R_f 0.10; system TAL—R_f 0.70 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).

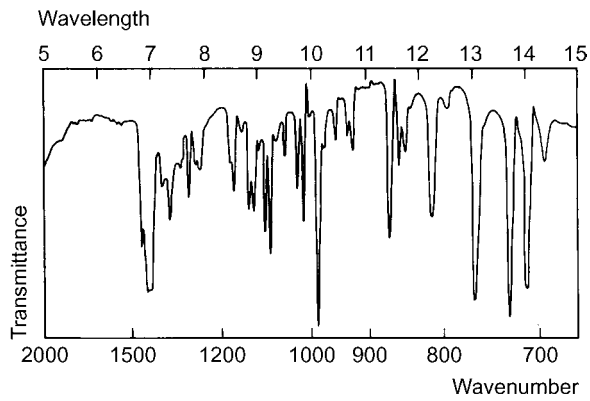
Gas Chromatography System GA—chlorcyclizine RI 2220, M (nor-) RI 2520; system GB—chlorcyclizine RI 2316, M (nor-) RI 2355; system GF—RI 2560.

High Performance Liquid Chromatography System HA—*k* 2.3; system HY—RI 340.

Ultraviolet Spectrum Aqueous acid—232 nm (A^1_1=581a).



Infrared Spectrum Principal peaks at wavenumbers 988, 725, 760, 706, 1087, 870 cm^{-1} chlorcyclizine hydrochloride (Nujol mull).



Mass Spectrum Principal ions at *m/z* 99, 56, 72, 165, 300, 228, 229, 242.

Quantification

Plasma (animal) **Colorimetry** For method for quantification of chlorcyclizine, cyclizine and their metabolites, see Kuntzman *et al.* [1965].

Tissues (animal) **Colorimetry** See Plasma Kuntzman *et al.* [1965].

Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the body. Metabolised by *N*-demethylation to form norchlorcyclizine and by *N*-oxidation. High concentrations of the *N*-desmethyl metabolite are found in the liver, lungs, kidney, and spleen. Slowly excreted in the urine; measurable amounts of norchlorcyclizine have been detected in the urine for up to 3 weeks after the cessation of chronic oral administration. About 0.5% of a dose is excreted in the urine as the *N*-oxide.

Therapeutic Concentration

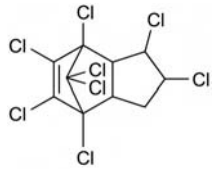
After a single oral dose of 2 mg/kg to 4 subjects, average peak plasma concentrations of about 0.05 mg/L and 0.03 mg/L were attained in 5 h for unchanged drug and norchlorcyclizine, respectively. After oral administration of 50 mg 3 times a day for 6 days, plasma concentrations of norchlorcyclizine of 0.05 to 0.11 mg/L were reported on the first day after the cessation of treatment and plasma concentrations of 0.02 to 0.04 mg/L were found on the 10th day after cessation of treatment [Kuntzman *et al.* 1973].

Half-life Plasma half-life, chlorcyclizine about 12 h, norchlorcyclizine 6 to 9 days.
Protein Binding Norchlorcyclizine, about 85 to 90%.
Dose 50 to 200 mg of chlorcyclizine hydrochloride daily.

Kuntzman R *et al.* (1965). Physiological distribution and metabolic inactivation of chlorcyclizine and cyclizine. *J Pharmacol Exp Ther* 149: 29–35.
Kuntzman R *et al.* (1973). Certain aspects of drug binding to nonplasma proteins as illustrated by studies with cyclizine, chlorcyclizine, and polymyxin B. *Ann N.Y Acad Sci* 226: 131–147.

Chlordane

Insecticide
$C_{10}H_6Cl_8$ = 409.8
CAS—57-74-9; 12789-03-6 (technical grade); 22212-52-8 (*cis*-); 5103-74-2 (*trans*-)
IUPAC Name 1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene
Synonym Chlordan
Proprietary Names *Corodane*; *Murphy Ant Killer*; *Nippon Ant Powder*; *Nizran*; *Sydane*; *Toxichlor*. It is an ingredient of *Cooper Ant Killer*.



Chemical Properties Technical chlordane is a mixture of chlorinated methano-indenes, the major components being the two stereoisomers *cis*-chlordane and *trans*-chlordane. The technical grade is an amber viscous liquid which is decomposed by alkalis. Insoluble in water; miscible with ethanol, chloroform, and ether. Log *P* (octanol/water), 6.3.
Thin-layer Chromatography System TB—R_f 0.72; system TE—R_f 0.87; system TF—R_f 0.72; system TAB—R_f 0.64; system TAC—R_f 0.44; system TAE—R_f 0.91.
Gas Chromatography System GA—RI 2020; system GK—*cis*-chlordane RRT 1.08, *trans*-chlordane RRT 1.06 (relative to caffeine).
High Performance Liquid Chromatography system HX—RI 1017; system HAO—*k* 0.00; system HAP—*k* 99.9.
Infrared Spectrum Principal peaks at wavenumbers 704, 1265, 1020, 1075, 1612, 826 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 373, 375, 377, 371, 44, 109, 75, 272 (*cis*-chlordane); 373, 375, 377, 371, 272, 237, 75, 65 (*trans*-chlordane).
Quantification
Blood **GC** For method, see Dale *et al.* [1966].
Disposition in the Body Readily absorbed from the gastrointestinal tract, lungs, and the skin. It is stored in adipose tissue. Oxychlordane has been detected in adipose tissue and breast milk.
Toxicity Severe toxicity may result after ingestion or skin contamination with >1 g, and fatalities have occurred after the ingestion of >2 g and after excessive skin contamination. The maximum permissible atmospheric concentration is 0.5 mg/m^3.

In a non-fatal poisoning case, a 4-year-old child who ingested an unknown amount of chlordane and developed intermittent convulsions had an initial serum concentration of 3.4 mg/L which decreased to 0.14 mg/L after 72 h; the rate of decline of the serum concentration was non-linear with a terminal half-life of 88 days. Urine samples obtained during the first 3 days after ingestion showed a decrease from 1.9 mg/L to 0.05 mg/L, but increased to 0.13 mg/L on the 35th day [Aldrich, Holmes 1969].

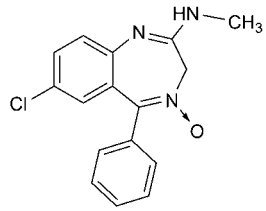
The following postmortem concentrations were reported in a fatality due to the ingestion of chlordane: blood 4.4 mg/L, urine 0.24 mg/L [Bost 1978].

A 66-year-old man who ingested about 400 mL of a 70% commercial solution and died after 40 h had the following postmortem tissue concentrations: blood 1.7 mg/L, fat 378 µg/g, kidney 14 µg/g, liver 43 µg/g, urine 0.6 mg/L [Baselt 2000].

Aldrich FD, Holmes JM (1969). Acute chlordane intoxication in a child. Case report with toxicological data. *Arch Environ Health* 19: 129–132.
Baselt RC (2000). *Disposition of Toxic Drugs and Chemicals in Man*, 5th edn. California: Biomedical Publications, 150–152.
Bost RO, Sunshine I (1978). *Bull Int Assoc Forensic Toxicol* 14(1): 30.
Dale WE *et al.* (1966). Hexane extractable chlorinated insecticides in human blood. *Life Sci* 5(1): 47–54.

Chlordiazepoxide

Anticonvulsant, 1,4-Benzodiazepine, Tranquilliser
$C_{16}H_{14}ClN_3O$ = 299.8
CAS—58-25-3
IUPAC Name 7-Chloro-4-hydroxy-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-imine
Synonyms Chlordiazepoxidum; 7-chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amine 4-oxide; clopoxide; methaminodiazepoxide.
Proprietary Names *Helogaphen*; *Libritabs*; *Librium* (tablets); *Multum*; *Omnalio*; *Reposans*; *Risolid*; *Silibrin*; *Tropium*. It is an ingredient of *Libraxin*; *Limbatril*; *Limbitrol*; *Menrium*.

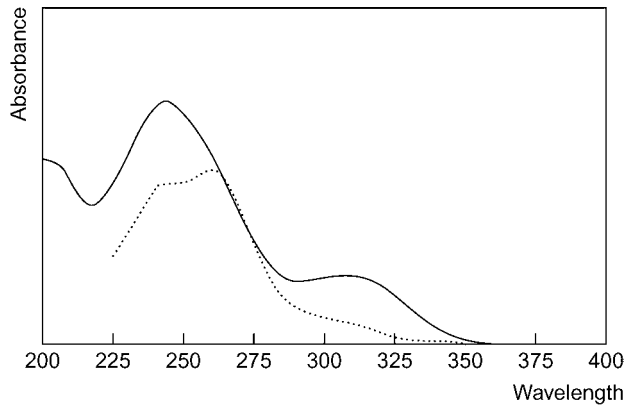


Chemical Properties Yellow crystalline powder, sensitive to sunlight. Mp 236° to 236.5°. Practically insoluble in water; soluble 1 in 50 of ethanol and 1 in 130 of ether; slightly soluble in chloroform. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005]. Chlordiazepoxide is unstable in blood at room temperature. Even in refrigerated samples originally of 5 mg/L the drug was not detectable within 1–2 weeks [Levine *et al.* 1983].

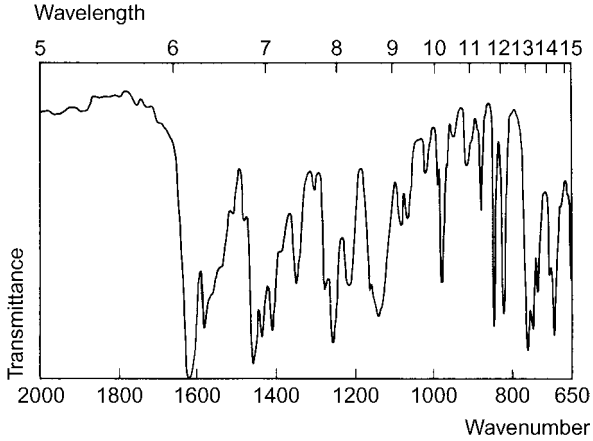
Chlordiazepoxide Hydrochloride

$C_{16}H_{14}ClN_3O$, HCl = 336.2
CAS—438-41-5
Proprietary Names *Ansiacal*; *A-Poxide*; *Balance*; *Benzodiapin*; *Cebrum*; *Corax*; *C-Tran*; *Disarim*; *Elenium*; *Equibral*; *Labican*; *Librium*; *Medilium*; *Mitran*; *Nack*; *Novopoxide*; *O.C.M.*; *Psichial*; *Psicoterina*; *Relaxil*; *Reliberan*; *Reposans*; *Seren Vita*; *SK-Lygen*; *Solium*; *Trilium*; *Tropium*; *Viansin*. It is an ingredient of *Clindex*; *Librax*; *Limbitrol*; *Menrium*.
Chemical Properties White or slightly yellowish crystalline powder. Mp 213°, with decomposition. Soluble 1 in 10 of water and 1 in 40 of ethanol; practically insoluble in chloroform and ether. pK_a 4.8 [Greenblatt *et al.* 1978]. Log *P* (octanol/water) 2.44.

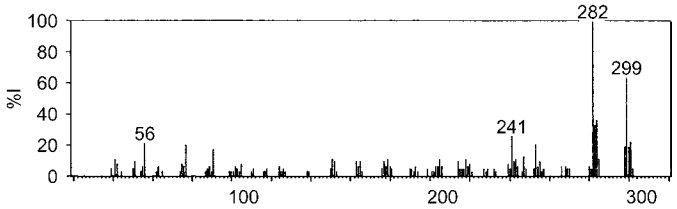
Colour Test Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.02; system TC—R_f 0.50; system TD—R_f 0.10; system TE—R_f 0.52; system TF—R_f 0.10; system TL—R_f 0.22; system TAD—R_f 0.53; system TAE—R_f 0.76; system TAF—R_f 0.77; system TAJ—R_f 0.48; system TAK—R_f 0.02; system TAL—R_f 0.79 (Dragendorff spray, positive; FPN reagent, yellow; acidified iodoplatinate solution, positive; Marquis reagent, yellow).
Gas Chromatography System GA—chlordiazepoxide RI 2795, M (nor-) RI 2452, M (demoxepam) RI 2529, nordiazepam RI 2490, oxazepam RI 2325; system GB—chlordiazepoxide RI 2981, M (nor-) RI 2679, M (demoxepam) RI 2806; system GG—chlordiazepoxide RI 3065, nordiazepam RI 3041, oxazepam RI 2803.
High Performance Liquid Chromatography System HI—chlordiazepoxide *k* 6.41, demoxepam *k* 2.42, M (nor-) *k* 4.47, nordiazepam *k* 8.00, oxazepam *k* 4.62; system HK—chlordiazepoxide *k* 2.87, demoxepam *k* 0.03, M (nor-) *k* 2.39, nordiazepam *k* 1.99, oxazepam *k* 0.73; system HX—RI 363; system HY—RI 285; system HZ—RT 3.2 min; system HAA—RT 15.2 min; system HAF—chlordiazepoxide RT 21.0 min; system HAL—chlordiazepoxide RT 7.7 min; system HAM—chlordiazepoxide RT 3.1 min; system HAX—chlordiazepoxide RT 6.9 min; system HAY—chlordiazepoxide RT 5.3 min; system HAZ—chlordiazepoxide *k* 1.68; system HBH—chlordiazepoxide *k* 6.68; system HBI—chlordiazepoxide *k* 1.65.
Ultraviolet Spectrum Aqueous acid—246 (A^1_1= 1112a), 308 nm; aqueous alkali—262 nm.



Infrared Spectrum Principal peaks at wavenumbers 1625, 760, 1260, 690, 1590, 850 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 282, 299, 284, 283, 241, 56, 301, 253; 285, 286, 269, 287, 241, 242, 77, 270 demoxepam; 285, 268, 284, 77, 286, 42, 287, 233 desmethylchlordiazepoxide; 242, 269, 270, 241, 243, 271, 244, 272 desmethyldiazepam; 257, 77, 268, 239, 205, 267, 233, 259 oxazepam.



Quantification

Blood **GC** Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD and ECD. Retention time: 6.16 min. Limit of detection, 10 µg/L ECD [Lillsunde, Seppala 1990]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (1.2 m × 3.2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 240°. ECD. Retention time: 9.95 min. Limit of detection, 0.2 mg/L [Peat, Kopjak 1979].

GC-MS Column: SE-30 WCOT vitreous silica capillary (25 m × 0.2 mm i.d.). Carrier gas: He, 22 psi. Temperature programme: 50° to 260° at 30°/min. EI ionisation at 70 eV. Limit of detection, 20–50 ng [Joyce *et al.* 1984].

HPLC Column: Bondapak C_{18} (30 cm × 4 mm i.d.). Mobile phase: methanol : 0.025 mol/L disodium hydrogen phosphate (pH 7.5; 58 : 42) or methanol : 0.025 mol/L disodium hydrogen phosphate (pH 7.5; 73 : 37), flow rate 2.4 mL/min. UV detection. Retention time: 12 and <5 min for the first and second mobile phases, respectively. Limit of detection, 0.2 mg/L [Peat, Kopjak 1979].

Note For 10 TLC methods for the detection of 21 anticonvulsants, see Gardner-Thorpe *et al.* [1971].

Plasma **TLC** Plates: silica gel F_{254} (20 × 20 cm). Solvent system: methanol : 12 N ammonium hydroxide (A, 100 : 1); *n*-heptane : chloroform : absolute methanol (B, 70 : 30 : 10), ethyl acetate : *n*-heptane : absolute ethanol (C, 70 : 30 : 10). R_f: 0.76, 0.45 and 0.38 for solvent system A, B and C, respectively. Limit of detection, 2.5 µg [Zingales 1971].

GC Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2 to 3 mL/min. Temperature programme: 200° for 1 min to 290° at 10 /min. NPD and ECD. Retention time: 6.16 min. Limit of detection, 10 µg/L [Lillsunde, Seppala 1990]. Column: Ultra 2 5% phenyl methyl silicone (25 m × 0.32 mm i.d.). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD and ECD. Limit of quantification, 5 µg/L [Gaillard *et al.* 1993]. See Blood [Peat, Kopjak 1979]. Column: 2% OV-17 on Chromosorb W HP 80/100 mesh (4′ × 2 mm i.d.). Carrier gas: He, 80 mL/min. Temperature: 275°. ECD. Retention time: ≈6 min. Limit of detection, 0.5 ng [Zingales 1971].

HPLC Column: Bondapak C_{18} (5 µm). Mobile phase: water : methanol : acetonitrile : acetic acid (pH 3.7; 51 : 28 : 16 : 0.2), flow rate 1.0 mL/min. UV detection (λ= 241 nm). Retention time: 10.1 min. Limit of detection, 75 µg/L [Garretty *et al.* 1998]. Column: LiChrosorb 10RP8 (25 cm × 4.6 mm i.d.). Mobile phase: water : methanol : tetraethylammonium (pH 6.0; 43 : 57 : 0.25), flow rate 2.0 mL/min. UV detection (λ= 254 nm). Retention time: 1.42 min relative to *N*-methylnitrazepam. Limit of detection, 0.02 mg/L [Willems *et al.* 1985]. Column: µBondapak (30 cm × 3.9 mm i.d.). Mobile phase: 450 mL methanol : acetonitrile (50 : 50), 550 mL water and 1 mL of 1 mol/L sodium acetate, flow rate 2.0 mL/min. UV detection (λ= 254 nm). Retention time: 11 min. Limit of detection, 50 µg/L [Divoll *et al.* 1982]. Column: LiChrosorb RP-8 (10 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L sodium acetate : methanol : acetonitrile (600 : 200 : 200), flow rate 2.0 mL/min. UV detection (λ= 240 nm). k': 11.2. Limit of detection, 20 µg/L [Vree *et al.* 1981]. Column: µBondapak C_{18} (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: methanol : isopropanol : 7.5 mmol/L acetate buffer (53 : 5 : 42), flow rate 0.9 mL/min. Electrochemical detection. Retention time: 12.3 min (k': 2.5) for chlordiazepoxide, 9.8 min (k': 1.8) for *N*-desmethylchlordiazepoxide. Limit of detection, 50 µg/L [Hackman, Brooks 1981]. Column: LiChrosorb RP-18 (250 × 4 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.1% ammonium carbonate (31 : 69), flow rate 2.0 mL/min. Retention time: 7.75 min. Limit of detection, 30 µg/L [Ascalone 1980]. See Blood [Peat, Kopjak 1979]. Column: Bondapak C_{18} Corasil (600 × 2.3 mm i.d., 35–52 µm). Mobile phase: acetonitrile : 0.1% aqueous ammonium carbonate (30 : 70), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Limit of detection, ≈0.1 mg/L [Skellern *et al.* 1978].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.2 mg/L (SIM), limit of detection, 0.01 mg/L (scan) [Kratzsch *et al.* 2004].

Serum **TLC** Plates: Quanta-Gram LQD (20 × 20 cm). Solvent system: acetone : dioxane : isopropanol : *n*-heptane : toluene : hexane (15 : 15 : 30 : 30 : 30 : 25). R_f: 0.48. Limit of quantification, 0.1 µg [Straughan *et al.* 1978]. Plates: silica gel GF (20 × 20 cm, 250 µm). Solvent system: chloroform : methanol : ammonium hydroxide (85 : 15 : 0.5). Development with 7 mol/L sulphuric acid followed by UV detection. Densitometry by fluorescence detection (λ_{ex}= 390 nm). R_f: 0.63, pale-blue spot. Limit of detection, 0.05 mg/L [Sun 1978a].

GC Column: 3% OV-17 Chromosorb W (HP) 80/100 mesh (3′ × 0.25" i.d.). Carrier gas: Ar: CH_4 (95 : 5), 66 mL/min. Temperature: 265°. ECD. Retention time: 5.1 min. Limit of detection, 0.05 mg/L [Kelly *et al.* 1979]. Column: 10% OV-1 on Chromosorb G HP 80/100 mesh (1.21 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 50 mL/min. Temperature: 270°. ECD. Retention time: 6.99 min. Limit of detection, 0.1 mg/L [Sun 1978b].

HPLC Column: TSK gel Super-ODS C_{18} reversed phase (100 × 4.6 mm i.d., 2 µm). Mobile phase: acetonitrile : 5 mmol/L sodium hydrogen phosphate (pH 6.0; 45 : 55), flow rate 0.65 mL/min. UV detection (λ= 254 nm). Retention time, 21.0 min. Limit of quantification, 1 µg/L [Tanaka *et al.* 1996; Tanaka *et al.* 1998] Column: C_{18} reversed phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium phosphate buffer (pH 6.0) containing 2.6 g/L pentane sulfonic acid : methanol : acetonitrile (45 : 35 : 20), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Limit of detection, 50 µg/L [Haver *et al.* 1986]. Column: C_{18} (250 × 4.6 mm i.d., 10 µm). Electrochemical detection. Limit of detection, 2 mg/L [Selavka *et al.* 1985]. Column: µBondapak C_{18} (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer, flow rate 3.0 mL/min. UV detection (λ= 210 nm). Retention time: 19.6 min. Limit of detection, 0.2 mg/L [Kabra *et al.* 1981]. Column: Ultrasphere ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 7 mmol/L dipotassium hydrogen phosphate (pH 3.7; 35 : 65), flow rate 2.0 mL/min. UV detection (λ= 242 nm). Retention time: 4.81 min. Limit of detection, 25 µg/L [Good, Andrews 1981]. Column: µBondapak C_{18} reversed phase (30 cm × 3.9 mm i.d.). Mobile phase: acetonitrile : methanol : acetate buffer (pH 5.0; 200 : 225 : 500), flow rate 2.0 mL/min. UV detection (λ= 240 nm). Retention time: 1.19 min relative to *N*-desmethylchlordiazepoxide. Limit of detection, 0.08 mg/L [Foreman *et al.* 1980].

Urine **TLC** Plates: Quanta-Gram LQD (20 × 20 cm). Solvent system: acetone : dioxane : isopropanol : *n*-heptane : toluene : hexane (15 : 15 : 30 : 30 : 30 : 25). R_f: 0.48. Limit of quantification, 0.1 µg [Straughan *et al.* 1978]. Plates: silica gel F_{254} (20 × 20 cm). Solvent system: chloroform twice followed by chloroform : acetone (1 : 1). UV detection (λ= 260 nm). R_f: 0.3. Limit of detection, 0.1–0.2 mg/L [Stronjny *et al.* 1977]. Plates: chromatoplates (250 µm). Solvent system: chloroform : acetone (A, 9 : 1), benzene (B), methanol (C), ethyl acetate : *n*-propanol : diethylamine (D, 70 : 30 : 1), ethylene chloride : methanol : concentrated aqueous ammonia (E, 90 : 10 : 1), toluene : acetone : concentrated aqueous ammonia (F, 50 : 50 : 1). UV detection, Dragendorff's reagent, Bratton-Marshall reagent or KI-*o*-tolidine. R_f: 0.15, 0.00, 0.56, 0.63, 0.48 and 0.55 for solvent system A to F, respectively. Light brown with UV light. Limit of detection, 5 (orange), 0.5 (purple-red) and 5 (brown) µg for each reagent, respectively [Sawada, Shinohara 1970].

GC-MS Column: DB-5 fused silica capillary cross-linked methyl silicone (12 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 250° for 3 min to 280° at 10°/min. Retention time: 4.90 min. Limit of detection not reported [Needleman, Porvaznik 1995]. Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 100° to 310° at 30 /min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

HPLC Column: Zorbax ODS (25 cm × 4.6 mm i.d., 5–6 µm). Mobile phase: 60 mmol/L ammonium hydroxide (pH 7.69) : 60 mmol/L acetic acid: acetonitrile 42 : 20 : 38 for 1 min to 0 : 25 : 75 at 5 min for 3 min to 42 : 20 : 38 at 8.1 min until 15 min), flow rate 2.0 mL/min. UV detection (λ = 254 nm). Retention time: ≈7 min. Limit of detection, 0.05 mg/L [Lensmeyer *et al.* 1982]. Column: LiChrosorb RP-8 (10 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L sodium acetate : methanol : acetonitrile (600 : 200 : 200), flow rate 2.0 mL/min. UV detection (λ= 240 nm). k': 11.2. Limit of detection, 20 µg/L [Vree *et al.* 1981].

Oral Fluid **GC-MS** Column: Agilent capillary (15 m × 0.25 mm i.d.). CI, SIM acquisition mode. Limit of quantification, 0.5 µg/L [Cone *et al.* 2007].

LC-MS Column: Zorbax Eclipse XDB C_{18} (50 × 4.6 mm i.d., 1.8 µm). Mobile phase: 20 mmol/L ammonium formate (pH 8.6) : acetonitrile (50 : 50), flow rate 0.2 mL/min for 6.5 min to 1 mL/min for 1.5 min to 0.2 mL/min for 2 min. ESI, MRM acquisition mode. Limit of quantification, 1 µg/L [Moore *et al.* 2007]. Column: Zorbax Bonus-RP (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol :

0.1% formic acid (50 : 50 for 0.5 min to 95 : 5 over 3 min for 1.3 min to 50 : 50 in 1 min for 2.2 min), flow rate 200 µL/min. APCI, positive ion mode. Limit of quantification, 1 µg/L, limit of detection, 0.5 µg/L [Ngwa *et al.* 2007].

Stomach Contents **TLC**. See Urine [Sawada, Shinohara 1970].

Liver **HPLC** Column: Ultrasphere ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.025 mol/L disodium hydrogen phosphate (pH 7.8, 60 : 40). UV detection (λ= 254 nm). Limit of detection not reported [Entwistle *et al.* 1986].

Note For a review of analytical methods for the measurement of 1,4-benzodiazepines, see Hailey [1974].

Disposition in the Body Readily absorbed after oral administration. Peak plasma concentrations are found after 1–2 h. Metabolised by demethylation and subsequent deamination to the active metabolites desmethylchlordiazepoxide and demoxepam. Demoxepam is further metabolised by hydrolysis with cleavage of the lactam ring and by reduction to desmethyldiazepam (nordazepam) followed by hydroxylation to oxazepam (desmethyldiazepam and oxazepam are also pharmacologically active). After a single oral or IV dose of 30-mg chlordiazepoxide, measurable plasma concentrations of *N*-desmethyldiazepam are reached 24–72 h after administration. When 10 mg chlordiazepoxide is administered 3 times daily, steady-state *N*-desmethydiazepam concentrations in plasma are reached after ~1 week of administration (range: 220–260 µg/L) [Dixon *et al.* 1976]. Approximately 60% of a dose is excreted in the urine and 10 to 20% is eliminated in the faeces; <1% of a dose is excreted in the urine unchanged, ~ 6% is excreted as demoxepam, and the remainder as ring-opened derivatives and glucuronide conjugates of oxazepam and other hydroxylated metabolites. Chlordiazepoxide passes into the cerebrospinal fluid and breast milk. It also crosses the placenta.

Therapeutic Concentration

In plasma, usually in the range 0.4 to 4 mg/L. Demoxepam and desmethyldiazepam may accumulate in the plasma of patients on chronic therapy; demoxepam is not normally detectable in plasma after a single dose of chlordiazepoxide.

After a single 50 mg IV dose of chlordiazepoxide to a healthy 72-year-old volunteer, the initial distribution half-life was 4.4 min, the elimination half-life was 18.3 h, the total volume of distribution was 0.32 L/kg, and the total clearance was 0.2 mL/min/kg [Divoll *et al.* 1982].

After the administration of a single 50 mg dose of chlordiazepoxide to a 50 kg female, a peak plasma concentration of ~2.25 mg/L was reached after 2 h [Straughan *et al.* 1978].

After daily oral administration of 10 mg three times a day to 8 subjects, mean steady-state plasma concentrations were: chlordiazepoxide 0.5–1.0 mg/L (mean 0.8), desmethylchlordiazepoxide 0.3–0.8 mg/L (mean 0.5), demoxepam 0.24–0.44 mg/L (mean 0.36) [Boxenbaum *et al.* 1977].

After a single oral dose of 20 mg to 6 subjects, peak plasma concentrations of 0.78–1.24 mg/L (mean 1.0) of chlordiazepoxide were attained in 2–6 h; desmethylchlordiazepoxide was detectable 5 h after the dose and peak plasma concentrations of 0.14–0.46 mg/L (mean 0.3) were attained in 8–24 h [Schwartz *et al.* 1971].

Toxicity Toxic reactions may be produced by plasma concentrations >3 mg/L; plasma concentrations in the region of 20 mg/L may produce coma or death, but fatalities caused by chlordiazepoxide alone are rare. Recoveries have occurred after the ingestion of single doses of about 2 g.

A 45-year-old female alcoholic was administered 5.2 g chlordiazepoxide over 4 days. Chlordiazepoxide therapy was stopped when she fell into a coma. Toxicity corresponded to the blood concentration of the metabolite demoxepam [Minder 1989].

The following postmortem chlordiazepoxide tissue concentrations were reported in a fatality in which death occurred 18–20 h after the ingestion of an unknown quantity of chlordiazepoxide: blood 26.4 mg/L, bile 39 mg/L, kidney 11 µg/g, liver 10 µg/g, spleen 9 µg/g, urine 7.8 mg/L [Moheni 1975].

The following peak plasma concentrations were observed in a comatose adult subject who had ingested 1 g: chlordiazepoxide 20 mg/L after 6 h, desmethylchlordiazepoxide 12 mg/L after 21 h, demoxepam 9 mg/L after 51 h [de Silva, D'Arconte 1969].

Bioavailability Almost 100%.

Half-life Plasma half-life, chlordiazepoxide 5–30 h (mean 15), demoxepam 14–95 h (mean 40); desmethyldiazepam about 40–100 h, but there is considerable inter-subject variation—see under Nordazepam.

Volume of Distribution 0.3–0.6 L/kg.

Clearance Plasma clearance, ≈0.5 mL/min/kg.

Saliva Plasma : saliva ratio, ≈33.

Protein Binding ≈96%.

Note For a review of the pharmacokinetics of chlordiazepoxide, see Greenblatt *et al.* [1978]. For a general review of benzodiazepines, see Miller and Gold [1990].

Dose In the treatment of anxiety 30 to 100 mg daily. For symptoms of alcohol withdrawal up to a maximum of 300 mg daily.

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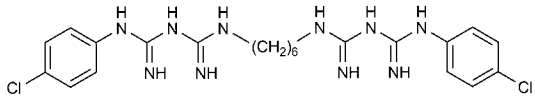
Chlorhexidine

Cationic Disinfectant
$C_{22}H_{30}Cl_2N_{10}$ = 505.5
CAS—55-56-1

IUPAC Name (1*E*)-2-[6-[[Amino-[[amino-[(4-chlorophenyl)amino]methylidene]amino]methylidene]amino]hexyl]-1-[amino-[(4-chlorophenyl)amino]methylidene]guanidine
Synonyms 1,6-Bis[*N*′-(*p*-chlorophenyl)-N^5-biguanido]hexane; 1,6-bis(N^5-*p*-chlorophenyl-*N*′-diguanido)hexane; *N*,*N*″ bis(4-chlorophenyl)-3,12-di-imino-2,4,11,13-tetra-azatetradecanedi-imidamide; 1,6-di(4′-chlorophenyldiguanido)hexane; 1,1′-hexamethylenebis[5-(*p*-chlorophenyl)biguanidine.
Proprietary Names *Hexol*. It is an ingredient of *Cetal* (liquid).



Chemical Properties Crystals. Mp 134°. pK_{a1} 10.8 (25°), pK_{a2} 10.3, pK_{a3} 2.2 [Lam *et al.* 1993]. Log *P* (octanol/water) 0.1. Stable in saliva at –20° for at least 2 months [Lam *et al.* 1993].

Chlorhexidine Diacetate

$C_{22}H_{30}Cl_2N_{10}$, $2C_2H_4O_2$ = 625.6
CAS—56-95-1
Proprietary Names *Chlorasept 2000*; *Nolvasan*.
Chemical Properties White to pale-cream microcrystalline powder. Mp 154° to 155°. When heated it decomposes with the production of trace amounts of 4-chloroaniline. Aqueous solutions slowly decompose. Soluble 1 in 55 of water and 1 in 15 of ethanol; soluble in glycerol and propylene glycol.

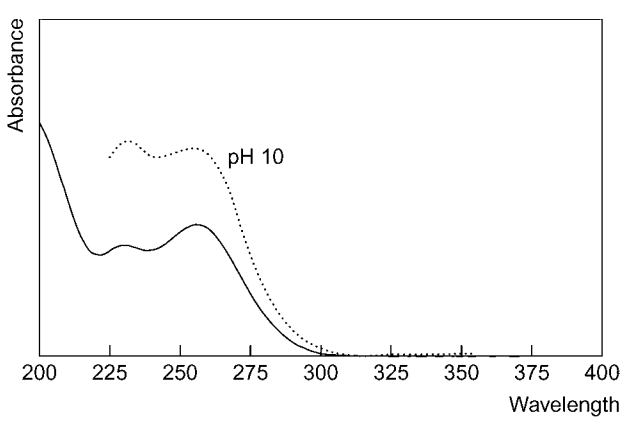
Chlorhexidine Digluconate

An aqueous solution containing 19 to 21% of $C_{22}H_{30}Cl_2N_{10}$, $2C_6H_{12}O_7$ = 897.8.
CAS—18472-51-0 (chlorhexidine gluconate)
Proprietary Names *Corsodyl*; *Dispray*; *Hibicare*; *Hibiclens*; *Hibidil*; *Hibiscrub*; *Hibisol*; *Hibitane (solution)*; *pHiso-MED*; *Rotersept*. It is an ingredient of *Cyteal*; *Eludril*; *Savloclens*; *Savlodil*; *Savlon (liquid)*; *Stomosol*.
Chemical Properties Colourless-to-pale-straw-coloured liquid that is affected by light. Weight per mL 1.06 to 1.07 g. Miscible with water, with up to 5 parts of ethanol, and with up to 3 parts of acetone.

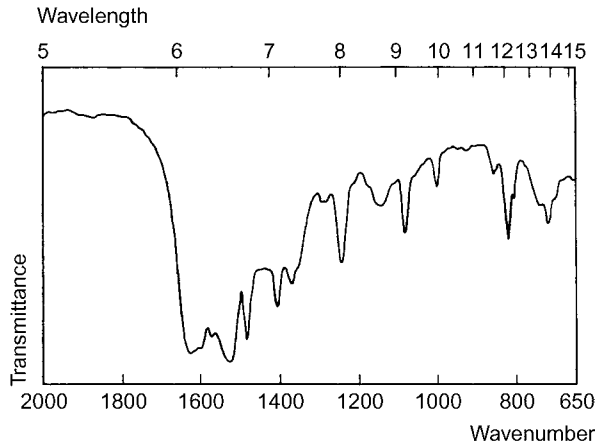
Chlorhexidine Hydrochloride

$C_{22}H_{30}Cl_2N_{10}$, 2HCl = 578.4
CAS—3697-42-5
Proprietary Names *Hibitane* (powder); *Lisium*; *Sterilon* (powder).
Chemical Properties Light-sensitive white crystalline powder. Mp 260° to 262°, with decomposition. Soluble 1 in 1700 of water, 1 in 450 of ethanol and 1 in 50 of propylene glycol; slightly soluble in methanol.

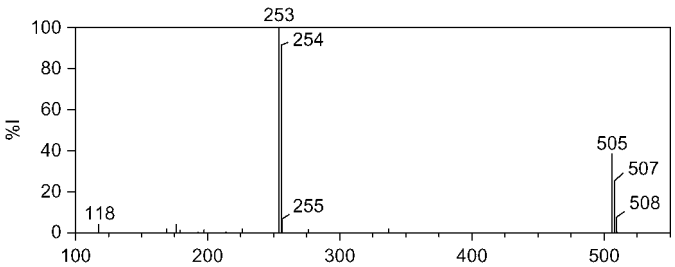
Colour Test Stir a small quantity on a white tile with a drop of bromine solution followed by a drop of sodium hydroxide solution—red.
Thin-layer Chromatography System TA—R_f 0.33; system TAE—R_f 0.01; system TB—R_f 0.00; system TE—R_f 0.13 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HAA—RT 13.5 min; system HX—RI 398; system HY—RI 305.
Ultraviolet Spectrum Aqueous acid—245 nm; aqueous alkali (pH 10)—232, 253 nm.



Infrared Spectrum Principal peaks at wavenumbers 1527, 1628, 1575, 1235, 820, 1080 cm^{-1} (KBr disk).



Mass Spectrum



Quantification

Blood **HPLC** Column: ODS reversed phase (10 µm). Mobile phase: 1 g/L toluene-4-sulfonic acid in methanol : water (65 : 35), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection, 1 mg/L [Huston *et al.* 1982].

LC-MS Column: TSKgel ODS-100V (50 × 2.0 mm, 5 µm). Mobile phase: acetonitrile : water : trifluoroacetic acid (65 : 35 : 0.1), flow rate 200 µL/min. ESI, positive or negative ion mode, SIM acquisition mode. Limit of quantification, 100 µg/L, limit of detection, 18.3 µg/L [Usui *et al.* 2006].

Serum **HPLC** Column: Capcell Pak C_{18} MG (250 × 3.0 mm i.d., 4.0 µm). Mobile phase: acetonitrile : water with 0.05% trifluoroacetic acid, 0.05% heptafluorobutyric acid and 0.1% triethylamine (40 : 60), flow rate 0.8 mL/min. UV detection (260 nm). Limit of detection, 0.05 mg/L [Kudo *et al.* 2002]. Column: LiChrospher 100 RP-18 (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L disodium hydrogen phosphate with 5 mmol/L 1-heptanesulfonic acid and 0.05 mol/L triethylamine (pH 2.5; 35 : 65). UV detection (λ = 260 nm). Limit of quantification, 0.5 mg/L [Pesonen *et al.* 1995]. See Blood [Huston *et al.* 1982].

Urine **HPLC** See Blood [Huston *et al.* 1982].

Oral Fluid **HPLC** Column: Shimpack CLC-C8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium pentanesulfonate in acetonitrile : 50 mmol/L sodium phosphate buffer (pH 3.0; 47 : 53), flow rate 1.0 mL/min. DAD. Limit of quantification, 50 µg/L [Tsuchiya *et al.* 1999]. Column: Ultrasphere ODS C_{18} (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L sodium acetate buffer and 5 mmol/L heptanesulfonic acid (pH 5.0; 40 : 60), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: 5.8–6.1 min. Limit of detection, 50 µg/L [Lam *et al.* 1993].

Skin **HPLC** LiChrospher 60 RP-select (250 × 4 mm i.d.). Mobile phase: acetonitrile : 30 mmol/L sodium acetate buffer (pH 3.3; 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: 3.4 min for chlorhexidine digluconate. Limit of detection, 50 µg/L [Carret *et al.* 1997].

Toxicity

A 28-year-old female was found dead with her left arm connected to an IV drip. The chlorhexidine concentration in her blood was 352 mg/L [Usui *et al.* 2006].

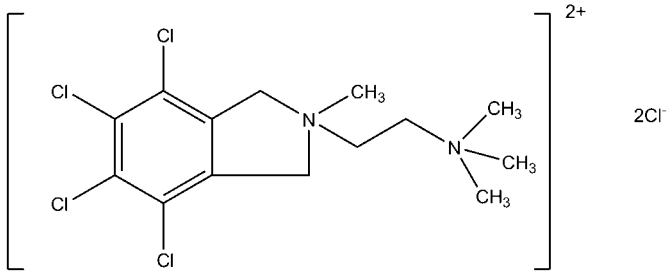
A 58-year-old Japanese female suffering from articular rheumatism underwent synovectomy on her finger. While in hospital she received an injection of antibiotics. Twenty minutes later she went into cardiac arrest, and after 100 min she died. A concentration of chlorhexidine was detected in the serum taken from the right side of her heart at a level of 39.5 mg/L only 27 h after death [Kudo *et al.* 2002].

Use Chlorhexidine gluconate is used in solutions of 0.01 to 0.5%.

Carret L *et al.* (1997). Kinetics of chlorhexidine on intact skin following a single application. *Pathol Biol (Paris)* 45: 737–740.
Huston CE *et al.* (1982). High-performance liquid chromatographic method for the determination of chlorhexidine. *J Chromatogr* 237: 457–464.
Kudo K *et al.* (2002). Toxicological analysis of chlorhexidine in human serum using HPLC on a polymer-coated ODS column. *J Anal Toxicol* 26: 119–122.
Lam YW *et al.* (1993). Sensitive high-performance liquid chromatographic assay for the determination of chlorhexidine in saliva. *J Chromatogr* 612: 166–171.
Pesonen T *et al.* (1995). Determination of chlorhexidine in saliva using high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 665: 222–225.
Tsuchiya H *et al.* (1999). High-performance liquid chromatographic analysis of chlorhexidine in saliva after mouthrinsing. *Caries Res* 33: 156–163.
Usui K *et al.* (2006). Determination of chlorhexidine (CHD) and nonylphenolethoxylates (NPEOn) using LC-ESI-MS method and application to hemolyzed blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 831: 105–109.

Chlorisondamine Chloride

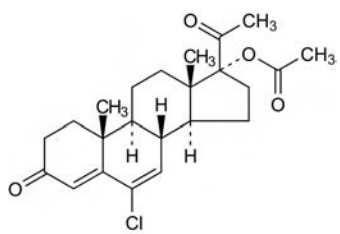
Quaternary Ammonium, Selective Nicotinic Receptor Antagonist, Antihypertensive
$C_{14}H_{20}Cl_6N_2 = 429.0$
IUPAC Name Ethylene-1-(4,5,6,7-tetrachloro-2-methylisoindolinium)-2-trimethylammonium dichloride
Synonym SU 3088
Proprietary Name *Ecolid*



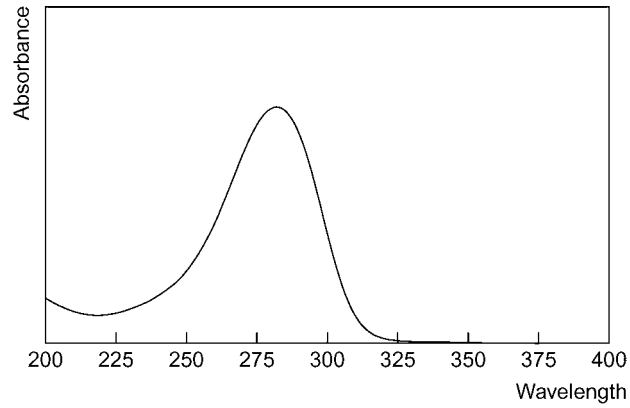
Chemical Properties Crystals. Mp 258° to 265° with decomposition. Soluble in water and ethanol.
Thin-layer Chromatography System T1—R_f 0.01 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—274, 282, 290 nm.
Infrared Spectrum Principal peaks at wavenumbers 1392, 964, 1480, 1177 cm^{-1} (KBr disk).
Dose Up to 200 mg daily.

Chlormadinone Acetate

Progestational Steroid
$C_{23}H_{29}ClO_4 = 404.9$
CAS—1961-77-9 (chlormadinone); 302-22-7 (acetate)
IUPAC Name [(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Acetyl-6-chloro-10,13-dimethyl-3-oxo-2,8,9, 11,12,14,15,16-octahydro-1H-cyclopenta[a]phenanthren-17-yl] acetate
Synonym 17-(Acetyloxy)-6-chloropregna-4,6-diene-3,20-dione
Proprietary Names *Chronosym*; *Cormin*; *Cyclonorm*; *Fertiletren*; *Gestafortin*; *Lutéran*; *Matrol*; *Traslan*.



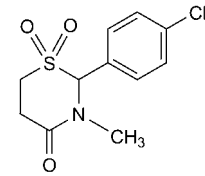
Chemical Properties A white to creamy-white, fluffy, crystalline powder. Mp 208° to 212°. Practically insoluble in water; soluble 1 in 160 of ethanol, 1 in 1.5 of chloroform, and 1 in 210 of ether. Log *P* (octanol/water), 4.0.
Gas Chromatography System GA—chlormadinone-H_2O RI 3340, chlormadinone acetate RI 3360.
High Performance Liquid Chromatography System HAA—Retention time 24.1 min (chlormadinone).
Ultraviolet Spectrum Methanol—285 nm (A_1^1=575b).



Infrared Spectrum Principal peaks at wavenumbers 1238, 1724, 1646, 1219, 881, 1597 cm^{-1} (KBr disk).
Dose Chlormadinone acetate has been given in doses of 2 to 10 mg daily.

Chlormezanone

Tranquilliser
$C_{11}H_{12}ClNO_3S = 273.7$
CAS—80-77-3
IUPAC Name 2-(4-Chlorophenyl)tetrahydro-3-methyl-4*H*-1,3-thiazin-4-one 1,1-dioxide
Synonyms Chlormethazanone; chlormezanonum; dichloromethazanone.
Proprietary Names *Trancopal*. It is an ingredient of *Arcanaflex*; *Besaprin*; *Beserol*; *Clormetadone*; *Lobak*; *Trancogesic*; *Trancoprin*.



Chemical Properties White crystalline powder. Mp 116.2° to 118.2°. Very slightly soluble in water; sparingly soluble in ethanol; freely soluble in acetone and chloroform. Log *P* (octanol/water) −0.26. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005]. Stable in plasma for at least 9 weeks when stored at −20° and after 3 freeze-thaw cycles. Extracts were stable at room temperature for 3 days [Schütz *et al.* 1997].
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.01; system TC—R_f 0.63; system TE—R_f 0.68; system TL—R_f 0.57; system TAE—R_f 0.84; system TAF—R_f 0.80; system TAJ—R_f 0.55; system TAK—R_f 0.45; system TAL—R_f 0.94.
Gas Chromatography System GA—chlormezanone RI 2199, M (4-chlorobenzoic acid) RI 1400, M (*N*-methyl-4-chlorobenzamide) RI 1555, art RI 1235; system GB—chlormezanone RI 2346, M (*N*-methyl-4-chlorobenzamide) RI 1596, art RI 1245.
High Performance Liquid Chromatography System HY—RI 334; system HAA—RT 15.5 min; system HAX—RT 6.0 min; system HAY—RT 5.3 min.
Column: Nucleosil RP C_{18} (120 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 85% orthophosphoric acid (35 : 65 : 0.1), flow rate 1.5 mL/min [Köppel *et al.* 1991].
Ultraviolet Spectrum Aqueous acid—258, 265 (A_1^1 = 23b), 272 nm; aqueous alkali—259 nm.
Infrared Spectrum Principal peaks at wavenumbers 1650, 1150, 1130, 1315, 880, 1090 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 152, 154, 42, 69, 174, 208, 153.
Quantification
Blood **GC** Column: HP-5 (25 m × 0.25 mm i.d., 0.33 µm). Temperature programme: 100° for 1 min to 300° at 10°/min. FID. Relative retention time: 1.12 min [Lo *et al.* 1997].
HPLC Column: ODS-Hypersil (200 × 2.1 mm i.d., 5 µm). Mobile phase: 2 mmol/L phosphate buffer (pH 3.2) : acetonitrile (95 : 5 to 50 : 50 at 20 min for 10 min to 95 : 5 at 31 min), flow rate 0.4 mL/min. DAD (λ = 210 nm). Relative retention time: 0.96 min [Lo *et al.* 1997]. Column: Nucleosil C_{18} (12 × 0.4 cm i.d., 5 µm). Mobile phase: acetonitrile : water: *o*-phosphoric acid (35 : 65 : 0.1) flow rate 1.5 mL/min. UV detection (λ = 225 nm). Limit of detection, 0.05 mg/L [Köppel *et al.* 1991].
Plasma **GC** Column: 3% OV-17 (2 m). Temperature programme: 100° to 300° at 10°/min. EI or CI. Limit of detection, 0.15 mg/L [Köppel *et al.* 1986]. Column: 5% EGS polyester on 80/100 Gas-Chrom Q (1 m × 3 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 100°. ^{63}N-ECD. Limit of detection, 0.1 mg/L [Ohya *et al.* 1980].

HPLC Column: reversed phase. Mobile phase: isocratic. UV detection ($\lambda = 230$ nm). Limit of quantification, 0.1 mg/L, limit of detection, 0.02 mg/L [Schütz *et al.* 1997]. Column: Nucleosil C_{18} (12 cm × 4 mm i.d., 7.5 µm). Mobile phase: methanol : water: 85% *o*-phosphoric acid (50 : 50 : 1), flow rate 1.0 mL/min. UV detection ($\lambda = 228$ nm). Retention time: 3.37 min. Limit of detection, 0.1 mg/L [Ali, Blume 1987].

Disposition in the Body Rapidly absorbed after oral administration, with peak plasma concentrations reached after 1 to 2 h. It appears to be metabolised by hydrolysis then oxidation to chlorobenzaldehyde and conjugation. Less than 5% of a dose is excreted in the urine as unchanged drug in 48 h; about 40% of a dose is excreted in the urine in 72 h as acidic metabolites (mainly 4-chlorohippuric acid).

Therapeutic Concentration

After a single 200-mg oral dose peak plasma concentrations ranged from 2.6 and 3.1 mg/L reached within 1–1.5 h [Schütz *et al.* 1997].

After a single oral dose of 200 mg to 5 subjects a mean peak plasma concentration of 1.86 mg/L was attained in 1 h. Repeated oral doses of 3 × 200 mg (12 subjects) or 4 × 400 mg (10 subjects) produced pre-dose chlormezanone plasma levels of 12.0 and 22.7 mg/L, respectively, after 5 days; levels were comparable after 10 days in 3 patients from each group. After a loading dose of 800 mg and repeated doses of 3 × 200 mg chlormezanone to 5 patients, plasma levels of 6.5, 8.9, 12.7, and 10.4 mg/L were determined after 2, 8, 16, and 36 h, respectively [Köppel *et al.* 1991].

After a single oral dose of 200 mg to 5 subjects, peak plasma concentrations of 2.5 to 3.4 mg/L (mean 2.8) were attained in ~4 h [Ohya *et al.* 1980].

After a single oral dose of 400 mg to 4 subjects, peak plasma concentrations of 4.9 to 9.9 mg/L (mean 7) were attained in 1 to 2 h; following oral doses of 150 mg three times a day for 3 days to 4 subjects, plasma concentrations of 5.8 to 8.4 mg/L (mean 6.8) were reported 2 h after the final dose [McChesney *et al.* 1967].

Toxicity Chlormezanone has been withdrawn from the market in many countries because of reports of serious skin reactions [Guillaume *et al.* 1987; Mahboob, Haroon 1998; Saiag *et al.* 1992].

A 39-year-old female was found in a comatose state 6 h after ingesting chlormezanone (possibly up to 12 g). A liver biopsy revealed diffuse hydropic degeneration and necrosis of hepatocytes. Chlormezanone levels 7 to 8 h post-ingestion and then every other day were as follows: 78.0, 45.9, 21.6 and 7.8 mg/L in serum and 3.8, 1.1, 0.5 mg/L then untraceable, in urine [Sheu *et al.* 1995].

A 29-year-old female experienced muscle spasm in the 27th week of pregnancy. She was given 600 mg of chlormezanone per day for 3 weeks. In the third week she complained of nausea and vomiting and jaundice appeared 3 days later. Four days later, the patient went into a coma. A healthy baby boy was delivered by Caesarean section and the following day an orthotopic liver transplant was carried out. The removed liver weighed 335 g. Mother and baby were in good health 26 months after the transplant [Bourliere *et al.* 1992].

In a fatality due to the combined ingestion of chlormezanone (possibly up to 11 g), diazepam (up to 200 mg) and nitrazepam (up to 80 mg), the following postmortem concentrations of chlormezanone were reported: femoral blood, 53 mg/L; brain 109 µg/g; liver, 88 µg/g and urine, 31 mg/L [Köppel *et al.* 1991].

A 14-year-old female ingested an unknown amount of ofloxacin, diphenhydramine and chlormezanone. Plasma concentrations of chlormezanone were 6 and 1.3 mg/L at 12 and 24 h post-ingestion, respectively [Koppel *et al.* 1990].

A 70-year-old female had received drug therapy for cervical vertebral syndrome and hypertension for 5 years. She had taken 3 g/day Pro G (15 mg propantheline bromide [Koppel *et al.* 1990], 778 mg magnesium silicate, 30 mg sodium copper chlorphylin, and 20 mg glycyrrhizin in 1 g granule), 5 mg/day diazepam, 600 mg/day Salyitamine (150 mg isopropylantipyrine, 40 mg allylisopropylacetylurea, 250 mg phenacetin, and 50 mg anhydrous caffeine per 600 mg granule), and 150 mg/day vitamin E. Approximately 4 months later, 200 mg/day chlormezanone was added, this was increased to 600 mg/day 2 months later. Approximately 2 weeks later she was admitted to hospital with abdominal pain and weight loss. She was diagnosed with hepatitis and chlormezanone therapy was stopped. She was discharged ~2 months later due to improvement of her symptoms [Ohsawa, Konishi 1986].

A 36-year-old female was admitted to hospital comatose after the ingestion of 7 g of chlormezanone; an initial plasma concentration of 60 mg/L was reported, which declined with a half-life of 29 h; the patient regained consciousness after 15 h [Armstrong *et al.* 1983].

Half-life Plasma half-life, ≈20 to 30 h.

Protein Binding ≈50%.

Note For a review of the pharmacokinetics of chlormezanone, see Gautier *et al.* [1990].

Dose 300 to 800 mg daily in divided doses.

Ali SL, Blume H (1987). Determination of chlormezanone in human plasma after administration of chlormezanone formulations. *Arzneimittelforschung* 37: 1396–1399.

Armstrong D *et al.* (1983). Chlormezanone poisoning. *Br Med J (Clin Res Ed)* 286: 845–846.

Bourliere M *et al.* (1992). Chlormezanone-induced fulminant hepatitis in a pregnant woman: successful delivery and liver transplantation. *J Gastroenterol Hepatol* 7: 339–341.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Gautier V *et al.* (1990). Pharmacokinetics of chlormezanone in healthy volunteers. *Therapie* 45: 315–319.

Guillaume JC *et al.* (1987). The culprit drugs in 87 cases of toxic epidermal necrolysis (Lyell's syndrome). *Arch Dermatol* 123: 1166–1170.

Koppel C *et al.* (1990). Central anticholinergic syndrome after ofloxacin overdose and therapeutic doses of diphenhydramine and chlormezanone. *J Toxicol Clin Toxicol* 28: 249–253.

Köppel C *et al.* (1991). Chlormezanone plasma and blood levels in patients after single and repeated oral doses and after suicidal drug overdose. *Eur J Drug Metab Pharmacokinet* 16: 43–47.

Köppel CT *et al.* (1986). Metabolism of chlormezanone in man. *Arzneimittelforschung* 36: 1116–1118.

Lo D *et al.* (1997). Acidic and neutral drugs screen in blood with quantitation using microbore high-performance liquid chromatography-diode array detection and capillary gas chromatography-flame ionization detection. *Forensic Sci Int* 90: 205–214.

Mahboob A, Haroon TS (1998). Drugs causing fixed eruptions: a study of 450 cases. *Int J Dermatol* 37: 833–838.

McChesney EW *et al.* (1967). Metabolism of chlormezanone in man and laboratory animals. *Biochem Pharmacol* 16: 813–826.

Ohsawa T, Konishi K (1986). Hepatitis associated with chlormezanone. *Drug Intell Clin Pharm* 20: 506.

Ohya K *et al.* (1980). Sensitive and selective method for the determination of chlormezanone in plasma by electron-capture gas chromatography. *J Chromatogr* 221: 67–74.

Saiag P *et al.* (1992). Drug-induced toxic epidermal necrolysis (Lyell syndrome) in patients infected with the human immunodeficiency virus. *J Am Acad Dermatol* 26: 567–574.

Schütz H *et al.* (1997). Relative bioavailability of 3 different chlormezanone 200 mg preparations after single dose oral administration. *Int J Clin Pharmacol Ther* 35: 112–116.

Sheu BS *et al.* (1995). Severe hepatocellular damage induced by chlormezanone overdose. *Am J Gastroenterol* 90: 833–835.

Chloroaniline

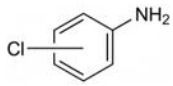
Insecticide

C_6H_6ClN = 127.6

CAS—27134-26-5

IUPAC Name 2-Chloroaniline

Synonyms Chlorobenzenamine; NSC-174207.



2-Chloroaniline

C_6H_6ClN = 127.6

CAS—95-51-2

Synonyms *o*-Chloroaniline; OCA.

Chemical Properties A pale amber liquid. Mp −1.9°. Bp 208.8°. It is practically insoluble in water (<0.1 g/100 mL at 20°) but soluble in acid and most organic solvents.

3-Chloroaniline

C_6H_6ClN = 127.6

CAS—108-42-9

Synonyms *m*-Chloroaniline; MCA.

Chemical Properties A colourless to light amber liquid. Mp −10.3°. Bp 230.5°. It is practically insoluble in water (<0.1 g/100 mL at 18°) but soluble in most common organic solvents (completely miscible in methanol and diethyl ether).

4-Chloroaniline

C_6H_6ClN = 127.6

CAS—106-47-8

Synonyms *p*-Chloroaniline; *p*-chlorophenylamine

Chemical Properties A white to pale yellow solid. Mp 72.5°. Bp 232.0°. It is practically insoluble in cold water (39 mg/100 mL) but soluble in hot water and freely soluble in alcohol, ether, acetone, and carbon disulphide.

Gas Chromatography System GA—4-chloroaniline RI 1210.

Quantification

Plasma **HPLC** Limit of detection, <6 µg/L for 3-chloroaniline, <14 µg/L for 4-chloroaniline [Marengo *et al.* 1999].

Disposition in the Body Chloroaniline is metabolised primarily to conjugated *p*-chloroaniline (*p*-CA) and 2-amino-5-chlorophenol (2-A-5-CP). Small amounts of 2,4-dichloroaniline (2,4-DCA) and *p*-chloroformanilide (*p*-CFA) have also been detected. All metabolites and the parent chemical are excreted mainly in urine with <1% present in faeces. Accumulation can occur in the liver.

Toxicity Hepatotoxicity, splenotoxicity, hematotoxicity, and nephrotoxicity.

Half-life Elimination half-lives of the metabolites: *p*-CA 2.4 h, 2-A-5-CP 1.7 h and 2,4-DCA 1.7 h.

Marengo E *et al.* (1999). Optimization of the separation of mono- and dichloroanilines in ion interaction high-performance liquid chromatography. *J Chromatogr A* 863(1): 1–11.

Chlorobutanol

Preservative

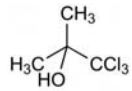
$C_4H_7Cl_3O$ = 177.5

CAS—57-15-8 (anhydrous); 6001-64-5 (hemihydrate)

IUPAC Name 1,1,1-Trichloro-2-methyl-2-propanol

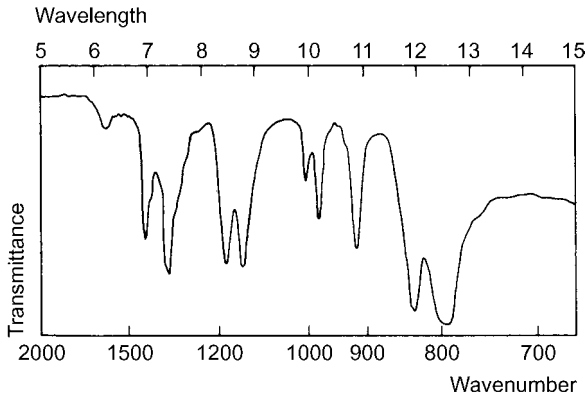
Synonyms Acetone-chloroforme; alcohol trichlorisobutylicus; chlorbutanol; chlorbutanolum hydratum; chlorbutol; chloretone; trichlorbutanolum.

Proprietary Names *Chloretone*; *Coliquifilm*; *Methaform*; *Sedaform*.



Chemical Properties Colourless or white crystals which are volatile at ordinary temperatures. Mp 78°; 97°. Bp about 167°. Soluble 1 in 130 of water, 1 in 1 of ethanol and 1 in 3 of chloroform; soluble in ether, acetone, and glacial acetic acid. Log *P* (octanol/water), 2.0.

Colour Test Fujiwara test—red.
Gas Chromatography System GA—RI 949; system GI—retention time 29.8 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 790, 833, 1145, 1186, 917, 980 cm^{-1} (Nujol mull).



Quantification
Blood **GC** ECD. Limit of detection, about 100 µg/L [Valentour, Sunshine 1975].
Urine **GC** See Blood [Valentour, Sunshine 1975].
Tissues **GC** See Blood [Valentour, Sunshine 1975].
Disposition in the Body Rapidly absorbed after oral administration, peak plasma concentrations being attained within 0.25 to 1 h. About 10% of a dose is excreted in the urine in 17 days mostly as the glucuronide and sulfate conjugates.
Toxicity

In a non-fatal case of chlorobutanol toxicity, a man who had been taking 1 to 1.5 g daily was admitted to hospital. An initial plasma concentration of about 100 mg/L was reported and the plasma half-life was found to be about 13 days [Borody *et al.* 1979].

In a fatality caused by chlorobutanol overdose, the following postmortem tissue concentrations were found: blood 64 mg/L, bile 123 mg/L, brain 161 µg/g, kidney 87 µg/g, liver 141 µg/g, spleen 120 µg/g, urine 31 mg/L [Valentour, Sunshine 1975].

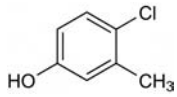
Half-life Plasma half-life, about 10 days.
Volume of Distribution About 3 L/kg.
Protein Binding About 50 to 60%.
Note For a report on the pharmacokinetics of chlorobutanol, see Tung *et al.* [1982].
Uses Chlorobutanol is used in a concentration of 0.5%. It was formerly given as a sedative in doses of 0.3 to 1.2 g.

Borody T *et al.* (1979). Chlorbutol toxicity and dependence. *Med J Aust* 1: 288.
Tung C *et al.* (1982). The pharmacokinetics of chlorbutol in man. *Biopharm Drug Dispos* 3: 371–378.
Valentour JC, Sunshine I (1975). Chlorobutanol poisoning. Report of a fatal case. *Z Rechtsmed* 77: 61–63.

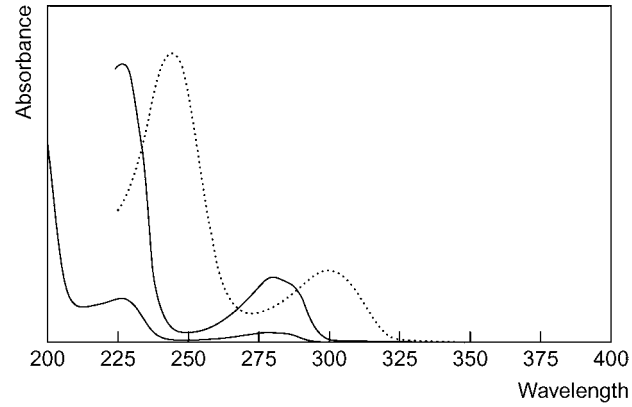
Chlorocresol

Disinfectant
C_7H_7ClO = 142.6
CAS—59-50-7

IUPAC Name 4-Chloro-3-methylphenol
Synonyms Chlorkresolum; parachlorometacresol; PCMC.
Proprietary Name *Wright's Vaporizing Fluid*



Chemical Properties Colourless crystals or crystalline powder. It is volatile in steam. Mp 55.5°. Soluble 1 in 260 of water, 1 in 50 of boiling water, and 1 in 0.4 of ethanol; freely soluble in acetone, benzene, chloroform, aqueous alkaline solutions, and ether. Solutions in water acquire a yellowish colour on exposure to light and air. pK_a 9.6 (25°). Log *P* (octanol/water), 3.1.
Gas Chromatography System GA—RI 1400.
High Performance Liquid Chromatography System HY—RI 455.
Ultraviolet Spectrum Aqueous acid—227, 279 nm (A^{1}_{1}=105a); aqueous alkali—244, 299 nm.

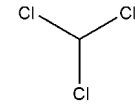


Use In concentrations of 0.1 to 0.2%.

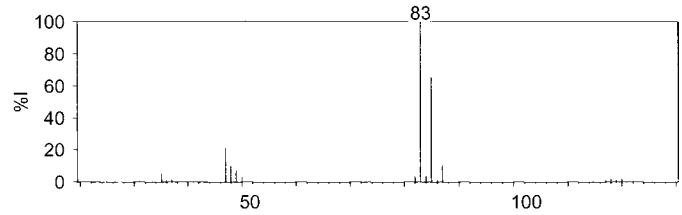
Chloroform

Anaesthetic (General)
$CHCl_3$ = 119.4
CAS—67-66-3

Synonyms Chloroformium anaesthesicum; chloroformum pro narcosi; trichloromethane.



Chemical Properties A colourless mobile volatile liquid. Bp 61° to 62°. Refractive index 1.4464. Chloroform (BP) contains 1–2% v/v of ethanol. Soluble 1 in 200 of water; miscible with dehydrated alcohol, benzene, and ether. Log *P* (octanol/water), 2.0.
Colour Tests Fujiwara test—red; palladium chloride—black; heat with a small amount of resorcinol in 2 mol/L sodium hydroxide—red; heat with a small amount of either α- or β-naphthol—blue.
Gas Chromatography System GA—RI 605; system GI—RT 6.2 min; system GAA—RI 603.
Infrared Spectrum Principal peaks at wavenumbers 714, 787, 1205, 1220, 928, 1515 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 83, 85, 47, 87, 48, 49, 35, 82.



Quantification
Blood **GC** Column: 15% silicone fluid MS 550 (1.8 m × 6.5 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 52.5°. FID. Limit of detection, 10 mg/L [Poobalasingam 1976]. Column: 2.5% OV-225 on 80/100 mesh (1.8 m (6 ft)). Carrier gas: N_2, 50 mL/min. Temperature: 50°. ECD. Limit of detection, 0.05 mg/L [Fry *et al.* 1972].

GC-MS See [Gottzein *et al.* 2010].

Urine **GC** See Blood [Fry *et al.* 1972].
Disposition in the Body Chloroform is almost completely absorbed after oral administration, rapidly absorbed after inhalation and subject to first-pass metabolism in the liver and lungs. It is rapidly distributed throughout the body and is taken up by adipose tissue. Between 20 and 70% of a dose is exhaled unchanged in 8 h and up to ~50% is exhaled as carbon dioxide in the same period; <0.01% of a dose is excreted in the urine.
Blood Concentration

After a single oral dose of 500 mg to 2 subjects, peak blood concentrations of ~1 and 5 mg/L were attained in 1 h [Fry *et al.* 1972].

A mean chloroform concentration in arterial blood of 173 mg/L was reported in spontaneously breathing subjects during surgical anaesthesia [Poobalasingham, Payne 1978].

Toxicity The minimum lethal dose is 10 mL by ingestion and the maximum permissible atmospheric concentration is 10 ppm. Exposure to air concentrations of 100–1000 ppm for short periods may cause discomfort and dizziness; concentrations of 7000 ppm or more will produce rapid loss of consciousness.

The following postmortem tissue concentrations were reported in 7 fatalities: blood (7 cases) 10–48 mg/L (mean, 32), brain (4 cases) 50.4–156 µg/g (mean, 101), kidney (3 cases) 16–27 µg/g (mean, 20), liver (6 cases) 6–86.2 µg/g (mean, 43.4) and urine (5 cases) 0–60 mg/L (mean, 15) [Bonnichsen, Maehly 1966; Giusti, Chiarotti 1981].

A case of chloroform poisoning in a 30-year-old male was reported with a blood concentration of 60 mg/kg and lung concentration of 14 mg/kg. [Kim *et al.* 1996].

Half-life Blood, ~1.5 h.

Volume of Distribution Blood, ~2–3 L/kg.

Clearance Blood, ~20 mL/min/kg.

Dose Concentrations of 2 to 4% of the vapour have been used for induction and 1 to 2% for maintenance of anaesthesia.

Bonnichsen R, Maehly AC (1966). Poisoning by volatile compounds. II. Chlorinated aliphatic hydrocarbons. *J Forensic Sci* 11: 414–427.

Fry BJ *et al.* (1972). Pulmonary elimination of chloroform and its metabolite in man. *Arch Int Pharmacodyn Ther* 196: 98–111.

Giusti GV, Chiarotti M (1981). Double 'suicide' by chloroform in a pair of twins. *Med Sci Law* 21: 2–3.

Gottzein AK *et al.* (2010). Qualitative screening for volatile organic compounds in human blood using solid-phase microextraction and gas chromatography–mass spectrometry. *J Mass Spectrom* 45: 391–397.

Kim NY *et al.* (1996). Two fatal cases of dichloromethane or chloroform poisoning. *J Forensic Sci* 41: 527–529.

Poobalasingam N (1976). Analysis of chloroform in blood. *Br J Anaesth* 48: 953–956.

Poobalasingham N, Payne JP (1978). The uptake and elimination of chloroform in man. *Br J Anaesth* 50: 325–329.

Chloromethylaniline

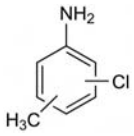
Insecticide

C_7H_8ClN = 141.6

CAS—95-81-8

IUPAC Name 2-Chloro-5-methylaniline

Synonyms Chlorotoluidine; 2-chloro-5-methylbenzeneamine; 6-chloro-*m*-toluidine.



Chemical Properties Mp 29.5°. Bp 228° to 229°. It is soluble in water. Log *P* (octanol/water), 2.27.

Mass Spectrum Principal ions at *m/z* 106, 141, 77, 143, 140, 142, 52, 79.

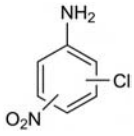
Chloronitroaniline

Insecticide

$C_6H_5ClN_2O_2$ = 172.6

CAS—41587-36-4

IUPAC Name *N*-Chloro-*N*-phenylnitramide



Chloronitrotoluene

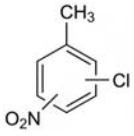
Pesticide

$C_7H_6ClNO_2$ = 171.6

CAS—25567-68-4

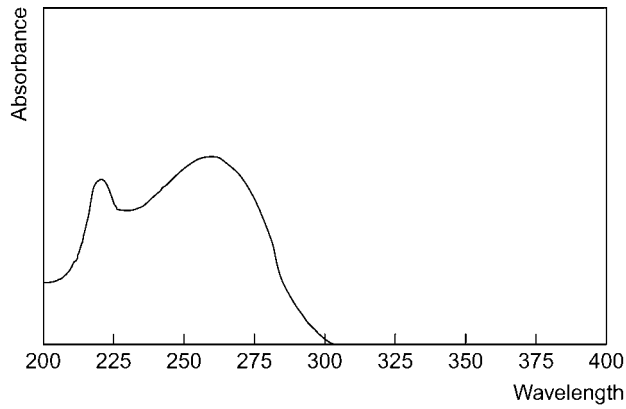
IUPAC Name [Chloro(nitro)methyl]benzene

Synonyms 1-(Chloromethyl)-4-nitrobenzene; α-chloro-*p*-nitrotoluene; *p*-nitrobenzylchloride.

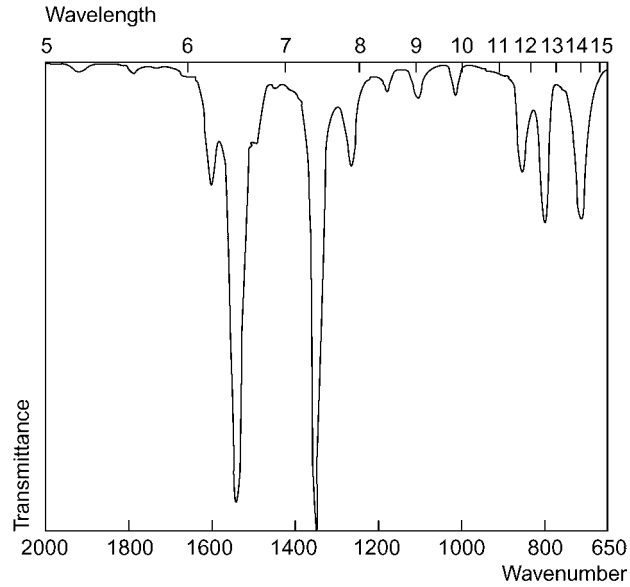


Chemical Properties A solid. Mp 71°. It is insoluble in water.

Ultraviolet Spectrum Aqueous solution—260 nm.



Infrared Spectrum Principal peaks at wavenumber 1353, 1541, 809, 719, 1263, 1609 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 136, 89, 171, 125, 63, 90, 78, 77.

m-Chlorophenylpiperazine

5-HT_{2C} Receptor Agonist, Arylpiperazine, Drug of Abuse

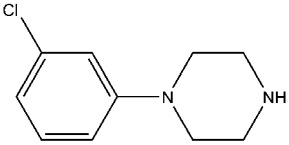
$C_{10}H_{13}ClN_2$ = 196.7

CAS—6640-24-0

IUPAC Name 1-(3-Chlorophenyl)piperazine

Synonyms Metachlorophenypiperazine; mCPP.

Street Names Arc-en-ciel; Arlequin; Duhovka; Rainbow; Regenboogies; X4.



Chemical Properties White powder. Soluble in water (7.31 g/L) [Meylan, Howard 1995]. pK_a 8.64 [Caccia 2007]. Log *P* (octanol/water), 2.11 [Caccia *et al.* 1981; Caccia 2007]. Metabolite of the antidepressants nefazodone, trazodone, etoperidone and mepiprazole.

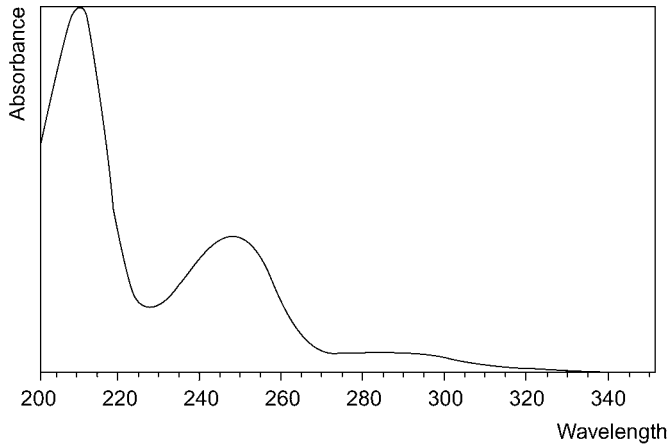
mCPP Hydrochloride

$C_{10}H_{13}ClN_2$, HCl = 233.2

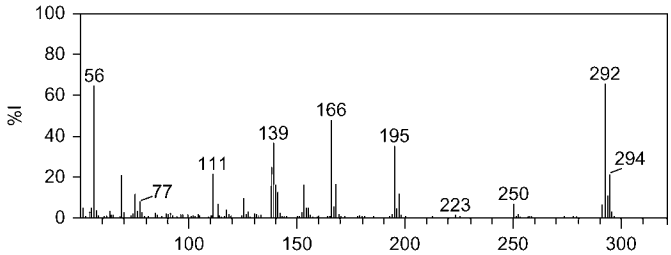
CAS—65369-76-8

Chemical Properties White powder.

Ultraviolet Spectrum Alkali—215, 250 nm [Suckow *et al* 1990].



Mass Spectrum Principal ions at *m/z* 292, 56, 166, 139, 195, 138, 111, 294 ((*N*)-trifluoroacetyl derivative) [Andriollo *et al* 1990].



Quantification

Plasma **GC-MS** Column: HP-5MS 5% phenylmethylsiloxane capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Retention times: mCPP 12.3, amfetamine 5.2 min, metamfetamine 6.5 min, ethylamfetamine 6.9 min, hydroxyamfetamine 7.8 min, *para*-methoxyamphetamine (PMA) 7.9 min, pholedrine 8.8 min, methylenedioxyamphetamine (MDA) 9.0 min, *N*-methyl-PMA (PMMA) 9.1 min, benzodioxolylbutanamine (BDB) 9.8 min, 4-methylthio-PMA (MTA) 9.9 min, benzylpiperazine 10.0 min, 1(3-trifluoromethylphenyl)piperazine 10.1 min, 3,4-methylenedioxymethamphetamine (MDMA) 10.2 min, methylenedioxyethylamphetamine (MDEA) 10.5 min, methyl-BDB (MBDB) 10.7 min, *p*-tolylpiperazine 10.9 min, 1-(4-methoxyphenyl)piperazine 12.3 min, 1-(3,4-methylenedioxybenzyl)-piperazine 13.6 min (all heptafluorobutyrate derivatives). Limit of quantification, 10 µ/L [Peters *et al.* 2003].

HPLC Column: LiChrospher 60 RP Select B (250 × 5.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer (pH 2, 36 : 64), flow rate 0.9 mL/min. DAD. Limit of quantification not reported [Staack *et al.* 2007]. Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% potassium dihydrogen phosphate (pH 2.5) : acetonitrile (77 : 23). Electrochemical detection. Limit of quantification, 5 µg/L; limit of detection, 2.5 µg/L [Ohkubo *et al.* 1995]. Column: TMS-bonded silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium phosphate monobasic (pH 3.2) : acetonitrile (82 : 18) containing 1.2 mL/L TEA and 20% heptanesulfonic acid solution, flow rate 1.8 to 2.0 mL/min. UV detection (λ = 214 nm). Retention times: mCPP ~7.0 min, *o*-tolylpiperazine ~6.0 min. Limit of detection, <3 µg/L [Suckow *et al.* 1990]. Column: TMS-bonded silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 3.0) : acetonitrile (73 : 27) containing 0.04 mol/L TEA and 0.02 mol/L heptanesulfonic acid, flow rate 1.5 mL/min. UV detection (λ = 214 nm). Retention times: mCPP 7.1 min, trazodone 12.8 min. Limit of detection, 5 µg/L [Miller, DeVane 1986].

Urine **GC-MS** Column: VF-5MS cross-linked methylsilicone capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, flow rate 1.0 mL/min. Temperature programme: 100° for 2 min, to 310° at 30°/min, for 12 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification not reported [Staack *et al.* 2007].

Other **GC** Rat Brain and Plasma. Column: 3% OV-17 on 80-100 mesh Supelcoport (2 m × 3.0 mm i.d.). Carrier gas: Ni, 35 mL/min. Temperature programme: 205°. Electrochemical detection (^{63}Ni). Retention time: 4.2 min (heptafluorobutyrate derivative). Limit of detection, 0.05 mg/L in plasma and 0.05 µg/g in brain [Caccia *et al.* 1981].

GC-MS Rat Urine. Column: HP-1 cross-linked methyl silicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV and PICI, full scan mode. Limit of detection, 50 µg/L [Staack, Maurer 2003]. Rat Brain and Plasma. Column: CP-SIL-19 CB fused silica capillary (15 m × 0.25 mm i.d.). Carrier gas: He, 0.4 bar. Temperature programme: 80° for 0.5 min to 220° at 35°/min to 240° at 8.5°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 µg/L in plasma and 12 ng/g in brain (*N*-trifluoroacetyl derivative) [Andriollo *et al.* 1990].

HPLC Human Liver Microsomes. Column: C_{18} (300 × 3.9 mm i.d.). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (35 : 65), flow rate 1.5 mL/min. UV detection (λ = 206 nm). Retention time: 3.7 min. Limit of quantification not reported [von Moltke *et al.* 1999]. Breast Milk Samples. Column: C_{18} (150 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 50 mmol/L potassium dihydrogen phosphate (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 205 nm). Retention time: 3.1 min. Limit of quantification not reported [Dodd *et al.* 1999].

Note See Trazodone and Nefazodone for further quantification methods.

Disposition in the Body In humans, metabolised to *p*-hydroxy-mCPP via CYP2D6 and subsequently conjugated. Urinary metabolites detected in rats include two hydroxy-mCPP isomers, *N*-(3-chlorophenyl)ethylenediamine, 3-chloroaniline, *N*-acetyl-3-chloroaniline, two hydroxyl-3-chloroaniline isomers, and two hydroxyl-3-chloroaniline isomers; however, hydroxylation of mCPP is the main metabolic step.

Therapeutic Concentration

Twelve healthy men (mean age 27 years) were administered either an infusion of 0.08 mg/kg mCPP (1 mL/min) or oral capsules containing 0.4 mg/kg mCPP after an overnight fast. The mean peak plasma concentration at the end of the infusion was reported as 31.4 µg/L, and for the oral administration 53.0 µg/L after 2.2 h; however, high inter-individual variations were observed [Feuchtl *et al.* 2004].

Single oral doses of mCPP (40 mg) or trazodone (100 mg) were given to 11 healthy volunteers (mean age 62.6 years). The mean plateau phase plasma concentrations of mCPP were 40.9 µg/L following mCPP administration and 14.6 µg/L following the trazodone dose [Lawlor *et al.* 1997].

Nine healthy male volunteers were administered mCPP, IV infused over 90 seconds in 0.06 and 0.08 mg/kg doses. Blood samples were collected for mCPP concentrations at 30, 45, 60, 90, 120, 150, 180 and 210 min following infusion. Mean blood levels reported for the 0.06 and 0.08 mg/kg doses were 12.6 µg/L and 16.5 µg/L, respectively [Kalus *et al.* 1992].

In a study investigating the pharmacokinetic profile of mCPP in a group of healthy volunteers, bioavailability after oral administration as well as clearance after IV administration varied ≈8-fold. This resulted in an oral AUC variation of >40-fold [Gijsman *et al.* 1998].

Toxicity Variability in CYP2D6 phenotype may lead to drug–drug interactions since it is involved in the metabolism of many other drugs, and may also explain the wide variation in the pharmacokinetics of mCPP. An intake of mCPP is differentiated from an intake of its precursor drugs by screening for unique urinary metabolites of the parent compound.

A 29-year-old woman (CYP2D6 intermediate metaboliser) admitted consuming cocaine intranasally, alcohol and paracetamol (acetaminophen). Blood and urine specimens were collected ~5.5 h after the last consumption of alcohol and cocaine. The blood alcohol concentration was found to be 0.87 g/L and plasma concentrations of benzoylecgonine (476 µg/L), ecgoninemethylester (56 µg/L) and ecgoninethylester (45 µg/L) confirmed the consumption of cocaine. However, mCPP was also detected in plasma at a relatively high concentration of ~16 µg/L, together with diltiazem (a known adulterant for cocaine). It is believed the cocaine consumed was adulterated with both diltiazem and mCPP. The high mCPP plasma concentration measured may have been the result of the subject's CYP2D6 phenotype and also the fact that cocaine is a CYP2D6 inhibitor [Staack *et al.* 2007].

A 29-year-old woman admitted consuming 3 multicoloured Arlequin tablets over a 5 h period. Thirty minutes after consuming the last tablet, she suffered from impaired vision, anxiety, and felt like she was overheating. Plasma and urine mCPP concentrations were reported as 3.2 mg/L and 2.3 mg/L, respectively. The patient was discharged 8 h later without apparent sequelae. In a clinical setting, a 28-year-old man treated for 13 years for obsessive–compulsive disorder, developed an adverse reaction 45 min after receiving an oral dose of 0.5 mg/kg mCPP. His blood mCPP concentration 90 min post-ingestion was only 124 µg/L. These 2 cases further highlight the inter-individual variability in the pharmacokinetics of mCPP [Lecompte *et al.* 2006].

Bioavailability Ranging from 10 to more than 100%.

Half-life Approximately 4.5 h (range 2.4 h to 6.8 h).

Volume of Distribution Approximately 2.65 L/kg (range 0.86 to 4.07).

Clearance Approximately 49.6 mL/h (range 11 mL/h to 92 mL/h).

Protein Binding Ranging from 66 to 74%.

Note For a review of the disposition and metabolism of mCPP and other 1-aryl-piperazines, see Caccia [2007]. For a study of the metabolism of mCPP in rats, see Staack and Maurer [2003] and Mayol *et al.* [1994].

Dose Used as a model reference compound in neurochemical studies of serotonin 5HT receptors (usual oral dose up to 0.75 mg/kg); however, because of the high variability in pharmacokinetics observed in humans, its applicability to such studies is now in question. Illicit mCPP is normally consumed orally with tablets containing anything from 8 to 80 mg. mCPP is also sometimes consumed in powder form and has been found in cocaine powder samples and is suspected to be a cutting agent and/or psychotropic enhancement agent.

Andriollo O *et al.* (1990). Assay of *m*-chlorophenylpiperazine in plasma and brain of rat by capillary gas chromatography–mass spectrometry. *J Chromatogr* 533: 215–223.

Caccia S (2007). *N*-Dealkylation of arylpiperazine derivatives: disposition and metabolism of the 1-aryl-piperazines formed. *Curr Drug Metab* 8: 612–622.

Caccia S *et al.* (1981). Determination of plasma and brain concentrations of trazodone and its metabolite, 1-*m*-chlorophenylpiperazine, by gas-liquid chromatography. *J Chromatogr* 210: 311–318.
Dodd S *et al.* (1999). Determination of nefazodone and its pharmacologically active metabolites in human blood plasma and breast milk by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 730: 249–255.
Feuchtl A *et al.* (2004). Pharmacokinetics of *m*-chlorophenylpiperazine after intravenous and oral administration in healthy male volunteers: implication for the pharmacodynamic profile. *Pharmacopsychiatry* 37: 180–188.
Gijsman HJ *et al.* (1998). Pharmacokinetic and pharmacodynamic profile of oral and intravenous *meta*-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289–295.
Kalus O *et al.* (1992). A dose–response study of intravenous *m*-chlorophenylpiperazine in normal subjects. *Psychopharmacology (Berl)* 106: 388–390.
Lawlor BA *et al.* (1997). Plasma levels of *m*-chlorophenylpiperazine following single oral dose administration of *m*-chlorophenylpiperazine and trazodone in human volunteers. *Biol Psychiatry* 41: 756–757.
Lecompte Y *et al.* (2006). Metachlorophenylpiperazine (mCPP): a new designer drug. *Therapie* 61: 523–530.
Mayol RF *et al.* (1994). Isolation and identification of the major urinary metabolite of *m*-chlorophenylpiperazine in the rat. *Drug Metab Dispos* 22: 171–174.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Miller RL, DeVane CL (1986). Analysis of trazodone and *m*-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography. *J Chromatogr* 374: 388–393.
Ohkubo T *et al.* (1995). High-performance liquid chromatographic determination of trazodone and 1-*m*-chlorophenylpiperazine with ultraviolet and electrochemical detector. *J Pharm Pharmacol* 47: 340–344.
Peters FT *et al.* (2003). Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom* 38: 659–676.
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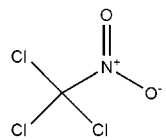
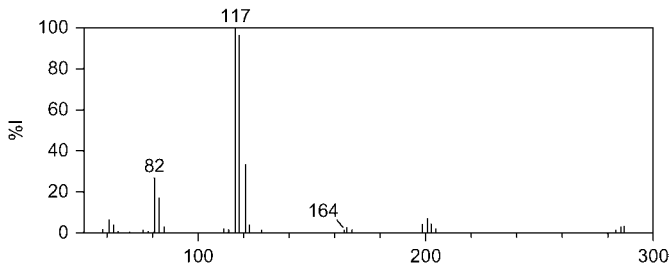
Chloropicrin

Fumigant, Nematocide, War Gas
CCl_3NO_2 = 164.4
CAS—76-06-2

IUPAC Name Trichloro(nitro)methane
Synonyms Acquinite; nitrochloroform; PS.
Proprietary Names *Larvacide 100*; *Picfume*.



Chemical Properties A colourless oily liquid, intense odour. Mp −69°. Bp 112°. Practically insoluble in water; miscible with benzene, absolute alcohol, and carbon disulfide; soluble in ether.
Mass Spectrum Principal peaks at *m*/*z* 117, 119, 121, 82, 164 [Gonmori *et al.* 1987].



Quantification

Lung **GC-MS** Column: 2% Silicone OV-17 on Uniport HP 80/100 mesh. Temperature: 66°. EI ionisation at 70 eV. Limit of detection, ~1.5 ng/g [Gonmori *et al.* 1987].
Other **GC-MS** Mouse Samples. Column: DB-5 (30 m × 0.25 mm i.d.). Temperature programme: 40° to 250° over 21 min. Limit of detection not reported [Sparks *et al.* 1997].

Disposition in the Body

Toxicity Potential symptoms of overexposure are irritation of eyes, skin, respiratory system; lacrimation; cough and pulmonary oedema; nausea and vomiting. See Centers for Disease Control and Prevention [2010].

A 37-year-old woman was exposed to chloropicrin inside a fumigation tent. Postmortem showed acute tracheitis with pulmonary congestion and oedema [Schneir *et al.* 2008].
An 18-year-old girl and a 21-year-old boy were sprayed with chloropicrin. The girl died of pulmonary oedema after 3 h and the boy recovered after 30 days in hospital [Gonmori *et al.* 1987].

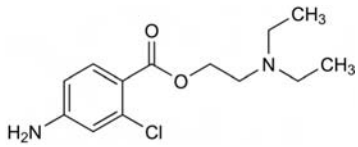
Note For reports of acute exposure, see Goldman *et al.* [1987], Centers for Disease Control and Prevention [2004a, 2004b].
Use Disinfecting cereals and grains; in synthesis, especially in the manufacture of methyl violet; as a fumigant, soil insecticide and war gas.

Centers for Disease Control and Prevention Brief report: exposure to tear gas from a theft-deterrent device on a safe: Wisconsin, December 2003. *MMWR Morb Mortal Wkly Rep* 53: 176–177.
Centers for Disease Control and Prevention Illness associated with drift of chloropicrin soil fumigant into a residential area: Kern County, California, 2003. *MMWR Morb Mortal Wkly Rep* 53: 740–742.
Centers for Disease Control and Prevention (2010). *NIOSH Pocket Guide to Chemical Hazards.* [Publication Number 2005-149.] Atlanta, GA: Centers for Disease Control and Prevention: 66.
Goldman LR *et al.* (1987). Acute symptoms in persons residing near a field treated with the soil fumigants methyl bromide and chloropicrin. *West J Med* 147: 95–98.
Gonmori K *et al.* (1987). A case of homicidal intoxication by chloropicrin. *Am J Forensic Med Pathol* 8: 135–138.
Schneir A *et al.* (2008). Systemic fluoride poisoning and death from inhalational exposure to sulfuryl fluoride. *Clin Toxicol (Phila)* 46: 850–854.
Sparks SE *et al.* (1997). Chloropicrin: reactions with biological thiols and metabolism in mice. *Chem Res Toxicol* 10: 1001–1007.

Chloroprocaine

Anaesthetic (Local)
$C_{13}H_{19}ClN_2O_2$ = 270.8
CAS—133-16-4

IUPAC Name 2-Diethylaminoethyl 4-amino-2-chlorobenzoate
Synonym 4-Amino-2-chlorobenzoic acid 2-(diethylamino)ethyl ester

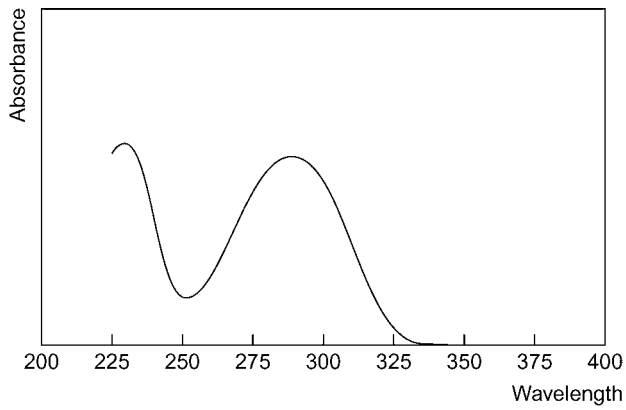


Chemical Properties pK_a 8.7. Log *P* (octanol/water), 2.9.

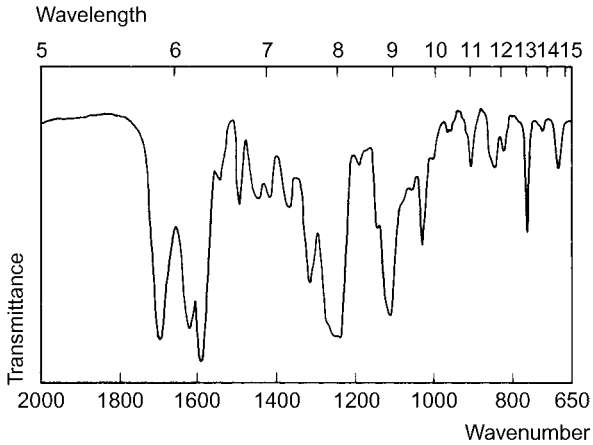
Chloroprocaine Hydrochloride

$C_{13}H_{19}ClN_2O_2$, HCl = 307.2
CAS—3858-89-7
Proprietary Name *Nesacaine*
Chemical Properties A white crystalline powder. Mp 176° to 178°. Soluble 1 in 20 of water and 1 in 100 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

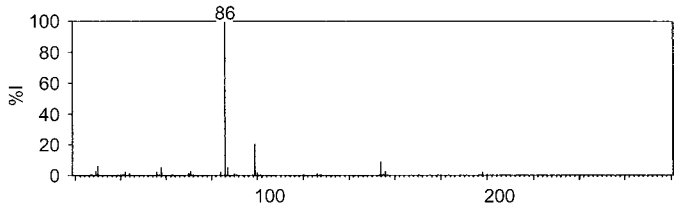
Colour Test Diazotisation—red.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.05; system TC—R_f 0.23; system TL—R_f 0.37; system TAJ—R_f 0.09; system TAK—R_f 0.00; system TAL—R_f 0.32 (acidified iodoplatinate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—RI 2241.
High Performance Liquid Chromatography System HQ—*k* 0.24; system HY—RI 250.
Ultraviolet Spectrum Aqueous acid—230 (A^{1}_{1}=330b), 288 nm (A^{1}_{1}=311b).



Infrared Spectrum Principal peaks at wavenumbers 1595, 1698, 1240, 1625, 1111, 1315 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 154, 30, 87, 58, 29, 156.



Quantification

Blood **TLC** For quantification of 4-amino-2-chlorobenzoic acid, see O'Brien *et al.* [1979].

HPLC Limit of detection, 5 µg/L for diethylaminoethanol [Meng *et al.* 1999].

Plasma **TLC** See Blood O'Brien *et al.* [1979].

GC FID. Limit of detection, 10 µg/L [O'Brien *et al.* 1979].

GC–MS Limit of detection, <2 µg/L [Kuhnert *et al.* 1981].

Serum **HPLC** See Blood [Meng *et al.* 1999].

Urine **TLC** See Blood O'Brien *et al.* [1979].

Disposition in the Body Rapidly hydrolysed in the plasma to 4-amino-2-chlorobenzoic acid and 2-diethylaminoethanol. About 50% of an IV dose is excreted in the urine in 90 min, mainly as an unidentified conjugate (possibly the glycine conjugate) of 4-amino-2-chlorobenzoic acid.

Therapeutic Concentration

After IV infusion of 250 mg over a period of 30 min to 3 subjects, peak plasma concentrations of 3.5 to 4.3 mg/L of 4-amino-2-chlorobenzoic acid were attained at the end of the infusion; chloroprocaine was not detectable in the plasma [O'Brien *et al.* 1979].

Toxicity The maximum safe IV or topical dose is 750 mg.

Dose Chloroprocaine hydrochloride is injected as a 0.5 to 3% solution.

Kuhnert BR *et al.* (1981). Measurement of 2-chloroprocaine in plasma by selected ion monitoring. *J Chromatogr* 224: 488–491.

Meng QC *et al.* (1999). High-performance liquid chromatographic analysis of the 2-chloroprocaine metabolite, diethylaminoethanol, in blood and serum. *Reg Anesth Pain Med* 24(3): 242–245.

O'Brien JE *et al.* (1979). Metabolism and measurement of chloroprocaine, an ester-type local anesthetic. *J.Pharm Sci* 68: 75–78.

Chloropyramine

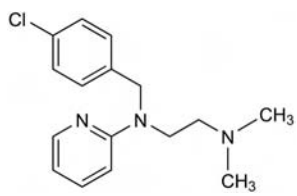
Antihistamine

$C_{16}H_{20}ClN_3$ = 289.8

CAS—59-32-5

IUPAC Name *N*-[(4-Chlorophenyl)methyl]-*N*′,*N*′-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine

Synonyms Chlortripelennamine; halopyramine.



Chemical Properties A yellow oil. Log *P* (octanol/water), 3.4.

Chloropyramine Hydrochloride

$C_{16}H_{20}ClN_3$, HCl = 326.3

CAS—6170-42-9

Proprietary Names *Alegan S*; *Avapena*; *Suprastin*; *Synopen*; *Synpen*.

Chemical Properties A white powder. Mp 172°. Freely soluble in water, ethanol, and chloroform; practically insoluble in ether.

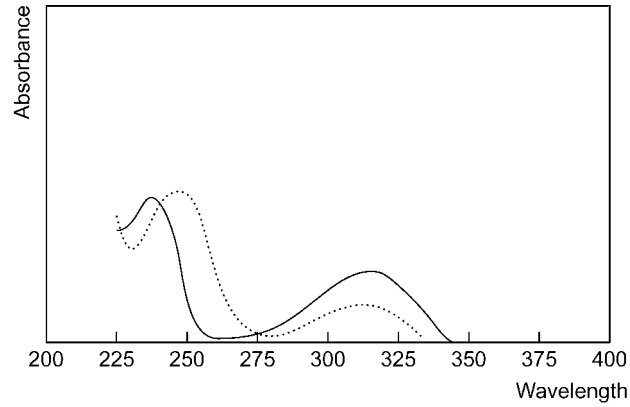
Colour Test Cyanogen bromide—yellow.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.41; system TC—R_f 0.28; system TE—R_f 0.63; system TL—R_f 0.17; system TAD—R_f 0.00; system TAE—R_f 0.22 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—chloropyramine RI 2190, M (N-desalkyl-) RI 1900, M (nor-) RI 2210, M (OH-)-AC RI 2440, M (N-desalkyl-)-AC RI 2160, M (nor-)-AC RI 2470, M (bis-nor-)-AC RI 2420.

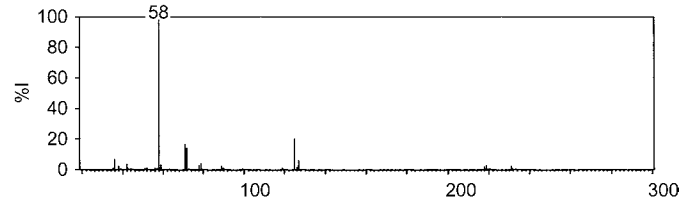
High Performance Liquid Chromatography System HA—*k* 4.2.

Ultraviolet Spectrum Aqueous acid—239 (A_1^1=515a), 315 nm; aqueous alkali—248, 313 nm.



Infrared Spectrum Principal peaks at wavenumbers 1494, 1598, 769, 1562, 1010, 1098 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 125, 71, 72, 36, 127, 79, 219.



Dose Chloropyramine hydrochloride has been given in doses of up to 150 mg daily.

Chloropyrilene

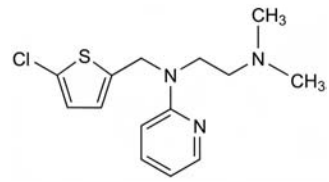
Antihistamine

$C_{14}H_{18}ClN_3S$ = 295.8

CAS—148-65-2

IUPAC Name N′-[(5-Chlorothiophen-2-yl)methyl]-N,N-dimethyl-N′-pyridin-2-ylethane-1, 2-diamine

Synonyms Chloromethapyrilene; chlorothen; *N*-[(5-chloro-2-thienyl)-methyl]-*N*′,*N*′-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine; chlorpyrilen; hista-chlorylene.



Chemical Properties A liquid. pK_a 8.4 (25°). Log *P* (octanol/water), 3.2.

Chloropyrilene Citrate

$C_{14}H_{18}ClN_3S$, $C_6H_8O_7$ = 488.0

CAS—148-64-1

Proprietary Names *Panta*; *Tagathen*.

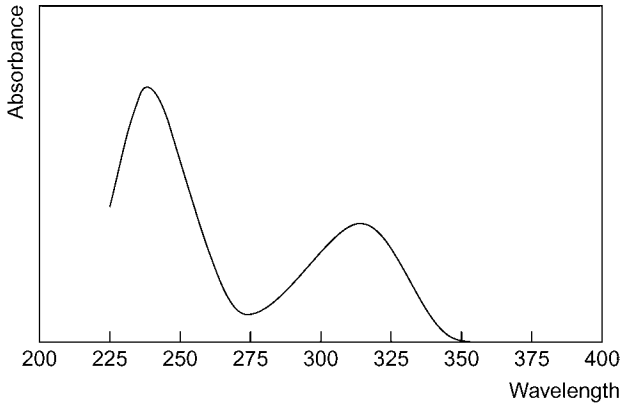
Chemical Properties A white crystalline powder. Mp 112° to 116°; on further heating it gradually solidifies and 125° to 140°, with decomposition. Soluble 1 in 35 of water and 1 in about 65 of alcohol; practically insoluble in benzene, chloroform, and ether.

Colour Tests Mandelin's test—violet→orange; Marquis test—violet.

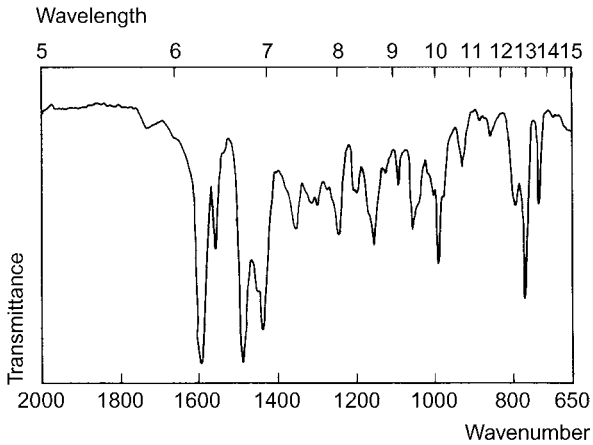
Thin-layer Chromatography System TA—R_f 0.53 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2133.

High Performance Liquid Chromatography System HA—*k* 4.0.
Ultraviolet Spectrum Aqueous acid—237 (A^1_1=520c), 312 nm; aqueous alkali—243 (A^1_1=676b), 308 nm (A^1_1=148b).



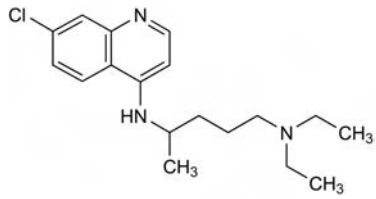
Infrared Spectrum Principal peaks at wavenumbers 1595, 767, 990, 1562, 1149, 1234 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 131, 72, 71, 79, 42, 30, 78.
Dose Up to 150 mg of chloropyrilene citrate daily.

Chloroquine

Antimalarial
$C_{18}H_{26}ClN_3$ = 319.9
CAS—54-05-7
IUPAC Name N^4-(7-Chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine
Synonyms Cloroquina; RP-3377.
Proprietary Names *Aralen*; *Artrichin*; *Bemaphate*; *Capquin*; *Nivaquine B*; *Paluken*; *Resoquine*; *Reumachlor*; *Sanoquin*.



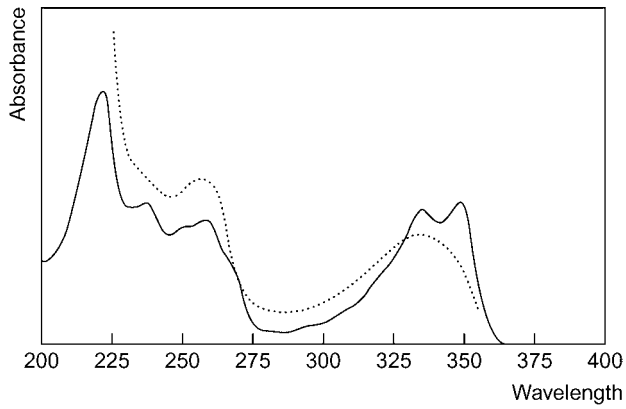
Chemical Properties A white or slightly yellow crystalline powder. Mp 87° to 92°. Very slightly soluble in water; soluble in chloroform and ether. pK_a 8.4, 10.8 (20°). Log *P* (octanol/water), 4.63. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Chloroquine Phosphate
$C_{18}H_{26}ClN_3,2H_3PO_4$ = 515.9
CAS—50-63-5
Synonyms Chingaminum; chlorochinum diphosphoricum; quingamine; SN-7618.
Proprietary Names *Anoclor*; *Aralen* (tablets); *Arechin*; *Avloclor*; *Chlorquin*; *Diroquine*; *Genocin*; *Heliopar*; *Imagon*; *Klorokin*; *Malaquin*; *Malarex*; *Malarivon*; *P-Roquine*; *Palux*; *Resochin(e)*; *Tresochin*; *Weimerquin*. It is an ingredient of *Aralis*; *Clopirim*; *Savarine*.
Chemical Properties A white powder, discolouring on exposure to light. There are 2 polymorphic forms, with Mp of 193° to 195° and at 215° to 218°. Freely soluble in water; very slightly soluble or practically insoluble in alcohol, in chloroform and in ether; very slightly soluble in methanol.

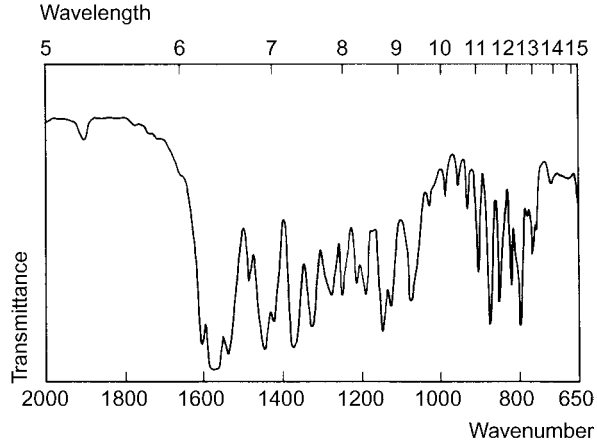
Chloroquine Hydrochloride
$C_{18}H_{26}ClN_3$, 2HCl = 392.8
CAS—3545-67-3

Chloroquine Sulfate
$C_{18}H_{26}ClN_3,H_2SO_4,H_2O$ = 436.0
CAS—132-73-0 (anhydrous)
Synonyms Chloroquini sulfas; chloroquine sulphate; RP-3377; Sulfato de cloroquina.
Proprietary Names *Daramal*; *Nivaquine*; *Plasmoquine*. It is an ingredient of *Daramal-Paludrine*.
Chemical Properties A white or almost white crystalline powder. Mp 205° to 210°. Requires protection from light. Freely soluble in water and methanol; very slightly soluble in alcohol; practically insoluble in ether.

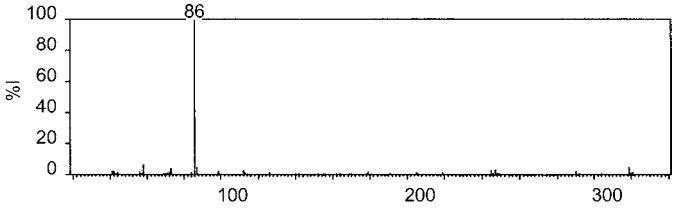
Colour Test Liebermann's reagent (100°)—orange.
Thin-layer Chromatography System TA—R_f 0.38; system TB—R_f 0.14; system TC—R_f 0.04; system TE—R_f 0.46; system TL—R_f 0.02; system TAD—R_f 0.00; system TAE—R_f 0.04; system TAF—R_f 0.14; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.04 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—RI 2605, M (desethyl-) RI 2555, M (desethyl-)-AC RI 3010; system GF—RI 3245.
High Performance Liquid Chromatography System HA—*k* 15.2 (tailing peak); system HX—RI 282; system HY—RI 246; system HZ—RT 2.1 min; system HAA—RT 5.4 min; system HAX—RT 12.7 min; system HAY—RT 3.6 min.
Ultraviolet Spectrum Aqueous acid—257, 329 nm (A^1_1= 600b), 343 nm (A^1_1= 625b); aqueous alkali—254 nm (A^1_1= 555b), 330 nm.



Infrared Spectrum Principal peaks at wave numbers 1573, 1538, 1612, 1155, 800, 870 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 58, 319, 87, 73, 247, 245, 112.



Quantification

Blood **GC** NSD. Limit of detection, 3 μg/L [Bergqvist, Eckerbom 1984].

HPLC Column: reversed phase. UV detection [Bell *et al.* 2007]. Column: XTerra. UV detection (λ= 254 nm). Limit of quantification, ~50 μg/L [Lejeune *et al.* 2007]. Column: XTerra RP18. Mobile phase: 20 μmol/L borate buffer (pH 9.0) : acetonitrile (60 : 40), flow rate 1 mL/min [Deng *et al.* 2006]. Column: C_{18}. Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 3) : perchloric acid (250: 747.5 : 2.5). Limit of quantification, 10 nmol/L [Minzi *et al.* 2003]. Fluorescence detection (λ_{ex}= 215 nm, no emission filter). Limit of quantification, 0.005 mg/L [Croes *et al.* 1994]. See also Houze *et al.* [1992].

Plasma **GC** See Blood [Bergqvist, Eckerbom 1984].

HPLC UV detection. Limit of detection, 20 μg/L [Yakasai 2006]. See Blood [Minzi *et al.* 2003]. See Dua *et al.* [1999] and Walker, Ademowo [1996]. Column: C_8 Lichrospher 60 RP select B. Mobile phase: water: acetonitrile : methanol (78 : 28 : 4) : 0.5 mol/L ammonium formate : 0.075 mol/L perchloric acid. UV detection (λ= 254 nm). Limit of quantification, 6 μg/L [Chaulet *et al.* 1994]. See Blood [Houze *et al.* 1992]. See also Alvan *et al.* [1982], Brown *et al.* [1982], Chaulet *et al.* [1993] and Zhong *et al.* [1993].

LC-MS Column: reversed phase. Mobile phase: 20 μmol/L ammonium formate : acetonitrile (both containing 0.5% formic acid). Limit of quantification, 0.15–3 μg/L [Hodel *et al.* 2009]. Column: Chirobiotic V. Mobile phase: methanol : acetonitrile : glacial acetic acid : diethylamine (90 : 10 : 0.5 : 0.5). ESI, MRM acquisition mode, positive ion mode [dos Santos Magalhaes, Sueli 2008].

Serum **HPLC** LIFD. Limit of detection, 1.9 fmol [Ibrahim *et al.* 2007]. Column: MZ Kromasil C_{18} (250 × 4 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : 0.1 mol/L ammonium acetate, (45 : 15 : 40), flow rate 1 mL/min. Fluorescence detection (λ_{ex}= 325 nm, λ_{em}= 375 nm). Limit of detection, 500 μ/L [Samanidou *et al.* 2005]. DAD [Volin 1995]. See Blood [Croes *et al.* 1994].

Urine **GC** See Blood [Bergqvist, Eckerbom 1984].

HPLC See Serum [Samanidou *et al.* 2005]. See Blood [Houze *et al.* 1992; Minzi *et al.* 2003]. See Plasma [Alvan *et al.* 1982; Walker, Ademowo 1996].

Liver **HPLC** Column: C_1. Mobile phase: methanol : water (70 : 30, v/v) : TEA (0.1% v/v). detection (λ_{ex}= 250 nm, λ_{em}= 380 nm). Limit of quantification, 78 nmol/L [Ducharme, Farinotti 1997].

Note For spectrofluometry for the quantification of chloroquine, see Adelusi, Salako [1980].

Disposition in the Body Chloroquine is rapidly absorbed after oral administration and widely distributed into body tissues. It accumulates in high concentrations in the kidneys, liver, lungs and spleen, and is strongly bound in melanin-containing cells (eyes and skin). It also crosses the placenta. Metabolic reactions include *N*-dealkylation and deamination followed by conjugation, possibly with glucuronic acid, of the carboxylic acid metabolites; the metabolites include monodesethyl- and didesethylchloroquine, 4-(7-chloroquinol-4-ylamino)pentan-1-ol, and 4-(7-chloroquinol-4-ylamino)pentanoic acid and its conjugate. Chloroquine is excreted slowly and may persist in tissues for prolonged periods; ~55% is excreted in the urine and approx. 10% is eliminated in the faeces in 90 days following therapy with 310 mg chloroquine phosphate daily for 14 days. The urinary excretion of unchanged drug is dependent upon urinary pH and larger amounts are excreted in acid urine than in alkaline urine; of the material excreted in the urine, ~70% is unchanged, 23% is monodesethylchloroquine, 1–2% is didesethylchloroquine and an unidentified metabolite, and 1–2% is conjugated carboxylic acid metabolites. Chloroquine and its monodesethyl metabolite are both distributed into breast milk.

Therapeutic Concentration In plasma, usually in the range 0.02–0.2 mg/L.

Following a single oral dose of 300 mg chloroquine to 11 subjects, peak plasma concentrations of chloroquine of 0.056–0.10 mg/L (mean, 0.08) and of monodesethylchloroquine of 0.01–0.02 mg/L were attained in 1–6 h [Gustafsson *et al.* 1983].

Healthy subjects received 3 different chloroquine regimens for 3 weeks: once weekly 300 mg (5 subjects), twice weekly 200 mg (4 subjects) and once daily 50 mg (5 subjects). All regimens produced peak and trough chloroquine concentrations above 16 μg/L after the first week, sufficient to suppress chloroquine-sensitive *Plasmodium falciparum* strains [Wetsteyn *et al.* 1995].

In 29 patients undergoing long-term chloroquine treatment for rheumatoid arthritis, steady-state blood concentrations varied considerably (0.0366–3.895 mg/L); concentrations of the 2 main metabolites also varied (bisdesethylchloroquine 0–0.267 mg/L, desethylchloroquine 0.0247–1.506 mg/L) [Augustijns *et al.* 1992].

Toxicity Doses as low as 1 g have caused deaths in children, and fatalities have occurred in adults after the ingestion of 3 to 44 g. Plasma concentrations greater than 0.6 mg/L may produce toxic effects and concentrations greater than 3 mg/L may be fatal.

Plasma concentrations in a 52-year-old woman who ingested 10 g chloroquine in a suicide attempt were 13.1 mg/L 3 h after ingestion and fell to 0.5 mg/L after 335 h. The chloroquine concentration in whole blood was 18.4 mg/L at 3 h and rose to a peak of 28.4 mg/L at 6–6.5 h after ingestion and then fell to 3.1 mg/L at 335 h. Maximum plasma and whole blood concentrations of desethylchloroquine were 1.48 mg/L and 3.7 mg/L, at 3 and 6 h, respectively [Boereboom *et al.* 2000].

A 32-year-old man died 3 days after ingesting an unknown quantitiy of chloroquine in a suicide attempt, despite receiving treatment. The chloroquine concentration on admittance to hospital was 6.5 mg/L in serum and 683.0 mg/L in urine. Postmortem concentrations were as follows: peripheral blood 9.2 mg/L, heart blood 26.4 mg/L, vitreous humour 5.3 mg/L, stomach content 2.7 mg/L and bile 18.2 mg/L [Keller *et al.* 1998].

In a 27-year-old man whose death was attributed to heart failure caused by chloroquine overdosage, the following tissue concentrations were reported: femoral blood 16.71 mg/L, urine 516.0 mg/L, bile 8410 mg/L, stomach contents 82 020 mg/L, duodenal contents 7550 mg/L, tracheal foam 50 370 mg/L, left lung 1170 μg/g, right lung 1270 μg/g, left kidney 1690 μg/g, right kidney 1690 μg/g, heart 520 μg/g, liver 1110 μg/g, pancreas 5510 μg/g, spleen 1000 μg/g, cerebral hemispheres 100 μg/g and cerebellum 80 μg/g; chloroquine was not detected in hair. The blood ethanol concentration was 350 mg/L, which could not have enhanced the effect of chloroquine [Kintz *et al.* 1988].

In 2 deaths from chloroquine overdosage in which ~3 g and 10 g had been ingested, the postmortem concentrations were blood 16 and 12.4 mg/L, kidney 70 and 300 μg/g, liver 175 and 344 μg/g, liver blood 90 and 44 mg/L, lung 38 and 98 μg/g, urine 20 and 68.4 mg/L; in the second of these cases the antemortem blood concentration was reported to be 8.6 mg/L [Robinson *et al.* 1970].

A 26-year-old woman died after ingesting an unknown quantity of chloroquine, possibly to induce abortion; postmortem concentrations were blood 4.2 mg/L, brain 3.8 μg/g, kidney 32.9 μg/g and liver 71 μg/g; alcohol was also present [Noirfalise 1978].

Bioavailability ~80–90%.

Half-life Plasma half-life, ~25–60 days.

Volume of Distribution Reported as 116–285 L/kg.

Clearance Plasma, ~3.7 mL/min/kg (after IV administration).

Distribution in Blood Plasma : whole blood ratio, ~0.3.

Protein Binding ~50–70%.

Note For reviews of the pharmacokinetics of chloroquine, see Ducharme, Farinotti [1996] and Krishna, White [1996].

Dose For an acute attack, the equivalent of 1.5 g chloroquine base over 3 days (900 mg on the first day); for prophylaxis, 300 mg once every 7 days.

Chloroquine base 300 mg is approx. equivalent to chloroquine phosphate 500 mg or chloroquine sulfate 400 mg; chloroquine base 40 mg is approx. equivalent to chloroquine hydrochloride 50 mg.

Adelusi SA, Salako LA (1980). Improved fluorimetric assay of chloroquine in biological samples. *J Pharm Pharmacol* 32: 711–712.

Alvan G *et al.* (1982). Determination of chloroquine and its desethyl metabolite in plasma, red blood cells and urine by liquid chromatography. *J Chromatogr* 229: 241–247.

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Bergqvist Y, Eckerbom S (1984). An improved gas chromatographic method for the simultaneous determination of chloroquine and two metabolites using capillary columns. *J Chromatogr* 306: 147–153.

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Brown ND *et al.* (1982). Determination of chloroquine and its de-ethylated metabolites in human plasma by ion-pair high-performance liquid chromatography. *J Chromatogr* 229: 248–254.

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Chaulet JF *et al.* (1993). Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography. *J Chromatogr* 613: 303–310.

Croes K *et al.* (1994). Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood. *J Anal Toxicol* 18: 255–260.

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Deng H *et al.* (2006). Sensitive fluorescence HPLC assay for AQ-13, a candidate aminoquinoline antimalarial, that also detects chloroquine and *N*-dealkylated metabolites. *J Chromatogr B Analyt Technol Biomed Life Sci* 833: 122–128.

dosSantos Magalhaes I, Sueli BP (2008). Enantioselective determination of chloroquine and its *N*-dealkylated metabolites in plasma using liquid-phase microextraction and LC-MS. *J Sep Sci* 31: 3106–3116.

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Ducharme J, Farinotti R (1997). Rapid and simple method to determine chloroquine and its desethylated metabolites in human microsomes by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 698: 243–250.

Gustafsson LL *et al.* (1983). Disposition of chloroquine in man after single intravenous and oral doses. *Br J Clin Pharmacol* 15: 471–479.

Hodel EM *et al.* (2009). A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 867–886.

Houze P *et al.* (1992). Simultaneous determination of chloroquine and its three metabolites in human plasma, whole blood and urine by ion-pair high-performance liquid chromatography. *J Chromatogr* 574: 305–312.

Ibrahim H *et al.* (2007). Very small injected samples to study chloroquine and quinine in human serum using capillary-LC and native fluorescence. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 481–487.

Keller T *et al.* (1998). Fatal chloroquine intoxication. *Forensic Sci Int* 96: 21–28.

Kintz P *et al.* (1988). Fatal chloroquine self-poisoning. *Hum Toxicol* 7: 541–543.

Krishna S, White NJ (1996). Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. *Clin Pharmacokinet* 30: 263–299.

Lejeune D *et al.* (2007). Simultaneous determination of monodesethylchloroquine, chloroquine, cycloguanil and proguanil on dried blood spots by reverse-phase liquid chromatography. *J Pharm Biomed Anal* 43: 1106–1115.

Minzi OM *et al.* (2003). High-performance liquid chromatographic method for determination of amodiaquine, chloroquine and their monodesethyl metabolites in biological samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 783: 473–480.
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Robinson AE *et al.* (1970). The distribution of chloroquine in man after fatal poisoning. *J Pharm Pharmacol* 22: 700–703.
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Wetsteyn JC *et al.* (1995). The pharmacokinetics of three multiple dose regimens of chloroquine: implications for malaria chemoprophylaxis. *Br J Clin Pharmacol* 39: 696–699.
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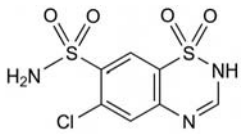
Chlorothiazide

Diuretic
$C_7H_6ClN_3O_4S_2$ = 295.7
CAS—58-94-6

IUPAC Name 6-Chloro-1,1-dioxo-4*H*-1λ^6,2,4-benzothiadiazine-7-sulfonamide
Synonym 6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; clorotiazida.
Proprietary Names *Azide*; *Chlotride*; *Diubram*; *Diuret*; *Diuril*; *Diurilix*; *Diurone*; *Saluric*. It is an ingredient of *Aldoclor* and *Diupres*.

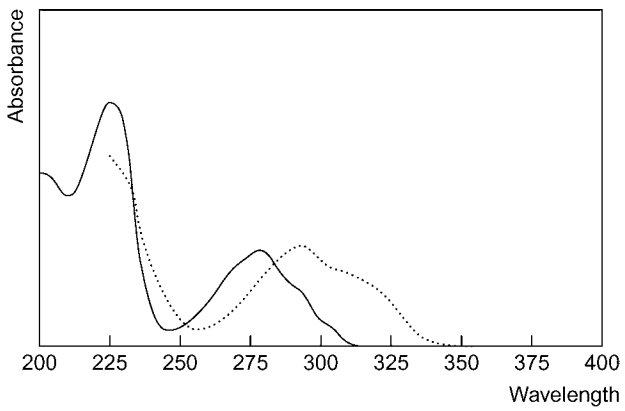


Chemical Properties A white crystalline powder. Mp about 340°, with decomposition. Very slightly soluble in water; practically insoluble in benzene, chloroform, and ether; soluble 1 in 650 of ethanol and 1 in 100 of acetone; freely soluble in dimethylformamide and dimethyl sulfoxide. pK_a 6.7, 9.5 (20°). Log *P* (ether/pH 7.4), −1.9.

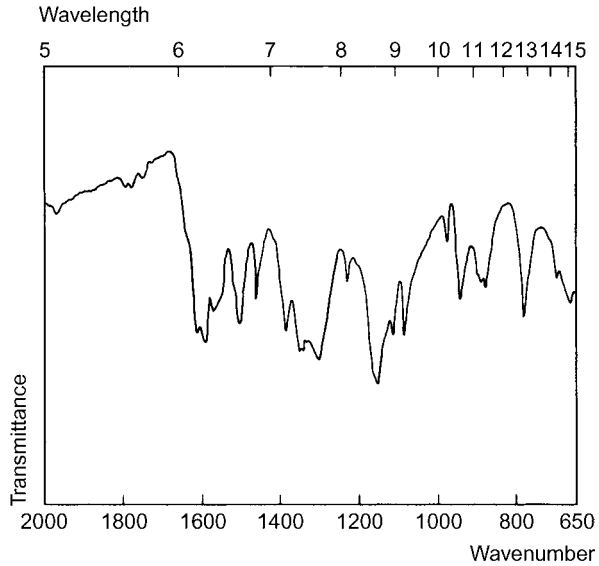
Chlorothiazide Sodium

$C_7H_5ClN_3NaO_4S_2$ = 317.7
CAS—7085-44-1
Synonym Sodium chlorothiazide
Proprietary Name *Diuril* (injection)
Chemical Properties A crystalline solid. Soluble in water.

Colour Tests Koppanyi–Zwikker test—blue; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.02; system TE—R_f 0.02; system TF—R_f 0.16; system TAD—R_f 0.11; system TAJ—R_f 0.11; system TAK—R_f 0.00; system TAL—R_f 0.41; system TAM—R_f 0.13 (mercuric chloride–diphenylcarbazone reagent, positive).
Gas Chromatography System GA—RI 1720; system GX—chlorothiazide-Me_3 retention time 6.6 min; system GY—chlorothiazide-Me_3 retention time 4.3 min.
High Performance Liquid Chromatography System HN—*k* 0.54; system HY—RI 239; system HZ—retention time 2.1 min.
Ultraviolet Spectrum Aqueous acid—278 nm (A^1_1=400a); aqueous alkali—292 nm (A^1_1=430a).



Infrared Spectrum Principal peaks at wavenumbers 1157, 1305, 1595, 1090, 1123, 1620 cm^{-1} (KBr disk).



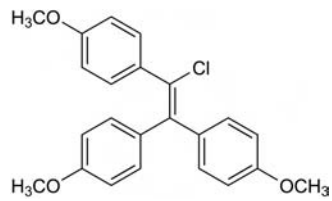
Quantification
Plasma **HPLC** UV detection. Limit of detection, 0.01 mg/L [de Vries, Voss 1993]. UV detection. Limit of detection, 10 µg/L, chlorothiazide or hydrochlorothiazide [Barbhaiya *et al.* 1981].
Serum **HPLC** Electrochemical detection. Limit of quantification, 5 µg/L [Richter *et al.* 1996].
Urine **HPLC** UV detection. Limit of detection, 1 mg/L [Farthing *et al.* 1998]. UV detection. Limit of detection, 0.2 mg/L [de Vries, Voss 1993]. UV detection. Limit of detection, 2 µg/L, chlorothiazide or hydrochlorothiazide [Barbhaiya *et al.* 1981].
Disposition in the Body Poorly and erratically absorbed after oral administration; bioavailability, about 20 to 30% after doses of 250 mg, decreasing with increasing dose. Chlorothiazide is not significantly metabolised and is excreted in the urine to a variable extent depending on the extent of absorption.
Therapeutic Concentration
Following single oral doses of 125, 250, and 500 mg to 12 subjects, mean peak plasma concentrations of 0.64, 0.92, and 1.3 mg/L were attained in about 1 h [Welling *et al.* 1982].
Twenty healthy males were administered 25 mg of hydrochlorothiazide daily for 11 days. The mean steady-state plasma concentration was 288 µg/L observed at 2 h [Weir *et al.* 1998].
Half-life Plasma half-life, about 1.5 h.
Protein Binding About 95%.
Dose 0.5 to 2 g daily.

Barbhaiya RH *et al.* (1981). High-pressure liquid chromatographic determination of chlorothiazide and hydrochlorothiazide in plasma and urine: preliminary results of clinical studies. *J Pharm Sci* 70: 291–295.
de Vries JX, Voss A (1993). Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography. *Biomed Chromatogr* 7(1): 12–14.
Farthing D *et al.* (1998). Simple method for determination of hydrochlorothiazide in human urine by high performance liquid chromatography utilizing narrowbore chromatography. *J Pharm Biomed Anal* 17(8): 1455–1459.
Richter K *et al.* (1996). New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection. *J Chromatogr A* 729(1–2): 293–296.
Weir SJ *et al.* (1998). Steady-state pharmacokinetics of diltiazem and hydrochlorothiazide administered alone and in combination. *Biopharm Drug Dispos* 19(6): 365–371.
Welling PG *et al.* (1982). *Curr Ther Res* 31: 379–385.

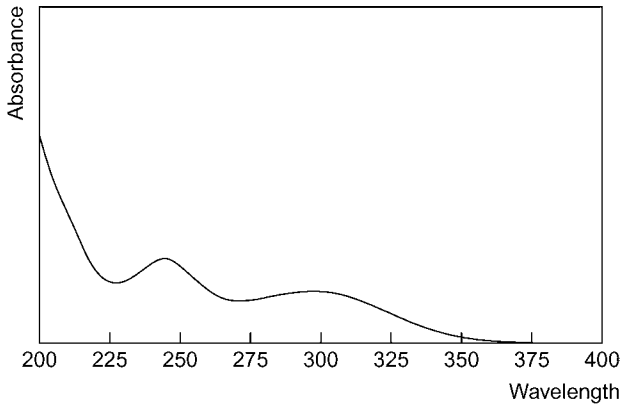
Chlorotrianisene

Oestrogen
$C_{23}H_{21}ClO_3$ = 380.9
CAS—569-57-3

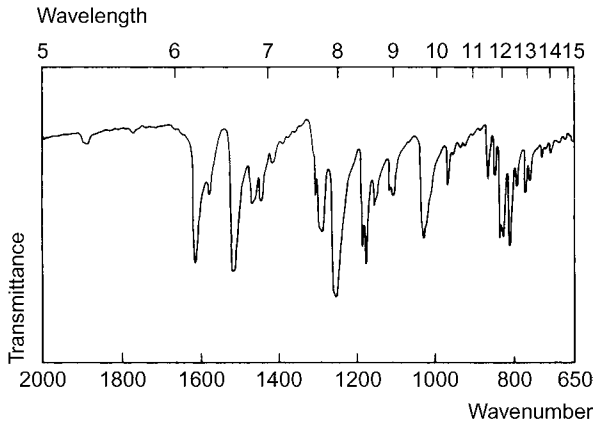
IUPAC Name 1-[1-Chloro-2,2-bis(4-methoxyphenyl)ethenyl]-4-methoxybenzene
Synonyms 1,1′,1″-(1-Chloro-1-ethenyl-2-ylidene)tris[4-methoxybenzene]; tri-*p*-anisylchloroethylene.
Proprietary Names *Anisene*; *Chlorotrisin*; *Merbentul*; *Tace*; *Triagen*.



Chemical Properties Small white crystals or crystalline powder. Mp about 118°. Soluble 1 in 4200 of water, 1 in 360 of ethanol, 1 in 7 of acetone, 1 in 1.5 of chloroform, and 1 in 28 of ether. Log *P* (octanol/water), 6.2.
Caution Chlorotrianisene is a powerful oestrogen. Contact with the skin or inhalation should be avoided.
Colour Tests Liebermann's reagent—green; sulfuric acid—violet.
Thin-layer Chromatography System TP—R_f 0.88; system TQ—R_f 0.77; system TR—R_f 0.98; system TS—R_f 0.92.
Gas Chromatography System GA—RI 2917.
Ultraviolet Spectrum Ethanol—247 (A^1_1=635a), 307 nm (A^1_1=410a).



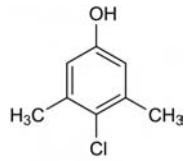
Infrared Spectrum Principal peaks at wavenumbers 1250, 1510, 1606, 1174, 813, 1184 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 380, 223, 382, 238, 152, 345, 215, 113.
Dose 12 to 24 mg daily.

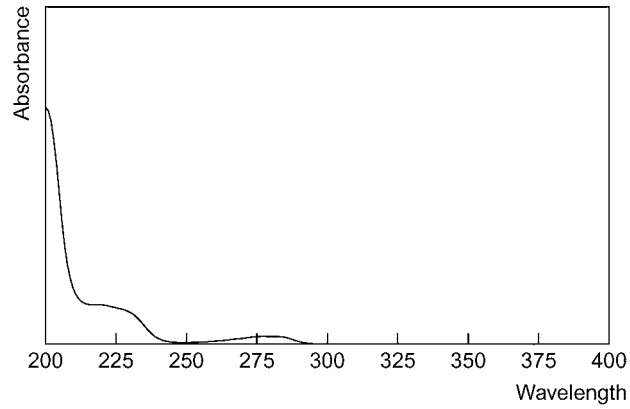
Chloroxylenol

Disinfectant
C_8H_9ClO = 156.6
CAS—88-04-0
IUPAC Name 4-Chloro-3,5-dimethylphenol
Synonyms Parachlorometaxylenol; PCMX.
Proprietary Names *Dettol*; *Metasep*.

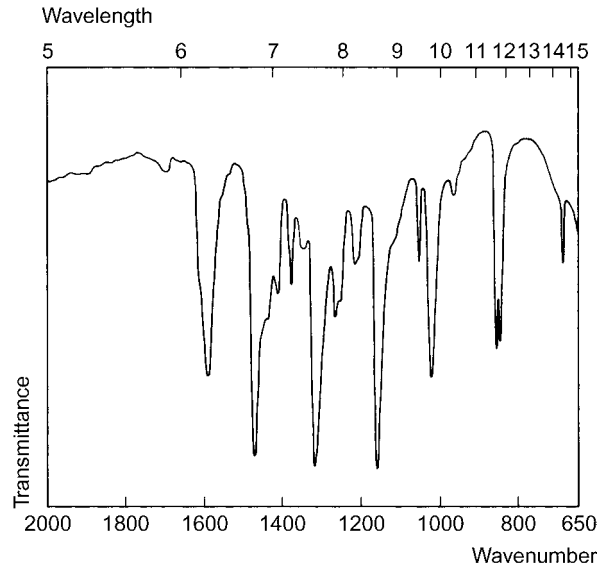


Chemical Properties White or cream-coloured crystals or crystalline powder. Volatile in steam. Mp 114° to 116°. Soluble 1 in 3000 of water, 1 in 200 of boiling water, and 1 in 1 of ethanol; soluble in benzene and in ether. pK_a 9.7 (25°). Log *P* (octanol/water), 3.3.
Colour Test Liebermann's reagent—black.

Gas Chromatography System GA—RI 1420.
High Performance Liquid Chromatography System HY—RI 511.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=83a); aqueous alkali—242 (A^1_1=650a), 296 nm.



Infrared Spectrum Principal peaks at wavenumbers 1158, 1317, 1590, 1020, 858, 848 cm^{-1} (KCl disk).

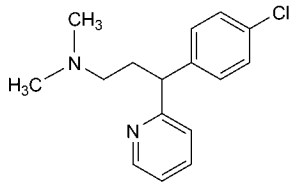


Disposition in the Body About 14% of ingested chloroxylenol is excreted in the urine as a glucuronide conjugate and 17% as the sulfate, together with traces of unchanged chloroxylenol; the amount of unchanged chloroxylenol excreted is increased if the urine is alkaline.
Toxicity
In a fatality caused by the ingestion of at least 100 mL of a solution containing 4.8% of chloroxylenol, postmortem chloroxylenol concentrations were as follows: blood 23 mg/L, liver 8 µg/g [Coutselinis, Boukis 1976].
A subject who had ingested about 17 g of chloroxylenol in solution was admitted to hospital 30 min afterwards and recovered after intensive treatment [Joubert *et al.* 1978].
Use Chloroxylenol is available in concentrations of about 5%; it is further diluted before use.

Coutselinis A, Boukis D (1976). Suicidal intoxication with Dettol (chloroxylenol) (a case report). *Med Sci Law* 16(3): 180.
Joubert P *et al.* (1978). Severe Dettol (chloroxylenol and terpineol) poisoning. *Brit Med J* 1: 890.

Chlorphenamine

Alkylamine, Antihistamine
$C_{16}H_{19}ClN_2$ = 274.8
CAS—132-22-9; 42882-96-2 (±)
IUPAC Name γ-(4-Chlorophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine
Synonyms Chlorpheniramine; chlorprophenpyridamine.
Proprietary Names *Alerdil*; *Bioflusin*; *Blendox*; *Cronal*; *Pollenase Antihistamine*; *Rimarin*.



Chemical Properties Oily liquid. Bp 142°. pK_{a1} 9.2, pK_{a2} 4.0 [Barnhart, Johnson 1977]. Log *P* (octanol/water) 3.38. Plasma samples at room temperature for 24 h were stable [Chen *et al.* 2004]. Plasma samples were stable for 24 h at room temperature and for 3 freeze-thaw cycles at −20° for 3 months. Plasma extracts at 4° showed contradictory results after 24 and 48 h [Celma *et al.* 2000]. Stock standard solutions prepared in methanol were stable for at least 3 months if kept at 4° [Hasegawa *et al.* 2006]. Stable in the mobile phase for 24 h at 10°, after 2 freeze–thaw cycles and at –20° for 75 days [Takagaki *et al.* 2002].

Chlorphenamine Maleate

$C_{16}H_{19}ClN_2$, $C_4H_4O_4$ = 390.9
CAS—113-92-8

Synonyms Chlorphenamini maleas; chlorpheniramine maleate.

Proprietary Names *Aller-Chlor*; *Allergex*; *Allergin*; *Allerphen*; *Allergy*; *Allergy Relief*; *Calimal*; *Chlo-Amine*; *Chlorhist*; *Chlor-Pro*; *Chlorpyrimine*; *Chlorspan*; *Chlor-Trimeton*; *Chlor-Tripolon*; *Histafen*; *Histalon*; *Piriject*; *Piriton*; *Teldrin*; *Trimeton*. It is an ingredient of many proprietary preparations—see Sweetman [2007].

Note The name Chloramin has been applied to tosylchloramide sodium.

Chemical Properties White crystalline powder. Mp 30° to 135°. Solubility in mg/mL at 25°: ethanol 330; chloroform 240; water 160; methanol 130. Slightly soluble in benzene and ether.

Dexchlorphenamine Maleate

CAS—25523-97-1 (dexchlorpheniramine); 2438-32-6 (dexchlorpheniramine maleate)

Synonyms Dexchlorpheniramine maleate; dexchlorphenamini maleas.

Proprietary Names *Phenamin*; *Polamin*; *Polaronil*; *Polaramin(e)*; *Rhiniramine*; *Trenelone*.

Chemical Properties White odourless crystalline powder. Protect from light. Soluble 1 in 1.1 water; 1 in 2 of alcohol; 1 in 1.7 chloroform and 1 in 2500 of ether; freely soluble in methyl alcohol and dichloromethane.

Colour Test Cyanogen bromide—orange.

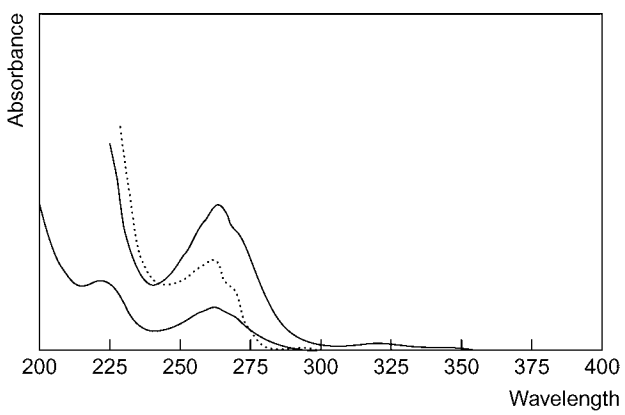
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.35; system TC—R_f 0.18; system TE—R_f 0.46; system TL—R_f 0.02; system TAE—R_f 0.12; system TAF—R_f 0.21; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.25 (Dragendorff spray, positive; FPN reagent, blue; acidified iodoplatinate solution, positive; Marquis reagent, violet; ninhydrin spray, positive).

Gas Chromatography System GA—chlorphenamine RI 1996, M (nor-) RI 2014, M (OH-)-AC RI 2405, M (bis-nor-)-AC RI 2535, M (desamino-OH-)-AC RI 2130, M (nor-)-AC RI 2530; system GB—chlorphenamine RI 2079, M (nor-) RI 2115, M (bis-nor-) RI 2065, M (nor-)-AC RI 2563; system GC—RI 2586; system GF—RI 2335.

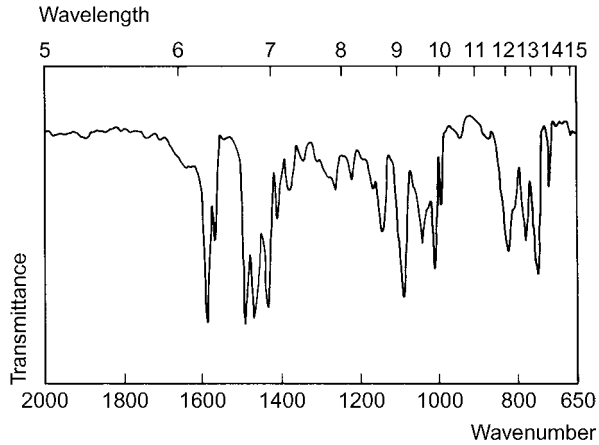
High Performance Liquid Chromatography System HA—*k* 3.9; system HX—RI 356; system HY—RI 264; system HZ—RT 3.5 min; system HAA—RT 12.9 min; system HAX—RT 10.8 min; system HAY—RT 5.3 min.

Column: Discovery cyanopropyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 7.5) : phosphate buffer (pH 2.0; 95 : 0 to 0 : 95) with 5% acetonitrile, flow rate 1.0 mL/min. UV detection (λ = 215 nm). Retention time: 14.5 min. Limit of quantification, 6 µg/L, limit of detection, 1.8 µg/L [Olmo *et al.* 2005].

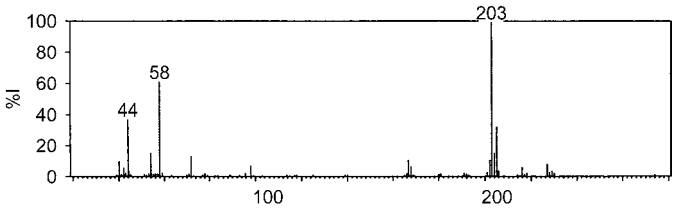
Ultraviolet Spectrum Aqueous acid—265 nm (A^1_1 = 302a); aqueous alkali—262 nm (A^1_1 = 205a).



Infrared Spectrum Principal peaks at wavenumbers 1585, 1086, 746, 1010, 830, 1562 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 203, 58, 44, 205, 54, 204, 72, 202.



Quantification

Blood **GC** Column: 10% SE-30 on 100/120 mesh Supelcoport (4′). Carrier gas: N_2, 40 mL/min. Temperature: 240°. FID. Retention time: 2.6 min [Reed 1981].

Plasma **GC** Column; cross-linked 50% phenylmethyl silicone (5 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 150° for 1 min to 240° at 10°/min for 1 min. NPD. Limit of detection, 0.3 µg/L [Masumoto *et al.* 1986].

GC-MS Column: DB-1MS fused silica capillary (30 m × 0.32 mm i.d., 0.25 um). Carrier gas: He, 2.0 mL/min. Temperature programme: 120° for 1 min to 300° at 20°/min. EI ionisation at 70 eV, positive ion mode. Limit of detection, 2 µg/L [Hasegawa *et al.* 2006].

HPLC Column: µBondapak C_{18} (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.05 mol/L ammonium phosphate (pH 2.5; 20 : 80). UV detection (λ = 254 nm). *k* 2.80. Retention time: 6.6 min. Limit of detection, 2 µg/L [Athanikar *et al.* 1979].

LC-MS Column: Diamonsil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (70 : 30 : 1), flow rate 0.65 mL/min. APCI, SRM acquisition mode. Limit of quantification, 0.2 µg/L [Chen *et al.* 2004]. Column: CYCLOBOND I β-cyclodextrin (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.25% diethylamine (pH 4.4) : acetonitrile : methanol (85 : 7.5 : 7.5), flow rate 0.5 mL/min. APEI, SIM acquisition mode. Limit of quantification, 0.25 µg/L; limit of detection, 0.13 µg/L [Fried *et al.* 2002]. Column: Develosil PhA (250 × 2.0 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate : acetonitrile-water (1 : 1, 45 : 55 for 5 min to 20 : 80 at 6.3 min to 10 min to 45 : 55), flow rate 0.3 mL/min for 5 min to 0.35 mL/min at 6.3 min to 0.3 mL/min at 10 min. ESI, positive ion mode, SIM acquisition mode. Retention time: 7.4 min. Limit of quantification, 0.52 µg/L [Takagaki *et al.* 2002]. Column: CYCLOBOND I β-cyclodextrin (250 × 4.6 mm, 5 µm). Mobile phase: 0.25% diethylamine (pH 4.4) : acetonitrile-methanol (50 : 50, 85 : 15), flow rate 0.5 mL/min. SIM acquisition mode. Retention time: 17.5 and 18.9 min for the (*R*)-(−) and (*S*)-(+)-enantiomers, respectively. Limit of detection, 0.05 µg/L [Yasuda *et al.* 2002]. Column: Kromasil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (80 : 20) with 0.5% formic acid and 1 mmol/L pentafluoropropionic anhydride, flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.2 µg/L [Celma *et al.* 2000].

Serum **GC** Column: 3% SP-2250 on 100/120 mesh Supelcoport (6′ × 3 mm i.d.). Carrier gas: N_2, 70 mL/min. Temperature: 195°. Limit of detection, 0.5 µg/L [Barnhart, Johnson 1977].

HPLC Column: Inertsil ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L potassium dihydrogen phosphate : acetonitrile (7 : 3) with or without 5 mmol/L SOS. UV detection (λ = 210 nm). Limit of quantification, 0.5 µg/L [Yamaguchi *et al.* 1994].

Urine **GC** Column: Chromosorb Q on 100/120 mesh (2 m × 0.64 cm o.d.). Carrier gas: N_2, 1.68 kg/cm^2. Temperature: 210°. Retention time: 6.0 min. Limit of detection, 0.5 mg/L [Ali, Beckett 1981].

HPLC Column: Nucleosil cyanopropyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : ethanol : diethylamine (96 : 4 : 0.05), flow rate 0.5 mL/min. UV detection (λ = 262 nm). Limit of quantification, 35 µg/L [Hiep *et al.* 1998]. See Plasma [Athanikar *et al.* 1979].

LC-MS Column: Sunfire C_8 (100 × 2.1 m i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate buffer (pH 3.5) : acetonitrile-methanol (2 : 1, 98 : 2 for 3.5 min to 80 : 20 at 4 min for 4 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 12.2 min. Limit of quantification, 12.5 ng/L, limit of detection, 10 µg/L [del Mar Ramirez Fernandez *et al.* 2007].

Bile **GC** See Blood [Reed 1981].

Oral Fluid **HPLC** See Plasma [Athanikar *et al.* 1979].
Stomach Contents **GC** See Blood [Reed 1981].
Brain **GC** See Blood [Reed 1981].
Kidney **GC** See Blood [Reed 1981].
Liver **GC** See Blood [Reed 1981].
Lung **GC** See Blood [Reed 1981].

Disposition in the Body Chlorphenamine maleate is readily and almost completely absorbed after oral administration; peak plasma concentrations are reached after 2.5 to 6 h. It is widely distributed in the body, including passage into the CNS. The major metabolites are monodesmethyl- and didesmethylchlorpheniramine. Approximately 35% of a single dose is excreted in the urine in 48 h; the 24-h excretion of unchanged drug accounts for ~3 to 10% of the dose, but this is increased by acidification of the urine and increased urinary flow, and decreased when the urine is alkaline; more non-polar metabolites appear to be excreted after IV rather than after oral administration. After daily oral administration, ~20% of a dose is excreted in the 24-h urine as unchanged drug, 20% as monodesmethylchlorpheniramine, and 5% as didesmethylchlorpheniramine. Less than 1% of a dose is eliminated in the faeces. Chlorphenamine inhibits CYP2D6-mediated reactions [He *et al.* 2002; Yasuda *et al.* 2002]

Therapeutic Concentration

Eighteen healthy volunteers who were administered a single oral dose of 4 mg chlorpheniramine maleate and 60 mg pseudoephedrine hydrochloride had a mean maximum plasma concentration of 7.25 ± 2.34 µg/L at 3.50 ± 1.79 h [Chen *et al.* 2004].

In 13 subjects who received a single oral dose of 8 mg (as test formulation tablets or as reference tablets), the maximum plasma concentrations were 0.022 and 0.0205 mg/L at 2.5 and 2.08 h for the test and reference preparations, respectively [Najjar *et al.* 1995].

After a single oral dose of 8 mg to 5 subjects, peak plasma concentrations of 0.01 to 0.04 mg/L (mean 0.02) were attained in 2 to 3 h; after repeated oral administration of 6 mg twice a day to 2 subjects, mean plasma steady-state concentrations of 0.02 and 0.03 mg/L were reported; the plasma concentration of monodesmethylchlorpheniramine averaged 0.008 mg/L and plasma concentrations of didesmethylchlorpheniramine were <0.003 mg/L [Huang *et al.* 1982].

After a single oral dose of 4, 8, or 12 mg chlorpheniramine peak serum concentrations were 5.5, 10.4, and 17.6 µg/L reached at 4 h [Barnhart, Johnson 1977].

Toxicity Toxic effects may be produced by plasma concentrations >20 mg/L.

Postmortem results from 47 pilot fatalities involving chlorphenamine revealed that where chlorphenamine was the sole agent present, tissue concentrations were as follows: blood 0.109 mg/L, liver 1.412 mg/L, lung 1.16 to 3.26 µg/g. In the remaining 31 cases, in which other drugs were also involved, the chlorphenamine concentration was reported as follows: blood 0.093 mg/L, liver 0.747 mg/L, lung 0.731 to 6.483 µg/g [Soper *et al.* 2000].

The following postmortem heart blood concentrations were reported in a fatality due to the ingestion of chlorphenamine, diphenhydramine, and guaifenesin: 0.2 mg/L, 8.8 mg/L and 27.4 mg/L. The cause of death was determined to be acute intoxication by the combined effects of the 3 drugs [Wogoman *et al.* 1999].

The following postmortem tissue concentrations were reported in a fatality due to the ingestion of chlorphenamine and alcohol in µg/mL: blood 1.1, bile 1.5, brain 2.5, kidney 1.4, liver 6.6, lung 5.2; a blood-alcohol concentration of 1200 µg/mL was also reported. The blood chlorphenamine concentration was ~65-times the therapeutic dose [Reed 1981].

Note For a case of atrioventricular block after an overdose of Decontabs (40 mg phenylpropanolamine, 10 mg phenylephrine, 5 mg chlorphenamine and 15 mg phenyltoloxamine per tablet), see Burton *et al.* [1985]. For a case of contact dermatitis from chlorphanamine in eye drops, see Tosti *et al.* [1990]. For a case of homicidal psychosis after cocaine abuse and chlorphenamine, see Strauss [1989].

Bioavailability Between 25 and 50%.

Half-life Values from 2 to 43 h have been reported; shorter half-life in children.

Volume of Distribution ≈3 to 5.9 L/kg.

Clearance Plasma clearance, ≈1.7 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ≈0.83.

Protein Binding ≈70%.

Note For a review of the pharmacokinetics of chlorphenamine, see Rumore [1984]. For a review of the pharmacokinetics of general H1-receptor antagonists, see Paton and Webster [1985].

Dose 12 to 24 mg of chlorphenamine maleate daily in divided doses; single doses of 8 to 12 mg have been given in sustained-release preparations. Up to 12 mg daily of dexpheniramine maleate is given in divided doses.

Ali HM, Beckett AH (1981). Rapid method for the determination of chlorpheniramine in urine. *J Chromatogr* 223: 208–212.

Athanikar NK *et al.* (1979). Chlorpheniramine I Rapid quantitative analysis of chlorpheniramine in plasma, saliva and urine by high-performance liquid chromatography. *J Chromatogr* 162: 367–376.

Barnhart JW, Johnson JD (1977). Simplified gas chromatographic method for the determination of chlorpheniramine in serum. *Anal Chem* 49: 1085–1086.

Burton BT *et al.* (1985). Atrioventricular block following overdose of decongestant cold medication. *J Emerg Med* 2: 415–419.

Celma C *et al.* (2000). Simultaneous determination of paracetamol and chlorpheniramine in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 870: 77–86.

Chen X *et al.* (2004). Simultaneous determination of chlorpheniramine and pseudoephedrine in human plasma by liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 18: 248–253.

del Mar Ramirez Fernandez M *et al.* (2007). Liquid chromatography-tandem mass spectrometry method for the simultaneous analysis of multiple hallucinogens, chlorpheniramine, ketamine, ritalinic acid, and metabolites, in urine. *J Anal Toxicol* 31: 497–504.

Fried KM *et al.* (2002). The enantioselective determination of chlorpheniramine and its major metabolites in human plasma using chiral chromatography on a beta-cyclodextrin chiral stationary phase and mass spectrometric detection. *J Pharm Biomed Anal* 27: 479–488.

Hasegawa C *et al.* (2006). Simultaneous determination of ten antihistamine drugs in human plasma using pipette tip solid-phase extraction and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 20: 537–543.

He N *et al.* (2002). Inhibitory effects of H1-antihistamines on CYP2D6- and CYP2C9-mediated drug metabolic reactions in human liver microsomes. *Eur J Clin Pharmacol* 57: 847–851.

Hiep BT *et al.* (1998). Determination of the enantiomers of chlorpheniramine and its main monodesmethyl metabolite in urine using achiral-chiral liquid chromatography. *J Chromatogr B Biomed Sci Appl* 707: 235–240.

Huang SM *et al.* (1982). Pharmacokinetics of chlorpheniramine after intravenous and oral administration in normal adults. *Eur J Clin Pharmacol* 22: 359–365.

Masumoto K *et al.* (1986). Simultaneous determination of codeine and chlorpheniramine in human plasma by capillary column gas chromatography. *J Chromatogr* 381: 323–329.

Najjar TA *et al.* (1995). Bioequivalence and pharmacokinetics of chlorpheneramine in healthy human volunteers. *Int J Clin Pharmacol Ther* 33: 619–622.

Olmo B *et al.* (2005). New approaches with two cyano columns to the separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds. *J Chromatogr B Analyt Technol Biomed Life Sci* 817: 159–165.

Paton DM, Webster DR (1985). Clinical pharmacokinetics of H1-receptor antagonists (the antihistamines). *Clin Pharmacokinet* 10: 477–497.

Reed D (1981). A fatal case involving chlorpheniramine. *Clin Toxicol* 18: 941–943.

Rumore MM (1984). Clinical pharmacokinetics of chlorpheniramine. *Drug Intell Clin Pharm* 18: 701–707.

Soper JW *et al.* (2000). Prevalence of chlorpheniramine in aviation accident pilot fatalities, 1991-1996. *Aviat Space Environ Med* 71: 1206–1209.

Strauss A (1989). Homicidal psychosis during the combined use of cocaine and an over-the-counter cold preparation. *J Clin Psychiatry* 50: 147.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

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Tosti A *et al.* (1990). Contact dermatitis due to chlorpheniramine maleate in eyedrops. *Contact Dermatitis* 22: 55.

Wogoman H *et al.* (1999). Acute intoxication with guaifenesin, diphenhydramine, and chlorpheniramine. *Am J Forensic Med Pathol* 20: 199–202.

Yamaguchi M *et al.* (1994). Sensitive high-performance liquid chromatographic determination of chlorpheniramine in human serum using column switching. *J Chromatogr B Biomed Appl* 661: 168–172.

Yasuda SU *et al.* (2002). The roles of CYP2D6 and stereoselectivity in the clinical pharmacokinetics of chlorpheniramine. *Br J Clin Pharmacol* 53: 519–525.

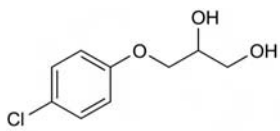
Chlorphenesin

Antifungal

$C_9H_{11}ClO_3 = 202.6$

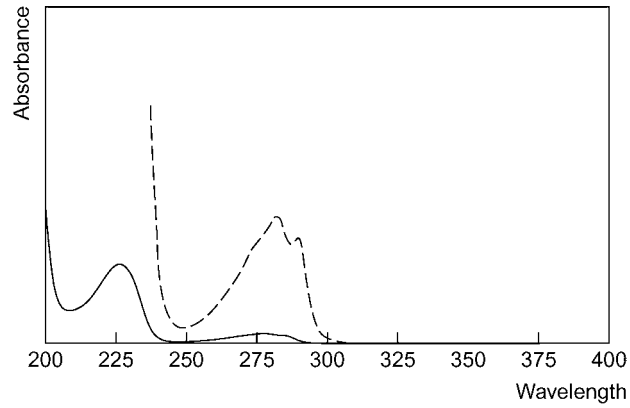
CAS—104-29-0

IUPAC Name 3-(4-Chlorophenoxy)-1,2-propanediol
Synonym *p*-Chlorophenyl α-glyceryl ether
Proprietary Names *Adermykon*; *Mycil*; *Soorphenesin*.

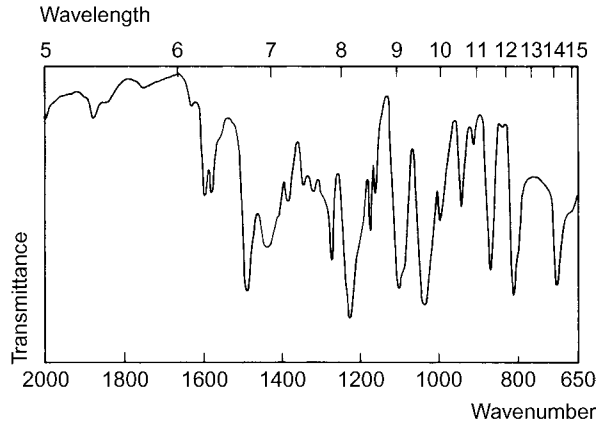


Chemical Properties White or pale cream-coloured crystals or crystalline aggregates. Mp 77° to 79°. Soluble 1 in 200 of water and 1 in 5 of ethanol; soluble in ether. Log *P* (octanol/water), 1.5.

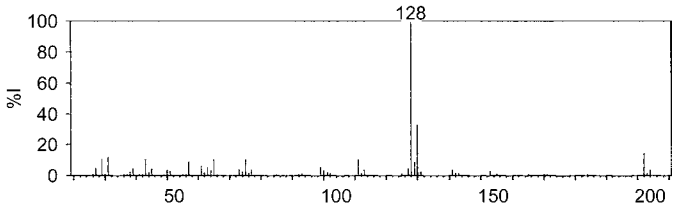
Colour Tests Liebermann's reagent—black; Mandelin's test—brown-green.
Thin-layer Chromatography System TA—R_f 0.82; system TE—R_f 0.62; system TAJ—R_f 0.38; system TAK—R_f 0.32; system TAL—R_f 0.86 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—chlorphenesin RI 1680, chlorphenesin-AC_2 RI 2070, chlorphenesin-AC RI 2030.
Ultraviolet Spectrum Methanol—281 (A_1^1=79b), 289 nm.



Infrared Spectrum Principal peaks at wavenumbers 1232, 1043, 818, 1109, 1490, 710 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 128, 130, 202, 31, 29, 65, 43, 111.

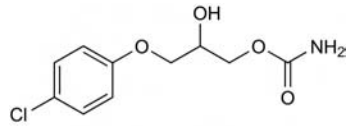


Use Topically in concentrations of 0.5 to 1%.

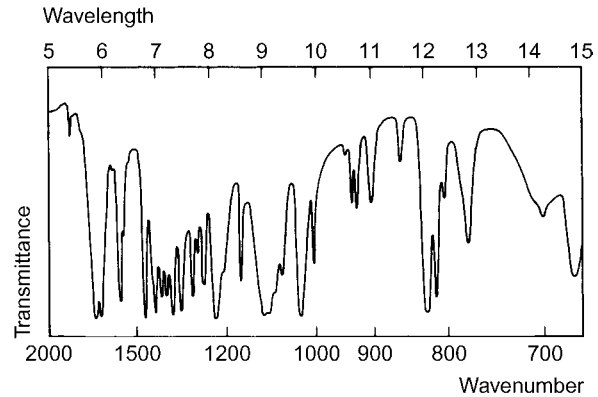
Chlorphenesin Carbamate

Muscle Relaxant
$C_{10}H_{12}ClNO_4$ = 245.7
CAS—886-74-8

IUPAC Name [3-(4-Chlorophenoxy)-2-hydroxypropyl] carbamate
Synonym 3-(4-Chlorophenoxy)-1,2-propanediol-1-carbamate
Proprietary Names *Maolate*; *Rinlaxer*.



Chemical Properties A white crystalline powder. Mp about 90°. Practically insoluble in water, benzene, and cyclohexane; readily soluble in ethanol, acetone, and ethyl acetate; soluble in chloroform. Log *P* (octanol/water), 0.8.
Thin-layer Chromatography System TA—R_f 0.82; system TB—R_f 0.00; system TD—R_f 0.11; system TE—R_f 0.52; system TF—R_f 0.34; system TAD—R_f 0.32; system TAE—R_f 0.87; system TAJ—R_f 0.31; system TAK—R_f 0.18; system TAL—R_f 0.80 (furfuraldehyde reagent, positive).
Gas Chromatography System GA—chlorphenesin RI 1680.
High Performance Liquid Chromatography System HX—RI 387; system HY—RI 348.
Ultraviolet Spectrum Ethanol—280 (A_1^1=75b), 288 nm (A_1^1=62b).
Infrared Spectrum Principal peaks at wavenumbers 1724, 1234, 1028, 1692, 1111, 824 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 128, 130, 202, 43, 129, 111, 75, 204.

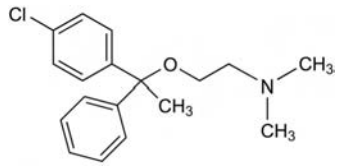
Quantification
Serum **GC** FID. Limit of detection, 500 µg/L [Kaiser, Shaw 1974].
Colorimetry See Forist, Judy [1971].
Disposition in the Body Readily absorbed after oral administration. About 85% of a dose is excreted in the urine as a glucuronide conjugate in 24 h together with small amounts of unchanged drug, and the oxidised metabolites, 4-chlorophenoxylactic acid, 4-chlorophenoxyacetic acid, and 4-chlorophenol.
Therapeutic Concentration
Following a single oral dose of 800 mg to 10 subjects, peak serum concentrations of 3.9 to 17.0 mg/L (mean 10) were attained in about 2 h; after daily oral dosing with 800 mg three times a day for 11 weeks to the same subjects, a mean peak serum concentration of 14.4 mg/L was reported about 2 h after the last dose [Stoll *et al.* 1974].
Half-life Plasma half-life, about 2 to 5 h.
Volume of Distribution About 1 L/kg.
Dose Initially 2.4 g daily, reduced to 1.6 g daily or less.

Forist AA, Judy RW (1971). Comparative pharmacokinetics of chlorphenesin carbamate and methocarbamol in man. *J Pharm Sci* 60: 1686–1688.
Kaiser DG, Shaw SR (1974). GLC determination of chlorphenesin carbamate in serum. *J Pharm Sci* 63: 1094–1097.
Stoll RG *et al.* (1974). Chlorphenesin carbamate serum levels during subchronic administration to human (normal) volunteers. *J Clin Pharmacol* 14: 520–524.

Chlorphenoxamine

Anticholinergic
$C_{18}H_{22}ClNO$ = 303.8
CAS—77-38-3

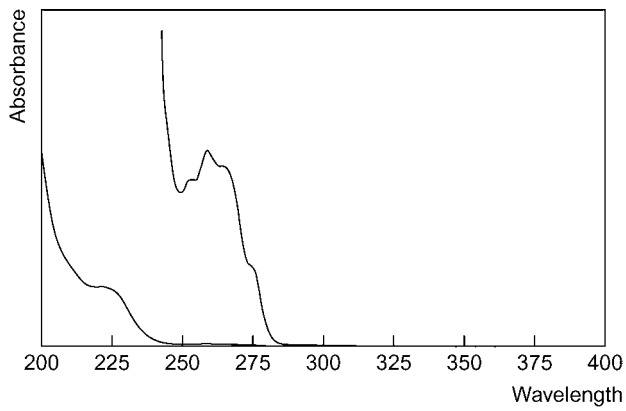
IUPAC Name 2-[1-(4-Chlorophenyl)-1-phenylethoxy]-*N*,*N*-dimethylethanamine



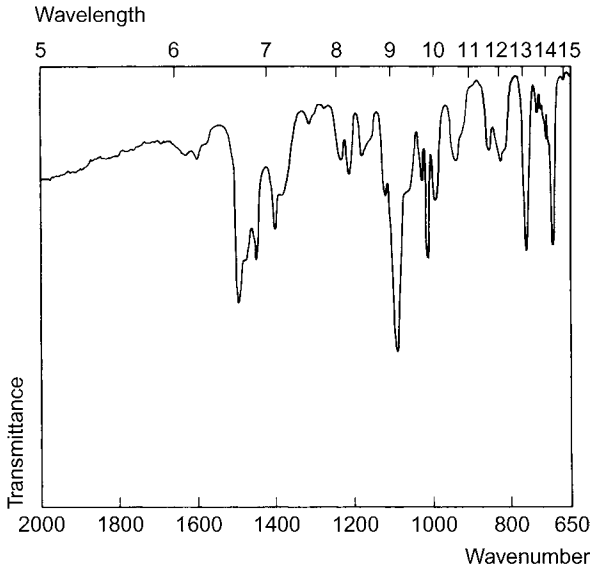
Chemical Properties An oil.

Chlorphenoxamine Hydrochloride
$C_{18}H_{22}ClNO$, HCl = 340.3
CAS—562-09-4
Proprietary Names *Clorevan*; *Phenoxene*; *Systral*.
Chemical Properties A white crystalline powder. Mp 128°. Soluble 1 in 2 of water, 1 in 1.8 of ethanol, and 1 in 1.5 of chloroform; practically insoluble in ether.

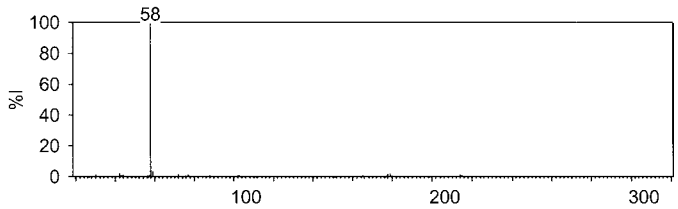
Colour Test Marquis test—yellow→green.
Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.47; system TC—R_f 0.36; system TE—R_f 0.70; system TL—R_f 0.17; system TAE—R_f 0.29; system TAJ—R_f 0.11; system TAK—R_f 0.05; system TAL—R_f 0.54 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—chlorphenoxamine RI 2080, M (OH-) RI 2470, M (OH-methoxy-carbinol-)-H_2O RI 2220; system GB—chlorphenoxamine RI 2190, M (nor-) RI 2205; system GF—RI 2365.
High Performance Liquid Chromatography System HA—*k* 2.9; system HY—RI 346.
Ultraviolet Spectrum Aqueous acid—259 nm (A_1^1=16b).



Infrared Spectrum Principal peaks at wavenumbers 1088, 1490, 1010, 760, 700, 990 cm^{-1} (chlorphenoxamine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 59, 179, 42, 178, 72, 77, 30.



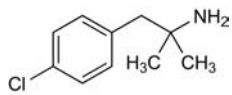
Dose 150 to 400 mg of chlorphenoxamine hydrochloride daily.

Chlorphentermine

Anorectic

$C_{10}H_{14}ClN$ = 183.7
CAS—461-78-9

IUPAC Name 1-(4-Chlorophenyl)-2-methylpropan-2-amine
Synonym 4-Chloro-α,α-dimethylbenzeneethanamine
Proprietary Names *Lucofen*; *Teramine*.

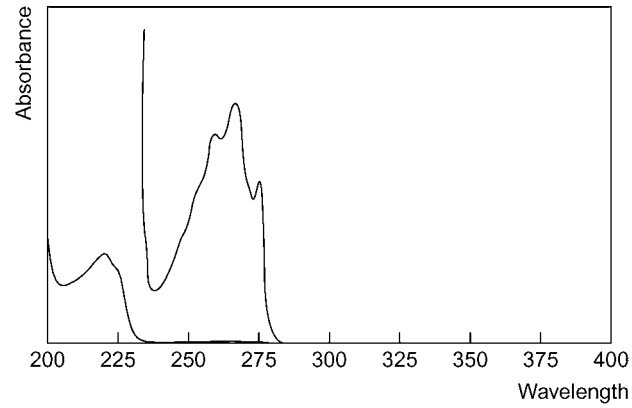


Chemical Properties A liquid. pK_a 9.6. Log *P* (octanol/water), 2.6.

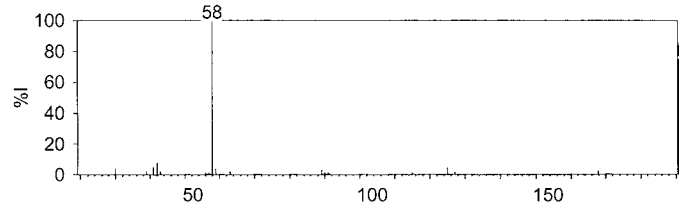
Chlorphentermine Hydrochloride

$C_{10}H_{14}ClN$, HCl = 220.1
CAS—151-06-4
Proprietary Name *Pre-Sate*
Chemical Properties A white or off-white crystalline powder. Mp about 234°. Soluble in water and ethanol; sparingly soluble in chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.18; system TC—R_f 0.17; system TE—R_f 0.48; system TL—R_f 0.08; system TAE—R_f 0.14; system TAF—R_f 0.77 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1344; system GB—RI 1393; system GC—RI 1725.
High Performance Liquid Chromatography System HA—*k* 0.9; system HC—*k* 0.82.
Ultraviolet Spectrum Aqueous acid—220, 259, 267 (A_1^1=11.6c), 274 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 818, 1493, 1092, 1020, 848, 754 cm^{-1} (chlorphentermine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 42, 41, 125, 59, 30, 89, 168.



Quantification
Blood **GC** FID. Limit of detection, 25 µg/L [Jun, Triggs 1970].
Urine **GC** FID. For quantification of chlorphentermine and metabolites, see Beckett, Bélanger [1977].
Disposition in the Body Absorbed after oral administration and metabolised mainly by *N*-oxidation and *N*-hydroxylation to nitro, nitroso and hydroxylamine derivatives. Excretion in the urine is dependent upon urinary pH, a much greater proportion of unchanged drug being excreted when the urine is acidic than when it is alkaline. In normal urine, about 17% of a dose is excreted unchanged and about 50% as *N*-oxidised metabolites in 48 h. If the urine is maintained at an acid pH, these proportions are approximately reversed, about 70% of a dose being excreted in 24 h. In alkaline urine <12% of a dose is excreted unchanged in 48 h.
Therapeutic Concentration
A single oral dose of 100 mg given to 4 subjects produced a mean peak blood concentration of about 0.32 mg/L in 4 h [Jun, Triggs 1970].
Half-life Plasma half-life, about 40 h.
Volume of Distribution About 2 to 3 L/kg.
Dose Usually the equivalent of 65 mg of chlorphentermine daily.

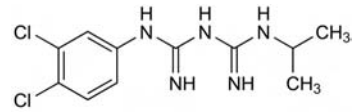
Beckett AH, Bélanger PM (1977). The metabolism, distribution and elimination of chlorphentermine in man. *Br J Clin Pharmacol* 4: 193–200.
Jun HW, Triggs EJ (1970). Blood levels of chlorphentermine in man. *J Pharm Sci* 59: 306–309.

Chlorproguanil

Antimalarial

$C_{11}H_{15}Cl_2N_5$ = 288.2
CAS—537-21-3

IUPAC Name (1*E*)-1-[Amino-(3,4-dichloroanilino)methylidene]-2-propan-2-ylguanidine
Synonym *N*-(3,4-Dichlorophenyl)-*N′*-(1-methylethyl)imidodicarbonimidic diamide; M-5943.



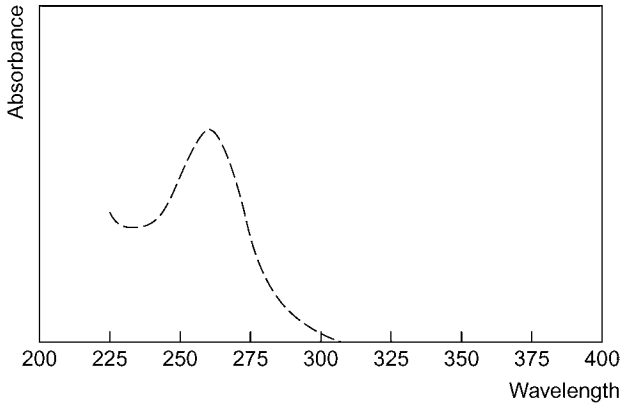
Chemical Properties Log *P* (octanol/water), 3.2.

Chlorproguanil Hydrochloride

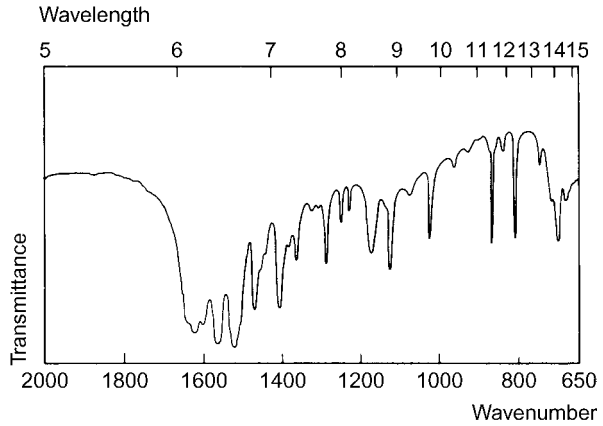
$C_{11}H_{15}Cl_2N_5$, HCl = 324.6
CAS—15537-76-5
Proprietary Name *Lapudrine*
Chemical Properties A white crystalline powder. Mp 246° to 247°. Soluble 1 in 100 of water and 1 in 50 of ethanol; practically insoluble in chloroform and ether.

Colour Tests To 10 mL of a saturated aqueous solution add 1 drop of 10% copper sulfate solution and 2.5 mL of dilute ammonia solution and shake well; add 5 mL of toluene and shake again—toluene layer is violet-red; dissolve 5 mg in 5 mL of a warm 1% solution of cetrimide in water, add 1 mL of sodium hydroxide solution and 1 mL of bromine solution—red.

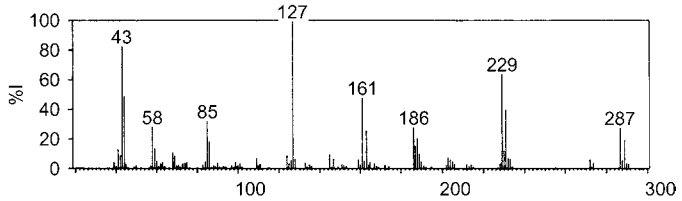
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.01 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1621.
Ultraviolet Spectrum Aqueous acid—250 nm (A^1_1=293c); methanol—260 nm (A^1_1=755b).



Infrared Spectrum Principal peaks at wavenumbers 1520, 1562, 1620, 1600, 1128, 1290 cm^{-1} (chlorproguanil hydrochloride, KBr disk).



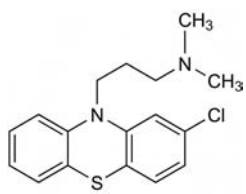
Mass Spectrum Principal ions at *m/z* 127, 43, 229, 44, 161, 231, 85, 186.



Dose 20 mg of chlorproguanil hydrochloride every 7 days.

Chlorpromazine

Tranquilliser
$C_{17}H_{19}ClN_2S$ = 319.9
CAS—50-53-3
IUPAC Name 3-(2-Chlorophenothiazin-10-yl)-*N,N*-dimethylpropan-1-amine
Synonyms 2601-A; 2-chloro-*N,N*-dimethyl-10*H*-phenothiazine-10-propanamine; HL-5746; RP-4560; SKF-2601-A.
Proprietary Names *Aminazine*; *Amplictil*; *Chlorderazin*; *Chlorpromados*; *Contomin*; *Esmind*; *Fenactil*; *Largactil* (suppositories); *Novomazina*; *Plegomazil*; *Promactil*; *Prozil*; *Thorazine* (suppositories); *Wintermin*.



Chemical Properties A white to creamy-white powder or waxy solid. It darkens on prolonged exposure to light. Mp 56° to 60°. Practically insoluble in water; soluble in 1 in 2 of ethanol, 1 in <1 of chloroform, and 1 in 1 of ether. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

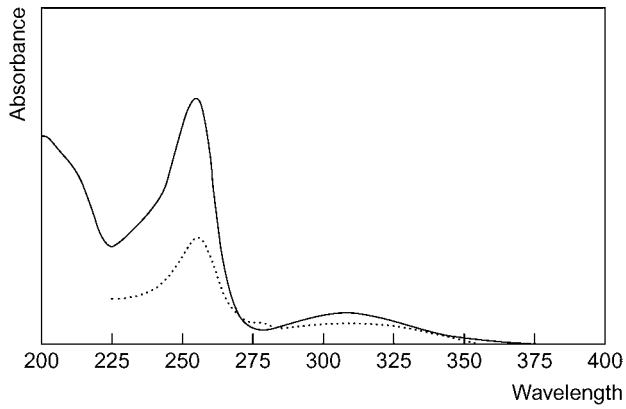
Chlorpromazine Embonate

$(C_{17}H_{19}ClN_2S)_2$, $C_{23}H_{16}O_6$ = 1026
Synonym Chlorpromazine pamoate
Proprietary Names *Chloractil*; *Chlorazin*; *Chlor-Promanyl*; *Chlorpromed*; *Clonazine*; *Hebanil*; *Hibanil*; *Hibernal*; *Klorproman*; *Klorpromex*; *Largactil*; *Lagatrex*; *Megaphen*; *Marazine*; *Matcine*; *Propaphenin*; *Prozin(e)*; *Taroctyl*; *Thorazine*; *Torazina*.
Chemical Properties A pale yellow powder. Very slightly soluble in water; soluble in acetone.

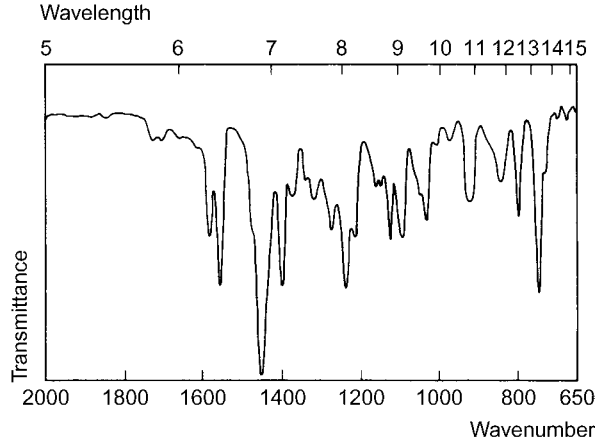
Chlorpromazine Hydrochloride

$C_{17}H_{19}ClN_2S$, HCl = 355.3
CAS—69-09-0
Synonym Aminazine
Proprietary Name *Largactil* (suspension)
Chemical Properties A white or creamy-white crystalline powder. It decomposes on exposure to air and light, becoming yellow, pink and finally violet. Mp with decomposition 179° to 180° (capillary), 194° to 196° (microblock). Soluble 1 in 2.5 of water; soluble in methanol, ethanol and chloroform; practically insoluble in ether and benzene. pK_a 9.3 (20°). Log *P* (octanol pH 7.4), 3.4.

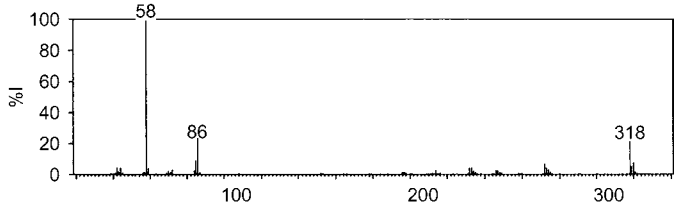
Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—red; FPN reagent—red; Liebermann's reagent—red-brown; Mandelin's test—green-→violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.45; system TC—R_f 0.35; system TE—R_f 0.70; system TL—R_f 0.17; system TAE—R_f 0.25; system TAF—R_f 0.45; system TAJ—R_f 0.11; system TAK—R_f 0.02; system TAL—R_f 0.47 (Dragendorff spray—positive; FPM reagent—pink; acidified iodoplatinate solution—positive; Marquis reagent—pink).
Gas Chromatography System GA—chlorpromazine RI 2413, M (nor-) RI 2480, M (didesmethyl-) RI 2480, M (sulfoxide) RI 2809, M (norsulfoxide) RI 2900, M (*N*-oxide) RI 2100, M (ring) RI 2100, M (OH-)-Me RI 2590, M (bisnor-)-AC RI 2990, M (nor-)-AC RI 3070; system GB—chlorpromazine RI 2618, M (nor-) RI 2656, M (didesmethyl-) RI 2646, M (sulfoxide) RI 3003, M (norsulfoxide) RI 3046, M (7-OH-) RI 2939, M (*N*-oxide) RI 2355; system GF—RI 2940; system GV—RT 16.6 min; system GW—RT 23.0 min.
High Performance Liquid Chromatography System HA—*k* 4.1, M (nor-) 2.2; system HX—RI 456; system HY—RI 350; system HZ—RT 9.1 min; system HAA—RT 16.0 min; system HAX—RT 17.0 min, M (sulfoxide) RT 8.4 min; system HAY—RT 7.8 min, M (sulfoxide) RT 4.3 min; system HAZ—*k* 2.64, M (sulfoxide) *k* 0.62.
Ultraviolet Spectrum Aqueous acid—269 nm (A^1_1 = 1025a). Chlorpromazine sulfoxide: aqueous acid—239 nm (A^1_1 = 1107b), 274 nm (A^1_1 = 343b), 300 nm (A^1_1 = 232b), 341 nm (A^1_1 = 168b).



Infrared Spectrum Principal peaks at wavenumbers 747, 1240, 1561, 1125, 1095, 1220 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 86, 318, 85, 320, 272, 319, 273.



C

Quantification

Blood **GC-MS** SID. Limit of detection, 250–500 ng/L [Hattori *et al.* 1992]. SIM acquisition mode. Limit of detection, μg/L range [Gruenke *et al.* 1985].

HPLC Column: Zorbax ODS. Mobile phase: methanol : water : tritely amine (pH 7.5, 75 : 24 : 0.7 : 0.3). DAD [Zhuo *et al.* 1997]. Column: Silica (300 × 4 mm i. d., 10 μm). Mobile phase: methanol : water (60 : 40) containing 0.2% ethanolamine, flow rate 1.5 mL/min. UV detection. Retention time: 6.24 min. Limit of quantification not reported [Allender *et al.* 1983].

LC-MS ESI, positive ion mode, SIM acquisition mode [Pufal, Sykutera 2008].

Plasma **GC-MS** Column: CBP1-bonded methyl silicone (12 m × 0.53 mm i.d., 1 μm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. Limit of detection, 5 μg/L [Tokunaga *et al.* 1996]. See Blood [Gruenke *et al.* 1985].

HPLC Column: Chromsep C_8 reverse phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer TEA (pH 3.0, 30 : 69.5 : 0.5), flow rate 1.0 mL/min. UV detection (λ = 238 nm). Retention time: 18 min. Limit of quantification, 2.5 μg/L, limit of detection, 0.8 μg/L [Mercolini *et al.* 2007]. Column: Cyano. Mobile phase: 0.1 mol/L ammonium acetate : acetonitrile (10 : 90). Limit of quantification, 0.25 μg/L [Cooper *et al.* 1983]. Electrochemical detection. Limit of detection, 0.25 μg/L [McKay *et al.* 1982]. UV detection. Limit of detection, 1 μg/L [Midha *et al.* 1981].

LC-MS Column: Discovery pentafluorophenylpropyl (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 3 mmol/L phosphate buffer (pH 1.9, 32 : 68), flow rate 0.5 mL/min. Electrochemical detection. Limit of quantification, 0.5 μg/L, limit of detection, 0.17 μg/L [Saracino *et al.* 2008].

Serum **GC-MS** Column: non-polar fused silica wide-bore capillary (15 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He. Temperature: 280°. FID. Limit of detection not reported [Ohashi *et al.* 2004]. NPD. Limit of quantification, 2.5 mg/L [Bailey, Guba 1979].

HPLC Column: Shim-Pack CLC-C_8 (250 × 4.6 mm i.d.). Mobile phase: methanol : sodium acetate (pH 4.1, 80 : 20), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.5 μg/L [Sobhi *et al.* 2007]. Column: reversed phase C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 300 : 200 : 500), flow rate 0.9 mL/min. Retention time: 22.3 min. UV detection (λ = 250 nm). Retention time: 22.3 min. Limit of quantification, 3.6 μg/L [Tanaka *et al.* 2007]. Column: reversed phase. UV detection (λ = 254 nm). Limit of quantification, 10 μg/L [Ohkubo *et al.* 1993].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.09 min. Limit of quantification, 0.27 μg/L [Kirchherr, Kühn-Velten 2006]. Column: Symmetry C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: methanol : 10 mmol/L ammonium acetate (10 : 90 for 5 min to 90 : 10 over 40 min for 10 min to 10 : 90 over 5 min for 20 min), flow rate 0.2 mL/min. ZQ single-quadrapole mass detector. Limit of detection, 1 μg/L [Miyaguchi *et al.* 2006].

Urine **GC-MS** Column: DB 5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Quadrupole MSD, SIM acquisition mode. Limit of detection, 5 μg/L [Olmos-Carmona, Hernandez-Carrasquilla 1999]. See Blood [Hattori *et al.* 1992].

HPLC See Serum [Sobhi *et al.* 2007].

LC-MS Column: Tandem LiChrosorb C_8 (150 × 4.6 mm i.d.)-LiChrosorb C_{18} (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : TEA (40 : 40 : 20 : 2), flow rate 0.5 mL/min. UV detection (λ = 250 nm). Limit of quantification, 102 μg/L, limit of detection, 30.6 μg/L [Cruz-Vera *et al.* 2009]. Column: KK-CARNU (100 × 4.6 mm i.d., 5 μm). Mobile phase: hexane-1 : 2-dichlormethane : absolute ethanol : trifluoracetic acid (400 : 150 : 100 : 1), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 250 nm, λ_{em} = 280 nm). Limit of quantification, 0.25 mg/L [Ponder, Stewart 1995].

Gastric Contents **HPLC** See Blood [Allender *et al.* 1983].

Milk **HPLC** See Serum [Ohkubo *et al.* 1993].

Oral Fluid **GC-MS** Column: methyl silicone capillary (16.5 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 0.8 mL/min. Temperature: 70°. EI ionisation, SIM acquisition mode (*m/z* 40 to 550). Limit of quantification, 1.0 μg/L, limit of detection, 0.4 μg/L. [Pujadas *et al.* 2007]. Column: HP-1 (300 × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature: 80°. Limit of quantification, 4.8 μg/L, limit of detection, 1.4 μg/L [Wylie *et al.* 2005].

Hair **GC-MS** Column: Saturn 2000 DB-5 capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: CH_4. Temperature: 100°. EI ionisation, SIM acquisition mode (*m/z* 50 to 500). Limit of detection, not reported [Shen *et al.* 2002].

Liver **HPLC** See Blood [Allender *et al.* 1983].

Disposition in the Body Chlorpromazine is readily absorbed after oral administration, with peak plasma levels occurring after 2–4 h; it undergoes first-pass metabolism in the gut wall, is extensively metabolised in the liver and excreted in the bile and urine. Metabolism is catalysed by the P450 isoenzyme CYP2D6 and includes sulfoxidation, *N*-demethylation, hydroxylation and *N*-oxidation; glucuronic acid conjugation and possibly ring fission also occur. There is some evidence that chlorpromazine may stimulate its own metabolism. A large number of metabolites have been isolated and some of the metabolites are active, particularly 7-hydroxychlorpromazine, although less so than the parent drug; several metabolites may be detected in plasma at concentrations similar to those of chlorpromazine during chronic treatment. Approximately 20 to 70% of an oral dose is excreted in the urine as metabolites, mostly conjugated, with 5% of the dose as the sulfoxide, and less than 1% as unchanged drug; approx. 5% of a dose is eliminated in the faeces as metabolites. Chlorpromazine metabolites have been detected in urine up to 18 months after discontinuation of long-term treatment. The monodesmethyl, 7-hydroxy and sulfoxide metabolites are taken up by erythrocytes together with traces of the parent drug and its *N*-oxide.

Chlorpromazine is widely distributed in the body. It crosses the blood–brain barrier, with levels in the brain higher than those in plasma. Chlorpromazine and its metabolites cross the placenta and are found in breast milk.

Therapeutic Concentration

A single oral dose of 150 mg given to 6 subjects produced peak plasma concentrations of 0.010–0.026 mg/L (mean 0.018) 3 h after administration [Hollister *et al.* 1970].

Following daily oral doses of 200–600 mg in 10 subjects, minimum steady-state plasma concentrations of 0.002–0.122 mg/L (mean 0.03) were reported; monodesmethyl and 7-hydroxychlorpromazine plasma concentrations averaged 16% and 30%, respectively, of the chlorpromazine plasma concentration, but there were considerable intersubject variations [Alfredsson *et al.* 1976].

Toxicity Severe toxic symptoms have occurred with doses of <0.1 g. Overdose of chlorpromazine is a fairly common occurrence, but fatalities are relatively rare. Blood concentrations in the region of 0.5–2 mg/L are associated with toxic effects, and concentrations of 2 mg/L or greater may be lethal.

In a review of eight fatal cases, blood concentrations of 3–35 mg/L (mean 17) and liver concentrations of 54–2110 μg/g (mean 366) were reported [Bonnichsen *et al.* 1970].

The following tissue distribution was reported in 2 fatalities: blood 4.2 and 6.7 mg/L, brain 126 and 148 μg/g, kidney 134 and 162 μg/g, liver 240 and 280 μg/g, urine 62 and 78 mg/L [Coutselinis *et al.* 1974].

A patient with renal failure died suddenly following the administration of chlorpromazine 780 mg daily for 5 days. Blood concentrations of 1.534 mg/L were reported [Dorson, Crismon 1988].

Bioavailability ≈20–30%, reduced during chronic therapy.

Half-life Plasma half-life, 7–120 h; mean values are usually in the range 15–30 h.

Volume of Distribution ≈21 L/kg.

Clearance ≈8.6 mL/min/kg from plasma.

Protein Binding ≈95–98%.

Dose Daily dosage of 75 to 300 mg of chlorpromazine hydrochloride; up to 1 g or more daily has been given to psychotic patients.

Alfredsson G *et al.* (1976). A mass fragmentographic method for the determination of chlorpromazine and two of its active metabolites in human plasma and CSF. *Psychopharmacology (Berl)* 48: 123–131.

Allender WJ *et al.* (1983). Extraction and analysis of chlorpromazine and its major metabolites in post mortem material by enzymic digestion and HPLC. *J Anal Toxicol* 7: 203–206.

Bailey DN, Guba JJ (1979). Gas-chromatographic analysis for chlorpromazine and some of its metabolites in human serum, with use of a nitrogen detector. *Clin Chem* 25: 1211–1215.

Bonnichsen R *et al.* (1970). Toxicological data on phenothiazine drugs in autopsy cases. *Z Rechtsmed* 67: 158–169.

Cooper JK *et al.* (1983). Subnanogram quantitation of chlorpromazine in plasma by high-performance liquid chromatography with electrochemical detection. *J Pharm Sci* 72: 1259–1262.

Coutselinis A *et al.* (1974). Fatal intoxication with chlorpromazine with special regard to the influence of putrefaction on its toxicological analysis. *Forensic Sci* 4: 191–194.

Cruz-Vera M *et al.* (2009). Determination of phenothiazine derivatives in human urine by using ionic liquid-based dynamic liquid-phase microextraction coupled with liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 37–42.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dorson PG, Crismon ML (1988). Chlorpromazine accumulation and sudden death in a patient with renal insufficiency. *Drug Intell Clin Pharm* 22: 776–778.

Gruenke LD *et al.* (1985). Determination of chlorpromazine and its major metabolites by gas chromatography/mass spectrometry: application to biological fluids. *Biomed Mass Spectrom* 12: 707–713.

Hattori H *et al.* (1992). Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 579: 247–252.

Hollister LE *et al.* (1970). Studies of delayed-action medication. V. Plasma levels and urinary excretion of four different dosage forms of chlorpromazine. *Clin Pharmacol Ther* 11: 49–59.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

McKay G *et al.* (1982). Gas chromatographic–mass spectrometric procedure for the quantitation of chlorpromazine in plasma and its comparison with a new high-performance liquid chromatographic assay with electrochemical detection. *J Chromatogr* 232: 275–282.

Mercolini L *et al.* (2007). Simultaneous analysis of classical neuroleptics, atypical antipsychotics and their metabolites in human plasma. *Anal Bioanal Chem* 388: 235–243.

Midha KK *et al.* (1981). High-performance liquid chromatographic assay for nanogram determination of chlorpromazine and its comparison with a radioimmunoassay. *J Pharm Sci* 70: 1043–1046.

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Zhuo X *et al.* (1997). [Determination of basic drugs in blood by RP-HPLC.]. *Fa Yi Xue Za Zhi* 13: 203–204.

Chlorpropamide

Antidiabetic

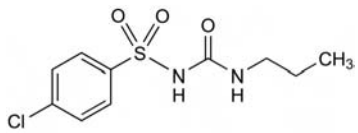
$C_{10}H_{13}ClN_2O_3S = 276.7$

CAS—94-20-2

IUPAC Name 1-(4-Chlorophenyl)sulfonyl-3-propylurea

Synonyms Chlorglypropamide; 4-Chloro-*N*-[(propylamino)carbonyl]benzenesulfonamide; P-607.

Proprietary Names *Chloromide*; *Chloronase*; *Diabetoral*; *Diabinese*; *Glymese*; *Insulase*; *Melitase*; *Mellinese*; *Novopropamide*; *Promide*; *Stabinol*.



Chemical Properties A white crystalline powder. Mp 126° to 130°. Practically insoluble in water; soluble 1 in 12 of ethanol, 1 in 5 of acetone, 1 in 9 of chloroform, and 1 in 200 of ether. pK_a 5.0 (20°). Log *P* (octanol/water), 2.3.

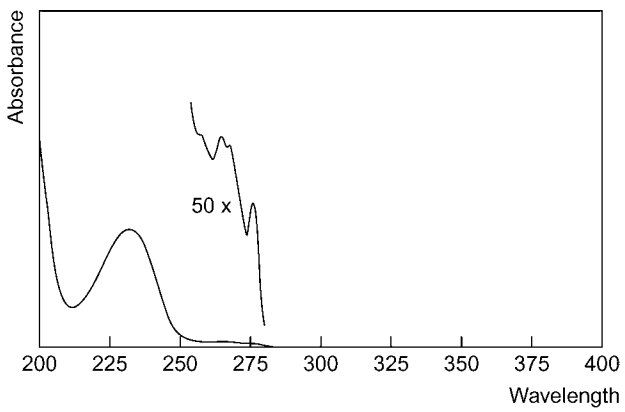
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.00; system TD—R_f 0.38; system TE—R_f 0.10; system TF—R_f 0.43; system TT—R_f 0.84; system TU—R_f 0.43; system TV—R_f 0.03; system TAD—R_f 0.49; system TAE—R_f 0.87; system TAF—R_f 0.88; system TAJ—R_f 0.65; system TAK—R_f 0.78; system TAL—R_f 0.06.

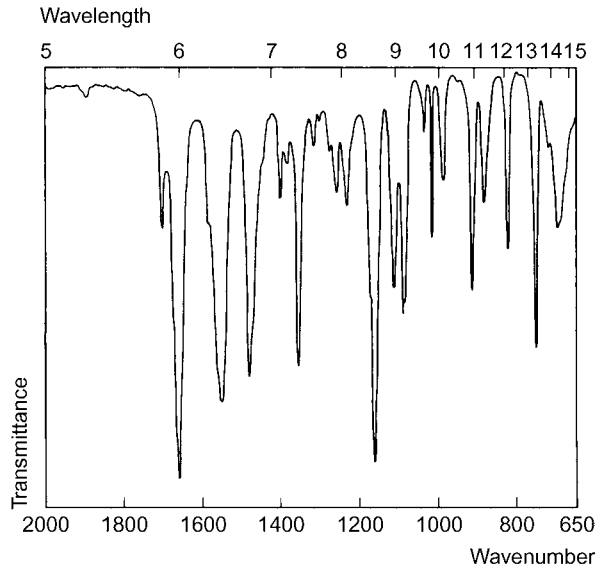
Gas Chromatography System GA—chlorpropamide RI 1791, chlorpropamide-Me RI 2165, chlorpropamide-Me_2 RI 2250.

High Performance Liquid Chromatography System HX—RI 450; system HY—RI 411 andRI 413; system HZ—retention time 5.0 min; system HAA—Retention time 17.7 min.

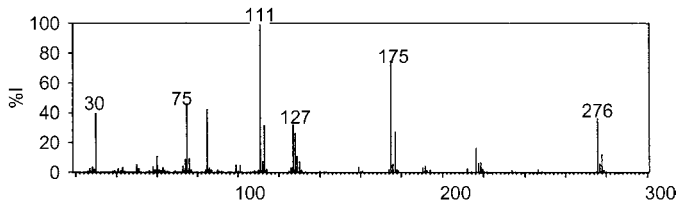
Ultraviolet Spectrum Methanolic acid—232 nm (A^1_1=598a).



Infrared Spectrum Principal peaks at wavenumbers 1661, 1159, 1553, 757, 1086, 909 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 111, 175, 75, 85, 30, 276, 127, 113.



Quantification

Blood **GC** ECD. Limit of detection, 1 mg/L for chlorpropamide or tolbutamide [Matin, Rowland 1973].

Plasma **GC** FID. Limit of detection, 50 µg/L for chlorpropamide or tolbutamide [Midha *et al.* 1976]. See Blood [Matin, Rowland 1973].

Serum **HPLC** UV detection. Chlorpropamide and tolbutamide. Limit of detection, 7 mg/L for chlorpropamide [Hill, Crechiolo 1978].

Urine **HPLC** UV detection. For method for chlorpropamide and metabolites using initial separation by thin-layer chromatography, see Taylor [1972].

Disposition in the Body Rapidly and completely absorbed after oral administration. The main metabolic reactions are hydroxylation at the 2- and 3-positions of the propyl substituent in the side-chain, *N*-dealkylation and hydrolysis to form the sulfonamide metabolite. About 80% of a single oral dose is excreted in the urine in 7 days. During chronic therapy, up to 100% of a dose is excreted in the urine in 24 h, with about 18% of the dose as unchanged drug, 2% as 4-chlorobenzenesulfonamide, 20% as 4-chlorobenzenesulfonylurea, 55% as 2-hydroxychlorpropamide, and 2% as 3-hydroxychlorpropamide.

Therapeutic Concentration In plasma, usually in the range 30 to 250 mg/L. Following single oral doses of 250 mg to 6 subjects, peak plasma concentrations of 23.9 to 39.4 mg/L (mean 28) were attained in 1 to 7 h. After daily oral doses of 250 to 500 mg to 4 subjects, steady-state plasma concentrations of 75.5 to 245.5 mg/L (mean 142) were reported; plasma concentrations of 4-chlorobenzenesulfonylurea and 2-hydroxychlorpropamide ranged from 3 to 6 mg/L and <1 to 9 mg/L, respectively [Taylor 1972].

Toxicity Prolonged hypoglycaemic coma has been reported after overdosage but fatalities are comparatively rare, although some instances of fatal blood dyscrasias have been reported. Peak plasma concentrations of 200 to 750 mg/L have been observed in comatose subjects.

Half-life Plasma half-life, 20 to 45 h (mean 35); increased in renal impairment.

Volume of Distribution About 0.1 to 0.3 L/kg.

Protein Binding 60 to 95%.

Dose 100 to 500 mg daily.

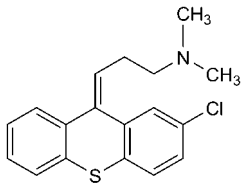
Hill RE, Crechiolo J (1978). Determination of serum tolbutamide and chlorpropamide by high-performance liquid chromatography. *J Chromatogr B, Biomed Appl* 145(1): 165–168.
Matin SB, Rowland M (1973). Determination of tolbutamide and chlorpropamide in biological fluids. *J Pharm Pharmacol* 25: 186–187.
Midha KK *et al.* (1976). GLC determination of plasma levels of intact chlorpropamide or tolbutamide. *J Pharm Sci* 65: 576–579.
Taylor JA (1972). Pharmacokinetics and biotransformation of chlorpropamide in man. *Clin Pharmacol Ther* 13: 710–718.

C

Chlorprothixene

Antipsychotic, Tranquilliser
$C_{18}H_{18}ClNS$ = 315.9
CAS—113-59-7

IUPAC Name (3*E*)-3-(2-Chlorothioxanthen-9-ylidene)-*N*,*N*-dimethylpropan-1-amine
Synonyms α-2-Chloro-10-(3-dimethylaminopropylidene)thiaxanthene; (*Z*)-2-chloro-*N*,*N*-dimethylthioxanthene-Δ9,γ-propylamine; *cis*-2-chloro-*N*,*N*-dimethyl-3-thioxanthen-9-ylidenepropylamine; (*Z*)-3-(2-chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethyl-1-propanamine; N-714; Ro-4-0403.
Proprietary Names *Taractan*; *Tarasan*; *Truxal*; *Truxaletten*. It is also an ingredient of *Silgastrin-T*.



Chemical Properties Pale-yellow crystalline powder. Mp 97° to 98°. Protect from light. Soluble 1 in 1700 of water, 1 in 29 of ethanol, 1 in 18 of acetone, 1 in 2 of chloroform and 1 in 14 of ether. Incompatible with acids and alkalis. pK_a 8.8 [Baselt 2008]. Log *P* (octanol/water) 5.18. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable in acetonitrile and serum samples for at least 6 months when stored at –20° [Bagli *et al.* 1994].

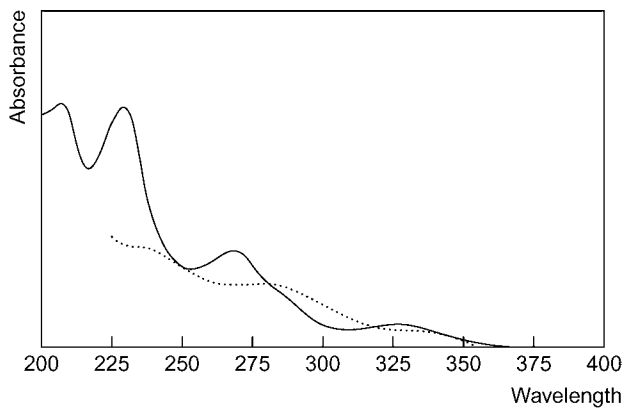
Chlorprothixene Hydrochloride

$C_{18}H_{18}ClNS$, HCl = 352.4
CAS—6469-93-8
Proprietary Names *Cloxan*; *Truquil*.
Chemical Properties White or almost-white crystalline powder. Mp 221°. Protect from light. Freely soluble in water at pH 6 to pH 6.5, slightly soluble in dichloromethane.

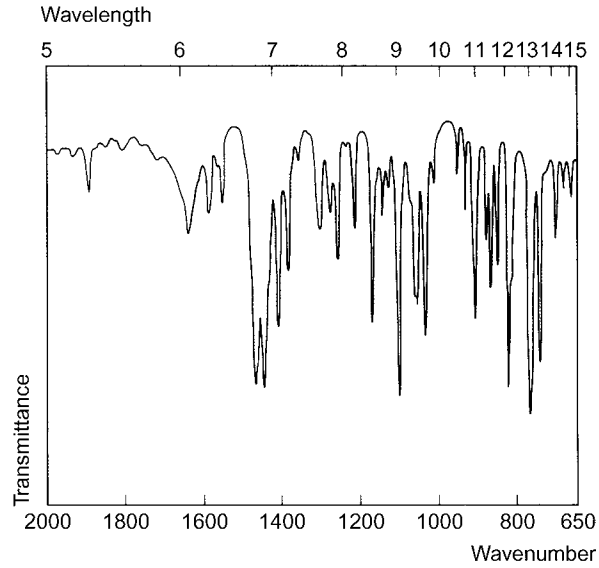
Chlorprothixene Mesilate

$C_{18}H_{18}ClNS$, CH_3SO_3H, H_2O = 430.0
Synonym Chlorprothixene mesylate

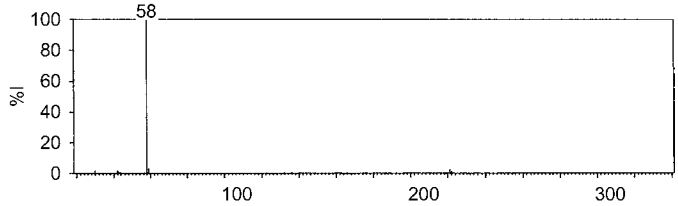
Colour Tests Formaldehyde–sulfuric acid—red (orange fluorescence under ultraviolet light); Liebermann's reagent—red; Mandelin's test—red; Marquis test—red-orange; sulfuric acid—orange (fluoresces under ultraviolet light).
Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.51; system TC—R_f 0.51; system TE—R_f 0.74; system TL—R_f 0.25; system TAE—R_f 0.34; system TAF—R_f 0.51; system TAJ—R_f 0.20; system TAK—R_f 0.08; system TAL—R_f 0.65 (acidified iodoplatinate solution, positive).
See [Zingales 1968].
Gas Chromatography System GA—chlorprothixene RI 2492, M (OH-dihydro-) isomer 1 RI 2750, M (OH-dihydro-) isomer 2 RI 2790, M (OH-methoxy-dihydro-) RI 2810, M (*N*-oxide)-$(CH_3)_2$NOH RI 2410, M (*N*-oxide sulfoxide-)-$(CH_3)_2$NOH I RI 2560, M (sulfoxide) RI 2720, Art (dihydro-) RI 2490, Art (Cl-thioxanthenone) RI 2260; system GB—chlorprothixene RI 2608; system GF—RI 2910.
High Performance Liquid Chromatography System HA—*k* 3.0; system HX—RI 459; system HY—RI 353; system HZ—RT 10.1 min; system HAX—RT 17.6 min; system HAY—RT 8.3 min.
Column: Nucleosil RP-100 CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : pyridine : 0.14 mol/L sodium acetate (pH 3.1, 698 : 2 : 300), flow rate 0.9 mL/min. Electrochemical detection. Retention time: 10.2 min [Bagli *et al.* 1994].
Ultraviolet Spectrum Aqueous acid—230 (A_1^1 = 1096b), 268, 325 nm [Allgen *et al* 1960].



Infrared Spectrum Principal peaks at wavenumbers 776, 1104, 832, 746, 1030, 1170 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 59, 221, 30, 42, 222, 255, 43.



Quantification

Blood **TLC** Plates: silica. Solvent system: benzene : acetone: 25% ammonia (1, 100 : 20 : 10), benzene : dioxane : 25% ammonia (2, 65 : 35 : 5), *n*-propanol: 25% ammonia (3, 100 : 1), *n*-butanol : acetic acid : water (4, 80 : 20 : 20). R_f: 0.78, 0.92, 0.61 and 0.49 for solvent systems 1, 2, 3 and 4, respectively. Limit of quantification, 0.5 mg/L [Christensen 1974].
Plasma **HPLC** Column: Supelco LC-PCN cyano (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L potassium dihydrogen phosphate (pH 4.5; 60 : 40), flow rate 2.0 mL/min. Retention time: 7.6 min. *k'*: 9.55. UV detection (λ = 229 nm). Limit of quantification, 5 µg/L [Brooks *et al.* 1985].
Serum **HPLC** Column: Nucleosil 100 CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : pyridine: 0.14 mol/L sodium acetate (pH 3.1; 698 : 2 : 300), flow rate 0.9 mL/min. Electrochemical detection. Retention time: 10.2 min. Limit of detection 0.5 µg/L [Bagli *et al.* 1994].
LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9; 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.1 min. Limit of quantification, 0.93 µg/L [Kirchherr, Kühn-Velten 2006].
Urine **TLC** See Blood [Christensen 1974].
Gastric Aspirate **GC-MS** Column: 3% QF-1 on Supelcoport 80/100 mesh (2.5 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 210°. EI ionisation at 70 eV. Limit of detection not reported [Pettersen *et al.* 1981].
Hair **GC** Column: AC-5 capillary (15 m × 0.25 mm i.d.). Temperature programme: 180° to 270° at 10°/min for 10 min. NPD. Limit of detection, 0.1–0.5 µg/g [Shen *et al.* 2002].
Liver **TLC** See Blood [Christensen 1974].
Disposition in the Body Readily absorbed after oral administration, but undergoes extensive first-pass metabolism. The major metabolite is the sulfoxide, but *N*-demethylation, *N*-oxidation and ring hydroxylation also occur [Breyer-Pfaff *et al.* 1985]. Chlorprothixene and its sulfoxide metabolites are distributed into breast milk. After daily oral doses, up to about 30% is excreted in the urine in 24 h as chlorprothixene sulfoxide, and up to ~40% is eliminated in the faeces, also as the sulfoxide [Allgen *et al.* 1960].

Therapeutic Concentration

Therapeutic doses range from 60 mg/day in ambulatory patients to 600 mg/day in institutionalised patients. A single oral dose of 50 mg produces blood concentrations of 0.04 mg/L 8 h after administration while 400 mg every 3 h resulted in concentrations ranging from 0.06 to 0.3 mg/L [de Silva, D'Arconte 1969].
Following a single oral dose of 30 mg to one subject, a peak blood concentration of ~0.01 mg/L was attained in 4 h [Raaflaub 1975].
After an overnight fast, 2 healthy male volunteers were administered a single oral dose of chlorprothixene. Maximum plasma concentrations of 5.3 and 8.8 µg/L were reached at 5 and 4 h, respectively [Brooks *et al.* 1985].

Oral administration of a single 100 mg dose of chlorprothixene, in the form of a solution (base), suspension (base) or tablets (hydrochloride), to 8 subjects, produced respective peak plasma chlorprothixene concentrations of 22.4 to 65.7 µg/L (mean 31.6) at 3.0 h, 13.3 to 70.6 µg/L (mean 24.2) at 2.88 h and 13.4 to 52.6 µg/L (mean 25.8) at 2.5 h [Bagli *et al.* 1996].

Toxicity The estimated minimum lethal dose is 2.5 g, although recovery has occurred after the ingestion of 12 g.

Two to 12 h after a suicidal ingestion of ~10 g of chlorprothixene, a 31-year-old female had the following plasma concentrations: chlorprothixene 10 mg/L, desmethylchlorprothixene 8 mg/L and chlorprothixene sulfoxide 1 mg/L. After 4-h haemoperfusion, the levels were: chlorprothixene 0.1 mg/L, desmethylchlorprothixene 0.1 mg/L and chlorprothixene sulfoxide was not detected [Köppel *et al.* 1987].

In 6 fatalities attributed to the ingestion of about 2.5 to 4 g of chlorprothixene, the following postmortem concentrations were reported: blood, chlorprothixene <0.1 to 0.4 mg/L, total metabolites <0.1 to 0.3 mg/L; liver, chlorprothixene 5 to 42 mg/L (mean 18), total metabolites <0.1 to 44 mg/L; in 1 case a urine concentration of total thioxanthenes of 15 mg/L was also reported; desmethylchlorprothixene was the major metabolite in the liver. Alcohol was also detected in 3 cases [Christensen 1974].

In a fatality involving the ingestion of up to 4 g of chlorprothixene, the postmortem tissue concentrations shown below were reported (mg/L):

	Chlorprothixene	*Chlorprothixene sulfoxide*
Blood	0.1	0.6
Bile	3.9	7.0
Urine	0.4	3.4

The stomach contents contained 340 mg chlorprothixene and 25 mg of the sulfoxide [Poklis *et al.* 1983].

Bioavailability ≈40%.

Half-life Plasma half-life, 8 to 12 h [Köppel *et al.* 1987] or 17 to 24 h [de Silva, D'Arconte 1969].

Volume of Distribution 11 to 23 L/kg [Köppel *et al.* 1987].

Clearance Plasma clearance, ≈14 to 20 mL/min/kg.

Protein Binding >99% [Köppel *et al.* 1987].

Dose Available as 10 to 100 mg tablets as the free base or a 100 mg/5 mL concentrate as the lactate or hydrochloride salts for oral administration; vials of 25 mg/2 mL as the hydrochloride salt are available for parenteral injection. The usual dose is 45 to 200 mg daily, oral or IM, in divided doses; up to 600 mg daily has been given.

Allgen LG *et al.* (1960). On the elimination of chlorprothixene in rat and man. *Experientia* 16: 325.

Bagli M *et al.* (1994). Quantification of chlorprothixene, levomepromazine and promethazine in human serum using high-performance liquid chromatography with coulometric electrochemical detection. *J Chromatogr B Biomed Appl* 657: 141–148.

Bagli M *et al.* (1996). Pharmacokinetics of chlorprothixene after single intravenous and oral administration of three galenic preparations. *Arzneimittelforschung* 46: 247–250.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, California, Biomedical Publications.

Breyer-Pfaff U *et al.* (1985). Phenolic metabolites of chlorprothixene in man and dog. *Drug Metab Dispos* 13: 479–489.

Brooks MA *et al.* (1985). Determination of chlorprothixene and its sulfoxide metabolite in plasma by high-performance liquid chromatography with ultraviolet and amperometric detection. *J Chromatogr* 337: 351–362.

Christensen H (1974). Chlorprothixene and its metabolites in blood, liver and urine from fatal poisoning. *Acta Pharmacol Toxicol (Copenh)* 34: 16–26.

de Silva JA, D'Arconte L (1969). The use of spectrophotofluorometry in the analysis of drugs in biological materials. *J Forensic Sci* 14: 184–204.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Köppel C *et al.* (1987). Hemoperfusion in severe chlorprothixene overdose. *Intensive Care Med* 13: 358–360.

Pettersen JE *et al.* (1981). Identification of 2-chlorothioxanthen-9-one in gastric aspirate in a case of chlorprothixene poisoning. *J Pharm Sci* 70: 812–813.

Poklis A *et al.* (1983). Chlorprothixene and chlorprothixene-sulfoxide in body fluids from a case of drug overdose. *J Anal Toxicol* 7: 29–32.

Raaflaub J (1975). On the pharmacokinetics of chlorprothixene in man. *Experientia* 31: 557–558.

Shen M *et al.* (2002). Detection of antidepressant and antipsychotic drugs in human hair. *Forensic Sci Int* 126: 153–161.

Zingales I (1968). Systematic identification of psychotropic drugs by thin layer chromatography. II. *J Chromatogr* 34: 44–51.

Chlorpyrifos

Organophosphate, Acaricide, Insecticide

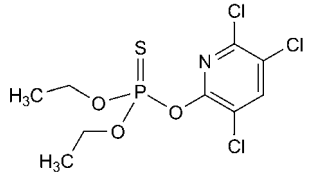
$C_9H_{11}Cl_3NO_3PS$ = 350.6

CAS—2921-88-2

IUPAC Name Phosphorothioic acid *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) ester

Synonyms Chlorpyrifos-ethyl; Dowco 179; ENT-27311.

Proprietary Names *Affront*; *Brodan*; *Bullet*; *Detmol UA*; *Dursban*; *Empire*; *Eradex*; *Ethion*; *Lorsban*; *Paqeant*; *Piridane*; *Pyrinex*; *Scout*; *Stipend*.



Chemical Properties Off-white to pale-yellow granular crystals. Mp 41° to 42°. It is practically insoluble in water (2 mg/L at 24°); readily soluble in organic solvents, acetone (6500 g/kg at 25°), benzene (7900 g/kg at 25°), chloroform (6300 g/kg at 25°), carbon disulphide (5900 g/kg at 25°), diethyl ether (5100 g/kg at 25°), xylene (5000 g/kg at 25°), methanol (450 g/kg at 25°), methylene chloride and ethylene dichloride. Log *P* (octanol/water) 4.96.

Chlorpyrifos-(Me)

$C_7H_7Cl_3NO_3PS$ = 322.5

CAS—5598-13-0

Synonyms Dowco 214; ENT-27520; NSC-60380; OMS-1155.

Proprietary Names *Douco 214*; *Dusban-methyl*; *Graincote*; *Noltron*; *Reldan*; *Smite*; *Turmar*; *Zertell*.

Chemical Properties Colourless, crystalline solid. Mp 45.5° to 46.5°. It is soluble in water (5 mg/L at 25°); very soluble in organic solvents; acetone (6400 g/kg at 24°), benzene (5200 g/kg at 24°), diethyl ether (4800 g/kg at 24°), chloroform (3500 g/kg at 24°), methanol (390 g/kg at 24°) and hexane (230 g/kg at 24°).

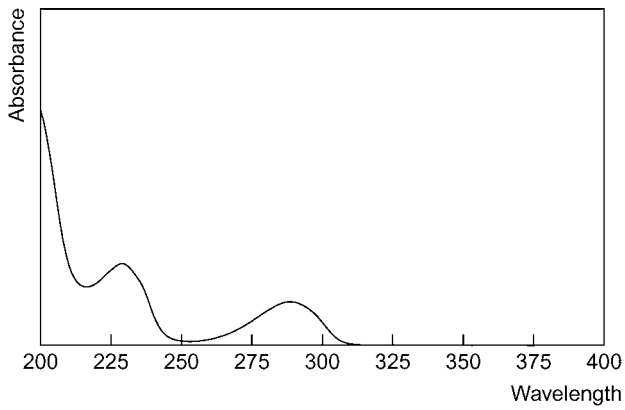
Thin-layer Chromatography System TE—chlorpyrifos R_f 0.99; system TX—chlorpyrifos R_f 0.64, chlorpyrifos-methyl R_f 0.56; system TY—chlorpyrifos R_f 0.95, chlorpyrifos-methyl R_f 0.89; system TAB—chlorpyrifos-methyl R_f 0.60; system TAC—chlorpyrifos-methyl R_f 0.35; system TAE—chlorpyrifos R_f 0.85.

Gas Chromatography System GA—chlorpyrifos RI 1955, chlorpyrifos-methyl RI 1847; system GKA—chlorpyrifos-methyl RI 1866; system GKB—chlorpyrifos-methyl RI 2060; system GKC—chlorpyrifos-methyl RI 2234.

Column: DB-17 (30 × 0.25 mm i.d., 0.5 µm). Temperature programme: 80° for 1 min, to 180° at 25°/min to 250° at 20°/min for 5 min to 320° for 2 min. Carrier gas: He. SIM acquisition mode. Retention time: 9.26 min [Brzak *et al.* 1998].

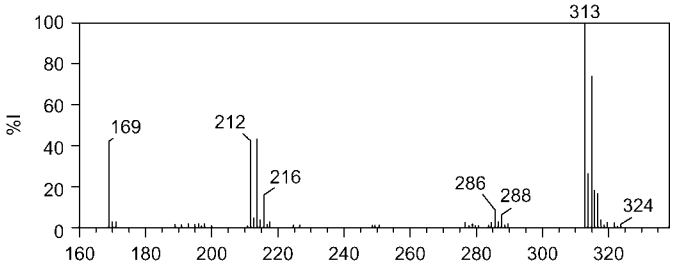
High Performance Liquid Chromatography System HAO—chlorpyrifos-methyl *k* 6.18.

Ultraviolet Spectrum Aqueous acid (acetonitrile)—230 and 289 nm.



Infrared Spectrum Principal peaks at wavenumber 1406, 1038, 850, 967, 1166, 1271 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 313, 315, 212, 169, 214, 216.



Quantification

Blood **GC** Column: 3% OV-17 and 8% QF-1 on Gas Chrom Q (2 m × 2 mm i.d.). Carrier gas: N_2, 200 mL/min. Temperature: 145°. FPD. Limit of quantification, 10 µg/L [Nolan *et al.* 1984].

GC-MS Column: DB5-MS 5% phenylmethylpolysiloxane (30 m × 250 µm i.d., 1 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection, 3.1–98.0 µg/L [Ostrea, Jr. *et al.* 2006]. Column: DB-17 capillary (30 m × 0.25 mm i. d., 0.5 µm). Carrier gas: He, 10 psi. Temperature programme: 80° for 1 min to 180° at 25°/min to 250° at 20°/min for 5 min to 320° at 40°/min for 2 min. NICI. Limit of quantification, 1 µg/L [Brzak *et al.* 1998].

Plasma **GC** Column: SP-2250-DB glass (6′ × 0.25" o.d.). Carrier gas: N_2, 30 mL/min. Temperature: 223°. NPD. Retention time: 2.58 min. Limit of quantification, 30 µg/L [Osterloh *et al.* 1983].

Serum **GC-MS** Column: fused silica cross-linked methylsilicone (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 22 psi. Temperature programme: 180° for 1 min to 255° at 15°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 83 µg/L, limit of detection, 25 µg/L [Martínez *et al.* 2004]. Column: DB-5 fused silica (30 m × 0.25 mm i.d.). Temperature programme: 40° for 1 min to 100° at 25°/min to 200° at 5°/min for 10 min. EI ionisation, SIM acquisition mode. Limit of detection not reported [Drevenkar et al 1993].

HPLC Column: Nucleosil $5C_{18}$ (15 cm × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (50 : 50), flow rate 1.0 mL/min. DAD (λ = 230 nm). Limit of detection, 0.61 mg/L [Cho *et al.* 1997].

LC-MS Column: Supelco Discovery C_{18} (50 × 2.1 mm i.d., 5 µm) followed by Supelco ABZ+ (100 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01% formic acid (65 : 35) followed by acetonitrile : 0.0025% formic acid (80 : 20). ESI, positive and negative ion modes. Retention time: 3.2 min. Limit of detection,1.5 µg/L [Sancho *et al.* 2000].

Urine **GC** Column: DB-5 (30 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 20 mL/min. Temperature: 145°. ECD. Limit of detection, 10 µg/L [Jitsunari *et al.* 1989]. See Blood [Nolan *et al.* 1984]. See Plasma [Osterloh *et al.* 1983].

GC-MS Column: HP35 cross-linked 35% diphenyldimethylpolysiloxane capillary (60 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.45 bar for 5 min to 2.0 bar at 0.035 bar/min to 2.25 bar at 0.07 bar/min. Temperature programme: 90° for 1 min to 125° at 25°/min for 1 min to 230° at 6°/min for 2 min to 280° at 30°/min for 12 min. EI ionisation at 70 eV. Limit of quantification, 0.1 µg/L, limit of detection, 0.05 µg/L [Koch, Angerer 2001]. Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 10 psi. Temperature programme: 100° for 1 min to 300° at 20°/min for 2 min. NICI, SIM acquisition mode. Limit of quantification, 1 µg/L, limit of detection, 0.2 µg/L [Ormand *et al.* 1999]. Column: J & W DB-5 fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1-2 mL/min. Temperature programme: 175° for 5 min to 275° at 25°/min. NCI at 70 eV, SIM acquisition mode. Limit of detection, 0.5 µg/L for TCP metabolite [Bartels, Kastl 1992].

HPLC See Serum. Limit of detection, 0.61 mg/L [Cho *et al.* 1997].

LC-MS See Serum. Limit of detection, 0.5 µg/L [Sancho *et al.* 2000].

Bile **GC** See Plasma [Osterloh *et al.* 1983].

CSF **GC** See Plasma [Osterloh *et al.* 1983].

Gastric Juice **GC** See Plasma [Osterloh *et al.* 1983].

Milk **GC** Column: 4% SE-30 and 6% OV-210 on 80/100 mesh Gas Chrom Q (6′ × 4 mm i.d.). Temperature: 230°. Carrier gas: N_2, 30 mL/min. FPD. Limit of detection not reported [Dogheim *et al.* 1996].

Brain **GC** See Plasma, limit of quantification, 80 ng/g [Osterloh *et al.* 1983].

Diaphragm **GC** See Brain [Osterloh *et al.* 1983].

Hair **GC-MS** See Blood. Limit of detection, 30.5–488.0 ng/g [Ostrea, Jr. *et al.* 2006].

Kidney **GC** See Brain [Osterloh *et al.* 1983].

Liver GC See Brain [Osterloh *et al.* 1983].

Other **GC** Food and Water Samples. See Milk [Dogheim *et al.* 1996].

GC-MS Handwipes. Column: DB-5 capillary (30 m × 0.25 mm). SIM acquisition mode. Limit of detection, 10 µg/L [Geno *et al.* 1996].

Disposition in the Body Chlorpyrifos is readily absorbed into the bloodstream from the gastrointestinal tract through the lungs and through the skin. After the formation of oxon by oxidative desulfuration, the major metabolites observed are 3,5,6-trichloro-2-pyridinol (TCP), diethylphosphate and diethylthio-phosphate. Chlorpyrifos and its metabolites are eliminated rapidly from the body via urine and faeces. Most of an ingested dose is recovered in urine as the metabolites whereas only 1% of the compound absorbed through dermal exposure is detected as metabolites in urine. No significant bioaccumulation has been observed.

Blood Concentration

Six healthy male Caucasian volunteers were administered a single oral dose of 0.5 mg/kg bodyweight chlorpyrifos followed by topical exposure of 0.5 mg/kg 4 weeks later. After both doses, blood chlorpyrifos concentrations of <30 µg/L were observed. The maximum TCP blood concentration was on average 930 µg/L (range, 510 to 1350 µg/L) 6 h after oral administration, and 63 µg/L (range: 29 to 122 µg/L) 24 h after topical administration [Nolan *et al.* 1984].

Toxicity Chlorpyrifos is very toxic and affects the central nervous, cardiovascular and respiratory systems. It is irritating to the skin and eyes. Doses up to 28 g may be fatal. No toxic effects have been observed with doses of 0.1 mg/kg body weight daily taken over 4 weeks. This dose is deemed the acceptable daily intake. A toxic dose of 300 mg/kg body weight has effects on the peripheral nervous system.

A pregnant 23-year-old attempted suicide wth chlorpyrifos-ethyl. The foetus had no cardiac activity. At postmortem the foetal blood had a concentration of 264 µg/L chlorpyrifos [Sebe *et al.* 2005].

A 26-year-old male ingested ~360 mL Dexol (6.7% chloropyrifos in 76.8% distillates), 360 mL of Ortho Weed-B-Gone M (10.8% diethylamine salts of 2,4-D, 11.6% MCPP and 77.6% aqueous inert ingredients) and a few granules of D-Con concentrate (0.025% warfarin). On hospital admission the gastric aspirate contained 17.2 µg/g chlorpyrifos. At postmortem chlorpyrifos tissue concentrations (ng/g) were as shown below.

Tissue	*Chlorpyrifos*
Brain grey matter (frontal lobe)	91.0
Brain white matter (frontal lobe)	574.6
Kidney	415.6
Liver	4187.0
Pancreas	2373.2

[Osterloh *et al.* 1983].

Note For an unusual case of chlorpyrifos ingestion by a 16-month-old female, see Mattingly *et al.* [2003]. For a review of 439 chlorpyrifos poisonings in Sri Lanka, see Eddleston *et al.* [2005] and >30,000 US suspected exposures including 8 fatalities, see Kingston *et al.* [1999]. For a case study of chlorpyrifos poisoning in pregnancy, see Solomon, Moodley [2007].

Half-life Chlorpyrifos, 1 day; TCP, 26.9 h (oral dose) and 30 h (dermal exposure).

Volume of Distribution TCP, 181 mL/kg.

Bartels MJ, Kastl PE (1992). Analysis of 3,5,6-trichloropyridinol in human urine using negative-ion chemical ionization gas chromatography-mass spectrometry. *J Chromatogr* 575: 69–74.

Brzak KA *et al.* (1998). Determination of chlorpyrifos, chlorpyrifos oxon, and 3,5,6-trichloro-2-pyridinol in rat and human blood. *J Anal Toxicol* 22: 203–210.

Cho Y *et al.* (1997). Determination of organophosphorous pesticides in biological samples of acute poisoning by HPLC with diode-array detector. *Chem Pharm Bull (Tokyo)* 45: 737–740.

Drevenkar V *et al.* (1993). Chlorpyrifos metabolites in serum and urine of poisoned persons. *Chem Biol Interact* 87: 315–322.

Dogheim SM *et al.* (1996). Monitoring of pesticide residues in human milk, soil, water, and food samples collected from Kafr El-Zayat Governorate. *J AOAC Int* 79: 111–116.

Eddleston M *et al.* (2005). Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. *Lancet* 366: 1452–1459.

Galera MM *et al.* (2006). Determination of nine pyrethroid insecticides by high-performance liquid chromatography with post-column photoderivatization and detection based on acetonitrile chemiluminescence. *J Chromatogr A* 1113: 191–197.

Geno PW *et al.* (1996). Handwipe sampling and analysis procedure for the measurement of dermal contact with pesticides. *Arch Environ Contam Toxicol* 30: 132–138.

Jitsunari F *et al.* (1989). Determination of 3,5,6-trichloro-2-pyridinol levels in the urine of termite control workers using chlorpyrifos. *Acta Med Okayama* 43: 299–306.

Kingston RL (1999). Chlorpyrifos: a ten-year US poison center exposure experience. *Vet Hum Toxicol* 41: 87–92.

Koch HM, Angerer J (2001). Analysis of 3,5,6-trichloro-2-pyridinol in urine samples from the general population using gas chromatography-mass spectrometry after steam distillation and solid-phase extraction. *J Chromatogr B Biomed.Sci.Appl.* 759: 43–49.

Martínez MA *et al.* (2004). Attempted suicide by ingestion of chlorpyrifos: identification in serum and gastric content by GC-FID/GC-MS. *J Anal Toxicol* 28: 609–615.

Mattingly JE *et al.* (2003). Intermediate syndrome after exposure to chlorpyrifos in a 16-month-old girl. *J Emerg Med* 25: 379–381.

Nolan RJ *et al.* (1984). Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol Appl Pharmacol* 73: 8–15.

Ormand JR *et al.* (1999). Semiautomated preparation of 3,5,6-trichloro-2-pyridinol in human urine using a Zymate XP laboratory robot with quantitative determination by gas chromatography-negative-ion chemical ionization mass spectrometry. *J Anal Toxicol* 23: 35–40.

Osterloh J *et al.* (1983). Toxicologic studies in a fatal overdose of 2,4-D, MCPP, and chlorpyrifos. *J Anal Toxicol* 7: 125–129.

Ostrea EMJr *et al.* (2006). Maternal hair–an appropriate matrix for detecting maternal exposure to pesticides during pregnancy. *Environ Res* 101: 312–322.

Sancho JV *et al.* (2000). Direct determination of chlorpyrifos and its main metabolite 3,5,6-trichloro-2-pyridinol in human serum and urine by coupled-column liquid chromatography/electrospray-tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 1485–1490.

Sebe A *et al.* (2005). Organophosphate poisoning associated with fetal death: a case study. *Mt Sinai J Med* 72: 354–356.

Solomon GM, Moodley J (2007). Acute chlorpyrifos poisoning in pregnancy: a case report. *Clin Toxicol.(Phila)* 45: 416–416.

Chlorquinaldol

Antibacterial, Antifungal

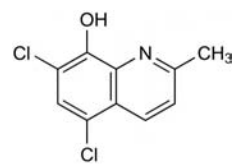
$C_{10}H_7Cl_2NO$ = 228.1

CAS—72-80-0

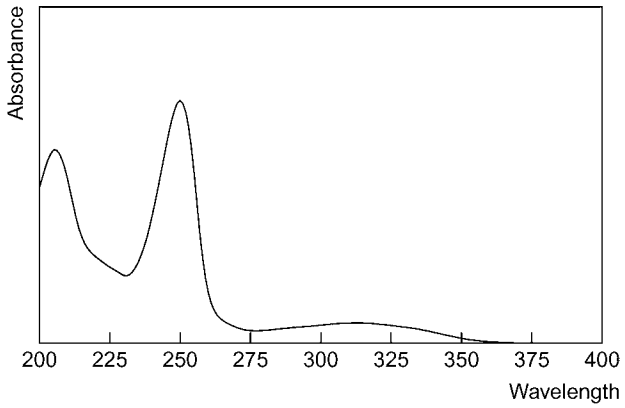
IUPAC Name 5,7-Dichloro-2-methyl-8-quinolinol

Synonyms Chlorchinaldol; hydroxydichloroquinaldine.

Proprietary Names *Afungil*; *Gyno-Sterosan*; *Gynothérax*; *Quesil*; *Siogen(o)*; *Siosteran*; *Soprosan*; *Sterosan*; *Steroxin*.



Chemical Properties A green or yellowish-brown crystalline powder. Mp about 113°. Practically insoluble in water; soluble 1 in 100 of ethanol, 1 in 20 of chloroform, and 1 in 33 of ether; soluble in acetone, benzene, and glacial acetic acid. Log *P* (octanol/water), 3.5.
Colour Test Ferric chloride—blue-green.
Thin-layer Chromatography System TA—R_f 0.71 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1802.
Ultraviolet Spectrum Aqueous acid—263 (A^1_1=2100b), 330 nm (A^1_1=132b); aqueous alkali—263 (A^1_1=1410b), 345 nm (A^1_1=186b); methanol—251 (A^1_1=1900b), 315 nm (A^1_1=146b).

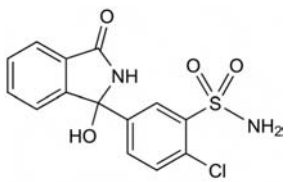


Infrared Spectrum Principal peaks at wavenumbers 1656, 1739, 1605, 758, 1111, 1153 cm^{-1} (KBr disk).
Uses Chlorquinaldol is used topically in a concentration of 3%; it has been used as 200 mg vaginal tablets.

Chlortalidone

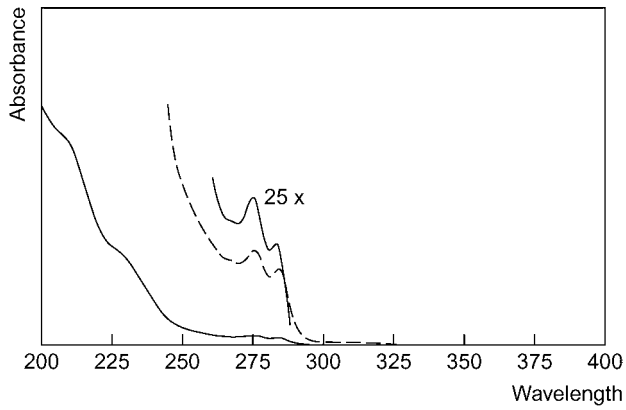
Diuretic
$C_{14}H_{11}ClN_2O_4S$ = 338.8
CAS—77-36-1

IUPAC Name 2-Chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl)benzenesulfonamide
Synonyms 2-Chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1*H*-isoindol-1-yl)-benzenesulfonamide; chlorthalidone; G-33182; phthalamudine.
Proprietary Names *Hydro-long*; *Hygroton*; *Igrolina*; *Novothalidone*; *Renon*; *Uridon*; *Urolin*; *Zambesil*. It is an ingredient of *Kalspare*, *Lopresoretic*, *Regroton*, and *Tenoret(ic)*.

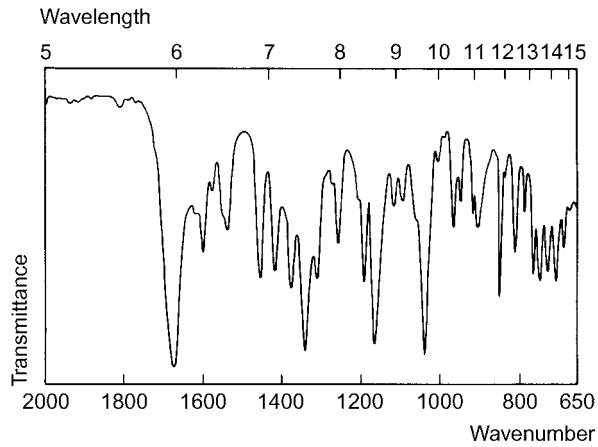


Chemical Properties A white or yellowish-white crystalline powder. Mp about 220°, with decomposition. Practically insoluble in water; soluble 1 in 150 of ethanol, 1 in 650 of chloroform, and 1 in 25 of methanol; slightly soluble in ether; soluble in solutions of alkali hydroxides. pK_a 9.4. Log *P* (octanol/water), 0.8.

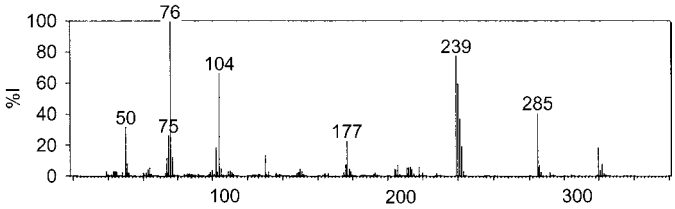
Colour Tests Koppanyi-Zwikker test—violet; Marquis test—yellow.
Thin-layer Chromatography System TD—R_f 0.04; system TE—R_f 0.42; system TF—R_f 0.40; system TAD—R_f 0.23; system TAE—R_f 0.88; system TAF—R_f 0.83; system TAJ—R_f 0.17; system TAK—R_f 0.10; system TAL—R_f 0.63.
Gas Chromatography System GA—chlortalidone RI 2145, chlortalidone-Me_4 RI 2630; system GX—chlortalidone-Me_4 retention time 7.7 min; system GY—chlortalidone-Me_4 retention time 4.8 min.
High Performance Liquid Chromatography System HN—*k* 1.28; system HX—RI 367; system HY—RI 308; system HZ—retention time 2.3 min.
Ultraviolet Spectrum Ethanol—275 (A^1_1=57a), 284 nm (A^1_1=45a).



Infrared Spectrum Principal peaks at wavenumbers 1685, 1033, 1160, 845, 1190, 1310 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 76, 239, 104, 240, 285, 241, 50, 75.



Quantification
Blood **HPLC** UV detection. Limit of detection, 100 µg/L [MacGregor *et al.* 1984]. UV detection. Limit of detection, 30 µg/L [Guelen *et al.* 1980].
Plasma **GC** AFID. Limit of detection, 10 µg/L [Fleuren, van Rossum 1978].
HPLC UV detection. Limit of quantification, 10 µg/L [Giachetti *et al.* 1997]. UV detection. Limit of detection, 30 µg/L [Guelen *et al.* 1980].
Urine **GC** AFID. See Plasma [Fleuren, van Rossum 1978].
HPLC UV detection (λ=214 nm). Limit of quantification, 0.1 mg/L, limit of detection, 0.02 mg/L [Salado *et al.* 1997]. See Blood [MacGregor *et al.* 1984]. See Blood [Guelen *et al.* 1980].
Erythrocytes **GC** AFID. See Plasma [Fleuren, van Rossum 1978].
Disposition in the Body Readily but incompletely absorbed after oral administration. It does not appear to be significantly metabolised. After a single dose, about 25 to 40% is excreted in the urine as unchanged drug and about 1% is eliminated in the bile; the quantity excreted in the urine appears to be dose-dependent. During daily therapy, about 50% of the daily dose is excreted unchanged in the urine in 24 h and about 25% is eliminated in the faeces.
Therapeutic Concentration
After single oral doses of 50 to 75 mg given to 7 subjects, peak plasma concentrations of 0.14 to 0.26 mg/L were attained in 1 to 3 h [Fleuren *et al.* 1979]. Following daily oral doses of 50 mg to 10 subjects, steady-state plasma concentrations of 0.2 to 1.4 mg/L (mean 0.5) were reported [Collste *et al.* 1976].
Bioavailability About 65%.
Half-life Plasma half-life, 35 to 70 h (mean, 48), increased in elderly subjects.
Volume of Distribution About 4 L/kg.
Clearance Plasma clearance, about 1 to 2 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, about 0.04 at therapeutic concentrations.
Protein Binding About 75%.
Note For a review of the pharmacokinetics of diuretics, see Beerman, Groschinsky-Grind [1980].

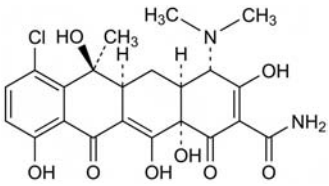
Dose Usually 50 mg daily or 100 to 200 mg on alternate days.

Beermann B, Groschinsky-Grind M (1980). Clinical pharmacokinetics of diuretics. *Clin Pharmacokinet* 5(3): 221–245.
Collste P *et al.* (1976). Interindividual differences in chlorthalidone concentration in plasma and red cells of man after single and multiple doses. *Eur J Clin Pharmacol* 9: 319–325.
Fleuren HL, van Rossum JM (1978). Determination of chlorthalidone in plasma, urine and red blood cells by gas chromatography with nitrogen detection. *J Chromatogr* 152(1): 41–54.
Fleuren HL *et al.* (1979). Absolute bioavailability of chlorthalidone in man: a cross-over study after intravenous and oral administration. *Eur J Clin Pharmacol* 15(1): 35–50.
Giachetti C *et al.* (1997). Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man. *J Chromatogr B Biomed Sci Appl* 698(1–2): 187–194.
Guelen PJM *et al.* (1980). *J Chromatogr B Biomed Appl* 181: 497–503.
MacGregor TR *et al.* (1984). Analysis of chlorthalidone in biological fluids by high-performance liquid chromatography using a rapid column cleanup procedure. *Ther Drug Monit* 6: 83–90.
Salado S *et al.* (1997). On-line solid-phase extraction and high-performance liquid chromatographic determination of chlorthalidone in urine. *J Chromatogr B Biomed Sci Appl* 690(1–2): 195–202.

Chlortetracycline

Antibacterial
$C_{22}H_{23}ClN_2O_8$ = 478.9
CAS—57-62-5

IUPAC Name (2*Z*,4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-[Amino(hydroxy)methylidene]-7-chloro-4-(dimethylamino)-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione
Synonym [4*S*-(4α,4aα,5aα,6β,12aα)]-7-Chloro-4-dimethylamino-1,4,4a,5,5a,6,11,-12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Proprietary Names *Acronize*; *Biomitsin*; *Orospray*.



Chemical Properties A yellow crystalline powder. Mp 168° to 169°. Very slightly soluble in water; very soluble in aqueous solutions above pH 8.5; slightly soluble in ethanol, acetone, ethyl acetate, and benzene; practically insoluble in ether. pK_a 3.3, 7.4, 9.3 (25°). Log *P* (octanol/pH 7.5), −0.9.

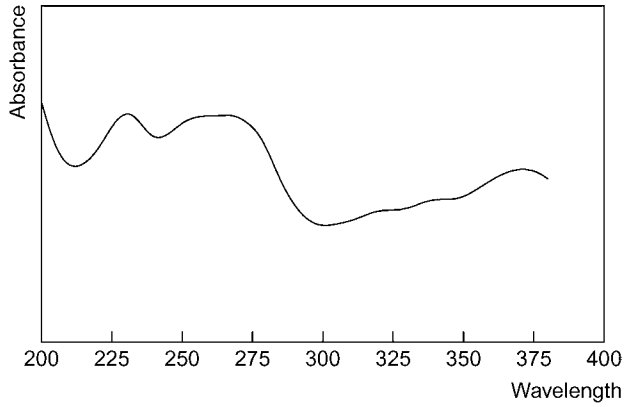
Chlortetracycline Calcium

CAS—5892-31-9
Chemical Properties A white powder. Practically insoluble in water.

Chlortetracycline Hydrochloride

$C_{22}H_{23}ClN_2O_8$, HCl = 515.3
CAS—64-72-2
Synonym Biomycin
Proprietary Names *Aureomycin(e)*; *Chlortet*. It is an ingredient of *Aureocort* and *Deteclo*.
Chemical Properties Yellow crystals. Mp about 210°, with decomposition. Soluble 1 in 75 to 1 in 110 of water and 1 in 250 to 1 in 560 of ethanol; practically insoluble in acetone, chloroform, and ether; soluble in solutions of alkali hydroxides and carbonates.

Colour Tests Benedict's reagent—red;formaldehyde–sulfuric acid—orange-brown; Mandelin's test—violet-brown→yellow; Marquis test—yellow→green; sulfuric acid—brown-blue.
Thin-layer Chromatography System TA—R_f 0.05, streaking (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HY—RI 280, RI 282.
Ultraviolet Spectrum Aqueous acid—266 (A^1_1=386a), 359 nm; aqueous alkali—253, 284, 346 nm.

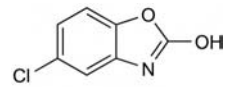


Infrared Spectrum Principal peaks at wavenumbers 1622, 1580, 1666, 1311, 1041, 1227 cm^{-1} (chlortetracycline hydrochloride, KBr disk). Polymorphism may occur.
Disposition in the Body Readily absorbed after oral administration but rapidly inactivated in the body. It is eliminated mainly by biliary excretion with only about 15% of a dose being excreted in the urine as unchanged drug.
Half-life Plasma half-life, about 6 h.
Protein Binding About 47%.
Dose 1 to 2 g of chlortetracycline hydrochloride daily.

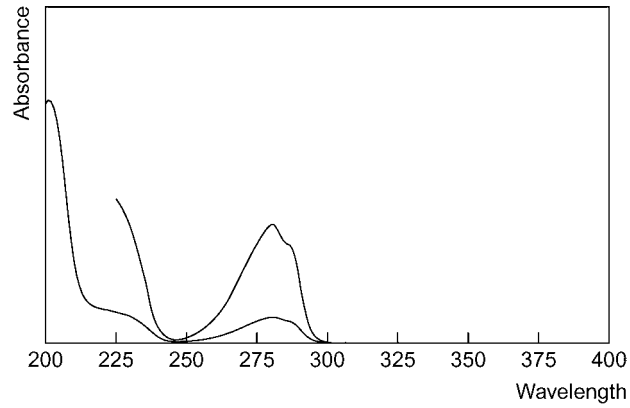
Chlorzoxazone

Muscle Relaxant
$C_7H_4ClNO_2$ = 169.6
CAS—95-25-0

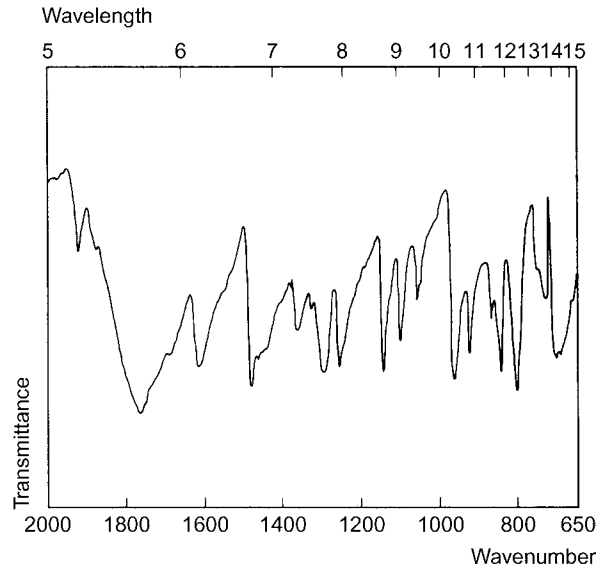
IUPAC Name 5-Chloro-3*H*-1,3-benzoxazol-2-one
Synonym Chlorobenzoxazolinone; 5-chloro-2(3*H*)-benzoxazolone.
Proprietary Names *Biomioran*; *Paraflex*. It is an ingredient of *Parafon*.



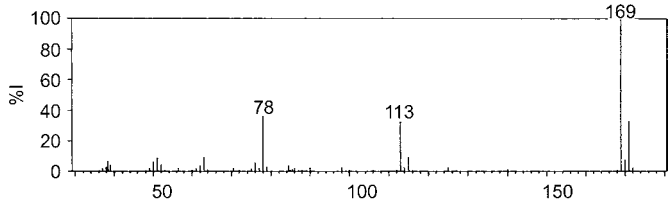
Chemical Properties Colourless crystals or white crystalline powder. Mp 190° to 194°. Sparingly soluble in water; soluble 1 in 20 of ethanol, 1 in 250 of chloroform, and 1 in 60 of ether; soluble in acetone, methanol, and isopropanol; freely soluble in aqueous solutions of alkali hydroxides and ammonia. pK_a 8.0 (20°). Log *P* (octanol/water), 1.6.
Colour Test Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.85; system TB—R_f 0.00; system TC—R_f 0.51; system TD—R_f 0.54; system TE—R_f 0.33; system TF—R_f 0.61; system TL—R_f 0.47; system TAD—R_f 0.56; system TAE—R_f 0.88; system TAF—R_f 0.90; system TAJ—R_f 0.51; system TAK—R_f 0.61; system TAL—R_f 0.92.
Gas Chromatography System GA—chlorzoxazone RI 1728, chlorzoxazone-Me RI 1750.
High Performance Liquid Chromatography System HX—RI 397.
Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1=306a); aqueous alkali—243 (A^1_1=580a), 287 nm (A^1_1=409a).



Infrared Spectrum Principal peaks at wavenumbers 1762, 801, 962, 1149, 840, 1300 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 169, 78, 171, 113, 115, 63, 51, 170.



Quantification

Plasma **GC-MS** Limit of quantification, 5 µg/L [Eap *et al.* 1998].

HPLC UV detection (λ=283 nm). Limit of quantification, 100 µg/L [Frye, Stiff 1996]. Chlorzoxazone and 6-hydroxychlorzoxazone. UV detection. Limit of detection, 80 ng [Honigberg *et al.* 1979].

Spectrofluorimetry Limit of detection, 60 µg/L [Stewart, Chan 1979].

Serum **HPLC** UV detection (λ=287 nm). Limit of quantification, 0.01 mg/L [Tanaka 1998]. UV detection (λ=230 nm). Limit of quantification, 0.5 mg/L, limit of detection, 0.1 mg/L [Haque, Stewart 1997].

Urine **HPLC** See Plasma [Frye, Stiff 1996].

Spectrofluorimetry Limit of detection, 130 µg/L, see Plasma [Stewart, Chan 1979].

Disposition in the Body Absorbed after oral administration and rapidly metabolised to 6-hydroxychlorzoxazone. Up to 90% of a dose is excreted in the urine in 48 h as conjugates of the 6-hydroxy metabolite; <1% is excreted as unchanged drug; 5-chloroacetanilide and 6-hydroxybenzoxazol-2(3*H*)-one have also been detected in urine.

Therapeutic Concentration

Following a single oral dose of 750 mg of chlorzoxazone and 900 mg of paracetamol, a mean peak plasma concentration of 36.3 mg/L of chlorzoxazone was attained in 0.7 h [Desiraju *et al.* 1983].

Half-life Plasma half-life, about 1 h.

Dose 0.75 to 3 g daily.

Desiraju RK *et al.* (1983). Pharmacokinetics of chlorzoxazone in humans. *J Pharm Sci* 72(9): 991–994.

Eap CB *et al.* (1998). Determination of chlorzoxazone and 6-hydroxychlorzoxazone in plasma by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 705(1): 139–144.

Frye RF, Stiff DD (1996). Determination of chlorzoxazone and 6-hydroxychlorzoxazone in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 686(2): 291–296.

Haque A, Stewart JT (1997). Direct injection analysis of chlorzoxazone and its major metabolite 6-hydroxychlorzoxazone in human serum using a semipermeable surface (SPS) HPLC column. *Biomed Chromatogr* 11(4): 236–239.

Honigberg IL *et al.* (1979). Liquid chromatography in pharmaceutical analysis X: Determination of chlorzoxazone and hydroxy metabolite in plasma. *J Pharm Sci* 68: 253–255.

Stewart JT, Chan C (1979). Fluorometric determination of chlorzoxazone in tablets and biological fluids. *J Pharm Sci* 68: 910–912.

Tanaka E (1998). Simultaneous determination of chlorzoxazone, indicator of CYP2E1, and its metabolite in human serum using a new reversed-phase chromatographic column of 2-microm porous microspherical silica-gel. *J Pharm Biomed Anal* 16(5): 899–904.

Cholesterol

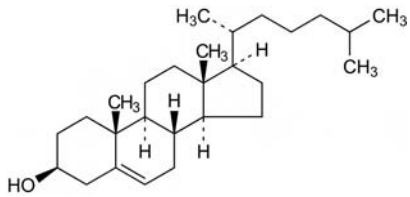
Sterol

$C_{27}H_{46}O$ = 386.7

CAS-57-88-5

IUPAC Name (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-Dimethyl-17-[(2*R*)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol

Synonyms (3β)-Cholest-5-en-3-ol; cholesterin.



Chemical Properties White or faintly yellow, pearly leaflets, needles, powder, or granules. It acquires a yellow to pale tan colour on prolonged exposure to light. Mp 147° to 150°. Practically insoluble in water; slowly soluble 1 in 100 of ethanol and 1 in 50 of dehydrated alcohol; soluble 1 in 2.8 of ether and 1 in 4.5 of chloroform; soluble in acetone, benzene and dioxan. Log *P* (octanol/water), 8.7.

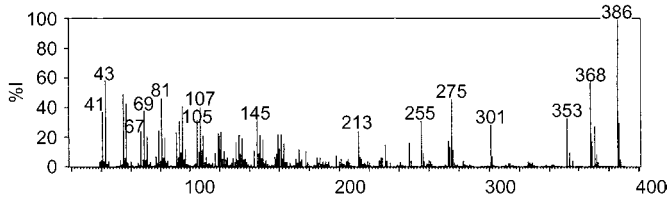
Colour Tests Antimony pentachloride—orange→brown; naphthol–sulfuric acid—yellow-brown/violet; sulfuric acid—orange; to a solution of 10 mg in 1 mL of chloroform add 1 mL of sulfuric acid; chloroform layer—red; sulfuric acid—green fluorescence; dissolve about 5 mg in 2 mL of chloroform, add 1 mL of acetic anhydride and 1 drop of sulfuric acid—pink→red→blue→green.

Thin-layer Chromatography System TB—R_f 0.22; system TC—R_f 0.64; system TD—R_f 0.61; system TE—R_f 0.76; system TF—R_f 0.60; system TL—R_f 0.69; system TAD—R_f 0.64; system TAE—R_f 0.84; system TAF—R_f 0.96; system TAJ—R_f 0.65; system TAK—R_f 0.78; system TAL—R_f 0.96; system TAM—R_f 0.96.

Gas Chromatography System GA—cholesterol RI 3088, cholesterol-H_2O RI 3030; system GE—RRT 1.35(relative to phenytoin).

Infrared Spectrum Principal peaks at wavenumbers 1059, 1022, 800, 959, 952, 839 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 386, 368, 43, 55, 275, 81, 57, 95.



Quantification

Semen **HPLC** Light-scattering detection [Grizard *et al.* 2000].

Grizard G *et al.* (2000). Separation and quantification of cholesterol and major phospholipid classes in human semen by high-performance liquid chromatography and light-scattering detection. *J Chromatogr Biomed B Sci Appl* 740(1): 101–107.

Choline

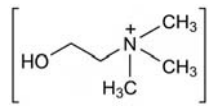
B Vitamin

$[C_5H_{14}NO]^+$ = 104.2

CAS—62-49-7 (cation)

IUPAC Name 2-Hydroxy-*N*,*N*,*N*-trimethylethanaminium

Proprietary Names Choline and its salts are ingredients of many preparations, see Sweetman [2009].



Chemical Properties A colourless, viscid, hygroscopic, strongly alkaline liquid. Very soluble in water and ethanol; practically insoluble in ether. pK_a 8.9.

Choline Bitartrate

$C_9H_{19}NO_7$ = 253.3

CAS—87-67-2

Synonym Choline acid tartrate

Chemical Properties A white, hygroscopic, crystalline powder. Very soluble in water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Choline Chloride

$C_5H_{14}ClNO$ = 139.6

CAS—67-48-1

Proprietary Name *Becholine*

Chemical Properties White, very hygroscopic crystals. Very soluble in water and ethanol; practically insoluble in chloroform and ether.

Choline Dihydrogen Citrate

$C_{11}H_{21}NO_8$ = 295.3

CAS—77-91-8

Synonym Choline citrate

Proprietary Name *Neurotropan*

Chemical Properties Colourless, translucent, hygroscopic crystals or white crystalline powder. Mp 105.0° to 107.5°. Soluble 1 in 1 of water and 1 in 45 of ethanol; practically insoluble in benzene, chloroform, and ether.

Choline Hydroxide

$C_5H_{15}NO_2$ = 121.2

Synonyms Bursine; fagine; gossypine; sincaline; vidine.

Chemical Properties Strongly alkaline liquid. Very soluble in water, alcohol. Insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.02; system TN—R_f 0.60; system TO—R_f 0.60 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

Ultraviolet Spectrum No significant absorption, 230–360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1636, 1620, 953, 1075, 1040, 860 cm^{-1} (choline chloride, thin film).

Quantification

Plasma **HPLC** Electrochemical detection [Fossati *et al.* 1994; Webb *et al.* 1993]. Choline and metabolites [Pomfret *et al.* 1989].

Urine **Colorimetry** [Eksborg, Persson 1971].

Peritoneal Dialysis Effluent **HPLC** See Plasma [Webb *et al.* 1993].

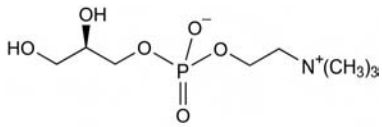
Tissues **HPLC** Choline and metabolites [Koc *et al.* 2002].

Disposition in the Body Choline is a metabolite of suxamethonium chloride and suxethonium bromide.
Dose Choline has been given in doses of 2 to 4 g daily.

Eksborg S, Persson BA (1971). Photometric determination of choline in biological objects by ion pair extraction technique. *Acta Pharm Suec* 8: 605–608.
Fossati T *et al.* (1994). Determination of plasma choline by high-performance liquid chromatography with a postcolumn enzyme reactor and electrochemical detection. *J Chromatogr B Biomed Appl* 656: 59–64.
Koc H *et al.* (2002). Quantitation of choline and its metabolites in tissues and foods by liquid chromatography/electrospray ionization-isotope dilution mass spectrometry. *Anal Chem* 74: 4734–4740.
Sweetman SC, ed. (2009). *Martindale: The Complete Drug Reference*, 36th edn. London: Pharmaceutical Press.
Pomfret EA *et al.* (1989). Measurement of choline and choline metabolite concentrations using high-pressure liquid chromatography and gas chromatography-mass spectrometry. *Anal Biochem* 180: 85–90.
Webb LE *et al.* (1993). A rapid high performance liquid chromatographic method for the analysis of choline in human plasma and peritoneal dialysis effluent: application in the assessment of choline loss in continuous ambulatory peritoneal dialysis. *Clin Biochem* 26: 173–177.

Choline Alfoscerate

Nootropic
$C_8H_{20}NO_6P = 257.2$
CAS—28319-77-9
IUPAC Name (*R*)-2-[[(2,3-Dihydroxypropoxy)hydroxyphosphinyl]oxy]-*N*,*N*,*N*-trimethylethanaminium hydroxide inner salt
Synonym L-α-Glycerylphosphorylcholine
Proprietary Names *Brezal*; *Delecit*; *Gliatilin*.



Chemical Properties White crystals. Mp 142.5°. Log *P* (octanol/water), −5.15.
High Performance Liquid Chromatography Column: RP cyano (Spheri-10, 10 µm, 30 × 4.6 mm), (bioreactor) anion-exchange cartridge (Aquapore AX-300, 7 µm, 30 × 2.1 mm i.d.). Mobile phase: 15 mmol/L sodium hydrogen phosphate and 0.5 mmol/L tetramethylammonium chloride solution, (pH 7). Electrochemical detection: platinum electrode against Ag/AgCl electrode at +0.5 V. Retention time: choline, 3.1 min [Abbiati *et al.* 566].
Quantification
Blood **GC-MS** Limit of quantification, 0.3 pg/L [Hasegawa *et al.*].
Plasma **HPLC** Column: RP cyano (Spheri-5, 5 µm, 100 × 4.6 mm i.d.), (bioreactor) anion-exchange cartridge (Aquapore AX-300, 7 µm, 30 × 2.1 mm i.d.). Mobile phase: 20 mmol/L sodium hydrogen phosphate and 10 mmol/L tetramethylammonium chloride solution, (pH 7.1). Flow rate, 0.7 mL/min. Electrochemical detection: platinum electrode at +300 mV. Retention time: choline, 5 min. Limit of quantification, 3.58 µg/L [Fossati *et al.* 1994].
Disposition in the Body Choline alfoscerate is completely absorbed after oral administration and is widely metabolised after IM or oral administration. It is rapidly cleaved by glycerylphosphorylcholine diesterase to glycerophosphate and choline, the main plasma metabolite, which is detectable until 6 to 8 h after administration.
Therapeutic Concentration
Twelve healthy males aged between 20 and 29 years were administered, IM, with a 1 g dose of L-α-glycerylphosphorylcholine. Choline levels in plasma reached 35.5 ± 3.5 µmol/L at 0.25 h and 35.8 ± 3.5 µmol/L at 0.5 h and mean endogenous plasma levels ranged from 10.6 to 12.0 µg/L [Gatti *et al.* 1992].
Half-life Terminal half-life of free choline derived from L-α-glycerylphosphorylcholine is 1.37 h.
Clearance 42.7 ± 2.7 µmol/l/h.

Abbiati G *et al.* (1991). High-performance liquid chromatographic assay of L-alpha-glycerophosphorylcholine using a two-step enzymic conversion. *J Chromatogr*,445–451.
Fossati T *et al.* (1994). Determination of plasma choline by high-performance liquid chromatography with a postcolumn enzyme reactor and electrochemical detection. *J Chromatogr* 656; Biomed. Appl.: 59–64.
Gatti G *et al.* (1992). A comparative study of free plasma choline levels following intramuscular administration of L-alpha-glycerylphosphorylcholine and citicoline in normal volunteers. *Int J Clin Pharm Ther Toxicol* 30(9): 331–335.
Hasegawa Y *et al.* (1982). Determination of picomole amounts of choline and acetylcholine in blood by gas chromatography-mass spectrometry equipped with a newly improved pyrolyzer. *J Chromatogr* 30(239): 335–342.

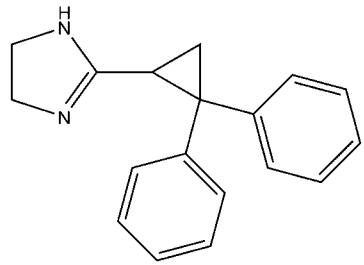
Chorionic Gonadotropin

Gonad Stimulating Hormone
Sialoglycoprotein = approx. 37000
CAS—9002-61-3
Synonyms CG; choriogonadotropin; gonadotrophinum chorionicum.
Proprietary Names *APL*; *Choragon*; *Chorex*; *Choron*; *Gonadotraphon LH*; *Gonasi HP*; *Gonic*; *Physex*; *Predalon*; *Pregnesin*; *Pregnyl*; *Primogonyl*; *Profasi*.
Chemical Properties Molecular structure. α-Subunit consists of 96 amino acids and 2 carbohydrate moieties, and the β-subunit consists of 149 amino acid residues at the C-terminal region. It is a white or almost white amorphous powder, which needs to be protected from light. It is soluble in water, aqueous glycerol and glycols; insoluble in anhydrous organic solvents.
Quantification
Serum **Fluoroimmunoassay** Limit of detection, 2.7 International Units (IU)/L [Cowan *et al.* 1991].
Urine **Fluoroimmunoassay** Limit of detection, 4.9 IU/L [Cowan *et al.* 1991].
Disposition in the Body Distributed primarily to the gonads. 20 to 30% of IM dose is excreted in urine within 5 to 6 days. The β-subunit of chorionic gonadotrophin is metabolised in the liver to a smaller component of molecular weight 12 000 to 17 500. Normally present in males and non-pregnant females <10 IU/L.
Therapeutic Concentration
Eighteen pituitary-suppressed healthy females, aged between 18 and 26 years, were administered with single-dose human chorionic gonadotrophin injections of 5000 and 10 000 IU (IM) and 10 000 IU (SC). Mean serum peak concentrations reached 156 IU/L, 307 IU/L and 339 IU/L, respectively, 20 h after injection [Mannaerts *et al.* 1998].
Toxicity Dose of 36 mg/kg (female) can cause reproductive side effects and changes in female fertility.
Half-life Blood concentrations decline in a biphasic manner. Half-lives between 6 and 11, and 23 and 38 h, respectively.
Clearance Metabolic clearance is 3.4 mL/min for men and 3.9 mL/min for women.
Dose Infertility treatment (female): single dose between 5000 and 10 000 IU, IM injection, followed by up to 3 repeat injections of 5000 IU each, within 9 days. Infertility treatment (male): single dose between 500 and 4000 units twice/three times a week. Prepubertal cryptorchidism treatment: single dose between 500 and 4000 IU three times a week, for 6 to 10 weeks. Delayed puberty treatment (male): initial dose of 500 to 1500 IU administered twice weekly, 4 to 6 weeks.

Cowan DA *et al.* (1991). Effect of administration of human chorionic gonadotrophin on criteria used to assess testosterone administration in athletes. *J Endocrinol* 131: 147–154.
Mannaerts BM *et al.* (1998). A randomized three-way cross-over study in healthy pituitary-suppressed women to compare the bioavailability of human chorionic gonadotrophin (Pregnyl) after intramuscular and subcutaneous administration. *Hum Reprod* 13(6): 1461–1464.

Cibenzoline

Antiarrhythmic, Sodium Channel Blocker
$C_{18}H_{18}N_2 = 262.4$
CAS—53267-01-9
IUPAC Name 2-[2,2-Di(phenyl)cyclopropyl]-4,5-dihydro-1*H*-imidazole
Synonyms Cifenline; 2-(2,2-diphenylcyclopropyl)-4,5-dihydro-1*H*-imidazole; (±)-2-(2,2-diphenylcyclopropyl)-2-imidazoline; 1-(2-Δ^2-imidazolinyl)-2,2-diphenylcyclopropane; Ro-22-7796; UP-33-901.



Chemical Properties Crystals. Mp 103° to 104°. Log *P* (octanol/water), 4.22 [Meylan, Howard 1995]. Stock solutions stable for at least 2 months at 4° [Kuhlkamp *et al.* 1990].

Cibenzoline Succinate

$C_{18}H_{18}N_2$, $C_4H_6O_4 = 380.4$
CAS—100678-32-8
Proprietary Names *Cibenol*; *Cipralam*; *Exacor*.

Quantification
Plasma **GC-MS** Column: Glass packed with 3% SP-2250 on 80 to 100 mesh Supelcoport (120 cm × 2 mm i.d.). Carrier gas: CH_4, 1.2 kg/m^2. SIM acquisition mode. Limit of detection, 1.0 µg/L [Min, Garland 1984].

HPLC Column: Zorbax SCX (250 × 4.6 mm i.d., 7–8 µm). Mobile phase: acetonitrile : 0.015 mol/L phosphate buffer (pH 6.0; 80 : 20), flow rate 1.5 mL/min. UV detection (λ = 214 nm). Limit of detection, 10.0 µg/L [Hackman *et al.* 1983].

Serum **HPLC** Column: Nucleosil CN reversed phase (125 × 4.6 mm i.d., 5 µm). Mobile phase: 971.5 mL water/25 mL PIC B-8 low UV reagent/1 mL butylamine/2.5 mL PIC D-4 reagent : acetonitrile (65 : 35), flow rate 1.1 mL/min. UV detection (λ = 214 nm). Limit of quantification, 10 ng/mL [Kuhlkamp *et al.* 1990].

Urine **HPLC** See Plasma. Limit of detection, 50.0 µg/L [Hackman *et al.* 1983]

Disposition in the Body Four main metabolites, M1 (*p*-hydroxycibenzoline), M2 (4,5-dehydrocibenzoline), and unknown metabolites M3 and M4 are formed by the action of CYP3A and CYP2D [Niwa *et al.* 2000].

Therapeutic Concentration

A study in 18 healthy volunteers investigated the effect of food on the bioavailability of 160 mg cibenzoline. Mean maximum plasma concentrations were 468 ± 121, 394 ± 123, 397 ± 110, 484 ± 159 ng/mL at 1.5 ± 0.4, 2.2 ± 1.0, 2.4 ± 1.1, 1.5 ± 0.7 h when the dose was administered in the fasting state, after a standard breakfast, midway through a standard breakfast, and before a standard breakfast, respectively. The rate of absorption was decreased slightly in the presence of food but this was not expected to be of clinical significance [Massarella *et al.* 1986a].

Twenty-five healthy male volunteers received a single dose of 160 mg cibenzoline followed 2 or 3 days later by 160 mg cibenzoline once every 12 h for 7 days. Mean maximum plasma concentrations were 454 ± 103 and 716 ± 187 ng/mL, respectively, at 1.6 ± 0.5 and 1.5 ± 0.5 h after the initial and final doses, respectively. The half-life following administration of the last dose was longer than that observed after the first dose, 9.7 and 8.4 h, respectively, because of a decrease in non-renal clearance [Massarella *et al.* 1986b].

Six patients with congestive heart failure and 5 healthy subjects were administered a 1 h infusion of 80 mg $^{15}N_2$-cibenzoline with a simultaneous 80 mg oral dose. Mean peak plasma concentrations were 313 ± 67 at 1.5 h and 327 ± 49 ng/mL at 1.4 h in healthy subjects and patients with heart failure, respectively [Massarella *et al.* 1987].

Thirty-four patients undergoing cardiac catheterisation received a single IV dose of 0.25, 0.5, 0.75, 1.0, or 1.2 mg/kg cibenzoline. The elimination half-life ranged from 45 min to 12 h, with a mean of 5 h 9 min ± 41 min. Cibenzoline had a dose-related negative inotropic effect and a similar haemodynamic effect to disopyramide [Humen *et al.* 1987].

Twelve healthy volunteers aged between 41 and 55 years (mean 47 years) were administered 160 mg cibenzoline concomitantly with 0.25 or 0.375 mg digoxin. The addition of cibenzoline did not significantly alter the pharmacokinetic profile of the digoxin regimen (levels not reported) [Khoo *et al.* 1988].

Sixteen male and female volunteers (27 to 67 years) were administered a single 130 mg oral dose of cibenzoline. Ten of the patients had renal impairment with a creatinine clearance <60 mL/min/70 kg and six were healthy with a creatinine clearnace of >60 mL/min/70 kg. The mean maximum plasma concentration was in the same range in all subjects (levels not reported). The elimination half-life increased with decreased renal function [Massarella *et al.* 1988].

Note For a study of cibenzoline in patients with frequent premature ventricular contractions see Holazo *et al.* [1986].

Toxicity

Three patients (aged 36, 68, and 80 years) were treated with 300 mg of cibenzoline and developed signs of toxicity (prolonged QTc, wide QRS, arrhythmias, hypotension, and hypoglycaemia). Plasma concentrations were 1944 to 2580 µg/L, 5 to 10 times the expected therapeutic concentration. All patients had severe renal dysfunction and after withdrawal of the drug, the elimination half-lives were 3 to 10 times longer than in patients with end-stage renal failure [Takahashi *et al.* 2002].

A 69-year-old man presented with severe general fatigue and progressive systemic muscle weakness. He had taken 300 mg cibenzoline a day for several years and at the end of June 2001 he began to feel generally fatigued with walking disturbance. By the beginning of July, he was unable to stand by himself and he felt short of breath. Once admitted to hospital he developed severe respiratory depression with increased serum BUN and creatinine. He was put on a respirator and the cibenzoline was replaced with mexiletine. His renal function improved and his spontaneous respiration and muscle movement were restored. He was released a month after admission [Inada *et al.* 2002].

An 80-year-old woman with permanent right ventricular pacing was admitted to hospital with general fatigue and clouding of consciousness. She had been taking cibenzoline succinate for 1 month. ECG revealed a prolonged QRS and QTc interval and pacing failure. Together with haemodynamic failure and liver and renal dysfunction, her symptoms were consistent with cibenzoline succinate poisoning. On day 4, charcoal haemoperfusion was performed because of the development of hypoglycaemia. After the haemoperfusion her symptoms resolved and she was discharged. Digoxin was prescribed [Aoyama *et al.* 1999].

Bioavailability Approximately 90% [Khoo *et al.* 1988].

Half-life Approximately 6 to 15 h [Khoo *et al.* 1988].

Volume of Distribution In healthy volunteers: 7.3 ± 3.4 and 6.5 ± 3.0 L/kg for an oral and IV dose, respectively. In patients with congestive heart failure, 5.4 ± 1.0 and 4.7 ± 1.1 L/kg for an oral and IV dose, respectively [Massarella *et al.* 1987].

Clearance

Disease state	*Treatment*	*Creatinine clearance (mL/min)*		
		Total	*Renal*	*Non-renal*
Chronic heart failure	80 mg oral	488 ± 207	289 ± 147	199 ± 120
	80 mg IV	414 ± 170	273 ± 140	141 ± 61
Healthy	80 mg oral	636 ± 240	385 ± 115	251 ± 167
	80 mg IV	536 ± 193	347 ± 113	189 ± 116
	160 mg oral	854 ± 179	420 ± 119	434 ± 165
	160 mg every 12 h for 7 days	653 ± 174	455 ± 121	198 ± 128

[Massarella *et al.* 1986b, 1988]

Dose 300 mg a day orally.

Aoyama N *et al.* (1999). Effect of charcoal hemoperfusion on clearance of cibenzoline succinate (cifenline) poisoning. *J Toxicol Clin Toxicol* 37: 505–508.

Hackman MR *et al.* (1983). Determination of cibenzoline in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 273: 347–356.

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Massarella JW *et al.* (1986b). Pharmacokinetics of cibenzoline after single and repetitive dosing in healthy volunteers. *J Clin Pharmacol* 26: 125–130.

Massarella JW *et al.* (1987). Effect of congestive heart failure on the pharmacokinetics of cibenzoline. *J Clin Pharmacol* 27: 187–192.

Massarella JW *et al.* (1988). Effect of renal impairment on the pharmacokinetics of cibenzoline. *Clin Pharmacol Ther* 43: 317–323.

Meylan W, Howard MPH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Min B, Garland H, W.A (1984). Quantitation of cibenzoline in human plasma by gas chromatography-negative ion chemical-ionization mass spectrometry. *J Chromatogr* 336: 403–409.

Niwa T *et al.* (2000). Stereoselective metabolism of cibenzoline, an antiarrhythmic drug, by human and rat liver microsomes: possible involvement of CYP2D and CYP3A. *Drug Metab Dispos* 28: 1128–1134.

Takahashi M *et al.* (2002). Extremely prolonged elimination of cibenzoline at toxic plasma concentrations in patients with renal impairments. *Ther Drug Monit* 24: 492–496.

Cicletanine

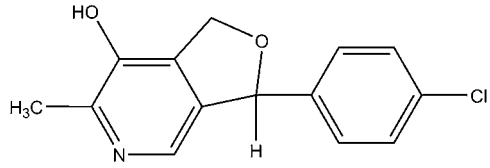
Antihypertensive, Diuretic, Furopyridine

$C_{14}H_{12}ClNO_2$ = 261.7

CAS—89943-82-8

IUPAC Name 3-(4-Chlorophenyl)-6-methyl-1,3-dihydro-furo[3,4-d]pyridin-7-ol

Synonyms Cicletanide; 3-(4-chlorophenyl)-1,3-dihydro-6-methylfuro[3,4-c]pyridine-7-ol; 3-(4-chlorophenyl)-6-methyl-1,3-dihydro-furo[3,4-c]pyridin-7-ol cicletanide; 1,3-dihydro-3-(4-chlorophenyl)-7-hydroxy-6-methylfuro[3,4-c]pyridine.



Cicletanine Hydrochloride

$C_{14}H_{12}ClNO_2$, HCl = 298.2

CAS—82747-56-6

Synonym BN-1270

Proprietary Names *Coverine*; *Justar*; *Secletan*; *Tenstaten*.

Chemical Properties White crystals. Mp 219° to 228°. Insoluble in water. There was no apparent effect of freezing at –20° over a 3 month period [Antoniewicz *et al.* 1992].

Quantification

Blood **HPLC** Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 10 µm) Mobile phase: methanol : 0.1 mol/L acetic acid (50 : 50), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Limit of detection, 20 µg/L [Cuisinaud *et al.* 1985].

Plasma **HPLC** Column: Nova-Pak reversed phase C_{18} (100 × 8 mm i.d., 4 µm). Mobile phase: 0.1 mol/L monobasic potassium phosphate (pH 2.5) : acetonitrile (75 : 25); flow rate 2.0 mL/min. Fluorescence detection ($\lambda_{ex} = 290$ nm, $\lambda_{em} > 370$ nm). Limit of quantification, 50 µg/L [Antoniewicz *et al.* 1992]. Column: Nova-Pak C_{18} (100 × 8.0 mm i.d., 4 µm). Mobile phase: acetonitrile : water (22 : 78) containing 0.02% isopropylamine (pH 2.4) with 0.085% phosphoric acid, flow rate 2.5 mL/min. UV detection ($\lambda = 220$ nm). Limit of detection, 10 µg/L [Prunonosa *et al.* 1992a]. See Blood [Cuisinaud *et al.* 1985].

CE Capillary: fused silica (57 cm total length, 75 µm i.d.). Buffer: 100 mmol/L sodium borate buffer (pH 8.6) : 25 mmol/L SDS containing 10% acetonitrile. UV detection ($\lambda = 214$ nm). Limit of detection, 20 µg/L [Prunonosa *et al.* 1992b]. Capillary: fused silica (57 cm total length, 75 µm i.d.). Buffer: 100 mmol/L sodium borate buffer (pH 8.6) : 100 mmol/L SDS : 25 mmol/L γ-cyclodextrins containing 10% acetonitrile. UV detection ($\lambda = 214$ nm). Limit of detection, 10 µg/L [Prunonosa *et al.* 1992a].

Urine **HPLC** See Blood. Limit of detection, 30 µg/L [Cuisinaud *et al.* 1985].

Oral Fluid **HPLC** See Blood [Cuisinaud *et al.* 1985].

Disposition in the Body Cicletanine undergoes glucuronidation and sulfation. Elimination is both renal and hepatic while urinary excretion of the unchanged drug is negligible.

Therapeutic Concentration

Ten healthy volunteers were administered a single 50 mg cicletanine dose of cicletanine. The mean peak plasma concentration was 1.78 ± 0.586 mg/L at 0.83 ± 0.8 h. The mean elimination half-life was 9.89 ± 4.32 h [Prunonosa *et al.* 1992c].

Eight healthy volunteers were administered 50 mg orally a day for 7 days. There was no significant difference between the parameters after the first dose and after repeated doses. The mean peak plasma concentration was 1.73 ng/L after the final dose [Peraire *et al.* 1991].

Forty-three patients with varying degrees of chronic renal failure were administered a single oral dose of 300 or 200 mg cicletanine. Six patients with moderate renal dysfunction were administered 200 mg a day orally for 30 days. In patients with severe renal dysfunction, the pharmacokinetics of cicletanine were significantly altered, with increased elimination half-life and tissue accumulation. Only minor pharmacokinetic changes were seen in patients with mild or moderate renal impairment, even after repeated administration of the drug. Mean maximum plasma concentrations were 20 ± 2, 14 ± 2 and 13 ± 2 µg/mL at 1.3 ± 0.1, 1.3 ± 0.2 and 0.9 ± 0.2 h, respectively, in control subjects, patients with mild renal failure, and patients with severe renal failure, respectively [Jungers 1988].

Note For a summary of the pharmacokinetics of cicletanine under various conditions, see Fredj [1988]. For a study of the interaction of cicletanine with tolbutamide in healthy volunteers, see Bayes *et al.* [1996].

Half-life Approximately 5 to 18 h.

Volume of Distribution In 8 subjects (weighing 75.3 ± 6.4 kg): 35.5 to 152.4 L [Peraire *et al.* 1991].

Clearance Oral and renal clearance 7.3 ± 2.5 and 0.026 ± 0.012 L/h, respectively.

Protein Binding 97.3% bound to serum proteins, 93.5% of which is to albumin [Zini *et al.* 1988].

Dose 50 to 100 mg orally daily.

Antoniewicz SM *et al.* (1992). Determination of cicletanine in human plasma by high-performance liquid chromatography using automated column switching. *J Chromatogr* 573: 93–98.

Bayes MC *et al.* (1996). A drug interaction study between cicletanine and tolbutamide in healthy volunteers. *Eur J Clin Pharmacol* 50: 381–384.

Cuisinaud G *et al.* (1985). High-performance liquid chromatographic determination of cicletanide, a new diuretic, in plasma, red blood cells, urine and saliva. *J Chromatogr* 341: 97–104.

Fredj G (). Clinical pharmacokinetics of cicletanine hydrochloride. *Drugs Exp Clin Res* 14: 181–188.

Jungers P (1988). Pharmacokinetics of cicletanine in patients with impaired renal function. *Drugs Exp Clin Res* 14: 189–194.

Peraire C *et al.* (1991). Multiple dose pharmacokinetic study of cicletanine in healthy volunteers. *Eur J Drug Metab Pharmacokinet* SpecNo3: 173–177.

Prunonosa J *et al.* (1992). Determination of cicletanine enantiomers in plasma by high-performance capillary electrophoresis. *J Chromatogr* 574: 127–133.

Prunonosa J *et al.* (1992). Comparison of high-performance liquid chromatography and high-performance capillary electrophoresis for the determination of cicletanine in plasma. *J Chromatogr* 581: 219–226.

Prunonosa J *et al.* (1992). Pharmacokinetic study of cicletanine in healthy volunteers. *Int J Clin Pharmacol Ther Toxicol* 30: 265–270.

Zini R *et al.* (1988). Cicletanine binding to human plasma proteins and erythrocytes, a particular HAS–drug interaction. *Life Sci* 43: 2103–2115.

Ciclosporin

Immunosuppressant

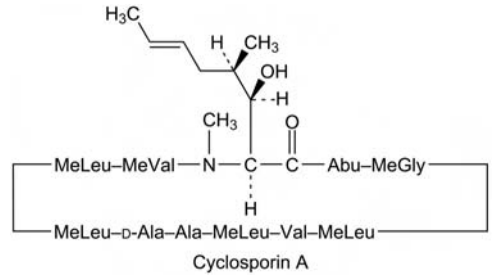
$C_{62}H_{111}N_{11}O_{12} = 1202.6$

CAS—59865-13-3

IUPAC Name 30-Ethyl-33-[(*Z*,1*S*,2*R*)-1-hydroxy-2-methylhex-4-enyl]-1,4,7,10,12,15,19,25,28-nonamethyl-6,9,18,24-tetrakis(2-methylpropyl)-3,21-di(propan-2-yl)-1,4,7,10,13,16,19,22,25,28,31-undecazacyclotritriacontane-2,5,8,11,14,17,20,23,26,29,32-undecone

Synonyms [*R*-[*RR**(E)]]-Cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L-α-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl); cyclosporin; cyclosporin A; cyclosporine.

Proprietary Names *Neoral*; *Sandimmun*; *Sandimmune*, *SangCya*.



Cyclosporin A

Chemical Properties A white or almost white powder. Soluble in methanol, ethanol, ether, chloroform and methylene chloride. It is practically insoluble in water and saturated hydrocarbons.

Quantification

Blood **HPLC** Column: C_8 (Alltima, 100 × 2.1 mm i.d., 5 µm) at 70°. Mobile phase: 72% methanol : 28% 50 mmol/L ammonium acetate buffer (pH 5.1), flow rate 0.3 mL/min. Electron spray tandem mass spectrometry detection. Retention time: 11.8 min. Limit of detection, 5 µg/L [Taylor *et al.* 1998]. Column: Spherisorb S5 CN (250-4 phasesep, 250 × 4.6 mm, 5 µm) at 50°. Mobile phase: hexane : isopropanol (90:10), flow rate 1.45 mL/min. UV detection (λ=212 nm). Retention times: ciclosporin, 8.9 min; metabolites, 11.0, 12.9 and 16.3 min. Limit of detection, 0.01 mg/L [Khoschsorur *et al.* 1997]. Column: Si 60 silica (250 × 4 mm, 5 µm) at 60°. Mobile phase: hexane : ethanol (85:15), flow rate 1.5 mL/min. UV detection (λ=210 nm). Retention time: 3.4 min . Limit of quantification, 25 µg/L [Poirier *et al.* 1994]. Column: RP-18 (RP Velosep, (400 × 3.2 mm, 3 µm). Mobile phase: 50% acetonitrile : 11% methanol : 39% 0.01 mol/L dipotassium hydrogen orthophosphate buffer (pH 6.5), flow rate 1.5 mL/min. UV detection (dual wavelength, 220 and 230 nm). Retention time: 2.5 min. Limit of detection, 45 µg/L [Salm *et al.* 1993].

Disposition in the Body After oral administration of conventional formulations absorption is very variable; absolute bioavailability can vary from <10% in liver transplant patients to up to 89% in some renal transplant patients. Peak blood and plasma concentrations are achieved about 3.5 h after an oral dose. Following administration of a microemulsion formulation, peak concentrations are achieved after about 1.5 to 2 h and bioavailability is greater than with conventional formulations although is still very variable. Ciclosporin is widely distributed throughout the body. In blood, the distribution is concentration dependent. It is extensively metabolised by CYP4503A mainly in the liver (some also in the gastro-intestinal tract and kidney) and excreted primarily in faeces via the bile; only about 6% is excreted in the urine. The low oral bioavailability of ciclosporin is due to first-pass metabolism in the gastro-intestinal wall rather than the liver. It crosses the placenta and is distributed into breast milk. Dialysis does not affect clearance significantly.

Therapeutic Concentration

Nine paediatric patients undergoing liver transplants, aged between 0.5 and 11 years, were administered with a 10.1 mg/kg IV dose over 24 h. The peak concentration was 0.834 mg/L after 4.4 h. The same children administered with an oral dose of 30 mg/kg reached peak plasma concentrations of 1.356 mg/L 2.6 h after ingestion [Dunn *et al.* 1995].

Fifty-four paediatric patients, with a mean age of 13.2 years, were administered with an oral 7.8 mg/kg dose under steady state conditions. Peak concentrations of 0.588 mg/L were reached 2.4 h after ingestion [Humbert *et al.* 1994].

Toxicity Nephrotoxicity is the major toxic effect; doses must be adjusted individually and kidney function must be monitored during therapy.

A 31-year-old woman accidentally received 5000 mg (92.6 mg/kg) of ciclosporin orally as a single dose on day 80 post-kidney transplant. She developed a severe headache, intractable vomiting and felt drunk. By 72 h post-ingestion all symptoms had subsided. At 18 h post dose serum-ciclosporin concentration was 2.6 mg/L. At 30 h post-dose, whole blood-ciclosporin concentration was 1.775 mg/L. There was little evidence of nephrotoxicity and no evidence of hepatotoxicity. There were no long-term sequelae [Schroeder *et al.* 1986].

A 43-year-old man with multiple sclerosis took 25 g of ciclosporin over an 8-day period when he misunderstood instructions. Symptoms included burning sensations in mouth and feet, altered taste, hyperaesthesia of the hands, sore gums, facial flushing, sensation of abdominal swelling, foot swelling, and mild stomach upset. Whole blood-ciclosporin concentration measured the day after discontinuation of ciclosporin was 1.778 mg/L. There was no evidence of hepatotoxicity or nephrotoxicity [Baumhefner *et al.* 1987].

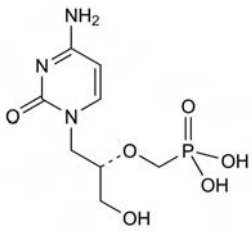
A 31-year-old man was given an overdose of 1.1 mg/kg/h for 33 h on day–19 post-kidney transplant. He developed severe abdominal pain that was considered to be due to the toxic levels of ciclosporin. There was also hepatotoxicity. Blood-ciclosporin concentration measured 10 h after stopping ciclosporin was 2 mg/L. It was estimated that concentrations were higher than 4 mg/L for the duration of the infusion. There was little evidence of nephrotoxicity [Kokado *et al.* 1989].

Half-life Terminal, ranged from 6.3 h (healthy adults); 20.4 h (patients with severe liver disease).
Volume of Distribution 2 to 5 L/kg; apparent, 13 L/kg.
Clearance Blood clearance, 0.71 L/h/kg (paediatrics); 0.34 L/h/kg (adults).
Distribution in Blood This is concentration and temperature dependent. Approximately 40% is taken up in erythrocytes and the remainder extensively binds plasma proteins. At high concentrations the binding to blood cells becomes saturated.
Protein Binding 90% (mostly to lipoprotein).
Note For reviews, see Cooney *et al.* [1997] and Holt, Johnston [1995].
Dose Organ transplantation: initially 10 to 15 mg/kg orally daily in two divided doses; the IV dose is one-third of the oral dose; maintenance dose, 2 to 6 mg/kg daily. Bone-marrow transplant: maintenance dose, 12.5 mg/kg orally daily (3 to 6 months). Psoriasis and atopic dermatitis: 2.5 to 5.0 mg/kg orally daily. Rheumatoid arthritis: 2.5 to 4.0 mg/kg orally daily. Nephrotic syndrome: 5 mg/kg orally daily (adults); 6 mg/kg (children).

Baumhefner RW *et al.* (1987). Huge cyclosporin overdose with favourable outcome. *Lancet* 2: 332.
Cooney GF *et al.* (1997). Cyclosporin pharmacokinetics in paediatric transplant recipients. *Clin Pharmacokinet* 32: 481–495.
Dunn SP *et al.* (1995). Absorption characteristics of a microemulsion formulation of cyclosporine in de novo pediatric liver transplant recipients. *Transplantation* 60(12): 1438–1442.
Holt DW, Johnston A (1995). Cyclosporin A: analytical methodology and factors affecting therapeutic drug monitoring. *Ther Drug Monit* 17: 625–630.
Humbert H *et al.* (1994). Steady-state pharmacokinetics of cyclosporine in renal transplant patients: does an influence of age or body weight exist? *Transplant Proc* 26: 2791–2797.
Khoschsorur G *et al.* (1997). Rapid, sensitive high-performance liquid chromatographic method for the determination of cyclosporin A and its metabolites M1, M17 and M21. *J Chromatogr B Biomed Sci Appl* 690: 367–372.
Kokado Y *et al.* (1989). An acute overdose of cyclosporine. *Transplantation* 47: 1096–1097].
Poirier JM *et al.* (1994). Cyclosporine in whole blood: drug monitoring difficulties and presentation of a reliable normal-phase liquid chromatographic assay. *Ther Drug Monit* 16: 388–389.
Salm P *et al.* (1993). A reliable high-performance liquid chromatography assay for high-throughput routine cyclosporin A monitoring in whole blood. *Ther Drug Monit* 15: 65–69.
Schroeder TJ *et al.* (1986). An acute overdose of cyclosporine. *Transplantation* 41: 406–409.
Taylor PJ *et al.* (1998). Microscale high-performance liquid chromatography-electrospray tandem mass spectrometry assay for cyclosporin A in blood. *J Chromatogr B Biomed Sci Appl* 705(2): 289–294.

Cidofovir

Antiviral
$C_8H_{14}N_3O_6P = 279.2$
CAS—113852-37-2
IUPAC Name [(2*S*)-1-(4-Amino-2-oxopyrimidin-1-yl)-3-hydroxypropan-2-yl] oxymethylphosphonic acid
Synonyms (*S*)-[[2-(4-Amino-2-oxo-1(2*H*)-pyrimidinyl)-1-(hydroxymethyl) ethoxy]methyl]-phosphonic acid; (*S*)-HPMPC; GS-504.
Proprietary Name *Vistide*

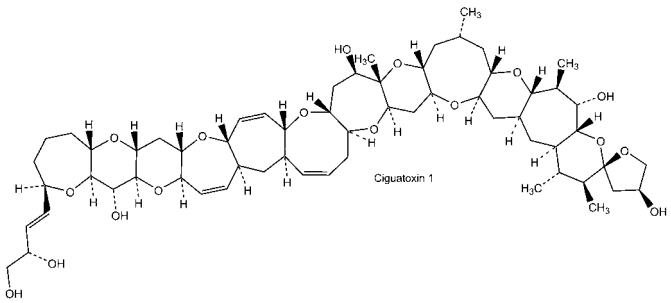


Chemical Properties A fluffy white powder. Mp 260° with decomposition. It is soluble in water at 170 g/l.
High Performance Liquid Chromatography Column: C_8 (Zorbax, 250 × 4.6 mm i.d., 5 µm). Mobile phase: (A) acetonitrile : 100 mmol/L phosphate buffer (pH 7.2) with 5 mM tetrabutylammonium dihydrogen phosphate (TBAHP) (5:95); (B) acetonitrile : 100 mmol/L phosphate buffer (pH 7.2) with 5 mM TBAHP (15:85). Linear gradient—100% (A) to 100% (B) over 9 min, flow rate 1.4 mL/min. UV detection (λ=274 nm). Retention time: 6.0 min.
Quantification
Plasma **HPLC** Column: ODS-2 (Prodigy, 100 × 4.6 mm, 5 µm). Mobile phase: 6 mmol/L Q12 and 12 mmol/L phosphoric acid in acetonitrile : water (30:70), final pH 3.0 to 3.1, flow rate 3 mL/min. Fluorescence detection ($\lambda_{ex} = 305$ nm, $\lambda_{em} = 370$ nm). Retention time: 3.1 min. Limit of detection, 5 µg/L [Eisenberg, Cundy 1996].
Serum **HPLC** Column: ODS-IP (Beckman Ultrasphere, 150 × 4.6 mm). Mobile phase: acetonitrile : methanol : water containing 5 mmol/L octyltriethylammonium phosphate (5:5:90), flow rate 2.0 mL/min. UV detection (λ= 274 nm). Retention time: 6.6 min. Limit of quantification, 0.22 mg/L [Cundy *et al.* 1995].
Urine **HPLC** Limit of quantification, 1.0 mg/L, see Serum [Cundy *et al.* 1995].
Disposition in the Body Cidofovir is administered IV. It is excreted largely unchanged in urine by glomerular filtration and tubular secretion. About 80 to 100% of a dose is recovered unchanged in urine over 24 h. No active metabolites are detected in serum. It is given concurrently with prehydration and oral probenecid to block the tubular secretion and thus reduce high concentrations in the kidney, since cidofovir is nephrotoxic.
Therapeutic Concentration
Thirty-nine HIV-infected males and 3 females, aged between 28 and 55 years, mean 39 years, were intravenously administered with 1 (no of subjects, n=5), 3 (n=10), 5 (n=2) or 10 (n=8) mg/kg cidofovir. Peak serum concentrations of the drug were 3.12, 7.34, 11.5 and 23.56 mg/L, respectively, for the 4 doses observed at the end of infusion. In the same study, cidofovir was also administered to the patients along with a high dose of probenecid. A 3 mg/kg dose of cidofovir produced a peak concentration of 8.08 mg/L; 5 mg/kg, 26.07 mg/L and 7.5 mg/kg dose, 42.95 mg/L, respectively [Cundy *et al.* 1995].
Toxicity Dose-dependent nephrotoxicity is the major adverse effect. A 5 mg/kg dose twice weekly is not well tolerated with resulting renal toxicity. (5 mg/kg weekly or 7.5 mg/kg every 3 weeks is feasible).
Half-life Plasma half-life of cidofovir, 2.2 h; active metabolite, cidofovir diphosphate, 17 to 65 h.
Volume of Distribution Steady state, mean, 490 mL/kg; 356 mL/kg (with probenecid).
Clearance Serum, mean, 148 mL/h/kg; 125 mL/h/kg (with probenecid).
Protein Binding <10% (*in vitro*) bound to plasma or serum proteins.
Note For a review of cidofovir, see Poklis *et al.* [1995].
Dose 5 mg/kg IV once every 2 weeks (maintenance dose) together with probenecid and prehydration.

Cundy KC *et al.* (1995). Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 39(6): 1247–1252.
Eisenberg EJ, Cundy KC (1996). High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides. *J Chromatogr B Biomed Appl* 679: 119–127.
Polis MA *et al.* (1995). Anticytomegaloviral activity and safety of cidofovir in patients with human immunodeficiency virus infection and cytomegalovirus viruria. *Antimicrob Agents Chemother* 39 (4): 882–886.

Ciguatoxins

Neurotoxin, Sodium Channel Activator
Synonym CTX



Chemical Properties Ciguatoxins are potent sodium channel activators found in a wide variety of fish; the toxins were ultimately traced to a dinoflagellate *Gambierdiscus* spp. Family of lipid-soluble polyether toxins responsible for ciguatera food poisoning; structural variations are associated with the oceanic region from which the dinoflagellate originates.

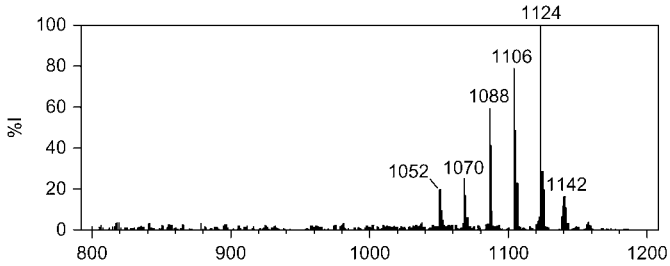
Ciguatoxin-1
$C_{60}H_{86}O_{19} = 1111.3$
CAS—11050-21-8
Synonym CTX-1
Chemical Properties White solid.

Ciguatoxin-2
$C_{60}H_{86}O_{18} = 1095.3$
CAS—142185-85-1
Synonym CTX-2
Chemical Properties White amorphous solid.

Ciguatoxin-3
$C_{60}H_{86}O_{18} = 1095.3$
CAS—139341-09-6
Synonym CTX-3
Chemical Properties White amorphous solid.

High Performance Liquid Chromatography Column: Ultrasphere ODS (250 × 2.1 mm i.d.). Mobile phase: 80 or 90% acetonitrile, flow rate 0.5 mL/min. Fluorescence detection ($\lambda_{ex} = 238$ nm, $\lambda_{em} = 244$ nm). Limit of detection, 0.5 to 1.0 ng [Dickey *et al.* 1992].

Mass Spectrum Principal ions at *m/z* 1124, 1106, 1088, 1070, 1052, 1142.



Quantification

Other **TLC** Australian and French Polynesian *Gambierdiscus toxicus.* Plates: silica gel 60. Solvent system: pentanol : pyridine : water (4 : 4 : 1) [Holmes *et al.* 1990]. Barracuda (*Sphyraena jello*) Flesh and Viscera. Plates: Silica gel 60 (0.25 mm). Solvent system: chloroform : methanol : 6 mol/L ammonium hydroxide (90 : 9.5 : 0.5). R_f value: 0.1 to 0.3 for flesh toxin; 0.08 to 0.25 for viscera toxin. Limit of detection not reported [Lewis, Endean 1984]. Spanish Mackerel (*Scomberomorus commersoni*). Plates: Silica gel 60 (0.25 mm). Solvent system: chloroform : methanol : 6 mol/L ammonium hydroxide (90 : 9.5 : 0.5). R_f value: 0.08 to 0.20. Limit of detection not reported [Lewis, Endean 1983].

HPLC Flesh of Fish. Column: Hamilton PRP-1 (5 μm). Mobile phase: acetonitrile : water (1 : 1). UV detection ($\lambda = 215$ nm). Limit of detection not reported [Lewis, Sellin 1992].

LC-MS Great Barracuda (*Sphyraena barracuda*). Column: Phenomenex Luna C8 (2) (150 × 2 mm, 5 μm). Mobile phase: water with 0.1% acetic acid : acetonitrile with 0.1% acetic acid (65 : 35 for 2 min to 20 : 80 at 30 min to 5 : 95 at 35 min for 5 min to 65 : 35 at 42 min for 5 min), flow rate 0.2 mL/min. MRM acquisition mode. Limit of quantification, 0.039 ppb [Dechraoui *et al.* 2005]. Column: Zorbax 300SB-C3 (150 × 2.1 mm i.d., 5 μm). Mobile phase: 0.1% formic acid : 90% acetonitrile with 0.1% formic acid (100 : 0 to 40 : 60 over 60 min to 0 : 100 for 2 min), flow rate 0.2 mL/min. API, positive ion mode. Limit of detection, 10 ng [Pottier *et al.* 2003]. Indian Ocean Reef Fish. Column: Zorbax 300SB-C3 (150 × 2.1 mm i.d.) or 300SB-C_{18} (150 × 2.1 mm i.d.). Mobile phase: 0.1% formic acid : 90% acetonitrile with 0.1% formic acid (100 : 0 to 30 : 70 over 70 min), flow rate 0.2 mL/min. ESI. Limit of detection not reported [Hamilton *et al.* 2002a]. Extracts of Red Bass (*Lutjanus bohar*) and Red Emperor (*Lutjanus sebae*). Column: Zorbax 300SB-C_{18} (150 × 2.1 mm i.d.). Mobile phase: acetonitrile : water : propan-2-ol (80 : 19 : 1 buffered with 0.1% formic acid), flow rate 0.2 mL/min. ESI. Limit of detection not reported [Hamilton *et al.* 2002b]. Crude Extracts of Fish. Column: Zorbax reversed phase C_3 (150 × 2.1 mm i.d.). Mobile phase: 0.1% formic acid : 90% acetonitrile with 0.1% formic acid (100 : 0 to 40 : 60 over 60 min to 0 : 100 for 2 min), flow rate 0.2 mL/min. API, positive ion mode. Limit of detection, 10 ng [Pottier *et al.* 2002a, b]. Column: Vydac TP52 (250 × 2.1 mm i.d., 5 μm). Mobile phase: 0.05% trifluoroacetic acid : 90% acetonitrile-0.05% trifluoroacetic acid (50 : 50 for 2 min to 0 : 100 over 25 min for 10 min), flow rate 150 μL/min. API, positive ion mode. Limit of detection, 0.04 ppb [Lewis *et al.* 1999]. Moray Eel (*Lycodontis javanicus*). Column: PRP-1 reversed phase (150 × 0.41 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid or 1 mmol/L ammonium acetate (pH 5.8) : 95% acetonitrile with 0.1% trifluoroacetic acid or 1 mmol/L ammonium acetate (50 : 50 for 10 min to 0 : 100 over 20 min for 20 min), flow rate 0.5 mL/min. API, positive ion mode. Limit of detection, ≈0.3 ppb [Lewis, Jones 1997]. Greater Amberjack (*Seriola dumerili*). Column: ODS-L80 J-sphere (250 × 2.0 mm i.d.). Mobile phase: 36% acetonitrile and 0.1% formic acid to 90% acetonitrile and 0.1% formic acid over 20 min, flow rate 200 μL/min. APCI. Retention time: 18.25 min. Limit of detection not reported [Poli *et al.* 1997]. Horse-eye Jack (*Caranx latus*). Column: Vydac 201 HS C_{18} (250 × 0.21 mm i.d.). Mobile phase: acetonitrile : 0.1% trifluoroacetic acid (1 : 1), flow rate 10 μL/min. API, positive ion mode. Limit of detection not reported [Vernoux, Lewis 1997]. Coral Cod (*Cephalopolis miniatus*). Column: PRP-1 reversed phase (150 × 41 mm i.d., 5 μm). Mobile phase: 0.1% trifluroacetic acid : 95% acetonitrile with 0.1% trifluoroacetic acid (50 : 50 for 10 min to 0 : 100 over 20 min for 20 min), flow rate 0.5 mL/min. API, positive ion mode. Limit of detection not reported [Lucas *et al.* 1997]. Moray Eel Viscera and Crude Fish Extracts. Column: C_{18} (5 μm). Mobile phase: acetonitrile : water (1 : 1), flow rate 1 mL/min. ESI, positive ion mode. Limit of detection, 1 ng [Lewis *et al.* 1994].

Note Ciguatoxin is often measured by mouse bioassay. The mouse bioassay defines the mouse unit (MU) as the minimum amount of toxin needed to kill a 20 g mouse within 24 h: 1 MU is equivalent to 72 μg ciguatoxin.

Disposition in the Body Ciguatera toxin appears to open and activate voltage-dependent sodium channels. This affects a global array of bodily functions. It has been reported that the Schwann cells surrounding peripheral nerve axons become oedematous, possibly modifying or impeding neural transmission [Levine 1995].

Toxicity Ciguatera fish poisoning (CFP) is a considerable cause of morbidity in tropical regions, affecting an estimated 50 000 people worldwide each year. Symptoms typically manifest 3–18 h after the ingestion of toxic fish, with gastrointestinal malaise usually occurring 6 h after ingestion and neurological manifestations occurring after 6–12 h. The most distinctive features of ciguatera are severe pruritus, hot/cold reversal (the 'dry ice sensation'), and tingling and numbness of the extremities. The gastrointestinal complaints usually abate within 1 to 3 days but neurological perturbations can last for 3 days to several years [Hahn, Capra 1992]. Ciguatera symptoms are highly variable between individuals and between regions, largely owing to the type of fish consumed [Anderson, Lobel 1987]. In very rare cases, delirium, coma, cardiac and respiratory failure, and death may occur [Levine 1995]. The LD_{50} values in mice via IP injection are 0.25, 2.3 and 0.9 μg/kg for CTX-1, CTX-2 and CTX-3, respectively, reached at a minimum time of 37, 53 and 60 min, respectively [Lewis *et al.* 1991].

A 47-year-old previously healthy woman was admitted to hospital after ingesting ~10 barracuda eggs. She presented with nausea, vomiting, watery diarrhoea and myalgias. Approximately 2 h after ingestion, she suffered from numbness and tingling of the lips and all 4 limbs, as well as severe headache and dizziness. She was treated with IV fluids as well as 5 mg atropine and a dopamine infusion because of her bradycardia and hypertension. Her symptoms improved but she remained bradycardic so required a continuous IV infusion of atropine totalling 40 mg over 2 days. She made a full recovery 26 months later [Hung *et al.* 2005].

A 71-year-old man was admitted to hospital with dyspnoea, severe nausea, vomiting, watery diarrhoea and myalgias. He had consumed 8 barracuda eggs 1 h previously. He developed headache, dizziness, weakness, dysuria, perioral paraesthesia and paraesthesia of the extremities. Sinus bradycardia and hypotension followed. He was treated with IV fluids, 5 mg atropine, and a dopamine infusion. Bradycardia persisted and he required a total of 30 mg atropine as a continuous infusion over 2 days. His paraesthesia resolved 2 months later [Hung *et al.* 2005].

A 45-year-old previously healthy woman presented with dizziness, nausea, vomiting, watery diarrhoea, perioral paraesthesia and myalgias. She had consumed 3 barracuda eggs ≈1 h previously. After treatment with IV fluids she was discharged 4 h later [Hung *et al.* 2005].

A 26-year-old white man was admitted to hospital after ingesting coral cod. He presented with weakness and listlessness and complained of nausea, vomiting and myalgia, with perioral and extremity paraesthesia. The patient was managed with IV fluids, metoclopramide, hyoscine, and paracetamol with codeine. He was discharged the following day, although still complaining of paraesthesia and dysaesthesia of the extremities [Lucas *et al.* 1997].

A 24-year-old Aboriginal woman was admitted to hospital with a 24 h history of vomiting and diarrhoea, abdominal cramps, listlessness and malaise. She had ingested coral cod 24 h previously. The patient was administered IV mannitol (1 g/kg) and additional IV fluid. The following day the patient was ambulant without cerebellar ataxia, fully alert, and afebrile The patient discharged herself before further examination was possible [Lucas *et al.* 1997].

Ciquatera poisoning occurs throughout the Caribbean and tropical Pacific regions but cases have also been reported in the more temperate climates of South Carolina and Texas, USA [Centers for Disease Control and Prevention 1998, 2006], the Canary Islands [Pérez-Arellano *et al.* 2005] and Avonmouth, UK [Kipping *et al.* 2006].

Note For a review on the epidemiology of ciguatera, see Glaziou and Legrand [1994].

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Cilazapril

ACE Inhibitor

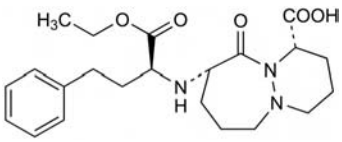
$C_{22}H_{31}N_3O_5.H_2O = 435.5$

CAS—92077-78-6

IUPAC Name (4*S*,7*S*)-7-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-6-oxo-1,2,3,4,7,8,9,10-octahydropyridazino[1,2-*a*]diazepine-4-carboxylic acid hydrate

Synonyms Cilazapril monohydrate; [1*S*-[1α,9α(*R**)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]octahydro-10-oxo-6*H*-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate; Ro-31-2848/006.

Proprietary Names *Dynorm*; *Inhibace*; *Initiss*; *Justor*; *Vascace*



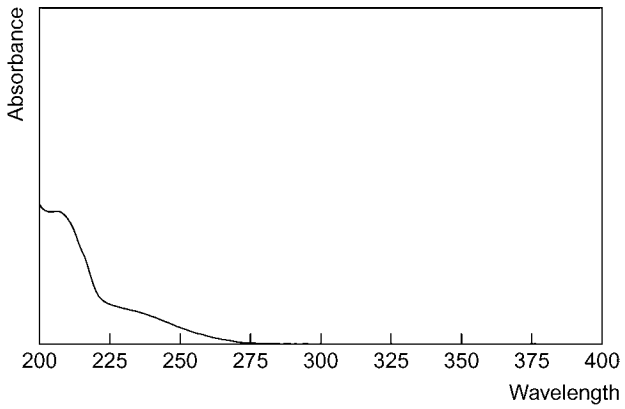
Chemical Properties Crystals (from aqueous ethanol). Mp 95° to 97°.

Thin-Layer Chromatography System TE—R_f 0.03; system TF—R_f 0.00.

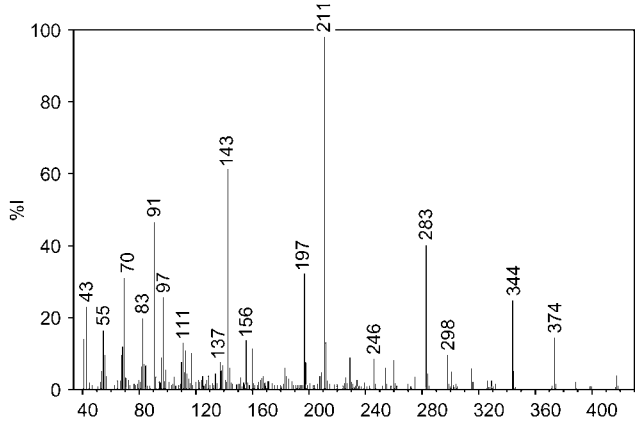
Gas Chromatography System GP—RI 3010 (cilazapril-Me); RI 2960 (M (cilazapril-Me_3)).

High Performance Liquid Chromatography System HX—RI 420; system HZ—cilazapril, retention time 4.5min; cilazaprilate,retention time 1.7 min; system HAA—retention time 14.4 min.

Ultraviolet Spectrum Principal peak at 208 nm.



Mass Spectrum Principal ions at *m/z* 211, 143, 91, 283, 197, 70, 344, 97.



Quantification

Plasma **Enzymic immunoassay** Limit of detection, 30 ng/L for cilazapril and 30 µg/L for cilazaprilate [Tanaka *et al.* 1987].

Serum **Enzymic immunoassay** See Plasma [Tanaka *et al.* 1987].

Urine **HPLC** Column: C_{18} (µBondapak, 300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : 5 mmol/L phosphoric acid (50:50), flow rate 1.0 mL/min. Electrochemical detection. Retention time: cilazapril, 5.1 min; cilazaprilate, 8.4 min. Limit of quantification, 0.17 mg/L for cilazapril and 0.14 mg/L for cilazaprilate. Limit of detection 50 µg/L and 40 µg/L, respectively [Prieto *et al.* 1998].

Pharmaceutical Products **HPLC** See Urine [Prieto *et al.* 1998].

Disposition in the Body After oral administration and rapid absorption, cilazapril is hydrolysed in the liver to its diacid, cilazaprilate (active ACE inhibitor), and no further metabolism occurs. Excretion is biphasic and occurs almost exclusively in the kidneys with ~53% cilazaprilate being recovered in urine after oral administration of cilazapril. Rapid elimination of cilazaprilate occurs with unbound drug eliminated within 8 h. Both cilazapril and cilazaprilate are removed to a limited extent by haemodialysis.

Therapeutic Concentration

Twelve healthy males, between 19 and 38 years old, were administered a single 2.5 mg oral dose of cilazapril at weekly intervals. A mean maximum plasma concentration of 0.082 mg/L at 0.83 h was observed for cilazapril and 0.036 mg/L at 1.7 h for cilazaprilate [Williams *et al.* 1989a]; [Williams *et al.* 1989b].

Toxicity Skin rashes and allergies have been noted in women administered the low toxic dose of 15 mg/kg at 22 week intervals. It is a poison by IV routes and moderately toxic by SC and IP routes.

Bioavailability About 60%.

Half-life Initial half-life of 1.3 to 1.8 h and a terminal phase elimination half-life of 40 to 50 h for cilazaprilate. Effective half-life (steady-state reached) is ~9 h.

Volume of Distribution Apparent mean volume of distribution for cilazaprilate is 29 to 46 L.

Clearance Plasma clearance, of cilazaprilate is 14 to 24 L/h, after a single oral dose of 2.5 to 10 mg cilazapril, and in patients with moderate essential hypertension clearance is 14 to 16 L/h, after a single oral dose of 5 to 20 mg.

Protein Binding 24%.

Dose An initial daily dose of 1.25 mg for 2 days; gradually increasing to 5 mg/day depending on blood pressure response. A single dose is usually between 2.5 and 5 mg/day.

Patients with creatinine clearance >40 mL/min, receive an initial daily dose of 1 mg; patients with clearance between 10 and 40 mL/min have an initial daily dose of 0.5 mg and following doses with a maximum of 2.5 mg. Patients with clearance <10 mL/min receive a 0.25 to 0.5 mg/day dose once or twice a week.

Prieto JA *et al.* (1998). Quantitative determination of the angiotensin-converting enzyme inhibitor cilazapril and its active metabolite cilazaprilat in pharmaceuticals and urine by high-performance liquid chromatography with amperometric detection. *J Chromatogr B Biomed Sci Appl* 714: 285–292.
Tanaka H *et al.* (1987). Enzyme immunoassay discrimination of a new angiotensin-converting enzyme (ACE) inhibitor, cilazapril, and its active metabolite. *J Pharm Sci* 76(3): 224–227.
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Cilostazol

Antithrombotic

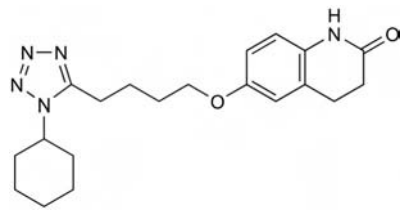
$C_{20}H_{27}N_5O_5 = 369.5$

CAS—73963-72-1

IUPAC Name 6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxyl]-3,4-dihydro-2(1*H*)-quinolinone

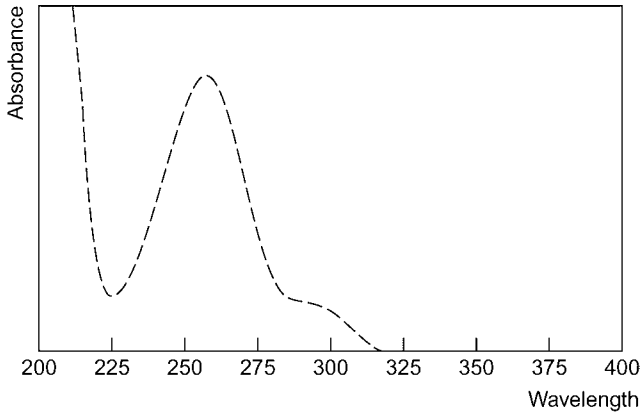
Synonyms OPC-21; OPC-13013.

Proprietary Names *Pletaal*; *Pletal*.

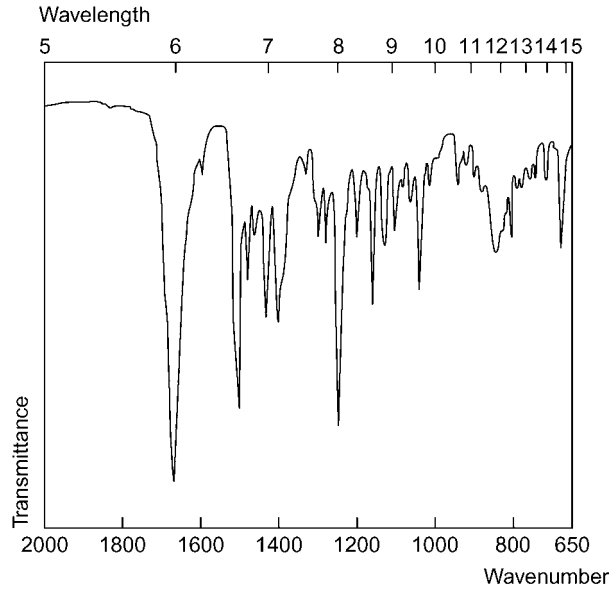


Chemical Properties White to off-white crystals or crystalline powder. Mp 160°. It is freely soluble in acetic acid, chloroform, *N*-methyl-2-pyrrolidone and DMSO; slightly soluble in methanol and ethanol; practically insoluble in ether, water, 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide. Log *P* (octanol/water), 4.04.

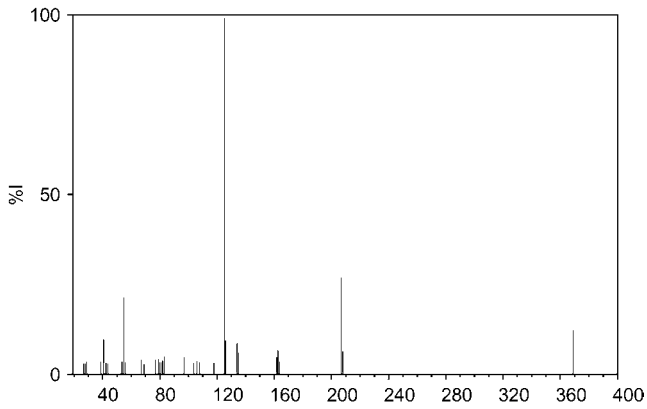
Ultraviolet Spectrum Methanol—257 nm.



Infrared Spectrum Principal peaks at wavenumbers 1667, 1505, 1246, 1039, 2932, 2867 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 125, 207, 55, 369, 41, 126, 134, 163.



Disposition in the Body Cilostazol is absorbed at a moderate rate after oral administration. Absorption is increased if the drug is administered with a high-fat meal. Mean peak plasma concentrations are observed 2.4 h after administration and the drug is extensively metabolised by hepatic cytochrome P450 enzymes (mainly 3A4). A number of metabolites are produced, two of which are active; 3,4-dehydrocilostazol (the most active metabolite accounting for at least 50% of the pharmacological activity) and 4′-*trans*-hydroxycilostazol. In plasma, 56% of the dose is detected as the unchanged drug, 15% 3,4-dehydrocilostazol and 4% 4′-*trans*-hydroxycilostazol. The primary elimination route is urine with 74% of the dose excreted in this way; <2% as the 3,4-dehydrocilostazol metabolite and 30% 4′-*trans*-hydroxycilostazol. No unchanged drug is detected in urine. It is extensively distributed in tissues.

Therapeutic Concentration

Twenty healthy non-smoking males, with a mean age of 24 years, were administered with single doses of 50, 100 and 200 mg. Twenty-six patients (male and female) with intermittent claudication (a complex of symptoms) resulting from peripheral arterial disease (PAD), with a mean age of 68 years, were administered with a dose of 100 mg. The mean peak plasma concentration of cilostazol was 1.223 mg/L for the healthy individuals and 1.332 mg/L for the patients which were observed 2.5 h (range 1 to 5) and 2 h (range 1 to 6) after administration, respectively. Mean peak concentrations for the metabolite OPC-13015 were 0.406 mg/L and 0.426 mg/L, respectively, for the healthy individuals and the patients. These levels were reached at about 3 h (range 1 to 6). Mean peak concentrations for the metabolite OPC-13213 were 0.125 mg/L and 0.224 mg/L, respectively, for the healthy individuals and the patients. These levels were reached at about 3 h (range 1 to 8 and 1 to 5, respectively) [Bramer *et al.* 1999].

Half-life 11 to 13 h.

Volume of Distribution 2.76 L/kg.

Clearance Oral, 0.18 L/h/kg. Relatively low plasma clearance.

Protein Binding Approximately 95 to 98%.

Dose The recommended dose is 100 mg daily but reduced to 50 mg if co-administered.

Bramer SL *et al.* (1999). Cilostazol pharmacokinetics after single and multiple oral doses in healthy males and patients with intermittent claudication resulting from peripheral arterial disease. *Clin Pharmacokinet* 37: 21–11.

Cimetidine

Histamine H_2-Receptor Antagonist

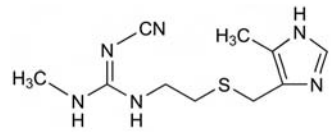
$C_{10}H_{16}N_6S = 252.4$

CAS—51481-61-9

IUPAC Name 1-Cyano-2-methyl-3-[2-[(5-methyl-1*H*-imidazol-4-yl)methylsulfanyl]ethyl] guanidine

Synonyms *N*-Cyano-*N*′-methyl-*N*″-[2-[[(5-methyl-1*H*-imidazol-4yl)methyl] thio]-ethyl]guanidine; SKF-92334.

Proprietary Names *Acibilin*; *Dyspanet*; *Edalene*; *Gimal*; *Gimetum*; *Peptol*; *Tagamet*; *Tratul*; *Ulcomet*; *Ulhys*.



Chemical Properties A crystalline powder. Mp 141° to 143°. Soluble 1 in about 88 of water. pK_a 6.8. Log *P* (octanol/water), 0.4.

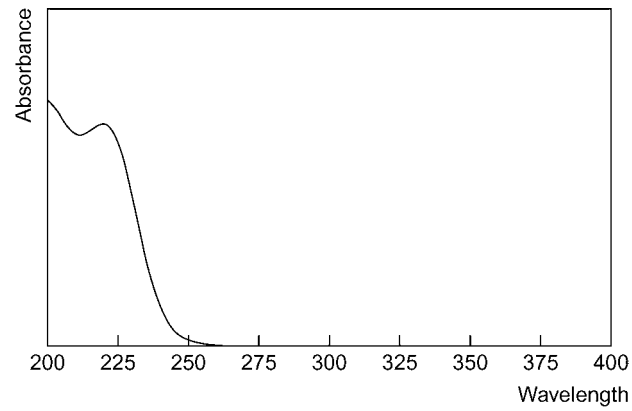
Colour Tests Nessler's reagent (100°)—black; sodium picrate (Steyn test)—red.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.00; system TC—R_f 0.09; system TE—R_f 0.27; system TL—R_f 0.12; system TAE—R_f 0.53; system TAF—R_f 0.55; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.08.

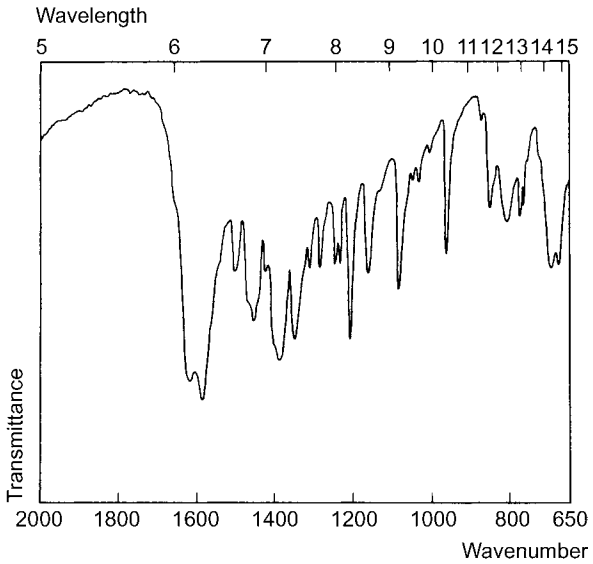
Gas Chromatography System GA—not eluted.

High Performance Liquid Chromatography System HA—*k* 0.4; system HX—RI 251; system HY—RI 226; system HZ—retention time 1.8 min; system HAA—retention time 3.6 min.

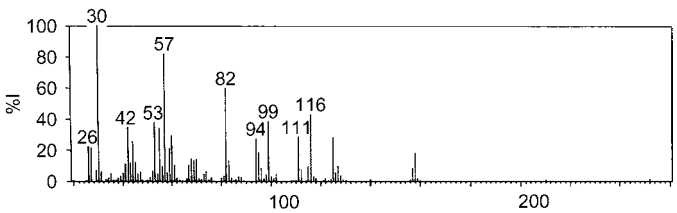
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1588, 1620, 1208, 1082, 1160, 1503 cm^{-1} (KBr disk). Polymorphism may occur.



Mass Spectrum Principal ions at *m/z* 30, 57, 82, 116, 99, 53, 55, 42.



Quantification

Plasma **HPLC** MS detection. Limit of quantification, 5 µg/L [Xu *et al.* 1999]. UV detection (λ=234 nm). Lower limit of quantification, 0.1 mg/L [Hempenius *et al.* 1998]. UV detection (λ=228 nm). Limit of quantification, 50 µg/L Limit of detection, 15 µg/L [Kelly *et al.* 1995]. UV detection. Limit of detection, 25 µg/L [Ching *et al.* 1984].

Serum **HPLC** UV detection. Cimetidine and metabolites, limit of detection, 50 µg/L for cimetidine [Ziemniak *et al.* 1981].

Urine **HPLC** See Plasma [Ching *et al.* 1984], and Serum [Ziemniak *et al.* 1981].

Disposition in the Body Rapidly absorbed after oral administration. About 50 to 80% of an IV dose is excreted in the urine as unchanged drug in 24 h, together with about 10% as the sulfoxide metabolite and about 5% as the 5-hydroxymethyl derivative. Up to about 10% of the dose is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 0.5 to 1.5 mg/L.

A single oral dose of 200 mg administered to 20 subjects resulted in peak blood concentrations of 0.6 to 1.9 mg/L (mean 1.1) in 1 to 2 h [Redolfi *et al.* 1979]. Following oral administration of 200 mg five times a day to 10 subjects, minimum steady-state plasma concentrations of 0.26 to 0.80 mg/L (mean 0.4) were reported; steady-state plasma concentrations of the sulfoxide metabolite ranged from 0.07 to 0.21 mg/L (mean 0.12) [Larsson 1982].

Toxicity Cimetidine appears to be relatively non-toxic; recovery has occurred after ingestion of 24 g in one dose, and ingestion of 60 g during 5 days caused no untoward effects. During chronic therapy, toxic effects are reportedly associated with trough plasma concentrations >1.3 mg/L.

In 3 cases of overdose caused by the ingestion of 5.2, 16, and 19.6 g of cimetidine, plasma concentrations of 37, 57, and 36 mg/L, respectively, were reported 2 to 3 h after ingestion; the subjects all recovered [Illingworth, Jarvie 1979].

In a fatality caused by the ingestion of cimetidine and diazepam, post-mortem blood concentrations of 110 mg/L of cimetidine and 5.8 mg/L of diazepam were reported [Hiss *et al.* 1982].

Bioavailability About 70% but there is considerable inter-subject variation.

Half-life Plasma half-life, 1 to 3 h.

Volume of Distribution About 1 to 2 L/kg.

Clearance Plasma clearance, about 9 mL/min/kg; considerably reduced in elderly patients.

Distribution in Blood Plasma : whole blood ratio, about 1.0.

Protein Binding 13 to 26%.

Note For a review of the pharmacokinetics of cimetidine, see Somogyi, Gugler [1983].

Dose 0.8 to 1.6 g daily; maximum of 2.4 g daily.

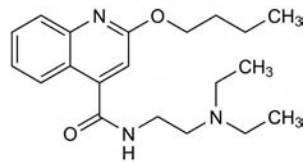
Ching MS *et al.* (1984). Liquid chromatographic analysis of cimetidine with procainamide as internal standard. *J Pharm Sci* 73: 1015.
Hempenius J *et al.* (1998). High-throughput solid-phase extraction for the determination of cimetidine in human plasma. *J Chromatogr B Biomed Sci Appl* 714: 361–368.
Hiss J *et al.* (1982). Fatal bradycardia after intentional overdose of cimetidine and diazepam. *Lancet* 2: 982.
Illingworth RN, Jarvie DR (1979). Absence of toxicity in cimetidine overdosage. *Br Med J* 1: 453–454.
Kelly MT *et al.* (1995). Determination of cimetidine in human plasma by high-performance liquid chromatography following liquid-liquid extraction. *J Chromatogr B Biomed Appl* 668(1): 117–123.
Larsson R (1982). The pharmacokinetics of cimetidine and its sulphoxide metabolite in patients with normal and impaired renal function. *Br J Clin Pharmacol* 13: 163–170.
Redolfi A *et al.* (1979). Blood level of cimetidine in relation to age. *Eur J Clin Pharmacol* 15: 257–261.
Somogyi A, Gugler R (1983). Clinical pharmacokinetics of cimetidine. *Clin Pharmacokinet* 8: 463–495.
Xu K *et al.* (1999). Quantitative analysis of cimetidine in human plasma using LC/APCI/SRM/MS. *Biomed Chromatogr* 13(7): 455–461.
Ziemniak JA *et al.* (1981). Liquid-chromatographic determination of cimetidine, its known metabolites, and creatinine in serum and urine. *Clin Chem* 27: 272–275.

C

Cinchocaine

Anaesthetic (Local)
$C_{20}H_{29}N_3O_2$ = 343.5
CAS—85-79-0

IUPAC Name 2-Butoxy-*N*-[2-(diethylamino)ethyl]-4-quinolinecarboxamide
Synonyms Cincainum; dibucaine.
Proprietary Name *Nupercainal*. It is an ingredient of many proprietary preparations—see Sweetman [2009].

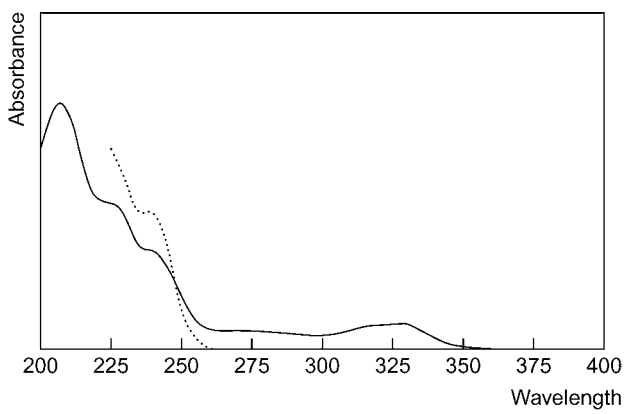


Chemical Properties A white, somewhat hygroscopic powder. Mp 64°. Soluble 1 in 4600 of water, 1 in less than 1 of ethanol and of chloroform, and 1 in 1.5 of ether. pK_a 7.5 (20°). Log *P* (octanol/water), 4.4.

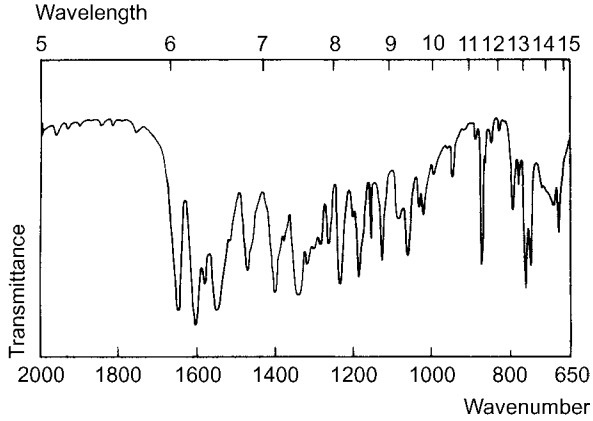
Cinchocaine Hydrochloride

$C_{20}H_{29}N_3O_2$, HCl = 379.9
CAS—61-12-1
Synonyms Cincaini chloridum; percainum; sovcainum.
Proprietary Names *Cincain*; *Doloposterine N*; *Nupercainal*; *Nupercaine*.
Chemical Properties Fine, white, crystals or white crystalline powder. Mp 90° to 98°, with decomposition. Soluble 1 in 0.5 of water; freely soluble in ethanol, acetone and chloroform; insoluble in ether and oils.

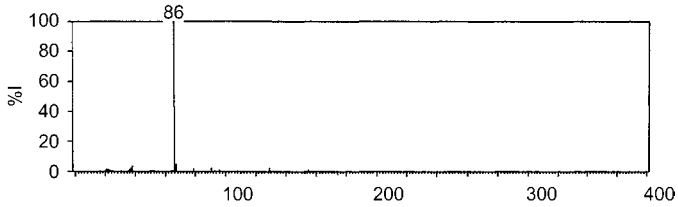
Colour Test Mercurous nitrate—black
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.25; system TC—R_f 0.34; system TE—R_f 0.67; system TL—R_f 0.35; system TAE—R_f 0.42 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2890; system GQ—retention time 10.4 min.
High Performance Liquid Chromatography System HA—*k* 1.9; system HR—*k* 5.51; system HY—RI 371.
Ultraviolet Spectrum Aqueous acid—247 (A^1_1=720a), 319 nm (A^1_1=260a); aqueous alkali—238, 325 nm.



Infrared Spectrum Principal peaks at wavenumbers 1598, 1643, 1540, 766, 1236, 1574 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 87, 58, 149, 111, 99, 57, 41 (no peaks above 210).



Quantification
Plasma **GC-MS** SIM. Limit of quantification, 100 µg/L. Limit of detection, 80 µg/L [Ohhima, Takayasu 1999].
Serum **GC-MS** Limit of detection, 1 µg/L [Alkalay *et al.* 1981].
Urine **GCMS** See Plasma [Ohhima, Takayasu 1999].

Disposition in the Body
Toxicity Cinchocaine is more toxic than procaine by injection, and more toxic than cocaine by local application.

An 18-month-old girl died 7 h after ingesting an estimated 150 mg (15 mg/kg) cinchocaine contained in a tube of ointment; cinchocaine levels were not obtained at autopsy. In a second fatality, involving a 2-year-old boy who ingested an unknown quantity of cinchocaine cream, the postmortem blood cinchocaine level was 1.3 mg/L. A third fatality was reported—a 21-month-old girl who ingested three-quarters of a 1 oz tube of cinchocaine ointment (~225 mg; 19 mg/kg)—in which death occurred 3 h after the ingestion; no autopsy was performed [Dayan *et al.* 1996].

In a fatality involving the ingestion of cinchocaine, the following postmortem tissue concentrations were reported: blood 0.6 mg/L, kidney 5.7 µg/g, liver 25 µg/g; ethanol was also detected in blood at a concentration of 1500 mg/L [Borkowski, Gubala 1976].

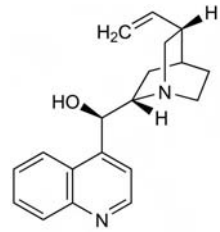
Half-life Plasma half-life, about 11 h.
Use Cinchocaine is used in concentrations of 0.1 to 1%, for surface anaesthesia.

Alkalay D *et al.* (1981). Quantitation of the local anesthetic dibucaine with gas chromatography / mass spectrometry. *Analyt Lett* 14: 1745–1756.
Borkowski T, Gubala W (1976). *Bull Int Assoc Forensic Toxicol* 12(3): 18.
Dayan PS *et al.* (1996). Fatal accidental dibucaine poisoning in children. *Ann Emerg Med* 28: 442–445.
Ohshima T, Takayasu T (1999). Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography-mass spectrometry with solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 726: 185–194.
Sweetman SC, ed. (2009). *Martindale: The complete drug reference*, 36th edn. London: Pharmaceutical Press.

Cinchonidine

Antimalarial
$C_{19}H_{22}N_2O = 294.4$
CAS—485-71-2

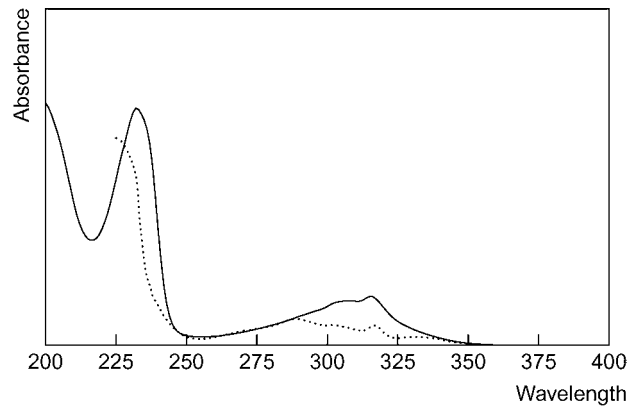
IUPAC Name (*R*)-[(2*S*,4*S*, 5*R*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-quinolin-4-ylmethanol
Synonyms (8α,9*R*)-Cinchonan-9-ol; cinchonatine; α-quinidine.



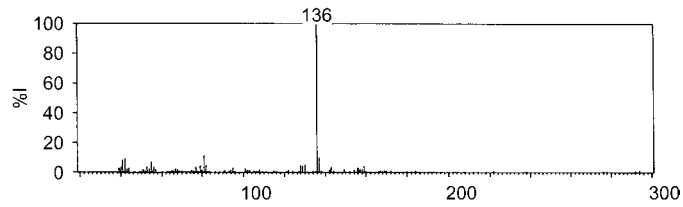
Chemical Properties An alkaloid present in the bark of various species of *Cinchona* (Rubiaceae). Cinchonidine is the (−)-stereoisomer of cinchonine; quinine is its (−)-6′-methoxy derivative. White crystals or powder. Mp about 210°, with decomposition. Practically insoluble in water; soluble 1 in 10 of ethanol; soluble in chloroform; moderately soluble in ether. pK_a 4.0, 8.2 (25°). Log *P* (octanol/water), 2.8.

Cinchonidine Sulfate
$(C_{19}H_{22}N_2O)_2, H_2SO_4, 7H_2O = 813.0$
CAS—524-61-8 (anhydrous)
Chemical Properties Colourless, shining, silky crystals. Mp about 207°. Soluble 1 in 100 of water and 1 in 60 of ethanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.06; system TC—R_f 0.08; system TE—R_f 0.44; system TL—R_f 0.06; system TAE—R_f 0.24; system TAF—R_f 0.55; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.70 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2590.
High Performance Liquid Chromatography System HA—*k* 3.1; system HX—RI 306; system HY—RI 214.
Ultraviolet Spectrum Aqueous acid—236 (A^1_1=1208a), 316 nm.



Infrared Spectrum Principal peaks at wavenumbers 754, 899, 1090, 800, 877, 1587 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 136, 81, 137, 42, 41, 55, 130, 128.

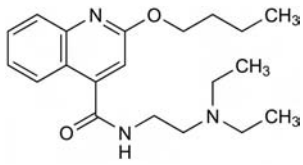


Disposition in the Body Metabolised by 2′-hydroxylation of the quinoline nucleus followed by 2-hydroxylation of the quinuclidine ring; both 2′-hydroxycinchonidine and 2,2′-dihydroxycinchonidine have been detected in urine.
Dose Cinchonidine sulfate was formerly given in doses of 60 to 600 mg.

Cinchonine

Antimalarial
$C_{19}H_{22}N_2O = 294.4$
CAS—118-10-5

IUPAC Name (*S*)-[(2*R*, 5*R*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-quinolin-4-ylmethanol
Synonym (9*S*)-Cinchonan-9-ol



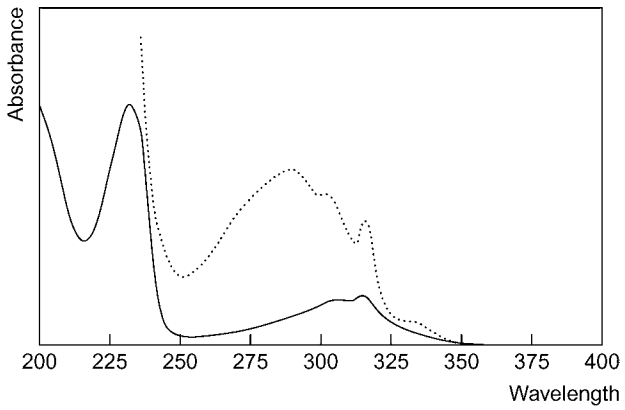
Chemical Properties An alkaloid present in the bark of various species of *Cinchona* (Rubiaceae). Cinchonine is the (+)-stereoisomer of cinchonidine; quinidine is its (+)-6′-methoxy derivative. White shining prisms or needles. Mp 264°. Practically insoluble in water; soluble 1 in 60 in ethanol, 1 in 110 of chloroform, and 1 in 500 of ether. pK_a 4.1, 8.2 (25°). Log *P* (octanol/water), 2.7.

Cinchonine Hydrochloride

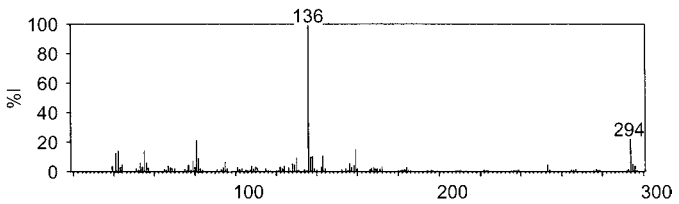
$C_{19}H_{22}N_2O$, HCl, $2H_2O$ = 366.9
CAS—5949-11-1 (anhydrous)

Chemical Properties White microcrystalline flakes or needles. Mp about 215°, with decomposition (anhydrous salt). Soluble 1 in 20 of water, 1 in 1.5 of ethanol, 1 in 20 of chloroform, and 1 in 300 of ether.

Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.06; system TC—R_f 0.12; system TE—R_f 0.44; system TL—R_f 0.05; system TAE—R_f 0.19; system TAF—R_f 0.61 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2590.
High Performance Liquid Chromatography System HX—RI 304; system HY—RI 209; system HAA—retention time 10.2 min.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1=1208a), 315 nm; aqueous alkali—288, 302, 315 nm.



Infrared Spectrum Principal peaks at wavenumbers 760, 1110, 1505, 1590, 990, 909 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 136, 294, 81, 159, 55, 42, 41, 143.

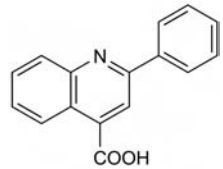


Disposition in the Body Rapidly and almost completely absorbed from the gastrointestinal tract, peak plasma concentrations being attained in 1 to 2 h. Metabolised by 2′-hydroxylation of the quinoline nucleus followed by 2-hydroxylation of the quinuclidine ring. About 55% of a dose is excreted as 2′-hydroxycinchonine, 22% as 2,2′-dihydroxycinchonine, and 5% as unchanged cinchonine.
Dose Cinchonine hydrochloride was formerly given in doses of 60 to 600 mg.

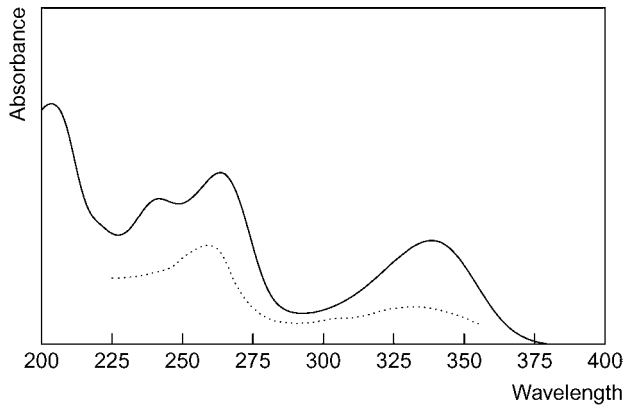
Cinchophen

Analgesic
$C_{16}H_{11}NO_2$ = 249.3
CAS—132-60-5

IUPAC Name 2-Phenyl-4-quinolinecarboxylic acid
Synonyms Acifenokinolin; phenylcinchoninic acid; quinophan.
Proprietary Names *Agotan*; *Atophan*; *Phenoquin*; *Quinophan*; *Rhematan*; *Tophol*; *Tophosan*.



Chemical Properties White or yellowish crystals or powder. Mp 213° to 216°. Practically insoluble in water; soluble 1 in 120 of ethanol, 1 in 40 of acetone, 1 in 400 of chloroform, and 1 in 100 of ether. Log *P* (octanol/water), 3.8.
Colour Tests Liebermann's reagent—red-orange; Marquis test—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.00; system TC—R_f 0.02; system TD—R_f 0.00; system TE—R_f 0.08; system TF—R_f 0.00; system TL—R_f 0.00; system TAD—R_f 0.07; system TAE—R_f 0.82; system TAF—R_f 0.72 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—243 (A^1_1=610b), 268, 344 nm; aqueous alkali—259, 330 nm.

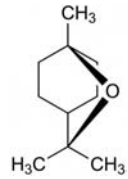


Infrared Spectrum Principal peaks at wavenumbers 759, 1250, 1698, 699, 731, 1199 cm^{-1} (KBr disk).
Disposition in the Body Readily absorbed after oral administration and extensively metabolised; <5% of a dose is excreted in the urine unchanged.
Half-life Plasma half-life, about 4 h.
Dose Cinchophen was formerly given in doses of 300 to 600 mg.

Cineole

Essential Oil
$C_{10}H_{18}O$ = 154.3
CAS—470-82-6

IUPAC Name 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane
Synonyms Cajuputol; eucalyptol.

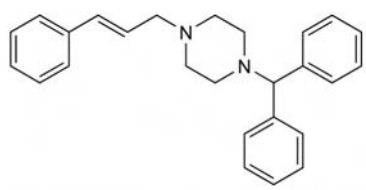


Chemical Properties Cineole is obtained from eucalyptus oil, cajuput oil, and other oils. A colourless liquid with an aromatic camphoraceous odour. Mass per mL 0.922 to 0.924 g. Fp 0°. Bp 176° to 177°. Refractive index (at 20°) 1.456 to 1.460. Practically insoluble in water; miscible with ethanol, chloroform, ether, and glacial acetic acid. Log *P* (octanol), 2.7.
Gas Chromatography System GB—RI 1063; system GO—retention time 11.2 min.
Infrared Spectrum Principal peaks at wavenumbers 980, 1075, 1219, 847, 1162, 1052 cm^{-1}.

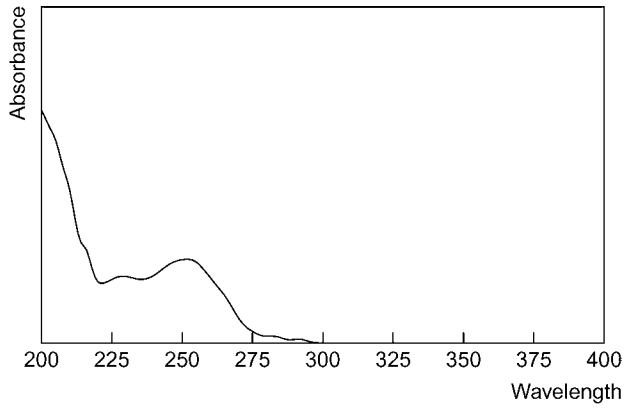
Cinnarizine

Antihistamine
$C_{26}H_{28}N_2$ = 368.5
CAS—298-57-7

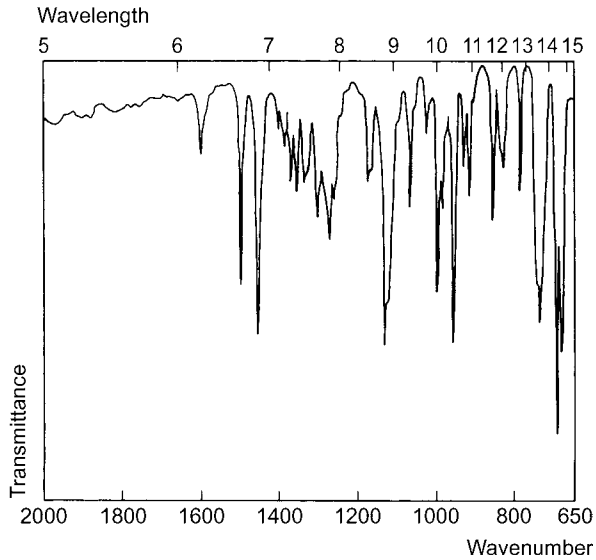
IUPAC Name 1-Benzhydryl-4-[(*E*)-3-phenylprop-2-enyl]piperazine
Synonyms 1-(Diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine; ginnipirine; S16-MD.
Proprietary Names *Aplactan*; *Aplexal*; *Carecin*; *Cerepar*; *Cinnacet*; *Gigantēn*; *Hilactan*; *Ixertol*; *Katoseran*; *Midronal*; *Olamin*; *Sepan*; *Stugeron*.



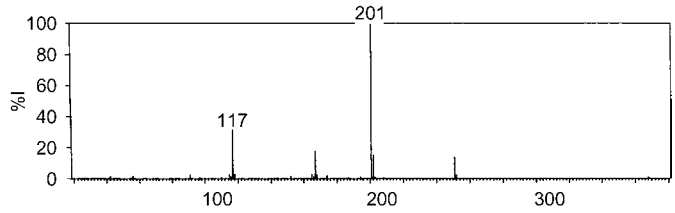
Chemical Properties Practically insoluble in water; soluble in dilute hydrochloric acid. Log *P* (octanol/water), 5.8.
Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.54; system TC—R_f 0.78; system TE—R_f 0.86; system TL—R_f 0.65; system TAE—R_f 0.79; system TAF—R_f 0.87 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—cinnarizine RI 3050, M (desalkyl-norcyclizine) RI 2120, M (benzophenone) (BPH) RI 1610, M (OH-benzophenone) isomer 1 RI 2065, M (OH-benzophenone) isomer 2 RI 2080, M (OH-methoxy-benzophenone) isomer 1 RI 2050, M (OH-methoxy-benzophenone) isomer 2 RI 2070, M (carbinol) RI 1750; system GB—cinnarizine RI 3233, M (desalkyl-) RI 2128, M (benzophenone) RI 1673.
High Performance Liquid Chromatography System HA—*k* 0.8; system HX—RI 560; system HZ—retention time 22.0 min; system HAA—retention time 19.3 min.
Ultraviolet Spectrum Aqueous acid—254 nm (A_1^1=584a).



Infrared Spectrum Principal peaks at wavenumbers 702, 691, 1138, 964, 740, 1000 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 201, 117, 167, 202, 251, 165, 118, 115.

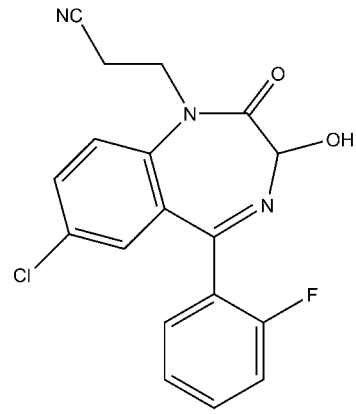


Quantification
Plasma **GC** AFID. Limit of detection, 0.5 µg/L [Woestenborgh et al. 1982].
HPLC UV detection. Limit of detection, 20 µg/L [Nitsche, Mascher 1982].
Serum **TLC** Limit of detection, 10 µg/L [Hassan *et al.* 2002].
HPLC UV detection (λ=250 nm). Limit of detection, 16 µg/L [Hassan *et al.* 2002].
Urine **GC** See Plasma [Woestenborgh et al. 1982].
Disposition in the Body
Therapeutic Concentration
Following a single oral dose of 50 mg to 6 subjects, a mean peak plasma concentration of 0.08 mg/L was attained in 2.3 h [Hundt *et al.* 1980].
Six young, healthy males were administered 75 mg cinnarizine as a tablet. Peak plasma drug concentrations reached a mean of 275 µg/L 3 h after dosing [Castaneda-Hernandez *et al.* 1993].
Half-life Plasma half-life, about 5 h.
Dose Usually 45 to 90 mg daily; doses of 150 to 225 mg daily are given in peripheral arterial disease.

Castaneda-Hernandez G *et al.* (1993). Pharmacokinetics of cinnarizine after single and multiple dosing in healthy volunteers. *Arzneimittelforschung* 43(5): 539–542.
Hassan SS *et al.* (2002). LC and TLC determination of cinnarizine in pharmaceutical preparations and serum. *J Pharm Biomed Anal* 28: 711–719.
Hundt HK *et al.* (1980). Determination of cinnarizine in plasma by high-performance liquid chromatography. *J Chromatogr* 183(3): 378–382.
Nitsche V, Mascher H (1982). Rapid high-performance liquid chromatographic assay of cinnarizine in human plasma. *J Chromatogr* 227(2): 521–525.
Woestenborgh R *et al.* (1982). Sensitive gas chromatographic method for the determination of cinnarizine and flunarizine in biological samples. *J Chromatogr* 232(1): 85–91.

Cinolazepam

Anticonvulsant, Hypnotic, Sedative, Skeletal Muscle Relaxant
$C_{18}H_{13}ClFN_3O_2$ = 357.8
CAS—75696-02-5
IUPAC Name 3-[7-Chloro-5-(2-fluorophenyl)-3-hydroxy-2-oxo-3*H*-1,4-benzodiazepin-1-yl]propanenitrile
Synonyms 7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-3-hydroxy-2-oxo-1*H*-1,4-benzodiazepine-1-propanenitrile; OX-373.
Proprietary Name *Gerodorm*



Chemical Properties Crystals. Mp 190° to 193°. Soluble in ethanol.
Quantification
Plasma **HPLC** Column: Lichrosorb RP-18 (125 × 4 mm i.d., 7 µm) or Polygosil 60–7 C_{18} (7.5 µm). Mobile phase: acetonitrile : 0.01 mol/L orthophosphoric acid (20 : 80; 30 : 70 for separation of optical isomeric glucuronides as well as free oxazepam and cinolazepam). UV detection (λ = 230 nm). Limit of detection not reported [Mascher *et al.* 1984].
Urine **HPLC** See Plasma [Mascher *et al.* 1984].
Note For a fluorometric method for the determination of cinolazepam in serum and urine, see Walash *et al.* [1994].

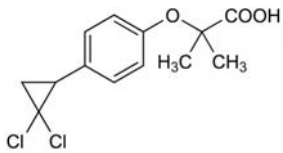
***Other* HPLC** Rabbit Plasma, Urine and Bile. See Plasma [Mascher *et al.* 1984].

Mascher H *et al.* (1984). Separation, isolation and identification of optical isomers of 1,4-benzodiazepine glucuronides from biological fluids by reversed-phase high-performance liquid chromatography. *J Chromatogr* 306: 231–239.
Walash MI *et al.* (1994). A selective fluorimetric method for the determination of some 1,4-benzodiazepine drugs containing a hydroxyl group at C-3. *J Pharm Biomed Anal* 12: 1417–1423.

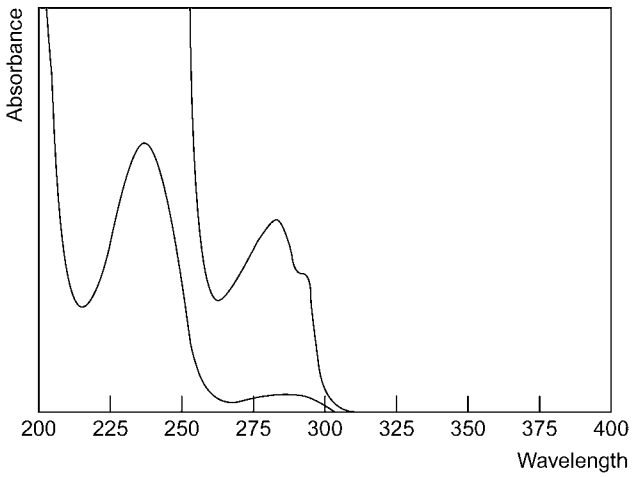
Ciprofibrate

Lipid-Regulating Agent
$C_{13}H_{14}Cl_2O_3 = 289.2$
CAS—52214-84-3

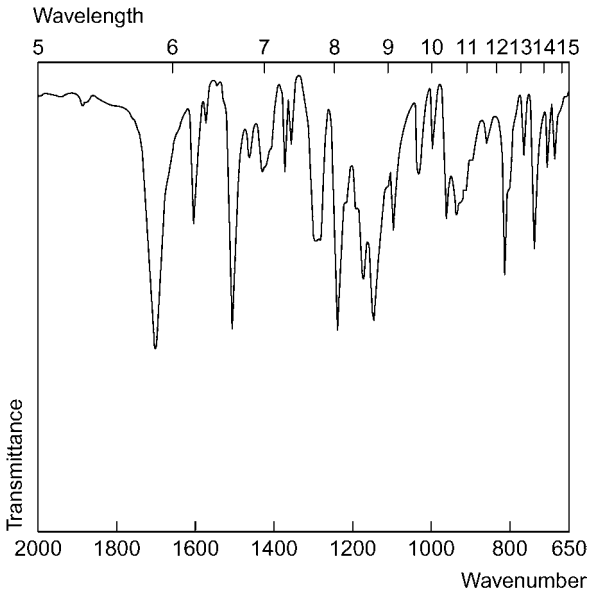
IUPAC Name 2-[4-(2,2-Dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid
Synonym Win-35833
Proprietary Names *Bi-Lipanor*; *Ciprol*; *Hyperlipen*; *Lipanor*; *Modalim*.



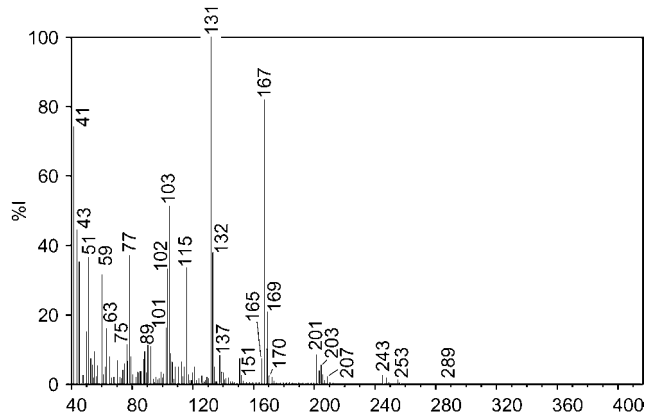
Chemical Properties A pale cream solid. Mp 114° to 116°. Log *P* (octanol/water), 3.94.
High Performance Liquid Chromatography System HAA—retention time 21.2 min.
Ultraviolet Spectrum Ethanol—232 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 131, 167, 41, 103, 43, 132, 77, 289.



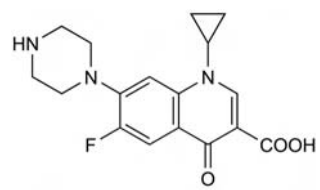
Quantification
***Plasma* HPLC** Column: RP Shimpack CLC-ODS RP-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L H_3PO_4 (55:45), flow rate 2.0 mL/min for the first 8 min then 2.5 mL/min until the end. UV detection (λ=232 nm). Retention time: 4.9 min. Limit of detection, 0.25 mg/L [Masnatta *et al.* 1996]. UV detection. Limit of detection, 0.69 mg/L [Park *et al.* 1982].
Disposition in the Body Ciprofibrate is readily absorbed after oral administration; peak plasma concentrations occur within 1 to 4 h. About 30 to 75% of a single dose is excreted in urine as the unchanged drug (20 to 25% of total excreted) and as glucuronide conjugates, in 72 h. It is not removed by dialysis.
Therapeutic Concentration
Group 1: Six patients with normal renal function, creatinine clearance 89 to 133 mL/min/1.73 m^2, aged 24 to 37 years; group 2: mild renal insufficiency, creatinine clearance 39 to 60 mL/min 1.73 m^2, aged 25 to 66 years; group 3: severe insufficiency, creatinine clearance 11 to 30 mL/min/1.73 m^2, aged 37 to 60 years; group 4: haemodialysis patients, 44 to 70 years old. Each group was administered with 100 mg ciprofibrate in a fasting state. The peak concentrations were 24, 22 and 17 mg/L for the first three groups observed at 1.3, 2.3 and 2.8 h [Ferry *et al.* 1989].
Half-life Plasma, 81 h in healthy individuals; 117 h (patients with mild renal failure; 154 h (patients undergoing haemodialysis); 172 h (severe renal failure).
Protein Binding 95% bound to albumin.
Note For reviews of ciprofibrate, see Betteridge [1993], and Turpin, Bruckert [1996].
Dose 100 mg daily.

Betteridge DJ (1993). Ciprofibrate–a profile. *Postgrad Med J* 69: 1S42–S49.
Ferry N *et al.* (1989). The influence of renal insufficiency and haemodialysis on the kinetics of ciprofibrate. *Br J Clin Pharmacol* 28(6): 675–681.
Masnatta LD *et al.* (1996). Determination of bezafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 687(2): 437–442.
Park GB *et al.* (1982). Determination of ciprofibrate in human plasma by high-performance liquid chromatography. *J Chromatogr* 227: 534–539.
Turpin G, Bruckert E (1996). Efficacy and safety of ciprofibrate in hyperlipoproteinaemias. *Atherosclerosis* 124: S83–S87.

Ciprofloxacin

Antibacterial
$C_{17}H_{18}FN_3O_3 = 331.4$
CAS—85721-33-1

IUPAC Name 1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid
Synonyms Bay-q-3939; 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.
Proprietary Names *Ciflox*; *Cipro*; *Ciprobay*; *Ciproxin*; *Ciproxine*; *Plenolyt*; *Septocipro*.



Chemical Properties Mp 255° to 257°, with decomposition. It is practically insoluble in water; very slightly soluble in ethanol and in methylene chloride; soluble in dilute acetic acid. Log *P* (octanol/water), 0.28.

C

Ciprofloxacin Hydrochloride

$C_{17}H_{18}FN_3O_3.HCl, H_2O = 385.8$
CAS—86483-48-9 (anhydrous); 86393-32-0 (monohydrate)
Synonym Bay-o-9867
Proprietary Names *Baycip*; *Belmacina*; *Catex*; *Ceprimax*; *Cetraxal*; *Ciflox*; *Ciloxan*; *Cipro*; *Ciprobay*; *Ciproxin*; *Ciproxine*; *Cunesin*; *Estecina*; *Flociprin*; *Globuce*; *Huberdoxina*; *Inkamil*; *Quipro*; *Rigoran*; *Sepcen*; *Septocipro*; *Tam*; *Velmonit*.
Chemical Properties Faintly yellowish to light yellow crystals. Mp 318° to 320°. It is sparingly soluble to soluble in water; slightly soluble in acetic acid and in methanol; very slightly soluble in ethanol; practically insoluble in acetone, in acetonitrile, in ethyl acetate, in hexane and in methylene chloride.

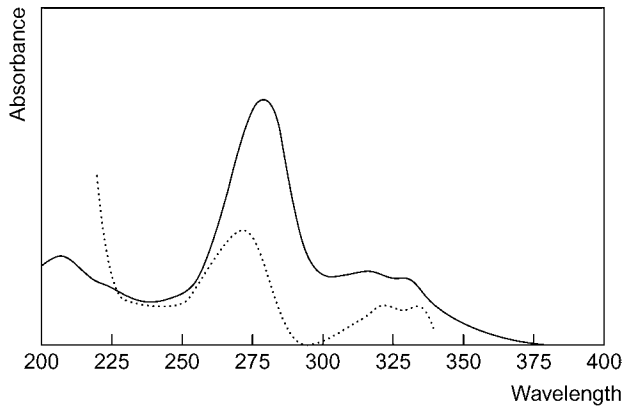
Ciprofloxacin Lactate

$C_{17}H_{18}FN_3O_3, C_3H_6O_3 = 421.4$
CAS—97867-33-9
Proprietary Names *Baycip*; *Ciprobay*; *Ciproxin*; *Ciproxine*; *Flociprin*; *Rigoran*; *Septocipro*; *Velmonit*.

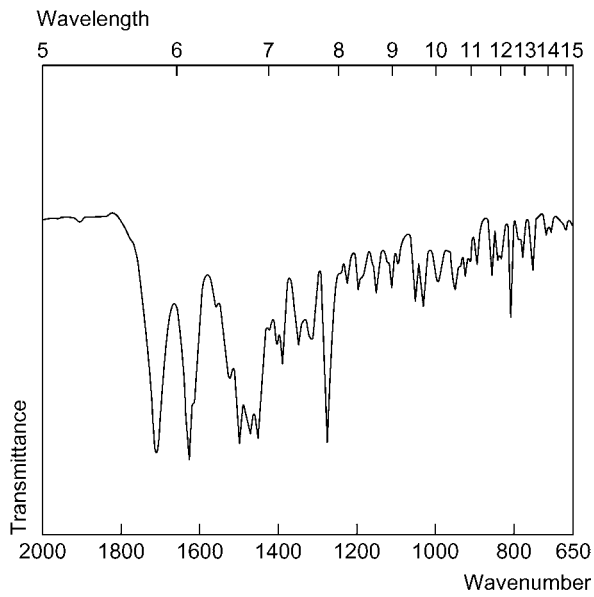
Thin-Layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.
High Performance Liquid Chromatography System HX—RI 318; system HY—RI 260; system HZ—retention time 1.8 min; system HAA—retention time 9.1 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer, (pH 3.5), flow rate 1 mL/min. UV diode array detection. Retention time: 1.1 min [Mills, Roberson 1993].

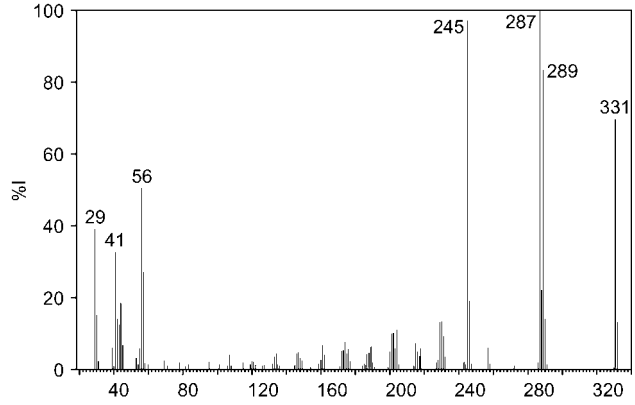
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—277, 315 nm; basic—272, 322, 334 nm.



Infrared Spectrum Principal peaks at wavenumbers 1703, 1619, 1267 cm^{-1} (KBr pellets).



Mass Spectrum Principal ions at *m/z* 287, 245, 289, 331, 56, 29, 41, 57.



Quantification

Blood **HPLC** Fluorescence detection. Limit of detection, 0.025 mg/L [Teja-Isavadharm *et al.* 1991].
Plasma **HPLC** Limit of quantification, 0.05 mg/L for ciprofloxacin and 0.01, 0.05 and 0.5 mg/L for its metabolites, M1, M2 and M3, respectively [Krol *et al.* 1995]. See Blood [Teja-Isavadharm *et al.* 1991].

CE Fluorescence detection. Limit of detection, 0.02 mg/L [Bannefeld *et al.* 1997].
Serum **HPLC** See Plasma [Krol *et al.* 1995]. UV detection. Limit of detection, 0.05 mg/L [Mack 1992]. UV detection. Limit of detection, 0.1 mg/L [Pou-Clave *et al.* 1991]. Fluorescence detection. Limit of detection, 0.02 mg/L [Myers, Blumer 1987]. Fluorescence detection. Limit of detection, 0.01 mg/L [Nilsson-Ehle 1987]. UV detection. Limit of detection, 0.06 mg/L [Vallée *et al.* 1986]. UV detection. Limit of detection, 0.01 mg/L [Jehl *et al.* 1985]. Fluorescence detection. Limit of detection, 0.08 mg/L [Nix *et al.* 1985].
Urine **HPLC** See Plasma [Krol *et al.* 1995]. See Serum [Mack 1992]; [Myers, Blumer 1987]; [Nilsson-Ehle 1987]; [Vallée *et al.* 1986]. Limit of detection, 0.5 mg/L [Jehl *et al.* 1985]; [Nix *et al.* 1985].
Bile **HPLC** UV detection. Limit of detection, 0.2 mg/L [Jehl *et al.* 1985].
Blister Fluid **HPLC** See Serum [Vallée *et al.* 1986].
Saliva **HPLC** See Serum [Mack 1992]; [Vallée *et al.* 1986].
Sputum **HPLC** See Serum [Mack 1992]; [Myers, Blumer 1987]; [Vallée *et al.* 1986].

Disposition in the Body Ciprofloxacin is rapidly and well absorbed after oral administration. It is widely distributed and it diffuses into CSF (cerebrospinal fluid); concentrations are usually <10% of peak plasma concentrations, although higher concentrations are found if the meninges are inflamed. It crosses the placenta and is distributed into breast milk. The drug is eliminated principally by urinary excretion (active tubular secretion as well as glomerular filtration). Non-renal clearance may account for about a third of elimination (hepatic metabolism, biliary excretion, and possibly transluminal secretion across intestinal mucosa). Active metabolites have been identified. About 40 to 50% of an oral dose is excreted unchanged in urine with about 15% excreted as metabolites. Up to 70% of a parenteral dose may be excreted unchanged within 24 h and 10% as metabolites. Faecal excretion over 5 days has accounted for 20 to 35% of an oral dose and 15% of an IV dose. Only small amounts are removed by haemodialysis or peritoneal dialysis.

Therapeutic Concentration The trough serum therapeutic concentration range is 0.05 to 0.5 mg/L and peak, 1 to 5 mg/L.

Single oral doses of 500 mg administered to 7 people undergoing orthopaedic surgery produced mean concentrations of 1.4 mg/L (serum), 0.4 µg/g (bone) and 1.1 µg/g (muscle) [Fong *et al.* 1986].

Toxicity

A 29-year-old woman was presented to hospital the day after ingesting 21 g of ciprofloxacin. She had vomited about 3 h after swallowing the tablets. The serum-ciprofloxacin concentration at presentation was 12 mg/L. 12 h later she developed oliguric acute renal failure. She made a full recovery following treatment with steroids, and hydration and acidification of urine [George *et al.* 1991].

Bioavailability 70%.
Half-life Plasma, 3.5 to 4.5 h.
Volume of Distribution 1.8 to 5.0 L/kg.
Clearance Plasma, 35 L/h.
Protein Binding 20 to 40%.
Note For reviews of ciprofloxacin, see Campoli-Richards *et al.* [1988] and Vance-Bryan *et al.* [1990].
Dose The equivalent of up to 1500 mg of ciprofloxacin daily by mouth; up to 800 mg daily may be given IV.

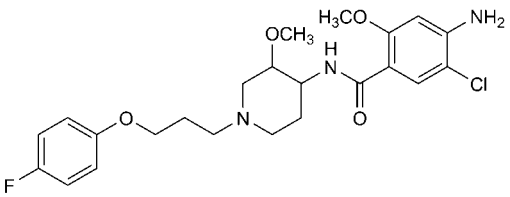
Bannefeld KH *et al.* (1997). Capillary electrophoresis with laser-induced fluorescence detection, an adequate alternative to high-performance liquid chromatography, for the determination of ciprofloxacin and its metabolite desethyleneciprofloxacin in human plasma. *J Chromatogr Biomed Sci Appl* 692: 453–459.
Campoli-Richards DM *et al.* (1988). Ciprofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 35: 373–447.
Fong IW *et al.* (1986). Ciprofloxacin concentrations in bone and muscle after oral dosing. *Antimicrob Agents Chemother* 29: 405–408.
George MJ *et al.* (1991). Acute renal failure after an overdose of ciprofloxacin. *Arch Intern Med* 151: 620.
Jehl F *et al.* (1985). High-performance liquid chromatographic method for determination of ciprofloxacin in biological fluids. *J Chromatogr* 339: 347–357.

Krol GJ *et al.* (1995). HPLC analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids. *J Pharm Biomed Anal* 14(1–2): 181–190.
Mack G (1992). Improved high-performance liquid chromatographic determination of ciprofloxacin and its metabolites in human specimens. *J Chromatogr* 582: 263–267.
Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 5: 104–105
Myers CM, Blumer JL (1987). High-performance liquid chromatography of ciprofloxacin and its metabolites in serum, urine and sputum. *J Chromatogr* 422: 153–164.
Nilsson-Ehle I (1987). Assay of ciprofloxacin and norfloxacin in serum and urine by high-performance liquid chromatography. *J Chromatogr* 416: 207–211.
Nix DE *et al.* (1985). Liquid-chromatographic determination of ciprofloxacin in serum and urine. *Clin Chem* 31: 684–686.
Pou-Clave L *et al.* (1991). Determination of ciprofloxacin in human serum by liquid chromatography. *J Chromatogr* 563: 211–215.
Teja-Isavadharm P *et al.* (1991). Measurement of ciprofloxacin in human plasma, whole blood, and erythrocytes by high-performance liquid chromatography. *Ther Drug Monit* 13: 263–267.
Vallée F *et al.* (1986). Determination of ciprofloxacin in biological samples by reversed-phase high performance liquid chromatography. *Ther Drug Monit* 8: 340–345.
Vance-Bryan K *et al.* (1990). Clinical pharmacokinetics of ciprofloxacin. *Clin Pharmacokinet* 19: 434–461.

Cisapride

Peristaltic Stimulant
$C_{23}H_{29}ClFN_3O_4 = 466.0$
CAS—81098-60-4

IUPAC Name 4-Amino-5-chloro-*N*-[1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide
Proprietary Names *Alimix*; *Arcasin*; *Cyprid*; *Fisiogastrol*; *Kelosal*; *Kinet*; *Prepulsid*; *Propulsid*; *Propulsin*; *Pulsitil*; *Trautil.*



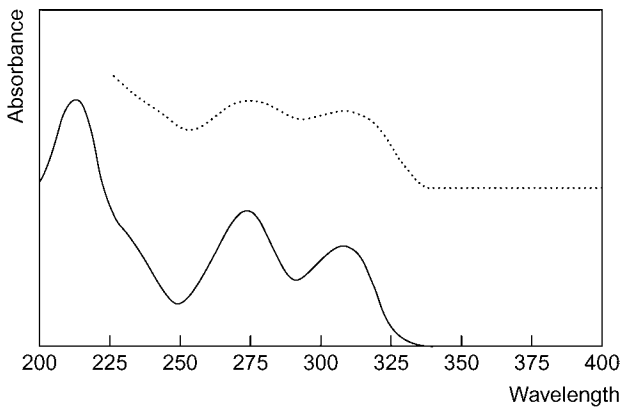
Chemical Properties White or almost-white powder; it exhibits polymorphism. It is practically insoluble in water; freely soluble in dimethylformamide; soluble in dichloromethane; sparingly soluble in methanol. pK_a 7.83. Log *P* (octanol/water) 3.96 [Woestenborghs *et al.* 1988]. Stable when extracted samples were reconstituted in mobile phase and stored at 20 ± 3° for 24 h. Stable in plasma stored at room temperature or 4° for at least 6 h, and at –20° for at least 48 h. Satisfactory stability was observed after 8 weeks at –70° [Preechagoon, Charles 1995]. Stable in suspension for 91 days at 4° and 28 days at 25° in darkened vials [Nahata *et al.* 1995]. Stable in plasma for at least 9 months at −20° [Woestenborghs *et al.* 1988].

Cisapride Monohydrate

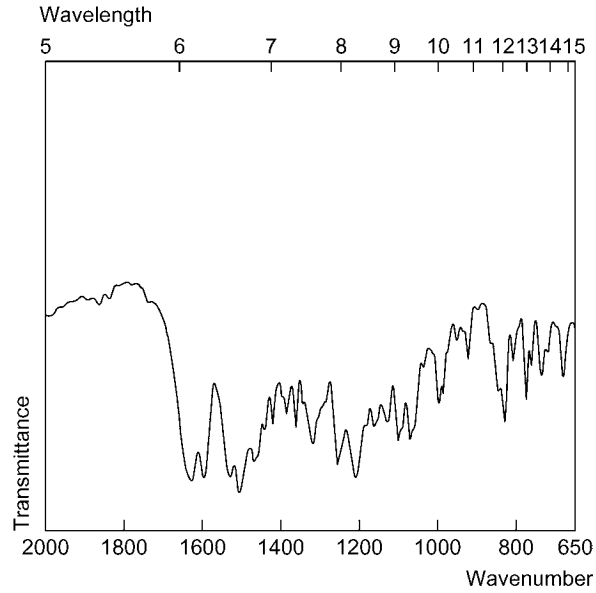
$C_{23}H_{29}ClFN_3O_4, H_2O = 484.0$
Synonym R-51619
Proprietary Names *Acenalin*; *Alimix*; *Cipril*; *Prepulsid*; *Propulsin*; *Risamal.*
Chemical Properties Crystals from 2-propanol. Mp 109.8°. Stable in plasma for at least 9 months if stored at –20° [Barone *et al.* 1987].

Thin-Layer Chromatography System TAE—R_f 0.66; system TB—R_f 0.00; system TE—R_f 0.62.
High Performance Liquid Chromatography System HAA—retention time 14.6 min; system HAX—retention time 10.9 min; system HAY—retention time 5.8 min; system HX—RI 431; system HY—RI 317; system HZ—retention time 5.2 min.

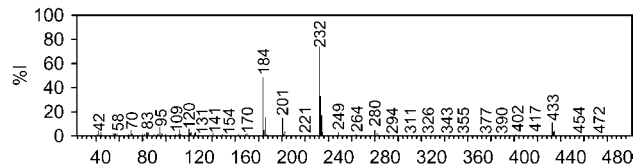
Column: Zorbax Rx-C8 (160 × 4.0 mm i.d., 5 µm). Mobile phase: water : triethylamine : acetonitrile (35 : 0.02 : 65), flow rate 1.0 mL/min. UV detection (λ = 276 nm). Retention time: 2.7 min [Nahata *et al.* 1995].
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—273, 307 nm; basic—275, 308 nm.



Infrared Spectrum Principal peaks at wavenumbers 1499, 1203, 823 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 232, 184, 233, 201, 186, 433, 95, 120.



Quantification

Plasma **HPLC** Column: Chiral (250 × 4.6 mm i.d.). Mobile phase: ethanol : hexane (35 : 65), flow rate 1.2 mL/min. UV detection (λ = 275 nm). Limit of quantification, 5 µg/L, limit of detection, 1 µg/L [Desta et al 2000]. Column: C_{18} RP NovaPak (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : methanol : 0.015 mol/L sodium phosphate buffer (pH 2.2; 68 : 19.4 : 12.6), flow rate 0.8 mL/min. UV detection (λ = 276 nm). *k* values: cisapride, 2.7 (retention time, 4.3 min); norcisapride, 3.7 (5.3 min). Limit of quantification, 10 µg/L for cisapride and norcisapride [Cisternino *et al.* 1998]. Column: C_8 Symmetry (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer (pH 5.2; 37 : 63), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 295 nm, λ_{em} = 350 nm). Limit of quantification, 8 µg/L; limit of detection, 5 µg/L [Preechagoon, Charles 1995]. Column: ODS-Hypersil (150 × 2.1 mm i.d., 5 µm). Mobile phase: water : acetonitrile (56 : 44), flow rate 0.8 mL/min. UV detection (λ = 276 nm). Retention time: 3.04 min. Limit of detection, 1 µg/L [Woestenborghs *et al.* 1988].

LC-MS Column: Hypersil C_{18} (500 × 4.6 mm i.d.). Mobile phase: methanol : 0.1 mol/L phosphate buffer (25 : 75 to 90 : 10). TIS, positive ion mode. Limit of detection, 1 µg/L [Lowry *et al.* 2003].

Serum **HPLC** Column: ODS Hypersil (150 × 4.6 mm i.d.). Mobile phase: 0.05 mol/L disodium hydrogen phosphate : acetonitrile (pH 8.4; 60 : 40), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 0.5 µg/L [de Condado *et al.* 2001].

Urine **HPLC** Column: Platinum EPS C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.02 mol/L sodium dihydrogen phosphate (45 : 55) containing 1 g/L triethylamine (pH 7.0), flow rate 1.3 mL/min. Fluorescence detection (λ_{ex} = 295 nm), λ_{em} = 300 nm). Limit of quantification, 50 µg/L [Addison *et al.* 1999].

Disposition in the Body Cisapride is readily absorbed after oral administration and the presence of food enhances absorption. It undergoes extensive metabolism in the liver and gut wall mainly via CYP3A4. This could affect neonates [Kearns *et al.*]. The main metabolic pathways are oxidative *N*-dealkylation, producing the major metabolite norcisapride, and aromatic hydroxylation to 3-fluoro-4-hydroxycisapride and 4-fluoro-2-hydroxycisapride. More than 90% of a dose is excreted as metabolites in urine and the faeces in ~equal amounts; 4 to 6% of a dose is recovered as the unchanged drug in the faeces and 0.2% in urine [Meuldermans *et al.* 1988]. A small amount is distributed into breast milk with ~0.1% of a dose being detected (milk : serum ratio, 0.045). It is not removed by haemodialysis.

Therapeutic Concentration

Mean peak plasma concentrations of 45 to 65 µg/L were reached about 1 to 2 h after administration of 10 mg given orally to healthy volunteers 15 min before food intake. Peak plasma concentrations of 49 µg/L were observed in the fasting state and 60 µg/L after the meal [Janssen Pharmaceutica data files].

Seventeen healthy females (age: 18 to 50 years) were administered 0.07 mg/kg cisapride. Mean peak plasma concentrations were 21.7 ± 8.3 μg/L at 1.29 ± 0.83 h [Lowry *et al.* 2003].

Ten male healthy volunteers were given 200 mL double-strength grapefruit juice 3 times a day. On day 3, subjects were given 10 mg cisapride with 200 mL double-strength grapefruit juice or water. Mean peak plasma concentrations of cisapride were 78.3 ± 23.6 μg/L at 2.5 h in the grapefruit juice phase compared with 43.2 ± 16.8 μg/L at 1.5 h in the control phase [Kivisto *et al.* 1999].

Eight elderly Japanese patients (age: 85 ± 6 years) were administered a single oral dose of 2.5 mg cisapride. They were also given 7.5 mg/day for the next 26 days. On day 1, the mean maximum plasma concentration was 10.7 ± 8.4 μg/L at 2.2 ± 1.2 h and on day 28 the mean maximum plasma concentration was 26.7 ± 7.6 μg/L at 2.8 ± 1.4 h [Yamamoto *et al.* 1998].

Twenty-two patients undergoing haemodialysis were administered a single oral dose of 20 mg cisapride. The mean maximum plasma concentration was 78.2 ± 20.9 μg/L reached at 2.0 ± 1.3 h. The terminal half-life was 9.6 ± 3.3 h, the volume of distribution was 4.8 ± 3.3 L/kg, and the total oral plasma clearance was 380 ± 161 mL/min [Gladziwa *et al.* 1991].

Toxicity Rare cases of cardiac arrhythmia, including ventricular tachycardia, ventricular fibrillation, torsade de pointes, and QT interval prolongation, have been reported [Bedu *et al.* 1997; Hanson *et al.* 1997].

A prolonged QT interval developed in 6 infants aged 11 to 70 days (weighing 1.2 to 4.0 kg) who were given cisapride 1.0 to 1.6 mg/kg daily. Correction of the QT interval occurred after cessation of cisapride in 5 of the infants and dose reduction to 0.8 mg/kg in another (plasma-cisapride concentrations were not reported) [Bedu *et al.* 1997].

Note For a case of movement disorders in a 3-year-old female while cisapride was being administered, see Elzinga-Huttenga *et al.* [2006]. For a case of an 8-month-old female with cisapride poisoning, see Gibly *et al.* [1997].

Bioavailability 35 to 40%.

Half-life Plasma, 7 to 10 h.

Volume of Distribution ≈24 L/kg [Gibly *et al.* 1997].

Clearance Plasma clearance, ≈100 mL/min.

Protein Binding 98% mainly to albumin [Gibly *et al.* 1997].

Note For a review of cisapride, see McCallum *et al.* [1988].

Dose Usually 5 to 10 mg three or four times daily.

Addison RS *et al.* (1999). A simple high-performance liquid chromatography assay for the major cisapride metabolite, norcisapride, in human urine. *J Chromatogr Sci* 37: 61–64.

Barone JA *et al.* (1987). Bioavailability of three oral dosage forms of cisapride, a gastrointestinal stimulant agent. *Clin Pharm* 6: 640–645.

Bedu A *et al.* (1997). Cisapride high dosage and long QT interval. *J Pediatr* 130: 164.

Cisternino S *et al.* (1998). Determination of cisapride and norcisapride in human plasma using high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 714: 395–398.

de Condado MC *et al.* (2001). Liquid chromatographic method with electrochemical detection for determination of cisapride in serum. *J AOAC Int* 84: 9–12.

Desta Z *et al.* (2000). Stereoselective determination of cisapride, a prokinetic agent, in human plasma by chiral high-performance liquid chromatography with ultraviolet detection: application to pharmacokinetic study. *J Chromatogr B Biomed.Sci.Appl* 744: 263–272.

Elzinga-Huttenga J (2006). Movement disorders induced by gastrointestinal drugs: two paediatric cases. *Neuropediatrics* 37: 102–106.

Gibly RL (1997). Cisapride poisoning. *Vet Hum Toxicol* 39: 231–233.

Gladziwa U *et al.* (1991). Pharmacokinetics and pharmacodynamics of cisapride in patients undergoing hemodialysis. *Clin Pharmacol Ther* 50: 673–681.

Hanson R *et al.* (1997). Cisapride-induced prolonged QT interval: too much of a good thing!. *J Pediatr* 130: 164–166.

Kearns GL *et al.* (2003). Cisapride disposition in neonates and infants: in vivo reflection of cytochrome P450 3A4 ontogeny. *Clin Pharmacol Ther* 74: 312–325.

Kivisto KT *et al.* (1999). Repeated consumption of grapefruit juice considerably increases plasma concentrations of cisapride. *Clin Pharmacol Ther* 66: 448–453.

Lowry JA *et al.* (2003). Cisapride: a potential model substrate to assess cytochrome P4503A4 activity in vivo. *Clin Pharmacol Ther* 73: 209–222.

McCallum RW *et al.* (1988). Cisapride. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use as a prokinetic agent in gastrointestinal motility disorders. *Drugs* 36: 652–681.

Meuldermans W *et al.* (1988). Excretion and biotransformation of cisapride in dogs and humans after oral administration. *Drug Metab Dispos* 16: 403–409.

Nahata MC *et al.* (1995). Stability of cisapride in a liquid dosage form at two temperatures. *Ann Pharmacother* 29: 125–126.

Preechagoon Y, Charles BG (1995). Analysis of cisapride in neonatal plasma using high-performance liquid chromatography with a base-stable column and fluorescence detection. *J Chromatogr B Biomed Appl* 670: 139–143.

Woestenborghs R *et al.* (1988). Determination of cisapride in plasma and animal tissues by high-performance liquid chromatography. *J Chromatogr* 424: 195–200.

Yamamoto T *et al.* (1999). Pharmacokinetic characteristics of cisapride in elderly patients. *Int J Clin Pharmacol Ther* 36: 432–434.

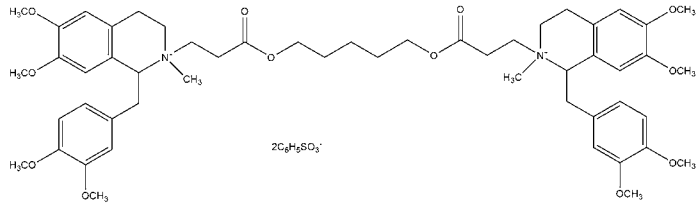
Cisatracurium

Skeletal Muscle Relaxant

$C_{53}H_{72}N_2O_{12} = 929.2$

IUPAC Name 5-[3-[(1*R*,2*S*)-1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl]propanoyloxy]pentyl 3-[(1*R*,2*R*)-1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl]propanoate

Synonym (1*R*,1′*R*,2*R*,2′*R*)-2,2′-[1,5-Pentanediylbis[oxy(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolinium]



Chemical Properties Log *P* (octanol/water), −2.12.

Cisatracurium Besilate

$C_{65}H_{82}N_2O_{18}S_2 = 1243.5$

CAS—96946-42-8

Synonyms Cisatracurium besylate; 51W89.

Proprietary Name *Nimbex dibenzenesulfonate*, .

Quantification

Plasma **HPLC** Fluorescence detection. Limit of quantification, 0.01 mg/L [Boyd *et al.* 1996].

Disposition in the Body After IV administration it is inactivated in plasma by Hofmann elimination, a non-enzymatic breakdown process (pH and temperature dependent) to form a monoquaternary acrylate metabolite and laudanosine, neither of which has neuromuscular blocking activity. This elimination process contributes to 77% of the clearance of the drug. The acrylate metabolite may also undergo further metabolism to its corresponding alcohol and then more laudanosine. Laudanosine (which may have some stimulatory action on the CNS (central nervous system) is further metabolised to desmethyl metabolites that are conjugated with glucuronic acid. Cisatracurium is excreted mainly as metabolites in urine and faeces; <10% is excreted as the unchanged drug. About 40% of a dose is eliminated in faeces.

Therapeutic Concentration

The mean peak plasma laudanosine concentration was 0.71 mg/L (range 0.21 to 1.26) following an infusion of cisatracurium at 0.19 (0.16 to 0.21) mg/kg/h in 6 intensive-care patients, critically ill, sedated and requiring neuromuscular blocking drug to assist mechanical ventilation (>18 years old). Following a mean atracurium infusion of 0.47 (range 0.3–0.74) mg/kg/h, the mean concentration was 2.31 mg/L (range 0.78 to 4.40) [Boyd *et al.* 1996].

The mean peak plasma concentration of laudanosine was 23.1 μg/L 4 min after a 0.1 mg/kg cisatracurium dose administered IV to 15 healthy subjects, with a mean age of 42.9 years (range 21.8 to 64.0) undergoing elective surgery. Seventeen patients with end-stage chronic renal failure, most of them scheduled for dialysis-access procedures with a mean age of 43.9 years (range 25.7 to 62.4), were administered with the same dose and mean concentrations were 0.031 mg/L at 150 min [Eastwood *et al.* 1995].

Peak plasma concentrations of laudanosine following administration of 0.1 mg/kg IV bolus of cisatracurium to 10 subjects ranged from 0.013–0.086 mg/L (mean 0.038 mg/L). Following administration of 0.2 mg/kg IV bolus to 10 subjects peak plasma-laudanosine concentrations ranged from 0.066–0.174 mg/L (mean 0.103 mg/L) [Lien *et al.* 1996].

Half-life Plasma, 22–35 min.

Volume of Distribution Steady state, 0.11–0.16 L/kg; increased in the elderly 17–37% and in patients with end-stage liver failure (21%).

Clearance Plasma, 4.6–5.7 mL/min/kg; decreases in those with renal failure and increases in patients with end-stage liver disease.

Note For a review of cisatracurium, see Bryson, Faulds [1997].

Dose The equivalent of 0.15 or 0.2 mg/kg of cisatracurium IV for intubation followed by 0.03 mg/kg every 20 min for maintenance.

Boyd AH *et al.* (1996). Comparison of the pharmacodynamics and pharmacokinetics of an infusion of cis-atracurium (51W89) or atracurium in critically ill patients undergoing mechanical ventilation in an intensive therapy unit. *Br J Anaesth* 76: 382–388.

Bryson HM, Faulds D (1997). Cisatracurium besilate. A review of its pharmacology and clinical potential in anaesthetic practice. *Drugs* 53: 848–866.

Eastwood NB *et al.* (1995). Pharmacokinetics of 1R-cis 1′R-cis atracurium besylate (51W89) and plasma laudanosine concentrations in health and chronic renal failure. *Br J Anaesth* 75: 431–435.

Lien CA *et al.* (1996). Pharmacokinetics of cisatracurium in patients receiving nitrous oxide/opioid/barbiturate anesthesia. *Anesthesiology* 84: 300–308.

Cisplatin

Antineoplastic

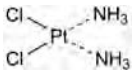
$(NH_3)_2.PtCl_2 = 300.1$

CAS—15663-27-1

IUPAC Name Azanide; dichloroplatinum(2+)

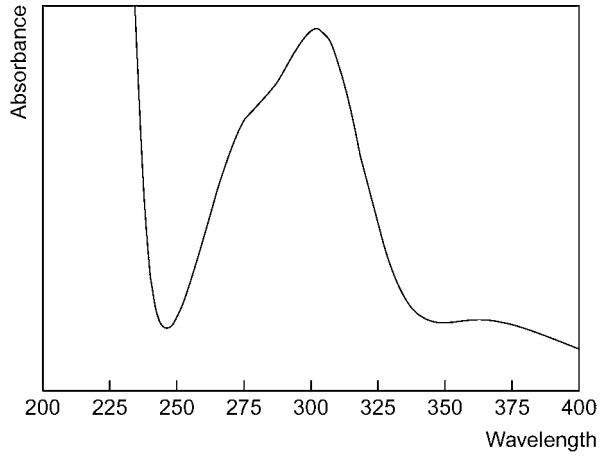
Synonyms CACP; CDDP; *cis*-DPP; cisplatinum; CPDC; DDP; *cis*-diamminedichloroplatinum; NSC-119875; Peyrone's salt; platinum diamminodichloride.

Proprietary Names *Abiplatin*; *Cisplatyl*; *Citoplatino*; *Lederplatin*; *Neoplatin*; *Norplatin*; *Placis*; *Platamine*; *Platiblastin*; *Platiblastine*; *Platinex*; *Platinol*; *Platistil*; *Platistin*; *Platosin*; *Randa*.

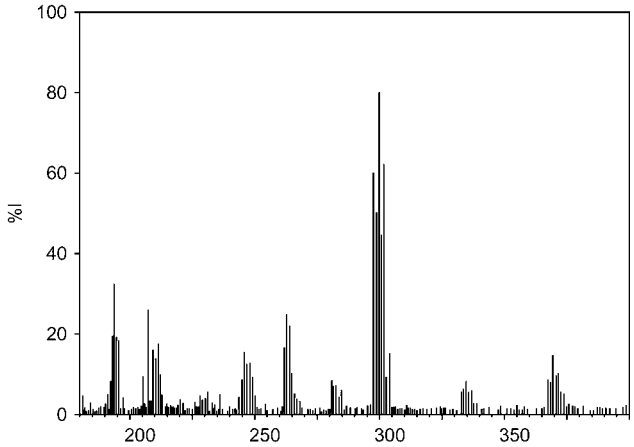


Chemical Properties A yellow powder or yellow or orange-yellow crystals. Mp 270°, with decomposition. It is slightly soluble in water (0.253 g/100 g at 25°); sparingly soluble in dimethylformamide; practically insoluble in ethanol and most common organic solvents, except *N,N*-dimethylformamide, *N,N*-dimethylacetamide and dimethylsulfoxide. Log *P* (octanol/water), −2.19.

Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—203 ($A^1_1 = 173$), 285 ($A^1_1 = 3.63$), 301 ($A^1_1 = 4.33$), 362 nm ($A^1_1 = 0.806$).



Mass Spectrum Principal ions at *m/z* 300, 302, 193, 207, 264, 246, 211, 370.



Quantification

Blood **HPLC** Column: Varian Micro-Pak SP C_{18} (150 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : water (40 : 30 : 30), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 4 ng per injection [Lanjwani *et al.* 2006].

Plasma **HPLC** Column: Nucleosil 5 SB (150 × 4.6 mm i.d.). Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 5.0, 11 : 9), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of quantification, 60 nmol/L [Verschraagen *et al.* 2002]. Column: anionic exchange resin (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L sodium chloride (85 : 15), flow rate 0.7 mL/min. UV detection (λ = 290 nm). Retention time: 11 min. Limit of detection, 0.08 mg/L [Kinoshita *et al.* 1990]. Column: Hypersil ODS (150 × 4.6 mm i.d., 5 µm). AES. Limit of detection, 0.035 mg/L [de Waal *et al.* 1987] Column: µBondapak C_{18} (150 × 3.9 mm i.d., 10 µm). Electrochemical detection. Limit of detection, 0.062 mg/L [Parsons, LeRoy 1986]. See also Andrews *et al.* [1984] and Reece *et al.* [1984].

LC-MS Column: µBondapak C_{18}. Mobile phase: 0.075 µmol/L SDS : methanol (pH 2.5, 93 : 7), flow rate 0.5 mL/min. Limit of quantification, 0.1 µmol/L [Bell *et al.* 2006].

Urine **HPLC** See Blood [Lanjwani *et al.* 2006]. See Plasma [Kinoshita *et al.* 1990].

Disposition in the Body Following IV administration, cisplatin concentrates in the liver, kidneys, and the large and small intestines. Penetration into CNS is poor. It is distributed into breast milk. The chlorine atoms in cisplatin undergo chemical displacement reactions with water and sulfhydryl groups (e.g. on proteins) rather than undergoing enzyme-catalysed metabolism. Excretion is mainly in urine but is incomplete and prolonged; up to 50% of a dose has been reported to be excreted in urine over 5 days and platinum may be detected in tissue for several months afterwards. There is some excretion in faeces via bile. Cisplatin is well absorbed following intraperitoneal administration.

Therapeutic Concentration The peak plasma therapeutic concentration is 1–5 mg/L (free fraction).

Toxicity The trough toxic concentration is 0.1 mg/L (free fraction).

A 59-year-old man received an accidental overdose of 300 mg/m^2 over 3 days. He developed severe emesis, myelosuppression, renal failure, mental deterioration, dim vision and hepatic toxicity. Three days later the plasma platinum concentration was 2.679 mg/L. Plasmapheresis was employed to reduce plasma platinum concentrations, and all symptoms resolved [Jung *et al.* 1995].

A 68-year-old woman received 280 mg/m^2 cisplatin without hydration when it was accidentally substituted for carboplatin. She developed severe emesis, myelosuppression, nephrotoxicity, ototoxicity, central neurotoxicity, visual loss and hepatic toxicity. On day 13 after the overdose, the plasma platinum concentration was 2.6 mg/L. Plasmapheresis was performed to reduce plasma platinum concentrations, but the patient required a kidney and bone-marrow transplant and remained deaf [Chu *et al.* 1993].

Half-life Plasma: unbound cisplatin 25–48 min, protein-bound platinum 58–73 h.

Volume of Distribution 11–12 L/m^2, also reported as 20–80 L.s.

Clearance Plasma, 15–16 L/h/m^2.

Protein Binding The majority is bound.

Dose Up to 120 mg/m^2 body-surface area by IV infusion as a single dose, or 15 to 20 mg/m^2 by infusion daily for 5 consecutive days, every 3 to 4 weeks.

Andrews PA *et al.* (1984). A high-performance liquid chromatographic assay with improved selectivity for cisplatin and active platinum (II) complexes in plasma ultrafiltrate. *Anal Biochem* 143: 46–56.

Bell DN *et al.* (2006). Specific determination of intact cisplatin and monohydrated cisplatin in human plasma and culture medium ultrafiltrates using HPLC on-line with inductively coupled plasma mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 837: 29–34.

Chu G *et al.* (1993). Massive cisplatin overdose by accidental substitution for carboplatin. Toxicity and management. *Cancer* 72: 3707–3714.

deWaal WA *et al.* (1987). Analysis of platinum species originating from *cis*-diamminedichloroplatinum(II) (cisplatin) in human and rat plasma by high-performance liquid chromatography with on-line inductively coupled plasma atomic emission spectrometric detection. *J Chromatogr* 407: 253–272.

Jung HK *et al.* (1995). A case of massive cisplatin overdose managed by plasmapheresis. *Korean J Intern Med* 10: 150–154.

Kinoshita M *et al.* (1990). High-performance liquid chromatographic analysis of unchanged *cis*-diamminedichloroplatinum (cisplatin) in plasma and urine with post-column derivatization. *J Chromatogr* 529: 462–467.

Lanjwani SN *et al.* (2006). High performance liquid chromatographic determination of platinum in blood and urine samples of cancer patients after administration of cisplatin drug using solvent extraction and *N,N′*-bis(salicylidene)-1,2-propanediamine as complexation reagent. *J Pharm Biomed Anal* 40: 833–839.

Parsons PJ, LeRoy AF (1986). Determination of cis-diamminedichloroplatinum(II) in human plasma using ion-pair chromatography with electrochemical detection. *J Chromatogr* 378: 395–408.

Reece PA *et al.* (1984). Sensitive high-performance liquid chromatographic assay for platinum in plasma ultrafiltrate. *J Chromatogr* 306: 417–423.

Verschraagen M *et al.* (2002). Simultaneous determination of intact cisplatin and its metabolite monohydrated cisplatin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 273–281.

Citalopram

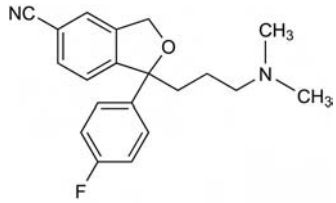
Antidepressant, Selective Serotonin Reuptake Inhibitor (SSRI)

$C_{20}H_{21}FN_2O$ = 324.4

CAS—59729-33-8

IUPAC Name 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile

Synonyms Lu 10-171; nitalapram.



Chemical Properties Log *P* (octanol/water), 3.74. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Citalopram Hydrobromide

$C_{20}H_{21}FN_2O$, HBr = 405.3

CAS—59729-32-7

Synonym Lu 10-171-B

Proprietary Names *Cipramil*; *Seropram*.

Chemical Properties A white to off-white, crystalline solid with Mp 186°. It is sparingly soluble in water; soluble in ethanol (96%); freely soluble in chloroform; practically insoluble in diethylether pK_a 9.5. Log *P* (octanol/phosphate buffer pH 7.4), 1.57.

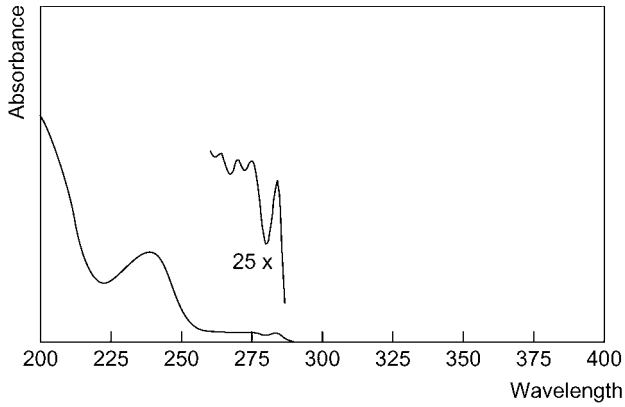
Thin-layer Chromatography System TB—R_f 0.22; system TE—R_f 0.60; system TAE—R_f 0.19.

Gas Chromatography System GB—citalopram RI 2499, M (nor) RI 2526; system GM—citalopram RRT 1.12 min, M (nor) RRT 1.23 min (relative to iprindole).

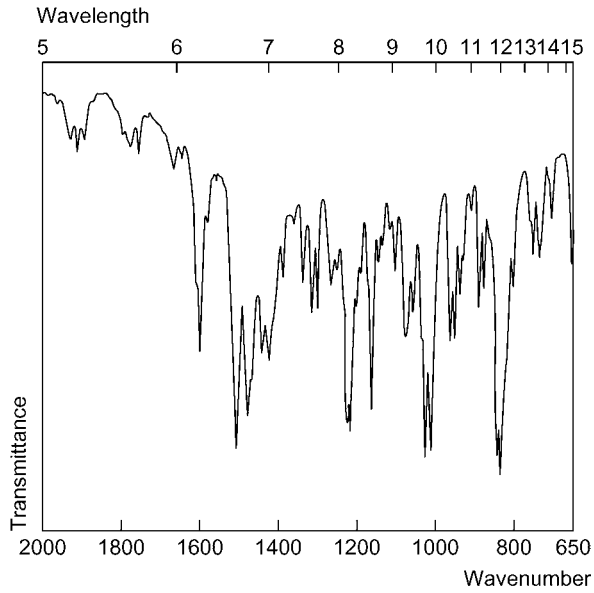
High Performance Liquid Chromatography System HX—RI 403; system HZ—citalopramRT 4.5 min, M (desmethyl) RT 3.7 min.

Column: C_{18} (Supelco, 250 × 4.6 mm i.d.). Mobile phase: 44 mmol/L potassium dihydrogen phosphate buffer containing 1.5 mL TEA in 1 L (pH 2.5) : acetonitrile (55 : 45), flow rate 1.5 mL/min. UV detection (λ = 240 nm). Retention time: 5.56 min for citalopram, 5.06 min for desmethylcitalopram, and 4.51 min for didesmethylcitalopram [Olesen, Linnet 1996].

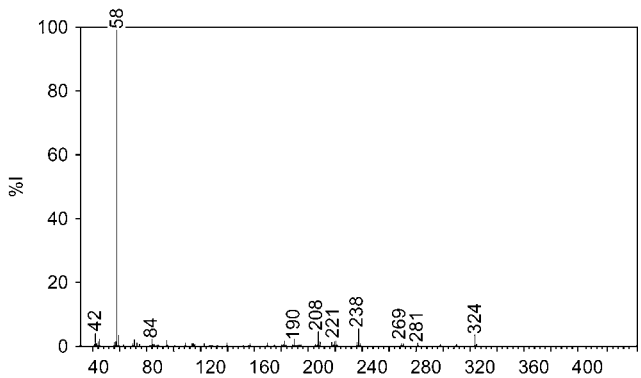
Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—205, 238 nm (hydrobromide); Methanol—209, 237, 270 nm.



Infrared Spectrum Principal peaks at wavenumbers 834, 1507, 1034, 1226, 1160, 1601 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 58, 238, 208, 42, 324, 190 (citalopram); *m/z* 44, 238, 310, 138, 208, 57 (desmethylcitalopram).



Quantification

Blood **GC** NPD. Limit of quantification, 60–786 μg/L, limit of detection, 18–236 μg/L [Martinez *et al.* 2004].

GC-MS [Wille *et al.* 2009].

Plasma **GC-MS** EI ionisation or CI, SIM acquisition mode. Limit of quantification, 5–12.5 μg/L (EI) 1–6.25 μg/L (CI) [Wille *et al.* 2007]. Column: Permabond SE-54-DF-0.25 fused silica capillary (25 m × 0.25 mm, 0.27 μm). Temperature programme: 160° to 260° at 30°/min. Carrier gas: He, 60 mL/min. Retention time (relative to amitriptyline): 1.27 for citalopram, 1.27 for didesmethylcitalopram, and 1.32 for desmethylcitalopram. Limit of quantification, 1 μg/L [Reymond *et al.* 1993].

HPLC Column: ODS (250 × 4.0 mm, 5 μm). Mobile phase: 0.4% tetramethylammonium chloride (pH 4.0) : acetonitrile (40 : 60), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 240 nm, λ_{em} = 308 nm). Limit of quantification, 0.5 μg/L [Unceta *et al.* 2010]. Column: Nucleosil 100 C_{18} (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : 45 mmol/L ammonium formate (pH 4.0, 50 : 50), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 245 nm, λ_{em} = 295 nm). Limit of quantification, 0.8 μg/L, limit of detection, 0.3 μg/L [Bagheri *et al.* 2008]. Column: Chiral-AGP. Mobile phase: 3 μmol/L *N*-dodecyl-*N*,*N*-dimethylammonio-3-propanesulfonate : 10 μmol/L hexanoic acid in phosphate buffer (pH 6.5). UV detection (λ = 240 nm). Limit of detection, 1 μg/L [Haupt 1996]. Column: acetylated β-Cyclobond (250 × 4.6 mm, 5 μm). Mobile phase: 20% acetonitrile or 65% methanol with 1% diethanolamine buffer (pH 6.1), flow rate 0.8 mL/min. Fluorometric detection λ_{ex} = 240 nm, λ_{em} = 296 nm). Retention time: (*S*)-(+)-citalopram 15.5 min, (*R*)-(−)-citalopram 16.6 min, (*S*)-(+)-desmethylcitalopram 12.2 min, (*R*)-(−)-desmethylcitalopram 12.9 min; (*S*)-(+)-didesmethylcitalopram 10.6 min; (*R*)-(−)-didesmethylcitalopram, 11.1 min. Limit of quantification, 3 μg/L [Rochat *et al.* 1995]. UV detection (λ = 239 nm). Limit of detection, 0.8 μg/L [Rop *et al.* 1990]. Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile : 0.025 mol/L potassium dihydrogen phosphate : water (45 : 50 : 5), flow rate 0.5 mL/min. UV detection (λ = 254 nm). Retention time: 11.3 min. Limit of detection, 4–5 μg/L [Rop *et al.* 1985].

Serum **HPLC** Column: LiChrospher CN (5 μm). Mobile phase: 8 mmol/L phosphate buffer (pH 6.4) : acetonitrile (50 : 50), flow rate 1.5 mL/min. UV detection (λ = 210 nm). Limit of detection, 6 μg/L [Greiner *et al.* 2007]. Monolithic C_{18} (50 × 4.6 mm). Mobile phase: methanol : 5 μmol/L acetate buffer at pH 3.9 [Kirchherr, Kühn-Velten 2006]. Column: reversed phase C_{18}. Mobile phase: acetonitrile : ammonium acetate buffer (pH 4). Limit of quantification, 1.2–54 nmol/L [Gutteck, Rentsch 2003]. Column: Nucleosil 100-Protect 1. Mobile phase: acetonitrile : potassium dihydrogenphosphate buffer. UV detection [Frahnert *et al.* 2003]. Column: C_{18}. Mobile phase: acetonitrile : buffer (30 : 70), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 300 nm) [Waschgler *et al.* 2002]. See also Olesen, Linnet [1996].

Urine **HPLC** See Plasma. Limit of quantification, 2.5 μg/L [Unceta *et al.* 2010].

Brain **GC-MS** See Blood [Wille *et al.* 2009].

HPLC See Plasma. Limit of quantification, 5.0 ng/g [Unceta *et al.* 2010].

Hair **GC-MS** See Blood [Wille *et al.* 2009].

Disposition in the Body Citalopram is readily absorbed after oral administration, with a bioavailability of ~80%; peak plasma concentrations occur after ~2–4 h. It is metabolised by *N*-demethylation, deamination and oxidation to desmethylcitalopram, didesmethylcitalopram, citalopram-*N*-oxide and a propionic acid derivative. The metabolites have some pharmacological activity although they do not contribute to the overall antidepressant effect of citalopram. Unlike some other selective serotonin reuptake inhibitors, it has only a mild inhibitory effect on CYP2D6. Approximately 12% of the daily dose is excreted unchanged in urine and an equal amount as the main active metabolite, desmethylcitalopram. Also present are an amino metabolite and a conjugated propionic acid derivative. Approximately 65% of a dose is unaccounted for and could possibly be present in faeces or undergo a number of different metabolic pathways. It is distributed into breast milk in very small amounts. Extensive tissue binding has been observed and accumulation can occur with repeated dosing.

Therapeutic Concentration The therapeutic concentration range is 20–200 μg/L.

Twelve healthy male volunteers, aged between 18 and 40 years, were administered citalopram 20 or 40 mg daily for 9 days. The mean plasma concentration was 91 μg/L (range, 45–126). The mean dimethylcitalopram concentration was 19–50 μg/L [Lader *et al.* 1986].

Patients suffering from severe depression were administered 20 mg citalopram daily for 12 weeks and the steady-state concentrations were 40 μg/L for citalopram, 12 μg/L for desmethylcitalopram and 2 μg/L for didesmethylcitalopram. For those given 40 mg daily, steady-state concentrations were 67, 22 and 4 μg/L for citalopram, desmethylcitalopram and didesmethylcitalopram, respectively [Montgomery *et al.* 1993].

Toxicity The lethal concentration is 500 μg/L.

Citalopram plasma concentrations were 6 times greater than the therapeutic concentrations in a patient who attempted suicide. There were no significant changes in level of consciousness, ECG or blood pressure [Pedersen *et al.* 1982].

A 41-year old Caucasian man was found dead at his home; he had a history of depression and suicide attempts. Surrounding the man was a number of medication bottles and a bottle of whiskey. Toxicological analysis showed that the man had ethanol, lormetazepam, cotinine, caffeine, moclobemide and citalopram in his urine and blood. Citalopram was detected in blood at a concentration of 4.47 mg/L and in urine at 19.7 mg/L. The metabolite desmethylcitalopam was found at concentrations of 0.42 mg/L and 1.22 mg/L in the 2 fluids, respectively. The cause of death was determined as the synergistic toxicity of moclobemide and citalopram; and death was ruled as multiple drug intoxication [Dams *et al.* 2001].

Bioavailability ≈80%.

Half-life Plasma: citalopram 33 h, desmethylcitalopram 48 h and didesmethylcitalopram, 96 h; half-life is longer in the elderly, 3.75 days.

Volume of Distribution 12–16 L/kg.

Clearance Plasma: 0.33 L/min in the young and 0.08 L/min in the elderly.

Protein Binding 50%.

Note For reviews of citalopram, see Baumann [1996], Milne, Goa [1991], and Preskorn [1997].

Dose The usual starting dose is 20 mg or, more frequently, 40 mg, which may be increased up to 60 mg citalopram daily, given as the hydrobromide.

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Pedersen OL *et al.* (1982). Citalopram, a selective serotonin reuptake inhibitor: clinical antidepressive and long-term effect: a phase II study. *Psychopharmacology (Berl)* 77: 199–204.

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Rop PP *et al.* (1990). Simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma by high-performance liquid chromatography after column extraction. *J Chromatogr* 527: 226–232.

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Citrinin

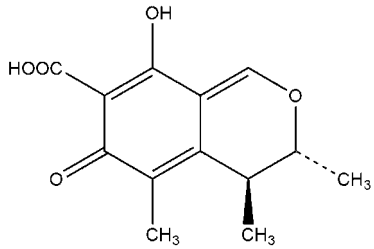
Mycotoxin

$C_{13}H_{14}O_5$=250.3

CAS—518-75-2

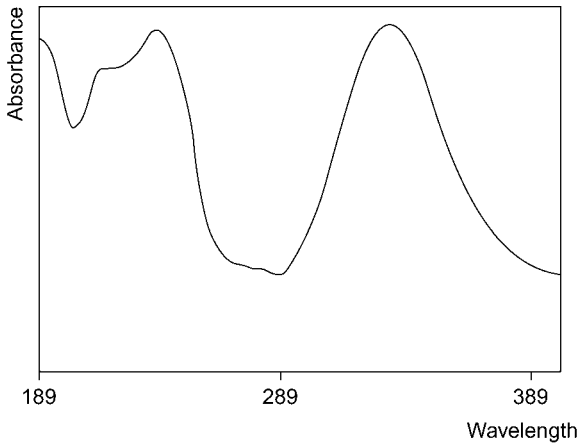
IUPAC Name (3*R*,4*S*)-7-(Dihydroxymethylidene)-3,4,5-trimethyl-3,4-dihydroisochromene-6,8-dione

Synonyms Antimycin; (3*R*,4*S*)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3*H*-isochromene-7-carboxylic acid.



Chemical Properties Mycotoxin first isolated from *Penicillium citrinum*. Lemon-yellow needles from alcohol. Mp 175° with decomposition. Strong acid. Practically insoluble in water. Soluble in alcohol, dioxane, and dilute alkali. Solutions change colour with changes in pH, from lemon-yellow at pH 4.6 to cherry-red at pH 9.9. Log *P* (octanol/ water), 0.45 [Meylan, Howard 1995].

Ultraviolet Spectrum 250, 331 nm [O'Neil *et al.* 2006].



Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: A) methanol : water; B) acetonitrile : water; C) tetrahydrofuran : water. Location reagent: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as shown in the table overleaf.

Quantification

Plasma **HPLC** Column: reversed phase (10 µm). Mobile phase: 0.25 N phosphoric acid : acetonitrile : 2-propanol (55 : 35 : 10). UV detection (λ= 340 nm). Limit of detection, 2–5 ng [Phillips *et al.* 1980].

Urine **HPLC** Column: C_8 reversed phase. Mobile phase: 0.25 N phosphoric acid : acetonitrile : 2-propanol (55 : 40 : 5). Fluorescence detection (λ_{ex}= 319 nm, λ_{em}= 460 nm). Limit of detection, 10 ppb [Orti *et al.* 1986].

Other **LC-MS** Peanut, Pistachio, Wheat, Maize, Cornflakes, Raisins, and Figs. ESI, positive ion mode, MRM acquisition mode. Retention time, 16.2 min. Limit of quantification, 10–200 µg/kg [Spanjer *et al.* 2008].

Toxicity LD_{50} (IP) in mice, rats: 35, 67 (mg/kg), respectively.

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O'Neil MJ, *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn, Whitehouse Station NJ: Merck Research Laboratories.

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	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	**60 : 40**	**0.24**	**0.71**	**60 : 40**	**N/A**	**0.95**	**N/A**	**N/A**	**N/A**
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
DON	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyl-DON	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
NIV	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989]

Citronella Oil

Monoterpene, Essential Oil, Insect Repellent, Antifungal
CAS—89998-15-2

Proprietary Names *Aulo Gelio Repelente*; *Buzz Away*; *Druide Insect Repellent*; *Kneipp Beruhigungs-Bad spezial*; *Mozzie Patch*; *Natrapel*; *Repelente Rep*; *Schupps Melissen Olbad*; *Valmarin Bad N*. It is also an ingredient in *Air Citronella*; *Amol*; *Apex Repel Natural*; *Aulo Repelente De Piojos*; *Apex Repel Natural*; *Baume du Chalet*; *Biokosma Embrocation*; *Biokosma Medizinalbad*; *Biokosma Red Point-Massagecreme*; *Carmolis*; *Citrosystem*; *Dr Fischers Melissengeist*; *esto-gast*; *Goanna Bite-Eze*; *Herzfluid*; *Kneipp Krauter Taschenkur Nerven und Schlaf N*; *Kneipp Sedativ-Bad*; *Melissengeist*; *Mistick Verde*; *Natural Zanzy*; *No-Bite*; *Novital*; *Pungino*; *Saltrates*; *Sansilla*; *Schupps Baldrian Sedativbad*; *Silvapin Aktiv-Tonic MMP*; *Snowfire*; *Treo*; *Valin Baldrian*; *Ysol 206*.

Chemical Properties The oil is obtained by steam distillation from the fresh or partially dried aerial parts of *Cymbopogon winterianus*. It contains 30.0 to 45.0% citronellal ($C_{10}H_{18}O$=154.3), 9.0 to 15.0% citronellol, 2.0 to 4.0% citronellyl acetate, <2.0% geranial, 20.0 to 25.0% geraniol ($C_{10}H_{18}O$=154.3), 3.0 to 8.0% geranyl acetate, 1.0 to 5.0% limonene, and <2.0% neral. A pale-yellow to brownish-yellow liquid with a very strong odour of citronellal. Soluble 1 in 4 of ethanol (80%), forming a clear or slightly opalescent solution. Citronella oil is volatile and may be isolated by distillation. Store in well-closed containers. Protect from light.

Quantification

Other **GC-MS** Plant Samples. Column: HP-5 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° to 240° at 3°/min, for 7 min. EI ionisation at 70 eV. Retention indices: β-myrcene 990, *cis*-β-ocimene 1034, *trans*-β-ocimene 1044, *trans*-geraniol 1254, geranyl acetate 1382, *trans*-caryophyllene 1414, (*E*,*E*)-farnesol 1720. Limit of quantification not reported [Duarte *et al.* 2007]. Leaf Samples. Column: BP21 fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 40° for 7 min to 190° at 5°/min for 20 min. EI ionisation at 70 eV. Retention times: limonene 10.1 min, citronellal 19.7 min, isolimonene 23.1 min, citronellol 26.8 min, geraniol 28.2 min, citronellyl acetate 32.1 min; 17 further constituents detected. Limit of quantification not reported [Batish *et al.* 2006]. Leaf Samples (*Cymbopogon citratus*). Column: DB5-MS 5% phenylmethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° to 280° at 3°/min for 30 min. EI ionisation at 70 eV. Retention indices: citronellol 1202, neral 1216, geraniol 1231, geranial 1253, hydroxyl-citronellal 1257, neryl acetate 1357; 21 further compounds detected. Limit of quantification not reported [Marongiu *et al.* 2006].

Note For a stable-isotope GC-MS method for the detection of citronellal and citral in essential oils, see Nhu-Trang *et al.* [2006]. For a colorimetric method for the identification of plant essential oil components, see Niu and Gilbert [2004].

Disposition in the Body Citronellal has been shown to undergo a cyclisation reaction in rabbits under the influence of hydrochloric acid in the stomach. The product, *p*-menthane-3,8-diol, is excreted as a glucuronide conjugate. Geraniol has been found to undergo a double ω-oxidation and also reduction of the 2,3-double bond, with 2 acids being produced.

Toxicity Hypersensitivity has been reported [Davids *et al.* 1978]. For a report of 5 non-fatal cases of poisoning with citronella oil in children, see Temple *et al.* [1991], no blood levels are presented. Report of a fatal poisoning in a 21-month-old child attributed to citronella oil [Mant 1961] is now in question. The child was administered a salt-water emetic, and postmortem findings included the presence of cerebral haemorrhages. These are consistent with salt intoxication [Temple *et al.* 1991].

Dose Used as a perfume and insect repellent. It is also used in aromatherapy.

Batish DR *et al.* (2006). Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. *Z Naturforsch [C]* 61: 465–471.

Davids MG *et al.* (1978). Contact dermatitis from an ostomy deodorant. *Contact Dermatitis* 4: 11–13.

Duarte MC *et al.* (2007). Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. *J Ethnopharmacol* 111: 197–201.

Mant AK (1961). Association proceedings VI. A case of poisoning by oil of citronella. *Med Sci Law* 1/2: 170–171.

Marongiu B *et al.* (2006). Comparative analysis of the oil and supercritical CO(2) extract of *Cymbopogon citratus* Stapf. *Nat Prod Res* 20: 455–459.

Nhu-Trang TT *et al.* (2006). Authenticity control of essential oils containing citronellal and citral by chiral and stable-isotope gas-chromatographic analysis. *Anal Bioanal Chem* 386: 2141–2152.

Niu C, Gilbert ES (2004). Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. *Appl Environ Microbiol* 70: 6951–6956.

Temple WA *et al.* (1991). Management of oil of citronella poisoning. *J Toxicol Clin Toxicol* 29: 257–262.

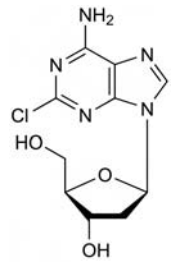
Cladribine

Antineoplastic
$C_{10}H_{12}ClN_5O_3$ = 285.7
CAS—4291-63-8

IUPAC Name (2*R*,3*S*,5*R*)-5-(6-Amino-2-chloropurin-9-yl)-2-(hydroxymethyl) oxolan-3-ol

Synonyms 2-CdA; 2-chloro-2′-deoxyadenosine; CldAdo; NSC-105014-F.

Proprietary Names *Leustat*; *Leustatin*; *Leustatine*.



Chemical Properties Crystals from water which soften at 210° to 215° and when solidified, turn brown. Also reported as crystals from ethanol with mp 220° (soften), that re-solidify, turn brown and do not melt <300°.

Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—265 nm; aqueous alkali (0.1 mol/L sodium hydroxide)—265 nm.

Quantification

Plasma **HPLC** Column: C_{18} (80 × 4.6 mm, 3 µm). Mobile phase: 10 mmol/L phosphate buffer, pH 3 with 11% methanol and 7% acetonitrile, flow rate 1 mL/min. UV detection (λ 265 nm). *k* value: cladribine, 4.8; 2-chloro-2′-deoxyadenosine (metabolite), 2.2. Limit of detection, 1 nmol/L [Albertioni *et al.* 1994]. Limit of detection, 1 µg/L [Liliemark *et al.* 1991].

LC-MS Column: Supelcosil LC-8-DB (330 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 5 mmol/L ammonium acetate (25:75), flow rate 1.0 mL/min. Tandem MS detection (positive ion mode). Retention time: 1.5 min. Limit of detection, 0.1 µg/L [Moyer *et al.* 1998].

Urine **HPLC** Limit of detection, 100 nmol/L, see Plasma [Albertioni *et al.* 1994].

Disposition in the Body Cladribine is extensively distributed after IV administration and undergoes metabolism in cells containing deoxycytidine kinase activity to form 2-chloro-2′-deoxyadenosine-5′-triphosphate. Other metabolites include, 5′-monophosphate and 2-chloroadenine (CAde), the major metabolite identified in plasma. It penetrates into CSF (cerebrospinal fluid); concentrations are about 25% of those in plasma. About 21 to 32% of an IV dose is excreted unchanged in urine within 24 h with 1.5% as CAde. After oral administration, 25% of the dose is excreted in urine with 3.8% the metabolite, CAde.

Therapeutic Concentration

A mean steady-state plasma concentration of 22.5 ng/L was reported during IV infusion of 0.14 mg/kg/day over 24 h for 5 days in 12 subjects. The mean peak concentration was 198 ng/L (range 70 to 381 ng/L) observed during the 2 h infusion, which decreased to the steady state levels with a mean of 6.3 h after the end of infusion. The volunteers were either diagnosed with chronic lymphocytic leukaemia, non-Hodgkins lymphoma or hairy-cell leukaemia [Liliemark, Juliusson 1991].

IV infusion of 8.9 mg/m^2/24 h for 5 days in 25 patients, aged between 8 months and 23 years, produced steady-state plasma concentrations ranging from 23.2 to 84.5 ng/L (mean 37.7 ng/L) at 12 h. Mean CSF concentration was 6.1 ng/L. These patients suffered from relapsed haematological malignancies, acute myeloid leukaemia or acute lymphoid leukaemia [Kearns *et al.* 1994].

Toxicity Cladribine has considerable bone-marrow toxicity (haemopoietic stem cell toxicity). In a phase I study, patients were administered with doses >0.26 mg/kg daily for 10 to 14 days. Both renal and central nervous system toxicity was observed, with some patients requiring haemodialysis due to severity of toxicity. Toxicity was not observed with doses of 0.9 mg/kg daily for 7 days [Beutler 1992].

Half-life Plasma, from 3 to 22 h

Volume of Distribution Approximately 9.2 to 12.7 L/kg; 356.6 L/m^2.

Clearance Plasma clearance, ~26 to 45 L/h/m^2; mean, 1.44 L/h/kg.

Protein Binding 20%.

Note For reviews of cladribine, see Beutler [1992], Bryson, Sorkin [1993], and Saven, Piro [1994].

For a review of the pharmacokinetics of cladribine, see Liliemark [1997].

Dose In chronic lymphocytic leukaemia 120 μg/kg daily for 5 consecutive days of a 28-day cycle, in hairy-cell leukaemia 90 μg/kg daily for 7 days.

Albertioni F *et al.* (1994). Analysis of 2-chloro-2′-deoxyadenosine in human blood plasma and urine by high-performance liquid chromatography using solid-phase extraction. *Ther Drug Monit* 16: 413–418.

Beutler E (1992). Cladribine (2-chlorodeoxyadenosine). *Lancet* 340: 952–956.

Bryson HM, Sorkin EM (1993). Cladribine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in haematological malignancies. *Drugs* 46: 872–894.

Kearns CM *et al.* (1994). Pharmacokinetics of cladribine (2-chlorodeoxyadenosine) in children with acute leukemia. *Cancer Res* 54: 1235–1239.

Liliemark J *et al.* (1991). Determination of 2-chloro-2′-deoxyadenosine in human plasma. *Biomed Chromatogr* 5: 262–264.

Liliemark J, Juliusson G (1991). On the pharmacokinetics of 2-chloro-2′-deoxyadenosine in humans. *Cancer Res* 51: 5570–5572.

Liliemark J (1997). The clinical pharmacokinetics of cladribine. *Clin Pharmacokinet* 32: 120–131.

Moyer MD *et al.* (1998). Determination of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Pharm Biomed Anal* 17(1): 45–51.

Moyer MD *et al.* (1998). Determination of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Pharm Biomed Anal* 17: 45–51.

Saven A, Piro LD (1994). 2-Chlorodeoxyadenosine: a newer purine analog active in the treatment of indolent lymphoid malignancies. *Ann Intern Med* 120: 784–791.

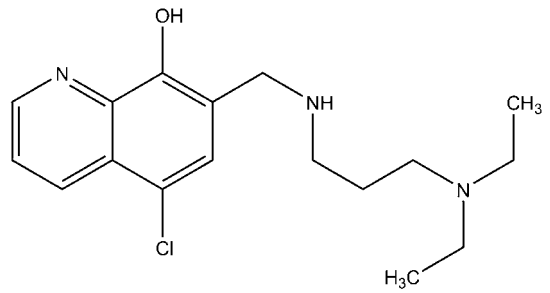
Clamoxyquin

Quinolinol, Antiamoebic

$C_{17}H_{24}ClN_3O$ = 321.9

CAS—2545-39-3

IUPAC Name 5-Chloro-7-[(3-diethylaminopropyl)aminomethyl]-8-hydroxyquinoline



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

Clamoxyquin Hydrochloride

$C_{17}H_{24}ClN_3O$, HCl = 358.4

CAS—4724-59-8

Synonyms ci 433; PAA 3854.

Chemical Properties Mp 160°. Soluble in water.

Clamoxyquin Pamoate

Chemical Properties The solubility of clamoxyquin pamoate in pH 7 0.1 mol/L phosphate buffer is 0.012%. Solutions of this drug (0.01%) in methanol or pH 7 phosphate buffer are stable for >2 weeks.

Thin-layer Chromatography System T1—R_f 0.02 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Clamoxyquin pamoate: Methanol—238, 289, 278, 300, 362 nm; aqueous alkali (0.1 N NaOH)—236, 288, 364, 300 nm.

Infrared Spectrum Principal peaks at wavenumbers 1387, 1347, 1444 cm^{-1} (KBr disk).

Disposition in the Body

Toxicity Clamoxyquin hydrochloride, LD_{50} (oral): in mice 891 mg/kg; clamoxyquin pamoate, LD_{50} (oral): in mice >2500 mg/kg.

Clarithromycin

Antibacterial

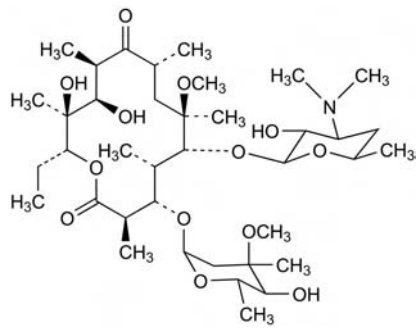
$C_{38}H_{69}NO_{13}$ = 748.0

CAS—81103-11-9

IUPAC Name (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione

Synonyms (2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-3-(2,6-Dideoxy-3-*C*,3-*O*-dimethyl-α-L-*ribo*hexopyranosyloxy)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethyl-9-oxo-5-(3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*hexopyranosyloxy)pentadecan-13-olide; 6-*O*-methylerythromycin, A-56268.

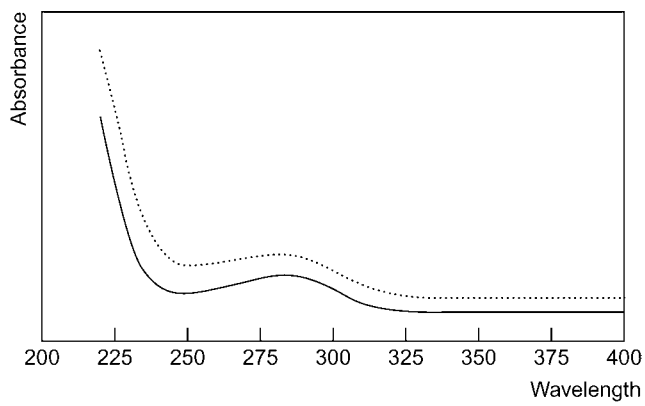
Proprietary Names *Biaxin*; *Biaxin HP*; *Biclar*; *Bremon*; *Cyllind*; *Klacid*; *Klaciped*; *Klaricid*; *Kofron*; *Macladin*; *Maclar*; *Mavid*; *Naxy*; *Veclam*; *Zeclar*.



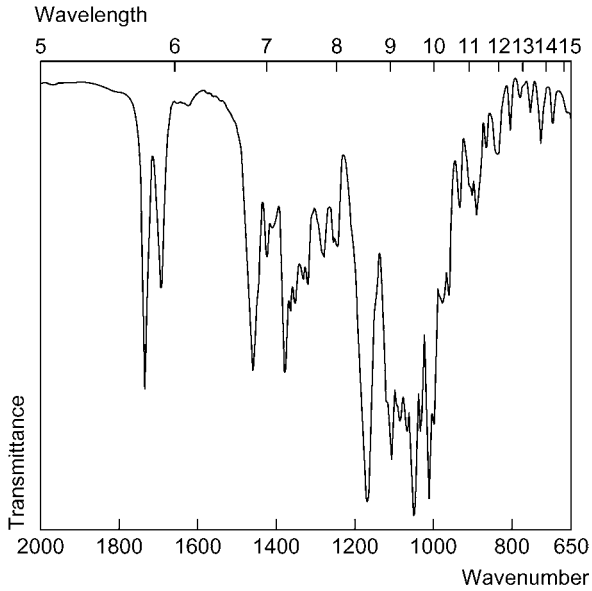
Chemical Properties A white to off-white crystalline powder. Crystals, mp 217° to 220° from chloroform and diisopropyl ether; mp 222° to 225° from ethanol. It is practically insoluble in water; soluble in acetone; slightly soluble in acetonitrile, ethanol and methanol.

High Performance Liquid Chromatography System HBA—retention time 15.7 min; system HBB—retention time 6.8 min.

Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—283 nm; basic—282 nm.



Infrared Spectrum Principal peaks at wavenumbers 1052, 1170, 1108, 1734 cm^{-1} (KBr pellet).



Quantification

Plasma **HPLC** Electrochemical detection. Limit of quantification, 0.1 mg/L [Taninaka *et a l.* 2000]. Column: C_{18} (Nucleosil 100-3, 150 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 15 mmol/L potassium dihydrogen phosphate buffer, (pH 6.0) (70:30), flow rate 1.2 mL/min. UV detection (λ=220 nm). Retention time: 10.2 min. Limit of quantification, 0.5 mg/L [Macek *et al.* 1999]. Electrochemical detection. Limit of detection, 0.03 mg/L [Kees *et al.* 1998]. Electrochemical detection. Clarithromycin and 14-hydroxyclarithromycin, limit of detection, 0.03 mg/L [Chu *et al.* 1991].

Serum **HPLC** Fluorescence detection (λ_{ex}=255 nm, λ_{em} = 315 nm). Limit of detection, 0.2 mg/L [Sastre Toraño, Guchelaar 1998].

Urine **HPLC** See Plasma [Chu *et al.* 1991].

Note For a review of methods for the determination of macrolides in biological fluids, see Kanfer *et al.* [1998].

Disposition in the Body Clarithromycin is rapidly absorbed from the gastrointestinal tract after oral administration with a bioavailability of about 55%. It undergoes first-pass metabolism. Pharmacokinetics are non-linear and dose-dependent with high doses producing disproportionate increases in plasma concentrations owing to saturation of metabolic pathways. Widely distributed and tissue concentrations exceed plasma concentrations. Readily enters leukocytes and macrophages. It is distributed into breast milk. Undergoes extensive metabolism in the liver by demethylation, hydroxylation and hydrolysis. The principal metabolite is 14-hydroxyclarithromycin and this also has antibacterial activity. At steady state, about 20% and 30%, respectively of a 250 mg or 500 mg dose is excreted in the urine as unchanged drug. Excretion in urine and faeces occurs in a dose-dependent manner: at low doses the two fractions are similar; at high doses urinary excretion is predominant. Not significantly removed by haemodialysis or peritoneal dialysis.

Therapeutic Concentration

Sixteen patients, aged between 31 and 67 years (mean 50.1 years) were administered with a single nasogastric dose of 500 mg clarithromycin suspension on days 1 and 4 with at least 72 h 'wash-out' between doses. The mean peak plasma concentration of clarithromycin was 2.1 mg/L on day 1 and 2.3 mg/L on day 4, observed at 3.5 and 3.3 h, respectively. The mean concentrations for the metabolite, 14-hydroxyclarithromycin, were 0.9 mg/L at 4.4 h and 1.0 mg/L at 4.3 h, on days 1 and 4, respectively [Fish, Abraham 1999].

Seventeen healthy males aged 18 to 40 years (mean 29 years) and 17 healthy males aged 20 to 39 years (mean 31 years) were administered with 250 and 500 mg doses, respectively, every 12 h for 7 doses after an overnight fast. The peak plasma concentrations for the 250 mg dose were 0.78 (0.4 to 1.22), 1.01 (0.67 to 1.67) and 1.14 (0.51 to 1.72) mg/L for a single dose and multiple dosing (dose 5 and 7), respectively. These were observed at 1.8, 2.1 and 2.0 (range of 0.5 to 4) h. For the 500 mg single dose, a concentration of 2.12 (0.78 to 3.54) mg/L was observed at 2 (1 to 3) h and for the multiple dosing 2.67 (095 to 4.71) mg/L at 2.6 (1 to 6) h for dose 5; and 2.85 (0.98 to 4.85) mg/L at 2.6 h (range, 1 to 4 h) for dose 7 [Chu *et al.* 1993].

Twelve healthy, young volunteers with mean age 23.2 (21 to 29) years and 12 healthy, elderly individuals (mean 73.6 years; range, 65 to 81 years) were administered with 500 mg clarithromycin every 12 h for 5 doses. The subjects were in fasting state and fasted for an additional 2 h after dosing. The mean drug peak plasma concentrations were 2.41 (range 1.58 to 4.09) mg/L and 3.28 (1.75 to 4.55) mg/L for the young and elderly, respectively, observed at ~2 h (slightly later in the elderly). Peak concentrations for the metabolite, 14-hydroxyclarithromycin, were 0.66 (0.29 to 1.06) mg/L for the young and 1.33 (0.72 to 2.05) mg/L for the elderly [Chu *et al.* 1992].

Bioavailability About 55%.

Half-life Plasma half-life is dose-dependent: clarithromycin about 3 to 4 h in subjects taking 250 mg twice daily and about 5 to 7 h in subjects taking 500 mg twice daily; 14-hydroxyclarithromycin 5 to 6 h in subjects taking 250 mg twice daily and about 7 h in subjects taking 500 mg twice daily. Plasma half-life is increased in subjects with impaired renal function: clarithromycin half-life is about 22 h and 14-hydroxyclarithromycin half-life is about 47 h when creatinine clearance is less than 30 mL/min.

Volume of Distribution Apparent, 175 L.

Clearance Total body, 29.2 to 58.1 L/h.

Protein Binding About 80% at therapeutic concentrations (free clarithromycin fraction increases at serum concentrations >1 mg/L suggesting saturation of binding process).

Note For a review of the pharmacokinetics of clarithromycin, see Fraschini *et al.* [1993]. For reviews of clarithromycin, see Peters, Clissold [1992] and Barradell *et al.* [1993].

Dose Usually 250 or 500 mg twice daily.

Barradell LB *et al.* (1993). Clarithromycin. A review of its pharmacological properties and therapeutic use in Mycobacterium avium-intracellulare complex infection in patients with acquired immune deficiency syndrome. *Drugs* 46: 289–312.

Chu SY *et al.* (1991). Simultaneous determination of clarithromycin and 14(R)-hydroxyclarithromycin in plasma and urine using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 571: 199–208.

Chu S *et al.* (1993). Single- and multiple-dose pharmacokinetics of clarithromycin, a new macrolide antimicrobial. *J Clin Pharmacol* 33(8): 719–726.

Chu SY *et al.* (1992). Clarithromycin pharmacokinetics in healthy young and elderly volunteers. *J Clin Pharmacol* 32: 1045–1049.

Fish DN, Abraham E (1999). Pharmacokinetics of a clarithromycin suspension administered via nasogastric tube to seriously ill patients. *Antimicrob Agents Chemother* 43(5): 1277–1280.

Fraschini F *et al.* (1993). Clarithromycin clinical pharmacokinetics. *Clin Pharmacokinet* 25: 189–204.

Kanfer I *et al.* (1998). Analysis of macrolide antibiotics. *J Chromatogr A* 812: 255–286.

Kees F *et al.* (1998). Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection. *J Chromatogr A* 812: 287–293.

Macek J *et al.* (1999). Determination of roxithromycin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B, Biomed Sci Appl* 723: 233–238.

Peters DH, Clissold SP (1992). Clarithromycin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic potential. *Drugs* 44: 117–164.

Sastre Toraño J, Guchelaar HJ (1998). Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J Chromatogr B, Biomed Sci Appl* 720: 89–97.

Taninaka C *et al.* (2000). Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *J Chromatogr B, Biomed Sci Appl* 738: 405–411.

Clefamide

Antiamoebic

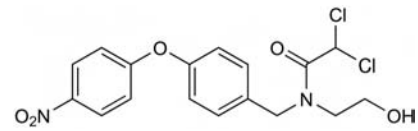
$C_{17}H_{16}Cl_2N_2O_5$ = 399.2

CAS—3576-64-5

IUPAC Name 2,2-Dichloro-*N*-(2-hydroxyethyl)-*N*-[[4-(4-nitrophenoxy)phenyl]methyl]-acetamide

Synonym Chlorophenoxamide

Proprietary Name *Mebinol*

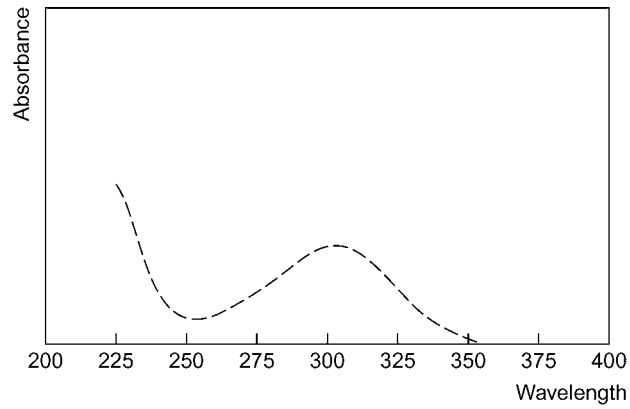


Chemical Properties A lemon-yellow crystalline powder. Mp 134° to 137°. Practically insoluble in water; soluble 1 in 100 of ethanol, 1 in 40 of acetone, and 1 in 80 of chloroform. Log *P* (octanol/water), 3.2.

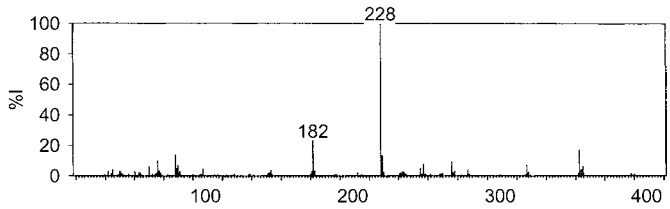
Colour Tests Mandelin's test—green→brown; Marquis test—yellow; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.00; system TC—R_f 0.56; system TL—R_f 0.68 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Ethanol—303 nm (A^1_1=310b).



Infrared Spectrum Principal peaks at wavenumbers 1244, 1666, 1510, 1595, 1078, 880 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 228, 182, 363, 88, 229, 76, 276, 257.

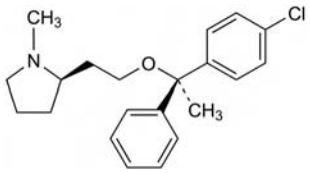


Disposition in the Body Poorly absorbed after oral administration.
Dose Clefamide has been given in doses of 1.5 to 2.25 g daily.

Clemastine

Antihistamine
$C_{21}H_{26}ClNO$ = 343.9
CAS—15686-51-8

IUPAC Name (2*R*)-2-[2-[(1*R*)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine
Synonyms Meclastine; mecloprodin.

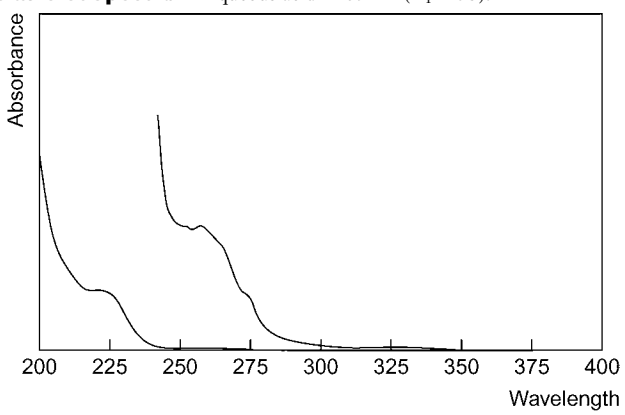


Chemical Properties Soluble in chloroform.

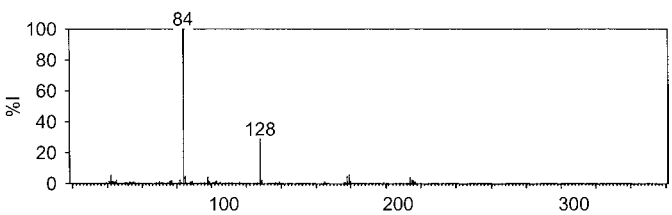
Clemastine Fumarate

$C_{21}H_{26}ClNO$, $C_4H_4O_4$ = 460.0
CAS—14976-57-9
Proprietary Names *Aller-eze*; *Alginan*; *Tavegil*; *Tavegyl*; *Tavist*; *Xolamin*.
Chemical Properties A white crystalline powder. Mp 177° to 178°. Slightly soluble in dilute acetic acid; soluble in methanol.

Colour Tests Liebermann's reagent—brown; Mandelin's test—yellow-brown; Marquis test—yellow (green rim); sulfuric acid—yellow (green rim).
Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.49; system TC—R_f 0.25; system TE—R_f 0.58; system TL—R_f 0.09; system TAE—R_f 0.88; system TAF—R_f 0.49 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—clemastine RI 2425, M (OH-methoxy-carbinol-)-H_2O RI 2220; system GF—RI 2710.
High Performance Liquid Chromatography System HA—*k* 3.7; system HX—RI 501; system HZ—retention time 14.0 min.
Ultraviolet Spectrum Aqueous acid—257 nm (A_1^1=27b).



Infrared Spectrum Principal peaks at wavenumbers 1090, 1011, 700, 763, 1121, 1210 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 84, 128, 179, 42, 85, 178, 214, 98.



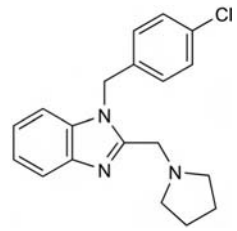
Quantification
Plasma **GC** NPD. Limit of detection, 0.06 µg/L [Davydova *et al.* 2000]. ECD. Limit of detection, 1 µg/L [Tham *et al.* 1978].
Disposition in the Body
Therapeutic Concentration
Following a single oral dose equivalent to 2 mg of clemastine to 12 subjects, peak plasma concentrations of about 0.002 mg/L were attained in 3 to 5 h [Tham *et al.* 1978].
Nineteen healthy men and women, aged 19 to 56 years, were orally administered a single 2.68 mg clemastine fumarate dose and in addition received the same dose three times daily for 3 days in a multidose study. The mean peak plasma concentrations reached were 0.75 µg/L for the single dose and 1.75 µg/L for the multiple dose. Each observed approximately 3.4 h after dosing [Davydova *et al.* 2000].
Dose The equivalent of 2 to 6 mg of clemastine daily.

Davydova NN *et al.* (2000). Determination of clemastine in human plasma by gas chromatography with nitrogen-phosphorus detection. *J Chromatogr B Biomed Sci Appl* 744(1): 177–181.
Tham R *et al.* (1978). Gaschromatography of clemastine. A study of plasma kinetics and biological effect. *Arzneimittelforschung* 28: 1017–1020.

Clemizole

Antihistamine
$C_{19}H_{20}ClN_3$ = 325.8
CAS—442-52-4

IUPAC Name 1-[(4-Chlorophenyl)methyl]-2-(1-pyrrolidinylmethyl)-1*H*-benzimidazole

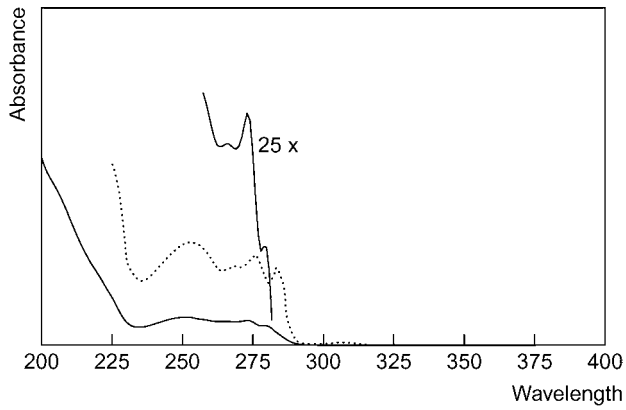


Chemical Properties Crystals. Mp 167°. Log *P* (octanol/water), 4.4.

Clemizole Hydrochloride

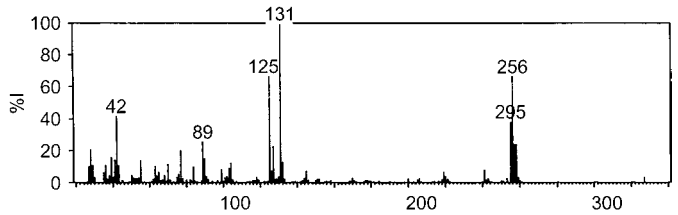
$C_{19}H_{20}ClN_3$, HCl = 362.3
CAS—1163-36-6
Proprietary Name *Allercur*
Chemical Properties A white crystalline powder. Mp about 246°. Sparingly soluble in water; soluble in ethanol and chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.78; system TB—R_f 0.33; system TC—R_f 0.69; system TE—R_f 0.78; system TL—R_f 0.52; system TAE—R_f 0.76; system TAF—R_f 0.73 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—clemizole RI 2620, M (oxo-) RI 2965.
High Performance Liquid Chromatography System HA—*k* 4.8 (tailing peak); system HX—RI 420.
Ultraviolet Spectrum Aqueous acid—275 nm (A_1^1=330a); aqueous alkali—254 (A_1^1=253b), 269, 275, 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 748, 765, 740, 833, 1111, 1010 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 131, 256, 125, 42, 255, 89, 258, 257.

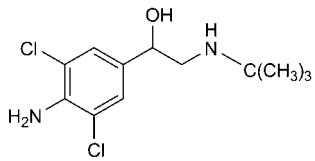


Dose 40 to 160 mg of clemizole hydrochloride daily.

Clenbuterol

β_2-*Adrenoceptor Agonist, Bronchodilator*
$C_{12}H_{18}Cl_2N_2O = 277.2$
CAS—37148-27-9

IUPAC Name 1-(4-Amino-3,5-dichlorophenyl)-2-(*tert*-butylamino)ethanol
Synonyms 4-Amino-3,5-dichloro-α-[[(1,1-dimethylethyl)amino]methyl]benzenemethanol; 4-amino-α-[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol; NAB-365.



Chemical Properties Log *P* (octanol/water) 2.47 [Prezelj *et al.* 2003].

Clenbuterol Hydrochloride

CAS—21898-19-1
IUPAC Name 1-(4-Amino-3, 5-dichlorophenyl)-2-(tert-butylamino)ethanol hydrochloride
Synonym NAB-365C
Proprietary Names *Broncodil*; *Clenasma*; *Contrasmina*; *Contraspasmin*; *Monores*; *Prontovent*; *Spiropent*; *Ventolase*; *Ventipulmin*.
Chemical Properties Colourless, microcrystalline powder. Mp 174° to 175.5°. Very soluble in water, methanol and ethanol; slightly soluble in chloroform; insoluble in benzene.

Thin-layer Chromatography System TAE—R_f 0.22 (clenbuterol), R_f 0.30 (M-hydroxy), R_f 0.87 (M2), R_f 0.87 (M3); system TB—R_f 0.13 (clenbuterol), R_f 0.01 (M-hydroxy), R_f 0.00 (M2), R_f 0.00 (M3); system TE—R_f 0.58 (clenbuterol), R_f 0.43 (M-hydroxy), R_f 0.02 (M2), R_f 0.06 (M3); system TF—R_f 0.00 (M2), R_f 0.19 (M3).

Plates: silica gel 60 (10 × 10 cm). Mobile phase: ethyl acetate : methanol : propionic acid (A, 8 : 1 : 1) and ethyl acetate : methanol : ammonia (B, 8.5 : 1 : 0.5). Developed with Ehrlich's reagent. R_f 0.40 (A) and 0.51 (B) [Courtheyn *et al.* 1991].

Gas Chromatography System GAI—RT 0.57 relative to 17α-methyl-5α-androstan-3β; system GA—RI 2100 (clenbuterol); RI 1895 (Art (-H_2O)) , RI 2160 (Art (-H_2O)).

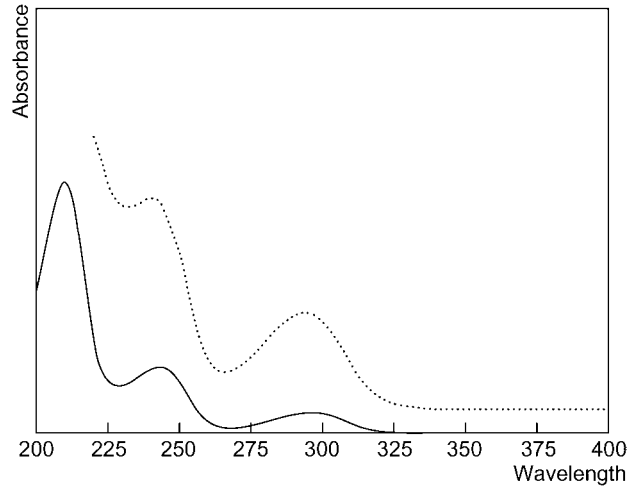
Column: HP Ultra 1 (25 m × 0.2 mm i. d., 0.11 μm). Temperature programme: 80° for 2 min, 20°/min up to 160°, then 2°/min to 190° and 30°/min to 300° for 10 min. Carrier gas: He, flow rate 1.0 ml/min. EI ionisation, SIM acquisition mode. Retention time: 15.4 min for the clenbuterol–mono-TMS (tetramethylsilane) derivative [Courtheyn *et al.* 1991].

Column: Equity 1701 (10 m × 0.1 mm i.d., 0.1 μm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° for 1 min to 300° at 55°/min for 2.1 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 0.06 min. Limit of detection, 1.5 μg/L [Brunelli *et al.* 2006].

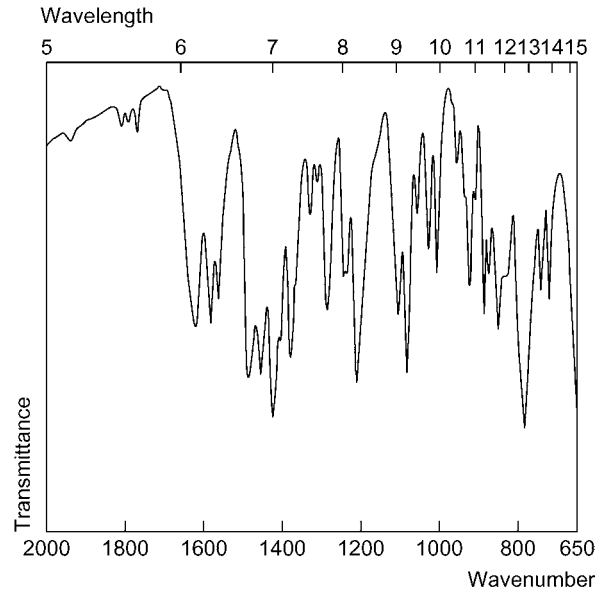
Column: 1% phenylmethylsilicone HP 1-MS (15 m × 0.25 mm i. d., 0.25 μm). Carrier gas: He, 1.1 ml/min. Temperature programme: 150° for 1 min to 215° at 15°/min to 300° at 35°/min for 2 min. FS mode. Retention time: 5.09 min [Abukhalaf *et al.* 2000].

High Performance Liquid Chromatography System HAW—*k* 6.1 (column (a)), *k* 5.0 (column (b)); system HAA—retention time 10.8 min; system HX—RI 326 clenbuterol-hydroxy-, RI 297 (M-hydroxy-), RI 346 ((M2)-hydroxy-), RI 419 ((M3)-hydroxy-); system HY—RI 282.

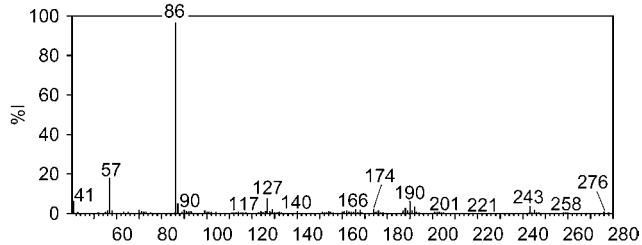
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—242, 295 nm; basic—240, 295 nm.



Infrared Spectrum Principal peaks at wavenumbers 1414, 774, 640 cm^{-1} (KBr pellets).



Mass Spectrum Principal ions at *m/z* 86, 57, 41, 127, 190, 243, 90, 174.



Quantification

Blood **GC-MS** Column: ZB5MS capillary (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 50° for 1.5 min to 300° at 20°/min for 4 min. Full scan mode. Retention time: 10.3 min. Limit of quantification, 5 μg/L, limit of detection, 2.5 μg/L [Wingert *et al.* 2008]. Column: DB-1 capillary (25 m × 0.2 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 300° at 25°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 100 μg/L [Black, Hansson 1999].

LC-MS Column: BDS Hypersil (15 cm × 2 mm i.d., 3 μm). Mobile phase: 2 mmol/L formic acid : acetonitrile (97 : 3 to 5 : 95 over 5 min), flow rate 250 μL/min. ESI, full MS. Retention time: 5.81 min. Limit of detection, 0.01 μmol/L [Yuen *et al.* 2005].

Plasma **GC-MS** Column: HP 1MS cross-linked 1% phenyl methylsilicone (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.1 mL/min. Temperature programme: 150° for 1 min to 215° at 15°/min to 300° at 35°/min for 2 min. SIM acquisition mode. Limit of quantification, 1.5 μg/L, limit of detection, 0.5 μg/L [Abukhalaf *et al.* 2000]. Column: OV 1701 fused silica capillary (25 m × 0.22 mm i.d., 0.11 μm). Carrier gas: He, 14 psi. Temperature programme: 180° to 230° at 8°/min to 290° at 20°/min. NICI at 100 eV. Limit of detection, 5 ng/L [Girault *et al.* 1990].

HPLC Column: Chirobiotic T (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile (70 : 30) containing 0.3% glacial acetic acid and 0.2% triethylamine, flow rate 1.0 mL/min. UV detection (λ = 246 nm). Retention time: 8.38 and 9.56 min for (–)-*R*- and (+)-*S*-clenbuterol, respectively. Limit of detection, <0.25 µmol/L [Aboul-Enein, Serignese 1999]. Column: Chirex 3022 (250 × 4.0 mm i.d.). Mobile phase: hexane : 1,2-dichloroethane : ethanol-5% trifluoroacetic acid (80 : 10 : 10), flow rate, 0.8 mL/min. UV detection (λ = 254 nm). Retention time: 9.73 and 11.63 min for *S*- and *R*-clenbuterol, respectively. Limit of quantification, 0.1 nmol [Abou-Basha, Aboul-Enein 1996].

LC-MS Column: NovaPak C_{18}. Mobile phase: acetonitrile : 1 mmol/L ammonium acetate buffer (pH 4.1; 90 : 10), flow rate 1.0 mL/min. APCI, positive ion mode, full scan or SIM acquisition mode. Limit of detection, 10 µg/L [Doerge *et al.* 1993].

Serum **HPLC** Column: Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 20 mmol/L phosphate buffer (pH 10; 10 : 50 : 40), flow rate 1.0 mL/min. UV detection (λ = 244 nm). Limit of quantification, 24 µg/L, limit of detection, 5 µg/L [Aresta *et al.* 2008]. Column: Chirex 3005 (250 × 4.6 mm i. d.). Mobile phase: *n*-hexane: 1,2-dichloroethane : methanol (54 : 38 : 8), flow rate 1.0 mL/min. UV detection (λ = 247 nm). Limit of detection, 0.47 and 1.04 µmol/L for *R*- and *S*-clenbuterol, respectively [Song *et al.* 2003].

LC-MS Column: Betasil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate- 8 mmol/L trifluoroacetic acid (pH 5.4) : acetonitrile (60 : 40), flow rate 0.8 mL/min. APCI, positive ion mode. Retention time: 4.7 min. Limit of detection, 1.0 µg/L [Hoffman *et al.* 2001]. Column: Pinkerton reversed phase (50 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium acetate : 0.01 mol/L triethylamine (pH 7.0), flow rate 1.0 mL/min. TSI. Limit of quantification, 0.5 µg/L [Hogendoorn *et al.* 1998].

Urine **GC-MS** See Serum. Limit of quantification, 32 µg/L, limit of detection, 9 µg/L [Aresta *et al.* 2008]. See Blood [Wingert *et al.* 2008]. Column: HP5 cross-linked phenyl methyl silicone (30 m). Carrier gas: He, 0.6 mL/min. Temperature programme: 125° for 2 min to 225° at 20°/min for 1 min to 320° at 10°/min for 10 min. EI ionisation. Limit of detection, 0.1 µg/L [Amendola *et al.* 2002]. Column: HP methylsiloxane fused silica capillary (17.5 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 100° for 2 min to 190° at 30°/min to 300° at 20°/min for 4 min. EI ionisation at 70 eV, scan or SIM acquisition mode. Retention time: 6.84 min. Limit of detection, 0.5 µg/L [Damasceno *et al.* 2002]. See plasma. Limit of quantification, 0.7 µg/L, limit of detection, 0.2 µg/L [Abukhalaf *et al.* 2000]. See Blood. Limit of detection, 500 µg/L [Black, Hansson 1999]. Column: Ultra-2% 5 phenyl methyl siloxane capillary (17 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 150° to 280° at 15°/min. EI ionisation at 70 eV, scan and SIM acquisition mode. Limit of detection, 0.02 µg/L [Keskin *et al.* 1998]. See Plasma [Abou-Basha, Aboul-Enein 1996]. Column: J & W DB-1 methylsilicone capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 100° for 1 min to 220° at 16°/min to 301° at 3.8°/min for 5.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Ayotte *et al.* 1996]. Column: DB-1 (15 m × 0.25 mm i.d.). Temperature programme: 150° for 1 min to 300° at 15°/min for 10 min. EI ionisation at 35 eV, positive ion mode, full scan or SIR mode. Retention time: 6.08 min. Limit of detection, <1 mg/L [Kingston *et al.* 1995]. See also [van Rhijn *et al.* 1995], [Polettini *et al.* 1993], [van Rhijn *et al.* 1993], [Polettini *et al.* 1991], [Förster *et al.* 1988], or [Schmid *et al.* 1988].

LC-MS Column: Purospher Star 18e (55 × 4.6 mm i.d., 3 µm). Mobile phase: 5 mmol/L ammonium acetate containing 0.1 acetic acid (pH 3.5) : acetonitrile (95 : 5 to 20 : 80 in 7 min), flow rate 1 mL/min. ESI, MRM acquisition mode. Limit of detection, 2 µg/L [Thevis *et al.* 2003].

Milk **LC-MS** Column: cyano (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% diethylamine and 0.1% trifluoroacetic acid : methanol (20 : 80), flow rate 2.0 mL/min. UV detection (λ = 230 nm) followed by APCI, positive ion mode, SIM acquisition mode. Limit of detection, 500 µg/L [Jones *et al.* 1999].

Hair **GC-MS** Column: HP5-MS 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.2 mL/min. Temperature programme: 110° for 1 min to 170° at 20°/min to 225° at 7°/min to 295° at 24°/min for 10 min. EI ionisation. Retention time: 12.6 min. Limit of detection, 2 ng/g [Kintz *et al.* 2000]. Column: CP-Sil 5 CB WCOT fused silica (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1.0 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 6.87 min. Limit of quantification, 0.1 µg/g, limit of detection, 0.02 µg/g [Deng *et al.* 1999]. Column: HP Ultra 1 cross-linked methylsilicone capillary (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 150° to 250° at 10°/min to 310° at 40°/min for 3 min. EI ionisation at 65 eV, SIM acquisition mode. Limit of detection, 4 ng/g, 0.8 ng/g with immunoaffinity chromatography [Machnik *et al.* 1999].

Other HPLC Animal Samples. Column: Nova-Pak C_{18} (15 cm × 4.6 mm i.d., 4 µm). Mobile phase: 0.025 mol/L sodium *n*-dodecyl sulfate 0.02 mol/L glacial acetic acid (pH 3.5) : acetonitrile (53 : 47), flow rate 1.3 mL/min. UV detection (λ = 493 nm). Limit of detection, 0.1 and 0.2 ng/g for liquid and solid samples, respectively [Courtheyn *et al.* 1991].

LC-MS Equine Urine. See Urine. Limit of detection, 10 µg/L [Thevis *et al.* 2003]. Bovine Liver. Column: Symmetry C_{18} (150 × 2.1 mm, 5 um). Mobile phase: methanol : 0.032% trifluoroacetic acid (22 : 78), flow rate 0.3 mL/min. ESI, full scan mode, positive ion mode. Relative retention time: 1.02 min. Limit of quantification, 0.21 ng/g, limit of detection, 0.11 ng/g [de Wasch *et al.* 1998].

Disposition in the Body Clenbuterol is rapidly and almost completely absorbed after oral administration, and extensively metabolised by first-pass sulfation. Excretion is mainly via the kidneys for IV administration and biotransformation for oral administration. No accumulation has been observed.

Therapeutic Concentration

Hair was collected from 4 pregnant females who had been treated with clenbuterol hydrochloride (3 × 20 µg/day). Concentrations ranged from 2 to 236 ng/g [Machnik *et al.* 1999].

Ten healthy volunteers were orally administered 20 µg clenbuterol. The mean maximum plasma concentration was 86.5 ± 18.1 ng/L reached at 2.35 ± 1.2 h [Girault *et al.* 1990].

Healthy males were orally administered 20, 40 and 80 µg doses of clenbuterol. Plasma levels reached maximum values of 0.1, 0.2 and 0.35 µg/L, respectively, within 2.5 h and lasted over 6 h. After repeated oral administration (twice a day), plasma levels reached 0.2 to 0.3 µg/L and 0.5 to 0.6 µg/L for doses of 20 and 40 µg, respectively [Yamamoto *et al.* 1985].

Toxicity Acute exposure has been associated with pulmonary oedema and respiratory failure [Schechter *et al.* 2007] as well as supraventricular tachycardia [Daubert *et al.* 2007].

In 12 heroin-related deaths the victims' urine tested positive for clenbuterol. Blood concentrations ranged from none detectable to 76 µg/L [Wingert *et al.* 2008].

A 28-year-old female accidentally ingested an unknown quantity of clenbuterol. Three hours after admission to hospital her serum clenbuterol concentration was 2.93 µg/L [Hoffman *et al.* 2001].

A 21-year-old body builder drank orange juice 'spiked' with 48 tablets (4.8 g) of clenbuterol and admitted with tachycardia, headache, dizziness, tremour, sweats, muscle weakness and agitation [Chodorowski, Sein 1997].

Note For other cases of clenbuterol toxicity after diamorphine use, see CDC [2005]. For cases of clenbuterol poisoning due to its unauthorised use in animal husbandry, see Salleras *et al.* [1995], Brambilla *et al.* [1997], Mitchell and Dunnavan [1998] or Barbosa *et al.* [2005].

Bioavailability 70 to 80% [Hoffman *et al.* 2001].

Volume of Distribution 5 L/kg [Schmid *et al.* 1988].

Half-life 25 to 39 h [Hoffman *et al.* 2001].

Protein Binding ≈89 to 98%, after a single dose of 80 µg [Yamamoto *et al.* 1985].

Dose A dose of 20 µg is administered by mouth twice daily and up to 40 µg has been reported. Clenbuterol hydrochloride can be used as an inhaler with a dosage of 20 µg three times a day.

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Clidinium Bromide

Anticholinergic

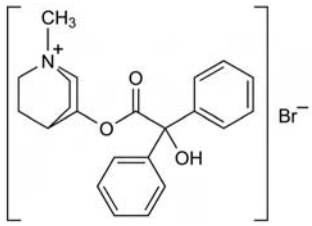
$C_{22}H_{26}BrNO_3$ = 432.4

CAS—7020-55-5 (clidinium); 3485-62-9 (bromide)

IUPAC Name (1-Methyl-1-azoniabicyclo[2.2.2]octan-3-yl) 2-hydroxy-2,2-diphenylacetate bromide

Synonyms 3-[(Hydroxydiphenylacetyl)oxy]-1-methyl-1-azoniabicyclo[2.2.2] octane bromide; Ro-2-3773.

Proprietary Names *Quarzan*. It is an ingredient of *Librax(in)*.



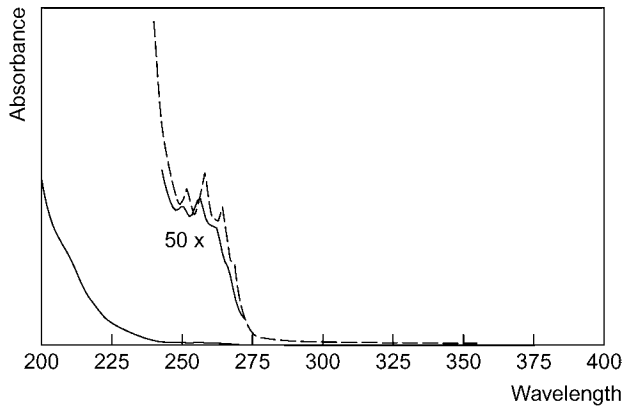
Chemical Properties A white crystalline powder. Mp about 242°. Soluble in water and ethanol; slightly soluble in ether. Log *P* (octanol/water), −0.5.

Colour Tests The following tests are performed on the nitrate: Liebermann's reagent—orange→brown; Mandelin's test—brown; Marquis test—orange→blue; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.00; system TE—R_f 0.01; system TF—R_f 0.70; system TAE—R_f 0.03 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 379; system HAA—retention time 13.3 min (clidinium).

Ultraviolet Spectrum Aqueous acid—253 (A^1_1=10b), 259 nm (A^1_1=11b); methanol—252, 258 (A^1_1=10.4a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1240, 769, 1190, 712, 1011 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 105, 77, 96, 183, 51, 42, 182, 94.

Disposition in the Body The major metabolite is 3-hydroxy-1-methylquinuclidinium bromide.

Dose 7.5 to 20 mg daily.

Clindamycin

Antibiotic

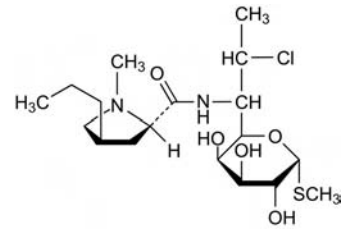
$C_{18}H_{33}ClN_2O_5S$ = 425.0

CAS—18323-44-9

IUPAC Name (2*S*,4*R*)-*N*-[(1*S*,2*S*)-2-Chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4, 5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide

Synonyms Chlorodeoxylincomycin; (7*S*)-chloro-7-deoxylincomycin; (2*S*-*trans*)-methyl-7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside; U-21251.

Note The name Clinimycin was formerly used for clindamycin and has also been used for a preparation of oxytetracycline.



Chemical Properties A yellow, amorphous solid. pK_a 7.7 (25°). Log *P* (octanol/water), 2.2.

Clindamycin Hydrochloride

$C_{18}H_{33}ClN_2O_5S$, HCl, H_2O = 479.5

CAS—21462-39-5 (anhydrous); 58207-19-5 (monohydrate)

Proprietary Names *Cleocin*; *Dalacin C*; *Dalacin(e)*; *Sobelin* (all as capsules).

Chemical Properties A white crystalline powder. Mp 141° to 143°. Soluble 1 in 2 of water and 1 in 200 of ethanol; freely soluble in methanol; very slightly soluble in chloroform.

Clindamycin Palmitate Hydrochloride

$C_{34}H_{63}ClN_2O_6S$, HCl = 699.9

CAS—36688-78-5 (palmitate); 25507-04-4 (palmitate hydrochloride)

Proprietary Names *Cleocin*; *Dalacin*; *Dalacin C*; *Sobelin* (all as granules for oral suspension).

Chemical Properties A white amorphous powder. It hydrolyses in solutions above pH 6.0. Freely soluble in water, ethanol, chloroform, and ether.

Clindamycin Phosphate

$C_{18}H_{34}ClN_2O_8PS$ = 505.0

CAS—24729-96-2

Proprietary Names *Cleocin*; *Dalacin*; *Dalacin C*; *Sobelin* (all as injection).

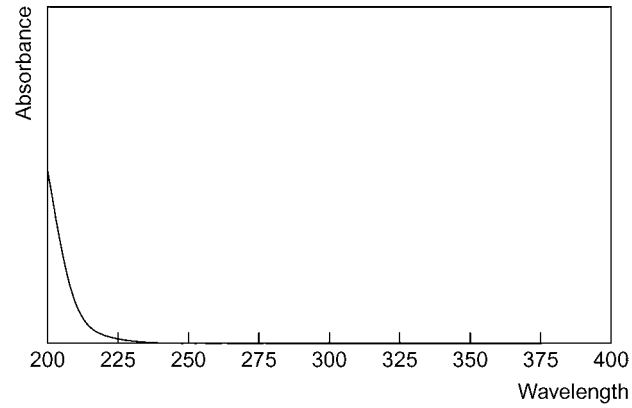
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 2.5 of water; slightly soluble in dehydrated alcohol; practically insoluble in chloroform and ether.

Colour Tests Palladium chloride—yellow; sodium nitroprusside (method 2)—violet.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.00; system TE—R_f 0.28; system TAE—R_f 0.81 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 354; system HY—RI 291; system HAA—retention time 12.0 min.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1513, 1664, 1080, 1050, 1302, 1250 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** MS–MS detection [Rechberger *et al.* 2003]. MS–MS detection. Limit of quantification, 0.05 mg/L [Yu *et al.* 1999].

Serum **HPLC** MS detection (*m/z* 405 to 425). Limit of quantification, 0.1 mg/L [Martens-Lobenhoffer, Banditt 2001].

Bone **HPLC** Limit of quantification, 0.1 µg/g, see Serum [Martens-Lobenhoffer, Banditt 2001].

Dose The equivalent of 0.6 to 1.8 g of clindamycin daily.

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Clioquinol

Antiamoebic

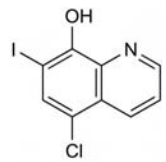
C_9H_5ClINO = 305.5

CAS—130-26-7

IUPAC Name 5-Chloro-7-iodo-8-quinolinol

Synonyms Chinoform; chloroiodoquine; cliochinolum; iodochlorhydroxyquin; iodochlorhydroxyquinoline; quiniodochlor; vioformo.

Proprietary Names *Amebil*; *Budoform*; *Eczecidin*; *Enteroquinol*; *Entero-Valodon*; *Entero-Vioform*; *Nioform*; *Rometin*; *Vioform*. It is an ingredient of *Oralcer* and *Unidiarea*.

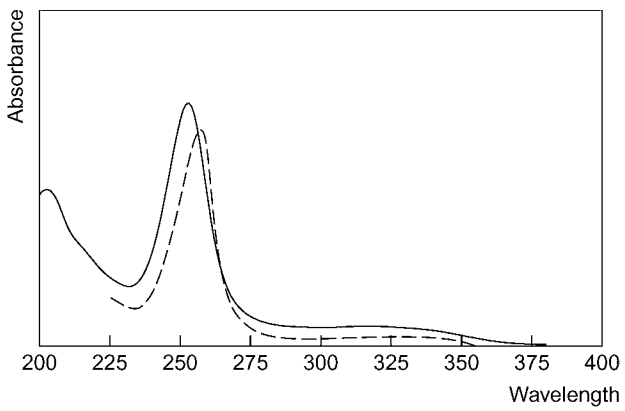


Chemical Properties A yellowish-white to brownish-yellow, voluminous powder. It darkens on exposure to light. Mp 178° to 179°, with decomposition. Almost insoluble in water, ethanol, and ether; soluble 1 in 43 of boiling ethanol, 1 in 128 of chloroform, 1 in 17 of boiling ethyl acetate, 1 in 170 of cold acetic acid, and 1 in 13 of boiling acetic acid; soluble in dimethylformamide. Log *P* (octanol/water), 3.5.

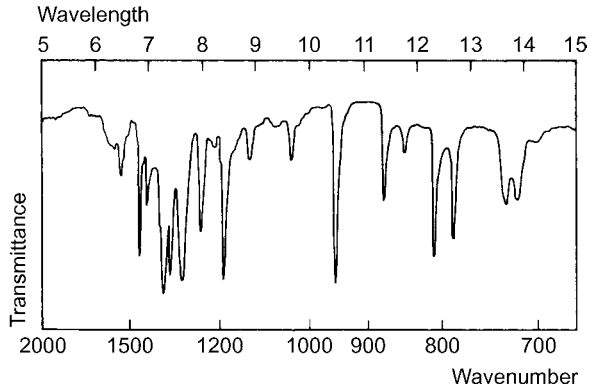
Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.00; system TC—R_f 0.05; system TE—R_f 0.30; system TL—R_f 0.09; system TAD—R_f 0.70; system TAE—R_f 0.40; system TAF—R_f 0.88.

Gas Chromatography System GA—RI 1975.

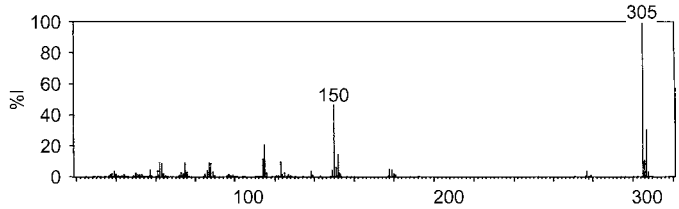
Ultraviolet Spectrum Methanol—255 (A_1^1=1196a), 325 nm.



Infrared Spectrum Principal peaks at wavenumbers 953, 1198, 808, 784, 1266, 728 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 305, 150, 307, 115, 152, 114, 306, 123.



Quantification

Plasma **GC** ECD. Limit of detection, 50 µg/L [Sioufi, Pommier 1981].

HPLC UV detection. Limit of detection, 600 µg/L [Hayakawa *et al.* 1982].

Urine **HPLC** See Plasma [Hayakawa *et al.* 1982].

Disposition in the Body Poorly absorbed after oral administration. About 25% of a dose is excreted in the urine in 24 h as glucuronide and sulfate conjugates.

Therapeutic Concentration

Following single oral doses of 250 and 1500 mg to 6 subjects, peak plasma concentrations of 1.7 to 8.3 mg/L (mean 5.2) and 14.6 to 34.8 mg/L (mean 23), respectively, were attained in 2 to 4 h [Jack, Riess 1973].

Half-life Plasma half-life, 11 to 14 h.

Dose Usually 0.75 to 1.5 g daily.

Hayakawa K *et al.* (1982). High-performance liquid chromatographic determination of clioquinol and its conjugates in biological materials. *J Chromatogr* 229(1): 159–165.

Jack DB, Riess W (1973). Pharmacokinetics of iodochlorhydroxyquin in man. *J Pharm Sci* 62: 1929–1932.

Sioufi A, Pommier F (1981). Gas chromatographic determination of clioquinol (Vioform) in human plasma. *J Chromatogr* 226(1): 219–223.

Clioxanide

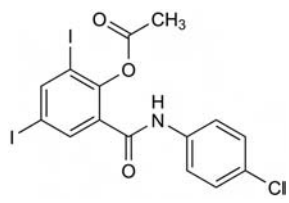
Anthelmintic (Veterinary)

$C_{15}H_{10}Cl_2NO_3$ = 541.5

CAS—14437-41-3

IUPAC Name [2-[(4-Chlorophenyl)carbamoyl]-4,6-diiodophenyl] acetate

Synonyms 2-(Acetyloxy)-*N*-(4-chlorophenyl)-3,5-diiodobenzamide; CI-633; SYD-230.



Chemical Properties A white crystalline powder. Mp 215° to 216°. Sparingly soluble in water; moderately soluble in ethanol and acetone; soluble in chloroform. Log *P* (octanol/water), 5.3.

Colour Tests Iodine test—positive; Mandelin's test—red-brown.

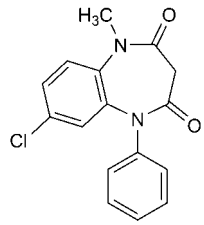
Thin-layer Chromatography System TA—R_f 0.79 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Ethanol—233 nm (A_1^1=625b); aqueous alkali—240 (A_1^1=561b), 282 (A_1^1=270b), 362 nm(A_1^1=172b).
Infrared Spectrum Principal peaks at wavenumbers 1190, 1647, 1520, 1309, 1764, 816 cm^{-1} (Nujol mull).

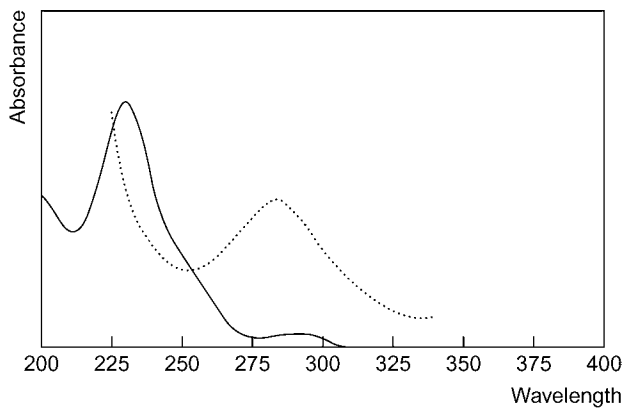
Clobazam

Anxiolytic, Anticonvulsant, Benzodiazepine, Tranquilliser
$C_{16}H_{13}ClN_2O_2$ = 300.7
CAS—22316-47-8

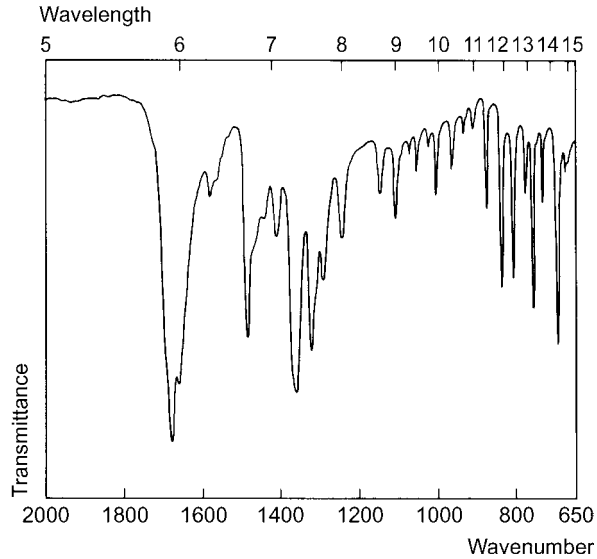
IUPAC Name 8-Chloro-5-methyl-1-phenyl-1,5-benzodiazepine-2,4-dione
Synonyms 7-Chloro-1-methyl-5-phenyl-1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)-dione; H-4723; HR-376; LM-2717; 1-phenyl-5-methyl-8-chloro-1,2,4,5-tetrahydro-2,4-dioxo-3*H*-1,5-benzodiazepine.
Proprietary Names *Castilium*; *Clarmyl*; *Clopax*; *Frisium*; *Noiafren*; *Urbadan*; *Urbanil*; *Urbanol*; *Urbanyl*.



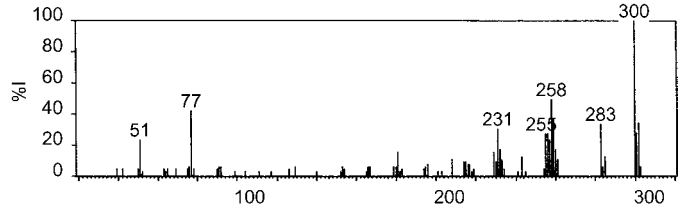
Chemical Properties White crystalline powder. M.p. 166° to 168°. Practically insoluble in water; sparingly soluble in ethanol; freely soluble in acetone and chloroform. Log *P* (octanol/water), 2.12. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Test Aromaticity (method 2)—colourless (heat for 3 min)/red.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.08; system TC—R_f 0.70; system TD—R_f 0.53; system TE—R_f 0.75; system TF—R_f 0.47; system TL—R_f 0.62; system TAD—R_f 0.70; system TAE—R_f 0.84; system TAF—R_f 0.85.
Gas Chromatography system GA—clobazam RI 2558, M (nor-) RI 2747, M (OH-) RI 3000, M (OH-MeO-) RI 3255; system GB—clobazam RI 2683, M (nor-) RI 2759; system GG—RI 3147.
High Performance Liquid Chromatography System HI—clobazam *k* 3.91, M (nor)*k* 3.06; system HK—clobazam *k* 0.03, M (nor-)*k* 0.01; system HX—clobazam RI 488; system HY—clobazam RI 455; system HZ—clobazam RT 6.2 min; system HAA—RT 19.2 min; system HAK—clobazam RT 9.3 min; system HAL—clobazam RT 7.8 min; system HAM—not detected; system HAX—clobazam RT 7.2 min; system HAY—clobazam RT 7.1 min; system HBH—clobazam *k* 4.14; system HBI—clobazam *k* 1.09.
Ultraviolet Spectrum Ethanol—228 (A_1^1 = 1450), 285 and 295 nm (A_1^1 = 75) [Hajdú *et al.* 1980]; aqueous acid—230 (A_1^1 = 1373b), 289 nm (A_1^1 = 76b); aqueous alkali—286 nm (A_1^1 = 193b).



Infrared Spectrum Principal peaks at wavenumbers 1684, 1664, 704, 1490, 764, 845 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 300, 258, 77, 259, 283, 302, 231, 256; desmethylclobazam 286, 244, 77, 218, 51, 217, 288, 215.



Quantification

Blood **GC** Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 6.28 min. Limit of detection not reported [Lillsunde, Seppala 1990].

HPLC Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 50 mmol/L potassium dihydrogen phosphate : acetonitrile (75 : 25 to 70 : 30 at 15 min to 65 : 35 at 25 min for 5 min), flow rate 0.8 mL/min. DAD. Limit of quantification, 10 µg/L [Dussy *et al.* 2006].

LC-MS Column: Symmetry C_{18} reversed phase (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : water (55 : 45 for 5 min to 65 : 35 over 12 min for 5 min), flow rate 0.25 mL/min. DAD (λ = 254 nm) followed by ESI, positive ion mode, SIR acquisition mode. Retention time: 6.7 min. Limit of quantification, 10.0 µg/L, limit of detection, 1.0 µg/L [Proença *et al.* 2004].

Plasma **GC** Column: Ultra 2 5% phenyl methyl silicone (25 m × 0.32 mm i.d.). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD and ECD. Relative retention time: 0.87. Limit of quantification, 2 µg/L [Gaillard *et al.* 1993]. Column: 3% CP-Sil on Chromosorb G HP, 100/120 mesh (0.9 m × 2.0 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 255°. ECD. Retention time: 4.2 min. Limit of detection, 7 nmol/L [Badcock, Zoanetti 1987]. Column: 10% OV-101 on 80/100 mesh Chromosorb (122 cm × 4 mm i.d.). Carrier gas: Ar:CH_4 (95 : 5), 25 mL/min. Temperature: 265°. ECD. Limit of detection, 3–5 µg/L [Greenblatt 1980].

GC-MS Column: CP-Sil 5 glass-coated capillary (25 m × 0.5 mm i.d.). Carrier gas: He, 0.6 bar. Temperature: 260°. ECD. Retention time: 4.3 min. Limit of quantification, 1 µg/L [Monjanel-Mouterde *et al.* 1994]. Column: cross-linked dimethylsilicone fused silica capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 bar. Temperature programme: 70° to 300° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 1–5 µg/L [Drouet-Coassolo *et al.* 1989]. Column: GP-2% SP-2510-DA on 100/120 mesh (0.5 m × 3.0 mm i.d.). Carrier gas: N_2, 80 mL/min. Temperature: 245°. ECD. Limit of detection, 5 µg/L [Riva *et al.* 1981].

HPLC Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: 10 mmol/L phosphate buffer (pH 3.5) : acetonitrile (70 : 30), flow rate 2.0 mL/min. UV detection (λ = 228 nm). Retention time: 2.4 min. Limit of quantification, 5 µg/L [Rouini *et al.* 2005]. Column: Hisep (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.18 mol/L ammonium acetate buffer (pH 2.5; 15 : 85), flow rate 2.0 mL/min. UV detection (λ = 254 nm). Retention time: 8.65 min. Limit of quantification, 0.5 mg/L, limit of detection, 0.16 mg/L [Pistos, Stewart 2003]. Column: RP C_8 Ultrasphere (250 × 4.6 mm, 5 µm). Mobile phase: 20 mmol/L acetate buffer (pH 5.5) : acetonitrile : triethylamine (70 : 30 : 0.01), flow rate 1.8 mL/min. Retention time: 13.5 min. Limit of detection, 6 µg/L [Bolner *et al.* 2001]. Column: Lichrosorb C_{18} (250 × 4.0 mm i.d., 10 µm). Mobile phase: methanol : acetonitrile : 0.1 mol/L potassium dihydrogen phosphate (pH 3.6; 23 : 20 : 57), flow rate

2 mL/min. Retention time: 12 min [Knapp *et al.* 1999]. Column: Novapak C_{18} (150 mm, 4.0 μm). Mobile phase: acetonitrile : methanol : 10 mmol/L dipotassium hydrogen phosphate (pH 3.7; 30 : 2 : 100), flow rate 1.5 mL/min. DAD (λ = 240 nm). Limit of detection, 15 nmol/L [Akerman 1996].

See also Lacroix *et al.* [1993], Streete *et al.* [1991], Dusci and Hackett [1987], Tomasini *et al.* [1985] and Brachet-Liermain *et al.* [1982].

Serum **GC** Column: HP-5 cross-linked phenyl methylsilicone capillary (5.0 m × 0.53 mm i.d., 2.65 μm). Carrier gas: He, 10 mL/min. Temperature: 250°. ECD. Retention time: 1.86 min. Limit of detection, 0.3 μmol/L [LeGatt, McIntosh 1993]. Column: SE-54 5% phenyl methyl silicone capillary (25 m × 0.31 mm i.d., 0.17 mm). Temperature programme: 200° to 270° at 10°/min. NPD. Limit of detection not reported [Bardy *et al.* 1991]. Column: SPB-1 wide bore capillary (30 m × 0.75 mm i.d.). Temperature: 120°. NPD. Limit of detection not reported [Fraser *et al.* 1988]. See Plasma [Badcock, Zoanetti 1987]. Column: 3% SP 2250 on 100/120 mesh Supelcoport (2 m × 2 mm i.d.). Temperature: 280°. NPD. Relative retention time: 0.88 min. Limit of detection, 2–5 μg/L [Arranz Peña, Sanenz Lope 1986]. Column: 2% OV 101 glass (1.2 m × 4 mm i.d., 120–150 μm). Carrier gas: He, 40 mL/min. Temperature: 240°. FID. Limit of detection, 5 μg/L [Hajdú *et al.* 1980].

HPLC See Blood [Dussy *et al.* 2006]. Column: Supelcosil LC-8-DB (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : potassium dihydrogen phosphate : hydrogen phosphate (440 : 540 : 20 : 0.4), flow rate 1.5 mL/min. UV detection (λ = 228 nm). Retention time: 6.6 min. Limit of quantification, 2 μg/L, limit of detection, 1.0 μg/L [Kunicki 2001]. See Plasma [Akerman 1996]. Column: Partisil II CCS/C_8 (100 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : 0.5 mmol/L potassium dihydrogen phosphate (40 : 59 : 1), flow rate 70 mL/h. UV detection (λ = 228 nm). Retention time: 8.6 min. Limit of detection, 10 μg/L [Gazdzik *et al.* 1989]. Column: RP-8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L sodium phosphate : acetonitrile (pH 3.0; 1 : 1), flow rate 1.6 mL/min. UV detection (λ = 228–232 nm). Limit of detection, 0.1 mg/L [Fraser *et al.* 1988]. Column: Supelco C_8 reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 1.75 mmol/L hydrochloric acid : 50 mmol/L sodium acetate (36 : 10 : 54), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Limit of detection, 0.5 μg/L [Zilli, Nisi 1986]. Column: Spherisorb ODS (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (53 : 47), flow rate 1.8 mL/min. UV detection (λ = 228 nm). Retention time: 2 min. Limit of detection, 50 μg/L [Ratnaraj *et al.* 1984].

CE Capillary: fused silica (total/effective length 47/40 cm, 50 μm i.d.). Buffer: 50 mmol/L sodium borate (pH 9.5) containing 18 mmol/L SDS : acetonitrile (86 : 14). UV detection (λ = 214 nm). Limit of quantification, 15 μg/L [Imazawa, Hatanaka 1997].

Urine **GC** See Plasma [Tomasini *et al.* 1985]. See Serum [Hajdú *et al.* 1980].

GC-MS Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

HPLC See Serum. Mobile phase: acetonitrile : water : potassium dihydrogen phosphate : hydrogen phosphate (360 : 580 : 60 : 0.4). Limit of quantification, 1 μg/L, limit of detection, 0.5 μg/L [Kunicki 2001].

Oral Fluid **GC** See Serum [Bardy *et al.* 1991]

LC-MS Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.1% formic acid (5 : 95 to 80 : 20 at 10 min), flow rate 200 μL/min. API, positive ion mode, MRM acquisition mode. Retention time: 11.7 min. Limit of quantification, 0.2 μg/L [Kintz *et al.* 2005].

Hair **LC-MS** Column: Phenomenex Luna phenyl hexyl (50 × 2 mm i.d., 3 μm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate (20 : 80 for 1 min to 90 : 10 at 4 min for 2 min to 20 : 80 at 7 min for 1 min). ESI. Limit of detection, 0.06 ng/g [Irving, Dickson 2007]. Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.1% formic acid (5 : 95 to 80 : 20 at 10 min), flow rate 200 μL/min. API, positive ion mode, MRM acquisition mode. Retention time: 11.7 min. Limit of quantification, 2 ng/g [Villain *et al.* 2005].

Liver **GC** See Serum [Fraser *et al.* 1988].

HPLC See Serum [Fraser *et al.* 1988].

Nails **LC-MS** See Hair [Irving, Dickson 2007].

Disposition in the Body Readily absorbed after oral administration; peak plasma concentrations reached after 1 to 4 h. Clobazam is highly lipophilic and rapidly crosses the blood–brain barrier. It is metabolised in the liver by demethylation and hydroxylation. Metabolites found in the serum include *N*-desmethylclobazam, which is thought to be active, 4′-hydroxyclobazam and 4′-hydroxydesmethylclobazam. Approximately 90% of a dose is excreted in the urine in 17 days and approximately 2% is eliminated in the faeces.

Therapeutic Concentration

After the oral administration of 10 mg clobazam to 14 healthy volunteers a mean peak plasma concentration of 173 ± 33.3 μg/L was reached at 1.4 ± 0.6 h [Rouini *et al.* 2005].

Fifteen patients with liver disease and 6 healthy volunteers were administered a single oral dose of 20 mg clobazam. Mean peak plasma concentrations were 350 ± 63, 230 ± 70, and 240 ± 113 μg/L in healthy volunteers, patients with viral hepatitis, and patients with cirrhosis, respectively. Peak concentrations were reached at 1.7 ± 0.8, 3 ± 1.9, and 2.5 ± 1.5 h in each group, respectively [Monjanel-Mouterde *et al.* 1994].

After a single oral dose of 20 mg, administered to 16 subjects, peak serum concentrations of 0.30 to 0.95 mg/L (mean 0.57) were attained in ~1.3 h [Ochs *et al.* 1984].

After daily oral doses of 10 mg to 7 young male and 6 elderly male subjects, the mean steady-state plasma concentrations for the 2 groups were: clobazam 0.12 to 0.25 mg/L (mean 0.17) and 0.17 to 0.41 mg/L (mean 0.3); desmethylclobazam 0.12 to 0.25 mg/L (mean 0.16) and 0.21 to 12.8 mg/L (mean 3.9), respectively. The corresponding steady-state plasma concentrations in 5 young female and 6 elderly female subjects, receiving the same dosage schedule, were: clobazam 0.19 to 0.27 mg/L (mean 0.23) and 0.16 to 0.32 mg/L (mean 0.23); desmethylclobazam 0.22 to 1.37 mg/L (mean 0.7) and 0.17 to 4.13 mg/L (mean 1.0), respectively [Greenblatt *et al.* 1983b].

After the administration of 0.3 to 1.6 mg/kg/day clozabam to 16 patients (age: 4 to 47 years) plasma concentrations ranged from 20 to 197 μg/L [Riva *et al.* 1981].

A healthy 30-year-old male was administered a single oral dose of 20 mg clobazam. A peak plasma concentration of 510 μg/L was reached after 1.5 h. Desmethylclozabam reached a peak concentration of 91 μg/L 48 h after dosing [Greenblatt 1980].

Seven healthy volunteers were administered clobazam for 10 days, 10 mg in the morning and 20 mg in the evening. On day 1, the mean maximum serum concentration of 0.31 mg/L was reached after 2 h. The elimination half-life was 8 h. *N*-Desmethylclobazam was only detected after 8 h at a concentration of 0.05 mg/L. A steady-state concentration of 0.90 ± 0.23 mg/L was reached on the 4th day. *N*-Desmethylclobazam reached a steady state serum concentration of 3.6 ± 1.3 mg/L on the 9th day [Hajdú *et al.* 1980].

Toxicity Up to 300 mg has been ingested without serious toxic effects.

A 49-year-old female was found dead. The blood concentration of clobazam was 3.9 mg/L [Proença *et al.* 2004].

Postmortem concentrations for clobazam in serum and liver in a 6-year-old male were 0.1 mg/L and 0.4 μg/g. Desmethylclobazam concentrations were also reported as 1.5 mg/L in serum, 8.6 μg/g in liver and 3.6 μg/g in the brain [Fraser *et al.* 1988].

Note For a case of clobazam causing toxic epidermal necrosis, see Redondo *et al.* [1996].

Half-life Plasma half-life, clobazam 10 to 58 h (mean 25), desmethylclobazam, ~40 h. The half-life may be prolonged considerably in elderly subjects.

Volume of Distribution ≈1 L/kg [Ochs *et al.* 1984].

Clearance Plasma, ≈0.5 mL/min/kg [Ochs *et al.* 1984].

Protein Binding ≈85 to 90% at therapeutic levels (0.1 to 1.6 mg/L) [Hajdú *et al.* 1980].

Note For a review of the pharmacokinetics of clobazam, see Brogden *et al.* [1980]. For a review of the pharmacokinetics of benzodiazepines, see Greenblatt *et al.* [1983a].

Dose Adults, usually 20 to 30 mg; in severe conditions up to 60 mg daily; children (3 years and over), no more than 30 mg daily.

Akerman KK (1996). Analysis of clobazam and its active metabolite norclobazam in plasma and serum using HPLC/DAD. *Scand J Clin Lab Invest* 56: 609–614.

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Bardy AH *et al.* (1991). Monitoring of concentrations of clobazam and norclobazam in serum and saliva of children with epilepsy. *Brain Dev* 13: 174–179.

Bolner A *et al.* (2001). Optimised determination of clobazam in human plasma with extraction and high-performance liquid chromatography analysis. *J Chromatogr B Biomed Sci Appl* 750: 177–180.

Brachet-Liermain A *et al.* (1982). Liquid chromatography determination of clobazam and its major metabolite *N*-desmethylclobazam in human plasma. *Ther Drug Monit* 4: 301–305.

Brogden RN *et al.* (1980). Clobazam: a review of its pharmacological properties and therapeutic use in anxiety. *Drugs* 20: 161–178.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Drouet-Coassolo C *et al.* (1989). Capillary gas chromatographic-mass spectrometric method for the identification and quantification of some benzodiazepines and their unconjugated metabolites in plasma. *J Chromatogr* 487: 295–311.

Dusci LJ, Hackett LP (1987). Simultaneous determination of clobazam, *N*-desmethyl clobazam and clonazepam in plasma by high performance liquid chromatography. *Ther Drug Monit* 9: 113–116.

Dussy FE *et al.* (2006). Quantification of benzodiazepines in whole blood and serum. *Int J Legal Med* 120: 323–330.

Fraser AD *et al.* (1988). Tissue distribution of ethosuximide and clobazam in a seizure related fatality. *J Forensic Sci* 33: 1058–1063.

Gaillard Y *et al.* (1993). Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction. *J Chromatogr* 622: 197–208.

Gazdzik WR *et al.* (1989). HPLC method for simultaneous determination of clobazam and *N*-desmethylclobazam in human serum, rat serum and rat brain homogenates. *Biomed Chromatogr* 3: 79–81.

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Clobenzorex

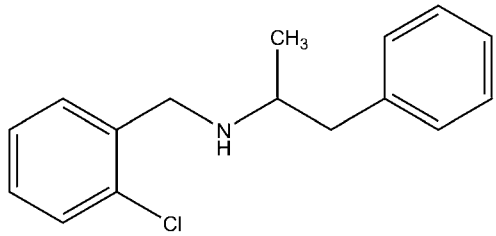
Anorectic

$C_{16}H_{18}ClN$ = 259.8

CAS—13364-32-4

IUPAC Name *N*-[(2-Chlorophenyl)methyl]-1-phenylpropan-2-amine

Synonyms (+)-*N*-(*o*-Chlorobenzyl)-α-methylphenethylamine; (+)-*N*-[(2-chlorophenyl)methyl]-α-methylbenzeneethanamine; (+)-*N*-[(2-chlorophenyl)methyl]-α-methylphenethylamine; *d*-*N*-(1-phenyl-2-propyl)-2-chlorobenzylamine.



Chemical Properties Colourless to light yellow solution. Bp 132° to 134°. Log *P* (octanol/water), 4.57 [Meylan, Howard 1995].

Clobenzorex Hydrochloride

$C_{16}H_{18}ClN$, HCl = 296.2

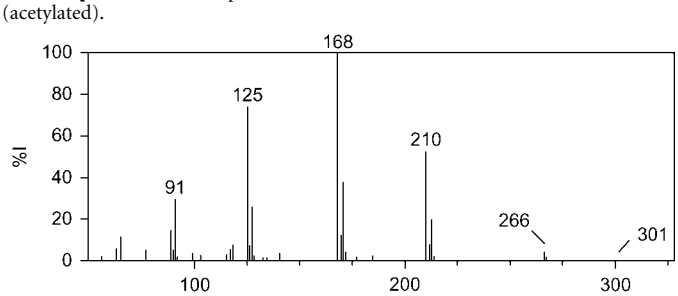
CAS—5843-53-8

Synonyms Ba-7205; SD-271-12.

Proprietary Names *Asenlix*; *Dinintel*; *Finedal*; *Itravil*; *Rexigen*.

Chemical Properties Crystals. Mp 182° to 183°. Soluble in water and ethanol; slightly soluble in methanol and chloroform.

Mass Spectrum Principal ions at *m/z* 168, 125, 210, 170, 91, 127, 212, 89 (acetylated).



Quantification

Urine **GC-MS** Column: HP-1 capillary (12 m × 0.2 mm i.d., 0.33 µm) or ZB-50 Phenomenex (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 180° at 20°/min to 250° at 28°/min with a 1 min final time. Limits of quantification and detection, 1 µg/L [Cody, Valtier 2001]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 80° for 1 min to 180° at 20°/min to 250° at 28°/min. Limit of detection, 10 µg/L for *p*-hydroxyclobenzorex; limits of quantification and detection for clobenzorex, 1 µg/L [Valtier, Cody 2000]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm) or DB-17 (30 m × 0.18 mm, 0.30 µm). Temperature programme: 80° for 1 min to 180° at 20°/min to 250° at 28°/min with 1 min final time. For DB-17, the same temperature programme only with a 2 min final time. Limit of detection, 1 µg/L [Cody, Valtier 1999]. Column: HP cross-linked methylsilicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.9 mL/min. Temperature programme: 100° to 310° at 30°/min, initial time 3 min, final time 8 min. Limit of detection, 10 µg/L [Maurer *et al.* 1997].

Other **GC-MS** Traditional Chinese Medicines. Column: HP-5MS fused silica 5% phenylmethylsilicone capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 65° for 1 min to 280° at 10°/min for 5 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [Ku *et al.* 1999].

CE Traditional Chinese medicines. Capillary (57 cm × 75 µm i.d.) Electrolyte buffer: 120 mmol/L sodium phosphate buffer (pH 2.1, NaH_2PO_4/H_3PO_4) : acetonitrile (85 : 15). UV detection (λ = 200 nm). Limit of detection, 200 µg/L [Ku *et al.* 1999].

Disposition in the Body Readily absorbed from the gastrointestinal tract. Eliminated as *p*-hydroxyclobenzorex (1.5 to 6% of administered dose) and amfetamine (15% of dose). *p*-Hydroxyclobenzorex is excreted much more rapidly than amfetamine. Other metabolites include *p*-hydroxyamfetamine, hippuric acid and conjugated clobenzorex, *p*-hydroxyclobenzorex, and *p*-hydroxyamfetamine.

Therapeutic Concentration

Five subjects were administered 30 mg clobenzorex for 7 days and individual urine samples were collected *ad hoc* for 14 days from the first day the drug was administered. Peak amfetamine urine concentrations were found at 82 to 168 h after the first dose and ranged from 2.9 to 4.7 mg/L. Peak concentrations of clobenzorex were found at 50 to 120 h after the first dose and ranged between 8 and 47 µg/L [Baden *et al.* 1999].

Note For a review of precursor compounds to amfetamine and metamfetamine, see Musshoff [2000]. For a study on the incorporation of amfetamine analogues into hair, see Nakahara and Kikura [1996].

EU regulatory authorities called for withdrawal of all anorectics from the market; still available in Mexico at the time of writing (2007).

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Clobetasol Propionate

Corticosteroid

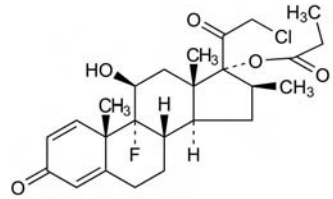
$C_{25}H_{32}ClFO_5$ = 467.0

CAS—25122-41-2 (clobetasol); 25122-46-7 (propionate)

IUPAC Name [(8*S*,9*R*,10*S*,11*S*,13*R*,14*S*,16*S*,17*R*)-13-(2-Chloroacetyl)-9-fluoro-11-hydroxy-10,16,17-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-17-yl] propanoate

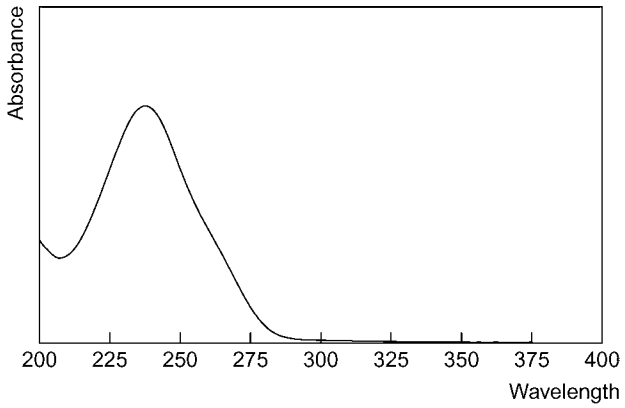
Synonym (11β,16β)-21-Chloro-9-fluoro-11,17-dihydroxy-16-methylpregna-1,4-diene-3,20-dione-17-propionate

Proprietary Names *Dermoval*; *Dermovat(e)*; *Dermoxin*.



Chemical Properties A white to creamy-white, crystalline powder. Mp 195.5° to 197°. Practically insoluble in water; soluble 1 in 100 of ethanol and 1 in 1000 of ether; soluble in acetone and chloroform. Log *P* (octanol/water), 3.5.

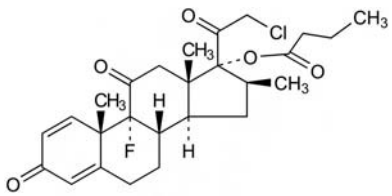
High Performance Liquid Chromatography System HY—RI 636.
Ultraviolet Spectrum Methanol—239 nm (A^1_1=390b).



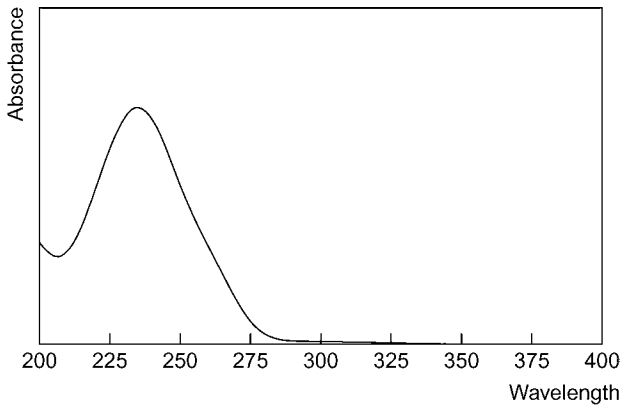
Infrared Spectrum Principal peaks at wavenumbers 1666, 1612, 1724, 884, 1063, 1010 cm^{-1}.
Use Topically in a concentration of 0.05%.

Clobetasone Butyrate

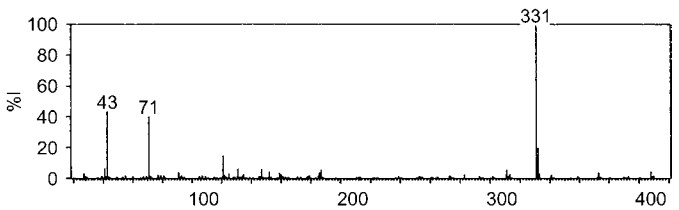
Corticosteroid
$C_{26}H_{32}ClFO_5$ = 479.0
CAS—54063-32-0 (clobetasone); 25122-57-0 (butyrate)
IUPAC Name [(8*S*,9*R*,10*S*,13*S*,14*S*,16*S*,17*R*)-17-(2-Chloroacetyl)-9-fluoro-10,13, 16-trimethyl-3,11-dioxo-7,8,12,14,15, 16-hexahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl] butanoate
Synonym (16β)-21-Chloro-9-fluoro-17-hydroxy-16-methylpregna-1,4-diene-3,11,20-trione-17-butyrate
Proprietary Names *Emovat(e)*; *Eumovate*. It is an ingredient of *Trimovate*.



Chemical Properties A white crystalline powder. Mp 90° to 100°. Practically insoluble in water; soluble in many organic solvents. Log *P* (octanol/water), 3.8.
Ultraviolet Spectrum Methanol—236 nm (A^1_1=330b).



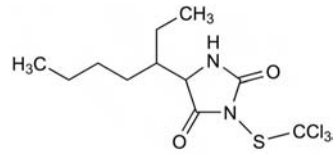
Mass Spectrum Principal ions at *m/z* 331, 43, 71, 332, 121, 147, 131, 41 (clobetasone).



Use Topically in a concentration of 0.05%.

Clodantoin

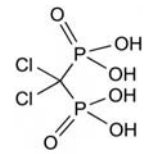
Antifungal
$C_{11}H_{17}Cl_3N_2O_2S$ = 347.7
CAS—5588-20-5
IUPAC Name 5-Heptan-3-yl-3-(trichloromethylsulfanyl)imidazolidine-2,4-dione
Synonyms Chlordantoin; 5-(1-ethylpentyl)-3-[(trichloromethyl)thio]-2,4-imidazolidinedione.
Proprietary Name *Sporostacin*



Chemical Properties Log *P* (octanol/water), 3.7.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1730, 1786, 730, 1059, 766, 1309 cm^{-1}.
Use Topically in a concentration of 1%.

Clodronic Acid

Calcium Regulator
$CH_4Cl_2O_6P_2$ = 244.9
CAS—10596-23-3
IUPAC Name [Dichloro(phosphono)methyl]phosphonic acid
Synonyms Clondronate; (dichloromethylene)bisphosphonic acid; DMDP.



Chemical Properties Mp 249° to 251°. Log *P* (octanol/water), 0.67.

Disodium Clodronate

$CH_2Cl_2Na_2O_6P_2$ = 288.9
CAS—22560-50-5
Synonyms Clodronate disodium; clodronate sodium; sodium clodronate; BM-06.011; Cl_2MDP.
Proprietary Names *Ascredar*; *Bonefos*; *Clasteon*; *Clastoban*; *Difosfonal*; *Lodronat*; *Loron*; *Lytos*; *Mebonat*; *Ostac*; *Ossiten*.

Quantification

Urine **GC-MS** Column: OV-1 (25 m × 0.31 mm i.d., 0.1 µm). Temperature: 120° to 230° at 10°/min. Injector temperature: 250°. Carrier gas: methane. MS detection (EI). Retention time: clodronate (TMS (trimethylsilyl) derivative), 7.2 min. Limit of detection, 0.025 mg/L [Auriola *et al.* 1989].

HPLC Column: anion exchange HPIC AS7 (250 × 4.0 mm i.d., 10 µm). Mobile phase: 30 mmol/L nitric acid solution, flow rate 1.0 mL/min. UV detection (λ=550 nm). k values: 7.16 (pH 2.7); 7.36 (pH 2.4); 5.82 (pH 2.1); 5.18 (pH 1.8). Limit of detection, 0.3 mg/L [Virtanen, Lajunen 1993].

Disposition in the Body Clodronate is poorly absorbed after oral administration and absorption is reduced by food, especially products containing calcium or other polyvalent cations; bioavailability is only 1 to 4% and varies between different formulations. It is not metabolised. Over 70% of an IV dose is excreted unchanged in the urine within 24 h, 5% in faeces, and the remainder is sequestered in bone tissue from where it is eliminated very slowly.

Therapeutic Concentration

Twenty patients with tumour-related bone disease of varying stages, 15 of which were women and 5 men with a mean age of 61 years (range 36 to 78), were administered with 300 mg clodronate over 2 to 3 h. The peak serum concentration was 11.8 mg/L after 2 h which decreased to 0.4 mg/L over 24 h [O'Rourke *et al.* 1994].

Six patients, 3 females and 2 males with Paget's disease and 1 male with prostatic cancer, were administered with 300 mg as a 3 h infusion daily for 5 consecutive days. A peak serum disodium clodronate concentration of 5.7 mg/L was observed in the males and 10.1 mg/L for the females [Hanhijarvi *et al.* 1989].

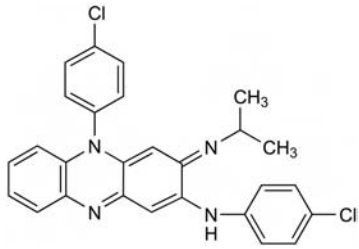
Bioavailability About 1 to 2%.
Half-life Plasma, 2 h; also reported as 5.6 h.
Volume of Distribution 0.2 to 0.7 L/kg.
Clearance Plasma, 5 to 15 L/h.
Protein Binding 36%.
Note For a review of clodronic acid, see Plosker, Goa [1994].
Dose Up to 3.20 g of disodium clodronate (in capsule form) daily orally or up to 2.08 g of disodium clodronate (in tablet form) daily. By IV infusion, 300 mg of disodium clodronate daily for not more than 10 days, or a single dose of 1.5 g infused over 4 h.

Auriola S *et al.* (1989). Analysis of (dichloromethylene) bisphosphonate in urine by capillary gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 7: 1623–1629.
Hanhijarvi H *et al.* (1989). Pharmacokinetics of disodium clodronate after daily intravenous infusions during five consecutive days. *Int J Clin Pharmacol Ther Toxicol* 27(12): 602–606.
O'Rourke N *et al.* (1994). *Drug Invest* 7: 26–33.
Plosker GL, Goa KL (1994). Clodronate. A review of its pharmacological properties and therapeutic efficacy in resorptive bone disease. *Drugs* 47: 945–982.
Virtanen V, Lajunen LHJ (1993). High-performance liquid chromatographic method for simultaneous determination of clodronate and some clodronate esters. *J Chromatogr* 617: 291–298.

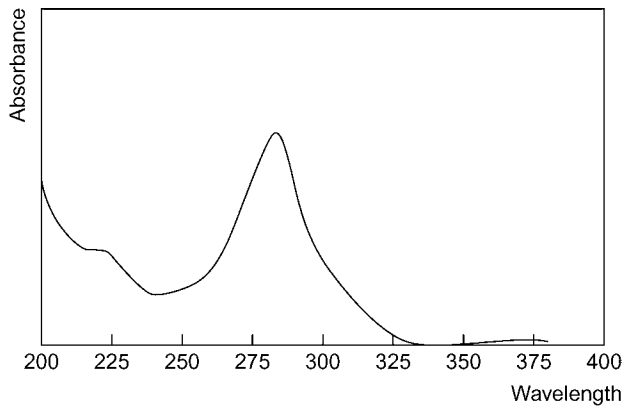
Clofazimine

Antileprotic
$C_{27}H_{22}Cl_2N_4$ = 473.4
CAS—2030-63-9

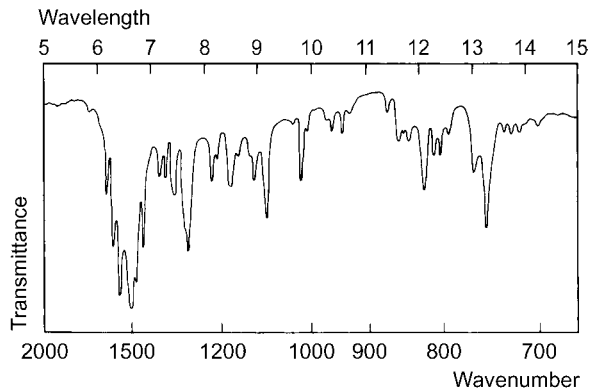
IUPAC Name *N*,5-Bis(4-chlorophenyl)-3-propan-2-yliminophenazin-2-amine
Synonyms B-663; *N*,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl) imino]-2-phenazinamine; G-30320; riminophenazine.
Proprietary Name *Lampren(e)*



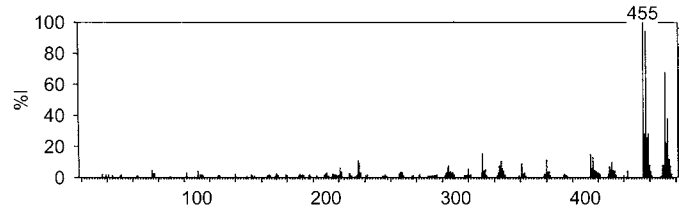
Chemical Properties Dark red crystals or orange-red microcrystalline powder. Mp 210°–212°. Practically insoluble in water; soluble 1 in 700 of ethanol, 1 in 15 of chloroform, and 1 in 1000 of ether; soluble in dilute acetic acid and dimethylformamide. pK_a 8.5. Log *P* (octanol/water), 7.7.
Colour Tests Mandelin's test—yellow-brown; Marquis test—violet; sulfuric acid—violet.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.57; system TC—R_f 0.59; system TE—R_f 0.86; system TL—R_f 0.68; system TAE—R_f 0.33; system TAF—R_f 0.86 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Methanolic acid—283 nm (A^1_1=1300b).



Infrared Spectrum Principal peaks at wavenumbers 1508, 1560, 1295, 1587, 747, 1083 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 455, 457, 472, 474, 459, 456, 458, 473.



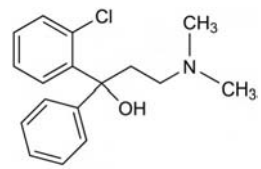
Quantification
Plasma **TLC** Limit of detection, 5 µg/L [Lanyi, Dubois 1982].
HPLC UV detection. Limit of detection, 10 µg/L [Peters *et al.* 1982].
Serum **HPLC** [Borner *et al.* 1999].
Urine **TLC** [Krishna *et al.* 1997].
LC-MS [Krishna *et al.* 1997].
Tissue **HPLC** See Serum [Borner *et al.* 1999].
Disposition in the Body Incompletely absorbed after oral administration. It is stored in body tissues and only very slowly excreted in the urine. 3-(*p*-Hydroxy) clofazimine and a 3-glucuronide conjugate have been detected in the urine, each accounting for <0.5% of a daily dose.
Therapeutic Concentration
Following single oral doses of 200 mg and 400 mg to 1 subject, peak plasma concentrations of 0.07 mg/L and 0.16 mg/L were attained in 8 and 4 h, respectively; plasma concentrations of clofazimine were still detectable at 264 h [Lanyi, Dubois 1982].
Dose 50 to 100 mg daily; 300 mg daily for lepra reactions.

Borner K *et al.* (1999). HPLC determination of clofazimine in tissues and serum of mice after intravenous administration of nanocrystalline or liposomal formulations. *Int J Antimicrob Agents* 11(1): 75–79.
Krishna DR *et al.* (1997). Characterization of clofazimine metabolites in humans by HPLC-electrospray mass spectrometry. *Arzneimittelforschung* 47: 303–306.
Lanyi Z, Dubois JP (1982). Determination of clofazimine in human plasma by thin-layer chromatography. *J Chromatogr* 232(1): 219–223.
Peters JH *et al.* (1982). Determination of clofazimine in plasma by high-performance liquid chromatography. *J Chromatogr* 229(2): 503–508.

Clofedanol

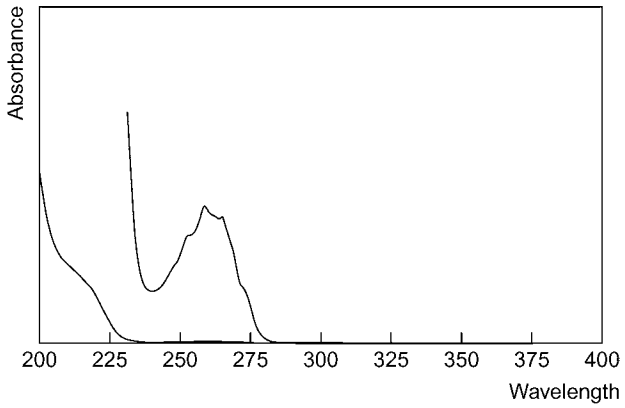
Cough Suppressant
$C_{17}H_{20}ClNO$ = 289.8
CAS—791-35-5

IUPAC Name 1-(2-Chlorophenyl)-3-(dimethylamino)-1-phenylpropan-1-ol
Synonym Clophedianol; 2-chloro-α-[2-(dimethylamino)ethyl]-α-phenylbenzenemethanol.

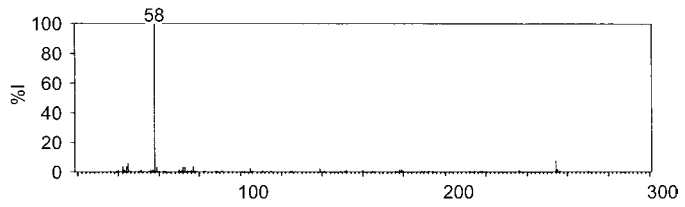


Chemical Properties Mp 120°. Log *P* (octanol/water), 3.5.

Clofedanol Hydrochloride
$C_{17}H_{20}ClNO$, HCl = 326.3
CAS—511-13-7
Proprietary Names *Detigon*; *Pectolitan*.
Chemical Properties Crystals. Mp 190° to 191°. Freely soluble in water and ethanol; sparingly soluble in ether, benzene, and ethyl acetate.
Colour Tests Mandelin's test—green-blue; Marquis test—red-violet→ brown.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.41; system TC—R_f 0.37; system TL—R_f 0.29; system TAE—R_f 0.32 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—clofedanol RI 2090, clofedanol-H_2O RI 2085, M (2-Cl-benzophenone) RI 1720, M (OH-)-H_2O RI 2130, M (aldehyde) RI 1900, M (nor-)-H_2O RI 2090.
Ultraviolet Spectrum Aqueous acid—259 (A^1_1=14.6a), 265 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 756, 700, 1035, 770, 1190, 735 cm^{-1}(KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 254, 45, 44, 77, 42, 59, 72.

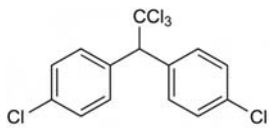


Dose 75 to 100 mg of clofedanol hydrochloride daily.

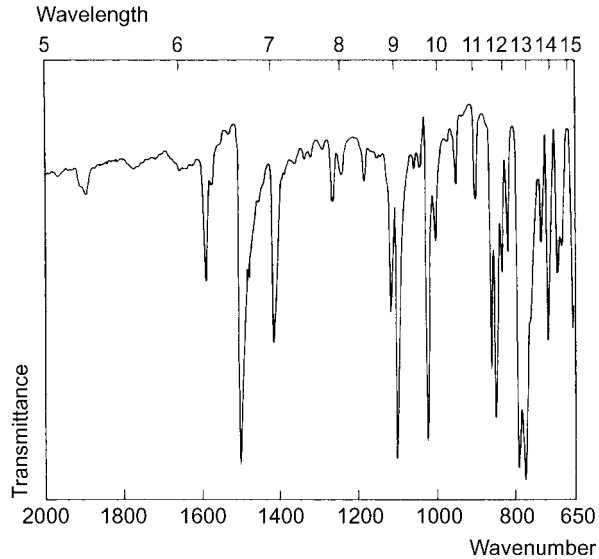
Clofenotane

Insecticide
$C_{14}H_9Cl_5$ = 354.5
CAS—50-29-3

Note Clofenotane contains about 70% of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (*pp′*-DDT) together with varying quantities of an isomer, 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (*op′*-DDT) and other related compounds.
Synonyms Chlorophenothane; chlorphenothanum; dicophane; DDT; dichlorodiphenyltrichloroethane; dichophanum; parachlorocidum; penticidum.



Chemical Properties White or nearly white crystals, small granules, flakes, or powder. Mp about 109°. The technical product is a waxy solid of indefinite melting point. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in 6 of boiling ethanol, 1 in 2.5 of acetone, 1 in 3.5 of chloroform, and 1 in 4 of ether. Log *P* (octanol/water), 6.9.
Colour Tests Liebermann's reagent—brown; nitric–sulfuric acid (Erdman's reagent—red→orange→green; heat a small quantity with a 0.5% solution of hydroquinone in sulfuric acid—red. Clofenotane does not react to the Fujiwara test.
Thin-layer Chromatography System TD—R_f 0.82; system TE—R_f 0.87; system TF—R_f 0.74; system TAD—R_f 0.80; system TAJ—R_f 0.95; system TAK—R_f 0.93; system TAL—R_f 0.97.
Gas Chromatography System GA— RI 2314 *pp′*-DDT, RI 2218 *op′*-DDT, RI 2140 *pp′*-DDE, RI 2090 *op′*-DDE; system GK— RRT 1.32 *pp′*-DDT, RRT 1.24 *op′*-DDT (both relative to caffeine).
Ultraviolet Spectrum Methanol—236 (A^1_1=510b), 267 nm (A^1_1=19b).
Infrared Spectrum Principal peaks at wavenumbers 775, 790, 1500, 1100, 1022, 850 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 235, 237, 165, 236, 75, 239, 82, 199 (*pp′*-DDT).
Quantification
Blood **GC** ECD. Clofenotane and metabolites, limit of detection, <1 mg/L [Radomski, Rey 1970].
Serum **GC** ECD. Clofenotane and metabolites [Dale *et al.* 1970].
Urine **GC** ECD. Dichlorodiphenylacetic acid (DDA), limit of detection, 50 pg [Della Fiorentina *et al.* 1978].
Tissues **GC** Limit of detection, <5 ng/g, see Blood [Radomski, Rey 1970].
Disposition in the Body Clofenotane dry powder is poorly absorbed from the gastrointestinal tract and is not absorbed through the skin. Solutions of clofenotane in oils are rapidly absorbed through intact skin and are also readily absorbed from the gastrointestinal tract. Clofenotane is metabolised to a small extent by dehydrochlorination to dichlorodiphenyldichloroethylene (DDE), which is less toxic; DDE does not appear to be metabolised further and is stored indefinitely in adipose tissues. Most of the DDE present in human fat is thought to result from preformed DDE taken in the diet rather than being due to ingestion of clofenotane. The major metabolic route for clofenotane is dechlorination to dichlorodiphenyldichloroethane (DDD) followed by degradation to dichlorodiphenylacetic acid (DDA) which is the major urinary excretion product. Urinary concentrations of DDA are indicative of storage of DDT in adipose tissue.
Blood Concentration

The following tissue concentrations of total DDT plus metabolites were reported in 35 industrially exposed subjects: blood 0.11 to 2.2 µg/mL (mean 0.6), fat 38 to 647 µg/g (mean 204), urine 0.05 to 3.4 µg/mL (mean 1.3); DDE accounted for about 40% of the material stored in fat and 6% of the urinary material compared to about 80% and 60%, respectively, reported for members of the general population [Laws *et al.* 1967].

Fat residues ranging from 0.04 to 17 µg/g (mean 2.6) of total DDT were reported in 236 subjects, without occupational exposure, in the United Kingdom in 1976–77 [Abbott *et al.* 1981].

Toxicity The estimated minimum lethal dose is 30 g but a single oral dose of 10 mg/kg may produce toxic symptoms; the maximum permissible atmospheric concentration is 1 mg/m^3 and the maximum acceptable daily intake is 5 µg/kg. The toxicity of some of the organic solvents, such as kerosene, used in the application of clofenotane has probably contributed to clofenotane fatalities.

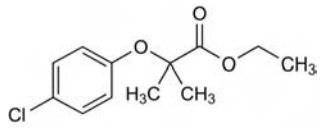
Abbott DC *et al.* (1981). Organochlorine pesticide residues in human fat in the United Kingdom 1976-7. *Brit Med J* 283: 1425–1428.
Dale WE *et al.* (1970). *J Assoc Off Anal Chem* 53: 1287–1292.
Fiorentina HD *et al.* (1978). Improved assay for the rapid determination of bis(p-chlorophenyl) acetic acid in human urine. *J Chromatogr* 157: 421–426.
Laws ER *et al.* (1967). Men with intensive occupational exposure to DDT. A clinical and chemical study. *Arch Environ Health* 15: 766–775.
Radomski JL, Rey A (1970). *J Chromatogr Sci* 8: 108–114pi .

Clofibrate

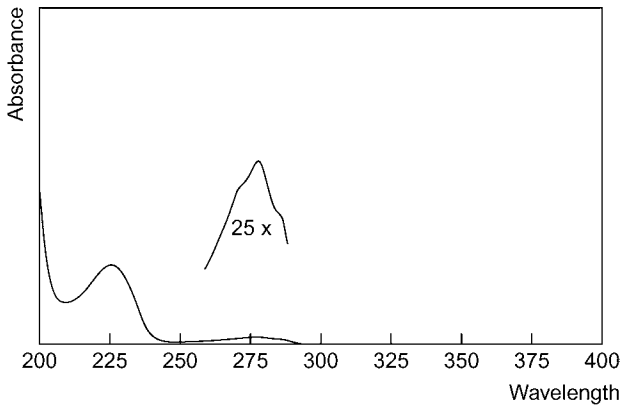
Lipid-Regulating Agent
$C_{12}H_{15}ClO_3$ = 242.7
CAS—637-07-0

IUPAC Name Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate
Synonyms 2-(4-Chlorophenoxy)-2-methylpropionoic acid ethyl ester; ethyl chlorophenoxyisobutyrate; ethyl clofibrate.

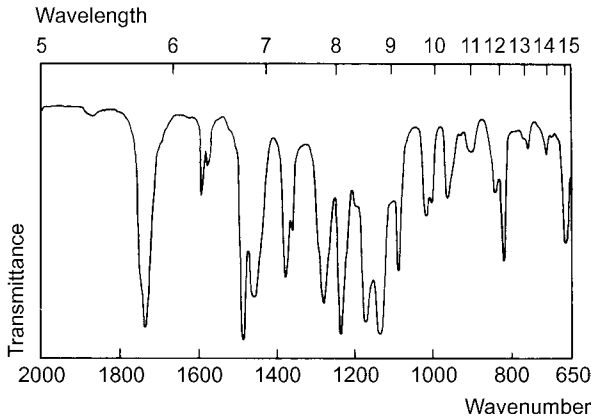
Proprietary Names *Amotril*; *Atheropront*; *Atromidin*; *Atromid-S*; *Azionyl*; *Claripex*; *Clofibral*; *Clofirem*; *Lipavlon*; *Liprin(al)*; *Normolipol*; *Novofibrate*; *Regelan*; *Skleromexe*.



Chemical Properties A clear, colourless to pale yellow liquid. Mass per mL 1.138 to 1.144 g. Practically insoluble in water; miscible with ethanol, acetone, chloroform, and ether. pK_a 3.0 (clofibric acid). Log *P* (octanol/water), 3.6.
Colour Tests Liebermann's reagent—violet-brown; Marquis test—blue (deepening).
Thin-layer Chromatography System TD—R_f 0.75; system TE—R_f 0.84; system TF—R_f 0.66; system TAD—R_f 0.71; system TAE—R_f 0.81; system TAJ—R_f 0.90; system TAK—R_f 0.80; system TAL—R_f 0.97.
Gas Chromatography System GA—clofibate RI 1542, M (clofibric acid) RI 1640.
High Performance Liquid Chromatography System HAA—retention time 18.3 min.
Ultraviolet Spectrum Dehydrated alcohol—280 (A^1_1=43b), 288 nm (A^1_1=31b).



Infrared Spectrum Principal peaks at wavenumbers 1140, 1238, 1735, 1175, 1282, 1090 cm^{-1} (KBr disk).



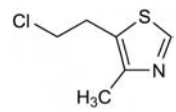
Mass Spectrum Principal ions at *m/z* 128, 130, 169, 87, 41, 129, 242, 171.
Quantification
Plasma **GC** FID. Limit of detection, 1 mg/L for clofibrate and clofibric acid [Wolf, Zimmerman 1980].

HPLC UV detection (λ=223 nm). Limit of quantification, clofibrate 3.6 and clofibric acid 4.9 mg/L, limit of detection, 1.1 mg/L and 1.5 mg/L, respectively [Barra *et al.* 1994]. UV detection. Limit of detection, 10 mg/L for clofibric acid and 1.5 mg/L for the glucuronide [Veenendaal, Meffin 1981]. UV detection. Limit of detection, 500 µg/L for clofibric acid [Bjornsson *et al.* 1977].
Urine **HPLC** See Plasma [Veenendaal, Meffin 1981; Bjornsson *et al.* 1977].
Saliva **HPLC** See Plasma [Bjornsson *et al.* 1977].
Disposition in the Body Readily and almost completely absorbed after oral administration. Rapidly hydrolysed by serum enzymes to clofibric acid, 2-(4-chlorophenoxy)-2-methylpropionic acid (active), which is conjugated with glucuronic acid. About 50 to 85% of a dose is excreted in the urine in 48 h, mostly as conjugated clofibric acid.
Therapeutic Concentration
After a single oral dose of 1 g, given to 8 subjects, peak plasma concentrations of 43 to 79 mg/L (mean 55) of clofibric acid were attained in 2 to 8 h [Chasseaud *et al.* 1974].
After a single oral dose of 2 g, given to 5 subjects, a mean plasma concentration of 151 mg/L of clofibric acid was attained in 4 to 6 h; a mean steady-state plasma concentration of 122 mg/L of clofibric acid was achieved after administering 1 g twice daily to 5 subjects [Gugler 1978].
Toxicity
A 15-year-old boy who took 24.5 g of clofibrate in a suicide attempt complained of headache, pain in the arms, and inability to walk, but no evidence of toxicity was found in the following 5 days [Greenhouse 1968].
Half-life Plasma half-life, clofibric acid 12 to 25 h but may be decreased when free fatty acid concentrations are high; longer half-lives have been reported after repeated doses, and in subjects with renal impairment.
Volume of Distribution Clofibric acid, about 0.1 to 0.2 L/kg.
Protein Binding Clofibric acid, about 95 to 98%. This may be reduced in the presence of high free fatty acid concentrations and is progressively reduced at plasma concentrations >50 mg/L.
Note For a summary of the clinical pharmacokinetics of clofibrate, see Sheiner *et al.* [1981].
Dose 20 to 30 mg/kg daily, orally.

Barra J *et al.* (1994). High-performance liquid chromatographic assay for the simultaneous determination of ethyl clofibrate and clofibric acid in plasma. Evaluation of plasma stability of ethyl clofibrate polylactic nanocapsules in human and rat plasmas. *J Chromatogr B Biomed Appl* 661 (1): 178–182.
Bjornsson TD *et al.* (1977). High-pressure liquid chromatographic analysis of drugs in biological fluids. IV. Determination of clofibrinic acid. *J Chromatogr* 137: 145–152.
Chasseaud LF *et al.* (1974). Plasma concentrations and bioavailability of clofibrate after administration to human subjects. *J Clin Pharmacol* 14: 382–386.
Greenhouse AH (1968). Attempted suicide with clofibrate. *JAMA* 204: 402–403.
Gugler R (1978). Clofibrate kinetics after single and multiple doses. *Clin Pharmacol Ther* 24: 432–438.
Sheiner LB *et al.* (1981). *J Pharmacokinet Biopharm* 9: 84–87.
Veenendaal JR, Meffin PJ (1981). The simultaneous analysis of clofibric acid and probenecid and the direct analysis of clofibric acid glucuronide by high-performance liquid chromatography. *J Chromatogr* 223(12) B Biomed. Appl.: 147–154.
Wolf MS, Zimmerman JJ (1980). Simultaneous GLC determination of clofibrate and clofibric acid in human plasma. *J Pharm Sci* 69: 92–93.

Clomethiazole

Hypnotic, Sedative
C_6H_8ClNS = 161.7
CAS—533-45-9
IUPAC Name 5-(2-Chloroethyl)-4-methylthiazole
Synonym Chlormethiazole
Proprietary Names *Distraneurin(e)*; *Hemineurin(e)*; *Heminevrin* (includes proprietary names of clomethiazole edisilate).

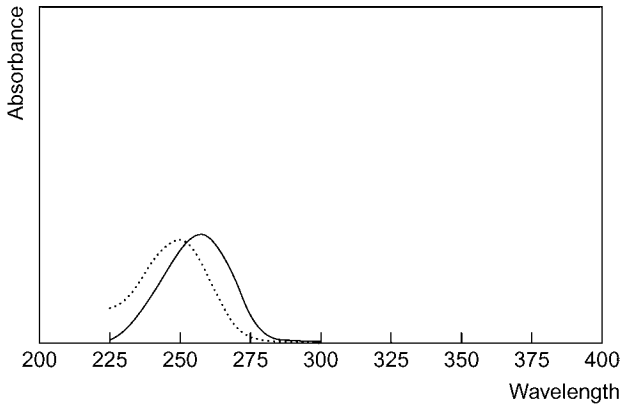


Chemical Properties A colourless to slightly yellow-brown, oily, viscous liquid. Soluble 1 in 100 of water; miscible with ethanol, chloroform, and ether. pK_a 3.2. Log *P* (octanol/water), 2.1. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

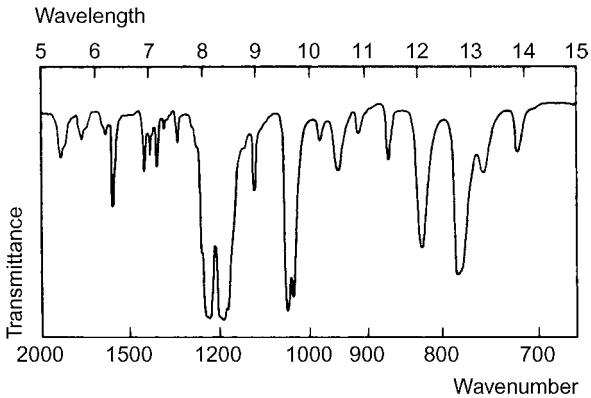
Clomethiazole Edisilate

$C_{14}H_{22}Cl_2N_2O_6S_4$ = 513.5
CAS—1867-58-9
Synonyms Chlormethiazole edisylate; chlormethiazole ethanedisulphonate.
Chemical Properties A white crystalline powder. Mp 126° to 129°. Freely soluble in water and warm ethanol; practically insoluble in ether.

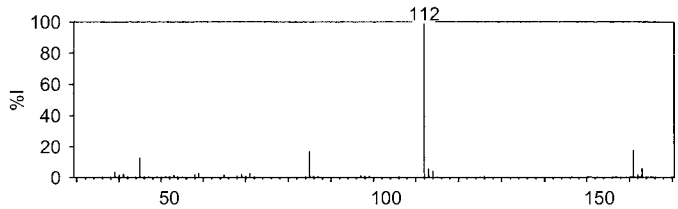
Colour Test Sodium nitroprusside (method 3)—violet.
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.44; system TC—R_f 0.69; system TE—R_f 0.76; system TL—R_f 0.58; system TAE—R_f 0.80; system TAF—R_f 0.85 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—clomethiazole RI 1227, M (1-OH-ethyl-) RI 1560, M (2-OH-) RI 1365, M (deschloro-) RI 1185, M (deschloro-2-OH-) RI 1160, M (deschloro-2-OH-ethyl-) RI 1380, M (deschloro-COOH-) RI 1235, M (deschloro-di-OH-) RI 1685; system GB—clomethiazole RI 1269.
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 395; system HY—RI 292; system HAA—retention time 16.0 min.
Ultraviolet Spectrum Aqueous acid—258 (A^1_1=288a); aqueous alkali—250 nm (A^1_1=270b).



Infrared Spectrum Principal peaks at wavenumbers 1190, 1227, 1041, 1031, 779, 823 cm^{-1} (clomethiazole edisilate, KBr disk).



Mass Spectrum Principal ions at *m/z* 112, 161, 85, 45, 163, 113, 114, 59.



Quantification

Blood **GC** AFID. Clomethiazole and two metabolites [Tsuei *et al.* 1980].

GC-MS Limit of detection, 1 µg/L [Jostell *et al.* 1978].

Plasma **GC** Limit of detection, 32 ng [Tsuei *et al.* 1980]. FID. Limit of detection, 50 µg/L for clomethiazole edisilate [Moore *et al.* 1975].

GC-MS See Blood [Jostell *et al.* 1978]. Clomethiazole and two metabolites, limit of detection, 1 µg/L for clomethiazole [Nation *et al.* 1977].

HPLC UV detection. Limit of detection, 50 µg/L [Hartley *et al.* 1987]. UV detection. Limit of detection, 200 µg/L [Hartley *et al.* 1983].

Urine **GC** See Plasma [Moore *et al.* 1975].

Disposition in the Body Rapidly absorbed after oral administration but extensively metabolised. A considerable number of metabolites have been identified in small quantities in urine. <5% of a dose is excreted unchanged in the urine, and up to about 14% is excreted as 4-methylthiazole-5-acetic acid. Other metabolites identified in the urine include 5-(1-hydroxy-2-chloroethyl)-4-methylthiazole and 5-(2-hydroxyethyl)thiazole-4-carbolactone (which appear to be more abundant than 4-methylthiazole-5-acetic acid), together with 5-acetyl-4-methylthiazole, 5-(1-hydroxyethyl)-4-methylthiazole, and 5-(2-hydroxyethyl)-4-methylthiazole in both free and conjugated forms. 5-Ethyl-2-hydroxy-4-methylthiazole has been found in the tissues following overdoses of clomethiazole. 5-(1-Hydroxyethyl)-4-methylthiazole and 5-acetyl-4-methylthiazole may be present in the plasma at concentrations similar to, or greater than, those of unchanged drug. Clomethiazole crosses the placenta and is found in breast milk.

Therapeutic Concentration In plasma, usually in the range 0.1 to 2.8 mg/L.

Following single oral doses of 192 mg to 6 subjects, peak plasma concentrations of 0.10 to 0.55 mg/L (mean 0.27) were attained in about 0.6 h [Pentikainen *et al.* 1980].

Toxicity Toxic effects are associated with plasma concentrations of 1.6 to 13 to 26 mg/L, and fatalities with concentrations of 8 to 50 to 170 mg/L.

In a report of 9 cases of fatal overdose, in which doses of about 10 to 50 g had been ingested, postmortem blood concentrations of 25 to 80 mg/L (mean 56) were found; in a further 6 fatalities due to clomethiazole and alcohol ingestion, postmortem blood concentrations ranged from 5 to 47 mg/L (mean 21) [Jakobsson, Möller 1972].

In a report of 2 cases of fatal overdose, postmortem concentrations were: blood 21 and 214 mg/L, liver blood 21 and 100 mg/L, bile 38 and 143 mg/L, urine 5 and 114 mg/L; in 3 further cases blood concentrations were reported to be 3.4, 50, and 75 mg/L [Robinson, McDowall 1979].

Note For a review of clomethiazole fatalities, see Klug, Schneider [1984].

Half-life Plasma half-life, about 3 to 7 h, increased in elderly subjects and in patients with liver disease.

Volume of Distribution About 3 to 12 L/kg (mean 8).

Clearance Plasma clearance, 10 to 25 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, about 1.2.

Protein Binding About 60 to 70%.

Note For a review of clomethiazole toxicity, see Houston *et al.* [1983].

Dose Clomethiazole as *Heminevrin (Astra)* is given in doses of 2 to 3 capsules daily. In alcohol withdrawal, an initial dose of 9 to12 capsules is given on the first day in a hospital or specialist centre, with dosage gradually reduced over the following 5 days.

Note Due to variations in bioavailability: for an equivalent therapeutic effect, one capsule (192 mg clomethiazole base ≡ 5 mL syrup (250 mg/5 mL clomethiazole edisilate) ≡ one tablet (500 mg edisilate).

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Hartley R *et al.* (1983). Determination of chlormethiazole in plasma by high-performance liquid chromatography. *J Chromatogr* 276(2): 471–477.

Hartley R *et al.* (1987). Improved high-performance liquid chromatographic procedure for the determination of chlormethiazole levels following solid-phase extraction from plasma. *J Chromatogr* 415: 357–364.

Houston A *et al.* (1983). Acute chlormethiazole poisoning in patients notified to the Poisons Unit, Guy's Hospital, 1978–1981. *Human Toxicol* 2: 361–369.

Jakobsson S, Möller M (1972). *Forensic Sci* 1114.

Jostell KG *et al.* (1978). Pharmacokinetics of clomethiazole in healthy adults. *Acta Pharmacol Toxicol (Copenh)* 43(3): 180–189.

Klug E, Schneider V (1984). [Clomethiazole poisoning]. *Z Rechtsmed* 93: 89–94.

Moore RG *et al.* (1975). Pharmacokinetics of chlormethiazole in humans. *Eur J Clin Pharmacol* 8: 353–357.

Nation RL *et al.* (1977). Plasma level of chlormethiazole and two metabolites after oral administration to young and aged human subjects. *Eur J Clin Pharmacol* 12: 137–145.

Pentikainen PJ *et al.* (1980). Pharmacokinetics of chlormethiazole in healthy volunteers and patients with cirrhosis of the liver. *Eur J Clin Pharmacol* 17: 275–284.

Robinson AE, McDowall RD (1979). Toxicological investigations of six chlormethiazole-related deaths. *Forensic Sci Int* 14: 49–55.

Tsuei SE *et al.* (1980). Simultaneous quantitation of chlormethiazole and two of its metabolites in blood and plasma by gas–liquid chromatography. *J Chromatogr* 182(1): 55–62.

Clomifene

Induction of Ovulation

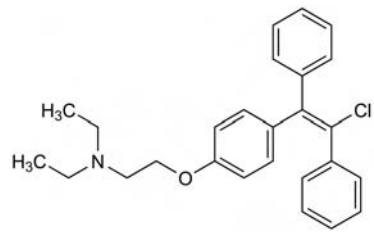
$C_{26}H_{28}ClNO$ = 406.0

CAS—911-45-5; 15690-57-0 (*E*-); 15690-55-8 (*Z*-)

IUPAC Name 2-[4-[(*Z*)-2-Chloro-1,2-diphenylethenyl]phenoxy]-*N*,*N*-diethylethanamine

Synonyms Chloramiphene; clomiphene.

Note A mixture of the *E* and *Z* isomers of 2-[4-(2-Chloro-1,2-diphenylethenyl) phenoxy]-*N*,*N*-diethylethanamine.



Chemical Properties Clomifene may be separated into its *E* and *Z* isomers, enclomifene and zuclomiphene. A white to pale yellow solid. Mp 111° to 115°. Sparingly soluble in water and ethanol; slightly soluble in chloroform. Log *P* (octanol/water), 6.7.

Clomifene Citrate

$C_{26}H_{28}ClNO$, $C_6H_8O_7$ = 598.1

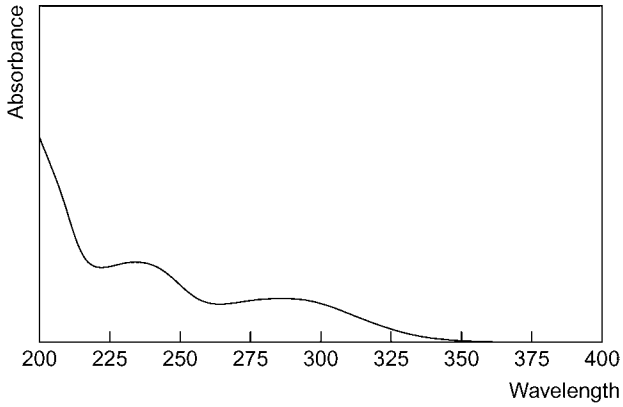
CAS—50-41-9; 7599-79-3 (*E*-); 7619-53-6 (*Z*-)

Proprietary Names *Clomid*; *Clomivid*; *Dyneric*; *Serophene*.

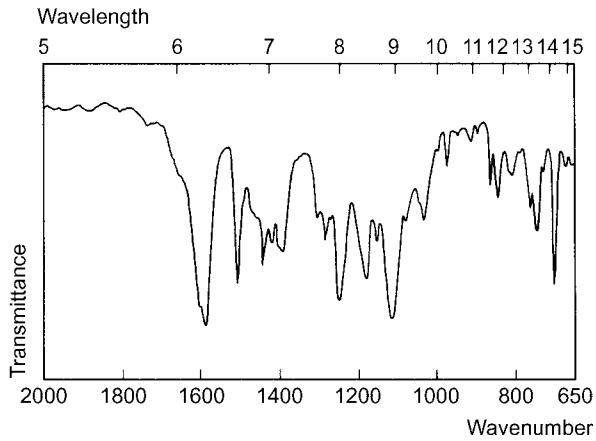
Chemical Properties A white to pale yellow powder. Mp 116.5° to 118°. Soluble 1 in 900 of water, 1 in 40 of ethanol, and 1 in 800 of chloroform; freely soluble in glacial acetic acid and methanol; insoluble in ether.

Colour Tests Liebermann's reagent—brown; Mandelin's test—violet →orange-brown; Marquis test—violet-brown.

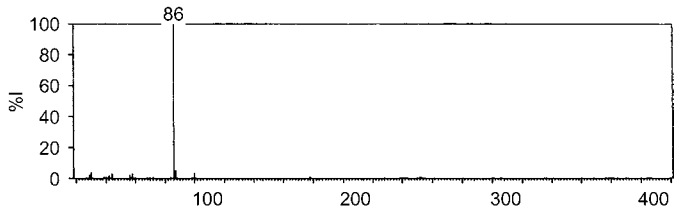
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.56; system TC—R_f 0.52; system TE—R_f 0.82; system TL—R_f 0.35; system TAE—R_f 0.30 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2930.
Ultraviolet Spectrum Aqueous acid—235 (A_1^1=464b), 292 nm (A_1^1=259b).



Infrared Spectrum Principal peaks at wavenumbers 1590, 1111, 1245, 1505, 700, 1170 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 86, 87, 100, 30, 58, 44, 42, 29.



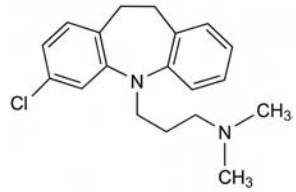
Quantification
Plasma **HPLC** Fluorescence detection (λ_{ex}=247 nm; λ_{em}=378 nm). Limit of quantification, 0.75 µg/L for *cis*-clomiphene and 1.25 µg/L for *trans*-clomiphene [Urmos *et al.* 1993]. Photolysis and fluorescence detection. Limit of detection, 350 ng/L [Harman *et al.* 1981].
Disposition in the Body Absorbed after oral administration and slowly excreted in the bile; enterohepatic circulation occurs.
Half-life Biological half-life, about 5 days.
Dose 50 mg of clomifene citrate daily, for 5 days; up to 250 mg daily has been given.

Harman PJ *et al.* (1981). High-performance liquid chromatographic determination of clomiphene using post-column on-line photolysis and fluorescence detection. *J Chromatogr* 225(1): 131–138.
Urmos I *et al.* (1993). Simple and rapid determination of clomiphene cis and trans isomers in human plasma by high-performance liquid chromatography using on-line post-column photochemical derivatization and fluorescence detection. *J Chromatogr* 617(1): 168–172.

Clomipramine

Antidepressant
$C_{19}H_{23}ClN_2$ = 314.9
CAS—303-49-1

IUPAC Name 3-(9-Chloro-5,6-dihydrobenzo[*b*][1]benzazepin-11-yl)-*N*,*N*-dimethylpropan-1-amine
Synonyms Chlorimipramine; 3-chloro-10,11-dihydro-*N*,*N*-dimethyl-5*H*-dibenz[*b*,*f*]azepine-5-propanamine; monochlorimipramine.

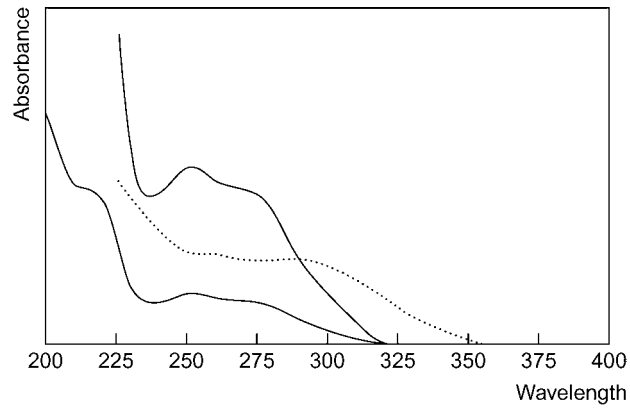


Chemical Properties Log *P* (octanol/water), 5.19 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

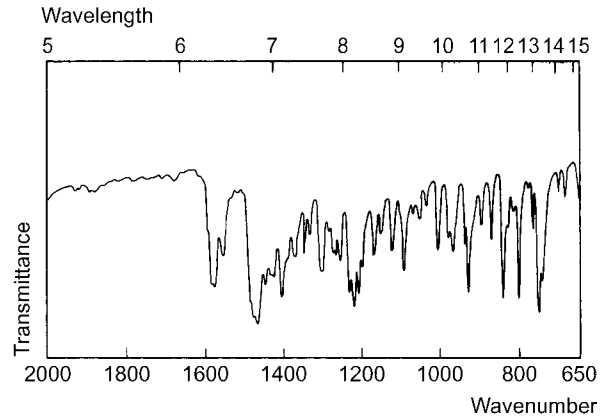
Clomipramine Hydrochloride

$C_{19}H_{23}ClN_2$, HCl = 351.3
CAS—17321-77-6
Proprietary Name *Anafranil*
Chemical Properties A white or slightly yellow crystalline powder. Mp approximately 192°. Soluble 1 in 8 of water, 1 in 5 of ethanol, 1 in 3 of chloroform and 1 in 100 of ether. Log *P* (octanol/water), 5.65 [Meylan, Howard 1995].

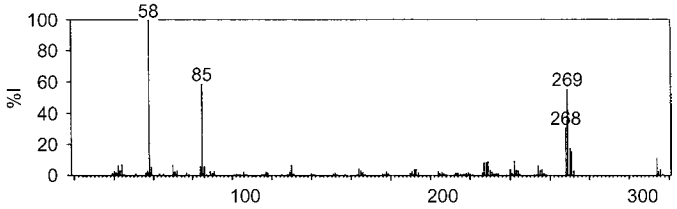
Colour Tests Forrest reagent—blue; FPN reagent—blue; Liebermann's reagent—blue; Mandelin's test—blue.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.53; system TC—R_f 0.34; system TE—R_f 0.72; system TAE—R_f 0.26; system TAF—R_f 0.54; system TAG—R_f 0.18 (acidified iodoplatinate solution, strong reaction).
Gas Chromatography System GA—clomipramine RI 2415, M (nor-) RI 2432, M (*N*-oxide) RI 2146, M (ring) RI 2230, M (8-OH-) RI 2727, M (8-OH-nor) RI 2762, M (2-OH-) RI 2569, M (10-OH-) RI 2574; system GB—clomipramine RI 2511, M (nor-) RI 2540, M (*N*-oxide) RI 2150, M (ring) RI 2335, M (8-OH-) RI 2843, M (8-OH-nor-) RI 2880, M (2-OH-) RI 2735, M (10-OH-) RI 2698; system GF—RI 2795; system GM—clomipramine RRT 1.172, M (nor-) RRT 1.374 (relative to iprindole).
High Performance Liquid Chromatography System HA—clomipramine *k* 3.4, M (monodesmethyl-) *k* 2.0; system HF—*k* 9.92; system HX—RI 462; system HY—RI 405; system HZ—RT 10.2 min; system HAA—RT 16.4 min.
Ultraviolet Spectrum Aqueous acid—251 nm (A_1^1 = 253a).



Infrared Spectrum Principal peaks at wavenumbers 752, 1225, 805, 842, 1212, 1237 cm^{-1} (clomipramine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 85, 269, 268, 270, 271, 314, 242; monodesmethylclomipramine 44, 71, 268, 227, 193, 42, 269, 229; 8-hydroxyclomipramine 58, 85, 86, 57, 84, 70, 43, 268.



Quantification

Blood **GC** Column: Chem Elut. NPD. Limit of quantification, 44–485 µg/L, limit of detection, 13–146 µg/L; Column: Bond Elut Certify. Limit of quantification, 25–223 µg/L, limit of detection, 8–67 µg/L [Martinez *et al.* 2003].

HPLC Column: silica gel. Mobile phase: ethanol : hexane : dichloromethane : diethylamine (30 : 62 : 8 : 5), flow rate 1.5 mL/min. UV detection. Limit of detection, 5 µg/L for clomipramine, 10 µg/L for monodesmethylclomipramine [Godbillon, Gauron 1981].

LC-MS Column: C_{18}. Mobile phase: acetonitrile : 0.1% trifluoroacetic acid (50 : 50), flow rate 0.4 mL/min [Pufal, Sykutera 2008]. Column: XTerra RP-18. Mobile phase: 4 mmol/L acetonitrile : ammonium formate buffer (pH 3.2). Limit of quantification, 2 µg/L [Titier *et al.* 2007]. Column: LiChroCART (125 × 3 mm i. d., 5 µm). Mobile phase: 0.1% formic acid : acetonitrile-0.1% formic acid (95 : 5, 95 : 5 for 2 min to 30 : 70 in 30 min for 2 min to 95 : 5 for 8 min), flow rate 0.4 mL/min. APCI, positive ion mode. Limit of quantification, 0.25 ng/mg, limit of detection, 0.12 ng/mg [Klys *et al.* 2005]

Plasma **GC** Column: 3% OV-17 on Chromosorb W, 100/120 (9 ft × 0.25 in o.d. (270 × 6.2 mm)). Carrier gas: 30 mL/min. Temperature: 275°. AFID. Limit of detection, 5 µg/L [Gifford *et al.* 1975].

HPLC Column: Kromasil C_8 (250 × 4 mm, 5 µm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (45 : 55), flow rate 1.5 m/min. UV detection (λ = 238 nm). Limit of detection, 0.1–0.6 ng [Samanidou *et al.* 2007]. Column: Lichrospher CN (250 × 4 mm, 5 µm) Mobile phase: 10 mmol/L dibasic potassium phosphate : acetonitrile : methanol (35 : 25 : 40), flow rate 1.5 mL/min. UV detector (λ = 214 nm). Limit of quantification, 5 µg/L [Pirola *et al.* 2002]. Mobile phase: water : acetonitrile (63 : 37) containing tetramethylethylenediamine and TEA. UV detection (λ = 254 nm). Limit of detection, 10 µg/L [Palego *et al.* 2001]. Column: Symmetry C_{18} (250 × 4.6 mm, 5 µg). DAD (λ = 200 to 450 nm) [Aymard *et al.* 1997]. Mobile phase: acetonitrile : aqueous sodium perchlorate (pH 2.5, 30 : 70). UV detection (λ = 220 nm) [Nielsen, Brosen 1993]. See also Diquet *et al.* [1993], Godbillon, Gauron [1981] and Spreux-Varoquaux *et al.* [1987].

LC-MS Column: Sunfire C_{18} IS (20 × 2.1 mm, 3.5 µm). Mobile phase: 2 mmol/L acetonitrile and ammonium formate (pH 3) [de Castro *et al.* 2008]. Limit of detection, 10 µg/L [de Castro *et al.* 2007]. Column: Inertsil C_8. Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. Limit of detection, 0.03–0.63 µg/mL [Shinozuka *et al.* 2006].

CE Capillary: Coated: alkylphosphonate-modified zirconia capillary electrochromatography. Buffer: 0.3 mol/L ammonium acetate (pH 4.5) : acetonitrile (65 : 35). Limit of detection, 11.4–51.5 µg/L [Wei *et al.* 2010].

Serum **GC** NPD. Limit of detection, 5 µg/L [Rao *et al.* 1994].

HPLC Column: Nucleosil 100-Protect 1. Mobile phase: acetonitrile : monobasic potassium phosphate buffer [Frahnert *et al.* 2003]. Column: LiChrospher CN (250 × 4.6 mm). Mobile phase: acetonitrile : 0.02 mol/L aqueous sodium perchlorate (pH 2.5, 38 : 62). UV detection (λ = 260 nm). Limit of quantification, 15 µg/L [Weigmann *et al.* 1998]. Column: silica gel. Mobile phase: methanol : water with butylamine. UV detection (λ = 254 nm). Limit of detection, 5–10 µg/L for clomipramine and metabolites [Coudoré *et al.* 1996].

LC-MS Column: Monolithic C_{18} (50 × 4.6 mm). Mobile phase: methanol : 5 mmol/L acetate buffer (pH 3.9). MRM acquisition mode [Kirchherr, Kühn-Velten 2006]. Turbulent flow chromatography [Sauvage *et al.* 2006]. Column: C_{18}. Mobile phase: acetonitrile : ammonium acetate buffer (pH 4). Limit of quantification, 1.2–54 nmol/L [Gutteck, Rentsch 2003].

Note For a fluorescence polarisation immunoassay, see Rao *et al.* [1994].

Urine **HPLC** Column: Kromasil C_8 (250 × 4 mm, 5 µm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (45 : 55), flow rate 1.5 mL/min. UV detection (λ = 238 nm). Limit of detection, 0.2–0.7 ng [Samanidou *et al.* 2007]. See Plasma [Nielsen, Brosen 1993].

LC-MS Column: reversed phase (5 µm). Mobile phase: phosphate buffer with tetraethylammonium chloride : acetonitrile (57 : 43). ECD. Limit of quantification, 0.2 µg/L [Spreux-Varoquaux *et al.* 1987].

CE See Plasma. Limit of detection, 3.7–17 µg/L [Wei *et al.* 2010].

Oral Fluid **LC-MS** See Plasma [de Castro *et al.* 2008].

Hair **LC-MS** See Blood. Limit of quantification 0.5 ng/mg, limit of detection, 0.5 ng/mg [Klys *et al.* 2005].

Note For a review of analytical methods see Scoggins *et al.* [1980].

Disposition in the Body Clomipramine is rapidly and completely absorbed after oral administration but undergoes extensive first-pass *N*-demethylation to the major active metabolite, monodesmethylclomipramine. Clomipramine and monodesmethylclomipramine are further metabolised by 8-hydroxylation, *N*-oxidation and conjugation. Approximately 10–15% of a dose is excreted in the urine in 24 h, of which <0.2% is unchanged clomipramine or monodesmethylclomipramine. A total of 45–60% of a dose is excreted in the urine over a period of 14 days and ~20–30% is slowly eliminated in the faeces.

Therapeutic Concentration Plasma concentrations of clomipramine and monodesmethylclomipramine vary considerably between individual patients. Steady-state concentrations of clomipramine are usually achieved in ~1–2 weeks, but monodesmethylclomipramine may continue to accumulate for 6 weeks or longer. 8-Hydroxyclomipramine and 8-hydroxydesmethylclomipramine have been detected in plasma at significant concentrations during high-dose chronic treatment.

Blood clomipramine concentration lower than 150 µg/L is usually associated with non-response; increasing levels to above 450 µg/L seldom improves therapeutic efficacy [Balant-Gorgia *et al.* 1991].

After a single oral dose of 50 mg, given to 5 subjects, peak plasma concentrations of clomipramine of 0.02–0.07 mg/L (mean 0.05) were attained in 2–4 h; peak plasma concentrations of monodesmethylclomipramine of 0.0005–0.012 mg/L (mean 0.005) were attained in 4–24 h [Jones, Luscombe 1976].

Steady-state plasma concentrations of 0.10–0.48 mg/L (mean 0.23) were reported after daily oral doses of 150 mg given to 17 subjects; the steady-state concentration of monodesmethylclomipramine was 0.24–0.96 mg/L (mean 0.45) [Montgomery *et al.* 1980].

After a single oral dose of 0.7 mg/kg given to 18 healthy subjects, peak plasma levels of clomipramine 43.8 ng/mL were attained in 2 h [Della Corte *et al.* 1993].

Clearance Clearance of clomipramine in Japanese patients was found to be 12.7 L/h and in Swedish patients 62.7 L/h. The difference was not accounted for by differences in body weight but was attributed to ethnic difference [Shimoda *et al.* 1999].

Toxicity Toxic effects have been associated with blood concentrations >0.4 mg/L for clomipramine plus monodesmethylclomipramine.

In 2 deaths caused by clomipramine, postmortem concentrations of clomipramine and monodesmethylclomipramine, respectively, were 1 mg/L and trace in blood, – and 4.5 µg/g in brain, 30 and 22 µg/g in liver, and 25 mg/L and – in urine [Haqqani, Gutteridge 1974].

In a fatality caused by clomipramine overdose, the following postmortem concentrations were reported for clomipramine and monodesmethylclomipramine, respectively: 0.54 and 0.58 mg/L in blood and 0.35 and 0.70 mg/L in urine [Meatherall *et al.* 1983].

In a fatality caused by clomipramine, alprazolam and ethanol, blood levels of clomipramine and desmethylclomipramine were 0.84 and 1.4 mg/L, respectively. Urine levels of clomipramine and desmethylclomipramine were 0.56 and 0.62 mg/L, respectively [Fraser *et al.* 1986]. Postmortem clomipramine concentrations ranged from 0.48–1.61 mg/L in the brain and 0.26–1.32 mg/L in the heart in this case.

A 31-year-old man suffering from mental illness died in hospital after a suicide attempt. Blood clomipramine concentration was 1.61 mg/L in heart and 0.48 mg/L in brain. Liver concentration was 3.95 mg/kg and 2.36 mg/kg in kidney. [Romano, Di Bono 1994].

In 10 cases, postmortem blood concentrations of 0.21–4.9 mg/L were observed and liver concentrations were 7.0–320.0 mg/kg [McIntyre *et al.* 1994].

A 41-year-old female suffering from depression was found dead and clomipramine concentrations were detected in blood at 3.3 mg/L and in liver at 280 mg/kg [McIntyre *et al.* 1994].

A 29-year-old man also suffering from depression showed concentrations of 1.8 mg/L and 320 mg/kg, in blood and liver, respectively [McIntyre *et al.* 1994].

In a case of fatal clomipramine toxicity, postmortem blood levels of clomipramine and norclomipramine were 9.49 and 1.10 µg/g, respectively. Levels of clomipramine and monodesmethylclomipramine in hair ranged from 1.86–7.6 ng/mg and from 4.13–9.71 ng/mg, respectively [Klys *et al.* 2005].

In a case of fatal poisoning involving clomipramine, chlorpromazine and flunitrazepam, the level of clomipramine in femoral blood was found to be 3.24 mg/L [Kinoshita *et al.* 2008].

For details of a fatality involving the ingestion of clomipramine and trimipramine, see Trimipramine.

For 5 further cases of clomipramine toxicity, including 3 fatalities, see Avella *et al.* [2004], Dale, Hole [1994], Ferrer-Dufol *et al.* [1998] and Stolk, van der Geest [1998].

Half-life Plasma half-life, 12–36 h (mean 21), considerably extended in overdose; desmethyl metabolite about 36 h; decreased in children.

Distribution in Blood Plasma : whole blood ratio, 1.2.

Volume of Distibution 12–17 L/kg.

Clearance Mean plasma clearance 73 L/h.

Protein Binding Clomipramine 90–95%; monodesmethylclomipramine 97–99%.

Note For a review of the pharmacokinetics of tricyclic antidepressants, see Molnar, Gupta [1980].

Dose Clomipramine hydrochloride, 10 to 150 mg daily. In severe depression may be increased to up to 250 mg daily or higher.

Avella J *et al.* (2004). Two cases involving clomipramine intoxication. *J Anal Toxicol* 28: 504–508.

Aymard G *et al.* (1997). Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection. *JChromatogr B Biomed Sci Appl* 700: 183–189.

Balant-Gorgia AE *et al.* (1991). Clinical pharmacokinetics of clomipramine. *Clin Pharmacokinet* 20: 447–462.
Coudoré F *et al.* (1996). Application of HPLC with silica-phase and reversed-phase eluents for the determination of clomipramine and demethylated and 8-hydroxylated metabolites. *J Anal Toxicol* 20: 101–105.
Dale O, Hole A (1994). Biphasic time-course of serum concentrations of clomipramine and desmethylclomipramine after a near-fatal overdose. *Vet Hum Toxicol* 36: 309–310.
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de Castro A *et al.* (2008). LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma: study of correlation between venlafaxine concentrations in both matrices. *J Pharm Biomed Anal* 48: 183–193.
Della Corte L *et al.* (1993). Pharmacokinetics of chlorimipramine, chlorpromazine and their *N*-dealkylated metabolites in plasma of healthy volunteers after a single oral dose of the parent compounds. *J Pharm Pharmacol* 45: 825–829.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Diquet B *et al.* (1993). Drug monitoring of clomipramine and desmethylclomipramine in depressed patients using a new liquid chromatographic assay. *Biomed Chromatogr* 7: 59–63.
Ferrer-Dufol A *et al.* (1998). Fatal serotonin syndrome caused by moclobemide–clomipramine overdose. *J Toxicol Clin Toxicol* 36: 31–32.
Frahnert C *et al.* (2003). Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. *J Chromatogr B Analyt Technol Biomed Life Sci* 794: 35–47.
Fraser AD *et al.* (1986). A fatality involving clomipramine. *J Forensic Sci* 31: 762–767.
Gifford LA *et al.* (1975). Sensitive method for the routine determination tricyclic antidepressants in plasma using a specific nitrogen detector. *J Chromatogr* 105: 107–113.
Godbillon J, Gauron S (1981). Determination of clomipramine or imipramine and their monodemethylated metabolites in human blood or plasma by high-performance liquid chromatography. *J Chromatogr* 204: 303–311.
Gutteck U, Rentsch KM (2003). Therapeutic drug monitoring of 13 antidepressant and five neuroleptic drugs in serum with liquid chromatography–electrospray ionization mass spectrometry. *Clin Chem Lab Med* 41: 1571–1579.
Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society.
Haqqani MT, Gutteridge DR (1974). Two cases of clomipramine hydrochloride (Anafranil) poisoning. *Forensic Sci* 3: 83–87.
Jones RB, Luscombe DK (1976). Single dose studies with clomipramine in normal subjects. *Postgrad Med J* 52: 62–68.
Kinoshita H *et al.* (2008). Forensic toxicological implication of an autopsy case of mixed drug overdose involving clomipramine, chlorpromazine and flunitrazepam. *Soud Lek* 53: 28–30.
Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.
Klys M *et al.* (2005). A fatal clomipramine intoxication case of a chronic alcoholic patient: application of postmortem hair analysis method of clomipramine and ethyl glucuronide using LC/APCI/MS. *Leg Med (Tokyo)* 7: 319–325.
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Palego L *et al.* (2001). Simultaneous analysis of clozapine, clomipramine and their metabolites by reversed-phase liquid chromatography. *Prog Neuropsychopharmacol Biol Psychiatry* 25: 519–533.
Pirola R *et al.* (2002). Simultaneous determination of clomipramine and its desmethyl and hydroxy metabolites in plasma of patients by high-performance liquid chromatography after solid-phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 205–210.
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Samanidou VF *et al.* (2007). Development of an HPLC method for the monitoring of tricyclic antidepressants in biofluids. *J Sep Sci* 30: 2391–2400.
Sauvage FL *et al.* (2006). A fully automated turbulent-flow liquid chromatography–tandem mass spectrometry technique for monitoring antidepressants in human serum. *Ther Drug Monit* 28: 123–130.
Scoggins BA *et al.* (1980). Measurement of tricyclic antidepressants. Part I. A review of methodology. *Clin Chem* 26: 5–17.
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Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.
Spreux-Varoquaux O *et al.* (1987). Determination of clomipramine and its hydroxylated and demethylated metabolites in plasma and urine by liquid chromatography with electrochemical detection. *J Chromatogr* 416: 311–319.
Stolk LM, van der Geest S (1998). Plasma concentrations after a clomipramine intoxication. *J Anal Toxicol* 22: 612–613.
Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.
Wei F *et al.* (2010). Combining poly(methacrylic acid–co-ethylene glycol dimethacrylate) monolith microextraction and octadecyl phosphonic acid-modified zirconia-coated CEC with field-enhanced sample injection for analysis of antidepressants in human plasma and urine. *Electrophoresis* 31: 714–723.
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Clomocycline

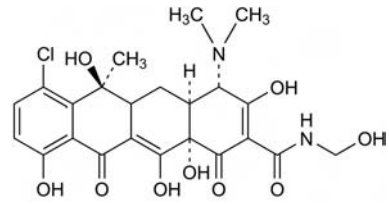
Antibacterial

$C_{23}H_{25}ClN_2O_9 = 508.9$

CAS—1181-54-0

IUPAC Name (2*Z*,4*S*,4*aS*,5*aS*,6*S*,12*aS*)-7-Chloro-4-(dimethylamino)-6,10,11,12*a*-tetrahydroxy-2-[hydroxy-(hydroxymethylamino)methylidene]-6-methyl-4,4*a*,5,5*a*-tetrahydrotetracene-1,3,12-trione

Synonyms 7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-*N*-(hydroxymethyl)-6-methyl-1,11-dioxo-2-naphthacenecarboxamide; chlormethylencycline; N^2-(hydroxymethyl)chlortetracycline; methylolchlortetracycline.



Chemical Properties A yellow powder. It is sensitive to light and air, and undergoes decomposition between 145° and 170° without a definite melting point. Log *P* (octanol/water), −1.5.

Clomocycline Sodium

$C_{23}H_{24}ClN_2NaO_9 = 530.9$

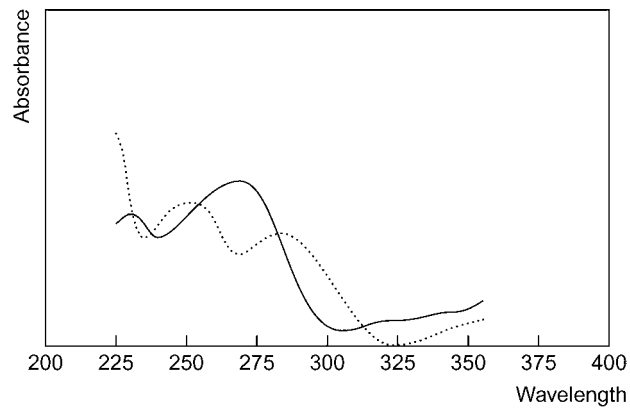
Proprietary Name *Megaclor*

Chemical Properties A yellow powder. It is sensitive to light and air, and undergoes decomposition without melting. Very soluble in water; soluble 1 in 250 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Benedict's reagent—red; formaldehyde–sulfuric acid—orange-brown; Liebermann's reagent—black; Mandelin's test—violet→brown; Marquis test—orange-brown; sulfuric acid—blue-black.

Thin-layer Chromatography System TA—R_f 0.05, streaking (acidified potassium permanganate solution, strong reaction).

Ultraviolet Spectrum Aqueous acid—229, 268 nm (A_1^1=350b); aqueous alkali—251, 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1634, 1501, 1307, 1205, 1235 cm^{-1} (clomocycline sodium, KBr disk).

Dose 0.68 to 1.36 g of clomocycline sodium daily.

Clonazepam

Anticonvulsant, Benzodiazepine

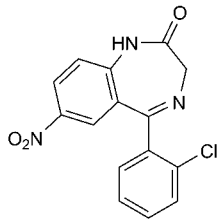
$C_{15}H_{10}ClN_3O_3 = 315.7$

CAS—1622-61-3

IUPAC Name 5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one

Synonyms 5-(2-Chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one; clonazepamum; Ro-5-4023.

Proprietary Names *Antelepsin*; *Clonapam*; *Clonex*; *Clonopin*; *Iktorivil*; *Kenoket*; *Klonopin*; *Paxam*; *Rivotril*.



Chemical Properties White to light-yellow crystalline powder. Mp 236.5° to 238.5°. Protect from light. Solubility in mg/mL at 25°: acetone 31, chloroform 15, methanol 8.6, ether 0.7, benzene 0.5, water <0.1. pK_{a1}, 1.5; pK_{a2},10.5. Log *P* (octanol/water) 2.41. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

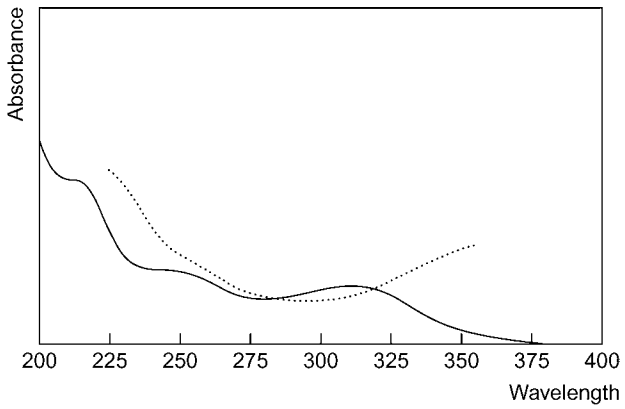
Colour Test Formaldehyde-sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.00; system TC—R_f 0.53; system TD—R_f 0.35; system TE—R_f 0.67; system TF—R_f 0.45; system TL—R_f 0.61; system TAD—R_f 0.56; system TAE—R_f 0.85; system TAF—R_f 0.87; system TAJ—R_f 0.50; system TAK—R_f 0.53; system TAL—R_f 0.91 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

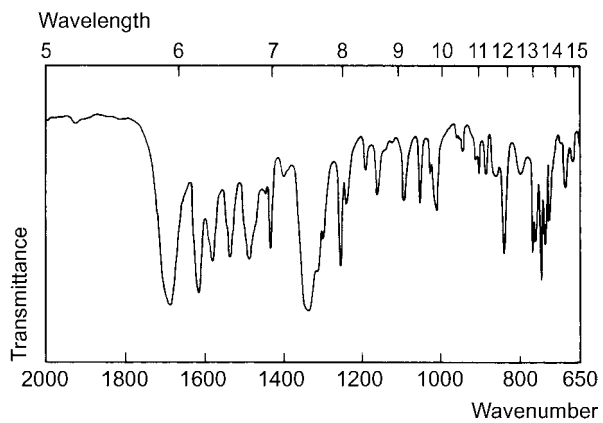
Gas Chromatography System GA—clonazepam RI 2823, M (7-amino-) RI 2890, M (amino-OH-) RI 2935; system GB—clonazepam RI 3000, clonazepam-TMS RI 2781, M (7-amino) RI 2996, M (7-amino-)-TMS_2 RI 2742; system GG—RI 3600.

High Performance Liquid Chromatography System HPLC 27—clonazepam RT 3.43 min, 7-acetamidoclonazepam RT 2.37 min; system HI—*k* 2.85; system HK—*k* 0.35; system HX—RI 465; system HY—RI 403; system HZ—RT 4.6 min; system HAA—RT 17.4 min; system HAF—clonazepam RT 5.30 min; system HAK—clonazepam RT 6.2 min; system HAL—clonazepam RT 4.3 min, 7-acetamidoclonazepam RT 1.45 min, 7-aminoclonazepam RT 1.55 min; system HAM—clonazepam RT 4.3 min; system HAX—clonazepam RT 6.5 min; system HAY—clonazepam RT 6.0 min; system HAZ—clonazepam *k* 0.79; system HBH—clonazepam *k* 2.92; system HBI—clonazepam *k* 0.79.

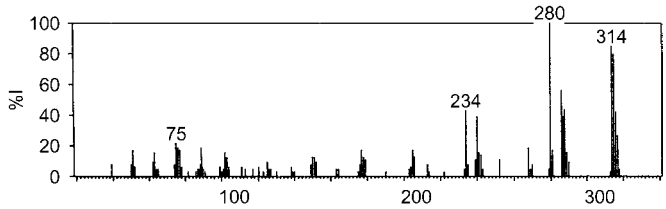
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1= 645b); methanol—245 (A_1^1= 460b), 309 nm (A_1^1= 360b).



Infrared Spectrum Principal peaks at wavenumbers 1685, 1610, 748, 1255, 1578, 1532 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 280, 314, 315, 286, 234, 288, 316, 240; 7-acetamidoclonazepam 43, 327, 299, 298, 292, 329, 328, 256; 7-aminoclonazepam 285, 256, 257, 258, 44, 287, 110, 220.



C

Quantification

Blood **HPLC** Column: LiChrospher Select B C_8 reversed phase (125 × 3.0 mm i.d., 5 µm). Mobile phase: 20 mmol/L potassium dihydrogen phosphate buffer (pH 2.1) : acetonitrile (30 : 70 to 35 : 65 at 30 min), flow rate 0.5 mL/min to 0.3 mL/min at 30 min. UV detection (λ = 254 nm). Limit of quantification, 50 µg/L, limit of detection, 15 µg/L [El Mahjoub, Staub 2001]. Column: Nova-Pak phenyl bonded (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 40 mmol/L potassium dihydrogen phosphate (pH 3.75; 28 : 72), flow rate 0.8 mL/min. UV–Vis detection (λ = 240 nm). Relative retention time: 1.99. Limit of quantification, 0.01 mg/L, limit of detection, 0.006 mg/L [Robertson, Drummer 1995].

Plasma **GC** Column: 3% OV-17 on Gas Chrom Q (1.2 m × 3 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 270°. ECD. Limit of detection, 2.5 µg/L [de Carvalho, Lanchote 1991]. Column: 3% SE-30 on 80/200 mesh Gas-Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 215°. FID and NPD. Retention time: 8 min. Limit of detection, 1 µg/L [Dhar, Kutt 1981]. Column: 3% OV-17 (10 m × 0.4 mm i.d.). Carrier gas: Ar : CH_3 (95 : 5), 10 mL/min. Temperature: 230°. ECD. Limit of detection, 1 µg/L [de Boer *et al.* 1978]. Column: OV-17 Gas Chrom Q on 80/100 mesh. ECD. Retention time: 6.9 min. Limit of detection, 0.5 µg/L [Berlin, Dahlström 1975].

GC-MS Column: HP-5 (12 m × 0.25 mm i.d., 0.33 µm). Carrier gas: He, 1 mL/min. Temperature programme: 180° to 300° at 30°/min. NCI, SIM acquisition mode. Limit of quantification, 0.25 µg/L, limit of detection, 0.1 µg/L [Song *et al.* 1996]. Column: O V-17 on Celite JJ CQ 100/120 mesh (0.9 m × 4.0 mm i.d.). Carrier gas: Ar : CH_3 (90 : 10), 80 mL/min. Temperature: 240°. EI ionisation at 70 eV. Limit of detection, 3.0 µg/L [Naestoft, Larsen 1974].

HPLC Column: Symmetry C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : phosphate buffer (1.09 g of potassium dihydrogen phosphate, pH 7.0; 50 : 50 to 70 : 30 at 15 min), flow rate 1.0 mL/min. UV detection (λ = 313 nm). Retention time: 6.20. Limit of detection, 2.0 µg/L [Le Guellac *et al.* 1998]. Column: C_{18} Velosep (100 × 3.2 mm i.d., 3 µm). Mobile phase: acetonitrile : glacial acetic acid : water (32 : 0.5 : 67.5), flow rate 0.5 mL/min. UV detection (λ = 0.5 mL/min). Retention time: 7.0 min. Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [Sallustio *et al* 1994]. Column: ODS C_{18} (150 × 4.0 mm i.d., 3 µm). Mobile phase: water : acetonitrile (1 : 1), flow rate 0.7 mL/min. UV detection (λ = 313 nm). Limit of detection, 4.0 µg/L [de Carvalho, Lanchote 1991]. Column: µBondapak Phenyl (30 cm × 4 mm i.d.). Mobile phase: acetonitrile : 45 mmol/L potassium dihydrogen phosphate buffer (pH 3.0; 40 : 60), flow rate 1.5 mL/min. UV detection (λ = 313 nm). Retention time: 6.0 min. Limit of detection, 5 µg/L [Dusci, Hackett 1987]. Column: Supelcosil LC-18 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L sodium acetate (pH 7.7; 35 : 65), flow rate 3.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 5 µg/L [Petters *et al.* 1984].

Serum **HPLC** Column: C_{18} reversed phase TSK gel Super-ODS (1, 100 × 4.6 mm i.d., 2 µm) or Hypersail ODS (2, 100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L sodium dihydrogen phosphate (pH 6.0; 45 : 55), flow rate 0.65 mL/min. UV detection (λ = 254 nm). Retention time: 5.3 and 18.2 min for columns 1 and 2, respectively. Limit of quantification, 5 µg/L [Tanaka *et al.* 1996; Tanaka *et al.* 1998]. Column: µ-Bondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : water (40 : 60), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 7.0 min. Limit of detection, 2.0 µg/L [Furuno *et al.* 1991]. Column: Supelco C_8 reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1.75 mmol/L hydrochloric acid : 50 mmol/L sodium acetate (36 : 10 : 54), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Limit of detection, 1.0 µg/L [Zilli, Nisi 1986].

Urine **GC-MS** Column; DB-17 capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 80 kPa. Temperature programme: 150° for 1 min to 230° at 10°/min for 5 min to 300° at 10°/min for 9 min. ECD. Limit of detection, 80 µg/L [Guan *et al.*]. Column: 5% phenyl methyl silicone (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.5 mL/min. Temperature programme: 240° to 260° at 25°/min to 300° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.1 min. Limit of detection, 0.1 mg/L [Black *et al.* 1994].

HPLC Column: Supelcosil C_{18} (5.0 cm × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (52 : 48), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 600 µg/L [Mullet, Pawliszyn 2002].

Hair **GC-MS** Column: HP-5MS capillary (30 m × 250 µm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 1 min to 310° at 30°/min for 3 min. NCI, SIM acquisition mode. Retention time: 10.8 min. Limit of quantification, 10 ng/g, limit of detection, 5 ng/g [Negrusz *et al.* 2000].

Disposition in the Body Rapidly absorbed after oral administration with peak plasma levels found after about 4 h. The major metabolic reaction is catalysed by CYP3A4, is reduction of the nitro group to 7-aminoclonazepam, followed by acetylation to 7-acetamidoclonazepam; 7-aminoclonazepam is slightly active and is found in plasma at concentrations similar to those of clonazepam. 3-Hydroxylation of clonazepam and its 2 metabolites may also occur, followed by conjugation with glucuronic acid or sulfate. Clonazepam crosses the placental barrier and is excreted in breast milk. Less than 1% of a dose is excreted unchanged in the urine in 24 h. Urinary excretion accounts for up to 70% of a dose, as both free and conjugated metabolites, over 7 days, of which about 50% consists of 7-amino-clonazepam and 7-acetamidoclonazepam.

Therapeutic Concentration In plasma, usually in the range 0.02–0.07 mg/L.

A single oral dose of 2 mg given to 8 subjects produced peak plasma concentrations of 0.007–0.024 mg/L (mean 0.017) in 1–4 h [Berlin, Dahlström 1975].

After daily oral dosing of 25 subjects with 6 mg per day, steady-state plasma concentrations of 0.029–0.075 mg/L of clonazepam, 0.023–0.137 mg/L of 7-aminoclonazepam, and <0.003–0.013 mg/L of 7-acetamidoclonazepam were reported [Naestoft, Larsen 1974].

In 4 children whose epilepsy was unsatisfactorily controlled with valproate therapy, a single oral dose of clonazepam 0.05 mg/kg produced peak plasma concentrations of 0.02485–0.1138 mg/L at 1–1.5 h; valproate concentrations remained within the therapeutic range and were unaffected by clonazepam [Wang, Wang 2002].

Toxicity Toxicity is associated with plasma concentrations >0.1 mg/L.

Bioavailability ≈98%.

Half-life Plasma half-life, 20–40 h.

Volume of Distribution ≈3 L/kg.

Distribution in Blood Plasma : whole blood ratio, 1.54

Clearance Plasma clearance, ≈1.5 mL/min/kg.

Protein Binding ≈86%.

Note For reviews of the pharmacokinetics of clonazepam, see Berlin and Dahlström [1976] and Pinder [1976].

Dose 4 to 8 mg daily.

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Dusci LJ, Hackett LP (1987). Simultaneous determination of clobazam, N-desmethyl clobazam and clonazepam in plasma by high performance liquid chromatography. *Ther Drug Monit* 9: 113–116.

El Mahjoub A *et al.* (2001). Determination of benzodiazepines in human hair by on-line high-performance liquid chromatography using a restricted access extraction column. *Forensic Sci Int* 123: 17–25.

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El Mahjoub A, Staub C (2001). Determination of benzodiazepines in human hair by on-line high-performance liquid chromatography using a restricted access extraction column. *Forensic Sci Int* 123: 17–25.

Furuno K *et al.* (1991). Clonazepam serum levels in epileptic patients determined simply and rapidly by high-performance liquid chromatography using a solid-phase extraction column. *Acta Med Okayama* 45: 123–127.

Guan F *et al.* (1999). Solid-phase microextraction and GC-ECD of benzophenones for detection of benzodiazepines in urine. *J Anal Toxicol* 23: 54–61.

Mullett WM, Pawliszyn J (2002). Direct determination of benzodiazepines in biological fluids by restricted-access solid-phase microextraction. *Anal Chem* 74: 1081–1087.

Naestoft J, Larsen NE (1974). Quantitative determination of clonazepam and its metabolites in human plasma by gas chromatography. *J Chromatogr* 93: 113–122.

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Petters I *et al.* (1984). Quantitation of clonazepam and its 7-amino and 7-acetamido metabolites in plasma by high-performance liquid chromatography. *J Chromatogr* 306: 241–248.

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Clonidine

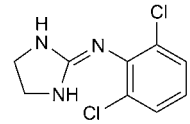
α_2 *Adrenoceptor Agonist, Antihypertensive*

$C_9H_9Cl_2N_3$ = 230.1

CAS—4205-90-7

IUPAC Name *N*-(2,6-Dichlorophenyl)-4,5-dihydro-1*H*-imidazol-2-amine

Synonyms Chlophazoline; ST-155-BS.



Chemical Properties White powder. Mp 130°. Soluble 1 in 8 of chloroform and 1 in 50 of ether. pK_a 8.3 [Baselt 2008], 8.2 [Nirogi *et al.* 2008]. Log *P* (octanol/water) 1.59. Stock solutions were stable at room temperature for 6 h and at 2 to 8° for 7 days. Clonidine was stable in plasma at room temperature for at least 4 h and for a minimum of 5 freeze-thaw cycles. Spiked plasma samples were stable for a minimum of 133 days when stored at –20°. Dry extract samples were stable for 19 h at 2 to 8° [Parekh *et al.* 2008]. No degradation was detected when plasma samples were stored at –20° for 12 days [Häring *et al.* 1988]. Stable in human plasma for 3 freeze-thaw cycles when stored below –50° and thawed at room temperature. Stable in plasma for 30 days at below –50°. Stock solutions are stable at room temperature for 6 and 22 h and at –4° for 30 days [Nirogi *et al.* 2008].

Clonidine Hydrochloride

$C_9H_9Cl_2N_3$, HCl = 266.6

CAS—4205-91-8

Synonym ST-155

Proprietary Names *Aruclonin*; *Atensina*; *Barclyd*; *Caprysin*; *Catanidin*; *Catapres (an)*; *Clonistada*; *Clonnirit*; *Dispaclonidin*; *Dixarit*; *Duraclon*; *Edolglau*; *Haemiton*; *Isoglaucon*; *Mirfat*; *Normopresan*; *Paracefan*; *Tenso-Timelets*. It is an ingredient of *Clorpres*; *Combipres*; *Combipresan* and *Dilapres*.

Chemical Properties White crystalline powder. Mp 305°. Soluble 1 in 13 of water, 1 in 25 of ethanol, 1 in 38 of dehydrated alcohol and 1 in 5000 of chloroform; practically insoluble in ether.

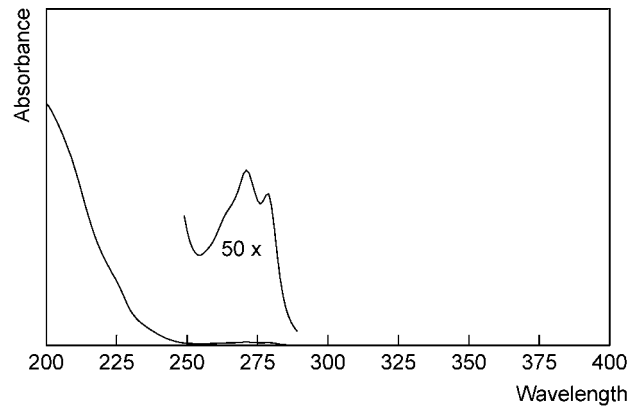
Colour Test Liebermann's reagent—yellow (→orange at 100°).

Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.08; system TC—R_f 0.31; system TE—R_f 0.70; system TL—R_f 0.53; system TAE—R_f 0.44; system TAF—R_f 0.76; system TAJ—R_f 0.09; system TAK—R_f 0.02; system TAL—R_f 0.51 (acidified iodoplatinate solution, positive).

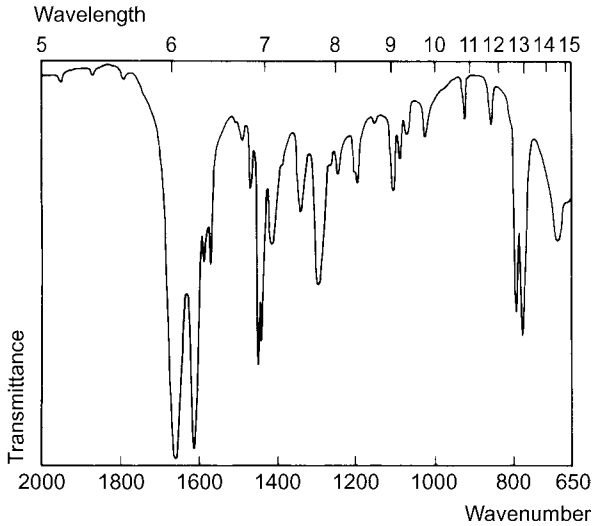
Gas Chromatography System GA—clonidine RI 2090, art (dichlorophenylisocyanate) RI 1350, art (dichlorophenylmethylcarbamate) RI 1500; system GB—clonidine RI 2165.

High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 258; system HY—RI 194; system HZ—RT 2.5 min; system HAA—RT 6.1 min; system HAX—RT 7.8 min; system HAY—RT 4.3 min.

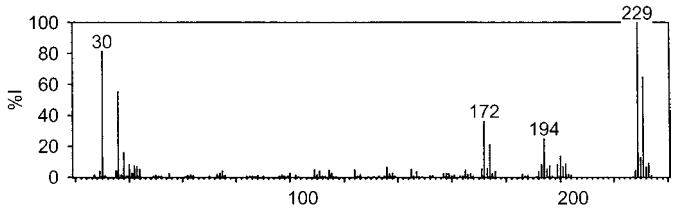
Ultraviolet Spectrum Aqueous acid—271 (A_1^1 = 21a), 278 nm.



Infrared Spectrum Principal peaks at wavenumbers 1653, 1610, 778, 795, 1295, 1568 cm^{-1} (clonidine hydrochloride) (KBr disk).



Mass Spectrum Principal ions at *m/z* 229, 30, 231, 172, 194, 174, 200, 230.



Quantification

Plasma **GC-MS** Column: Ultra-1 bonded phase fused silica (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 103 kPa. Temperature programme: 50° for 1 min to 300° at 40°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: ≈9.75 min. Limit of detection 0.025 µg/L [Yamahata *et al.* 1994]. Column: SE-54 capillary (30 m × 0.3 mm i.d., 0.05 µm). Carrier gas: He, 40 cm/s. Temperature programme: 100° for 1 min to 300° at 6°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 1 µg/L [Arrendale *et al.* 1988]. Column: CP Sil-5 fused silica capillary (25 m × 0.23 mm i.d., 0.13 µm). Carrier gas: He, 12 psi. Temperature programme: 180° to 250° at 10°/min to 300° at 20°/min. NICI. Limit of detection, 5 ng/L [Girault, Fourtillan 1988]. Column: OV-1 Oribond fused silica (25 m × 0.25 mm i.d.). Temperature programme: 60° for 1 min to 150° to 275° at 25°/min. NICI, SIM acquisition mode. Limit of detection, 10 ng/L [Häring *et al.* 1988]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (1.8 m × 2.0 mm i.d.). Carrier gas, He, 15 mL/min. Temperature: 270°. ECD, CI, positive/negative ion mode. Limit of quantification, 10 ng/L [Murray, Davies 1984]. Column: 3% OV-17 on Gas-Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 15 mL/min. CI, SIM acquisition mode. Retention time: 2.3 min. Limit of quantification, 100 ng/L [Murray *et al.* 1981]. Column: 3% OV-17 on Gas-Chrom Q 80/100 (2 mm × 3 mm i.d.). Carrier gas: Ar : CH3 (95 : 5). ECD. Limit of detection, 100 ng/L [Edlund 1980]. Column: 3% OV-17 Chromosorb 100/120 (1.8 m × 2.0 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature: 175°. ECD. Retention time: 5.0 min. Limit of detection, 25 ng/L [Chu *et al.* 1979].

LC-MS Column: Inertsil ODS-3 (50 × 3.0 mm i.d., 3 µm). Mobile phase: 5 mmol/L ammonium formate buffer (pH 2.8) : methanol (10 : 90), flow rate 0.25 mL/min. TIS, MRM acquisition mode, positive ion mode. Limit of quantification, 10 ng/L [Nirogi *et al.* 2008]. Column: Hypersil Hypurity C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L ammonium acetate : acetonitrile (20 : 80), flow rate 0.4 mL/min. TIS, positive ion mode, MRM acquisition mode. Retention time: 2.1 min. Limit of quantification, 50 ng/L [Parekh *et al.* 2008]. Column: Inertsil ODS3 or Betasil silica (30 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water : formic acid (10 : 90 : 1 to 70 : 30 : 1 at 2 min to 10 : 90 : 1 at 2.1 min (Inertsil column) or 90 : 10 : 1 to 50 : 50 : 1 at 1.0 min for 1.1 min to 90 : 10 : 1 at 2.1 min (Betasil column), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 500 ng/L [Naidong *et al.* 2002].

Serum **GC-MS** Column: DB-5 capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 70° for 1.0 min to 200° at 25°/min to 300° at 10°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 87.5 ng/L, limit of detection, 26.3 ng/L [Wenzl *et al.* 2002].

LC-MS Column: BetaBasic C4 (100 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20), flow rate 350 µL/min. ESI, CID, SRM acquisition mode. Retention time: 3.44 min. Limit of quantification, 0.1 µg/L, limit of detection, 0.01 µg/L [Müller *et al.* 2007].

Urine **GC** See Plasma [Chu *et al.* 1979].

GC-MS See Plasma [Murray *et al.* 1981].

Other **HPLC** Reservoirs for IV Drips. Column: Hibar RT LiChrospher RP-18 (12.5 × 4.0 mm i.d., 5 µm). Mobile phase: 2.2 mmol/L potassium dihydrogen phosphate and 16 mmol/L disodium hydrogen phosphate (pH 7.9) : methanol (35 : 65) with 3.5 mmol/L sodium dodecylsulfate, flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 3.9 min. Limit of detection not reported [Wulf *et al.* 1994].

Disposition in the Body Well absorbed after oral administration; peak plasma concentrations reached within 3 to 5 h. Approximately 40 to 60% of a single dose is excreted in the urine as unchanged drug in 24 h, and ~20% of a dose is eliminated in the faeces in 4 days, probably via the enterohepatic circulation. Several inactive metabolites have been detected in urine in small quantities. Clonidine is absorbed through the skin, the therapeutic concentration being achieved 2 or 3 days after application of a transdermal delivery system, and it is maintained for ≈8 h after removal. Clonidine crosses the placental barrier.

Therapeutic Concentration

In 31 cancer patients given 0.030 mg/h clonidine by continuous epidural infusion for 14 days, steady-state plasma concentrations of 0.00022 to 0.00623 mg/L (mean 0.00219) on day 8 and 0.00061 to 0.00651 mg/L (mean 0.0025) on day 15 were reported; no accumulation in the plasma appeared to occur [Boswell *et al.* 1997].

After oral administration of clonidine 0.075 mg every 12 h for 3 days to 8 subjects, a peak plasma concentration of 0.00039 mg/L was attained at 75 h after the seventh dose. In the same subjects, when clonidine was applied transdermally to the chest wall for 3 days (as a patch containing 6 mg), the peak plasma concentration was not significantly different (0.00030 at 68 h) [Fujimura *et al.* 1994].

After rectal administration of 0.0025 mg/kg clonidine to 10 infants (aged 14 to 48 month), peak plasma concentrations of 0.00062 to 0.00088 mg/L (mean 0.00077) were obtained at 29 to 70 min (mean 52) [Lonnqvist *et al.* 1994].

After the administration of a single oral dose of 300 µg clonidine peak plasma concentrations of ≈700 ng/L were reached after 3 h [Häring *et al.* 1988].

Toxicity Serious toxic effects have been reported after ingestion of doses of 0.4 to 4 mg by children and 4 to 11 mg by adults, but recovery is usually rapid. Clonidine patches have a programmed delivery of 0.1, 0.2, or 0.3 mg per day for 7 days. The patches contain 2.5, 5.0, and 7.5 mg clonidine, respectively. They can be extremely toxic or even fatal to the paediatric population [Behrman, Goertemoeller 2007].

A 5-year-old child weighing 17.5 kg ingested an overdose of 50 mg clonidine hydrochloride, the serum concentration was 0.064 mg/L 17 h later, the highest reported in a paediatric patient. After supportive treatment and administration of atropine and naloxone, the child recovered and was discharged without sequelae 42 h after admission. The overdose resulted from a pharmacy compounding error so that the child ingested 5 mL of a medication containing ~50 mg of clonidine instead of 0.05 mg/5 mL [Romano, Dinh 2001].

A 9-month-old child was admitted to hospital with a reduced level of consciousness and within a few hours developed apnoea and pinpoint pupils. The child was discharged 36 h after admission after treatment with naloxone. Further investigation led to the theory that the evening before admission, the elderly babysitter's clonidine patch had accidentally fallen into the baby's playpen where the baby subsequently sucked on it (despite the babysitter's denial of involvement); the baby's clonidine level was determined to be 0.011 mg/L but the results were not available until nearly 2 weeks after discharge [Kraft 1998].

Two females were accidentally given doses of 25 mg instead of 0.025 mg; plasma concentrations of 0.025 and 0.027 mg/L were reported on admission and at 24 h, respectively, and decreased with a half-life of 22 h; the subjects recovered with treatment [Oliver Rotellar *et al.* 1981].

A 28-year-old female ingested 30 to 70 clonidine tablets (150 µg each). She was admitted to hospital and 16 h later her plasma clonidine concentration was 4.7 µg/L. Forty hours after the overdose her plasma concentration was 2.1 µg/L [Lilja *et al.* 1984].

In a fatality due to clonidine overdose, the following postmortem concentrations were reported: blood 0.023 mg/L, brain 0.024 µg/g, kidney 0.086 µg/g [Lukkari *et al.* 1983].

Note There are several reported cases of overdose with clonidine in children and adults. For a case study of overdose after the ingestion of just 3 × 0.2 mg tablets, see Anagnos and Mazyck [1984]. For cases of iatrogenic overdose in children, see Meyer and Cambray [2008] or Suchard and Graeme [2002]. For 2 cases of overdose in children after clonidine administration by transdermal patch, see Broderick-Cantwell [1999]. For a review of the pharmacology of clonidine overdose, see Conner and Watanabe [1979] or [Seger 2002].

Bioavailability 75 to 95 %.

Half-life Plasma half-life, 10 to 25 h.

Volume of Distribution 2.1 to 5.6 L/kg

Clearance 200 to 250 mL/min [Marruecos *et al.* 1983].

Protein Binding ≈20 to 40%.

Note For a review of the pharmacokinetics of clonidine, see Lowenthal *et al.* [1988].

Dose Usually 0.2 to 1.2 mg of clonidine hydrochloride daily; doses of 1.8 mg or more daily may be given.

Anagnos DJ, Mazyck EM (1984). Clonidine overdose from minimal ingestion. *Ala Med* 53: 26–27.

Arrendale RF *et al.* (1988). Determination of clonidine in human plasma by cold on-column injection capillary gas chromatography-selected-ion monitoring-mass spectrometry. *J Chromatogr* 432: 165–175.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, California: Biomedical Publications.

Behrman A, Goertemoeller S (2007). A sticky situation: toxicity of clonidine and fentanyl transdermal patches in pediatrics. *J Emerg Nurs* 33: 290–293.

Boswell G *et al.* (1997). Plasma concentrations and disposition of clonidine following a constant 14-day epidural infusion in cancer patients. *Clin Ther* 19: 1024–1030.
Broderick-Cantwell JJ (1999). Case study: accidental clonidine patch overdose in attention-deficit/hyperactivity disorder patients. *J Am Acad Child Adolesc Psychiatry* 38: 95–98.
Chu LC *et al.* (1979). Determination of submicrogram quantities of clonidine in biological fluids. *J Pharm Sci* 68: 72–74.
Conner CS, Watanabe AS (1979). Clonidine overdose: a review. *Am J Hosp Pharm* 36: 906–911.
Edlund PO (1980). Determination of clonidine in human plasma by glass capillary gas chromatography with electron-capture detection. *J Chromatogr* 187: 161–169.
Fujimura A *et al.* (1994). Comparison of the pharmacokinetics, pharmacodynamics, and safety of oral (Catapres) and transdermal (M-5041T) clonidine in healthy subjects. *J Clin Pharmacol* 34: 260–265.
Girault J, Fourtillan JB (1988). Quantitative measurement of clonidine in human plasma by combined gas chromatography/electron capture negative ion chemical ionization mass spectrometry. *Biomed Environ Mass Spectrom* 17: 443–448.
Häring N *et al.* (1988). Gas chromatographic/mass spectrometric determination of clonidine in body fluids. Application to pharmacokinetics. *Arzneimittelforschung* 38: 404–407.
Kraft ME (1998). A 9-month-old with bradycardia and periodic apnea. *J Emerg Nurs* 24: 457–459.
Lilja M *et al.* (1984). Hypertension after clonidine overdose. A case report. *Ann Clin Res* 16: 10–12.
Lonnqvist PA *et al.* (1994). Pharmacokinetics of clonidine after rectal administration in children. *Anesthesiology* 81: 1097–1101.
Lowenthal DT *et al.* (1988). Clinical pharmacokinetics of clonidine. *Clin Pharmacokinet* 14: 287–310.
Lukkari I *et al.* (1983). Lethal clonidine poisoning. *TIAFT Bulletin* 17: 13–14.
Marruecos L *et al.* (1983). Clonidine overdose. *Crit Care Med* 11: 959–960.
Meyer C, Cambray R (2008). One hundred times the intended dose of caudal clonidine in three pediatric patients. *Paediatr Anaesth* 18: 888–890.
Müller C *et al.* (2007). Sensitive and convenient method for the quantification of clonidine in serum of pediatric patients using liquid chromatography/tandem mass spectrometry. *J Chromatogr A* 1139: 221–227.
Murray S, Davies DS (1984). Bis(trifluoromethyl)aryl derivatives for drug analysis by gas chromatography electron capture negative ion chemical ionization mass spectrometry. Application to the measurement of low levels of clonidine in plasma. *Biomed Mass Spectrom* 11: 435–440.
Murray S *et al.* (1981). The measurement of clonidine in human plasma and urine by combined gas chromatography mass spectrometry with ammonia chemical ionization. *Biomed Mass Spectrom* 8: 500–502.
Naidong W *et al.* (2002). Simultaneous development of six LC-MS-MS methods for the determination of multiple analytes in human plasma. *J Pharm Biomed Anal* 28: 1115–1126.
Nirogi R *et al.* (2008). Liquid chromatography tandem mass spectrometry method for the quantification of clonidine with LLOQ of 10 pg/mL in human plasma. *Biomed Chromatogr* 22: 992–1000.
Oliver Rotellar JA *et al.* (1981). Clonidine in thousand-fold overdose. *Lancet* 1: 1312.
Parekh SA *et al.* (2008). Rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of clonidine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 867: 172–178.
Romano MJ, Dinh A (2001). A 1000-fold overdose of clonidine caused by a compounding error in a 5-year-old child with attention-deficit/hyperactivity disorder. *Pediatrics* 108: 471–472.
Seger DL (2002). Clonidine toxicity revisited. *J Toxicol Clin Toxicol* 40: 145–155.
Suchard JR, Graeme KA (2002). Pediatric clonidine poisoning as a result of pharmacy compounding error. *Pediatr Emerg Care* 18: 295–296.
Wenzl T *et al.* (2002). Determination and quantification of clonidine in human blood serum. *J Biochem Biophys Methods* 53: 131–139.
Wulf H *et al.* (1994). The stability of mixtures of morphine hydrochloride, bupivacaine hydrochloride, and clonidine hydrochloride in portable pump reservoirs for the management of chronic pain syndromes. *J Pain Symptom Manage* 9: 308–311.
Yamahata T *et al.* (1994). Determination of clonidine in human plasma by gas chromatography-electron-impact mass spectrometry. *J Chromatogr B Biomed Appl* 653: 92–97.

Clonitazene

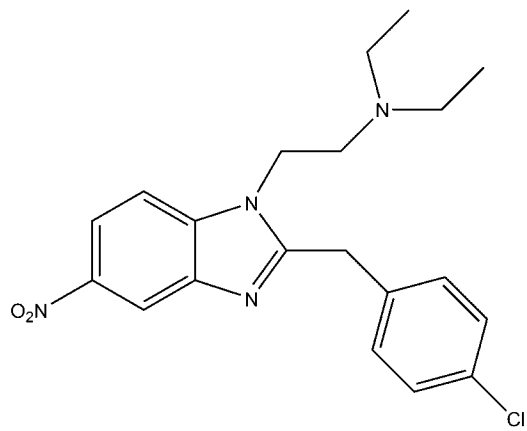
Narcotic

$C_{20}H_{23}ClN_4O_2$ = 386.9

CAS—3861-76-5

IUPAC Name 2-(*p*-Chlorobenzyl)-1-(2-diethylaminoethyl)-5-nitrobenzimidazole

Synonym Clonitazine



Chemical Properties Mp 75.5°. Log *P* (octanol/water) 5.18 [Meylan, Howard 1995].

Colour Tests Ammonium molybdate test—faint grey-blue (limit of detection, 1.0 µg); Vitali's test—faint brown/faint brown/faint brown (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.70 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—232, 285 nm.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

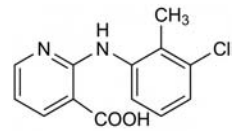
Clonixin

Analgesic

$C_{13}H_{11}ClN_2O_2$ = 262.7

CAS—17737-65-4

IUPAC Name 2-[(3-Chloro-2-methylphenyl)amino]-3-pyridinecarboxylic acid



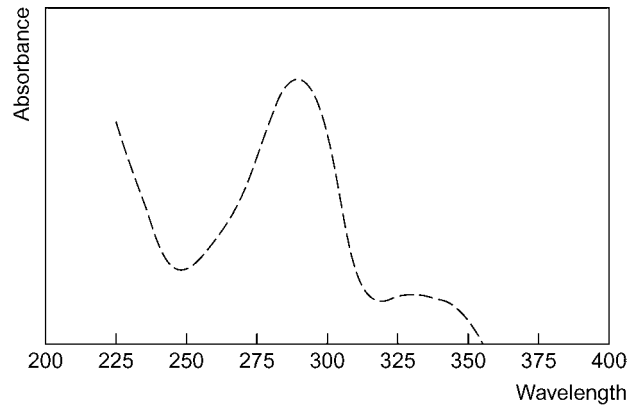
Chemical Properties A colourless or cream-coloured solid. Mp about 234°. Log *P* (octanol/water), 4.6.

Thin-layer Chromatography System TG—R_f 0.30; system TAJ—R_f 0.40; system TAK—R_f 0.14; system TAL—R_f 0.80 (Ludy Tenger reagent, orange).

Gas Chromatography System GD—Methyl derivativeRRT 1.61 (relative to n-$C_{16}H_{34}$).

High Performance Liquid Chromatography System HV—RRT 0.87 (relative to meclofenamic acid); system HY—RI 345.

Ultraviolet Spectrum Methanol—289 (A^{1}_{1}=950b), 335 nm.



Quantification

Plasma **HPLC** Limit of quantification, 10 µg/L [Bica *et al.* 2000].

Urine **HPLC** Limit of quantification, 20 µg/L [Bica *et al.* 2000].

Disposition in the Body Rapidly absorbed after oral administration. Metabolised by hydroxylation to 4′-hydroxy-, 5′-hydroxy-, and 2′-hydroxymethyl derivatives. About 60% of a dose is excreted in the urine in 24 h.

Therapeutic Concentration

Following a single oral dose of 750 mg to 12 subjects, peak serum concentrations of 29 to 66 mg/L (mean 46) were attained in about 1.7 h [Furst, Paulus 1975].

Half-life Plasma half-life, about 1.5 h.

Dose Clonixin has been given in doses of 600 mg.

Bica A *et al.* (2000). Determination of clonixin in plasma and urine by reversed-phase high-performance liquid chromatography. *J Chromatogr A* 889: 135–141.
Furst DE, Paulus HE (1975). Lack of effect of rheumatoid arthritis on clonixin metabolism. *Clin Pharmacol Ther* 17: 622–626.

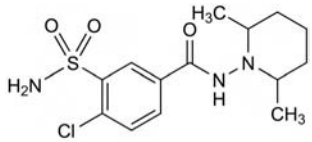
Clopamide

Antihypertensive, Diuretic

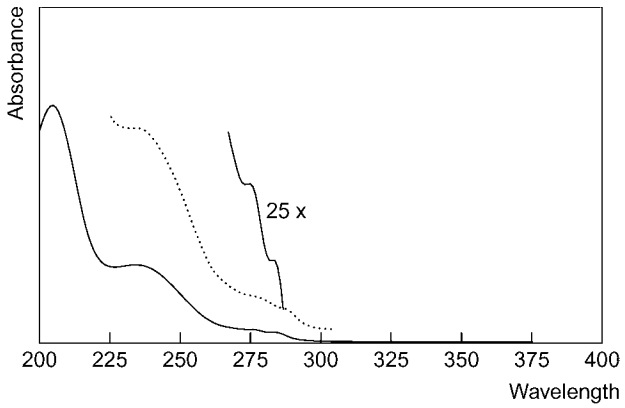
$C_{14}H_{20}ClN_3O_3S$ = 345.9

CAS—636-54-4

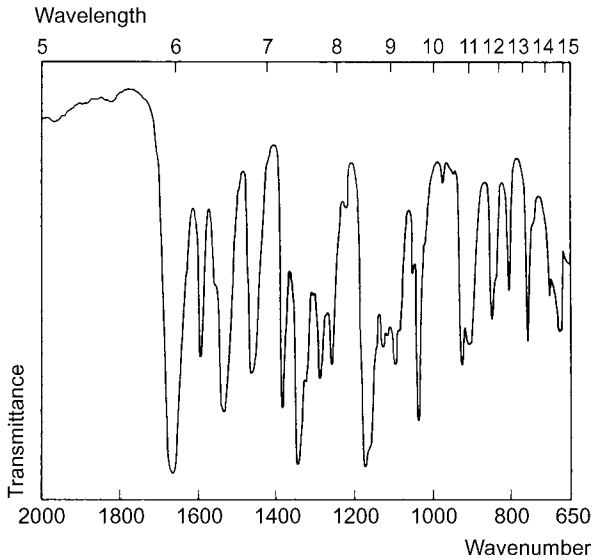
IUPAC Name 4-Chloro-*N*-[(2*R*,6*S*)-2,6-dimethylpiperidin-1-yl]-3-sulfamoylbenzamide
Synonyms *rel*-3-(Aminosulfonyl)-4-chloro-*N*-[(2*R*,6*S*)-2,6-dimethyl-1-piperidinyl]benzamide; chlosudimeprimyl; DT-327.
Proprietary Names *Brinaldix*. It is an ingredient of *Viskaldix*.



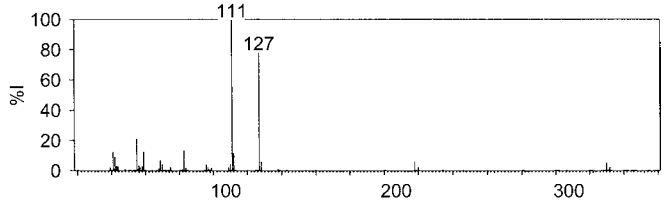
Chemical Properties A white crystalline powder. Mp about 246°. Soluble 1 in about 250 of water, 1 in about 100 of dehydrated alcohol, 1 in about 250 of chloroform, and 1 in 35 of methanol. Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 0.06 [Demme *et al.* 2005].
Colour Tests Koppanyi-Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.79; system TD—R_f 0.19; system TE—R_f 0.55; system TF—R_f 0.38; system TAD—R_f 0.39 (acidified iodoplatinate solution, strong reaction).
Gas Chromatography System GA—clopamide not eluted, clopamide-Me_2 RI 2805, clopamide-ME_3 RI 2600, clopamide-Art (-SO_2NH) RI 2195; system GX—clopamide-Me_2 retention time 7.0 min.
High Performance Liquid Chromatography System HN—*k* 4.01; system HX—RI 377; system HY—RI 310.
Ultraviolet Spectrum Aqueous acid—242 nm (A^1_1=346b); methanol—232, 277 (A^1_1=48b), 286 nm (A^1_1=36b).



Infrared Spectrum Principal peaks at wavenumbers 1663, 1172, 1038, 1532, 1285, 928 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 111, 127, 55, 83, 59, 41, 112, 42; clopamide-Me_2 *m/z* 111, 112, 127, 55, 139, 358; clopamide-Me_3 *m/z* 372, 374, 387, 373, 264, 245; clopamide-Art (-SO_2NH) *m/z* 111, 127, 139, 83, 96, 251.



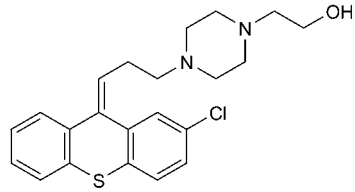
Quantification
Plasma **GLC-MS** Limit of detection, 10 mg/L [Stuber *et al.* 1989].
Disposition in the Body
Therapeutic Concentration
Eight healthy volunteers were administered 5 mg clopamide and a mean peak plasma concentration of 197 mg/L was reached after 1.1 h [Stuber *et al.* 1989].
Dose 20 to 60 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Stuber W *et al.* (1989). Gas-liquid chromatography-mass spectroscopy determination of clopamide in plasma. *J Pharm Sci* 78(8): 679–682.

Clopenthixol

Thioxanthene, Dopamine Antagonist, Tranquilliser
$C_{22}H_{25}ClN_2OS$ = 401.0
CAS—982-24-1

IUPAC Name 2-[4-[3-(2-Chlorothioxanthen-9-ylidene)propyl]piperazin-1-yl] ethanol
Synonyms 4-[3-(2-Chloro-9*H*-thioxanthen-9-ylidene)propyl]-1-piperazineethanol; cloperphenthixan; zuclopenthixol.



Chemical Properties Colourless syrup. Sparingly soluble in ether. Readily soluble in methanol. Log *P* (octanol/water) 4.2. Extraction yield (chlorobutane), 1 [Demme *et al* 2005].

Clopenthixol Decanoate

$C_{32}H_{43}ClN_2O_2S$ = 555.2
Proprietary Name *Clopixol* (injection)
Chemical Properties Yellowish oily liquid. Practically insoluble in water; soluble in ethanol, chloroform, and ether.

Clopenthixol Hydrochloride

$C_{22}H_{25}ClN_2OS$, 2HCl = 473.9
CAS—633-59-0
Proprietary Names *Ciatyl*; *Clopixol* (tablets); *Sordinol*.
Chemical Properties Crystals. Mp ≈257°, with decomposition. Freely soluble in water, sparingly soluble in ethanol.

Colour Tests Formaldehyde-sulfuric acid—red (orange fluorescence under UV light); Liebermann's reagent—red; sulfuric acid—orange (fluoresces under UV light).
Thin-layer Chromatography System TA—R_f 0.56; system TAE—R_f 0.45; system TL—R_f 0.11; system TB—R_f 0.07; system TC—R_f 0.32; system TE—R_f 0.44 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—Clopenthixol *cis*-isomer (zuclopenthixol) RI 3400, clopenthixol-AC RI 3460, M (*N*-oxide)-$C_6H_{14}N_2O_2$- RI 2410, M (desalkyl-)-AC RI 3490, M (desalkyl-dihydro-)-AC RI 3450, Art-(Cl-thioxanthenone) RI 2260.
High Performance Liquid Chromatography System HX—RI 448; system HY—RI 411.

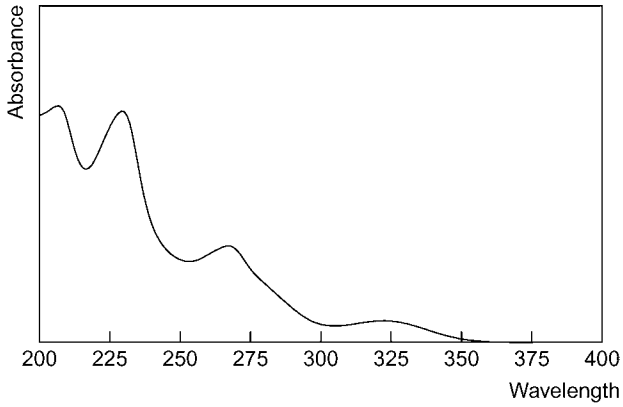
Column: Kromasil Si 100 (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : sodium dihydrogen phosphate buffer (pH 3.5; 30 : 70 for calixarene phases, 35 : 65 for resorcinarene phase), flow rate 1.0 mL/min. UV detection (λ = 225 nm). *k'*: 3.6-14.46 for calixarene phases, 5.41 for resorcinarene phase [Sokoließ *et al.* 2002].

Column: LiChrosorb RP-18 ODS silica (120 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : 0.2 mol/L potassium phosphate buffer (pH 4.0; 60 : 35 : 5) with 2.5 mmol/L DTMA bromide and 5 mmol/L sodium decanesulfonate, flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: ≈24 and 17 min for *cis*- and *trans*-clopenthixol, respectively [Helboe 1990].

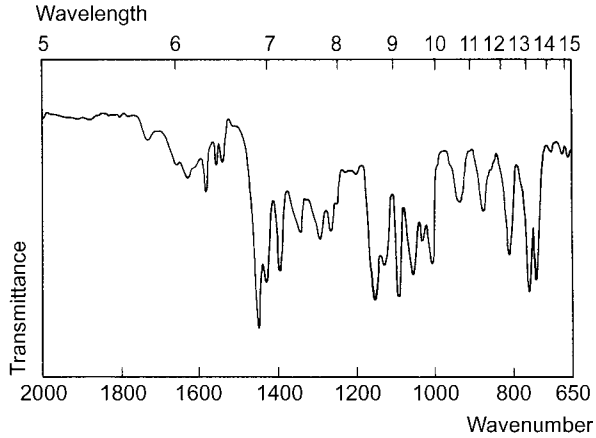
Column: Spherisorb (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: ethyl acetate : methanol : 3% ammonia (85 : 15 : 1), flow rate 1 mL/min. UV detection (λ = 260 nm). Retention time: 8.02 and 8.89 min for *cis*- and *trans*-clopenthixol, respectively. Limit of detection, 1 mg/L [Li Wan Po *et al.* 1979].

C

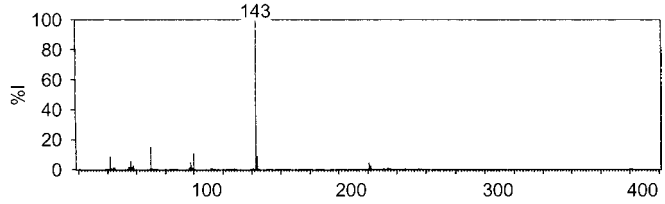
Ultraviolet Spectrum Aqueous acid—231 ($A_1^1 = 891$a), 269, 325 nm.



Infrared Spectrum Principal peaks at wavenumbers 1150, 1090, 757, 740, 1052, 1123 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 143, 70, 100, 144, 42, 56, 98, 221.



Quantification

Blood **GC-MS** Column: BPX5 capillary (15 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 30 mL/min. Temperature programme: 70° for 1 min to 220° at 15°/min to 260° at 5°/min to 330° at 25°/min. EI ionisation at 70 eV, MSD. Retention time: 22.26 and 22.43 min for the *trans*(*E*)- and *cis*(*Z*)-isomers, respectively [Rop 2001].

HPLC Column: Symmetry (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.1 mol/L ammonium acetate (30 : 30 : 40), flow rate 1.0 mL/min. DAD (λ = 230 nm). Limit of quantification, 40 and 45 µg/L for the *trans*(*E*)- and *cis*(*Z*)-isomers, respectively [Rop 2001]. Column: NovaPak C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : 10 mmol/L potassium dihydrogen phosphate (65 : 5 : 30), flow rate 0.80 mL/min. DAD (λ = 228 nm). Retention time: 8.98 min for *cis*(*Z*)-clopenthixol, 10.21 min for *trans*(*E*)-clopenthixol. Limit of detection, 7 µg/L for both isomers [Tracqui *et al.* 1997].

Column: Symmetry C_{18} (150 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile: 0.1% formic acid (10 : 90 to 90 : 10 in 10 min to 10 : 90 in 1 min), flow rate 0.5 mL/min. ESI, full scan mode. Limit of detection, 1 µg/L [Kollroser *et al.* 2001].

Plasma **HPLC** Column: Microsorb MV C reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 25 mmol/L phosphate buffer (pH 2.2; 35 : 65), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 1 µg/L, limit of detection, 0.3 µg/L [Pucci *et al.* 2003]. Column: Phenomenex Luna C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 40 mmol/L phosphate buffer (pH 7.5) : acetonitrile (52 : 48). UV detection (λ = 258 nm). Retention time: 7.12 min. Limit of detection, 1.0 nmol/L [Jaanson *et al.* 2002]. Column: Spherisorb S5 CN (120 × m i.d.). Mobile phase: acetonitrile : 0.2 mol/L potassium phosphate (pH 6.5) : water (36 : 5 : 59) containing dodecyl-*N,N,N*-trimethylammonium bromide, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 260 nm; λ_{em} = 435 nm). Limit of detection, 0.05 µg/L, 0.2 µg/L for metabolite [Hansen, Hansen 1994].

Serum **HPLC** Column: Spherisorb S5CN (150 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate buffer : methanol (1 : 9), flow rate 0.6 mL/min. UV detection (λ = 256 nm). Limit of quantification, 2 µg/L [Angelo, Petersen 2001]. Column: Spherisorb S 5 W (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: *n*-heptane : 2-propanol : concentrated ammonia : water (85 : 15 : 0.4 : 0.2), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.5 µg/L for clopenthixol and 2.5 µg/L for des-(2-hydroxyethyl)clopenthixol [Aaes-Jørgensen 1980].

LC-MS Column: Nucleosil C_{18} (300 × 4.0 mm i.d.). Mobile phase: methanol : 0.2% ammonia (75 : 25), flow rate 0.6 mL/min. EI/CI. Retention time: 8.38 min. Limit of detection, 1.0 µg/L [Tas *et al.* 1986]

Urine **HPLC** See Plasma [Hansen, Hansen 1994]. See Blood [Rop 2001]. See Blood [Tracqui *et al.* 1997].

Gastric Contents **HPLC** See Blood [Rop 2001]. See Blood [Tracqui *et al.* 1997].

Vitreous Humour **HPLC** See Blood [Tracqui *et al.* 1997].

Brain **HPLC** See Blood [Tracqui *et al.* 1997].

Kidney **HPLC** See Blood [Tracqui *et al.* 1997].

Liver **HPLC** See Blood [Tracqui *et al.* 1997].

Lung **HPLC** See Blood [Tracqui *et al.* 1997].

Skeletal Muscle **HPLC** See Blood [Tracqui *et al.* 1997].

Disposition in the Body Clopenthixol decanoate is slowly absorbed after injection to give peak serum concentrations of clopenthixol and the des-(2-hydroxyethyl) metabolite after approximately 3 to 7 days. Clopenthixol, the des-(2-hydroxyethyl) metabolite, and their sulfoxide metabolites have been detected in urine together with small amounts of clopenthixol glucuronide [Khan 1969]. A significant part is played by CYP2D6 in the systemic elimination of clopenthixol [Jaanson *et al.* 2002].

Therapeutic Concentration

After the administration of a single IM dose of 50 mg clopenthixol to 19 acutely disturbed psychotic Asian patients, serum concentrations were 19.9 ± 2.8, 31.5 ± 4.5 and 17.8 ± 2.9 µg/L at 24, 48, and 72 h, respectively [Tan *et al.* 1993].

Nine healthy male volunteers were administered a 30 mg dose of clopenthixol (*cis*(*Z*)/*trans*(*E*)-clopenthixol 1/2) followed by a 10 mg dose of *cis*(*Z*)-clopenthixol after at least a week. The serum concentration curves of *cis*(*Z*)-clopenthixol were equal after both doses [Aaes-Jørgensen 1981].

A mean minimum steady-state serum concentration of 0.004 mg/L of clopenthixol was reported in 24 subjects, who had been receiving average doses of 200 mg IM every 4 weeks, for 5 to 6 months [Dencker *et al.* 1980].

After an oral dose of 30 mg of clopenthixol to 1 subject, a peak serum concentration of about 0.005 mg/L of *cis*-clopenthixol was attained in about 3 h; the peak concentration of the inactive *trans*-isomer was slightly higher and was attained after 4 h; the serum concentrations declined slowly and could be measured after 48 h; only traces of the des-(2-hydroxyethyl) metabolite were detected [Aaes-Jørgensen 1980].

Toxicity

A 39-year-old female with bipolar affective disorder was found dead in her room. Clopenthixol concentrations in her peripheral and heart blood were 0.60 and 0.68 mg/L, respectively [Kollroser *et al.* 2001].

A 26-year-old male with mental illness and drug addiction was found dead. The total clopenthixol in peripheral blood was 0.455 mg/L, 0.7 mg/L in cardiac blood, 31.56 mg/L in gastric contents and 0.735 mg/L in urine. The *cis*(Z)-isomer of the drug was found at a concentration of 0.455, 29.88 and 0.505 mg/L in cardiac blood, gastric contents and urine, respectively. *Trans*(E)-isomer were found at concentrations of 0.245, 1.68 and 0.23 mg/L, respectively [Rop 2001].

A 16-year-old female was found dead. The *cis*(Z)-clopenthixol concentrations were 0.391, 0.957 and 105.4 mg/L, 0.75, 1.413, 5.21 and 0.12 ng/g in blood, urine, gastric contents, liver, kidney, lung and brain, respectively. The *trans*(E)-clopenthixol concentrations were 0.275, 0.832 and 7.430 mg/L, and 0.837, 2.380, 9.758 and 0.058 ng/g, respectively [Tracqui *et al.* 1997].

A patient took 200 to 300 mg bromazepam, 2.5 g of clopenthixol and an unspecified amount of reserpine in an attempted suicide. Serum samples taken on the following 7 consecutive days showed that the clopenthixol concentrations decreased from 0.9 to 0.04 mg/L [Tas *et al.* 1986].

Note For a case of a 14-year-old female with neuroleptic malignant syndrome following clopethixol intake, see Erermis *et al.* [2007].

Volume of Distribution 15 to 20 L/kg [Tracqui *et al.* 1997].

Dose The equivalent of 20 to 150 mg of clopenthixol daily; up to 250 mg daily has been given.

Aaes-Jørgensen T (1980). Specific high-performance liquid chromatographic method for estimation of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol and a *N*-dealkyl metabolite. *J Chromatogr* 183: 239–245.

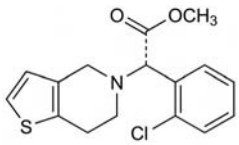
Aaes-Jørgensen T (1981). Serum concentrations of *cis*(*Z*)- and *trans*(*E*)-clopenthixol after administration of *cis*(*Z*)-clopenthixol and clopenthixol to human volunteers. *Acta Psychiatr Scand Suppl* 294: 64–69.

Angelo HR, Petersen A (2001). Therapeutic drug monitoring of haloperidol, perphenazine, and zuclopenthixol in serum by a fully automated sequential solid phase extraction followed by high-performance liquid chromatography. *Ther Drug Monit* 23: 157–162.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Dencker SJ *et al.* (1980). Clopenthixol and flupenthixol depot preparations in outpatient schizophrenics. Serum levels and clinical outcome. *Acta Psychiatr Scand Suppl* 279: 55–63.
Erermis S *et al.* (2007). Zuclopenthixol-induced neuroleptic malignant syndrome in an adolescent girl. *Clin Toxicol (Phila)* 45: 277–280.
Hansen BB, Hansen SH (1994). Determination of zuclopenthixol and its main *N*-dealkylated metabolite in biological fluids using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection. *J Chromatogr B Biomed Appl* 658: 319–325.
Helboe P (1990). Controlling the retention of clopenthixol and other basic drug substances by reversed-phase ion-pair chromatography on bonded-phase materials using two counter-ions of opposite charge. *J Chromatogr* 523: 217–225.
Jaanson P *et al.* (2002). Maintenance therapy with zuclopenthixol decanoate: associations between plasma concentrations, neurological side effects and CYP2D6 genotype. *Psychopharmacology (Berl)* 162: 67–73.
Khan AR (1969). Some aspects of clopenthixol metabolism in rats and humans. *Acta Pharmacol Toxicol(Copenh)* 27: 202–212.
Kollroser M *et al.* (2001). HPLC-ESI-MS/MS determination of zuclopenthixol in a fatal intoxication during psychiatric therapy. *Forensic Sci Int* 123: 243–247.
Li Wan Po A, Irwin WJ (1979). A high performance liquid chromatographic assay of *cis*- and *trans*-isomers of tricyclic neuroleptic drugs. *J Pharm Pharmacol* 31: 512–516.
Pucci V *et al.* (2003). Liquid chromatographic analysis of the *cis*(*Z*)- and *trans*(*E*)-isomers of clopenthixol in human plasma using a novel solid phase extraction procedure. *J Chromatogr B Analyt Technol Biomed Life Sci* 792: 313–321.
Rop PP (2001). Concentrations of *cis*(*Z*)-clopenthixol and *trans*(*E*)-clopenthixol in a lethal case involving zuclopenthixol, diazepam, and cyamemazine. *J Anal Toxicol* 25: 348–352.
Sokoließ T *et al.* (2002). Separation of *cis*- and *trans*-isomers of thioxanthene and dibenz[*b*,*e*]oxepin derivatives on calixarene- and resorcinarene-bonded high-performance liquid chromatography stationary phases. *J Chromatogr A* 948: 309–319.
Tan CH *et al.* (1993). Clinical evaluation and serum concentration of zuclopenthixol acetate in psychotic Asian patients: a single-dose preliminary study. *Ther Drug Monit* 15: 108–112.
Tas AC *et al.* (1986). LC/MS determination of bromazepam, clopenthixol, and reserpine in serum of a non-fatal case of intoxication. *J Anal Toxicol* 10: 46–48.
Tracqui A *et al.* (1997). HPLC-DAD and HPLC-MS findings in fatality involving (*Z*)-*cis*-clopenthixol (zuclopenthixol). *J Anal Toxicol* 21: 314–318.

Clopidogrel

Antiplatelet
$C_{16}H_{16}ClNO_2S$ = 321.8
CAS—113665-84-2; 94188-84-8

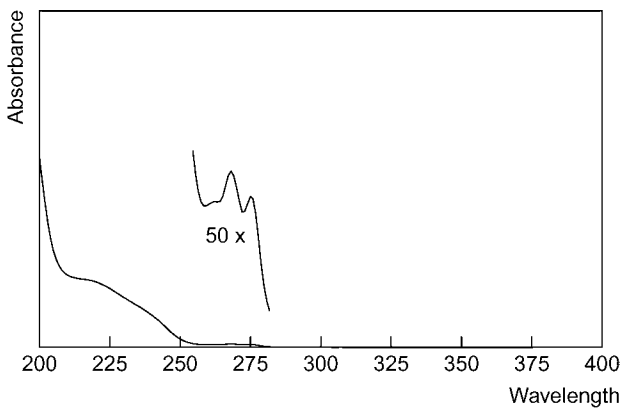
IUPAC Name Methyl (2*S*)-2-(2-chlorophenyl)-2-(6,7-dihydro-4*H*-thieno[3, 2-*c*]pyridin-5-yl)acetate
Synonyms (α*S*)-α-(2-Chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5-(4*H*)-acetic acid methyl ester; SR-25990.



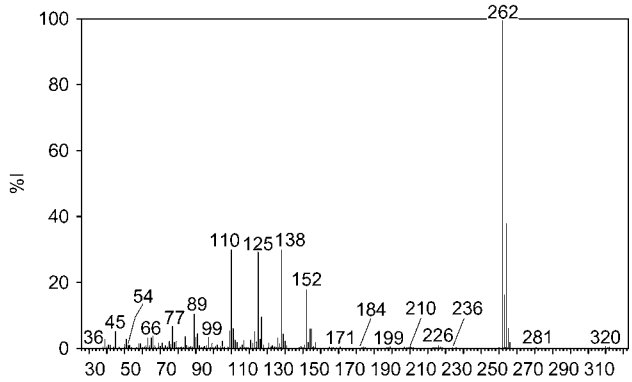
Clopidogrel Bisulfate

$C_{16}H_{16}ClNO_2S$, H_2SO_4 = 419.9
CAS—120202-66-6
Synonyms Clopidogrel bisulphate; clopidogrel hydrogen sulphate; SR-25990C
Proprietary Name *Plavix*
Chemical Properties A white to off-white powder. Mp 184°. It is practically insoluble in water at neutral pH; freely soluble in water at pH 1, and in methanol; sparingly soluble in methylene chloride; practically insoluble in ethyl ether.

Ultraviolet Spectrum Aqueous acid—270, 278, 308 nm.



Mass Spectrum Principal ions at *m/z* 262, 264, 110, 138, 125, 152, 89, 77 (clopidogrel bisulfate).



Quantification

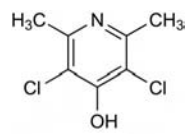
Plasma **GC-MS** Column: DB1 (30 m × 0.25 mm i.d., 0.1 µm). Temperature programme: 120° then up to 290° at 30°/min, held for 3 min. Injector temperature: 290°. Carrier gas: He, flow rate 40 cm/s. MS detection (CI (chemical ionisation), FS (full scan) mode). Retention time: clopidogrel (carboxylic acid derivative), 6.5 min. Limit of quantification, 5 µg/L [Lagorce *et al.* 1998].
Serum **GC-MS** See Plasma [Lagorce *et al.* 1998].
Disposition in the Body After oral administration, clopidogrel is rapidly, but incompletely (~50%), absorbed. It is extensively metabolised in the liver to produce two metabolites, the main circulating metabolite (85%) being the inactive carboxylic acid derivative. Clopidogrel is oxidised to 2-oxo-clopidogrel with further hydrolysis to produce the active thiol derivative, which has not yet been identified in plasma. Clopidogrel and its metabolites are equally excreted in urine and faeces.
Therapeutic Concentration
Twelve healthy males were administered with single oral doses of 50, 75, 100 and 150 mg. Plasma concentrations of the parent drug were very low, less than the quantification limit (0.00025 mg/L) and peak concentrations of the inactive metabolite reached ~1.6, 2.9, 3.1 mg/L, respectively, within 0.8 to 1 h. Steady state values (between 0.8 and 0.11 mg/L) were reached by the eighth day (75 mg/day) [Caplain *et al.* 1999].
Toxicity A single case of deliberate overdose, during clinical trials, has been reported. A 34-year-old woman swallowed a single dose of 1050 mg (equivalent to 14 standard 75 mg tablets) clopidogrel with no associated undesirable effects. No special therapy was needed.
Half-life The elimination half-life of the inactive metabolite is ~8 h.
Clearance 10 mg/L/h.
Protein Binding Clopidogrel and its inactive metabolite bind plasma proteins 98% and 94%, respectively.
Dose A single oral daily dose of 75 mg.

Caplain H *et al.* (1999). Pharmacokinetics of clopidogrel. *Semin Thromb Hemost* 25: 25–28.
Lagorce P *et al.* (1998). Assay method for the carboxylic acid metabolite of clopidogrel in human plasma by gas chromatography-mass spectrometry. *J Chromatogr B, Biomed Sci Appl* 720: 107–117.

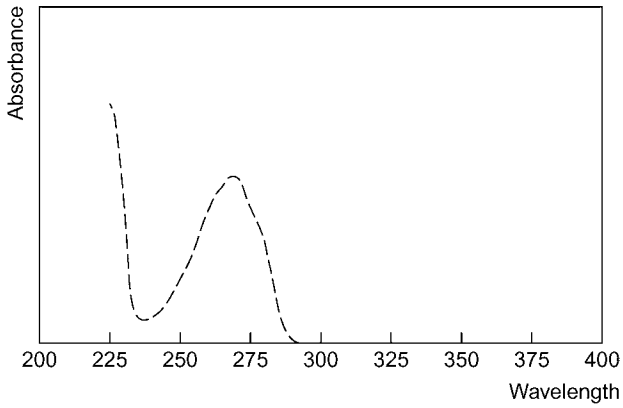
Clopidol

Coccidiostat (Veterinary)
$C_7H_7Cl_2NO$ = 192.0
CAS—2971-90-6

IUPAC Name 3,5-Dichloro-2,6-dimethyl-4-pyridinol
Synonyms Clopindol; meticlorpindol.
Proprietary Name *Coyden*



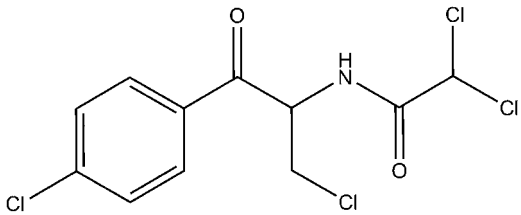
Chemical Properties A white crystalline powder. Mp 320°. Practically insoluble in water; slightly soluble in ethanol; soluble in methanol. Log *P* (octanol/water), 2.7.
Ultraviolet Spectrum Ethanol—269 nm (A_1^1=460b).



Infrared Spectrum Principal peaks at wavenumbers 1538, 1504, 753, 1618, 762, 1095 cm^{-1} (KBr disk).

Cloponone

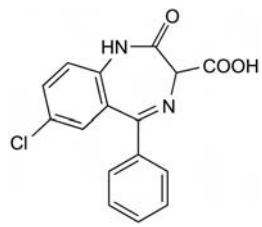
Antibacterial, Antimycotic
$C_{11}H_9C_{l4}NO_2 = 329.0$
CAS—15301-50-5
IUPAC Name 2,2-Dichloro-*N*-[3-chloro-1-(4-chlorophenyl)-1-oxopropan-2-yl]acetamide
Synonyms β-*p*-Dichloro-α-dichloroacetamidopropiophenone; K 374.
Proprietary Names It is an ingredient of *Ginetris* and *Golaval*.



Chemical Properties A white crystalline powder. Slightly soluble in ethanol; soluble in methanol and chloroform. Cloponone is extracted by chloroform from aqueous acid solutions.
Thin-layer Chromatography System T1—R_f 0.76 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.55 relative to diphenhydramine; retention time 0.35 relative to codeine.
Ultraviolet Spectrum 0.1 N sulfuric acid—261 nm (E1%, 1cm 466); minimum at 232 nm.
Infrared Spectrum Principal peaks at wavenumbers 1667, 1689, 1536 cm^{-1}.

Clorazepic Acid

Tranquilliser
$C_{16}H_{11}ClN_2O_3 = 314.7$
CAS—23887-31-2
IUPAC Name 7-Chloro-2,3-dihydro-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-carboxylic acid



Chemical Properties pK_a 3.5, 12.5. Log *P* (octanol/water), 2.05.

Clorazepate Dipotassium

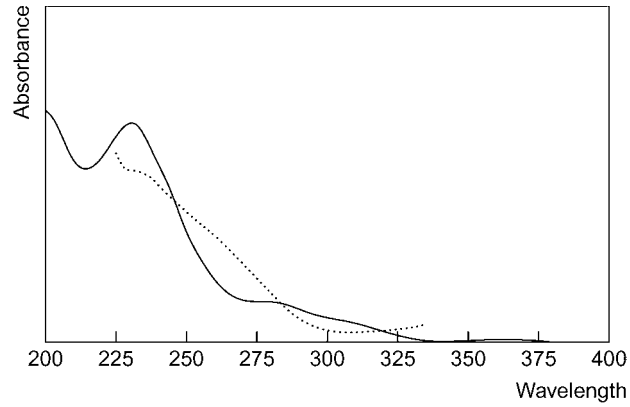
$C_{16}H_{10}ClKN_2O_3$, KOH = 408.9
CAS—57109-90-7
Synonyms Abbott 35616; AH-3232; CB-4306; clorazepate dipotassium; potassium clorazepate.
Proprietary Names *Belseren*; *Clorazecaps*; *Clorazetabs*; *Dorken*; *Gen-Xene*; *Medipax*; *Mendon*; *Nansius*; *Novo-Clopate*; *Transene*; *Tranxen(e)*; *Tranxilen(e)*; *Tranxilium*. It is an ingredient of *Dorken* and *Noctran*.

Chemical Properties A fine off-white powder. Freely soluble in water, very poorly soluble in ethanol. Practically insoluble in ether and chloroform. In aqueous solution, it converts to the monopotassium salt.

Clorazepate Monopotassium

$C_{16}H_{10}ClKN_2O_3 = 352.8$
CAS—5991-71-9
Synonym CB-4311
Proprietary Name *Azene*
Chemical Properties A fine, off-white powder. Very soluble in water; practically insoluble in organic solvents.

Colour Test Formaldehyde–sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.84; system TB—R_f 0.03; system TC—R_f 0.56; system TD—R_f 0.34; system TE—R_f 0.68; system TF—R_f 0.46; system TL—R_f 0.60; system TAD—R_f 0.57; system TAE—R_f 0.83; system TAF—R_f 0.87.
Gas Chromatography System GA—clorazepic acid RI 2457, nordazepam RI 2490, oxazepam RI 2325; system GB—clorazepic acid RI 2618, nordazepam RI 2625, oxazepam RI 2438; system GG—clorazepic acid RI 3125, nordazepam RI 3041, oxazepam RI 2803.
High Performance Liquid Chromatography System HI—*k* 1.17; system HK—*k* 2.00; system HX—RI 475; system HY—RI 388; system HZ—retention time 5.6 min; system HAA—clorazepate retention time 18.4 min; system HBH—*k* 8.22; system HBI—*k* 1.84.
Ultraviolet Spectrum Dipotassium clorazepate: aqueous acid—237 (A^1_1=747b), 287 nm.



Infrared Spectrum Principal peaks at wavenumbers 1597, 1548, 1300, 702, 1230, 830 cm^{-1} (dipotassium clorazepate) (KBr disk).
Mass Spectrum Principal ions at *m/z* 242, 43, 270, 269, 241, 103, 243, 76.
Quantification
Blood **GC** ECD. Clorazepic acid and desmethyldiazepam. Limit of detection, 4 µg/L [Brooks *et al.* 1977].
Plasma **HPLC** UV detection. Clorazepate dipotassium and desmethyldiazepam [Colin *et al.* 1983].
Urine **GC** ECD. Clorazepic acid and desmethyldiazepam. Limit of detection, 5 µg/L for clorazepic acid and desmethyldiazepam, 25 µg/L for oxazepam [Brooks *et al.* 1977].
Disposition in the Body Rapidly decarboxylated below pH 4 to the active metabolite desmethyldiazepam (nordazepam), and thus is absorbed mainly as desmethyldiazepam after oral administration; this is then hydroxylated to form oxazepam which is conjugated with glucuronic acid. Up to about 10% of a dose is excreted in urine in 24 h, mainly as oxazepam glucuronide, together with small amounts of unchanged clorazepate and conjugated desmethyldiazepam. Oxazepam glucuronide is still detectable in urine 12 days after a single dose. Small amounts of clorazepic acid and its metabolites are excreted in breast milk.
Therapeutic Concentration
Following a single oral dose of 15 mg to 4 subjects, peak blood concentrations of desmethyldiazepam of 0.14 to 0.18 mg/L (mean 0.16) were attained in 0.5 to 1 h; peak blood-clorazepate concentrations of 0.03 to 0.11 mg/L (mean 0.06) were reported at about 1 h [Abruzzo *et al.* 1977].
Average steady-state serum concentrations of 0.40 to 0.61 mg/L (mean 0.48) of desmethyldiazepam were reported in 7 patients receiving daily oral doses of 0.6 mg/kg [Wilensky *et al.* 1978].
Toxicity Fatalities from overdosage are rare and recovery from an overdose of 600 mg has been reported.
Half-life Plasma half-life, clorazepate about 2 h, desmethyldiazepam about 40 to 100 h but there is considerable intersubject variation—see under Nordazepam.
Volume of Distribution Desmethyldiazepam 0.5 to 2.5 L/ kg, increased in elderly subjects.
Clearance Plasma clearance, desmethyldiazepam about 0.1 to 0.3 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, desmethyldiazepam 1.7.
Protein Binding Desmethyldiazepam about 91%.
Dose 7.5 to 22.5 mg of dipotassium clorazepate daily; up to 60 mg daily has been given.

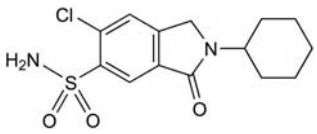
Abruzzo CW *et al.* (1977). Changes in the oral absorption characteristics in man of dipotassium clorazepate at normal and elevated gastric pH. *J Pharmacokinet Biopharm* 5: 377–390.

Brooks MA *et al.* (1977). Determination of clorazepate and its major metabolites in blood and urine by electron capture gas-liquid chromatography. *J Chromatogr* 135: 123–131.
Colin P *et al.* (1983). High-performance liquid chromatography determination of dipotassium clorazepate and its major metabolite nordiazepam in plasma. *J Chromatogr* 273(2): 367–377.
Wilensky AJ *et al.* (1978). Clorazepate kinetics in treated epileptics. *Clin Pharmacol Ther* 24: 22–30.

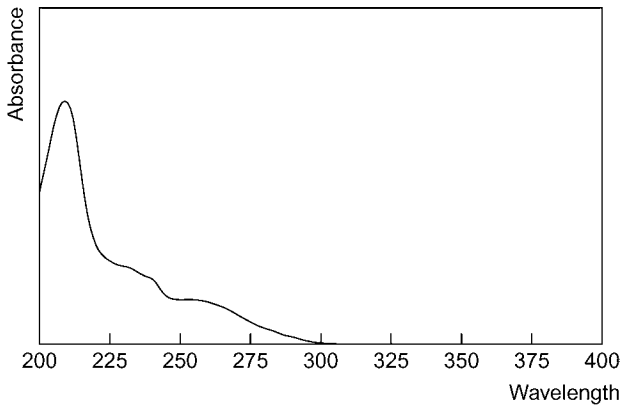
Clorexolone

Diuretic
$C_{14}H_{17}ClN_2O_3S$ = 328.8
CAS—2127-01-7

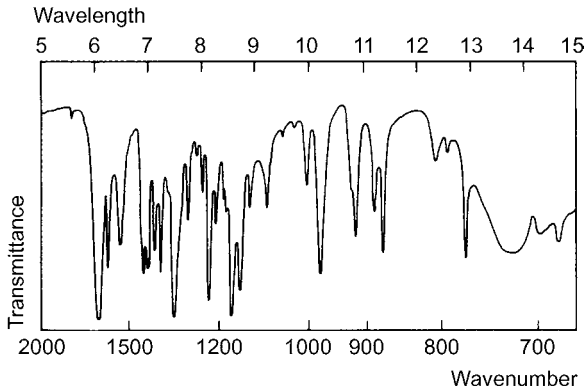
IUPAC Name 6-Chloro-2-cyclohexyl-2,3-dihydro-3-oxo-1*H*-isoindole-5-sulfonamide
Proprietary Names *Flonatril*; *Nefrolan*.



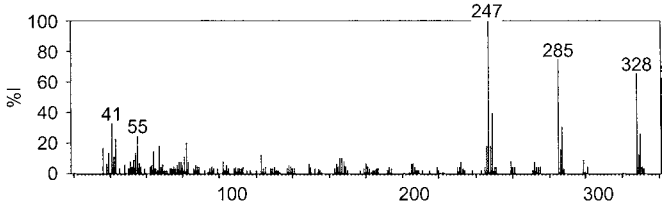
Chemical Properties A white crystalline powder. Mp 266° to 268°. Very slightly soluble in water; soluble in dimethylformamide, methanol, and solutions of alkali hydroxides. Log *P* (octanol/water), 2.0.
Colour Test Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.76; system TD—R_f 0.31; system TE—R_f 0.60; system TF—R_f 0.51; system TAD—R_f 0.47; system TAE—R_f 0.79 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3194.
High Performance Liquid Chromatography System HN—*k* 7.26; system HY—RI 391.
Ultraviolet Spectrum Ethanol—252 nm (A_1^1=246a).



Infrared Spectrum Principal peaks at wavenumbers 1661, 1172, 1234, 1149, 978, 1613 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 247, 285, 328, 249, 41, 287, 330, 55.

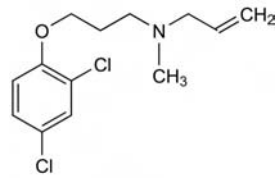


Disposition in the Body Absorbed after oral administration. It is almost completely excreted in the urine in 48 h as three cyclohexane ring-hydroxylated metabolites.
Dose 10 to 100 mg daily.

Clorgiline

Antidepressant
$C_{13}H_{15}Cl_2NO$ = 272.2
CAS—17780-72-2

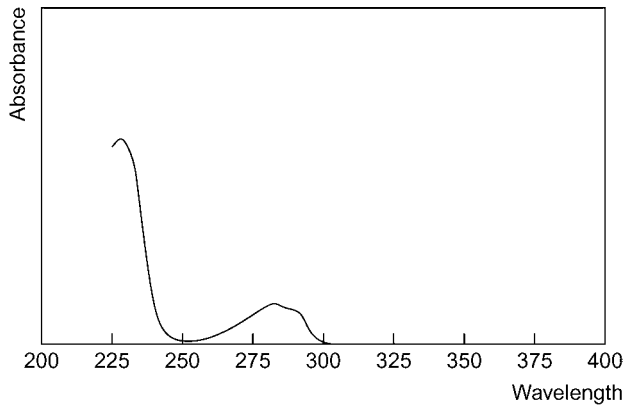
IUPAC Name *N*-[3-(2,4-Dichlorophenoxy)propyl]-*N*-methylprop-2-ynylamine
Synonym Clorgyline



Clorgiline Hydrochloride

$C_{13}H_{15}Cl_2NO$, HCl = 308.6
CAS—17780-75-5
Chemical Properties A white crystalline powder. Mp 102° to 103°. Very soluble in water; soluble in chloroform.

Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.42; system TC—R_f 0.70; system TL—R_f 0.59 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1883.
Ultraviolet Spectrum Aqueous acid—228 (A_1^1=310b), 282 nm.

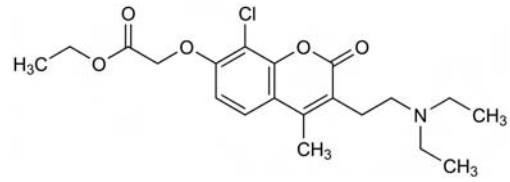


Infrared Spectrum Principal peaks at wavenumbers 1280, 1250, 1055, 1099, 801, 1124 cm^{-1}.
Dose Clorgiline hydrochloride has been tried in doses of 10 to 30 mg daily.

Cloricromen

Antithrombotic, Vasodilator (Coronary)
$C_{20}H_{26}ClNO_5$ = 395.9
CAS—68206-94-0

IUPAC Name Ethyl 2-[8-chloro-3-(2-diethylaminoethyl)-4-methyl-2-oxochromen-7-yl] oxyacetate
Synonyms AD_6; 8-chlorocarbocromen; [[8-chloro-3-[2-(diethylamino)ethyl]-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl]oxy]acetic acid ethyl ester.



Chemical Properties Crystals. Mp 147° to 148° (from ethyl acetate).

Cloricromen Hydrochloride

$C_{20}H_{25}ClNO_5$, HCl = 432.3
CAS—74697-28-2
Proprietary Names *Cromocap*; *Proendotel*.

Chemical Properties Mp 219° to 220°.

Quantification

Biological Samples **HPLC** Fluorescence detection (λ_{ex}=310 nm; λ_{em}=390 nm) [Mariot *et al.* 1992].

Note For studies on the molecular aspects of cloricromen distribution in human platelets and its pharmacological effects, see Travagli *et al.* [1989].

Dose Orally, 100 mg two or three time daily or IV in a dose of 30 mg daily.

Mariot R *et al.* (1992). Determination of the coumarin derivative cloricromene acid in rabbit plasma and platelets. *J Chromatogr* 576: 143–148.

Travagli RA *et al.* (1989). Molecular aspects of cloricromene (AD6) distribution in human platelets and its pharmacological effects. *Thromb Res* 54: 327–338.

Clorprenaline

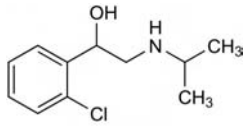
Sympathomimetic

$C_{11}H_{16}ClNO$ = 213.7

CAS—3811-25-4

IUPAC Name 2-Chloro-α-[[(1-methylethyl)amino]methyl]benzenemethanol

Synonyms Chlorprenaline; isoprofenamine; isoprophenamine.



Chemical Properties Soluble in chloroform. Log *P* (octanol/water), 1.82.

Clorprenaline Hydrochloride

$C_{11}H_{16}ClNO$, HCl, H_2O = 268.2

CAS—6933-90-0 (anhydrous); 5588-22-7 (monohydrate)

Proprietary Names *Broncon*; *Clopinerin*; *Conselt*; *Fusca*; *Katulein*; *Pentadoll*; *Restanolon*.

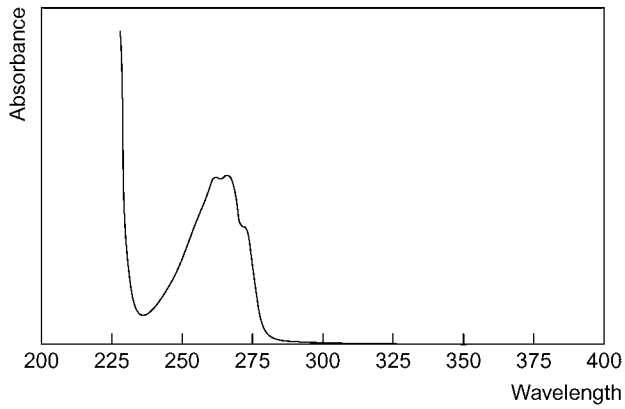
Chemical Properties White crystals. Mp 163° to 164° (monohydrate). Soluble in water.

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.18; system TC—R_f 0.15; system TL—R_f 0.20 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 1604; system GB—RI 1614; system GC—RI 1880.

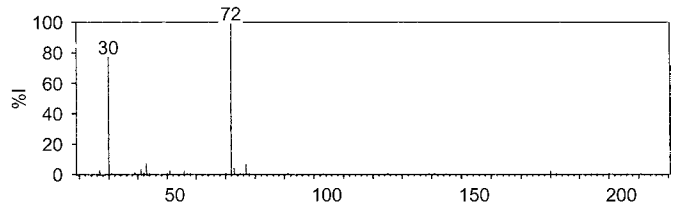
High Performance Liquid Chromatography System HA—*k* 1.1.

Ultraviolet Spectrum Aqueous acid—262, 266 nm (A^1_1=10.1b).



Infrared Spectrum Principal peaks at wavenumbers 1075, 775, 1587, 1041, 1030, 1123 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 72, 30, 43, 77, 73, 51, 41, 27.



Quantification

Plasma **HPLC** Clorprenaline enantiomers, limit of quantification, 1 ng [Miwa *et al.* 1991].

Dose Clorprenaline hydrochloride has been given in doses of 30 to 80 mg daily.

Miwa T *et al.* (1991). Application of an ovomucoid-conjugated polymer column for the enantiospecific determination of chlorprenaline concentrations in plasma. *J Chromatogr* 566: 163–171.

Clospipramine

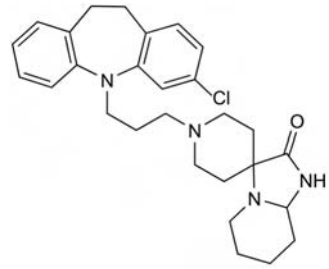
Antipsychotic

$C_{28}H_{35}ClN_4O$ = 479.1

CAS—89419-40-9

IUPAC Name 1′-[3-(3-Chloro-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepin-5-yl)propyl]-hexahydrospiro[imidazo[1,2-*a*]pyridine-3(2*H*),4′-piperidin]-2-one

Synonym Mosapramine.



Clospipramine Dihydrochloride

$C_{28}H_{35}ClN_4O.2HCl$ = 552.0

CAS—98043-60-8

Synonym Mosapramine dihydrochloride.

Proprietary Names *Cremin*; *Y-516*.

Chemical Properties A white to slightly creamy white, odourless crystalline powder. Mp 271°. It is freely soluble in formic acid; slightly soluble in water, methanol, ethanol and dimethylformamide; practically insoluble in acetonitrile, acetic anhydride and ether.

Quantification

Plasma **HPLC** UV detection (λ=257 nm). Limit of detection, 1 μg/L [Ishigooka *et al.* 1989].

Urine **HPLC** Limit of detection, 20 μg/L, see Plasma [Ishigooka *et al.* 1989].

Disposition in the Body Clospipramine dihydrochloride is metabolised, mainly in the liver, by hydrogenation at the spiroamine site and hydroxylation at the iminodibenzyl ring to produce two metabolites. Excretion is mainly via bile but the drug has also been recovered in faeces and urine (rate urinary excretion <0.5%).

Therapeutic Concentration

Five healthy males, aged between 22 and 44 years, were administered orally with single doses of 6.25, 12.5 and 25 mg. Plasma concentrations reached a maximum of 7.9 ± 1.7 μg/L within 6.0 ± 1.4 h. Plasma concentrations are dose-dependent and decrease in a monophasic manner [Ishigooka *et al.* 1989].

Half-life 15 h.

Clearance 130 to 170 L/h.

Dose Usual dose of 30 to150 mg/day is administered as three separate doses. Dose may be increased to 300 mg/day.

Ishigooka J *et al.* (1989). Pharmacokinetic study of iminodibenzyl antipsychotic drugs, clocapramine and Y-516 in dog and man. *Psychopharmacology* 97: 303–308.

Clostebol

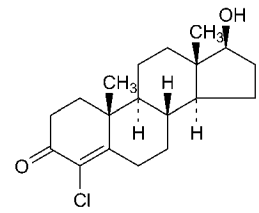
Anabolic Steroid

$C_{19}H_{27}ClO_2$ = 322.87

CAS—1093-58-9

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-4-Chloro-17-hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one

Synonyms (17β-4-Chloro-17-hydroxyandrost-4-en-3-one; 4-chlorotestosterone.



Chemical Properties Crystals from acetone and hexane. Mp 188° to 190. Log *P* (octanol/water) 3.76.

Clostebol Acetate

$C_{21}H_{29}ClO_3$ = 364.9

CAS—855-19-6

Synonyms Chlortestosterone acetate; 4-chlorotestosterone acetate.

Proprietary Names *Alfa-Trofodermine*; *Macrobin*; *Megagrisent Mono*; *Steranabol*; *Trofodermin*; *Trofoseptine*.

Chemical Properties Crystals from methanol. Mp 228° to 230°. Soluble in ethanol.

Colour Test Zimmerman test—blue.

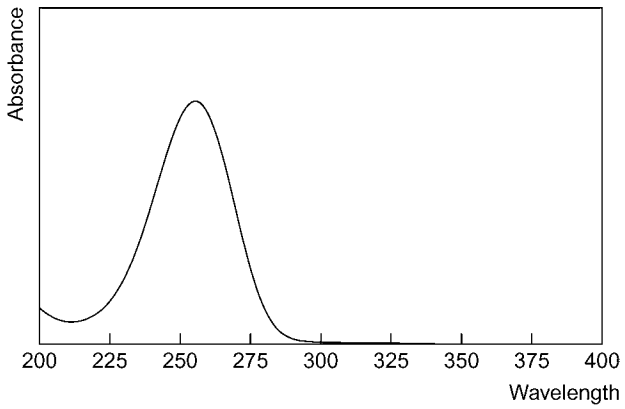
Gas Chromatography System GA—clostebol acetate RI 2965, clostebol acetate-TMS RI 2870, clostebol enol-TMS_2 RI 2830, clostebol-HCl-Ac RI 2700, clostebol-HCl-TMS RI 2675, clostebol-HCl enol-TMS_2 RI 2640.

Column: HP1 methyl silicone (0.2 mm i.d., 0.33 µm). Column temperature: 280°. Carrier gas: He, 0.9 mL/min. Detection: mass spectrometer. RI: 3023 [Mills, Roberson 1993].

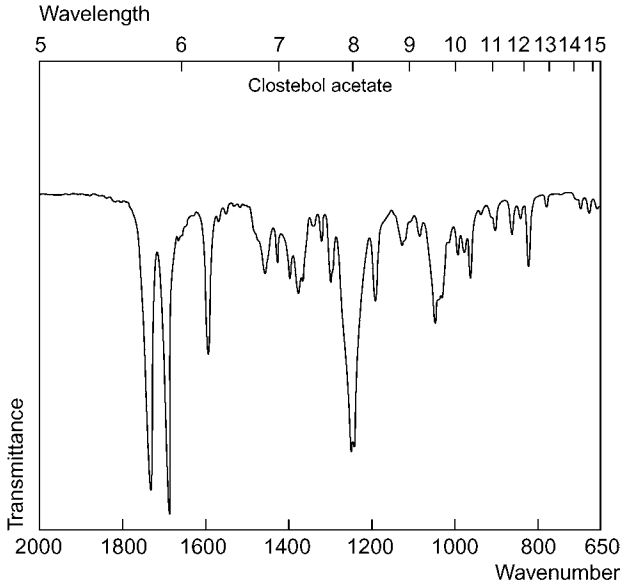
High Performance Liquid Chromatography System HAR—clostebol acetate RRT 1.90 (relative to testosterone).

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer (pH 3.5; 90 : 10), flow rate 1 mL/min. DAD. Retention time: 4.6 min [Mills, Roberson 1993].

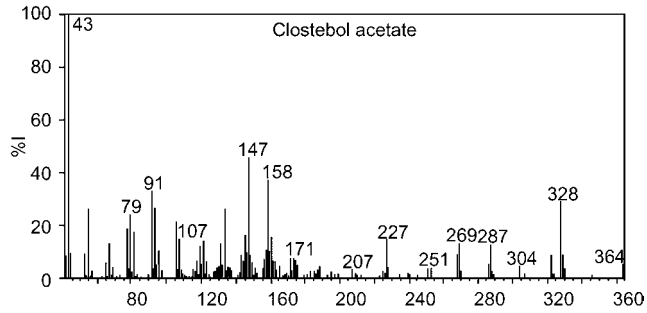
Ultraviolet Spectrum Clostebol acetate: Aqueous acid (ethanol)—254 nm.



Infrared Spectrum Clostebol acetate: Principal peaks at wavenumbers 1727, 1683, 1251 cm^{-1} (KBr pellets).



Mass Spectrum Clostebol acetate: Principal ions at *m/z* 43, 147, 158, 91, 328, 55, 93, 79.



Quantification

Urine **GC-MS** EI ionisation, SIM acquisition mode. Retention time: 21.3 min. Limit of quantification, 0.8 µg/L [Walshe *et al.* 1998]. Column: HP Ultra 2 fused silica cross-linked with 5% phenylmethylsilicone (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 26 cm/s. Temperature programme: 100° for 1 min to 230° at 40°/min to 280° at 5/min for 15 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 15.6 min [Debruyckere *et al.* 1992; Debruyckere *et al.* 1994].

Note For an ELISA for clostebol, see Crabbe *et al.* [2003].

Disposition in the Body A high percentage of clostebol acetate is absorbed after oral administration and possibly inactivated. Clostebol is metabolised to 4-chloro-Δ^4-androstene-3α-ol-17-one, which can be detected in urine along with additional 17-ketisteroid metabolites and 4-chlorotestosterone alcohol. Free 4-chlorotestosterone acetate can be detected in faeces but never in urine [Castegnaro, Sala 1973]. It is distributed in breast milk.

Dose 30 mg dose administered weekly (IM injection) or 15 mg two or three times daily (orally).

Castegnaro E, Sala G (1973). Absorption and metabolism of 4-chlorotestosterone acetate by oral route. *Steroids Lipids Res* 4: 184–192.

Crabbe P *et al.* (2003). Screening of clostebol and its metabolites in bovine urine with ELISA and comparison with GC-MS results in an interlaboratory study. *J Anal Toxicol* 27: 213–220.

Debruyckere G *et al.* (1992). Clostebol-positive urine after consumption of contaminated meat. *Clin Chem* 38: 1869–1873.

Debruyckere G *et al.* (1994). Gas-chromatographic-mass spectrometric confirmation of a clostebol metabolite in urine. *Anal Chim Acta* 291: 155–160.

Mills TI, Roberson JC (1993). *Instrumental Data for Drug Analysis*, Vol. 4-5, 2nd edn. Boca Raton, FL: CRC Press.

Walshe M *et al.* (1998). Studies on the determination of chlorotestosterone and its metabolites in bovine urine. *Analyst* 123: 2687–2691.

Clotiapine

Tranquilliser

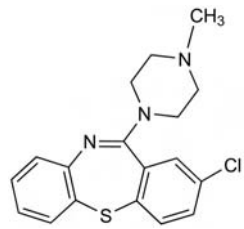
$C_{18}H_{18}ClN_3S$ = 343.9

CAS—2058-52-8

IUPAC Name 8-Chloro-6-(4-methylpiperazin-1-yl)benzo[*b*][1,4]benzothiazepine

Synonyms 2-Chloro-11-(4-methyl-1-piperazinyl)dibenzo[*b,f*][1,4]thiazepine; clothiapine.

Proprietary Names *Entumin(e)*



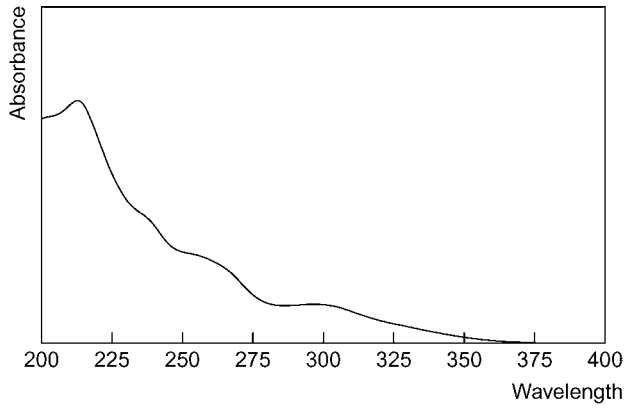
Chemical Properties Crystals. Mp 116° to 122°. Soluble 1 in 10 of hydrochloric acid. Log *P* (octanol/water), 3.8.

Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.41; system TC—R_f 0.59; system TL—R_f 0.23 (acidified iodoplatinate solution, positive).

Gas chromatography System GA—clotiapine RI 2712, M (OH-)-AC RI 3000, M (nor-)-AC RI 3030, M (nor-OH-)-AC_2 RI 3400, M (oxo-) RI 3030; system GB—clotiapine RI 2833, M (nor-) RI 2882.

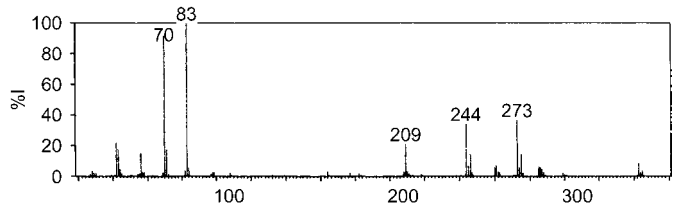
High Performance Liquid Chromatography System HY—RI 348.

Ultraviolet Spectrum Aqueous acid—213, 297 nm.



Infrared Spectrum Principal peaks at wavenumbers 1596, 1570, 1548, 1295, 757, 1234 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 83, 70, 273, 244, 209, 42, 71, 43.

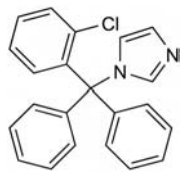


Dose Clotiapine has been given in doses of 40 to 120 mg daily.

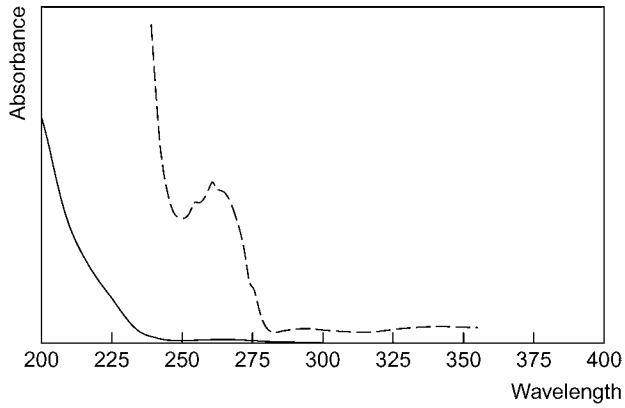
Clotrimazole

Antifungal
$C_{22}H_{17}ClN_2$ = 344.8
CAS—23593-75-1

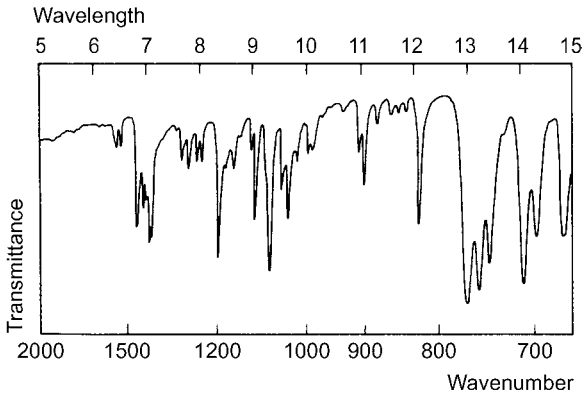
IUPAC Name 1-[(2-Chlorophenyl)diphenylmethyl]-1*H*-imidazole
Synonym Chlortritylimidazol
Proprietary Names *Canesten*; *Eparol*; *Gyne-Lotrimin*; *Lotrimin*; *Mycelex*; *Trimysten*.



Chemical Properties A white to pale yellow crystalline powder. Mp 147° to 149°. Slightly soluble in water, benzene, and toluene; freely soluble in ethanol; soluble in acetone, chloroform, and ethyl acetate; slightly soluble in ether. Log *P* (octanol/water), 6.3.
Thin-layer Chromatography System TE—R_f 0.76; system TAD—R_f 0.68; system TAE—R_f 0.80.
Gas Chromatography System GA—RI 2800.
Ultraviolet Spectrum Methanol—254, 260 nm (A_1^1=20b).



Infrared Spectrum Principal peaks at wavenumbers 765, 752, 708, 1075, 741, 1205 cm^{-1} (KBr disk).

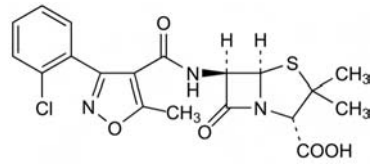


Mass Spectrum Principal ions at *m/z* 277, 279, 165, 278, 241, 239, 242, 240.
Uses Topically in a concentration of 1 or 2% or as vaginal tablets.

Cloxacillin

Antibiotic
$C_{19}H_{18}ClN_3O_5S$ = 435.9
CAS—61-72-3

IUPAC Name (2*S*,5*R*,6*R*)-6-[[[3-(2-Chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

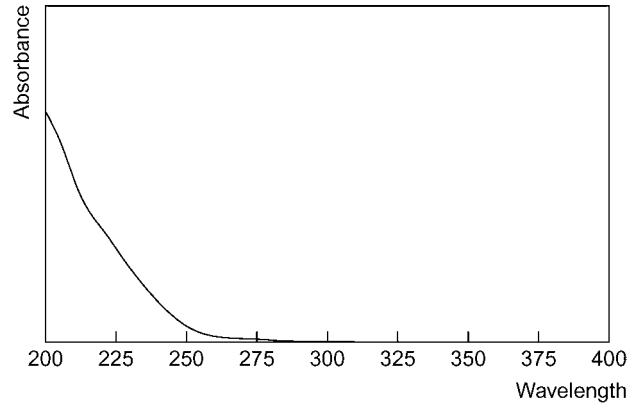


Chemical Properties pK_a 2.7 (25°). Log *P* (octanol/water), 2.5.

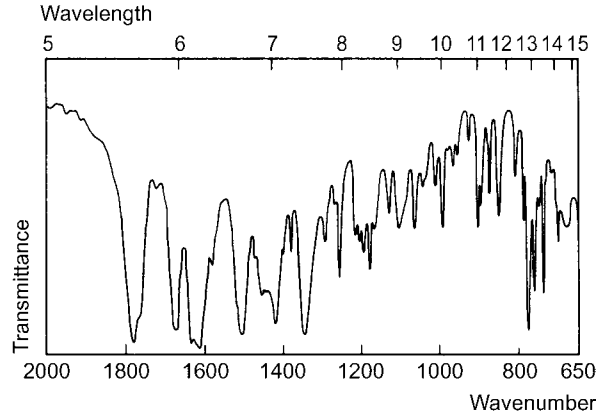
Cloxacillin Sodium

$C_{19}H_{17}ClN_3NaO_5S$, H_2O = 475.9
CAS—642-78-4 (anhydrous); 7081-44-9 (monohydrate)
Proprietary Names *Austrastaph*; *Bactopen*; *Cloxapen*; *Cloxilean*; *Cloxypen*; *Ekvacillin*; *Novocloxin*; *Orbenin(e)*; *Staphybiotic*; *Tegopen*. It is an ingredient of *Ampiclox*.
Chemical Properties A white, hygroscopic, crystalline powder. Mp 170° with decomposition. Soluble 1 in 2.5 of water, 1 in 30 of ethanol, and 1 in 500 of chloroform. Soluble in pyridine and ethylene glycol.

Ultraviolet Spectrum No significant absorption between 200 and 400 nm.



Infrared Spectrum Principal peaks at wavenumbers 1598, 1620, 1765, 1495, 1659, 771 cm^{-1} (cloxacillin sodium, KBr disk).



Quantification
Serum **HPLC** UV detection. Limit of detection, 50 mg/L [Teare *et al.* 1982]. UV detection. Limit of detection, 500 mg/L [Soldin *et al.* 1980].
Urine **HPLC** See Serum [Teare *et al.* 1982].
Disposition in the Body Incompletely absorbed after oral administration. About 35% of an oral dose is excreted in the urine unchanged in 12 h, together with about 11% as penicilloic acid; about 10% of a dose is excreted in the bile.
Half-life Plasma half-life, about 0.5 to 1.5 h.
Volume of Distribution About 0.1 L/kg.
Protein Binding About 94%.
Dose Usually the equivalent of 2 g of cloxacillin daily.

Soldin SJ *et al.* (1980). A rapid high performance liquid chromatographic procedure for the analysis of cloxacillin and/or nafcillin in serum. *Ther Drug Monit* 2: 417–422.

Teare FW *et al.* (1982). High-pressure liquid chromatographic assay of cloxacillin in serum and urine. *J Pharm Sci* 71: 938–941.

Clozapine

Antipsychotic, Benzodiazepine

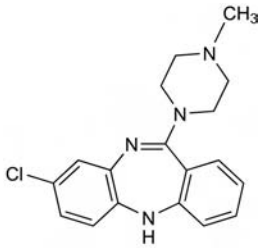
$C_{18}H_{19}ClN_4 = 326.8$

CAS—5786-21-0

IUPAC Name 3-Chloro-6-(4-methylpiperazin-1-yl)-5*H*-benzo[*c*][1,5]benzodiazepine

Synonyms 8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine; clozapina; clozapinum; HF-1854; klotsapiini; klozapin; klozapina; klozapinas.

Proprietary Names *Clopine*; *Clopsine*; *Clozaril*; *Denzapine*; *Elcrit*; *FazaClo*; *Froidir*; *Leponex*; *Zaponex*.



Chemical Properties Yellow crystals. Mp 183° to 184°. Soluble in chloroform; slightly soluble in acetone, acetonitrile, ethyl acetate and ethanol; practically insoluble in water. pK_{a1} 3.70, pK_{a2} 7.60. Log *P* (octanol/water), 3.23. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable in processed samples for 24 h in the autosampler, after 3 freeze-thaw cycles and at −20° for 1 month. Serum samples were stable at room temperature for 6 h [Ming, Heathcote 2009].

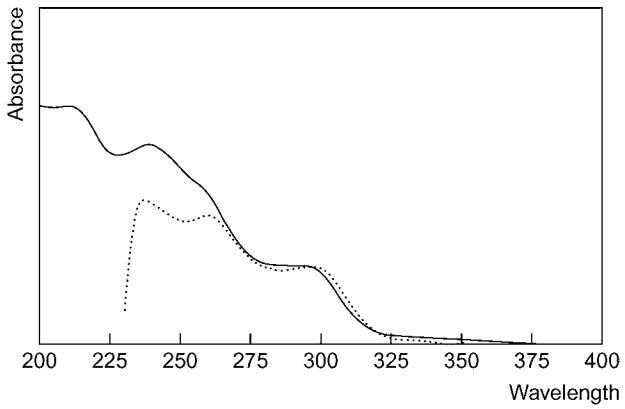
Colour Tests Formaldehyde–sulfuric acid—yellow; nitric acid, cold—red.

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.04; system TC—R_f 0.38; system TD—R_f 0.04; system TE—R_f 0.55; system TF—R_f 0.05; system TL—R_f 0.17; system TAD—R_f 0.29; system TAE—R_f 0.42.

Gas Chromatography System GA—clozapine RI 2895; M (nor-) RI 3105; M (nor-acetyl-) RI 3490; system GB—clozapine RI 3024; M (nor-) RI 3092; M (nor-acetyl-) RI 3609; system GG—RI 3455.

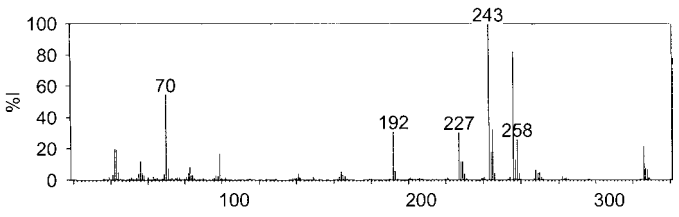
High Performance Liquid Chromatography System HX—RI 368; system HY—RI 284; system HZ—RT 3.9 min; system HAX—RT 10.9 min; system HAY—RT 5.2 min.

Ultraviolet Spectrum Aqueous acid—245, 297 nm; aqueous alkali—238, 261, 297 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1560, 758, 1136, 1101, 1117 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 243, 256, 70, 245, 192, 227, 258, 326.



Quantification

Blood **GC** Column: cross-linked fused silica (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Retention time: 12.8 min. Limit of quantification, 122–218 µg/L, limit of detection, 37–66 µg/L [Sanchez de la Torre *et al.* 2005].

GC-MS Limit of detection, 25 ng/mL [Melent'ev, Kurochkina 1999].

HPLC Column: Kromasil Ultrabase C_{18} (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 7.0, 48 : 52), UV detection (λ = 254 nm). Limit of quantification, 20 µg/L for clozapine and norclozapine and 30 µg/L for clozapine *N*-oxide [Guitton *et al.* 1997].

Plasma **GC** Column: HP-5890 (30 m × 0.53 mm i.d., 1 µm). Temperature: 260°. NPD. Limit of detection, 30 µg/L [Ulrich *et al.* 1999]. Column: Heliflex Drug Three (15 m). Carrier gas; N_2, 14 mL/min. Temperature programme; 230° for 1 min to 295° at 12°/min for 1 min. NPD. Retention time: 6.04 min. Limit of quantification, 35 µg/L [Jennison *et al.* 1995; Patrick, Markowitz 1997]. See Blood [Guitton *et al.* 1997].

GC-MS Column: OV-1701 (8 m × 0.32 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature: 260°. EI ionisation at 40 eV Limit of quantification, 1 µg/L for clozapine and 5 µg/L for norclozapine [Bondesson, Lindström 1988].

HPLC Column: C_8 reversed phase. Mobile phase: acetonitrile : 34 mmol/L phosphate buffer containing 0.3% TEA (pH 2.0, 29 : 71). UV detection (λ = 254 nm). Retention time: 5.9 min. Limit of quantification, 20 µg/L, limit of detection, 7 µg/L [Mercolini *et al.* 2007a]. Column: C_8 reversed phase. Mobile phase: aqueous phosphate buffer containing TEA (pH 3.0) : acetonitrile (70 : 30). UV detection (λ = 238 nm). Limit of quantification, <2.6 µg/L for clozapine and metabolites, limit of detection, <0.9 µg/L [Mercolini *et al.* 2007b]. Column: C_6 phenyl (150 × 2 mm, 3 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 5) : acetonitrile : methanol (5 : 3 : 2). UV detection (λ = 254 nm). Limit of quantification, 100 µg/L for clozapine and 50 µg/L for norclozapine [Rosland *et al.* 2007]. Column: XTerra MS C_{18}. UV detection. Limit of quantification, 15 µg/L [Garay Garcia *et al.* 2003]. Column: C(8). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 3.8). DAD (λ = 260, 280 and 240 nm). Limit of quantification, 5 µg/L [Titier *et al.* 2003]. See also Akerman [1997], Avenoso *et al.* [1998], Chung *et al.* [1993] Coudoré *et al.* [1999], Edno *et al.* [1997], Freeman *et al.* [1996], Johansen *et al.* [1997], Liu *et al.* [2001], Llerena *et al.* [2001], Lovdahl *et al.* [1991] and McCarthy *et al.* [1995].

LC-MS Column: reversed phase. Mobile phase: gradient (pH 8.1). SIM acquisition mode. Linit of detection, 2 µg/L [Choong *et al.* 2009]. Column: Macherey-Nagel C_{18} (125 × 2 mm, 3 µm). Mobile phase: 2.7 mmol/L formic acid-10 mmol/L ammonium acetate : acetonitrile (53 : 47), flow rate 0.16 mL/min. ESI, SIR acquisition mode. Limit of quantification, 20–1000 µg/L [Zhou *et al.* 2004]. Column: LiChroCART Superspher 60 RP Select B (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 at 8 min to 60 : 40 at 9.5 min for 0.5 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min at 9.5 min to 0.4 mL/min. APCI, SIM acquisition mode or scan mode. Limit of quantification, 0.05 mg/L (SIM mode), limit of detection, <0.02 mg/L (scan mode) [Kratzsch *et al.* 2003]. Column: Symmetry C_{18} (150 × 30 mm, 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (20 : 80), flow rate 0.5 mL/min [Kollroser, Schober 2002].

CE Capillary: coated alkylphosphonate-modified zirconia CEC. Buffer: 0.3 mol/L ammonium acetate (pH 4.5) : acetonitrile (65 : 35). Limit of detection, 11.4–51.5 µg/L [Wei *et al.* 2010]. Capillary: untreated fused silica (31.2/20 cm, total/effective length, 50 µm). Buffer: 400 mmol/L phosphate buffer (pH 3.0) containing 50% ethylene glycol. UV detection (λ = 214 nm). Limit of detection, 5 µg/L for clozapine and norclozapine, 10 µg/L for clozapine *N*-oxide [Ho *et al.* 2004].

Serum **TLC** Plates: HPTLC silica gel F 254. Solvent: chloroform : methanol (9 : 1). Densiometry (290 nm). Limit of quantification, 50 µg/L, limit of detection, 30 µg/L [Mennickent *et al.* 2007].

GC See Plasma [Jennison *et al.* 1995]. Column: DB5 (15 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 6 mL/min. Temperature: 250°. NSD. Limit of quantification, 1–2 µg/L [Richter 1988].

HPLC Column: ODS Hypersil C_{18} (250 × 4.6 mm, 5 µm). UV detection (λ = 254 nm). Limit of quantification, 10–50 µg/L [Sachse *et al.* 2006]. Column: Nucleosil 100-Protect 1. Mobile phase: acetonitrile : potassium dihydrogen phosphate buffer [Frahnert *et al.* 2003]. Column: C_{18}. Mobile phase: acetonitrile : buffer (30 : 70), flow rate 1.5 mL/min. UV detection (λ = 260 nm) and fluorescence detection (λ_{ex} = 227, λ_{em} = 300 nm) [Waschgler *et al.* 2002]. Column: C_{18} ODS Hypersil (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : water : tetramethylethylenediamine (pH 6.5, 37 : 62.6 : 0.4). UV detection (λ = 254 nm). Limit of quantification, 10–30 µg/L [Weigmann *et al.* 1997; Weigmann *et al.* 2001]. Column: C_{18} ODS Hypersil (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : water (40 : 60) buffered with 0.4% tetramethylethylenediamine (pH 6.5). Column: C_8. Mobile phase: acetonitrile : methanol : 10 mmol/L dipotassium hydrogen phosphate (pH 3.7, 30 : 2 : 100), flow rate 1.5 mL/min. DAD (λ = 220 nm). Limit of detection, 15 nmol/L [Akerman 1997]. See also Gupta [1995], McCarthy *et al.* [1995], Schulz *et al.* [1995] and Weigmann, Hiemke [1992].

LC-MS Column: ACQUITY BEH C_{18} (50 × 2.1 mm, 1.7 µm). Mobile phase: methanol : 0.2% ammonium hydroxide (50 : 50 for 0.4 min to 20 : 80 at 0.5 min until 1.4 min to 0 : 100 at 1.5 min for 1.3 min to 50 : 50 at 2.9 min for 0.6 min), flow rate 0.40 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 10 µg/L [Ming, Heathcote 2009]. Column: monolithic C_{18} (50 × 4.6 mm). Mobile phase: methanol-5 mmol/L acetate buffer (pH 3.9). MRM acquisition mode [Kirchherr, Kühn-Velten 2006].

Urine **GC-MS** Limit of detection, 30 ng/mL [Melent'ev, Kurochkina 1999].

HPLC See Serum [Weigmann *et al.* 1997].

CE See Plasma. Limit of detection, 3.7–17 µg/L [Wei *et al.* 2010].

Hair **LC-MS** Column: RP-C8-select B (125 × 2 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium formate with 0.1% formic acid (pH 3) : acetonitrile with

0.1% formic acid (90 : 10 to 70 : 30 at 6.6 min to 30 : 70 at 26.6 min to 10 : 90 at 33.3 min). MRM acquisition mode. Limit of quantification, 0.051 ng/mg, limit of detection, 0.017 ng/mg [Weinmann *et al.* 2002].

Disposition in the Body Clozapine undergoes a moderate first-pass metabolism and is almost completely metabolised before excretion via *N*-demethylation, *N*-oxidation, oxidation of the chlorine-containing ring and thiomethyl conjugation. Metabolism is catalysed by P450 isoenzyme CYP1A2. Only the desmethyl metabolite shows any pharmacological activity but it is considerably weaker and of shorter duration than that of the parent drug. Only trace amounts of unchanged drug are detected in the urine and faeces. Approximately 50% of the administered dose is excreted as metabolites in the urine and 30% in the faeces.

Therapeutic Concentration

After administration of an oral dose of 200 mg clozapine to 10 schizophrenic patients, average peak plasma concentrations of 0.249–0.386 mg/L were attained in 1.0–6.3 h (mean 3 h) [Cheng *et al.* 1988].

In 14 patients, the oral clearance and half-life of clozapine after administration of a single dose of 100 mg was 55.4 L/h and 13.7 h, respectively. The mean AUCs for clozapine and norcloazapine were 2389.9 and 751.1 μg.h/L, respectively [Lin *et al.* 1994].

In 12 patients where dose was titrated over 10–14 days to a target dose of 6 mg/kg daily, the mean clozapine plasma concentration achieved after 5 days at the target dose was 0.584 mg/L; a wide variation was apparent (±0.417 mg/L) [Bennett, Keck 1996].

After administration of clozapine at a dosage of up to 500 mg daily for 12 weeks to 45 schizophrenic patients, mean steady-state plasma concentrations of clozapine and norclozapine were reported to be 0.472 and 0.201 mg/L, respectively, in those who responded to treatment and 0.328 and 0.156 mg/L in those unresponsive to treatment. A cut-off value of 0.350–0.400 mg/L distinguished responders from non-responders, although the incidence of adverse effects was twice as high at concentrations above 0.350 mg/L compared with those below [Spina *et al.* 2000].

Twenty-six subjects treated with clozapine 200–700 mg daily for psychosis had concentrations of 0.030–1.016 mg/L in plasma (22 subjects), 0.17–34.24 μg/g in hair (23 subjects) and 0.049–5.609 μg/patch in sweat (20 subjects); a better dose–concentration relationship was observed between daily dose and hair or sweat concentrations than plasma concentration [Cirimele *et al.* 2000].

In children aged 9–16 years, serum levels of clozapine and its metabolites norclozapine and clozapine *N*-oxide were 289, 410 and 63 μg/L, respectively. In contrast to these findings, norclozapine levels in adults are usually 10 to 25% less than clozapine levels. Dose-normalised clozapine serum levels in children were found to be similar to those reported in adults [Frazier *et al.* 2003].

Plasma clozapine levels in Asian and Caucasian patients were found to be similar; however, the mean clozapine daily dose was 176 mg in the Asian patients but 433 mg in Caucasian patients. Therefore, Asian patients had more than twice the effective clozapine concentration–dose ratio [Ng *et al.* 2005]. See also Lee *et al.* [2009].

Patients with plasma clozapine levels >0.25 mg/L were found to be more likely to have moderate to severe side effects than patients with plasma levels <0.25 mg [Yusufi *et al.* 2007].

Toxicity

A 25-year-old man who died several hours after the ingestion of an estimated 2 g clozapine had the following postmortem tissue concentrations: blood 0.00581 mg/L, liver 42.9 μg/g, urine 0.0113 mg/L, gastric contents 6.5 mg (total); the antemortem blood concentration was 0.00194 mg/L at approximately 4 h after the ingestion [Meeker *et al.* 1992].

In 3 fatalities involving clozapine, tissue concentrations were reported as follows: 3.6, 2.6 and 13 μg/g in blood and 28, 19 and 85 μg/g in the liver. The concentrations of desmethylclozapine were: –, 1.1 and 3.1 μg/g in the blood and –, 18 and 50 μg/g in the liver [Worm *et al.* 1993].

The following body-fluid concentrations were reported in a 15-year-old girl who died following ingestion of an unknown quantity of 100 mg clozapine tablets in a suicide attempt:

	Clozapine (mg/L)	*N-Desmethylclozapine (mg/L)*
Antemortem peripheral blood	9.4	0.6
Postmortem peripheral blood	8.8	0.5
Heart blood	12.0	1.5
Cerebrospinal fluid	0.2	ND
Vitreous humour	1.3	ND
Stomach content	2410	172
Bile	1844	75

[Keller *et al.* 1997]

A 19-year-old woman who survived despite ingesting 5 g clozapine had a plasma concentration of 3.8 mg/L 2.5 h after the ingestion. The only symptoms were somnolence with intermittent periods of agitation and a mild anticholinergic syndrome [Broich *et al.* 1998].

For a further report of a fatal overdose with clozapine and orphenadrine, see Fucci *et al.* [2001].

A 10-year-old-girl who accidently ingested clozapine presented with dramatic changes in mental status and progressive alteration of consciousness. Urine analysis 24 h after ingestion found clozapine 500 μg/L. Full recovery occurred after ~55 h [Borzutzky *et al.* 2003].

For reports of a neonatal death and a case of delayed peristalsis in a neonate following clozapine self-poisoning in late pregnancy, see Klys *et al.* [2007] and Novikova *et al.* [2009], respectively.

For a report of clozapine poisoning in a child as a result of Munchausen syndrome by proxy, see Bartsch *et al.* [2003].

In a case of sudden unexpected death in a patient who had been taking clozapine 300 mg daily for ~6 weeks, no clozapine was found in the patient's stomach at postmortem 8 h after death. This was consistent with reports of non-compliance with medication on the previous day. However, clozapine levels in cardiac blood were found to be 4500 μg/L (>1300 μg/L is considered toxic). The high blood level was attributed to postmortem drug redistribution rather than overdose [Kerswill, Vicente 2003]. For comment, see de Leon, Simpson [2004].

For reports of toxic clozapine levels during acute urinary-tract infection and during inflammatory reactions, see Jecel *et al.* [2005] and Pfuhlmann *et al.* [2009], respectively.

In an analysis of clozapine poisoning in the UK/Eire between 1992 and 2003, there were 7 fatal and 5 non-fatal overdoses in patients who were poorly or non-adherent to clozapine or who had taken clozapine prescribed for someone else. Postmortem blood concentrations were 8.2 mg/L (clozapine) and 1.9 mg/L (norclozapine), and the clozapine : norclozapine ratio was 4.4. Postmortem plasma levels were 3.9 mg/L (clozapine) and 0.4 mg/L (norclozapine), and clozapine : norclozapine ratio was 7.6. In 54 other patients who died while taking clozapine, postmortem blood levels were 1.9 mg/L (clozapine) and 1.4 mg/L (norclozapine), and the clozapine : norclozapine ratio was 1.5. Median postmortem increase in clozapine and norclozapine levels compared with the most recent values before death were 489% and 371%, respectively. It was concluded that clozapine poisoning cannot be diagnosed from postmortem blood levels alone, but that a comparison with antemortem levels provides further diagnostic information [Flanagan *et al.* 2005].

For other analyses of clozapine self-poisoning and poisoning, see Reith *et al.* [1998] and Sliundin *et al.* [2007], respectively.

Bioavailability ≈50–60%.

Half-life 6 to 17 h.

Volume of Distribution 2–7 L/kg. Also reported as 1.6 L/kg.

Clearance 6.1 mL/min/kg.

Protein Binding ≈95%.

Note For a review of the pharmacokinetics and pharmacodynamics of clozapine, see Jann *et al.* [1993] and Mauri *et al.* [2007]. For discussions on therapeutic drug monitoring in clozapine therapy, see Freeman, Oyewumi [1997], Olesen [1998] and Ulrich *et al.* [2003],

Dose In schizophrenia, initially 12.5 mg once or twice daily, increased gradually to 300–450 mg daily; maximum daily dose 900 mg; usual maintenance dose 250–450 mg daily.

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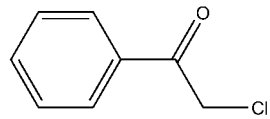
CN Gas

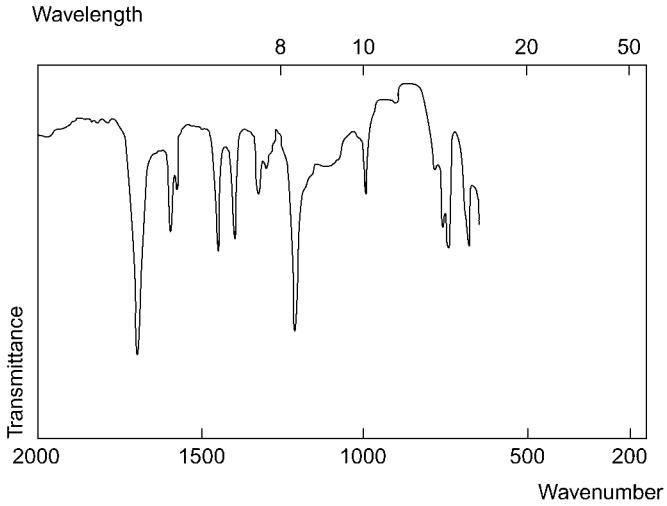
Phenylketone, Riot Control Agent
$C_8H_7ClO = 154.6$
CAS—532-27-4

IUPAC Name 2-Chloro-1-phenylethanone
Synonyms α-Chloroacetophenone; ω-chloroacetophenone; 1-chloroacetophenone; 2-chloroacetophenone; phenacyl chloride; phenylchloromethylketone; tear gas.
Proprietary Names *Chemical Mace*; *CN*; *Mace*; *Mace CN* (defense spray formulations); *CN Smoke Mix 206 Long Range Projectile*; *CN Smoke Mix 219/70 Yard Projectile*; *Pyrotechnic CN Agent* (pyrotechnic formulations).



Chemical Properties Colourless-to-grey crystalline solid with a floral odour. Mp 58° to 59°; also reported as 54° and 56.5°. Bp 244° to 245°; also reported as 247°. Practically insoluble in water; freely soluble in alcohol, ether, benzene [O'Neil *et al.* 2006]. Log *P* (octanol/water), 1.93 [Meylan, Howard 1995]. Stable in storage for 2 weeks in the dark at 25°; a 4% decrease is observed after storage at 60° [National Toxicology Program 1990]. Hydrolysis of CN is very slow in water or when alkali is added; therefore, CN is difficult to decompose under practical conditions [Blain 2003]. CN is an SN_2 alkylating agent that reacts directly with nucleophilic sites [Ballantyne, Swanston 1978].
Thin-layer Chromatography Plates: precoated silica gel (0.25 mm). Solvent systems: (1) toluene or (2) hexane : dioxane (88 : 12). Locating reagent 2,4-dinitrophenylhydrazine in hydrochloric acid-ethanol and UV detection ($\lambda = 254$ nm). R_f value: not reported. Limit of quantification not reported [National Toxicology Program 1990].

Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 105, 77, 51.

Disposition in the Body Little is known on the uptake and distribution of CN following exposure. It is postulated that CN is converted to an electrophilic metabolite that reacts with sulfhydryl-containing enzymes, such as lactic dehydrogenase and nucleophilic sites of macromolecules.

Toxicity CN is the most toxic lachrymator (when compared with others in its class) used in tear gas formulations, causing burning in the eyes, nose and throat as well as a stinging or burning sensation on exposed skin. These symptoms occur almost instantaneously on exposure and generally resolve within ≈20 min. However, severe and permanent cornea injury has been demonstrated in laboratory animals. Odour threshold by humans is 0.035 ppm, the nose irritation threshold is 0.034 ppm, and the eye irritation threshold is 0.022 ppm [Amoore, Hautala 1983]. Human ocular irritancy thresholds and toxicity estimates are reported as follows: irritancy threshold 1 mg/m^3, intolerable concentration 5 mg/m^3, lethal concentration (10 min exposure) 850 mg/m^3 [Olajos, Salem 2001]. The inhalation toxicity of chemical warfare agents, military chemicals and riot control agents is, by convention, expressed by the notation Ct. It is defined as the product of the concentration in mg/m^3 multiplied by the exposure time (t) in minutes (mg·min/m^3). The terms LCt_{50} and ICt_{50} describe the airborne dosages that are lethal (L) or incapacitating (I) to 50% of the exposed population. The minimal irritant concentration estimate in humans is 0.3 to 1 mg·min/m^3 with LCt_{50} and ICt_{50} estimates reported as from 8500 to 25 000 mg·min/m^3 (also as 7000 mg·min/m^3) and 20 to 50 mg·min/m^3, respectively. Animal LCt_{50} (mg·min/m^3) values: rat 8750 (also reported as 3700), mouse 18 200 (also reported as 73 500), rabbit 11 480, guinea pig 13 140 (also reported as 3500). Animal LD_{50} (mg/kg) values: rat 127 (oral), 36 (IP), 41 (IV); mouse 81 (IV); rabbit 118 (oral), 31 (IV); guinea pig 158 (oral), 17 (IP). [Ballantyne, Swanston 1978; National Toxicology Program 1990; Olajos, Salem 2001; Punte *et al.* 1962]. Volunteers exposed to airborne concentrations of CN responded to its short-term irritant properties after 1 min of exposure at 210 mg/m^3; 2 min of exposure at 120 mg/m^3, or 3 min of exposure at 90 mg/m^3. The maximum safe inhaled dose of CN for humans is estimated at 500 mg·min/m^3 [Punte *et al.* 1962]. CN has accounted for at least 5 deaths resulting from pulmonary injury and/or asphyxia [Chapman, White 1978; Stein, Kirwan 1964]. OSHA air monitoring/exposure levels for CN are 0.05 ppm for the permissible exposure limit (PEL) and 15 mg/m^3 for the immediately dangerous to life and health (IDLH) limit. Owing to its poor water solubility, CN formulations usually contain solvents as carriers as well as some kind of propellant gas. It is worth noting that some solvents may contribute to unwanted effects of CN use, such as corneal erosion, skin blistering, or even neurotoxicity [Olajos, Stopford 2004].

Note For a review and assessment of the toxicology and carcinogenesis of CN in rats and mice, see National Toxicology Program [1990]. For reviews of riot control agents, see Blain [2003]; Hu *et al.* [1989]; Olajos and Salem [2001]; Olajos and Stopford [2004].

Dose Used as riot control agent, having replaced adamsite (DM).

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Cocaine

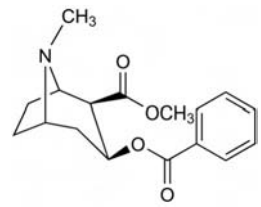
Anaesthetic (Local)

$C_{17}H_{21}NO_4$ = 303.4

CAS—50-36-2

IUPAC Name Methyl-(1*S*,3*S*,4*R*,5*R*)-3-benzoyloxy-8-methyl-8-azabicyclo-[3.2.1]octane-4-carboxylate

Synonyms Cocaina; ethyl-[1*R*-(exo,exo)]-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate; methyl benzoylecgonine. For names used to describe various forms of cocaine, see Sweetman [2007].



Chemical Properties An alkaloid obtained from coca, the dried leaves of *Erythroxylum coca* and other species of *Erythroxylum* (Erythroxylaceae) or by synthesis from ecgonine. A white crystalline, slightly volatile, powder. Mp 98°. Soluble 1 in 600 of water (1 in 270 of water at 80°), 1 in 6.5 of ethanol, 1 in 0.7 of chloroform and 1 in 3.5 of ether; also soluble in acetone, ethyl acetate and carbon disulfide. pK_a 8.7 [Needham *et al.* 2000], 8.6 (20°). Log P (octanol/water), 2.3. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Hydrolysed when stored at −16° [Javaid *et al.* 1978]. Brain tissue samples show degradation of 48% when stored at 4° for 1 month and 90% when stored for 2 months. Traces of cocaine were still detectable after 144 days storage at 4° [Hernandez *et al.* 1994].

Cocaine Hydrochloride

$C_{17}H_{21}NO_4$, HCl = 339.8

CAS—53-21-4

Synonyms Cocainium chloratum; cocaini hydrochloridum.

Chemical Properties Hygroscopic colourless crystals or white crystalline powder. Mp ~195°, with decomposition. Soluble 1 in 0.4 of water, 1 in 3.2 of cold alcohol, 1 in 2 of hot alcohol and 1 in 12.5 of chloroform; also soluble in glycerol and acetone; practically insoluble in ether.

Colour Tests Cobalt thiocyanate—blue; *p*-dimethylaminobenzaldehyde (100° for 3 min)—red.

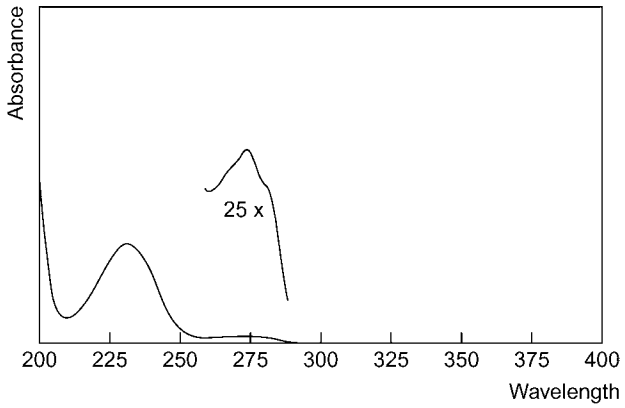
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.45; system TC—R_f 0.47; system TE—R_f 0.77; system TL—R_f 0.54; system TAE—R_f 0.35; system TAF—R_f 0.30; system TAJ—R_f 0.13; system TAK—R_f 0.00; system TAL—R_f 0.02 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).

Plate: silica 60 F_{254} (10 × 10 cm). Mobile phase: hexane : toluene : diethylamine (65 : 20 : 5). UV detection. R_f 0.36 [Antonilli *et al.* 2001].

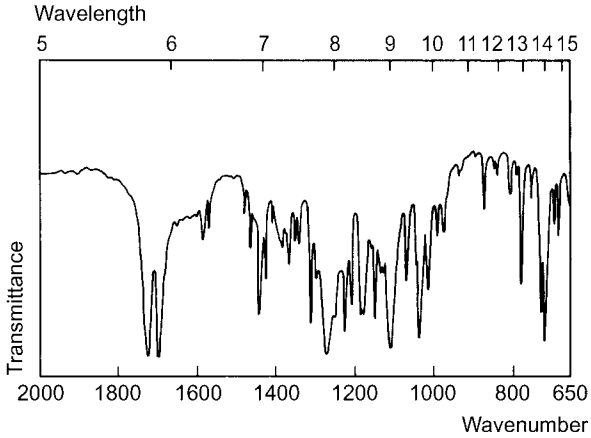
Gas Chromatography System GA—cocaine RI 2187, M (benzoylecgonine) RI 2570, M (ecgonine) not eluted, M (anhydroecgonine methylester) RI 1280, M (methylecgonine) RI 1472, M (nor-) RI 2162, M (*m*-OH-) RI 2460, M (OH-methoxy-) RI 2670, M (cocaethylene) RI 2250, M (nor-cocaethylene) RI 2115; system GB—cocaine RI 2289, M (ecgonine) not eluted, M (anhydroecgonine methylester) RI 1430, M (norecgonine) RI 1472, M (methylecgonine) RI 1530, M (ethylecgonine) RI 1602, M (nor-) RI 2259, M (benzoylecgonine) RI 2663, M (*m*-OH-) RI 2608, M (*p*-OH-) RI 2650, M (OH-methoxy-) RI 2729, M (cocaethylene) RI 2345, M (norcocaethylene) RI 2317, M (OH-cocaethylene) RI 2709, M (OH-methoxycocaethylene) RI 2779, M (methoxycocaethylene) RI 2663; system GF—RI 2550; system GQ—RT 7.85 min; system GAK—RT 12.7 min.

High Performance Liquid Chromatography System HA—cocaine k 2.8, M (benzoylecgonine) k 0.9 (tailing peak), M (ecgonine) k 1.1; system HQ—cocaine k 2.68, M (benzoylecgonine) k 5.68; system HX—RI 348; system HY—RI 289; system HZ—RT 3.3 min; system HAA—RT 11.9 min; system HAX—RT 10.0 min; system HAY—RT 5.0 min; system HBC—RT 5.2 min.

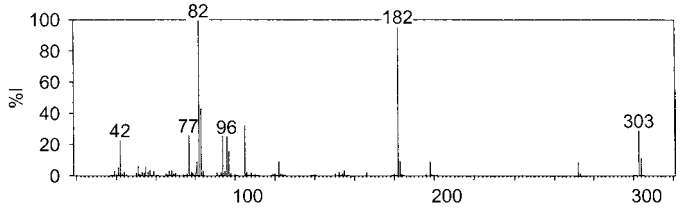
Ultraviolet Spectrum Aqueous acid—233 (A^1_1 = 430a), 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1710, 1738, 1275, 1110, 712, 1037 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 82, 182, 83, 105, 303, 77, 94, 96 (cocaine); 124, 82, 168, 77, 105, 42, 94, 83 (benzoylecgonine).



Quantification

Blood **GC** Column: 5% OV-225 on Gas-Chrom Q 100/120 mesh (3.0 m [10′]). Temperature: 110°. ECD. Limit of detection not reported [Javaid *et al.* 1978]. AFID. Limit of detection, 0.02 mg/L [Dvorchik *et al.* 1977]

GC-MS SIM acquisition mode [Fleming *et al.* 2010]. SIM acquisition mode. Limit of detection, 25 µg/L [Cardona *et al.* 2006]. Limit of detection, 0.5–4.0 µg/L [Paul *et al.* 2005]. FID. Limit of detection, 71 µg/L [Fernandez *et al.* 2004]. Column: DB-35 ms (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 2 µg/L [Gunnar *et al.* 2004]. See also Abusada *et al.* [1993]; Aderjan *et al.* [1993]; Chasin, Midio [2000]; Corburt, Koves [1994]; and Smirnow, Logan [1996].

HPLC Column: Varian Pursuit C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: methanol : ammonium formate. Limit of quantification, 0.0005–0.01 mg/kg [Bjork *et al.* 2010]. Column: Zorbax C_{18}. Mobile phase: formic acid : water : acetonitrile. MRM acquisition mode. Limit of quantification, 0.008 mg/kg [Johansen, Bhatia 2007]. Column: Spherisorb 5 RP 8S (100 × 2.1 mm i.d., 5 µm). Mobile phase: water : acetonitrile containing 0.1% formic acid and 2 mmol/L ammonium formate (50 : 50), flow rate 400 µL/min. ESI, MRM acquisition mode. Limit of quantification, 5 µg/L [Cailleux *et al.* 1999].

LC-MS Column: Gemini C6-Phenyl (50 × 3.0 mm i.d., 5 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (100 : 0 for 3.01 min to 98 : 2 at 3.05 min to 10 : 90 at 7.35 min until 8 min to 100 : 0 at 9.15 min until 10 min), flow rate 0.9 mL/min to 1.0 mL/min at 3.05 min until 10 min. TIS, positive ion mode, MRM acquisition mode. Limit of quantification, 8 µg/L, limit of detection, 3 µg/L [Jagerdeo *et al.* 2008].

Plasma **GC** Column: HP Ultra-2 (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 200° for 2 min to 280° at 30°/min until 11 min. NPD. Limit of quantification, 4 µg/L [Virag *et al.* 1994]. See Blood [Dvorchik *et al.* 1977; Javaid *et al.* 1978].

GC-MS Column: 5% phenylmethylsiloxane (12 m × 200 µm i.d., 0.33 µm). Temperature programme: 90° for 2 min to 215° at 30°/min for 5 min to 260° at 30°/min. SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 19 µg/L [Alvarez *et al.* 2007]. See Blood [Gunnar *et al.* 2004]. Column: DB-1 (15 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 15 mL/min. Temperature programme: 140° to 300° at 16°/min for 10 min. EI ionisation, positive ion mode, SIM acquisition mode (*m/z* 82). Limit of quantification, 100 µg/L. Limit of detection, 80 µg/L [Ohshima, Takayasu 1999]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 1 min to 220° at 35°/min for 0.25 min to 250° at 10°/min for 3 min. SIM acquisition mode. Limit of detection, 1 µg/L [Wang *et al.* 1994]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° to 220° at 35°/min for 15 s to 250° at 10°/min for 15 s to 260° at 3.5°/min for 15 s. Limit of detection, 1 µg/L [Cone *et al.* 1994]. See also Abusada *et al.* [1993].

HPLC DAD. Limit of detection, 0.05 mg/L [Fernandez *et al.* 2007]. See Blood [Cailleux *et al.* 1999]. Column: Lichrospher RP18 (125 × 4 mm i.d.). Mobile phase: methanol (pH 7) : phosphate buffer (70 : 30), flow rate 0.4 mL/min to 0.7 mL/min at 6 min to 1.0 mL/min at 8.0 min to 0.7 mL/min at 10 min to 0.7 mL/min at 11.0 min to 0.6 mL/min at 12 min to 0.4 mL/min. UV detection (λ = 235 nm). Retention time: 8.8 min. Limit of detection, 12.5 µg/L [Fernandez *et al.* 1996]. See also Evans, Morarity [1980] and Tagliaro *et al.* [1994].

LC-MS TIS, positive ion mode [Sergi *et al.* 2009]. Column: Zorbax Eclipse XDB-C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate buffer : acetonitrile-methanol (1 : 1, 99 : 1 for 3 min to 20 : 80 at 4 min for 11 min), flow rate 0.3 mL/min. TIS, MRM acquisition mode. Limit of quantification, 14.4 µg/L, limit of detection, 4.1 µg/L [Klingmann *et al.* 2001]. Column: fused silica (500 × 0.1 mm i.d.) followed by C_{18} (30 × 4.6 mm i.d., 3 µm). Mobile phase: 25 mmol/L ammonium acetate in methanol water (50 : 50), flow rate 1.0 mL/min. APCI, MRM acquisition mode. Limit of detection, 2 µg/L [Sosnoff *et al.* 1996].

Serum **GC-MS** Limit of quantification, 25 µg/L [Maresova *et al.* 2008]. See Blood [Gunnar *et al.* 2004]. See Weinmann *et al.* [2000].

Urine **GC** Limit of quantification, 5 µg/L, limit of detection, 20 µg/L [Farina *et al.* 2002]. See Blood [Javaid *et al.* 1978].

GC-MS Column: Oasis hydrophilic-lipophilic balance (20 × 3.9 mm i.d.) [Brunetto *et al.* 2010]. See Blood [Fleming *et al.* 2010]. Column: HP-5 (30 m × 250 µm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 1 min to 190° for 1 min at 30°/min to 260° at 4°/min for 4 min to 290° for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 13.8 min. Limit of quantification, 20 ng/g, limit of detection, 5 ng/g [Lopez *et al.* 2009]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 3 min to 300° at 20°/min for 3 min. Limit of quantification, 100 µg/L, limit of detection, 50 µg/L [Saito *et al.* 2007]. See Blood [Fernandez *et al.* 2004; Paul *et al.* 2005; Wang *et al.* 1994]. See also Aderjan *et al.* [1993], Cardenas *et al.* [1996], Cone *et al.* [1994], De Giovanni, Strano Rossi [1994], de la Torre *et al.* [1995], Elsohly *et al.* [1998], Gaillard, Pepin [1997], Garside *et al.* [1997], Gerlits [1993], Hall *et al.* [1999], Karacic, Skender [2000], Lombardero *et al.* [1993], Ohshima, Takayasu [1999], and Yonamine, Saviano[2006].

HPLC Limit of detection, 0.5 µg/L [Jeanville *et al.* 2001]. Column: LiChroCART-LiCrospher 100 RP-18 (250 × 4 mm i.d., 5 µm). Mobile phase: 0.045 mol/L ammonium acetate solution in water-methanol-acetonitrile (80 : 10 : 10) : 0.045 mol/L ammonium acetate solution in water-methanol-acetonitrile (20 : 40 : 40, 100 : 0 to 47.2 : 52.8 in 20 min), flow rate 1 mL/min. UV detection, (λ = 235 nm). Retention time: 14.8 min. Limit of quantification, 200 µg/L [Antonilli *et al.* 2001]. See Blood [Cailleux *et al.* 1999]. See also Clauwaert *et al.* [1996], Nakashima *et al.* [1992], Nishikawa *et al.* [1994], and Roy *et al.* [1992].

LC-MS Limit of quantification, 25 µg/L [Shakleya *et al.* 2010]. Column: XDB-C_8 (50 × 2.1 mm i.d., 1.8 µm). Mobile phase: 20 mmol/L ammonium formate (pH 2.7) : methanol-acetonitrile (50 : 50). Flow rate 270 µL/min. Limit of detection, 1.0 µg/L [Langman *et al.* 2009]. Column: Restek (30 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium formate (pH 3.0). ESI, CID, MRM acquisition mode. Limit of quantification, 5.3 µg/L, limit of detection, 1.6 pg [Needham *et al.* 2000]. See Plasma. Limit of detection, 5 µg/L [Fernandez *et al.* 1996].

Amniotic Fluid **HPLC** See Moore *et al.* [1992].

Breast Milk **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 1 min to 220° at 30°/min for 0.5 mL/min to 330° at 20°/min. SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 2.5 ng/mL [Winecker *et al.* 2001].

Sweat **GC-MS** Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Limit of quantification, 2.5 ng/patch, limit of detection, 1.25 ng/patch [Huestis *et al.* 1999].

Meconium **GC-MS** See Urine [Lopez *et al.* 2009]. Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 6 psi. Temperature programme: 50° for 1 min to 280° at 33°/min. Limit of detection, 11 µg/L [Lombardero *et al.* 1993]. See Blood [Abusada *et al.* 1993]. Limit of quantification, <0.25 µg/g [Clark *et al.* 1992].

HPLC Column: C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.01 mol/L sodium dihydrogen phosphate buffer (pH 2.0)-58 µmol/L tetrabutylammonium hydroxide : acetonitrile (87 : 13), flow rate 1.0 mL/min. Limit of quantification, 50 ng/g [Murphey *et al.* 1993].

Oral Fluid **LC-MS** See Plasma [Sergi *et al.* 2009].

GC-MS See Plasma [Cone *et al.* 1994; Wang *et al.* 1994].

Bone **GC-MS** See McGrath, Jenkins [2009].

Brain **GC-MS** Column: DB 5 MS (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 115° for 1.3 min to 290° at 15°/min for 2 min. EI ionisation, full scan mode. Limit of quantification, 50 ng/g, limit of detection, 25 ng/g [Hernandez *et al.* 1994].

HPLC See Browne *et al.* [1991].

Hair **GC-MS** See Gambelunghe *et al.* [2005]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 130° to 270° at 25°/min to 280° at 6°/min to 300° at 35°/min. PICI. Limit of quantification, 0.05 ng/mg, limit of detection, 0.01 ng/mg [Bourland *et al.* 2000]. Column: RTX 5 5% diphenylpolysiloxane: 95% dimethylpolysiloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 1 min to 300° at 50°/min for 6 min. SIM acquisition mode. Limit of detection, 0.2 ng/mg [Skender *et al.* 2002]. See Brewer *et al.* [2001], Girod, Staub [2000], Hold *et al.* [1998a], Hold *et al.* [1998b], Kintz, Mangin [1995], Pichini *et al.* [1999]. See Plasma [Moeller *et al.* 1993; Wang *et al.* 1994].

HPLC Column: Hypersil BDS-C_{18} (150 × 2.1 mm i.d., 3 μm). Mobile phase: 45 mmol/L ammonium acetate-methanol : acetonitrile (84 : 8 : 8) : methanol-acetonitrile-water (42 : 42 : 16, 100 : 0 for 7 min to 65 : 35 in 27 min for 3 min), flow rate 0.2 mL/min. Fluorescence detection (λ_{ex}= 242 nm, λ_{em}= 315 nm). Limit of detection, 0.2 ng/mg [Clauwaert *et al.* 2000]. Column: Hypersil BDS-C_{18} (125 × 2.1 mm i.d., 3 μm). Mobile phase: ammonium acetate buffer : methanol : acetonitrile. Fluorescence detection (λ_{ex}= 242 nm, λ_{em}= 315 nm) [Clauwaert *et al.* 1998]. Column: Polystyrene divinylbenzene (5 μm). Mobile phase: 0.1 mol/L potassium phosphate (pH 3.0) : methanol : tetrahydrofuran (70 : 25 : 5), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 230 nm, λ_{em}= 315 nm). Limit of detection, 1.5 μg/L [Tagliaro *et al.* 1993].

LC-MS Column: Zorbax SB-Phenyl (100 × 2.1 mm i.d., 3.5 μm). 25 mmol/L formic acid : 100% acetonitrile (98 : 2 to 80 : 20 for 3 min to 10 : 90 in 4 min for 2 min), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.0125 ng/mg [Hegstad *et al.* 2008].

Disposition in the Body Cocaine is normally only used as a surface anaesthetic in the eye, ear, nose and throat because of the possibility of systemic toxic effects when given by other routes. Addicts may inject it or use it as a snuff; it is, however, increasingly misused in its smokeable form ('crack cocaine') owing to the rapid absorption of the substance from the lungs. It is less toxic when taken orally owing to hydrolysis in the gastrointestinal tract. The main metabolites are benzoylecgonine, ecgonine and ecgonine methyl ester, all of which are inactive; some norcocaine, an active metabolite, may also be produced; other metabolites which have been reported are ethylecgonine, hydroxycocaine and methylecgonidine. Approximately 1–9% of a daily IV dose of 120 mg cocaine is excreted unchanged in the urine, together with 35–55% as benzoylecgonine. The excretion of unchanged drug is increased when the urine is acid; some ecgonine may also be present. After an intranasal dose of 1.5 mg/kg, up to 4% of the dose is excreted in the urine unchanged in 24 h, and 16–36% of the dose is excreted as benzoylecgonine. Approximately 30–60% of an oral, intranasal or intravenous dose is excreted in the urine as ecgonine methyl ester. No unchanged drug is eliminated in the faeces. Cocaine crosses the placenta and is found in breast milk.

Therapeutic Concentration

Six males with a history of cocaine abuse were administered with a single cocaine dose by the following routes: 25 mg cocaine hydrochloride IV, 32 mg cocaine hydrochloride intranasal and 42 mg cocaine base (smoked). Peak plasma concentrations of benzoylecgonine (BZE), ecgonine methyl ester (EME), norcocaine (NCOC), benzoylnorecgonine (BNE), *meta*- and *para*-hydroxycocaine (*m*-HOCOC, *p*-HOCOC), *meta*- and *para*-hydroxybenzoylecgonine (*m*-HOBZE, *p*-HOBZE) and anhydroecgonine methyl ester (AEME) were determined by GC–MS and were as follows:

	IV (n = 6)		*Intranasal (n = 6)*		*Smoked (n = 5)*	
	Peak conc. (ng/mL)	*Time (h)*	*Peak conc. (ng/mL)*	*Time (h)*	*Peak conc. (ng/mL)*	*Time (h)*
Cocaine	775	3.9	412	5.1	707	2.6
BZE	15611	5.6	13681	7.8	9395	4.1
EME	4968	5	5831	5	3193	4.1
NCOC	19	5.1	11	4.3	4	3.3
BNE	255	0.6	409	8.5	160	5.1
m-HOCOC	43	0.08	47	5.3	48	3.7
p-HOCOC	6	0.01	7	4.6	1	4.8
m-HOBZE	131	0.45	208	10.2	131	6.9
p-HOBZE	247	0.43	353	7.5	258	4.1
AEME	0	n/a	0	n/a	23	2.3

Total percentage dose excreted in urine for the IV, intranasal and smoked routes were 57.1, 45.4 and 24.9,%, respectively [Cone *et al.* 1998].

Ten volunteers were administered with single and multiple doses of cocaine; IV and by inhalation. On 4 separate study days, the volunteers smoked 2 doses of 25 mg cocaine with a 14–min interval (day 1), smoked 2 doses of 50 mg cocaine (day 2), were administered 2 doses of IV 16 mg cocaine with a 14–min period between (day 3), and were again given 2 doses of IV 32 mg cocaine (day 4). Peak plasma concentrations of cocaine and benzoylecgonine were determined and were as follows:

		Cocaine	*Benzoylecgonine*
IV			
2 × 16 mg	peak (mg/L)	0.23	0.24
	time (min)	18	90
2 × 32 mg	peak (mg/L)	0.47	0.4
	time (min)	18	90
Smoked			
2 × 25 mg	peak (mg/L)	0.21	0.2
	time (min)	18	60
2 × 50 mg	peak (mg/L)	0.38	0.35
	time (min)	18	90

After multiple dosing in 3 volunteers (316–mg dose administered over 90 min), the peak plasma cocaine concentration was 1.2 mg/L [Isenschmid *et al.* 1992].

Toxicity The estimated minimum lethal dose is 1.2 g but susceptible persons have died from doses as small as 30 mg when applied to mucous membranes; addicts may be able to tolerate up to 5 g a day. Toxic effects have been noted with blood concentrations in the range 0.25–5 mg/L and fatalities have occurred with concentrations of 1 mg/L or more.

Postmortem concentrations of cocaine and benzoylecgonine in a 27-year-old man who died following the rupture in his gastrointestinal tract, of 4 cocaine-containing packages were reported as: blood 4.0 and 17.0 mg/L, urine 152.0 and 512 mg/L, bile 99.8 and 54.0 mg/L, vitreous humour 7.1 and 5.8 mg/L, brain 7.5 and 3.5 mg/g, and hair 55.5 and 27.7 μg/g [Furnari *et al.* 2002].

A 17-year-old male drug dealer died ~90 min after swallowing a package containing cocaine; the following postmortem tissue concentrations were reported for cocaine, benzoylecgonine and ecgonine methyl ester, respectively: blood 98.1, 86.1 and 51.4 mg/L, liver 128.1, 134.3 and 20.3 μg/g, kidney 92.5, 8.6 and 6.2 μg/g, brain 99.1, 32.1 and 17.6 μg/g, spleen 72.6, 4.7 and 6.9 μg/g, lung 121.3, 89.4 and 36.4 μg/g, and urine 10, 3.3 and 4.2 mg/L [Fineschi *et al.* 2002].

A teenage drug courier who died after a 12–h flight was found to have 24 packages of cocaine in her intestinal tract, several of which had burst or become damaged. Her postmortem blood concentration of cocaine was 104 mg/L [Patel 1996].

A blood cocaine concentration of 330 mg/L was found in a 26-year-old female who died as a result of cocaine intoxication; the concentrations of benzoylecgonine and ecgonine methyl ester were 50 and 18 mg/L, respectively. Such high concentrations suggested a massive administration but the manner of death was undetermined [Peretti *et al.* 1990].

Half-life Plasma half-life, 0.7–1.5 h (dose dependent).

Volume of Distribution ~1–3 L/kg.

Clearance Plasma clearance, 10–32 mL/min/kg (dose dependent).

Dose Cocaine hydrochloride is used in concentrations of 1 to 25%, for local anaesthesia.

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Codeine

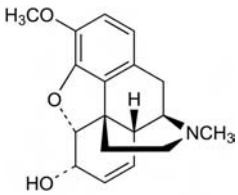
Narcotic Analgesic

$C_{18}H_{21}NO_3,H_2O = 317.4$

CAS—76-57-3 (anhydrous); 6059-47-8 (monohydrate)

IUPAC Name (5α,6α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol monohydrate

Synonyms Codeinum; methylmorphine; metilmorfina; morphine methyl ether.
Proprietary Names *Codicaps*; *Eulyptan*; *Pneumogenol*.



Chemical Properties An alkaloid obtained from opium or prepared by methylation of morphine. Colourless crystals or white crystalline powder that effloresces slowly in dry air and is affected by light. Mp 154° to 156°. Soluble 1 in 120 of water, 1 in 15 of boiling water, 1 in 2 of ethanol, 1 in 0.5 of chloroform, 1 in 13 of benzene and 1 in 18 of ether; freely soluble in amyl alcohol, methanol and dilute acids; almost insoluble in petroleum ether or in solutions of alkali hydroxides. pK_a 8.2 (20°). Log *P* (octanol/water pH 7.4), 0.6. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005].

Codeine Hydrochloride

$C_{18}H_{21}NO_3$, HCl,$2H_2O$ = 371.9
CAS—1422-07-7 (anhydrous)
Chemical Properties A white crystalline powder. Mp ~280°, with some decomposition. Soluble 1 in 20 of water, 1 in 180 of ethanol and 1 in 800 of chloroform.

Codeine Phosphate

$C_{18}H_{21}NO_3$,H_3PO_4,$^1/_2H_2O$ = 406.4
CAS—52-28-8 (anhydrous); 41444-62-6 (hemihydrate); 5913-76-8 (sesquihydrate)
Synonyms Codeine phosphate hemihydrate; codeini phosphas; methylmorphine phosphate.
Proprietary Names *Actacode*; *Bromophar*; *Bronchodine*; *Bronchosedal*; *Codant*; *Codedrill*; *Codenfan*; *Codicompren*; *Evacode*; *Galcodine*; *Glottyl*; *Paderyl*; *Toularynx*; *Tricodein*; *Tryasol*; *Tussoret*. Codeine phosphate is an ingredient of many proprietary preparations [Sweetman 2007].
Chemical Properties Small colourless crystals or white crystalline powder. Soluble 1 in 4 of water, 1 in 450 of ethanol and 1 in 125 of boiling ethanol; practically insoluble in chloroform and ether.

Codeine Sulfate

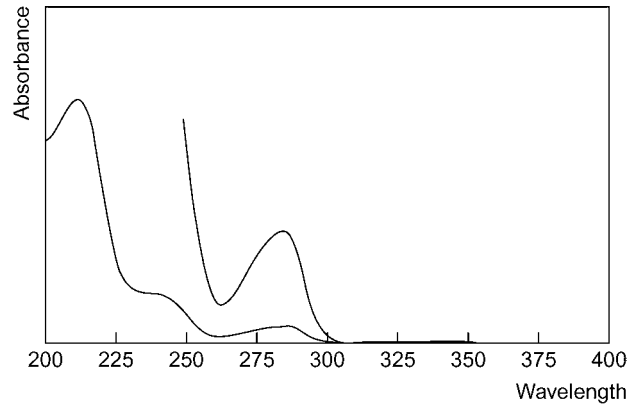
$(C_{18}H_{21}NO_3)_2$,H_2SO_4,$3H_2O$ = 750.9
CAS—1420-53-7 (anhydrous); 6854-40-6 (trihydrate)
Synonym Codeine sulphate
Chemical Properties White crystals or crystalline powder. Soluble 1 in 30 of water and 1 in 1300 of ethanol; practically insoluble in chloroform and ether.

Colour Test Liebermann's reagent—black; Mandelin's test—green; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.06; system TC—R_f 0.18; system TE—R_f 0.35; system TL—R_f 0.03; system TAE—R_f 0.21; system TAF—R_f 0.22; system TAJ—R_f 0.10; system TAK—R_f 0.00; system TAL—R_f 0.26 (acidified iodoplatinate solution—positive; Dragendorff Spray—positive; Marquis test—violet).
Gas Chromatography System GA—RI 2375, codeine-AC RI 2503, codeine-PFP RI 2430, codeine-trifluroacetic acid RI 2280, codeine-TMS RI 2520, M (nor-)RI 2388, M (nor-)-AC_2RI 2945, M (morphine) RI 2445; system GB—RI 1511, codeine-AC RI 2645, codeine-TMS RI 2592, M (nor-) RI 2535, M (nor-)-TMS_2 RI 2631; system GC—RI 2681, M (morphine) RI 2542; system GF—RI 2860; system GM—RRT 1.519, codeine-ACRI 1.449 (both relative to iprindole).
Gas Chromatography-Mass Spectrometry Column: HP-5 capillary 95% dimethylsiloxane, 5% diphenylsiloxane (25 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 2 min to 280° at 20°/min for 10 min. Carrier gas: 1 mL/min. SIM acquisition mode (*m/z* 282, 395). Retention time: 13.18 min [Pragst *et al.* 1999].
High Performance Liquid Chromatography system HA—*k* 4.8 (tailing peak), M (morphine) RI 3.8 (tailing peak), M (nor-) RI 3.1 (tailing peak); system HC—*k* 1.21, M (morphine) RI 1.30, M (nor-) 3.51; System HS—*k* 1.90, M (morphine) RI 5.16;system HX—RI 266; system HY—RI 237; system HZ—RT 1.9 min; system HAA—RT 5.0 min; system HAM—not detected; system HAX—RT 6.1 min; system HAY—RT 3.4 min.

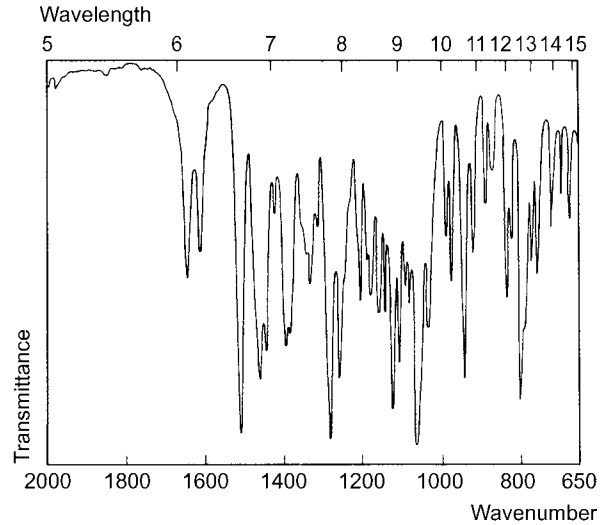
Column: Basic C_8 (150 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium phosphate dibasic (pH 5.8, 8 : 92), flow rate 1.0 mL/min. Fluorescence detection, (λ_{ex} = 214 nm, λ_{em} = 345 nm). Retention time: 15 min [Weingarten *et al.* 1995].

Column: µPorasil (300 × 3.9 mm i.d., 10 µm). Mobile phase: 5 mmol/L sodium acetate buffer (pH 3.75) : acetonitrile (25 : 75), flow rate 1.2 mL/min. ECD. Retention time: 8.24 min (*k*, 3.76) [Liaw *et al.* 1998].

Column: Hypersil BDS-C_{18} (100 × 46 mm i.d., 3 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 3) : acetonitrile (93 : 7), flow rate 1.0 mL/min. UV detection (λ = 212 nm). Retention time: 6.4 min for codeine, 5.5 min for norcodeine [Pascual, Sanagustin 1999].
Ultraviolet Spectrum Aqueous acid—285 nm (A^1_1 = 55a). No alkaline shift.



Infrared Spectrum Principal peaks at wave numbers 1052, 1268, 1500, 1111, 793, 934 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* (codeine) 299, 42, 162, 124, 229, 59, 300, 69; (morphine) 285, 162, 42, 215, 286, 124, 44, 284; (norcodeine) 285, 81, 215, 148, 286, 164, 110, 115.

Quantification

Blood **GC** NPD. Limit of detection, 100 µg/L [Lee, Lee 1991]. Column: Borosilicate OV-1 (25 m × 0.36 mm i.d.). Carrier gas: He, 35 cm/s. Temperature: 220°. ECD. Limit of detection, 5 µg/L [Edlund 1981].

GC-MS Temperature programme: 150° for 0.5 min to 320° at 25°/min for 3 min. SIM acquisition mode. Retention time: 4.65 min. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 305° at 10°/min for 0.5 min. EI ionisation. Limit of quantification, 10 µg/L, limit of detection, 2 µg/L [Meatherall 2005]. Column: Chromabond DB-1 C_{18} (30 m × 25 mm i.d., 0.25 µm). Carrier gas: He, 30 mL/min. Temperature programme: 100° to 300° at 20°/min. TIC, SIM acquisition mode. Limit of detection, <5 µg/L [Geier *et al.* 1996]. Column: OV1 (12 m × 0.2 mm i.d). Temperature programme: 150° for 2 min to 220° at 40°/min for 6 min. MSD. Retention time: 8.2 min. Limit of detection, <1 µg/L [Musshoff, Daldrup 1993]. Column: HP Ultra1 cross-linked methyl silicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 120° for 30 s to 220° at 40°/min to 244° at 4°/min to 300° at 40°/min. EI ionisation at 70 eV. Limit of detection, 0.05 µmol/L [Krogh *et al.* 1993]. See also Gjerde *et al.* [1991].

HPLC Column: Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: methanol : ammonium formate. Limit of quantification, 0.0005–0.01 mg/kg [Bjork *et al.* 2010]. Column: ODS-3 (150 × 3 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium formate (pH 3.0) : acetonitrile (95 : 5 for 5 min to 80 : 20 in 5 min for 5 min), flow rate 0.4 mL/min. ESI, SIM acquisition mode. Limit of quantification, 1 µg/L [Dienes-Nagy *et al.* 1999]. Column: Superspher RP-18 ODS (125 × 3 mm i.d., 4 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium formate buffer (pH 3.0, 5 : 95), flow rate 0.6 mL/min for 4 min to 1.1 mL/min in 3 min for 10 min. APCI, SIM acquisition mode. Limit of detection, 2.5 µg/L [Bogusz *et al.* 1997]. Column: Nova-Pak phenyl (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L sodium dihydrogen orthophosphate (pH 6.6, 10 : 90), flow rate 1.2 mL/min. UV detection (λ = 210 nm). Retention time: 19.2 min. Limit of detection, 0.06 mg/L [Crump *et al.* 1994].

LC-MS Column: Nova-Pak CN (100 × 3.9 mm i.d., 4 μm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate buffer (pH 3.0, 15 : 85). ESI, MRM acquisition mode. Limit of quantification, 2 μg/L, limit of detection, 1 μg/L [Coles *et al.* 2007].

Plasma **GC** Column: DB-17 fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 130° for 2 min to 300° at 8°/min. NPD. Retention time: 23.9 min. Limit of detection, 2.5 μg/L [Seno *et al.* 1995]. See Blood [Edlund 1981].

GC-MS See Blood [Geier *et al.* 1996]. Column: HP-1 (12 m × 0.25 mm i.d., 0.33 μm). Carrier gas: He, 5 psi. Temperature programme: 100° for 1 min to 250° at 20°/min to 257° at 2°/min. NICI. Limit of detection, <1 pg [Watson *et al.* 1995]. Limit of detection, 5 μg/L [Wang *et al.* 1994]. See Blood [Krogh *et al.* 1993].

HPLC Column: C_{18}.(150 × 2.1 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : 1% formic acid (70 : 10 : 20), flow rate 0.4 mL/min. SIM acquisition mode, *m/z* 300/165, positive ion mode. Limit of quantification, 0.2 μg/L [Liao *et al.* 2009]. Column: XTerra RP8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer (pH 6.5, 10 : 90 to 2 : 98 at 5 min to 20 : 80 at 7 min to 35 : 65 at 10 min to 50 : 50 at 15 min for 5 min to 10 : 90 at 22 min), flow rate 0.7 mL/min to 0.8 mL/min at 7 min until 20 min to 0.7 mL/min. DAD (λ= 285 nm). Limit of detection, 0.05 mg/L [Fernandez *et al.* 2007]. Column: XTerra RP8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : phosphate buffer (pH 6.53, 10 : 90 to 15 : 85 at 5 min to 20 : 80 at 7 min to 35 : 65 at 10 min to 50 : 50 at 15 min for 5 min to 10 : 90 at 22 min), flow rate 0.7 mL/min to 0.8 mL/min at 7 min until 20 min to 0.7 mL/min at 22 min. DAD (λ= 285 nm). Limit of detection, 0.032 mg/L [Fernandez *et al.* 2006]. Column: Hypersil BDS-C_{18} (100 × 4.6 mm i. d., 3 μm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 3.0) : acetonitrile (93 : 7), flow rate 1 mL/min. UV detection (λ= 212 nm). Retention time: 6.4 min. Limit of quantification, 1.56 μg/L, limit of detection, 0.47 μg/L [Pascual, Sanagustin 1999]. Column: MicroPorasil. Mobile phase: 5 mmol/L sodium acetate buffer (pH 3.75) : acetonitrile (25 : 75), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 8.24 min. Limit of detection, 0.14 μg/L [Liaw *et al.* 1998].

See also Chari *et al.* [1991], Freiermuth, Plasse [1997], He *et al.* [1998], Svensson *et al.* [1995], Vree, Verwey-van Wissen [1992] and Weingarten *et al.* [1995].

LC-MS See Blood [Coles *et al.* 2007].

Serum **GC-MS** Limit of quantification, 25 μg/L [Maresova *et al.* 2008]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 80° for 1 min to 200° at 20°/min to 240° at 5°/min to 310° at 30°/min for 6 min. SIM acquisition mode. Limit of quantification, 3.4 μg/L, limit of detection, 3.0 μg/L [Weinmann *et al.* 2000] Column: DB-5 dimethylpolysiloxane (25 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 100 kPa. Temperature programme: 150° for 1 min to 250° at 35°/min for 4 min to 300° at 30°/min. NICI. Limit of quantification, 0.15 μg/L [Hofmann *et al.* 1999]. See Blood [Musshoff, Daldrup 1993].

HPLC Column: Supelcosil LC-Si (250 × 2.1 mm i.d., 5 μm). Mobile phase: water : methanol : acetonitrile : formic acid (59.8 : 5.2 : 34.65 : 0.35), flow rate 230 μL/min. SIM acquisition mode. Limit of quantification, 4 μg/L [Zuccaro *et al.* 1997]. See Blood [Bogusz *et al.* 1997].

LC-MS See Blood [Coles *et al.* 2007].

Urine **GC** Column: EC-5 5% phenylpolysiloxane, 95% methylpolysiloxane (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2.0 mL/min. Temperature programme: 110° for 1 min to 260° at 32°/min. NPD. Limit of detection, 1.5 μg/L [Raikos *et al.* 2009]. Column: HP5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 200° for 0.5 min to 300° at 50°/min for 1.2 min. Retention time: 3.15 min. Limit of detection, 50 μg/L [Meadway *et al.* 2002]. See Blood [Seno *et al.* 1995]. Column: Chrompack (25 m × 0.32 mm i.d., 0.12 μm). Carrier gas: N_2, 1.3 mL/min. Temperature programme: 120° to 200° at 10°/min to 250° at 2°/min. NSD or FID. Limit of detection, 4 μg/L [Vu-Duc, Vernay 1990].

GC-MS See Blood [Goldberger *et al.* 2010]. See Serum [Hofmann *et al.* 1999]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 290° at 10°/min for 0.5 min. Limit of quantification, 25 μg/L, limit of detection, 10 μg/L [Meatherall 1999]. See CSF [Pragst *et al.* 1999].Column: DB-5MS (15 m × 0.25 mm i.d.). Carrier gas: He, 1.4 mL/min. Temperature programme: 120° for 0.3 min to 235° at 30°/min for 2 min. Limit of detection, 6 μg/L [Paul *et al.* 1999]. See also Broussard *et al.* [1997], Cremese *et al.* [1998], O'Neal, Poklis [1997] and Wang *et al.* [1994].

HPLC Column: Nova-Pak phenyl (150 × 3.9 mm i.d., 5 μm). Mobile phase: acetonitrile : 10 mmol/L sodium dihydrogen phosphate (pH 6.6, 10 : 90), flow rate 1.2 mL/min. Dual ultraviolet spectrophotometric and electrochemical detection. Retention time: 19.2 min. Limit of detection, 0.05 mg/L [Gerostamoulos *et al.* 1993]. See Plasma [Chari *et al.* 1991; He *et al.* 1998; Svensson *et al.* 1995; Vree, Verwey-van Wissen 1992]. See Blood [Bogusz *et al.* 1997].

LC-MS See Blood [Coles *et al.* 2007].

Bile **GC** See Blood [Lee, Lee 1991].

HPLC See Blood. Limit of detection, 0.2 mg/L [Crump *et al.* 1994].

CSF **GC-MS** Column: HP-5 (25 m × 0.25 mm i.d., 0.25 μm). Carrier gas: 1 mL/min. Temperature programme: 120° for 2 min to 280° at 20°/min for 10 min. SIM acquisition mode (*m/z* 282, 395). Limit of quantification, 1 μg/L [Pragst *et al.* 1999].

HPLC See Blood [Bogusz *et al.* 1997].See Plasma [Chari *et al.* 1991].

Meconium **LC-MS** See Blood [Coles *et al.* 2007].

Oral Fluid **GC-MS** Column: DB-5 (25 m × 0.2 mm i.d., 0.33 mm). Carrier gas: 1.5 mL/min. Temperature programme: 150° for 1.0 min to 245° at 20°/min for 8 min to 290° at 50°/min. EI ionisation. Limit of quantification, 2 μg/L, limit of detection, 2 μg/L [Jones *et al.* 2002]. See Plasma [Wang *et al.* 1994].

Vitreous Humour **GC-MS** See CSF [Pragst *et al.* 1999].

HPLC See Blood [Bogusz *et al.* 1997].

Hair **GC-MS** See Oral Fluid. Limit of quantification, 0.32 ng/mg, limit of detection, 0.03 ng/mg [Jones *et al.* 2002]. Column: 100% poly(dimethylsiloxane) (30 m × 0.245 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 80° for 1 min to 120° at 50°/min to 300° at 20°/min for 11 min. SIM acquisition mode. Limit of detection not reported [Brewer *et al.* 2001]. Column: Restek Rtx-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 260° at 15°/min to 290° at 7°/min for 6 min. Limit of detection, 0.01 ng/mg [Pichini *et al.* 1999]. Column: DB-1 capillary (30 m × 0.32 mm i.d., 1.0 μm). Carrier gas: He, 8 psi. Temperature programme: 120° for 0.1 min to 270° at 20°/min to 310° at 10°/min for 1.4 min. PICI, SIM acquisition mode (*m/z* 372). Limit of quantification, 0.5 mg/g [Hold *et al.* 1998]. Column: DB-5 MS capillary (30 m × 0.25 mm i.d.). Carrier gas: He, 7 psi. Temperature programme: 175° for 1 min to 300° at 15°/min for 0.5 min. Limit of detection, 10 pg [Wilkins *et al.* 1995]. See also Kintz, Mangin [1995] and Wang *et al.* [1994].

HPLC Column: silica-based C_{18} (150 × 4.6 mm i.d., 3 μm). Mobile phase: 20 μmol/L monobasic sodium phosphate : acetonitrile (90 : 10 to 50 : 50). Electrochemical detection. Retention time: 15.53 min. Limit of detection, 18 pg [Achilli *et al.* 1996].

LC-MS Column: Zorbax SB-Phenyl (2.1 × 100 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 25 μmol/L formic acid (5 : 95). ESI, positive ion mode [Hegstad *et al.* 2008].

Note For a review of the analysis of opioids by GC-MS, see Wasels, Belleville [1994].

Disposition in the Body

Codeine is well absorbed after oral administration, with peak plasma levels occurring after ~1 h; it is metabolised in the liver by *O*-demethylation involving P450 isozyme CYP2D6 to form morphine, *N*-demethylation to form norcodeine and conjugation to form glucuronides and sulfates of both unchanged drug and its metabolites. After an oral dose, ~86% is excreted in the urine in 24 h; of the excreted material, 40–70% is free or conjugated codeine, 5–15% is free or conjugated morphine, 10–20% is free or conjugated norcodeine and trace amounts may be free or conjugated normorphine; some of the dose is excreted in the bile and trace amounts are found in the faeces; unchanged drug accounts for 6–8% of the dose in urine in 24 h but this may increase to ~10% when the urinary pH is decreased. After an IM dose, 15–20% is excreted unchanged in acid urine in 24 h. Small amounts of hydrocodone, norhydrocodone, 6α-hydrocodol and 6β-hydrocodol have also been detected in urine.

Therapeutic Concentration

Thirteen healthy adult volunteers with a mean age of 36.5 years (range, 33–44) were administered 150 mg of the sustained-release formulation of codeine (Contin) every 12 h for 5 doses and an immediate-release codeine phosphate dose of 60 mg every 4 h for the first 3 doses followed by 30 mg every 4 h for 12 doses. After a single dose, the peak plasma concentration was 217.8 μg/L (range, 112.1–375.7) for the sustained-release formulation and 138.8 μg/L (range, 49.7–221.3) for the immediate release. These concentrations were observed at 2.3 h (range, 1.0–4.5) and 1.1 h (range, 0.5–2.0), respectively. The mean steady-state concentrations were 263.8 μg/L (range, 172.9–453.0) and 222.9 μg/L (range, 129.8–274.6), respectively, for the sustained-release and immediate-release formulations of codeine, and were attained at 3.2 h (range, 1.5–5.0) and 1.1 h (range, 0.5–1.5), respectively [Band *et al.* 1994].

Eight healthy male and female volunteers, aged 20–50 years, were administered an oral dose of 30 mg codeine phosphate (equivalent to 22 mg codeine). The mean peak plasma concentrations for codeine, codeine-6-phosphate and morphine 3-glucuronide, respectively, were 66.9 μg/L (range, 34.0–118.8), 968.0 μg/L (range, 693.0–1518.0) and 53.5 μg/L (range, 32.2–71.3). These concentrations were observed after 2 h [Vree, Verwey-van Wissen 1992].

In 11 women undergoing hysterectomy and using patient-controlled analgesia as a means of administering codeine, there was a nine-fold variation in the minimum plasma concentrations consistent with pain relief (40–350 μg/L). Two patients did not experience pain relief, one of whom had been classified as a poor metaboliser of dextromethorphan. In the 9 responding patients, the effective dose varied from 4.8–25.3 mg/h [Persson *et al.* 1995].

Toxicity The estimated minimum lethal dose is 800 mg, but codeine is much less toxic than morphine and death directly attributable to codeine is rare; in most fatalities involving codeine, other drugs and/or alcohol are also present. Drug addicts may use doses up to 10 times normal before showing toxic effects, while children may show toxicity with only 1/20th of the dose.

Of 107 drug-related deaths involving codeine, it was considered to be the major poison in six. The mean total concentration of codeine in femoral blood was 4.0 mg/L (range, 2.1–8.0), with the mean concentration of free codeine being 1.3 mg/L (range, 0.4–2.8). In the remaining 101 cases involving a combination of codeine and other drugs (most often paracetamol, diazepam, salicylates or ethanol), the mean total and free codeine blood concentrations were 1.8 mg/L (range, 0.04–26.00) and 0.82 mg/L (range, 0.02–9.00), respectively. In the absence of other contributing factors, total and free codeine concentrations of >2.0 and >0.4 mg/L, respectively, may be sufficient to cause death [Gerostamoulos *et al.* 1996].

In 39 deaths in which codeine was implicated, the postmortem blood concentrations for codeine ranged from <0.1–8.8 mg/L (mean, 1.2) and for morphine from <0.1–0.7 mg/L (mean, 0.16); in 22 of these deaths, the following concentrations ranges (mean) in other tissues and fluids were reported:

	Codeine	*Morphine*
Bile (mg/L)	0.5–43 (11)	<0.1–119 (33)
Kidney (μg/g)	<0.1–36.3 (7)	<0.1–12.4 (2)
Liver (μg/g)	<0.1–45 (5)	<0.1–6.4 (2)
Urine (mg/L)	3.2–229 (60)	<0.1–69.8 (20)

In all cases, other drugs were also present [Nakamura *et al.* 1976].

In 2 deaths involving the ingestion of large numbers of codeine tablets, postmortem blood concentrations of codeine of 15 and 48 mg/L were reported; urine concentrations were 155 and 370 mg/L; in both cases alcohol had also been ingested and in one case a low concentration of salicylate was also found in the blood [Peat, Sengupta 1977].

Bioavailability ~50%.

Half-life Plasma half-life, 2–4 h.

Volume of Distribution ~2.5–3.5 L/kg.

Clearance Plasma clearance, 10–15 mL/min/kg.

Protein Binding ~7–25%

Dose For pain relief, 30 to 60 mg every 4 h to a usual maximum of 240 mg daily.

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Codeine *N*-oxide

Narcotic

$C_{18}H_{21}NO_4 = 315.4$

IUPAC Name Codeine *N*-oxide

Synonym Genocodeine

Colour Tests Ammonium molybdate test—green→blue (limit of detection, 0.1 μg); sulfuric acid–formaldehyde test—deep purple (limit of detection, 0.1 μg); Vitali's test—yellow/faint orange/purple→chocolate-brown (limit of detection, 0.1 μg).

Thin-layer Chromatography System T1—R_f 0.23 (location reagent acidified iodoplatinate spray, positive reaction).

Quantification

Other **HPLC** Elixirs. Column: μBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: buffer (0.015 mol/L 1-butane sulfonic acid sodium salt, 0.015 mol/L potassium

phosphate monobasic, 2 mL triethylamine/L water, pH 4.8) : methanol (80 : 20), flow rate 2.0 mL/min. UV detection ($\lambda = 214$ nm). Retention times: codeine *N*-oxide 6.4 min, codeine phosphate 4.9 min, codeinone 8.8 min. Limit of detection, 0.25 wt.% with respect to parent compound [Sisco *et al.* 1986]. Tablets and Capsules. Column: μBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: buffer (0.015 mol/L potassium phosphate monobasic, 2 mL triethylamine/L water, pH 2.35) : methanol (93 : 7), flow rate 3.0 mL/min. UV detection ($\lambda = 214$ nm). Retention times: codeine *N*-oxide 4.2 min, codeine 5.3 min, codeinone 8.8 min. Limit of quantification not reported, sensitivity factors reported [Sisco *et al.* 1985].

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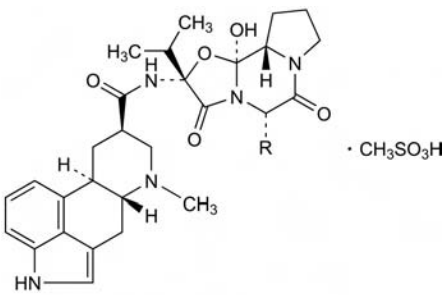
Codergocrine Mesilate

Vasodilator

CAS—11032-41-0 (codergocrine); 8067-24-1 (mesilate)

Synonyms Co-dergocrine methanesulfonate; DEA; DHAE; dihydroergotoxine mesilate; dihydrogenated ergot alkaloids; hydrogenated ergot alkaloids.

Proprietary Names *Circanol*; *Deapril-ST*; *Hydergin(e)*; *Progeril*.



Dihydroergocornine	$R = CH(CH_3)_2$
Dihydroergocristine	$R = CH_2C_6H_5$
Dihydro-α-ergocryptine	$R = CH_2CH(CH_3)_2$
Dihydro-β-ergocryptine	$R = CH(CH_3)CH_2CH_3$

Chemical Properties A mixture in equal proportions of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5$, $CH_4O_3S = 659.8$), dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5$, $CH_4O_3S = 707.8$), and α- and β-dihydroergocryptine mesilates ($C_{32}H_{43}N_5O_5$, $CH_4O_3S = 673.8$) in the ratio 2:1. A white to yellowish-white powder. Mp 196° to 206°, with decomposition. Soluble 1 in 50 of water, 1 in 30 of ethanol, 1 in 10 of acetone, and 1 in 100 of chloroform; practically insoluble in ether. pK_a 6.9 (24°).

Thin-layer Chromatography Codergocrine: system TA—R_f 0.66; system TB—R_f 0.01; system TC—R_f 0.48; system TL—R_f 0.29; system TM—R_f 0.64.

High Performance Liquid Chromatography System HP—dihydro-ergocristine *k* 18.3, dihydroergocryptine *k* 15.9.

Infrared Spectrum Principal peaks at the following wavenumbers (all KBr disk):

Dihydroergocornine 1633, 1662, 1720, 1705, 1558, 768 cm^{-1}.
Dihydroergocristine 1635, 1728, 1510, 1215, 760, 1540 cm^{-1}.
Dihydroergocryptine mesilate 1633, 1210, 1172, 1048, 1675, 1732 cm^{-1}.

Mass Spectrum Principal ions at the following *m/z*:

Codergocrine 70, 154, 125, 41, 43, 155, 42, 225.
Dihydroergocornine 70, 71, 269, 154, 195, 55, 59, 57.
Dihydroergocristine 125, 70, 91, 153, 41, 244, 43, 71.
Dihydroergocryptine 154, 70, 155, 167, 223, 225, 349, 153.

Quantification

Plasma **HPLC** Fluorescence detection. Limit of detection, 700 ng/L [Zecca *et al.* 1983].

Radioimmunoassay Limit of detection, 10 ng/L [Loh, Woodcock 1983].

Disposition in the Body Poorly absorbed after oral administration.

Therapeutic Concentration

Following a single oral dose of 4.5 mg to 8 subjects, peak plasma concentrations of 0.3 to 1.1 μg/L (mean 0.0006) were attained in 0.2 to 1.2 h [Woodcock *et al.* 1982].

Bioavailability 5 to 12%.

Half-life Plasma half-life, 8 to 25 h (mean 13).

Volume of Distribution 10 to 20 L/kg.

Clearance Plasma clearance, 20 to 30 mL/min/kg.

Dose Up to 4.5 mg daily, orally; 0.75 to 3 mg daily, sublingually.

Loh W, Woodcock BG (1983). Specificity and precision of a radioimmunoassay of dihydroergotoxine in plasma. *Arzneimittelforschung* 33: 568–570.
Woodcock BG *et al.* (1982). Dihydroergotoxine kinetics in healthy men after intravenous and oral administration. *Clin Pharmacol Ther* 32: 622–627.
Zecca L *et al.* (1983). Determination of dihydroergocristine and dihydroergotamine in plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 272(2): 401–405.

Colchicine

Gout Suppressant

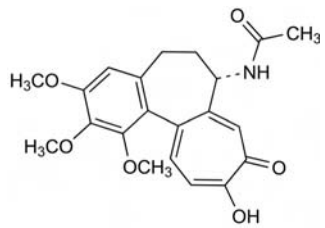
$C_{22}H_{25}NO_6 = 399.4$

CAS—64-86-8

IUPAC Name *N*-[(7*S*)-5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]-heptalen-7-yl]acetamide

Synonym Colchicinum

Proprietary Names *Colchineos*; *Colchiquim*; *Colcin*; *Colgout*; *Coluric*; *Ticolcin*. It is also an ingredient of *ColBenemid*, *Colchimax*, *Gripponyl*, and *Proben-C*.



Chemical Properties An alkaloid obtained from the corm and seeds of the meadow saffron *Colchicum autumnale* (Liliaceae) and other *Colchicum* species. Pale yellow to greenish-yellow crystals or amorphous scales or powder, darkening on exposure to light. Mp 142° to 150°. Soluble 1 in about 20 of water, but moderately concentrated aqueous solutions may deposit crystals of a sesquihydrate, which is less soluble in cold water than the anhydrous alkaloid. Freely soluble in ethanol and chloroform; soluble 1 in 220 of ether and 1 in 100 benzene; practically insoluble in petroleum ether. pK_a 12.35 (20°). Log *P* (octanol/water), 1.3. Extraction yield (chlorobutane), 0.13 [Demme *et al.* 2005].

Colour Tests Liebermann's reagent—green; Mandelin's test—green; Marquis test—yellow

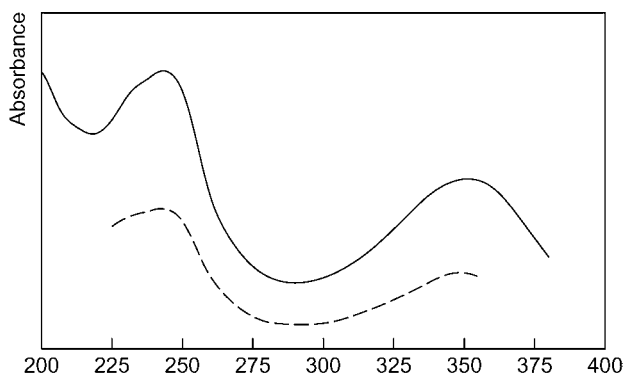
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.00; system TC—R_f 0.37; system TE—R_f 0.33; system TL—R_f 0.12; system TAE—R_f 0.69; system TAF—R_f 0.63; system TAJ—R_f 0.39; system TAK—R_f 0.08; system TAL—R_f 0.73 (van Urk reagent, yellow).

Gas Chromatography System GA—RI 3200.

High Performance Liquid Chromatography System HA—*k* 0.2; system HX—RI 382; system HY—RI 327; system HZ—retention time 2.1 min; system HAX—retention time 5.1 min; system HAY—retention time 4.1 min.

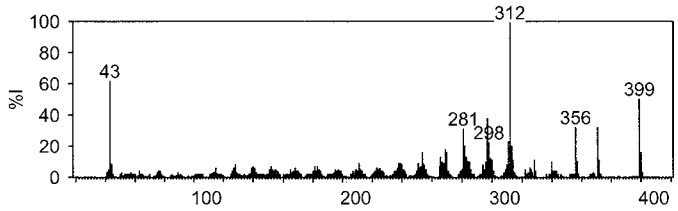
Column: RP C_{18} (Novapak, 150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L potassium dihydrogen phosphate (with 5 μmol/L pentane sulfonic acid, pH 6.0) : methanol : acetonitrile (60:26.6:13.4), flow rate 0.8 mL/min. UV detection (λ=245 nm). Retention time: 6.6 min [McIntyre *et al.* 1994].

Ultraviolet Spectrum Ethanol—243 (A^1_1=730a), 350 nm (A^1_1=425a).



Infrared Spectrum Principal peaks at wavenumbers 1250, 1550, 1588, 1610, 1090, 1655 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 312, 43, 399, 297, 356, 281, 371, 311.



Quantification

Blood **HPLC** UV detection (λ=350 nm). Limit of detection, 1.0 µg/L [Kintz *et al.* 1997]. UV detection. Limit of detection, 10 µg/L [Caplan *et al.* 1980].

HPLC-MS Limit of detection, 0.6 µg/L [Tracqui *et al.* 1996].

Plasma **Spectrofluorimetry** [Bourdon, Galliot 1976].

HPLC UV detection. Limit of detection, 5 µg/L [Jarvie *et al.* 1979].

HPLC-MS See Blood [Tracqui *et al.* 1996].

Radioimmunoassay Comparison of three RIAs. Limit of detection, 0.2 µg/L [Sabouraud *et al.* 1994].

Serum **HPLC** (λ=254 nm). Limit of detection, 0.4 µg/L for desacetylcolchicine and 4.0 µg/L for colchicine [Ko *et al.* 1990].

Radioimmunoassay Limit of detection, 350 ng/L [Scherrmann *et al.* 1980].

Urine **Spectrofluorimetry** See Plasma [Bourdon, Galliot 1976].

HPLC See Serum [Ko *et al.* 1990].

HPLC-MS See Blood [Tracqui *et al.* 1996].

Tissue **HPLC** Column: C_{18} ODS (Hypersil, 125 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.03 mol/L ammonium sulfate (with 1% TEA, pH 3.0); (25:75) for 5 min, gradient to (40:60) in 5 min, held for 10 min; flow rate 1 mL/min. IS: methylclonazepam. UV detection (λ=340 nm). Retention time(s): colchicine, 8.1 min; IS, 18.0 min. Limit of detection, 5 ng/g [Dehon *et al.* 1999].

Disposition in the Body Readily absorbed after oral administration with peak plasma concentrations reached within 2 h. It is partially deacetylated in the liver to desmethylcolchicine. Most of the drug is excreted in the faeces but 10 to 20% is excreted in the urine. Colchicine is excreted in the bile and undergoes enterohepatic circulation. Colchicine is distributed in the kidney, liver, and spleen, but little is found in the heart, skeletal muscle, and brain.

Therapeutic Concentration

In 30 patients treated for familial Mediterranean fever with colchicine 0.5 to 2 mg daily, there was a large inter-subject variability in plasma colchicine concentration (0.13 to 1.75 µg/L 24 h after a dose of 1 mg daily) [Chappey *et al.* 1994].

After administration of a single oral dose of 1 mg colchicine to 8 patients with chronic liver disease, a mean peak plasma concentration of 3.6 µg/L (range 0.4 to 10.0) was attained at 2.16 h. Repeated administration resulted in comparable values. Concentrations in bile were higher (2.025 mg/L) [Rudi *et al.* 1994].

Steady-state serum concentrations of 0.3 to 2.4 µg/L were reported after daily oral doses of 1 mg given to 8 subjects [Halkin *et al.* 1980].

Toxicity The minimum lethal dose in man is about 6 mg (although recovery has occurred after much larger doses).

A 73-year-old man was hospitalised 8 h after receiving a 1 mg IV dose of colchicine for gouty arthritis and died 10 h later; he had consumed 8 × 0.6 mg colchicine tablets over the previous 8 days. The following postmortem colchicine tissue concentrations were reported: cardiac blood 50 µg/L, vitreous humour 10 µg/L, liver 0.575 µg/g, bile 12 000 µg/L, gastric contents 4.4 µg in 60 g [Jones *et al.* 2002].

After ingestion of about 40 flowers of *Colchicum autumnale* L., a 44-year-old man was admitted 2 h later with nausea, vomiting, and abdominal pains followed by diarrhoea after 14 h. Maximal colchicine levels were 4.34 µg/L at 13 h in plasma and 5.43 µg/L at 16 h in erythrocytes [Danel *et al.* 2001].

In a reported fatality following the ingestion of colchicine tablets, a 42-year-old man had the following levels in blood and postmortem tissues: plasma, 4.5 µg/L (after 24 h); kidney, 396 µg/g; liver, 347 µg/g; heart, 334 µg/g; lung, 58 µg/g; muscle, 10 µg/g; and brain, 5 µg/g [Dehon *et al.* 1999].

In a suicidal overdose resulting from the ingestion of colchicine tablets, the femoral blood level was 62 µg/L and the maximum concentration found in bile was 2921 µg/L [Kintz *et al.* 1997].

Half-life Plasma half-life, about 1 h.

Volume of Distribution About 0.7 to 2 L/kg.

Protein Binding About 30 to 50%.

Dose For an acute attack of gout, 1 mg initially, followed by 500 µg every 2 or 3 h; maximum total dose of 6 mg; a second course may be taken after an interval of at least 3 days.

Bourdon R, Galliot M (1976). [Determination of colchicine in biological fluids]. *Ann Biol Clin (Paris)* 34: 393–401.

Caplan YH *et al.* (1980). A fatal overdose with colchicine. *J Anal Toxicol* 4: 153–155.

Chappey O *et al.* (1994). Colchicine concentration in leukocytes of patients with familial Mediterranean fever. *Br J Clin Pharmacol* 38: 87–89.

Danel VC *et al.* (2001). Self-poisoning with Colchicum autumnale L. flowers. *J Toxicol Clin Toxicol* 39: 409–411.

Dehon B *et al.* (1999). Colchicine poisoning: report of a fatal case with body fluid and post-mortem tissue analysis by high-performance liquid chromatography. *Biomed Chromatogr* 13: 235–238.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Halkin H *et al.* (1980). Colchicine kinetics in patients with familial Mediterranean fever. *Clin Pharmacol Ther* 28: 82–87.

Jarvie D *et al.* (1979). Estimation of colchicine in a poisoned patient by using high performance liquid chromatography. *Clin Toxicol* 14: 375–381.

Jones GR *et al.* (2002). Application of LC-MS analysis to a colchicine fatality. *J Anal Toxicol* 26: 365–369.

Kintz P *et al.* (1997). Colchicine poisoning: report of a fatal case and presentation of an HPLC procedure for body fluid and tissue analyses. *J Anal Toxicol* 21: 70–72.

Ko RJ *et al.* (1990). Determination of the antimitotic agents N-desacetylcolchicine, demecolcine and colchicine in serum and urine. *J Chromatogr* 525: 411–418.

McIntyre IM *et al.* (1994). Death following colchicine poisoning. *J Forensic Sci* 39: 280–286.

Rudi J *et al.* (1994). Plasma kinetics and biliary excretion of colchicine in patients with chronic liver disease after oral administration of a single dose and after long-term treatment. *Scand J Gastroenterol* 29: 346–351.

Sabouraud A *et al.* (1994). Radioimmunoassay of colchicine with antisera exhibiting variable cross-reactivity. *Ther Drug Monit* 16: 179–185.

Scherrmann JM *et al.* (1980). A sensitive radioimmunoassay for colchicine. *J Pharm Pharmacol* 32: 800–802.

Tracqui A *et al.* (1996). High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. *J Chromatogr B Biomed Appl* 675(2): 235–242.

Colecalciferol

Vitamin

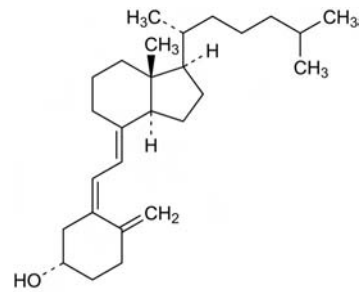
$C_{27}H_{44}O$ = 384.6

CAS—67-97-0

IUPAC Name (1*S*,3*Z*)-3-[(2*E*)-2-[(1*R*,3*aS*,7*aR*)-7*a*-Methyl-1-[(2R)-6-methylheptan-2-yl]-2,3,3*a*,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexan-1-ol

Synonyms Activated 7-dehydrocholesterol; cholecalciferol; (3β,5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3-ol; vitamin D_3.

Proprietary Names *D-Mulsin*; *D_3-Vicotrat forte*; *Neo-Dohyfral D_3*.



Chemical Properties White crystals. It is affected by air and light, and should be stored in a cool place in hermetically sealed containers in which the air has been replaced by an inert gas. Solutions in volatile solvents are unstable. Mp about 84°. Practically insoluble in water; freely soluble in ethanol, chloroform, and ether. Log *P* (octanol/water), 10.2.

Colour Test To a 10% solution in chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid—red→violet→blue-green.

Gas Chromatography System GA—colecalciferol RI 3150, colecalciferol-H_2O RI 3130.

Ultraviolet Spectrum Ethanol—265 nm (A_1^1=480a).

Infrared Spectrum Principal peaks at wavenumbers 1052, 894, 901, 967, 862, 1075 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection. Colecalciferol, ergocalciferol and the 25-hydroxy metabolites, limit of detection, 500 ng/L [Jones 1978].

Disposition in the Body Well absorbed after oral administration and subject to enterohepatic circulation; decreased absorption may occur in subjects with impaired liver and biliary function. Metabolised by hydroxylation to active metabolites. The major metabolite is 25-hydroxycolecalciferol which is formed in the liver. This is further metabolised by 1α- or 24-hydroxylation in the kidneys. Most of a dose is excreted in the bile and eliminated in the faeces; about 25% of a dose is excreted as conjugates. Unchanged colecalciferol does not appear to be excreted in the urine.

Blood Concentration Normal serum concentrations of 25-hydroxycolecalciferol are about 15 to 40 µg/L, but there are considerable inter-subject and seasonal variations.

Half-life Plasma half-life, about 1 to 2 days; after IV administration a terminal elimination half-life of about 18 days has been reported.

Protein Binding Bound to some extent to globulins and to lipoproteins.

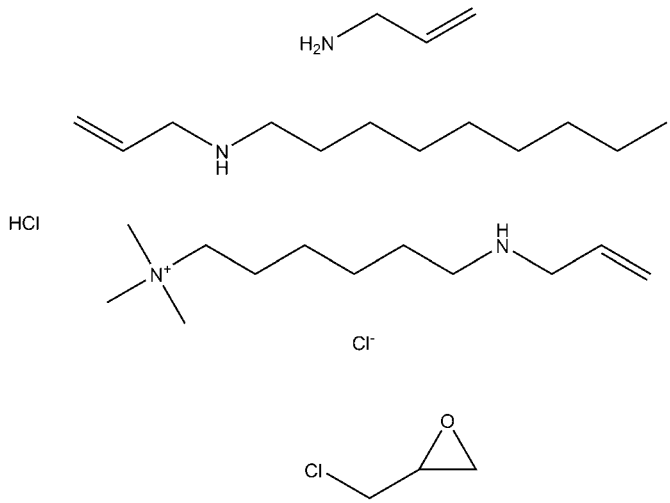
Dose 0.025 to 5 mg daily.

Jones G (1978). Assay of vitamins D2 and D3, and 25-hydroxyvitamins D2 and D3 in human plasma by high-performance liquid chromatography. *Clin Chem* 24: 287–298.

Colesevelam Hydrochloride

Antihyperlipidaemic, Bile Acid Sequestrant
$C_{31}H_{66}Cl_2N_4O$, HCl = 618.3
CAS—182815-44-7

IUPAC Names 2-(Chloromethyl)oxirane; prop-2-en-1-amine; *N*-prop-2-enyldecan-1-amine; trimethyl-[6-(prop-2-enylamino)hexyl]azanium; chloride; hydrochloride.
Synonyms GT-31-104; *N,N,N*-trimethyl-6-(2-propenylamino)-1-hexanaminium chloride polymer with (chloromethyl)oxirane, 2-propen-1-amine, and *N*-2-propenyl-1-decanamine, hydrochloride.
Proprietary Names *CholestaGel*; *WelChol*.



Disposition in the Body Colesevelam forms non-absorbable complexes with bile acids and is excreted entirely through the gastrointestinal tract.
Therapeutic Concentration Sixteen healthy volunteers were administered non-radiolabelled colesevelam hydrochloride 1.9 g twice daily for 4 weeks, followed by a single dose of 2.4 g [^{14}C]colesevelam (480 pCi). Total recovery of [^{14}C]colesevelam was 0.05 ± 0.01 and $74 \pm 27\%$ in urine and faeces, respectively. The mean faecal recovery was $82 \pm 16\%$. The mean maximum concentration of 0.165 ± 0.1 µg equiv/g was reached at 72 h after the administered dose [Heller *et al.* 2002]. Colesevelam has been shown to have no effect on the pharmacokinetics of lovastatin when co-administered at dinner [Donovan *et al.* 2002].

Colesevelam hydrochloride was co-administered with fenofibrate to investigate its effect on fenofibrate metabolism. Co-administration of 3750 mg colesevelam with 160 mg fenofibrate resulted in an approximate 20% reduction in the mean peak plasma concentration of fenofibric acid. There were no significant differences in the time to reach maximum plasma concentration, elimination rate constant or elimination half-life [Jones *et al.* 2004].

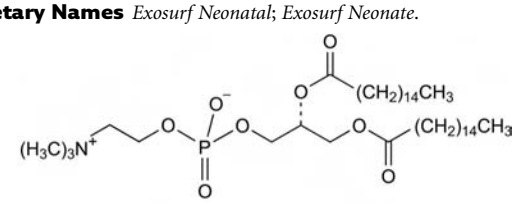
Dose 3.75 g daily orally alone or divided into 2, may be increased to 4.375 g. When combined with statins the dose is usually 2.5 to 3.75 g daily.

Donovan JM *et al.* (2002). Effect of colesevelam on lovastatin pharmacokinetics. *Ann Pharmacother* 36: 392–397.
Heller DP *et al.* (2002). Absorption of colesevelam hydrochloride in healthy volunteers. *Ann Pharmacother* 36: 398–403.
Jones MR *et al.* (2004). Effect of colesevelam HCl on single-dose fenofibrate pharmacokinetics. *Clin Pharmacokinet* 43: 943–950.

Colfosceril Palmitate

Lung Surfactant
$C_{40}H_{80}NO_8P$ = 734.0
CAS—63-89-8

IUPAC Name [(2*R*)-2,3-Di(hexadecanoyloxy)propyl] 2-(trimethylazaniumyl) ethyl phosphate
Synonyms Dipalmitoyl phosphatidylcholine; DPCC; (7*R*)-4-hydroxy-*N,N,N*-trimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,5,9-trioxa-4-phosphapentacosan-1-aminium inner salt 4-oxide.
Proprietary Names *Exosurf Neonatal*; *Exosurf Neonate*.



Chemical Properties A sterilised lyophilised powder. Mp 234° to 235°. Readily soluble in chloroform, hot di-isobutyl ketone, hot dioxane. Solubility at 22° to 23° (g/100 mL): ethanol 1.5, ether 0.02, acetone 0.02, pyridine 1.1, acetic acid 4.0, methanol 1.4. Log *P* (octanol/water), 9.81.
Thin-layer Chromatography Plate: silica gel (LK5, 20 × 20 cm). Mobile phase: chloroform : methanol : 1-propanol : potassium chloride (0.25%) : ethyl acetate (25:13:25:9:25 mL). Reference compounds: phosphatidylcholine, sphingomyelin, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and phosphatidyl-glycerol. Detection by spraying with cupric acetate (3%):phosphoric acid (8%). Rf 0.35.

Plate: silica gel (LK5, 20 × 20 cm). Mobile phase: chloroform : methanol : 2-propanol : potassium chloride (0.25%) : triethylamine (30:9:25:6:18 mL). Rf 0.26.

Plate: LHPK (HPTLC) (10 × 10 cm). Mobile phase: chloroform : methanol : 2-propanol : water : triethylamine (30:9:27:8:25 mL). Rf 0.23.

Plate: LHPK (HPTLC) (10 × 10 cm). Mobile phase: chloroform : ethanol : water : triethylamine (30:34:7:35 mL). Rf 0.21 [Touchstone *et al.* 1980].

Quantification
Endotracheal Tube Aspirate **HPLC** Column: ODS silica (Apex II, 25 cm). Mobile phase: 40 mmol/L choline chloride in methanol : water (92.5:7.5), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=340 nm, λ_{em}=460 nm). Retention time: colfosceril palmitate, 39.7 min. Limit of detection, 0.2 ng/L [Ashton *et al.* 1992].
Disposition in the Body Colfoscerol palmitate is absorbed from the alveolus into the lung tissue where it is reused for further phospholipid synthesis and secreted into the alveolus as new surfactant. Metabolism occurs and the products are incorporated into the body pools. Elimination is mainly through expired air with a small amount in faeces and urine.
Therapeutic Concentration

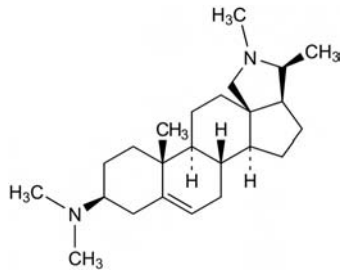
Twenty-two infants of less than 34 weeks' gestation and ventilated from day 1 for respiratory distress syndrome, were administered two doses of 67.5 mg colfosceril palmitate (DPPC) in the first 24 h. Percentage DPPC (of total phosphatidylcholine) reached 100% within 2 days [Ashton *et al.* 1992].

Toxicity Associated with cytotoxicity.
Half-life Mean half-life of the phosphatidylcholine component, 30 h.
Dose A single dose of 67.5 mg/kg birth weight of colfoscerol palmitate is administered. Doses may be repeated 12 and 24 h later.

Ashton MR *et al.* (1992). Turnover of exogenous artificial surfactant. *Arch Dis Child* 67: 383–387.
Touchstone JC *et al.* (1980). *Lipids* 15(1): 61–62.

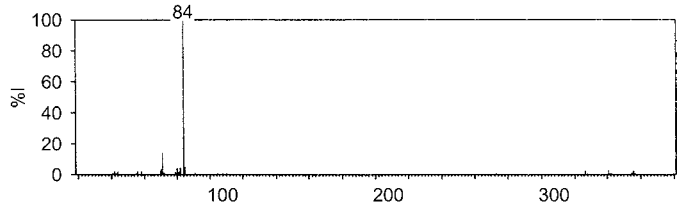
Conessine

Antiamoebic
$C_{24}H_{40}N_2$ = 356.6
CAS—546-06-5
Synonym (3β)-*N,N*-Dimethylcon-5-enin-3-amine



Chemical Properties An alkaloid obtained from the seeds of *Holarrhena antidysenterica* (Apocynaceae). A white crystalline powder. Mp 127° to 129°. Sparingly soluble in water. Log *P* (octanol/water), 5.1.

Conessine Hydrobromide
$C_{24}H_{40}N_2$, 2HBr = 518.4
CAS—5913-82-6
Synonym Conessine bromide
Chemical Properties White acicular crystals or microcrystalline powder. Mp 340°, with decomposition. Soluble 1 in 5 of water and 1 in 11 of ethanol (90%); very slightly soluble in ether; practically insoluble in petroleum ether.
Colour Tests Mandelin's test—yellow; Marquis test—yellow→orange.
Thin-layer Chromatography System TA—R_f 0.28; system TB—R_f 0.49; system TC—R_f 0.03; system TL—R_f 0.03 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Mass Spectrum Principal ions at *m/z* 84, 71, 85, 82, 80, 341, 70, 356.

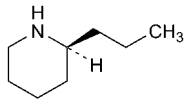


Dose Conessine hydrobromide was formerly given in doses of 300 to 500 mg daily.

Coniine

Alkaloid
$C_8H_{17}N = 127.2$
CAS—458-88-8

IUPAC Name (2*S*)-2-Propylpiperidine
Synonyms Cicutine; conicine; conine.



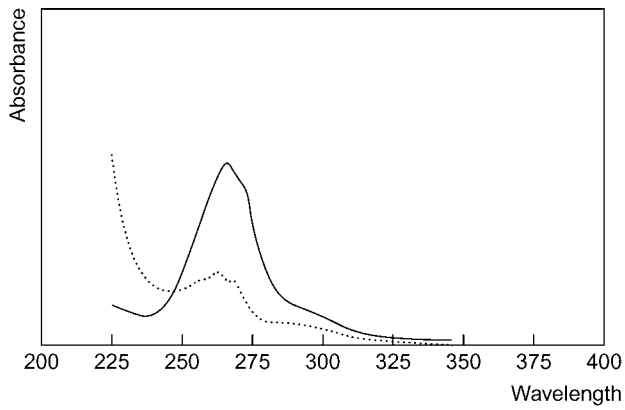
Chemical Properties An alkaloid obtained mainly from the fruits and leaves of hemlock, *Conium maculatum* (Umbelliferae). An almost colourless volatile liquid. Fp –2°. Bp 166°. Soluble 1 in 100 of water; miscible with ethanol and ether; slightly soluble in chloroform. pK_a 11.0 (25°). Log *P* (octanol/water) 2.6. In extracts, coniine was stable at high and low concentrations for >24 h, before and after 3 freeze-thaw cycles and after storage at −20° for 1 month [Beyer *et al.* 2007].

Coniine Hydrobromide

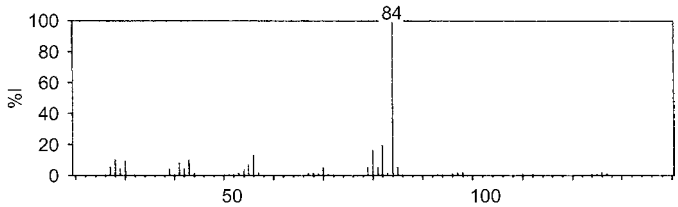
$C_8H_{17}N$, HBr = 208.1
CAS—637-49-0

Chemical Properties Colourless crystals. Mp ≈211°. Soluble 1 in 2 of water and 1 in 3 of ethanol; soluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.26; system TAE—R_f 0.05; system TAF—R_f 0.70; system TL—R_f 0.05; system TB—R_f 0.39; system TC—R_f 0.13; system TE—R_f 0.37 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 214.
Ultraviolet Spectrum Aqueous acid—266 nm (A_1^1 = 6b); aqueous alkali—262, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1033, 1007, 1575, 1300, 1078, 1139 cm^{-1} (coniine hydrobromide (Nujol mull)).
Mass Spectrum Principal ions at *m/z* 84, 82, 80, 56, 43, 28, 30, 41.



Quantification

Plasma **LC-MS** Column: LiChroCART (125 × 2 mm i.d.). Mobile phase: 50 mmol/L aqueous ammonium formate (pH 3.5) : acetonitrile (90 : 10 for 2 min to 20 : 80 at 5 min for 2 min to 90 : 10 at 10 min), flow rate 0.4 mL/min for 2 min to 0.6 mL/min for 5 min to 0.4 mL/min for 3 min. APCI, positive ion mode, SIM acquisition mode or tandem MS, ESI, MRM acquisition mode. Limit of quantification, 50 and 0.1 µg/L for single stage MS and tandem MS, respectively [Beyer *et al.* 2007].
Other **GC-MS** Plants, Hay and Cattle Urine. Column: HP-1 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 psi. Temperature programme: 40° for 2 min to 80° at 20°/min to 90° at 5°/min to 200° at 45°/min. Limit of detection not reported [Galey *et al.* 1992]. Chicken, Quail and Turkey Tissue. Column: DB-17 capillary (30 m × 0.25 mm i.d.). Temperature programme: 50° for 10 min to 240° at 10°/min. EI ionisation at 70 eV and CI. Limit of detection, 0.58 mg/L [Frank, Reed 1990].

HPLC Plant Extracts. Column: Betasil C_{18} (100 × 2.1 mm i.d.). Mobile phase: 20 mmol/L ammonium acetate : methanol (50 : 50), flow rate 0.5 mL/min. UV detection. Limit of detection not reported [Lee *et al.* 2008].

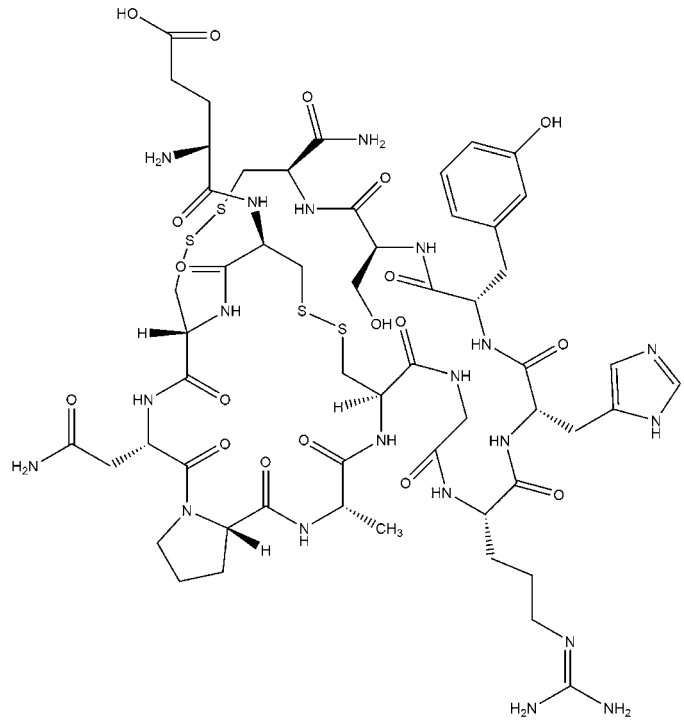
Disposition in the Body

Toxicity Coniine is well absorbed from the gastrointestinal tract and is very poisonous; the estimated minimum lethal dose is 150 mg and toxic symptoms may occur after ingestion of 60 mg. Death may occur within 30 min or be delayed 3 to 12 h. Coniine resembles nicotine in its peripheral action but produces more pronounced paralysis of the CNS and of the skeletal muscle nerve-endings.
Note For a review of the clinical manifestations of accidental poisoning by hemlock, see Rizzi *et al.* [1991]. For a non-fatal case of a 4-year-old male who ingested poison hemlock, see Frank *et al.* [1995]. For a review of 11 cases of hemlock poisoning, see Rizzi *et al.* [1989] and for cases of acute renal failure after hemlock poisoning, see Scatizzi *et al.* [1993].

Beyer J *et al.* (2007). Detection and validated quantification of toxic alkaloids in human blood plasma–comparison of LC-APCI-MS with LC-ESI-MS/MS. *J Mass Spectrom* 42: 621–633.
Frank AA, Reed WM (1990). Comparative toxicity of coniine, an alkaloid of *Conium maculatum* (poison hemlock), in chickens, quails, and turkeys. *Avian Dis* 34: 433–437.
Frank BS *et al.* (1995). Ingestion of poison hemlock (*Conium maculatum*). *West J Med* 163: 573–574.
Galey FD *et al.* (1992). Toxicosis in dairy cattle exposed to poison hemlock (*Conium maculatum*) in hay: isolation of Conium alkaloids in plants, hay, and urine. *J Vet Diagn Invest* 4: 60–64.
Lee ST *et al.* (2008). Separation and measurement of plant alkaloid enantiomers by RP-HPLC analysis of their Fmoc-Alanine analogs. *Phytochem Anal* 19: 395–402.
Rizzi D *et al.* (1989). Rhabdomyolysis and acute tubular necrosis in coniine (hemlock) poisoning. *Lancet* 2: 1461–1462.
Rizzi D *et al.* (1991). Clinical spectrum of accidental hemlock poisoning: neurotoxic manifestations, rhabdomyolysis and acute tubular necrosis. *Nephrol Dial Transplant* 6: 939–943.
Scatizzi A *et al.* (1993). Acute renal failure due to tubular necrosis caused by wildfowl-mediated hemlock poisoning. *Ren Fail* 15: 93–96.

Conotoxins

Neurotoxin



Chemical Properties Conotoxins are potent neurotoxins produced by the venom of the marine cone snail, genus *Conus*. They are peptides of 10 to 30 amino acid residues, typically with one or more disulfide bond. There have been five conotoxins identified so far:

Toxin	*Target*
α	Acetylcholine receptors
δ	Inhibits the inactivation of voltage-activated calcium channels (VACC)
	Inhibits potassium channels
µ	Inhibits VACC in muscles
ω	Inhibits *N*-type VACC

Ziconitide

Analgesic/Calcium Channel Blocker/Neuroprotective
$C_{102}H_{172}N_{36}O_{32}S_7 = 2639.13$
CAS—107452-89-1

Synonyms ω-Conotoxin MVIIA; ω-conopeptide MVIAA; SNX-111; CI-1009.
Proprietary Name *Prialt* (Elan).
Chemical Properties An ω-toxin derived from *Conus magus*. Acts as a selective N-type VACC blocker.
Use As a pharmacological tool to target VACC; licensed by FDA for the relief of intrathecal pain.

Copper

Metal
Cu = 63.55
CAS—7758-98-7

Chemical Properties Reddish solid. Mp 1083°. Bp 2595°. Insoluble in water. Valencies: Cu(O), Cu(+1), Cu(+2), Cu(+3). Found in nature in the elemental form and also in many minerals: cuprite, malachite, azurite, chalcopyrite, chalcocite and bornite. Extensively used in all industries and many applications because of its durability, ductility, malleability, and electrical and thermal conductivity.

Copper Sulfate

$CuSO_4$ = 159.6
Synonyms Blue copperas; blue stone; blue vitriol; copper (II) sulphate; cupric sulphate; Roman vitriol; Salzburg vitriol.
Chemical Properties Blue crystals. Decomposes at 560°. Very soluble in water; soluble in methanol; slightly soluble in ethanol. Used in metal finishing, mineral froth flotation, wood preservatives, water treatment, fungicides, algicides, petroleum refining.

Colour Tests Applicable to gastric contents and scene residues. Place a small volume (0.1 mL) of sample on to a filter paper to give a spot of an approximate diameter of 1 cm. Expose the spot to concentrated ammonia fumes and add 0.1 mL of a methanolic solution of dithiooximide (10 g/L)—Copper salts give an olive-green stain. Chromium salts also give a green stain, but usually before the dithiooximide is added. Several other metals give yellow-brown or red-brown colours. Limit of detection, 1 mg/L.

Confirmatory test Place 0.1 mL of the sample in a spotting-tile well and add 0.05 mL of 0.01 mol/L hydrochloric acid. Mix 0.1 mL of ammonium mercurithiocyanate reagent (prepared by mixing 8 g of mercuric chloride and 9 g of ammonium thiocyanate in 100 mL of water) with 0.1 mL of zinc acetate solution (10 g/L) and add to the sample in the well.—Copper salts form a violet precipitate of zinc mercurithiocyanate. Limit of detection, 50 mg/L

Quantification

Specimen Collection Blood—5 mL, K-EDTA tube; urine—sample of 24 h urine collection (for investigation of copper-related disease).
Blood **DPASV** Limit of detection, 2 µg/L [Moreno *et al.* 1999].

AAS Gas flow: 4.5 L/min. Acetylene: 1.1 L/min (λ = 324.8 nm). Limit of detection not reported [Piekoszewski *et al.* 2000]. Gas flow: 2.3 mL/min. Hollow cathode lamp (λ = 324.7 nm). Limit of detection not reported [Chatterjee *et al.* 1994].

ETAAS Dry cycle: 78° for 40 s to 82° for 10 s to 86° for 5 s. Char cycle: 600° for 20 s. Atomisation cycle: 2600° for 7 s. Carrier gas: Ar, 60 mL/min. Zeeman AA mode (λ = 324.8 nm). Limit of detection, 1.3 µg/L [Liska *et al.* 1985].

FAAS Oxidant: air, 1.60 kg/cm^2. Fuel: acetylene, 0.30 kg/cm^2. Zeeman AA mode (λ = 324.8 nm). Flame continuous aspiration, microsampler/peak area, or microsampler/peak height mode. Limit of detection, 4.1, 10.3 and 25.3 µg/L for each mode, respectively [Liska *et al.* 1985].

ICP-AES Limit of detection, 10 µg/kg [Dinya *et al.* 2005]. Meinhard or Babington nebuliser (λ = 324.8 nm). Limit of detection, 1.7 and 2.2 µg/L for each nebuliser, respectively [Prohaska *et al.* 2000].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 1.13 L/min. Nebuliser gas: 1.0 mL/min. Limit of detection not reported [Rainska *et al.* 2007]. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 5 µg/L [De Boer *et al.* 2004].
Plasma **ETAAS** See Blood [Liska *et al.* 1985].

FAAS See Blood [Liska *et al.* 1985].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 0.6 L/min. Nebuliser gas: 0.93 L/min. Limit of detection not reported [Venelinov *et al.* 2004].
Serum **ETAAS** See Blood [Liska *et al.* 1985].

FAAS See Blood [Liska *et al.* 1985]. Perkin-Elmer hollow cathode lamp (λ = 324.8 nm). Limit of detection, not reported [Weinstock, Uhlemann 1981].

ICP-AES Plasma gas: Ar, 12 L/min. Nebuliser gas: Ar, 0.5 L/min. Nebuliser pressure: 2.3 bar. (λ = 324.8nm). Limit of detection, 0.02 µmol/L [Chappuis *et al.* 1992].

ICP-MS Plasma gas: Ar, 11.0 L/min. Auxiliary gas: Ar, 1.4 L/min. Nebuliser gas: Ar, 0.9 to 1.0 L/min. Limit of detection, 0.03 µg/L [Gercken, Barnes 1991]. Plasma gas: 13 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 0.72 L/min. Limit of detection not reported [Vanhoe *et al.* 1989].
Oral Fluid **ICP-MS** Plasma gas: 13 L/min. Auxillary gas: 0.55 L/min. Nebuliser gas: 0.1 L/min. Limit of detection, 0.04 µg/L [Menegario *et al.* 2001].
Urine **ETAAS** Dry cycle: 110° at 1 s for 20 s to 130° in 15 s for 20 s. Char cycle: 1250° in 30 s for 15 s; change gas from Ar to 5% H_2 in Ar for 5 s. Atomisation cycle: 2300° for 5 s; normal gas, Ar 250 mL/min; purge gas, 5% H_2 in Ar 250 mL/min. Hollow cathode lamp (λ = 324.8 nm). Limit of detection, 0.08 µg/L [Lin, Huang 2001]. Dry cycle: 110° at 7 s for 13 s. Char cycle: 900° in 7 s for 23 s. Atomisation cycle: 2700° in 2 s for 10 s, gas flow: 10 mL/min (λ = 324.8 nm). Limit of detection not reported [Halls *et al.* 1981].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 1.13 L/min. Nebuliser gas: 1.0 mL/min. Limit of detection, not reported [Rainska *et al.* 2007]. See Blood. Limit of detection, 2.5 µg/L [De Boer *et al.* 2004].
Note For a direct spectrophotometric method for determining copper in urine, see Jerònimo *et al.* [2004].
Bone **FAAS** Carrier gas: air-acetylene (λ = 324.8 nm). Limit of detection not reported [Baranowska *et al.* 1995].
Hair **AAS** Gas flow: 2.3 mL/min. Hollow cathode lamp (λ = 324.7 nm). Limit of detection not reported [Chatterjee *et al.* 1994].

ETAAS Dry cycle: 80° to 120° in 10 s. Char cycle: 300° to 400° at 10 s. Atomisation cycle: 2700° to 2800° in 5 s. Carrier gas: 200 mL/min. Hitachi model 180-50, S.N.5721-2 (λ = 193.8 nm). Limit of detection, not reported [Kazi *et al.* 2006].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 0.8 L/min. Nebuliser gas: 0.8 L/min. Limit of detection not reported [Samanta *et al.* 2004].
Note For a study following the trace element hair analysis of one man over 20 years, see Klevay *et al.* [2004].
Liver **ETAAS** Dry cycle: 120° at 30 s for 10 s, Ar, 3.0 L/min. Char cycle: 700° in 30 s for 10 s, Ar, 3.0 L/min. Atomisation cycle: 2600° in 1 s for 7 s, gas stop. Zinc hollow cathode lamp (λ= 324.8 nm). Limit of detection not reported [Treble *et al.* 1998]
Nail **ICP-MS** See Hair [Samanta *et al.* 2004].
Teeth **ICP-AES** Perkin-Elmer Plasma 40 Spectrometer (λ = 324.8 nm). Limit of detection not reported [Chew *et al.* 2000].
Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000a].
Colostrum **ETAAS** Dry cycle: 50° to 120° at 20 s for 20 s. Char cycle: 120° to 900° in 30 s for 30 s. Atomisation cycle: 1600° for 7 s. Limit of detection, 0.71 µg/L [Turan *et al.* 2001].
Milk **ICP-AES** Cooling gas: 13 L/min. Auxiliary gas: 0.5 L/min. Meinhard nebuliser (λ = 324.8 nm). Limit of detection, 0.012 mg/kg [Silva *et al.* 1997].

ICP-MS Plasma gas: 12 to 13 L/min. Auxiliary gas: 0.9 to 1.0 L/min. Sample gas: 1.0 to 1.2 L/min. Limit of detection, 180 µg/L [Krachler *et al.* 2000b].
Ascitic Fluid **ETAAS** Dry cycle: 60° to 90° in 15 s for 5 s to 100° in 10 s for 5 s to 110° in 15 s for 5 s to 140° for 5 s, 200 mL/min. Char cycle: 140° to 800° in 10 s for 20 s, 100 mL/min. Atomisation cycle: 2400° for 4 s. Lamp current: 10.0 mA (λ = 324.8 nm). Limit of detection, 0.4 µg/L [Milacic, Benedik 1999; Scancar *et al.* 1999].
Iliac Crest **ETAAS** Dry cycle: 60° to 90° in 10 s for 5 s to 100° in 10 s for 5 s to 150° in 10 s, 200 mL/min. Char cycle: 150° to 1000° in 10 s for 20 s, 100 mL/min. Atomisation cycle: 2700° for 4 s. Lamp current: 10.0 mA (λ = 324.8 nm). Limit of detection, 0.4 µg/L [Scancar *et al.* 2000].
Thyroid Tissue **FAAS** ATI UNICAM hollow cathode lamp. Limit of quantification, 70 ppb [Yaman, Akdeniz 2004].
Other **DPASV** Yemeni Khat. Limit of detection, 2.8 µg/kg [Matloob 2003].

ETAAS Eggs and Chicken Feed. Dry cycle: 120° at 1 s for 50 s. Char cycle: 1400° in 1 s for 30 s to 20° in 1 s for 5 s; Ar, 300 mL/min. Atomisation cycle: 2300° for 5 s (gas stop). Limit of detection, 0.74 mg/kg [Fakayode, Olu-Owolabi 2003]. Cocaine samples. Char cycle: 1300°. Atomisation cycle: 2400° (λ = 324.8 nm). Limit of detection not reported [Bermejo-Barrera *et al.* 1999].

ICP-AES Argentine wine. Outer gas: 8.5 L/min. Auxiliary gas: 1.0 L/min. Nebuliser gas: 1.0 L/min. (λ = 324.7 nm). Limit of detection, 40 ng/L [Lara *et al.* 2005].

ICP-MS Meals from Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 46 µg/kg [Noel *et al.* 2003].
Note For a study characterising copper in the uterine fluid of patients using the copper T-380A intrauterine device, see Arancibia *et al.* [2003].

Disposition in the Body Copper is absorbed from the gastrointestinal tract bound to amino acids or as ionic copper. Average absorption efficiencies in healthy adults range from 25 to 60%, although numerous factors affect this process, including the amount of copper in the diet, competition with other metals (zinc, iron and cadmium), and age. Roughly 80% of absorbed copper is excreted into the bile. Intestinal excretion into the bowel accounts for a further 18% and 2 to 4% appears in the urine. A small proportion of the copper taken into the liver is tightly bound to ceruloplasmin and this form accounts for over 90% of the copper present in serum. Copper is an essential element required for the normal functioning of at least 30 enzymes. The ability of copper to cycle between an oxidised state and a reduced state is used by cuproenzymes involved in redox reactions. However, it is this very property which poses a potential toxicity risk, as the transitions between oxidation states can generate superoxide radicals and hydroxyl radicals.
Normal Concentrations Serum—0.7 to 1.6 mg/L (11 to 25 µmol/L); urine—< 50 µg/day (0.8 µmol/day); brain—5.1 to 8.3 mg/kg; kidney—1.2 to 3.1 mg/kg; liver—3.0 to 9.5 mg/kg; muscle—0.6 to 1.0 mg/kg; spleen—0.2 to 2.1 mg/kg.
Concentrations in Copper-Related Liver Disease (Wilson's Disease) Serum—<0.4 mg/L; urine—>100 µg/day.
Toxicity Acute occupational inhalation of copper fumes or copper dust causes respiratory problems and aching muscles. In a group of workers occupationally

exposed to copper, serum levels averaged 1.26 mg/L [Cohen 1974]. Chronic occupational copper poisoning leads to nausea, vomiting, nervous disorders and hepatomegaly, and serum copper levels of 0.8 to over 2.0 mg/L have been reported [Suciu *et al.* 1977]. In cases of severe copper poisoning following ingestion of soluble salts (10 to 20 g), blood copper concentrations average ~8 mg/L [Chuttani *et al.* 1965].

An 18-month-old boy was admitted to hospital 1 h after dinking a solution containing ~3 g cupric sulfate. His serum copper concentration was 16.5 mg/L, which decreased to 2.3 mg/L 24 h later [Walsh *et al.* 1977].

In 27 patients with acute copper sulfate poisoning, 11 developed renal failure. In these 11 patients serum copper concentrations ranged from 1.15 to 3.9 mg/L [Chugh *et al.* 1977].

A person ingested an unknown quantity of wood-preserving solution containing large amounts of copper sulfate and sodium dichromate, with a smaller but substantial amount of an arsenic compound. Concentrations of copper in various tissues were reported as follows:

Tissue	*Concentration (μg/kg)*
Blood	5.5
Brain	63
Heart	3.7
Kidney	17.5
Liver	56
Lung	11.2
Spleen	3.8
Stomach	33
Urine	1.5 (mg/L)

[Cross *et al.* 1979].

A 58-year-old white woman died 18 h after admission to hospital. Postmortem examination revealed in the stomach 275 US coins, amounting to $14.84. Kidney and liver copper concentrations were 963 and 1160 mg/kg, respectively. Concentrations of nickel, chromium,and lead were within normal ranges [Yelin *et al.* 1987].

A 58-year-old man was found dead. Copper concentrations in tissues at postmortem were as follows:

Tissue	*Concentration (mg/kg or mg/L)*
Bile	2.8
Blood	13.8
Kidney	41.4
Liver	35.1
Lung	33.7
Stomach contents	2988

Concentrations of zinc and cadmium were within the normal range [Kurisaki *et al.* 1988].

A 48-year-old woman went to a witch-doctor (*iNyanga*) where she was administered a concoction of salt, vinegar, sugar, methylated ethanol and copper sulfate. She lapsed into a coma and died ~48 h later. During this time she was given additional medication that may have contained more copper sulfate. At postmortem, her blood copper concentration was 42 mg/L [Lamont, Duflou 1988].

An 11-year-old girl accidentally drank a solution containing 29 mg/L copper sulfate. Copper concentrations in her stomach contents were 240 mg/L, antemortem serum 25 mg/L, antemortem blood 16 mg/L, and postmortem blood 66 mg/L [Gulliver 1991; Mucklow 1997].

A 47-year-old man attempted suicide by injecting copper sulfate, both IV and SC. He was given haemodialysis for acute renal failure. His serum and dialysate copper levels were as follows:

Time	*Serum copper*		*Dialysate copper*	
	Pre-dialysis (μg/L)	*Post-dialysis (μg/L)*	*Pre-dialysis (μg/L)*	*Post-dialysis (μg/L)*
Day 1	180	165	20	25
Day 3	165	168	28	23

[Oldenquist, Salem 1999].

A 25-year-old woman ingested ≈25 diazepam 2.5 mg tablets. She was given 2.5 g cupric sulfate in 1.75 L water as an emetic. She died 3 days later. Postmortem copper concentrations were as follows:

Tissue	*Concentration (mg/kg or mg/L)*
Whole blood	5.31
Brain	1.1
Colon wall	0.3
Gastric contents	4.6
Gastric wall	1.1
Intestinal contents	12.6
Jejunal wall	1.5
Kidney	8.9
Liver	19.0

[Liu *et al.* 2001].

Note For a study of copper levels in the CSF of patients with multiple sclerosis, see Melo *et al.* [2003]; in the blood, serum and red blood cells of patients with motor neuron disease, see Pamphlett *et al.* [2001].·Rahil-Khazen *et al.* [2002] have studied trace element levels in the postmortem tissue of 30 Norwegians. For a case of suicide by ingesting a chromated copper arsenate wood preservative, see Hay *et al.* [2000]. For a report warning against preparing acidic drinks in copper urns, see Gill, Bhagat [1999]. For concentrations in the urine of glass-manufacturing workers, see Apostoli *et al.* [1998] and Arai *et al.* [1994]. For a review of the detection and monitoring of disorders of copper and other trace elements, see Taylor [1996]; for a study on the environmental influences on the trace element content of teeth, see Brown *et al.* [2004]. For a residential exposure to copper naphthenate, see Bluhm *et al.* [1992]. For 4 fatal poisonings following the ingestion of 'spiritual water', see Akintonwa *et al.* [1989]. For a case study on the fatal accidental ingestion of Clinitest (20 mg copper sulfate, 300 mg citric acid, 232.5 mg sodium hydroxide and 80 mg sodium carbonate), see O'Connor *et al.* [1984].

Half-life 26 days.

Volume of Distribution 2.0 L/kg.

Distribution in Blood Serum : erythrocyte ratio, 1.2.Lamont and Duflou, 1988

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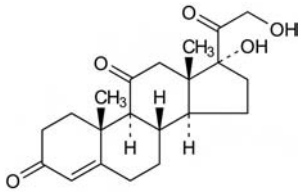
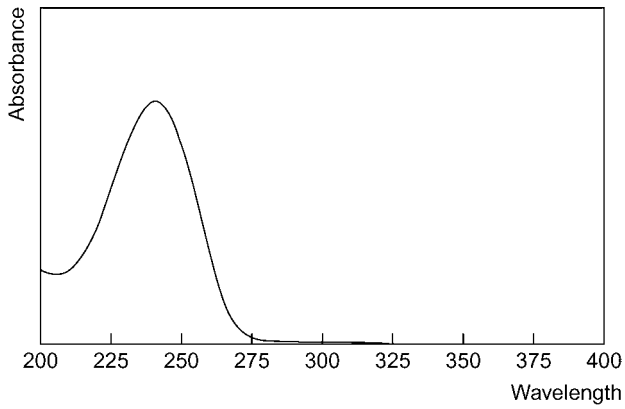


Chemical Properties Crystals. Mp 217° to 224°, with some decomposition. Very slightly soluble in water; fairly soluble in ethanol and acetone; sparingly soluble in chloroform and ether. Log *P* (octanol/water), 1.5.

Cortisone Acetate

$C_{23}H_{30}O_6 = 402.5$
CAS—50-04-4
Synonym Cortisone 21-Acetate
Note The name Cortisol is also applied to hydrocortisone.
Proprietary Names *Adreson*; *Cortate*; *Cortelan*; *Cortisol*; *Cortison*; *Cortistab*; *Cortisyl*; *Cortone*; *Cortone Acetate*; *Sterop*.
Chemical Properties A white crystalline powder. Mp 235° to 238°, with decomposition. Soluble 1 in 5000 of water, 1 in 300 to 1 in 350 of ethanol, and 1 in 4 of chloroform; slightly soluble in ether.

Colour Tests Naphthol–sulfuric acid—orange-brown/orange; sulfuric acid—yellow (green fluorescence under UV light).
Thin-layer Chromatography Cortisone acetate: system TA—R_f 0.90; system TB—R_f 0.03; system TE—R_f 0.68; system TP—R_f 0.72; system TQ—R_f 0.28; system TR—R_f 0.55; system TS—R_f 0.00; system TAE—R_f 0.87; system TAJ—R_f 0.51; system TAK—R_f 0.09; system TAL—R_f 0.83; system TAM—R_f 0.91 (DPST solution).
High Performance Liquid Chromatography System HT—*k* 2.4; system HY—RI 372.
Ultraviolet Spectrum Cortisone acetate: ethanol—240 nm (A_1^1=390a).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1660, 1235, 1720, 1275, 1750 cm^{-1} (cortisone acetate, KBr disk).
Quantification
Plasma **HPLC** Fluorescence detection. Limit of detection, 0.1 mg/L [Shibata *et al.* 1998].
Urine **HPLC** MS/MS. Limit of detection, 2 mg/L [Taylor *et al.* 2002] and see Plasma.
Hair **HPLC** MS. Limit of detection, 0.03 to 0.17 mg/g [Cirimele *et al.* 2000].
Dose 25 to 50 mg of cortisone acetate daily.

Cirimele V *et al.* (2000). Identification of ten corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry. *Forensic Sci Int* 107: 381–388.
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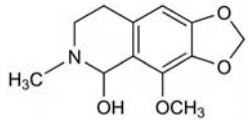
Cortisone

Corticosteroid
$C_{21}H_{28}O_5 = 360.4$
CAS—53-06-5
IUPAC Name (8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Hydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[*a*]phenanthrene-3,11-dione
Synonyms Compound E; 11-dehydro-17-hydroxycorticosterone; 17,21-dihydroxypregn-4-ene-3,11,20-trione.

Cotarnine

Haemostatic
$C_{12}H_{15}NO_4 = 237.3$
CAS—82-54-2
IUPAC Name 4-Methoxy-6-methyl-7,8-dihydro-5*H*-[1,3]dioxolo[4,5-*g*]isoquinolin-5-ol
Synonym 5,6,7,8-Tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-ol

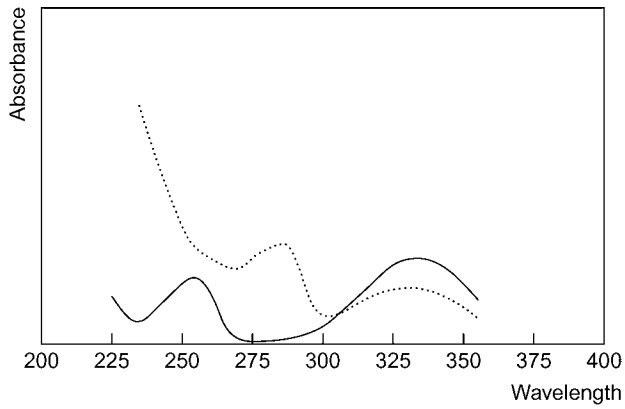


Chemical Properties An alkaloid obtained by oxidising noscapine with nitric acid. Crystals. Mp 132° to 137°, with decomposition. Slightly soluble in water; soluble in dilute acids, ethanol, chloroform, benzene, and ether. Log *P* (octanol/water), 0.6.

Cotarnine Chloride

$C_{12}H_{14}ClNO_3$, $2H_2O$ = 291.7
CAS—10018-19-6 (anhydrous); 16210-52-9 (dihydrate)
Synonyms Cotarnine hydrochloride; stypticine.
Chemical Properties A pale yellow deliquescent powder. Mp 197°. When heated to decomposition, highly toxic fumes are evolved. Soluble in water, ethanol, and chloroform; practically insoluble in ether.

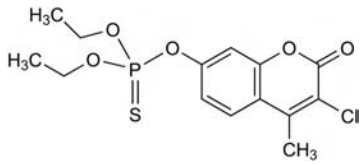
Colour Tests Liebermann's reagent—black; Mandelin's test—red-orange→brown.
Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.38; system TC—R_f 0.01; system TE—R_f 0.26; system TL—R_f 0.00; system TAE—R_f 0.01; system TAF—R_f 0.22 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1808.
High Performance Liquid Chromatography System HA—*k* 8.2 (tailing peak).
Ultraviolet Spectrum Aqueous acid—253 (A_1^1=582b), 332 nm (A_1^1=666b); aqueous alkali—286, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1605, 1086, 1490, 1655, 1505, 1300 cm^{-1} (cotarnine chloride, KCl disk).
Dose Cotarnine chloride was formerly given in doses of 20 to 100 mg.

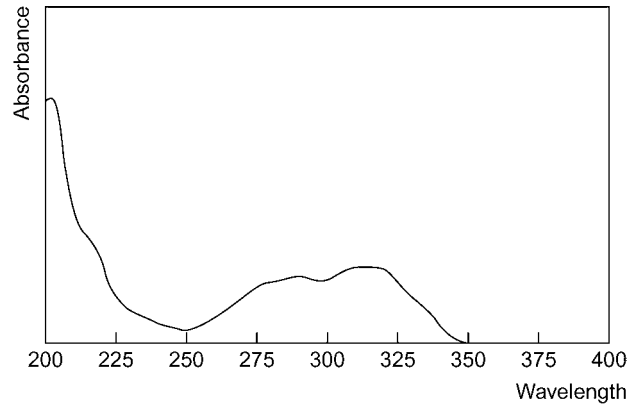
Coumaphos

Ectoparasiticide, Insecticide, Nematocide
$C_{14}H_{16}ClO_5PS$ = 362.8
CAS—56-72-4
IUPAC Name 3-Chloro-7-diethoxyphosphinothioyloxy-4-methylchromen-2-one
Synonyms Bayer 21/199; coumafos; ENT 17957; OMS 485; phosphorothioic acid *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) *O*,*O*-diethyl ester.
Proprietary Names *Agridip*; *Asunthol*; *Baymix*; *Checkmite*; *Co-Ral*; *Diolice*; *Meldane*; *Muscatox*; *Negasunt*; *Perizin*; *Resitox*; *Suntol*; *Umbethion*.

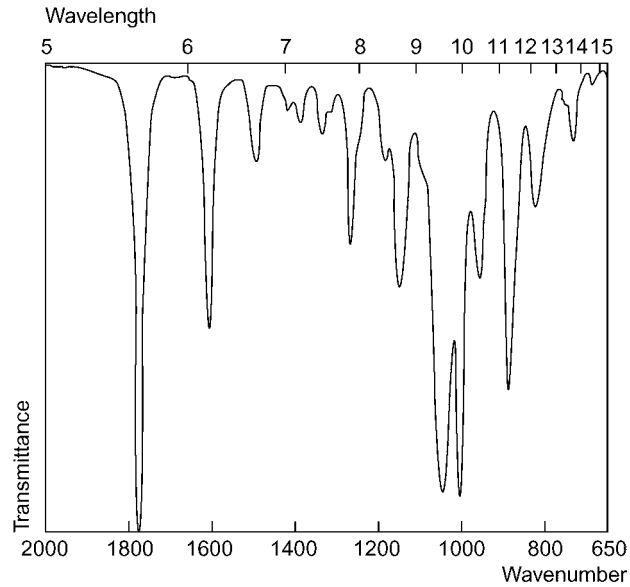


Chemical Properties A white to tan to grey-coloured crystalline solid. Mp 95° to 92° in the technical state). It is practically insoluble in water; limited solubility in most organic solvents; soluble in chloroform. Log *P* (octanol/water), 4.13.
Thin-layer Chromatography System TX—R_f 0.27; system TY—R_f 0.61.
Gas Chromatography System GA—RI 2573.

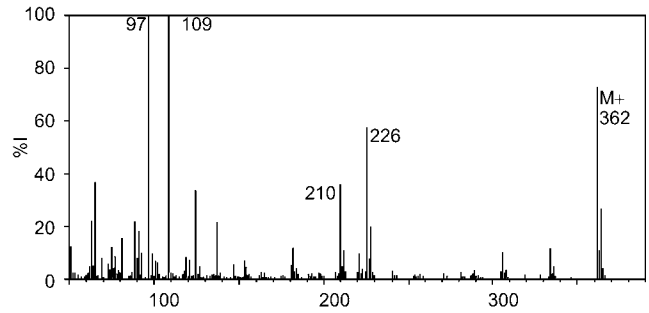
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1774, 1046, 1004, 888, 1609, 1151 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 97, 109, 362, 226, 65, 210, 125, 137.

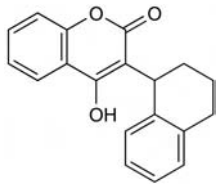


Disposition in the Body Coumaphos is readily absorbed through the skin. It is rapidly broken down in the body into non-toxic products which are eliminated via urine (the majority) and faeces. There is no evidence of bioaccumulation.
Toxicity Coumaphos is highly toxic if ingested or inhaled, and is moderately toxic via skin contact and if administered via IP routes.

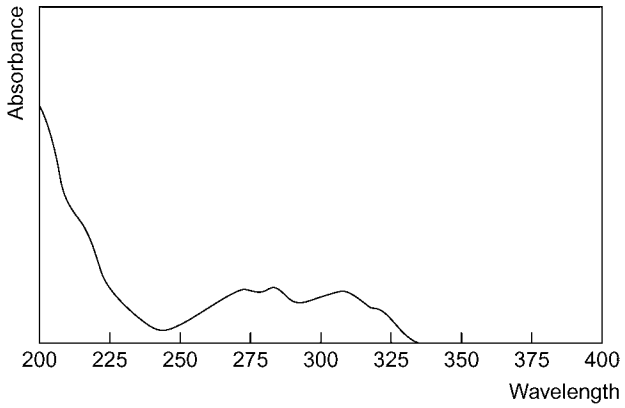
Coumatetralyl

Rodenticide
$C_{19}H_{16}O_3$ = 292.3
CAS—5836-29-3

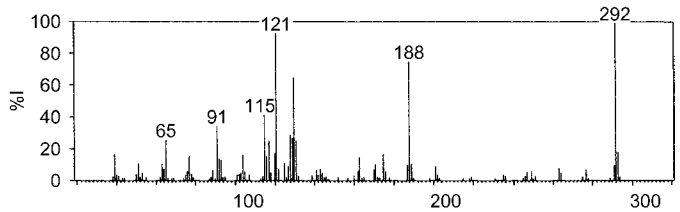
IUPAC Name 2-Hydroxy-3-(1,2,3,4-tetrahydronaphthalen-1-yl)chromen-4-one
Synonym 4-Hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin
Proprietary Name *Racumin*



Chemical Properties A yellowish-white crystalline powder. Mp 172° to 176°. Practically insoluble in water; soluble in ethanol and acetone; slightly soluble in ether. pK_a 4.8. Log *P* (octanol/water), 3.5.
Thin-layer Chromatography System TD—R_f 0.73; system TE—R_f 0.13; system TF—R_f 0.74; system TX—R_f 0.14; system TY—R_f 0.30 (location under UV light, pink fluorescence; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—coumatetralyl RI 2635, coumatetralyl-HY RI 2250, coumatetralyl-Me-HY RI 2300, coumatetralyl isomer-1-Me RI 2655, coumatetralyl isomer-2-Me RI 2690, M (OH-) isomer-1-Me RI 2910, M (OH-) isomer-2-Me_2 RI 2925, M (OH-) isomer-3-Me_2 RI 2935, M (OH-) isomer-4-Me_2 RI 2990, M (di-OH-) isomer-1-Me_2 RI 3005, M (OH-methoxy-)-Me_2 RI 3070, M (di-OH-) isomer-2-Me_2 RI 3085, M (di-OH-) isomer-3-Me_3 RI 3105, M (tri-OH-)-H_2O-Me_2 RI 3175.
High Performance Liquid Chromatography System HY—RI 598; system HAA—retention time 5.0 min.
Ultraviolet Spectrum Aqueous acid—273, 283, 309 nm; aqueous alkali—311 nm.



Infrared Spectrum Principal peaks at wavenumbers 1615, 762, 740, 1685, 1250, 1570 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 292, 121, 188, 130, 115, 91, 128, 129.



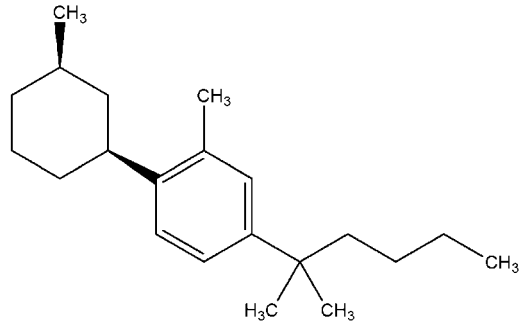
Quantification
Blood **TLC** Limit of quantification, 0.5 mg/L. Limit of detection, 0.2 mg/L [Berny *et al.* 1995].
Serum **HPLC** Fluorescence detection (λ_{ex}=318 nm; λ_{em}=390 nm). 1 mg/L [Chalermchaikit *et al.* 1993].
Liver **TLC** Limit of detection, 0.2 mg/g, see Blood [Berny *et al.* 1995].
HPLC 1 ng/g, see Serum [Chalermchaikit *et al.* 1993].

Berny PJ *et al.* (1995). Anticoagulant poisoning in animals: a simple new high-performance thin-layer chromatographic (HPTLC) method for the simultaneous determination of eight anticoagulant rodenticides in liver samples. *J Anal Toxicol* 19(7): 576–580.
Chalermchaikit T *et al.* (1993). Simultaneous determination of eight anticoagulant rodenticides in blood serum and liver. *J Anal Toxicol* 17(1): 56–61.

CP 47,497

Analgesic, Cannabinoid Agonist
$C_{21}H_{34}O_2$ = 318.5
CAS—70434-82-1

IUPAC Name 1-[(1*R*,3*S*)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol



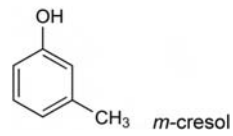
Chemical Properties Scientific research tool developed by Pfizer as a potent agonist of the cannabinoid CB1 receptor. It is the main active ingredient of the herbal incense known as Spice, specifically the 1,1-dimethyloctyl homologue. It is illegal in France and Germany.
Mass Spectrum Principal peak at *m/z* 301.
Quantification
Blood **LC-MS** MRM acquisition mode. Limit of quantification, 0.6 µg/L, limit of detection, 0.2 µg/L [Neukamm *et al.* 2009].
Urine **LC-MS** Enhanced product ion scan. Limit of detection not reported [Kraemer *et al.* 2009].
GC-MS EI ionisation. Limit of detection, not reported [Kraemer *et al.* 2009].
Hair **LC-MS** See Blood [Neukamm *et al.* 2009].
Other **LC-MS** Herbal Products. Column: UPLC HSS T3 (100 × 2.1 mm i.d., 1.8 µm). Mobile phase: 0.1% formic acid in acetonitrile : water, flow rate 0.3 mL/min. Limit of detection not reported [Uchiyama *et al.* 2009].

Kraemer T *et al.* (2009). Studies on the metabolism of JWH-018 and of a homologue of CP 47,497, pharmacologically active ingredients of different misused incense ('Spice') using GC-MS and LC-MS techniques. *Ann Toxicol Anal* 21(Suppl 1): S21–S22.
Neukamm MA *et al.* (2009). Quantitative determination of the active 'Spice' ingredient JWH-018 in blood and hair by liquid chromatography–tandem mass spectrometry. *Ann Toxicol Anal* 21: S1–21.
Uchiyama N *et al.* (2009). Identification of cannabinoid analogs as new type of designer drugs in herbal products. *Ann Toxicol Anal* 21: S1-53–S1-54.

Cresol

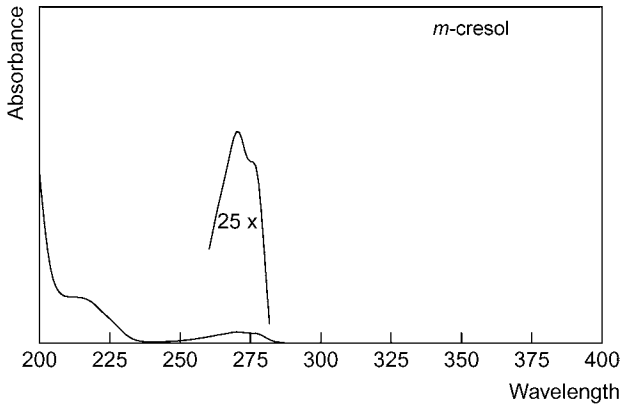
Disinfectant
$C_{21}H_{24}O_3$ = 324.4
CAS—1319-77-3; 95-48-7 (*o*-cresol); 108-39-4 (*m*-cresol); 106-44-5 (*p*-cresol)

Synonyms Cresylic acid; tricresol.
Proprietary Name It is an ingredient of *Lyseptol*.
Note The use of the name *Lysol* is limited. In some countries it is a trademark applied to a product of different composition.



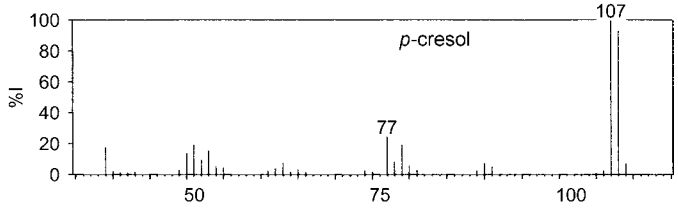
Chemical Properties Cresol is a mixture of *o*-, *m*-, and *p*-cresols ($CH_3C_6H_4OH$ = 108.1), in which the *m*-isomer predominates, and of other phenols obtained from coal tar. An almost colourless to pale brownish-yellow liquid, becoming darker with age or on exposure to light. Mass per mL 1.029 to1.044 g. Bp 195° to 205°. Almost completely soluble 1 in 50 of water; miscible with ethanol, benzene, chloroform, glycerol, petroleum ether, and ether. Soluble in solutions of fixed alkali hydroxides. pK_a *m*-cresol 10.1, *o*-cresol 10.3, *p*-cresol 10.3 (25°). Log *P* (octanol/water), 2.0.
Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; potassium dichromate—brown (*o*-cresol 30 s, *m*-cresol 2 min); heat with about an equal quantity of phthalic anhydride and a few drops of sulfuric acid until the mixture is orange-brown; cool the mixture with a few drops of water and make alkaline with sodium hydroxide solution—red with *o*-cresol and blue-violet with *m*-cresol
Thin-layer Chromatography System TAJ—*m*-cresol RF 69, *o*-cresol RF 73, *p*-cresol RF 66; system TAK—*m*-cresol RF 78, *o*-cresol RF 82, *p*-cresol RF 78; system TAL—RF 96 *m*-, *o*-, *p*-cresol.
Gas Chromatography System GA—*m*-cresol RI 1065, *o*-cresol RI 1040, *p*-cresol RI 1060, *p*-cresol-AC RI 1110.

Ultraviolet Spectrum Aqueous alkali—239 (A^{1}_{1}=949b), 290 nm (A^{1}_{1}=251b); ethanol—275 (A^{1}_{1}=163b), 279 nm (A^{1}_{1}=145b).



Infrared Spectrum Principal peaks at wavenumbers 1209, 816, 1515, 741, 1176, 1105 cm^{-1} (*p*-cresol (Nujol mull)).

Mass Spectrum Principal ions at *m/z* 107, 108, 77, 79, 51, 39, 53, 50 (*p*-cresol).



Quantification

Blood **GC** FID. Limit of detection 20 mg/L [Bruce *et al.* 1976].

Serum **HPLC** Fluorescence detection (λ_{ex}=284 nm; λ_{em}=310 nm). Limit of detection *p*-cresol 0.14 mg/L [De Smet *et al.* 1998].

Urine **GC** FID [Amorim *et al.* 1997]. ECD [Dills *et al.* 1997] and see Blood.

HPLC UV detection (λ=270 nm). Limit of detection, 0.8 mg [Birkett *et al.* 1995]. UV detection (λ=271 nm). Limit of detection, 0.2 mg/L [Schlatter, Astier 1995].

Biological Fluids **GC** [Yashiki *et al.* 1990].

Faeces **HPLC** See Urine [Birkett *et al.* 1995].

Tissues **GC** See Blood [Bruce *et al.* 1976].

Disposition in the Body Absorbed after ingestion, and through the skin and mucous membranes. It is metabolised by conjugation and oxidation; *p*-cresol is endogenously produced in normal subjects, and may be present in urine at concentrations of 20 to 200 mg/L (mainly in conjugated form).

Toxicity The estimated minimum lethal dose is 2 g, and the maximum permissible atmospheric concentration is 5 ppm.

In 2 fatalities due to the ingestion of cresol, the following postmortem tissue concentrations were reported: blood 71, 190 mg/L; brain 2.8, – μg/g; kidney 396, – μg/g; liver 900, 480 μg/g; urine –, 304 mg/L [Bruce *et al.* 1976].

A 65-year-old man with schizophrenia died 15 min after he ingested a large volume of saponated cresol solution. Free *p*-cresol was detected in heart blood at a concentration of 458.8 mg/L and *m*-cresol at 957.3 mg/L; and the glucuronic acid conjugated forms at 38.2 and 85.6 mg/L, respectively. 250 mL of a cresol-odour-emitting fluid was also present in the stomach [Monma-Ohtaki *et al.* 2002].

A 46-year-old man ingested 100 mL of saponated cresol solution and was admitted to hospital. The serum concentrations of *p*-cresol and *m*-cresol were 43.3 mg/g and 73.8 mg/g, respectively, and the total concentration of cresol was 117 mg/g. Although levels were in the fatal ranges the individual recovered after hospital treatment [Yashiki *et al.* 1990].

Amorim LC *et al.* (1997). Determination of o-cresol by gas chromatography and comparison with hippuric acid levels in urine samples of individuals exposed to toluene. *J Toxicol Environ Health* 50: 401–407.

Birkett AM *et al.* (1995). Simple high-performance liquid chromatographic analysis of phenol and p-cresol in urine and feces. *J Chromatogr B Biomed Appl* 674(2): 187–191.

Bruce AM *et al.* (1976). Cresol poisoning. *Med Sci Law* 16: 171–176.

De Smet R *et al.* (1998). A sensitive HPLC method for the quantification of free and total p-cresol in patients with chronic renal failure. *Clin Chim Acta* 278(1): 1–21.

Dills RL *et al.* (1997). Quantitation of o-, m- and p-cresol and deuterated analogs in human urine by gas chromatography with electron capture detection. *J Chromatogr B Biomed Sci Appl* 703(1–2): 105–113.

Monma-Ohtaki J *et al.* (2002). An autopsy case of poisoning by massive absorption of cresol a short time before death. *Forensic Sci Int* 126(1): 77–81.

Schlatter J, Astier A (1995). Rapid determination of O- and P-cresol isomers in urine from workers exposed to toluene by high-performance liquid chromatography using a graphitized carbon column. *Biomed Chromatogr* 9(6): 302–304.

Yashiki M *et al.* (1990). Gas chromatographic determination of cresols in the biological fluids of a non-fatal case of cresol intoxication. *Forensic Sci Int* 47(1): 21–29.

Yashiki M *et al.* (1990). Gas chromatographic determination of cresols in the biological fluids of a non-fatal case of cresol intoxication. *Forens Sci Int* 47(1): 21–29.

CR Gas

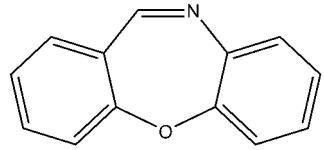
Benzodiazepine, Riot Control Agent

$C_{13}H_9ON$ = 195.2

CAS—257-07-8

IUPAC Name Dibenz[b][1,4]oxazepine

Synonyms Dibenz[b,f][1,4]oxazepine; dibenzoxazepine; tear gas.



Chemical Properties Odourless pale yellow solid; also reported as white solid. Mp 72°. Bp 335°. Sparingly soluble in water (3.73 mg/L). Soluble in organic solvents including chlorinated organics. Chemically stable in organic solvents. Log *P* (octanol/water), 3.49. CR hydrolyses slowly in water. CR riot control agent formulations generally consist of 0.1% CR dissolved in a solution of 80 parts of propylene glycol and 20 parts water. It may persist for prolonged periods in the environment because of the aqueous stability of benzodiazepines in aqueous media [Meylan, Howard 1995; Olajos, Salem 2001]. CR is the parent compound of the antipsychotic drug loxapine [Blain 2003].

Disposition in the Body CR metabolism has not been elucidated in humans owing to the very high sensitivity of humans to the irritant properties of CR. Animal studies have shown that aerosols of CR are rapidly absorbed from the respiratory tract, with a plasma half-life after inhalation exposure of ~5 min. It is readily absorbed by the cornea and corneal homogenates and metabolised to a lactam derivative. Studies in rats have shown that CR is rapidly absorbed from the gastrointestinal tract, undergoes hepatic metabolism, biliary secretion, enterohepatic circulation, and renal excretion. The major metabolic pathway of CR in the rat is oxidation to its more toxic lactam form, subsequent ring hydroxylation, sulfate conjugation and urinary excretion.

Toxicity CR is a potent sensory irritant of low toxicity. It is less toxic than CS or CN by all routes of exposure. Human ocular irritancy thresholds and toxicity estimates are reported as follows: irritancy threshold 0.002 mg/m^3, intolerable concentration 1 mg/m^3, lethal concentration (10 min exposure) 10 000 mg/m^3 [Olajos, Salem 2001]. The inhalation toxicity of chemical warfare agents, military chemicals, and riot control agents is, by convention, expressed by the notation Ct. It is defined as the product of the concentration in mg/m^3 multiplied by the exposure time (*t*) in minutes (mg·min/m^3). The terms LCt_{50} and ICt_{50} describe the airborne dosages that are lethal (L) or incapacitating (I) to 50% of the exposed population. The minimal irritant concentration estimate in humans is 0.002 mg·min/m^3, with LCt_{50} and ICt_{50} estimates reported as >100 000 mg·min/m^3 and ≈1 mg·min/m^3, respectively. Animal LCt_{50} (mg·min/m^3) values: mouse 169 500 to 203 600; rat 139 000 to 428 400; rabbit 160 000 to 169 000; guinea pig 169 500 [Olajos, Salem 2001]. Animal LD_{50} (mg/kg) values: rat 5900 (oral), 766 (IP), 68 (IV); mouse 112 (IV), 4000 (oral); rabbit 1760 (oral), 47 (IV); guinea pig 629 (oral), 463 (IP) [Ballantyne, Swanston 1978].

Note For reviews of riot control agents, see Blain [2003]; Hu *et al.* [1989]; Olajos and Salem [2001]; and Olajos and Stopford [2004].

Ballantyne B, Swanston DW (1978). The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). *Arch Toxicol* 40: 75–95.

Blain PG (2003). Tear gases and irritant incapacitants. 1-chloroacetophenone, 2-chlorobenzylidene malononitrile and dibenz[b,f]-1,4-oxazepine. *Toxicol Rev* 22: 103–110.

Hu H *et al.* (1989). Tear gas: harassing agent or toxic chemical weapon? *JAMA* 262: 660–663.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Olajos E, Salem JH (2001). Riot control agents: pharmacology, toxicology, biochemistry and chemistry. *J Appl Toxicol* 21: 355–391.

Olajos EJ, Stopford W (2004). *Riot Control Agents: Issues in Toxicology, Safety and Health Care*. Boca Raton, FL: CRC Press.

Cropropamide

Respiratory Stimulant

$C_{13}H_{24}N_2O_2$ = 240.3

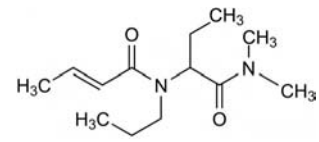
CAS—633-47-6 (cropropamide); 8015-51-8 (prethcamide)

IUPAC Name 2-[[(*E*)-But-2-enoyl]-propylamino]-*N*,*N*-dimethylbutanamide

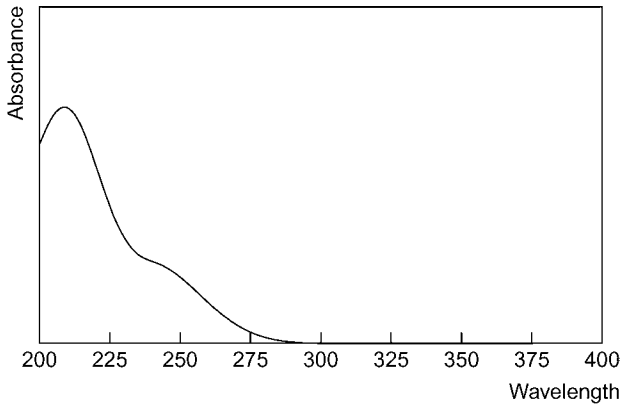
Synonym *N*-[1-[(Dimethylamino)carbonyl]propyl]-*N*-propyl-2-butenamide

Note It is an ingredient of Prethcamide, which is a mixture of equal parts (by weight of) cropropamide and crotetamide.

Proprietary Names It is an ingredient of *Micoren* and of *Respirot*.



Chemical Properties A liquid. Easily soluble in water and ether; miscible with ethanol. Log *P* (octanol/water), 1.6.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.29; system TC—R_f 0.69; system TE—R_f 0.74; system TL—R_f 0.57; system TAE—R_f 0.25; system TAF—R_f 0.83 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1738.
High Performance Liquid Chromatography System HZ—retention time 3.3 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

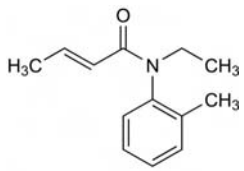


Infrared Spectrum Principal peaks at wavenumbers 1654, 1617, 1219, 1282, 1098, 1123 cm^{-1} (KBr disk).
Dose Prethcamide (cropropamide and crotetamide) is usually given in doses of 1.2 to 1.6 g daily.

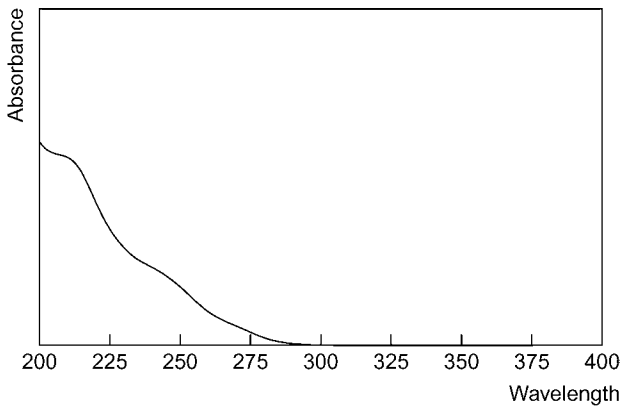
Crotamiton

Acaricide, Antipruritic
$C_{13}H_{17}NO = 203.3$
CAS—483-63-6

IUPAC Name *N*-Ethyl-*N*-(2-methylphenyl)-2-butenamide
Proprietary Names *Crotamitex*; *Eurax*; *Euraxil*.



Chemical Properties A colourless or pale yellow, oily liquid. Soluble 1 in 400 of water; miscible with ethanol, ether, and methanol. Log *P* (octanol/water), 2.7.
Thin-layer Chromatography System TA—R_f 0.83; system TE—R_f 0.83; system TAE—R_f 0.84 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1600.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

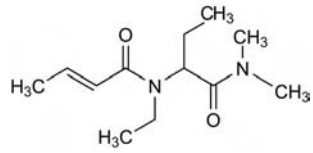


Infrared Spectrum Principal peaks at wavenumbers 1656, 1623, 1235, 1280, 1316, 1590 cm^{-1} (thin film).
Use Topically in a concentration of 10%.

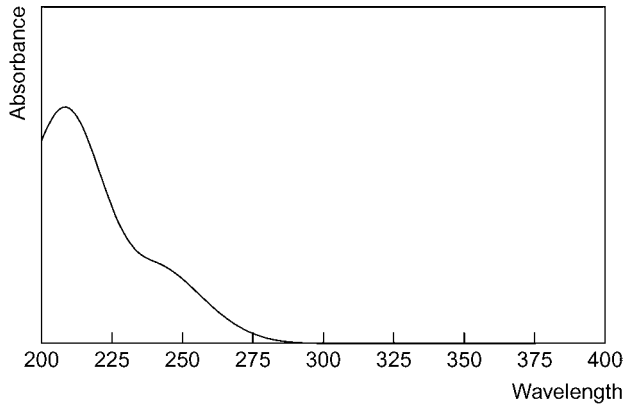
Crotetamide

Respiratory Stimulant
$C_{12}H_{22}N_2O_2 = 226.3$
CAS—6168-76-9 (crotetamide); 8015-51-8 (prethcamide)

IUPAC Name 2-[[(*E*)-But-2-enoyl]-ethylamino]-*N*,*N*-dimethylbutanamide
Synonyms Crotethamide; *N*-[1-[(dimethylamino)carbonyl]propyl]-*N*-ethyl-2-butenamide.
Proprietary Names It is an ingredient of *Micoren* and of *Respirot*.



Chemical Properties A liquid. Easily soluble in water and ether; miscible with ethanol. Log *P* (octanol/water), 1.1.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.28; system TC—Rf 67; system TE—R_f 0.69; system TL—R_f 0.55; system TAE—R_f 0.83; system TAF—R_f 0.79 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1688.
High Performance Liquid Chromatography System HX—RI 366; system HZ—retention time 2.6 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

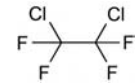


Infrared Spectrum Principal peaks at wavenumbers 1655, 1617, 1234, 1098, 1282, 1123 cm^{-1} (KBr disk).
Dose See under Cropropamide.

Cryofluorane

Aerosol Propellent, Refrigerant
$CClF_2{\cdot}CClF_2 = 170.9$
CAS—76-14-2

IUPAC Name 1,2-Dichloro-1,1,2,2-tetrafluoroethane
Synonyms Dichlorotetrafluoroethane; propellent 114; refrigerant 114; tetrafluorodichloroethane.
Proprietary Names *Arcton 33*; *Arcton 114*.

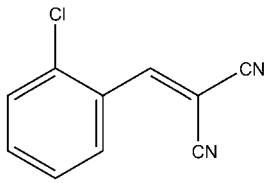


Chemical Properties A colourless non-flammable gas which, when liquefied by compression, forms a clear colourless liquid. Bp about 3.5°. In the liquid state it is practically immiscible with water, but miscible with dehydrated alcohol. Log *P* (octanol/water), 2.8.
Gas Chromatography System GA—RI 361; system GI—Retention time 2.0 min.
Mass Spectrum Principal ions at *m/z* 85, 135, 87, 137, 31, 101, 100, 50.

CS Gas

Organonitrile, Riot Control Agent
$C_{10}H_5ClN_2 = 188.6$
CAS—2698-41-1

IUPAC Name [(2-Chlorophenyl)methylene]propanedinitrile
Synonyms *o*-Chlorobenzalmalononitrile; *o*-chlorobenzylidenemalononitrile; β,β-dicyano-*o*-chlorostyrene.
Proprietary Names *Paralyzer*. It is also an ingredient of *Sabre*.



Chemical Properties White crystalline solid with pungent pepper-like odour. Mp 95° to 96°. Bp 310° to 315°. Sparingly soluble in water; soluble in acetone, dioxane, methylene chloride, ethyl acetate, benzene [O'Neil *et al.* 2006]. Log *P* (octanol/water), 2.75 [Meylan, Howard 1995]. CS can be disseminated as a dry powder (by thermal or explosive methods), via spraying of the molten material, or in solution with inorganic solvents. CS exists in three forms: CS (pure form), CS1 and CS2 (mixtures of the crystalline agent and an aerogel). CS2 contains 95% micronised CS, 5% Cab-o-Sil and 1% hexamethyldisilazane. CS hydrolyses slowly to *o*-chlorobenzaldehyde and malononitrile. Chemically, CS is the most persistent of the lachrymatory agents and will adsorb on to most porous surfaces [Olajos, Salem 2001]. CS is an SN_2 alkylating agent that reacts directly with nucleophilic sites [Ballantyne, Swanston 1978].
Note For a study of the thermal degradation products of CS at elevated temperatures, see Kluchinsky *et al.* [2002].
Mass Spectrum Principal ions at *m/z* 153, 183, 126, 75, 50, 161, 99, 137.

Quantification

Other **TLC** Rat Urine. Plates: aluminium coated with silica gel H-60 (20 × 20 cm). Solvent system: dichloromethane : methanol : acetic acid (20 : 4 : 1). UV detection (with radioactive ink). R_f values: 2-chlorobenzaldehyde, 0.91; 2-chlorohippuric acid, 0.55; 2-chlorobenzylmercapturic acid, 0.63; 2-chlorobenzoic acid, 0.76; 2-chlorobenzyl alcohol, 0.85. Limit of quantification not reported [Rietveld *et al.* 1988].

GC-MS CS Canisters. Column: HP5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 37 cm/s. Temperature programme: 80° for 2 min to 275° at 15°/min. EI ionisation at 70 eV. Retention times: CS, 15.5 min; 2-chlorobenzaldehyde, 7.7 min; CS epoxide, 13.6 min; 3-quinolinecarbonitrile, 15.1 min; CS isomer, 16.4 min. Limit of quantification not reported [Smith *et al.* 2002].

Disposition in the Body Animal and human studies have shown that CS is readily absorbed from the respiratory tract and rapidly distributed by the blood throughout the body. CS and its main metabolites in blood (dihydro-CS and 2-chlorobenzaldehyde) have extremely short half-lives (CS blood half-life <30 s). Elimination of CS follows first-order kinetics, almost spontaneously hydrolysing to malononitrile, 2-chlorobenzyl malononitrile, 2-chlorobenzaldehyde, 2-chlorohippuric acid, thiocyanate and cyanide. CS and its metabolites can be detected in blood following inhalation exposure, but only after high doses. Intravenous and intragastric studies have shown that the majority of the administered dose is eliminated in the urine. Major urinary metabolites include 2-chlorohippuric acid, 1-*O*-(2-chlorobenzyl)glucuronic acid, 2-chlorobenzyl cysteine and 2-chlorobenzoic acid. Theories have been postulated that some of the toxic effects attributed to CS may arise from the conversion of CS to cyanide but this does not seem likely since a 1-min CS exposure to an intolerable level (10 mg/m^3) produces less cyanide than 2 inhalations of a cigarette. Furthermore, significant amounts of free cyanide do not appear in the plasma.

Toxicity CS produces harassing effects principally on the eyes (excessive tearing and discomfort), which usually resolve within 20 min. Human ocular irritancy thresholds and toxicity estimates are reported as follows: irritancy threshold 0.004 mg/m^3, intolerable concentration 3 mg/m^3, lethal concentration (10 min exposure) 2500 mg/m^3 [Olajos, Salem 2001]. The inhalation toxicity of chemical warfare agents, military chemicals, and riot control agents is, by convention, expressed by the notation Ct. It is defined as the product of the concentration in mg/m^3 multiplied by the exposure time (*t*) in minutes (mg·min/m^3). The terms LCt_{50} and ICt_{50} describe the airborne dosages that are lethal (L) or incapacitating (I) to 50% of the exposed population. The minimal irritant concentration estimate in humans is 0.004 mg·min/m^3, with LCt_{50} and ICt_{50} estimates reported as from 25 000 to 150 000 mg·min/m^3 and 5 mg·min/m^3, respectively. The maximum tolerable concentration in humans is estimated at <10 mg/m^3 (also as 3 mg/m^3) and the lethal concentration 2500 mg/m^3. Animal LCt_{50} values (mg·min/m^3) from acute exposures to CS dispersed from 10% CS in methylene dichloride: rats 1 004 000; mice 627 000; guinea pigs 46 000; doses of up to 30 000 were not lethal to monkeys. Animal LCt_{50} values (mg·min/m^3) from exposures to CS dispersed from thermal grenades: rats 164 000; guinea pigs 36 000. LCt_{50} values (mg·min/m^3) for acute exposure to CS2: rats 68 000; guinea pigs 49 000; dogs 70 000; monkeys 74 000. [Olajos, Salem 2001]. LD_{50} in rats (mg/kg): 28 (IV), 48 (IP), 1366 (oral) [Ballantyne, Swanston 1978]. CS is 10 times more potent as a lachrymator than CN (1-chloroacetophenone) but is less toxic [Blain 2003]. OSHA air monitoring/exposure levels for CS are 0.05 ppm for the permissible exposure limit (PEL) and 2 mg/m^3 for the immediately dangerous to life (IDLH) limit. Owing to its poor water solubility, CS-containing formulations usually comprise solvents as carriers as well as some kind of propellant gas. It is worth noting that some solvents may contribute to unwanted effects of CS use, such as corneal erosion, skin blistering, or even neurotoxicity. At the time of writing, in spite of its extensive use, there have been no verified causes of death in humans following CS application [Olajos, Stopford 2004].

Six male volunteers were exposed to CS at concentrations of 0.5 to 1.5 mg/m^3 for 90 min. Neither CS nor 2-chlorobenzaldehyde was detected in the blood, and only in 1 volunteer was a trace of 2-chlorobenzyl malononitrile detected [Leadbeater 1973].

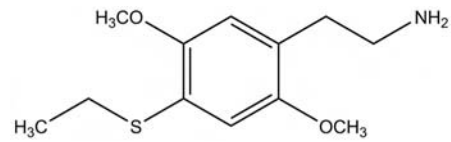
Note For a study of absorption, metabolism, and toxicokinetics of 2-chlorobenzaldehyde in rats, see Rietveld *et al.* [1983] and Rietveld *et al.* [1988]. For reviews of riot control agents, see Blain [2003]; Hu *et al.* [1989]; Olajos, Salem [2001]; and Olajos and Stopford [2004]. For a review and assessment of the toxicology and carcinogenesis of CS2 in rats and mice, see National Toxicology Program [1990]. For a study of the metabolism of CS in rats, see Brewster *et al.* [1987].
Dose Used as riot control agent having replaced CN gas (chloroacetophenone), which, in turn, replaced adamsite (DM).

Ballantyne B, Swanston DW (1978). The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). *Arch Toxicol* 40: 75–95.
Blain PG (2003). Tear gases and irritant incapacitants. 1-chloroacetophenone, 2-chlorobenzylidene malononitrile and dibenz[b,f]-1,4-oxazepine. *Toxicol Rev* 22: 103–110.
Brewster K *et al.* (1987). The fate of 2-chlorobenzylidene malononitrile (CS) in rats. *Xenobiotica* 17: 911–924.
Hu H *et al.* (1989). Tear gas: harassing agent or toxic chemical weapon. *JAMA* 262: 660–663.
Kluchinsky TAJr *et al.* (2002). Formation of 2-chlorobenzylidenemalononitrile (CS riot control agent) thermal degradation products at elevated temperatures. *J Chromatogr A* 952: 205–213.
Leadbeater L (1973). The absorption of *ortho*-chlorobenzylidenemalononitrile (CS) by the respiratory tract. *Toxicol Appl Pharmacol* 25: 101–110.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
National Toxicology Program NTP toxicology and carcinogenesis studies of 2-chloroacetophenone (CAS No, 532-27-4) in F344/N rats and B6C3F1 mice (inhalation studies). *Natl Toxicol Program Tech Rep Ser* 379: 1–191.
Olajos EJ, Salem H (2001). Riot control agents: pharmacology, toxicology, biochemistry and chemistry. *J Appl Toxicol* 21: 355–391.
Olajos EJ, Stopford W (2004). *Riot Control Agents: Issues in Toxicology, Safety and Health Care*. Boca Raton, FL: CRC Press.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.
Rietveld EC *et al.* (1983). 2-Chlorobenzylmercapturic acid, a metabolite of the riot control agent 2-chlorobenzylidene malononitrile (CS) in the rat. *Arch Toxicol* 54: 139–144.
Rietveld EC *et al.* (1988). Percutaneous absorption of ^{14}C-labelled 2-chlorobenzaldehyde in rats, Metabolism and toxicokinetics. *Eur J Drug Metab Pharmacokinet* 13: 231–240.
Smith PA *et al.* (2002). Traditional sampling with laboratory analysis and solid phase microextraction sampling with field gas chromatography/mass spectrometry by military industrial hygienists. *AIHA J (Fairfax, Va)* 63: 284–292.

2C-T-2

5-HT$_{2A}$ Receptor Agonist, Hallucinogen, Phenethylamine
$C_{12}H_{19}NO_2S$ = 241.4
CAS—207740-24-7

Synonyms 2,5-Dimethoxy-4-ethylthiophenethylamine; 4-ethylsulfanyl-2,5-dimethoxyphenethylazan; 4-ethylthio-2,5-dimethoxyphenethylamine.

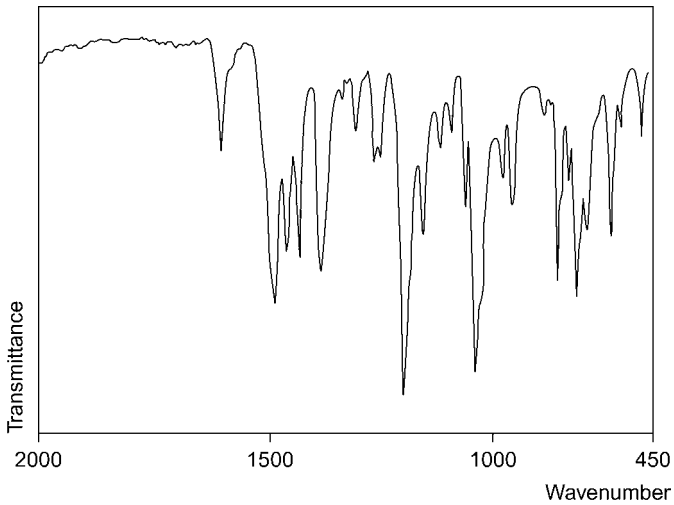


Chemical Properties Bp 120° to 130°.

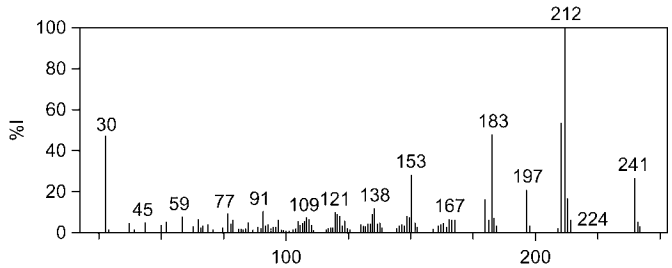
2C-T-2 Hydrochloride

$C_{12}H_{19}NO_2S$, HCl = 277.9
Synonym 2,5-Dimethoxy-4-ethylthiophenethylamine hydrochloride; 4-ethylsulfanyl-2,5-dimethoxyphenethylazan hydrochloride; 4-ethylthio-2,5-dimethoxyphenethylamine hydrochloride
Chemical Properties White crystalline solid. Mp 180°. No indication of instability of processed samples over a time period of 32 h. When the samples were freeze-thawed, the ratio of the means was within 90 and 110% for all analytes at high concentrations [Habrdova *et al.* 2005].

Colour Test Marquis test—light pink-orange.
Gas Chromatography-Mass Spectrometry Column: HP-5MS cross-linked phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 1 min to 200° at 20°/min for 8.84 min. EI ionisation at 70 eV. Limit of detection, 5.1 mg/L [Tsai *et al.* 2006].
Capillary Electrophoresis Column: fused silica capillary (total/effective length: 96/90 cm for sweeping MEKC and 100/94 cm for stacking MEKC, 50 µm i.d.). Lamp: Xe. Fluorescence detection (λ_{ex} = 300 nm, λ_{em} = 340 nm). Limit of detection, 28.9 and 9.66 µg/L for sweeping and stacking MEKC, respectively [Tsai *et al.* 2006].
Infrared Spectrum Principal peaks at wavenumber 736, 809, 1036, 1384, 1489, 1204 cm^{-1} (hydrochloride).



Mass Spectrum Principal ions at *m/z* 72, 43, 30, 56, 151, 221, 41, 98 (HFB derivative).



Quantification

Plasma **GC-MS** Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 1.1 mL/min. Temperature programme: 80° for 0.5 min to 310° at 30°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 µg/L [Habrdova *et al.* 2005].

Other **GC-MS** Microsomes. Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 50° for 3 min to 310° at 40°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Theobald, Maurer 2007]. Rat Urine. Column: HP-1 capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm) Carrier gas: He, 1 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV, full scan mode. Limit of detection, 20 µg/L [Theobald *et al.* 2005]. Synthesised Sample. Column: Ultra-1 (12 m × 0.22 mm i.d, 0.25 µm). Temperature programme: 100° to 280° at 10°/min. EI ionisation. Limit of detection not reported [Poortman-van der Meer 1999].

CE Mouse Urine, Spleen and Thymus. Column: Fused silica capillary (50 or 75 µm i.d.). UV detection (λ= 290 nm). Limit of detection, 4500, 5000, 19.2 and 9.1 µg/L for CZE, MEKC, stacking MEKC and sweeping MEKC, respectively [Chiu *et al.* 2004].

Disposition in the Body Rat studies have indicated the presence of 4 metabolites in the urine; 2-(4-ethylthio-2,5-dimethoxyphenyl)-ethanol (M_r 242), 4-ethylthio-2,5-dimethoxyphenyl acetic acid (M_r 256), 1-acetamino-2-(2-hydroxy-4-ethylthio-5-methoxyphenyl)-ethane (M_r 269), and 1-acetamino-2-(2-methoxy-4-ethylthio-5-hydroxyphenyl)-ethane [Lin *et al.* 2003]. There are no published human data.

Toxicity At the time of writing (2007), there are no published data regarding non-fatal or fatal 2C-T-2 intoxication. In addition, there is no published pharmacological and toxicological information in humans. Subjective reports indicate it is a stimulant with hallucinogenic properties [Shulgin, Shulgin 1991].

Dose Threshold, 5 mg; light dose 10 to 15 mg; common dose 16 to 32 mg; strong dose 32 to 48 mg [Shulgin, Shulgin 1991].

Chiu YC *et al.* (2004). The bioactivity of 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) and its detection in rat urine by capillary electrophoresis combined with an on-line sample concentration technique. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 127–133.

Habrdova V *et al.* (2005). Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom* 40: 785–795.

Lin LC *et al.* (2003). Identification of 2,5-dimethoxy-4-ethylthiophenethylamine and its metabolites in the urine of rats by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 798: 241–247.

Poortman-van der Meer AJ (1999). The synthesis of 2,5-dimethoxy-4-ethylthiophenethylmine (2C-T-2). A case report. *J Clandestine Lab Invest Chemist Assoc* 9: 17–20.

Shulgin, A, Shulgin, A. (1991) *PiHKAL: A Chemical Love Story.* Berkeley, CA: Transform Press.

Theobald D, Maurer S, HH (2007). Identification of monoamine oxidase and cytochrome P450 isoenzymes involved in the deamination of phenethylamine-derived designer drugs (2C-series). *Biochem Pharmacol* 73: 287–297.

Theobald DS *et al.* (2005). New designer drug 2,5-dimethoxy-4-ethylthio-beta-phenethylamine (2C-T-2): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J Mass Spectrom* 40: 1157–1172.

Tsai CC *et al.* (2006). Optimization of the separation and on-line sample concentration of phenethylamine designer drugs with capillary electrophoresis-fluorescence detection. *J Chromatogr A* 1101: 319–323.

2C-T-7

5-HT_2 Receptor Agonist, Hallucinogen, Phenethylamine

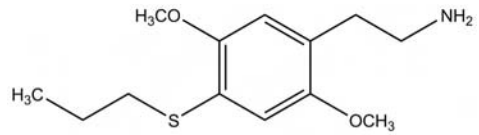
$C_{13}H_{21}NO_2S$=255.4

CAS—207740-26-9

IUPAC Name 2-(2,5-Dimethoxy-4-propylsulfanylphenyl)ethanamine

Synonyms 2,5-Dimethoxy-4-propylthiophenethylamine; 2-[2,5-dimethoxy-4-(propylthio)phenyl]ethanamine.

Street Names 7th Heaven; 7-Up; Beautiful; Lucky 7; Red Raspberry; T-7; Tripstasy; Tweety bird mescaline. Blue tablets with a 'Ying-Yang' logo containing 2C-T-7 have been sold as Blue Mystic in Dutch Smartshops.



Chemical Properties Mp 140° to 150°.

2C-T-7 Hydrochloride

$C_{13}H_{21}NO_2S$, HCl = 291.9

Synonyms 2-(2,5-Dimethoxy-4-propylsulfanylphenyl)ethanamine hydrochloride; 2,5-dimethoxy-4-propylthiophenethylamine hydrochloride; 2-[2,5-dimethoxy-4-(propylthio)phenyl]ethanamine hydrochloride.

Chemical Properties White crystalline solid. 2C-T-7 is stable for approximately 70 days in sodium fluoride-preserved blood when stored at 4° [Curtis *et al.* 2003].

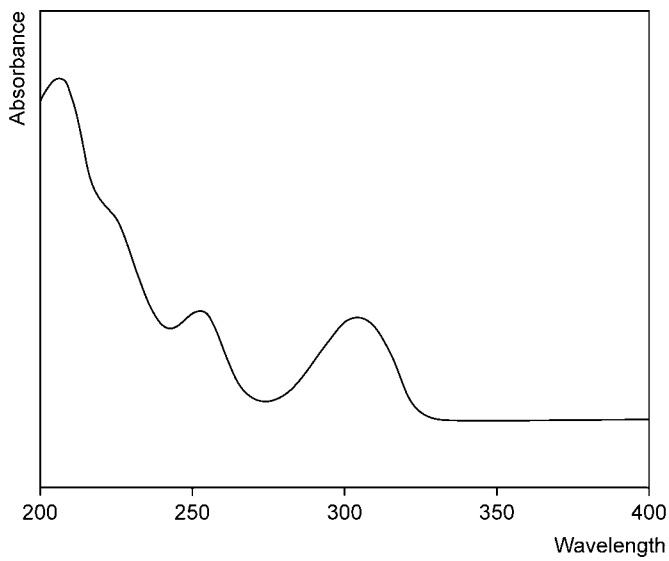
Colour Tests Marquis test—slow/weak 'salmon orange' (pink, orange red); Mecke's reagent—dark orange→dark purple-ish orange; sodium nitroprusside reagent—cherry red colour [Zimmerman 2001].

Gas Chromatography-Mass Spectrometry Column: HP-5MS cross-linked phenylmethylysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 1 min to 200° at 20°/min for 8.84 min. EI ionisation at 70 eV. Limit of detection, 5.11 mg/L [Tsai *et al.* 2006]. Column: HP-1 (12 m × 0.20 mm i.d., 0.33 µm). Temperature programme: 150° to 260° at 20°/min, for 2 min. MSD. Retention time: 3.75 min [Zimmerman 2001].

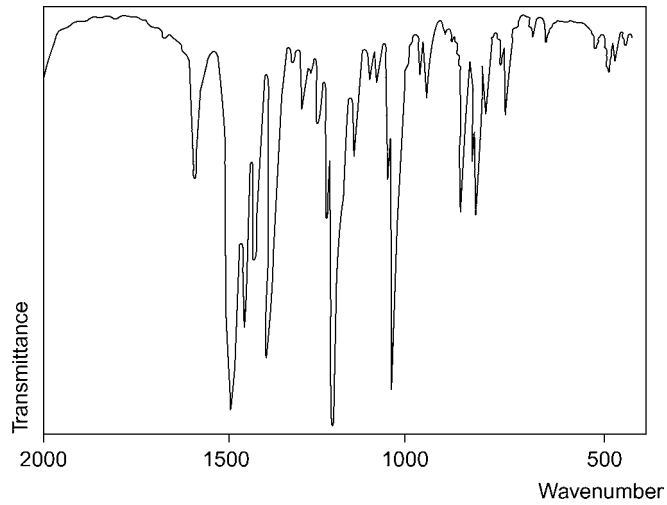
Gas Chromatography Column: HP-1 capillary (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° to 300° at 20°/min. NPD [Curtis *et al.* 2003].

Capillary Electrophoresis Column: fused silica capillary (total/effective length: 96/90 cm for sweeping MEKC and 100/94 cm for stacking MEKC, 50 µm i.d.). Lamp: Xe. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 340 nm). Limit of detection, 562 and 10.7 µg/L for sweeping and stacking MEKC, respectively [Tsai *et al.* 2006].

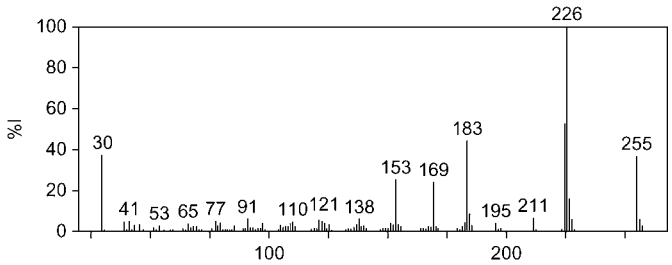
Ultraviolet Spectrum Methanol—206, 253 and 304 nm.



Infrared Spectrum Principal peaks at wavenumber 1208, 1499, 1392, 1040, 850, 811 cm^{-1} [Zimmerman 2001].



Mass Spectrum Principal ions at *m/z* 226, 225, 183, 30, 255, 153, 169, 227.



Quantification

Blood **GC-MS** Column: Phenomex ZBI (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 10°/min to 300° at 60°/min for 14 min. EI ionisation, SIM acquisition mode. Retention time: 12.9 min. Limit of detection, 5–10 µg/L [Vorce, Sklerov 2004]. Column: HP-1MS capillary (15 m × 0.25 mm i.d). Temperature programme: 120° to 300° at 20°/min. EI ionisation, SIM acquisition mode. Retention time: 11.3 min. Limit of quantification, 15.6 µg/L, limit of detection, 6.25 µg/L [Curtis *et al.* 2003].

Urine **GC-MS** See Blood [Vorce, Sklerov 2004]. See Blood [Curtis *et al.* 2003].

Other **GC-MS** Rat Urine. Column: HP-1 cross-linked methylsilicone capillary (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 310° at 30°/min. Full scan mode, EI ionisation at 70 eV. Limit of detection, 10 µg/L [Theobald *et al.* 2005].

Toxicity 2C-T-7 is believed to have been involved in 4 deaths worldwide but only 1 case has been published. 2C-T-7 can produce both CNS stimulation and hallucinogenic effects. Acute toxicity may present as nausea, vomiting and headache. Because of its potential inhibition of the action of monoamine oxidase, 2C-T-7 may adversely interact with monoamine oxidase inhibitor medication.

In a fatality where 2C-T-7 was identified 1 year after death, 2C-T-7 was found to be 57 µg/L in postmortem heart blood, 100 µg/L in femoral blood, 1.12 mg/L in urine and 0.854 mg/kg in liver. No other drugs were detected. Toxic symptoms included hallucinations, vomiting, convulsions, respiratory depression, and finally cardiac arrest. Studies indicated 2C-T-7 concentrations began to decline after 70 days at 4°; therefore it was suggested that the concentrations measured were likely to have been higher at the time of death [Curtis *et al.* 2003].

Curtis B *et al.* (2003). Postmortem identification and quantitation of 2,5-dimethoxy-4-n-propylthiophenethylamine using GC-MSD and GC-NPD. *J Anal Toxicol* 27: 493–498.

Tsai CC *et al.* (2006). Optimization of the separation and on-line sample concentration of phenethylamine designer drugs with capillary electrophoresis-fluorescence detection. *J Chromatogr A* 1101: 319–323.

Theobald DS *et al.* (2005). New designer drug 2,5-dimethoxy-4-ethylthio-beta-phenethylamine (2C-T-2): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J Mass Spectrom* 40: 1157–1172.

Vorce SP, Sklerov JH (2004). A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray-MS. *J Anal Toxicol* 28: 407–410.

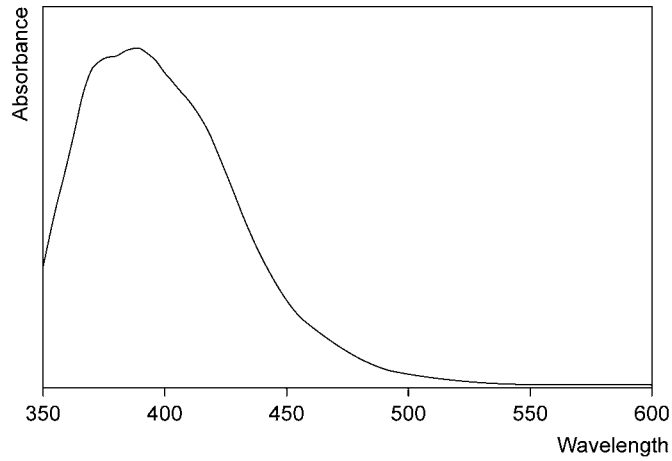
Zimmerman MM (2001). The identification of 2,5-dimethoxy-4-(N)-propylthiophenethylamine (2C-T-7). *Microgram* 34: 169–173.

Cyanide

Electron Transport Chain Inhibitor
CN = 26.02
CAS—57-12-5

IUPAC Name Cyanide
Synonyms Cyanide (1−); cyanide anion; cyanide ion; cyanides; isocyanide.

Chemical Properties A white solid with a faint almond odour. Water solubility 9.54 mg/L (25°). Log P (octanol/water), −0.69.
Colour Test Sodium Picrate (Steyn test)—orange-red/red; limit of detection, 25–135 µg/L.
Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 27, 26, 12, 28, 13, 14 (HCN).
Quantification

Blood **GC** Column: GasPRO (60 m × 0.32 mm i.d.). Carrier gas: He, 1.4 mL/min. Temperature programme: 130° for 1 min to 150° at 2°/min to 260° at 8°/min for 15 min. NPD. Limit of quantification, <4 µg/L, limit of detection, <3 µg/L [Boadas-Vaello *et al.* 2008]. Column: PoraBOND U fused silica (30 m × 0.32 mm i.d., 7 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 90° for 5 min to 140° at 10°/min. Retention time: 1.9 min. NPD. Limit of detection, 50 µg/L [Gambaro *et al.* 2007]. Column: HP-PLOT Q capillary (15 m × 0.32 mm i.d., 20 µm). Carrier gas: He, 3 mL/min. Temperature programme: 30° for 1.5 min to 190° at 35°/min for 1 min. NPD. Limit of detection, 30 µg/L [Calafat, Stanfill 2002]. Column: Supel-Q PLOT fused silica (30 m × 0.32 mm i.d.). Column temperature: −30° for 1 min to 160° at 10°/min. Carrier gas: He, 3.0 mL/min. NPD or EI ionisation, SIM acquisition mode. Retention time: 9.0 min. [Ishii *et al.* 1998].

GC-MS Column: CPSIL-19 CB (50 m × 0.32 mm i.d., 1.2 µm). Carrier gas: N_2, 20 psi. µECD. Limit of quantification, 0.01 mg/L [Felby 2009]. Column: HP-PLOT Q-bonded polystyrene–divinylbenzene (15 m × 0.32 mm i.d., 20 µm). Carrier gas: He, 2.1 mL/min. Temperature programme: −15° for 1.5 min to 240° at 40°/min for 1 min. SIM acquisition mode. Retention time: ~4.7 min. Limit of detection, 0.007 µg/g [Murphy *et al.* 2006]. Column: capillary with polar stationary phase. SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 6 µg/L (both in aqueous solutions) [Frison *et al.* 2006]. Carrier gas: He. Temperature programme: 40° for 1 min to 240° at 25°/min for 5 min. Limit of quantification, 1 µmol/L, limit of detection, 0.3 µmol/L [Dumas *et al.* 2005]. Column: CP Sil 8B methyl silicone (50 m × 0.23 mm, 1.2 µm). Temperature: 60°. Carrier gas: He, flow rate 2 mL/min. ECD. Retention time: cyanogen chloride, 2.7 min [Odoul *et al.* 1994]. See also Maseda *et al.* [1990].

HPLC Column: RP-18 (Hypersil ODS, 5 µm, 100 × 2.1 mm i.d.). Mobile phase: deionised water : acetonitrile (92 : 8), 0.4 mL/min flow rate. Fluorescence detection (λ_{ex} = 418 nm, λ_{em} = 460 nm). Retention time: 1.7 min for the 1-cyano-2-substituted benzoisoindole derivative. Limit of detection, 2 µg/L [Felscher, Wulfmeyer 1998]

LC-MS Column: NovaPak C_{18}. Mobile phase: 2 mmol/L ammonium acetate (pH 3.0). Negative ion mode. Limit of quantification, 15 µg/L, limit of detection, 5 µg/L [Tracqui *et al.* 2002].

CE Capillary: fused silica (64.5/56.0 cm total/effective length, 75 µm). Mobile phase: 0.1 mol/L β-alanine (pH 3.5). DAD (λ = 232 nm). Limit of quantification, 9 µmol/L, limit of detection, 3 µmol/L [Papežová, Glatz 2006]. Capillary: fused silica (117/55/108.5 cm total/length to fluorescence detector/length to UV detector, 75 µm i.d.). Mobile phase: 20 mmol/L borax buffer (pH 6.0) : methanol (8 : 2). Fluorescence detection (λ_{ex} = 418 nm, λ_{em} = 460 nm) or UV detection (λ = 250 nm). Limit of detection, 0.1 µg/L [Chinaka *et al.* 2001].

Plasma **GC-MS** Column: Rtx-5 fused silica (10 m × 0.18 mm i.d., 0.2 µm). Carrier gas: He, 1.01 mL/min. Temperature programme: 60° for 1 min to 180° at 15°/min for 1 min. EI ionisation at 70 eV, scan or SIM acquisition mode. Limit of detection, 40 µg/L [Liu *et al.* 2009]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 1 min to 230° at 15°/min to 300° at 30°/min for 1 min. MSD, SIM acquisition mode. Limit of quantification, 50 µg/L [Logue *et al.* 2005].

Note For a bioassay for the detection of cyanide in serum see Fonong [1987].

Urine **GC-MS** See Plasma. Limit of detection, 10 µg/L [Liu *et al.* 2009]. See Plasma [Logue *et al.* 2005].

HPLC Column: Kromasil KR 100-5 C_{18} (250 × 4.6 mm, 5 µm). Mobile phase: methanol : 50 mmol/L *o*-phosphoric acid (pH 2.3, 1 : 1.3), flow rate 0.5 mL/min. Fluorometric detection (λ_{ex} = 385 nm, λ_{em} = 476 nm). Retention time: 12 min for the *N*-carbamylcysteine–DACM derivative, 22 and 24 min for the cysteine-DACM derivative. Limit of detection, 0.3 µmol/L [Lundquist *et al.* 1995].

CE Capillary: fused silica (44/36 cm total/effective length, 50 µm) coated with cetyltrimethylammonium bromide. UV detection (λ = 254 nm). Limit of detection, 0.01 µmol/L [Meng *et al.* 2009]. Capillary: fused silica (25/18 cm total/effective length, 75 µm i.d.). Running buffer: 20 mmol/L sulfuric acid (pH 3) with 1 mmol/L hexamethonium bromide. UV detection (λ = 254 nm). Limit of detection, 0.08 µmol/L [Jermak *et al.* 2006].

Oral Fluid **CE** See Urine [Jermak *et al.* 2006; Meng *et al.* 2009].

Disposition in the Body Cyanide is metabolised to thiocyanate after reacting with a sulfur donor, for example thiosulfate. Up to 80% of a cyanide dose is converted this way. An alternative metabolic pathway is the formation of 2-aminothiazoline-4-carboxylic acid when cyanide reacts with cystine (when exposure rate exceeds cyanide-to-thiocyanate conversion rate). Cyanide is excreted through the lungs and kidneys.

Therapeutic Concentration In healthy subjects, endogenous cyanide concentration is approximately 8.41 ± 3.09 µg/L [Ishii *et al.* 1998].

A smoker can have cyanide levels of ≥500 µg/L in whole blood. For normal red blood cell cyanide, levels can be <26 µg/L. The blood therapeutic concentration is 1–12 µg/L.

Toxicity Cyanide can be fatal at plasma concentrations of 260 µg/L and above. The toxic concentration is 500 µg/L; lethal concentration, 4–5 mg/L but also reported to be as low as 1 mg/L.

A woman who attempted suicide by subcutaneous injection of cyanide had serum cyanide levels of 4.6 mg/L [Prieto *et al.* 2005].

Half-life Cyanide has a half-life of 0.7–2.1 h.

Volume of Distribution 0.4 L/kg.

Distribution in Blood Cyanide is concentrated in the erythrocytes. 98% of cyanide is found in red blood cells and 2% in plasma.

Protein Binding Binds to methemoglobin, present in haemoglobin.

Boadas-Vaello P *et al.* (2008). Determination of cyanide and volatile alkylnitriles in whole blood by headspace solid-phase microextraction and gas chromatography with nitrogen phosphorus detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 870: 17–21.

Calafat AM, Stanfill SB (2002). Rapid quantitation of cyanide in whole blood by automated headspace gas chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 131–137.

Chinaka S *et al.* (2001). High-sensitivity analysis of cyanide by capillary electrophoresis with fluorescence detection. *Anal Sci* 17: 649–652.

Dumas P *et al.* (2005). Isotope dilution-mass spectrometry determination of blood cyanide by headspace gas chromatography. *J Anal Toxicol* 29: 71–75.

Felby S (2009). Determination of cyanide in blood by reaction head-space gas chromatography. *Forensic Sci Med Pathol* 5: 39–43.

Felscher D, Wulfmeyer M (1998). A new specific method to detect cyanide in body fluids, especially whole blood, by fluorimetry. *J Anal Toxicol* 22: 363–366.

Fonong T (1987). Enzyme method for the spectrophotometric determination of micro-amounts of cyanide. *Analyst* 112: 1033–1035.

Frison G *et al.* (2006). An improved method for cyanide determination in blood using solid-phase microextraction and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 20: 2932–2938.

Gambaro V *et al.* (2007). Blood cyanide determination in two cases of fatal intoxication: comparison between headspace gas chromatography and a spectrophotometric method. *J Forensic Sci* 52: 1401–1404.

Ishii A *et al.* (1998). Determination of cyanide in whole blood by capillary gas chromatography with cryogenic oven trapping. *Anal Chem* 70: 4873–4876.

Jermak S *et al.* (2006). Headspace single-drop microextraction with in-drop derivatization and capillary electrophoretic determination for free cyanide analysis. *Electrophoresis* 27: 4538–4544.

Liu G *et al.* (2009). Rapid determination of cyanide in human plasma and urine by gas chromatography–mass spectrometry with two-step derivatization. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 3054–3058.

Logue BA *et al.* (2005). Determination of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine and plasma by gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 237–244.

Lundquist P *et al.* (1995). Analysis of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine by high-performance liquid chromatography. *Anal Biochem* 228: 27–34.

Maseda C *et al.* (1990). [Determination of blood cyanide using head-space gas chromatography with electron capture detection]. *Nihon Hoigaku Zasshi* 44: 131–136.

Meng L *et al.* (2009). Simultaneous derivatization and extraction of free cyanide in biological samples with home-made hollow fiber-protected headspace liquid-phase microextraction followed by capillary electrophoresis with UV detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 3645–3651.

Murphy KE *et al.* (2006). Determination of cyanide in blood by isotope-dilution gas chromatography–mass spectrometry. *Clin Chem* 52: 458–467.

Odoul M *et al.* (1994). Specific determination of cyanide in blood by headspace gas chromatography. *J Anal Toxicol* 18: 205–207.

Papežová K, Glatz Z (2006). Determination of cyanide in microliter samples by capillary electrophoresis and in-capillary enzymatic reaction with rhodanese. *J Chromatogr A* 1120: 268–272.

Prieto I *et al.* (2005). Acute cyanide poisoning by subcutaneous injection. *Emerg Med J* 22: 389–390.

Tracqui A *et al.* (2002). Determination of blood cyanide by HPLC-MS. *J Anal Toxicol* 26: 144–148.

Cyanocobalamin

Haemopoietic Vitamin

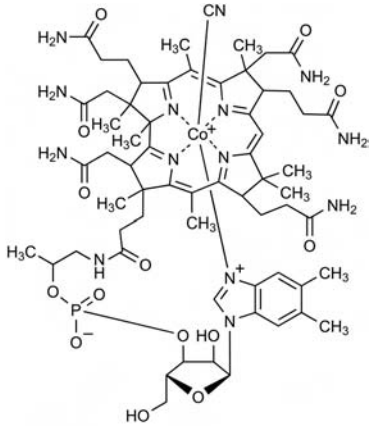
$C_{63}H_{88}CoN_{14}O_{14}P$ = 1355.4

CAS—68-19-9

IUPAC Name 5,6-Dimethylbenzimidazolyl cyanocobamide

Synonyms Cobamin; cycobemin; vitamin B_{12}.

Proprietary Names *Anacobin*; *Bedoz*; *Berubigen*; *Betalin 12*; *Bio-12*; *Cyanabin*; *Cytacon*; *Cytamen*; *Hepacon-B12*; *Neo-Rubex*; *Redisol*; *Rubesol*; *Rubion*; *Rubramin*; *Ruvite*; *Sytobex*. It is an ingredient of *Ce-Cobalin*, *Hepanorm* and *Reactivan*.



Chemical Properties Dark red hygroscopic crystals or powder. Soluble 1 in 80 of water and 1 in 180 of ethanol (90%); practically insoluble in chloroform and ether. Log *P* (octanol/water), 3.6.

High Performance Liquid Chromatography System HAA—retention time 3.8 min.

Ultraviolet Spectrum Water—278 (A^1_1=119a), 361 (A^1_1=207a), 550 nm (A^1_1=63a).

Infrared Spectrum Principal peaks at wavenumbers 1660, 1497, 1575, 1070, 1150, 1220 cm^{-1} (KBr disk).

Dose Initially 0.25 to 1 mg IM, on alternate days.

Cyanogen Bromide

Reagent for Synthesis of Cyanamides

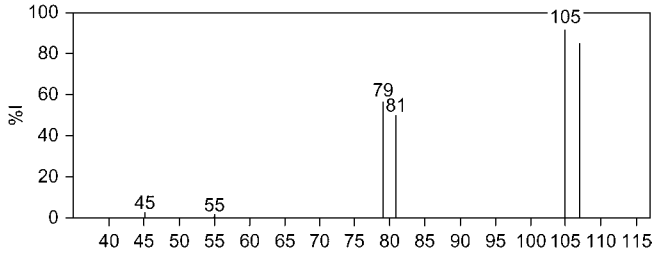
CNBr = 105.9

CAS—506-68-3

Synonyms Bromine cyanide; CBrN.

Chemical Properties Crystalline solid, decomposition in the presence of moisture. Vapours are highly irritant and very poisonous. Mp 52°. Bp 61° to 62°. Freely soluble in water, alcohol and ether. Aqueous solutions of alkalis decompose it to alkali cyanide and alkali bromide. Log *P* (octanol/water), −0.29 [Meylan, Howard 1995].

Mass Spectrum Principal ions at *m/z* 105, 79, 81, 45, 55 [Yang, Shang 2005].



Quantification

Other **GC** Water. Column: DB-1701 fused silica (30 m × 320 µm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 30° to 35° at 5°/min to 220° at 10°/min. ECD. Limit of detection, 41 ng/L [Cancho *et al.* 2000].

GC-MS Environmental Samples. EI ionisation at 70 eV, MSD. Limit of detection, 1.7 µg/L [Yang, Shang 2005].

Cancho B *et al.* (2000). Simultaneous determination of cyanogen chloride and cyanogen bromide in treated water at sub-microg/L levels by a new solid-phase microextraction–gas chromatography–electron-capture detection method. *J Chromatogr A* 897: 307–315.

Meylan W, Howard M, PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Yang X, Shang C (2005). Quantification of aqueous cyanogen chloride and cyanogen bromide in environmental samples by MIMS. *Water Res* 39: 1709–1718.

Cyanogen Chloride

Reagent for Synthesis of Cyanamides, Military Poison Gas

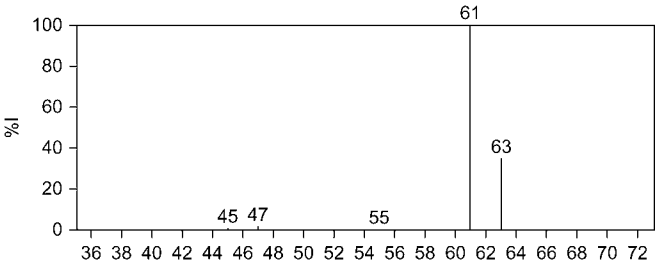
CNCl = 61.5

CAS—506-77-4

Synonym CClN

Chemical Properties Liquid. Vapours are highly irritant and very poisonous. Mp −6°. Bp 13.8°. Soluble in water, alcohol and ether. Log *P* (octanol/water), −0.29 [Meylan, Howard 1995].

Mass Spectrum Principal ions at *m/z* 61, 63, 47, 45, 55 [Yang, Shang 2005].



Quantification

Other **GC** Water. Column: DB-1701 fused silica (30 m × 320 µm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 30° to 35° at 5°/min to 220° at 10°/min. ECD. Limit of detection, 77 ng/L [Cancho *et al.* 2000].

GC-MS Environmental Samples. EI ionisation at 70 eV, MSD. Limit of detection, 1.7 µg/L [Yang, Shang 2005].

Cancho B *et al.* (2000). Simultaneous determination of cyanogen chloride and cyanogen bromide in treated water at sub-microg/L levels by a new solid-phase microextraction–gas chromatography–electron-capture detection method. *J Chromatogr A* 897: 307–315.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Yang X, Shang C (2005). Quantification of aqueous cyanogen chloride and cyanogen bromide in environmental samples by MIMS. *Water Res* 39: 1709–1718.

Cyclamic Acid

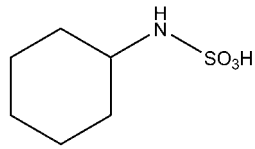
Cyclic Sulfamic Acid, Sweetener

$C_6H_{13}NO_3S$ = 179.2

CAS—100-88-9

IUPAC Name *N*-Cyclohexylsulfamic acid

Synonyms Cyclam. acid; E952; hexamic acid.



Chemical Properties White crystalline powder. Mp 175°. Soluble 1 in 7.5 of water, 1 in 3 of ethanol, 1 in 250 of chloroform, and 1 in 4 of propylene glycol. Cyclamic acid and its salts are intense sweetening agents. In dilute solutions (≤0.17%), sodium cyclamate is approximately 30-times as sweet as sucrose (but this factor decreases at higher concentrations). When the concentration approaches 0.5%, a bitter taste becomes noticeable. It is stable to heat. pKa 1.9.

Calcium Cyclamate

$C_{12}H_{24}CaN_2O_6S_2$, $2H_2O$ = 432.6

CAS—139-06-0 (anhydrous calcium cyclamate); 5897-16-5 (calcium cyclamate dihydrate)

IUPAC Name Calcium *N*-cyclohexylsulfamate dihydrate

Synonyms Calcium calc. cyclam.; calcium cyclohexanesulfamate; ciclamato de calcio; cyclamate calcium; E952.

Proprietary Name *Sucaryl Calcium*

Chemical Properties White crystals or crystalline powder. Soluble 1 in 4 of water and 1 in 50 of ethanol; almost insoluble in ether and chloroform.

Potassium Cyclamate

$C_6H_{12}NO_3SK$ = 217.3

CAS—7758-04-5

IUPAC Name Potassium *N*-cyclohexysulfamate

Synonyms Cyclamate potassium; HSDB 1239; monopotassium cyclohexanesulfamate; potassium cyclohexanesulfamate.

Sodium Cyclamate

$C_6H_{12}NNaO_3S$ = 201.2

CAS—139-05-9

IUPAC Name Sodium *N*-cyclohexylsulfamate

Synonyms

Ciclamato de sodio; cyclamate sodium; E952; natrii cyclamas; natrio ciklamatas; nátrium-ciklamát; natrium-cyklamát; natriumcyklamat; natriumsyklamaatti; siklamat sodyum; sod. cyclam.; sodium cyclohexanesulphamate; sodium, cyclamate de.

Proprietary Names *Dulceryl*; *Finn Cristal*; *Kaldil-Diet*; *Rondo*; *Sucaryl Sodium*; *Sucrin*; *Sukar-Sin*; *Tadalin*.

Chemical Properties White crystals or crystalline powder. Soluble 1 in 5 of water and 1 in 250 of ethanol; almost insoluble in ether and chloroform. A 10% solution in water has a pH of 5.5 to 7.5.

Thin-layer Chromatography Plates: glass plates (10 × 10 cm or 10 × 20 cm) were coated with 0.25 mm of Avicel SF (other experiments included coating the plates with polyamide, silica gel, alumina or DEAE-cellulose). Solvent systems: A) ethyl acetate : concentrated ammonia : acetone (1 : 1 : 8); B) dimethylformamide : ethanol : water (5 : 4 : 1); C) dioxane : pyridine : water (7 : 2 : 1); D) pyridine : ethanol : water (6 : 3 : 1); E) tetrahydrofuran : pyridine : water (6 : 3 : 1). Location reagents: 1) spray with 0.1 % Pinacryptol Yellow solution in 95% ethanol, dry in the dark for 10 min and examine under UV light; cyclamate appears as an orange fluorescent spot on a light-greenish-blue background, saccharin is a non-fluorescent orange spot, dulcin a dark violet spot; 2) after exposure to bromine vapour, spray with 0.1% ethanolic solution of fluorescein, air-dry, spray with 2% ethanolic solution of naphthylethylenediamine; cyclamate appears as a yellow spot, saccharin is yellowish pink, dulcin is yellowish orange on a dull orange background; 3) spray with 1 part of 0.1% ethanolic solution of Methyl Red and 2 parts of phosphate buffer solution (pH 7.0); cyclamate and saccharin give reddish orange spots on a yellow background; 4) spray with solution of silver nitrate (0.17 g) in 1 mL water and mixed with 5 mL of 10% ammonia, diluted to 200 mL with ethanol, then spray with 0.01% ethanolic pyrogallol; cyclamate appears as a transient white spot on a brown background. R_f values were reported as follows:

	Solvent system				
	A	***B***	***C***	***D***	***E***
Cyclamate	0.15	0.74	0.29	0.47	0.31
Saccharin	0.31	0.83	0.39	0.64	0.41
Dulcin	0.82	0.91	0.80	0.79	0.89

Limits of detection (µg) were reported as follows:

	Method of detection			
	1	***2***	***3***	***4***
Cyclamate	1.0	2.0	4.0	8.0
Saccharin	0.2	1.0	1.0	1.0
Dulcin	0.2	1.0	–	–

[Nagasawa *et al.* 1970]

Plates: glass plates (10 × 20 cm) coated with silica Gel G in water (1 : 2), 0.25-µm thickness, activated at 110° for 1 h. Solvent system: ethyl acetate : isopropyl alcohol : acetone : methanol : water (50 : 15 : 15 : 4 : 16). Location reagent bromine vapour and 0.05 g sodium fluorescein in 10 mL absolute alcohol. R_f 0.56. Quantification with fluorescence detection (λ = 540 nm). Limit of quantification not reported [Das *et al.* 1970].

Quantification

Urine **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L monoammonium phosphate (pH 3.5; 60 : 40), flow rate 1.0 mL/min. UV detection (λ = 335 nm). Retention time: cyclohexylamine 10 min (trinitrophenyl derivative). Limit of detection, 0.11 ppm for cyclamate, 0.06 ppm for cyclohexylamine [Casals *et al.* 1996].

Other **HPLC** Food Samples. Column: C_{18} (250 × 3.0 mm i.d., 5 µm). Mobile phase: buffer preparation, 4 mL formic acid in 5 L water, adjusted to pH 4.5 with 12.5 mL triethylamine; methanol-buffer-acetone (69 : 24 : 7) : methanol-buffer-acetone (11 : 82 : 7; 0 : 100 for 4 min to 53 : 47 over 7 min to 100 : 0 over 12 min for 1 min), flow rate 0.5 mL/min. ELS detection. Retention times: cyclamic acid ≈5.5 min, acesulfame potassium ≈3.8 min, saccharin ≈4.2 min, aspartame ≈11 min, sucralose ≈12.5 min, dulcin ≈13.5 min, alitame ≈15.0 min, neohesperidine dihydrochalcone ≈17.5 min, neotame ≈22.0 min. Limit of quantification, 26 mg/kg for cyclamic acid, aspartame, neotame, saccharin, sucralose, 25 mg/kg for neohesperidine dihydrochalcone, 27 mg/kg for alitame, 29 mg/kg for acesulfame chicken, 43 mg/kg for dulcin; limit of detection, 13 mg/kg for cyclamic acid, acesulfame potassium, alitame, aspartame, neodulcin, saccharin and sucralose, 11 mg/kg for neohesperidine dihydrochalcone; 30 mg/kg for dulcin [Wasik *et al.* 2007]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (4 : 1), flow rate 1.0 mL/min. UV detection (λ = 314 nm). Limit of quantification not reported [Horie *et al.* 2007]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L phosphate buffer (pH 7.0) : methanol (3 : 2), flow rate 1.0 mL/min with Methyl Red (pH 7.0), flow rate 30 µmol/L. UV-Vis detection (λ = 433 nm). Retention times: sodium cyclamate 6 min, saccharin 4 min, aspartame 10 min. Limit of quantification not reported [Choi *et al.* 2000]. Column: C_{18} (300 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.02 mol/L potassium dihydrogen phosphate (3 : 97), flow rate 1.3 mL/min. Post-column ion-pair extraction and absorbance detection (λ = 546 nm). Retention time: 10 min. Limit of detection, 1 to 2 mg/L [Lawrence 1987].

LC-MS Food Samples. Column: C_{18} (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol: 0.1% aqueous formic acid (4 : 1), flow rate 0.2 mL/min. ESI, negative ion mode. Limit of quantification not reported [Horie *et al.* 2007].

CE Food Samples. Column: uncoated fused silica capillary (total/effective length: 64.5/56 cm, 75 µm i.d.). Running buffer: 1.0 mmol/L hexadecyltrimethylammonium bromide and 10 mmol/L potassium sorbate. UV detection (λ = 300 nm). Migration time: 4 min. Limit of detection, 5 mg/kg for syrups, jams, and drinks and 10 mg/kg for pickles and confectionery [Horie *et al.* 2007].

Note For the detection of cyclamic acid using GC with packed columns, see Weston and Wheals [1970] and Nagasawa *et al.* [1975].

Disposition in the Body When administered as the sodium or calcium salt in humans, cyclamic acid has been found to be largely excreted unchanged, mainly in the urine and, to a lesser extent, in the faeces. However, there is some evidence that a very small proportion may be converted to cyclohexylamine, which is reported to be toxic. Inter-individual differences have been observed with regard to the ability to metabolise cyclamate: >70% of the human population cannot metabolise cyclamate, whereas only 3–5% of the population metabolise >20% of their daily intake.

Toxicity Single 1-g doses of sodium and calcium cyclamate have been administered IV without ill effect and up to 12 g of calcium cyclamate has been administered orally, the only side-effect being the production of soft stools. The use of cyclamates as artificial sweeteners in food, soft drinks, and artificial sweetening tablets was at one time prohibited in Great Britain and some other countries because of concern about the metabolite cyclohexylamine. However, after reappraisal their use is now allowed.

The WHO has set an estimated acceptable daily intake for sodium and calcium cyclamate, expressed as cyclamic acid, at up to 11 mg/kg body-weight. In Europe, a temporary acceptable daily intake for sodium and calcium cyclamate, expressed as cyclamic acid, has been set at up to 1.5 mg/kg body-weight. LD_{50} (mouse, IP): 1.15 g/kg; LD_{50} (mouse, IV): 4.8 g/kg; LD_{50} (mouse, oral): 17 g/kg; LD_{50} (rat, IP): 1.35 g/kg; LD_{50} (rat, IV): 3.5 g/kg; LD_{50} (rat, oral): 15.25 g/kg.

Half-life Cyclamic acid 8 h, cyclohexylamine 4 h.

Volume of Distribution Cyclohexylamine 2.05 to 2.90 L/kg.

Clearance Cyclohexylamine 511 to 757 mL/min (renal).

Protein Binding Cyclohexylamine 33%.

Note For absorption/excretion studies of cyclamic acid and cyclohexylamine in humans and other species, see Renwick and Williams [1972] and Eichelbaum *et al.* [1974].

Casals I *et al.* (1996). Quantification of cyclamate and cyclohexylamine in urine samples using high-performance liquid chromatography with trinitrobenzenesulfonic acid pre-column derivatization. *J Chromatogr A* 750: 397–402.

Choi MM *et al.* (2000). Determination of cyclamate in low-calorie foods by high-performance liquid chromatography with indirect visible photometry. *Analyst* 125: 217–220.

Das DK *et al.* (1970). Detection and estimation of cyclamate by thin-layer chromatography. *J Chromatogr* 52: 354–356.

Eichelbaum M *et al.* (1974). Pharmacokinetics, cardiovascular and metabolic actions of cyclohexylamine in man. *Arch Toxikol* 31: 243–263.

Horie M *et al.* (2007). Rapid determination of cyclamate in foods by solid-phase extraction and capillary electrophoresis. *J Chromatogr A* 1154: 423–428.

Lawrence JF (1987). Use of post-column ion-pair extraction with absorbance detection for the liquid chromatographic determination of cyclamate and other artificial sweeteners in diet beverages. *Analyst* 112: 879–881.

Nagasawa K *et al.* (1975). Gas chromatographic determination of micro-amounts of cyclamates. *J Chromatogr* 111: 51–56.

Nagasawa K *et al.* (1970). Separation and detection of synthetic sweetners by thin-layer chromatography. *J Chromatogr* 52: 173–176.

Renwick AG, Williams RT (1972). The metabolites of cyclohexylamine in man and certain animals. *Biochem J* 129: 857–867.

Wasik A *et al.* (2007). Simultaneous determination of nine intense sweeteners in foodstuffs by high performance liquid chromatography and evaporative light scattering detection–development and single-laboratory validation. *J Chromatogr A* 1157: 187–196.

Weston RE, Wheals BB (1970). The determination of cyclohexylamine by electron-capture gas chromatography. *Analyst* 95: 680–682.

Cyclandelate

Vasodilator

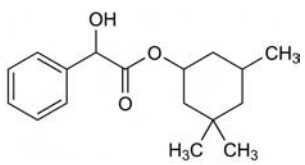
$C_{17}H_{24}O_3$ = 276.4

CAS—456-59-7

IUPAC Name (3,3,5-Trimethylcyclohexyl) 2-hydroxy-2-phenylacetate

Synonym α-Hydroxybenzeneacetic acid 3,3,5-trimethylcyclohexyl ester

Proprietary Names *Cyclobral*; *Cyclospasmol*; *Spasmocyclon*.



Chemical Properties A white amorphous powder, which may sublime on storage into a crystalline form, resembling cotton wool. Mp 55.0° to 56.5°. Practically insoluble in water; soluble 1 in about 1 of ethanol; very soluble in chloroform and ether; freely soluble in acetonitrile, ethyl acetate, dimethylformamide and toluene. Log *P* (octanol/water), 4.1.

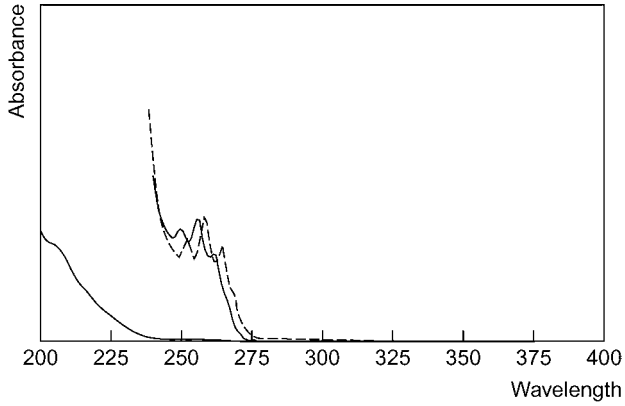
Colour Tests Liebermann's reagent—orange; Marquis test—orange (slow).

Thin-layer Chromatography System TB—R_f 0.37; system TD—R_f 0.74; system TE—R_f 0.80; system TF—R_f 0.77; system TAD—R_f 0.73; system TAE—R_f 0.87; system TAF—R_f 0.95; system TAJ—R_f 0.81; system TAK—R_f 0.71; system TAL—R_f 0.95.

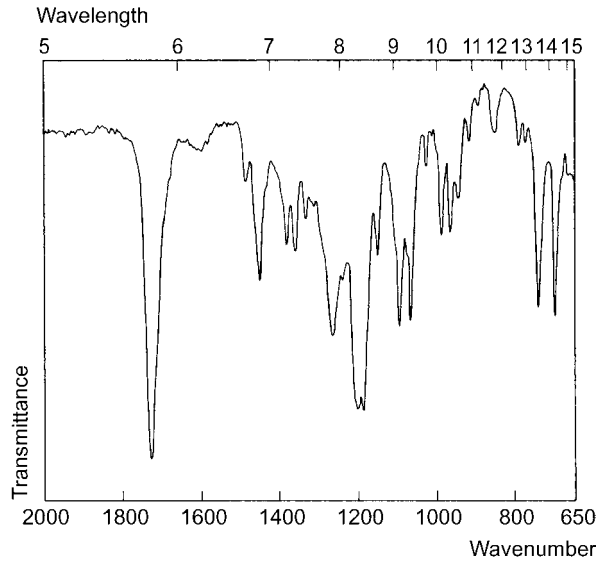
Gas Chromatography System GA—RI 1903.

High Performance Liquid Chromatography System HAA—retention time 26.4 min.

Ultraviolet Spectrum Methanol—252, 258 (A_1^1=7.6b), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1734, 1192, 1212, 1274, 1104, 1074 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 107, 69, 125, 83, 79, 55, 41, 77.

Dose 1.6 g daily.

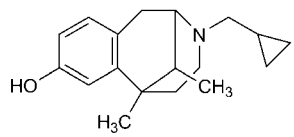
Cyclazocine

Narcotic Antagonist

$C_{18}H_{25}NO$ = 271.4

CAS—3572-80-3

IUPAC Name 3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol



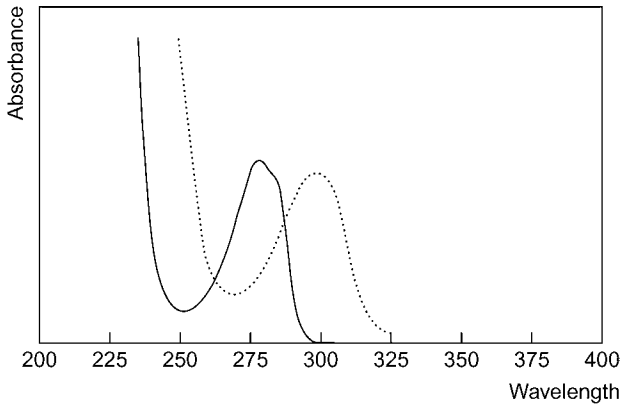
Chemical Properties Crystals. Mp 201° to 204°. Soluble in chloroform and dilute acetic acid. Log *P* (octanol/pH 7.4) 1.3.

Colour Tests Liebermann's reagent—black; Mandelin's test—green; Marquis test—brown→green.

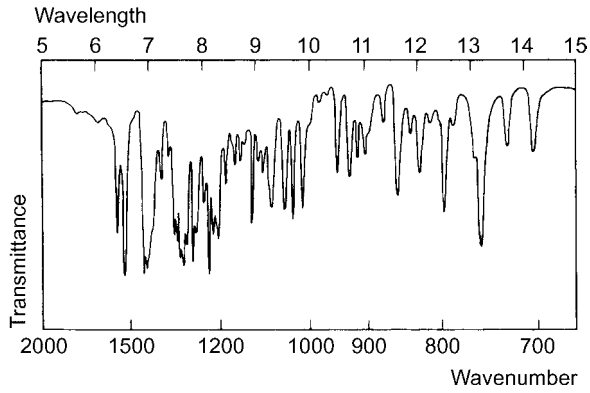
Thin-layer Chromatography System TA—R_f 0.53; system TAE—R_f 0.24; system TAF—R_f 0.74; system TAG—R_f 0.25; system TB—R_f 0.15; system TC—R_f 0.13; system TE—R_f 0.65 (acidified iodoplatinate solution, strong reaction).

High Performance Liquid Chromatography System HA—*k* 2.1; system HY—RI 289.

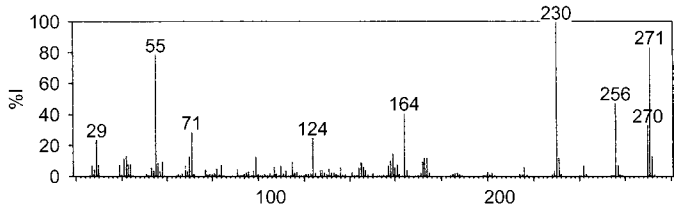
Ultraviolet Spectrum Aqueous acid—277 nm; aqueous alkali—237, 298 nm.



Infrared Spectrum Principal peaks at wavenumbers 1570, 1255, 1304, 754, 1227, 1241 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 230, 271, 55, 256, 164, 270, 71, 124.



Quantification

Plasma **GC** Column: 3% OV-1 on Gas Chrom Q (180 cm × 4 mm i.d.). Temperature: 210°. FID. Retention time: 5.7 min. Limit of detection, 0.05–0.12 mg/L [Medzihradsky, Dahlstrom 1975].

GC-MS Column: glass (91.44 cm). Carrier gas: CH_4, 10 mL/min. Temperature: 260°. CI, SIM acquisition mode. Retention time: ≈0.9 min. Limit of quantification, 110 ng/L, limit of detection, 20 ng/L [Peterson *et al.* 1979].

Urine **GC** Column: 3% OV-17 on Chromosorb W-HP 80/100 mesh. Carrier gas: N_2, 40 mL/min. Temperature: 270°. FID. Limit of detection, 1 mg/L [Digregorio, O'Brien, 1974]. Column: 3% SE-30 on Gas-Chrom Q 80/100 mesh (6′ × 2 mm i.d.). Carrier gas: He, 30–35 mL/min. Temperature 210°–220°. FID. Limit of detection, 10 ng/mL [Kaiko, Inturrisi 1974].

Disposition in the Body Absorbed after oral administration. Approximately 20% of a dose is excreted in the urine unchanged together with 24% as conjugated cyclazocine, 4% as norcyclazocine, and 11% as conjugated norcyclazocine.

Dose Cyclazocine has been given in maintenance doses of about 4 mg daily; doses of 10 mg have been given to extend the effect to 48 h.

Digregorio GJ, O'Brien (1974). Chromatographic detection of narcotic antagonists in human urine. *J Chromatogr* 101: 424–427.

Kaiko RF, Inturrisi CE (1974). The quantitation of cyclazocine and its metabolites in human urine by use of gas-liquid chromatography. *J Chromatogr* 100: 63–72.

Medzihradsky F, Dahlstrom J (1975). Concurrent determination of narcotic drugs in plasma by gas-liquid chromatography. *Pharmacol Res Commun* 07: 55–69.

Peterson JE *et al.* (1979). Analysis of cyclazocine in plasma. *J Pharm Sci* 68: 1447–1450.

Cyclizine

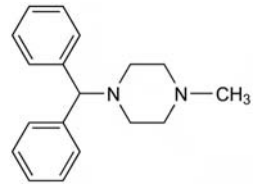
Antihistamine, Antiemetic

$C_{18}H_{22}N_2$ = 266.4

CAS—82-92-8

IUPAC Name 1-Benzhydryl-4-methylpiperazine

Synonyms Compound 47-83; 1-diphenylmethyl-4-methylpiperazine; Wellcome preparation 47-83.



Chemical Properties A white or creamy-white light-sensitive crystalline powder. Mp 105.5° to 107.5° (from petroleum ether). Practically insoluble in water; soluble 1 in 6 of ethanol, 1 in 1 of chloroform, and 1 in 6 of ether. Dissolves in most organic solvents and in dilute acids. pK_a 2.4, 7.8. Log *P* (octanol/water), 2.97.

Cyclizine Hydrochloride

$C_{18}H_{22}N_2$, HCl = 302.8

CAS—303-25-3

Synonym Cyclizinium chloride

Proprietary Names *Covamet*; *Echnatol*; *Emitex*; *Fortravel*; *Marezine (tablets)*; *Marzine*; *Medazine*; *Nauzine*; *Norizine*; *Ryccard*; *Triazine*; *Valoid (tablets)*. It is an ingredient of *Cyclimorph*, *Diconal*, *Igril*, *Megral*, *Migral*, *Migril*, *Vertipam*, and *Wellconal*.

Chemical Properties A white crystalline powder, or small colourless crystals. Mp about 285°, with decomposition. Soluble 1 in about 125 of water, 1 in about 120 of ethanol, and 1 in 75 of chloroform; practically insoluble in ether.

Cyclizine Lactate

$C_{18}H_{22}N_2$, $C_3H_6O_3$ = 356.5

CAS—5897-19-8

Proprietary Names *Marezine* (injection); *Valoid* (injection).

Chemical Properties Freely soluble in water.

Cyclizine Tartrate

$C_{18}H_{22}N_2$, $C_4H_6O_6$ = 416.5

Colour Tests Liebermann's reagent—orange; Marquis test—yellow

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.48; system TC—R_f 0.41; system TE—R_f 0.68; system TL—R_f 0.16; system TAE—R_f 0.39; system TAF—R_f 0.52; system TAJ—R_f 0.23; system TAK—R_f 0.11; system TAL—R_f 0.72 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, yellow).

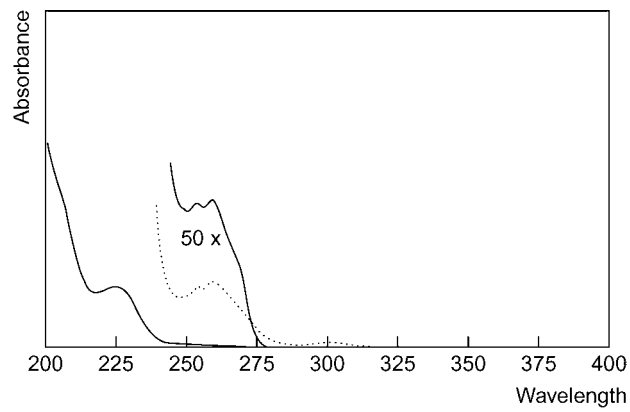
Gas Chromatography System GA—cyclizine RI 2025, M (nor-) RI 2120, M (benzophenone) RI 1610; system GB—cyclizine RI 2104, M (nor-) RI 2128, M (benzophenone) RI 1673; system GC—RI 2348; system GF—RI 2320.

Gas Chromatography-Mass Spectrometry

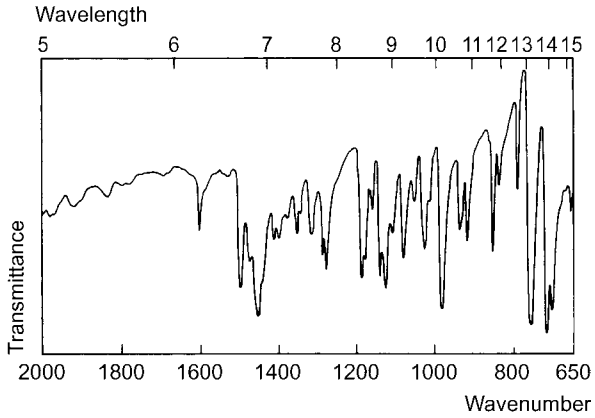
Column: OV-17 fused silica (15 m × 0.25 mm i.d., 0.2 μm). Temperature programme: 100° to 260° at 10°/min for 11 min. Injector temperature: 230°. IS: diphenhydramine. TIC, *m/z*56 and 58 for cyclizine and diphenhydramine, respectively. Retention time: cyclizine, 12.2 min; diphenhydramine, 10.5 min [Backer *et al.* 1989].

High Performance Liquid Chromatography System HA—cyclizine *k* 2.9, norcyclizine *k* 2.2; system HX—RI 405; system HZ—retention time 4.8 min; system HAX—retention time 12.4 min; system HAY—retention time 5.8 min.

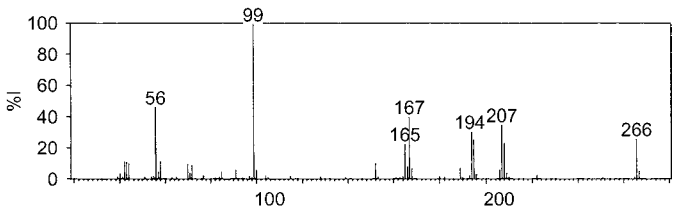
Ultraviolet Spectrum Aqueous acid—257, 262 (A_1^1=28a), 268 nm; aqueous alkali—260 nm (A_1^1=16b).



Infrared Spectrum Principal peaks at wavenumbers 716, 756, 701, 984, 1125, 1496 cm^{-1} (cyclizine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 99, 56, 167, 207, 194, 266, 195, 165.



Quantification

Blood **GC** AFID. Limit of detection, 10 µg/L [Griffin, Baselt 1984].

Plasma **GC** AFID. Cyclizine and norcyclizine. Limit of detection, 10 µg/L for cyclizine [Land *et al.* 1981].

Serum **HPLC** Column: ODS C_{18} (Techil, 150 × 4.1 mm i.d., 5 µm), at 30°. Mobile phase: 0.05 mol/L phosphate buffer (pH 3.0) : acetonitrile (70:30), flow rate 1 mL/min. IS: chlorcyclizine hydrochloride. Electrochemical detection. Retention time: norcyclizine, 5 min; cyclizine, 6 min; IS, 12 min. Limit of detection, 1 µg/L [Walker, Kanfer 1995].

Urine **GC** See Plasma [Land *et al.* 1981]. See Blood. Limit of detection, 5 µg/L [Griffin, Baselt 1984].

HPLC See Serum [Walker, Kanfer 1995].

Disposition in the Body Cyclizine is absorbed from the gastrointestinal tract with an onset of action within 2 h. It is extensively metabolised by *N*-demethylation to form the relatively inactive norcyclizine, a metabolite that is widely distributed throughout the tissues and especially in lungs, kidneys, liver, and spleen. <1% of the total oral dose is eliminated in the urine.

Therapeutic Concentration

Following a single oral dose of 50 mg of cyclizine hydrochloride to 1 subject, a peak blood concentration of 0.069 mg/L was attained in 2 h [Griffin, Baselt 1984].

On the first day after termination of therapy with oral doses of 50 mg thrice daily to 4 subjects, plasma concentrations of norcyclizine were in the range of 0.004–0.022 mg/L (mean 0.014) [Kuntzman *et al.* 1967].

Toxicity

In a fatality resulting from a cyclizine overdose, 80 mg/L cyclizine in blood was detected in a 17-year-old female. Norcyclizine was not detected [Backer *et al.* 1989].

In a fatality attributed principally to cyclizine, postmortem concentrations were: blood 15 mg/L, liver 108 µg/g; doxepin was also detected at concentrations of 0.7 mg/L and 6 µg/g in the blood and liver, respectively [Lewin *et al.* 1981].

For case histories involving the injection of cyclizine with dipipanone, see under Dipipanone.

Half-life Plasma half-life, cyclizine about 24 h.

Protein Binding Norcyclizine about 60%.

Dose Adults, up to 150 mg of cyclizine hydrochloride daily; children (6 to 12 years), up to 75 mg daily, in divided doses.

Backer RC *et al.* (1989). Fatality resulting from cyclizine overdose. *J Anal Toxicol* 13: 308–309.

Griffin DS, Baselt RC (1984). Blood and urine concentrations of cyclizine by nitrogen-phosphorus gas-liquid chromatography. *J Anal Toxicol* 8: 97–99.

Kuntzman R *et al.* (1967). Importance of tissue and plasma binding in determining the retention of norchlorcyclizine and norcyclizine in man, dog and rat. *J Pharmacol Exp Ther* 158: 332–339.

Land G *et al.* (1981). Determination of cyclizine and norcyclizine in plasma and urine using gas–liquid chromatography with nitrogen selective detection. *J Chromatogr* 222(1): 135–140.

Lewin JF *et al.* (1981). *Bull Int Assoc Forensic Toxicol* 16(2): 23–24.

Walker RB, Kanfer I (1995). Sensitive high-performance liquid chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine, in biological fluids using coulometric detection. *J Chromatogr B Biomed Appl* 672(1): 172–177.

Cyclobarbital

Hypnotic, Barbiturate

$C_{12}H_{16}N_2O_3 = 236.3$

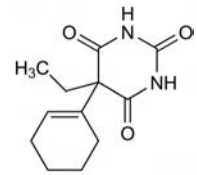
CAS—52-31-3

IUPAC Name 5-(Cyclohexen-1-yl)-5-ethyl-1,3-diazinane-2,4,6-trione

Synonyms Ciclobarbital; cyclobarbitalum; cyclobarbitone; 5-(1-cyclohexen-1-yl)-5-ethyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; ethylhexabital; hexemalum; tetrahydrophenobarbital.

Note The name ciclobarbital has been applied to hexobarbital.

Proprietary Names *Cavonyl*; *Cyclodorm*; *Cyklodorm*; *Fanodormo*; *Irifan*; *Namuron*; *Palinum*; *Phanodorm*; *Phanodorn*; *Philodorm*; *Prälumin*; *Pro-Sonil*; *Sonaform*.



Chemical Properties A white crystalline powder, which gradually decomposes on storage. Mp 171° to 174°. Soluble 1 in 800 of water, 1 in 5 of ethanol, 1 in 20 of chloroform, and 1 in 20 of ether. pK_a 7.6 (20°). Log *P* (octanol/water), 1.77.

Cyclobarbital Calcium

$(C_{12}H_{15}N_2O_3)_2Ca = 510.6$

CAS-143-76-0

Synonyms Ciclobarbital calcium; cyclobarbitone Calcium; hexemalcalcium.

Proprietary Name It is an ingredient of *Domidorm*.

Chemical Properties A white or slightly yellowish, crystalline powder. Soluble 1 in 70 of water, 1 in 500 of 95% alcohol. Practically insoluble in ether and chloroform.

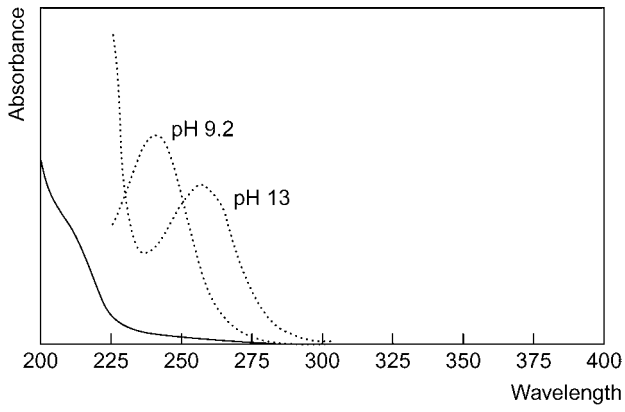
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown-red/green.

Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.40; system TF—R_f 0.64; system TH—R_f 0.59; system TAD—R_f 0.58; system TAE—R_f 0.88 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).

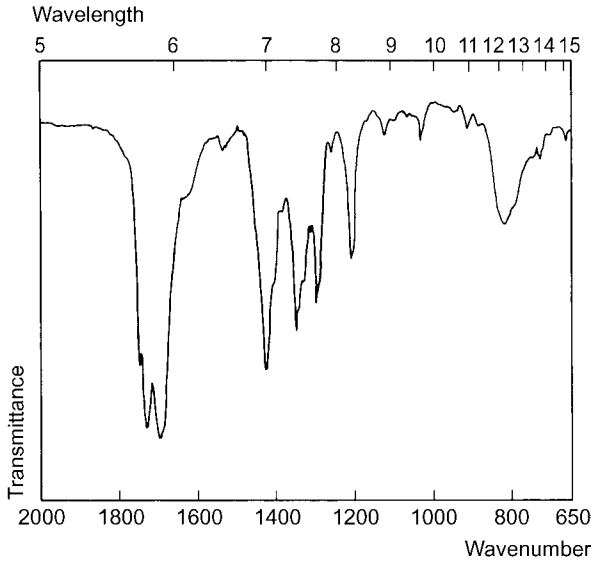
Gas Chromatography System GA—cyclobarbital RI 1955, cyclobarbital-Me_2 RI 1845, M (oxo-) RI 2190, M (oxo-)-Me_2 RI 2050; system GF—RI 2825; system GAJ—cyclobarbital RRT 1.142 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HG—*k* 5.25; system HH—*k* 2.61; system HX—RI 384; system HY—RI 352; system HZ—retention time 3.2 min.

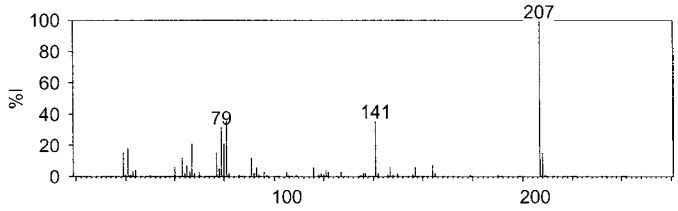
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A_1^1=410a); 1 mol/L sodium hydroxide (pH 13)—256 nm (A_1^1=320b).



Infrared Spectrum Principal peaks at wavenumbers 1693, 1725, 1745, 1300, 1210, 830 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 207, 141, 81, 79, 67, 80, 41, 77.

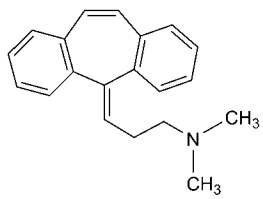


Quantification See under Amobarbital.
Disposition in the Body Rapidly absorbed after oral administration. The main metabolic reaction appears to be oxidation to ketocyclobarbital [5-(3-oxocyclohex-1-enyl)-5-ethylbarbituric acid]. <10% of a dose is excreted in the urine unchanged.
Therapeutic Concentration In plasma, usually in the range 2 to 10 mg/L. After a single oral dose of 300 mg of the calcium salt, given to 6 subjects, peak plasma concentrations of 7.4 to 10.3 mg/L (mean 8.7) were attained in 0.7 to 2.5 h [Breimer, Winten 1976].
Toxicity The estimated minimum lethal dose is 2 g. Severe toxic effects are associated with blood concentrations greater than 10 mg/L.
Half-life Plasma half-life, 8 to 17 h (mean 12), increased in subjects with liver disease.
Volume of Distribution About 0.5 L/kg.
Clearance Plasma clearance, about 0.5 mL/min/kg.
Protein Binding About 70%.
Dose 100 to 400 mg of cyclobarbital calcium, as a hypnotic.

Breimer DD, Winten MA (1976). Pharmacokinetics and relative bioavailability of cyclobarbital calcium in man after oral administration. *Eur J Clin Pharmacol* 9: 443–450.

Cyclobenzaprine

Tricyclic Amine, Skeletal Muscle Relaxant
$C_{20}H_{21}N$ = 275.4
CAS—303-53-7
IUPAC Name 3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine
Synonyms CBZ; MK-130; proheptatriene.

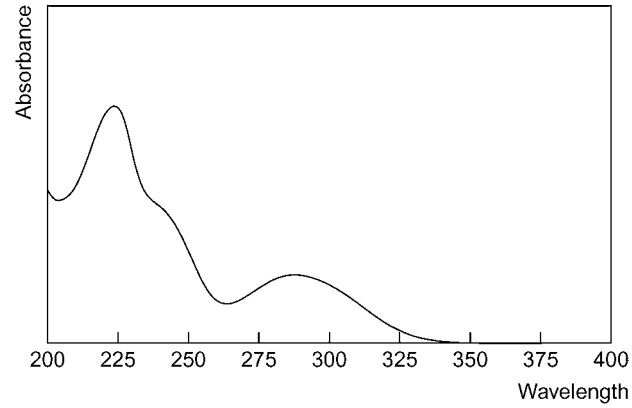


Chemical Properties pK_a 8.5 [Baselt 2008]. Log *P* (octanol/water) 4.8 [Meylan, Howard 1995]. Stable in urine and plasma when stored at –20° for 1 and 3 months, respectively [Constanzer *et al.* 1995].

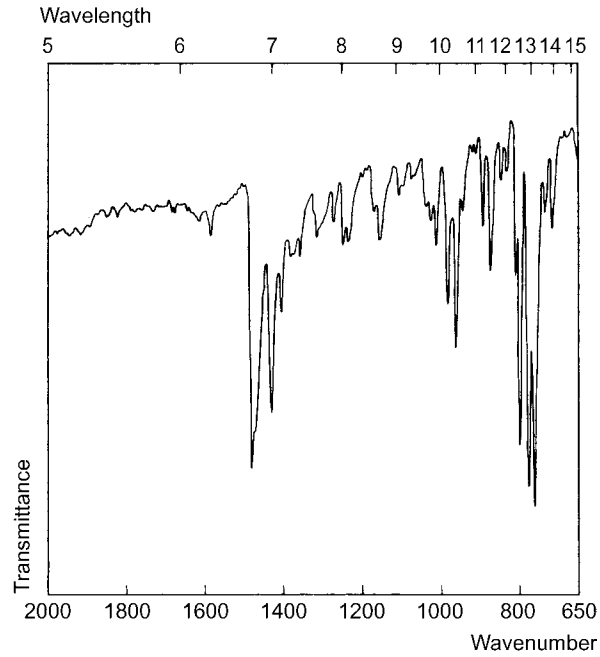
Cyclobenzaprine Hydrochloride

$C_{20}H_{21}N$, HCl = 311.9
CAS—6202-23-9
Proprietary Names *Flexeril*; *Lisseril*.
Chemical Properties White crystalline powder. Mp 215° to 219°. Freely soluble in water and ethanol; sparingly soluble in isopropanol; slightly soluble in chloroform and methylene chloride; practically insoluble in hydrocarbons.

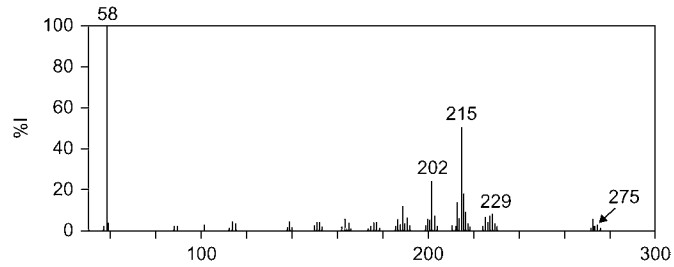
Thin-layer Chromatography System TE—R_f 0.69.
Gas Chromatography System GA—RI 2195.
High Performance Liquid Chromatography System HY—RI 378.
Column: octyl-bonded phase on silica (25 × 0.46 mm i.d.). Mobile phase: acetonitrile : 0.6% potassium dihydrogen phosphate (pH 3.0; 75 : 25), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: ≈7 min. Limit of detection, 5.0 mg/L [Heinitz 1982].
Ultraviolet Spectrum Aqueous acid—290 nm (A_1^1 = 407b) No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 764, 778, 800, 968, 988, 880 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 59, 42, 215, 202, 57, 189, 43.



Quantification
Blood **HPLC** Column: PE HS-5 silica. Mobile phase: methanol : 1 mol/L ammonium nitrate : 1 mol/L ammonium hydroxide (3925 : 25 : 37.5), flow rate 1.0 mL/min. DAD. Retention time: 4.09 min. Limit of detection, 1.5 mg/L [Winek, Jr. *et al.* 1999].

Plasma **GC** Column: DB-5 J&W 1% OV-17 on Gas-Chrom Q 80/100 mesh (40 m, 0.25 μm). Carrier gas: He, 2.068 bars. Temperature programme: 140° to 230° at 50°/min to 245° at 4°/min to 300° at 50°/min for 3 min. NPD. Limit of quantification, 1.0 μg/L [Constanzer *et al.* 1985]. Column: 3% OV-17 on Gas-Chrom Q 100/120 mesh (6′ × 0.08" i.d.). Carrier gas: He, 50 mL/min. Temperature: 240°. NPD. Limit of detection, 2 μg/L [Hucker, Stauffer 1976a]. Column: 1.5% OV-17 on Gas Chrom Q 80/100 mesh (1.8 m × 4.0 mm i.d.). Carrier gas: He, 100 mL/min. Temperature: 218°. FID. Retention time: 2.8 min. Limit of detection, 10 μg/L [Hucker, Stauffer 1976b].

GC-MS Column: Ultra-1 fused silica capillary (15 m × 0.2 μm i.d., 0.33 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 180° to 220° at 25°/min. EI ionisation, full scan mode. Retention time: ≈8.5 min. Limit of detection not reported [Wong *et al.* 1995].

HPLC Column: Supelcosil LC-PCN (15 cm × 4.7 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : 0.01 mol/L phosphate buffer (600 : 150 : 250), flow rate 2.0 mL/min. UV detection (λ = 254 nm). Relative retention time: 0.46. Limit of detection, 3 μg/L [Wong *et al.* 1995]. Column: BDS C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.085% phosphoric acid (pH 6.5; 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 226 nm). Retention time, 7.8 min. Limit of quantification, 0.5 μg/L [Constanzer *et al.* 1995].

LC-MS Column: BDS C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (90 : 10) containing 0.1% formic acid and 10 mmol/L ammonium acetate, flow rate 1.0 mL/min. APCI. Retention time: 1.9 min. Limit of quantification, 0.1 μg/L [Constanzer *et al.* 1995].

Serum **HPLC** Column: LC-PCN (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : 10 mmol/L phosphate buffer (pH 7.1; 60 : 15 : 25), flow rate 2 mL/min. UV detection (λ = 214 to 254 nm). Limit of detection, 10 μg/L [Puopolo, Flood 1987].

Urine **GC** See Plasma. Column: DB-5 J&W 1% OV-17 on Gas-Chrom Q 80/100 mesh (20 m, 0.25 μm). Temperature programme: 140° to 230° at 50°/min to 245° at 4°/min for 2 min. Limit of quantification, 0.2 mg/L [Constanzer *et al.* 1985]. See Plasma [Hucker, Stauffer 1976b].

HPLC See Plasma. Mobile phase: acetonitrile : 0.085% phosphoric acid (pH 6.5; 43 : 57). Retention time, 9.6 min. Limit of quantification, 10 μg/L [Constanzer *et al.* 1995].

LC-MS See Plasma [Constanzer *et al.* 1995].

Other **GC** Rat Urine. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (2 m × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 270°. FID. Limit of detection not reported [Belvedere *et al.* 1976].

GC-MS Rat Urine. Column:. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (2 m × 4 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 270°. EI ionisation at 70 eV. Limit of detection not reported [Belvedere *et al.* 1976].

Disposition in the Body Irregularly absorbed after oral administration, and extensively metabolised. The major metabolites are a glucuronide conjugate and 10,11-dihydroxynortriptyline; monodesmethylcyclobenzaprine is a minor metabolite. Approximately 50% of an oral dose is excreted in the urine in 5 days, with less than 1% as unchanged drug. Approximately 14% of a dose is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 40 mg to 4 subjects, peak plasma concentrations of about 0.027 mg/L were attained in 4 h; after oral doses of 20 mg three times a day to 9 subjects, a mean peak plasma concentration of 0.034 mg/L was reported 6 h after the first dose on the fourth day [Hucker *et al.* 1977].

Toxicity Cyclobenzaprine should be used with caution in patients receiving other serotonin-enhancing drugs due to the possibility of serotonin syndrome [Day, Jeanmonod 2008; Keegan *et al.* 2006].

Case 1: A 56-year-old female was found dead at home. Postmortem femoral blood concentrations of cyclobenzaprine and diazepam were 0.96 and 0.3 mg/L, respectively.

Case 2: a 37-year-old male took a handful of cyclobenzaprine tablets. He was found dead 5 h later. His femoral blood cyclobenzaprine concentration was 0.8 mg/L and ethanol concentration was 0.174 g/dL [Spiller, Cutino 2003].

Steady-state plasma concentrations were twice as high in elderly subjects after consumption of oral doses of 5 mg cyclobenzaprine twice daily when compared with healthy young subjects. Subjects with mild hepatic insufficiency also had a twofold increase in steady-state concentrations compared with healthy controls [Winchell *et al.* 2002].

A 35-year-old female was found dead in the bath. Cyclobenzaprine was found at a concentration of 1.79 mg/L in the blood along with ethanol at 21.5 mg/L [Winek, Jr. *et al.* 1999].

A 19-year-old male died 7 h after admission to hospital after being found with several medicine bottles. Cyclobenzaprine was found in his blood at a concentration of 0.3 mg/L as well as phenylpropanolamine (2.5 mg/L), chlorpheniramine (0.2 mg/L), lidocaine (6.6 mg/L), phenytoin (19 mg/L) and ibuprofen (130 mg/L). Cause of death was intoxication by cyclobenzaprine and ibuprofen [Levine *et al.* 1993].

In a fatality in which cyclobenzaprine was the suspected cause of death, the following postmortem tissue concentrations were reported: blood 0.46 mg/L, bile 12.0 mg/L, liver 12.4 μg/g, urine 3.2 mg/L; low concentrations of morphine and diazepam were also detected [Beck, Lamoreaux 1979].

Note For a case of paranoid psychosis after self-administration of cyclobenzaprine, see O'Neil *et al.* [2000].

Bioavailability ≈55% [Winchell *et al.* 2002].

Half-life Plasma half-life, ≈1 to 3 days [Linden *et al.* 1983].

Clearance 689 mL/min [Winchell *et al.* 2002].

Protein Binding ≈93% [Linden *et al.* 1983].

Dose Usually 30 mg (as 10 mg tablets) of cyclobenzaprine hydrochloride daily; maximum of 60 mg daily.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, CA: Biomedical Publications.

Beck BK, Lamoreaux T (1979). Cyclobenzaprine fatality. *TIAFT Bulletin* 14: 27–28.

Belvedere G *et al.* (1976). Identification of 10 11-epoxide and other cyclobenzaprine metabolites isolated from rat urine. *J Pharm Sci* 65: 815–821.

Constanzer M *et al.* (1995). Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. *J Chromatogr B Biomed Appl* 666: 117–126.

Constanzer ML *et al.* (1985). Determination of cyclobenzaprine in plasma and urine using capillary gas chromatography with nitrogen-selective detection. *J Chromatogr* 339: 414–418.

Day LT, Jeanmonod RK (2008). Serotonin syndrome in a patient taking Lexapro and Flexeril: a case report. *Am J Emerg Med* 26: 1069–3.

Heinitz ML (1982). Determination of cyclobenzaprine in tablets by high-performance liquid chromatography. *J Pharm Sci* 71: 656–658.

Hucker HB, Stauffer SC (1976a). Gas-liquid chromatographic determination of nanogram amounts of cyclobenzaprine in plasma using a nitrogen detector. *J Chromatogr* 124: 164–168.

Hucker HB, Stauffer SC (1976b). GLC determination of cyclobenzaprine in plasma and urine. *J Pharm Sci* 65: 1253–1255.

Hucker HB *et al.* (1977). Plasma levels and bioavailability of cyclobenzaprine in human subjects. *J Clin Pharmacol* 17: 719–727.

Keegan MT *et al.* (2006). Serotonin syndrome from the interaction of cyclobenzaprine with other serotoninergic drugs. *Anesth Analg* 103: 1466–1468.

Levine B *et al.* (1993). A multiple drug intoxication involving cyclobenzaprine and ibuprofen. *Am J Forensic Med Pathol* 14: 246–248.

Linden CH *et al.* (1983). Cyclobenzaprine overdosage. *J Toxicol Clin Toxicol* 20: 281–288.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

O'Neil BA *et al.* (2000). First episode psychosis following cyclobenzaprine use. *Can J Psychiatry* 45: 763–764.

Puopolo PR, Flood JG (1987). Detection of interference by cyclobenzaprine in liquid-chromatographic assays of tricyclic antidepressants. *Clin Chem* 33: 819–820.

Spiller HA, Cutino L (2003). Fatal cyclobenzaprine overdose with postmortem values. *J Forensic Sci* 48: 883–884.

Winchell GA *et al.* (2002). Cyclobenzaprine pharmacokinetics, including the effects of age, gender, and hepatic insufficiency. *J Clin Pharmacol* 42: 61–69.

Winek CLJr *et al.* (1999). Drowning due to cyclobenzaprine and ethanol. *Forensic Sci Int* 100: 105–108.

Wong EC *et al.* (1995). Potential interference of cyclobenzaprine and norcyclobenzaprine with HPLC measurement of amitriptyline and nortriptyline: resolution by GC-MS analysis. *J Anal Toxicol* 19: 218–224.

Cyclofenil

Induction of Ovulation

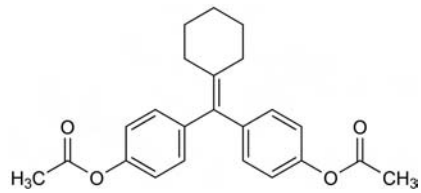
$C_{23}H_{24}O_4$ = 364.4

CAS—2624-43-3

IUPAC Name 4-[[4-(Acetyloxy)phenyl]cyclohexylidenemethyl]phenol acetate

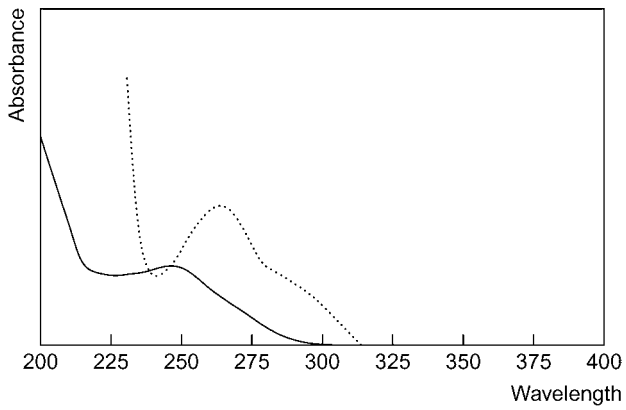
Synonyms F-6066; H-3452; ICI-48213.

Proprietary Names *Fertodur*; *Ondogyne*; *Ondonid*; *Rehibin*; *Sexovid*.



Chemical Properties A white crystalline powder. Mp 137° to 140°. Practically insoluble in water; soluble 1 in 250 of ethanol, 1 in 2 of chloroform and 1 in 30 of ether. Log *P* (octanol/water), 5.4.

Ultraviolet Spectrum Acid ethanol—247 nm; alkaline ethanol—262 nm.



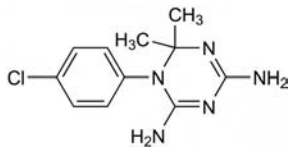
Infrared Spectrum Principal peaks at wavenumbers 1204, 1219, 1754, 917, 1162, 1492 cm^{-1}.

Dose 200 to 400 mg daily; doses of 800 mg daily have been given in 5-day courses.

Cycloguanil

Antimalarial
$C_{11}H_{14}ClN_5 = 251.7$
CAS—516-21-2

IUPAC Name 1-(4-Chlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine
Synonyms Chlorguanide triazine; D-20; M-10580; TCl.

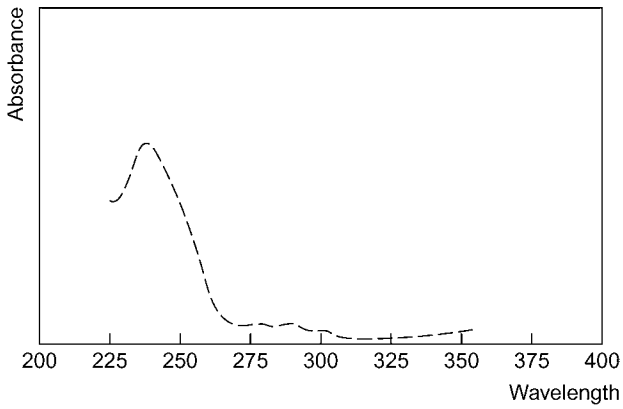


Chemical Properties Crystals. Mp 146°. Log *P* (octanol/water), 1.0.

Cycloguanil Embonate

$(C_{11}H_{14}ClN_5)_2, (C_{23}H_{16}O_6) = 891.8$
CAS—609-78-9
Synonym Cycloguanil pamoate
Chemical Properties A pale greenish-yellow, crystalline powder. Mp 231° to 234°. Practically insoluble in water; sparingly soluble in dimethylformamide.

High Performance Liquid Chromatography System HAA—retention time 10.8 min.
Ultraviolet Spectrum Methanol—237 (A_1^1=2250b), 278, 289 (A_1^1=180b), 301 nm.



Infrared Spectrum Principal peaks at wavenumbers 1673, 1515, 1628, 1570, 760, 1224 cm^{-1} (KBr disk).
Quantification See also under Proguanil.
Blood **HPLC** UV detection. Limit of quantification, 15 mg/L [Kolawole *et al.* 1995].
Plasma **HPLC** Limit of detection, 1 mg/L [Kusaka *et al.* 1996].
Urine **HPLC** Limit of detection, 5 mg/L [Kusaka *et al.* 1996].
Disposition in the Body Slowly absorbed after IM administration and excreted mainly in the urine.

Cycloguanil is the active metabolite of proguanil.

Dose The equivalent of 5 to 6 mg/kg of cycloguanil, IM, every 4 months.

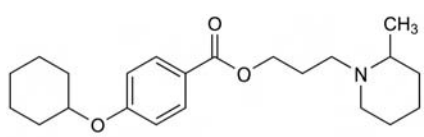
Kolawole JA *et al.* (1995). Determination of proguanil and metabolites in small sample volumes of whole blood stored on filter paper by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 674(1): 149–154.

Kusaka M *et al.* (1996). Simultaneous measurement of proguanil and its metabolites in human plasma and urine by reversed-phase high-performance liquid chromatography, and its preliminary application in relation to genetically determined S-mephenytoin 4′-hydroxylation status. *Am J Trop Med Hyg* 54(2): 189–196.

Cyclomethycaine

Anaesthetic (Local)
$C_{22}H_{33}NO_3 = 359.5$
CAS—139-62-8 (cyclomethycaine); 537-61-1 (hydrochloride)

IUPAC Name 3-(2-Methylpiperidin-1-yl)propyl 4-cyclohexyloxybenzoate
Synonym 4-(Cyclohexyloxy)benzoic acid 3-(2-methyl-1-piperidinyl)propyl ester

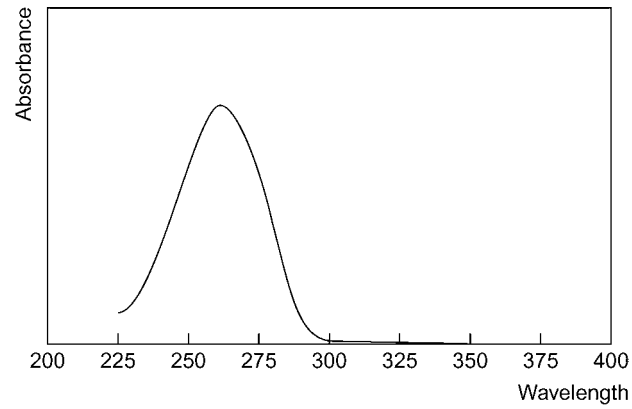


Chemical Properties A white crystalline powder. Sparingly soluble in water, ethanol and ether; very slightly soluble in chloroform. Log *P* (octanol/water), 6.2.

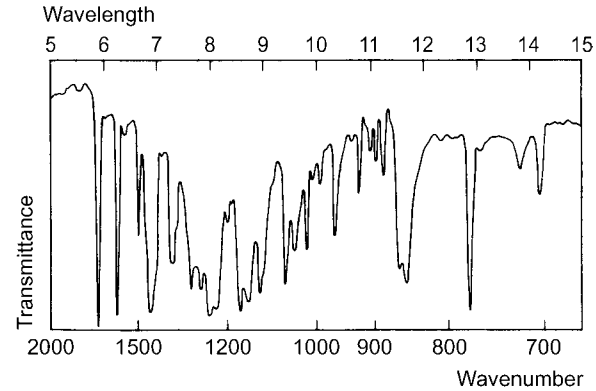
Cyclomethycaine Sulfate

$C_{22}H_{33}NO_3, H_2SO_4 = 457.6$
CAS—50978-10-4
Proprietary Name *Surfacaine*
Chemical Properties A white crystalline powder. Mp 162° to 166°. Soluble 1 in 50 of water, 1 in 50 of ethanol and 1 in about 227 of chloroform.

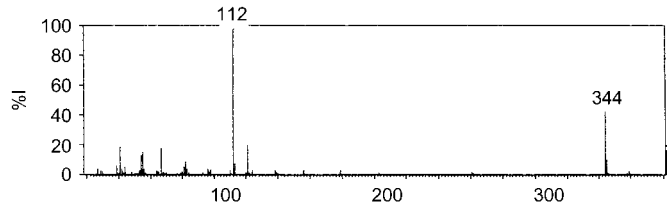
Colour Test Mandelin's test—green→brown.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.55; system TC—R_f 0.36; system TL—R_f 0.25 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HR—*k* 10.31; system HY—RI 413.
Ultraviolet Spectrum Aqueous acid—261 nm (A_1^1=508a).



Infrared Spectrum Principal peaks at wavenumbers 1704, 1605, 1253, 775, 1170, 1235 cm^{-1} (cyclomethycaine sulfate, Nujol mull).



Mass Spectrum Principal ions at *m/z* 112, 344, 121, 41, 67, 55, 54, 345.

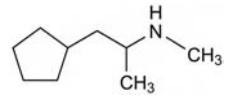


Use Cyclomethycaine sulfate has been used in concentrations of 0.5 to 1%.

Cyclopentamine

Sympathomimetic
$C_9H_{19}N = 141.3$
CAS—102-45-4

IUPAC Name 1-Cyclopentyl-*N*-methylpropan-2-amine
Synonyms Cyclopentadrin; *N*,α-dimethylcyclopentaneethanamine.



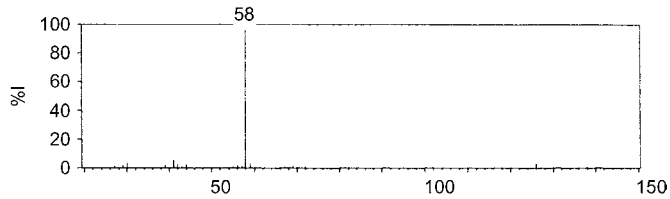
Chemical Properties Log *P* (octanol/water), 3.0.

Cyclopentamine Hydrochloride

$C_9H_{19}N, HCl = 177.7$
CAS—3459-06-1

Synonym Cyclopentaminium chloride
Proprietary Name It is an ingredient of *Co-Pyronil*.
Chemical Properties A white crystalline powder. Mp 113° to 115°. Soluble 1 in 1 of water, 1 in 2 of ethanol, and 1 in 1 of chloroform; slightly soluble in ether.

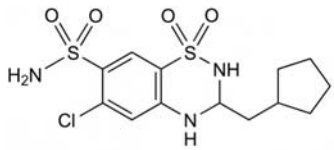
Thin-layer Chromatography System TA—R_f 0.20; system TB—R_f 0.32; system TC—R_f 0.10; system TE—R_f 0.66; system TL—R_f 0.02; system TAE—R_f 0.06; system TAF—R_f 0.68 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Ninhydrin spray, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—cyclopentamine RI 1230, cyclopentamine-AC RI 1680.
High Performance Liquid Chromatography System HA—*k* 1.7.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1585, 1020, 1050, 1100, 1082, 115 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 58, 41, 30, 126, 59, 69, 56, 44.



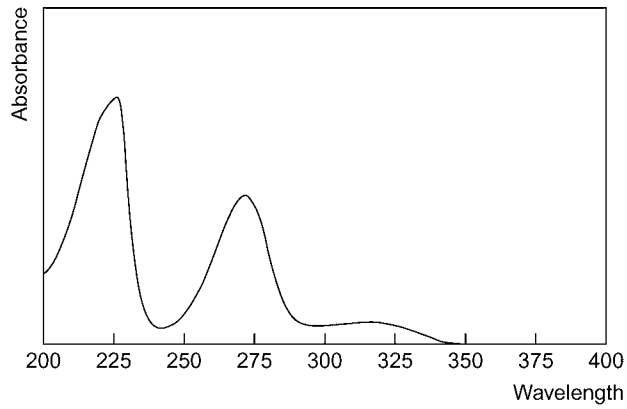
Disposition in the Body
Toxicity The estimated minimum lethal dose for children up to 2 years is 200 mg when applied to the mucous membranes; the minimum lethal dose for adults may be at least 10–times greater.
Dose Cyclopentamine hydrochloride has been used as a 0.5 or 1% nasal solution. It has been given in doses of 25 mg IM, or 5 to 10 mg IV.

Cyclopenthiazide

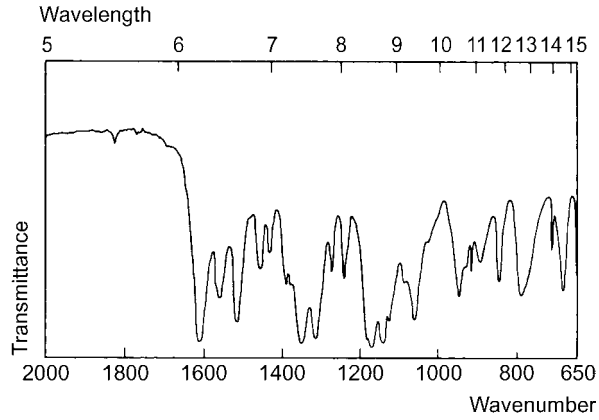
Diuretic
$C_{13}H_{18}ClN_3O_4S_2$ = 379.9
CAS—742-20-1
IUPAC Name 6-Chloro-3-(cyclopentylmethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
Proprietary Names *Navidrex*. It is an ingredient of *Trasidrex*.



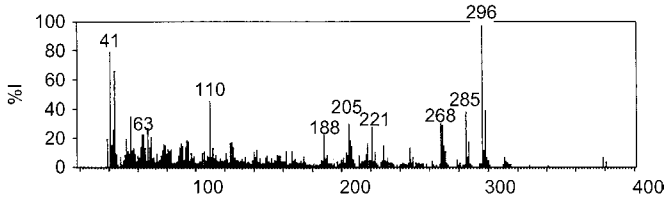
Chemical Properties A white powder. Mp 235°, with decomposition. Practically insoluble in water; soluble 1 in 12 of ethanol and 1 in 600 of chloroform; soluble in ether. Log *P* (octanol/water), 1.3.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—brown-green; sulfuric acid—yellow.
Thin-layer Chromatography System TD—R_f 0.21; system TE—R_f 0.66; system TF—R_f 0.62; system TAD—R_f 0.27.
Gas Chromatography System GY—cyclopenthiazide-Me_4 retention time 6.3 min.
High Performance Liquid Chromatography System HN—*k* 16.45; system HY—RI 453.
Ultraviolet Spectrum Aqueous acid—274 (A^1_1=556a), 319 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1168, 1138, 1605, 1309, 1511, 1060 cm^{-1} (KBr disk).



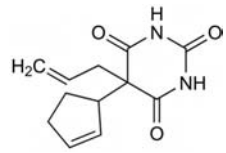
Mass Spectrum Principal ions at *m/z* 296, 41, 44, 110, 298, 285, 55, 268.



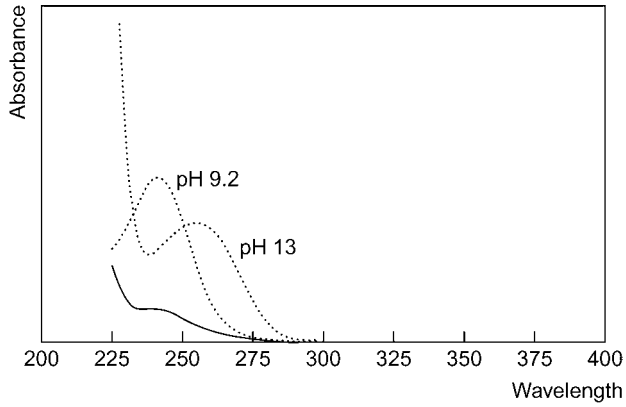
Dose 250 to 500 µg daily; maximum of 1.5 mg daily.

Cyclopentobarbital

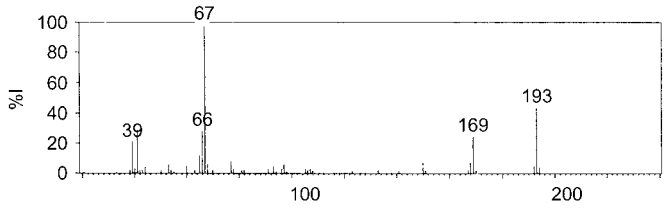
Sedative, Barbiturate
$C_{12}H_{14}N_2O_3$ = 234.3
CAS—76-68-6
IUPAC Name 5-Cyclopent-2-en-1-yl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione
Synonyms Cyclopentenylallyl barbituric acid; 5-(2-cyclopenten-1-yl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; cyclopentobarbitone.
Proprietary Name *Cyclopal*



Chemical Properties Crystals. Mp 139° to 140°. Slightly soluble in cold water; moderately soluble in hot water; freely soluble in ethanol and organic solvents. pK_a 7.8. Log *P* (octanol/water), 1.5.
Colour Test Vanillin reagent—brown-red/green.
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.39; system TF—R_f 0.65; system TH—R_f 0.62; system TAD—R_f 0.59; system TAE—R_f 0.90; system TAJ—R_f 0.66; system TAK—R_f 0.63; system TAL—R_f 0.90.
Gas Chromatography System GA—cyclopentobarbital RI 1865; cyclopentobarbital-Me_2 RI 1775.
High Performance Liquid Chromatography System HG—*k* 6.00; system HH—*k* 3.84; system HX—RI 391; system HY—RI 352.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—241 nm (A^1_1=411a); 1 mol/L sodium hydroxide (pH 13)—254 nm (A^1_1=306b).



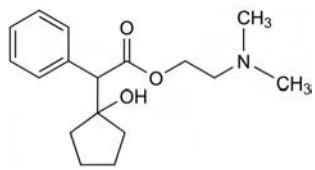
Infrared Spectrum Principal peaks at wavenumbers 1700, 1755, 1212, 815, 1319, 848 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 67, 193, 66, 41, 169, 39, 65, 77.



Dose Cyclopentobarbital has been given in a dose of 100 to 200 mg.

Cyclopentolate

Anticholinergic
$C_{17}H_{25}NO_3 = 291.4$
CAS—512-15-2
IUPAC Name 2-Dimethylaminoethyl 2-(1-hydroxycyclopentyl)-2-phenylacetate
Synonym α-(1-Hydroxycyclopentyl)benzeneacetatic acid 2-(dimethylamino) ethyl ester

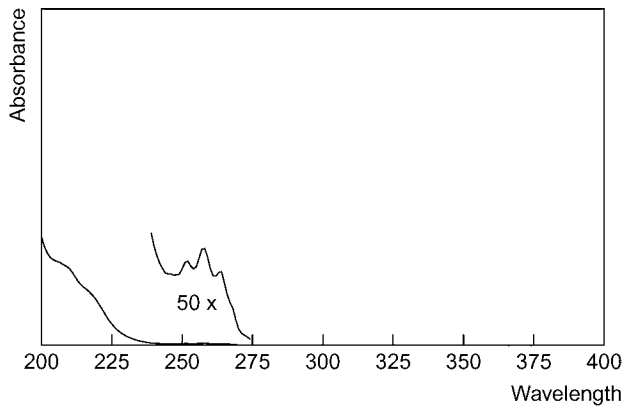


Chemical Properties pK_a 7.9. Log *P* (octanol/water), 2.5.

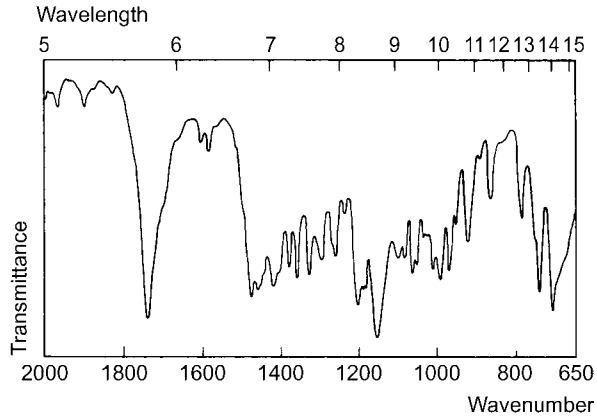
Cyclopentolate Hydrochloride

$C_{17}H_{25}NO_3$, HCl = 327.9
CAS—5870-29-1
Proprietary Names *Cyclogyl*; *Cyclopen*; *Mydplegic*; *Mydrilate*; *Skiacol P.O.S*; *Zyklolat*.
Chemical Properties A white crystalline powder. Mp 135° to 138°. Soluble 1 in less than 1 of water and 1 in 5 of ethanol; practically insoluble in ether.

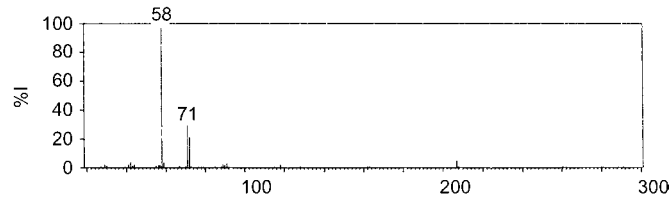
Colour Test Mandelin's test—brown.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.32; system TC—R_f 0.39; system TE—R_f 0.64; system TL—R_f 0.26; system TAE—R_f 0.46; system TAJ—R_f 0.23; system TAK—R_f 0.02; system TAL—R_f 0.42.
Gas Chromatography System GA—cyclopentolate RI 2022, cyclopentolate-H_2O RI 2000; system GB—cyclopentolate RI 2092, cyclopentolate-H_2O RI 1551.
High Performance Liquid Chromatography System HA—*k* 1.6 (tailing peak); system HX—RI 353; system HY—RI 287; system HZ—retention time 3.2 min.
Ultraviolet Spectrum Aqueous acid—252 (A^1_1=5a), 258 (A^1_1=6a), 264 nm (A^1_1=5a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1148, 1733, 704, 1199, 735, 990 cm^{-1} (cyclopentolate hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 71, 72, 207, 42, 91, 59, 118; cyclopentolate-H_2O *m/z* 58, 71, 91, 115, 129, 273.



Disposition in the Body

Therapeutic Concentration

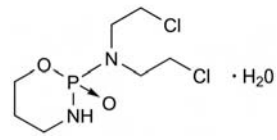
Eight healthy volunteers were administered 30 mL 1% cyclopentolate hydrochloride or cyclopentolate polygalacturonate in saline or acetate buffer in one eye. Peak plasma concentrations of 3 mg/L were reached within 30 min with both formulations [Lahdes *et al.* 1993].

Half-life Elimination, 111 min.
Use Cyclopentolate hydrochloride is used as a 0.5 to 1% ophthalmic solution.

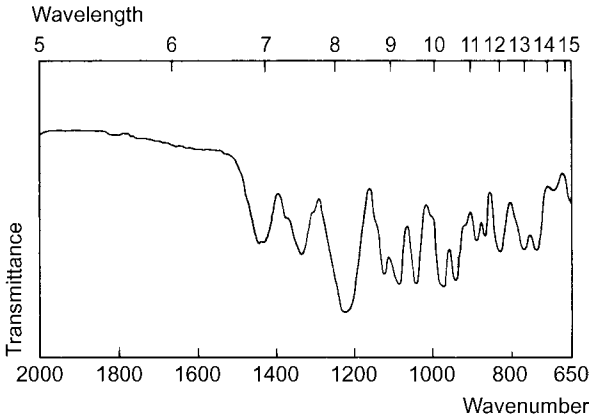
Lahdes K *et al.* (1993). Plasma concentrations and ocular effects of cyclopentolate after ocular application of three formulations. *Br J Clin Pharmacol* 35(5): 479–483.

Cyclophosphamide

Antineoplastic
$C_7H_{15}Cl_2N_2O_2P$, H_2O = 279.1
CAS—50-18-0 (anhydrous); 6055-19-2 (monohydrate)
IUPAC Name *N*,*N*-Bis(2-chloroethyl)-2-oxo-1,3,2λ^5-oxazaphosphinan-2-amine
Synonym *N*,*N*-Bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine-2-oxide monohydrate
Proprietary Names *Cytoxan*; *Endoxan(a)*; *Procytox*; *Sendoxan*.



Chemical Properties A fine, white, crystalline powder which discolours on exposure to light. Mp 41° to 45°. It liquefies upon loss of its water of crystallisation. In aqueous solutions, at temperatures above 30°, hydrolysis occurs with removal of chlorine. Soluble 1 in 25 of water and 1 in 1 of ethanol; slightly soluble in benzene, ethylene glycol, carbon tetrachloride, and dioxane; sparingly soluble in ether and acetone. Log *P* (octanol/water), 0.6.
Colour Test Phosphorus test—yellow precipitate.
Thin-layer Chromatography System TAE—R_f 0.74.
Gas Chromatography System GA—cyclophosphamide RI 2065, cyclophosphamide-HCl RI 1975.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1225, 1044, 975, 1088, 945, 1128 cm^{-1} (KBr disk).



Quantification

Plasma **GC** NPD. Limit of quantification, 5 mg/L. Limit of detection, 1 mg/L Huitema *et al.* 2001]. NPD [Kalhorn *et al.* 1999]. AFID. Cyclophosphamide metabolites (phosphoramide mustard and nornitrogen mustard) [Juma *et al.* 1980]. AFID. Limit of detection, 10 µg/L [Van den Bosch, De Vos 1980]. AFID. Limit of detection, 10 µg/L [Facchinetti *et al.* 1978].

HPLC Fluorescence detection (λ_{ex}=350 nm; λ_{em}=550 nm). Limit of quantification, 60 µg/L [Griskevicius *et al.* 2002]. UV detection (λ = 276 nm). Limit of quantification, 50 µg/L. Limit of detection, 40 µg/L [Huitema *et al.* 2000]. Limit of detection, 15 µg/L and 30 µg/L metabolites [Baumann *et al.* 1999].

Urine **GC** See Plasma [Facchinetti *et al.* 1978].

GC-MS Limit of detection, >0.25 mg/L [Sessink *et al.* 1993].

HPLC-MS Cyclophosphamide, 4-ketocyclophosphamide and carboxyphosphamide, limit of detection, 10 pg [Bahr, Schulten 1981].

Disposition in the Body Well absorbed after oral administration. The parent drug is inactive, but is converted into active metabolites by the liver. The drug and metabolites are widely distributed in the body. It is oxidised to 4-hydroxycyclophosphamide, which is in equilibrium with aldophosphamide, both of which are cytotoxic. 4-Hydroxycyclophosphamide can be oxidised further to 4-ketocyclophosphamide (inactive). Aldophosphamide may be converted to the cytotoxic metabolites phosphoramide mustard and acrolein, or oxidised to inactive carboxyphosphamide. Carboxyphosphamide may be converted to nornitrogen mustard, an active alkylating agent at acid pH. Up to 95% of a dose is excreted in the urine, mostly during the first 48 h, with up to 25% as unchanged drug and the rest as metabolites. Small amounts are excreted in the bile and faeces.

Therapeutic Concentration

After a single IV dose of 9.4 mg/kg to a patient, a plasma concentration of about 26 mg/L of cyclophosphamide was attained immediately after injection, and this fell to about 13 mg/L after 2 h. After single IV doses of 9 to 12 mg/kg and 40 mg/kg, peak plasma concentrations of unbound alkylating metabolites of about 0.3 to 2 mg/L (8 studies) and about 2 to 6 mg/L (7 studies), respectively, were achieved, usually after 2 to 3 h [Bagley, Jr*et al.* 1973].

Six male and female patients, mean age 52.8 years, were administered an IV bolus of 0.3 to 0.75 (mean, 0.55) g/m^2 cyclophosphamide. 1 h after dose, the mean serum concentration of *R*-cyclophosphamide was 13.49 mg/L and *S*-cyclophosphamide 12.89 mg/L; and 2.08 and 2.25 mg/L 20 h after dosing [Corlett, Chrystyn 1996].

Bioavailability >75%.

Half-life Plasma half-life, about 3 to 12 h (mean 7).

Volume of Distribution About 0.7 L/kg.

Clearance Plasma clearance, about 1 mL/min/kg.

Saliva Plasma : saliva ratio, about 1.6.

Protein Binding Cyclophosphamide about 12 to 24%, alkylating metabolites about 50 to 60%.

Note For a review of the pharmacokinetics of cyclophosphamide, see Grochow, Colvin [1979].

Dose Regimens usually range from 2 to 6 mg/kg daily, orally or intravenously, to 60 to 80 mg/kg as a single IV dose every 3 to 4 weeks.

Bagley Jr CM *et al.* (1973). Clinical pharmacology of cyclophosphamide. *Cancer Res* 33(2): 226–233.

Bahr USchulten HR (1981). Isolation, identification and determination of cyclophosphamide and two of its metabolites in urine of a multiple sclerosis patient by high pressure liquid chromatography and field desorption mass spectrometry. *Biomed Mass Spectrom* 8(11): 553–557.

Baumann F *et al.* (1999). Determination of cyclophosphamide and its metabolites in human plasma by high-performance liquid chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 729(1–2): 297–305.

Corlett SA, Chrystyn H (1996). High-performance liquid chromatographic determination of the enantiomers of cyclophosphamide in serum. *J Chromatogr B Biomed Sci Appl* 682(2): 337–342.

Facchinetti T *et al.* (1978). Simple and sensitive method for the determination of cyclophosphamide by means of a nitrogen–phosphorus-selective detector. *J Chromatogr* 145(2): 315–318.

Griskevicius L *et al.* (2002). Simple method based on fluorescent detection for the determination of 4-hydroxycyclophosphamide in plasma. *Ther Drug Monit* 24(3): 405–409.

Grochow LB, Colvin M (1979). Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet* 4: 380–394.

Huitema AD *et al.* (2001). Sensitive gas chromatographic determination of the cyclophosphamide metabolite 2-dechloroethylcyclophosphamide in human plasma. *J Chromatogr B Biomed Sci Appl* 757: 349–357.

Huitema AD *et al.* (2000). Simple and selective determination of the cyclophosphamide metabolite phosphoramide mustard in human plasma using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 745: 345–355.

Juma FD *et al.* (1980). The pharmacokinetics of cyclophosphamide, phosphoramide mustard and nor-nitrogen mustard studied by gas chromatography in patients receiving cyclophosphamide therapy. *Br J Clin Pharmacol* 10: 327–335.

Kalhorn TF *et al.* (1999). Analysis of cyclophosphamide and five metabolites from human plasma using liquid chromatography-mass spectrometry and gas chromatography-nitrogen-phosphorus detection. *J Chromatogr B Biomed Sci Appl* 732(2): 287–298.

Sessink PJ *et al.* (1993). Determination of cyclophosphamide in urine by gas chromatography-mass spectrometry. *J Chromatogr* 616(2): 333–337.

Van den Bosch N, De Vos D (1980). Some aspects of the gas–liquid chromatographic analysis of cyclophosphamide in plasma. *J Chromatogr* 183(1): 49–56.

Cycloserine

Antibiotic, Tuberculostatic

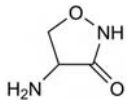
$C_3H_6N_2O_2$ = 102.1

CAS—68-41-7

IUPAC Name D-4-Amino-3-isoxazolidinone

Synonym D-Cycloserine

Proprietary Names *Closina*; *Seromycin*.

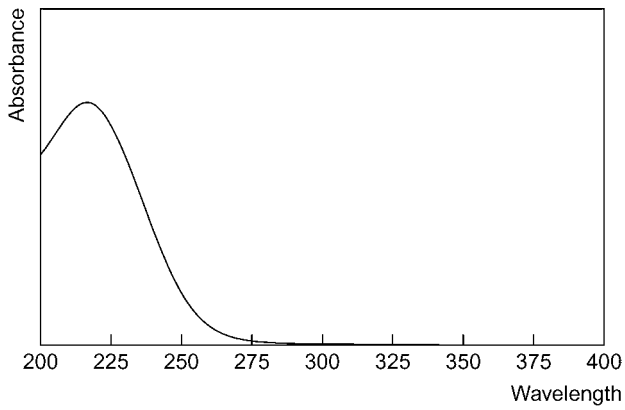


Chemical Properties An antimicrobial substance produced by the growth of certain strains of *Streptomyces orchidaceus* or *S. garyphalus*, or obtained by synthesis. A white or pale yellow, hygroscopic, crystalline powder. It is unstable in neutral or acid solutions. Mp 155° to 156°, with decomposition. Soluble 1 in 10 of water and 1 in 50 of ethanol; slightly soluble in chloroform, ether, and propylene glycol. pK_a 4.5, 7.4 (25°). Log *P* (octanol/water), −1.7.

Colour Test Mercurous nitrate—black.

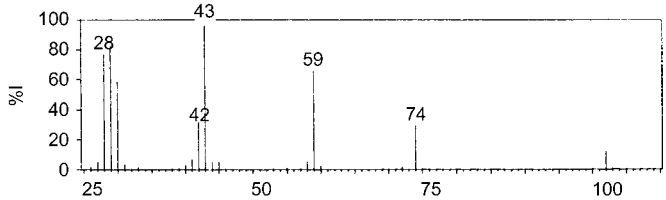
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.01; system TC—R_f 0.01; system TL—R_f 0.02 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1577, 1550, 1526, 1620, 934, 877 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 43, 29, 28, 59, 30, 42, 74, 102



Quantification

Plasma **HPLC** Fluorescence detection (λ_{ex}=381 nm; λ_{em}=450 nm). Limit of detection, 10 mg/L [David *et al.* 2001].

Disposition in the Body Readily absorbed after oral administration and widely distributed throughout body fluids and tissues. About 65% of a dose is excreted in the urine unchanged in 72 h, mostly in the first 24 h.

Therapeutic Concentration

After oral administration of 750 mg, divided into three 6-hourly doses to 11 subjects, followed by a single dose of 500 mg the next morning, peak serum concentrations of 22 to 34 mg/L (mean 28) were reported 2 to 4 h after the last dose [Mattila *et al.* 1969].

Toxicity

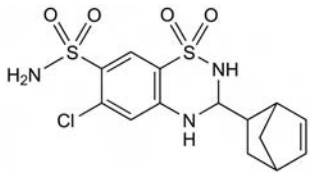
A female subject who ingested a non-fatal overdose of 3 g, had a peak plasma concentration of 91 mg/L on admission to hospital; following

peritoneal dialysis for 20 h, the plasma concentration declined to 25 mg/L [Atkins *et al.* 1965].
Half-life Plasma half-life, 4 to 30 h (mean 10).
Protein Binding <20%.
Dose 0.25 to 1 g daily.

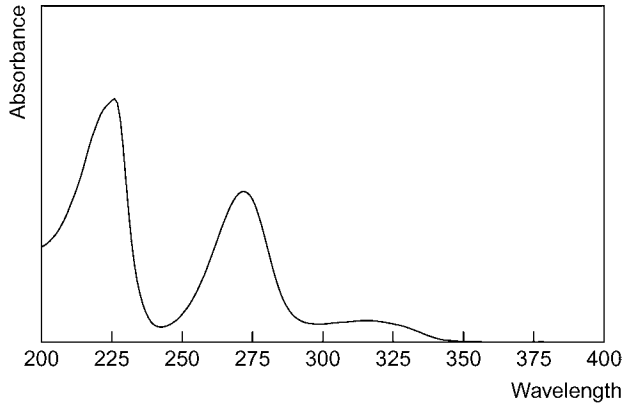
Atkins R *et al.* (1965). Acute poisoning by cycloserine. *Brit Med J* 1: 907–908.
David V *et al.* (2001). Determination of cycloserine in human plasma by high-performance liquid chromatography with fluorescence detection, using derivatization with p-benzoquinone. *J Chromatogr B Biomed Sci Appl* 761(1): 27–33.
Mattila MJ *et al.* (1969). Serum levels, urinary excretion, and side-effects of cycloserine in the presence of isoniazid and p-aminosalicylic acid. *Scand J Resp Dis* 50(4): 291–300.

Cyclothiazide

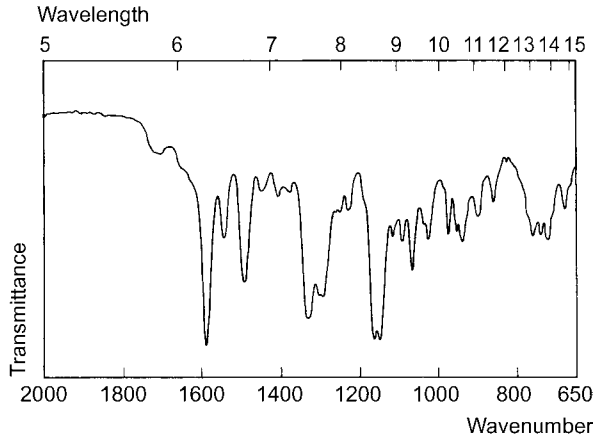
Diuretic
$C_{14}H_{16}ClN_3O_4S_2$ = 389.9
CAS—2259-96-3
IUPAC Name 3-Bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
Proprietary Names *Anhydron*; *Doburil*.



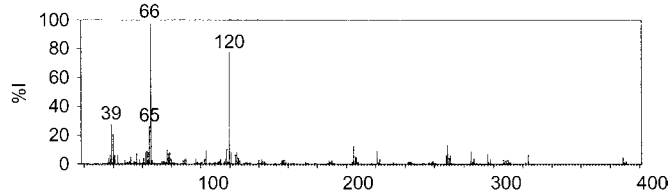
Chemical Properties A white powder. Mp 217° to 225°. Practically insoluble in water; soluble 1 in 70 of ethanol and 1 in 30 of methanol; freely soluble in acetone; practically insoluble in chloroform and ether. Log *P* (octanol/water), 2.0.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—brown; sulfuric acid—orange→red-brown.
Thin-layer Chromatography System TA—R_f 0.77; system TD—R_f 0.18; system TE—R_f 0.59; system TF—R_f 0.60; system TAD—R_f 0.26 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HN—*k* 10.78,*k* 11.91, and *k* 12.81; system HY—RI 433.
Ultraviolet Spectrum Aqueous acid—272 (A^1_1=549b), 314 nm.



Infrared Spectrum Principal peaks at wavenumbers 1596, 1153, 1168, 1307, 1500, 1075 cm^{-1} (KBr disk).



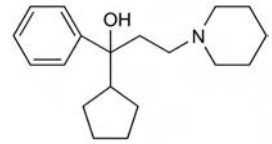
Mass Spectrum Principal ions at *m/z* 66, 120, 39, 65, 269, 205, 118, 77.



Dose 1 to 2 mg daily; up to 6 mg daily may be given.

Cycrimine

Anticholinergic
$C_{19}H_{29}NO$ = 287.4
CAS—77-39-4
IUPAC Name 1-Cyclopentyl-1-phenyl-3-piperidinopropan-1-ol

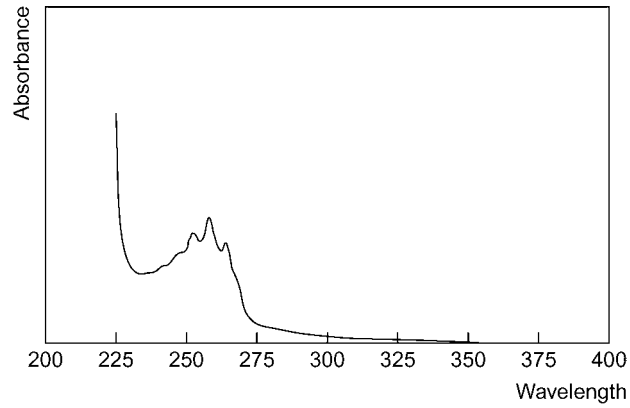


Chemical Properties Mp 90° to 96°. Log *P* (octanol/water), 2.0 (hydrochloride).

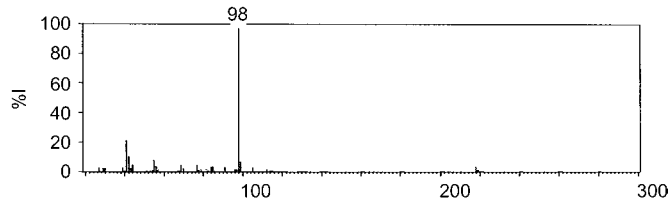
Cycrimine Hydrochloride

$C_{19}H_{29}NO$, HCl = 323.9
CAS—126-02-3
Synonym Cycriminium chloride
Proprietary Name *Pagitane Hydrochloride*
Chemical Properties A white solid. Mp about 242°, with decomposition. Soluble 1 in 175 of water, 1 in 50 of ethanol, and 1 in 35 of chloroform; practically insoluble in ether.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—red-brown; Marquis test—orange→red.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.67; system TC—R_f 0.61; system TL—R_f 0.60 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2114.
Ultraviolet Spectrum Aqueous acid—251, 256 (A^1_1=6b), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 696, 1117, 755, 751, 1030, 1298 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 41, 42, 55, 99, 77, 69, 44.



Quantification

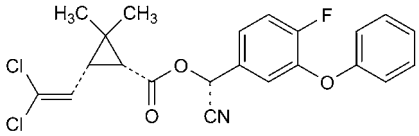
Biological Fluids **GC-MS** Flame thermionic detection. Limit of detection, 50 to 100 ng/L [Owen *et al.* 1989].
Dose Usually 3.75 to 20 mg of cycrimine hydrochloride daily.

Owen JA *et al.* (1989). Capillary gas chromatography of trihexyphenidyl, procyclidine and cycrimine in biological fluids. *J Chromatogr* 494: 135–142.

Cyfluthrin

Pyrethroid Insecticide
$C_{22}H_{18}Cl_2FNO_3 = 434.3$
CAS—68359-37-5

IUPAC Name Cyano-(4-fluoro-3-phenoxy-phenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate
Synonyms β-Cyfluthrin; BAY FCR 1272; BAY-VI-1704; cyfoxylate; FCR 1272; FCR 4545.
Proprietary Names *Attatox*; *Baythroid*; *Baythroid H*; *Bulldock*; *Contur*; *Eulan SP*; *Laser*; *Renounce*; *Responsar*; *Solfac*; *Tempo*; *Tempo H*.

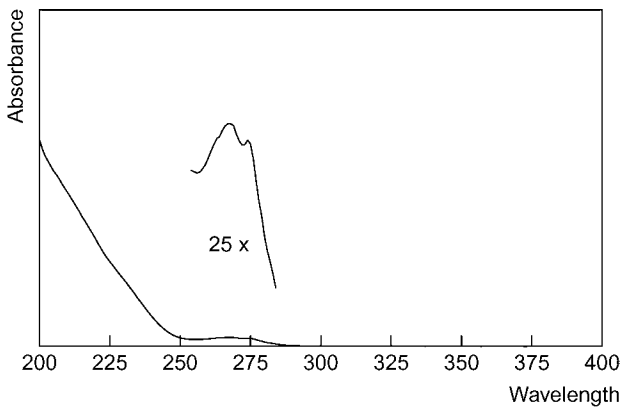


Chemical Properties Yellowish-brown oil. Mp 60°. It is soluble in water (0.002 g/L at 20°), 2-propanol (20 to 50 g/L), methylene chloride (>200 g/L), hexane (10 to 20 g/L) and toluene. Log *P* (octanol/water) 5.95. Stock solutions were stable at –18° for at least 6 months. Urine samples were stable at –21° for >1 year. Derivatised samples were stable for 1 month at 4° [Leng, Gries 2005]. Unstable in plasma [Leng *et al.* 1997a].
Thin-layer Chromatography System TX—R_f 0.37; system TY—R_f 0.89.
Gas Chromatography System GA—RI 2749.
Gas Chromatography-Mass Spectrometry Column: HP-5MS cross-linked 5% diphenyl 95% dimethylpolysiloxane. Carrier gas: He, 1.0 mL/min. Temperature: 180° for 2 min to 280° at 5°/min. Column: BGB-172 20% tert-butyldimethylsilyl-β-cyclodextrin in 15% diphenyl and 85% dimethylpolysiloxane. Carrier gas: He, 1.0 mL/min. Temperature programme: 160° for 2 min to 220° at 1°/min for 60 min to 230° at 5°/min. ECD or MSD, EI ionisation at 70 eV, full scan mode. Details regarding retention time are shown in the table below.

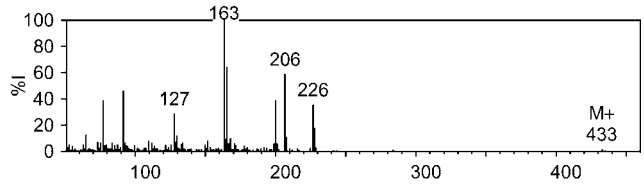
Configuration				*Retention time (min)*	
1C	3C	αC	1C/3C	HP-5MS	BGB-172
R	*R*	*R*	*cis*	32.8	121.3
S	*S*	*S*	*cis*	32.8	123.2
R	*S*	*R*	*trans*	33.1	126.9
S	*R*	*S*	*trans*	33.1	126.9
R	*R*	*S*	*cis*	33.3	129.2
S	*S*	*R*	*cis*	33.3	131.2
R	*S*	*S*	*trans*	33.5	132.3
S	*R*	*R*	*trans*	33.5	132.3

[Liu, Gan 2004]

High Performance Liquid Chromatography Column: chiral stationary phase on stainless steel (250 × 4.6 mm i.d.). Mobile phase: *n*-hexane : 1,2-dichloromethane : 2-propanol (96.8 : 3 : 0.2), flow rate 1.0 mL/min. UV and CD detection (λ = 230 nm). k'_1/k'_2: 10.45/11.07, 12.80/13.15, 13.54/14.13, 15.83/17.22 for (α*R*)(*1R*)*cis*/(α*S*)(*1S*)*cis*, (α*R*)(*1S*)*cis*/(α*S*)(*1R*)*cis*, (α*R*)(*1R*)*trans*/(α*S*)(*1S*)*trans*, and (α*R*)(*1S*)*trans*/(α*S*)(*1R*)*trans*, respectively [Tan *et al.* 2007]. See also Girelli *et al.* [2002].
Ultraviolet Spectrum



Mass Spectrum Principal peaks at *m/z* 163, 206, 226, 127



Quantification
Blood **GC-MS** Column: DB5-MS 5% phenylmethylpolysiloxane (30 m × 250 µm i.d., 1 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection, 3.1 to 98.0 µg/L [Ostrea, Jr. *et al.* 2006]. Column: DB-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 3 min to 200° at 10°/min for 5 min to 240° at 5°/min for 45 min. SIM acquisition mode. Limit of quantification, 2 µg/L, limit of detection, 1 µg/L [Ramesh, Ravi 2004a]. Column: Column: DB-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 3 min to 200° at 10°/min for 5 min to 240° at 5°/min for 45 min. NCI, SIM acquisition mode. Limit of quantification, 20 ng/L, limit of detection, 5 ng/L [Ramesh, Ravi 2004b].
Plasma **GC-MS** ECD. Limit of detection, 5 µg/L for DCCA and FPBA [Leng *et al.* 1997a].
Serum **GC-MS** See Blood [Ramesh, Ravi 2004b].
Urine **GC-MS** Column: Rtx 65 medium polar fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 60° for 1 min to 150° at 8°/min to 300° at 30°/min for 5 min. EI ionisation at 70 eV, positive or negative ion mode. Limit of detection, 0.05 µg/L for *cis*- and *trans*-DCCA and 0.02 µg/L for FPBA with positive ion mode, 0.02 µg/L for *cis*- and *trans*-DCCA, and 0.005 µg/L for FPBA with negative ion mode [Leng, Gries 2005]. Column: 5% phenyl methylpolysiloxane (60 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 21 p.s.i. Temperature programme: 90° for 1 min to 150° at 6°/min for 7 min to 200° at 10°/min for 12 min to 270° at 20°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 33.2 min for FPBA. Limit of detection, 0.5 µg/L for FPBA [Angerer, Ritter 1997]. MSD. Limit of detection, 0.5 µg/L for DCCA and FPBA [Leng *et al.* 1997a]. Column: DB 5 MS fused silica capillary (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 100 kPa. Temperature programme: 70° for 1 min to 140° at 20°/min for 1 min to 270° at 10°/min for 1 min. EI ionisation at 70 eV, MID acquisition mode. Limit of detection, 0.5 µg/L [Leng *et al.* 1997b].
Meconium **GC-MS** Column: DB-5MS 5% phenyl methyl polysiloxane capillary (30 m × 0.25 mm i.d., 1 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 1 min to 280° at 10°/min for 10 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.39 µg/g [Bielawski *et al.* 2005].
Hair **GC-MS** See Blood. Limit of detection, 30.5–488.0 ng/g [Ostrea, Jr. *et al.* 2006].
Other **GC-MS** Water and Fish. Column: DB5 or DB17 MS (60m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 1 min to 210° at 15°/min for 10 min to 290° at 2°/min for 14 min. ECD. Limit of detection, 2 ng/L in water and 3.0 ng/g in fish [Mekebri *et al.* 2008]. White Wine. Column: SLB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 30 cm/s. Temperature programme: 40° to 300° at 3°/min. EI ionisation, scan acquisition mode. Limit of detection, 124 µg/L [Mondello *et al.* 2008]. Vegetables. Column: SPB-5 fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° to 200° at 8°/min to 210° at 1°/min to 270° at 1.2°/min. ITMS, SIM acquisition mode. Limit of quantification, 21 ng/g, limit of detection, 10 ng/g [González-Rodriguez *et al.* 2008]. Soil. Column: HP-5 capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 2 min to 210° at 20°/min to 270° at 3°/min for 7 min. EI ionisation at 70 eV or NCI or ECD (for ECD carrier gas: N_2, 1.3 mL/min), full scan, SIM or MS/MS acquisition mode. Limit of detection, 1.9, 1.3 and 0.47 ng/g for EI in full scan, SIM and MS/MS mode, respectively, 0.44, and 0.18 ng/g for NCI in full scan and SIM and 2.6 ng/g for ECD [Esteve-Turrillas *et al.* 2006]. Bovine Fat. Column: DB-608 (30 m × 0.53 mm i.d., 0.83 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1.5 min to 280° at 20°/min for 27 min. ECD. Retention time: 20.2, 20.35 and 20.61 min. Limit of quantification, 38.9 µg/kg, limit of detection, 11.7 µg/kg [Akre, MacNeil 2006]. Water, Vegetable and Fruit. Column: HP-5 MS 5% phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 5 min to 265° at 30°/min to 300° at 4°/min for 4.42 min. EI ionisation at 50 eV, SIM acquisition mode. Limit of detection, 2.0 µg/L and 0.025 µg/g [Beltran *et al.* 2003]. Fruit and Vegetables. Column: DB-1 capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 80° for 1 min to 250° at 25°/min for 30 min to 270° at 15°/min for 2 min to 80° at 25°/min. ECD. Column: HP MS-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.4 mL/min. Temperature programme: 80° for 1 min to 250° at 25°/min for 23 min to 270° at 25°/min for 2 min. EI ionisation at 70 eV. Limit of quantification, 0.01 and 0.02 µg/g for ECD and EI ionisation, respectively [Sannino *et al.* 2003]. Fats and Oils. CBP-1 methyl silicone fused silica capillary (25 m × 0.2 mm i.d., 0.25 µm). Carrier gas: N_2, 1.2 mL/mmin. Temperature: 250°. ECD. Limit of detection, 2 µg/L [Ramesh, Balasubramanian 1998]. Vegetables. Column: HP 50 + cross-linked 50% phenyl 50% methylsilicone fused silica (15 m × 0.53 mm, 1 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 2 min to 180° at 10°/min to 270° at 5°/min

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for 20 min. ECD. Limit of detection, 0.1 μg/g [Di Muccio *et al.* 1997]. Column: HP-1 (5 m × 0.53 mm i.d., 2.65 μm). Carrier gas: CH_4:Ar, 5.0 mL/min. Temperature programme: 230° for 2 min to 260° at 2°/min for 5 min. ECD. Retention time: 13.7 min. Limit of detection, 3–30 ng/g [Pang *et al.* 1994].

HPLC Soil. Column: Chiralcel OD (250 × 4.6 mm i.d., 10 μm). Mobile phase: *n*-hexane : isopropanol (100 : 0.1), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, <0.03 μg/g [Li *et al.* 2008]. Tomato. Column: Gemini C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (66 : 34 for 18 min to 75 : 25 in 1 min to 90 : 10 in 1 min), flow rate 1.4 mL/min for 18 min to 1.5 mL/min. Chemiluminescence detection. Limit of quantification, 0.044 mg/L, limit of detection, 0.017 mg/L [Galera *et al.* 2006]. Soil. Column: silica gel (250 × 4.6 mm i.d., 5 μm). Mobile phase: *n*-hexane : 2-propanol (100 : 0.06), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 0.05 μg/g [Li *et al.* 2003]. Column: glycidoxypropylsilanised silica gel (150 × 4.6 mm i.d., 5 μm).UV detection (λ = 230 nm). Limit of detection, <0.01 mg/L [Dondi *et al.* 1999].

Disposition in the Body Cyfluthrin is rapidly metabolised in the liver via plasma esterase hydrolysis and metabolites include *cis*-(3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid (*cis*-DCCA), *trans*-DCCA and 4-fluoro-3-phenoxybenzoic acid (FPBA). It is eliminated fairly quickly in urine (~60%) and faeces (~20%) within 24 h. 98% of the dose is eliminated within 48 h.

Toxicity Moderately toxic and irritant to human skin, especially facial skin. Toxic effects have a delay of 1 to 2 h after exposure.

A 64-year-old male was found unconscious at home with 1 empty bottle and 1 half-filled bottle containing emulsion (with 35 mL cyfluthrin). Analysis showed that there was a concentration of 10 mg/L cyfluthrin in his blood, and 14 mg cyfluthrin in his stomach, 0.004 mg/g cyfluthrin in the liver, and 7 mg/L in his urine [Hon *et al.* 1988].

Note For a review of the neurobehavioural toxicology of pyrethroid insecticides, see Wolansky and Harrill [2008].

Half-life *cis*-DCCA, 6.9 h; *trans*-DCCA, 6.2 h; FPBA, 5.3 h.

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Cyprenorphine

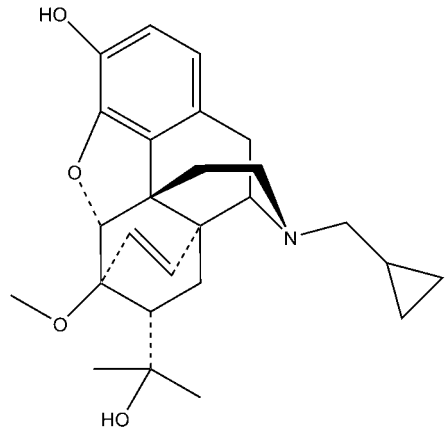
Opioid Derivative, Narcotic Antagonist

$C_{26}H_{33}NO_4$ = 423.6

CAS—4406-22-8

IUPAC Name *N*-Cyclopropylmethyl-7,8-dihydro-7α-(1-hydroxy-1-methylethyl)-O6-methyl-6,14-endo-ethenomorphine

Synonyms *N*-Cyclopropylmethyl-19-methylnororvinol; M 285.



Chemical Properties Mp 234°. Soluble 1 in 2500 of water; readily soluble in ethanol, ether, and chloroform. Extracted by organic solvents from aqueous alkaline solutions.

Cyprenorphine Hydrochloride

Chemical Properties White crystalline powder. Mp 254° to 255°. Soluble 1 in 30 of water and 1 in 16 of ethanol.

Colour Tests Ammonium molybdate test—bluish-purple→blue→dull green (limit of detection, 0.1 μg); sulfuric acid–formaldehyde test—bluish-grey→yellow-brown (limit of detection, 0.1 μg); Vitali's test—faint orange (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.75 (location reagents: acidified iodoplatinate spray, positive reaction; Marquis reagent, grey).

Ultraviolet Spectrum Aqueous acid (0.1 N hydrochloric acid)—287 nm; aqueous alkali (0.1 N sodium hydroxide)—301 nm.

Note Cyprenorphine is a highly potent narcotic antagonist with actions similar to those of nalorphine. It has been used to antagonise the effects of acetorphine and etorphine.

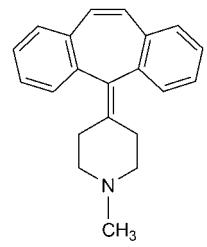
Cyproheptadine

5-HT Receptor Antagonist, Antihistamine

$C_{21}H_{21}N$ = 287.4

CAS—129-03-3

Synonym 4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine



Chemical Properties Crystals. Mp ≈113°. Log *P* (octanol/water) 4.69 [Sangster 1997], 4.92 [Gil-Agusti *et al.* 2001], (octanol/pH 7.4), 3.2. Stock solutions in methanol were stable for at least 3 months if stored at 4° [Hasegawa *et al.* 2006].

Cyproheptadine Hydrochloride

$C_{21}H_{21}N$, HCl, $1^1/_2H_2O$ = 350.9

CAS—969-33-5 (anhydrous); 41354-29-4 (sesquihydrate)

Proprietary Names *Antegan*; *Nuran*; *Periactin(e)*; *Periactinol*; *Vimicon*.

Chemical Properties White to slightly yellow, crystalline powder. Mp 214° to 216°, with decomposition. Soluble 1 in 275 of water, 1 in 35 of ethanol, 1 in about 16 of chloroform and 1 in 1.5 of methanol; practically insoluble in ether.

Colour Tests Mandelin's test—violet-brown; Marquis test—grey-green; sulfuric acid—violet.

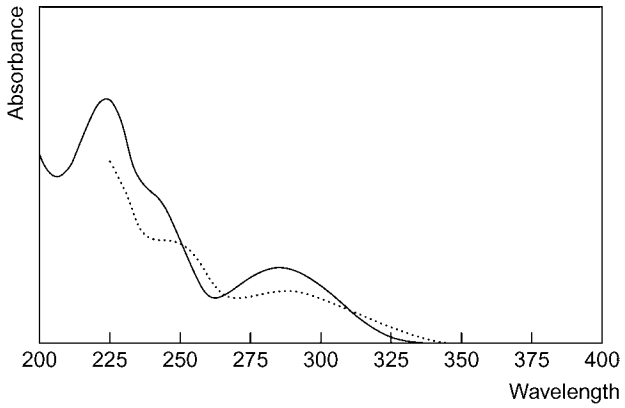
Thin-layer Chromatography System TA—R_f 0.51; system TAE—R_f 0.30; system TAF—R_f 0.50; system TL—R_f 0.13; system TB—R_f 0.45; system TC—R_f 0.44; system TE—R_f 0.64 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—cyproheptadine RI 2355, M (OH-) RI 3060, M (nor-) RI 2400, M (oxo-) RI 2960, M (nor-OH-) RI 2450; system GB—cyproheptadine RI 2460, M (nor-OH-)-H_2O RI 2608; system GC—RI 2307; system GF—RI 2710.

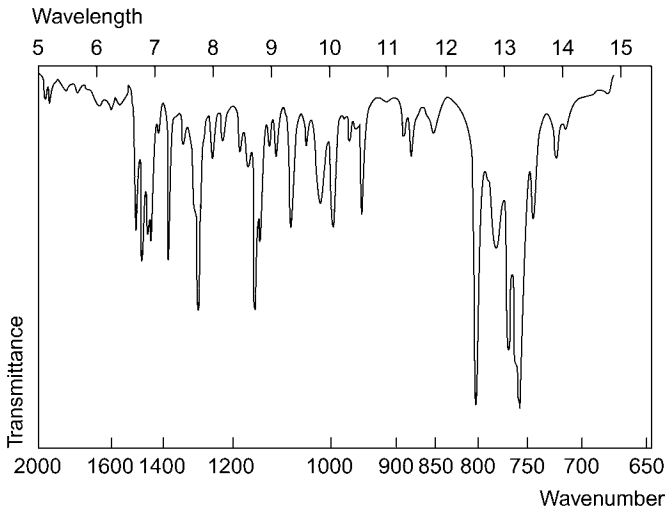
High Performance Liquid Chromatography System HA—*k* 3.2; system HAA—RT 15.0 min; system HY—RI 354; system HZ—RT 6.5 min.

Column: ODS-2 (120 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (60 : 40). UV detection (λ = 285 nm). Retention time: 8.7 min. Limit of detection, 65 µg/L [Gil-Agusti *et al.* 2001].

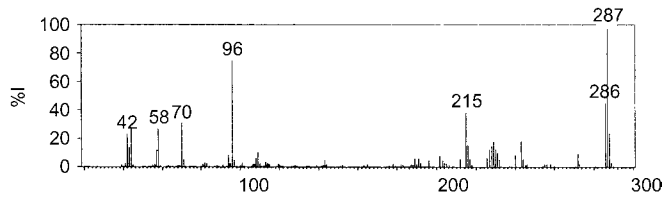
Ultraviolet Spectrum Aqueous acid—286 nm (A_1^1 = 433a); aqueous alkali—284 nm (A_1^1 = 377b).



Infrared Spectrum Principal peaks at wavenumbers 777, 756, 815, 786, 1640, 960 cm^{-1} (cyproheptadine hydrochloride), (KBr disk).



Mass Spectrum Principal ions at *m/z* 287, 96, 286, 215, 70, 44, 58, 42; M (OH^-) *m/z* 303, 203, 202, 217, 304, 205; M (nor-) *m/z* 273, 215, 216, 231, 229, 272; M (oxo-) *m/z* 229, 215, 202, 242, 301, 258; M (nor-OH-)-H_2O *m/z* 271, 272, 270, 165, 193, 241.



Quantification

Blood **GC-MS** Column: HP-5 fused silica capillary (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 200° at 30°/min to 260° at 10°/min to 300° at 20°/min for 8 min. EI ionisation. Limit of detection, 0.13 mg/L [Levine *et al.* 1998].

Plasma **GC** Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (91 cm × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 250°. NPD. Retention time: 2.4 min. Limit of detection, 6.25 µg/L [Hucker, Hutt 1983b]. Column: 3% SP-2250 Supelcoport on 80/100 mesh (0.91 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 230°. Retention time, 4.2 min. Limit of detection, 3 µg/L [Hucker, Hutt 1983a].

GC-MS Column: DB-1MS fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 120° for 1 min to 300° at 20°/min. EI ionisation at 70 eV, positive ion mode. Limit of detection, 0.2 µg/L [Hasegawa *et al.* 2006].

Urine **GC** See Plasma [Hucker, Hutt 1983a].

GC-MS See Blood [Levine *et al.* 1998]. Column: HP capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1988].

HPLC Column: octyl-ODP (150 × 6 mm i.d.). Mobile phase: acetate buffer : methanol (56 : 44, pH 3.6), flow rate 1.4 mL/min. UV detection (λ = 254 nm). Retention time: 8.3 min. Limit of quantification 50 µg/L, limit of detection, 15 µg/L [Kountourellis, Ebete 1995].

Bile **GC-MS** See Blood [Levine *et al.* 1998].

CSF **GC-MS** See Blood [Levine *et al.* 1998].

Gastric Contents **GC-MS** See Blood [Levine *et al.* 1998].

Kidney **GC-MS** See Blood [Levine *et al.* 1998].

Liver **GC-MS** See Blood [Levine *et al.* 1998].

Other **HPLC** Rat Tissue. Column: Partisil 10/25 ODS C_{18}. Mobile phase: methanol : 0.03mol/L potassium dihydrogen phosphate (60 : 40), flow rate 1.4mL/min. UV detection (λ = 210 nm). Limit of detection, 0.5 µg/g [Chow, Fischer 1987].

Disposition in the Body Absorbed after oral administration and extensively distributed throughout the tissues. Metabolised by aromatic-ring hydroxylation, *N*-demethylation, heterocyclic-ring oxidation, and glucuronic-acid conjugation; the major metabolite in urine is a quaternary ammonium glucuronide conjugate. About 67 to 77% of a dose is excreted in the urine in 6 days, the remainder being eliminated in the faeces. Of the excreted material, 58 to 65% is conjugated with glucuronic acid, 9 to 11% is conjugated with sulfate, 20 to 26% is excreted as polar material not hydrolysable by glucuronidase or sulfatase, and ~5% is unconjugated.

Therapeutic Concentration

After giving 8 mg cyproheptadine via the oral and sublingual route to 5 subjects, mean maximum plasma concentrations of 30.0 and 4.0 µg/L were reached at 4.0 and 9.6 h, respectively [Gunja *et al.* 2004].

After a single oral dose of 5 mg to 2 subjects, peak plasma concentrations of cyproheptadine metabolites of 0.036 and 0.05 mg/L were attained in 6 to 9 h; unchanged drug was not detected [Hintze *et al.* 1975].

Toxicity

A 28-year-old male was found dead. The heart blood concentration of cyproheptadine was 0.46 mg/L and ethanol was detected at 0.9 g/L. Cyproheptadine was also detected at concentrations of 8.1 mg/L, 1.8 mg/kg, 7.6 mg/kg, 0.75 mg/L and 2.3 mg in bile, kidney, liver, urine and stomach contents, respectively [Levine *et al.* 1998].

Half-life 16 h [Paton, Webster 1985].

Dose 12 to 16 mg of anhydrous cyproheptadine hydrochloride daily; maximum of 32 mg daily.

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Gunja N *et al.* (2004). A comparison of the pharmacokinetics of oral and sublingual cyproheptadine. *J Toxicol Clin Toxicol* 42: 79–83.

Hasegawa C *et al.* (2006). Simultaneous determination of ten antihistamine drugs in human plasma using pipette tip solid-phase extraction and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 20: 537–543.

Hintze KL *et al.* (1975). Disposition of cyproheptadine in rats, mice, and humans and identification of a stable epoxide metabolite. *Drug Metab Dispos* 3: 1–9.

Hucker HB, Hutt JE (1983a). Determination of cyproheptadine in plasma and urine by GLC with a nitrogen-sensitive detector. *J Pharm Sci* 72: 1069–1070.

Hucker HB, Hutt JE (1983b). GLC determination of (-)-1-cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine in human plasma and urine. *J Pharm Sci* 72: 815–817.

Kountourellis JE, Ebete KO (1995). Reversed-phase high performance liquid chromatographic determination of cyproheptadine from urine by solid-phase extraction. *J Chromatogr B Biomed Appl* 664: 468–471.

Levine B *et al.* (1998). A cyproheptadine fatality. *J Anal Toxicol* 22: 72–74.

Maurer H, Pfleger K (1988). Identification and differentiation of alkylamine antihistamines and their metabolites in urine by computerized gas chromatography-mass spectrometry. *J Chromatogr* 430: 31–41.

Paton DM, Webster DR (1985). Clinical pharmacokinetics of H1-receptor antagonists (the antihistamines). *Clin Pharmacokinet* 10: 477–497.

Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* London: John Wiley and Sons.

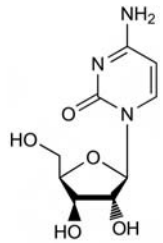
Cytarabine

Antineoplastic

$C_9H_{13}N_3O_5$ = 243.2

CAS—147-94-4

IUPAC Name 4-Amino-1-[(2*R*,3*S*,4*S*,5*R*)-3, 4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]pyrimidin-2-one
Synonyms 4-Amino-1-β-D-arabinofuranosyl-2(1*H*)-pyrimidinone; arabinosylcytosine; Ara-C; cytosine arabinoside.
Proprietary Names *Alexan*; *Aracytin(e)*; *Cytosar*; *Cytosar-U*; *Udicil*.

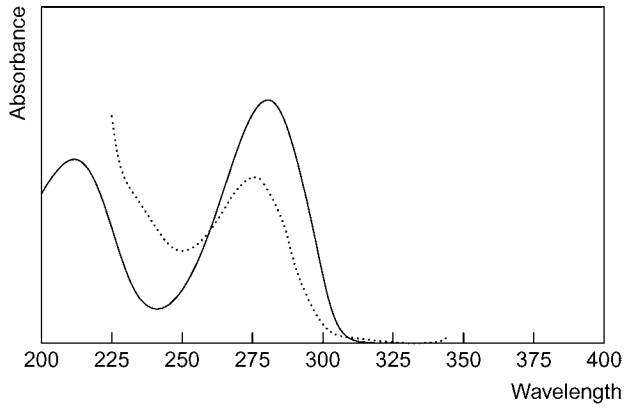


Chemical Properties A white crystalline powder. Mp 212° to 213°. Soluble 1 in 10 of water, 1 in 1000 of ethanol, and 1 in 1000 of chloroform. pK_a 4.3. Log *P* (octanol/water), −2.5.

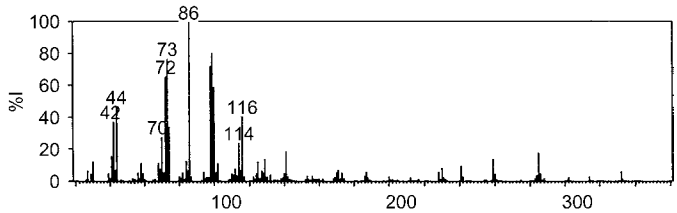
Cytarabine Hydrochloride

$C_9H_{13}N_3O_5$, HCl = 279.7
CAS—69-74-9
Chemical Properties A white crystalline powder. Soluble 1 in 1 of water; less soluble in organic solvents.

Thin-layer Chromatography System TA—R_f 0.05; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.01; system TAD—R_f 0.00; system TAE—R_f 0.69 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1=555a); aqueous alkali—274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1075, 1027, 1145, 1109, 1178, 1002 cm^{-1} (KBr disk).
Mass Spectrum

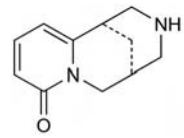


Quantification
Plasma **GC** AFID. Limit of detection, 40 µg/L [Boutagy, Harvey 1978].
HPLC UV detection. Limit of detection, 20 µg/L [Sinkule, Evans 1983]. UV detection. Limit of detection, 20 µg/L [Bury, Keary 1978].
Urine **HPLC** See Plasma [Sinkule, Evans 1983].
Cerebrospinal Fluid **HPLC** See Plasma [Sinkule, Evans 1983].
Dose 2 to 4 mg/kg daily, IV.

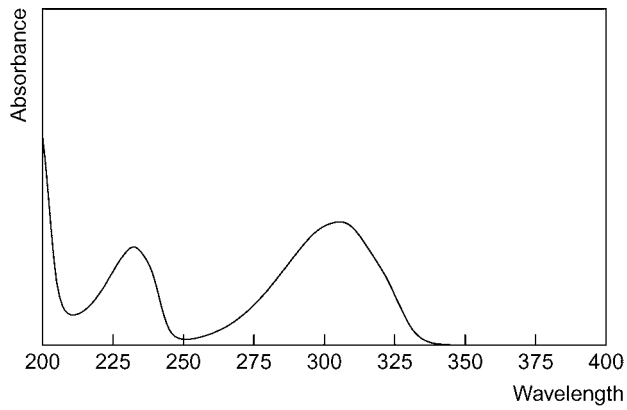
Boutagy J, Harvey DJ (1978). Determination of cytosine arabinoside in human plasma by gas chromatography with a nitrogen-sensitive detector and by gas chromatography–mass spectrometry. *J Chromatogr* 146(2): 283–296.
Bury RW, Keary PJ (1978). Determination of cytosine arabinoside in human plasma by high-pressure liquid chromatography. *J Chromatogr* 146(2): 350–353.
Sinkule JA, Evans WE (1983). High-performance liquid chromatographic assay for cytosine arabinoside, uracil arabinoside and some related nucleosides. *J Chromatogr* 274: 87–93.

Cytisine

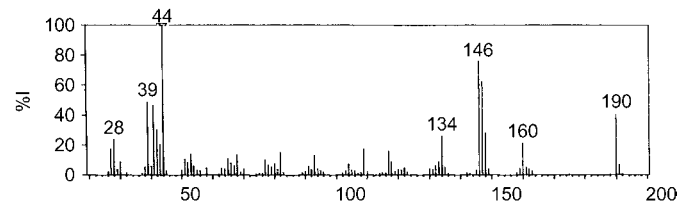
Alkaloid
$C_{11}H_{14}N_2O$ = 190.2
CAS—485-35-8
Synonyms Baptitoxine; cytiton; (1*R*)-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one; laburnine; sophorine; ulexine.



Chemical Properties An alkaloid found in all parts of the laburnum, *Laburnum anagyroides* (Leguminosae), particularly in the pods and seeds, and in some other leguminous plants. A white or slightly yellowish crystalline powder. Mp 154° to 157°. Soluble 1 in 1.3 of water, 1 in 13 of acetone, 1 in 30 of benzene, 1 in 10 of ethyl acetate, 1 in 3.5 of ethanol, and 1 in 2 of chloroform; practically insoluble in petroleum ether. Log *P* (octanol/water), 0.6.
Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.01; system TC—R_f 0.10; system TL—R_f 0.02 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—232, 302 nm (A^1_1=344b).



Infrared Spectrum Principal peaks at wavenumbers 1650, 1540, 1565, 1140, 790, 1311 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 44, 146, 147, 39, 41, 190, 42, 148.



Quantification
Blood **UV-Vis** [Richards, Stephens 1970].
Disposition in the Body
Toxicity Cytisine is a highly toxic alkaloid which resembles nicotine in its actions. Fatalities have occurred after the ingestion of laburnum seeds, but recovery is more usual.

In a fatality due to the ingestion of laburnum seeds (estimated amount ingested 50 mg), a blood concentration of 6.8 mg/L of cytisine was reported; the stomach contained 23 laburnum pods [Richards, Stephens 1970].

Note For reviews of laburnum poisoning in children, see Mitchell [1951], and Bramley, Goulding [1981].

Bramley A, Goulding R (1981). Laburnum "poisoning". *Brit Med J* 283: 1220–1221.
Mitchell RG (1951). Laburnum poisoning in children; report on ten cases. *Lancet* 2: 57–58.
Richards HG, Stephens A (1970). A fatal case of laburnum seed poisoning. *Med Sci Law* 10(4): 260–266.

Dactinomycin

Antineoplastic

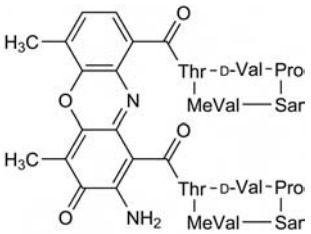
$C_{62}H_{86}N_{12}O_{16}$ = 1255.5

CAS—50-76-0

IUPAC Name 2-Amino-4,6-dimethyl-3-oxo-1-*N*,9-*N*-bis[7,11,14-trimethyl-2,5,9,12, 15-pentaoxo-3,10-di(propan-2-yl)-8-oxa-1,4,11,14-tetrazabicyclo[14.3.0]nonadecan-6-yl]phenoxazine-1,9-dicarboxamide

Synonyms Actinomycin C_1; actinomycin D; [2-amino-*N*,*N*′-bis[hexadecahydro-2,5,9-trimethyl-6,13-bis(1-methylethyl)-1,4,7,11,14-pentaoxo-1*H*-pyrrolo-[2,1-i][1,4,7,10,13]oxatetra-azacyclohexadecin-10-yl]-4,6-dimethyl-3-oxo]-3*H*-phenoxazine-1,9-dicarboxamide; meractinomycin; NSC-3053.

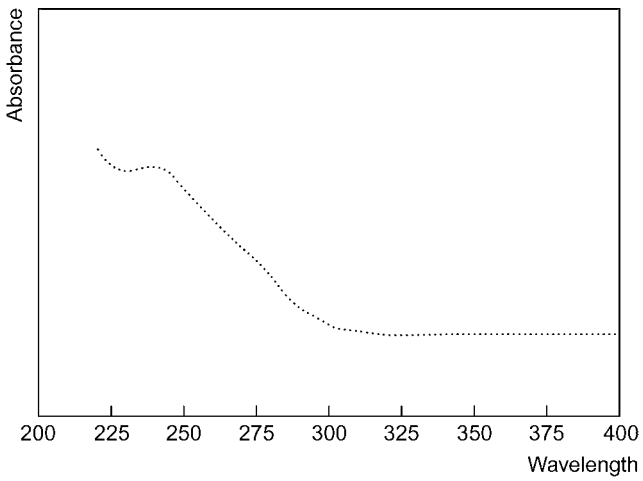
Proprietary Names *Cosmegen*



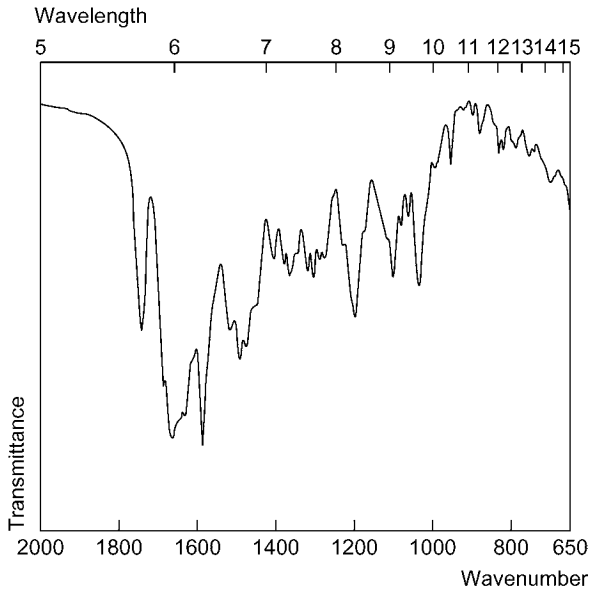
Chemical Properties A bright red, somewhat hygroscopic, crystalline powder. Crystals from absolute alcohol. Mp 241.5° to 243°, with decomposition. Dilute solutions are very sensitive to light. It is soluble in water at 10°, slightly soluble in water at 37°; freely soluble in ethanol; very slightly soluble in ether. Log *P* (octanol/water), −0.91.

High Performance Liquid Chromatography Column: ODS Hypersil Si-10 (300 × 4.0 mm i.d., 10 µm). Mobile phase: methanol with 1% NHOH : methylene chloride (2:98), flow rate 2 mL/min. UV detection. Retention time: 5.0 min [Mills, Roberson 1993].

Ultraviolet Spectrum Ethanol—240 nm.



Infrared Spectrum Principal peaks at wavenumbers 1745, 1668, 1590, 1195 cm^{-1} (KBr pellets).



Disposition in the Body Dactinomycin is rapidly distributed following IV administration and concentrates in bone marrow and nucleated cells. It does not cross the blood–brain barrier. The drug undergoes minimal metabolism and is slowly excreted in urine (about 10% as unchanged drug) and via bile in faeces (about 50% as the unchanged drug).

Half-life Plasma, 36 h.

Dose Usually 500 µg IV daily for a maximum of 5 days.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 1: 580–581

Danazol

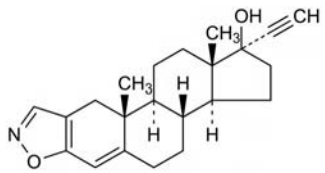
Androgen

$C_{22}H_{27}NO_2$ = 337.5

CAS—17230-88-5

Synonyms 17α-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17β-ol; win-17757.

Proprietary Names *Azol*; *Cyclomen*; *Danatrol*; *Danazant*; *Danocrine*; *Danokrin*; *Danol*; *Ladazol*; *Mastodanatrol*; *Winobanin*.



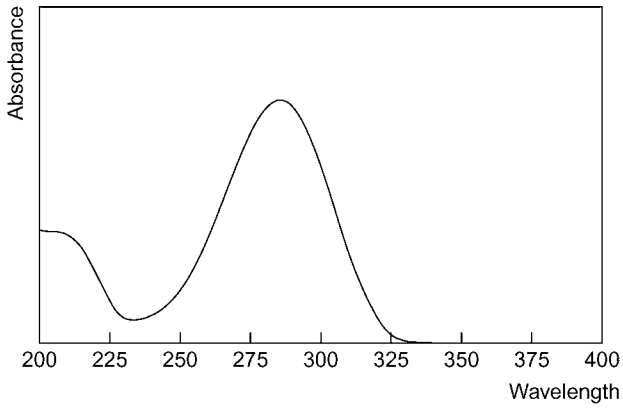
Chemical Properties A white or pale yellow crystalline powder. Mp 224.4° to 226.8°, with decomposition. It is practically insoluble in water and in light petroleum; sparingly soluble in ethanol and in benzene; freely soluble in chloroform; slightly soluble in ether; soluble in acetone. Log *P* (octanol/water), 4.21.

Gas Chromatography Column: 3% OV-1 Chromosorb WHP 80/100 mesh (4 feet × $\frac{1}{4}$ inch). Temperature: 280°. Carrier gas: N_2, flow rate 32 mL/min. Detection: flame ionisation. Retention index: 3126 [Mills, Roberson JC 1993].

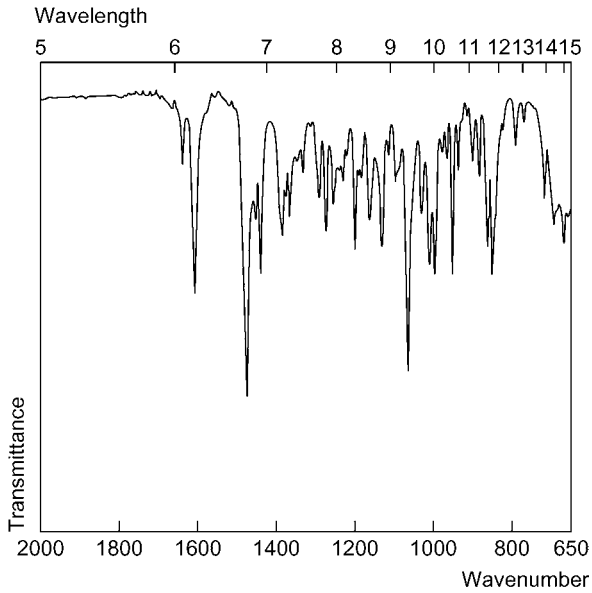
High Performance Liquid Chromatography System HY—RI 711.

Column: ODS Hypersil Si-10 (300 × 4.0 mm i.d., 10 µm). Mobile phase: methanol with 1% NHOH : methylene chloride (10:90), flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: 5.5 min [Mills, Roberson JC 1993].

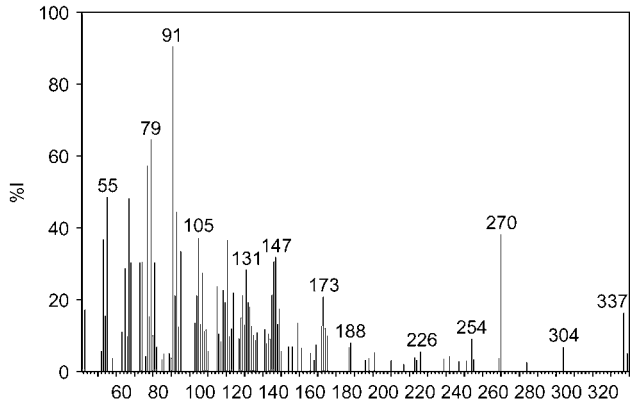
Ultraviolet Spectrum Ethanol—228, 254 nm.



Infrared Spectrum Principal peaks at wavenumbers 1597, 1470, 1062, 843 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 91, 79, 77, 55, 67, 93, 270, 105, 147, 131, 337.



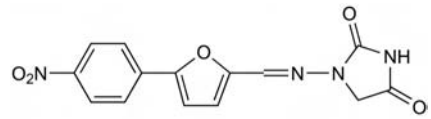
Quantification
Plasma **HPLC** UV detection. Limit of detection, 2 µg/L [Hooper *et al.* 1987].
Serum **HPLC** Column: (1)-C_8 (Spherisorb, 150 × 3.9 mm i.d., 5 µm); (2)-ODS-2 (Spherisorb, 150 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : water (55:45), flow rate 1.6 mL/min. UV detection (λ=285 nm). Retention time: 9.5 min. Limit of detection, 1 µg/L [Selinger *et al.* 1990]. UV detection. Limit of detection, 1.5 µg/L [Nygard *et al.* 1987].
Disposition in the Body Danazol is absorbed following oral administration; absorption is increased markedly if taken with food. It is metabolised in the liver to inactive metabolites, including 2-hydroxymethylethisterone, that are then excreted in urine.

Therapeutic Concentration
Twenty-four healthy females, aged between 18 and 41 years (mean 26 years), were administered with 50, 100, 200 or 400 mg doses after an overnight fast, for 3 weeks. Steady state plasma concentrations were 83, 115, 183 and 193 µg/L, respectively, observed after 1 to 2 h [Potts *et al.* 1980].
Fifteen healthy, sterile, pre-menopausal females, aged 18 to 45 years, were orally administered with a single dose of 200 mg danazol on two separate occasions, 14 days apart. Mean peak serum concentrations reached 51.5 µg/L on the first treatment at 2.3 h and 54.3 µg/L at 2.7 h on the second occasion [Selinger *et al.* 1990].
Fifteen healthy young males, aged 18 to 26 years (mean 22.3 years), were administered with 400 mg danazol following an overnight fast; two different formulations were used. The peak plasma concentrations ranged from 34.1 to 170 (mean 73.6) µg/L for formulation produced by Alphapharm and 30.8 to 143 (mean 69.6) µg/L for the Winthrop formulation. These were observed at 2 h and 2.47 h, respectively, for the two formulations [Hooper *et al.* 1991].
Half-life Plasma half-life, 4.5 h (healthy women); 9.4 h (healthy males).
Clearance Total body, mean, 710 L/h (healthy males).
Note For a review of danazol, see Potts *et al.* [1980].
Dose Up to 800 mg daily.

Hooper WD *et al.* (1987). Sensitive and selective assay of danazol in plasma by high-performance liquid chromatography. *J Chromatogr* 416: 347–352.
Hooper WD *et al.* (1991). Single oral dose pharmacokinetics and comparative bioavailability of danazol in humans. *Biopharm Drug Dispos* 12: 577–582.
Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 1: 582–583
Nygard GA *et al.* (1987). Analysis of danazol in serum using high-performance liquid chromatography. *J Chromatogr* 415: 438–444.
Potts GO *et al.* (1980). Pharmacology and pharmacokinetics of danazol. *Drugs* 19: 321–330.
Selinger K *et al.* (1990). A liquid chromatographic method for the determination of danazol in human serum. *J Pharm Biomed Anal* 8(1): 79–84.

Dantrolene

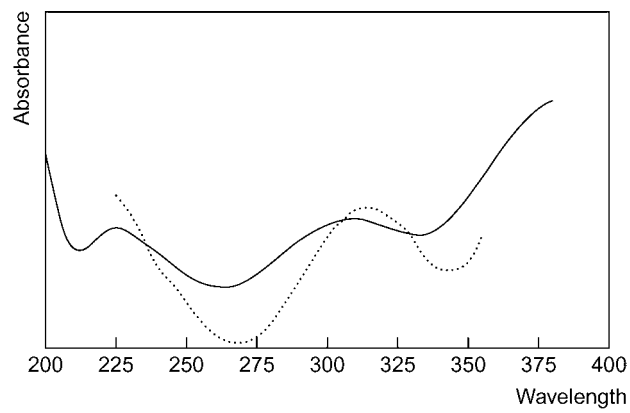
Muscle Relaxant
$C_{14}H_{10}N_4O_5 = 314.3$
CAS—7261-97-4
IUPAC Name 1-[[[5-(4-Nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinedione



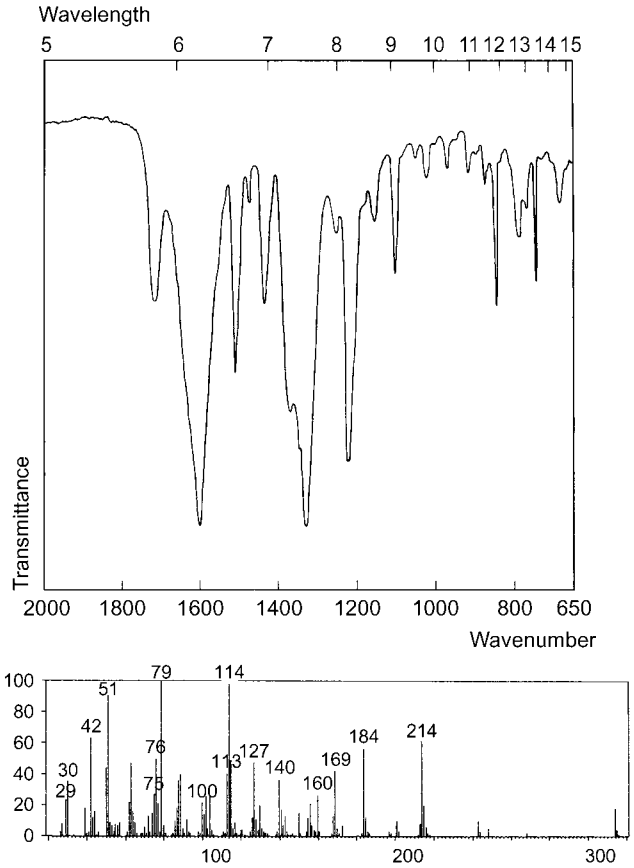
Chemical Properties Crystals. Mp 279° to 280°. Practically insoluble in water. pK_a 7.5. Log *P* (octanol/water), 1.7.

Dantrolene Sodium
$C_{14}H_9N_4NaO_5.3\frac{1}{2}H_2O = 399.3$
CAS—14663-23-1 (anhydrous); 24868-20-0 (hemiheptahydrate)
Proprietary Names *Dantamacrin*; *Dantrium*.
Chemical Properties An orange powder. Slightly soluble in water; its solubility increases in alkaline solution. Soluble 1 in 40 to 1 in 50 of acetone, and 1 in 25 of propylene glycol.

Thin-layer Chromatography System TD—R_f 0.19; system TE—R_f 0.09; system TF—R_f 0.36; system TAD—R_f 0.50; system TAJ—R_f 0.46; system TAK—R_f 0.24; system TAL—R_f 0.90.
High Performance Liquid Chromatography System HZ—retention time 4.0 min; system HAX—retention time 6.0 min; system HAY—retention time 5.2 min.
Ultraviolet Spectrum Dantrolene sodium: aqueous alkali—314 nm (A^1_1=487b).



Infrared Spectrum Principal peaks at wavenumbers 1600, 1225, 1510, 850, 1713, 1108 cm^{-1} (dantrolene sodium, KBr disk).



Quantification

Blood **Spectrofluorimetry** Limit of detection, 100 µg/L [Hollifield, Conklin 1973].

Plasma **Spectrofluorimetry** See Blood [Hollifield, Conklin 1973].

HPLC Metabolite [Wuis *et al.* 1990]. UV detection. Dantrolene, 5-hydroxydantrolene and *p*-acetamidodantrolene, limit of detection, 20 mg/L [Wuis *et al.* 1982].

Urine **Spectrofluorimetry** See Blood [Hollifield, Conklin 1973].

HPLC See Plasma [Wuis *et al.* 1990]; [Wuis *et al.* 1982].

Bile **Spectrofluorimetry** Limit of detection, 400 mg/L [Hollifield, Conklin 1973].

Tissues **Spectrofluorimetry** see Bile [Hollifield, Conklin 1973].

Disposition in the Body Slowly and incompletely absorbed after oral administration. Metabolised by oxidation to 5-hydroxydantrolene, which is active, and by reduction followed by acetylation to form the *p*-acetamido derivative. About 20% of an oral dose is excreted in the urine as metabolites, mainly the 5-hydroxy derivative; <5% of a dose is excreted in the urine as unchanged drug; up to about 50% of a dose may be excreted in the bile.

Therapeutic Concentration

Following a single oral dose of 100 mg to 8 subjects, peak plasma concentrations of 0.7 to 1.7 mg/L (mean 1.2) of dantrolene and 0.2 to 0.5 mg/L (mean 0.4) of 5-hydroxydantrolene were attained in about 1 to 8 h and 1 to 22 h, respectively [Meyler *et al.* 1979].

In 4 subjects receiving daily oral doses of 25 to 50 mg 2 to 4 times a day, plasma-dantrolene concentrations were fairly stable and remained between about 0.03 and 0.1 mg/L; concentrations in an additional 2 subjects fluctuated between about 0.03 and 0.2 mg/L; plasma concentrations of 5-hydroxydantrolene and *p*-acetamidodantrolene ranged from about 0.1 to 0.3 mg/L [Vallner *et al.* 1979].

Toxicity Fatalities due to liver damage have been reported.

Half-life Plasma half-life, dantrolene 4 to 22 h (mean 9), 5-hydroxydantrolene 8 to 29 h (mean 16).

Note For a review of the pharmacokinetics of dantrolene, see Pinder *et al.* [1977].

Dose Initially 25 mg of dantrolene sodium daily, increased to a maximum of 400 mg daily.

Hollifield RD, Conklin JD (1973). Determination of dantrolene in biological specimens containing drug-related metabolites. *J Pharm Sci* 62: 271–274.

Meyler WJ *et al.* (1979). Relationship between plasma concentration and effect of dantrolene sodium in man. *Eur J Clin Pharmacol* 16: 203–209.

Pinder RM *et al.* (1977). Dantrolene sodium: a review of its pharmacological properties and therapeutic efficacy in spasticity. *Drugs* 13: 3–23.

Vallner JJ *et al.* (1979). *Curr Ther Res* 25: 79–91.

Wuis EW *et al.* (1990). Determination of a dantrolene metabolite, 5-(p-nitrophenyl)-2-furoic acid, in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 526: 575–580.

Wuis EW *et al.* (1982). Simultaneous determination of dantrolene and its metabolites, 5-hydroxydantrolene and nitro-reduced acetylated dantrolene (F 490), in plasma and urine of man and dog by high-performance liquid chromatography. *J Chromatogr* 231: 401–409.

Dantron

Purgative

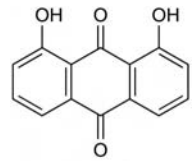
$C_{14}H_8O_4 = 240.2$

CAS—117-10-2

IUPAC Name 1,8-Dihydroxy-9,10-anthracenedione

Synonyms Antrapurol; chrysazin; chryzacin; danthron; dianthon; dioxyanthrachinonum.

Proprietary Names *Dorbane*; *Duolax*; *Modane*; *Roydan*. It is an ingredient of *Dorbanex*, *Dorbantyl*, *Doxidan*, *Normacol X*, and *Normax*.



Chemical Properties An orange crystalline powder. Mp 193° to 197°. Almost insoluble in water; soluble 1 in 2500 of ethanol, 1 in 30 of chloroform, and 1 in 500 of ether; very slightly soluble in aqueous solutions of alkali hydroxides. Log *P* (octanol/water), 3.9.

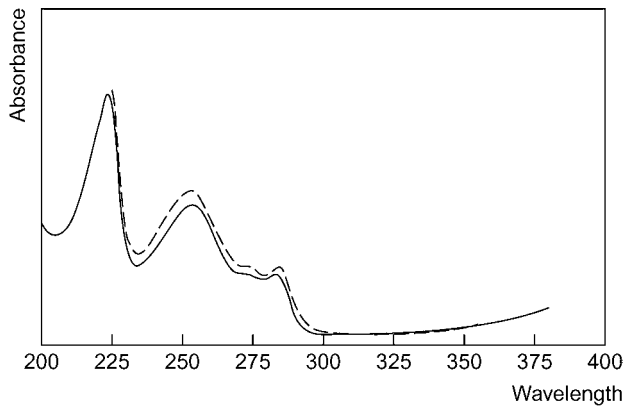
Colour Test When dissolved in sulfuric acid—red; dilution with water—yellow precipitate.

Thin-layer Chromatography System TD—R_f 0.80; system TE—R_f 0.43; system TF—R_f 0.69; system TAD—R_f 0.78.

Gas Chromatography System GA—dantron-Me RI 2435, dantron-Me_2 RI 2475, dantron-AC RI 2460, dantron-AC_2 RI 2595, dantron-TMS RI 2465, dantron-TMS_2 RI 2530; system GB—dantron-TMS RI 2574, dantron-TMS_2 RI 2611.

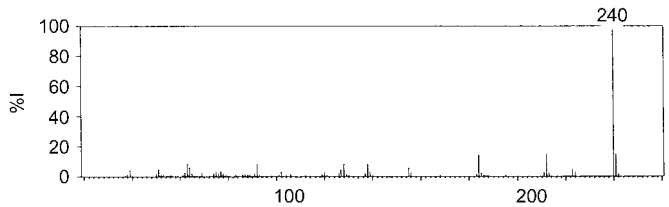
High Performance Liquid Chromatography System HY—RI 603.

Ultraviolet Spectrum Methanol—252 (A^{1}_{1}=915a), 283 nm; alkaline methanol—280 nm (A^{1}_{1}=510a).



Infrared Spectrum Principal peaks at wavenumbers 1264, 740, 1620, 1597, 1152, 1197 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 240, 212, 241, 184, 138, 92, 128, 63.



Dose 50 to 150 mg daily.

Dapsone

Antileprotic

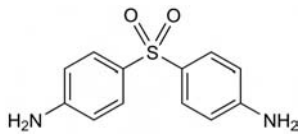
$C_{12}H_{12}N_2O_2S = 248.3$

CAS—80-08-0

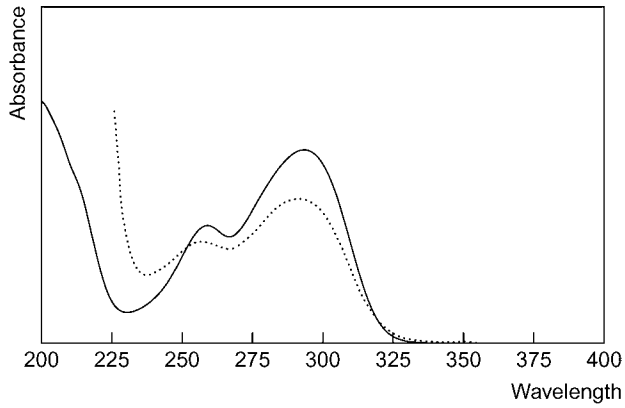
IUPAC Name 4-(4-Aminophenyl)sulfonylaniline

Synonyms DADPS; DDS; diaphenylsulfone; disulone; 4,4′-sulfonylbisbenzeneamine; sulphonyldianiline.

Proprietary Names *Avlosulfon*; *Cryosulfone*; *Diphenasone*; *Disulone*; *Eporal*; *Navophone*. It is an ingredient of *Maloprim*.



Chemical Properties A white or slightly yellowish-white crystalline powder. It discolours on exposure to light but this is not accompanied by significant decomposition. Mp 175° to 181°. Soluble 1 in 7000 of water and 1 in about 36 of ethanol; soluble in acetone and dilute mineral acids. pK_a 1.3, 2.5. Log *P* (octanol/water), 2.0.
Gas Chromatography System GA—RI 2880.
High Performance Liquid Chromatography System HY—RI 298; system HZ—retention time 2.6 min; system HAA—retention time 12.6 min.
Ultraviolet Spectrum Aqueous acid—288 nm (A^1_i=350b); aqueous alkali—256, 292 nm.

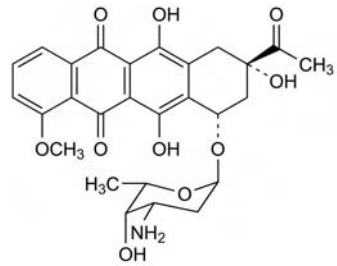


Infrared Spectrum Principal peaks at wavenumbers 1150, 1276, 1592, 1107, 685, 1633 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 108, 248, 140, 65, 92, 141, 109, 80.
Quantification
Blood **HPLC** Limit of quantification, 20 mg/L for dapsone and 15 mg/L for monoacetyldapsone [Ronn *et al.* 1995].
Plasma **TLC** Limit of detection, 20 mg/L [Ahmad, Rogers 1980].
GC ECD. Dapsone and monoacetyldapsone [Burchfield *et al.* 1973].
HPLC UV detection (λ = 295 nm). Limit of detection, 0.002 mg/L for dapsone, 0.047 mg/L for hydroxylaminodapsone [Kwadijk, Torano 2002]. UV detection. Dapsone and monoacetyldapsone. Limit of detection, 5 mg/L [Jones, Ovenell 1979].
Serum **HPLC** Electrochemical detection. UV detection (λ = 295 nm). Limit of detection, 25 mg/L [Moncrieff 1994]. UV detection. Dapsone and monoacetyldapsone. Limit of detection, 200 mg/L [Zuidema *et al.* 1980].
Saliva **TLC** See Plasma [Ahmad, Rogers 1980].
HPLC Limit of detection, 0.2 mg/L [Moncrieff 1994].
Other **HPLC** Raw Material, Dosage Forms, Biological Fluids. Limit of detection, 10 pg using fluorescence detection, 250 pg using UV detection [Mannan *et al.* 1977].
Disposition in the Body Slowly and almost completely absorbed after oral administration and widely distributed throughout the body. It is acetylated to monoacetyldapsone, the extent of which is genetically determined. It is also metabolised by *N*-oxidation to mono-*N*-hydroxydapsone, together with glucuronic acid and sulfate conjugation of dapsone and the metabolites. Enterohepatic recycling occurs. About 70–90% of a dose is excreted in the urine, about 10–20% as unchanged drug, about 50% as dapsone conjugates, and about 30% as *N*-oxidation products (mostly conjugated). Small amounts are eliminated in the faeces. About 30–50% of a single dose is excreted in the urine within 24 h and more than 70% in 3 days, but there is wide individual variation.
Therapeutic Concentration
After a single oral dose of 50 mg to 6 subjects, plasma concentrations of dapsone were 1.02 to 1.44 mg/L (mean 1.24) in 3 slow acetylators, and 1.03 to 1.48 mg/L (mean 1.23) in 3 rapid acetylators, after 4 h. The concentrations of monoacetyldapsone were 0.15 to 0.38 mg/L (mean 0.25) in the slow acetylators and 0.63 to 1.66 mg/L (mean 0.98) in the rapid acetylators, after 4 h [Biggs, Levy 1971].
Toxicity Toxic effects are likely to occur at serum concentrations above 10 mg/L. In 1 case of fatal overdosage, a 22-month-old child died 55 h after ingesting about 5 g of dapsone. The concentrations in the plasma and cerebrospinal fluid were 150 mg/L and 33 mg/L, respectively, 42 h after ingestion. Postmortem concentrations were: blood 135 mg/L, brain 90 µg/g, liver 165 µg/g, and urine 400 mg/L [Davies 1950].
Half-life Plasma half-life, 10–50 h (mean 24).
Protein Binding Dapsone 50–80%, monoacetyldapsone 98–100%.
Dose 50 to 100 mg daily.

Ahmad RA, Rogers HJ (1980). Plasma and salivary pharmacokinetics of dapsone estimated by a thin layer chromatographic method. *Eur J Clin Pharmacol* 17: 129–133.
Biggs JT, Levy L (1971). *Proc Soc Exp Biol Med* 137: 692–695.
Burchfield HP *et al.* (1973). Gas chromatographic methods for analysis of sulfone drugs used in leprosy chemotherapy. *Anal Chem* 45: 916–920.
Davies R (1950). Fatal poisoning with udolac (diaminodiphenylsulphone). *Lancet* 1: 905–906.
Jones CR, Ovenell SM (1979). Determination of plasma concentrations of dapsone, monoacetyl dapsone and pyrimethamine in human subjects dosed with maloprim. *J Chromatogr* 163(2): 179–185.
Kwadijk S, Torano JS (2002). High-performance liquid chromatographic method with ultraviolet detection for the determination of dapsone and its hydroxylated metabolite in human plasma. *Biomed Chromatogr* 16(3): 203–208.
Mannan CA *et al.* (1977). High-speed liquid chromatographic analysis of dapsone and related compounds. *J Pharm Sci* 66: 1618–1623.
Moncrieff J (1994). Determination of dapsone in serum and saliva using reversed-phase high-performance liquid chromatography with ultraviolet or electrochemical detection. *J Chromatogr B Biomed Sci Appl* 654(1): 103–110.
Ronn AM *et al.* (1995). High-performance liquid chromatography determination of dapsone, monoacetyldapsone, and pyrimethamine in filter paper blood spots. *Ther Drug Monit* 17(1): 79–83.
Zuidema J *et al.* (1980). Rapid high-performance liquid chromatographic method for the determination of dapsone and monoacetyldapsone in biological fluids. *J Chromatogr* 182(1): 130–135.

Daunorubicin

Antineoplastic
$C_{27}H_{29}NO_{10}$ = 527.5
CAS—20830-81-3
IUPAC Name (7*S*,9*S*)-9-Acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dione
Synonyms (8*S*-*cis*)-8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione; daunomycin; leukaemomycin C; RP-13057; rubidomycin.
Proprietary Name *Cerubidin*



Chemical Properties Mp 208° to 209°. pK_a 10.3. Log *P* (octanol/water), 1.83.

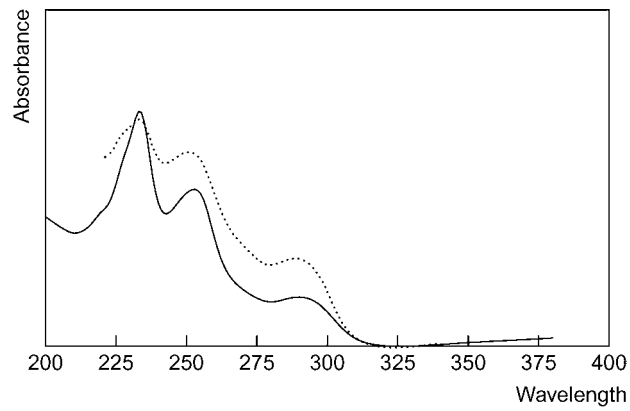
Daunorubicin Hydrochloride

$C_{27}H_{29}NO_{10}$,HCl = 564.0
CAS—23541-50-6
Proprietary Names *Cerubidine*; *Daunoblastin*; *Ondena*.
Chemical Properties An orange-red, hygroscopic crystalline powder. Decomposes at 188° to 190°. It is freely soluble in water, methanol and aqueous alcohol; slightly soluble in ethanol; very slightly soluble in chloroform; practically insoluble in acetone, chloroform, ether and benzene.

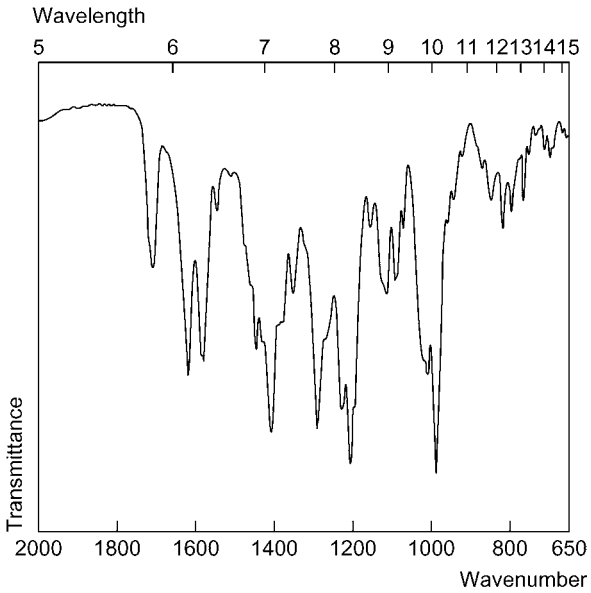
Daunorubicin Lactate

$C_{27}H_{29}NO_{10}$,$C_6H_8O_7$ = 719.6
Proprietary Name *Daunoxome*

High Performance Liquid Chromatography Column: ODS Hypersil Si-10 (300 × 4 mm i.d., 10 µm). Mobile phase: methanol with 1% NHOH : methylene chloride (10:90), flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: 4.0 min [Mills, Roberson 1993].
Ultraviolet Spectrum Ethanol—234, 251, 288 nm



Infrared Spectrum Principal peaks at wavenumbers 1703, 1618, 1576, 1407 cm^{-1} (KBr pellets).



Mass Spectrum Principal ions at *m/z* 43, 398, 337, 72, 309, 95, 362, 217.

Quantification

Plasma **HPLC** Fluorescence detection. Daunorubicin and metabolites, limit of detection, 5 µg/L [Galettis *et al.* 1994]. Fluorescence detection. Daunorubicin and metabolites, detection range, 200 to 900 µg/L in plasma [de Jong *et al.* 1990]. Electrochemical detection. Daunorubicin and metabolites, limit of detection, <10 µg/L in plasma [Akpofure *et al.* 1982].

Other Biological Fluids **HPLC** See Plasma [Akpofure *et al.* 1982].

Tissue **HPLC** See Plasma [de Jong *et al.* 1990].

Disposition in the Body Conventional formulations of daunorubicin are rapidly distributed into body tissues following IV administration, particularly liver, lungs, kidneys, spleen and heart. It does not cross the blood–brain barrier. It is rapidly metabolised in the liver and excreted in bile (about 40%) and urine as the unchanged drug and metabolites. The major metabolite, daunorubicinol, has antineoplastic activity. A liposomal formulation of daunorubicin is also available that differs in its pharmacokinetic characteristics. Following IV administration it remains largely confined within the vascular fluid volume and therefore plasma clearance is higher. The values below apply to the conventional formulation.

Therapeutic Concentration

Patients diagnosed with a variety of disseminated solid malignancies with normal renal function, were administered either with an 80 mg/m^2 IV dose or with 120 mg/m^2. Plasma concentrations after 15, 30 and 60 min were 400, 290 and 250 µg/L, respectively, for the 80 mg/m^2 dose and 650, 400 and 320 µg/L for the 120 mg/m^2 dose [Alberts *et al.* 1971].

Toxicity Cardiotoxicity, both acute and chronic, is the main major dose-limiting adverse effect. It is more likely to occur if the total cumulative dose exceeds 550 mg/m^2 in adults or 300 mg/m^2 in children.

Daunorubicin concentrations were measured at autopsy in 2 subjects who died 16 and 19 h after receiving doses of 120 mg/m^2 and 140 mg/m^2, respectively. The following concentrations of the two doses in different organs were: 18.4 and 38 µg/g (kidney), 10.0 and 13.0 µg/g (spleen), 8.3 and 12.0 µg/g (liver), 6.5 and 10.0 µg/g (lung), 4.4 and 6.5 µg/g (heart), 4.0 and 7.3 µg/g (ileum) [Alberts *et al.* 1971].

Half-life Plasma, daunorubicin, 18.5 h; daunorubicinol, 26.7 h.

Volume of Distribution Approximately 1000 L; 1055 L or 619 L/m^2 (80 mg/m^2); 897 L or 511 L/m^2 (120 mg/m^2).

Clearance Plasma clearance, 230 mL/min.

Dose Usually up to 45 mg/m^2 body surface IV daily to a maximum cumulative dose of 550 mg/m^2.

Akpofure C *et al.* (1982). Quantitation of daunorubicin and its metabolites by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 232: 377–383.

Alberts DS *et al.* (1971). The pharmacokinetics of daunomycin in man. *Clin Pharmacol Ther* 12: 96–104.

de Jong J *et al.* (1990). Sensitive method for the determination of daunorubicin and all its known metabolites in plasma and heart by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 529: 359–368.

Galettis P *et al.* (1994). Daunorubicin pharmacokinetics and the correlation with P-glycoprotein and response in patients with acute leukaemia. *Br J Cancer* 70: 324–329.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 1: 588–589

Debrisoquine

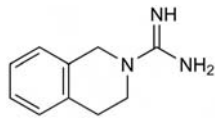
Antihypertensive

$C_{10}H_{13}N$ = 175.2

CAS—1131-64-2

IUPAC Name 3,4-Dihydro-2(1*H*)-isoquinolinecarboximidamide

Synonym Isocaramidine



Chemical Properties pK_a 11.9. Log *P* (octanol/water), 0.8.

Debrisoquine Sulfate

$(C_{10}H_{13}N_3)_2.H_2SO_4$ = 448.5

CAS—581-88-4

Proprietary Name *Declinax*

Chemical Properties A white crystalline powder. Mp about 274°, with decomposition. Soluble 1 in 40 of water; very slightly soluble in ethanol; practically insoluble in chloroform and ether.

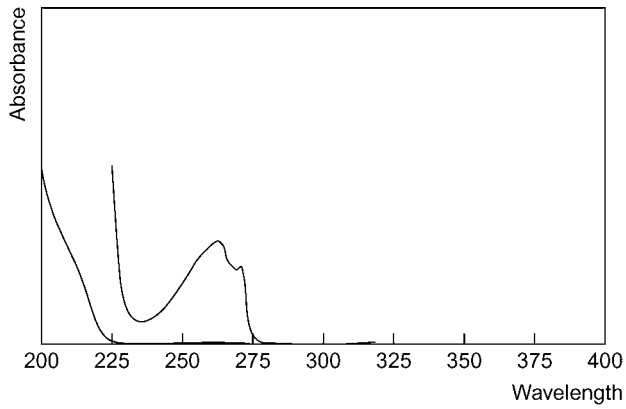
Colour Tests Mandelin's test—green; Marquis test—red-brown.

Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00; system TAJ—R_f 0.00; system TAK—R_f 0.03; system TAL—R_f 0.36 (acidified iodoplatinate solution, positive).

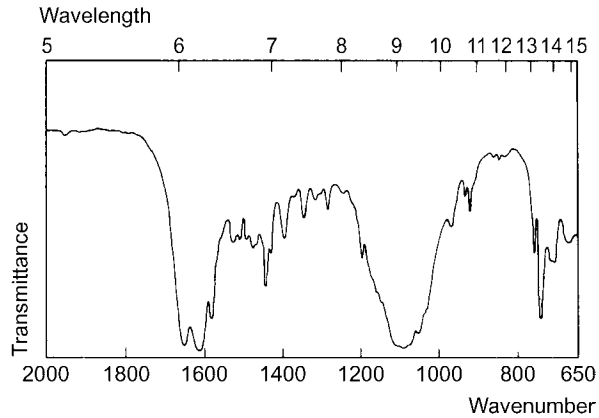
Gas Chromatography System GA—not eluted; system GB—not eluted.

High Performance Liquid Chromatography System HA—*k* 1.2; system HY—RI 245.

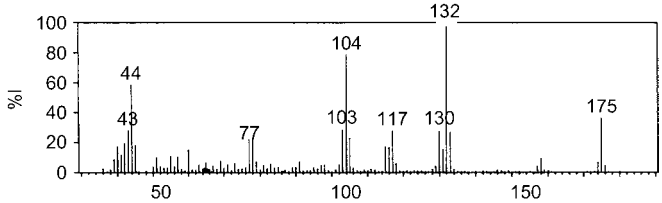
Ultraviolet Spectrum Aqueous acid—262 (A_1^1=17.7a), 270 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1614, 1090, 1650, 1583, 750, 715 cm^{-1} (debrisoquine sulfate, KBr disk).



Mass Spectrum Principal ions at *m/z* 132, 104, 44, 175, 103, 117, 43, 130.



Quantification
Blood **GC** AFID. Debrisoquine and 4-hydroxydebrisoquine, limit of detection, 3 µg/L [Lennard *et al.* 1977].
Plasma **GC** See Blood [Lennard *et al.* 1977].
GC-MS Limit of detection, 1 µg/L for debrisoquine and 5 µg/L for 4-hydroxydebrisoquine [Malcolm, Marten 1976].
Urine **GC** ECD [Idle *et al.* 1979] and see Blood [Lennard *et al.* 1977].
HPLC [Pereira *et al.* 2000]. Fluorescence detection (λ_{ex}=208 nm; λ_{em}=562 nm). Limit of quantification, 50 µg/L. Limit of detection, 30 µg/L for drug and 20 µg/L for metabolite [Frye, Branch 1996; Bozkurt *et al.* 1993].
Saliva **GC** See Blood [Lennard *et al.* 1977].
Disposition in the Body Rapidly absorbed after oral administration. The main metabolic reaction is 4-hydroxylation; aromatic hydroxylation at positions 5, 6, 7, and 8 also occurs and acidic ring-opened metabolites are also formed. There is no evidence of glucuronic acid conjugation in man. The extent of 4-hydroxylation of debrisoquine is genetically determined. About 75% of a dose is excreted in the urine in 24 h. Excretion of unchanged drug ranges from 8 to 80% of a dose. About 90% of the population are extensive metabolisers of debrisoquine and, in these subjects, about 10 to 40% of a dose is excreted as 4-hydroxydebrisoquine; the remaining 10% are poor metabolisers and excrete less than 2% as the 4-hydroxy metabolite. Acidic ring-opened metabolites account for up to 15% of a dose and the 5-, 6-, 7-, and 8-hydroxy metabolites for about 7%. About 12% of a dose is eliminated in the faeces.
Therapeutic Concentration
Following a single oral dose of 20 mg to 3 subjects, peak plasma concentrations of 0.010, 0.013, and 0.024 mg/L of debrisoquine were reported at 1.5 to 3.5 h; peak concentrations of the 4-hydroxy metabolite averaging 0.035 mg/L were attained in 1.5 to 2.5 h. The 4-hydroxy metabolite was eliminated more rapidly than debrisoquine and was not detectable after 24 h [Silas *et al.* 1978].
Maximum steady-state plasma concentrations of 0.015 to 0.18 mg/L (mean 0.08), and 0 to 0.05 mg/L (mean 0.02) for debrisoquine and 4-hydroxydebrisoquine respectively, were reported in 13 patients receiving daily oral doses of 40 mg [Silas *et al.* 1977].
Half-life Plasma half-life, debrisoquine about 16 to 30 h, 4-hydroxydebrisoquine about 10 h.
Distribution in Blood Plasma : whole blood ratio, 0.45.
Protein Binding About 25%.
Dose The equivalent of 10 to 120 mg of debrisoquine daily; up to 300 mg or more daily may be given.

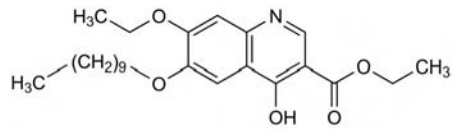
Bozkurt A *et al.* (1993). Determination of debrisoquine and 4-hydroxydebrisoquine in urine by high-performance liquid chromatography with fluorescence detection after solid-phase extraction. *J Pharm Biomed Anal* 11(8): 745–749.
Frye RF, Branch RA (1996). Improved high-performance liquid chromatographic determination of debrisoquine and 4-hydroxydebrisoquine in human urine following direct injection. *J Chromatogr B Biomed Appl* 677(1): 178–182.
Idle JR *et al.* (1979). The metabolism of [14C]-debrisoquine in man. *Br J Clin Pharmacol* 7: 257–266.
Lennard MS *et al.* (1977). Determination of debrisoquine and its 4-hydroxy metabolite in biological fluids by gas chromatography with flame-ionization and nitrogen-selective detection. *J Chromatogr* 133: 161–166.
Malcolm SL, Marten TR (1976). Determination of debrisoquin and its 4-hydroxy metabolite in plasma by gas chromatography/mass spectrometry. *Anal Chem* 48: 807–809.
Pereira VA *et al.* (2000). A micromethod for quantitation of debrisoquine and 4-hydroxydebrisoquine in urine by liquid chromatography. *Braz J Med Biol Research* 33(5): 509–514.
Silas JH *et al.* (1978). The disposition of debrisoquine in hypertensive patients. *Br J Clin Pharmacol* 5: 27–34.
Silas JH *et al.* (1977). Why hypertensive patients vary in their response to oral debrisoquine. *Brit Med J* 1: 422–425.

Decamethonium Bromide

Muscle Relaxant
$C_{16}H_{38}Br_2N_2$ = 418.3
CAS—156-74-1 (decamethonium); 541-22-0 (bromide)
Synonyms C-10; *N,N,N,N′,N′,N′*-hexamethyl-1,10-decanediaminium dibromide.
Proprietary Name *Syncurine*
Chemical Properties A white, hygroscopic, crystalline powder. Mp about 265°, with decomposition. Freely soluble in water and ethanol; very slightly soluble in chloroform; practically insoluble in ether. Log *P* (octanol/water), −4.8.
Thin-layer Chromatography System TA—R_f 0.00; system TN—R_f 0.56; system TO—R_f 0.16. System TA—R_f 0.00 decamethonium; system TB—R_f 0.00 decamethonium; system TC—R_f 0.00 decamethonium; system TE—R_f 0.00 decamethonium; system TL—R_f 0.00 decamethonium; system TAE—R_f 0.00 decamethonium; system TAF—R_f 0.02 decamethonium (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HX—RI 56 (decamethonium).
Ultraviolet Spectrum Aqueous acid—227 nm (A^1_1=584b).
Infrared Spectrum Principal peaks at wavenumbers 1627, 910, 965, 1030, 1136, 1063 cm^{-1} (KBr disk).
Dose Initially 2 to 2.5 mg by IV injection.

Decoquinate

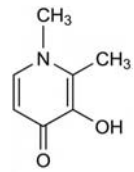
Coccidiostat (Veterinary)
$C_{24}H_{35}NO_5$ = 417.5
CAS—18507-89-6
IUPAC Name 6-Decyloxy-7-ethoxy-4-hydroxy-3-quinolinecarboxylic acid ethyl ester
Synonym M&B 15497
Proprietary Name *Deccox*



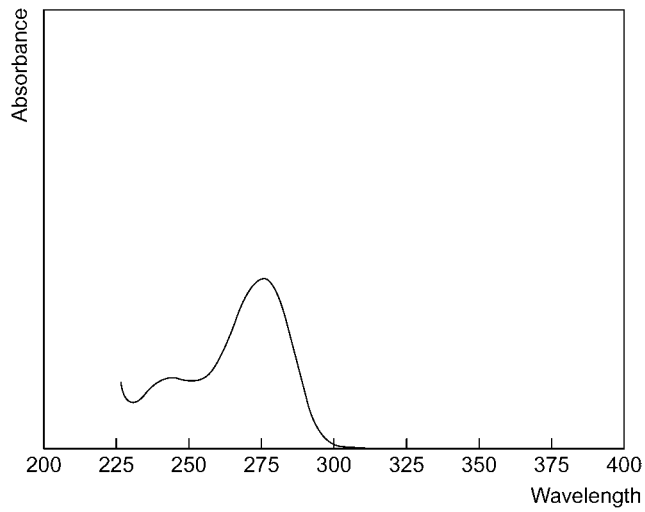
Chemical Properties A cream to buff-coloured microcrystalline powder. Mp about 242°. Practically insoluble in water and ethanol; slightly soluble in chloroform and ether; soluble in acetone. Log *P* (octanol/water), 7.8.
Colour Tests Liebermann's reagent—orange (slow); Mandelin's test—red-brown.
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Methanolic acid—265 nm (A^1_1=1000b).
Infrared Spectrum Principal peaks at wavenumbers 1695, 1613, 1497, 1263, 1205, 1558 cm^{-1} (KBr disk).

Deferiprone

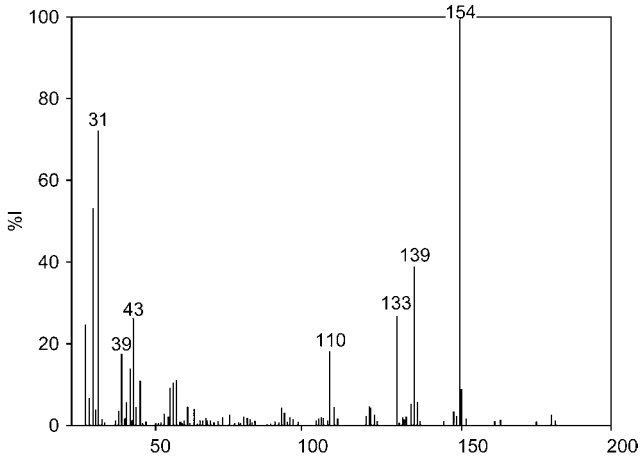
Iron Chelator
$C_7H_9NO_2$ = 139.2
CAS—30652-11-0
IUPAC Name 3-Hydroxy-1,2-dimethylpyridin-4-one
Synonyms BRN-1447108; CP20; 1,2-dimethyl-3-hydroxypyrid-4-one; L1.
Proprietary Name *Kelfer*



Chemical Properties Mp 271° to 273°. Maximum water solubility of 16–18 g/L at 24°. pK_a 3.5. Log *P* (octanol/water), −0.77; log *P* (*n*-octanol/Tris HCl, pH 7.4), 0.0009.
High Performance Liquid Chromatography Column: Hypercarb-PGC (100 × 4.6 mm i d., 7 µm). Mobile phase: 10 mmol/L phosphate buffer (pH 2.9) containing 2 mmol/L EDTA and acetonitrile (88 : 12). UV detection (λ=275 nm). Retention time: 4 min [Singh *et al.* 1991].
Ultraviolet Spectrum Aqueous acid—279 nm.



Mass Spectrum Principal ions at *m/z* (Deferiprone-*O*-methylated metabolite) 154, 31, 29, 139, 133, 43, 27.



Quantification

Plasma **HPLC** Column: Hypercarb (Shandon, 100 × 4.7 mm i.d., 7 µm). Mobile phase: 10 mmol/L disodium phosphate and 2 mmol/L EDTA (pH 3), flow rate 1.0 mL/min. UV detection (λ=280 nm). Retention time: 5.38 min. Limit of detection, 0.5 mg/L [Klein *et al.* 1991].

Serum **Bioassay** Limit of detection, 0.5 mg/L [Pope *et al.* 1997].

Urine **HPLC** UV detection (λ=280 nm). Limit of detection, 120 mg/L [Kontoghiorghes *et al.* 1990].

Saliva **Bioassay** See Serum [Pope *et al.* 1997].

Disposition in the Body Deferiprone is rapidly absorbed and >85% of an administered dose is metabolised in the liver by glucuronidation to a conjugate lacking chelating properties. Deferiprone is excreted mainly in urine (~80% recovered) as the parent drug, its conjugate and bound to iron. Only a small proportion of the drug is found unchanged in urine within 24 h. Excretion of the glucuronide is slower than that of the parent drug. Other metabolites include an *O*-methyl metabolite, 2-hydroxylmethyl metabolite and its glucuronide.

Therapeutic Concentration

Four males and 5 females with homozygous β-thalassemia, aged between 11 and 18.5 years, were administered with a mean dose of 75.7 ± 1.73 mg/kg deferiprone daily for 3–13 months. A mean peak plasma concentration of 11.3 mg/L was reached within 1 h [Pope *et al.* 1997].

Half-life 1–2.5 h.

Volume of Distribution Steady state, 1.55–1.73 L/kg; healthy volunteers, 72.8 L/kg; patients with β-thalassemia, 115.1 L/kg.

Clearance Healthy volunteers, 34.8 L/h; patients with β-thalassemia, 39.6 L/h.

Protein Binding 24.8–42.6% depending on dose administered.

Dose A usual dose of 25 mg/kg is administered three times a day, with a maximum of 100 mg/kg daily.

Klein J *et al.* (1991). A high-performance liquid chromatographic method for the measurement of the iron chelator 1,2-dimethyl-3-hydroxypyridin-4-one in human plasma. *Ther Drug Monit* 13: 51–54.

Kontoghiorghes GJ *et al.* (1990). Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. *Clin Pharmacol Ther* 48: 255–261.

Pope E *et al.* (1997). Salivary measurement of deferiprone concentrations and correlation with serum levels. *Ther Drug Monit* 19: 95–97.

Singh S *et al.* (1992). Urinary metabolic profiles in human and rat of 1,2-dimethyl- and 1,2-diethyl-substituted 3-hydroxypyridin-4-ones. *Drug Metab Dispos* 20(2): 256–261.

Deflazacort

Antiinflammatory

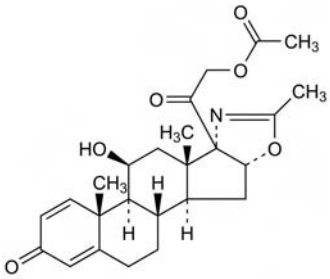
$C_{25}H_{31}NO_6$ = 441.5

CAS—14484-47-0

IUPAC Name (11β,16β)-21-(Acetyloxy)-11-hydroxy-2′-methyl-5′*H*-pregna-1,4-dieno[17,16-*d*]oxazole-3,20-dione

Synonyms L5458; DL-458-IT; MDL-458.

Proprietary Names *Azacort*; *Calcort*; *Deflan*; *Dezacor*; *Flantadin*; *Lantadin: Oxazacort*; *Zamane*.

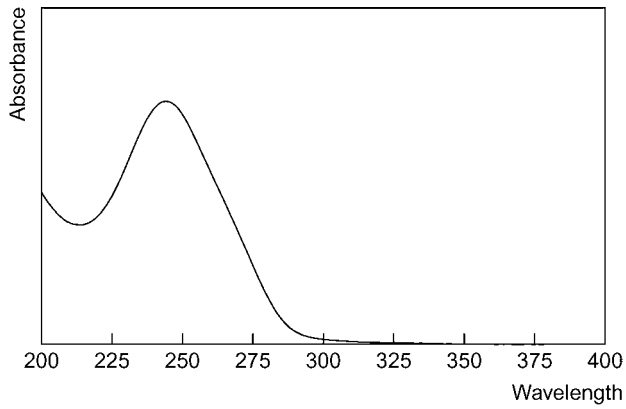


Chemical Properties White crystals (acetone:hexane). Mp 255° to 256.5°. Log *P* (octanol/water), 1.31.

High Performance Liquid Chromatography

Column: ODS (Hypersil, 250 × 4.6 mm i. d., 5 µm). Temperature 40°. Mobile phase: isopropanol : 0.05 mol/L acetate buffer (solvent A, pH 4.5) (10:90). Solvent A composition decreased from 90% to 30% for 25 min and held for 5 min, increased to 90% for 5 min and held for 15 min. Retention time: 21-hydroxydeflazacort, 6.1 min; deflazacort-6-β-hydroxide, 28.3 min [Hitara *et al.* 1994].

Ultraviolet Spectrum Methanol—242 nm.



Quantification

Plasma **HPLC** UV detection. Detection range, 1.0 to 500 µg/L for metabolite, 21-desacetyldeflazacort [Rao *et al.* 1996].

Urine **HPLC** Column: 5-ODS (Hypersil, 200 × 4.6 mm i.d., 5 µm at 30°, or Spherisorb, 150 × 4.6 mm i.d., 5 µm at 20°). Mobile phase: water : tetrahydrofuran (77:23) flow rate 1.0 mL/min (Hypersil) or (80:20) 1.2 mL/min flow rate (Spherisorb). UV detection (λ=245 nm). k values: deflazacort, 15.5 (Hypersil), 13.8 (Spherisorb); 21-hydroxydeflazacort, 4.6, 3.8. Limit of detection, 0.4 µg/L [Santos-Montes *et al.* 1994].

Disposition in the Body Deflazacort is well absorbed from the gastrointestinal tract and is immediately converted by plasma esterases to its active metabolite, 21-desacetyldeflazacort with further metabolism to deflazacort-6-β-hydroxide. Elimination is mainly via the kidneys, with 70% of the administrated dose recovered in the urine and the remaining 30% in faeces.

Therapeutic Concentration

Twenty-three healthy males, ~25 years old, were orally administered with 3, 6 and 36 mg doses of deflazacort (tablets and aqueous suspensions). Peak plasma concentrations of active metabolite, 21-desacetyldeflazacort, were 10.4 ± 5.0, 19.8 ± 7.5, 132.6 ± 52.5 µg/L, respectively, and were reached within 1.5 to 2 h [Rao *et al.* 1996].

Bioavailability Approximately 68%.

Half-life 1.9 to 2.3 h.

Volume of Distribution Deflazacort-6-β-hydroxide, 83 L.

Clearance 83.9 ± 24.5 (36 mg dose) to 97.1 ± 47.7 L/h (3 mg dose).

Protein Binding 40%

Note For a review of pharmacokinetics data see Rao *et al.* [1996].

Dose By oral administration, initial dose up to 120 mg/kg body weight and a usual maintenance dose of 3 to 18 mg/kg. Between 0.25 and 1.5 mg/kg body weight for children.

Hirata H *et al.* (1994). Simultaneous determination of deflazacort metabolites II and III, cortisol, cortisone, prednisolone and prednisone in human serum by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 658(1): 55–61.

Rao N *et al.* (1996). An investigation of the dose proportionality of deflazacort pharmacokinetics. *Biopharm Drug Dispos* 17: 753–760.

Santos-Montes A *et al.* (1994). Extraction and high-performance liquid chromatographic separation of deflazacort and its metabolite 21-hydroxydeflazacort. Application to urine samples. *J Chromatogr B Biomed Appl* 657: 248–253.

Dehydrochloromethyltestosterone

Anabolic Steroid

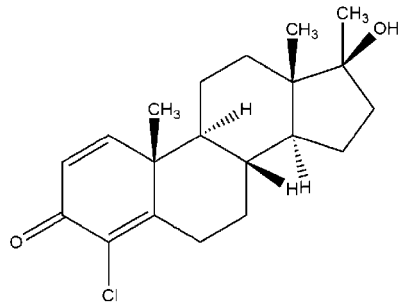
$C_{20}H_{27}O_2Cl$ = 334.9

CAS—2446-23-3

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-4-Chloro-17-hydroxy-10,13,17-trimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-3-one

Synonyms Chlorodehydromethyltestosterone; 4-chloro-17β-hydroxy-17α-methylandrost-1,4-dien-3-one; turinabol.

Proprietary Name *Oral-Turinabol*



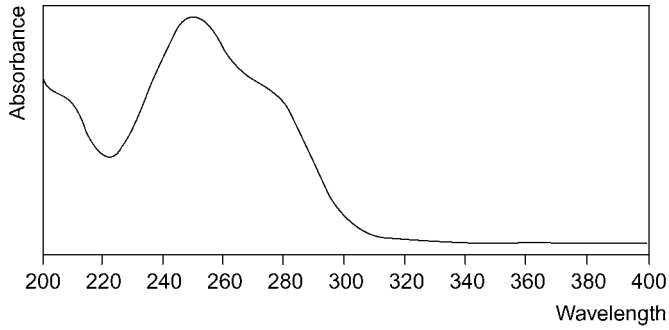
Note Prohormone of the anabolic steroid boldenone.

Gas Chromatography Column: HP-5 5% phenylmethylsilicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 175° for 1 min to 280° at 15°/min. FID. Limit of detection not reported [Wisniewski, Hays 2006].

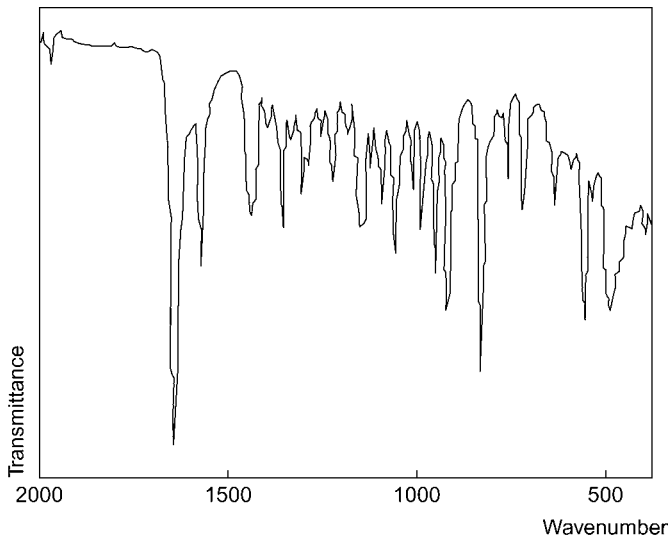
Gas Chromatography-Mass Spectrometry Column: HP-5 5% phenylmethylsilicone (15 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 0.5 min to 300° at 30°/min for 1 min. EI ionisation, scanning acquisition mode. Limit of detection not reported [Wisniewski, Hays 2006]

High Performance Liquid Chromatography Column: Waters Xterra RP18 (150 × 4.6 mm i.d., 3.5 µm). Mobile phase: water : acetonitrile (80 : 20 for 3 min to 55 : 45 at 5 min for 8 min to 35 : 65 at 16 min for 10 min to 10 : 90 at 31 min for 9 min). UV detection (λ = 225 nm). Limit of detection not reported [Wisniewski, Hays 2006]

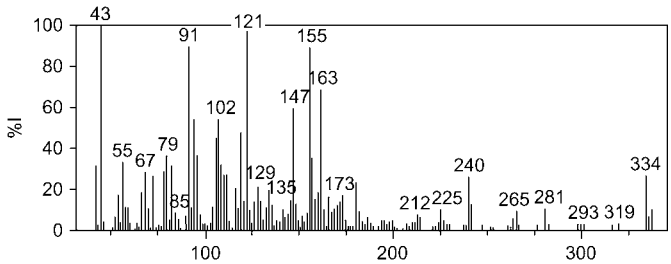
Ultraviolet Spectrum Peak at 250 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 240, 478, 143, 455, 373, 333, 263 (di-TMS derivative).



Quantification

Urine **GC-MS** Column 1: HP5 fused silica capillary cross-linked 5% phenylmethylsilicone SE54 (17 m × 0.2 mm i.d., 0.3 µm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. Column 2: Fused silica capillary column cross-linked methyl silicone OV1 (20 m × 0.25 mm i.d., 0.33 µm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. EI ionisation at 70 eV. Limit of detection not reported [Schanzer *et al.* 1992]. Column: Carlo Erba Model 2900 capillary SE-54 (30 m × 0.32 mm i.d.) Carrier gas: He, 3.0 mL/min. Temperature programme: 100° for 10 min to 200° at 25°/min to 280° at 2.5°/min. EI ionisation at 25 eV. Limit of detection, 1 µg/L [Durbeck *et al.* 1983].

HPLC Column: Merck Lichrosorb (R) RP18 (100 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (30 : 70 to 80 : 20 after 20 min), flow rate 1.0 mL/min. Limit of detection not reported [Schanzer *et al.* 1992]

LC-MS Column: LiChroCART Purospher RP C_{18} (125 × 3 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate–0.01% acetic acid : 90% methanol (50 : 50 to 0 : 100 at 15 min for 3 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.4 µg/L for the metabolite, 6β-hydroxy-4-chloro-dehydromethyltestosterone [Leinonen *et al.* 2004]

Disposition in the Body Urinary metabolites include the epimer 6β-hydroxy-, 16β-dihydroxy-, 6,12-dihydroxy-, and the 6β,16β-dihydroxy metabolites.

Therapeutic Concentration

A healthy male volunteer (32 years old, 62 kg) was administered 20 mg dehydrochloromethyltestosterone orally. No parent compound was detected in urine over 72 h after dosage. However, the 17β-methyl epimer was detected and the total excretion reported was 0.18% of the applied dose [Schanzer *et al.* 1992].

A single dose of 25 mg oral turinabol was administered to male adults (26 to 48 years). Urine samples were collected every 2 h after administration for at least a week. The main urinary metabolites included 6β-hydroxyturinabol, 16β-hydroxyturinabol, and 6β,16β-dihydroxyturinabol, making up about 15% of the administered dose. The fate of the remaining 85% was unknown but was assumed to be partly stored in fat or muscular tissues since, under certain stress situations, it may be remobilised and excreted even after a long period of time (6 to 12 months) [Durbeck *et al.* 1983].

Toxicity

A 32-year-old man underwent radical orchiectomy for a tumour on the right testicle. Upon histological examination the tumour was identified as an intratesticular leiomyosarcoma. The patient reported a 5-year history of the systematic use of high-dose dehydrochloromethyltestosterone (Oral-Turinabol) that began when the patient was 18 years of age and ceased 9 years before presentation [Froehner *et al.* 1999].

Dose 40 or 60 mg per day, taken in divided doses.

Durbeck HW *et al.* (1983). GC and capillary column GC/MS determination of synthetic anabolic steroids. II. 4-chloro-methandienone (oral turinabol) and its metabolites. *J Chromatogr Sci* 21: 405–410.

Froehner M *et al.* (1999). Intratesticular leiomyosarcoma in a young man after high dose doping with oral-turinabol: a case report. *Cancer* 86: 1571–1575.

Leinonen A *et al.* (2004). Screening of free 17-alkyl-substituted anabolic steroids in human urine by liquid chromatography-electrospray ionization tandem mass spectrometry. *Steroids* 69: 101–109.

Schanzer W *et al.* (1992). 17-Epimerization of 17alpha-methyl anabolic steroids in humans: metabolism and synthesis of 17alpha-hydroxy-17beta-methyl steroids. *Steroids* 57: 537–550.

Wisniewski ES, Hays PA (2006). Dehydrochlormethyltestosterone: an analytical profile. *Microgram Bull* 3987.

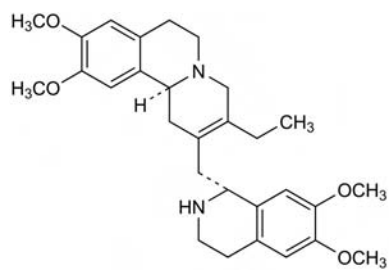
Dehydroemetine

Antiprotozoal

$C_{29}H_{38}N_2O_4$ = 478.6

CAS—4914-30-1

IUPAC Name 2,3-Didehydro-6′,7′,10,11-tetramethoxyemetan



Chemical Properties Log *P* (octanol/water), 5.2.

Dehydroemetine Hydrochloride

$C_{29}H_{38}N_2O_4$,2HCl = 551.6

CAS—2228-39-9

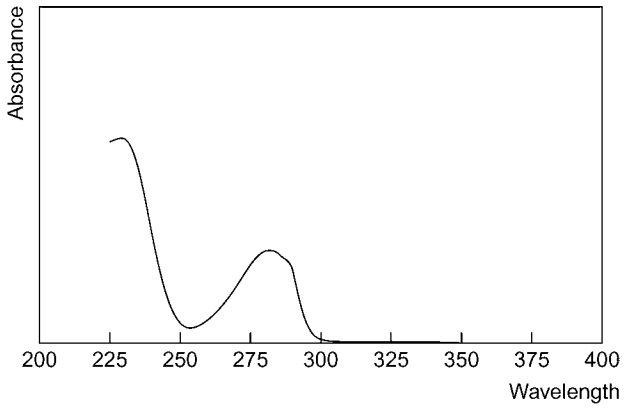
Proprietary Name *Dametine*

Chemical Properties A white crystalline powder. Soluble 1 in 30 of water.

Colour Test Marquis test—orange.

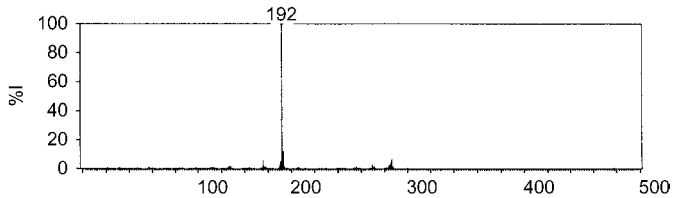
Thin-layer Chromatography System TA—R_f 0.43; system TB—R_f 0.06; system TC—R_f 0.21; system TL—R_f 0.02 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—230 (A^{1}_{1}=290a), 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 1121, 1222, 1508, 1252, 1205, 1022 cm^{-1} (dehydroemetine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 192, 193, 287, 176, 191, 286, 270, 285.



Dose 60 to 180 mg of dehydroemetine hydrochloride daily, IM.

Dehydroepiandrosterone

Endogenous Steroid

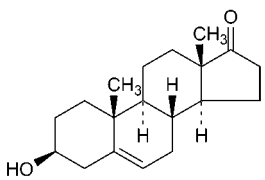
$C_{19}H_{28}O_2$ = 288.4

CAS—53-43-0

IUPAC Name 3β-3-Hydroxyandrost-5-en-17-one

Synonyms Dehydroisoandrosterone; DHEA; prasterone.

Proprietary Names *Astenile*; *Deandros*; *Diandrone*; *Psicosterone*.



Chemical Properties Dimorphous needles with Mp 140° to 141° or leaflets with Mp 152° to 153°. Soluble in water (solubility is 63.45 mg/L at 25°), benzene, alcohol, ether; sparingly soluble in chloroform and petroleum ether. Log *P* (octanol/water) 3.23 [Hansch *et al.* 1995]. Stable in saliva after 3 freeze-thaw cycles and for at least 6 months at –20° [Higashi *et al.* 2007b].

Dehydroepiandrosterone Sodium Sulfate

$C_{19}H_{27}NaO_5S$ = 390.5

CAS—1099-87-2

Synonyms DHA-S sodium; DHEAS; prasterone sodium sulfate; sodium dehydroepiandrosterone sulfate.

Proprietary Name *Mylis*

Chemical Properties White crystalline powder. Mp 154°. Soluble in methanol; slightly soluble in water and absolute ethanol; practically insoluble in acetone, chloroform and benzene. Stable in plasma when incubated with lithium heparin or EDTA for 16 h at 37° [Jones, James 1985].

Dehydroepiandrosterone Sodium Sulfate Dihydrate

$C_{19}H_{27}NaO_5S,2H_2O$ = 426.6

Dehydroepiandrosterone Sulfate

$C_{19}H_{28}O_5S$ = 368.5

CAS—651-48-9

Synonyms DHA-S; dehydroepiandrosterone sulfate; prasterone sulfate.

Dehydroepiandrosterone Enanthate

$C_{26}H_{40}O_3$ = 400.6

CAS—23983-43-9

Synonyms Dehydroepiandrosterone heptanoate; prasterone enanthate; prasterone heptanoate.

Thin-layer Chromatography Plate: silica gel G-Merck (20 × 20 cm). Mobile phase: hexane : ether : acetic acid (1 : 9 : 0.1). Developed with colour developing reagents. R_f 0.60 [Frye *et al.* 2000].

Gas Chromatography System GA—DHEA RI 2530, DHEA-H_2O RI 2595, DHEA enol-TMS_2 RI 2580; system GAR—DHEA RT 11.8 min.

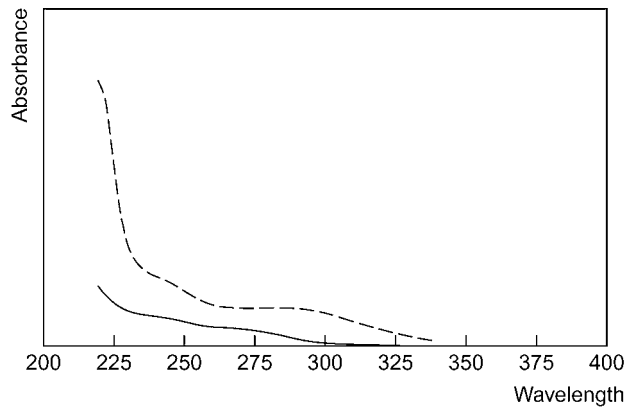
Column: 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 150° for 1 min to 295° at 30°/min for 8 min. Carrier gas: He, 1.0 mL/min. EI ionisation. Retention time: dehydroepiandrosterone, 7.04 min; testosterone, 7.38 min [Kintz *et al.* 1999]

Gas Chromatography-Mass Spectrometry Column: HP1 methyl silicone (0.2 mm, 0.33 µm). Temperature: 280°. Carrier gas: He, 0.9 mL/min. RI: 2588 [Mills, Roberson 1993].

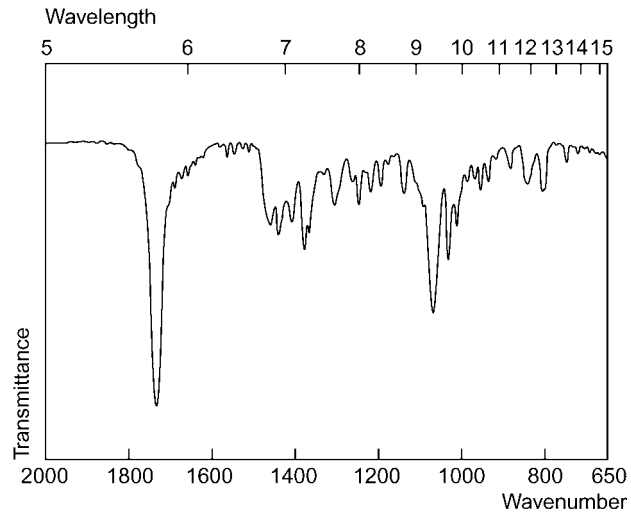
High Performance Liquid Chromatography System HATb—RRT 1.13 (relative to testosterone).

Column: ODS Hypersil (100 × 4.6 mm, 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer (pH 3.5; 90 : 10), 1 mL/min flow rate. DAD. Retention time: 2.4 min. [Mills, Roberson 1993].

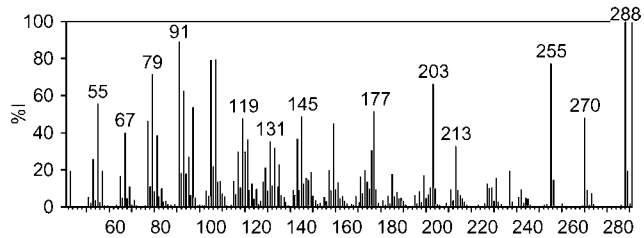
Ultraviolet Spectrum



Infrared Spectrum Principal peak at wavenumbers 1725, 1372, 1062 cm^{-1} (KBr pellets).



Mass Spectrum Principal ions at *m/z* 288, 91, 107, 105, 255, 203, 93, 55.



Quantification

Plasma **GC-MS** Column: 5% phenylmethylsilicone (30 m × 4.6 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 120° for 3 min to 310° at 40°/min to 120° at 10°/min. EI ionisation at 70 eV, full scan or SIM acquisition mode. Limit of quantification, 2 nmol/L, limit of detection, 0.8 nmol/L [Diallo *et al.* 2004]. Column: Phenomenex ZEBRON ZB-50 (15 m × 0.25 mm i.d., 0.15 µm). Carrier gas:

He. Temperature programme: 180° for 3.25 min to 270° at 40°/min to 280° at 5°/min to 310° at 40°/min for 2.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Hert *et al.* 2004]. Column: OV-1 fused silica (25 m × 0.15 mm i.d., 0.1 µm). Carrier gas: He, 1.8 bar. Temperature programme: 50° for 6 min to 230° at 30°/min for 2 min to 290° at 3°/min. EI ionisaton at 70 eV, MSD, SIM acquisition mode. Limit of detection, 10 pg on column [Wudy *et al.* 1992]. FAB, negative ion mode. Limit of detection, 500 µg/L for DHEA-S [Gaskell *et al.* 1983].

HPLC Column: Zorbax C_{18} (150 × 4.6 mm i.d., 3.5 µm). Mobile phase: 5.0 mmol/L tetrabutylammonium dihydrogenphosphate (pH 3.4) : acetonitrile : methanol (60 : 10 : 30 to 55 : 10 : 35 at 15 min to 15 : 10 : 75 at 17 min for 1 min to 60 : 10 : 30 at 20 min), flow rate 1.0 mL/min. UV detection (λ = 240 nm). Limit of quantification 10 µg/L, limit of detection, 3 µg/L [Marwah *et al.* 1999].

LC-MS Column: Shodex MS Pak PK-2A (10 × 2.0 mm i.d.) followed by Capcell Pak C_{18} UG 120 (150 × 1.5 mm i.d., 5 µm). Mobile phase: 0.1% triethylamine (pH 6.4) : 0.1% triethylamine in 90% acetonitrile (100 : 0 for 10 min to 70 : 30 at 10.1 min to 50 : 50 at 15 min to 20 : 80 at 20 min for 2 min to 70 : 30 at 25 min for 5 min), flow rate 100 µL/min. ESI, negative ion mode. Limit of detection, 5 nmol/L for DHEA-S [Cho *et al.* 2006].

Serum **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 180° to 235° at 10°/min to 310° at 6°/min for 4.15 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 12.65 min. Limit of quantification, 0.1 µg/L, limit of detection, 0.09 µg/L [Magnisali *et al.* 2008]. Column: Zebron ZB-50 (15 m × 0.25 mm i.d., 0.15 µm). Temperature programme: 120° for 1 min to 220° at 40°/min to 240° at 2.9°/min to 310° at 40°/min for 2 min. SIM acquisition mode. Retention time: 7.1 min. Limit of detection, 2.7 pg [Hill *et al.* 2005].

HPLC Column: Wakosil 5C4 (300 × 4.0 mm i.d.). Mobile phase: acetonitrile : water (70 : 30), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 495 nm, λ_{em} = 516 nm). Retention time: 7.69 min. Limit of detection, 1.2 pg/L [Katayama *et al.* 1998].

LC-MS Column: Develosil ODS-HG-5 (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium formate (30 : 70), flow rate 0.15 mL/min. ESI, negative ion mode. Limit of quantification, 0.05 mg/L for DHEA-S [Mitamura *et al.* 2003]. Column: Econosphere C_{18} (150 × 4 mm i.d., 5 µm). Mobile phase: methanol : 0.2 mol/L ammonium acetate (60 : 40). TSI, negative ion mode, SIM acquisition mode. Limit of detection, 670 µg/L [Shackleton *et al.* 1990].

Urine **GC-MS** Column: HP-1 fused silica cross-linked methylsilicone (17 m × 0.2 mm i.d., 0.11 mm). Carrier gas: He, 40 cm/s. Temperature programme: 180° for 0.3 min to 231° at 3/min to 310° at 30°/min for 1.07 min. Retention time: ≈13 min. Limit of detection not reported [Uralets, Gillette 2000]. Column: HP-1 methylsilicobe fused silica (17 or 25 m × 0.22 mm, 0.11 µm). Temperature programme: 170° to 230° at 3.0°/min to 300° at 30°/min for 3.67 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Dehennin *et al.* 1998].

LC-MS Column: XBridge C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 1% acetic acid : acetonitrile (74 : 26 to 70.5 : 29.5 in 14 min for 16 min., flow rate 1.0 mL/min. ESI, negative ion mode, full scan mode. Limit of detection, 5.0 µg/L [Strahm *et al.* 2008].

Oral Fluid **GC-MS** Column: 1% OV-1 on Gas Chrom Q 100/120 (3 m × 3 mm i.d.) or OV-1 glass (25 m × 0.25 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 250°. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.9 nmol/L for DHEA-S [Finlay *et al.* 1982].

LC-MS Column: J'sphere ODS H-80 (150 × 2.0 mm i.d., 4 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (1 : 1), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 25 ng/L, 100 ng/L for DHEA-S, limit of detection, 13 ng/L, [Higashi *et al.* 2007a; Higashi *et al.* 2007b].

Hair **GC-MS** Column: HP-5MS 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 295° at 30°/min for 8 min. EI ionisation. Limit of detection, 0.5 ng/g [Kintz *et al.* 1999]. Column: cross-linked methylsilicone (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 121 kPa. Temperature programme: 220° for 2 min to 240° at 4°/min for 5 min to 310° at 15°/min for 3.33 min. EI ionisation at 70 eV, MSD, scan and SIM acquisition mode. Relative retention time: 0.69. Limit of detection, 0.2 ng/g [Choi, Chung 1999].

Other **GC-MS** Microsomal Fractions. Column: DB-17MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 14 psig. Temperature programme: 100° for 0.5 min to 325° at 30°/min to 325° at 2°/min for 5 min. EI ionisation at 70 eV, full mass scan. Limit of detection not reported [Miller *et al.* 2004].

Note For a review of FAB-MS for the measurement of steroids, see [Gaskell 1990].

Disposition in the Body DHEA and DHEA-S are widely distributed throughout the body. DHEA is metabolised to androsterone and etiocholanolone, the most abundant metabolites found in urine after a single oral dose. Other metabolites include androstenedione, a product from 3β-hydrosteroid dehydrogenase (HD), and testosterone (17β-HD). Men and women metabolise DHEA and DHEA-S differently, which may result in varying concentrations of other androgenic and estrogenic steroids being detected in the body.

Therapeutic Concentration

Six healthy men and 7 women were administered with a single and multiple doses of 200 mg dehydroepiandrosterone. Men: peak plasma concentrations of 22.4 ± 1.6 µg/L DHEA, 7015 ± 625 µg/L DHEA-S were reached within 1.3 ± 0.6 h and 3.3 ± 1.8 h, respectively. Women: peak plasma concentrations of 27.2 ± 2.9 µg/L DHEA and 7467 ± 645 µg/L DHEA-S were seen 1.4 ± 0.7 and 2.1 ± 0.7 h after administration. After multiple dosing, peak plasma concentrations and time to peak remained relatively constant [Frye *et al.* 2000].

Toxicity Dehydroepiandrosterone sodium sulfate and its dihydrate have low toxicity at 4 mg/kg with gastrointestinal and skin effects, for example, rashes and haemorrhages. It is moderately toxic by IP and SC routes, and can be a poison by the IV route.

Half-life DHEA: (men) 6 to 7.5 h, (women) 7 to 11.5 h; DHEA-S: (men and women) 20 to 27 h.

Dose In general, 25 to 50 mg daily is recommended as a supplementation dose for a normal population, but body builders are recommended up to 1000 mg daily by some suppliers.

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Delapril

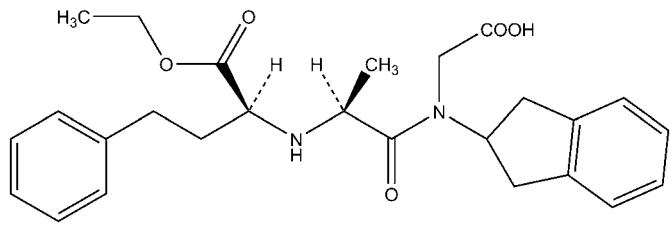
ACE Inhibitor, Antihypertensive

$C_{26}H_{32}N_2O_5$ = 452.5

CAS—83435-66-9

IUPAC Name 2-[2,3-Dihydro-1*H*-inden-2-yl-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]amino]acetic acid

Synonyms Alindapril; *N*-[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine; *N*-[*N*-[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-*N*-(indan-2-yl)glycine; ethyl (*S*)-2-[[(*S*)-1-[(carboxymethyl)-2-indanylcarbamoyl]ethyl]amino]-4-phenylbutyrate; indalapril.



Delapril Hydrochloride

$C_{26}H_{32}N_2O_5$,HCl = 489.0
CAS—83435-67-0
Synonyms CV-3317; REV-6000A.
Proprietary Names *Adecut*; *Beniod*; *Cupressin*; *Delacard*; *Delaket*; Delakete; *Trinordiol*.
Chemical Properties Colourless plates. Mp 166° to 170°. Log *P* (octanol/water), 4.77 [ACD 2007]. Stable in human plasma at room temperature for at least 4 h and for at least 3 months frozen at -15° [Ito *et al.* 1985].

Quantification

Serum **HPLC** Column: LS-410 octadecyl bonded silica (300 × 4 mm i.d., 10 µm). Mobile phase: 0.1 mol/L potassium dihydrogen phosphate : acetonitrile (55 : 45), flow rate 0.7 mL/min. UV detection (λ = 210 nm). Limit of quantification, 10 µg/L [Ito *et al.* 1985].
Urine **HPLC** See Serum. Limit of quantification, 50 µg/L [Ito *et al.* 1985].
Disposition in the Body Delapril is converted to an active diacid derivative (M1), which, in turn, is converted to an active 5-hydroxyindane diacid (M3). The diketopiperazine metabolite (M2) is inactive. Urinary excretion of delapril and its metabolites is 55.7% of the administered dose, mainly as M1 (21.4%) and M3 (30.4%). M1 is more lipophilic than the other ACE inhibitors, with a log *P* (octanol/water), of 1.97. After the single oral administration of increasing doses of delapril (3 to 60 mg) to healthy volunteers, it is rapidly absorbed. There was a correlation between the dose and the levels of M1. Urinary excretion of delapril and its metabolites is 55.7% of the administered dose, mainly as M1 (21.4%) and M3 (30.4%). Food intake has little effect on the absorption of the drug [Razzetti, Acerbi 1995].

Therapeutic Concentration

Eighteen healthy male volunteers (age 21 to 45 years; weight 57 to 87 kg) and 16 healthy elderly subjects (age 65 to 73 years; weight, 51 to 97 kg) were administered 30 mg delapril or 10 mg manidipine in combination or a fixed combination tablet of the same doses. Mean maximum plasma concentrations of delapril, M1, M2 and M3 were 194 ± 70, 382 ± 149, 132 ± 23 and 171 ± 40 µg/L attained at 1.0, 1.75, 2.0 and 2.0 h, respectively, after the extemporary combination. Mean maximum plasma concentrations of delapril, M1, M2, and M3 were 170 ± 68, 365 ± 116, 138 ± 31 and 173 ± 44 µg/L at 1.0, 1.5, 1.75 and 2.0 h, respectively, after the fixed combination. Mean peak plasma concentrations in young and elderly subjects after single and repeated doses were as follows:

	Single dose		*Repeated dose*	
	Young	*Elderly*	*Young*	*Elderly*
Delapril				
C_{max} (µg/L)	173 ± 69	138 ± 81	199 ± 85	169 ± 101
t_{max} (h)	1.0	1.5	1.0	1.0
M1				
C_{max} (µg/L)	357 ± 127	387 ± 140	365 ± 170	452 ± 202
t_{max} (h)	1.5	2.0	1.25	2.0

[Stockis *et al.* 2003]

Six healthy volunteers were administered 60 mg delapril. High plasma levels of M1 were observed followed by M3. Levels of M2 and unchanged delapril were low. The mean maximum plasma concentrations of M1 and M3 were 1.2 and 0.43 mg/L at 1.39 and 1.67 h, respectively [Razzetti, Acerbi 1995].

Sixteen healthy male volunteers (mean age, 26.3 years; mean weight, 71.3 kg) were administered 30 mg delapril and 2.5 mg indapamide once or once daily for 7 days. Mean maximum plasma concentrations of M1, M2 and M3 were 895, 142 and 259 µg/L, attained at 1, 1.41 and 1.47 h, respectively, after the single dose and 1073, 166 and 242 µg/L, attained at 0.9, 1.27 and 1.25 h, respectively, after multiple doses [Hutt *et al.* 1994].

Food and renal impairment have been shown to have no effect on the pharmacokinetics of the prodrug, but renal impairment influences the plasma profiles of the active metabolites. The plasma concentrations and area under curve values of M1 and M3 are higher in patients with renal impairment than in healthy volunteers [Minamisawa *et al.* 1990; Onoyama *et al.* 1988; Singlas, Fillastre 1991].

The repeated administration of 30 mg delapril for 7 days to 9 elderly patients with essential hypertension resulted in increased plasma concentrations and AUCs compared with healthy adults, although this could have been caused by age-related decreases in renal and hepatic function. Mean peak plasma concentrations for delapril, M1 and M3 were 489 ± 139, 635 ± 56 and 229 ± 21 ng/mL attained at 1.1 ± 0.1, 1.2 ± 0.1 and 1.9 ± 0.1 h, respectively [Shionoiri *et al.* 1987].

Note For an update and review of the pharmacokinetics of ACE inhibitors, see Song and White [2002].
Half-life 0.3, 1.21 and 1.4 h for delapril, M1 and M3, respectively [Kelly, O'Malley 1990].

Dose 30 to 60 mg daily in 2 separate doses.

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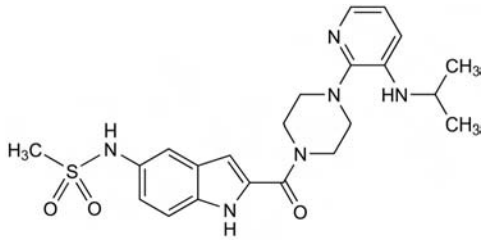
Delavirdine

Antiviral
$C_{22}H_{28}N_6O_3S$ = 456.6
CAS—136817-59-9
IUPAC Name 1-[3-[(1-Methylethyl)amino]-2-pyridinyl]-4-[[5-[(methylsulfonyl)amino]-1*H*-indol-2-yl]carbonyl]piperazine
Synonym U-90152



Chemical Properties Crystals from ethyl acetate and hexane. Mp 226° to 228°. pK_a 4.5.

Delavirdine Monomethanesulfonate

$C_{22}H_{28}N_6O_3S.CH_4O_3S$ = 552.7
CAS—147221-93-0
Synonyms Delavirdine mesylate; U-90152S.
Proprietary Name *Rescriptor*

High Performance Liquid Chromatography System HAE—retention time 6.30 min.

Column: cyano (DuPont Zorbax SB CN). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 6) : acetonitrile (67:33). Fluorescence detection (λ_{ex}=295 nm, λ_{em}=418 nm). Retention time: delavirdine, 7.9 min; desalkyl delavirdine, 2.7 min [Borin *et al.* 1997].

Quantification

Plasma **HPLC** UV detection (λ=260 nm). Limit of quantification, 75 µg/L [Proust *et al.* 2000]. Column: Zorbax SB C_{18} (75 × 4.6 mm, 3.5 µm). Mobile phase: 25 mmol/L citrate buffer : acetonitrile (82:18), (pH 2.7). flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}=300 nm, λ_{em}=425 nm). Retention time: 19.2 min. Limit of quantification, 50 µg/L. Limit of detection, 10 µg/L [Veldkamp *et al.* 1999].
Serum **HPLC** UV detection. Limit of detection, 110 µg/L [Simon *et al.* 2001].
Disposition in the Body Delavirdine is rapidly absorbed after oral administration and is extensively metabolised in the liver by the cytochrome P450 3A isoenzymes. Absorption is at least 45%. It is excreted as the metabolites in urine and faeces with <5% as the unchanged drug in urine.

Therapeutic Concentration

Thirteen HIV (human immunodeficiency virus)-positive volunteers, with a mean CD4 count of 260 cells/mm^3 (range 192 to 402 cells/mm^3) and mean age of 39 years (range, 24 to 50 years) were administered 300 mg delavirdine mesylate orally every 8 h for 30 days, 2 h after or 1 h before meals. Steady state concentrations were observed by day 11 and averaged 19 µg/L for

delavirdine and 2.6 µg/L for desalkyl delavirdine. For the parent drug, peak plasma concentrations reached 31 (22 to 44) µg/L on day 15, 26 (20 to 36) µg/L on day 16 and 28 (18 to 40) µg/L on day 30. These concentrations were observed at 1.0 (1.0 to 1.5), 2.0 (0.75 to 2.5) and 0.75 to 1.0 h, respectively. For desalkyl delavirdine, peak concentrations were 3.2 (2.9 to 4.2), 2.8 (2.3 to 4.0) and 2.8 (2.5 to 4.5) µg/L for days 15, 16 and 30 observed at approx. 2.5 h (0.75 to 6.0 h) [Borin *et al.* 1997].

Half-life 2 to 11 h.

Protein Binding 98%.

Dose The usual dose is 400 mg three times daily.

Borin MT *et al.* (1997). Effect of fluconazole on the steady-state pharmacokinetics of delavirdine in human immunodeficiency virus-positive patients. *Antimicrob Agents Chemother* 41(9): 1892–1897.

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Deltamethrin

Ectoparasiticide, Insecticide

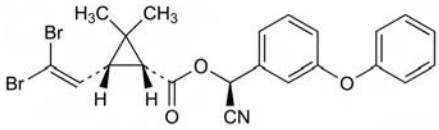
$C_{22}H_{19}Br_2NO_3 = 505.2$

CAS—52918-63-5

IUPAC Name *(S)*-α-Cyano-3-phenoxybenzyl-(1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

Synonyms Decamethrin; deltamethrine; esbecythrin; FMC-45498; NRDC-161; OMS 1998; RU-22974.

Proprietary Names *Butofin*; *Butoss*; *Butox*; *Cislin*; *Crackdown*; *Cresus*; *Decis*; *Decis-Prime*; *Delsekte*; *Hunter*; *K-Othrin*; *K-Otek*.



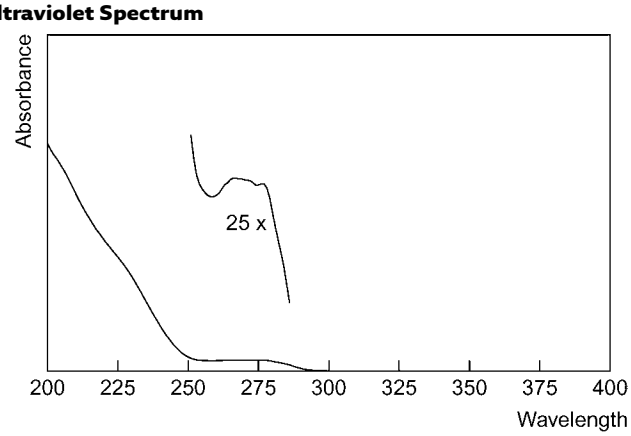
Chemical Properties A colourless, crystalline powder or white/slightly beige powder. Mp 98° to 101°. It is soluble in kerosene and isoalkanes (<0.5 g/100 g at 20°), isopropanol (<0.6 g/100 g at 20°), ethanol (1.5 g/100 g at 20°), xylene (25 g/100 g at 20°), methylene chloride (70 g/100 g at 20°), toluene (250 g/L at room temperature), acetone (500 g/L at room temperature), benzene and dimethyl sulfoxide (450 g/L at room temperature), cyclohexane (750 g/L at room temperature), and dioxane (900 g/L at room temperature); practically insoluble in water (2 µg/L at 20°). Log *P* (octanol/water), 4.6 (25°).

Thin-layer Chromatography System TX—R_f 0.40; system TY—R_f 0.90; system TZ—R_f 0.95; system TAA—R_f 0.72.

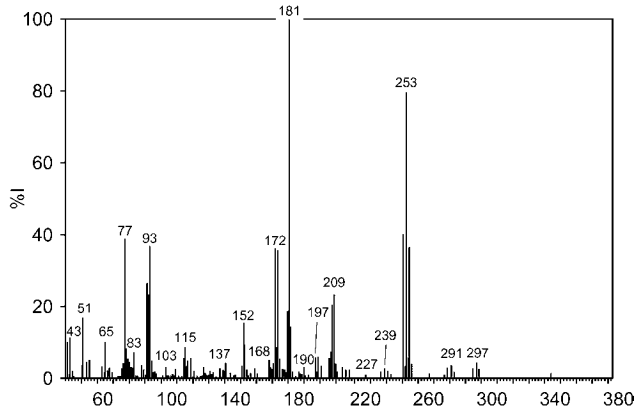
Plate: silica gel G (0.25 mm). Mobile phase: cyclohexane:toluene (6:4). Developed by spraying with 20% sodium hydroxide solution, 1% copper (II) acetate solution, and 1% phosphomolybdic acid solution, and 0.1% o-toluidine reagent 5 min later. Rf 0.31 [Patil *et al.* 1992].

Gas Chromatography System GA—deltamethrin RI 2900, M (COOH-)-Me RI 1540, M (desacyl-)-HCN RI 1700, M (desacyl-)-Me RI 2590; system GKD—retention time 5.8 min.

Ultraviolet Spectrum



Mass Spectrum



Quantification

Plasma **GC** Limit of detection, 2 mg/L [Junting, Chuichang 1991].

Urine **GC** Limit of detection, 200 mg/L [Wu *et al.* 1994] and see Plasma.

GC-MS Limit of detection for the metabolites, 0.3 to 0.5 µg/L [Angerer, Ritter 1997]. Limit of detection, 0.2 µg/L [Kuhn *et al.* 1996].

Disposition in the Body Deltamethrin is hydrolysed by liver microsomal enzymes to three metabolites: 3-(2,2-dibromovinyl)-2,2-cyclopropane carboxylic acid, 3-phenoxy-benzylhydroxy-ethyl acetate, and 3-phenoxylbenzoic acid. All three metabolites have been detected in urine.

Toxicity There have been many cases of dermal deltamethrin poisoning due to inadequate handling of the chemical during agricultural use. Cases of accidental and suicidal poisoning have also been observed with oral doses between 2 and 250 mg/kg. A dose of 100 to 250 mg/kg can result in coma, even though short-lived (15 to 20 min). The allowed daily intake is 0.01 mg/kg.

Angerer J, Ritter A (1997). Determination of metabolites of pyrethroids in human urine using solid-phase extraction and gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 695: 217–226.

Junting L, Chuichang F (1991). Solid phase extraction method for rapid isolation and clean-up of some synthetic pyrethroid insecticides from human urine and plasma. *Forensic Sci Int* 51: 89–93.

Kuhn KH *et al.* (1996). *Chromatographia* 43(5/6)285.

Patil VB *et al.* (1992). Thin-layer chromatographic detection of pyrethroid insecticides containing a nitrile group. *Analyst* 117(1): 75–76.

Wu YQ *et al.* (1994). Determination of pyrethroids in human urine by gas chromatography. *Biomed Env Sci* 7(3): 216–221.

Demecarium Bromide

Anticholinesterase

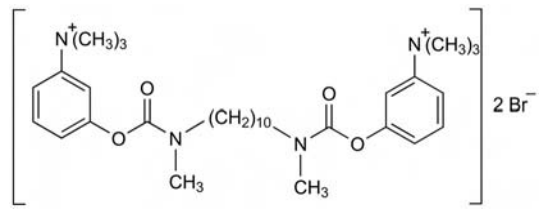
$C_{32}H_{52}Br_2N_4O_4 = 716.6$

CAS—56-94-0

IUPAC Name Trimethyl-[3-[methyl-[10-[methyl-[3-(trimethylazaniumyl)phenoxy] carbonylamino]decyl]carbamoyl]oxyphenyl]azanium dibromide

Synonyms BC-4; 3,3′-[1,10-decanediylbis[(methylimino)carbonyloxy]]bis[*N,N,N*-trimethylbenzeneaminium]dibromide.

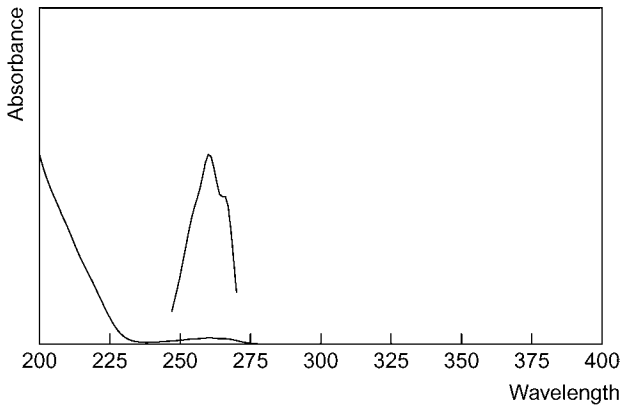
Proprietary Names *Humorsol*; *Tosmilen(e)*.



Chemical Properties A white or slightly yellow, slightly hygroscopic, crystalline powder. Mp 162° to 167°, with decomposition. Freely soluble in water and ethanol; sparingly soluble in acetone; insoluble in ether. Log *P* (octanol/water), −1.8.

Thin-layer Chromatography System TA—R_f 0.00 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—259 (A^{1}_{1}=14b), 265 nm. No alkaline shift.

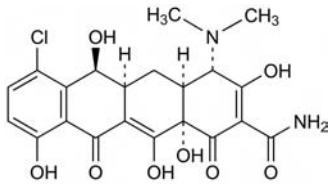


Infrared Spectrum Principal peaks at wavenumbers 1724, 1216, 1149, 1178, 1124, 943 cm^{-1} (KBr disk).
Use As a 0.25 to 0.5% ophthalmic solution.

Demeclocycline

Antibacterial
$C_{21}H_{21}ClN_2O_8$ = 464.9
CAS—127-33-3

IUPAC Name (2*Z*,4*S*,4a*S*,5a*S*,6*S*, 12a*S*)-2-Amino(hydroxy)methylidene]-7-chloro-4-(dimethylamino)-6,10,11,12*a*-tetrahydroxy-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-1,3,12-trione
Synonyms [4*S*-(4α,4aα,5aα,6β,12aα)]-7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide; demethylchlortetracycline; DMCT; RP-10192.
Proprietary Name *Ledermycin* (drops and syrup)

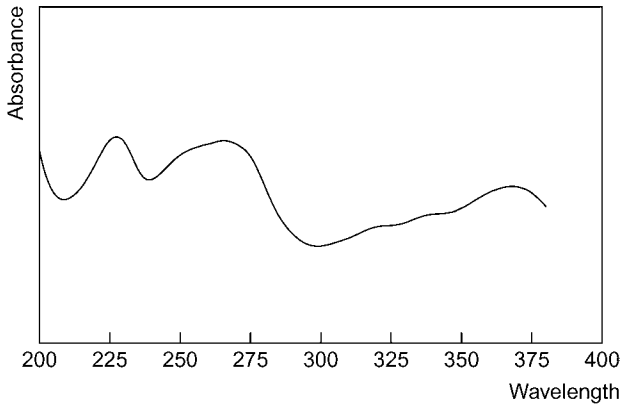


Chemical Properties A yellow crystalline powder. Sparingly soluble in water; soluble 1 in 200 of ethanol and 1 in 40 of methanol; soluble in dilute hydrochloric acid and in solutions of alkali hydroxides and carbonates. pK_a 3.3, 7.2, 9.3 (25°). Log *P* (octanol/pH 7.5), −1.3.

Demeclocycline Hydrochloride

$C_{21}H_{21}ClN_2O_8$,HCl = 501.3
CAS—64-73-3

Proprietary Names *Declomycin*; *Ledermycin(e)* (capsules and tablets). It is an ingredient of *Declostatin* and *Deteclo*.
Chemical Properties A yellow, amphoteric, crystalline powder. Soluble 1 in 30 to 1 in 60 of water, 1 in 45 to 1 in 200 of ethanol and 1 in 50 of methanol; practically insoluble in chloroform and ether; soluble in aqueous solutions of alkali hydroxides and carbonates.
Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—orange→brown-red; Mandelin's test—violet-brown; Marquis test—yellow→green; Nessler's reagent—brown; sulfuric acid—brown-blue.
Thin-layer Chromatography System TA—R_f 0.05, streaking; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.04 (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HY—RI 273.
Ultraviolet Spectrum Aqueous acid—268 nm (A^1_1=398a); aqueous alkali—241 nm (A^1_1=364b), 276 nm (A^1_1=304b), 388 nm (A^1_1=385b).

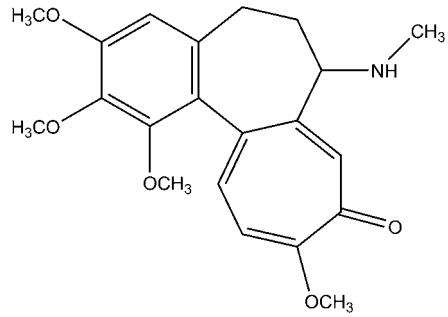


Infrared Spectrum Principal peaks at wavenumbers 1616, 1573, 1660, 1190, 1298, 1315 cm^{-1} (KBr disk).
Disposition in the Body Readily absorbed after oral administration and slowly excreted in the urine.
Half-life Plasma half-life, about 10 to 15 h.
Protein Binding 40 to 90%.
Dose Usually 600 mg of demeclocycline hydrochloride daily; up to 1.2 g daily has been given.

Demecolcine

Coumarin, Antimitotic
$C_{21}H_{25}NO_5$ = 371.4
CAS—477-30-5

IUPAC Name Deacetylmethylcolchicine
Synonyms Colchamine; desacetylmethylcolchicine; 6,7-dihydro-1,2,3,10-tetramethoxy-7-(methylamino)benzo[a]heptalen-9(5*H*)-one; *N*-methyl-*N*-desacetylcolchicine; omaine; Santavy's substance F.
Proprietary Name *Colcemid*



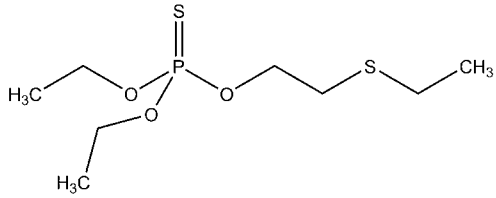
Chemical Properties Demecolcine is an alkaloid isolated from the corms of the meadow saffron, *Colchicum autumnale* (Liliaceae). Pale-yellow crystals. Mp 186°. Soluble 1 in 50 of water; soluble in ethanol, ether and chloroform. Extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—green→yellow (limit of detection, 0.05 μg); ammonium vanadate test—brown-purple→yellow (limit of detection, 0.025 μg); Vitali's test—yellow→purple/brown/red-violet (limit of detectio,n 0.05 μg).
Thin-layer Chromatography System T1—R_f 0.56 (location reagent *p*-dimethylaminobenzaldehyde spray, positive reaction).
High Performance Liquid Chromatography Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L potassium dihydrogen phosphate buffer with 5 mmol/L 1-pentanesulfonic acid : methanol : acetonitrile (60 : 26.6 : 13.4, pH 6.0). UV detection (λ = 254 nm and 350 nm). Retention time: 8.2 min. Limit of quantification not reported [Ko *et al.* 1990].
Infrared Spectrum Principal peaks at wavenumbers 1241 or 1562, 1582, 1090 cm^{-1} (KBr disk).
Quantification
Other **HPLC** Plant Samples (Jordanian *Colchicum* species). Column: C_{18} LichroCart (125 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 3% aqueous acetic acid (10 : 90 for 11 min to 60 : 40 over 4 min to 10 : 90 over 5 min), flow rate 1.0 mL/min. UV detection (λ = 245 nm). Retention times: (–)-demecolcine 8.8 min, (–)-cochicine 10.5 min. Limit of quantification not reported [Alali *et al.* 2007]. Corns, Stems and Leaves of *Colchicum auntumnale* L. species. Column: C_{18} (250 × 4.6 mm i.d., 10 μm). Mobile phase: 0.02 mol/L phosphate buffer (pH 7.5) : methanol-acetonitrile-0.02 mol/L phosphate buffer (pH 7.5; 38 : 35 : 27; 50 : 50 for 10 min to 20 : 80 over 6 min for 5 min), flow rate 1.0 mL/min. UV detection (λ = 353 nm). Limit of quantification, <1 mg/kg for dried corms or stems and <3 mg/kg for dried leaves [Vicar *et al.* 1993].
Dose Up to 10 mg daily.

Alali FQ *et al.* (2007). Determination of (–)-demecolcine and (–)–colchicine content in selected Jordanian *Colchicum* species. *Pharmazie* 62: 739–742.
Ko RJ *et al.* (1990). Determination of the antimitotic agents *N*-desacetylcolchicine, demecolcine and colchicine in serum and urine. *J Chromatogr* 525: 411–418.
Vicar J *et al.* (1993). Changes in colchicine and demecolcine content during vegetation period of colchicum autumnale L. *Acta Univ Palacki Olomuc Fac Med* 136: 5–7.

Demeton-*O*

Anticholinesterase, Insecticide, Organophosphate
$C_8H_{19}O_3PS_2$ = 258.3
CAS—8065-48-3 (demeton)

IUPAC Name Diethyl 2-ethylthioethyl phosphorothionate
Synonyms Demeton (mixture of demeton-O and demeton-S); mercaptofos; phosphorothioic acid O,O-diethyl O-[2-(ethylthio)ethyl] ester.
Proprietary Names *Systox* (mixture of demeton-O and demeton-S).



Chemical Properties Oily liquid. $Bp_{1.2}$ 128°. Extracted by organic solvents from aqueous acid or alkaline solutions.

Demeton-*O*-methyl

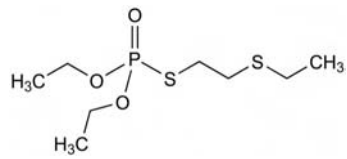
Synonyms Demeton-methyl (mixture of demeton-O-methyl and demeton-S-methyl); SPOC compound; thionate.
Proprietary Name *Metasystox* (mixture of demeton-O-methyl and demeton-S-methyl).
Chemical Properties Yellowish-brown oil. $Bp_{0.15}$ 74°. Soluble 1 in about 3000 of water. In solution or upon storage, 30% of demeton-O-methyl is transformed into demeton-S-methyl. Extracted by organic solvents from aqueous acid or alkaline solutions.

Thin-layer Chromatography System T16—R_f 0.60 demeton-O (location reagent bromophenol blue spray followed by 5% aqueous acetic acid spray, mauve).
Gas Chromatography System GA—RI 1576 demeton-O.
Disposition in the Body Demeton-O and demeton-O-methyl are very readily absorbed by all routes; they have a particularly high percutaneous toxicity. They exert a direct inhibitory action on cholinesterase and are rapidly hydrolysed in tissues.
Toxicity The estimated acute lethal dose in humans is 5 g, but exposure to as little as 300 mg daily may be dangerous.

Demeton-*S*

Insecticide
$C_8H_{19}O_3PS_2 = 258.3$
CAS—126-75-0

IUPAC Name 1-Diethoxyphosphorylsulfanyl-2-ethylsulfanylethane
Synonyms *O,O*-Diethyl-*S*-2-ethylthioethyl phosphorothioate; isosystox; mercaptofos teolovy.



Chemical Properties A colourless oil. Soluble 1 in 500 of water; soluble in most organic solvents. Log *P* (octanol/water), 2.09 [Meylan, Howard 1995].

Demeton-*S*-(Me)

$C_6H_{15}O_3PS_2 = 230.3$
CAS—919-86-8
Synonyms Metaisosystox; methyl-mercaptofos teolovy.
Note The name demeton-methyl used to be applied to a mixture of demeton-*S*-methyl and demeton-*O*-methyl but this mixture has been replaced by demeton-*S*-methyl.
Proprietary Names *Azotox*; *Demetox*; *DSM*; *Duratox*; *Metasystox 55*.
Chemical Properties A pale yellow oil of low viscosity. In solution or upon storage, demeton-*S*-methyl is oxidised to the sulfoxide and the sulfone. Refractive index 1.5065. Soluble 1 in 300 of water; soluble in most organic solvents.

Colour Test Palladium chloride—brown-orange; phosphorus test—yellow.
Thin-layer Chromatography system TE—(-methyl) R_f 0.81; system TX—(-methyl) R_f 0.18; system TY—(-methyl) R_f 0.13; system TAE—(-methyl) R_f 0.86.
Gas Chromatography System GA—demeton-S RI 1684, demeton-S-methyl RI 1628, demeton-S-methylsulfone RI 1866, demeton-S-methylsulfoxide RI 1860; system GK—RRT 0.70 (relative to caffeine).
High Performance Liquid Chromatography System HY—demeton-S-methyl RI 353.
Infrared Spectrum Principal peaks at wavenumbers 1020, 1250, 775, 793, 826, 1190 cm^{-1} (demeton-S-methyl).
Mass Spectrum Principal ions at *m/z* 88, 60, 109, 142, 79, 47, 111, 61 (demeton-S-methyl).
Disposition in the Body Demeton-S and demeton-S-methyl are very readily absorbed by all routes and have a particularly high toxicity following percutaneous absorption. They are rapidly hydrolysed in the tissues to the corresponding sulfoxide and sulfone.
Toxicity Demeton-S and demeton-S-methyl exert a direct inhibitory action on cholinesterase. The estimated minimum lethal dose is 5 g but exposure to 300 mg daily may be dangerous.

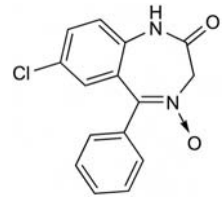
In a fatality due to the ingestion of demeton-S-methyl, the following postmortem tissue concentrations were reported: blood 10 mg/L, bile 58 mg/L, kidney 5 µg/g, liver 17 µg/g and urine 121 mg/L [Hall, Carson 1970].

Hall RA, Carson ED (1970). *TIAFT Bull* 76.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

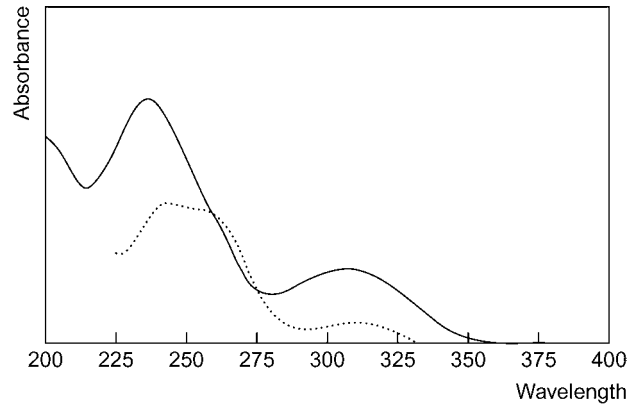
Demoxepam

Tranquilliser
$C_{15}H_{11}ClN_2O_2 = 286.7$
CAS—963-39-3

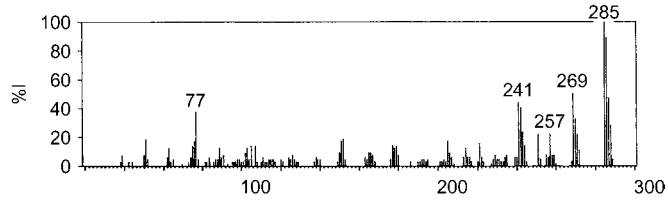
IUPAC Name 7-Chloro-4-hydroxy-5-phenyl-3*H*-1,4-benzodiazepin-2-one
Synonyms Chlordiazepoxide lactam; 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide; demossepam.



Chemical Properties A white crystalline powder. Soluble in chloroform. pK_a 4.5, 10.6. Log *P* (octanol/pH 7.4), 1.5.
Colour Test Formaldehyde–sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.00; system TC—R_f 0.35; system TD—R_f 0.15; system TE—R_f 0.41; system TF—R_f 0.24; system TL—R_f 0.51; system TAD—R_f 0.42; system TAE—R_f 0.81; system TAF—R_f 0.83 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—demoxepam RI 2529, M oxazepam RI 2325; system GB—demoxepam RI 2806, M oxazepam RI 2438; system GG—demoxepam RI 3043, M oxazepam RI 2803.
High Performance Liquid Chromatography System HI—demoxepam *k* 2.42, M oxazepam *k* 4.62; system HK—demoxepam *k* 0.03, M oxazepam *k* 0.73; system HX—RI 416; system HY—RI 341; system HZ—RT 3.1 min.
Ultraviolet Spectrum Aqueous acid—237, 305 nm; aqueous alkali—243, 255, 310 nm; methanol—236 ($A^1_1 = 1340$b), 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1678, 690, 1240, 1265, 755, 717 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 285, 286, 269, 287, 241, 242, 77, 270.



Quantification

Blood **TLC** Spectrofluorometry. Limit of detection, 0.1–0.2 µg [Stronjny *et al.* 1977].
Plasma **TLC** See Blood [Stronjny *et al.* 1977].
HPLC Limit of detection, 0.075–0.125 mg/L [Garretty *et al.* 1998]. Column: C_{18} reversed phase. Limit of detection, 0.05 mg/L [Divoll *et al.* 1982].
Serum **TLC** Fluorescence densiometry ($\lambda = 430$ nm). Limit of detection, 0.01 mg/L [Sun 1978].

GC-MS Column: 5% OV-225 on 80/100 mesh Gas Chrom Q (1.21 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 50 mL/min. Temperature: 265°. ECD. Retention time: 4.4 min. Limit of detection, 0.8 ng/injection [Sun, Hoffman 1978].

HPLC Column: C_{18} reverse-phase. Mobile phase: phosphate buffer (pH 6.0) : methanol : acetonitrile (45 : 35 : 20). UV detection (λ = 254 nm). Limit of detection, 20 mg/L [Haver *et al.* 1986]. Column: C_{18} reversed phase. Mobile phase: 60 mmol/L ammonium acetate (pH 7.69) : 60 mmol/L acetic acid (pH 2.8) : acetonitrile. Limit of detection, 0.05–0.10 mg/L [Lensmeyer *et al.* 1982]. Column: reversed phase. Retention time, ~2 min. Limit of detection, ~100 pg [Brinkman *et al.* 1981]. Column: reversed phase. UV detection. Limit of quantification, 50 µg/L [Good, Andrews 1981]. Column: reversed phase. Mobile phase: acetonitrile : methanol : acetate buffer (pH 5.0, 200 : 225 : 500), flow rate 2 mL/min. UV detection (λ = 240 nm) [Foreman *et al.* 1980].

Urine **LC-MS** Column: REMEDi HS. Limit of detection, 250 ng [Essien *et al.* 1996].

Disposition in the Body Demoxepam is well absorbed after oral administration. Approximately 10% of a dose is excreted in the urine in 24 h and ~60% in 7 days, including ~27% as unchanged demoxepam and ~5% as conjugated oxazepam. Other urinary metabolites include the 5-(4-hydroxyphenyl) and 9-hydroxy derivatives. Approximately 15% of the dose is eliminated in the faeces in 7 days, with ~2% as desmethyldiazepam.

Demoxepam is a metabolite of chlordiazepoxide.

Therapeutic Concentration

Following a single oral dose of 20 mg of demoxepam given to 6 subjects, peak plasma concentrations of 0.50–0.74 µg/mL (mean 0.6) were attained in 2–8 h [Schwartz *et al.* 1971].

Half-life Plasma half-life, 14–95 h (mean 40).

Brinkman UA *et al.* (1981). Liquid chromatography of demoxepam and phenothiazines using a post-column photochemical reactor and fluorescence detection. *J Chromatogr* 217: 463–471.

Divoll M *et al.* (1982). Liquid chromatographic determination of chlordiazepoxide and metabolites in plasma. *Pharmacology* 24: 261–266.

Essien H *et al.* (1996). Use of direct-probe mass spectrometry as a toxicology confirmation method for demoxepam in urine following high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 683: 199–208.

Foreman JM *et al.* (1980). Simultaneous assay of diazepam, chlordiazepoxide, *N*-desmethyldiazepam, *N*-desmethylchloridazepoxide, and demoxepam in serum by high performance, liquid chromatography. *Clin Biochem* 13: 122–125.

Garretty DJ *et al.* (1998). Micro-extraction of chlordiazepoxide and its primary metabolites, desmethylchlordiazepoxide and demoxepam, from plasma and their measurement by liquid chromatography. *Ann Clin Biochem* 35: 528–533.

Good TJ, Andrews JS (1981). The use of bonded-phase extraction columns for rapid sample preparation of benzodiazepines and metabolites from serum for HPLC analysis. *J Chromatogr Sci* 19: 562–566.

Haver VM *et al.* (1986). Simplified high performance liquid chromatographic method for the determination of clonazepam and other benzodiazepines in serum. *Ther Drug Monit* 8: 352–357.

Lensmeyer GL *et al.* (1982). Liquid-chromatographic procedure for simultaneous analysis for eight benzodiazepines in serum. *Clin Chem* 28: 2274–2278.

Schwartz MA *et al.* (1971). Biological half-life of chlordiazepoxide and its metabolite, demoxepam, in man. *J Pharm Sci* 60: 1500–1503.

Stronjny N *et al.* (1977). Determination of chlordiazepoxide, diazepam, and their major metabolites in blood or plasma by spectrophotodensitometry. *J Chromatogr* 143: 363–374.

Sun SR (1978). Quantitative determination of chlordiazepoxide and its metabolites in serum by fluorescence TLC–densitometry. *J Pharm Sci* 67: 639–641.

Sun SR, Hoffman DJ (1978). Rapid GLC determination of chlordiazepoxide and metabolite in serum using on-column methylation. *J Pharm Sci* 67: 1647–1648.

Denatonium Benzoate

Alcohol Denaturant

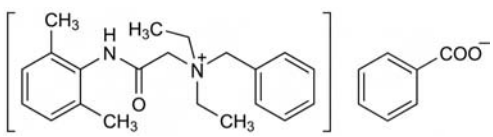
$C_{28}H_{34}N_2O_3$ = 446.6

CAS—3734-33-6

IUPAC Name Benzyl-[2-(2,6-dimethylanilino)-2-oxoethyl]-diethylazanium benzoate

Synonym *N*-[2-[(2,6-Dimethylphenyl)amino]-2-oxoethyl]-*N*,*N*-diethylbenzenemethanaminium benzoate

Proprietary Name *Bitrex*



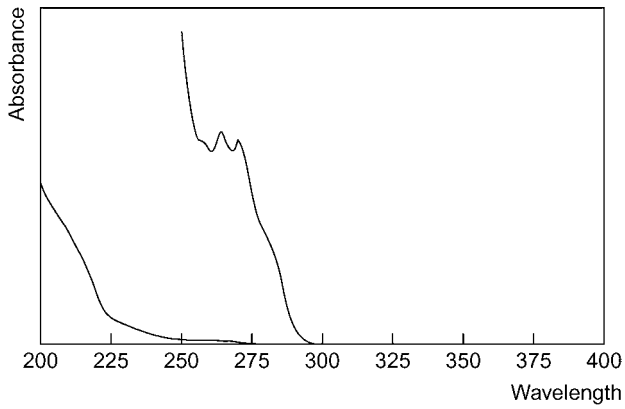
Chemical Properties A white crystalline powder. Mp 166° to 170°. Soluble in water; soluble 1 in about 2 of ethanol and 1 in about 3 of chloroform; sparingly soluble in acetone; practically insoluble in ether. Log *P* (octanol/water), 1.8.

Colour Test Mandelin's test—violet.

Thin-layer Chromatography System TA—R_f 0.10; system TB—R_f 0.00; system TE—R_f 0.03; system TF—R_f 0.25; system TAE—R_f 0.01 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 422.

Ultraviolet Spectrum Aqueous acid—263 (A^1_1=36b), 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1550, 1596, 1680, 719, 704, 757 cm^{-1}.

Denopamine

β_1-Adrenoceptor Agonist, Cardiotonic

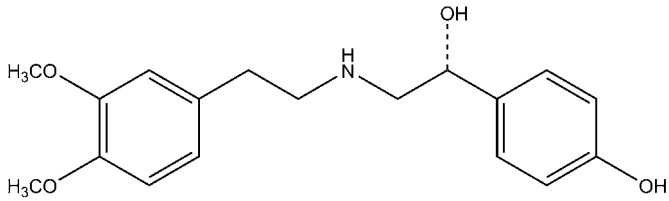
$C_{18}H_{23}NO_4$ = 317.4

CAS—71771-90-9

IUPAC Name 4-[(1*R*)-2-[2-(3,4-Dimethoxyphenyl)ethylamino]-1-hydroxyethyl] phenol

Synonyms (−)-(*R*)-α-[[(3,4-Dimethoxyphenethyl)amino]methyl]-*p*-hydroxybenzyl alcohol; (α*R*)-α-[[[2-(3,4-dimethoxyphenyl)ethyl]amino]methyl]-4-hydroxybenzenemethanol; (−)-(*R*)-1-(4-hydroxyphenyl)-2-(3,4-dimethoxyphenethylamino) ethanol; TA-064.

Proprietary Names *Carguto*; *Kalgut*.



Chemical Properties pK_{a1} 8.2 [Nishi *et al.* 1995], pK_{a2} 10.0 [Ishibuchi *et al.* 1997]. Log *P* (octanol/water), 1.73 [ACD 2007].

l-Denopamine Hydrochloride

$C_{18}H_{23}NO_4$,HCl = 353.9

Chemical Properties Crystals from isopropanol. Mp 138° to 139.5°.

dl-Denopamine Hydrochloride

Chemical Properties Crystals from isopropanol and isopropyl ether. Mp 164° to 167°.

High Performance Liquid Chromatography Column: ULTRON ES-CD, cyclodextrin immobilised chiral stationary phases (150 × 6 mm i.d., 5 µm). Mobile phase: tetrahydrofuran with 50 mmol/L phosphate buffer (pH 4.6), flow rate 1 mL/min. UV detection (λ = 220 nm). Limit of detection not reported [Nishi *et al.* 1995].

Capillary Electrophoresis Capillary: uncoated fused silica (total/effective length 50/37 cm, 75 µm i.d.). Buffer: acidic solution containing uncharged cyclodextrins. DAD (λ = 210 to 220 nm). Limit of detection not reported [Ishibuchi *et al.* 1997]. Capillary: fused silica (total/effective length 57/50 cm, 75 µm i.d.). DAD (λ = 210 to 220 nm). Limit of detection, not reported [Nishi *et al.* 1995].

Mass Spectrum Principal ions at *m/z* 252 and 297 (*O*-TMS derivative).

Quantification

Plasma **GC-MS** Column: 1.5% OV-1 on 100/120 Mesh Gas Chrom Q or 1.5% OV-17 on 100/120 mesh Shimalite W (500 × 3 mm i.d.). Carrier gas: He, 35 mL/min. EI ionisation at 30 eV, SIM acquisition mode. Limit of detection not reported [Suzuki *et al.* 1983].

HPLC Column: Macherey Nagel Nucleosil 5C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L dipotassium hydrogen phosphate (pH 5.5; 27 : 100), flow rate 0.9 mL/min. Electrochemical detection. Limit of detection, 2 µg/L [Tagawa *et al.* 1990]. Column: Waters radial Pack C_{18} (100 × 8 mm i.d., 10 µm). Mobile phase: water : methanol : acetonitrile (16 : 3 : 1) containing 1% ethanol and 2% triethylamine, flow rate 1.5 mL/min. UV detection (λ = 280 nm). Limit of detection not reported [Suzuki *et al.* 1983].

Urine **GC-MS** Column: cross-linked methylsilicone fused silica (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 80 mL/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Suzuki *et al.* 1985]. See Plasma [Suzuki *et al.* 1983].

HPLC See Plasma [Suzuki *et al.* 1983].

Disposition in the Body Denopamine undergoes oxidative 3′- or 4′-*O*-demethylation and/or *m*-hydroxylation followed by *m*- or *p*-catechol *O*-methyltransferase methylation to form 5 metabolites. Approximately 30 to 40% of an orally administered dose is excreted in the urine within 24 h as free and conjugated denopamine and conjugates of the 5 metabolites.

Therapeutic Concentration

The time to peak plasma concentration was the only pharmacokinetic value that was significantly different when 9 healthy male volunteers were compared with 11 haemodialysis patients. This could be attributed to intestinal oedema caused by water retention or forced immobility during haemodialysis. Mean peak plasma concentrations were as follows:

Patients	C_{max} (μg/L)	t_{max} (h)	$t_{1/2}$ (h)
Haemodialysis	14.9 ± 3.7	3.0 ± 0.5	4.1 ± 0.8
Interdialysis	19.1 ± 9.5	2.9 ± 0.9	6.1 ± 0.7
Healthy volunteers	15.5 ± 1.6	0.9 ± 0.2	4.0 ± 0.9

[Nakahama *et al.* 1991]

In healthy volunteers administered denopamine, the plasma concentrations increased rapidly, reaching a maximum at approximately 0.5 to 2 h, and declined with an elimination half-life of approx. 4 h. Mean peak plasma concentrations were as follows:

Treatment	C_{max} (μg/L)	t_{max} (h)	$t_{1/2}$ (h)
2 × 5 mg tablet	19.2 ± 2.4	0.83 ± 0.17	4.02 ± 0.88
1 × 10 mg tablet	15.5 ± 1.6	0.89 ± 0.16	4.00 ± 0.93
5% fine granules (200 mg)	16.9 ± 1.3	0.50 ± 0.00	3.67 ± 0.61

[Tagawa *et al.* 1990].

The minimal effective plasma level (5.5 to 8.8 μg/L) can be obtained by the oral administration of 10 mg three times daily at 15 to 30 min after administration [Bito *et al.* 1988].

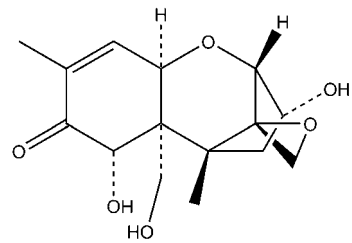
Half-life Approximately 4 h.

ACD (2007) *ACD/LogP DB.* Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).
Bito K *et al.* (1988). Clinicopharmacological studies of a newly synthesized cardiotonic agent (TA-064) in patients with congestive heart failure. *Clin Cardiol* 11: 334–339.
Ishibuchi K *et al.* (1997). Enantiomer separation of denopamine by capillary electrophoresis with charged and uncharged cyclodextrins. *Electrophoresis* 18: 1007–1012.
Nakahama H *et al.* (1991). Pharmacokinetics of denopamine in hemodialysis patients. *Ren Fail* 13: 37–41.
Nishi H *et al.* (1995). Enantiomeric separation of denopamine by capillary electrophoresis and high-performance liquid chromatography using cyclodextrins. *J Pharm Biomed Anal* 13: 1483–1492.
Suzuki T *et al.* (1983). Metabolism of a new cardiotonic agent, (−)-alpha-(3,4-dimethoxyphenethylaminomethyl)-4-hydroxybenzyl alcohol (TA-064), in man. *O*-Demethylation and ring hydroxylation. *Drug Metab Dispos* 11: 377–386.
Suzuki T *et al.* (1985). Improved separation of the denopamine metabolites using capillary column gas chromatography–mass spectrometry. *Chem Pharm Bull (Tokyo)* 33: 2549–2552.
Tagawa K *et al.* (1990). Determination of denopamine in human and dog plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 529: 500–506.

Deoxynivalenol

Epoxysesquiterpenoid, Mycotoxin
$C_{15}H_{20}O_6$ = 296.3
CAS—51481-10-8

IUPAC Name 12,13-Epoxy-3,7,15-trihydroxytrichothec-9-en-8-one
Synonyms C09747; dehydronivalenol; desoxynivalenol; DON; LMPR01030038; vomitoxin.



Chemical Properties Non-fluorescent type B trichothecene mycotoxin is associated primarily with *Fusarium graminearum* and *F. culmorum*, both of which are important plant pathogens. Surveys have shown that DON occurs predominantly in grains such as wheat, barley, oats, rye, and maize, and less often in rice, sorghum and triticale [WHO/JECFA 2002]. Fine needles from ethyl acetate/petroleum ether. Mp 151° to 153° [O'Neil *et al.* 2006]. Log *P* (octanol/water), −0.71 [Weiss *et al.* 2003]. When an aqueous solution of DON was treated with 10 mg of activated carbon, between 1.83 and 98.93% of the DON was sequestered. Neither hydrated sodium calcium aluminosilicate or sepiolate were effective in binding DON [Galvano *et al.* 1998]. In degradation studies, DON and nivalenol (NIV) solution tests were shown to be relatively stable in buffer solutions over the pH range 1–10. Quite harsh conditions (pH 12, high salt concentration, 80°, prolonged exposure) were needed to achieve substantial breakdown [Lauren, Smith 2001]. Solutions of DON and NIV in acetonitrile were shown to be stable at temperatures up to 25° for at least 24 months, while long-term storage of DON and NIV in ethyl acetate or as thin-film at temperatures above freezing should be avoided [Widestrand, Pettersson 2001]. The use of low-dose gamma irradiation to destroy DON present on grain was shown to be unsuitable because of the high irradiation dose required for destruction of the toxin (>50 kGy) [O'Neill *et al.* 1993].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagent: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ = 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values were reported as follows:

	Mobile phase solvent ratio, R_f value								
	A	*RP-18*	*KC-18*	*B*	*RP-18*	*KC-18*	*C*	*RP-18*	*KC-18*
DON	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyl-DON	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
NIV	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Aflatoxin B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxin B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxin G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxin G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

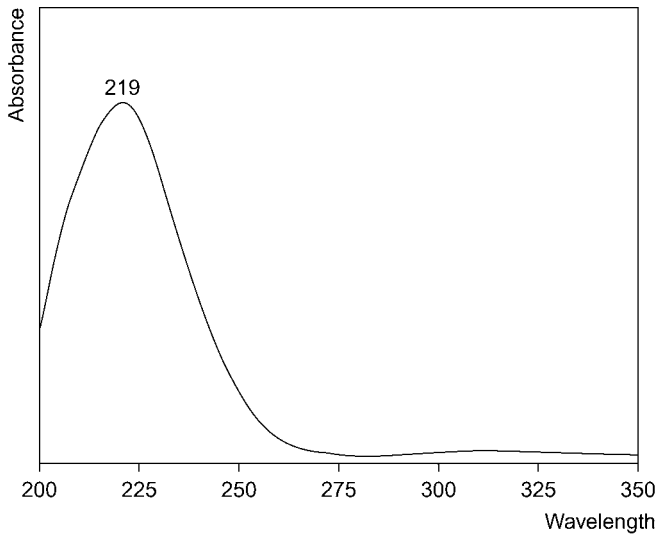
[Abramson *et al.* 1989].

Gas Chromatography-Mass Spectrometry Column: HP-5MS (30 m × 0.25 mm, 0.25 μm). Carrier gas: He, 2.0 mL/min. Temperature programme: 80° for 2 min to 200° at 35°/min for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode (FID also used). Retention time: 17.5 min (*N*,*O*-bis(trimethylsilyl)acetamide : trimethylchlorosilane : *N*-trimethylsilylimidazole derivative). Limit of quantification not reported [Krska *et al.* 2004]. Column: DB-5MS (30 m × 0.5 mm, 0.25 μm). Temperature programme: 150° for 1 min to 280° at 3°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention times: DON 9.7 min, 7-acetyl-DON 9.8 min, 4-acetyl-NIV 9.8 min, 3-acetyl-NIV 9.88 min, 15-acetyl-NIV 9.94 min, NIV 10.0 min, 3,15-diacetyl-DON 10.5 min, 4,15-diacetyl-NIV 10.7 min (TMS-ether derivatives). Limit of quantification not reported [Rodrigues-Fo *et al.* 2002]. Column: BP-1 methylsilicone (25 m × 0.33 mm i.d., 0.5 μm). Carrier gas: He, 3.0 mL/min. Temperature programme: 150° to 300° at 15°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 5.7 min (trifluoroacetyl ester derivative). Limit of detection, 10 pg [Wreford, Shaw 1988].

High Performance Liquid Chromatography Column: C_{18} (150 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : water (1 : 1), flow rate 0.4 mL/min. UV detection (λ = 219 nm). Limit of quantification not reported [Krska *et al.* 2004].

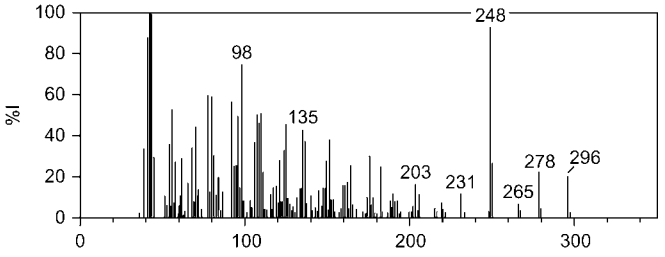
Liquid Chromatography-Mass Spectrometry Column: C_8 (150 × 3.0 mm i.d., 5 μm). Mobile phase: methanol : water both containing 0.1 mol/L ammonium acetate (20 : 80 to 80 : 20 over 18 min), flow rate 0.5 mL/min. ESI, positive ion mode, full-scan and SIM acquisition mode, MRM acquisition mode. Limit of quantification not reported [Krska *et al.* 2004].

Ultraviolet Spectrum Ethanol—218 nm[Krska *et al.* 2004; Kuronen 1989].



Infrared Spectrum See Krska *et al.* [2004].

Mass Spectrum



Quantification

Blood **GC-MS** Column: BP-5 (12 m × 0.22 mm i.d., 0.25 μm). Carrier gas: He, 8 psi. Temperature programme: 90° for 2 min to 180° at 20°/min to 240° at 5°/min for 2 min. CI, negative ion mode, SIM acquisition mode. Limit of detection, 2 to 7 ppb for DON, NIV, T-2, HT-2, T-2 tetraol, diacetoxyscirpenol, scirpentriol, 15-MAS (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986].

Urine **GC-MS** Column: BP-1 methylsilicone (25 m × 0.2 mm i.d., 0.25 μm). Carrier gas: He, 15 psi. Temperature programme: 160° for 1 min to 275° at 10°/min, for 5 min. EI ionisation at 70 eV, positive ion mode, SIM. Limit of detection, DON, NIV, T-2, HT-2, T-2 tetraol, diacetoxyscirpenol, scirpentriol and 2 to7 ppb for15-MAS (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986].

LC-MS Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol (10 : 90 to 20 : 90 over 45 min), flow rate 1.0 mL/min. ESI, positive ion mode, SIR. Retention time: 15.8 min. Limit of detection, 4 μg/L [Meky *et al.* 2003].

Faeces **GC** Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 40 cm/s. Temperature programme: 50° for 2 min to 205° at 40°/min for 10 min to 222° at 1°/min to 280° at 10°/min for 5 min. ECD (^{63}Ni). Limit of quantification not reported (*N*,*O*-bis(trimethylsilyl)acetamide : trimethylchlorosilane : *N*-trimethylsilylimidazole derivative) [Sundstol, Pettersson 2003].

Other **TLC** Maize Samples. Plates: silica gel. Solvent system: chloroform : methanol (94 : 6). Location reagent 20% aluminium chloride in methanol. Heat for 15 min at 110° to 120°. UV detection (λ = 365 nm). Limit of quantification not reported [Schaafsma *et al.* 1998]. Wheat and Maize Samples. Plates: precoated silica gel 60 (20 × 20 cm), dipped in 15% aluminium chloride solution, air-dried, activated at 105° for 1 h. Solvent system: chloroform : acetone: propan-2-ol (8 : 1 : 1). Plate heated at 120° for 7 min. UV detection. R_f 0.6 (blue fluorescent spot). Limit of quantification, 40 μg/kg in wheat and 100 μg/kg in maize [Fernandez *et al.* 1994; Trucksess *et al.* 1984].

GC Pig Plasma and Urine. Column: glass packed with 3% OV-17 on 100-120 mesh Supelcoport (1.8 m × 2.0 mm i.d.). Carrier gas: He, 35 mL/min. Temperature programme: 165° isothermal. ECD (Ni^{63}). Retention times: DON 6 min, T-2 tetraol 5 min, scirpenetriol 4.5 min (trifluoroacetic acid derivatives). Limit of detection, <25 ppb [Rood *et al.* 1986]. Bovine Urine and Faeces, Milk Samples. Column: glass packed with 3% OV-17 on 100-120 mesh Supelcoport (1.8 m × 2.0 mm i.d.). Carrier gas: Ar-CH_4 (95 : 5), 35 mL/min. Temperature programme: 220° isothermal. ECD (Ni^{63}). Limit of detection, 50 ppb for DON and its de-epoxy metabolite (TMS derivatives) in urine and faeces; 1 μg/L in milk [Dahlem *et al.* 1986; Swanson *et al.* 1986].

GC-MS Soy Sauce Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 140° for 2 min to 275° at 7°/min for 2 min to 290° at 30°/min for 5 min. Limit of detection, 7 μg/kg for DON, 15-acetyl-DON and T-2 triol; between 3 and 14 μg/kg for 3-acetyl-DON, NIV, fusarenone-X, T-2, HT-2, T-2 tetraol, scirpenetriol, 15-monoacetoxyscirpenol, diacetoxyscirpenol and neosolaniol [Schollenberger *et al.* 2007]. Food Samples. Column: Rtx-200 (60 m × 0.25 mm i.d., 0.1 μm). Carrier gas: He, 1.3 mL/min. Temperature programme: 115° for 5 min, to 125° at 50°/min to 300° at 5°/min for 10 min. EI at 70 eV, positive ion mode. Retention times: DON 24.6 min, 3-acetyl-DON 28.8 min, 15-acetyl-DON 28.3 min, NIV 26.9 min, fusarenone-X 26.4 min, diacetoxyscirpenol 29.4 min, neosolaniol 30.1 min, HT-2 33.7 min, T-2 34.5 min. Limit of quantification, DON 0.30 μg/kg, 3-acetyl-DON 0.26 μg/kg, 15-acetyl-DON 0.19 μg/kg, NIV 0.28 μg/kg, fusarenone-X 0.16 μg/kg, diacetoxyscirpenol 0.36 μg/kg, neosolaniol 0.37 μg/kg, HT-2 0.26 μg/kg, T-2 0.18 μg/kg [Schothorst *et al.* 2005]. Grain Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 80° to 245° at 60°/min for 3 min to 260° at 3°/min to 270° at 10°/min for 7 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of quantification, 30 μg/kg for DON, 3-acetyl-DON, fusarenone-X and diacetoxyscirpenol; 20 μg/kg for HT-2 and T-2; 30 μg/kg for nivalenol (*N*,*O*-bis (trimethylsilyl)acetamide : trimethylchlorosilane : *N*-trimethylsilylimidazole derivatives) [Jestoi *et al.* 2004]. Food Samples. Column: 5% phenylmethyl siloxane capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 70° to 170° at 25°/min to 300° at 5°/min for 2 min. Limit of detection, 7 ng/g (trimethylsilylimidazole : trimethylchlorosilane derivative) [Cirillo *et al.* 2003]. Barley and Wheat Samples. Column: fused silica capillary (30 m × 0.25 mm i.d.). Carrier gas: He, 2.0 mL/min. Temperature programme: 120° for 5 min to 280 at 8°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.5 ng for DON, 0.25 ng for NIV (TMS : ether derivatives) [Yumbe-Guevara *et al.* 2003]. Fungal Cultures. Column: HP-5 (30 m × 0.25 mm i.d., 0.10 μm). Carrier gas: He, 40 cm/s. Temperature programme: 80° for 1 min to 160° at 40°/min to 205° at 4°/min to 240° at 8°/min to 300° at 40°/min for 3 min. Electron ionisation, positive ion mode and CI, negative ion mode. Retention indices for pentafluoropropionyl (PFP) derivatives: DON-PFP_3 1418, nivalenol-PFP_4 1279, 15-acetyl-DON-PFP_2 1634, 3-acetyl-DON-PFP_2 1640. Limit of detection, 15 to 30 pg (EI), 10 to 30 pg (CI) [Nielsen, Thrane 2001]. Barley Samples. Column: Rtx-200 (30 m × 0.25 μm i.d., 0.25 μm). Carrier gas: He, 0.75 mL/min. Temperature programme: 90° for 0.2 min to 210° at 30°/min to 300° at 6°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention times: DON 12.3 min, 3-acetyl-DON 13.8 min, fusarenone-X 14.8 min, diacetoxyscirpenol 14.0 min, 15-MAS 13.8 min, T-2 17.9 min, scirpentriol 12.9 min, zearalenone 17.6 min. Limit of detection, 0.1 to 0.5 mg/kg [Onji *et al.* 1998]. Beer Samples. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 22 psi. Temperature programme: 80° for 1 min to 180° at 50°/min to 280° at 5°/min for 5 min. EI ionisation at 42 eV, positive ion mode, SIM acquisition mode. Limit of detection, DON 0.1 to 1.5 μg/L, NIV 0.01 to 0.3 μg/L, α- and β-zearalenol 2.5 to 3.0 μg/L, zearalenone 1.5 to 2.0 μg/L [Scott *et al.* 1993]. 'Yellow Rain' Powder Sample. Column: glass packed with 3% OV-17 on 100-120 mesh Chromosorb (1.0 m × 2.0 mm i.d.). Carrier gas: He, 25 mL/min. Temperature programme: 180° to 260° at 12°/min for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention times: DON 2.3 min, T-2 6.2 min, HT-2 5.5 min, zearalenone 6.5 min, diacetoxyscirpenol 4.0 min (TMS derivatives). Limit of quantification not reported [Rosen, Rosen 1982].

HPLC *In vitro* Gastric Juice Samples. Column: C_{18} (100 × 3.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (10 : 90), flow rate 0.2 mL/min. UV detection (λ = 218 nm). Limit of quantification, 0.05 mg/L [Sabater-Vilar *et al.* 2007]. Apical or basolateral media samples. Column: C_{18} (100 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile (90 : 10), flow rate 0.6 mL/min. UV detection (λ = 220 nm). Retention time: 4.6 min. Limit of quantification, media from the upper compartment 10 μg/L, media from the lower compartment 20 μg/L; limit of detection, media from the upper compartment 3 μg/L, media from the lower compartment; 6 μg/L [Sergent *et al.* 2006]. Grain Samples. Column: C_{18} (100 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water (12 : 88), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Limit of quantification, 5 μg/kg [Ramirez *et al.* 2006]. Column: C_{18} (250 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (65 : 35) containing 0.75% acetic acid, flow rate 1.0 mL/min.

D

Fluorescence detection ($\lambda_{ex} = 292$ nm, $\lambda_{em} = 425$ nm). Limit of detection, 0.4 μg/kg for DON and HT-2, 0.2 μg/kg for NIV and diacetoxyscirpenol, 1.0 μg/kg for15-acetyl-DON and 3-acetyl-DON, 0.6 μg/kg for fusarenone-X and T-2 (coumarin-3-carbonyl derivatives) [Dall'Asta *et al.* 2004; Mateo *et al.* 2001]. Column: C_{18} (10 μm). Mobile phase: deoxygenated methanol : 40 mmol/L borate buffer (35 : 65), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 3.6 min. Limit of detection, 25 μg/L [Sylvia *et al.* 1986]. Wheat Samples. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol: water (23 : 77), flow rate 1.0 mL/min. UV detection ($\lambda = 229$ nm). Retention time: 10.2 min. Limit of detection, 30 ppb [Chang *et al.* 1984].

LC-MS Corn Silage Samples. Column: C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: acetonitrile : water acidified with 0.5% acetic acid (pH 3; 5 : 95 to 50 : 50 over 15 min to 80 : 20 over 10 min), flow rate 0.3 mL/min. ESI, positive and negative ion modes, SIM acquisition mode. Retention times: DON 6.9 min, aflatoxin B_1 15.4 min, citrinin 16.7 min, fumonisin B_1 15.1 min, gliotoxin 14.2 min, ochratoxin A 20.3 min, zearalenone 20.4 min. Limit of quantification, DON 5 ppb, gliotoxin 6.5 ppb, zearalenone 20 ppb; for aflatoxin B_1, citrinin, fumonisin B_1 and ochratoxin A1.5 ppb [Richard *et al.* 2007]. Food Samples. Column: C_{80} (150 × 2.1 mm i.d., 4 μm). Mobile phase: methanol : water (10 : 90 for 1 min to 80 : 20 over 26 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Limit of detection, DON and norDON A 2.5 μg/kg, norDON B and C 1 μg/kg [Bretz *et al.* 2006]. Fungal cultures. Column: C_{18} (150 × 3.0 mm i.d., 3.5 μm). Mobile phase: water : methanol–10 mmol/L ammonium acetate–20 μmol/L sodium acetate (70 : 30 to 50 : 50 over 1 min to 40 : 60 over 9.5 min to 15 : 85 over 1 min for 7 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: DON 5.6 min, NIV 3.9 min, de-epoxy-DON 7.0 min, verrucarol 7.7 min, neosolaniol 6.7 min, aflatoxin G_2 9.4 min, aflatoxin B_2 11.4 min, aflatoxin G_1 10.5 min, aflatoxin B_1 12.5 min, diacetoxyscirpenol 12.7 min, ochratoxin A 16.8 min, T-2 toxin 17.6 min, verrucarin A 17.5 min, roridin A 17.7 min, zearalenone 18.9 min, sterigmatocystin 19.5 min. Limit of detection, DON 15 μg/L, other compounds 0.04 to 75 μg/L [Delmulle *et al.* 2006]. Pig Urine. Column: Synergi Polar-RP (150 × 4.6 mm i.d., 4 μm). Mobile phase: water : acetonitrile : methanol (82 : 9 : 9 to 40 : 60 : 0 over 25 min), flow rate 1.0 mL/min. APCI, negative ion mode, SIM acquisition mode. Retention times: DON 5.4 min, de-epoxy metabolite 7 min. Limit of quantification, DON 25 ng/g, de-epoxy metabolite 25 ng/g [Razzazi-Fazeli *et al.* 2003]. Wheat Samples. Column: C_{18} (125 × 2.0 mm i.d., 3 μm). Mobile phase: methanol : water (25 : 75 to 2 : 98 over 12 min), flow rate 0.25 mL/min. API, positive ion mode, full-scan. Limit of quantification, DON and neosolaniol 50 ppb, NIV and 15-acetyl-DON 100 ppb, fusarenone-X 40 ppb, 3-acetyl-DON 25 ppb, diacetoxyscirpenol 20 ppb, HT-2 10 ppb, T-2 60 ppb [Berger *et al.* 1999] Rat Urine and Faeces. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol : 0.1 mol/L ammonium acetate (20 : 80 to 70 : 30 over 20 min for 5 min), flow rate 1.5 mL/min. TSI, positive and negative ion modes, multiple ion detection. Limit of detection, DON 150 pg, de-epoxy-DON 300 pg, scirpentriol 150 pg, de-epoxy-scirpentriol 300 pg, MAS 50 pg [Voyksner *et al.* 1987].

Note For an LC-MS method for the quantification of multiple mycotoxins in a variety of nuts and dried fruits, see Spanjer *et al.* [2008]; in infant foods, see Lattanzio *et al.* [2008]. For the determination of 39 mycotoxins in wheat and maize by LC-MS, see Sulyok *et al.* [2006]; for type A and B trichothecenes in cereals by LC-MS, see Klotzel *et al.* [2005]; for 17 mycotoxins using HPLC-DAD, see Kuronen [1989]; Jimenez, Mateo [1997]. For a purity assessment study of mycotoxin standards comparing UV, HPLC-UV, GC-ECD, GC-FID, GC-MS, LC-MS, DSC, NMR and FT-IR methods, see Krska *et al.* [2005]. For an ELISA method used for screening beers on the European market, see Papadopoulou-Bouraoui *et al.* [2004]. For a method using supercritical fluid extraction coupled with HPLC or GC-ECD for the analysis of DON and related compounds in wheat samples, see Josephs *et al.* [1998].

Disposition in the Body At the time of writing, there is no DON toxicokinetic information available in humans. Animal studies have shown that the oral bioavailability of trichothecenes is generally low as a result of physiological instability and first-pass metabolism. After oral administration of DON to rats, the major metabolic route is de-epoxidation to the corresponding methylene derivative and glucuronidation, generally to less toxic substances. DON and its metabolites are primarily excreted via faeces but also occur in the urine (65% and 25%, respectively, within 4 days).

Toxicity LD_{50} in mice (mg/kg): 70.0 [Yoshizawa, Morooka 1975]; also reported as 76.7 [Morooka *et al.* 1972]. Provisional maximum tolerable daily intake (PMTDI) 1 μg/kg bodyweight [WHO/JECFA 2002].

> Urine samples were collected from 11 individuals from Linxian County, China, a rural Chinese population with a staple diet of wheat and maize. It is also an area known for its high rates of oesophageal cancer and studies suggest that this disease may be etiologically associated with mycotoxin exposure. The mean levels of DON detected in the urine samples were 37 μg/L and 12 μg/L from the high- and low-risk areas for DON exposure and oesophageal cancer, respectively. From animal studies and assuming a 60 kg person produces 1 L urine/day, and a 40% recovery of DON in human samples, the levels detected in the high- and low-risk populations may represent a daily exposure ranging from 1.9 to 13.0 and 0.6 to 2.5 μg/kg, respectively [Meky *et al.* 2003].

Note For an overview of some major mycotoxins, including DON, see Richard [2007]. For a review of DON toxicology and potential effects on humans, see Pestka and Smolinski [2005]; Rotter *et al.* [1996]; Sudakin [2003]. For proceedings of a conference on trichothecenes with a special focus on DON, see ISLI Europe Natural Toxin Task Force [2004].

Dose It has been reported that DON (and more generally, trichothecenes) has been used as an agent of biological or chemical warfare ('yellow rain') [Mirocha *et al.* 1983; Rosen, Rosen 1982; Stark 2005].

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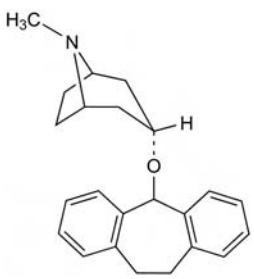
Deptropine

Antihistamine

$C_{23}H_{27}NO = 333.5$

CAS—604-51-3

Synonyms Dibenzheptropine; *endo*-3-[(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)oxy]-8-methyl-8-azabicyclo[3.2.1]octane.



Chemical Properties Log *P* (octanol/water), 5.3.

Deptropine Citrate

$C_{23}H_{27}NO,C_6H_8O_7 = 525.6$

CAS—2169-75-7

Proprietary Names *Brontine*. It is an ingredient of *Brontisol*.

Chemical Properties A white to off-white microcrystalline powder. Very slightly soluble in water and ethanol; soluble 1 in 100 of methanol; practically insoluble in chloroform and ether.

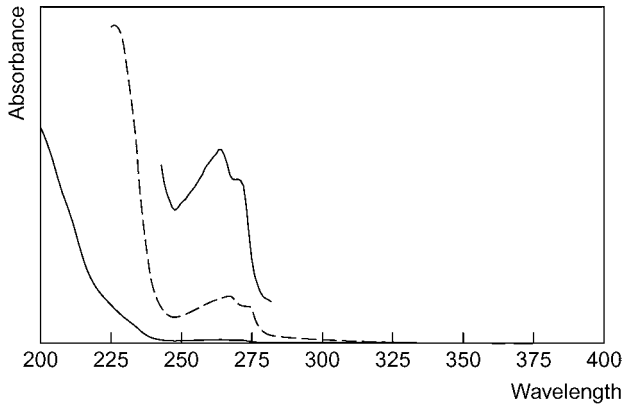
Colour Tests Mandelin's test—yellow; Marquis test—yellow.

Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 0.26; system TC—R_f 0.04; system TE—R_f 0.36; system TL—R_f 0.01; system TAE—R_f 0.01 (acidified potassium permanganate solution, positive).

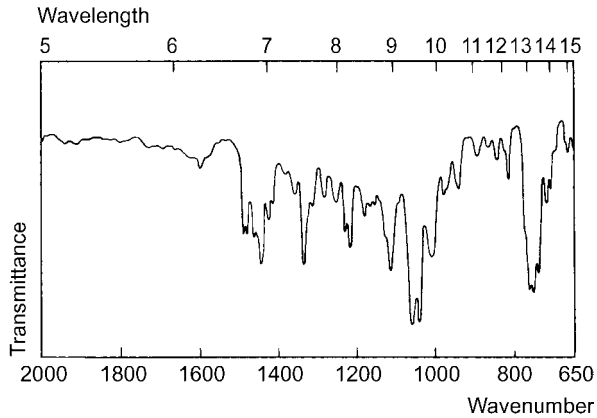
Gas Chromatography System GA—RI 2615.

High Performance Liquid Chromatography System HA—*k* 5.0 (tailing peak); system HX—RI 471; system HZ—retention time 10.3 min.

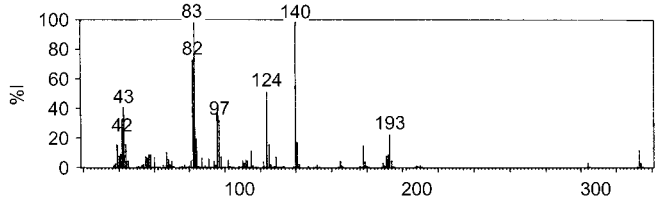
Ultraviolet Spectrum Methanol—266 (A_1^1=18.6b), 272 nm.



Infrared Spectrum Principal peaks at wavenumbers 1059, 1040, 751, 760, 738, 1114 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 83, 140, 82, 124, 43, 96, 97, 42.



Dose 2 mg of deptropine citrate daily.

Dequalinium Chloride

Antibacterial, Antifungal

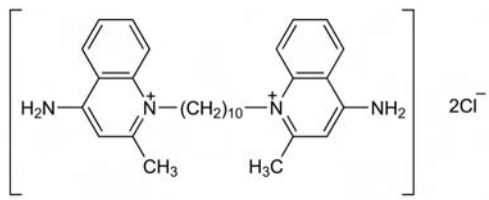
$C_{30}H_{40}Cl_2N_4 = 527.6$

CAS—6707-58-0 (dequalinium); 522-51-0 (chloride)

IUPAC Name 1-[10-(4-Amino-2-methylquinolin-1-ium-1-yl)decyl]-2- methylquinolin-1-ium-4-amine dichloride

Synonyms Decalinium chloride; decaminum; 1,1′-(1,10-decanediyl)bis-[4-amino-2-methylquinolinium chloride].

Proprietary Names *Dequadin*; *Dequavagyn*; *Labosept*; *Optipect Halstabletten*; *Phylletten*; *Sorot*.



Chemical Properties A creamy-white powder. Mp about 315°, with decomposition. Slightly soluble in water; soluble 1 in 30 of boiling water, 1 in 60 of methanol and 1 in 200 of propylene glycol. Log *P* (octanol/water), 4.3.

Dequalinium Acetate

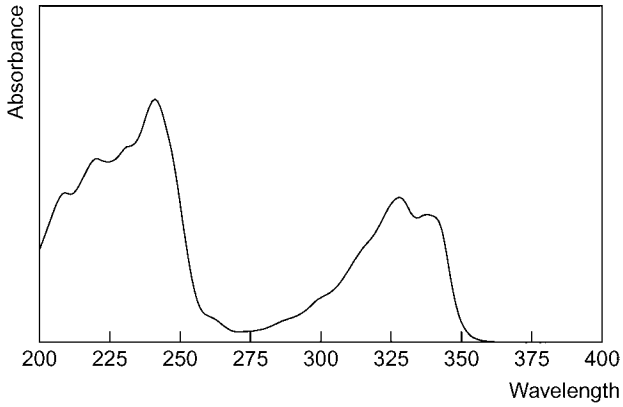
$C_{34}H_{46}N_4O_4 = 574.8$
CAS—4028-98-2

Chemical Properties A white or pinkish-buff, slightly hygroscopic powder. Mp about 280°, with decomposition. Soluble 1 in 2 of water and 1 in 12 of ethanol.

Colour Tests Aromaticity (method 2)—red/brown-violet; Liebermann's reagent—yellow (→orange at 100°).

Thin-layer Chromatography System TA—R_f 0.03 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Water—240 nm (A_1^1=812b), 326 nm (A_1^1=469b), 335 nm (A_1^1=406b); aqueous acid—241, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1605, 1660, 765, 1560, 1540, 1307 cm^{-1} (KCl disk).

Dose Lozenges containing 250 µg of dequalinium chloride are available.

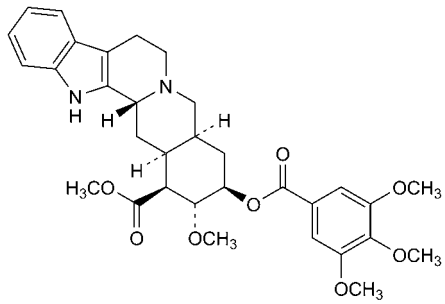
Deserpidine

Antihypertensive

$C_{32}H_{38}N_2O_8 = 578.7$
CAS—131-01-1

Synonyms Canescine; 11-demethoxyreserpine; methyl-(3β,16β,17α,18β,20α)-17-methoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]-3,20-yohimban-16-carboxylate.

Proprietary Names *Harmonyl*. It is an ingredient of *Enduronyl*.



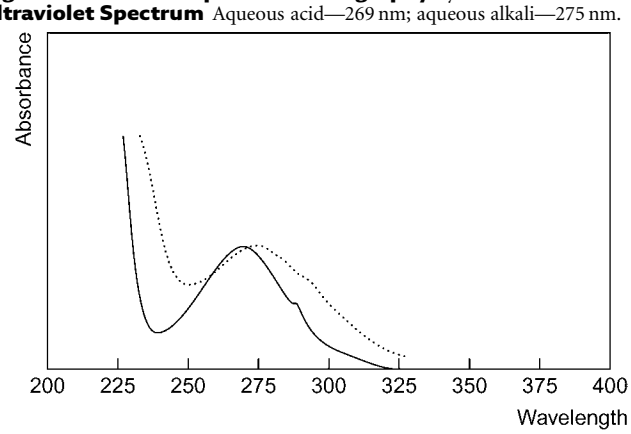
Chemical Properties An alkaloid isolated from the root of *Rauwolfia canescens* (Apocynaceae). Crystals. Mp 230° to 234°, with decomposition. Practically insoluble in water; soluble in hot ethanol and chloroform. Log *P* (octanol/water) 3.2.

Colour Tests Mandelin's test—blue→green; Marquis test—grey-green.

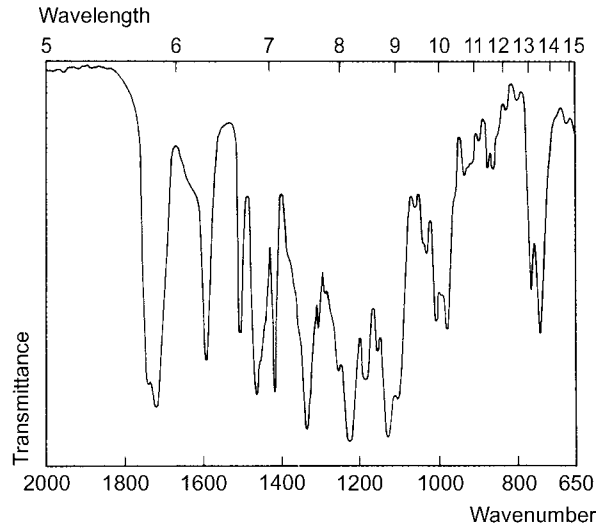
Thin-layer Chromatography System TA—R_f 0.72; system TAE—R_f 0.73; system TAG—R_f 0.66; system TB—R_f 0.03; system TC—R_f 0.77; system TE—R_f 0.81 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HA—*k* 0.4.

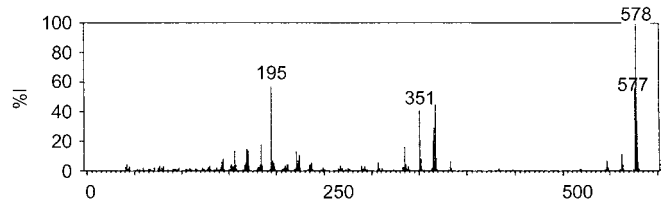
Ultraviolet Spectrum Aqueous acid—269 nm; aqueous alkali—275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1225, 1127, 1715, 1100, 1184, 1590 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 578, 195, 577, 367, 351, 579, 366, 365.



Dose 0.25 to 1 mg daily.

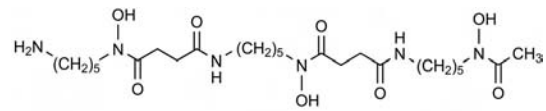
Desferrioxamine

Chelating Agent

$C_{25}H_{48}N_6O_8 = 560.7$
CAS—70-51-9

IUPAC Name *N*′-[5-[[4-[[5-(Acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]-hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide

Synonyms Deferoxamine; DFM; DFOM.



Chemical Properties Log *P* (octanol/water), −2.5.

Desferrioxamine Mesilate

$C_{25}H_{48}N_6O_8,CH_3SO_3H = 656.8$
CAS—138-14-7

Synonyms Deferoxamine mesilate; desferrioxamine B mesilate; desferrioxamine methanesulfonate.

Proprietary Name *Desferal*

Chemical Properties A white to cream-coloured powder. Soluble 1 in 5 of water and 1 in 20 of ethanol; practically insoluble in dehydrated alcohol, chloroform and ether.

Colour Test Mandelin's test—blue→violet.

Thin-layer Chromatography System TA—R_f 0.08, streaking (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1628, 1195, 1562, 1265, 1149, 1041 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection (λ=430 nm). Limit of quantification, 1.0 mg/L desferrioxamine, 5.0 mg/L ferrioxamine, limit of detection, 0.313 mg/L desferrioxamine, 0.039 mg/L ferrioxamine [Kraemer, Breithaupt 1998].

Disposition in the Body Poorly absorbed after oral administration. It specifically chelates ferric iron to an octahedral complex, ferrioxamine, which is readily excreted in the urine. Theoretically, 100 mg of desferrioxamine can chelate approximately 8.5 mg of iron. About 13 to 65% of a ^{59}Fe-labelled dose is excreted in the urine in 24 h.

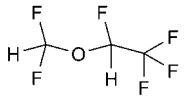
Dose In acute iron poisoning: 5 g of desferrioxamine mesilate by mouth, with 2 g IM, and up to 15 mg/kg/h by IV infusion.

Kraemer HJ, Breithaupt H (1998). Quantification of desferrioxamine, ferrioxamine and aluminoxamine by post-column derivatization high-performance liquid chromatography. Non-linear calibration resulting from second-order reaction kinetics. *J Chromatogr B Biomed Sci Appl* 710: 191–204.

Desflurane

Anaesthetic (General)
$C_3H_2F_6O = 168.0$
CAS—57041-67-5

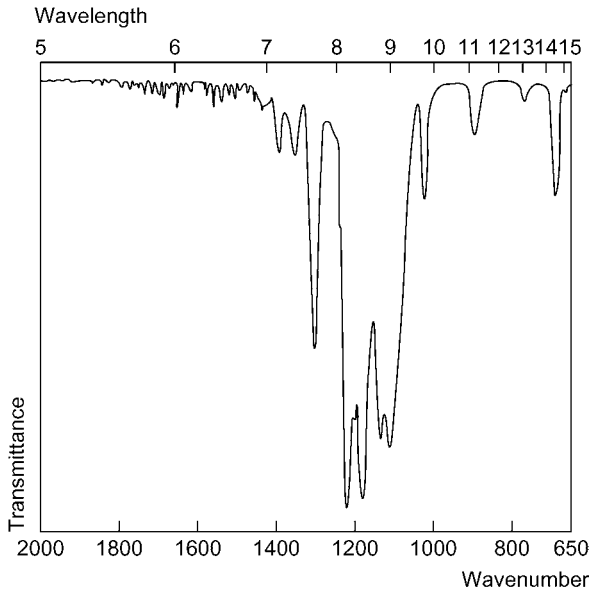
IUPAC Name 2-(Difluoromethoxy)-1,1,1,2-tetrafluoroethane
Synonym (±)-2-(Difluoromethyl)-1,2,2,2-tetrafluoroethyl ether
Proprietary Name *Suprane*



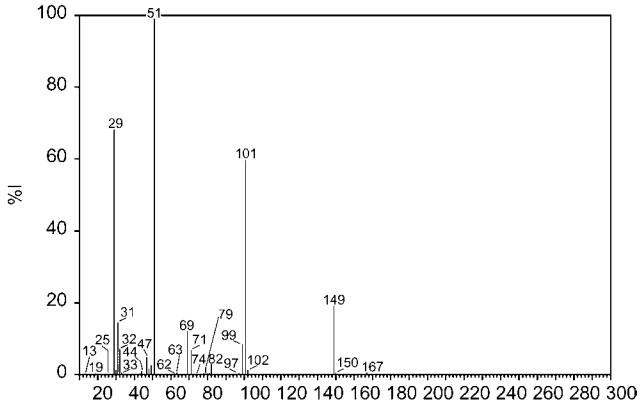
Chemical Properties A non-flammable colourless volatile liquid with a slight non-pungent odour. Mp −126°. Bp 23.5°. Density 1.44. Log *P* (oil/gas) 18.7 (37°), Log *P* (blood/gas), 0.424 (37°) [Caldwell 1994], 0.45 [Chidiac 2004], Log *P* (octanol/water), 1.20, Log *P* (fat/blood), 27.2, Log *P* (brain/blood), 1.29 [Yasuda *et al.* 1989]. Stable in soda lime [Pihlainen, Ojanpera 1998].

Gas Chromatography Column: Octakis (3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin on polysiloxane SE-54 (1 m × 24 mm i.d. or 1 m × 6 mm i.d.). Carrier gas: N_2, 1 or 3 bar for the first and second column, respectively. Temperature: 100°. FID [Juza *et al.* 1997].

Infrared Spectrum Principal peaks at wavenumbers 1219, 1180, 1112, 1135, 1304, 696 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 51, 29, 101, 149, 31, 69, 99, 32.



Quantification
Blood **GC** Column: HP-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: N_2, 3.0 mL/min. Temperature programme: 40° to 200° at 15°/min for 4.33 min. FID. Limit of detection not reported [Lu *et al.* 2004]. Column: PoraPLOT Q (25 m × 0.32 mm, 10 µm). Carrier gas: He, 3.8 mL/min. Temperature programme: 30° for 2 min to 250° at 15°/min for 5 min. FID, FTIR. Reference compound: diethyl ketone. Relative retention time: 0.65. Limit of detection, 0.13 mg/L [Ojanperä *et al.* 1998].

GC-MS Column: DB-5 capillary (60 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 40 cm/s. Temperature programme: 35° for 3.5 min to 120° at 40°/min for 0.68 min. EI ionisation at 70 eV, full scan and SIM acquisition modes. Retention time: 2.82 min. Limit of quantification, 9.2 mg/L, limit of detection, 2.3 mg/L [Yang *et al.* 2001].

Disposition in the Body Absorption, distribution and elimination after inhalation are more rapid than for other halogenated anaesthetics such as isoflurane or halothane. This is due to the low blood/gas coefficient of desflurane. About 0.02% of administered desflurane is metabolised in the liver; trifluoroacetic acid has been detected in the serum and urine of patients given desflurane. The majority of desflurane is excreted mainly unchanged through the lungs. A very small amount diffuses through the skin.

Therapeutic Concentration
The mean maximum serum concentration of trifluoroacetic acid in 13 subjects was 0.38 µmol/L at 24 h after exposure to desflurane for a mean of 6.81 h. Concentrations remained elevated for 6 days after exposure. [Sutton *et al.* 1991].

Toxicity Desflurane use is associated with carbon monoxide production and sympathetic activation [Berry *et al.* 1999; Umbrain *et al.* 2002].

Note For adult cases of desflurane-induced hepatotoxicity, see Martin *et al.* [1995], Berghaus *et al.* [1999], Chung *et al.* [2003] or Tung *et al.* [2005]. For a paediatric case of hepatotoxicity in a 15-month-old with Mobius syndrome, see Côté and Bouchard [2007].

Clearance Plasma, 4.6 L/min; pulmonary, 4.11 L/min.

Note For reviews of desflurane, see Koblin [1992] and Caldwell [1994]. For a review of the analytical toxicology of fluorinated inhalation anaesthetics, see Pihlainen and Ojanpera [1998].

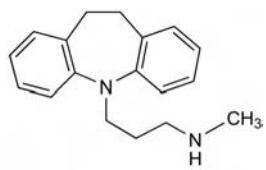
Dose Using a calibrated vaporiser, concentrations of 4 to 11% are required for induction of anaesthesia. For maintenance, concentrations between 2 and 6% (in nitrous oxide); 2.5 to 8.5% (in oxygen or oxygen-enriched air); maximum, 17%.

Berghaus TM *et al.* (1999). Hepatotoxicity following desflurane anesthesia. *Hepatology* 29: 613–614.
Berry PD *et al.* (1999). Severe carbon monoxide poisoning during desflurane anesthesia. *Anesthesiology* 90: 613–616.
Caldwell JE (1994). Desflurane clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 27: 6–18.
Chidiac EJ (2004). Desflurane vs. sevoflurane—a review. *Middle East J Anesthesiol* 17: 791–810.
Chung PC *et al.* (2003). Reproducible hepatic dysfunction following separate anesthesia with sevoflurane and desflurane. *Chang Gung Med J* 26: 357–362.
Côté G, Bouchard S (2007). Hepatotoxicity after desflurane anesthesia in a 15-month-old child with Mobius syndrome after previous exposure to isoflurane. *Anesthesiology* 107: 843–845.
Juza M *et al.* (1997). Preparative enantiomer separation of the inhalation anesthetics enflurane, isoflurane and desflurane by gas chromatography on a derivatized gamma-cyclodextrin stationary phase. *J Chromatogr A* 769: 119–127.
Koblin DD (1992). Characteristics and implications of desflurane metabolism and toxicity. *Anesth Analg* 75: S10–S16.
Lu CC *et al.* (2004). Pharmacokinetics of desflurane uptake into the brain and body. *Anaesthesia* 59: 216–221.
Martin JL *et al.* (1995). Hepatotoxicity after desflurane anesthesia. *Anesthesiology* 83: 1125–1129.
Ojanperä I *et al.* (1998). Identification limits for volatile organic compounds in the blood by purge-and-trap GC-FTIR. *J Anal Toxicol* 22: 290–295.
Pihlainen K, Ojanpera I (1998). Analytical toxicology of fluorinated inhalation anaesthetics. *Forensic Sci Int* 97: 117–133.
Sutton TS *et al.* (1991). Fluoride metabolites after prolonged exposure of volunteers and patients to desflurane. *Anesth Analg* 73: 180–185.
Tung D *et al.* (2005). Severe desflurane hepatotoxicity after colon surgery in an elderly patient. *Can J Anaesth* 52: 133–136.
Umbrain V *et al.* (2002). Desflurane: a reappraisal. *Acta Anaesthesiol Belg* 53: 187–191.
Yang NC *et al.* (2001). Simultaneous determination of fluorinated inhalation anesthetics in blood by gas chromatography-mass spectrometry combined with a headspace autosampler. *J Chromatogr B Biomed Sci Appl* 759: 307–318.
Yasuda N *et al.* (1989). Solubility of I-653, sevoflurane, isoflurane, and halothane in human tissues. *Anesth Analg* 69: 370–373.

Desipramine

Antidepressant
$C_{18}H_{22}N_2 = 266.4$
CAS—50-47-5

IUPAC Name 10,11-Dihydro-*N*-methyl-*5H*-dibenz[*b,f*]azepine-5-propanamine
Synonyms Desmethylimipramine; DMI.

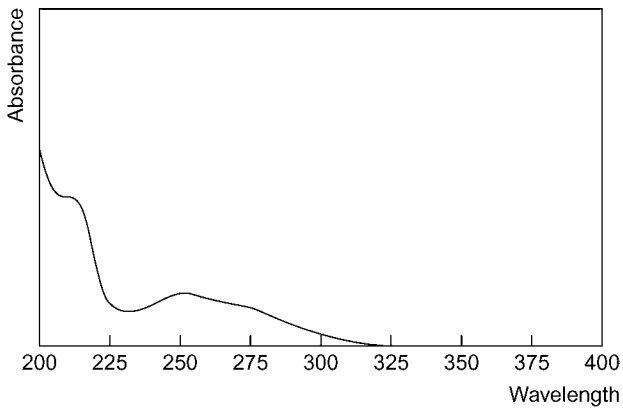


Chemical Properties pK_a 10.4 [Sangster 1997]. Log *P* (octanol/water), 4.9 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

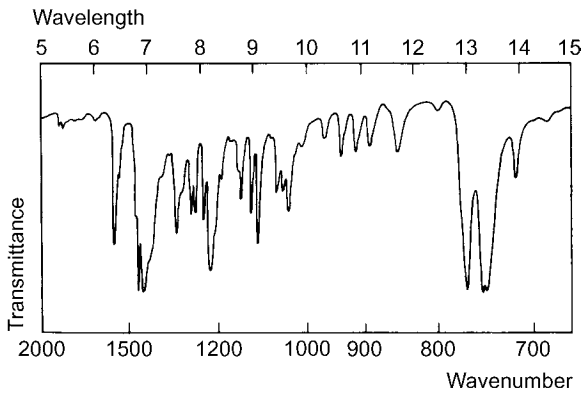
Desipramine Hydrochloride

$C_{18}H_{22}N_2$,HCl = 302.8
CAS—58-28-6
Synonyms Desipramini hydrochloridum; EX-4355; G-35020; JB-8181; NSC-114901; RMI-9384A.
Proprietary Names *Deprexan*; *Norpramin*; *Nortimil*; *Pertofran(e)*; *Petylyl*.
Chemical Properties A white crystalline powder. Mp 215° to 216°. Soluble 1 in 20 of water, 1 in 20 of ethanol and 1 in ~4 of chloroform; practically insoluble in ether; freely soluble in methanol, 10.2 (24°). Log *P* (octanol/water), 2.94 [Meylan, Howard 1995], (octanol/pH 7.4), 1.4.

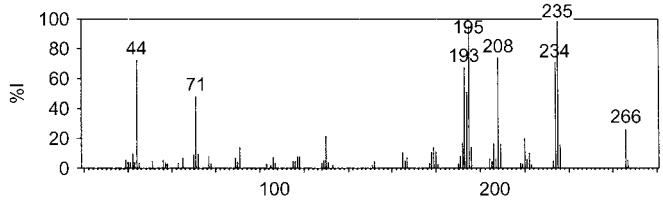
Colour Test Forrest reagent—blue; Mandelin's test—yellow→blue.
Thin-layer Chromatography System TA—R_f 0.26; system TB—R_f 0.19; system TC—R_f 0.11; system TE—R_f 0.40; system TL—R_f 0.03; system TAE—R_f 0.07; system TAF—R_f 0.71; system TAJ—R_f 0.07; system TAK—R_f 0.23; system TAL—R_f 0.72 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; FPN reagent, blue; Marquis test, blue).
Gas Chromatography System GA—desipramine RI 2235, M (2-OH-) RI 2553, M (ring) RI 1930, M (ring-OH-) RI 2240, M (ring-di-OH-) RI 2600, M (acetyl-) RI 2670, M (ring-OH-methoxy-) RI 2390; system GB—desipramine RI 2338, M (2-OH-) RI 2669, M (10-OH-) RI 2521, M (ring) RI 2014, M (OH-ring) RI 2335, M (di-OH-) RI 2995, M (acetyl-) RI 2811, M (OH-methoxy-) RI 2749; system GM—desipramine RRT 0.896 (relative to iprindole); system GS—RT 18.7 min.
High Performance Liquid Chromatography System HA—desipramine *k* 2.1, didesmethylimipramine *k* 1.3, M (2-OH-) *k* 1.2; system HF—*k* 3.60; system HX—RI 424; system HY—RI 361; system HZ—RT 5.9 min; system HAA—RT 14.9 min; system HAM—RT 9.1 min; system HAX—RT 13.0 min; system HAY—RT 6.3 min; system HAZ—desipramine *k* 1.52, M (2-OH-) *k* 0.39.
Ultraviolet Spectrum Aqueous acid—250 nm (A^1_1 = 308a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 746, 741, 763, 1230, 1590, 1104 cm^{-1} (desipramine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 235, 195, 208, 44, 234, 193, 194, 71; 2-hydroxydesipramine 44, 209, 211, 250, 210, 224, 42, 251.



Quantification See also under Imipramine.
Blood **GC** Column: cross-linked methylsilicone (25 m × 4.6 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 130 µg/L, limit of detection, 39 µg/L [Martinez *et al.* 2002].
Plasma **GC** Column: 5% phenyl methyl silicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 150° to 300° at 10°/min. NPD. Relative retention time: 0.923. Limit of quantification, 13.3 µg/L, limit of detection, 4.0 µg/L [de la Torre *et al.* 1998]. Column: DB-17 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 260° for 1 min. NPD detection. Limit of quantification, 90 µg/L [Ulrich, Martens 1997]. Carrier gas: N_2, 17 mL/min. Temperature: 243°. AFID. Limit of detection, 1 µg/L [Antal *et al.* 1980].
GC-MS Column: Ultra-1 cross-linked methyl silicone (8 m × 0.31 mm i.d., 0.17 mm). Carrier gas: He, 28 cm/s. Temperature programme: 150° to 240° at 30°/min for 7 min to 300° at 10°/min. Limit of quantification, 25 µg/L [Way *et al.* 1998].
HPLC Column: Nova-Pack C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 0.02 mol/L TEA (pH 5.5, 35 : 65), flow rate 1.0 mL/min. UV detection (λ = 215 nm). Limit of detection, 1 µg/L [Bakkali *et al.* 1999]. Column: Nova-Pak C_{18}. Mobile phase: phosphate buffer with 50% acetonitrile and ~0.2% diethylamine (pH 8). UV detection (λ = 242 nm). Limit of detection, 20 µg/L [Theurillat, Thormann 1998]. Column: Reversed phase. Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen phosphate (pH 6.0, 30 : 70), flow rate 2 mL/min. Electrochemical detection. Limit of quantification, 3 µg/L [Chen *et al.* 1997]. Column: Supelco PCN. Mobile phase: acetonitrile : methanol : 0.015 mol/L phosphate buffer (120 : 35 : 100). Limit of quantification, 25 nmol/L [Elm, Hansen 1995]. Column: reversed phase C_{18}. Electrochemical detection. Limit of detection, 0.3 µg/L [Koyama *et al.* 1993]. See also Foglia *et al.* [1991] and Queiroz *et al.* [1995].
LC-MS Column: Sunfire C_{18} IS (20 m × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate (pH 3). ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 µg/L [de Castro *et al.* 2008]. Column: RP-18 column (150 × 2.1 mm, 5 µm). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 5.5) : acetonitrile (50 : 50). Limit of detection, 100 ng/L [Alves *et al.* 2007]. Column: Inertsil C_8. Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. Limit of detection, 30–630 µg/L [Shinozuka *et al.* 2006]. Column: SB-C_{18} (15 m × 2.1 mm i.d., 3 µm). Mobile phase: 3 mmol/L ammonium acetate (pH 3.3) : acetonitrile (66 : 34), flow rate 1.4 mL/min. API-TOF, positive ion mode. Limit of quantification, 2 µg/L [Zhang *et al.* 2000].
Serum **GC** See Plasma [Antal *et al.* 1980].
HPLC Column: octyl reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3, 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 254 nm). k': 2.282. Limit of detection, ~10 µg/L [Segatti *et al.* 1991].
Urine **HPLC** See Plasma [Chen *et al.* 1997].
Disposition in the Body Desipramine is well absorbed after oral administration. It is metabolised by aromatic hydroxylation and possibly hydroxylamine formation; *N*-demethylation to form didesmethylimipramine may occur to a minor extent. 2-Hydroxydesipramine, the major metabolite, is active. Less than 5% of a dose is excreted in urine in 24 h as unchanged drug; the urinary excretion of unchanged drug is pH dependent and is increased in acidic urine; the hydroxylated metabolites are excreted mainly as glucuronide conjugates. The extent of 2-hydroxylation is genetically determined. Desipramine is the major active metabolite of imipramine and lofepramine.
Therapeutic Concentration Plasma concentrations vary considerably between individual subjects.

After administration of a single oral dose of 50 mg to 4 subjects, peak plasma-desipramine concentrations of 0.008–0.015 mg/L (mean 0.012) were attained in 3–6 h; peak plasma concentrations of 2-hydroxydesipramine of 0.011–0.013 mg/L were attained in ~2–4 h [DeVane *et al.* 1981].

Following daily oral doses of ~2.5 mg/kg to 47 subjects, minimum steady-state plasma concentrations of 0.02–0.88 mg/L (mean 0.17) desipramine and 0.007–0.13 mg/L (mean 0.04) 2-hydroxydesipramine were reported [Bock *et al.* 1983].

Of 34 elderly patients treated for depression with desipramine for 4 weeks at a fixed dosage regimen, 12 non-responders received a second period of treatment at an increased dose. In 16 patients aged over 74 years, a daily dose of 2.45 mg/kg resulted in a mean plasma level of 0.118 mg/L for desipramine and 0.072 mg/L for hydroxydesipramine; in 45 patients aged less than 60 years (including some patients from previously published work), a daily dose of 2.6 mg/kg resulted in a mean plasma level of 0.142 mg/L for desipramine and 0.041 mg/L for hydroxydesipramine. A plasma concentration of 0.105 mg/L significantly separated responders from non-responders, although the response rate was still low relative to rates in prior studies of younger patients [Nelson *et al.* 1995].

Toxicity Plasma concentrations >0.4 mg/L may produce toxic effects. Fatalities are comparatively rare but may occur with plasma concentrations >10 mg/L.

Recovery has occurred in 2 subjects after the ingestion of up to 2 g; peak plasma concentrations of 1.56 mg/L and 1.58 mg/L were reported [Biggs *et al.* 1977].

In a fatal case, a blood concentration of 3 mg/L and a liver concentration of 140 µg/g were reported [Baselt, Cravey 1977].

The following postmortem concentrations were found after the death of a 2-year-old child: blood 8 mg/L, liver blood 22 mg/L, bile 67 mg/L, urine 2 mg/L [Robinson *et al.* 1979].

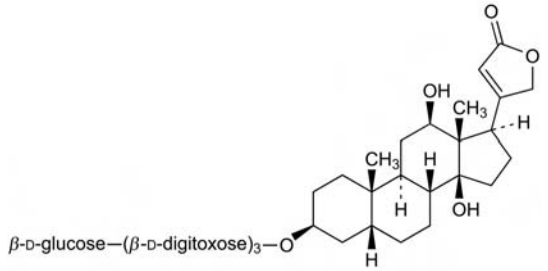
Note For a review of tricyclic antidepressant overdose, see Kerr *et al.* [2001].
Half-life Plasma half-life, 10–35 h.
Protein Binding ≈70–90%.
Note For a review of the clinical pharmacokinetics of imipramine and desipramine, see Sallee, Pollock [1990]. For a review of the pharmacokinetics of tricyclic antidepressants see Molnar, Gupta [1980].
Dose Desipramine hydrochloride 25 to 200 mg daily; up to 300 mg daily has been given.

Alves C *et al.* (2007). Analysis of tricyclic antidepressant drugs in plasma by means of solid-phase microextraction–liquid chromatography–mass spectrometry. *J Mass Spectrom* 42: 1342–1347.
Antal E *et al.* (1980). Technical considerations in the gas chromatographic analysis of desipramine. *J Chromatogr* 183: 149–157.
Bakkali A *et al.* (1999). Solid-phase extraction with liquid chromatography and ultraviolet detection for the assay of antidepressant drugs in human plasma. *Talanta* 49: 773–783.
Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–103.
Biggs JT *et al.* (1977). Tricyclic antidepressant overdose: incidence of symptoms. *JAMA* 238: 135–138.
Bock JL *et al.* (1983). Desipramine hydroxylation: variability and effect of antipsychotic drugs. *Clin Pharmacol Ther* 33: 322–328.
Chen AG *et al.* (1997). Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 693: 153–158.
de Castro A *et al.* (2008). LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. *J Pharm Biomed Anal* 48: 183–193.
de la Torre R *et al.* (1998). Quantitative determination of tricyclic antidepressants and their metabolites in plasma by solid-phase extraction (Bond-Elut TCA) and separation by capillary gas chromatography with nitrogen–phosphorous detection. *Ther Drug Monit* 20: 340–346.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.
DeVane CL *et al.* (1981). Desipramine and 2-hydroxy-desipramine pharmacokinetics in normal volunteers. *Eur J Clin Pharmacol* 19: 61–64.
Elm T, Hansen EL (1995). Simultaneous determination of lofepramine and desipramine by a high-performance liquid chromatographic method used for therapeutic drug monitoring. *J Chromatogr B Biomed Appl* 665: 355–361.
Foglia JP *et al.* (1991). Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection. *J Chromatogr* 572: 247–258.
Hansch C *et al.* (1995). Exploring QSAR: *Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.
Kerr GW *et al.* (2001). Tricyclic antidepressant overdose: a review. *Emerg Med J* 18: 236–241.
Koyama E *et al.* (1993). Simultaneous high-performance liquid chromatography–electrochemical detection determination of imipramine, desipramine, their 2-hydroxylated metabolites, and imipramine *N*-oxide in human plasma and urine: preliminary application to oxidation pharmacogenetics. *Ther Drug Monit* 15: 224–235.
Martinez MA *et al.* (2002). Simultaneous determination of viloxazine, venlafaxine, imipramine, desipramine, sertraline, and amoxapine in whole blood: comparison of two extraction/cleanup procedures for capillary gas chromatography with nitrogen–phosphorus detection. *J Anal Toxicol* 26: 296–302.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Molnar G, Gupta RN (1980). Plasma levels and tricyclic antidepressant therapy. Part 2. Pharmacokinetic, clinical and toxicologic aspects. *Biopharm Drug Dispos* 1: 283–305.
Nelson JC *et al.* (1995). Desipramine treatment of major depression in patients over 75 years of age. *J Clin Psychopharmacol* 15: 99–105.
Queiroz RH *et al.* (1995). Simultaneous HPLC analysis of tricyclic antidepressants and metabolites in plasma samples. *Pharm Acta Helv* 70: 181–186.
Robinson AE *et al.* (1979). Tricyclic and tetracyclic antidepressant drugs: forensic toxicology of some autopsy cases. *J Anal Toxicol* 3: 3–13.
Sallee FR, Pollock BG (1990). Clinical pharmacokinetics of imipramine and desipramine. *Clin Pharmacokinet* 18: 346–364.
Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry*. Chichester, UK: Wiley.
Segatti MP *et al.* (1991). Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis. *J Chromatogr* 536: 319–325.
Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.
Theurillat R, Thormann W (1998). Monitoring of tricyclic antidepressants in human serum and plasma by HPLC: characterization of a simple, laboratory developed method via external quality assessment. *J Pharm Biomed Anal* 18: 751–760.
Ulrich S, Martens J (1997). Solid-phase microextraction with capillary gas–liquid chromatography and nitrogen–phosphorus selective detection for the assay of antidepressant drugs in human plasma. *J Chromatogr B Biomed Sci Appl* 696: 217–234.
Way BA *et al.* (1998). Isotope dilution gas chromatographic-mass spectrometric measurement of tricyclic antidepressant drugs: utility of the 4-carbethoxyhexafluorobutyryl derivatives of secondary amines. *J Anal Toxicol* 22: 374–382.
Zhang H *et al.* (2000). Atmospheric pressure ionization time-of-flight mass spectrometry coupled with fast liquid chromatography for quantitation and accurate mass measurement of five pharmaceutical drugs in human plasma. *J Mass Spectrom* 35: 423–431.

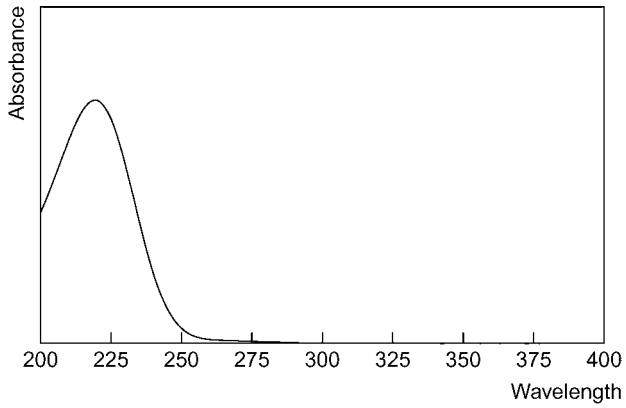
Deslanoside

Cardiac Glycoside
$C_{47}H_{74}O_{19}$ = 943.1
CAS—17598-65-1

IUPAC Name 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-12,14-Dihydroxy-3-[(2*R*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-6-methyl-5-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[*a*]phenanthren-17-yl]-2*H*-furan-5-one
Synonyms Deacetyl-lanatoside C; desacetyl-lanatoside C; (3β,5β,12β)-3-[(*O*-β-D-Glucopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxycard-20(22)-enolide.
Proprietary Names *Cedilanid-D*; *Cedilanid(e)*; *Desace*.
Note The name Cedilanid(e) is also applied to preparations of lanatoside C.



Chemical Properties White hygroscopic crystals or crystalline powder. Mp about 220°. Practically insoluble in water; soluble 1 in 2500 of ethanol and 1 in 200 of methanol; very slightly soluble in chloroform; practically insoluble in ether.
Colour Test Dissolve 2 to 3 mg in 5 mL of a solution containing 0.5 mL of a 9% ferric chloride solution and 100 mL of acetic acid; underlay with 5 mL of sulfuric acid—intense blue in the acetic acid layer and a brown ring free from red at the junction of the two liquids (see also Lanatoside C). (Perchloric acid solution, followed by examination under UV light, blue fluorescence; *p*-anisaldehyde reagent, blue.)
Ultraviolet Spectrum Principal peak at 221 nm.



Disposition in the Body Incompletely absorbed after oral administration and metabolised to digoxin. After IV administration, about 80% of a single dose is excreted in the urine in 72 h, mostly as unchanged drug, and about 10% of a dose is eliminated in the faeces. During maintenance treatment, about 30% of a daily dose is excreted in the 24-h urine. After oral administration digoxin accounts for about 50% of the total urinary excretion material.

Deslanoside is a metabolite of lanatoside C.

Therapeutic Concentration

After an IV dose of 400 µg given to 5 subjects, a mean plasma concentration of 0.03 mg/L was reported at 15 min, declining to 0.001 mg/L at 48 h; after an oral dose of 800 µg given to 5 subjects, a mean peak plasma concentration of 0.003 mg/L was attained in 2 h [Marzo *et al.* 1982].

Half-life Plasma half-life, about 33 h.
Distribution in Blood Plasma : whole blood ratio, 1.4.
Protein Binding Weakly bound.
Dose For rapid digitalisation, 0.8 to 1.6 mg intravenously.

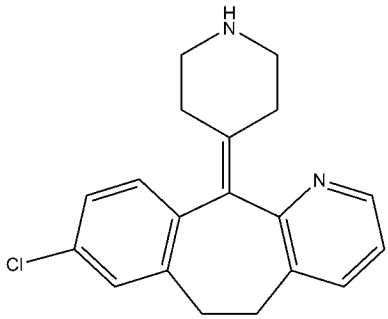
Marzo A *et al.* (1982). Pharmacokinetics of deslanoside C-3H administered orally to healthy volunteers. *Farmaco Prat* 37(1): 28–37.

Desloratadine

Antihistamine, Histamine H_1-Antagonist, Piperidine Derivative
$C_{19}H_{19}ClN_2$ = 310.8
CAS—100643-71-8

IUPAC Name
Synonyms 8-Chloro-6,11-dihydro-11-(4-piperdinylidene)-5*H*-benzo[5,6]cyclohepta[1,2-b]pyridine; descarboethoxyloratadine; desloratadin; desloratadina; Sch-34117.

Proprietary Names *Aerius*; *Aviant*; *Azomyr*; *Claramax*; *Desalex*; *Deslorat*; *Esparflin*; *Frenaler*; *Hexaler*; *Mailen*; *Neo Larmax*; *Neoclaritine*; *Neohysticlar*; *Novo Alerpriv*; *Rinaid*; *Rinofilax*. It is also an ingredient in *Clarinex-D*.

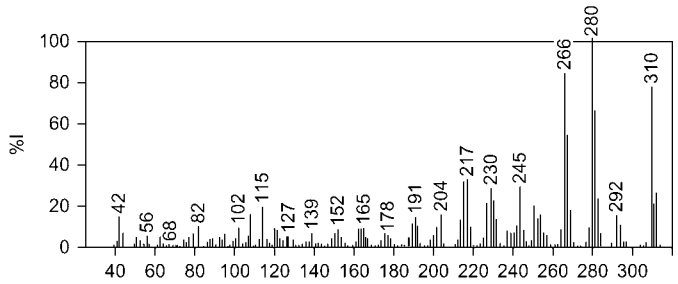


Chemical Properties Crystals. Mp 150° to 151°. Slightly soluble in water; very soluble in ethanol, propylene glycol. Log *P* (octanol/water), 3.2 [Wishart 2006]. Stable in stock solutions for up to 274 days. Stable in human plasma samples for 162 h at room temperature [Shen *et al.* 2006].

High Performance Liquid Chromatography Column: Diamonsil BDS C_{18} (150 × 5.0 mm i.d., 5 µm). Mobile phase: methanol : 0.03 mol/L heptanesulfonic acid sodium : glacial acetic acid (70 : 30 : 4), flow rate 1.0 mL/min. UV detection (λ = 247 nm). Retention time: 5.4 min. Limit of quantification, 0.1 mg/L [Qi *et al.* 2005].

Mass Spectrum

Prinipal ions at *m/z* 280, 266, 310, 281, 267, 217, 216, 245.



Quantification

Plasma **LC-MS** Column: Betasil Silica-100 (100 × 2.0 mm i.d., 5 µm). Mobile phase: 25 mmol/L ammonium acetate in 1% formic acid : 25 mmol/L ammonium acetate in methanol (15 : 85), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.4 min. Limit of quantification, 0.1 ng/L [Shen *et al.* 2007]. Column: C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate : methanol-acetonitrile (3 : 2; 50 : 50), flow rate 0.2 mL/min. Retention time: desloratadine 1.42 min, 3-hydroxydesloratadine 1.19 min. Limit of quantification, 0.05 µg/L for desloratadine and 3-hydroxydesloratadine [Xu *et al.* 2007]. Column: C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium formate with 0.2% formic acid : 10 mmol/L ammonium formate in methanol with 0.2% formic acid (20 : 80 for 0.5 min to 90 : 10 over 2.8 min for 0.5 min), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.955 pg for desloratadine, 1.05 pg for 3-hydroxydesloratadine[Shen *et al.* 2006]. Column: C_{18} (50 × 2.1 mm i.d., 1.7 µm). Mobile phase: 10 mmol/L ammonium formate with 0.2% formic acid : 10 mmol/L ammonium formate in methanol with 0.2% formic acid (20 : 80 for 0.25 min to 90 : 10 over 1.4 min for 0.25 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, desloratadine 478 pg, 3-hydroxyloratadine 525 pg [Shen *et al.* 2006]. Column: C_{18} (50 × 4.6 mm i.d.). Mobile phase: acetonitrile : 3 mmol/L ammonium acetate (80 : 20), flow rate 0.8 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 2.37 min. Limit of quantification, 0.05 µg/L [Yeh *et al.* 2004]. See also [Yang *et al.* 2003].

Serum **HPLC** Column: Cyanopropyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 12 mmol/L ammonium acetate (pH 3.55; 45 : 55), flow rate 1.5 mL/min. UV detection (λ = 243 nm). Limit of quantification, 0.15 µg/L; limit of detection, 0.045 µg/L [Emara *et al.* 2007].

Other **HPLC** Tablets. Column: Diamonsil BDS C_{18} (150 × 5.0 mm i.d., 5 µm). Mobile phase: methanol : 0.03 mol/L heptanesulfonic acid sodium : glacial acetic acid (70 : 30 : 4), flow rate 1.0 mL/min. UV detection (λ = 247 nm). Retention time: 5.4 min. Limit of quantification, 0.1 mg/L [Qi *et al.* 2005]. Canine Plasma. Column: Hypersil CN (150 × 5.0 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile: 0.01 mol/L potassium dihydrogen phosphate (pH 5.5, 35 : 35 : 30), flow rate 0.8 mL/min. UV detection (λ = 241 nm). Retention time: desloratadine 9.5 min, *N*-methyldesloratadine 11.0 min. Limit of quantification, 5 µg/L [Liu *et al.* 2004].

Disposition in the Body Desloratadine is the major, active metabolite of loratadine. It is rapidly absorbed following oral administration, with peak plasma concentrations occurring after ≈3 h. It is extensively metabolised to its principal active metabolite, 3-hydroxydesloratadine, which is subsequently deactivated by glucuronidation. Other metabolites include the 5- and 6-hydroxy forms of desloratadine, which are further conjugated. The CYP isoenzyme involved in the metabolism has not yet been identified but CYP3A4 and CYP2D6 are unlikely to be extensively involved. Approximately 40% of an administered dose is excreted as metabolites in urine and 45% in the faeces. Desloratadine is excreted into breast milk. Although ≈20% of African Americans and 6 to 7% of the general population are slow metabolisers of desloratadine, the clinical significance of this is unknown.

Therapeutic Concentration

Two groups of children (group A: aged 2 to 5 years; group B: aged 6 to 11 years) were administered desloratadine syrup (0.5 mg/L) orally via syringe. Group A were administered the equivalent of 1.25 mg desloratadine and group B 2.5 mg. Peak plasma concentrations for desloratadine and 3-hydroxydesloratadine as well as some pharmacokinetic parameters were reported as follows:

Parameter	*Group A*	*Group B*
Desloratadine		
C_{max} (µg/L)	2.68	2.23
Time (h)	3.17	3.67
Half-life (h)	16.4	19.4
Clearance (L/h)	35.8	73.7
Volume of distribution (L)	707	1619
3-Hydroxydesloratadine		
C_{max} (µg/L)	0.644	0.764
Time (h)	4.89	4.44
Half-life (h)	26.2	28.1

[Gupta *et al.* 2007a].

Two groups of subjects (group A: 9 with normal hepatic function, group B: 12 with moderate hepatic impairment) were administered desloratadine 5 mg once daily for 10 days. Peak plasma concentrations of desloratadine and 3-hydroxydesloratadine on day 10 were as follows:

	Group A	*Group B*
Desloratadine		
C_{max} (µg/L)	6.47	7.94
Time (h)	5.61	4.13
3-Hydroxydesloratadine		
C_{max} (µg/L)	1.20	1.30
Time (h)	5.72	5.75

Healthy volunteers in Group A were phenotyped as poor metabolisers and normal metabolisers. Plasma concentrations on day 10 were reported as:

	Normal	*Poor*
Desloratadine		
C_{max} (µg/L)	3.88	11.7
Time (h)	4.75	7.33
3-Hydroxydesloratadine		
C_{max} (µg/L)	1.69	0.219
Time (h)	5.42	6.33

In a separate analysis of the data, in subjects with moderate hepatic impairment, the mean peak plasma concentration of desloratadine on day 10 of African American subjects was ≈1.5 times that found in Caucasian subjects. In healthy African American volunteers, the increase was approximately 3-fold when compared with healthy Caucasian subjects [Gupta *et al.* 2007b].

Eight healthy Chinese men (aged 23 years) were administered a single oral dose of 10 mg desloratadine. The mean peak plasma concentration was 6.8 µg/L after 1.85 h [Yeh *et al.* 2004].

In a dose-ranging study, 20 healthy male volunteers (3 Caucasians, 17 African Americans) were administered single doses of desloratadine: 5, 7.5, 10 or 20 mg. Peak plasma concentrations were 2.18, 3.03, 3.80, and 8.08 µg/L for the escalating doses, respectively, all attained within 3 h [Gupta *et al.* 2002].

Toxicity Desloratadine was a 4-fold weaker inhibitor of the intestinal wall p-glycoprotein (pGp) transport system *in vitro* than loratadine and, therefore, has a lower potential for intestinal pGp-mediated drug interactions [Cayen *et al.* 2000].

Half-life Approximately 21 h.

Volume of Distribution Approximately 3643 L.

Clearance Approximately 120 L/h.

Protein Binding Approximately 82 to 87%.

Dose Given by mouth in a dose of 5 mg once daily.

Cayen M *et al.* (2000). Effect of desloratidine on p-glycoprotein transport system (MDR1) [Abstract]. *Allergy* 55282.

Emara S *et al.* (2007). Direct injection liquid chromatographic technique for simultaneous determination of two antihistaminic drugs and their main metabolites in serum. *J AOAC Int* 90: 384–390.

Gupta S *et al.* (2002). Desloratadine demonstrates dose proportionality in healthy adults after single doses. *Clin Pharmacokinet* 41: 11–6.

Gupta S *et al.* (2007). Pharmacokinetics of desloratadine in children between 2 and 11 years of age. *Br J Clin Pharmacol* 63: 534–540.

Gupta SK *et al.* (2007). Multiple-dose pharmacokinetics and safety of desloratadine in subjects with moderate hepatic impairment. *J Clin Pharmacol* 47: 1283–1291.

Liu L *et al.* (2004). High-performance liquid chromatographic method for the bioequivalence evaluation of desloratadine fumarate tablets in dogs. *J Pharm Biomed Anal* 34: 1013–1019.

Qi M *et al.* (2005). Determination of desloratadine in drug substance and pharmaceutical preparations by liquid chromatography. *J Pharm Biomed Anal* 38: 355–359.

Shen JX *et al.* (2006). Orthogonal extraction/chromatography and UPLC, two powerful new techniques for bioanalytical quantitation of desloratadine and 3-hydroxydesloratadine at 25 pg/mL. *J Pharm Biomed Anal* 40: 689–706.

Shen JX *et al.* (2007). Simultaneous determination of desloratadine and pseudoephedrine in human plasma using micro solid-phase extraction tips and aqueous normal-phase liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 21: 3145–3155.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Xu HR *et al.* (2007). Simultaneous determination of desloratadine and its active metabolite 3-hydroxydesloratadine in human plasma by LC/MS/MS and its application to pharmacokinetics and bioequivalence. *J Pharm Biomed Anal* 45: 659–666.

Yang L *et al.* (2003). Validation of a sensitive and automated 96-well solid-phase extraction liquid chromatography–tandem mass spectrometry method for the determination of desloratadine and 3-hydroxydesloratadine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sc.i* 792: 229–240.

Yeh GC *et al.* (2004). Pharmacokinetics and bioequivalence study of a generic desloratadine tablet formulation in healthy male volunteers. *Arzneimittelforschung* 54: 166–170.

Desmetryne

Herbicide

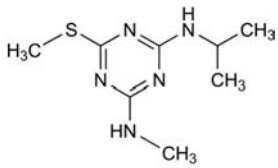
$C_8H_{15}N_5S = 213.3$

CAS—1014-69-3

IUPAC Name 4-*N*-Methyl-6-methylsulfanyl-2-*N*-propan-2-yl-1,3,5-triazine-2,4-diamine

Synonym 2-Isopropylamino-4-methylamino-6-methylthio-1,3,5-triazine

Proprietary Name *Semeron 25 WP*

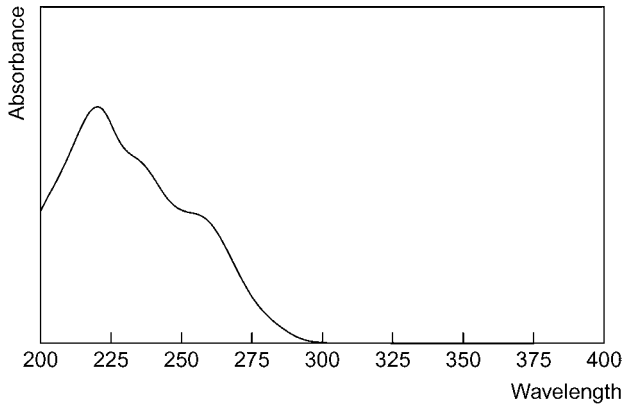


Chemical Properties A white crystalline solid. Mp 84° to 86°. Very slightly soluble in water; readily soluble in organic solvents. pK_a 4.0. Log *P* (octanol/water), 2.4.

Thin-layer Chromatography System TA—R_f 0.73; system TX—R_f 0.21; system TY—R_f 0.16 (Dragendorff spray, positive).

Gas Chromatography System GA—RI 1800; system GK—RRT 0.95 (relative to caffeine).

Ultraviolet Spectrum Aqueous acid—258 nm (A_1^1=450b).



Infrared Spectrum Principal peaks at wavenumbers 1531, 1587, 1258, 806, 1292, 1163 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 213, 57, 58, 198, 82, 171, 43, 99.

Desomorphine

Narcotic Analgesic

$C_{17}H_{21}NO_2 = 271.4$

CAS—427-00-9

IUPAC Name 4,5-Epoxy-3-hydroxy-*N*-methylmorphinan

Synonym Dihydrodesoxymorphine

Chemical Properties Forms rectangular plates from an acetone : water mixture. Mp 189° (anhydrous base). Soluble in acetone and ethyl acetate.

Desomorphine Hydrobromide

Proprietary Name *Permonid*

Colour Tests Ammonium molybdate test—deep purple→blue→green→yellow (limit of detection, 0.1 µg); ammonium vanadate test—grey-purple (limit of detection, 1.0 µg); sulfuric acid–formaldehyde test—purple (limit of detection, 0.1 µg); Vitali's test—yellow/yellow/orange.

Thin-layer Chromatography System T1—R_f 0.29 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—retention time 0.78 relative to codeine; system G4—retention time 0.66 relative to codeine.

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—277 nm, inflexions at 225 nm and 282 nm.

Toxicity The estimated minimum lethal dose in man is ~200 mg. Desomorphine has been reported to have greater addiction-producing properties than morphine.

Desonide

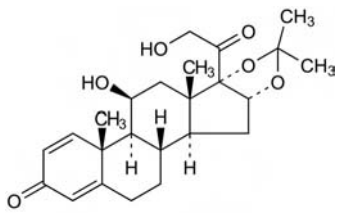
Antiinflammatory Corticosteroid

$C_{24}H_{32}O_6 = 416.5$

CAS—638-94-8

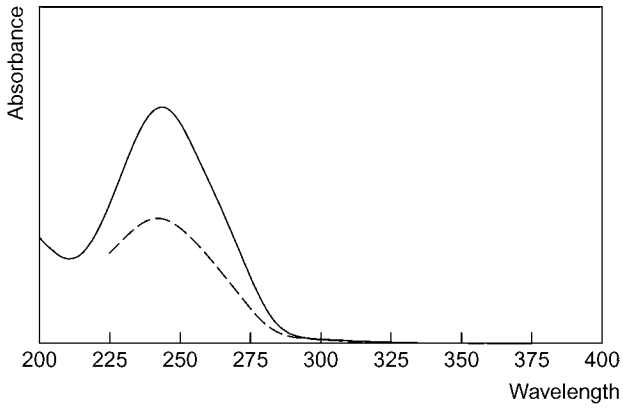
Synonyms Desfluorotriamcinolone acetonide; (11β,16α)-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione; 16-hydroxyprednisolone-16,17-acetonide.

Proprietary Names *Apolar*; *Locapred*; *Tridesilon*; *Tridésonit*.



Chemical Properties White powder or crystals. Log *P* (octanol/water), 2.8.

Ultraviolet Spectrum Methanol—243 nm (A_1^1=450b).

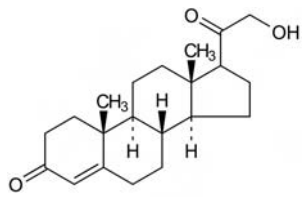


Infrared Spectrum Principal peaks at wavenumbers 1639, 1052, 1612, 1086, 1265, 1694 cm^{-1}.
Use Topically as a 0.05% cream or ointment.

Desoxycortone

Corticosteroid
$C_{21}H_{30}O_3$ = 330.5
CAS—64-85-7

IUPAC Name (8*S*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-(2-Hydroxyacetyl)-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonyms Decortone; deoxycorticosterone; deoxycortone; desoxycorticosterone; 21-hydroxypregn-4-ene-3,20-dione; 21-hydroxyprogesterone.



Chemical Properties Crystals. Mp 141° to 142°. Freely soluble in ethanol and acetone. Log *P* (octanol/water), 2.9.

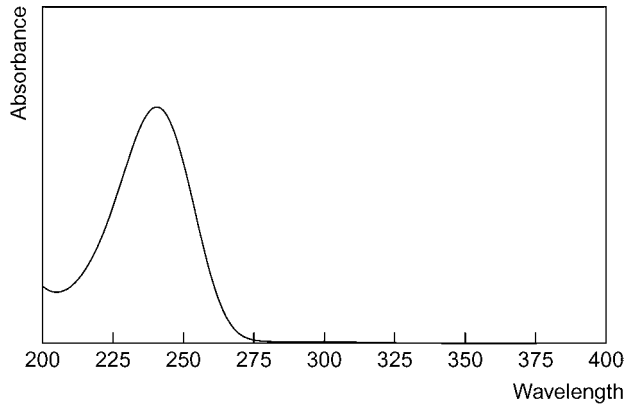
Desoxycortone Acetate

$C_{23}H_{32}O_4$ = 372.5
CAS—56-47-3
Proprietary Names *Cortiron*; *Doca*; *Percorten*; *Syncortyl*. It is an ingredient of *Plex-Hormone*.
Chemical Properties Colourless crystals or white or creamy-white crystalline powder. Mp 155° to 161°. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in 30 of acetone, 1 in 1.5 of chloroform and 1 in 170 of ether; slightly soluble in dioxane and propylene glycol.

Desoxycortone Pivalate

$C_{26}H_{38}O_4$ = 414.6
CAS—808-48-0
Synonyms Desoxycortone trimethylacetate; desoxycorticosterone pivalate.
Proprietary Name *Percorten M*
Chemical Properties A white or creamy-white crystalline powder. Mp 198° to 206°. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 350 to 1 in 500 of ethanol, 1 in 3 of chloroform and 1 in 60 of dioxan; slightly soluble in ether.

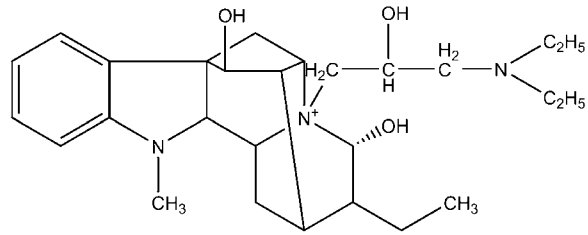
Colour Tests Antimony pentachloride—orange; naphthol-sulfuric acid—orange-red/blue-black; sulfuric acid—yellow (green-yellow fluorescence under ultraviolet light).
Thin-layer Chromatography Desoxycortone acetate: system TP—R_f 0.86; system TQ—R_f 0.52; system TR—R_f 0.98; system TS—R_f 0.95; system TAJ—R_f 0.78; system TAK—R_f 0.71; system TAL—R_f 0.96; system TAM—R_f 0.91 (DPST solution).
Ultraviolet Spectrum Desoxycortone acetate: ethanol—240 nm (A_1^1=445a).



Infrared Spectrum Principal peaks at wavenumbers 1242, 1667, 1744, 1718, 1203, 1610 cm^{-1} (desoxycortone acetate, KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 55, 299, 91, 147, 79, 253, 271 (desoxycortone acetate).
Dose Usually 1 to 5 mg of desoxycortone acetate daily, intramuscularly; sublingual doses of 2 to 10 mg daily have also been given.

Detajmium

Antiarrhythmic
$C_{27}H_{42}N_3O_3$ = 456.7
CAS—47719-70-0
Synonym 4-[3-(Diethylamino)-2-hydroxypropyl]ajmalinium

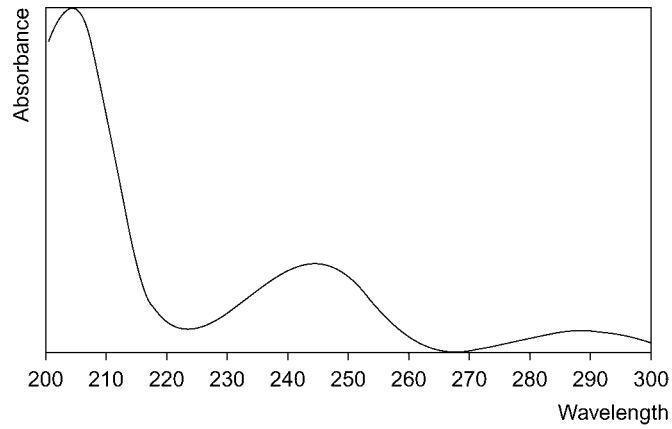


Chemical Properties Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].
Note Commercially available detajmium is a mixture of 4 stereoisomers.

Detajmium Bitartrate

$C_{31}H_{47}N_3O_9,H_2O$ = 623.7
CAS—53862-81-0 (monohydrate); 33774-52-6 (anhydrous).
Synonym 4-[3-(Diethylamino)-2-hydroxypropyl]ajmalinium hydrogen tartrate monohydrate
Proprietary Name *Tachmalcor*

Ultraviolet Spectrum 205, 245 nm [Tenczer *et al.* 1994].



Mass Spectrum Principal ions at *m/z* 86, 365, 369, 112, 144, 196, 158, 366.
Quantification
Blood **GC-MS** Column: Fused quartz silica SE-54 capillary (25 m × 0.32 mm i.d., 0.3 µm). Carrier gas: He, 1.7 mL/min. Temperature programme: 75° to 300° at 15°/min. EI ionisation. Limit of detection not reported [Tenczer *et al.* 1994].

HPLC Column: Lichrospher 60 RP Select B (125 × 4 mm i.d.). Mobile phase: acetonitrile : 0.07% orthophosphoric acid (16 : 84), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 296 nm, λ_{em} = 358 nm). Limit of quantification, 50 µg/L [Tenczer *et al.* 1994].

Serum **HPLC** Column: RP18 (125 × 4 mm i.d., 5 µm) Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 3.5; 26 : 74), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 247 nm, λ_{em} = 353 nm). Limit of quantification, 1 µg/L [Oertel *et al.* 1999].

Disposition in the Body Metabolised by hydroxylation of the indole ring with subsequent *O*-methylation, reduction as well as oxidation of the C_{21} hydroxy function and cleavage of the *N*-alkyl side chain.

Toxicity

A 14-year-old female collapsed with asystolia after ingestion of an unknown amount of detajmium. Postmortem examination revealed a detajmium concentration of 12 mg/L. Six further cases of detajmium fatalities had the following blood concentrations:

	Age (years)	*Sex*	*Detajmium in blood (mg/L)*
Case 1	23	Female	35
Case 2	18	Female	12
Case 3	42	Female	25
Case 4	36	Female	1.5
Case 5	71	Male	2.0
Case 6	16	Female	28

[Tenczer *et al.* 1994].

Dose Orally, from 75 to 300 mg detajmium bitartrate daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Oertel R *et al.* (1999). Sensitive method for the determination of the antiarrhythmic drug detajmium in serum by solid-phase extraction and high-performance liquid chromatography with fluorimetric detection. *J Chromatogr A* 846: 217–222.
Tenczer J *et al.* (1994). Fatal poisoning with detajmium: identification of detajmium and its metabolites and artifacts by gas chromatography-mass spectrometry and quantification by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 661: 47–55.

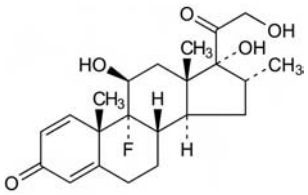
Dexamethasone

Corticosteroid
$C_{22}H_{29}FO_5$ = 392.5
CAS—50-02-2

IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-3-one

Synonyms Desamethasone; 9α-fluoro-16α-methylprednisolone; (11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Proprietary Names *Aeroseb-Dex*; *Decaderm*; *Decadron (elixir and tablets)*; *Decalix*; *Decaspray*; *Dexacortisyl*; *Dexalocal*; *Dexasone (tablets)*; *Dexone*; *Dezone (tablets)*; *Fortecortin (tablets)*; *Hexadrol (tablets)*; *Maxidex*; *Millicorten*; *Miral*; *Oradexon (tablets)*. It is an ingredient of *Maxitrol*.



Chemical Properties Dexamethasone is a synthetic glucocorticoid and is an isomer of betamethasone. A white crystalline powder. Mp 268° to 271°, with decomposition. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 42 of ethanol and 1 in 165 of chloroform; soluble in acetone; sparingly soluble in methanol; very slightly soluble in ether. Log *P* (octanol/water), 1.8.

Dexamethasone Acetate

$C_{24}H_{31}FO_6$ = 434.5
CAS—1177-87-3 (anhydrous); 55812-90-3 (monohydrate)

Synonym Dexamethasone 21-acetate
Proprietary Names *Decadron-LA*; *Dexacen-La-8*; *Dexasone L.A.*; *Fortecortin (suspension for injection)*.
Chemical Properties A white powder. Mp about 225°, with decomposition. Practically insoluble in water; soluble 1 in 40 of ethanol, 1 in 25 of dehydrated alcohol, 1 in 33 of chloroform, and 1 in 1000 of ether; freely soluble in acetone and methanol.

Dexamethasone Isonicotinate

$C_{28}H_{32}FNO_6$ = 497.6
CAS—2265-64-7
Synonym Dexamethasone 21-isonicotinate
Proprietary Names *Auxison(e)*. It is an ingredient of *Dexa-Rhinaspray*.
Chemical Properties Crystals. Mp 250° to 252°.

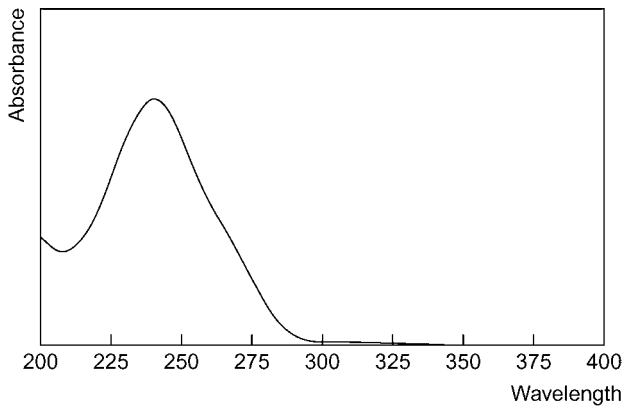
Dexamethasone Sodium Phosphate

$C_{22}H_{28}FNa_2O_8P$ = 516.4
CAS—2392-39-4
Synonym Sodium 9α-fluoro-16α-methylprednisolone 21-phosphate
Proprietary Names *Dalaron*; *Decadron (injection)*; *Decasone*; *Dexacen-4*; *Dexasone (injection)*; *Dezone (injection)*; *Fortecortin (injection)*; *Hexadrol (injection)*; *Novadex*; *Oradexon (injection)*; *Savacort-D*; *Turbinaire Decadron*.
Chemical Properties A white or slightly yellow, very hygroscopic, crystalline powder. Mp 233° to 235°. Soluble 1 in 2 of water; sparingly soluble in dehydrated alcohol; practically insoluble in chloroform and ether.

Dexamethasone Tebutate

$C_{28}H_{39}FO_6$ = 490.6
CAS—24668-75-5
Synonyms Dexamethasone butylacetate; dexamethasone TBA; dexamethasone tertiary butylacetate.
Proprietary Name *Decadron-TBA*
Chemical Properties Practically insoluble in water; soluble in ethanol and acetone.

Colour Tests Antimony pentachloride—green; naphthol-sulfuric acid—yellow-green/yellow; sulfuric acid—orange-pink.
Thin-layer Chromatography System TP—R_f 0.32; system TQ—R_f 0.08; system TR—R_f 0.00; system TS—R_f 0.00; system TAJ—R_f 0.38; system TAK—R_f 0.07; system TAL—R_f 0.75; system TAM—R_f 0.66 (DPST solution).
Gas Chromatography System GA—RI 2970.
High Performance Liquid Chromatography System HT—*k* 4.8; system HY—RI 381; system HZ—retention time 3.4 min; system HAA—retention time 13.1 min.
Ultraviolet Spectrum Methanol—240 nm (A_1^1=385a).



Infrared Spectrum Principal peaks at wavenumbers 1663, 896, 1622, 1695, 1052, 1603 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 121, 122, 315, 43, 147, 223, 135, 41.
Quantification
Plasma **GC–MS** Limit of detection, 0.1 µg/L [Girault *et al.* 1990].

HPLC UV detection (λ=240 nm). Limit of quantification, 15 µg/L [Schild, Charles 1994]. UV detection. Limit of detection, 10 ng [Cham *et al.* 1980].

Serum **HPLC** For method, see McWhinney *et al.* [1996].
Urine **HPLC** See Serum McWhinney *et al.* [1996].
Synovial fluid **GC–MS** See Plasma [Girault *et al.* 1990].
Tissues **GC–MS** See Plasma [Girault *et al.* 1990].

HPLC See Plasma [Cham *et al.* 1980].

Disposition in the Body Rapidly absorbed after oral administration. Up to 65% of a dose is excreted in the urine in 24 h, mainly as metabolites.

Therapeutic Concentration

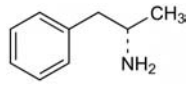
Seven extremely low birth weight infants, mean gestational age 25.6 weeks, suffering from bronchoplumonary dysplasia, were administered an IV bolus dose of 0.369 mg/kg dexamethasone sodium phosphate. A mean peak concentration of 250.5 µg/L was reached [Charles *et al.* 1993].

Half-life Plasma half-life, about 2 to 5 h.
Volume of Distribution About 1 L/kg.
Distribution in Blood Plasma : whole blood ratio, 1.2.
Protein Binding About 67%.
Dose 0.5 to 9 mg daily.

Cham B *et al.* (1980). High performance liquid chromatographic assay of dexamethasone in plasma and tissue. *Ther Drug Monit* 2: 373–377.
Charles B *et al.* (1993). Pharmacokinetics of dexamethasone following single-dose intravenous administration to extremely low birth weight infants. *Dev Pharmacol Ther* 20(3–4): 205–210.
Girault J *et al.* (1990). A rapid and highly sensitive method for the quantitative determination of dexamethasone in plasma, synovial fluid and tissues by combined gas chromatography/negative ion chemical ionization mass spectrometry. *Biomed Environ Mass Spectrom* 19(5): 295–302.
McWhinney BC *et al.* (1996). Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine. *Clin Chem* 42: 979–981.
Schild PN, Charles BG (1994). Determination of dexamethasone in plasma of premature neonates using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 658: 189–192.

Dexamfetamine

Central Stimulant, Anorectic
$C_9H_{13}N$ = 135.2
CAS—51-64-9
IUPAC Name (α*S*)-α-Methylbenzene-ethanamine
Synonyms Dexamphetamine, dextroamphetamine.
Proprietary Names It is an ingredient of *Biphetamine* and *Durophet*.
Note For analytical data and quantification, see under Amfetamine.



Chemical Properties pK_a 9.9. Log *P* (octanol/water), 1.8.

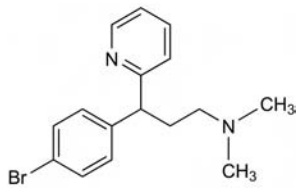
Dexamfetamine Phosphate
$C_9H_{13}N,H_3PO_4$ = 233.2
CAS—7528-00-9
Synonyms Dextroamphetamine phosphate; monobasic dextroamphetamine phosphate.
Chemical Properties A white crystalline powder. Soluble 1 in 20 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Dexamfetamine Sulfate
$(C_9H_{13}N)_2,H_2SO_4$ = 368.5
CAS—51-63-8
Synonym Dextro amfetamine sulfate
Proprietary Names *Dexampex*; *Dexedrine*; *Diphylets*; *Ferndex*. It is an ingredient of *Dexamyl* and *Eskatrol*.
Chemical Properties A white or almost white crystalline powder. Mp >300°. Soluble 1 in 9 to 1 in 10 of water and 1 in 800 of ethanol; practically insoluble in ether.

Disposition in the Body Well absorbed after oral administration. It is approximately twice as potent as amfetamine. For information on metabolism, excretion and pharmacokinetics, see under Amfetamine.
Dose 5 to 60 mg dexamphetamine sulfate, daily.

Dexbrompheniramine

Antihistamine
$C_{16}H_{19}BrN_2$ = 319.2
CAS—132-21-8
IUPAC Name (+)-γ-(4-Bromophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine



Chemical Properties An oily liquid.

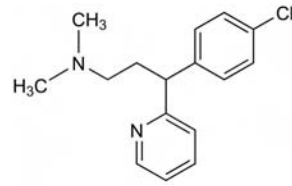
Dexbrompheniramine Maleate
$C_{16}H_{19}BrN_2,C_4H_4O_4$ = 435.3
CAS—2391-03-9
Proprietary Names It is an ingredient of *Disophrol*, *Drixoral* and *Halin*.
Chemical Properties A white crystalline powder. Mp 103° to 113°. Soluble 1 in 1.2 of water, 1 in 2.5 of ethanol and 1 in 2 of chloroform; very slightly soluble in ether. For analytical data, see under Brompheniramine.

Disposition in the Body See under Brompheniramine. Dexbrompheniramine is approximately twice as potent as brompheniramine.
Toxicity
The following postmortem concentrations of dexbrompheniramine were reported in a fatality involving the ingestion of dexbrompheniramine and 3 other drugs: blood 0.2 mg/L, liver 4.5 µg/g [Baselt, Gross 1977].
Dose Usually 8 mg of dexbrompheniramine maleate daily; up to 18 mg daily has been given in sustained-release preparations.

Baselt RC, Gross EM (1977). *J Anal Toxicol* 1: 168–170.

Dexchlorphenamine

Antihistamine
$C_{16}H_{19}ClN_2$ = 274.8
CAS—25523-97-1
IUPAC Name (3*S*)-3-(4-Chlorophenyl)-*N*,*N*-dimethyl-3-pyridin-2-ylpropan-1-amine
Synonyms (+)-γ-(4-Chlorophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine; dexchlorpheniramine.



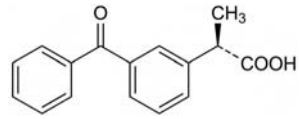
Chemical Properties An oily liquid. Soluble in chloroform.

Dexchlorphenamine Maleate
$C_{16}H_{19}ClN_2,C_4H_4O_4$ = 390.9
CAS—2438-32-6
Proprietary Names *Destral*; *Polaramin(e)*.
Chemical Properties A white crystalline powder. Mp 113° to 115°. Soluble 1 in 1.1 of water, 1 in 2 of ethanol and 1 in 1.7 of chloroform; very slightly soluble in ether. For analytical data, see under Chlorphenamine.

Disposition in the Body See under Chlorphenamine. Dexchlorphenamine is more potent than chlorphenamine.
Dose 6 to 8 mg of dexchlorphenamine maleate daily; up to 18 mg daily has been given in sustained-release preparations.

Dexketoprofen

NSAID
$C_{16}H_{14}O_3$ = 254.3
CAS—22161-81-5
IUPAC Name (*S*)-(+)-2-(3-Benzoylphenyl)propionic acid



Chemical Properties A white to off-white crystalline powder. Mp 92° to 97°. Practically insoluble in water; freely soluble in alcohol, acetone and dichloromethane.

Dexketoprofen Trometamol
$C_{16}H_{14}O_3.C_4H_{11}NO_3$ = 375.4
CAS—156604-79-4
Proprietary Names *Enantyrum*; *Ketesse*; *Nosatal*; *Quiralem*; *Sympal*; *Viaxel*.
Chemical Properties A white crystalline powder. Mp 103° to 107°. Very soluble in water, methanol and dimethyl sulfoxide; fairly soluble in ethanol; slightly soluble in isopropyl alcohol; virtually insoluble in chloroform, ethyl ether and hexane. For analytical data, see under Ketoprofen.

Quantification
Plasma **HPLC** Limit of quantification, 0.01 mg/L (reverse-phase column), 0.08 mg/L (chiral column) [Barbanoj *et al.* 1998].
Disposition in the Body Dexketoprofen is rapidly absorbed and metabolised to produce an acyl-glucuronide conjugate metabolite, which is easily hydrolysed in plasma and alkali conditions. The drug is excreted in urine as the conjugate and also

as the unaltered form when the glucuronide undergoes hydrolysis, so the metabolic transfer is reversed. Between 73% and 82% of a dose is recovered in urine within the first 12 h.

Therapeutic Concentration

Nine healthy males and 9 females, aged between 19 and 34 years, were administered a single dose of dexketoprofen (25 mg) and a single dose of dexketoprofen trometamol (25 mg), equivalent to 25 mg dexketoprofen. For dexketoprofen, peak plasma concentrations of 2.02 ± 0.69 mg/L were reached within 0.5 to 3 h. For dexketoprofen trometamol, peak concentrations were 3.70 ± 0.72 mg/L 0.25 to 0.75 h after administration [Barbanoj *et al.* 1998].

Toxicity Gastrointestinal toxicity can occur with potentially life threatening bleeding and perforations.

Half-life 1.2 to 2.5 h.

Volume of Distribution Approximately 0.25 L/kg.

Clearance Approximately 0.10 L/kg/h.

Protein Binding 99.2%.

Dose The usual dose is 12.5 mg every 4 to 6 h or 25 mg every 8 h. A maximum dose of 75 mg daily may be administered. In the elderly, a maximum dose of 50 mg daily may be given.

Barbanoj MJ *et al.* (1998). Pharmacokinetics of dexketoprofen trometamol in healthy volunteers after single and repeated oral doses. *J Clin Pharmacol* 38: 33S–40S.

Dexmedetomidine

Analgesic, Anxiolytic, Sedative

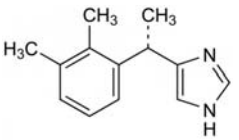
$C_{13}H_{16}N_2 = 200.3$

CAS—113775-47-6

IUPAC Name 4-[(1*S*)-1-(2,3-Dimethylphenyl)ethyl]-*1H*-imidazole

Synonyms D-form of medetomidine; MPV-1440; (*S*)-medetomidine.

Proprietary Name *Precedex*



Quantification

Serum **GC–MS** Limit of quantification, 0.01 µg/L [Vuorilehto *et al.* 1989].

Disposition in the Body

Therapeutic Concentration

Nine healthy male volunteers, mean age 23 years, were fasted overnight and supplied with a standard light breakfast prior to treatment with a transdermal patch (625 µg dexmedetomide base) which was placed on the forehead for 12 h or an IV dose of dexmedetomide hydrochloride. The IV dose was administered at a constant-rate infusion of 2 µg/kg for over 5 min. The mean peak plasma concentration for the transdermal patch was 0.51 µg/L (range 0.21–0.79 µg/L) observed at 6 (3–8) h. For the IV dose, a peak concentration of ~3.5 µg/L was reached by the end of the infusion [Kivistö *et al.* 1994].

Six healthy males, 21–23 years old, were administered 0.5, 1.0 and 1.5 µg/kg doses of dexmedetomide after an overnight fast and standard light breakfast. At least 48 h was allowed between each dose. The peak plasma concentrations were 0.137, 0.257 and 0.335 µg/L for the 3 doses observed at 1.66, 1.57 and 1.63 h [Scheinin *et al.* 1992].

Half-life Mean, 2.1 h.

Volume of Distribution Apparent, mean, 2.30 L/kg.

Clearance Total plasma, mean, 0.80 L/min.

Kivistö KT *et al.* (1994). Pharmacokinetics and pharmacodynamics of transdermal dexmedetomidine. *Eur J Clin Pharmacol* 46(4): 345–349.

Scheinin H *et al.* (1992). Pharmacodynamics and pharmacokinetics of intramuscular dexmedetomidine. *Clin Pharmacol Ther* 52: 537–546.

Vuorilehto L *et al.* (1989). Picogram level determination of medetomidine in dog serum by capillary gas chromatography with negative ion chemical ionization mass spectrometry. *J Chromatogr* 497: 282–287.

Dexpanthenol

Vitamin B Activity

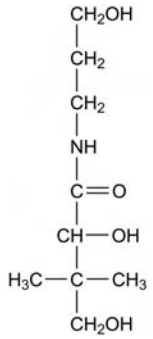
$C_9H_{19}NO_4 = 205.3$

CAS—81-13-0

IUPAC Name 2,4-Dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutanamide

Synonyms Dextro-pantothenyl alcohol; pantothenol.

Proprietary Names *Bepanthen(e)*; *Ilopan*; *Motilyn*; *Panthoderm*.



Chemical Properties A clear, colourless or slightly yellow, hygroscopic, viscous liquid. Freely soluble in water, ethanol and methanol; soluble 1 in 70 of chloroform and 1 in 200 of ether. Log *P* (octanol/water), −1.9.

Thin-layer Chromatography System TA—R_f 0.70 (acidified potassium permanganate solution, positive, developing slowly).

Gas Chromatography System GA—RI 1807.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1629, 1035, 1064, 1520, 1282, 1250 cm^{-1} (thin film).

Dose Dexpanthenol has been given in doses of 250 to 500 mg intramuscularly.

Dexrazoxane

Cardioprotective

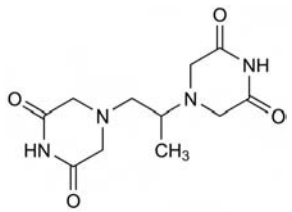
$C_{11}H_{16}N_4O_4 = 268.3$

CAS—24584-09-6

IUPAC Name 4-[(2*S*)-2-(3,5-Dioxopiperazin-1-yl)propyl]piperazine-2,6-dione

Synonyms ADR-529; ICRF-187; (+)-(*S*)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione; NSC-169780; 2,6-piperazinedione.

Proprietary Names *Cardioxane*; *Eucardian*; *Zinecard*.



Chemical Properties A whitish crystalline powder. Mp 191° to 197°. Sparingly soluble in water (approx. 14 g/L) and 0.1 mol/L hydrochloric acid; slightly soluble in ethanol, methanol; practically insoluble in non-polar organic solvents. pK_a 2.1. Log *P* (octanol/water), 0.025.

High Performance Liquid Chromatography Column: ODS2 (LKB Spherisorb, 3 µm). Mobile phase: ammonium formate (0.15 mol/L, pH 7.4) : methanol, composition increased linearly from 0 to 8% in 10 min, then to 80% in 20 min. flow rate 1 mL/min. Retention times: dexrazoxane, 17.1 min; diacid–diamide derivative, 3.7 min; monoacid–monoamide derivatives, 4.8 min; 4.1 min [Burke *et al.* 1991].

Disposition in the Body Dexrazoxane is distributed rapidly throughout the body and is hydrolysed by dihydropyrimidine amidohydrolase (DHPase) to 1-ring and 2-ring open EDTA (ethylenediaminetetra-acetate)-like products. Metabolites (active) include a diacid–diamide cleavage product and the two monoacid–monoamide ring products. Dexrazoxane is excreted in urine as the unchanged drug and the metabolites. Approximately 42% of a dose is recovered in urine.

Therapeutic Concentration

Mean peak plasma concentrations can be detected at the end of a 15-min infusion and reach 0.0365 mg/L with a 500 mg/m^2 dose. [Zinecard (dexrazoxane for injection) prescribing information.]

Toxicity Haematological toxicity can occur, caused by chemotherapy, but is more common in dexrazoxane-treated patients. Dose-limiting leucopenia has been observed with doses >4000 mg/m^2 dexrazoxane (on its own) but there is no evidence of cumulative toxicity.

Half-life 2.1 to 2.5 h.

Volume of Distribution 22.0 to 22.4 L/m^2 (or 0.5 to 1.3 L/kg), tends to be higher in children compared with adults.

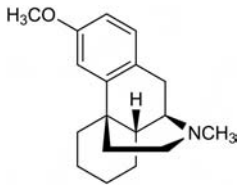
Clearance Between 6.25 and 7.88 L/h/m^2, tends to be higher in children than adults.
Protein Binding <2%.
Dose In the USA: dexrazoxane is administered with doxorubicin or epirubicin in a 10:1 ratio; 500 mg/m^2 dose of dexrazoxane with 50 mg/m^2 doxorubicin or 1000 mg/m^2 with 100 mg/m^2 epirubicin. In Europe: the ratio is 20:1 with a maximum dose of 1000 mg/m^2 each cycle.

Burke TG *et al.* (1991). Characterization of the aqueous decomposition products of (+)1,2-bis(3,5-dioxopiperazinyl-1-yl)-propane (ICRF-187) by liquid chromatographic and mass spectral analysis. *J Pharm Sci* 80: 338–340.
Pharmacie and UpJohn Company, Zinecard (dexrazoxane for injection) prescribing information.

Dextromethorphan

Antitussive, Cough Suppressant
$C_{18}H_{25}NO = 271.4$
CAS—125-71-3

IUPAC Name (+)-3-Methoxy-17-methylmorphinan
Synonyms Deoxydihydrothebacodine; methorphan.

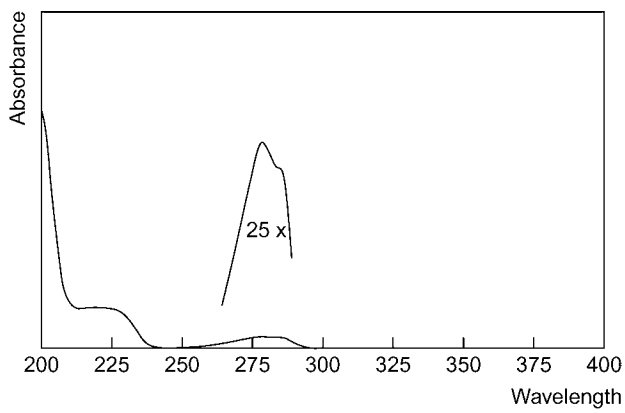


Chemical Properties An almost white or slightly yellow crystalline powder. Mp 109° to 113°. Practically insoluble in water; freely soluble in chloroform. Log *P* (octanol/water), 3.97 [Meylan, Howard 1995].

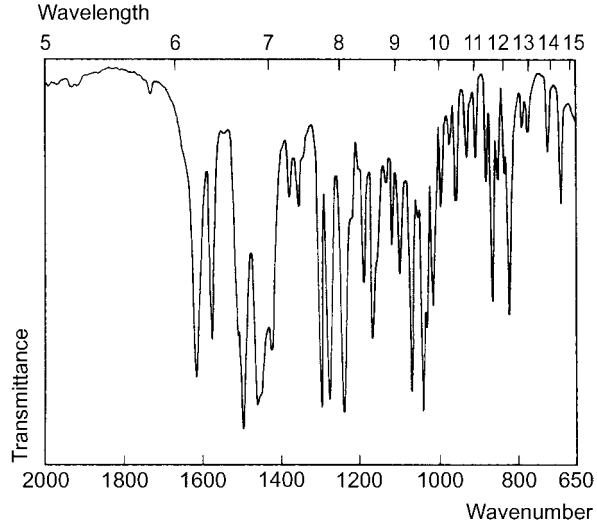
Dextromethorphan Hydrobromide

$C_{18}H_{25}NO,HBr,H_2O = 370.3$
CAS—125-69-9 (anhydrous); 6700-34-1 (monohydrate)
Synonyms Ro-1-5470/5; demorphan hydrobromide.
Proprietary Names *Benylin Adult*; *Benylin DM*; *Benylin Dry Coughs Non-Drowsy*; *Benylin Pediatric*; *Creo-Terpin*; *Delsym*; *DexAlone*; *Diabe-Tuss DM*; *Franolyn Sedative*; *Hold DM*; *Nirolex for Dry Coughs*; *Pertussin*; *Robitussin for Dry Coughs*; *Robitussin Junior*; *Silphen DM*; *Strepsils Cough*; *Suppress*; *Trocal*; *Vicks Vapospray for Dry Coughs*. It is an ingredient of many proprietary preparations, see Sweetman [2007].
Chemical Properties A white crystalline powder. Mp 122° to 124°, with decomposition. Soluble in water: 1 in 60 to 1 in 65 at 25°, 1 in 20 at 50°, 1 in 4 at 85°; also soluble 1 in 10 of glycerol and 1 in 10 of ethanol; freely soluble in chloroform with the separation of water; practically insoluble in ether. pK_a 8.3.

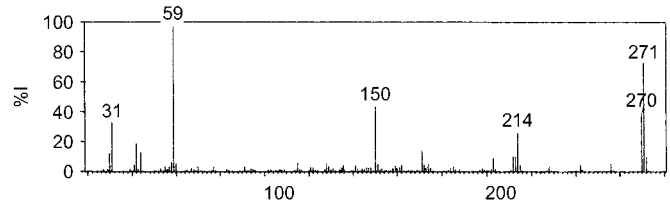
Colour Tests Aromaticity (method 2)—yellow/orange-red; Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.42; system TC—R_f 0.18; system TE—R_f 0.47; system TL—R_f 0.06; system TAE—R_f 0.10; system TAF—R_f 0.42 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—dextromethorphan RI 2138, dextrorphan RI 2240.
High Performance Liquid Chromatography System HA—dextromethorphan 5.6 (tailing peak), dextrorphan 4.7 (tailing peak); system HX—RI 377; system HY—RI 298; system HAA—RT 13.3 min.
Ultraviolet Spectrum Aqueous acid—278 nm ($A_1^1 = 70$a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1496, 1242, 1040, 1300, 1280, 1070 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 59, 271, 150, 270, 31, 214, 42, 171; dextrorphan 59, 257, 150, 256, 31, 76, 42, 157.



Quantification

Blood **LC-MS** [Liang *et al.* 2009].
Plasma **GC-MS** Limit of detection, 10 ng/L [Bagheri *et al.* 2005].

HPLC Column: phenyl. Mobile phase: methanol : acetonitrile : 10 mmol/L potassium dihydrogen phosphate buffer (pH 3.5; 20 : 30 : 50, with the addition of 0.02% TEA). Limit of quantification, 1 nmol/L [Lin *et al.* 2007]. Column: C_{18} Tracer Excel (150 × 4.0 mm i.d., 3 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate buffer (pH 3)-methanol-tetrahydrofuran (68.5 : 31 : 0.5) : methanol-tetrahydrofuran (93.25 : 6.75). Fluorometric detection. Limit of quantification, 10 µg/L [Afshar *et al.* 2004]. Column: Spherisorb CN (250 × 4.6 mm i.d., 3 µm). Fluorescence detection (λ_{ex} = 220 nm, λ_{em} = 305 nm). Limit of quantification, 5 µg/L [Härtter *et al.* 1996]. Limit of detection, 20 µg/L. Fluorescence detection [Gillilan *et al.* 1980].

LC-MS Limit of quantification, 10 ng/L [Eichhold *et al.* 2007], 5 ng/L [Eichhold *et al.* 1997a]. 50 ng/L [Eichhold *et al.* 1997b].

Note For spectrofluorimetric quantification of dextrophan, a metabolite of dextromethorphan, see Ramachander *et al.* [1977].
Serum **GC** Limit of detection, 1 µg/L, ECD [Barnhart, Massad 1979].
Urine **GC** Column: HP-1 capillary (17 m × 0.22 mm). FID. Limit of quantification, 0.37 µmol/L, limit of detection, 0.30 µmol/L [Wu *et al.* 2003].

GC-MS Limit of detection, 10 ng [Xu *et al.* 1993].

HPLC Column: reversed phase C_{18} (150 × 4.6 mm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 6.0, 60 : 40), flow rate 1 mL/min. UV detection (λ = 210 nm). Limit of quantification, 1.6 mg/L, limit of detection, 0.5 mg/L [Santagati *et al.* 2005]. Column: phenyl and nitrile. Mobile phase: aqueous solution containing 1.5% acetic acid and 0.1% TEA : acetonitrile (75 : 25). Fluorescence detection. Limit of detection, 0.01 µmol/L [Bendriss *et al.* 2001]. Column: C-4. Fluorescence detection. Limit of quantification, ~50 µg/L [Mistry *et al.* 1998]. Column: phenyl. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 310 nm). Limit of detection, 0.023 mg/L [Cai *et al.* 1997]. Column: Alltima C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 1 mmol/L SDS in acetonitrile : 0.01 mol/L sodium dihydrogen phosphate (pH 2.5, 40.5 : 59.5). Fluorescence detection. Limit of quantification, 25 µg/L [Bartoletti *et al.* 1996]. See also Jones *et al.* [1996], Lam, Rodriguez [1993], Marshall *et al.* [1992] and Park *et al.* [1984].

LC-MS Column: Keystone Prism (50 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : water containing 0.1% trifluroacetic acid (pH 3, 5 : 65 or 27 : 73), flow rate 0.4 mL/min. Limit of quantification, 1 µg/L [Constanzer *et al.* 2005].
Liver **GC** Column: capillary. ECD [Salsali *et al.* 1999].

Disposition in the Body Dextromethorphan is well absorbed after oral administration. It is metabolised by *N*- and *O*-demethylation followed by sulfate or glucuronic acid conjugation; *O*-Demethylation involves P450 isoenzyme CYP2D6. The major metabolite is dextrorphan, (+)-3-hydroxy-*N*-methylmorphinan, which has some activity; (+)-3-hydroxymorphinan has also been identified. Approximately 50% of a dose is excreted in the urine in 24 h, mostly as the glucuronide and sulfate conjugates of the metabolites; approx. 8% of a dose is excreted as unchanged drug in 6 h.

Therapeutic Concentration

Following a single oral dose of 30 mg dextromethorphan to 6 subjects, a mean peak plasma concentration of 0.38 mg/L conjugated dextrorphan was attained in 2 h [Ramachander *et al.* 1977].

After an oral dose of 20 mg dextromethorphan hydrobromide given to 12 subjects, peak serum concentrations of <0.001–0.008 mg/L (mean 0.0018) dextromethorphan were attained in ~2.5 h [Barnhart, Massad 1979].

Toxicity The estimated minimum lethal dose is 0.5 g.

Of 2 cases of death following dextromethorphan intake in young people, the first was by suicide.

In the first case, an 18-year-old girl was found dead, with 2 empty bottles that had contained 30 mg tablets of dextromethorphan in her handbag (the quantity of tablets was not stated). The following concentrations in blood and liver, respectively, were reported: dextromethorphan 9.2 and 31.2 µg/g, dextrorphan 2.9 and 11.5 µg/g.

In the second case, it was not known whether the overdose was intentional. In a 27-year-old man, the following concentrations in blood and liver, respectively, were reported: dextromethorphan 3.3 and 230 µg/g, dextrorphan 1.5 and 29.2 µg/g [Rammer *et al.* 1988].

Half-life Reported as 3.2–3.6 h in rapid metabolisers.

Volume of Distribution Reported as 255–316 L/kg

Dose Up to 120 mg of dextromethorphan hydrobromide daily in divided doses.

Afshar M *et al.* (2004). Simple chromatography method for simultaneous determination of dextromethorphan and its main metabolites in human plasma with fluorimetric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 802: 317–322.

Bagheri H *et al.* (2005). Sol-gel-based solid-phase microextraction and gas chromatography–mass spectrometry determination of dextromethorphan and dextrorphan in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 818: 147–157.

Barnhart JW, Massad EN (1979). Determination of dextromethorphan in serum by gas chromatography. *J Chromatogr* 163: 390–395.

Bartoletti RA *et al.* (1996). High performance liquid chromatography determination of dextromethorphan and its metabolites in urine using solid-phase extraction. *J Pharm Biomed Anal* 14: 1281–1286.

Bendriss EK *et al.* (2001). High-performance liquid chromatography assay for simultaneous determination of dextromethorphan and its main metabolites in urine and in microsomal preparations. *J Chromatogr B Biomed Sci Appl* 754: 209–215.

Cai WM *et al.* (1997). High performance liquid chromatographic determination of dextromethorphan and its metabolite in human urine. *Yao Xue Xue Bao* 32: 861–864.

Constanzer ML *et al.* (2005). Determination of dextromethorphan and its metabolite dextrorphan in human urine using high performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry: a study of selectivity of a tandem mass spectrometric assay. *J Chromatogr B Analyt Technol Biomed Life Sci* 816: 297–308.

Eichhold TH *et al.* (1997). Determination of dextromethorphan and dextrorphan in human plasma by liquid chromatography/tandem mass spectrometry. *J Mass Spectrom* 32: 1205–1211.

Eichhold TH *et al.* (1997). Highly sensitive high-performance liquid chromatographic-tandem mass spectrometric method for the analysis of dextromethorphan in human plasma. *J Chromatogr B Biomed Sci Appl* 698: 147–154.

Eichhold TH *et al.* (2007). Simultaneous determination of dextromethorphan, dextrorphan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 43: 586–600.

Gillilan R *et al.* (1980). *Anal Lett* 13: 381–387.

Härtter S *et al.* (1996). Automated determination of dextromethorphan and its main metabolites in human plasma by high-performance liquid chromatography and column switching. *Ther Drug Monit* 18: 297–303.

Jones DR *et al.* (1996). Quantification of dextromethorphan and metabolites: a dual phenotypic marker for cytochrome P450 3A4/5 and 2D6 activity. *J Chromatogr B Biomed Appl* 678: 105–111.

Lam YW, Rodriguez SY (1993). High-performance liquid chromatography determination of dextromethorphan and dextrorphan for oxidation phenotyping by fluorescence and ultraviolet detection. *Ther Drug Monit* 15: 300–304.

Liang X *et al.* (2009). Study of dried blood spots technique for the determination of dextromethorphan and its metabolite dextrorphan in human whole blood by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 799–806.

Lin SY *et al.* (2007). Simultaneous analysis of dextromethorphan and its three metabolites in human plasma using an improved HPLC method with fluorometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 859: 141–146.

Marshall PS *et al.* (1992). Determination of dextromethorphan and its *O*-demethylated metabolite from urine. *Ther Drug Monit* 14: 402–407.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Mistry B *et al.* (1998). A sensitive assay of metoprolol and its major metabolite alpha-hydroxymetoprolol in human plasma and determination of dextromethorphan and its metabolite dextrorphan in urine with high performance liquid chromatography and fluorometric detection. *J Pharm Biomed Anal* 16: 1041–1049.

Park YH *et al.* (1984). Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. *J Pharm Sci* 73: 24–29.

Ramachander G *et al.* (1977). Determination of dextrorphan in plasma and evaluation of bioavailability of dextromethorphan hydrobromide in humans. *J Pharm Sci* 66: 1047–1048.

Rammer L *et al.* (1988). Fatal intoxication by dextromethorphan: a report on two cases. *Forensic Sci Int* 37: 233–236.

Salsali M *et al.* (1999). Analysis of dextrorphan, a metabolite of dextromethorphan, using gas chromatography with electron-capture detection. *J Pharmacol Toxicol Methods* 41: 143–146.

Santagati NA *et al.* (2005). Simultaneous determination of phenytoin and dextromethorphan in urine by solid-phase extraction and HPLC-DAD. *J Sep Sci* 28: 1157–1162.

Sweetman S, ed. (2007). *Martindale, The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Wu YJ *et al.* (2003). Determination of dextromethorphan and its metabolite dextrorphan in human urine by capillary gas chromatography without derivatization. *J Chromatogr B Analyt Technol Biomed Life Sci* 784: 219–224.

Xu YX *et al.* (1993). [Analysis of dextromethorphan and its metabolites in human urine by using gas chromatography–mass spectrometry.]. *Yao Xue Xue Bao* 28: 156–159.

Dextromoramide

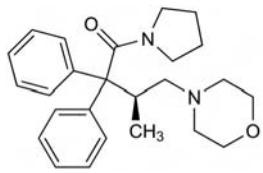
Narcotic Analgesic

$C_{25}H_{32}N_2O_2$=392.5

CAS—357-56-2

IUPAC Name 1-[(3*S*)-3-Methyl-4-(4-morpholinyl)-1-oxo-2,2-diphenylbutyl] pyrrolidine

Synonyms Dextrodiphenopyrine; *d*-moramid; pyrrolamidol; *R*-875; SKF-5137.



Chemical Properties A white amorphous or microcrystalline powder. Mp 180° to 184°. Practically insoluble in water; solubility (g/100 mL): in ethanol 50, in methanol 40, in acetone 50, in ethyl acetate 40, in benzene 5, in chloroform 5; slightly soluble in ether.

Dextromoramide Tartrate

$C_{25}H_{32}N_2O_2,C_4H_6O_6$ = 542.6

CAS—2922-44-3

Synonyms Dextromoramide acid tartrate; dextromoramide hydrogen tartrate; dextromoramidi tartras.

Proprietary Names *Dimorlin*; *Jetrium*; *Palfium*; *Palphium*.

Chemical Properties A white amorphous or crystalline powder. Mp 189° to 192°, with slight decomposition. Soluble 1 in 1 of ethanol, 1 in 1 of acetone, 1 in 25 of water, and 1 in 40 of methanol; slightly soluble in chloroform and isopropyl alcohol; very slightly soluble in ether. Log *P* (octanol/water), 3.61.

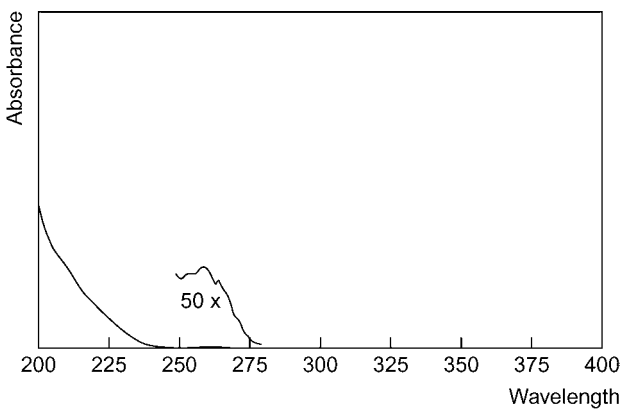
Colour Test Liebermann's reagent (100°)—green.

Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.42; system TC—R_f 0.71; system TE—R_f 0.79; system TL—R_f 0.60; system TAE—R_f 0.72; system TAF—R_f 0.78 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).

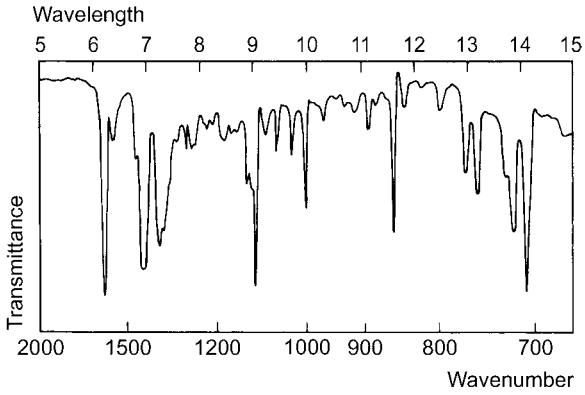
Gas Chromatography System GA—dextromoramide RI 2940, M (OH-) RI 3095, M (OH-)-AC RI 3210, M (methoxy-) RI 3269; system GB—dextromoramide RI 3094, M (OH-) RI 3310; system GC—RI 3625.

High Performance Liquid Chromatography System HA—*k* 0.7; system HC—*k* 0.09; system HX—RI 440; system HY—RI 390; system HAA—RT 15.8 min.

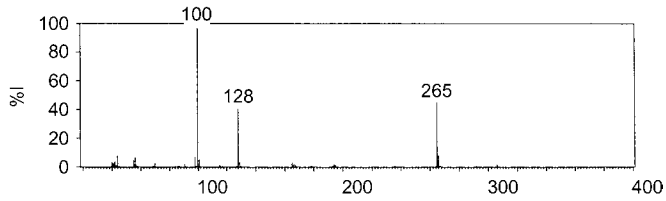
Ultraviolet Spectrum Aqueous acid—254, 259 (A_1^1 = 10.6a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1623, 703, 1107, 717, 858, 1002 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 100, 265, 128, 266, 44, 98, 56, 101.



Quantification

Blood **HPLC** DAD [Rop *et al.* 1993]. UV detection (λ= 215 nm) [Rop *et al.* 1992].

Plasma **GC** NSD. Limit of quantification, 10 µg/L [Kintz *et al.* 1990b].

GC-MS Column: BP1 capillary (25 m). SIM acquisition mode. Limit of detection, 0.5 µg/L [Kintz *et al.* 1990a].

HPLC Column: Supelcosil LC-ABZ. Mobile phase: 25 mmol/L potassium dihydrogen phosphate buffer (pH 2.5) : acetonitrile (80 : 20). UV detection (λ= 206 nm) [Ufkes *et al.* 1998]. See Blood [Rop *et al.* 1992].

Urine **GC-MS** See Plasma. Limit of detection, 0.3 µg/L[Kintz *et al.* 1990a].

Disposition in the Body Dextromoramide is absorbed after oral administration. Metabolic reactions may include *N*-oxidation, hydroxylation and amide hydrolysis; it is possible that 2-hydroxydextromoramide is a major metabolite. Excreted in the urine, partly as unchanged drug.

Therapeutic Concentration

In 3 patients with cancer who received IV treatment with 5 mg dextromoramide, one to three times daily, the plasma level on the fourth day of treatment was 0.01385 mg/L just before the first daily dose and 0.08428 mg/L just after dosing. In 1 patient, the whole blood concentration was undetectable just before the dose and was 0.076 mg/L 30 min after dosing [Rop *et al.* 1992].

Toxicity The estimated minimum lethal dose is 500 mg. Fatalities have occurred at blood concentrations greater than 0.04 mg/L.

In a fatality resulting from the abuse of dextromoramide and propoxyphene, the postmortem whole blood concentrations were 0.194 mg/L dextromoramide, 0.614 mg/L propoxyphene and 1.1 mg/L norpropoxyphene [Rop *et al.* 1993].

A 38-year-old man was found in his car suffering from a heart attack. Serum analysis confirmed the presence of dextromoramide (Palfium), methadone and lidocaine (lignocaine). The serum concentrations at admission to the hospital were 1.9 mg/L dextromoramide, 0.4 mg/L methadone, and 0.4 mg/L lidocaine [Brewer 1990].

In 2 fatalities involving overdose resulting from the abuse of dextromoramide, the whole blood concentrations were 0.9843 mg/L and 0.871 mg/L [Kintz *et al.* 1989a].

The following postmortem tissue concentrations were reported in a 31-year-old female who died after IV injection of dextromoramide: blood 1.526 mg/L, bile 0.494 mg/L, gastric contents (30 mL) 0.018 mg, kidney 0.131 µg/g, brain 0.065 µg/g, liver 0.391 µg/g; no other drugs were detected [Kintz *et al.* 1989b].

Dose The equivalent of 5 to 20 mg of dextromoramide, repeated as necessary.

Brewer E (1990). A dextromoramide-related fatality. *J Forensic Sci* 35: 483–489.

Kintz P *et al.* (1989a). Fatal intoxication by dextromoramide: a report on two cases. *J Anal Toxicol* 13: 238–239.

Kintz P *et al.* (1989b). Toxicological findings after fatal dextromoramide injection. *J Toxicol Clin Toxicol* 27: 385–388.

Kintz P *et al.* (1990a). Determination of dextromoramide by capillary gas chromatography and electron impact mass spectrometry. *J Anal Toxicol* 14: 252–253.

Kintz P *et al.* (1990b). Simultaneous screening and quantification of several nonopiate narcotic analgesics and phencyclidine in human plasma using capillary gas chromatography. *Methods Find Exp Clin Pharmacol* 12: 193–196.

Rop PP *et al.* (1992). Determination of dextromoramide in plasma and whole blood using high-performance liquid chromatography with ultraviolet absorbance detection. *J Chromatogr* 573: 87–92.

Rop PP *et al.* (1993). Simultaneous determination of dextromoramide, propoxyphene and norpropoxyphene in necropsic whole blood by liquid chromatography. *J Chromatogr* 615: 357–364.

Ufkes JG *et al.* (1998). Determination and pharmacokinetics of dextromoramide in methadone maintenance therapy. *Pharm World Sci* 20: 83–87.

Dextropropoxyphene

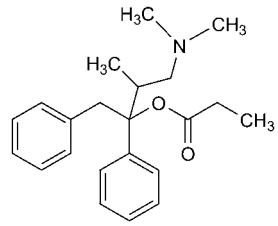
Narcotic Analgesic

$C_{22}H_{29}NO_2$ = 339.5

CAS—469-62-5

IUPAC Name [(2*S*,3*R*)-4-(Dimethylamino)-3-methyl-1,2-di(phenyl)butan-2-yl] propanoate

Synonyms (α*S*)-α-[(1*R*)-2-(Dimethylamino)-1-methylethyl]-α-phenylbenzene ethanol propanoate; propoxyphene.



Chemical Properties Crystals. Mp 75° to 76°. Very slightly soluble in water. pK_a 6.3. Log *P* (octanol/water) 4.2 [Hansch *et al.* 1995]. Unaffected by strong-alkali treatment [Amalfitano *et al.* 1996].

Dextropropoxyphene Hydrochloride

$C_{22}H_{29}NO_2$,HCl = 375.9

CAS—1639-60-7

Synonym Propoxyphene hydrochloride

Proprietary Names *Algaphan*; *Algodex*; *Antalvic*; *Daraphen*; *Darvon*; *Depronal*; *Depronal SA*; *Develin*; *Dolene*; *Dolocap*; *Erantin*; *Mardon*; *Novopropoxyn*; *Pro-65*; *Proxagesic*; *SK-65*; *642 Tablets*. It is an ingredient of *Cosalgesic*; *Dextrogesic*; *Distalgesic*.

Chemical Properties White or slightly yellow powder. Mp 163° to 169°. Soluble 1 in 0.3 of water, 1 in 1.5 of ethanol, and 1 in 0.6 of chloroform; soluble in acetone; practically insoluble in benzene and ether.

Dextropropoxyphene Napsilate

$C_{22}H_{29}NO_2$,$C_{10}H_8O_3S$,H_2O = 565.7

CAS—17140-78-2 (anhydrous); 26570-10-5 (monohydrate)

Synonym Propoxyphene napsilate

Proprietary Names *Darvon-N*; *Doloxene*. It is an ingredient of *Darvocet-N*; *Distalgesic Soluble*; *Dolasan* and *Napsalgesic*.

Chemical Properties White powder. Mp 158° to 165°. Practically insoluble in water; soluble 1 in 13 to 1 in 15 of ethanol, and 1 in 3 of chloroform; soluble in acetone and methanol.

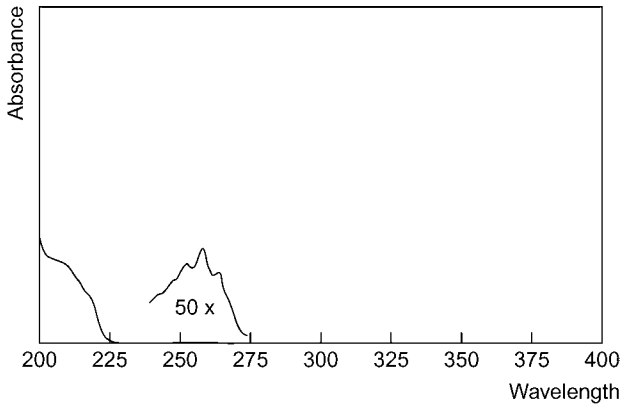
Colour Tests Liebermann's reagent—brown; Mandelin's test—grey-brown; Marquis test—black-violet→green.

Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.59; system TC—R_f 0.55 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, violet).

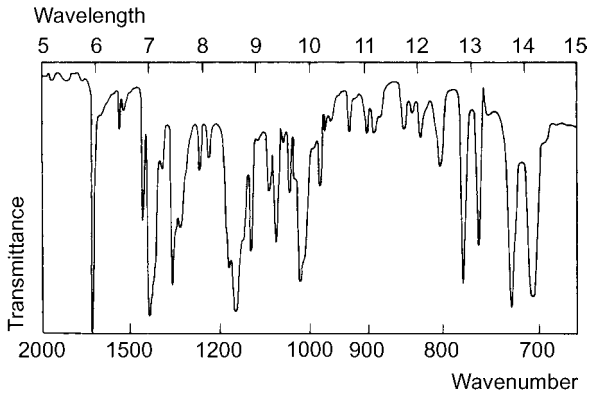
Gas Chromatography System GA—dextropropoxyphene RI 2188, norpropoxyphene RI 2214, M (nor-amide) RI 2526, M (nor-*N*-proponyl-) RI 2400, M (nor-)-AC RI 2365; system GB—dextropropoxyphene RI 2268norpropoxyphene RI 2487, M (nor-amide) RI 2673, M (nor-N-proponyl-) RI 2514; system GAS—dextropropoxyphene RI 1938; system GC—dextropropoxyphene RI 2173; system GF—dextropropoxyphene RI 2370, norpropoxyphene RI 3025; system GM—RT relative to iprindole: dextropropoxyphene RT 1.220, norpropoxyphene RT 1.248, M (nor-amide) RT 1.969, M (nor-*N*-proponyl-) RT 1.300.

High Performance Liquid Chromatography System HA—dextropropoxyphene *k* 1.9, norpropoxyphene *k* 1.3; system HAA—RT 15.8 min; system HC—*k* 0.19; system HY—RI 374; system HZ—RT 7.6 min.

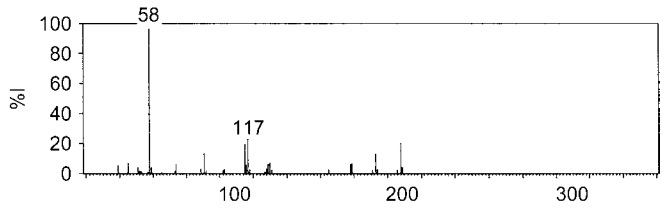
Ultraviolet Spectrum Aqueous acid—252, 257 (A^1_1 = 12a), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1175, 725, 704, 776, 1026 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 58, 117, 208, 115, 193, 91, 179, 130 (no peaks above 210); norpropoxyphene44, 208, 117, 58, 193, 130, 57, 29.



Quantification

Blood **GC-MS** Column: CP SIL 8 CB (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme; 130° for 1 min to 10° at 25°/min for 6.8 min to 280° at 25°/min for 2.2 min. SIM acquisition mode. Retention time: 9.5 min. Limit of detection, 0.07 mg/L [Gaillard, Pépin 1998].

Plasma **GC** Column: 3% OV-17 on 100/120 mesh Chromsorb Q (1.8 m × 2.0 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 235°. NPD. Limit of detection, 6 µg/L [Kintz *et al.* 1990]. Column: 3% SP-2250 on Supelcoport 100/120 mesh (1.2 m × 2 mm i.d.). Carrier gas: H_2, 30 mL/min. Temperature programme: 210° for 4 min to 260° at 8°/min for 2 min. NSD. Relative retention time: 0.6, 1.1 for NDPX. Limit of detection, 50 µg/L, 100 µg/L for NDPX [Margot *et al.* 1983]. Column: 3% OV-17 Chromosorb W AW DMCS, 80/100 mesh (1.8 m × 2.0 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 180°. FID. Retention time: 12.1 min, 82.0 min for norpropoxyphene. Limit of quantification, 20 µg/L [Cleemann 1977]. Column: 3% SE-30 Gas-Chrom Q 80/100 mesh (6′ × 2 mm i.d.). Carrier gas: He, 33 mL/min. Temperature: 216°. FID. Limit of detection, 0.04 µg on column [Verebely, Inturrisi 1973].

HPLC Column: µ-Bondapak C_{18} (30 cm × 3.9 mm i.d.). Mobile phase: acetonitrile : 2 mmol/L sulfuric acid (1 : 1), flow rate 1.5 mL/min. UV detection (λ = 205 nm). Retention time: 5.7 min. Limit of detection, 20 µg/L [Kunka *et al.* 1985].

Urine **GC** Column: DB-5 MS 5% phenyl silicone (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 100° to 280° at 15°/min for 5 min. FID. Relative retention time: 0.905. Limit of quantification, 90 µg/L [Amalfitano *et al.* 1996]. See Plasma [Margot *et al.* 1983]. See Plasma [Verebely, Inturrisi 1973].

Breast Milk **HPLC** See Plasma [Kunka *et al.* 1985].

Hair **GC-MS** See Blood. Limit of detection, 0.05 µg/g [Gaillard, Pépin 1998].

HPLC Column: Select B (125 × 4 mm i.d). Mobile phase: acetonitrile : phosphate buffer (pH 2.3; 24 : 76), flow rate 1.5 mL/min. UV detection (λ = 200 to 360 nm). Limit of detection, <1.0 µg/g for dextropropoxyphene and 1.5 µg/g for norpropoxyphene [Mersch *et al.* 1997].

Note For an article on the thermal decomposition of propoxyphene during GC analysis, see Millard *et al.* [1980].

Disposition in the Body Readily and completely absorbed but undergoes considerable first-pass metabolism; bioavailability about 40%. Rapidly distributed and concentrated in the brain, lungs, liver and kidneys. The main metabolic reaction is *N*-demethylation which produces norpropoxyphene (NDPX), the major metabolite, and also dinorpropoxyphene which is dehydrated to cyclic dinorpropoxyphene. Norpropoxyphene has less than half the activity of dextropropoxyphene. In 24 h, about 35% of a dose is excreted in urine with about 13% of the dose as norpropoxyphene and up to 5% as unchanged drug. A total of 60–70% of a dose is excreted in urine in about 5 days; about 18% of the dose may be eliminated in the faeces over the same period.

Therapeutic Concentration In plasma, usually in the range 0.05 to 0.75 mg/L. Peak plasma concentrations of dextropropoxyphene of 0.17 to 0.37 mg/L (mean 0.23) were attained 2 h after administration of a single oral dose of 130 mg to 6 subjects; peak norpropoxyphene concentrations of 0.19 to 0.42 mg/L (mean 0.27) were attained in 4 h [Verebely, Inturrisi 1974].

After daily oral dosing of 195 mg to 3 subjects, steady-state plasma concentrations were: dextropropoxyphene 0.24 to 0.75 mg/L (mean 0.4); norpropoxyphene 0.6 to 3.0 mg/L (mean 1.5) [Verebely, Inturrisi 1973].

Toxicity The estimated minimum lethal dose is 0.5 g. Blood concentrations in the region of 1 mg/L are likely to cause toxic reactions; concentrations of ≥2 mg/L may be lethal. In fatalities in which dextropropoxyphene is involved, it is common to find much greater concentrations in the liver and lungs than in the blood. Addicts can ingest 10 times the normal dosage before showing toxicity, whereas children show toxic symptoms after only 1/20 of the normal dose.

In a review of several fatalities, blood concentrations in 23 cases ranged from 1 to 60 mg/L (mean 13) and liver concentrations in 73 cases ranged from 5 to 550 µg/g (mean 97) [Cravey *et al.* 1974].

In 6 fatalities attributed to dextropropoxyphene overdose, the postmortem tissue concentrations, mg/L or µg/g (mean, *N*) shown below were reported.

	Dextropropoxyphene	*Norpropoxyphene*
Blood	1.1–4.9 (3.5, *6*)	1.4–5.9 (3.5, *6*)
Brain	2.8–25.2 (14, *4*)	4–9.1 (6, *4*)
Liver	5.7–229 (117, *6*)	24.2–73.2 (50, *6*)
Urine	1.5–60.2 (17, *5*)	1.6–287 (68, *5*)

[McBay 1976].

Thirty hair samples from deceased drug addicts were analysed and the mean dextropropoxyphene concentration was 8.7 µg/g (range, 0.24 to 27.41) and mean norpropoxyphene 24.1 µg/g (range, 0.36 to 68.94) [Gaillard, Pépin 1998].

Half-life Plasma half-life, dextropropoxyphene 8 to 24 h (mean 15), norpropoxyphene 20 to 50 h (mean 29).

Volume of Distribution ≈16 L/kg.

Clearance Plasma clearance, ≈15 mL/min/kg.

Protein Binding ≈70 to 80%.

Note For a review of dextropropoxyphene pharmacokinetics and overdose, see [Young 1983].

Dose Usually 195 to 260 mg of dextropropoxyphene hydrochloride, or 300 to 400 mg of the napsilate, daily.

Amalfitano G *et al.* (1996). Gas chromatographic quantitation of dextropropoxyphene and norpropoxyphene in urine after solid-phase extraction. *J Anal Toxicol* 20: 547–554.

Cleemann M (1977). Gas chromatographic determination of propoxyphene and norpropoxyphene in plasma. *J Chromatogr* 132: 287–294.

Cravey RH *et al.* (1974). Incidence of propoxyphene poisoning: a report of fatal cases. *J Forensic Sci* 19: 72–80.

Gaillard Y, Pépin G (1998). Gas chromatographic-mass spectrometric quantitation of dextropropoxyphene and norpropoxyphene in hair and whole blood after automated on-line solid-phase extraction Application in twelve fatalities. *J Chromatogr B Biomed Sci Appl* 709: 69–77.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants.* Washington DC: American Chemical Society.

Kintz P *et al.* (1990). Simultaneous determination of dextropropoxyphene, norpropoxyphene and methaqualone in plasma by gas chromatography with selective nitrogen detection. *J Toxicol Clin Exp* 10: 89–94.

Kunka RL *et al.* (1985). Liquid chromatographic determination of propoxyphene and norpropoxyphene in plasma and breast milk. *J Pharm Sci* 74: 103–104.

Margot PA *et al.* (1983). Capillary and packed column GC determination of propoxyphene and norpropoxyphene in biological specimens: analytical problems and improvements. *J Chromatogr Sci* 21: 201–204.

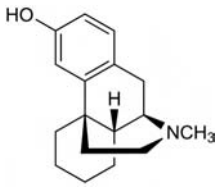
McBay AJ (1976). Propoxyphene and norpropoxyphene concentrations in blood and tissues in cases of fatal overdose. *Clin Chem* 22: 1319–1321.

Mersch F *et al.* (1997). Quantification of dextropropoxyphene and its metabolite by HPLC in hair of overdose cases. *Forensic Sci Int* 84: 237–242.

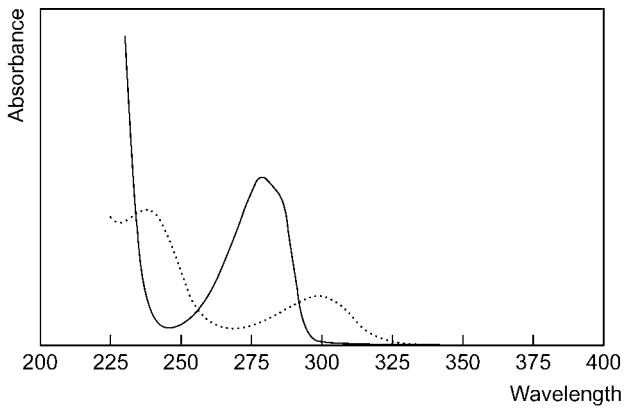
Millard BJ *et al.* (1980). Thermal decomposition of propoxyphene during GLC analysis. *J Pharm Sci* 69: 1177–1179.
Verebely K, Inturrisi CE (1973). The simultaneous determination of propoxyphene and norpropoxyphene in human biofluids using gas-liquid chromatography. *J Chromatogr* 75: 195–205.
Verebely K, Inturrisi CE (1974). Disposition of propoxyphene and norpropoxyphene in man after a single oral dose. *Clin Pharmacol Ther* 15: 302–309.
Young RJ (1983). Dextropropoxyphene overdosage Pharmacological considerations and clinical management. *Drugs* 26: 70–79.

Dextrorphan

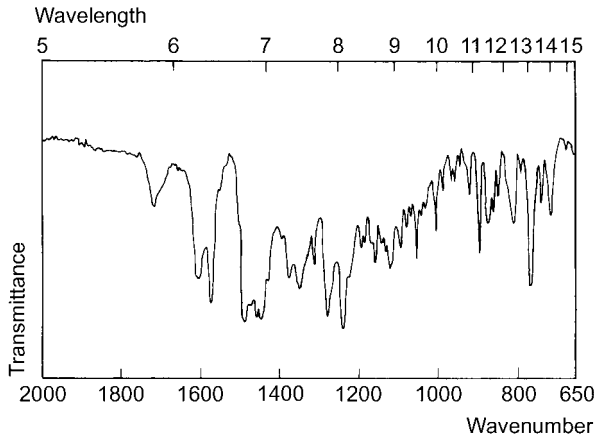
Cough Suppressant
$C_{17}H_{23}NO$ = 257.4
CAS—125-73-5
Synonyms (+)-17-Methylmorphinan-3-ol; Ro-1-6794.



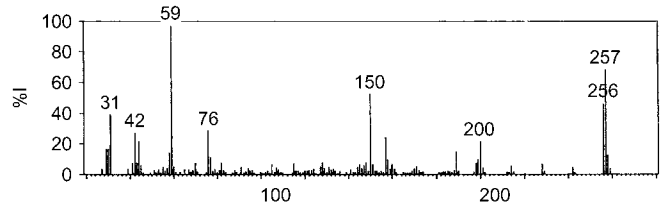
Chemical Properties Crystals. Mp 198° to 199°. Log *P* (octanol/water), 3.1.
Thin-layer Chromatography System TA—R_f 0.35; system TB—R_f 0.14; system TC—R_f 0.04; system TE—R_f 0.42; system TL—R_f 0.03; system TAE—R_f 0.10; system TAF—R_f 0.49 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—dextrorphan RI 2230, dextrorphan-AC RI 2280, dextrorphan-PFP RI 2060, dextrorphan-TFA RI 2015, dextrorphan-TMS RI 2230, M (nor-) RI 2241, M (nor-)-AC_2 RI 2710, M (OH-)-AC_2 RI 2555; system GB—dextrorphan RI 2323, M (nor-) RI 2328.
High Performance Liquid Chromatography System HA—*k* 4.7 (tailing peak); system HX—RI 325.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=79a); aqueous alkali—240 (A^1_1=339a), 299 nm (A^1_1=119a).



Infrared Spectrum Principal peaks at wavenumbers 1240, 1495, 1280, 1580, 756, 1610 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 59, 257, 150, 256, 31, 76, 42, 157; M (nor-) 243, 157, 136, 198, 200, 242; M (OH-)-AC_2 357, 231, 356, 355, 298, 315.

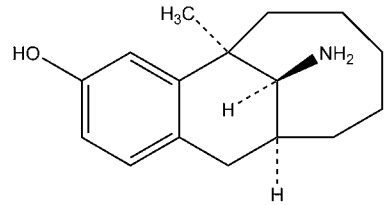


Quantification See under Dextromethorphan.
***Plasma* HPLC** MS/MS detection. Limit of quantification, 5 ng/L [Eichhold *et al.* 1997]. Fluorescence detection (λ_{ex}=280 nm; λ_{em}=315 nm). Limit of detection, 20 µg/L [Stavchansky *et al.* 1995].
***Urine* HPLC** Fluorescence detection (λ_{ex}=280 nm; λ_{em}=310 nm). Limit of detection, 0.02 mg/L for dextromethorphan [Lam, Rodriguez 1993].
Disposition in the Body Dextrorphan is the major metabolite of dextromethorphan but has less antitussive activity.

Eichhold TH *et al.* (1997). Determination of dextromethorphan and dextrorphan in human plasma by liquid chromatography/tandem mass spectrometry. *J Mass Spectrom* 32(11): 1205–1211.
Lam YW, Rodriguez SY (1993). High-performance liquid chromatography determination of dextromethorphan and dextrorphan for oxidation phenotyping by fluorescence and ultraviolet detection. *Ther Drug Monit* 15(4): 300–304.
Stavchansky S *et al.* (1995). Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection. *J Pharm Biomed Anal* 13(7): 919–925.

Dezocine

Narcotic Analgesic, Opioid Receptor Antagonist
$C_{16}H_{23}NO$ = 245.4
CAS—53648-55-8
Synonyms (5*R*,11*S*,13*S*)-13-Amino-5,6,7,8,9,10,11,12-octahydro-5-methyl-5,11-methanobenzocyclodecen-3-ol; Wy-16225.
Proprietary Name *Dalgan*



Chemical Properties Solid. Log *P* 3.97 [Wishart 2006].

Dezocine Hydrobromide

$C_{16}H_{23}NO,HBr$ = 326.3
Chemical Properties Crystalline powder. Mp 269° to 270°. Soluble in water.

Quantification

***Plasma* HPLC** Column: µBondapak C_{18} (120 × 3.9 mm i.d., 10 µm). Mobile phase: water : methanol : butanol : phosphoric acid (650 : 289 : 10 : 1), flow rate 0.8 to 1.0 mL/min. Electrochemical detection. Limit of detection, 1 to 2 µg/L [Locniskar, Greenblatt 1986].
Disposition in the Body Readily absorbed following IM administration. It appears to be metabolised in the liver and excreted mainly as the glucuronide conjugate in the urine.

Therapeutic Concentration

Pharmacokinetics of IV dezocine and the bioavailability of IM and SC dezocine were evaluated in healthy male volunteers. In study 1, 12 volunteers were administered 5, 10 or 20 mg IV doses over a period of 5 min. In study 2, 24 volunteers were administered 10 mg IV or IM dezocine. In the third study, 24 volunteers were given 10 mg dezocine IV or SC. Results of study 1 are summarised below:

	Dose		
	5 mg	***10 mg***	***20 mg***
Half-life (h)	2.60	2.60	2.80
Volume of distribution (L/kg)	11.8	11.2	9.40
Total clearance (L/h/kg)	3.13	2.96	2.49

In the second study, the mean peak serum concentration after IM injection reached 18.9 µg/L after 0.57 h. In study 3, the mean peak serum concentration after SC injection reached 11.1 µg/L after 1.2 h [Locniskar *et al.* 1986].

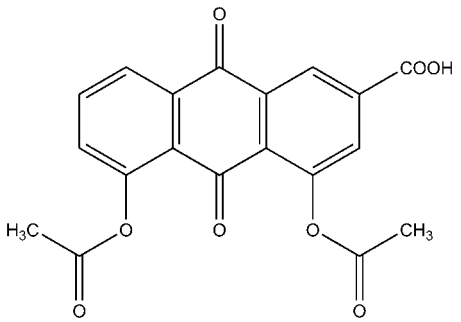
Bioavailability Approximately 97%.
Half-life Approximately 2.7 h.
Volume of Distribution Approximately 10.8 L/kg.
Clearance Approximately 2.86 L/h/kg.
Protein Binding 91.6 ± 0.8%.
Dose Dezocine is administered in opioid-dependent subjects maintained on oral methadone (30 mg daily) as IM injections ranging from 7.5 to 60 mg.

Locniskar A, Greenblatt DJ (1986). Determination of ciramadol and dezocine, two new analgesics, by high-performance liquid chromatography using electrochemical detection. *J Chromatogr* 374: 215–220.
Locniskar A *et al.* (1986). Pharmacokinetics of dezocine, a new analgesic: effect of dose and route of administration. *Eur J Clin Pharmacol 30*,121–123.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

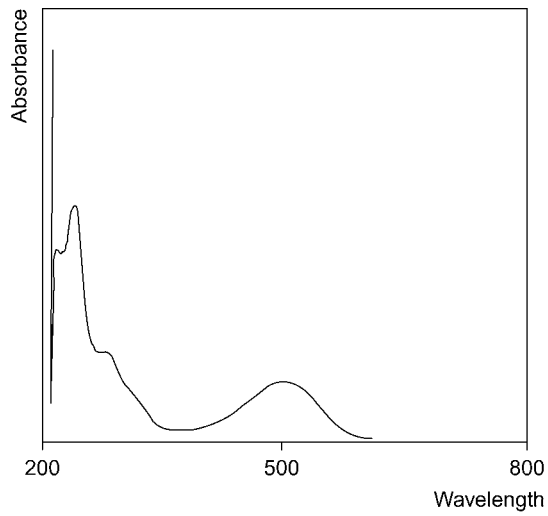
Diacerein

Antiarthritic, Antiinflammatory, Interleukin I Inhibitor
$C_{19}H_{12}O_8$ = 368.3
CAS—13739-02-1

IUPAC Name 4,5-Diacetyloxy-9,10-dioxoanthracene-2-carboxylic acid
Synonyms 4,5-bis(Acetyloxy)-9,10-dihydro-9,10-dioxo-2-anthracenecarboxylic acid; DAR; diacerhein; 1,8-diacetoxy-3-carboxyanthraquinone; diacetylrhein; 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthroic acid diacetate; SF-277.
Proprietary Names *Artrodar*; *Fisiodar*.



Chemical Properties Yellow plates. Mp 217° to 218°. Log *P* (octanol/water), 2.42 [Meylan, Howard 1995], 1.99 [ACD 2007]. At room temperature, degradation to rhein proceeds stoichiometrically. In acid-stressed samples, 40% of the drug at room temperature and 85 to 90% at 37° degrades to rhein. Stable to the effect of temperature and UV light; 73.5% of diacerein was recovered after oxidative stress [Giannellini *et al.* 2005].
High Performance Liquid Chromatography Column: RP Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L phosphoric acid : methanol (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Giannellini *et al.* 2005].
Liquid Chromatography-Mass Spectrometry Column: RP Luna C_8 (50 × 2.0 mm i.d., 3 µm). Mobile phase: 0.05% formic acid in acetonitrile : 0.05% formic acid (10 : 90 to 90 : 10 at 25 min), flow rate 0.2 mL/min. Limit of detection not reported [Giannellini *et al.* 2005].
Ultraviolet Spectrum 0.1 mol/l NaOH—241, 277 and 502 nm [Borgmann *et al.* 2007].



Quantification
Plasma **HPLC** Column: Nucleosil C_{18}. Mobile phase: acetonitrile : McIlvaine buffer (0.1 mol/L citric acid : 0.2 mol/L disodium phosphate [98 : 2], pH 2.2; 47 : 53), flow rate 1.0 mL/min. Photometric detection (λ = 432 nm). Limit of detection, 0.1 mg/L [Debord *et al.* 1994].
Urine **HPLC** See Plasma [Debord *et al.* 1994].
Disposition in the Body Readily absorbed following IM administration. It appears to be metabolised in the liver to the active metabolite rhein and excreted mainly as the glucuronide conjugate in the urine. Rhein is a naturally occurring hydroxyanthroquinone present in rhubarb.
Therapeutic Concentration
A study involving 12 healthy subjects and 16 patients with various degrees of renal failure was undertaken to determine the influence of renal function on the pharmacokinetics of diacerein. Group I consisted of 6 males and 6 females (aged 41 to 59 years; weight 48 to 84 kg) with normal creatinine clearance of between 68 and 111 mL/min. Group II contained 1 female and 7 males with mild renal insufficiency (aged 31 to 67 years; weight 50 to 95 kg), creatinine clearance 33 to 56 mL/min. Group III contained 2 females and 6 males with severe renal insufficiency (aged 35 to 63 years; weight 47 to 93 kg), creatinine clearance 10 to 27 mL/min. Each group fasted overnight and received a single oral dose of 50 mg diacerein. Peak plasma concentrations of rhein did not change significantly relative to the degree of impairment as seen in the following:

	Rhein plasma concentration		
	Group I	***Group II***	***Group III***
Mean peak concentration (mg/L)	3.2	3.6	3.5
t_{max} (h)	2.2	2.7	2.7

[Debord *et al.* 1994].

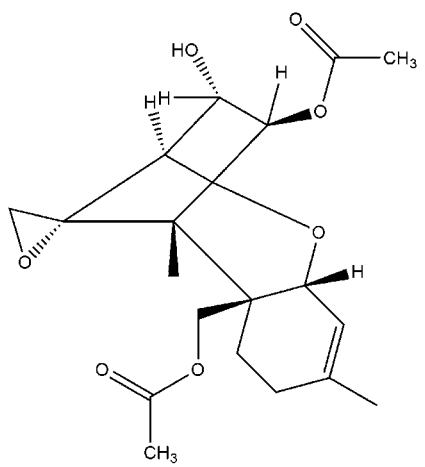
Toxicity For a case report of fatal hepatitis from the administration of diacerein, see Renan *et al.* [2001] (no levels reported).
Bioavailability Rhein, approx. 35%.
Half-life In healthy subjects, 4.3 h; 7 to 8 h with repeated administration [Nicolas *et al.* 1998].
Volume of Distribution Rhein, approx. 13.3 L (28 subjects weighing 47–95 kg) [Debord *et al.* 1994].
Clearance Rhein, approx. 0.13 L/h.
Protein Binding Approximately 99%.
Dose 50 mg twice daily orally. Doses should be halved in patients with creatinine clearance less than 30 mL/min.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).
Borgmann SH *et al.* (2007). Direct spectrophotometric determination of diacerhein in capsules. *Pharmazie* 62: 483–485.
Debord P *et al.* (1994). Influence of renal function on the pharmacokinetics of diacerein after a single oral dose. *Eur J Drug Metab Pharmacokinet* 19: 13–19.
Giannellini V *et al.* (2005). A validated HPLC stability-indicating method for the determination of diacerhein in bulk drug substance. *J Pharm Biomed Anal* 39: 776–780.
Meylan W, Howard M, P.H (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Nicolas P *et al.* (1998). Clinical pharmacokinetics of diacerein. *Clin Pharmacokinet* 35: 347–359.
Renan X *et al.* (2001). [Case report of fatal hepatitis from diacerein.]. *Therapie* 56: 190–191.

Diacetoxyscirpenol

12,13-Epoxytrichothecene, Mycotoxin
$C_{19}H_{26}O_7$ = 366.4

Synonyms Anguidin; anguidine; 4,15-di-*O*-acetylscirpenol; diazetoxyscirpenol; 4,15-diacetoxyscirpenol; 4,15-diacetoxyscirpen-3-ol; scirpenetriol 4,15-diacetate.



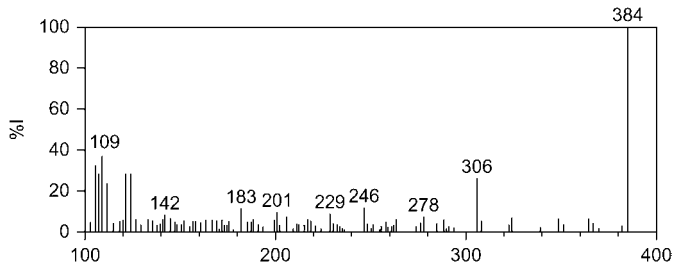
	?Mobile phase solvent ratio, R_f value								
	A	*RP-18*	*KC-18*	*B*	*RP-18*	*KC-18*	*C*	*RP-18*	*KC-18*
Aflatoxin B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxin B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxin G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxin G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	**65 : 35**	**0.31**	**0.42**	**65 : 35**	**0.63**	**0.59**	**N/A**	**N/A**	**N/A**
Deoxynivalenol	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyldeoxynivalenol	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Nivalenol	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as follows:

Ultraviolet Spectrum No UV absorption [Sydenham *et al.* 1996].

Mass Spectrum Principal peaks at *m/z* 384, 109, 306, 246, 183, 201 [Sydenham *et al.* 1996].



Quantification

Blood **GC-MS** Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 35 cm/s. Temperature programme: 75° for 2 min to 300° at 10°/min. CI, SIM acquisition mode. Limit of detection, 30 pg [D'Agostino *et al.* 1986].

Urine **GC-MS** Column: BP-1 (25 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 15 psi. Temperature programme: 160° for 1 min to 275° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 2–5 µg/L [Black *et al.* 1986].

Other **GC-MS** Food. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 180° at 8°/min to 250°. EI ionisation at 70 eV. Limit of detection not reported [Yoshizawa 2001]. *Fusarium* Isolates. Column: Restek Rtx1 (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 150° for 2 min to 300° at 20°/min. EI ionisation at 40 eV, SIR and MRM acquisition mode. Limit of detection, low pg [Raza *et al.* 1993].

HPLC Dust. Column: Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase: 30% dichloromethane in hexane : 50% propan-2-ol in dichloromethane (95 : 5 to 50 : 50 over 20 min), flow rate 1.2 mL/min. UV detection (λ= 260 nm). Limit of detection, <0.4 ng/mg [Smoragiewicz *et al.* 1993].

LC-MS Food (peanuts, pistachios, wheat, maize, cornflakes, raisins, figs). Column: Alltima C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (90 : 10 to 30 : 70 at 12 min for 4 min to 10 : 90 at 17.5 min for 2.5 min to 90 : 10 at 21 min for 4 min), flow rate 0.3 mL/min. ESI. MRM acquisition mode, positive ion mode. Limit of detection, 25 µg/kg [Spanjer *et al.* 2008]. Wheat Flour. Column: Nucleosil C_{18} (125 × 2 mm i.d., 3 µm). Mobile phase: methanol : water (25 : 75 to 98 : 2 at 12 min for 5 min to 25 : 75 for 1 min), flow rate 250 µL/min. APCI, positive ion mode. Limit of quantification, 70 pg, limit of detection, 20 pg [Berger *et al.* 1999].

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Diamfenetide

Anthelmintic (Veterinary)

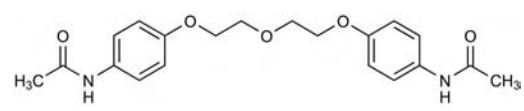
$C_{20}H_{24}N_2O_5$ = 372.4

CAS—36141-82-9

IUPAC Name *N*-[4-[2-[2-(4-Acetamidophenoxy)ethoxy]ethoxy]phenyl]acetamide

Synonyms Diamphenethide; *N*,*N*′-[oxybis(2,1-ethanediyloxy-4,1-phenylene)] bisacetamide.

Proprietary Name *Coriban*

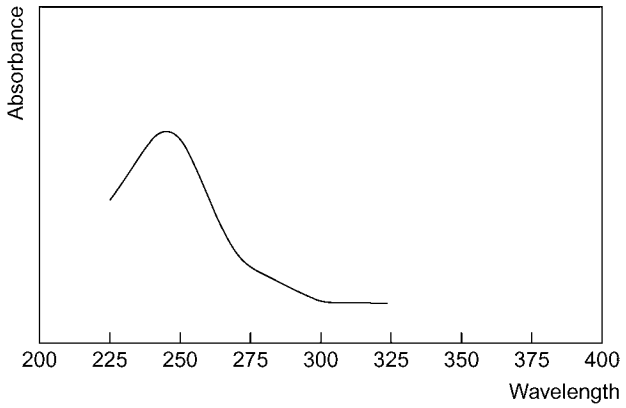


Chemical Properties A white to pale buff-coloured powder. Practically insoluble in water; soluble 1 in 160 of ethanol, 1 in 500 of chloroform and 1 in 150 of methanol; practically insoluble in ether. Log *P* (octanol/water), 1.8.

Colour Test Mandelin's test—grey-green.

Thin-layer Chromatography System TA—R_f 0.81 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—244 nm.

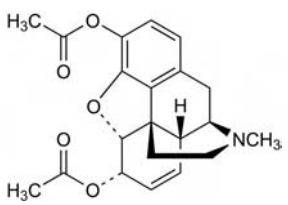


Infrared Spectrum Principal peaks at wavenumbers 1653, 1508, 1529, 1238, 1136, 1597 cm^{-1} (KBr disk).

Diamorphine

Narcotic Analgesic
$C_{21}H_{23}NO_5$ = 369.4
CAS—561-27-3

IUPAC Name (5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol diacetate (ester)
Synonyms Acetomorphine; diacetylmorphine; heroin.

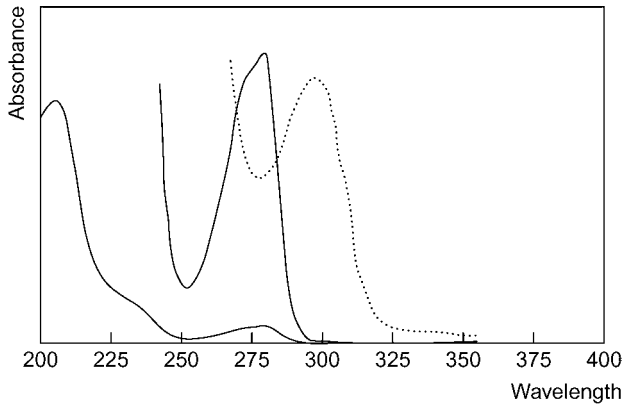


Chemical Properties White crystals. Mp 173°. It is rapidly hydrolysed by alkalis. Soluble 1 in 1700 of water, 1 in 31 of ethanol, 1 in 1.5 of chloroform and 1 in 100 of ether. pK_a 7.6 (23°) Log *P* (ether/water pH 7.0), 0.2.

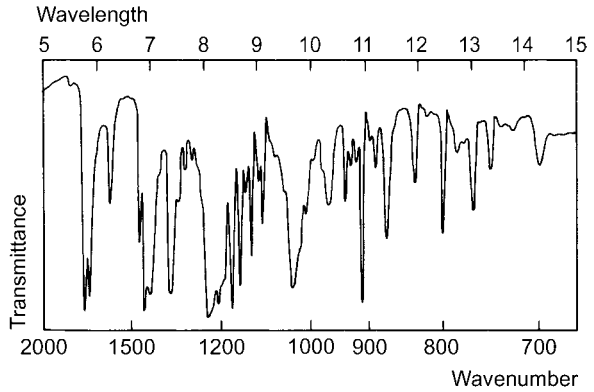
Diamorphine Hydrochloride

$C_{21}H_{23}NO_5$,HCl,H_2O = 423.9
CAS—1502-95-0 (anhydrous)
Proprietary Names *Diagesil*; *Diamorf*.
Chemical Properties An almost white crystalline powder. Mp 243° to 244°. Soluble 1 in 2 of water, 1 in 11 of ethanol and 1 in 1.6 of chloroform; insoluble in ether. Diamorphine hydrolyses in aqueous solution to 3-*O*- and 6-*O*-monoacetylmorphine and morphine to a significant extent at room temperature.

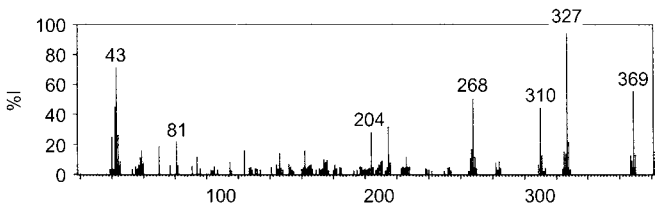
Colour Test Liebermann's reagent—black; Mandelin's test—blue-grey; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.15; system TC—R_f 0.38; system TE—R_f 0.49; system TL—R_f 0.04; system TAE—R_f 0.26; system TAF—R_f 0.33; system TAJ—R_f 0.25; system TAK—R_f 0.05; system TAL—R_f 0.64 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—violet).
Gas Chromatography System GA—RI 2615, M (6-monoacetylmorphine) RI 2525, M (3-monoacetylmorphine) RI 2495, M (morphine) RI 2445; system GB—RI 2769, M (6-monoacetylmorphine) RI 2646, M (3-monoacetylmorphine) RI 2625, M (morphine) RI 2564; system GAK—RT 14.2 min.
High Performance Liquid Chromatography System HA—*k* 3.0 (tailing peak), M (6-monoacetylmorphine) *k* 3.6 (tailing peak), M (morphine) 3.8 (tailing peak); system HC—*k* 0.66, M (6-monoacetylmorphine) *k* 0.80, M (morphine) *k* 1.30; system HS—*k* 0.35, M (6-monoacetylmorphine) *k* 1.00, M (morphine) *k* 5.16; system HX—RI 340; system HY—RI 282; system HAX—RT 7.9 min; system HAY—RT 4.1 min; system HBC—RT 2.9 min.
Ultraviolet Spectrum Aqueous acid—279 nm (A^{1}_{1}= 46a); aqueous alkali—299 nm (A^{1}_{1}= 69a)



Infrared Spectrum Principal peaks at wave numbers 1245, 1764, 1178, 1215, 911, 1736 cm^{-1} (diamorphine hydrochloride, Nujol mull). Two polymorphic forms may occur.



Mass Spectrum Principal ions at *m/z* (diamorphine) 327, 43, 369, 268, 310, 42, 215, 204; (6-monoacetylmorphine) 327, 268, 42, 43, 215, 44, 328, 269; (morphine) 285, 162, 42, 215, 286, 124, 44, 284.



Quantification

Blood **GC** Column: 2% OV-17 (2.13 m × 4.0 mm i.d.). Carrier gas: N_2, 65 mL/min. Temperature: 250°. AFID. Limit of detection, 100 µg/L [Smith, Cole 1975].

GC-MS Column: XTerra C_{18}. MRM acquisition mode, positive ion mode. Limit of quantification, 0.0007–0.02 mg/L [Karinen *et al.* 2009]. Column: 95% dimethylpolysiloxane, 5% diphenylpolysiloxane Rtx (15 m × 25 mm i.d., 0.1 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 150° for 1 min to 200° at 12.5°/min for 15 s to 290° at 30°/min for 4 min. SIM acquisition mode. Limit of quantification, 1 µg/L [Goldberger *et al.* 1993].

HPLC Column: LiChrosorb Si-60 (300 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol (pH 7, 75 : 25), flow rate 80 mL/h. UV detection (λ= 218 nm). Limit of detection, 12.5 µg/L [Umans *et al.* 1982].

LC-MS Column: Gemini C_{18} reversed phase (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile : ammonium acetate (pH 3.2, 15 : 85 for 9 min to 30 : 70 in 13 min to 80 : 20 in 10 min to 95 : 5 in 1 min), flow rate 150 µL/min. TIS, MRM acquisition mode. Limit of quantification, 0.008 mg/L, limit of detection, 0.002 mg/L [Gergov *et al.* 2009].

Plasma **GC-MS** Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 1 min to 220° at 35°/min for 0.25 min to 250° at 10°/min for 3 min. MSD, SIM acquisition mode. Limit of detection, 1 µg/L [Wang *et al.* 1994]. See Blood [Goldberger *et al.* 1993].

HPLC Column: Spherisorb C18 ODS-2 (125 × 2 mm i.d., 3 µm). Mobile phase: water containing 8.5 g *o*-phosphoric acid (85%) and 0.39 g hexylamine/

1000 g : acetonitrile-water containing 8.5 g *o*-phosphoric acid and 1.1 g hexylamine/1000 g (98 : 2 to 97.5 : 2.5 at 2.5 min to 82.5 : 17.5 at 2.8 min to 97.5 : 2.5 in 3.5 min to 92.5 : 7.5 at 6 min to 85.5 : 14.5 at 7.5 min to 75 : 25 at 11 min), flow rate 0.2 mL/min. DAD (λ= 210 nm). Limit of quantification, 25 µg/L [Bourquin *et al.* 1997].

LC-MS Column: Zorbax reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate (pH 4.0) : acetonitrile (97 : 3 for 2 min to 87 : 13 at 2.6 min to 84.5 : 15.5 at 8 min to 20 : 80 at 8.1 min until 11 min to 97 : 3 at 11.1 min until 15 min), flow rate 1.0 mL/min. TIS, MRM acquisition mode. Retention time: 8.0 min. Limit of quantification, 5 µg/L [Rook *et al.* 2005].

Serum **HPLC** Column: Supelcosil LC-Si (250 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : water : formic acid (59.8 : 5.2 : 34.65 : 0.35), flow rate 230 µL/min. API. Limit of quantification, 0.5 µg/L [Zuccaro *et al.* 1997].

Urine **GC-MS** See Plasma [Wang *et al.* 1994]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 120° for 1 min to 220° at 20°/min to 260° at 5°/min to 280° at 20°/min for 2 min. EI ionisation, SIM acquisition mode. Limit of detection, 50 µg/L [De Giovanni, Strano Rossi 1994]. See Blood [Goldberger *et al.* 1993].

HPLC Column: Silica (200 × 2 mm i.d., 3 µm). Mobile phase: dichloromethane : pentane : diethylamine : methanol. Limit of detection, 4–20 µg/L [Low, Taylor 1995]. Column: Supelcosil LC-18-DB (5 µm). Mobile phase: 0.1 mol/L sodium hydrogen phosphate : methanol (pH 7.3, 67.5 : 32.5), flow rate 1.0 mL/min Electrochemical detection. Retention time: 8.22 min. Limit of detection, 250 µg/L [Sawyer *et al.* 1988].

LC-MS See Blood [Gergov *et al.* 2009].

Meconium **GC-MS** See Salem *et al.* [2001].

Oral Fluid **GC-MS** See Plasma [Wang *et al.* 1994]. See Blood [Goldberger *et al.* 1993].

Brain **GC-MS** See Blood. Limit of quantification, 0.002–0.06 µg/g [Karinen *et al.* 2009].

Hair **GC-MS** Column: Restek Rtx-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 260° at 15°/min to 290° at 7°/min for 6 min. Limit of quantification, 0.02 ng/mg [Pichini *et al.* 1999]. See Plasma. Limit of detection, 0.1 ng/mg [Wang *et al.* 1994]. See Polettini *et al.* [1993].

LC-MS Column: Zorbax phenyl (50 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile-methanol-20 µmol/L formate buffer (pH 3.0, 10 : 10 : 80) : acetonitrile-methanol-20 µmol/L formate buffer (pH 3.0, 35 : 35 : 30; 100 : 0 to 35 : 65 from 0.5 to 0.7 min to 100 : 0 in 2 min), flow rate 0.25 mL/min. ESI. Limit of detection not reported [Kronstrand *et al.* 2004].

Other **GC** Illicit Heroin Samples. Column: 2.5% OV-1 or SE-52 on 80/100 mesh Chromosorb G (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 240° for 2 min to 280° at 24°/min for 6 min. Limit of detection not reported [Machata, Vycudilik 1980].

HPLC Column: µBondapak C_{18} (25 × 4.6 cm i.d.). Mobile phase: acetonitrile : water : phosphoric acid with 0.02 mol/L methanesulfonic acid (pH 2.2, 12 : 87 : 1), flow rate 3.0 mL/min. UV detection (λ= 254 nm). Limit of detection, 0.5 g/L [Lurie *et al.* 1982].

Disposition in the Body Diamorphine is readily absorbed after oral administration or by injection. Following injection, it is rapidly hydrolysed to 6-monoacetylmorphine in blood and then more slowly metabolised to morphine, which is the major active metabolite; normorphine is also formed to a minor extent. Small quantities of codeine are occasionally seen in the urine of addicts, but this is thought to arise from the presence of acetylcodeine as an impurity in illicit heroin samples. The blood concentration of morphine depends on the route of administration of the drug, the dose, time since the last dose and the individual's metabolism. Orally administered diamorphine undergoes extensive first-pass metabolism to morphine. All metabolites may be conjugated with glucuronic acid. Up to 80% of a dose is excreted in the urine in 24 h, mainly as morphine 3-glucuronide, with ≈5–7% of the dose as free morphine, 1% as 6-monoacetylmorphine, 0.1% as unchanged drug and trace amounts of other metabolites. After inhalation, 14–20% of the dose appears in the urine; morphine metabolites are excreted in the bile. Diamorphine crosses the blood–brain barrier within 15–20 s and achieves relatively high brain levels; 68% of an IV dose is absorbed into the brain.

Therapeutic Concentration Because of its rapid hydrolysis, diamorphine is difficult to detect in plasma. Morphine plasma concentrations are usually in the range 0.01–0.07 mg/L.

Twenty-six newborn premature neonates, with a gestational age of 26–40 weeks (mean, 30.7), were administered with a loading dose of 50 µg/kg diamorphine over a 30 min period followed by a 15 µg/kg/h IV infusion for 14–149 h (mean, 60.2). The mean steady-state plasma morphine concentration after the 15 µg/kg/h dose was 62.5 µg/L (range, 20–98) [Barrett *et al.* 1991].

Nineteen newborn infants of 24–41 weeks of gestation (mean, 29.7) who were on ventilation were administered with a loading dose of 50 µg/kg or 200 µg/kg diamorphine followed by 15 µg/kg/h as an IV infusion. The mean steady-state plasma morphine concentration was 86 µg/L after the 50 µg/kg and 200 µg/kg doses. The steady-state morphine concentration was 86 µg/L (ranges, 20–215 for the 50 µg/kg loading dose; 14–146 for the 200 µg/kg dose). For morphine 3-glucuronide, steady state was 703 µg/L (ranges 135–1237 and 45 to 1401 for the 2 loading doses, respectively) and for morphine 6-glucuronide, 48 µg/L (ranges, 35–91 and 11–120 µg/L, respectively) [Barrett *et al.* 1996].

Toxicity Diamorphine is 2–3 times more potent than morphine. The estimated minimum lethal dose is 200 mg, but addicts may be able to tolerate up to 10 times as much; fatalities have occurred after doses of 10 mg.

A 17-year-old pregnant girl, a known drug abuser, was found dead in a public toilet with needle puncture marks. A postmortem carried out 48 h after death showed massive brain and lung oedema associated with an acute intoxication. In her femoral vein blood a 6-monoacetylmorphine concentration of 4 µg/L was detected as well as 280 µg/L morphine and 20 µg/L codeine. In her urine 1170 µg/L of 6-monoacetylmorphine was found. These were all consistent with a recent heroin intake. 6-Monoacetylmorphine, morphine, morphine 3-glucuronide and codeine were all detected in the fetus's amniotic fluid, brain and lung at high concentrations [Skopp *et al.* 1998].

A 40-year-old man with a long history of drug abuse was found dead after an overdose of heroin. Heroin was detected at a concentration of 0.109 mg/L in blood and 17 ng/g in gastrointestinal contents. Its metabolite, 6-monoacetylmorphine, was found at concentrations of 0.168 mg/L and 12 ng/g, respectively, and morphine at 1.14 mg/L and 425 ng/g, respectively. Morphine was also found in urine at a concentration of 3.65 mg/L [Rop *et al.* 1997].

Of 10 deaths resulting from heroin overdoses via non-injection routes, 4 involved inhalation ('chasing'), 4 involved nasal administration ('snorting') and 2 involved oral ingestion. In 1 case, the person was observed to smoke a heroin cigarette and then snort heroin immediately before losing conciousness. In 9 of the cases, venipuncture marks were detected at postmortem. The median blood morphine concentration was 0.31 mg/L (range, 0.06–0.99). Drugs other than morphine were also detected in 7 cases (alcohol (4), cocaine (2), dothiepin, methadone and paracetamol (1 each)) [Darke, Ross 2000].

A 46-year-old man died after injecting heroin into his penis. The concentration of morphine in body fluids at postmortem were blood 0.68 mg/L, urine 0.49 mg/L, bile 0.32 mg/L and vitreous humour 0.062 mg/L; ethanol was also detected in these fluids [Winek *et al.* 1999].

Of 10 deaths involving heroin body-packers, four had blood concentrations <1 mg/L. In five others, concentrations were 4.4, 6.7, 35.8, 39.4 and 52.6 mg/L. One victim, who died of peritonitis, had no detectable morphine in the blood [Wetli *et al.* 1997].

Half-life Plasma half-life, diamorphine ≈3 min, morphine ≈2–3 h.

Volume of Distribution Morphine ≈3–5 L/kg.

Clearance Plasma clearance, morphine ≈15–20 mL/min/kg; newborn infants (24–41 weeks of gestation), 4.6 mL/min/kg.

Protein Binding Morphine ≈20–35%.

Note For a review of the pharmacokinetics and pharmacodynamics of smoked heroin, see Jenkins *et al.* [1994]; for a review of the metabolism and excretion of diamorphine, see Boerner [1975]. For a review of fatal heroin overdose, see Darke, Zador [1996]; for reports of heroin use when driving, see Ceder, Jones [2002].

Dose For acute pain, 5 to 10 mg diamorphine hydrochloride every 4 h by SC or IM injection.

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Pichini S *et al.* (1999). Determination of opiates and cocaine in hair as trimethylsilyl derivatives using gas chromatography–tandem mass spectrometry. *J Anal Toxicol* 23: 343–348.

Polettini A *et al.* (1993). Rapid and highly selective GC/MS/MS detection of heroin and its metabolites in hair. *Forensic Sci Int* 63: 217–225.

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Salem MY *et al.* (2001). GC-MS determination of heroin metabolites in meconium: evaluation of four solid-phase extraction cartridges. *J Anal Toxicol* 25: 93–98.

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Diampromide

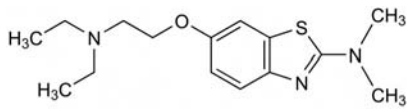
Narcotic
$C_{21}H_{28}N_2O$ = 324.5
CAS—552-25-0

IUPAC Name *N*-[2-(Methylphenethylamino)propyl]propionanilide
Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.
Colour Test Sulfuric acid–formaldehyde test—orange (limit of detection, 1.0 μg).
Thin-layer Chromatography System T1—R_f 0.42 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—225, 257, 263 nm.

Diamthazole

Antifungal
$C_{15}H_{23}N_3OS$ = 293.4
CAS—95-27-2

IUPAC Name 6-[2-(Diethylamino)ethoxy]-*N*,*N*-dimethyl-2-benzothiazolamine
Synonyms Amycazol; dimazole.

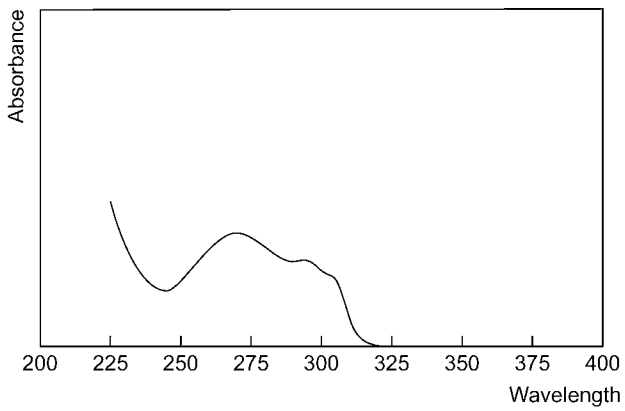


Chemical Properties Log *P* (octanol/water), 2.5 (hydrochloride).

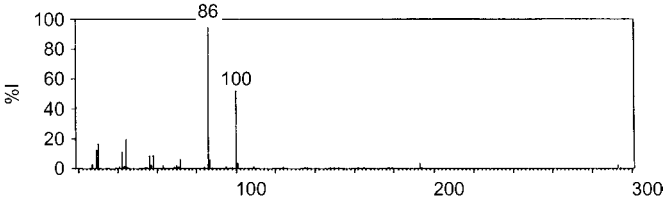
Diamthazole Hydrochloride

$C_{15}H_{23}N_3OS,2HCl$ = 366.4
CAS—136-96-9
Proprietary Name *Asterol*
Chemical Properties A white, hygroscopic, crystalline powder. Mp 240° to 243°. Soluble in water, ethanol and methanol; very slightly soluble in chloroform and ether.

Colour Test Liebermann's reagent—green.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.30; system TC—R_f 0.30 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—270 (A^1_1=370b), 293 nm.



Infrared Spectrum Principal peaks at wavenumbers 1015, 1041, 667, 1548, 1602, 1190 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 100, 44, 30, 29, 42, 58, 56.

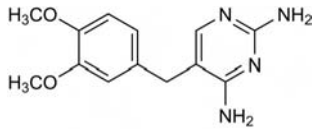


Use Diamthazole hydrochloride has been used in a concentration of 5%.

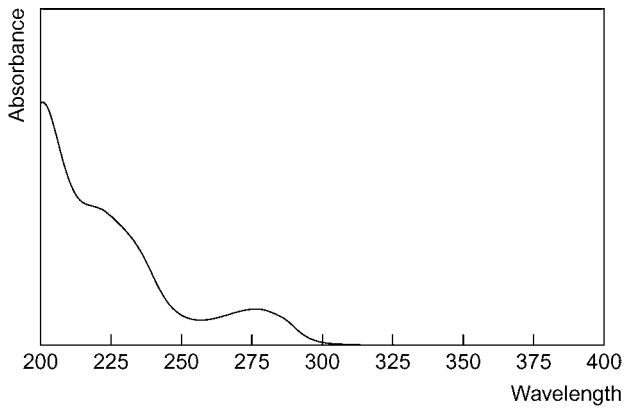
Diaveridine

Coccidiostat (Veterinary)
$C_{13}H_{16}N_4O_2$ = 260.3
CAS—5355-16-8

IUPAC Name 5-[(3,4-Dimethoxyphenyl)methyl]-2,4-pyrimidinediamine
Proprietary Name It is an ingredient of *Saquadil*.



Chemical Properties A white or creamy-white crystalline powder. Mp 233°. Very slightly soluble in water and ethanol; soluble 1 in 600 of chloroform. Log *P* (octanol/water), 1.0.
Colour Tests Mandelin's test—green; Marquis test—grey→violet-brown.
Thin-layer Chromatography System TA—R_f 0.58 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HAA—retention time 7.0 min.
Ultraviolet Spectrum Aqueous acid—276 nm (A^1_1=300a).

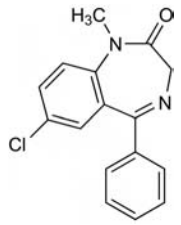


Infrared Spectrum Principal peaks at wavenumbers 1630, 1645, 1598, 1250, 1510, 1570 cm^{-1} (KBr disk).

Diazepam

Anxiolytic, Benzodiazepine, Tranquilliser
$C_{16}H_{13}ClN_2O$ = 284.8
CAS—439-14-5

IUPAC Name 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-one
Synonyms Diazepamum; LA-III; NSC-77518; Ro-5-2807; Wy-3467.
Proprietary Names *Antenex*; *Anxicalm*; *Apozepam*; *Dialar*; *Diapam*; *Diazemuls*; *Diazep*; *Ducene*; *Faustan*; *Gewacalm*; *Hexalid*; *Lamra*; *Medipam*; *Novazam*; *Novodipam*; *Pro-Pam*; *Psychopax*; *Rimapam*; *Serenack*; *Stesolid*; *Tensium*; *Tranquase*; *Umbrium*; *Valaxona*; *Valclair*; *Valiquid*; *Valium*; *Vivol*.



Chemical Properties A white or yellow crystalline powder. Mp 125° to 126°. Slightly soluble in water; soluble 1 in 25 of ethanol, 1 in 2 of chloroform, and 1 in 39 of ether. pK_{a1} 3.5 [Krogh *et al.* 1997], pK_{a2}3.3 (20°). Log *P* (octanol/water), 2.8

[Capella-Peiró *et al.* 2002], 3.08 [Mullett, Pawliszyn 2001], (octanol/water pH 7.4), 2.7. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

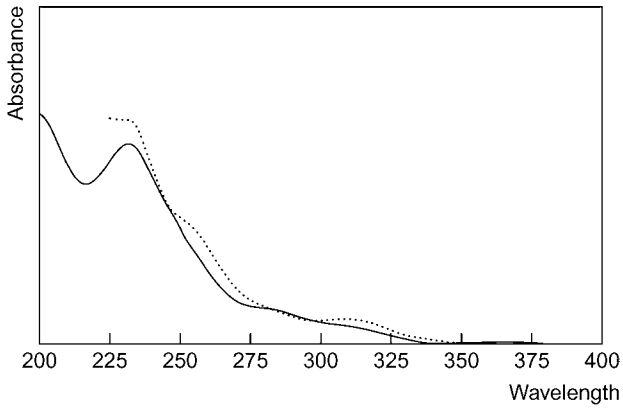
Colour Test Formaldehyde-sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.27; system TC—R_f 0.73; system TD—R_f 0.58; system TE—R_f 0.76; system TF—R_f 0.49; system TL—R_f 0.59; system TAD—R_f 0.72; system TAE—R_f 0.82; system TAF—R_f 0.85; system TAJ—R_f 0.67; system TAK—R_f 0.48; system TAL—R_f 0.96 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; FPN reagent—yellow; Marquis test—yellow).

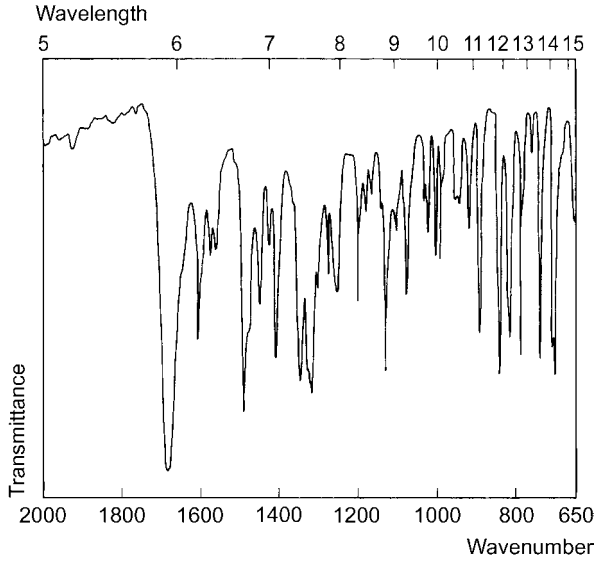
Gas Chromatography System GA—diazepam RI 2428, M (nordazepam[desmethyldiazepam or nordiazepam]) RI 2490, M (oxazepam) RI 2325, M (temazepam) RI 2595; system GB—diazepam RI 2556, M (nordazepam) RI 2625, M (oxazepam) RI 2438, M (temazepam) RI 2727; system GF—RI 3045; system GG—diazepam RI 2940, M (nordazepam) RI 3041, M (oxazepam) RI 2803, M (temazepam) RI 3125. (Nordazepam, oxazepam and temazepam are metabolites of diazepam).

High Performance Liquid Chromatography System HA—diazepam *k* 0.1, M (nordazepam) *k* 0.2; system HI—diazepam *k* 9.47, M (nordazepam) *k* 8.00, M (oxazepam) *k* 4.62, M (temazepam) *k* 5.68; system HJ—diazepam *k* 2.29; system HK—diazepam *k* 2.49, M (nordazepam) *k* 1.99, M (oxazepam) *k* 0.73, M (temazepam) *k* 0.60; system HX—RI 528; system HY—RI 429; system HZ—RT 8.4 min; system HAA—RT 20.3 min; system HAF—RT 29.8 min; system HAL—RT 13.2 min; system HAM—diazepam RT 10.3 min, M (oxazepam) RT 3.4 and M (oxazepam) RT 4.4 min; system HAX—diazepam RT 7.7 min, M (nordazepam) RT 6.7 min, M (oxazepam) RT 6.0 min, M (temazepam) RT 8.9 min; system HAY—diazepam RT 8.8 min, M (nordazepam) RT 6.8 min, M (oxazepam) RT 4.5 min, M (temazepam) RT 6.7 min; system HAZ—*k* 2.25; system HBH—diazepam *k* 10.4, M (nordazepam) *k* 8.97, M (oxazepam) *k* 5.42, M (temazepam) *k* 6.80; system HBI—diazepam *k* 2.29, M (nordazepam) *k* 1.89, M (oxazepam) *k* 1.25, M (temazepam) *k*1.49.

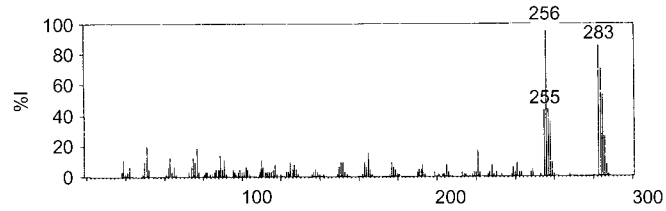
Ultraviolet Spectrum Aqueous acid—242 (A^1_1= 1020a), 284, 366 nm.



Infrared Spectrum Principal peaks at wavenumbers 1681, 1313, 705, 840, 1125, 740 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 256, 283, 284, 285, 257, 255, 258, 286 (diazepam); 242, 269, 270, 241, 243, 271, 244, 272 (nordazepam); 257, 77, 268, 239, 205, 267, 233, 259 (oxazepam); 271, 273, 300, 272, 256, 77, 255, 257(temazepam).



Quantification

Blood **GC** Columns: DB-1 and DB-1701 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 28° at 30°/min to 230° at 2°/min to 300° at 30°/min for 1 min or 120° for 1 min to 230° at 40°/min to 280° at 8°/min for 9 min. ECD. Limit of detection, 0.084 or 0.096 µmol/L [Gjerde *et al.* 1992]. Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 5.57 min. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: 100% methylsiloxane or 5% phenylsiloxane-95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 25°/min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification and limit of detection, 117 and 38.6 µg/L, respectively [Papoutsis *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 25 µg/L [Tiscione *et al.* 2008]. Column: DB-5 cross-linked 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 2 min to 180° at 30°/min to 280° at 5°/min for 19 min. Full scan mode. Retention time: 21.1 min. Limit of detection, 0.05 mg/L [Paterson *et al.* 2004]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 10 µg/L [Pirnay *et al.* 2002]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation [Papoutsis *et al.* 2010]. Column: BPX5 capillary (15 m × 0.32 mm i.d., 0.25 µm). SIM acquisition mode. Limit of detection, 0.2–20 µg/L [Inoue *et al.* 2000].

HPLC Column: Chromolith Performance (RP-18e) (100 × 4.6 mm). Mobile phase: 35 mmol/L phosphate buffer (pH 2.1) : acetonitrile (70 : 30), flow rate 2 mL/min. DAD (λ= 220 nm). Limit of quantification, 30 µg/L [Bugey, Staub 2004]. Column: Lichrospher select B (125 × 3 mm). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate (pH 2.1, 30 : 70 to 35 : 65 at 30 min), flow rate 0.5 mL/min to 0.3 mL/min at 30 min. DAD (λ= 254 nm). Limit of quantification, 30 µg/L, limit of detection, 10 µg/L [El Mahjoub, Staub 2001a]. Column: Lichrospher Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 2.1) : acetonitrile (65 : 35), flow rate 0.3 mL/min. UV detection (λ= 220 nm). Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [El Mahjoub, Staub 2000a].

LC-MS Column: Acquity BEH Phenyl (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (80 : 20 for 0.25 min to 65 : 35 over 2.25 min for 2.5 min to 20 : 80 over 1 min to 80 : 20 over 0.01 min for 1.4 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of detection not reported [Gunn *et al.* 2010]. Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5 to 50 : 25 : 25 at 7 min to 10 : 45 : 45 at 27 min for 3 min to 95 : 5 : 5 at 31 min), flow rate 0.45 mL/min. APCI. Limit of quantification, 2 µg/L [Dussy *et al.* 2006]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 0.006 mol/L formic acid (pH 3.0) : methanol (70 : 30 to 60 : 40 at 5 min to 50 : 50 in 25 min to 40 : 60 at 30 min for 5 min to 70 : 30 at 36 min for 9 min), flow rate 0.2 mL/min. Limit of quantification, 13.34.0 µg/L, limit of detection, 4.0 µg/L [Smink *et al.* 2004].

Plasma **GC** NPD. Limit of detection, 20–115 nmol/L. [Ugland *et al.* 2000]. Column: CPSIL 8 CB (25 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 150° to 230° at 40°/min for 2 min to 250° at 5°/min for 1 min to 300° at 15°/min for 3 min. NPD. Limit of detection, 0.01 µmol/L [Reubsaet *et al.* 1998]. Column: DB-1 methyl silicone (30 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature: 300°. NPD. Limit of detection, 100 nmol/L [Krogh *et al.* 1997]. Column: Ultra 2 5% phenyl methyl silicone (25 m × 0.32 mm i.d., 0.52 µm). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD. Retention time: 0.722. Limit of quantification, 2 µg/L [Gaillard *et al.* 1993].

GC-MS Column: HP-1 capillary (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° for 2 min to 310° at 40°/min for 2.5 min. EI ionisation, SIM acquisition mode. Retention time: 6.68 min. Limit of quantification, 0.05 mg/L [Peters *et al.* 2005].

HPLC Column: LC-18 DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 µmol/L potassium dihydrogen phosphate buffer solution (pH 6.0) : methanol : diethyl ether (55 : 40 : 5), flow rate 0.8 mL/min. UV detection (λ= 245 nm). Limit of quantification, 30 and 50 µg/L [Borges *et al.* 2009]. Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol : water (65 : 35), flow rate 1.0 mL/min. UV detection (λ= 230 nm) [Liang *et al.* 2009]. Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.).

Mobile phase: 10 µmol/L phosphate buffer (pH 2.5) methanol : acetonitrile (63 : 10 : 27), flow rate 2 mL/min. Limit of quantification, 2 µg/L [Rouini *et al.* 2008]. Column: Kromasil C_8 (250 × 5 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.05 mol/L ammonium acetate. Limit of quantification, 0.07–1.57 mg/L, limit of detection, 0.02–0.47 mg/L [Uddin *et al.* 2008].

See also Atta-Politou *et al.* [1999], Azzam *et al.* [1998], El Mahjoub, Staub [2000b], Kamali [1993], Lacroix *et al.* [1993], Muchoh *et al.* [2001], Mullett, Pawliszyn [2001], Samanidou *et al.* [2007], [Iwase *et al.* [1994].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Retention time: 7.75 min. Limit of quantification, 20 µg/L, limit of detection, 10 µg/L [Marin *et al.* 2008; Marin, McMillin 2010]. Column: XTerraMS C8 (100 × 2.1 mm, i.d., 3.5 µm). Mobile phase: 2 mmol/L ammonium formate buffer (pH 3) : acetonitrile (99 : 1 to 98 : 2 from 2 to 3 min to 5 : 95 at 3.1 min until 7.5 min to 99 : 1 at 8 min for 3 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 7 to 7.5 min. Limit of quantification, 1 µg/L, limit of detection, 0.1 µg/L [Abbara *et al.* 2008]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.01 mg/L [Kratzsch *et al.* 2004].

Serum **GC-MS** Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 280° at 10°/min for 20 min. EI ionisation at 70 eV. Retention time: 23.1 min. Limit of quantification, 0.025 mg/L, limit of detection, <0.025 mg/L [Maresova *et al.* 2008]. Column: SPB-1 (30 m × 0.75 mm i.d., 1.0 µm). Carrier gas: He, 0.2 bar. Temperature programme: 230° for 2 min to 280° at 7°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 3.0 ng/L, limit of detection, 2.0 ng/L [Duthel *et al.* 1992].

HPLC See Blood [Dussy *et al.* 2006]. Column: Symmetry Shield RP8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen phosphate (40 : 60), flow rate 0.9 mL/min. UV detection (λ = 230 nm). Limit of detection, 1.0 µg/L [He *et al.* 2005]. Column: Kromasil C_{18}(150 × 4.6 mm i.d., 5 µm). Mobile phase: butanol : 0.06 mol/L SDS (pH 7, 5 : 95), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 10 µg/L [Capella-Peiró *et al.* 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (52 : 48), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 46 µg/L [Mullett, Pawliszyn 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (54 : 46), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 150.0 µg/L, limit of detection, 45.0 µg/L [Mullett, Pawliszyn 2001]. See Blood [El Mahjoub, Staub 2000a]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: flow rate 1.5 mL/min. DAD (λ = 254 nm). Limit of quantification, 127.6 µg/L, limit of detection, 35.7 µg/L [Ahrens *et al.* 2000]. Column: TSK gel Super-ODS (100 × 4.6 mm i.d., 2 µm) or Hypersil ODS-C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 µmol/L sodium dihydrogen phosphate (pH 6, 45 : 55), flow rate 0.65 mL/min. UV detection (λ = 254 nm) [Tanaka *et al.* 1996]. Column: Supelcosil C_8 DB 5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 0.5 mol/L monobasic potassium phosphate (370 : 600 : 30), flow rate 1.65 mL/min. UV detection (λ = 219 nm). Limit of quantification, 1 µg/L [Welk 1996]. Column: Chromspher C_8 (100 × 3.0 mm i.d., 5 µm). Mobile phase: methanol : water (20 : 80 for 2 min to 30 : 70 at 2.2 min until 4 min to 40 : 60 at 4.4 min until 4.5 min to 43 : 57 at 5 min to 45 : 55 at 6 min to 52 : 48 at 7 min to 58 : 42 at 9.5 min to 75 : 25 at 10.5 min for 5 min to 20 : 80 at 15.3 min until 19 min), flow rate 0.7 mL/min. DAD (λ = 450 nm). Limit of quantification, 30 µg/L, limit of detection, 10 µg/L [Lambert *et al.* 1995]. See also Goldnik *et al.* [1993].

LC-MS See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Unison UK-C_{18} RP ODS (150 × 2 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 over 20 min to 20 : 80 over 5 min), flow rate 0.25 mL/min. MRM acquisition mode. Limit of quantification, 6.8 µg/L, limit of detection, 2.0 µg/L [Nakamura *et al.* 2009]. Column: LiChrospher 60-RP select B (100 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : water : acetonitrile (1 : 1 : 1), flow rate 100 µL/min. APCI, SRM acquisition mode. Limit of quantification, 2 µg/L [Kleinschnitz *et al.* 1996].

Urine **GC** See Plasma [Ugland *et al.* 2000]. Column: DB-17 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 80 kPa. Temperature programme: 150° for 1 min to 230° at 10°/min for 5 min to 300° at 10°/min for 9 min. ECD. Limit of detection, 160 µg/L [Guan *et al.* 1999]. See Plasma. Limit of detection, 0.01–0.45 µmol/L [Reubsaet *et al.* 1998]. Column: DB-17 (15 m). Carrier gas: N_2. Temperature: 225°. ECD. Retention time: 2.07 min. Limit of detection, 0.13 ng [Beischlag, Inaba 1992].

HPLC See Plasma [Uddin *et al.* 2008]. Column: Inertsil C_8 (250 × 4 mm i.d., 5 µm). Mobile phase: ammonium acetate : 0.05 mol/L methanol : acetonitrile (33 : 57 : 10). UV detection (λ = 240 nm). Limit of detection, 2.6–12.6 ng [Samanidou *et al.* 2007]. Column: Hypersil C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.04 mol/L phosphate (pH 4)-0.4% octylamine : acetonitrile (73 : 27, 70 : 30, 65; 35 or 60 : 40), flow rate 1 mL/min. DAD (λ = 240 nm). Retention time: 17.9 min. Limit of detection not reported [Segura *et al.* 2001]. Hypersil BDS RP_{18} (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: methanol : acetonitrile : 0.05 mol/L potassium dihydrogen phosphate (pH 3.5, 50 : 10 : 40), flow rate 1.2 mL/min. UV detection (λ = 232 nm). Retention time: 11.6 min. Limit of quantification, 10 µg/L [Azzam *et al.* 1998]. See Serum [Kleinschnitz *et al.* 1996]. Column: LiChrospher 100 RP-18(e) (250 × 4 mm i.d., 5 µm). Mobile phase: water : methanol : triethylamine (pH 5.5, 70 : 30 : 0.1), flow rate 0.7 mL/min. UV detection (λ = 240 nm). Limit of detection, 2 µg/L [Chiba *et al.* 1995].

GC-MS See Blood [Goldberger *et al.* 2010].

LC-MS Column: Hypurity C_8 (150 × 3 mm i.d.). Mobile phase: 4 mmol/L ammonium acetate (pH 6.8) in methanol-water (5 : 95) : 1% propan-2-ol, 0.05% formic acid in methanol (100 : 0 for 1 min to 0 : 100 at 3 min for 1.5 min to 100 : 0 over 0.1 min for 1.4 min). TIS, MRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 2.5 µg/L [Glover, Allen 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 from 3.01 to 6 min to 100 : 0 from 6.01 to 10 min for 5 min), flow rate 0.9 mL/min for 3 min to 0.3 mL/min until 10 min to 0.9 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.1 µg/L [Umezawa *et al.* 2008]. Column: Symmetry C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 5, 10 : 90 to 30 : 70 at 5 min to 80 : 20 at 9 min to 10 : 90 at 9.1 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.01 µmol/L [Hegstad *et al.* 2006]. See Serum [Kleinschnitz *et al.* 1996].

CE-MS Running buffer: methanol : water (50 : 50) containing 0.1% formic acid, flow rate 0.5 mL/min. ESI, TOF. Limit of quantification, 12.5 µg/L, limit of detection, 1.1 µg/L [Blas, McCord 2008].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Oral Fluid **HPLC** See Plasma [Uddin *et al.* 2008]

Hair **GC-MS** Column: HP-Ultra 2 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 70° for 2 min to 220° at 25°/min to 255° at 5°/min to 300° for 7 min. EI ionisation at 70 eV. Retention time: 13.1 min. Limit of detection, 0.01 ng/mg. [Yegles *et al.* 1997].

HPLC Column: Lichrospher select-B (125 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate (pH 2.1, 30 : 70 to 35 : 65 in 30 min), flow rate 0.5 mL/min to 0.3 mL/min at 30 min. UV detection (λ = 254 nm). Limit of quantification, 0.3–0.45 ng/mg, limit of detection, 0.2 ng/mg [El Mahjoub, Staub 2001b].

LC-MS Mobile phase: 3 mmol/L ammonium formate and 0.001% formic acid in water : acetonitrile (65 : 35 to 20 : 80 after 13 min to 10 : 90 at 13.5 min until 16.5 min to 65 : 35 until 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.22 ng/30 mg, limit of detection, 0.13 ng/30 mg [Miller *et al.* 2006].

Disposition in the Body Diazepam is rapidly and completely absorbed after oral administration, with peak plasma level occurring within ~30–90 min. The main metabolic reactions are *N*-demethylation, 3-hydroxylation and glucuronic acid conjugation. The major active metabolite is desmethyldiazepam (nordazepam), which accumulates during chronic dosing; other metabolites include oxazepam and temazepam, both of which are active. The formation of nordazepam and oxazepam is catalysed by the P450 isozymes CYP2C19 and CYP3A. Only small traces of unchanged diazepam are excreted in the urine and the relative amounts of metabolites are variable and appear to be dose dependent. Approximately 70% of a dose is excreted in the urine, mainly as oxazepam glucuronide and conjugated nordazepam, together with smaller amounts of conjugated temazepam. Approximately 10% of the dose may be eliminated in the faeces. Diazepam and its metabolites cross the blood–brain barrier and the placenta; they are also found in breast milk.

Diazepam is a metabolite of ketazolam and medazepam.

Therapeutic Concentration In plasma, usually in the range 0.1–2.5 mg/L. After discontinuation of chronic therapy, concentrations of nordazepam may be substantially higher than diazepam and both unchanged drug and metabolite are still detectable 7 days after cessation of dosing.

Following a single oral dose of 10 mg to 4 subjects, peak blood concentrations of 0.14–0.19 mg/L (mean, 0.15) were attained in 1–1.5 h; average peak concentrations of 0.03 mg/L of nordazepam were attained after 24 h [Kaplan *et al.* 1973].

After chronic daily oral dosing of 5 mg twice daily to 15 subjects, steady-state plasma concentrations were: diazepam 0.09–0.37 mg/L (mean, 0.23), nordazepam 0.13–0.46 mg/L (mean, 0.29), oxazepam 0.01–0.03 mg/L (mean, 0.02), temazepam 0.01–0.05 mg/L (mean, 0.03) [Zingales 1973].

After intranasal administration of diazepam to nine healthy subjects (20 mg/mL solution; 100 µL into one nostril ≡ 2 mg dose), a mean serum plasma concentration of 0.039 mg/L was attained in 18 min; this equated to 32.9% of the concentration that was attained 10 min after the same dose given IV to the same subjects [Gizurarson *et al.* 1999].

Toxicity Toxic effects may be produced by blood concentrations >1.5 mg/L; fatalities caused by diazepam alone are rare but may occur at blood concentrations >5 mg/L.

In a review of 914 drug-related deaths in which diazepam was involved, it was found to be the sole cause of death in only 2 cases; postmortem concentrations of diazepam in these 2 cases were blood 5 and 19 mg/L, liver 13 µg/g in the first case [Finkle *et al.* 1979].

In a fatality from diazepam and alcohol ingestion, postmortem tissue diazepam concentrations were: blood 1.3 mg/L, bile 4.5 mg/L, brain 2.4 µg/g, kidney 11.7 µg/g, liver 11.4 µg/g, urine 6.6 mg/L [Simon 1976].

Half-life Plasma half-life, diazepam 20–100 h (mean, 48); nordazepam, ~40–100 h but there is considerable intersubject variation (see Nordazepam monograph). The plasma half-life appears to be increased in elderly subjects and neonates, and in subjects with liver disease; sex differences have also been suggested.

Volume of Distribution Diazepam and nordazepam, 0.5–2.5 L/kg; increased in elderly subjects.
Clearance Plasma clearance, diazepam ~0.3–0.5 mL/min/kg, nordazepam ~0.1–0.3 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, diazepam 1.8, nordazepam 1.7.
Protein Binding Diazepam 98–99%, nordazepam ~97%.
Note For reviews of the clinical pharmacokinetics of diazepam, see Friedman *et al.* [1992] and Mandelli *et al.* [1978].
Dose Usually 5 to 30 mg daily.

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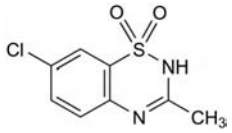
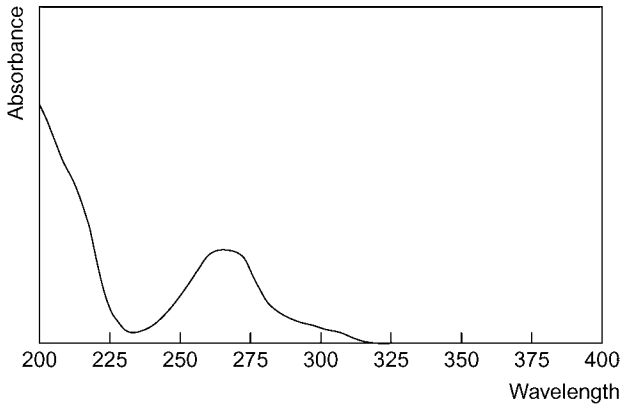
Diazoxide

Antihypertensive, Hyperglycaemic
$C_8H_7ClN_2O_2S = 230.7$
CAS—364-98-7

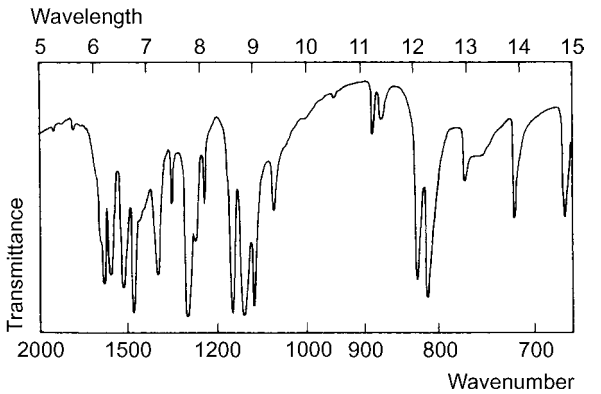
IUPAC Name 7-Chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide
Synonym SRG-95213
Proprietary Names *Eudemine*; *Hyperstat*; *Hypertonalum*; *Proglicem*; *Proglycem*.



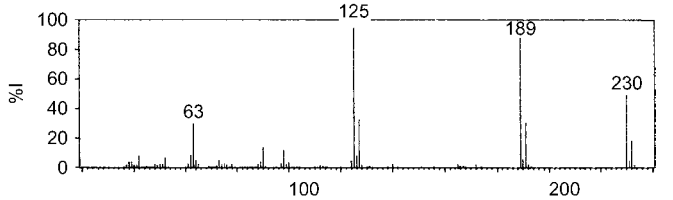
Chemical Properties A white or creamy-white crystalline powder. Mp 330° to 331°. Practically insoluble in water, chloroform and ether; soluble 1 in 250 of ethanol; very soluble in solutions of alkali hydroxides; freely soluble in dimethylformamide. pK_a 8.7. Log *P* (octanol/water), 1.2.
Thin-layer Chromatography System TA—R_f 0.82; system TB—R_f 0.01; system TC—R_f 0.28; system TE—R_f 0.20; system TL—R_f 0.41; system TAE—R_f 0.84; system TAJ—R_f 0.37; system TAK—R_f 0.13; system TAL—R_f 0.75.
Gas Chromatography System GA—not eluted; system GB—not eluted.
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 368; system HY—RI 299.
Ultraviolet Spectrum Aqueous alkali—280 nm (A^1_1=585a).



Infrared Spectrum Principal peaks at wavenumbers 1139, 1294, 1166, 1495, 1112, 812 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 125, 189, 230, 127, 191, 63, 232, 90.



Quantification
Plasma **TLC** UV detection. For method for diazoxide and metabolites, see Pruitt *et al.* [1974].
GC–MS Limit of detection, 10 µg/L [Sadee *et al.* 1973].
HPLC UV detection. Limit of detection, 100 µg/L [Vree *et al.* 1979].
Urine **TLC** See Plasma [Pruitt *et al.* 1974].
GC–MS See Plasma [Sadee *et al.* 1973].
HPLC See Plasma [Vree *et al.* 1979].
Faeces **TLC** See Plasma [Pruitt *et al.* 1974].
Disposition in the Body Readily absorbed after oral administration. Metabolised by oxidation to the 3-hydroxymethyl derivative which is conjugated with sulfate or further metabolised to the 3-carboxy derivative. About 90% of a dose is excreted in the urine in 5 to 6 days, of which 6 to 50% is unchanged drug; the 3-hydroxymethyl and 3-carboxy metabolites each account for about 20 to 30% of the excreted material; the amount of unchanged drug excreted in the urine appears to be reduced in hypertensive patients; about 2% of a dose is eliminated in the faeces.
Therapeutic Concentration In plasma, usually in the range 15 to 50 mg/L. Following a single oral dose of 300 mg to 2 subjects, peak plasma concentrations of about 15 to 20 mg/L were attained in 3 to 6 h [Pruitt *et al.* 1973].
Toxicity Toxic effects may be associated with plasma concentrations greater than 100 mg/L.
Half-life Plasma half-life, adults, about 20 to 70 h; appears to be lower in children.
Volume of Distribution About 0.2 to 0.3 L/kg.
Clearance Plasma clearance, about 0.1 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, about 1.7.
Protein Binding About 90%; decreased in subjects with renal impairment.
Note For a review of the pharmacokinetics of diazoxide, see Pearson [1977].
Dose In the treatment of intractable hypoglycaemia: initially 5 mg/kg daily, orally. In hypertensive crises: 300 mg intravenously.

Pearson RM (1977). Pharmacokinetics and response to diazoxide in renal failure. *Clin Pharmacokinet* 2: 198–204.
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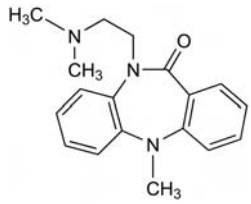
Sadee W *et al.* (1973). *J Pharmacokinet Biopharm* 1: 295–305.
Vree TB *et al.* (1979). Rapid determination of diazoxide in plasma and urine of man by means of high-performance liquid chromatography. *J Chromatogr* 164: 228–234.

Dibenzepin

Antidepressant
$C_{18}H_{21}N_3O$ = 295.4
CAS—4498-32-2

IUPAC Name 5-(2-Dimethylaminoethyl)-11-methylbenzo[*b*][1,4]benzodiazepin-6-one
Synonym 10-[2-(Dimethylamino)ethyl]-5,10-dihydro-5-methyl-11*H*-dibenzo[*b*,*e*]-[1,4]diazepin-11-one

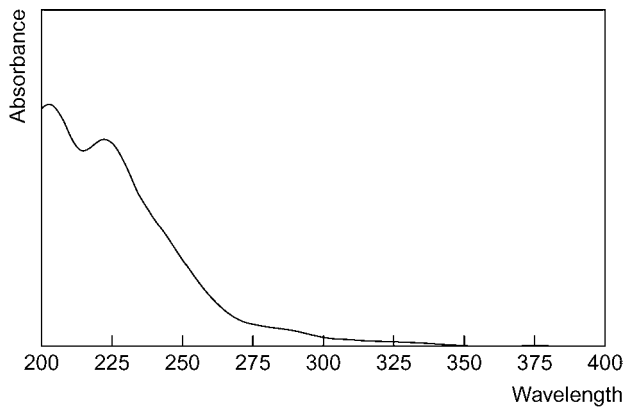


Chemical Properties Mp 116° to 117°. Log *P* (octanol/pH 7.4), 1.7. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

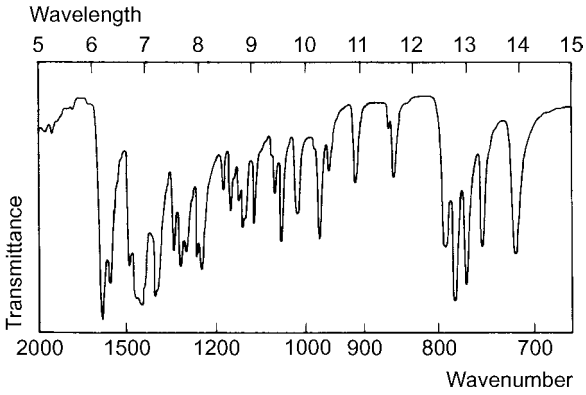
Dibenzepin Hydrochloride

$C_{18}H_{21}N_3O$,HCl = 331.8
CAS—315-80-0
Proprietary Names *Deprex*; *Écatril*; *Noveril*. Deprex is also used as a proprietary name for amitriptyline hydrochloride.

Chemical Properties A colourless, fine crystalline powder. Mp 238°. Soluble 1 in 16 of water; soluble in ethanol and chloroform.
Colour Test Mandelin's test—green.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.22; system TC—R_f 0.35; system TE—R_f 0.55; system TL—R_f 0.14; system TAE—R_f 0.38; system TAF—R_f 0.22 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—dibenzepin RI 2450, M (nor-) RI 2449, M (di-nor) RI 2406, M (ter-nor-) RI 2680, M (N_5-desmethyl-) RI 2455, M (OH-) isomer-1-AC RI 2600, M (OH-) isomer-2-AC RI 2770; system GB—dibenzepin RI 2566; system GF—RI 2885; system GM—dibenzepin RRT 1.735 (relative to iprindole).
High Performance Liquid Chromatography System HA—*k* 2.8; system HF—*k* 0.50; system HX—RI 361; system HY—RI 300.
Ultraviolet Spectrum Aqueous acid—203, 223 nm.



Infrared Spectrum Principal peaks at wavenumbers 1639, 775, 761, 1603, 1250, 1316 cm^{-1} (dibenzepin hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 224, 209, 71, 225, 72, 210, 180.
Quantification
Blood **GC** AFID. Limit of detection, 300 ng/g, dibenzepin and desmethyl metabolites [Schlicht, Gelbke 1978].

UV Spectrophotometry Limit of detection, 500 ng/g, dibenzepin and desmethyl metabolites [Christensen, Felby 1975].
Plasma **HPLC** MS-MS detection. For method, see Kollroser and Schober [2002].
Urine **GC** See Blood [Schlicht, Gelbke 1978].

UV spectrophotometry See Blood [Christensen, Felby 1975].
Tissues **GC** See Blood [Schlicht, Gelbke 1978].

UV spectrophotometry See Blood [Christensen, Felby 1975].
Disposition in the Body Readily absorbed after oral administration; rapidly and extensively metabolised, mainly by *N*-demethylation. About 20 to 30% of a dose is excreted in the urine as free and conjugated desmethyl metabolites in 24 h, together with about 1% of the dose as unchanged drug.
Therapeutic Concentration

During daily oral treatment with doses of 8 mg/kg of dibenzepin hydrochloride given to 12 patients for 22 days, mean plasma concentrations of 0.18 mg/L of dibenzepin and 0.28 mg/L of *N*-monodesmethyldibenzepin (demethylated in the side-chain) were reported, determined 4 h after a dose. Plasma concentrations declined slightly during therapy [Gauch, Modestin 1973].

Toxicity The estimated minimum lethal dose is about 3 g; doses greater than 1.5 g may result in serious poisoning. Patients liable to arrhythmias are particularly susceptible to toxic effects.

The following tissue disposition of total dibenzepin and metabolites was reported in 6 suicides due to the ingestion of dibenzepin: blood 23 to 147 µg/g (mean 73), liver 255 to 566 µg/g (mean 386), urine 63 to 695 µg/g (mean 230, 4 cases) [Christensen, Felby 1975].

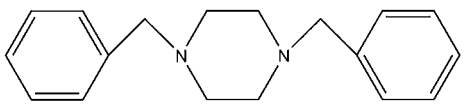
In a fatality due to the ingestion of 3.6 g of dibenzepin, the following postmortem tissue concentrations were reported for dibenzepin and total desmethyl metabolites, respectively: blood 23, – mg/L; bile 113, 137 mg/L; brain 42, 12 µg/g; kidney 63, 38 µg/g; liver 130, 134 µg/g; urine 350, 258 mg/L [Schlicht, Gelbke 1978].

Half-life Plasma half-life, about 4 h (dibenzepin plus desmethyl metabolites).
Dose 240 to 560 mg of dibenzepin hydrochloride daily.

Christensen H, Felby S (1975). Dibenzepine and its metabolites in blood, muscle, liver, vitreous body and urine from fatal poisoning. *Acta Pharmacol Toxicol (Copenh)* 37(5): 393–401.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Gauch R, Modestin J (1973). [Pharmacokinetics of dibenzepin]. *Arzneimittelforschung* 23: 687–690.
Kollroser M, Schober C (2002). Simultaneous determination of seven tricyclic antidepressant drugs in human plasma by direct-injection HPLC-APCI-MS-MS with an ion trap detector. *Ther Drug Monit* 24(4): 537–544.
Schlicht HJ, Gelbke HP (1978). Gas chromatographic determination of dibenzepine and its basic metabolites in biological material. *J Chromatogr* 166: 599–603.

Dibenzylpiperazine

Arylpiperazine, Drug of Abuse
$C_{18}H_{22}N_2 = 266.4$
CAS—2298-55-7
IUPAC Name 1,4-Dibenzylpiperazine
Synonym DBZP

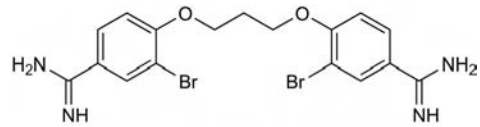


Chemical Properties Log *P* (octanol/water), 3.1 [National Institutes of Health 2005]. Dibenzylpiperazine is usually found as an impurity in samples of the recreational stimulant benzylpiperazine (BZP). Its presence is indicative of low quality or badly manufactured BZP, either because the synthesis reaction temperature is too high or because of an excess of benzyl chloride in the reaction mixture.
Disposition in the Body
Toxicity At the time of writing, there are no reports of DBZP use (or abuse). It is not believed to have any stimulant effects although this has not been confirmed through appropriate testing.

National Institutes of Health (2005). *PubChem Compound: Dibenzylpiperazine*. Bethesda, MD: National Institutes of Health and US National Library of Medicine. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=200601 (accessed 15 November 2009).

Dibrompropamidine

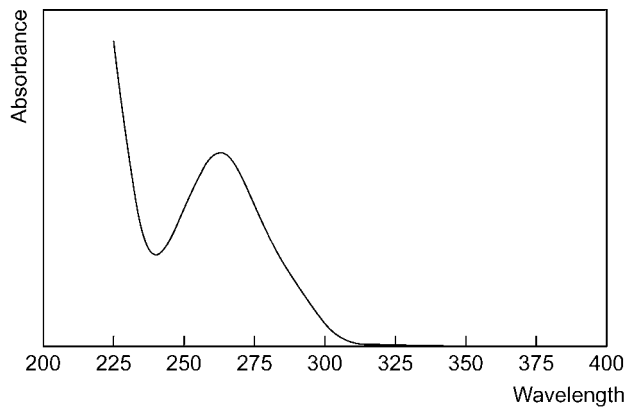
Antibacterial, Antifungal
$C_{17}H_{18}Br_2N_4O_2 = 470.2$
CAS—496-00-4
IUPAC Name 3-Bromo-4-[3-(2-bromo-4-carbamimidoylphenoxy)propoxy] benzenecarboximidamide
Synonym 4,4′-[1,3-Propanediylbis(oxy)]bis(3-bromobenzenecarboximidamide)



Dibrompropamidine Isetionate

$C_{17}H_{18}Br_2N_4O_2,2C_2H_6O_4S = 722.4$
CAS—614-87-9
Proprietary Names *Brolene* (eye ointment); *Brulidine*.
Chemical Properties A white crystalline powder. Mp 226°. Soluble 1 in 2 of water and 1 in 60 of ethanol; soluble in glycerol; practically insoluble in chloroform and ether.

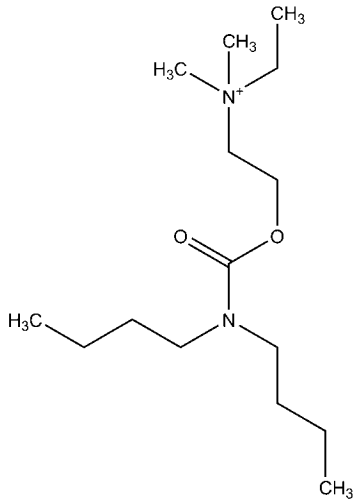
Colour Tests Aromaticity (method 2)—yellow/brown; Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.01 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—261 nm (A^{1}_{1}=512a).



Infrared Spectrum Principal peaks at wavenumbers 1190, 1654, 1266, 1047, 1600, 1306 cm^{-1} (dibrompropamidine isetionate, KBr disk).
Use Dibrompropamidine isetionate is used in a concentration of 0.15%.

Dibutoline

Quaternary Ammonium, Parasympatholytic, Antispasmodic
$(C_{15}H_{33}N_2O_2)_2 = 546.8$
CAS—21962-82-3
IUPAC Name (2-Dibutylcarbamoyloxy)ethyl-ethyl-dimethylammonium

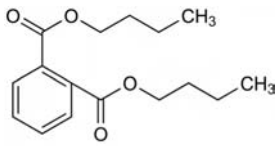


Dibutoline Sulfate
$(C_{15}H_{33}N_2O_2)_2,SO_4 = 642.9$
IUPAC Name 2-(Dibutylcarbamoyloxy)ethyl-ethyl-dimethylazanium sulfate
Synonym (Ethyl(2-hydroxyethyl)dimethylammonium) sulfate bis (dibutylcarbamate)
Proprietary Name *Dibuline sulfate*
Chemical Properties Extremely hygroscopic powder. Aqueous solutions decompose on heating at 100°. Soluble in water and benzene.

Colour Tests Ammonium molybdate test—faint blue-grey (limit of detection, 1.0 µg); sulfuric acid–formaldehyde test—red (limit of detection, 1.0 µg); Vitali's test—faint yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.02 (location reagent acidified iodoplatinate spray, positive reaction).
Disposition in the Body Poorly absorbed from the gastrointestinal tract.
Toxicity The lethal dose in humans is probably between 10 and 100 mg/kg. Its effect is weaker and of shorter duration than that of atropine.
Dose Up to 100 mg daily, IM or SC.

Dibutyl Phthalate

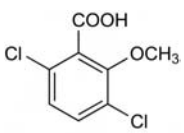
Insect Repellent
$C_{16}H_{22}O_4 = 278.3$
CAS—84-74-2
IUPAC Name Dibutyl benzene-1,2-dicarboxylate
Synonyms 1,2-Benzenedicarboxylic acid dibutyl ester; butyl phthalate; DBP.



Chemical Properties A clear, colourless or faintly yellow, somewhat viscous liquid. Mass per mL about 1.045 g. Bp 340°. Refractive index 1.492 to 1.495. Soluble 1 in 2500 of water; very soluble in ethanol, ether, acetone and benzene. Log *P* (octanol/water), 4.5.
Gas Chromatography System GA—RI 1913.
Ultraviolet Spectrum Methanol—274 nm (A^{1}_{1}=45b).
Mass Spectrum Principal ions at *m/z* 149, 41, 29, 57, 56, 104, 32, 65.

Dicamba

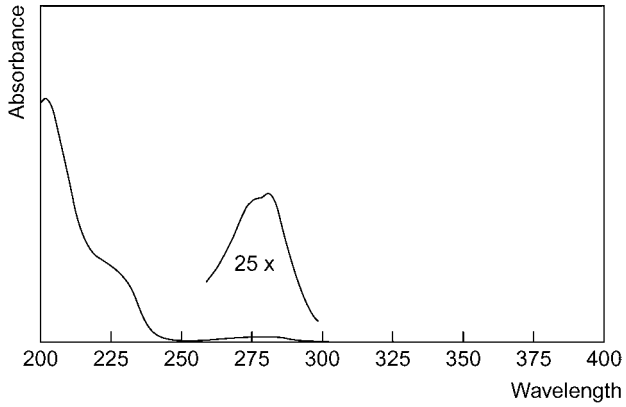
Herbicide
$C_8H_6Cl_2O_3 = 221.0$
CAS—1918-00-9
IUPAC Name 3,6-Dichloro-2-methoxybenzoic acid.
Synonym Dianat
Proprietary Names *Banfel*; *Banvel*; *Banvel-CST*; *Banvel-D*; *Banvel XG*; *Dianat*; *Dicazin*; *Fallowmaster*; *Killes*; *Mediben*; *Metambane*; *Tracker*; *Trooper*; *Velsicol*. It is also an ingredient in *Banlene* and *Cambilene*.



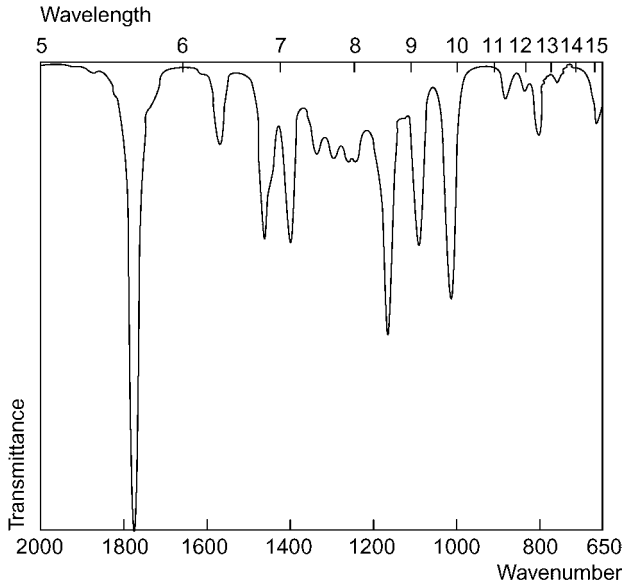
Chemical Properties Pure dicamba is a white crystalline solid and the technical acid is a pale buff crystalline solid. Mp 114° to 116°. Soluble in ethanol (922 g/L at 25°), cyclohexane (916 g/L at 25°), acetone (810 g/L at 25°), dichloromethane (260 g/L at 25°), toluene (130 g/L at 25°) and xylene (78 g/L at 25°); very slightly soluble in dioxane and water (8.3 g/L at 25°). pK_a 1.97 (also reported as 1.90 and 1.87 at 25°). Log *P* (octanol/water), 2.21.
Thin-layer Chromatography System TX—R_f 0.41; system TY—R_f 0.70; system TAB—R_f 0.04; system TAC—R_f 0.05.
Gas Chromatography System GA—dicamba RI 1795, 2,5-dichloromethoxybenzene RI 1200, dicamba-Me RI 1525; system GK—dicamba RRT 0.49 (relative to caffeine).

Column: DB-1 (2.0 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120°, held for 0.3 min, to 180° at 15°/min. Carrier gas: H_2, 1.9 mL/min. FID. Retention time: 1.3 min [Liu *et al.* 1994].

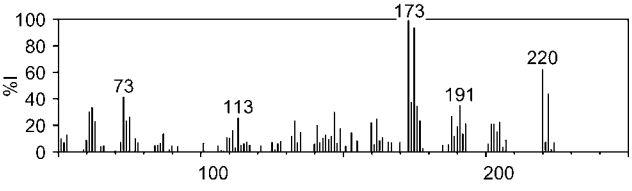
High Performance Liquid Chromatography Column: RP C_{18} Econosphere (150 × 4.6 mm i.d., 5 µm). Mobile phase: (A:B) acetonitrile : acetic acid solution (2%). Elution programme: (A:B) (25:75) for 5 min, to (40:60) over 10 min, held 5 min, flow rate 1.5 mL/min. Retention time: 6.1 min [Larson, Houglum 1991].
Ultraviolet Spectrum Aqueous solution (neutral)—274; acetonitrile—205, 230, 278 nm.



Infrared Spectrum Principal peaks at wavenumber 1778, 1169, 1019, 1094, 1402 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 173, 175, 220, 73, 222, 174, 191, 62.



Quantification
Urine **GC** ECD. Limit of detection of dicamba, 0.017 mg/L [Grover *et al.* 1985].

Solution **HPLC** UV detection (λ=274 nm). Limit of detection, <1 mg/L [Fogarty *et al.* 1994].

Disposition in the Body Dicamba is rapidly and almost completely absorbed into the bloodstream from the gastro-intestinal tract. It is distributed to tissues around the body and does not bioaccumulate. There is no evidence that it is absorbed through the skin. Dicamba is excreted mainly in urine as the unmetabolised state but can also be detected in faeces.

Toxicity Dicamba is moderately toxic by ingestion and slightly toxic by inhalation and dermal exposure. It can be irritating, corrosive and cause severe and permanent damage to the eyes. The Environmental Protection Agency (EPA) has established a Lifetime Health Advisory (LHA) level of 200 µg/L of dicamba in drinking water.

A 61-year-old woman committed suicide by ingesting an unknown quantity of Killes, containing 9 g/L dicamba. Postmortem toxicological analysis showed that the woman's blood dicamba concentration was 170 mg/L, 140 mg/L in her bile, and in her liver a concentration of <100 mg/kg was found. (2,4-Dichlorophenoxy) acetic acid and mecoprop were also detected [Fraser *et al.* 1984].

Protein Binding Dicamba is highly protein bound in plasma.

Fogarty AM *et al.* (1994). Determination of dicamba by reverse-phase HPLC. *J Liq Chromatogr* 17 (12): 2667–2674.

Fraser AD *et al.* (1984). Toxicologic studies in a fatal overdose of 2,4-D, mecoprop, and dicamba. *J Forensic Sci* 29(4): 1237–1241.

Grover R *et al.* (1985). Procedure for the determination of 2,4-D and dicamba in inhalation, dermal, hand-wash, and urine samples from spray applicators. *J Environ Sci Health B* 20(1): 113–128.

Larson RD, Houglum JE (1991). *J Assoc Off Anal Chem* 74(4): 679–681.

Liu Z *et al.* (1994). Comprehensive two-dimensional gas chromatography for the fast separation and determination of pesticides extracted from human serum. *Anal Chem* 66: 3086–3092.

Dichloralphenazone

Hypnotic, Sedative

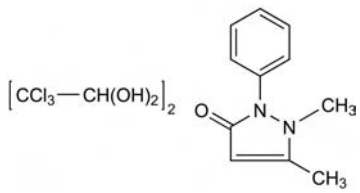
$C_{15}H_{18}Cl_6N_2O_5$ = 519.0

CAS—480-30-8

IUPAC Name 1,5-Dimethyl-2-phenylpyrazol-3-one; 2,2,2-trichloroethane-1,1-diol

Synonym Dichloralantipyrine

Proprietary Names *Bonadorm*; *Chloralol*; *Welldorm*. It is an ingredient of *Midrid* and *Paedo-Sed*.



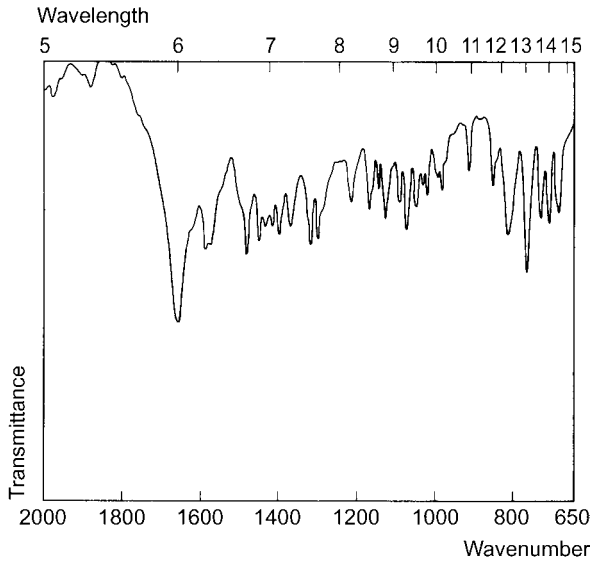
Chemical Properties A complex of cloral hydrate and phenazone. A white microcrystalline powder. Mp 64° to 67°. Soluble 1 in 10 of water, 1 in 1 of ethanol and 1 in 2 of chloroform; soluble in dilute acids. Log *P* (octanol/water), 0.6.

Note It is decomposed by dilute alkalis with the liberation of chloroform. In aqueous and ethanolic solutions it dissociates into cloral hydrate and phenazone.

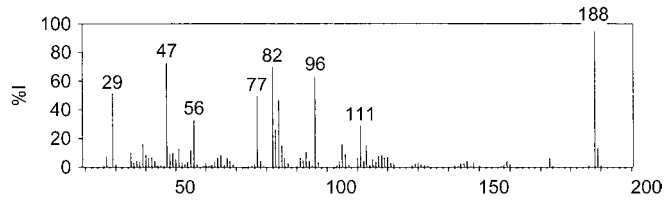
Gas Chromatography System GA—dichloralphenazone RI 1855, M (4-OH-phenazone) RI 1855.

Ultraviolet Spectrum Phenazone: aqueous acid—230 nm (A_1^1=590b); aqueous alkali—242 (A_1^1=494a), 256 nm.

Infrared Spectrum Principal peaks at wavenumbers 1656, 767, 1587, 1562, 1315, 1298 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 188, 47, 82, 96, 29, 77, 84, 56.



Quantification See under Chloral Hydrate and Phenazone.

Disposition in the Body After administration, dichloralphenazone acts as a mixture of cloral hydrate and phenazone and reference should be made to the entries under these substances.

Toxicity

Blood concentrations of 20 mg/L of trichloroethanol and 40 mg/L of phenazone were reported in a comatose subject following an overdose of dichloralphenazone; the subject eventually recovered [Armstrong, Stone 1972].

Dose 0.65 to 1.95 g daily.

Armstrong RJ, Stone HM (1972). *Bull Int Assoc Forensic Toxicol* 8(4): 8–9.

Dichlorodifluoromethane

Aerosol Propellent, Refrigerant

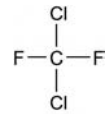
CCl_2F_2 = 120.9

CAS—75-71-8

IUPAC Name Dichloro(difluoro)methane

Synonyms Difluorodichloromethane; propellent 12; refrigerant 12.

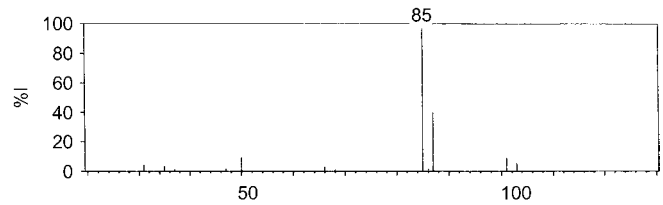
Proprietary Names *Arcton 12*. It is an ingredient of *PR Spray* and *Skefron*.



Chemical Properties A colourless non-flammable gas which, when liquefied by compression, forms a clear colourless liquid. Fp −155°. Bp about −29.8°. Log *P* (octanol/water), 2.2.

Gas Chromatography System GA—RI 305; system GI—retention time 0.9 min.

Mass Spectrum Principal ions at *m/z* 85, 87, 101, 50, 103, 31, 66, 35.



Dichlorophen

Anthelmintic

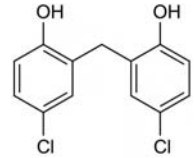
$C_{13}H_{10}Cl_2O_2$ = 269.1

CAS—97-23-4

IUPAC Name 4-Chloro-2-[(5-chloro-2-hydroxyphenyl)methyl]phenol

Synonyms Di-phenthane-70; 2,2′-methylenebis[4-chlorophenol].

Proprietary Names *Anthiphen*; *Dicestal (vet.)*; *Ovis*; *Plath-Lyse*; *Wespuril*.



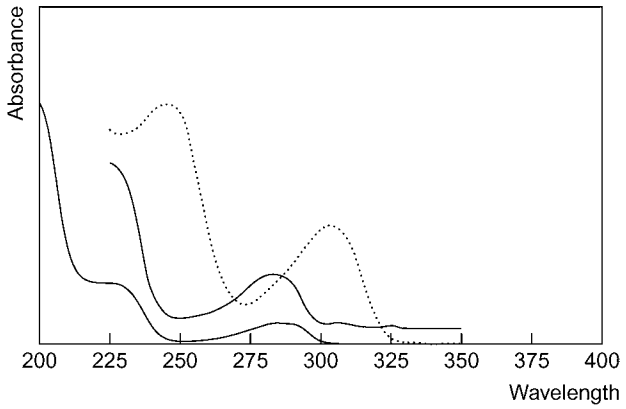
Chemical Properties A white or slightly cream-coloured powder. Mp about 175°. Practically insoluble in water; soluble 1 in 1 of ethanol and 1 in less than 1 of ether; soluble in methanol, isopropyl ether and petroleum ether; sparingly soluble in toluene. pK_a 7.6. Log *P* (octanol/water), 4.3.

Colour Tests Aromaticity (method 2)—yellow/brown; Folin–Ciocalteu reagent—blue; Liebermann's reagent—violet-brown; Millon's reagent—red.

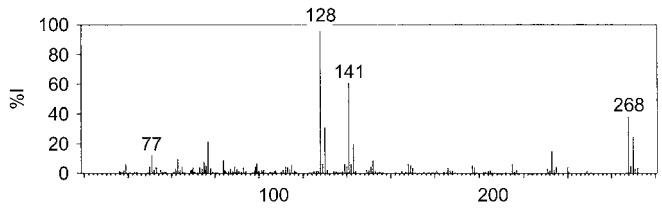
Thin-layer Chromatography System TD—R_f 0.59; system TE—R_f 0.34; system TF—R_f 0.67; system TX—R_f 0.12; system TY—R_f 0.16; system TAD—R_f 0.57; system TAE—R_f 0.91.

Gas Chromatography System GA—dichlorophen RI 2140, dichlorophen-AC_2 RI 2250, dichlorophen-Et_2 RI 2225, dichlorophen-Me_2 RI 2245.

High Performance Liquid Chromatography System HY—RI 591.
Ultraviolet Spectrum Aqueous acid—282 nm; aqueous alkali—245 (A^1_1=650a), 304 nm.



Infrared Spectrum Principal peaks at wavenumbers 1226, 817, 1111, 1162, 1245, 1170 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 128, 141, 268, 130, 270, 77, 143, 233.



Disposition in the Body

Toxicity

An acute fatality was reported with a blood concentration of 9.77 mg/L dichlorophen. No other drugs or alcohol were detected [Kintz *et al.* 1997].

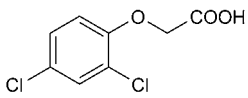
Dose Usually 6 g daily for 2 or 3 days; a single dose of 9 g has also been given.

Kintz P *et al.* (1997). Acute fatal poisoning with dichlorophen. *Int J Legal Med* 110(2): 95–96.

Dichlorophenoxyacetic Acid

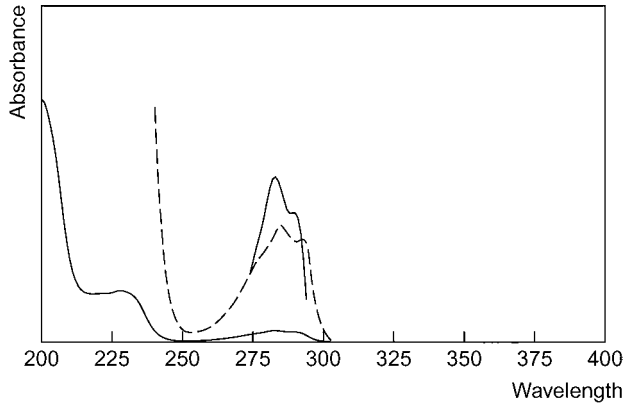
Herbicide
$C_8H_6Cl_2O_3$ = 221.0
CAS—94-75-7

IUPAC Name 2-(2,4-Dichlorophenoxy)acetic acid
Synonym 2,4-D
Proprietary Names *Animex*; *Cornox D*; *Destox*; *Dicotox*; *Dioweed*; *Dormone*; *Fernimine*; *For-ester*; *Iso-planatox*; *Palormone D*; *Silvapron D*; *Syford*; *Verdone*; *Vigon-DC*. It is an ingredient of *Econal*; *Herbatox*; *Killex*; *Nettle Ban*; *Selex*; *Spontox*; *Stancide BWK 75*; *Tributon*.

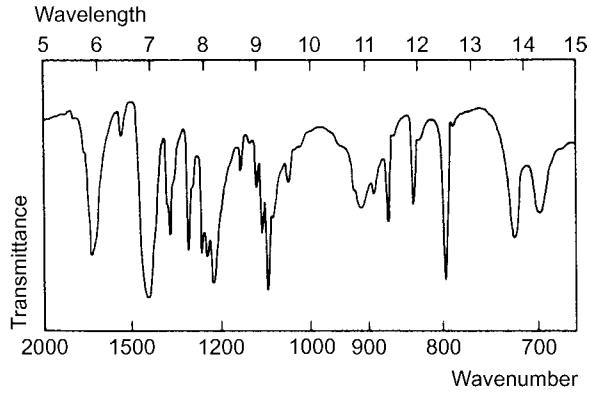


Chemical Properties Corrosive white powder. Mp ≈140°. Almost insoluble in water; soluble in aqueous solutions of alkalis and in alcohols. pK_a 3.3 [Baselt 2008; Prescott *et al.* 1979], 2.6 [Flanagan, Ruprah 1989], 2.7. Log *P* (octanol/water) 2.8 [Hansch *et al.* 1995]. Stable in urine at ambient temperature for 48 h [Hughes *et al.* 2001]. Stable in diluted urine samples when kept at –20° for 6 months [Baker *et al.* 2000]. Standard solutions in deionised water or methanol (1 : 1) show some decomposition after 5–6 weeks at room temperature. This can be overcome by preparing the solutions in Tris buffer : methanol (1 : 1). Standard equine-serum-matrix solutions were stable for at least a year at –5° to –20° [Flanagan, Ruprah 1989].
Thin-layer Chromatography System TD—R_f 0.04; system TE—R_f 0.04; system TF—R_f 0.06; system TX—R_f 0.00; system TY—R_f 0.02; system TAB—R_f 0.02; system TAC—R_f 0.03.
Gas Chromatography System GA—2,4-D RI 1800, M (2,4-dichlorophenol)RI 1320, 2,4-D-Me RI 1593, 2,4-D-butyl ester RI 1840, 2,4-D-isobutyl ester RI 1805, 2,4-D-isopropyl ester RI 1700, 2,4-Dichlorophenoxybutyric acid-Me RI 1835; system GK—RT 0.65 relative to caffeine. system GK—dichlorophenoxyacetic acid iso-ocitol ester RT 1.12, dichlorophenoxyacetic acid iso-ocitol ester RT 1.18, both relative to caffeine.

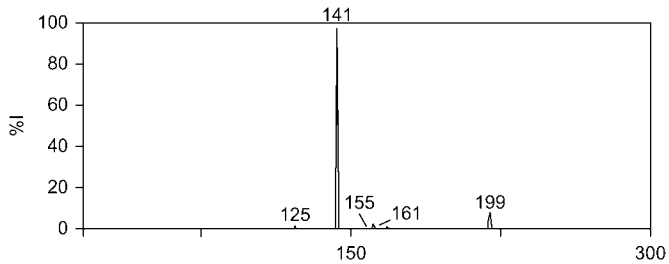
Ultraviolet Spectrum Ethanol—284 (A^1_1 = 95b), 292 nm.



Infrared Spectrum Principal peaks at wavenumbers 1090, 1230, 795, 1264, 1247, 1724 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 199, 45, 175, 145, 111, 109, 234, 133 (dichlorophenoxyacetic acid methyl ester).



Quantification

Blood **GC-MS** Column: DB-5 capillary (30 m × 0.53 mm i.d.). Carrier gas: He, 20.0 mL/min. EI ionisation at 70 eV. Limit of detection not reported [Jorens *et al.* 1995].

HPLC Column: Spherisorb S5 Phenyl (250 × 5 mm i.d.). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate (pH 3.5) : acetonitrile (3 : 1), flow rate 1.8 mL/min. UV detection (λ = 240 nm). *k'*: 0.8. Limit of detection, 20 mg/L [Flanagan, Ruprah 1989]. Column: RP-8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate (pH 3.2) : acetonitrile : *n*-nonylamine (550 : 450 : 0.6), flow rate 1.6 mL/min. UV detection (λ = 280 nm). Limit of quantification, 0.5 mg/L [Fraser *et al.* 1984]. Column: Ultrasphere ODS (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : phosphate buffer (pH 6.8; 14 : 10 : 76), flow rate 1.7 mL/min. UV detection (λ = 227 nm). Retention time: 6.08 min. Limit of detection, 1 mg/L [Osterloh *et al.* 1983].

Plasma **GC** Column: 10% OV17 Gaschrom Q (4′ × .025"). Carrier gas: N_2, 60 mL/min. Temperature: 200°. Limit of detection not reported [Prescott *et al.* 1979]. Column: 3% SE-30 on 100/120 varaport 30 (150 × 3.2mm i.d.). Temperature: 175°. FID. Retention time: 9 min. Limit of detection not reported [Kohli *et al.* 1974].

HPLC Column Supersphere 100 reversed phase (125 × 4 mm i.d.). Mobile phase: acetonitrile : triethylammonium phosphate buffer (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 284 nm). *k'*: 5.18. Limit of detection, 0.5 mg/L [Keller *et al.* 1994]. See Blood [Flanagan, Ruprah 1989].

Urine **GC** Column: Chromopak CP Sil 5 (30 m × 0.32 mm i.d., 0.4 μm) or Chromopack CP Sil 8 (50 m × 0.32 mm i.d., 0.4 μm). Carrier gas: He, 2.0 mL/min. Temperature programme: 50° for 1 min to 200° at 30°/min for 5 min to 250° at 5°/min for 10 min (first column) or 50° for 1 min to 270° at 25°/min for 30 min. Limit of detection, 1.0 μg/L [Aprea *et al.* 1997]. Column: 5% Dexil 300 on 80/100 Chromosorb W HP mesh (1.83 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 40 mL/min. Temperature: 210°. ECD. Retention time: 4.0 min for the methyl ester. Limit of detection, 50 μg/L [Grover *et al.* 1985]. Column: 4% SE-30 and 6% QF1 on Chromsorb W 80/100 mesh (150 × 0.6 cm o.d.). Carrier gas: N_2, 30 mL/mi. Temperature: 190°. ECD. Limit of detection, 30 μg/L [Vural, Burgaz 1984]. Column: 100/120 mesh Ultra-Bond 20 M (1.5 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 40 mL/min. Temperature: 150°. ECD. Retention time: 3.5 and 4.0 min for the methyl and *n*-butyl ester, respectively. Limit of detection, 100 μg/L [Smith, Hayden 1979]. See Plasma. Temperature: 160°. Retention time: 19 min [Kohli *et al.* 1974]. Column: 20% OV-101 Gas-Chrom Q 60/80 mesh. Carrier gas: N_2, 60 mL/min. Temperature: 175°. Retention time: ≈5 min. Limit of detection, 3.75 ng/g [Shafik *et al.* 1971].

GC-MS Column: HP-5 5% diphenol 95% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 1 min to 280° at 8°/min. MSD, SIM acquisition mode. Limit of quantification, 5 μg/L [Hughes *et al.* 2001]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 120° to 300° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.75 μg/L [Thompson, Treble 1996]. See Blood [Jorens *et al.* 1995].

HPLC Column: Hypersil C_{18} BDS (15 cm × 300 μm i.d., 3 μm). Mobile phase: 0.8% aqueous phosphoric acid solution : methanol (60 : 40 for 15 min to 30:70 for 30 min), flow rate 0.8 μL/min. DAD (λ = 232 nm). Limit of quantification, 19.3 μg/L, limit of detection, 5.8 μg/L [Rosales-Conrado *et al.* 2008]. Column: Supelco LC8 (25 cm × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : phosphate buffer (pH 3.2; 25 : 75 to 33 : 67 at 1.14%/min twice for 5 min to 25 : 75 at 2.67%/min), flow rate 2.0 mL/min. DAD (λ = 230 nm). Limit of detection, 15 μg/L [Aprea *et al.* 1997]. See Blood [Flanagan, Ruprah 1989]. See Blood [Fraser *et al.* 1984].

LC-MS Column: C_{18} (50 × 2.1 mm i.d., 4 μm). Mobile phase: 0.5% acetic acid : 0.5% acetic acid in methanol (80 : 20 to 5 : 95 in 6 min for 0.5 min to 80 : 20 for 2 min), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection, 0.05 μg/L [Lindh *et al.* 2008]. Column: Betasil hexylphenyl (100 × 4.6 mm i.d., 3 μm). Mobile phase: 0.1% acetic acid in water : 0.1% acetic acid in acetonitrile (47 : 53 for 5 min to 15 : 85 at 6 min to 0 : 100 at 7 min for 2 min to 47 : 53 for 3 min), flow rate 0.5 mL/min. MRM acquisition mode, negative ion mode. Limit of detection, 54 ng/L [Norrgran *et al.* 2006]. Column: Aquasil C_8 (100 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1% acetic acid : 0.1% acetic acid in methanol (70 : 30 to 0 : 100 at 8.1 min), flow rate 1.0 mL/min. APCI, positive and negative ion mode. Limit of detection, 0.29 μg/L [Baker *et al.* 2000]. Column: Partisil 5 ODS-3 (25 cm × 4.6 mm). Mobile phase: acetonitrile : 0.2% glacial acetic acid (60 : 40), flow rate 1.0 mL/min. APCI, negative ion mode. Limit of detection, 0.43 μg/L [Beeson *et al.* 1999].

Bile **HPLC** See Blood [Fraser *et al.* 1984]. See Blood [Osterloh *et al.* 1983].

CSF **HPLC** See Blood [Osterloh *et al.* 1983].

Brain **HPLC** See Plasma [Keller *et al.* 1994]. See Blood [Osterloh *et al.* 1983].

Diaphragm **HPLC** See Blood [Osterloh *et al.* 1983].

Heart **HPLC** See Plasma [Keller *et al.* 1994]. See Blood [Osterloh *et al.* 1983].

Kidney **HPLC** See Plasma [Keller *et al.* 1994]. See Blood [Osterloh *et al.* 1983].

Liver **HPLC** See Plasma [Keller *et al.* 1994]. See Blood [Fraser *et al.* 1984]. See Blood [Osterloh *et al.* 1983].

Lung See Plasma [Keller *et al.* 1994].

Muscle See Plasma [Keller *et al.* 1994].

Pancreas See Plasma [Keller *et al.* 1994].

Other **LC-MS** Solid Media. Column: Zorbax Eclipse XDB C_8 (50 × 2.1 mm i.d., 5 μm). Mobile phase: 0.2% acetic acid in water : 0.2% acetic acid in methanol (90 : 10 to 10 : 90 to 90 : 10), flow rate, 0.3 mL/min. TSI, MRM acquisition mode, negative ion mode. Limit of detection, 2.9 μg/L [Gardner *et al.* 2005].

Disposition in the Body Poorly absorbed through the skin. Readily absorbed, distributed and excreted without metabolism. After ingestion, ~80% is excreted in the urine unchanged in 4 days, and ~13% is excreted as conjugates [Kancir *et al.* 1988].

Blood Concentration

After ingestion of 5 mg/kg by 6 subjects, average peak plasma concentrations of ≈35 mg/L were attained in 7 to 24 h [Kohli *et al.* 1974].

A 26-year-old male ingested ~75 mL of Verdone ~10 h before admission to hospital. Serum and urine 2,4-D concentrations were as shown in the table below.

Hours post-ingestion	*Serum concentration (mg/L)*	*24-h Urine excretion (mg)*	*Renal clearance (mL/min)*
11	79.6		
35	50.5	1460	0.2
59	31.0	770	0.17
83	15	900	0.41
107	11	630	0.39
131	7	1380	1.40

[Wells *et al.* 1981].

A 39-year-old deliberately ingested the contents of a lemonade bottle that was later shown to contain 10% 2,4-D and 20% mecoprop. Plasma concentration on admission was 400 mg/L and there was no appreciable decline over the next 2 days. Renal clearance of 2,4-D ranged from 0.14 mL/min at a urine pH of 5.1 to 63 mL/min at pH 8.3 [Prescott *et al.* 1979].

Toxicity The estimated minimum lethal dose is 7 g. The maximum permissible atmospheric concentration is 10 mg/m^3 and the maximum acceptable daily intake is 300 μg/kg. Di-chlorophenoxyacetic acid is very irritating to the eyes, nose and throat.

The following postmortem tissue concentrations have been reported in 4 fatalities involving ingestion of dichlorophenoxyacetic acid: blood 126 to 826 mg/L (mean 447, 4 cases), bile 573 mg/L (1 case), brain 13 and 66 μg/g (2 cases), kidney 62 to 82 μg/g (mean 69, 3 cases), liver 21 to 183 μg/g (mean 134, 4 cases), urine 111 and 264 mg/L (2 cases) [Coutselinis *et al.* 1977; Nielsen *et al.* 1965]; [Ryall, 1978]; [M. Watson, 1978].

A 35-year-old male ingested 50 g Celatox Gazon (100 g/L 2,4-D and 400 g/L MCPP). Total serum chlorophenoxy herbicide concentrations were 605 and 155 mg/L at 4 and 41 h postadmission, respectively. He made a full recovery [Berthelot-Moritz *et al.* 1997].

A 60-year-old male drank 0.5 L of 'Animex' (480 g/L 2,4-D) and died 5.5 h later. Blood and urine concentrations of 2,4-D were 192 and 3.8 mg/L, respectively [Jorens *et al.* 1995].

A 49-year-old male ingested an unknown amount of dichlorophenoxyacetic acid solution (500 g/L) and later died after hospital treatment. The concentrations of the herbicide in lung, liver, kidney, heart, pancreas, muscle, brain and stomach contents were 533, 386, 370, 361, 298, 273 and 185 mg/kg and 125 mg/L, respectively. The herbicide was detected in blood before treatment at a concentration of 389 mg/L and in plasma at 750 mg/L. [Keller *et al.* 1994].

A 61-year-old female ingested an unknown quantity of Killex (100 g/L 2,4-D, 50 g/L mecoprop and 9 g/L dicamba). At postmortem, her blood, urine and bile concentrations of 2,4-D were 520, 70, and 340 mg/L and her liver concentration was 540 mg/kg [Fraser *et al.* 1984].

A 26-year-old male ingested ~360 mL Dexol (6.7% chloropyrifos in 76.8% distillates), 360 mL of Ortho Weed-B-Gone M (10.8% diethylamine salts of 2,4-D, 11.6% MCPP and 77.6% aqueous inert ingredients), and a few granules of D-Con concentrate (0.025% warfarin). On admission the gastric aspirate contained 108.2 μg/g 2,4-D. At postmortem 2,4-D tissue concentrations (μg/g) were as detailed below.

Tissue	*2,4-D*
CSF	96.7
Brain grey matter (frontal lobe)	186.4
Brain white matter (frontal lobe)	298.5
Brain stem	254.8
Whole blood	389.5
Bile	154.8
Liver	293.5
Pancreas	220.9
Left ventricle	301.2
Diaphragm	283.1
Kidney	315.0

[Osterloh *et al.* 1983].

Note For 3 cases of survival following serum concentrations of 0.37 to 1.77 μg/L, see Durakovic *et al.* [1992].

For reviews of poisoning due to chlorophenoxy herbicides, see Bradberry *et al.* [2004] or Bradberry *et al.* [2000].

Half-life Plasma half-life, ≈12 to 33 h.

Volume of Distribution 0.1 to 0.2 L/kg [Bradberry *et al.* 2004].

Clearance Urine pH-dependent [Prescott *et al.* 1979].

Distribution in Blood Plasma : whole blood ratio, 1.80 to 2.27 [Flanagan, Ruprah 1989].

Protein Binding Extensive to albumin [Bradberry *et al.* 2004].

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Dichlorvos

Insecticide

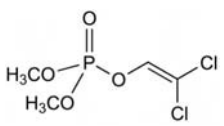
$C_4H_7Cl_2O_4P$ = 221.0

CAS—62-73-7

IUPAC Name 2,2-Dichloroethenyl dimethyl phosphate

Synonyms DDVP; dichlorfos; dichlorophos; 2,2-dichlorovinyl dimethyl phosphate; dichlorovos; ENT-20738; NSC-6738; OMS-14; SD-1750.

Proprietary Names *Astrobat*; *Atgard*; *Canogard*; *Dedevap*; *Dichlorman*; *Divipan*; *Doom*; *Equigard*; *Equigal*; *Estrosol*; *Herkol*; *Nogos*; *Nuvan*; *Prosvit*; *Task*; *Vapona*; *Verdisol.*



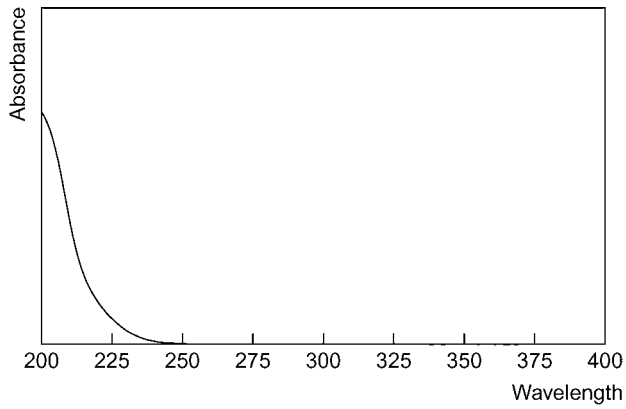
Chemical Properties A colourless to amber, oily liquid. Mp <25°. Bp 140° at 20 mmHg. It is soluble in water (8000 mg/L at 20°) and glycerol (0.5 g/100 mL); very soluble in dichloromethane, propan-2-ol and toluene; soluble in ethanol, chloroform, acetone and kerosene; miscible with many organic solvents and aerosol propellents; moderately soluble in diesel oil, kerosene, isoparaffinic hydrocarbons and mineral oils. Log *P* (octanol/water), 1.47. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Thin-layer Chromatography System TE—R_f 0.83; system TX—R_f 0.20; system TY—R_f 0.20; system TZ—R_f 0.75; system TAA—R_f 0.36; system TAB—R_f 0.00; system TAC—R_f 0.00.

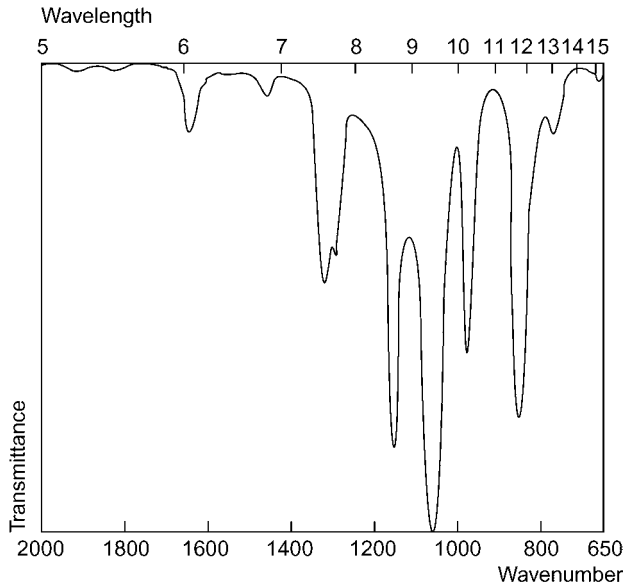
Gas Chromatography System GA—RI 1275; system GK—RRT 0.23 (relative to caffeine).

High Performance Liquid Chromatography System HAO—*k* 5.22; system HAP—*k* 0.86.

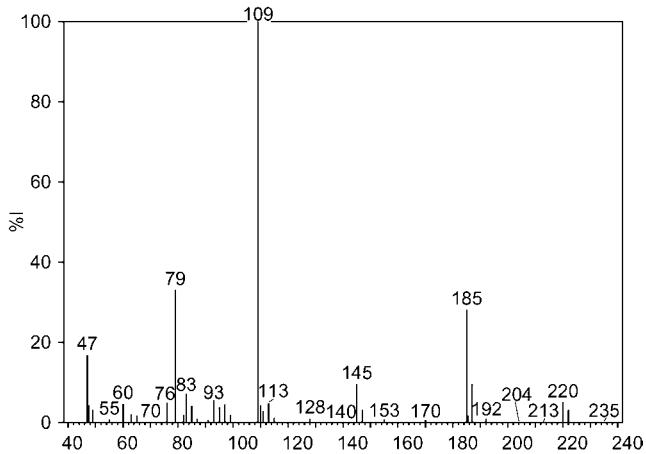
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1061, 1158, 850, 978, 1320, 2971 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 109, 185, 15, 79, 47, 145, 187, 29.



Quantification

Blood **GC-MS** Column: Supelco PTE 5 5% phenylsiloxane 95% methylsiloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 70 kPa. Temperature programme: 60° for 2 min to 90° at 40°/min to 250° at 15°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 mg/L, limit of detection, 0.275 mg/L [Abe *et al.* 2008]. Column: HP-5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 1 min to 290° at 10°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, >0.025 mg/g, limit of detection, 0.1 µg/g [Musshoff *et al.* 2002]. Limit of detection, 150 µg/L [Takayasu *et al.* 2001]. Column: Ultra-1 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 45 kPa.

Temperature programme: 80° for 1 min to 100° at 50°/min for 0.3 min to 113° at 1.5°/min for 0 min to 300° at 70°/min for 2.0 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 μg/L [Heinig *et al.* 2000]. Column: 5% phenylsiloxane 95% dimethylsilixane (12.5 m × 0.32 mm i.d.). Carrier gas: He, 5 mL/min. Temperature programme: 80° for 30 s to 130° at 70°/min. NPD. Limit of detection, 50 nmol/L [Villén *et al.* 1990].

Plasma **HPLC** Column: C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: 1 mmol/L OSA : methanol (70 : 30; pH 3.0), flow rate 1.5 mL/min. UV detection (λ = 210 nm). Retention time: 44.2 min. Limit of detection, 40 μg/L [Unni *et al.* 1992].

Serum **HPTLC** Dichlorvos and other pesticides [Yan *et al.* 2006].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 70 kPa. Temperature programme: 60° for 2 min to 110° at 40°/min to 170° at 12°/min to 300° at 40°/min for 3.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, <0.06 mg/L [Tarbah *et al.* 2004]. Limit of detection, 60 μg/L for the metabolite dimethylphosphate [Tarbah *et al.* 1998].

HPLC Column: C_{18}. Mobile phase: acetonitrile : water. DAD (λ = 230 nm). Limit of detection, 0.05–6.8 mg/L for dichlorvos and other organophosphorus pesticides [Cho *et al.* 1997].

LC-MS Column: C_{18}. Mobile phase: 10 mmol/L ammonium formate : methanol. Limit of quantification, 0.25–1.25 mg/L for dichlorvos and other organophosphorus pesticides [Inoue *et al.* 2007].

Urine **GC-MS** See Blood [Abe *et al.* 2008; Heinig *et al.* 2000; Takayasu *et al.* 2001].

HPLC See Serum [Cho *et al.* 1997].

Stomach Contents **GC-MS** See Blood [Abe *et al.* 2008].

Kidney **GC-MS** See Blood [Abe *et al.* 2008].

Liver **GC-MS** See Blood [Abe *et al.* 2008].

Lung **GC-MS** See Blood [Abe *et al.* 2008].

Disposition in the Body Dichlorvos is easily absorbed through the skin. It is absorbed after ingestion and rapidly moves to the liver where it is quickly detoxified by degrading enzymes found in tissues and blood plasma. It is distributed in tissues, especially kidneys, liver, stomach and intestines. It is rapidly metabolised and eliminated from the body via expired air and urine. One metabolite is dimethylphosphate, which is common for some of the organophosphate compounds, and another is dichloroacetaldehyde. It does not accumulate in tissues.

Toxicity Dichlorvos is highly toxic by inhalation, dermal absorption and ingestion. Severe poisoning will affect the CNS. A lethal oral dose of 400 mg/kg bodyweight has been reported and a toxic dose of 300 mg/kg. The allowed daily intake is 0.04 mg/kg.

A 72-year-old woman was found dead after ingesting dichlorvos. A 500 mL bottle of dichlorvos was found beside the body (concentration 75%). There was 250 mL of a volatile fluid in the stomach, which was equivalent to 300 g dichlorvos. Dichlorvos was detected in the spleen (3340 μg/g), heart (815 μg/g), urine (4.5 mg/L), blood (29 mg/L), brain (9.7 μg/g), lung (81 μg/g), kidney (80 μg/g) and liver (20 μg/g) [Shimizu *et al.* 1996].

A 54-year-old man was found dead after ingesting dichlorvos (possibly 82 g; 1 g/kg). Levels of dichlorvos in cardiac blood was ~4 times higher than in peripheral blood. Levels in the kidneys and lungs were lower and it was not found in the liver. The stomach contents was 38 g [Abe *et al.* 2008].

Thirteen patients were admitted to hospital after eating wheat bagels that had been deliberately contaminated with dichlorvos. Mean serum acetylcholinesterase levels were 2945 U/L (range, 470–7830) [Kavalci *et al.* 2009].

For other reports of poisoning with dichlorvos, see Güloglu *et al.* [2004], Kumar *et al.* [2009], and Yucel *et al.* [2008].

Half-life ~25 min.

Clearance Oral clearance in healthy volunteers is ~1558 L/h.

Abe E *et al.* (2008). A fatal dichlorvos poisoning: concentrations in biological specimens. *J Forensic Sci* 53: 997–1000.

Cho Y *et al.* (1997). Determination of organophosphorous pesticides in biological samples of acute poisoning by HPLC with diode-array detector. *Chem Pharm Bull (Tokyo)* 45: 737–740.

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Heinig R *et al.* (2000). Development, validation and application of assays to quantify metrifonate and 2,2-dichlorovinyl dimethylphosphate in human body fluids. *J Chromatogr B Biomed Sci Appl* 741: 257–269.

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Shimizu K *et al.* (1996). Tissue distribution of DDVP after fatal ingestion. *Forensic Sci Int* 83: 61–66.

Takayasu T *et al.* (2001). Rapid analysis of pesticide components, xylene, *o*-dichlorbenzene, cresol and dichlorvos, in blood and urine by pulse heating-gas chromatography–mass spectrometry. *LegMed (Tokyo)* 3: 157–161.

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Tarbah FA *et al.* (2004). Acute poisoning with phosphamidon: determination of dimethyl phosphate (DMP) as a stable metabolite in a case of organophosphate insecticide intoxication. *J Anal Toxicol* 28: 198–203.

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Yan HF *et al.* (2006). [High performance thin layer chromatography quality assessment of Rogor, methyl parathion, methamidophos and dichlorvos in serum]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 24: 437–438.

Yucel I *et al.* (2008). Suicide attempt with injection of insecticide in both wrists. *Orthopedics* 31: 174.

Diclofenac

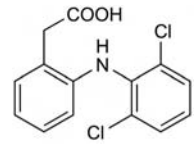
Analgesic, Antiinflammatory, Antipyretic

$C_{14}H_{11}Cl_2NO_2$ = 296.2

CAS—15307-86-5

IUPAC Name 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid

Synonyms Diclophenac; [*o*-(2,6-Dichloroanilino)phenyl]acetic acid.



Chemical Properties Crystals. Mp 156° to 158°. pK_a 4.2. Log *P* (octanol/water), 4.5. Extraction yield (chlorobutane), 0.2 [Demme *et al* 2005].

Diclofenac Sodium

$C_{14}H_{10}Cl_2NNaO_2$ = 318.1

CAS—15307-79-6

Synonyms Diclofenacum natricum; GP-45840.

Proprietary Names *Acoflam*; *Arthrotec*; *Cataflam*; *Dicloflex*; *Diclomax*; *Diclotard*; *Diclovol*; *Diclozip*; *Econac*; *Flamatak*; *Flamrase*; *Flexotard*; *Isclofen*; *Lofensaid*; *Motifene*; *Pennsaid*; *Rheumatac*; *Rhumalgan*; *Slofenac*; *Solaraze*; *Volraman*; *Volsaid*; *Voltaren(e)*; *Voltarol*.

Chemical Properties Crystals. Mp 283° to 285°. Solubility at 25° (mg/mL): deionised water (pH 5.2) >9; methanol >24; acetone 6; acetonitrile <1; cyclohexane <1.

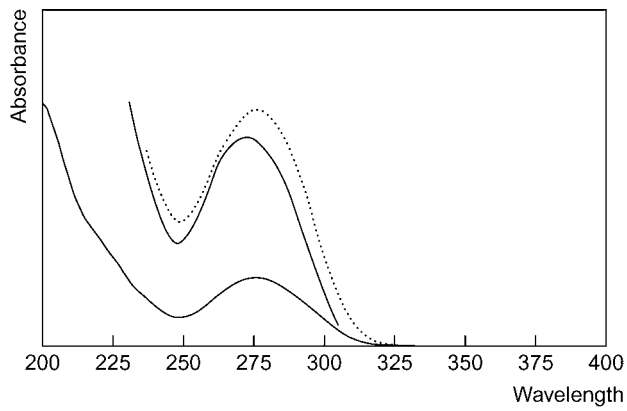
Colour Tests Liebermann's reagent—red-brown; Mandelin's test—red-brown; Marquis test—brown (slow).

Thin-layer Chromatography System TA—R_f 0.90; system TD—R_f 0.25; system TE—R_f 0.12; system TF—R_f 0.27; system TG—R_f 0.29; system TAD—R_f 0.47; system TAE—R_f 0.90; system TAJ—R_f 0.40; system TAK—R_f 0.64; system TAL—R_f 0.84 (chromic acid solution—red).

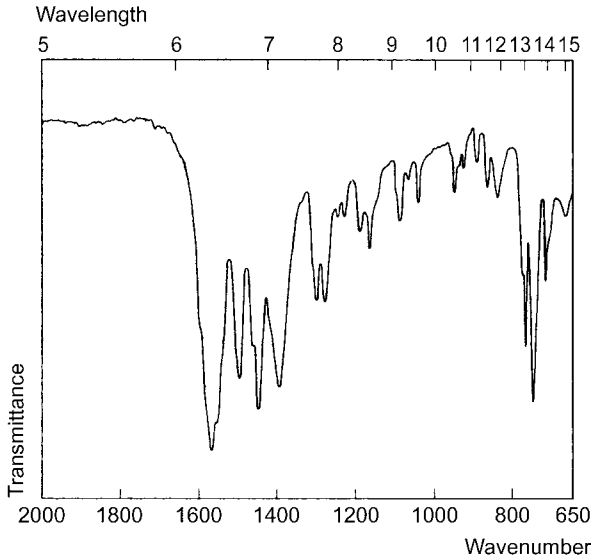
Gas Chromatography System GA—diclofenac RI 2271, M (-Me) RI 2195, M (-Me_2) RI 2220, M (OH--Me_2) RI 2460; system GB—diclofenac RI 2231; system GD—M (-Me) RRT 1.42 (relative to *n*-$C_{16}H_{34}$); system GL—M (-2Me) RI 2200; M (OH-)-Me_2 RI 2460.

High Performance Liquid Chromatography System HD—*k* 11.5; system HV—RT 0.85 min (relative to meclofenamic acid); system HX—RI 616; system HY—RI 592; system HZ—RT 14.8 min; system HAA—RT 22.1 min; system HAX—RT 8.7 min; system HAY—RT 10.0 min.

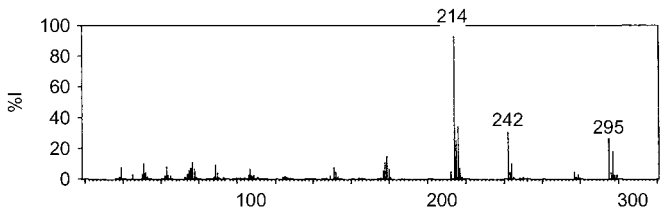
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1 = 309b); aqueous alkali—275 nm (A_1^1 = 351b)



Infrared Spectrum Principal peaks at wavenumbers 1572, 756, 1504, 775, 1286, 1308 cm^{-1} (diclofenac sodium, KBr disk).



Mass Spectrum Principal ions at *m/z* 214, 216, 242, 295, 215, 297, 179, 178.



Quantification

Plasma **GC** ECD. Limit of detection, 100 µg/L [Ikeda *et al.* 1980].

GC-MS Column: HP-1. Carrier gas: He. SIM acquisition mode. Limit of quantification, 1 µg/L, limit of detection, 0.2 µg/L [Borenstein *et al.* 1996]. SIM acquisition mode. Limit of detection, 2 µg/L [Del Puppo *et al.* 1991]. Limit of detection, 100 ng/L [Sioufi *et al.* 1991].

HPLC UV detection (λ= 276 nm). Limit of quantification, 50 µg/L [Rigato *et al.* 2009]. Limit of detection, 5 µg/L [Arcelloni *et al.* 2001]. Column: Capcell Pak MF Ph-1 (35 × 2 mm i.d.) and phenylhexyl column (100 × 2 mm i.d.). Mobile phase: acetonitrile : 0.02 mol/L potassium phosphate (pH 7; 33 : 67). Limit of detection, 10 µg/L [Lee *et al.* 2000]. UV detection (λ= 282 nm). Limit of detection, 3 µg/L [Hanses *et al.* 1995]. Column: C_{18} Spherisorb. Mobile phase: acetonitrile : 0.1 mol/L sodium acetate (pH 6.3, 35 : 65,). UV detection (λ= 278 nm). Retention time, 2.8 min. Limit of detection, 20 µg/L [Avgerinos *et al.* 1993]. See also Blagbrough *et al.* [1992], Brunner, Luders [1991], Chan *et al.* [1982]<Query: Chan ref missing; please add>, Giagoudakis, Markantonis [1998], Miller [1993], Santos *et al.* [1992], Wiese, Hermansson [1991], Zecca *et al.* [1991].

Serum **HPLC** Column: Regis SPS 100 RP-8 (150 × 4.6 mm i.d., 5 µm) and LiChrospher 100 RP_{18} (5 µm). Fluorescence detection (λ_{ex}=286 nm, λ_{em}= 360 nm). Limit of detection, 1 µg/L [Kuhlmann, Krauss 1997]. Limit of detection, 10 µg/L [Zhang *et al.* 1994]. Column: Spherisorb S5 ODS-2. Mobile phase: methanol : 0.05 mol/L phosphate buffer (43 : 57). Fluorescence detection (λ_{ex}= 282 nm, λ_{em}= 365 nm). Limit of quantification, 20 µg/L [Moncrieff 1992].

Urine **GC-MS** Limit of detection, 10–50 µg/L [Maurer *et al.* 2001]. See Plasma [el Haj *et al.* 1999; Sioufi *et al.* 1991]. ECD [Schneider, Degen 1981].

HPLC Column: reversed phase ODS. Mobile phase: 10 mmol/L acetate buffer (pH 4.0) : acetonitrile (58 : 42). UV detection (λ= 210 nm). Limit of quantification, 20 µg/L, limit of detection, <7 µg/L [Bakkali *et al.* 1999]. Column: reversed phase Inertsil ODS-2. Mobile phase: phosphate buffer : acetonitrile (pH 5.0). UV detection (λ= 230 or 320 nm). Limit of quantification, 50 µg/L [Hirai *et al.* 1997]. Column: reversed phase. Mobile phase: ascorbic acid. Limit of quantification, 400 µg/L [Sawchuk *et al.* 1995].

Aqueous Humour **HPLC** Column: Regis SPS 100 RP_8 (150 × 4.6 mm i.d., 5 µm). ECD. Limit of detection, 500 ng/L [Kuhlmann *et al.* 1998]. Column: reversed phase octyl. Mobile phase: acetic acid : acetonitrile : TEA. UV detection. Limit of detection, 0.3 ng [Riegel, Ellis 1994]. See Serum [Kuhlmann, Krauss 1997].

CSF **HPLC** See Plasma [Zecca *et al.* 1991].

Sinovial Fluid **HPLC** See Plasma [Blagbrough *et al.* 1992].

Disposition in the Body Diclofenac is well absorbed after oral administration but undergoes significant first-pass metabolism. Up to approx. 70% of a dose is excreted in the urine in 3 days, including 20–40% as glucuronide and sulfate conjugates of the major metabolite 4′-hydroxydiclofenac (active) and up to ~15% as conjugates of unchanged diclofenac. Other metabolites identified in the urine include 5-hydroxydiclofenac (~12% of the dose), 3′-hydroxydiclofenac and 4′,5-dihydroxydiclofenac. Approximately 10–20% of a dose is excreted in the bile as 4′-hydroxydiclofenac and <5% as unchanged drug. Diclofenac enters synovial fluid, where peak levels are attained ~2–4 h after peak plasma levels. Diclofenac is excreted in breast milk but in quantities so small that no adverse effects on breast-fed infants would be expected.

Therapeutic Concentration

Following oral administration of 50 mg diclofenac sodium three times a day to four subjects, peak plasma concentrations of 0.1 to 2.2 mg/L (mean 0.8) diclofenac and 0.3 to 2.0 mg/L (mean 1.2) 4′-hydroxydiclofenac were reported 3 h after a dose; peak concentrations in synovial fluid of 0.1 to 0.6 mg/L (mean 0.3) diclofenac and 0.2 to 1.0 mg/L (mean 0.6) 4′-hydroxydiclofenac were attained in 3 h; concentrations in synovial fluid exceeded those in plasma after 4 h [Fowler *et al.* 1983].

Diclofenac was administered as a single oral 150 mg dose to 6 healthy subjects, 6 patients with chronic active hepatitis and 6 patients with alcoholic cirrhosis. Peak plasma concentrations of diclofenac, 3′-hydroxydiclofenac, 4′-hydroxydiclofenac and 3′-hydroxy-4′,5-methoxydiclofenac of 5.699, 0.412, 2.613 and 0.385 mg/L, respectively, were attained at 0.63, 0.79, 0.88 and 5.8 h, respectively, in the healthy subjects. The equivalent respective values in patients with hepatitis were 6.574, 0.392, 2.786 and 0.306 mg/L at 0.42, 0.58, 0.50 and 4.0 h; in patients with cirrhosis, they were 11.59, 0.264, 2.481 and 0.191 mg/L at 0.33, 1.04, 0.75 and 7.0 h [Lill *et al.* 2000].

In 20 healthy subjects given a single oral dose of diclofenac 100 mg as two different sustained-release tablets, peak plasma concentrations of 1.161 and 0.799 mg/L were attained in 4.2 and 4.5 h [Zmeili *et al.* 1996].

Toxicity

A 19-year-old man was admitted to hospital following the ingestion of 1.5 g diclofenac sodium and 4 g chlormezanone. Plasma diclofenac concentrations of 60.1 mg/L and 0.19 mg/L were reported at 7 and 15 h, respectively; chlormezanone could not be determined. The subject recovered after ~2 days [Netter *et al.* 1984].

Bioavailability ~50–60%.

Half-life Plasma half-life, ~1–2 h; synovial fluid half-life 3–6 h.

Volume of Distribution 0.17 L/kg.

Clearance ~4 mL/min/kg from plasma.

Protein Binding >99%.

Note For reviews of diclofenac see Brogden *et al.* [1980], Davies, Anderson [1997] and Shintaku *et al.* [2009].

Dose 75 to 150 mg of diclofenac sodium daily in divided doses.

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Shintaku K *et al.* (2009). Transplacental pharmacokinetics of diclofenac in perfused human placenta. *Drug Metab Dispos* 37: 962–968.
Sioufi A *et al.* (1991). Determination of diclofenac in plasma and urine by capillary gas chromatography–mass spectrometry with possible simultaneous determination of deuterium-labelled diclofenac. *J Chromatogr* 571: 87–100.
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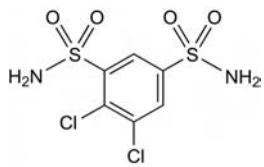
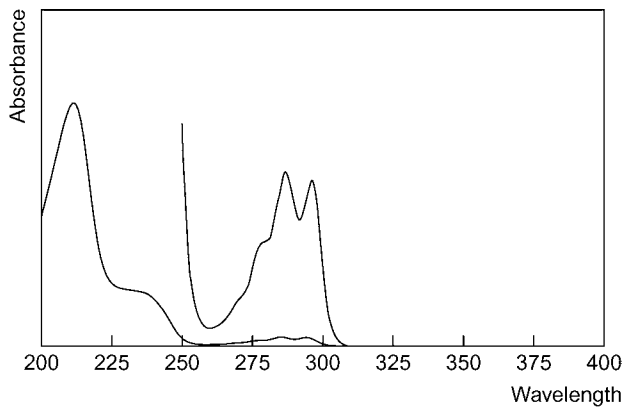
Diclofenamide

Carbonic Anhydrase Inhibitor
$C_6H_6Cl_2N_2O_4S_2$ = 305.2
CAS—120-97-8

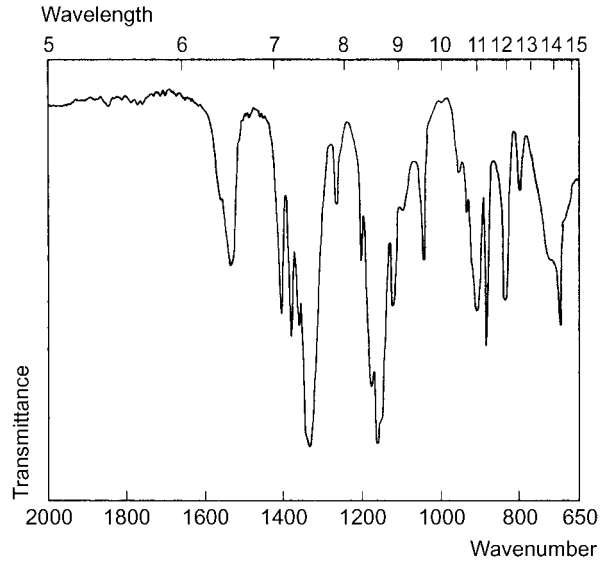
IUPAC Name 4,5-Dichloro-1,3-benzenedisulfonamide
Synonyms DCPA; dichlorophenamide; dichlorphenamide.
Proprietary Names *Daranide*; *Oralcon*; *Oratrol*.



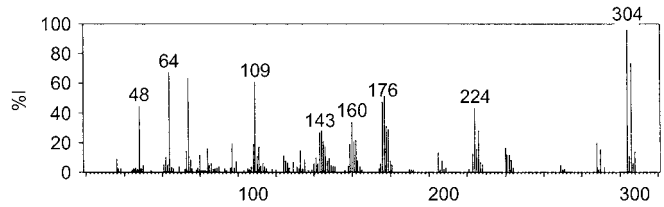
Chemical Properties A white crystalline powder. Mp about 240°. Practically insoluble in water and chloroform; soluble 1 in 30 of ethanol; slightly soluble in ether; freely soluble in pyridine; soluble in solutions of alkali hydroxides and carbonates. pK_a 7.4, 8.6. Log *P* (ether/pH 7.4), 1.0.
Colour Test Koppanyi-Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.82; system TD—R_f 0.14; system TE—R_f 0.33; system TF—R_f 0.64; system TAD—R_f 0.23 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—286 (A_1^1=60a), 296 nm.



Infrared Spectrum Principal peaks at wavenumbers 1162, 1176, 882, 702, 907, 1123 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 304, 306, 64, 74, 109, 177, 176, 48.



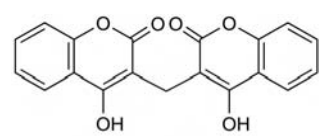
Quantification
Serum (rabbit) **GC** ECD. Limit of detection, 20 µg/L [Schmitt *et al.* 1979].
Dose 100 to 200 mg daily in the treatment of glaucoma.

Schmitt C *et al.* (1979). Electron-capture GLC assay of dichlorphenamide. *J Pharm Sci* 68: 381–383.

Dicoumarol

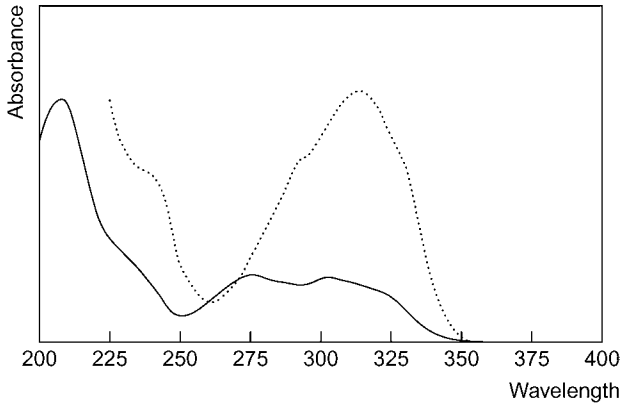
Anticoagulant
$C_{19}H_{12}O_6$ = 336.3
CAS—66-76-2

IUPAC Name 2-Hydroxy-3-[(2-hydroxy-4-oxochromen-3-yl)methyl]chromen-4-one
Synonyms Bishydroxycoumarin; dicoumarin; dicumarol; melitoxin; 3,3′-methylenebis[4-hydroxy-2*H*-1-benzopyran-2-one].
Proprietary Name *Dufalone*

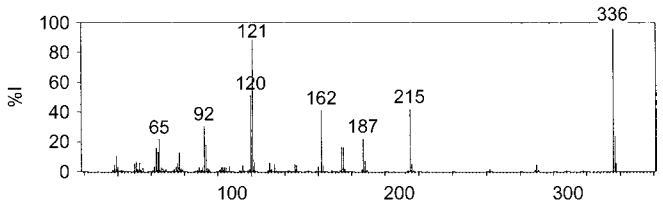


Chemical Properties A white or creamy-white crystalline powder. Mp 287° to 293°. Practically insoluble in water, ethanol and ether; slightly soluble in benzene and chloroform; freely soluble in solutions of alkali hydroxides. pK_a 4.4, 8.0. Log *P* (octanol/water), 2.1.
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.18; system TE—R_f 0.30; system TF—R_f 0.32; System TAD—R_f 0.33; system TAE—R_f 0.88; system TAJ—R_f 0.60; system TAK—R_f 0.80; system TAL—R_f 0.96 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 599.

Ultraviolet Spectrum Aqueous alkali—314 nm (A^1_1=756a).



Mass Spectrum Principal ions at *m/z* 336, 121, 120, 215, 162, 92, 337, 187.



Quantification

Plasma **UV spectrophotometry** Limit of detection, 2 mg/L [Nagashima *et al.* 1968].

Disposition in the Body Slowly and irregularly absorbed after oral administration. It is extensively metabolised with <1% of a dose being excreted in the urine as unchanged drug.

Therapeutic Concentration

After a single oral dose of 150 mg to 8 subjects, a mean peak plasma concentration of 16.7 mg/L was attained in 12 h [Solomon, Schrogie 1967].

Following daily oral administration of 2 mg/kg to 3 subjects, plasma concentrations of 44 to 59 mg/L (mean 53) were reported on the 6th day [Vesell, Page 1968].

Toxicity Fatalities, usually due to severe haemorrhage, have been reported following daily oral doses of 100 mg or more.

Half-life Plasma half-life, dose-dependent and may range from 7 to 100 h.

Protein Binding >99%.

Dose Maintenance, 25 to 150 mg daily.

Nagashima R *et al.* (1968). Comparative pharmacokinetics of coumarin anticoagulants. I. Unusual interaction of bishydroxycoumarin with plasma proteins–development of a new assay. *J Pharm Sci* 57: 58–67.

Solomon HM, Schrogie JJ (1967). The anticoagulant response to bishydroxycoumarin. I. The role of individual variation. *Clin Pharmacol Ther* 8: 65–69.

Vesell ES, Page JG (1968). Genetic control of dicumarol levels in man. *J Clin Invest* 47: 2657–2663.

Dicycloverine

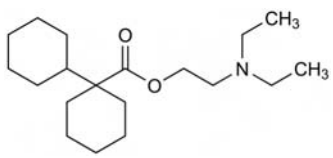
Anticholinergic

$C_{19}H_{35}NO_2$ = 309.5

CAS—77-19-0

IUPAC Name 2-Diethylaminoethyl 1-cyclohexylcyclohexane-1-carboxylate

Synonyms [1,1′-Bicyclohexyl]-1-carboxylic acid 2-(diethylamino)ethyl ester; dicyclomine.



Chemical Properties Log *P* (octanol/water), 3.98 (hydrochloride).

Dicycloverine Hydrochloride

$C_{19}H_{35}NO_2$,HCl = 346.0

CAS—67-92-5

Proprietary Names *Antispas*; *Bentyl*; *Bentylol*; *Byclomine*; *Clomin*; *Dibent*; *Diclomin*; *Dicomin*; *Dilomine*; *Formulex*; *Lomine*; *Medicyclomine*; *Merbentyl*; *Notensyl*; *Optimal*; *Or-Tyl*; *Wyovin*. It is an ingredient of *Anacidron*, *Diarrest*, *Kolanticon*, *Kolantyl* and *Ovol*.

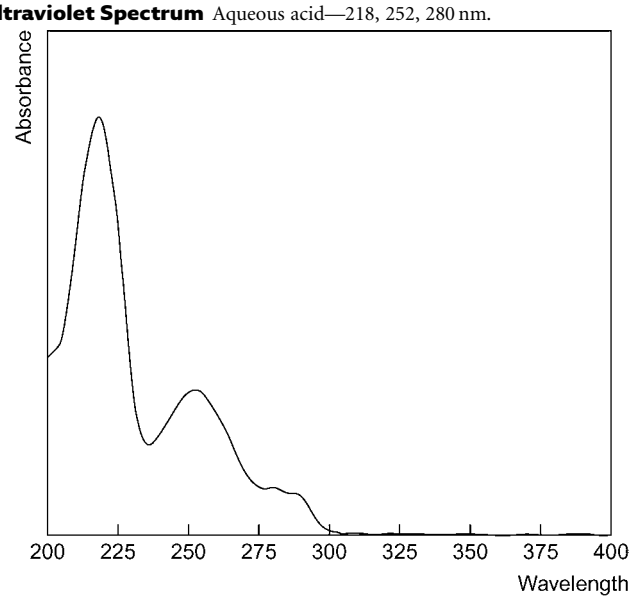
Chemical Properties A white crystalline powder. Mp 164° to 166° (from butanone). Soluble 1 in 20 of water, 1 in 5 of ethanol and 1 in 2 of chloroform; very slightly soluble in ether.

Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.67; system TC—R_f 0.64; system TE—R_f 0.84; system TL—R_f 0.54; system TAE—R_f 0.55; system TAJ—R_f 0.42; system TAK—R_f 0.25; system TAL—R_f 0.84 (acidified iodoplatinate solution, positive).

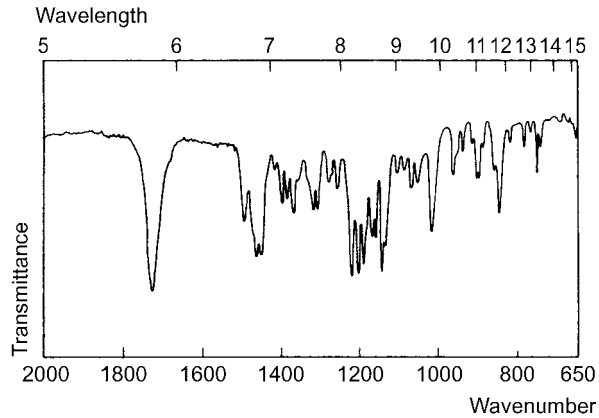
Gas Chromatography System GA—RI 2111; system GB—RI 2175; system GF—RI 2265.

High Performance Liquid Chromatography System HA—*k* 1.1; system HY—RI 575.

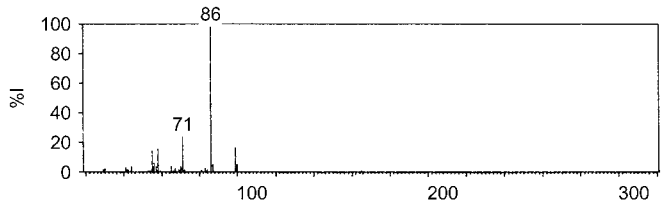
Ultraviolet Spectrum Aqueous acid—218, 252, 280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1714, 1214, 1136, 1197, 1184, 1155 cm^{-1} (dicycloverine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 71, 99, 58, 55, 56, 100, 87.



Quantification

Plasma **GC** NSD. For method, see Walker *et al.* [1987]. AFID. Limit of detection, 1 µg/L [Meffin *et al.* 1973].

Disposition in the Body Rapidly absorbed after oral administration. About 80% of a dose is excreted in the urine and 10% is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 20 mg to 1 subject, a peak plasma concentration of about 0.02 mg/L was attained in about 1.5 h; following administration of 40 mg to the same subject as a sustained-release preparation, a maximum plasma concentration of about 0.08 mg/L was reported after about 2.5 h [Meffin *et al.* 1973].

Toxicity Fatalities have been reported in children after the ingestion of about 0.2 g of a sustained-release preparation.

A blood concentration of 0.505 mg/L (nearly 10 times the reported adult therapeutic level) was reported in a fatality involving an infant [Garriott *et al.* 1984].

Half-life Plasma half-life, about 5 h.

Dose 30 to 80 mg of dicycloverine hydrochloride daily; in the USA doses of up to 160 mg daily in divided doses have been recommended.

Garriott JC *et al.* (1984). Two cases of death involving dicyclomine in infants. Measurement of therapeutic and toxic concentrations in blood. *J Toxicol Clin Toxicol* 22: 455–462.

Meffin PJ *et al.* (1973). Determination of dicyclomine in plasma by gas chromatography. *Anal Chem* 45: 1964–1966.

Walker BJ *et al.* (1987). Quantitative analysis of dicyclomine in human plasma by capillary gas chromatography and nitrogen-selective detection. *J Chromatogr* 416: 150–153.

Didanosine

Antiviral

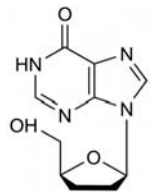
$C_{10}H_{12}N_4O_3 = 236.2$

CAS—69655-05-6

IUPAC Name 9-[(2*R*,5*S*)-5-(Hydroxymethyl)oxolan-2-yl]-3*H*-purin-6-one

Synonyms DDI; ddI; ddIno; 2′,3′-dideoxyinosine; dideoxyinosine; NSC-612049; BMY-40900.

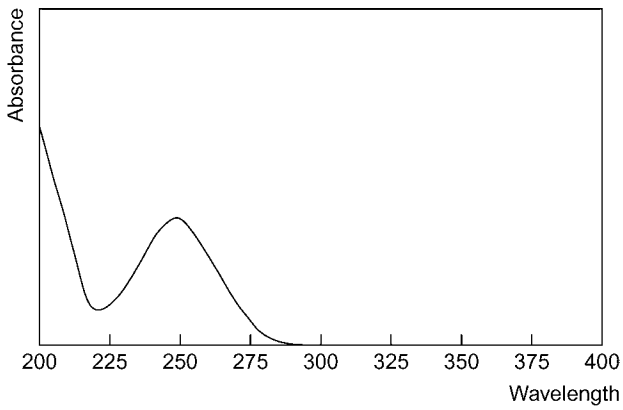
Proprietary Name *Videx*



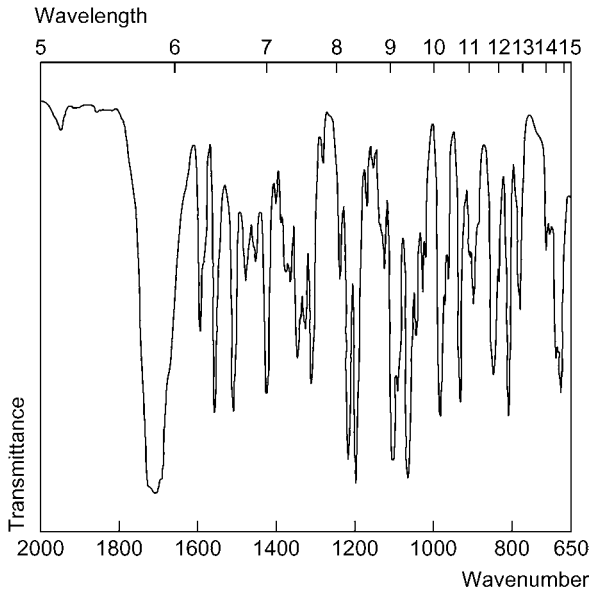
Chemical Properties A white crystalline powder. Mp 160° to 163°. Unstable in acidic solutions. pK_a 9.12. Log *P* (octanol/water), −1.24.

High Performance Liquid Chromatography System HAD—*k* 3.20.

Ultraviolet Spectrum Aqueous acid (pH 2)—248 nm; (ethanol)—246, 250 nm; aqueous alkali (pH 12)—254 nm.



Infrared Spectrum



Quantification

Plasma **HPLC** Limit of quantification, 10 μg/L [Aymard *et al.* 2000]. Column: NovaPak (100 × 8 mm, i.d., 4 μm). Mobile phase: 5% acetonitrile in 0.1% heptafluorobutyric acid in deionised, distilled water, flow rate 2.0 mL/min. UV detection (λ=252 and 260 nm). Retention time: 8.4 min. Limit of detection, 0.1 μg/L [Carpen *et al.* 1990]. UV detection. Limit of detection, 50 μg/L [Frijus-Plessen *et al.* 1990]. Column: ODS Ultrasphere (250 × 4.6 mm, 5 μm); (urine) C_8 Zorbax (250 × 4.6 mm, 5 μm). Mobile phase (plasma): 15% methanol in 50 mmol/L potassium phosphate buffer containing 0.05% TEA (pH 4.0), flow rate 0.7 mL/min; (urine) 3.9% methoxyethanol in 18.3 mmol/L potassium phosphate buffer (pH 7.2), 1.0 mL/min. UV detection (λ=254 nm). Retention time: (plasma) 11.2 min; (urine) 15.3 min. Limit of detection, 25 μg/L [Knupp *et al.* 1990].

Serum **HPLC** UV detection. Limit of detection, 120 μg/L [Simon *et al.* 2001]. UV detection. Limit of detection, 25 μg/L, free didanosine [Rosell-Rovira *et al.* 1996].

Urine **HPLC** See Plasma [Carpen *et al.* 1990]. See Plasma. Limit of detection, 1000 μg/L [Knupp *et al.* 1990].

Cerebrospinal Fluid **HPLC** See Plasma [Carpen *et al.* 1990].

Disposition in the Body Didanosine is rapidly absorbed and reduced by 50% in the presence of food. It is hydrolysed in the acid medium of the stomach and is therefore given orally with pH buffers or antacids. Concentrations in CSF are about 20% of those in plasma 1 h after IV infusion. It crosses the placenta; is detected in amniotic fluid and fetal blood and crosses the blood–brain barrier. The drug is extensively metabolised to the active antiviral metabolite dideoxyadenosine triphosphate, as well as uric acid. Acid hydrolysis may also occur to produce hypoxanthine. About 35 to 60% of a dose is excreted unchanged in urine by glomerular filtration and active tubular secretion. It is partially cleared by haemodialysis but not by peritoneal dialysis.

Therapeutic Concentration Mean peak plasma concentrations of 1.6 mg/L (range 0.6 to 2.9 mg/L) are produced after a single dose of 375 mg of a buffered powder for oral solution. Mean peak plasma concentrations of 1.6 mg/L (range 0.5 to 2.6 mg/L) are produced after administration of a single dose of 300 mg of a buffered chewable/dispersible tablet.

Groups of 3 to 4 patients diagnosed having AIDS (acquired immunodeficiency syndrome) or poor-prognosis AIDS-related complex were administered 0.2, 0.4, 0.8, 1.6, 3.2 or 6.4 mg/kg IV doses over 90 min or the equivalent oral doses. Peak concentrations were 0.6, 1.5, 1.8, 4.6, 10.5 and 18.5 μmol/L for the IV doses and 0.3, 0.8, 1.2, 2.8, 6.2 and 9.2 μmol/L for the oral doses, respectively [Hartman *et al.* 1990].

Toxicity

In phase I clinical studies, 12 out of 92 patients suffered from peripheral neuropathy (the most frequent serious toxic effect), which is dose related, when administered doses of 12 mg/kg daily for 6 weeks. This is believed to be due to accumulation of the drug above concentrations of 1250 mg/kg. Pancreatitis, in some cases fatal, has developed during therapy with didanosine; 8 out of 92 patients being treated with >12.5 mg/kg daily for 5 to 8.5 months suffered from pancreatitis [Faulds, Brogden 1992].

A 36-year-old man with Gilbert Syndrome, AIDS and a history of severe myelosuppression when administered with zidovudine was treated with 12 mg/kg once daily didanosine instead. Treatment was stopped after 15 weeks owing to side effects but was resumed after 6 weeks. Three months after treatment was restarted, the man was admitted to hospital with nausea, anorexia and abdominal pain. A CT scan showed he had a fatty liver with an enlarged globular pancreas. Renal dysfunction was observed the day before he died, caused by fulminent hepatic failure (a complication of treatment) [Lai *et al.* 1991].

Bioavailability Bioavailability is substantially reduced by administration with or after food and varies considerably between patients; it is reported to range from 20 to 40% depending on the formulation used.

Half-life Plasma, didanosine, 30 min to 4 h; dideoxyadenosine triphosphate, more than 12 h; increased in patients with renal impairment.

Volume of Distribution Mean, 1.01 L/kg in adults (range 0.76 to 1.29 L/kg); 24 L/m^2 in children.

Clearance Plasma clearance, 600 to 800 mL/min in adults; 510 mL/min/m^2 in children. Total body, 0.08 L/kg/h.

Protein Binding <5%.

Note For reviews of didanosine, see Shelton *et al.* [1992], Faulds and Brogden [1992], Lipsky [1993], and Perry and Balfour [1996].

For a review of the pharmacokinetics of antiviral nucleoside analogues, see Morse *et al.* [1993].

Dose Adults with body weight ≥60 kg: 200 mg (tablets) or 250 mg (solution) every 12 h; ≤60 kg: 125 mg (tablets) or 167 mg (solution). Children: 25 to 100 mg (tablet) or 31 to 125 mg (powder formulation) twice daily, depending on body surface area.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 744: 227–240.

Carpen ME *et al.* (1990). High-performance liquid chromatographic method for analysis of 2′,3′-dideoxyinosine in human body fluids. *J Chromatogr* 526: 69–75.

Faulds D, Brogden RN (1992). Didanosine. A review of its antiviral activity, pharmacokinetic properties and therapeutic potential in human immunodeficiency virus infection. *Drugs* 44: 94–116.

Frijus-Plessen N *et al.* (1990). Determination of 3′-azido-3′-deoxythymidine, 2′,3′-dideoxycytidine, 3′-fluoro-3′-deoxythymidine and 2′,3′-dideoxyinosine in biological samples by high-performance liquid chromatography. *J Chromatogr* 534: 101–107.

Hartman NR *et al.* (1990). Pharmacokinetics of 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine in patients with severe human immunodeficiency virus infection. *Clin Pharmacol Ther* 47(5): 647–654.

Knupp CA *et al.* (1990). Quantitation of didanosine in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 533: 282–290.

Lai KK *et al.* (1991). Fulminant hepatic failure associated with 2′,3′-dideoxyinosine (ddI). *Ann Intern Med* 115(4): 283–284.

Lipsky JJ (1993). Zalcitabine and didanosine. *Lancet* 341: 30–32.
Morse GD *et al.* (1993). Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin Pharmacokinet* 24: 101–123.
Perry CM, Balfour JA (1996). Didanosine. An update on its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV disease. *Drugs* 52: 928–962.
Rosell-Rovira ML *et al.* (1996). Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography. *J Chromatogr B Biomed Appl*,89–92.
Shelton MJ *et al.* (1992). Didanosine. *Ann Pharmacother* 26: 660–670.
Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

Dieldrin

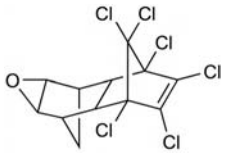
Insecticide

$C_{12}H_8Cl_6O$ = 380.9

CAS—60-57-1 (HEOD)

Proprietary Name *Dilstan EC-15*

Note Dieldrin contains about 85% of HEOD, (1aα,2β,2aα,3β,6β,6aα,7β,7aα)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7 : 3,6-dimethanonaphth[2,3-*b*]oxirene, the remaining 15% being mainly chlorinated organic compounds related to HEOD.



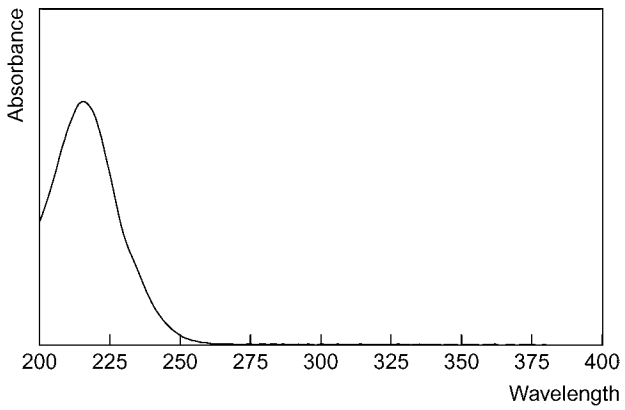
Chemical Properties A light-tan, flaky, crystalline solid. Mp 176° to 177°. Practically insoluble in water; soluble 1 in 4 of ethanol, 1 in 40 of carbon tetrachloride and 1 in 1 of methanol; moderately soluble in chloroform. Log *P* (octanol/water), 5.4.

Colour Tests Nitric–sulfuric acid (Erdman's reagent)—pink; sulfuric acid–fuming sulfuric acid—red.

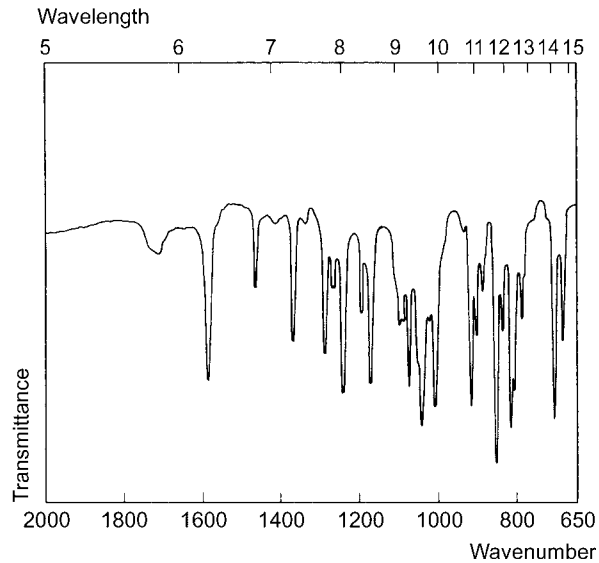
Thin-layer Chromatography System TB—R_f 0.60; system TE—R_f 0.89; system TF—R_f 0.74; system TX—R_f 0.65; system TY—R_f 0.87; system TAB—R_f 0.51; system TAC—R_f 0.27; system TAE—R_f 0.89.

Gas Chromatography System GA—RI 2110; system GK—RRT 1.13(relative to caffeine).

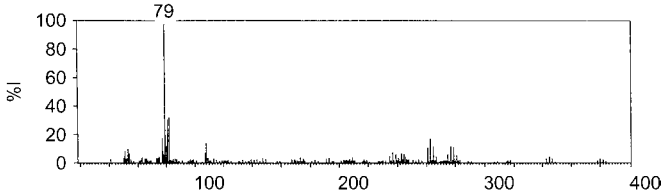
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 848, 811, 1038, 700, 1005, 912 cm^{-1} (KCl disk).



Mass Spectrum Principal ions at *m/z* 79, 82, 81, 263, 77, 108, 265, 80.



Quantification

***Blood* GC** ECD. For method, see Steentoft [1979].

***Plasma* GC** ECD. Limit of detection, 0.1 to 0.7 µg/L [Saady, Poklis 1990].

***Serum* GC** See Plasma [Saady, Poklis 1990].

GC–MS For method, see Dmitrovic *et al.* [2002].

***Tissues* GC** See Blood [Steentoft 1979].

Disposition in the Body Both dieldrin powder and solutions are readily absorbed after oral administration, through the lungs and through intact skin. Dieldrin is selectively stored in body fat and persists for several weeks after cessation of exposure. It is eliminated in the faeces mainly as unknown hydrophilic metabolites; a small amount is excreted in the urine as metabolites but very little as unchanged dieldrin.

Dieldrin is a metabolite of aldrin.

Blood Concentration Blood concentrations averaging 0.001 mg/L have been reported in subjects with no occupational exposure to dieldrin.

Serum concentrations ranging from 0.001 to 0.137 mg/L (mean 0.02) were reported in 71 industrially exposed workers, and fat concentrations of 0.6 to 32 µg/g (mean 6) were reported in 28 of these subjects [Hayes, Curley 1968].

Steady-state blood concentrations of about 0.007 and 0.02 mg/L were reported in 2 groups of 3 subjects who received doses of 0.05 mg and 0.21 mg daily for 2 years; maximum fat concentrations of 1.3 to 1.6 µg/g and 2.2 to 4.9 µg/g were reported in the same subjects [Hunter *et al.* 1969].

Fat residues in 236 non-exposed subjects were reported to be in the range <0.01 to 0.5 µg/g (mean 0.1) [Abbott *et al.* 1981].

Toxicity The estimated minimum lethal dose is 5 g; ingestion of 10 mg/kg may produce toxic effects. The maximum permissible atmospheric concentration is 0.25 mg/m^3 and the maximum acceptable daily intake is 0.1 µg/kg. Blood concentrations greater than 0.15 µg/mL are usually toxic. Several fatalities due to accidental or deliberate ingestion of dieldrin have been reported.

A serum concentration of 0.27 mg/L and a fat concentration of 47 µg/g were reported 3 days after ingestion of dieldrin by a 4-year-old boy who survived [Garrettson and Curley 1969].

In a fatality due to the ingestion of dieldrin, postmortem blood and liver concentrations of 0.5 mg/L and 29 µg/g, respectively, were reported [Steentoft 1979].

Half-life Blood half-life, 50 to 170 days (mean 97).

Distribution in Blood Plasma : whole blood ratio, about 1.5.

Protein Binding >99%.

Abbott DC *et al.* (1981). Organochlorine pesticide residues in human fat in the United Kingdom 1976-7. *BMJ* 283: 1425–1428.
Dmitrovic J *et al.* (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicol Lett* 134: 253–258.
Garrettson LK, Curley A (1969). Dieldrin: studies in a poisoned child. *Arch Environ Health* 19: 814–822.
Hayes WJ, Curley A (1968). Storage and excretion of dieldrin and related compounds. Effect of occupational exposure. *Arch Environ Health* 16(2): 155–162.
Hunter CG *et al.* (1969). Pharmacodynamics of dieldrin (HEOD). Ingestion by human subjects for 18 to 24 months, and postexposure for eight months. *Arch Environ Health* 18: 12–21.
Saady JJ, Poklis A (1990). Determination of chlorinated hydrocarbon pesticides by solid-phase extraction and capillary GC with electron capture detection. *J Anal Toxicol* 14(5): 301–304.
Steentoft A (1979). A case of fatal dieldrin poisoning. *Med Sci Law* 19: 268–269.

Dienestrol

Oestrogen

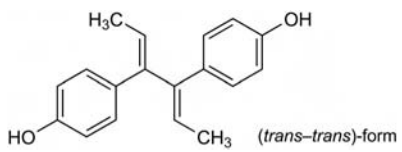
$C_{18}H_{18}O_2$ = 266.3

CAS—84-17-3; 13029-44-2 (*E*,*E*-)

IUPAC Name 4-[(2*E*,4*E*)-4-(4-Hydroxyphenyl)hexa-2,4-dien-3-yl]phenol

Synonyms Dehydrostilbestrol; dienoestrol; 4,4′-(1,2-diethylidene-1,2-ethanediyl)bisphenol; oestrodienolum.

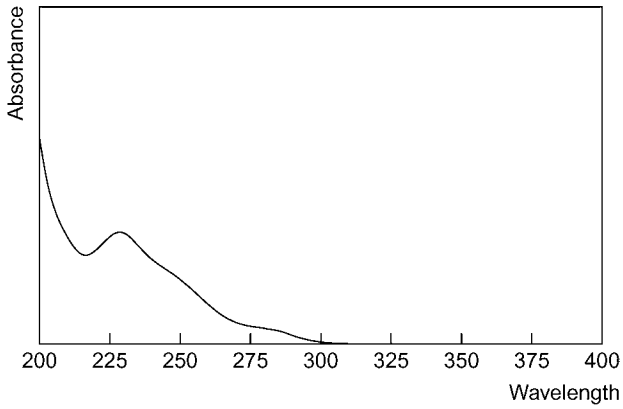
Proprietary Names *Cycladiene*; *DV*; *Estraguard*; *Hormofemin*.



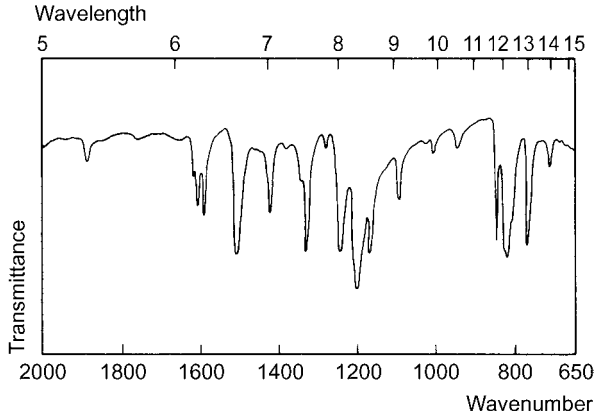
Chemical Properties White crystals or crystalline powder. Mp 227° to 234°. Practically insoluble in water; soluble 1 in 8 of ethanol, 1 in 5 of acetone and 1 in 15 of ether; freely soluble in propylene glycol; soluble in chloroform and aqueous solutions of alkali hydroxides. Log *P* (octanol/water), 5.3.

Caution Dienestrol is a powerful oestrogen. Contact with the skin or inhalation should be avoided.

Colour Tests Antimony pentachloride—red; naphthol–sulfuric acid—orange-brown/yellow; sulfuric acid—orange-red; dissolve about 0.5 mg in 0.2 mL of acetic acid, add 1 mL of phosphoric acid, and heat on a water-bath for 3 min—violet, becoming slightly more blue on dilution with 3 mL of acetic acid (this test distinguishes dienestrol from diethylstilbestrol, which produces a yellow colour).
Thin-layer Chromatography System TP—R_f 0.72; system TQ—R_f 0.25; system TR—R_f 0.34; system TS—R_f 0.05, streaking may occur.
High Performance Liquid Chromatography System HY—RI 535.
Ultraviolet Spectrum Principal peak at 230 nm.



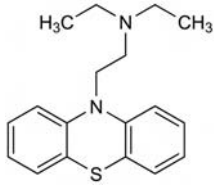
Infrared Spectrum Principal peaks at wavenumbers 1206, 827, 1173, 1510, 1248, 775 cm^{-1} (KBr disk).



Disposition in the Body Readily absorbed after oral administration. It is excreted in the urine mainly as a glucuronide conjugate.
Dose Dienestrol has been given orally in doses of 0.5 to 30 mg daily. It is used as a 0.01 to 0.025% vaginal cream.

Diethazine

Anticholinergic
$C_{18}H_{22}N_2S$ = 298.4
CAS—60-91-3
IUPAC Name *N*,*N*-Diethyl-10*H*-phenothiazine-10-ethanamine
Synonyms Eazamine; RP-2987.

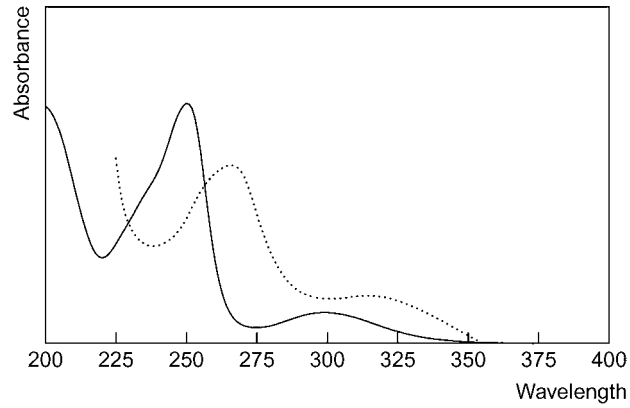


Chemical Properties An oily liquid. pK_a 9.1 (20°). Log *P* (octanol/water), 5.9.

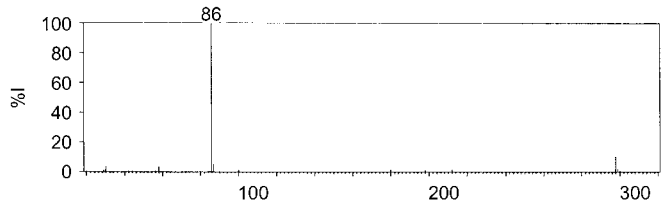
Diethazine Hydrochloride
$C_{18}H_{22}N_2S$,HCl = 334.9
CAS—341-70-8

Chemical Properties A white or slightly cream-coloured crystalline powder. Mp 184° to 186°. Soluble 1 in 5 of water, 1 in 6 of ethanol and 1 in 5 of chloroform; practically insoluble in ether.

Colour Tests Formaldehyde–sulfuric acid—red-violet; Forrest reagent—red; FPN reagent—orange; Mandelin's test—green→violet (excess reagent); Marquis test—violet-brown.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.57; system TC—R_f 0.51; system TE—R_f 0.77; system TL—R_f 0.39; system TAE—R_f 0.33; system TAF—R_f 0.54 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2377.
High Performance Liquid Chromatography System HA—*k* 3.4; system HAX—retention time 15.1 min; system HAY—retention time 7.4 min.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1=1200b), 298 nm; aqueous alkali—265 nm.



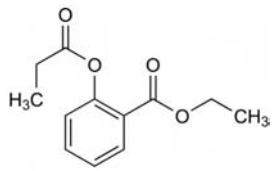
Infrared Spectrum Principal peaks at wavenumbers 748, 1245, 1587, 1282, 1562, 1219 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 298, 87, 30, 58, 299, 212, 180.



Dose Diethazine hydrochloride has been given in doses of 0.15 to 1.5 g daily.

Diethyl Phthalate

Plasticiser, Solvent
$C_{12}H_{14}O_4$ = 222.2
CAS—84-66-2
IUPAC Name Diethyl-1,2-benzenedicarboxylate
Synonym Ethyl phthalate

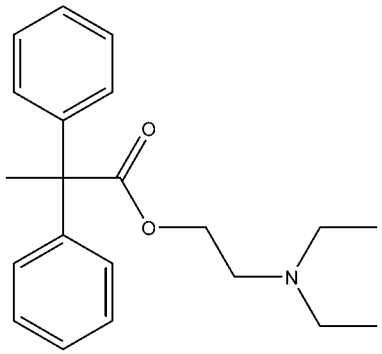


Chemical Properties A clear, colourless, somewhat viscous liquid. Mass per mL about 1.117 g. Bp about 295°. Refractive index 1.500 to 1.505. Practically insoluble in water; miscible with ethanol and ether. Log *P* (octanol/water), 2.4.
Gas Chromatography System GA—RI 1564.
Infrared Spectrum Principal peaks at wavenumbers 1733, 1287, 1086, 1132, 744, 1044 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 149, 177, 150, 65, 76, 105, 176, 104.

Diethylaminoethyl Diphenylpropionate

Parasympatholytic
$C_{21}H_{27}NO_2$ = 325.5

IUPAC Name 2-diethylaminoethyl 2,2-diphenylpropanoate
Synonyms Aprophen; diethylaminoethyl diphenylpropionate; 2-diethylaminoethyl αβ-diphenylpropionate.



Diethylaminoethyl Diphenylpropionate Hydrochloride

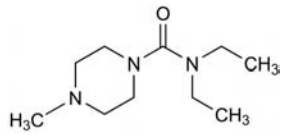
Proprietary Names *Spasmadryl*. It is an ingredient of *Sedalby tablets*.
Chemical Properties White crystalline powder. Mp 111° to 112°. Very soluble in water, soluble in ethanol, and sparingly soluble in ether.

Colour Test Vitali's test— -/-/purple (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.64 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid, maxima at 252 (E1%, 1 cm 4.3) and 264.5 nm (E1%, 1 cm 3.2).
Disposition in the Body In rats, intravenously administered aprophen is rapidly cleared from the blood, and with a half-life of 4 min [Brown *et al.* 1993].

Brown ND *et al.* (1993). Synthesis and antimuscarinic activity of 2-[*N*-(ethyl)-(*N*-beta-hydroxyethyl)]aminoethyl 2,2-diphenylpropionate, a metabolite of aprophen. *J Pharm Sci* 82: 563–564.

Diethylcarbamazine

Antifilarial
$C_{10}H_{21}N_3O$ = 199.3
CAS—90-89-1
IUPAC Name *N*,*N*-Diethyl-4-methyl-1-piperazinecarboxamide

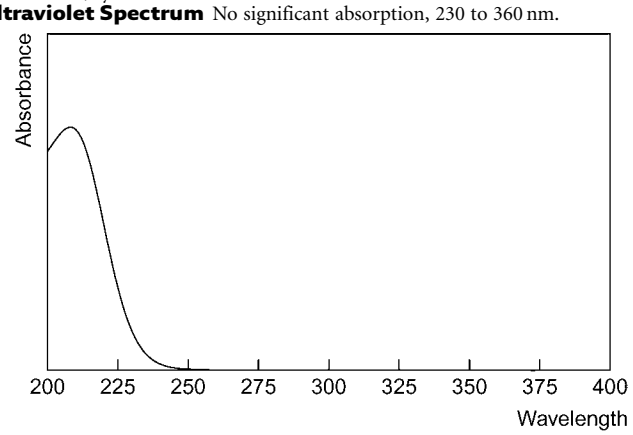


Chemical Properties A crystalline powder. Mp 47° to 49°. Soluble in water, ethanol, chloroform and ether. pK_a 7.7 (20°). Log *P* (octanol/water), 0.4.

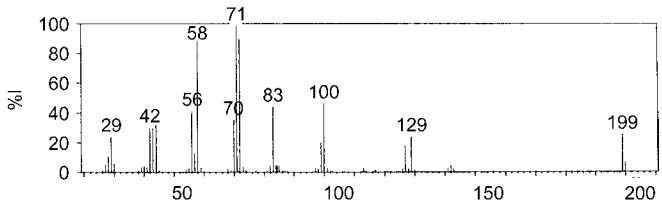
Diethylcarbamazine Citrate

$C_{10}H_{21}N_3O,C_6H_8O_7$ = 391.4
CAS—1642-54-2
Synonyms Diethylcarbamazine acid citrate; ditrazini citras.
Proprietary Names *Banocide*; *Hetrazan*; *Notézine*.
Chemical Properties A white, crystalline, slightly hygroscopic powder. Mp 141° to 143°. Freely soluble in water; soluble 1 in 35 of ethanol; practically insoluble in acetone, benzene, chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.17; system TC—R_f 0.26; system TL—R_f 0.05 (Dragendorff spray, positive).
Gas Chromatography System GA—RI 1497.
High Performance Liquid Chromatography System HA—*k* 1.4; system HX—RI 219; system HY—RI 192.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1618, 1258, 1217, 1712, 1105, 1149 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 71, 72, 58, 100, 83, 56, 70, 44.



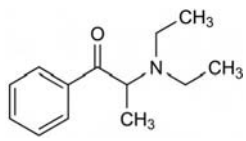
Quantification

Plasma **GC** FID. Limit of quantification, 70 µg/L, limit of detection, 60 µg/L [Miller, Fleckenstein 2001]. NSD. Limit of detection, 4 µg/L [Lee *et al.* 1997]. AFID. Limit of detection, 10 µg/L [Allen *et al.* 1979].
Urine **GC** See Plasma [Allen *et al.* 1979].
Biological fluids **Immunoassay** For method, see Mitsui *et al.* [1996].
Disposition in the Body Readily absorbed after oral administration. About 50% of a dose is excreted in the urine as unchanged drug and 10% as the *N*-oxide metabolite in 48 h; the excretion of unchanged drug is pH-dependent, being increased in acidic urine (pH 5) but decreasing to less than 10% in alkaline urine (pH 8).
Therapeutic Concentration
Following a single oral dose of 50 mg to 5 subjects, peak plasma concentrations of 0.08 to 0.20 mg/L were attained in about 1 to 2 h; a secondary rise in plasma concentrations was observed at 3 to 9 h [Edwards *et al.* 1981].
Half-life Plasma half-life, about 10 h under alkaline urinary pH conditions or 3 h if an acidic urinary pH is maintained.
Protein Binding Not significantly bound.
Dose 1 to 6 mg/kg of diethylcarbamazine citrate daily, orally.

Allen GD *et al.* (1979). Determination of 1-diethylcarbamoyl-4-methylpiperazine (diethylcarbamazine) in human plasma and urine. *J Chromatogr* 164(4): 521–526.
Edwards G *et al.* (1981). Diethylcarbamazine disposition in patients with onchocerciasis. *Clin Pharmacol Ther* 30: 551–557.
Lee S *et al.* (1997). Specific gas chromatographic analysis of diethylcarbamazine in human plasma using solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 704(1–2): 181–185.
Miller *Jnr.*JR, Fleckenstein L (2001). Gas chromatographic assay of diethylcarbamazine in human plasma for application to clinical pharmacokinetic studies. *J Pharm Biomed Anal* 26: 665–674.
Mitsui Y *et al.* (1996). Development of a competitive enzyme-linked immunosorbent assay for diethylcarbamazine. *Trop Med Int Health* 1(4): 528–534.

Diethylpropion

Anorectic
$C_{13}H_{19}NO$ = 205.3
CAS—90-84-6
IUPAC Name 2-(Diethylamino)-1-phenyl-1-propanone
Synonym Amfepramone



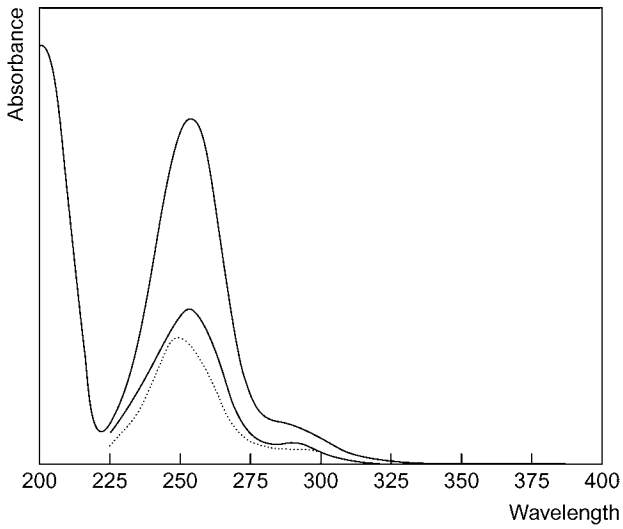
Chemical Properties Log *P* (octanol/water), 3.04. A white to off-white, fine, crystalline powder. Mp 168°, with decomposition. Soluble 1 in 0.5 of water, 1 in 3 of ethanol, and 1 in 3 of chloroform; practically insoluble in ether.

Diethylpropion Hydrochloride

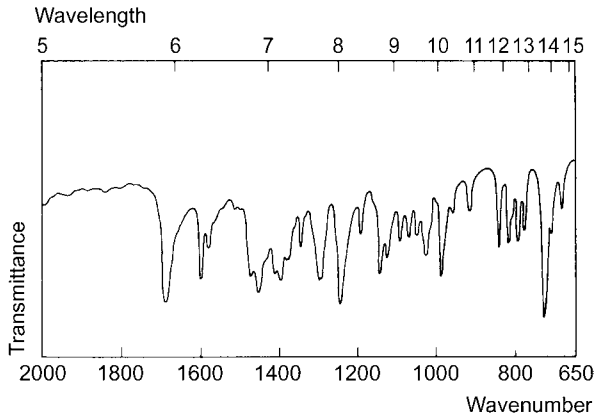
$C_{13}H_{19}NO,HCl$ = 241.8
CAS—134-80-5
Proprietary Names *Anorex*; *Atractil*; *Dobesin*; *Dietec*; *D.I.P.*; *Dualid S*; *Hipofagin S*; *Ifa Norex*; *Inibex S*; *Moderatan Diffucap*; *Neobes*; *Prefamone*; *Regenon*; *Tenuate Dospan*. The name *Tepanil* is also applied to phenylpropanolamine hydrochloride.

Colour Test Liebermann's reagent—yellow.
Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.62; system TC—R_f 0.63; system TE—R_f 0.85; system TL—R_f 0.64; system TAE—R_f 0.55; system TAF—R_f 0.56; system TAJ—R_f 0.44; system TAK—R_f 0.02; system TAL—R_f 0.35 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, violet-brown).
Gas Chromatography System GA—diethylpropion RI 1486, M (phenylpropanolamine) RI 1360; system GB—diethylpropion RI 1532, M (phenylpropanolamine) RI 1352, M (diethylnorephedrine) RI 1599, M (ethylnorephedrine) RI 1457, M (*N*-desethyl-) RI 1423, M (*N*-didesethyl-) RI 1338; system GC—diethylpropion RI 1715, M (norephedrine) RI 1383; system GF—diethylpropion RI 1655.
High Performance Liquid Chromatography System HA—diethylpropion *k* 1.7, M (norephedrine) *k* 0.9; system HC—diethylpropion *k* 0.16, M (norephedrine) *k* 0.70; system HY—RI 230; system HZ—retention time 2.8 min.

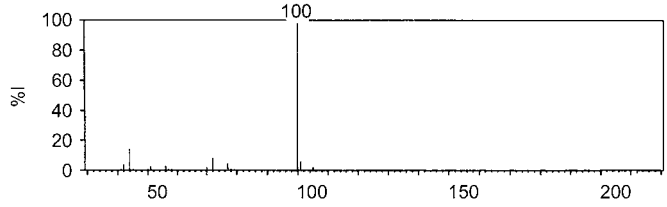
Ultraviolet Spectrum Aqueous acid—253 nm (A_1^1=673a); aqueous alkali—246 nm.



Infrared Spectrum Principal peaks at wavenumbers 701, 1230, 1682, 1287, 973, 1594 cm^{-1} (diethylpropion hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 100, 44, 72, 101, 77, 56, 42, 105 (no peaks above 110); norephedrine 44, 77, 79, 51, 45, 42, 107, 105.



Quantification

Plasma **GC–MS** Limit of detection, 600 ng/L [Wright *et al.* 1975].

HPLC For method of quantification for diethylpropion enantiomers, see Mey *et al.* [1998].

Urine **GC** FID. Limit of detection, 300 µg/L, diethylpropion and metabolites [Testa, Beckett 1972].

Disposition in the Body Readily absorbed after oral administration. Metabolised by *N*-dealkylation, reduction, deamination and *N*-hydroxylation primarily to active metabolites; keto reduction is stereoselective, resulting in the formation of *threo*-hydroxylated metabolites; glucuronide formation also occurs along with the formation of hippuric and mandelic acids. About 80 to 90% of a dose is excreted in the urine. The amount excreted in the urine is reduced when the urine is alkaline. Of the urinary excreted material, *N*-ethylaminopropiophenone, norephedrine (phenylpropanolamine) and hippuric acid are the main metabolites together with small amounts of unchanged drug, aminopropiophenone, *N*-diethylnorephedrine and *N*-ethylnorephedrine. Diethylpropion crosses the blood–brain barrier and the placenta. The drug and its metabolites are distributed into breast milk.

Therapeutic Concentration

Following a single oral dose of 75 mg to 5 subjects, a mean peak plasma concentration of 0.007 mg/L was attained in 0.5 h; total concentrations of the monodesethyl and didesethyl metabolites reached an average peak of 0.19 mg/L at 2 h [Wright *et al.* 1975].

Toxicity The estimated minimum lethal doses are 200 mg for a child and 2 g for an adult.

The following disposition was reported in a case of fatal overdose resulting from the injection of illicit diethylpropion tablets: blood 5.4 mg/L, bile 14.4 mg/L, kidney 0.9 µg/g, liver 0.9 µg/g, injection site 43.2 µg/g [Fysh, Taylor 1978].

Half-life Derived from urinary excretion data, 1.5 to 3 h in subjects whose urine is acidic.

Dose Usually 75 mg of diethylpropion hydrochloride daily.

Fysh RR, Taylor JF (1978). *Bull Int Assoc Forensic Toxicol* 14(2): 16–17.

Mey B *et al.* (1998). Kinetics of racemization of (+)- and (–)-diethylpropion: studies in aqueous solution, with and without the addition of cyclodextrins, in organic solvents and in human plasma. *Chirality* 10: 307–315.

Testa B, Beckett AH (1972). Studies on the metabolism of diethylpropion. I. Analytical procedure. *J Chromatogr* 71: 39–54.

Wright GJ *et al.* (1975). The objective and timing of drug disposition studies, appendix III. Diethylpropion and its metabolites in the blood plasma of the human after subcutaneous and oral administration. *Drug Metab Rev* 4: 267–276.

Diethylstilbestrol

Antineoplastic (Hormonal)

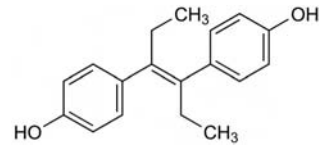
$C_{18}H_{20}O_2$ = 268.4

CAS—56-53-1

IUPAC Name 4-[(*E*)-4-(4-Hydroxyphenyl)hex-3-en-3-yl]phenol

Synonyms DES; 4,4′[(*1E*)-1,2-diethyl-1,2-ethenediyl]bisphenol; diethylstilboestrol; stilbol.

Proprietary Names *Apstil*; *Distilbene*.



Chemical Properties A white crystalline powder. Mp 169° to 172°. Practically insoluble in water; soluble 1 in 5 of ethanol, 1 in 200 of chloroform and 1 in 3 of ether; soluble in acetone and methanol; soluble in fatty oils and alkali hydroxides.

Caution Diethylstilbestrol is a powerful oestrogen. Contact with the skin or inhalation should be avoided.

Diethylstilbestrol Diphosphate

$C_{18}H_{22}O_8P_2$ = 428.3

CAS—522-40-7

Synonyms Fosfestrol; Phosphoestrolum.

Proprietary Names *Honvan*; *Stilphostrol (tablets)*.

Note Fosfestrol Sodium (the tetra-sodium salt of diethylstilbestrol diphosphate) is available under the proprietary names *Honvan*, *Honvol*, *Ronvan*, *ST-52* and *Stilphostrol* (injection).

Chemical Properties An off-white crystalline powder which decomposes at about 204° to 206°. Sparingly soluble in water; soluble in ethanol and dilute alkalis. Log *P* (octanol/water), 5.1.

Diethylstilbestrol Dipropionate

$C_{24}H_{28}O_4$ = 380.5

CAS—130-80-3

Proprietary Names *Cyren B*; *Dibestil*.

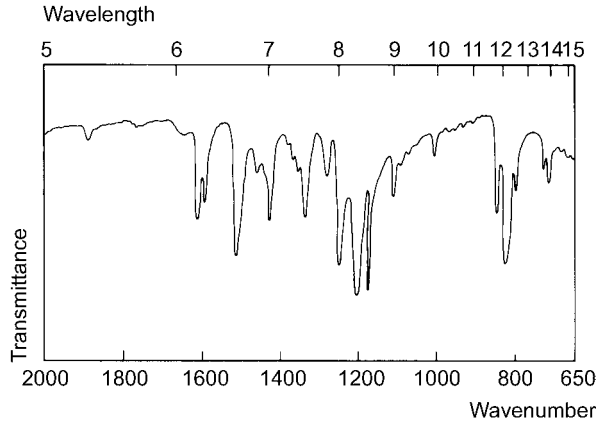
Chemical Properties Colourless crystals or white crystalline powder. Mp 104°. Slightly soluble in water and dilute mineral acids; soluble 1 in 100 of ethanol (90%) and 1 in 6 of ether; freely soluble in acetone and chloroform.

Colour Tests Antimony pentachloride—red; naphthol–sulfuric acid—yellow/orange; sulfuric acid—orange; dissolve about 0.5 mg in 0.2 mL of acetic acid, add 1 mL of phosphoric acid acid, and heat on a water-bath for 3 min—yellow, disappearing on dilution with 3 mL of acetic acid. (This test distinguishes diethylstilbestrol from dienestrol, which produces a violet colour.)

Thin-layer Chromatography System TB—R_f 0.03; system TE—R_f 0.73; system TF—R_f 0.68; system TP—R_f 0.65, streaking may occur; system TQ—R_f 0.10, streaking may occur; system TR—R_f 0.18, streaking may occur; system TS—R_f 0.03; system TAE—R_f 0.92; system TAF—R_f 0.92.

Gas Chromatography System GA—RI 2298.

High Performance Liquid Chromatography System HX— 592; system HY—RI 559; system HAA—retention time 20.9 min.
Ultraviolet Spectrum Ethanol—241 nm (A^1_1=600a).
Infrared Spectrum Principal peaks at wavenumbers 1205, 1176, 833, 1250, 1512, 1610 cm^{-1} (KBr disk).

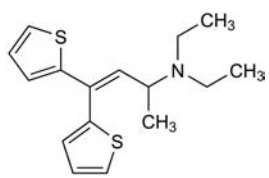


Mass Spectrum Principal ions at *m/z* 107, 145, 268, 238, 121, 133, 159, 224.
Quantification
Blood **GC** FID. For method, see Kohrman and MacGee [1977].
Plasma **GC** See Blood [Kohrman, MacGee 1977].
HPLC UV detection. For method, see Newport *et al.* [1980].
Serum **GC** See Blood [Kohrman, MacGee 1977].
Urine **HPLC** See Plasma [Newport *et al.* 1980].
Bile **GC** See Blood [Kohrman, MacGee 1977].
Tissues **GC** See Blood [Kohrman, MacGee 1977].
HPLC See Plasma [Newport *et al.* 1980].
Dose 0.1 to 2 mg daily; doses of 15 mg daily may be given.

Kohrman KA, MacGee J (1977). Simple and rapid gas-liquid chromatographic determination of diethylstilbestrol in biological specimens. *J Assoc Off Anal Chem* 60: 5–8.
Newport GD *et al.* (1980). *J Liq Chromatogr* 3: 1053–1070.

Diethylthiambutene

Narcotic Analgesic (Veterinary)
$C_{16}H_{21}NS_2$ = 291.5
CAS—86-14-6
IUPAC Name 7-[Pyridin-1-ium-3-yl(pyrrolidin-1-ium-1-yl)methyl]quinolin-1-ium-8-ol
Synonyms *N*,*N*-Diethyl-1-methyl-3,3-di-2-thienylallylamine; thiambutene; 191C49.

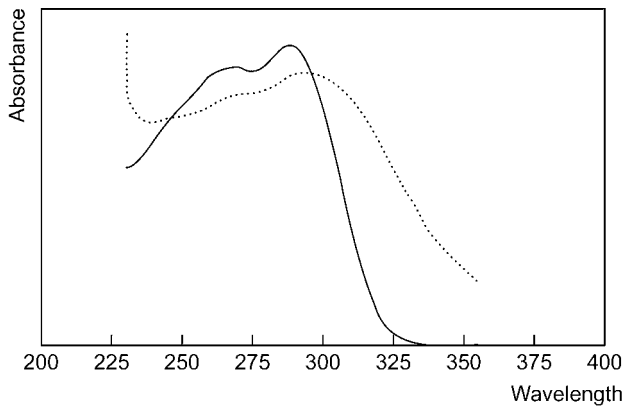


Chemical Properties Log *P* (octanol/water), 4.5.

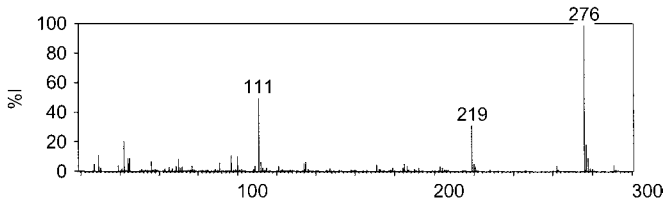
Diethylthiambutene Hydrochloride

$C_{16}H_{21}NS_2$,HCl = 327.9
CAS—132-19-4
Chemical Properties A white crystalline powder. Mp 152° to 153°. Soluble 1 in 2 of water and 1 in 1 of ethanol; practically insoluble in ether.

Colour Tests Liebermann's reagent (100°)—orange; Mandelin's test—green→ green-blue; Marquis test—violet; sulfuric acid—orange; cold nitric acid gives a pink-brown colour changing to green.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.60; system TC—R_f 0.43; system TE—R_f 0.81; system TL—R_f 0.43; system TAE—R_f 0.40 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, red-violet).
Gas Chromatography System GA—RI 2008.
High Performance Liquid Chromatography System HA—*k* 2.0.
Ultraviolet Spectrum Aqueous acid—268, 288 nm (A^1_1=362a); aqueous alkali—295 nm.

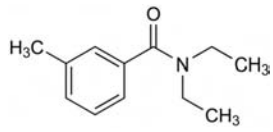


Infrared Spectrum Principal peaks at wavenumbers 716, 743, 851, 833, 1244, 1618 cm^{-1} (diethylthiambutene hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 276, 111, 219, 42, 277, 97, 29, 100.



Diethyltoluamide

Insect Repellent
$C_{12}H_{17}NO$ = 191.3
CAS—134-62-3
IUPAC Name *N*,*N*-Diethyl-3-methylbenzamide
Synonym DEET
Proprietary Names *Autan*; *Detamide*; *Dieltamid*; *Flypel*; *Metadelphene*; *Off*; *Repel*.



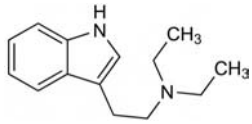
Chemical Properties A colourless or faintly yellow liquid. Weight per mL 0.997 to 1.000 g. Practically insoluble in water; freely soluble in ethanol, benzene, chloroform and ether; sparingly soluble in petroleum ether. Log *P* (octanol/water), 2.2.
Thin-layer Chromatography System TA—R_f 0.73 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1583.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1629, 1284, 794, 1305, 1581, 1095 cm^{-1} (KBr disk).
Quantification
Urine **GC–MS** For method for quantification of diethyltoluamide and metabolites, see Wu *et al.* [1979].
Disposition in the Body
Toxicity
Two patients ingested large amounts of a concentrated (between 47.5 and 95%) insect repellent containing DEET. Serum concentrations were 168 mg/L and 240 mg/L [Tenenbein 1987].
Use As a 50 to 75% solution.

Tenenbein M (1987). Severe toxic reactions and death following the ingestion of diethyltoluamide-containing insect repellents. *JAMA* 258(11): 1509–1511.
Wu A *et al.* (1979). *J High Resolut Chromatogr Chromatogr Commun* 2: 558–562.

Diethyltryptamine

Hallucinogen
$C_{14}H_{20}N_2 = 216.3$
CAS—61-51-8

IUPAC Name *N*,*N*-Diethyl-2-(1*H*-indol-3-yl)ethanamine
Synonyms DET; *N*,*N*-diethyl-1*H*-indole-3-ethanamine; *N*,*N*-diethyltryptamine.

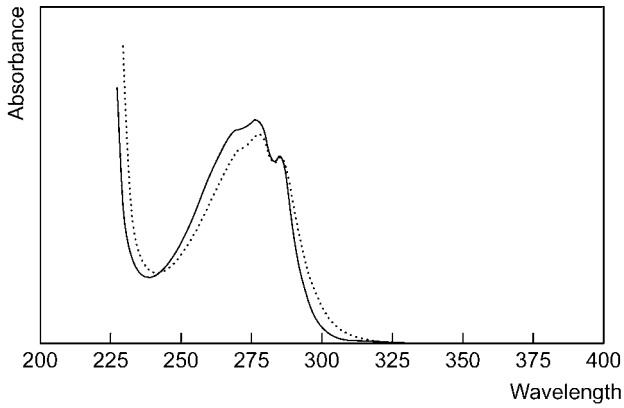


Chemical Properties An orange oily liquid. Soluble in ethanol and chloroform.

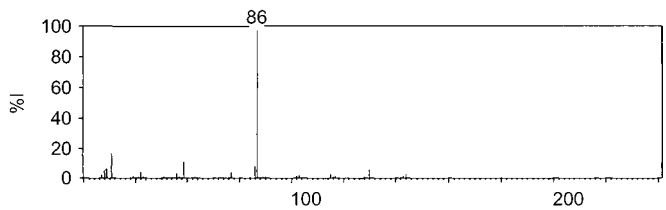
Diethyltryptamine Hydrochloride

Chemical Properties A white crystalline powder. Mp 87° to 89°. Soluble in water.

Colour Tests Mandelin's test—grey-green→yellow; Marquis test—yellow→brown.
Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.15; system TC—R_f 0.10; system TE—R_f 0.63; system TL—R_f 0.11; system TAE—R_f 0.14; system TAF—R_f 0.56; system TAJ—R_f 0.02; system TAK—R_f 0.03; system TAL—R_f 0.41 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—brown).
Gas Chromatography System GA—RI 1910.
High Performance Liquid Chromatography System HY—RI 276.
Ultraviolet Spectrum Aqueous acid—278 (A^1_1= 270a), 287 nm.

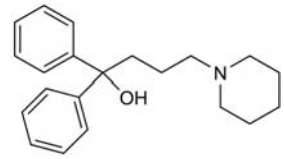


Infrared Spectrum Principal peaks at wavenumbers 740, 1111, 1063, 1086, 1234, 724 cm^{-1}.
Mass Spectrum Principal ions at *m*/*z* 86, 30, 58, 29, 130, 87, 77, 42



Note For a report on the effects and metabolism of diethyltryptamine, see Szara *et al.* [1966]. For a method of separating and detecting tryptamines in urine including diethyltryptamine, see Wang *et al.* [2009].

Szara S *et al.* (1966). Psychological effects and metabolism of *N*,*N*-diethyltryptamine in man. *Arch Gen Psychiatry* 15: 320–329.
Wang MJ *et al.* (2009). Optimization of separation and online sample concentration of *N*,*N*-dimethyltryptamine and related compounds using MEKC. *J Sep Sci* 32: 441–445.

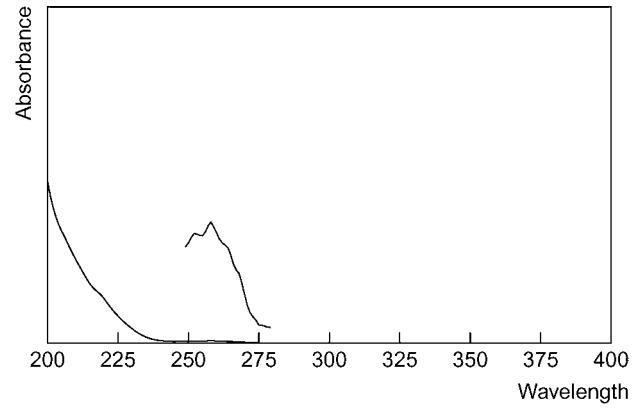


Chemical Properties Mp 103° to 104°. Log *P* (octanol/water), 4.7.

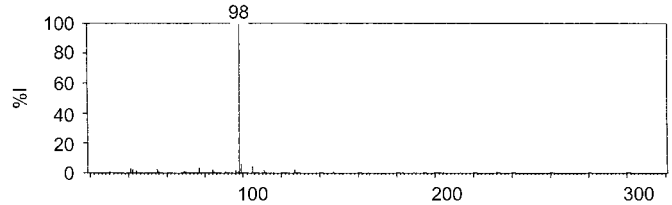
Difenidol Hydrochloride

$C_{21}H_{27}NO,HCl = 345.9$
CAS—3254-89-5
Proprietary Names *Cephadol*; *Normavom*; *Vontril*; *Vontrol*.
Chemical Properties A white crystalline powder. Mp 214° to 221°, with decomposition. Soluble in water, ethanol, and chloroform; freely soluble in methanol; practically insoluble in ether, benzene, and petroleum ether.

Colour Tests Liebermann's reagent—brown; Mandelin's test—yellow; Marquis test—yellow; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.56; system TC—R_f 0.45; system TE—R_f 0.91; system TL—R_f 0.51; system TAJ—R_f 0.24; system TAK—R_f 0.13; system TAL—R_f 0.67 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2384.
High Performance Liquid Chromatography System HAX—retention time 12.1 min; system HAY—retention time 6.1 min.
Ultraviolet Spectrum Aqueous acid—252, 258 nm.



Infrared Spectrum Principal peaks at wavenumbers 697, 701, 749, 773, 1123, 1219 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m*/*z* 98, 99, 105, 77, 55, 41, 127, 111.



Disposition in the Body Absorbed from the gastro-intestinal tract; peak blood concentrations are attained in 1.5 to 3 h. It is excreted in the urine and faeces.
Toxicity

Analysis of 21 cases of difenidol overdose showed that the average amount ingested was 222.5 mg (range, 25 to 800); adults ingested 150 to 700 mg, children ingested 25 to 800 mg. Toxicity was severe in 4 children, one of whom died (a $2^1/_2$-year-old boy who ingested 225 mg (15 mg/kg)) [Yang, Deng 1998.]

Dose The equivalent of 25 to 50 mg of difenidol every 4 h; maximum of 300 mg daily.

Yang CC, Deng JF (1998). Clinical experience in acute overdosage of diphenidol. *J Toxicol Clin Toxicol* 36: 33–39.

Difenidol

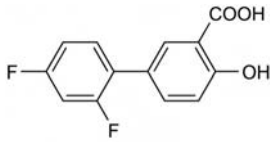
Antiemetic
$C_{21}H_{27}NO = 309.4$
CAS—972-02-1

IUPAC Name α,α-Diphenyl-1-piperidinebutanol
Synonym Diphenidol
Proprietary Names *Dicavin*; *Hemitiken*; *Laudifen*; *Nautrol*; *Normitrol*; *Serratol*; *Vernausin*.

Diflunisal

Analgesic
$C_{13}H_8F_2O_3 = 250.2$
CAS—22494-42-4

IUPAC Name 5-(2,4-Difluorophenyl)-2-hydroxybenzoic acid
Synonym 2′,4′-Difluoro-4-hydroxy-[1,1′-biphenyl]-3-carboxylic acid
Proprietary Names *Aflogos*; *Artrodol*; *Biartac*; *Difludol*; *Diflusal*; *Dolobid*; *Dolobis*; *Donobid*; *Fluniget*.



Chemical Properties White crystals or crystalline powder. Mp about 212°. Sparingly soluble in water; soluble in ethanol, ether, and dilute alkalis. Log *P* (octanol/water), 4.4.

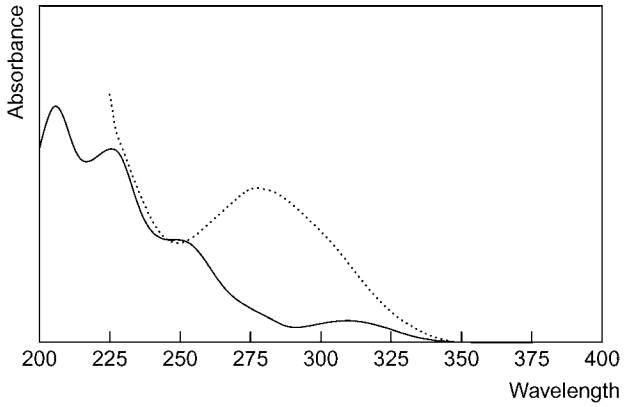
Colour Tests Ferric chloride—violet; Folin–Ciocalteu reagent—blue; McNally's test—violet

Thin-layer Chromatography System TD—R_f 0.08; system TE—R_f 0.16; system TF—R_f 0.05; system TG—R_f 0.37; system TAD—R_f 0.18; system TAE—R_f 0.89; system TAJ—R_f 0.06; system TAK—R_f 0.69; system TAL—R_f 0.69 (chromic acid solution, blue-grey).

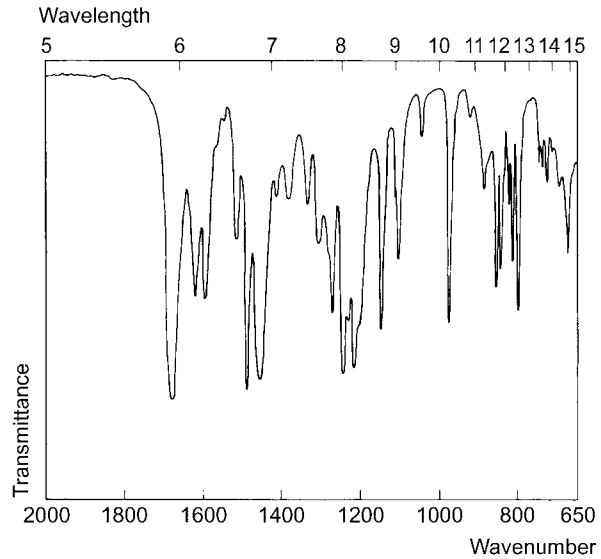
Gas Chromatography System GA—diflunisal RI 2095, diflunisal-Me RI 2050, diflunisal-Me_2 RI 1990; system GD—RRT 1.20 of methyl derivative (relative to n-$C_{16}H_{34}$).

High Performance Liquid Chromatography System HD—*k* 4.1; system HV—RRT 0.77 (relative to meclofenamic acid); system HX—RI 508; system HY—RI 583; system HZ—retention time 5.4 min; system HAX—retention time 5.9 min; system HAY—retention time 6.0 min.

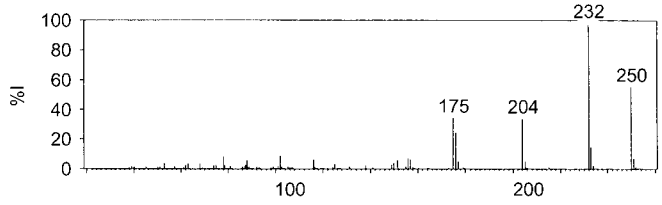
Ultraviolet Spectrum Acid methanol—251 (A^1_1=560a), 315 nm (A^1_1=130a); aqueous alkali—277 nm.



Infrared Spectrum Principal peaks at wavenumbers 1683, 1492, 1248, 1220, 1150, 978 cm^{-1} (KBr disk). Two polymorphic forms may occur.



Mass Spectrum Principal ions at *m/z* 232, 250, 175, 204, 176, 233, 102, 78.



Quantification

Plasma **GC** FID. Limit of detection, 1 mg/L [Tocco *et al.* 1975].

HPLC UV detection. For method for quantification of diflunisal and other NSAIDs in overdose, see Streete [1989]. Fluorescence detection. Limit of detection, 50 µg/L [Ray, Day 1983].

Spectrofluorimetry Limit of detection, 500 ng [Tocco *et al.* 1975].

Serum **HPLC** See Plasma [Streete 1989]. For method for quantification of diflunisal and its glucuronides, see Hansen-Moller *et al.* [1987].

Urine **GC** See Plasma [Tocco *et al.* 1975].

GC–MS For method for quantification of diflunisal and some NSAIDs, see el Haj *et al.* [1999].

HPLC Fluorescence detection. Diflunisal and its glucuronide and sulfate conjugates. Limit of detection, 0.1 mg/L for diflunisal [Loewen *et al.* 1989]. See Serum [Hansen-Moller *et al.* 1987]. For method for quantification of diflunisal glucuronides, see Musson *et al.* [1985]. See Plasma [Ray, Day 1983].

Disposition in the Body Well absorbed after oral administration. Up to about 80% of a dose is excreted in the urine in 24 h, mostly as the ester and ether glucuronides. <3% of a dose is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 500 mg given to 5 subjects, peak plasma concentrations of 58 to 92 mg/L (mean 77) were attained in 2 h [Verbeeck *et al.* 1979].

Following oral administration of 500 mg twice daily to 6 subjects, steady-state plasma concentrations of 66 to 183 mg/L were reported [Wählin-Boll *et al.* 1981].

After administration of diflunisal as either a single oral dose of 1000 mg daily or two doses of 500 mg daily to 13 subjects, mean peak plasma concentrations of 186 and 150 mg/L, respectively, were achieved in 2.5 and 1.9 h. Steady-state plasma concentrations were 118 and 116 mg/L in the respective groups [Mojaverian *et al.* 1985].

Toxicity Diflunisal appears to be relatively non-toxic.

A 47-year-old woman went into a coma after ingesting 29 g of diflunisal but subsequently recovered uneventfully [Upadhyay, Gupta 1978].

In a fatality involving the ingestion of diflunisal, the following postmortem tissue concentrations were reported: blood 260 mg/L, bile 71 mg/L, kidney 350 µg/g, liver 400 µg/g, stomach contents 34 g, urine 78 mg/L [Levine *et al.* 1987].

Half-life Plasma half-life, about 5 to 12 h (dose-dependent).

Volume of Distribution About 0.1 L/kg.

Clearance Plasma clearance, about 0.1 mL/min/kg.

Protein Binding About 99%.

Note For a review of the pharmacokinetics of diflunisal, see Brogden *et al.* [1980].

Dose 0.5 to 1 g daily.

Brogden RN *et al.* (1980). Diflunisal: a review of its pharmacological properties and therapeutic use in pain and musculoskeletal strains and sprains and pain in osteoarthritis. *Drugs* 19: 84–106.

el Haj BM *et al.* (1999). The GC/MS analysis of some commonly used non-steriodal anti-inflammatory drugs (NSAIDs) in pharmaceutical dosage forms and in urine. *Forensic Sci Int* 105: 141–153.

Hansen-Moller J *et al.* (1987). Reversed-phase high-performance liquid chromatographic assay for the simultaneous determination of diflunisal and its glucuronides in serum and urine. Rearrangement of the 1-O-acylglucuronide. *J Chromatogr* 420: 99–109.

Levine B *et al.* (1987). A diflunisal related fatality: a case report. *Forensic Sci Int* 35: 45–50.

Loewen GR *et al.* (1989). High-performance liquid chromatographic method for the simultaneous quantitation of diflunisal and its glucuronide and sulfate conjugates in human urine. *J Pharm Sci* 78: 250–255.

Mojaverian P *et al.* (1985). Steady-state disposition of diflunisal: once- versus twice-daily administration. *Pharmacotherapy* 5(6): 336–339.

Musson DG *et al.* (1985). Assay methodology for quantification of the ester and ether glucuronide conjugates of diflunisal in human urine. *J Chromatogr* 337: 363–378.

Ray JE, Day RO (1983). High-performance liquid chromatographic analysis of diflunisal in plasma and urine: application to pharmacokinetic studies in two normal volunteers. *J Pharm Sci* 72: 1403–1405.

Streete PJ (1989). Rapid high-performance liquid chromatographic methods for the determination of overdose concentrations of some non-steroidal anti-inflammatory drugs in plasma or serum. *J Chromatogr* 495: 179–193.

Tocco DJ *et al.* (1975). Physiological disposition and metabolism of 5-(2′,4′-difluorophenyl)salicyclic acid, a new salicylate. *Drug Metab Dispos* 3: 453–466.

Upadhyay HP, Gupta SK (1978). Diflunisal (Dolobid) overdose. *BMJ* 2: 640.

Verbeeck R *et al.* (1979). Biotransformation of diflunisal and renal excretion of its glucuronides in renal insufficiency. *Br J Clin Pharmacol* 7: 273–282.

Wählin-Boll E *et al.* (1981). High-pressure liquid chromatographic determination of acetylsalicylic acid, salicylic acid, diflunisal, indomethacin, indoprofen and indobufen. *Eur J Clin Pharmacol* 20: 375–378.

Digitoxin

Cardiac Glycoside

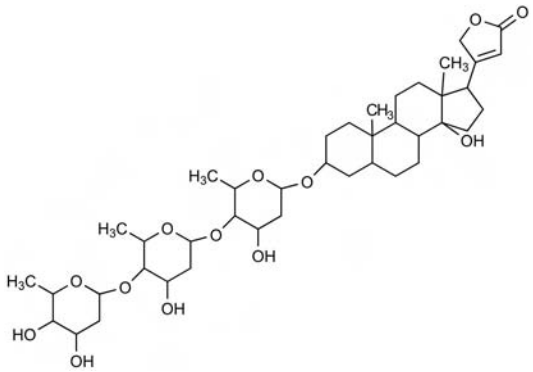
$C_{41}H_{64}O_{13}$ = 764.9

CAS—71-63-6

IUPAC Name (3β,5β)-3-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide

Synonyms Digitaline cristallisée; digitoxoside.

Proprietary Names *Asthenthilo*; *Coramedan*; *Crystodigin*; *Digimerck*; *Digitaline*; *Digitox*; *Digitrin*; *Ditaven*; *Nativelle*; *Purodigin*; *Tardigal*; *Variplastic*



Chemical Properties A glycoside obtained from suitable species of the genus *Digitalis* (Scrophulariaceae). A white or pale buff-coloured, microcrystalline powder. Mp ~256°. Practically insoluble in water; soluble 1 in 60 of ethanol, 1 in 40 of chloroform, 1 in 400 of ethyl acetate; sparingly soluble in ether, petroleum ether and methanol; freely soluble in a mixture of equal volumes of chloroform and methanol. Log *P* (octanol/water), 1.8.

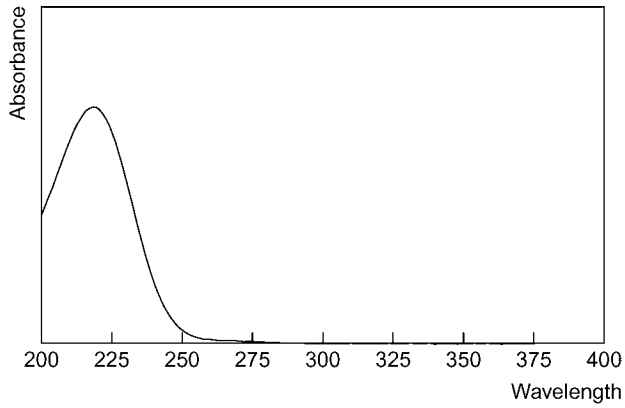
Colour Test Antimony pentachloride—yellow-brown-black-violet.

Thin-layer Chromatography System TAD—R_f 0.42; system TAE—R_f 0.88; system TD—R_f 0.03; system TE—R_f 0.36; system T_f—R_f 0.10; system TK—R_f 0.72 (perchloric acid solution, followed by examination under UV light, red fluorescence; *p*-anisaldehyde reagent, blue).

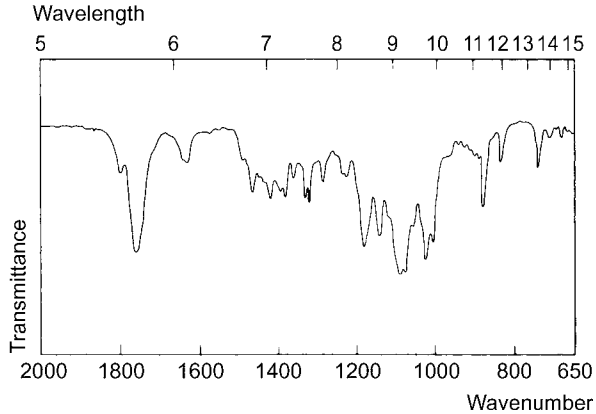
Gas Chromatography System GA—RI 1902.

High Performance Liquid Chromatography System HAA—RT 18.7 min; system HM—*k* 5.40; system HY—RI 468.

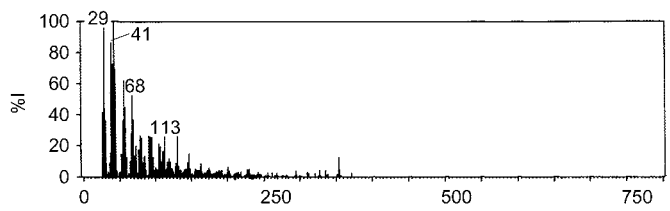
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1072, 1058, 1010, 1740, 1168, 990 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 29, 39, 41, 45, 57, 68, 58.



Quantification

Blood **HPLC** Column: Atlantis dC_{18} (50 × 2.1 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate (pH 3.1) and acetonitrile (100%), flow rate 0.3 mL/min [Oiestad *et al.* 2009]. Digoxin and other cardiac glycosides [Guan *et al.* 1999].

Plasma **HPLC** Colum: NovaPak C_{18} (150 × 2.0 mm i.d., 4 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate (pH 3.0, 1 : 3), flow rate 200 µL/min. Limit of detection, 0.15–0.6 µg/L [Tracqui *et al.* 1997].

Serum **TLC** Limit of detection, 1–2 ng [Faber 1977].

Note For a radioimmunoassay for the detection of digitoxin, see Nore *et al.* [1980]; for a fluoroimmunoassay, see Al Hakiem *et al.* [1982].

Urine **HPLC** Column: Nucleosil RP-5C_{18} (250 × 4 mm i.d., 5 µm). Mobile phase: methanol : water containing PIC B7 reagent (66 : 34 increasing the methanol by 0.4%/min for 5 min followed by 0.58%/min for 12 min followed by 75 : 25 for 8 min). UV detection (λ = 222 nm). Limit of detection, 0.5 mg/L [Santos *et al.* 1987]. See Blood [Guan *et al.* 1999].

Tissues **HPLC** Column: Nucleosil C_{18} (250 × 4.0 mm i.d., 10 µm). Mobile phase: acetonitrile : methanol : water (20 : 20 : 60), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: ~12 min. Limit of detection, 10 ng [Plum, Daldrup 1986].

Disposition in the Body Digitoxin is readily absorbed after oral administration; bioavailability is >90%. High concentrations are found in the kidney, ventricular myocardium, liver and skeletal muscle. It is extensively metabolised by hydrolysis to digitoxigenin bisdigitoxoside, digitoxigenin monodigitoxoside and digitoxigenin, and by hydroxylation to digoxin and the corresponding digoxigenin derivatives, all of which are active. Epimerisation to the inactive metabolites epidigitoxigenin and epidigoxigenin, followed by glucuronide conjugation, also occurs; dihydrodigitoxin has also been detected in plasma. Approximately 60–80% of a single dose is excreted in the urine over a period of 3 weeks, mainly as metabolites, and up to 20% is eliminated in the faeces. The excretion of digitoxin and its active and inactive metabolites is very variable and is independent of dose and route of administration. Approximately 20–50% of a dose appears to be excreted as unchanged drug in urine and faeces in variable proportions, and the amount of unchanged drug in the urine has been reported to be greater during maintenance treatment than in single-dose studies. Digitoxin is a metabolite of acetyldigitoxin.

Therapeutic Concentration In serum, usually in the range 0.01–0.03 mg/L.

After a single oral dose of 20 µg/kg, given to 6 children, peak serum concentrations of 0.02–0.05 mg/L were attained in 1.5–2.5 h [Larsen, Storstein 1983].

Following daily oral doses of 0.1 mg to 7 subjects, steady-state serum concentrations of 0.009–0.026 mg/L (mean 0.017–mg/L) were reported [Haustein 1981].

Toxicity The estimated minimum lethal dose is 3 mg. Toxic effects are usually associated with serum concentrations of ~0.03 mg/L or more.

In a 65-year-old woman who died almost 15 h after ingesting 7 mg digitoxin, the plasma concentration just before death was 0.212 mg/L [Krappweis *et al.* 1996].

Following a massive digitoxin overdose in a 20-month-old girl, an initial serum level of 0.629 mg/L was reported. After 6 doses of digoxin-specific antibody fragments, digitoxin was undetectable in the serum but reappeared on days 6 and 7 [Schmitt *et al.* 1994].

A subject who ingested 10 mg digitoxin recovered following treatment with activated charcoal; a plasma concentration of 0.26 mg/L was reported 4 h after the dose, decreasing to 0.027 mg/L at 80 h [Pond *et al.* 1981].

Half-life Plasma half-life, 3–16 days (mean 7 days).

Volume of Distribution 0.4–0.8 L/kg (mean 0.6 L/kg); increased in children.

Clearance ≈0.04 mL/min/kg from plasma; increased in children.

Distribution in Blood Plasma : whole blood ratio, ~1.7.

Protein Binding ≈95%.

Note For a review of the clinical pharmacokinetics of digitoxin see Perrier *et al.* [1977].

Dose Maximum initial total dose of 1.6 mg over 1–2 days; maintenance, 50 to 200 µg daily.

Al Hakiem MH *et al.* (1982). Fluoroimmunoassay of digitoxin in serum. *Clin Chem* 28: 1364–1366.

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Krappweis J *et al.* (1996). Digitoxin intoxication with lethal outcome. *Eur J Med Res* 1: 551–553.

Larsen A, Storstein L (1983). Digitoxin kinetics and renal excretion in children. *Clin Pharmacol Ther* 33: 717–726.

Nore AK *et al.* (1980). Digitalis glycosides in serum, urine, and cerebrospinal fluid, determined with a commercial radioimmunoassay. *Clin Chem* 26: 321–323.

Oiestad EL *et al.* (2009). Determination of digoxin and digitoxin in whole blood. *J Anal Toxicol* 33: 372–378.

Perrier D *et al.* (1977). Clinical pharmacokinetics of digitoxin. *Clin Pharmacokinet* 2: 292–311.

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Santos SR *et al.* (1987). Simultaneous analysis of digitoxin and its clinically relevant metabolites using high-performance liquid chromatography and radioimmunoassay. *J Chromatogr* 419: 155–164.
Schmitt K *et al.* (1994). Massive digitoxin intoxication treated with digoxin-specific antibodies in a child. *Pediatr Cardiol* 15: 48–49.
Tracqui A *et al.* (1997). High-performance liquid chromatography–ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. *J Chromatogr B Biomed Sci Appl* 692: 101–109.

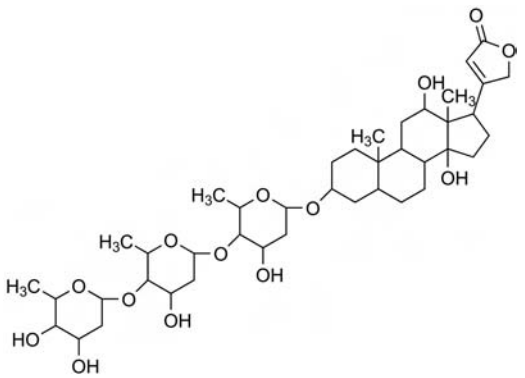
Digoxin

Cardiac Glycoside
$C_{41}H_{64}O_{14}$ = 780.9
CAS—20830-75-5

IUPAC Name 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-3-[(2*R*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-4,5-Dihydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one

Synonyms 3β-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl) oxy]-12β,14β-dihydroxy-5β-card-20(22)-enolide, digoxinum; digoxosidum.

Proprietary Names *Digacin*; *Digomal*; *Digosin*; *Digoxin(e) Nativelle*; *Dilanacin*; *Dogoxine*; *Eudigox*; *Grexin*; *Hemigoxine Nativelle*; *Lanacardin*; *Lanacrist*; *Lanicor*; *Lanoxin(e)*; *Lanoxicaps*; *Lenoxin*; *Malpluxin*; *Neo-Dioxanin*; *Novodigal*; *Prodigox*; *Purgoxin*; *Rougoxin*; *Toloxin*.



Chemical Properties Crystals. A glycoside obtained from the leaves of *Digitalis lanata* (Scrophulariaceae). Colourless crystals or a white powder. Mp ~240°, with decomposition. Practically insoluble in water, dehydrated alcohol, ether, acetone and ethyl acetate; soluble 1 in 122 of ethanol (80%) and 1 in 4 of pyridine; slightly soluble in chloroform; freely soluble in a mixture of equal volumes of chloroform and methanol. Log*P* (octanol/water), 1.26.

Metildigoxin

$C_{42}H_{66}O_{14}$ = 795.0
CAS—30685-43-9

IUPAC Name 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-12,14-Dihydroxy-3-[(2*R*,4*S*,5*S*, 6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*, 6*R*)-4-hydroxy-5-methoxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-6- methyloxan-2-yl]oxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16, 17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one

Synonyms 3β,5β,12β)-3-{[2,6-Dideoxy-4-*O*-methyl-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl]oxy}-12,14-dihydroxycard-20(22)-enolide; medigoxin; β-methyldigoxin, 4-*O*-methyldigoxin.

Proprietary Names *Cardiolan*; *Lanirapid*; *Lanitop*; *Miopat*.

Chemical Properties Crystals. Mp 227° to 231°.

α-Acetyldigoxin

$C_{43}H_{66}O_{15}$ = 823.0
CAS—5511-98-8

Proprietary Names *Lanatilin*; *Sandolanid*.

Chemical Properties Prisms. Mp 225° with decomposition. Very sparingly soluble in ethyl acetate.

β-Acetyldigoxin

CAS—5355-48-6

Proprietary Names *Corotal*; *Digostad*; *Digotab*; *Digox*; *Gladixol N*; *Kardiamed*; *Longdigox*; *Novodigal* (β-acetyldigoxin or digoxin); *Stillacor*.

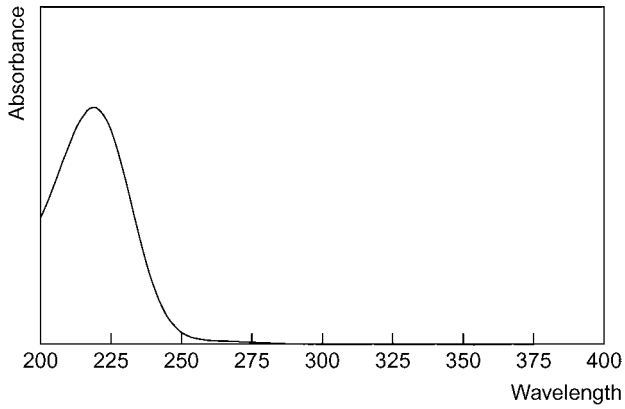
Chemical Properties Needles. Sparingly soluble in ethyl acetate.

Colour Test Antimony pentachloride—yellow→brown→black-violet.

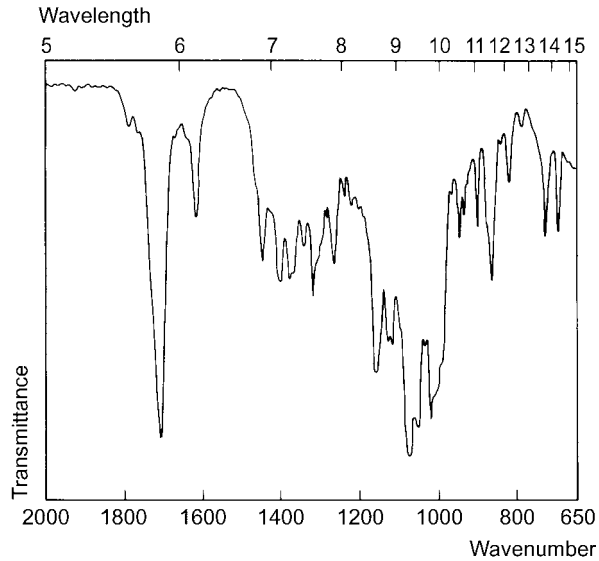
Thin-layer Chromatography System TD—R_f 0.01; system TE—R_f 0.33; system TF—R_f 0.05; system TAD—R_f 0.28; system TAE—R_f 0.85 (perchloric acid solution, followed by examination under UV light, blue fluorescence; *p*-anisaldehyde reagent, blue).

High Performance Liquid Chromatography System HM—*k* 11.3; system HY—RI 347; system HAA—RT 13.9 min.

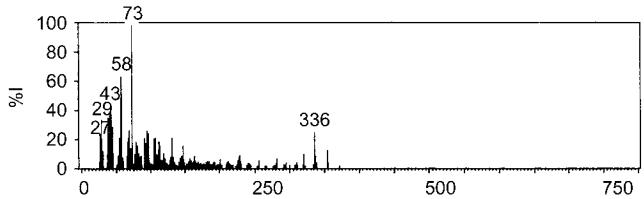
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1075, 1709, 1055, 1020, 1160, 1110 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m*/z 73, 58, 57, 43, 41, 39, 29, 45 (no peaks above 360).



Quantification

Blood **LC-MS** Column: Mightysil RP-18 (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile-2 mmol/L ammonium acetate (20 : 80) : acetonitrile-2 mmol/L ammonium acetate (80 : 20; 100 : 0 for 2 min to 0 : 100 at 12 min for 3 min to 100 : 0 at 20 min), flow rate 0.2 mL/min. ESI or APCI, positive ion mode, TIC or SIM acquisition mode. Limit of quantification, 0.05 µg/L [Guan *et al.* 1999].

Note For a radioimmunoassay, see Fletcher *et al.* [1979].

Plasma **LC-MS** Column: Zorbax SB-$C_{18.}$ Mobile phase: methanol : 0.1 % formic acid in 10 µmol/L sodium acetate (55 : 45), flow rate 1.0 mL/min. ESI. Retention time: 1.9 min. Limit of quantification, 0.5 µg/L [Vlase *et al.* 2009]. Column: Nova-Pak C_{18} (150 mm × 2.0 mm i.d., 4 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate (pH 3.0, 1 : 3), flow rate 200 µL/min. Retention time: 8.08 min. Limit of detection, 0.15–0.6 µg/L [Tracqui *et al.* 1997].

Note For a radioimmunoassay, see Nelson *et al.* [1979].

Serum **HPLC** Column: Spherisorb (150 × 4.6 mm i.d., 3 µm). Mobile phase: hexane : methylene chloride : acetonitrile : methanol (36 : 6.3 : 5.2 : 0.2), flow rate

1.6 mL/min. Fluorescence detection (λ_{ex}= 217 nm, λ_{em}= 340 nm). Limit of detection, 0.25 µg/L [Tzou *et al.* 1995]. Immunochemical detection. Limit of detection, 160 pg/mL [Oosterkamp *et al.* 1994].

Note For a radioimmunoassay, see Butler *et al.* [1982].

Urine **HPLC** Column: Partisil 10 ODS (250 × 4.6 mm i.d.). Mobile phase: methanol : water (35 : 65 to 45 : 55), flow rate 2.0 mL/min. UV detection (λ= 220 nm). Retention time: 21.9 min. Limit of detection, 278 ng [Gault *et al.* 1980]

LC-MS See Blood [Guan *et al.* 1999].

Disposition in the Body The absorption of digoxin after oral administration is variable and subject to bioavailability differences. Absorption occurs mainly in the small intestine and is delayed in the presence of food. Digoxin is rapidly distributed throughout the body and <20% of the total digoxin in the body is located in blood. High concentrations are found in the heart, brain and kidneys, but the skeletal muscles form the largest digoxin store. Concentrations in the myocardium are higher than in plasma. Digoxin is also found in the CSF and it crosses the placenta. Small amounts of digoxin are excreted in breast milk. Digoxin is metabolised by stepwise removal of the sugar moieties to form digoxigenin, which is further metabolised to inactive metabolites and these may be excreted in the free or conjugated form. Reduction to dihydrodigoxin, which is relatively inactive, also occurs. Up to 80% of a dose is excreted in the urine in 7 days, with 27% of the dose in the first 24 h; the remainder is eliminated in the faeces via the bile. In most patients, 80–90% of the material excreted in the urine is unchanged, up to 10% is in the dihydro form, and a small amount includes digoxigenin and the mono- and bisdigitoxosides. In ~10% of patients, however, between 20 and 55% is excreted as metabolites, mainly dihydrodigoxin. Of the material excreted in the bile, ~50% is unchanged, ~25% is digoxin bisdigitoxoside, ~25% is digoxin monodigitoxoside and ~1% is digoxigenin.

Digoxin is a metabolite of deslanoside, digitoxin, lanatoside C and metildigoxin.

Therapeutic Concentration In serum, usually in the range 0.001–0.0025 mg/L. Blood concentrations are significantly lower in hyperthyroidism but increased in hypothyroidism and in patients with renal failure; children tolerate higher serum concentrations than adults.

In a post-hoc analysis of a trial involving 3782 subjects, a serum digoxin concentration in the range 0.0005–0.0008 mg/L was found to be optimal for treating heart failure in men with a left ventricular ejection fraction of 45% or less; the daily dose of digoxin was in the range 0.125–0.500 mg, with 75% of subjects receiving 0.250 mg daily [Rathore *et al.* 2003].

Steady-state plasma concentrations of 0.0004–0.0021 mg/L (mean 0.0011) were reported in 14 patients on an oral daily dosage of 0.125–0.25 mg [Gayes *et al.* 1978].

Following a single oral dose of 0.25 mg to 6 subjects, a mean peak serum concentration of 0.001 mg/L was attained in 1 h [Panisset *et al.* 1973].

Toxicity Toxic effects are usually associated with serum concentrations of 1.4–7.0 µg/L (mean 2.8) and fatalities with concentrations of 1.5–30 µg/L (mean 10).

In an overdose fatality involving digoxin in a suicidal 82-year-old man, blood concentrations of 0.0122–0.0132 mg/L were reported approximately 2.5 h after ingestion of the drug [Rodriguez-Calvo *et al.* 2002].

Total and free serum digoxin concentrations of 0.0171 and 0.0124 mg/L, respectively, were reported in a 2-year-old child 4 h after ingestion of 22.5–23 mg digoxin. Following gastrointestinal decontamination and treatment with digoxin-specific Fab fragments, serum levels reduced to 0.00011 mg/L within 40 min [Kearns *et al.* 1989].

The following distribution was observed in a 3-day-old child who received a total of 4 mg digoxin and died several hours later: blood 0.03 mg/L, brain 0.0009 µg/g, kidney 0.13 µg/g and liver 0.034 µg/g [Selesky *et al.* 1977].

In 7 deaths involving ingestion of digoxin, postmortem 'serum' concentrations of 0.007–0.024 mg/L (mean 0.013) were reported [Moffat 1974].

In a fatality involving IM injection of 1 mg digoxin in an infant, a postmortem 'serum' concentration of 0.071 mg/L was reported [Moffat 1974].

Half-life Plasma half-life, ~20–50 h, prolonged in subjects with renal impairment.

Bioavailability Approximately 70% from tablets BP or USP specifications, 80% from elixirs, and over 90% from liquid-filled soft gelatin capsules.

Volume of Distribution ~5–10 L/kg.

Clearance Plasma clearance, ~1–4 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio: 0.93.

Protein Binding ~22–30%.

Note For reviews of the pharmacokinetics of digoxin, see Aronson [1980], Iisalo [1977], Mooradian [1988] and van der Vijgh, Oe [1977].

Dose 125 to 500 µg daily. For rapid digitalisation, an initial dose of 0.75 to 1.5 mg may be given.

Aronson JK (1980). Clinical pharmacokinetics of digoxin 1980. *Clin Pharmacokinet* 5: 137–149.

Butler VPJr *et al.* (1982). The development and application of a radioimmunoassay for dihydrodigoxin, a digoxin metabolite. *J Pharmacol Exp Ther* 221: 123–131.

Fletcher SM *et al.* (1979). Radioimmunoassay of cardiac glycosides in haemolysed blood: derivation of serum levels. *J Forensic Sci Soc* 19: 183–188.

Gault MH *et al.* (1980). High-performance liquid chromatographic method for isolation of tritiated digoxin and metabolites in urine. *J Chromatogr* 182: 465–472.

Gayes JM *et al.* (1978). Cerebrospinal fluid digoxin concentrations in humans. *J Clin Pharmacol* 18: 16–20.

Guan F *et al.* (1999). Identification and quantification of cardiac glycosides in blood and urine samples by HPLC/MS/MS. *Anal Chem* 71: 4034–4043.

Iisalo E (1977). Clinical pharmacokinetics of digoxin. *Clin Pharmacokinet* 2: 1–16.

Kearns GL *et al.* (1989). Pharmacokinetics and efficacy of digoxin specific Fab fragments in a child following massive digoxin overdose. *J Clin Pharmacol* 29: 901–908.

Moffat AC (1974). Interpretation of post mortem serum levels of cardiac glycosides after suspected overdosage. *Acta Pharmacol Toxicol (Copenh)* 35: 386–394.

Mooradian AD (1988). Digitalis: an update of clinical pharmacokinetics, therapeutic monitoring techniques and treatment recommendations. *Clin Pharmacokinet* 15: 165–179.

Nelson HA *et al.* (1979). Isolation by high-performance liquid chromatography and quantitation by radioimmunoassay of therapeutic concentrations of digoxin and metabolites. *J Chromatogr* 163: 169–177.

Oosterkamp AJ *et al.* (1994). Bioanalysis of digoxin and its metabolites using direct serum injection combined with liquid chromatography and on-line immunochemical detection. *J Chromatogr B Biomed Appl* 653: 55–61.

Panisset JC *et al.* (1973). Comparative bioavailability of two oral preparations of digoxin in healthy volunteers. *CMAJ* 109: 700–702.

Rathore SS *et al.* (2003). Association of serum digoxin concentration and outcomes in patients with heart failure. *JAMA* 289: 871–878.

Rodriguez-Calvo MS *et al.* (2002). Report of a suicidal digoxin intoxication: a case report. *Med Sci Law* 42: 265–268.

Selesky M *et al.* (1977). Digoxin concentrations in fatal cases. *J Forensic Sci* 22: 409–417.

Tracqui A *et al.* (1997). High-performance liquid chromatography–ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. *J Chromatogr B Biomed Sci Appl* 692: 101–109.

Tzou MC *et al.* (1995). Specific and sensitive determination of digoxin and metabolites in human serum by high performance liquid chromatography with cyclodextrin solid-phase extraction and precolumn fluorescence derivatization. *J Pharm Biomed Anal* 13: 1531–1540.

van der Vijgh WJ, Oe PL (1977). Pharmacokinetic aspects of digoxin in patients with terminal renal failure III. Effect of heparin. *Int J Clin Pharmacol Biopharm* 15: 560–562.

Vlase L *et al.* (2009). A new, high-throughput high-performance liquid chromatographic/mass spectrometric assay for therapeutic level monitoring of digoxin in human plasma. *J AOAC Int* 92: 1390–1395.

Dihydralazine

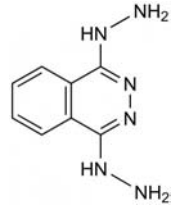
Antihypertensive

$C_8H_{10}N_6$ = 190.2

CAS—484-23-1

IUPAC Name (4-Hydrazinylphthalazin-1-yl)hydrazine

Synonyms Dihydrallazine; 2,3-dihydro-1,4-phthalazinedione dihydrazone.



Chemical Properties Orange crystalline needles. Mp about 180° with decomposition. Log *P* (octanol/water), −1.8.

Dihydralazine Sulfate

$C_8H_{10}N_6,H_2SO_4$ = 288.3

CAS—7327-87-9

Proprietary Names *Depressan*; *Dihyzin*; *Nepresol*; *Nepressol*.

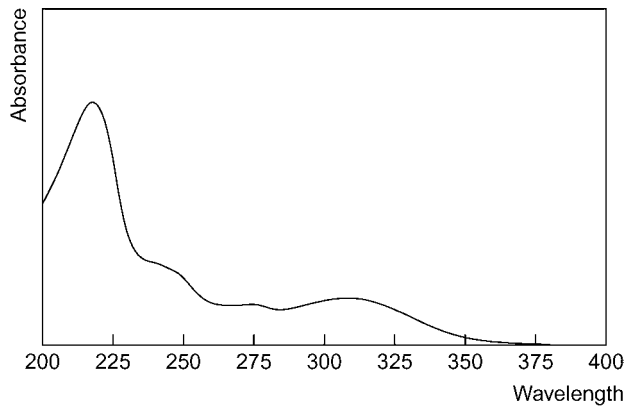
Chemical Properties A white to slightly yellow crystalline powder. Mp 233° with decomposition. Slightly soluble in water; practically insoluble in ethanol, chloroform and methanol.

Colour Tests Mandelin's test—yellow; Nessler's reagent—black.

Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.34; system TC—R_f 0.02; system TE—R_f 0.18; system TL—R_f 0.01; system TAE—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 39.

Ultraviolet Spectrum Aqueous acid—240, 274, 306 nm (A^1_1=323a).



Infrared Spectrum Principal peaks at wavenumbers 1608, 1575, 1537, 1136, 769, 1100 cm^{-1} (KBr disk).

Quantification

Plasma **GC** ECD. Limit of detection, 5 µg/L, dihydralazine plus acid-labile hydrazones [Degen *et al.* 1982].

Dose Dihydralazine sulfate has been given in doses of 12.5 to 150 mg daily.

Degen PH *et al.* (1982). Determination of apparent dihydralazine in plasma by gas–liquid chromatography and electron-capture detection. *J Chromatogr* 233: 375–380.

Dihydrocodeine

Narcotic Analgesic

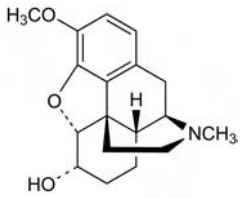
$C_{18}H_{23}NO_3 = 301.4$

CAS—125-28-0

IUPAC Name (5α,6α)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-ol

Synonyms Dihydroneopine; drocode; hydrocodeine.

Proprietary Names *Remedacen*; *Dihydrocodeine*; *Polistirex*



Chemical Properties Crystals. Mp 112° to 113°. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].

Dihydrocodeine Phosphate

$C_{18}H_{23}NO_3,H_3PO_4 = 399.4$

CAS—24204-13-5

Chemical Properties A white to yellowish-white crystalline powder. Soluble 1 in 3 of water; slightly soluble in ethanol.

Dihydrocodeine Tartrate

$C_{18}H_{23}NO_3,C_4H_6O_6 = 451.5$

CAS—5965-13-9

Synonyms Dihydrocodeine acid tartrate; dihydrocodeine bitartrate; drocode bitartrate; hydrocodeine bitartrate.

Proprietary Names *Codicontin*; *Codilol*; *Contugesic*; *DF 118*; *DHC*; *Dicodin*; *Didor*; *Fortuss*; *Hydol*; *Hydrocodeinon*; *Paracodin(a)*; *Paracodine*; *Rikodeine*; *Tiamon Mono*; *Tosidrin*. It is an ingredient of *DHC Plus*, *Galake*, *Panlor DC*, *Paramol*, *Remedeine* and *Synalgos-DC*.

Chemical Properties Colourless crystals or white crystalline powder. Mp 192° to 193° (anhydrous), 186° to 190° (commercial medicinal grade). Soluble 1 in 4.5 of water; sparingly soluble in ethanol; practically insoluble in ether. pK_a 8.8 (25°). Log *P* (ether/pH 7.0), −1.5.

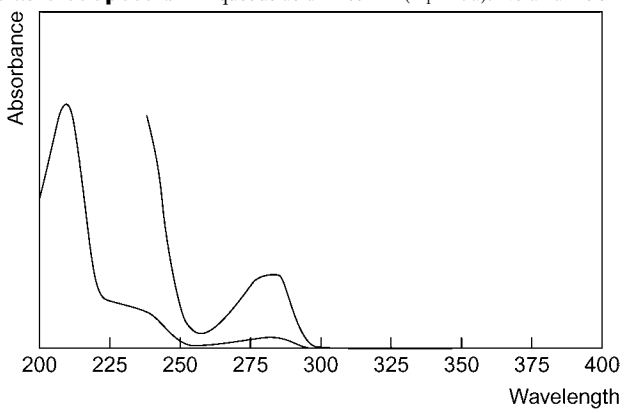
Colour Test Mandelin's test—grey-green; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.26; system TB—R_f 0.08; system TC—R_f 0.13; system TE—R_f 0.29; system TL—R_f 0.02; system TAE—R_f 0.11; system TAF—R_f 0.19; system TAJ—R_f 0.06; system TAK—R_f 0.00; system TAL—R_f 0.38 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis reagent—blue-violet.)

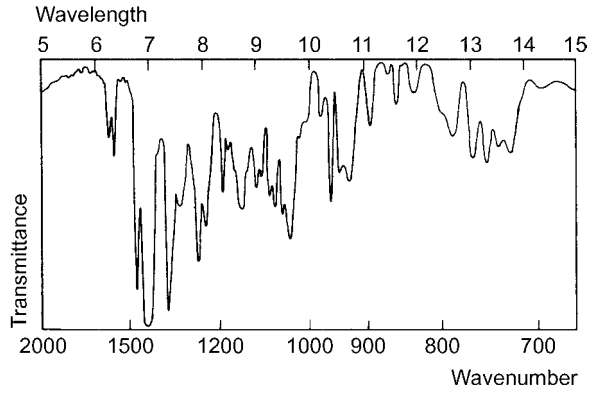
Gas Chromatography System GA—RI 2390, dihydrocodeine-AC RI 2445, dihydrocodeine-PFP RI 2360, dihydrocodeine-TFA RI 2265, dihydrocodeine-TMS RI 2480, M (nor-)-AC_2 RI 2750; system GB—RI 2511, dihydrocodeine-TMS RI 2496, M (nor-) RI 2599, M (nor-)-TMS_2 RI 2559; system GC—RI 2702; system GF—RI 2840 system GM—RRT 1.493 (relative to iprindole).

High Performance Liquid Chromatography System HA—*k* 7.2 (tailing peak); system HAA—RT 4.7 min,; system HC—*k* 2.50; system HX—RI 261; system HY—RI 208; system HZ—RT 2.0 min.

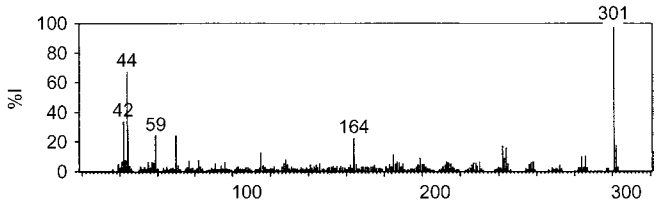
Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1 = 47a). No alkaline shift.



Infrared Spectrum Principal ions at wave numbers 1495, 1271, 1040, 1250, 1055, 1149 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 301, 44, 42, 59, 164, 70, 302, 242.



Quantification

Blood **GC** Column: HP-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 0.7 mL/min. Temperature: 320°. EI ionisation. Limit of quantification, 10 ng/mL [Mykkänen *et al.* 2000]. Column: 3% SE-30 on Gas Chrom Q (1.8 m × 0.63 mm (6 ft × 0.25 in) o.d.) or 3% OV-17 on Gas Chrom Q (1.2 m × 0.63 mm (4 ft × 0.25 in) o.d.) or 3% OV-1 on Gas chrom Q (1.2 m × 0.63 mm (4 ft × 0.25 in) o.d.) or 4% XE-60 on Gas Chrom Q (1.2 m × 0.63 mm (4 ft × 0.25 in) o.d.). Carrier gas: 30, 50, 40 and 40 mL/min, respectively. Temperature: 220°, 235°, 200° and 240°, respectively. FID. Retention time: 8.8, 10.1, 4.0, and 3.6 min, respectively. Limit of detection, 0.8 mg/L [Peat, Sengupta 1977].

GC-MS Column: DB-1 fused silica column (30 m × 25 mm i.d., 0.25 µm). Carrier gas: H_2, 30 mL/min. Temperature: 300°. Limit of detection, <5 ng/mL [Geier *et al.* 1996]. Column: OV1 (12 m × 0.2 mm i.d.). Temperature programme: 150° for 2 min to 220° at 40°/min for 6 min. EI ionisation, SIM acquisition mode. Retention time: 7.92 min. Limit of detection, <1 µg/L [Musshoff, Daldrup 1993].

HPLC Column: Nucleosil 100-5 C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 25 mmol/L TEA phosphate (4 : 96), flow rate 1 mL/min. Fluorescence detection (λ= 220 nm) [Klinder *et al.* 1999].

LC-MS Column: Synergi Polar (150 × 2 mm i.d). Mobile phase: 1 mmol/L ammonium formate and 0.1% formic acid (pH 2.7) : 70% acetonitrile-1 mmol/L ammonium formate and 0.1% formic acid (pH 3.8). ESI, positive ion mode, MRM acquisition mode. Limit of detection not reported [Taylor, Elliott 2009]. Column: Phenomenex Synergi. Mobile phase: 10 mmol/L ammonium formate : acetonitrile (pH 3). Limit of quantification 0.5–4.09 ng/mL, limit of detection 0.16–1.2 ng/mL [Al Asmari, Anderson 2007]

Plasma **GC** Column: DB-17 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 22 cm/s. Temperature: 280°. SID (comparison with NPD). Limit of detection, 2.5 µg/L [Seno *et al.* 1995]. Column: 3% OV-17 on 80/100 mesh Chromosorb W-HP (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 260°. Limit of quantification, 20 µg/L [Cowan *et al.* 1988]

LC-MS See Blood [Taylor, Elliott 2009].

GC-MS See Blood [Geier *et al.* 1996].

Serum **GC-MS** Column: DB-5 capillary column (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2. Temperature: 300°. Limit of quantification, 2 µg/L for dihydrocodeine and 40 ng/L for dihydromorphine [Hofmann *et al.* 1995]. See Blood [Musshoff, Daldrup 1993].

Urine **HPLC** Colum: Hypersil (200 × 2 mm i.d., 3 µm). Mobile phase: dichloromethane : pentane : methanol : diethylamine (29.8 : 65 : 5.5 : 0.5), flow rate 0.4 mL/min. UV detection (λ= 280 nm,). Limit of detection, 4–20 µg/L (comparison with enzyme immunoassay) [Low, Taylor 1995]. Limit of quantification, 2 mg/L[Cowan *et al.* 1988].

GC See Plasma [Seno *et al.* 1995]. See Blood [Peat, Sengupta 1977].

GC-MS Column: HP5MS capillary column (30 m × 0.25 mm i.d., 0.25 m). Carier gas: H_2, 1.5 mL/min. Temperature: 200° to 300° [Meadway *et al.* 2002]

Bile **GC** See Blood [Peat, Sengupta 1977].

Liver **GC** See Blood [Peat, Sengupta 1977].

Hair **HPLC** Column: C_{18} silica-based (150 × 4.6 mm i.d., 3 µm). Mobile phase: 20 mmol/L monobasic sodium phosphate-acetonitrile (pH 7.0; 90 : 10) : 20 mmol/L monobasic sodium phosphate-acetonitrile (pH 7.0; 50 : 50) (93 : 7 for 11.5 min to 50 : 50 at 19.5 min for 5 min to 90 : 10 at 34.5 min), flow rate 0.8 mL/min. Electrochemical detection. Limit of detection, 48 pg [Achilli *et al.* 1996].

GC-MS Column: Ultra-2 cross-linked 5% phenylmethylsiloxane (12 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 250° to 280° at 5°/min. Limit of detection, 30 pg [Sachs *et al.* 1993].

Note For methods of quantification of biological samples by HPLC for dihydrocodeine and drugs of abuse and their metabolites, see Moeller *et al.* [1998].

Disposition in the Body Dihydrocodeine undergoes extensive first-pass metabolism in the gut wall or liver. Metabolism includes *N*-demethylation to form nordihydrocodeine, *O*-demethylation producing dihydromorphine, 6-keto reduction and conjugation. After an oral dose of 52 mg, 20–30% is excreted in the urine in 24 h; this is increased to ≈35% when the urine is acid. After an IM dose of ≈43 mg under conditions of controlled acidic urinary pH, 40–60% is excreted in the urine in 24 h. Approximately 30–45% of the urinary excreted material is conjugated.

Therapeutic Concentration

Following a single oral dose of 60 mg dihydrocodeine to 12 subjects, mean peak plasma concentrations of dihydrocodeine of 341 nmol/L (range, 312–370) and of dihydromorphine of 4.7 nmol/L (range, 3.7–5.8) were attained in ≈5 h; multiple dosing at 60 mg twice daily for 2 days, then 90 mg twice daily for 2 days, and then 120 mg twice daily for 2 days produced minimum mean steady-state dihydrocodeine concentrations of 186 nmol/L (range, 157–215), 203 nmol/L (range, 242–345) and 443 nmol/L (range, 367–518), respectively [Ammon *et al.* 1999].

Following single oral doses of 30 mg and 60 mg to 7 subjects, mean peak plasma concentrations of 0.072 and 0.146 mg/L, were attained in 1.7 h respectively; the corresponding peak plasma concentrations of acidic metabolites were 0.56 and 1.48 mg/L, respectively [Rowell *et al.* 1983].

Toxicity The estimated minimum lethal dose is 0.5 g, but addicts may be able to tolerate up to 10 times as much, whereas 25 mg may be fatal in children.

In a 16-year-old man who died from drowning and cold exposure following a drug overdose, the following concentrations of diphenhydramine, free dihydrocodeine and total dihydrocodeine, respectively, were found ≈9 days after death: femoral venous blood 1.89, 3.27 and 3.30 mg/L, right cardiac chamber blood 0.294, 0.237 and 0.240 mg/L, urine 22.6, 37.3 and 43.1 mg/L, stomach 0.029, 0.018 and 0.024 mg and femoral muscle 0.270, 0.246 and 0.314 μg/g [Moriya, Hashimoto 2001].

In 3 deaths involving dihydrocodeine, the femoral blood concentrations of dihydrocodeine and its metabolites at postmortem were: dihydrocodeine 18.45, 1.92 and 2.36 mg/L; dihydrocodeine 6-glucuronide 7.18, 2.68 and 1.61 mg/L; nordihydrocodeine 2.21, 0.27 and 0.12 mg/L; dihydromorphine 0.21, 0.18 and 0.16 mg/L; dihydromorphine 3-glucuronide 1.08, 1.04 and 0.31 mg/L; and dihydromorphine 6-glucuronide 0.08, 0.08 and 0.07 mg/L. In the first 2 cases, diazepam and nordiazepam were also detected and in the third chloral hydrate and trichlorethanol had also been ingested [Klinder *et al.* 1999].

A 30-year-old man who had been undergoing long-term substitution therapy with dihydrocodeine died of unkown causes. At postmortem, 3.5 h after death, the following tissue concentrations were found (in addition to diazepam and nordazepam)

In 4 deaths caused by ingestion of dihydrocodeine, postmortem blood concentrations of 7.2–12.0 mg/L (mean, 9.0) were reported [Paterson 1985].

In a death attributed to an oral overdose of dihydrocodeine, the following postmortem tissue concentrations were reported: blood 12 mg/L, bile 5340 mg/L, liver 620 μg/g and urine 570 mg/L; chlordiazepoxide was also detected. In a second case in which the death of an addict occurred rapidly after an IV overdose of dihydrocodeine, the postmortem concentrations were blood 720 mg/L, bile 3 mg/L, liver blood 364 mg/L, and urine 7 mg/L; pentobarbital and paracetamol were also detected [Peat, Sengupta 1977].

Half-life Plasma half-life, ≈4 h.

Bioavailability ≈20%

Volume of Distribution ≈1 L/kg.

Clearance Plasma clearance, ≈4 mL/min/kg.

Dose 30 to 60 mg of dihydrocodeine tartrate every 4 to 6 h up to maximum of 240 mg daily.

Achilli G *et al.* (1996). Determination of illicit drugs and related substances by high-performance liquid chromatography with an electrochemical coulometric-array detector. *J Chromatogr A* 729: 273–277.

Al Asmari AI, Anderson RA (2007). Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 31: 394–408.

Ammon S *et al.* (1999). Pharmacokinetics of dihydrocodeine and its active metabolite after single and multiple oral dosing. *Br J Clin Pharmacol* 48: 317–322.

Cowan DA *et al.* (1988). Two assays for dihydrocodeine in plasma and in urine and their use to determine the bioavailability of a controlled-release product. *J Pharm Sci* 77: 606–609.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Geier A *et al.* (1996). Evaluation of a solid-phase extraction procedure for the simultaneous determination of morphine, 6-monoacetylmorphine, codeine and dihydrocodeine in plasma and whole blood by GC/MS. *Int J Legal Med* 109: 80–83.

Hofmann U *et al.* (1995). Simultaneous determination of dihydrocodeine and dihydromorphine in serum by gas chromatography–tandem mass spectrometry. *J Chromatogr B Biomed Appl* 663: 59–65.

Klinder K *et al.* (1999). The detection of dihydrocodeine and its main metabolites in cases of fatal overdose. *Int J Legal Med* 112: 155–158.

Low AS, Taylor RB (1995). Analysis of common opiates and heroin metabolites in urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 663: 225–233.

Meadway C *et al.* (2002). A rapid GC-MS method for the determination of dihydrocodeine, codeine, norcodeine, morphine, normorphine and 6-MAM in urine. *Forensic Sci Int* 127: 136–141.

Moeller MR *et al.* (1998). Determination of drugs of abuse in blood. *J Chromatogr B Biomed Sci Appl* 713: 91–109.

Moriya F, Hashimoto Y (2001). Postmortem diffusion of drugs from the bladder into femoral venous blood. *Forensic Sci Int* 123: 248–253.

Musshoff F, Daldrup T (1993). Evaluation of a method for simultaneous quantification of codeine, dihydrocodeine, morphine, and 6-monoacetylmorphine in serum, blood, and postmortem blood. *Int J Legal Med* 106: 107–109.

Mykkänen S *et al.* (2000). GCD quantitation of opiates as propionyl derivatives in blood. *J Anal Toxicol* 24: 122–126.

Paterson SC (1985). Drug levels found in cases of fatal self-poisoning. *Forensic Sci Int* 27: 129–133.

Peat MA, Sengupta A (1977). Toxicological investigations of cases of death involving codeine and dihydrocodeine. *Forensic Sci* 9: 21–32.

Rowell FJ *et al.* (1983). Pharmacokinetics of intravenous and oral dihydrocodeine and its acid metabolites. *Eur J Clin Pharmacol* 25: 419–424.

Sachs H *et al.* (1993). Determination of dihydrocodeine in hair of opiate addicts by GC/MS. *Int J Legal Med* 105: 247–250.

Seno H *et al.* (1995). Gas chromatography with surface ionization detection: a highly sensitive method for determining underivatized codeine and dihydrocodeine in body fluids. *J Chromatogr B Biomed Appl* 673: 189–195.

Skopp G *et al.* (1998). Postmortem distribution of dihydrocodeine and metabolites in a fatal case of dihydrocodeine intoxication. *Forensic Sci Int* 95: 99–107.

Taylor K, Elliott S (2009). A validated hybrid quadrupole linear ion-trap LC-MS method for the analysis of morphine and morphine glucuronides applied to opiate deaths. *Forensic Sci Int* 187: 34–41.

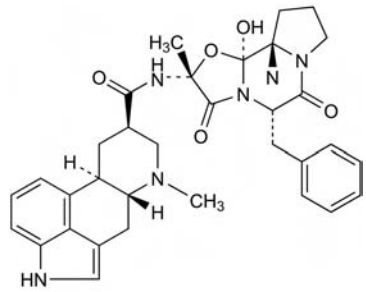
Dihydroergotamine

Treatment of Migraine

$C_{33}H_{37}N_5O_5 = 583.7$

CAS—5111-12-6

Synonym 9,10-Dihydro-12′-hydroxy-2′-methyl-5′-(phenylmethyl)ergotaman-3′,6′,18-trione



Chemical Properties White crystals. Mp 239°. Practically insoluble in water; sparingly soluble in ethanol, methanol, benzene and chloroform. pK_a 6.9 (24°).

Dihydroergotamine Mesilate

$C_{33}H_{37}N_5O_5,CH_3SO_3H = 679.8$

CAS—6190-39-2

	Left femoral vein (whole blood) (mg/L)	*Left ventricle (whole blood) (mg/L)*	*Liver (μg/g)*	*Kidney (μg/g)*	*Cerebrum (μg/g)*
Dihydrocodeine	1.910	2.695	1.282	10.557	0.802
Dihydrocodeine 6-glucuronide	1.689	1.759	1.955	27.609	–
N-Nordihydrocodeine	0.199	0.226	0.364	4.938	1.369
Dihydromorphine	0.152	0.182	—	2.080	–
Dihydromorphine 3-glucuronide	0.658	0.580	–	–	–
Dihydromorphine 6-glucuronide	0.093*	0.132*	–	1.330	–

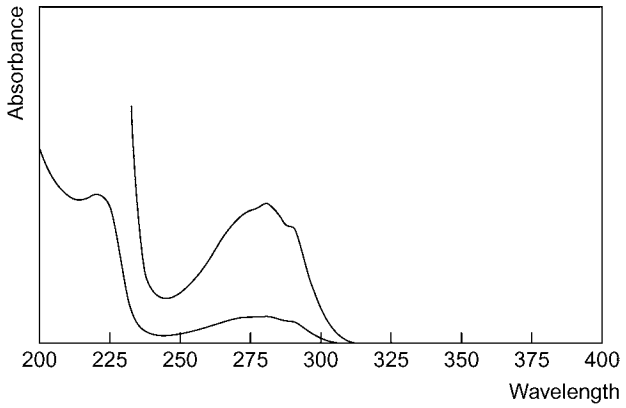
[a]*In serum [Skopp *et al.* 1998].

Synonym Dihydroergotamine methanesulphonate
Proprietary Names *DET MS*; *D.H.E. 45*; *Dihydergot*; *Ikaran*; *Séglor*; *Tonopres*.
Chemical Properties A white to slightly yellowish, or an off-white to slightly red, microcrystalline powder. Soluble 1 in 125 of water, 1 in 90 of ethanol, 1 in 175 of chloroform and 1 in 2600 of ether; soluble in acetone.

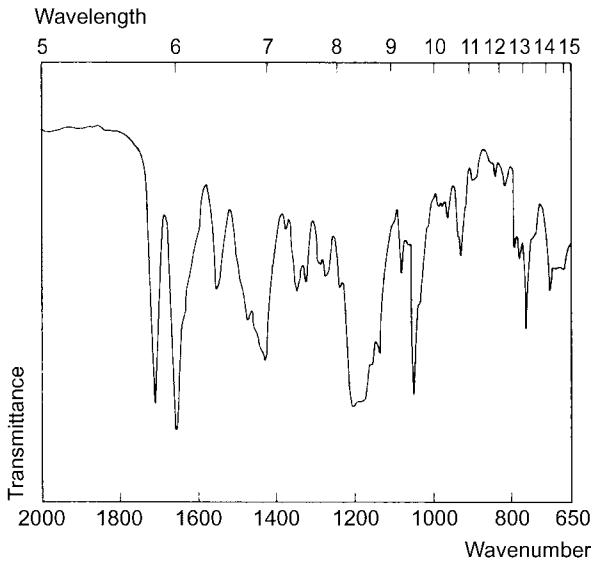
Dihydroergotamine Tartrate

$(C_{33}H_{37}N_5O_5)_2,C_4H_6O_6 = 1317.4$
CAS—5989-77-5
Chemical Properties Colourless crystals or a white crystalline powder. Mp about 203°, with decomposition. Very slightly soluble in water; sparingly soluble in ethanol; soluble in pyridine.

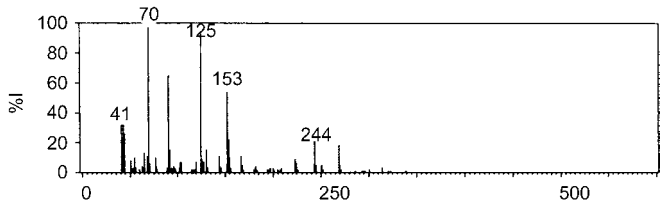
Colour Tests *p*-Dimethylaminobenzaldehyde—violet; Mandelin's test—violet-brown; Marquis test—grey-brown.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.01; system TC—R_f 0.28; system TE—R_f 0.42; system TL—R_f 0.14; system TM—R_f 0.40; system TAE—R_f 0.58; system TAJ—R_f 0.33; system TAK—R_f 0.03; system TAL—R_f 0.84 (Van Urk reagent, blue).
Gas Chromatography System GA—RI 2315.
High Performance Liquid Chromatography System HA—*k* 0.6; system HP—*k* 11.4; system HX—RI 411; system HZ—retention time 3.4 min.
Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=108a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1210, 1712, 1053, 1140, 768 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 70, 125, 91, 153, 43, 41, 44, 244 (no peaks above 340).



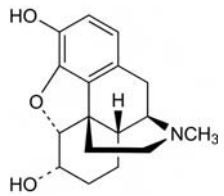
Quantification

Plasma **HPLC** Fluorescence detection. Limit of quantification, 0.1 µg/L [Murday *et al.* 1999]. Fluorescence detection (λ_{ex}=277 nm, λ_{em}=348 nm). Limit of detection, 0.08 µg/L [Romeijn *et al.* 1997]. Fluorescence detection. Limit of detection, 0.1 mg/L, dihydroergotamine and metabolites [Humbert *et al.* 1987].
Radioimmunoassay For method, see Rosenthaler and Munzer [1979].
Urine **HPLC** See Plasma [Humbert *et al.* 1987].
Disposition in the Body Poorly absorbed after oral administration and undergoes extensive first-pass metabolism with considerable intersubject variation. After oral administration <5% of a dose is excreted in the urine unchanged in 24 h, compared to about 11% after intravenous administration. The major metabolite is the 8′-hydroxy derivative, which is active.
Therapeutic Concentration
Following single oral doses of 10, 20 and 30 mg to 6 subjects, mean plasma concentrations of about 0.0002, 0.0006 and 0.001 mg/L, respectively, were attained in about 0.5 to 1 h [Little *et al.* 1982].
Note For a study of the pharmacokinetics of dihydroergotamine administered by nasal spray, see Humbert *et al.* [1996].
Bioavailability <5%.
Half-life Plasma half-life, about 2 to 4 h; a longer terminal elimination half-life of about 20 to 30 h has also been reported.
Volume of Distribution 6 to 23 L/kg (mean 14).
Clearance Plasma clearance, 7 to 22 mL/min/kg (mean 15).
Note For a review of the current uses of ergotamine and dihydroergotamine, see Tfelt-Hansen [2001].
Dose 1 to 3 mg of dihydroergotamine mesilate, repeated if necessary to a maximum of 10 mg daily.

Humbert H *et al.* (1987). Determination of sub-nanogram amounts of dihydroergotamine in plasma and urine using liquid chromatography and fluorimetric detection with off-line and on-line solid-phase drug enrichment. *J Chromatogr* 417: 319–329.
Humbert H *et al.* (1996). Human pharmacokinetics of dihydroergotamine administered by nasal spray. *Clin Pharmacol Ther* 60: 265–275.
Little PJ *et al.* (1982). Bioavailability of dihydroergotamine in man. *Br J Clin Pharmacol* 13: 785–790.
Murday M *et al.* (1999). Determination of dihydroergotamine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 735: 151–157.
Romeijn SG *et al.* (1997). Simplified solid-phase extraction method for determination of dihydroergotamine in rabbit and human serum using high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 692: 227–232.
Rosenthaler J, Munzer H (1976). 9-10-dihydroergotamine: production of antibodies and radioimmunoassay. *Experientia* 32(2): 234–236.
Tfelt-Hansen P (2001). Ergotamine, dihydroergotamine: current uses and problems. *Curr Med Res Opin* 17: 1S30–S34.

Dihydromorphine

Narcotic Analgesic
$C_{17}H_{21}NO_3 = 287.4$
CAS—509-60-4
Synonym (5α,6α)-4,5-Epoxy-17-methylmorphinan-3,6-diol

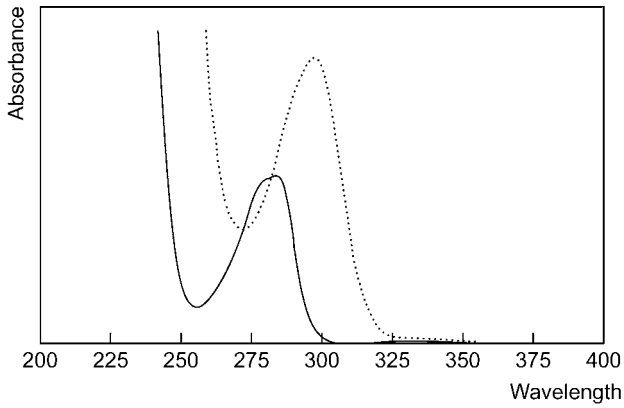


Chemical Properties Crystals. White crystals. Mp of the hydrated compound, 157°, with decomposition. Practically insoluble in water; soluble in acetone, ethanol and chloroform.

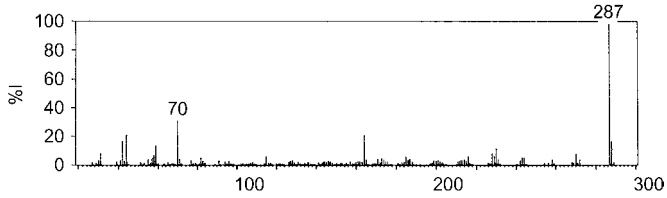
Dihydromorphine Hydrochloride

$C_{17}H_{21}NO_3,HCl = 323.8$
CAS—1421-28-9
Chemical Properties Crystals. Very soluble in water; sparingly soluble in ethanol. pK_a 8.6. Log *P* (octanol/water pH 7.4), −1.0.

Colour Tests Mandelin's test—grey; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.25; system TB—R_f 0.02; system TC—R_f 0.03; system TE—R_f 0.18; system TL—R_f 0.01; system TAE—R_f 0.12 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—violet).
Gas Chromatography System GA—dihydromorphine RI 2400, dihydromorphine-AC_2 RI 2545, dihydromorphine-PFP_2 RI 2330, dihydromorphine-TMS_2 RI 2520, M (nor-)-AC_3 RI 2790; system GB—dihydromorphine RI 2527, dihydromorphine-TMS_2 RI 2518; system GC—RI 2504.
High Performance Liquid Chromatography System HA—*k* 5.7 (tailing peak); system HC—*k* 2.75; system HX—RI 237; system HY—RI 156.
Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1 = 45a); aqueous alkali—297 nm (A_1^1 = 82b).



Infrared Spectrum Principal peaks at wavenumbers 1245, 760, 940, 747, 1085, 1145 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 287, 70, 44, 164, 42, 288, 59, 230.



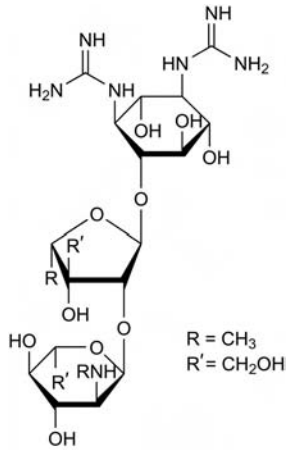
Quantification
Blood **LC-MS** Column: Phenomenex Synergi. Mobile phase: 10 mmol/L ammonium formate: acetonitrile (pH 3). Limit of quantification, 0.5–4.09 ng/mL, limit of detection, 0.16–1.2 ng/mL [Al Asmari, Anderson 2007].
Serum **GC-MS** Column: DB-5 capillary column (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2. Temperature: 300°. Limit of quantification, 2 ng/mL for dihydrocodeine and 40 pg/mL for dihydromorphine [Hofmann *et al.* 1995].

Al Asmari AI, Anderson RA (2007). Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 31: 394–408.
Hofmann U *et al.* (1995). Simultaneous determination of dihydrocodeine and dihydromorphine in serum by gas chromatography–tandem mass spectrometry. *J Chromatogr B Biomed Appl* 663: 59–65.

Dihydrostreptomycin

Antibiotic
$C_{21}H_{41}N_7O_{12}$ = 583.6
CAS—128-46-1

IUPAC Name 2-[(1*R*,2*R*,3*S*,5*R*,6*S*)-3-(Diaminomethylideneamino)-4-[(2*R*,3*R*,4*R*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-(methylamino)oxan-2-yl]oxy-4-hydroxy-4-(hydroxymethyl)-5-methyloxolan-2-yl]oxy-2,5,6-trihydroxycyclohexyl]guanidine
Synonym *O*-2-Deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-(hydroxymethyl)-α-L-lyxofuranosyl-(1→4)-*N*,*N*′-bis-(aminoimino-methyl)-D-streptamine



Chemical Properties pK_a 7.8. Log *P* (octanol/water), −7.5.

Dihydrostreptomycin Sulfate
$(C_{21}H_{41}N_7O_{12})_2,3H_2SO_4$ = 1461.4
CAS—5490-27-7

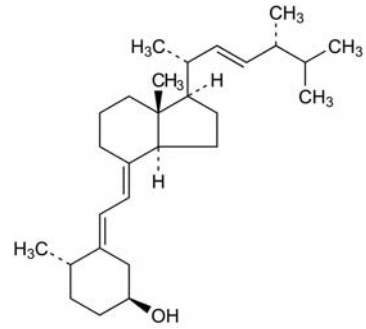
Proprietary Names It is an ingredient of *Citrocil*, *Cilinafosal DHD Estrep*, *Estrefen*, *Estreptoenterol*, *Fluocal com Pectina*, *Salitanol Estreptomicina* and *Sulfintestin Neomicina*.
Chemical Properties A white hygroscopic solid. Mp 255° to 265° with decomposition. Very soluble in water; practically insoluble in ethanol, chloroform and ether.

Colour Tests Nessler's reagent—yellow→brown; palladium chloride—brown (slow).
Thin-layer Chromatography System TA—R_f 0.01; system TAE—R_f 0.00 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1111, 1666, 1052, 1724, 1250, 1515 cm^{-1}.
Dose The equivalent of 0.5 to 1 g of dihydrostreptomycin has been given daily, intramuscularly.

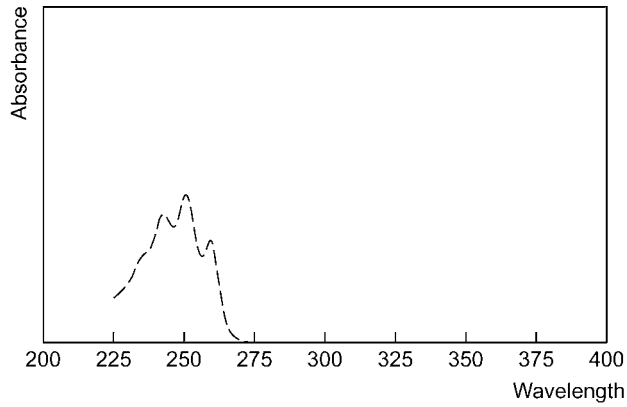
Dihydrotachysterol

Vitamin D Activity
$C_{28}H_{46}O$ = 398.7
CAS—67-96-9

IUPAC Name 3-[2-[1-(5,6-Dimethylhept-3-en-2-yl)-7*a*-methyl-2,3,3*a*,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylcyclohexan-1-ol
Synonyms Dichysterol; (3β,5*E*,7*E*,10α,22*E*)-9,10-secoergosta-5,7,22-trien-3-ol.
Proprietary Names *AT 10*; *Calcamine*; *Dihydral*; *Dygratyl*; *Hytakerol*; *Parterol*; *Tachyrol*.



Chemical Properties Colourless crystals or white crystalline powder. Mp 123° to 129°. May also occur in a form melting at about 113°. Practically insoluble in water; soluble 1 in 20 of ethanol, 1 in 0.7 of chloroform and 1 in 3 of ether. Log *P* (octanol/water), 10.4.
Ultraviolet Spectrum Methanol—242, 251 (A_1^1=990a), 261 nm.



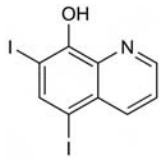
Infrared Spectrum Principal peaks at wavenumbers 1056, 967, 1018, 1009, 870, 980 cm^{-1} (KBr disk).
Dose Initially 0.25 to 2.5 mg daily.

Diiodohydroxyquinoline

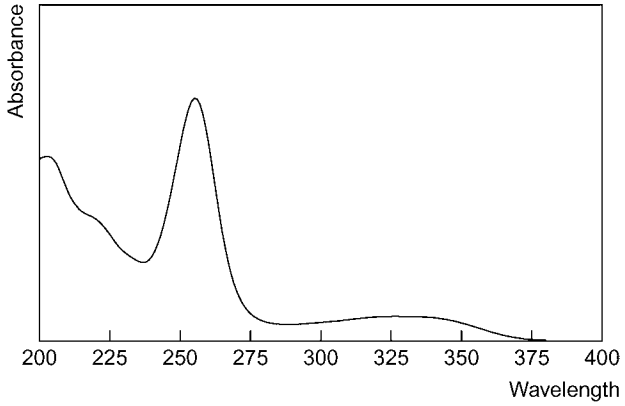
Antiamoebic
$C_9H_5I_2NO$ = 397.0
CAS—83-73-8

IUPAC Name 5,7-Diiodo-8-quinolinol
Synonyms Diiodohydroxyquin; diodoxyquinoléine; iodoquinol.
Proprietary Names *Amabagyl*; *Antidifar*; *Carsuquin*; *Diameb*; *Diodolina*; *Diodoquin*; *Direxiode*; *Diyomex*; *Diyosul*; *Diyowil*; *Drioquilen*; *Dysetrin*; *Embequin*;

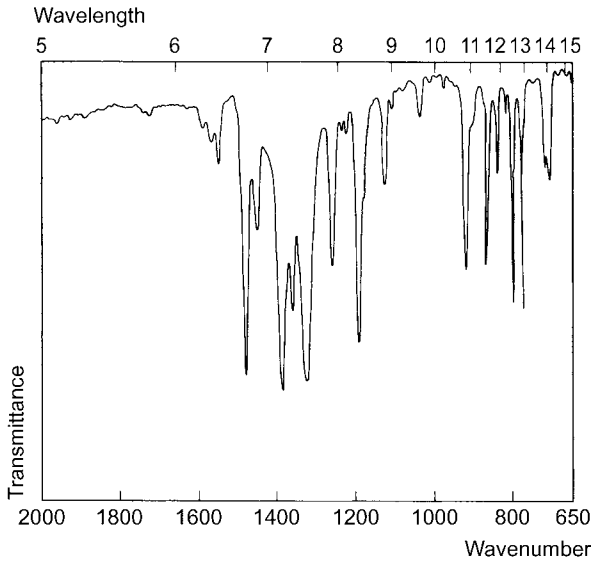
Entero-Diyod; *Entodiba*; *Flanoquin*; *Floraquin*; *Moebiquin*; *Sebaquin*; *Versamiv*; *Yodoxin*; *Yopin*.



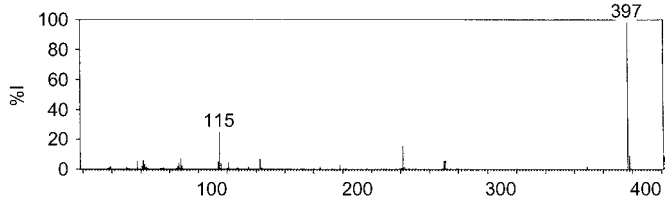
Chemical Properties A light yellowish to tan-coloured microcrystalline powder, not readily wetted in water. Mp 200° to 215°, with decomposition. Almost insoluble in water; sparingly soluble in ethanol, acetone and ether; soluble in hot pyridine and hot dioxane. Log *P* (octanol/water), 4.0.
Colour Test Heat a few crystals with 1 mL of sulfuric acid—violet iodine vapour.
Thin-layer Chromatography System TD—R_f 0.17; system TE—R_f 0.28; system TF—R_f 0.19; system TAD—R_f 0.72.
Ultraviolet Spectrum Dilute 5 mL of a 0.01% solution in dioxan to 100 mL with dehydrated alcohol—258 nm (A_1^1=1060b).



Infrared Spectrum Principal peaks at wavenumbers 1204, 781, 806, 920, 1265, 869 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 397, 115, 242, 398, 88, 143, 271, 62.



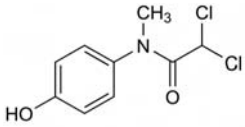
Quantification
Urine **Colorimetry** Limit of detection, 10 mg/L [Berggren, Hansson 1968].
Disposition in the Body Incompletely and irregularly absorbed from the small intestine. About 5% of a dose is excreted in the urine as the glucuronide conjugate in 10 h.

Dose Usually 1.8 g daily.

Berggren L, Hansson O (1968). Absorption of intestinal antiseptics derived from 8-hydroxyquinolines. *Clin Pharmacol Ther* 9: 67–70.

Diloxanide

Antiamoebic
$C_9H_9Cl_2NO_2$ = 234.1
CAS—579-38-4
IUPAC Name 2,2-Dichloro-*N*-(4-hydroxyphenyl)-*N*-methylacetamide
Synonym Diloxan

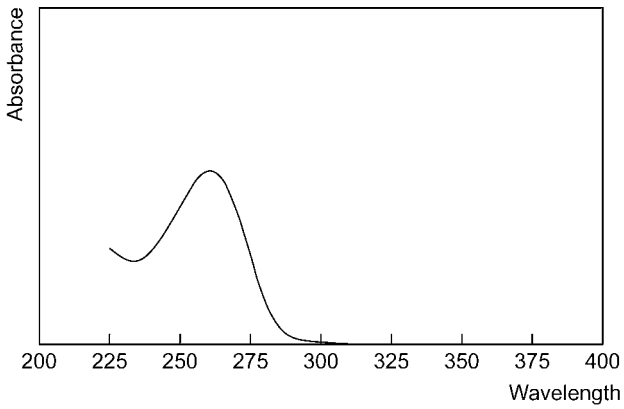


Chemical Properties A white crystalline powder. Mp 175°. Slightly soluble in water; soluble 1 in 8 of ethanol, 1 in 35 of chloroform and 1 in 66 of ether. Log *P* (octanol/water), 1.8.

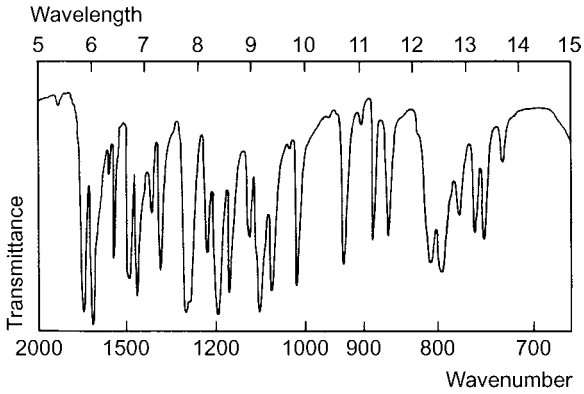
Diloxanide Furoate

$C_{14}H_{11}Cl_2NO_4$ = 328.2
CAS—3736-81-0
Proprietary Names *Furamid(e)*. It is an ingredient of *Entamizole*.
Chemical Properties A white crystalline powder. Mp 114° to 116°. Very slightly soluble in water; soluble 1 in 100 of ethanol, 1 in 2.5 of chloroform and 1 in 130 of ether.

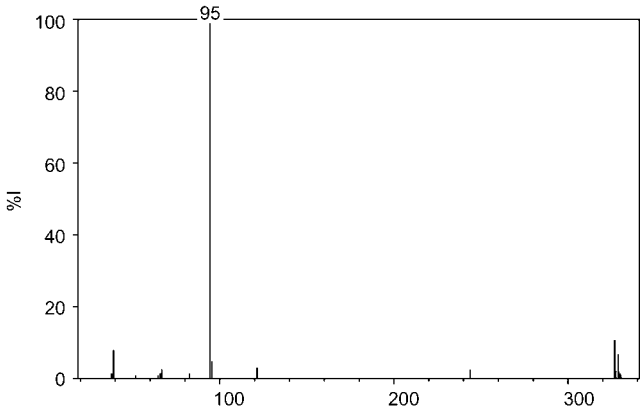
Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—yellow.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.16; system TC—R_f 0.74; system TL—R_f 0.67 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2420.
High Performance Liquid Chromatography System HY—RI 500.
Ultraviolet Spectrum Diloxanide furoate: aqueous acid—262 nm (A_1^1=224b); ethanol—258 nm (A_1^1=705a). Diloxanide: ethanol—278 nm (A_1^1=104a).



Infrared Spectrum Principal peaks at wavenumbers 1678, 1197, 1093, 1290, 1727, 1167 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 95, 327, 39, 329, 96, 122, 244, 67 (diloxanide furoate).

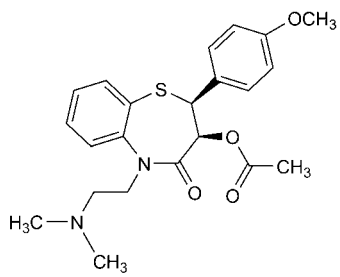


Disposition in the Body After oral administration diloxanide furoate is hydrolysed to diloxanide and then readily absorbed; it is excreted in the urine and faeces.
Dose 1.5 g of diloxanide furoate daily.

Diltiazem

Antianginal, Antiarrhythmic, Antihypertensive, Calcium Channel Blocker
$C_{22}H_{26}N_2O_4S = 414.5$
CAS—42399-41-7

IUPAC Name [(2*S*,3*S*)-5-(2-Dimethylaminoethyl)-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate
Synonyms (2*S*-*cis*)-3-(Acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5*H*)-one; (+)-*cis*-5-[2-(dimethylamino)-ethyl]2,3-dihydro-3-hydroxy-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one acetate (ester).
Proprietary Names *Dilzem*; *Surecaps*.



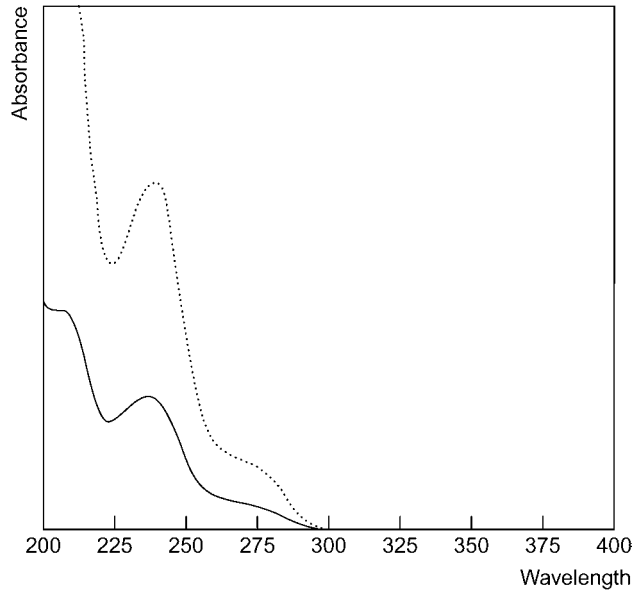
Chemical Properties pK_a ≈8.0 [Rutledge *et al.* 1993], 7.7 [Rosell *et al.* 1993; Ververs *et al.* 1990]. Log *P* (octanol/water) 2.79 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Diltiazem is stable in whole blood at room temperature for 4 h, at –20° for 1 month, and after 3 freeze-thaw cycles [Li *et al.* 2008]. Plasma samples were stable after 5 freeze-thaw cycles, after 4 months at –20° and for 48 h at room temperature [Georgita *et al.* 2008]. Stock solutions in methanol were stable at –20° for 1 month. Plasma samples were stable at 4° for 24 h, at room temperature for 24 h and after 3 freeze-thaw cycles [Li *et al.* 2007]. Plasma samples were stable in the autosampler for 2, 12, and 24 h, after 1 and 2 freeze-thaw cycles, and after 1 month at –20° [Zendelovska *et al.* 2003]. Stable in plasma after 2 freeze-thaw cycles [Quiroga *et al.* 2001]. Plasma sample extracts were stable for at least 3 days at room temperature. Spiked plasma samples and *ex-vivo* samples were stable at –80° for 16 months and 31 months, respectively [Carignan *et al.* 1995]. Diltiazem and desacetyldiltiazem were found to be stable in serum for up to 8 weeks at –20°, whereas some degradation was observed after 7 days at 4° [Paczkowski *et al.* 1995]. Diltiazem and metabolites were stable in plasma for at least 24 h in the autosampler [Ascalone *et al.* 1994]. Stable in plasma at –20° for 30 days [Rutledge *et al.* 1994]. Stock solutions stored at –25° were stable for 6 months [Rustum 1989]. There was no degradation in spiked plasma samples until day 7 when stored at 4°. Diltiazem was very stable in plasma frozen at –20° for at least 6 weeks [Dubé *et al.* 1988]. Samples stored in bovine plasma were stable for at least 3 months at –20° in the dark [Bhamra *et al.* 1987].
Note For a study of stability of diltiazem and its metabolites in plasma during storage, see Yeung *et al.* [1991] and in different biological fluids, see McLean *et al.* [1991]. For a study of diltiazem stability in whole blood, see Koves *et al.* [1998].

Diltiazem Hydrochloride

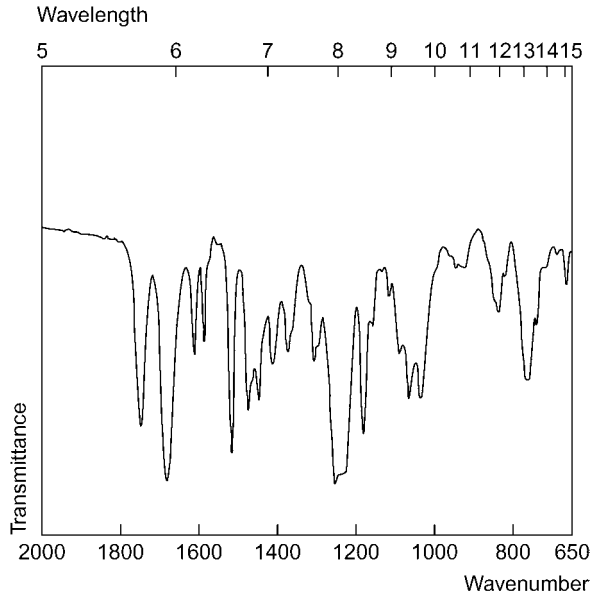
$C_{22}H_{26}N_2O_4S,HCl = 451.0$
CAS—33286-22-5
Synonyms CRD-401; diltiazem hydrochloridum; latiazem hydrochloride; MK-793.

Proprietary Names *Adizem*; *Altiazem*; *Angiozem*; *Angitil*; *Calazem*; *Calcicard*; *Cardizem*; *Dilacar*; *Dilcardia*; *Herbesser*; *Slozem*; *Tiazac*; *Tildiem*; *Viazam*; *Zemtard*.
Chemical Properties White, odourless, crystalline powder or small crystals. It is freely soluble in water, chloroform, dichloromethane, formic acid and methyl alcohol; sparingly soluble in dehydrated alcohol; practically insoluble in ether. Mp 207.5° to 212.0°. Log *P* (octanol/water) 19.4 [Cassidy *et al.* 1988].

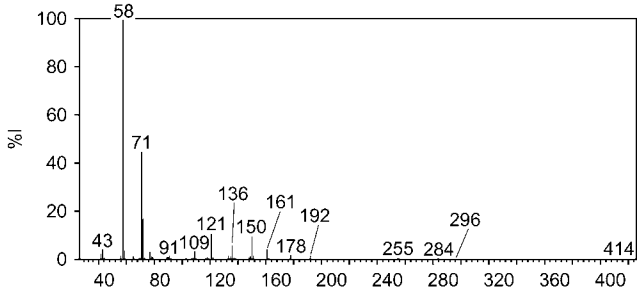
Thin-layer Chromatography System TAE—R_f 0.47; system TE—R_f 0.56.
Plates: Silica (Merck 60). Mobile phase: ethyl acetate : methanol : ammonia (80 : 10 : 10). Reference compound: diltiazem hydrochloride R_f 0.54 [Devarajan, Dhavse 1998].
Gas Chromatography System GA—diltiazem RI 2949, M (desacetyl-) RI 2990, M (*O*-desmethyl-) RI 3050; system GB—diltiazem RI 3076, M (desacetyl-) RI 3092, M (*O*-desmethyl-) RI 3147, M (*N*-desmethyl-) RI 3114.
High Performance Liquid Chromatography System HAV—diltiazem *k* 6.1, deacetyldiltiazem *k* 3.6, desmethyldiltiazem *k* 5.1; system HAA—RT 14.0 min; system HAX—diltiazem RT 11.1 min, desacetyldiltiazem RT 9.3 min; system HAY—diltiazem RT 5.4 min, desacetyldiltiazem RT 4.6 min; system HY—RI 361; system HZ—RT 4.5 min.
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—236 nm; basic—237 nm.



Infrared Spectrum Principal peaks at 2393, 1679, 1743, 839, 781, 3056 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 58, 71, 414, 284, 296, 150, 121, 344.



Quantification

Blood **GC** Column: TC-5 5% phenyl methyl silicone (15 m × 0.53 mm, 1.5 µm). Carrier gas: He, 15 kPa. Temperature programme: 150° for 2 min to 300° at 20°/min for 10.5 min. FTD. Limit of detection, 0.01 mg/L [Moriya, Hashimoto 2004]. Column: Restek RTX-50 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min for 12 min to 3 mL/min over 0.5 min for 3.5 min. Temperature programme: 220° for 3 min to 280° at 10°/min for 2 min to 300° at 40°/min for 5.5 min. NPD. Limit of detection, 60 µg/L [Engelhart *et al.* 1997].

GC-MS Column: HP Ultra-2 cross-linked fused silica capillary (12 m × 0.2 mm i.d., 0.33 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 2 min to 200° at 50°/min to 260° at 5°/min to 290° at 10°/min for 2 min. MSD. Limit of detection not reported [Romano *et al.* 2002].

HPLC Column: Supelcosil LC-DP (250 × 4.6 mm i.d.,). Mobile phase: acetonitrile : tetraethylammonium buffer (pH 3.4) : 3.7 mmol/L potassium phosphate (25 : 5 : 10), flow rate 0.6 mL/min. UV detection (λ= 229 nm). Limit of detection, 0.02 mg/L [Koves *et al.* 1998]. Column: Partisil 10 ODS C_{18}. Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 3.2; 60 : 40), flow rate 3.0 mL/min. UV detection (λ = 230 nm). Retention time: 4.8 min. Limit of detection not reported [Kaliciak *et al.* 1992]. Column: PRP-1 (150 × 4.1 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.01 mol/L dibasic potassium phosphate and 5.0 mmol/L tetrabutylammonium hydroxide (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 µg/L [Rustum 1989].

LC-MS Column: Hypersil BDS C_{18} (50 × 2.1 mm i.d., 3 µm). Mobile phase: methanol : 2 mmol/L ammonium acetate (95 : 5), flow rate 200 µL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.16 min. Limit of quantification, 2 µg/L, limit of detection, 0.5 µg/L [Li *et al.* 2008]. Column: Atlantis dC_{18} (150 × 2.1 mm i.d., 3.0 µm). Mobile phase: 10 mmol/L ammonium formate (pH 3.1) : acetonitrile (90 : 10 to 10 : 90 at 10 min for 3 min), flow rate, 0.3 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 0.015 µmol/L, limit of detection, 0.005 µmol/L [Kristoffersen *et al.* 2007].

Plasma **GC** Column: 5% phenyl polymethylsiloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature: 265°. ECD. Limit of detection, 3 µg/L [Grech-Belanger *et al.* 1987]. Column: 3% OV 7 on Gas Chrom Q 80/100 (2 m × 2 mm i.d.). Carrier gas: Ar : CH_4, 60 mL/min. Temperature: 245°. ECD. Limit of detection, 2 µg/L [Clozel *et al.* 1984a]. Column: Chromosorb W 80/100 mesh 1% OV-17 (2 m × 4 mm i.d.). Carrier gas: He, 50 mL/min. NPD. Retention time: 7.20 min. Limit of detection, 10 µg/L [Rovei *et al.* 1977].

GC-MS Column: 20% OV-17 on Chromosorb WHP (1.5 m). Carrier gas; Ar : CH_4 (95 : 5), 120 mL/min. Temperature: 320°. Retention time: 3.5 min. Limit of detection, 5 µg/L [Ochs, Knüchel 1984].

HPLC Column: Spherisorb C_{18} (250 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : 2.8 mmol/L triethylamine (80 : 20), flow rate 1.2 mL/min. UV detection (λ= 239 nm). Limit of detection, 3 µg/L [Li *et al.* 2003]. Column: Lichrospher 60 RP-select B (125 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.025 mol/L potassium dihydrogen phosphate (pH 5.5; 35 : 65), flow rate 1.5 mL/min. UV detection (λ= 215 nm). Limit of quantification, 20 µg/L, limit of detection, 5 µg/L [Zendelovska *et al.* 2003]. Column: Lichrocart RP-18 (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : 0.04 mol/L ammonium acetate : acetonitrile (29 : 38 : 23) with 0.04% triethylamine (pH 7.4), flow rate 1.2 mL/min. UV detection (λ= 240 nm). Limit of quantification, 20 µg/L [Quiroga *et al.* 2001]. Column: Hypersil BDS C8 (20 cm × 4.0 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate (pH 2.9) : triethylamine : acetonitrile : methanol (600 : 2 : 280 : 90), flow rate 1.0 mL/min. UV detection (λ= 238 nm). Retention time: 13.7, 7.5, 6.4 and 3.9 min for diltiazem, M_1, M_A, and M_2, respectively. Limit of detection, 5 µg/L for diltiazem, 2.5 µg/L for M_A, M_1 and M_2, respectively [Christensen *et al.* 1999]. Column: Lichrospher 60-5 select B. Mobile phase: methanol : ethanol : water (5:3 : 2). Limit of quantification, 5 µg/L [Scheiwe *et al.* 1996]. Column: Supelcosil C_{18} R-P (250 × 4.6 mm, i.d., 5 µm). Mobile phase: methanol : 0.04 mol/L ammonium acetate buffer : acetonitrile (38 : 36 : 26), containing 0.08% triethylamine (pH 7.5), flow rate 1.2 mL/min. UV detection (λ= 237 nm). Retention time: 3.6, 4.1, 4.7, 5.6, 7.8, 9.4 and 10.6 min for metabolites M_6, M_B, M_4, M_X, M_2, M_A and M_1:, respectively, diltiazem, 13.1 min. Limit of detection, <10 mg/L [Yeung *et al.* 1996].

See also [Carignan *et al.* 1995], [Fauville *et al.* 1995], [Ascalone *et al.* 1994], [Rutledge *et al.* 1994], [Sigusch *et al.* 1994], [Rosell *et al.* 1993], [Rutledge *et al.* 1993], [Hussain *et al.* 1992], [Christrup *et al.* 1992], [De Bernardis *et al.* 1992], [Jensen, Larsen 1991], [Ververs *et al.* 1990], [Boulieu *et al.* 1990], [Ascalone, Flaminio 1989], [Yeung *et al.* 1989], [Dubé *et al.* 1988], [Boucher *et al.* 1989], [Zhao, Chow 1989].

LC-MS Column: Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.2% triethylamine (pH 3.5) : acetonitrile (75 : 25), flow rate 0.8 mL/min. ESI, positive ion mode. Limit of quantification, 1.0 µg/L [Georgita *et al.* 2008]. Column: Capcell Pak C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water both containing 0.02% formic acid (5 : 95 to 50 : 50 at 3.5 min to 95 : 5 in 0.5 min to 5 : 95 in 0.5 min for 3 min), flow rate 0.3 mL/min. TIS, positive ion mode. Retention time: 4.1 min. Limit of quantification, 1 µg/L [Li *et al.* 2007]. Column: Inertsil C_8 (50 × 3 mm i.d., 50 µm). Mobile phase: methanol-10 mmol/L ammonia (pH 2.75; 20 : 80) : methanol-10 mmol/L ammonia (pH 2.75; 85, 15, 100 : 0 to 0 : 100 at 6 min to 0 : 100 at 7 min to 100 : 0 over 0.5 s), flow rate 0.4 mL/min. APCI, SIM acquisition mode. Limit of quantification, 1 µg/L [Molden *et al.* 2003]. Column: Inertsil C_8 (50 × 3 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonia (pH 2.75; 20 : 80 to 85 : 15 at 6 min. APCI, SIM acquisition mode. Limit of quantification, 1 µg/L [Molden *et al.* 2002b].

Serum **GC-MS** Column: HP-17 (12 m). Carrier gas: 15 mL/min. Temperature: 280°. NPD. Retention time: 4.9 min. Limit of detection, 0.2 mg/L [Beno, Nemeth 1991]. Column: 1% OV-17 on 100-120 mesh Chromosorb W (glass, 2 m × 3 mm, i.d.). Temperature: 280°. Carrier gas: N_2, 50 mL/min. Reference compound: flurazepam. ECD. Retention time: 4.2 min. Limit of detection, 2 mg/L [Alebic-Kolbah, Plavšic 1990].

HPLC Column: Supelcosil LC-CN (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water: 0.5 mol/L potassium dihydrogen phosphate buffer (pH 4.8; 400 : 584 : 16), flow rate 1.6 mL/min. UV detection (λ = 240 nm). Limit of detection, 3 µg/L [Paczkowski *et al.* 1995]. Column: Nucleosil C_{18} (120 × 4 mm i.d., 5 µm). Mobile phase: 50 mmol/L dihydrogen ammonium phosphate : acetonitrile (pH 5; 40 : 60) with 0.25% trietilamine, flow rate 1.0 mL/min. Limit of quantification, 8 µg/L [Saenz-Campos *et al.* 1995]. Column: LiChrosorb RP-8 (300 × 4.1 mm i.d., 10 µm). Mobile phase: 40% acetonitrile in 0.01 mol/L dibasic sodium phosphate and 0.1% triethanolamine dissolved in ultra-pure deionised water (pH 3), flow rate, 1.2 mL/min. UV detection (λ= 237 nm). Retention time: 5.6 min. Limit of detection, 2.5 mg/L [Chaudhary *et al.* 1993]. Column: Shimpak-CLS-ODS/H (25 cm × 4.6 mm, 5 µm). Mobile phase: acetonitrile : 0.1% triethylamine (1 : 1), flow rate 1.5 mL/min. UV detection (λ= 254 nm). Retention time: 6.0 min. Limit of detection not reported [Sarveswar Rao, Rambhau 1993]. Column: Spherisorb S5W (250 × 5 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium perchlorate in methanol (pH 7.6), flow rate 2.0 mL/min. UV detection (λ= 240 nm). Limit of detection, 10 µg/L for diltiazem, 20 µg/L for desacetyldiltiazem [Bhamra *et al.* 1987].

Urine **GC** See Blood [Moriya, Hashimoto 2004]. See Blood [Engelhart *et al.* 1997].

GC-MS Column: HP-1 (12.5 m × 0.2 mm, 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 1 min to 190° at 25°/min for 3 min to 280° at 10°/min for 12 min. FID. Retention time: 18.9 min. Limit of quantification, 0.1 mg/L [Kalin *et al.* 1994]. See Serum [Beno, Nemeth 1991]. Column: HP capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, scan mode. Limit of detection not reported [Maurer 1990]. Column: Durabond-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.5 kg/cm^2. Temperature: 280°. PIEI or NICI. Limit of detection not reported [Sugawara *et al.* 1988a].

HPLC See Blood [Kaliciak *et al.* 1992]. Column: C_{18} (10 cm × 8 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.15 mol/L phosphate buffer (pH 6.0) : water (10 : 90 : 0 to 25 : 25 : 50 at 15 min to 40 : 20 : 40 at 30 min to 60 : 13.3 : 26.7 at 40 min), flow rate 2.0 mL/min. UV detection (λ = 238 nm). Limit of detection not reported [Sugawara *et al.* 1988b]. Column: Spherisorb ODS C_{18} (25 × 0.46 cm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 3; 72 : 28), flow rate 2.5 mL/min for 5.5 min to 3.0 mL/min until 13 min. Retention time: 10.09 min. Limit of detection, 0.1 mg/L [Clozel *et al.* 1984b].

Bile **GC** See Blood [Moriya, Hashimoto 2004]. See Blood [Engelhart *et al.* 1997].

GC-MS See Blood [Romano *et al.* 2002].

CSF **GC** See Blood [Moriya, Hashimoto 2004].

Gastric Contents **GC** See Blood [Moriya, Hashimoto 2004]. See Blood [Engelhart *et al.* 1997].

Pericardial Fluid **GC** See Blood [Moriya, Hashimoto 2004].

Vitreous Humour **GC** See Blood [Moriya, Hashimoto 2004]. See Blood [Kaliciak *et al.* 1992].

Brain **GC** See Blood. Limit of detection, 0.04 µg/g [Moriya, Hashimoto 2004]

GC-MS See Blood [Romano *et al.* 2002].

Cardiac Tissue **GC** See Brain [Moriya, Hashimoto 2004].

GC-MS See Blood [Romano *et al.* 2002].

HPLC Column: Spherisorb C_6 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.25 mol/L potassium acetate buffer (pH 4; 60 : 40), flow rate, 1 mL/min. UV detection (λ= 237 nm) Retention time: 4.7 min. Limit of quantification, 0.3 µg/g [Läer *et al.* 1997].

Kidney **GC** See Brain [Moriya, Hashimoto 2004]. See Blood [Engelhart *et al.* 1997].

GC-MS See Blood [Romano *et al.* 2002].

Liver **GC** See Brain [Moriya, Hashimoto 2004]. See Blood [Engelhart *et al.* 1997].

GC-MS See Blood [Romano *et al.* 2002].

HPLC See Blood [Kaliciak *et al.* 1992].

Lung **GC** See Brain [Moriya, Hashimoto 2004].

GC-MS See Blood [Romano *et al.* 2002].

Muscle **GC** See Brain [Moriya, Hashimoto 2004].

Spleen **GC** See Blood [Engelhart *et al.* 1997].

Disposition in the Body After oral administration, diltiazem is rapidly absorbed (~90%) from the gastrointestinal tract and undergoes extensive first-pass hepatic metabolism via deacetylation, *N*-demethylation, *O*-demethylation and oxidative deamination. *O*- and *N*-demethylation are mediated by CYP2D6 and CYP3A4, respectively [Molden *et al.* 2002a]. Pharmacologically active diltiazem metabolites are markedly accumulated in individuals with a deficient CYP2D6-metabolising phenotype [Molden *et al.* 2002b]. Metabolites are A_1, M_A, M_B, M_X, M_1, M_2, M_4 and M_6. 2–4% of the administered dose appears unchanged in the urine. Metabolites are excreted via bile, urine and the faeces, and it can be found in breast milk.

Therapeutic Concentration Average optimum dose 180–360 mg daily with a blood therapeutic level 0.05–0.20 mg/L [Erickson *et al.* 1991]. The serum therapeutic concentration range is 0.05–0.4 mg/L.

Twenty-four healthy volunteers were administered a single oral dose of 120 mg diltiazem. A mean peak plasma concentration of 179.8 µg/L was reached in 2.3 h [Georgita *et al.* 2008].

Eight healthy male Chinese volunteers were administered a single oral dose of 60 mg diltazem hydrochloride slow-release formulation. The mean peak plasma concentration of 118.5 µg/L was reached in 3.1 h [Li *et al.* 2003].

Sixteen healthy men were divided into 2 groups. After 2 days of placebo, Group A received 240 mg diltiazem once daily for 7 days, 480 mg diltiazem once daily for 7 days, then 720 mg diltiazem once daily for 7 days. Group B received 180 mg diltiazem once daily for 7 days, 360 mg once daily for 7 days, then 720 mg for 7 days. The daily diltiazem dose was given as a combination of 120 mg cardizem CD capsules and matching placebo while group B received a combination of 180 mg Cardizem CD tablets and matching placebo. Mean pharmacokinetic parameters were as shown below.

Six healthy males and 6 females were orally administered a single 120 mg dose of diltiazem. Peak serum levels reached 0.194 ± 0.094 and 0.181 ± 0.048 mg/L for male and females, respectively, within 2.2 ± 0.8 h [Saenz-Campos *et al.* 1995].

A healthy volunteer was administered a single oral dose of 60 mg diltazem. The maximal concentration of diltazem was 53 µg/L reached at 2 h [Paczkowski *et al.* 1995].

Pharmacokinetic analysis of a study in which cirrhotic patients and controls received long-term diltiazem, 30 or 60 mg thrice daily, showed no change in absorption but a decrease in elimination, probably due to impaired oxidative metabolism in cirrhotic patients [Kurosawa *et al.* 1990].

Toxicity The toxic dose for adults is 2 g and 6 mg/kg for children, and affects the skin, for example, rashes [Scherschun *et al.* 2001]. It is a poison by SC, IV and IP routes and moderately toxic by ingestion. Overdose is associated with bradycardia with or without atrioventricular conduction defects and hypotension. A toxic serum concentration is 0.8 mg/L and a concentration of 2–6 mg/L has been associated with fatalities, although survival after ingestion of doses as large as 12 g have been reported [Fauville *et al.* 1995].

A 19-year-old male ingested an unknown quantity of drugs amongst which 3.6 g of diltiazem was unaccounted for. The maximum concentration of diltiazem was 577 µg/L at ≈7 h postingestion. This coincided with the start of haemodialysis. He made a full recovery [Roberts *et al.* 2008].

A 65-year-old male accidentally ingested 6 of his 360 mg diltiazem sustained-release tablets (2160 mg). An antemortem blood sample drawn at 11.5 h post-ingestion had a concentration of 2.9 mg/L. He was pronounced dead 17 h post-ingestion and postmortem central blood had a concentration of 6 mg/L and the peripheral blood had 5 mg/L [Cantrell, Williams 2005].

A 54-year-old female with intracerebral haemorrhage received 30 mg diltazem hydrochloride per day. She died 1.5 years later. A 48-year-old male with cerebral haemorrhage received a Herbesser injection of 50 mg diltazem hydrochloride. He died 38 h later. Postmortem concentrations were as shown above.

	Diltiazem concentration (mg/L or mg/kg)	
Sample	***Case 1***	***Case 2***
Blood		
Pulmonary arteries	2.12	1.04
Pulmonary veins	1.89	1.42
Left cardiac chambers	NA	0.908
Right cardiac chambers	0.253	0.592
Aorta	0.703	1.01
Inferior vena cava	0.182	0.595
Right femoral vein	0.07	0.46
CSF	0.037	NA
Right vitreous humour	NA	0.116
Pericardial fluid	0.510	0.925
Bile	1.46	4.64
Urine	0.998	1.46
Cerebrum	0.635	0.459
Cerebellum	0.545	0.769
Left lung	4.33	3.51
Right lung	5.73	3.39
Myocardium	2.45	1.6
Liver	0.82	3.21
Right kidney	0.796	1.18
Right iliopsoas muscle	0.148	NA
Right femoral muscle	NA	0.548
Gastric contents	108	5.04

[Moriya, Hashimoto 2004]

A 16-year-old female ingested 12 g of sustained-release diltiazem tablets. The peak concentration of diltiazem was approximately 5750 mg/L at 20 h post-ingestion. The girl made a full recovery [Durward *et al.* 2003].

A 60-year-old male ingested 40 slow-release 200 mg diltiazem tablets. Postmortem concentrations (mg/mL or mg/g) of diltiazem and M_1 were as shown below.

Sample	***Diltiazem***	***Desacetyldiltiazem***
Blood	31	9.7
Brain	33.1	13.7
Lung	179.5	47.5
Heart	41.8	10
Liver	182	47.3
Kidney	49.2	22.6
Bile	294.9	29.4

[Romano *et al.* 2002]

A 54-year-old angina sufferer self-medicated with six 180 mg diltiazem slow-release tablets. He was admitted to hospital with sinus bradycardia and cardiogenic shock. He developed acidosis, anuria, type-I respiratory failure and persistent hypotension. His diltiazem serum concentration 23 h after admission was 1.23 mg/L [Satchithananda *et al.* 2000].

A 38-year-old white male was found comatose. His peak serum diltiazem concentration was 3.7 mg/L measured at 8.8 h after admission to hospital [Snook *et al.* 2000].

A 78-year-old female was found dead. Diltiazem concentrations in her blood, urine, bile, and gastric contents were 5.9, 11.7, 4.0, and 2.8 mg/L, respectively [Engelhart *et al.* 1997].

A 22-year-old female ingested 7.2 g of slow-release diltiazem (30 Dilzem 240 mg depot capsules). The maximum serum concentration of diltiazem was 3171 µg/L reached 16.5 h post-ingestion. She made a full recovery [Luomanmäki *et al.* 1997].

	Group A			***Group B***		
Parameter	240 mg (n= 8)	480 mg (n= 8)	720 mg (n= 6)	180 mg (n= 8)	360 mg (n= 8)	720 mg (n= 6)
C_{max} (µg/L)	166.4 (54)	361.8 (32)	608.3 (38)	73.2 (25)	176.6 (36)	529.2 (32)
T_{max} (h)	9.8 (32)	7.9 (20)	8.0 (19)	9.8 (32)	11.3 (19)	9.0 (30)
C_{min} (µg/L)	42.5 (53)	106.4 (49)	239.5 (37)	20.3 (61)	66.7 (40)	188.9 (32)
$AUC_{(0-24)}$ (h · µg/L)	2410 (50)	5335 (38)	10167 (37)	1092 (35)	2953 (36)	8398 (24)
CL_o (L/h)	121 (45)	100 (32)	79 (35)	183 (34)	135 (33)	89 (21)

[Robbins-Weilert *et al.* 1999]

A 63-year-old male was admitted to hospital because of pain extending from his right groin to the toes and shortness of breath. On day 5 after admission his urine diltiazem was 9 times the therapeutic amount at 12.7 mg/L. He made a full recovery [Chan *et al.* 1996].

After the ingestion of 4.2 g diltiazem, a 30-year-old male had a plasma diltiazem concentration of 2.32 mg/L at ≈5 h following ingestion. By 70 h post-ingestion the plasma diltiazem concentration had fallen to 0.316 mg/L [Fauville *et al.* 1995].

A suicidal 39-year-old male was found dead and the coroner decided it was due to cardiac failure. Toxicological analysis of urine and blood from around his heart was requested because he had been threatening to commit suicide the previous night. Analysis showed that he had >30-times the recommended therapeutic dose of diltiazem: 6.9 and 4.7 mg/L in blood and urine, respectively. [Kalin *et al.* 1994].

A 51-year-old took an overdose of 150 60 mg diltiazem tablets (≈9000 mg). Diltiazem and M_1 concentrations were as shown below.

Time (h)	*Diltiazem (μg/L)*	*Desacetyldiltiazem (μg/L)*
2.5	4528	954
10.5	3577	872
18.5	2348	802
26.5	1520	434
34.5	923	288

[Connolly *et al.* 1993]

Case 1: A 21-year-old male took 25 diltiazem 60 mg tablets in combination with propranolol. At postmortem his diltiazem blood concentration was 1.5 mg/L. Case 2: A 68-year-old male was found dead in bed. At postmortem he had 5400 mg in his stomach and a blood concentration of 2.5 mg/L. Case 3: A woman was found dead in bed. She had 3300 mg diltiazem in her stomach and a blood concentration of 4.0 mg/L. Case 4: A 58-year-old male was found dead in bed. Postmortem examination revealed stomach contents of 650 mg with a blood concentration of 8.0 mg/L [Roper *et al.* 1993].

An elderly female was found dead having committed suicide. Ditiazem concentrations were: blood (inferior vena cava), 6.7 mg/L, urine, 5.4 mg/L, vitreous humour, 5.5 mg/L and liver, 79 mg/kg [Kaliciak *et al.* 1992].

A 25-year-old female with a history of cocaine and alcohol abuse ingested an unknown quantity of diltiazem and metoclopramide. Serum and urine dialtiazem concentrations were as shown below.

Time post-admission (h)	*Diltiazem (mg/L)*
Serum	
1.0	8.49
3.0	7.18
5.5	5.34
8.25	3.57
14.5	1.91
Urine	
1.0	4.48

[Beno, Nemeth 1991]

A 38-year-old female ingested 900 mg diltiazem. The peak plasma diltiazem concentration was 1.72 mg/L at 7 h, with an elimination half-life of 10.2 h [Roberts *et al.* 1991].

A 50-year-old took 98 60 mg diltiazem tablets with alcohol. The peak plasma diltiazem concentration reached 6090 μg/L at 4 h after admission to hospital [Ferner *et al.* 1989].

A 58-year-old took 10.8 g diltiazem and was admitted with hypotension and complete heart block. Dopamine, isoprenaline and calcium chloride were required to maintain blood pressure. ECG converted to sinus rhythm after 31 h. Plasma concentrations were 1.67 mg/L 43 h after oral administration and decreased to 0.012 mg/L over the following 55 h [Malcolm *et al.* 1986].

Note For a case of IV diltiazem for the treatment of hyperkalaemia resulting in a fatal outcome, see Moser *et al.* [1996]. For a case of rhabdomyolysis as a result of concomitant diltiazem with atorvastatin, see Lewin III *et al.* [2002].

Half-life 2–12 h [Caillé *et al.* 1991], 13.3 h [Luomanmäki *et al.* 1997], 24–48 h [Durward *et al.* 2003].

Bioavailability ≈30–40% [Bianchetti *et al.* 1991]. Lower after early-morning administration [Sarveswar Rao, Rambhau 1993].

Volume of Distribution 3–13 L/kg [Caillé *et al.* 1991], 11.1 L/kg [Ochs, Knüchel 1984]. Mean apparent volume of distribution at steady state also reported as 360–391 L.

Clearance Total clearance, 12–21 mL/min/kg after oral administration and 16 mL/min/kg after IV injection; also reported as 65 L/h.

Distribution in Blood 14–23% unbound drug in serum.

Protein Binding 77–93%, mainly to α_1-acid glycoprotein [Caillé *et al.* 1991], 30–40% to albumin.

Note For a review of the pharmacology, pharmacokinetics and efficacy of diltiazem, see Chaffman and Brogden [1985].

Dose For angina pectoris: initial dose of 60 mg diltiazem hydrochloride 3 times a day, which can be increased to 360 mg a day if necessary (Reduced to 60 mg twice daily for the elderly). For hypertension: initial dose of 60 to 120 mg twice daily, which can be increased, if necessary, to 360 mg/kg daily. For cardiac arrhythmia: 0.25 mg/kg body weight followed by 0.35 mg/kg, after 15 min, if required.

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Rutledge DR *et al.* (1994). Liquid chromatographic determination of celiprolol, diltiazem, desmethyldiltiazem and deacetyldiltiazem in plasma using a short alkyl chain silanol deactivated column. *J Pharm Biomed Anal* 12: 135–140.
Saenz-Campos D *et al.* (1995). Gender related pharmacokinetics of diltiazem in healthy subjects. *Int J Clin Pharmacol Ther* 33: 397–400.
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Satchithananda DK *et al.* (2000). Lesson of the week Unrecognised accidental overdose with diltiazem. *BMJ* 321: 160–161.
Scheiwe MW *et al.* (1996). Bioequivalence and relative bioavailability of a new diltiazem sustained release formulation. *Arzneimittelforschung* 46: 960–963.
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Sugawara Y *et al.* (1988a). Metabolism of diltiazem. I. Structures of new acidic and basic metabolites in rat, dog and man. *J Pharmacobiodyn* 11: 211–223.
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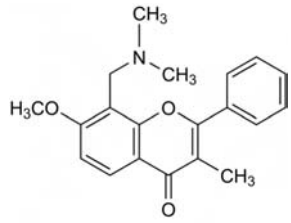
Yeung PK *et al.* (1991). Stability of diltiazem and its metabolites in plasma during storage. *Ther Drug Monit* 13: 369–374.
Yeung PK *et al.* (1996). Steady-state plasma concentrations of diltiazem and its metabolites in patients and healthy volunteers. *Ther Drug Monit* 18: 40–45.
Zendelovska D *et al.* (2003). High-performance liquid chromatographic determination of diltiazem in human plasma after solid-phase and liquid-liquid extraction. *Anal Bioanal Chem* 376: 848–853.
Zhao H, Chow MS (1989). Analysis of diltiazem and desacetyldiltiazem in plasma using modified high-performance liquid chromatography: improved sensitivity and reproducibility. *Pharm Res* 6: 428–430.

Dimefline

Respiratory Stimulant
$C_{20}H_{21}NO_3 = 323.4$
CAS—1165-48-6
IUPAC Name 8-[(Dimethylamino)methyl]-7-methoxy-3-methyl-2-phenyl-4*H*-1-benzopyran-4-one

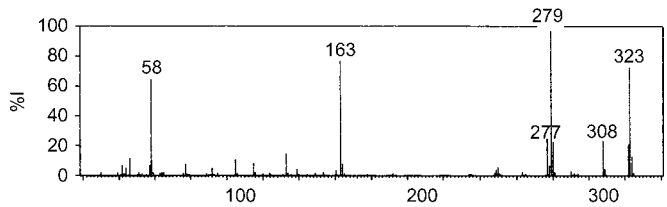


Chemical Properties Log *P* (octanol/water), 3.6.

Dimefline Hydrochloride

$C_{20}H_{21}NO_3,HCl = 359.9$
CAS—2740-04-7
Proprietary Name *Remeflin*
Chemical Properties A white crystalline powder. 213° with decomposition. Soluble in water and ethanol; practically insoluble in chloroform and ether.

Colour Test Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.15; system TC—R_f 0.48; system TL—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2555.
Ultraviolet Spectrum Aqueous acid—309 nm (A_1^1=950b).
Infrared Spectrum Principal peaks at wavenumbers 1637, 1278, 1602, 1081, 699, 1136 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 279, 163, 323, 58, 277, 308, 280, 322.

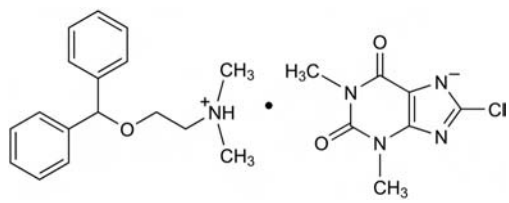


Quantification
Urine **GC** Nitrogen specific detection. Limit of detection, 10 µg/L [Delbeke, Debackere 1991].
Dose Dimefline hydrochloride has been given in doses of 16 to 48 mg daily.

Delbeke FT, Debackere M (1991). The influence of diuretics on the excretion and metabolism of doping agents–V. Dimefline. *J Pharm Biomed Anal* 9: 23–28.

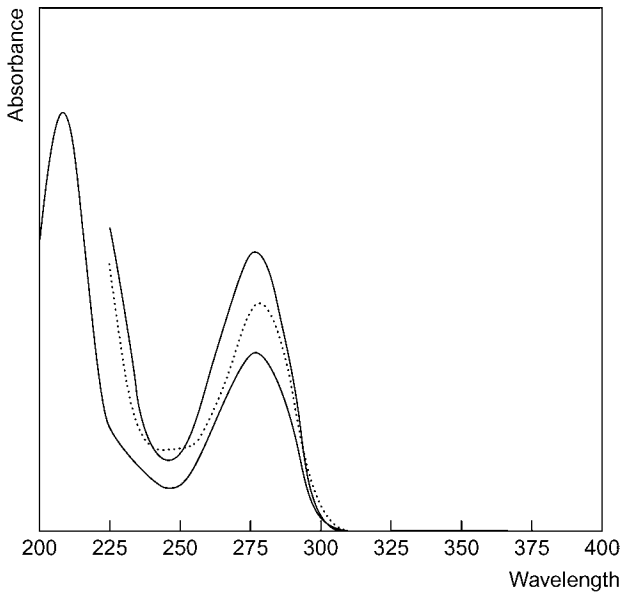
Dimenhydrinate

Antihistamine
$C_{17}H_{21}NO,C_7H_7ClN_4O_2 = 470.0$
CAS—523-87-5
IUPAC Name 8-Chloro-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione compound with 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (1:1)
Synonyms Chloranautine; diphenhydramine teoclate; diphenhydramine theoclate; dimenhydrinatum.
Proprietary Names *Amosyt*; *Antemin*; *Calm-X*; *Dimen*; *Dimetabs*; *Dinate*; *Dramamine*; *Dramanate*; *Dramilin*; *Dymenate*; *Emedyl*; *Gravol*; *Hydrate*; *Lomarin*; *Marmine*; *Motozina*; *Nauseatol*; *Nausex*; *Nausicalm*; *Reisegold*; *Reisetabletten-ratiopharm*; *RubieMen*; *Superpep*; *Tega-Vert*; *Travamine*; *Travel-Gum*; *Trawell*; *Triptone*; *Vagomine*; *Valontan*; *Vertab*; *Vertigo-Vomex*; *Vertirosan*; *Vomacur*; *Xamamina*.

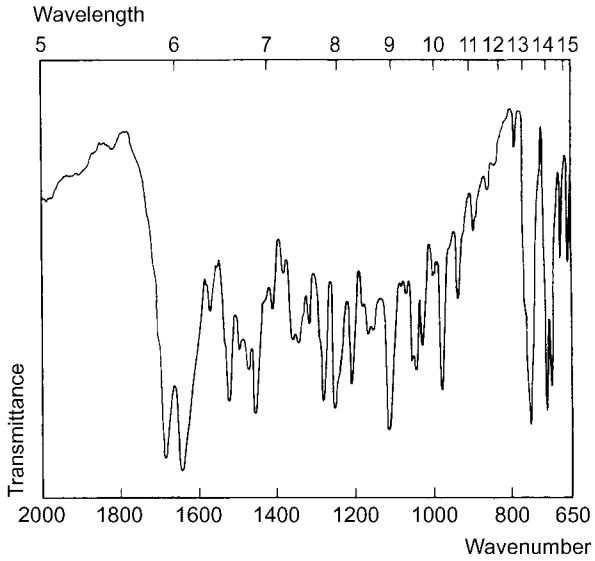


Chemical Properties A white crystalline powder. Mp 102° to 107°. Solubility

in water about 3 mg/mL; soluble 1 in 2 of ethanol and 1 in 2 of chloroform; soluble in benzene; almost insoluble in ether. Log *P* (octanol/water), −0.4.
Thin-layer Chromatography System TA—R_f 0.55 and R_f 0.88; system TB—R_f 0.45 and R_f 0.00; system TC—R_f 0.33 and R_f 0.10; system TE—R_f 0.68 and R_f 0.02; system TL—R_f 0.15 and R_f 0.10; system TAE—R_f 0.28 and R_f 0.87; system TAF—R_f 0.48 and R_f 0.46.
Gas Chromatography System GA—RI 1844.
High Performance Liquid Chromatography System HY—RI 182.
Ultraviolet Spectrum Aqueous acid—208, 276 nm ($A^{1\%}_{1cm}$=268a); aqueous alkali—278 nm.



Infrared Spectrum Principal peaks at wavenumbers 1640, 1685, 1118, 755, 712, 1255 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 73, 45, 43, 57, 167, 44, 165.
Quantification
Serum **HPLC** UV detection. Limit of detection, 50 µg/mL [Skofitsch, Lembeck 1983].
Toxicity

A 19-year-old female died after ingesting 5 g of dimenhydrinate in a suicidal gesture; within an hour of the ingestion, status epilepticus and ventricular dysrhythmias presented. Diphenhydramine concentrations in the urine were 10.8 mg/L [Winn, McDonnell 1993].

Dose 50 to 100 mg three or four times daily.

Farrell M *et al.* (1991). Response of life threatening dimenhydrinate intoxication to sodium bicarbonate administration. *J Toxicol Clin Toxicol* 29: 527–535.
Skofitsch G, Lembeck F (1983). Serum levels of dimenhydrinate. Determination by HPLC with UV detection after intake of dimenhydrinate in a coated chewing gum dragee. *Arzneimittelforschung* 33: 1674–1676.
Winn RE, McDonnell KP (1993). Fatality secondary to massive overdose of dimenhydrinate. *Ann Emerg Med* 22: 1481–1484.

Dimenoxadole

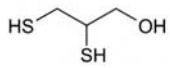
Diphenylacetic Acid Derivative, Narcotic Analgesic
$C_{20}H_{25}NO_3$ = 327.4
CAS—509-78-4

IUPAC Name 2-Dimethylaminoethyl α-ethoxy-α,α-diphenylacetate
Synonym Dimenoxadol
Proprietary Name *Estocin*
Chemical Properties Mp 170° to 172°.
Colour Tests Ammonium molybdate test—orange→dull purple (limit of detection, 0.1 µg); ammonium vanadate test—orange→dull purple (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—orange→greenish-blue (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.60 (location reagent acidified iodoplatinate spray, positive reaction).
Toxicity LD_{50} (oral): in mice 700 to 800 mg/kg.

Dimercaprol

Chelating Agent
$C_3H_8OS_2$ = 124.2
CAS—59-52-9

IUPAC Name 2,3-Dimercapto-1-propanol
Synonyms BAL; British anti-lewisite; dimercaptopropanol.
Proprietary Name *Sulfactin*

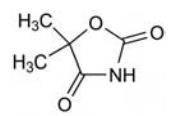


Chemical Properties A clear, colourless, or slightly yellow liquid. Relative density 1.239 to 1.259. Refractive index 1.568 to 1.574. Soluble 1 in 20 of water; miscible with ethanol, ether and methanol; soluble in vegetable oils. pK_a 8.6 (25°). Log *P* (octanol/water), 0.2.
Colour Test Palladium chloride—yellow.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1022, 1050, 990, 1270, 1235, 1170 cm^{-1} (thin film).
Dose 2.5 to 4 mg/kg, intramuscularly, up to 6 times daily as an antidote to arsenic, gold and mercury poisoning.

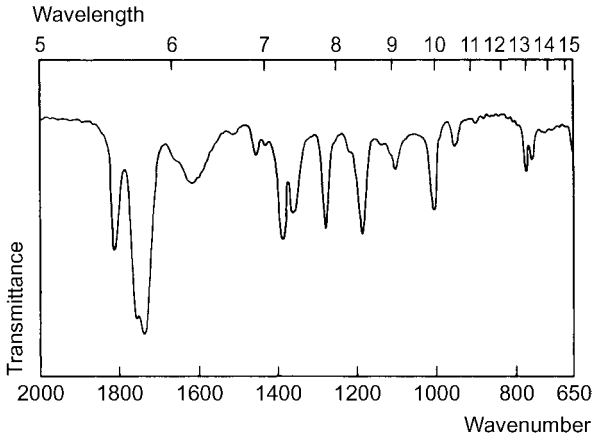
Dimethadione

Anticonvulsant
$C_5H_7NO_3$ = 129.1
CAS—695-53-4

IUPAC Name 5,5-Dimethyl-2,4-oxazolidinedione



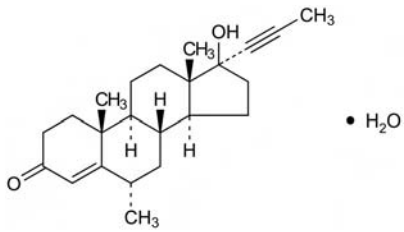
Chemical Properties Crystals. Mp 76° to 77°. pK_a 6.1 (37°). Log *P* (octanol/water), 0.2.
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.
Gas Chromatography System GA—RI 1060.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1740, 1760, 1815, 1188, 1280, 1007 cm^{-1}.



Quantification See under Trimethadione.
Disposition in the Body Dimethadione is the active metabolite of trimethadione. It is very slowly excreted in the urine over a period of several days, the rate of excretion being increased in alkaline urine.
Toxicity Toxic effects may be associated with plasma concentrations greater than 1 g/L.
Half-life Derived from urinary excretion data, 6 to 13 days.

Dimethisterone

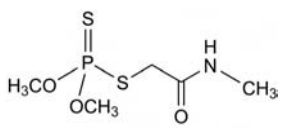
Progestational Steroid
$C_{23}H_{32}O_2,H_2O = 358.5$
CAS—79-64-1 (anhydrous); 41354-30-7 (monohydrate)
IUPAC Name (6*S*,8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-6,10,13-trimethyl-17-prop-1-ynyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-one hydrate
Synonyms 6α,21-Dimethylethisterone; (6α,17β)-17-hydroxy-6-methyl-17-(1-propynyl)androst-4-en-3-one monohydrate.



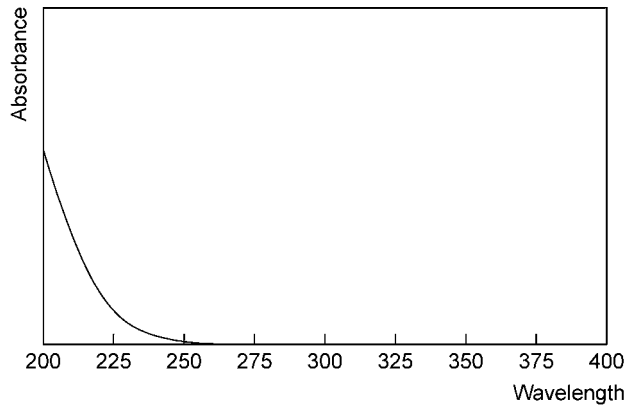
Chemical Properties A white crystalline powder. Mp 102°. Practically insoluble in water; soluble 1 in 3 of ethanol, 1 in 0.7 of chloroform and 1 in 1 of pyridine; slightly soluble in acetone. Log *P* (octanol/water), 4.4.
Colour Tests Antimony pentachloride—brown; naphthol–sulfuric acid—red-brown/brown-green; sulfuric acid—orange-red.
Thin-layer Chromatography System TP—R_f 0.80; system TQ—R_f 0.42; system TR—R_f 0.91; system TS—R_f 0.95.
Ultraviolet Spectrum Dimethisterone anhydrous: dehydrated alcohol—240 nm (A_1^1=467a).
Infrared Spectrum Principal peaks at wavenumbers 1658, 1605, 1275, 876, 1281, 1241 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 67, 137, 91, 138, 55, 79, 41, 95.
Dose Dimethisterone has been given in doses of 15 mg daily.

Dimethoate

Insecticide
$C_5H_{12}NO_3PS_2 = 229.3$
CAS—60-51-5
IUPAC Name 2-Dimethoxyphosphinothioylsulfanyl-*N*-methylacetamide
Synonyms Fosfamid; phosphorodithioic acid *O*,*O*-dimethyl-*S*-[2-(methylamino)-2-oxoethyl] ester.
Proprietary Names *Cygon*; *Fostion MM*; *Rogor E*; *Roxion*.



Chemical Properties A white solid. Mp 52° to 52.5°. Soluble 1 in 40 of water; freely soluble in most organic solvents except saturated hydrocarbons. Log *P* (octanol/water), 0.8. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].
Colour Tests Palladium chloride—yellow; phosphorus test—yellow.
Thin-layer Chromatography System TE—R_f 0.99; system TL—R_f 0.01; system TW—R_f 0.19; system TX—R_f 0.04; system TY—R_f 0.04; system TZ—R_f 0.37; system TAA—R_f 0.08; system TAB—R_f 0.03; system TAE—R_f 0.80.
Gas Chromatography System GA—dimethoate RI 1725, M (OH-) RI 1430, M (COOH-)-Me RI 1400; system GK—dimethoate RRT 0.86 (relative to caffeine).
High Performance Liquid Chromatography System HY—RI 289; system HZ—retention time 2.8 min.
Ultraviolet Spectrum

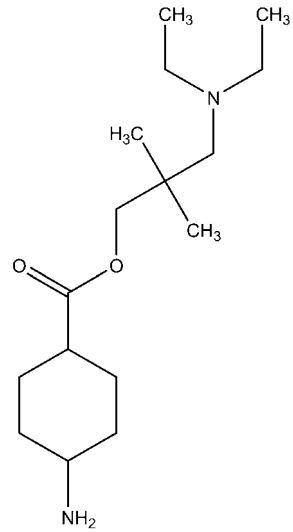


Infrared Spectrum Principal peaks at wavenumbers 1010, 1666, 819, 666, 1538, 1176 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 87, 93, 125, 58, 47, 63, 79, 42.
Quantification
Blood **GC** FPD. For method, see Kojima *et al.* [1990].
Disposition in the Body
Toxicity
In a 57-year-old male who became semi-comatose after ingesting not more than 22 g of formothion, blood levels of dimethoate at ~1.5 and 6 h after ingestion were 21.4 and 12.7 μg/g, respectively [Kojima *et al.* 1990].

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Kojima T *et al.* (1990). Determination of dimethoate in blood and hemoperfusion cartridge following ingestion of formothion: a case study. *Forensic Sci Int* 48: 79–88.

Dimethocaine

Anaesthetic (Local)
$C_{16}H_{26}N_2O_2 = 278.4$
CAS—94-15-5
IUPAC Name [3-(Diethylamino)-2,2-dimethylpropyl] 4-aminobenzoate
Synonym 3-*ρ*-Aminobenzoyloxy-*N*,*N*-diethyl-2,2-dimethyl-propylamine



Chemical Properties A white crystalline powder.

Dimethocaine Hydrochloride
Proprietary Name *Larocain(e)*

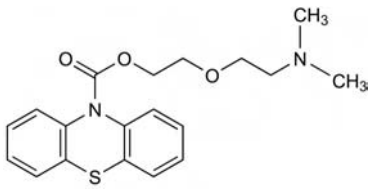
Chemical Properties Minute crystals or powder. Mp 196° to 197°. Soluble 1 in 3 of water and 1 in 10 of ethanol; insoluble in ether. Dimethocaine is extracted by chloroform from alkaline solutions.

Colour Tests Ammonium vanadate test—faint grey rim (limit of detection, 0.1 µg); Vitali's test—pale yellow/bright yellow (limit of detection, 0.25 µg). Dimethocaine may be diazotised and coupled with alkaline β-naphthol to give a red colour.
Thin-layer Chromatography System T1—R_f 0.69 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 2.00 relative to diphenhydramine; retention time 0.48 relative to codeine; System G4/225—retention time 1.29 relative to diphenhydramine; retention time 0.13 relative to codeine.
Toxicity LD_{50} (SC): in mice 300 mg/kg.

Dimethoxanate

Cough Suppressant
$C_{19}H_{22}N_2O_3S = 358.5$
CAS—477-93-0

IUPAC Name 2-(2-Dimethylaminoethyloxy)ethyl phenothiazine-10-carboxylate
Synonym 10*H*-Phenothiazine-10-carboxylic acid 2-[2-(dimethylamino)ethoxy] ethyl ester

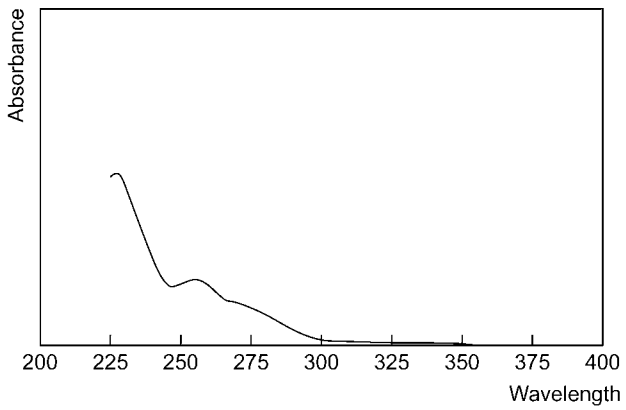


Chemical Properties Log *P* (octanol/water), 4.1.

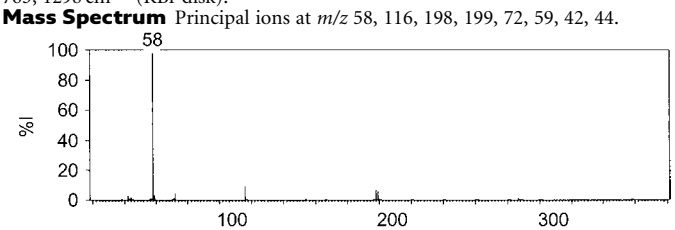
Dimethoxanate Hydrochloride

$C_{19}H_{22}N_2O_3S,HCl = 394.9$
CAS—518-63-8
Proprietary Name *Cotrane*
Chemical Properties Crystals. Mp 161° to 163°, with decomposition.

Colour Tests Mandelin's test—green→brown; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.39; system TB—R_f 0.18; system TC—R_f 0.24; system TE—R_f 0.49; system TL—R_f 0.06; system TAE—R_f 0.21; system TAF—R_f 0.38; system TAJ—R_f 0.10; system TAK—R_f 0.84; system TAL—R_f 0.49 (Dragendorff spray, positive; FPN reagent, violet; acidified iodoplatinate solution, positive; Marquis reagent, brown; ninhydrin spray, positive).
Gas Chromatography System GA—RI 2029.
High Performance Liquid Chromatography System HA—*k* 5.8 (tailing peak).
Ultraviolet Spectrum Aqueous acid—254 nm (A_1^1=24b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1718, 1317, 1217, 1250, 763, 1298 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 116, 198, 199, 72, 59, 42, 44.

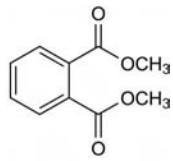


Dose Dimethoxanate hydrochloride has been given in doses of 75 to 200 mg daily.

Dimethyl Phthalate

Insect Repellent
$C_{10}H_{10}O_4 = 194.2$
CAS—131-11-3

IUPAC Name Dimethyl benzene-1,2-dicarboxylate
Synonyms 1,2-Benzenedicarboxylic acid dimethyl ester; DMP; methyl phthalate.
Proprietary Names *Avolin*; *Fermine*; *Mipax*; *Palitinol M*.

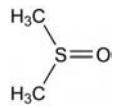


Chemical Properties A colourless liquid. Mass per mL 1.186 to 1.192 g. Bp about 280°, with decomposition. Refractive index 1.515 to 1.517. Soluble 1 in 250 of water; miscible with ethanol, ether, chloroform and most organic solvents; practically insoluble in petroleum ether and other paraffin hydrocarbons. Log *P* (octanol/water), 1.6.
Gas Chromatography System GA—RI 1406.
High Performance Liquid Chromatography System HAA—retention time 17.0 min.
Ultraviolet Spectrum Ethanol—275 nm (A_1^1=75b).
Mass Spectrum Principal ions at *m/z* 163, 77, 164, 135, 194, 76, 92, 50.
Use Topically in concentrations of at least 40%.

Dimethyl Sulfoxide

Solvent
C_2H_6OS=78.13
CAS—67-68-5

IUPAC Name Methylsulfinylmethane
Synonyms Dimethyl sulphoxide; dimexide; DMSO; methyl sulfoxide.
Proprietary Names *Deltan*; *Dolicur*; *Dolmoso*; *Kemsol*; *Rheumabene*; *Rimso-50*. It is an ingredient of *Herpid* and *Iduridin*.



Chemical Properties A colourless, hygroscopic, viscous liquid. Mp not lower than 18.3°. Bp 189° to 192°. Refractive index 1.478 to 1.479. Soluble in water, ethanol, acetone, benzene, chloroform, ether and most organic solvents. Log *P* (octanol/water), −1.4.
Colour Test 1% Potassium Permanganate Solution—decolourises; cautiously add 1.5 mL dropwise to 2.5 mL of hydriodic acid cooled in ice and filter rapidly—unstable violet residue that is soluble in chloroform giving a red solution.
Gas Chromatography System GAA—RI 784.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Mass Spectrum Principal ions at m/z 63, 78, 45, 61, 46, 62, 48, 47.
Quantification
Plasma **GC** FID detection [Paulin *et al.* 1966]. Column: (640 × 0.2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 155° for 3 min to 170° at 30°/min for 3.5 min. FID. Limit of detection, 10 mg/L [Garretson, Aitchison 1982]. FID detection [Mehta *et al.* 1986].

GC-MS Column: (1.07 m × 2.0 mm i.d.) Temperature: 170°. SIM acquisition mode [Garretson, Aitchison 1982].
Serum **GC** See Plasma [Garretson, Aitchison 1982]. FID detection [Hucker *et al.* 1967].
CSF **GC** See Plasma [Garretson, Aitchison 1982]. See Plasma [Paulin *et al.* 1966].
Urine **GC** See Serum [Hucker *et al.* 1967]. See Plasma [Garretson, Aitchison 1982; Mehta *et al.* 1986].

GC-MS See Plasma [Garretson, Aitchison 1982]. Column: DB-WAX (30 m × 0.25 mm i.d., 0.5 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 1 min to 230° at 10°/min. Limit of detection, 0.04 mg/L [Takeuchi *et al.* 2010].
Disposition in the Body Dimethyl sulfoxide is readily absorbed after injection or after oral or percutaneous administration and widely distributed throughout the body. It is oxidised to dimethyl sulfone. After an oral dose, ~30–70% is excreted in the urine unchanged and ~20% as the sulfone; after percutaneous administration, ~13% is excreted unchanged and ~18% as the sulfone; ~3% of a dose is eliminated through the lungs as dimethyl sulfide.
Therapeutic Concentration
After an oral dose of 1 g/kg to 6 subjects, peak serum concentrations of dimethyl sulfoxide of 1029–3380 mg/L (mean, 2200) were attained in 1–4 h, and peak concentrations of the sulfone metabolite of 260–600 mg/L were reported after ~72 h [Hucker *et al.* 1967].

After percutaneous administration of 1 g/kg to 2 subjects, peak serum concentrations of ~500 mg/L dimethyl sulfoxide and 350 mg/L of the sulfone metabolite were reported after 4–8 h and 36–72 h, respectively [Hucker *et al.* 1967].

The mean peak plasma dimethyl sulfoxide concentration in 10 subjects undergoing peripheral blood stem cell transplants (cryopreserved with 10% DMSO v/v; patients received infusions containing 254–824 mmol (over 20–120 min)) was 19.1 mmol/L (0.5 mg/L) 5 min after the end of the infusion. Plasma dimethyl sulfone concentrations increased during the first 24 h, plateaued at 4.4 mmol/L and remained at this level until 48 h. Dimethyl sulfoxide concentrations were at steady state by 5 min and remained between 3 and 5 mmol/L for 48 h [Egorin *et al.* 1998].

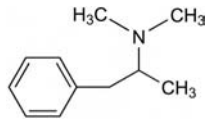
Toxicity Dimethyl sulfoxide has low systemic toxicity but gives local toxic effects.
Half-life Plasma: dimethyl sulfoxide ~10–20 h, sulfone metabolite ~70 h.

Egorin MJ *et al.* (1998). Plasma concentrations and pharmacokinetics of dimethylsulfoxide and its metabolites in patients undergoing peripheral-blood stem-cell transplants. *J Clin Oncol* 16: 610–615.
Garretson SE, Aitchison JP (1982). Determination of dimethyl sulfoxide in serum and other body fluids by gas chromatography. *J Anal Toxicol* 6: 76–81.
Hucker HB *et al.* (1967). Studies on the absorption, excretion and metabolism of dimethylsulfoxide (DMSO) in man. *J Pharmacol Exp Ther* 155: 309–317.
Mehta AC *et al.* (1986). Rapid gas chromatographic determination of dimethyl sulphoxide and its metabolite dimethyl sulphone in plasma and urine. *J Chromatogr* 383: 400–404.
Paulin HJ *et al.* (1966). Determination of dimethyl sulfoxide in plasma and cerebrospinal fluid by gas–liquid chromatography. *Anal Chem* 38: 651–652.
Takeuchi A *et al.* (2010). Determination of dimethyl sulfoxide and dimethyl sulfone in urine by gas chromatography–mass spectrometry after preparation using 2,2-dimethoxypropane. *Biomed Chromatogr* 24: 465–471.

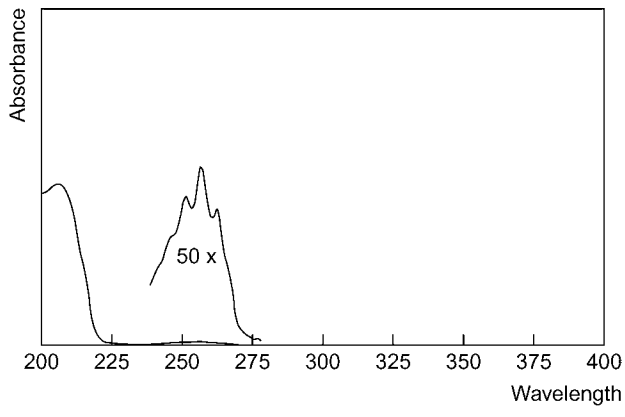
Dimethylamfetamine

Central Stimulant, Phenethylamine
$C_{11}H_{17}N$ = 163.3
CAS—4075-96-1

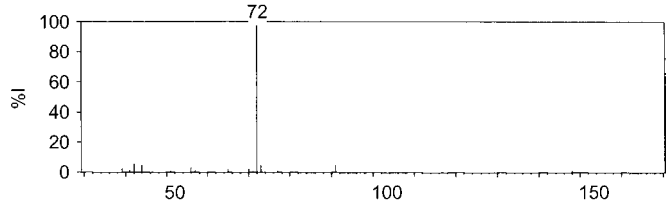
IUPAC Name *N,N*-Dimethyl-2-phenylpropan-1-amine
Synonyms Dimethylamphetamine; *N,N,*α-trimethylphenethylamine.



Chemical Properties pK_a 9.8.
Gas Chromatography System GA—RI 1235; system GC—RI 1429.
High Performance Liquid Chromatography System HB—*k* 11.08; system HC—*k* 1.89.
Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 72, 91, 73, 44, 42, 56, 70, 65.



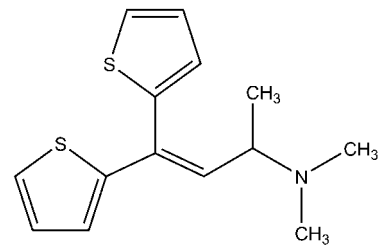
Quantification
Urine **LC-MS** Column: CAPCELL PAK C_{18} MG II (150 × 2 mm, 5 µm). Mobile phase: 5 mmol/L ammonium formate (pH 4.0) : acetonitrile, flow rate 230 µL/min. Limit of detection, <1.95 µg/L for dimethylamfetamine and other amfetamines [Kim *et al.* 2008]. Column: Alltech Platinum EPS C_{18}. Mobile phase: 0.01 mol/L ammonium formate (pH 3.0) : acetonitrile (77 : 23), flow rate 0.2 mL/min. Dimethylamfetamine, metamfetamine and their metabolites [Cheng *et al.* 2007]. Column: Asahipak GS-320HQ (300 × 7.6 mm i.d., 1 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate buffer (pH 8.5, 30 : 70), flow rate 0.6 mL/min. ESI, SIM acquisition mode. Limit of detection, 1 µg/L for dimethylamfetamine, dimethylamfetamine *N*-oxide and metamfetamine and 3 µg/L for amfetamine [Sato *et al.* 2002]. Column: SCX (150 × 2.0 mm i.d.). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 4; 65 : 35), flow rate 0.2 mL/min. Limit of detection, 5 µg/L for dimethylamfetamine *N*-oxide, 5 µg/L for dimethylamfetamine, 50 µg/L for amfetamine, and 10 µg/L for metamfetamine [Katagi *et al.* 2000].
Disposition in the Body Dimethylamfetamine is metabolised to metamfetamine. For a study on the metabolism of dimethylamfetamine, see Inoue, Suzuki [1987].

Cheng WC *et al.* (2007). A rapid and convenient LC/MS method for routine identification of methamphetamine/dimethylamphetamine and their metabolites in urine. *Forensic Sci Int* 166: 1–7.
Inoue T, Suzuki S (1987). The metabolism of dimethylamphetamine in rat and man. *Xenobiotica* 17: 965–971.
Katagi M *et al.* (2000). Discrimination of dimethylamphetamine and methamphetamine use: simultaneous determination of dimethylamphetamine-*N*-oxide and other metabolites in urine by high-performance liquid chromatography–electrospray ionization mass spectrometry. *J Anal Toxicol* 24: 354–358.
Kim JY *et al.* (2008). Simultaneous determination of methamphetamine, 3,4-methylenedioxy-*N*-methylamphetamine, 3,4-methylenedioxy-*N*-ethylamphetamine, *N,N*-dimethylamphetamine, and their metabolites in urine by liquid chromatography–electrospray ionization-tandem mass spectrometry. *Arch Pharm Res* 31: 1644–1651.
Sato M *et al.* (2002). Analysis of dimethylamphetamine and its metabolites in human urine by liquid chromatography–electrospray ionization-mass spectrometry with direct sample injection. *Forensic Sci Int* 128: 146–154.

Dimethylthiambutene

Thienyl Opioid Derivative, Narcotic
$C_{14}H_{17}NS_2$ = 263.4
CAS—524-84-5

IUPAC Name *N,N*-Dimethyl-1-methyl-3,3-di-2-thienylallylamine
Synonyms 338C48; 3-dimethylamino-1,1-bis(2-thienyl)-1-butene; 3-dimethylamino-1,1-di(2′-thienyl)but-1-ene; *N,N*-dimethyl-4,4-di-2-thienyl-3-buten-2-amine; NIH-4542; *N,N,*1-trimethyl-3,3-di-2-thienylallylamine.



Chemical Properties Viscous oil. $Bp_{0.05}$ 123° to 125°. Bp_3 157° to 158°. Soluble in ether and chloroform [O'Neil *et al.* 2006]. Log *P* (octanol/water) 3.53 [Meylan, Howard 1995]. Extracted by organic solvents from aqueous alkaline solutions.

Dimethylthiambutene Hydrochloride

$C_{14}H_{17}NS_2$,HCl = 299.9
Proprietary Names *Aminobutene*; *Dimethibutin*; *Kobaton*; *Ohton*; *Takaton*.
Chemical Properties Mp 168° to 169°. Soluble in water and chloroform.

Colour Tests Ammonium molybdate test—orange-brown→pale green (limit of detection, 0.1 µg); ammonium vanadate test—brown→blue-green (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—purple→brown (limit of detection, 0.1 µg); Vitali's test—red→green/brown/dark brown (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.55 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.10 relative to diphenhydramine; system G4—retention time 0.74 relative to diphenhydramine.
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—286, 268, 227 nm.
Disposition in the Body
Toxicity LD_{50} (oral): in mice 199 mg/kg.
Dose Most often used in veterinary medicine.

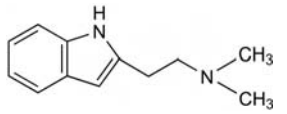
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals,* 14th edn. Whitehouse Station, NJ: Merck & Co., Inc.

Dimethyltryptamine

Hallucinogen
$C_{12}H_{16}N_2$ = 188.3
CAS—61-50-7

IUPAC Name *N,N*-Dimethyl-1*H*-indole-3-ethanamine

Synonyms Businessman's trip; *N,N*-dimethyltryptamine; DMT.

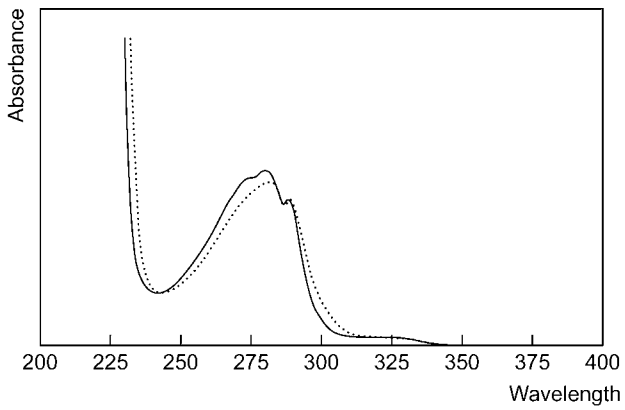


Chemical Properties Crystals. An active principle obtained from the seeds and leaves of *Piptadenia peregrina* (Mimosaceae). Mp 44.6° to 46.8°. Freely soluble in dilute acetic acid and dilute mineral acid.

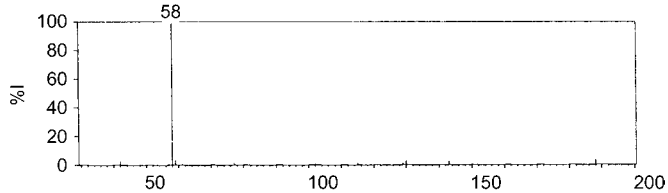
Dimethyltryptamine Hydrochloride

Chemical Properties A white crystalline powder. Mp 165° to 168°. Soluble in water. pK_a 8.7 (ethanol–water). Log *P* (octanol/water), 1.9.

Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.09; system TC—R_f 0.09; system TE—R_f 0.50; system TL—R_f 0.06; system TAE—R_f 0.14; system TAF—R_f 0.39 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; Marquis test, grey-brown; Van Urk reagent, blue).
Gas Chromatography System GA—RI 1753.
High Performance Liquid Chromatography System HY—RI 228.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1 = 327a), 288 nm.



Infrared Spectrum Principal peaks at wavenumbers 743, 1113, 1235, 1050, 812, 1010 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 188, 130, 59, 42, 143, 129, 115.



Quantification

Blood **GC** Column: DB-1 fused silica capillary (30 m × 0.32 mm i.d., 0.2 µm). Carrier gas: H_2, 3 or 3.5 mL/min. Temperature: 230° and 280°. SID. Limit of detection, 0.5 µg/L [Ishii *et al.* 1997].

GC-MS Column: SE-30 (18 m × 0.33 mm i.d.). Carrier gas: He, 2 mL/min. Temperature: 200°. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 ng/L [Walker *et al.* 1979].

Plasma **GC** Column: 5% phenyl methyl silicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: H_2, 0.7 mL/min. Temperature programme: 70° for 1 min to 120° at 30°/min to 280° at 20°/min. Limit of quantification, 1.6 µg/L [Yritia *et al.* 2002].

HPLC Column: Supelcosil LC-DB-8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.1 mol/L ammonium acetate (pH 6.9, 20 : 20 : 60), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 232 nm, λ_{em} = 351 nm or λ_{ex} = 340 nm, λ_{em} = 495 nm). Limit of quantification, 2 µg/L [Callaway *et al.* 1996].

Urine **GC** See Blood [Ishii *et al.* 1997].

GC-MS Column: 1% OV-101 on 80/100 mesh Gas-Chrom Q. Temperature: 190°. EI ionisation, MID. Limit of detection, 100 ng/L [Räisänen, Kärkkäinen 1979].

LC-MS EI ionisation at 70 eV. SRM acquisition mode. Limit of detection, 2–10 µg/L [Björnstad *et al.* 2009]. Column: Brownlee O Spheri-5 RP-18 (100 × 1.0 mm i.d., 5 µm). Mobile phase: methanol : water (50 : 50) with 0.2% formic acid, flow rate 40 µL/min. ESI, MRM acquisition mode. Limit of detection, 0.1 µg/L [Forsström *et al.* 2001].

Disposition in the Body Dimethyltryptamine is inactive when taken orally. After IM injection it is rapidly and extensively metabolised, primarily to indol-3-ylacetic acid. Approximately 33% of a dose is excreted in the urine in 6 h as free and conjugated (glucuronide) indol-3-ylacetic acid; <0.1% of a dose is excreted unchanged in the urine in 24 h.

Plasma concentrations of endogenous dimethyltryptamine are generally <0.001 mg/L.

Fifteen male volunteers, between the ages of 26 and 48 years, were administered 2 mL/kg of hoasca tea, an Amazonian sacramental beverage. The tea alkaloid content was determined to be as follows: DMT 0.24 mg/mL, harmine 1.70 mg/mL, harmaline 0.20 mg/mL and tetrahydroharmine 1.07 mg/mL. The mean peak plasma concentration of dimethyltryptamine was 15.8 µg/L, reached after 107.5 min, which coincided with peak times of psychoactivity [Callaway *et al.* 1999].

After IM injection of 0.7 mg/kg to 11 subjects, peak blood concentrations averaged 0.1 mg/L at 0.17 h, coinciding with the maximum psychoactive effects [Kaplan *et al.* 1974].

Toxicity Dimethyltryptamine produces hallucinations and perceptual distortion similar to the effects of lysergide.

Björnstad K *et al.* (2009). Bioanalytical and clinical evaluation of 103 suspected cases of intoxications with psychoactive plant materials. *Clin Toxicol (Phila)* 47: 566–572.

Callaway JC *et al.* (1996). Quantitation of *N,N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *J Anal Toxicol*,20,492–497.

Callaway JC *et al.* (1999). Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *J Ethnopharmacol* 65: 243–256.

Forsström T *et al.* (2001). Determination of potentially hallucinogenic *N*-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 61: 547–556.

Ishii A *et al.* (1997). A simple and sensitive quantitation of *N,N*-dimethyltryptamine by gas chromatography with surface ionization detection. *J Anal Toxicol* 21: 36–40.

Kaplan J *et al.* (1974). Blood and urine levels of *N,N*-dimethyltryptamine following administration of psychoactive dosages to human subjects. *Psychopharmacologia* 38: 239–245.

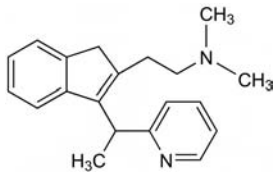
Räisänen M, Kärkkäinen J (1979). Mass fragmentographic quantification of urinary *N,N*-dimethyltryptamine and bufotenine. *J Chromatogr* 162: 579–584.

Walker RW *et al.* (1979). Improved selective ion monitoring mass-spectrometric assay for the determination of *N,N*-dimethyltryptamine in human blood utilizing capillary column gas chromatography. *J Chromatogr* 162: 539–546.

Yritia M *et al.* (2002). Determination of *N,N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of Ayahuasca. *J Chromatogr B Analyt Technol Biomed Life Sci* 779: 271–281.

Dimetindene

Antihistamine
$C_{20}H_{24}N_2$ = 292.4
CAS—5636-83-9
IUPAC Name *N,N*-Dimethyl-3-[1-(2-pyridinyl)ethyl]-1*H*-indene-2-ethanamine
Synonyms Dimethindene; dimethpyrindene; dimethylpyrindene.

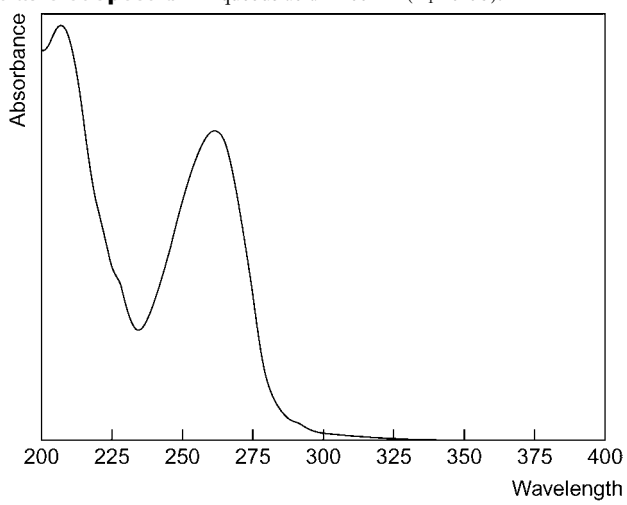


Chemical Properties Log *P* (octanol/water), 5.0. Extraction yield (chlorobutane), 0.98 [Demme *et al.* 2005].

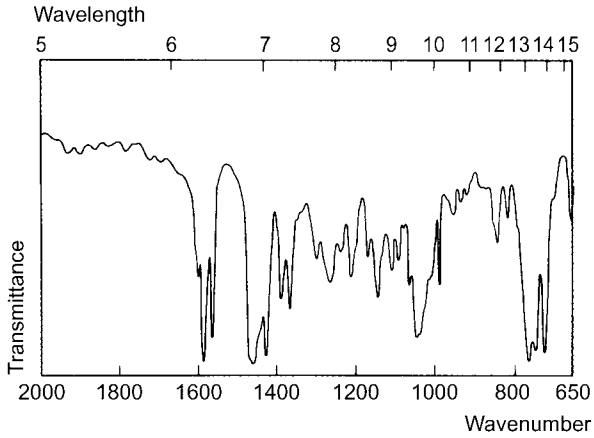
Dimetindene Maleate

$C_{20}H_{24}N_2,C_4H_4O_4$ = 408.5
CAS—3614-69-5
Proprietary Names *Fenistil*; *Neostil*. It is an ingredient of *Vibrocil*.
Chemical Properties A white to off-white crystalline powder. Mp 159° to 161°. Soluble 1 in 63 of water, 1 in 185 of ethanol and 1 in 10 of chloroform; practically insoluble in ether.

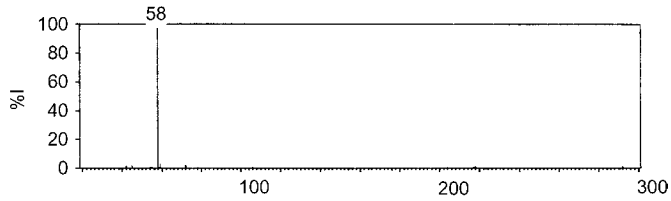
Colour Tests Cyanogen bromide—orange-pink; Mandelin's test—green; Marquis test—violet→blue.
Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.36; system TC—R_f 0.13; system TE—R_f 0.47; system TL—R_f 0.06; system TAE—R_f 0.10; system TAJ—R_f 0.04; system TAK—R_f 0.00; system TAL—R_f 0.40 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2275; system GB—RI 2376; system GC—RI 2669.
High Performance Liquid Chromatography System HA—*k* 5.1; system HX—RI 338; system HY—RI 288.
Ultraviolet Spectrum Aqueous acid—260 nm (A^1_1=613b).



Infrared Spectrum Principal peaks at wavenumbers 763, 1590, 724, 747, 1570, 1050 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 58, 59, 72, 45, 292, 218, 42.



Quantification

Plasma **GC–MS** For method, see Kauert *et al.* [1993].
Serum **GC** FID. Limit of detection, 10 µg/L [Wermeille, Huber 1982].
Urine **GC** FID. Limit of detection, 10 µg/L [Wermeille, Huber 1982].

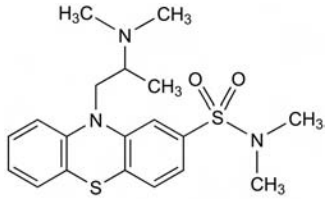
HPLC For method for quantification of dimetindene and its major metabolites, see Prien and Blaschke [1997]. Limit of detection, <5 nmol/L [Chollet *et al.* 1993]. For method for quantification of dimetindene and *N*-desmethyldimetindene, see Radler and Blaschke [1991].
Tears **HPLC** Limit of detection, <12 µg/L [Chollet *et al.* 1998].
Dose Up to 6 mg of dimetindene maleate daily.

Chollet D *et al.* (1993). High-performance liquid chromatographic method for the determination of dimethindene in urine. *J Chromatogr* 629: 89–93.
Chollet DF *et al.* (1998). Determination of dimethindene in human tears by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 707: 334–347.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Kauert G *et al.* (1993). Quantification of dimethindene in plasma by gas chromatography-mass fragmentography using ammonia chemical ionization. *J Chromatogr* 617: 318–323.
Prien D, Blaschke G (1997). Studies of the metabolism of the antihistaminic drug dimethindene by high-performance liquid chromatography and capillary electrophoresis including enantioselective aspects. *J Chromatogr B Biomed Sci Appl* 688: 309–318.
Radler S, Blaschke G (1991). Enantioselective determination of dimethindene in urine after oral administration of racemic dimethindene. *J Chromatogr* 567: 229–239.
Wermeille MM, Huber GA (1982). Gas–liquid chromatographic determination of free dimethindene in human serum and urine at low concentrations. *J Chromatogr* 228: 187–194.

Dimetotiazine

Antihistamine
$C_{19}H_{25}N_3O_2S_2$ = 391.6
CAS—7456-24-8

IUPAC Name 10-[2-(Dimethylamino)propyl]-*N*,*N*-dimethyl-10*H*-phenothiazine-2-sulfonamide
Synonyms Dimethiotazine; dimethothiazine; fonazine.



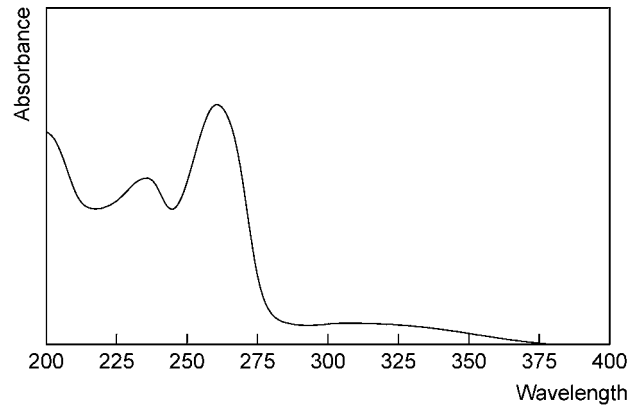
Chemical Properties Log *P* (octanol/water), 3.5.

Dimetotiazine Mesilate

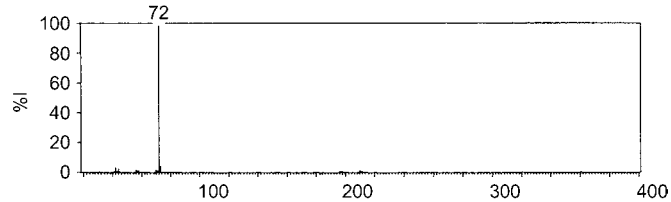
$C_{19}H_{25}N_3O_2S_2,CH_3SO_3H$ = 487.7
CAS—7455-39-2; 13115-40-7

Proprietary Names *Banistyl*; *Bonpac*; *Calsekin*; *Migristene*; *Neomestine*; *Promaquid*; *Yoristen*.
Chemical Properties A white crystalline powder. Mp about 175°. Very soluble in water; practically insoluble in ether.

Colour Tests Formaldehyde–sulfuric acid—yellow; Forrest reagent—red; FPN reagent—red; Mandelin's test—red-violet; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.13; system TC—R_f 0.48; system TL—R_f 0.28; system TAE—R_f 0.43 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—dimetotiazine RI 3060, M (nor-) RI 3150, M (OH-)-AC RI 3200, M (bis-nor-)-AC RI 3380, M (nor-)-AC RI 3360; system GB—RI 3096.
High Performance Liquid Chromatography System HA—*k* 2.1.
Ultraviolet Spectrum Aqueous acid—235, 262 nm (A^1_1=675a); aqueous alkali—268 nm (A^1_1=808b).



Infrared Spectrum Principal peaks at wavenumbers 1205, 1190, 1057, 1160, 1220, 717 cm^{-1} (dimetotiazine mesilate, KBr disk).
Mass Spectrum Principal ions at *m/z* 72, 73, 320, 71, 70, 56, 210, 198.

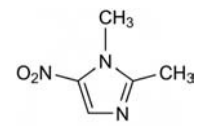


Dose Usually the equivalent of 60 to 120 mg of dimetotiazine daily.

Dimetridazole

Antiprotozoal (Veterinary)
$C_5H_7N_3O_2$ = 141.1
CAS—551-92-8

IUPAC Name 1,2-Dimethyl-5-nitro-1*H*-imidazole
Proprietary Names *Dimetrasol*; *Emtryl*; *Unizole*.



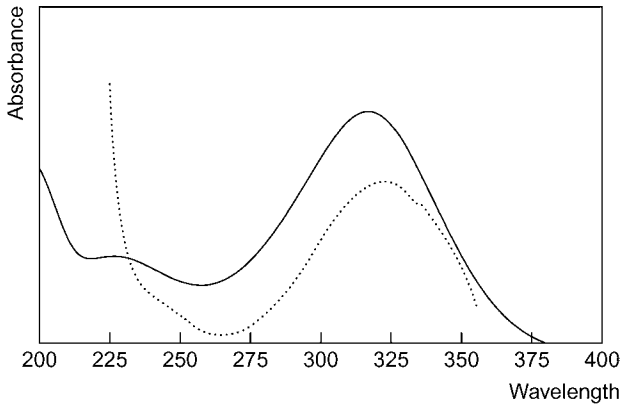
Chemical Properties An almost white to brownish-yellow powder, which darkens on exposure to light. Mp 138° to 141°. Sparingly soluble in cold water; soluble 1 in 30 of ethanol, 1 in 5 of chloroform and 1 in 170 of ether. Log *P* (octanol/water), 0.3.

Dimetridazole Hydrochloride

$C_5H_7N_3O_2$,HCl = 177.6
CAS—25332-20-1
Chemical Properties Crystals. Mp 195°. Freely soluble in water and ethanol; sparingly soluble in acetone.

Colour Test Methanolic potassium hydroxide—violet (when boiled).
Thin-layer Chromatography System TA—R_f 0.63 (acidified potassium permanganate solution, strong reaction).
Gas Chromatography System GA—RI 1353.
High Performance Liquid Chromatography System HAA—retention time 10.0 min andretention time 10.1 min.

Ultraviolet Spectrum Aqueous acid—317, 226 nm; aqueous alkali—322 nm (A_1^1=500b).

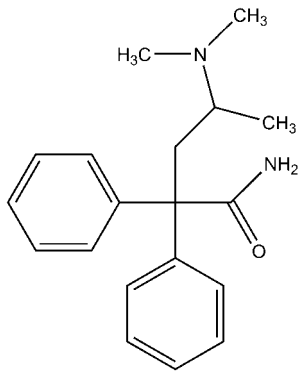


Infrared Spectrum Principal peaks at wavenumbers 1181, 1258, 1515, 825, 746, 1119 cm^{-1} (KBr disk).

Dimevamide

Acetamide, Antimuscarinic
$C_{19}H_{24}N_2O$ = 296.4
CAS—60-46-8

IUPAC Name α-[2-(Dimetylamino)propyl]-α-phenylbenzeneacetamide
Synonyms Aminopentamide; BL 139; valemeramide.
Proprietary Name *Kantrexil*



Chemical Properties The racemate crystallises as long prisms from dilute ethanol. Mp 183° to 184°. Practically insoluble in water. Extracted by organic solvents from aqueous alkaline solutions. Log *P* (octanol/water) 2.75 [Meylan, Howard 1995].

Dimevamide Sulfate

$C_{19}H_{24}N_2O,H_2SO_4$ = 394.5
CAS—35144-63-9 (xH_2SO_4)
Proprietary Name *Centrine*
Chemical Properties White crystalline powder. Mp 178° to 181°. The racemate is usually employed in medicine. Soluble in water and ethanol.

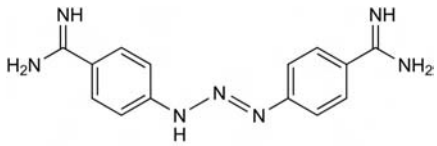
Thin-layer Chromatography System T1—R_f 0.30 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—253, 259, 265 nm.
Infrared Spectrum Principal peaks at wavenumbers 695, 750 or 1370 cm^{-1} (KBr disk).
Quantification
Urine **GC** Columns: glass column packed with 3% SE 30 or 3% OV17 on Chromosorb 750 (1 m × 2.0 mm i.d.). Carrier gas: N, 40 mL/min, H, 25 mL/min, air, 350 mL/min. Temperature: 230°. FID. Limit of detection 5 µg/L [Serfontein, de Villiers 1976].
Disposition in the Body
Therapeutic Concentration
Five adult male volunteers were administered 300 µg dimevamide in tablets of 100 µg. Dimevamide was detected (but not quantified) in the urine of all volunteers when tested 24 h post-administration [Serfontein, de Villiers 1976].
Dose Up to 2 g daily.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Serfontein WJ, deVilliers LS (1976). Monitoring aminopentamide urinary excretion by means of multiple 'microphase' extraction - a rapid method for the extraction and concentration of small amounts of lipophilic drugs from large volumes of biological fluids without distillation. *S Afr J Med Sci* 41: 221–228.

Diminazene

Antiprotozoal, Antibacterial
$C_{14}H_{15}N_7$ = 281.3
CAS—536-71-0

IUPAC Name 4-[2-(4-Carbamimidoylphenyl)iminohydrazinyl]benzenecarboximidamide
Synonym 4,4′-(1-Triazene-1,3-diyl)bis(benzenecarboximidamide)

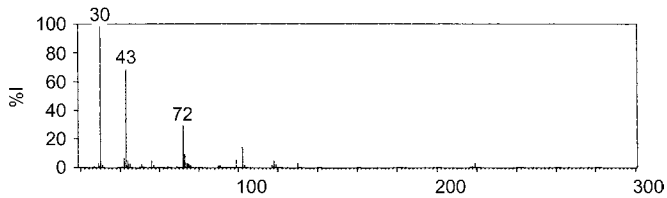


Chemical Properties Log *P* (octanol/water), 1.5 (aceturate).

Diminazene Aceturate

$C_{22}H_{29}N_9O_6,4H_2O$ = 587.6
CAS—908-54-3 (anhydrous)
Proprietary Names *Azidin*; *Berenil*; *Ganasag*; *Pirocide*.
Chemical Properties A yellow powder. Mp 217°, with decomposition. Soluble 1 in 14 of water; slightly soluble in ethanol; very slightly soluble in chloroform and ether.

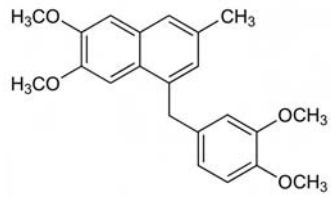
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00 (aceturate) (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Diminazene aceturate: aqueous acid—257 nm (A_1^1=305a); aqueous alkali—247 nm (broad) (A_1^1=239b).
Infrared Spectrum Principal peaks at wavenumbers 1610, 1635, 1588, 1171, 1267, 1198 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 30, 43, 72, 102, 73, 42, 118, 99.



Dimoxyline

Antispasmodic
$C_{22}H_{25}NO_4$ = 367.4
CAS—147-27-3

IUPAC Name 1-(4-Ethoxy-3-methoxybenzyl)-6,7-dimethoxy-3-methylisoquinoline
Synonym Dioxyline



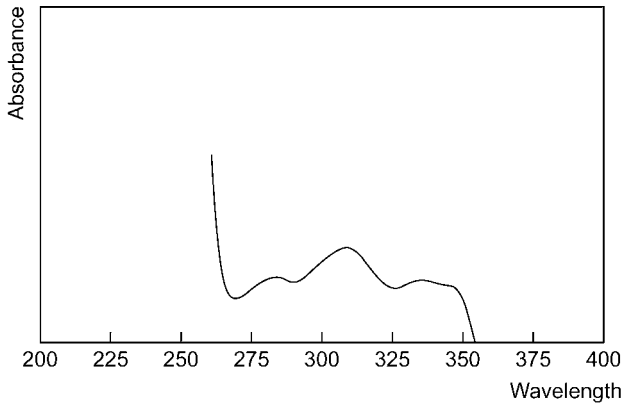
Chemical Properties Crystals. Mp 124° to 125°. Log *P* (octanol/water), 4.7.

Dimoxyline Phosphate

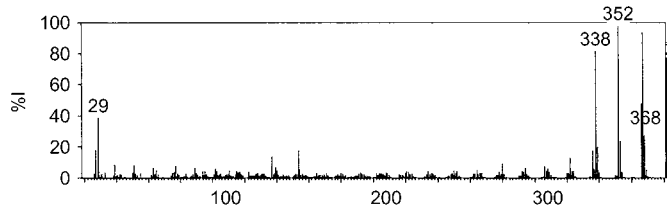
$C_{22}H_{25}NO_4,H_3PO_4$ = 465.4
CAS—5667-46-9
Proprietary Names *Paveril Phosphate*; *Paverona*.
Chemical Properties A white crystalline powder. Mp about 198°, with decomposition. Soluble 1 in 25 of water and 1 in 320 of ethanol.

Colour Tests Mandelin's test—green; Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.16; system TC—R_f 0.75; system TE—R_f 0.87; system TL—R_f 0.58; system TAJ—R_f 0.64; system TAK—R_f 0.10; system TAL—R_f 0.93 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2895.
Ultraviolet Spectrum Aqueous acid—285, 309 (A_1^1=179b), 335 nm.



Infrared Spectrum Principal peaks at wavenumbers 1504, 1247, 1208, 1145, 1225, 865 cm^{-1} (KCl disk).
Mass Spectrum Principal ions at *m/z* 352, 367, 338, 366, 29, 368, 353, 339.

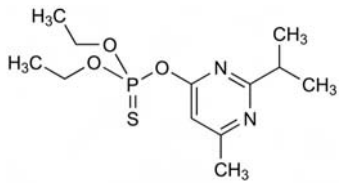


Disposition in the Body Absorbed from the gastrointestinal tract. Metabolised in the liver and excreted in the urine.
Dose 0.3 to 1.6 g of dimoxyline phosphate daily.

Dimpylate

Insecticide
$C_{12}H_{21}N_2O_3PS$ = 304.4
CAS—333-41-5

IUPAC Name Diethoxy-(6-methyl-2-propan-2-ylpyrimidin-4-yl)oxy-sulfanylidene-λ^5-phosphane
Synonyms Diazinon; phosphorothioic acid *O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]ester.
Proprietary Names *Basudin*; *Diazitol*.



Chemical Properties The pure compound is a colourless liquid; the technical grade is a pale to dark brown liquid. It is stable in alkaline solutions, but is slowly hydrolysed in water and dilute acids. Slightly soluble in water; miscible with ethanol, ether and petroleum oils. Log *P* (octanol/water), 3.8.
Colour Tests Palladium chloride—orange-brown; phosphorus test—yellow.
Thin-layer Chromatography System TE—R_f 0.39; system TF—R_f 0.64; system TW—R_f 0.82; system TX—R_f 0.47; system TY—R_f 0.50; system TZ—R_f 0.76; system TAA—R_f 0.21; system TAB—R_f 0.30; system TAC—R_f 0.20; system TAE—R_f 0.81.
Gas Chromatography System GA—RI 1760; system GK—RRT 0.79(relative to caffeine).
High Performance Liquid Chromatography System HAA—retention time 25.8 min; system HX—RI 618; system HY—RI 628; system HZ—retention time 33.2 min.
Ultraviolet Spectrum Hexane—248 nm (A_1^1=170b).
Infrared Spectrum Principal peaks at wavenumbers 1020, 1587, 971, 1562, 823, 1159 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 137, 179, 152, 93, 153, 199, 97, 43.
Quantification
Postmortem Tissues **GC** FID or ECD. For method of quantification, see Poklis *et al.* [1980].
Disposition in the Body Rapidly absorbed from the lungs and through the skin.
Toxicity The estimated minimum lethal dose is 25 g.

In 2 fatalities due to dimpylate ingestion, postmortem tissue concentrations were: blood 0.7, 33 mg/L, brain 12, 62 µg/g, kidney 3 - µg/g, liver 30, 345 µg/g, adipose tissue 37 - µg/g [Wall 1981].

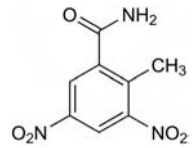
In a fatality attributed to dimpylate, the following postmortem tissue concentrations were reported: blood 277 mg/L, bile 200 mg/L, brain 2 µg/g, kidney 0.1 µg/g, liver 4 µg/g [Poklis *et al.* 1980].

Poklis A *et al.* (1980). A fatal diazinon poisoning. *Forensic Sci Int* 15: 135–140.
Wall WH (1981). *Bull Int Assoc Forensic Toxicol* 16(2): 27–28.

Dinitolmide

Coccidiostat (Veterinary)
$C_8H_7N_3O_5$ = 225.2
CAS—148-01-6

IUPAC Name 2-Methyl-3,5-dinitrobenzamide
Synonyms Dinitrotoluamide; methyldinitrobenzamide; zoalene.
Proprietary Name *Zoamix*

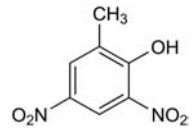


Chemical Properties A cream-coloured to light tan-coloured powder. Mp 177° to 181°. Practically insoluble in water; soluble 1 in 100 of ethanol, 1 in 15 of acetone, 1 in 650 of chloroform and 1 in 850 of ether. Log *P* (octanol/water), 0.2.
Colour Tests Methanolic potassium hydroxide—green; Nessler's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.75 (acidified iodoplatinate solution, positive, developing slowly).
Infrared Spectrum Principal peaks at wavenumbers 1527, 1672, 1612, 738, 1595, 1289 cm^{-1} (KBr disk).

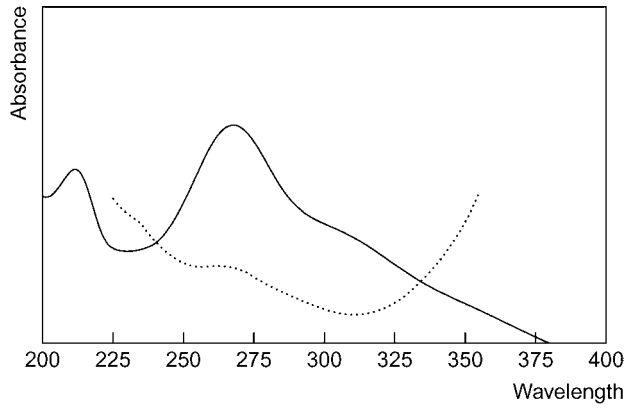
Dinitro-orthocresol

Insecticide, Herbicide
$C_7H_6N_2O_5$ = 198.1
CAS—534-52-1

IUPAC Name 2-Methyl-4,6-dinitrophenol
Synonyms Dinitrocresol; dinitrol; ditrosol; DN; DNC; DNOC; KIII; KIV.
Proprietary Names *Effusan*; *Lipan*; *Selinon*; *Sinox*.



Chemical Properties Yellowish crystals. Mp 86°. Soluble 1 in 7500 of water; readily soluble in alkaline aqueous solutions, ether, acetone, ethanol and most organic solvents; sparingly soluble in petroleum ether. pK_a 4.3 (21°). Log *P* (octanol/water), 2.1.
Caution It is explosive and is usually moistened with up to 10% of water to reduce the hazard.
Colour Test Methanolic potassium hydroxide—orange (100°).
Thin-layer Chromatography System TF—R_f 0.25; system TX—R_f 0.06; system TY—R_f 0.38.
Gas Chromatography System GA—RI 1660. system GK—RRT 0.74, Dinitro-orthocresol methyl ether (relative to caffeine).
Ultraviolet Spectrum Aqueous acid—271 nm (A_1^1=631b); aqueous alkali—265 nm (A_1^1=251b).



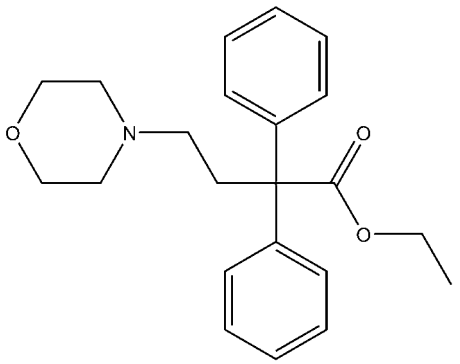
Infrared Spectrum Principal peaks at wavenumbers 1605, 1232, 1090, 1218, 1546, 1156 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 182, 165, 89, 90, 212, 51, 65, 91 (dinitro-orthocresol methyl ether).

Quantification
Blood **Colorimetry** For method, see Fenwick and Parker [1955].
Tissues **Colorimetry** See Blood [Fenwick, Parker 1955].
Disposition in the Body Absorbed through the skin, by inhalation, or by accidental ingestion. It acts as a cumulative poison causing an increase in the metabolic rate, and it is eliminated slowly over a period of weeks. After ingestion, <5% is excreted unchanged in the urine.
Toxicity The acute toxic dose is about 200 mg and the maximum permissible atmospheric concentration is 0.2 mg/m^3. Blood concentrations greater than about 50 mg/L are associated with severe toxicity.
Note For a review of poisoning by dinitro-orthocresol, see Bidstrup *et al.* [1952].

Bidstrup PL *et al.* (1952). *Lancet* 1: 794–795.
Fenwick ML, Parker VH (1955). *Analyst* 80: 774–776.

Dioxaphetyl Butyrate

Narcotic Analgesic
$C_{22}H_{27}NO_3$ = 353.3
CAS—467-86-7
IUPAC Name Ethyl 4-morpholino-4-yl-2,2-di(phenyl)butanoate
Synonym Ethyl γ-morpholino-α,α-diphenylbutyrate



Chemical Properties Mp 68.5° to 70°. Bp 170° to 180°. Soluble in water (24.5 mg/L). Log *P* (octanol/water) 3.97 [Meylan, Howard 1995]. Extracted by organic solvents from aqueous alkaline solutions.

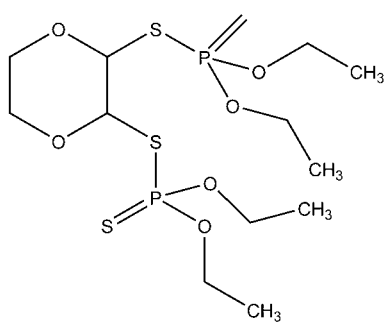
Dioxaphetyl Butyrate Hydrochloride
Synonym Amigalon
Proprietary Name *Spasmoxale*
Chemical Properties Mp 167.5° to 168.5°.

Thin-layer Chromatography System T1—R_f 0.72 (location reagent acidified iodoplatinate spray, positive reaction).

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Dioxathion

Organophosphate, Insecticide, Acaricide
$C_{12}H_{26}O_6P_2S_4$ = 456.5
CAS—78-34-2
IUPAC Name (3-Diethoxyphosphinothioylsulfanyl-1,4-dioxan-2-yl)sulfanyl-diethoxy-sulfanylidenephosphorane
Synonyms *S*,*S*′-1,4-Dioxane-2,3-diyl O,O,O′,O′-tetraethyl bis(dithiophosphate); *S*,*S*″-1,4-dioxane-2,3-diylbis(O,O-diethylphosphorothiolothionate); phosphorodithoic acid.
Proprietary Names *Bercotox*; *Delnav*; *Hercules AC 528*; *Kavadel*; *Navadel*; *Polythion*.

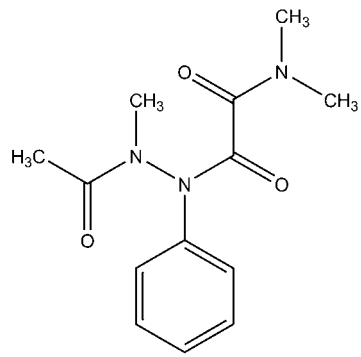


Chemical Properties Dark amber-coloured liquid or powder with a garlic odour which should be stored in well-closed containers in a cool place. Mp −20°. Miscible with ethanol, acetone, benzene, and xylene. Practically insoluble in water (1.55 mg/L). Log *P* (octanol/water) 3.45 [Meylan, Howard 1995]. Dioxathion undergoes photo-oxidation on silica gel surfaces to form corresponding oxons and various cleavage products from the phosphorus- and non-phosphorus-containing moieties [Harned, Casida 1976]. Extracted by organic solvents from aqueous acid solutions.
Thin-layer Chromatography Plates: precoated silica gel 60F254 (20 × 20 cm, 0.25 mm). Solvents systems: A) benzene : hexane (17 : 3); B) isopropyl ether : ethanol (19 : 1); C) hexane : ethyl acetate : methanol (12 : 5 : 2); D) ethyl acetate : methanol : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine; 2,6-dibromo-*N*-chloro-p-benzoquinone imine. R_f values: trans-dioxathion A) 0.31, B) 0.62, C) 0.62, D) 0.84; cis-dioxathion A) 0.26, B) 0.59, C) 0.61, D) 0.84. Limit of quantification not reported [Harned, Casida 1976].
Quantification
Other **TLC** Rat Urine and Faeces. Plates: precoated silica gel $60F_{254}$ (20 × 20 cm, 0.25 mm). Solvents systems: A) benzene : hexane (17 : 3); B) isopropyl ether : ethanol (19 : 1); C) hexane : ethyl acetate : methanol (12 : 5 : 2); D) ethyl acetate : methanol : water. R_f values: *trans*-dioxathion A) 0.31, B) 0.62, C) 0.62, D) 0.84; *cis*-dioxathion A) 0.26, B) 0.59, C) 0.61, D) 0.84. Limit of quantification not reported [Harned, Casida 1976].
Disposition in the Body Dioxathion depresses cholinesterase activity in mammals but, if applied externally in the recommended concentrations, it has a good margin of safety. When dioxathion is absorbed, it is rapidly metabolised and leaves insignificant residues in tissues.
Toxicity Very toxic when inhaled, swallowed, or spilled on the skin. The recommended strength of washes for weekly dipping or spraying of infested cattle is 0.05%. LD_{50} (IP, mg/kg): mice *trans*-dioxathion >125; *cis*-dioxathion 38 to 75 [Harned, Casida 1976].

Harned WH, Casida JE (1976). Dioxathion metabolites, photoproducts, and oxidative degradation products. *J Agric Food Chem* 24: 689–699.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Dioxyamidopyrine

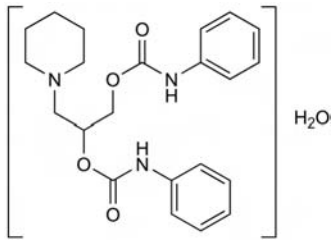
Analgesic
$C_{13}H_{17}N_3O_3$ = 263.3
IUPAC Name 2-[(Acetyl-methylamino)-phenylamino]-*N*,*N*-dimethyl-2-oxoacetamide
Synonyms β-Acetyl-β-methyl-α-dimethyloxamoylphenylhydrazine; dioxoaminopyrine; dioxyaminopyrine; dioxypyramidon.
Proprietary Name *Triamid*



Chemical Properties A translucent crystalline powder. Mp 105° to 106°. Soluble 1 in 2 of water; soluble in ethanol and chloroform. Dioxyamidopyrine is extracted by chloroform from aqueous alkaline solutions.
Colour Test Vitali's test—orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.69 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.60 relative to diphenhydramine, retention time 0.36 relative to codeine; system G4/225—retention time 5.00 relative to diphenhydamine, retention time 0.51 relative to codeine.
Ultraviolet Spectrum Methanol—236 nm (E1%, 1cm 264).

Diperodon

Anaesthetic (Local)
$C_{22}H_{27}N_3O_4,H_2O$ = 415.5
CAS—101-08-6 (anhydrous); 51552-99-9 (monohydrate)
IUPAC Name [2-(Phenylcarbamoyloxy)-3-piperidin-1-ylpropyl] *N*-phenylcarbamate
Synonyms Diperocaine; 3-piperidinopropylene bis(phenylcarbamate) monohydrate.
Proprietary Name *Diothane*



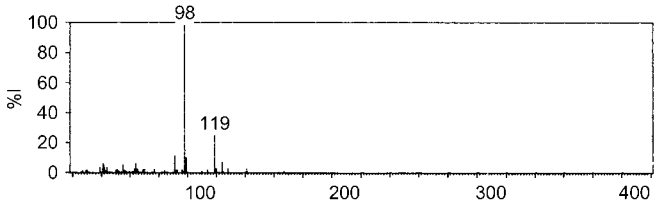
Chemical Properties A white to cream-coloured powder. Practically insoluble in water; soluble 1 in 3 of ethanol, 1 in 10 of chloroform, 1 in 4 of ether and 1 in 1 of methanol. Log *P* (octanol/water), 2.6 (hydrochloride).

Diperodon Hydrochloride

$C_{22}H_{27}N_3O_4,HCl = 433.9$
CAS—537-12-2

Chemical Properties A white crystalline powder. Mp about 198°. Soluble 1 in 100 of water; soluble in ethanol; slightly soluble in acetone; practically insoluble in ether.

Colour Tests Liebermann's reagent—brown; Mandelin's test—red→green.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.15; system TC—R_f 0.58; system TL—R_f 0.66 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2370.
High Performance Liquid Chromatography System HR—*k* 2.48.
Ultraviolet Spectrum Aqueous acid—232 nm (A^1_1=1320b).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1705, 1530, 1600, 1305, 1050 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 119, 91, 99, 124, 64, 41, 55.



Quantification

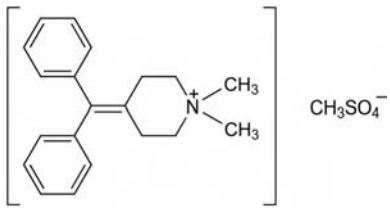
Blood **HPLC** Limit of detection, 5 µg/mL [Cizmarik *et al.* 1996].
Use Diperodon has been applied topically in a concentration of 1%.

Cizmarik J *et al.* (1996). Study of local anaesthetics. Part 133. Determination of diperodon in blood serum in vitro by the HPLC method. *Acta Pol Pharm* 53: 167–169.

Diphemanil Metilsulfate

Anticholinergic
$C_{20}H_{24}N,CH_3SO_4 = 389.5$
CAS—62-97-5

IUPAC Name 4-(Diphenylmethylene)-1,1-dimethylpiperidinium methyl sulfate
Synonyms Diphemanil methylsulfate; diphenmethanil methylsulfate; vagophemanil methylsulfate.
Proprietary Name *Prantal*

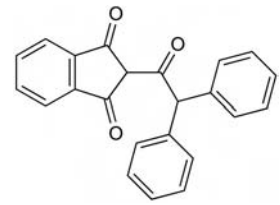


Chemical Properties A white, hygroscopic, crystalline powder. Mp 194° to 195°. Soluble 1 in 33 of water, 1 in 33 of ethanol and 1 in 33 of chloroform; very slightly soluble in ether. Log *P* (octanol/water), 2.6.
Colour Tests Liebermann's reagent—brown; Mandelin's test—brown; Marquis test—orange-red; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 708, 1250, 1230, 1010, 765, 699 cm^{-1} (KBr disk).
Dose 400 to 800 mg daily.

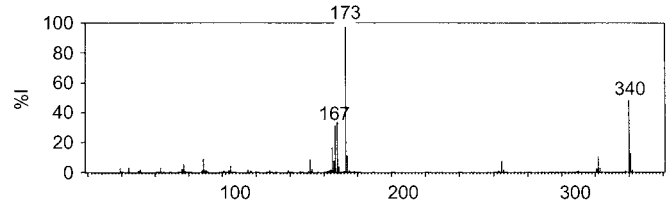
Diphenadione

Anticoagulant, Rodenticide
$C_{23}H_{16}O_3 = 340.4$
CAS—82-66-6

IUPAC Name 2-(Diphenylacetyl)-1*H*-indene-1,3(2*H*)-dione
Synonym Diphacinone
Proprietary Name *Diphacin (rodenticide)*



Chemical Properties Yellow crystals or crystalline powder. Mp 144° to 150°. Practically insoluble in water; soluble 1 in less than 100 of ethanol and chloroform; soluble in ether, acetone and glacial acetic acid; slightly soluble in benzene. Log *P* (octanol/water), 4.8.
Colour Tests Liebermann's reagent—orange-brown; methanolic potassium hydroxide—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TD—R_f 0.11; system TE—R_f 0.46; system TF—R_f 0.33; system TAD—R_f 0.53; system TAJ—R_f 0.51; system TAK—R_f 0.74; system TAL—R_f 0.91 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2934.
Ultraviolet Spectrum Aqueous acid—336 nm (A^1_1=675b). No alkaline shift.
Mass Spectrum Principal ions at *m/z* 173, 340, 168, 167, 165, 341, 174, 322.

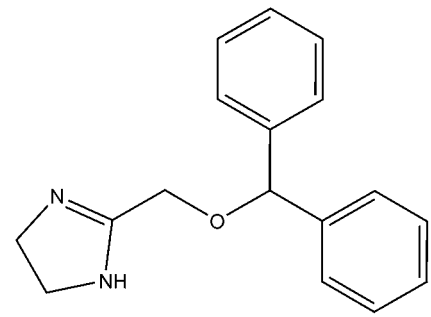


Dose Maintenance, 2.5 to 5 mg daily.

Diphenazoline

Antihistamine
$C_{17}H_{18}N_2O = 266.3$

IUPAC Name 2-[Di(phenyl)methoxymethyl]-4,5-dihydro-1*H*-imidazole
Synonyms 2-Diphenylmethoxymethyl-2-imidazoline; MG 322.



Chemical Properties Mp 102° to 103°. Soluble in chloroform. Diphenazoline is extracted by chloroform from aqueous alkaline solutions.

Diphenazoline Hydrochloride

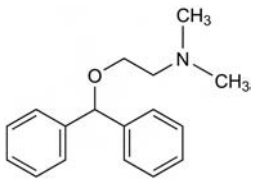
Proprietary Names *Antadril*; *Antadryl*; *Anthradil.*
Chemical Properties A white crystalline powder. Mp 205° to 207°. Soluble in water and ethanol.

Colour Tests Ammonium molybdate test—bright yellow (limit of detection, 0.1 µg); ammonium vanadate test—bright yellow (limit of detection, 0.1 µg); formaldehyde test—bright yellow (limit of detection, 0.1 µg); sulfuric acid test—bright yellow (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.27 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 3.33 relative to diphenhydramine, retention time 0.75 relative to codeine; system G4/225—retention time 0.85 relative to codeine.

Diphenhydramine

Antihistamine
$C_{17}H_{21}NO = 255.4$
CAS—58-73-1

IUPAC Name 2-Diphenylmethoxy-*N*,*N*-dimethylethanamine
Synonym Benzhydramine



Note Diphenhydramine is an isomer of phenyltoloxamine.
Chemical Properties pK_a 8.98 [Sangster 1997]. Log *P* (octanol/water), 3.27 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

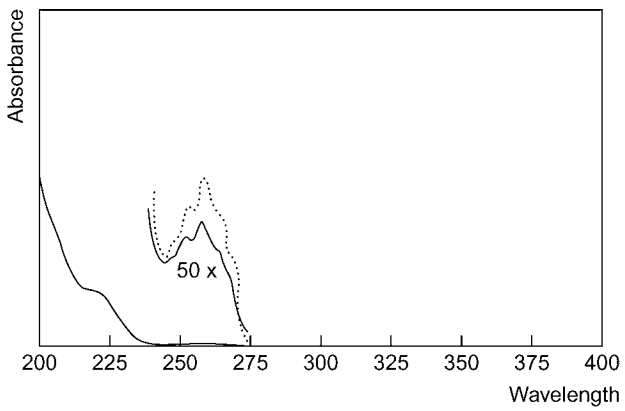
Diphenhydramine Acefyllinate

$C_{17}H_{21}NO,2C_9H_{10}N_4O_4 = 731.8$
CAS—6888-11-5
Synonyms Benzhydramine di(acefyllinate); bietanautine.
Proprietary Name *Nautamine*

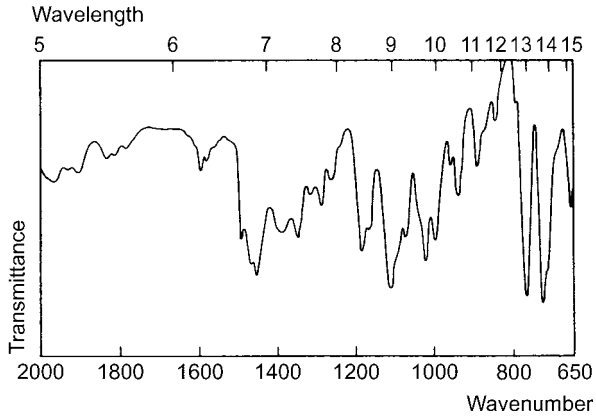
Diphenhydramine Hydrochloride

$C_{17}H_{21}NO,HCl = 291.8$
CAS—147-24-0
Synonyms Benzhydramine hydrochloride; dimedrolum.
Proprietary Names *Aller-Dryl*; *Aller-eze*; *Allermax*; *Banophen Allergy*; *Benadryl*; *Bidramine*; *Dermamycin*; *Diphen*; *Diphenhist*; *Dormin*; *Dreemon*; *Dytuss*; *Genahist*; *Histergan*; *Hyrexin*; *Medinex*; *Nightcalm*; *Nytol*; *Paxidorm*; *Pheramin*; *Siladryl*; *Silphen*; *Sleep Aid*; *Sleepeaze*; *Sleepia*; *Sominex*; *Somnium*; *Tusstat*; *Twilite*. It is an ingredient of many proprietary preparations [Sweetman 2007].
Chemical Properties A white crystalline powder that slowly darkens on exposure to light. Mp 166° to 170°. Soluble 1 in 1 of water, 1 in 2 of ethanol, 1 in 50 of acetone, and 1 in 2 of chloroform; very slightly soluble in benzene and in ether. Log *P* (octanol/water), 3.11 [Meylan, Howard 1995].

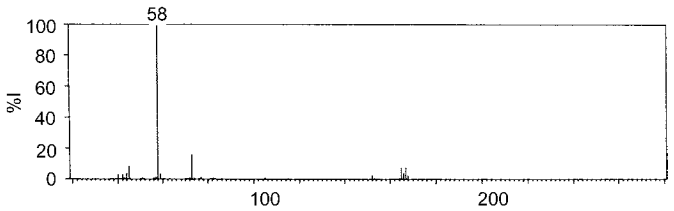
Colour Test Liebermann's reagent—brown-orange; Mandelin's test—yellow; Marquis test—yellow; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.44; system TC—R_f 0.33; system TE—R_f 0.65; system TL—R_f 0.15; system TAE—R_f 0.27; system TAF—R_f 0.48 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; FPN reagent, pink; Marquis test, yellow; ninhydrin spray, positive).
Gas Chromatography System GA—diphenhydramine RI 1870, M (nor-) RI 1520, M (nor-acetyl-) RI 2265, M (di-nor-acetyl) RI 2240, M (methoxy-) RI 2010, M (diphenylmethanol) RI 1645, M (OH-) RI 1890, M (di-OH-) RI 1895, M (desamino-OH-) RI 1760, M (benzophenone) (BPH) RI 1610, M (OH-BPH isomer 1) RI 2065, M (OH-BPH) isomer 2 RI 2080, M (OH-BPH isomer 1) RI 2050, M (OH-methoxy-BPH) isomer 2 RI 2070; system GB—diphenhydramine RI 1928, M (nor-) RI 1922, M (nor-acetyl-) RI 2360, M (di-nor-acetyl-) RI 2240, M (desamino-) RI 1883, M (methoxy-) RI 2239, M (diphenylmethane) RI 1465, M (diphenylmethanol) RI 1645, M (4-phenylmethylphenol) RI 1780; system GC—RI 2378; system GF—RI 2105.
High Performance Liquid Chromatography System HA—*k* 3.3; system HX—RI 393; system HY—RI 336; system HAX—RT 12.2 min; system HAY—RT 6.0 min.
Ultraviolet Spectrum Aqueous acid—252, 257 nm ($A_1^1 = 17a$).



Infrared Spectrum Principal peaks at wavenumbers 713, 754, 1103, 1017, 1180, 991 cm^{-1} (diphenhydramine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 73, 45, 167, 165, 166, 44, 152 (no peaks above 200); didesmethyldiphenhydramine 30, 167, 45, 168, 165, 183, 166, 152; monodesmethyldiphenhydramine 44, 167, 168, 165, 183, 105, 152, 77.



Quantification

Blood **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3 mL/min. Temperature programme: 170° for 1 min to 280° at 10°/min. FID. Limit of detection, 100 µg/L [Nishikawa *et al.* 1997].Column: DB-1 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8/min. NPD. Retention time: 13.7 min. Limit of detection, 100–250 ng/L [Hattori *et al.* 1992]. FID [Backer *et al.* 1977].
Serum **GC** AFID. Limit of detection, 2.5 µg/L [Lutz *et al.* 1983].
Urine **HPLC** See Fischer, Breyer-Pfaff [1995].
GC See Blood. Limit of detection, 62 µg/L [Nishikawa *et al.* 1997]. See Blood [Backer *et al.* 1977; Hattori *et al.* 1992].
Tissues See Blood [Backer *et al.* 1977].
Disposition in the Body Diphenhydramine is readily absorbed after oral administration but undergoes extensive first-pass metabolism by *N*-dealkylation and oxidative deamination to form monodesmethyl and didesmethyl metabolites and diphenylmethoxyacetic acid, which may be conjugated with glutamine or glycine. Up to 65% of a dose is excreted in the urine in 96 h. The major urinary metabolite appears to be diphenylmethoxyacetic acid in free or conjugated form; very little is excreted as unchanged drug. Diphenhydramine crosses the placenta and is excreted in breast milk.
Therapeutic Concentration In plasma, usually in the range 0.1–1.0 µg/mL.
Following a single oral dose of 100 mg diphenhydramine hydrochloride to 4 subjects, peak plasma concentrations of 0.08–0.16 mg/L (mean 0.11) were obtained 2–4 h after administration; total amine concentrations were ~50% higher because of the presence of *N*-dealkylated metabolites. After single oral doses of 100 mg to a further 4 subjects, the concentration of acidic metabolites increased over a period of 24 h to ~2 mg/L. After multiple oral doses of 50 mg diphenhydramine hydrochloride 4 times daily to 4 subjects, maximum steady-state plasma diphenhydramine concentrations of 0.10–0.27 mgL were reported; the maximum steady-state amine concentrations were between 0.17 and 0.37 mg/L [Glazko *et al.* 1974].
Toxicity The estimated minimum lethal dose is 3 g. Toxic effects may be produced by plasma concentrations >1 mg/L.
In a 16-year-old male who died from drowning and cold exposure following a drug overdose, the following concentrations of diphenhydramine, free dihydrocodeine and total dihydrocodeine, respectively, were found ~9 days after death: femoral venous blood 1.89, 3.27 and 3.30 mg/L; right cardiac chamber blood 0.294, 0.237 and 0.240 mg/L; urine 22.6, 37.3 and 43.1 mg/L; stomach 0.029, 0.018 and 0.024 mg; and femoral muscle 0.270, 0.246 and 0.314 µg/g [Moriya, Hashimoto 2001].
A 35-year-old woman who underwent emergency charcoal haemoperfusion and haemodialysis 4.5 h after ingesting 16 g (~20 mg/kg) diphenhydramine, had blood concentrations of 3.3 mg/L immediately before haemoperfusion and 2.56 mg/L immediately after [Mullins *et al.* 1999].
In 2 fatalities attributed to diphenhydramine, the following postmortem tissue concentrations were reported (mg/L or µg/g):

	Diphenhydramine	*Diphenylmethoxyacetic acid*
Blood	19.7, 6.9	3.0, 15.3
Heart blood	50, 14.7	3.6, 19
Kidney	114, –	–, –
Liver	260, –	–, –
Lung	460, –	–, –
Urine	34.9, 376	0.3, 20.4

D

In the first case death occurred within 3 h whereas the second subject survived for 12 h after ingestion [Aderjan *et al.* 1982].

In 4 cases of suicidal diphenhydramine overdose, blood levels ranged from 5–35 mg/L [Karch 1998].

In 4 deaths from diphenhydramine overdose, the following postmortem concentrations were obtained: blood 8–31 mg/L (mean 20), brain 8 and 32 µg/g (2 cases), kidney 13 µg/g (1 case), liver 23–47 µg/g (mean 34), urine 40 and 6.4 mg/L (2 cases) [Backer *et al.* 1977; Baselt, Cravey 1977; Peclet *et al.* 1981].

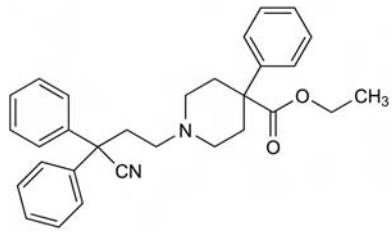
Half-life Plasma half-life, 2.4–9.3 h.
Bioavailability ~50–70%.
Volume of Distribution 4.5–8 L/kg.
Clearance Plasma clearance, ~6–10 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, ~1.3.
Saliva Plasma : saliva ratio, ~3.
Protein Binding ~80%.
Dose Diphenhydramine hydrochloride 75 to 200 mg daily in divided doses.

Aderjan R *et al.* (1982). [Poisoning by diphenhydramine: forensic toxicologic interpretation of analytic results]. *Z Rechtsmed* 88: 263–270.
Backer *et al.* (1977). Diphenhydramine suicide: case report. *J Anal Toxicol* 1: 227–228.
Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–103.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.
Fischer D, Breyer-Pfaff U (1995). Comparison of procedures for measuring the quaternary *N*-glucuronides of amitriptyline and diphenhydramine in human urine with and without hydrolysis. *J Pharm Pharmacol* 47: 534–538.
Glazko AJ *et al.* (1974). Metabolic disposition of diphenhydramine. *Clin Pharmacol Ther* 16: 1066–1076.
Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants.* Washington DC: American Chemical Society.
Hattori H *et al.* (1992). Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 581: 213–218.
Karch SB (1998). Diphenhydramine toxicity: comparisons of postmortem findings in diphenhydramine-, cocaine-, and heroin-related deaths. *Am J Forensic Med Pathol* 19: 143–147.
Lutz D *et al.* (1983). Quantitative determination of diphenhydramine and orphenadrine in human serum by capillary gas chromatography. *J Clin Chem Clin Biochem* 21: 595–597.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *JPharm Sci* 84: 83–92.
Moriya F, Hashimoto Y (2001). Postmortem diffusion of drugs from the bladder into femoral venous blood. *Forensic Sci Int* 123: 248–253.
Mullins M (1999). Life-threatening diphenhydramine overdose treated with charcoal hemoperfusion and hemodialysis. *Ann Emerg Med* 33: 104–107.
Nishikawa M *et al.* (1997). Simple analysis of diphenylmethane antihistaminics and their analogues in bodily fluids by headspace solid-phase microextraction–capillary gas chromatography. *J Chromatogr Sci* 35: 275–279.
Peclet C *et al.* (1981). Diphenhydramine intoxication. *TIAFT Bull* 16: 26–27.
Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* Chichester, UK: Wiley.
Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Diphenoxylate

Narcotic Antidiarrhoeal
$C_{30}H_{32}N_2O_2$ = 452.6
CAS—915-30-0

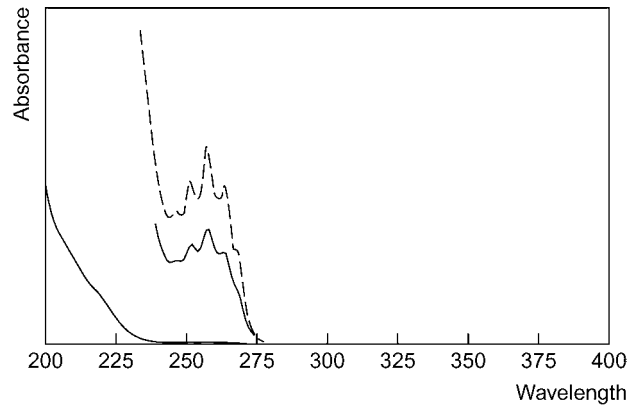
IUPAC Name Ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate
Synonym 1-(3-Cyano-3,3-diphenylpropyl)-4-phenyl-4-piperidinecarboxylic acid ethyl ester



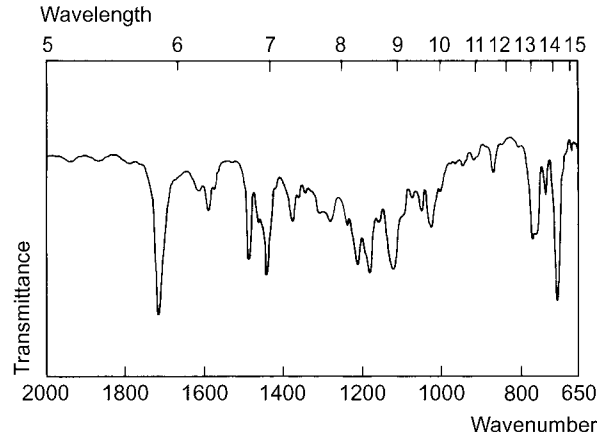
Diphenoxylate Hydrochloride
$C_{30}H_{32}N_2O_2$,HCl = 489.1
CAS—3810-80-8

Proprietary Names *Dhamotil*; *Diarphen*; *Diarsed*; *Diastop*; *Dilomil*; *Eldox*; *Erlotyl*; *Intard*; *Lofenoxal*; *Logen*, *Lomotil*; *Lonox*; *Lotharin*; *Reasec*; *Remodil*; *Retardin*; *Tropergen*.
Note Preparations of diphenoxylate hydrochloride usually contain subclinical amounts of atropine sulfate in an attempt to prevent abuse by deliberate overdosage.
Chemical Properties A white crystalline powder. Mp 220.5° to 222°. Sparingly soluble in water; soluble 1 in 50 of ethanol and 1 in 2.5 of chloroform; soluble in methanol; practically insoluble in ether.

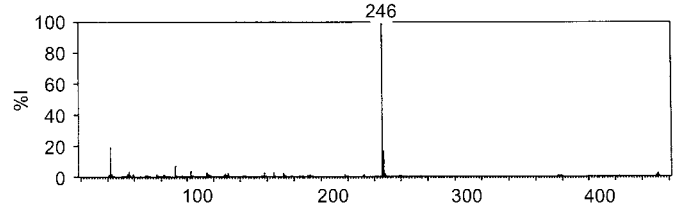
Chemical Properties pK_a 7.1. Log *P* (octanol/water), 6.3.
Colour Test Sodium picrate (Steyn test)—orange.
Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.42; system TC—R_f 0.84; system TE—R_f 0.87; system TL—R_f 0.70; system TAE—R_f 0.90; system TAF—R_f 0.92 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, orange).
Gas Chromatography System GA—RI 3514; system GB—RI 3670; system GM—not eluted.
High Performance Liquid Chromatography System HA—diphenoxylate *k* 0.2, diphenoxylic acid *k* 0.6 (tailing peak); system HY—RI 385; system HZ—retention time 27.4 min; system HAY—retention time 14.1 min.
Ultraviolet Spectrum Methanol—252, 258 (A_1^1=14a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1728, 697, 1183, 1120, 1215, 1495 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 246, 42, 247, 91, 103, 165, 115, 56; diphenoxylic acid 218, 42, 219, 91, 165, 155, 193, 115.



Quantification
Plasma **GC** TCD. For method, see Al Ragheb *et al.* [1982].
GC–MS Limit of detection, 20 mg/L diphenoxylic acid [Ford *et al.* 1976].
Urine **GC** See Plasma [Al Ragheb *et al.* 1982].
Disposition in the Body Rapidly absorbed after oral administration but it is usually administered together with a small quantity of atropine and this may delay absorption, especially with high doses. It is extensively metabolised by hydrolysis,

hydroxylation and conjugation with glucuronic acid. The major metabolites are diphenoxylic acid (difenoxin), which is active, and hydroxydiphenoxylic acid in both free and conjugated forms. About 14% and 50% of a dose, respectively, is excreted in the urine and faeces in 96 h; <0.1% of a dose is excreted in the urine as unchanged drug in 24 h.

Therapeutic Concentration

After an oral dose of 5 mg, peak plasma concentrations of diphenoxylate of about 0.01 mg/L were attained in 2 h and peak concentrations of diphenoxylic acid of about 0.04 mg/L were attained in the same period [Karim *et al.* 1972].

Toxicity Diphenoxylate has addiction-producing properties. The estimated minimum lethal dose is 0.2 g although recovery has occurred after the ingestion of 0.75 g.

A postmortem blood concentration of 0.34 µg/mL was reported in a $3^1/_2$-year-old child who died after ingesting diphenoxylate [Al Ragheb *et al.* 1982].

Half-life Plasma half-life, diphenoxylate about 2.5 h, diphenoxylic acid about 4 h.

Volume of Distribution About 4 L/kg.

Dose Initially 10 mg of diphenoxylate hydrochloride, followed by 5 mg every 6 h.

Al Ragheb SA *et al.* (1982). A case of fatal Lomotil overdosage. *Med Sci Law* 22(3): 210–214.
Ford GC *et al.* (1976). The measurement of diphenoxylic acid in plasma following administration of diphenoxylate. *Biomed Mass Spectrom* 3: 45–47.
Karim A *et al.* (1972). Pharmacokinetics and metabolism of diphenoxylate in man. *Clin Pharmacol Ther* 13: 407–419.

Diphenyl

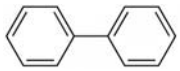
Fungistatic

$C_{12}H_{10}$ = 154.2

CAS—92-52-4

IUPAC Name 1,1′-Biphenyl

Synonym Phenylbenzene



Chemical Properties A white crystalline powder. Mp 69° to 71°. Bp 254° to 255°. Practically insoluble in water; soluble in ethanol and ether. Log *P* (octanol/water), 4.0.

Gas Chromatography System GA—RI 1389.

Infrared Spectrum Principal peaks at wavenumbers 740, 710, 1573, 1175, 1018, 918 cm^{-1} (KBr disk).

Diphenylprolinol

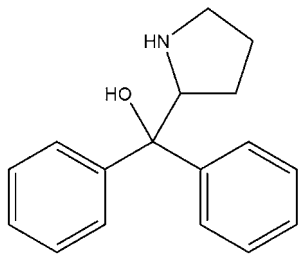
Dopamine and Noradrenaline Reuptake Inhibitor

$C_{17}H_{19}NO_2$ = 253.3

CAS—22348-32-9

IUPAC Name Diphenyl(pyrrolidin-2-yl)methanol

Synonym CID204386; D2PM; (*S*)-(−)-1,1-diphenylprolinol; diphenyl-pyrrolidin-2-ylmethanol; diphenyl(2-pyrrolidinyl)methanol; diphenyl(pyrrolidin-2-yl) methanol; HMS1439N22; Maybridge3_003124; JFD 01912; MolPort-002-041-205; Oprea1_549580.



Disposition in the Body

Toxicity A 21-year-old man ingested 3 tablets of 'Head Candy' and presented with chest pain. His serum concentration of diphenylprolinol was 0.17 mg/L while his glaucine levels were 0.1 mg/L [Lidder *et al.* 2008]

Lidder S *et al.* (2008). Cardiovascular toxicity associated with recreational use of diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]). *J Med Toxicol* 4: 167–169.

Diphenylpyraline

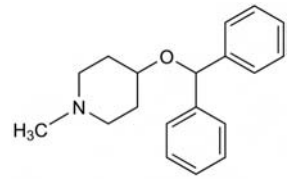
Antihistamine

$C_{19}H_{23}NO$ = 281.4

CAS—147-20-6

IUPAC Name 4-Benzhydryloxy-1-methylpiperidine

Synonyms 4-(Diphenylmethoxy)-1-methylpiperidine; diphenylpyrilene.



Chemical Properties Log *P* (octanol/water), 3.9.

Diphenylpyraline Hydrochloride

$C_{19}H_{23}NO,HCl$ = 317.9

CAS—132-18-3

Proprietary Names *Arbid-N*; *Dayfen*; *Diafen*; *Hispril*; *Histryl*; *Histyn*; *Kolton (gel)*; *Lergoban*; *Lergobine*. It is an ingredient of *Eskornade*.

Chemical Properties A white crystalline powder. Mp 204° to 209°. Soluble 1 in 1 of water, 1 in 3 of ethanol and 1 in 2 of chloroform; soluble in isopropanol; practically insoluble in ether and benzene.

Diphenylpyraline Hydrobromide

$C_{19}H_{23}NO,HBr$ = 362.3

CAS—132-18-3

Chemical Properties Crystals Mp 201° to 202°. Soluble in water and ethanol

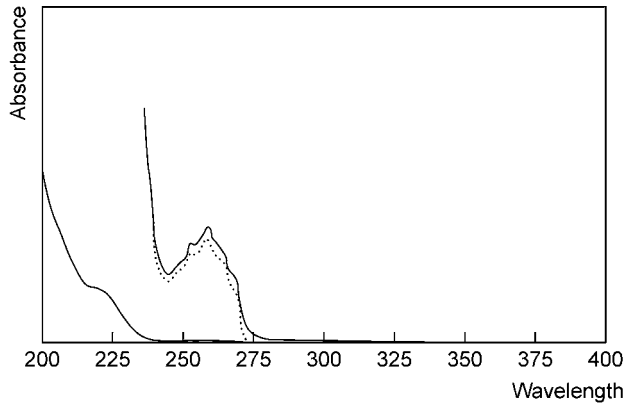
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—yellow; Marquis test—yellow.

Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.42; system TC—R_f 0.28; system TE—R_f 0.68; system TL—R_f 0.08; system TAE—R_f 0.23; system TAF—R_f 0.49; system TAJ—R_f 0.61; system TAK—R_f 0.50; system TAL—R_f 0.92 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, brown; ninhydrin spray, positive).

Gas Chromatography System GA—diphenylpyraline RI 2100, M (benzophenone) (BPH) RI 1610, M (OH-benzophenone-) isomer-1 RI 2065, M (OH-benzophenone-) isomer 2 RI 2080, M (OH-methoxy-benzophenone-) isomer-1 RI 2050, M (OH-methoxy-benzophenone-) isomer-2 RI 2070; system GB—RI 2128; system GC—RI 2447; system GF—RI 2405.

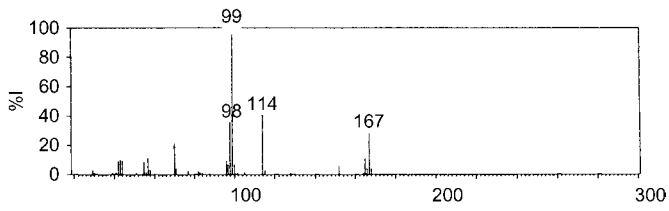
High Performance Liquid Chromatography System HA—*k* 3.3 (tailing peak); system HX—RI 401.

Ultraviolet Spectrum Aqueous acid—253, 258 nm (A^1_1=17a).



Infrared Spectrum Principal peaks at wavenumbers 1070, 709, 1050, 696, 765, 1101 cm^{-1} (diphenylpyraline hydrochloride, KCl disk).

Mass Spectrum Principal ions at *m/z* 99, 114, 98, 167, 70, 165, 57, 43.



Quantification

Blood **GC** FID. Limit of detection, 76–473 mg/L, diphenylpyraline and other antihistamines [Nishikawa *et al.* 1997].

Plasma **HPLC** UV detection. Limit of detection, 15 mg/L [Ebete, Koundourellis 1996].

Urine **GC** Limit of detection, 13–186 mg/L, see Blood [Nishikawa *et al.* 1997]. FID. For method, see Graham and Bolt [1974].

HPLC See Plasma [Ebete, Koundourellis 1996].

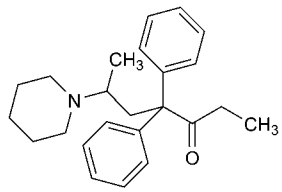
Disposition in the Body Absorbed after oral administration. <10% of a dose is excreted in the urine as unchanged drug. Possible metabolites include the *N*-oxide and *N*-desmethyldiphenylpyraline.
Half-life Derived from urinary excretion data, 24 to 40 h.
Dose 10 to 20 mg of diphenylpyraline hydrochloride daily.

Ebete KO, Koundourellis JE (1996). Determination of diphenylpyraline in plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 677: 319–323.
Graham G, Bolt AG (1974). Half-life of diphenylpyraline in man. *J Pharmacokinet Biopharm* 2: 191–195.
Nishikawa M *et al.* (1997). Simple analysis of diphenylmethane antihistaminics and their analogues in bodily fluids by headspace solid-phase microextraction-capillary gas chromatography. *J Chromatogr Sci* 35: 275–279.

Dipipanone

Narcotic Analgesic
$C_{24}H_{31}NO = 349.5$
CAS—467-83-4

IUPAC Name 4,4-Di(phenyl)-6-piperidin-1-ylheptan-3-one
Synonyms 4,4-Diphenyl-6-(1-piperidinyl)-3-heptanone; phenylpiperone; piperidyl methadone; piperidylamidone.



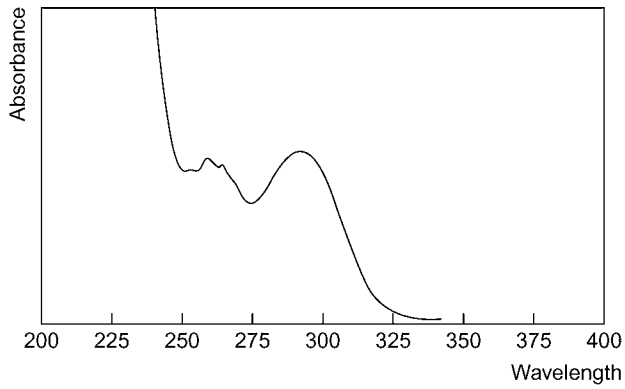
Chemical Properties pK_a 8.5(25°). Log *P* (octanol/water) 5.5 [Meylan, Howard 1995].

Dipipanone Hydrochloride

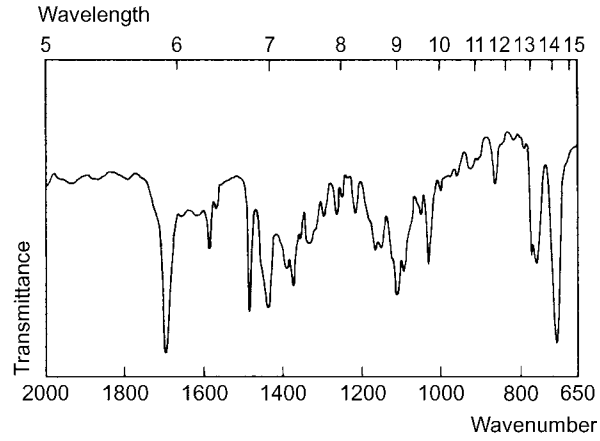
$C_{24}H_{31}NO,HCl,H_2O = 404.0$
CAS—856-87-1
Proprietary Names It is an ingredient of *Diconal*; *Wellconal*.
Chemical Properties White crystalline powder. Mp 123° to 126° (also reported to be 126° to 127°). Soluble 1 in 40 of water, 1 in 1.5 of ethanol, and 1 in 6 of acetone; practically insoluble in ether.

Colour Test Mandelin's test—green→blue.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.67; system TC—R_f 0.33; system TE—R_f 0.87; system TL—R_f 0.70; system TAE—R_f 0.27; system TAF—R_f 0.72 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2474; system GB—RI 2586; system GC—RI 2894; system GF—RI 2710; system GM—RRT 1.309 (relative to iprindole).
High Performance Liquid Chromatography System HA—*k* 2.2; system HC—*k* 1.61; system HX—RI 500; system HY—RI 363.

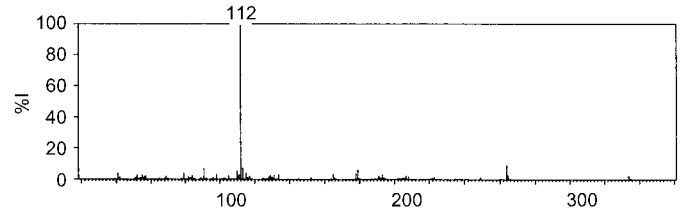
Column: SGE CPS-Hypersil (100 × 2.1 mm i.d., 5 µm). Mobile phase: 0.025 mol/L phosphate buffer (pH 4.0)-isopropyl alcohol-acetonitrile (94 : 3 : 3) : 0.025 mol/L phosphate buffer (pH 4.0)-isopropyl alcohol-acetonitrile (50 : 25 : 25, 80 : 20 for 5 min to 55 : 45 at 5.1 min until 20 min to 80 : 20 at 21 min for 9 min), flow rate 0.2 mL/min. UV detection (λ = 205 nm). Limit of detection, 2 ng on column [Roy, Jefferies 1990].
Ultraviolet Spectrum Aqueous acid—259, 265, 293 nm ($A_1^1 = 15.6$a).



Infrared Spectrum Principal peaks at wavenumbers 1708, 698, 1491, 1110, 1092, 1030 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 112, 264, 113, 91, 179, 110, 178, 115.



Quantification

Plasma **GC** Column: SPB5 fused silica capillary (15 m × 0.25 mm i.d., 1 µm). Carrier gas: N_2, 1 mL/min. Temperature: 250°. NPD. Limit of detection, 1 µg/L [Cathapermal, Caddy 1988]. Column: BP-1 dimethylsiloxane (15 m × 0.32 mm i.d., 0.5 µm). Carrier gas: He, 60 cm/s. Temperature programme: 80° for 2.5 min to 230° at 40°/min to 250° at 5°/min for 1.5 min. TSD. Retention time: 10.11 min. Limit of detection, 1 mg/L [Paterson 1988].
Urine **GC** See Plasma [Cathapermal, Caddy 1988]. See Plasma [Paterson 1988].
Disposition in the Body Absorbed after oral administration with rapid onset of action. It is metabolised in the liver and excreted in the urine and faeces. Prolonged use of dipipanone may produce dependence of the morphine type.
Toxicity The estimated minimum lethal dose is 0.1 g.

In 2 fatalities after IV injection of Diconal (dipipanone with cyclizine), the postmortem tissue concentrations (mg/L or µg/g) shown below were reported.

	Cyclizine	*Dipipanone*
Blood	2.0, 0.5	6.2, 0.5
Bile	20, –	–, –
Liver	3.0, –	5.2, –
Urine	0.5, 9.5	–, 18.5

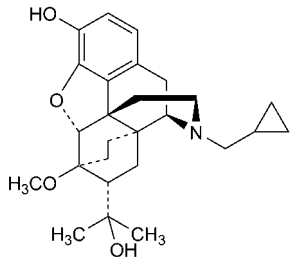
In 34 addicts, urinary concentrations of free dipipanone ranged from 0 to 5.2 mg/L (mean 1.6) [Sheehan *et al.* 1979].
Dose 10 to 30 mg of dipipanone hydrochloride every 6 h.

Cathapermal S, Caddy B (1988). Capillary gas chromatographic determination of cyclizine and dipipanone in biological fluids. *Analyst* 113: 385–388.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Paterson S (1988). Measurement of dipipanone using capillary gas chromatography. *J Chromatogr* 424: 152–157.
Roy IM, Jefferies TM (1990). Performance evaluation of an aqueous-organic phase separator for post-column reactions in high-performance liquid chromatography, and its application to the enhanced detection of some basic drugs of abuse. *J Pharm Biomed Anal* 8: 831–835.
Sheehan TMT *et al.* (1979). The Abuse of Preparations Containing Dipipanone and Cyclizine. In: JS Oliver, ed., *Forensic Toxicology (Proceedings of the European Meeting of the International Association of Forensic Toxicologists)*. London: Croom Helm.

Diprenorphine

Narcotic Antagonist (Veterinary)
$C_{26}H_{35}NO_4$ = 425.6
CAS—14357-78-9

Synonym (5α,7α-17-(Cyclopropylmethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-dimethyl-6,14-ethenomorphinan-7-methanol

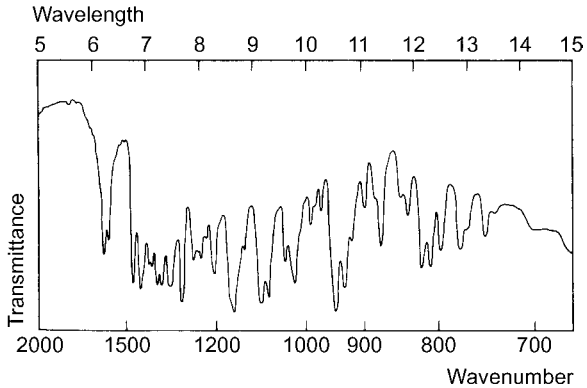


Chemical Properties White microcrystalline powder. Mp 190° to 192°.

Diprenorphine Hydrochloride

$C_{26}H_{35}NO_4$,HCl = 462.0
CAS—16808-86-9
Proprietary Name *Revivon*
Chemical Properties White crystalline powder. Soluble 1 in 30 of water, 1 in 160 of ethanol and 1 in 2500 of chloroform; practically insoluble in ether.

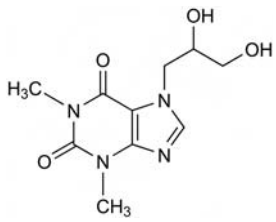
Colour Tests Liebermann's reagent—black; Mandelin's test—grey; Marquis test—grey-violet.
Thin-layer Chromatography System TA—R_f 0.70 (acidified iodoplatinate solution, positive).
Gas Chromatography System GB—RI 3385; system GM—not eluted.
High Performance Liquid Chromatography System HA—*k* 0.6; system HY—RI 301.
Ultraviolet Spectrum Aqueous acid—230 (A^1_1 = 158b), 286 nm (A^1_1 = 36a); aqueous alkali—246 (A^1_1 = 184b), 299 nm (A^1_1 = 58a).
Infrared Spectrum Principal peaks at wavenumbers 1160, 947, 1311, 1092, 1078, 934 cm^{-1} (KBr disk).



Diprophylline

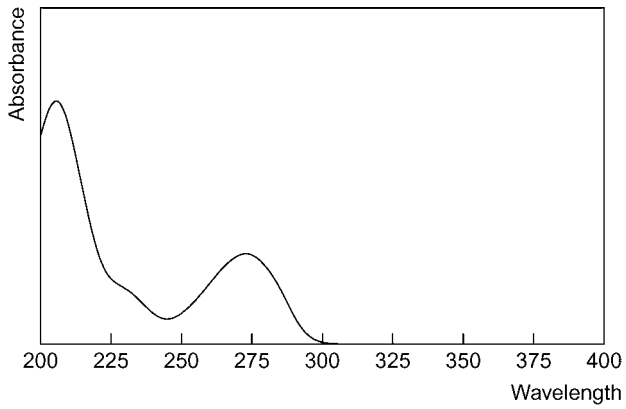
Xanthine Bronchodilator
$C_{10}H_{14}N_4O_4$ = 254.2
CAS—479-18-5

IUPAC Name 7-(2,3-Dihydroxypropyl)-1,3-dimethylpurine-2,6-dione
Synonyms 7-(2,3-Dihydroxypropyl)-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione; dihydroxypropyltheophyllinum; diprophyllinum; dyphylline; glyphyllinum; hyphylline.
Proprietary Names *Asthmolysin*; *Austrophyllin*; *Dilor*; *Droxine*; *Dyflex*; *Dylix*; *Isophyllen*; *Katasma*; *Lufyllin*; *Neothyllin(e)*; *Neufil*.

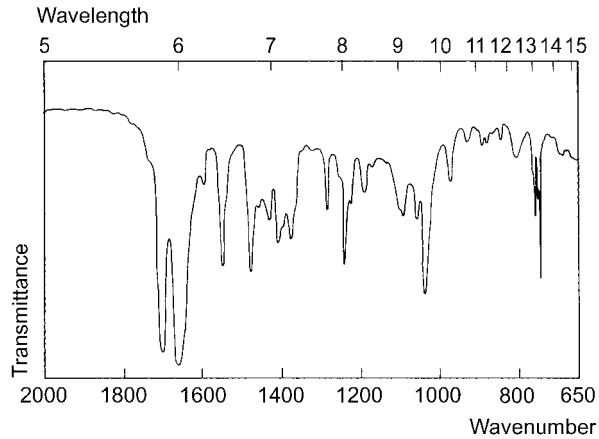


Chemical Properties A white crystalline powder. Mp 158°. Soluble 1 in 3 of water and 1 in 50 of ethanol; slightly soluble in chloroform; practically insoluble in ether. Log *P* (octanol/water), −1.5.

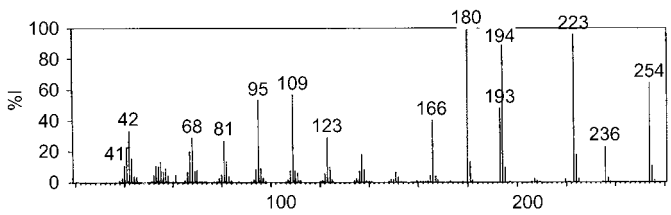
Colour Test Amalic acid test—yellow/violet.
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.00; system TC—R_f 0.12; system TE—R_f 0.25; system TL—R_f 0.12; system TAE—R_f 0.70; system TAF—R_f 0.59 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—diprophylline RI 2280, diprophylline-AC_2 RI 2455.
High Performance Liquid Chromatography System HX—RI 275; system HY—RI 227; system HAA—retention time 3.6 min.
Ultraviolet Spectrum Aqueous acid—206, 273 nm (A^1_1=380a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1700, 1039, 747, 1562, 1234 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 180, 223, 194, 254, 109, 95, 193, 166.



Quantification

Plasma **HPLC** Limit of detection, 25 µg/L [Gisclon *et al.* 1979a].
Serum **GC** FID. For method, see Shihabi and Dave [1977].
HPLC UV detection. For method for quantification of diprophylline and doxophylline, see Tagliaro *et al.* [1990]. UV detection. Limit of detection, 0.25 mg/L, diprophylline and theophylline [Kester *et al.* 1987]. UV detection. Limit of detection, 1 mg/L [Paterson 1982].
Urine **HPLC** For method, see Gisclon *et al.* [1979a].
Saliva **GC** See Plasma [Shihabi, Dave 1977].
HPLC Limit of detection, 50 µg/L [Gisclon *et al.* 1979a].
Disposition in the Body Rapidly and almost completely absorbed after oral administration. About 83% of a dose is excreted in the urine as unchanged drug in 24 h; <1% of a dose is eliminated in the faeces. It is also rapidly absorbed from IM injection sites. Diprophylline is excreted in breast milk.
Therapeutic Concentration In plasma, usually in the range 10 to 20 mg/L. After a single oral dose of 1.2 g to 6 subjects, peak plasma concentrations of 18 to 29 mg/L (mean 22) were attained in about 0.5 h and peak saliva concentrations of 7 to 17 mg/L (mean 12) were reported at 0.7 h [Gisclon *et al.* 1979b].
Toxicity Plasma concentrations greater than 20 µg/mL may produce toxic effects.
Bioavailability About 90%.
Half-life Plasma half-life, about 2 h.

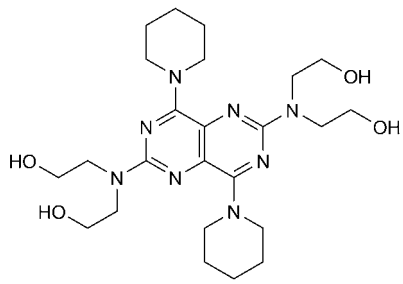
Volume of Distribution About 0.8 L/kg.
Clearance Plasma clearance, about 5 mL/min/kg.
Dose 15 mg/kg body weight every 6 h.

Gisclon L *et al.* (1979a). Saliva, urine and plasma analysis of dyphylline via HPLC. *Res Commun Chem Pathol Pharmacol* 23(3): 523–531.
Gisclon LG *et al.* (1979b). Pharmacokinetics of orally administered dyphylline. *Am J Hosp Pharm* 36: 1179–1184.
Kester MB *et al.* (1987). Microassay for the simultaneous determination of theophylline and dyphylline in serum by high-performance liquid chromatography. *J Chromatogr* 416: 91–97.
Paterson N (1982). High-performance liquid chromatographic method for the determination of diprophylline in human serum. *J Chromatogr* 232(2): 450–455.
Shihabi ZK, Dave RP (1977). Gas-chromatographic determination of dyphylline in serum and saliva. *Clin Chem* 23: 942–943.
Tagliaro F *et al.* (1990). Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum. *Clin Chem* 36: 113–115.

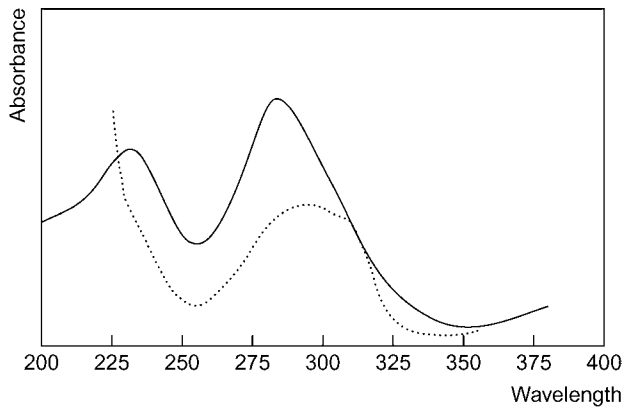
Dipyridamole

Antithrombotic, Antianginal Vasodilator, Phosphodiesterase Inhibitor
$C_{24}H_{40}N_8O_4 = 504.6$
CAS—58-32-2

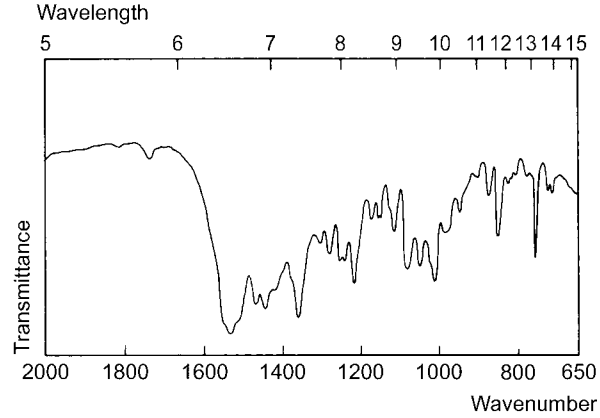
IUPAC Name 2-[[2-[Bis(2-hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-6-yl]-(2-hydroxyethyl)amino]ethanol
Synonym 2,2′,2″,2‴-[(4,8-Di-1-piperidinylpyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo]tetrakisethanol
Proprietary Names *Agremol*; *Atrombin*; *Cardoxin*; *Cerebrovase*; *Cleridium*; *Coronarine*; *Corosan*; *Curantyl N*; *Dipyridan*; *Dipyrin*; *Fluxocor*; *Miosen*; *Modaplate*; *Natyl*; *Novo-Dipiradol*; *Perkod*; *Persantin(e)*; *Plato*; *Posanin*; *Procardin*; *Procor*; *Protangix*; *Pytazen*.



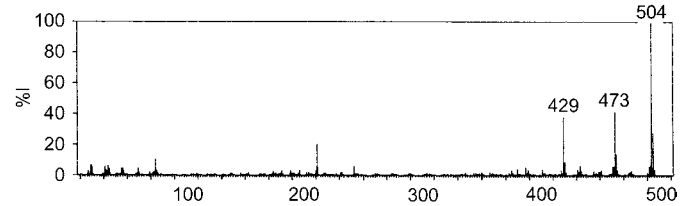
Chemical Properties Intensely yellow crystalline powder. Solutions have a yellowish-blue fluorescence. Mp 164° to 167°. Slightly soluble in water; very soluble in methanol, ethanol, and chloroform; soluble in dilute acids; practically insoluble in ether. pK_a 6.4 [Wolfram, Bjornsson 1980]. Log *P* (octanol/water) 2.7. Stock solutions and plasma samples were stable at room temperature for 6 h, at –20° for 15 days, in the autosampler (15°) for 24 h and after 3 freeze-thaw cycles [Wang *et al.* 2008]. Stock solutions were stable at room temperature and 2 to 8° for a minimum of 8 h and 6 days, respectively. Dipyridamole in control human plasma was stable for at least 7.5 h at room temperature and for a minimum of 3 freeze-thaw cycles. Spiked plasma samples were stable at –20° and –70° for a minimum of 93 days. Stability in the autosampler (5°) was determined to be 23.5 h [Yadav *et al.* 2008]. Freshly prepared standard solutions degrade in daylight. Samples prepared with deproteinised serum were stable for several days [Pedersen 1979].
Colour Tests Mandelin's test—violet; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.00; system TC—R_f 0.37; system TE—R_f 0.44; system TL—R_f 0.42; system TAE—R_f 0.82; system TAF—R_f 0.87 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1640.
High Performance Liquid Chromatography System HA—*k* 0.2; system HAA—RT 13.2 min; system HX—RI 393; system HY—RI 335; system HZ—RT 3.0 min.
Ultraviolet Spectrum Acid methanol—230, 285 nm ($A_1^1 = 650a$); aqueous alkali—295 nm.



Infrared Spectrum Principal peaks at wavenumbers 1526, 1214, 1010, 1076, 1041, 1251 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 504, 473, 429, 505, 221, 474, 84, 430.



Quantification

Blood **HPLC** Column: μBondapak C_{18} (30 × 0.39 cm i.d., 10 μm). Mobile phase: methanol : water (65 : 35) both containing 0.005 mol/L 1-heptanesulfonic acid sodium salt and 0.1% acetic acid, flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 285 nm, λ_{em} = 470 nm). Retention time: 5.1 min. Limit of detection not reported [Wolfram, Bjornsson 1980].
Plasma **HPLC** Column: Ultrasphere XL ODS (70 × 4.6 mm i.d., 3 μm). Mobile phase: methanol : 0.02 mol/L ammonium acetate buffer (pH 5.0; 65 : 35), flow rate 1.5 mL/min. UV detection (λ = 280 nm). Retention time: 4.57 min. Limit of detection, 10 μg/L [Barberi *et al.* 1991]. Column: Merck C_{18} (10 × 3 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.01 mol/L monobasic potassium buffer (pH 7) with 0.1 N sodium hydroxide (45 : 55), flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 285 nm, λ_{em} = 470 nm). Retention time: 3.5 min. Limit of detection, 5 μg/L [Ricevuti *et al.* 1991]. Column: ODS Hypersil. Mobile phase: methanol : 0.01 mol/L tris hydrochloride buffer (pH 8.6; 85 : 15). Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 460 nm). Retention time: 6 min. Limit of detection, 0.01 mg/L [Dresse *et al.* 1982]. Column: μBondapak C_{18} (30 × 0.39 cm i.d., 10 μm). Mobile phase: acetonitrile : 0.01 mol/L sodium phosphate buffer (pH 7.0; 50 : 50), flow rate 1.5 mL/min. UV detection (λ = 285 nm). Retention time: 5.5 min. Limit of detection, 5 μg/L [Williams *et al.* 1981]. Column: Ultrasphere C_{18} (25 cm × 0.46 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer containing 0.01 mol/L *N,N,N,N*-tetramethylethylene diamine (33 : 67), flow rate 2.5 mL/min. UV detection (λ = 280 nm). Retention time: 7.5 min. Limit of detection, 2 μg/L [Rosenfeld *et al.* 1982]. See Blood [Wolfram, Bjornsson 1980]. Column: Lichrosorb RP-18 (125 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 0.2 mol/L Tris hydrochloride buffer (80 : 20), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 415 nm, λ_{em} = 478 nm). Limit of detection, <5 μg/L [Schmid *et al.* 1979].
LC-MS Column: Shimadzu VP-ODS-C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: methanol : water (pH 4.25; 70 : 30), flow rate 2.0 mL/min. ESI, negative ion mode. Retention time: 5.7 min. Limit of quantification, 10 μg/L, limit of detection, 1 μg/L [Wang *et al.* 2008]. Column: Gemini C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water (90 : 10) containing 0.1% formic acid, flow rate 1.0 mLmin. ESI, TIS, positive ion mode, MRM acquisition mode. Limit of detection, 5.1 μg/L [Yadav *et al.* 2008].
Serum **HPLC** Column: μBondapak C_{18} (30 × 0.39 cm i.d., 10 μm). Mobile phase: methanol : 0.02 mol/L sodium acetate (pH 4.0; 75 : 25), flow rate 2.0 mL/min. UV detection (λ = 280 nm). Retention time: ≈7.5 min. Limit of detection, 8 μg/L [Pedersen 1979].
Note For a spectrofluorimetic method for the detection of dipyridamole, see Steyn [1979].

Disposition in the Body Readily absorbed after oral administration. Eliminated mainly in the faeces; excretion may be delayed due to enterohepatic circulation. Small amounts are excreted in the urine as the glucuronide conjugate.

Therapeutic Concentration

After the administration of 75 mg dipyridamole to 67 healthy male subjects a mean maximum plasma concentration of 2000 ± 764 μg/L was reached at 0.77 ± 0.3 h [Yadav *et al.* 2008].

After oral administration of 50 mg three times a day to 10 subjects, maximum steady-state plasma concentrations of 0.4–2.6 mg/L (mean 1.4) were reported; trough concentrations were 0.1–1.5 mg/L (mean 0.7). In 10 subjects given 75 mg twice daily, the corresponding maximum and minimum steady-state concentrations were 0.5–4 mg/L (mean 1.7) and 0.1–2.6 mg/L (mean 0.9) [Mahony *et al.* 1983].

Two healthy male volunteers were administered 2 × 25 mg Persantine tablets following a 12 h fast. Mean peak plasma concentrations reached ≈517 µg/L at ≈1 h [Williams *et al.* 1981].

A healthy volunteer was administered a single oral dose of 25 mg dipyridamole. The peak plasma concentration of ≈300 µg/L was reached after ≈1 h [Schmid *et al.* 1979].

After a single oral dose of 100 mg given to 4 subjects, peak serum concentrations of ≈2 mg/L were attained in 1 h [Nielsen-Kudsk, Pedersen 1979].

Toxicity

A 23-year-old female who suffered profound shock after deliberately ingesting 1750 mg of dipyridamole was successfully treated with aminophylline and dopamine [Chen *et al.* 1994].

Half-life Plasma half-life, ≈12 h.

Volume of Distribution ≈2.5 L/kg.

Clearance Plasma clearance, ≈2 mL/min/kg. Gastric pH has an impact on gastric emptying in elderly patients with a higher pH resulting in a slower emptying [Russell *et al.* 1994].

Protein Binding >90% [Szebeni, Weinstein 1991].

Dose 300 to 600 mg daily (antithrombotic); 150 mg daily (antianginal).

Barberi M *et al.* (1991). Sensitive determination of free and plasma protein-bound dipyridamole by high-performance liquid chromatography. *J Chromatogr* 565: 511–515.

Chen ZC *et al.* (1994). Profound shock resulting from a large dose of dipyridamole. *Int J Cardiol* 46: 75–78.

Dresse A *et al.* (1982). Pharmacokinetics of oral dipyridamole (Persantine) and its effect on platelet adenosine uptake in man. *Eur J Clin Pharmacol* 23: 229–234.

Mahony C *et al.* (1983). Plasma dipyridamole concentrations after two different dosage regimens in patients. *J Clin Pharmacol* 23: 123–126.

Nielsen-Kudsk F, Pedersen AK (1979). Pharmacokinetics of dipyridamole. *Acta Pharmacol Toxicol (Copenh)* 44: 391–399.

Pedersen AK (1979). Specific determination of dipyridamole in serum by high-performance liquid chromatography. *J Chromatogr* 162: 98–103.

Ricevuti G *et al.* (1991). Pharmacokinetics of dipyridamole-beta-cyclodextrin complex in healthy volunteers after single and multiple doses. *Eur J Drug Metab Pharmacokinet* 16: 197–201.

Rosenfeld J *et al.* (1982). High-performance liquid chromatographic determination of dipyridamole. *J Chromatogr* 231: 216–221.

Russell TL *et al.* (1994). pH-related changes in the absorption of dipyridamole in the elderly. *Pharm Res* 11: 136–143.

Schmid J *et al.* (1979). Rapid, sensitive determination of dipyridamole in human plasma by high-performance liquid chromatography. *J Chromatogr* 163: 239–243.

Steyn JM (1979). Spectrofluorimetric determination of dipyridamole in serum–a comparison of two methods. *J Chromatogr* 164: 487–494.

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Wang N *et al.* (2008). Simultaneous determination of dipyridamole and salicylic acid in human plasma by high performance liquid chromatography-mass spectrometry. *Biomed Chromatogr* 22: 149–156.

Williams C *et al.* (1981). High-performance liquid chromatographic assay for plasma dipyridamole monitoring. *J Chromatogr* 225: 225–230.

Wolfram KM, Bjornsson TD (1980). High-performance liquid chromatographic analysis of dipyridamole in plasma and whole blood. *J Chromatogr* 183: 57–64.

Yadav M *et al.* (2008). Effect of collision-activated dissociation gas and collision energy on the fragmentation of dipyridamole and its rapid and sensitive liquid chromatography/electrospray ionization tandem mass spectrometric determination in human plasma. *Rapid Commun Mass Spectrom* 22: 511–518.

Dipyrone

Analgesic, Antiinflammatory, Antipyretic

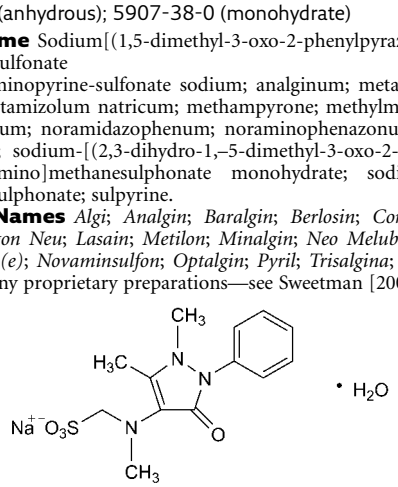
$C_{13}H_{16}N_3NaO_4S,H_2O = 351.4$

CAS—68-89-3 (anhydrous); 5907-38-0 (monohydrate)

IUPAC Name Sodium[(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)-methylamino]methanesulfonate

Synonyms Aminopyrine-sulfonate sodium; analginum; metamizole; metamizole sodium; metamizolum natricum; methampyrone; methylmelubrin; natrium novaminsulfonicum; noramidazophenum; noraminophenazonum; novamidazofen; NSC-73205; sodium-[(2,3-dihydro-1,–5-dimethyl-3-oxo-2-phenyl-1*H*-pyrazol-4-yl)methylamino]methanesulphonate monohydrate; sodium noramidopyrine methanesulphonate; sulpyrine.

Proprietary Names *Algi*; *Analgin*; *Baralgin*; *Berlosin*; *Conmel*; *Dolemicin*; *Dolocalma*; *Inalgon Neu*; *Lasain*; *Metilon*; *Minalgin*; *Neo Melubrina*; *Nevralgina*; *Nolotil*; *Novalgin(e)*; *Novaminsulfon*; *Optalgin*; *Pyril*; *Trisalgina*; *Unagen*. It is an ingredient of many proprietary preparations—see Sweetman [2007].



Chemical Properties White or yellowish-white crystalline powder. Mp 172°. Soluble 1 in 1.5 of water and 1 in 30 of ethanol; practically insoluble in ether, acetone, benzene and chloroform.

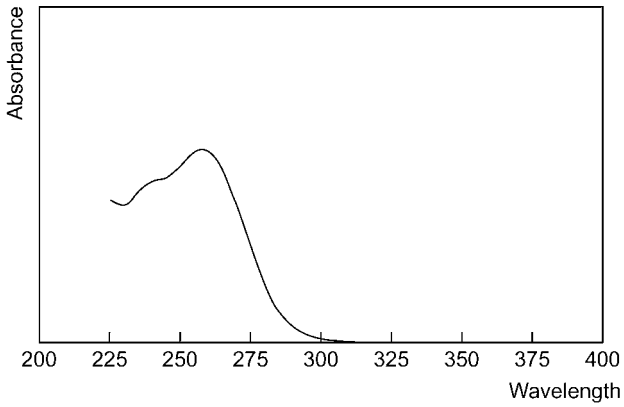
Colour Tests Ferric chloride—violet; Folin–Ciocalteu reagent—blue; Liebermann's reagent (100°)—blue; Mandelin's test—brown; nitrous acid—transient blue.

Thin-layer Chromatography System TA—R_f 0.84; system TB—R_f 0.00; system TC—R_f 0.01; system TD—R_f 0.00; system TE—R_f 0.02; system TF—R_f 0.00; system TL—R_f 0.02; system TAD—R_f 0.02; system TAE—R_f 0.85; system TAF—R_f 0.59 (acidified iodoplatinate solution, white).

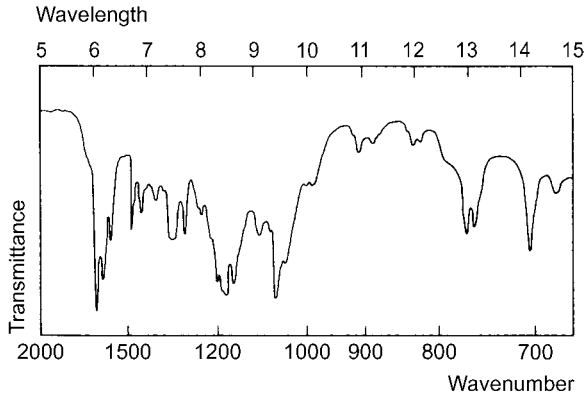
Gas Chromatography System GA—M (bis-desalkyl-) RI 1955, M (desalkyl-)-AC RI 2395; system GB—dipyrone RI 2069.

High Performance Liquid Chromatography System HD—*k* 0.1; system HW—*k* 0.45; system HX—RI 316; system HY—RI 194; system HZ—RT 1.4 min.

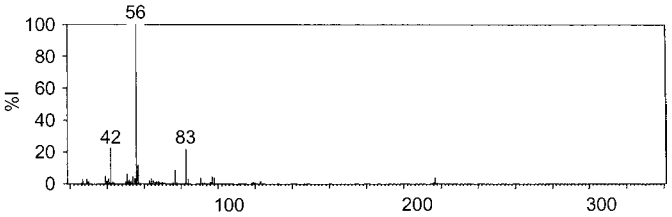
Ultraviolet Spectrum Aqueous acid—258 nm (A^1_1 = 266a).



Infrared Spectrum Principal peaks at wavenumbers 1672, 1064, 1179, 1163, 1208, 1639 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 56, 42, 83, 57, 77, 51, 97, 54.



Quantification

Plasma **HPLC** Column: OD-MP Spheri-5 RP-18 (10 cm × 4.6 mm i.d.). Mobile phase: 0.1 mol/L SDS with 2.5% pentanol, flow rate 1.0 mL/min. UV detection (λ = 262 nm). Limit of detection, 14.4 and 18 mg/L for FAA and AA, respectively [Carretero *et al.* 1995]. Column: Spherisorb ODS (125 × 4.5 mm i.d., 5 µm). Mobile phase: 0.05 mol/L TEAP buffer (pH 2.8) : acetonitrile : methanol (100 : 10 : 8), flow rate 1.1 mL/min. UV detection (λ = 265 nm). Limit of detection, 0.1 mg/L [Damm 1989]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : 0.01 mol/L sodium acetate (pH 3.0; 8 : 92), flow rate 1.6 mL/min. UV detection (λ = 257 nm). Retention time: 14, 17, 21, and 25 min for MAA, AA, FAA and AAA, respectively. Limit of detection, 0.1 mg/L [Katz *et al.* 1984]. Column: µBondapak C_{18} (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: water : methanol : acetic acid (75 : 20 : 5), flow rate 1.0 mL/min. UV detection (λ = 254 and 280 nm). Limit of quantification, 1 mg/L for dipyrone and 250 ng/mL for 4-methylaminophenazone Asmardi, Jamali 1983].

Urine **HPLC** See Plasma. Limit of detection, 2 mg/L [Damm 1989]. Column: Spherisorb ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methnol : triethylamine : acetic acid (70.9 : 27.7 : 0.9 : 0.5), flow rate 1.0 mL/min. UV detection

($\lambda = 254$ nm). Retention time: 22.2 min for MMA. Limit of detection, 30 µg/L [Agúndez *et al.* 1994]. See Plasma [Asmardi, Jamali 1983].

***Other* HPLC** Human Liver Microsomes. Column: Lichrospher 60 RP (125 × 3.0 mm i.d., 5 µm). Mobile phase: methanol : 0.05 mol/L sodium acetate buffer (pH 2.2; 1 : 99 to 4 : 96 at 6 min to 7 : 93 at 13 min to 9 : 91 at 18 min for 8 min to 1 : 99 at 27 min), flow rate 0.9 mL/min. UV detection ($\lambda = 257$ nm). Limit of quantification, 50 µg/L for all metabolites [Geisslinger *et al.* 1996].

Disposition in the Body Rapidly hydrolysed by desmethane-sulfonation in the gastrointestinal tract after oral administration to the active metabolite 4-methylamino-antipyrine (MMA). MMA undergoes incomplete oxidative demethylation to 4-formylamino-antipyrine (FAA) and demethylation to 4-amino-antipyrine (AA), which in turn is acetylated to form 4-acetylamino-antipyrine (AAA). About 70% of a dose is excreted in the urine in 24 h as metabolites. Dipyrone metabolites are excreted in breast milk.

Therapeutic Concentration

In 12 subjects given single oral doses of a test and reference preparation of dipyrone 1 g, the mean peak plasma concentrations of 4-methylamino-antipyrine, 4-formylamino-antipyrine, 4-amino-antipyrine, and 4-acetylamino-antipyrine were 17.19 and 16.92 mg/L (test and reference), 1.6 and 1.84 mg/L, 2.94 and 2.89 mg/L, and 1.93 and 1.86 mg/L at 1.42 and 1.46 h, 6.2 and 7.0 h, 4.4 and 4.4 h, and 11.3 and 11.6 h, respectively. Four of the subjects were rapid and 8 were slow acetylators; the peak plasma concentration (or the test preparation) differed significantly between the 2 acetylator types only for 4 amino-antipyrine (16.98 vs 18.52 for slow vs rapid acetylators) and 4-acetyl-amino-antipyrine (1.38 vs 4.38 mg/L) [Bacracheva *et al.* 1995].

The formation and urinary excretion of dipyrone metabolites was measured in 12 healthy volunteers following a single oral 1.0 g dose of dipyrone. The group consisted of 3 slow and 9 rapid acetylators. Renal clearance of all metabolites was similar for both phenotypes. Formation of the metabolites AA and AAA were significantly different between the 2 phenotypes: AA, 0.25 (slow) vs 0.1 mL/min/kg (rapid) AAA, 0.75 vs 7.53 mL/min/kg (rapid) [Zylber-Katz *et al.* 1992].

A single oral dose of 1 g dipyrone sodium was administered to 12 healthy volunteers. Pharmacokinetic values for the main metabolites are shown below.

	MAA	*AA*	*FAA*	*AAA*
C_{max} (mg/L)	12.2 ± 1.4	1.5 ± 0.8	2.4 ± 0.5	1.8 ± 0.6
t_{max} (h)	1.3 ± 0.4	4.4 ± 0.9	5.8 ± 1.3	15.0 + 6.4
$t_{1/2}$(h)	2.7 ± 0.5	3.7 ± 1.3	11.2 ± 1.5	9.5 + 1.5
CL_{ren} (mL/min)	5.0 ± 1.8	37.8 ± 12.8	48.5 ± 4.5	60.6 + 8.3

[Damm 1989].

After a single oral dose of 1 g given to 12 slow acetylators and 11 rapid acetylators (determined with reference to dapsone), the mean peak plasma concentrations (mg/L, time in hours) were as shown below.

	Slow acetylators	*Rapid acetylators*
4-Acetylaminophenazone	1.6 (16.1)	4.4 (10.0)
4-Aminophenazone	2.7 (6.7)	1.6 (3.2)
4-Formylaminophenazone	1.8 (7.7)	2.5 (6.6)
4-Methylaminophenazone	10.0 (1.6)	11.0 (1.2)

[Levy *et al.* 1984]

Toxicity Dipyrone is rarely used in the US due to an association with agranulocytosis. Other adverse reactions include skin rashes, anaphylaxis, thrombocytopaenia, hepatocellular damage, and CNS symptoms. The estimated minimum lethal dose is 5 g, but fatalities from acute poisoning are rare.

A 56-year-old male died after ingesting up to 20 g dipyrone, 2 g baclofen and 0.15 g oxybutynin; terazosin, aspirin, defibrotide, and laxatives also may have been ingested. Postmortem, carried out a few hours after death, revealed dipyrone, baclofen and oxybutynin concentrations, respectively, of 106, 669 and 0.1 mg/L in the blood and 774 mg/L, 215 mg/L, and trace amounts in the urine [De Giovanni, d'Aloja 2001].

Note For a case of a death after the IV administration of Avafortan (24 mg/mL avapyrazone and 240 mg/mL dipyrone) see Fosseus and Straughan [1983].

Half-life Plasma half-life, 4-acetylamino-antipyrine about 10.6 h, 4-amino-antipyrine ≈5 h, 4-formylamino-antipyrine ≈10 h, and 4-methylamino-antipyrine ≈3.3 h.

Dose 0.5 to 4 g daily in divided doses.

Agúndez JA *et al.* (1994). Determination of aminopyrine, dipyrone and its metabolites in urine by high-performance liquid chromatography. *Ther Drug Monit* 16: 316–322.

Asmardi G, Jamali F (1983). High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids. *J Chromatogr* 277: 183–189.

Bacracheva N *et al.* (1995). Comparative bioavailability of two oral metamizole formulations. Influence of the acetylation phenotype. *Arzneimittelforschung* 45: 282–285.

Carretero I *et al.* (1995). Determination of antipyrine metabolites in human plasma by solid-phase extraction and micellar liquid chromatography. *Analyst* 120: 1729–1732.

Damm D (1989). Simultaneous determination of the main metabolites of dipyrone by high-pressure liquid chromatography. *Arzneimittelforschung* 39: 1415–1417.

De Giovanni N, d'Aloja E (2001). Death due to baclofen and dipyrone ingestion. *Forensic Sci Int* 123: 26–32.

Fosseus CG, Straughan JL (1983). Sudden death due to intravenous avapyrazone and dipyrone. *S Afr Med J* 64: 81.

Geisslinger G *et al.* (1996). High-performance liquid chromatographic analysis of dipyrone metabolites to study their formation in human liver microsomes. *Pharm Res* 13: 1272–1275.

Katz EZ *et al.* (1984). Simultaneous determination of dipyrone metabolites in plasma by high-performance liquid chromatography. *J Chromatogr* 305: 477–484.

Levy M *et al.* (1984). Plasma kinetics of dipyrone metabolites in rapid and slow acetylators. *Eur J Clin Pharmacol* 27: 453–458.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Zylber-Katz E *et al.* (1992). Formation and excretion of dipyrone metabolites in man. *Eur J Clin Pharmacol* 42: 187–191.

Diquat

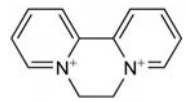
Herbicide

$C_{12}H_{12}N_2 = 184.2$

CAS—2764-72-9

IUPAC Name 6,7-Dihydrodipyrido[1,2-*a*:2′,1′-*c*]pyrazinediium ion

Synonym Deiquat



Note Unless otherwise stated, the analytical information given below refers to diquat dibromide. For information on the monoene and diene reduction products for GC-MS analysis, see under Quaternary ammonium herbicides in Chapter 16.

Diquat Dibromide

$C_{12}H_{12}Br_2N_2 = 344.0$

CAS—85-00-7

Proprietary Names *Aquacide*; *Reglone*. It is an ingredient of *Pathclear* and *Weedol*.

Chemical Properties Yellow crystals. Mp ~335°. Soluble in water. Log *P* (octanol/water), 2.4.

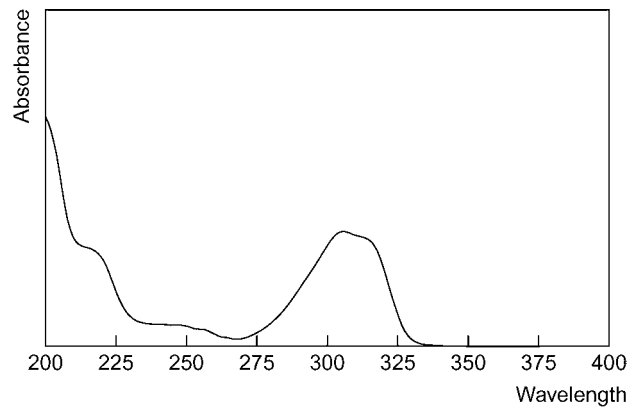
Colour Test Sodium dithionite—green.

Thin-layer Chromatography System TA—R_f 0.00; system TAB—R_f 0.00; system TAC—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.00; system TL—R_f 0.00 (acidified iodoplatinate solution, positive).

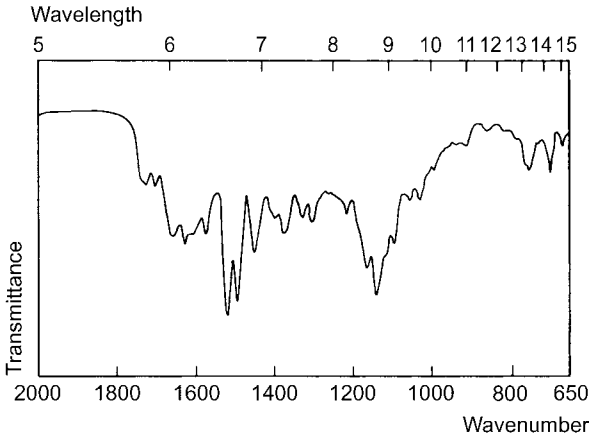
Gas Chromatography System GA—diquat dibromide not eluted; system GK—monoene reduction product RT 0.40 min; diene reduction product RT 0.49 min (both relative to caffeine).

High Performance Liquid Chromatography System HX—RI 00; system HAO—diquat dibromide*k* 99.9; system HAP—diquat dibromide *k* 0.00.

Ultraviolet Spectrum Aqueous acid—310 nm (A^1_1 = 518b)



Infrared Spectrum Principal peaks at wavenumbers 1522, 1498, 1140, 1166, 1097, 1632 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* (monoene reduction product) 83, 108, 96, 54, 111, 192, 84, 55; (diene reduction product) 54, 108, 81, 190, 111, 135, 83, 80 .

Quantification

Blood **HPLC** Column: μ-Bondapack C_{18} (300 × 3.8 mm). Mobile phase: deionised water : octane sulfonic acid : acetonitrile : orthophosphoric acid : diethylamine : tetrahydrofuran, flow rate 1.0 mL/min. UV detection (λ = 315 nm) [Madhu *et al.* 1995].

GC FID [Kawase *et al.* 1984].

Serum **HPLC** UV detection (λ = 391 nm). Limit of quantification, 100 μg/L [Hara *et al.* 2007]. Quantification of paraquat and diquat [Nakagiri *et al.* 1989].

Urine **HPLC** See Blood [Madhu *et al.* 1995]. See Serum [Nakagiri *et al.* 1989]. UV detection. Limit of detection, <1 mg/L [Gill *et al.* 1983].

GC See Blood [Kawase *et al.* 1984].

Bile **HPLC** See Blood [Madhu *et al.* 1995].

Kidney **HPLC** See Blood [Madhu *et al.* 1995].

Liver **HPLC** See Blood [Madhu *et al.* 1995].

Biological Materials **HPLC** Column: Sep-Pak C_{18}. Mobile phase: 0.5% potassium bromide : 5% methanol solution : TEA (1 mL/L). UV detection (λ = 310 nm). Limit of detection, 0.05 μg/g [Ito *et al.* 1993].

Disposition in the Body Diquat is absorbed from the gastrointestinal tract and slightly absorbed through the skin.

Toxicity Diquat produces similar adverse effects to those of paraquat except that it does not seem to cause the progressive fibrosis in the lungs. A fatality after ingestion of ≈2 g has been reported and the ingestion of ≈20–50 mL of a 20% concentrate has caused death within 2–7 days. The maximum permissible atmospheric concentration is 0.5 mg/m^3 (as the dibromide) and the maximum acceptable daily intake (as the dichloride) is 5 μg/kg.

A 37-year-old man died 26 h after ingesting 300 mL diquat solution (equivalent to 60 g diquat ion) in a suicide attempt. Four hours after ingestion, the concentration of diquat in the serum was 64 mg/L. Postmortem tissue concentrations were (μg/g): kidney 4.5, lung 3.4, liver 2.3, brain 1.6, heart 1.1 [Hantson *et al.* 2000].

A 44-year-old woman ingested an unknown quantity of diquat and died ≈16 h after being found. The following postmortem concentrations were reported: kidney 11.6 μg/g and liver 5.1 μg/g [Pannell, Thomson 1981].

A young man drank several mouthfuls of a 20% concentrate of diquat and died 7 days later. The following postmortem concentrations were reported: blood 0.6 mg/L, heart 0.11 μg/g, kidney 1.19 μg/g, liver 0.33 μg/g, lung 0.56 μg/g and spleen 1.04 μg/g [Schönborn *et al.* 1971].

Note For a review of diquat poisoning see Jones, Vale [2000].

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Dirithromycin

Antibacterial, Macrolide

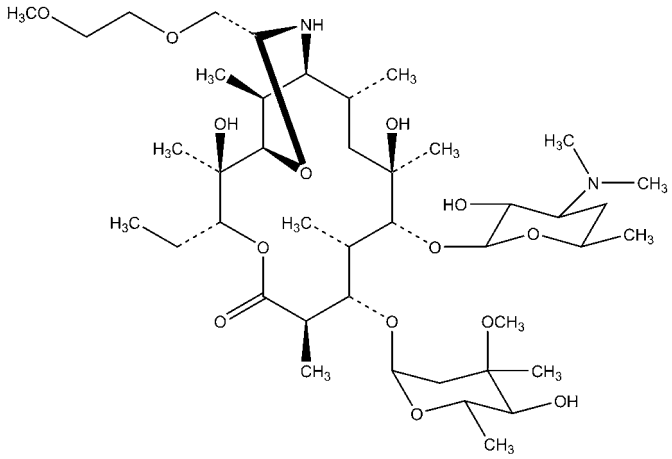
$C_{42}H_{78}N_2O_{14}$ = 835.1

CAS—62013-04-1

IUPAC Name (1*S*,2*R*,4*R*,5*R*,6*S*,7*S*,8*R*,11*R*,12*R*,15*R*,17*S*)-5-[(2*S*,3*R*,4*S*,6*R*)-4-Dimethylamino-3-hydroxy-6-methyloxan-2-yl]oxy-11-ethyl-4,12-dihydroxy-7-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-15-(2-methoxyethoxymethyl)-2,4,6,8,12,17-hexamethyl-10,14-dioxa-16-azabicyclo[11.3.1]heptadecan-9-one

Synonyms AS-E 136; [9*S*(*R*)]-9-Deoxo-11-deoxy-9,11-[imino[2-(2-methoxyethoxy)ethylidene]oxy]erythromycin; (1*R*,2*R*,3*R*,6*R*,7*S*,8*S*,9*R*,10*R*,12*R*,13*S*,15*R*,17*S*)-7-[(2,6-dideoxy-3-C-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)oxy]-3-ethyl-2,10-dihydroxy-15-[(2-methoxyethoxy)methyl]-2,6,8,10,12,17-hexamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-4,16-dioxa-14-azabicyclo[11.3.1]heptadecan-5-one; LY-237216.

Proprietary Names *Dimac*; *Dynabac*; *Noriclan*; *Nortron*; *Unibac*; *Valodin*.



Chemical Properties Crystals. Mp 186° to 189°. pK_a 9.0 in 66% aqueous dimethyl fluoride [O'Neil *et al.* 2006].

Thin-layer Chromatography Plates: Silica gel 60F_{254}. Mobile phase: *n*-pentane : dichloromethane : methanol : concentrated ammonia (40 : 60 : 10 : 0.6). UV detection (λ = 254 nm) or molybdatophosphoric acid hydrate spray with oven at 120° for 7 min [Firl *et al.* 1990].

Liquid Chromatography-Mass Spectrometry Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : propan-2-ol : 0.2 mol/L ammonium acetate buffer (pH 8.5): water (160 : 165 : 50 : 625), flow rate 1.0 mL/min. ESI, positive ion mode, CI . Limit of quantification or detection not reported [Diana *et al.* 2006].

Disposition in the Body Readily absorbed following oral administration and undergoes non-enzymatic hydrolysis to its active metabolite, erythromycylamine within 35 min of administration. It does not induce or inhibit CYP-dependent oxidative metabolism of other drugs [Jain, Danziger 2004]. Erythromycylamine accumulates in polymorphonuclear leucocytes to a greater extent than does erythromycin [Geerdes-Fenge *et al.* 1997]. Approximately 62 to 81% of an orally administered dose is eliminated in the faeces, predominantly as erythromycylamine, with ≈1.2 to 2.9% recovered in urine. The concentration of biologically active products in lung tissue, bronchial mucosa and bronchial secretions is 20 to 40 times the simultaneous plasma concentration. This has also been reported in samples of nasal mucosa, and tonsillar and prostatic tissue.

Therapeutic Concentration Following daily doses of 500 mg dirithromycin for 5 days the mean concentration in the bronchial mucosa was 1.7 ± 0.8 mg/kg [Bergogne-Berezin 1993]. Multiple 500 mg doses resulted in mean nasal mucosa concentrations of 1.86 ± 0.54 mg/kg. A mean concentration of 1.06 ± 0.48 mg/kg was found in tonsillar tissue 12 h after a 500 mg dose had been administered: 56% of this concentration was maintained for 24 h after the last dose [Benson *et al.* 1996].

Twelve healthy male volunteers received 4 single oral doses of 250, 500, 750 and 1000 mg dirithromycin on separate occasions. Peak plasma concentrations were not detected following an oral dose of 250 mg but measured 0.29 ± 0.21, 0.64 ± 0.44 and 0.41 ± 0.24 mg/L for the 500, 750 and 1000 mg doses, respectively, attained at 4 h independent of dose. After the IV administration of 100 mg to the same volunteers, the peak plasma concentration was 2.37 ± 0.28 mg/L [Sides *et al.* 1993].

Following oral administration of a single 500 mg dose of dirithromycin to 8 healthy volunteers and to 16 patients with cirrhosis (8 with class A (mild) and 8 with class B (moderate) cirrhosis according to Pugh's and Child's classification), mean peak serum concentrations obtained 3 to 4 h after administration were as follows:

Twenty patients suffering from an acute exacerbation of mild chronic obstructive pulmonary disease were administered 500 mg oral dirithromycin daily for 5 days. Dirithromycin concentrations in bronchial

Group	*C_{max} (mg/L)*	*Half-life (h)*
Healthy	0.29	23.3
Class A patients	0.48	35.2
Class B patients	0.52	39.5

[Mazzei *et al.* 1999].

secretions, bronchial mucosa and epithelial lining fluid were 2.67, 2.71 and 2.37 mg/L, respectively, measured 4 h after the final dose. At all time periods, concentrations of dirithromycin in secretions, bronchial mucosa, and epithelial lining fluid were greater than the concentration in serum [Matera *et al.* 1997].

Forty-six patients suffering from chronic bronchitis were administered 500 mg oral dirithromycin for 5 days. Dirithromycin concentrations in several sample types were measured at 24, 48, and 72 h after the last dose and were reported as follows:

Sample	*24 h*	*48 h*	*72 h*
Serum (mg/L)	0.17	0.05	0.05
Bronchial mucosa (mg/kg)	6.51	6.61	5.67
Bronchial secretions (mg/L)	1.26	0.64	0.84

[Leroyer *et al.* 1998].

Bioavailability Approximately 6 to 14%.
Half-life Dirithromycin, ~23 h; erythromycylamine, 30 to 44 h.
Volume of Distribution Erythromycylamine, 800 to 7500 L.
Clearance Erythromycylamine, 15 to 30 L/h.
Protein Binding Approximately 19%, principally to α_1-acid glycoprotein.
Dose 500 mg once daily by mouth.

Benson JM *et al.* (1996). Tonsillar tissue penetration of dirithromycin after multiple doses. *J Clin Pharmacol* 36: 832–837.
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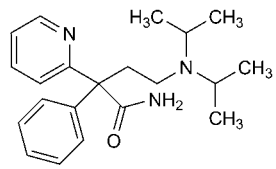
Leroyer C *et al.* (1998). Dirithromycin concentrations in bronchial mucosa and secretions. *Respiration* 65: 381–385.
Matera MG *et al.* (1997). Pulmonary concentrations of dirithromycin and erythromycin during acute exacerbation of mild chronic obstructive pulmonary disease. *Eur Respir J* 10: 98–103.
Mazzei T *et al.* (1999). Pharmacokinetics of dirithromycin in patients with mild or moderate cirrhosis. *Antimicrob Agents Chemother* 43: 1556–1559.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.
Sides GD *et al.* (1993). Pharmacokinetics of dirithromycin. *J Antimicrob Chemother* 31(SupplC): 65–75.

Disopyramide

Antiarrhythmic
$C_{21}H_{29}N_3O = 339.5$
CAS—3737-09-5

IUPAC Name 4-[Di(propan-2-yl)amino]-2-phenyl-2-pyridin-2-ylbutanamide
Synonyms α-2-[Bis(1-methylethyl)amino]ethyl]-α-phenyl-2-pyridineacetamide; DP.
Proprietary Names *Dicorynan*; *Dimodan*; *Disofarin*; *Disonorm*; *Durbis* (capsules); *Isomide*; *Isorythm*; *Ritmodan*; *Rythmodan* (capsules).

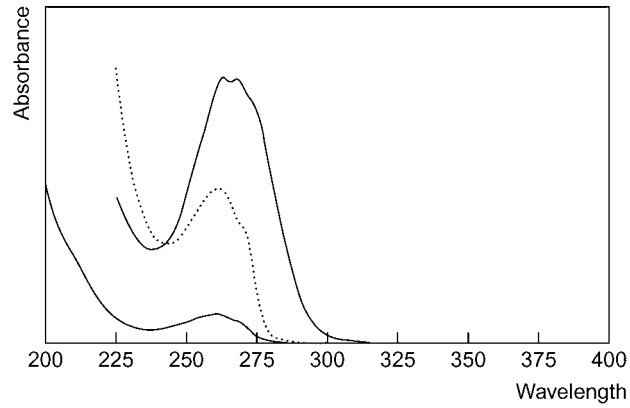


Chemical Properties White powder. Mp ≈95°. Soluble 1 in 200 of water, 1 in 10 of ethanol, 1 in 5 of chloroform and 1 in 5 of ether. pK_a 10.4 [Karim *et al.* 1982], 8.4. Log P (chloroform/water), 3.1 (pH 7.2) [Karim *et al.* 1982], (octanol/water),2.6. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005]. Stock racemic DP and MND solutions prepared in methanol containing 0.01 mol/L HCl in the concentration range 1 to 500 mg/L were stable for at least 3 months if stored at –20° [Jabor *et al.* 2001]. Stable in plasma for at least 1 month if stored at –20° and 4° [Charette *et al.* 1983]. Stable if incubated at 37° for 1 h or stored in human plasma at –20° for 86 days [Karim *et al.* 1982]. Stable in plasma for at least 48 h [Johnston, McHaffie 1978].

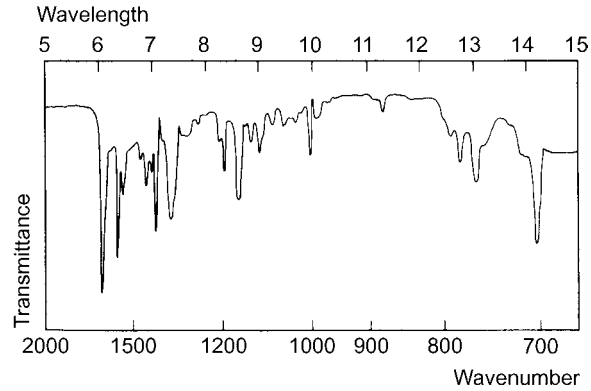
Disopyramide Phosphate

$C_{21}H_{29}N_3O,H_3PO_4 = 437.5$
CAS—22059-60-5
Proprietary Names *Dicorantil*; *Dirythmin*; *Dirytmin*; *Diso-Duriles*; *Disomet*; *Disonorm*; *Durbis (injection)*; *Isomide*; *Isorythm*; *Norpace*; *Ritmodan*; *Ritmoforine*; *Rythmical*; *Rythmodan* (injection and tablets); *Rythmodul*.
Chemical Properties White powder. Mp ≈205°, with decomposition. Soluble 1 in 20 of water and 1 in 50 of ethanol; practically insoluble in chloroform and ether.

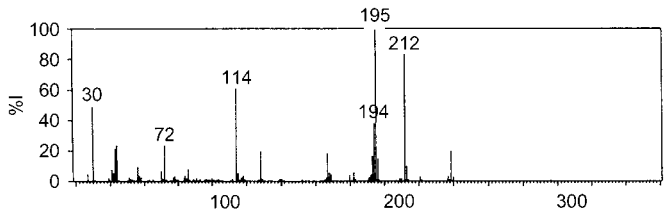
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.07; system TC—R_f 0.08; system TE—R_f 0.60; system TL—R_f 0.13; system TAE—R_f 0.09; system TAF—R_f 0.07.
Gas Chromatography System GA—disopyramide RI 2505, disopyramide-CHNO RI 2030, M (*N*-desalkyl-CHNO-)-AC RI 2330, M (*N*-desalkyl-)-AC RI 2640, M (bis-desalkyl--NH_3 RI 2245; system GB—disopyramide RI 2608, M (*N*-desalkyl-) RI 2286; system GF—RI 2910.
High Performance Liquid Chromatography System HA—disopyramide *k* 2.4, *N*-monodesisopropyldisopyramide *k* 1.8; system HAA—RT 11.4 min; system HX—RI 345; system HY—RI 281; system HZ—RT 3.0 min.
Ultraviolet Spectrum Aqueous acid—263, 269 (A_1^1 = 199a); aqueous alkali—261 nm.



Infrared Spectrum Principal peaks at wavenumbers 1664, 1585, 697, 1163, 1562, 760 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 195, 212, 114, 30, 194, 72, 44, 43 (no peaks above 240) See Anderson *et al.* [1980].



Quantification

Blood **GC** Column: SE-54 fused silica capillary (12 m × 0.32 mm i.d.). Carrier gas: He, 4.0 mL/min. Temperature programme: 80° for 1 min to 300° at 20°/min for 5 min. NPD, positive ion mode. Limit of detection, 1 mg/L [Singer, Mozayani 1995]. Column: 50% phenyl methyl silicone fused silica capillary (10 m × 0.54 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 200° for 0.5 min to 280° at 10°/min for 5 min. NPD. Limit of detection, 0.3 mg/L [Sathyavagiswaran 1987]. Column: 3% OV-17 on Chromosorb W-HP 80/100 mesh (2′ × 4 mm i.d.). Carrier gas: He, 50 mL/min. Temperature: 230°. FID. Limit of detection not reported [Michalek *et al.* 1982]. Column: 3% OV-17 on 100/120 mesh Gas-Chrom Q (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 260°. Retention time: ≈1.5 min. Limit of detection, 5 mg/L [Doedens, Forney 1978].

HPLC Column: Vydac TP 101 (30 cm × 4.6 mm i.d.). Mobile phase: methylene chloride : methanol : ammonium hydroxide (85 : 15 : 0.5), flow rate 1.0 mL/min. Limit of detection, 0.5 mg/L [Anderson *et al.* 1980].

Plasma **GC** Column: 3% OV7 phenylmethyl silicone on HP Chrom W 100/120 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature programme: 190° for 3 min to 280° at 3°/min for 10 min. FID. Limit of detection, 25 µg/L [Quaglio *et al.* 1995]. Column: 3% OV-17 (1 m × 3 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 190° to 250° at 4°/min. FID. Retention time ≈23 min. Limit of detection not reported [Sakata *et al.* 1988]. Column: 5% phenylmethyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 1 mL/min. Temperature programme: 160° to 195° at 5°/min. NPD Retention time: 10.1 min. Limit of detection, 0.05 mg/L [Kapil *et al.* 1984; Kapil *et al.* 1985]. Column: 3% SE 30 Supelcoport 80/100 mesh (1 m × 6 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 260°. Retention time: 1.6 min. Limit of detection not reported [Johnston, Hamer 1981].

See also Bredesen [1980]; Aitio [1979]; Foster, Reid [1979]; Hayler, Flanagan [1978]; Johnston, McHaffie [1978].

HPLC Column: Chiralpak AD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : ethanol (91 : 9), with 0.1% diethylamine, flow rate 1.2 mL/min. UV detection (λ = 270 nm). Limit of quantification, 12.5 µg/L [Bortocan *et al.* 2000]. Column: Capcell Pak CN (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer (pH 2.5) : acetonitrile (80 : 20), flow rate 1.0 mL/min. UV detection (261 nm). Retention time: 8.8 min. Limit of detection, 0.03 mg/L [Sagawa *et al.* 1997]. Column: LiChrosorb RP-18 (250 × 4 mm i.d., 10 µm). Mobile phase: acetonitrile : methanol : phosphate buffer (pH 3.2; 6 : 1 : 3), flow rate 1.0 mL/min. UV detecton (λ = 269 nm). Retention time: 6.08 min. Limit of detection not reported [Witek *et al.* 1994]. Column: Chiralcel OF (50 × 4.6 mm i.d.). Mobile phase: *n*-hexane : 2-propanol : diethylamine (80 : 20 : 0.1), flow rate 0.6 mL/min. UV detection (λ = 261 nm). Limit of detection, 0.01 and 0.025 mg/L for S-DP and other analytes, respectively [Echizen *et al.* 1991]. Column: Spherisorb hexyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate (pH 4.0; 40 : 60), flow rate 1.5mL/min. UV detection (λ = 254 nm). Limit of quantification, 0.5 mg/L [Verbesselt *et al.* 1991]. Column: chiral cellulose triphenylcarbamate (50 × 4.6 mm i.d.). Mobile phase: *n*-hexane : 2-propanol (80 : 20) with diethylamine, flow rate 0.6 mL/min. UV detection (λ = 261 nm). Retention time: 5.6 min for *S*(+)disopyramide. Limit of detection, 0.01 mg/L for *S*(+)disopyramide [Echizen *et al.* 1990].

See also Takahashi *et al.* [1990]; Enquist, Hermansson [1989]; Le Corre *et al.* [1988]; Dusci, Hackett [1985]; Bridges, Jennison [1983]; Charette *et al.* [1983]; Ahokas *et al.* [1980]; Draper *et al.* [1979]; Nygard *et al.* [1979]; Ilett *et al.* [1978]; Meffin *et al.* [1977].

CE Column: fused silica capillary (42 cm × 75 µm i.d.). Buffer: 20 mmol/L sodium acetate buffer (pH 5.0) containing 0.2% sulfated β-CD. UV detection (λ = 214 nm). Limit of quantification, 62.5 µg/L for (–)-(*R*)-DP, (+)-(*S*)-DP and (–)-(*R*)-MND, 125 µg/L for (+)-(*S*)-MND [Jabor *et al.* 2001].

Serum **GC** Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (1.8 m × 2 mm i.d.) or 2% SP-2250 or 2% OV-101 on Chromosorb W-HP 100/120 mesh (1.2 m × 2 mm i.d.). Carrier gas: He, 40 mL/min for 3% OV-17, N_2, 30 mL/min for 2% OV-101 and SP-2250. Temperature: 260°, 230° and 250° for each column, respectively. NPD. Limit of detection not reported [Vasiliades *et al.* 1979a]. Column: 2% SP-2250 on Chromosorb W-HP 100/120 mesh (1.2 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature: 250°. NSD. Limit of detection, <0.25 mg/L [Vasiliades *et al.* 1979b]. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (0.6 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 250°. NSD. Retention time: 0.9 min. Limit of quantification, 0.25 mg/L [Brien *et al.* 1983]. See Blood [Hayler, Flanagan 1978]. Column: 3% OV-17 on Gas-Chrom Q on 100/120 mesh (3′ × 2 mm i.d.). Carrier gas: N_2, 30mL/min. Temperature: 255°. NPD. Limit of detection, 0.5 mg/L [Duchateau *et al.* 1975].

GC-MS Column: Column: 2% SP-2250 on Chromosorb W-HP 100/120 mesh (1.2 m × 2 mm i.d). Temperature programme: 240° for 3.5 min to 260° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, <0.25 mg/L [Vasiliades *et al.* 1979b; Vasiliades *et al.* 1979a]

HPLC Column: Shim-Pack CLD-ODS. Mobile phase: 0.044 mol/L phosphate buffer (pH 2.6)-0.5% triethylamine : acetonitrile (70 : 30), flow rate 1.2 mL/min. Retention time: 5.5 min. Limit of detection, 50 µg/L [Tsuchishita *et al.* 2008]. Column: Finepac $SILC_{18}$ (150 × 4.6mm i.d.). Mobile phase: acetonitrile : 0.03 mol/L potassium dihydrogen phosphate (pH 3.0) : methanol (35 : 59 : 5), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: ≈8 min. Limit of detection, 0.1 and 0.05 mg/L for DP and MND, respectively [Masuhara *et al.* 1995]. Column: Shandon ODS reversed phase (125 × 4.6 mm i.d., 5 µm). Mobile phase: water-PIC-B8 reagent-triethylamine (974 : 25 : 1) with 0.0125 mol/L 1-octanesulfonic acid : acetonitrile (70 : 30), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.05 mg/L for disopyramide and its active metabolite mono-*N*-dealkylated disopyramide [Mayer *et al.* 1991]. Column: cyanopropyl (10 µm). Mobile phase: 5 mmol/L disodium hydrogenphosphate (pH 7.9) : acetonitrile : methanol (5 : 12 : 3), flow rate 3.0 mL/min. Limit of detection, 5 mg/L [Chen *et al.* 1987]. Column: Supelcosil CN (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 25 mmol/L sodium phosphate (pH 6.8), flow rate 2.0 mL/min. UV detection (λ = 215 nm). Limit of detection, 0.3 µmol/L for disopyramide, lidocaine and their active monodealkylated metabolites [Angelo *et al.* 1986].

See also Proelss, Townsend [1986]; Taylor *et al.* [1986]; Wang *et al.* [1986]; Swezey, Ponzo [1984]; Kabra *et al.* [1981]; Wesley, Lasky [1981]; Flood *et al.* [1980]; Broussard, Frings [1979]; Lima [1979]; Vasiliades *et al.* [1979b].

Urine **GC** See Blood [Singer, Mozayani 1995]. See Blood [Michalek *et al.* 1982]. See Blood [Hayler, Flanagan 1978].

HPLC See Plasma [Bortocan *et al.* 2000]. See Plasma [Echizen *et al.* 1990]. See Plasma [Enquist, Hermansson 1989]. See Plasma [Charette *et al.* 1983]. Column: µ-Bondapak CN (0.25 m × 0.5 cm). Mobile phase: 4 g sodium acetate, 40 g acetic acid and 150 mL methanol per L water (pH 3.5). UV detection (λ = 1.9 mL/min). Limit of detection, 50 µg/L [Lima 1979]. See plasma [Meffin *et al.* 1977].

Bile **GC** See Blood [Singer, Mozayani 1995]. See Blood [Sathyavagiswaran 1987].

HPLC See Blood [Anderson *et al.* 1980].

CSF **GC** See Plasma [Sakata *et al.* 1988].

Oral Fluid **HPLC** See Plasma [Sagawa *et al.* 1997]. See Urine [Lima 1979].

Stomach Contents **GC** See Blood [Singer, Mozayani 1995]. See Blood [Michalek *et al.* 1982].

Brain **GC** See Plasma [Sakata *et al.* 1988]. See Blood [Michalek *et al.* 1982].

Heart **GC** See Plasma [Sakata *et al.* 1988].

Kidney **GC** See Plasma [Sakata *et al.* 1988].

HPLC See Blood [Anderson *et al.* 1980].

Liver **GC** See Blood [Singer, Mozayani 1995]. See Plasma [Sakata *et al.* 1988]. See Blood [Sathyavagiswaran 1987]. See Blood [Michalek *et al.* 1982].

HPLC See Blood [Anderson *et al.* 1980].

Lung **GC** See Plasma [Sakata *et al.* 1988].

Spleen **GC** See Plasma [Sakata *et al.* 1988]. See Blood [Sathyavagiswaran 1987].

Disposition in the Body

Rapidly and almost completely absorbed after oral administration and widely distributed throughout the body; bioavailability about 80% [Taylor, Pappas 1986]. The major metabolite, mono-*N*-dealkyldisopyramide (MND), is about one-half as active as disopyramide. Conversion of disopyramide to MND is mainly catalysed by CYP3A4. Up to about 50 to 60% of a dose is excreted in the urine in 5 days as unchanged drug, with about 20 to 30% as the desalkyl metabolite; about 10 to 15% of a dose is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 2.8 to 7.5 mg/L.

After the administration of a 100 mg racemic mixture of DP and MND to a healthy volunteer, the pharmacokinetic values shown below were measured.

Parameter	*(+)-(S)-DP*	*(–)-(R)-DP*	*(+)-(S)-MND*	*(–)-(R)-MND*
C_{max}(mg/L)	1.41	0.81	0.06	0.05
t_{max}(h)	2.00	2.00	3.00	4.00
$t_{1/2}$ (h)	9.10	12.10	11.00	9.00

[Bortocan *et al.* 2000]

After daily oral doses averaging 6 mg/kg to 63 subjects, mean steady-state plasma concentrations of 3.3 mg/L for disopyramide and 0.99 mg/L for the desalkyl metabolite were reported; plasma concentrations were higher in 17 subjects with renal dysfunction (mean 4.3), but lower in subjects concurrently taking enzyme-inducing drugs [Aitio 1981].

After a single oral dose of 100 mg of disopyramide phosphate to 6 subjects, peak plasma concentrations of 1.8 to 3.6 mg/L (mean 2.4) were attained in about 2 h [Ranney *et al.* 1971].

Toxicity Toxic effects are usually associated with plasma concentrations >8 mg/L. Case 1: A 16-year-old ingested an unknown quantity of Rythmodan tablets. The plasma disopyramide concentration was 4.3 mg/L 9 h post-ingestion. Case 2: A 26-year-old female ingested ~40 × 150 mg Norpace capsules. Postmortem blood contained 114 mg/L. Case 3: A 24-year-old female ingested an unknown quantity of Rythmodan. After ~3 h the plasma concentration was 35 mg/L. Case 4: A 17-year-old male took an unknown amount of disopyramide. The plasma concentration 10 h before death was 25.5 mg/L. At postmortem blood and stomach contents contained 34 mg/L and 2.3 g/L, respectively. Case 5: A 35-year-old male ingested an estimated 68 capsules of 100 mg rythmodan. His plasma concentration 2 h before death was 8.3 mg/L and at postmortem it was 8.5 mg/L [Hayler *et al.* 1978].

A 19-year-old female deliberately ingested 25 capsules of disopyramide phosphate 150 mg (Norpace). Despite intensive resuscitation the patient died. Plasma disopyramide concentration on admission was 29 mg/L [Powell *et al.* 1978].

A 44-year-old female took an overdose of disopyramide. Concentrations in her blood, liver, kidney and bile were 26.6, 35.8, 147, and 349 mg/L, respectively [Anderson *et al.* 1980].

In a fatality due to the ingestion of about 15 g of disopyramide, the following postmortem tissue concentrations were reported: blood 57 mg/L, brain 29 µg/g, liver 115 µg/g, urine 1500 mg/L [Michalek *et al.* 1982].

In 2 cases of suicide by means of disopyramide ingestion, the postmortem blood concentrations were 146 mg/L (amount ingested unknown) and 63 mg/L (3.6 g apparently ingested) [Sathyavagiswaran 1987].

An 11-year-old feamle was anaesthetised with lidocaine. Shortly afterwards she suffered respiratory arrest. Ten hours after the spinal anaesthesia she died. At postmortem the concentrations of disopyramide shown below were found.

	Concentration (mg/L or µg/g)
Plasma	15.5
CSF	3.6
Brain	2.8
Lung	18.0
Heart	14.6
Liver	22.9
Spleen	17.8
Kidney	57.7

[Sakata *et al.* 1988]

In a 4-year-old child who died after accidental ingestion of disopyramide and sulindac, postmortem tissue concentrations were (for disopyramide and sulindac, respectively): blood 41.3 and 12.2 mg/L, bile 435 and 1251 mg/L, liver 98.2 and 12.4 µg/g, gastric content 15 and 70 mg, urine 430 and 29.8 mg/L [Singer, Mozayani 1995].

Note For a case of acute hepatotoxicity after disopyramide, see Antonelli *et al.* [1984].

Half-life Plasma half-life, disopyramide 3 to 11 h (dose-dependent), increased in subjects with renal [Nagura *et al.* 1991] or hepatic impairment [Baselt 2008]; desalkyl metabolite about 13 h.

Volume of Distribution ≈0.6 L/kg (dose-dependent); unbound drug ≈2 L/kg [Giacomini *et al.* 1982].

Distribution Milk : plasma ratio 0.9 [Barnett *et al.* 1982].

Clearance Plasma clearance, ≈0.5 to 2 mL/min/kg (dose-dependent); unbound drug ≈6 mL/min/kg [Giacomini *et al.* 1982].

Protein Binding Dominated by α_1-acid glycoprotein and to some extent abumin [Echizen *et al.* 1991; Lima 1987]. In plasma, disopyramide ≈35 to 82% (concentration-dependent), desalkyl metabolite ≈30 to 70% (concentration-dependent); considerable intersubject genetic variation occurs [Hervé *et al.* 1996; Kuroda *et al.* 2001].

Note For reviews of disopyramide, see Heel *et al.* [1978]and Karim *et al.* [1982].

Dose 300 to 800 mg daily.

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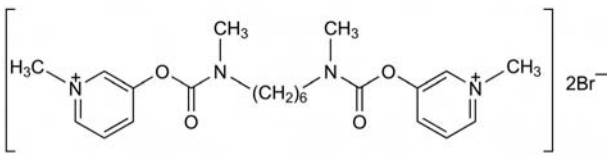
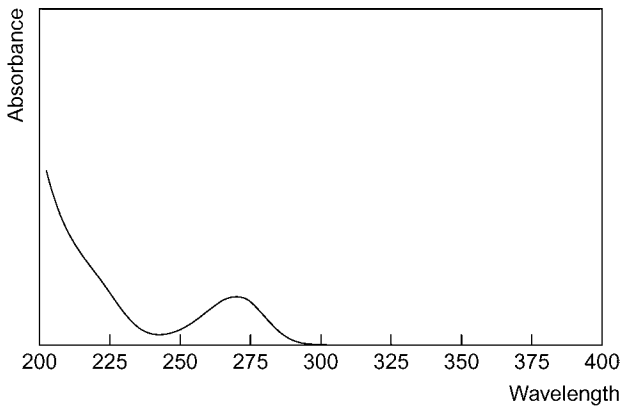
Distigmine Bromide

Anticholinesterase
$C_{22}H_{32}Br_2N_4O_4 = 576.3$
CAS—15876-67-2

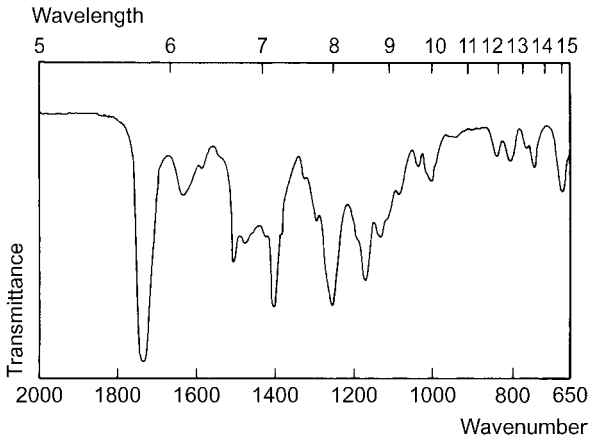
Synonyms Bispyridostigmine bromide; hexamarium bromide; 3,3′-[1,6-hexanediylbis[(methylimino)carbonyl]oxy]bis[1-methylpyridinium]dibromide.
Proprietary Names *Tonus*; *Ubretid*.



Chemical Properties A crystalline powder. Decomposes at 149°. Freely soluble in water. Log *P* (octanol/water), −0.4.
Thin-layer Chromatography System TA—R_f 0.00; system TE—R_f 0.03; system TAE—R_f 0.02 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—270 nm (A^1_1=160a).



Infrared Spectrum Principal peaks at wavenumbers 1735, 1255, 1170, 1507, 1135, 1295 cm^{-1} (KBr disk).

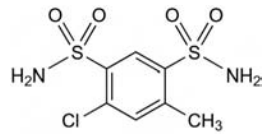


Disposition in the Body Poorly absorbed after oral administration. <5% of an oral dose is excreted in the urine in 24 h, compared to 50% of an IM dose.
Dose 5 to 20 mg daily, orally.

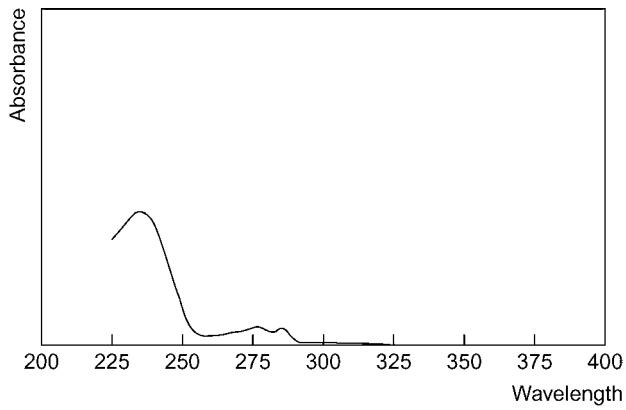
Disulfamide

Diuretic
$C_7H_9ClN_2O_4S_2 = 284.7$
CAS—671-88-5

IUPAC Name 4-Chloro-6-methyl-1,3-benzenedisulfonamide
Synonym Disulphamide
Proprietary Names *Disamide*; *Natirene*.



Chemical Properties A white or creamy-white crystalline powder. Mp 260°. Soluble 1 in 500 of cold water, 1 in 50 of boiling water, 1 in 50 of ethanol and 1 in 1000 of ether; slightly soluble in chloroform. Log *P* (octanol/water), 1.0.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1=336b), 276, 285 nm.

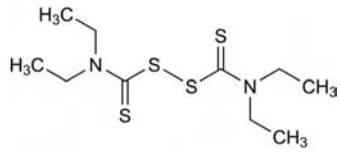


Infrared Spectrum Principal peaks at wavenumbers 1320, 1172, 1552, 965, 675, 1550 cm^{-1} (KBr disk).
Dose Disulfamide has been given in doses of 100 to 200 mg daily.

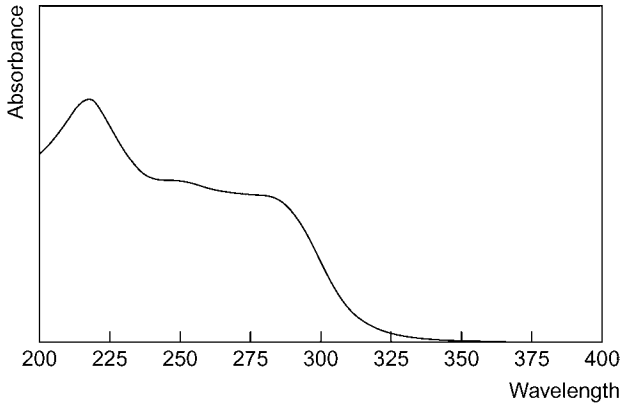
Disulfiram

Alcohol Deterrent
$C_{10}H_{20}N_2S_4 = 296.5$
CAS—97-77-8

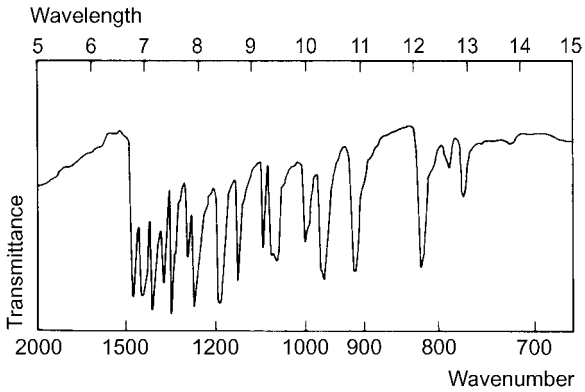
IUPAC Name Diethylcarbamothioylsulfanyl *N*,*N*-diethylcarbamodithioate
Synonyms Éthyldithiourame; tetraethylthioperoxydicarbonic diamide; TTD.
Proprietary Names *Antabus(e)*; *Difaram*; *Esperal*; *Etabus*; *Etiltox*; *Refusal*; *Tetradin*.



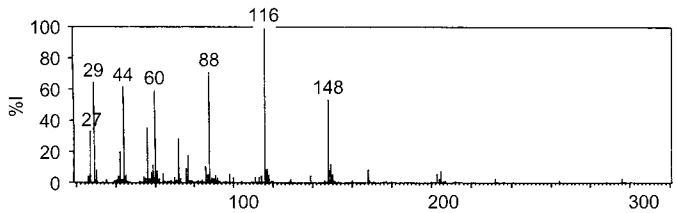
Chemical Properties A white powder. Mp 70°. Practically insoluble in water; soluble 1 in 65 of ethanol, 1 in 2 of chloroform and 1 in 20 of ether; soluble in acetone, benzene and carbon disulfide. Log *P* (octanol/water), 3.9.
Colour Tests Palladium chloride—orange; Dissolve 50 mg in 5 mL of ethanol and add 1 mL of potassium cyanide solution—yellow→green→blue-green.
Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.21; system TC—R_f 0.78; system TE—R_f 0.78; system TL—R_f 0.69; system TAE—R_f 0.78; system TAF—R_f 0.81.
Gas Chromatography System GA—RI 2141(on packed column, not eluted on capillary column).
High Performance Liquid Chromatography System HX—RI 741; system HY—RI 734; system HAA—retention time 25.1 min.
Ultraviolet Spectrum Principal peak at 219 nm.



Infrared Spectrum Principal peaks at wavenumbers 1267, 1193, 1492, 1147, 966, 913 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 116, 88, 29, 44, 60, 148, 56, 27.



Quantification

Blood **GC** Head-space analysis and FPD. Limit of detection, 10 µg/L, disulfiram and metabolites [Sauter, von Wartburg 1977].

Plasma **HPLC** For method for quantification of disulfiram and its metabolites, see Johansson [1986]. UV detection. Limit of detection, 50 µg/L, disulfiram and dithiocarbamate metabolites [Masso, Kramer 1981]. UV detection. Limit of detection, 200 µg/L, disulfiram and metabolites [Jensen, Faiman 1980].

Urine **HPLC** See Plasma [Jensen, Faiman 1980].

Disposition in the Body Disulfiram produces hypersensitivity to alcohol by inhibiting the oxidation of acetaldehyde, the primary metabolite of alcohol; the concentrations of acetaldehyde in the blood and expired air are increased by 5 to 10 times. Disulfiram is incompletely absorbed after oral administration; the maximum effect usually occurs about 12 h after ingestion, possibly because it is highly lipid soluble and is likely to be initially localised in fat. The metabolites include diethyldithiocarbamic acid, methyl diethyldithiocarbamate, diethylamine, carbon disulfide and inorganic sulfate; glucuronide conjugation may occur. Up to about 20% of a dose is eliminated unchanged in the faeces, up to 50% of a dose may be excreted from the lungs as carbon disulfide, and the remainder is slowly excreted as metabolites in the urine; about 20% of a dose is still present in the body up to 7 days after ingestion.

Therapeutic Concentration

Following a single oral dose of 250 mg to 15 subjects, mean peak plasma concentrations of 0.38 mg/L of disulfiram, 0.77 mg/L of diethyldithiocarbamic acid, 0.30 mg/L of methyl diethyldithiocarbamate, and 1.7 mg/L of diethylamine were attained in about 9 h; a mean peak plasma concentration of 22 mg/L of carbon disulfide was reported at 6 h; there was considerable intersubject variability in the plasma concentrations [Faiman *et al.* 1984].

Toxicity Severe toxic reactions may occur after administration of disulfiram if the blood concentration of acetaldehyde is greater than 5 mg/L; with very high concentrations of alcohol in the blood the administration of as little as 0.5 to 1 g of disulfiram has proved fatal in some cases. Fatalities due to disulfiram-induced hepatitis have been reported and there have also been reports of severe phenytoin intoxication after simultaneous administration.

In a fatality due to ingestion of disulfiram and alcohol, postmortem blood concentrations of 8 mg/L of sodium diethyldithiocarbamate and 9600 mg/L of ethanol were reported [Martens, Heyndrickx 1975].

Half-life Plasma half-life, disulfiram about 7 h, diethyldithiocarbamic acid about 15 h, carbon disulfide about 9 h.

Note For a review of disulfiram, see Brien and Loomis [1983] and Johansson [1992].

Dose 800 mg on the first day, reducing to a maintenance dose of 100 to 200 mg daily.

Brien JF, Loomis CW (1983). Disposition and pharmacokinetics of disulfiram and calcium carbimide (calcium cyanamide). *Drug Metab Rev* 14(1): 113–126.
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Disulfoton

Insecticide

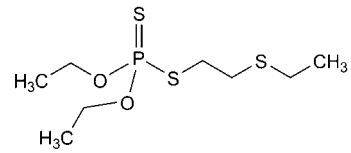
$C_8H_{19}O_2PS_3 = 274.4$

CAS—298-04-4

IUPAC Name Diethoxy-(2-ethylsulfanylethylsulfanyl)-sulfanylidene-λ^5-phosphane

Synonyms Bay-19639; Bay-S276; *O,O*-diethyl-*S*-ethylmercaptoethyldithiophosphate; dithiodemeton; ENT-23347; ethylthiodemeton; thiodemeton; thiometon-ethyl.

Proprietary Names *Dimaz*; *Disipton*; *Dithiosystox*; *Di-syston*; *Disystox*; *Ekatin TD*; *Frumen AL*; *Frumin G*; *Frumin AL*; *Solvigram*; *Solvirex*; *Terraclor Super-X*.



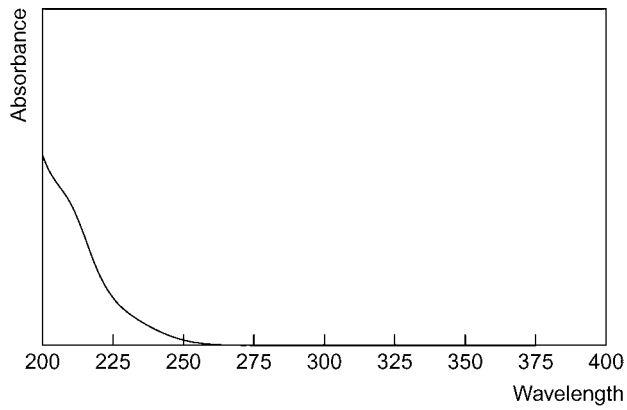
Chemical Properties Pale-yellow liquid or solid. Mp 108°. Bp 62° at 0.01 mm Hg. Insoluble in water (25 mg/L at 23°, 16.3 mg/L at 20°); soluble in most organic solvents and fatty oils. Log *P* (octanol/water) 4.02 [Hansch *et al.* 1995].

Thin-layer Chromatography System TX—R_f 0.58; system TY—R_f 0.89; system TAB—R_f 0.52; system TAC—R_f 0.35.

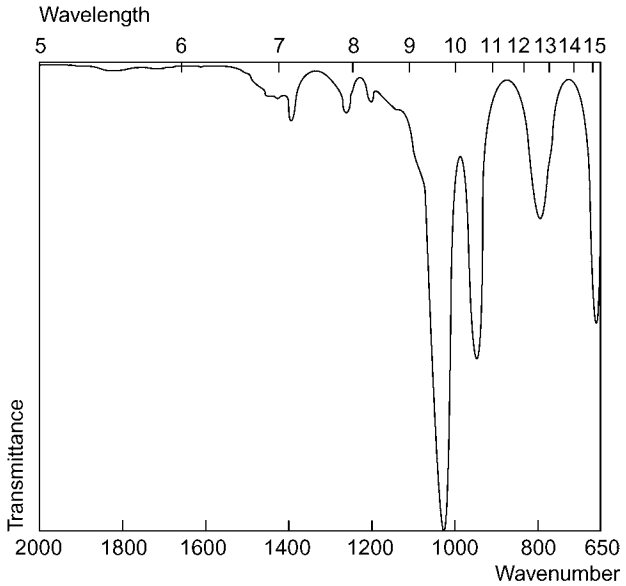
Gas Chromatography System GA—disulfoton RI 2749, M (sulphone) RI 2077, M (sulphoxide) RI 1303; system GK—disulfoton RRT 0.86 (relative to caffeine); system GC1—RI 1776; system GC2—RI 1906; system GC3—RI 2080.

High Performance Liquid Chromatography System HAO—*k* 0.10; system HAP—*k* 6.48; system HY—RI 691.

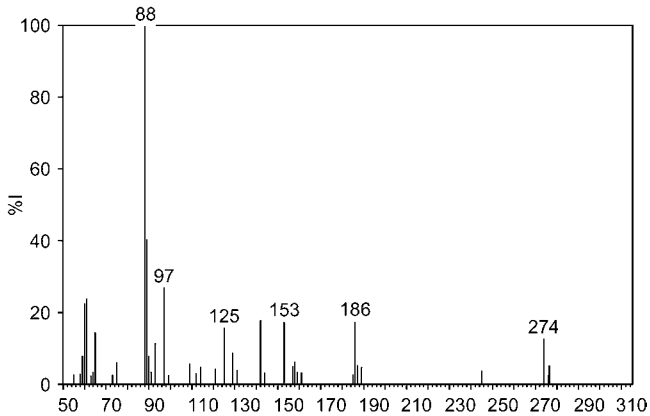
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1031, 952, 666, 798, 2983, 2941 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 89, 29, 97, 60, 61, 27, 65, 125.



Quantification

Blood **GC** Column: NEUTRA BOND-1 fused silica capillary (15 m × 0.53 mm i.d., 2.0 µm). Carrier gas: N_2, 25 mL/min. Temperature: 200°. FPD. Limit of detection, 0.2 ng [Yashiki *et al.* 1990].

GC-MS Column: HP-5MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 1 min to 290° at 10°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.01 µg/g [Musshoff *et al.* 2002]. Column: NEUTRA BOND-1 fused silica capillary (15 m × 0.53 mm i.d., 2.0 µm). Carrier gas: He, 20 mL/min. Temperature programme: 0° to 280° at 8°/min. EI ionisation at 70 eV. Retention time: 13.1 min. Limit of detection not reported [Yashiki *et al.* 1990].

Plasma **GC** Column: 10% Apiezon Grease L on Chromosorb W AW DMCS 60/80 mesh (1.5 m × 3.4 mm i.d.). Carrier gas: He, 66 mL/min. Temperature: 220°. Retention time: 4.0 min. FTD. Limit of detection not reported [Futagami *et al.* 1995].

Urine **GC** See Blood [Yashiki *et al.* 1990].

GC-MS See Blood [Yashiki *et al.* 1990].

Stomach Contents **GC** See Blood [Yashiki *et al.* 1990].

GC-MS See Blood [Yashiki *et al.* 1990].

Disposition in the Body Disulfoton is rapidly absorbed after oral ingestion from the gastrointestinal tract. It is metabolised to the sulfoxide and sulfone metabolites, and corresponding phosphorothioate metabolites and derivatives of *O,O*-diethylhydrophosphate and 2-ethylthioethylmercaptan. It is excreted mainly via urine (≈80%) and also via the faeces and expired air.

Toxicity Disulfoton is very highly toxic by all routes of exposure. The allowed daily intake is 0.002 mg/kg body weight and a blood disulfoton level of 1.45 nmol/g is associated with fatality. A lethal adult dose is estimated to be between 500 mg and 4 g.

A 75-year-old female was admitted to hospital after an attempted suicide. She had ingested a large amount of granular Di-syston, containing 5% disulfoton (an organophosphate). On admission, the total disulfoton and metabolites plasma concentration was 1.095 mg/L which decreased to 505 µg/L after gastric lavage. A peak concentration (1.322 mg/L) was not reached, however, until 56 h post-ingestion, because of the prolonged absorption of the organophosphate from the granules present in the stomach. These concentrations indicate a severe level of disulfoton intoxication [Futagami *et al.* 1995].

An 87-year-old male was admitted to hospital after ingesting three or four heaped tablespoonfuls of Di-Syston (disulfoton) dissolved in water. He was unconscious, had difficulty breathing and did not respond well to painful stimuli, but made a full recovery after treatment. The concentration of disulfoton in his blood was 25.4 ng/g and the total of metabolites was 4.92 nmol/g (corresponding to 1.35 µg/g disulfoton), indicating a severe level of disulfoton intoxication. The concentration of the pesticide in the stomach contents on admission to hospital was 32.9 µg/g [Yashiki *et al.* 1990].

Futagami K *et al.* (1995). Acute organophosphate poisoning after disulfoton ingestion. *J Toxicol Clin Toxicol* 33: 151–155.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Musshoff F *et al.* (2002). Simple determination of 22 organophosphorous pesticides in human blood using headspace solid-phase microextraction and gas chromatography with mass spectrometric detection. *J Chromatogr Sci* 40: 29–34.

Yashiki M *et al.* (1990). Determination of disulfoton and its metabolites in the body fluids of a Di-Syston intoxication case. *Forensic Sci Int* 48: 145–154.

Dithiazanine Iodide

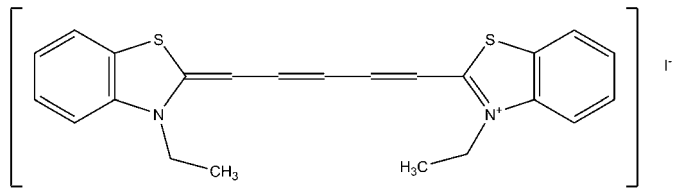
Anthelmintic, Organocyanide

$C_{23}H_{23}N_2S_2,I = 518.5$

CAS—541-73-8

IUPAC Name 3-Ethyl-2-[(3E,5Z)-5-(3-ethyl-1,3-benzothiazol-2-ylidene)penta-1,3-dienyl]-1,3-benzothiazol-3-ium iodide

Synonyms 3,3′-Diethylthiadicarbocyanine iodide; DTDCI; 3-ethyl-2-[5-(3-ethylbenzothiazolidin-2-ylidene)penta-1,3-dienyl]benzothiazolium iodide.



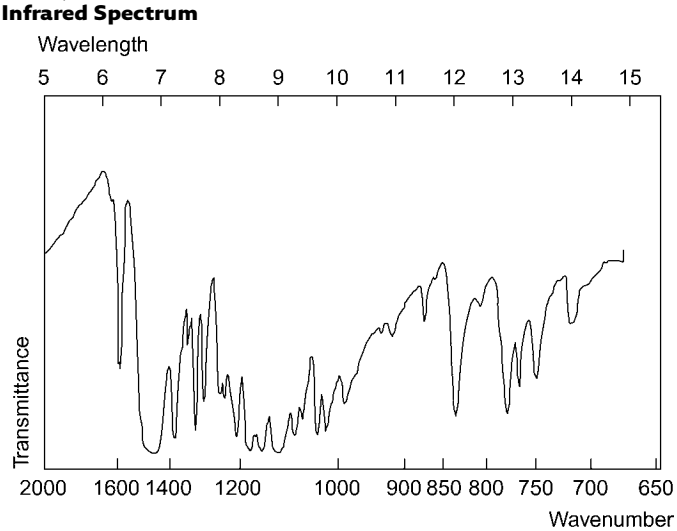
Proprietary Names *Delvex*; *Telmid*.

Chemical Properties Dark-greenish crystalline powder. Mp 248° with decomposition. Almost insoluble in water; very slightly soluble in ethanol; insoluble in ether.

High Performance Liquid Chromatography Column: TSKgel ODS $80T_M$ (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 25 mmol/L imidazole buffer (pH 6.5; 60 : 40) containing 10 mmol/L sodium 1-propanesulfonate, flow rate 1.0 mL/min. Peroxyoxalate chemiluminescence detection. Retention times: DTDCI ≈15 min, methylene blue ≈2 min, pyridine ≈3 min, oxazine ≈5 min. Limit of detection, 0.19 fmol [Kimoto *et al.* 1996].

Ultraviolet Spectrum Ethanol—327, 276 nm with inflexions at about 255 and 364 nm; methanol—652 nm.

Infrared Spectrum



Disposition in the Body Not readily absorbed from the gastrointestinal tract. An effective concentration level of the dye may be attained in the faeces, which it stains a bluish-green colour.

Toxicity Dithiazanine is very toxic when absorbed from the gastrointestinal tract. Its use should be discontinued if the skin or sclera become blue. LD_{50} (oral, mg/kg): in dogs 200, in mice 4 to 6, and in rats 165 to 192.

A 3-year-old girl who was given 150 mg of dithiazanine daily for 5 days died on the sixth day. Dithiazanine was shown spectrophotometrically to be present in the liver in a concentration of 0.1 mg% (1 μg/g) but none was demonstrated in the blood. The intestinal mucosa and contents, and the surface of the kidney, were green; the heart and liver showed some green discolouration [Abadie, Samuels 1965].

Dose Up to 600 mg daily, also used as a near-infrared dye.

Abadie SH, Samuels M (1965). A fatality associated with dithiazine iodide therapy. *JAMA* 192: 326–327.

Kimoto K *et al.* (1996). Sensitive detection of near-infrared fluorescent dyes using high-performance liquid chromatography with peroxyoxalate chemiluminescence detection system. *Biomed Chromatogr* 10: 189–190.

Dithranol

Dermatological Agent

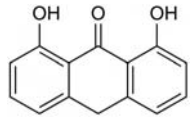
$C_{14}H_{10}O_3$ = 226.2

CAS—1143-38-0

IUPAC Name 1,8-Dihydroxy-9(10*H*)-anthracenone

Synonyms Anthralin; dioxyanthranol.

Proprietary Names *Anthra-Derm*; *Anthraforte*; *Anthrascalp*; *Anthranol*; *Antranol*; *Desmoline*; *Dithrocream*; *Drithocreme*; *Dritho-Scalp*; *Lasan*; *Micanol*; *Psoricreme*; *Psoriderm*; *Timicolid*. It is an ingredient of *Psoradrate* and *Psorin*.



Chemical Properties A yellow to yellowish-brown crystalline powder. Mp 176° to 181°. Practically insoluble in water; slightly soluble in ethanol, glacial acetic acid and ether; soluble in acetone, benzene and chloroform. Log *P* (octanol/water), 4.4.

Caution Dithranol is a powerful irritant and should be kept away from the eyes and tender parts of the skin.

Dithranol Triacetate

$C_{20}H_{16}O_6$ = 352.3

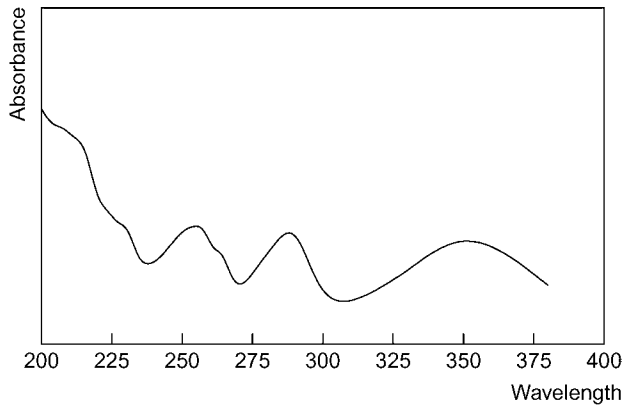
CAS—16203-97-7

Synonym Dithranol acetate

Proprietary Name *Exolan (cream)*

High Performance Liquid Chromatography System HY—RI 703.

Ultraviolet Spectrum Aqueous alkali—276 nm (A^1_1=490b); methanol—256 (A^1_1=467b), 288 (A^1_1=358b), 360 nm (A^1_1=418b).



Infrared Spectrum Principal peaks at wavenumbers 1598, 1278, 1615, 1222, 1168, 1635 cm^{-1} (KCl disk).

Use Topically in concentrations of 0.05 to 1%.

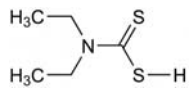
Ditiocarb

Immunomodulator, Chelating Agent

$C_5H_{10}NS_2$ = 148.3

IUPAC Name Diethyldithiocarbamic acid

Synonyms Dithiocarb; diethylcarbamodithioc acid.



Chemical Properties Log *P* (octanol/water), −1.43.

Ditiocarb Sodium

$C_5H_{10}NNaS_2$ = 171.3

CAS—148-18-5

Synonyms ACS; DEDC; DEDK; DeDTC; DDTC; DTC; diethylcarbamodithioc acid sodium salt; dithiocarb sodium; DRG-0066; SDDC; sodium diethyldithiocarbamate; dithiocarb.

Proprietary Name *Imuthiol*

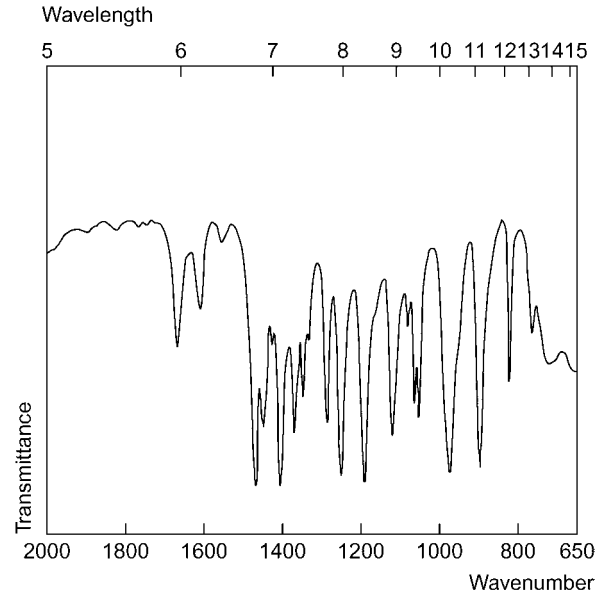
Chemical Properties A white, slightly brown or slightly pink crystalline powder. Mp 95°. Soluble in water (≥10 g/100 mL at 14°).

Ditiocarb Sodium Trihydrate

$C_5H_{10}NNaS_2.3H_2O$ = 225.3

Chemical Properties A powder comprising thin, irregular, plate-like crystals. Mp 94° to 102°. Freely soluble in water; soluble in ethanol, methanol and acetone; insoluble in ether and benzene.

Infrared Spectrum (Ditiocarb sodium) Principal peaks at wavenumbers 1477, 1202, 984, 914 cm^{-1} (KBr pellets).



Quantification

***Plasma* HPLC** Limit of detection, 0.5 mg/L [Awni *et al.* 1994].

Disposition in the Body Ditiocarb is well absorbed and metabolised in the liver to its glucuronide, methyl ester and diethylamine metabolites, carbon disulfide and sulfate ions. Excretion is via urine with carbon disulfide being excreted in exhaled breath. Approximately 80% of an oral dose is detected in plasma 20 min after administration. Ditiocarb is eliminated rapidly from the body with 60% of a dose being excreted within 3 h.

Therapeutic Concentration

Eighteen healthy male volunteers, aged between 18 and 35 years, of white, black and Native American origin were administered with either a 200 mg/m^2/h dose of DDTC or 400 mg/m^2/h, over 4 h. Mean steady state concentrations of diethyldithiocarbamate were 0.027 mg/L for the lower dose and 0.075 mg/L for the higher dose [Awni *et al.* 1994].

Half-life Approximately 20 min.

Volume of Distribution Mean, 0.287 L/kg (200 mg/m^2/h dose); 0.211 L/kg (400 mg/m^2/h dose).

Clearance Mean plasma clearance, 25.5 mL/min/kg (200 mg/m^2/h dose); 16.9 mL/min/kg (400 mg/m^2/h dose).

Awni WM *et al.* (1994). A dose-ranging pharmacokinetics study of sodium diethyldithiocarbamate in normal healthy volunteers. *J Clin Pharmacol* 34: 1183–1190.

Diuron

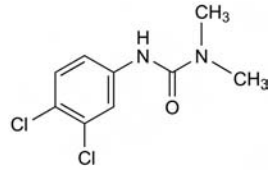
Herbicide

$C_9H_{10}Cl_2N_2O$ = 233.1

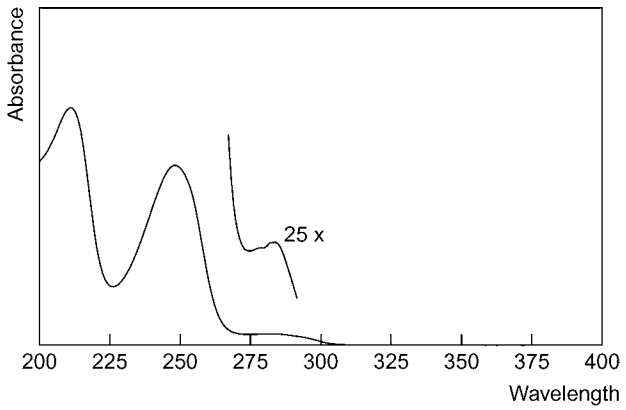
CAS—330-54-1

IUPAC Name *N*′-(3,4-Dichlorophenyl)-*N*,*N*-dimethylurea

Proprietary Names *Karmex*; *Urox D*. It is an ingredient of *Dexuron* and *Krovar*.



Chemical Properties Crystals. Mp 158° to 159°. Practically insoluble in water; soluble in acetone; sparingly soluble in hydrocarbon solvents. Log *P* (octanol/water), 2.7.
Colour Tests Liebermann's reagent—orange; Marquis test—pink-orange.
Gas Chromatography System GA—diuron RI 1850, diuron-ME RI 1880, 3,4-dichloroaniline RI 1405; system GK—3,4-dichloroaniline RRT 0.36 and 3,4-dichlorophenyl isocyanate RRT 0.13 (both relative to caffeine).
High Performance Liquid Chromatography System HY—RI 417; system HZ—retention time 5.8 min; system HAA—retention time 18.5 min.
Ultraviolet Spectrum Ethanol—250 (A^1_1=1124b), 287 nm.

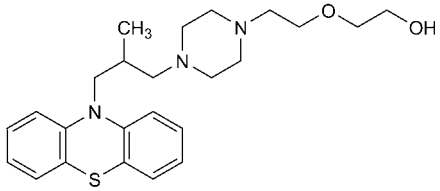


Mass Spectrum Principal ions at *m/z* 72, 44, 73, 42, 232, 187, 124, 45.
Quantification
Blood **HPLC** For method for quantification of diuron and its metabolites, see Van Boven *et al.* [1990].
Urine **GC** MS detection. Limit of detection, 0.03 µg/L, dichloroanilines as markers for diuron and other herbicides, some pesticides and fungicides [Wittke *et al.* 2001]. ECD detection. Limit of detection, 0.05 µg/L, dichloroanilines as markers for diuron and other herbicides, some pesticides, and fungicides [Wittke *et al.* 2001].
HPLC See Blood [Van Boven *et al.* 1990].

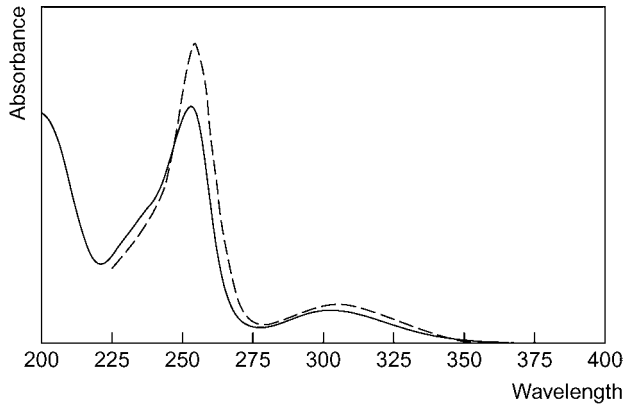
Van Boven M *et al.* (1990). HPLC analysis of diuron and metabolites in blood and urine. *J Anal Toxicol* 14: 231–234.
Wittke K *et al.* (2001). Determination of dichloroanilines in human urine by GC-MS, GC-MS-MS, and GC-ECD as markers of low-level pesticide exposure. *J Chromatogr B Biomed Sci Appl* 755: 215–228.

Dixyrazine

Tranquilliser
$C_{24}H_{33}N_3O_2S$ = 427.6
CAS—2470-73-7
IUPAC Name 2-[2-[4-(2-Methyl-3-phenothiazin-10-ylpropyl)piperazin-1-yl]ethoxy]ethanol
Synonyms 2-(2-{4-[2-Methyl-3-(10*H*-phenothiazin-10-yl)propyl]-1-piperazinyl}ethoxy)ethanol; UCB-3412.
Proprietary Name *Esucos*



Chemical Properties White to slightly greyish or yellowish powder. Very slightly soluble in water; soluble in ethanol, chloroform and ether. pK_a 7.8 (25°). Log *P* (octanol/water), 3.3 [Meylan, Howard 1995].
Thin-layer Chromatography System TAE—R_f 0.47; system TE—R_f 0.49.
Gas Chromatography System GA—dixyrazine RI 3220, M (phenothiazine) RI 2020, dixyrazine-AC RI 3530, M (*N*-desalkyl-)-AC RI 3355, M (*O*-desalkyl-)-AC RI 3350, M (amino-)-AC RI 2765.
Ultraviolet Spectrum Methanol—255 (A^1_1 = 980b), 305 nm.

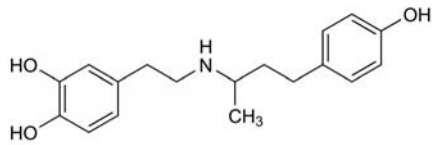


Mass Spectrum Principal ions at *m/z* 212, 42, 187, 45, 70, 180, 56, 98.
Quantification
Plasma **GC-MS** Column: SE-30 glass capillary (7 m). Carrier gas: He, 3.5 mL/min. Temperature: 280°. EI ionisation at 70 eV. Limit of detection, 400 ng/L [Brante *et al.* 1981].
Serum **GC-MS** See Plasma [Brante *et al.* 1981].
HPLC Column: Sphere-5 C_{18} (25 cm × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 1.0% triethylamine-phosphate buffer (pH 4.5; 37 : 13 : 50), flow rate 0.6 mL/min. UV detection (λ = 254 nm). Retention time: 4.5 min. Limit of detection, 1.0 nmol/L [Liedholm *et al.* 1985].
Bioavailability Low in the fasting state, increased to 2 to 29% following food [Liedholm *et al.* 1985].
Half-life 3.4 h after IV administration.
Volume of Distribution 5.9 L/kg.
Clearance >1200 mL/min.
Dose 20 to 75 mg daily.

Brante G *et al.* (1981). Gas chromatographic—mass spectrometric determination of dixyrazine in human blood. *Eur J Clin Pharmacol* 20: 307–310.
Liedholm H *et al.* (1985). Pharmacokinetics of dixyrazine: low bioavailability, improved by food intake. *Drug Nutr Interact* 3: 87–92.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Dobutamine

Sympathomimetic
$C_{18}H_{23}NO_3$ = 301.4
CAS—34368-04-2
IUPAC Name 4-[2-[[3-(4-Hydroxyphenyl)-1-methylpropyl]amino]ethyl]-1,2-benzenediol

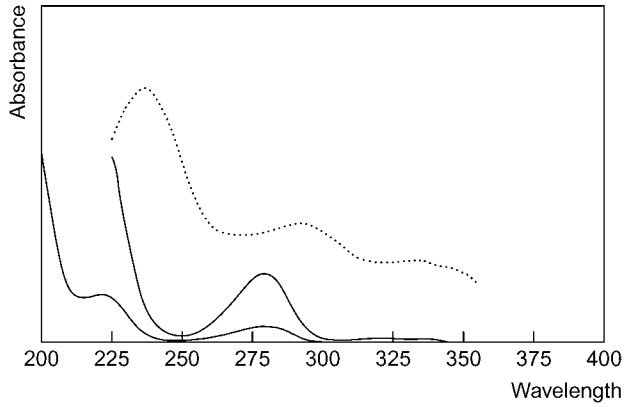


Chemical Properties pK_a 9.5.

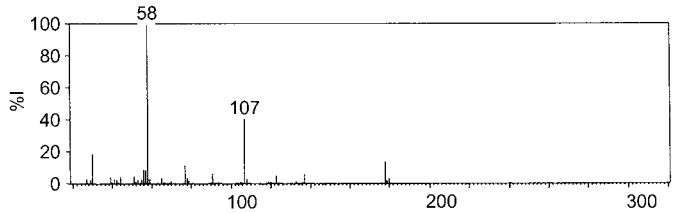
Dobutamine Hydrochloride

$C_{18}H_{23}NO_3$,HCl = 337.8
CAS—49745-95-1
Proprietary Names *Butamine*; *Cryobutol*; *Dobucard*; *Dobuject*; *Dobutam*; *Dobutina*; *Dobutrex*; *Inotrex*; *Kardion*; *Oxiken*; *Posiject*.
Chemical Properties A white powder. Mp 184° to 186°. Sparingly soluble in water and ethanol; soluble in methanol and pyridine.

Colour Tests Ammoniacal silver nitrate—black; *p*-dimethylaminobenzaldehyde—orange/violet; ferric chloride—green; folin–ciocalteu reagent—blue; methanolic potassium hydroxide—brown-pink; Millon's reagent—red; Nessler's reagent—black; palladium chloride—orange→brown; potassium dichromate—green-brown.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.49; system TL—R_f 0.03; system TAE—R_f 0.87.
Gas Chromatography System GA—dobutamine-AC_4 RI 3495, M (*N*-desalkyl-*O*-methyl-)-AC_2 RI 2070, M (*N*-desalkyl-*O*-methyl-)-AC RI 2330, M (*O*-methyl-) RI 3200, M (*O*-methyl-)-AC_2 RI 3100, M (*O*-methyl-)-AC_3 RI 3350.
High Performance Liquid Chromatography System HX—RI 308; system HAX—retention time 6.6 min; system HAY—retention time 3.8 min.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=131b); aqueous alkali—236, 292 nm.



Infrared Spectrum Principal peaks at wavenumbers 1520, 1280, 1266, 1190, 1200, 1220 cm^{-1} (dobutamine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 107, 30, 178, 77, 56, 57, 137.

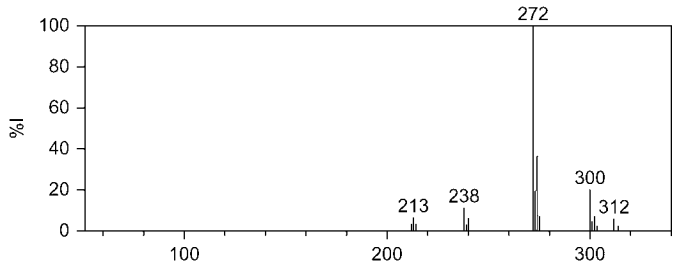


Quantification
Plasma **HPLC** Fluorescence detection. Limit of detection, 5 µg/L [Leflour *et al.* 1994]. Electrochemical detection. Limit of detection, <0.1 µg/L [Husseini *et al.* 1993]. Fluorescence detection. Limit of detection, 0.3 to 0.8 pg [Alberts *et al.* 1992]. Fluorescence detection. For method, see Knoll and Brandl [1986]. Electrochemical detection. Limit of detection, 1 µg/L [Dixon *et al.* 1985].
Urine **HPLC** See Plasma [Alberts *et al.* 1992].
Disposition in the Body Inactive after oral administration. After IV administration, it is rapidly metabolised by glucuronide conjugation and 3-*O*-methylation to inactive metabolites which are excreted in the urine, mostly in the first 2 h.
Half-life Plasma half-life, about 2 min.
Dose Usually 2.5 to 10 µg/kg/min of dobutamine hydrochloride, by IV infusion.

Alberts G *et al.* (1992). Simultaneous determination of catecholamines and dobutamine in human plasma and urine by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 583: 236–240.
Dixon R *et al.* (1985). Cardiotonic agents: a simple HPLC procedure for the quantitation of dobutamine and a new congener in plasma. *Res Commun Chem Pathol Pharmacol* 48: 313–316.
Hardee GE, Lai JW (1983). *Anal Lett (Part B)* 16: 69–75.
Husseini H *et al.* (1993). Rapid and sensitive assay of dobutamine in plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr* 620: 164–168.
Knoll R, Brandl M (1986). [Determination of dobutamine levels in human plasma–methodological and clinical aspects]. *Anasth Intensivther Notfallmed* 21: 34–37.
Leflour C *et al.* (1994). Solid phase extraction and high performance liquid chromatographic determination of dobutamine in plasma of dialysed patients. *Biomed Chromatogr* 8: 309–312.

DOC

Psychedelic
$C_{11}H_{17}ClNO_2 = 230.7$
IUPAC Name 4-Chloro-2,5-dimethoxyamfetamine
Synonyms 4-Chloro-2,5-DMA; 2,5-dimethoxy-4-chloroamfetamine.
Chemical Properties DOC has a stereocentre and *R*(−)-DOC is reported to be the more active stereoisomer.
Mass Spectrum Principal peaks at *m/z* 212, 86, 271, 197, 185, 156 (AC) [Ewald *et al.* 2008].

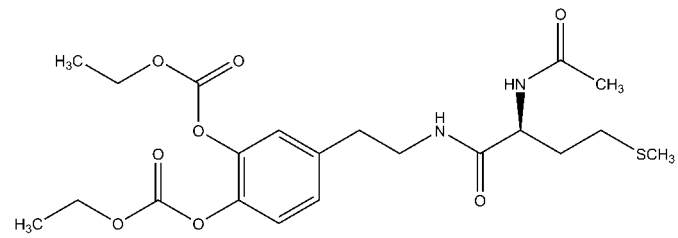


Quantification
Other **GC-MS** Rat Urine. Column: HP-1 cross-linked methyl silicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min. EI ionisation at 70 eV, positive ion mode, full scan mode; CI, positive ion mode. Retention index: 2055 (acetyl derivative). Limit of quantification not reported [Ewald *et al.* 2008].
Disposition in the Body Studies in rats have shown that DOC is metabolised by *O*-demethylation at position 2 or 5 of the phenyl ring and subsequently by glucuronidation and/or sulfation.
Toxicity Typically, entheogenic effects of DOC in humans are reported to last between 12–24 h. Onset of the drug is relatively long, developing over a period of 3–5 h. After several more hours, effects begin to decline, but residual stimulation is sometimes observed [Shulgin, Shulgin 1991].
Note For a study of the metabolism and detection of DOC in rats, see Ewald *et al.* [2008].
Dose Reported as 1.5 to 3 mg.

Ewald AH *et al.* (2008). Metabolism and toxicological detection of the designer drug 4-chloro-2,5-dimethoxyamphetamine in rat urine using gas chromatography-mass spectrometry. *Anal Bioanal Chem* 390: 1837–1842.
Shulgin, A, Shulgin, A. (1991) *PiHKAL: A Chemical Love Story.* Berkeley, CA: Transform Press.

Docarpamine

Cardiotonic, Dopaminergic Agent, Dopamine Prodrug
$C_{21}H_{30}N_2O_8S = 470.5$
CAS—74639-40-0
IUPAC Name [5-[2-[[(2*S*)-2-Acetamido-4-methylsulfanylbutanoyl]amino] ethyl]-2-ethoxycarbonyloxyphenyl] ethyl carbonate
Synonyms (−)-(*S*)-2-Acetamido-*N*-(3,4-dihydroxyphenethyl)-4-(methylthio) butyramide bis(ethyl carbonate) ester; 4-[2-[[(2*S*)-2-(acetylamino)-4-(methylthio)-1-oxobutyl]amino]ethyl]-1,2-phenylene diethyl ester; TA-870; TA-8704.
Proprietary Name *Tanadopa*



Chemical Properties Crystals. Mp 85° to 90°. Crystalline powder. Mp 105° to 108°. Slightly soluble in water; readily soluble in ethanol.
High Performance Liquid Chromatography Column: Cosmosil 5C-18-MS (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 4.2; 0 : 100 for 2 min to 75 : 25 over 7.5 min for 5.5 min or 40 : 60), flow rate 1.5 mL/min. UV detection. Retention times: 11.4 min (gradient) or 6.5 min (isocratic) [Sugiyama *et al.* 2000].
Quantification
Plasma **HPLC** Desethoxy metabolite. Column: ODS (250 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L phosphate buffer (pH 3.0) containing 0.1 mmol/L sodium EDTA : methanol : acetonitrile (70 : 25 : 5), flow rate 1.2 mL/min. Electrochemical detection (700 mV). Limit of detection not reported [Yoshikawa *et al.* 1990a].
Other **HPLC** Dog Blood. Column switching method. Electrochemical detection. Limit of quantification, 2 µg/L [Yoshikawa *et al.* 1990b].
Disposition in the Body Readily absorbed following IM or oral administration. It appears to be rapidly metabolised to its desethoxy form in plasma, which is then converted to dopamine. Dopamine is known to be metabolised by MAO and catechol *O*-methyltransferase to form 3,4-dihydroxyphenylacetic acid and homovanillic acid as well as other metabolites.
Therapeutic Concentration
Ten infants (age 1–4 months, weight 2.95–5.16 kg) with symptoms of CHF were administered 3 doses of oral docarpamine (range 15.0–20.4 mg/kg) at intervals of 8 h. Plasma concentrations of free dopamine were measured at 1, 2, and 3 h after the first dose; mean values reported were 37.9, 37.8, and 11.5 µg/L, respectively [Tomita *et al.* 1996].

In an adult clinical study, a single oral dose administration of 750 mg docarpamine resulted in a peak plasma concentration of free dopamine of 54.1 µg/L [Ogawa *et al.* 1991].

Two groups of 3 volunteers were administered either 0.75 or 1.5 g oral docarpamine. A third group was administered IV dopamine at 1 and 3 µg/kg/min for 20 min successively. Mean peak plasma concentrations of free dopamine in the 0.75 and 1.5 g oral dosage groups were reported as 63 and 127 µg/L, respectively, after 1.6 h. Free dopamine levels in the IV group were reported as 8.4, 31.3 and 0.8 µg/L at 20, 40 and 60 min, respectively. In the 0.75 g oral docarpamine group, the mean peak plasma concentration of the desethoxy form of docarpamine was 176 µg/L after 1.4 h [Yoshikawa *et al.* 1990a].

Dose Usual oral dose of 2.25 g daily, in three divided doses.

Ogawa H *et al.* (1991). Clinical efficacy of TA-870, a new oral dopamine in patients with tapering of dopamine: multiclinical co-operative study. *Jpn J Pharmacol Ther* 19: 1907–1931.

Sugiyama T *et al.* (2000). Selection of mobile phase in high-performance liquid chromatographic determination for medicines. *Biol Pharm Bull* 23: 274–278.

Tomita H *et al.* (1996). Plasma concentration and acute clinical effects of docarpamine, orally active dopamine prodrug, in infants. *Acta Paediatr Jpn* 38: 440–443.

Yoshikawa M *et al.* (1990a). Disposition of a new orally active dopamine prodrug, *N*-(*N*-acetyl-L-methionyl)-*O*,*O*-bis(ethoxycarbonyl) dopamine (TA-870) in humans. *Drug Metab Dispos* 18: 212–217.

Yoshikawa M *et al.* (1990b). A new method for the high performance liquid chromatographic determination of TA-870, a dopamine prodrug (catechol ester compound). *Biomed Chromatogr* 4: 181–187.

Docetaxel

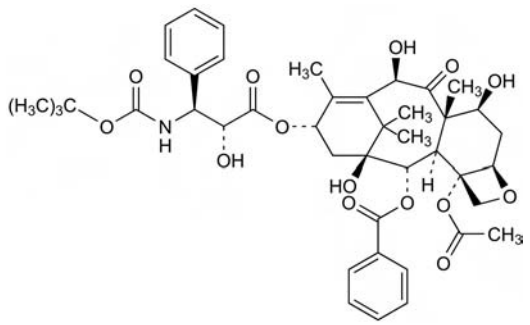
Antineoplastic

$C_{43}H_{53}NO_{14}$ = 807.9

CAS—114977-28-5

Synonyms RP-56976; *N*-debenzoyl-*N*-*tert*-butoxycarbonyl-10-deacetyl taxol; (α*R*,β*S*)-β-[[(1,1-dimethylethoxy)carbonyl]amino]-α-hydrobenzenepropanoic acid (2*aR*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-12b-(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl ester.

Proprietary Name *Taxotere*



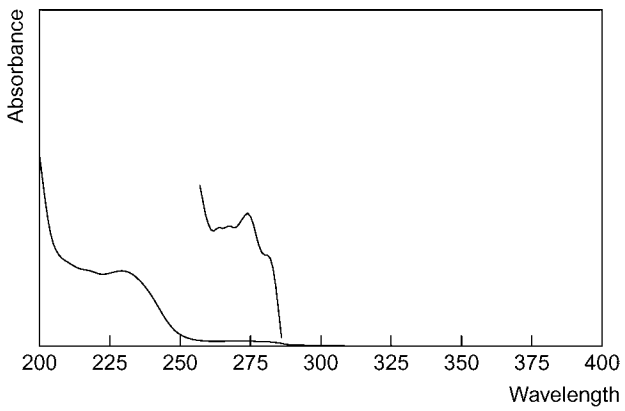
Chemical Properties A white to almost white powder. Mp 232°.

Docetaxel Trihydrate

$C_{43}H_{59}NO_{17}$ = 861.9

CAS—148408-66-6

Ultraviolet Spectrum Aqueous acid (ethanol)—230, 275, 283 nm.



Quantification

Plasma **HPLC** Column: RP-CSC-C_8 Nucleosil (150 × 4.6 mm i.d., 5 µm). Mobile phase: 30 mmol/L phosphate buffer (pH 3.0) : acetonitrile (47:53), flow rate 1.3 mL/min. UV detection (λ=227 nm). Retention time: 7.2 min. Limit of quantification, 5 µg/L, limit of detection, 2.5 µg/L [Rouini *et al.* 1998].Column: Inertsil ODS-80A (150 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : tetrahydrofuran : ammonium hydroxide (37.5 : 60: 2.5 : 0.1, pH 6.0), flow rate 1.0 mL/min. UV detection (λ=230 nm). Retention time: 8.5 min. Limit of quantification, 10 µg/L [Loos *et al.* 1997].

Disposition in the Body Docetaxel is rapidly distributed throughout the body into body tissue and is extensively metabolised by the hepatic cytochromes of the CYP3A group. Excretion is mainly in faeces (75%) as 1 major and 3 minor inactive metabolites and a very low amount of the unchanged drug.

Therapeutic Concentration

Four patients with solid tumours, both male and female, were administered with an IV dose of 100 mg/m^2 docetaxel over 1–2 h. A peak plasma concentration of 2.41 mg/L was reached by the end of infusion [Extra *et al.* 1993].

In another study, 7 patients administered with a 100 mg/m^2 dose reached peak plasma concentrations of 3.67 mg/L [da Costa*et al.* 1992].

Toxicity The maximum tolerated dose is 90 mg/m^2 as a 24-h infusion. Development of severe peripheral neurotoxicity has been observed in 4.1% of patients.

Half-life Approximately 11 h.

Volume of Distribution 95–150 L/m^2 (from various studies), also reported as 113 L.

Clearance 17–22 L/h/m^2 (as reported in various literature).

Distribution in Blood Little interaction with red blood cells.

Protein Binding >95%.

Dose Doses between 55 and 100 mg/m^2 body surface are administered for 1 h every 3 weeks; the greater dose is the usual dose. Lower doses are given if adverse reactions are observed during treatment. Patients with hepatic impairment: 75 mg/m^2.

da Costa L *et al.* (1992). Immunoscintigraphy in Hodgkin's disease and anaplastic large cell lymphomas: results in 18 patients using the iodine radiolabeled monoclonal antibody HRS-3. *Ann Oncol* 3: 453–57.

Extra JM *et al.* (1993). Phase I and pharmacokinetic study of Taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. *Cancer Res* 53: 1037–1042.

Loos WJ *et al.* (1997). Sensitive determination of docetaxel in human plasma by liquid-liquid extraction and reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 693: 437–441.

Rouini MR *et al.* (1998). A rapid reversed phase high performance liquid chromatographic method for the determination of docetaxel (Taxotere) in human plasma using a column switching technique. *J Pharm Biomed Anal* 17: 1243–1247.

Docusate Sodium

Anionic Surfactant

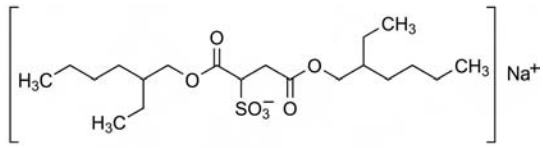
$C_{20}H_{37}NaO_7S$ = 444.6

CAS—10041-19-7 [1,4-bis(2-ethylhexyl) sulfosuccinate]; 577-11-7

IUPAC Name Sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate

Synonyms Dioctyl sodium sulfosuccinate; DSS; sodium 1,4–bis(2–ethylhexyl) sulfosuccinate; sodium dioctyl sulphosuccinate.

Proprietary Names *Colace*; *Coloxyl*; *Dialose*; *Dioctyl*; *Disonate*; *Docusoft*; *Docusol*; *DOK*; *Modane Soft*; *Molcer*; *Norgalax*; *Regulex*; *Silace*; *Waxsol*. It is an ingredient of *Capsuvac*, *Klyx*, *Migraleve*and *Normax*.



Chemical Properties White, hygroscopic, waxy masses or flakes. Slowly soluble 1 in 70 of water, higher concentrations forming a thick gel; soluble 1 in 3 of ethanol, 1 in 1 of chloroform and 1 in 1 of ether; soluble in carbon tetrachloride, petroleum ether, naphtha, xylene, dibutyl phthalate, liquid petrolatum, acetone and vegetable oils. Log *P* (octanol/water), 6.1.

Docusate Calcium

$C_{40}H_{74}CaO_{14}S_2$ = 883.2

CAS—128-49-4

Synonym Dioctyl calcium sulfosuccinate

Proprietary Names *Surfak*. It is an ingredient of *Doxidan*.

Chemical Properties A white amorphous solid. Soluble 1 in 3300 of water; freely soluble in ethanol, chloroform and ether; soluble in mineral and vegetable oils and liquid polyethylene glycol; practically insoluble in glycerol.

Docusate Potassium

$C_{20}H_{37}KO_7S$ = 460.7

CAS—7491-09-0

Synonym Dioctyl potassium sulfosuccinate

Proprietary Names *Dialose*; *Kasof*.

Chemical Properties A white amorphous solid. Sparingly soluble in water; soluble in ethanol; very soluble in light petroleum.

Colour Tests To 5 mL of a 0.1% solution add 1 mL of dilute sulfuric acid, 10 mL of chloroform and 0.2 mL of dimethyl yellow solution and shake—the chloroform layer is coloured red. Add 50 mg of cetrimide and shake—the colour of the chloroform layer changes to yellow.

Infrared Spectrum Principal peaks at wavenumbers 1724, 1250, 1052, 1020, 1612, 1086 cm^{-1}.

Disposition in the Body Absorbed after oral administration and excreted in the bile. It is excreted in breast milk.

Dose 50 to 500 mg daily as a laxative.

Dodecyl Gallate

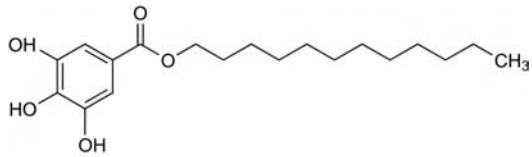
Antioxidant

$C_{19}H_{30}O_5$ = 338.4

CAS—1166-52-5

IUPAC Name Dodecyl 3,4,5-trihydroxybenzoate

Synonym Lauril gallate
Proprietary Names *Progallin LA*. It is an ingredient of *Embanox 7*.

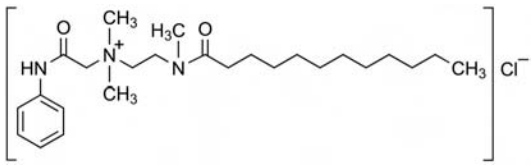


Chemical Properties A white or creamy-white powder. Mp 96° to 97.5°. Practically insoluble in water; soluble 1 in 3.5 of ethanol, 1 in 60 of chloroform and 1 in 4 of ether.
Colour Tests Ammoniacal silver nitrate—black; ferric chloride—blue; Folin–Ciocalteau reagent—blue; methanolic potassium hydroxide—orange; Nessler's reagent—black.
Ultraviolet Spectrum Methanol—275 nm (A_1^1=300b).

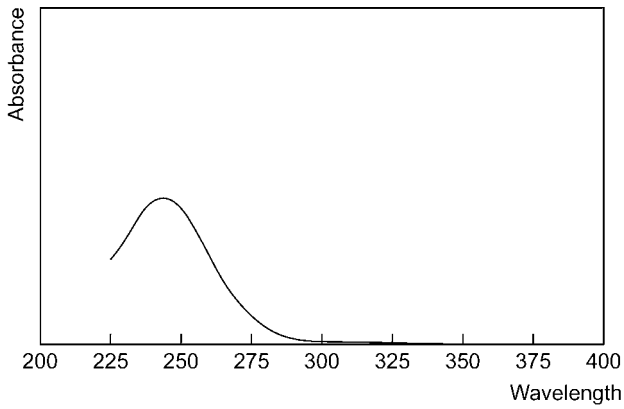
Dofamium Chloride

Cationic Disinfectant
$C_{25}H_{44}ClN_3O_2$ = 454.1
CAS—54063-35-3

IUPAC Name (2-Anilino-2-oxoethyl)-[2-[dodecanoyl(methyl)amino]ethyl]-dimethylazanium chloride
Synonyms Dimethyl [2-(*N*-methyldodecanamido)ethyl][(phenylcarbamoyl) methyl]ammonium chloride; phenamylium chloride.



Chemical Properties A white crystalline powder. Soluble in water.
Colour Test Mandelin's test—red→brown.
Thin-layer Chromatography System TA—R_f 0.03 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1676, RI 1898 and RI 1974.
Ultraviolet Spectrum Aqueous acid—244 nm (A_1^1=242b).

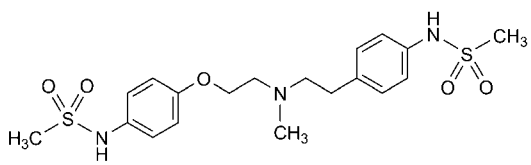


Infrared Spectrum Principal peaks at wavenumbers 1670, 1626, 760, 1538, 1592, 1298 cm^{-1}.

Dofetilide

Antiarrhythmic
$C_{19}H_{27}N_3O_5S$ = 441.6
CAS—115256-11-6

IUPAC Name *N*-[4-[2-[2-[4-(Methanesulfonamido)phenyl]ethyl-methyl-amino]ethoxy]phenyl]methanesulfonamide
Synonyms 1-(4-methanesulfonamidophenoxy)-2-[*N*-(4-methanesulfonamido-phenethyl)-*N*-methylamino]ethane; *N*-[4-[2-[Methyl[2-[4-[(methylsulfonyl)amino]-phenoxy]ethyl]amino]ethyl]phenyl]methanesulfonamide; UK-68798.
Proprietary Name *Tikosyn*



Chemical Properties White to off-white crystalline solid. Mp 161°. Very slightly soluble in water and propan-2-ol; soluble in 0.1 mol/L sodium hydroxide, acetone and 0.1 mol/L hydrochloric acid. pK_a 7.0; 9.0; 9.6. Log *P* (pH 7.4) 0.96.
Quantification
Urine **HPLC** Column: Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : ammonium phosphate buffer (pH 7.0; 35 : 65), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: dofetilide, 8.5 min; *N*-desmethyl metabolite, 7.5 min. Limit of detection, 2.5 µgL [Walker *et al.* 1991].
Disposition in the Body Dofetilide is almost completely absorbed after oral administration and peak plasma concentrations are observed after 3 h. The presence of food increases the time taken to reach these concentrations but does not alter the oral bioavailability. Steady-state concentrations are reached within 2 to 3 days. It is metabolised in the liver, by *N*-oxidation and *N*-deakylation, to essentially inactive metabolites. Approximately 80% of an administered dose is excreted in urine; mostly as the unchanged drug and the rest as inactive or minimal activity metabolites. No quantifiable amounts of metabolites have been detected in plasma but five have been detected in urine.
Therapeutic Concentration
Eighteen male and female patients with coronary artery disease, mean age 55 years (range, 42 to 65 years) were administered doses of 1.5, 3.0 and 4.5 µg/kg dofetilide at a constant infusion rate over 10 min. The mean peak plasma concentrations were 1.74 (0.70 to 2.51) µg/L, 3.35 (2.7 to 3.86) µg/L and 5.11 (3.25 to 6.47) µg/L for the three doses. These concentrations were observed at the end of the infusion period [Sedgwick *et al.* 1991].

Ten healthy males (mean age, 23.4 years; range, 19 to 30 years) were administered 0.5 mg dofetilide orally and 0.5 mg IV as a continuous infusion over a 30-min period. A wash-out period of 1 week was allowed between treatments. The peak plasma concentrations were 1.9 (range, 1.3 to 2.4) µg/L for the oral dose, observed at 2.6 (1.5 to 4.0) h, and 6.0 (3.7 to 8.2) µg/L for the IV dose [Le Coz *et al.* 1995].

Healthy males aged 18 to 45 years (mean, 25 years) were divided into three groups and treated with dofetilide. Group 1: 1.0 mg daily (0.33 mg three times a day) and 0.5 mg twice daily; group 2: 1.5 mg daily (0.5 mg three times daily) and 0.75 mg twice daily; group 3: 2.5 mg daily (1.25 mg twice daily) and 0.83 mg three times daily. Each volunteer participated in a 5-day dosing period with a 6-day washout in between. For group 1, the peak plasma concentrations were 1.7 µg/L for the 0.33 mg dose on day 1 and 2.64 µg/L for the 0.5 mg dose. These concentrations were observed at 2.29 and 2.00 h, respectively. On day 5, the concentrations were 3.04 and 3.80 µg/L for the two doses, observed at 2 h. For group 2, peak plasma concentrations were 2.26 µg/L for the 0.5 mg dose on day 1 and 3.54 µg/L for the 0.75 mg dose, both observed at 2.13 h. On day 5, the concentrations were 4.78 and 5.23 µg/L for the 2 doses, observed at 2.0 and 1.88 h, respectively. For group 3, concentrations reached 3.55 µg/L for the 0.83 mg dose on day 1 and 5.47 µg/L for the 1.25 mg dose. These were observed at 2.29 and 1.57 h, respectively. On day 5, concentrations were 7.71 and 10.07 µg/L for the 2 doses, observed at 2.14 and 1.57 h, respectively. Steady-state concentrations were reached by day 3 [Allen *et al.* 2000].
Toxicity
In some patients being treated with dofetilide, it can cause an abnormal heartbeat which in rare instances can result in death. Symptoms are fast beating of the heart, dizziness and fainting which usually occur in the first few days of treatment [American Society of Health-System Pharmacists 2007].

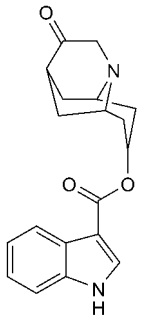
Above the recommended therapeutic dose, 500 µg twice daily, the degree of QT prolongation and the subsequent risk of cardiac arrhythmia is considered too high [Allen *et al.* 2000].
Half-life Plasma elimination, 6.2 to 9.7 h; half-life increases with renal impairment.
Bioavailability 92 to 99%.
Volume of Distribution 226 to 249 L; 3.8 L/kg.
Clearance Systemic clearance, 0.35 L/h/kg; plasma, 4.7 mL/min/kg [Sedgwick *et al.* 1991]; 20.8 to 28.2 L/h; clearance decreases with renal impairment.
Protein Binding 60 to 70%.
Dose The recommended oral dose is 500 µg twice daily (normal renal function); 250 µg twice daily (creatinine clearance, 40 to 60 mL/min); 125 µg twice daily (creatinine clearance, 20 to 40 mL/min).

Allen MJ *et al.* (2000). The pharmacokinetics and pharmacodynamics of oral dofetilide after twice daily and three times daily dosing. *Br J Clin Pharmacol* 50: 247–253.
American Society of Health-System Pharmacists (2007). *Dofetilide*. Baltimore, MD: AHFS Consumer Medication Information.
LeCoz F *et al.* (1995). Pharmacokinetic and pharmacodynamic modeling of the effects of oral and intravenous administrations of dofetilide on ventricular repolarization. *Clin Pharmacol Ther* 57: 533–542.
Sedgwick M *et al.* (1991). Pharmacokinetic and pharmacodynamic effects of UK-68,798, a new potential class III antiarrhythmic drug. *Br J Clin Pharmacol* 31: 515–519.
Walker DK *et al.* (1991). Liquid-liquid extraction and high-performance liquid chromatography for the determination of a novel antidysrhythmic agent (UK-68, 798) in human urine. *J Chromatogr* 568: 475–480.

Dolasetron

5-HT_3 Receptor Antagonist, Antiemetic
$C_{19}H_{20}N_2O_3$ =324.4
CAS—115956-12-2

Synonyms 1*H*-Indole-3-carboxylic acid (2α,6α,8α,9^a,β)-octahydro-3-oxo-2,6-methano-2*H*-quinolizin-8-yl ester; MDL-73147.

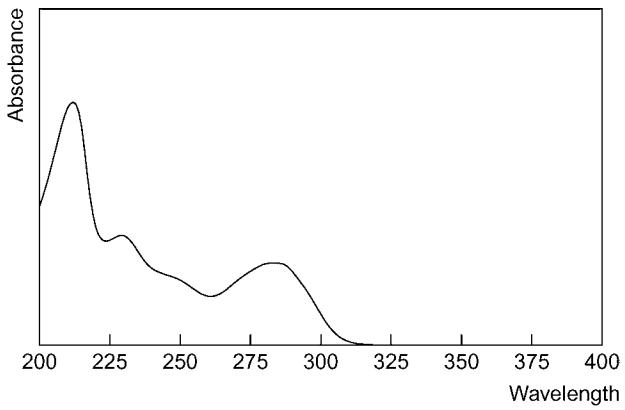


Chemical Properties Log *P* (octanol/water) 2.35 [Johnson *et al.* 2003].

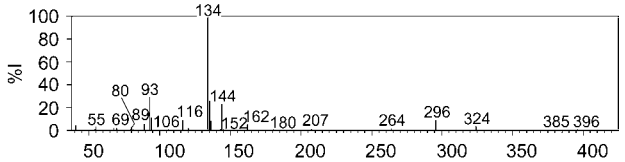
Dolasetron Methanesulfonate

$C_{19}H_{20}N_2O_3.CH_3SO_3H = 420.5$
CAS—115956-13-3
Synonyms Dolasetron mesylate; MDL-73147EF.
Proprietary Name *Anzemet*
Chemical Properties White to off-white powder. Mp 278°. Freely soluble in water and propylene glycol; slightly soluble in ethanol and normal saline. Stable for at least 90 days if stored at 3–5 or 23–25° in Ora-Plus and strawberry syrup (1 : 1) or Ora sweet SF [Johnson *et al.* 2003].

Ultraviolet Spectrum Aqueous acid (ethanol)—229, 282 nm.



Mass Spectrum Principal ions at *m/z* 134, 93, 135, 144, 116, 296, 162, 324.



Quantification

Plasma **GC-MS** Column: DB-5 (7.5 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 10–11 psi. Temperature programme: 170° for 1 min to 270° at 20°/min for 2 min. PCI. Retention time: 6.45 min. Limit of quantification, 2 μg/L [Gillespie *et al.* 1993].

HPLC Column: C_{18}. Mobile phase: acetonitrile : 0.05 mol/L ammonium acetate (pH 7.5; 24 : 76), flow rate 0.8 mL/min. UV detection (λ = 280 nm). Retention time: 6.9 min. Limit of detection not reported [Johnson *et al.* 2003]. Column: OVM (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 25 mmol/L ammonium acetate (pH 7.2; 16 : 84), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 274 nm, λ_{em} = 345 nm). Limit of quantification, 1.7 μg/L. [McElvain *et al.* 1997]. Column: Ultrasphere IP C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L sodium dihydrogenphosphate (pH 2.5) : *n*-butanol : acetonitrile (89 : 6 : 5) flow rate 0.7 mL/min. Fluorescence detection (λ_{ex} = 285 nm, λ_{em} = 345 nm). Retention time: 7.6 min for dolasetron mesylate (retention factor, 3.2); 8.7 min for hydrodolasetron (3.9). Limit of quantification, 10 nmol/L for dolasetron, 5 nmol/L for MDL 74156 [Huebert *et al.* 1996]. Column: CN Spherisorb (150 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : 0.05 mol/L ammonium acetate buffer (pH 7.5; 24 : 76), flow rate 0.8 mL/min. UV detection (λ = 280 nm). Retention time: 7.2 min. Limit of quantification, 10 μg/L [Gillespie *et al.* 1993].

Urine **HPLC** See Plasma. Limit of quantification, 5 nmol/L for dolasetron and MDL 74156 [Huebert *et al.* 1996]. Ultron ES-OVM (15 × 0.46 cm i.d.). Mobile phase: acetonitrile : 0.025 mol/L sodium hydrogen phosphate (pH 6.0; 10 : 90), flow rate 1.0 mL/min. UV detection (λ = 281 nm). Limit of detection not reported [Dow, Berg 1995]. Column: SCD-100 (250 × m i.d., 5 μm). Mobile phase: 0.05 mol/L dipotassium hydrogen phosphate : acetonitrile (70 : 30) with 0.8% triethylamine (pH 7.5), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Limit of detection, 100 dpm [Reith *et al.* 1995]. Column: Nucleosil C_4 (250 × 4.6 mm, 5 μm). Mobile phase: acetonitrile- 0.05 mol/L ammonium acetate (pH 7.4; 10 : 90): acetonitrile-0.05 mol/L ammonium acetate (pH 7.4; 60 : 40, 100 : 0 to 0 : 100 over 30 min), flow rate 1.0 mL/min. UV detection (λ = 280 mn). Retention time (*k*): hydrodolasetron, 18.9 min (8.95). Limit of quantification, 200 μg/L [Sanwald *et al.* 1994].

LC-MS Column: Zorbax C_8 (25 cm × 0.32 mm i.d., 5 μm). Mobile phase: acetonitrile-1% acetic acid (5 : 95) : acetonitrile-1% acetic acid (95 : 5, 100 : 0 to 50 : 50 in 50 min), flow rate 2 μL/min. ESI. Limit of detection not reported [Ackermann *et al.* 1996].

Other **HPLC** Human Liver Microsomes. Column: Nucleosil C_4 (250 × 4.6 mm, 5 μm). Mobile phase: acetonitrile-20 mmol/L ammonium acetate (pH 6.0; 10 : 90) : acetonitrile-20 mmol/L ammonium acetate (pH 6.0; 60 : 40, 100 : 0 to 30 : 70 over 30 min), flow rate 1.0 mL/min. UV detection (λ = 280 mn). Limit of detection, 0.25 nmol/L [Sanwald *et al.* 1996].

Disposition in the Body Dolasetron is rapidly absorbed and subsequently metabolised to hydrodolasetron (with the most clinically relevant activity) by carbonyl reductase. The metabolite appears rapidly in plasma with peak concentrations appearing within the hour; the parent drug is rarely detected. Food does not affect the abosorption of the drug. Hydrodolasetron undergoes additional hydroxylation (CYP2D6) and *N*-oxidation (by flavin mono-oxygenase and CYP3A). It is excreted in urine as hydrodolasetron (53% of administered IV dose) and other metabolites including hydroxylated glucuronides and *N*-oxide. Approximately 66% of an administered dose is detected in urine and the remainder in the faeces.

Therapeutic Concentration

After a single IV dose of 2.4 mg/kg dolasetron mesylate to elderly healthy subjects, a mean maximum plasma concentration of 1465 ± 770 μg/L was observed [Dempsey *et al.* 1996].

Group 1: 6 healthy volunteers with a mean age 41.5 years (range, 34 to 53 years); group 2: 7 patients with mild hepatic impairment (Child–Pugh class A), mean age 40.7 (range, 34 to 46 years); group 3: 4 patients with moderate to severe impairment (class B or C1), mean age 48.5 (range, 42 to 52) years. All participants had creatinine clearance >70 mL/min and were fasted overnight before treatment. Each subject was administered a single 150 mg oral and intravenous dose of dolasetron mesylate over a 10-min infusion period. A 7-day washout period was allowed between the two doses. For the oral doses of the drug, mean peak plasma dolasetron concentrations were 6 (range, 0 to 12) μg/L, 37 (range, 0 to 138) μg/L and 214 (range, 137 to 289) μg/L for groups 1, 2 and 3, respectively. These concentrations were observed between 0.25 and 1.0 h. The mean peak hydrodolasetron concentrations were 347 (range, 170 to 526) μg/L, 387 (range, 258 to 542) μg/L and 410 (range, 374 to 466) μg/L for the 3 groups, observed between 0.5 and 2.0 h. After IV administration of dolasetron, peak plasma concentrations were 3055 (range, 2358 to 5584) μg/L for group 1, 2098 (range, 792 to 4025) μg/L for group 2 and 3299 (range, 2371 to 4871) μg/L for group 3. These concentrations were observed at 0.18 h (range, 0.1 to 0.28 h). Peak hydrodolasetron concentrations were 424 (range, 339 to 492) μg/L, 473 (range, 375 to 610) μg/L and 396 (range, 278 to 602) μg/L for the 3 groups, respectively, observed at 0.25 to 1.0 h [Stubbs *et al.* 1997].

Group 1: 12 male and female patients with mild-to-moderate renal impairment (creatinine clearance, 41 to 80 mL/min), mean age 62.8 years; group 2: 12 patients with moderate-to-severe renal impairment (creatinine clearance, 11 to 40 mL/min), mean age, 50.7 years; group 3: 12 patients, mean age 51.8 years, with end-stage renal impairment undergoing chronic haemodialysis (creatinine clearance ≤10 mL/min); group 4: 24 healthy male volunteers (creatinine clearance, >80 mL/min), mean age 23.8 years. All subjects were fasted overnight and administered 200 mg dolasetron mesylate orally and IV as a 10-min infusion, with a 7-day washout period between doses. Peak plasma hydrodolasetron concentrations were 775.7, 812.8, 866.5 and 646.9 μg/L for groups 1, 2, 3 and 4, respectively, after the IV dose. These concentrations were observed at 0.72, 0.77, 0.69 and 0.67 h, respectively. After the oral dose, concentrations were 742.7, 680.9, 700.8 and 601.2 μg/L at 0.81, 0.79, 0.72 and 0.74 h, respectively, for the 4 groups [Dimmitt *et al.* 1998].

Twenty-four healthy male volunteers were administered 200 mg dolasetron mesylate after an overnight fast or after a high-fat breakfast. The mean maximum plasma concentrations were 581.9 and 411.0 μg/L reached at 1.11 and 1.8 h in the fasted and fed states, respectively [Lippert *et al.* 1998].

Toxicity Symptoms of acute toxicity include tremors, depression and convulsions. Dolasetron has been associated with electrocardiogram interval changes; magnitude and frequency dependent on the concentration of the metabolite, hydrodolasetron, in the blood. This can lead to cardiovascular effects including heart block and cardiac arrhythmias.

Note For a case report of dolasetron leading to prolonged QTc interval and severe hypotension, see Rochford *et al.* [2007].

Half-life Hydrodolasetron, 8.1 h.

Bioavailability 75%.

Volume of Distribution 1.4 L/kg (patients with mild-to-moderate renal impairment); 1.5 L/kg (moderate to severe renal impairment); 3.65 L/kg (end-stage renal impairment); 1.03 L/kg (healthy individuals). Hydrodolasetron, 5.8 L/kg.

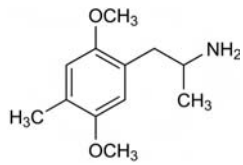
Clearance Systemic clearance, 55.7 mL/min/kg (patients with mild-to-moderate renal impairment); 117.5 mL/min/kg (moderate to severe renal impairment); 66.1 mL/min/kg (end-stage renal impairment); 114.9 mL/min/kg (healthy individuals). Apparent clearance, hydrodolasetron, 9.4 to 13.4 mL/min/kg.
Protein Binding 69 to 77%.
Dose
Oral Adults, 100 mg 1 h before chemotherapy or within 2 h before surgery. Children (2 to 16-years-old), 1.8 mg/kg body weight within 1 h of chemotherapy; a maximum of 100 mg; alternatively, 1.2 mg/kg body weight within 2 h before surgery.
By injection Adults, 100 mg 30 min before chemotherapy; alternatively, 12.5 mg 15 min before anaesthesia has finished before surgery. Children (2 to 16-years-old), 1.8 mg/kg 30 min before chemotherapy, maximum 100 mg; alternatively, 0.35 mg/kg 15 min before the end of anaesthesia, maximum 12.5 mg.

Ackermann BL *et al.* (1996). Application of packed capillary liquid chromatography/mass spectrometry with electrospray ionization to the study of the human biotransformation of the antiemetic drug dolasetron. *J Mass Spectrom* 31: 681–689.
Dempsey E *et al.* (1996). Pharmacokinetics of single intravenous and oral doses of dolasetron mesylate in healthy elderly volunteers. *J Clin Pharmacol* 36: 903–910.
Dimmitt DC *et al.* (1998). Pharmacokinetics of oral and intravenous dolasetron mesylate in patients with renal impairment. *J Clin Pharmacol* 38: 798–806.
Dow J, Berg C (1995). Stereoselectivity of the carbonyl reduction of dolasetron in rats, dogs, and humans. *Chirality* 7: 342–348.
Gillespie TA *et al.* (1993). Determination of dolasetron and its reduced metabolite in human plasma by GC-MS and LC. *J Pharm Biomed Anal* 11: 955–962.
Huebert ND *et al.* (1996). Simultaneous measurement of dolasetron and its major metabolite, MDL 74,156, in human plasma and urine. *J Chromatogr B Biomed Appl* 685: 291–297.
Johnson CE *et al.* (2003). Stability of dolasetron in two oral liquid vehicles. *Am J Health Syst Pharm* 60: 2242–2244.
Lippert C *et al.* (1998). The effect of food on the bioavailability of dolasetron mesylate tablets. *Biopharm Drug Dispos* 19: 17–19.
McElvain JS *et al.* (1997). Validation of a reversed-phase HPLC method for directly quantifying the enantiomers of MDL 74,156, the primary metabolite of dolasetron mesylate, in human plasma. *J Pharm Biomed Anal* 15: 513–521.
Reith MK *et al.* (1995). Human metabolism of dolasetron mesylate, a 5-HT3 receptor antagonist. *Drug Metab Dispos* 23: 806–812.
Rochford M *et al.* (2007). Dolasetron overdose resulting in prolonged QTc interval and severe hypotension: a case report and literature review. *Emerg Med J* 24: 515–517.
Sanwald P *et al.* (1994). Simultaneous measurement of the major metabolites of dolasetron mesilate in human urine using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 661: 101–107.
Sanwald P *et al.* (1996). Use of electrospray ionization liquid chromatography-mass spectrometry to study the role of CYP2D6 in the *in vitro* metabolism of 5-hydroxytryptamine receptor antagonists. *J Chromatogr B Biomed Appl* 678: 53–61.
Stubbs K *et al.* (1997). Pharmacokinetics of dolasetron after oral and intravenous administration of dolasetron mesylate in healthy volunteers and patients with hepatic dysfunction. *J Clin Pharmacol* 37: 926–936.

DOM

Hallucinogen
$C_{12}H_{19}NO_2$ = 209.3
CAS—15588-95-1

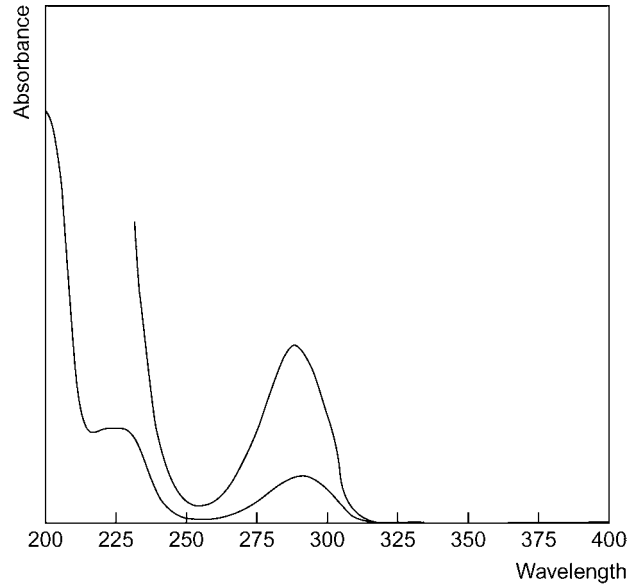
IUPAC Name 1-(2,5-Dimethoxy-4-methylphenyl)propan-2-amine
Synonyms 2,5-Dimethoxy-α,4-dimethylbenzene-ethanamine; 2,5-dimethoxy-4-methylamfetamine; 2,5-dimethoxy-4-metamfetamine; STP (STP has been used as a synonym for DOM and DOM is reported to be the active principle of the hallucinogenic preparation known as STP).



Chemical Properties Mp 60.5° to 61.0°. Practically insoluble in water; soluble in chloroform. Log *P* (octanol/water), 2.2.

DOM Hydrochloride
$C_{12}H_{19}NO_2$,HCl = 245.7
CAS—15589-00-1
Chemical Properties White crystals from propan-2-ol/ether. Mp 189° to 189.5°.

Colour Test Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.15; system TC—R_f 0.17; system TE—R_f 0.41; system TAE—R_f 0.09; system TL—R_f 0.16; system TAF—R_f 0.76 (Dragendorff Spray—positive; FPN reagent—positive; acidified iodoplatinate solution—positive; Marquis test—yellow; Ninhydrin (spray)—positive; acidified potassium permanganate—positive).
Gas Chromatography System GA—DOM RI 1616, M (-PFP) RI 1730, M (-AC) RI 2020, M (-AC_2) RI 2090, M (art, formyl) RI 1565, M (*O*-desmethyl-)(-PFP_2) RI 1780, M (OH--AC_2) RI 2260, M (desamino-oxo-OH-)(-PFP_2) RI 2045, M (desamino-oxo-OH-)(-AC_2) RI 2560; system GB—RI 1652.
High Performance Liquid Chromatography System HC—*k* 1.13; system HX—RI 340.
Ultraviolet Spectrum Aqueous acid—289 nm (A^1_1=221b)



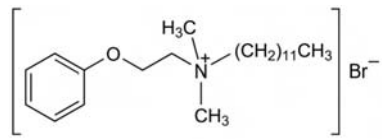
Infrared Spectrum Principal peaks at wavenumbers 1208, 1041, 1541, 1515, 1634, 1094 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 44, 166, 151, 57, 43, 91, 135, 209.
Quantification **GC** Column: (1) 10% Apiezon L plus 2% potassium hydroxide, or (2) Carbowax 20M plus 2% potassium hydroxide, both on Chromosorb W/AW on 80/100 mesh. Carrier gas: N_2, 30 mL/min. Temperature: 165°. Retention time: 12.4 and 22.5 min for 4-methyl-2,5 dimethoxyamfetamine on columns 1 and 2, respectively. Limit of detection not reported [Canfield *et al.* 1977].
Other **GC-MS** Rat brain. Column: HP-5 MS methyl silicone (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 20 mL/min. Temperature programme: 80° for 2 min to 170° at 20°/min to 225° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 100 ng/g [Eckler *et al.* 2001].
Disposition in the Body Approximately 20% of an ingested dose is excreted in the urine unchanged in 24 h; peak urinary excretion occurs 3 to 6 h after ingestion.

Canfield DV *et al.* (1977). Gas chromatographic analysis of amphetamine derivatives and morpholine-related drugs. *J Forensic Sci* 22: 429–433.
Eckler JR *et al.* (2001). A sensitive method for determining levels of [−]-2,5,-dimethoxy-4-methylamphetamine in the brain tissue. *J Pharmacol Toxicol Meth* 46: 37–43.

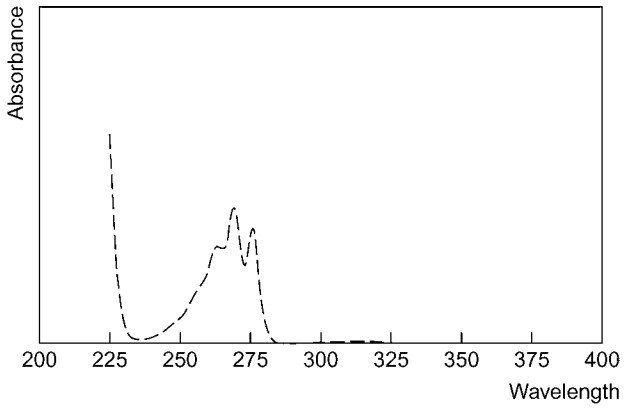
Domiphen Bromide

Cationic Disinfectant
$C_{22}H_{40}BrNO$ = 414.5
CAS—13900-14-6 (domiphen); 538-71-6 (bromide)

IUPAC Name Dodecyl-dimethyl-(2-phenoxyethyl)azanium bromide
Synonyms *N*,*N*-Dimethyl-*N*-(2-phenoxyethyl)-1-dodecanaminium bromide; phenododecinium bromide.
Proprietary Names *Antiseptique Pastilles*; *Bradoral*; *Bronchodex Pastilles*; *Neobradoral*; *Oraseptic*. It is an ingredient of *Bradosol*.



Chemical Properties Colourless or faintly yellow crystalline flakes. Mp 112° to 113°. Soluble 1 in less than 2 of water and of ethanol, and 1 in 30 of acetone; soluble in ethyl acetate and chloroform; very slightly soluble in benzene. Log *P* (octanol/water), 4.2.
Colour Test Dissolve 10 mg in 10 mL of water, add 0.1 mL of a 0.5% aqueous eosin solution and 100 mL of water—pink.
Thin-layer Chromatography System TA—R_f 0.05 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2310.
High Performance Liquid Chromatography System HY—RI 506 (domiphen).
Ultraviolet Spectrum Methanol—268 (A^1_1=30a), 277 nm.



Infrared Spectrum Principal peaks at wavenumbers 1228, 752, 1500, 1600, 689, 1080 cm^{-1} (KBr disk).

Dose Lozenges containing 500 µg of domiphen bromide are available.

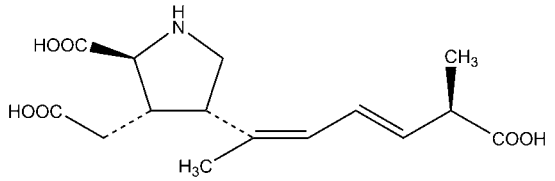
Domoic Acid

Glutamatergic Agonist, Kainoid, Neurotoxin

$C_{15}H_{21}NO_6 = 311.3$

CAS—14277-97-5

IUPAC Name (2*S*,3*S*,4*S*)-2-Carboxy-4-[(1*Z*,3*E*,5*R*)-5-carboxy-1-methyl-1,3-hexanedienyl]-3-pyrrolidine-acetic acid



Chemical Properties Excitory amino acid belonging to the kainoid class of compounds principally isolated from the red alga *Chondria armata* Okamura, Rhodomelaceae, known in Japanese as 'domoi'. It can also be produced by a number of marine organisms (plankton) such as diatoms of the genus *Pseudonitzschia*. Ten isomers of domoic acid (isodomoic acids A to H and domoic acid 5′-diastereomer) have been identified in marine samples [Wright *et al.* 1990; Zaman *et al.* 1997]. Isodomoic acids are minor consitutents relative to domoic acid and the isomers are not always present in contaminated shellfish. Domoic acid can be converted to isodomoic acids when exposed to UV light or heat. Domoic acid and its isomers do not degrade under ambient temperatures or when exposed to light in sterile saline solution [Johannessen 2000]. However, domoic acid has been shown to decompose under acidic conditions (50% loss in 1 week at pH 3) [Quilliam *et al.* 1989].

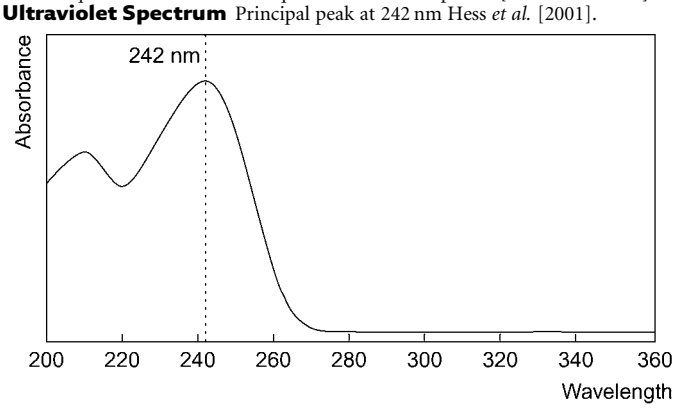
Domoic Acid Dihydrate

Chemical Properties Mp 217°. Soluble in water, acetic acid; insoluble in methanol, ethanol, chloroform, acetone, benzene [O'Neil *et al.* 2006]. pK_a 2.10, 3.72, 4.93, 9.82 [Pineiro *et al.* 1999].

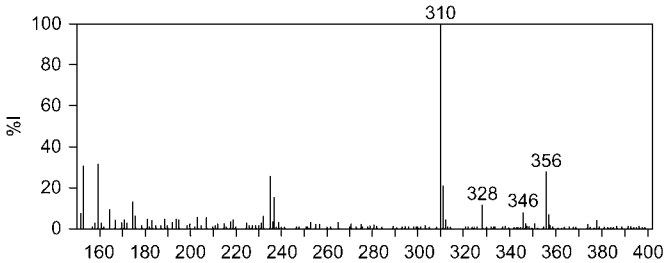
High Performance Liquid Chromatography Column: C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 2.87 mmol/L aqueous phosphoric acid : acetonitrile (86 : 14), flow rate 1.0 mL/min. UV detection (λ = 242 nm). Limit of quantification not reported [Johannessen 2000].

Liquid Chromatography-Mass Spectrometry Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : aqueous 0.01 mol/L trifluoroacetic acid containing 0.01% heptafluorobutyric acid (5 : 95 to 40 : 60 over 12 min to 70 : 30 over 5 min for 10 min), flow rate 0.7 mL/min. API, positive ion mode, MRM acquisition mode. Retention times: domoic acid 12 min, saxitoxin 7.5 min, okadaic acid 24 min. Limit of quantification not reported [Dahlmann *et al.* 2003]. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% aqueous formic acid : 0.1% formic acid in acetonitrile (95 : 5 for 3 min to 5 : 95 over 10 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification not reported [Burns *et al.* 2007].

Ultraviolet Spectrum Principal peak at 242 nm Hess *et al.* [2001].



Mass Spectrum Principal ions at *m/z* 73, 75, 692, 115, 95, 147(*N*-trifluoroacetyl-*O*-*tert*-butyldimethylsilyl derivative).



Quantification

Serum **HPLC** Column: Partisil ODS-3 (250 × 4.1 mm i.d., 10 µm). Mobile phase: acetonitrile : water (pH 3.0; 10.5 : 89.5), flow rate 1.5 mL/min for 19 min and 3.0 mL/min for 11 min. Retention time: 11.4 min. Limit of quantification, 0.2 mg/L [Blanchard, Tasker 1990].

Other **TLC** Shellfish Tissue. Plates: silica gel $60F_{254}$ (10 × 20 cm, 250 µm). Solvent system: butanol : acetic acid : water (3 : 1 : 1). UV detection (λ = 254 nm) and spray with ninhydrin reagent. R_f value: 0.45 (distinct yellow colour). Limit of detection, 0.3 µg/spot [Quilliam *et al.* 1998].

GC-MS Mussel Samples. Column: DB-1 fused silica capillary (20 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 100° to 300° at 10°/min. EI ionisation at 70 eV, SIR. Retention time: 20.1 min (trifluoroacetyl-*O*-*tert*-butyldimethylsilyl derivative). Limit of quantification, 5 mg/kg [Pleasance *et al.* 1990].

HPLC Seawater. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (38 : 62) with 0.05% trifluoroacetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 470 nm, λ_{em} = 530 nm). Limit of detection, 120 ng/L (7-fluoro-4-nitro-2,1,3-benzoxadiazole derivative) [Chan *et al.* 2007]. Cuttlefish samples. Column: Nucleosil C_{18} (125 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% aqueous formic acid (10 : 90), flow rate 0.45 mL/min. UV detection (λ = 242 and 262 nm). Retention times: domoic acid 7.4 min, isodomoic acid 6.2 min, 5′-diastereomer 8.3 min. Limit of quantification, 0.2 µg/g [Costa *et al.* 2005]. Shellfish Tissue. Column: C_{18} (100 × 4.6 mm i.d.). Mobile phase: 5 mmol/L phosphate buffer (pH 2.7) : acetonitrile (9 : 1), flow rate 0.5 mL/min. Chemilumiscence detection and UV detection (λ = 242 nm). Limit of detection, 0.4 µg/L [Kodamatani *et al.* 2004]. Blue Mussel Extract. Column: C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05% aqueous trifluoroacetic acid (5 : 95 to 35 : 65 over 15 min for 5 min), flow rate 1 mL/min. UV detection (λ = 242 nm). Retention time: 12.6 min. Limit of detection, 0.5 mg/L [Yu *et al.* 2004]. Whale Faeces. Column: Vydac C_{18} (25 × 2.1 mm i.d.). Mobile phase: water : acetonitrile : trifluoroacetic acid (90 : 10 : 0.1). UV detection (λ = 242 nm). Limit of detection, 0.2 µg/g [Lefebvre *et al.* 2002]. Scallop Tissue Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% trifluoroacetic acid in 10% aqueous acetonitrile, flow rate 1.5 mL/min. UV detection (λ = 242 nm). Limit of detection, 0.2 µg/g [Hess *et al.* 2001]. Phytoplankton (*Pseudonitzschia australis*). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (40 : 60) with 0.1% trifluoroacetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 470 nm, λ_{em} = 530 nm). Retention time: 10 min (7-fluoro-4-nitro-2,1,3-benzoxadiazole derivative). Limit of quantification, 1 µg/g [James *et al.* 2000]. Sea Lion Faeces and Anchovies. Column: Vydac C_{18} (25 × 2.1 mm i.d.). Mobile phase: water : methanol : trifluoroacetic acid (90 : 10 : 0.1), flow rate 0.3 mL/min. DAD (λ = 242 and 280 nm). Retention time: 9.5 min. Limit of detection, 0.1 mg/L [Lefebvre *et al.* 1999]. Red alga (*Chondria armata*). Column: C_{18} (250 × 4.0 mm i.d.). Mobile phase: 0.1% trifluoroacetic acid in 10% aqueous acetonitrile, flow rate 1.2 mL/min. UV detection (λ = 242 nm). Retention times: domoic acid 17.9 min, isodomoic acid 15.0 min, isodomoic acid A 16.0 min, isodomoic acid B 21.5 min, isodomoic acid G 23.0 min, isodomoic acid F 25.9 min, isodomoic acid H 31.7 min. Limit of quantification not reported [Zaman *et al.* 1997]. Crab and Razor Clam. Column: C_{18} Vydac (25 × 2.1 mm i.d.). Mobile phase: water : acetonitrile : trifluoroacetic acid (90 : 10 : 0.1), flow rate 0.3 mL/min. UV detection (λ = 242 nm). Retention time: 8.0 min. Limit of quantification, not reported [Hatfield *et al.* 1994]. Rat Urine, Serum and Faeces. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.2% formic acid and 12% acetonitrile in water (pH 3.0), flow rate 0.5 mL/min. Retention time: ~7.0 min. Limit of detection, 0.1 µg/g [Lawrence *et al.* 1994]. Rat Serum and Urine. Column: µBondapak C_{18} (300 mm, 10 µm). Mobile phase: acetonitrile : 0.2% aqueous trifluoroacetic acid (10 : 90), flow rate 1.5 mL/min. UV detection (λ = 242 nm). Retention time: 13.5 min. Limit of quantification not reported [Suzuki, Hierlihy 1993]. Shellfish Tissue. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: aceonitrile : water (pH 2.5, 15 : 85 to 80 : 20 over 20 min), flow rate 1.0 mL/min. UV detection (λ = 242 nm). Retention time: 6.1 min. Limit of detection, 5 to 10 µg/g [Lawrence, Menard 1991].

LC-MS Seawater and Phytoplankton. Column: C_{18} (150 × 2.0 mm i.d., 5 µm). Mobile phase: water : acetonitrile with 0.1% formic acid (95 : 5 for 2 min to 60 : 40 over 13 min to 5 : 95 over 2 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 12 min. Limit of quantification, 0.05 µg/L [Wang *et al.* 2007]. Shellfish Samples. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (1 : 9) with 0.035% trifluroacetic acid (pH 2.5), flow rate 0.2 mL/min. ESI, APCI, APPI or APCI/APPI, positive ion mode, SRM acquisition mode. Retention time: 8.2 min. Limit of detection, ESI 0.2 µg/g, APCI 1.8 µg/g, APPI 1.2 µg/g, APCI/APPI 1.2 µg/g [Pardo *et al.* 2007]. Shellfish Tissue. Column: TSK-gel amide-80 (250 × 2.0 mm i.d., 5 µm). Mobile phase: water : 95% acetonitrile in water-2 mmol/L ammonium formate-3.6 mmol/L formic acid

(25 : 75), flow rate 0.2 mL/min. ESI, positive and negative ion modes, SIM and MRM acquisition modes. Retention time: 5.0 min. Limit of detection, 63 μg/kg (positive mode), 190 μg/kg (negative mode) [Ciminiello *et al.* 2005]. Greenshell Mussel, Pacific Oyster, New Zealand Cockle and Scallop Roe. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile-water (1 : 9) : acetonitrile–water (9 : 1) : 33 mmol/L aqueous ammonium hydroxide and 500 mmol/L aqueous formic acid. (85 : 5 : 10 for 2 min to 5 : 85 : 10 over 11 min for 12 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.05 mg/kg [McNabb *et al.* 2005]. Bovine Serum and Urine. Column: SphereClone ODS2 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 1% acetic acid in water : 1% acetic acid in methanol : acetonitrile (23 : 57 : 20), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Retention time: 9.9 min. Limit of detection, 5 μg/kg [Tor *et al.* 2003]. Shellfish. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: methanol : water : 40 mmol/L ammonium hydroxide (90 : 33 : 10) with 0.5 mol/L formic acid, flow rate 0.2 mL/min. API, positive ion mode, MRM acquisition mode. Retention time: 5.8 min. Limit of quantification, 0.5 mg/kg; limit of detection, 0.15 mg/kg [Holland *et al.* 2003]. Shellfish Tissue. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water with 0.05% trifluoroacetic acid (5 : 95 to 40 : 60 over 25 min), flow rate 0.2 mL/min. DAD. Retention time: 18.1 min. Limit of detection, 25 μg/L (MS^1), 14 μg/L (MS^2), 8 μg/L (MS^3) [Furey *et al.* 2001]. Scallop Tissue Samples. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: water-50 mmol/L formic acid-2 mmol/L ammonium formate : acetonitrile-water (95 : 5)-50 mmol/L formic acid-2 mmol/L ammonium formate (85 : 15), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of detection, 0.4 μg/g [Hess *et al.* 2001].

CE Shellfish Samples. Column: bare fused silica (75 cm × 50 μm i.d.). UV detection (λ = 242 nm). Buffer: 20 mmol/L sodium tetraborate (pH 9.0) : acetonitrile (9 : 1). Migration times: domoic acid 9.0 min, isodomoic acid E 8.8 min. Limit of quantification, 150 μg/kg [Zhao *et al.* 1997].

Note For a review of HPLC-UV methods comparing different stationary phases for the analysis of domoic acid in mussels, see Lawrence *et al.* [1991] and Lawrence *et al.* [1989].

Disposition in the Body

Toxicity Domoic acid binding studies to the glutamate receptor have shown that domoic acid has a strong affinity for subclasses of the kainite receptor. These are widely distributed in the mammalian brain but are particularly concentrated in the CA3 region of the hippocampus in humans. It appears that the domoic acid-glutamate receptor interaction mediates the toxic response. Following the 1987 amnesic shellfish poisoning (ASP) outbreak, the Canadian authorities imposed an action limit for domoic acid in mussels of 20 μg/g mussel flesh, which when exceeded would result in closure of shellfish harvesting areas [Jeffery *et al.* 2004]. Ingestion of as little as 60 mg domoic acid is sufficient to induce gastro-intestinal illness. Similarly, ingestion of 270 mg domoic acid results in neurological effects [Perl *et al.* 1990].

Note For proceedings of a conference on domoic acid-induced ASP, see Liston [1990]. For a review of domoic acid, see Jeffery *et al.* [2004]. For a study of the renal clearance of domoic acid in the rat, see Suzuki, Hierlihy [1993].

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Donepezil

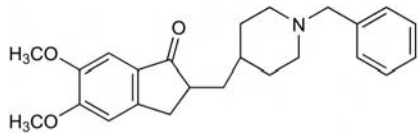
Nootropic

$C_{24}H_{29}NO_3$ = 379.5

CAS—120014-06-4

IUPAC Name 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one

Synonyms 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl] methyl]-1*H*-inden-1-one; ER-4111.



Donepezil Hydrochloride

$C_{24}H_{29}NO_3$,HCl = 416.0

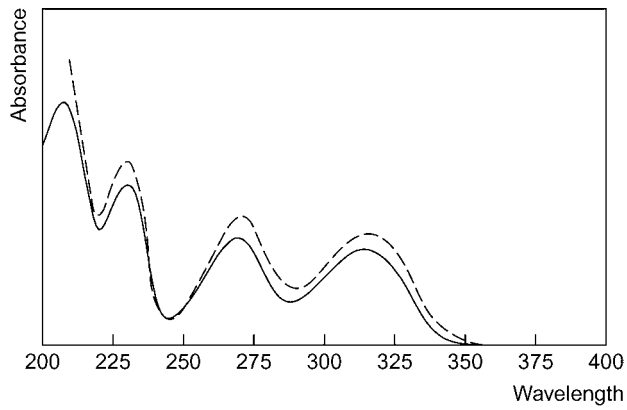
CAS—120011-70-3

Synonyms BNAG; E2020.

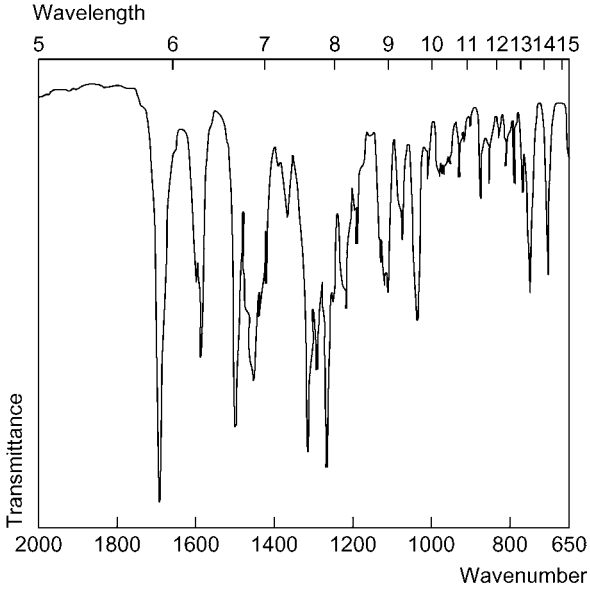
Proprietary Names *Aricept*; *Memac*.

Chemical Properties A white to off-white solid with Mp 224°. It is soluble in water (55 g/L at 25°); freely soluble in chloroform and glacial acetic acid. pK_a 8.90.

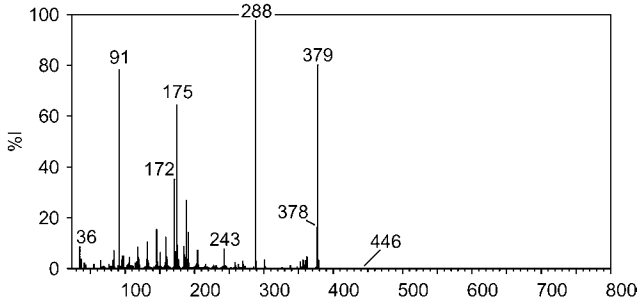
Ultraviolet Spectrum Aqueous acid—230, 271, 316 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1605, 1589, 1500, 749, 702 cm^{-1}.



Mass Spectrum Principal peaks at *m/z* 288, 379, 91, 175, 172, 178, 378, 243.



Quantification

Plasma **HPLC** Column: short C_{30}. Mobile phase: 25 mmol/L citric acid-50 mmol/L disodium hydrogen phosphate (pH 6.0) : acetonitrile (73 : 27) containing 3.5 mmol/L sodium 1-octanesulfonate. Fluorescence detection (λ_{ex}= 325 nm; λ_{em}= 390 nm). Limit of detection, 0.2 µg/L [Nakashima *et al.* 2006]. Column: C_{18} STR ODS-II (150 × 4.6 mm, 5 µm). Mobile phase: 0.02 mol/L phosphate buffer (pH 4.6) : 6 mol/L perchloric acid : acetonitrile (59.5: 0.5: 40), flow rate 1.0 mL/min. UV detection (λ= 315 nm). Limit of detection, 3 µg/L [Yasui-Furukori *et al.* 2002]. Column: Nucleosil 5 C_{18}(250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 5 mmol/L SDS (pH 3.5, 60 : 40). UV detection. Retention time: donepezil hydrochloride, 7 min. Limit of detection, 0.5 µg/L [Ohnishi *et al.* 1993].

LC-MS Column: Betabasic-C(8) (100 × 4.6 mm, 5 µm). Mobile phase: methanol : water : formic acid (90 : 9.97 : 0.03). ESI, positive ion mode. Limit of quantification, 0.15 µg/L [Shah *et al.* 2009]. Column: reversed phase C_{18}. YMC Pack ODS-A (50 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (82 : 18), flow rate 700 µL/min. TIS. Limit of quantification, 2.5 µg/L [Apostolou *et al.* 2008]. Column: C_{18}.ODS-A (50 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 5.0, 82 : 18), flow rate 0.7 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 µg/L [Apostolou *et al.* 2007]. Column: Waters Nova-Pak C_{18} (150 × 3.9 mm, 4 µm). Mobile phase: 0.2% formic acid in 20 mmol/L ammonium acetate : methanol : acetonitrile (pH 3.43, 63 : 20 : 17). TIS, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 µg/L [Patel *et al.* 2008]. Column: Aquasil C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 1% formic acid (70 : 10 : 20), flow rate 0.3 mL/min. ESI, SRM acquisition mode. Limit of quantification, 0.1 µg/L [Xie *et al.* 2006]. See also Lu *et al.* [2003], Lu *et al.* [2004], Matsui *et al.* [1999] and Matsui *et al.* [1995].

HILIC-MS Column: Atlantis HILIC silica. Mobile phase: acetonitrile : 50 mmol/L ammonium formate (pH 4.0, 85 : 15). SRM acquisition mode. Limit of quantification, 0.1 µg/L [Park *et al.* 2008].

Disposition in the Body Donepezil is well absorbed from the gastrointestinal tract after oral administration and is partially metabolised in the liver by cytochrome P450. Four major metabolites are produced by *O*-dealkylation followed by hydroxylation and partial subsequent glucuronidation or hydrolysis; the major metabolites are known to be active. There are also several minor metabolites. Over 10 days, 57% of a single dose is recovered as metabolites in urine and 15% in faeces; 17% of the drug remains unchanged and is excreted in urine; 28% is unrecovered, possibly owing to accumulation.

Therapeutic Concentration

Forty-eight healthy males (non-smokers) were administered a single dose of donepezil, 2, 4 or 6 mg. Maximum plasma concentrations reached 3.2 ± 0.6, 6.9 ± 0.7, 11.6 ± 2.8 µg/L, respectively. Plasma concentration rose in proportion to the dose (the same for multiple dose studies). These concentrations were reached within 1.7–6.6 h [Rogers, Friedhoff 1998].

For plasma levels with multiple dose donepezil, see Rogers *et al.* [1998].

Toxicity

A 79-year-old woman who took an overdose of 45 mg donepezil had a plasma level of 54.6 µg/L on admission to hospital. Plasma donepezil levels gradually decreased to normal limits in ~90 h [Yano *et al.* 2003].

Another report of an overdose occurred in a 79-year-old patient who was inadvertently given 50 mg donepezil instead of 5 mg. The main adverse effect was bradycardia, which responded to atropine [Shepherd *et al.* 1999].

For a further report of donepezil overdose, see Greene *et al.* [1999].

Half-life Elimination, ~50–60 h (single dose), 75 h (steady state) and 104 h (elderly).

Volume of Distribution 13.6–14.8 L/kg.

Clearance Body clearance, 0.12 L/h/kg.

Protein Binding ~95%.

Dose A 5 mg dose is administered once daily. After 4 to 6 weeks, the dose can be increased to 10 mg daily, if necessary.

Note For the metabolism and elimination of donepezil, see Tiseo *et al.* [1998]; for a further pharmacokinetic study, see Mihara *et al.* [1993].

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Dopamine

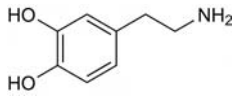
Sympathomimetic

$C_8H_{11}NO_2$ = 153.2

CAS—51-61-6

IUPAC Name 4-(2-Aminoethyl)-1,2-benzenediol

Synonym 3-Hydroxytyramine



Chemical Properties Crystals. pK_a 8.8, 10.6 (20°). Log *P* (octanol/water), −1.0.

Dopamine Hydrochloride

$C_8H_{11}NO_2$,HCl = 189.6
CAS—62-31-7

Proprietary Names *Abbodop*; *Cordodopa*; *Dopamex*; *Dopaminex*; *Docard*; *Dopmin*; *Drynalken*; *Drynalquin*; *Dynatra*; *Dynos*; *Giludop*; *Inopin*; *Inovan*; *Intropin*; *Inotropisa*; *Medopa*; *Revimine*; *Revivan*; *Zetarina*.

Chemical Properties A white crystalline powder. Mp about 241°, with decomposition. Freely soluble in water; soluble in methanol and ethanol; practically insoluble in ether, petroleum ether, chloroform, benzene and toluene.

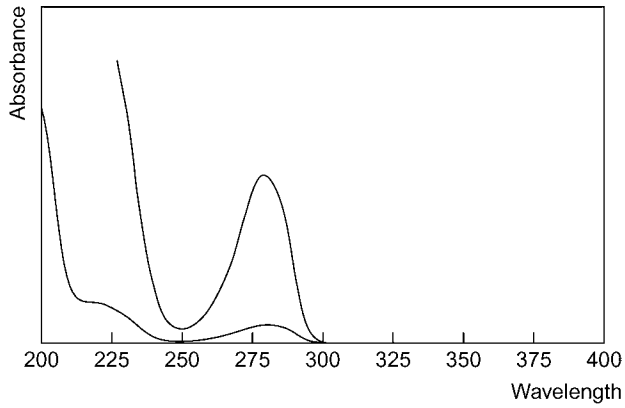
Colour Tests Ammoniacal silver nitrate—orange-brown/black; *p*-dimethylaminobenzaldehyde—orange/violet; ferric chloride—green; Folin–Ciocalteau reagent—blue; Marquis test—brown-violet; methanolic potassium hydroxide—blue→orange→brown; Nessler's reagent—black; potassium dichromate—green-→brown (30 s).

Thin-layer Chromatography System TA—R_f 0.18, streaking; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.43; system TL—R_f 0.00; system TAE—R_f 0.14; system TAF—R_f 0.59; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.07 (acidified potassium permanganate solution, positive).

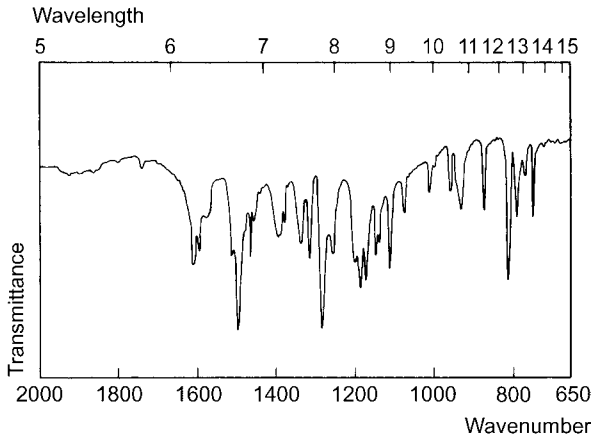
Gas Chromatography System GA—dopamine RI 2175, M (*O*-methyl-)-AC_2 RI 2070.

High Performance Liquid Chromatography System HA—*k* 2.7 (tailing peak); system HZ—retention time 1.7 min.

Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=178a).



Infrared Spectrum Principal peaks at wavenumbers 1503, 1287, 1190, 1174, 813, 1115 cm^{-1} (KBr disk).



Quantification See under Levodopa.

Disposition in the Body Dopamine is a naturally occurring catecholamine and is considered to be the metabolic precursor of noradrenaline. It is inactivated after oral administration. It is excreted mainly as 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid); noradrenaline (norepinephrine) and 3-methoxytyramine are also metabolites.

Dopamine is a metabolite of levodopa.

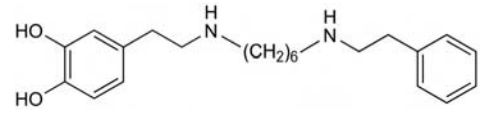
Dose Initially 2 to 5 μg/kg/min of dopamine hydrochloride, by IV infusion.

Dopexamine

β-*Adrenoceptor Agonist*
$C_{22}H_{32}N_2O_2$ = 356.5
CAS—86197-47-9

IUPAC Name 4-[2-[6-(Phenethylamino)hexylamino]ethyl]benzene-1,2-diol

Synonyms FPL-60278; 4-[2-[[6-(phenylethylamino)hexyl]amino]ethyl] pyrocatechol.



Chemical Properties Log *P* (octanol/water), 4.27.

Dopexamine Dihydrochloride

$C_{22}H_{32}N_2O_2$,2HCl = 429.4
CAS—86484-91-5

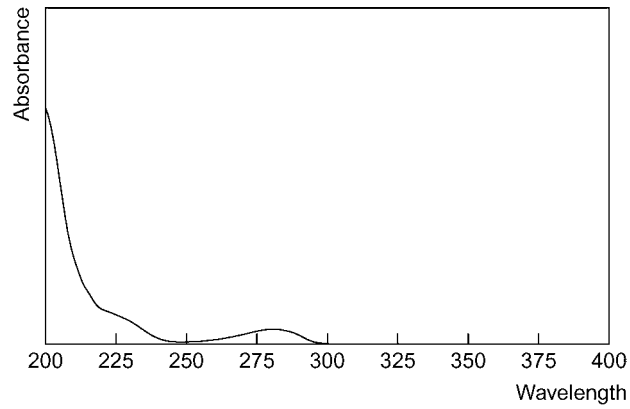
Synonym FPL-60278AR

Proprietary Name *Dopacard*

Thin-Layer Chromatography System TB—R_f 0.00; system TE—R_f 0.16; system TAE—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 289.

Ultraviolet Spectrum



Quantification

Blood **HPLC** Columns: ODS silica Hypersil (250 × 4.9 mm i.d., 5 μm). Temperature: 37° to 43°. Mobile phase: methanol : 4.04 g/L sodium heptane-1--sulfonate, 1 mmol/L disodium EDTA, 10 ml/L orthophosphoric acid and 22.5 ml/L di-isopropylamine (49:51). flow rate 1.2 mL/min. ECD. Retention time: dopexamine hydrochloride, 7.0 to 7.3 min. Limit of quantification, 5 μg/L [Baker *et al.* 1995].

Disposition in the Body Dopexamine is extensively metabolised by *O*-methylation of the catechol-hydroxy moiety and subsequent sulfate conjugation. Elimination from blood occurs quickly. Two pharmacologically inactive metabolites are produced and both are excreted in urine and faeces, along with the unchanged drug. The major metabolite is the 2-methoxy, 1-sulfate metabolite and accounts for >90% of an administered dose. Over 12 days, 50% drug is excreted in urine and 20% in faeces.

Therapeutic Concentration

Ten healthy volunteers were administered dopexamine hydrochloride (total dose 180 μg/kg) over 60 min by IV infusion. There was a proportional increase in plasma concentration with a peak at 124 mg/L after 1 h. Plasma concentrations rapidly decreased when the infusion was stopped [Fitton, Benfield 1990].

Toxicity Overdose effects are short lived owing to short half-life with pharmacological actions including tachycardia, palpitations, tremors, nausea, vomiting and anginal pain.

Half-life 7 min; 11 min in patients with cardiac failure.

Clearance Plasma clearance, 36 mL/min/kg.

Dose An initial dose of 0.5 μg/kg body weight/min is administered, which can be increased up to 1 μg/kg body weight/min, if necessary. Further increases occur in 0.5 to 1.0 μg/kg/min increments at intervals of not less than 15 min with a maximum dose of 6 μg/kg body weight/min.

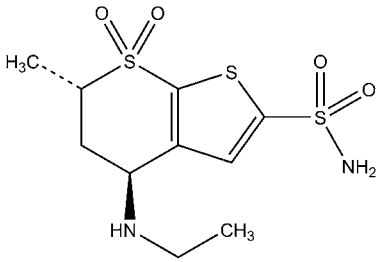
Baker PR *et al.* (1995). Determination of dopexamine hydrochloride in human blood by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* 667(2): 283–290.

Fitton A, Benfield P (1990). Dopexamine hydrochloride. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in acute cardiac insufficiency. *Drugs* 39(2): 308–330.

Dorzolamide

Antiglaucoma, Carbonic Anhydrase Inhibitor, Sulfonamide
$C_{10}H_{16}N_2O_4S_3 = 324.4$
CAS—120279-96-1

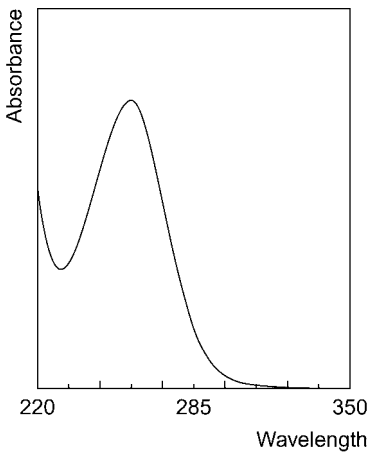
IUPAC Name (4*R*,6*R*)-4-Ethylamino-6-methyl-7,7-dioxo-5,6-dihydro-4*H*-thieno[5,4-b]thiopyran-2-sulfonamide
Synonyms (4*S*,6*S*)-4-(Ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-b] thiopyran-2-sulfonamide 7,7-dioxide; L-671152.



Dorzolamide Hydrochloride

$C_{10}H_{16}N_2O_4S_3,HCl = 360.9$
CAS—130693-82-2
Synonyms MK-0507; MK-507.
Proprietary Name *Trusopt*. It is also an ingredient of *Cosopt*.
Chemical Properties A white to off-white crystalline powder. Mp 283° to 285°. Soluble in water. pK_a 7.8 [Remko, der Lieth 2004], 8.4 [Supuran *et al.* 2003]. Log *P* (octanol/water), 0.71 [Remko, der Lieth 2004].

Thin-layer Chromatography Ophthalmic solution. Plates: Silica gel GF_{254} (20 × 20 cm, 0.25 mm). Solvent system: methanol : 25% ammonia (100 : 1.5). UV detection (λ = 254 nm). R_f 0.67 [Bebawy 2002].
High Performance Liquid Chromatography Ophthalmic solution. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : phosphate buffer (pH 2.5; 8 : 10 : 85), flow rate 1.2 mL/min. UV detection (λ = 250 nm). Retention time: 4.1 min. Limit of quantification, 4 mg/L [Erk 2003].
Ultraviolet Spectrum Ophthalmic solution—Principal peak at 250.3 nm. Limit of detection, 34 µg/L [Erk 2002].



Quantification

Blood **HPLC** Column: C_8 (250 × 4.6 mm i.d., 5 µm) and C_{18} (50 × 4.6 mm i.d., 3 µm) in series. Mobile phase: acetonitrile : water-0.085% phosphoric acid-1.6 mg/mL sodium octanesulfonate (25 : 75), flow rate 1 mL/min. UV detection (λ = 252 nm). Limit of quantification, 5 µg/L [Matuszewski *et al.* 1994a].
Plasma **HPLC** Column: C_8 (250 × 4.6 mm i.d., 5 µm) and C_{18} (50 × 4.6 mm i.d., 3 µm) in series. Mobile phase: acetonitrile : water-0.085% phosphoric acid-1.6 mg/mL sodium octanesulfonate (25 : 75), flow rate 1 mL/min. UV detection (λ = 252 nm). Limit of quantification, 5 µg/L [Matuszewski *et al.* 1994a].
LC-MS Column: Hypersil base-deactivated cyano (100 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate-0.1% trifluoracetic acid (35 : 65), flow rate 0.6 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 µg/L [Constanzer *et al.* 1997].
Urine **HPLC** Column: C_8 (250 × 4.6 mm i.d., 5 µm) and C_{18} (50 × 4.6 mm i.d., 3 µm) in series. Mobile phase: acetonitrile : water-0.085% phosphoric acid-1.6 mg/mL sodium octanesulfonate (24 : 76), flow rate 1 mL/min. UV detection (λ = 252 nm). Limit of quantification, 5 µg/L [Matuszewski *et al.* 1994a].
Aqueous Humour **HPLC** Column: C_8 (125 × 4.0 mm i.d.). Mobile phase: acetonitrile : 0.085% phosphoric acid-2.1 mg/mL octanesulfonic acid (25 : 75). UV detection (λ = 252 nm). Retention time: 13.5 min. Limit of quantification, 100 µg/L, limit of detection, 50 µg/L [Schmitz *et al.* 1999].
Other **HPLC** Animal Tissues. Column: C_{18}. Mobile phase: acetonitrile : 1% TEA solution (pH 3.5; 7 : 93), flow rate 1 mL/min. UV detection (λ = 254 nm). Retention time: 16.5 min. Limit of quantification not reported [Inoue *et al.* 2004].

Note For methods describing the chiral separation of the stereoisomers of dorzolamide, see Matuszewski *et al.* [1994b]; Matuszewski and Constanzer [1992]. For a Raman spectroscopy method for the detection of dorzolamide in animal tissues, see Bauer *et al.* [1999].
Disposition in the Body The rate and extent of drug absorption after topical application is influenced by losses caused by tear turnover and blinking, and by protein binding in tears. Only a small part of the instilled drug becomes available for absorption. Studies have reported absorption rates of 50 to 73% of the applied drug. The drug then reaches the systemic circulation via drainage through the nasolacrimal duct and is subsequently absorbed from the nasopharyngeal mucosa or goes directly through the conjuctival blood vessels. Dorzolamide is metabolised to *N*-deethyldorzolamide in the systemic circulation via CYP2B1/2, CYP2E1 and CYP3A2. Steady-state concentrations are achieved after 6, 12 and 18 months of treatment. It binds strongly to human erythrocytes, more precisely to the enzymes carbonic anhydrase I and II, which are mainly located in red blood cells (>90%). Dorzolamide and its metabolite are excreted predominantly via the renal route.

Therapeutic Concentration

Thirty-two patients scheduled for routine cataract surgery were administered a drop of a 2% solution (equivalent to 0.76 mg) of dorzolamide. Maximum aqueous humour concentrations were reached 1 to 2 h after administration. The mean concentration was 1 mg/L after ~2 h, 0.7–1.0 mg/L after 4–6 h, and ~0.2 mg/L after 12 h [Schmitz *et al.* 1999].

Ten patients with open-angle glaucoma or ocular hypertension were administered a drop (30 µL) of a 2% solution of dorzolamide every 8 h (~4 mg/day) for 6 months. Red cell concentrations for dorzolamide and its metabolite were 23.0 and 4.9 µmol/L, respectively. Mean daily urine concentration for dorzolamide plus metabolite was 1.7 mg/day, although large variations were observed (range 0.1 to 2.9 mg/day) [Maren *et al.* 1997].

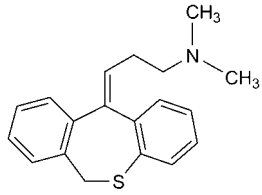
Toxicity For a case report of acidosis in a neonate, see Morris *et al.* [2003]; for a review on dorzolamide-induced thrombocytopenia, see Martin, Danese [2001].
Half-life Approximately 130 days.
Clearance Approximately 5.4 L/h.
Dose As eye drops containing 2% of the base. When used as monotherapy, the usual dose is one drop three times daily; a twice daily regimen is recommended when used in conjunction with a β-blocker.

Bauer NJ *et al.* (1999). Non-invasive assessment of ocular pharmacokinetics using confocal Raman spectroscopy. *J Ocul Pharmacol Ther* 15: 123–134.
Bebawy LI (2002). Application of TLC-densitometry, first-derivative UV-spectrophotometry and ratio derivative spectrophotometry for the determination of dorzolamide hydrochloride and timolol maleate. *J Pharm Biomed Anal* 27: 737–746.
Constanzer ML *et al.* (1997). Low level determination of dorzolamide and its de-ethylated metabolite in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *J Pharm Biomed Anal* 15: 1001–1008.
Erk N (2002). Simultaneous determination of dorzolamide HCl and timolol maleate in eye drops by two different spectroscopic methods. *J Pharm Biomed Anal* 28: 391–397.
Erk N (2003). Voltammetric and HPLC determination of dorzolamide hydrochloride in eye drops. *Pharmazie* 58: 870–873.
Inoue J *et al.* (2004). Effects of dorzolamide hydrochloride on ocular tissues. *J Ocul Pharmacol Ther* 20: 1–13.
Maren TH *et al.* (1997). Ocular absorption, blood levels, and excretion of dorzolamide, a topically active carbonic anhydrase inhibitor. *J Ocul Pharmacol Ther* 13: 23–30.
Martin XD, Danese M (2001). Dorzolamide-induced immune thrombocytopenia: a case report and literature review. *J Glaucoma* 10: 133–135.
Matuszewski BK, Constanzer ML (1992). Indirect chiral separation and analyses in human biological fluids of the stereoisomers of a thienothiopyran-2-sulfonamide (TRUSOPT), a novel carbonic anhydrase inhibitor with two chiral centers in the molecule. *Chirality* 4: 515–519.
Matuszewski BK *et al.* (1994). Determination of MK-507, a novel topically effective carbonic anhydrase inhibitor, and its de-ethylated metabolite in human whole blood, plasma, and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 653: 77–85.
Matuszewski BK *et al.* (1994). Anall chiral separation of the stereoisomers of a novel carbonic anhydrase inhibitor and its deethylated metabolite, and the assignment of absolute configuration of the human metabolite and chiral degradation products. *Pharm Res* 11: 449–454.
Morris S *et al.* (2003). Topical dorzolamide and metabolic acidosis in a neonate. *Br J Ophthalmol* 87: 1052–1053.
Remko M, der Lieth CW (2004). Theoretical study of gas-phase acidity, pK_a, lipophilicity, and solubility of some biologically active sulfonamides. *Bioorg Med Chem* 12: 5395–5403.
Schmitz K *et al.* (1999). Population pharmacokinetics of 2% topical dorzolamide in the aqueous humor of humans. *Invest Ophthalmol Vis Sci* 40: 1621–1624.
Supuran CT *et al.* (2003). Carbonic anhydrase inhibitors. *Med Res Rev* 23: 146–189.

Dosulepin

Antidepressant
$C_{19}H_{21}NS = 295.4$
CAS—113-53-1

IUPAC Name (3*Z*)-3-(6*H*-Benzo[*c*][1]benzothiepin-11-ylidene)-*N*,*N*-dimethylpropan-1-amine
Synonyms 3-Dibenzo[*b*,*e*]thiepin-11(6*H*)-ylidene-*N*,*N*-dimethyl-1-propanamine; dothiepin.



Chemical Properties Mp 55° to 57°. Log *P* (octanol/pH 7.4), 2.8 [Ilett *et al.* 1992], (octanol/water) 4.49 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Unstable under conditions of putrefaction [Pounder *et al.* 1994].

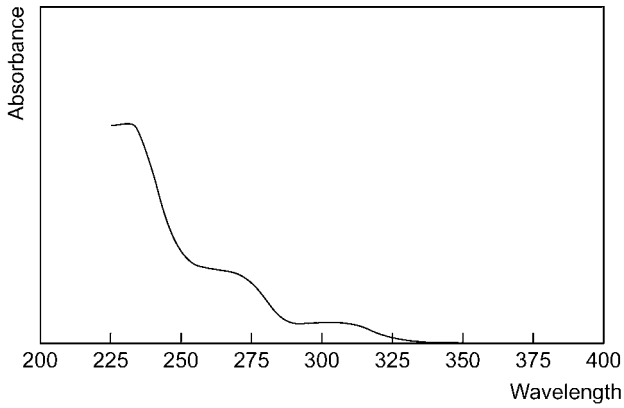
Dosulepin Hydrochloride

$C_{19}H_{21}NS,HCl = 331.9$
CAS—897-15-4
Synonym Dothiepin hydrochloride
Proprietary Names *Dopin*; *Dopress*; *Dothapax*; *Dothep*; *Jardin*; *Prepadine*; *Prothiaden*; *Protiaden(e)*; *Thaden*; *Xerenal.*
Chemical Properties White to faintly yellow crystalline powder. Mp 218° to 221°. Soluble 1 in 2 of water, 1 in 8 of ethanol and 1 in 2 of chloroform; practically insoluble in ether.

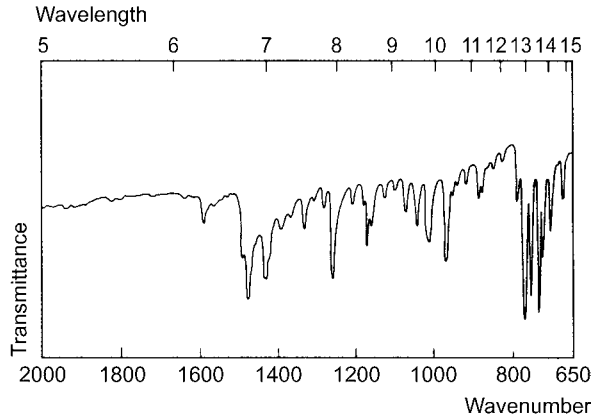
Colour Tests Liebermann's reagent—red-brown; Mandelin's test—green; Marquis test—brown; sulfuric acid—violet.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.49; system TC—R_f 0.42; system TE—R_f 0.65; system TF—dosulepin-*S*-oxide R_f 0.00; system TL—R_f 0.16; system TAE—R_f 0.27; system TAF—R_f 0.41 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—dosulepin RI 2380, M (OH-) RI 2500, M (nor-) RI 2421, M (*N*-oxide) RI 2100, M (OH-*N*-oxide) RI 2130, M (sulfoxide) RI 2392, M (norsulfoxide) RI 2421; system GB—dosulepin RI 2486, M (nor-) RI 2507, M (sulfoxide) RI 2533, M (norsulfoxide) RI 2839; system GF—dosulepin RI 2770, dosulepin sulfoxide RI 2820; system GM—dosulepin RRT 1.259, M (nor-) RRT 1.450 (both relative to iprindole).
High Performance Liquid Chromatography System HA—dosulepin *k* 3.2, dosulepin sulfoxide *k* 4.6 (tailing peak), monodesmethyldosulepin *k* 2.2; system HF—*k* 3.60; system HX—RI 428; system HY—RI 367; system HZ—RT 5.7 min.

Column: Hypersil (250 × 4.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.15 mol/L sodium hexane sulfonic acid (60 : 40), flow rate 2.0 mL/min. UV detection (λ = 260 nm). Limit of detection 1.9 mg/L [Pawlak, Clark 1989].

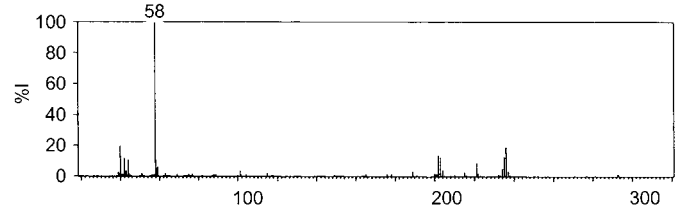
Column: Spherisorb (25 cm × 4.6 mm i.d., 5 µm). Mobile phase ethyl acetate : methanol : 3% ammonia (85 : 15 : 1), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: 8.54 and 9.05 min for the *cis* and *trans*-isomers, respectively. Limit of detection, 1.0 mg/L [Li Wan Po, Irwin 1979].
Ultraviolet Spectrum Aqueous acid—230 (A^1_1 = 770a), 303 nm.



Infrared Spectrum Principal peaks at wavenumbers 763, 727, 747, 1252, 963, 717 cm^{-1} (dosulepin hydrochloride), (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 236, 40, 202, 235, 203, 42, 44; desmethyldosulepin 44, 204, 203, 202, 41, 221, 57, 55; dosulepin sulfoxide 58, 44, 31, 59, 57, 42, 45, 40.



Quantification

Blood **GC-MS** Column: DB-5 cross-linked 5% phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 2 min to 180 at 30°/min to 280° at 5°/min for 19 min. Full scan mode. Retention time: 20.1 min. Limit of quantification, 0.05 mg/L [Paterson *et al.* 2004]. Column: HP-5 MS fused silica 95% dimethyl 5% diphenyl polysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 180° to 200° at 5°/min for 2 min to 220° at 5°/min for 5 min to 240° at 5°/min and to 320° at 50°/min. Retention time: 16.5 min. MSD, SIM acquisition mode. Limit of detection, 0.5 mg/L [Keller *et al.* 2000]. Column: 3% OV-101 on GasChrom W HP 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 210° to 230° at 4°/min. EI ionisation at 70 eV. Limit of detection, 1 µg/L [Maguire *et al.* 1981b].

HPLC Column: Novapak C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : 10 mmol/L potassium hydrogen phosphate buffer (pH 2.6; 65 : 5 : 30), flow rate 0.8 mL/min. DAD. Limit of detection not reported [Cirimele *et al.* 1995]. Column: Chromspher C_8 (10 × 0.3 cm i.d., 5 µm). Mobile phase: methanol : water (30 : 70 to 75 : 25 in 5 min), flow rate 0.7,L/min. DAD (λ = 230 nm). Retention time: ≈10.5 min, 10.7 min dosulepin-sulfoxide. Limit of detection not reported [Lambert *et al.* 1994]. Column: Apex II ODS silica (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L sodium dihydrogen phosphate buffer (pH 3.0) : acetonitrile (60 : 40), flow rate 1.5 mL/min. UV detection (λ = 255 nm). Limit of detection not reported [Pounder *et al.* 1994]. Column: Spheri-5 RP-18 (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L sodium dihydrogen phosphate : diethylamine (pH 8.0; 40 : 57.5 : 2.5), flow rate 2.0 mL/min. UV detection (λ = 220 or 254 nm). Retention time: 14.6 min. Limit of detection, 0.05 mg/L [McIntyre *et al.* 1993].

Plasma **GC** Column: 3% OV-17 on Chromosorb W 100/120 mesh (9′ × 0.25" o.d.). Carrier gas: 30 mL/min. Temperature: 275°. AFID. Limit of detection, 4.0 ng [Gifford *et al.* 1975].

GC-MS Column: 3% OV-17 on GasChrom Q 100/120 mesh (1 m × 2 mm i.d.). Carrier gas: CH_4, 8-10 mL/min. Temperature: 220°. EI/CI. Limit of detection, 0.5 µg/L [Crampton *et al.* 1980].

HPLC Column: Ultrasphere C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L 1-octanesulfonic acid-0.5 mmol/L *N,N,N,N*-tetramethylethylene diame (pH 2.5; 35: 65, flow rate 2 mL/min. UV detection (λ = 230 nm). Retention time: 10.8 min. Limit of detection not reported [Hackett *et al.* 1998]. Column: µBondapak phenyl (30 cm × 4 mm i.d.). Mobile phase: acetonitrile : hydrogen phosphate : sodium chloride (35 : 0.01 : 0.01), flow rate 1.8 mL/min. UV detection (λ = 230 nm). Limit of quantification, 2 µg/L [Ilett *et al.* 1992]. Column: cyano (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L dipotassium hydrogen orthophosphate buffer (pH 7.0; methanol (50 : 30 : 20), flow rate 1.7 mL/min. UV detection (λ = 240 nm). Retention time: 4.6 min. Limit of detection, 50 µg/L [Taylor *et al.* 1992]. Column: Partisil 10 ODS (25 cm × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.5% aqueous potassium dihydrogen phosphate (pH 3.0; 35 : 65), flow rate 2.0 mL/min. UV detection (λ = 231 nm). Retention time: 9 min. Limit of detection, 10 µg/L [Brodie *et al.* 1977].

LC-MS Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.1 mg/L, limit of detection, 0.08 mg/L [Shinozuka *et al.* 2006]. Column: Symmetry C_{18} (150 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (28 : 72 for 4 min to 70 : 30 in 1 min for 3 min to 28 : 72 in 0.7 min), flow rate 0.6 mL/min. APCI, positive ion mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Kollroser, Schober 2002].

Serum **GC-MS** Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 280° at 10°/min for 20 min. EI ionisation at 70 eV. Retention time: 22.5 min. Limit of detection, 0.025 mg/L, limit of detection, <0.025 mg/L [Maresova *et al.* 2008].

HPLC Column: Super-Octyl TSK gel (100 × 4.6 mm i.d., 2 µm) or Hypersil MOS-C_8 (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 20 mmol/L potassium dihydrogen phosphate (pH 7.0; 60 : 40), flow rate 0.6 mL/min. UV detection (λ = 254 nm). Retention time: 10.6 and 19.7 min for the TSK and Hypersil columns, respectively. Limit of quantification, 0.05 µg/L on the TSK column [Tanaka *et al.* 1997]. See Plasma [Brodie *et al.* 1977].

Urine **GC-MS** Column: HP-1 capillary (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [Bickeboeller-Friedrich, Maurer 2001]. See Blood [Keller *et al.* 2000].

HPLC See Blood [Cirimele *et al.* 1995]. See Serum [Tanaka *et al.* 1997]. See Blood [Lambert *et al.* 1994]. See Blood [Pounder *et al.* 1994]. Column: µBondapak C_{18} (15 cm × 0.4 cm i.d., 10 µm). Mobile phase: 0.015 mol/L phosphate buffer : acetonitrile : 1 mol/L dimethylamine (50 : 50 : 1), flow rate 2.0 mL/min. UV detection (λ = 210 nm). Retention time: 47.1 min. Limit of detection, 0.1 mg/L [Kawahara *et al.* 1987].

Bile **HPLC** See Blood [Keller *et al.* 2000]. See Blood [Cirimele *et al.* 1995]. See Blood [Pounder *et al.* 1994].

Gastric Contents **HPLC** See Blood [Keller *et al.* 2000]. See Blood [Cirimele *et al.* 1995]. See Blood [Lambert *et al.* 1994].

Milk **HPLC** See Plasma [Ilett *et al.* 1992].

Vitreous Humour **HPLC** See Blood [Keller *et al.* 2000]. See Blood [Pounder *et al.* 1994].

Brain **HPLC** See Serum [Tanaka *et al.* 1997]. See Blood [Cirimele *et al.* 1995].

Hair **GC** Column: BP-5 (12 m × 0.53 mm i.d., 1.0 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 100° for 2 min to 310° at 10°/min for 10 min. NPD. Limit of detection, 0.1 to 0.25 µg/g [Couper *et al.* 1995].

GC-MS Column: HPS-5MS 5% phenyl 95% methyl siloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 295° at 30°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 11.08 min. Limit of detection not reported [Cirimele *et al.* 1995].

Heart **HPLC** See Blood [Cirimele *et al.* 1995]. See Blood [Pounder *et al.* 1994].

Kidney **HPLC** See Blood [Cirimele *et al.* 1995]. See Blood [Lambert *et al.* 1994].

Liver **HPLC** See Serum [Tanaka *et al.* 1997]. See Blood [Cirimele *et al.* 1995]. See Blood [Lambert *et al.* 1994]. See Blood [Pounder *et al.* 1994].

Lung **HPLC** See Blood [Pounder *et al.* 1994].

Muscle **HPLC** See Blood [Pounder *et al.* 1994].

Other **HPLC** Column. Rat Brain. Ultrasphere ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L acetate buffer : acetonitrile : perchloric acid : trichloroacetic acid (50 : 50 : 2 : 1.5, pH 2.0), flow rate 1.5 mL/min. ECD. Limit of detection not reported [Shibanoki *et al.* 1987].

Disposition in the Body Well absorbed after oral administration. The main metabolic reactions are demethylation to desmethyldosulepin (northiaden) and oxidation to dosulepin sulfoxide. About 50 to 60% of a dose is excreted in the urine in 24 h, mainly as metabolites, and 15 to 40% of a dose is eliminated in the faeces in the same period. Enterohepatic circulation has been reported. Small amounts may be excreted in breast milk [Ilett *et al.* 1992].

Therapeutic Concentration Plasma concentrations vary considerably between individuals.

After a single oral dose of 75 mg dosulepin to 9 depressed patients a mean maximum plasma concentration of 49 ± 27 µg/L was reached at 3 ± 1.2 h [Maguire *et al.* 1983].

Eleven patients were administered a single oral dose of 75 mg dosulepin on the first day of the placebo period. On the following 4 days the patients received placebo but after that a single dose of 150 mg dosulepin was administered daily for the next 28 days. There was considerable inter-individual variation. Plasma concentrations at week 4 ranged from 16 to 179 µg/L dosulepin, 126 to 849 µg/L for dosulepin *S*-oxide and 1 to 53 µg/L desmethyldosulepin [Maguire *et al.* 1982].

After a single oral dose of 75 mg to 7 subjects, peak plasma concentrations of 47 (33 to 71) µg/L of dosulepin were reached in 2 to 5 h. Dosulepin *S*-oxide was the major metabolite reaching a peak level of 81 (34 to 150) ug/L at 5 (4 to 6) h. In comparison, desmethyldosulepin reached a peak concentration of only 10 (3 to 21) µg/L at 5 (4 to 9) h. The mean elimination half-life of dosulepin *S*-oxide was 19 (13 to 35) h while that for desmethyldosulepin was 33 (22 to 60) h. [Maguire *et al.* 1981a]

After a single oral dose of 75 mg dosulepin HCl to 6 healthy male volunteers a mean peak plasma concentration of 61.6 µg/L reached at 3 h [Crampton *et al.* 1980].

After oral administration of 25 mg given 3 times a day to 5 subjects for 12 days, minimum steady-state serum concentrations of 17 to 65 µg/L (mean 37) of dosulepin, and <0.01 to 47 µg/L (mean 15) of desmethyldosulepin, were reported [Nakra *et al.* 1977].

Toxicity Fatalities have been associated with blood concentrations of 1 to 5 to 19 mg/L.

A 36-year-old female was admitted to hospital 3 h after the ingestion of 2 to 3 g of dosulepin with alcohol. After 24 h, following treatment by haemoperfusion, she appeared to be recovering but toxic symptoms reappeared after 48 h followed by death 56 h after ingestion. The plasma concentrations shown below were reported (mg/L).

Hours after ingestion	*Dosulepin*	*Desmethyldosulepin*
4.5	2.51	0
6.0	1.88	0.13
17.5	1.07	0.32
57.5[a]	4.54	1.28

[a]postmortem
[b][Bloodworth *et al.* 1984]

In 8 cases of overdose with dosulepin, all had high initial levels of dosulepin (0.819 to 3.851 mg/L), dosulepin-*S*-oxide (0.655 to 2.162 mg/L), nordosulepin (0.088 to 0.422 mg/L), and nordosulepin-*S*-oxide (0.176 to 0.530 mg/L); all patients survived following treatment with repeated-dose activated charcoal [Ilett *et al.* 1991].

A 22-year-old female was found dead beside a canal. At postmortem her dosulepin concentrations in her blood, urine, stomach, liver, and kidney were 2.1, 9.7, 130, 2.19, 1.4 mg/L or µg/g, respectively [Lambert *et al.* 1994].

In 2 cases of suicide involving dosulepin ingestion (765 and 1875 mg, respectively), initial blood concentrations were 0.26 to 1.85 and 4.08 to 23.98 mg/L. Pulmonary artery concentrations rose over 18 h (0.32 rising to 0.9, and 6.54 rising to 19.53 mg/L) with peripheral blood concentrations remaining stable. Postmortem tissue concentrations in the respective cases were: liver 4.3 and 52 µg/g, heart 2.92 and 16.8 µg/g, lung 18.6 and 73.9, skeletal muscle 1.1 and 8.98 µg/g. Mean dosulepin levels in thoracic blood rose from 0.43 mg/L at 0 h postmortem to 1.73 at 8 h and then fell to 0.61 mg/L at 12 h, probably reflecting redistribution from the lungs [Pounder *et al.* 1994].

The following postmortem tissue concentrations were reported in a 22-year-old male suspected of ingesting up to 4.5 g of dosulepin (mg/L or µg/g): blood 5.75, urine 7.42, bile 110, gastric contents 373, liver 45.2, kidney 10.4, heart 16/0, brain 2.77, hair of head 1.89, axillary hair 1.17 [Cirimele *et al.* 1995].

In a 32-year-old male who committed suicide by ingesting an unknown quantity of dosulepin, the following postmortem tissue concentrations of dosulepin and desmethyldosulepin, respectively, were reported: peripheral blood 7.28 and 2.03 mg/L, heart blood 6.98 and 1.63 mg/L, urine 12.58 and 3.71 mg/L, vitreous humour 0.28 and 0.02 mg/L, stomach contents 65.41 and 1.01 mg/L, bile 12.24 and 1.06 mg/L; diazepam and desmethyldiazepam were also detected in tissues [Keller *et al.* 2000].

Note For reviews of CNS and cardiovascular effects of TCA poisoning, see Bateman [2005] and Thanacoody and Thomas [2005], respectively. For a case of serotonin syndrome after an overdose of dosulepin in a 13-year-old female, see Radomski [1998]. For a case of myocardial infarction after the ingestion of 1875 mg dosulepin, see Arya *et al.* [2004].

Bioavailability ≈30% after first pass metabolism [Pounder *et al.* 1994].

Half-life Plasma half-life, dosulepin 14 to 40 h (mean 22), desmethyldosulepin 22 to 60 h (mean 33), dosulepin sulfoxide 13 to 35 h (mean 19) [Maguire *et al.* 1981a].

Clearance 1.36 (0.88 to 1.8) L/kg/h [Maguire *et al.* 1981a].

Volume of Distribution 83.1 and 85.4 L/kg for females and males, respectively [Pounder *et al.* 1994], 45 (20 to 92) L/kg [Maguire *et al.* 1981a], 18.8 to 195.5 L/kg [Keller *et al.* 2000].

Distribution in Blood Plasma : whole blood ratio, ≈1.4 [Maguire *et al.* 1981a].

Note For a review of the pharmacokinetics of tricyclic antidepressants, see Molnar and Gupta [1980].

Dose 75 to 150 mg of dosulepin hydrochloride daily; up to 225 mg daily has been given.

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Couper FJ *et al.* (1995). Detection of antidepressant and antipsychotic drugs in postmortem human scalp hair. *J Forensic Sci* 40: 87–90.

Crampton EL *et al.* (1980). Chemical ionisation mass fragmentographic measurement of dothiepin plasma concentrations following a single oral dose in man. *J Chromatogr* 183: 141–148.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

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Lambert W *et al.* (1994). A fatal case of trazodone and dothiepin poisoning: toxicological findings. *J Anal Toxicol* 18: 176–179.
Li Wan Po A, Irwin WJ (1979). A high performance liquid chromatographic assay of *cis*- and *trans*-isomers of tricyclic neuroleptic drugs. *J Pharm Pharmacol* 31: 512–516.
Maguire KP *et al.* (1981a). Metabolism and pharmacokinetics of dothiepin. *Br J Clin Pharmacol* 12: 405–409.
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Doxacurium Chloride

Muscle Relaxant

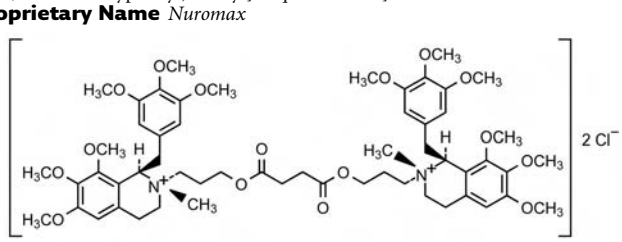
$C_{56}H_{78}Cl_2N_2O_{16}$ = 1106.1

CAS—106819-53-8 (meso isomer); 83348-52-1 (total racemate)

IUPAC Name 1-*O*-[3-[(1*R*,2*S*)-6,7,8-Trimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl]propyl] 4-*O*-[3-[(1*S*,2*R*)-6,7,8-trimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl]propyl] butanedioate dichloride

Synonyms BW-A938U; [1α,2β(1′*S**,2′*R**)]-2,2′-[(1,4-dioxo-1,4-butanediyl)bis(oxy-3,1-propanediyl)]bis[1,2,3,4-tetrahydro-6,7,8-trimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]isoquinolinium] dichloride.

Proprietary Name *Nuromax*



Chemical Properties An amorphous solid which is soluble in water.

Quantification

Plasma **HPLC** UV detection. Limit of quantification, 4 μg/L [Gariepy *et al.* 1993]. Column: S5C1 Spherisorb (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L monobasic potassium phosphate (pH 3) : acetonitrile containing 0.5% methanol (30:70), flow rate 1.0 mL/min. UV detection (λ=210 nm). Retention time: 6.7 min. Limit of quantification, 10 μg/L [DeAngelis *et al.* 1990].

Urine **HPLC** See Plasma [Gariepy *et al.* 1993]. See Plasma [DeAngelis *et al.* 1990].

Disposition in the Body Doxacurium is excreted in urine and bile, mostly as the unchanged parent drug. It is distributed primarily in extracellular fluids as it is a large polar compound. It is not metabolised by plasma cholinesterase or hepatic enzymes. The drug is rapidly cleared from the body and is eliminated mainly in urine (70%) and also via bile. In healthy individuals, 24 to 38% of a dose is recovered unchanged in urine within 6 to 12 h after administration.

Therapeutic Concentration

Nine healthy, young subjects (aged 19 to 39 years) and 9 healthy, elderly subjects (70 to 83 years) were administered 30 μg/kg doxacurium chloride (equivalent to 25 μg/kg doxacurium base) after induction of anaesthesia. In the younger subjects the mean plasma concentration reached 337 μg/L at 2 min and then declined to 5 μg/L over 360 min after injection. In the elderly subjects, however, their peak concentration of 336 μg/L declined to 8 μg/L after 480 min [Gariepy *et al.* 1993].

Half-life Approximately 1.7 to 2 h (healthy individuals); 3.7 h (patients with renal insufficiency).

Volume of Distribution Steady state, 0.23 (0.12 to 0.29) L/kg.

Clearance Plasma, 2.5 mL/kg/min.

Protein Binding Approximately 28 to 34%.

Dose The initial dose is 50 μg/kg body weight intravenously, followed by a maintenance dose of 5 to 10 μg/kg. A dose of 80 μg/kg may also be used.

DeAngelis R *et al.* (1990). High-performance liquid chromatographic analysis of doxacurium, a new long-acting neuromuscular blocker. *J Chromatogr* 525(2): 389–400.
Gariepy LP *et al.* (1993). Influence of aging on the pharmacokinetics and pharmacodynamics of doxacurium. *Clin Pharmacol Ther* 53(3): 340–347.

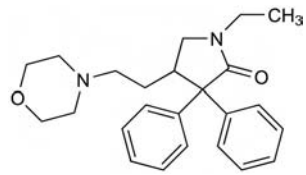
Doxapram

Respiratory Stimulant

$C_{24}H_{30}N_2O_2$ = 378.5

CAS—309-29-5

IUPAC Name 1-Ethyl-4-[2-(4-morpholinyl)ethyl]-3,3-diphenyl-2-pyrrolidinone



Chemical Properties Log *P* (octanol/water), 3.4.

Doxapram Hydrochloride

$C_{24}H_{30}N_2O_2$,HCl,H_2O = 433.0

CAS—113-07-5 (anhydrous); 7081-53-0 (monohydrate)

Proprietary Names *Dopram*; *Doxapril*; *Stimulexin*.

Chemical Properties A white crystalline powder. Mp about 220°. Soluble 1 in 50 of water; sparingly soluble in ethanol; slightly soluble in chloroform; practically insoluble in ether.

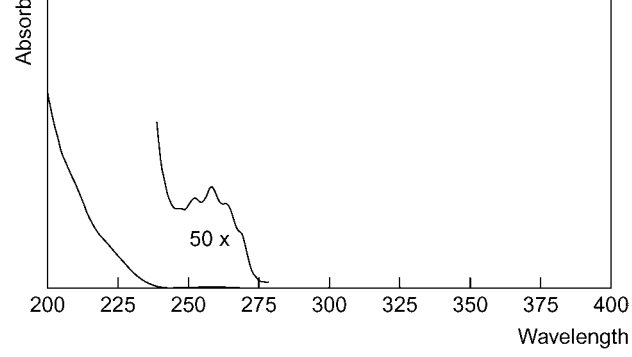
Colour Tests Liebermann's reagent—orange; Mandelin's test—blue.

Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.20; system TC—R_f 0.70; system TE—R_f 0.72; system TL—R_f 0.54; system TAE—R_f 0.69; system TAJ—R_f 0.60; system TAK—R_f 0.03; system TAL—R_f 0.73 (acidified iodoplatinate solution, positive).

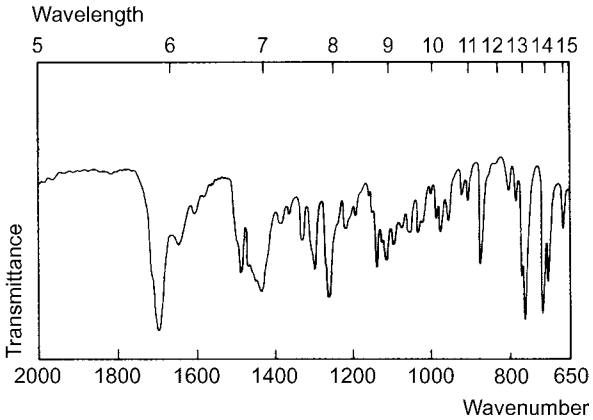
Gas Chromatography System GA—RI 2906; system GB—doxapram RI 3046, M (keto-) RI 3018; system GC—RI 2230.

High Performance Liquid Chromatography System HA—*k* 0.4; system HY—RI 312; system HZ—retention time 3.2 min; system HAX—retention time 8.7 min; system HAY—retention time 4.6 min.

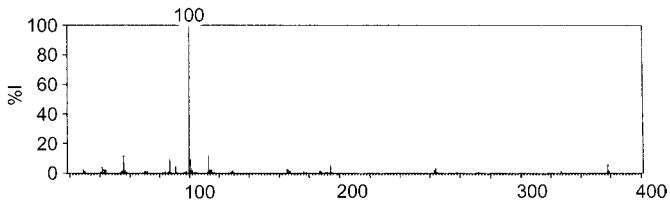
Ultraviolet Spectrum Aqueous acid—253, 259 (A^1_1=12c), 265 nm.



Infrared Spectrum Principal peaks at wavenumbers 1683, 753, 710, 1253, 696, 764 cm^{-1} (doxapram hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 100, 113, 56, 101, 87, 378, 194, 91.



Quantification

Blood **GC** MS detection. For method, see Nichol *et al.* [1980].

Plasma **GC** See Blood [Nichol *et al.* 1980]. AFID. Limit of detection, 10 µg/L [Robson, Prescott 1977].

Urine **GC** FID. For method, see Nichol *et al.* [1980].

Disposition in the Body Readily absorbed after oral administration. The major metabolite is the 2-ketomorpholino derivative.

Therapeutic Concentration

Following an IV injection of 1.5 mg/kg to 6 subjects, plasma concentrations of 1.6 to 4.3 mg/L (mean 2.6) were reported after 2 min declining to 0.6 to 1.5 mg/L (mean 0.9) at 2 h. Following IV infusion of 6.5 mg/kg to 6 subjects, peak plasma concentrations of 3.3 to 5.2 mg/L (mean 4.1) were reported at the end of the infusion; peak concentrations of the 2-ketomorpholino metabolite of 1.1 to 2.0 mg/L (mean 1.6) were attained 0.2 to 2 h after the end of the infusion [Clements *et al.* 1979].

In 18 premature infants, a steady-state plasma concentration of approx. 1.5 mg/L was achieved by IV administration of a loading dose of 5.5 mg/kg and a maintenance infusion of 1 mg/h [Jamali *et al.* 1988].

Bioavailability About 60%.

Half-life Plasma half-life, about 7 h.

Volume of Distribution About 3 L/kg.

Clearance Plasma clearance, about 5 mL/min/kg.

Dose 1 to 1.5 mg/kg of doxapram hydrochloride by IV injection.

Clements JA *et al.* (1979). The disposition of intravenous doxapram in man. *Eur J Clin Pharmacol* 16: 411–416.

Jamali F *et al.* (1988). Doxapram dosage regimen in apnea of prematurity based on pharmacokinetic data. *Dev Pharmacol Ther* 11: 253–257.

Nichol H *et al.* (1980). Quantitation of doxapram in blood, plasma and urine. *J Chromatogr* 182: 191–200.

Robson RH, Prescott LF (1977). Rapid gas–liquid chromatographic estimation of doxapram in plasma. *J Chromatogr* 143(5): 527–529.

Doxazosin

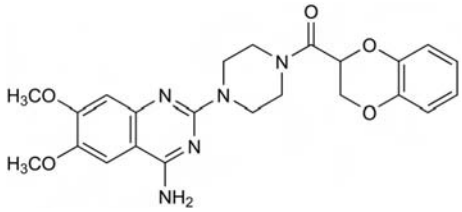
Antihypertensive

$C_{23}H_{25}N_5O_5$ = 451.5

CAS—74197-85-8

IUPAC Name [4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(2,3-dihydro-1,4-benzodioxin-3-yl)methanone

Synonyms 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl)carbonyl]piperazine; UK-33274.



Chemical Properties Log *P* (octanol/water), 2.09. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Doxazosin Hydrochloride

$C_{23}H_{25}N_5O_5$,HCl = 488.0

CAS—70918-01-3

Chemical Properties A crystalline powder. Mp 289° to 290°.

Doxazosin Mesilate

$C_{23}H_{25}N_5O_5$,CH_3SO_3H = 547.6

CAS—77883-43-3

Synonyms Doxazosin mesylate; UK-33274-27.

Proprietary Names *Aldafil*; *Benur*; *Cardenalin*; *Cardular*; *Cardura*; *Carduran*; *Dedralen*; *Diblocin*; *Normothen*; *Progandol*; *Prostadilate*; *Supressin*.

Chemical Properties It is freely soluble in dimethylsulfoxide; soluble in dimethylformamide; slightly soluble in methanol, ethanol, and water (0.8% solubility at 25°); very slightly soluble in acetone and methylene chloride.

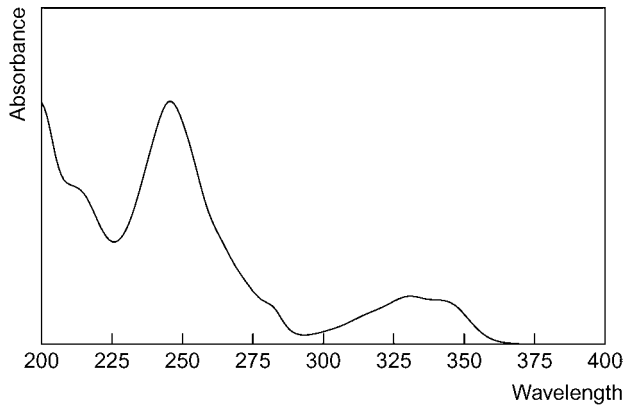
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.73; system TAE—R_f 0.71.

Gas Chromatography System GB—RI 3054.

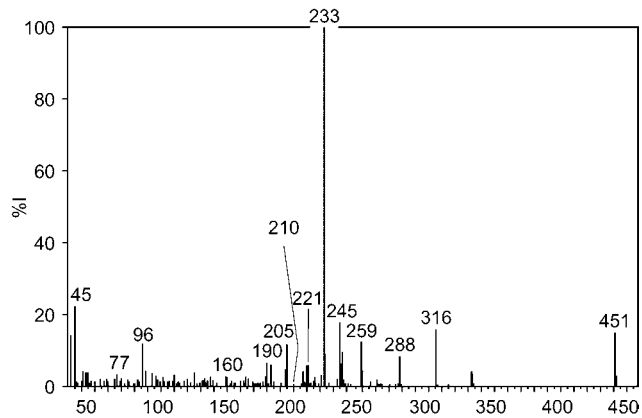
High Performance Liquid Chromatography System HX—RI 403.

Column: ODS Spherisorb (125 × 5 mm i.d., 5 µm). Mobile phase: methanol : 0.02 mol/L tetramethyl ammonium chloride / 0.01 mol/L sodium heptane sulfonate buffer (70:30), flow rate 1.0 mL/min. Retention time: 5.2 min [Kaye *et al.* 1986].

Ultraviolet Spectrum Aqueous acid—246, 331 nm (doxazosin); ethanol —250, 275, 282 nm (doxazosin mesilate).



Mass Spectrum Principal ions at *m/z* 233, 45, 245, 316, 451, 259, 42, 96.



Quantification

Plasma **HPLC** Limit of quantification, 0.2 µg/L [Vashi *et al.* 1996]. Limit of determination, 1 µg/L [Jackman *et al.* 1991].

Disposition in the Body Doxazosin is well absorbed and extensively metabolised in the liver by *O*-demethylation or hydroxylation. Enterohepatic recycling occurs as a large amount of the drug is excreted in faeces as its metabolites and a small amount of the unchanged drug. The remainder of the drug can be found in urine, ~9%. 16% of an oral dose is found as the 6-*O*-demethylation metabolite, 7% as the 7-*O*-demethylation metabolite, 5% as the 6′-hydroxy metabolite and 7% as the 7′-hydroxymetabolite. Other metabolites include 2-piperazinyl and 2-amino compounds.

Therapeutic Concentration

Five men and 5 women patients with essential hypertension were administered with a 1 mg dose of doxazosin. Peak plasma concentrations of 15.7 ± 2.7 µg/L were reached within 2.1 ± 0.4 h. After multiple dosing of the same dosage, over 2 weeks, peak concentrations reached 19.3 ± 2.3 µg/L still within 1.9 ± 0.2 h [Kaye *et al.* 1986].

Toxicity

A 32-year-old woman with chronic renal failure and depression took a 60 mg dose of doxazosin and died owing to a grand mal seizure resulting

from hypotension. Blood analysis showed a doxazosin level of 0.9 g/L [www.Rxlist.com]

Bioavailability 65%.

Half-life 16 to 22 h.

Volume of Distribution 1.01 L/kg (in young adults, aged ~29 years); 1.71 L/kg (elderly, aged 71 years).

Clearance 1.2 mL/min/kg.

Protein Binding Approximately 98%.

Dose Initially a dose of 1 mg daily is administered, which can be increased over time to a maximum of 16 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Jackman GP *et al.* (1991). Validation of a solid-phase extraction high-performance liquid chromatographic assay for doxazosin. *J Chromatogr* 566: 234–238.

Kaye B *et al.* (1986). The metabolism and kinetics of doxazosin in man, mouse, rat and dog. *Br J Clin Pharmacol* 21: 19S–25S.

Vashi V *et al.* (1996). Effect of time of administration on the pharmacokinetics and tolerance of doxazosin in healthy male volunteers. *J Clin Pharmacol* 36: 325–331.

Doxepin

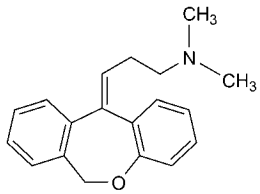
Tertiary Tricyclic Antidepressant, Histamine H_1- and H_2-Antagonist

$C_{19}H_{21}NO = 279.4$

CAS—1668-19-5

IUPAC Name (3*E*)-3-(6*H*-Benzo[*c*][1]benzoxepin-11-ylidene)-*N*,*N*-dimethylpropan-1-amine

Synonym Mixture of the *cis*-and *trans*-isomers of 3-dibenz[*b*,*e*]oxepin-11(6*H*)-ylidene-*N*,*N*-dimethyl-1-propanamine



Chemical Properties Oily liquid. pK_a 9.0 (25°). Log *P* (octanol/pH 7.4), 2.4 [Wozniakiewicz *et al.* 2008], (octanol/water), 4.29 [Sangster 1997], (hexane/water), 4.13 [Kemp *et al.* 1985]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stock solutions were stable for 4 months at 4°. Spiked plasma samples were stable for at least 1 month at –18° and following 4 freeze-thaw cycles [Samanidou *et al.* 2007]. Plasma samples were stable for 6 weeks at –20°, after 3 freeze-thaw cycles, for 48 h at ≤20° for 24 h at 4° [Geister *et al.* 2001]. Urine and plasma samples were stable after 2 freeze-thaw cycles (–20°), after 2 days at 4° and when reconstituted in the mobile phase and stored at room temperature for up to 16.5 h [Yan *et al.* 1997]. Plasma samples were stable at –20° for at least 2 weeks [Dilger *et al.* 1988]. Standards kept at 4° deteriorated within 1 week while standards stored at –20° were stable for at least 4 months [Wilson *et al.* 1977]. Plasma samples were stable for at least 1 week if stored at –20° [Biggs *et al.* 1976].

Doxepin Hydrochloride

$C_{19}H_{21}NO,HCl = 315.8$

CAS—1229-29-4

Synonyms NSC-108160; P-3693A.

Proprietary Names *Anten*; *Aponal*; *Deptran*; *Desidoxepin*; *Doneurin*; *Doxal*; *Gilex*; *Mareen*; *Quitaxon*; *Sinequan*; *Sinquan(e)*; *Xepin*; *Zonalon*.

Chemical Properties White crystalline powder. Mp 184° to 186°, 188° to 189°. Soluble 1 in 1.5 of water, 1 in 1 of ethanol and 1 in 2 of chloroform; very slightly soluble in ether.

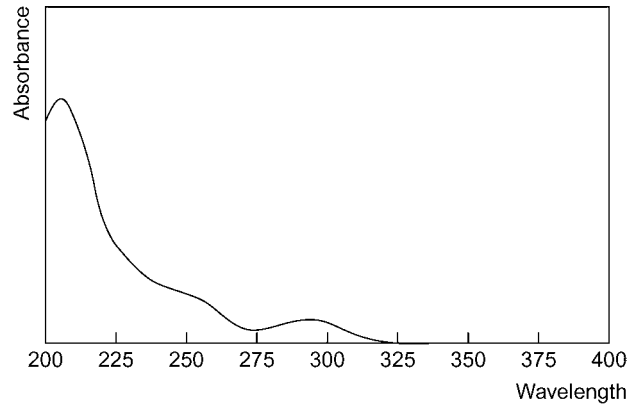
Colour Tests Liebermann's reagent—black; Mandelin's test—brown; Marquis test—brown; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.48; system TC—R_f 0.37; system TE—R_f 0.63; system TL—R_f 0.13; system TAE—R_f 0.24; system TAF—R_f 0.45; system TAF—R_f 0.45; system TAJ—R_f 0.23; system TAK—R_f 0.14; system TAL—R_f 0.71 (acidified iodoplatinate solution, positive).

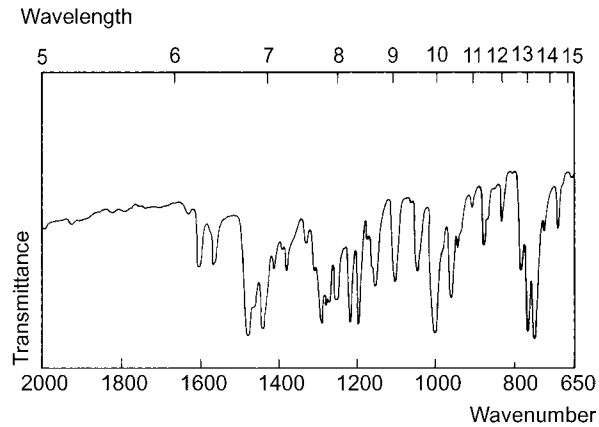
Gas Chromatography System GA—doxepin (*cis* and *trans*) RI 2220, M (*cis*-*N*-oxide) RI 1970, M (*cis*-nor-) RI 2245, M (*trans*-nor-) RI 2245, M (*cis*-OH-) RI 2535, M (*trans*-OH-) RI 2560, M (*cis*-nor-OH-) RI 2540, M (OH-dihydro-) RI 2530; system GB—doxepin (*cis* isomer) RI 2301; doxepin (*trans* isomer) RI 2321, M (*cis*-*N*-oxide) RI 2077, M (*trans*-*N*-oxide) RI 2081, M (*cis*-nor) RI 2333; M (*trans*-nor) RI 2339; M (*cis*-OH-) RI 2528, M (*trans*-OH-) RI 2544, M (*cis*-nor-OH-) RI 2644; M (*trans*-nor-OH-) RI 2671; system GF—RI 2570; system GM—doxepin (*cis* isomer) RRT 0.788; doxepin (*trans* isomer) RRT 0.823; M (*cis*-nor-) RRT 0.830, M (*trans*-nor-) RRT 0.933 (all relative to iprindole); system GS—doxepin (*cis* isomer) RT 17.2, doxepin (*trans* isomer) RT 17.6 min, chloramitriptyline (IS) RT 21.2 min.

High Performance Liquid Chromatography System HA—doxepin *k* 3.7, monodesmethyldoxepin *k* 2.2; system HF—*k* 2.27; system HX—RI 404; system HY—RI 316; system HZ—doxepin RT 5.0 min, desmethyl doxepin RT 4.6 min; system HAA—RT 14.1 min; system HAX—RT 12.9 min; system HAY—RT 6.1 min.

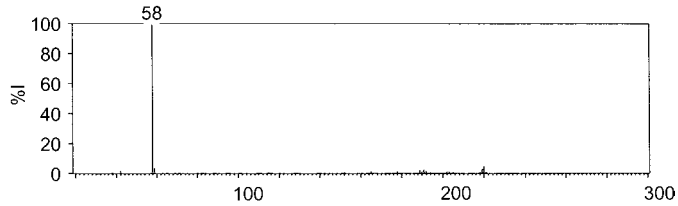
Ultraviolet Spectrum 0.1 N sulfuric acid—292 nm ($A_1^1 = 231a$) [Norheim 1973].



Infrared Spectrum Principal peaks at wavenumbers (doxepin hydrochloride) 1477, 750, 1439, 768, 1006, 1198, 1290, 1219 cm^{-1}, (KBr disk) [Norheim 1973].



Mass Spectrum Principal ions at *m/z* 58, 220, 219, 59, 191, 189, 42, 205.



Quantification

Blood **GC** Column: HP cross-linked methylsilicone (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 23 µg/L, limit of detection, 7 µg/L [Martinez *et al.* 2004]. Column: 3.5% FFAP on 100/120 mesh Supelcoport or 3% OV-17 on 100/120 mesh chromosorb W-HP (1.2 m × 1.4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 238° or 243°. FID. Retention time: 17.4 and 19.1 min for *cis*- and *trans*-doxepin, respectively on the first column, 10.6 min for the second column. Limit of detection, 25 µg/L [Cordonnier *et al.* 1983]. Column: 3% SE30 on Diatomite CLS 100/120 mesh (5′ × 1/8" i.d.) or 5% SE30 on 60/80 mesh AW Chromasorb W DMCS (6′ × 1/8" i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 200°. FID. Relative retention time, 0.64. Limit of detection not reported [Oliver, Watson 1974]. Column: 3% SE-30 on Varaport 30 100/120 mesh (5′ × 1/8" i.d.) or 3% OV-17 on Chromosorb G80 to 80 mesh support (6′ × 2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 220° or 270°, respectively. Retention time: 3.9 min on SE-30, 5.3 min on OV-17. Limit of detection, <10 ng [Norheim 1973].

GC-MS Column: 10% OV-17. Carrier gas: He, 30 mL/min. Temperature programme: 180° to 300° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Norheim 1973].

LC-MS Column: XTerra RP18 (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2; 5 : 95 for 2 min to 20 : 80 in 1 min to 30 : 70 over 12 min for 2 min to 5 : 95 in 30 s for 2.5 min), flow rate 0.15 mL/min. ESI, MRM acquisition mode, positive ion mode. Retention time: 9.4 min. Limit of quantification, 2 µg/L [Titier *et al.* 2007].

Plasma **GC** Column: 3% SP-2250 on 80/100 mesh Supelcoport (6′ × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 230°. NPD. Retention time: 4.6 min. Limit of detection, 0.5 µg/L [Abernethy *et al.* 1981]. Column: OV-17 (20 m × 0.5 mm i.d.) or 3% OV-17 on 80/100 mesh Gas Chrom Q (1.8 m × 2 mm i.d.). Carrier

gas: H_2, 4 mL/min for the former, He, 30 mL/min for the latter. Temperature: 230°. NPD. Limit of detection, 2 µg/L [Rosseel *et al.* 1978]. Column: 1% OV-17 plus 2% OV 225 Chromosorb WHP on 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 215°. FID. Relative retention time: 0.87. Limit of detection, 10 µg/L [Dorrity, Jr. *et al.* 1977]. Column: 3% OV-225 on 100/120 mesh Gas Chrom Q (1.83 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature: 215°. FID. Limit of detection, 10 µg/L [O'Brien, Hinsvark 1976].

GC-MS Column: 3% OV-17 on 100/120 Gas-Chrom Q (1.1 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 220°. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 1 µg/L [Davis *et al.* 1983]. Column: 3% OV-225 on 80/100 mesh Chromosorb W (HP) (6′ × 1/8" i.d.). Carrier gas: He, 30 mL/min. Temperature: 205°. EI ionisation at 70 eV. Limit of detection, 0.4 mg/L [de Groot *et al.* 1978]. Column: 3% SP-2250DB on 100/120 mesh Supelcoport (15 m × 2 mm i.d.). Carrier gas: CH_4, 20 mL/min. Temperature: 205°. CI mode. Relative retention time: 1.7. Limit of detection, 10 µg/L [Jenkins, Friedel 1978]. Column: 3% OV-1 on 100/120 Gas Chrom Q (1 m × 2 mm i.d.). Carrier gas: CH_4: Ar (5 : 95), 30 mL/min. Temperature: 240°. EI ionisation at 70 eV. Limit of detection, 2.5 µg/L [Wallace *et al.* 1978]. Column: 3% OV-225 on 100/120 mesh Gas Chrom Q (1.5 m × 2.0 mm i.d.). Carrier gas: CH_4, 16 mL/min. Temperature: 220°. CI. Retention time: 3.6 and 3.91 min for *cis*- and *trans*-doxepin, respectively. Limit of detection, 5 µg/L [Wilson *et al.* 1977]. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (50 cm × 6 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 180° to 220°. EI ionisation at 70 eV. Retention time: 2–3.5 min. Relative retention time: 0.56. Limit of detection, 10 µg/L [Biggs *et al.* 1976].

HPLC Column: Kromasil C_8 (250 × 4mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (45 : 55), flow rate 1.5 mL/min. UV detection (λ = 238 nm). Retention time: 3.5 min. Limit of quantification, 0.2 ng, limit of detection, 0.07 ng [Samanidou *et al.* 2007]. Column: Ultrasphere C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L 1-octanesulfonic acid-0.5 mmol/L *N,N,N,N*-tetramethylethylene diame (pH 2.5; 35: 650) flow rate 2 mL/min. UV detection (λ = 230 nm). Retention time: 7.7 min. Limit of detection not reported [Hackett *et al.* 1998]. Column: Spherisorb (150 × 4.5 mm i.d., 3 µm). Mobile phase: hexane : methanol : nonylamine (95 : 5 : 0.3), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of quantification, 1 µg/L for *cis* and *trans*-doxepin [Yan *et al.* 1997]. Column: Beckmann ultrasphere C_{18} (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water mixture (methanol : 0.25 mol/L potassium dihydrogen phosphate : triethylamine : orthophosphoric acid [58 : 10 : 0.4 : 0.24]), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 1.8 mg/L [Dunn *et al.* 1994]. Column: Hypersil Si 100 (250 cm × 4.6 mm i.d.). Mobile phase: water : acetonitrile : tetraethylammonium perchlorate (pH 8.4; 400 : 1200 : 1.2), flow rate 0.95 mL/min. UV detection (λ = 230 nm). Limit of quantification, 0.426 µg/L for *trans*-doxepin [Dilger *et al.* 1988].

See also Kemp *et al.* [1985], Sutfin *et al.* [1984], Visser *et al.* [1984], Faulkner, Lee [1983], Sonsalla *et al.* [1982], Bannister *et al.* [1981], Kabra *et al.* [1981], Vandemark *et al.* [1978].

LC-MS Column: Chromolith Speed ROD C18 (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol: 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 4.43 µg/L Kirchherr, Kühn-Velten 2006. Column: Symmetry C_{18} (150 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (28 : 72 for 4 min to 70 : 30 in 1 min for 3 min to 28 : 72 in 0.7 min), flow rate 0.6 mL/min. APCI, positive ion mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Kollroser, Schober 2002]. Column: GROM SIL 120 Cyano-3cp (60 × 4.6 mm i.d., 5 µm). Mobile phase: ammonium carbaminate : acetonitrile, flow rate 0.6 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 59 ng/L [Geister *et al.* 2001]. Column: Phenomenex Luna C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water : 0.05% formic acid (600 : 400 : 1), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.87 and 1.94 min for doxepin and desmethydoxepin, respectively. Limit of quantification, 0.32 and 0.18 µg/L for doxepin and desmethydoxepin, respectively [Badenhorst *et al.* 2000]. Column: SB-C_{18} Mac Mod (15 × 2.1 mm i.d., 3 µm). Mobile phase: 3 mmol/L ammonium acetate (pH 3.0) : acetonitrile (66 : 34), flow rate 1.4 mL/min. TIS, positive ion mode, SRM acquisition mode. Limit of quantification, 1 µg/L [Zhang *et al.* 2000].

CE Column: fused silica capillary (total/effective length 71.5/50 cm × 50 µm i.d.). Mobile phase: 25 mmol/L dodecyltrimethylammonium bromide with 2 mol/L urea in 37.5 mmol/L phosphate buffer (pH 8.0). UV detection (λ = 254 nm). Limit of detection, 5–10 µg/L [Lee *et al.* 1993].

Serum **GC** Column: SPB-1 100% polymethylsiloxane (60 m × 0.75 mm i.d., 1.0 µm). Carrier gas: He, 8 mL/min. Temperature: 260°. NSD. Limit of detection, 25 µg/L [Rifai *et al.* 1988]. Column: 3% OV-25 on Gas-Chrom Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 15 mL/min. Temperature: 245°. NPD. Relative retention time: 4.0. Limit of detection, 10 µg/L [Bredesen *et al.* 1981]. Column: 3% OV-225 on 100/120 mesh (1 m × 2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature programme: 250° at 2°/min for 40 h. Retention time: 0.69 and 0.74 for *cis*- and *trans*-doxepin, respectively. Limit of detection, 5–10 µg/L [Kristinsson 1981]. Column: 3% OV-17 100/120 mesh Gas-Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 235° for 6 min to 280° at 32°/min for 4 min. FID or NPD. Retention time: 4.1 min. Limit of detection, 3 µg/L [Vasiliades *et al.* 1979].

GC-MS Column: DB-1701 (15 m × 0.32 mm i.d., 1 µm). Carrier gas: He, 1 mL/min. Temperature programme: 170° for 1 min to 230° at 20°/min to 250° at 5°/min. CI, positive ion mode. Limit of detection, 0.5 µg/L [Ghabrial *et al.* 1991]. Column: 2% SP-2250 Chromosorb W-HP 100/120 mesh (1.2 m × 2.0 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 220° for 4 min to 250° at 30°/min for 10–15 min. EI ionisation at 70 eV. Limit of detection, 1 ng/L [Vasiliades 1980].

HPLC Column: Spheri-5 C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphoric buffer (pH 2.36; 1 : 1), flow rate 1 mL/min. DAD (λ = 254 nm). Limit of quantification, 0.04 mg/L, limit of detection, 0.01 mg/L [Wozniakiewicz *et al.* 2008]. Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogenphosphate (pH 7.0) : acetonitrile (60 : 40), flow rate, 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 18.3 min. Limit of detection not reported [Frahnert *et al.* 2003]. Column: Super-Octyl TSK gel (100 × 4.6 mm i.d., 2 µm) or Hypersil MOS-C_8 (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 20 mmol/L potassium dihydrogen phosphate (pH 7.0; 60 : 40), flow rate 0.6 mL/min. UV detection (λ = 254 nm). Retention time: 8.5 and 10.3 min for the TSK and Hypersil columns, respectively. Limit of quantification, 0.05 µg/L on the TSK column [Tanaka *et al.* 1997]. Column: silica 80Å (100 × 6 mm i.d., 3 µm). Mobile phase: 0.025 mol/L dibasic sodium phosphate (pH 3.0) : acetonitrile (80 : 20) with 0.021 mol/L *n*-nonylamine (pH 7.4 to 7.8), flow rate 1.6 mL/min. UV detection. Retention time: 8.35, 8.83, 14.7 and 16.4 min for *Z*-desmethyldoxepin, *E*-desmethyldoxepin, *Z*-doxepin, and *E*-doxepin, respectively. Limit of quantification, 10 µg/L for doxepin and 5 µg/L for desmethyldoxepin [Adamczyk *et al.* 1995]. Column: Nucleosil 100 CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.01 mol/L potassium phosphate buffer (pH 6.8; 188 : 5778 : 235). UV detection (λ = 214 nm). Limit of detection, 10 µg/L [Rao *et al.* 1994].

See also Emm *et al.* [1987], Lin, Frade [1987], Messiha [1986], Beierle, Hubbard [1983], Koteel *et al.* [1982], Sonsalla *et al.* [1982], Bannister *et al.* [1981], Kabra *et al.* [1981], Thoma *et al.* [1979], Proelss *et al.* [1978]

LC-MS Column: Uptisphere (12.5 cm × 2 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0)-acetonitrile : acetonitrile (100 : 0), flow rate 200 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 22 nmol/L [Gutteck, Rentsch 2003].

Urine **GC** See Blood [Cordonnier *et al.* 1983]. See Blood [Oliver, Watson 1974]. See Blood [Norheim 1973]. Column: 3% SE-30 on Chromosorb W HP 80/100 mesh (6′ × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 220° FID. Retention time: 5.0 min. Limit of detection, 1 mg/L [Dusci, Hackett 1971].

GC-MS Column: CP-SIL 5CB (10 m × 0.15 mm i.d., 0.12 µm). Carrier gas: H_2, 1 mL/min. Temperature programme: 110° for 0.5 min to 250° at 40°/min to 280° at 60°/min. SIM acquisition mode. Retention time: 3.32 min. Limit of quantification, 100 µg/L, limit of detection, 50 µg/L [Rana *et al.* 2008]. See Plasma [de Groot *et al.* 1978]. See Blood [Norheim 1973].

HPLC See Plasma. Limit of quantification, 0.65 ng, limit of detection, 2.0 ng [Samanidou *et al.* 2007]. See Serum [Tanaka *et al.* 1997]. See Plasma [Yan *et al.* 1997]. See Plasma. Limit of detection, 0.19 mg/L [Dunn *et al.* 1994]. Column: Cyano (25 cm × 9.4 mm i.d.). Mobile phase: ethanol : water (1 : 1), 3 mL/min. UV detection (λ = 254 nm). Retention time: 5.5 min for doxepin N^+-glucuronide. Limit of detection, 5 mg/L [Luo *et al.* 1991]. Column: Zorbax CN (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol: 0.1 mol/L ammonium acetate (84 : 8 : 8), flow rate 1.6 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Shu *et al.* 1990a]. Column: Spherisorb nitrile (15 × 0.46 cm i.d., 3 µm). Mobile phase: water : acetonitrile-methanol (75 : 25, 95 : 5 for 1 min to 0 : 100 in 16 min for 4 min), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: ≈5 min for glucuronide metabolites. Limit of detection not reported [Shu *et al.* 1990b].

Bile **GC-MS** See Plasma [de Groot *et al.* 1978].

HPLC See Plasma. Limit of detection, 15 mg/L [Dunn *et al.* 1994].

Gastric Contents **HPLC** See Plasma. Limit of detection, 1.5 g/L [Dunn *et al.* 1994].

Milk **HPLC** Column: MOS- Hypersil C_8 reversed phase (100 × 2 mm i.d., 3 µm). Mobile phase: 0.02 mol/L monobasic potassium phosphate : *N,N*-dimethyloctylamine (pH 6.5) : acetonitrile (66.0.085 : 34), flow rate 0.5 mL/min. UV detection (λ = 242 nm). Retention time: 6.74 min. Limit of quantification, 2.0 µg/L, limit of detection, 1.25 µg/L [Hostetter *et al.* 2004]. See Plasma [Kemp *et al.* 1985].

Vitreous Humour **HPLC** Column: Zorbax cyanopropyl (25 cm × 4.6 mm i.d., 5–6 µm). Mobile phase: 0.5 mol/L acetic acid : acetonitrile: *n*-butylamine (60 : 40 : 0.0022), flow rate 2.5 mL/min. UV detection (λ = 254 nm). Limit of detection, 16.7 µg/L [Evenson, Engstrand 1989].

Brain **GC** See Blood [Oliver, Watson 1974].

HPLC See Serum [Tanaka *et al.* 1997].

Hair **GC** Column: AC-5 capillary (15 m × 0.25 mm i.d.). Temperature programme: 180° to 270° at 10°/min for 10 min. NPD. Limit of detection, 0.1–0.5 µg/g [Shen *et al.* 2002].

GC-MS Column: DB-5 (30 m × 0.25 mm i.d.). Carrier gas: CH_4. Temperature programme: 100° for 2 min to 150° at 10°/min to 280° at 25°/min. CI, full scan mode. Retention time: 10.2 min [Shen *et al.* 2002]. Column: HP-5MS (30 m × 250 µm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 130° for 1 min to 280° at 12°/min for 3 min. SIM acquisition mode. Retention time: 11.48 min. Limit of quantification, 0.25 µg/g [Negrusz *et al.* 1998].

Heart **GC** See Blood [Cordonnier *et al.* 1983].

Kidney **GC** See Blood [Cordonnier *et al.* 1983]. See Blood [Oliver, Watson 1974].

Liver **GC** See Blood [Cordonnier *et al.* 1983]. See Blood [Oliver, Watson 1974]. See Blood [Norheim 1973].

GC-MS See Blood [Norheim 1973].

HPLC See Serum [Tanaka *et al.* 1997].

Other **GC-MS** Human Liver Microsomes. Column: DB-17 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 35 cm/s. Temperature programme: 150° for 1 min to to 270° at 15°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.6 and 10.75 min for *trans*-doxepin and *cis*-doxepin, respectively. Limit of detection, 50 mol/L [Haritos *et al.* 1999].

LC-MS Foodstuffs. Column: Luna C_8 100-Å (100 × 2.0 mm i.d., 3 µm). Mobile phase: 0.1% formic acid : 0.1% formic acid in acetonitrile (95 : 5 for 3 min to 5 : 95 at 18 min for 1 min to 95 : 5 over 0.5 min for 2.5 min. ESI, positive ion mode, SIM acquisition mode. Retention time: 10.4 min. Limit of detection not reported [Zuckschwerdt *et al.* 2008].

Note For a radioimmunoassay method in plasma and serum, see Virtanen *et al.* [1980].

Disposition in the Body Well absorbed after oral administration, but undergoes extensive first-pass demethylation; bioavailability 15 to 45%. Other metabolic reactions include *N*-oxidation, aromatic hydroxylation, and glucuronic acid conjugation [Luo *et al.* 1991; Shu *et al.* 1990b]. Monodesmethyldoxepin is an active metabolite. CYP2D6 is a major oxidative enzyme involved in the metabolism of doxepin [Haritos *et al.* 2000], whereas *N*-demethylation of doxepin is catalysed by CYP2C19 [Koski *et al.* 2007]. Doxepin is excreted in the urine, chiefly as metabolites and conjugated metabolites; <1% of a dose is excreted in the urine as unchanged drug in 24 h. It is also absorbed through the skin if applied topically. It crosses the blood–brain barrier and the placenta. Doxepin and its active metabolite desmethyldoxepin are excreted in breast milk, whereas only desmethyldoxepin is detectable in the plasma of the infant after 43 days [Kemp *et al.* 1985].

Therapeutic Concentration In plasma, usually in the range 0.05 to 0.15 mg/L.

After a single oral dose of 75 mg given to 7 subjects, peak plasma concentrations of 0.01 to 0.05 mg/L (mean 0.03) of doxepin were attained in 2 to 4 h and peak plasma concentrations of 0.005 to 0.014 mg/L (mean 0.01) of monodesmethyldoxepin were reported at 2 to 10 h [Ziegler *et al.* 1978].

Seven male patients, 4 with dysthymic disorders and 3 with non-psychomotor major depressive disorders were administered a single oral daily dose of 150 mg doxepin at bedtime for up to 3 weeks. Plasma clearance ranged from 0.7 to 1.15 L/h/kg and volume of distribution ranged between 12.7 to 30.2 L/kg. Approximately 70% of the oral dose was estimated to undergo first-pass metabolism [Faulkner *et al.* 1983].

After a single oral dose of 100 mg doxepin to 30 subjects the mean peak plasma concentration of *trans*-doxepin was ≈13 µg/L reached at 3 to 4 h. The desmethyldoxepin concentration reached ≈4 µg/L which slowly decreased to 0.5 µg/L at 72 h after dosing. The mean terminal elimination half-life was calculated to be ≈11 and 20 h for *trans*-doxepin and metabolite, respectively [Dilger *et al.* 1988].

After a single oral dose of 100 mg doxepin to 20 healthy volunteers the maximum doxepin concentration ranged from 6.79 to 18.57 µg/L at ≈2 h. The maximum desmethyldoxepin concentration was between 6.33 and 17.85 µg/L [Badenhorst *et al.* 2000].

Analysis of data from a serum-level databank from German and US sources showed the mean steady-state serum concentration of doxepin plus desmethyldoxepin to be 0.089 (±0.075) mg/L following a daily dose of 143 (±30) mg doxepin [Leucht *et al.* 2001].

A retrospective analysis of data from 114 psychiatric patients treated with doxepin 25 to 400 mg daily for 22 to 306 days (92% by the oral route; 8% by the IV route) revealed doxepin plasma concentration to be 40 to 1057 nmol/L (mean 276.23) and desmethyldoxepin to be 141 to 1627 nmol/L (mean 251) [Meyer-Barner *et al.* 2002].

Toxicity Doses >500 mg are likely to produce severe toxic effects and the estimated minimum lethal dose is 1 g. Blood concentrations >0.1 mg/L may be associated with toxic reactions and fatalities have been associated with blood concentrations of 1 to 8 to 18 mg/L.

A 35-year-old female was found dead. At postmortem the concentration of doxepin hydrochloride in blood, liver and urine was 1.9, 32, and 1.6 mg/g [Norheim 1973].

In 5 deaths due to over-dosage of doxepin, the postmortem blood concentrations ranged from 9 to 19 mg/L (mean 13 mg/L) and in 3 of these cases, liver concentrations of 71, 75, and 500 µg/g were reported [Oliver, Watson 1974].

In a report of 4 fatalities attributed to overdose of doxepin, the postmortem blood and tissue concentrations, mg/L or µg/g (mean), shown below were found.

	Doxepin	*Monodesmethyldoxepin*
Blood	0.7–29 (9.3)	0.1–6.2 (1.7)
Bile	38–195 (95)	1.0–19 (7.1)
Brain	9–21 (14)	1.5–22 (7.2)
Kidney	3.3–19 (12)	0.5–9.0 (3.0)
Liver	22–38 (32)	1.2–20 (7.5)
Urine	2.1–12 (7.5)	0.7–6.4 (2.8)

[de Groot *et al.* 1978]

A 24-year-old male ingested at least 2500 mg doxepin and 500 mg chlorazepate. The postmortem concentrations of *cis* and *trans*-doxepin (mg/L or mg/kg) are shown below.

	Cis and **trans***-doxepin*		*Desmethyldoxepin*	
Sample	Concentration	Ratio	Concentration	Ratio to doxepin
Standard	—	16 : 84	—	—
Blood	3.3	10 : 90	—	—
Urine	1.4	8 : 92	—	—
Kidney	4.3	11 : 89	0.52	12 : 88
Liver	63.8	13 : 87	4.47	7 : 93
Left ventricle	3.6	9 : 91	0.10	3 : 97
Ventricular septum	2.7	9 : 91	0.46	17 : 83

[a][Cordonnier *et al.* 1983]

A 5% doxepin hydrochloride cream was applied to a 2.5-year-old male with severe eczema. He suffered a grand mal seizure and was admitted to hospital. The serum doxepin concentration was 382 µg/L and nordoxepin was 141 µg/L. Eight days later his serum doxepin concentration was 119 µg/L [Vo *et al.* 1995].

A 43-year-old Finnish male was found dead. Postmortem revealed 2.4 and 2.9 mg/L of doxepin and nordoxepin in his femoral venous blood, respectively. CYP2D6 genotype analysis revealed *3 and *4, which translates to a total absence of the CYP2D6 enzyme. The CYP2C19 genotype was determined as *1/*1 (i.e. alleles *2 and *3 not found), corresponding to extensive metabolism of CYP2C19 substrates such as doxepin. The doxepin-to-nordoxepin ratio was 0.83. Cause of death was fatal doxepin poisoning [Koski *et al.* 2007].

In a young female fatality, femoral blood drug concentrations were: paroxetine 0.176 mg/L, doxepin 82.12 mg/L, and desmethyldoxepin 0.34 mg/L [Musshoff *et al.* 1999].

Note For a study of doxepin-induced contact dermatitis, see Shelley *et al.* [1996] and for doxepin poisoning in a child see Walter and Kauffman [1980].

Half-life Plasma half-life, doxepin 8.2 to 24.5 h (mean 16.8), desmethyldoxepin 33.2 to 80.7 h (mean 51.3) [Ziegler *et al.* 1978]. Urinary *cis*/*trans* ratio range 0.08 to 3.06 [Ghabrial *et al.* 1991].

Bioavailability 15 to 45%

Volume of Distribution 9.1 to 33.3 L/kg, mean 20.2 L/kg [Ziegler *et al.* 1978]; ≈20 to 24 L/kg.

Clearance Plasma clearance, 0.93 (0.69 to 1.02) L/h/kg [Faulkner *et al.* 1983].

Distribution in Blood Plasma: whole blood ratio, doxepin ≈0.8 but there is considerable intersubject variation; monodesmethyldoxepin ≈0.6.

Protein Binding ≈80%.

Note For a review of the pharmacokinetics of tricyclic antidepressants, see Molnar and Gupta [1980].

Dose The equivalent of 30 to 300 mg of doxepin daily by mouth.

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Doxercalciferol

Antihyperparathyroid, Vitamin D Activity

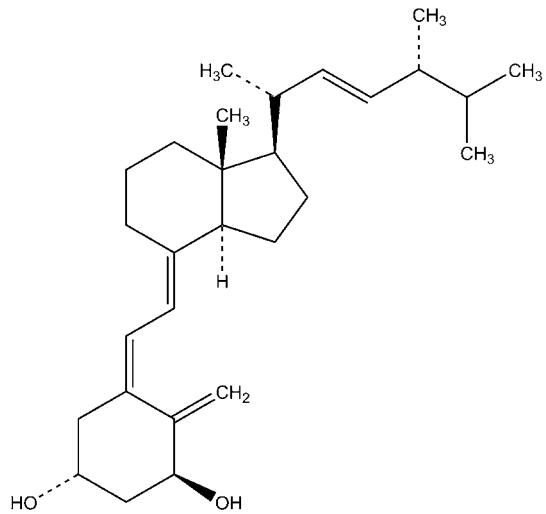
$C_{28}H_{44}O_2 = 412.6$

CAS—54573-75-0

IUPAC Name (1*R*,3*S*,5*Z*)-5-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-1-[(*E*,2*R*,5*R*)-5,6-Dimethylhept-3-en-2-yl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol

Synonyms 1-Hydroxyergocalciferol; 1α-hydroxyvitamin D_2; 1α-OH-D_2; (1α,3β,5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),22-tetraene-1,3-diol.

Proprietary Name *Hectorol*



Chemical Properties Crystals. Mp 138° to 140°.

Quantification

Plasma **HPLC** Column: Zorbax-SIL (250 × 4.6 mm i.d.). Mobile phase: hexane : methylene chloride : propan-2-ol (47.9 : 47.9 : 4.2). Liquid scintillation detection. Limit of detection, 1 µg/L [Upton *et al.* 2003].

Serum **HPLC** See Plasma [Upton *et al.* 2003].

Disposition in the Body Doxercalciferol is not active when it is absorbed in the intestine or after injection. After intestinal absorption, it is metabolised within

liver microsomes to 1-25-dihydroxy vitamin D_2 (1-25-OH-D_2), the major active metabolite, and 1-24-OH-D_2, via CYP27. Hence, doxercalciferol is considered to be a prohormone.

Therapeutic Concentration

Twenty-two osteopenic women with normal kidney and liver function were administered 5 µg IV and 2 and 5 µg oral doses of doxercalciferol with a wash-out period of 1 week between each dosing regimen. Peak plasma concentrations of 1-25-OH-D_2 per µg of dose were 10.2, 5.0, and 3.5 ng/L for 5 µg IV and 2 µg and 5 µg oral doses, respectively, attained after 8, 11 and 11 h, respectively [Upton *et al.* 2003].

Twenty-four healthy volunteers were administered a 5 µg oral dose of doxercalciferol every 48 h for 5 doses. After a 14-day wash-out period, they were administered a 15 µg oral dose every 48 h for 5 doses. Peak plasma concentrations of 1-25-OH-D_2 per µg of dose were 6.0 and 4.5 ng/L for the 5 and 15 µg doses, respectively, reached after 11 h for both doses [Upton *et al.* 2003].

Orally administered doxercalciferol (10 µg every other day for 5 days, then 5 µg every other day for 5 days) was administered to 11 patients on chronic haemodialysis. The mean peak serum concentration of 1-25-OH-D_2 was 38 ng/L after 12 h [Bailie, Johnson 2002].

Bioavailability Approximately 42% for 1-25-OH-D_2.

Half-Life Approximately 34 h.

Protein Binding Highly bound to plasma proteins.

Dose Adult starting dose, 10 µg orally three times weekly for a total weekly dose of 30 µg.

Bailie GR, Johnson CA (2002). Comparative review of the pharmacokinetics of vitamin D analogues. *Semin Dial* 15: 352–357.

Upton RA *et al.* (2003). Pharmacokinetics of doxercalciferol, a new vitamin D analogue that lowers parathyroid hormone. *Nephrol Dial Transplant* 18: 750–758.

Doxofylline

Bronchodilator, Theophylline Derivative

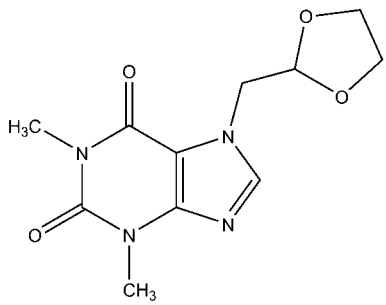
$C_{11}H_{14}N_4O_4 = 266.3$

CAS—69975-86-6

IUPAC Name 7-(1,3-Dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione

Synonyms ABC-12/3; 7-(1,3-dioxolan-2-yl-methyl)-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione doxophylline; dioxyfilline.

Proprietary Names *Ansimar*; *Maxivent*; *Ventax*.



Chemical Properties Crystals. Mp 144° to 145.5°. Soluble in water, acetone, ethyl acetate, benzene, chloroform, dioxane, hot methanol and hot ethanol. Practically insoluble in ethyl ether or petroleum ether.

Quantification

Plasma **HPLC** Column: RP silica particles derivatised with glycine–phenylalanine–phenylalanine (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L phosphate buffer (pH 6.8), flow rate 0.3 mL/min. UV detection (λ = 275 nm). Retention time: 13.9 min. Limit of detection, >1 mg/L [Tagliaro *et al.* 1990]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.01 mol/L phosphate buffer (pH 3.0; 5 : 8 : 87), flow rate 1.2 mL/min. UV detection (λ = 273 nm). Retention time: ~7 min. Limit of detection, 10 µg/L [Lagana *et al.* 1990].

Serum **HPLC** See Plasma [Tagliaro *et al.* 1990]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm) Mobile phase: acetonitrile : methanol : 0.01 mol/L phosphate buffer (pH 3.0; 5 : 8 : 87), flow rate 1.2 mL/min. UV detection (λ = 273 nm). Retention time and limit of quantification not reported [Bologna *et al.* 1990].

LC-MS Column: Amazon C_{18}. Mobile phase: formic acid (pH 2.5) : acetonitrile (10 : 90). API, MRM acquisition mode. Retention time: 1.46 min. Limit of quantification, 1 µg/L [Sreenivas *et al.* 2008].

Other **HPLC** Tablets and Syrup. Column: Lichrosorb C_{18} (250 mm, 10 µm). Mobile phase: acetonitrile : water (30 : 70), flow rate 1.5 mL/min. UV detection (λ = 273 nm). Retention time: 2.6 min. Limit of quantification, 5 mg/L [Badini *et al.* 1982].

Disposition in the Body Rapidly absorbed after oral administration, remaining in the blood for 6 h and completely excreted in the urine within 12 h. The drug circulates unchanged and the only metabolite detectable in serum is β-hydroethyltheophylline (also the main metabolite in urine).

Therapeutic Concentration The lower limit of the therapeutic concentration is approx. 12 µg/L in serum. The upper limit has not been determined, but in patients with serum concentrations higher than 30 µg/L no significant side effects were observed [Villani *et al.* 1997].

A group of 6 healthy volunteers were administered a 400 mg oral dose of doxophylline. A mean peak plasma concentration of 2.88 mg/L was reached at 1 h [Lagana *et al.* 1990].

Doxophylline was administered under fasting conditions to 6 patients with chronic bronchitis as a single IV dose of 100 mg over 10 min. A mean peak serum concentration of 2.5 mg/L was reached at 0.1 h but levels were very variable between patients (range 0.97 to 4.12 mg/L) [Bologna *et al.* 1990].

Eight patients with chronic bronchitis were administered a twice daily 400 mg oral dose of doxophylline for 5 days. A mean peak serum concentration of 15.2 mg/L was reached at 1 h [Bologna *et al.* 1990].

Bioavailability Approximately 63%.

Half-life Approximately 90 min, also reported as 8 h.

Volume of Distribution Approximately 267 L.

Clearance Approximately 0.5 L/min.

Dose Orally, up to 1200 mg daily. It may also be given by slow IV injection.

Badini C *et al.* (1982). Assay of 2-(7′-theophyllinmethyl)-1,3-dioxolane using HPLC. *Farmaco (Prat)* 37: 320–324.

Bologna E *et al.* (1990). Oral and intravenous pharmacokinetic profiles of doxofylline in patients with chronic bronchitis. *J Int Med Res* 18: 282–288.

Lagana A *et al.* (1990). Solid phase extraction and high performance liquid chromatographic determination of doxophylline in plasma. *Biomed Chromatogr* 4: 205–207.

Sreenivas N *et al.* (2008). Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of doxofylline in human serum: application to a clinical pharmacokinetic study. *Biomed Chromatogr* 22: 654–661.

Tagliaro F *et al.* (1990). Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum. *Clin Chem* 36: 113–115.

Villani F *et al.* (1997). Oral doxophylline in patients with chronic obstructive pulmonary disease. *Int J Clin Pharmacol Ther* 35: 107–111.

Doxorubicin

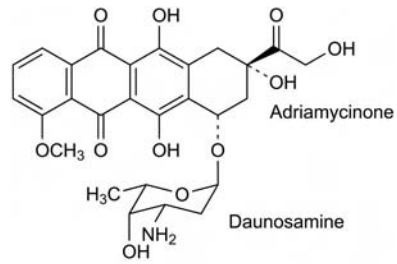
Antineoplastic

$C_{27}H_{29}NO_{11} = 543.5$

CAS—23214-92-8

IUPAC Name (7*S*,9*S*)-7-[(2*R*,4*S*,5*S*,6*S*)-4Amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7*H*-tetracene-5, 12-dione

Synonyms Adriamycin; (8*S*-*cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione; 14-hydroxydaunorubicin.



Chemical Properties An antibiotic isolated from *Streptomyces peucetius* var. *caesius*. Mp 229° to 231°. pK_a 8.2, 10.2. Log *P* (octanol/water), 1.3.

Doxorubicin Hydrochloride

$C_{27}H_{29}NO_{11}$,HCl = 580.0

CAS—25316-40-9

Proprietary Names *Adriamycin*; *Adriblastin(a)*; *Adriblastine*; *Adrim*; *Adrimedac*; *Caelyx*; *Doxil*; *DOXO-cell*; *Doxolem*; *Doxorubin*; *Doxotec*; *Farmiblastina*; *Fauldoxo*; *Ribodoxo-L*; *Rubex*.

Chemical Properties An orange-red, hygroscopic, crystalline powder. Mp 204° to 205°, with decomposition. Soluble in water, methanol and aqueous alcohols; soluble 1 in 75 of ethanol; practically insoluble in acetone, benzene, chloroform, ethyl ether, petroleum ether, ether and other organic solvents.

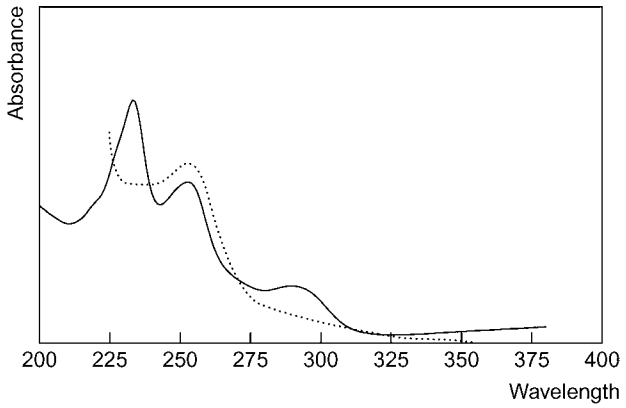
Caution Doxorubicin hydrochloride is irritant; avoid contact with skin and mucous membranes.

Colour Tests Mandelin's test—green; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.12; system TE—R_f 0.00; system TF—R_f 0.00 (visible red streak).

High Performance Liquid Chromatography System HX—RI 370; system HAA—retention time 12.1 min.

Ultraviolet Spectrum Aqueous acid—233, 253, 290 nm; aqueous alkali—253 nm; methanol—233 (A^{1}_{1}=702b), 253, 290 nm.



Infrared Spectrum Principal peaks at wavenumbers 1282, 990, 1010, 1587, 1612, 1204 cm^{-1} (doxorubicin hydrochloride).

Quantification

Plasma **HPLC** Fluorescence detection. Limit of detection, <1 µg/L, doxorubicin and doxorubicinol [de Bruijn *et al.* 1999]. Electrochemical detection. Limit of detection, <1 µg/L, doxorubicin, epirubicin and principal metabolites [Ricciarello *et al.* 1998]. Fluorescence detection. Limit of detection, 1 to 2 nmol/L, doxorubicin and its metabolites [Andersen *et al.* 1993]. Fluorescence detection. Limit of detection, <25 µg/L [Cox *et al.* 1991]. Fluorescence detection. For method for quantification of doxorubicin and metabolites, see Beijnen *et al.* [1991]. UV detection. Limit of detection, 10 µg/L, doxorubicin and daunorubicin [Mikan *et al.* 1990].

Tissues **HPLC** See Plasma [Cox *et al.* 1991].

Dose 1.2 to 2.4 mg/kg, intravenously, as a single dose every 3 weeks.

Andersen A *et al.* (1993). A sensitive and simple high-performance liquid chromatographic method for the determination of doxorubicin and its metabolites in plasma. *Ther Drug Monit* 15: 455–461.

Beijnen JH *et al.* (1991). HPLC determination of doxorubicin, doxorubicinol and four aglycone metabolites in plasma of AIDS patients. *J Pharm Biomed Anal* 9: 995–1002.

Cox SK *et al.* (1991). Determination of adriamycin in plasma and tissue biopsies. *J Chromatogr* 564: 322–329.

de Bruijn P *et al.* (1999). Determination of doxorubicin and doxorubicinol in plasma of cancer patients by high-performance liquid chromatography. *Anal Biochem* 266: 216–221.

Mikan A *et al.* (1990). High performance liquid chromatography determination of doxorubicin and daunorubicin in plasma using UV detection and column switching. *Biomed Chromatogr* 4: 154–156.

Ricciarello R *et al.* (1998). Simultaneous determination of epirubicin, doxorubicin and their principal metabolites in human plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Sci Appl* 707: 219–225.

Doxycycline

Antibiotic

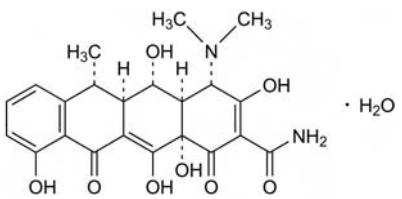
$C_{22}H_{24}N_2O_8,H_2O$ = 462.5

CAS—564-25-0 (anhydrous); 17086-28-1 (monohydrate)

IUPAC Name (2*Z*,4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-2-[Amino(hydroxy)methylidene]-4-(dimethylamino)-5,10,11,12*a*-tetrahydroxy-6-methyl-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-1,3, 12-trione

Synonyms [4*S*-(4α,4aα,5α,5aα,6α,12aα)]-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate; doxycycline monohydrate.

Proprietary Names *Cyclodox*; *Doximed*; *Doxy*; *Doxybene*; *Doxycline*; *Doxyderma*; *Doxydoc*; *Doxy-HP*; *Doxymerck*; *Doxymono*; *Doxysol*; *Doxystad*; *Idocyklin*; *Vibradox*; *Vibramycin-D*; *Vibramycine N*; *Vibra-S*; *Vibravenös*.



Chemical Properties A yellow crystalline powder. Very slightly soluble in water; sparingly soluble in ethanol; practically insoluble in chloroform and ether; freely soluble in dilute acids and alkali hydroxides. pK_a 3.5, 7.7, 9.5 (20°). Log *P* (octanol/water), −0.02.

Doxycycline Hydrochloride

$C_{22}H_{24}N_2O_8,HCl,{}^{1}/_{2}C_2H_5OH,{}^{1}/_{2}H_2O$ = 512.9

CAS—10592-13-9 ($C_{22}H_{24}N_2O_8,HCl$); 24390-14-5 ($C_{22}H_{24}N_2O_8,HCl,{}^{1}/_{2}C_2H_5OH,{}^{1}/_{2}H_2O$)

Synonyms Doxycycline hyclate; doxycyclini chloridum.

Proprietary Names *Atridox*; *Demix*; *Doxi Crisol*; *Doxi Sergo*; *Doxibiotic*; *Doxiclat*; *Doxylar*; *Doximycin*; *Doxin(a)*; *Doxine*; *Doxitab*; *Doxiten*; *Doxitin*; *Doxy*; *Doxybene*; *Doxybiocin*; *Doxycap*; *Doxychel*; *Doxyclin(e)*; *Doxycyl*; *Doxylar*; *Dumoxin*; *Idocyklin*; *Periostat*; *Ramysis*; *Vibramycin(e)*; *Vibra-Tabs*; *Vibraveineuse*; *Vibravenos*.

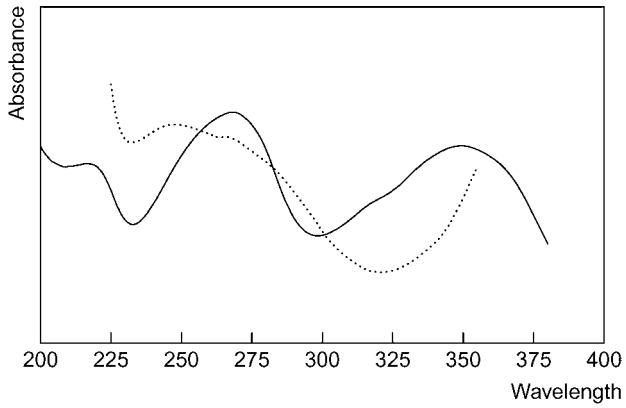
Chemical Properties A yellow crystalline powder. Mp about 200°, with decomposition. Soluble 1 in 3 of water, 1 in 60 of ethanol and 1 in 4 of methanol; practically insoluble in chloroform and ether.

Colour Tests Benedict's reagent—red; formaldehyde–sulfuric acid—yellow; Mandelin's test—green→yellow; Marquis test—yellow; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.12, streaking; system TAE—R_f 0.88 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HY—RI 291.

Ultraviolet Spectrum Aqueous acid—269 (A^{1}_{1}=412b), 346 nm.



Infrared Spectrum Principal peaks at wavenumbers 1580, 1613, 1660, 1244, 1220, 1040 cm^{-1} (doxycycline hydrochloride, KBr disk).

Quantification

Plasma **HPLC** UV detection. Limit of detection, 3 µg/L [Zarghi *et al.* 2001]. Limit of detection, 0.125 mg/L [Axisa *et al.* 2000]. UV detection. Limit of detection, <25 µg/L [Prevosto *et al.* 1995].

Serum **HPLC** UV detection. Limit of detection, 50 µg/L [De Leenheer, Nelis 1979].

Urine **HPLC** UV detection. Limit of detection, <25 µg/L [Prevosto *et al.* 1995]. See Serum [De Leenheer, Nelis 1979].

Tissue **HPLC** See Plasma [Axisa *et al.* 2000].

Disposition in the Body Readily and almost completely absorbed after oral administration; peak plasma concentrations are attained in about 2 h. It does not appear to be significantly metabolised. About 40% of a dose is excreted in the urine unchanged in 72 h (about 24% in the first 24 h).

Half-life Plasma half-life, about 22 h.

Protein Binding About 82 to 90%.

Note For a review of doxycycline, see Cunha *et al.* [1982].

Dose The equivalent of 100 to 200 mg of doxycycline daily; a 1-day course of 300 to 600 mg has also been given.

Axisa B *et al.* (2000). Simple and reliable method of doxycycline determination in human plasma and biological tissues. *J Chromatogr B Biomed Sci Appl* 744: 359–365.

Cunha BA *et al.* (1982). Doxycycline. *Ther Drug Monit* 4: 115–135.

De Leenheer AP, Nelis HJC (1979). Doxycycline determination in human serum and urine by high-performance liquid chromatography. *J Pharm Sci* 68: 999–1002.

Prevosto JM *et al.* (1995). Determination of doxycycline in human plasma and urine samples by high performance liquid chromatography. Application for drug monitoring in malaria chemoprophylaxis. *Ann Biol Clin (Paris)* 53: 29–32.

Zarghi A *et al.* (2001). Rapid high-performance liquid chromatographic method for determination of doxycycline in human plasma. *Boll Chim Farm* 140: 112–114.

Doxylamine

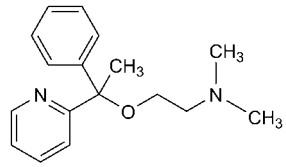
Antihistamine

$C_{17}H_{22}N_2O$ = 270.4

CAS—469-21-6

IUPAC Name *N,N*-Dimethyl-2-(1-phenyl-1-pyridin-2-ylethoxy)ethanamine

Synonyms *N,N*-Dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine; histadoxylamine.



Chemical Properties Liquid. Bp ≈140°. Soluble in acids. pK_a 4.4, 9.2. Log *P* (octanol/water), 2.37 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

D

Doxylamine Succinate

$C_{17}H_{22}N_2O,C_4H_6O_4 = 388.5$
CAS—562-10-7

Synonym Doxylamine hydrogen succinate

Proprietary Names *Decapryn*; *Donormyl*; *Dormidina*; *Dozile*; *Duebien*; *Gittalun*; *Hewedormir forte*; *Hoggar N*; *Lidene*; *Mereprine*; *Munleit*; *Noctyl*; *Restavit*; *Restwel*; *Sanalepsi N*; *Sedaplus*; *Somnil*; *Unisom*. It is an ingredient of many proprietary preparations—see Sweetman [2007].

Chemical Properties White or creamy-white powder. Mp 100° to 104°. Soluble 1 in 1 of water, 1 in 2 of ethanol, 1 in 2 of chloroform and 1 in 370 of ether; slightly soluble in benzene. Log *P* (octanol/water) −1.07 [Meylan, Howard 1995].

Colour Tests Cyanogen bromide—orange-pink; Liebermann's reagent—red-orange; Marquis test—violet; sulfuric acid—pink.

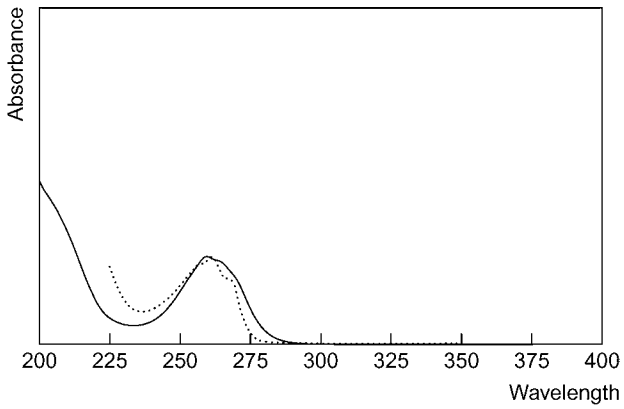
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.41; system TC—R_f 0.10; system TE—R_f 0.60; system TL—R_f 0.09; system TAE—R_f 0.12 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—doxylamine RI 1910, M (carbinol)-H_2O RI 1560, M (OH-)-AC RI 2300, M (OH-carbinol-)-AC RI 2980, M (OH-methoxy-)-AC RI 2320; M (bis-nor-)-AC RI 2280, M (desamino-OH-)-AC RI 1960; M (nor-)-AC RI 2340; system GB—doxylamine RI 1970, M (nor-) RI 1974, M (carbinol)-H_2O RI 1670; system GF—RI 2170.

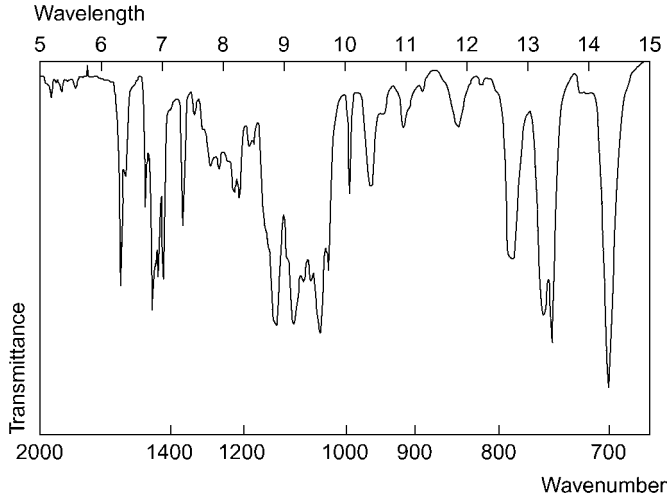
High Performance Liquid Chromatography System HA—*k* 4.4; system HY—RI 259; system HAA—RT 11.1 min.

Column: ODS-2 (120 × 4.6 mm i.d., 5 m). Mobile phase: methanol : water (60 : 40). UV detection (λ = 285 nm). Retention time: 2.1 min. Limit of detection, 35 µg/L [Gil-Agusti *et al.* 2001].

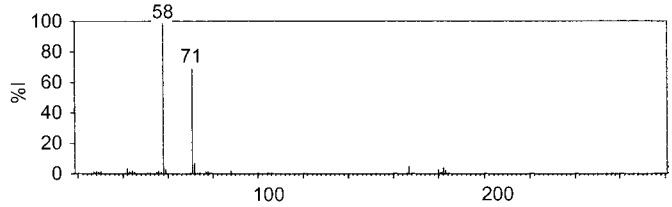
Ultraviolet Spectrum Aqueous acid—261 nm (A_1^1 = 335a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 700, 1590, 1123, 1086, 1041, 751 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 71, 72, 167, 182, 42, 180, 59.



Quantification

Blood **GC** Column: DB-1 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. NPD. Retention time: 14.3 min. Limit of detection, 100-250 ng/L [Hattori *et al.* 1992]. Column: SE-30 Chrom W HP 80/100 mesh (6′ × 0.08" i.d.). Carrier gas: He, 50 psi. Temperature programme: 115° for 3 min to 260° at 16°/min for 12 min. FID. Limit of detection, 1 mg/L [Bayley *et al.* 1975].

GC-MS Column: 2% SE-30 silicone oil on 100/120 mesh Chromosorb (2 m × 0.6 cm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 220° to 270° at 10°/min. EI ionisation at 70 eV or CI. Limit of quantification, 5–10 mg/L [Cailleux *et al.* 1981].

Plasma **GC** Column: 3% SP-2250 Supelcoport 80/100 mesh (3.05 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 230°. NPD. Retention time: 4.2 min. Limit of detection, 1 µg/L [Friedman, Greenblatt 1985].

GC-MS Column: DB-5 (15 m × 0.25 mm i.d., 1.0 µm). Temperature programme: 150° for 1 min to 310° at 20°/min for 0.2 min. Limit of detection, 1.2 mg/L [Siek, Dunn 1993]. Limit of detection 100 ng/mL [Cailleux *et al.* 1981].

HPLC Column: Ultrasphere ODS C_{18} (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : triethylamine : water (pH 5.3; 420 : 4 : 1000), flow rate 1.0 mL/min. UV detection. Limit of detection, 1.2 mg/L [Siek, Dunn 1993]. Column: µPorasil (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: methanol-ammonium hydroxide-ammonium chloride (57 : 2 : 1) : chloroform : acetonitrile (1 : 8 : 1), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 14.6 min. Limit of detection, 5 µg/L [Kohlhof *et al.* 1983].

Urine **GC** See Blood [Hattori *et al.* 1992]. Column: 5% Dexsil 300 on Chromasorb W HP 80/100 mesh (183 cm × 2 mm i.d.). Carrier gas: He, 25 mL/min. Temperature: 260°. NPD. Retention time, 3.2 min. Limit of detection, 100 µg/L [Thompson, Jr. *et al.* 1982].

GC-MS Column: HP cross-linked methylsilicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Tempertature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, scan mode. Limit of detection not reported [Maurer, Pfleger 1988]. Column: 3% OV-1 on Gas Chrom Q 100/120 mesh (1.2 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 150–300° at 0–16°/min. EI ionisation at 70 eV. Limit of detection not reported [Ganes, Midha 1987].

HPLC Column: Ultrasphere cyano (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: diethylamine : 2 mol/L ammonium acetate : water : acetonitrile (0.1 : 4 : 196 : 800). UV detection (λ = 254 nm). Limit of detection not reported [Ganes, Midha 1987].

Peritoneal Fluid **GC** See Blood [Bayley *et al.* 1975].

Kidney **GC** See Blood [Bayley *et al.* 1975].

Liver **GC** See Blood [Bayley *et al.* 1975].

Lung **GC** See Blood [Bayley *et al.* 1975].

Spleen **GC** See Blood [Bayley *et al.* 1975].

Disposition in the Body Absorbed after oral administration with 60 to 80% of a dose recovered in urine within 24 h. *N*-Desmethyldoxylamine, *N,N*-didesmethyldoxylamine and their *N*-acetyl conjugates have been identified in human urine following a single oral dose [Ganes, Midha 1987].

Therapeutic Concentration

Twenty-five healthy female volunteers aged 20 to 35 years were administered a single oral dose of 25 mg doxylamine succinate. Thirteen of the subjects with a mean age of 27.6 years (range, 24–35) had been taking low-dose oestrogen-containing oral contraceptive steroids (50 µg of ethinyl oestradiol) for a minimum of 3 months. Mean maximum plasma concentrations were 102.9 and 100.2 µg/L at 2.4 and 1.9 h, respectively for the control group and the oral contraceptive users [Luna *et al.* 1989].

After a single oral dose of 25 mg in 16 subjects, a peak plasma level of 99 µg/L was obtained after 2.4 h [Friedman, Greenblatt 1985].

Toxicity

A 3-year-old male ingested ~100 Bendectin (10 mg dicyclomine hydrochloride, 10 mg doxylamine succinate and 10 mg pyridoxine hydrochloride). At postmortem the concentrations (mg/L) shown below were found.

	Doxylamine	*Dicyclomine*	*Pyridoxine*
Blood	12	N.D.	N.D.
Peritoneal fluid	18	3	2
Liver	14	1	4
Kidney	22	3	2
Lung	25	1	4
Spleen	20	1	2

N.D. Not determined
[a][Bayley *et al.* 1975].

A 20-year-old male was found dead in his home. Tissue distribution of doxylamine and pyrilamine (mg/L) were as shown below.

In 109 cases of doxylamine mono-intoxication, about 60% of the cases involved ingestion of 250 to 1000 mg (10- to 40-times the single therapeutic dose). For 23 of the cases, about 80% of doxylamine plasma levels were in the range 0.5 to 5.0 mg/L [Köppel *et al.* 1987].

	Doxylamine	*Pyrilaminne*
Blood	0.7	7.0
Urine	17.0	80.0
Bile	3.2	82.8
Gastric contents, total mg	5	147

[a][Wu Chen *et al.* 1983].

In a fatality involving doxylamine overdose, the blood concentration was 1.2 mg/L, with a nordoxylamine concentration of 0.52 mg/L; the concentration of doxylamine plus metabolites in urine was 25 mg/L [Siek, Dunn 1993].

A 24-year-old male took an overdose of 250 doxylamine succinate tablets. The plasma concentration of doxylamine was 13.3 mg/L, equivalent to ≈6250 mg. Despite evidence of massive rhabdomyelosis he was discharged after 16 days [Soto *et al.* 1993].

In 2 cases of suicide by means of doxylamine ingestion, the following tissue distribution was reported at postmortem: blood 140 and 100 mg/L (in case 1 and case 2, respectively), urine 180 mg/L and not reported, stomach contents 500 and 900 mg, liver 80 and 300 µg/g [Bockholdt *et al.* 2001].

Note Doxylamine overdose has been associated with severe rhabdomyelosis [Leybishkis *et al.* 2001].

Half-life Elimination half-life, ≈10 h [Köppel *et al.* 1987].

Clearance 217 mL/min [Köppel *et al.* 1987].

Dose Usually up to 150 mg of doxylamine succinate daily; usual hypnotic dose, 25 mg at night.

Bayley M *et al.* (1975). Fatal overdose from Bendectin. *Clin Pediatr (Phila)* 14: 507–509.

Bockholdt B *et al.* (2001). Suicide through doxylamine poisoning. *Forensic Sci Int* 119: 138–140.

Cailleux A *et al.* (1981). Identification and quantitation of neutral and basic drugs in blood by gas chromatography and mass spectrometry. *J Chromatogr Sci* 19: 163–176.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Friedman H, Greenblatt DJ (1985). The pharmacokinetics of doxylamine: use of automated gas chromatography with nitrogen-phosphorus detection. *J Clin Pharmacol* 25: 448–451.

Ganes DA, Midha KK (1987). Identification in *in vivo* acetylation pathway for *N*-dealkylated metabolites of doxylamine in humans. *Xenobiotica* 17: 993–999.

Gil-Agusti M *et al.* (2001). Quantitation of antihistamines in pharmaceutical preparations by liquid chromatography with a micellar mobile phase of sodium dodecyl sulfate and pentanol. *J AOAC Int* 84: 1687–1694.

Hattori H *et al.* (1992). Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 581: 213–218.

Kohlhof KJ *et al.* (1983). Analysis of doxylamine in plasma by high-performance liquid chromatography. *J Pharm Sci* 72: 961–962.

Köppel C *et al.* (1987). Poisoning with over-the-counter doxylamine preparations: an evaluation of 109 cases. *Hum Toxicol* 6: 355–359.

Leybishkis B *et al.* (2001). Doxylamine overdose as a potential cause of rhabdomyolysis. *Am J Med Sci* 322: 48–49.

Luna BG *et al.* (1989). Doxylamine and diphenhydramine pharmacokinetics in women on low-dose estrogen oral contraceptives. *J Clin Pharmacol* 29: 257–260.

Maurer H, Pfleger K (1988). Screening procedure for the detection of alkanolamine antihistamines and their metabolites in urine using computerized gas chromatography-mass spectrometry. *J Chromatogr* 428: 43–60.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Siek TJ, Dunn WA (1993). Documentation of a doxylamine overdose death: quantitation by standard addition and use of three instrumental techniques. *J Forensic Sci* 38: 713–720.

Soto LF *et al.* (1993). Severe rhabdomyolysis after doxylamine overdose. *Postgrad Med* 93: 227–229.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Thompson HCJr *et al.* (1982). Trace analysis of doxylamine succinate in animal feed, human, urine, and wastewater by GC using a rubidium-sensitized nitrogen detector. *J Chromatogr Sci* 20: 373–380.

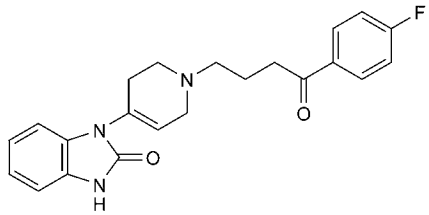
Wu Chen NB *et al.* (1983). The general toxicology unknown, II. A case report: doxylamine and pyrilamine intoxication. *J Forensic Sci* 28: 398–403.

Droperidol

Neuroleptic, Tranquilliser

$C_{22}H_{22}FN_3O_2 = 379.4$

CAS—548-73-2



IUPAC Name 1-[1-[4-(4-Fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-2*H*-benzimidazol-2-one

Proprietary Names *Dehydrobenzperidol*; *Dridol*; *Droleptan*; *Inapsine*; *Paxical*; *Sintodian.* It is an ingredient of *Innovar* and *Thalamonal.*

Chemical Properties White to light tan-coloured, amorphous or microcrystalline powder that gradually darkens on exposure to light. Mp 145° to 146.5°. Practically insoluble in water; soluble 1 in 140 of ethanol, 1 in 4 of chloroform, and 1 in 500 of ether. pK_a 7.5. Log *P* (octanol/water) 3.5. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

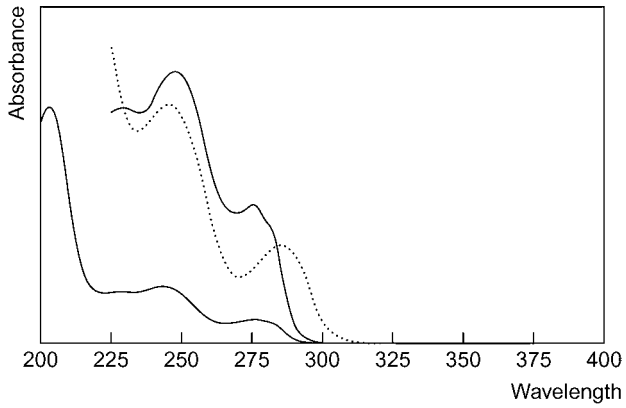
Colour Test Mandelin's test—blue→green.

Thin-layer Chromatography System TA—R_f 0.67; system TAE—R_f 0.71; system TAF—R_f 0.73; system TL—R_f 0.36; system TB—R_f 0.02; system TC—R_f 0.48; system TE—R_f 0.58 (acidified iodoplatinate solution, positive).

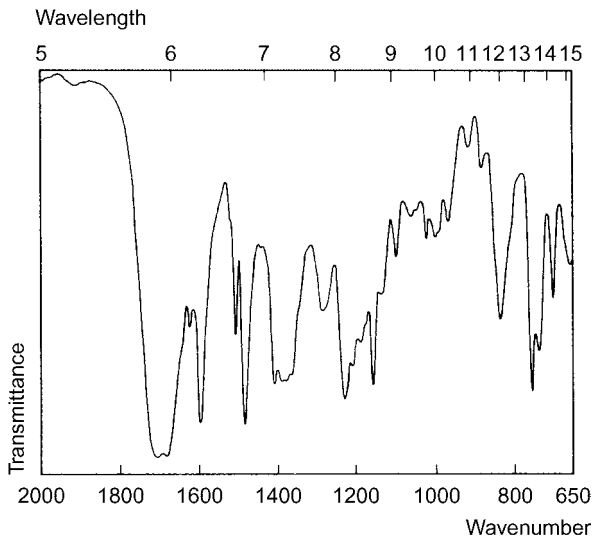
Gas Chromatography System GA—droperidol-AC_2 RI 3430, droperidol-Me-AC_2 RI 3370 , M (benzimidazolone-AC_2) RI 1950, M (benzimidazolone-AC_2) RI 1730; system GB—droperidol RI not eluted.

High Performance Liquid Chromatography System HA—*k* 0.6; system HAA—RT 21.2 min; system HX—RI 385; system HY—RI 323; system HZ—RT 3.5 min.

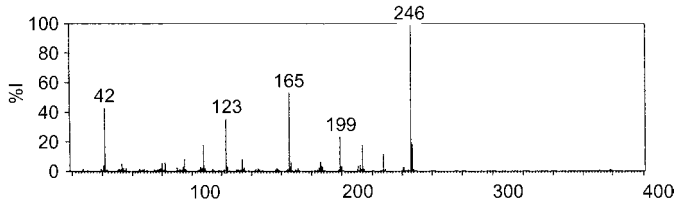
Ultraviolet Spectrum Aqueous acid—248 ($A^1_1 = 443$a), 276 nm; aqueous alkali—246, 288 nm.



Infrared Spectrum Principal peaks at wavenumbers 1685, 1705, 1595, 1230, 752, 1156 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 246, 165, 42, 123, 199, 247, 214, 108.



D

Quantification

Blood **HPLC** Column: Brownlee Spheri (220 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer : acetonitrile (pH 3.75; 40 : 60), flow rate 1.2 mL/min. UV detection (λ = 248 nm). Retention time: 4.4 min. Limit of quantification, 4.0 µg/L [Tan, Boniface 1990].

Plasma **GC** Column: 2% OV3 on HP Chrom W AW DMCS 80/100 mesh or 3% Dexil 300 on HP Chrom W AW DMCS 80/100 mesh. Carrier gas N_2, 35 mL/min. Temperature programme: 230° to 285° at 2°/min or 250° to 290° at 3°/min. Limit of detection, 30 µg/L [Quaglio *et al.* 1982].

HPLC Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% phosphoric acid (240 : 760), flow rate 0.5 mL/min to 1.5 mL/min at 8 min to 1.65 min at 20 min. UV detection (λ = 250 nm). Limit of detection, 1 µg/L [Higashi *et al.* 2006]. Column: Spherisorb Nitrile S5CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer (pH 2.4) : acetonitrile : ethanol (65 : 20 : 15), flow rate 1.4 mL/min. UV detection (λ = 200 nm). Retention time: 4.06 min. Limit of quantification, 2 µg/L, limit of detection, 0.5 µg/L [Kumar *et al.* 1996]. Column: Novapak C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate buffer (pH 6.7; 45 : 55), flow rate 1.0 mL/min. Retention time: 4.8 min. Limit of detection, 20 µg/L [Guichard *et al.* 1993]. Column: 100 RP 18 silica (120 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer (pH 2.9)-ethanol-acetonitile (70 : 22 : 8) : 2-propanol (98.5 : 1.5 for 2.5 min to 94 : 6 at 10 min to 90 : 10 at 10.1 min until 15 min to 98.5 : 1.5 at 15.5 min), flow rate 0.8 mL/min. DAD (λ = 254 and 226 nm). Retention time: ≈3.5 min. Limit of quantification, 450 ng/L [Wilhelm, Kemper 1990].

Disposition in the Body Absorbed after oral administration. Approximately 75% of a dose is excreted in the urine with <10% as unchanged drug; about 22% of a dose is eliminated in the faeces [Cressman *et al.* 1973].

Toxicity

A 29-year-old male died following the administration of a therapeutic dose (5 mg IM) of droperidol. Concentrations of droperidol were 11 µg/L in his heart blood and 40 and 46 µg/L in other blood samples [Tan, Boniface 1990].

Half-life Plasma half-life, ≈2–3 h.

Protein Binding ≈85–90%.

Dose 5 to 20 mg every 4 to 8 h.

Cressman WA *et al.* (1973). Absorption, metabolism and excretion of droperidol by human subjects following intramuscular and intravenous administration. *Anesthesiology* 38: 363–369.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Guichard J *et al.* (1993). Simultaneous high-performance liquid chromatographic assay of droperidol and flunitrazepam in human plasma, Application to haemodilution blood samples collected during clinical anaesthesia. *J Chromatogr* 612: 269–275.

Higashi Y *et al.* (2006). Simultaneous analysis of haloperidol, its three metabolites and two other butyrophenone-type neuroleptics by high performance liquid chromatography with dual ultraviolet detection. *Biomed Chromatogr* 20: 166–172.

Kumar K *et al.* (1996). Determination of droperidol in plasma by liquid chromatography. *J Pharm Biomed Anal* 14: 1529–1533.

Quaglio MP *et al.* (1982). Determination of benperidol, droperidol and pimozide in human plasma by GLC. *Boll Chim Farm* 121: 276–284.

Tan ST, Boniface PJ (1990). High-performance liquid chromatography of droperidol in whole blood. *J Chromatogr* 532: 181–186.

Wilhelm D, Kemper A (1990). High-performance liquid chromatographic procedure for the determination of clozapine, haloperidol, droperidol and several benzodiazepines in plasma. *J Chromatogr* 525: 218–224.

Dropropizine

Cough Suppressant

$C_{13}H_{20}N_2O_2$ = 236.3

CAS—17692-31-8

IUPAC Name 3-(4-Phenyl-1-piperazinyl)-1,2-propanediol

Proprietary Names *Atossion*; *Catabex*; *Domutussina*; *Eritos*; *Ribex*; *Tosofren*; *Tussiflex D*; *Vibral*.

Chemical Properties A white crystalline powder. Mp 108° to 109°. Soluble in chloroform and in dilute acetic acid. Log *P* (octanol/water), 0.1.

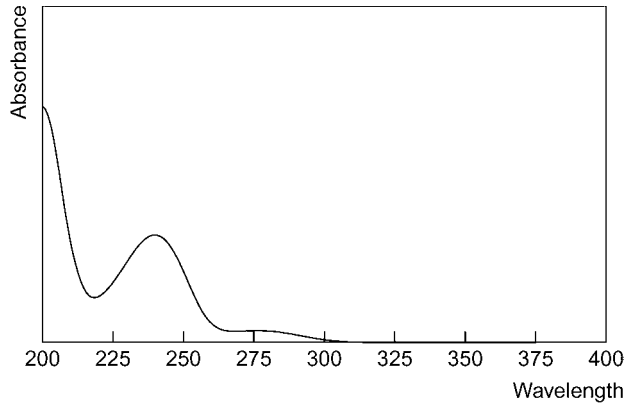
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—orange (slow); nitric acid, cold gives a red colour.

Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.01; system TE—R_f 0.34; system TAE—R_f 0.59 (acidified iodoplatinate solution, strong reaction).

Gas Chromatography System GA—dropropizine RI 2205, dropropizine-AC_2 RI 2430, dropropizine-AC RI 2390.

High Performance Liquid Chromatography System HX—RI 240; system HAA—retention time 7.2 min.

Ultraviolet Spectrum Aqueous acid—238 nm (A^1_1=330a).



Infrared Spectrum Principal peaks at wavenumbers 1595, 1238, 1500, 760, 1060, 925 cm^{-1} (KBr disk).

Dose Dropropizine has been given in doses of 90 to 120 mg daily.

Drospirenone

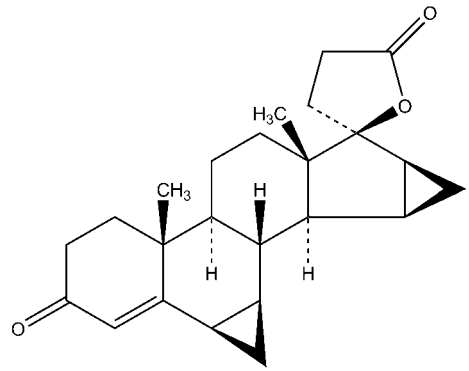
Aldosterone Antagonist, Contraceptive, Progestogen, Steroid Hormone

$C_{24}H_{30}O_3$ = 366.5

CAS—67392-87-4

Synonyms Dihydrospirorenone; 6β,7β,15β,16β-dimethylene-3-oxo-4-androstene-[17(β-1′)-spiro-5′]-perhydrofuran-2′-one; 6β,7β,15β,16β-dimethylen-3-oxo-17α-pregn-4-ene-21,17-carbolactone; (6*R*,7*R*,8*R*,9*S*,10*R*,13*S*,14*S*,15*S*,16*S*,17*S*)-1,3′,4′,6,6a,7,8,9,10,11,12,13,14,15,15a,16-hexadecahydro-10,13-dimethylspiro-[17*H*-dicyclopropa[6,7:15,16]cyclopenta[a]phenanthrene-17,2′(5′*H*)-furan]-3,5′(2*H*)-dione; ZK-30595.

Proprietary Names *Diva-Total*. It is also an ingredient of *Allurene*; *Angeliq*; *Belanette*; *Dahlia*; *Damsel*; *Diva*; *Divina*; *Equifem*; *Femelle*; *Gadofem*; *Isis Fe*; *Isis*; *Jasmine*; *Jasminelle*; *Kala*; *Kirumelle*; *Liofora*; *Maxima*; *Petibelle*; *Yadine*; *Yarine*; *Yasmin*; *Yasminelle*; *YAZ*; *Yira*; *Yirala*.



Chemical Properties Mp 201.3°.

Ultraviolet Spectrum Methanol—265 nm.

Quantification

Plasma **TLC** Plates: Silica gel 60F_{254} (20 x 20, 0.25 µm). Solvent system: chloroform : methanol (96 : 4, run twice) then cut the upper half of the plate and run in toluene : *n*-hexane (50 : 50). Detection: Zeiss scanner (KM 3) in remission mode. R_f values not reported. Limit of quantification not reported [Krause, Jakobs 1982].

HPLC Column: LiChrosorb C_{18} (250 x 4.6 mm i.d., 10 µm). Mobile phase: methanol : water-0.01 mol/L SDS-acetic acid (2 mL/L; 60 : 40), flow rate 2 mL/min. UV detection (λ = 254 nm). Retention time: 13.4 min. Limit of detection, <5 µg/L [Krause, Jakobs 1982].

Disposition in the Body Rapidly absorbed following oral administration with peak plasma concentrations occurring within 1 to 2 h and steady state achieved after ~7 daily administrations of a 3 mg dose. It is extensively metabolised, mostly independently of CYP enzymes although minor metabolism by CYP3A4 occurs. The 2 main plasma metabolites are the acid form of drospirenone and 4,5-dihydrodrospirenone-3-sulfate, neither of which is pharmacologically active. Excretion

of drospirenone is nearly complete after 10 days with trace amounts excreted as unchanged drospirenone in urine and faeces. At least 20 metabolites are observed in urine and faeces.

Therapeutic Concentration

In a study investigating the effects of renal function on the pharmacokinetics of drospirenone, 3 groups of patients were administered 1 tablet of 3 mg drospirenone daily for 14 days. The 3 groups were defined as normal renal function (creatine clearance (Cl_{CR}) >80 mL/min, Group A), mild renal impairment (CL_{CR} >50 to 80 mL/min, Group B), and moderate renal impairment (Cl_{CR} 30 to 50 mL/min, Group C). Peak serum concentrations were reported as follows:

Six healthy young women were administered a single tablet containing 3 mg drospirenone and 30 µg ethinylestradiol prior to a standard breakfast.

Parameter	*Group A*	*Group B*	*Group C*
C_{max} (µg/L)	35.8	39.6	42.4
Time (h)	4.0	2.0	2.0
$t_{1/2}$ (h)	33.6	32.4	42.8
Clearance (mL/min)	91.0	87.3	66.6

[Schurmann *et al.* 2006].

The mean peak drospirenone serum concentration was 30.8 µg/L after 2.5 h and corresponding values in breast milk were 13.5 µg/L after 2.8 h. The half-life of drospirenone in breast milk was reported as 11.7 h [Blode *et al.* 2001].

A total of 13 healthy women were administered a tablet containing 3 mg drospirenone and 30 µg ethinyl estradiol daily for 21 days followed by a 7-day tablet-free interval for 13 cycles. The mean peak serum drospirenone concentration following the first dose was 36.9 µg/L within 1 to 2 h. Mean peak serum concentrations measured after last tablet administration in treatment cycles 1, 6, 9, and 13 were reported as follows:

Parameter	*Cycle 1*	*Cycle 6*	*Cycle 9*	*Cycle 13*
C_{max} (µg/L)	87.5	84.2	81.3	78.7
Time (h)	1.7	1.8	1.6	1.6

[Blode *et al.* 2000].

Bioavailability Approximately 76%.
Half-life Approximately 30 to 40 h.
Volume of Distribution Approximately 4 L/kg.
Clearance Approximately 91 mL/min in healthy subjects.
Protein Binding Approximately 97% (not bound to sex-hormone-binding globulin or corticosteroid-binding globulin).
Note For a review of drospirenone, see Rapkin and Winer [2007].
Dose Used as the progestogenic component of a combined oral contraceptive in doses of 3 mg daily, and as the progestogenic component of menopausal HRT in a dose of 0.5 or 2 mg daily.

Blode H *et al.* (2001). Transfer of drospirenone to breast milk after a single oral administration of 3 mg drospirenone + 30 microg ethinylestradiol to healthy lactating women. *Eur J Contracept Reprod Health Care* 6: 167–171.

Blode H *et al.* (2000). A 1-year pharmacokinetic investigation of a novel oral contraceptive containing drospirenone in healthy female volunteers. *Eur J Contracept Reprod Health Care* 5: 256–264.

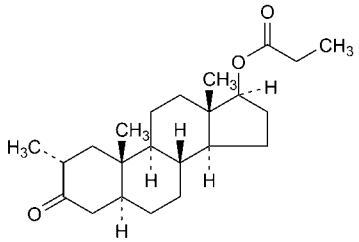
Krause W, Jakobs U (1982). Determination of plasma levels of spirorenone, a new aldosterone antagonist, and one of its metabolites by high-performance liquid chromatography. *J Chromatogr* 230: 37–45.

Rapkin AJ, Winer SA (2007). Drospirenone: a novel progestin. *Expert Opin Pharmacother* 8: 989–999.

Schurmann R *et al.* (2006). Effect of drospirenone on serum potassium and drospirenone pharmacokinetics in women with normal or impaired renal function. *J Clin Pharmacol* 46: 867–875.

Drostanolone Propionate

Anabolic Steroid
$C_{23}H_{36}O_3$ = 360.5
CAS—58-19-5 (drostanolone); 521-12-0 (propionate)
IUPAC Name [(2*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-2,10,13-Trimethyl-3-oxo-1,2,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl] propanoate
Synonyms Dromostanolone propionate; (2α,5α,17β)17-hydroxy-2-methyl-androstan-3-one propionate.
Proprietary Names *Drolban*; *Masterid*; *Masteril*; *Masteron*; *Permastril*.



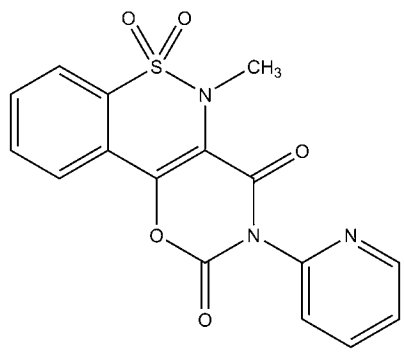
Chemical Properties White to creamy-white crystalline powder. Mp 127° to 133°. Practically insoluble in water; soluble 1 in 30 of ethanol, 1 in 2 of chloroform and 1 in 20 of ether. Log *P* (octanol/water) 4.98 [Meylan, Howard 1995].
Gas Chromatography System GA—drostanolone RI 2555, drostanolone-AC RI 2700, drostanolone-TMS RI 2575, drostanolone-enol-TMS_2 RI 2625, drostanolone propionate RI 2985; system GAI—RRT 0.974 (relative to 17α-methyl-5α-androstan-3β,17β-diol).
Infrared Spectrum Principal peaks at wavenumbers 1715, 1200, 1735, 1035, 1087, 1265 cm^{-1} (KBr disk).
Quantification **GC-MS** Column: DB1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.8 mL/min. Temperature programme: 120° for 0.5 min to 180° at 30°/min to 230° at 4°/min to 233° at 1°/min to 265° at 9°/min for 10 min. MSD. Limit of detection not reported [de Boer *et al.* 1992].
Dose Usually 100 mg thrice weekly, by IM injection.

deBoer D *et al.* (1992). The methyl-5 alpha-dihydrotestosterones mesterolone and drostanolone; gas chromatographic/mass spectrometric characterization of the urinary metabolites. *J Steroid Biochem Mol Biol* 42: 411–419.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Droxicam

Analgesic, Antiinflammatory, Antipyretic, NSAID, Prostaglandin Inhibitor
$C_{16}H_{11}N_3O_5S$ = 357.3
CAS—90101-16-9
IUPAC Name 5-Methyl-3-(2-pyridyl)-2*H*,5*H*-1,3-oxazino[5,6-c]-[1,2]benzothiazine-2,4(3*H*)-dione 6,6-dioxide
Proprietary Names *Dobenam*; *Drogelon*; *Droxar*; *Ferpan*; *Ombolan*; *Pensatron*.



Chemical Properties Droxicam is a prodrug of piroxicam.
Quantification
Plasma **HPLC** Column: LiChrospher 60 RP-Select B (250 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : water : acetic acid (48 : 45 : 7), flow rate 1.1 mL/min. UV detection (λ = 340 nm). Limit of quantification, 20 µg/L for piroxicam [Maya *et al.* 1995a, 1995b]. Column: Lichrosorb DIOL (25 × 0.46 mm i.d., 5 µm). UV detection (λ = 314 nm) Limit of detection not reported [Martinez *et al.* 1988]. Column: µBondapak cyano (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : water : acetic acid (25 : 70 : 5), flow rate 1.2 mL/min. UV detection (λ = 365 nm). Retention time: 4.8 min for piroxicam. Limit of detection, 0.5 mg/L for piroxicam [Twomey *et al.* 1980].
Disposition in the Body Hydrolysis of droxicam to piroxicam appears to take place in the acid conditions of the stomach and gastrointestinal tract. The

bioavailability of droxicam is equivalent to that of piroxicam but mean peak plasma concentrations are reached more slowly with a t_{max} of ~7 h as opposed to 2 to 3 h [Olkkola *et al.* 1994]. Although the biotransformation of droxicam is a pH-dependent process, drugs that increase gastrointestinal pH, such as antacids and ranitidine, do not appear to alter the absorption or pharmacokinetic disposition of droxicam [Bartlett *et al.* 1992a].

Therapeutic Concentration Droxicam at a dose of 20 mg/day has been shown in clinical trials to be efficacious in relieving the symptoms of spinal osteoarthritis [Bohl *et al.* 1990], rheumatoid arthritis [Rodriguez-de-la-Serna *et al.* 1991; Sanchez Andrada, Rodriguez Valverde 1991], and degenerative joint disease [Schuetz *et al.* 1991].

Twenty healthy volunteers were administered droxicam at a dose of 20 mg a day for 20 days. After 20 days, the steady-state plasma concentration was achieved in all volunteers, ranging from 4.21 to 13.19 mg/L [Martinez *et al.* 1988].

Fouteen healthy male volunteers (aged 22 ± 4 years; weight 60.9 ± 8.8 kg) were administered a single dose of 10 mg droxicam. Mean peak plasma concentrations were 0.82 ± 0.15 mg/L at 6.1 ± 3.5 h. Another group of 14 healthy men (aged 28 ± 5 years; weight 68.2 ± 4.3 kg) was administered 10 mg/day for 20 consecutive days. The steady-state mean plasma concentration was 2.06 ± 0.42 mg/L reached at 8.2 ± 6.0 h [Martinez *et al.* 1989].

Eight healthy male volunteers were administered a single 20 mg dose of droxicam to investigate the effect of gastric emptying on droxicam pharmacokinetics. The absorption rate of droxicam was modified but the bioavailability was unchanged in conditions of altered gastric emptying [Sanchez *et al.* 1989].

When 10 elderly healthy volunteers were administered a single dose of 20 mg droxicam, the absorption, distribution and excretion did not differ significantly from young healthy volunteers [Hosie *et al.* 1991].

Twenty-five healthy volunteers were administered single (20 mg) or multiple (20 mg/day for 30 days) doses of droxicam. After a single dose, the mean peak concentration of piroxicam was 1.41 ± 0.25 mg/L at 15 ± 7 h. After multiple doses the mean peak plasma concentration at steady state was 6.23 ± 2.36 mg/L [Bartlett *et al.* 1992b].

Twelve healthy male volunteers were administered 20 mg droxicam alone or in combination with the antacid Mucal powder. Mean maximum plasma concentrations of 2.08 and 1.85 µg/L were reached at 7.08 and 8.17 h, respectively [Maya *et al.* 1995b].

Note For a review of the pharmacokinetic profile of droxicam, see Martinez and Sanchez [1991]; for a review of the adverse events seen with droxicam in the early clinical trials, see Sanchez *et al.* [1991].

Toxicity Droxicam was withdrawn from the market in December 1994 because of adverse effects on the liver [Lapeyre-Mestre *et al.* 2006]. The ulcerogenic dose of droxicam in 50% of the population is 57 mg/kg [Jane, Rodriguez de la Serna 1991].

Bioavailability As for piroxicam

Half-life Approximately 66 h following a single dose, 42 h after multiple doses.

Protein Binding Extensively (above 96%) plasma protein bound.

Bartlett A *et al.* (1992). The effect of antacid and ranitidine on droxicam pharmacokinetics. *J Clin Pharmacol* 32: 1115–1119.

Bartlett A *et al.* (1992). Cross-over study of the bioavailability of a new NSAID (droxicam) versus piroxicam in healthy volunteers following single and multiple dose administration. *Eur J Drug Metab Pharmacokinet* 17: 195–199.

Bohl D *et al.* (1990). A clinical trial comparing a new NSAID (droxicam) and piroxicam in spinal osteoarthritis. *Int J Clin Pharmacol Ther Toxicol* 28: 416–419.

Hosie J *et al.* (1991). A pharmacokinetic study to evaluate the profile of droxicam in elderly healthy volunteers after a single oral dose of 20 mg. *Eur J Rheumatol Inflamm* 11: 45–49.

Jane F, Rodriguez de la Serna A (1991). Droxicam: a pharmacological and clinical review of a new NSAID. *Eur J Rheumatol Inflamm* 11: 3–9.

Lapeyre-Mestre M *et al.* (2006). Non-steroidal anti-inflammatory drug-related hepatic damage in France and Spain: analysis from national spontaneous reporting systems. *Fundam Clin Pharmacol* 20: 391–395.

Martinez L, Sanchez J (1991). Pharmacokinetic profile of droxicam. *Eur J Rheumatol Inflamm* 11: 10–14.

Martinez L *et al.* (1988). Comparative study of the multiple dose pharmacokinetics and the tolerance of a new NSAID (droxicam) versus piroxicam in healthy volunteers. *Methods FInd Exp Clin Pharmacol* 10: 729–737.

Martinez L *et al.* (1989). Single and multiple dose pharmacokinetics of a new NSAID (droxicam) in healthy volunteers. *Eur J Drug Metab Pharmacokinet* 14: 303–307.

Maya MT *et al.* (1995a). A rapid method for the determination of piroxicam in plasma by high-performance liquid chromatography. *J Pharm Biomed Anal* 13: 319–322.

Maya MT *et al.* (1995b). A comparative bioavailability study to estimate the influence of an antacid on droxicam pharmacokinetics. *Eur J Drug Metab Pharmacokinet* 20: 275–279.

Olkkola KT *et al.* (1994). Pharmacokinetics of oxicam nonsteroidal anti-inflammatory agents. *Clin Pharmacokinet* 26: 107–120.

Rodriguez de la Serna A *et al.* (1991). Comparative double-blind study of droxicam (new NSAID) versus indomethacin in rheumatoid arthritis. *Eur J Rheumatol Inflamm* 11: 35–44.

Sanchez Andrada S, Rodriguez Valverde V (1991). A double-blind randomised controlled trial of droxicam versus indomethacin in rheumatoid arthritis. *Eur J Rheumatol Inflamm* 11: 15–20.

Sanchez J *et al.* (1989). The influence of gastric emptying on droxicam pharmacokinetics. *J Clin Pharmacol* 29: 739–745.

Sanchez J *et al.* (1991). Adverse events with droxicam in the early clinical trials. *Eur J Rheumatol Inflamm* 11: 50–58.

Schuetz E *et al.* (1991). Therapeutic activity, clinical and gastric tolerance of 20 mg daily dose of droxicam in comparison with piroxicam in patients with degenerative joint disease. *Eur J Rheumatol Inflamm* 11: 21–28.

Twomey TM *et al.* (1980). Analysis of piroxicam in plasma by high-performance liquid chromatography. *J Chromatogr* 183: 104–108.

Droxidopa

Dopaminergic Agent

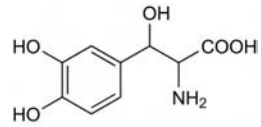
$C_9H_{11}NO_5$ = 213.2

CAS—23651-95-8

IUPAC Name 2-Amino-3-(3,4-dihydroxyphenyl)-3-hydroxypropanoic acid

Synonyms L-*threo*-3-(3,4-Dihydroxyphenyl)serine; *threo*-β,3-dihydroxy-L-tyrosine; L-DOPS; L-*threo*-DOPS; threo-dopaserine.

Proprietary Name *Dops*



Chemical Properties Crystals from ethanol and ether. Mp 232° to 235°, with decomposition.

High Performance Liquid Chromatography Column: C_{18} Cp MicroSpher (3 µm, 100 × 4.6 mm), at 35°. Mobile phase: 0.025 mol/L disodium hydrogen phosphate, 0.347 mmol/L sodium dodecyl sulfate, 0.269 mmol/L EDTA : methanol (90:10), (pH 2.1). ECD. Retention time: D,L-*threo*-DOPS, 4.1 min.

Quantification

Plasma **HPLC** Column: C_{18} Cp MicroSpher (3 µm, 100 × 4.6 mm). Mobile phase: (A) 0.05 mol/L sodium phosphate (pH 6.5); (B) 20 mL tetrahydrofuran : 10 mL methanol : 1 L sodium phosphate. Elution programme: 0 to 100% B in 0 to 1 min, followed by isocratic elution with B, flow rate 1 mL/min. Fluorescence detection (λ_{ex}=344 nm, λ_{em}=442 nm). Retention time: D-*threo*-DOPS derivative (*o*-phthaldialdehyde and *N*-acetyl-L-cysteine), 7.14 min; L-*threo*-DOPS derivative, 7.90 min. Limit of detection, 0.01 mg/L (liquid–liquid extraction method), 0.02 mg/L (deproteinisation method) [Boomsma *et al.* 1988]. Column: ODS-T Yanapak (250 × 4 mm i.d.), 7C_{18} Nucleosil or 7ODS-H Chemcosorb. Mobile phase: potassium phosphate (0.1 M, pH 3.10), flow rate 0.8 mL/min. ECD. Retention time: 3.5 min. Limit of detection, 0.15 mg/L [Suzuki *et al.* 1982].

Urine **HPLC** See Plasma [Suzuki *et al.* 1982].

Disposition in the Body Absorption of droxidopa is relatively slow after oral administration and it is converted to (–)-noradrenaline [(–)-norepinephrine] by aromatic L-amino acid decarboxylase, which is distributed in various tissues including the peripheral sympathetic nerves. Maximum plasma concentration is reached after 3 h. It is excreted in urine unchanged (20% of an oral dose is recovered within 12 h of administration) and as noradrenaline (norepinephrine), with ~1.5% of a dose being recovered over 24 h.

Therapeutic Concentration

Five healthy males and 2 women, aged between 25 and 41 years, were administered a single 300 mg dose of droxidopa after overnight fasting. Peak plasma concentrations of droxidopa were reached within 3 h after administration at 1.50 ± 0.31 mg/L. A considerable amount remained in the blood 12 h after administration [Suzuki *et al.* 1982].

Boomsma F *et al.* (1988). Determination of D,L-threo-3,4-dihydroxyphenylserine and of the D- and L-enantiomers in human plasma and urine. *J Chromatogr* 427: 219–227.

Suzuki T *et al.* (1982). Pharmacokinetic studies of oral L-threo-3,4-dihydroxyphenylserine in normal subjects and patients with familial amyloid polyneuropathy. *Eur J Clin Pharmacol* 23: 463–468.

Duloxetine

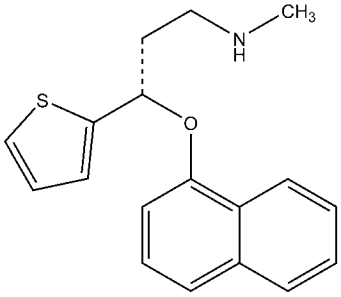
Amine, Antidepressant, SSNRI, Treatment of Urinary Incontinence

$C_{18}H_{19}NOS$ = 297.4

CAS—116539-59-4

IUPAC Name (3*S*)-*N*-Methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine

Synonyms LY-248686; (+)-*N*-methyl-3-(1-naphthalenyloxy)-3-(2-thienyl)propanamine; (γ*S*)-*N*-methyl-γ-(1-naphthalenyloxy)-2-thiophenepropanamine; (+)-(*S*)-*N*-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine.

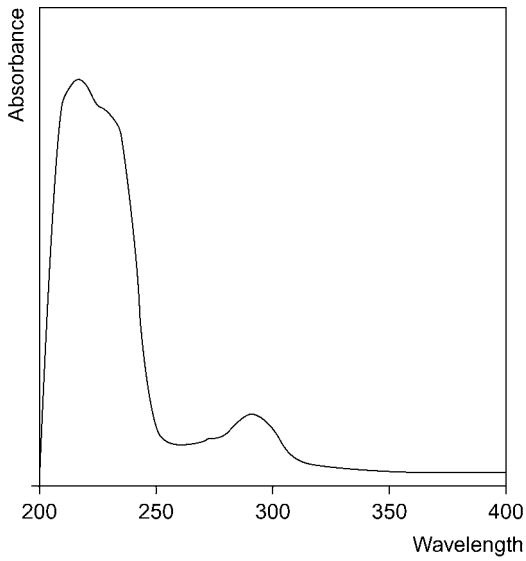


Chemical Properties pK_a 10.02. Log *P* (octanol/water), 4.23 [Berezhkovskiy 2006]. Stock solutions stable for up to 1 month and stable in plasma for up to 20 days at –20° [Ma *et al.* 2007].

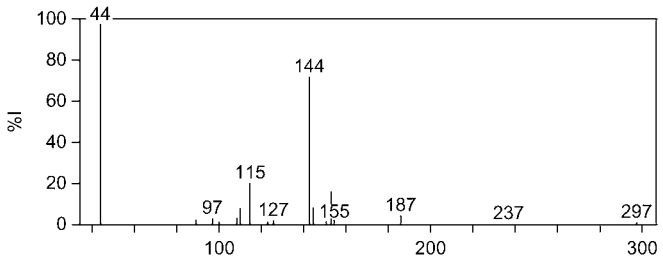
Duloxetine Hydrochloride

$C_{18}H_{19}NOS,HCl = 333.9$
CAS—136434-34-9
Proprietary Names *Ariclaim*; *Cymbalta*; *Duxetin*; *Xeristar*; *Yentreve*.
Chemical Properties White solid. pK_a in dimethylformide : water (66 : 34) 9.6 [O'Neil *et al.* 2006].

High Performance Liquid Chromatography Column: Zorbax SB-CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-25 mmol/L potassium dihydrogen phosphate (pH 3.0, 20 : 80) : acetonitrile-25 mmol/L potassium dihydrogen phosphate (pH 3.0, 75 : 25; 100 : 0 to 70 : 30 over 15 min to 0 : 100 over 12 min), flow rate 1.0 mL/min. Retention time: ~21 min. Limit of quantification not reported [Jansen *et al.* 1998].
Ultraviolet Spectrum Principal peak at 210 nm.



Mass Spectrum Principal ions at *m/z* 44, 144, 115, 154 [Anderson *et al.* 2006].



Quantification

Blood **GC-MS** Column: HP-5 (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 140° to 300° at 10°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.05 mg/L, limit of detection, 0.03 mg/L [Anderson *et al.* 2006].
Plasma **HPLC** Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer–0.3% TEA (pH 3.0; 40 : 60), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: ~5 min. Limit of quantification, 2.0 µg/L, limit of detection, 0.7 µg/L [Mercolini *et al.* 2007]. Column: LiChrospher 60 RP-select B (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile-methanol (92 : 8) : 0.25 mol/L sodium acetate buffer (pH 4.5; 35 : 65), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 16.0 min. Limit of quantification, 5 µg/L [Malfara *et al.* 2007].
LC-MS Column: C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 3.5; 45 : 55), flow rate 0.3 mL/min. API, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 µg/L [Senthamil Selvan *et al.* 2007]. Column: Hypersil-Hypurity C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 20 mmol/L ammonium acetate (pH 3.5; 42 : 20 : 38), flow rate 0.24 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: ~2.0 min. Limit of quantification, 0.8 µg/L [Ma *et al.* 2007].
Urine **GC-MS** See Blood [Anderson *et al.* 2006].
Bile **GC-MS** See Blood [Anderson *et al.* 2006].
Gastric Contents **GC-MS** See Blood [Anderson *et al.* 2006].
Vitreous Humour **GC-MS** See Blood [Anderson *et al.* 2006].
Liver **GC-MS** See Blood [Anderson *et al.* 2006].
Disposition in the Body Rapidly absorbed following oral administration. There is an approximate 2 h lag until absorption begins, with peak plasma concentrations reached 6 h post-administration and steady-state levels reached after 3 days. When given with food, the absorption rate decreases by about 10%. There is a one-third reduction in duloxetine bioavailability in smokers compared with non-smokers owing to an effect on CYP1A4. Duloxetine is metabolised in the liver by CYP2D6 and CYP1A2 to form multiple oxidative and conjugated inactive metabolites. Metabolites found in plasma include 4-hydroxyduloxetine glucuronide and 5-hydroxy-6-methoxyduloxetine sulfate. These are largely excreted in the urine (72%) and to a lesser extent in faeces (18.5%). Unchanged duloxetine accounts for only ~3% of the circulating plasma concentration. Duloxetine and its metabolites cross the placental barrier and are distributed into breast milk. It is also worth noting that both CYP2D6 and CYP1A2 show racial differences such that 1% of Asians (Chinese, Japanese, Korean), 5–10% of Caucasians and 0–18% of Africans do not have the CYP2D6 enzyme. Chinese women and Africans have lower CYP1A2 activity than Caucasians. This may impact on duloxetine pharmacokinetics.
Therapeutic Concentration Reported as 20–80 µg/L [Muller *et al.* 2004] and also as 21–114 µg/L [Anderson *et al.* 2006].

Seven healthy subjects and 6 patients with moderate liver cirrhosis were administered a 20 mg dose of duloxetine hydrochloride as an enteric-coated capsule following an overnight fast. All participants were Caucasians and were genotyped as CYP2D6 extensive metabolisers. Mean peak plasma concentrations (C_{max}) and time to C_{max} for duloxetine, its 4-hydroxyduloxetine glucuonide conjugate (M1), and 5-hydroxy-6-methoxyduloxetine sulfate conjugate (M2) were reported as follows:

Significant differences in duloxetine values for plasma clearance, volume of distribution, and half-life were noted between the 2 groups, with values for cirrhotic patients at 24.1 L/h, 1704 L, and 48 h, compared with 160 L/h, 2909 L, and 13.5 h, respectively, in healthy subjects [Suri *et al.* 2005].

Twelve healthy non-smoking, non-drinking Chinese volunteers were administered 22.4 (low), 44.8 (middle), and 67.2 mg (high) oral dosages of duloxetine hydrochloride in 3 studies with a washout period of 14 days between studies. Mean peak plasma concentrations for the 3 groups were reported as follows:

Parameter	*Low dose*	*Middle dose*	*High dose*
C_{max} (µg/L)	22.7	42.3	69.6
Time (h)	6.1	6.4	6.3
Clearance (L/h)	58.3	67.8	55.7

[Ma *et al.* 2007].

The potential pharmacokinetic differences of duloxetine in Chinese, Caucasian and Japanese subjects were investigated in a multicentre study. All participants were administered a single oral dose of 60 mg duloxetine

Parameter	*Duloxetine*		*M1*		*M2*	
	Cirrhotic patients	*Healthy subjects*	*Cirrhotic patients*	*Healthy subjects*	*Cirrhotic patients*	*Healthy subjects*
C_{max} (µg/L)	14.6	13.8	35.0	118.0	19.6	96.7
Time (h)	6	3.5	7	5	6	4

Parameter	*Chinese subjects*	*Caucasian subjects*	*Japanese subjects*
C_{max} (μg/L)	36.3	39.8	46.0
Time (h)	6.0	6.0	6.0
Clearance (L/h)	102	104	80.7
Volume of distribution (L)	1728	1617	1495
Half-life (h)	10.3	9.78	11.1

hydrochloride and the following pharmacokinetic parameters were reported:

The difference in the C_{max} values obtained in Japanese subjects was attributed to a 15% lower mean bodyweight in these subjects [Tianmei *et al.* 2007].

Toxicity Duloxetine is both a substrate for, and a potent inhibitor of, CYP2D6. Caution needs to be exercised when duloxetine is co-administered with other potent inhibitors of this enzyme (paroxetine, fluoxetine) as significantly high concentrations of duloxetine may result.

In a review of deaths where duloxetine was detected alongside other medication, duloxetine postmortem concentrations in 4 such cases were reported as follows:

Further cases and full case histories are reported in Anderson *et al.* [2006]. The above represent the first postmortem cases to be reported but more data are required to establish clear postmortem therapeutic/toxic levels [Anderson *et al.* 2006].

Bioavailability Approximately 50% (range 30–80%).

Half-life Approximately 13.5 h in healthy subjects; up to 48 h in cirrhotic patients.

Volume of Distribution Approximately 1943 L (range 803–3065 L).

Clearance Approximately 114 L/h (range 44–214 L/h).

Protein Binding 96%, primarily to albumin and α_1-acid glycoprotein.

Dose Given by mouth as the hydrochloride although doses are expressed in terms of the base; duloxetine hydrochloride 22.5 mg is equivalent to ≈20 mg duloxetine. Used in the treatment of depression in usual doses of 20 or 30 mg twice daily, or 60 mg once daily. In the treatment of diabetic peripheral neuropathic pain, the usual dosage is 60 mg once daily. It is also used in the treatment of moderate to severe stress urinary incontinence in women. Usual initial dosage is 40 mg twice daily; however, some patients may benefit from an initial dosage of 20 mg twice daily for 2 weeks before increasing to 40 mg twice daily.

Anderson D *et al.* (2006). A first look at duloxetine (Cymbalta) in a postmortem laboratory. *J Anal Toxicol* 30: 576–580.

Berezhkovskiy LM (2006). Determination of drug binding to plasma proteins using competitive equilibrium binding to dextran-coated charcoal. *J Pharmacokinet Pharmacodyn* 33: 595–608.

Jansen PJ *et al.* (1998). Characterization of impurities formed by interaction of duloxetine HCl with enteric polymers hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate. *J Pharm Sci* 87: 81–85.

Ma N *et al.* (2007). Determination of duloxetine in human plasma via LC/MS and subsequent application to a pharmacokinetic study in healthy Chinese volunteers. *Clin Chim Acta* 380: 100–105.

Malfara WR *et al.* (2007). Reliable HPLC method for therapeutic drug monitoring of frequently prescribed tricyclic and nontricyclic antidepressants. *J Pharm Biomed Anal* 44: 955–962.

Mercolini L *et al.* (2007). HPLC analysis of the novel antidepressant duloxetine in human plasma after an original solid-phase extraction procedure. *J Chromatogr B Analyt Technol Biomed Life Sci* 856: 81–87.

Muller WE *et al.* (2004). Selektive serotonin- und noradrenalin-wiederaufnahmehemmer (SSNRI). Antidepressiva mit dualem wirkungsmechanismus. *Psychopharmakotherapie* 11: 71–75.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Senthamil Selvan P *et al.* (2007). Determination of duloxetine in human plasma by liquid chromatography with atmospheric pressure ionization–tandem mass spectrometry and its application to pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 858: 269–275.

Suri A *et al.* (2005). Duloxetine pharmacokinetics in cirrhotics compared with healthy subjects. *Int J Clin Pharmacol Ther* 43: 78–84.

Tianmei S *et al.* (2007). Pharmacokinetics and tolerability of duloxetine following oral administration to healthy Chinese subjects. *Clin Pharmacokinet* 46: 767–775.

Dutasteride

5α-Reductase Inhibitor, Antiandrogen, Treatment of BPH

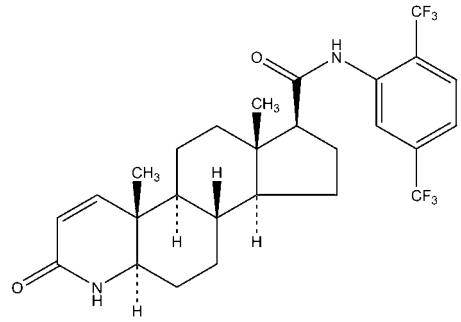
$C_{27}H_{30}F_6N_2O_2$ = 528.5

CAS—164656-23-9

IUPAC Name (1*S*,3a*S*,3b*S*,5a*R*,9a*R*,11a*S*)-*N*-[2,5-bis(Trifluoromethyl)phenyl]-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f] quinoline-1-carboxamide

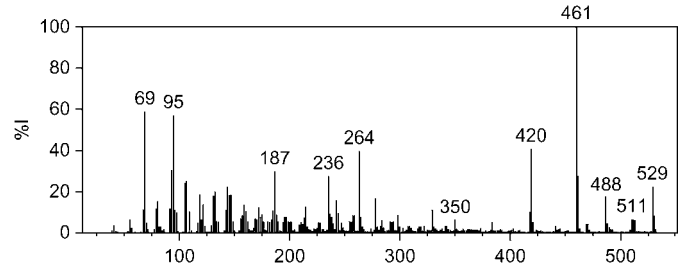
Synonyms GG-745; GI-198745; 17β-*N*-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-4-aza-5α-androst-1-en-3-one; (4a*R*,4b*S*,6a*S*,7*S*,9a*S*,9b*S*,11a*R*)-*N*-[2,5-bis(trifluoromethyl)phenyl)]-2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11,11a-tetradecahydro-4a,6a-dimethyl-2-oxo-1*H*-indeno[5,4f]quinoline-7-carboxamide.

Proprietary Names *Avodart*; *Avolve*; *Duagen*.



Chemical Properties A white to pale yellow powder. Mp 242° to 250°. Insoluble in water. Soluble in ethanol (44 g/L), methanol (64 g/L), and polyethylene glycol 400 (3 g/L). Log *P* (octanol/water), 7.17 [Wishart 2006].

Mass Spectrum Principal ions at *m/z* 461, 69, 95, 264, 420, 187, 236.



Quantification

Plasma **LC-MS** Column: Waters Xterra MS C_{18} (50 × 3 mm i.d.). Mobile phase: 10 mmol/L ammonium formate : acetonitrile (pH 3.0; 15 : 85), flow rate 0.6 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 100 ng/L [Ramakrishna *et al.* 2004].

Note For a study of the mass spectral fragmentation reactions of dutasteride and related compounds, see Burinsky *et al.* [2001].

Disposition in the Body Dutasteride is extensively metabolised to 4′-hydroxydutasteride and 6-hydroxydutasteride, primarily by CYP3A4. The 1,2-dihydroxy-, 6,4′-dihydroxy- and 15-hydroxy-metabolites have also been detected in serum. It is mainly excreted in faeces as the unchanged drug (5%) and as the metabolites (40%). Only trace amounts are found in urine. Therefore, approx. 55% of the drug is unaccounted for. Approximately 11.5% of the serum concentration can partition into semen. After oral administration of a 0.5 mg dose, the time to reach a peak serum concentration is 2 to 3 h. When the drug is administered with food, this concentration decreases by 10 to 15%. The drug can be detected in serum up to 4 to 6 months after completion of treatment.

Case No.	*Central blood (mg/L)*	*Femoral blood (mg/L)*	*Vitreous humour (mg/L)*	*Liver (mg/kg)*	*Gastric Contents (mg total)*	*Bile (mg/L)*	*Urine (mg/L)*	*Cause of death*
4	0.30	0.19	0.11	2.2	0.67	–	0.47	Multiple drug intoxication
7	0.22	–	0.06	1.3	0.08	1.3	0.17	Morphine intoxication
9	0.17	0.10	–	1.2	0.39	1.1	0.07	Multiple drug intoxication
12	0.23	0.20	0.09	4.4	–	0.69	0.08	Methadone intoxication

[Anderson *et al.* 2006].

Therapeutic Concentration

Forty-eight healthy males aged between 20 and 57 years (median 37 years) and weighing between 56.3 and 102 kg received single doses (0.01 to 40 mg) of dutasteride or placebo. At high doses, there was a high volume of distribution (511 L) and a low linear clearance (0.58 L/h), which combined to result in a half-life of up to 5 weeks. The value of the peak plasma concentration increased proportionally with dose (levels not reported) and doses ranging from 0.1 to 10 mg/day were clinically effective [Gisleskog *et al.* 1999].

Toxicity Plasma concentrations may increase in the presence of CYP3A inhibitors such as verapamil, cimetidine, diltiazem, or ketoconozole. Dutasteride does not appear to interact with warfarin, digoxin, colestyramine, tamsulosin, or tarazosin.
Note For a review of dutasteride, see Brown and Nuttall [2003].
Bioavailibility Approximately 60%.
Half-life Steady state, 5 weeks.
Volume of Distribution 511 L.
Clearance 0.58 L/h.
Protein Binding 99%, plasma albumin and 96.6%, α_1-acid glycoprotein.
Dose 0.5 mg daily.

Brown CT, Nuttall MC (2003). Dutasteride: a new 5-alpha reductase inhibitor for men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. *Int J Clin Pract* 57: 705–709.

Burinsky DJ *et al.* (2001). Mass spectral fragmentation reactions of a therapeutic 4-azasteroid and related compounds. *J Am Soc Mass Spectrom* 12: 385–398.

Gisleskog PO *et al.* (1999). The pharmacokinetic modelling of GI198745 (dutasteride), a compound with parallel linear and nonlinear elimination. *Br J Clin Pharmacol* 47: 53–58.

Ramakrishna NV *et al.* (2004). Selective and rapid liquid chromatography–tandem mass spectrometry assay of dutasteride in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 809: 117–124.

Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Duteplase

Thrombolytic
$C_{2736}H_{4174}N_{914}O_{824}S_{46}$ = 64529.1
CAS—120608-46-0

Synonyms Extrinsic plasminogen activator; fibrinokinase; 245-L-methionine plasminogen activator (human tissue type 2-chain form protein moiety); SM-9527; t-PA; TPA.
Proprietary Names *Actilyse*; *Actiplas*; *Prolysis*; *Solcot*; *Tiplagen*.
Chemical Properties Practically insoluble in solutions of low ionic strength at neutral pH.
Disposition in the Body
Therapeutic Concentration

Twenty-four patients with suspected acute myocardial infarction were divided into two dosing groups. Group 1: 9 patients, mean age 61 years, were administered a mean dose of 115.7 mg duteplase as an IV infusion at a rate of 33.3 mL/min for a mean of 81.3 min. Heparin was also administered intravenously as a bolus within 30 min of the start of the duteplase dose and continued for 48 h after the end of duteplase treatment. Group 2: 15 patients, mean age 53 years, were administered a lytic dose of 1.2 mg/kg (dose adjusted to nearest 5 kg body weight) duteplase over 60 min at an infusion rate of 30 mL/h, followed by a 0.12 mg/kg bolus over 1 min and a maintenance infusion of 0.69 mg/kg over 3 h at 5.6 mL/h. Heparin was also administered before duteplase treatment and for 3 h after. The mean total duteplase dose was 153.4 mg. The steady state plasma antigen concentration was 2335 µg/L for group 1 and 2319 µg/L after the lytic dose for group 2 and 434 µg/L after the maintenance dose. For the individuals in group 1, the mean time to reach 75% of the steady state concentration was 18 min and 27 min to reach 90%. For group 2, 75% of the steady state concentration was reached in 14 min and 90% in 22 min [Koster *et al.* 1991].

Toxicity High doses of duteplase are associated with an increased risk of serious bleeding.

A 47-year-old woman with acute myocardial infarction was treated with 162.5 mg oral aspirin, 0.4 million units/kg bolus duteplase, then 0.36 million units/kg over the first hour, followed by 0.7 million units/kg/h for 3 h and 12 500 units of subcutaneous heparin. This was the thrombolytic protocol. 9 h after duteplase was administered, the woman became hypotensive and tachycardic. She also developed a splenic haemorrhage and rupture resulting in removal of the spleen [Blankenship, Indeck 1991].

Half-life Mean, 8 min.
Volume of Distribution Mean, 8.5 L.
Clearance Mean, 685 mL/min.

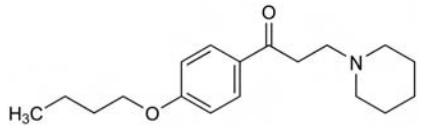
Blankenship J, Indeck M (1991). Splenic hemorrhage after tissue plasminogen activator for acute myocardial infarction. *N Engl J Med* 325: 969.

Koster RW *et al.* (1991). The pharmacokinetics of recombinant double-chain t-PA (duteplase): effects of bolus injection, infusions, and administration by weight in patients with myocardial infarction. *Clin Pharmacol Ther* 50(3): 267–277.

Dyclonine

Anaesthetic (Local)
$C_{18}H_{27}NO_2$ = 289.4
CAS—586-60-7

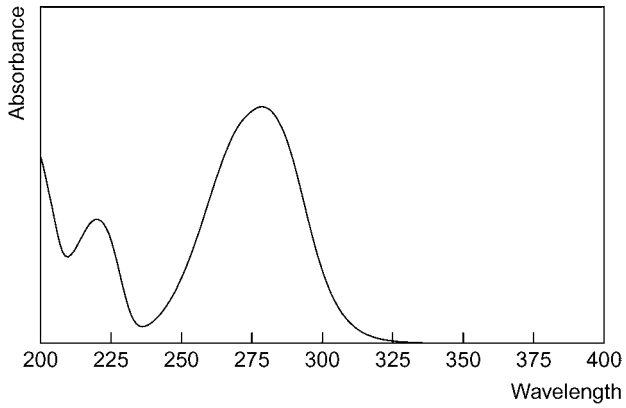
IUPAC Name 1-(4-Butoxyphenyl)-3-(1-piperidinyl)-1-propanone



Dyclonine Hydrochloride

$C_{18}H_{27}NO_2$,HCl = 325.9
CAS—536-43-6
Synonym Dyclonine chloride
Proprietary Names *Dyclone*; *Sucrets*.
Chemical Properties White crystals or white crystalline powder. Mp 175° to 176°. Soluble 1 in 60 of water, 1 in 24 of ethanol and 1 in 2.3 of chloroform; soluble in acetone; practically insoluble in ether.

Colour Tests Aromaticity (method 2)—yellow-orange; Liebermann's reagent (100°)—orange.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.49; system TC—R_f 0.40; system TL—R_f 0.25 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1678.
High Performance Liquid Chromatography System HR—*k* 2.78; system HY—RI 347.
Ultraviolet Spectrum Aqueous acid—282 nm (A^1_1=580b).

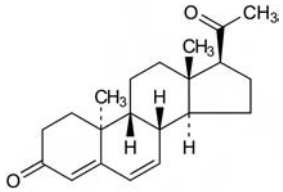


Infrared Spectrum Principal peaks at wavenumbers 1178, 1605, 1229, 1672, 1575, 965 cm^{-1} (dyclonine hydrochloride, KBr disk).
Use Dyclonine hydrochloride is applied as a 0.5 or 1% solution.

Dydrogesterone

Progestational Steroid
$C_{21}H_{28}O_2$ = 312.5
CAS—152-62-5

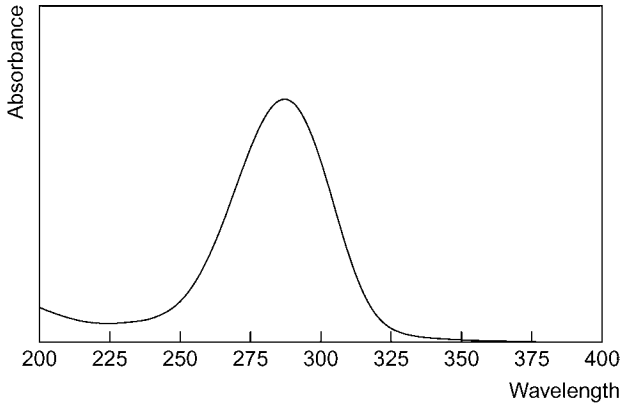
IUPAC Name (8*S*,9*R*,10*S*,13*S*,14*S*,17*S*)-17-Acetyl-10,13-dimethyl-1,2,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthren-3-one
Synonyms Dehydroprogesterone; didrogesteron; isopregnenone; (9β,10α)-pregna-4,6-diene-3,20-dione.
Proprietary Names *Biphaston*; *Dufaston*; *Duphaston*; *Gynorest*. It is an ingredient of *Femapak* and *Femoston*.



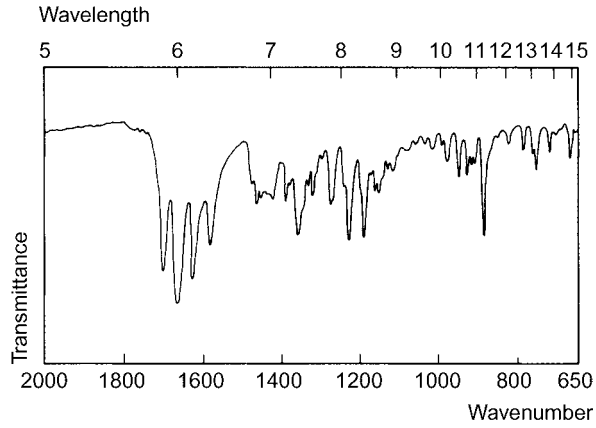
Chemical Properties A white to pale yellow crystalline powder. Mp 167° to 171°. Practically insoluble in water; soluble 1 in 40 to 1 in 52 of ethanol, 1 in 17 of acetone, 1 in 2 of chloroform and 1 in 140 of ether. Log *P* (octanol/water), 3.4.
Colour Tests Antimony pentachloride—orange; naphthol–sulfuric acid—orange-red; sulfuric acid—orange (green-yellow fluorescence under ultraviolet light).

Thin-layer Chromatography System TP—R_f 0.86; system TQ—R_f 0.53; system TR—R_f 0.96; system TS—R_f 0.98 (*p*-toluenesulfonic acid solution, positive).

Ultraviolet Spectrum Dehydrated alcohol—286 nm (A_1^1=838a).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1622, 1697, 1581, 1232, 1197 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 91, 227, 268, 312, 79, 55, 77.

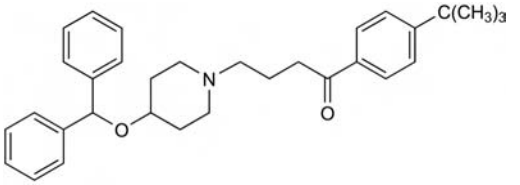
Disposition in the Body Rapidly absorbed after oral administration. The major metabolites are 20α-hydroxydydrogesterone and 21-hydroxydydrogesterone. About 50% of a dose is excreted in the urine in 24 h, as metabolites.

Dose Usually 20 to 30 mg daily.

Ebastine

Antihistamine
$C_{32}H_{39}NO_2 = 469.7$
CAS—90729-43-4

IUPAC Name 4-(4-Benzhydryloxypiperidin-1-yl)-1-(4-tert-butylphenyl)butan-1-one
Synonyms 1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(diphenylmethoxy)-1-piperidin-yl]-1-butanone; LAS-W-090.
Proprietary Names *Bastel*; *Bromselon*; *Ebastel*; *Evastel*; *Kestine*.



Chemical Properties pK_a 10.32.

Ebastine Fumarate

$C_{32}H_{39}NO_6,C_4H_4O_4 = 585.7$
Chemical Properties Crystals from ethanol. Mp 197° to 198°.

Quantification

Plasma **HPLC** Column: ODS (250 × 4.0 mm i.d., 10 µm). Mobile phase: methanol : 0.02 mol/L sodium acetate : glacial acetic acid : water (380:20:5.5:100), flow rate 2 mL/min. UV detection. Retention time: carebastine metabolite, 7.3 min. Limit of detection, 0.02 mg/L, carebastine [Vincent *et al.* 1988].
Disposition in the Body Ebastine is extensively metabolised in the liver into the active metabolite, carebastine, a carboxylic acid metabolite. As metabolism is extensive, there is little parent drug present in plasma. Ebastine is excreted mainly in urine with 40% of a dose being recovered over a 24-h period. Approximately 6% can be detected in faeces.
Therapeutic Concentration

Nine healthy normotensive male volunteers, aged between 19 and 33 years, were administered with a 10 mg and a 50 mg dose of ebastine. Peak plasma concentrations of 0.099 ± 0.029 mg/L and 0.417 ± 0.0546 mg/L were reached within 3.61 ± 1.06 and 3.70 ± 0.76 h, respectively [Vincent *et al.* 1988].

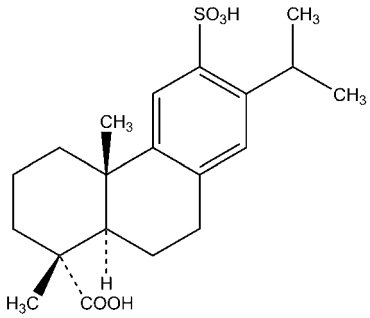
Bioavailability Increases with consumption of food.
Half-life 13 to 16 h.
Protein Binding Approximately 98%.
Dose A usual dose of 10 mg daily is administered to adults, with a maximum of 20 mg. For children over 6 years, 5 mg daily is given.

Vincent J *et al.* (1988). The pharmacokinetics, antihistamine and concentration-effect relationship of ebastine in healthy subjects. *Br J Clin Pharmacol* 26: 497–502.

Ecabet

Antiulcerative
$C_{20}H_{28}O_5S = 380.5$
CAS—33159-27-2

IUPAC Name (1*R*,4a*S*,10a*R*)-1,4a-Dimethyl-7-propan-2-yl-6-sulfo-2,3,4,9,10,10a-hexahydrophenanthrene-1-carboxylic acid
Synonyms Dehydro-6-sulfoabietic acid; 13-isopropyl-12-sulfopodocarpa-8,11,13-trien-15-oic acid; (1*R*,4a*S*,10a*R*)-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-6-sulfo-1-phenanthrenecarboxylic acid; 12-sulfodehydroabietic acid.



Ecabet Sodium

$C_{20}H_{27}NaO_5S,5H_2O = 492.6$
CAS—86408-72-2
Synonym TA-2711
Proprietary Name *Gastrom*
Chemical Properties Mp >300°.

Quantification

Other **TLC** Animal Tissues. Silica gel $60F_{254}$. Solvent system: chloroform: methanol (2 : 1). R_f values not reported [Ito *et al.* 1991].

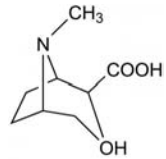
HPLC Animal Tissues. Column: ODS. Mobile phase: acetonitrile–tetrahydrofuran (7 : 3) : 0.1 mol/L phosphate buffer (pH 2.2; 20 : 80 for 1 min to 35 : 65 over 15 min for 4 min). UV detection (λ = 238 nm). Retention time, limit of quantification and limit of detection not reported [Ito *et al.* 1991].
Disposition in the Body Ecabet inhibits peptic activity and only a small amount of an oral dose transfers into circulating blood. Its cytoprotective effect is caused by direct action on the gastric mucosa. It is bound to proteins by hydrophobic interactions.
Bioavailability Approximately 97%, mainly to albumin.
Dose 1 g orally twice daily

Ito Y *et al.* (1991). Metabolic fate of a new anti-ulcer drug (+)-(1*R*,4a*S*,10a*R*)- 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1- methylethyl)-6-sulfo-1-phenanthrenecarboxylic acid 6-sodium salt pentahydrate (TA-2711). I. Disposition, metabolism and protein binding in rats and dogs. *J Pharmacobiodyn* 14: 533–546.

Ecgonine

Alkaloid
$C_9H_{15}NO_3 = 185.2$
CAS—481-37-8

IUPAC Name [1*R*-(*exo*,*exo*)]-3-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid
Synonym Laevo-ecgonine

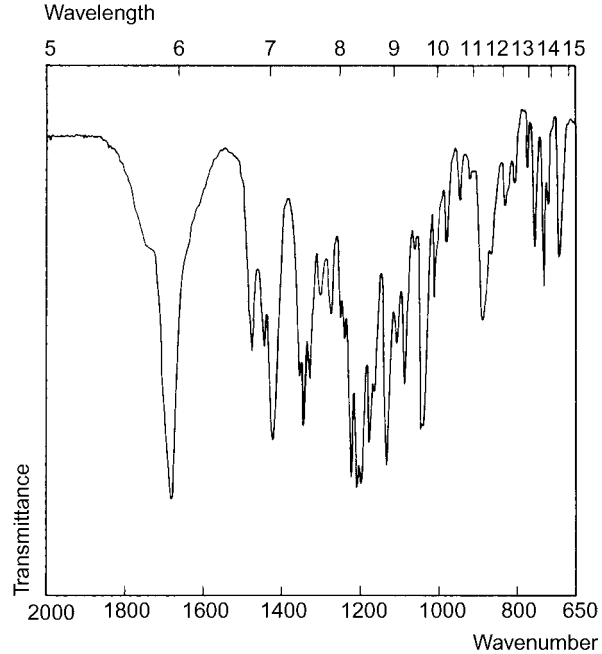


Chemical Properties Mp 198°, with decomposition; the dry substance melts at 205°. Soluble in water; slightly soluble in ethanol; sparingly soluble in acetone, ether, benzene, chloroform and petroleum ether. pK_a 2.8, 11.1. Log *P* (octanol/water), −3.8.

Ecgonine Hydrochloride

$C_9H_{15}NO_3,HCl = 221.7$
CAS—5796-31-6
Chemical Properties Triclinic plates from water. Mp 246°.

Thin-layer Chromatography System TA—R_f 0.17; system TAL—R_f 0.05 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 1.1.
Ultraviolet Spectrum Ethanol—275 nm.
Infrared Spectrum Principal peaks at wavenumbers 1688, 1210, 1200, 1223, 1134, 1179 cm^{-1} (ecgonine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 82, 97, 42, 83, 96, 57, 94, 55.
Quantification See also under Cocaine.
Plasma **HPLC–MS** For method for quantification of ecgonine and cocaine and some other metabolites, see Klingmann *et al.* [2001].
Urine **HPLC** Fluorescence detection. For method for quantification of ecgonine methyl ester and other cocaine metabolites, see Roy *et al.* [1992].

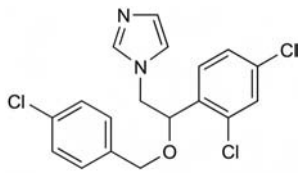
LC–MS Ecgonine methyl ester, benzoylecgonine and cocaine. Limit of detection, 0.5 µg/L, ecgonine methyl ester [Jeanville *et al.* 2001]. Ecgonine methyl ester and cocaine. Limit of detection, ecgonine methyl ester 2.8 pg [Needham *et al.* 2000]. For method for quantification of ecgonine, ecgonine methyl ester and other cocaine metabolites, see Nishikawa *et al.* [1994].
Note In blood, plasma, or serum: methods for monitoring drugs of abuse in cases of suspected driving under the influence of drugs [Moeller, Kraemer 2002].
Disposition in the Body Ecgonine and its methyl ester are metabolites of cocaine.

Roy IM *et al.* (1992). Analysis of cocaine, benzoylecgonine, ecgonine methyl ester, ethylcocaine and norcocaine in human urine using HPLC with post-column ion-pair extraction and fluorescence detection. *J Pharm Biomed Anal* 10: 943–948.
Nishikawa M *et al.* (1994). The analysis of cocaine and its metabolites by liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (LC/APCI-MS). *Forensic Sci Int* 66: 149–158.
Needham SR *et al.* (2000). Performance of a pentafluorophenylpropyl stationary phase for the electrospray ionization high-performance liquid chromatography-mass spectrometry-mass spectrometry assay of cocaine and its metabolite ecgonine methyl ester in human urine. *J Chromatogr B Biomed Sci Appl* 748: 77–87.
Jeanville PM *et al.* (2001). Rapid confirmation/quantitation of ecgonine methyl ester, benzoylecgonine, and cocaine in urine using on-line extraction coupled with fast HPLC and tandem mass spectrometry. *J Anal Toxicol* 25: 69–75.
Klingmann A *et al.* (2001). Analysis of cocaine, benzoylecgonine, ecogonine methyl ester, and ecgonine by high-pressure liquid chromatography-API mass spectrometry and application to a short-term degradation study of cocaine in plasma. *J Anal Toxicol* 25: 425–430.
Moeller MR, Kraemer T (2002). Drugs of abuse monitoring in blood for control of driving under the influence of drugs. *Ther Drug Monit* 24: 210–221.

Econazole

Antifungal
$C_{18}H_{15}Cl_3N_2O$ = 381.7
CAS—27220-47-9

IUPAC Name 1-[2-[(4-Chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole

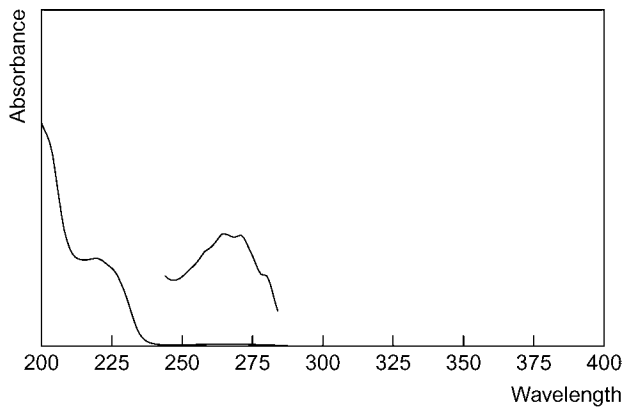


Chemical Properties Mp 86.8°.

Econazole Nitrate

$C_{18}H_{15}Cl_3N_2O,HNO_3$ = 444.7
CAS—24169-02-6; 68797-31-9
Proprietary Names *Dermazol(e)*; *Eccelium*; *Eco Mi*; *Ecodergin*; *Ecoderm*; *Ecorex*; *Ecostatin*; *Ecosteril*; *Ecotam*; *Ecreme*; *Epi-Pevaryl*; *Gyno-Pevaryl*; *Ifenac*; *Micoespec*; *Micoseptil*; *Micostyl*; *Pevalip*; *Pevaryl*; *Sebolith*; *Spectazole*. It is an ingredient of *Econacort*.
Chemical Properties A white crystalline powder. Mp about 164°, with decomposition. Very slightly soluble in water and ether; soluble 1 in 125 of ethanol, 1 in 60 of chloroform and 1 in 25 of methanol.

Thin-layer Chromatography System TA—R_f 0.80; system TB—R_f 0.09; system TC—R_f 0.61; system TE—R_f 0.75; system TAE—R_f 0.78; system TAF—R_f 0.80.
Gas Chromatography System GA—RI 3550.
High Performance Liquid Chromatography System HX—RI 526; system HY—RI 385; system HAA—retention time 20.1 min.
Ultraviolet Spectrum Methanolic acid—265 (A^1_1=12b), 271 (A^1_1=12.5a), 280 nm (A^1_1=7.6a).



Infrared Spectrum Principal peaks at wavenumbers 1090, 1310, 805, 825, 1010, 1040 cm^{-1} (econazole nitrate, KBr disk).
Quantification
Plasma **HPLC** UV detection. Limit of detection, 200 µg/L [Brodie *et al.* 1978].
Serum **HPLC** For method for quantification of econazole and other antifungal drugs, see Ng *et al.* [1996].
Disposition in the Body About 10% of a topical dose is absorbed and after vaginal application about 5% of the dose is absorbed. Following oral administration, about 40% of the dose is excreted in the urine and 30% is eliminated in the faeces, in 5 days.
Blood Concentration

A mean peak plasma concentration of 2.6 mg/L has been reported 2.5 h after oral administration of 250 mg of econazole to 8 subjects [Heel *et al.* 1978].

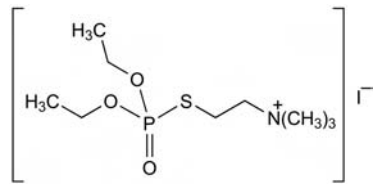
Uses Econazole nitrate is applied topically in a concentration of 1%, or as pessaries.

Brodie RR *et al.* (1978). High-performance liquid chromatographic determination of the antimycotic agent, econazole in plasma. *J Chromatogr* 155: 209–213.
Heel RC *et al.* (1978). Econazole: a review of its antifungal activity and therapeutic efficacy. *Drugs* 16 (3): 177–201.
Ng TK *et al.* (1996). Rapid high performance liquid chromatographic assay for antifungal agents in human sera. *J Antimicrob Chemother* 37(3): 465–472.

Ecothiopate Iodide

Anticholinesterase
$C_9H_{23}INO_3PS$ = 383.2
CAS—6736-03-4 (ecothiopate); 513-10-0 (iodide)

IUPAC Name 2-Diethoxyphosphorylsulfanylethyl(trimethyl)azanium iodide
Synonyms Diethoxyphosphinylthiocholine iodide; 2-[(diethoxyphosphinyl) thio]-*N*,*N*,*N*-trimethylethanaminium iodide; echothiopate iodide; echothiophate iodide; ecostigmine iodide.
Proprietary Name *Phospholine Iodide*

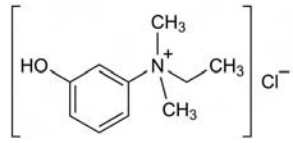


Chemical Properties A white, hygroscopic, crystalline powder. Mp 138° to 124.5°. Soluble in water, alcohol and chloroform. Log *P* (octanol/water), −2.2.
Colour Test Palladium chloride—orange.
Thin-layer Chromatography System TA—R_f 0.02. (Acidified iodoplatinate solution, positive.)
Use As a 0.03 to 0.25% ophthalmic solution.

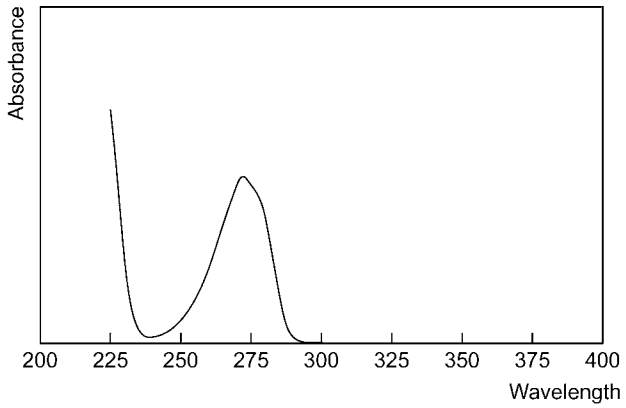
Edrophonium Chloride

Anticholinesterase
$C_{10}H_{16}ClNO$ = 201.7
CAS—312-48-1 (edrophonium); 116-38-1 (chloride)

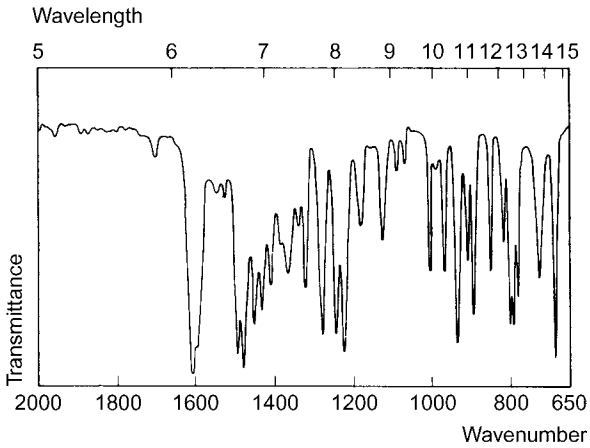
IUPAC Name Ethyl-(3-hydroxyphenyl)-dimethylazanium chloride
Synonym *N*-Ethyl-3-hydroxy-*N*,*N*-dimethylbenzenaminium chloride
Proprietary Names *Anticude*; *Camsilon*; *Enlon*; *Reversol*; *Tensilon*.



Chemical Properties A white crystalline powder. Mp 165° to 170°, with decomposition. Soluble 1 in 0.5 of water and 1 in 5 of ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), −3.0.
Colour Test Mandelin's test—blue-green.
Thin-layer Chromatography System TA—R_f 0.07 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—273 nm (A^1_1=110a); aqueous alkali—240 (A^1_1=550b), 294 nm (A^1_1=170b).



Infrared Spectrum Principal peaks at wavenumbers 1609, 690, 1217, 1495, 934, 1280 cm^{-1} (KBr disk).



Disposition in the Body

Therapeutic Concentration

Following IV injections of 100 µg/kg to 5 subjects, plasma concentrations of 0.8 to 3.9 mg/L (mean 1.6) were reported at 2 min, declining to 0.038 to 0.056 mg/L (mean 0.048) at 1 h [Calvey *et al.* 1976].

Toxicity The estimated minimum lethal dose is 100 mg.

Half-life Plasma half-life, about 0.5 h.

Dose 2 to 10 mg intravenously, in the diagnosis of myasthenia gravis.

Calvey TN *et al.* (1976). Plasma concentration of edrophonium in man. *Clin Pharmacol Ther* 19: 813–820.

Efavirenz

Antiviral

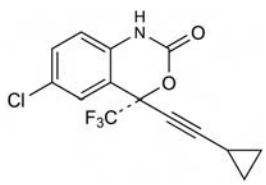
$C_{14}H_9ClF_3NO_2$ = 315.7

CAS—154598-52-4

IUPAC Name (*S*)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one

Synonyms DMP-266; L-743726.

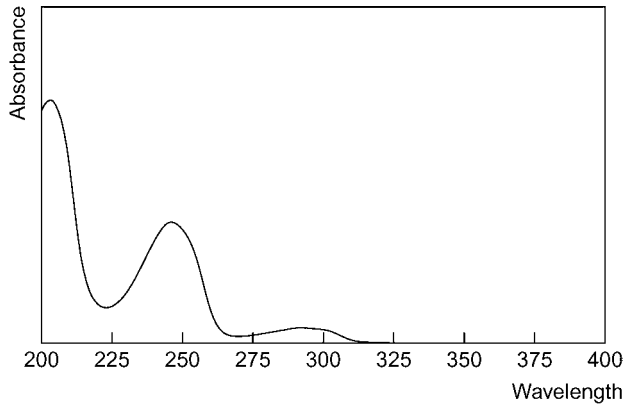
Proprietary Name *Sustiva*



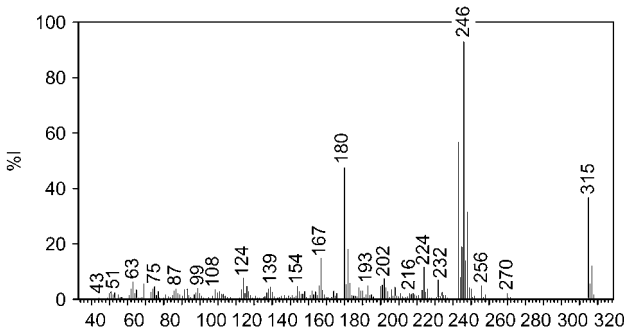
Chemical Properties A white to slightly pink crystalline powder, which is practically insoluble in water (<10 g/L). pK_a 10.2.

High Performance Liquid Chromatography System HAC—*k* 8.5; system HAE—retention time 28.6 min.

Ultraviolet Spectrum Principal peaks at 247, 293 nm.



Mass Spectrum Principal ions at *m/z* 246, 243, 180, 315, 248, 245, 182, 167.



Quantification

Plasma **HPLC** UV detection (λ=241 nm). Limit of quantification, 0.05 mg/L [Aymard *et al.* 2000]. Column: C_{18} Zorbak SB (3.5 µm, 150 × 4.6 mm i.d.). Mobile phase: 25 mmol/L phosphate buffer: acetonitrile (53:47), (pH 7.5), flow rate 1.5 mL/min. UV detection (λ=246 nm). Retention time: 10.2 min. Limit of detection, 0.01 mg/L [Veldkamp *et al.* 1999]. UV detection (λ=247 nm). Limit of detection, 0.1 mg/L [Villani *et al.* 1999].

Serum **HPLC** UV detection. Limit of detection, 0.062 mg/L [Simon *et al.* 2001].

Disposition in the Body

Efavirenz is metabolised by the cytochrome P450 system to hydroxylated metabolites, which can undergo subsequent glucuronidation to inactive metabolites. 14 to 34% of an administered dose can be recovered in urine as the metabolites and 16 to 61% in faeces as the parent drug. Efavirenz is known to cross the blood–brain barrier.

Therapeutic Concentration

A single oral dose between 100 and 1600 mg was administered to uninfected volunteers and peak plasma concentrations between 0.51 and 2.9 mg/L were observed. These levels were reached after 5 h. A 600 mg dose administered to HIV-infected patients, however, produced a peak plasma concentration of 4.1 mg/L, which was reached within 3 to 5 h [DuPont Pharmaceutical Company 1998].

Toxicity A mild to moderate rash with blistering and shedding is the main toxicological effect. Two incidents have been noted, where two patients accidentally took a total dose of 1200 mg and showed an increase in nervous system symptoms and involuntary muscle contractions.

Half-life The estimated half-life for a single dose is 52 to 76 h and 40 to 55 h for multiple doses.

Clearance Mean plasma clearance, 0.18 L/h/kg.

Protein Binding >99%, mainly albumin.

Dose Adult: 600 mg daily. Child: between 200 and 400 mg daily depending on body weight (13 to 39 kg), >40 kg: adult dose.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

DuPont Pharmaceutical Company (1998, Sept. 17). Sustiva (Efavirenz capsules) Prescribing Information.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

Veldkamp AI *et al.* (1999). Quantitative determination of efavirenz (DMP 266), a novel non-nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 734(1): 55–61.

Villani P *et al.* (1999). High-performance liquid chromatography method for analyzing the antiretroviral agent efavirenz in human plasma. *Ther Drug Monit* 21(3): 346–350.

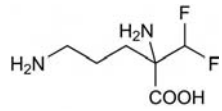
Eflornithine

Antineoplastic, Antiprotozoal

$C_6H_{12}F_2N_2O_2$ = 182.2

CAS—67037-37-0

IUPAC Name 2,5-Diamino-2-(difluoromethyl)pentanoic acid
Synonyms DFMO; 2-(difluoromethyl)-D,L-ornithine; MDL-71782; RMI-71782.



Eflornithine Hydrochloride

$C_6H_{13}ClF_2N_2O_2 = 218.7$
CAS—68278-23-9
Chemical Properties Crystalline powder. Mp 183°.

Eflornithine Hydrochloride Monohydrate

$C_6H_{15}ClF_2N_2O_3 = 236.6$
CAS—96020-91-6
Synonym MDL-71782A
Proprietary Name *Ornidyl*
Chemical Properties Crystalline powder. Mp 183°.

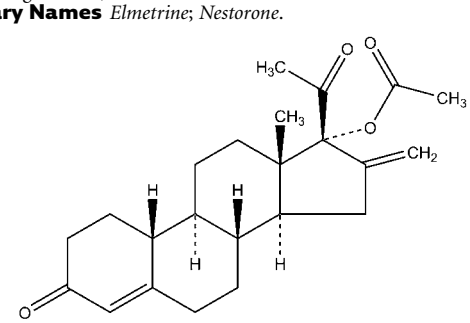
Quantification

Serum **HPLC** Column: RP C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phases: (A) 10 mmol/L sodium acetate (pH 4.18) : tetrahydrofuran (95:5); (B) acetonitrile : tetrahydrofuran (90:10). Elution program: (A:B) (100:0) for 2 min, to (63.3:36.7) in 19 min, to (0:100) for 2 min at 1.5 mL/min, back to initial conditions in 2 min, held for 3 min. UV detection (λ=330 nm). Retention time: 14.7 min. Limit of detection, 10 mg/L [Cohen *et al.* 1989].
Note For an ELISA for the quantification of eflornithine, see Gunaretna *et al.* [1994].
Disposition in the Body Eflornithine is absorbed (~60%) after oral administration and undergoes decarboxylation. >80% of the dose is excreted in urine, 40 to 45% of which remains unchanged. The drug is distributed into central spinal fluid.
Therapeutic Concentration
Six healthy men, aged between 21 and 31 years, were administered an oral dose of 10 and 20 mg/kg body weight eflornithine, and a 10 mg/kg IV dose. Peak plasma concentrations of 31.4 to 51.0 nmol/L were reached, within 2 to 6 h, for the 10 mg/kg oral dose and 27.4 to 137.5 nmol/L for the 20 mg/kg dose (1.5 to 4 h) [Haegele *et al.* 1981].
Toxicity Ototoxicity can occur and loss of hearing has been observed with a cumulative dose. Low toxicity via ingestion and IP routes.
Bioavailability Oral bioavailability, 55%.
Half-life 3 to 4 h.
Volume of Distribution 0.33 to 0.37 L/kg.
Clearance Total body clearance, 1.17 mL/min/kg.
Dose A dose of 100 mg/kg body weight is administered every 6 h, IV, for at least 14 days, which may be followed by a 300 mg/kg body weight daily dose (orally) for 3 to 4 weeks. Dose is decreased for patients with impaired renal function.

Cohen JL *et al.* (1989). High-pressure liquid chromatographic analysis of eflornithine in serum. *J Pharm Sci* 78(2): 114–116.
Gunaratna PC *et al.* (1994). Pharmacokinetic studies of alpha-difluoromethylornithine in rabbits using an enzyme-linked immunosorbent assay. *J Pharm Biomed Anal* 12(10): 1249–1257.
Haegele KD *et al.* (1981). Kinetics of alpha-difluoromethylornithine: an irreversible inhibitor of ornithine decarboxylase. *Clin Pharmacol Ther* 30: 210–215.

Elcometrine

Contraceptive, Progestogen, Progestational Steroid
$C_{23}H_{30}O_4 = 370.5$
CAS—7759-35-5
IUPAC Name [(8*R*,9*S*,10*R*,13S,14*S*,17*R*)-17-Acetyl-13-methyl-16-methylidene-3-oxo-2,6,7,8,9,10,11,12,14,15-decahydro-1*H*-cyclopenta[a]phenanthren-17-yl] acetate
Synonyms 17-(Acetyloxy)-16-methylene-19-norpregn-4-ene-3,20-dione; 16-methylene-17α-acetoxy-19-norpregn-4-ene-3,20-dione; 16-methylene-17α-acetoxy-19 norprogesterone; ST-1435.
Proprietary Names *Elmetrine*; *Nestorone*.



Chemical Properties Crystals. Mp 178° to 179°.

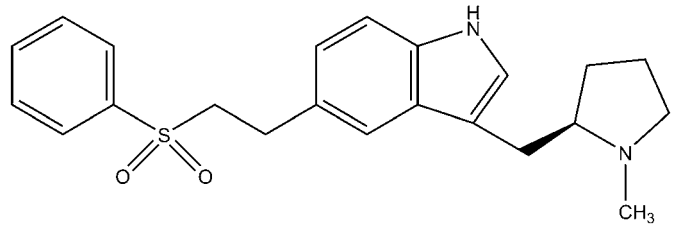
Quantification

Serum **HPLC** Column: Lichrosorb C_{18} (240 × 4.0 mm i.d.). Mobile phase: acetonitrile : water : methanol (35 : 35 : 30), flow rate 1 mL/min. UV detection (λ = 245 nm). Retention time: 9 min. Limit of quantification and limit of detection not reported [Heikinheimo *et al.* 1994].
Disposition in the Body Elcometrine is poorly absorbed by the oral route and rapidly metabolised during hepatic first-pass metabolism. It has a very short half-life because of its inability to bind to sex-hormone-binding globulin or to cortisol-binding globulin, transport proteins that prolong the biological half-life of many steroid hormones.
Therapeutic Concentration
A group of 25 postpartum nursing women were administered an implant containing 50 mg elcometrine. Mean serum concentrations were reported as 171.5 ng/L (463 pmol/L) on day 15 and 141.2 ng/L (381 pmol/L) on day 75. Elcometrine mean serum concentrations were also measured on day 75 in breast milk and in serum from babies and were reported as 373 pmol/L and 19.3 pmol/L, respectively [Coutinho *et al.* 1999].
After oral administration of 100 µg of elcometrine in fasting women, the mean peak plasma concentration reached 156.7 ng/L (423 pmol/L) after 10 min. This level was reduced by 50% at 60 min [Noe *et al.* 1993].
Six healthy postpartum women were administered 20 mg elcometrine parenterally. After 2 weeks, mean plasma and breast milk concentrations were measured by radioimmunoassay and reported as 62 and 38 µg/L, respectively [Lahteenmaki *et al.* 1990].
Bioavailability Approximately 12%.
Half-life Biphasic, 0.13 h and 14.6 h.
Volume of Distribution Approximately 263 L.
Clearance Approximately 126 L/h.

Coutinho EM *et al.* (1999). Use of a single implant of elcometrine (ST-1435), a nonorally active progestin, as a long acting contraceptive for postpartum nursing women. *Contraception* 59: 115–122.
Heikinheimo O *et al.* (1994). The progestin ST 1435—rapid metabolism in man. *Contraception* 50: 275–289.
Lahteenmaki PL *et al.* (1990). Milk and plasma concentrations of the progestin ST-1435 in women treated parenterally with ST-1435. *Contraception* 42: 555–562.
Noe G *et al.* (1993). Pharmacokinetics and bioavailability of ST 1435 administered by different routes. *Contraception* 48: 548–556.

Eletriptan

5-HT$_1$ Receptor Agonist, Antimigraine
$C_{22}H_{26}N_2O_2S = 382.5$
CAS—143322-58-1
IUPAC Name 3-[[(2*R*)-1-Methylpyrrolidin-2-yl]methyl]-5-(2-phenylsulfonylethyl)-1*H*-indole
Synonyms 5-[2-(Benzenesulfonyl)ethyl]3-(1-methylpyrrolidin-2(*R*)-ylmethyl)-1H-indole; 3-[[(2*R*)-1-methyl-2-pyrrolidinyl]methyl]-5-[2-(phenylsulfonyl)ethyl]-1H-indole; 3-[(1-methylpyrrolidin-2-yl)methyl]-5-(2-phenylsulfonylethyl)-1H-indole; UK-116044.



Chemical Properties pK_a 9.2 [Cooper *et al.* 1999]. Log *P* (octanol/water), 3.58 [Wishart 2006].

Eletriptan Hydrobromide

$C_{22}H_{26}N_2O_2S,HBr = 463.4$
CAS—177834-92-3
Proprietary Name *Relpax*
Chemical Properties A white to light pale coloured powder. Readily soluble in water.

Quantification

Plasma **HPLC** Column (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 500 mmol/L potassium phosphate buffer (pH 3.5) : water (30 : 6 : 64), flow rate 1.0 mL/min. UV detection (λ = 225 nm). Limit of quantification, 0.5 µg/L [Cooper *et al.* 1999].
Oral Fluid **HPLC** Column (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 500 mmol/L potassium phosphate buffer (pH 3.5) : water (30 : 6 : 64), flow rate 1.0 mL/min. UV detection (λ = 225 nm). Limit of quantification, 0.5 µg/L [Cooper *et al.* 1999].
Disposition in the Body Readily absorbed after oral administration, with peak plasma concentrations reached 1.5 h after dosing (2 h in patients with migraine). It is primarily metabolised by CYP3A4, with a small contribution (<10%) from other CYP enzymes. The active *N*-demethylated metabolite makes up 10 to 20% of the plasma concentration of the drug. Only about 10% of a dose is cleared via the renal route. Eletriptan does not inhibit or induce any CYP enzymes

and, therefore, is unlikely to cause clinically important interactions or to alter the plasma levels of other drugs.

Therapeutic Concentration

In a low-dose range study, 8 healthy male volunteers were administered oral doses of 7.5 and 20 mg eletriptan with a washout period of 7 days between doses. Mean peak plasma concentrations were 23.9 μg/L for the 7.5 mg dose and 61.5 μg/L for the 20 mg dose, attained at 0.8 h and 1 h, respectively. In a high-dose range study, 12 healthy male volunteers were administered oral doses of 30 and 120 mg eletriptan with a washout period of 7 days. Mean peak plasma concentrations were 64.8 μg/L for the 30 mg dose and 369 μg/L for the 120 mg dose, attained at 0.8 h and 1.4 h, respectively [Milton *et al.* 2002].

Five groups of healthy adult males were randomised to receive eletriptan as a single oral dose of 10 mg ($n = 7$), 30 mg ($n = 10$), 60 mg ($n = 10$), 90 mg ($n = 11$), or 120 mg ($n = 10$). Mean peak plasma and saliva concentrations were measured and reported as follows:

	10 mg	*30 mg*	*60 mg*	*90 mg*	*120 mg*
Peak plasma concentration (μg/L)	13	57	151	181	285
Time (h)	1.0	1.25	1.0	1.25	1.25
Peak saliva concentration (μg/L)	NA	17	47	48	166
Time (h)	NA	2.0	2.0	2.0	1.0

NA, not available [Shah *et al.* 2002].

Note The different phases of the menstrual cycle have no clinically significant effect on the pharmacokinetics, safety or tolerability of eletriptan in healthy volunteers [Shah *et al.* 2001].

Bioavailability Absolute, 50%.

Half-life Approximately 4 h; 5.7 h in the elderly.

Volume of Distribution Approximately 138 L.

Protein Binding Approximately 85%.

Dose Eletriptan hydrobromide 40 mg once daily. If required, a second tablet can be taken if directed by a healthcare professional but no more than 2 tablets should be taken in any 24 h period.

Cooper JD *et al.* (1999). Determination of eletriptan in plasma and saliva using automated sequential trace enrichment of dialysate and high-performance liquid chromatography. *J Pharm Biomed Anal* 21: 787–796.

Milton KA *et al.* (2002). Pharmacokinetics, pharmacodynamics, and safety of the 5-HT(1B/1D) agonist eletriptan following intravenous and oral administration. *J Clin Pharmacol* 42: 528–539.

Shah AK *et al.* (2001). Pharmacokinetics and safety of oral eletriptan during different phases of the menstrual cycle in healthy volunteers. *J Clin Pharmacol* 41: 1339–1344.

Shah AK *et al.* (2002). The pharmacokinetics and safety of single escalating oral doses of eletriptan. *J Clin Pharmacol* 42: 520–527.

Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Emamectin

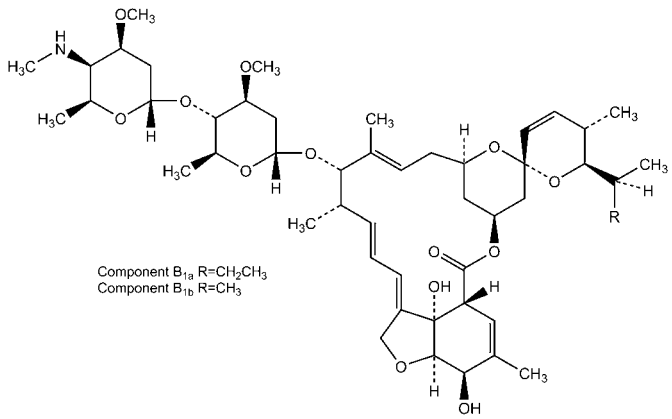
Insecticide, Macrocyclic Lactone

$C_{49}H_{75}NO_{13} : C_{48}H_{73}NO_{13}$ (9 : 1) = 886.13; 872.11

CAS—137335-79-6 (components); 121124-29-6 (major); 121424-52-0 (minor)

IUPAC Name 4′-Epi-(methylamino)-4′-deoxyavermectin B_{1a} and 4′-epi-(methylamino-4′-deoxyavermectin B_{1b} (mixture 9 : 1)

Synonyms (4′*R*)-5-*O*-Demethyl-4′-deoxy-4′-(methylamino)avermectin A1a and (4′*R*)-5-*O*-demethyl-25-de(1-methylpropyl)-4′deoxy-4′-(methylamino)-25-(1-methylethyl)avermectin A1a (mixture 9 : 1).



Emamectin Benzoate

CAS—137512-74-4

IUPAC Name 4′-Deoxy-4′-*epi*-*N*-methylaminoavermectin B_1 benzoate

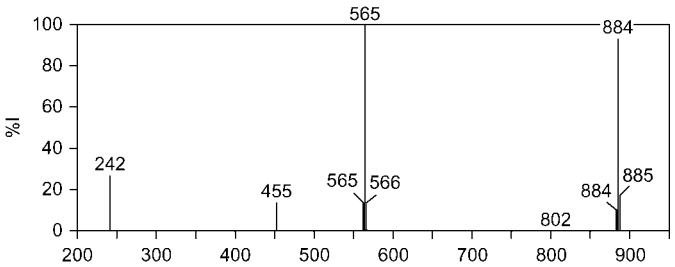
Synonym MK-244

Proprietary Names *Affirm*; *Proclaim*; *Slice*.

Chemical Properties 1.1% form is an off-white crystalline powder. Mp 141° to 146°. Freely soluble in chloroform and methanol. Solubility 0.32 mg/mL at pH 5 (aqueous), 0.024 mg/mL at pH 7 (aqueous); insoluble in hexane. pK_{a1} 4.2; pK_{a2} 7.6. Log *P* (octanol/aqueous phosphate), 3.0 (pH 5.1); 5.0 (pH 7.0) [O'Neil *et al.* 2006].

Ultraviolet Spectrum 10% Methanol—244 nm.

Mass Spectrum Principal ions at *m/z* 565, 884, 242, 885, 566, 455.



Quantification

Plasma **LC-MS** Column: Hypersil C_8 (50 × 2.0 mm i.d., 5 μm). Mobile phase: 20 mmol/L ammonium acetate (pH 4.8) : acetonitrile (60 : 40 for 0.5 min to 0 : 100 over 1.5 min for 1.5 min), flow rate 300 μL/min. API, positive ion mode, CID. Limit of quantification, 1.0 μg/L [Pereira, Chang 2004].

Other **LC-MS** Food. Column: C_8 (50 × 4.6 mm i.d., 1.8 μm). Mobile phase: acetonitrile : 0.2% ammonium formate (50 : 50 to 0 : 100 over 5 min), flow rate 200 μL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3.8 min. Limit of quantification, 0.05 to 0.1 μg/kg [Hernando *et al.* 2007]. Milk. Column: C_{18} (100 × 3.0 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 10 mmol/L TEA (75 : 25), flow rate 350 μL/min. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 0.85 μg/L; limit of detection, 0.35 μg/L [Durden 2007]. Lobster Tissue. Column: ODS/B (250 × 3.0 mm i.d., 5 μm). Mobile phase: acetonitrile : acetonitrile-water (90 : 10) : acetonitrile-methanol (90 : 10; 80 : 20 : 0 for 10 min to 0 : 0 : 100 over 1 min for 8 min), flow rate 1 mL/min. Fluorescence detection ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 470$ nm). Retention time: emamectin, 8.0 min; desmethylaminoemamectin, 5.7 min (methylimidazole–trifluoroacetic acid derivatives). Limit of quantification, 3.32 ng/g; limit of detection, 1.1 ng/g [Tauber *et al.* 2006]. Fish. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (94 : 6), flow rate 2.2 mL/min. Fluorescence detection ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 470$ nm). Retention time: ~5 min (methylimidazole-trifluoroacetic acid derivative). Limit of quantification, 1.5 ng/g; limit of detection, 0.5 ng/g [van de Riet *et al.* 2001].

Note For a methodology review for the determination of macrocyclic lactone residues in biological matrices, see Danaher *et al.* [2006].

Disposition in the Body In salmon, unchanged emamectin is the major residue found in muscle and skin tissue, accounting for 98% of total residue at 12 h. The major metabolite found in tissue is *N*-desmethylemamectin.

Toxicity

A 67-year-old man (weight 75 kg), previously in good health attempted suicide by drinking ~500 mL of 2.15% w/w emamectin benzoate in 2,6-bis (1,1-dimethylethyl)-4-methylphenol and 1-hexanol. This corresponded to ~25.6 mg/kg emamectin benzoate. He developed a transient gastrointestinal upset with endoscopy-proven gastric erosion, mild CNS depression, and aspiration pneumonia. Emamectin blood levels were not measured and the patient was discharged after 1 week with no sequelae [Yen, Lin 2004].

Danaher M *et al.* (2006). Review of methodology for the determination of macrocyclic lactone residues in biological matrices. *J Chromatogr B Analyt Technol Biomed Life Sci* 844: 175–203.

Durden DA (2007). Positive and negative electrospray LC-MS-MS methods for quantitation of the antiparasitic endectocide drugs, abamectin, doramectin, emamectin, eprinomectin, ivermectin, moxidectin and selamectin in milk. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 134–146.

Hernando MD *et al.* (2007). Fast separation liquid chromatography–tandem mass spectrometry for the confirmation and quantitative analysis of avermectin residues in food. *J Chromatogr A* 1155: 62–73.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Pereira T, Chang SW (2004). Semi-automated quantification of ivermectin in rat and human plasma using protein precipitation and filtration with liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 18: 1265–1276.

Tauber R *et al.* (2006). Liquid chromatography/fluorescence method for emamectin B1a and desmethylamino-emamectin B1a residues in lobster tissue. *J AOAC Int* 89: 1672–1676.

van deRiet JM *et al.* (2001). Simultaneous determination of emamectin and ivermectin residues in Atlantic salmon muscle by liquid chromatography with fluorescence detection. *J AOAC Int* 84: 1358–1362.

Yen TH, Lin JL (2004). Acute poisoning with emamectin benzoate. *J Toxicol Clin Toxicol* 42: 657–661.

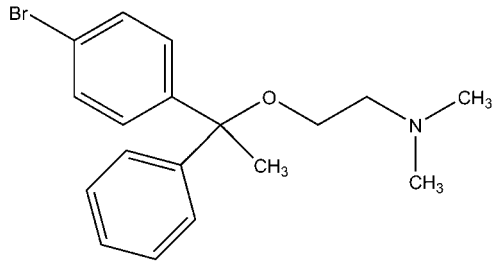
Embramine

Antiemetic, Antihistamine

$C_{18}H_{22}BrNO$ = 348.3

CAS—3565-72-8

IUPAC Name 2-[1-(4-Bromophenyl)-1-phenylethoxy]-*N*,*N*-dimethylethanamine
Synonym *p*-Bromo-α-methylbenzhydryl-2-dimethylaminoethyl ether

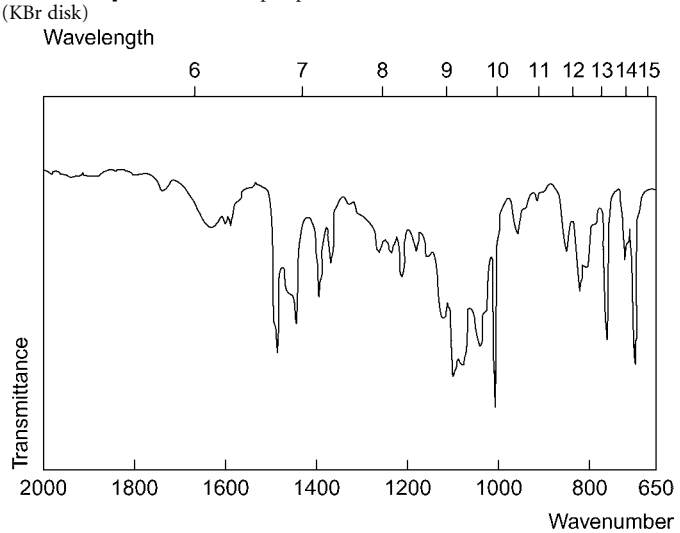


E

Embramine Hydrochloride

$C_{18}H_{22}BrNO,HCl = 384.7$
CAS—13977-28-1
Synonym Mebrophenhydramine
Proprietary Names *Mebryl*; *Medrin*.
Chemical Properties Crystals from acetone–ether. Mp 150° to 152°. Log *P* (octanol/water), 5.70 [Meylan, Howard 1995].

Colour Tests Ammonium molybdate test—yellow→green→blue (limit of detection, 0.1 µg); ammonium vanadate test—orange-brown (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—orange (limit of detection, 0.1 µg); Vitali's test—dull orange/dull orange/dull orange (limit of detection., 0.25 µg).
Thin-Layer Chromatography System TA—R_f 0.54; system TB—R_f 0.50; system TC—R_f 0.32; system TL—R_f 0.17.
Gas Chromatography System GA—RI 2185.
Ultraviolet Spectrum 0.05 mol/L sulfuric acid—226 ($A^1_1 = 400$), 258 nm ($A^1_1 = 18$).
Infrared Spectrum Principal peaks at wavenumbers 1009, 1095, 699 cm^{-1} (KBr disk)



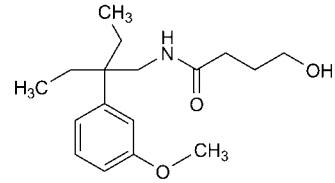
Disposition in the Body Embramine is rapidly absorbed form the gastrointestinal tract. Six hours after a large dose, high levels of the drug are found in the liver, lungs and spleen, with lower levels in the brain and heart.
Toxicity Long-term administration of large doses to dogs and cats has shown it to have negligible toxicity. The LD_{50} value in mice via oral dosing is 330 mg/kg
Dose Up to 150 mg daily. Adults: to prevent motion sickness one tablet (25 mg) is usually sufficient to be taken 15 to 30 min before departure; the full effect is achieved within 1 h. During long-lasting travel half to one tablet (12.5 to 25 mg) is taken at 2 to 3 h intervals as required. For treatment of motion sickness, the recommended dose is one to three tablets (25 to 75 mg). Repeated administration should be considered individually because of the long-lasting effect of the drug. The recommended maximum daily dose is six tablets. To prevent radiation sickness one tablet (25 mg) is given 1 h before exposure to radiation and one tablet (25 mg) at approx. 6 h after exposure. In other indications, the usual therapeutic dosage is 12.5 to 25 mg given two to three times daily. Children aged 2 to 6 years: the recommended dose is 6.25 mg (quarter of a tablet) one or two times daily. Children aged 6 to 15 years: the recommended dose is 6.25–12.5 mg (quarter to half a tablet) one to two times daily.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

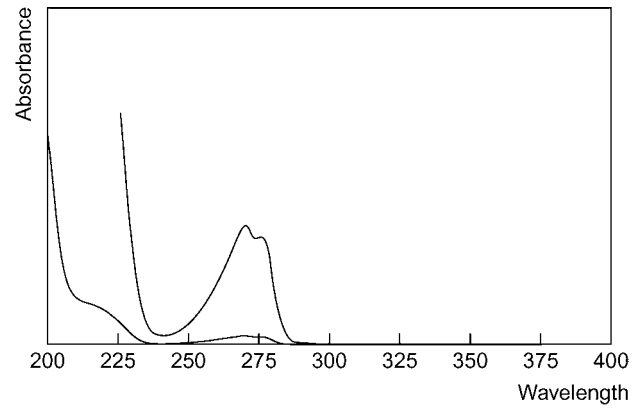
Embutramide

Narcotic Analgesic
$C_{17}H_{27}NO_3 = 293.4$
CAS—15687-14-6

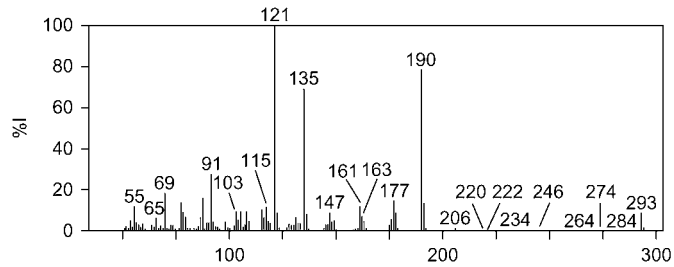
IUPAC Name *N*-[2-Ethyl-2-(3-methoxyphenyl)butyl]-4-hydroxybutanamide
Synonym *N*-(β,β-Diethyl-*m*-methoxyphenethyl)-4-hydroxybutyramide
Proprietary Names It is an ingredient of *Tanax* (veterinary) and *T 61* (veterinary).



Chemical Properties Crystals. Mp 70°. Stock solutions were stable at room temperature for 36 h, at 4° for 2 months and at –20° for 3 months. Spiked serum samples were stable for 1 month when stored at 4° [Fidani *et al.* 2008]. Samples stored at 4° for 6 months showed substantial degradation whereas samples stored at –20° for 6 months were stable [Huo *et al.* 1994].
Colour Tests Mandelin's test—brown; Marquis test—blue.
Thin-layer Chromatography System TA—R_f 0.72 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HAA—RT 17.4 min.
Ultraviolet Spectrum Aqueous acid—271 ($A^1_1 = 50$b), 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1652, 1248, 1037, 1595, 1562, 1063 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 121, 135, 190, 91, 293, 161, 177 [Giorgi *et al* 2001; Abe *et al* 2004].



Quantification

Blood **GC-MS** Column: HP-5 MS capillary (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 295° at 30°/min for 5 min. MSD, SIM acquisition mode. Limit of detection, 1 mg/L [Kintz *et al.* 2002]. Column: Mega OV-1 fused silica capillary (15 m × 0.18 mm i.d., 0.1 mm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 295° at 15°/min for 10 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of quantification, 250 µg/L [Giorgi *et al.* 2001].

HPLC Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 25 mmol/L phosphate buffer (pH 3.8; 30 : 70 to 20 : 80 in 18 min), flow rate 1.0 mL/min. DAD (λ = 273 nm). Retention time: 10.4 min. Limit of quantification, 0.6 mg/L, limit of detection, 0.2 mg/L [Abe *et al.* 2004]. Column: Spherisorb ODS2 C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (pH 4.7; 65 : 35), flow rate 0.9 mL/min. UV detection (λ = 273 nm). Limit of quantification, 2.5 mg/L [Giorgi *et al.* 2001].

Kidney **GC-MS** See Blood [Giorgi *et al.* 2001].

HPLC See Blood [Giorgi *et al.* 2001].

Liver **GC-MS** See Blood [Giorgi *et al.* 2001].

HPLC See Blood [Giorgi *et al.* 2001].

Other **GC** Dog Blood. Column: Ultra 2 5% phenyl methyl silicone (25 m × 0.25 mm i.d., 0.17 µm). Carrier gas: He, 0.67 mL/min. Temperature programme: 150° for 2 min to 280° at 10°/min for 5 min. NPD. Limit of detection, 40 µg/L [Huo *et al.* 1994].

GC-MS Meat and Bone Meal. Column: CP-Sil 5 CB-MS capillary (15 m × 0.25 mm i.d., 0.1 μm). Carrier gas: He. Temperature programme: 90° for 2 min to 200° at 12.5°/min to 300° at 30°/min. EI ionisation at 70 eV or PICI. Limit of quantification, ≈200 μg/kg, limit of detection, 50 and 20 μg/kg for EI and CI, respectively [Hooijerink *et al.* 1998]. Porcine Tissues. Column: DB-5 (15 m × 0.25 mm, 0.2 μm). Carrier gas: 1 mL/min. Temperature programme: 215° to 275° at 25°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 25 ng [Braselton *et al.* 1988].

LC-MS Equine Serum. Column: Ciano-phase Luna (150 × 2 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid : methanol (60 : 40), flow rate 0.25 mL/min. ESI, positive ion mode, full scan mode. Limit of quantification, 1 μg/L, limit of detection, 0.3 μg/L [Fidani *et al.* 2008].

Note For a UV spectrophotometry method for the measurement of ebutramide in blood and urine, see Bertol *et al.* [1983]

Disposition in the Body

Toxicity The lethal dose in mammals is between 50 and 180 mg/kg [Abe *et al.* 2004].

In 3 fatalities caused by the injection of a preparation containing embutramide and mebezonium iodide, the postmortem concentrations (mg/L) shown below were reported.

	Embutramide	*Mebezonium iodide*
Blood	3.0, 15.5, 12.1	4.5, 6.0, 7.5
Urine	2.0, 6.3, 4.5	0.8, 2.0, 1.8

[a][Bertol *et al.* 1983].

A 37-year-old Caucasian female was found dead. Two empty T-61 50 mL (dimethylformmaide containing 200 mg/mL embutramide, 50 mg/mL mebezonium iodide and 5 mg/mL tetracine) bottles were next to her. Blood and liver concentrations of embutramide were 31 and 12 ppm, respectively [Smith, Lewis 1989].

A 58-year-old veterinarian died after ingesting about 30 mL of T-61 solution (a mixture of embutramide, mebezonium and tetracaine; used for animal euthanasia). The embutramide concentration in postmortem femoral blood was 43.0 mg/L [Kintz *et al.* 2002].

A 40-year-old veterinarian was found dead with an IV 50 mL bottle of Tanax tied to his arm. It was empty, indicating that 10 g embutramide was administered. A blood concentration of 90 mg/L embutramide was measured [Abe *et al.* 2004].

Volume of Distribution 1.6 L/kg [Abe *et al.* 2004].

Abe E *et al.* (2004). A rapid and sensitive high-performance liquid chromatography method for determination of embutramide (a Tanax or T61 component) in human blood with photodiode-array UV detection. *J Anal Toxicol* 28: 118–121.

Bertol E *et al.* (1983). Analytical toxicological studies in cases of suicide by injection of Tanax, a veterinary euthanasia agent. *J Pharm Biomed Anal* 1: 373–377.

Braselton WE *et al.* (1988). Determination of embutramide in mammalian tissues. *Vet Hum Toxicol* 30: 536–539.

Fidani M *et al.* (2008). Development of a liquid chromatography-tandem mass spectrometry method for an euthanasic veterinarian drug: Tanax. *J Pharm Biomed Anal* 48: 902–908.

Giorgi M *et al.* (2001). Comparison of HPLC and GC-MS methods for determination of embutramide (a component of Tanax or T-61) in biological specimens. *J Anal Toxicol* 25: 323–327.

Hooijerink D *et al.* (1998). Determination of embutramide and pentobarbital in meat and bone meal by gas chromatography-mass spectrometry. *Analyst* 123: 2513–2516.

Huo JZ *et al.* (1994). Determination of embutramide in biological matrices by gas chromatography with nitrogen-phosphorus detection. *J Chromatogr B Biomed Appl* 661: 69–74.

Kintz P *et al.* (2002). Blood investigation in a fatality involving the veterinary drug T-61. *J Anal Toxicol* 26: 529–531.

Smith RA, Lewis D (1989). Suicide by ingestion of T-61. *Vet Hum Toxicol* 31: 319–320.

Emedastine

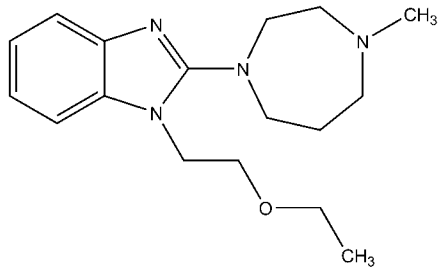
Antihistamine

$C_{17}H_{26}N_4O = 302.4$

CAS—87233-61-2

IUPAC Name 1-(2-Ethoxyethyl)-2-(4-methyl-1,4-diazepan-1-yl)benzimidazole

Synonym 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-1H-benzimidazole



Emedastine Difumarate

$C_{17}H_{26}N_4O,2C_4H_4O_4 = 534.6$

CAS—87233-62-3

Synonyms KB-2413; LY-188695.

Proprietary Name *Emadine*

Chemical Properties Crystals from ethanol. Mp 148° to 151°. pK_{a1} 4.51, pK_{a2} 8.48 [Alcon Laboratories 2003]. Log *P* (octanol/water), 2.03 [Wishart 2006]. When stored at 4 to 30°, the ophthalmic solution is stable for 24 months. Discoloured solutions should be discarded. There were no stability related problems during the routine analysis of samples for pharmacokinetic, bioavailabilty, or bioequivalence studies [Tian *et al.* 2007].

Ultraviolet Spectrum Peaks at 206, 227, 280 and 286 nm.

Quantification

Plasma **LC-MS**Column: Phenomex Luna CN 100A (150 × 2.0 mm, 5 μm). Mobile phase: methanol : 20 mmol/L ammonium acetate (pH 4.0; 80 : 20), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 0.05 μg/L; limit of detection, 0.01 μg/L [Tian *et al.* 2007].

Other **HPLC** Rat Skin. Column: Intersil ODS-2 (150 × 5 mm i.d., 5 μm). Mobile phase: 0.025 mol/L phosphate buffer (pH 2.4) containing 0.25% sodium lauryl sulfate : acetonitrile (50 : 50), flow rate 1.2 mL/min. UV detection (λ = 280 nm). Limit of detection, 500 μg/L [Harada *et al.* 2005].

Disposition in the Body

Following oral administration, emedastine is metabolised by the liver to form 5- and 6-hydroxyemedastine. These metabolites are further oxidised to form 5′-oxo analogues. Emedastine *N*-oxide is a minor metabolite. Approximately 44% of an oral dose is recovered in the urine within 24 h. Approximately 3.6% of an oral dose is excreted in the urine as the parent compound, but the 5- and 6-hydroxyemedastine metabolites and their conjugates are recovered in urine.

Therapeutic Concentration

Six healthy males and 6 healthy females (mean ages 35 ± 4 and 30 ± 5 years, respectively) were included in a study to investigate the pharmacokinetics of emedastine. Volunteers were involved in either a single-dose study, where subjects received either 2, 4 or 8 mg of drug, a multiple-dose study, where subjects received drug for 5 consecutive days at 2 mg twice daily, or a food-effect study, where subjects were either given a light meal 4 h after administration or a meal directly afterwards (see table below).

Note For pharmacokinetic and pharmacodynamics parameters in healthy volunteers, see Brunner *et al.* [2002] or Jansen *et al.* [2000]. For pharmacokinetic parameters in renally impaired patients, see Joukhadar *et al.* [2001], in combination with alcohol, see Vermeeren *et al.* [2002], and in combination with ketoconazole, see Herranz *et al.* [2001].

Half-life Approximately 3 to 4 h [Alcon Laboratories 2003].

Dose Instilled twice daily as a 0.05% solution for the symptomatic relief of allergic conjunctivitis. Orally in doses of 2 to 4 mg daily in two divided doses for allergic rhinitis, urticaria and pruritic skin disorders.

Alcon Laboratories (2003). Emadine (emadastine difumarate) ophthalmic solution 0.05% prescribing information. Hemel Hempstead, UK: Alcon Laboratories.

Brunner M *et al.* (2002). Pharmacokinetic and mass balance study of unlabelled and ^{14}C-labelled emedastine difumarate in healthy volunteers. *Xenobiotica* 32: 761–770.

Harada S *et al.* (2005). Estimation of in vivo percutaneous absorption of emedastine from bile excretion data using a deconvolution method. *Drug Metab Pharmacokinet* 20: 331–336.

	Single dose			*Multiple dose*	*Food intake*	*Fasting*
	2 mg PO	*4 mg PO*	*8 mg PO*	*2 mg bid*	*2 mg PO*	*2 mg PO*
$t_{1/2}$ (h)	6.66 ± 0.55	6.6 ± 0.98	6.71 ± 1.4	8.81 ± 4.28	6.16 ± 2.58	6.72 ± 3.85
t_{max} (h)	3.83 ± 1.7	3.75 ± 1.54	4.17 ± 0.94	3.42 ± 1.08	5.00 ± 1.28	4.00 ± 0.74
C_{max} (h)	5.13 ± 1.73	9.64 ± 3.66	20.64 ± 6.32	9.24 ± 4.75	2.67 ± 1.06	5.81 ± 1.33
$AUC_{0-\infty}$	54.6 ± 15.6	96.0 ± 47.4	248 ± 118	—	23.2 ± 13.2	42.9 ± 16.6

[Tian *et al.* 2007].

Herranz U *et al.* (2001). Emedastine-ketoconazole: pharmacokinetic and pharmacodynamic interactions in healthy volunteers. *Int J Clin Pharmacol Ther* 39: 102–109.
Jansen B *et al.* (2000). Pharmacokinetics and pharmacodynamics of the novel H_1-receptor antagonist emedastine in healthy volunteers. *Eur J Clin Pharmacol* 55: 837–841.
Joukhadar C *et al.* (2001). Pharmacokinetics of emedastine difumarate, a new anti-histaminic agent in patients with renal impairment. *Eur J Clin Pharmacol* 56: 905–910.
Tian Y *et al.* (2007). High-performance liquid chromatography-electrospray ionization–mass spectrometric determination of emedastine difumarate in human plasma and its pharmacokinetics. *J Chromatogr Sci* 45: 158–164.
Vermeeren A *et al.* (2002). Effects of emedastine and cetirizine, alone and with alcohol, on actual driving of males and females. *J Psychopharmacol* 16: 57–64.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

E

Emepronium Bromide

Anticholinergic

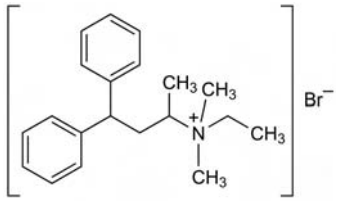
$C_{20}H_{28}BrN = 362.4$

CAS—27892-33-7 (emepronium); 3614-30-0 (bromide)

IUPAC Name 4,4-Diphenylbutan-2-yl-ethyl-dimethylazanium bromide

Synonym *N*-Ethyl-*N*,*N*-α-trimethyl-γ-phenylbenzenepropanaminium bromide

Proprietary Names *Cetiprin*; *Uro-Ripirin*.



Chemical Properties A white crystalline powder. Mp 204°. Soluble in water, ethanol and chloroform. Log *P* (octanol/water), 0.5.

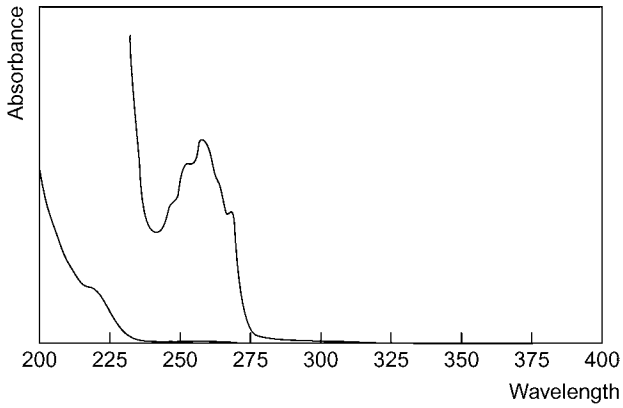
Colour Test Liebermann's reagent—brown.

Thin-layer Chromatography System TA—R_f 0.05; system TB—R_f 0.00; system TE—R_f 0.02; system TAE—R_f 0.03 (acidified iodoplatinate solution, strong reaction).

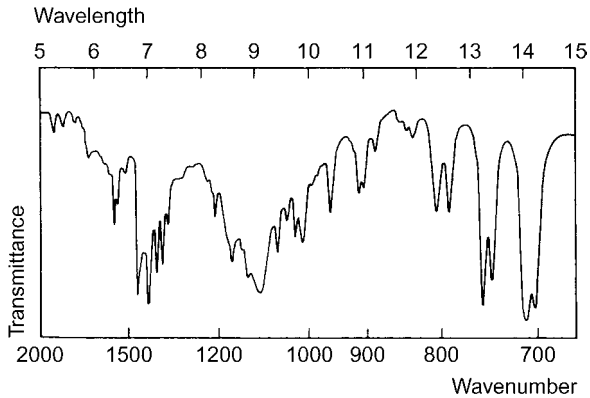
Gas Chromatography System GA—RI 1973.

High Performance Liquid Chromatography System HA—*k* 5.2; system HX—RI 420.

Ultraviolet Spectrum Aqueous acid—253, 258 (A^1_1=13a), 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 708, 699, 752, 1102, 742, 1134 cm^{-1} (KBr disk).



Quantification

Serum **GC** ECD. Limit of detection, 200 ng/L [Hartvig *et al.* 1976].

Disposition in the Body Poorly absorbed after oral administration; <5% of a dose is excreted in the urine in 24 h, partly as metabolites. After IM administration, about 30% of a dose is excreted in the urine in 3 days and 45% is eliminated in the faeces in the same period.

Therapeutic Concentration

Following a single oral dose of 150 mg to 5 subjects, peak serum concentrations of 0.017 to 0.10 mg/L were attained in about 2 h [Vessman *et al.* 1970].

Half-life Plasma half-life, about 7 to 11 h.

Dose 200 to 600 mg daily.

Hartvig P *et al.* (1976). Electron-capture GC determination of subnanogram amounts of emepronium bromide in serum. *J Pharm Sci* 65: 1707–1709.
Vessman J *et al.* (1970). Gas chromatography and electron capture detection of benzophenone formed by chromic acid oxidation. 2. Determination of emepronium (Cetiprin), a quaternary ammonium compound in serum. *Acta Pharm Suec* 7: 363–372.

Emetine

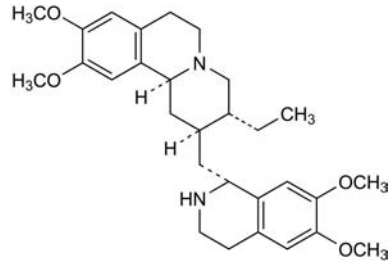
Antiamoebic

$C_{29}H_{40}N_2O_4 = 480.6$

CAS—483-18-1

IUPAC Name (2*S*,3*R*,11b*S*)-2-[[(1*R*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl]methyl]-3-ethyl-9,10-dimethoxy-2,3,4,6,7,11*b*-hexahydro-1*H*-benzo[*a*]quinolizine

Synonyms Ipecine; methylcephaëline; 6′,7′,10,11-tetramethoxyemetan.



Chemical Properties An alkaloid present in ipecacuanha, the dried root, or rhizome and roots, of *Cephaëlis ipecacuanha* (= *Uragoga ipecacuanha*) (Rubiaceae) or *C. acuminata*. A white amorphous powder. Mp 74°. Slightly soluble in water; soluble in ethanol, chloroform and ether. pK_a 7.4, 8.3 (25°). Log *P* (octanol/water), 5.2.

Emetine Hydrochloride

$C_{29}H_{40}N_2O_4,2HCl,\ 7H_2O = 679.7$

CAS—316-42-7 (anhydrous); 7083-71-8 (hydrate)

Synonym Emetine dihydrochloride

Proprietary Names It is an ingredient of *Cophylac*, *Ipeca*, *Sano Tuss* and *Spirbon*.

Chemical Properties A white or very slightly yellow, crystalline powder. Mp 235° to 255° with decomposition, after drying. Soluble 1 in 8 of water, 1 in 12 of ethanol (90%) and 1 in 4 of chloroform; practically insoluble in ether.

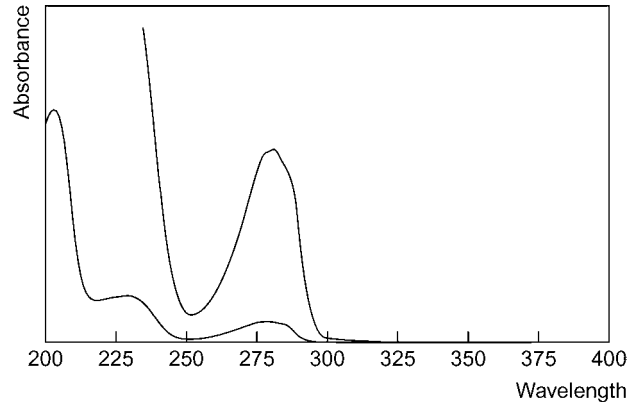
Colour Test Liebermann's reagent—black.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.13; system TC—R_f 0.34; system TE—R_f 0.53; system TL—R_f 0.12; system TAE—R_f 0.19; system TAJ—R_f 0.09; system TAK—R_f 0.02; system TAL—R_f 0.58 (acidified iodoplatinate solution, positive).

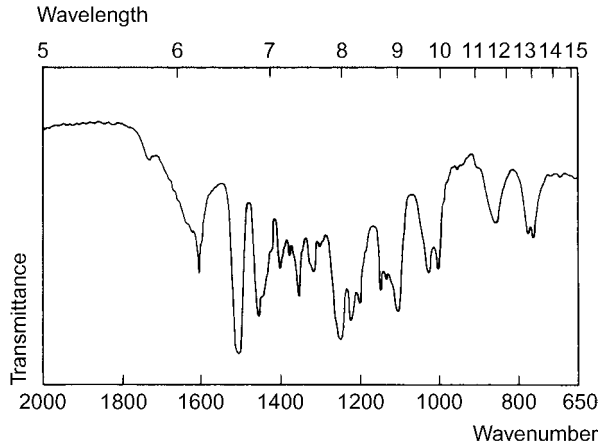
Gas Chromatography System GA—RI 2505.

High Performance Liquid Chromatography System HA—*k* 7.1 (tailing peak); system HX—RI 310; system HAA—retention time 9.4 min.

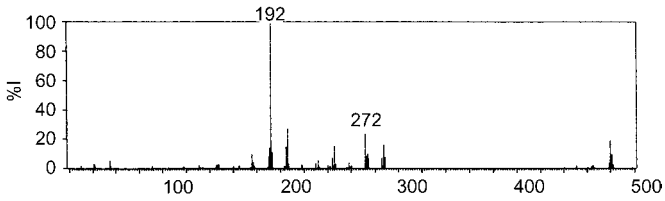
Ultraviolet Spectrum Aqueous acid—230 (A^1_1=341a), 282 nm (A^1_1=158a).



Infrared Spectrum Principal peaks at wavenumbers 1514, 1256, 1228, 1110, 1200, 1150 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 192, 206, 272, 480, 288, 246, 205, 191.



Quantification

Blood **HPLC** Fluorescence detection. Limit of detection, 5 µg/L [Crouch *et al.* 1984].

Plasma **HPLC** Fluorescence detection. Emetine and cephaëline. Limit of detection, <2.5 µg/L for emetine [Asano *et al.* 2001]. Fluorescence detection. Limit of detection, 10 µg/L [Bannister *et al.* 1979].

Urine **HPLC** See Plasma. Limit of detection, <5 µg/L for emetine [Asano *et al.* 2001]. See Blood [Crouch *et al.* 1984].

Disposition in the Body Rapidly absorbed after oral administration and after injection; concentrated in the liver, kidneys, lungs and spleen. It is excreted only very slowly in the urine and detectable concentrations are found up to 60 days after treatment.

Toxicity The minimum lethal dose is 150 mg.

In a fatality which occurred after chronic ingestion of about 70 mg of emetine daily for 3 months, the following postmortem tissue concentrations were reported: blood 2.4 µg/g, bile 1.9 µg/g, kidney 7.4 µg/g, liver 14 µg/g [Adler *et al.* 1980].

Dose Up to 60 mg of emetine hydrochloride daily, SC or IM, for no longer than 10 days.

Adler AG *et al.* (1980). Death resulting from ipecac syrup poisoning. *JAMA* 243(19): 1927–1928.

Asano T *et al.* (2001). High-performance liquid chromatographic assay with fluorescence detection for the determination of cephaeline and emetine in human plasma and urine. *J Chromatogr B Biomed Sci Appl* 757: 197–206.

Bannister SJ *et al.* (1979). High-performance liquid chromatographic analysis of emetine after oxidative activation to a fluorescent product. *J Chromatogr* 176: 381–390.

Crouch DJ *et al.* (1984). Quantitative analysis of emetine and cephaeline by reversed-phase high performance liquid chromatography with fluorescence detection. *J Anal Toxicol* 8: 63–65.

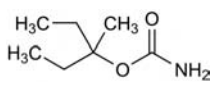
Emylcamate

Tranquilliser, Muscle Relaxant

$C_7H_{15}NO_2$ = 145.2

CAS—78-28-4

IUPAC Name 3-Methyl-3-pentanol carbamate



Chemical Properties A white crystalline powder. Mp 56°. Soluble 1 in 250 of water; freely soluble in benzene, ethanol, ether and glycol ethers. Log *P* (octanol/water), 1.8.

Gas Chromatography System GA—RI 1105.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1690, 1610, 1041, 1170, 1150, 1010 cm^{-1} (KCl disk).

Mass Spectrum Principal ions at *m/z* 73, 43, 84, 55, 69, 41, 44, 85.

Dose Emylcamate was formerly given in doses of 600 to 800 mg daily.

Enalapril

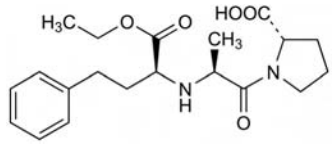
Antihypertensive

$C_{20}H_{28}N_2O_5$ = 376.5

CAS—75847-73-3

IUPAC Name (2*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl] pyrrolidine-2-carboxylic acid

Synonym (*S*)-1-[*N*-[1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline



Chemical Properties pK_a 2.97 and 5.35 (25°) (maleate). Log *P* (octanol/water), 2.45. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Enalapril Maleate

$C_{20}H_{28}N_2O_5,C_4H_4O_4$ = 492.5

CAS—76095-16-4

Synonyms L-154; MK-421; 739-001D.

Proprietary Names *Amprace*; *Bitensil*; *Cardiovet*; *Enacard*; *Enaloc*; *Enapren*; *Glioten*; *Hipoartel*; *Innovace*; *Lotrial*; *Olivin*; *Pres*; *Renitec*; *Reniten*; *Renivace*; *Vasotec*; *Xanef*.

Chemical Properties A white to off-white crystalline, odourless powder. Mp 143° to 144.5°. Solubility in water is 0.025 g/mL, alcohol 0.08 g/mL, methanol 0.2 g/mL and dimethyl formamide >400 g/L. It is, also, slightly soluble in semi-polar organic solvents; practically insoluble in non-polar organic solvents.

Enalaprilat

$C_{18}H_{24}N_2O_5,2H_2O$ = 384.4

CAS—76420-72-9 (anhydrous); 84680-54-6 (dihydrate)

Synonyms Enalaprilic acid; MK-422.

Chemical Properties A white to nearly white, hygroscopic crystalline powder, needles from water, Mp 148° to 151°. It is soluble in water (1 in 200), methyl alcohol (1 in 68), dimethyl formamide (1 in 40); slightly soluble in isopropyl alcohol; very slightly soluble in acetone, alcohol and hexane; practically insoluble in chloroform.

Colour Test Bromothymol blue (acidic aqueous, pH 2: $CHCl_3$)—yellow.

Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.00; system TE—R_f 0.00(enalapril), R_f 0.00(enalaprilat); system TF—R_f 0.00(enalapril), R_f 0.00(enalaprilat); system TAD—R_f 0.00; system TAE—R_f 0.85.

Plate: silica gel (Analtech GF, Whatman KLF or E Merck G60). Mobile phase: chloroform: methanol: acetic acid (90:10:1). Enalapril, Rf 0.6; enalaprilat, Rf 0.

Plate: silica gel (Analtech GF, Whatman KLF or E Merck G60). Mobile phase: n-butanol: water: acetic acid (3:1:1). Enalapril, Rf 0.7; enalaprilat, Rf 0.35.

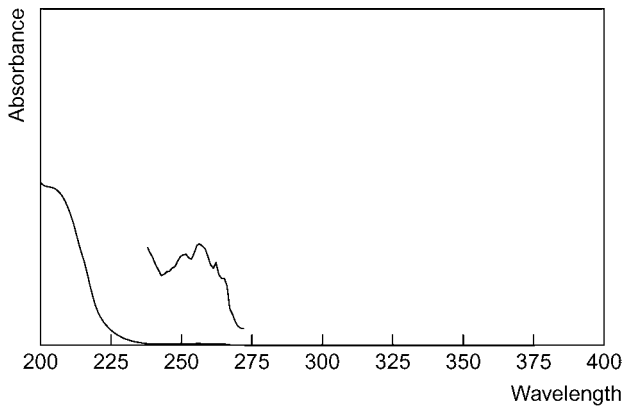
Plate: silica gel (Analtech GF, Whatman KLF or E Merck G60). Mobile phase: n-butanol: toluene: water: acetone: acetic acid (1:1:1:1). Enalapril, Rf 0.55; enalaprilat, Rf 0.45 [Ip, Brenner 1987].

Gas Chromatography System GA—RI 2620enalapril, RI 2650 enalapril-ME; system GP—RI 2675 enalapril-ME, RI 2680 M (enalaprilat)-ME3, RI 2735 M (enalaprilat-H_2O)-ME.

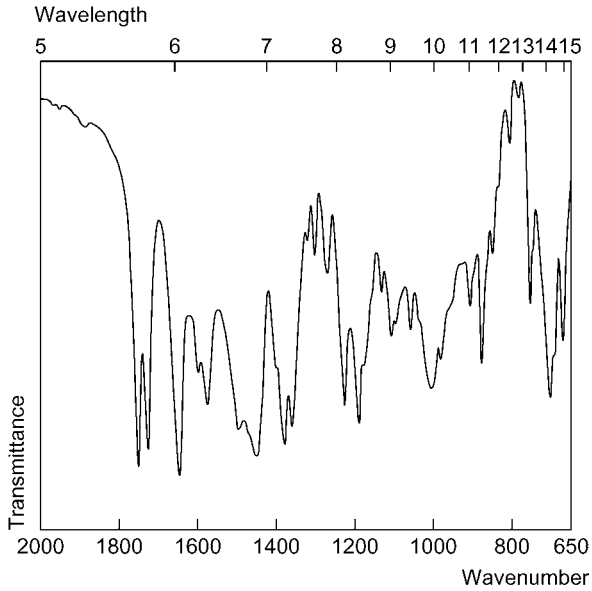
High Performance Liquid Chromatography System HX—RI 201; system HZ—retention time 1.5 min (enalaprilat); system HAA—retention time 3.4 min.

Column: Hypersil ODS (250 × 4.5 mm i.d., 5 µm). Mobile phase: 20 mmol/L sodium heptanesulfonate (pH 2.5) : acetonitrile (5% THF) (63:37), flow rate 1.0 mL/min. UV detection (λ=215 nm). Retention time: 10 min [Bonazzi *et al.* 1997].

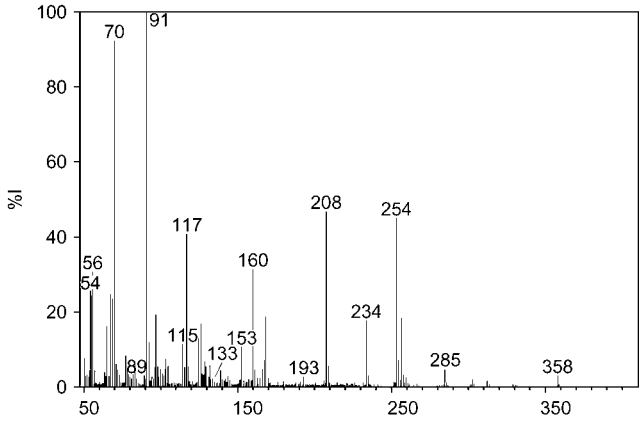
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—257, 268 nm; (0.1 mol/L phosphate buffer, pH 4.5: methanol (80:20)—229 nm (enalapril maleate).



Infrared Spectrum Principal peaks at wavenumbers 1750, 1645, 1425, 1390, 1187 cm^{-1} (maleate).



Mass Spectrum Principal ions at *m/z* 91, 70, 208, 254, 117, 56, 160, 54 (maleate).



Quantification

Plasma **GC–MS** Column: silica (DB-1, 10 m × 0.25 mm i.d., 0.25 µm). Temperature programme: held at 150° for 1 min, and increased to 280° at 30°/min. Injector temperature: 280°. Carrier gas: He. MS detection (NICI, SIM at m/z 302, enalapril; 288 enalaprilat). Reference compound: RS-5139. Retention time: enalapril 4.58 min; enalaprilat 4.49 min. Limit of detection, 0.2 µg/L and 2 µg/L for enalapril and enalaprilat, respectively [Shioya *et al.* 1992].

Serum **Fluoroimmunoassay** Limit of detection, 0.5 µg/L [Yuan, Gilbert 1996].

Urine **GC–MS** See Plasma [Shioya *et al.* 1992].

Disposition in the Body After oral administration, 60% of a dose is absorbed, and rapidly and extensively hydrolysed in the liver to enalaprilat. Peak plasma concentrations of enalaprilat are achieved 3 to 4 h after oral dose. Enalapril is excreted in urine as the metabolite, enalaprilat and the rest in faeces as the unchanged drug. After a single oral dose of 20 mg enalapril, enalapril and enalaprilat can be detected in breast milk with a concentration of 1 to 2.3 µg/L for the latter. Enalaprilat is removed by haemodialysis and peritoneal dialysis.

Therapeutic Concentration The serum therapeutic concentration range is 0.01 to 0.05 mg/L for the metabolite, desethylenalapril.

Eighteen healthy, normotensive subjects: 9 males, aged between 21 and 39 years, 6 males and 3 females, aged between 66 to 76 years, were administered 10 mg enalapril maleate after an overnight fast. Peak plasma concentrations of 128 ± 63 nmol/L enalaprilat were reached, within 4 h, for the younger group and 199 ± 105 nmol/L, within 5 h, for the more elderly group [MacDonald *et al.* 1993].

Toxicity Treatment with enalapril can result in renal failure, with the possibility of death. Severe hypotension is the main toxic effect and loss of hearing has also been reported by some.

Bioavailability Oral bioavailability, 53 to 74% (enalapril) and 36 to 44% (enalaprilat).

Half-life Elimination half-life is ~2 h for enalapril and ≥38 h for enalaprilat (also reported as 11 h).

Volume of Distribution 1.7 L/kg.

Protein Binding 50 to 60% bound.

Dose An initial dose of 5 mg daily is administered, followed by a maintenance dose of 10 to 20 mg daily. The maximum dose is 40 mg daily. In patients with renal impairment, or those also taking a diuretic and the elderly, an initial dose of 2.5 mg daily is administered.

Bonazzi D *et al.* (1997). Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). *J Pharm Biomed Anal* 16(3): 431–438.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Ip DP, Brenner GS. Brittain HG, ed.(1987). *Analytical Profiles of Drug Substances and Excipients*. New York: Academic Press, 16: 207–243

MacDonald NJ *et al.* (1993). The effects of age on the pharmacokinetics and pharmacodynamics of single oral doses of benazepril and enalapril. *Br J Clin Pharmacol* 36: 205–209.

Shioya H *et al.* (1992). Determination of enalapril and its active metabolite enalaprilat in plasma and urine by gas chromatography/mass spectrometry. *Biomed Chromatogr* 6(2): 59–62.

Yuan AS, Gilbert JD (1996). Time-resolved fluoroimmunoassay for the determination of lisinopril and enalaprilat in human serum. *J Pharm Biomed Anal* 14(7): 773–781.

Enallylpropymal

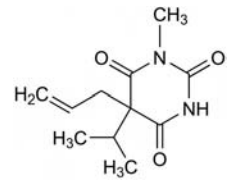
Barbiturate

$C_{11}H_{16}N_2O_3 = 224.3$

CAS—1861-21-8

IUPAC Name 1-Methyl-5-propan-2-yl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

Synonym 1-Methyl-5-(1-methylethyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione



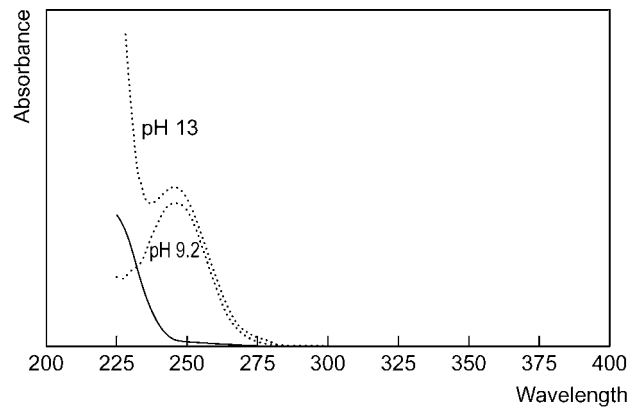
Chemical Properties Crystals. Mp 56° to 57°. Soluble in organic solvents. Log *P* (octanol/water), 1.6.

Thin-layer Chromatography System TD—R_f 0.71; system TE—R_f 0.58; system TF—R_f 0.71; system TH—R_f 0.87; system TAD—R_f 0.70.

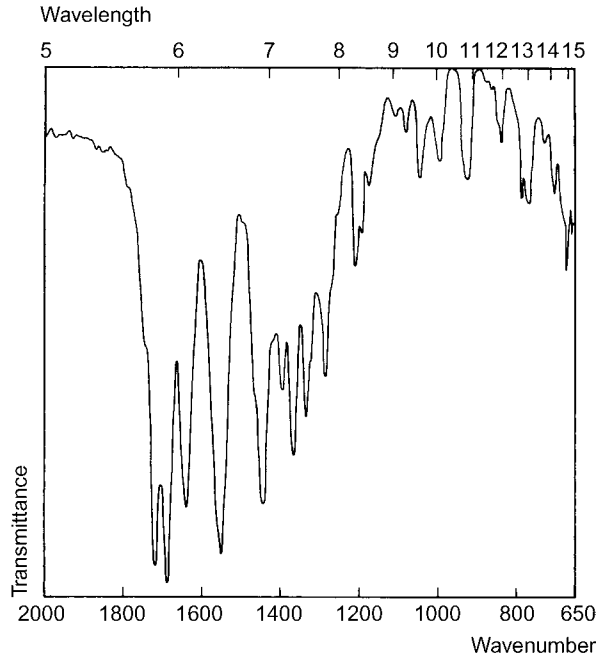
Gas Chromatography System GA—enallylpropymal RI 1560; enallylpropymal-Me_2 RI 1520.

High Performance Liquid Chromatography System HG—*k* 8.65; system HH—*k* 6.96; system HY—RI 394.

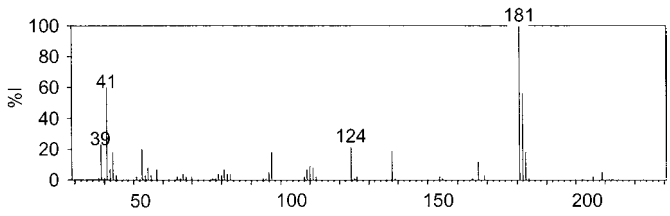
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—245 nm (A_1^1=329b); 1 mol/L sodium hydroxide (pH 13)—244 nm (A_1^1=362b).



Infrared Spectrum Principal peaks at wavenumbers 1689, 1718, 1550, 1640, 1285, 672 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 181, 41, 182, 39, 124, 53, 138, 97.



Encainide

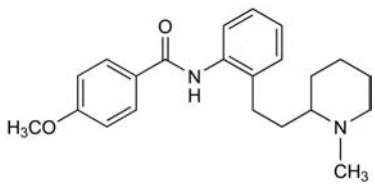
Antiarrhythmic

$C_{22}H_{28}N_2O_2$ = 352.5

CAS—66778-36-7; 37612-13-8

IUPAC Name 4-Methoxy-*N*-[2-[2-(1-methyl-2-piperidinyl)ethyl]phenyl] benzamide

Synonym MJ-9067-1



Chemical Properties pK_a 10.2. Log *P* (octanol/water), 4.24.

Encainide Hydrochloride

$C_{22}H_{28}N_2O_2$,HCl = 388.9

CAS—66794-74-9

Synonym MJ-9067

Proprietary Names *Enkade*; *Enkaid*.

Chemical Properties Crystalline powder. Mp 131.5° to 132.5°. It is freely soluble in water; slightly soluble in ethanol; insoluble in heptane.

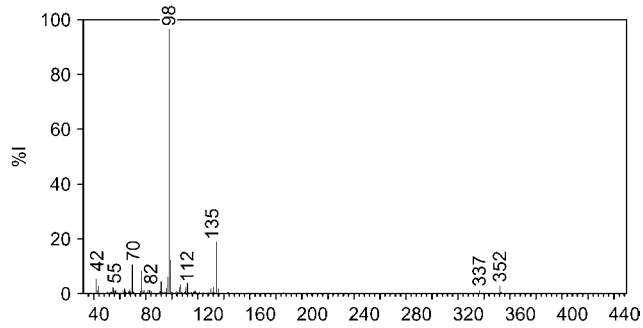
Thin-layer Chromatography System TB—R_f 0.28; system TE—R_f 0.54; system TAE—R_f 0.16.

Gas Chromatography System GA—RI 3016.

High Performance Liquid Chromatography System HX—RI 363; system HAX—retention time 9.4 min; system HAY—retention time 4.9 min.

Column: silica Resolve (150 mm × 4.6 mm i.d., 5 μm), (guard) 10 mm pre-column packed with same material. Mobile phase: methanol : water : methanesulfonic acid : triethylamine (400:4:0.02:0.02). Internal standard (IS): 4-methyl-propanolol. UV detection (λ=270 nm). Retention time: IS, 3.6 min; NDE, 4.5 min; ODE, 5.6 min; MODE, 6.3 min; encainide, 7.0 min [Poirier *et al.* 1990].

Mass Spectrum Principal ions at *m/z* 98, 135, 99, 70, 77, 97, 42, 112.



Quantification

***Plasma* HPLC** Column: phenyl μBondapak (300 × 3.9 mm i.d., 10 μm). Mobile phase: ethanol : water : methanesulfonic acid (500:30:0.1), flow rate 1.5 mL/min. UV detection (λ=254 nm). Retention time: encainide, 13.2 min; N-desmethyl encainide, 9.1 min; 3-methoxy-o-desmethyl encainide, 6.0 min; o-desmethyl encainide, 5.3 min. Limit of detection, 10 μg/L [Selinger, Crawhall 1989]. Column: silica Zorbax (250 × 4 mm i. d., 5 to 6 μm). Mobile phase: absolute ethanol : water : methanesulfonic acid (500:60:0.2), flow rate 1.0 mL/min. UV detection (λ=254 nm). Retention time: encainide, 9.29 min; 3-methoxy-o-desmethyl encainide (MODE), 8.15 min; o-desmethyl encainide (ODE), 7.11 min; N-desmethyl encainide (NDE), 6.00 min. Limit of detection, 5 μg/L [Bartek *et al.* 1988].

***Urine* HPLC** See Plasma. Limit of detection, 25 μg/L [Bartek *et al.* 1988].

Disposition in the Body Encainide is well absorbed and extensively metabolised in the liver to two active metabolites, (ODE) and (MODE). Inactive metabolites include (NDE) and sulfate and glucuronide conjugates. The extent of metabolism varies between individuals because there are extensive metabolisers and poor metabolisers. Approximately 7% of the Caucasian and Black population are poor metabolisers. Excretion varies between these individuals but is mainly via urine with approx. 30% of a dose present in faeces. 39% of an administered dose is found in the urine of poor metabolisers but only 4.9% for extensive metabolisers with 10.6% ODE and 3.6% MODE.

Therapeutic Concentration The serum therapeutic concentration range is 60 to 280 μg/L for the metabolite, MODE and 100 to 300 μg/L for ODE.

Eight healthy individuals, extensive and poor metabolisers, were administered a 50 mg dose encainide every 8 h. The extensive metabolisers reached plasma concentrations of 18 ± 6 μg/L encainide, 115 ± 11 μg/L ODE, 94 ± 10 μg/L MODE and <10 μg/L NDE. The poor metabolisers, however, had plasma concentrations of 450 μg/L encainide, 12 μg/L ODE and 80 μg/L NDE. No MODE was detected.

Six patients with hepatic disease were also studied with a dose of 50 mg encainide. Plasma concentrations of 63 ± 9 μg/L encainide, 87 ± 8 μg/L ODE and 113 ± 81 μg/L MODE were reached. No NDE was detected [Roden *et al.* 1988].

Toxicity Severe and potentially fatal ventricular tachycardia and cardiac arrest, congestive heart failure and seizures. Toxicity observed with ODE concentrations >300 μg/L.

Bioavailability 30% (extensive metabolisers) and 88% (poor metabolisers).

Half-life For encainide, 2.5 h (extensive metabolisers) and 8 to 11 h (poor metabolisers). For ODE, 4 to 8 h and MODE, 12 to 20 h.

Volume of Distribution Approximately 265 L.

Protein Binding Encainide is bound 70 to 78%, ODE 75 to 85% and MODE 92%.

Dose A usual dose of 100 to 200 mg daily is administered. In patients with renal clearance 10 to 50 mL/min: dose reduced by 50%, clearance <10 mL/min: initially 25 mg daily, which can be increased to a maximum of 150 mg daily.

Bartek MJ *et al.* (1988). Analysis of encainide and metabolites in plasma and urine by high-performance liquid chromatography. *Ther Drug Monit* 10(4): 446–452.

Poirier JM *et al.* (1990). Analysis of encainide and its three major metabolites in plasma by column liquid chromatography. *J Chromatogr* 534: 223–227.

Roden DM *et al.* (1988). Clinical pharmacokinetics of encainide. *Clin Pharmacokinet* 14: 141–147.

Selinger K, Crawhall JC (1989). Determination of encainide and its metabolites by high-performance liquid chromatography. *J Pharm Biomed Anal* 7(3): 355–359.

Endosulfan

Insecticide

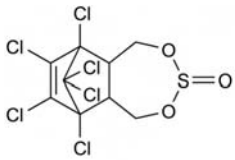
$C_9H_6Cl_6O_3S$ = 406.9

CAS—115-29-7; 959-98-8 (α-); 33213-65-9 (β-)

IUPAC Name 6,7,8,9,10,10-Hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide

Synonyms Benzoepin; 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-yl-enebismethylene sulphite.

Proprietary Names *Malix*; *Thiodan*; *Thionex*.

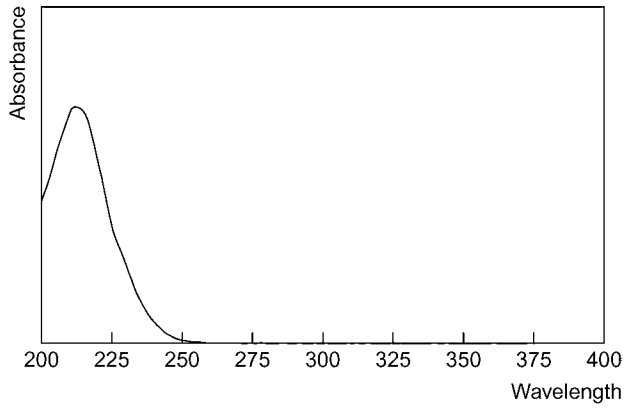


Chemical Properties Endosulfan is a mixture of 2 stereoisomers: the α-isomer, Mp 108° to 110°, accounts for ~70% of the technical grade and the β-isomer, Mp 208° to 210°, for ~30%. The technical grade is a brownish crystalline solid. Mp 70° to 100°. Practically insoluble in water; soluble in most organic solvents. Log *P* (octanol/water), 3.8.

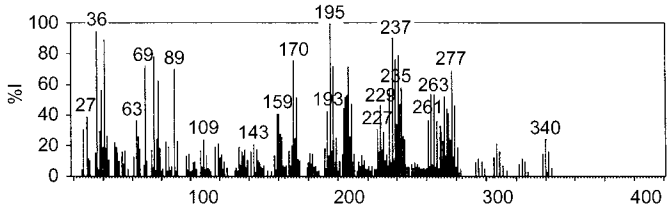
Thin-layer Chromatography System TX—R_f 0.40; system TY—R_f 0.77; system TZ—R_f 0.77; system TAA—R_f 0.95.

Gas Chromatography System GA—endosulfan RI 2090, M (sulfate) RI 2260, endosulfan II RI 2175; system GK—RRT 1.10 (relative to caffeine).

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 195, 36, 237, 41, 241, 75, 239, 170.



Quantification

Blood **GC-MS** SIM acquisition mode. Limit of detection, 0.1 ng/L [Ramesh, Ravi 2003]. ECD. Limit of detection, 1.0 μg/L [Ramesh, Ravi 2002]. Column: Ultra-1 (25 m × 0.33 mm i.d., 200 μm). Carrier gas: He, 1.7 mL/min. Temperature programme: 60° for 3 min to 290° at 12°/min for 10 min. EI ionisation. Limit of quantification, 0.3 μg/L, limit of detection, 0.09 μg/L [Garcia-Repetto *et al.* 2001]. Column: OV-1 3% on Chromosorb G AW 80/100 mesh. Carrier gas: N_2, 50 mL/min. Temperature: 200° or 175°. ECD or FID. Limit of detection, 15 μg [Coutselinis *et al.* 1976].

Serum **GC-MS** Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. EI ionisation. Limit of quantification, 0.25–2.00 μg/L, limit of detection, 0.05–0.62 μg/L [Moreno Frias *et al.* 2004]. Column: DB-5MS 5% phenylmethylpolysiloxane or DB-1MS 100% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 80° for 21 min to 290° at 10°/min for 9 min. EI ionisation or NCI, SIM acquisition mode. Retention time: 16.2 min. Limit of quantification, 0.05 μg/L [Dmitrovic *et al.* 2002]. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: N_2. ECD. Limit of quantification, 0.25–1.0 μg/L, limit of detection, 0.05–0.62 μg/L [Martinez Vidal *et al.* 2002]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 36.6 cm/s. Temperature programme: 60° for 1.5 min to 220° at 50°/min to 300° at 5°/min. EI ionisation at 70 eV. Limit of quantification, 26–68 ng/L, limit of detection, 6–19 ng/L [Arrebola *et al.* 2001]. Limit of detection, 0.03–5.0 μg/L [Martinez Vidal *et al.* 2000].

Milk **GC-MS** See Serum [Moreno Frias *et al.* 2004].

Lipid **GC-MS** See Serum [Moreno Frias *et al.* 2004].

Tissues **GC** See Blood [Coutselinis *et al.* 1976].

Disposition in the Body

Toxicity

In a death involving the ingestion of endosulfan dispersed in 55% xylene, the following concentrations of endosulfan were reported: blood 30 mg/L, liver 20 μg/g, kidney 2.0 μg/g and brain 0.3 μg/g; 0.5 g endosulfan and 0.4 g xylene were found in 50 mL stomach contents [Bernardelli, Gennari 1987].

In 6 reported cases of acute endosulfan intoxication, the 1 fatality had a blood endosulfan level of 2.85 mg/L compared with a mean of 0.48 mg/L in the survivors [Blanco-Coronado *et al.* 1992].

A 21-year-old woman who was 5 months pregnant ingested an unknown amount of endosulfan; the foetus died. Maternal blood levels were 0.47 μg/g. Highest levels of endosulfan were found in the liver and foetal kidneys [Sancewicz-Pach *et al.* 1997].

In a 43-year-old man who died 4 days after non-accidental ingestion of 100 mL of endosulfan (18 g, 260 mg/kg), the postmortem tissue concentrations of α-endosulfan, β-endosulfan and endosulfan sulfate, respectively, were adipose tissue 4.105 μg/g, –, –; liver <0.050, <0.055 and 3.034 μg/g; brain 0.080, 0.069 and 1.348 μg/g; kidney 0.059, <0.050 and 0.393 μg/g; and stomach contents 3.541, 1.391 and <0.080 μg/g. Blood concentrations 2.5 h after ingestion were 644, 101 and 874 μg/L, respectively, and declined to 106, 27 and 347 μg/L, respectively, at 7.5 h [Boereboom *et al.* 1998].

Cerebral oedema and cardiac failure developed following acute endosulfan poisoning in 2 subjects, 1 of whom died. Peak serum level of endosulfan in the survivor was 0.12 mg/L ~23 h after ingestion, whereas in the subject who died the peak blood level was 0.86 mg/L ~25 h after ingestion [Eyer *et al.* 2004].

In a retrospective study of 52 patients who presented with endosulfan toxicity, 16 patients died. Ingestion of endosulfan 35 g or more was associated with an increased risk of death [Moon, Chun 2009].

In a study on occupational exposure to pesticides on farms, mean baseline serum levels of endosulfan were 530 μg/L, which was considered high. After 1 day of spraying, serum levels of endosulfan increased by an average of 60 μg/L in workers applying the pesticide. Lower increases occurred in workers not directly involved in the spraying [Dalvie *et al.* 2009].

Note For other cases of endosulfan poisoning, see Brandt *et al.* [2001], Coutselinis *et al.* [1978], Demeter *et al.* [1977], Kucuker *et al.* [2009], Ramaswamy *et al.* [2008] and Satar *et al.* [2009].

For reviews of the characteristics of endosulfan poisoning, see Durukan *et al.* [2009] and Karatas *et al.* [2006].

Arrebola FJ *et al.* (2001). Analysis of endosulfan and its metabolites in human serum using gas chromatography–tandem mass spectrometry. *J Chromatogr Sci* 39: 177–182.
Bernardelli BC, Gennari MC (1987). Death caused by ingestion of endosulfan. *J Forensic Sci* 32: 1109–1112.
Blanco-Coronado JL *et al.* (1992). Acute intoxication by endosulfan. *J Toxicol Clin Toxicol* 30: 575–583.
Boereboom FT *et al.* (1998). Nonaccidental endosulfan intoxication: a case report with toxicokinetic calculations and tissue concentrations. *J Toxicol Clin Toxicol* 36: 345–352.
Brandt VA *et al.* (2001). Exposure to endosulfan in farmers: two case studies. *Am J Ind Med* 39: 643–649.
Coutselinis A *et al.* (1976). Separation and identification of the insecticide "endosulfan" from biological materials. *Forensic Sci* 8: 251–254.
Coutselinis A *et al.* (1978). Concentration levels of endosulfan in biological material (report of three cases). *Forensic Sci* 11: 75.
Dalvie MA *et al.* (2009). Pesticide exposure and blood endosulfan levels after first season spray amongst farm workers in the Western Cape, South Africa. *J Environ Sci Health B* 44: 271–277.
Demeter J *et al.* (1977). Toxicological analysis in a case of endosulfan suicide. *Bull Environ Contam Toxicol* 18: 110–114.
Dmitrovic J *et al.* (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicol Lett* 134: 253–258.
Durukan P *et al.* (2009). Experiences with endosulfan mass poisoning in rural areas. *Eur J Emerg Med* 16: 53–56.
Eyer F *et al.* (2004). Acute endosulfan poisoning with cerebral edema and cardiac failure. *J Toxicol Clin Toxicol* 42: 927–932.
Garcia-Repetto R *et al.* (2001). New method for determination of ten pesticides in human blood. *J AOAC Int* 84: 342–349.
Karatas AD *et al.* (2006). Characteristics of endosulfan poisoning: a study of 23 cases. *Singapore Med J* 47: 1030–1032.
Kucuker H *et al.* (2009). Fatal acute endosulfan toxicity: a case report. *Basic Clin Pharmacol Toxicol* 104: 49–51.
Martinez Vidal JL *et al.* (2000). Trace determination of alpha- and beta-endosulfan and three metabolites in human serum by gas chromatography electron capture detection and gas chromatography tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 939–946.
Martinez Vidal JL *et al.* (2002). Determination of endocrine-disrupting pesticides and polychlorinated biphenyls in human serum by GC-ECD and GC-MS-MS and evaluation of contributions to the uncertainty of the results. *Anal Bioanal Chem* 372: 766–775.
Moon JM, Chun BJ (2009) Acute endosulfan poisoning: a retrospective study. *Hum Exp Toxicol* 28: 309-316.
Moreno Frias M *et al.* (2004). Determination of organochlorine compounds in human biological samples by GC-MS/MS. *Biomed Chromatogr* 18: 102–111.
Ramaswamy S *et al.* (2008). Endosulfan poisoning with intravascular hemolysis. *J Emerg Med* 34: 295–297.
Ramesh A, Ravi PE (2002). A rapid and sensitive analytical method for the quantification of residues of endosulfan in blood. *J Environ Monit* 4: 190–193.
Ramesh A, Ravi PE (2003). Determination of residues of endosulfan in human blood by a negative ion chemical ionization gas chromatographic/mass spectrometric method: impact of long-term aerial spray exposure. *Pest Manag Sci* 59: 252–258.
Sancewicz-Pach K *et al.* (1997). Acute pesticides poisonings in pregnant women. *Przegl Lek* 54: 741–744.
Satar S *et al.* (2009). Unintentional endosulfan poisoning. *Bratisl Lek Listy* 110: 301–303.

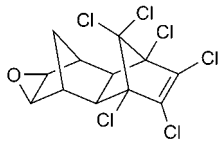
Endrin

Insecticide

$C_{12}H_8Cl_6O$ = 380.9

CAS—72-20-8

Synonym (1*a*α,2β,2*a*,β,3α,6α,6*a*β,7β,7*a*α)-3,4,5,6,9,9-Hexachloro-1*a*,2,2*a*,3,6,6*a*,7,7*a*-octahydro-2,7 : 3,6-dimethanonaphth[2,3-*b*]oxirene



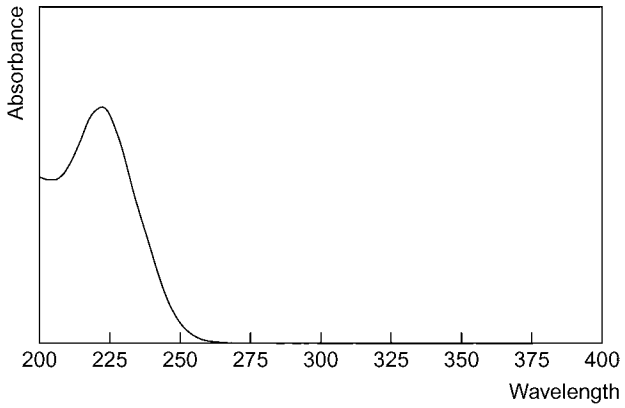
Chemical Properties White crystalline solid. Mp 245°, with decomposition. Practically insoluble in water; sparingly soluble in ethanol. Log *P* (octanol/water) 5.2.

Colour Tests Nitric–sulfuric acid—pink; sulfuric acid–fuming sulfuric Acid—pink-orange.

Thin-layer Chromatography System TAB—R_f 0.55; system TAC—R_f 0.35; system TX—R_f 0.71; system TY—R_f 0.90.

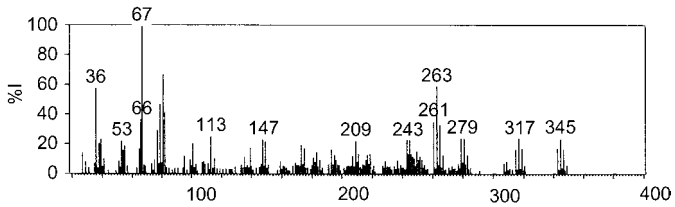
Gas Chromatography System GA—RI 2183; system GK—RT 1.19 relative to caffeine.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 851, 750, 724, 1049, 889, 1010 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 67, 81, 263, 36, 79, 82, 261, 265.



Quantification

Blood **GC** Column: TC-1 dimethylsilicone (15 m × 0.53 mm i.d., 1.5 µm) or TC-17 50% phenylmethyl silicone (15 m × 0.53 mm i.d., 1 µm). Carrier gas: N_2, 15 kPa. Temperature programme: 100° or 150° for 0 or 2 min, respectively to 260° at 10°/min for 27 min. FTD or FID. Limit of detection, 25 µg/L [Moriya, Hashimoto 1999].

GC-MS Column: BP 5 fused silica capillary (12 m × 0.22 mm i.d.). Carrier gas: He, 2.6 mL/min. Temperature programme: 60° to 280° at 30°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Kintz *et al.* 1992].

Serum **GC** Column: 5% phenyl methyl siloxane (30 m × 0.25 µm). Carrier gas: N_2, 30 mL/min. Temperature programme: 60° to 190° at 25°/min to 280° at 5°/min for 2 min. ECD. Limit of quantification, 1.4 µg/L [Delgado *et al.* 2002].

GC-MS Column: DB-5MS 5% phenylmethylpolysiloxane or DB-1MS 100% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 80° to 290° at 10°/min for 9 min. CI, negative ion mode, or EI ionisation. Retention time: 17.2 min. Limit of quantification, 0.25 µg/L [Dmitrovic *et al.* 2002].

Urine **GC** See Blood [Moriya, Hashimoto 1999] Column: 3% OV-225 Gas-Chrom Q 100/120 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 145 mL/min. Temperature: 225°. Retention time: 1.3 and 3.25 min for 12-ketoendrin and anti-12-hydroxyendrin. Limit of detection 0.0048 mg/L [Baldwin, Hutson 1980].

GC-MS See Blood [Kintz *et al.* 1992].

Bile **GC** See Blood [Moriya, Hashimoto 1999].

GC-MS See Blood [Kintz *et al.* 1992].

CSF **GC** See Blood [Moriya, Hashimoto 1999].

Milk **GC** Column: SPB-608 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 150° for 2 min to 200° at 15°/min to 290° at 5°/min for 10 min. ECD. Retention time: 24.9 min. [Al Saleh *et al.* 1998]. Column: 1.5% OV-17 or 1.9% OV-210 0n Gas-Chrom Q 80/100 mesh (6′ × 4 mm i.d.). Carrier gas: N_2, 80 mL/min. Temperature: 210°. Limit of detection not reported [Dogheim *et al.* 1996].

Pericardial Fluid **GC** See Blood [Moriya, Hashimoto 1999].

Stomach Contents **GC** See Blood [Moriya, Hashimoto 1999].

Vitreous Humour **GC** See Blood [Moriya, Hashimoto 1999].

GC-MS See Blood [Kintz *et al.* 1992].

Adipose Tissue **GC-MS** Column. DB-5 fused silica capillary (30 m × 0.32 mm i.d, 1.0 µm). Carrier gas: He, 1–2 mL/min. Temperature programme: 80° for 1 min to 190° at 30°/min to 270° at 2°/min for 15 min to 290° at 15°/min for 5 min. Limit of detection, 10 µg/kg [Djordjevic *et al.* 1994].

Cerebrum **GC** See Blood. Limit of detection, 0.1 mg/kg [Moriya, Hashimoto 1999].

Femoral Muscle **GC** See Cerebrum [Moriya, Hashimoto 1999].

Kidney **GC** See Cerebrum [Moriya, Hashimoto 1999].

Liver **GC** See Cerebrum [Moriya, Hashimoto 1999].

Lung **GC** See Cerebrum [Moriya, Hashimoto 1999].

Myocardium **GC** See Cerebrum [Moriya, Hashimoto 1999].

Spleen **GC** See Cerebrum [Moriya, Hashimoto 1999].

Note For a colorimetric method for the detection of endrin in blood, urine or tissues, see Sane and Kamat [1981].

Disposition in the Body The major metabolite in the rat is *anti*-12-hydroxyendrrin, which is eliminated in the bile as the glucuronide but is excreted non-conjuated in the faeces. 12-Ketoendrin is a minor metabolite [Baldwin, Hutson 1980].

Toxicity

Endrin concentrations in blood 4 h, 6 days, and 11 days after ingestion of 12 g by a 49-year-old male were 450, 86 and 71 µg/L, respectively; corresponding dieldrin concentrations were 60, 19 and 19 µg/L. The man died on the 11th day following ingestion and at postmortem the tissue : blood ratios were as shown below.

Tissue	*Endrin concentration (mg/kg)*	*Ratio tissue/blood*
Adipose tissue	89.5	1260
Blood	0.071	1.0
Brain	0.89	12.5
Heart	0.87	12.3
Kidney	0.55	7.7
Liver	1.32	18.6

[Runhaar *et al.* 1985]

In a fatality attributed to the ingestion of endrin by an 86-year-old female, postmortem tissue concentrations, approximately 20 h after death, were (mg/L or µg/g): femoral vein blood 0.353, left cardiac chamber blood 0.615, thoracic aorta blood 0.542, bile 2.06, myocardium 0.467, cerebrum 1.93, cerebrospinal fluid 0.515, vitreous humour 1.67, left hilus of lung 6.20, right hilus of lung 1.19, liver 13.8, kidney none detected, right femoral muscle 2.08 and a stomach content total of 66 mg [Moriya, Hashimoto 1999].

A 21-year-old Caucasian female was found dead at home. The distribution of endrin (mg/L) was as shown below.

Tissue	*Endrin (mg/L)*
Blood	544.9
Bile	780.5
Stomach contents	47351.6
Urine	6.2

[Kintz *et al.* 1992]

Postmortem tissue concentrations from individuals from Pakistan, Qatar, and the Netherlands were as shown below.

Tissue	*Pakistan*	*Qatar*	*Netherlands*
Adipose	4010	N.D.	89500
Brain	1680	N.D.	890
Kidney	1760	116	100
Liver	1430	685	1320
Small intestine	13690	N.D.	N.D.
Stomach wall	N.D.	160	N.D.

N.D. Not determined [Rowley *et al.* 1987].

Note For reports of toxicity following shipments of endrin-contaminated flour, see Weeks [1967] or Coble *et al.* [1967]. For possible cases of endrin contamination of taquitos, see the Centre for Disease Control (CDC) [1989] or Waller *et al.* [1992].

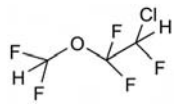
Al Saleh I *et al.* (1998). Residue levels of organochlorinated insecticides in breast milk: a preliminary report from Al-Kharj, Saudi Arabia. *J Environ Pathol Toxicol Oncol* 17: 37–50.

Baldwin MK *et al.* (1980). Analysis of human urine for a metabolite of endrin by chemical oxidation and gas-liquid chromatography as an indicator of exposure to endrin. *Analyst* 105: 60–65.
Centre for Disease Control (CDC) (1989). Endrin poisoning associated with taquito ingestion–California. *MMWR Morb Mortal Wkly Rep* 38: 345–347.
Coble Y *et al.* (1967). Acute endrin poisoning. *JAMA* 202: 489–493.
Delgado IF *et al.* (2002). Serum levels of organochlorine pesticides and polychlorinated biphenyls among inhabitants of Greater Metropolitan Rio de Janeiro, Brazil. *Cad Saude Publica* 18: 519–524.
Djordjevic MV *et al.* (1994). Assessment of chlorinated pesticides and polychlorinated biphenyls in adipose breast tissue using a supercritical fluid extraction method. *Carcinogenesis* 15: 2581–2585.
Dmitrovic J *et al.* (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicol Lett* 134: 253–258.
Dogheim SM *et al.* (1996). Monitoring of pesticide residues in human milk, soil, water, and food samples collected from Kafr El-Zayat Governorate. *J AOAC Int* 79: 111–116.
Kintz P *et al.* (1992). A high endrin concentration in a fatal case. *Forensic Sci Int* 54: 177–180.
Moriya F, Hashimoto Y (1999). Comparative studies on tissue distributions of organophosphorus, carbamate and organochlorine pesticides in decedents intoxicated with these chemicals. *J Forensic Sci* 44: 1131–1135.
Rowley DL *et al.* (1987). Convulsions caused by endrin poisoning in Pakistan. *Pediatrics* 79: 928–934.
Runhaar EA *et al.* (1985). A case of fatal endrin poisoning. *Hum Toxicol* 4: 241–247.
Sane RT, Kamat SS (1981). Colorimetric determination of endrin in biological materials. *Forensic Sci Int* 18: 63–66.
Waller K *et al.* (1992). Seizures after eating a snack food contaminated with the pesticide endrin. The tale of the toxic taquitos. *West J Med* 157: 648–651.
Weeks DE (1967). Endrin food-poisoning. A report on four outbreaks caused by two separate shipments of endrin-contaminated flour. *Bull World Health Organ* 37: 499–512.

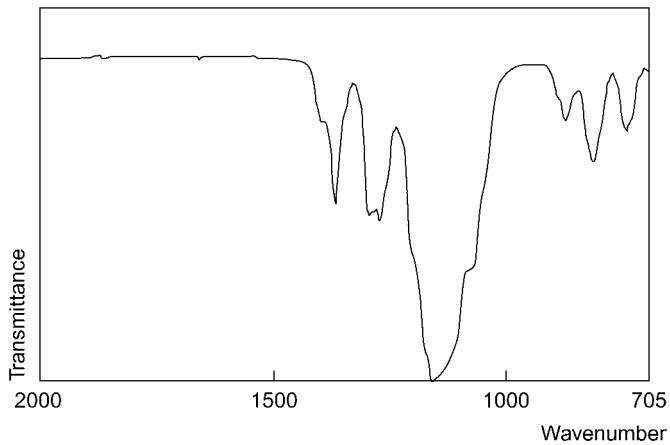
Enflurane

Anaesthetic (General)
$C_3H_2ClF_5O = 184.5$
CAS—13838-16-9

IUPAC Name 2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane
Synonyms Anaesthetic compound No. 347; 2-chloro-1,1,2-trifluoroethyl difluoromethyl ether; methylflurether; NSC-115944.
Proprietary Names *Alyrane*; *Efrane*; *Enfran*; *Enlirane*; *Ethrane*; *Etrane*.



Chemical Properties A colourless volatile liquid. Bp 56.5°. Relative density 1.516 to 1.519. Miscible with other organic liquids including fats and oils. Log *P* (octanol/water), 2.1.
Gas Chromatography System GA—RI 462; system GI—RT 8.3 min.
Infrared Spectrum



Quantification

Blood **GC** Column: Rtx-volatile fused silica capillary (30 m × 0.32 mm i.d., 1.5 µm). Carrier gas: He, 2 mL/min. Temperature programme: -40° for 1 min to 70° at 10°/min to 250° at 20°/min for 4 min. FID. Limit of detection, 10 µg/L [Kojima *et al.* 2001]. Column: DB-WAX (30 m × 0.53 mm i.d., 1.0 µm). Carrier gas: He, 20 mL/min. Temperature: 35°. FID. Limit of detection, 200 ng/0.25 g [Ise *et al.* 1997]. Column: SE-30 5% on Varaport 30 100/120 mesh (6 ft × 0.125 in o.d.). Temperature programme: 80° for 2 min to 200° at 50°/min for 1 min to 80°. TCD. Retention time: 0.8 min. Limit of detection, 1.5 µg/L [Miller, Gandolfi 1979].

GC-MS Column: DB-5 capillary (60 m × 0.25 mm i.d., 0.25 µm). Limit of quantification, ~20 mg/L [Yang *et al.* 2001]. Column: HP-WAX (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 35° for 5 min to 85° at 10°/min. Limit of detection, 200 ng/0.25 g [Ise *et al.* 1997].

Plasma **GC-MS** Column: DB-1 (30 m × 0.53 mm i.d., 5.0 µm). Carrier gas: He, 15 mL/min. Temperature: 60°. EI ionisation, positive ion mode, SIM acquisition mode. Limit of detection, 0.2 mg/L [Saito *et al.* 1995].
Adipose Tissue **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Brain **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Kidney **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Liver **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Lung **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Skeletal Muscle **GC** See Blood [Ise *et al.* 1997].
GC-MS See Blood [Ise *et al.* 1997].
Spleen **GC** See Blood. Limit of detection, 300 ng/0.25 g [Ise *et al.* 1997].
GC-MS See Blood. Limit of detection, 300 ng/0.25 g [Ise *et al.* 1997].
Reviews Toxicological and occupational/therapeutic monitoring of enflurane, desflurane, isoflurane and sevoflurane; a review of analytical methods, particularly GC methods with head space, purge and trap, or pulse heating extraction [Pihlainen, Ojanperä 1998].

In vaporisers, exhaled and inhaled gas mixtures, body fluids and tissues: enflurane, halothane, isoflurane and nitrous oxide, a review of GC methods [Uyanik 1997].

Disposition in the Body Enflurane is readily absorbed on inhalation. Approximately 50% of a dose is exhaled unchanged in 18 h and more than 80% is exhaled in 5 days. Less than 5% of a dose is excreted in the urine as non-volatile metabolites. Difluoromethoxydifluoroacetic acid has been identified as a urinary metabolite. Enflurane metabolism may be mainly catalysed by P450 isoform CYP2E1 [Kharasch *et al.* 1994].

Therapeutic Concentration

In 10 subjects receiving anaesthesia with 0.5–2.5% enflurane, a mean peak venous blood concentration of 95 mg/L was attained in 30 min, declining to 0.5 mg/L at 90 min after discontinuation of anaesthesia [Corall *et al.* 1977].

In 26 obese and 8 non-obese subjects given pharmacologically equivalent doses of enflurane (~2 h of monitored anaesthetic care), arterial enflurane reach maximal levels 3 times faster in obese than in non-obese subjects. The enflurane blood–gas partition coefficient was 30% lower in obese subjects and the rate at which inorganic fluoride appeared in serum of obese subjects (5.5 µmol/L/h) was twice that seen in non-obese subjects. Maximum serum fluoride occurred at 2 h post-anaesthesia and was 60% higher in obese subjects (27.8 vs 17 µmol/L), indicating increased biotransformation in obese subjects [Miller *et al.* 1980].

A mean peak serum fluoride concentration of 0.2 mg/L was attained 3 h after induction in 18 subjects receiving 0.6–2% of enflurane [Duvaldestin *et al.* 1981].

In 26 subjects undergoing cardiac surgery requiring hypothermic cardiopulmonary bypass, pre-bypass enflurane blood concentrations following delivered enflurane concentrations of 0.5%, 0.8% and 1% were 48, 52 and 115 mg/L, respectively. During hypothermia (28°), corresponding enflurane levels were 44, 56 and 145 mg/L, respectively, indicating that during cooling with enflurane at a concentration of 1% (but not with 0.5% and 0.8%) significant increases in blood levels occurred [Goucke *et al.* 2007].

Toxicity

A 21-year-old man found dead after apparent accidental intoxication with enflurane following its abuse had the following tissue concentrations $3^1/_2$ days later: blood 130 mg/L, brain 350 mg/L, SC fat 100 mg/L [Jacob *et al.* 1989].

An anaesthetist who was sniffing from an enflurane-moistened handkerchief before a road traffic accident was found to have an enflurane blood level of 2.92 mg/L, which was considered to have impaired his driving [Musshoff *et al.* 2002].

For a fatal enflurane poisoning by recreational inhalation, see Walker, Morano [1990].

Half-life ~36 h.
Dose For induction of anaesthesia, up to 4.5% of the vapour by inhalation; maintenance, 0.5 to 3% [Sweetman 2007].

Corall IM *et al.* (1977). Enflurane (Ethrane) anaesthesia in man: metabolism and effects on biochemical and haematological variables. *Br J Anaesth* 49: 881–885.
Duvaldestin P *et al.* (1981). Enflurane anesthesia and antipyrine metabolism. *Clin Pharmacol Ther* 29: 61–64.
Goucke CR *et al.* (2007). Blood concentrations of enflurane before, during, and after hypothermic cardiopulmonary bypass. *J Cardiothorac Vasc Anesth* 21: 218–223.
Ise H *et al.* (1997). Simple and rapid determination of enflurane in human tissues using gas chromatography and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 698: 97–102.
Jacob B *et al.* (1989). Fatal accidental enflurane intoxication. *J Forensic Sci* 34: 1408–1412.
Kharasch ED *et al.* (1994). Clinical enflurane metabolism by cytochrome P450 2E1. *Clin Pharmacol Ther* 55: 434–440.
Kojima T *et al.* (2001). Sensitive determination of four general anaesthetics in human whole blood by capillary gas chromatography with cryogenic oven trapping. *J Chromatogr B Biomed Sci Appl* 762: 103–108.
Miller MS, Gandolfi AJ (1979). A rapid, sensitive method for quantifying enflurane in whole blood. *Anesthesiology* 51: 542–544.

Miller MS *et al.* (1980). Disposition of enflurane in obese patients. *J Pharmacol Exp Ther* 215: 292–296.
Musshoff F *et al.* (2002). An unusual case of driving under the influence of enflurane. *Forensic Sci Int* 128: 187–189.
Pihlainen K, Ojanperä I (1998). Analytical toxicology of fluorinated inhalation anaesthetics. *Forensic Sci Int* 97: 117–133.
Saito K *et al.* (1995). Determination of the volatile anesthetics halothane, enflurane, isoflurane, and sevoflurane in biological specimens by pulse-heating GC-MS. *J Anal Toxicol* 19: 115–119.
Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35 edn. London: Pharmaceutical Press.
Uyanik A (1997). Gas chromatography in anaesthesia. I. A brief review of analytical methods and gas chromatographic detector and column systems. *J Chromatogr B Biomed Sci Appl* 693: 1–9.
Walker FB, Morano RA (1990). Fatal recreational inhalation of enflurane. *J Forensic Sci* 35: 197–198.
Yang NC *et al.* (2001). Simultaneous determination of fluorinated inhalation anesthetics in blood by gas chromatography–mass spectrometry combined with a headspace autosampler. *J Chromatogr B Biomed Sci Appl* 759: 307–318.

Enoxacin

Antibiotic, Fluoroquinolone

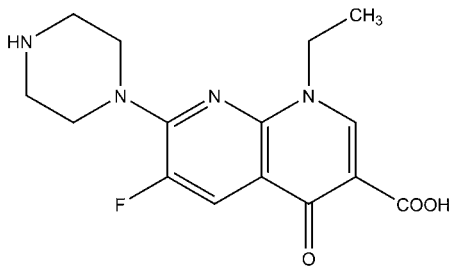
$C_{15}H_{17}FN_4O_3 = 320.3$

CAS—74011-58-8

IUPAC Name 1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-[1,8]naphthyridine-3-carboxylic acid

Synonyms AT-2266; CI-919; 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid; PD-107779.

Proprietary Names *Abenox*; *Comprecin*; *Flumark*; *Penetrex*.



Chemical Properties Crystals. Mp 220° to 224°. Soluble in water. pK_{a1} 6.3, pK_{a2} 8.7 [Jaehde *et al.* 1995; Neugebauer *et al.* 2005]. Log *P* (octanol/water), −0.20 [Sangster 1997], 0.387 [Wishart 2006]. Stock solutions stored refrigerated at 4° were stable for 2 months [Samanidou *et al.* 2005]. Stability was verified in serum refrigerated for 6 months [Samanidou *et al.* 2003]. Plasma and tissue extracts were stable for 4 days at 4° [Hamel *et al.* 1998].

Enoxacin Sesquihydrate

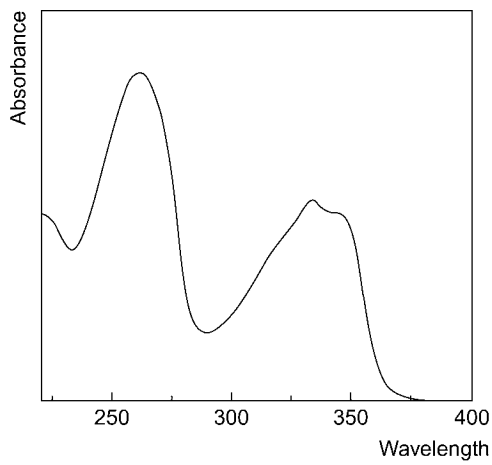
$C_{15}H_{17}FN_4O_3, {}^3/_2H_2O = 347.3$

CAS—84294-96-2

Proprietary Names *Bactidan*; *Enoxen*; *Enoxor*; *Gyramid*.

High Performance Liquid Chromatography Column: PerfectSil Target ODS-3 (250 × 4 mm i.d., 5 µm) Mobile phase: 0.1% trifluoroacetic acid : acetonitrile : methanol (80 : 10 : 10 for 10 min to 80 : 20 : 0 at 20 min to 45 : 55 : 0 at 30 min for 3 min to 80 : 10 : 10 at 33.1 min), flow rate 1.2 mL/min. DAD (λ = 275 nm). Limit of detection, 0.2 mg/L [Samanidou *et al.* 2005].

Ultraviolet Spectrum Aqueous acid—268, 219, 338 nm.



Infrared Spectrum Principal peaks at wavenumbers 2400 to 2000, 1615 cm^{-1} (KBr disk).

Quantification

***Plasma* HPLC** Column: C_{18} Nucleosil (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 95 mmol/L citric acid–35 mmol/L ammonium perchloride (pH 2.1) : tetrabutylammonium hydroxide (209 : 788 : 3), flow rate 0.9 mL/min. UV detection (λ = 340 nm). Limit of quantification, 20 µg/L [Hamel *et al.* 1998]. Column: reversed phase (125 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L citric acid buffer containing 22 mmol/L ammonium perchlorate : acetonitrile containing 5 mmol/L ion pairing reagent (88 : 12). UV detection (λ = 340 nm). Limit of quantification, 41.9 µg/L [Well *et al.* 1998]. Column: reversed phase Waters C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.08 mol/L potassium dihydrogen phosphate and 6 mmol/L tetrabutylammonium hydroxide (pH 2.5 in 1 L of dH_2O, plus 350 mL methanol), flow rate 2.0 mL/min. UV detection (λ = 268 nm). Limit of detection, 50 µg/L [Zhai *et al.* 1995]. Column: Spherisorb ODS II C_{18} (5 µm). Mobile phase: acetonitrile : 0.1 mol/L citric acid–40 mmol/L ammonium perchlorate–5 mmol/L tetrabutylammonium hydroxide (12 : 88 to 14 : 86), flow rate 1.0 to 2.0 mL/min. UV detection (λ= 340 nm). Limit of quantification, 39 µg/L [Jaehde *et al.* 1995].

***Serum* HPLC** Column: Nova-Pak C_{18} (150 × 3.9 mm i.d.). Mobile phase: 30 mmol/L dipotassium hydrogen phosphate trihydrate (pH 3.0) : tetrahydrofuran (97 : 3). Fluorometric detection (λ_{ex} = 277 nm, λ_{em} = 409 nm). Limit of detection, 4.9 µg/L [Espinosa-Mansilla *et al.* 2005]. Column: Kromasil 100 C_8 (250 × 4 mm i. d., 5 µm). Mobile phase: acetonitrile : methanol : 0.4 mol/L citric acid (7 : 15 : 78), flow rate 1.2mL/min. UV detection (λ = 275 nm). Limit of detection, 1 µg/L [Samanidou *et al.* 2003].

***Oral Fluid* HPLC** Column: reversed phase Waters C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.08 mol/L potassium dihydrogen phosphate and 6 mmol/L tetrabutylammonium hydroxide (pH 2.5 in 1 L of dH_2O, plus 350 mL methanol), flow rate 2.0 mL/min. UV detection (λ= 268 nm). Limit of detection, 50 µg/L [Zhai *et al.* 1995].

***Urine* LC-MS** Column: YMC/3-4-5 (50 × 4.0 mm i.d., 3 µm). Mobile phase: acetonitrile : water with 0.02 mol/L formic acid (pH 2.75; 2 : 98 to 57 : 43 in 10 min), flow rate 1 mL/min. ESI, MRM acquisition mode. Retention time: 3.68 min. Limit of detection not reported [Volmer *et al.* 1997].

HPLC Column: Nova-Pak C_{18} (150 × 3.9 mm i.d.). Mobile phase: 30 mmol/L dipotassium hydrogen phosphate trihydrate (pH 3.0) : tetrahydrofuran (96 : 4). Fluorometric detection (λ_{ex} = 277 nm, λ_{em} = 409 nm). Limit of detection, 4.9 µg/L [Espinosa-Mansilla *et al.* 2005]. Column: reversed phase (125 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L citric acid buffer containing 22 mmol/L ammonium perchlorate : acetonitrile containing 5 mmol/L ion pairing reagent (88 : 12). UV detection (λ = 340 nm). Limit of quantification, 99.1 µg/L [Well *et al.* 1998]. See Plasma. Limit of quantification, 1.95 mg/L [Jaehde *et al.* 1995].

***Nasal Secretions* HPLC** See Plasma. Limit of quantification, 78 µg/L [Jaehde *et al.* 1995].

***Tissues* HPLC** Column: C_{18} Nucleosil column (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 95 mmol/L citric acid–35 mmol/L ammonium perchloride (pH 2.1) tetrabutylammonium hydroxide (209 : 788 : 3), flow rate 0.9 mL/min. UV detection (λ = 340 nm). Limit of quantification, 50 µg/kg [Hamel *et al.* 1998].

***Other* LC-MS** Cow M ilk. See Urine. Limit of quantification, 2 mg/L, limit of detection, 2 mg/L [Volmer *et al.* 1997].

CE Pig Muscle. Capillary: uncoated fused silica (48.5 cm × 50 µm i.d., 40.0 cm to detector). Buffer: 25 mmol/L sodium dihydrogen phosphate with 25 mmol/L borax with 25 mmol/L boric acid (pH 9.0). DAD (λ = 280 nm). Limit of quantification, 0.075 mg/kg, limit of detection, 0.023 mg/kg [Sun *et al.* 2007].

Disposition in the Body Approximately 60% of enoxacin is excreted unchanged in the urine [Hamel *et al.* 1998] and another 10 to 15% is excreted as an active metabolite (3-oxoenoxacin).

Therapeutic Concentration

After an overnight fast, 11 healthy male volunteers were administered a single dose of 428 mg enoxacin IV over 60 min. Mean peak plasma concentrations of enoxacin and 3′-oxoenoxacin were 4.42 mg/L and 0.44 mg/L, respectively. Mean peak saliva concentrations for the same analytes were very similar at 4.14 mg/L and 0.11 mg/L, respectively. Mean peak enoxacin concentrations in tears and sweat were measured as 0.72 and 0.35 mg/L, respectively [Jaehde *et al.* 1995].

Bioavailability Approximately 90%.

Half-life Plasma, 5.1 h; oral fluid, 3.75 h; nasal secretions, 5.13 h; tears, 6.03 h and sweat, 8.27 h.

Volume of Distribution Steady state, 191 L.

Clearance 360 mL/min.

Protein Binding Approximately 51%.

Dose 200 to 400 mg twice daily.

Espinosa-Mansilla A *et al.* (2005). HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: application to urine and serum. *J Chromatogr B Analyt Technol Biomed Life Sci* 822: 185–193.
Hamel B *et al.* (1998). Reversed-phase high-performance liquid chromatographic determination of enoxacin and 4-oxo-enoxacin in human plasma and prostatic tissue. Application to a pharmacokinetic study. *J Chromatogr A* 812: 369–379.
Jaehde U *et al.* (1995). Distribution kinetics of enoxacin and its metabolite oxoenoxacin in excretory fluids of healthy volunteers. *Antimicrob Agents Chemother* 39: 2092–2097.
Neugebauer U *et al.* (2005). Vibrational spectroscopic characterization of fluoroquinolones. *Spectrochim Acta A Mol Biomol Spectrosc* 61: 1505–1517.
Samanidou VF *et al.* (2003). Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC. *Anal Bioanal Chem* 375: 623–629.

Samanidou VF *et al.* (2005). Validation of a novel HPLC sorbent material for the determination of ten quinolones in human and veterinary pharmaceutical formulations. *J Sep Sci* 28: 2444–2453.
Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* Chichester, UK: Wiley.
Sun HW *et al.* (2007). Effective separation and simultaneous determination of seven fluoroquinolones by capillary electrophoresis with diode-array detector. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 145–151.
Volmer DA *et al.* (1997). Study of 4-quinolone antibiotics in biological samples by short-column liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Anal Chem* 69: 4143–4155.
Well M *et al.* (1998).*et al.* Urinary bactericidal activity and pharmacokinetics of enoxacin versus norfloxacin and ciprofloxacin in healthy volunteers after a single oral dose. *Int J Antimicrob Agents* 10: 31–38.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.
Zhai S *et al.* (1995). Simultaneous determination of theophylline, enoxacin and ciprofloxacin in human plasma and saliva by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 669: 372–376.

Enoxaparin

Anticoagulant
CAS—74011-58-8

IUPAC Name 1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,8-naphthyridine-3-carboxylic acid
Synonyms Enoxaparine; low molecular weight Heparin; PK10169; RP-54563.
Proprietary Name *Lovenox*
Note Low molecular weight fragment of depolymerised heparin = ~4000–6000 Da.
Chemical Properties Obtained by peroxide fragmentation of heparin sodium.

Enoxaparin Sodium

CAS—9041-08-1
Synonyms Enoxaparinum natricum; heparin sodium.
Proprietary Names *Clexane*; *Decipar*; *Klexane*; *Lovenox*; *Plancina*; *Trombenox*; *Ultraparin*.

Quantification

Plasma **Bioassay** For method for bioassay of antifactor II_a activity, see Larsen *et al.* [1978].
Disposition in the Body Enoxaparin is rapidly and completely absorbed after SC injection with a peak plasma activity reached in 1–5 h. It is metabolised in the liver by desulfation and depolymerisation. It is excreted via the kidneys and is found unchanged and as its metabolites in urine. Enoxaparin is poorly absorbed after oral administration.
Therapeutic Concentration
Twelve healthy volunteers were administered with single doses of 20, 40, 60 and 80 mg enoxaparin. Peak plasma concentrations were between 1.6 and 7.5 mg/L and were reached between 2.3 and 4.0 h [Frydman *et al.* 1988].
Toxicity Large oral doses should not lead to any serious effects. Accidental overdosing, by parenteral administration, may lead to haemorrhagic problems with severe bleeding. Risk of bleeding increases with plasma concentrations of antifactor X_A >0.4 units/mL.
Bioavailability About 92%.
Half-life 4 to 5 h, 6 to 7 h in the elderly.
Volume of Distribution Between 5.2 and 9.3 L.
Clearance Total body clearance varies from 0.8 to 1.9 L/h.
Protein Binding Enoxaparin binds endogenous plasma proteins, for example, histidine-rich glycoprotein and fibronectin.
Note For a review of the pharmacokinetics of enoxaparin, see Noble *et al.* [1995]. For a review of low-molecular-weight heparins, see Cziraky and Spinler [1993].
Dose Dose ranges from 1 mg (100 International Units, IU)/kg body weight every 12 h with increases in 0.5 to 1 mg/kg body weight increments to a single dose of 40 mg (2000 IU) once daily.

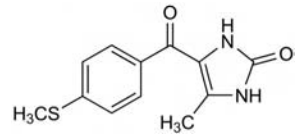
Cziraky MJ, Spinler SA (1993). Low-molecular-weight heparins for the treatment of deep-vein thrombosis. *Clin Pharm* 12: 892–899.
Frydman AM *et al.* (1988). The antithrombotic activity and pharmacokinetics of enoxaparine, a low molecular weight heparin, in humans given single subcutaneous doses of 20 to 80 mg. *J Clin Pharmacol* 28: 609–618.
Larsen ML *et al.* (1978). Assay of plasma heparin using thrombin and the chromogenic substrate H-D-Phe-Pip-Arg-pNA (S-2238). *Thromb Res* 13(2): 285–288.
Noble S *et al.* (1995). Enoxaparin. A reappraisal of its pharmacology and clinical applications in the prevention and treatment of thromboembolic disease. *Drugs* 49: 388–410.

Enoximone

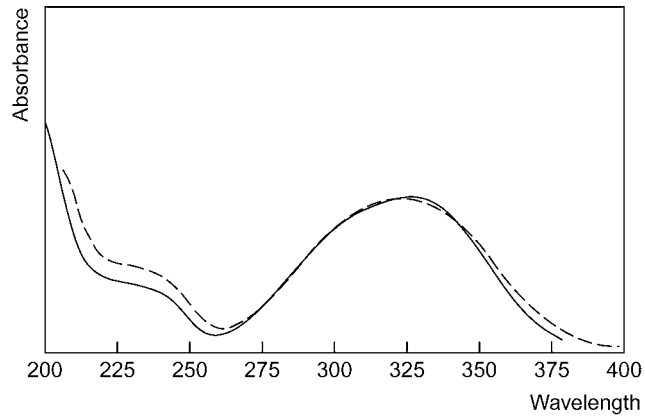
Vasodilator, Inotrope
$C_{12}H_{12}N_2O_2S$ = 248.3
CAS—77671-31-9

IUPAC Name 4-Methyl-5-(4-methylsulfanylbenzoyl)-1,3-dihydroimidazol-2-one
Synonyms 1,3-Dihydro-4-methyl-5-[4-(methylthio)benzoyl]-2*H*-imidazol-2-one; fenoximone; MDL-17043; MDL-19438; RMI-17043; YMDL-17043.

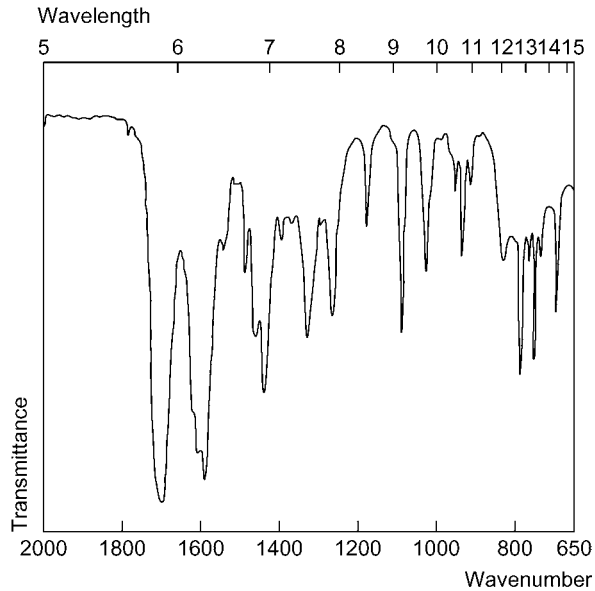
Proprietary Names *Perfan*; *Perfane*.



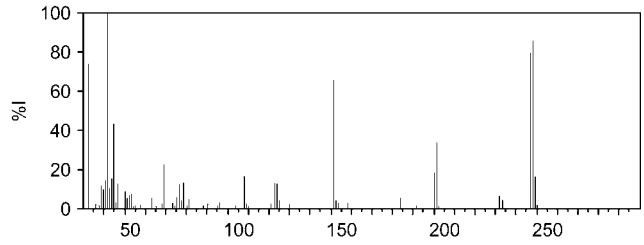
Chemical Properties Crystalline powder. Mp 255° to 258° with decomposition. Log *P* (octanol/water), 1.86.
High Performance Liquid Chromatography Column: C_{18} ODS Ultraspher (150 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (86:14) for 3 min, to (10:90) in 6 min, back to original conditions in 1 min, flow rate 1 mL/min. UV detection (λ=365 nm). Internal standard (IS): MDL 82249. Retention times: enoximone, 8.25 min; enoximone sulfoxide, 2.47 min; IS, 7.75 min [Tarral *et al.* 1990].
Ultraviolet Spectrum Aqueous acid—327 nm.



Infrared Spectrum Principal peaks at wavenumbers 1700, 1610, 1435, 1325, 830, 1090 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 42, 248, 247, 32, 151, 45, 201, 69.



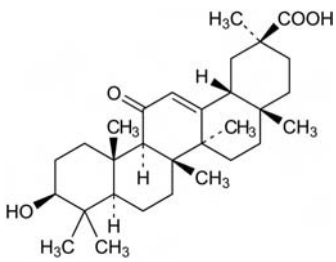
Quantification
Plasma **HPLC** UV detection (λ=340 nm). Limit of quantification, 0.005 and 0.01 mg/L for enoximone and the metabolites, respectively [Morita *et al.* 1995].
Serum **HPLC** UV detection (λ=335 nm). Limit of detection, 0.01 mg/L for enoximone and metabolite [Cooper, Turnell 1986]. Limit of quantification, is 0.05 mg/L for enoximone and sulfoxide metabolite [Chan *et al.* 1984].
Urine **HPLC** UV detection (λ=340 nm). Limit of quantification, 0.5 and 1.0 mg/L for enoximone and the metabolites, respectively [Morita *et al.* 1995].
Disposition in the Body Following IV injection or infusion it undergoes metabolism in the liver to an active sulfoxide metabolite (piroximone). It is excreted primarily via the kidney, as metabolites in urine. After an IV dose, ~70% is excreted in urine as metabolites and 1% as the unchanged drug.
Therapeutic Concentration
Twenty infants, aged between 0.6 and 49.7 weeks (median 6.0 weeks), were administered a loading dose of 1 mg/kg enoximone over 2 min, followed by an infusion of 10 µg/kg/min for a median time of 97 h (24 to 572 h). The infants were already anaesthetised for weaning from cardiopulmonary bypass. The maximum plasma concentration ranged between 0.708 and 6.893 mg/L (median 1.536 mg/L) [Booker *et al.* 2000].
Twenty-three healthy male Japanese volunteers with a mean age of 22.0 years (range, 20 to 27 years) were administered either a 0.25, 0.5 or 1.0 mg/kg single IV bolus dose at 2-weekly intervals; or a single 2.0 mg/kg dose; or a single 1.0 mg/kg bolus dose on day 1 followed by 3-hourly interval doses on day 2; or a single 10 µg/kg/min continuous infusion over a 4-h period. In the first part of the study, the peak concentrations for the 0.25, 0.5, 1.0 and 2.0 mg/kg dose were 0.31, 1.82, 3.40 and 5.38 mg/L, respectively observed at 0.06, 0.01 and 0.0 h. The sulfoxide metabolite concentrations were 0.23, 0.53, 1.08 and 1.82 mg/L at 0.25, 0.25, 0.17 and 0.19 h for the four doses, respectively. The multiple dosing with a 1 mg/kg dose produced peak concentrations of 2.35 mg/L at 3.21 h. The single 10 µg/kg/min dose produced concentrations of 0.51 mg/L at 4.0 h [Morita *et al.* 1995].
Toxicity Severe supraventricular and ventricular arrhythmias can occur.
Half-life In healthy volunteers, 1 to 4 h, in patients with heart failure, 6 h (bolus injections), 8 h (continuous infusion); infants (0.6 to 49.7 weeks) 1.4 to 10.9 h (median 6.4); sulfoxide, 2.9 to 17.7 h (median 7.4).
Volume of Distribution 1.1 to 3.6 L/kg; 1.4 to 15.5 L/kg (median 3.8) (infants aged 0.6 to 49.7 weeks).
Clearance Plasma clearance, 3.7 to 13.0 mL/min/kg; 2.4 to 18.9 mL/min/kg (median 9.2) (infants aged 0.6 to 49.7 weeks).
Protein Binding 85%.
Dose Maximum of 24 mg/kg IV in 24 h.

Booker PD *et al.* (2000). Enoximone pharmacokinetics in infants. *Br J Anaesth* 85(2): 205–210.
Chan KY *et al.* (1984). Simultaneous analysis of a new cardiotonic agent, MDL 17,043, and its major sulfoxide metabolite in plasma by high-performance liquid chromatography. *J Chromatogr* 306: 249–256.
Cooper JD, Turnell DC (1986). Automatic preparation of human serum samples for analysis of the drug enoximone and its sulphoxide metabolite using high-performance liquid chromatography. *J Chromatogr* 380(1): 109–116.
Morita S *et al.* (1995). Pharmacokinetics of enoximone after various intravenous administrations to healthy volunteers. *J Pharm Sci* 84(2): 152–157.
Tarral E *et al.* (1990). [Determination of enoximone and its principle metabolite in serum and urine using high pressure liquid chromatography]. *Therapie* 45: 1–6.

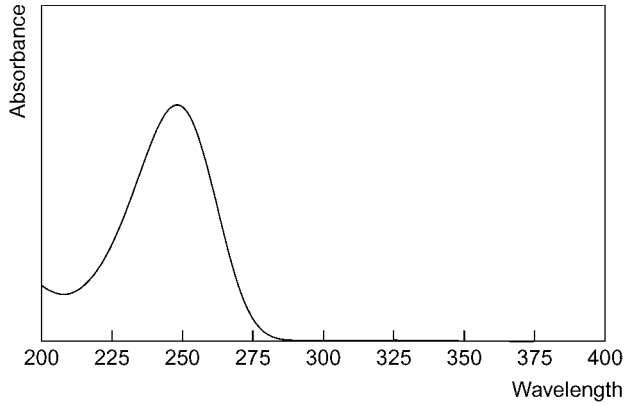
Enoxolone

Dermatological Agent
$C_{30}H_{46}O_4$ = 470.7
CAS—471-53-4

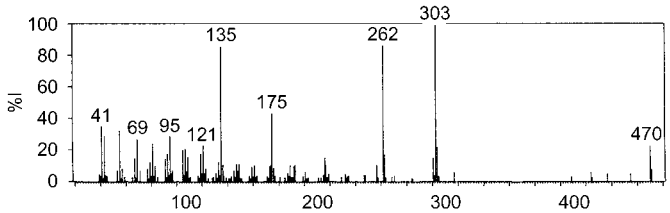
IUPAC Name (2*S*,4a*S*,6a*R*,6a*S*,6b*R*,8a*R*,10*S*,12a*S*,14b*R*)-10-Hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-3,4,5,6,6a,7,8,8a,10,11,12, 14b-dodecahydro-1*H*-picene-2-carboxylic acid
Synonyms Glycyrrhetic acid; glycyrrhetinic acid; (3β,20β)-3-hydroxy-11-oxo-olean-12-en-29-oic acid.
Proprietary Names *Arthrodont*; *PO 12*. It is an ingredient of *Gelclair*.



Chemical Properties A white or faintly cream-coloured powder. Mp 296°. Very sparingly soluble in water; soluble in ethanol, ether, pyridine and acetic acid; freely soluble in chloroform and dioxane; practically insoluble in petroleum ether. Log *P* (octanol/water), 6.9.
Colour Tests Antimony pentachloride—orange-brown→violet; naphthol–sulfuric acid—red-brown/orange; sulfuric acid—yellow
Thin-layer Chromatography System TD—R_f 0.21; system TE—R_f 0.07; system TF—R_f 0.46; system TAD—R_f 0.47.
Ultraviolet Spectrum Aqueous acid—254 nm; aqueous alkali—260 nm (A^1_1=338b).



Mass Spectrum Principal ions at *m/z* 303, 262, 135, 175, 41, 55, 43, 95.

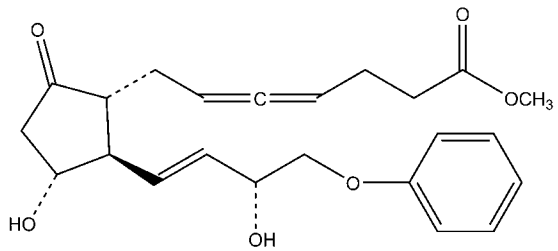


Disposition in the Body Enoxolone is a metabolite of carbenoxolone.
Use Has been used topically in a concentration of 2%.

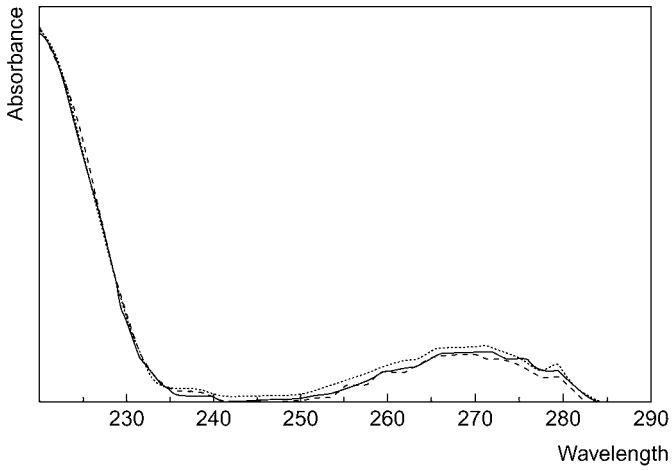
Enprostil

Antiulcerative, Prostaglandin
$C_{23}H_{28}O_6$ = 400.5
CAS—73121-56-9

IUPAC Name Methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*,3*R*)-3-hydroxy-4-(phenoxy)but-1-enyl]-5-oxocyclopentyl]hepta-4,5-dienoate
Synonyms rel-7-[(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*R*)-3-hydroxy-4-phenoxy-1-butenyl]-5-oxocyclopentanyl]-4,5-heptadienoic acid methyl ester; (*dl*)-9-keto-11α,15α-dihydroxy-16-phenoxy-17,18,19,20-tetranorprosta-4,5,13-*trans*-trienoic acid methyl ester; methyl (*E*)-(11*R*,15*R*)-11,15-dihydroxy-9-oxo-16-phenoxy-17,18,19,20-tetranorprosta-4,5,13-trienoate; methyl 7-{(*E*)-(3*R**)-3-hydroxy-2-[(1*R**,2*R**,3*R**)-3-hydroxy-4-phenoxybut-1-enyl]-5-oxocyclopentyl}hepta-4,5-dienoate; RS-84135.
Proprietary Names *Camleed*; *Fundyl*; *Gadrin(e)*; *Gardrin*.



Chemical Properties White to off-white waxy solid. Soluble in alcohol, propylene glycol and propylene carbonate. Slightly soluble in water. Log *P* (octanol/water), 1.45 [ACD 2007].
High Performance Liquid Chromatography Column: Partisil 5 ODS-3 (100 × 4.6 mm i.d., 5 µm). Mobile phase: tetrahydrofuran : methanol : 1 mmol/L phosphate buffer (pH 6.5; 15 : 30 : 55), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 8 min. Limit of quantification, 4 µmol/L [Kenley *et al.* 1986a].
Ultraviolet Spectrum Tetrahydrofuran : methanol : phosphate buffer—268 nm Kenley *et al.* [1986a].



Quantification

Plasma **HPLC** Column: Spheri-5 normal phase silica (100 × 4.6 mm i.d., 5 µm) followed by Column: NC2 Spheri-5 silica (220 × 4.6 mm i.d., 5 µm). Mobile phase: dichloromethane : acetonitrile (20 : 70); or Column: Chemcosorb 7CN reversed phase (250 × 4.6 mm i.d., 7 µm). Mobile phase: methanol : water : acetic acid (40 : 60 : 0.1), flow rate 1.0 mL/min. Fluorometric detection (λ_{ex} = 325 nm). Limit of quantification, 5 ng/L [Kiang *et al.* 1991].

Other **HPLC** Capsules. Column 1: Sphericel C_{18} (300 × 4.6 mm i.d., 10 µm). Mobile phase 1: water, flow rate 1.5 mL/min. Column 2: Partisil 10 cation exchange (250 × 4.6 mm i.d., 10 µm). Mobile phase 2: tetrahydrofuran : methanol : 1.0 mmol/L ammonium phosphate buffer (pH 2.4; 15 : 35 : 50), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 10.8 min. Limits of quantification and detection not reported [Kenley *et al.* 1986b].

Disposition in the Body Eliminated predominantly via the urine, with approx. 53% excreted after 48 h. After 144 h, a further 34% is eliminated via the faeces.

Therapeutic Concentration

Plasma concentrations in subjects administered an oral dose of 70 µg enprostil were measured at 15, 30, 60, 90 and 120 min post-dose and reported as 24, 113, 27, 21 and 7 µg/L, respectively. The plasma level at 3 h post-dose was below the limit of quantification of the HPLC–LIFD method used [Kiang *et al.* 1991].

Following a single oral dose of 1 µg/kg [^{3}H]enprostil administered to 4 healthy volunteers, plasma concentrations of 0.94 µg·equiv/L (enprostil plus unidentified metabolites) were reached within 30 to 60 min [Goa, Monk 1987].

Note For a study on the efficacy of enprostil combined with cimetidine compared with cimetidine alone in treating gastric ulcers, see Murata *et al.* [2005].

Half-life Approximately 34 h.

Dose In acute treatment of adults with duodenal or gastric ulcers, 35 µg orally, twice daily for at least 4 weeks.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Goa KL, Monk JP (). Enprostil. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of peptic ulcer disease. *Drugs* 34: 539–559.

Kenley RA *et al.* (1986a). Stability-specific HPLC analysis of the antiulcer prostaglandin, enprostil, in a soft elastic gelatin capsule formation. *J Liq Chromatogr* 9: 3577–3595.

Kenley RA *et al.* (1986b). Multidimensional column-switching liquid chromatographic method for dissolution testing of enprostil soft elastic gelatin capsules. *J Pharm Sci* 75: 999–1002.

Kiang CH *et al.* (1991). Determination of femtomole/milliliter concentrations of enprostil acid in human plasma using high-performance liquid chromatography-laser-induced fluorescence detection. *J Chromatogr* 567: 195–212.

Murata H *et al.* (2005). Combination of enprostil and cimetidine is more effective than cimetidine alone in treating gastric ulcer: prospective multicenter randomized controlled trial. *Hepatogastroenterology* 52: 1925–1929.

Entacapone

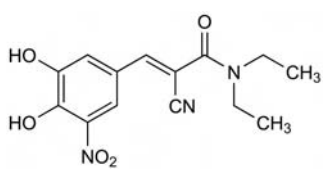
Dopaminergic Agent, Antiparkinsonian

$C_{14}H_{15}N_3O_5$ = 305.3

CAS—130929-57-6

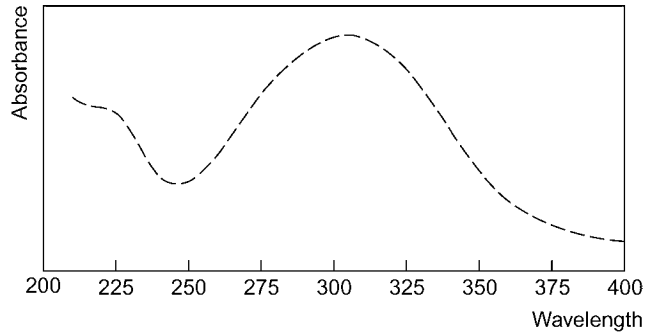
IUPAC Name (E)-2-Cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)acrylamide

Proprietary Names *Comtan*; *Comtess*.

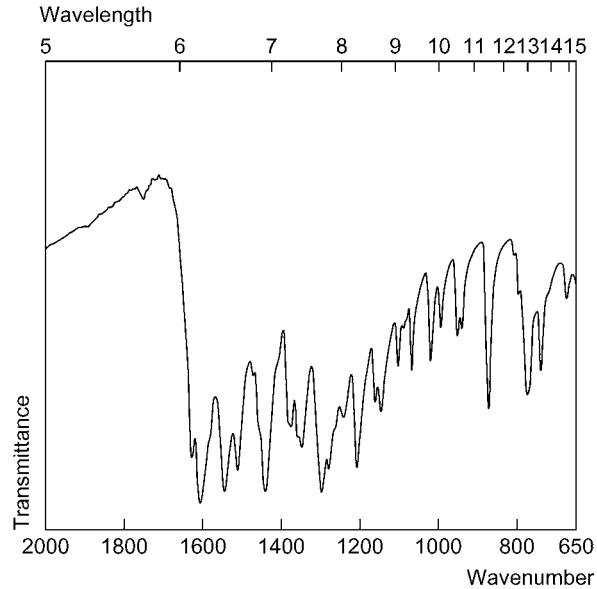


Chemical Properties Powder. Mp 153° to 156°. pK_a 4.5. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

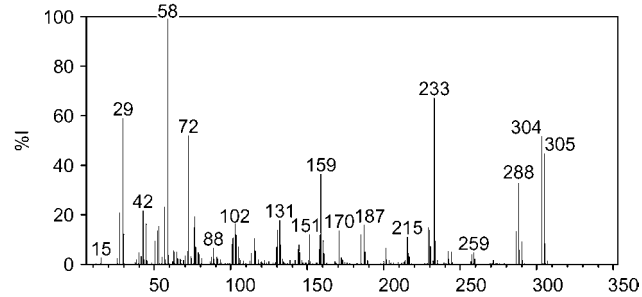
Ultraviolet Spectrum Neutral—306 nm.



Infrared Spectrum Principal peaks at wavenumbers 2209, 1630, 1610, 1537 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 58, 233, 29, 304, 72, 305, 159, 288.



Quantification

Plasma **HPLC** Amperometric detection. Limit of quantification, 0.01 mg/L [Karlson, Wikberg 1992].

Urine **HPLC** Column: ODS-2 silica Spherisorb (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L sodium dihydrogen phosphate buffer and 10 mmol/L citric acid buffer (pH 2.2) : methanol (55:45), flow rate 1.0 mL/min. UV detection (λ=310 nm). Retention time: entacapone, 9.5 min; (*Z*)-entacapone, 5.8 min. Limit of detection, 0.5 mg/L [Wikberg *et al.* 1993].

Disposition in the Body Entacapone is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism. Entacapone is converted to its (*cis*)-isomer, (*Z*)-entacapone, the main metabolite in plasma, followed by direct glucuronidation to inactive glucuronide conjugates. Four metabolites have been observed—M3, M6, M8 and M10. Elimination is mainly via faeces (80 to 90%) and the remainder in urine as glucuronide conjugates and (*Z*)-isomer.

Therapeutic Concentration

Twelve healthy males were administered single oral doses of entacapone: 5, 25, 50, 100, 200, 400 and 800 mg. Peak plasma concentrations ranged from 62 to 7280 µg/L, with increasing dose, and were reached between 0.46 and 0.88 h [Keranen *et al.* 1994].

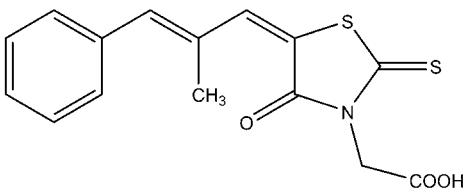
Toxicity No special hazard to humans, anaemia observed in repeated dose toxicity studies.
Bioavailability Approximately 35% (oral).
Half-life Elimination half-life, $t_{1/2}$ (α-phase) 0.27 to 0.37 h and (β-phase) 1.59 to 3.10 h.
Volume of Distribution 181 L.
Clearance Total clearance, 800 mL/min.
Protein Binding Extensively binds to plasma proteins and serum albumin ~98%.
Dose A usual dose of 200 mg entacapone, up to ten times a day, with a maximum of 2000 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Karlsson M, Wikberg T (1992). Liquid chromatographic determination of a new catechol-O-methyltransferase inhibitor, entacapone, and its Z-isomer in human plasma and urine. *J Pharm Biomed Anal* 10(8): 593–600.
Keranen T *et al.* (1994). Inhibition of soluble catechol-O-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. *Eur J Clin Pharmacol* 46 (2): 151–157.
Wikberg T *et al.* (1993). Identification of major metabolites of the catechol-O-methyltransferase inhibitor entacapone in rats and humans. *Drug Metab Dispos* 21: 81–92.

Epalrestat

Antidiabetic, Thiazolidine
$C_{15}H_{13}NO_3S_2 = 319.4$
CAS—82159-09-9

IUPAC Name 2-[(5*Z*)-5-[(*E*)-2-Methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl]acetic acid
Synonyms 3-Carboxymethyl-5-(2-methylcinnamylidene)rhodanine; 5-[(*E*,*E*)-β-methylcinnamylidene]-4-oxo-2-thioxo-3-thiazolidine-acetic acid; (5*Z*)-5-[(2*E*)-2-methyl-3-phenyl-2-propenylidene]-4-oxo-2-thioxo-3-thiazolidineacetic acid; ONO-2235.
Proprietary Names *Kinedak*; *Sorbistat*.



Chemical Properties Crystals from ethanol–water. Mp 210° to 217°.

Epalrestat *N*-Methyl-D-Glucamine
$C_{22}H_{30}N_2O_8S_2 = 514.6$
Chemical Properties Crystals from methanol. Mp 163° to 165°.

Disposition in the Body Metabolised to mono- and dihydroxyphenylepalrestat as sulfate conjugates and, together with unchanged drug, these are present in both serum and urine. There is some evidence that epalrestat binds to serum albumin.

Therapeutic Concentration

Single oral doses of 50, 100 and 200 mg epalrestat were administered to 7 healthy male volunteers. Mean peak plasma concentrations of the drug and its metabolites were as follows:

Dose (mg)	*Compound*	*C_{max} (mg/L)*	*Time (h)*
50	Epalrestast	3.9	1
	4-OH Metabolite	0.5	2
	3,4-$(OH)_2$ Metabolite	–	–
100	Epalrestast	7.4	2
	4-OH Metabolite	1.2	2
	3,4-$(OH)_2$ Metabolite	0.4	2
200	Epalrestast	13.6	2
	4-OH Metabolite	2.10	2
	3,4-$(OH)_2$ Metabolite	0.70	2

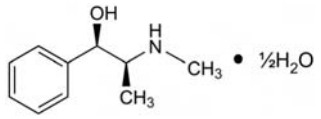
The half-life was in the range 0.8 to 1.1 h [Steele *et al.* 1993].
Half-life Approximately 1 h.
Dose Recommended, 50 mg three times daily before meals.

Steele JW *et al.* (1993). Epalrestat. A review of its pharmacology, and therapeutic potential in late-onset complications of diabetes mellitus. *Drugs Aging* 3: 532–555.

Ephedrine

Sympathomimetic
$C_{10}H_{15}NO,½H_2O = 174.2$
CAS—50906-05-3

IUPAC Name (1*R*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrate
Synonyms Hydrated ephedrine; (α*R*)-α-[(1*S*)-1-(methylamino)ethyl]benzenemethanol hemihydrate.
Proprietary Names It is an ingredient of *Franolyn Expectorant* and *Letigen*.



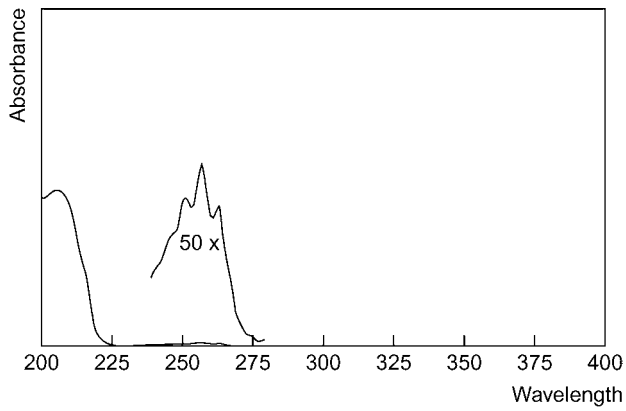
Chemical Properties An alkaloid obtained from species of *Ephedra*, or prepared synthetically. Colourless crystals or white crystalline powder or granules that decompose on exposure to light. Mp 40° (hemihydrate); in warm weather it slowly volatilises. Soluble 1 in 20 of water and 1 in <1 of ethanol; soluble in chloroform with turbidity owing to separation of water; soluble in ether. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].

Anhydrous Ephedrine
$C_{10}H_{15}NO = 165.2$
CAS—299-42-3
Synonym Ephedrine
Proprietary Names *Ephed 20th*; *Kondon's Nasal*; *Tendrin*.
Chemical Properties Unctuous deliquescent colourless crystals, or white crystalline powder. It rapidly absorbs carbon dioxide and decomposes on exposure to light. Mp 38.1°. Soluble 1 in 20 of water; very soluble in ethanol; soluble in chloroform; freely soluble in ether.

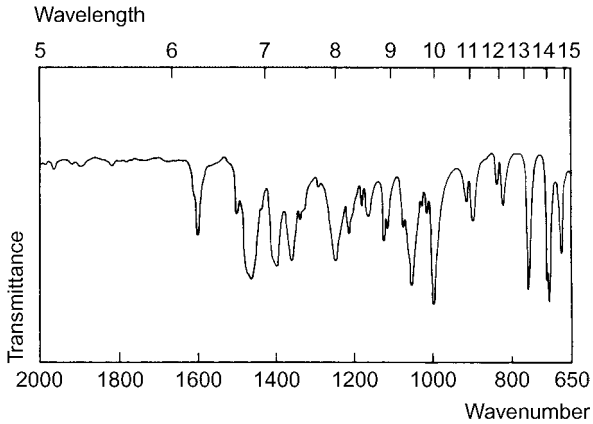
Ephedrine Hydrochloride
$C_{10}H_{15}NO,HCl = 201.7$
CAS—50-98-6
Synonyms Ephedrine chloride; ephedrinium chloratum; l-ephedrinum hydrochloricum.
Proprietary Names *CAM*; it is an ingredient of many proprietary preparations [Sweetman 2007].
Chemical Properties Colourless crystals or white crystalline powder. Mp 217° to 220°. Soluble 1 in 3 to 1 in 4 of water and 1 in 14 to 1 in 17 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

Ephedrine Sulfate
$(C_{10}H_{15}NO)_2,H_2SO_4 = 428.5$
CAS—134-72-5
Proprietary Names It is an ingredient of *Franol Plus*.
Chemical Properties Fine white crystals or powder that darken on exposure to light. Mp 245° with decomposition [O'Neil *et al.* 2006]. Soluble 1 in 1.3 of water and 1 in 90 of ethanol. pK_a 9.6 (25°). Log *P* (octanol/water), 1.1.

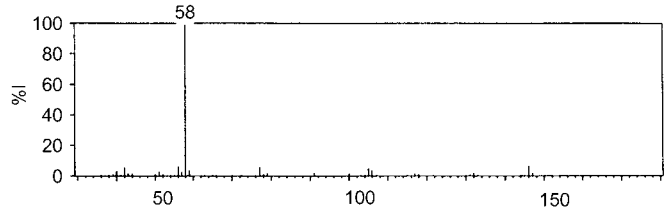
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.30; system TB—R_f 0.05; system TC—R_f 0.05; system TE—R_f 0.25; system TL—R_f 0.01; system TAE—R_f 0.10; system TAF—R_f 0.64; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.29 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive; Dragendorff spray, positive; Marquis test, brown; ninhydrin spray, positive).
Gas Chromatography System GA—ephedrine RI 1365, M (-TFA_2) RI 1345, M (-PFP_2) RI 1370, M (-TMS_2) RI 1620, M (-AC_2) RI 1795, M (nor-) RI 1360, M (nor-)-TFA_2 RI 1355, M (nor-)-PFP_2 RI 1380, M (nor-)-TMS_2 RI 1555, M (nor-)-AC_2 RI 1805, M (OH-) RI 1875, M (OH-)-AC_3 RI 2145; system GB—ephedrine RI 1410, M (nor-) RI 1356; system GC—ephedrine RI 1467, M (nor-) RI 1383.
High Performance Liquid Chromatography System HA—ephedrine *k* 1.0, M (nor-) *k* 0.9; system HB—ephedrine *k* 5.68, M (nor-) *k* 3.87; system HC—ephedrine *k* 1.79, M (nor-) *k* 0.70; system HX—RI 221; system HY—RI 227; system HZ—RT 2.1 min; system HAA—RT 5.7 min; system HAM—ephedrine not detected.
Ultraviolet Spectrum Aqueous acid—251, 257 ($A^1_1 = 12a$), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 994, 699, 754, 1049, 1242, 670 cm^{-1} (ephedrine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 146, 56, 105, 77, 42, 106, 40; norephedrine 44, 77, 79, 51, 45, 42, 107, 105.



Quantification

Blood **GC-MS** Column: 5% phenyl silicone capillary (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 20 mL/min. Temperature programme: 70° to 175° at 25°/min to 280° at 50°/min. SIM acquisition mode [Backer *et al.* 1997].

LC-MS Column: XBridge Shield RP-18 (250 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium bicarbonate (pH 10.5, 100 : 0 to 40 : 60 at 3.5 min to 80 : 20 at 7.5 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.2 µg/L [Qiu *et al.* 2008].

Plasma **GC** Column: 3% phenyl cyanopropyl methyl silicone on 100/120 mesh (0.9 m × 2.0 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 93 mL/min. Temperature: 235°. ECD. Limit of detection, 2 µg/L [Midha *et al.* 1979].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 50° for 0.5 min to 200° at 30°/min to 300° at 10°/min for 5 min. EI ionisation, SIM acquisition mode. Limit of detection, 2–5 µg/L [Frison *et al.* 2005]. Limit of detection, 0.82 µg/L [Nakano *et al.* 2000].

HPLC Column: C_{18} (150 × 4.6 mm. Mobile phase: acetonitrile : water (52 : 48). Fluorescence detection (λ = 264 and 313 nm). Limit of detection, 2 µg/L for ephedrine and 5 µg/L for norephedrine [Aymard *et al.* 2000]. Column: reversed phase. Mobile phase: 0.6% phosphate buffer (pH 6.5) : methanol (3 : 8). Fluorescence detection. Limit of detection, 0.5 ng [Shao *et al.* 1995].

LC-MS Column: Zorbax SB-C_{18} (150 × 4.6 mm). Mobile phase: methanol : water : formic acid (80 : 20 : 0.5), flow rate 0.5 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.5 µg/L [Ren *et al.* 2006]. Column: Hypersil Phenyl BDS (50 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium formate with 0.1% formic acid : 10 mmol/L ammonium formate with 0.1% formic acid (100 : 0 to 80 : 20 over 7 min to 0 : 100 over 0.5 min for 1 min to 100 : 0 for 3.5 min). APCI, positive ion mode, SRM acquisition mode. Limit of quantification, 1 µg/L [Jacob *et al.* 2004]. Column: YMC phenyl LC (250 × 2 mm, 5 µm). Mobile phase: 3.8 g ammonium acetate : 20 mL glacial acetic acid: 30 mL acetonitrile: to 1000 mL with water. Limit of quantification, 100 µg/L for ephedrine alkaloids [Trujillo, Sorenson 2003].

CE Buffer: 0.1 mol/L phosphate electrolyte (pH 2.5) : acetonitrile (90 : 10). Limit of detection, 5.3 µg/L [Wei *et al.* 2007].

Serum **CE** UV detection (λ = 192 nm). Limit of detection, 0.15–0.25 µg/L [Fang *et al.* 2006a].

Urine **GC** Column: Rtx–5 5% diphenyl 95% dimethylpolysiloxane (15 m × 0.25 mm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 100° to 105° at 0.5°/min to 118° at 2°/min to 280° at 50°/min for 2 min. NPD. Retention time: 14.8 min. Limit of detection, 4 mg/L [Van Eenoo *et al.* 2001]. See Plasma [Midha *et al.* 1979].

GC-MS Column: 5% phenylmethyl silicone (17 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 85° for 1.5 min to 270° at 15°/min to 290° at 50°/min for 2.5 min. EI ionisation at 70 eV, scan mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Strano-Rossi *et al.* 2008]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 3 min to 300° at 20°/min for 3 min. MSD. Retention time: 7.67 min. Limit of quantification, 12.5 µg/L, limit of detection, 5 µg/L [Saito *et al.* 2007]. Column: HP Ultra 2 5% diphenyl 95% dimethylsiloxane (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperatue programme: 80° for 2 min to 150° at 10°/min to 310° at 10°/min for 1 min. EI ionisation at 70 eV, MSD, scan mode. Limit of detection, 5.0 mg/L [Spyridaki *et al.* 2001]. See Plasma [Frison *et al.* 2005].

HPLC Column: Nucleosil-100 C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.03 mol/L sodium phosphate buffer (pH 3.0, 7 : 93 to 30 : 70 in 10 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Bagheri *et al.* 2008]. Column: Hypersil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L TEA and 20 mmol/L potassium dihydrogen phosphate (pH 3) : acetonitrile (88 : 12 for 6 min to 45 : 55 at 25 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 0.05 mg/L [Zhang *et al.* 2008]. Column: Spherisorb C_{18}. Mobile phase: tetraethylammonium phosphate : methanol. UV detection (λ = 206 nm). Limit of detection, 0.1–0.3 mg/L [Chan *et al.* 2005]. Column: Hypersil BDS C_{18} (150 × 3 mm i.d., 3 µm). Mobile phase: 3% acetonitrile in 0.1% sulfuric acid. Retention time: 11.9 min. Limit of quantification, 5.5 mg/L [Gmeiner *et al.* 2002]. Column: Symmetry Shield (compared with other columns). Mobile phase: 50 mmol/L phosphate buffer : 25 mmol/L TEA, flow rate 1.5 mL/min to 2.0 mL/min in 10 min. UV detection (λ = 215 nm). Limit of detection, 0.3 mg/L [Imaz *et al.* 2000]. Column: Sep Spherisorb ODS 1. Mobile phase: tetraethylammonium phosphate : methanol. UV detection (λ = 214 nm). Limit of detection, 0.5 mg/L [van der Merwe *et al.* 1994]. See also Imaz *et al.* [1993].

LC-MS Column: C_8. Mobile phase: water : acetonitrile (98 : 2) containing 0.1% HAc and 0.01% trifluroacetic acid. Limit of detection, 2.5–5 mg/L [Deventer *et al.* 2009]. Column: Hypersil GOLD (100 × 2.1 mm i.d., 5 µm). Mobile phase: 1% acetonitrile in 10 mmol/L formic acid : 60% acetonitrile in 10 mmol/L formic acid (100 : 0 to 0 : 100 at 10 min to 100 : 0 for 4 min), flow rate 200 µL/min. ESI, positive ion mode. Limit of quantification, 5 µg/L [Björnstad *et al.* 2009]. Limit of detection, 1 mg/L [Trujillo, Sorenson 2003]. See Blood [Qiu *et al.* 2008]. See Plasma [Jacob *et al.* 2004].

CE Buffer: 0.1 mol/L phosphate electrolyte (pH 2.5): acetonitrile (90 : 10). Limit of detection, 8 µg/L [Wei *et al.* 2007]. Limit of detection, 3 µg/L for ephedrine and 5 µg/L for pseudoephedrine [Fang *et al.* 2006b]. See Serum [Fang *et al.* 2006a].

Note For an ion mobility spectroscopy method for the analysis of ephedrine, see Lokhnauth, Snow [2005].

Oral Fluid **GC-MS** See Urine. SIM acquisition mode [Strano-Rossi *et al.* 2008].

Brain **GC-MS** See Blood [Backer *et al.* 1997].

Hair **GC-MS** See Plasma. Limit of detection, 0.1–0.2 µg/g [Frison *et al.* 2005]. Column: V-1 capillary (equivalent). Temperature programme: 60° to 280° at 20°/min. Limit of detection, <50 pg derivatised ephedrine [Nakahara, Kikura 1997].

Kidney **GC-MS** See Blood [Backer *et al.* 1997].

Liver **GC-MS** See Blood [Backer *et al.* 1997].

Disposition in the Body

Ephedrine is readily absorbed after oral or percutaneous administration. It is metabolised by *N*-demethylation to norephedrine (phenylpropanolamine) and by oxidative deamination followed by conjugation. It accumulates in the liver, lungs, kidneys, spleen and brain. Approximately 90% of a dose is excreted in the urine in 24 h, with ~55–75% of the dose as unchanged drug, 8–20% as norephedrine and 4–13% as deaminated metabolites such as benzoic acid, hippuric acid and 1-phenylpropane-1,2-diol. In acidic urine, excretion of unchanged drug is increased slightly, whereas in alkaline urine, ~20–35% of the dose is excreted unchanged and the proportion of norephedrine is increased.

Ephedrine is a metabolite of methylephedrine.

Therapeutic Concentration In plasma, usually in the range 0.035–0.08 mg/L.

After a single oral dose of 22 mg of ephedrine hydrochloride to 10 subjects, peak plasma concentrations of 0.04–0.14 mg/L (mean 0.08) were attained; after daily oral doses of 33 mg of ephedrine hydrochloride to 10 subjects, peak plasma concentrations of 0.07–0.12 mg/L (mean 0.08) were obtained [Costello *et al.* 1975].

Ephedrine toxicity has been linked to nutritional supplements containing *Ephedra sinica* (ma huang) and a number of deaths have occurred. The pharmcokinetics of 3 commercially available ma huang products (mean ephedrine content per dose: 27, 25.6 and 23.6 mg, respectively) were compared with those of ephedrine hydrochloride capsules (25 mg dose) in 10 subjects. The mean peak plasma concentrations of ephedrine (and the time at which the peak occurred) were ephedrine hydrochloride 86 µg/L (at 2.81 h) and the ma huang products 100.1 µg/L (at 2.68 h), 86.2 µg/L (at 2.61 h) and 73.4 µg/L (at 3.05 h), respectively [Gurley *et al.* 1998].

A single 25-mg dose of ephedrine may result in urine levels above 10 mg/L if tested within ~8 h after administration [Tseng *et al.* 2006].

For the pharmacodynamics and pharmacokinetics of single oral and nasal doses of ephedrine, see Berlin *et al.* [2001].

Toxicity The estimated minimum lethal dose in children up to 2 years of age is 200 mg and for adults 2 g, but fatalities are rare. Single doses of up to 400 mg have been given without causing serious toxic effects.

In a 28-year-old woman whose death was attributed to the ingestion of ephedrine with suicidal intent, the following postmortem tissue concentrations were reported: blood 11 mg/L, liver 24 µg/g, kidney 14 µg/g and brain 8.9 µg/g. Amitriptyline was also detected [Backer *et al.* 1997].

In a review of 127 autopsies where ephedrine was identified postmortem, blood ephedrine levels were found to be <0.49 mg/L in 50%, with a range of 0.07–11.73 mg/L in trauma victims and 0.02–12.35 mg/L in non-trauma cases. Norephedrine and pseudoephedrine were present in the blood of 22.8% and 6.3%, respectively. More than 88% of decedents tested positive for other drugs [Blechman *et al.* 2004].

For reports of sudden deaths related to ephedrine toxicity from a ma huang-containing drink, see Theoharides [1997] and Karch [1999]. For reports of 3 deaths after overdoses with Letigen (caffeine 200 mg/ephedrine 20 mg), see Hedetoft *et al.* [1999] and Kanstrup, Petersen [2003].

Half-life Plasma half-life, 3–6 h but may be increased when the urine is alkaline and decreased when it is acid.

Clearance Renal clearance 0.34 L/min.

Volume of Distribution 181 L.

Dose Ephedrine hydrochloride or sulfate 90 to 180 mg daily orally in divided doses; in concentrations of 0.5 to 1% in nasal drops.

Aymard G *et al.* (2000). Sensitive determination of ephedrine and norephedrine in human plasma samples using derivatization with 9-fluorenylmethyl chloroformate and liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 25–31.

Backer R *et al.* (1997). Fatal ephedrine intoxication. *J Forensic Sci* 42: 157–159.

Bagheri H *et al.* (2008). Liquid–liquid–liquid microextraction followed by HPLC with UV detection for quantitation of ephedrine in urine. *J Sep Sci* 31: 3212–3217.

Berlin I *et al.* (2001). Pharmacodynamics and pharmacokinetics of single nasal (5 mg and 10 mg) and oral (50 mg) doses of ephedrine in healthy subjects. *Eur J Clin Pharmacol* 57: 447–455.

Björnstad K *et al.* (2009). A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1162–1168.

Blechman KM *et al.* (2004). Demographic, pathologic, and toxicological profiles of 127 decedents testing positive for ephedrine alkaloids. *Forensic Sci Int* 139: 61–69.

Chan KH *et al.* (2005). Simultaneous quantification of six ephedrines in a Mahwang preparation and in urine by high-performance liquid chromatography. *Biomed Chromatogr* 19: 337–342.

Costello JF *et al.* (1975). Pharmocokinetics of ephedrine in asthmatics receiving acute and chronic treatment. *Br J Clin Pharmacol* 2: 180–181.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

Deventer K *et al.* (2009). Development and validation of an LC-MS/MS method for the quantification of ephedrines in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 369–374.

Fang H *et al.* (2006a). Centrifuge microextraction coupled with on-line back-extraction field-amplified sample injection method for the determination of trace ephedrine derivatives in the urine and serum. *Anal Chem* 78: 6043–6049.

Fang H *et al.* (2006b). Solid-phase microextraction coupled with capillary electrophoresis to determine ephedrine derivatives in water and urine using a sol-gel derived butyl methacrylate/silicone fiber. *Talanta* 68: 979–986.

Frison G *et al.* (2005). Gas chromatography/mass spectrometry determination of amphetamine-related drugs and ephedrines in plasma, urine and hair samples after derivatization with 2,2,2-trichloroethyl chloroformate. *Rapid Commun Mass Spectrom* 19: 919–927.

Gmeiner G *et al.* (2002). Quantification of ephedrines in urine by column-switching high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 768: 215–221.

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Nakano M *et al.* (2000). [GC-MS determination of l-ephedrine and d-pseudoephedrine in human plasma]. *Yakugaku Zasshi* 120: 583–586.

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Ren S *et al.* (2006). [Simultaneous determination of ephedrine and chlorpheniramine in human plasma by a highly sensitive liquid chromatography–tandem mass spectrometric method]. *Yao Xue Xue Bao* 41: 188–192.

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Shao G *et al.* (1995). Quantitative analysis of (l)-ephedrine and (d)-pseudoephedrine in plasma by high-performance liquid chromatography with fluorescence detection. *Yao Xue Xue Bao* 30: 384–389.

Spyridaki MH *et al.* (2001). Determination of ephedrines in urine by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl*,758,311–314.

Strano-Rossi S *et al.* (2008). Parallel analysis of stimulants in saliva and urine by gas chromatography/mass spectrometry: perspectives for 'in competition' anti-doping analysis. *Anal Chim Acta* 606: 217–222.

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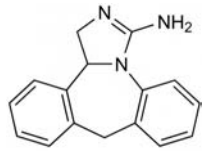
Epinastine

Antihistamine

$C_{16}H_{15}N_3 = 249.3$

CAS—80012-43-7

Synonyms 9,13b-Dihydro-1*H*-dibenz[*c*,*f*]imidazo[1,5-*a*]azepin-3-amine; WAL-801.



Chemical Properties A crystalline powder. Mp 205° to 208°. pK_a 11.2. Log *P* (octanol/water), 3.51; log *P* (octanol/aqueous buffer, pH 7.4 (20°)), −0.70.

Epinastine Hydrobromide

$C_{16}H_{15}N_3,HBr = 330.2$

Chemical Properties A crystalline powder. Mp 284° to 286°.

Epinastine Hydrochloride

$C_{16}H_{16}ClN_3 = 285.8$

CAS—108929-04-0

Synonym WAL-801-Cl

Proprietary Names *Alesion*, *Flurinol*.

Chemical Properties A crystalline powder. Mp 273° to 275°. It is freely soluble in water.

Quantification

Plasma **HPLC** Column: 5C18-MS Cosmosil (150 × 4.6 mm i.d.). Mobile phase: triethylamine (0.3%, pH 4.5) : methanol (64:36), flow rate 1.3 mL/min. UV detection (λ=220 nm). Internal standard (IS): diphenidol. Retention time: epinastine, 8.1 min; IS, 14.8 min. Limit of detection, 4 µg/L for epinastine hydrochloride [Ohtani *et al.* 1996].

Disposition in the Body Epinastine is excreted mainly via urine and faeces unchanged.

Dose A usual dose of 20 mg daily is administered.

Ohtani H *et al.* (1996). Quantitative determination of epinastine in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 683(2): 281–284.

Epirubicin

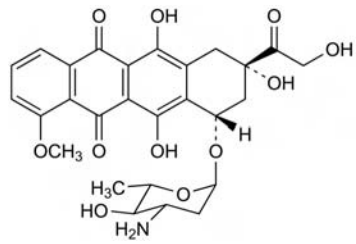
Antineoplastic

$C_{27}H_{29}NO_{11} = 543.5$

CAS—56420-45-2

IUPAC Name (7*S*,9*S*)-7-[(2*R*,4S,5*R*,6*S*)-4-Amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9, 11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dione

Synonyms (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione; 4′-epiadriamycin; 4′-epi-DX; 4′-Epidoxorubicin; IMI-28; pidorubicin.



Epirubicin Hydrochloride

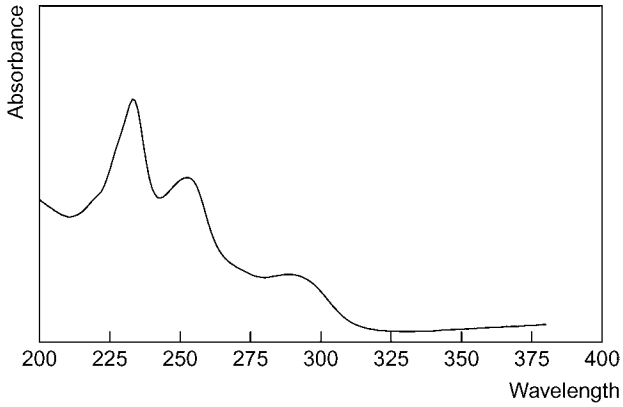
$C_{27}H_{29}NO_{11},HCl = 580.0$

CAS—56390-09-1

Proprietary Names *Farmorubicin*; *Farmorubicina*; *Farmorubicine*; *Pharmorubicin*.

Chemical Properties Red–orange crystals. Mp 185°, with decomposition.

Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.
High Performance Liquid Chromatography System HX—RI 379.
Ultraviolet Spectrum Principal peaks at 233, 253, 292 nm.



Quantification

Plasma **HPLC** Fluorescence detection. Epirubicin and metabolites. Limit of detection, 5 µg>L for epirubicin [Barker *et al.* 1996]. Fluorescence detection (λ_{ex}=480 nm, λ_{em}=580 nm). Limit of detection, 1 µg>L [Dobbs, Twelves 1991].

Disposition in the Body Pharmacokinetics vary widely between patients. Epirubicin is rapidly and extensively distributed into body tissues after IV administration. High concentrations occur in tumour tissues. It also concentrates in red blood cells and therefore concentrations in blood are substantially higher than those in plasma. It does not cross the blood–brain barrier. Epirubicin undergoes extensive metabolism, primarily in the liver (but also in other organs and cells including red blood cells), to form epirubicinol (13-hydroxyepirubicin) which has a very low degree of cytotoxic activity and appreciable amounts of glucuronide derivatives. 7-Deoxyaglycones have also been detected in plasma of patients treated with epirubicin. Epirubicinol is eliminated mainly in bile. About 10 to 20% of a dose is recovered in urine within 48 h as the unchanged drug, epirubicinol, or as the glucuronide metabolite. 40% is recovered in bile in 72 h.

Therapeutic Concentration The serum therapeutic concentration range is 10 to 50 µg/L.

Twenty-seven patients with nasopharyngeal carcinomas, were administered an IV dose of 75 mg/m^2 epirubicin rapidly. Peak plasma concentrations of >1860 µg/L were observed by the end of infusion [Hu *et al.* 1989].

Toxicity Myelosuppression is the major acute dose-limiting toxicity and cardiotoxicity is the most important cumulative dose-limiting toxicity.

Half-life Plasma, 30 to 40 h.

Volume of Distribution Between 13 and 52 L/kg. Steady state, 1000 L/m^2.

Clearance Plasma clearance, 50 L/h/m^2; 0.9 L/min.

Note For reviews of epirubicin, see Plosker and Faulds [1993] and Coukell and Faulds [1997].

For a review of the pharmacokinetics of epirubicin, see Robert [1994].

Dose Usually 60 to 90 mg/m^2 as a single dose IV every 3 weeks. A total cumulative dose of 0.9 to 1 g/m^2 should not generally be exceeded because of the risk of cardiotoxicity.

Barker IK *et al.* (1996). Determination of plasma concentrations of epirubicin and its metabolites by high-performance liquid chromatography during a 96-h infusion in cancer chemotherapy. *J Chromatogr B Biomed Appl* 681(2): 323–329.

Coukell AJ, Faulds D (1997). Epirubicin. An updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of breast cancer. *Drugs* 53: 453–482.

Dobbs NA, Twelves CJ (1991). Measurement of epidoxorubicin and its metabolites by high-performance liquid chromatography using an advanced automated sample processor. *J Chromatogr* 572: 211–217.

Hu OY *et al.* (1989). Pharmacokinetic and pharmacodynamic studies with 4′-epi-doxorubicin in nasopharyngeal carcinoma patients. *Cancer Chemother Pharmacol* 24(5): 332–337.

Plosker GL, Faulds D (1993). Epirubicin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cancer chemotherapy. *Drugs* 45: 788–856.

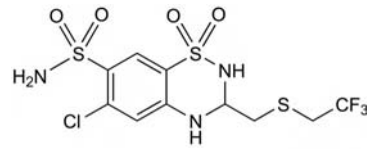
Robert J (1994). Clinical pharmacokinetics of epirubicin. *Clin Pharmacokinet* 26: 428–438.

Epithiazide

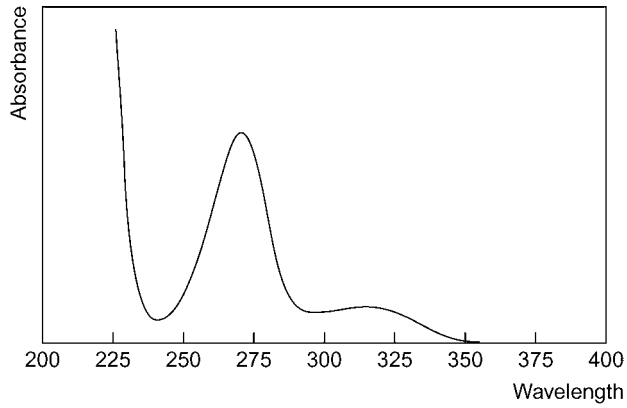
Diuretic

$C_{10}H_{11}ClF_3N_3O_4S_3$ = 425.8
CAS—1764-85-8

IUPAC Name 6-Chloro-3,4-dihydro-3-(2,2,2-trifluoroethylthiomethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide
Synonym Epitizide
Proprietary Name It is an ingredient of *Dyta-Urese*.



Chemical Properties A white crystalline powder. Mp 206° to 207°. Practically insoluble in water; soluble in alkaline solutions. Log *P* (octanol/water), 0.8.
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.13; system TE—R_f 0.44; system TF—R_f 0.62; system TAD—R_f 0.25; system TAE—R_f 0.88; system TAJ—R_f 0.22; system TAK—R_f 0.05; system TAL—R_f 0.63.
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 457.
Ultraviolet Spectrum Aqueous acid—272, 315 nm.



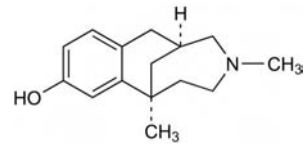
Infrared Spectrum Principal peaks at wavenumbers 1176, 1314, 1167, 1126, 1603, 1269 cm^{-1} (Nujol mull).
Dose 8 to 12 mg daily.

Eptazocine

Analgesic

$C_{15}H_{21}NO$ = 231.3
CAS—72522-13-5

Synonym (*1S*)-2,3,4,5,6,7-Hexahydro-1,4-dimethyl-1,6-methano-1*H*-4-benzazonin-10-ol



Eptazocine Hydrobromide

$C_{15}H_{22}BrNO$ = 312.2
CAS—72150-17-5
Synonym ST-2121
Proprietary Name *Sedapain*
Chemical Properties A crystalline powder. Mp 207° to 210°.

High Performance Liquid Chromatography Column: C_{18}-MS Cosmosil (150 × 4.6 mm i.d., 5 µm), at 25°. Mobile phase: 0.07 mol/L sodium phosphate buffer (pH 3) containing 5 mmol/L sodium heptylsulfonate : methanol (55:45), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex}=278 nm, λ_{em}=324 nm). Internal standard (IS): methyleptazocine. Retention time: eptazocine, 4.9 min; IS, 7.2 min [Suzuki *et al.* 2000].

Suzuki T *et al.* (2000). Pharmacokinetic/pharmacodynamic relationship of eptazocine, a narcotic-antagonist analgesic, in rats. *Biol Pharm Bull* 23: 1504–1510.

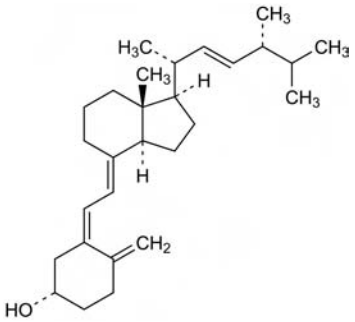
Ergocalciferol

Vitamin

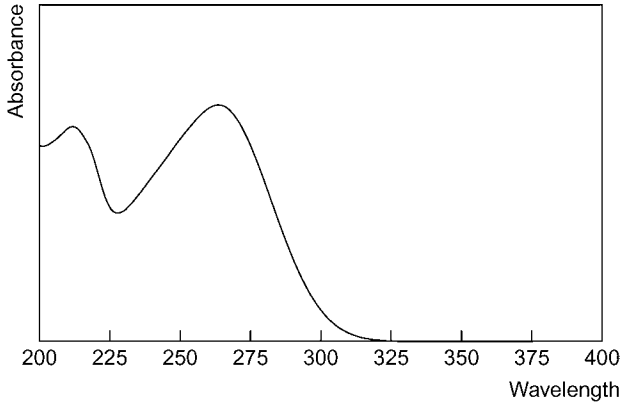
$C_{28}H_{44}O$ = 396.7
CAS—50-14-6

IUPAC Name 3-[2-[1-(5,6-Dimethylhept-3-en-2-yl)-7*a*-methyl-2,3,3*a*,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexan-1-ol
Synonyms Calciferol; irradiated ergosterol; (3β,5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol; viosterol; vitamin D_2.
Proprietary Names *Drisdol*; *Endo D*; *Ostelin*; *Radiostol*; *Sterogyl*; *Vita-D-Grin*.

Note Ergocalciferol is also used as a rodenticide. It is an ingredient of *Sorexa C.R.*



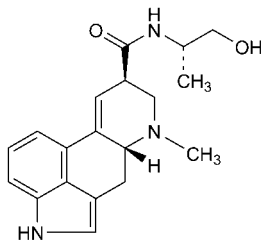
Chemical Properties Colourless or slightly yellow crystals, or white or slightly yellow crystalline powder. Mp 113° to 119°. Practically insoluble in water; soluble 1 in 2 of ethanol, 1 in 10 of acetone, 1 in 0.7 of chloroform and 1 in 2 of ether. Log *P* (octanol/water), 10.4.
High Performance Liquid Chromatography System HY—RI 276.
Ultraviolet Spectrum Ethanol—265 nm (A^1_1=475b).



Infrared Spectrum Principal peaks at wavenumbers 973, 1059, 1079, 1712, 893, 1000 cm^{-1} (KBr disk).
Quantification See under Colecalciferol.
Disposition in the Body Well absorbed after oral administration; absorption may be decreased with impaired liver and biliary function, and extensive enterohepatic circulation occurs. Metabolised by 25-hydroxylation in the liver followed by 1α- or 24-hydroxylation in the kidney; possibly conjugated with glucuronic acid or sulfate. Excreted mainly in the bile together with small amounts of metabolites in the urine; unchanged ergocalciferol does not appear to be excreted in the urine.
Blood Concentration Normal serum concentrations of 25-hydroxyergocalciferol are about 0.01 to 0.04 mg/L, but there are considerable inter-subject and seasonal variations.
Half-life About 40 days.
Protein Binding In blood, bound to α- and β-lipoproteins.
Dose 0.025 to 5 mg daily.

Ergometrine

Ergot Alkaloid
$C_{19}H_{23}N_3O_2$ = 325.4
CAS—60-79-7
Synonyms [8β(*S*)]-9,10-Didehydro-*N*-(2-hydroxy-1-methylethyl)-6-methylergoline-8-carboxamide; ergobasine; ergonovine.



Chemical Properties Colourless crystals. Solutions in water give a blue fluorescence. Mp 162°. Slightly soluble in water; more soluble in ethanol; freely soluble in ethyl acetate and acetone; sparingly soluble in chloroform. pK_a 6.8 (20°) [O'Neil MJ *et al.* 2006]. Log *P* (ether) 0.52 [Meylan, Howard 1995], −0.9. Methylergometrine was very unstable when kept in direct sunlight, with half-lives of 15 and 35 min at pH 2 and pH 7, respectively. There was no degradation under fluorescent light [Smith, Molinaro 1988]. Methylergometrine stock solutions were stable for 60 days at –50° [Edlund 1981].

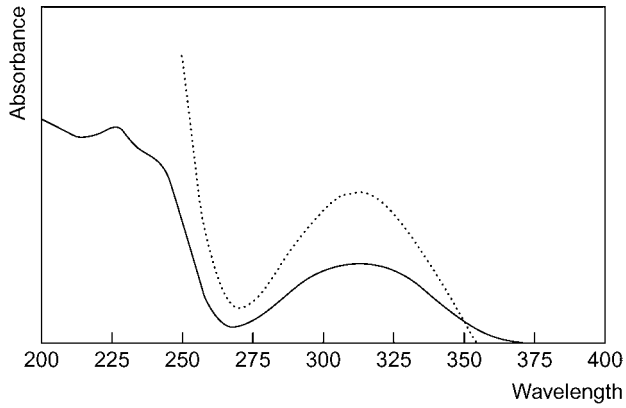
Ergometrine Maleate

$C_{19}H_{23}N_3O_2,C_4H_4O_4$ = 441.5
CAS—129-51-1
Synonyms Ergobasine maleate; ergometrinhydrogenmaleat; ergonovine bimaleate; ergonovine maleate; ergostetrine maleate; ergotocine maleate.
Proprietary Names *Ergotrate.* It is an ingredient of *Syntometrine.*
Chemical Properties A white or yellowish, slightly hygroscopic, microcrystalline powder. It darkens with age and on exposure to light. Solutions in water and ethanol give a blue fluorescence. Mp 167°, with decomposition. Soluble 1 in 36 of water and 1 in 100 of ethanol; practically insoluble in chloroform and ether.

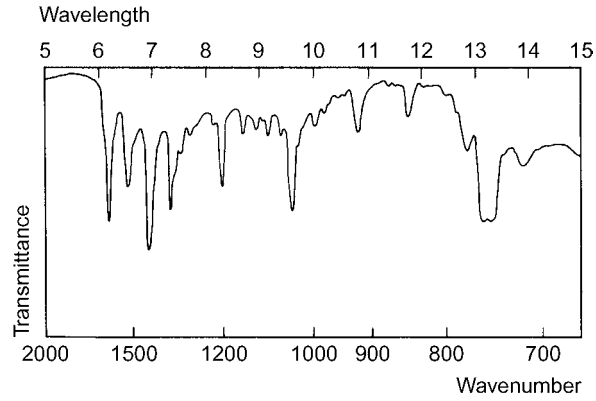
Ergometrine Tartrate

$(C_{19}H_{23}N_3O_2)_2,C_4H_6O_6$ = 800.9
CAS—129-50-0
Synonym Ergonovinum tartaricum
Chemical Properties White or slightly reddish-yellow, very light, matted masses of acicular crystals. Slightly soluble in water; soluble in ethanol; slightly soluble in chloroform and ether.

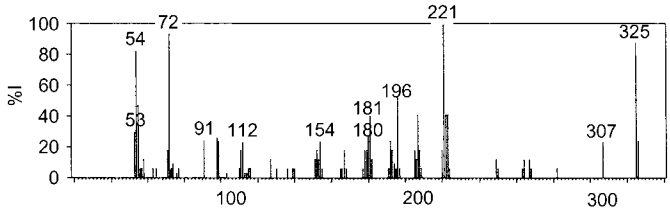
Colour Tests *p*-Dimethylaminobenzaldehyde—violet; Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.57; system TAE—R_f 0.62; system TAF—R_f 0.60; system TL—R_f 0.08; system TB—R_f 0.00; system TC—R_f 0.12; system TE—R_f 0.33; system TL—R_f 0.08; system TM—R_f 0.26 (Van Urk reagent, blue).
High Performance Liquid Chromatography System HA—*k* 0.4; system HP—*k* 0.50; system HX—RI 292.
Ultraviolet Spectrum Aqueous acid—313 nm (A^1_1 = 240a); aqueous alkali—310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1634, 754, 748, 1044, 1212, 1541 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 221, 72, 325, 54, 196, 55, 207, 181.



Quantification

***Plasma* HPLC** Column: Spherisorb 5-ODS (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.067 mol/L potassium dihydrogen phosphate-diethylamine (1 : 1) : acetonitrile (60 : 40), flow rate, 1.2 mL/min. Fluorescence detection (λ_{ex} = 315 nm, λ_{em} = 430 nm). Retention time: 8.61 min for methylergometrine. Limit of quantification, 150 ng/L for methylergometrine [de Groot *et al.* 1995]. Column: Spherisorb 5-ODS (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.067 mol/L potassium dihydrogenphosphate-diethylamine (1 : 1) : acetonitrile (65 : 35), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 315 nm, λ_{em} = 430 nm). Retention time: 5.26 min. Limit of quantification, 75 ng/L [de Groot *et al.* 1993]. Column: Supelcosil LC-8 (octyl, 250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L potassium phosphate (pH 7.0; 3 : 7), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 315 nm, λ_{em} = 440 nm). Retention time: 4.5 min for methylergometrine. Limit of quantification, 20 ng/L, limit of detection, 5 ng/L, both for methylergometrine [Smith, Molinaro 1988]. Column: ODS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L ammonium carbonate (30 : 70 or 50 : 50). Fluorescence detection (λ_{ex} = 328 nm, λ_{em} = 320 nm). Limit of detection, 100 ng/L for methylergometrine [Edlund 1981].

Disposition in the Body More rapidly absorbed from the gastrointestinal tract than ergotamine. Metabolised by hydroxylation and glucuronic acid conjugation and possibly *N*-demethylation. Peak plasma concentrations are attained within 60–90 min and are more than tenfold those achieved with an equivalent dose of ergotamine. It is less toxic than ergotamine. Ergometrine has been reported to be detected unchanged in urine up to 8 h after injection, the maximum concentration usually occurring 2 to 3 h after an injection. It is mainly excreted in the bile as 12-hydroxyergometrine glucuronide.

Therapeutic Concentration

Six male volunteers were administered an oral dose of 0.2 mg ergometrine maleate. A mean peak plasma concentration of 0.16 ug/L was reached after 54 min [de Groot *et al.* 1994].

After a single oral dose of ergometrine maleate (0.2 mg = 0.147 mg base) to one healthy male volunteer, a peak plasma concentration of 1.32 µg/L was reached after 12 min [de Groot *et al.* 1993].

Toxicity A dose of 0.2 mg, if inadvertently given to a neonate, is ~20-times the dose applicable to an adult [Edwards 1971]. For cases of ergometrine being inadvertently given to neonates, see Baum *et al.* [1996], Yalaburgi and Mohapatra [1982], Kenna [1972], Brereteon-Stiles *et al.* [1972] and Edwards [1971].

Note For a fatality following ergometrine IM following birth, see Devitt *et al.* [1970]. See also Whitfield and Salfield [1980], Asgaonkar *et al.* [1987], Dargaville and Campbell [1998], De Costa [2002], Aeby *et al.* [2003] and Bangh *et al.* [2005].

Volume of Distribution 73.4 ± 22.01 [de Groot *et al.* 1994].

Bioavailability 1 [de Groot *et al.* 1993], 34–117% [de Groot *et al.* 1994].

Half-life 1.4 h [de Groot *et al.* 1993].

Dose 0.4 to 1.5 mg of ergometrine maleate daily, orally; doses of 200 to 500 µg are given IM.

Aeby A *et al.* (2003). Methylergometrine poisoning in children: review of 34 cases. *J Toxicol Clin Toxicol* 41: 249–253.

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Yalaburgi SB, Mohapatra KC (1982). Accidental administration of syntometrine to a neonate resulting in death. *East Afr Med J* 59: 698–700.

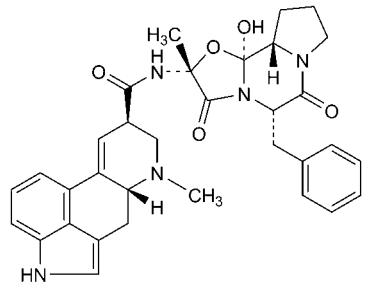
Ergotamine

α-Adrenoceptor Antagonist, Ergot Alkaloid

$C_{33}H_{35}N_5O_5$ = 581.7

CAS—113-15-5

Synonym (5′α)-12′-Hydroxy-2′-methyl-(phenylmethyl)ergotaman-3′,6′,18-trione



Chemical Properties Hygroscopic crystals which darken and decompose on exposure to air, heat and light. Mp 212° to 214°, with decomposition. Almost insoluble in water; slightly soluble in ethanol; freely soluble in chloroform, pyridine and glacial acetic acid; moderately soluble in ethyl acetate; slightly soluble in benzene. pK_a 6.4 (24°). Log *P* (octanol/water) 2.53 [Meylan, Howard 1995]. Ergotamine undergoes spontaneous epimerisation at C-8 to ergotaminine, epimerisation at C-2 in acidic solutions to form 'aci-compounds'. Addition of water to the 9 : 10 double bond can occur, catalysed by UV light. The indole is susceptible to oxidation by oxygen [Edlund 1981].

Ergotamine Tartrate

$(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ = 1313.4

CAS—379-79-3

Proprietary Names *Enxak*; *Ergodryl Mono*; *Ergokapton*; *Ergomar*; *Ergostat*; *Ergotan*; *Gynergen(e)*; *Lingraine*; *Migrexa*. It is an ingredient of *Bellergal*; *Cafergot*; *Dolatrin*; *Migril*; *Wigraine*.

Chemical Properties Colourless crystals or white or yellowish-white crystalline powder. Mp about 180°, with decomposition. Soluble 1 in about 500 of water and 1 in 500 of ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water) 2.5 [Meylan, Howard 1995].

Colour Tests *p*-Dimethylaminobenzaldehyde—violet; Mandelin's test—violet-brown; Marquis test—brown.

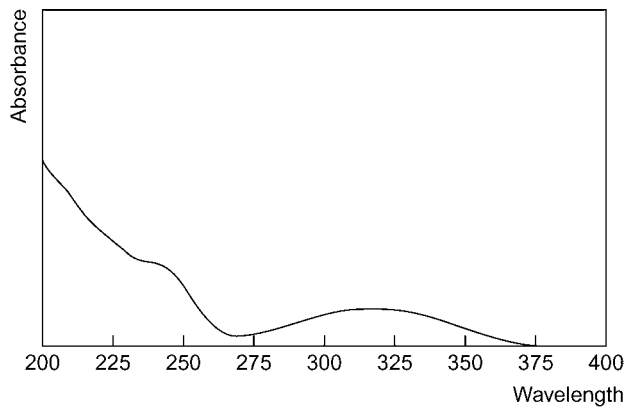
Thin-layer Chromatography System TA—R_f 0.63; system TAE—R_f 0.68; system TAF—R_f 0.64; system TL—R_f 0.22; system TB—R_f 0.01; system TC—R_f 0.34; system TE—R_f 0.44; system TL—R_f 0.23; system TM—R_f 0.48 (Van Urk reagent, blue).

Gas Chromatography System GA—RI 2366.

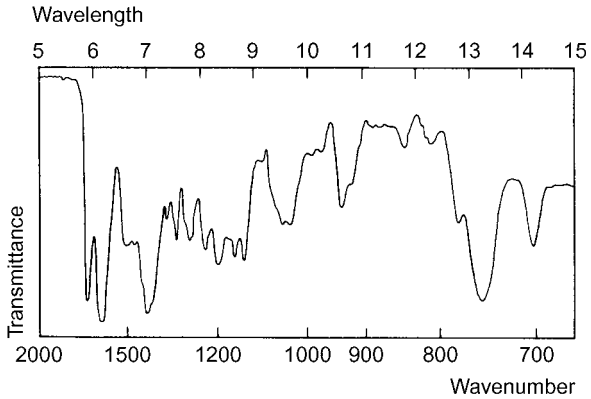
High Performance Liquid Chromatography System HA—*k* 0.4; system HP—*k* 9.58; system HX—RI 416; system HZ—RT 3.4 min.

Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : water : triethylamine (60 : 40 : 0.1), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 3.6 min. Limit of quantification, 0.5 mg/L, limit of detection, 0.35 mg/L [Elbarbry *et al.* 2007].

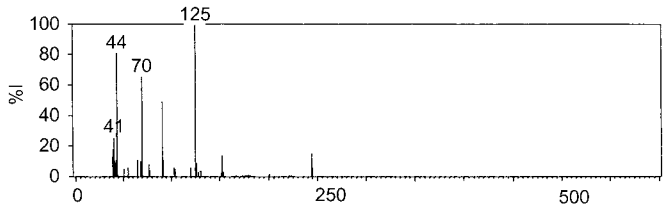
Ultraviolet Spectrum Aqueous acid—316 nm (A^1_1 = 133a); aqueous alkali—310 nm (A^1_1 = 148b).



Infrared Spectrum Principal peaks at wavenumbers 1631, 1712, 750, 1208, 1136, 1160 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 125, 44, 70, 91, 41, 40, 244, 153 (no peaks above 250).



Quantification

Blood **LC-MS** Column: Luna CN (150 × 2.1 mm i.d., 5 µm). Mobile phase: water-0.1% formic acid-2 mmol/L ammonium formate (pH 3.0) : acetonitrile-0.1% formic acid-2 mmol/L ammonium formate (80 : 20 to 20 : 80 in 8 min for 4 min), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 10 ng/L, limit of detection, 5 ng/L [Favretto *et al.* 2007].

Plasma **GC-MS** Column: 5% phenyl 95% methyl silicone fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.034 bar. Temperature programme: 60° to 280° at 35°/min for 10 min. EI ionisation. Limit of detection, 50 ng/L [Feng *et al.* 1992].

HPLC Column: ODS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L ammonium carbonate (30 : 70 or 50 : 50). Fluorescence detection ($\lambda_{ex} = 328$ nm, $\lambda_{em} = 320$ nm). Limit of detection, 100 ng/L [Edlund 1981].

Note For a radioimmunoassay method for the detection of ergot alkaloids in plasma, see Schran *et al.* [1979] or Ala-Hurula *et al.* [1979].

Serum **GC-MS** See Plasma [Feng *et al.* 1992].

Urine **LC-MS** See Blood [Favretto *et al.* 2007].

Hair **LC-MS** See Blood. Limit of quantification, 15 pg/mg, limit of detection, 10 pg/mg [Favretto *et al.* 2007].

Disposition in the Body Poorly absorbed after oral administration; the extent and rate of absorption is increased if caffeine is administered concurrently. Ergotamine is rapidly metabolised in the liver and is excreted mainly in the bile as metabolites; only traces of unchanged drug are excreted in the urine or faeces. Metabolised by CYP3A4. Ergotamine increases peristalsis of the gastrointestinal tract [Zavaleta *et al.* 2001].

Therapeutic Concentration

After a single oral dose of 2 mg of ergotamine tartrate administered to 11 subjects, peak plasma concentrations of 0.13 to 0.91 µg/L (mean 0.59) were attained in 0.5 to 3 h; following an IM dose of 0.5 mg given to 10 subjects, peak plasma concentrations of 0.88 to 3.6 µg/L (mean 2.07) were attained in ~0.5 h [Ala-Hurula *et al.* 1979].

After an IM injection of ergotamine tartrate 0.5 mg (3.6 to 3.9 µg/kg) into 10 subjects with migraine, the steady-state plasma concentration resulting in 50% of maximal effect was calculated to be 0.24 µg/L [Tfelt-Hansen, Paalzow 1985].

Toxicity Ergotamine is highly toxic; in large repeated doses it can produce all the symptoms of ergot poisoning. Fatal poisoning has occurred after the oral administration of 26 mg of ergotamine over several days, and also after single injections of only 0.5 to 1.5 mg.

A 40-year-old female with ergotism after ergotamine tartrate ingestion had a blood concentration of 320 ng/L and a urine concentration of 100 ng/L. Ergotamine concentration was 24 and 15 pg/mg in proximal and distal hair, respectively [Favretto *et al.* 2007].

In 12 cases of non-fatal ergotamine overdose, plasma concentrations of 0 to 3.1 µg/L (mean 0.82) were reported 1.7 to 5 h after ingestion [Lamb *et al.* 1983].

A 17-year-old primigravida in her 35th week of pregnancy took 10 tablets of Migril (≈20 mg ergotamine tartrate). The foetus was in cephalic presentation and the heart rate was 170 bpm. Foetal death was diagnosed ≈8 h later. The girl was successfully treated with nifedipine for MI [Au *et al.* 1985].

A 14-month-old male swallowed a total of 12 mg ergotamine tartrate. Symptoms included severe dyspnoea, pyrexia, haemorrhagic gastritis, rapid onset of cerebral oedema followed by death [Jones, Williams 1966].

Note For cases of ergotism (arterial insuffiency in the extremities, also known as Holy Fire or St Anthony's fire) following therapeutic doses of ergotamine tartrate, see Enge and Sivertssen [1965], Voyvodic and Hayward [1996], Tay and Chee [1998], Sintenie *et al.* [1992], Doig *et al.* [1985], Weaver *et al.* [1989], Fitzgerald [1978], Matthews and Havill [1979] and MacGuire and Cassidy [1984]. For a case report of recurrent ergotism, see Curry, Jr. and Yalamanchili [1978]. For a case of facial ischaemia caused by ergotism, see Lazarides *et al.* [1992]. For a case of renal impairment following ergotamine, see Lund [1992]. There is a possible interaction between erythromycin and ergotamine Ghali *et al.* [1993] or Karam *et al.* [2000]. For interactions of ergotamine with ritonavir, see Baldwin and Ceraldi [2003]; Blanche *et al.* [1999]; Caballero-Granado *et al.* [1997]; Pardo *et al.* [2003]; Tribble *et al.* [2002]; or Vila *et al.* [2001]. For a case of pancreatitis and hepatitis after ergotamine ingestion, see Deviere *et al.* [1987]. For a review of ergotism, see Harrison [1978]. For a case of ergotamine poisoning in a paraplegic patient, see Lenger [1984]. See also Molkara *et al.* [2006], Aydogan *et al.* [2005], Garcia *et al.* [2000], Piquemal *et al.* [1998], Wilke *et al.* [1997], Flaherty and Bates [1996], Voyvodic and Hayward [1996], Fincham *et al.* [1985], Merhoff and Porter [1974], London *et al.* [1970], Yao *et al.* [1970] or Glazer *et al.* [1966] for case studies.

Half-life Biphasic, α phase, 2.7 h, β phase 21 h [Kanto 1983].

Bioavailability 1.2% (0.5 to 2.2) for inhalaton, 1.8% (0.6 to 3.5%) for suppositories and 1.5% (0.6 to 2.6%) for effervescent tablets [Ekbom *et al.* 1983], 47% for IM (28 to 61%) [Meyler 1996]

Volume of Distribution ≈2 L/kg, 141 L [Kanto 1983].

Clearance Plasma clearance, ≈5 to 18 mL/min/kg (mean 11).

Note For a review of ergometrine efficacy in migraine, see Perrin [1985].

Dose Not more than 8 mg of ergotamine tartrate in a day or 12 mg in any one week, as shown in the table below.

Recommended dosage for ergotamine

Route	Single dose	Maximum per attack	Maximum per week
Oral	1–2 mg	6 mg	12 mg
Sublingual	2 mg	6 mg	12 mg
Rectal	2 mg	4 mg	8 mg
Inhalaton	0.36 mg (1 inhalation)	2.16 mg (6 inhalations)	12 mg (33 inhalations)
Parenteral	0.25–0.5 mg	0.5 mg	1.0 mg

[Curry Jr, Yalamanchili 1978]

Note For a review of ergotamine and its use in migrane, see Saxena and de Deyn [1992].

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Lenger R (1984). Ergot poisoning in paraplegia. *Paraplegia* 22: 42–44.
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Sintenie JB *et al.* (1992). Misleading cause of acute arterial insufficiency: ergotamine intoxication. Case report. *Eur J Surg* 158: 189–190.
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Tfelt-Hansen P, Paalzow L (1985). Intramuscular ergotamine: plasma levels and dynamic activity. *Clin Pharmacol Ther* 37: 29–35.
Tribble MA *et al.* (2002). Fatal ergotism induced by an HIV protease inhibitor. *Headache* 42: 694–695.
Vila A *et al.* (2001). Clinical ergotism induced by ritonavir. *Scand J Infect Dis* 33: 788–789.
Voyvodic F, Hayward M (1996). Case report: upper extremity ischaemia secondary to ergotamine poisoning. *Clin Radiol* 51: 589–591.
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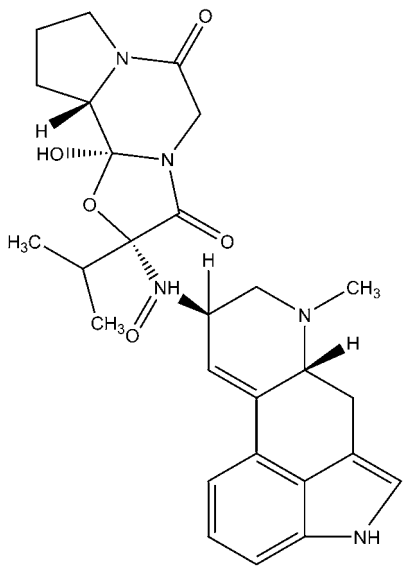
Ergotoxine

Ergot Derivative

$C_{28}H_{33}N_5O_5 = 519.6$

CAS—8006-25-5

Synonym Ecboline



Chemical Properties Ergotoxine is a 1 : 1 : 1 mixture of the 3 isomorphous alkaloids: ergocornine, ergocristine and ergocryptine. Crystals. Mp 190° with decomposition. Almost insoluble in water and petroleum ether; very soluble in alcohol, acetone, chloroform and ethyl acetate; slightly soluble in ether.

Ergotoxine Esilate

$C_{31}H_{39}N_5O_5,C_2H_5SO_{3H} = 671.8$

CAS—8047-28-7

Synonym Ergotoxine ethanesulfonate

Chemical Properties Colourless crystals that decompose at 209°. Almost insoluble in water; slightly soluble in ethanol; soluble in methanol. Solid and solutions are sensitive to light and air.

Ergotoxine Phosphate

Chemical Properties Clusters of needles, decomposing at 187°. Soluble in 320 parts of water, 15 parts boiling alcohol.

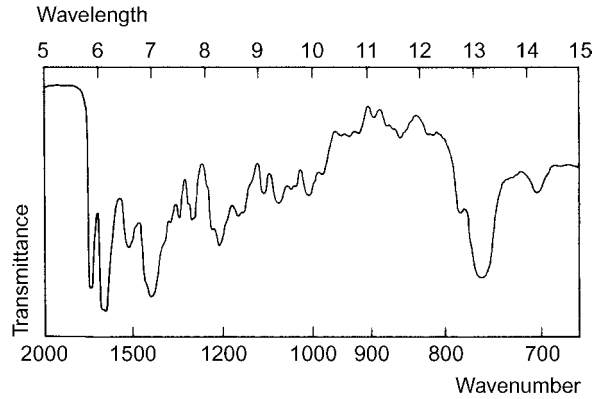
Colour Test *p*-Dimethylaminobenzaldehyde—violet.

Thin-layer Chromatography System TA—R_f 0.66; system TL—R_f 0.48; system TB—R_f 0.01; system TC—R_f 0.62; system TL—R_f 0.48; system TM—R_f 0.67 (acidified potassium permanganate solution, strong reaction; Van Urk reagent, blue).

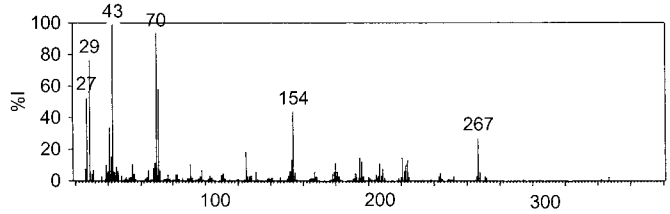
Gas Chromatography System GA—ergocristine RI 2495, ergocryptine RI 2184.

High Performance Liquid Chromatography System HA—ergocornine *k* 0.4, ergocristine *k* 0.3, ergocryptine *k* 0.4; system HP—ergocornine *k* 10.2, ergocristine *k* 17.3, ergocryptine *k* 15.2.

Infrared Spectrum Principal peaks at wavenumbers 1639, 1721, 758, 1534, 1212, 1294 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 70, 29, 71, 27, 154, 41, 267; ergocornine 43, 70, 71, 54, 44, 154, 267, 55; ergocristine 70, 125, 71, 91, 153, 267, 154, 221; ergocryptine 43, 70, 71, 154, 41, 209, 69, 267.



Disposition in the Body

Therapeutic Concentration

Ten healthy volunteers (6 males, 4 females) aged between 30 and 68 years were administered 1 mL of 3H-dihydroergotoxine solution or 1 of 3H-DH-ergotoxine tablet (equivalent to 1 mg Hydergine) orally. The drug was also infused IV at a dose of 0.5 mg. Urinary excretion percentages were as shown in the table below.

Time (h)	*Tablet oral (1 mg)*	*Solution oral (1 mg)*	*Infusion IV (0.5 mg)*
0–4	0.55 ± 0.08	0.51 ± 0.06	3.4 ± 0.44
0–8	1.18 ± 0.11	1.13 ± 0.08	4.89 ± 0.55
0–16	1.44 ± 0.14	1.23 ± 0.07	5.91 ± 0.9
0–24	1.74 ± 0.2	1.59 ± 0.14	6.77 ± 1.26
0–48	2.01 ± 0.3	1.74 ± 0.15	8.12 ± 1.31
0–72	2.09 ± 0.33	1.77 ± 0.15	8.45 ± 1.11

[a][Loddo *et al.* 1976].

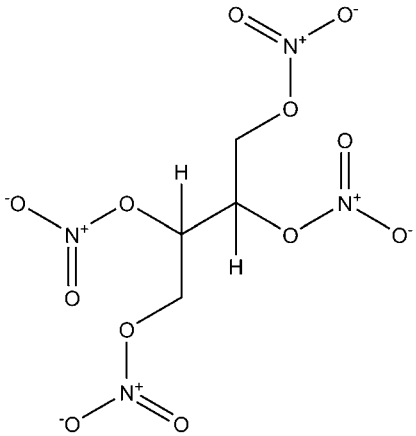
Half-life 5.2 to 8.7 h after a single oral or sublingual dose [Kanto 1983].
Dose Ergotoxine esilate was formerly given parenterally in doses of 0.5 to 1 mg.

Kanto J (1983). Clinical pharmacokinetics of ergotamine, dihydroergotamine, ergotoxine, bromocriptine, methysergide, and lergotrile. *Int J Clin Pharmacol Ther Toxicol* 21: 135–142.
Loddo P *et al.* (1976). Urinary elimination of dihydro-ergotoxine after oral administration and intravenous infusion. *Boll Chim Farm* 115: 570–574.

Erythrityl Tetranitrate

Vasodilator (Coronary)
$C_4H_6(NO_3)_4$ = 302.1
CAS—142435-64-1

IUPAC Name 1,3,4-Trinitrooxybutan-2-yl nitrate
Synonyms Erithritol tetranitrate; erythrol nitrate of tetranitrate; erythrotetranitral; nitro-erythrite; nitroerythrol; tetranitrol.
Proprietary Names *Cardilate*. It is an ingredient of *Dolorin* and *Erythin*.



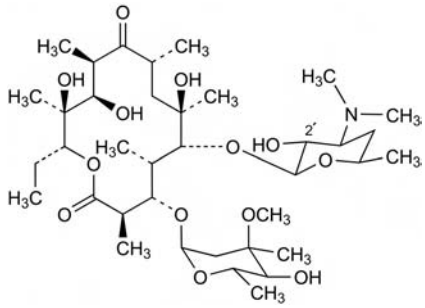
Chemical Properties Colourless crystals which are explosive unless mixed with an inert substance. Mp ≈61°. Almost insoluble in water; soluble in ethanol, ether and glycerin. Extracted by organic solvents from aqueous acid or alkaline solutions.
Quantification
Other **HPLC** Various Tablet Formulations. Column: C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : water (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention times: erythrityl tetranitrate (ET) ≈32 min, isosorbide dinitrate (ID) ≈10 min, nitroglycerin (internal standard) ≈15 min, pentaerythritol tetranitrate (PT) ≈25 min. Limit of quantification, 0.6, 0.2 and 0.4 µg, for ET, ID and PT, respectively [Olsen, Scroggins 1984].
Disposition in the Body Acts more slowly than glyceryl trinitrate or amyl nitrite, but has a much longer duration of action. After oral administration, the maximum effect is attained in 20 to 30 min and lasts for 3 to 4 h. Erythrityl tetranitrate releases nitrite ions into the bloodstream, but the amount does not appear to be sufficient to account for the pharmacological effect of the drug.
Toxicity Prolonged use may result in methaemoglobinaemia.
Dose Up to 60 mg.

Olsen CS, Scroggins HS (1984). High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms. *J Pharm Sci* 73: 1303–1304.

Erythromycin

Antibiotic
$C_{37}H_{67}NO_{13}$ = 733.9
CAS—114-07-8 (erythromycin A)

IUPAC Name (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione
Proprietary Names *Abboticine*; *Ak-Mycin*; *Akne-Mycin*; *ATS*; *Del-Mycin*; *E-Base*; *E/Gel*; *Emgel*; *Emu-V*; *E-Mycin*; *Eratrex*; *Eromycin*; *Eryacne*; *Eryc*; *Erycen*; *Erycette*; *Erycinum*; *Eryderm*; *Erygel*; *Erymax*; *Ery-Tab*; *Erythrocin*; *Erythromid*; *Ilosone*; *Ilotycin*; *Novo-Rythro*; *PCE*; *Retcin*; *Robimycin*; *Rommix*; *Ronmix*; *Staticin*; *Stiemycin*; *Theramycin Z*; *Toloryth*; *T-Stat*.



Chemical Properties Erythromycin and its salts are ingredients of many preparations—see Sweetman [2009]. An antimicrobial substance produced by the growth of certain strains of *Streptomyces erythreus*. It is a mixture consisting largely of erythromycin A with lesser amounts of erythromycins B and C. White or slightly yellow, slightly hygroscopic, crystals or powder. Mp 135° to 140°. Soluble 1 in 1000 of water, less soluble at higher temperatures; soluble 1 in 5 of ethanol; freely soluble in acetone, chloroform, acetonitrile and ethyl acetate; moderately soluble in ether, ethylene dichloride and amyl acetate. pK_a 8.9. Log *P* (octanol/water), 3.1.

Erythromycin Estolate
$C_{40}H_{71}NO_{14}$,$C_{12}H_{26}O_4S$ = 1056.4
CAS—3521-62-8
Synonyms Erythromycin propionate laurilsulfate; propionyl erythromycin laurilsulfate.
Proprietary Names *E-Mycin*; *Erymin*; *Eromycin*; *Ilosone*.
Chemical Properties A white crystalline powder. Mp 135° to 140°, with decomposition. Practically insoluble in water; soluble 1 in 2 of ethanol, 1 in 15 of acetone and 1 in 10 of chloroform.

Erythromycin Ethylsuccinate
$C_{43}H_{75}NO_{16}$ = 862.1
CAS—41342-53-4; 1264-62-6
Proprietary Names *Abboticin(e)*; *Arpimycin*; *EES*; *E-Mycin*; *Eromycin*; *Ery*; *Erymax*; *Erymin*; *Ery-Ped*; *Erythrocin(e) Erythroped*.
Chemical Properties A white or slightly yellow crystalline powder. Very slightly soluble in water; freely soluble in ethanol and chloroform.

Erythromycin Gluceptate
$C_{37}H_{67}NO_{13}$,$C_7H_{14}O_8$ = 960.1
CAS—23067-13-2
Synonym Erythromycin glucoheptonate
Proprietary Name *Ilotycin*
Chemical Properties A white, slightly hygroscopic powder. Mp 95° to 140°. Freely soluble in water, ethanol, methanol, acetone, propylene glycol and dioxane; slightly soluble in chloroform; practically insoluble in ether, carbon tetrachloride, toluene and benzene.

Erythromycin Lactobionate
$C_{37}H_{67}NO_{13}$,$C_{12}H_{22}O_{12}$ = 1092
CAS—3847-29-8
Proprietary Names *Abboticin*; *Eryc*; *Erycinum*; *Erymax*; *Erythrocin(e)*.
Chemical Properties White or slightly yellow crystals or powder. Mp 145° to 150°. Freely soluble in water, ethanol and methanol; slightly soluble in chloroform and ether.

Erythromycin Propionate
$C_{40}H_{71}NO_{14}$ = 790.0
CAS—134-36-1
Proprietary Names *Ery*; *Propiocine*.
Chemical Properties A white powder. Very slightly soluble in water; readily soluble in acetone, ethyl acetate, ethanol and chloroform.

Erythromycin Stearate
$C_{37}H_{67}NO_{13}$,$C_{18}H_{36}O_2$ = 1018.4
CAS—643-22-1
Proprietary Names *Abboticin(e)*; *Eramycin*; *Eromycin*; *Erotab*; *Erycin*; *Erycinum*; *Erythrocin(e)*.
Chemical Properties White or slightly yellow crystals or powder. Practically insoluble in water; slightly soluble in ethanol, chloroform and ether.

Colour Test Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.11; system TE—R_f 0.46; system TAE—R_f 0.24; system TAJ—R_f 0.04; system TAK—R_f 0.02; system TAL—R_f 0.42.
Infrared Spectrum Principal peaks at wavenumbers 1050, 1168, 1074, 1010, 1108, 1734 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** Electrochemical detection. For method of quantification, see Toreson and Eriksson [1995].
HPLC–MS Limit of detection, <0.5 µg/L [Li *et al.* 1998].
Serum **HPLC** Fluorescence detection. For method for quantification of erythromycin and other macrolide antibiotics, see Sastre Toraño and Guchelaar [1998].

Derivatisation and fluorescence detection. Limit of detection, <10 µg/L for erythromycin and erythromycin ethylsuccinate [Tsuji 1978].

Gastric Juice **HPLC** See Plasma [Toreson, Eriksson 1995].

Review Analysis of macrolide antibiotics [Kanfer *et al.* 1998].

Disposition in the Body Erythromycin base is destroyed by gastric acid; erythromycin stearate is hydrolysed in the intestine and absorbed as free erythromycin; the estolate is absorbed as the ester and then hydrolysed. About 5 to 10% of a dose is excreted in the urine unchanged and large amounts of unchanged drug are excreted in the bile.

Therapeutic Concentration

Following single oral doses of 250 mg, 500 mg and 1 g of a preparation containing enteric-coated pellets of erythromycin base to 23 subjects, mean peak serum concentrations of 1.9, 3.8 and 6.5 mg/L, respectively, were attained in about 2 h. After a single oral dose of 500 mg of erythromycin stearate to the same subjects, a mean peak serum concentration of 2.9 mg/L was attained in about 2 h [Josefsson *et al.* 1982].

Following oral administration of 250 mg of erythromycin four times a day to 16 subjects, maximum and minimum steady-state plasma concentrations of 1.7 and 0.45 mg/L were reported on the third day, compared with 1.34 and 0.58 mg/L respectively, following repeated oral administration of erythromycin estolate [DiSanto *et al.* 1980].

Half-life Plasma half-life, about 1 to 3 h (dose-dependent).

Volume of Distribution About 0.7 L/kg.

Clearance Plasma clearance, about 7 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.43.

Protein Binding Erythromycin about 70 to 80%, erythromycin propionate about 90 to 99%, erythromycin stearate about 90%.

Dose 1 to 4 g daily.

DiSanto AR *et al.* (1980). Comparative bioavailability evaluation of erythromycin base and its salts and esters. I. Erythromycin estolate capsules versus enteric-coated erythromycin base tablets. *J Clin Pharmacol* 20: 437–443.

Josefsson K *et al.* (1982). Dose-related pharmacokinetics after oral administration of a new formulation of erythromycin base. *Br J Clin Pharmacol* 13: 685–691.

Kanfer I *et al.* (1998). Analysis of macrolide antibiotics. *J Chromatogr A* 812: 255–286.

Li YX *et al.* (1998). Sensitive determination of erythromycin in human plasma by LC-MS/MS. *J Pharm Biomed Anal* 16(6): 961–970.

Sastre Toraño JS, Guchelaar HJ (1998). Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 720: 89–97.

Sweetman SC, ed. (2009). *Martindale: The Complete Drug Reference*, 36 edn. London: Pharmaceutical Press.

Toreson H, Eriksson BM (1995). Determination of erythromycin in gastric juice and blood plasma by liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Appl* 673: 81–89.

Tsuji K (1978). Fluorimetric determination of erythromycin and erythromycin ethylsuccinate in serum by a high-performance liquid chromatographic post-column, on-stream derivatization and extraction method. *J Chromatogr* 158: 337–348.

Escitalopram

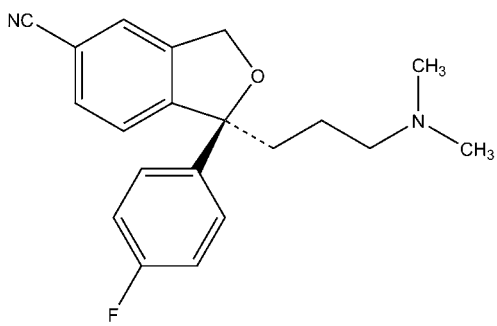
Antidepressant, Phthalane Derivative, Selective Serotonin Reuptake Inhibitor (SSRI)

$C_{20}H_{21}FN_2O = 324.4$

CAS—128196-01-0

IUPAC Name (1*S*)-1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3*H*-2-benzofuran-5-carbonitrile

Synonyms *S*-Citalopram; (+)-(*S*)-1-[3-(dimethylamino)propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonitrile.



Chemical Properties Log *P* (octanol/water), 4.22 [Wishart 2006]. Stable in plasma for 36 days at –70° and for 8 h at room temperature. Working solutions of escitalopram stable for 30 days at 2 to 8° [Singh *et al.* 2004].

Escitalopram Oxalate

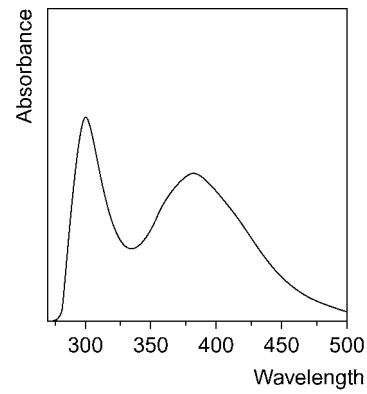
$C_{20}H_{21}FN_2O,C_2H_2O_4 = 414.4$

CAS—219861-08-2

Synonyms *S*-Citalopram oxalate; Lu-26-054/0.

Proprietary Names *Aramix*; *Cipralex*; *Citalax*; *Ectiban*; *Entact*; *Esertia*; *Ipran*; *Lexapro*; *Lextor*; *Meridian*; *Neozentius*; *Recita*; *S-Citadep*; *Seroplex*; *Sipralexa*.

Ultraviolet Spectrum Neutral—305, 384 nm.



Quantification

Blood **HPLC** Column: acetylated β-cyclodextrin (250 × 4.6 mm i.d.). Mobile phase: methanol : 100 mmol/L citric acid TEA (pH 6.3, 55 : 45), flow rate 0.8 mL/min. Fluorescence detection ($\lambda_{ex} = 240$ nm, $\lambda_{em} = 300$ nm). Retention times: escitalopram 15.6 min, desmethylescitalopram 12.5 min, didesmethylescitalopram 10.8 min; (*R*)-citalopram 17 min, (*R*)-desmethylcitalopram 13.2 min, (*R*)-didesmethylcitalopram 11.3 min. Limit of quantification, 2 µg/kg for all analytes [Holmgren *et al.* 2004].

Plasma **HPLC** Column: Merck RP Select B C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (40 : 60) containing 0.01% sodium chloride and 0.01% hydrogen phosphate, flow rate 1.6 mL/min. UV detection ($\lambda = 210$ nm). Limit of detection, 1 µg/L [Rampono *et al.* 2000]. Column: Chirobiotic vancomycin (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetic acid : TEA (99.9 : 0.055 : 0.060), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 240$ nm, $\lambda_{em} = 296$ nm). Retention time: escitalopram 12.6 min, desmethylescitalopram 13.7 min, didesmethylescitalopram 9.7 min; (*R*)-citalopram 11.5 min, (*R*)-desmethylcitalopram 10.5 min, (*R*)-didesmethylcitalopram 8.0 min. Limit of quantification, 5 µg/L for escitalopram, (*R*)-citalopram and desmethyl metabolites; 7.5 µg/L for didesmethyl metabolites [Kosel *et al.* 1998]. Column: Chiral-AGP (100 × 4.0 mm i.d.). Mobile phase: 10 mmol/L hexanoic acid and 3.0 mmol/L SB-12 in phosphate buffer (pH 6.5), flow rate 0.90 mL/min. UV detection ($\lambda = 240$ nm). Retention time: escitalopram 10.7 min, (*R*)-citalopram 8.8 min. Limits of quantification, escitalopram 2.55 µg/L, (*R*)-citalopram 2.31 µg/L [Haupt 1996]. Column: Cyclobond (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 1% diethylamine buffer (pH 6.1; 65 : 35), flow rate 0.8 mL/min. Fluorescence detection ($\lambda_{ex} = 240$ nm, $\lambda_{em} = 296$ nm). Retention time: escitalopram 15.5 min, desmethylescitalopram 12.2 min, didesmethylescitalopram 10.6 min, (*R*)-citalopram 16.6 min, (*R*)-desmethylcitalopram 12.9 min, (*R*)-didesmethylcitalopram 11.1 min. Limit of quantification, 3 µg/L for all analytes [Rochat *et al.* 1995a]. Column: Chiralcel OD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : propan-2-ol (93.5 : 6.5) containing 0.2% diethylamine, flow rate 0.4 mL/min for 16 min, to 1.0 mL/min for 1 min. Fluorescence detection ($\lambda_{ex} = 240$ nm, $\lambda_{em} = 296$ nm). Retention times: escitalopram 21 min, desmethylescitalopram 25 min; didesmethylescitalopram 31 min; (*R*)-citalopram 20 min; (*R*)-desmethylcitalopram 28 min; (*R*)-didesmethylcitalopram 35.5 min. Limit of quantification, 25 µg/L for citalopram enantiomers, 5 µg/L for desmethylcitalopram and didesmethylcitalopram enantiomers. For analysis of citalopram propionic acid enantiomers: Mobile phase: hexane : propan-2-ol (50 : 50), flow rate 0.65 mL/min. Retention times: escitalopram propionic acid 12.5 min; (*R*)-citalopram propionic acid 10.5 min. Limit of quantification, 5 µg/L [Rochat *et al.* 1995b].

LC-MS Column: Chiralcel OD-R (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : methanol : water (30 : 30 : 40) containing 0.05% diethylamine, flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: escitalopram 10.5 min, desmethyl metabolite 11.5 min. Limit of quantification, 0.1 µg/L for all analytes [Rocha *et al.* 2007]. Column: ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 2.0 mmol/L ammonium acetate (pH 5.0, 46 : 54), flow rate 1.0 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 1.0 µg/L [Singh *et al.* 2004].

Serum **HPLC** Column: β-cyclodextrin (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L citric acid buffer (pH 6.0) with 30–40% methanol, flow rate 1.0-1.5 mL/min. Fluorescence detection ($\lambda_{ex} = 240$ nm, $\lambda_{em} = 296$ nm). Retention times: escitalopram ~38 min, desmethylescitalopram approx. 23 min, didesmethylescitalopram approx. 17 min; (*R*)-citalopram ~30 min, (*R*)-desmethylcitalopram approx. 20 min, (*R*)-didesmethylcitalopram ~15 min. Limits of quantification, 15.4, 8.1 and 3.4 nmol/L for both enantiomers of citalopram, desmethylcitalopram and didesmethylcitalopram, respectively [Sidhu *et al.* 1997].

Urine **HPLC** See Serum [Sidhu *et al.* 1997].

CE Column: fused silica capillary (50 cm, 75 µm i.d.). Buffer: 20 mmol/L sodium dihydrogen phosphate/disodium hydrogen phosphate buffer (pH 5) with 0.2%

carboxymethylated-γ-cyclodextrin and 0.05% hydroxypropylmethylcellulose. UV detection (λ = 205 nm). Limit of quantification, 230 µg/L for escitalopram, 270 µg/L for desmethyl/didesmethyl metabolites, 200 µg/L for *N*-oxide metabolite [Berzas-Nevado *et al.* 2006].

CE-MS Column: fused silica capillary (total/effective length: 55/42 cm, 75 µm i.d.) with 5 cm filled with solution of 575 µL of 3-(trimethoxysilyl)propyl methacrylate and 100 µL of 0.12 mol/L hydrochloric acid, irradiated at 365 nm for 4.5 min. Buffer: 5 mmol/L ammonium acetate (pH 6.8) : acetonitrile (30 : 70). TOF, ESI. Limit of quantification, 34 ng/L, limit of detection, 10 ng/L [Johannesson, Bergquist 2007].

***Milk* HPLC** See Plasma [Rampono *et al.* 2000].

Note For the fluorometric quantification of escitalopram in plasma, see Serebruany *et al.* [2007].

Disposition in the Body Rapidly absorbed after oral administration with peak plasma concentrations reached within 4 h of dosing. Escitalopram is predominantly metabolised in the liver to less lipophilic compounds that are readily excreted in urine. Biotransformation occurs via oxidative metabolism with *N*-demethylation to desmethylescitalopram ((*S*)-desmethylcitalopram) and didesmethylescitalopram ((*S*)-didesmethylcitalopram), deamination and dehydrogenation to a propionic acid derivative, and also *N*-oxidation and glucuronide conjugation. *N*-Demethylation of escitalopram is mediated by CYP3A4 (~37%), CYP2C19 (~35%) and, to a lesser extent, CYP2D6 (~28%). Studies have shown that genetic polymorphism of CYP2C19 affects plasma concentrations of escitalopram and its desmethyl metabolite. Poor metabolisers have higher plasma concentrations of escitalopram and lower concentrations of the desmethyl metabolite, although renal clearance and urinary recovery of the compounds do not differ significantly between the 2 phenotypes. Further demethylation of desmethylescitalopram to didesmethylescitalopram is mediated by CYP2D6 and an unknown non-CYP-mediated reaction. Monoamine oxidases A and B and aldehyde oxidase may mediate metabolism in the brain. Biotransformation of escitalopram to its propionic acid metabolite occurs via deamination mediated by MAOs A and B followed by aldehyde oxidase-mediated oxidation. Elimination of escitalopram and its metabolites is mainly via renal excretion with only a small fraction voided in the faeces. Only 8 to 10% of a dose is excreted unchanged. There is no conversion of escitalopram to the *R*-isomer after oral administration in either plasma or urine. It is distributed into breastmilk.

Therapeutic Concentration

A group of healthy volunteers were administered escitalopram as an IV infusion (10 mg over 60 min) or oral formulation (single 20 mg oral dose and 10 or 30 mg/day for 24 days). Mean peak plasma concentrations of escitalopram and its desmethyl metabolite (M1) for the different regimen were reported as:

Dose:	*10 mg IV*	*20 mg oral*	*10 mg/day oral for 24 days*	*30 mg/day oral for 24 days*
Escitalopram				
C_{max} (µg/L)	19.0	18.8	20.6	64.4
Time (h)	0.99	3.0	3.9	4.1
M1				
C_{max} (µg/L)	1.4	3.4	7.4	19.4
Time (h)	36.5	14.0	7.5	6.0

[Sogaard *et al.* 2005].

Twenty 20 healthy male subjects were administered either 10 mg/day escitalopram or 20 mg/day citalopram for 10 days. Mean peak plasma concentrations and concentrations at 5.5 h and 53.5 h post-dose were reported as:

	C_{max} (µg/L)	*Time (h)*	$C_{5.5\,h}$ (µg/L)	$C_{53.5\,h}$ (µg/L)
(*S*)-Citalopram				
Escitalopram 10 mg	16.7	5.5	16.7	4.6
Citalopram 20 mg	16.8	7.8	15.8	4.8
(*R*)-Citalopram				
Citalopram 20 mg	27.7	7.8	24.9	12.3

A higher serotonin transporter occupancy was found after escitalopram dosing compared to citalopram despite similar plasma concentrations of the *S*-enantiomer. This can be explained by the attenuating effect of (*R*)-citalopram on the occupancy of (*S*)-citalopram at the serotonin transporter [Klein *et al.* 2007].

The transfer of escitalopram into breastmilk was studied in a group of 8 breastfeeding women who were being treated for postnatal depression with a median dose of 10 mg/day escitalopram. Blood samples were taken just before the morning dose of escitalopram and at 2, 4 and 6 h after dosing. Mean peak concentrations of escitalopram and its desmethyl metabolite in breastmilk and plasma were reported as:

	Breastmilk		*Plasma*	
	C_{max} (µg/L)	*Time (h)*	C_{max} (µg/L)	*Time (h)*
Escitalopram	78	5.5	35	3.4
Metabolite	27	4.8	10	2.1

The absolute escitalopram infant dose was estimated as 7.6 µg/kg/day or 3.9% of the maternal dose [Rampono *et al.* 2006].

Toxicity Escitalopram is a weak or negligible inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, suggesting that it has low potential to cause significant interactions with drugs that are substrates of these isoenzymes. However, the co-administration of desipramine (a CYP2D6 substrate) to a high-dose regimen of escitalopram resulted in a 40% increase in the peak plasma concentration and a 100% increase in the AUC of desipramine.

Bioavailability Approximately 80%.

Half-life Approximately 27 to 32 h.

Volume of Distribution 1100 L.

Clearance Approximately 36.0 L/h; renal 2.7 L/h.

Protein Binding Approximately 56%.

Note For a review of the clinical pharmacokinetics of escitalopram, see Rao [2007].

Dose Given orally as the oxalate although doses are expressed in terms of the base; escitalopram oxalate 12.8 mg is equivalent to approx. 10 mg of escitalopram.

In the treatment of depression, the usual dose is 10 mg once daily increased, after at least a week, to a maximum of 20 mg once daily if necessary.

It is also used in the treatment of panic disorder with or without agoraphobia. Initial doses are 5 mg once daily, increased after a week to 10 mg once daily; further increases up to a maximum of 20 mg daily may be necessary in some patients.

Berzas-Nevado JJ *et al.* (2006). Enantiomeric screening of racemic citalopram and metabolites in human urine by entangled polymer solution capillary electrophoresis: an innovatory robustness/ruggedness study. *Electrophoresis* 27: 905–917.

Haupt D (1996). Determination of citalopram enantiomers in human plasma by liquid chromatographic separation on a Chiral-AGP column. *J Chromatogr B Biomed Appl* 685: 299–305.

Holmgren P *et al.* (2004). Enantioselective analysis of citalopram and its metabolites in postmortem blood and genotyping for CYD2D6 and CYP2C19. *J Anal Toxicol* 28: 94–104.

Johannesson N, Bergquist J (2007). Rapid on-line extraction and quantification of escitalopram from urine using sol-gel columns and mass spectrometric detection. *J Pharm Biomed Anal* 43: 1045–1048.

Klein N *et al.* (2007). Higher serotonin transporter occupancy after multiple dose administration of escitalopram compared to citalopram: an [^{123}I]ADAM SPECT study. *Psychopharmacology (Berl)* 191: 333–339.

Kosel M *et al.* (1998). Analysis of the enantiomers of citalopram and its demethylated metabolites using chiral liquid chromatography. *J Chromatogr B Biomed Sci Appl* 719: 234–238.

Rampono J *et al.* (2000). Citalopram and demethylcitalopram in human milk; distribution, excretion and effects in breast fed infants. *Br J Clin Pharmacol* 50: 263–268.

Rampono J *et al.* (2006). Transfer of escitalopram and its metabolite demethylescitalopram into breastmilk. *Br J Clin Pharmacol* 62: 316–322.

Rao N (2007). The clinical pharmacokinetics of escitalopram. *Clin Pharmacokinet* 46: 281–290.

Rocha A *et al.* (2007). Enantioselective analysis of citalopram and demethylcitalopram in human and rat plasma by chiral LC-MS/MS: application to pharmacokinetics. *Chirality* 19: 793–801.

Rochat B *et al.* (1995). Analysis of enantiomers of citalopram and its demethylated metabolites in plasma of depressive patients using chiral reverse-phase liquid chromatography. *Ther Drug Monit* 17: 273–279.

Rochat B *et al.* (1995). Determination of the enantiomers of citalopram, its demethylated and propionic acid metabolites in human plasma by chiral HPLC. *Chirality* 7: 389–395.

Serebruany V *et al.* (2007). Fluorimetric quantitation of citalopram and escitalopram in plasma: developing an express method to monitor compliance in clinical trials. *Clin Chem Lab Med* 45: 513–520.

Sidhu J *et al.* (1997). Steady-state pharmacokinetics of the enantiomers of citalopram and its metabolites in humans. *Chirality* 9: 686–692.

Singh SS *et al.* (2004). Liquid chromatography–electrospray ionisation mass spectrometry method for the determination of escitalopram in human plasma and its application in bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 209–215.

Sogaard B *et al.* (2005). The pharmacokinetics of escitalopram after oral and intravenous administration of single and multiple doses to healthy subjects. *J Clin Pharmacol* 45: 1400–1406.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

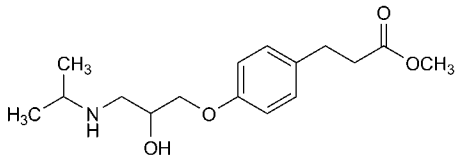
Esmolol

β-*Blocker*

$C_{16}H_{25}NO_4$ = 295.4

CAS—84057-94-3

IUPAC Name 4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy]benzenepropanoic acid methyl ester



Chemical Properties Esmolol is an oil which gradually becomes crystalline at room temperature. Mp 48° to 50°. pK_a 9.5 [Fan *et al.* 1991]. Log *P* (octanol/water, pH 7), 0.42. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005]. Esmolol in methylene chloride extracts was stable for 1 year at –20° [Achari *et al.* 1988]. Esmolol is unstable in blood at room temperature with a half-life of ≈27 min, but the addition of NaF improves stability [Zuppa *et al.* 2003].

E

Esmolol Hydrochloride

$C_{16}H_{25}NO_4,HCl = 331.8$

CAS—81161-17-3

Synonym Asl-8052

Proprietary Name *Brevibloc*

Chemical Properties A white to off-white crystalline powder that is relatively hydrophilic. Mp 85° to 86°. It is very soluble in water; freely soluble in alcohol. For the stability of esmolol hydrochloride in IV solutions, see Baaske *et al.* [1994].

Gas Chromatography System GB—esmolol RI 2311, Art RI 2395.

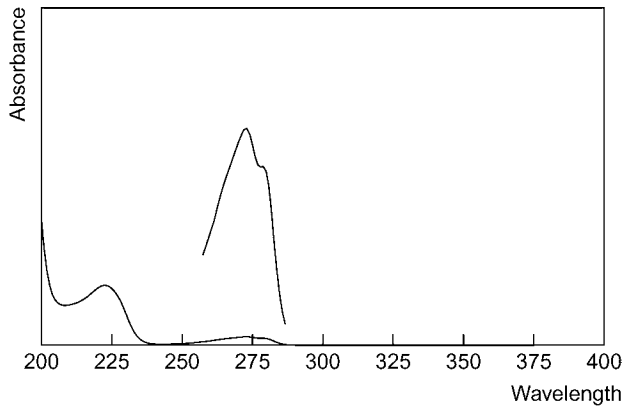
High Performance Liquid Chromatography Column: μBondapak C_{18} (30 cm × 3.9 mm). Mobile phase: 0.005 mol/L monobasic potassium phosphate : methanol : acetonitrile (65 : 20 : 15), flow rate 2.0 mL/min. UV detection (λ = 214 nm). Limit of detection not reported [Baaske *et al.* 1994].

Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm) Mobile phase: phosphate buffer (pH 6.2)-acetonitrile (pH 6.0; 65 : 35): 10 mmol/L sodium acetate-acetonitrile-glacial acetic acid (pH 3.4; 91 : 8 : 1), flow rate 2 mL/min. UV detection (λ = 229 nm). Retention time: 6.1 min for esmolol with the first mobile phase; 10.8 min for de-esterified metabolite with the second system. Limit of detection, 0.025 and 1 mg/L for esmolol and metabolite, respectively [Achari *et al.* 1986a].

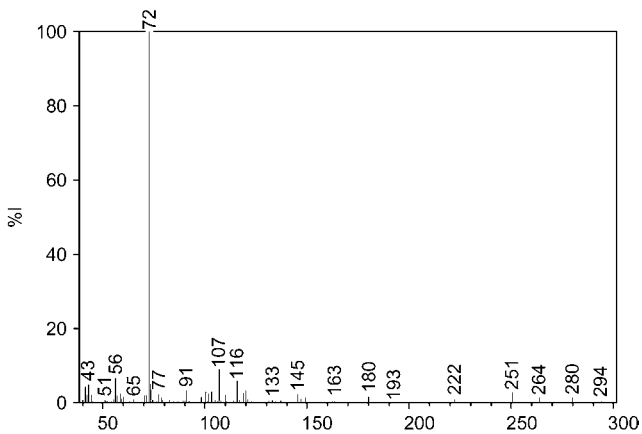
Column: cyano-bonded silica (30 cm × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile : glacial acetic acid : 0.068% sodium acetate trihydrate (150 : 10 : 840), flow rate 2 mL/min. UV detection (λ = 280 nm). Retention time: 7.71 min for esmolol hydrochloride, 5.14 min for esmolol. Limit of quantification, ≈10 mg/L [Lee *et al.* 1984].

Column: Radial-Pak CN (100 × 5 mm i.d., 10 μm). Mobile phase: methanol : 0.06 mol/L potassium dihydrogen phosphate buffer : triethylamine, (pH 3.15; 25 : 75 : 0.1), flow rate 1.8 mL/min. UV detection (λ = 221 nm). Retention time: 2.6 min. [Fan *et al.* 1991].

Ultraviolet Spectrum Aqueous acid (pH 2.38)—192, 220, 276 nm.



Mass Spectrum Principal ions at *m/z* 72, 107, 116, 251, 43, 56, 91, 102.



Quantification

Blood **GC-MS** Column: 3% SP-2250 on Supelcoport 100/200 mesh (2 m × 2 mm i.d.). Temperature: 230°. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 2.5 μg/L [Sum, Yacobi 1984].

HPLC Column: Radial-Pak CN (10 cm × 5 mm, 10 μm). Mobile phase: methanol : 0.06 mol/L potassium dihydrogenphosphate buffer : triethylamine (pH 3.15; 25 : 75 : 0.1), flow rate 1.8 mL/min. UV detection (λ = 221 nm). Retention time: 2.6 min. Limit of quantification, 10 μg/L, limit of detection, 5 μg/L [Fan *et al.* 1991]. Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phases: 0.01 mol/L monobasic potassium phosphate (pH 2.45) : methanol (63 : 37) flow rate 1.5 mL/min. UV detection (λ = 229 nm). Retention time: 4.1 min. Limit of detection, 25 μg/L [Achari *et al.* 1988]. Column: μBondapak C_{18} (15 cm × 4 mm i.d.). UV detection (λ = 229 nm). Limit of detection, 50 μg/L [Sintetos *et al.* 1987]. Column: μBondapak phenyl (30 cm × 3.9 mm, 10 μm). Mobile phase: 0.01 mol/L sodium acetate : acetonitrile : glacial acetic acid (pH 3.7; 87 : 12 : 1), flow rate 2.0 mL/min. UV detection (λ = 280 nm). Retention time: 23.4 min, 7.3 min for metabolite. Limit of detection, 1.0 mg/L [Stampfli *et al.* 1984]. Column: μBondapak (25 cm × 4.6 mm i.d.). Mobile phase: 0.01 mol/L sodium acetate : acetic acid : acetonitrile (pH 3.5; 87 : 1 : 12), flow rate 2.0 mL/min. UV detection (λ = 280 nm). Limit of detection not reported [Sum *et al.* 1983].

Plasma **HPLC** Column: Zorbax C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer (pH 4.5; 55 : 45), flow rate 0.75 mL/min. UV detection (λ = 224 nm). Retention time: 6.8, 7.7, 16.2 and 19.2 min for (—)-*S*-esmolol, (+)-*R*-esmolol, (—)-*S*-acid metabolite and (+)-*R*-acid metabolite, respectively. Limit of detection, 0.008 and 0.006 mg/L for each enantiomer and the acid metabolites, respectively [Tang *et al.* 2004a]. Limit of quantification, 0.035 mg/L, limit of detection, 0.003 mg/L [Tang *et al.* 2004b].

LC-MS Column: XTerra MSC18 (50 × 2.1 mm i.d., 3 μm). Mobile phase: water-0.05% formic acid-1% methanol : methanol-formic acid (95 : 5 to 20 : 80 at 5 min for 3 min to 95 : 5 at 9 min for 6 min), flow rate 250 μL/min. ESI. Retention time: 5.7 min. Limit of quantification, 2 μg/L [Zuppa *et al.* 2003].

Serum **LC-MS** Column: Zorbax SB-C_{18} (150 × 2.1 mm, 3.5 μm). Mobile phase: acetonitrile : 0.05 mol/L ammonium acetate (pH 6.4; 10 : 90 to 50 : 50 at 15 min), flow rate 0.7 mL/min. ESI, SIM acquisition mode. Retention time: 10.02 min. Limit of detection, 23 μg/L [Buszewski *et al.* 2009].

Urine **HPLC** Column: μBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: phosphate buffer (pH 6.6) : acetonitrile : 13% phosphoric acid (65 : 35 : 0.05), flow rate 2.0 mL/min. UV detection (λ = 229 nm). Retention time: 6.1 min. Limit of detection, 0.025 and 1.0 mg/L for esmolol and metabolite, respectively [Achari *et al.* 1986a; Achari *et al.* 1986b].

Disposition in the Body Esmolol is rapidly hydrolysed in red blood cells by esterases. An acidic de-esterified metabolite (with very little β-blocking activity), methanol and several inactive metabolites are produced. Excretion is mainly via urine, primarily as the de-esterified metabolite (71 to 83%) and <2% of the drug appearing unchanged; the rest are inactive metabolites. The remainder of the dose can be recovered in the faeces. Only small amounts of the metabolite are removed by dialysis. The metabolite accumulates in patients with renal failure.

Therapeutic Concentration

Eight healthy male volunteers aged between 21 and 27 years were administered continuous IV doses of 50, 150 and 400 μg/kg/min for 2 h. Peak plasma concentrations of 77.9 mg/L ASL-8123 (mean) were reached within 26 min after infusion was stopped. Steady-state concentrations of the parent drug esmolol reached 1.59 mg/L (mean) [Yacobi *et al.* 1983].

Nine male volunteers with cirrhosis and 3 healthy males, aged between 23 and 65 years, were administered 200 μg/kg/min esmolol over 4 h. Mean steady-state esmolol concentrations were 1.68 mg/L for the healthy individuals and 1.41 mg/L for those with cirrhosis. These concentrations were observed during infusion and rapidly decreased once the infusion was complete. Mean concentrations of the metabolite were 39.2 and 56.0 mg/L for the healthy individuals and cirrhosis patients, respectively. These were seen after ~4 h [Buchi *et al.* 1987].

Twelve healthy male volunteers aged between 22 and 44 years were administered an initial dose of 30 mg followed by a subsequent larger IV bolus dose. Peak esmolol concentrations were measured in the blood samples drawn within 2 min of the bolus. In 4 subjects receiving the highest dose (150 mg), peak blood esmolol concentrations were 0.868, 1.16, 1.25, and 1.47 mg/L. Blood concentrations remained within the measurable range for <15 min. In the 4 subjects who received doses of 100 and 150 mg, peak metabolite concentrations were measured 90 min after the 150 mg dose in 3 subjects and at 120 min in the fourth subject. Peak concentrations were 2.89, 3.54, 3.59, and 3.86 mg/L [Sintetos *et al.* 1987].

Toxicity Massive accidental overdosing has occurred due to dilution errors, which has resulted in cardiac arrest, death and permanent disability. A concentration between 12.5 to 50 mg/kg is associated with fatalities.

Half-life ≈9 min for esmolol [Sum *et al.* 1983]; increases in patients with liver failure, up to 16 min; 3.7 h for the metabolite.

Volume of Distribution 1.0 to 2.4 L/kg; increases in patients with renal failure, up to 3.4 L/kg; 0.41 L/kg (metabolite) [Sum *et al.* 1983].

Clearance Plasma clearance, 20 L/kg/h; 0.08 L/h/kg or 1.28 mL/min/kg (metabolite); 285 mL/min/kg [Sum *et al.* 1983].

Protein Binding Esmolol is 55% bound and the metabolite 10%.

Note For a general review of esmolol, see Benfield and Sorkin [1987].

Dose An initial loading dose of 500 μg/kg body weight is administered. A maintenance dose of 50 μg/kg/min is used, which can be increased (if necessary) to a maximum of 200 μg/kg/min.

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Tang YH *et al.* (2004b). Stereoselective RP-HPLC determination of esmolol enantiomers in human plasma after pre-column derivatization. *J Biochem Biophys Methods* 59: 159–166.

Yacobi A *et al.* (1983). Esmolol: a pharmacokinetic profile of a new cardioselective beta-blocking agent. *J Pharm Sci* 72: 710–711.

Zuppa AF *et al.* (2003). Liquid chromatography-electrospray mass spectrometry (LC-MS) method for determination of esmolol concentration in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 796: 293–301.

Esomeprazole

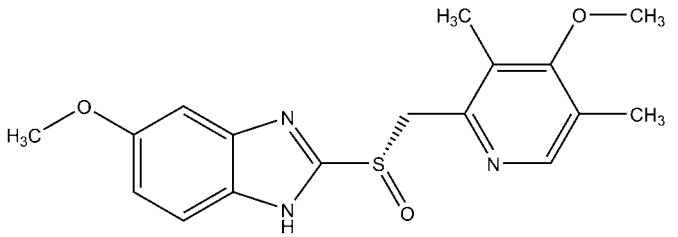
Antiulcerative, Benzimidazole, Proton Pump Inhibitor

$C_{34}H_{36}N_6O_6S_2 = 345.4$

CAS—119141-88-7

IUPAC Name 6-Methoxy-2-[(*R*)-(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1*H*-benzimidazole

Synonyms H199/18; 5-methoxy-2-{(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl}benzimidazole; perprazole.



Chemical Properties Mp 155°. Very slightly soluble in water. Log *P* (octanol/water), 1.078 [Wishart 2006].

Esomeprazole Magnesium

$C_{34}H_{36}MgN_6O_6S_2,3H_2O = 767.2$

CAS—217087-09-7

Proprietary Names *Axagon*; *Axiago*; *Esomac*; *Esomax*; *Esopral*; *Esoz*; *Inexium*; *Nexium*; *Nexiam*; *Sompraz.*

High Performance Liquid Chromatography Column: Zorbax C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : phosphate buffer (pH 7.3; 35 : 65). Limit of quantification not reported [Bladh *et al.* 2007].

Quantification

Plasma **HPLC** Column: Hypersil BDS C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 7.4; 30 : 70), flow rate 1.0 mL/min. UV detection (λ = 284 nm). Limit of quantification not reported [Agarwal *et al.* 2007]. Column: LiChrospher 100 Diol. Mobile phase: ammonium hydroxide : methanol : water : isohexane : ethyl acetate (0.05 : 8.0 : 0.8 : 55 : 37.15). UV detection (λ = 302 nm). Retention time: esomeprazole 3.5 min, esomeprazole sulfone 4.5 min, 5-hydroxyesomeprazole 8.0 min. Limit of quantification, 25 nmol/L for esomeprazole and esomeprazole sulfone; 50 nmol/L for 5-hydroxyesomeprazole [Hassan-Alin *et al.* 2006]. Column: Amylase tris(3,5-dimethylphenylcarbamate) coated (20% w/w) on to APS-Nucleosil (150 × 4.6 mm i.d., 7 μm). Mobile phase: acetonitrile : water (60 : 40), flow rate 0.5 mL/min. UV detection (λ = 302 nm). Retention time: esomeprazole 20 min, (*R*)-omeprazole 25 min. Limit of quantification, 0.05 mg/L; limit of detection, 6.3 μg/L for esomeprazole and (*R*)-omeprazole [Cass *et al.* 2003]. Column: α_1-acid glycoprotein (100 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 10 mmol/L sodium phosphate (pH 5.5; 12 : 88), flow rate 0.9 mL/min. UV detection (λ = 302 nm). Limit of detection, 25 nmol/L for esomeprazole and 5-hydroxyesomeprazole [Tybring *et al.* 1997].

LC-MS Column: Hypersil BDS C_8 (50 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : formic acid : 0.1 mol/L ammonium acetate : water (250 : 1 : 100 : 645; pH 3.8), flow rate 0.75 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: esomeprazole 3.3 min, 5-hydroxyomeprazole 1.7 min, omeprazole sulfone 5.5 min. Limit of quantification, 20 nmol/L for all analytes [Hultman *et al.* 2007]. Column: Chiralpak AD (50 × 4.6 mm i.d., 10 μm). Mobile phase: concentrated acetic acid : ethanol : acetonitrile : isohexane (0.004 : 30.0 : 1.0 : 68.006), flow rate 1.0 mL/min. ESI, MRM acquisition mode. Retention time: esomeprazole 3 min, (*R*)-omeprazole 4.5 min. Limit of quantification, 10 nmol/L [Stenhoff *et al.* 1999].

Serum **LC-MS** Column: ReproSil Chiral-CA (250 × 2.0 mm i.d., 5 μm). Mobile phase: propan-2-ol-acetic acid-diethylamine (100 : 4 : 1) : hexane (10 : 90 to 15 : 85 over 10 min for 1 min), flow rate 0.35 mL/min. APPI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 μg/L for esomeprazole and (*R*)-omeprazole, 2.5 μg/L for 5-hydroxyesomeprazole and (*R*)-5-hydroxyomeprazole, limit of detection, 0.2 μg/L for esomeprazole and (*R*)-omeprazole, 1 μg/L for 5-hydroxyesomeprazole and (*R*)-5-hydroxyomeprazole [Martens-Lobenhoffer *et al.* 2007].

Disposition in the Body Rapidly absorbed after oral doses, with peak plasma levels occurring after ≈1 to 2 h. It is acid labile and an enteric-coated formulation has been developed. Food delays and decreases the absorption of esomeprazole, but this does not significantly change its effect on intragastric acidity. It is extensively metabolised in the liver by CYP2C19 to hydroxy- and desmethyl-metabolites. These have no effect on gastric acid secretion. The remainder is metabolised by CYP3A4 to esomeprazole sulfone. Other minor metabolites include 5-*O*-desmethylesomeprazole, 3-hydroxyesomeprazole, and esomeprazole sulfide. With repeated dosing, there is a decrease in first-pass metabolism and systemic clearance, probably caused by an inhibition of the CYP2C19 isoenzyme. However, there is no accumulation during once daily use. Almost 80% of an oral dose is eliminated as metabolites in the urine, the remainder in the faeces. Less than 1% of the parent compound is found unchanged in urine.

Therapeutic Concentration

Two groups of healthy volunteers were administered esomeprazole as an IV infusion and/or oral formulation. In studies A and B, subjects received IV doses of 20 mg and 40 mg esomeprazole, respectively, on day 1. After a washout period of 5 to 14 days, the same doses (20 mg as a solution and 40 mg as a capsule) were given orally for 5 days and then again IV on day 6. Blood samples for determination of esomeprazole in plasma were taken up to 12 h (study A) or 24 h (study B) post-dose after the first and second IV doses and on day 1 and day 5 of oral dosing. Peak plasma concentrations for the 20 mg dose were reported as:

	C_{max} (μmol/L)
IV route	
1st dose	2.51
2nd dose	2.67
Oral route	
Day 1	1.86
Day 5	2.65

Peak plasma concentrations for the 40 mg dose were reported as:

	C_{max} (μmol/L)
IV route	
1st dose	5.53
2nd dose	6.91
Oral route	
Day 1	2.38
Day 5	4.64

Corresponding values for esomeprazole sulfone and 5-hydroxyesomeprazole following oral administration of the 40 mg dose were:

Metabolite	*C_{max} (μmol/L)*
Esomeprazole sulfone	
Day 1	0.76
Day 5	1.71
5-Hydroxyesomeprazole	
Day 1	0.29
Day 5	0.28

[Hassan-Alin *et al.* 2000].

Twelve healthy male volunteers were administered 5, 10 or 20 mg oral esomeprazole or 20 mg omeprazole once daily for 5 days. Peak plasma concentrations were reported as:

Dose	*Esomeprazole*			*Omeprazole*
	5 mg	*10 mg*	*20 mg*	*20 mg*
Day 1				
C_{max} (μmol/L)	0.35	0.79	1.68	0.62
Time (h)	0.31	0.30	0.38	1.94
Day 5				
C_{max} (μmol/L)	0.42	0.98	2.55	1.00
Time (h)	0.31	0.33	0.29	1.23

[Andersson *et al.* 2001].

In a bioequivalence study where 49 healthy male Caucasians were administered a single oral dose of 40 mg esomeprazole as a tablet or a tablet encapsulated in hard gelatine, peak plasma concentrations were reported as:

	Tablet	*Tablet in hard gelatine*
C_{max} (mg/L)	1.02	1.03
Time (h)	1.5	1.5
Half-life (h)	1.02	1.03

[Talpes *et al.* 2005].

Two group of young patients (group A: 1–5 years; group B: 6–11 years) with symptoms of gastro-oesophageal reflux disease were administered oral doses of 5, 10 or 20 mg esomeprazole. Peak plasma concentrations were reported as:

Dose	*Group A*		*Group B*	
	5 mg	*10 mg*	*10 mg*	*20 mg*
C_{max} (μmol/L)	0.62	2.98	1.77	3.73
Time (h)	1.33	1.44	1.79	1.75
Half-life (h)	0.42	0.74	0.88	0.73
Clearance (L/h)	19.4	6.0	7.8	9.2

[Zhao *et al.* 2006].

Toxicity Since intragastric pH will increase with esomeprazole treatment, the absorption of drugs with pH-sensitive absorption may be affected (e.g. digoxin and ketoconazole). Esomeprazole has the potential to inhibit CYP2C19, thus altering pharmacokinetics of drugs such as diazepam, phenytoin and warfarin. However, it does not seem to interact with drugs that are metabolised by CYP1A2, CYP2A6, CYP2C9, CYP2D6 or CYP2E1 [Andersson *et al.* 2001]. Studies on drug interactions between esomeprazole, amoxicillin and clarithromycin have shown that the combination treatment with these drugs results in higher plasma levels of esomeprazole compared with an esomeprazole-alone regimen. Plasma levels of amoxicillin and clarithromycin were similar irrespective of whether the drugs were administered alone or in combination with esomeprazole [Hassan-Alin *et al.* 2006]. In a case report, a 96% reduction in atazanavir trough plasma concentration was observed where a patient with HIV was taking a combination of 300 mg atazanavir and 100 mg ritonavir every morning plus 40 mg esomeprazole every evening [Kiser *et al.* 2006].
Bioavailability Increases with both dose and repeated administration to ≈68 and 89% for doses of 20 and 40 mg, respectively.
Half-life Approximately 1.3 h.
Volume of Distribution Steady state, 15.1 L.
Clearance Approximately 11.3 L/h.
Protein Binding Approximately 97%.
Dose Given as the magnesium or sodium salt but doses are calculated in terms of esomeprazole. Esomeprazole magnesium 22.2 mg and esomeprazole sodium 21.3 mg are each equivalent to ≈20 mg of esomeprazole. Usual dose is between 20 and 40 mg daily.

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Martens-Lobenhoffer J *et al.* (2007). Enantioselective quantification of omeprazole and its main metabolites in human serum by chiral HPLC–atmospheric pressure photoionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 301–307.
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Tybring G *et al.* (1997). Enantioselective hydroxylation of omeprazole catalyzed by CYP2C19 in Swedish white subjects. *Clin Pharmacol Ther* 62: 129–137.
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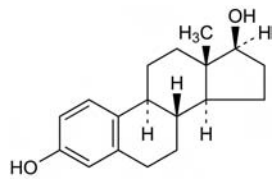
Estradiol

Oestrogen
$C_{18}H_{24}O_2$ = 272.4
CAS—50-28-2
IUPAC Name (8*R*,9*S*,13*S*,14*S*,17*S*)-13-Methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,17-diol
Synonyms Beta-estradiol; dihydrofolliculine; dihydrotheelin; dihydroxyoestrin; (17β)-estra-1,3,5(10)-triene-3,17-diol; oestradiol.
Proprietary Names *Estrace*; *Farmacyrol*; *Gynoestryl*; *Oestrogel*; *Progynon*. It is an ingredient of *Hormonin* and *Trisequens*.



Chemical Properties White or creamy-white, hygroscopic crystals or crystalline powder. Mp 173° to 179°. Almost insoluble in water; soluble 1 in 28 of ethanol, 1 in 17 of acetone, 1 in 435 of chloroform and 1 in 150 of ether; soluble in dioxan and solutions of alkali hydroxides. Log *P* (octanol/water), 4.0.

Estradiol Benzoate

$C_{25}H_{28}O_3$ = 376.5
CAS—50-50-0
Synonyms Estradiol monobenzoate; oestradiol benzoate.
Proprietary Names *Benzo-Gynoestryl 5*; *Benztrone*; *Gynécormone*; *Oestramine*; *Ovocyclin M*; *Progynon B*. It is an ingredient of *Mixogen* (injection) and *Plex-Hormone* (injection).
Chemical Properties Colourless crystals or white or creamy-white crystalline powder. Mp 190° to 198°. Practically insoluble in water; slightly soluble in ethanol; soluble 1 in 5 of chloroform and 1 in 150 of ether; soluble in acetone and dioxan.

Estradiol Cipionate

$C_{26}H_{36}O_3$ = 396.6
CAS—313-06-4
Synonyms Estradiol cyclopentylpropionate; oestradiol cipionate.

Proprietary Names *Depo-Estradiol Cipionate*; *Neoginon Depositum*.
Chemical Properties A white crystalline powder. Mp 149° to 153°. Practically insoluble in water; soluble 1 in 40 of ethanol and 1 in 7 of chloroform; soluble in acetone, ether and dioxan.

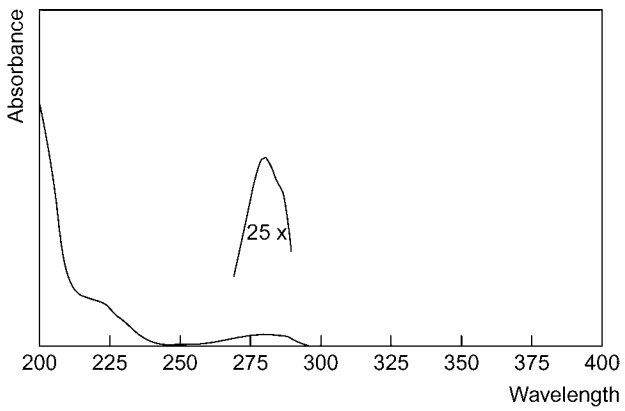
Estradiol Undecylate

$C_{29}H_{44}O_3$ = 440.7
CAS—3571-53-7
Synonyms Estradiol undecanoate; oestradiol undecylate.
Proprietary Names *Progynon Depot*; *Progynon-Retard*.

Estradiol Valerate

$C_{23}H_{32}O_3$ = 356.5
CAS—979-32-8
Synonym Oestradiol valerate
Proprietary Names *Delestrogen*; *Femogex*; *Östrogynol sine*; *Primogyn Depot*; *Progynon Depot*; *Progynova*. It is an ingredient of *Cyclacur* and *Cyclo-Progynova*.
Chemical Properties A white crystalline powder. Mp 144° to 145°. Practically insoluble in water; soluble in dioxan and methanol.

Colour Tests Antimony pentachloride—brown; Liebermann's reagent—black; naphthol–sulfuric acid—blue-green, yellow dichroism/orange; sulfuric acid—yellow (green fluorescence under ultraviolet light).
Thin-layer Chromatography Estradiol benzoate: system TP—R_f 0.79; system TQ—R_f 0.32; system TR—R_f 0.96; system TS—R_f 0.79. Estradiol: system TD—R_f 0.61; system TE—R_f 0.78; system TF—R_f 0.58; system TAD—R_f 0.64; system TAJ—R_f 0.55; system TAK—R_f 0.58; system TAL—R_f 0.91; system TAM—R_f 0.09.
Gas Chromatography System GA—RI 2659.
High Performance Liquid Chromatography System HX—RI 896; system HY—RI 456; system HAA—retention time 18.2 min.
Ultraviolet Spectrum Estradiol benzoate: ethanol—231 nm (A^1_1=490b).



Infrared Spectrum Principal peaks at wavenumbers 1245, 1054, 1227, 1493, 1276, 821 cm^{-1} (KBr disk).
Quantification
Serum **HPLC** Fluorescence detection. Limit of detection, 0.14 µg/L [DeSilva *et al.* 1996]. Fluorescence detection (comparison with radioimmunoassay). For method of quantification for estradiol and estriol, see Kondo *et al.* [1990].

HPLC–RIA Limit of detection, 0.64 µg/L and 1.04 µg/L for estradiol and estrone, respectively [Yasui *et al.* 1999].

MS For method of quantification for estradiol, estrone and other sex hormones (comparison with radioimmunoassay), see Dorgan *et al.* [2002].

Urine **GC–MS** For method of quantification for estradiol, estriol and estrone, see Xiao, McCalley [2000].
Disposition in the Body
Therapeutic Concentration

Mean serum estradiol concentrations in 25 subjects given 2 mg estradiol, 1 mg estriol and 1 mg norethisterone acetate for 4 months to 6 years were 138 (range, 53 to 279) ng/L; concentrations remained relatively even, reaching a broad maximum between 2 and 14 h after intake [Klehr-Bathmann, Kuhl 1995].

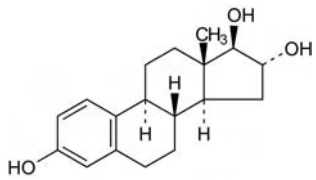
Following application of estradiol from a transdermal gel to 23 subjects at a dose of 0.5, 1.0 and 1.5 mg daily, mean peak serum levels of 39.0, 67.3 and 158.5 ng/L, respectively, were attained at 7, 6, and 3 h [Järvinen *et al.* 2000].

Dose Up to 2 mg daily, orally.

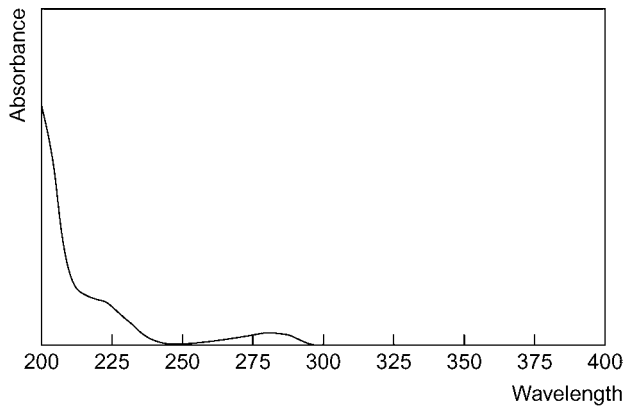
DeSilva KH *et al.* (1996). Pyrene sulphonyl chloride as a reagent for quantitation of oestrogens in human serum using HPLC with conventional and laser-induced fluorescence detection. *Biomed Chromatogr* 10: 318–324.
Dorgan JF *et al.* (2002). Measurement of steroid sex hormones in serum: a comparison of radioimmunoassay and mass spectrometry. *Steroids* 67: 151–158.
Järvinen A *et al.* (2000). Effect of dose on the absorption of estradiol from a transdermal gel. *Maturitas* 35: 51–56.
Klehr-Bathmann I, Kuhl H (1995). Formation of ethinylestradiol in postmenopausal women during continuous treatment with a combination of estradiol, estriol and norethisterone acetate. *Maturitas* 21: 245–250.
Kondo Z *et al.* (1990). Measurement of serum unconjugated estriol and estradiol by high-performance liquid chromatography. *J Clin Lab Anal* 4(6): 410–413.
Xiao X, McCalley D (2000). Quantitative analysis of estrogens in human urine using gas chromatography/negative chemical ionisation mass spectrometry. *Rapid Commun Mass Spectrom* 14: 1991–2001.
Yasui T *et al.* (1999). Combination of automatic HPLC-RIA method for determination of estrone and estradiol in serum. *J Clin Lab Anal* 13: 266–272.

Estriol

Oestrogen
$C_{18}H_{24}O_3$ = 288.4
CAS—50-27-1
IUPAC Name (8*R*,9*S*,13*S*,14*S*,16*R*,17*R*)-13-Methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,16,17-triol
Synonyms (16α,17β)-Estra-1,3,5(10)-triene-3,16,17-triol; oestriol; theelol.
Proprietary Names *Hormomed*; *Ovestin*. It is an ingredient of *Hormonin* and *Trisequens*.



Chemical Properties A white crystalline powder. Mp about 280°. Practically insoluble in water; soluble 1 in about 500 of ethanol; soluble in acetone, chloroform, dioxan and ether; freely soluble in pyridine and in solutions of fixed alkali hydroxides. pK_a 10.4. Log *P* (octanol/water), 2.4.
Colour Tests Antimony pentachloride—brown; Liebermann's reagent—black; naphthol–sulfuric acid—green, yellow dichroism/orange; sulfuric acid—no initial colour (yellow-green fluorescence under ultraviolet light).
Gas Chromatography System GA—RI 2955.
High Performance Liquid Chromatography System HAA—retention time 13.1 min.
Ultraviolet Spectrum Ethanol—280 nm.



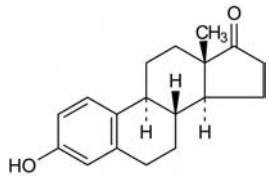
Infrared Spectrum Principal peaks at wavenumbers 1228, 1067, 917, 1147, 1250, 1605 cm^{-1} (Nujol mull).
Quantification See also Estradiol.
Serum **HPLC** UV and electrochemical detection. Limit of detection, 2.5 µg/L and 295 µg/L for estriol and estriol 3-sulfate, respectively [Tagawa *et al.* 1999].
Urine **HPLC** UV detection. For method of quantification, see Ke and Yuan [1989].
Dose 0.25 to 1 mg daily.

Ke L, Yuan YS (1989). Determination of estriol and creatinine in urine by high performance liquid chromatography. *Biomed Chromatogr* 3: 196–198.
Tagawa N *et al.* (1999). Simultaneous determination of estriol and estriol 3-sulfate in serum by column-switching semi-micro high-performance liquid chromatography with ultraviolet and electrochemical detection. *J Chromatogr B, Biomed Sci Appl* 723: 39–45.

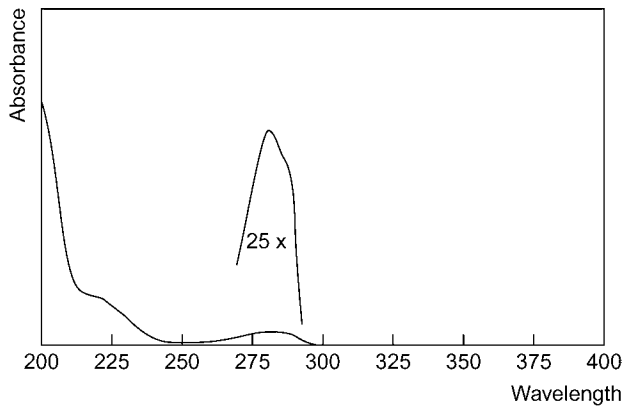
Estrone

Oestrogen
$C_{18}H_{22}O_2$ = 270.4
CAS—53-16-7

IUPAC Name (8*R*,9*S*,13*S*,14*S*)-3-Hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-one
Synonyms Folliculin; 3-hydroxyestra-1,3,5(10)-trien-17-one; ketohydroxyoestrin; oestrone.
Proprietary Names *Cristallovar*; *Femogen*; *Kolpon*; *Oestrilin* (vaginal preparations). It is an ingredient of *Hormonin*.



Chemical Properties Colourless crystals or white to creamy-white crystalline powder. Mp about 260°, with decomposition. Practically insoluble in water; soluble 1 in 250 of ethanol, 1 in 50 of acetone and 1 in 110 of chloroform; soluble in dioxan, pyridine, fixed alkali hydroxide solutions; slightly soluble in ether. Log *P* (octanol/water), 3.1.
Colour Tests Antimony pentachloride—brown; Liebermann's reagent—black; naphthol–sulfuric acid—green, yellow dichroism/orange; sulfuric acid—green-yellow (green fluorescence under ultraviolet light).
Thin-layer Chromatography System TB—R_f 0.09; system TE—R_f 0.76; system TF—R_f 0.68; system TAE—R_f 0.87; system TAJ—R_f 0.63; system TAK—R_f 0.66; system TAL—R_f 0.91; system TAM—R_f 0.95.
Gas Chromatography System GA—RI 2612.
High Performance Liquid Chromatography System HX—RI 544; system HY—RI 521.
Ultraviolet Spectrum Methanol—280 nm (A_1^1=78a).

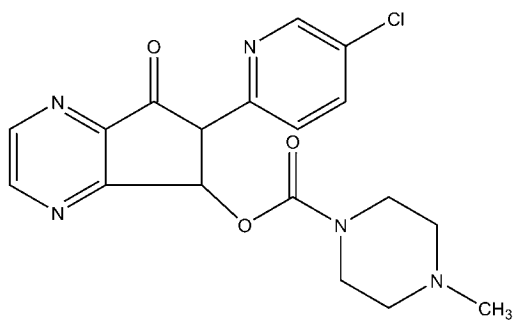


Infrared Spectrum Principal peaks at wavenumbers 1282, 820, 1709, 1244, 921, 1493 cm^{-1}.
Quantification See under Estradiol.
Dose Estrone has been given in doses of 0.1 to 5 mg daily by IM injection.

Eszopiclone

Cyclopyrrolone, GABA Receptor Agonist, Hypnotic, Sedative
$C_{17}H_{17}ClN_6O_3$ = 388.8
CAS—138729-47-2

IUPAC Name [(7*S*)-6-(5-chloropyridin-2-yl)-5-oxo-7*H*-pyrrolo[3,4-*b*]pyrazin-7-yl] 4-methylpiperazine-1-carboxylate
Synonyms (+)-(5*S*)-6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate; (+)-zopiclone; (*S*)-zopiclone.
Proprietary Names *Inductal*; *Lunesta*.



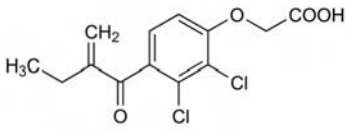
Chemical Properties (+)-Isomer of zopiclone. Solid. Very slightly soluble in water. Log *P* (octanol/water), 0.8 [Wishart 2006].
Thin-layer Chromatography Plates: Polygram Sil G/UV_{254}. Solvent system: dichloromethane : methanol (92.5 : 7.5). UV detection (λ= 365 nm) with acidified iodoplatinate reagent spray. R_f 0.3 for eszopiclone, 0.08 for (*S*)-*N*-desmethyleszopiclone. Limit of quantification not reported [Mannaert, Daenens 1996].
High Performance Liquid Chromatography Column: Chiralpak AS (250 × 4.6 mm i.d.). Mobile phase: *n*-hexane : ethanol : diethylamine (60 : 40 : 0.1 for eszopiclone; 55 : 45 : 0.1 for *N*-desmethyleszopiclone), flow rate 0.5 mL/min. UV detection (λ= 305 nm). Retention time: eszopiclone 14.6 min, (*S*)-*N*-desmethylzopiclone 15.8 min. Limit of quantification not reported [Mannaert, Daenens 1996].
Quantification
Plasma **HPLC** Column: cellulose carbamate (250 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : ethanol : methanol (55 : 30 : 15) with 1% diethylamine, flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}= 305 nm, λ_{em}= 470 nm). Retention time: ≈22 min. Limit of quantification not reported [Fernandez *et al.* 1993a]. Column: cellulose carbamate (250 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : ethanol (40 : 60), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 470 nm). Retention time: ≈11 min. Limit of quantification not reported [Fernandez *et al.* 1991].
Urine **CE** Column: uncoated fused silica capillary (total/effective length: 47/40 cm, 50 µm). Buffer: 100 mmol/L phosphate buffer (pH 2.75) with 16.3 mmol/L β-cyclodextrin. Fluorescence detection (λ_{ex}= 325 nm, λ_{em} = 450 nm). Retention time: eszopiclone 14.1 min, (*S*)-*N*-desmethylzopiclone 13.7 min, (*S*)-zopiclone-*N*-oxide 15.8 min. Limit of quantification not reported [Hempel, Blaschke 1996].
Oral Fluid **CE** See Urine [Hempel, Blaschke 1996].
Disposition in the Body Eszopiclone exhibits similar pharmacokinetics to zopiclone. It is rapidly absorbed after oral administration, with peak plasma concentrations reached within 1.6 h. It is extensively metabolised by CYP3A4 and CYP2E1 pathways via oxidation and demethylation. The primary plasma metabolites are (*S*)-zopiclone-*N*-oxide and (*S*)-*N*-desmethylzopiclone, the latter is pharmacologically active. It is widely distributed in body tissues, including the brain, and is eliminated in urine (75% as metabolites, <7% as parent compound), saliva,; and breast milk.
Therapeutic Concentration
Twelve healthy male Caucasian volunteers were administered a single oral dose of 15 mg of racemic zopiclone. Mean peak plasma concentrations of eszopiclone and (*R*)-zopiclone were 87.3 and 44.0 µg/L attained at 98.8 and 88.8 min, respectively [Fernandez *et al.* 1993b].
Toxicity The starting dose should be reduced in patients taking potent inhibitors of CYP3A4; a dose not exceeding 1 mg is recommended. This may then be increased to 2 mg. Eszopiclone and lorazepam decrease each other's peak plasma concentrations by 22%. Co-administration of 3 mg eszopiclone to subjects receiving 400 mg ketoconazole, a potent inhibitor of CYP3A4, resulted in a 2.2-fold increase in exposure to eszopiclone. It does not appear to inhibit CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4.
Bioavailability Approximately 80%.
Half-life Approximately 6.5 h.
Volume of Distribution 98.6 L.
Clearance 11.7 L/h.
Distribution in Blood Blood : plasma ratio, <1.
Protein Binding Approximately 52–59%.
Note For a review of eszopiclone, see Najib [2006].
Dose The usual dose is 2 mg orally immediately before sleep; if appropriate, the dose may be started at or increased to 3 mg. In elderly patients who have difficulty falling asleep, the initial dose is 1 mg; this may be increased to 2 mg. For elderly patients who have difficulty staying asleep, the starting dose is 2 mg. The starting dose of eszopiclone should be reduced to 1 mg before sleep in patients with severe hepatic impairment. No dose adjustment is necessary in patients with mild to moderate impairment.

Fernandez C *et al.* (1991). Determination of zopiclone enantiomers in plasma by liquid chromatography using a chiral cellulose carbamate column. *J Chromatogr* 572: 195–202.
Fernandez C *et al.* (1993a). Determination of the enantiomers of zopiclone and its two chiral metabolites in urine using an automated coupled achiral–chiral chromatographic system. *J Chromatogr* 617: 271–278.
Fernandez C *et al.* (1993b). Pharmacokinetics of zopiclone and its enantiomers in Caucasian young healthy volunteers. *Drug Metab Dispos* 21: 1125–1128.
Hempel G, Blaschke G (1996). Enantioselective determination of zopiclone and its metabolites in urine by capillary electrophoresis. *J Chromatogr B Biomed Appl* 675: 139–146.
Mannaert E, Daenens P (1996). Semi-preparative chiral resolution of zopiclone and *N*-desmethylzopiclone. *J Pharm Biomed Anal* 14: 1367–1370.
Najib J (2006). Eszopiclone, a nonbenzodiazepine sedative-hypnotic agent for the treatment of transient and chronic insomnia. *Clin Ther* 28: 491–516.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Etacrynic Acid

Diuretic
$C_{13}H_{12}Cl_2O_4$ = 303.1
CAS—58-54-8

IUPAC Name [2,3-Dichloro-4-(2-methylene-1-oxobutyl)phenoxy]acetic acid
Synonyms Etacrynsäure; ethacrynic acid.
Proprietary Names *Edecril*; *Edecrin(e)* (tablets); *Hydromedin* (tablets); *Reomax*; *Uregyt*.

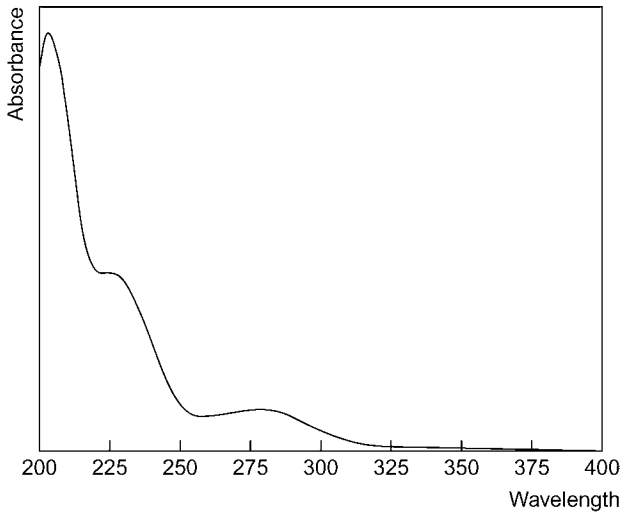


Chemical Properties A white crystalline powder. Mp about 121° to 122°. Sparingly soluble in water and aqueous acids; soluble 1 in 1.6 of ethanol, 1 in 6 of chloroform and 1 in 3.5 of ether. pK_a 3.5 (20°). Log *P* (octanol/water), 3.7.
Caution Etacrynic acid, especially in the form of dust, is irritating to the skin, eyes and mucous membranes.

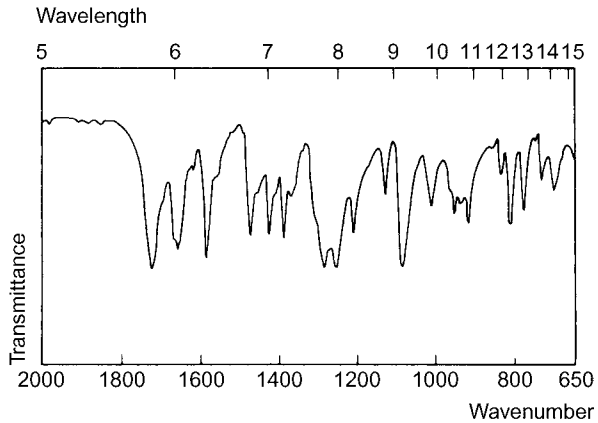
Etacrynate Sodium

$C_{13}H_{11}Cl_2NaO_4$ = 325.1
CAS—6500-81-8
Synonyms Etacrynate sodium; sodium etacrynate.
Proprietary Names *Edecrin(e)* (injection); *Hydromedin* (injection); *Reomax*.

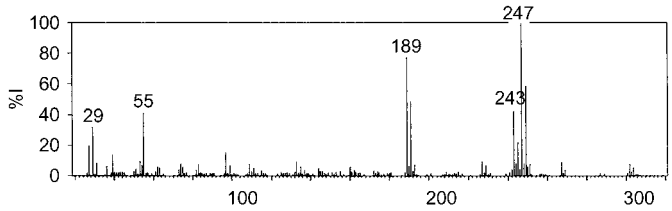
Thin-layer Chromatography System TA—R_f 0.96; system TD—R_f 0.03; system TE—R_f 0.05; system TF—R_f 0.02; system TAD—R_f 0.05; system TAE—R_f 0.71; system TAJ—R_f 0.05; system TAK—R_f 0.42; system TAL—R_f 0.57.
Gas Chromatography System GA—etacrynic acid, not eluted, etacrynic acid-Me, RI 2195; system GX—etacrynic acid-Me, retention time 4.0 min; system GY—etacrynic acid-Me, retention time 3.3 min.
High Performance Liquid Chromatography System HX—RI 521; system HY—RI 497.
Ultraviolet Spectrum Acid methanol—270 nm (A_1^1=115a).



Infrared Spectrum Principal peaks at wavenumbers 1726, 1249, 1279, 1077, 1586, 1661 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 247, 189, 249, 191, 243, 55, 29, 245; etacrynic acid-Me *m/z* 261, 263, 243, 245, 281, 316.

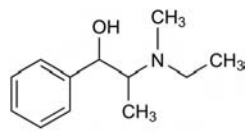


Quantification
Plasma **GC–MS** Limit of detection, 10 to 20 µg/L [Stuber *et al.* 1982].
HPLC UV detection. Etacrynic acid and its conjugated metabolite. Limit of detection, <20 µg/L for etacrynic acid [Voith *et al.* 1995]. UV detection. Limit of detection, 0.1 mg/L [LaCreta *et al.* 1991].
Urine **HPLC** See Plasma [Voith *et al.* 1995].
Disposition in the Body Readily absorbed after oral administration and distributed to the liver and kidneys. After IV administration, 60% is excreted in the urine and 30% in the bile in 24 h; urinary excretion is pH-dependent and the material excreted in the urine consists of unchanged drug, a cysteine conjugate and a third unstable metabolite, in approx. equal proportions.
Toxicity Thrombocytopenia and fatal agranulocytosis have been reported during chronic treatment.
Half-life Plasma half-life, 0.5 to 1 h.
Protein Binding Significantly bound.
Dose Usually 50 to 150 mg daily; maximum of 400 mg daily.

LaCreta FP *et al.* (1991). High-performance liquid chromatographic determination of ethacrynic acid in human plasma. *J Chromatogr* 571: 271–276.
Stuber W *et al.* (1982). Determination of ethacrynic and tienilic acid in plasma by gas–liquid chromatography - mass spectrometry. *J Chromatogr Biomed Appl* 227: 193–198.
Voith B *et al.* (1995). New specific and sensitive HPLC-assays for ethacrynic acid and its main metabolite–the cysteine conjugate–in biological material. *J Pharm Biomed Anal* 13: 1373–1382.

Etafedrine

Sympathomimetic
$C_{12}H_{19}NO$ = 193.3
CAS—7681-79-0; 48141-64-6
IUPAC Name 2-[Ethyl(methyl)amino]-1-phenylpropan-1-ol
Synonyms Ethylephedrine; α-[1-(ethylmethylamino)ethyl]benzenemethanol.

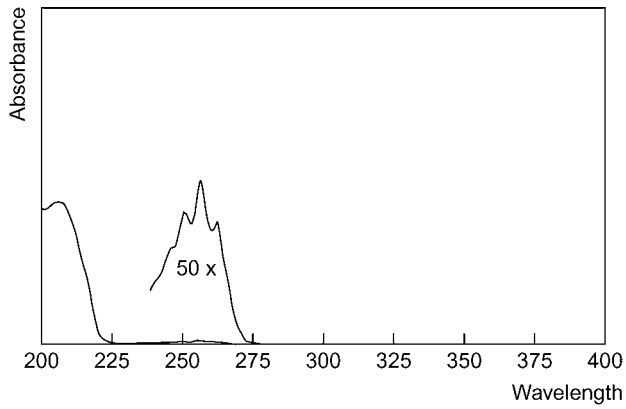


Chemical Properties Log *P* (octanol/water), 1.4.

Etafedrine Hydrochloride

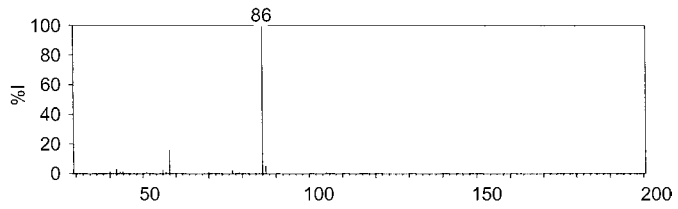
$C_{12}H_{19}NO,HCl$ = 229.8
CAS—5591-29-7
Proprietary Names It is an ingredient of *Calmydone*, *Dalmacol*, and some *Nethaprin* and *Revenil* preparations.
Chemical Properties Crystals. Mp 183° to 184°. Soluble 1 in 1.5 of water and 1 in 8 of ethanol.

Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.35; system TC—R_f 0.09; system TE—R_f 0.56; system TL—R_f 0.15; system TAE—R_f 0.14; system TAJ—R_f 0.04; system TAK—R_f 0.06; system TAL—R_f 0.49 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1519; system GB—RI 1510; system GC—RI 1737.
High Performance Liquid Chromatography System HA—*k* 1.9.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=10a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 697, 1051, 1020, 1000, 740, 1115 cm^{-1} (KBr disk).

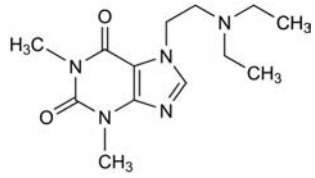
Mass Spectrum Principal ions at *m/z* 86, 58, 87, 42, 56, 77, 44, 43.



Dose 80 to 200 mg of etafedrine hydrochloride daily.

Etamiphylline

Xanthine Bronchodilator
$C_{13}H_{21}N_5O_2$ = 279.3
CAS—314-35-2
IUPAC Name 7-[2-(Diethylamino)ethyl]-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione
Synonym Etamphyllin

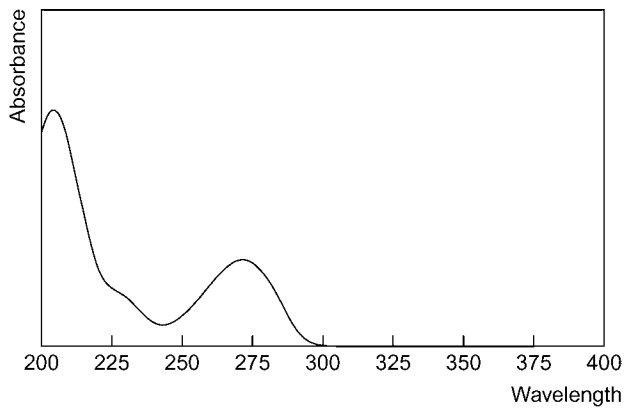


Chemical Properties A waxy solid. Mp 75°. Very soluble in water and acetone; slightly soluble in ethanol and ether. Log *P* (octanol/water), 0.8.

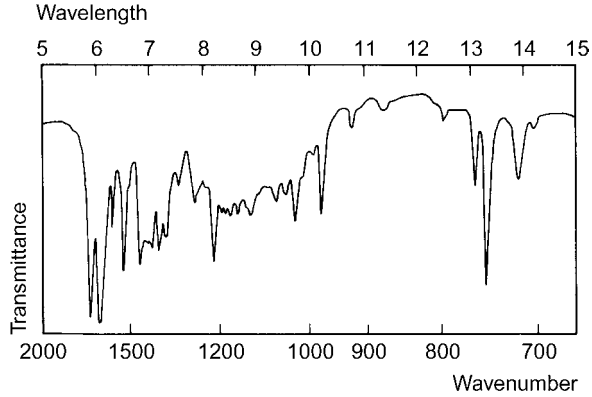
Etamiphylline Camsilate

$C_{23}H_{37}N_5O_6S$ = 511.6
CAS—19326-29-5
Synonym Diétamiphylline camphosulfonate
Proprietary Names *Camphophyline*. It is an ingredient of *Longtussin* and *Solufilina Sedante*.
Chemical Properties A white crystalline powder. Mp 174°. Very soluble in water; soluble in ethanol and chloroform; very slightly soluble in ether.

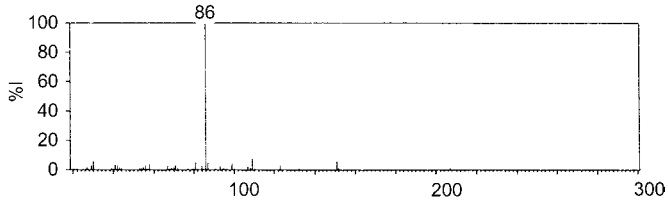
Colour Test Amalic acid test—yellow/pink.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.12; system TC—R_f 0.39; system TE—R_f 0.74; system TL—R_f 0.17; system TAJ—R_f 0.28; system TAK—R_f 0.02; system TAL—R_f 0.44 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—etamiphylline RI 2210, M (desethyl-)-AC RI 2560.
High Performance Liquid Chromatography System HA—*k* 1.2.
Ultraviolet Spectrum Water—274 nm (A_1^1=320b).



Infrared Spectrum Principal peaks at wavenumbers 1656, 1706, 748, 1543, 1220, 1600 cm^{-1}.



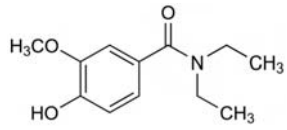
Mass Spectrum Principal ions at *m/z* 86, 109, 30, 151, 87, 81, 99, 58.



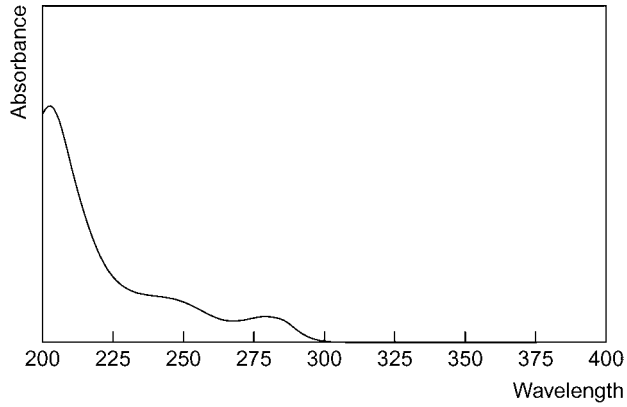
Dose 0.3 to 1.2 g of etamiphylline camsilate daily.

Etamivan

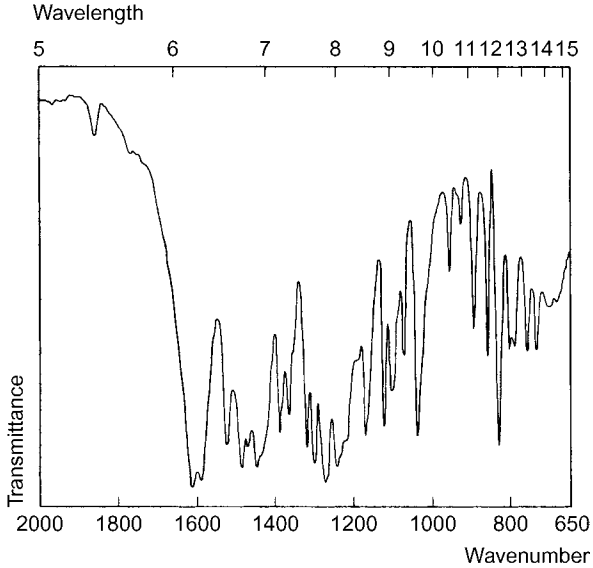
Respiratory Stimulant
$C_{12}H_{17}NO_3$ = 223.3
CAS—304-84-7
IUPAC Name *N*,*N*-Diethyl-4-hydroxy-3-methoxybenzamide
Synonyms Ethamivan; vanillic acid diethylamide; vanillic diethylamide.
Proprietary Names *Cardiovanil*; *Emivan*; *Vandid*.



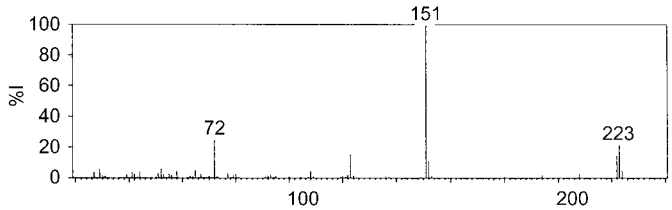
Chemical Properties A white crystalline powder. Mp 94° to 99°. Soluble 1 in 100 of water, 1 in 2 of ethanol, 1 in 1.5 of chloroform and 1 in 50 of ether. Log *P* (octanol/water), 1.1.
Colour Tests Ferric chloride—green; Folin–Ciocalteu reagent—blue; Liebermann's reagent—black
Thin-layer Chromatography System TA—R_f 0.94; system TD—R_f 0.38; system TE—R_f 0.41; system TF—R_f 0.35; system TAD—R_f 0.59; system TAE—R_f 0.85; system TAJ—R_f 0.60; system TAK—R_f 0.48; system TAL—R_f 0.96.
Gas Chromatography System GA—etamivan RI 1900, etamivan-AC RI 1970; system GC—RI 2409.
Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=153a).



Infrared Spectrum Principal peaks at wavenumbers 1608, 1270, 1585, 1240, 1300, 1543 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 151, 72, 223, 123, 222, 152, 52, 29.



Quantification
Urine **HPLC** For method of quantification for etamivan and other drugs of abuse, see Ma *et al.* [1998].
Dose Etamivan has been given IV in doses of up to 10 mL of a 5% solution; doses of 40 to 240 mg daily have been given orally.

Ma C *et al.* (1998). Studies on analytical method for 10 drugs of abuse in urine using HPLC. *Yao Xue Xue Bao* 33: 764–767.

Etanercept

Antiinflammatory, TNFα-Fusion Protein
CAS—185243-69-0

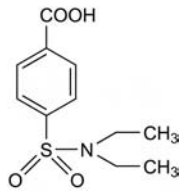
Synonyms Human tumour necrosis factor receptor p75 Fc fusion protein; Rhu-TNFR:Fc; 1-235-tumour necrosis factor receptor (human) fusion protein with 236-467-immunoglobulin G_1 (human γ_1-chain Fc fragment).
Proprietary Name *Enbrel*
Chemical Properties Dimer of 1–235 TNF receptor (human) fusion protein with 236-467-immunoglobin G_1. pK_a 7.89 [Wishart 2006]. Log P (octanol/water), −0.529 [Wishart 2006].
Disposition in the Body Eliminated by the reticuloendothelial system of the liver or spleen.
Therapeutic Concentration
In a single-dose study, 26 healthy volunteers were administered 25 mg etanercept by SC injection into the abdomen. Serum samples were collected for 21 days and analysed by ELISA. The mean peak serum concentration was 1.46 mg/L after 51 h [Korth-Bradley *et al.* 2000].
Eleven patients with heart failure were administered a dose of 12 mg/m^2(maximum dose 25 mg) etanercept by SC injection twice weekly for 12 weeks. The mean serum concentration was 2.34 mg/L, reached after 46 h [Soran *et al.* 2001].
Note For a study of the pharmacokinetics of etanercept in patients with psoriasis, see Nestorov *et al.* [2006].
Bioavailability Approximately 58%.
Half-life 68 ± 19 h.
Volume of Distribution 12 ± 6 L.
Clearance Apparent clearance, 132 ± 85 mL/h.
Dose Usually 25 mg SC, twice weekly.

Korth-Bradley JM *et al.* (2000). The pharmacokinetics of etanercept in healthy volunteers. *Ann Pharmacother* 34: 161–164.
Nestorov I *et al.* (2006). Pharmacokinetics of subcutaneously administered etanercept in subjects with psoriasis. *Br J Clin Pharmacol* 62: 435–445.
Soran O *et al.* (2001). The pharmacokinetics of etanercept in patients with heart failure. *Br J Clin Pharmacol* 51: 191–192.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Etebenecid

Uricosuric
$C_{11}H_{15}NO_4S$ = 257.3
CAS—1213-06-5

IUPAC Name 4-Diethylsulfamoylbenzoic acid
Synonym Ethebenecid

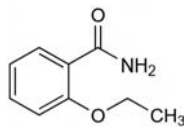


Chemical Properties Crystals. Mp 192° to 194°. pK_a 3.3 (25°). Log *P* (octanol/water), 1.9.
Ultraviolet Spectrum Methanol—246 nm (A^1_1=460b).
Infrared Spectrum Principal peaks at wavenumbers 1694, 729, 1162, 1282, 1298, 934 cm^{-1}.
Dose Etebenecid has been given in doses of 1 to 3 g daily.

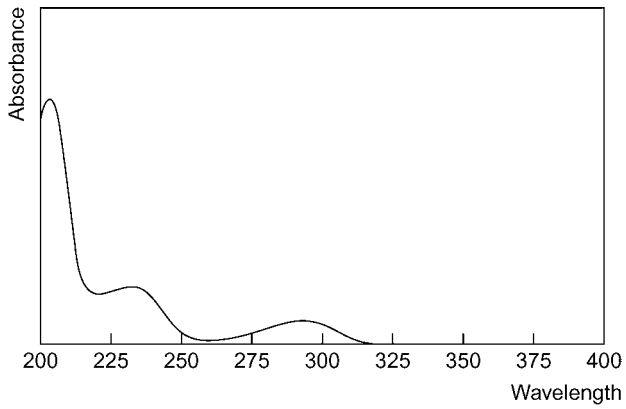
Etenzamide

Analgesic
$C_9H_{11}NO_2$ = 165.2
CAS—938-73-8

IUPAC Name 2-Ethoxybenzamide
Synonyms Aethoxybenzamidum; ethenzamide; ethoxybenzamide; ethylsalicylamide; HP-209; salicylamide *O*-ethyl ether.
Proprietary Names *Simil NF*; *Trancalgyl*. It is an ingredient of *Algopriv*; *Antifohnon-N*; *Cephyl*; *Coldadolin*; *Dolmix*; *Glutisal*; *Helopyrin*; *Katagrip*; *Kolton grippale N*; *Nicaphlogyl*; *Nisicur*; *Seltoc*; *Seranex sans codeine*.

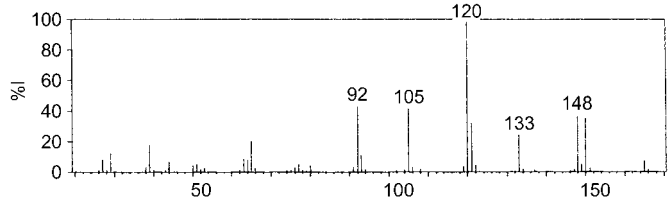


Chemical Properties A white crystalline powder. Mp 132° to 134°. Practically insoluble in cold water; slightly soluble in boiling water and in ether; soluble in ethanol and acetone; freely soluble in chloroform. Log *P* (octanol/water), 0.8.
Colour Tests Liebermann's reagent—brown; Mandelin's test—green-brown; Marquis test—red; Nessler's reagent—brown-orange (weak).
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.03; system TC—R_f 0.59; system TE—R_f 0.76; system T—R_f 0.55; system TAE—R_f 0.87 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—etenzamide RI 1560, M (desethyl)/salicylamide RI 1414, M (desethyl-)-AC RI 1660.
High Performance Liquid Chromatography System HD—*k* 0.55; system HW—*k* 4.60; system HY—RI 303.
Ultraviolet Spectrum Aqueous acid—234 (A^1_1=540c), 293 nm.



Infrared Spectrum Principal peaks at wavenumbers 1634, 1233, 753, 1276, 1114, 1587 cm^{-1} (KBr disk).

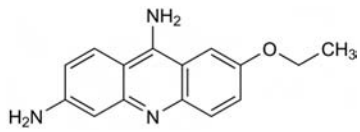
Mass Spectrum Principal ions at *m/z* 120, 92, 105, 148, 150, 121, 133, 65.



Dose Etenzamide has been given in doses of up to 4 g daily in divided doses.

Ethacridine

Disinfectant
$C_{15}H_{15}N_3O = 253.3$
CAS—442-16-0
IUPAC Name 7-Ethoxy-3,9-acridinediamine

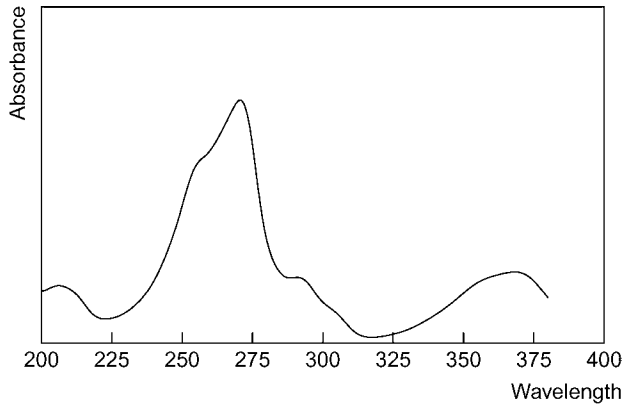


Chemical Properties Orange crystals. Mp 226°. Log *P* (octanol/water), 2.1.

Ethacridine Lactate

$C_{15}H_{15}N_3O,C_3H_6O_3 = 343.4$
CAS—1837-57-6
Synonyms Acrinol; acrinol lactate; aethacridinium lacticum; ethacrindini lactas; lactoacridine.
Proprietary Names *Metifex*; *Rivanol*; *Urocridin*; *Uroseptol*; *Vucine*.
Chemical Properties A yellow crystalline powder. It forms yellow fluorescent solutions. Mp about 245°, with decomposition. Slowly soluble 1 in 15 of water, 1 in 9 of boiling water and 1 in about 150 of ethanol.

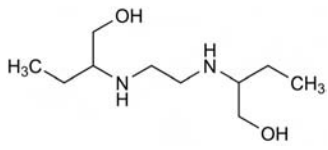
Colour Test Marquis test—orange→red.
Thin-layer Chromatography System TA—R_f 0.60; system TAE—R_f 0.07 (van Urk reagent, yellow).
Ultraviolet Spectrum Aqueous acid—268 nm (A_1^1=2062a).



Infrared Spectrum Principal peaks at wavenumbers 1113, 1630, 1495, 1226, 1591, 1034 cm^{-1} (KBr disk).
Dose Ethacridine lactate has been given, orally, in doses of 200 to 600 mg daily.

Ethambutol

Antituberculosis
$C_{10}H_{24}N_2O_2 = 204.3$
CAS—74-55-5
IUPAC Name (2*S*)-2-[2-[[(2*S*)-1-Hydroxybutan-2-yl]amino]ethylamino]butan-1-ol
Synonyms EMB; 2,2′-(1,2-ethanediyldiimino)bis-1-butanol.

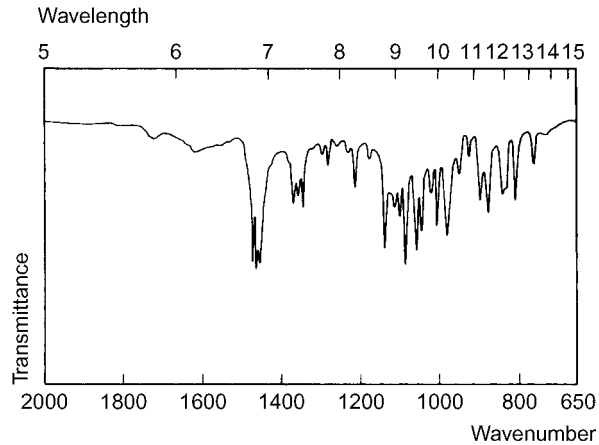


Chemical Properties Mp 87.5° to 88.8°. Sparingly soluble in water; soluble in chloroform and methylene chloride. pK_a 6.3, 9.5 (20°). Log *P* (octanol/water), −0.4.

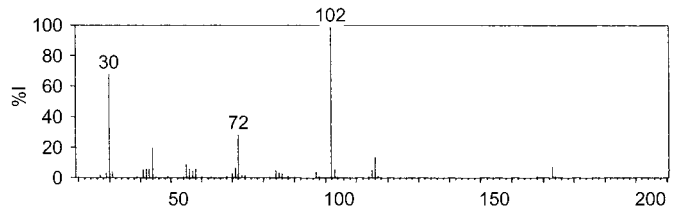
Ethambutol Hydrochloride

$C_{10}H_{24}N_2O_2,2HCl = 277.2$
CAS—1070-11-7
Proprietary Names *Dexambutol*; *EMB*; *Etapiam*; *Etibi*; *Lambutol*; *Miambutol*; *Myambutol*; *Mycrol*; *Myrin*; *Oributol*; *Servabutol*; *Tambutec*; *Tobutol*; *Tubetam*; *Turresis*. It is an ingredient of *Mynah*.
Chemical Properties A white, crystalline, hygroscopic powder. Mp 198.5° to 200.3° also reported as 201.8° to 202.6°. Soluble 1 in 1 of water, 1 in 4 of ethanol, 1 in 850 of chloroform and 1 in 9 of methanol; very slightly soluble in ether.

Thin-layer Chromatography System TA—R_f 0.30; system TB—R_f 0.03; system TC—R_f 0.02; system TE—R_f 0.76; system TL—R_f 0.02; system TAD—R_f 0.00; system TAE—R_f 0.12; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.20 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1090, 1061, 1142, 987, 1050, 1009 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 102, 30, 72, 44, 116, 55, 173, 71.



Quantification

Plasma **GC** ECD. Limit of detection, 10 µg/L [Lee, Wang 1980].
GC–MS Limit of detection, 36 µg/L [Holdiness *et al.* 1981].
HPLC UV detection. Limit of detection, <0.2 mg/L [Chenevier *et al.* 1998]. Fluorescence detection. Limit of detection, <10 µg/L [Breda *et al.* 1996].
HPLC–MS Limit of detection, 0.05 mg/L [Conte *et al.* 2002].
Urine **HPLC** See Plasma [Breda *et al.* 1996].
Bronchoalveolar Lavage Fluid **HPLC–MS** Limit of detection, 0.005 mg/L [Conte *et al.* 2002].
Alveolar Cells **HPLC–MS** See Bronchoalveolar Lavage Fluid [Conte *et al.* 2002].
Disposition in the Body Readily absorbed after oral administration. About 50 to 70% of a dose is excreted in the urine as unchanged drug in 24 h, and up to 15% may be excreted as inactive aldehyde and carboxylic acid metabolites; up to 20% of an oral dose may be eliminated in the faeces.
Therapeutic Concentration In plasma, usually in the range 2.5 to 6.5 mg/L. After a single oral dose of 15 mg/kg to 6 subjects, peak plasma concentrations of 3.2 to 5.6 mg/L (mean 4) were attained in 2 to 4 h [Lee *et al.* 1977].
Toxicity Toxic effects are usually associated with plasma concentrations >6 mg/L. In a fatality caused by the ingestion of ethambutol and rifampicin, the following postmortem concentrations were reported for ethambutol and rifampicin, respectively: blood 84 and 182 mg/L, urine 6800 and 3300 mg/L; low concentrations of ethanol were also detected [Jack *et al.* 1978].
Half-life Plasma half-life, about 10 to 15 h.
Clearance Plasma clearance, about 9 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 0.6 to 0.9.
Protein Binding About 10 to 40% (concentration-dependent).
Dose 15 to 25 mg/kg of ethambutol hydrochloride daily; 50 mg/kg has been given twice weekly.

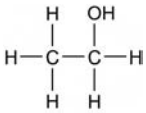
Breda M *et al.* (1996). Determination of ethambutol in human plasma and urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr A* 729: 301–307.

Chenevier P *et al.* (1998). Determination of ethambutol in plasma by high-performance liquid chromatography after pre-column derivatization. *J Chromatogr B Biomed Sci Appl* 708: 310–315.
Conte JE *et al.* (2002). A high-pressure liquid chromatographic-tandem mass spectrometric method for the determination of ethambutol in human plasma, bronchoalveolar lavage fluid, and alveolar cells. *J Chromatogr Sci* 40: 113–118.
Holdiness MR *et al.* (1981). *J Chromatogr* 224: 415–422.
Jack DB (1978). Fatal rifampicin-ethambutol overdosage. *Lancet* 2: 1107–1108.
Lee CS *et al.* (1977). Kinetics of oral ethambutol in the normal subject. *Clin Pharmacol Ther* 22: 615–621.
Lee CS, Wang LH (1980). Improved GLC determination of ethambutol. *J Pharm Sci* 69: 362–363.

Ethanol

CNS Depressant
$CH_3CH_2OH = 46.07$
CAS—64-17-5

IUPAC Name Ethanol
Synonyms Alcohol; ethyl alcohol.



Chemical Properties Ethanol of the *British Pharmacopoeia* is Dehydrated Alcohol (Absolute Alcohol), which contains 99.4–100% of C_2H_6O, sp.gr. 0.7904–0.7935 (20°/20°). Rectified Spirit is ethanol (90%). In the *United States Pharmacopeia*, Alcohol contains 94.9–96.0% of C_2H_6O, sp.gr. 0.812–0.816 (15.56°). A clear, colourless, mobile, volatile, readily inflammable, hygroscopic liquid. Ethanol (96%) boils at ~78°. Miscible with water, chloroform and ether. pK_a, 15.9 (25°). Log *P* (octanol/water), −0.3.

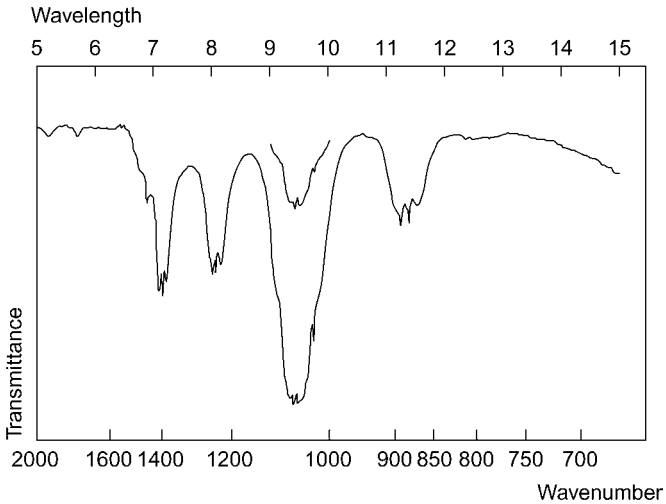
Industrial Denatured Alcohol

Synonym IDA
Chemical Properties A mixture of ethanol, of an appropriate strength, 95 parts by volume with wood naphtha 5 parts by volume.
Note Two other classes of methylated spirit are recognised in the United Kingdom: completely denatured alcohol (CDA) and denatured ethanol. Mineralised methylated spirits is ethanol of an appropriate strength, 90 parts by volume mixed with wood naphtha 9.5 parts by volume and crude pyridine 0.5 parts by volume, and to every 2000 L of this mixture is added 7.5 L of mineral naphtha (petroleum oil) and 3.0 g methyl violet. Denatured ethanol is ethanol, of a strength not less than 85%, 98 parts by volume mixed with propan-2-ol 2 parts by volume, and to the resulting mixture is added denatonium benzoate 10 µg/mL, or solid quassin 120 µg/mL, or sucrose octa-acetate 4000 µg/mL.

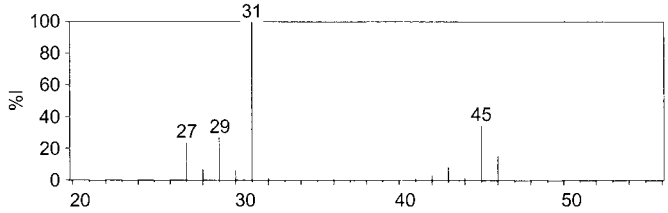
Surgical Spirit (BP) is a mixture of castor oil 2.5 mL, diethyl phthalate 2 mL and methyl salicylate 0.5 mL, diluted to 100 mL with industrial denatured alcohol.

Rubbing Alcohol (USP) is a mixture of acetone 8 volumes, methyl isobutyl ketone 1.5 volumes and alcohol 100 volumes; the mixture contains not less than 3550 µg/mL of sucrose octa-acetate or 14 µg/mL of denatonium benzoate.

Colour Test Potassium dichromate (method 2)—green.
Gas Chromatography System GA—RI 421; system GI—RT 1.9 min; system GAA—RI 427.
Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 31, 45, 29, 27, 46, 43, 30, 42.



Quantification

Blood **GC** Column: Autosystem XL (60 m × 0.53 mm i.d., 1 µm). Carrier gas: N_2. Limit of detection not reported [Büyük *et al.* 2009]. FID. Limit of detection, <0.75 mg/L for ethanol and <0.85 mg/L for acetaldehyde [Pontes *et al.* 2009]. Comparison of alkyl nitrite GLC and direct vapour phase [Barinskaia *et al.* 2006]. Column: capillary. FID [Kristoffersen *et al.* 2006]. Column: capillary. Limit of detection, 5.6 mg/L [Maleki *et al.* 2006]. Postmortem alcohol. FID [De Martinis *et al.* 2006]. Postmortem alcohol. Limit of detection, 1 mg/L [De Martinis, Martin 2002]. See also Goto, Ikemoto [1991], [Macchia *et al.* [1995], McCarver-May, Durisin [1997], Tangerman [1997], Tsukamoto *et al.* [1998], Watanabe-Suzuki *et al.* [1999], Zuba *et al.* [2002a] and Zuba *et al.* [2002b].

GC-MS Postmortem ethanol [Maeda *et al.* 2006]. Scan mode. Limit of detection, 0.2 mg/L, limit of quantification, 2 mg/L [Wasfi *et al.* 2004].

HPLC ELSD to detect phosphatidylethanol [Aradottir, Olsson 2005]. Column: LiChrospher 100 RP-18 (250 × 4 mm i.d., 5 µm). Mobile phase: 14.7 mmol/L potassium dihydrogen phosphate : 8.76 mmol/L dipotassium hydrogen phosphate : acetonitrile (15 : 45 : 40), flow rate 1.5 mL/min. UV detection (λ= 276 nm). Limit of quantification, 17 mg/L, limit of detection, 76 mg/L [Pellegrino *et al.* 1999].

LC-MS Column: Luna C_8 (50 × 2 mm i.d., 3 µm). Mobile phase: 2 mmol/L ammonium acetate : methanol-acetone (95 : 5; 25 : 75 for 3 min to 0 : 100 at 9 min for 3 min to 25 : 75 at 13 min for 2 min), flow rate 0.4 mL/min. ESI. Limit of detection, 20 µg/L for phosphatidylethanol [Gnann *et al.* 2009]. Column: C_8 (2.1 mm). ESI. Limit of detection, 1 µg/L for phosphatidylethanol [Tolonen *et al.* 2005]. Alcohol dehydrogenase method. Limit of quantification, 24 mg/L, limit of detection, 7 mg/L [Kristoffersen, Smith-Kielland 2005; Kristoffersen *et al.* 2005]. Limit of detection, 500 mg/L [Poklis, Mackell 1982].

Plasma **GC** See Blood [Kristoffersen *et al.* 2006]. Column: HP-FFAP cross-linked capillary (50 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 200 kPa. Temperature: 70°. FID. Retention time: 7.1 min. Limit of detection, 10 mg/L [Macchia *et al.* 1995].

GC-MS Column: Porapak Q 80/100 mesh (6 ft × 0.125 in i.d.). Carrier gas: He, 50 mL/min. Temperature: 170° Limit of detection, 5 ng [Pereira *et al.* 1974].

Serum **GC** Comparison with enzymatic method [Winek *et al.* 2004]. Limits of detection, 0.1 mg/L for ethanol and acetaldehyde [Tangerman 1997]. See Plasma [Macchia *et al.* 1995].

HPLC See [Pellegrino *et al.* 1999].

LC-MS ESI. Limit of quantification, 0.2 µmol/L for ethylglucuronide) and 0.4 µmol/L for ethyl sulfate, limit of detection, 0.04 µmol/L for ethyl glucuronide and 0.08 µmol/L for ethylsulfate [Morini *et al.* 2007].

Urine **GC** Column: Stabilwax capillary (30 m × 0.53 mm, 1.0 µm). Carrier gas: He. FID Limit of quantification, 0.5 mg/L [Zilly *et al.* 2003]. Column: fused silica glass capillary bonded with PEG-20M or fused silica glass capillary of Pora PLOT Q. Ethanol and acetaldehyde [Tsukamoto *et al.* 1998]. Column: Poraplot Q (10 m × 0.32 mm i.d.). Carrier gas: He, 2.6 mL/min. Temperature: 250°. FID. Limit of quantification, 10 mg/L, limit of detection, 8 mg/L [Corrêa, Pedroso 1997].

See Blood [Barinskaia *et al.* 2006; De Martinis, Martin 2002; De Martinis *et al.* 2006; Maleki *et al.* 2006; Pontes *et al.* 2009]. See Serum [Tangerman 1997]. See Plasma [Macchia *et al.* 1995].

GC-MS See Plasma [Pereira *et al.* 1974]. Alcohol dehydrogenase method. Limit of detection, 12 mg/L, limit of quantification, 42 mg/L [Kristoffersen, Smith-Kielland 2005; Kristoffersen *et al.* 2005].

Faecal Supernatants **GC** See Serum [Tangerman 1997].

Oral Fluid **GC** See Blood [Barinskaia *et al.* 2006; Maleki *et al.* 2006]. See Plasma [Macchia *et al.* 1995].

Synovial Fluid **GC** See Blood [Büyük *et al.* 2009].

Vitreous Humour **GC** See Blood [De Martinis *et al.* 2006; De Martinis, Martin 2002; Pontes *et al.* 2009].

Bone Marrow **GC-MS** See Blood [Maeda *et al.* 2006].

Expired Air **GC** Limits of detection, 0.1 nmol/L to a few nmol/L [Qin *et al.* 1997]. Column: Poraplot Q (10 m × 0.32 mm i.d.). Carrier gas: He, 50 kPa. Temperature programme: 40° for 3 min to 175° at 50°/min. SIM acquisition mode. Ion-trap detection. Limit of detection, 5 pg [Ghoos *et al.* 1989].

Note Analysers. For recent reports of the analysis of breath alcohol with breath alcohol analysers, see Watterson, Ellefsen [2009] (Intoxilyzer 8000C), Lindberg *et al.* [2007]. For a comparison of blood and breath measurements (Intoxilyzer 5000), see Jones *et al.* [1992].

Other **GC** Mouse Liver Cells. Column: Porapak Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: He, 37 mL/min. Temperature: 130°. FID. Retention time: 3.5 min [Mendenhall *et al.* 1980].

Reviews For a review of interpretation of results of ethanol analysis in postmortem specimens, see Kugelberg, Jones [2007]. For physical methods of quantification with sampling from different body fluids, see Swift [2003]; in blood, see Cravey, Jain [1974]; and in breath, see Mason, Dubowski [1976] and Jain, Cravey [1974]. For

early reviews of alcohol analysis by chemical and infrared methods, see Jain, Cravey [1972a] and by gas chromatography, see Jain, Cravey [1972b].

Disposition in the Body Ethanol is readily absorbed from the stomach and small intestine after oral administration but absorption may be delayed by the presence of food; it is rapidly distributed throughout the body fluids and is metabolised in the liver by alcohol dehydrogenase to acetaldehyde and then further oxidised to acetate and carbon dioxide. More than 90% is metabolised and is excreted by the lungs and in the urine, saliva, sweat and other secretions, together with unchanged alcohol. The body can metabolise ~10–15 mL/h. The rate of metabolism may be accelerated following repeated excessive use.

Blood Concentration

After administration of 15, 30, 45 and 60 mL of 95% ethanol to 8 fasting subjects, peak blood concentrations of 25–290 mg/L (mean 199), 180–620 mg/L (mean 440), 410–860 mg/L (mean 680) and 630–1120 mg/L (mean 860), respectively were attained in 0.3, 0.5, 0.8 and 1 h [Wilkinson *et al.* 1977a]. See also Wilkinson *et al.* [1977b].

After administration of 0.5 g/kg of ethanol to 4 subjects, blood acetaldehyde concentrations of 0.02–0.06 mg/mL were reported after 40–80 min [Christensen *et al.* 1981].

In a study in subjects with high blood alcohol levels, the mean blood concentration was 4.05 g/L and the rate of elimination was 0.33 g/L/h (range 0.02–0.62). It is possible that there is ultra-rapid elimination of blood alcohol in those with very high alcohol levels, possibly through induction of CYP2E1 ethanol metabolism [Jones 2008].

For a comparison of blood and breath alcohol levels with tissue levels and the contribution of alcohol pharmacokinetics to observed differences, see Ridder *et al.* [2009].

Toxicity The minimum lethal dose is ~500 mL of 50% spirit ingested in ~1 h. In the UK, it is illegal to be in charge of a motor vehicle with a concentration >800 mg/L in the blood, 1070 mg/L in the urine or 35 mg/L in the breath. Toxic effects are associated with blood concentrations of 840–4500 mg/L (mean 2400), and subjects with concentrations of 3000 mg/L or more are considered to be clinically drunk. Blood concentrations of 2250–6030 mg/L (mean 4000) have been associated with fatalities. The maximum permissible atmospheric concentration is 1000 ppm.

In 10 fatalities caused by the ingestion of ethanol, the following postmortem tissue concentrations were reported: blood 4230–17 660 mg/L (mean 7410), bile 1250–27 950 mg/L (mean 7860), brain 3100–9120 μg/g (mean 4430), kidney 2850–10 430 μg/g (mean 4830), liver 2450–11 610 μg/g (mean 4470) and urine 4850–9400 mg/L (mean 6190) [Christopoulos *et al.* 1973].

There was an unusual distribution of ethanol in a 48-year-old man with a history of alcoholism who was found dead: femoral blood (2 samples) 2.57 and 2.73 g/L, heart blood 6.43 g/L, vitreous humour 7.63 g/L, urine 840 μg/g, bile 6.16 g/L, liver 2.50 mg/g and gastric contents 2.47 g/53 g. It was proposed that agonal or postmortem aspiration of ethanol-rich vomitus and postmortem fermentation could account for the elevated concentrations in heart blood and bile; high vitreous ethanol could have resulted from diffusion in the agonal phase or postmortem from gastric aspirate in the carpet [Singer, Jones 1997].

Analysis of the blood of 169 acutely poisoned alcoholics on hospital admission showed the mean blood ethanol concentrations to be 3.14 g/L (range 0.76–6.6) [Zuba *et al.* 2002a; Zuba *et al.* 2002b].

In a study in 59 subjects, breath alcohol levels were determined at hourly intervals using an Alcotest 7110 MK IIIA analyser. Mean peak alcohol levels were 0.456 mg/L. The mean breath alcohol elimination rate was 0.082 mg/L. The mean elimination rate in females of 0.087 mg/L was significantly higher than that in males (0.078 mg/L) [Pavlic *et al.* 2007].

In a study to determine levels of drugs in postmortem femoral blood from 24 876 examinations representing all causes of death, alcohol was the most common psychoactive compound found, being present in 8108 (33%), at mean, median and highest concentrations of 1.43, 1.20 and 8.0 g/L, respectively [Jones, Holmgren 2009].

Mean blood alcohol levels of 1.86 g/L were found in 104 young patients (mean age 16.2 years) admitted to an emergency department with acute alcohol intoxication over a 12 month period; 21.2% were slightly intoxicated, 75.8% moderately and 3% severely [Sanz Marcos *et al.* 2009].

In a study of blood alcohol levels in suicides over a 10-year period in Sweden (11 441 cases), 34% (37% males, 31% females) had consumed alcohol before death. Mean blood alcohol levels were 1.34 g/L in men and 1.25 g/L in women. Many were heavily intoxicated, with 90th percentiles of blood alcohol levels in the range 2.3–2.8 g/L [Holmgren, Jones 2010].

Half-life Plasma half-life is dose dependent.

Volume of Distribution ~0.6 L/kg.

Distribution in Blood Plasma : whole blood ratio, 1.2.

Saliva Plasma : saliva ratio, ~0.93.

Protein Binding No significant binding.

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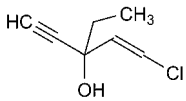
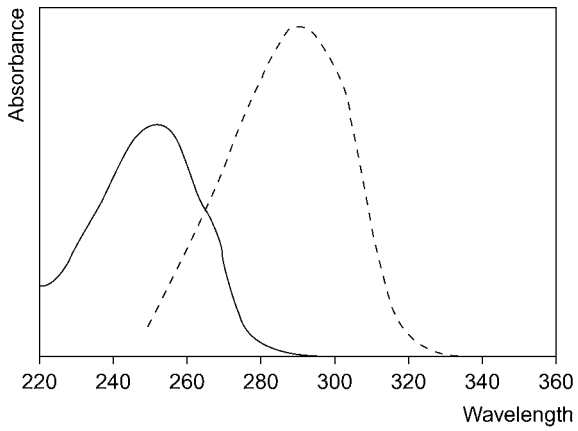
Ethchlorvynol

Hypnotic
C_7H_9ClO = 144.6
CAS—113-18-8

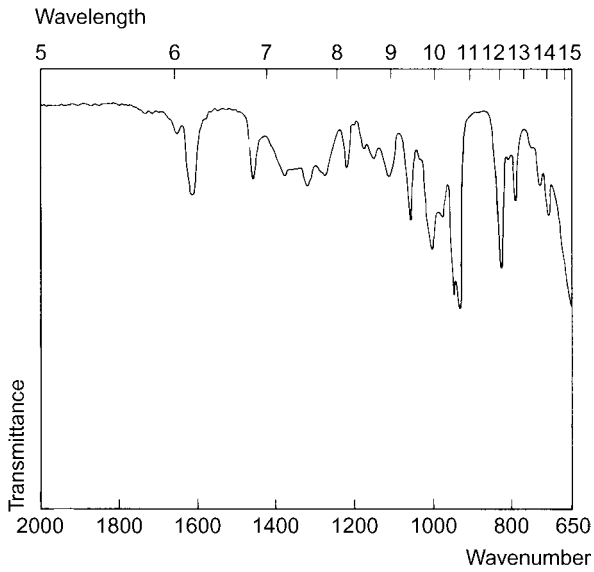
IUPAC Name 1-Chloro-3-ethyl-1-penten-4-yl-3-ol
Synonyms β-Chlorovinyl ethyl ethynyl carbinol; E-ethchlorvynol; ethyl B-chlorvynol.
Proprietary Name *Placidyl*



Chemical Properties A colourless to yellow liquid. This slightly viscous liquid darkens on exposure to air and light. Weight per mL ≈1.072 g. Refractive index at 20°, 1.4770 to 1.4805. Practically immiscible with water; miscible with ethanol, chloroform, ether and most other organic solvents. Log *P* (octanol/water), 1.6.
Colour Test Ammoniacal silver nitrate—white/yellow precipitate.
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.81; system TF—R_f 0.74; system TAD—R_f 0.82; system TAE—R_f 0.87.
See Peel and Freimuth [1972].
Gas Chromatography System GA—RI 1015; system GB—RI 1060; system GI—RT 35.2 min.
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 935, 950, 830, 1010, 1063, 980 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 115, 117, 89, 53, 109, 51, 91, 39.
Quantification
Blood **GC** Column: 3% OV-17 on 100/200 Chromasorb WHB (PE, 6′). Carrier gas: He, 52 mL/min. Temperature: 80°. FID. Limit of detection not reported [Winek *et al.* 1989]. Column: 3% OV-1 GasChrom W on 80/100 mesh (6′ × 4 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 100°. Retention time: 1.9 min. Limit of detection, 1 mg/L [McCurdy 1977]. Column: SE-30. Carrier gas: He, 35 mL/min. Temperature: 80°. Limit of detection not reported [White, Graves 1974]. Column: 2.5% XE-60 on AW-DMCS Chromosorb G, 80/100 mesh (1.5 m × 4 mm i.d.). Carrier gas: Ar, 70 mL/min. Temperature: 100°. FID. Retention time: 13.5 min. Limit of detection, 20 mg/L [Gibson, Wright 1972]. Column: Chromosorb W 6% diethylene glycol (6′ × 1/8 "i.d.). Carrier gas: He, 60 mL/min. Temperature: 168°. FID. Limit of detection, 0.1 µg [Hedley-Whyte, Laasberg 1969]. Column: 1% Hi-Eff 3-BP on 80/100 mesh Gas Chrom Q (6′ × 1/4" i.d.). Carrier gas: He, 35 mL/min. Temperature: 110°. FID. Retention time: 3 min. Limit of detection, 4.5 µg/L [Maes *et al.* 1969].
Plasma **GC** Column: 2% Carbowax 20M 5% HOH on HP Chromosorb W 80/100 mesh. Carrier gas: N_2, 60 mL/min. Temperature: 140°. FID. Retention time: 0.69. Limit of detection, 2 mg/L [Flanagan *et al.* 1978]. Column: 2% Carbowax 20M and 5% KOH on HP Chromosorb W 80/100 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 200°. FID. Relative retention time: 0.69. Limit of detection, 2 mg/L [Flanagan, Lee 1977].
HPLC Ultrasphere ODS C_{18} (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (5 : 95 to 22 : 78 in 24 min to 45 : 55 in 10 min for 5 min to 5 : 95 for 5 min). Flow rate 3.0 mL/min. UV detection (λ = 210 nm). Retention time: 16.5 min. Limit of detection, 2 mg/L [Kabra *et al.* 1981].
Serum **GC** See Plasma [Flanagan *et al.* 1978]. Column: 5% NPGS on AW-DMCS 100/120 mesh (1.52 m × 0.32 m i.d.). Carrier gas: N_2, 50 to 60 mL/min. Temperature: 120°. ECD. Limit of detection, 0.25 mg/L [Cummins *et al.* 1971]. Column: Silicone L-40 20% on acid-washed Chromosorb V 80/100 mesh (5 ′ × 1/8″ i.d.). Carrier gas: N_2, 22 mL/min. Temperature: 125°. FID. Retention time: 3 to 4 min. Limit of detection, 5 mg/L [Robinson 1968].
HPLC See Plasma [Kabra *et al.* 1981].
Urine **GC** See Blood [Winek *et al.* 1989]. See Plasma [Flanagan *et al.* 1978]. See Plasma [Flanagan, Lee 1977]. See Blood [McCurdy 1977]. See Blood [Gibson, Wright 1972]. See Serum [Cummins *et al.* 1971]. See Blood [Hedley-Whyte, Laasberg 1969]. See Blood [Maes *et al.* 1969]. See Serum [Robinson 1968].
Bile **GC** See Blood [Winek *et al.* 1989]. Column: 0.2% Carbowax 1500 on 80/100 mesh (6′ × 1/8″ i.d.). Carrier gas: He, 30 mL/min. Temperature: 150°. FID. Limit of detection not reported [Winek *et al.* 1988]. See Blood [Maes *et al.* 1969].
Gastric Contents **GC** See Blood [Winek *et al.* 1989]. See Plasma [Flanagan *et al.* 1978]. See Blood [McCurdy 1977]. See Blood [Maes *et al.* 1969].
Vitreous Humour **GC** See Blood [Winek *et al.* 1989].
Brain **GC** See Blood [Maes *et al.* 1969].
Kidney **GC** See Blood [Winek *et al.* 1989]. See Blood [Maes *et al.* 1969].
Liver **GC** See Blood [Winek *et al.* 1989]. See Blood [Maes *et al.* 1969].
Spleen **GC** See Blood [Maes *et al.* 1969].
Other **GC** Rabbit Blood and Bone Marrow. Column: 3% OV-17 on 100/120 mesh Chromosorb W HP (6′). Carrier gas: He, 52 mL/min. Temperature: 80°. FID. Limit of detection not reported [Winek *et al.* 1981].
Note For an evaluation of gas chromatographic and colorimetric methods, see Bridges and Jennison [1984]. For a colourimetric method, see Kaistha and Tadrus [1978]. For a UV spectrophotometric method for detection in blood, serum and urine, see Wallace *et al.* [1974].
Disposition in the Body Rapidly and completely absorbed after oral administration (60 to 90 min [Lynn *et al.* 1979;Yell 1990]). It is extensively metabolised; the major metabolite found in the blood and urine of poisoned patients is 1-chloro-3-ethynylpent-1-ene-3,4-diol [Kelner, Bailey 1983]; other metabolites include 1-chloro-3-ethynylpent-1-ene-3,5-diol and 1-chloro-3-ethylpent-1-ene-3,4-diol. Ethchlorvynol is highly localised in tissues (particularly adipose tissue) and released very slowly. Less than 0.1% of a dose is excreted in the urine in 24 h as unchanged drug or its glucuronide conjugate [Cummins *et al.* 1971]. Ethchlorvynol crosses the placenta. It is not known whether it is the parent compound or a metabolite that is responsible for the peculiar pungent breath odour frequently noticed in intoxicated patients [Bertino, Jr., Reed 1986].
Therapeutic Concentration The therapeutic concentraion in plasma is usually in the range 5 to 20 mg/L. After a single oral dose of 500 mg to 8 subjects, a mean peak plasma concentration of 6.5 mg/L was attained in 1 h [Cummins *et al.* 1971].
Toxicity The estimated minimum lethal dose is 15 g, although death has occurred after the acute ingestion of 2.5 g [Gary, Tresznewsky 1983]. Plasma concentrations greater than about 20 mg/L are associated with toxic effects [Kelner, Bailey 1983]. Prolonged use of ethchlorvynol may lead to dependence of the barbiturate–alcohol type. Ethchlorvynol overdose can result in pressure necrosis [Chamberlain *et al.* 1990].

In a review of 13 fatalities due to overdosage with ethchlorvynol, postmortem blood concentrations were in the range 14 to 400 mg/L (mean, 119 mg/L) [Rehling 1967].

A 36-year-old male attempted suicide. The initial plasma concentration of ethchlorvynol was 60 mg/L, and this decreased to 2.5 mg/L by the ninth day. The urinary concentration decreased from 6.5 to 1.1 mg/L. The initial whole-blood concentration was 90 mg/L and, in the peritoneal dialysate, the concentration was 7.2 mg/L [Hedley-Whyte, Laasberg 1969].

A 27-year-old male was admitted to hospital in grade-III coma. On admission, his blood contained 51 mg/L ethchlrovinol, 30 mg/L cyclobarbitone and 12 mg/L methaqualone. He made a complete recovery after haemodialysis [Gibson, Wright 1972].

In 3 fatalities due to ethchlorvynol, the following postmortem tissue concentrations were reported: blood, 85, 22 and 66 mg/L; brain, 57, not tested, 285 µg/g; kidney, 54, 63, and 860 µg/g; liver 70, 60, and 507 µg/g; adipose tissue 1040 and 142 µg/g (2 cases) [Cravey, Baselt 1968; Winek *et al.* 1989].

Toxicological analysis of body fluids and tissues of a 54-year-old female fatality who had been embalmed revealed an ethchlorvynol concentration of 112 mg/L in the bile; phenobarbital (32.8 mg/L) was also found in the bloody fluid from the heart [Winek *et al.* 1988].

The following 2 cases of fatal poisoning the following tissue concentrations (mg/100 mL or mg/100 g) were measured:

Tissue	*Case 1*	*Case 2*
Blood	15.4	15.4
Urine	1.0	N.D.
Bile	16.0	13.5
Gastric contents	11.3	4.5
Brain	8.3	14.8
Kidney	2.2	7.2
Liver	2.7	10.6
Spleen	4.6	9.7

N.D. Not determined. [Maes *et al.* 1969]

In 2 fatalities involving ethchlorvynol overdose, the following postmortem tissue concentrations were reported: blood, 198.2 and 29.0 mg/L; urine, 261.8 and 10.6 mg/L; bile, 361.8 and 22.7 mg/L; eye fluid, 10.0 and not reported; liver, 620.0 and 21.3 µg/g; kidney, not reported and 17.3 µg/g; and stomach contents (total), 130 000 and 533 mg. In case 1, ethanol, diazepam, and nordiazepam were also detected; in case 2, diazepam, nordiazepam, and hydrocodone were also present [Winek *et al.* 1989].

Note For ethchlorvynol concentrations in non-fatal (51) and fatal (38) ingestions, see Bailey and Shaw [1990].

Half-life Distribution half-life, ≈1 to 3 h and elimination half-life of 10 to 25 h [Gary, Tresznewsky 1983], increased to 21 to 105 h in overdose cases [Bertino, Jr., Reed 1986; Yell 1990] reduced by haemodialysis [Forycki *et al.* 1985].

Volume of Distribution ≈2 to 3 L/kg; 4 L/kg [Bertino, Jr., Reed 1986; Mack 1983; Yell 1990]; 270 to 300 L [Lynn *et al.* 1979]; 3.0 to 4.0 [Forycki *et al.* 1985].

Clearance 1 to 6 mL/min [Lynn *et al.* 1979].

Distribution in Blood Ratio of plasma : whole blood ≈1.1.

Protein Binding ≈55% [Forycki *et al.* 1985]; 62% [Yell 1990].

Dose 0.2 to 1 g, as a hypnotic.

Bailey DN, Shaw RF (1990). Ethchlorvynol ingestion in San Diego County: a 14-year review of cases with blood concentrations and findings. *J Anal Toxicol* 14: 348–352.

Bertino JS,Jr Reed MD (1986). Barbiturate and nonbarbiturate sedative hypnotic intoxication in children. *Pediatr Clin North Am* 33: 703–722.

Bridges RR, Jennison TA (1984). Analysis of ethchlorvynol (Placidyl): evaluation of a comparison performed in a clinical laboratory. *J Anal Toxicol* 8: 263–268.

Chamberlain JM *et al.* (1990). Pressure necrosis following ethchlorvynol overdose. *Am J Emerg Med* 8: 467–468.

Cravey RH, Baselt RC (1968). Studies of the body distribution of ethchlorvynol. *J Forensic Sci* 13: 532–536.

Cummins LM *et al.* (1971). Serum and urine levels of ethchlorvynol in man. *J Pharm Sci* 60: 261–263.

Flanagan RJ, Lee TD (1977). Rapid micro-method for the measurement of ethchlorvynol in blood plasma and in urine by gas-liquid chromatography. *J Chromatogr* 137: 119–126.

Flanagan RJ *et al.* (1978). Analysis of chlormethiazole, ethchlorvynol and trichloroethanol in biological fluids by gas-liquid chromatography as an aid to the diagnosis of acute poisoning. *J Chromatogr* 153: 473–479.

Forycki Z *et al.* (1985). Tranquilizers, analgetics and antidepressants in patients treated with hemodialysis. *Blood Purif* 3: 109–119.

Gary NE, Tresznewsky O (1983). Clinical aspects of drug intoxication: barbiturates and a potpourri of other sedatives, hypnotics, and tranquilizers. *Heart Lung* 12: 122–127.

Gibson PF, Wright N (1972). Ethchlorvynol in biological fluids: specificity of assay methods. *J Pharm Sci* 61: 169–171.

Hedley-Whyte J, Laasberg LH (1969). Ethchlorvynol poisoning: gas liquid chromatography in management. *Anesthesiology* 30: 107–111.

Kabra PM *et al.* (1981). Rapid method for screening toxic drugs in serum with liquid chromatography. *J Anal Toxicol* 5: 177–182.

Kaistha KK, Tadrus R (1978). Qualitative detection of placidyl (ethchlorvynol) alone or in combination with poly-drugs in drug abuse urine screening programs using ion-exchange paper and/or liquid-liquid extraction. *J Chromatogr* 161: 287–290.

Kelner MJ, Bailey DN (1983). Ethchlorvynol ingestion: interpretation of blood concentrations and clinical findings. *J Toxicol Clin Toxicol* 21: 399–408.

Lynn RI *et al.* (1979). Resin hemoperfusion for treatment of ethchlorvynol overdose. *Ann Intern Med* 91: 549–553.

Mack RB (1983). Death, the ultimate cure for insomnia—Placidyl overdose. *N C Med J* 44: 803–804.

Maes R *et al.* (1969). The gas chromatographic determination of selected sedatives (ethchlorvynol, paraldehyde, meprobamate, and Carisoprodol) in biological material. *J Forensic Sci* 14: 235–254.

McCurdy HH (1977). Quantitation of ethchlorvynol by gas chromatography. *J Anal Toxicol* 1: 164–165.

Peel HW, Freimuth HC (1972). Methods for the detection and determination of ethchlorvynol in biological tissue. *J Forensic Sci* 17: 688–692.

Rehling CJ (1967). Poison residues in human tissues. In: Stolman A, ed. *Progress in Chemical Toxicology*, vol. 3. New York: Academic Press, pp. 363-386.

Robinson DW (1968). Method for determining ethchlorvynol in urine and serum by gas chromatography. *J Pharm Sci* 57: 185–186.

Wallace JE *et al.* (1974). Spectrophotometric determination of ethchlorvynol in biologic specimens. *Clin Chem* 20: 159–162.

White JM, Graves MH (1974). The detection of sedative-hypnotic drugs in the impaired driver. *J Chromatogr Sci* 12: 219–224.

Winek CL *et al.* (1981). A comparative study of ethchlorvynol levels in blood versus bone marrow. *Forensic Sci Int* 17: 197–202.

Winek CL *et al.* (1988). Determination of ethchlorvynol in body tissues and fluids after embalmment. *Forensic Sci Int* 37: 161–166.

Winek CL *et al.* (1989). Body distribution of ethchlorvynol. *J Forensic Sci* 34: 687–690.

Yell RP (1990). Ethchlorvynol overdose. *Am J Emerg Med* 8: 246–250.

Ether

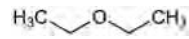
Anaesthetic (General)

$(C_2H_5)_2O = 74.12$

CAS—60-29-7

IUPAC Name Ethoxyethane

Synonyms Diethyl ether; diethyl oxide; ethyl ether; ethyl oxide; 1,1′-oxybisethane.

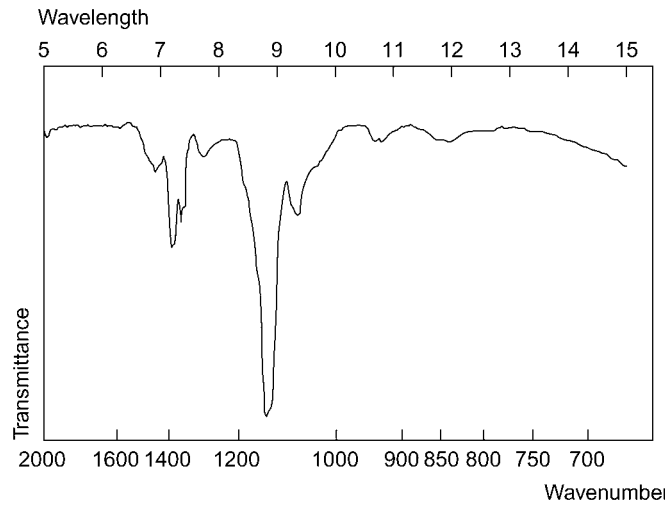


Chemical Properties A clear, colourless, volatile, inflammable, very mobile liquid. Bp 34° to 36°. Soluble 1 in 10 to 1 in 12 of water; miscible with ethanol, chloroform, benzene and petroleum ether. pK_a −3.6. Log *P* (octanol/water), 0.9. Anaesthetic Ether (BP) is highly purified ether containing not more than 0.002% of a stabiliser such as propyl gallate or hydroquinone. Solvent Ether (BP) is not so highly purified and is without a stabiliser.

Caution Ether is very volatile and inflammable, and mixtures of its vapour with oxygen, nitrous oxide or air at certain concentrations are explosive. It should not be used in the presence of an open flame or any electrical apparatus liable to produce a spark; precautions should be taken against the production of static electrical discharge. Explosive peroxides are generated by the atmospheric oxidation of solvent ether and it is dangerous to distil a sample which contains peroxides.

Gas Chromatography System GA—RI 515; system GI—RT 5.9 min; system GAA—RI 499.

Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 31, 59, 29, 45, 74, 27, 41, 43.

Quantification

Blood **GC-MS** Column: Rtx-1 capillary (60 m × 0.32 mm i.d., 3.0 µm). Carrier gas: He, 1.5 mL/min. Temperature: 100°. SIM acquisition mode. Limit of quantification, 10 mg/L [Cox *et al.* 2006].

Urine **GC-MS** See Blood [Cox *et al.* 2006].

Bile **GC-MS** See Blood [Cox *et al.* 2006].

Kidney **GC-MS** See Blood [Cox *et al.* 2006].

Liver **GC-MS** See Blood [Cox *et al.* 2006].

Disposition in the Body Ether is absorbed into the circulation after inhalation; the blood: gas partition coefficient is high (~12). Approximately 90% of a dose is slowly exhaled unchanged and very little is metabolised; a small amount may be excreted unchanged in the urine. Acetaldehyde is believed to be a minor metabolite.

Therapeutic Concentration In plasma, during anaesthesia, usually in the range 500–1500 mg/L.

Toxicity The estimated minimum lethal oral dose is 30 mL; the maximum permissible atmospheric concentration is 400 ppm. Atmospheric concentrations of 2000 ppm may cause dizziness and 100 000 ppm may be rapidly fatal.

In 4 elderly persons who died after being given ether with other medication, ether concentrations (mg/L or µg/g) immediately after the operation were: blood 600, 3750, 2880, 190; brain 700, 610, 2720, 310; liver 400, 1280, 260, 230; and lung 200, 1580, 210, –. In the second patient, the urine contained 340 mg/L [Campbell 1960].

A 24-year-old man became severely comatose after attempting suicide by injecting 5 mL ether into his left cubital vein. Ether was identified by gas chromatography [Serrano *et al.* 1999].

A 49-year-old man who committed suicide using plastic bag suffocation and ether inhalation was found to have blood levels of ether of 1277 mg/L. As this was within the levels used in anaesthesia, it was concluded that death was caused by asphyxiation [Athanaselis *et al.* 2002].

For other reports of suicide involving ether, see Bauer, Denk [1985] and Winek *et al.* [1970]. For the report of the death of a small child from ether poisoning, see Rittner *et al.* [1984]; for other reports of poisoning with ether, see Lechat [1981a] and Lechat [1981b]. For another case report and review of the literature, see Kernbach-Wighton *et al.* [1998].

Dose For induction of anaesthesia, 10 to 20% of the vapour by inhalation; maintenance, 3 to 10%.

Athanaselis S *et al.* (2002). Asphyxial death by ether inhalation and plastic-bag suffocation instructed by the press and the Internet. *J Med Internet Res* 4: E18.

Bauer G, Denk W (1985). [Catamnesis of a suicide: death by asphyxiation in a plastic bag under the effect of ether while listening to music]. *Crisis* 6: 46–56.

Campbell, JE (1960). Deaths associated with anesthesia. *J Forensic Sci* 5: 501-549.

Cox D *et al.* (2006). Distribution of ether in two postmortem cases. *J Anal Toxicol* 30: 635–637.

Kernbach-Wighton G *et al.* (1998). [Phenomenology of ether administration. With a review of the literature]. *Arch Kriminol* 202: 87–94.

Lechat P (1981a). [Ethyl ether poisoning]. *Bull Acad Natl Med* 165: 277–280.

Lechat P (1981b). [Poisoning by ether and other volatile solvents]. *Bull Acad Natl Med* 165: 281–282.

Rittner C *et al.* (1984). [Death of a small child as a result of ether poisoning]. *Arch Kriminol* 173: 103–108.

Serrano N *et al.* (1999). Ether suicide poisoning by intravenous injection. *Intensive Care Med* 25: 337–338.

Winek CL *et al.* (1970). Suicide with plastic bag and ethyl ether. *Lancet* i: 365.

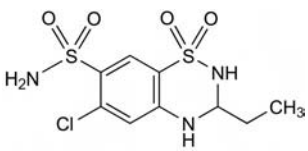
Ethiazide

Diuretic

$C_9H_{12}ClN_3O_4S_2$ = 325.8

CAS—1824-58-4

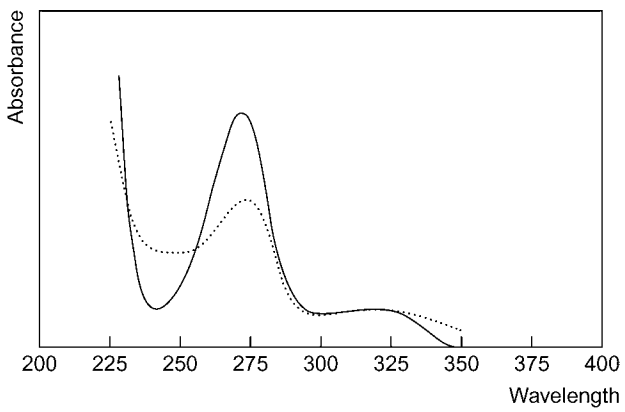
IUPAC Name 6-Chloro-3-ethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide



Chemical Properties Crystals. Mp 269° to 270°. Log *P* (octanol/water), 0.3.

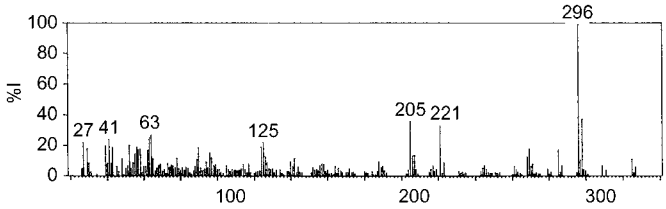
Thin-layer Chromatography System TD—R_f 0.11; system TE—R_f 0.50; system TF—R_f 0.50; system TL—R_f 0.22.

Ultraviolet Spectrum Aqueous acid—272 nm (A_1^1=345b).



Infrared Spectrum Principal peaks at wavenumbers 1163, 1172, 1603, 1312, 781, 1510 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 296, 298, 205, 221, 64, 63, 41, 125.



Dose Usually 5 to 10 mg daily.

E

Ethinamate

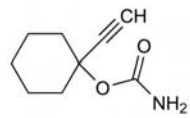
Hypnotic

$C_9H_{13}NO_2$ = 167.2

CAS—126-52-3

IUPAC Name 1-Ethynylcyclohexanol carbamate

Proprietary Names *Valamin*; *Valmid*; *Valmidate*.



Chemical Properties A white powder. Mp 96° to 98°. Soluble 1 in 400 of water and 1 in about 3 of ethanol; freely soluble in chloroform and ether. Log *P* (octanol/water), 1.9.

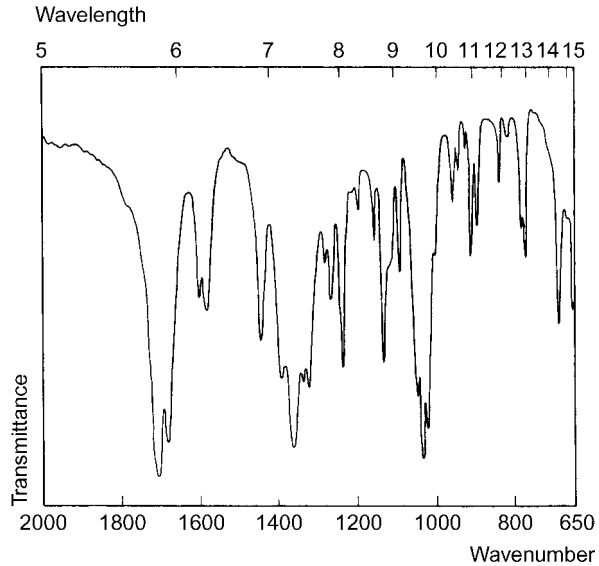
Colour Test Ammoniacal silver nitrate—yellow/brown.

Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.05; system TD—R_f 0.49; system TE—R_f 0.76; system TF—R_f 0.59; system TAD—R_f 0.58; system TAE—R_f 0.86; system TAF—R_f 0.87; system TAJ—R_f 0.58; system TAK—R_f 0.69; system TAL—R_f 0.91 (furfuraldehyde reagent, positive; acidified potassium permanganate solution, positive).

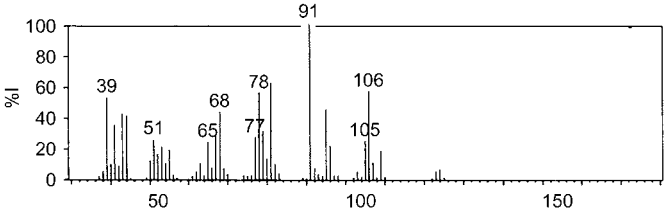
Gas Chromatography System GA—RI 1365.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1713, 1041, 1694, 1030, 1052, 1250 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 81, 106, 78, 39, 95, 68, 43.



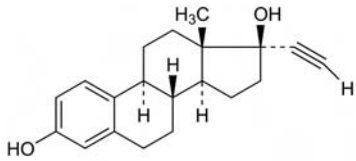
Quantification
Plasma **GC** FID. Ethinamate and *trans*-4-hydroxyethinamate. Limit of detection, 500 µg/L for ethinamate [Kleber *et al.* 1977].
Urine **GC** See Plasma [Kleber *et al.* 1977].
Disposition in the Body Rapidly absorbed after oral administration. Metabolised by hydroxylation to 4-hydroxyethinamate, which exists as stereoisomers, and conjugated with glucuronic acid. About 40% of a dose is excreted in the urine in 24 h as *trans*-4-hydroxyethinamate or its glucuronide conjugate; only a small amount is excreted unchanged in the urine; 3- and 2-hydroxyethinamate have also been detected in urine.
Therapeutic Concentration In plasma, usually in the range 5 to 10 mg/L.
After a single oral dose of 1 g to 8 subjects, peak blood concentrations of 3.8 to 11.7 mg/L (mean 6.5) were attained in 1 to 2 h [Clifford *et al.* 1974].
Toxicity The estimated minimum lethal dose is 15 g but recovery has occurred after ingestion of as much as 28 g. Prolonged use of ethinamate may lead to dependence of the barbiturate–alcohol type. Blood concentrations >100 mg/L have been associated with fatalities.
Half-life Plasma half-life, about 2 h.
Dose 0.5 to 1 g, as a hypnotic.

Clifford JM *et al.* (1974). Absorption and clearance of secobarbital, heptabarbital, methaqualone, and ethinamate. *Clin Pharmacol Ther* 16: 376–389.
Kleber JW *et al.* (1977). GLC determination of ethinamate and its hydroxy derivative in biological fluids. *J Pharm Sci* 66: 992–994.

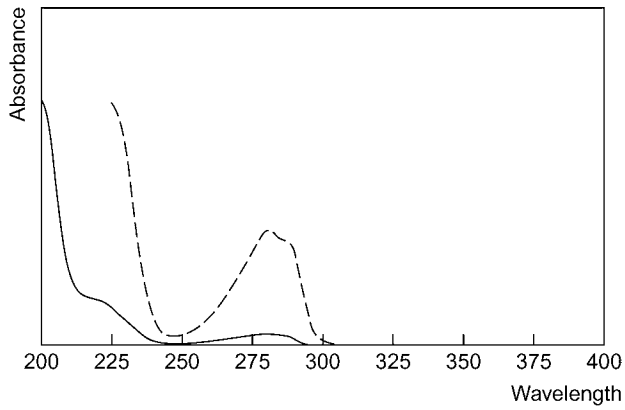
Ethinylestradiol

Oestrogen
$C_{20}H_{24}O_2$ = 296.4
CAS—57-63-6

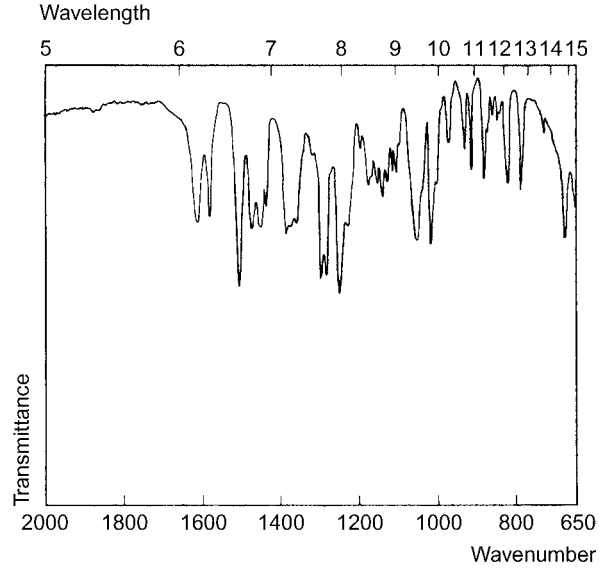
IUPAC Name (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-13-methyl-7,8,9,11,12,14,15, 16-octahydro-6*H*-cyclopenta[*a*]phenanthrene-3,17-diol
Synonyms Ethinyl estradiol; ethinyloestradiol; etinilestradiol; (17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol.
Proprietary Names *Estigyn*; *Estinyl*; *Etifollin*; *Farmacyrol*; *Feminone*; *Gynolett*; *Lynoral*; *Primogyn C*; *Progynon C*; *Turisteron*.



Chemical Properties Ethinylestradiol is an ingredient of many oral contraceptives, see Sweetman [2009]. A fine, white to slightly yellowish-white, crystalline powder. There are two forms, one melts at 141° to 146° and the other at 182° to 184°. Practically insoluble in water; soluble 1 in 5 of acetone, 1 in 4 of dioxane, 1 in 6 of ethanol, 1 in 20 of chloroform and 1 in 4 of ether. Log *P* (octanol/water), 3.7.
Caution Ethinylestradiol is a powerful estrogen. Contact with the skin or inhalation should be avoided.
Colour Tests Ammoniacal silver nitrate—white precipitate/yellow; antimony pentachloride—brown→black; Liebermann's reagent—black; naphthol–sulfuric acid—brown-red/pink; sulfuric acid—red-orange.
Thin-layer Chromatography System TA—R_f 0.90; system TB—R_f 0.04; system TE—R_f 0.71; system TP—R_f 0.72; system TQ—R_f 0.30; system TR—R_f 0.40; system TS—R_f 0.40, streaking may occur; system TAE—R_f 0.86; system TAJ—R_f 0.55; system TAK—R_f 0.58; system TAL—R_f 0.91; system TAM—R_f 0.94.
Gas Chromatography System GA—RI 2719.
High Performance Liquid Chromatography System HX—RI 539.
Ultraviolet Spectrum Ethanol—281 nm (A^1_1=70a).



Infrared Spectrum Principal peaks at wavenumbers 1252, 1505, 1298, 1285, 1020, 1060 cm^{-1} (KBr disk).



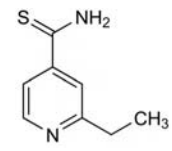
Mass Spectrum Principal ions at *m/z* 213, 160, 159, 296, 133, 145, 212, 157.
Quantification
Plasma **HPLC** Limit of detection, <2 ng/L [Tacey *et al.* 1994].
Radioimmunoassay For method, see Agasan *et al.* [1994].
Serum **Radioimmunoassay** For comparison with GC–MS, see Kuhnz *et al.* [1993].
Disposition in the Body
Therapeutic Concentration Application of a 20 cm^2 contraceptive patch, releasing ethinylestradiol 20 µg and norelgestromin 150 µg daily, to the abdomen, arm, buttock or torso of 37 subjects produced respective mean peak plasma ethinylestradiol concentrations of 58.7, 69.5, 66.3 and 71.2 ng/L at 48 h; corresponding peak plasma concentrations of norelgestromin (at 72 h) were 0.88, 1.18, 1.17 and 1.07 µg/L) [Abrams *et al.* 2002].
Dose 10 to 150 µg daily; up to 2 mg daily may be given.

Abrams LS *et al.* (2002). Pharmacokinetics of a contraceptive patch (Evra/Ortho Evra) containing norelgestromin and ethinyloestradiol at four application sites. *Br J Clin Pharmacol* 53: 141–146.
Agasan AL *et al.* (1994). Development of a radioimmunoassay method for ethynylestradiol in plasma using a monoclonal antibody. *J Immunol Methods* 177: 251–260.
Kuhnz W *et al.* (1993). Radioimmunological analysis of ethinylestradiol in human serum. Validation of the method and comparison with a gas chromatographic/mass spectrometric assay. *Arzneimittelforschung* 43: 16–21.
Sweetman SC, ed. (2009). *Martindale: The Complete Drug Reference*, 36 edn. London: Pharmaceutical Press.
Tacey RL *et al.* (1994). Development of a highly sensitive and specific assay for plasma ethinylestradiol using combined extraction, liquid chromatography and radioimmunoassay. *J Pharm Biomed Anal* 12: 1303–1310.

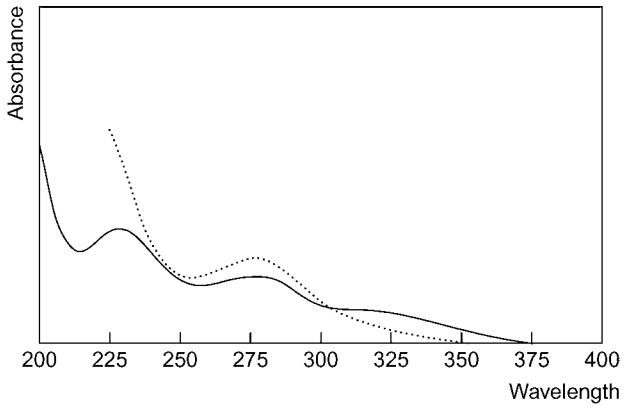
Ethionamide

Antituberculosis
$C_8H_{10}N_2S$ = 166.2
CAS—536-33-4

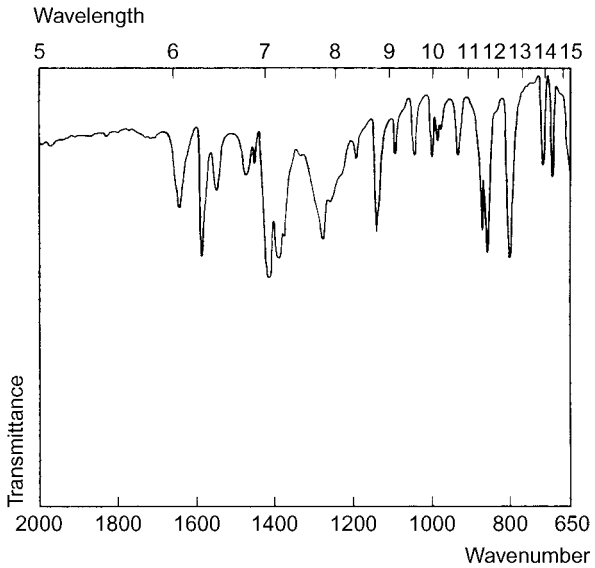
IUPAC Name 2-Ethyl-4-pyridinecarbothioamide
Synonym Etionamida
Proprietary Names *Ethatyl*; *Trescatyl*.



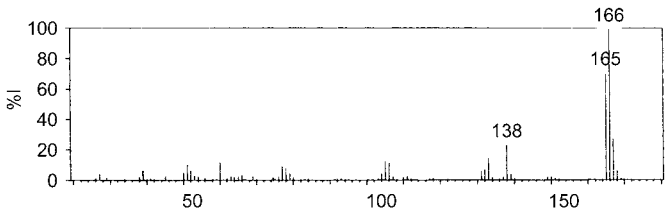
Chemical Properties A bright-yellow crystalline powder, darkening on exposure to light. Mp 158° to 165°. Very sparingly soluble in water; freely soluble in pyridine; soluble 1 in 30 of ethanol, 1 in 45 of acetone, 1 in 350 of chloroform and 1 in 600 of ether; sparingly soluble in methanol. Log *P* (octanol/water), 1.5.
Colour Tests Nessler's reagent—brown-orange; palladium chloride—brown
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.00; system TC—R_f 0.36; system TL—R_f 0.55 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1756.
Ultraviolet Spectrum Aqueous acid—230 (A^1_1=635a), 275 nm; aqueous alkali—277 nm.



Infrared Spectrum Principal peaks at wavenumbers 1588, 808, 865, 1275, 1140, 880 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 166, 165, 167, 138, 133, 105, 60, 106.



Quantification

Plasma **HPLC** UV detection. Limit of detection 10 µg/L [Jenner, Ellard 1981].
HPLC–MS Limit of detection, 0.05 mg/L [Conte *et al.* 2001].

Serum **HPLC** Ethionamide and other antitubercular drugs. Limit of detection, <94 µg/L [Gennaro *et al.* 2001]. For method, see Peloquin *et al.* [1991]. See Plasma [Jenner, Ellard 1981].

Urine **HPLC** See Plasma [Jenner, Ellard 1981].

Bronchoalveolar Lavage **HPLC–MS** Limit of detection, 0.005 mg/L [Conte *et al.* 2001].

Alveolar Cells **HPLC–MS** See Bronchoalveolar Lavage [Conte *et al.* 2001].

Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the body. Peak plasma concentrations are attained 2 to 3 h after a dose. It is extensively metabolised and is excreted in the urine mainly as metabolites with little unchanged drug. The metabolites include ethionamide sulfoxide, 2-ethylisonicotinic acid and 2-ethylisonicotinamide.

Therapeutic Concentration

Administration of a single oral 500 mg dose of ethionamide to 16 subjects resulted in a mean maximum plasma concentration of 2.3 mg/L (range, 0.99 to 6.10) at 1.7 h (0.75 to 3.00) in the fasting state; this was not statistically changed when ethionamide was given with food, orange juice or antacids [Auclair *et al.* 2001].

Dose 0.5 to 1 g daily.

Auclair B *et al.* (2001). Pharmacokinetics of ethionamide administered under fasting conditions or with orange juice, food, or antacids. *Antimicrob Agents Chemother* 45: 810–814.

Conte JE *et al.* (2001). High-performance liquid chromatographic-tandem mass spectrometric method for the determination of ethionamide in human plasma, bronchoalveolar lavage fluid and alveolar cells. *J Chromatogr B Biomed Sci Appl* 753(2): 343–353.

Gennaro MC *et al.* (2001). Ion interaction reagent reversed-phase high-performance liquid chromatography determination of anti-tuberculosis drugs and metabolites in biological fluids. *J Chromatogr B Biomed Sci Appl* 754: 477–486.

Jenner PJ, Ellard GA (1981). High-performance liquid chromatographic determination of ethionamide and prothionamide in body fluids. *J Chromatogr Biomed Appl* 225: 245–251.

Peloquin CA *et al.* (1991). Improved high-performance liquid chromatographic assay for the determination of ethionamide in serum. *J Chromatogr* 563: 472–475.

Ethisterone

Progestational Steroid

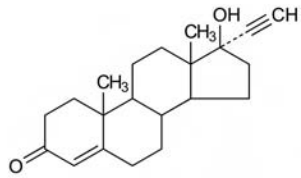
$C_{21}H_{28}O_2 = 312.4$

CAS—434-03-7

IUPAC Name 17-Ethynyl-17-hydroxy-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-one

Synonyms Aethisteron; anhydrohydroxyprogesterone; ethinyltestosterone; 17α-hydroxypregn-4-en-20-yn-3-one; praegnin; pregneninolone; pregnin.

Proprietary Names *Etherone*; *Gestone-Oral*; *Lutocyclin*; *Lutocyclol*; *Ora-Lutin*; *Progesterol*; *Pranone*; *Syngestrotabs*; *Trosinone*.

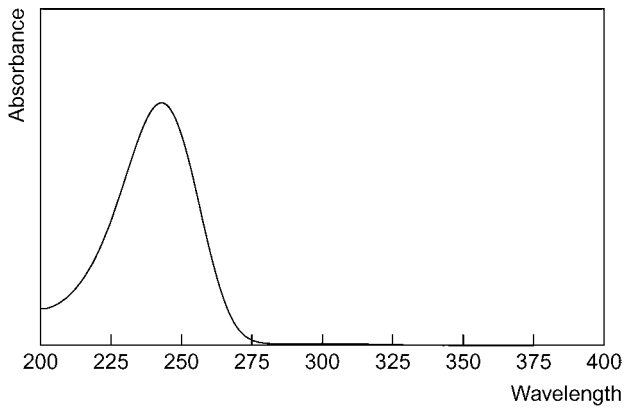


Chemical Properties A white, slightly hygroscopic, crystalline powder. Mp 272° to 276°. Practically insoluble in water; soluble 1 in 1000 of ethanol, 1 in 750 of acetone, 1 in 110 of chloroform, 1 in 3000 of ether and 1 in 35 of pyridine. Log *P* (octanol/water), 3.1.

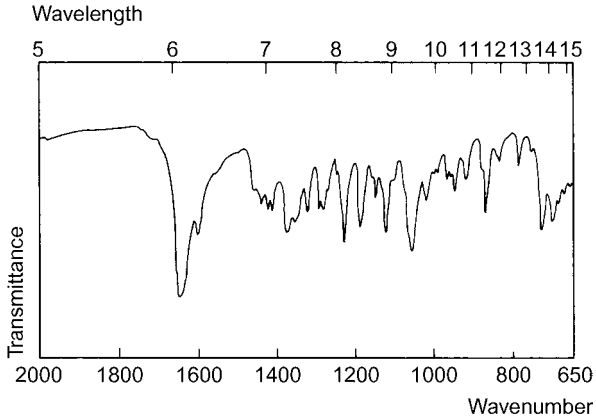
Thin-layer Chromatography System TP—R_f 0.78; system TQ—R_f 0.39; system TR—R_f 0.80; system TS—R_f 0.00.

High Performance Liquid Chromatography System HATb—RRT 0.84 (relative to testosterone).

Ultraviolet Spectrum Dehydrated alcohol—240 nm (A_1^1=520a).



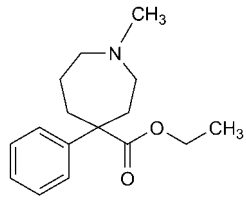
Infrared Spectrum Principal peaks at wavenumbers 1660, 1060, 1235, 1612, 1127, 723 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 122, 121, 91, 147, 161, 43, 107, 120.
Dose 25 to 100 mg daily.

Ethoheptazine

Narcotic Analgesic
$C_{16}H_{23}NO_2$ = 261.4
CAS—77-15-6
IUPAC Name Ethylhexahydro-1-methyl-4-phenyl-1*H*-azepine-4-carboxylate
Synonym Heptacyclazine

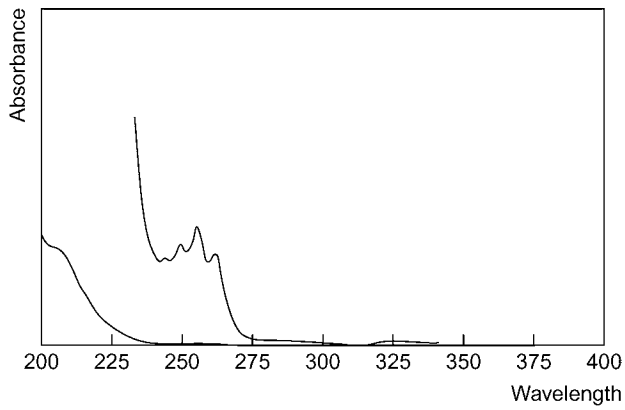


Chemical Properties Liquid. pK_a 8.5. Log *P* (octanol/water), 3.5.

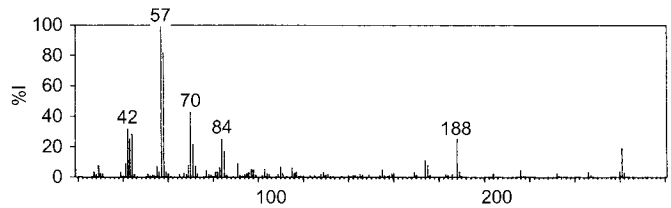
Ethoheptazine Citrate

$C_{16}H_{23}NO_2,C_6H_8O_7$ = 453.5
CAS—6700-56-7
Proprietary Names *Zactane*. It is an ingredient of *Equagesic*.
Chemical Properties White powder. Mp ≈140°. Soluble in water and ethanol; practically insoluble in ether.

Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.40; system TAE—R_f 0.12; system TAF—R_f 0.41; system TL—R_f 0.04; system TB—R_f 0.45; system TC—R_f 0.19; system TE—R_f 0.55 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, orange; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1857; system GB—RI 1923; system GAS—RI 1882; system GC—RI 1630; system GF—RI 2110.
High Performance Liquid Chromatography System HA—*k* 3.3; system HC—*k* 1.55; system HX—RI 359.
Ultraviolet Spectrum Aqueous acid—251, 257 nm (A_1^1 = 8c), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1727, 1193, 1094, 695, 1248, 1030 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 57, 58, 70, 42, 44, 188, 84, 43.



Quantification

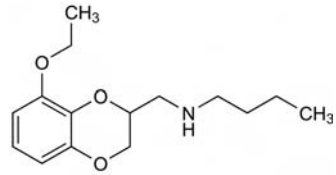
Blood **GC** Column: 3% OV-17 on Chromosorb W HP (2 m × 3.2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 225°. FID. Limit of detection, 1.0 mg/L [Drost *et al.* 1983].
Brain **GC** See Blood [Drost *et al.* 1983].
Kidney **GC** See Blood [Drost *et al.* 1983].
Liver **GC** See Blood [Drost *et al.* 1983].
Spleen **GC** See Blood [Drost *et al.* 1983].

Disposition in the Body Readily absorbed after oral administration; peak blood concentrations are attained within 1 h of a dose. It is extensively metabolised and excreted in the urine.
Toxicity The estimated minimum lethal dose is 1 g.
In a fatality caused by the ingestion of an unknown amount of ethoheptazine, the following postmortem concentrations were reported: blood, 15 µg/g; brain, 4.5 µg/g; kidney, 2.4 µg/g; liver, 10.0 µg/g; spleen, 3.1 µg/g [Drost *et al.* 1983].
Dose 225 to 600 mg of ethoheptazine citrate daily.

Drost RH *et al.* (1983). Determination of ethoheptazine in human post mortem material. *J Chromatogr* 277: 352–355.

Ethomoxane

Tranquilliser
$C_{15}H_{23}NO_3$ = 265.4
CAS—3570-46-5
IUPAC Name *N*-Butyl-8-ethoxy-1,4-benzodioxan-2-ylmethylamine

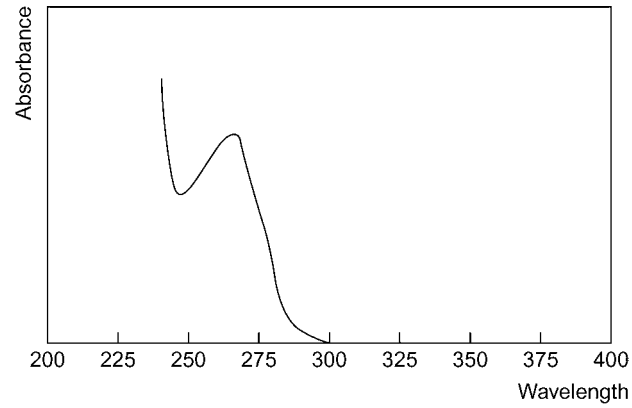


Chemical Properties A liquid.

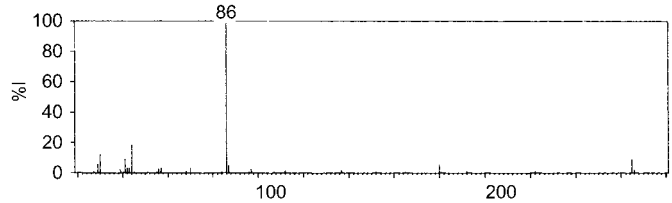
Ethomoxane Hydrochloride

$C_{15}H_{23}NO_3$,HCl = 301.8
CAS—6038-78-4
Chemical Properties Crystals. Mp 196° to 197°.

Colour Tests Mandelin's test—red-brown; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.34; system TC—R_f 0.47; system TL—R_f 0.36 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1975.
Ultraviolet Spectrum Aqueous acid—267 nm (A_1^1=23.5a).



Infrared Spectrum Principal peaks at wavenumbers 1120, 1279, 1594, 1493, 1097, 1252 cm^{-1} (KBr disk).

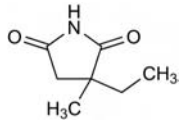


Mass Spectrum Principal ions at *m/z* 86, 44, 30, 265, 41, 180, 29, 87.

Ethosuximide

Anticonvulsant
$C_7H_{11}NO_2$ = 141.2
CAS—77-67-8
IUPAC Name 3-Ethyl-3-methyl-2,5-pyrrolidinedione
Synonym Ethosuccimide

Proprietary Names *Emeside*; *Ethymal*; *Petinimid*; *Petnidan*; *Pyknolepsinum*; *Simatin*; *Suxilep*; *Suxinutin*; *Zarondan*; *Zarontin*.



Chemical Properties A white powder or waxy solid. Mp 46° to 52°. Soluble 1 in 4.5 of water and 1 in less than 1 of ethanol, chloroform and ether. pK_a 9.5 (20°). Log *P* (octanol/water), 0.4. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].

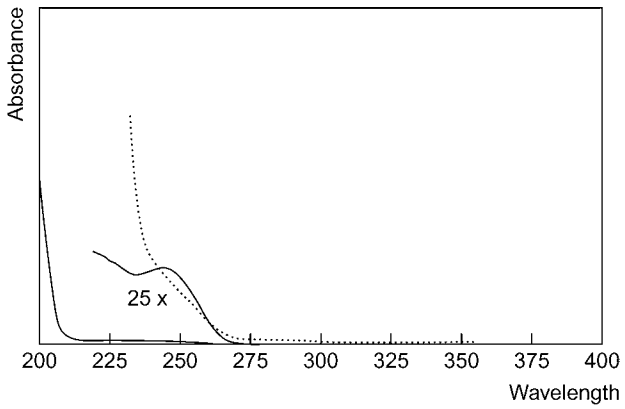
Colour Test Koppanyi–Zwikker test—violet.

Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.05; system TD—R_f 0.50; system TE—R_f 0.66; system TF—R_f 0.53; system TAD—R_f 0.59; system TAE—R_f 0.84.

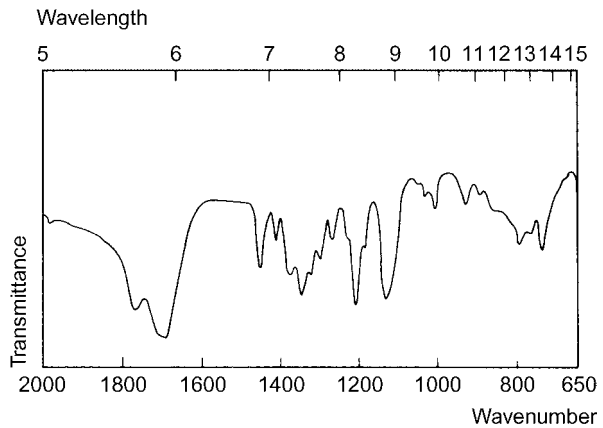
Gas Chromatography System GA—ethosuximide RI 1205, M (3-OH-) RI 1322, M (OH-ethyl-) RI 1370, M (oxo-) RI 1270; system GB—ethosuximide RI 1258, M (OH-ethyl-) RI 1436, M (3-OH-) RI 1395; system GE—ethosuximide RRT 0.18 (relative to phenytoin); system GAJ—ethosuximide RRT 0.453 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HE—*k* 0.91; system HX—RI 301; system HY—RI 276; system HZ—retention time 2.3 min; System HAA—retention time 10.5 min.

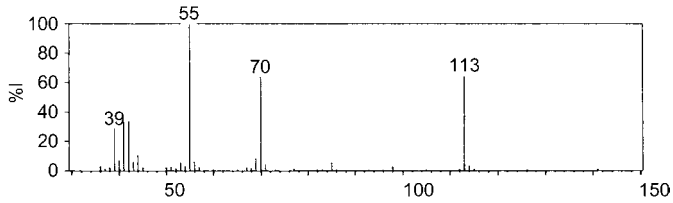
Ultraviolet Spectrum Aqueous acid—244 nm (A_1^1=9.9b).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1777, 1208, 1130, 1303, 730 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 113, 70, 55, 42, 41, 39, 85, 69; M (OH-ethyl-) 113, 85, 98, 69, 71, 142; M (3-OH-) 71, 86, 129, 139, 142, 157; M (oxo-) 70, 155, 55, 113, 69, 98.



Quantification

Blood **GC–MS** For method for quantification of ethosuximide and other antiepileptic drugs, see Speed *et al.* [2000].

Plasma **GC** ECD. Limit of detection, 500 µg/L [Wallace *et al.* 1979]. FID. Limit of detection, 2.5 mg/L, ethosuximide and phensuximide [van der Kleijn *et al.* 1973].

GC–MS Limit of detection, <2.5 mg/L, ethosuximide enantiomers [Sghendo *et al.* 2002].

HPLC Limit of detection, about 0.9 µmol/L [Chen *et al.* 1999]. UV detection. For method for quantification of ethosuximide and other antiepileptic drugs, see Matar *et al.* [1999]. UV detection. Limit of detection, about 2 mg/L, ethosuximide and other anticonvulsants [Christofides, Fry 1980].

Serum **GC** See Plasma [Wallace *et al.* 1979].

HPLC See Plasma [Christofides, Fry 1980]. UV detection. Limit of detection, 1 mg/L, ethosuximide and other anticonvulsants [Kabra *et al.* 1978].

Urine **GC** See Plasma [van der Kleijn *et al.* 1973].

GC–MS See Plasma [Sghendo *et al.* 2002].

Liver **GC–MS** See Blood [Speed *et al.* 2000].

Disposition in the Body Readily absorbed after oral administration. Metabolised by hydroxylation to 3-hydroxyethosuximide and 1-hydroxyethylethosuximide; the latter is further oxidised to produce 2-acetyl-2-methylsuccinimide; an additional metabolite, 2-carboxymethyl-2-methylsuccinimide has also been reported. None of the metabolites is pharmacologically active. About 25% of a dose is excreted in the urine in 24 h as 1-hydroxyethylethosuximide glucuronide and about 14% as the unconjugated metabolite. Up to about 20% of a dose is excreted as unchanged drug in the urine over a 9 day period.

Therapeutic Concentration In plasma, usually in the range 40 to 100 mg/L.

After a single oral dose of 500 mg to 5 children, peak plasma concentrations of 28 to 51 mg/L (mean 39) were attained in 3 to 7 h [Buchanan *et al.* 1969].

Steady-state serum concentrations of 31 to 68 mg/L (mean 49) were reported in 5 young adults receiving daily oral doses of 1 g [Solow, Green 1971].

Toxicity The estimated minimum lethal dose is 5 g. Toxic effects may be observed at plasma concentrations greater than about 100 mg/L.

In a fatality involving ethosuximide, the following postmortem tissue concentrations were reported: blood 250 mg/L, liver 280 µg/g, urine 120 mg/L; phenobarbital was also detected [Rousseau *et al.* 1980].

Half-life Plasma half-life, 40 to 60 h in adults and about 30 h in children.

Volume of Distribution About 0.7 L/kg.

Saliva Plasma : saliva ratio, about 1.1.

Protein Binding Not significantly bound.

Dose 0.5 to 2 g daily.

Buchanan RA *et al.* (1969). Absorption and elimination of ethosuximide in children. *J Clin. Pharmacol* 9: 393–398.

Chen SH *et al.* (1999). Trace analysis of ethosuximide in human plasma with a chemically removable derivatizing reagent and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 729: 111–117.

Christofides JA, Fry DE (1980). Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin. Chem* 26: 499–501.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Kabra PM *et al.* (1978). *J Anal Toxicol* 2: 127–133.

Matar KM *et al.* (1999). Liquid chromatographic determination of six antiepileptic drugs and two metabolites in microsamples of human plasma. *Ther Drug Monit* 21: 559–566.

Rousseau M *et al.* (1980). *Bull Int Assoc Forensic Toxicol* 15(2): 5–6.

Sghendo L *et al.* (2002). A sensitive gas chromatographic/mass spectrometric method for the resolution and quantification of ethosuximide enantiomers in biological fluids. *J Chromatogr B Anal Technol Biomed Life Sci* 772: 307–315.

Solow EB, Green JB (1971). The determination of ethosuximide in serum by gas chromatography. Preliminary results of clinical application. *Clin Chim Acta* 33: 87–90.

Speed DJ *et al.* (2000). Analysis of six anticonvulsant drugs using solid-phase extraction, deuterated internal standards, and gas chromatography-mass spectrometry. *J Anal Toxicol* 24: 685–690.

van der Kleijn E *et al.* (1973). Gas chromatographic determination of ethosuximide and phensuximide in plasma and urine of man. *J Pharm Pharmacol* 25: 324–327.

Wallace JE *et al.* (1979). Electron-capture gas-liquid chromatographic determination of ethosuximide and desmethylmethsuximide in plasma or serum. *Clin Chem* 25: 252–255.

Ethotoin

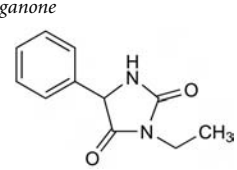
Anticonvulsant

$C_{11}H_{12}N_2O_2$ = 204.2

CAS—86-35-1

IUPAC Name 3-Ethyl-5-phenyl-2,4-imidazolidinedione

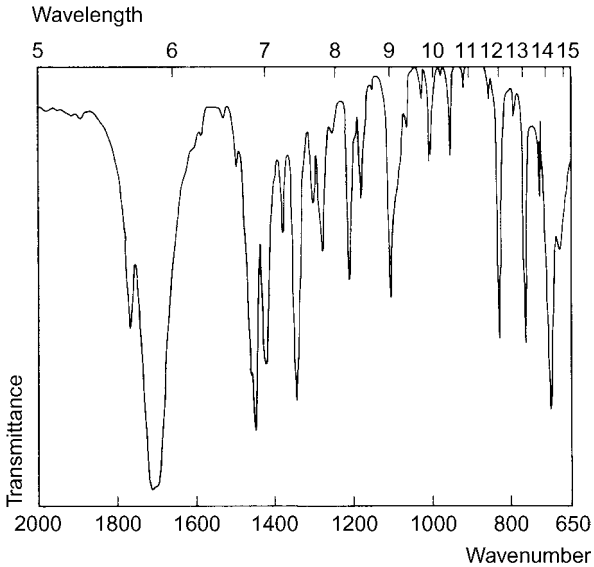
Proprietary Name *Peganone*



Chemical Properties A white crystalline powder. Mp about 90°. Sparingly soluble in cold water, more soluble in hot water; soluble 1 in 4 of dehydrated alcohol, 1 in 1.5 of chloroform and 1 in 25 of ether; freely soluble in benzene and dilute aqueous solutions of alkali hydroxides. pK_a 8.5. Log *P* (octanol/water), 1.0.

Thin-layer Chromatography System TA—R_f 0.88; system TD—R_f 0.53; system TE—R_f 0.71; system TF—R_f 0.54; system TAD—R_f 0.60; system TAJ—R_f 0.61; system TAK—R_f 0.66; system TAL—R_f 0.91.

Gas Chromatography System GA—RI 1800; system GB—RI 1751; system GE—RRT 0.57(relative to phenytoin); system GAJ—RRT 0.940(relative to methylphenobarbital).
High Performance Liquid Chromatography System HE—*k* 2.81.
Ultraviolet Spectrum Dehydrated alcohol—259 (A^{1}_{1}=11b), 265 nm (A^{1}_{1}=8b).
Infrared Spectrum Principal peaks at wavenumbers 1716, 700, 760, 820, 1780, 1100 cm^{-1} (KBr disk).

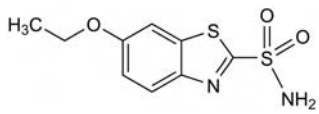


Mass Spectrum Principal ions at *m/z* 104, 105, 204, 77, 78, 133, 51, 132.
Quantification
Serum **GC** FID. Limit of detection, 1 mg/L [Larsen, Naestoft 1974].
Urine **GC–MS** Limit of detection, 5 mg/L, ethotoin and some metabolites [Naestoft, Larsen 1977].
Disposition in the Body Readily absorbed after oral administration. Extensively metabolised by *N*-desethylation and ring cleavage, 5-hydroxylation and *p*-hydroxylation of the phenyl ring. During chronic therapy, <5% of the dose is excreted in the urine in 24 h as unchanged drug, together with 5 to 14% of the dose as desethylethotoin, 17 to 35% as 5-hydroxyethotoin, 14 to 32% as conjugated *p*-hydroxyethotoin and small amounts of other phenyl-substituted oxidation products. 2-Phenylhydantoic acid may account for about 10% of the dose.
Therapeutic Concentration In plasma, usually in the range 6 to 20 mg/L.
Following single oral doses of 500, 1500 and 2500 mg to 5 subjects, peak plasma concentrations of 9 to 15 mg/L (mean 12), 30 to 59 mg/L (mean 42) and 37 to 75 mg/L (mean 54), respectively, were attained in about 1.5, 3.5 and 3.5 h [Meyer *et al.* 1983].
During daily oral dosing with 30 mg/kg to 7 subjects, minimum steady-state plasma concentrations of 4.5 to 14 mg/L (mean 10) were reported; following daily oral treatment with 60 mg/kg to 6 subjects, minimum steady-state plasma concentrations of 14 to 50 mg/L (mean 30) were reported [Sjö *et al.* 1975].
Toxicity The estimated minimum lethal dose is 5 g.
Half-life Plasma half-life, 3 to 11 h (dose-dependent).
Dose Initially 1 g daily, increasing to 2 to 3 g daily.

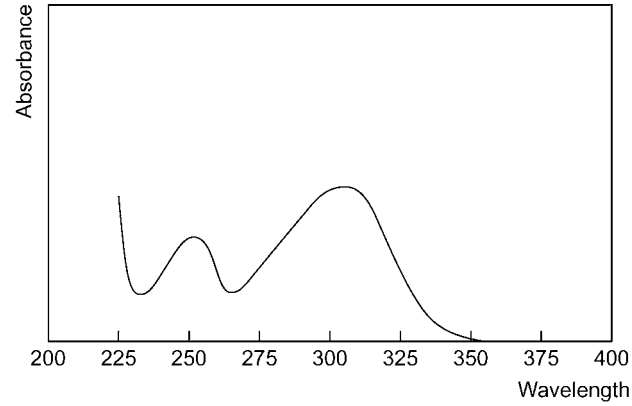
Larsen NE, Naestoft J (1974). *J Chromatogr* 92: 157–161.
Meyer MC *et al.* (1983). Nonlinear ethotoin kinetics. *Clin Pharmacol Ther* 33: 329–334.
Naestoft J, Larsen NE (1977). Mass fragmentographic quantitation of ethotoin and some of its metabolites in human urine. *J Chromatogr Biomed Appl* 143: 161–169.
Sjö *et al.* (1975). Dose-dependent kinetics of ethotoin in man. *Clin Exp Pharmacol Physiol* 2(3): 185–192.

Ethoxzolamide

Carbonic Anhydrase Inhibitor, Diuretic
$C_9H_{10}N_2O_3S_2$ = 258.3
CAS—452-35-7
IUPAC Name 6-Ethoxy-2-benzothiazolesulfonamide
Synonym Ethoxyzolamide
Proprietary Names *Cardrase*; *Ethamide*; *Glaucotensil*; *Redupresin*.



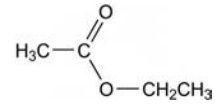
Chemical Properties A white or slightly yellow crystalline powder. Mp 189° to 195°. Practically insoluble in water; slightly soluble in ethanol, chloroform and ether. pK_a 8.1. Log *P* (octanol/pH 7.4), 2.2.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—yellow; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.76; system TD—R_f 0.43; system TE—R_f 0.43; system TF—R_f 0.65; system TAD—R_f 0.51; system TAJ—R_f 0.51; system TAK—R_f 0.48; system TAL—R_f 0.91 (Marquis reagent, positive).
Gas Chromatography System GA—RI 2578.
Ultraviolet Spectrum Aqueous acid—253, 304 nm (A^{1}_{1}=96a).



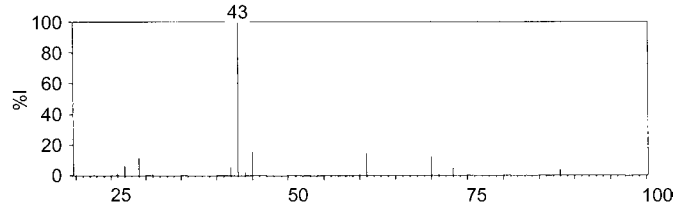
Infrared Spectrum Principal peaks at wavenumbers 1170, 1490, 1263, 824, 1224, 1036 cm^{-1} (KBr disk).
Dose In the treatment of glaucoma, 250 to 500 mg daily.

Ethyl Acetate

Flavouring Agent, Solvent
$C_4H_8O_2$ = 88.10
CAS—141-78-6
Synonym Acetic ether

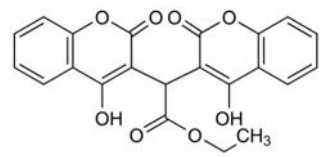


Chemical Properties A colourless flammable liquid. Sp. gr. 0.894 to 0.898. Bp 77°. Soluble 1 in 15 of water; miscible with acetone, ethanol, chloroform and ether. Log *P* (octanol/water), 0.7.
Gas Chromatography System GA—RI 596; system GI—retention time 9.4 min.
Mass Spectrum Principal ions at *m/z* 43, 61, 45, 70, 29, 27, 73, 42.



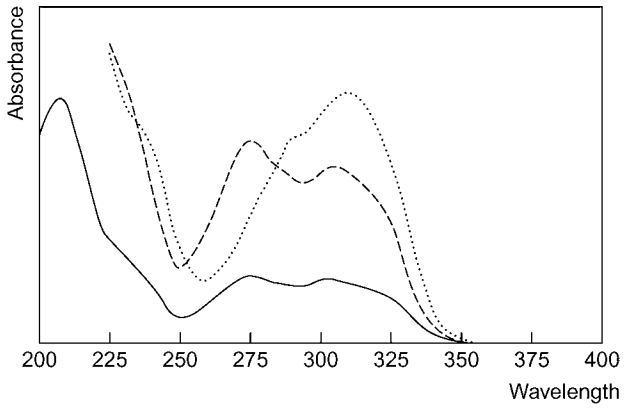
Ethyl Biscoumacetate

Anticoagulant
$C_{22}H_{16}O_8$ = 408.4
CAS—548-00-5
IUPAC Name Ethyl 2,2-bis(2-hydroxy-4-oxochromen-3-yl)acetate
Synonyms Aethylis biscoumacetas; BOEA; ethyldicoumarol; ethyl-4-hydroxy-α-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)-2-oxo-2*H*-1-benzopyran-3-acetate; neodicumarinum.
Proprietary Names *Pelentan*; *Stabilène*; *Tromexan*.

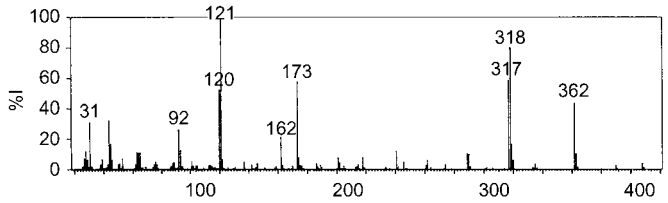


Chemical Properties A white to yellowish-white, fine crystalline powder. There are two forms, one melts at 154° to 157° and the other at 177° to 182°. Practically insoluble in water; soluble 1 in 20 of acetone; slightly soluble in alcohol and ether; soluble in chloroform. pK_a 3.1. Log *P* (octanol/water), 1.9.

Colour Test Sulfuric acid—orange-yellow.
Thin-layer Chromatography System TD—R_f 0.04; system TE—R_f 0.24; system TF—R_f 0.32; system TAD—R_f 0.21 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Water—275 (A^1_1=449b), 303 nm; aqueous alkali—311 nm (A^1_1=622b).



Infrared Spectrum Principal peaks at wavenumbers 1653, 1600, 768, 1560, 754, 1730 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 121, 318, 317, 173, 120, 362, 44, 31.

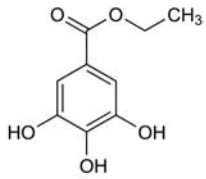


Quantification
Plasma **HPLC** UV detection. Limit of detection, 100 µg/L [Arman, Jamali 1983].
Serum **HPLC–MS** For method for quantification of ethyl biscoumacetate and its metabolite 7-hydroxyethyl bicoumacetate, see Pospisil *et al.* [1992].
Disposition in the Body Readily absorbed after oral administration and extensively metabolised. About 5 to 15% of a dose is excreted in the urine as hydroxyethyl biscoumacetate and only traces are excreted unchanged.
Therapeutic Concentration
Following a single oral dose of 300 mg to 4 subjects, peak plasma concentrations of 10.2 to 12.4 mg/L (mean 11.2) were attained in 1 to 1.5 h [Arman, Jamali 1983].
After a single 300 mg oral dose of ethyl biscoumacetate to 10 subjects, peak plasma concentrations of 4.46 mg/L were reached in 1.62 h; equivalent values for the main metabolite, 7-hydroxyethyl biscoumacetate, were 12.74 mg/L in 3.29 h [Perlik, Patzelova 1994].
Half-life Plasma half-life, about 1 to 2 h.
Protein Binding About 90%.
Dose Maintenance, 300 to 600 mg daily.

Arman M, Jamali F (1983). High-performance liquid chromatographic determination of ethyl biscoumacetate in human plasma. *J Chromatogr Biomed Appl* 272: 406–410.
Perlik F, Patzelova V (1994). Pharmacokinetics of ethyl biscoumacetate and its metabolite 7-hydroxy ethyl biscoumacetate in healthy volunteers. *Int J Clin Pharmacol Ther* 32: 622–624.
Pospisil J *et al.* (1992). Determination of ethyl biscoumacetate and its metabolite 7-hydroxyethyl biscoumacetate in human serum by high-performance liquid chromatography and mass spectrometry. *J Chromatogr* 574: 71–75.

Ethyl Gallate

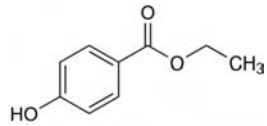
Antioxidant
$C_9H_{10}O_5$ = 198.2
CAS—831-61-8
IUPAC Name Ethyl-3,4,5-trihydroxybenzoate
Proprietary Name *Progallin A*



Chemical Properties A white to creamy-white crystalline powder. Mp 151° to 154°. Soluble 1 in 500 of water, 1 in 3 of ethanol, 1 in 3 of ether and 1 in 3 of propylene glycol. Log *P* (octanol/water), 1.3.
Colour Test Ferric chloride—violet-black→blue-black.
Ultraviolet Spectrum Methanol—275 nm (A^1_1=540b).

Ethyl Hydroxybenzoate

Preservative
$C_9H_{10}O_3$ = 166.2
CAS—120-47-8
IUPAC Name Ethyl-4-hydroxybenzoate
Synonyms Aethylum hydroxybenzoicum; ethylis paraoxybenzoas; ethylparaben.
Proprietary Names *Nipagin A*; *Solbrol A*.



Chemical Properties Colourless crystals or white crystalline powder. Mp 116°. Soluble 1 in 1500 of water, 1 in 2 of ethanol, 1 in 1.2 of acetone, 1 in 10 of chloroform and 1 in 3.5 of ether. pK_a 8.3 (25°). Log *P* (octanol/water), 2.5.

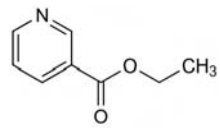
Sodium Ethyl Hydroxybenzoate

$C_9H_9NaO_3$ = 188.2
CAS—35285-68-8
Proprietary Name *Nipagin A Sodium*
Chemical Properties A white hygroscopic crystalline powder. Soluble 1 in 2 of water and 1 in 3 of ethanol (50%).

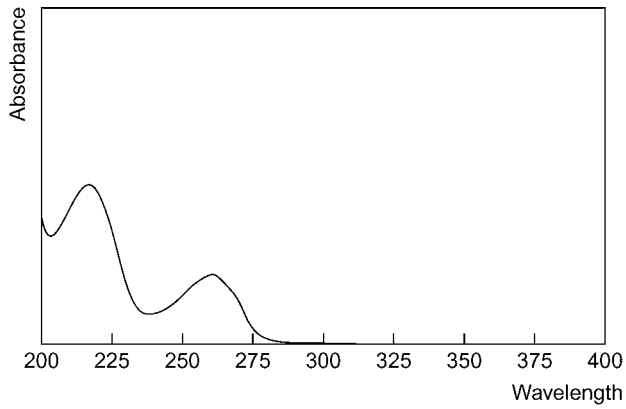
Colour Test Millon's reagent—red.
Thin-layer Chromatography System TD—R_f 0.64; system TE—R_f 0.46; system TF—R_f 0.66; system TAD—R_f 0.57; system TAE—R_f 0.88.
Gas Chromatography System GA—RI 1580.
Ultraviolet Spectrum Aqueous acid—254 nm (A^1_1=956b); aqueous alkali—295 nm (A^1_1=1390b).
Infrared Spectrum Principal peaks at wavenumbers 1290, 1673, 1170, 1240, 1610, 1590 cm^{-1} (KBr disk).

Ethyl Nicotinate

Vasodilator (Topical)
$C_8H_9NO_2$ = 151.2
CAS—614-18-6
IUPAC Name Ethyl pyridine-3-carboxylate
Proprietary Names It is an ingredient of *PR Heat Spray*, *Thermal*, *Thermocutan*, *Transvane* and *Transvasin*.



Chemical Properties A liquid. Mp 8° to 9°. Bp 225°. Very soluble in water, ethanol and ether. pK_a 3.4 (20°). Log *P* (octanol/water), 1.3.
Thin-layer Chromatography System TA—R_f 0.00 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—261 nm (A^1_1=341a).

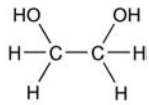


Infrared Spectrum Principal peaks at wavenumbers 1286, 1724, 1110, 740, 1591, 1025 cm^{-1} (KBr disk).
Use Topically in concentrations of 1 to 2%.

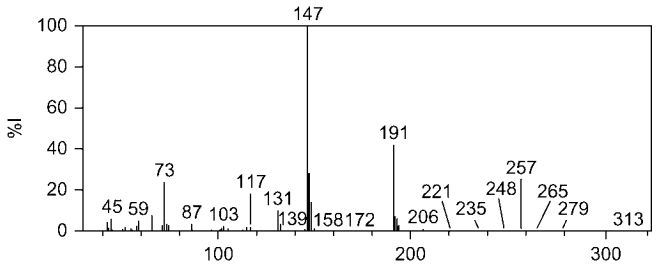
Ethylene Glycol

Antifreeze, Solvent
$C_2H_6O_2 = 62.07$
CAS—107-21-1

IUPAC Name Ethane-1,2-diol
Synonyms 1,2-Ethanediol; ethylene alcohol; glycol.



Chemical Properties A colourless, hygroscopic, syrupy liquid. Weight per volume ~1.114 g/mL. Fp ~-13°. Bp ~197°. Miscible with water, glycerol, acetic acid, ethanol and acetone; slightly soluble in ether; practically insoluble in benzene. pK_a 15.0. Log *P* (octanol/water), −1.4.
Gas Chromatography System GA—RI 798; system GI—RT 17.0 min (tailing peak).
Mass Spectrum Principal ions at *m/z* 147, 191, 73, 131, 45, 59, 87, 117.



Quantification

Blood **GC** Column: Chrompack CP Wax 57-CB (25 m × 0.32 mm i.d., 0.2 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 110° to 130° at 6°/min for 0.5 min to 250° at 30°/min for 2 min. FID. Limit of detection, 0.05 g/L [Jonsson *et al.* 1989].

GC-MS Column: 5% diphenylpolysiloxane, 95% dimethyl polysiloxane Rxi-5 ms (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 1 min to 125° at 25°/min to 160° at 5°/min to 220° at 25°/min. EI ionisation. Limit of detection, 25 mg/L for ethylene glycol and glycolic acid [Rosano *et al.* 2009]. Column: DB-5MS 5% phenylsiloxane, 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min Temperature programme: 80° for 1 min to 140° at 5°/min to 240° at 40°/min for 5 min. EI ionisation, scan mode. Limit of quantification, 1.3 mg/L, limit of detection, 0.7 mg/L [Gembus *et al.* 2002].

Plasma **GC** Column: Porapak Q 50/80 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 100° to 210° at 2°/min. Limit of detection, 0.4 µmol/L [Cheung, Lin 1987]. Column: 3% OV-25 on 100/200 mesh Chromasorb W (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 70°. FID. Retention time: 1.8 min. Limit of detection, 50 mg/L [Robinson, Reive 1981].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 36 cm/min. Temperature programme: 50° for 1 min to 100° at 10°/min to 230° at 40°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.39 mg/L, limit of detection, 0.12 mg/L, for ethylene glycol [Van Hee *et al.* 2004]. Column: HP capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 200° at 10°/min. EI ionisation at 70 eV. Limit of quantification, 0.1 g/L, limit of detection, 0.01 g/L [Maurer, Kessler 1988; Maurer *et al.* 2001].

Serum **Spectrofluorimetry** Limit of detection, 50 mg/L [Meola *et al.* 1980].

GC Column: Rtx-200 capillary (30 m × 0.53 mm i.d., 3 µm). Carrier gas: he, 80.1 mL/min. Temperature programme: 40° for 1 min to 260° at 70°/min. FID. Retention time: 2.59 min. Limit of detection, 10 mg/L [Williams *et al.* 2000]. Column 1: Packed column (1.8 m × 2 mm) packed with 80/100 HayeSep R. Limit of detection, 2 mmol/L. Column 2: wide-bore capillary Rtx-200 (Restek) (30 m × 0.53 mm i.d., 3 µm). Carrier gas: N_2, 45 mL/min. Temperature programme: 90° to 205° at 10°/min for 2 min. Limit of detection, 0.1 mmol/L [Livesey *et al.* 1995].

GC-MS See Plasma [Van Hee *et al.* 2004]. See Blood [Gembus *et al.* 2002]. Column: Ultra-2 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 80° for 0.5 min to 225° at 25°/min. SIM acquisition mode. Limit of quantification, 10 mg/L for ethylene glycol and glycolic acid [Porter *et al.* 1999].

Urine **GC-MS** See Plasma [Van Hee *et al.* 2004].

Note For a review of assay methods see Doedens [1983].

Disposition in the Body Ethylene glycol is metabolised initially to glycoaldehyde and subsequently to lactic acid and oxalic acid. Calcium oxalate crystals are deposited in the kidneys and some oxalate may be excreted in the urine together with unchanged ethylene glycol.

Toxicity Ethylene glycol itself is probably non-toxic and the serious toxic effects are caused by the metabolites. In adults, the minimum lethal dose is ~100 mL, although survival has been reported after ingestion of 250 mL. Toxic effects are usually associated with plasma concentrations greater than 500 mg/L. The maximum permissible atmospheric concentration of ethylene glycol vapour is 100 ppm.

A 51-year-old man ingested 600 mL of antifreeze solution but recovered after treatment. A blood concentration of 6500 mg/L ethylene glycol was reported 2.5 h after ingestion; pharmacokinetic studies indicated that the volume of distribution of ethylene glycol was ~0.8 L/kg and the plasma half-life was ~3 h [Peterson *et al.* 1981].

A 36-year-old man was admitted to hospital 6 h after ingesting 500 mL of an antifreeze mixture containing ethylene glycol and methanol; initial serum concentrations were 1900 mg/L for ethylene glycol and 1130 mg/L for methanol; the subject recovered after treatment with ethanol and haemodialysis [Vites *et al.* 1984].

A 27-year-old man ingested 2.25 L of Prestone antifreeze in a suicide attempt. His serum ethylene glycol concentration was 995 mg/L [Eder *et al.* 1998].

A 58-year-old man ingested ~56 mL of half-strength antifreeze. His serum ethylene glycol concentration was 7.91 g/L on admission [Eder *et al.* 1998].

The plasma ethylene glycol concentration in a 36-year-old man who ingested ~3 L of ethylene glycol antifreeze in a suicide attempt was 18.89 g/L. Despite this very high level, the man survived after haemodialysis and ethanol infusion [Johnson *et al.* 1999].

Five hours after ingesting an unknown amount of ethylene glycol, the plasma concentration in a 23-year-old man was 1162 mg/L. The man died 27 h after ingestion. Just before death, the plasma ethylene glycol concentration was 359 mg/L and concentrations in postmortem tissue homogenates 70 h after death were: right and left kidney 227.6 and 258.5 mg/L, respectively; right and left lung 310 and 281.7 mg/L, respectively; heart 57.6 mg/L; liver 299.8 mg/L; spleen 310 mg/L; brain 134.5 mg/L; gastric contents 859.2 mg/L; bile 785.6 mg/L; pleural fluid 700.7 mg/L; and pancreas not detected [Hantson *et al.* 2002].

In a fatal case of ethylene glycol poisoning, in which it was estimated that the subject consumed 1034 g, the concentrations in blood, urine and vitreous humour were 23.4, 22.61 and 10.28 g/L, respectively [Garg *et al.* 2009].

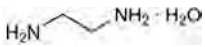
In 12 fatalities involving ethylene glycol poisoning, levels in postmortem blood ranged from 58–7790 mg/L (mean, 1830). Glycolic acid levels were 810–1770 mg/L (mean, 1360) [Rosano *et al.* 2009].

For other cases of ethylene glycol poisoning, see Jorens [2009] and Lovric *et al.* [2007]. For a review of ethylene glycol poisoning, see Leth, Gregersen [2005]; for review of the management of ethylene glycol poisoning, see Brent [2001] and Scalley *et al.* [2002]. For a rapid qualitative test for suspected ethylene glycol poisoning, see Long *et al.* [2008].

Brent J (2001). Current management of ethylene glycol poisoning. *Drugs* 61: 979–988.
Cheung ST, Lin WN (1987). Simultaneous determination of methanol, ethanol, acetone, isopropanol and ethylene glycol in plasma by gas chromatography. *J Chromatogr* 414: 248–250.
Doedens DJ (1983). Methods for the determination of ethylene glycol. *Vet Hum Toxicol* 25: 96–101.
Eder AF *et al.* (1998). Ethylene glycol poisoning: toxicokinetic and analytical factors affecting laboratory diagnosis. *Clin Chem* 44: 168–177.
Garg U *et al.* (2009). A fatal case involving extremely high levels of ethylene glycol without elevation of its metabolites or crystalluria. *Am J Forensic Med Pathol* 30: 273–275.
Gembus V *et al.* (2002). Determination of glycols in biological specimens by gas chromatography–mass spectrometry. *J Anal Toxicol* 26: 280–285.
Hantson P *et al.* (2002). Determination of ethylene glycol tissue content after fatal oral poisoning and pathologic findings. *Am J Forensic Med Pathol* 23: 159–161.
Johnson B *et al.* (1999). Emergency department hemodialysis in a case of severe ethylene glycol poisoning. *Ann Emerg Med* 33: 108–110.
Jonsson JA *et al.* (1989). Determination of ethylene glycol in postmortem blood by capillary gas chromatography. *J Anal Toxicol* 13: 25–26.
Jorens PG (2009). Ethylene glycol poisoning and lactate concentrations. *J Anal Toxicol* 33: 395.
Leth PM, Gregersen M (2005). Ethylene glycol poisoning. *Forensic Sci Int* 155: 179–184.
Livesey JF *et al.* (1995). Simultaneous determination of alcohols and ethylene glycol in serum by packed- or capillary-column gas chromatography. *Clin Chem* 41: 300–305.
Long H *et al.* (2008). A rapid qualitative test for suspected ethylene glycol poisoning. *Acad Emerg Med* 15: 688–690.
Lovric M *et al.* (2007). Ethylene glycol poisoning. *Forensic Sci Int* 170: 213–215.
Maurer H, Kessler C (1988). Identification and quantification of ethylene glycol and diethylene glycol in plasma using gas chromatography–mass spectrometry. *Arch Toxicol* 62: 66–69.
Maurer HH *et al.* (2001). Validated gas chromatographic-mass spectrometric assay for determination of the antifreezes ethylene glycol and diethylene glycol in human plasma after microwave-assisted pivalylation. *J Chromatogr B Biomed Sci Appl* 754: 401–409.
Meola JM *et al.* (1980). Fluorometry of ethylene glycol in serum. *Clin Chem* 26: 1709.
Peterson CD *et al.* (1981). Ethylene glycol poisoning: pharmacokinetics during therapy with ethanol and hemodialysis. *N Engl J Med* 304: 21–23.
Porter WH *et al.* (1999). Simultaneous determination of ethylene glycol and glycolic acid in serum by gas chromatography–mass spectrometry. *J Anal Toxicol* 23: 591–597.
Robinson DW, Reive DS (1981). A gas chromatographic procedure for quantitation of ethylene glycol in postmortem blood. *J Anal Toxicol* 5: 69–72.
Rosano TG *et al.* (2009). Ethylene glycol and glycolic acid in postmortem blood from fatal poisonings. *J Anal Toxicol* 33: 508–513.
Scalley RD *et al.* (2002). Treatment of ethylene glycol poisoning. *Am FamPhysician* 66: 807–812.
Van Hee P *et al.* (2004). Analysis of gamma-hydroxybutyric acid, DL-lactic acid, glycolic acid, ethylene glycol and other glycols in body fluids by a direct injection gas chromatography–mass spectrometry assay for wide use. *Clin Chem Lab Med* 42: 1341–1345.
Vites NP *et al.* (1984). Recovery after potentially lethal amount of anti-freeze. *Lancet* i: 562.
Williams RH *et al.* (2000). Simultaneous detection and quantitation of diethylene glycol, ethylene glycol, and the toxic alcohols in serum using capillary column gas chromatography. *J Anal Toxicol* 24: 621–626.

Ethylenediamine Hydrate

Pharmaceutical Adjuvant
$C_2H_8N_2,H_2O = 78.1$
CAS—107-15-3 (anhydrous); 6780-13-8 (monohydrate)

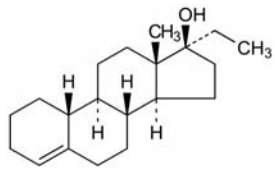


Chemical Properties A clear, colourless or slightly yellow, strongly alkaline liquid. It is hygroscopic and absorbs carbon dioxide from the air. It solidifies on cooling to a crystalline mass (Mp 10°). Mass per mL about 0.96 g. Bp about 120°. Miscible with water and ethanol; soluble 1 in 130 of chloroform; slightly soluble in ether. pK_a 7.2, 10.0 (20°). Log *P* (octanol/water), −2.0 (anhydrous).
Caution It is irritant to the skin and mucous membranes.
Colour Test Dissolve 1 mL in 5 mL of water and to 3 drops of this solution add 2 mL of a 1% solution of copper sulfate and shake—violet-blue.
Mass Spectrum Principal ions at *m/z* 30, 42, 43, 27, 44, 29, 31, 41.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 50 µg/L [Cotgreave, Caldwell 1983].
Urine **HPLC** For method, see Cotgreave and Caldwell [1983].
Disposition in the Body Absorbed after oral administration and rapidly metabolised.
Toxicity The maximum permissible atmospheric concentration is 10 ppm.
Half-life Plasma half-life, about 0.5 to 1 h.
Use Ethylenediamine hydrate is used in the manufacture of aminophylline and in the preparation of aminophylline injections.

Cotgreave IA, Caldwell J (1983). Studies on aminophylline disposition I. A rapid and sensitive HPLC assay for ethylenediamine in plasma and urine. *Drug Dispos* 4(1): 53–62.

Ethylestrenol

Anabolic Steroid
$C_{20}H_{32}O = 288.5$
CAS—965-90-2
IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Ethyl-13-methyl-2,3,6,7,8,9,10,11,12,14,15,16-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-ol
Synonyms Ethyloestrenol; (17α)-19-norpregn-4-en-17-ol.
Proprietary Names *Durabolin-O*; *Maxibolin*; *Orabolin*; *Orgabolin*; *Orgaboral*.



Chemical Properties A white crystalline powder. Mp 76° to 78°. Practically insoluble in water; soluble 1 in 9 of ethanol, 1 in 2 of chloroform and 1 in 6 of ether. Log *P* (octanol/water), 5.5.
Thin-layer Chromatography System TP—R_f 0.79; system TQ—R_f 0.50; system TR—R_f 0.94; system TS—R_f 0.99.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 975, 994, 964, 1299, 1142, 1165 cm^{-1} (Nujol mull).
Dose Usually 2 to 4 mg daily.

Ethylisobutrazine

Tranquilliser
$C_{20}H_{26}N_2S = 326.5$
IUPAC Name 3-(2-Ethylphenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine
Synonyms Ethotrimeprazine; 2-ethyl-10-(3-dimethylamino-2-methylpropyl)-phenothiazine; ethymemazine; RP 6484.
Proprietary Name *Diquel*
Chemical Properties A white crystalline powder. Ethylisobutrazine is extracted by organic solvents from aqueous alkaline solutions.

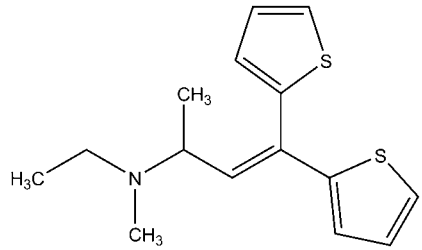
Ethylisobutrazine Hydrochloride

Proprietary Names *Nuital*; *Sergetyl*.
Chemical Properties Mp 160° to 163°. Soluble in water, ethanol, chloroform, methanol and acetone; practically insoluble in ether.

Colour Tests Ammonium molybate test—deep purple (limit of detection, 0.25 µg); ammonium vanadate test—green; sulfuric acid test—purple (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—purple (limit of detection, 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.60 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid—maxima at 251 nm (E1%, 1 cm 760) and 300 nm (E1%, 1 cm 115); minima at 222 nm and 275 nm.
Dose Up to 40 mg daily.

Ethylmethylthiambutene

Narcotic
$C_{15}H_{19}NS_2 = 277.5$
CAS—441-61-2
IUPAC Name *N*-Ethyl-*N*-methyl-4,4-di(thiophen-2-yl)but-3-en-2-amine
Synonyms 1C50; emethibutin; ethylmethiambutene; *N*-ethyl-*N*,1-dimethyl-3,3-di-2-thienylallylamine; *N*-ethyl-*N*,1-dimethyl-3,3-di-2-thienyl-2-propenamine; 3-ethylmethylamino-1,1-di(2′-thienyl)but-1-ene; *N*-ethyl-*N*-methyl-4,4-di-2-thienyl-3-buten-2-amine; NIH-5145.



Chemical Properties Practically insoluble in water (61.7 mg/L). Log *P* (octanol/water) 4.02 [Meylan, Howard 1995]. Extracted by organic solvents aqueous alkaline solutions.

Ethylmethylthiambutene Hydrochloride

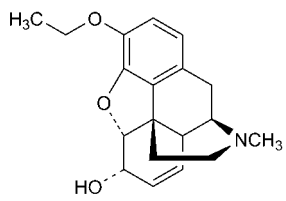
$C_{15}H_{19}NS_2,HCl = 314$
Chemical Properties Crystals. Mp 137° to 138°.

Colour Tests Ammonium molybdate test—orange-brown→pale green (limit of detection, 0.1 µg); ammonium vanadate test—green→greenish-blue (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—purple-brown (limit of detection, 0.1 µg); Vitali's test—red→green/brown/brown.
Thin-layer Chromatography System T1—R_f 0.58 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.30 relative to diphenhydramine; system G4—retention time 0.80 relative to diphenhydramine.
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—268, 285, 226 nm.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Ethylmorphine

Narcotic Analgesic
$C_{19}H_{23}NO_3 = 313.4$
CAS—76-58-4
Synonym 7,8-Didehydro-4,5-epoxy-3-ethoxy-17-methylmorphinan-6-ol



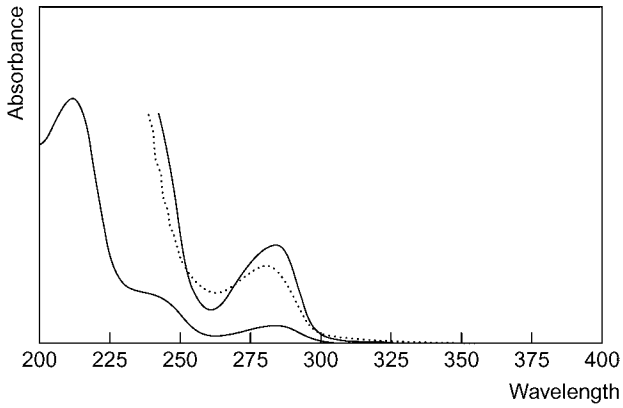
Chemical Properties Crystals. Mp 199° to 201°. pK_a 8.2 (20°) [Baselt 2008]. Log *P* (octanol/water), 1.77 [Meylan, Howard 1995].

Ethylmorphine Hydrochloride

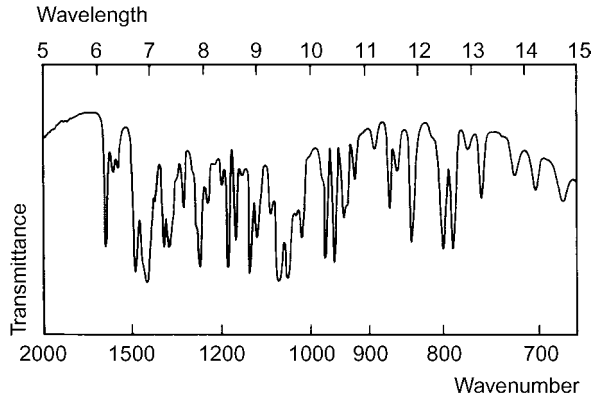
$C_{19}H_{23}NO_3,HCl,\ 2H_2O = 385.9$
CAS—125-30-4 (anhydrous); 6746-59-4 (dihydrate)
Synonym Chlorhydrate de Codéthyline
Proprietary Name It is an ingredient of *Natirose*.
Chemical Properties White crystalline powder. Mp ≈123°, with decomposition. Soluble 1 in 10 of water, 1 in 25 of ethanol, 1 in 1 of warm ethanol, and 1 in 250 of chloroform; slightly soluble in ether.

Colour Test Marquis test—yellow→violet→black.
Thin-layer Chromatography System TA—R_f 0.40; system TAE—R_f 0.21; system TAF—R_f 0.26; system TL—R_f 0.06; system TB—R_f 0.07; system TC—R_f 0.22; system TE—R_f 0.36 (Dragendorff spray, positive acidified iodoplatinate solution, positive; Marquis reagent, blue-violet).
Gas Chromatography System GA—ethylmorphine RI 2420, ethylmorphine-AC RI 2530, ethylmorphine-PFP RI 2430, ethylmorphine-TFA RI 2320, ethylmorphine-TMS RI 2540, M (nor-)-AC_2 RI 2930.
High Performance Liquid Chromatography System HA—*k* 3.7 (tailing peak); system HC—*k* 1.06; system H—*k* 1.45; system HX—RI 291; system HY—RI 244.

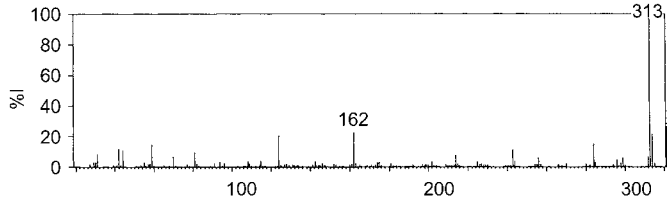
Ultraviolet Spectrum Aqueous acid—284 nm ($A_1^1 = 48a$); aqueous alkali—281 nm.



Infrared Spectrum Principal peaks at wavenumbers 1064, 1045, 1129, 1499, 1185, 1264 cm^{-1} (Ethylmorphine hydrochloride, Nujol mull).



Mass Spectrum Principal ions at *m/z* 313, 162, 314, 124, 284, 59, 42, 243; ethylmorphine-AC *m/z*355, 296, 327, 234, 268, 204; ethylmorphine-PFP *m/z* 296, 459, 280, 266, 402, 430; ehtylmorphine-TFA *m/z*296, 409, 380, 280, 352, 266; ethylmorphine-TMS *m/z*385, 192, 146, 196, 234, 357; M (nor-)-AC_2 *m/z*87, 209, 237, 383, 341, 181.



Quantification

Blood **GC** Column. CP-Sil 8 Chrompak (25 m × 0.32 mm i.d., 0.61 µm). Carrier gas: He, 0.8 bar. Temperature programme: 220° for 2 min to 260° at 5°/min for 2 min to 300° at 10°/min for 14 min. NPD. Retention time: 13.3 min. Limit of detection, 0.5 mg/L [Demedts *et al.* 1983].

GC-MS Column: HP-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 150° for 1 min to 220° at 30°/min to 320° at 10°/min for 1 min. EI ionisation, SIM acquisition mode. Retention time: 10.7 min. Limit of quantification, 0.01 mg/L [Mykkänen *et al.* 2000]. Column: Restek RTX-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carier gas: He, 10 psi. Temperature programme: 85° for 1 min to 220° at 30°/min to 260° at 3°/min to 275° at 15°/min for 5 min. EI ionisation. Relative retention time: 0.877. Limit of detection not reported [Balíková, Maresová 1998]. Column: BP 5 SGE capillary (12 m × 0.22 mm i.d.). Carrier gas: He, 1.8 mL/min. Temperature programme: 60° to 310° at 30°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Kintz *et al.* 1994]. Column: HP Ultra 1 cross-linked methylsilicone capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 120° for 30 s to 220° at 40°/min to 244° at 4°/min to 300° at 40°/min for 3.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.05 µmol/L [Krogh *et al.* 1993]. Column: Bond Elut C_{18} methylsilicon (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.4 mL/min. Temperature programme: 120° for 0.5 min to 230° at 25°/min to 270 at 12°/min to 320° at 25°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 6.7 min. Limit of detection, 0.025 µmol/L [Gjerde *et al.* 1991].

Plasma **GC-MS** See Blood [Krogh *et al.* 1993].

GC Column: BP 10 SGE (25 m × 0.22 mm i.d.). Carrier gas: He, 3.2 mL/min. Temperature: 240°. NPD. Retention time: 10.67 min. Limit of detection, 9.0 µg/L [Kintz *et al.* 1989].

Urine **GC** See Plasma [Kintz *et al.* 1989]. Column: HP cross-linked methylsilicone (12.5 m × m i.d., 0.33 µm). Carrier gas: He, 30 mL/min. Temperature programme: 120° for 0.5 min to 200° at 30°/min to 240° at 5°/min to 300° at 40°/min. NPD, EI ionisation at 70 eV, SIM acquisition mode. Relative retention time: 0.84. Limit of detection, 0.25 µmol/L [Christophersen *et al.* 1987].

GC-MS See Blood [Kintz *et al.* 1994].

HPLC Column: Chromolith Flash RP-18e (25 × 4.6 mm i.d.). Mobile phase: 10 to 40% methanol in an aqueous solution adjusted to pH 2.0 with trifluoroacetic acid over 6 min, flow rate 1 mL/min. FIA. Limit of detection, 3 nmol/L [Adcock *et al.* 2007].

LC-MS Column: Luna C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 25 mmol/L formic acin containing 1% acetonitrile : 25 mmol/L formic acid containing 90% acetonitrile (100 : 0 to 77 : 23 in 5 min to 0 : 100 at 6 min to 100 : 0 for 7 min), flow rate 0.3 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 126 µg/L, limit of detection, 38 µg/L [Gustavsson *et al.* 2007].

CE Column: fused silica capillary (80 cm × 50 µm i.d.). Running buffer: methanol: water: acetic acid (60 : 39 : 1), flow rate 3 µL/min. ESI, positive ion mode. Limit of detection not reported [Wey *et al.* 2000].

Bile **GC-MS** See Blood [Kintz *et al.* 1994].

Gastric Contents **GC** See Blood [Demedts *et al.* 1983].

GC-MS See Blood [Kintz *et al.* 1994].

Vitreous Humour **GC-MS** See Blood [Kintz *et al.* 1994].

Brain **GC-MS** See Blood [Kintz *et al.* 1994].

Hair **GC-MS** Column: BP-5 SGE 5% phenyl 95% methyl siloxane (12 m × 0.22 mm i.d.). Carrier gas: He, 1.8 mL/min. Temperature programme: 60° to 310° at 30°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, ≈0.1 to 0.8 µg/g [Kintz, Mangin 1995].

HPLC Column: C_{18} NBS (150 × 4.6 mm i.d., 3 µm). Mobile phase: 20 mmol/L monobasic sodium phosphate-acetonitrile (pH 7.0; 90 : 10) : 20 mmol/L monobasic sodium phosphate-acetonitrile (pH 7.0; 50 : 50, 93 : 7 for 11.5 min to 50 : 50 at 19.5 min for 5 min to 10 : 90 at 34.5 min), flow rate 0.8 mL/min. Electrochemical detection. Limit of detection, 201 pg [Achilli *et al.* 1996].

LC-MS Column: Zorbax phenyl (50 × 2.1 mm i.d.). Mobile phase: acetonitrile-methanol-20 mmol/L formate buffer (pH 3.0; 10 : 1080) : acetonitrile-methanol-20 mmol/L formate buffer (pH 3.0; 35 : 35 : 30; 100 : 0 to 35 : 65 from 0.5 to 7 min to 100 : 0 for 2 min), flow rate 0.25 mL/min. ESI, MRM acquisition mode. Limit of quantification, 13 ng/g, limit of detection, 4 ng/g [Kronstrand *et al.* 2004].

Heart **GC-MS** See Blood [Kintz *et al.* 1994].

Kidney **GC-MS** See Blood [Kintz *et al.* 1994].

Liver **GC** See Blood [Demedts *et al.* 1983].

GC-MS See Blood [Kintz *et al.* 1994].

Lung **GC-MS** See Blood [Kintz *et al.* 1994].

Muscle **GC-MS** See Blood [Kintz *et al.* 1994].

Small Intestine **GC** See Blood [Demedts *et al.* 1983].

Disposition in the Body Absorbed after oral administration. It is metabolised by *N*-demethylation to norethylmorphine and by *O*-de-ethylation to morphine. The *O*-de-ethylation is catalysed by CYP2D6 and the *N*-demethylation by CYP3A4 [Liu *et al.* 1995]. 3-*O*-Ethylmorphin-6-yl-β-D-glucopyranosiduronic acid (ethylmorphine-6-glucuronide) is assumed to be the major metabolite of ethylmorphine in humans [Bugge *et al.* 1995].

Therapeutic Concentration Ten healthy male volunteers were given a single dose of Cosylan cough mixture. The median urinary recovery was 77% over 48 h. Two subjects phenotypically classified as poor metabolisers were genotypically CYP2D6A/wt and CYP2D6D/wt [Aasmundstad *et al.* 1995].

Toxicity The estimated minimum lethal dose is 500 mg.

A 27-year-old male was found vomiting in a park; he died before the emergency services arrived. His blood ethylmorphine concentration was 15.6 mg/L [Balíková, Maresová 1998].

In a death attributed to ingestion of ethylmorphine, the concentrations of the drug measured at postmortem were as described below.

Sample	*Concentration (µg/L or ng/g)*
Femoral blood	488
Cardiac blood	347
Urine	9488
Bile	681
Gastric contents	14454
Vitreous humour	469
Kidney	1809
Lung	329
Brain	158
Heart	253
Liver	1006
Psoas muscle	174

[Kintz *et al.* 1994].

Half-life 2 to 3 h [Baselt 2008], 2.5 to 6.2 [Popa *et al.* 1998].

Volume of Distribution 3 to 4 L/kg [Baselt 2008].

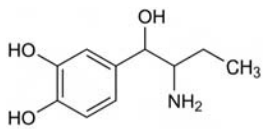
Aasmundstad TA *et al.* (1995). Biotransformation and pharmacokinetics of ethylmorphine after a single oral dose. *Br J Clin Pharmacol* 39: 611–620.

Achilli G *et al.* (1996). Determination of illicit drugs and related substances by high-performance liquid chromatography with an electrochemical coulometric-array detector. *J Chromatogr A* 729: 273–277.
Adcock JL *et al.* (2007). A hybrid FIA/HPLC system incorporating monolithic column chromatography. *Anal Chim Acta* 600: 136–141.
Balíková M, Maresová V (1998). Fatal opiates overdose. Toxicological identification of various metabolites in a blood sample by GC-MS after silylation. *Forensic Sci Int* 94: 201–209.
Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, California, Biomedical Publications.
Bugge A *et al.* (1995). Synthesis of ethylmorphine-6-glucuronide: a metabolite of ethylmorphine in man. *Acta Chem Scand* 49: 380–384.
Christophersen AS *et al.* (1987). Identification of opiates in urine by capillary column gas chromatography of two different derivatives. *J Chromatogr* 422: 117–124.
Demedts P *et al.* (1983). Application of the combined use of fused silica capillary columns and NPD for the toxicological determination of codeine and ethylmorphine in a human overdose case. *J Anal Toxicol* 7: 113–115.
Gjerde H *et al.* (1991). Evaluation of a method for simultaneous quantification of codeine, ethylmorphine and morphine in blood. *Forensic Sci Int* 51: 105–110.
Gustavsson E *et al.* (2007). Validation of direct injection electrospray LC-MS/MS for confirmation of opiates in urine drug testing. *J Mass Spectrom* 42: 881–889.
Kintz P, Mangin P (1995). Simultaneous determination of opiates, cocaine and major metabolites of cocaine in human hair by gas chromotography/mass spectrometry (GC/MS). *Forensic Sci Int* 73: 93–100.
Kintz P *et al.* (1994). Ethylmorphine concentrations in human samples in an overdose case. *Arch Toxicol* 68: 210–211.
Kintz P *et al.* (1989). Simultaneous identification and quantification of several opiates and derivatives by capillary gas chromatography and nitrogen selective detection. *Z Rechtsmed* 103: 57–62.
Krogh M *et al.* (1993). Automated sample preparation by on-line dialysis and trace enrichment. Analysis of morphine, 6-monoacetylmorphine, codeine, ethylmorphine and pholcodine in plasma and whole blood by capillary gas chromatography and capillary gas chromatography-mass spectrometry. *J Chromatogr* 621: 41–48.
Kronstrand R *et al.* (2004). Screening for drugs of abuse in hair with ion spray LC-MS-MS. *Forensic Sci Int* 145: 183–190.
Liu Z *et al.* (1995). Evidence for a role of cytochrome P450 2D6 and 3A4 in ethylmorphine metabolism. *Br J Clin Pharmacol* 39: 77–80.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Mykkänen S *et al.* (2000). GCD quantitation of opiates as propionyl derivatives in blood. *J Anal Toxicol* 24: 122–126.
Popa C *et al.* (1998). Morphine formation from ethylmorphine: implications for drugs-of-abuse testing in urine. *J Anal Toxicol* 22: 142–147.
Wey AB *et al.* (2000). Analysis of codeine, dihydrocodeine and their glucuronides in human urine by electrokinetic capillary immunoassays and capillary electrophoresis-ion trap mass spectrometry. *J Chromatogr A* 895: 133–146.

Ethylnoradrenaline

Sympathomimetic
$C_{10}H_{15}NO_3$ = 197.2
CAS—536-24-3

IUPAC Name 4-(2-Amino-1-hydroxybutyl)-1,2-benzenediol
Synonym Ethylnorepinephrine

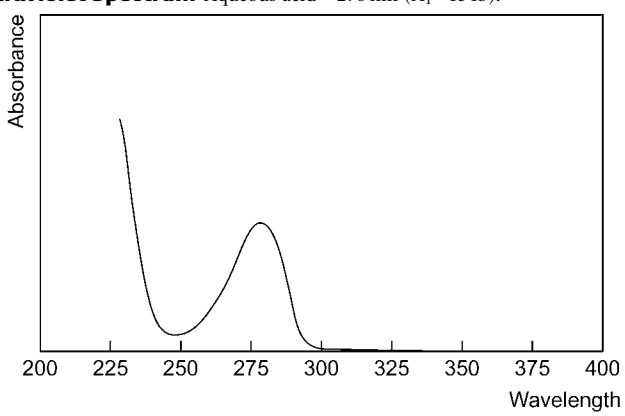


Chemical Properties pK_a 8.4 (25°).

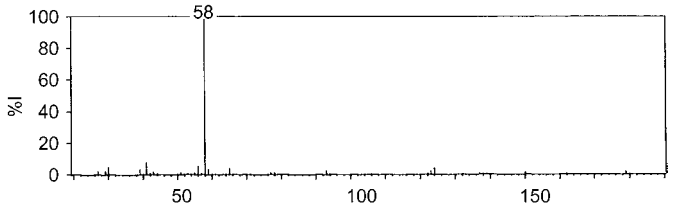
Ethylnoradrenaline Hydrochloride

$C_{10}H_{15}NO_3$,HCl = 233.7
CAS—3198-07-0
Proprietary Name *Bronkephrine*
Chemical Properties A crystalline solid. Mp 199° to 200°, with decomposition. Soluble in water.

Colour Tests Ferric chloride—green; Mandelin's test—orange; Marquis test—orange→brown; Nessler's reagent—black.
Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.01; system TC—R_f 0.02; system TE—R_f 0.15; system TL—R_f 0.24; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.15 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—278 nm (A^{1}_{1}=154b).



Infrared Spectrum Principal peaks at wavenumbers 1500, 1529, 1280, 1265, 1052, 1600 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 41, 56, 30, 124, 65, 59, 93.

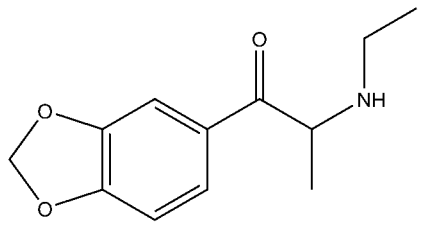


Dose 1 to 2 mg of ethylnoradrenaline hydrochloride, SC or IM.

E

Ethylone

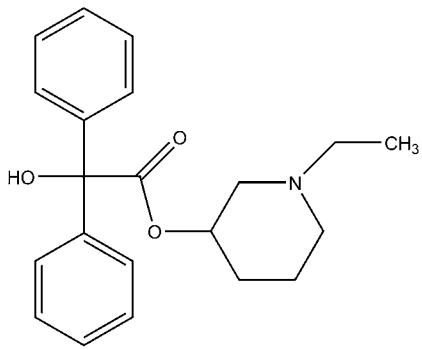
Cathinone Derivative, Phenethylamine, Stimulant
$C_{12}H_{15}NO_3$ = 221.2
IUPAC Name 2-Ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one
Synonym bk-MDEA



Chemical Properties Cathinone analogue of 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA).
Dose Reported to be less potent than methylone.

Ethylpiperidyl Benzilate

Hallucinogen
$C_{21}H_{25}NO_3$ = 339.4
IUPAC Name (1-Ethylpiperidin-3-yl) 2-hydroxy-2,2-di(phenyl)acetate
Synonym 1-Ethylpiperid-3-yl benzilate



Chemical Properties Bp 194° to 198° at 0.12 to 0.18 mmHg. Ethylpiperidyl benzilate is extracted by organic solvents from aqueous alkaline solutions.

Ethylpiperidyl Benzilate Hydrochloride

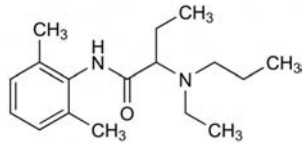
Synonym JB 318
Chemical Properties A white crystalline powder. Mp 186° to 192°. Ethylpiperidyl benzilate is extracted by organic solvents from aqueous alkaline solutions.

Thin-layer Chromatography System T1—R_f 0.73 (location reagent acidified iodoplatinate spray, strong reaction).
Ultraviolet Spectrum Methanol—maxima at 252 (E1%, 1 cm 10), 258 (E1%, 1 cm 11) and 264 nm (E1%, 1 cm 9); inflexion at 268 nm; minima at 249 nm, and 263 nm (hydrochloride).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1716, 692 cm^{-1} (hydrochloride).
Toxicity LD_{50} (oral): in mice 34 mg/kg and in rats 25 mg/kg.

Etidocaine

Anaesthetic (Local)
$C_{17}H_{28}N_2O$ = 276.4
CAS—36637-18-0

IUPAC Name *N*-(2,6-Dimethylphenyl)-2-(ethylpropylamino)butanamide

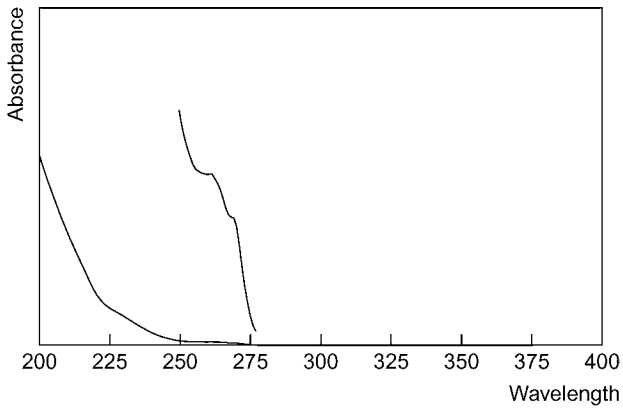


Chemical Properties A white crystalline powder. Mp 87° to 91°. Soluble 1 in about 7000 of water; freely soluble in organic solvents. pK_a 7.9. Log *P* (octanol/water), 3.7.

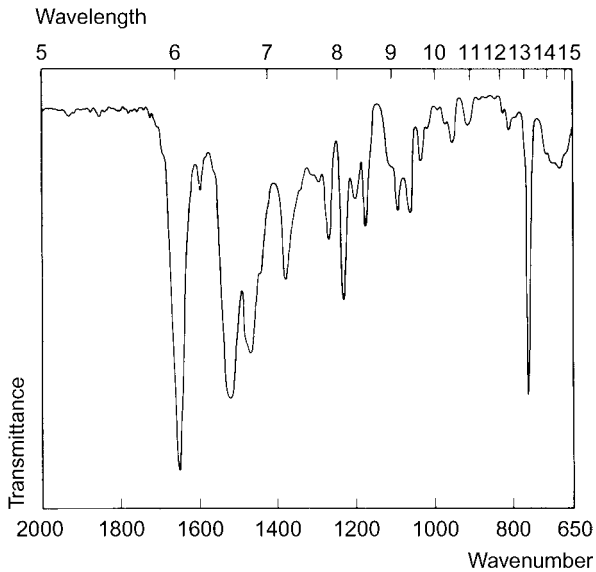
Etidocaine Hydrochloride

$C_{17}H_{28}N_2O,HCl = 312.9$
CAS—36637-19-1
Proprietary Name *Duranest*
Chemical Properties A white crystalline powder. Mp 202° to 205°, with decomposition. Soluble 1 in 10 of water and 1 in 5 of chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.91; system TE—R_f 0.75; system TAE—R_f 0.80; system TAJ—R_f 0.66; system TAK—R_f 0.19; system TAL—R_f 0.75.
Gas Chromatography System GA—RI 2040.
Ultraviolet Spectrum Aqueous acid—262 (A_1^1=17a), 271 nm (A_1^1=14a).



Infrared Spectrum Principal peaks at wavenumbers 1650, 1520, 765, 1235, 1272, 1180 cm^{-1} (KBr disk).



Quantification
Plasma **GC** FID. Limit of detection, 20 µg/L, etidocaine and *N*-desalkyl metabolites [Morgan *et al.* 1977].

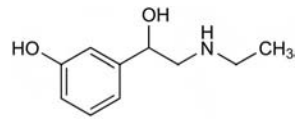
HPLC UV detection. For method for quantification of etidocaine and other local anaesthetics, see Drewe *et al.* [1997]. UV detection. Limit of detection, about 30 µg/L, etidocaine and other amide local anaesthetics [Adams *et al.* 1989].
Serum **GC** Nitrogen–phosphorus detection. Etidocaine and other drugs including local anaesthetics. Limit of detection, 10 µg/L for etidocaine [Coyle, Denson 1986].
Urine **GC** See Plasma [Morgan *et al.* 1977].
Biological Samples **GC** Nitrogen-sensitive detection. For method for quantification of etidocaine and other local anaesthetics, see Lau *et al.* [1991].

Disposition in the Body Rapidly absorbed into the circulation after epidural administration. Extensively metabolised by *N*-dealkylation, hydrolysis and ring hydroxylation. Desethyletidocaine and despropyletidocaine are detectable in plasma. <1% of a dose is excreted in the urine unchanged in 48 h.
Toxicity Plasma concentrations >2 mg/L may be associated with toxic effects.
Half-life Plasma half-life, after IV administration, about 2.5 h, increased to about 6 h after epidural administration.
Volume of Distribution About 2 L/kg.
Clearance Plasma clearance, about 15 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.7.
Protein Binding About 94%.
Note For a review of the pharmacokinetics of local anaesthetics, see Tucker and Mather [1979].
Dose Etidocaine hydrochloride is administered by injection as 0.5 to 1.5% solutions; maximum dose, 300 mg.

Adams HA *et al.* (1989). *Reg Anesth* 12: 53–57.
Coyle DE, Denson DD (1986). Simultaneous measurement of bupivacaine, etidocaine, lidocaine, meperidine, mepivacaine, and methadone. *Ther Drug Monit* 8: 98–101.
Drewe J *et al.* (1997). High-performance liquid chromatographic method for an automated determination of local anaesthetics in human plasma. *J Chromatogr B Biomed Sci Appl* 691: 105–110.
Lau OW *et al.* (1991). Gas-liquid chromatographic determination and pharmacological studies of six clinically-used local anesthetics. *Methods Find Exp Clin Pharmacol* 13: 475–481.
Morgan DJ *et al.* (1977). Disposition and placental transfer of etidocaine in pregnancy. *Eur J Clin Pharmacol* 12: 359–365.
Tucker GT, Mather LE (1979). Clinical pharmacokinetics of local anaesthetics. *Clin Pharmacokinet* 4: 241–278.

Etilefrine

Sympathomimetic
$C_{10}H_{15}NO_2 = 181.2$
CAS—709-55-7
IUPAC Name 3-[2-(Ethylamino)-1-hydroxyethyl]phenol
Synonyms Ethyladrianol; α-[(ethylamino)methyl]-3-hydroxybenzenemethanol; ethylnorphenylephrine.

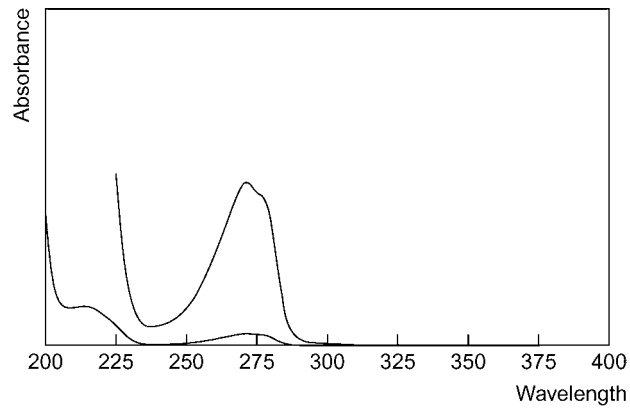


Chemical Properties A white crystalline powder. Mp 147° to 148°. Soluble 1 in 25 of water and 1 in 15 of ethanol. pK_a 9.0, 10.2 (25°). Log *P* (octanol/water), 0.3.

Etilefrine Hydrochloride

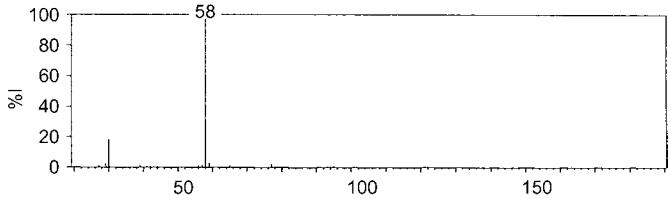
$C_{10}H_{15}NO_2,HCl = 217.7$
CAS—534-87-2
Proprietary Names *Adrenam*; *Bioflutin-N*; *Cardanat*; *Cardialgine*; *Circupon RR*; *Circuvit-E*; *Effortil*; *Efortil*; *Efxine*; *Hyprosia*; *Thomasin*; *Tonus-forte-Tablinen*; *Tri-Effortil*.
Chemical Properties A white crystalline powder. Mp 121°. Freely soluble in water; soluble in ethanol; practically insoluble in chloroform.

Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—grey→green→brown; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.41; system TB—R_f 0.02; system TC—R_f 0.02; system TE—R_f 0.22; system TL—R_f 0.03; system TAE—R_f 0.15; system TAF—R_f 0.74 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—etilefrine RI 1685, etilefrine-AC_3 RI 2150, etilefrine-Me_2-AC RI 2000.
High Performance Liquid Chromatography System HX—RI 118; system HY—RI 142.
Ultraviolet Spectrum Aqueous acid—272 nm (A_1^1=102a); aqueous alkali—237 (A_1^1=491b), 290 nm (A_1^1=167a).



Infrared Spectrum Principal peaks at wavenumbers 1597, 1068, 797, 1305, 1279, 1222 cm^{-1} (etilefrine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 30, 59, 77, 29, 95, 65, 57.

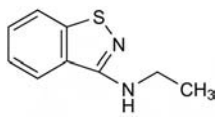


Dose Etilefrine hydrochloride has been given in doses of 15 to 50 mg daily.

Etisazole

Antifungal (Veterinary)
$C_9H_{10}N_2S$ = 178.3
CAS—7716-60-1

IUPAC Name *N*-Ethyl-1,2-benzisothiazol-3-ylamine



Chemical Properties A white to slightly brown crystalline powder. Mp 78°. Log *P* (octanol/water), 2.3.

Etisazole Hydrochloride

$C_9H_{10}N_2S$,HCl = 214.7
CAS—7716-59-8

Proprietary Name *Netrosylla*

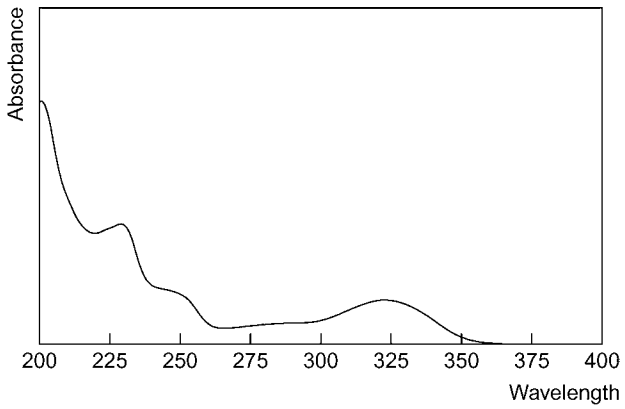
Chemical Properties A crystalline powder. Mp 182° to 186°. Sparingly soluble in water; freely soluble in ethanol and methanol; slightly soluble in chloroform and ether.

Colour Test Liebermann's reagent—red-brown.

Thin-layer Chromatography System TA—R_f 0.74 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1668.

Ultraviolet Spectrum Aqueous acid—232 (A^{1}_{1}=1200b), 323 nm.



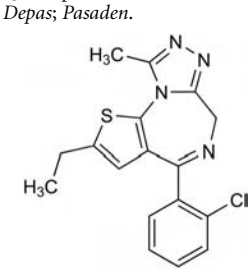
Infrared Spectrum Principal peaks at wavenumbers 1608, 1314, 1292, 772, 706, 1111 (KBr disk).

Etizolam

Anxiolytic
$C_{17}H_{15}ClN_4S$ = 342.9
CAS—40054-69-1

Synonyms AHR-3219; 4-(2-chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo-[4,3-*a*][1,4]diazepine; Y-7131.

Proprietary Names *Depas*; *Pasaden*.



Chemical Properties A white crystalline powder with Mp 147° to 148°. It is insoluble in water; soluble in ethanol and chloroform. Log *P* (octanol/water), 3.1.

Thin-layer Chromatography System TE—R_f 0.52; system TAE—R_f 0.72.

Plate: silica gel 60 F254 (10 × 10 cm). Mobile phase 1: cyclohexane : toluene : diethylamine (75:15:10); mobile phase 2: chloroform : methanol (90:10); mobile phase 3: chloroform : acetone (80:20). Dragendorff's reagent was used to visualise the spots. Rf 4 (mobile phase 1); Rf 45 (mobile phase 2); Rf 13 (mobile phase 3) [Otsubo *et al.* 1995].

Gas Chromatography System GA—RI 2980.

High Performance Liquid Chromatography System HAF—retention time 18.4 min.

Ultraviolet Spectrum Aqueous acid (ethanol)—243 nm; aqueous acid—250, 294, 362 nm; aqueous alkali—243 nm.

Infrared Spectrum Principal peaks at wavenumbers 764, 1410, 1615, 1380, 1040, 1524 cm^{-1} (KBr disc).

Mass Spectrum Principal ions at *m/z* 342, 18, 28, 266, 313, 224, 239, 45.

Quantification

Blood **HPLC** UV detection (λ=254 nm). Limit of quantification, 5 µg/L [Tanaka *et al.* 682].

Plasma **HPLC** Limit of detection, 2.5 µg/L [Fracasso *et al.* 1991].

Urine **HPLC** Limit of detection, 1 µg/L [Fracasso *et al.* 1991].

Disposition in the Body Etizolam is rapidly absorbed after oral administration and is almost entirely eliminated by non-renal pathways. Peak plasma concentrations are reached within 0.5 to 2 h. There is little accumulation of the drug in the body and elimination is by biotransformation of the drug; mainly microsomal oxidation to produce hydroxylated derivatives which are subsequently conjugated. The main metabolite is α-hydroxyetizolam (20% of which is in the unconjugated form) and is pharmacologically active as opposed to the other metabolites which are not.

Therapeutic Concentration

Six healthy males, aged between 20 and 32 years (mean 26 years), were administered a 0.5 mg etizolam after an overnight fast. A peak plasma concentration of 8.3 µg/L was observed 0.9 h after dosing. Multiple dosing of 0.5 mg every 12 h for 1 week was also carried out. Peak plasma concentrations for the parent drug and its metabolite, α-hydroxyetizolam, were 9.3 µg/L and 9.9 µg/L, respectively observed at 1.2 and 2.5 h [Fracasso *et al.* 1991].

Half-life Approximately 3.4 h.

Volume of Distribution 0.9 L (after a single 0.5 mg dose); usually referred to as 1.0 L/kg.

Clearance Oral, between 10.4 and 19.3 (mean 13.8) L/h (after a single 0.5 mg dose).

Protein Binding Approximately 93%.

Dose 1.5 to 3 mg daily.

Fracasso C *et al.* (1991). Single and multiple dose pharmacokinetics of etizolam in healthy subjects. *Eur J Clin Pharmacol* 40: 181–185.

Otsubo K *et al.* (1995). Rapid and sensitive detection of benzodiazepines and zopiclone in serum using high-performance thin-layer chromatography. *J Chromatogr B Biomed Appl* 669(2): 408–412.

Tanaka E *et al.* (1996). Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-microns porous microspherical silica gel. *J Chromatogr B Biomed Appl* 682(1): 173–178.

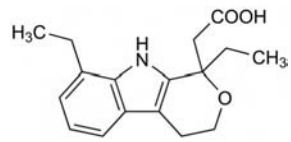
Etodolac

Antiinflammatory
$C_{17}H_{21}NO_3$ = 287.4
CAS—41340-25-4

IUPAC Name 2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid

Synonyms AY-24236;: CCRIS 3923; 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic acid; etodolic acid; NSC-282126.

Proprietary Names *Edolan*; *Lodine*; *Ramoder*; *Tedolan*; *Ultradol*; *Zedolac*.



Chemical Properties A white to almost-white crystalline powder. It is practically insoluble in water; freely soluble in alcohol, chloroform, dimethyl sulfoxide and aqueous polyethylene glycol. Mp 145° to 148°. pK_a 4.65. Log *P* (octanol/water), 3.93; (pH 7.4), 11.4.

Gas Chromatography System GL—RI 2225(etodolac-ME).

Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, flow rate 0.9 mL/min. Detection: mass spectrometer. Retention index: 2333 [Mills, Roberson 1993].

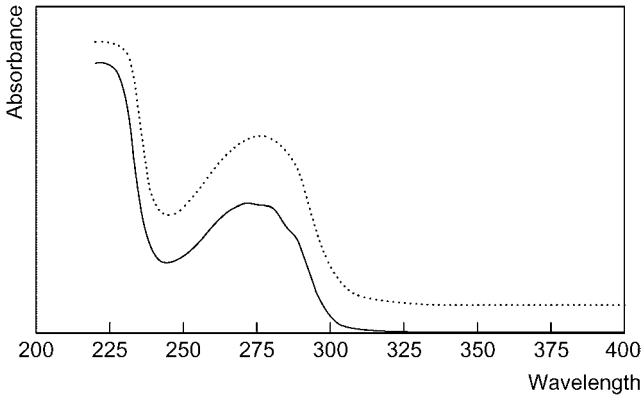
High Performance Liquid Chromatography System HAA—retention time 21.5 min.

Column: RP-18 LiChrosorb (250 × 4.0 mm i.d., 7 µm), (guard) (40 × 4.0 mm). Mobile phase: aqueous acetic acid (1%) : acetonitrile (50:50), flow rate 1.3 mL/min. UV detection (λ=227 nm). Retention time: 8.5 min [Koupai-Abyazani *et al.* 1999].

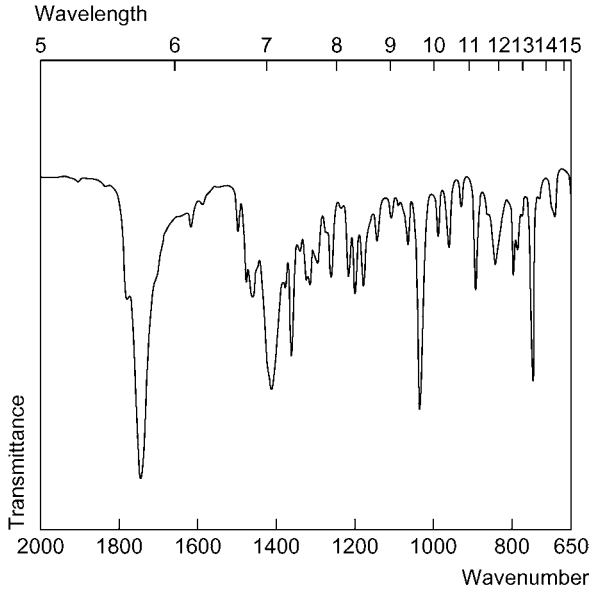
Column: silica (Partisil 5 Silica, 5 µm, 250 × 4.6 mm i.d.), (guard) (5 cm, packed with 30 to 38 µm silica-coated glass beads). Mobile phase: hexane : ethyl acetate : isopropanol (85:15:0.2), flow rate 2 mL/min. UV detection (λ=280 nm). Retention time: R-enantiomer of etodolac, 21.9 min; S-enatiomer, 29.5 min [Jamali *et al.* 1988].

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, 1 mL/min flow rate. UV (λ=280 nm). Retention time: 2.8 min [Mills, Roberson 1993].

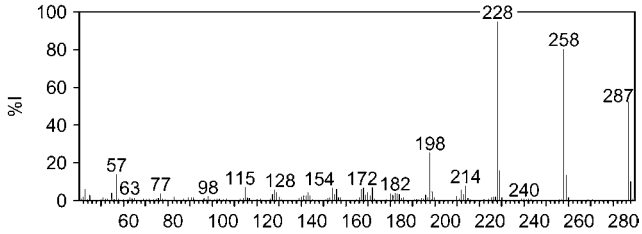
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—272 nm; basic—274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1746, 1412, 1034, 748 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 228, 258, 287, 198, 57, 229, 259, 214.



Quantification

***Serum* HPLC** UV detection (λ=227 nm). Limit of quantification, 0.04 mg/L, limit of detection, 0.03 mg/L [Koupai-Abyazani *et al.* 1999].

***Urine* HPLC** UV detection (λ=227 nm). Limit of quantification, 0.4 mg/L, limit of detection, 0.3 mg/L [Koupai-Abyazani *et al.* 1999].

Disposition in the Body Etodolac is well absorbed after oral administration and extensively metabolised in the liver, via hydroxylation followed by conjugation and acyl glucuronidation. The drug is easily distributed into synovial fluid. Etodolac is primarily excreted in urine but also in faeces. 1% of a dose is detected unchanged in urine with hydroxylated etodolac (46%), glucuronidated etodolac (13%), hydroxylated metabolites (5%), hydroxylated glucuronide metabolites (20%) and 4-ureidoetodolac metabolite.

Therapeutic Concentration The serum therapeutic concentration range is 20 to 50 mg/L.

Fourteen healthy male volunteers, with a mean age of 27 years, were administered a 400 mg dose of etodolac (in a solution and as the sustained release formulation). Peak plasma concentrations of 36.8 mg/L were reached within 0.55 h and 7.5 mg/L within 7.9 h, respectively [Dey *et al.* 1989].

Toxicity Gastrointestinal toxicity including bleeding and perforation has been reported with overdosing as well as a case of coma.

Bioavailability 80 to 100%.

Half-life 7 h.

Volume of Distribution 0.3 to 0.5 L/kg.

Clearance 40 to 47 mL/h/kg.

Protein Binding 99%.

Dose Patient with body weight >60 kg: a usual dose of 200 to 400 mg is administered every 6 to 8 h with a maximum of 1200 g daily. Patient <60 kg: maximum 20 mg/kg body weight daily.

Dey M *et al.* (1989). *Int J Pharm* 49: 121–128.

Jamali F *et al.* (1988). Application of a stereospecific high-performance liquid chromatography assay to a pharmacokinetic study of etodolac enantiomers in humans. *J Pharm Sci* 77(11): 963–966.

Koupai-Abyazani MR *et al.* (1999). Etodolac in equine urine and serum: determination by high-performance liquid chromatography with ultraviolet detection, confirmation, and metabolite identification by atmospheric pressure ionization mass spectrometry. *J Anal Toxicol* 23: 200–209.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press 6: 80–81.

Etofenamate

Analgesic

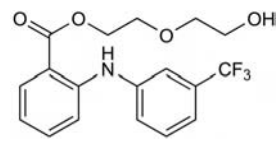
$C_{18}H_{18}F_3NO_4$ = 369.3

CAS—30544-47-9

IUPAC Name 2-(2-Hydroxyethoxy)ethyl 2-[3-(trifluoromethyl)anilino]benzoate

Synonym 2-[[3-(Trifluoromethyl)phenyl]amino]benzoic acid 2-(2-hydroxyethoxy)ethyl ester

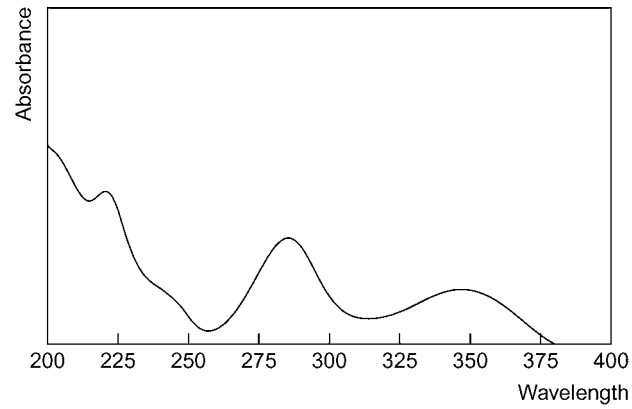
Proprietary Names *Activon*; *Afrolate*; *Algesalona*; *Bayro*; *Etofen*; *Fenogel*; *Flexium*; *Flogoprofen*; *Reumon*; *Rheuma-Gel*; *Rheumon*; *Traumalix*; *Traumon*; *Zenavan*.



Chemical Properties A pale-yellow viscous liquid. Practically insoluble in water; soluble in organic solvents. Log *P* (octanol/water), 4.2.

Thin-layer Chromatography System TE—R_f 0.78; system TAE—R_f 0.89.

Ultraviolet Spectrum Aqueous acid—284 (A^1_1=287b), 349 nm (A^1_1=165b).



Quantification

***Plasma* GC** FID. For method of quantification, see Dell *et al.* [1981].

***Urine* GC** See Plasma [Dell *et al.* 1981].

***Tissues* GC** See Plasma [Dell *et al.* 1981].

Disposition in the Body Well absorbed from the gastrointestinal tract and through the skin. The unchanged drug is found in small amounts in urine, together with hydroxylated derivatives of etofenamate and flufenamic acid.

Therapeutic Concentration

Following a single oral dose of 300 mg to 6 subjects, a mean peak plasma concentration of 10.0 mg/L was attained in 1 h; following topical administration of 6 g to 6 subjects, a mean peak plasma concentration of 0.15 mg/L was attained in 2 h [Dell *et al.* 1977].

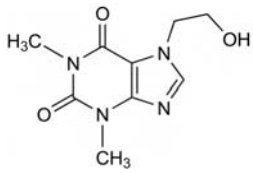
Half-life Plasma half-life, about 1.6 h after ingestion and about 3.3 h after topical administration.
Use Topically in a concentration of 5%.

Dell HD *et al.* (1977). [On biochemistry and pharmacokinetics of etofenamate/studies in man (author's transl)]. *Arzneimittelforschung* 27: 1322–1325.
Dell HD *et al.* (1981). [Gas-liquid chromatographic determination of etofenamate/ Determination, method and use in biological material (author's transl)]. *Arzneimittelforschung* 31(1): 17–21.

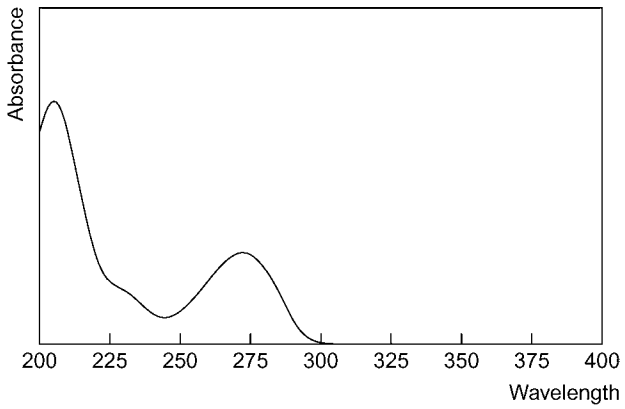
Etofylline

Xanthine Bronchodilator
$C_9H_{12}N_4O_3$ = 224.2
CAS—519-37-9

IUPAC Name 7-(2-Hydroxyethyl)-1,3-dimethylpurine-2,6-dione
Synonyms Aethophyllinum; 3,7-dihydro-7-(2-hydroxyethyl)-1,3-dimethyl-1*H*-purine-2,6-dione; hydroxyethyltheophyllinum; oxyetophylline.
Proprietary Names *Actophlem*; *Alcophyllex*; *Apoplectal*; *Bio-Phyllin*; *Coroverlan*; *Dilinct*; *Flebo Stop*; *Instenon*; *Oxyphylline*; *Perphyllon*; *Solphyllex*; *Solphyllin*; *Theophen*; *Theo-Talusin*.



Chemical Properties A white crystalline powder. Mp 161° to 166°. Freely soluble in water; moderately soluble in ethanol; sparingly soluble in chloroform; practically insoluble in ether. Log *P* (octanol/water), −0.8.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.38; system TF—R_f 0.06; system TAE—R_f 0.66.
Gas Chromatography System GA—etophylline RI 2125, etophylline-AC RI 2200, etophylline clofibrate RI 3125.
High Performance Liquid Chromatography System HX—RI 289.
Ultraviolet Spectrum Aqueous acid—270 nm (A^1_1=416b).

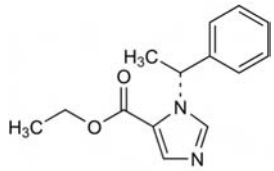


Dose Up to 1.5 g daily.

Etomidate

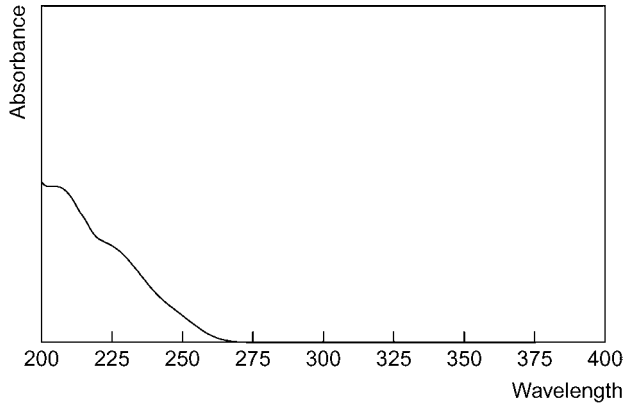
Anaesthetic (General)
$C_{14}H_{16}N_2O_2$ = 244.3
CAS—33125-97-2

IUPAC Name Ethyl 3-(1-phenylethyl)imidazole-4-carboxylate
Synonym 1-[(1*R*)-1-Phenylethyl]-1*H*-imidazole-5-carboxylic acid ethyl ester
Proprietary Names *Amidate*; *Hypnomidat(e)*; *Radenarcon*.

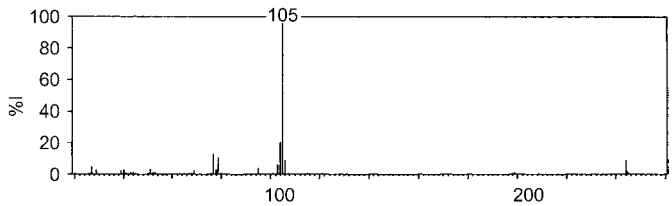


Chemical Properties A white or yellowish crystalline or amorphous powder. Mp about 67°. Solubility in water at 25°, 0.0045 mg in 100 mL; soluble in chloroform, methanol, ethanol, propylene glycol and acetone. pK_a 4.2. Log *P* (octanol/water), 3.0. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.26; system TC—R_f 0.71; system TE—R_f 0.73; system TL—R_f 0.52; system TAE—R_f 0.78; system TAF—R_f 0.85.
Gas Chromatography System GA—etomidate RI 1870, M (COOH-)-Me RI 1840.
High Performance Liquid Chromatography System HX—RI 475; system HY—RI 417.
Ultraviolet Spectrum Water—242 nm (A^1_1=450b).



Infrared Spectrum Principal peaks at wavenumbers 1212, 1708, 712, 1112, 1132, 664 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 105, 104, 77, 79, 244, 106, 108, 27.



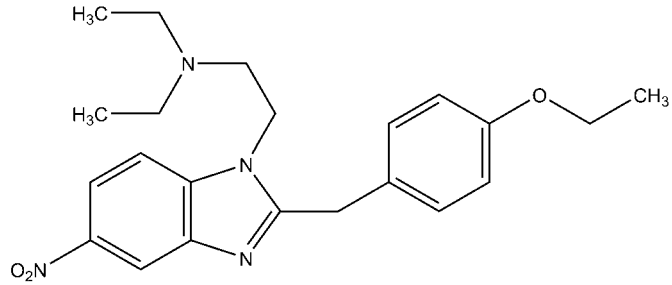
Quantification
Plasma **GC** AFID. Limit of detection, 5 µg/L [de Boer *et al.* 1979].
GC–MS Limit of detection, 1 µg/L [Van Hamme *et al.* 1977].
HPLC For method of quantification, see McIntosh and Rajewski [2001]. UV detection. Limit of detection, 2 µg/L [Ellis, Beck 1982].
Disposition in the Body Rapidly distributed after IV injection. It is metabolised in the liver by hydrolysis and *N*-dealkylation to inactive metabolites. About 90% of a dose is excreted in the urine as the carboxylic acid derivative, together with mandelic acid and benzoic acid; <5% is excreted as unchanged drug.
Therapeutic Concentration
After IV injection of 0.3 mg/kg to 8 subjects, plasma concentrations of 0.22 to 0.41 mg/L (mean 0.32) were reported at 4 min [Van Hamme *et al.* 1978].
Half-life Plasma half-life, 2 to 11 h (mean 5).
Volume of Distribution About 4 to 5 L/kg.
Clearance Plasma clearance, 9 to 18 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 0.62.
Protein Binding About 75%.
Dose 300 µg/kg IV, with supplementary doses of 100 to 200 µg/kg, as required.

de Boer AG *et al.* (1979). Assay of etomidate in plasma by capillary gas chromatography with nitrogen-selective detection. *J Chromatogr Biomed Appl* 162: 591–595.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Ellis EO, Beck PR (1982). Determination of etomidate in human plasma by high-performance liquid chromatography. *J Chromatogr Biomed Appl* 232: 207–211.
McIntosh MP, Rajewski RA (2001). A simple and efficient high-performance liquid chromatographic assay for etomidate in plasma. *J Pharm Biomed Anal* 24: 689–694.
Van Hamme MJ *et al.* (1977). Mass fragmentographic determination of plasma etomidate concentrations. *J Pharm Sci* 66: 1344–1346.
Van Hamme MJ *et al.* (1978). Pharmacokinetics of etomidate, a new intravenous anesthetic. *Anesthesiology* 49: 274–277.

Etonitazene

Opiate, Narcotic Analgesic
$C_{22}H_{28}N_4O_3$ = 396.5
CAS—911-65-9

IUPAC Name 2-[2-[(4-Ethoxyphenyl)methyl]-5-nitrobenzimidazol-1-yl]-*N*,*N*-diethylethanamine
Synonyms 1-[(2-Diethylamino)ethyl]-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole; 2-*p*-ethoxybenzyl-1-(2-diethylaminoethyl)-5-nitrobenzimidazole; 2-[(4-ethoxyphenyl)methyl]-*N*,*N*-diethyl-5-nitro-1*H*-benzimidazole-1ethanamine; etonitazine.



Chemical Properties White crystalline powder. Slightly soluble in water (0.582 mg/L). Soluble in dilute hydrochloric acid. Log *P* (octanol/water) 5.11 [Meylan, Howard 1995]. Extracted by organic solvents from aqueous alkaline solutions.

Etonitazene Hydrochloride

$C_{22}H_{28}N_4O_3$,HCl = 432.9

Chemical Properties Mp 162° to 164° [O'Neil *et al.* 2006].

Thin-layer Chromatography System T1—R_f 0.70 (location reagent acidified iodoplatinate spray, positive reaction). Plates: fluorescent silica. Solvent system: methanol. Detection using fluorescence quenching (λ_{ex} = 254 nm). R_f value: 0.82. Limit of quantification not reported [Gabor, Leader 1980].

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—230, 282 nm.

Quantification

Note For the quantitative determination of etonitazene using chemical reduction and fluorescence detection, see Gabor and Leader [1980].

Dose Used as an analgesic having morphine-like activity. It is about 3 orders of magnitude more potent than morphine.

Gabor G, Leader H (1980). Quantitative determination of etonitazene. *Anal Biochem* 106: 377–379.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Etoposide

Antineoplastic

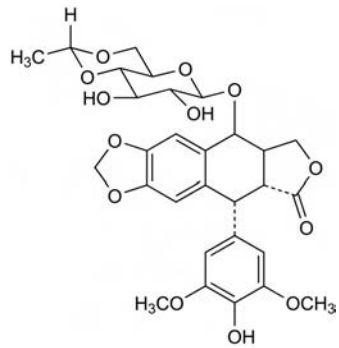
$C_{29}H_{32}O_{13}$ = 588.6

CAS—33419-42-0

IUPAC Name (5*S*,5*aR*,8*aR*,9*R*)-5-[[(2*R*,4*aR*,6*R*,7*R*,8*R*,8*aS*)-7,8-Dihydroxy-2-methyl-4,4*a*,6,7,8,8*a*-hexahydropyrano[3,2-*d*][1,3]dioxin-6-yl]oxy]-9-(4-hydroxy-3,5-dimethoxyphenyl)-5*a*,6,8*a*,9-tetrahydro-5*H*-[2]benzofuro[6,5-*f*][1,3]benzodioxol-8-one

Synonyms EPEG; 9-[(4,6-*O*-ethylidene-β-D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one; NSC-141540; VP-16; VP-16-213.

Proprietary Names *Celltop*; *Eposin*; *Exitop*; *Lastet*; *Toposar*; *Vepesid*; *Vepeside*.



Chemical Properties A fine white, or almost white, crystalline powder. Mp 236° to 251°. It is practically insoluble or very slightly soluble in water; slightly soluble in ethanol, in chloroform, in dichloromethane, in ethyl acetate, and in methylene chloride; sparingly soluble in methanol. pK_a 9.8. Log *P* (octanol/water), 0.60.

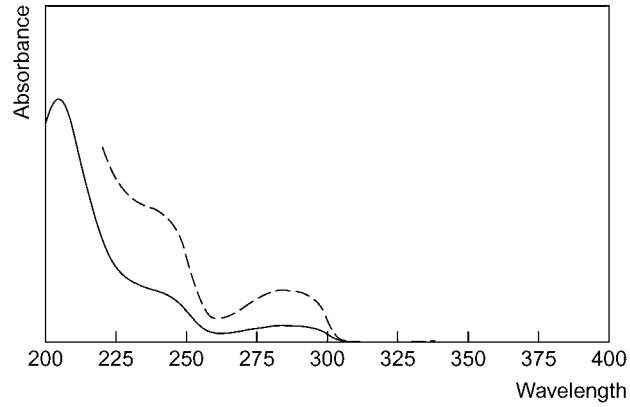
Etoposide Phosphate

$C_{29}H_{33}O_{16}P$ = 668.6

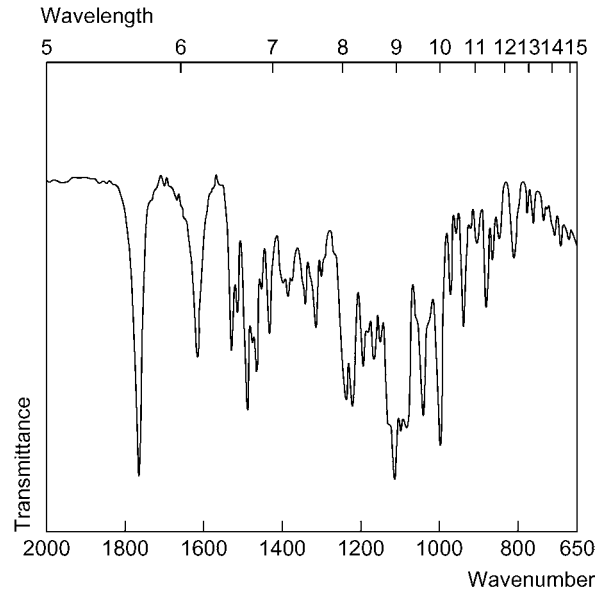
Proprietary Name *Etopophos*

High Performance Liquid Chromatography Column: ODS Hypersil Si-10 (300 × 4 mm i.d., 10 µm). Mobile phase: methanol with 1% NHOH : methylene chloride (2:98), flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: 4.6 min [Mills, Roberson 1993].

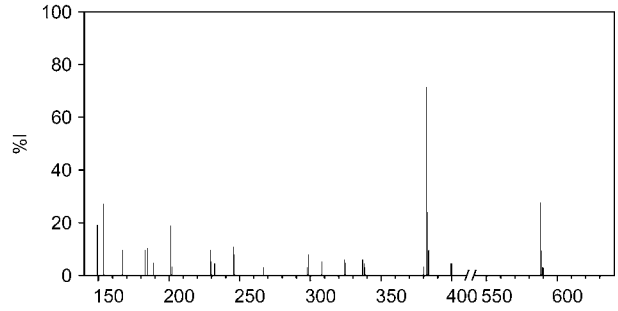
Ultraviolet Spectrum Methanol—283 nm (A^1_1=72.2); ethanol—284, 291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1775, 1610, 1515, 1485, 1250 cm^{-1}.



Mass Spectrum



Quantification

Plasma **HPLC** Column: phenyl µBondapak (300 × 3.9 mm, 10 µm). Mobile phase: acetonitrile : water : glacial acetic acid (35:64:1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=288 nm, λ_{em}=328 nm). Internal standard (IS): teniposide. Retention times: etoposide, 6.5 min; IS, 18 min. Limit of detection, 50 µg/L [Robieux *et al.* 1996]. Electrochemical detection. Limit of detection, 0.01 µg/L [el-Yazigi, Martin 1987]. Electrochemical detection. Limit of detection, 10 µg/L [Duncan *et al.* 1986]. UV detection. Limit of detection, 100 µg/L [Harvey *et al.* 1985]. Electrochemical detection. Limit of detection, 5 µg/L [Littlewood *et al.* 1984]. Fluorescence detection. Limit of detection, 0.008 µg [Werkhoven-Goewie *et al.* 1983].

Serum **HPLC** See Plasma [Werkhoven-Goewie *et al.* 1983].

Urine **HPLC** See Plasma [Harvey *et al.* 1985]. See Plasma [Werkhoven-Goewie *et al.* 1983].

Disposition in the Body Pharmacokinetics show wide interindividual variation. Absorption is variable after oral administration, but on average about 50% of a dose is absorbed. Peak plasma concentrations occur 0.5 to 3 h after oral administration. Undergoes rapid distribution. It is metabolised to several metabolites,

including a hydroxy acid metabolite, glucuronide and sulfate conjugates, and an *O*-demethylated compound. Excreted in urine and via bile in the faeces (a small amount) as unchanged drug and metabolites; 30 to 50% of a dose is excreted in urine over 72 h as the unchanged drug and 20% as metabolites. Crosses the blood–brain barrier poorly; concentrations in CSF about 1 to 10% of those in plasma. It is distributed into breast milk.

Therapeutic Concentration The trough serum therapeutic concentration range is 2 to 6 mg/L and peak, 8 to 14 mg/L.

Twelve patients with biopsy-proven cancer, <65 years old, were split into four groups and administered either 400, 500, 600 or 800 mg/m^2 daily via a 2- to 3-h infusion. The maximum plasma-etoposide concentration following infusion of 400 mg/m^2 for 3 consecutive days ranged from 26 to 53 mg/L, 27 to 73 mg/L for the 500 mg/m^2 dose, 42 to 114 mg/L for 600 mg/m^2 and 72 mg/L for the single patient administered 800 mg/m^2. These concentrations were observed during the infusion period [Hande *et al.* 1984].

Fifteen adult patients with various malignancies, aged between 25 and 74 years, were administered 80 to 120 mg/m^2 etoposide over 30 to 60 min. All patients were receiving polychemotherapy as well. A mean peak concentration of 24.2 mg/L was observed [Pfluger *et al.* 1987].

Toxicity Etoposide doses of 2.4 to 3.5 mg/m^2 administered IV over 3 days caused severe mucositis and myelotoxicity. Metabolic acidosis and cases of severe hepatotoxicity have also been reported with doses above the recommended [Bristol Myers Squibb Pharmaceuticals Ltd].

The dose-limiting toxicity is dose-related myelosuppression.

Half-life Plasma half-life, 3 to 19 h.

Volume of Distribution Adults, 7 to 17 L/m^2; children, 5 to 10 L/m^2.

Clearance Plasma, adult, 15 to 35 mL/min/m^2 (0.9 to 2.1 L/h/m^2); children, 20 to 40 mL/min/m^2 (1.2 to 2.4 L/h/m^2).

Protein Binding About 94%.

Note For reviews of etoposide, see Henwood and Brogden [1990] and Clark and Slevin [1987].

Dose From 120 mg/m^2 up to 240 mg/m^2 of etoposide IV daily for 5 days. Oral dose is twice the IV dose.

Bristol Myers Squibb Pharmaceuticals Ltd., prescribing data

Clark PI, Slevin ML (1987). The clinical pharmacology of etoposide and teniposide. *Clin Pharmacokinet* 12: 223–252.

Duncan GF *et al.* (1986). High-performance liquid chromatographic method for the determination of etoposide in plasma using electrochemical detection. *J Chromatogr* 380(2): 357–365.

el-Yazigi A, Martin CR (1987). Improved assay for etoposide in plasma by radial-compression liquid chromatography with electrochemical detection. *Clin Chem* 33: 803–805.

Hande KR *et al.* (1984). Pharmacokinetics of high-dose etoposide (VP-16-213) administered to cancer patients. *Cancer Res* 44: 379–382.

Harvey VJ *et al.* (1985). High-performance liquid chromatography of etoposide in plasma and urine. *J Chromatogr* 339(2): 419–423.

Henwood JM, Brogden RN (1990). Etoposide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in combination chemotherapy of cancer. *Drugs* 39: 438–490.

Littlewood TJ *et al.* (1984). High-performance liquid chromatographic determination of etoposide in plasma using electrochemical detection. *J Chromatogr* 336(2): 434–437.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press 1: 908–909

Pfluger KH *et al.* (1987). Drug monitoring of etoposide (VP16-213). Correlation of pharmacokinetic parameters to clinical and biochemical data from patients receiving etoposide. *Cancer Chemother Pharmacol* 20: 59–66.

Robieux I *et al.* (1996). Determination of unbound etoposide concentration in ultrafiltered plasma by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr B Biomed Appl* 686(1): 35–41.

Werkhoven-Goewie CE *et al.* (1983). Automated liquid chromatographic analysis of the antitumorigenic drugs etoposide (VP 16-213) and teniposide (VM 26). *J Chromatogr* 276: 349–57.

Etoricoxib

Analgesic, COX-2 Inhibitor

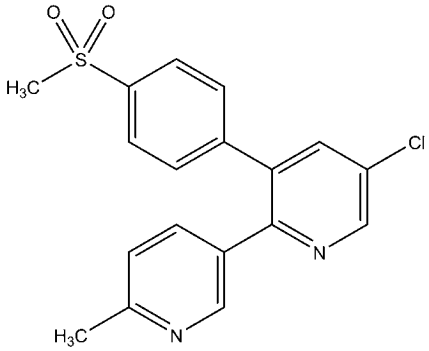
$C_{18}H_{15}ClN_2O_2S = 358.8$

CAS—202409-33-4

IUPAC Name 5-Chloro-6′-methyl-3-[4-(methylsulfonyl)-phenyl]-2,3′-bipyridine

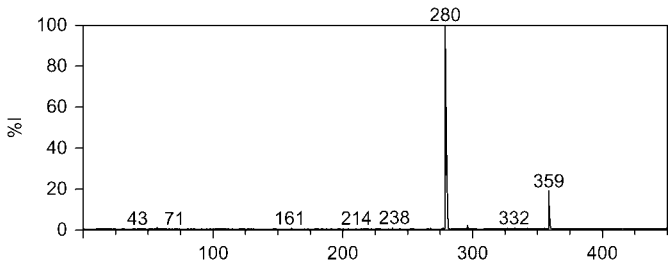
Synonyms L-791456; MK-0663.

Proprietary Name *Arcoxia*



Chemical Properties White solid. Mp 127° to 128°. pK_a 4.5. Stability was established for 21 days at –20°, in frozen plasma at –20° for at least 3 freeze–thaw cycles, over a period of 24 h at 5°, and on the benchtop for 8 h [Pavan Kumar *et al.* 2006]. Stability of etoricoxib stored at -80° including 2 freeze–thaw cycles was >90%. Standard solutions were found to be stable at –80° for at least 2 months and a minimum of 2 weeks when stored protected from light at room temperature [Werner *et al.* 2005]. Stock solutions were stable for at least 6 months when stored under light-protected conditions at 4°. Etoricoxib was stable in plasma for at least 30 days if stored at –70° as well as after 3 freeze–thaw cycles and 24 h room temperature storage [Ramakrishna *et al.* 2005].

Mass Spectrum Principal ions at *m/z* 280, 359, 238, 161.



Quantification

Plasma **HPLC** Column: Kromasil KR 100-5 C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water–acetonitrile (5 : 95) : methanol–water (90 : 10; 100 : 0 : 0 at 0 min, 90 : 0 : 10 at 2 min, 50 : 20 : 30 at 9 min, 30 : 50 : 20 at 25 min, 10 : 85 : 5 at 35 min, 100 : 0 : 0 at 36 min until 45 min), flow rate 1.0 mL/min. UV detection ($\lambda = 235$ nm). Limit of quantification, 0.1 mg/L [Pavan Kumar *et al.* 2006]. Column: Waters symmetry C_{18} (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : water (42 : 58), flow rate 1.2 mL/min. UV detection ($\lambda = 284$ nm). Limit of quantification, 5 µg/L [Ramakrishna *et al.* 2005]. Column: Prism RP (150 × 4.6 mm). Mobile phase: acetonitrile : water (45 : 55) in 10 mmol/L acetate buffer (pH 4). Fluorescence detection ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 375$ nm). Limit of quantification, 500 µg/L [Matthews *et al.* 2001].

LC-MS Column: Nucleosil C_8 guard column (120-5, 8 × 3 mm). Mobile phase: methanol : water (50 : 50), flow rate 300 µL/min. APCI source, SRM acquisition mode. Retention time: 1.05 min. Limit of quantification, 5 µg/L [Werner *et al.* 2005]. Limit of quantification, 0.2 µg/L [Brautigam *et al.* 2003]. Limit of quantification, 0.5 µg/L [Rose *et al.* 2002].

Urine **HPLC** Column: Prism RP (150 × 4.6 mm). Mobile phase: acetonitrile : water (45 : 55) in 10 mmol/L acetate buffer (pH 4). Fluorescence detection ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 375$ nm). Limit of quantification, 500 µg/L [Matthews *et al.* 2001].

Disposition in the Body Eliminated primarily as metabolites in urine, with <1% of an administered dose recovered as the unchanged drug. Renal and hepatic impairments do not significantly affect the absorption and distribution of the drug. It is metabolised via 6′-methyl oxidation and 1′-*N*-oxidation, yielding 1 major compound, a 6′-carboxylic acid metabolite, and 4 minor compounds: etoricoxib 1′-*N*-oxide, 6′-hydroxymethyletoricoxib, its 1′-*N*-oxide and its glucuronide. These metabolites do not inhibit COX-1 and do not significantly contribute to the inhibition of COX-2. They are formed by the following P450 enzymes: CYP3A4 (major role), CYP2C9, CYP2D6, CYP1A2, and CYP2C19. In addition, oxidation of 6′-hydroxymethyletoricoxib to its corresponding carboxylic acid requires human liver cytosol and NAD^+. Excretion is preliminary via the renal route with 70% (IV) and 60% (oral) of the dose excreted in urine. Faecal elimination accounts for around 20% for either route of administration.

Therapeutic Concentration

Sixty healthy male and female subjects and ten patients with mild hepatic insufficiency were administered simultaneous single 5 mg IV doses of [$^{13}C_6$] etoricoxib (day 1) and 60 mg oral doses of etoricoxib once daily for 21 days. A further 10 patients with moderate hepatic insufficiency were administered the same regimen except the oral doses were administered every other day for 21 days. The mean peak plasma concentrations after the single dose were 1.69, 1.65, and 1.68 mg/L for the groups, respectively. These were observed at 1.06, 1.75, and 1.75 h, respectively. After dosing 60 mg to 152 healthy subjects and 6 patients with mild hepatic insufficiency, peak plasma concentrations were 1.11 and 1.10 mg/L, respectively, attained after 1.01 and 1.25 h, respectively [Agrawal *et al.* 2003a].

Twelve healthy male and female subjects were administered a single oral dose of 120 mg etoricoxib in a fasting state and after a meal with high fat content. The mean peak plasma concentrations were 2.43 ± 0.66 and 1.56 ± 0.36 mg/L, respectively, observed at 1.0 (0.5 to 2.0) and 3.0 (0.5 to 10.0) h, respectively. Another 12 healthy subjects were administered single oral doses of 30, 60, 120 and 240 mg etoricoxib. The mean peak plasma concentrations were 0.53 ± 0.14, 1.27 ± 0.36, 2.14 ± 0.6 and 4.37 ± 1.2 mg/L, attained at 1.8 (0.4 to 4.0), 1.0 (0.5 to 1.5), 1.5 (0.5 to 2.0) and 1.3 (0.5 to 2.0) h, respectively. Twenty-four healthy subjects were also administered 120 mg single dose and multiple oral doses (120 mg daily for 10 days). After the single dose, the mean peak plasma concentration was 2.05 ± 0.67 mg/L at

1.5 h (0.5 to 24.0) and was 3.59 ± 1.31 mg/L after the last dose during multiple dosing, attained at 1.3 h (0.5 to 4.0) [Agrawal *et al.* 2003b].

Toxicity On 27 April 2007, the FDA issued a 'non-approvable letter' that stated that Merck are required to provide more study data to prove that the benefits of etoricoxib outweigh the risks before it will receive FDA approval.

Bioavailability 100%.

Half-life 27 h.

Clearance 49 mL/min.

Protein Binding 91.9% (healthy); 86 to 88% (renal impairment).

Volume of Distribution 119 L at steady state

Dose 60 to 120 mg daily.

Agrawal NG *et al.* (2003). Pharmacokinetics of etoricoxib in patients with hepatic impairment. *J Clin Pharmacol* 43: 1136–1148.

Agrawal NG *et al.* (2003). Single- and multiple-dose pharmacokinetics of etoricoxib, a selective inhibitor of cyclooxygenase-2, in man. *J Clin Pharmacol* 43: 268–276.

Brautigam L *et al.* (2003). Determination of etoricoxib in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionisation. *J Chromatogr B Analyt Technol Biomed Life Sci* 788: 309–315.

Matthews CZ *et al.* (2001). High-throughput, semi-automated determination of a cyclooxygenase II inhibitor in human plasma and urine using solid-phase extraction in the 96-well format and high-performance liquid chromatography with post-column photochemical derivatization-fluorescence detection. *J Chromatogr B Biomed Sci Appl* 751: 237–246.

Pavan Kumar VV *et al.* (2006). Simultaneous quantitation of etoricoxib, salicylic acid, valdecoxib, ketoprofen, nimesulide and celecoxib in plasma by high-performance liquid chromatography with UV detection. *Biomed Chromatogr* 20: 125–132.

Ramakrishna NV *et al.* (2005). Validated liquid chromatographic ultraviolet method for the quantitation of etoricoxib in human plasma using liquid–liquid extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 816: 215–221.

Rose MJ *et al.* (2002). Simultaneous determination of unlabeled and carbon-13-labeled etoricoxib, a new cyclooxygenase-2 inhibitor, in human plasma using HPLC-MS/MS. *J Pharm Sci* 91: 405–416.

Werner U *et al.* (2005). A liquid chromatography–mass spectrometry method for the quantification of both etoricoxib and valdecoxib in human plasma. *Biomed Chromatogr* 19: 113–118.

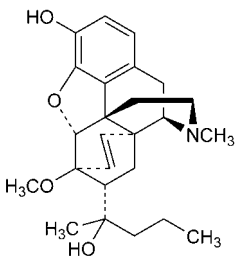
Etorphine

Narcotic Analgesic (Veterinary)

$C_{25}H_{33}NO_4$ = 411.5

CAS—14521-96-1

Synonyms (–)-[5α,7α(*R*)]-4,5-Epoxy-3-hydroxy-6-methoxy-α,17-dimethyl-α-propyl-6,14-ethenomorphinan-7-methanol; M-99; 19-propylorvinol; (6*R*,7*R*,14*R*)-7,8-dihydro-7-(1-hydroxy-1-methylbutyl)-6-*O*-methyl-6,14-ethenomorphine.



Chemical Properties Mp 214° to 217°. Soluble 1 in 30 000 of water; freely soluble in ethanol, chloroform and ether. Log *P* (octanol/pH 7.4), 1.9 (octanol/water) 2.79 [Sangster 1997].

Caution It is dangerous to smell or taste this material.

Etorphine Hydrochloride

$C_{25}H_{33}NO_4$,HCl = 448.0

CAS—13764-49-3

Proprietary Name It is an ingredient of *Immobilon*.

Chemical Properties White microcrystalline powder. Mp 266° to 267°. Soluble 1 in 40 of water, 1 in 30 of ethanol and 1 in 2200 of chloroform; practically insoluble in ether.

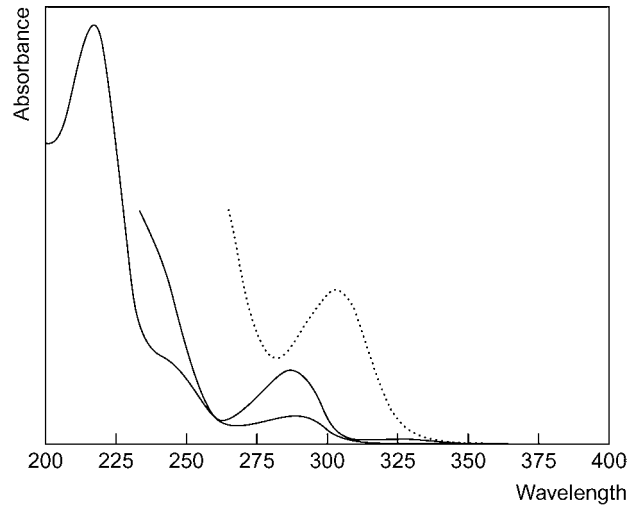
Colour Test Marquis test—blue-grey→yellow-brown.

Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.08; system TC—R_f 0.61; system TE—R_f 0.72; system TL—R_f 0.63; system TAE—R_f 0.84; system TAJ—R_f 0.52; system TAK—R_f 0.00; system TAL—R_f 0.60 (acidified iodoplatinate solution, positive; Marquis reagent, grey; acidified potassium permanganate solution, positive).

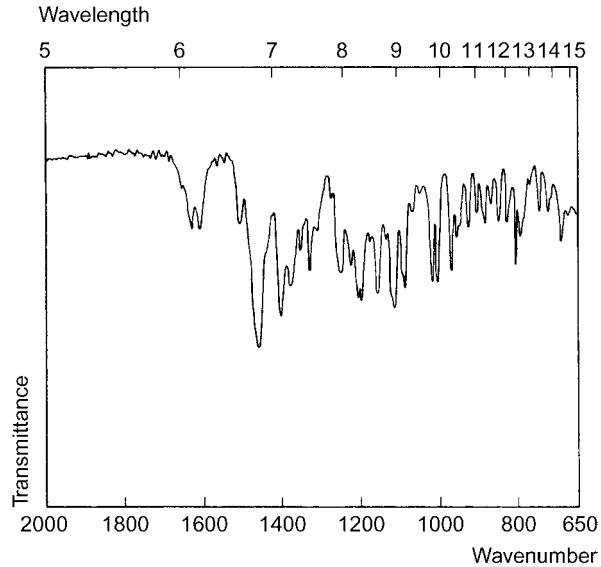
Gas Chromatography System GA—RI 3033; system GB—RI 3211; system GM—not eluted.

High Performance Liquid Chromatography System HA—*k* 0.6; system HC—*k* 1.11; system HX—RI 344; system HY—RI 292.

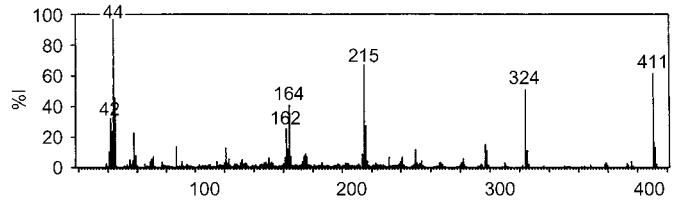
Ultraviolet Spectrum Aqueous acid—289 (A_1^1 = 37a); aqueous alkali—302 nm (A_1^1 = 65a).



Infrared Spectrum Principal peaks at wavenumbers 1113, 1195, 1205, 1155, 1088, 1005 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 44, 215, 411, 324, 45, 164, 42, 216.



Quantification

Blood **HPLC** Column; Spherisorb S5OD/CN (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (0 : 100 to 70 : 30 in 15 min for 3 min, or 20 : 80), flow rate 2 mL/min. DAD (λ = 200–595 nm). Limit of quantification, 2 µg/L [Elliott, Hale 1999].

Urine **GC** Column: 3% OV-17 on 80/100 mesh Gas Chrom Q (3′ × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 230°. FID. Retention time: 17 min. Limit of detection, 0.1 mg/L [Gorodetzky, Kullberg 1975].

Other **GC-MS** Equine Urine. Column: CP-Sil 5 Chrompak (25 m × 0.22 mm). Carrier gas: He, 2.0 mL/min. Temperature programme: 60° to 120° at 20°/min to 285° at 8°/min. EI ionisation, SIM acquisition mode. Limit of detection, 0.1 µg/L [Bonnaire *et al.* 1989].

Disposition in the Body

Toxicity It is up to 1000-times more potent than morphine; several cases of poisoning (including onefatality) have been reported after accidental pricking of the skin with an injection needle [Goodrich 1977; Volans, Whittle 1976].

A 54-year-old veterinary surgeon whose death was suspected to be due to parenteral administration of etorphine had the following postmortem tissue concentrations: femoral blood 14.5 µg/L, heart blood 23.5 µg/L, urine 0 µg/L [Elliott, Hale 1999].

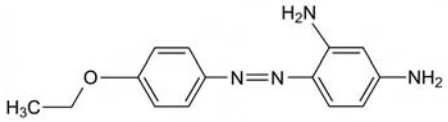
Note For a case of etorphine poisoning, see Brink and Erasmus [2003] or for overdose, see Sterken *et al.* [2004].
Use With acepromazine as a sedative for the control of large animals, and with methotrimeprazine (levomepromazine) as a neuroleptanalgesic in small animals.

Bonnaire Y *et al.* (1989). GC/MS confirmatory method for etorphine in horse urine. *J Anal Toxicol* 13: 193–196.
Brink CF, Erasmus J (2003). Etorphine poisoning. *S Afr Med J* 93: 761–762.
Elliott SP, Hale KA (1999). Analysis of etorphine in postmortem samples by HPLC with UV diode-array detection. *Forensic Sci Int* 101: 9–16.
Goodrich PG (1977). Accidental self-injection. *Vet Rec* 100: 458–459.
Gorodetzky CW, Kullberg MP (1975). Etrophine in man. II. Detectability in urine by common screening methods. *Clin Pharmacol Ther* 17: 273–276.
Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* London: John Wiley and Sons.
Sterken J *et al.* (2004). Intentional overdose of Large Animal Immobilon. *Eur J Emerg Med* 11: 298–301.
Volans GN, Whittle BA (1976). Letter: Accidental injection of Immobilon. *Br Med J* 2: 472–473.

Etoxazene

Analgesic
$C_{14}H_{16}N_4O$ = 256.3
CAS—94-10-0

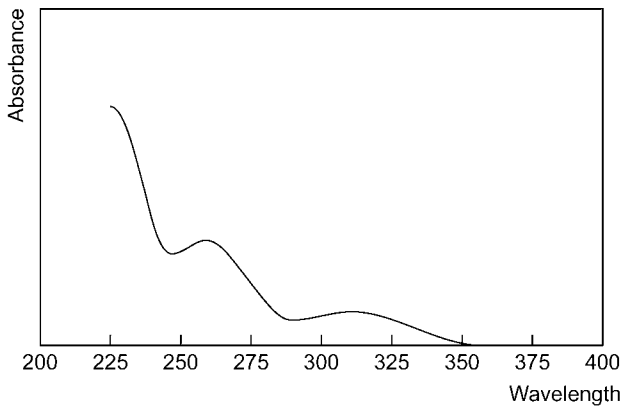
IUPAC Name 4-(4-Ethoxyphenylazo)benzene-1,3-diyldiamine
Synonyms *p*-Ethoxychrysoidine; ethoxazene.



Chemical Properties Mp 117° to 120°. Log *P* (octanol/water), 2.7.

Etoxazene Hydrochloride
$C_{14}H_{16}N_4O,HCl$ = 292.8
CAS—2313-87-3
Proprietary Name *Serenium*
Chemical Properties A reddish powder. Practically insoluble in water; soluble in boiling water and in ethanol.

Colour Test Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.00; system TC—R_f 0.56; system TL—R_f 0.67; system TAJ—R_f 0.57; system TAK—R_f 0.02; system TAL—R_f 0.70 (Dragendorff spray, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—261 (A^1_1=308b), 310 nm.

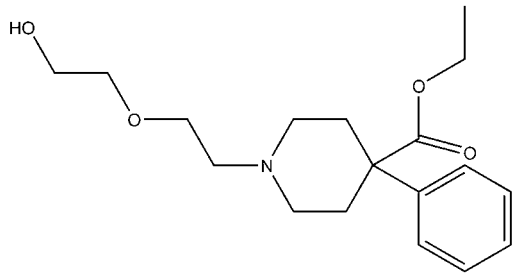


Infrared Spectrum Principal peaks at wavenumbers 1245, 1624, 1635, 1600, 1185, 1501 cm^{-1} (KBr disk).
Dose Etoxazene hydrochloride has been given in doses of 300 mg daily.

Etoxeridine

Phenylpiperidine, Narcotic
$C_{18}H_{27}NO_4$ = 321.4
CAS—469-82-9

IUPAC Name Ethyl 1-[2-(2-hydroxyethoxy)ethyl]-4-phenylpiperidine-4-carboxylate
Synonyms Aténorax; atenos; carbetidine; UC 2073; UCB 2073; Wy 2039.

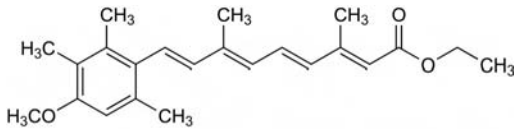


Chemical Properties White crystalline powder. Soluble in dilute hydrochloric acid. Extracted by organic solvents from aqueous alkaline solutions.
Thin-layer Chromatography System T1—R_f 0.64 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 0.85 (relative to codeine); system G4—retention time 1.05 (relative to codeine).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—258, 252, 264 nm.

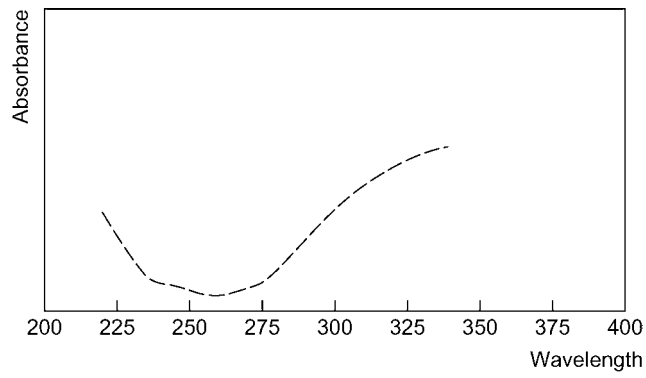
Etretinate

Dermatological Agent
$C_{23}H_{30}O_3$ = 354.5
CAS—54350-48-0

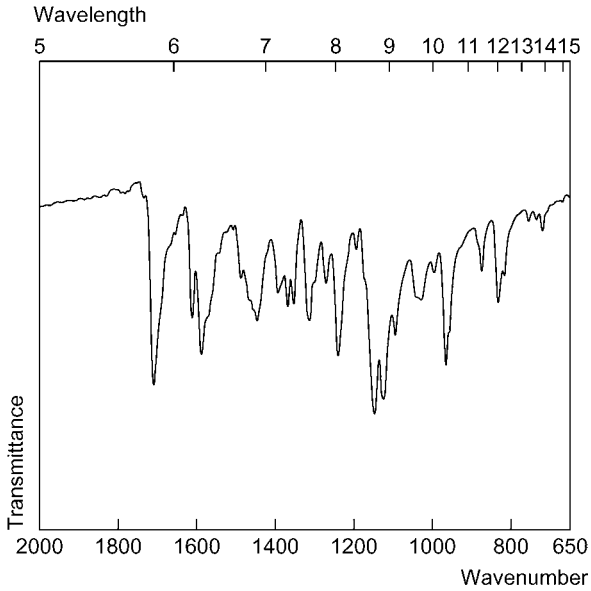
IUPAC Name Ethyl (2*E*,4*E*,6*E*,8*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4, 6,8-tetraenoate
Synonyms (*all-E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid ethyl ester; Ro-10-9359.
Proprietary Names *Tegison*; *Tigason*.



Chemical Properties Crystals. Mp 104° to 105°.
Gas Chromatography Column: methyl silicone (HP1, 0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. Detection: mass spectrometer. Retention index: 2761 [Mills, Roberson 1993].
High Performance Liquid Chromatography Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 1 mL/min. UV detection (λ=365 nm). Retention time: 3.4 min [Mills, Roberson 1993].
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1703, 1140, 957 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** UV detection (λ=350 nm). Limit of detection, 3 μg/L [De Leenheer *et al.* 1990]. UV detection (λ=360 nm). Limit of quantification, 2 μg/L [Wyss, Bucheli 1988]. Column: Nucleosil 5 C_{18} (250 × 4.6 mm), steel. Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (80:20), flow rate 2.5 mL/min. UV diode array detection. Retention time: 6.8 min. Limit of detection, 1 μg/L [Jakobsen *et al.* 1987]. UV detection. Limit of detection, 4 μg/L [Paravicini, Busslinger 1983]. UV detection. Limit of detection, 25 μg/L [Besner *et al.* 1982].

Disposition in the Body Etretinate is rapidly absorbed after an initial lag time of ~1 h following oral administration. Peak plasma concentrations occur after about 2 to 6 h. Absorption is increased by administration with milk or fatty food. The drug undergoes significant first-pass metabolism (hydrolysis) and the active metabolite is acitretin (the carboxyl acid, Ro 10-1670) which may itself be metabolised to etretinate. Subsequent metabolism occurs to inactive 13-*cis*-acitretin (Ro 13-7652, the major metabolite in blood following multiple dosing) and other metabolites. Etretinate accumulates in adipose tissue (especially in liver and subcutaneous fat) after repeated dosing; detectable concentrations have been found in serum up to 3 years after discontinuation of therapy. Up to 75% of a dose is excreted via bile in faeces mainly as the unchanged drug, acitretin, β-glucuronides and other minor metabolites (18 metabolites have been identified in total). It is also excreted in urine as metabolites. No intact drug is present in urine. It crosses the placenta and is distributed into breast milk.

Therapeutic Concentration

Peak plasma concentrations of 180 to 353 μg/L (mean 238 μg/L) occurred after 3 to 3.5 h in patients with severe psoriasis (aged between 41 and 72 years) who had been taking 40 mg of etretinate daily for 3 months. The drug was administered after an overnight fast and also patients fasted for 3 h after dosing [Larsen *et al.* 1987].

Toxicity Etretinate is teratogenic at therapeutic doses.

Bioavailability 40%.

Half-life Plasma half-life, 120 days.

Volume of Distribution 40 L/kg (measured after oral administration).

Distribution in Blood Blood : plasma ratio, 0.60. Red blood cell : plasma, 0.11.

Protein Binding >99% (mainly to lipoproteins).

Dose Initially, 0.75 to 1 mg/kg per day with a maximum of 1.5 mg/kg, orally. 75 mg daily should not be exceeded.

Besner JG *et al.* (1982). High-performance liquid chromatography of Ro 10-9359 (tigason) and its metabolite Ro 10-1670 in human plasma. *J Chromatogr* 231: 467–472.

De Leenheer AP *et al.* (1990). High-performance liquid chromatographic determination of etretinate and all-trans- and 13-cis-acitretin in human plasma. *J Chromatogr* 500: 637–642.

Jakobsen P *et al.* (1987). Simultaneous determination of the aromatic retinoids etretin and etretinate and their main metabolites by reversed-phase liquid chromatography. *J Chromatogr* 415: 413–418.

Larsen FG *et al.* (1987). Single dose pharmacokinetics of etretin and etretinate in psoriatic patients. *Pharmacol Toxicol* 61(2): 85–88.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press 5: 180–181

Paravicini U, Busslinger A (1983). Determination of etretinate and its main metabolite in human plasma using normal-phase high-performance liquid chromatography. *J Chromatogr* 276: 359–366.

Wyss R, Bucheli F (1988). Quantitative analysis of retinoids in biological fluids by high-performance liquid chromatography using column switching. II. Simultaneous determination of etretinate, acitretin and 13-cis-acitretin in plasma. *J Chromatogr* 431: 297–307.

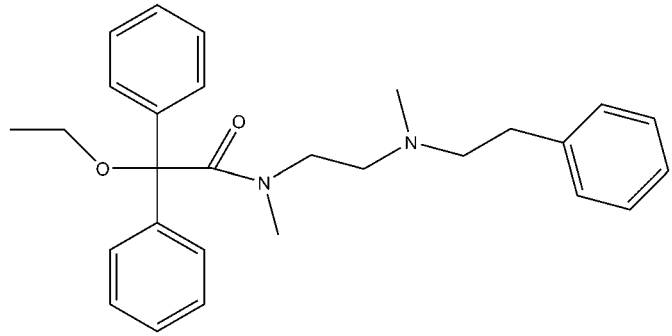
Etymide

Acetamide, Analgesic

$C_{28}H_{34}N_2O_2$ = 430.6

IUPAC Name 2-Ethoxy-*N*-methyl-*N*-[2-(methyl-(2-phenylethyl)amino)ethyl]-2,2-di(phenyl)acetamide

Synonyms Carbiphene; etomide; SQ 10269.



Chemical Properties Extracted by organic solvents from aqueous alkaline solutions.

Etymide Hydrochloride

Proprietary Names *Bandol*; *Jubalon*.

Colour Tests Ammonium molybdate test—orange→blue (limit of detection, 0.1 μg).

Thin-layer Chromatography System T1—R_f 0.72 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—258, 263 nm, and an inflexion at ~270 nm.

Dose Up to 450 mg daily has been given.

Etynodiol Diacetate

Progestational Steroid

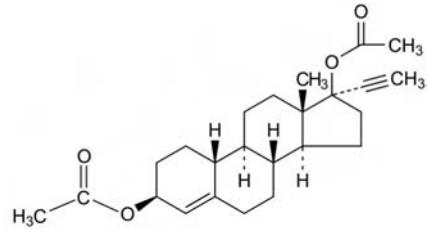
$C_{24}H_{32}O_4$ = 384.5

CAS—1231-93-2 (etynodiol); 297-76-7 (diacetate)

IUPAC Name [(3*S*,8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Acetyloxy-17-ethynyl-13-methyl-2,3,6,7,8,9,10,11,12,14,15,16-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl] acetate

Synonym (3β,17α)-19-Norpregn-4-en-20-yne-3,17-diol diacetate

Proprietary Names *Femulen*; *Lutométrodiol*. It is an ingredient of *Conova 30*, *Demulen*, *Normex*, *Ovulen* and *Zovia*.



Chemical Properties A white crystalline powder. Mp 126° to 131°. Very slightly soluble in water; soluble 1 in 15 of ethanol, 1 in 1 of chloroform and 1 in 3.5 of ether. Log *P* (octanol/water), 3.6 (etynodiol).

Thin-layer Chromatography System TB—R_f 0.11; system TE—R_f 0.71; system TF—R_f 0.57; system TP—R_f 0.83; system TQ—R_f 0.61; system TR—R_f 0.95; system TS—R_f 0.99; system TAE—R_f 0.89.

Gas Chromatography System GA— RI 2445 and RI 2779.

High Performance Liquid Chromatography System HX—RI 549.

Infrared Spectrum Principal peaks at wavenumbers 1252, 1226, 1746, 1737, 1012, 1028 cm^{-1} (KBr disk).

Dose 0.5 to 6 mg daily.

Eucatropine

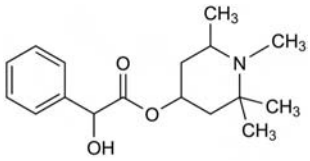
Anticholinergic

$C_{17}H_{25}NO_3$ = 291.4

CAS—100-91-4

IUPAC Name (1,2,2,6-Tetramethylpiperidin-4-yl) 2-hydroxy-2-phenylacetate

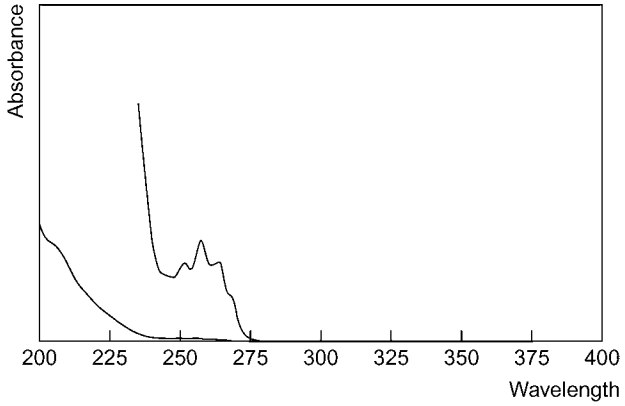
Synonym α-Hydroxybenzeneacetic acid 1,2,2,6-tetramethyl-4-piperidinyl ester



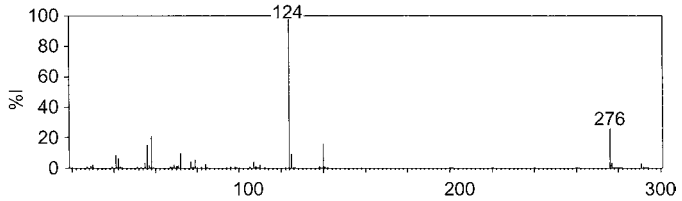
Chemical Properties Log *P* (octanol/water), 1.9.

Eucatropine Hydrochloride
$C_{17}H_{25}NO_3$,HCl = 327.9
CAS—536-93-6
Chemical Properties A white granular powder. Mp 183° to 186°. Very soluble in water; freely soluble in ethanol and chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.18; system TC—R_f 0.13; system TE—R_f 0.60; system TL—R_f 0.12; system TAJ—R_f 0.03; system TAK—R_f 0.02; system TAL—R_f 0.33 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2026.
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=6.8a), 264 nm.



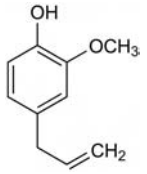
Infrared Spectrum Principal peaks at wavenumbers 1720, 1235, 1740, 1295, 1145, 1262 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 124, 276, 58, 140, 56, 72, 125, 41.



Use Eucatropine hydrochloride is used as a 2 to 10% ophthalmic solution.

Eugenol

Essential Oil
$C_{10}H_{12}O_2$ = 164.2
CAS—97-53-0
IUPAC Name 2-Methoxy-4-(2-propenyl)phenol
Synonyms 4-Allylguaiacol; caryophyllic acid; eugenic acid.

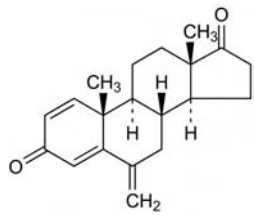


Chemical Properties Eugenol is the principal constituent of clove oil. A colourless or pale-yellow liquid with an odour of clove. It darkens in colour with age or on exposure to air. Mass per mL 1.064 to 1.068 g. Bp 255°. Refractive index, at 20°, 1.540 to 1.542. Practically insoluble in water; soluble 1 in 2 of ethanol (70%); miscible with ethanol, chloroform and ether; soluble in glacial acetic acid and in aqueous fixed alkali hydroxide solutions. pK_a 9.8 (20°). Log *P* (octanol/water), 2.3.
Thin-layer Chromatography System TA—R_f 0.95; system TE—R_f 0.87; system TAJ—R_f 0.85; system TAK—R_f 0.78; system TAL—R_f 0.96.
Gas Chromatography System GA—RI 1368; system GB—RI 1380.
High Performance Liquid Chromatography System HY—RI 430.
Ultraviolet Spectrum Aqueous alkali—246 (A^1_1=552b), 296 nm (A^1_1=262b); ethanol—232 (A^1_1=406b), 282 nm (A^1_1=193b).
Mass Spectrum Principal ions at *m/z* 164, 149, 131, 137, 103, 77, 133, 165.
Quantification
Serum **HPLC** For method of quantification, see Fischer and Dengler [1990].
Urine **GC–MS** For method for quantification of eugenol and other methoxyphenols, see Dills *et al.* [2001].
HPLC See Serum [Fischer, Dengler 1990].
Bile **HPLC** See Serum [Fischer, Dengler 1990].

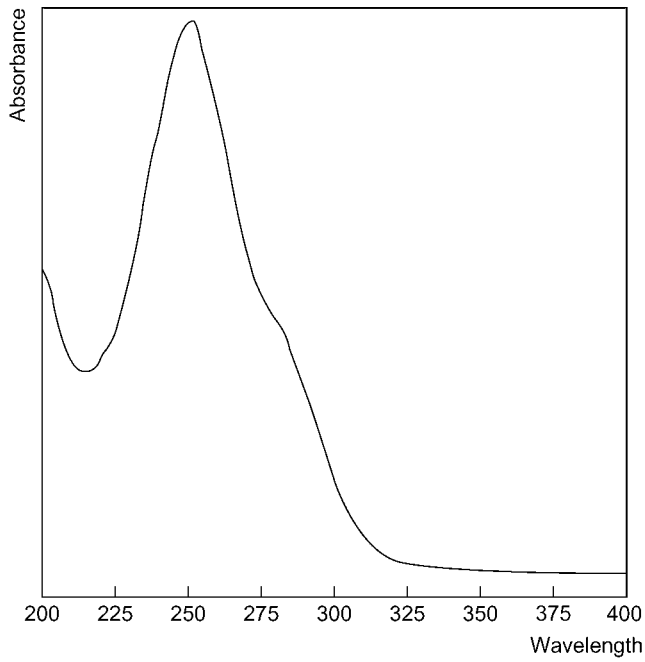
Dills RL *et al.* (2001). Measurement of urinary methoxyphenols and their use for biological monitoring of wood smoke exposure. *Environ Res* 85: 145–158.
Fischer IU, Dengler HJ (1990). Sensitive high-performance liquid chromatographic assay for the determination of eugenol in body fluids. *J Chromatogr* 525: 369–377.

Exemestane

Aromatase Inhibitor, Antineoplastic
$C_{20}H_{24}O_2$ = 296.4
CAS—107868-30-4
IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-Dimethyl-6-methylidene-7,8,9,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthrene-3,17-dione
Synonyms FCE-24304; 6-methylenandrosta-1,4-diene-3,17-dione.
Proprietary Name *Aromasin*



Chemical Properties A white to slightly yellow crystalline powder. It is freely soluble in *N*,*N*-dimethylformamide; soluble in methanol; practically insoluble in water.
Ultraviolet Spectrum Aqueous acid (pH 4)—251 nm.



Quantification
Plasma **HPLC** Column: Zorbax SB C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 100% acetonitrile, flow rate 1 mL/min. MS–MS detection. Retention time: 2.3 min. Limit of quantification, 0.05 µg/L [Cenacchi *et al.* 2000]. Column: RP18 Lichrocart (125 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L potassium dihydrogen phosphate (pH 4.5) (35:65), flow rate 1.5 mL/min. UV detection (λ=247 nm). Retention time: exemestane, 14 min; 17-dihydroexemestane, 10 min. Limit of quantification, 10 µg/L [Breda *et al.* 1993].
Disposition in the Body Exemestane is rapidly absorbed after oral administration; more rapidly in women with breast cancer compared with those without. The presence of food can enhance absorption. It is extensively distributed into body tissues and is cleared from systemic circulation mainly by metabolism. The drug

undergoes extensive metabolism, in the liver, by oxidation and reduction to inactive or active metabolites which are less active than the parent drug including the 17-dihydro metabolite. Plasma levels can increase, by up to 40%, when the drug is administered with a high-fat meal. Plasma levels of the metabolite, 17-hydroxyexemestane are less than one tenth of the corresponding unchanged drug level. Exemestane is excreted, equally, in both urine and faeces with <1% of the dose being detected unchanged in urine.

Therapeutic Concentration

Thirty-two healthy post-menopausal women were administered multiple doses of 1.0, 2.5, 5.0 and 10.0 mg daily (8 volunteers each dose) for 7 days. Peak plasma concentrations were generally reached within 1 to 2 h and the concentrations measured on day 7 were 0.83, 2.18, 7.29 and 11.04 µg/L for the four doses, respectively [Persiani *et al.* 1995].

Fifteen healthy post-menopausal women, with a mean age of 60 years (range 48 to 75 years) and a mean time from menopause of 12.3 years (2 to 36 years), were administered single doses of 50, 200, 400 and 800 mg exemestane 15 to 30 min after a high lipid breakfast. Subjects were observed for 2 weeks at each dose level before it was increased. Peak plasma concentrations, observed between 1 and 2 h, were 27, 221, 343 and 414 µg/L for the doses, respectively [Evans *et al.* 1992].

Half-life Approximately 24 to 27 h.

Clearance Total, 517 L/h.

Distribution in Blood Distribution of the drug and its metabolites into blood cells is negligible.

Protein Binding 90%, especially albumin and α_1-acid glycoprotein.

Dose 25 mg once daily.

Breda M *et al.* (1993). Determination of exemestane, a new aromatase inhibitor, in plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 620(2): 225–231.
Cenacchi V *et al.* (2000). LC-MS-MS determination of exemestane in human plasma with heated nebulizer interface following solid-phase extraction in the 96 well plate format. *J Pharm Biomed Anal* 22: 451–460.
Evans TR *et al.* (1992). Phase I and endocrine study of exemestane (FCE 24304), a new aromatase inhibitor, in postmenopausal women. *Cancer Res* 52: 5933–5939.
Persiani S *et al.* (1995). *Eur J Cancer* 31 (Suppl.5)S198.

Exifone

Nootropic

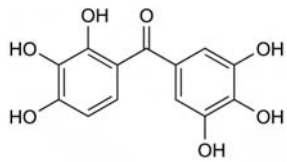
$C_{13}H_{10}O_7$ = 278.2

CAS—52479-85-3

IUPAC Name (2,3,4-Trihydroxyphenyl)(3,4,5-trihydroxyphenyl)methanone

Synonym 4-Galloylpyrogallol

Proprietary Name *Adlone*



Chemical Properties A pale-yellow crystalline powder. Mp 270°. Log *P* (octanol/water), 1.89.

High Performance Liquid Chromatography Column: C_{18} RCM NovaPak (4 µm, 100 × 5 mm i.d.). Mobile phase: acetonitrile : 300 mmol/L orthophosphoric acid (pH 2.2) (15:85), flow rate 0.9 mL/min. Electrochemical detection. Retention time: 8 min [Descombe *et al.* 1989].

Quantification

Plasma **HPLC** Limit of quantification, 1.0 ng/L [Descombe *et al.* 1989].

Urine **HPLC** See Plasma [Descombe *et al.* 1989].

Toxicity

In a reported fatality, a 74-year-old woman administered with 600 mg/day of exifone for memory loss developed hepatitis after 5 months of treatment [Pateron *et al.* 1990].

Dose 200 mg three times daily with a maximum of 1200 mg daily.

Descombe JJ *et al.* (1989). Determination of exifone in human plasma and urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 496: 345–353.
Pateron D *et al.* (1990). [Fatal hepatitis probably caused by exifone (Adlone)]. *Gastroenterol Clin Biol* 14: 294–295.

Ezetimibe

Antihyperlipidaemic, 2-Azetidinone, Cholesterol Absorption Inhibitor

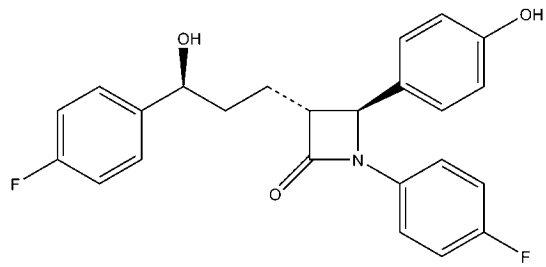
$C_{24}H_{21}F_2NO_3$ = 409.4

CAS—163222-33-1

IUPAC Name (3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one

Synonyms (3*R*,4*S*)-1-(*p*-Fluorophenyl)-3-[(3*S*)-3-(*p*-fluorophenyl)-3-hydroxypropyl]-4-(*p*-hydroxyphenyl)-2-azetidinone; Sch-58235.

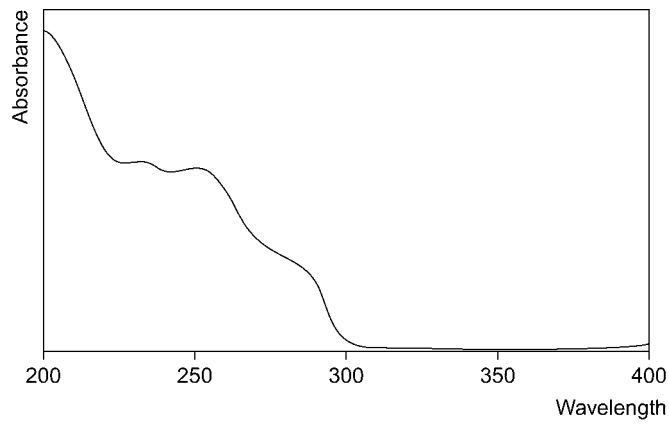
Proprietary Names *Acotral*; *Alin*; *Alipas*; *Cerclerol*; *Cetrakam*; *Coracil*; *Ezetrol*; *Ixacor*; *Lipimibe*; *Nalecol*; *Sinterol*; *Trilip*; *Vadel*; *Zetia*; *Zient.* It is also an ingredient of *Adacai*; *Alipas Duo*; *Ampliar Duo*; *Ateroclar Duo*; *Inegy*; *Vytorin*; *Zintrepid*.



Chemical Properties White solid. Mp 164° to 166°. pK_a 9.66. Log *P* (octanol/water), 4.39 [Oswald *et al.* 2006a]. For degradation studies of ezetimibe under various stress conditions, see Chaudhari *et al.* [2007] and Singh *et al.* [2006].

High Performance Liquid Chromatography Column: Lichrospher C_8 (250 × 4.0 mm i.d., 4 µm). Mobile phase: 0.02 mol/L ammonium acetate (pH 7.0) : acetonitrile (70 : 30 to 0 : 100 over 80 min), flow rate 1.0 mL/min. UV detection (λ = 250 nm). Retention time: ezetimibe 26.9 min; degradation products I, II, III, IV, V, VII and VII, 12.9, 16.5, 23.3, 29.2, 30.8, 43.0 and 50.5 min, respectively [Singh *et al.* 2006]. Column: Kromasil 100 C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: water-0.05% 1-heptane sulfonic acid (pH 6.8) : acetonitrile (30 : 70), flow rate 0.5 mL/min. UV detection (λ = 232 nm). Limit of quantification, 0.5 mg/L [Sistla *et al.* 2005].

Ultraviolet Spectrum Chaudhari et al [2007]



Quantification

Plasma **HPLC** Column: Capcell Pak C_{18} MG (50 × 2 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (70 : 30 to 100 : 0 over 0.1 min for 1.4 min to 30 : 70 over 0.1 min for 3.4 min), flow rate 0.25 mL/min. TSI, negative ion mode, MRM acquisition mode. Limit of quantification, unchanged ezetimibe 0.02 µg/L, total ezetimibe 0.25 µg/L [Li *et al.* 2006].

LC-MS Column: Spherisorb ODS-2 (100 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : 0.025 mol/L ammonium acetate (90 : 10), flow rate 1.5 mL/min. API, positive ion mode, SRM acquisition mode. Limit of quantification, unchanged ezetimibe 1.0 µg/L, total ezetimibe 5.02 µg/L [Patrick *et al.* 2002].

Serum **LC-MS** Column: C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water (60 : 40), flow rate 0.2 mL/min. APCI, negative ion mode, MRM acquisition mode. Limit of quantification, unchanged ezetimibe 0.1 µg/L, total ezetimibe 1.0 µg/L [Oswald *et al.* 2006b].

Urine **LC-MS** See Serum. Limit of quantification, unchanged ezetimibe or total ezetimibe 25 µg/L [Oswald *et al.* 2006b]. See Plasma [Patrick *et al.* 2002].

Faeces **LC-MS** See Serum. Limit of quantification, unchanged ezetimibe or total ezetimibe 100 µg/L [Oswald *et al.* 2006b]. See Plasma [Patrick *et al.* 2002].

Other **HPLC** Tablets. Column: LiChrospher 100 C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : methanol (pH 4.0; 60 : 25 : 15), flow rate 1.5 mL/min. UV detection (λ = 238 nm). Retention time: 2.04 min. Limit of quantification, 3.0 mg/L; limit of detection, 0.7 mg/L [Chaudhari *et al.* 2007]. Human Liver Microsomes and Rat Bile. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water-acetonitrile (10 : 90) : water-methanol (10 : 90; 100 : 0 : 0 to 90 : 0 : 10 over 2 min, to 50 : 20 : 30 over 7 min, to 50 : 25 : 25 over 3 min, to 30 : 50 : 20 over 13 min, to 10 : 85 : 5 over 3 min for 7 min), flow rate 1.0 mL/min. UV detection (λ = 250 nm). Retention time: ezetimibe 29.0 min, ezetimibe glucuronide 24.2 min, ezetimibe ketone glucuronide 27.8 min, ezetimibe ketone 30.6 min. Limit of quantification, 0.02 mg/L [Basha *et al.* 2007].

Disposition in the Body Rapidly absorbed when given orally and undergoes extensive conjugation in the small intestine and liver to an active glucuronide metabolite, which is the main circulating form. The major metabolic pathway consists of glucuronidation of the 4-hydroxyphenyl group by uridine 5′-diphosphate-glucuronosyltransferase isoenzymes. The parent drug and its conjugate are then transported through portal vessels to the liver, where ezetimibe undergoes further glucuronidation and subsequent biliary secretion into the intestine, forming further minor metabolites. After an oral dose, ~78% is excreted in the faeces, mainly as ezetimibe, and approx. 11% is excreted in the urine, mainly as the glucuronide. Ezetimibe undergoes enterohepatic recycling.

Therapeutic Concentration

Eight healthy volunteers were administered either ezetimibe alone (two 10 mg fast-release tablets) or concomitantly with a 600 mg tablet of rifampin. Mean peak plasma concentrations and time to peak concentration for ezetimibe and its glucuronide were reported as follows:

	Ezetimibe		*Glucuronide*	
	Without rifampin	*With rifampin*	*Without rifampin*	*With rifampin*
C_{max} (μg/L)	4.7	12.0	107	282
Time (h)	4.8	1.9	1.1	2.7

The differences observed in plasma concentrations are thought to be due to the inhibition of hepatic organic anion-transporting polypeptides by rifampin [Oswald *et al.* 2006b].

Eight healthy male volunteers were given a single dose of 20 mg [^{14}C] ezetimibe as a capsule. Peak plasma concentrations of free, conjugated and total ezetimibe were 5.21, 61.2 and 64.2 μg/L, respectively, reached after 9.9, 2.3 and 2.3 h, respectively. On average, ~89% of the administered dose was excreted in urine (11%) and faeces (78%) by 240 h post-administration. The major metabolite observed in plasma was ezetimibe glucuronide; ezetimibe was not observed in plasma samples taken 24 h post-dose. This was also the case in urine; however, additional minor metabolites were observed including the 4-hydroxyphenyl glucuronide conjugate of the ketone analogue of ezetimibe. In faeces, unconjugated ezetimibe accounted for approx. 96% of the chromatographic radioactivity together with another minor metabolite resulting from the oxidation of the benzylic hydroxyl group to a ketone [Patrick *et al.* 2002].

Half-life Approximately 22 h for both ezetimibe and its glucuronide conjugate.

Protein Binding >90% for both ezetimibe and its glucuronide conjugate.

Note For a review of ezetimibe, see Kosoglou *et al.* [2005].

Dose Given orally in a usual dose of 10 mg once daily.

Basha SJ *et al.* (2007). Concurrent determination of ezetimibe and its phase I and II metabolites by HPLC with UV detection: quantitative application to various in vitro metabolic stability studies and for qualitative estimation in bile. *J Chromatogr B Analyt Technol Biomed Life Sci* 853: 88–96.

Chaudhari BG *et al.* (2007). Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of simvastatin and ezetimibe from their combination drug products. *J AOAC Int* 90: 1242–1249.

Kosoglou T *et al.* (2005). Ezetimibe: a review of its metabolism, pharmacokinetics and drug interactions. *Clin Pharmacokinet* 44: 467–494.

Li S *et al.* (2006). Liquid chromatography–negative ion electrospray tandem mass spectrometry method for the quantification of ezetimibe in human plasma. *J Pharm Biomed Anal* 40: 987–992.

Oswald S *et al.* (2006a). A LC-MS/MS method to quantify the novel cholesterol lowering drug ezetimibe in human serum, urine and feces in healthy subjects genotyped for *SLCO1B1*. *J Chromatogr B Analyt Technol Biomed Life Sci* 830: 143–150.

Oswald S *et al.* (2006b). Disposition and sterol-lowering effect of ezetimibe are influenced by single-dose coadministration of rifampin, an inhibitor of multidrug transport proteins. *Clin Pharmacol Ther* 80: 477–485.

Patrick JE *et al.* (2002). Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male subjects. *Drug Metab Dispos* 30: 430–437.

Singh S *et al.* (2006). Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay. *J Pharm Biomed Anal* 41: 1037–1040.

Sistla R *et al.* (2005). Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J Pharm Biomed Anal* 39: 517–522.

Famciclovir

Antiviral

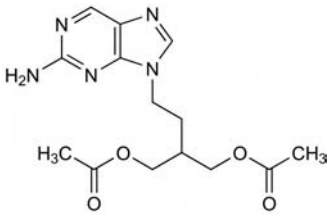
$C_{14}H_{19}H_5O_4 = 321.3$

CAS—104227-87-4

IUPAC Name [2-(Acetyloxymethyl)-4-(2-aminopurin-9-yl)butyl] acetate

Synonyms 9-[4-Acetoxy-3-(acetoxymethyl)but-1-yl]-2-aminopurine; BRL-42810; FCV.

Proprietary Names *Famvir*; *Vectavir*.

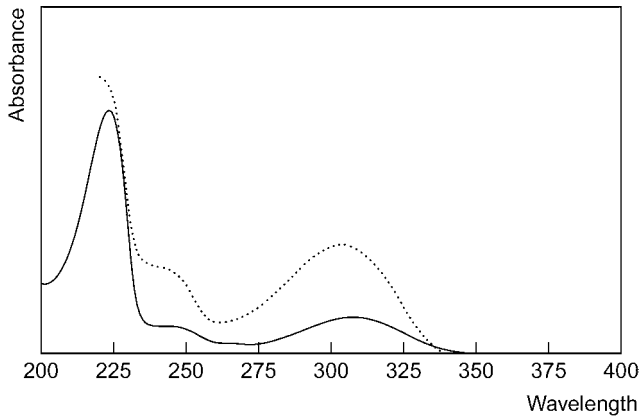


Chemical Properties A white to pale-yellow solid. Mp 102° to 104°. It is soluble in water (>25% at 25°), acetone and methanol; sparingly soluble in ethanol and isopropanol. Log *P* (octanol/water, pH 4.8), 1.09; log *P* (octanol/buffer pH 7.4), 2.08.

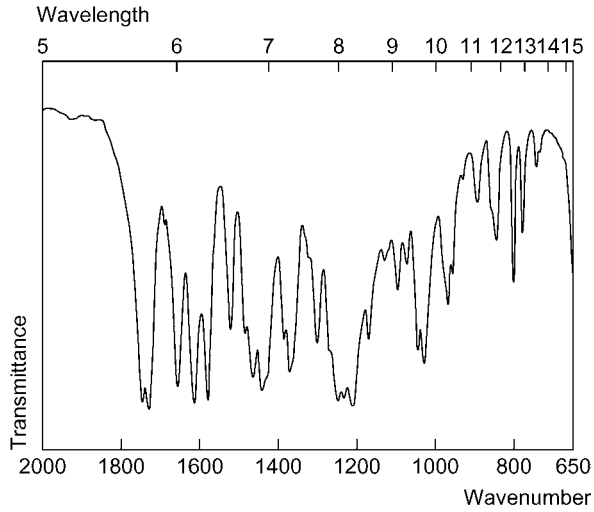
Gas Chromatography Column: methyl silicone (HP1, 0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. MS detection. Retention index: 2552 [Mills, Roberson 1993].

High Performance Liquid Chromatography Column: ODS Apex 1 (100 × 4.6 mm i.d., 3 µm). Mobile phase: (A)-methanol : 0.01 mol/L disodium hydrogen orthophosphate (pH 7.0) (7:93); (B)-methanol : 0.01 mol/L disodium hydrogen orthophosphate (pH 7.0) (35:65). Elution programme: (A:B) (100:0) to (0:100) in 4 min, held for 1.5 min, back to initial conditions in 1 min, flow rate 2.0 mL/min. UV detection: dual wavelength (λ_1=254 nm, λ_2=305 nm). Retention time: penciclovir (metabolite), 1.6 min; 6-deoxyfamciclovir precursor, 2.6 min; famciclovir, 6.0 min [Winton *et al.* 1990].

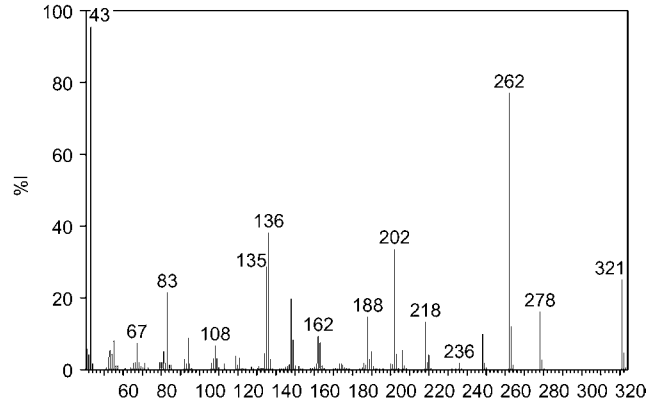
Ultraviolet Spectrum Aqueous acid—224, 246, 310 nm; basic—304 nm.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1616, 1216 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 262, 136, 202, 135, 321, 83, 148.



Quantification

***Plasma* HPLC** Column: ODS Apex 1 (100 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.01 mol/L sodium phosphate buffer (pH 7.0) (5:95), flow rate 1.0 mL/min. UV detection: dual wavelength (λ_1=254 nm, λ_2=305 nm). Retention time: penciclovir, 7.8 min; 6-deoxyfamciclovir precursor, 14.0 min. Limit of quantification, 0.2 mg/L [McMeekin *et al.* 1992].

***Urine* HPLC** See Plasma. Limit of quantification, 10 mg/L [McMeekin *et al.* 1992].

Disposition in the Body Famciclovir is rapidly absorbed after oral administration and converted, by deacetylation, in blood, and oxidation (aldehyde oxidase), in the liver, to penciclovir (BR-39123) and several inactive metabolites. Virtually no famciclovir is detected in plasma or urine. Penciclovir is converted by intracellular virus-induced thymine kinase to penciclovir triphosphate which is responsible for the antiviral activity. The drug is excreted, primarily, in urine as the metabolite, penciclovir, and the 6-deoxy precursor, BRL-42359. 73% of penciclovir can be detected in urine over 24 h and the remainder appears in faeces. Elimination is reduced in patients with renal impairment.

Therapeutic Concentration

Twenty healthy male volunteers, with a mean age of 36 years, were administered single oral doses of 125, 250, 500 and 750 mg famciclovir, after 10 h fasting. The peak plasma concentrations of penciclovir: 0.84, 1.59, 3.34 and 5.09 mg/L, respectively, were reached within 0.5 to 0.75 h. Concentrations reached below 0.2 mg/L within 6 h for the 125 and 250 mg doses, and within 10 h for the higher doses [Pue *et al.* 1994].

Bioavailability Approximately 77%.

Half-life 2 to 3 h.

Volume of Distribution Steady state, 0.98 to 1.08 L/kg; metabolite, penciclovir, 1.5 L/kg.

Clearance Approximately 0.48 L/h/kg.

Distribution in Blood The blood : plasma ratio for the metabolite, penciclovir is ~1.0.

Protein Binding The metabolite, penciclovir is <20% bound.

Dose The usual dose administered is between 250 and 500 mg every 8 h. Dosing interval is extended in patients with renal impairment.

McMeekin JR *et al.* (1992). *Anal Proc* 29: 178–180.
Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press 6: 82–83
Pue MA *et al.* (1994). Linear pharmacokinetics of penciclovir following administration of single oral doses of famciclovir 125, 250, 500 and 750 mg to healthy volunteers. *J Antimicrob Chemother* 33: 119–127.
Winton CF *et al.* (1990). *Anal Proc* 27: 181–182.

Famotidine

Histamine H_2-Receptor Antagonist, Antiulcerative

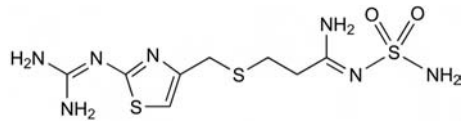
$C_8H_{15}N_7O_2S_3 = 337.5$

CAS—76824-35-6

IUPAC Name 3-[[[2-[(Aminoiminomethyl)amino]-4-thiazolyl]methyl]thio]-*N*-(aminosulfonyl)propanimidamide

Synonyms Famotidinum; L643341; MK-208; YM-11170.

Proprietary Names *Amfamox*; *Brolin*; *Dispromil*; *Famodil*; *Famodine*; *Famosan*; *Famoxal*; *Ganor*; *Gastor*; *Gastropen*; *Ifada*; *Lecedil*; *Motiax*; *Pepcid*; *Pepcidac*; *Pepcidin*; *Pepcidine*; *Pepdine*; *Pepdul*; *Ulcusan*; *Ulfinol*.



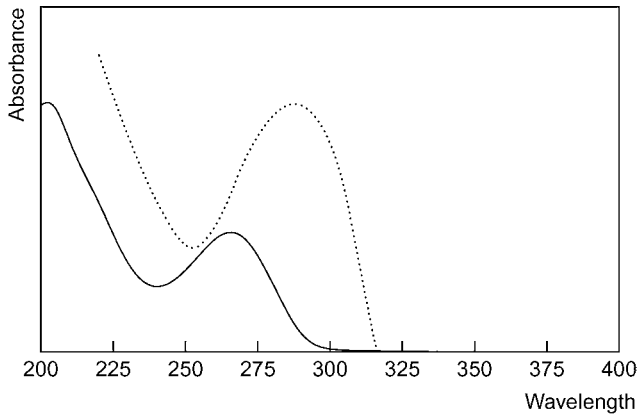
Chemical Properties A white to pale yellowish white crystalline powder with Mp 163° to 164°. Protect from light. It is very slightly soluble in water (0.1% at 20°) and dehydrated alcohol; practically insoluble in acetone, alcohol, chloroform (< 0.01%, 20°), ether and ethyl acetate (< 0.01%, 20°); slightly soluble in methyl alcohol; freely soluble in dimethylformamide (80%, 20°) and glacial acetic acid (50%, 20°). It dissolves in dilute mineral acids. pK_a 7.06. Log *P* (octanol/water), −0.64.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.25; system TAE—R_f 0.53.

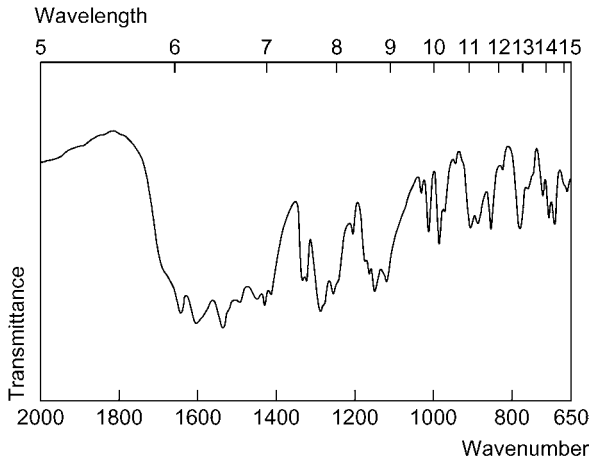
High Performance Liquid Chromatography System HX—RI 233;.system HY —RI 191; system HZ—retention time 1.8 min; system HAA—retention time 3.5 min.

Column: LC-8 Supelcosyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.016 mol/L phosphoric acid (10:10:80), flow rate 1.0 mL/min. UV detection (λ=254 nm). Retention time: 9.5 min [Carlucci *et al.* 1988].

Ultraviolet Spectrum Aqueous acid—265 nm; basic—286 nm.



Infrared Spectrum Principal peaks at wavenumbers 1638, 1535, 1290, 781 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 188, 155, 113, 43, 45, 85, 69, 146.

Quantification

***Plasma* HPLC** Column: ODS-3 Prodigy (250 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 2.5 g/L heptanesulfonic acid in 20 mmol/L sodium acetate buffer (pH 4.7) (23:77), flow rate 1.0 mL/min. Internal standard (IS): cimetidine. UV detection (λ=267 nm). Retention time: 8.4 min; IS, 6.8 min. Limit of detection, 0.075 mg/L [Dowling, Frye 1999]. UV detection (λ=267 nm). Limit of quantification, 1 µg/L [Zhong, Yeh 1998]. UV detection (λ=267 nm). Limit of quantification, 5 µg/L [Wanwimolruk *et al.* 1991]. UV detection (λ=267 nm). Limit of quantification, 0.005 mg/L [Vincek *et al.* 1985].

***Urine* HPLC** See Plasma. Limit of detection, 1 mg/L [Dowling, Frye 1999]. See Plasma. Limit of quantification, 0.5 mg/L [Vincek *et al.* 1985].

Disposition in the Body After IV administration, the plasma famotidine concentration–time profile exhibits a biexponential decay. Famotidine is readily but incompletely absorbed after oral administration. There are three formulations available (tablet, capsule and suspension), which appear to be bioequivalent. Only a small amount of the drug is metabolised in the liver to famotidine *S*-oxide and the remainder is excreted unchanged in urine (~67%). The drug is widely distributed in body tissues and can be detected in breast milk. Famotidine interacts neither with the hepatic oxidative drug metabolism nor with tubular secretion of other commonly used therapeutic agents such as theophylline, warfarin and procainamide. Coadministration of potent antacids reduces the oral absorption of famotidine by 20 to 30%. A prolongation of elimination half-life and a decrease in total body clearance and renal clearance are observed in patients with renal failure, indicating that dosage adjustment may be necessary in patients who have renal insufficiency. No significant changes occur in famotidine disposition in patients with cirrhosis indicating that no reduction in drug dosage should be necessary in these patients. The pharmacokinetics and pharmacodynamics of IV famotidine appear to be similar in both children over the age of 1 year and adults.

Therapeutic Concentration The serum therapeutic concentration range is 0.02 to 0.06 mg/L.

Eight healthy males, aged between 22 and 27 years, were administered a single 40 mg dose of famotidine, after an overnight fast. Peak plasma levels of 0.156 ± 0.022 mg/L were reached within 3 h [Barzaghi *et al.* 1989].

Ten young patients, aged between 5 and 19 days, were administered a 0.5 mg/kg body weight infusion over 15 min. Peak concentrations of 0.641 mg/L were reached by the end of administration [James *et al.* 1998].

Plasma concentrations following IV continuous infusion of 40 (group A) or 20 (group B) mg/day over more than two days, were analysed in 10 adult patients. The mean age, dose and plasma concentration were 64.8 years, 0.81 mg/kg/day and 0.141 mg/L in group A, and 54.2 years, 0.34 mg/kg/day and 0.045 mg/L in group B, respectively [Espitia *et al.* 1992].

Toxicity Convulsions and mental deterioration have been reported in the elderly with renal failure owing to grossly elevated plasma and cerebrospinal fluid concentrations of the drug. Toxic epidermal necrolysis can also occur. Since 1992, there have been 60 reports of serious blood dyscrasias.

Bioavailability 20 to 66%.

Half-life Distribution half-life is ~0.18 to 0.5 h in adults. Elimination half-life is ~3 h in adults, which can increase to 24 h in patients with suppressed urine excretion. The elimination half-life for infants is ~10 h. Elimination half-life is 3.3 h in paediatric patients with normal kidney function, aged between 2 and 7 years.

Volume of Distribution In adults: 0.94 to 1.33 L/kg; in infants: 0.82 L/kg. In paediatric patients (aged between 2 and 7 years): 1.4 L/kg.

Clearance 0.19 to 0.43 L/min. In patients with end-stage renal failure: 0.034 L/min. In paediatric patients with normal kidney function: 0.3 L/h/kg.

Protein Binding 15 to 22%.

Dose A usual dose of 20 to 40 mg is administered once or twice daily. A paediatric dosage for IV famotidine is 0.3 to 0.5 mg/kg every 8 to 12 h similarly as adults. Patients with impaired renal function: dose is reduced or dose interval extended.

Barzaghi N *et al.* (1989). Impaired bioavailability of famotidine given concurrently with a potent antacid. *J Clin Pharmacol* 29: 670–672.

Carlucci G *et al.* (1988). Determination of famotidine in plasma, urine and gastric juice by high-performance liquid chromatography using disposable solid-phase extraction columns. *J Pharm Biomed Anal* 6(5): 515–519.

Dowling TC, Frye RF (1999). Determination of famotidine in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 732: 239–243.

Espitia VR *et al.* (1992). Identical 24-hour intragastric pH response to low continuous infusion doses of famotidine in active gastric ulcer patients. *Intern Med (Tokyo, Japan)* 31: 299–303.

James LP *et al.* (1998). Pharmacokinetics and pharmacodynamics of famotidine in infants. *J Clin Pharmacol* 38: 1089–1095.

Vincek WC *et al.* (1985). Analytical method for the quantification of famotidine, an H2-receptor blocker, in plasma and urine. *J Chromatogr* 338: 438–443.

Wanwimolruk S *et al.* (1991). Sensitive high-performance liquid chromatographic determination of famotidine in plasma. Application to pharmacokinetic study. *J Chromatogr* 572: 227–238.

Zhong L, Yeh KC (1998). Determination of famotidine in human plasma by high performance liquid chromatography with column switching. *J Pharm Biomed Anal* 16: 1051–1057.

Famphur

Organophosphate, Cholinesterase Inhibitor, Insecticide

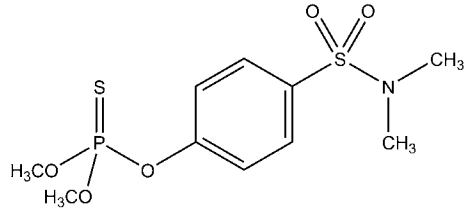
$C_{10}H_{16}NO_5PS_2$ = 325.3

CAS—52-85-7

IUPAC Name 4-Dimethoxyphosphinothioyloxy-*N,N*-dimethylbenzenesulfonamide

Synonyms American cyanamid 38023; ANC-38023; *O,O*-dimethyl-*O*-*p*-dimethylbenzenesulfonamide ester with phosphorothioic acid *O,O*-dimethyl ester; ENT-25,644; *O*-ester with *p*-hydroxy-*N,N*-dimethylbenzenesulfonamide; famfos; famophos; phosphorothioic acid *O*-[4-[(dimethylamino)sulfonyl]phenyl] *O,O* -dimethyl ester; phosphorothioic acid *O,O* -dimethyl ester.

Proprietary Names *Bash*; *Bo-Ana*; *Dovip*; *Warbex*.



Chemical Properties Crystalline powder. Mp 53°. Very soluble in chloroform and carbon tetrachloride; soluble in water (109 mg/L at 25°). Log *P* (octanol/water), 2.23 [Hansch *et al.* 1995].

Quantification

***Other* GC** Animal Tissues. Column: 10% OV-101 on 80–100 mesh Chromosorb (1.22 m × 4 mm i.d.). Carrier gas: N_2, 240 mL/min. Temperature: 235°. FPD. Retention time: 2.2 min. Limit of detection, 25 ng/L [Ivey 1976].

GC-MS Animal Tissues. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 80° for 0.5 min to 180° at 17°/min for 1 min to 250° at 10°/min for 2 min. EI ionisation at 70 eV. Retention time: 17 min. Limit of quantification not reported [Braselton *et al.* 2000].

Disposition the Body

Toxicity Poisonous by oral and IM routes and moderately toxic through the skin.

Dose Used as a systemic ectoparasiticide in veterinary practice; it is applied topically to the host animal.

Braselton WE *et al.* (2000). Gas chromatography/mass spectrometry identification and quantification of isazophos in a famphur pour-on and in bovine tissues after a toxic exposure. *J Vet Diagn Invest* 12: 15–20.

Hansch C *et al.* (1995). The expanding role of quantitative structure–activity relationships (QSAR) in toxicology. *Toxicol Lett* 79: 45–53.

Ivey MC (1976). Gas–liquid chromatographic determination of famphur and its oxygen analog in tissues of reindeer and cattle. *J Assoc Off Anal Chem* 59: 261–263.

F

Famprofazone

Analgesic

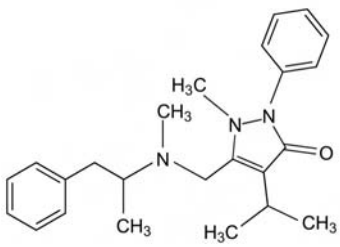
$C_{24}H_{31}N_3O = 377.5$

CAS—22881-35-2

IUPAC Name 1-Methyl-5-[[methyl(1-phenylpropan-2-yl)amino]methyl]-2-phenyl-4-propan-2-ylpyrazol-3-one

Synonym 4-Isopropyl-1-methyl-5-[*N*-methyl-*N*-(α-methylphenethyl)aminomethyl]-2-phenyl-4-pyrazolin-3-one

Proprietary Name It is an ingredient of *Gewodin*.



Chemical Properties A white crystalline powder. Mp 132° to 133°. Practically insoluble in water; slightly soluble in dilute acetic acid.

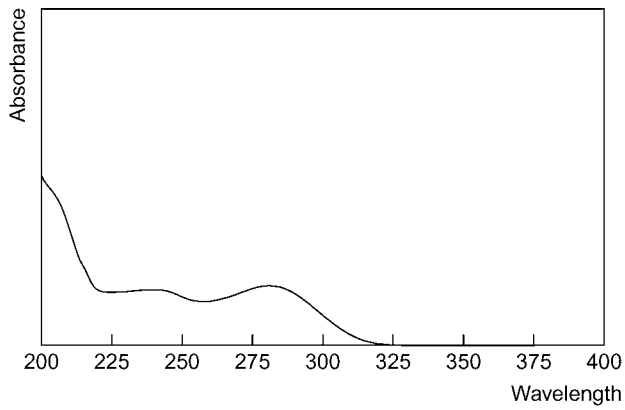
Colour Tests Liebermann's reagent—red-orange; Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.37; system TC—R_f 0.74; system TE—R_f 0.87; system TL—R_f 0.67; system TAE—R_f 0.90 (acidified iodoplatinate solution, positive).

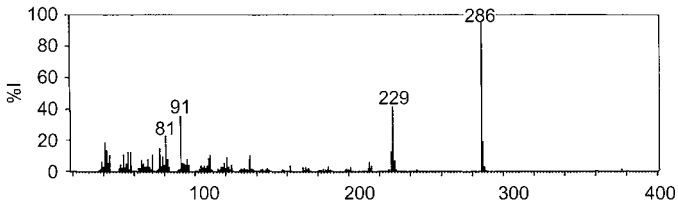
Gas Chromatography System GA—Famprofazone RI 2965, M (OH-propyphenazone) RI 2410, M (OH-propyphenazone)-AC RI 2240, M (metamfetamine) RI 1175.

High Performance Liquid Chromatography System HD—*k* 2.5.

Ultraviolet Spectrum Aqueous acid—243, 279 nm (A_1^1=248a).



Mass Spectrum Principal ions at *m/z* 286, 229, 91, 81, 287, 41, 77, 228.



Disposition in the Body

Therapeutic Concentration

After a single 100 mg dose of famprofazone to 2 subjects, the plasma concentration of metamfetamine was 24 to 44 µg/L over 2 to 12 h and declined to 10 µg/L in one subject and an undetectable level in the other after 24 h [Oh *et al.* 1992].

Dose Famprofazone has been given in doses of up to 150 mg daily.

Oh ES *et al.* (1992). Plasma and urinary concentrations of methamphetamine after oral administration of famprofazone to man. *Xenobiotica* 22(3): 377–384.

Fasudil

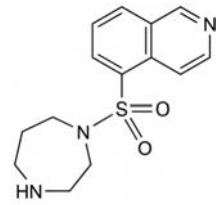
Vasodilator

$C_{14}H_{17}N_3O_2S = 291.4$

CAS—103745-39-7

IUPAC Name 5-(1,4-Diazepan-1-ylsulfonyl)isoquinoline

Synonym Hexahydro-1-(5-isoquinolinylsulfonyl)-1*H*-1,4-diazepine



Fasudil Hydrochloride

$C_{14}H_{17}N_3O_2S,HCl = 327.8$

CAS—105628-07-7

Synonyms AT-877; HA-1077.

Proprietary Names *Eril*; *Fadil*.

Chemical Properties A white crystalline powder with Mp 219.3°. It is soluble in water up to 2×10^{-2} mol/L at pH 5.0 to 7.0.

Fazadinium Bromide

Muscle Relaxant

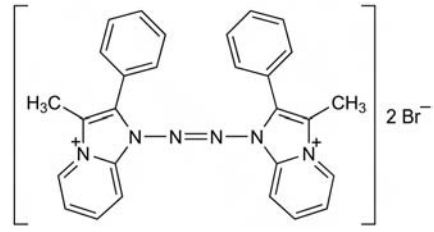
$C_{28}H_{24}Br_2N_6 = 604.3$

CAS—36653-54-0 (fazadinium); 49564-56-9 (bromide)

IUPAC Name (*E*)-bis(3-Methyl-2-phenylimidazo[1,2-*a*]pyridin-4-ium-1-yl) diazene dibromide

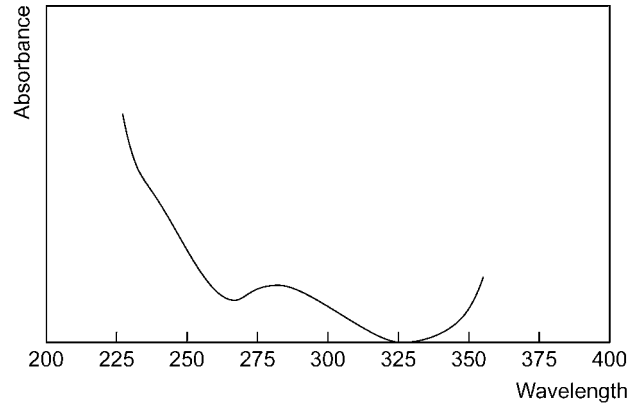
Synonym 1,1′-Azobis[3-methyl-2-phenylimidazo[1,2-*a*]pyridinium] dibromide

Proprietary Name *Fazadon*



Chemical Properties A yellow solid. Mp 215°. Soluble in water.

Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1=188a).



Infrared Spectrum Principal peaks at wavenumbers 1280, 1220, 1160, 1250, 760, 780 cm^{-1} (Nujol mull).

Quantification

Plasma **Spectrofluorimetry** Limit of detection, 50 µg/L [Pastorino 1978].

Disposition in the Body About 50% of a dose is excreted in the urine in 24 h, mainly unchanged. Extensive biliary excretion has been reported.

Therapeutic Concentration

Following an IV dose of 70 mg to 5 subjects, the mean plasma concentration was >10 mg/L at 2 min and 2.2 mg/L at 1 h [D'Souza *et al.* 1979].

Half-life Plasma half-life, about 1 h.

Volume of Distribution About 0.3 L/kg.

Clearance Plasma clearance, about 3 mL/min/kg.

Protein Binding About 17%.

Dose Initially, the equivalent of 0.75 to 1 mg/kg of fazadinium, intravenously.

D'Souza J *et al.* (1979). 125I-labelled Rose Bengal in the quantitative estimation of fazadinium and other quaternary ammonium compounds in biological fluids. *J. Pharm. Pharmacol* 31(6): 416–418.

Pastorino AM (1978). Fluorimetric determination and pharmacokinetic studies of fazadinium bromide in dogs. *Arzneimittelforschung* 28: 1728–1730.

Felbamate

Anticonvulsant

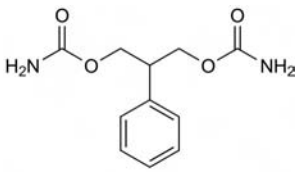
$C_{11}H_{14}N_2O_4 = 238.2$

CAS—25451-15-4

IUPAC Name (3-Carbamoyloxy-2-phenylpropyl) carbamate

Synonyms ADD-03055; 2-phenyl-1,3-propanediol dicarbamate; W-554.

Proprietary Names *Felbamyl*; *Felbatol*; *Taloxa*; *Taloxoral*.



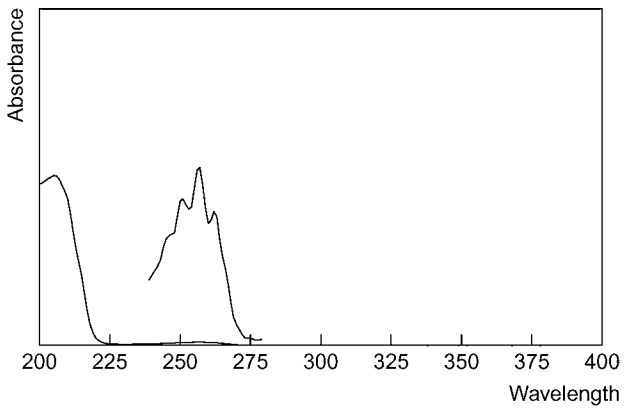
Chemical Properties A white, odourless powder with Mp 151° to 152°. It is sparingly soluble in water, methanol, ethanol, acetone, and chloroform; freely soluble in dimethylsulfoxide, 1-methyl-2-pyrrolidinone, and dimethylformamide. Log *P* (octanol/water), 0.76.

Gas Chromatography System GB—RI 1475.

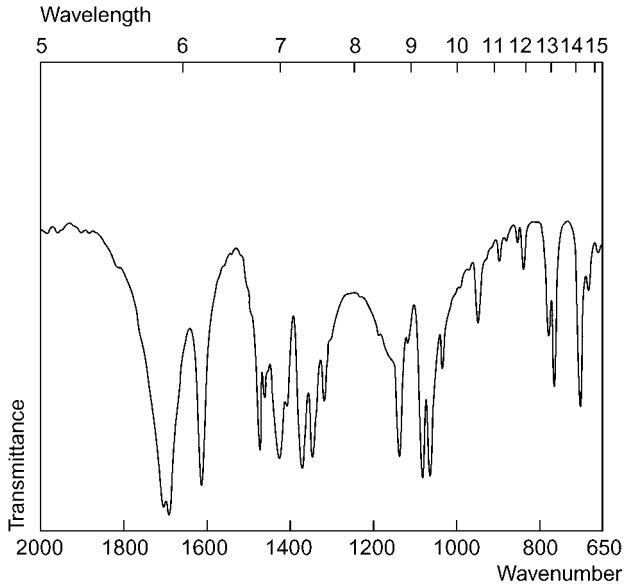
Column: DB-1 (30 m × 0.25 mm i.d., 0.25 µm). Temperature: 240°. Injector and detector temperatures: 280° and 320°, respectively. Carrier gas: H_2, 15 psi inlet pressure. Detection: FID. IS: 2-methyl-2-phenyl-1,3-propanedioldicarbamate. Retention time(s): felbamate, 2.29 min; IS, 2.50 min [Poquette 1995].

High Performance Liquid Chromatography Column: ODS-2 Spherisorb (150 × 4.6 mm i.d., 3 µm). Mobile phase: potassium dihydrogen phosphate (4.2 g in 850 mL water (pH 4.0)) and 150 mL acetonitrile, flow rate 1.0 mL/min. IS: 2-methyl-2-phenyl-1,3-propanedioldicarbamate. Retention time: felbamate, 11.0 min; 2-hydroxyfelbamate, 4.5 min; p-hydroxyfelbamate, 3.2 min; monocarbamate derivative, 7.1 min; IS, 18.8 min [Romanyshyn *et al.* 1994].

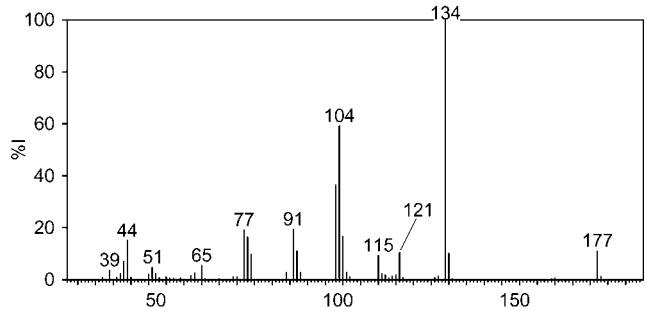
Ultraviolet Spectrum Aqueous acid—251, 256, 262 nm; basic—246, 251, 256, 262 nm.



Infrared Spectrum Principal peaks at wavenumbers 1693, 1614, 1082 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 134, 104, 103, 91, 77, 78, 105, 44.



Quantification

Serum **GC–MS** Column: methylsilicone HP (10 m × 0.53 mm i.d., 0.33 µm). Temperature programme: held at 170° for 0.1 min, increased to 280° at 10°/min, held for 2 min. Injector and detector temperatures: 235° and 300°, respectively. Carrier gas: He, flow rate 16.4 mL/min, hydrogen 4 mL/min, and air 75 mL/min. Detection: nitrogen selective. IS: methylfelbamate. Retention time: felbamate, 3.71 min; IS, 4.11 min. Limit of detection and quantification, 5 mg/L [Gur *et al.* 1995].

HPLC Column: C_{18} Zorbax (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : tetrahydrofuran : phosphate buffer (210:410:500:2880), flow rate 1.5 mL/min. UV detection (λ=254 nm). IS: methylfelbamate. Retention time: felbamate, 5.0 min; IS, 7.0 min. Limit of detection and quantification, 5 mg/L [Gur *et al.* 1995].

Disposition in the Body Felbamate is well absorbed (~90%) after oral administration and is partly metabolised in the liver by hydroxylation and conjugation, to produce active and inactive metabolites. The drug is excreted in urine unchanged (40 to 50%), as unidentified metabolites and conjugates (40%) and known active metabolites (15%), including 2-hydroxyfelbamate, *p*-hydroxyfelbamate, a monocarbamate derivative, and 3-carbamoyloxy-2-phenyl propionic acid. <5% of the dose is excreted in faeces.

Therapeutic Concentration The therapeutic plasma concentration is between 30 and 80 µg/L.

A study was carried out with 24 healthy, elderly volunteers (male and female), aged between 66 and 78 years, and 11 younger volunteers, 18 to 45 years. Low dosage regimen: a single dose of 600 mg felbamate was administered on day 1, followed by 600 mg every 12 h (days 6 to 7) and 600 mg every 12 h (days 8 to 14) for a multiple dosing study. High dosage regimen: a single 1200 mg dose was administered on day 1 followed by 600 mg every 12 h and 1200 mg every 12 h, on the respective days. For the elderly group, peak plasma concentrations of 13.0 and 22.0 µg/L were reached for the low and high single doses, respectively. For the younger volunteers, 8.9 and 19.6 µg/L were reached, respectively. All peak concentrations were reached within 2 h of administration. After multiple dosing, peak plasma concentrations reached 32.7 and 78.5 µg/L for the low and high doses, respectively (young) and 41.1 and 75.5 µg/L, respectively for the elderly [Richens *et al.* 1997].

Toxicity

Acute liver failure has been reported with 4 fatalities out of 8 cases [Schering-Plough 1994] and aplastic anaemia with 2 out of 10 cases resulting in death [US report 1994].

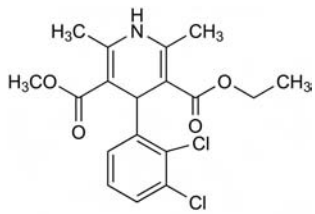
Half-life Elimination half-life, 14 to 23 h.
Volume of Distribution 0.76 to 0.81 L/kg.
Clearance 25 mL/min/kg (single dose), 41 mL/min/kg (repeated dose).
Distribution in Blood Equilibrates freely between plasma and whole blood.
Protein Binding 22 to 36%.
Dose An initial dose of 1.2 g daily is administered to adults with a maximum 3.6 g daily. In children: an initial dose of 15 mg/kg body weight, maximum 45 mg/kg. Dose may be adjusted for patients with renal dysfunction.

Gur P *et al.* (1995). Chromatographic procedures for the determination of felbamate in serum. *J Anal Toxicol* 19: 499–503.
Poquette MA (1995). Isothermal gas chromatographic method for the rapid determination of felbamate concentration in human serum. *Ther Drug Monit* 17(2): 168–173.
Richens A *et al.* (1997). Single and multiple dose pharmacokinetics of felbamate in the elderly. *Br J Clin Pharmacol* 44: 129–134.
Romanyshyn LA *et al.* (1994). Simultaneous determination of felbamate and three metabolites in human plasma by high-performance liquid chromatography. *Ther Drug Monit* 16(1): 83–89.
Schering-Plough manufacturers report (1994).
US Food and Drug Administration report (1994).

Felodipine

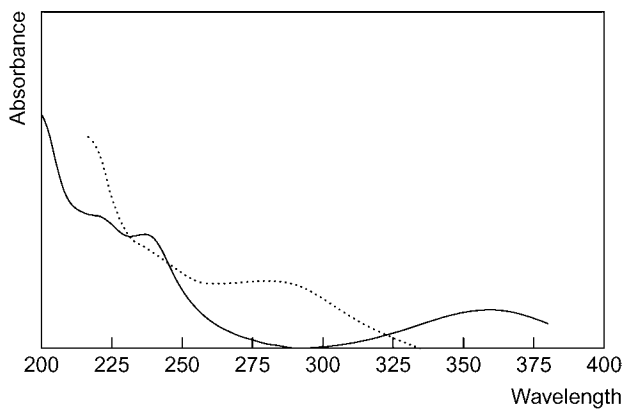
Antihypertensive
$C_{18}H_{19}Cl_2NO_4 = 384.3$
CAS—72509-76-3; 86189-69-7

IUPAC Name 5-*O*-Ethyl 3-*O*-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate
Synonyms 4-(2,3-Dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid ethyl methyl ester; felodipinum; H-154/82.
Proprietary Names *Agon*; *Feloday*; *Felodur*; *Fensil*; *Flodil*; *Hydac*; *Modip*; *Munobal*; *Perfudal*; *Plendil*; *Preslow*; *Prevex*; *Renedil*; *Spendil*.

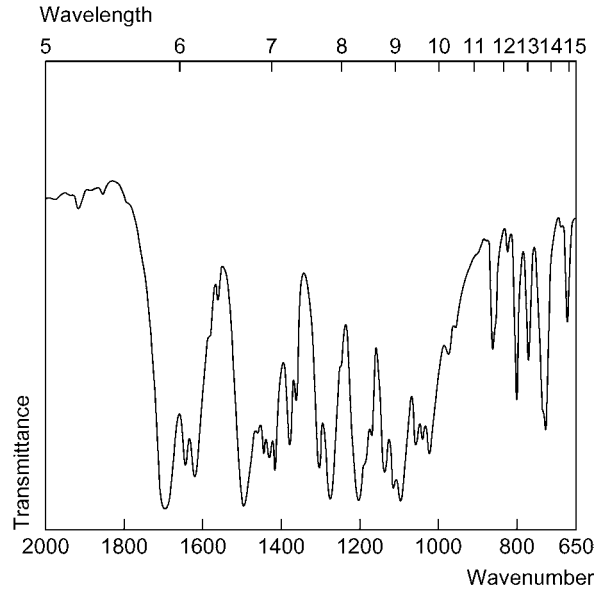


Chemical Properties A white to light-yellow crystalline powder. Mp 145°. Protect from light. It is practically insoluble in water (19.17 mg/L at 25°); freely soluble in acetone, absolute alcohol, methyl alcohol and dichloromethane. Log *P* (octanol/water), 3.86. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].
Thin-layer Chromatography System TB—R_f 0.02; system TE—R_f 0.77; system TF—R_f 0.60; system TAE—R_f 0.87.
Gas Chromatography System GB—RI 2793; system GP—RI 2235 M ((dehydro-desethyl)-ME); RI 2280 M (dehydro-).

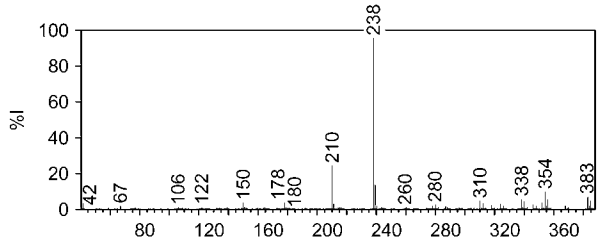
Column: methyl silicone HP1 (0.2 mm i.d., 0.33 μm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. Detection: mass spectrometer. Retention index: 2647 [Mills, Roberson 1993].
High Performance Liquid Chromatography System HX—RI 690; system HZ—retention time 25.8 min; system HAA—retention time 24.4 min.
Ultraviolet Spectrum Aqueous acid—237, 359 nm; basic—281 nm.



Infrared Spectrum Principal peaks at wavenumbers 1698, 1496, 1206, 1099 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 238, 210, 239, 354, 383, 338, 178, 310.



Quantification
Plasma **GC** Limit of quantification, 0.1 μg/L for felodipine, 0.3 μg/L for its metabolite [Soons *et al.* 1990]. Column: silica (25 m × 0.32 mm i.d.), silanised with diphenyltetramethyldisilazane. Temperature: programmed: 8°/min to 270°. Injector and detector temperatures: 270° and 350°, respectively. Carrier gas: He at 1.2 bar pressure. Detection: electron capture, ^{63}Ni. Retention time: 14.8 min [Ahnoff 1984]. Limit of detection, 0.4 to 0.8 μg/L [Ahnoff 1984].

HPLC Column: RP-select B LiChrospher 60 (250 × 4.0 mm i.d., 5 μm). Mobile phase: A-methanol : 0.05 mol/L phosphate buffer (pH 3.5) (45:55); B-methanol: 0.05 mol/L phosphate buffer (pH 3.5) (80:20), flow rate 1.15 mL/min. Elution program: (A:B) (100:0) for 17 min, to (0:100) in 5 min, held for 5 min. UV detection (λ=220 nm). Internal standard (IS): 3,5-pyridine dicarboxylic acid. Retention time: felodipine metabolites, M3, 10.4 min; M4, 17.4 min; M6, 9.4 min; M7, 15.1 min; IS, 13.2 min. Limit of detection, 8 μg/L [Gabrielsson *et al.* 1992]. Limit of detection, 5 μg/L for each enantiomer [Soons *et al.* 1990].
Disposition in the Body Felodipine is almost completely absorbed after oral administration and undergoes extensive first-pass metabolism in the liver, by microsomal cytochrome P450 3A enzyme, to form a pyridine analogue (1,4-dihydropyridine goes to inactive dehydrofelodipine). Subsequent oxidation results in ~15 inactive metabolites. 70% of a dose is excreted in urine, with <0.5% unchanged, and the rest in faeces, as metabolites.
Therapeutic Concentration The serum therapeutic concentration range is 1 to 8 μg/L.

A total of 140 volunteers: men and women, healthy and hypertensive (all women), aged between 20 and 39 years (70 people), 40 and 59 (30 people) and 60 and 80 years (40 people) were administered doses of 5 mg twice daily and 10 mg twice daily. The duration of the treatment varied from 6 to 30 days. Peak plasma concentrations increased with age where levels of 0.019 ± 0.009, 0.023 ± 0.010, 0.030 ± 0.0129 nmol/L were reached for the 20 to 39 years, 40 to 59, 60 to 80 age groups, respectively. All peak levels were reached within 2 h [Blychert *et al.* 1991].

Twelve healthy males, mean age 25 years (range, 20 to 34 years) were administered with 20 mg (racemate) felodipine. Peak plasma concentrations for the racemate drug were 30.3 (13.6 to 47.6) μg/L; for (*S*)-felodipine, 20.4 (0.53 to 32.7) μg/L and (*R*)-felodipine, 9.77 (5.07 to 15.6) μg/L. All concentrations were observed at a mean of 58 min (range 19 to 180) [Soons *et al.* 1993].

Toxicity The serum toxic concentration range is 10 to 15 μg/L. Overdosing may cause excessive peripheral vasodilation with marked hypotension and possibly bradycardia.
Bioavailability 15%.
Half-life 11 to 16 h (oral administration in normal patients); 2 h (patients with renal failure).
Volume of Distribution Apparent volume of distribution, 10 L/kg (normal patients); 5.6 L/kg (patients with cirrhosis).
Clearance Systemic plasma clearance, about 0.8 L/min.
Protein Binding Approximately 99%, mainly albumin.

Dose An initial dose of 5 mg daily is given followed by a usual maintenance dose of 2.5 to 10 mg. Elderly and patients with impaired liver function: initial dose of 2.5 mg daily.

Ahnoff M (1984). Determination of felodipine in plasma by capillary gas chromatography with electron capture detection. *J Pharm Biomed Anal* 2: 519–526.

Blychert E *et al.* (1991). A population study of the pharmacokinetics of felodipine. *Br J Clin Pharm* 31: 15–24.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Gabrielsson M *et al.* (1992). Determination of four carboxylic acid metabolites of felodipine in plasma by high-performance liquid chromatography. *J Chromatogr* 573(2): 265–274.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press 6: 86–87.

Soons PA *et al.* (1990). Enantioselective determination of felodipine and other chiral dihydropyridine calcium entry blockers in human plasma. *J Chromatogr* 528(2): 343–356.

Soons PA *et al.* (1993). Stereoselective pharmacokinetics of oral felodipine and nitrendipine in healthy subjects: correlation with nifedipine pharmacokinetics. *Eur J Clin Pharmacol* 44(2): 163–169.

Fenamiphos

Nematocide

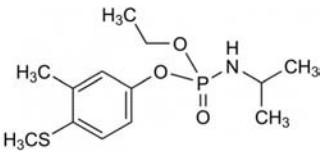
$C_{13}H_{22}NO_3PS = 303.4$

CAS—22224-92-6

IUPAC Name *N*-[Ethoxy-(3-methyl-4-methylsulfanylphenoxy)phosphoryl] propan-2-amine

Synonyms B-68138; Bay 68138; Bayer 68138; (1-methylethyl)phosphoramidic acid ethyl 3-methyl-4-(methylthio)phenyl ester; phenamiphos; SRA-3886.

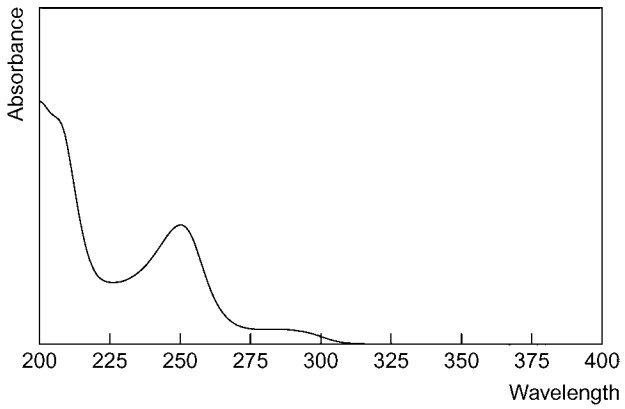
Proprietary Name *Nemacur*



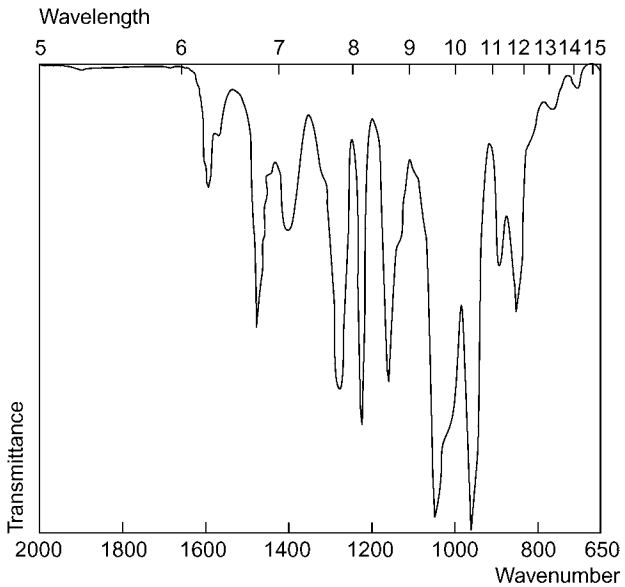
Chemical Properties A colourless, crystalline solid or a tan, waxy solid (technical state). Mp 49°. It is soluble in water (329 mg/L at 20°), 2-propanol, dichloromethane, toluene, and isopropanol. pK_a 10.5 (25°). Log *P* (octanol/water), 3.25.

Gas Chromatography System GA—RI 2020.

Ultraviolet Spectrum Principal peak at 250 nm.



Infrared Spectrum Principal peaks at wavenumber 967, 1049, 1226, 1278, 1158, 1473 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 303, 154, 217, 44, 288, 80, 260, 153.

Disposition in the Body Fenamiphos is readily absorbed after oral ingestion (~95%) and after inhalation, via the lungs. It is rapidly broken down within the body to produce the two major oxidative metabolites—fenamiphos sulfoxide and fenamiphos sulfone—which may be followed by subsequent hydrolysis and conjugation. These are excreted in urine. The majority of a dose is recovered in urine 15 h after exposure.

Toxicity Fenamiphos is highly toxic if ingested orally and by inhalation. It can also cause acute dermal toxicity if exposed and significant eye damage at acute exposure levels. The allowed daily intake is 0.5 µg/kg body weight.

Fenbendazole

Anthelmintic

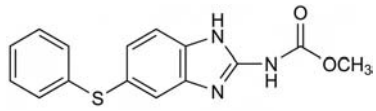
$C_{15}H_{13}N_3O_2S = 299.3$

CAS—43210-67-9

IUPAC Name Methyl *N*-(6-phenylsulfanyl-1*H*-benzimidazol-2-yl)carbamate

Synonym [5-(Phenylthio)-1*H*-benzimidazol-2-yl]carbamic acid methyl ester

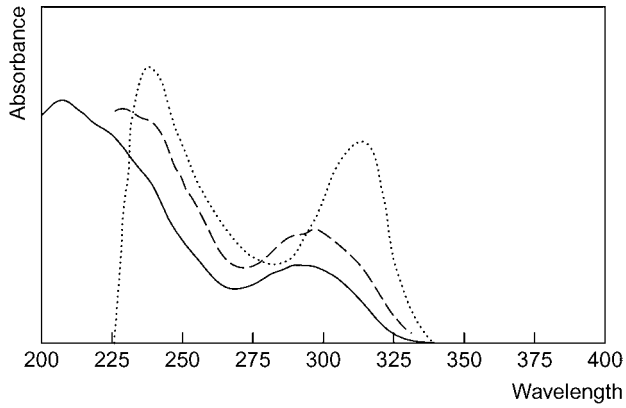
Proprietary Names *Axilur*; *Curazole*; *Fencare*; *Fenzol*; *Flexadin*; *Forazole*; *Granofen*; *Multiwurma-F*; *Orazole*; *Panacur*; *Parazole*; *Safe-Guard*; *Wormaway Feben*; *Zerofen* (all vet.).



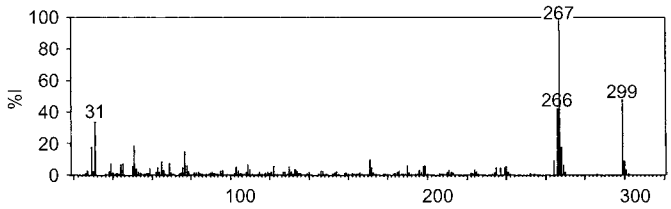
Chemical Properties A light brownish-grey crystalline powder. Mp 233°, with decomposition. Insoluble in water; freely soluble in dimethyl sulfoxide. Log *P* (octanol/water), 3.8.

High Performance Liquid Chromatography System HY—RI 370.

Ultraviolet Spectrum Aqueous acid—289, 302 nm; aqueous alkali—238, 312 nm; ethanol—296 nm (A^1_1=416b).



Mass Spectrum Principal ions at *m/z* 267, 299, 266, 31, 51, 268, 29, 77.



Dose Fenbendazole has been given in doses of 1 to 1.5 g.

Fenbufen

Analgesic

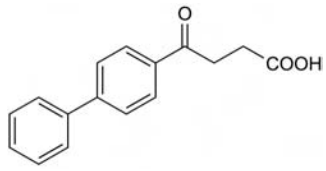
$C_{16}H_{14}O_3 = 254.3$

CAS—36330-85-5

IUPAC Name 4-Oxo-4-(4-phenylphenyl)butanoic acid

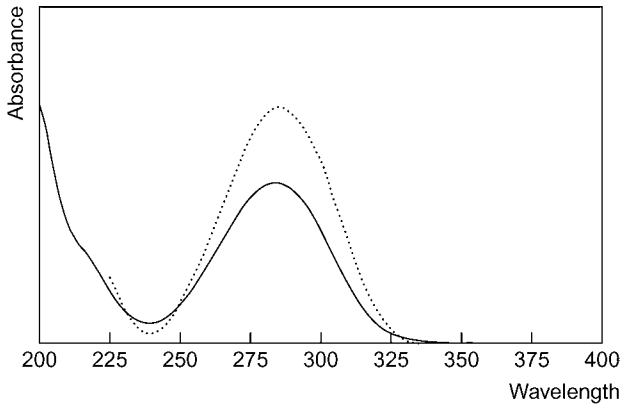
Synonym γ-Oxo-[1,1′-biphenyl]-4-butanoic acid

Proprietary Names *Cincopal*; *Cinopal*; *Lederfen*; *Reugast*.

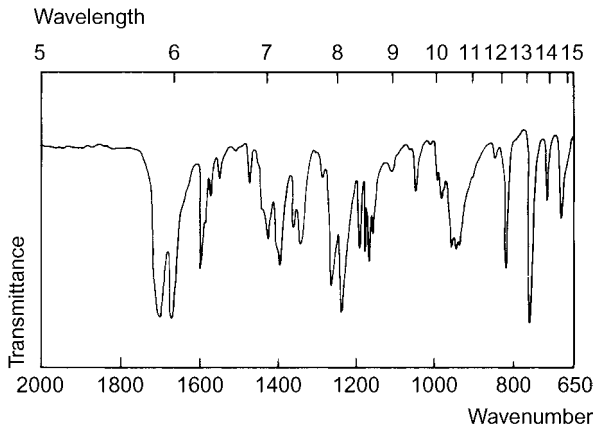


Chemical Properties Crystals. Mp about 180°. pK_a 4.5. Log *P* (octanol/water), 3.2.

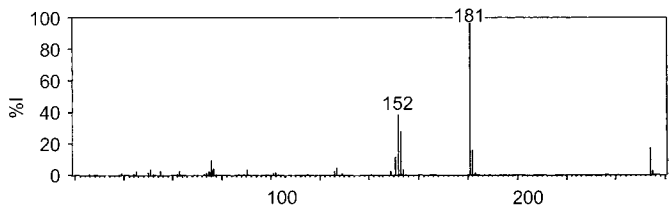
Colour Tests Liebermann's reagent—red-brown; Marquis test—orange→brown; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.92; system TD—R_f 0.18; system TE—R_f 0.04; system TF—R_f 0.30; system TG—R_f 0.09; system TAD—R_f 0.39; system TAJ—R_f 0.43; system TAK—R_f 0.68; system TAL—R_f 0.91.
Gas Chromatography System GA—fenbufen RI 3078, fenbufen-Me RI 2315; System GD—methyl derivative RRT 1.79 (relative to n-$C_{16}H_{34}$); system GL—fenbufen-Me RI 1975, M (acetic acid OH-)-Me_2 RI 2190.
High Performance Liquid Chromatography System HD—*k* 4.0; system HV—RRT 0.81 (relative to meclofenamic acid); system HX—RI 520; system HY—RI 461; system HAA—retention time 19.3 min.
Ultraviolet Spectrum Aqueous alkali—285 nm (A^1_1= 835a).



Infrared Spectrum Principal peaks at wavenumbers 764, 1674, 1708, 1242, 1268, 829 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 181, 152, 153, 254, 182, 151, 76, 127; fenbufen-Me *m/z* 181, 152, 153, 182, 268, 237; M (acetic acid OH-)-Me_2 *m/z* 197, 256, 154, 128, 152, 198.



Quantification
Plasma **GC** FID. Limit of detection, 50 µg/L for fenbufen, 100 µg/L for metabolites (i) and (ii) (see under Disposition in the Body) [Cuisinaud *et al.* 1978].

HPLC UV detection. Fenbufen, rufloxacin and felbinac. Limit of detection, 0.3 mg/L for fenbufen [Carlucci *et al.* 1996].
Serum **HPLC** Limit of detection, 300 to 800 µg/L for fenbufen and other NSAIDs [Haque, Stewart 1999]. Fluorescence detection. Limit of detection, 10 µg/L for fenbufen and its metabolites [Siluveru, Stewart 1996]. UV detection. Limit of detection, 500 µg/L for fenbufen and metabolites (i) and (ii) [Fleitman *et al.* 1982]. UV detection. Limit of detection, 500 µg/L for fenbufen and metabolites [Van Lear *et al.* 1978].
Urine **HPLC** UV detection. Fenbufen and other NSAIDs. Limit of detection, 0.05 mg/L for fenbufen [Hirai *et al.* 1997]. See Serum. Limit of detection, 1 µg/L for fenbufen and metabolites [Van Lear *et al.* 1978].
Disposition in the Body Readily absorbed after oral administration, and extensively metabolised to active metabolites. A number of metabolites have been identified including (i) 4-(biphenyl-4-yl)-4-hydroxybutyric acid, (ii) biphenyl-4-ylacetic acid, (iii) (4′-hydroxybiphenyl-4-yl)acetic acid, and (iv) 4-hydroxy-(4′-hydroxybiphenyl-4-yl)butyric acid. About 40% of a dose is excreted in the urine in 24 h. The major urinary metabolites are metabolite (iii), about 11% of the dose, and metabolite (iv), about 17% of the dose; metabolites (i) and (ii) are excreted in amounts less than about 3% of the dose. Very little unchanged drug is excreted in the urine. <2% of the dose is eliminated in the faeces in 24 h.

Therapeutic Concentration

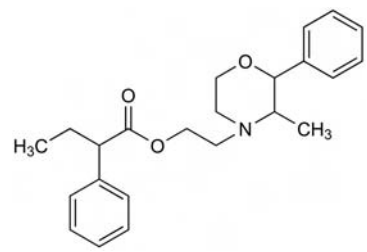
After a single oral dose of 600 mg administered to 3 subjects, mean peak serum concentrations of 8.1 mg/L of fenbufen and 64.9 mg/L of metabolite (i) were attained in about 2 h. A mean peak serum concentration of 13.2 mg/L of metabolite (ii) was attained in about 8 h [Chiccarelli *et al.* 1980].

Half-life Plasma half-life, fenbufen and metabolites (i) and (ii), about 10 h.
Protein Binding Fenbufen and metabolite (ii), >99%; metabolite (i), about 98%.
Note For a review of the pharmacokinetics of fenbufen, see Brogden *et al.* [1981].
Dose 600 to 900 mg daily.

Brogden RN *et al.* (1981). Fenbufen: a review of its pharmacological properties and therapeutic use in rheumatic diseases and acute pain. *Drugs* 21: 1–22.
Carlucci G *et al.* (1996). Simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography. *J Chromatogr Biomed Appl* 682: 315–319.
Chiccarelli FS *et al.* (1980). Metabolic and pharmacokinetic studies with fenbufen in man. *Arzneimittelforschung* 30: 728–735.
Cuisinaud G *et al.* (1978). Gas chromatographic determination of 3-(4-biphenylylcarbonyl) propionic acid (fenbufen) and two metabolites in human plasma. *J Chromatogr* 148: 509–513.
Fleitman JS *et al.* (1982). High-performance liquid chromatography assay for fenbufen and two serum metabolites. *J Chromatogr Biomed Appl* 228: 372–376.
Haque A, Stewart JT (1999). Direct injection HPLC analysis of some non-steroidal anti-inflammatory drugs on restricted access media columns. *Biomed Chromatogr* 13: 51–56.
Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692(2): 375–388.
Siluveru M, Stewart JT (1996). Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using solid-phase extraction and on-line post-column ultraviolet irradiation and fluorescence detection. *J Chromatogr Biomed Appl* 682: 89–94.
Van Lear GE *et al.* (1978). Quantitation of the anti-inflammatory agent fenbufen and its metabolites in human serum and urine using high-pressure liquid chromatography. *J Pharm Sci* 67: 1662–1664.

Fenbutrazate

Anorectic
$C_{23}H_{29}NO_3$ = 367.5
CAS—4378-36-3
IUPAC Name 2-(3-Methyl-2-phenylmorpholin-4-yl)ethyl 2-phenylbutanoate
Synonyms α-Ethylbenzeneacetic acid 2-(3-methyl-2-phenyl-4-morpholinyl) ethyl ester; phenbutrazate.



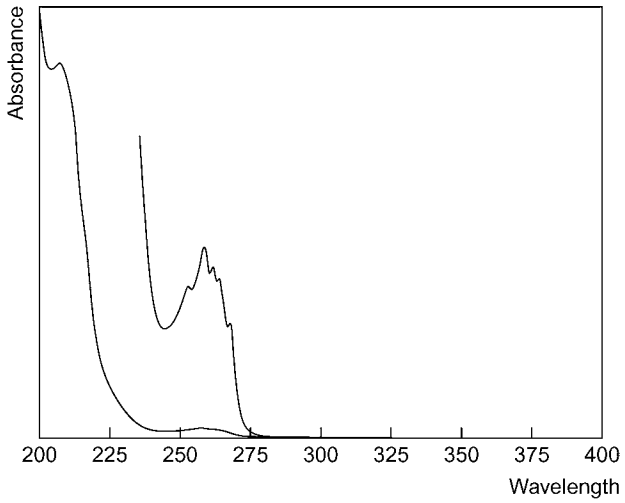
Chemical Properties A viscous oil. Soluble in methanol. Log *P* (octanol/water), 4.4.

Fenbutrazate Hydrochloride

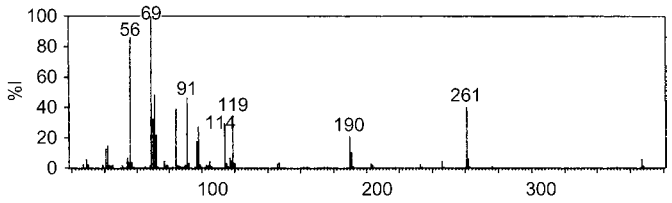
$C_{23}H_{29}NO_3$,HCl = 403.9
CAS—6474-85-7
Synonym Phenbutrazate hydrochloride
Chemical Properties A fine white crystalline powder. Mp 154°. Slightly soluble in water; soluble in ethanol and acetone; practically insoluble in ether and benzene.

Colour Tests Liebermann's reagent—red-orange; Marquis test—yellow (slow).

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.47; system TC—R_f 0.78; system TE—R_f 0.86; system TL—R_f 0.67; system TAE—R_f 0.88 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2670.
High Performance Liquid Chromatography System HA—*k* 0.3.
Ultraviolet Spectrum Aqueous acid—252, 257 (A^1_1=10b), 261, 267 nm.



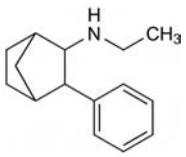
Infrared Spectrum Principal peaks at wavenumbers 1715, 1100, 1200, 690, 760, 1020 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 69, 56, 71, 91, 261, 84, 119, 70.



Dose Fenbutrazate hydrochloride has been given in doses of 40 to 60 mg daily.

Fencamfamin

CNS Stimulant
$C_{15}H_{21}N$ = 215.3
CAS—1209-98-9
IUPAC Name *N*-Ethyl-3-phenylbicyclo[2.2.1]heptan-2-amine



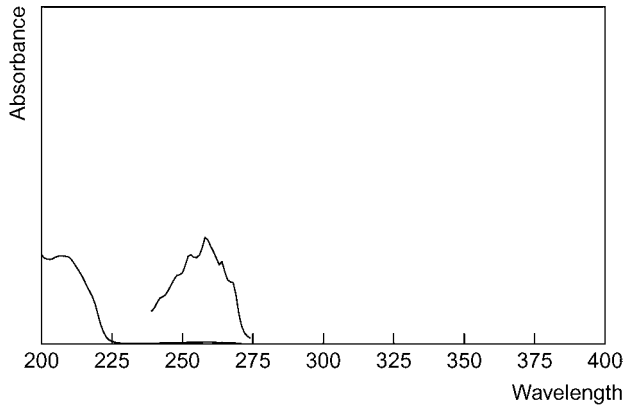
Chemical Properties Practically insoluble in water; soluble in chloroform. pK_a 8.7 (25°). Log *P* (octanol/water), 3.2.

Fencamfamin Hydrochloride

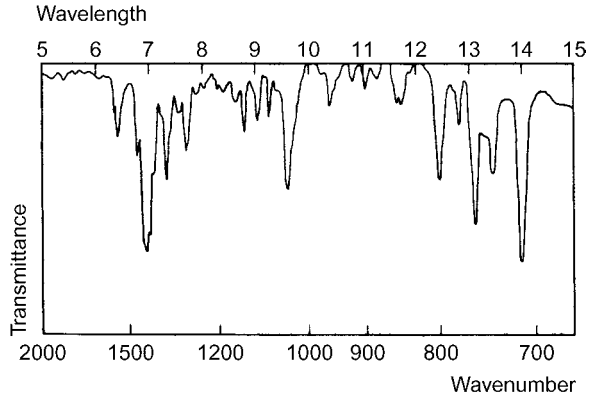
$C_{15}H_{21}N,HCl$ = 251.8
CAS—2240-14-4
Proprietary Name It is an ingredient of *Reactivan*.
Chemical Properties A white crystalline solid. Mp 192°. Freely soluble in water, ethanol, methanol and chloroform; slightly soluble in benzene; practically insoluble in ether.

Colour Tests Liebermann's reagent—red-orange; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.62; system TC—R_f 0.34; system TE—R_f 0.77; system TL—R_f 0.30; system TAE—R_f 0.21 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—fencamfamin RI 1675, fencamfamin-AC RI 2085, fencamfamin-TFA RI 1970, fencamfamin-TMS RI 1780, fencamfamin-PFP RI 1755, M (desethyl-)-AC RI 2005, M (desethyl-OH-)-AC_2 RI 2305; system GB—RI 1737; system GC—RI 2180.
High Performance Liquid Chromatography System HA—*k* 1.3; system HC—*k* 0.72; system HX—RI 354; system HY—RI 309.

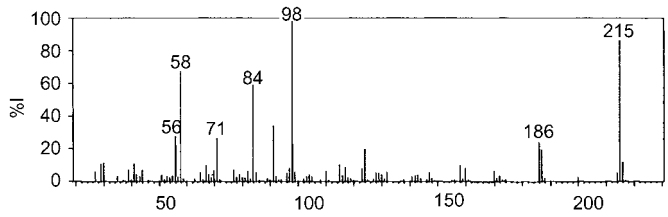
Ultraviolet Spectrum Aqueous acid—253, 259 (A^1_1=10a), 265, 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 708, 756, 1042, 797, 738, 1492 cm^{-1} (fencamfamin hydrochloride (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 98, 215, 58, 84, 91, 56, 71, 186; fencamfamin-AC *m/z* 170, 142, 58, 97, 91, 84; M (desethyl-)-AC *m/z* 170, 142, 91, 171, 115, 229; M (desethyl-OH-)-AC_2 *m/z* 142, 168, 228, 91, 119, 287.



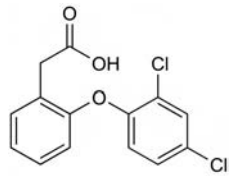
Quantification
Urine **GC** ECD. Limit of detection, 40 µg/L for fencamfamin, 50 µg/L for the desethyl metabolite [Delbeke, Debackere 1981].
Disposition in the Body Absorbed after oral administration. Metabolised by de-ethylation. About 10 to 30% of a dose is excreted in the urine as unchanged drug and desethylfencamfamin in 3 days. The extent and rate of excretion is dependent on the urinary pH.
Half-life Derived from urinary excretion data, about 16 h.
Dose Usually 30 mg of fencamfamin hydrochloride daily.

Delbeke FT, Debackere M (1981). Detection and metabolism of fencamfamine and the influence of acetazolamide on its urinary excretion. *Biopharm Drug Dispos* 2: 17–30.

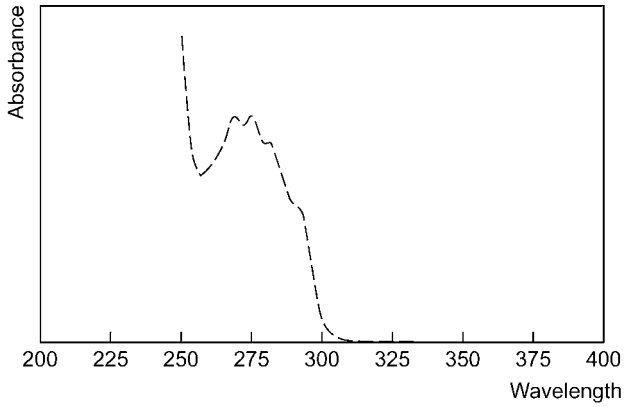
Fenclofenac

Analgesic
$C_{14}H_{10}Cl_2O_3$ = 297.1
CAS—34645-84-6

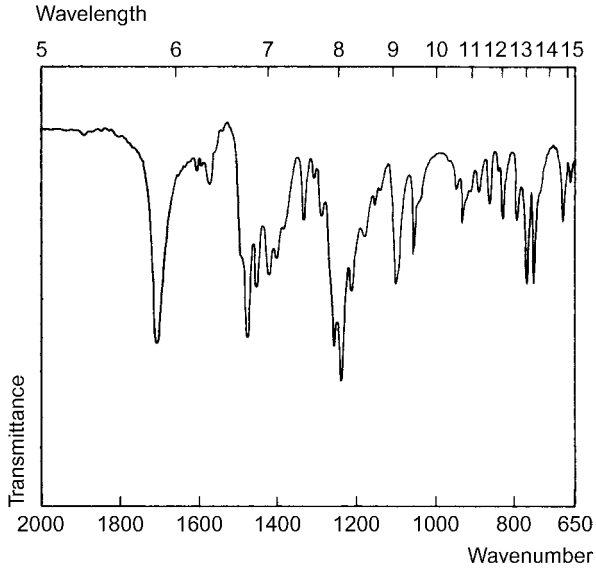
IUPAC Name [2-(2,4-Dichlorophenoxy)phenyl]acetic acid



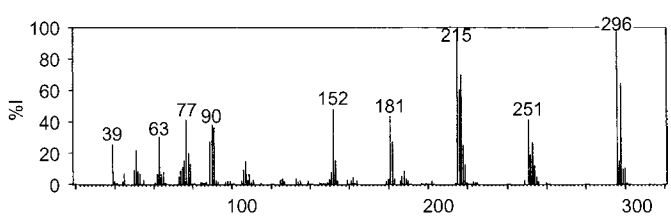
Chemical Properties A white powder. Mp about 136°. Slightly soluble in water, but less soluble in acid solutions; freely soluble in most organic solvents. pK_a 5.5. Log *P* (octanol/water), 4.8.
Colour Tests Liebermann's reagent—orange (slow), (→brown at 100°); Marquis test—red (slow).
Thin-layer Chromatography System TG—R_f 0.20.
Gas Chromatography System GD—methyl derivative RRT 1.55 and RRT 1.26 (relative to n-$C_{16}H_{34}$).
High Performance Liquid Chromatography System HV—RRT 0.91 (relative to meclofenamic acid).
Ultraviolet Spectrum Methanol—270 (A^1_1=72b), 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1242, 1258, 1707, 1215, 1100, 772 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 296, 215, 217, 298, 216, 152, 181, 251.



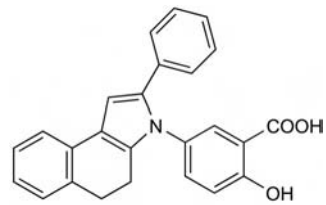
Quantification
Plasma **GC** ECD. Limit of detection, 300 µg/L [Henson *et al.* 1980].

Disposition in the Body Well absorbed after oral administration. >90% of a dose is excreted in the urine in the form of conjugates of the drug and hydroxylated metabolites.
Therapeutic Concentration
A single oral dose of 600 mg to 9 subjects produced a mean peak plasma concentration of 63 mg/L in 3 to 4 h. Daily oral doses of 1200 mg to 5 subjects produced a mean steady-state plasma concentration of 87 mg/L [Henson *et al.* 1980].
Following daily oral doses of 10 to 25 mg/kg to 18 children for 3 weeks, maximum steady-state plasma concentrations of 52 to 372 mg/L (mean 120) were reported 2 to 8 h after a dose [Mäkelä *et al.* 1983].
Toxicity Fenclofenac was used in rheumatic disorders but it had a high incidence of adverse effects, especially skin reactions, and was withdrawn from the market.
Half-life Plasma half-life, 15 to 40 h (mean 26).
Volume of Distribution About 0.2 L/kg.
Distribution in Blood Plasma : whole blood ratio, about 1.7.
Protein Binding About 99%.
Dose 0.6 to 1.2 g daily.

Henson R *et al.* (1980). Pharmacokinetics of fenclofenac following single and multiple doses. *Eur J Drug Metab Pharmacokinet* 5(4): 217–223.
Mäkelä AL *et al.* (1983). Pharmacokinetics of fenclofenac in children with juvenile rheumatoid arthritis. *Eur J Clin Pharmacol* 25(3): 381–388.

Fendosal

Analgesic
$C_{25}H_{19}NO_3$ = 381.4
CAS—53597-27-6
IUPAC Name 2-Hydroxy-5-(2-phenyl-4,5-dihydrobenzo[*e*]indol-3-yl)benzoic acid
Synonym 5-(4,5-Dihydro-2-phenyl-3*H*-benz[*e*]indol-3-yl)-2-hydroxybenzoic acid
Proprietary Name *Alnovin*

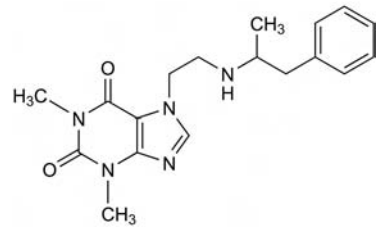


Chemical Properties A yellow powder. Mp 239° to 241°. Practically insoluble in water; soluble 1 in 50 of ethanol; slightly soluble in alkaline solutions and in propylene glycol. pK_a 3.1.
Thin-layer Chromatography System TA—R_f 0.95; system TE—R_f 0.22; system TAJ—R_f 0.05; system TAK—R_f 0.68; system TAL—R_f 0.83.
Ultraviolet Spectrum Aqueous alkali—292 nm (A^1_1=564b).
Infrared Spectrum Principal peaks at wavenumbers 1675, 1242, 1500, 1615, 1298, 765 cm^{-1} (KBr disk).
Quantification
Plasma **Fluorescence spectrophotometry** Limit of detection, 0.1 mg/L [Hill *et al.* 1980].
Dose Fendosal has been given in doses of 200 to 400 mg.

Hill HM *et al.* (1980). Rapid fluorimetric procedure for the analysis of fendosal in plasma and data following oral dosing. *Biopharm Drug Dispos* 1: 97–102.

Fenetylline

Xanthine Stimulant
$C_{18}H_{23}N_5O_2$ = 341.4
CAS—3736-08-1
IUPAC Name 1,3-Dimethyl-7-[2-(1-phenylpropan-2-ylamino)ethyl]purine-2,6-dione
Synonyms Amfetyline; 3,7-dihydro-1,3-dimethyl-7-[2-[(1-methyl-2-phenylethyl) amino]ethyl]-1*H*-purine-2,6-dione; 7-ethyltheophylline amfetamine; fenethylline.



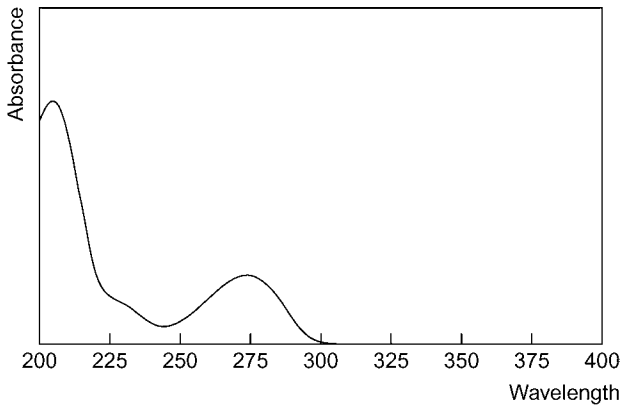
Chemical Properties Practically insoluble in water; soluble in chloroform. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Fenetylline Hydrochloride

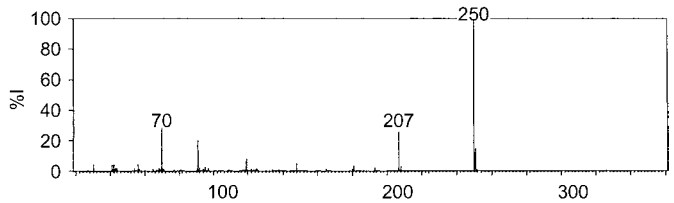
$C_{18}H_{23}N_5O_2$,HCl = 377.9
CAS—1892-80-4

Synonyms Fenethylline hydrochloride; H814; R-720-11.
Proprietary Names *Captagon*; *Fitton*.
Chemical Properties A white crystalline powder. Mp 227° to 229° and 237° to 239°. Soluble in water.

Colour Tests Amalic acid test—pink-orange/violet; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.03; system TC—R_f 0.45; system TE—R_f 0.54; system TL—R_f 0.14; system TAE—R_f 0.44 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—fenetylline RI 2830, fenetylline-AC RI 3110, M (N-desalkyl)-AC RI 2480, M (amfetamine) RI 1125, M (etofylline) RI 2125; system GB—fenetylline RI 2900.
High Performance Liquid Chromatography System HC—*k* 0.27; system HX—RI 336; system HY—RI 277.
Ultraviolet Spectrum Aqueous acid—275 nm (A_1^1=242a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1664, 1705, 1600, 1546, 746, 1219 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 250, 70, 207, 91, 251, 119, 148, 56.



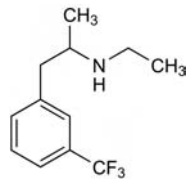
Quantification
Hair **MS–GC** For method of quantification, see Kikura and Nakahara [1997].
Dose Fenetylline hydrochloride has been given in doses of 25 to 100 mg daily to treat narcolepsy.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Kikura R, Nakahara Y (1997). Hair analysis for drugs of abuse. XVI. Disposition of fenethylline and its metabolite into hair and discrimination between fenethylline use and amphetamine use by hair analysis. *J Anal Toxicol* 21: 291–296.

Fenfluramine

Anorectic
$C_{12}H_{16}F_3N$ = 231.3
CAS—458-24-2

IUPAC Name N-Ethyl-1-[3-(trifluoromethyl)phenyl]propan-2-amine
Synonym *N*-Ethyl-α-methyl-3-(trifluoromethyl)benzeneethanamine



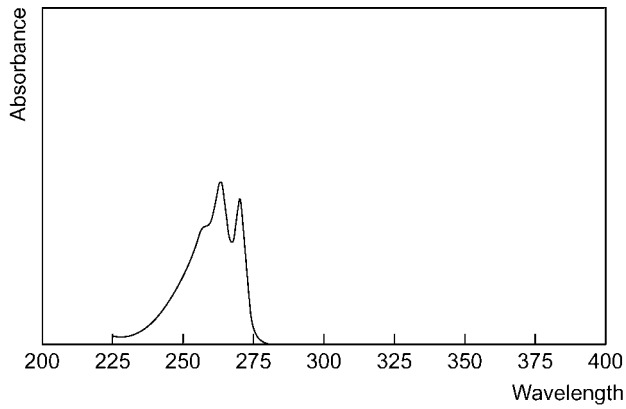
Chemical Properties Practically insoluble in water; soluble in chloroform. pK_a 9.1 (25°). Log *P* (octanol/water), 3.4.

Fenfluramine Hydrochloride

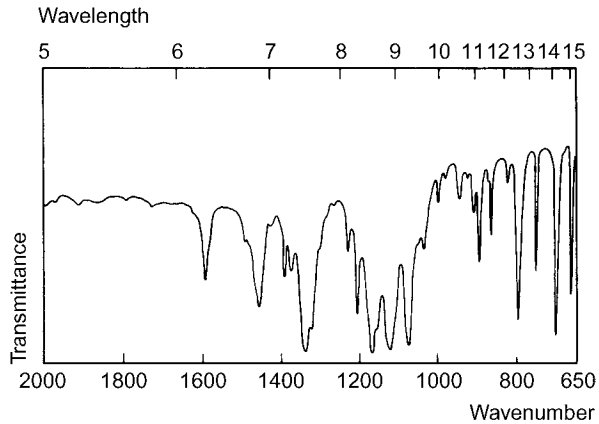
$C_{12}H_{16}F_3N$,HCl = 267.7
CAS—404-82-0

Proprietary Names *Dima-Fen*; *Pesos*; *Ponderal*; *Ponderax*; *Pondimin*.
Chemical Properties A white crystalline powder. Mp 168° to 172°. Soluble 1 in 20 of water, 1 in 10 of ethanol and 1 in 10 of chloroform; practically insoluble in ether.

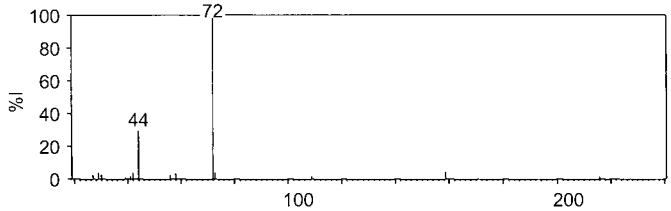
Colour Test Liebermann's reagent (100°)—yellow.
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.42; system TC—R_f 0.16; system TE—R_f 0.61; system TL—R_f 0.11; system TAE—R_f 0.20; system TAJ—R_f 0.07; system TAK—R_f 0.25; system TAL—R_f 0.68 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—fenfluramine RI 1230, norfenfluramine RI 1133, fenfluramine-TFA RI 1455, fenfluramine-PFP RI 1455, fenfluramine-AC RI 1580, norfenfluramine-AC RI 1510; system GB—fenfluramine RI 1252, norfenfluramine RI 1157; system GC—fenfluramine RI 1621, norfenfluramine RI 1470.
High Performance Liquid Chromatography System HA—fenfluramine *k* 1.3, norfenfluramine *k* 1.0; system HC—*k* 0.88; system HX—RI 371; system HY—RI 315; system HZ—retention time 3.9 min; system HAA—retention time 13.1 min.
Ultraviolet Spectrum Aqueous acid—264 (A_1^1=22a), 271 nm.



Infrared Spectrum Principal peaks at wavenumbers 1165, 1116, 1070, 698, 793, 1202 cm^{-1} (fenfluramine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 44, 159, 73, 58, 42, 109, 56; norfenfluramine 44, 42, 159, 43, 45, 184, 41, 109.



Quantification
Blood **GC–MS** Fenfluramine, amfetamine and metamfetamine. Limit of detection, 5 µg/L for fenfluramine [Namera *et al.* 2000].
Plasma **GC** Nitrogen-specific detection. For method of quantification for D-fenfluramine and D-norfenfluramine, see Richards *et al.* [1989]. ECD. For method of quantification for enantiomers of fenfluramine and norfenfluramine see, Srinivas *et al.* [1988]. ECD. Limit of detection, 5 µg/L for fenfluramine, 100 ng/L for norfenfluramine [Midha *et al.* 1979].

HPLC Fluorescence detection. For method of quantification for DL-fenfluramine, DL-norfenfluramine and phentermine, see Kaddoumi *et al.* [2001].

Urine **GC** See Plasma [Richards *et al.* 1989]. ECD. For method of quantification for fenfluramine and norfenfluramine, see Midha *et al.* [1979].

GC–MS For method of quantification for fenfluramine and amfetamine-related drugs, see Namera *et al.* [2002].

Disposition in the Body Readily absorbed after oral administration and accumulates in the tissues. The major metabolite in the blood is the *N*-desethyl derivative, norfenfluramine, which is active. It is also metabolised by oxidation to *m*-trifluoromethylbenzoic acid which is conjugated with glycine to form *m*-trifluoromethylhippuric acid. The rate of elimination is influenced by urinary pH and urinary flow. In acidic urine about 23% of a dose is excreted unchanged and about 17% as norfenfluramine in 48 h; the remainder consists of *m*-trifluoromethylhippuric acid; in alkaline urine about 2% is excreted as unchanged drug and norfenfluramine; when the urinary pH is not controlled, 3 to 10% may be excreted as unchanged drug and 3 to 14% as norfenfluramine. Up to 5% of a dose is eliminated in the faeces as fenfluramine and norfenfluramine.

Therapeutic Concentration In plasma, usually in the range 0.05 to 0.15 mg/L. There is considerable inter-subject variation in plasma concentrations and it has been reported that the therapeutic effect (weight loss) is greater in those patients who can tolerate higher plasma concentrations (more than 0.2 mg/L) [Innes *et al.* 1977].

A single oral dose of 60 mg administered to 5 subjects, resulted in a mean plasma concentration of 0.06 mg/L of fenfluramine in 2 to 4 h and 0.016 mg/L of norfenfluramine in 4 to 6 h. Steady-state plasma concentrations of 0.04 to 0.12 mg/L of fenfluramine were attained in 3 to 4 days after daily administration of 60 mg, in divided doses, to 6 subjects; concentrations of norfenfluramine were similar [Campbell 1971].

Toxicity In adults the minimum lethal dose is probably in excess of 2 g but for young children as little as 200 mg may cause death. Toxic effects may be produced when the plasma concentration is greater than about 0.5 mg/L, and death has occurred at concentrations above 6 mg/L.

Three children aged 6 years, 3 years, and 1 year 9 months ingested between them about 4 g of fenfluramine hydrochloride. The youngest and the oldest child died; postmortem blood concentrations were 6.5 and 16 mg/L respectively and liver concentrations were 48 and 136 µg/g; a urine concentration of 60 mg/L was found in the younger child [Gold *et al.* 1969].

A 13-year-old boy died after ingesting 2 g of fenfluramine hydrochloride; postmortem concentrations, mg/L or µg/g, were:

[Fleisher, Campbell 1969].

	Fenfluramine	*Norfenfluramine*
Blood	6.5	0.75
Bile	64.5	10.2
Brain	42	5.3
Kidney	27.1	1.5
Liver	49	8.5
Urine	89	10

In a fatality involving the suicidal ingestion of fenfluramine, a blood concentration of 7.46 mg/L was reported. Chronic fenfluramine use was demonstrated by the presence of the drug in hair (14.1 µg/g) [Kintz, Mangin 1992].

Half-life Plasma half-life, 11 to 30 h (mean 20).

Distribution in Blood Plasma : whole blood ratio, about 0.74.

Protein Binding About 30%.

Note For reviews of fenfluramine, see Pinder *et al.* [1975] and Vivero *et al.* [1998]. For a review of fenfluramine poisoning, see Von Mühlendahl and Krienke [1979].

Dose Initially 40 mg of fenfluramine hydrochloride daily, increasing to 60 to 120 mg daily.

Campbell DB, Turner P (1971). Plasma concentrations of fenfluramine and its metabolite, norfenfluramine, after single and repeated oral administration. *Br J Pharmacol* 43(2): 465P–466P.

Fleisher MR, Campbell DB (1969). Fenfluramine overdosage. *Lancet* 2: 1306–1307.

Gold RG *et al.* (1969). Fenfluramine overdosage. *Lancet* 2: 1306.

Innes JA *et al.* (1977). Plasma fenfluramine levels, weight loss, and side effects. *Br Med J* 2: 1322–1325.

Kaddoumi A *et al.* (2001). Fluorometric determination of DL-fenfluramine, DL-norfenfluramine and phentermine in plasma by achiral and chiral high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 763: 79–90.

Kintz P, Mangin P (1992). Toxicological findings after fatal fenfluramine self-poisoning. *Hum Exp Toxicol* 11(1): 51–52.

Midha KK *et al.* (1979). *Can J Pharm Sci* 14: 18–21.

Namera A *et al.* (2000). Simple and simultaneous analysis of fenfluramine, amphetamine and methamphetamine in whole blood by gas chromatography-mass spectrometry after headspace-solid phase microextraction and derivatization. *Forensic Sci Int* 109: 215–223.

Namera A *et al.* (2002). Automated headspace solid-phase microextraction and in-matrix derivatization for the determination of amphetamine-related drugs in human urine by gas chromatography-mass spectrometry. *J Chromatogr Sci* 40: 19–25.

Pinder RM *et al.* (1975). Fenfluramine: a review of its pharmacological properties and therapeutic efficacy in obesity. *Drugs* 10(4): 241–323.

Richards RP *et al.* (1989). The measurement of d-fenfluramine and its metabolite, d-norfenfluramine in plasma and urine with an application of the method to pharmacokinetic studies. *Xenobiotica* 19: 547–553.

Srinivas NR *et al.* (1988). Enantioselective gas chromatographic assay with electron-capture detection for dl-fenfluramine and dl-norfenfluramine in plasma. *J Chromatogr* 433: 105–117.

Vivero LE *et al.* (1998). A close look at fenfluramine and dexfenfluramine. *J Emerg Med* 16(2): 197–205.

Von Mühlendahl KE, Krienke EG (1979). Fenfluramine poisoning. *Clin Toxicol* 14(1): 97–106.

Fenimide

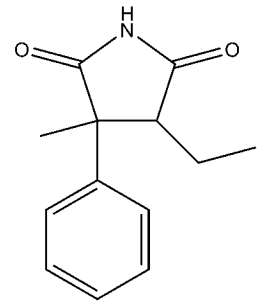
Succinimide, Antipsychotic

$C_{13}H_{15}NO_2 = 217.3$

CAS—60-45-7

IUPAC Name 4-Ethyl-3-methyl-3-phenylpyrrolidine-2,5-dione

Synonyms CI-419; α-ethyl-α′-methyl-α′-phenylsuccinimide; fenetimide; fenimid.



Chemical Properties White crystalline powder. Insoluble in water; soluble in ethanol and chloroform. Extracted by organic solvents from aqueous acid or alkaline solutions.

Thin-layer Chromatography System T1—R_f 0.75 (location reagents: strongly acid iodoplatinate spray, positive reaction; iodine-carbon tetrachloride spray, positive reaction).

Ultraviolet Spectrum Ethanol—253, 259, 248 and 265 nm.

Fenitrothion

Organophosphate, Cholinesterase Inhibitor, Insecticide

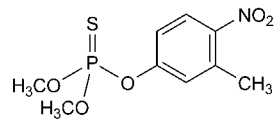
$C_9H_{12}NO_5PS = 277.2$

CAS—122-14-5

IUPAC Name Dimethoxy-(3-methyl-4-nitrophenoxy)-sulfanylidene-λ^5-phosphane

Synonyms AC-47300; Bayer 41831; Bayer S 5660; ENT-25715; MEP; OMS-45; OMS-223; phosphorothioic acid *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) ester; S-5660; S-1102-A; metathion.

Proprietary Names *Accothion*; *Agrothion*; *Cyfen*; *Cytel*; *Cyten*; *Dicofen*; *Etalene*; *Fenitox*; *Folithion*; *Micromite*; *Novathion*; *Nuvanol*; *Pestroy*; *Sumithion*; *Verthion*.



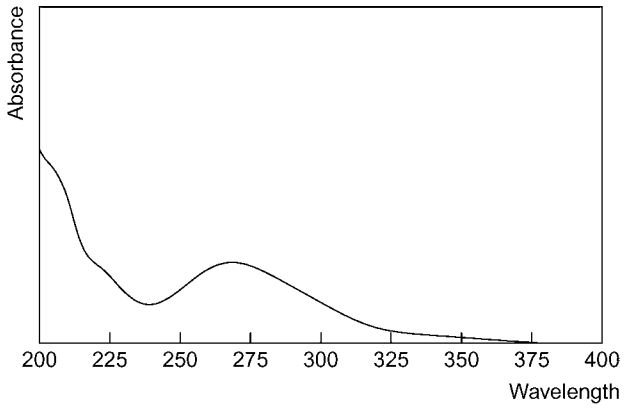
Chemical Properties A yellow to brown oil. Mp 3.4°. Bp 118° at 0.05 mmHg. It is practically insoluble in water (14 mg/L at 30°); has low solubility in aliphatic hydrocarbons; soluble in most organic solvents including alcohol esters, ketones, aromatic hydrocarbons and chlorinated hydrocarbons. It has a solubility in hexane of 42 g/kg at 20 to 25°; dichloromethane, methanol and xylene >1000 g/kg; propan-2-ol 193 g/kg. Log *P* (octanol/water), 3.43.

Thin-layer Chromatography System TX—R_f 0.32; system TY—R_f 0.76; system TZ—R_f 0.82; system TAA—R_f 0.65; system TAB—R_f 0.50; system TAC—R_f 0.17.

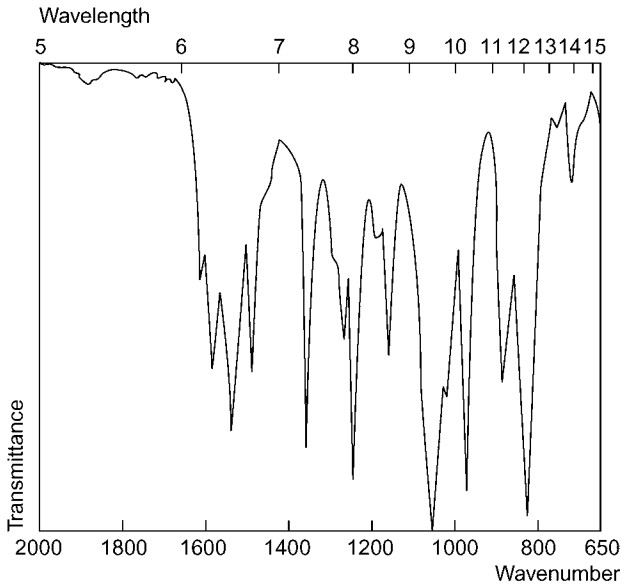
Gas Chromatography System GA—RI 1905; system GK—RRT 1.01 (relative to caffeine); system GKA—RI 1944; system GKB—RI 2112; system GKC—RI 2278.

High Performance Liquid Chromatography System HAP—*k* 2.86; system HAO—*k* 0.22.

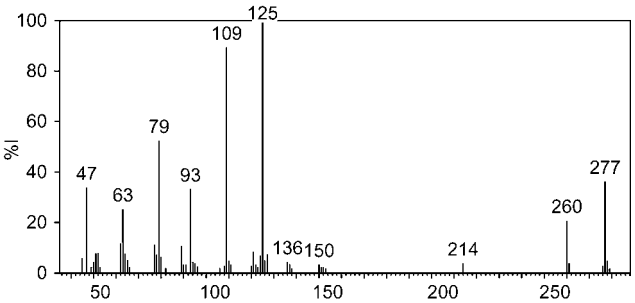
Ultraviolet Spectrum Aqueous acid (acetonitrile)—268 nm.



Infrared Spectrum Principal peaks at wavenumber 1055, 830, 971, 1243, 1359, 1539 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 125, 109, 79, 277, 47, 93, 63, 260.



Quantification

Blood **GC** Column: Shimadzu HiCap-CBP 1 (10 m × 0.53 mm i.d., 1.0 µm). Carrier gas: N_2, 20 mL/min. Temperature: 160°. FID or FPD. Limit of detection not reported [Kojima *et al.* 1989]. Column: 3% OV-17 on Chromosorb G 60/80 mesh (1 m × 2.6 cm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 240°. FPD. Limit of detection, 0.1 ng [Yashiki *et al.* 1986].

GC-MS Column: Shimadzu HiCap-CBP 1 (12 m × 0.53 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 100° to 280° at 8°/min. EI ionisation at 70 eV or CI at 150 eV. Limit of detection not reported [Kojima *et al.* 1989].

HPLC Column: Novapak C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : water (4 : 6), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 0.1 mg/L [Ageda *et al.* 2006].

Serum

Note For a HPTLC method for the detection of fenirothion, see Futagami *et al.* [1997].

GC-MS Column: DB-5MS 5% phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 1 min to 180° at 30°/min to 300° at 4°/min for 2 min. EI ionisation at 70 eV. Retention time: 10.6 min. Limit of quantification, 3.3 µg/L, limit of detection, 1.1 µg/L [Pitarch *et al.* 2003].

HPLC Column: Nucleosil 5C_{18} (15 cm × 4 mm i.d.). Mobile phase: acetonitrile : water (50 : 50), flow rate 1.0 mL/min. DAD (λ = 230 nm). Retention time: 9.54 min. Limit of quantification, 0.15 mg/L, limit of detection, 0.15 mg/L [Cho *et al.* 1997].

LC-MS Column: XTerra MS C_{18} (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate : methanol (100 : 0 to 0 : 100 in 3 min for 6.5 min to 100 : 0 at 10 min), flow rate 0.3 mL/min. APCI, positive or negative ion mode. Retention time: 5.61 min. Limit of quantification, 0.25 mg/L, limit of detection, 0.125 mg/L [Inoue *et al.* 2007].

Urine **GC** See Blood [Kojima *et al.* 1989]. See Blood [Yashiki *et al.* 1986].

HPLC See Serum [Cho *et al.* 1997]. Column: Chemcosorb ODS (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (44 : 56) containing 0.1% acetic acid, flow rate 0.5 mL/min. UV detection (λ = 315 nm). Limit of detection, 3.4 g/L. [Chang, Lin 1995].

LC-MS Column: Discovery C_{18} (50 × 2.1 mm i.d., 5 µm) followed by Supelco ABZ+ (100 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01% formic acid in water followed by acetonitrile: 0.0025% formic acid, flow rate 300 µL/min. ESI, MRM acquisition mode, negative ion mode. Retention time: 2.2 min for 3-methyl-4-nitrophenol. Limit of detection, <1 µg/L [Sancho *et al.* 2002].

Other **GC** Drinking Water. Column: Octadecyl C_{18}. Limit of detection, 0.01 µg/L [Badach *et al.* 2007].

LC-MS Mlik-based Infant Formulas. Column: HP-5MS 5% diphenyl 95% dimethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° to 180° at 80°/min for 10 min. Retention time: 21.7 min. Limit of quantification, 0.2 µg/kg, limit of detection, 0.06 µg/kg [Mezcua *et al.* 2007].

Disposition in the Body

Fenitrothion is rapidly absorbed after ingestion. It is metabolised to 3-methyl-4-nitrophenol, aminofenitrothion, aminofenitroxon, acetyl-aminofenitroxon and *S*-methylfenitrothion. It is distributed among various tissues. All metabolites have been detected in urine and excretion mainly occurs within the first 12 h; 50–70% of a dose is excreted in urine within 24 h as the metabolites. There is no evidence to show that fenitrothion accumulates within the body, but it is stored in fat tissues and it is possibly released under stress conditions.

Toxicity

Allowed daily intake is 0.003 mg/kg body weight. A blood concentration of 260 ng/g causes low-to-mild poisoning. The low toxic dose reported for acute oral toxicity in a female is 800 mg/kg.

A 43-year-old male attempted suicide by ingesting ≈100 mL 5% fenitrothion and acetate emulsion. Serum concentrations on hospital admission were 7.2 and 4.5 mg/L for fenitrothion and acetate, respectively [Inoue *et al.* 2007].

A 23-year-old male was admitted to hospital after an attempted suicide. He had ingested 50 mL of fenitrothion emulsion (5%) but vomited soon after. A blood fenitrothion concentration of 169.5 ng/g was observed 3 h after ingestion. He made a full recovery and was discharged from hospital 3 days after admission. [Kojima *et al.* 1989].

A 56-year-old male attempted suicide by ingesting 60 mL of 50% fenitrothion emulsion. He was treated with haemodialysis and haemoperfusion 5 h later. After 60 min his symptoms gradually improved but 4 days after the initial ingestion, cholinergic symptoms recurred and he died 2 days later despite immediate treatment. Toxicological analysis showed that distribution within tissues and organs was high, with the highest concentration in fat (59 mg/kg wet weight, which is 10-times greater than in other organs, suggesting a slow release of the pesticide from fat). [Yoshida *et al.* 1987].

Half-life

4.5 h.

Ageda S *et al.* (2006). The stability of organophosphorus insecticides in fresh blood. *Leg Med (Tokyo)* 8: 144–149.

Badach H *et al.* (2007). Pesticide content in drinking water samples collected from orchard areas in central Poland. *Ann Agric Environ Med* 14: 109–114.

Chang MJ, Lin RS (1995). Biological monitoring of exposure to fenitrothion by high performance liquid chromatography. *Bull Environ Contam Toxicol* 55: 29–35.

Cho Y *et al.* (1997). Determination of organophosphorous pesticides in biological samples of acute poisoning by HPLC with diode-array detector. *Chem Pharm Bull (Tokyo)* 45: 737–740.

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Pitarch E *et al.* (2003). Rapid multiresidue determination of organochlorine and organophosphorus compounds in human serum by solid-phase extraction and gas chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem* 376: 189–197.

Sancho JV *et al.* (2002). Different quantitation approaches for xenobiotics in human urine samples by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 16: 639–645.

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Yoshida M *et al.* (1987). A case of acute poisoning with fenitrothion (Sumithion). *Hum Toxicol* 6: 403–406.

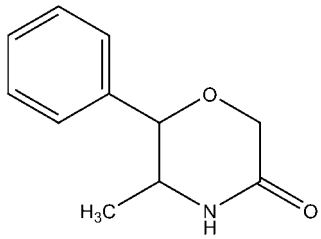
Fenmetramide

Antidepressant

$C_{11}H_{13}NO_2 = 191.2$

IUPAC Name 5-Methyl-6-phenylmorpholin-3-one

Synonyms Fenmetramid; McN 1075; 3-methyl-5-oxo-2-phenylmorpholine.



Chemical Properties A white crystalline powder. Slightly soluble in dilute acetic acid. Fenmetramide is extracted by organic solvents from aqueous alkaline solutions.

Colour Test Sulfuric acid-formaldehyde test—dull orange→brown (limit of detection, 0.1 µg).

Thin-layer Chromatography System T1—R_f 0.68 (location reagent Marquis reagent, strong reaction).

Fenofibrate

Antihyperlipidaemic, Fibric Acid Derivative

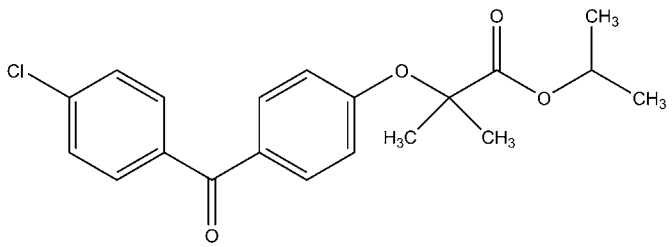
$C_{20}H_{21}ClO_4 = 360.8$

CAS—49562-28-9

IUPAC Name Propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate

Synonyms 2-[4-(4-Chlorobenzoyl)-phenoxy]-2-methylpropanoic acid 1-methylethyl ester; isopropyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropionate; LF-178; procetofen; procetofene.

Proprietary Names *Ankebin*; *Antara*; *Apo-Feno*; *Craveril*; *Docfenofi*; *Elasterin*; *Fegenor*; *Fenardin*; *Fenobrate*; *Fenofitop*; *Fenogal*; *Fenolip*; *Fenotard*; *Fenox*; *Fulcro*; *Grofibrat*; *Hypolip*; *Lipanthyl*; *Lipantil*; *Lipcor*; *Lipidil*; *Lipirex*; *Lipoclar*; *Lipofen*; *Lipofene*; *Liposit*; *Lipsin*; *Lofibra*; *Minuslip*; *Nolipax*; *Procetoken*; *Protolipan*; *Qualipantyl*; *Secalip*; *Sclerofin*; *Tricor*; *Triglide*; *Trolip*.



Chemical Properties Crystals from propan-2-ol. Mp 80° to 81°. Practically insoluble in water; slightly soluble in ethanol and methanol; soluble in acetone, ether, benzene and chloroform. A white or almost white crystalline powder. Mp 79° to 82°. Practically insoluble in water; slightly soluble in alcohol; very soluble in dichloromethane. Log *P* (octanol/water), 5.19 [Meylan, Howard 1995]. Protect from light.

Ultraviolet Spectrum Neutral—292, 215, 260 nm.

Quantification

Plasma **HPLC** Column: polar reversed phase (250 × 4.6 mm i.d., 4 µm). Mobile phase: 20 mmol/L ammonium acetate buffer : propan-2-ol : acetonitrile (56 : 24 : 20) with 0.1% formic acid, flow rate 0.75 mL/min. UV detection (λ = 285 nm). Limit of quantification, fenofibric acid 0.16 mg/L; limit of detection, fenofibric acid 0.05 mg/L [Straka *et al.* 2007]. Column: Uptisphere OBD (125 × 3.0 mm i.d., 3 µm). Mobile phase: 1% acetic acid : acetonitrile (50 : 50), flow rate 0.5 mL/min. UV detection (λ = 292 nm). Retention time: fenofibric acid 9.0 min. Limit of quantification, 0.03 mg/L [Sauron *et al.* 2006]. Column: C_{18} (250 × 4.0 mm i.d.). Mobile phase: acetonitrile : 0.02 mol/L phosphoric acid buffer (pH 3.4; 55 : 45). UV detection (λ = 287 nm). Limit of detection, 0.1 mg/L [Yun *et al.* 2006]. Column: Nucleosil C_8 (125 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : 0.04 mol/L phosphoric acid buffer (60 : 40), flow rate 0.80 mL/min. UV detection (λ = 288 nm). Retention time: fenofibric acid 6.3 min. Limit of quantification, 120 µg/L; limit of detection, 36 µg/L [Streel *et al.* 2000]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L phosphoric acid buffer (55 : 45), flow rate 2.0 mL/min for 6 min to 2.5 mL/min over 2 min for 9 min. UV detection (λ = 287 nm). Retention time: fenofibric acid 5.5 min. Limit of quantification, 0.25 mg/L [Masnatta *et al.* 1996]. See also Nobilis *et al.* [1998] and Doser *et al.* [1996].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid : acetonitrile (45 : 55), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: fenofibric acid 4.7 min. Limit of quantification, 0.5 µg/L [Trivedi *et al.* 2005].

Urine **TLC** Plates: silica gel F_{254} (0.2 mm). Solvent system: toluene : ethyl acetate : glacial acetic acid (20 : 10 : 1). UV detection (λ = 254 nm). R_f: fenofibrate 0.93, fenofibric acid 0.46, fenofibryl glucuronide 0.00. Solvent system: chloroform : methanol : glacial acetic acid (24 : 8 : 1). UV detection (λ = 254 nm). R_f: fenofibrate 0.98, fenofibric acid 0.97, fenofibryl glucuronide 0.50. Limit of quantification not reported [Weil *et al.* 1990].

HPLC Column: Partisil 10 ODS-3 (250 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : 10 mmol/L sodium phosphate buffer containing 5 mmol/L tetraheptylammonium bromide (pH 6.0; 77 : 23), flow rate 1.0 mL/min. UV detection (λ = 300 nm). Retention times: fenofibric acid 18.5 min, fenofibryl glucuronide 14.5 min. Limit of quantification, fenofibric acid 0.3 mg/L [Liu *et al.* 1991]. Column: Nucleosil C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L phosphate buffer (pH 7.5; 45 : 55) containing 5 mmol/L tetrabutylammonium bromide, flow rate 1.0 mL/min. UV detection (λ = 290 nm). Retention time: fenofibryl glucuronide 9.3 min. Limit of quantification not reported [Weil *et al.* 1990].

Faeces **TLC** See Urine [Weil *et al.* 1990].

HPLC See Urine [Weil *et al.* 1990].

Other **HPLC** Lipoprotein Fractions. Column: LichroCart C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water : concentrated acetic acid (60 : 40 : 1), flow rate 1 mL/min. UV detection (λ = 285 nm). Retention time: fenofibric acid 3.6 min. Limit of quantification not reported [Nobilis *et al.* 1998].

Disposition in the Body Fenofibrate is the oral prodrug of fenofibric acid. The micronised oral formulation of fenofibrate is readily absorbed from the gastrointestinal tract when taken with food; absorption is substantially reduced if given after an overnight fast. However, a new nanoparticle formulation offers equivalent absorption and pharmacokinetics even when administered independently of food. It is rapidly hydrolysed to its active metabolite fenofibric acid. Fenofibric acid is excreted mainly in the urine (~60%), mainly as the glucuronide conjugate but also as a reduced form of fenofibric acid and its glucuronide. Approximately 25% of a dose is excreted in the faeces. It is not removed by haemodialysis.

Therapeutic Concentration

A group of 24 healthy volunteers was administered a 250 mg sustained-release capsule of fenofibrate after a high fat meal (group A) or under fasted conditions (group B). Mean peak plasma concentrations of fenofibric acid for groups A and B were 9.15 mg/L and 2.39 mg/L, respectively, reached after 8.1 h and 6.4 h, respectively. A significant difference in the apparent volume of distribution was noted between the 2 groups, with a value of 30.2 L for group A and 134.7 L for group B [Yun *et al.* 2006].

A group of 45 healthy volunteers was administered a novel nanoparticle oral formulation tablet of 145 mg fenofibrate after a high fat meal, low fat meal, or under fasted conditions. Mean peak fenofibric acid plasma concentrations for the three regimens were 7.96, 7.96 and 7.94 mg/L, respectively, attained after 4.3, 3.6 and 2.3 h, respectively. This demonstrates pharmacokinetic equivalence when the drug is administered with or without food. In a previous study with a micronised fenofibrate 160 mg tablet, the effect of administration with food resulted in almost a 3-fold increase in the mean peak plasma concentration of fenofibric acid [Sauron *et al.* 2006].

In a bioequivalence study, a group of 24 healthy volunteers was administered either micronised or semi-solid formulations of fenofibrate at two doses: 67 mg and 200 mg. Peak plasma concentrations of fenofibric acid in the 67 mg study were 4.1 mg/L for both formulations, achieved at 5.1 h for the semi-solid dose and 6.3 h for the micronised dose. In the 200 mg study, peak plasma concentrations of fenofibric acid were 9.6 mg/L at 4.6 h for the semi-solid dose and 10.1 mg/L at 6.2 h for the micronised dose [Sonet *et al.* 2002].

Toxicity Fenofibrate has low potential for drug–drug interactions. Fenofibrate and fenofibric acid are weak inhibitors of CYP2C19 and CYP2A6 and mild to moderate inhibitors of CYP2C9.

Bioavailability Micronised fenofibrate, 100%.

Half-life In healthy volunteers, 19 to 27 h; in patients with renal failure, 143 h.

Volume of Distribution Approximately 0.89 L/kg

Clearance In the elderly, 1.2 L/h; in young adults, 1.1 L/h.

Protein Binding Fenofibric acid, ~99%, primarily to albumin.

Dose Given orally, usually in a micronised form that has improved bioavailability. The usual initial dosage is 43 to 200 mg daily, as a single dose or in three divided doses depending on the formulation. The dose should be adjusted according to response to a maximum dosage of 267 mg daily. Children may be given a dose of 67 mg per 20 kg bodyweight daily. A modified-release preparation is also available and is given in a dose of 54 to 160 mg daily (equivalent to 67 to 200 mg of micronised fenofibrate).

It may also be given in the non-micronised form; 67 mg of micronised fenofibrate is therapeutically equivalent to ≈100 mg of the non-micronised form. The usual initial dose is 300 mg daily in divided doses with food. Doses may be adjusted according to response to between 200 and 400 mg daily. Children may be given 5 mg/kg daily.

Doser K *et al.* (1996). Comparative steady state study with 2 fenofibrate 250 mg slow release capsules. An example of bioequivalence assessment with a highly variable drug. *Int J Clin Pharmacol Ther* 34: 345–348.

Liu HF *et al.* (1991). Urinary glucuronide excretion of fenofibric and clofibric acid glucuronides in man. Is it polymorphic? *Eur J Clin Pharmacol* 41: 153–159.

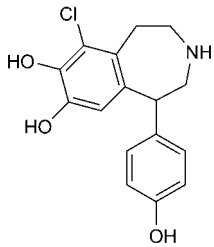
Masnatta LD *et al.* (1996). Determination of bezafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 687: 437–442.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Nobilis M *et al.* (1998). Distribution of fenofibric acid in lipoprotein fractions of patients. *Eur J Drug Metab Pharmacokinet* 23: 287–294.
Sauron R *et al.* (2006). Absence of a food effect with a 145 mg nanoparticle fenofibrate tablet formulation. *Int J Clin Pharmacol Ther* 44: 64–70.
Sonet B *et al.* (2002). Randomised crossover studies of the bioequivalence of two fenofibrate formulations after administration of a single oral dose in healthy volunteers. *Arzneimittelforschung* 52: 200–204.
Straka RJ *et al.* (2007). Determination of fenofibric acid concentrations by HPLC after anion exchange solid-phase extraction from human serum. *Ther Drug Monit* 29: 197–202.
Streel B *et al.* (2000). Determination of fenofibric acid in human plasma using automated solid-phase extraction coupled to liquid chromatography. *J Chromatogr B Biomed Sci Appl* 742: 391–400.
Trivedi RK *et al.* (2005). Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC-MS/MS with electrospray ionization: assay development, validation and application to a clinical study. *J Pharm Biomed Anal* 39: 661–669.
Weil A *et al.* (1990). The metabolism and disposition of ^{14}C-fenofibrate in human volunteers. *Drug Metab Dispos* 18: 115–120.
Yun HY *et al.* (2006). The effects of food on the bioavailability of fenofibrate administered orally in healthy volunteers via sustained-release capsule. *Clin Pharmacokinet* 45: 425–432.

Fenoldopam

Dopamine D_1 Receptor Agonist, Antihypertensive
$C_{16}H_{16}ClNO_3 = 305.8$
CAS—67227-56-9

IUPAC Name 6-Chloro-1-(4-hydroxyphenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol
Synonyms 6-Chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol; SK&F 82526.



Chemical Properties Log *P* (octanol/water), 2.39. Undergoes rapid decomposition in water. Stable for 3 months at 4° dissolved in 0.05 mol/L acetic acid. 0.5% Ascorbic acid also stabilises fenoldopam in samples of plasma and urine. Stable in plasma at –20° for at least 1 month with the addition of ascorbic acid [Boppana *et al.* 1984].

Fenoldopam Hydrobromide
$C_{16}H_{17}BrClNO_3 = 386.7$
Chemical Properties Mp 277°.

Fenoldopam Methanesulfonate
$C_{17}H_{20}ClNO_6S = 401.9$
CAS—67227-57-0
Synonyms Fenoldopam mesylate; SKF-82526-J.
Proprietary Name *Corlopam*
Chemical Properties Mp 274°. Water solubility is 4003 mg/L at 25°.

Quantification
***Plasma* HPLC** Column: Aquapore butylsilica (22 cm × 2.1 mm i.d., 7 µm). Mobile phase: Methanol : acetonitrile : citrate-acetate buffer (pH 5.6) : water (75 : 85 : 140 : 210), flow rate 0.3 mL/min. Electrochemical detection. Limit of detection, 0.5 µg/L [Boppana *et al.* 1992]. Column: Ultrasphere octadecyl silic (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : citrate-acetate buffer (pH 3.2; 18 : 82), flow rate 1. mL/min. Retention time: 8.7 min. Limit of detection, 25 ng/L [Boppana *et al.* 1989]. Column: Ultrasphere octadecyl silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : citrate-acetate buffer (pH 4.0; 20 : 80), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 4 µg/L [Boppana *et al.* 1986]. Column: Ultrasphere octadecyl silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : citrate-acetate buffer (pH 4.0; 20 : 80), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 8.7 min. Limit of quantification, 50 ng/L [Boppana *et al.* 1984]. Column: Partisil ODS-3 5/25 (5 µm). Mobile phase: citrate-acetate buffer : acetonitrile (92 : 8), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 12.7 min. Limit of detection, 50 ng/L [Osborne, Randolph 1983].
***Urine* HPLC** See Plasma [Boppana *et al.* 1986]. See Plasma [Boppana *et al.* 1984].

Disposition in the Body Fenoldopam is extensively metabolised by conjugation: glucuronidation, sulfation and methylation. Metabolites include fenoldopam 7-sulfate and 8-sulfate, and are excreted predominantly in urine (90%) and faeces (10%). Approximately 4% of a dose is excreted unchanged in urine. Fenolodpam has poor bioavailability, ~6%, by oral administration; <0.005% of the drug crosses the blood–brain barrier.

Therapeutic Concentration
Sixteen male and 2 female patients with New York Heart Association Class-III heart failure aged between 33 and 67 years were administered a 100-mg dose of fenoldopam. The dose was administered with food or after a 12 h fast. Peak plasma concentrations of 26.5 and 10.9 µg/L fenoldopam were reached for the fasted and fed patients within 1.3 to 2 h, respectively. Fenoldopam 7-sulfate and fenoldopam 8-sulfate were also detected at concentrations of 1357 and 689 µg/L, respectively, in the fasted patients and 1411 and 707 µg/L in the fed subjects. These concentrations were reached between 2.4 and 3.6 h after administration [Blanchett *et al.* 1991].

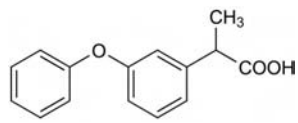
Toxicity Reported to be 6-times as potent as dopamine in causing vasodilation.
Bioavailability ≈6%.
Half-life ≈5 to 10 min.
Volume of Distribution 0.23 L/kg (0.025 µg/kg body weight/min dose) to 0.66 L/kg (0.25 and 0.5 µg/kg body weight/min).
Clearance 1.49 L/h/kg (0.025 µg/kg body weight/min dose) to 2.29 L/h/kg (0.5 µg/kg body weight/min dose).
Protein Binding ≈88%.
Dose IV: usual dose of 0.1 to 1.6 µg/kg body weight/min is administered and adjusted in increments of 0.05 to 1.0 µg/kg body weight. Oral: 100 mg two-to-four times daily.

Blanchett DG *et al.* (1991). The effect of food on pharmacokinetics and pharmacodynamics of fenoldopam in class III heart failure. *Clin Pharmacol Ther* 49: 449–456.
Boppana VK *et al.* (1984). Determination of fenoldopam (SK&F 82526) and its metabolites in human plasma and urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 317: 463–474.
Boppana VK *et al.* (1986). Use of a post-column immobilized beta-glucuronidase enzyme reactor for the determination of diastereomeric glucuronides of fenoldopam in plasma and urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 353: 231–247.
Boppana VK *et al.* (1989). Simplified procedures for the determination of fenoldopam and its metabolites in human plasma by high-performance liquid chromatography with electrochemical detection: comparison of manual and robotic sample preparation methods. *J Chromatogr* 487: 385–399.
Boppana VK *et al.* (1992). Determination of the enantiomers of fenoldopam in human plasma by reversed-phase high-performance liquid chromatography after chiral derivatization. *J Chromatogr* 592: 317–322.
Osborne VL, Randolph WC (1983). Analysis of SK&F 82526 in plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 255: 491–496.

Fenoprofen

Analgesic
$C_{15}H_{14}O_3 = 242.3$
CAS—31879-05-7

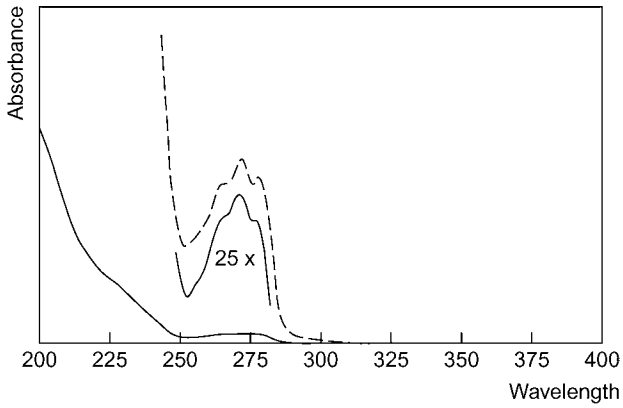
IUPAC Name 2-(3-Phenoxyphenyl)propanoic acid
Synonym α-Methyl-3-phenoxybenzeneacetic acid



Chemical Properties pK_a 4.5 (25°). Log *P* (octanol/pH 7.4), 0.8.

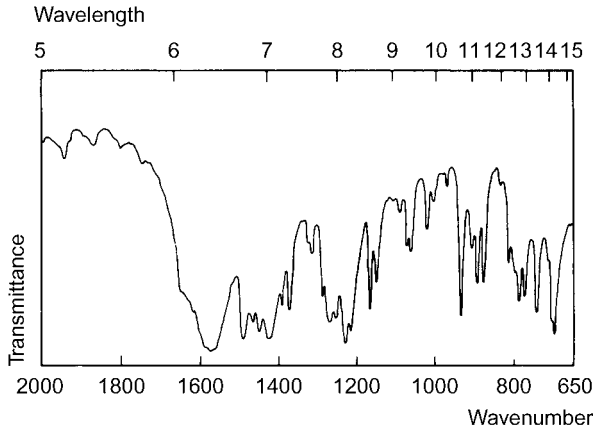
Fenoprofen Calcium
$(C_{15}H_{13}O_3)_2Ca,2H_2O = 558.6$
CAS—34597-40-5 (anhydrous); 53746-45-5 (dihydrate)
Proprietary Names *Fenopron*; *Fepron*; *Nalfon*; *Nalgésic*; *Progesic*; *Trandor.*
Chemical Properties A white crystalline powder. Mp 105° to 110°. Soluble 1 in 400 to 1 in 500 of water, 1 in 15 of ethanol and 1 in 300 of chloroform.

Colour Tests Liebermann's reagent—red-brown; Marquis test—pink.
Thin-layer Chromatography System TA—R_f 0.96; system TD—R_f 0.42; system TE—R_f 0.06; system TF—R_f 0.38; system TG—R_f 0.16; system TAD—R_f 0.50; system TAJ—R_f 0.58; system TAK—R_f 0.78; system TAL—R_f 0.76 (Ludy Tenger reagent, orange).
Gas Chromatography System GA—fenoprofen RI 2016, fenoprofen-Me RI 1906; system GD—methyl derivative RRT 1.31 (relative to n-$C_{16}H_{34}$); system GL—fenoprofen-Me RI 1970, M (OH-)-Me_2 RI 2130.
High Performance Liquid Chromatography System HD—*k* 7.9; system HX—RI 574; system HY—RI 524; system HZ—retention time 10.9 min; system HAA—retention time 21.2 min; system HAX—retention time 8.0 min; system HAY—retention time 8.9 min.
Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=72a); methanol—273 (A^1_1=72a), 280 nm.

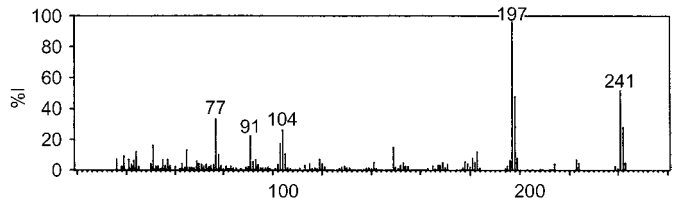


F

Infrared Spectrum Principal peaks at wavenumbers 1562, 1225, 696, 1211, 1268, 1248 cm^{-1} (fenoprofen calcium, KBr disk).



Mass Spectrum Principal ions at *m/z* 197, 241, 198, 77, 242, 104, 91, 103; fenoprofen-Me *m/z* 197, 256, 198, 257, 91, 103; M (OH-)-Me_2 *m/z* 286, 227, 287, 123, 91, 152.



Quantification

Plasma **GC** FID. Limit of detection, 250 µg/L [Nash *et al.* 1971].

HPLC UV detection. Fenoprofen, ibuprofen and ketoprofen. Limit of detection, 0.25 mg/L for fenoprofen [Menzel-Soglowek *et al.* 1990]. UV detection. For method of quantification for fenoprofen enantiomers and metabolites, see Volland *et al.* [1990]. UV detection. For method of quantification for fenoprofen enantiomers, see Mehvar and Jamali [1988]. UV detection. For method of quantification for fenoprofen and other NSAIDs, see Owen *et al.* [1987]. UV detection. Limit of detection, 500 µg/L [Bopp *et al.* 1981].

Urine **HPLC** See Plasma [Volland *et al.* 1990]. See Plasma [Mehvar, Jamali 1988].

Disposition in the Body Readily absorbed after oral administration. About 90% of a dose is excreted in the urine in 24 h, about 3% of the excreted material being unchanged drug, about 45% fenoprofen glucuronide, about 42% 4-hydroxyfenoprofen glucuronide and about 2% free 4-hydroxyfenoprofen. About 2% of a dose is eliminated in the faeces in 24 h.

Therapeutic Concentration

After a single oral dose of 250 mg administered to 4 subjects, peak plasma concentrations of 23 to 31 mg/L (mean 27) were attained in 0.5 to 2 h [Rubin *et al.* 1971].

Toxicity Recovery has been reported after the ingestion of about 60 g.

A 17-year-old girl presented with coma, metabolic acidosis, hypotension and respiratory depression within 4 h of ingesting 24 to 36 g of fenoprofen as a suicidal gesture; treatment led to recovery within 3 days [Kolodzik *et al.* 1990].

Half-life Plasma half-life, 2 to 3 h.

Volume of Distribution About 0.1 L/kg.

Protein Binding About 99%.

Note For a review of the pharmacological properties of fenoprofen, see Brogden *et al.* [1977].

Dose The equivalent of 0.9 to 2.4 g of fenoprofen daily.

Bopp RJ *et al.* (1981). High-performance liquid chromatographic assay for fenoprofen in human plasma. *J Pharm Sci* 70: 507–509.

Brogden RN *et al.* (1977). Alclofenac: a review of its pharmacological properties and therapeutic efficacy in rheumatoid arthritis and allied rheumatic disorders. *Drugs* 13: 241–265.

Kolodzik JM *et al.* (1990). Nonsteroidal anti-inflammatory drugs and coma: a case report of fenoprofen overdose. *Ann Emerg Med* 19: 378–381.

Mehvar R, Jamali F (1988). Stereospecific high-performance liquid chromatographic (HPLC) assay of fenoprofen enantiomers in plasma and urine. *Pharm Res* 5: 53–56.

Menzel-Soglowek S *et al.* (1990). Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral alpha 1-acid glycoprotein column. *J Chromatogr* 532: 295–303.

Nash JF *et al.* (1971). GLC determination of dl-2-(3-phenoxyphenyl)propionic acid (Fenoprofen) in human plasma. *J Pharm Sci* 60: 1062–1064.

Owen SG *et al.* (1987). Rapid high-performance liquid chromatographic assay for the simultaneous analysis of non-steroidal anti-inflammatory drugs in plasma. *J Chromatogr* 416: 293–302.

Rubin A *et al.* (1971). Physiological disposition of fenoprofen in man. I. Pharmacokinetic comparison of calcium and sodium salts administered orally. *J Pharm Sci* 60: 1797–1801.

Volland C *et al.* (1990). Stereoselective analysis of fenoprofen and its metabolites. *J Chromatogr* 534: 127–138.

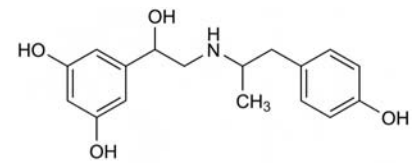
Fenoterol

Sympathomimetic

$C_{17}H_{21}NO_4 = 303.4$

CAS—13392-18-2

IUPAC Name 5-[1-Hydroxy-2-[[2-(4-hydroxyphenyl)-1-methylethyl]amino] ethyl]-1,3-benzenediol



Chemical Properties pK_a 8.5, 10.0. Log *P* (octanol/water), 1.2.

Fenoterol Hydrobromide

$C_{17}H_{21}NO_4,HBr = 384.3$

CAS—1944-12-3

Proprietary Names *Berotec*; *Dosberotec*; *Partusisten*. It is an ingredient of *Duovent*.

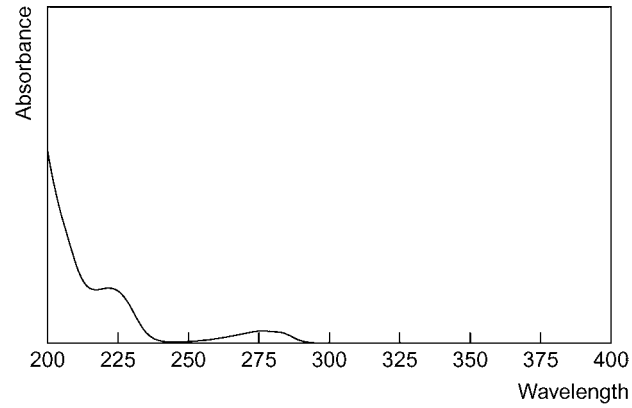
Chemical Properties A white crystalline powder. Mp about 230°, with decomposition. Soluble 1 in 10 of water and 1 in 11 of ethanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.25; system TF—R_f 0.00; system TL—R_f 0.04; system TAE—R_f 0.38; system TAF—R_f 0.81.

Gas Chromatography System GA—fenoterol RI 1416, fenoterol-H_2O-AC_4 RI 3440.

High Performance Liquid Chromatography System HA—*k* 0.7; system HX—RI 287; system HY—RI 246; system HAX—retention time 5.7 min; system HAY—retention time 3.4 min.

Ultraviolet Spectrum Aqueous acid—275 nm (A_1^1=107a); aqueous alkali—295 nm (A_1^1=190b).



Infrared Spectrum Principal peaks at wavenumbers 1605, 1510, 700, 1160, 1575, 1200 cm^{-1} (fenoterol hydrobromide, KBr disk).

Quantification

Blood **GC–MS** Limit of detection, <1 µg/L, fenoterol, salbutamol and terbutaline [Couper, Drummer 1996].

***Plasma* HPLC** Fluorescence detection. For method for quantification, see Meineke *et al.* [2002]. Fluorescence detection. Limit of detection, <376 ng/L [Kramer, Blaschke 2001].

Disposition in the Body Rapidly but incompletely absorbed after inhalation or oral administration. It undergoes extensive first-pass metabolism by sulfate conjugation. About 35% of an oral dose is excreted in the urine in 24 h, mainly as the inactive sulfate conjugate, and <2% as unchanged drug; about 40% of an oral dose is eliminated in the faeces.

Therapeutic Concentration

After oral administration of 5 mg of tritiated fenoterol to 8 subjects, peak plasma radioactivity equivalent to about 0.04 mg/L of fenoterol was attained in 2 h; most of the radioactivity was due to metabolites. Following inhalation of a 200 µg or 500 µg metered-dose by 3 subjects, peak plasma radioactivity equivalent to about 0.0003 to 0.0004 mg/L of fenoterol was reported [Buchelt, Rominger 1972].

Fenoterol was given by IV infusion to 5 subjects at a dose of 0.5, 1.0 or 2.0 µg/min over 3 h. Steady-state plasma concentrations of 385, 636 and 980 ng/L, respectively, were reached within 90 to 120 min [Warnke *et al.* 1992].

In 20 healthy subjects receiving an IV infusion of fenoterol 1 µg/min for 4 h, steady-state plasma concentrations were reached by 2 h later (median 0.89 µg/L, range, 0.72 to 1.16 µg/L). The maximum plasma concentration of fenoterol glucuronide was 0.88 µg/L (range, 0.74 to 0.97) at the end of the infusion and that of fenoterol sulfate was 0.65 µg/L (0.49 to 0.70). A 10 mg oral dose every 20 min up to a total dose of 30 mg in the same subjects produced a median peak plasma-fenoterol level of 0.54 µg/L (range, 0.25 to 0.69), with the concentrations of fenoterol glucuronide and sulfate reaching a maximum of 14.3 µg/L (range 8.7 to 17.9) and 92 µg/L (87 to 105), respectively, at about 2 h [Hildebrandt *et al.* 1994].

Half-life Plasma half-life, about 6 to 7 h.

Note For a review of fenoterol see, Svedmyr [1985].

Dose Up to 360 µg of fenoterol hydrobromide every 4 h, by aerosol inhalation; 15 to 20 mg daily has been given orally.

Buchelt L, Rominger KL (1972). *Med Proc* 18: 15–20.

Couper FJ, Drummer OH (1996). Gas chromatographic-mass spectrometric determination of beta 2-agonists in postmortem blood: application in forensic medicine. *J Chromatogr B Biomed Appl* 685(2): 265–272.

Hildebrandt R *et al.* (1994). Fenoterol metabolism in man: sulphation versus glucuronidation. *Xenobiotica* 24(1): 71–77.

Kramer S, Blaschke G (2001). High-performance liquid chromatographic determination of the beta2-selective adrenergic agonist fenoterol in human plasma after fluorescence derivatization. *J Chromatogr B Biomed Sci Appl* 751(1): 169–175.

Meineke I *et al.* (2002). Determination of fenoterol in human plasma by HPLC with fluorescence detection after derivatization. *J Pharm Biomed Anal* 29: 147–152.

Svedmyr N (1985). Fenoterol: a beta2-adrenergic agonist for use in asthma. Pharmacology, pharmacokinetics, clinical efficacy and adverse effects. *Pharmacotherapy* 5(3): 109–126.

Warnke K *et al.* (1992). The pharmacokinetics of the beta 2-adrenoceptor agonist fenoterol in healthy women. *Eur J Clin Pharmacol* 43: 663–665.

Fenpipramide

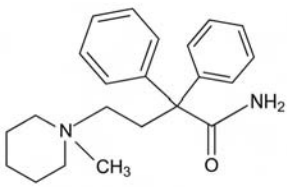
Antispasmodic (Veterinary)

$C_{21}H_{26}N_2O = 322.4$

CAS—77-01-0

IUPAC Name 2,2-Diphenyl-4-piperidinobutyramide

Synonym Fenpiverinium



Chemical Properties White crystals. Mp 188°. Practically insoluble in water; soluble in chloroform.

Fenpipramide Hydrochloride

$C_{21}H_{26}N_2O,HCl,\ H_2O = 376.9$

CAS—14007-53-5 (anhydrous)

Proprietary Name It is an ingredient of *Efosin*.

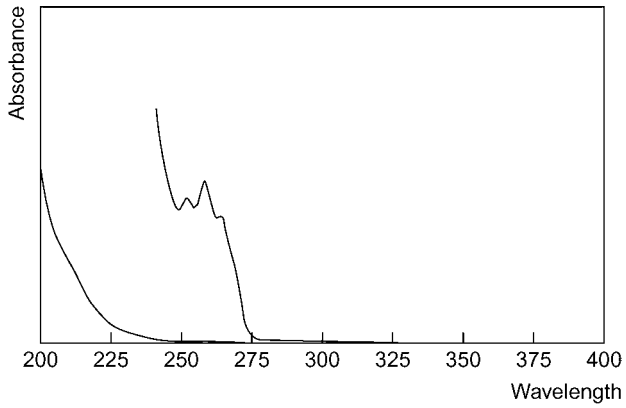
Chemical Properties A white crystalline powder. Soluble in water, ethanol and chloroform.

Colour Test Liebermann's reagent—orange.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.03; system TC—R_f 0.16; system TE—R_f 0.48; system TL—R_f 0.15; system TAE—R_f 0.25 (acidified iodoplatinate solution, positive).

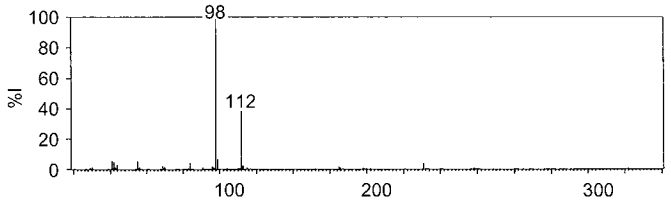
Gas Chromatography System GA—RI 2690.

Ultraviolet Spectrum Aqueous acid—253, 259 (A^1_1=12a), 265 nm.



Infrared Spectrum Principal peaks at wavenumbers 1618, 1684, 695, 712, 702, 764 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 98, 112, 99, 55, 42, 41, 211, 84.



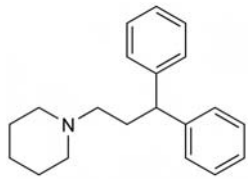
Fenpiprane

Antispasmodic (Veterinary)

$C_{20}H_{25}N = 279.4$

CAS—3540-95-2

IUPAC Name 1-(3,3-Diphenylpropyl)piperidine



Chemical Properties Crystals. Mp 41° to 42.5°. Practically insoluble in water; soluble in chloroform.

Fenpiprane Hydrochloride

$C_{20}H_{25}N,HCl = 315.9$

CAS—3329-14-4

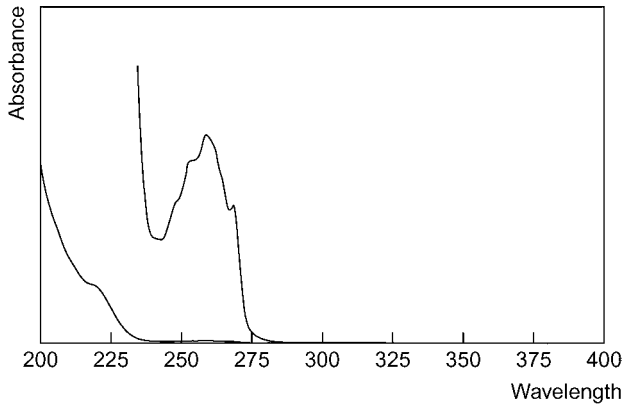
Proprietary Names *Aspasan*. It is an ingredient of *Efosin*.

Chemical Properties A white crystalline powder. Mp 216° to 217°. Soluble in water and chloroform.

Colour Tests Liebermann's reagent—brown; Mandelin's test—green; Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.61 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—259 (A^1_1=16.7a), 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1492, 1631, 1092, 698, 1010, 1600 cm^{-1} (KBr disk).

Fenproporex

Anorectic

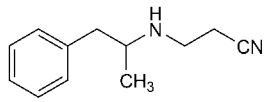
$C_{12}H_{16}N_2$ = 188.3

CAS—15686-61-0; 18305-29-8 (hydrochloride)

IUPAC Name 3-(1-Phenylpropan-2-ylamino)propanenitrile

Synonyms *N*-2-Cyanoethylamphetamine; 3-[(1-methyl-2-phenylethyl)amino] propanenitrile.

Proprietary Names *Antiobes*; *Delgafen*; *Desobesi-M*; *Dicel*; *Drenur*; *Fenisec*; *Gacilin*; *Igrasmin*; *Ifa Dex*; *Ifa Diety*; *Inobesin*; *Lipomax*; *Nobese*; *Obisin*; *Pesex-R*; *Solvolip*; *Tegisec* (includes proprietary names of the hydrochloride salt).



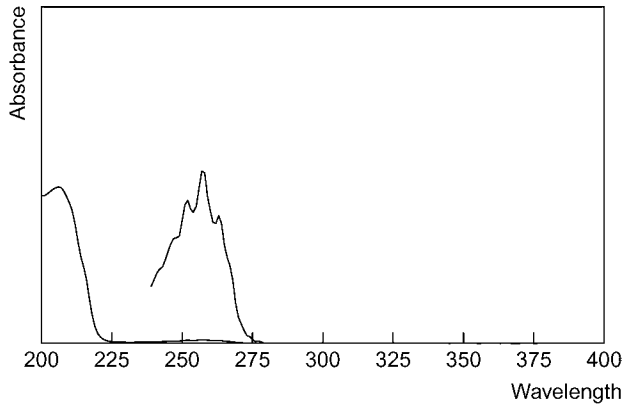
Chemical Properties Log *P* (octanol/water), 1.7.

Thin-layer Chromatography System TE—R_f 0.77.

Gas Chromatography System GA—fenproporex RI 1585, fenproporex-TFA RI 1705, fenproporex-PFP RI 1685, fenproporex-AC RI 1915; system GB—fenproporex RI 1648.

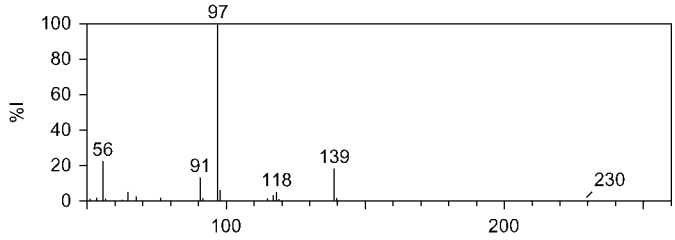
High Performance Liquid Chromatography System HY—RI 226; system HAA—RT 19.3 min.

Ultraviolet Spectrum Aqueous acid—252, 257, 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 745, 1583, 697, 1019, 707, 1139 cm^{-1} (Fenproporex hydrochloride), (KBr disk).

Mass Spectrum Principal ions at *m/z* 97, 56, 91, 68, 132, 173; fenproporex-AC *m/z* 97, 56, 139, 91, 118, 65 [Kraemer *et al* 2000]; fenproporex-PFP *m/z* 243, 118, 190, 56, 91, 202; fenproporex-TFA *m/z* 193, 118, 140, 91, 56, 152.



Quantification

Blood **GC** Column: DB-17 (15 m × 0.32 mm i.d., 0.15 µm). Carrier gas: He, 17.5 psi. Temperature programme: 130° for 1 min to 300° at 15°/min for 1 min. NPD. Limit of detection, 1 µg/L [Bell *et al.* 2001].

GC-MS Column: HP cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of detection, 50 µg/L [Kraemer *et al.* 2000].

Urine **GC** See Blood [Bell *et al.* 2001].

Gastric Contents **GC** See Blood [Bell *et al.* 2001].

Liver **GC** See Blood [Bell *et al.* 2001].

Disposition in the Body

Toxicity

In a fatality attributed to fenproporex in a young adult, the following tissue concentrations were found for fenproporex and amfetamine, respectively: inferior vena cava blood, 0.90 and 0.084 mg/L; urine, 1.2 and 0.94 mg/L; and total gastric content, 120 and 0.14 mg; other drugs detected in the blood included diazepam (0.54 mg/L), nordiazepam (0.46 mg/L), diphenhydramine (0.12 mg/L) and gamma hydroxybutyric acid (1100 mg/L) [Bell *et al.* 2001].

Dose Fenproporex has been given as the hydrochloride in usual doses equivalent to 20 mg of the base daily.

Bell RR *et al.* (2001). A contemporaneous finding of fenproporex in a polydrug suicide. *J Anal Toxicol* 25: 652–656.

Kraemer T *et al.* (2000). Studies on the metabolism and toxicological detection of the amphetamine-like anorectic fenproporex in human urine by gas chromatography-mass spectrometry and fluorescence polarization immunoassay. *J Chromatogr B Biomed Sci Appl* 738: 107–118.

Fentanyl

Narcotic Analgesic

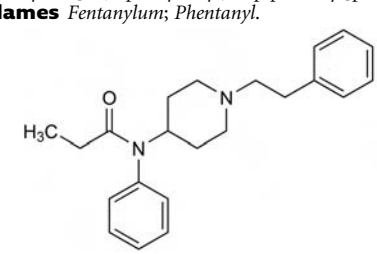
$C_{22}H_{28}N_2O$ = 336.5

CAS—437-38-7

IUPAC Name *N*-[1-Phenethylpiperidin-4-yl)-*N*-phenylpropanamide

Synonym *N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]propanamide

Proprietary Names *Fentanylum*; *Phentanyl.*



Chemical Properties Crystals. Mp 83° to 84°. Sparingly soluble in water. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Fentanyl Citrate

$C_{22}H_{28}N_2O,C_6H_8O_7$ = 528.6

CAS—990-73-8

Synonyms Fentanyli citras; McN-JR-4263-49; R-4263

Proprietary Names *Actiq*; *Durogesic*; *Fentanest*; *Haldid*; *Leptanal*; *Sintenyl*; *Sublimaze*; *Tanyl*. It is an ingredient of *Fentazin (vet.)*; *Hypnorm (vet.)*; *Inoval*; *Innovar*; *Leptofen*; *Marcain with Fentanyl*; *Thalamonal*.

Chemical Properties White granules or a white glistening crystalline powder. Mp 149° to 151°. Soluble 1 in 40 of water, 1 in 140 ethanol, 1 in 350 of chloroform, and 1 in 10 of methanol; slightly soluble in ether. Log *P* (octanol/water pH 7.4), 2.3.

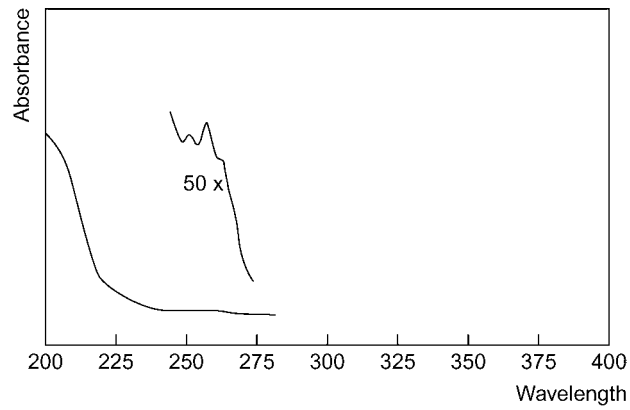
Colour Test Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.43; system TC—R_f 0.74; system TE—R_f 0.78; system TL—R_f 0.58; system TAE—R_f 0.70; system TAF—R_f 0.77 (acidified iodoplatinate solution, positive).

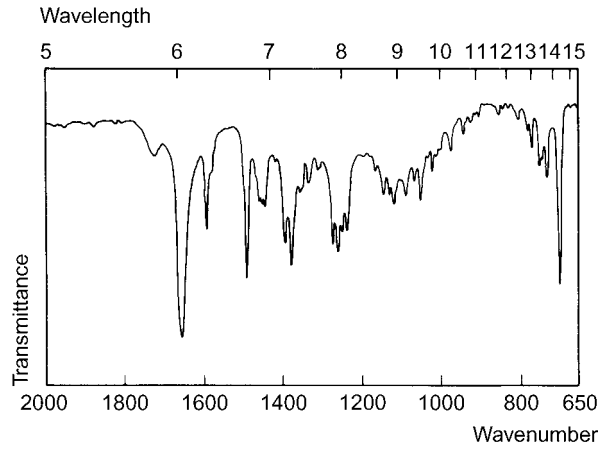
Gas Chromatography System GA—RI 2720; system GB—RI 2899.

High Performance Liquid Chromatography System HA—*k* 0.8; system HC—*k* 1.11; system HX—RI 373; system HY—RI 299; system HAA—RT 14.2 min; system HAX—RT 11.4 min; system HAY—RT 6.0 min.

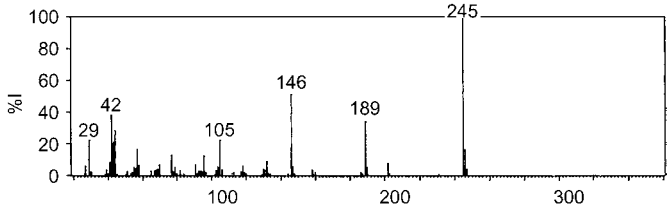
Ultraviolet Spectrum Aqueous acid—251, 257 (A^1_1 = 13a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1660, 701, 1493, 1263, 1273, 1236 cm^{-1} (KBr disk, see below).



Mass Spectrum Principal ions at *m/z* 245, 146, 42, 189, 44, 105, 29, 43.



Quantification

Blood **GC** NPD. Limit of detection not reported [Kowalski *et al.* 1987].

GC-MS Column: HP-5MS. SIM acquisition mode. Limit of detection, 2.5 μg/L [Wong *et al.* 2008]. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 6 mL/min. Temperature: 270°. SIM acquisition mode. Limit of detection not reported [Martin *et al.* 2006]. Column: HP-5 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 3.0 mL/min. SIM acquisition mode. Limit of detection, 1.67 μg/L [Anderson, Muto 2000].

LC-MS Column: Genesis C_{18} reversed phase (100 × 2.0 mm i.d., 3.0 μm). Mobile phase: acetronitrile : 0.1% formic acid (15 : 85 for 9 min to 30 : 70 over 13 min to 80 : 20 over 10 min to 95 : 5 for 1 min), flow rate 150 μL/min. Quadrapole MS detection, positive ion mode, MRM acquisition mode. Retention time: 13.3 min. Limit of detection, 0.08 μg/L [Gergov *et al.* 2009]. Column: XterraMS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.15% formic acid : 0.15% acetonitrile (90 : 10 for 2 min to 1 : 100 over 6 min for 5 min to 90 : 10 in 1 min), flow rate 0.2 mL/min. MS/MS detection, positive ion mode, MRM acquisition mode. Limit of detection not reported [Coopman *et al.* 2007]. Column: LiChriCART LiChrospher 60 reversed phase select B. Mobile phase: 0.1% formic acid in water : acetonitrile. Limit of quantification, 0.25 μg/L, limit of detection, 0.05 μg/L [Skulska *et al.* 2007].

Plasma **GC** Column: HP-1 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature: 220°. Quadrapole MS detection, EI ionisation, SIM acquisition mode. Limit of detection, 0.03 μg/L [Bagheri *et al.* 2007]. Column: HP Ultra (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 120 kPa. SIM acquisition mode. Limit of quantification, 0.01 μg/L [Moisés *et al.* 2005]. AFID detection. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.55 mL/min. MS detection, SIM acquisition mode. Limit of detection, 20 ng/L [Fryirs *et al.* 1997]. Limit of detection, 3.3 μg/L [Van Rooy *et al.* 1981].

HPLC Column: Shim-pack CLC-C_8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02% perchloric acid : methanol (48 : 52), flow rate 1 mL/min. Limit of detection, 0.1 μg/L [Ebrahimzadeh *et al.* 2008]. MS-MS fluorescence detection [Nitsun *et al.* 2006]. Column: Spherisorb silica (250 × 4.5 mm i.d., 5 μm). Mobile phase: methanol : 0.02% perchloric acid (30 : 70). UV detection (λ = 200 nm). Limit of detection, 200 ng/L [Portier *et al.* 1999]. Column: Econosphere reverse-phase (250 × 4.6 mm i.d., 5 μm). Mobile phase: potassium dihydrogen phosphate adjusted with 85% orthophosphoric acid : acetonitrile (65 : 35), flow rate 1.4 mL/min. UV detection (λ = 195 nm). Limit of quantification, 2.0 μg/L, limit of detection, 0.25 μg/L [Kumar *et al.* 1996].

LC-MS Column: YMC Pro C_{18} (50 × 2 mm i.d.). Mobile phase: acetonitrile : 5 mmol/L formic acid (18 : 82). Triple quadrapole detection, positive ion mode. Limit of detection, 0.02 μg/L [Huynh *et al.* 2005]. Column: Betasil silica (50 × 3 mm i.d., 2 μm). Mobile phase: acetonitrile : water containing formic acid (90 : 10 to 50 : 50 over 2 min to 90 : 10 over 2.1 min), flow rate 0.5 mL/min. Limit of quantification, 40 ng/L [Naidong *et al.* 2002].

Serum **GC** Column: Chrompack fused silica (10 m × 0.32 mm i.d.). Carrier gas: He, 5 mL/min. TSD detection. Limit of detection, 0.25 μg/L [Woestenborghs *et al.* 1987].

Urine **GC-MS** See Blood [Wong *et al.* 2008]. Column: HP-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 20 mL/min. SIM acquisition mode. Limit of detection, 3 μg/L [Poklis, Backer 2004]. Column: DB35-MS (30 m × 20.25 mm i.d., 0.15 μm). Carrier gas: He, 2.5 mL/min. EI ionisation. Limit of detection, 2.5 ng/L [Van Nimmen *et al.* 2004]. See Blood [Anderson, Muto 2000]. Limit of detection, 0.3 μg/L [Valaer *et al.* 1997].

HPLC See Plasma [Ebrahimzadeh *et al.* 2008]. DAD [Klinke, Linnet 2007].

LC-MS See Blood. Limit of detection, 0.10 μg/L [Gergov *et al.* 2009]. See Blood [Coopman *et al.* 2007]. See Blood. Limit of quantification, 2.1 μg/L, limit of detection, 1.9 μg/L [Skulska *et al.* 2007]. Column: YMC Pro C_{18} (50 × 2 mm i.d.). Mobile phase: acetonitrile : 5 mmol/L formic acid (2 : 98 to 30 : 70 over 4 min for 1 min). Triple quadrapole detection, positive ion mode. Limit of detection, 0.1 μg/L [Huynh *et al.* 2005].

Bile **GC-MS** See Blood [Anderson, Muto 2000].

LC-MS See Blood [Coopman *et al.* 2007].

Gastric Contents **GC-MS** See Blood [Anderson, Muto 2000].

LC-MS See Blood [Coopman *et al.* 2007].

Milk **HPLC** See Plasma [Nitsun *et al.* 2006].

Oral Fluid **GC-MS** Column: DB-35ms (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2.0 mL/min. Temperature: 90°. MS detection, EI ionisation, SIM acquisition mode. Retention time: 7.33 min. Limit of quantification, 5.42 μg/L [Gunnar *et al.* 2005].

Sweat **LC-MS** See Hair. Limit of quantification, 0.09 ng per half patch, limit of detection, 0.04 ng per half patch [Schneider *et al.* 2008].

Vitreous Humour **GC-MS** See Blood [Anderson, Muto 2000].

LC-MS See Blood [Coopman *et al.* 2007].

Hair **GC-MS** Column: 5% phenyl 95% methyl silicone DB-5 (15 m × 0.25 mm i.d., 0.25 μm). MSD, EI ionisation. Limit of quantification, 5 ng/g [[Moore *et al.* 2008]. Column: HP5-MS 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas, He, 1.0 mL/min. Temperature programme: 100° for 1 min to 295° at 30°/min for 5 min. MS-MS detection, EI ionisation. Retention time: 11.9 min. Limit of quantification, 1 ng/g [Kintz *et al.* 2005].

LC-MS Column: Xterra MS C_{18} (150 × 3.9 mm i.d., 5 μm). Mobile phase: 20 mmol/L ammonium acetate (pH 3.8) : acetonitrile (62 : 38), flow rate 1.0 mL/min. Retention time: 5.59 min. MS-MS detection, EI ionisation. Limit of quantification, 0.06 μg/g, limit of detection, 0.03 μg/g [Schneider *et al.* 2008]. MS-MS detection [Musshoff *et al.* 2007].

Kidney **GC-MS** See Blood [Anderson, Muto 2000].

LC-MS See Blood [Coopman *et al.* 2007].

Liver **GC-MS** See Blood [Anderson, Muto 2000].

LC-MS See Blood [Coopman *et al.* 2007].

Disposition in the Body Fentanyl is rapidly metabolised in the liver. Two metabolites, norfentanyl and despropionylfentanyl, have been detected in plasma at concentrations similar to those of fentanyl. Approximately 70% of a dose is excreted in the urine in 72 h, mostly as metabolites, with ~10–20% of a dose being excreted as unchanged drug in 48 h. Approximately 9% of a dose is eliminated in the faeces. Fentanyl crosses the placenta and small amounts may be found in breast milk.

Therapeutic Concentration

Following IV injection of 200 μg to 6 subjects, plasma concentrations of ~2 μg/L were reported after 2 min [Michiels *et al.* 1977].

Following a single IV dose of 60 μg/kg to 5 subjects, plasma concentrations of 30–200 μg/L (mean 0.1) were reported at 1 min, decreasing to 10 μg/L at 1 h [Bovill, Sebel 1980].

After 10 subjects received 200 μg fentanyl by IV injection or 2000 μg of 50% free and 50% liposome-encapsulated fentanyl by aerosol inhalation, the mean peak plasma fentanyl level was significantly greater for the IV route (4.67 ± 1.87 at 3.6 min vs 1.15 ± 0.36 μg/L at 22.7 min); but at 8 and 24 h after administration the plasma levels were greater following inhalation (0.16 ± 0.10 vs 0.25 ± 0.14 μg/L at 8 h, and 0.05 ± 0.06 vs 0.12 ± 0.16 μg/L at 24 h, for IV and inhalation, respectively) [Hung *et al.* 1995].

In 10 children receiving treatment with fentanyl transdermal patches, the time to reach peak plasma concentration was 18 to >66 h for patches releasing 25 μg/h fentanyl. Peak plasma concentrations of 0.91 μg/L were obtained with 25 μg//h patches and 39.3 μg//L with 200 μg/h patches [Collins *et al.* 1999].

Administration of fentanyl (10 to 15 μg/kg) via an oral transmucosal delivery system to 17 children resulted in peak plasma concentrations of 1.03 ± 0.31 μg/L. The children were thought to have swallowed a large fraction of the dose, leading to a relatively late and variable peak concentration time of 53 ± 40 min [Wheeler *et al.* 2002].

Toxicity The estimated minimum lethal dose is 2 mg.

The following postmortem tissue concentrations were reported in a fatality resulting from the self-administration of fentanyl (in μg/L or μg/g): serum 17.7, blood 27.5, urine 92.7, bile 58.2, liver 0.0775, kidney 0.0415, brain 0.0302, lung 0.0834, stomach 31.6. A partly filled syringe containing 2800 μg/L fentanyl was found near the scene of death [Chaturvedi *et al.* 1990].

In a review of 25 fatalities involving fentanyl transdermal patches, the following post-mortem tissue concentrations were reported (in μg/L or μg/g): heart blood 1.89–139 (23 cases), femoral blood 3.1–43 (13 cases), vitreous humour 2–20 (4 cases), liver 0.0058–0.613 (22 cases), bile 3.5–262 (15 cases), urine 2.9–895 (19 cases), gastric 0–122 μg total (17 cases), spleen 0.0078–0.079 (3 cases), kidney 0.011 (1 case), lung 0.031 (1 case) [Anderson, Muto 2000].

An 83-year-old female who was found dead with three 100 μg/h fentanyl patches on her chest had the following tissue concentrations at postmortem: blood 25 μg/L, brain 0.054 μg/g, heart 0.094 μg/g, kidney 0.069 μg/g, liver 0.104 μg/g [Edinboro *et al.* 1997].

A postmortem serum fentanyl concentration of 2 μg/L was reported in a 35-year-old woman who had intravenously injected the contents of a trandermal fentanyl patch (5 mg, shared with another person who survived); 0.16 g/L ethanol was also found in the serum [Reeves, Ginifer 2002].

A 63-year-old man who was found dead with 20 fentanyl patches of different strengths with a total dose of 1350 μg/h had the following tissue concentrations at postmortem: femoral blood 0.0949 μg/g, heart blood (left) 0.0459 μg/g, heart blood (right) 0.0748 μg/g, urine 101 μg/L, bile 468 μg/L, gastric contents 745 μg/L, CSF 78.4 μg/L, vitreous humour 133 μg/L [Wiesbrock *et al.* 2008].

Half-life Plasma half-life, ~3.7 h (dose dependent: also increased in the elderly and premature infants and during cardiopulmonary by-pass surgery).

Volume of Distribution Approximately 4 L/kg.

Clearance Plasma clearance, ~13 mL/min/kg.

Protein Binding ~80%.

Dose For analgesia in surgery, initially, the equivalent of 50 to 200 μg fentanyl. IV; supplementary doses of 50 μg. With assisted ventilation, an initial dose of 300 to 3500 μg may be given.

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Fenthion

Cholinesterase Inhibitor, Acaricide, Insecticide

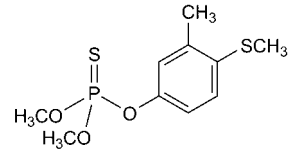
$C_{10}H_{15}O_3PS_2$ = 278.3

CAS—55-38-9

IUPAC Name Dimethoxy-(3-methyl-4-methylsulfanylphenoxy)-sulfanylidene-λ^5-phosphane

Synonyms Bay 29493; bayer 29493; *O,O*-dimethyl-*o*-(4-methylthio)-*m*-tolyl] phosphorothioate; DMTP; ENT25540; MPP; mercaptophos; OMS2; phosphorothioic acid *O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] ester; S-1752.

Proprietary Names *Baycid*; *Baytex*; *Entex*; *Lebaycid*; *Lysoff*; *Queletox*; *Spotten*; *Talodex*; *Tiguvon*.



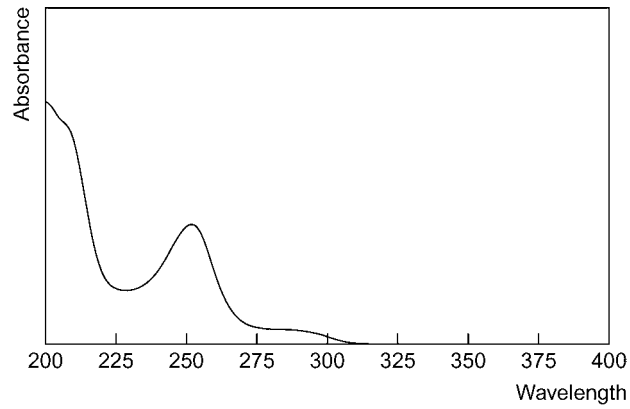
Chemical Properties Pure fenthion is a colourless liquid. The technical form is an oily yellowish-brown liquid. Mp 7.5°. Bp 87° at 0.01 mmHg. It is practically insoluble in water; readily soluble in methanol, ethanol, acetone, 2-propanol, dichloromethane, toluene and most organic solvents, including alcohol, ethers, esters and halogenated aromatics; slightly soluble in hexane. Log *P* (octanol/water), 4.09 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable through 3 freeze-thaw cycles and after 3 weeks at –30° [Inoue *et al.* 2007].

Thin-layer Chromatography System TX—R_f 0.41; system TY—R_f 0.81; system TZ—R_f 0.90; system TAA—R_f 0.68.

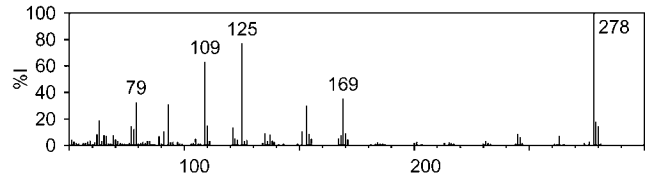
Plates: silica gel. Solvent system: benzene : methanol (9 : 1). Developed by spraying with 0.2% palladium chloride solution. R_f 0.9 [Meyer *et al.* 1998].

Gas Chromatography System GA—RI 1938.

Ultraviolet Spectrum Methanol—229 nm; aqueous acid—252 nm.



Mass Spectrum Principal ions at *m/z* 278, 125, 109, 169, 93.



Quantification

Blood **GC** Column: DB-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.4 mL/min. Temperature programme: 180° for 3 min to 230° at 10°/min for 35 min to 270° at 5°/min for 5 min. Retention time: 33.6 min. Limit of detection, 1 ppb [Tsatsakis *et al.* 2002]. Column: HP-1 fused silica (10 m × 0.53 mm i.d., 2.65 µm). Carrier gas: He, 15 mL/min. Temperature programme: 60° for 2 min to 250° at 10°/min for 8 min. NPD. Retention time: 14.16 min. Limit of quantification, 0.5 µg/L, limit of detection, 0.15 µg/L [Garcia-Repetto *et al.* 2001]. Column: DB-5 (15 m × 0.32 mm i.d., 1.5 µm). Carrier gas: He, 6.1 mL/min. Temperature programme: 120° to 240° at 10°/min. FID. Limit of detection not reported [Tsatsakis *et al.* 1996]. Column: Chromosorb W/HP 8/100 mesh 3% OV-17 (1/2" o.d. × 2 mm i.d.) Carrier gas: N_2, 2.5 mL/min. Temperature: 200°. FPD. Limit of detection, 0.1 µg/L [Brunetto *et al.* 1992].

GC-MS Column: HP-5MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 1 min to 290° at 10°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.01 µg/g [Musshoff *et al.* 2002]. Column: SGE BPX5 (25 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Column temperature: 60° to 120° at 30°/min to 220° at 10°/min to 290° at 5°/min to 300° for 3 min. EI ionisation, SIM acquisition mode. Retention time: 14.3 min. Limit of quantification, 0.1 mg/L [Meyer *et al.* 1998].

HPLC Column: Aluspher RP-select B (125 × 4.0 mm i.d., 5 µm). Mobile phase: 0.0125 mol/L sodium hydroxide in methanol : 0.0125 mol/L sodium hydroxide in water (10 : 90 for 5 min to 90 : 10 over 15 min for 5 min), flow rate 1.0 mL/min. UV detection (λ = 250 nm). Retention time: 18.3 min. Limit of quantification, 0.25 mg/L [Meyer *et al.* 1998].

LC-MS Column: Luna C_{18} reversed phase (30 × 2.0 mm i.d., 3 µm). Mobile phase: 2 mmol/L ammonium acetate buffer containing 1 mL/L formic acid in water (pH 2.8) : 2 mmol/L ammonium acetate buffer containing 1 mL/L formic acid in methanol (60 : 40 to 20 : 80 at 1.5 min to 60 : 40 at 6 min), flow rate 0.4 mL/min. ESI,

positive ion mode, SRM acquisition mode. Limit of quantification, 0.5 µg/L [Salm *et al.* 2009].

Plasma **GC** Column: 3% OV-17 on Chromosorb WAWDMCS 100/120 mesh (2 m × 1/8" i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 230°. Limit of detection, 3 µg/L [Mahieu *et al.* 1982].

Serum

Note For a HPTLC method for the detection of fenthion, see Futagami *et al.* [1997].

HPLC Column: Nucleosil $5C_{18}$ (15 cm × 4 mm i.d.). Mobile phase: acetonitrile : water (50 : 50), flow rate 1.0 mL/min. DAD (λ = 230 nm). Retention time: 14.1 min. Limit of quantification, 0.25 mg/L, limit of detection, 0.13 mg/L [Cho *et al.* 1997].

LC-MS Column: XTerra MS C_{18} (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate : methanol (100 : 0 to 0 : 100 in 3 min for 6.5 min to 100 : 0 at 10 min), flow rate 0.3 mL/min. APCI, positive or negative ion mode. Retention time: 5.61 min. Limit of quantification, 1.25 mg/L, limit of detection, 1 mg/L [Inoue *et al.* 2007].

Urine **GC** See Blood [Tsatsakis *et al.* 2002].

HPLC See Serum [Cho *et al.* 1997].

Vitreous Humour **GC** See Blood [Tsatsakis *et al.* 1996].

Adipose Tissue **GC** See Blood [Tsatsakis *et al.* 1996].

Brain **GC** See Blood [Tsatsakis *et al.* 1996].

Kidney **GC** See Blood [Tsatsakis *et al.* 1996].

Liver **GC** See Blood [Tsatsakis *et al.* 1996].

Orchis **GC** See Blood [Tsatsakis *et al.* 1996].

Thyroid **GC** See Blood [Tsatsakis *et al.* 1996].

Disposition in the Body Fenthion is readily absorbed into the bloodstream via the digestive tract, lungs and skin, and is systematically distributed. It is rapidly metabolised to weak active products by several pathways, the major pathway being by hepatic hydrolysis. It is eliminated through urine and faeces as the hydrolysis products. Fenthion is stored in fat and its release is delayed for metabolism.

Toxicity Fenthion is moderately toxic after ingestion and dermal exposure, and is slightly toxic if inhaled. The allowed daily intake is 1 µg/kg. A lethal dose of 50 mg/kg has been established, but the route of administration is not known. Acute respiratory failure defined as intermediate syndrome begins 24–96 h after the cholinergic crisis [Karademir *et al.* 1990; Sedgwick, Senanayake 1997; van den Neucker *et al.* 1991]. Delayed neuropathy can follow 2 to 3 weeks later with the inhibition of the neuropathy target esterase [Serrano, Fedriani 1997].

A 67-year-old male was admitted to hospital. His blood and urine fenthion concentrations were 2.7 and 0.5 mg/L, respectively, at the time of hospital admission. He was discharged 43 days later. A 13-year-old male was spraying crops with fenthion. On admission to hospital his blood concentration was 0.95 mg/L. After 5 days he was breathing by himself and he was discharged from hospital 4 days later [Tsatsakis *et al.* 2002].

A 66-year-old female was found dead in her bathroom, a suicide case that was not suspected to be fenthion poisoning. After toxicological analysis, a fenthion blood concentration of 3.8 mg/L was detected, and it was determined that fenthion ingestion was the cause of death. A total amount of 17 g of fenthion was found in the stomach and 203 g/g in the liver [Meyer *et al.* 1998].

A 69-year-old farmer ingested 200 mL Lebaycid. Postmortem concentrations of fenthion were 1.7, 4.8, 23.1, 16.8, 135.2 5.8, 7.1, or 13.8 mg/L or µg/g in the vitreous humour, blood, kidney, liver, fat, orchis, thyroid and brain, respectively [Tsatsakis *et al.* 1996].

A 41-year-old male was admitted to hospital having ingested an unknown quantity of fenthion. On admission, 20 h after the ingestion, he had a blood fenthion concentration of 0.27 mg/L. This increased on the first day to 0.78 mg/L. He died on day 7 from a refractory cardiovascular collapse [Brunetto *et al.* 1992].

A 43-year-old male ingested 30 mL of Lebaycid (–18 g fenthion). On admission to hospital his blood fenthion concentration was 71 µg/L, and 3 h later it was 102 µg/L [Mahieu *et al.* 1982].

Note Several cases in which extrapyramidal manifestations complicated the organophosphorus intoxication are reported in Senanayake and Sanmuganathan [1995]. For a case of prolonged toxicity after fenthion ingestion, see Merrill and Mihm [1982] or Borowitz [1988]. For a case of fenthion taken during pregnancy, see Karalliedde *et al.* [1988]. Cases of SC injection of fenthion are reported in Bala *et al.* [2008], Hadimioglu *et al.* [2002], Premaratna *et al.* [2001], Serrano and Fedriani [1997].

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Fenticlor

Antifungal

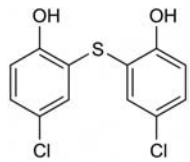
$C_{12}H_8Cl_2O_2S$ = 287.2

CAS—97-24-5

IUPAC Name 4-Chloro-2-(5-chloro-2-hydroxyphenyl)sulfanylphenol

Synonym 2,2′-Thiobis[4-chlorophenol]

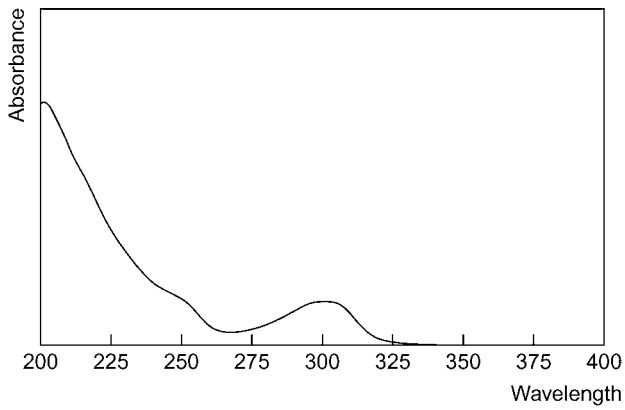
Proprietary Names *Novex*. It is an ingredient of *Dermisdin*.



Chemical Properties A white crystalline powder. Mp 176°. Practically insoluble in water; freely soluble in ethanol; soluble in aqueous solutions of sodium hydroxide. Log *P* (octanol/water), 4.6.

Thin-layer Chromatography System TAE—R_f 0.91.

Ultraviolet Spectrum Principal peak at 300 nm.



Infrared Spectrum Principal peaks at wavenumbers 1269, 825, 1209, 818, 889, 1104 cm^{-1}.

Use Fenticlor has been applied topically in concentrations of up to 2%.

Fenyramidol

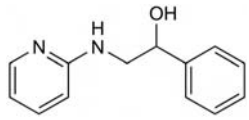
Analgesic, Muscle Relaxant

$C_{13}H_{14}N_2O$ = 214.3

CAS—553-69-5

IUPAC Name 1-Phenyl-2-(pyridin-2-ylamino)ethanol

Synonyms Phenyramidol; α-[(2-pyridinylamino)methyl]benzenemethanol.

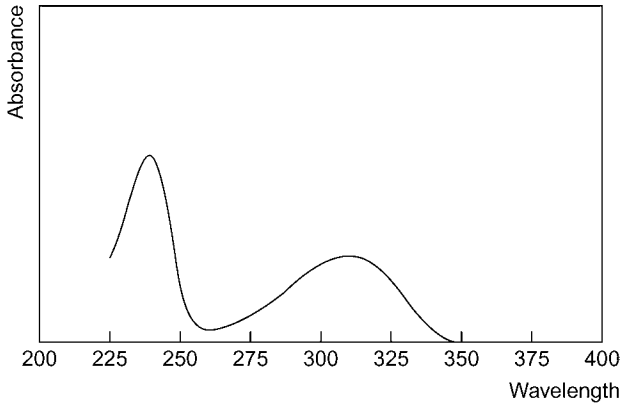


Chemical Properties Crystals. Mp 82° to 85°. pK_a 5.9. Log *P* (octanol/water), 1.7.

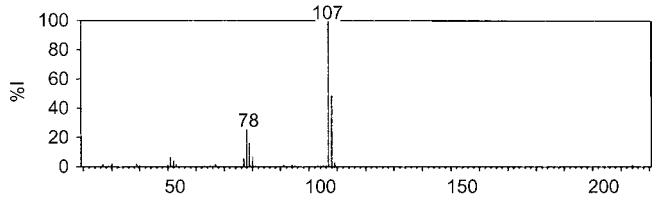
Fenyramidol Hydrochloride

$C_{13}H_{14}N_2O,HCl = 250.7$
CAS—326-43-2
Synonyms Phenyramidol hydrochloride; IN-511; MJ-505; NSC-17777.
Proprietary Name *Cabral*
Chemical Properties A white crystalline powder. Mp 140° to 142°. Freely soluble in water; soluble in ethanol.

Colour Tests Cyanogen bromide—orange-pink; Mandelin's test—blue; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.08; system TC—R_f 0.52; system TE—R_f 0.76; system TL—R_f 0.59; system TAE—R_f 0.80; system TAF—R_f 0.86 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1960.
High Performance Liquid Chromatography System HX—RI 282.
Ultraviolet Spectrum Aqueous acid—237 (A^1_1=666a), 309 nm.



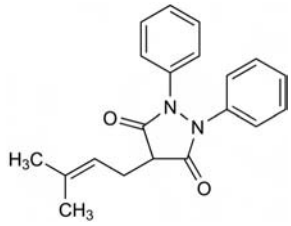
Infrared Spectrum Principal peaks at wavenumbers 1613, 1524, 700, 768, 1058, 752 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 107, 108, 78, 79, 80, 77, 51, 52.



Dose Up to 3.2 g of fenyramidol hydrochloride daily.

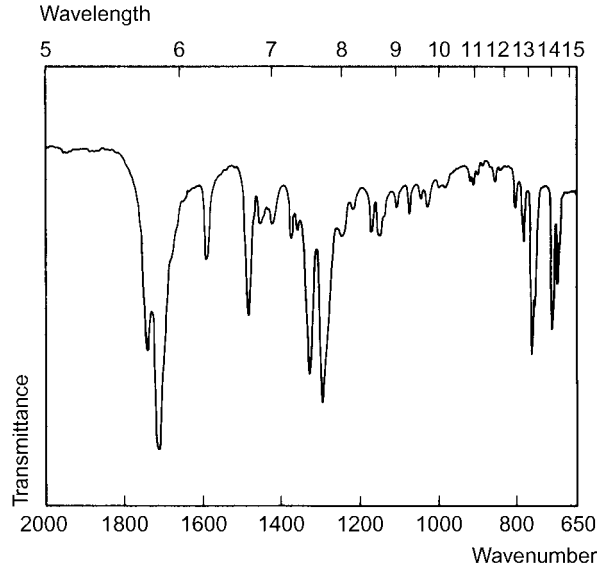
Feprazone

Analgesic
$C_{20}H_{20}N_2O_2 = 320.4$
CAS—30748-29-9
IUPAC Name 4-(3-Methyl-2-butenyl)-1,2-diphenyl-3,5-pyrazolidinedione
Synonyms Phenylprenazone; prenazone.
Proprietary Names *Brotazona*; *Methrazone*; *Reuflodol*; *Zepelan*; *Zepelin*.

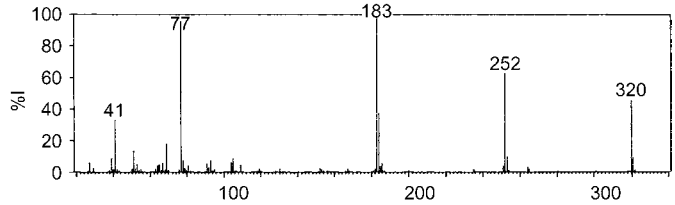


Chemical Properties A white crystalline powder. Mp about 157°. Practically insoluble in water; slightly soluble in cyclohexane, ethanol and ether; very soluble in acetone and chloroform; sparingly soluble in acetonitrile and benzene. Log *P* (octanol/water), 3.9.

Colour Tests *p*-Dimethylaminobenzaldehyde—red; Liebermann's reagent—brown-orange (→brown at 100°).
Thin-layer Chromatography System TE—R_f 0.19; system TG—R_f 0.45; system TAE—R_f 0.92 (chromic acid solution, yellow; Ludy Tenger reagent, orange; mercurous nitrate spray, positive).
Gas Chromatography System GA—RI 2380; system GD—methyl derivative RRT 1.81 (relative to n-$C_{16}H_{34}$); system GF—RI 2800.
High Performance Liquid Chromatography System HV—RRT 0.92 (relative to meclofenamic acid).
Ultraviolet Spectrum Methanolic alkali—266 nm (A^1_1=700b).
Infrared Spectrum Principal peaks at wavenumbers 1715, 1300, 767, 1745, 715, 703 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 183, 77, 252, 320, 184, 41, 69, 51.

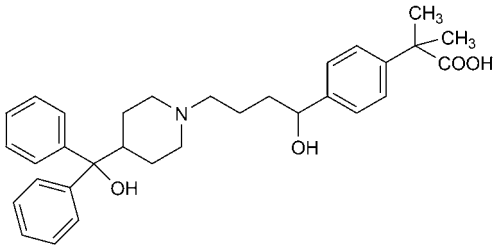


Quantification
Plasma **HPLC** UV detection. Limit of detection, 100 µg/L feprazone, 200 µg/L 4-(3-hydroxymethyl)feprazone [Spahn, Mutschler 1982].
Disposition in the Body Absorbed after oral administration. Metabolised by hydroxylation to 4-(3-hydroxymethyl)feprazone. <1% of a dose is excreted unchanged in the urine.
Therapeutic Concentration
Following a single oral dose of 400 mg to 8 subjects, peak plasma concentrations of 30.2 to 54.7 mg/L (mean 40.6) were attained in 4 to 8 h; peak plasma concentrations of 1.09 to 3.60 mg/L (mean 1.9) of the 4-(3-hydroxymethyl) metabolite were reported at about 27 h [Spahn, Mutschler 1985].
Half-life Plasma half-life, 6 to 30 h (mean 20).
Protein Binding 90 to 99%.
Dose 200 to 600 mg daily.

Spahn H, Mutschler E (1982). Determination of feprazone and one of its metabolites in human plasma after high-performance liquid chromatographic or thin-layer chromatographic separation. *J Chromatogr Biomed Appl* 232: 145–153.
Spahn H, Mutschler E (1985). The influence of aluminium hydroxide on the bioavailability of feprazone. Single dose study. *Arzneimittelforschung* 35: 167–169.

Fexofenadine

Antihistamine
$C_{32}H_{39}NO_4 = 501.7$
CAS—83799-24-0
IUPAC Name 2-[4-[1-Hydroxy-4-[4-[hydroxy(diphenyl)methyl]piperidin-1-yl]butyl]phenyl]-2-methylpropanoic acid
Synonyms Carboxyterfenadine; α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]benzene acetic acid; MDL-16455 M-I; terfenadine carboxylate.



Chemical Properties White to off-white crystalline powder. Mp 142° to 143°. Log *P* (octanol/water), 2.81 [Meylan, Howard 1995]. Plasma samples were stable at –80° for 6 months [Miura *et al.* 2007]. Stable in human plasma for 30 days at −20° ± 5° or –70° or below for 3 freeze-thaw cycles for 4 h at room temperature, and for 48 h in the autosampler at 5°. Fexofenadine stock solutions were stable for 6 h at room temperature and for 30 days at 5° [Yamane *et al.* 2007]. Fexofenadine in human plasma is stable toward 3 freeze-thaw cycles [Fu *et al.* 2004].

Fexofenadine Hydrochloride

$C_{32}H_{39}NO_4$,HCl = 538.1

CAS—138452-21-8

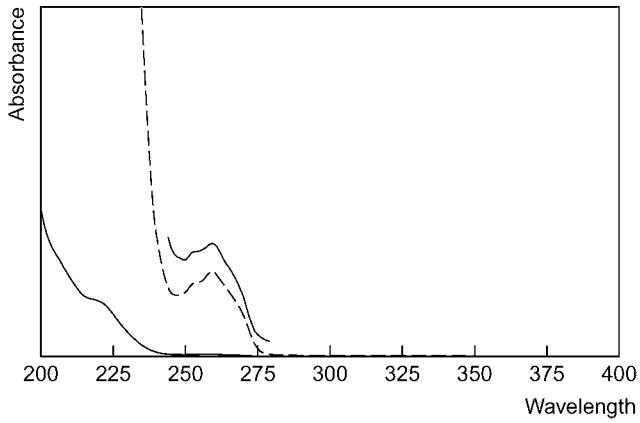
Synonym Terfenadine carboxylate hydrochloride

Proprietary Names *Allegra*; *Telfast*.

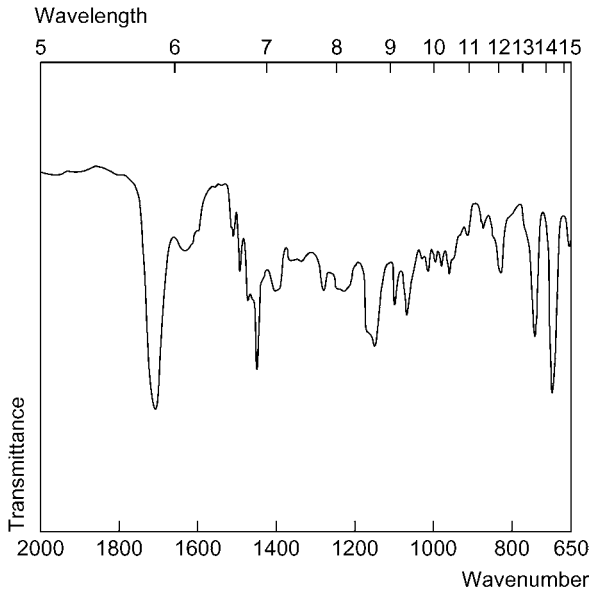
Chemical Properties White to off-white crystalline powder which is freely soluble in methanol and ethanol; slightly soluble in chloroform and water; insoluble in hexane.

High Performance Liquid Chromatography Column: Resolvosil BSA-7 (150 × 4 mm i.d., 5 µm). Mobile phase: 2-propanol : 0.08 mol/L sodium phosphate (pH 8.0; 1.5 : 98.5), flow rate 0.4 mL/min. UV detection (λ = 210 or 254 nm). Retention time: (*S*)-fexofenadine 10.2 min, (*R*)-fexofenadine 14.4 min [Chan *et al.* 1991].

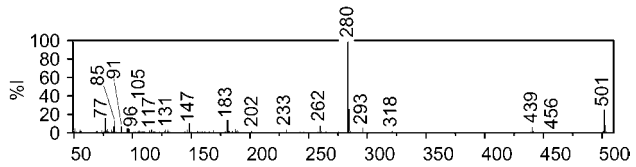
Ultraviolet Spectrum



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 280, 281, 501, 262, 105, 85.



Quantification

Plasma **HPLC** Column: Chiral CD-Ph (250 × 4.6 mm i.d.). Mobile phase: 0.5% potassium dihydrogen phosphate (pH 3.5) : acetonitrile (65 : 35), flow rate 0.5 mL/min. UV detection (λ = 220 nm). Limit of quantification, 25 µg/L, limit of detection, 12.5 µg/L [Miura *et al.* 2007].

LC-MS Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.03% formic acid : acetonitrile (40 : 60), flow rate 1.0 mL/min. TIS, MRM acquisition mode. Limit of quantification, 1 µg/L [Nirogi *et al.* 2007]. Column: XBridge C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate (91 : 9), flow rate 0.6 mL/min. ESI (TIS), MRM acquisition mode. Limit of quantification, 10 ng/L [Yamane *et al.* 2007] Column: Agilent Zorbax Eclipse XDB C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L formic acid (25 : 75 for 3 min to 60 : 40 at 4 min for 1.25 min to 95 : 5 briefly before 25 : 75), flow rate 0.25 mL/min. MSD, SIM acquisition mode. Retention time: 5.7 min. Limit of detection, 1 µg/L [Kharasch *et al.* 2005]. Column: Ultra IBD (50 × 3.2 mm i.d., 3 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate : formic acid (90 : 10 : 0.1). APCI, MRM acquisition mode. Limit of detection, 1 µg/L [Fu *et al.* 2004]. Column: Inertsil ODS-80A (150 × 4.6 mm i.d., 5 µm). Mobile phase 0.05 mol/L potassium dihydrogen phosphate buffer-acetonitrile-methanol (60 : 35 : 10) : 0.05 mol/L potassium dihydrogen phosphate buffer-acetonitrile (40 : 60, 100 : 0 to 0 : 100 in 10 min to 100 : 0 at 17 min), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 220 nm, λ_{em} = 290 nm). Retention time: 4.5 min. Limit of quantification, 1.0 µg/L [Uno *et al.* 2004].

See also Hofmann *et al.* [2002].

Serum **HPLC** Column: Shimpack cyanopropyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 12 mmol/L ammonium acetate (pH 4.85; 50 : 50), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Limit of quantification, 0.15 µg/L, limit of detection, 0.045 µg/L [Emara *et al.* 2007].

Urine **HPLC** Column: Cyclobond I (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.014 mol/L sodium perchlorate (75 : 25), flow rate 0.2 mL/min. UV detection (λ = 210 or 254 nm). Limit of detection not reported [Chan *et al.* 1991].

LC-MS See Plasma. Limit of quantification, 20 µg/L [Hofmann *et al.* 2002].

Disposition in the Body Fexofenadine is rapidly absorbed after oral administration, and only 5% of a dose is metabolised by the intestinal mucosa, 0.5 to 1.5 % undergoes hepatic biotransformation. The drug is mainly excreted in the faeces unchanged (80%) and 12% in urine [Mason *et al.* 1999]. Fexofenadine is a metabolite of terfenadine and therefore has been detected in breast milk after terfenadine administration. Fexofenadine does not cross the blood–brain barrier.

Therapeutic Concentration After the administration of 60 mg racemic fexofenadine to 3 healthy volunteers, the pharmacokinetic values shown below were measured.

Parameter	*(R)-Fexofenadine*	*(S)-Fexofenadine*
C_{max} (µg/L)	178 ± 220	121 ± 185
$t_{1/2}$ (h)	5.3 ± 2.0	4.0 ± 1.8
$AUC_{0-\infty}$ (ng h/mL)	979 ± 720	564 ± 555

[Miura *et al.* 2007]

Following a single oral dose of 60 mg fexofenadine a mean maximum plasma concentration of 275 µg/L was reached at 2 h [Yamane *et al.* 2007].

Seventy subjects (18 to 44 years) were administered 180 mg fexofenadine HCl/240 mg pseudoephedrine HCl extended-release or 180 mg fexofenadine HCl/240 mg pseudoephedrine HCl. Mean peak plasma concentrations of 569.4 and 561.6 µg/L and 631.3 and 584.6 µg/L fexofenadine were reached after the first dose and the seventh dose for each treatment group, respectively [Howard *et al.* 2005].

Four healthy adults were administered a single oral dose of 180 mg fexofenadine. Mean peak plasma concentration was 734.5 ± 261.3 µg/L at 1.5 ± 0.6 h [Hofmann *et al.* 2002].

Following a single oral dose of 80 mg fexofenadine, the mean maximum plasma concentration was 248.7 and 442.6 µg/L reached at 2.6 and 2.7 h in healthy subjects and renally impaired subjects [Mason *et al.* 1999].

Twenty-four healthy adult volunteers (19 to 45 year of age) were administered a single oral dose every 12 h for 7 days. A 60 mg dose produced peak plasma concentrations of 0.29 mg/L; a 120 mg dose produced 0.6 mg/L; and a 240 mg dose produced 1.53 mg/L. All levels were reached within 1.02 to 1.33 h [Robbins *et al.* 1998].

Toxicity Clinical data indicate that fexofenadine is non-sedating even at high doses [Philpot 2000].

A 67-year-old male was admitted to hospital after fainting. An abnormally long QTc time was seen on electrocardiography (ECG). He had started taking

180 mg fexofenadine every day approximately two months earlier. Fexofenadine treatment was stopped on hospital admission and the QTc time shortened. An everyday dose of 180 mg was restarted, and the QTc time increased again. After 11 days of treatment with fexofenadine, he suffered tachycardia and fibrillation. He was successfully defibrillated and treatment permanently stopped. The QTc time decreased [Pinto *et al.* 1999].

Note For a case of fexofenadine exacerbating psoriasis, see Saraswat and Saraswat [2006].

Half-life 14 to 18 h.

Volume of Distribution 5.4 to 5.8 L/kg.

Clearance 14 to 18 mL/min/kg.

Protein Binding 60 to 70%.

Dose A usual dose of 120 mg daily is administered but is reduced to 60 mg in patients with renal impairment. The maximum dose is 180 mg daily.

Chan KY *et al.* (1991). Direct enantiomeric separation of terfenadine and its major acid metabolite by high-performance liquid chromatography, and the lack of stereoselective terfenadine enantiomer biotransformation in man. *J Chromatogr* 571: 291–297.

Emara S *et al.* (2007). Direct injection liquid chromatographic technique for simultaneous determination of two antihistaminic drugs and their main metabolites in serum. *J AOAC Int* 90: 384–390.

Fu I *et al.* (2004). Determination of fexofenadine in human plasma using 96-well solid phase extraction and HPLC with tandem mass spectrometric detection. *J Pharm Biomed Anal* 35: 837–846.

Hofmann U *et al.* (2002). Determination of fexofenadine in human plasma and urine by liquid chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 227–233.

Howard DR *et al.* (2005). Single-dose and steady-state bioequivalence of fexofenadine and pseudoephedrine combination tablets compared with individual formulations in healthy adults. *Curr Med Res Opin* 21: 769–776.

Kharasch ED *et al.* (2005). Evaluation of first-pass cytochrome P4503A (CYP3A) and P-glycoprotein activities using alfentanil and fexofenadine in combination. *J Clin Pharmacol* 45: 79–88.

Mason J *et al.* (1999). The systemic safety of fexofenadine HCl. *Clin Exp Allergy* 29(suppl3): 163–170.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Miura M *et al.* (2007). Determination of fexofenadine enantiomers in human plasma with high-performance liquid chromatography. *J Pharm Biomed Anal* 43: 741–745.

Nirogi RV *et al.* (2007). Quantification of fexofenadine in human plasma by liquid chromatography coupled to electrospray tandem mass spectrometry using mosapride as internal standard. *Biomed Chromatogr* 21: 209–216.

Philpot EE (2000). Safety of second generation antihistamines. *Allergy Asthma Proc* 21: 15–20.

Pinto YM *et al.* (1999). QT lengthening and life-threatening arrhythmias associated with fexofenadine. *Lancet* 353: 980.

Robbins DK *et al.* (1998). Dose proportionality and comparison of single and multiple dose pharmacokinetics of fexofenadine (MDL 16455) and its enantiomers in healthy male volunteers. *Biopharm Drug Dispos* 19: 455–463.

Saraswat A, Saraswat M (2006). Pustular exacerbation of psoriasis due to fexofenadine. *Clin Exp Dermatol* 31: 477–478.

Uno T *et al.* (2004). Liquid chromatographic determination of fexofenadine in human plasma with fluorescence detection. *J Pharm Biomed Anal* 35: 937–942.

Yamane N *et al.* (2007). Microdose clinical trial: quantitative determination of fexofenadine in human plasma using liquid chromatography/electrospray ionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 858: 118–128.

Finasteride

Azasteroid, 5α-Reductase Inhibitor, Treatment of BPH

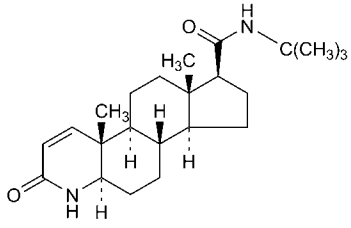
$C_{23}H_{36}N_2O_2 = 372.6$

CAS—98319-26-7

IUPAC Name (1*S*,3a*S*,3b*S*,5a*R*,9a*R*,9b*S*,11a*S*)-*N*-tert-Butyl-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide

Synonyms (5α,17β)-*N*-(1,1-Dimethylethyl)-3-oxo-4-aza-5-androst-1-ene-17-carboxamide; *N*-(2-methyl–2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide; MK-906; MK-0906.

Proprietary Names *Andozac*; *Chibro-Proscar*; *Finastid*; *Proscar*; *Prostide*; *Propecia*; *Urprosan*.



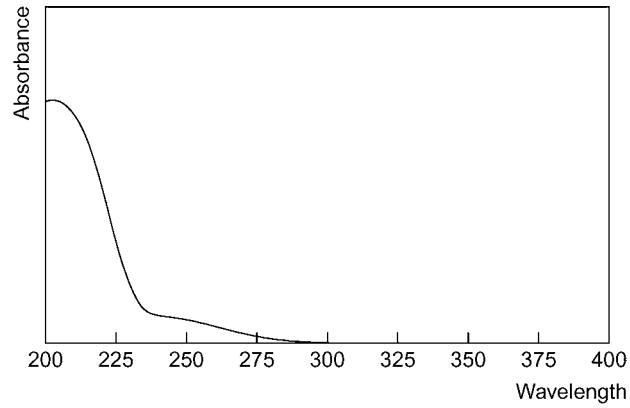
Chemical Properties White to off-white crystalline solid. Mp 252° to 254°, also reported to be 257°. It is freely soluble in chloroform, DMSO, ethanol, methanol and *n*-propanol; sparingly soluble in propylene glycol and polyethylene glycol 400; very slightly soluble in hydrochloric acid (0.1 mol/L) and sodium hydroxide (0.1 mol/L); practically insoluble in water (11.68 mg/L at 25°). Log *P* (octanol/water), 3.03 [Hansch *et al.* 1995]. Plasma samples were stable at −18° for at least 6 weeks [Ptácek *et al.* 2000]. Solutions stored at 4° were stable for at least 6 months [Takano, Hata 1996].

Thin-layer Chromatography Plate: silica. Solvent system: chloroform : methanol : water : ammonium hydroxide (55 : 35 : 6 : 4). Finasteride ω-oic acid R_f 0.58.

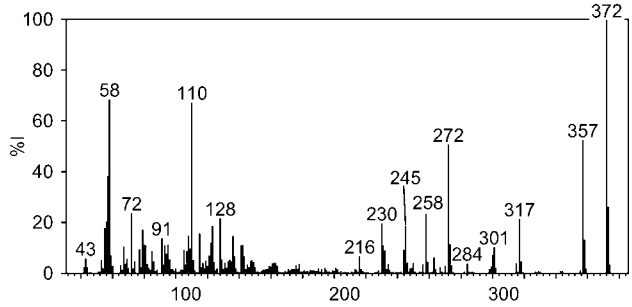
Plate: silica. Solvent system: ethyl acetate : 2-butanol : formic acid (90 : 8 : 2). Finasteride ω-oic acid R_f 0.43. [Carlin *et al.* 1992].

High Performance Liquid Chromatography Column: Zorbax C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (26 : 39 : 35), flow rate 0.9 to 1.0 mL/min. UV detection (λ = 210 nm). Retention time: ω-hydroxyfinasteride 6.5 min, finasteride 13.4 min, 4-*n*-methylfinasteride 21.0 min. [Carlin *et al.* 1992].

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 372, 58, 110, 357, 272, 57, 373, 72.



Quantification

Plasma **GC-MS** Column: 100% methylsilicone phase (12 m × 0.22 mm i.d., 0.33 µm). Carrier gas: He, 35 kPa. Temperature programme: 70° for 1 min, to 200° at 30°/min for 1 min to 280° at 10°/min for 10 min to 300° at 20°/min for 5 min. SIM acquisition mode. Retention time: 19.7 min. Limit of detection, 0.05 µg/L [Guarna *et al.* 1995].

HPLC Column: Nucleosil 100-3 C_{18} (150 × 3.2 mm i.d., 3 µm). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate buffer (40 : 60), flow rate 0.6 mL/min. UV detection (λ = 210 nm). Retention time: 17 min. Limit of quantification 4.08 µg/L [Ptácek *et al.* 2000]. Column: RP symmetry (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.04 mol/L orthophosphoric acid (pH 4.0; 45 : 55), flow rate 1.2 mL/min. UV detection (λ = 215 nm). Retention time: 14.6 min. Limit of detection, 5 µg/L [Carlucci, Mazzeo 1997]. Column: Inertsil ODS-2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile water (10 : 90, 25 : 75, 70 : 30 or 45 : 55), flow rate 1.0 or 1.1 mL/min. UV detection (λ = 210 nm). Limit of quantification, 1 µg/L [Takano, Hata 1996]. Column: Zorbax C_8 (250 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (26 : 39 : 35), flow rate 0.9 to 1.0 mL/min. UV detection (λ = 210 nm). Limit of quantification, 5–10 µg/L [Carlin *et al.* 1988;Carlin *et al.* 1992]. Column: Altex RP-8 (15 × 0.46 cm, 5 µm) with Analytichem RP-18 (5 × 0.46 cm, 3 µm). Mobile phase: methanol : acetonitrile : water (6 : 5 : 7), flow rate 1.0 mL/min. DAD (λ = 210 nm). Retention time: 13.1 min. Limit of quantification, 1 µg/L [Constanzer *et al.* 1991].

LC-MS Column: Luna C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: 0.1% formic acid : methanol (88 : 12 for 2 min to 10 : 90 over 1 min for 7 min), flow rate 0.2 mL/min. SRM acquisition mode, positive ion mode. Limit of quantification, 0.5 µg/L [Lundahl *et al.* 2009]. Column: SymmetryShield RP18 (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium acetate-0.1% formic acid : acetonitrile (58 : 42 to 49.5 : 50.5 over 3.5 min to 70 : 30 for 2.5 min to 58 : 42 at 6.01 min for 6.99 min), flow rate 200 µL/min. SIM acquisition mode. Limit of quantification, 1 µg/L [Chen *et al.* 2008]. Column: Hypurity C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (46 : 54) containing 0.1% acetic acid and 0.1% TFA, flow rate 0.2 mL/min. ESI, SIM acquisition mode. Limit of quantification, 0.2 µg/L [Guo *et al.* 2007]. Column: reversed phase. Mobile phase: acetonitrile : 1 mmol/L ammonium acetate (75 : 25), flow rate 200 µL/min. API. Limit of quantification, 0.5 µg/L [Almeida *et al.* 2005]. Column: reversed phase C_{18} (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (80 : 20) with 10 mmol/L formic acid, flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.6 min. Limit of quantification, 0.5 µg/L [de Menezes *et al.* 2001]. Column: Hypersil C_{18} (50 × 2 mm i.d., 3 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate and 0.1% formic acid (50 : 50 or 90 : 10), flow rate 0.2 mL/min. Retention time: 1.0 or 1.7 min. TIS. Limit of detection, 25 ng/L [Matuszewski *et al.* 1998] Column: C_{18} (33 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (70 : 30), flow rate

1.0 mL/min. APCI, positive ion mode. Limit of quantification, 200 ng/L [Constanzer *et al.* 1994].

Urine **LC-MS** See Plasma. Limit of quantification, 2.0 µg/L [Lundahl *et al.* 2009]. Column: Discovery Supelco C_{18} (150 × 2.1 mm i.d., 5 µm) or Zorbax C_{18} (50 × 2.1 mm i.d., 1.8 µm). Mobile phase: 0.1% acetic acid : acetonitrile with 0.1% acetic acid (85 : 15 to 40 : 60 in 7 min and 100 : 0 in 14 min), flow rate, 0.25 mL/min. CID, MRM acquisition mode. Limit of detection, 30 µg/L [Mazzarino, Botré 2006].

Bile **LC-MS** See Plasma. Limit of quantification, 2.5 µg/L [Lundahl *et al.* 2009].

Disposition in the Body Finasteride is well absorbed after oral administration and metabolised in the liver by oxidation to a (17)-monocarboxylic acid metabolite (18.5% of the dose) and ω-hydroxylated finasteride. There is virtually no unchanged drug. The metabolites are excreted in urine (approximately 40%) and faeces (50 to 65%). The drug is widely distributed throughout the body and crosses the blood–brain barrier.

Therapeutic Concentration

A 5 mg dose of finasteride was orally administered to 24 healthy volunteers. The mean maximum plasma concentration was 56.4 ± 8.7 µg/L reached at 1.8 ± 0.4 h [Guo *et al.* 2007].

Twenty-four healthy adult males were administered a single oral dose of 5 mg finasteride. A mean maximum plasma concentration of 35.9 µg/L was reached at 2 h [Almeida *et al.* 2005].

Twenty-six healthy male volunteers were administered 2 x 5 mg oral finasteride. The plasma concentration reached a maximum of ≈60 µg/L at 2 h [Ptácek *et al.* 2000].

Thirty-two healthy males aged between 28 and 53 years were administered with single doses of 5, 10, 20, 50 and 100 mg finasteride after an overnight fast. Peak plasma concentrations increased with dose: 38.1, 81.5, 147.1, 442.0 and 835.5 µg/L, respectively. The time to reach this peak increased from 1.8 to 2.8 h. The same volunteers were also administered with multiple doses of 10 mg daily for 7 days. On day 1 peak plasma concentrations of 82.5 µg/L were reached followed by 88.7 and 96.2 µg/L for days 4 and 7, respectively. Peak concentrations were reached between 3.7 and 4.5 h after administration [Ohtawa *et al.* 1991].

After a single oral dose of 50, 200 or 400 mg a peak plasma concentration of 0.29 ± 0.02, 0.92 ± 0.08 and 2.05 ± 0.19 mg/L were reached at 4, 2 to 4, and 4 to 6 h, respectively [Carlin *et al.* 1988].

Toxicity For a case of solitary fixed drug eruption after finasteride administration see Oyama, Kaneko [2009].

Bioavailability Mean bioavailability has been reported to be 63 and 80%.

Half-life ≈6 h (individuals aged <60 years); 8 h (>70 years). 17.3 ± 3.5 or 13.4 ± 0.73 [Carlin *et al.* 1988]; 5.71 h [Almeida *et al.* 2005].

Volume of Distribution 44 to 96 L.

Clearance 165 mL/min.

Protein Binding 90%.

Dose A usual dose of 1 mg daily is recommended. The maximum dose is 5 mg.

Almeida A *et al.* (2005). Bioequivalence study of two different coated tablet formulations of finasteride in healthy volunteers. *Arzneimittelforschung* 55: 218–222.

Carlin JR *et al.* (1988). High-performance liquid chromatographic determination of *N*-(2-methyl-2-propyl)-3-oxo-4-aza-5 alpha-androst-1-ene-17 beta-carboxamide, a 4-azasteroid, in human plasma from a phase I study. *J Chromatogr* 427: 79–91.

Carlin JR *et al.* (1992). Disposition and pharmacokinetics of [14C]finasteride after oral administration in humans. *Drug Metab Dispos* 20: 148–155.

Carlucci G, Mazzeo P (1997). Finasteride in biological fluids: extraction and separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 693: 245–248.

Chen X *et al.* (2008). Development and validation of an LC-MS assay for finasteride and its application to prostate cancer prevention trial sample analysis. *J Chromatogr Sci* 46: 356–361.

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Constanzer ML *et al.* (1994). Picogram determination of finasteride in human plasma and semen by high-performance liquid chromatography with atmospheric-pressure chemical-ionization tandem mass spectrometry. *J Chromatogr B Biomed Appl* 658: 281–287.

de Menezes FG *et al.* (2001). Bioequivalence study of finasteride. Determination in human plasma by high-pressure liquid chromatography coupled to tandem mass spectrometry. *Arzneimittelforschung* 51: 145–150.

Guarna A *et al.* (1995). Synthesis of 5,6,6-[2H3]finasteride and quantitative determination of finasteride in human plasma at picogram level by an isotope-dilution mass spectrometric method. *J Chromatogr B Biomed Appl* 674: 197–204.

Guo FQ *et al.* (2007). A rapid, simple, specific liquid chromatographic-electrospray mass spectrometry method for the determination of finasteride in human plasma and its application to pharmacokinetic study. *J Pharm Biomed Anal* 43: 1507–1513.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Lundahl A *et al.* (2009). The effect of St. John's wort on the pharmacokinetics, metabolism and biliary excretion of finasteride and its metabolites in healthy men. *Eur J Pharm Sci* 36: 433–443.

Matuszewski BK *et al.* (1998). Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. *Anal Chem* 70: 882–889.

Mazzarino M, Botré F (2006). A fast liquid chromatographic/mass spectrometric screening method for the simultaneous detection of synthetic glucocorticoids, some stimulants, anti-oestrogen drugs and synthetic anabolic steroids. *Rapid Commun Mass Spectrom* 20: 3465–3476.

Ohtawa M *et al.* (1991). Pharmacokinetics and biochemical efficacy after single and multiple oral administration of *N*-(2-methyl-2-propyl)-3-oxo-4-aza-5 alpha-androst-1-ene-17 beta-carboxamide, a new type of specific competitive inhibitor of testosterone 5 alpha-reductase, in volunteers. *Eur J Drug Metab Pharmacokinet* 16: 15–21.

Oyama N, Kaneko F (2009). Solitary fixed drug eruption caused by finasteride. *J Am Acad Dermatol* 60: 168–169.

Ptácek P *et al.* (2000). Determination of finasteride in human plasma by liquid-liquid extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 738: 305–310.

Takano T, Hata S (1996). High-performance liquid chromatographic determination of finasteride in human plasma using direct injection with column switching. *J Chromatogr B Biomed Appl* 676: 141–146.

Flavoxate

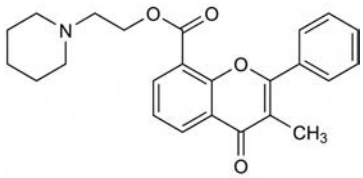
Antispasmodic

$C_{24}H_{25}NO_4$ = 391.5

CAS—15301-69-6

IUPAC Name 2-Piperidin-1-ylethyl 3-methyl-4-oxo-2-phenylchromene-8-carboxylate

Synonym 3-Methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylic acid 2-(1-piperidinyl)ethyl ester



Chemical Properties Soluble in ethanol and chloroform. pK_a 7.3. Log *P* (octanol/water), 4.9.

Flavoxate Hydrochloride

$C_{24}H_{25}NO_4$,HCl = 427.9

CAS—3717-88-2

Proprietary Names *Bladderon*; *Bladuril*; *Cleanxate*; *Flavorin*; *Genurin*; *Spasuret*; *Spasuri*; *Urispadol*; *Urispas*; *Uronid*; *Uroxate*.

Chemical Properties A creamy-white crystalline powder. Mp 230° to 236°. Soluble 1 in 150 of water, 1 in 500 of ethanol, 1 in 40 of chloroform and 1 in 120 of methanol; practically insoluble in ether.

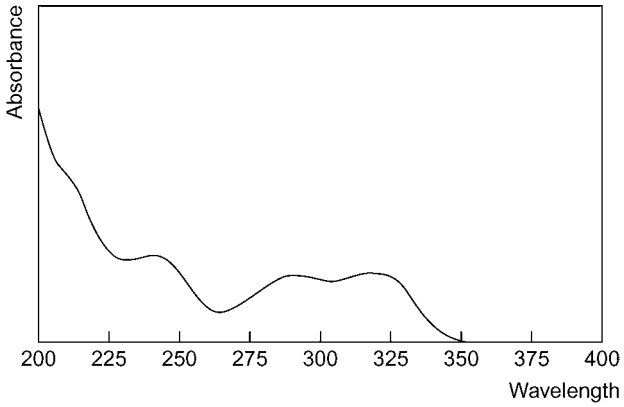
Colour Test Liebermann's reagent—yellow.

Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.36; system TC—R_f 0.67; system TE—R_f 0.77; system TL—R_f 0.45; system TAE—R_f 0.48; system TAJ—R_f 0.52; system TAK—R_f 0.71; system TAL—R_f 0.92 (acidified iodoplatinate solution, strong reaction).

Gas Chromatography System GA—not eluted.

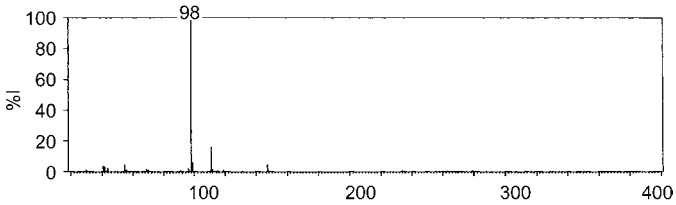
High Performance Liquid Chromatography System HA—*k* 2.2; system HAX—retention time 11.3 min; system HAY—retention time 6.0 min.

Ultraviolet Spectrum Aqueous acid—241 ($A^{\frac{1}{1}}$=360b), 293, 320 nm.



Infrared Spectrum Principal peaks at wavenumbers 1633, 1121, 1718, 1255, 759, 1272 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 98, 111, 99, 147, 55, 41, 42, 96.



Dose 300 to 800 mg of flavoxate hydrochloride daily.

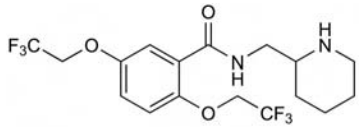
Flecainide

Antiarrhythmic

$C_{17}H_{20}F_6N_2O_3$ = 414.3

CAS—54143-55-4; 99495-87-1 (±)

IUPAC Name *N*-(2-Piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide



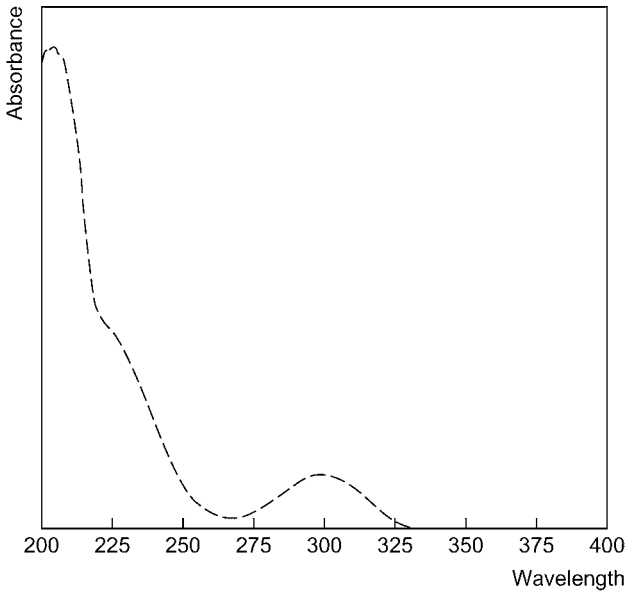
Chemical Properties A white, granular solid. Mp 105° to 107°. pK_a 9.3 (flecainide acetate). Log *P* (octanol/water), 3.8. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Flecainide Acetate
$C_{17}H_{20}F_6N_2O_3,C_2H_4O_2 = 474.4$
CAS—54143-56-5
Synonym R-818
Proprietary Names *Almarytm*; *Apocard*; *Aristocor*; *Corflene*; *Flecaine*; *Tambocor.*

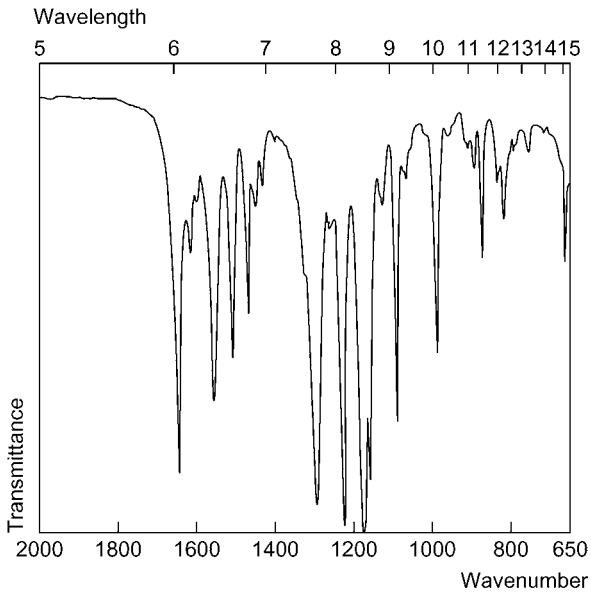
F

Chemical Properties A white to slightly off-white granular solid. Mp 145° to 147°. It is soluble in water (48.4 g/L at 37°) and ethanol (300 g/L at 37°); freely soluble in dilute acetic acid; practically insoluble in dilute hydrochloric acid.

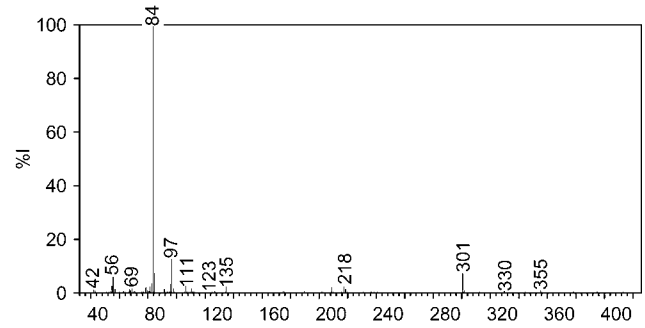
Thin-layer Chromatography System TB—R_f 0.06; system TE—R_f 0.49; system TAE—R_f 0.28.
Gas Chromatography System GA—RI 2250 (flecainide); RI 2500 (Art (formyl)); system GB—RI 2351 (flecainide); RI 2240 (Art (formyl)).
High Performance Liquid Chromatography System HX—RI 419; system HY—RI 355; system HZ—retention time 5.2 min.
Ultraviolet Spectrum Ethanol—205 (A^1_1=521), 230 (A^1_1=219), 300 (A^1_1=59) nm.



Infrared Spectrum Principal peaks at wavenumbers 1169, 1221, 1291, 1637, 1083, 1549 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 84, 97, 301, 56, 85, 96, 218, 82.



Quantification
Blood **HPLC** Fluorescence detection. Limit of detection, 20 µg/L [Bhamra *et al.* 1984].
Plasma **HPLC** Column: RP C_6 Spherisorb (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate (pH 3) (60:40), flow rate 1.5 mL/min. Internal standard (IS): S-15277. UV detection (λ=300 nm) or fluorescence detection (λ_{ex}=300 nm, λ_{em}=370 nm). Retention time: flecainide, 8.39 min; IS, 6.17 min. Limit of detection, 50 µg/L [Verbesselt *et al.* 1991]. Column: RP C_{18} Radial-Pak (100 × 8 mm, 5 µm). Mobile phase: methanol : 25% ammonia (99.9:0.1), flow rate 0.7 mL/min. IS: N-(2-piperidinylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide hydrochloride. Fluorescence detection (λ_{ex}=293 nm, λ_{em}=340 nm). Retention time: flecainide, 7.2 min; IS, 8.3 min. Limit of detection, 10 µg/L [Plomp *et al.* 1986]. See Blood [Bhamra *et al.* 1984]. Fluorescence detection. Limit of detection, 3 µg/L. UV detection. Limit of detection, 50 µg/L [Chang *et al.* 1984].
Serum **HPLC** See Blood [Bhamra *et al.* 1984].
Disposition in the Body Flecainide is rapidly and almost completely absorbed after oral administration and peak plasma concentrations occur after 0.5 to 6 h. It is extensively metabolised (subject to genetic polymorphism) mainly to *m*-O-dealkylated flecainide and the *m*-O-dealkylated lactam of flecainide, which both have some activity. *m*-O-dealkylated flecainide makes up to 20% of the drug's anti-arrhythmic activity. CYP2D6 is involved in its metabolism. Flecainide is excreted mainly in urine (about 10 to 50% as the unchanged drug and the remainder as metabolites, depending on type of administration). About 5% is excreted in faeces. Excretion of flecainide is decreased in alkaline urine. Haemodialysis removes only about 1% of unchanged flecainide. It crosses the placenta and is distributed into breast milk.
Therapeutic Concentration The trough serum therapeutic concentration range is 0.45 to 0.9 mg/L and peak, 0.75 to 1.25 mg/L.

Trough plasma-flecainide concentrations at steady state in 13 individuals, aged between 28 and 72 years (average, 53.7 years), taking 100, 200 and 250 mg of flecainide orally twice a day were 0.295 and 0.314 mg/L (2 subjects), 0.390 to 0.814 mg/L (5 subjects), and 0.753 and 1.387 mg/L, respectively. All participants suffered from coronary artery disease, heart disease or primary rhythm disorder [Anderson *et al.* 1981].

Normal, healthy volunteers were administered 200 mg and peak concentrations of 0.341 mg/L were observed at 2.4 h [Hall 1984].

Twenty men and one woman, aged 33 to 74 years, with impaired renal function (10 of whom had end-stage renal disease requiring haemodialysis) were administered a single, oral dose of 200 mg flecainide acetate. 7 to 14 days after this single dose, each patient received 100 mg flecainide acetate every 12 or 24 h for 10 days. Mean peak flecainide acetate concentrations were 0.33 mg/L, after 3.3 h, following the single dose and 0.687 mg/L, after 2.7 h, for the multiple dosing regimen [Forland *et al.* 1988].

Toxicity Overdosing with flecainide is potentially life threatening and can cause or worsen supraventricular or ventricular arrhythmias and heart failure. Plasma levels above 0.7 to 1.0 mg/L are associated with increased likelihood of adverse experiences; the toxic concentration range is 1.5 to 3.0 mg/L.

Three subjects receiving flecainide developed dose-related blurring of vision at plasma concentrations of 0.713 to 0.906 mg/L (mean 0.802 mg/L). Three other subjects who required plasma concentrations >0.7 mg/L for efficacy reported no adverse effects [Duff *et al.* 1981].

A young woman attempted to commit suicide and was found with no effective blood pressure and in extreme cardiac suffering. A flecainide concentration of 5.4 mg/L was observed (therapeutic range 0.2 to 1.0 mg/L) and during 10 h of cardiopulmonary bypass support, this reduced to 1.4 mg/L. Blood pressure and effective cardiac rhythm eventually returned and treatment was continued until drug levels decreased further. Unfortunately, the woman died, however, owing to severe neurological damage which occurred at the time of overdose [Yasui *et al.* 1997].

Bioavailability 90 to 95%.
Half-life Plasma half-life, 7 to 23 h; 19 h (patients with arrhythmia); 26 to 49 h (patients with renal/hepatic dysfunction); 50 h (patients with congestive heart failure).
Volume of Distribution Steady state, 5 to 13.4 L/kg; average 5.5 to 8.7 L/kg for IV administration and 10 L/kg for oral.
Clearance Plasma, 4.6 to 12.1 mL/min/kg after an IV dose of 0.6 to 1.7 mg/kg; 4.1 to 17 mL/min/kg after an oral dose of 60 to 240 mg.
Protein Binding 32 to 58%.
Note For reviews of flecainide, see Holmes and Heel [1985] and Conard and Ober [1984].

Dose IV administration: initially 2 mg/kg body weight is administered for the first 30 min, followed by 1.5 mg/kg/h for the first hour and maintained at 0.1 to 0.25 mg/kg/h for subsequent hours. The infusion period should not exceed 24 h and the maximum dose is 600 mg. For patients with severe renal impairment (creatinine clearance <35 mL/min/1.73 m^2). Oral administration: initial dose is 50 to 100 mg twice daily, with a maximum of 300 to 400 mg daily. Flecainide is not recommended to children under 12 years old.

Anderson JL *et al.* (1981). Oral flecainide acetate for the treatment of ventricular arrhythmias. *N Engl J Med* 305: 473–477.

Bhamra RK *et al.* (1984). High-performance liquid chromatographic method for the measurement of mexiletine and flecainide in blood plasma or serum. *J Chromatogr* 307: 439–444.

Chang SF *et al.* (1984). Application of a bonded-phase extraction column for rapid sample preparation of flecainide from human plasma for high-performance liquid chromatographic analysis–fluorescence or ultraviolet detection. *Ther Drug Monit* 6: 105–111.

Conard GJ, Ober RE (1984). Metabolism of flecainide. *Am J Cardiol* 53: 41B–51B.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Duff HJ *et al.* (1981). Suppression of resistant ventricular arrhythmias by twice daily dosing with flecainide. *Am J Cardiol* 48: 1133–1140.

Forland SC *et al.* (1988). Flecainide pharmacokinetics after multiple dosing in patients with impaired renal function. *J Clin Pharmacol* 28(8): 727–735.

Holmes B, Heel RC (1985). Flecainide. A preliminary review of its pharmacodynamic properties and therapeutic efficacy. *Drugs* 29: 1–33.

Plomp TA *et al.* (1986). Measurement of flecainide plasma concentrations by high performance liquid chromatography with fluorescence detection. *J Anal Toxicol* 10(3): 102–106.

Hall RJ (1984). Aortic valve disease. *Eur Heart J* 5: A135–139.

Verbesselt R *et al.* (1991). High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Ther Drug Monit* 13: 157–165.

Yasui RK *et al.* (1997). Flecainide overdose: is cardiopulmonary support the treatment? *Ann Emer Med* 29(5): 680–682.

Floctafenine

Analgesic

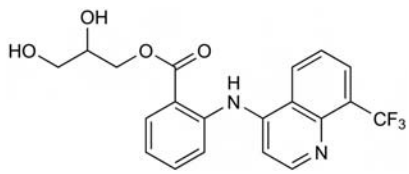
$C_{20}H_{17}F_3N_2O_4$ = 406.4

CAS—23779-99-9

IUPAC Name 2,3-Dihydroxypropyl 2-[[8-(trifluoromethyl)quinolin-4-yl]amino]benzoate

Synonym 2-[[8-(Trifluoromethyl)-4-quinolinyl]amino]benzoic acid 2,3-dihydroxypropyl ester

Proprietary Names *Idalon*; *Idarac*.



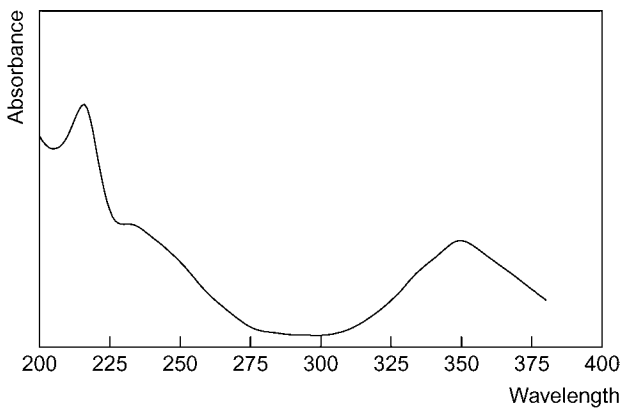
Chemical Properties A yellowish-white powder. Mp 175° to 179°. Insoluble in water; soluble in ethanol and acetone; very slightly soluble in chloroform, ether and methylene chloride; freely soluble in dimethylformamide and pyridine. Log *P* (octanol/water), 4.0.

Thin-layer Chromatography System TE—R_f 0.85; system TAE—R_f 0.85.

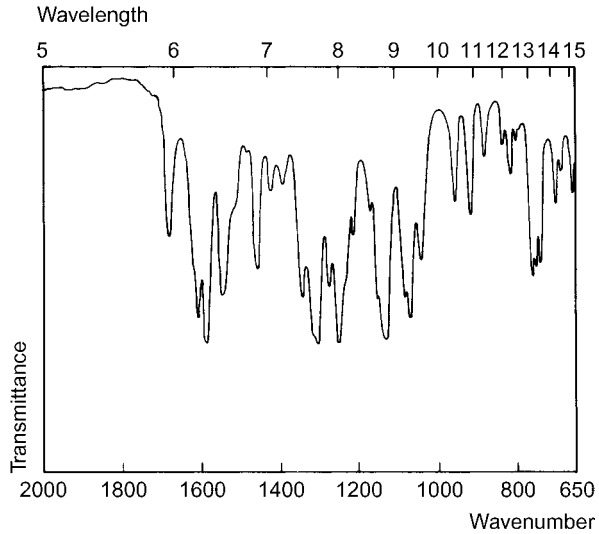
Gas Chromatography System GA—floctafenine RI 3132, floctafenine-Me RI 2433.

High Performance Liquid Chromatography System HZ—retention time 4.4 min; system HAA—retention time 17.2 min; system HAX—retention time 6.6 min; system HAY—retention time 5.8 min.

Ultraviolet Spectrum Aqueous acid—233 (A^1_1=547a), 348 nm (A^1_1=450a); aqueous alkali—249 (A^1_1=392b), 359 nm (A^1_1=390b).



Infrared Spectrum Principal peaks at wavenumbers 1585, 1305, 1252, 1131, 1605, 1070 cm^{-1} (KBr disk).



Quantification

Blood **TLC–spectrophotometry** For method of quantification for floctafenic acid and hydroxyfloctafenic acid, see Lynn *et al.* [1979].

Plasma **HPLC** UV detection. Limit of detection, 100 µg/L floctafenine, 50 µg/L floctafenic acid [Abdel-Hay, Gharaibeh 1992]. UV detection. Floctafenine and glafenine. Limit of detection, about 3.2 µmol/L for floctafenine [Tracqui *et al.* 1988].

Urine **TLC–spectrophotometry** See Blood [Lynn *et al.* 1979].

Disposition in the Body Absorbed after oral administration. It is rapidly metabolised to floctafenic acid, which is thought to be responsible for most of the analgesic activity. About 25% of a dose is excreted in the urine as floctafenic acid and hydroxyfloctafenic acid in 48 h.

Therapeutic Concentration

A single oral dose of 400 mg administered to 7 subjects produced peak blood concentrations of floctafenic acid of 0.9 to 3.8 mg/L (mean 2.0) in 0.5 to 2.5 h [Lynn *et al.* 1979].

Protein Binding Floctafenic acid, extensively bound.

Dose 0.8 to 1.6 g daily.

Abdel-Hay MH, Gharaibeh AM (1992). High-performance liquid chromatographic determination of floctafenine and its major metabolite, floctafenic acid in plasma. *J Clin Pharm Ther* 17: 31–35.

Lynn RK *et al.* (1979). The metabolism of floctafenin in man and rodents. *J Clin Pharmacol* 19: 20–30.

Tracqui A *et al.* (1988). Simultaneous determination of glafenine and floctafenine in human plasma using high-performance liquid chromatography. *Ann Biol Clin (Paris)* 46: 665–667.

Flosequinan

Antihypertensive

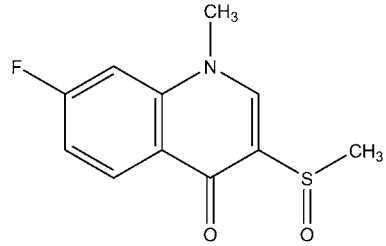
$C_{11}H_{10}FNO_2S$ = 239.3

CAS—76568-02-0

IUPAC Name 7-Fluoro-1-methyl-3-methylsulfinylquinolin-4-one

Synonyms BTS-49465; flosequinon; 7-fluoro-1-methyl-3-(methylsulfinyl)-4(1*H*)-quinolinone.

Proprietary Name *Manoplax*



Chemical Properties Crystals. Mp 226° to 228°. Log *P* (octanol/water), 2.72 [ACD 2007]. Standard solutions frozen at –20° were found to be stable for at least 6 months [Slegowski *et al.* 1988].

Quantification

Plasma **HPLC** Column: Cosmosil 5C_{18}. UV detection (λ = 254 nm). Limit of detection, 5 µg/L for flosequinan and its metabolite [Sakai *et al.* 1993]. Column: Cellulose tris-3,5-dimethylphenylcarbamate on silica gel. Mobile phase: ethanol : methanol (22 : 78). UV detection (λ= 320 nm). Limit of detection, 5 µg/L [Kashiyama *et al.* 1994]. Column: Waters C_{18} Nova-Pak (15 cm × 3.9 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile (73 : 20 : 7), flow rate 1.2 mL/min. UV detection (λ= 254 nm). Limit of detection, 50 µg/L [Slegowski *et al.* 1988].

Column: Lichrosorb RP8 (25 cm × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water (3 : 1). UV detection (λ = 254 nm). Retention times: flosequinan, 6.0 min; sulfone metabolite, 6.8 min. Limit of detection not reported [Wynne *et al.* 1985].

Urine **HPLC** Column: Cosmosil $5C_{18}$. UV detection (λ = 254 nm). Limit of detection, 50 µg/L for flosequinan and 10 µg/L for its metabolite [Sakai *et al.* 1993]. See Plasma. Limit of detection, 2.5 mg/L [Slegowski *et al.* 1988]. See Plasma [Wynne *et al.* 1985].

Oral Fluid **HPLC** See Plasma [Wynne *et al.* 1985].

Disposition in the Body Rapidly absorbed following oral administration and rapidly cleared from the systemic circulation, primarily via metabolic oxidation to the corresponding sulfone. The major active metabolite is 7-fluoro-1-methyl-3 methylsulfinyl-4-quinolone (BTS 53554), which is cleared primarily by the renal route [Wynne *et al.* 1985]. Approximately 1% of the administered dose is excreted unchanged in the urine [Gallo *et al.* 1993].

Therapeutic Concentration Flosequinan is a direct-acting arteriovenous vasodilator that was used as an adjunct to the conventional treatment of heart failure, but it was withdrawn from the market after findings of excess mortality [Kamali, Edwards 1995].

Twenty patients with chronic cardiac failure had a mean maximum plasma concentration of 2.52 mg/L at 1.4 h after administration of 100 mg flosequinan. After 36 days, there was a small increase in the maximum plasma concentration to 3.21 mg/L. The elimination half-life decreased from 6.4 to 4.9 h. The $AUC_{0-\infty}$ and the elimination rate constant (k_{el}) remained unchanged [Nicholls *et al.* 1996].

Ten patients with compromised hepatic function were administered a single oral dose of 100 mg flosequinan. The mean peak plasma concentration was 0.98 ± 0.52 mg/L at 35.4 ± 27.4 h, compared with 1.84 ± 0.26 mg/L at 7.0 ± 3.1 h in healthy subjects. Adverse events were reported by 5 of the subjects, including headache and syncope [Hinson *et al.* 1994].

Twenty patients with severe renal dysfunction were administered a single oral dose of 100 mg flosequinan. Mean peak plasma concentration was 1.37 ± 0.67 mg/L at 1.6 ± 1.4 h. Renal clearance was decreased by approx. 20% compared with healthy volunteers. The active metabolite reached a mean peak plasma concentration of 2.22 ± 0.58 mg/L at 10.9 ± 5.9 h [Gallo *et al.* 1993].

A study in elderly patients with congestive heart failure investigated the effects of a single oral dose of 50 mg flosequinan (8 patients) compared with once-daily treatment with 25 mg for 2 weeks. After a single dose, the mean peak plasma concentration was 1.17 mg/L, attained at 2.5 h. The mean peak plasma concentration of the active metabolite was 1.44 mg/L at 20.3 h. Flosequinan did not accumulate but the active metabolite accumulated gradually reaching steady state after approx. 2 weeks [Sakai *et al.* 1993].

Six healthy male volunteers were administered a single oral dose of 200 mg flosequinan. Mean maximum plasma concentrations for flosequinan and its metabolite were 3.19 and 3.63 mg/L, respectively, attained at 0.5 and 6.0 h, respectively [Wynne *et al.* 1985].

Note For a study investigating the concomitant administration of flosequinan and digoxin, see Rau *et al.* [1994]; for a review of the pharmacology of flosequinan, see Yates [1991].

Half-life Flosequinan 1.6 h; sulfone metabolite 37.6 h.

Dose Patients taking 100 mg flosequinan a day were found to have a significantly increased risk of dying; the manufacturer recommended reducing the dose to 75 or 50 mg a day.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Gallo BV *et al.* (1993). Pharmacokinetic profile of flosequinan in patients with compromised renal function. *J Pharm Sci* 82: 282–285.

Hinson JL *et al.* (1994). Pharmacokinetics, safety, and tolerability of flosequinan in patients with hepatic dysfunction. *J Pharm Sci* 83: 382–385.

Kamali F, Edwards C (1995). Possible role of metabolite in flosequinan-related mortality. *Clin Pharmacokinet* 29: 396–403.

Kashiyama E *et al.* (1994). Stereospecific and simultaneous high-performance liquid chromatographic assay of flosequinan and its metabolites in human plasma. *J Chromatogr* 652: 179–185.

Nicholls DP *et al.* (1996). Pharmacokinetics of flosequinan in patients with heart failure. *Eur J Clin Pharmacol* 50: 289–291.

Rau R *et al.* (1994). Effects of concurrent administration of flosequinan and digoxin on the pharmacokinetics of each drug. *Arzneimittelforschung* 44: 300–304.

Sakai M *et al.* (1993). Pharmacokinetics of flosequinan in elderly patients with chronic congestive heart failure. *Eur J Clin Pharmacol* 44: 387–389.

Slegowski MB *et al.* (1988). Simplified high-performance liquid chromatographic determination of flosequinan and its metabolite in plasma, serum and urine. *J Chromatogr* 425: 227–232.

Wynne RD *et al.* (1985). The pharmacokinetics and haemodynamics of BTS 49 465 and its major metabolite in healthy volunteers. *Eur J Clin Pharmacol* 28: 659–664.

Yates DB (1991). Pharmacology of flosequinan. *Am Heart J* 121: 974–983.

Fluanisone

Tranquilliser

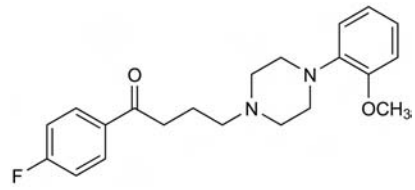
$C_{21}H_{25}FN_2O_2 = 356.4$

CAS—1480-19-9

IUPAC Name 1-(4-Fluorophenyl)-4-[4-(2-methoxyphenyl)-1-piperazinyl]-1-butanone

Synonym Haloanisone

Proprietary Names *Sedalande*. It is an ingredient of *Hypnorm* (vet.).



Chemical Properties An almost white to buff-coloured crystalline powder. Mp 67.5° to 68.5°. Practically insoluble in water; soluble 1 in 12 of ethanol, 1 in 1 of chloroform and 1 in 22 of ether. Log *P* (octanol/water), 3.6.

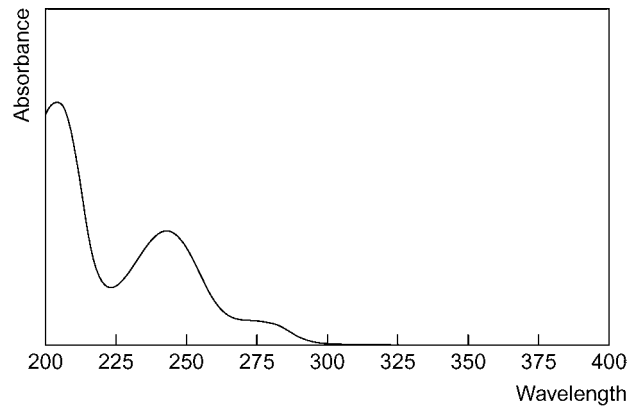
Colour Test Mandelin's test—brown.

Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.39; system TC—R_f 0.68; system TE—R_f 0.82; system TF—R_f 0.23; system TL—R_f 0.60; system TAE—R_f 0.67; system TAF—R_f 0.75 (acidified potassium permanganate solution, positive).

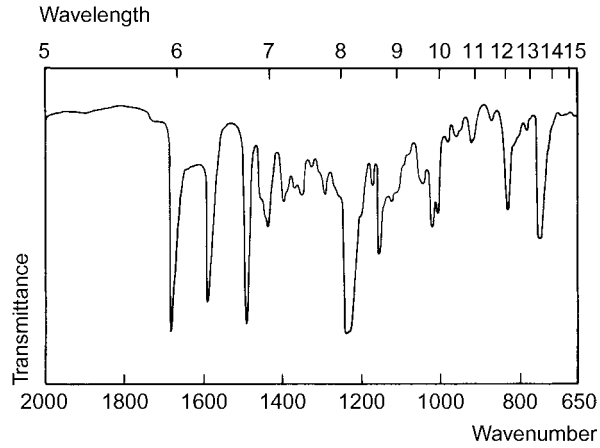
Gas Chromatography System GA—fluanisone RI 2785, M (*O*-desmethyl-) RI 2715.

High Performance Liquid Chromatography System HX—RI 423; system HY—RI 349.

Ultraviolet Spectrum Acid isopropyl alcohol—243 nm (A_1^1=550a).



Infrared Spectrum Principal peaks at wavenumbers 1690, 1235, 1497, 1598, 1155, 750 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 205, 218, 123, 356, 219, 162, 95, 190; M (*O*-desmethyl-) *m/z* 194, 165, 123, 342, 338, 134.

Dose Fluanisone has been given in doses of 5 to 7.5 mg daily.

Fluclorolone Acetonide

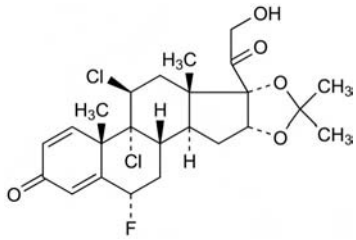
Corticosteroid

$C_{24}H_{29}Cl_2FO_5 = 487.4$

CAS—3693-39-8

Synonyms (6α,11β,16α)-9,11-Dichloro-6-fluoro-21-hydroxy-16,17-[(1-methylethylidine)bis(oxy)]-pregna-1,4-diene-3,20-dione; flucloronide.

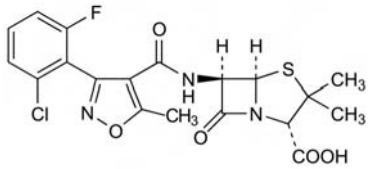
Proprietary Names *Cutanit*; *Topilar*.



Chemical Properties A white crystalline powder. Mp about 245°, with decomposition. Practically insoluble in water; soluble in ethanol, chloroform and methanol. Log *P* (octanol/water), 4.2.
Ultraviolet Spectrum Methanol—236 nm (A^{1}_{1}=242b).
Infrared Spectrum Principal peaks at wavenumbers 1666, 1086, 1052, 1063, 1724, 1639 cm^{-1}.
Use Topically as a 0.025% cream or ointment.

Flucloxacillin

Antibiotic
$C_{19}H_{17}ClFN_3O_5S$ = 453.9
CAS—5250-39-5
IUPAC Name (2*S*,5*R*,6*R*)-6-[[[3-(2-Chloro-6-fluorophenyl)-5-methyl-4-isoxazolyl]-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Synonym Floxacillin
Proprietary Names *Flucloxil*; *Staphlex*.

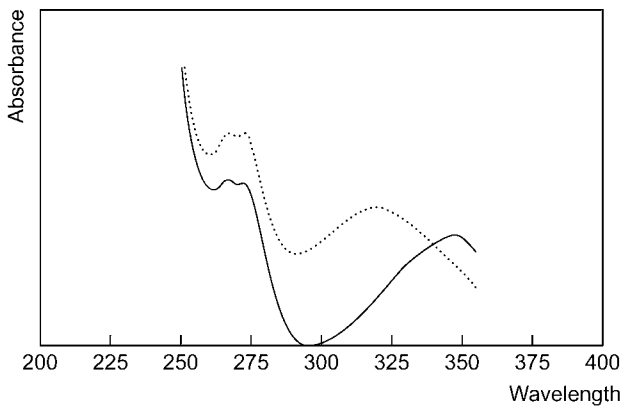


Chemical Properties pK_a 2.7. Log *P* (octanol/water), 2.6.

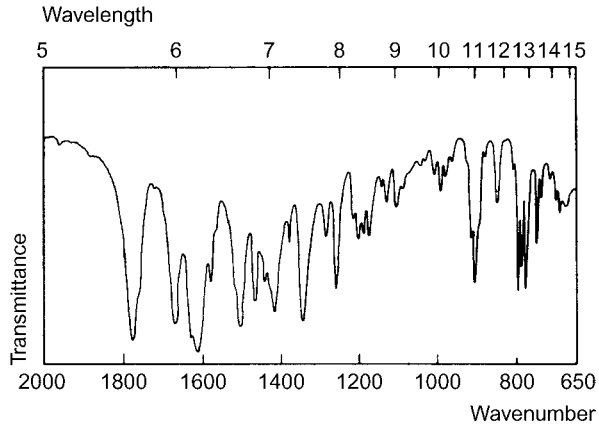
Flucloxacillin Sodium

$C_{19}H_{16}ClFN_3NaO_5S,H_2O$ = 493.9
CAS—1847-24-1 (anhydrous); 34214-51-2 (monohydrate)
Proprietary Names *Betabiotic*; *Cloxillin*; *Evercid*; *Flopen*; *Floxapen* (also as the magnesium salt); *Flucil*; *Flucillin*; *Flucinal*; *Fluclomix*; *Fluclon*; *Flucloxin*; *Fluoclox*; *Galfloxin*; *Geriflox*; *Heracillin*; *Ladropen*; *Stafoxil*; *Staphlex*; *Staphycid* (also as the magnesium salt); *Staphylex*; *Zoxin*. It is an ingredient of *Magnapen*.
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 1 of water, 1 in 8 of ethanol, 1 in 8 of acetone and 1 in 2 of methanol.

High Performance Liquid Chromatography System HY—RI 469.
Ultraviolet Spectrum Aqueous acid—268, 274, 344 nm; aqueous alkali—268, 274, 318 nm.



Infrared Spectrum Principal peaks at wavenumbers 1603, 1767, 1622, 1495, 1660, 794 cm^{-1} (flucloxacillin sodium, KBr disk).

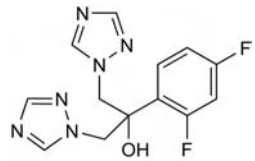


Quantification
***Plasma* HPLC** Limit of detection, 50 µg/L [Charles *et al.* 1994]. Flucloxacillin, cloxacillin and dicloxacillin. Limit of detection, 0.1 mg/L for flucloxacillin [Hung *et al.* 1988].
Disposition in the Body
Therapeutic Concentration
After a single oral dose of flucloxacillin sodium (500 mg) to 7 elderly subjects, the time to reach a mean peak plasma concentration of 15.3 mg/L (range ± 5.9) was 1.2 h; corresponding values for the 5-hydroxymethyl metabolite were 2.58 mg/L (±2.20) at 2.17 h. IV administration of the same dose to the same subjects produced mean maximum plasma concentrations of flucloxacillin and its 5-hydroxymethyl metabolite, of 69.0 mg/L (±21.2) at 0.14 h and 3.36 mg/L (±2.72) at 1.77 h, respectively [Gath *et al.* 1995].
Dose The equivalent of 1 to 2 g of flucloxacillin daily.

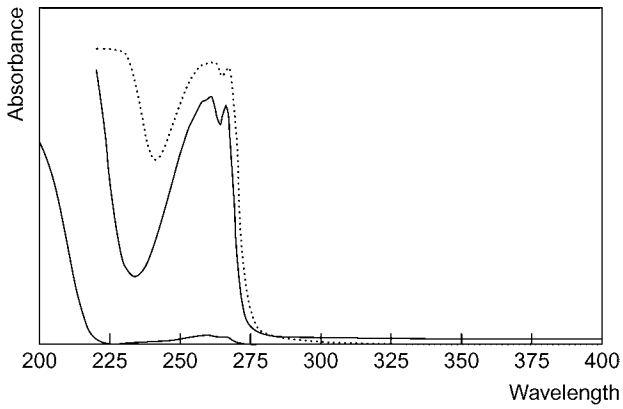
Charles BG *et al.* (1994). Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column. *J Chromatogr Biomed Appl* 660: 186–190.
Gath J *et al.* (1995). Pharmacokinetics and bioavailability of flucloxacillin in elderly hospitalized patients. *J Clin Pharmacol* 35: 31–36.
Hung CT *et al.* (1988). Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design. *J Chromatogr* 425: 2: 331–341.

Fluconazole

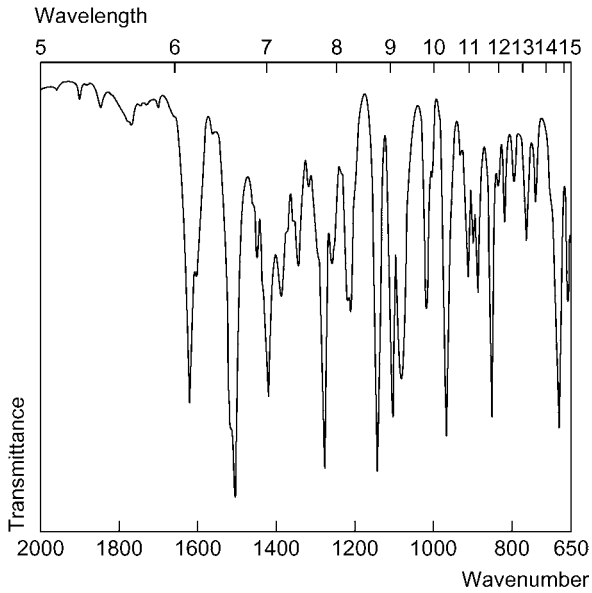
Antifungal
$C_{13}H_{12}F_2N_6O$ = 306.3
CAS—86386-73-4
IUPAC Name 2-(2,4-Difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol
Synonyms α-(2,4-Difluorophenyl)-α-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-1,2,4-triazole-1-ethanol; UK-49858.
Proprietary Names *Biozolene*; *Diflucan*; *Elazor*; *Fungata*; *Lavisa*; *Loitin*; *Solacap*; *Triflucan*.



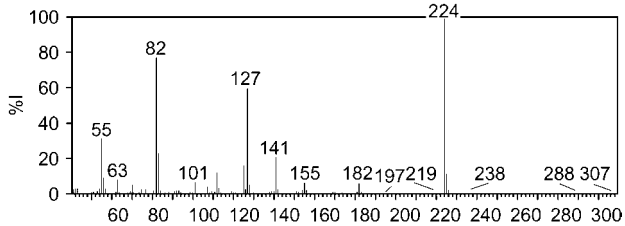
Chemical Properties A white crystalline solid. Mp 138° to 140°. It is slightly soluble in water and saline. pK_a 2.03 (37°). Log *P* (octanol/pH 7.4), 1.0. Extraction yield (chlorobutane), 0.1 [Demme *et al.* 2005].
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.35; system TAE—R_f 0.67.
High Performance Liquid Chromatography System HAA—retention time 11.4 min; system HX—RI 340; system HY—RI 289.
Ultraviolet Spectrum Aqueous acid—261, 266 nm; basic—261, 267 nm.



Infrared Spectrum Principal peaks at wavenumbers 1507, 1279, 1144, 680, 1620, 966 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 224, 82, 127, 55, 83, 141, 125, 113.



Quantification

Plasma **GC** Column: 1-methylsilicone gum (10 m × 0.53 mm i.d.). Temperature: 220°. Injector and detector temperatures: 250° and 300°, respectively. Carrier gas: argon : methane (95:5), flow rate 5 mL/min. Detection: electron capture. IS: medazepam. Retention time: fluconazole, 4.35 min; IS, 6.40 min. Limit of detection, 0.003 mg/L [Beijnen *et al.* 1991].

HPLC Column: C_{18} (Genesis, 150 × 4.6 mm i.d., 4 µm). Temperature: 40°. Mobile phase: water : acetonitrile (60:40) with 5 mmol/L acetic acid, pH 3.7, flow rate 0.9 mL/min. IS: metronidazole. MS detection (APCI (atmospheric pressure chemical ionisation), MRM (multiple reaction monitoring) at m/z 306.9 to 219.8 for fluconazole and 171.8 to 127.7 for IS.). Retention time: fluconazole, 3.1 min; IS, 2.8 min. Limit of quantification, 0.005 mg/L [Moraes *et al.* 1999].

Bioassay Limit of detection, 2 mg/L [Rex *et al.* 1991]. Limit of detection, 0.2 mg/L [Van't Wout *et al.* 1988].

Serum **GC** Column: 5% methylphenyl : 95% dimethylpolysiloxane, silica DB5 (15 m × 0.32 mm i.d., 0.25 µm). Temperature: 230°. Injector and detector temperatures: 280° and 285°, respectively. Carrier gas: H_2, flow rate 25 mL/min. Detection: nitrogen-selective. Internal standard (IS): medazepam. Retention time: fluconazole, 1.5 min; IS, 3.2 min. Limit of detection, 0.05 mg/L [Debruyne *et al.* 1988].

HPLC Column: C_{18} Adsorbosphere (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L tris(hydroxymethyl)aminomethane phosphate (pH 7) : acetonitrile (75:25), flow rate 1.0 mL/min. UV detection (λ=210 nm). IS: UK-48134. Retention time: fluconazole, 6.6 min; IS, 9.0 min. Limit of detection, 0.1 mg/L [Inagaki *et al.* 1992].

Bioassay See Plasma [Van't Wout *et al.* 1988].

Disposition in the Body Fluconazole is well absorbed after oral administration and is widely distributed throughout the body. It penetrates all body fluids and has been detected in breast milk, joint fluid, saliva, sputum, vaginal fluid and peritoneal fluid. 80% of the parent drug is excreted unchanged in urine and 11% as metabolites.

Therapeutic Concentration Multiple dosing increases peak plasma concentrations and steady state concentrations are reached between 6 and 10 days. The serum therapeutic concentration range is 5 to 15 mg/L.

Ten AIDS patients and 10 healthy volunteers were administered, IV, over 20 min with 100 mg fluconazole. Peak plasma concentrations reached 2.9 ± 0.9 and 2.8 ± 0.4 mg/L for the AIDS and healthy patients, respectively [Yeates *et al.* 1994].

Toxicity The toxic serum concentration is 20 mg/L.

Case: A 56-year-old woman was found dead at home. Toxicological investigation of her blood found a fluconazole concentration of 29.18 mg/L and an acenocoumarol concentration of 0.72 mg/L. Fluconazole inhibited the cytochrome P450 enzymes and thus decreased the clearance of acenocoumarol, resulting in death owing to the haemorrhaging effects of the drug [Kintz *et al.* 1995].

Half-life Approximately 30 h (range from 20 to 50 h); increased in patients with impaired renal function.

Bioavailability Approximately 90%.

Volume of Distribution Apparent volume of distribution, 0.7 to 1.0 L/kg.

Clearance In adults: approx. 0.23 mL/min/kg; 5 to 15 years old: 0.58 mL/min/kg; 9 months to 13 years old: 0.455 mL/min/kg.

Protein Binding Approximately 12%.

Dose Doses range from 50 to 400 mg daily, depending on symptoms and severity. Patients with renal impairment: reduced doses. Children: 3 mg/kg to 12 mg/kg daily (> 4 weeks old), every 72 h (< 2 weeks) and every 48 h (between 2 and 4 weeks).

Beijnen JH *et al.* (1991). Gas chromatographic analysis, with electron capture detection, of the antifungal drug fluconazole in microvolumes of human plasma. *J Pharm Biomed Anal* 9: 1173–1175.

Debruyne D *et al.* (1988). Determination of fluconazole in biological fluids by capillary column gas chromatography with a nitrogen detector. *J Pharm Sci* 77: 534–535.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Inagaki K *et al.* (1992). Determination of fluconazole in human serum by solid-phase extraction and reversed-phase high-performance liquid chromatography. *Ther Drug Monit* 14: 306–311.

Kintz P *et al.* (1995). *TIAFT Bulletin Case Notes* 25(4): .

Moraes LA *et al.* (1999). Fluconazole bioequivalence study: quantification by tandem mass spectrometry. *Ther Drug Monit* 21: 200–207.

Rex JH *et al.* (1991). Standardization of a fluconazole bioassay and correlation of results with those obtained by high-pressure liquid chromatography. *Antimicrob Agents Chemother* 35: 846–850.

Van't Wout JW *et al.* (1988). A prospective study of the efficacy of fluconazole (UK-49,858) against deep-seated fungal infections. *J Antimicrob Chemother* 21: 665–672.

Yeates RA *et al.* (1994). The pharmacokinetics of fluconazole after a single intravenous dose in AIDS patients. *Br J Clin Pharmacol* 38: 77–79.

Flucytosine

Antifungal

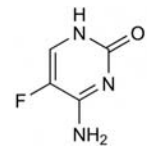
$C_4H_4FN_3O$ = 129.1

CAS—2022-85-7

IUPAC Name 4-Amino-5-fluoro-2(1*H*)-pyrimidinone

Synonym 5-Fluorocytosine

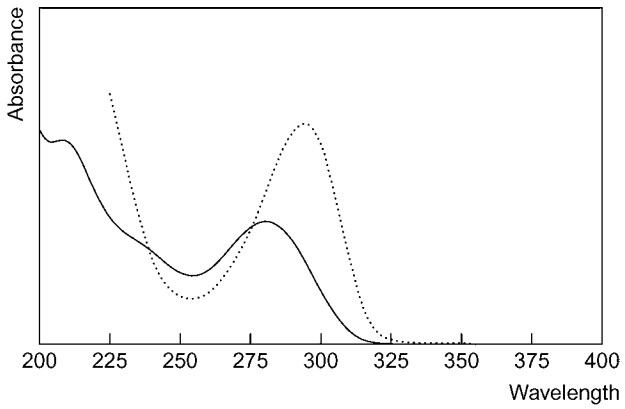
Proprietary Names *Alcobon*; *Ancobon*; *Ancotil*.



Chemical Properties A white crystalline powder. Mp 295° to 297°, with decomposition. Soluble 1 in 67 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether. pK_a 2.9, 10.7. Log *P* (octanol/water), −1.4.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.09; system TAE—R_f 0.57.

High Performance Liquid Chromatography System HX—RI 72; system HZ—retention time 1.5 min; system HAA—retention time 3.1 min.
Ultraviolet Spectrum Aqueous acid—286 nm (A_1^1=709a); aqueous alkali—293 nm.



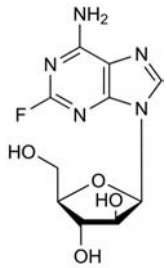
Infrared Spectrum Principal peaks at wavenumbers 1676, 1638, 1548, 1230, 1516, 1211 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** Diode-array detection. For method for quantification of flucytosine and fluorouracil, see Torano *et al.* [2001].
Serum **HPLC** See Plasma [Torano *et al.* 2001]. Limit of detection, 80 to 625 µg/L for flucytosine and other antifungal drugs [Ng *et al.* 1996]. For comparison with bioassays, see St-Germain *et al.* [1989]; Hulsewede [1994].
Dose 350 to 200 mg/kg daily.

Hulsewede JW (1994). Comparison of high-performance liquid chromatography and bioassay for the determination of 5-fluorocytosine in serum. *Zentralbl Bakteriol* 281: 513–518.
Ng TK *et al.* (1996). Rapid high performance liquid chromatographic assay for antifungal agents in human sera. *J Antimicrob Chemother* 37(3): 465–472.
St-Germain G *et al.* (1989). Performance characteristics of two bioassays and high-performance liquid chromatography for determination of flucytosine in serum. *Antimicrob Agents Chemother* 33: 1403–1405.
Torano JS *et al.* (2001). Simultaneous determination of flucytosine and fluorouracil in human plasma by high-performance liquid chromatography. *Biomed Chromatogr* 15: 89–94.

Fludarabine

Antineoplastic
$C_{10}H_{12}FN_5O_4$ = 285.2
CAS—21679-14-1

IUPAC Name (2*R*,3*S*,4*S*,5*R*)-2-(6-Amino-2-fluoropurin-9-yl)-5-(hydroxymethyl)oxolane-3, 4-diol
Synonyms 9-β-D-Arabino-furanosyl-2-fluoro-9*H*-purin-6-amine; 2-F-ara-A; 2-fluorovidarabine; NSC-118218; NSC-118218-H.

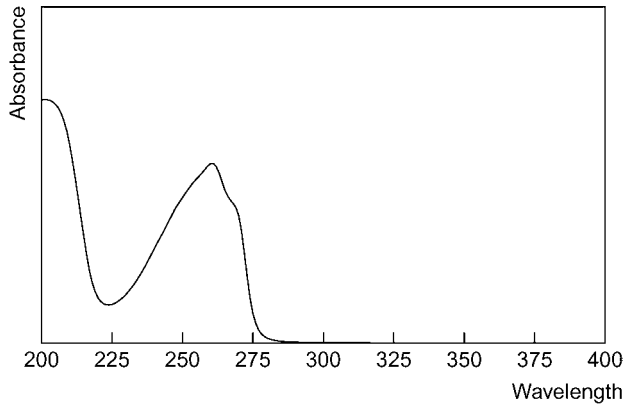


Chemical Properties Crystals from ethanol and water. Mp 260°. It is sparingly soluble in water and organic solvents. Log *P* (octanol/water), −1.18.

Fludarabine Phosphate

$C_{10}H_{13}FN_5O_7P$ = 365.2
CAS—75607-67-9
Synonyms Fludarabine-5′-monophosphate; 2-F-ara-AMP; 2-fluoro-AMP; NSC-312887; NSC-328002.
Proprietary Names *Beneflur*; *Fludara*.

Ultraviolet Spectrum Principal peak at 258 nm.



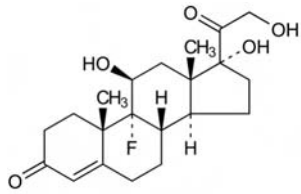
Quantification
Plasma **HPLC** Column: RP-18 LiChrosorb (200 × 4 mm i.d., 7 µm). Mobile phase: methanol : phosphate buffer (pH 4.15) (20:80), flow rate 1.0 mL/min. Internal standard (IS): mercaptopurine. UV detection (λ=254 nm). Retention time: fludarabine, 6.3 min; IS, 3.3 min. Limit of detection, 50 µg/L [Misztal *et al.* 1996]. Fluorescence detection. Limit of quantification, 0.6 µg/L [Kemena *et al.* 1991].
Urine **HPLC** Column: C-18 Ultrasphere (5 µm, 250 × 4.6 mm). Mobile phase: methanol : 10 mmol/L $(NH_4)H_2PO_4$ (pH 4.15) (6:94), flow rate 1.0 to 1.2 mL/min. UV detection (λ=254 nm). Retention time: 55 min. Limit of detection, 0.0125 ng/L [Hersh *et al.* 1986].
Disposition in the Body Fludarabine phosphate is rapidly dephosphorylated to fludarabine after IV administration and is taken up by lymphocytes where it is rephosphorylated, by kinase, to active fludarabine triphosphate (2-F-ara-ATP). Peak plasma concentrations of 2-F-ara-ATP are observed ~4 h after administration and then undergo triphasic clearance. Excretion is mainly via the kidneys with ~60% of a dose being excreted in urine within 24 h. Considerable variations in pharmacokinetics have been observed between individuals.
Therapeutic Concentration
A single dose of 25 mg 2-F-ara-AMP/m^2 was administered, by infusion, over 30 min, to a number of cancer patients. Maximum plasma concentrations of 2-F-ara-A reached 3.5 to 3.7 µg/L by the end of the infusion. Moderate accumulation was observed after the fifth dose to 4.4 to 4.8 µg/L [Schering Health Care Ltd]
Toxicity High doses of fludarabine phosphate are associated with irreversible central nervous system toxicity with symptoms such as blindness, coma and even death. Also, myelosuppression, pulmonary toxicity, ototoxicity, severe thrombocytopenia and neutropenia, owing to bone marrow suppression, have also been observed. The main intracellular metabolite, 2-F-ara-ATP, has cytotoxicity activity.
Overdose case: the usual dose of fludarabine phosphate, for chronic lymphocytic leukaemia, was administered and 6 months into treatment, progressive cerebral dysfunction due to leucoencephalopathy developed. The patient slipped into a coma and died 8 weeks later [Zabernigg *et al.* 1994].
Bioavailability Systemic bioavailability of 2-F-ara-ATP, 50 to 65%.
Half-life Initial half-life is 1 to 2 h and terminal half-life ranges from 10 to 30 h.
Volume of Distribution Steady state, 2.4 ± 1.6 L/kg (83 ± 55 L/m^2).
Clearance Plasma clearance, 2.2 ± 1.2 mL/min/kg (79 ± 40 mL/min/kg).
Protein Binding No tendency to bind proteins.
Dose A usual dose of 25 to 40 mg fludarabine phosphate/m^2 body surface is administered over 30 min, intravenously, daily for 5 days and courses repeated every 28 days. Dosage is reduced with severe or persistent myelosuppression and mild to moderate renal impairment (by up to 50%).

Hersh MR *et al.* (1986). Pharmacokinetic study of fludarabine phosphate (NSC 312887). *Cancer Chemother Pharmacol* 17: 277–280.
Kemena A *et al.* (1991). A sensitive fluorescence assay for quantitation of fludarabine and metabolites in biological fluids. *Clin Chim Acta* 200: 95–106.
Misztal G *et al.* (1996). Determination of fludarabine phosphate in human plasma using reversed phase high-performance liquid chromatography. *Pharmazie* 51: 733–734.
Schering Health Care Limited data
Zabernigg A *et al.* (1994). Late-onset fatal neurological toxicity of fludarabine. *Lancet* 344: 1780.

Fludrocortisone

Corticosteroid
$C_{21}H_{29}FO_5$ = 380.5
CAS—127-31-1

IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-9-Fluoro-11, 17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-1,2,6,7,8,11,12,14,15, 16-decahydrocyclopenta[*a*]phenanthren-3-one
Synonyms 9α-Fluorohydrocortisone; (11β)-9-fluoro-11,17,21-trihydroxypregn-4-ene-3, 20-dione.
Proprietary Name *Astonin-H*

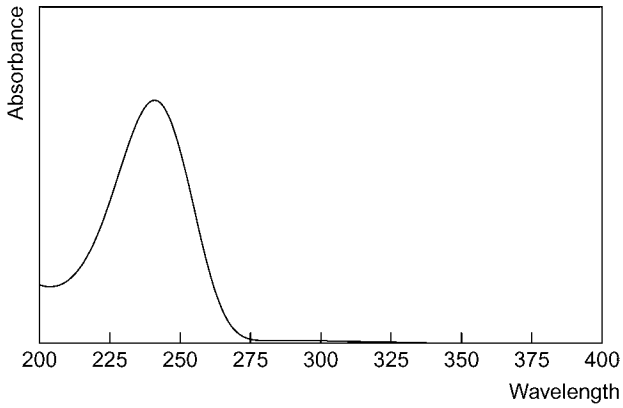


Chemical Properties Crystals which decompose between 260°. Very soluble in water. Log *P* (octanol/water), 1.7.

Fludrocortisone Acetate

$C_{23}H_{31}FO_6 = 422.5$
CAS—514-36-3
Synonym 9α-Fluorohydrocortisone 21-acetate
Proprietary Names *Florinef*. It is an ingredient of *Panotile*.
Chemical Properties A white to pale-yellow, hygroscopic, crystalline powder. Mp 233° to 234° (occasionally 205° to 208° with resolidification on further heating then Mp 226° to 228°). A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in 50 of chloroform and 1 in 250 of ether.

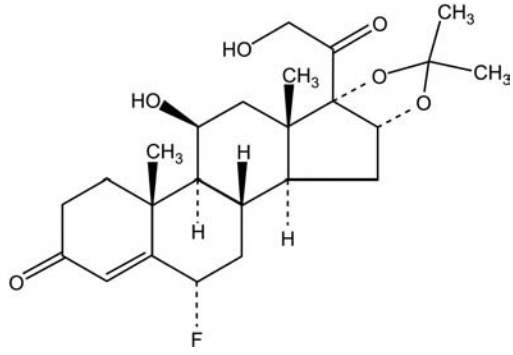
Colour Tests Antimony pentachloride—orange; naphthol–sulfuric acid—violet/brown; sulfuric acid—orange (green fluorescence under ultraviolet light).
Thin-layer Chromatography Fludrocortisone acetate: system TA—R_f 0.90; system TE—R_f 0.86; system TP—R_f 0.58; system TQ—R_f 0.12; system TR—R_f 0.30; system TS—R_f 0.00. Fludrocortisone: system TAJ—R_f 0.55; system TAK—R_f 0.35; system TAL—R_f 0.91; system TAM—R_f 0.90 (DPST solution).
Ultraviolet Spectrum Fludrocortisone acetate: dehydrated alcohol—240 nm (A^1_1=405a).



Infrared Spectrum Principal peaks at wavenumbers 1651, 1271, 1714, 1736, 1246, 1041 cm^{-1} (fludrocortisone acetate, KBr disk).
Dose Usually 100 to 300 μg of fludrocortisone acetate daily.

Fludroxycortide

Corticosteroid
$C_{24}H_{33}FO_6 = 436.5$
CAS—1524-88-5
Synonyms Fluorandrenolone; (6α,11β,16α)-6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione; 6α-fluoro-16α-hydroxyhydrocortisone 16,17-acetonide; flurandrenolide; flurandrenolone.
Proprietary Names *Cordran*; *Drenison*; *Haelan*; *Sermaka*.

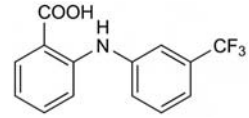


Chemical Properties A white, fluffy, crystalline powder. Mp 247° to 255°. Practically insoluble in water and ether; soluble 1 in 72 of ethanol, 1 in 10 of chloroform and 1 in 25 of methanol. Log *P* (octanol/water), 3.0.

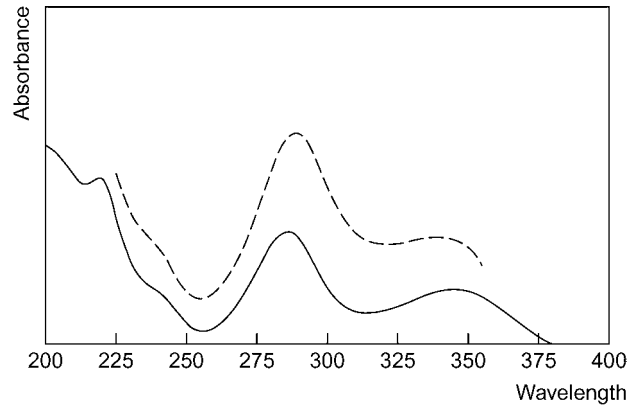
Colour Tests Naphthol–sulfuric acid—green, brown dichroism/yellow; sulfuric acid—yellow (green fluorescence under ultraviolet light).
Ultraviolet Spectrum Methanol–235 nm ($A^1_{1=}$ = 324a).
Infrared Spectrum Principal peaks at wavenumbers 1675, 1701, 1059, 1045, 1095, 1079 cm^{-1} (KBr disk).
Use Topically in concentrations of 0.0125 to 0.05%.

Flufenamic Acid

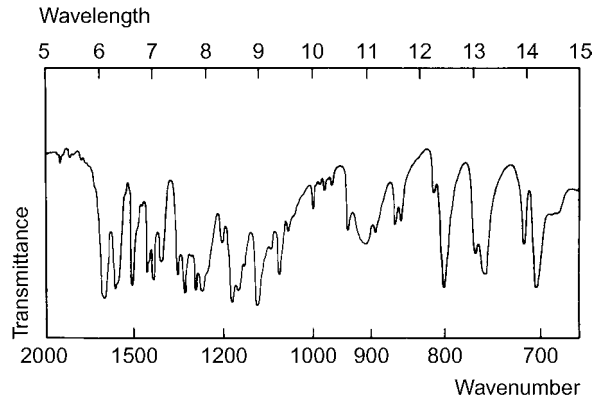
Analgesic
$C_{14}H_{10}F_3NO_2 = 281.2$
CAS—530-78-9
IUPAC Name 2-[[3-(Trifluoromethyl)phenyl]amino]benzoic acid
Proprietary Names *Achless*; *Ansatin*; *Arlef*; *Dignodolin*; *Fullsafe*; *Meralen*; *Paraflu*; *Parlef*; *Rheuma Lindofluid*; *Sastridex*; *Surika*; *Tecramine*.



Chemical Properties A pale-yellow crystalline powder. Mp 124° to 125°, with resolidification and 134° to 136°. Practically insoluble in water; soluble 1 in 4 of ethanol, 1 in 7 of chloroform and 1 in 3 of ether. pK_a 3.9. Log *P* (octanol/water), 5.2.
Thin-layer Chromatography System TA—R_f 0.96; system TE—R_f 0.18; system TG—R_f 0.37; system TAE—R_f 0.84; system TAJ—R_f 0.55; system TAK—R_f 0.78; system TAL—R_f 0.95 (chromic acid solution, blue).
Gas Chromatography System GA—RI 1950; system GD—methyl derivative RRT 1.26 (relative to n-$C_{16}H_{34}$); system GL—flufenamic acid-Me RI 1875, M (OH-)-Me_2 RI 2115.
High Performance Liquid Chromatography System HD—*k* 19.7; system HV—RRT 1.00 (relative to meclofenamic acid); system HX—RI 671; system HY—RI 667.
Ultraviolet Spectrum Methanol—288 (A^1_1=593a), 339 nm.



Infrared Spectrum Principal peaks at wavenumbers 1115, 1176, 1653, 1284, 1265, 1600 cm^{-1} (KBr disk). Five polymorphic forms occur.



Mass Spectrum Principal ions at *m/z* 263, 281, 166, 92, 145, 167, 235, 139; flufenamic acid-Me *m/z* 263, 295, 235, 166, 264, 92; M (OH-)-Me_2 *m/z* 325, 293, 278, 250, 223, 202.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 1 mg/L for flufenamic acid and mefenamic acid [Lin *et al.*1980].

Urine **HPLC** UV detection. Flufenamic acid, mefenamic acid and tolfenamic acid. Limit of detection, about 3.5 ng for flufenamic acid [Mikami *et al.* 2000].

Disposition in the Body Readily absorbed after oral administration. Metabolised by hydroxylation and glucuronic acid conjugation. About 50% of a dose is excreted in the urine in 72 h and about 36% is eliminated in the faeces. The material excreted in the urine consists mainly of conjugated flufenamic acid and free and conjugated 4′-hydroxyflufenamic acid with smaller amounts of the 5′-hydroxy and dihydroxy derivatives.

Therapeutic Concentration

Following oral administration of 200 mg three times daily for 4 days to 10 subjects, plasma concentrations of 0.3 to 17 mg/L (mean 6.4) were reported 2 h after the morning dose [Buchanan *et al.* 1969].

Half-life Plasma half-life, about 3 h.

Protein Binding Extensively bound.

Dose 400 to 600 mg daily.

Buchanan RA *et al.* (1969). The breast milk excretion on flufenamic acid. *Curr Ther Res* 11: 533–538.

Lin CK *et al.* (1980). Determination of two fenamates in plasma by high-performance liquid chromatography. *J Pharm Sci* 69: 95–97.

Mikami E *et al.* (2000). Simultaneous analysis of anthranilic acid derivatives in pharmaceuticals and human urine by high-performance liquid chromatography with isocratic elution. *J Chromatogr B Biomed Sci Appl* 744: 81–89.

Flugestone

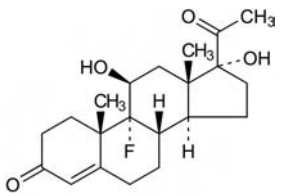
Progestational Steroid (Veterinary)

$C_{21}H_{29}FO_4 = 364.5$

CAS—337-03-1

IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-17-Acetyl-9-fluoro-11,17-dihydroxy-10,13-dimethyl-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[*a*]phenanthren-3-one

Synonyms 9α-Fluoro-11β,17α-dihydroxypregn-4-ene-3,20-dione; flurogestone.



Chemical Properties Log *P* (octanol/water), 2.0 (flugestone acetate).

Flugestone Acetate

$C_{23}H_{31}FO_5 = 406.5$

CAS—2529-45-5

Proprietary Names *Chronogest*; *Ovakron*.

Chemical Properties A white or creamy-white powder. Mp 266° to 269°. Very slightly soluble in water; soluble 1 in 23 of ethanol, 1 in 2.5 of chloroform and 1 in 100 of methanol.

Ultraviolet Spectrum Flugestone acetate: methanol—238 nm (A_1^1=425b).

Flumazenil

Benzodiazepine Antagonist

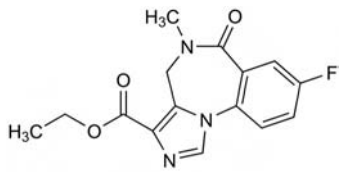
$C_{15}H_{14}FN_3O_3 = 303.3$

CAS—78755-81-4

IUPAC Name Ethyl 8-fluoro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1, 4]benzodiazepine-3-carboxylate

Synonyms Flumazepil; 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1, 5-*a*][1,4]-benzodiazepine-3-carboxylic acid ethyl ester; Ro-15-1788.

Proprietary Names *Anexate*; *Lanexat*; *Mazicon*; *Romazicon*.

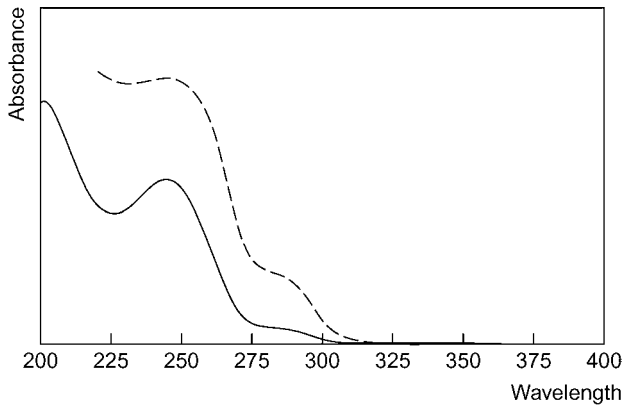


Chemical Properties A white to off-white crystalline compound. Mp 201° to 203°. Insoluble in water; slightly soluble in dilute acidic solutions. pK_a 1.8. Log *P* (octanol/water): 1.0. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005].

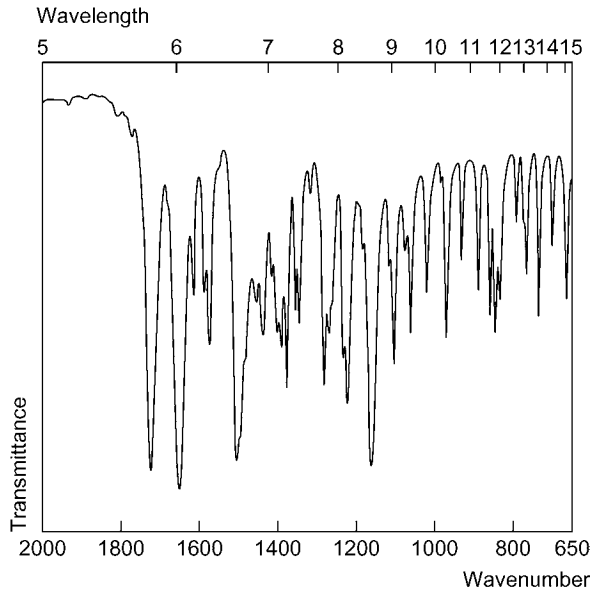
Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.03; system TC—R_f 0.63; system TD—R_f 0.30; system TE—R_f 0.61; system TF—R_f 0.14; system TL—R_f 0.44; system TAD—R_f 0.61; system TAE—R_f 0.76; system TAF—R_f 0.72.

High Performance Liquid Chromatography System HX—RI 387; system HY—RI 327; system HZ—retention time 2.6 min.

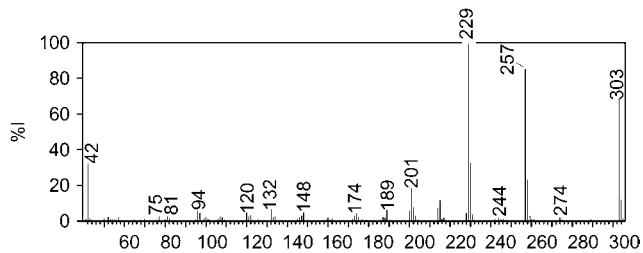
Ultraviolet Spectrum Aqueous base—244 nm.



Infrared Spectrum Principal peaks at wavenumbers 1724, 1652, 1161 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 229, 257, 303, 42, 230, 258, 201, 215.



Quantification

Plasma **GC** Column: RTX-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Temperature: 260°. Injector and detector temperature: 260° and 300°, respectively. Carrier gas: He, 103 kPa inlet pressure. Internal standard (IS): clinazolam. Detection: nitrogen–phosphorus. Retention time: flumazenil, 7 min; IS, 12 min. Limit of detection, 3 μg/L [Fisher *et al.* 1995]. NSD. Limit of detection, 0.05 μg/L [Zell, Timm, 1986].

GC-MS Limit of quantification, 1 μg/L [Song *et al.* 1995]. Limit of quantification, 0.5 μg/L [Fukuda *et al.* 1989].

HPLC Column: CP-microsphere C_{18} (100 × 4.6 mm i.d., 3 μm). Mobile phase: methanol : aqueous sodium dihydrogen phosphate monohydrate (0.3%, pH 3.9) (34.75:65.25), flow rate 1.5 mL/min. IS: Ro 15-3505. UV detection (λ=245 nm). Retention time: flumazenil, 4.86 min; IS, 6.17 min. Limit of quantification, 0.5 μg/L [Breimer *et al.* 1991]. UV detection (λ=220 nm). Limit of quantification, 2 μg/L, limit of detection, 0.3 μg/L [Vletter *et al.* 1990]. Column: C_{18} RP cartridge NovaPak (100 × 8 mm, 4 μm). Mobile phase: 32% acetonitrile : 0.04 mol/L sodium hydrogen phosphate buffer (with 1 mL/L TEA (triethylamine), pH 7.2) (32:68), flow rate 1.5 mL/min. UV detection (λ=220 nm). IS: flurazepam. Retention time: flumazenil, 3.8 min; IS, 18.5 min. Flumazenil and metabolites, limit of detection, 4 μg/L [Chan, Jones 1993].

Urine **HPLC** See Plasma [Chan, Jones 1993].

Disposition in the Body Flumazenil is rapidly and well absorbed after oral administration, but undergoes extensive first-pass hepatic metabolism. It is therefore given by IV administration. It is rapidly distributed into the brain. Flumazenil is extensively metabolised in the liver to an inactive carboxylic acid form and excreted predominantly in urine (<1% is excreted unchanged). Metabolites include *N*-desmethylflumazenil, *N*-desmethylflumazenil acid and flumazenil acid. Some (5 to 10%) is excreted via bile in faeces.

Therapeutic Concentration The trough serum therapeutic concentration range is 10 to 50 µg/L and peak, 200 to 300 µg/L.

Six healthy male volunteers, aged between 28 and 42 years, were administered intravenously a dose of 2.5 mg over 30 s. The effective plasma concentration was 60 µg/L during the infusion which stayed constant until 5 min after infusion had ended. It then rapidly declined to 3 µg/L over a 3 h period [Klotz *et al.* 1984].

Bioavailability Approximately 16%.

Half-life Plasma half-life, 0.7 to 1.3 h.

Volume of Distribution 0.9 to 1.1 L/kg.

Clearance Plasma clearance, 0.8 to 1.0 L/h/kg; 31 to 78 L/h. Blood, 43 L/h.

Distribution in Blood Blood : plasma ratio is 0.8 to 1.3.

Protein Binding 50% (of which about two-thirds is bound to albumin).

Note For a review of the pharmacokinetics of flumazenil, see Klotz and Kanto [1988].

Dose Usually, 300 to 600 µg as an IV injection; 100 to 400 µg/h may be given by IV infusion.

Breimer LT *et al.* (1991). Pharmacokinetics and EEG effects of flumazenil in volunteers. *Clin Pharmacokinet* 20: 491–496.

Chan K, Jones RD (1993). Simultaneous determination of flumazenil, midazolam and metabolites in human biological fluids by liquid chromatography. *J Chromatogr* 619: 154–160.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fisher LE *et al.* (1995). Simultaneous determination of midazolam and flumazenil concentrations in human plasma by gas chromatography. *J Chromatogr Biomed Appl* 665: 217–221.

Fukuda EK *et al.* (1989). Quantitation of the benzodiazepine antagonist flumazenil in human plasma by gas chromatography-mass spectrometry. *J Chromatogr* 491: 97–106.

Klotz U, Kanto J (1988). *Clin Pharmacokinet* Pharmacokinetics and clinical use of flumazenil (Ro 15-1788). 14(1): 1–12.

Klotz U *et al.* (1984). Pharmacokinetics of the selective benzodiazepine antagonist Ro 15-1788 in man. *Eur J Clin Pharmacol* 27: 115–117.

Song D *et al.* (1995). Determination of flumazenil in plasma by gas chromatography-negative ion chemical ionization mass spectrometry. *J Chromatogr Biomed Appl* 663: 263–273.

Vletter AA *et al.* (1990). High-performance liquid chromatographic assay to determine midazolam and flumazenil simultaneously in human plasma. *J Chromatogr* 530: 177–185.

Zell M, Timm U (1986). Highly sensitive assay of benzodiazepine antagonist in plasma by capillary gas chromatography with nitrogen-selective detection. *J Chromatogr* 382: 175–188.

Flumetasone

Corticosteroid

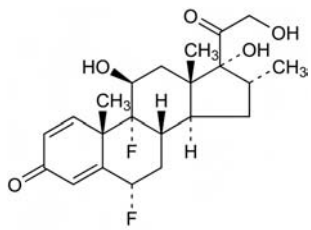
$C_{22}H_{28}F_2O_5 = 410.5$

CAS—2135-17-3

IUPAC Name (6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-6,9-Difluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-3-one

Synonyms 6α-Dexamethasone; (6α,11β,16α)-6,9-difluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione; flumethasone.

Proprietary Name *Flucort* (vet.)



Chemical Properties Log *P* (octanol/water), 1.9.

Flumetasone Pivalate

$C_{27}H_{36}F_2O_6 = 494.6$

CAS—2002-29-1

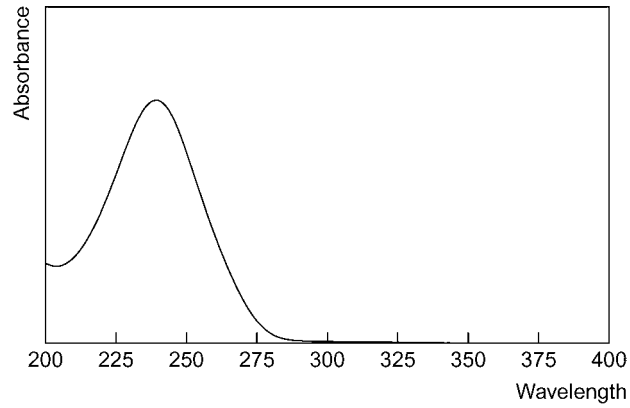
Synonym Flumetasone trimethylacetate

Proprietary Names *Cerson*; *Locacorten*; *Locorten(e)*.

Chemical Properties A white crystalline powder. Practically insoluble in water; soluble 1 in 89 of ethanol, 1 in 350 of chloroform and 1 in 2800 of ether.

Thin-layer Chromatography System TAM—R_f 0.64.

Ultraviolet Spectrum Methanol—238 nm (A^1_1=438a).



Infrared Spectrum Principal peaks at wavenumbers 1664, 1623, 901, 1733, 1139, 1160 cm^{-1} (flumetasone pivalate, KBr disk).

Use Flumetasone pivalate is used topically in a concentration of 0.02%.

Flunitrazepam

Benzodiazepine, Hypnotic

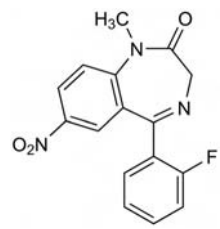
$C_{16}H_{12}FN_3O_3 = 313.3$

CAS—1622-62-4

IUPAC Name 5-(2-Fluorophenyl)-1-methyl-7-nitro-3*H*-1,4-benzodiazepin-2-one

Synonyms Flunitrazepamum; Ro-5-4200.

Proprietary Names *Absint*; *Darkene*; *Flunimerck*; *Fluninoc*; *Flunipam*; *Flupam*; *Fluscand*; *Fluserin*; *Flutraz*; *Hypnodorm*; *Hypnor*; *Insom*; *Narcozep*; *Rohipnol*; *Rohypnol*; *Roipnol*; *Ronal*; *Somnubene*; *Valsera*.



Chemical Properties An almost white to pale yellow crystalline solid. Mp ~170°. Sparingly soluble in water; soluble 1 in 172 ethanol, 1 in 3 of chloroform, 1 in 300 of ether and 1 in 100 of methanol. pK_a 1.8. Log *P* (octanol/water), 2.1. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

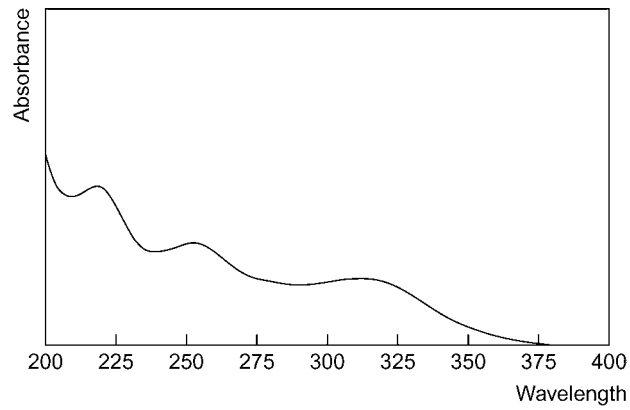
Colour Test Formaldehyde-sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.10; system TC—R_f 0.72; system TD—R_f 0.54; system TE—R_f 0.74; system TF—R_f 0.47; system TL—R_f 0.63; system TAD—R_f 0.72; system TAE—R_f 0.80; system TAF—R_f 0.82.

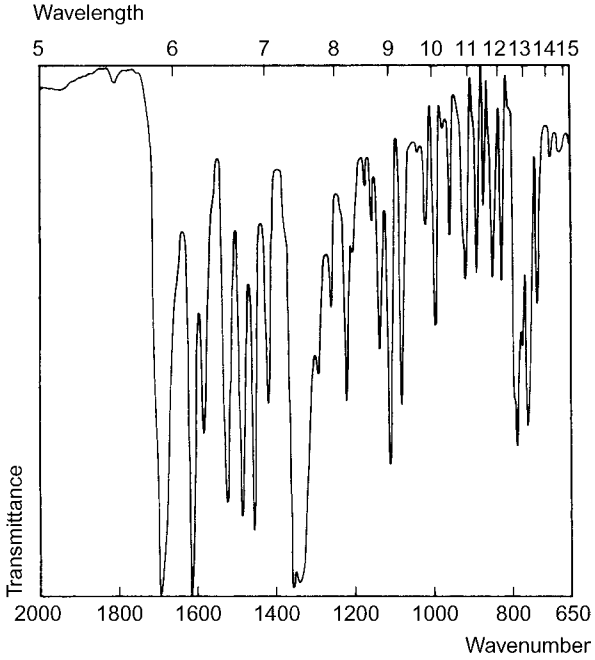
Gas Chromatography System GA—flunitrazepam RI 2600, M (nor-) RI 2720, M (7-amino-) RI 2723, M (nor-amino-) RI 2825; system GB—flunitrazepam RI 2744, M (nor-) RI 2816, M (nor-)-TMS RI 2622, M (7-amino-) RI 2804, M (7-amino-)-TMS RI 2836; system GG—RI 3190.

High Performance Liquid Chromatography System HI—*k* 3.15; system HK—*k* 0.47; system HX—RI 483; system HY—RI 305; system HZ—RT 5.6 min; system HAA—RT 18.6 min; system HAL—flunitrazepam RT 6.2 min, M (nor) RT 3.7 min; system HAZ—flunitrazepam *k* 0.86, M (nor-) *k* 0.65, M (7-amino) *k* 0.22; system HBH—*k* 3.34; system HBI—*k* 0.86.

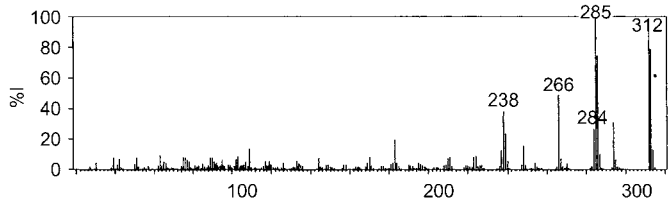
Ultraviolet Spectrum Methanol—252 (A^1_1= 516a), 308 nm (A^1_1 = 332a).



Infrared Spectrum Principal peaks at wavenumbers 1697, 1620, 1490, 1528, 1107, 783 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 285, 312, 313, 286, 266, 238, 294, 284 (flunitrazepam); 269, 240, 241, 268, 270, 107, 121, 213 (7-amino-1-desmethylflunitrazepam); 283, 44, 255, 282, 254, 284, 264, 256 (7-aminoflunitrazepam); 298, 271, 299, 224, 272, 270, 252, 280 (desmethylflunitrazepam).



Quantification

Blood **GC** Columns: DB-1 and DB-1701 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 28° at 30°/min to 230° at 2°/min to 300° at 30°/min for 1 min or 120° for 1 min to 230° at 40°/min to 280° at 8°/min for 9 min. ECD. Limit of detection, 0.015 µmol/L (DB-1701) [Gjerde *et al.* 1992]. Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2 to 3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 6.28 min. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: HP-1 (12 m × 0.22 mm i.d., 0.33 µm). Carrier gas: He, 0.5 mL/min. EI, SIM acquisition mode. Limit of detection, 0.1 µg/L [Elian 1999]. Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 36.2 cm/s. SIM acquisition mode. Limit of detection, 5 µg/L [Elsohly *et al.* 1999]. Column: HP-5MS (12 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 150° for 5 min to 300° at 30°/min. SIM acquisition mode. Limit of quantification, 1.0 µg/L [Hackett, Elian 2006]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation. Limit of quantification, 1.58 µg/L, limit of detection, 0.52 µg/L [Papoutsis *et al.* 2010].

HPLC UV detection (λ= 240 nm). Limit of detection, 1 to 8 µg/L [Robertson, Drummer 1995]. Column: LiChrospher RP-Select B C_8 (8 m × 4 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 2.1) : acetonitrile (65 : 35), flow rate 0.3 mL/min. UV detection (λ= 220 nm). Limit of quantification, 103.5 µg/L, limit of detection, 3.5 µg/L [El Mahjoub, Staub 2000]. Column: LiChrospher RP-Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 2.1) : acetonitrile (94 : 6). DAD detection (λ= 254 nm). Limit of detection not reported [El Mahjoub, Staub 2001a]. Column: Retek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5), flow rate 0.45 mL/min. DAD. Limit of quantification, 3 µg/L [Dussy *et al.* 2006]. Column: RP-Synergi C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : trifluoroacetic acid, flow rate 1 mL/min. DAD (λ= 250 nm). Limit of quantification, 5 µg/L [Hackett, Elian 2006].

LC-MS Column: ODS. Mobile phase: acetonitrile : 50 mmol/L ammonium formate buffer (45 : 55, pH 3.0). APCI, SIM acquisition mode. Limit of detection, 0.2 µg/L [Bogusz *et al.* 1998]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: formic acid : methanol (30 : 60), flow rate 0.2 mL/min. MS-MS detection. Limit of quantification, 0.7 µg/L, limit of detection, 0.2 µg/L [Smink *et al.* 2004]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : formate buffer (10 : 90), flow rate 0.2 mL/min. ESI mode, MRM acquisition mode. Limit of quantification, 1.0 µg/L [Laloup *et al.* 2005]. Column: XBridge Shield (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetate buffer (pH 5.0) : 20 mmol/L acetonitrile (67 : 33), flow rate 200 µL/min. APPI mode, SIM acquisition mode. Limit of quantification, 3 µg/L [Marchi *et al.* 2009].

Plasma **GC** ECD. Limit of detection, 0.5 µg/L [Sumirtapura *et al.* 1982]. See Blood [Lillsunde, Seppälä 1990]. Limit of detection, 0.01–0.48 mmol/L [Reubsaet *et al.* 1998].

GC-MS See Blood [Elsohly *et al.* 1999]. Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 43 cm/min. Temperature programme: 180° for 0.5 min to 260° at 20°/min for 1 min to 280° at 30°/min for 8 min. SIM acquisition mode. Limit of quantification, 1.0 µg/L, limit of detection, 10 µg/L [Snyder *et al.* 2001].

HPLC UV detection. Limit of detection, 1 µg/L [Vree *et al.* 1977]. Column: Nova-Pak C_{18}. Mobile phase: acetonitrile : 10 mmol/L ammonium acetate buffer (pH 6.7, 45 : 55). Limit of detection, 10 µg/L [Guichard *et al.* 1993]. Column: LiCrospher 60 RP-Select B (250 × 4 mm i.d.). Mobile phase: acetonitrile : 0.02 mmol/L phosphate buffer (pH 2.0, 36 : 64), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Limit of detection, 1 µg/L [Deinl *et al.* 1998]. Column: Kromasil C_8 (250 × 5 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.05 mmol/L ammonium acetate (14 : 32 : 54 for 0.01 min to 13 : 32 : 55 at 9 min to 11 : 28 : 61 at 11 min to 40 : 28 : 32 at 12 min), flow rate 1.1 mL/min. DAD detection (λ= 240 nm). Limit of quantification, 1.52 µg/L, limit of detection, 0.46 µg/L [Uddin *et al.* 2008]. Column: LC-18 DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate (pH 6.0) : methanol : diethyl ether (55 : 40 : 5), flow rate 0.8 mL/min. UV detection (λ= 245 nm). Limit of quantification, 30 µg/L [Borges *et al.* 2009].

LC-MS Column: Merck LiChroCART (125 × 2 mm i.d.) Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min). ACPI mode, SIM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 5 µg/L [Kratzsch *et al.* 2004]. Column: XTerra RP 18 (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid, flow rate 0.25 mL/min. ESI. Limit of detection, 0.5 µg/L [Quintela *et al.* 2005].

Serum **GC-MS** See Plasma [Snyder *et al.* 2001]. Column DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 290° at 20°/min. MS-MS detection. Limit of detection, 60 µg/L [Terada *et al.* 2003].

HPLC See Plasma [Deinl *et al.* 1998]. Column: LiChrospher 60 RP-Select B (25 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mmol/L phosphate buffer (pH 2.0, 36 : 64), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Limit of detection, 1 µg/L [Deinl *et al.* 1999]. See Blood [Dussy *et al.* 2006; El Mahjoub, Staub 2000].

LC-MS See Blood [Bogusz *et al.* 1998]. Column: Unison UK-C_{18} reversed phase (150 × 2 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate : 0.1% formic acid (70 : 30 over 20 min to 20 : 80 over 5 min), flow rate 0.25 mL/min. MRM acquisition mode. Limit of quantification, 1.0 µg/L, limit of detection, 3.2 µg/L [Nakamura *et al.* 2009]. ESI mode. Limit of quantification, 2 µg/L [Kleinschnitz *et al.* 1996].

Urine **GC** See Plasma [Reubsaet *et al.* 1998].

GC-MS Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 43 cm/s. Temperature programme: 180° to 260° at 20°/min to 280° at 30°/min for 3 min. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Elsohly *et al.* 1997]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 1 min to 200° at 20°/min to 300° at 15°/min. Limit of detection, 5 µg/L [Augsburger *et al.* 1998]. See Plasma [Snyder *et al.* 2001]. See Blood [Hackett, Elian 2006].

HPLC See Serum [Kleinschnitz *et al.* 1996]. UV detection (λ= 254 nm). Column: LiChrospher 60 RP-Select B (25 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol–water (90 : 10) : acetonitrile–0.05 mol/L phosphate buffer (pH 2.0, 36 : 64), flow rate 0.5, 0.05 and 1.0 mL/min. Limit of detection, <2 µg/L [Deinl *et al.* 1997]. See Plasma [Deinl *et al.* 1998]. See Blood [Hackett, Elian 2006]. See Plasma. Limit of quantification, 0.38 µg/L, limit of detection, 0.11 µg/L [Uddin *et al.* 2008].

LC-MS See Blood [Bogusz *et al.* 1998]. See Blood. Limit of quantification, 10 µg/L [Laloup *et al.* 2005]. Column: ACQUITY UPLC C_{18} (50 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.05% formic acid in 10 mmol/L ammonium formate : 0.05% formic acid in acetonitrile (95 : 5 to 35 : 65 over 2 min to 5 : 95 for 5 min to 95 : 5). MRM acquisition mode. Limit of detection, 0.5 µg/L [Forsman *et al.* 2009].

Oral Fluid **GC-MS** Column: HP-1 (30 m × 250 µm i.d., 0.25 µm). Carrier gas: He, 2.1 mL/min. Temperature programme: 60° to 310° at 30°/min. SIM mode. Limit of quantification, 0.1 µg/L, limit of detection, 0.05 µg/L [Samyn *et al.* 2002]. Column: Ultra 1 (16.5 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 0.8 mL/min. SIM mode. Limit of quantification, 10.9 µg/L, limit of detection, 3.6 µg/L [Pujadas *et al.* 2007].

HPLC See Plasma. Limit of quantification, 0.39 µg/L, limit of detection, 0.12 µg/L [Uddin *et al.* 2008].

LC-MS Column: XTerra RP C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid, flow rate 0.25 mL/min. ESI mode. Limit of detection, 0.5 µg/L [Quintela *et al.* 2005]. Column: Zorbax Eclipse XDB C_{18} (50 × 4.6 mm i.d., 1.8 µm). Mobile phase: 20 mmol/L ammonium formate : acetonitrile (50 : 50), flow rate 0.2 mL/min. MRM mode. MS-MS detection. Limit of quantification, 0.5 µg/L [Moore *et al.* 2007]. Column: Zorbax Bonus RP C_{14} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : 0.1% formic acid, flow rate 200 µL/min. MRM acquisition

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mode. MS-MS detection. Limit of quantification, 0.1 μg/L, limit of detection, 0.02 μg/L [Ngwa *et al.* 2007].

Hair **GC-MS** Column: HP5-MS 5% phenylsiloxane, 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. NCI mode, SIM acquisition mode. Retention time: 10.5 min. Limit of detection, 15 pg/mg [Cirimele *et al.* 1997]. Column: HP-5 MS (30 m × 250 μm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° to 310° at 30°/min. NCI mode. Limit of quantification, 2.5 ng/g, limit of detection, 1.5 ng/g [Negrusz *et al.* 1999].

HPLC Column: LiChrospher RP-Select B C_8 (125 × 3 mm i.d., 5 μm). Mobile phase: 30 mmol/L phosphate buffer : acetonitrile (pH 2.1, 95 : 5). DAD (λ = 254 nm). Limit of detection, 0.2 μg/g [El Mahjoub and Staub 2001b].

LC-MS Column: Zorbax Phenyl (50 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile-methanol-20 mmol/L formate buffer (pH 3.0, 10 : 10 : 80) : acetonitrile-methanol-20 mmol/L formate buffer (35 : 35 : 30, 100 : 0 to 35 : 65 at 7 min to 100 : 0 for 2 min), flow rate 0.25 mL/min. MRM acquisition mode. Retention time: 5.85 min. Limit of quantification, 0.025 ng/mg [Kronstrand *et al.* 2002]. See Blood [Laloup *et al.* 2005].

Disposition in the Body Flunitrazepam is readily absorbed after oral administration. It is extensively metabolised in the liver to desmethylflunitrazepam, which has some activity, and 7-aminoflunitrazepam, which is inactive; other metabolites include 3-hydroxyflunitrazepam, 3-hydroxy-7-acetamidoflunitrazepam, and 7-amino-1-desmethylflunitrazepam. Flunitrazepam is excreted in the urine almost entirely as metabolites, both free and conjugated, with <1% as unchanged drug. Approximately 10% of a dose is eliminated in the faeces. Flunitrazepam crosses the placental barrier and it is excreted in breast milk.

Therapeutic Concentration

After single oral doses of 1 mg and 2 mg flunitrazepam given to 11 and 5 subjects, respectively, mean peak plasma concentrations of 0.0025 and 0.015 mg/L, respectively, were attained in ~1 h [Clarke *et al.* 1980].

Following oral doses of 2 mg daily for 28 days given to 7 subjects, a mean peak plasma concentration of ~0.02 mg/L was reported 3 h after the last dose [Wickstrøm *et al.* 1980].

Infusion of flunitrazepam over 20 min (0.2 mg/kg) to 25 neonates and 6 infants produced peak plasma concentrations of 0.077–1.028 mg/L (mean, 243) [Pariente-Khayat *et al.* 1999].

Toxicity

Of 8 deaths involving flunitrazepam, four had 7-aminoflunitrazepam levels of 0.45 mg/L (where the fatality involved flunitrazepam alone) and 4 had 7-aminoflunitrazepam levels of 0.16 mg/L (where the fatality involved flunitrazepam and ethanol); in the latter group, the mean ethanol concentration was 1.6 g/L. Levels of flunitrazepam were low, suggesting that 7-aminoflunitrazepam was produced postmortem [Drummer *et al.* 1993].

Half-life Plasma half-life, 3 h (distribution); 16–35 h (elimination).

Bioavailability 80–90%.

Volume of Distribution Approximately 3.5–5.5 L/kg.

Clearance Plasma clearance, ~2 mL/min/kg.

Protein Binding Approximately 77–80%.

Dose 0.5 to 2 mg, as a hypnotic.

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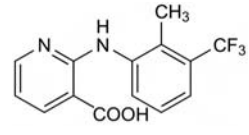
Flunixin

Analgesic

$C_{14}H_{11}F_3N_2O_2$ = 296.2

CAS—38677-85-9

IUPAC Name 2-[[2-Methyl-3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid



Chemical Properties Crystals. Mp 226° to 228°.

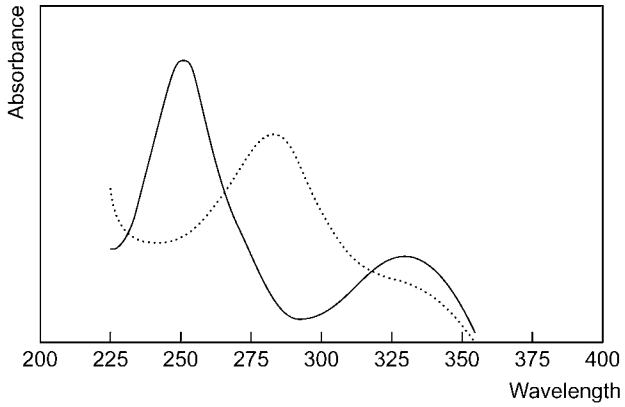
Flunixin Meglumine

$C_{14}H_{11}F_3N_2O_2.C_7H_{17}NO_5$ = 491.5

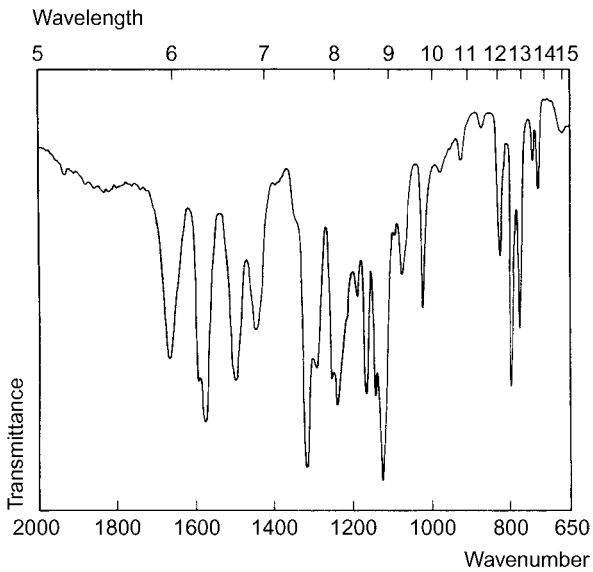
CAS—42461-84-7

Proprietary Names *Banamine*; *Binixin*; *Cronyxin*; *Equileve*; *Equi-Phar Equigesic*; *Finadyne*; *Flumeglumine*; *Flu-Nix*; *Flunixamine*; *Meflosyl*; *Resprixin*; *Suppressor* (all veterinary).
Chemical Properties Colourless crystals. Mp 135° to 139°. Soluble in water.

Thin-layer Chromatography System TA—R_f 0.96; system TE—R_f 0.12; system TG—R_f 0.33; system TAJ—R_f 0.37; system TAK—R_f 0.20; system TAL—R_f 0.83 (Ludy Tenger reagent, orange).
Gas Chromatography System GD—methyl derivative RRT 1.39 (relative to n-$C_{16}H_{34}$).
High Performance Liquid Chromatography System HV—RRT 0.99 (relative to meclofenamic acid); system HY—RI 414.
Ultraviolet Spectrum Aqueous acid—252, 327 nm; aqueous alkali—281 nm (A^1_1=490b).



Infrared Spectrum Principal peaks at wavenumbers 1122, 1315, 1572, 1237, 1142, 1165 cm^{-1}.



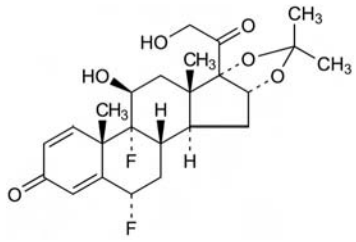
Quantification
Other **GC** Equine urine. NPD. Limit of detection, 74 µg/L [Johansson, Anler 1988].
HPLC Bovine plasma. Limit of detection, <7 µg/L [Odensvik, Johansson 1995]. Equine plasma or inflammatory exudates. UV detection. Limit of detection, 50 µg/L [Higgins *et al.* 1987]. Equine plasma. UV detection. Limit of detection, 50 µg/L [Hardee *et al.* 1982].

Hardee GE *et al.* (1982). *J Liq Chromatogr* 5: 1991–2003.
Higgins AJ *et al.* (1987). Measurement of flunixin in equine inflammatory exudate and plasma by high performance liquid chromatography. *Equine Vet J* 19: 303–306.
Johansson M, Anler EL (1988). Gas chromatographic analysis of flunixin in equine urine after extractive methylation. *J Chromatogr* 427: 55–66.
Odensvik K, Johansson IM (1995). High-performance liquid chromatography method for determination of flunixin in bovine plasma and pharmacokinetics after single and repeated doses of the drug. *Am J Vet Res* 56: 489–495.

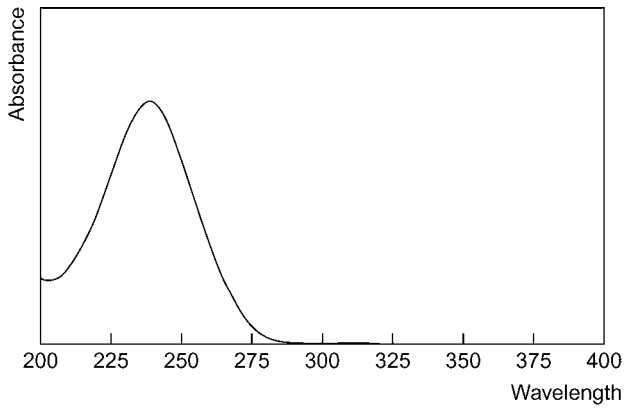
Fluocinolone Acetonide

Corticosteroid
$C_{24}H_{30}F_2O_6$ = 452.5
CAS—67-73-2

Synonyms (6α,11β,16α)-6,9-Difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-pregna-1,4-diene-3,20-dione; 6α,9α-difluoro-16α-hydroxyprednisolone acetonide.
Proprietary Names *Alfa-Fluorone*; *Boniderma*; *Capex*; *Cortamide*; *Cortoderm*; *Dermalar*; *DermaSmoothe/FS*; *Dermobeta*; *Dermoline*; *Flucinar*; *Flunolone-V*; *Fluocortan*; *Fluoderm*; *Fluonid*; *Flurosyn*; *Gelidina*; *Jellin*; *Jellisoft*; *Synalar*; *Synamol*; *Synandone*; *Synemol*.



Chemical Properties A white crystalline powder. Mp about 270°, with decomposition. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 26 of dehydrated alcohol, 1 in 10 of acetone, 1 in 15 to 1 in 25 of chloroform and 1 in 350 of ether; soluble in methanol. Log *P* (octanol/water), 2.5.
Colour Tests Naphthol–sulfuric acid—green/yellow; sulfuric acid—yellow (green fluorescence under ultraviolet light).
Thin-layer Chromatography System TP—R_f 0.42; system TQ—R_f 0.08; system TR—R_f 0.10; system TS—R_f 0.01; system TAM—R_f 0.68. (DPST solution.)
High Performance Liquid Chromatography System HY—RI 491 (fluocinolone).
Ultraviolet Spectrum Ethanol—240 nm (A^1_1=360a).

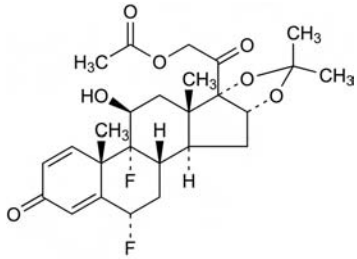


Infrared Spectrum Principal peaks at wavenumbers 1669, 1074, 1629, 910, 1056, 1615 cm^{-1} (KBr disk).
Use Topically in concentrations of 0.01 to 0.2%.

Fluocinonide

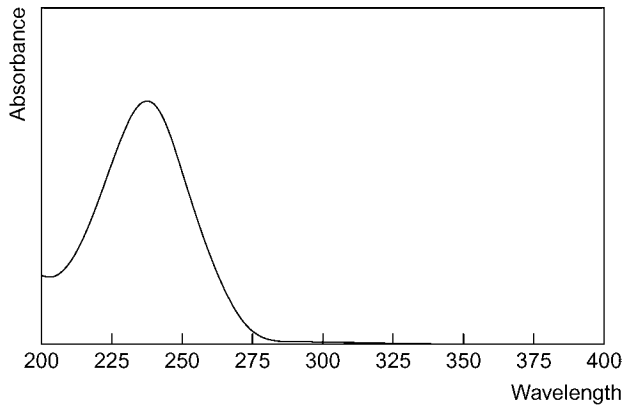
Corticosteroid
$C_{26}H_{32}F_2O_7$ = 494.5
CAS—356-12-7

Synonyms (6α,11β,16α)-21-(Acetyloxy)-6,9-difluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione; fluocinolone acetonide 21-acetate.
Proprietary Names *Cusigel*; *Flu-21*; *Fluonex*; *Gelisyn*; *Klariderm*; *Korticoid*; *Lidemol*; *Lidex*; *Lyderm*; *Lydonide*; *Metosyn*; *Novoter*; *Tiamol*; *Topsym*; *Topsymin*; *Topsyn(e)*; *Vasoderm*.



Chemical Properties A white to cream-coloured crystalline powder. Mp about 300°, with decomposition. Practically insoluble in water; soluble 1 in 70 of ethanol, 1 in 10 of acetone and 1 in 10 of chloroform; very slightly soluble in ether. Log *P* (octanol/water), 3.2.

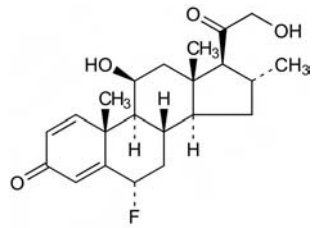
Ultraviolet Spectrum Methanol—240 nm (A^1_1=360a).



Infrared Spectrum Principal peaks at wavenumbers 1670, 1240, 1730, 1750, 1070, 1635 cm^{-1} (KBr disk).
Use Topically in a concentration of 0.05%.

Fluocortolone

Corticosteroid
$C_{22}H_{29}FO_4$ = 376.5
CAS—152-97-6
IUPAC Name (6*S*,8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,16*R*,17*S*)-6-Fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthren-3-one
Synonyms (6α,11β,16α)-6-Fluoro-11,21-dihydroxy-16-methylpregna-1,4-diene-3,20-dione; 6α-fluoro-16α-methyldehydrocorticosterone.
Proprietary Names *Ultralan* (tablets). It is an ingredient of *Ultralanum*.



Chemical Properties A white crystalline powder. Mp 188° to 190.5°. Practically insoluble in water; soluble 1 in 120 of ethanol; soluble in chloroform and ether. Log *P* (octanol/water), 2.1.

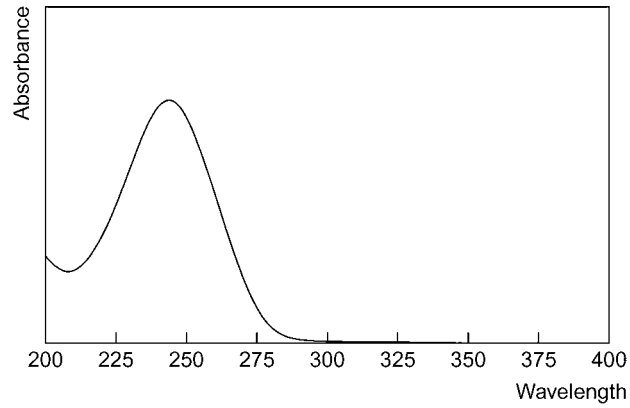
Fluocortolone Hexanoate

$C_{28}H_{39}FO_5$ = 474.6
CAS—303-40-2
Synonym Fluocortolone caproate
Proprietary Names It is an ingredient of *Ultradil Plain*, *Ultralan* (cream and lotion) and *Ultralanum*.
Chemical Properties A white to creamy-white crystalline powder. Mp 242° to 245°. Practically insoluble in water and ether; very slightly soluble in ethanol and methanol; soluble 1 in 18 of chloroform.

Fluocortolone Pivalate

$C_{27}H_{37}FO_5$ = 460.6
CAS—29205-06-9
Synonym Fluocortolone trimethylacetate
Proprietary Names It is an ingredient of *Ultradil Plain*, *Ultralan* (cream and lotion) and *Ultralanum Plain* (cream).
Chemical Properties A white to creamy-white crystalline powder. Mp 187°. Almost insoluble in water; soluble 1 in 36 of ethanol and 1 in 3 of chloroform; freely soluble in dioxan; slightly soluble in ether.

Colour Tests Antimony pentachloride—green-yellow; naphthol–sulfuric acid—red-brown/red-brown; sulfuric acid—orange-yellow.
Thin-layer Chromatography Fluocortolone: system TE—R_f 0.50; system TF—R_f 0.28. Fluocortolone hexanoate: system TP—R_f 0.79; system TQ—R_f 0.39; system TR—R_f 0.88; system TS—R_f 0.00. (*p*-Toluenesulfonic acid solution, positive.) Fluocortolone pivalate: system TP—R_f 0.78; system TQ—R_f 0.35; system TR—R_f 0.89; system TS—R_f 0.58.
Gas Chromatography System GA—fluocortolone RI 3225, fluocortolone-AC_2 RI 3400, fluocortolone-AC RI 3420.
Ultraviolet Spectrum Methanol—242 nm (A^1_1=429a).



Infrared Spectrum Principal peaks at wavenumbers 1658, 1163, 1622, 1722, 1176, 1747 cm^{-1} (fluocortolone hexanoate, KBr disk); 1662, 1159, 1725, 1619, 1605, 1285 cm^{-1} (fluocortolone pivalate, KBr disk).
Disposition in the Body
Therapeutic Concentration
Administration of 20, 50 and 100 mg oral doses of fluocortolone to 9 subjects at weekly intervals resulted in mean maximum plasma concentrations of 199, 419, and 812 mg/L, respectively, 1 to 2 h after dosing [Tauber *et al.* 1986].
Dose Fluocortolone has been given orally in doses of 5 to 60 mg daily.

Tauber U *et al.* (1986). Fluocortolone: pharmacokinetics and effect on plasma cortisol level. *Eur J Clin Pharmacol* 30: 433–438.

Fluorides

Anion

Fluoroacetic Acid

$C_2H_3FO_2$ = 78.0
CAS—144-49-0
Synonyms Fluoroethanoic acid; gifblaar poison.
Chemical Properties Crystals. Mp 33°. Bp 165°.

Hydrofluoric Acid

HF = 20.01
CAS—7664-39-3
Synonym Fluorohydric acid
Chemical Properties Solution of hydrogen fluoride gas in water. Colourless or almost colourless, fuming liquid. Miscible with water. Weak acid, pK_a 3.19 [O'Neil *et al.* 2006]. Attacks glass or stoneware, dissolving the silica. Keep in plastic, lead, wax, or paraffin paper bottles. Poisonous, handle with care.

Sodium Fluoride

FNa = 41.9
CAS—7681-49-4
Proprietary Names *Chemifluor*; *Duraphat*; *Florocid*; *Fluoros*; *Flura-Drops*; *Karidium*; *Lemoflur*; *Luride-SF*; *Ossalin*; *Ossin*; *Osteo-F*; *Osteofluor*; *Slow-Fluoride*; *Villiaumite*; *Zymafluor*.
Chemical Properties Cubic or tetragonal crystals. Mp 993°. Bp 1704°. Soluble in water; insoluble in alcohol. Used in the treatment of osteoporosis, as an insecticide, as a constituent of vitreous enamel and glass mixes, in electroplating, in the fluoridation of drinking water, as disinfectant for fermentation apparatus in breweries and distilleries, as an anticoagulant, as preservative for biological specimens.

Cryolite

AlF_6Na_3 = 209.9
CAS—15096-52-3
Synonyms Ice spar; kryolith; sodium aluminium fluoride.
Chemical Properties Snow-white, semi-opaque masses, vitreous fracture. Mp 1000°. Soluble in concentrated sulfuric acid. Used as a pesticide.

Stannous Fluoride

F_2Sn = 156.7
CAS—7783-47-3
Proprietary Names *Fluoristan*; *Tin Difluoride*.
Chemical Properties Monoclinic, lamellar plates. Mp 213°. Soluble in water. Used in dentistry.

Sodium Fluorosilicate

Na_2SiF_6 = 188.1
CAS—16893-85-9
Synonyms Disodium hexafluorosilicate; disodium silicofluoride; silicon sodium fluoride; sodium fluosililcate.
Proprietary Names *Prodan*; *Salufer*.
Chemical Properties White granular powder. Decomposes at red heat. Slightly soluble in water; insoluble in alcohol. Used in enamels, as insecticide, rodenticide,

fluoridating agent for drinking water, intermediate in production of synthetic cryolite.

Quantification

Note For the detection of fluoride ions in plasma, serum or urine using an ion-selective electrode, see Itai and Tsunoda [2001]; Fuchs *et al.* [1975]; Neefus *et al.* [1970]. For a comparison of methods for measuring fluorine in biological and environmental samples, see Venkateswarlu [1990].

Disposition in the Body Soluble fluoride compounds are rapidly and completely absorbed following oral exposure. Fluoride is believed to replace the hydroxyl ion and possibly the bicarbonate ion associated with hydroxyapatite – a mineral phase during formation of bone. Once absorbed, a portion of the fluoride is deposited in the skeleton, and most of the remainder is excreted in the urine, with smaller amounts in faeces, sweat and saliva, within 24 h. A fraction of the circulating inorganic fluoride acts as an enzyme inhibitor because it forms metal–fluoride–phosphate complexes that interfere with the activity of those enzymes requiring a metal ion cofactor. It is a general inhibitor of the energy production system of the cell.

Toxicity

A 65-year-old skilled worker sustained third-degree burns to his face after being splashed with hydrofluoric acid. His serum, urine, pericardium fluid and thoracic cavity fluid fluoride concentrations were 6.38, 6.60, 6.17 and 5.37 mg/L [Takase *et al.* 2004].

A 56-year-old man mistook the contents of a peanut butter jar, which contained a glass-etching compound including 20% ammonium bifluoride and 13% sodium bifluoride. The postmortem blood fluoride concentration was 19 mg/L (normal range, 0.2 to 0.6 mg/L) [Randall, Fraser 1994].

A 14-month-old boy spilled a bottle of rust remover and sustained 11% total body surface burns to both anterior thighs and knees and the right scapular region. The rust remover contained 4.4 mol/L hydrofluoric acid, equivalent to an 8% solution. On admission, his serum fluoride concentration was 21.3 mg/L (normal range, 0 to 0.5 mg/L) [Bordelon *et al.* 1993].

A 13-month-old boy ingested soldering flux containing potassium fluoride. He died ~12 h later. Postmortem fluoride concentrations in blood, liver, stomach and rib were 2, 3, 30 and 300 mg/L, respectively. There were multiple small (<1 cm) cardiac rhabdomyomas throughout both ventricular walls [Byard 1997; Byard *et al.* 1991].

A 33-year-old woman was found dead having ingested a cockroach powder based on sodium fluoride. The following concentrations were found at postmortem:

Tissue	*Concentration (mg/L or mg/kg)*
Bile	3.4
Stomach contents	2225
Kidney	16
Liver	8.6
Urine	295

[Poklis, Mackell 1989].

Note For a review of fluoride toxicity from ingestion of dental products, see Shulman and Wells [1997].

Half-life 49–72 days.

Bioavailability Nearly 100% when ingested orally in the fasting state [Ekstrand *et al.* 1990; Poklis, Mackell 1989].

Bordelon BM *et al.* (1993). Systemic fluoride toxicity in a child with hydrofluoric acid burns: case report. *J Trauma* 34: 437–439.

Byard RW (1997). Significant coincidental findings at autopsy in accidental childhood death. *Med Sci Law* 37: 259–262.

Byard RW *et al.* (1991). Incidental cardiac rhabdomyomas: a significant finding necessitating additional investigation at the time of autopsy. *J Forensic Sci* 36: 1229–1233.

Ekstrand J *et al.* (1990). Pharmacokinetics of fluoride in man and its clinical relevance. *J Dent Res* 69 (SpecNo): 550–555.

Fuchs C *et al.* (1975). Fluoride determination in plasma by ion selective electrodes: a simplified method for the clinical laboratory. *Clin Chim Acta* 60: 157–167.

Itai K, Tsunoda H (2001). Highly sensitive and rapid method for determination of fluoride ion concentrations in serum and urine using flow injection analysis with a fluoride ion-selective electrode. *Clin Chim Acta* 308: 163–171.

Neefus JD *et al.* (1970). The determination of fluoride in urine using a fluoride-specific ion electrode. *Am Ind Hyg Assoc J* 31: 96–99.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Poklis A, Mackell MA (1989). Disposition of fluoride in a fatal case of unsuspected sodium fluoride poisoning. *Forensic Sci Int* 41: 55–59.

Randall BB, Fraser BJ (1994). Peanut butter and fatal fluoride poisoning. A case of mistaken identity. *Am J Forensic Med Pathol* 15: 40–43.

Shulman JD, Wells LM (1997). Acute fluoride toxicity from ingesting home-use dental products in children, birth to 6 years of age. *J Public Health Dent* 57: 150–158.

Takase I *et al.* (2004). Fatality due to acute fluoride poisoning in the workplace. *Leg Med (Tokyo)* 6: 197–200.

Venkateswarlu P (1990). Evaluation of analytical methods for fluorine in biological and related materials. *J Dent Res* 69(SpecNo): 514–521.

Fluoroacetamide

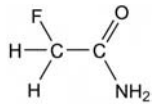
Rodenticide

$FCH_2CONH_2 = 77.1$

CAS—640-19-7

IUPAC Name 2-Fluoroacetamide

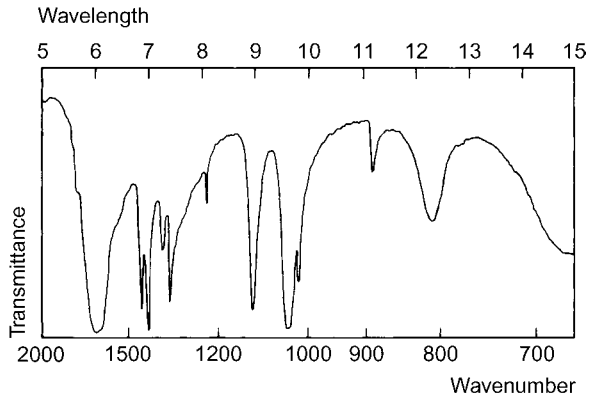
Synonyms Compound 1081; fluoroacetic acid amide.



Chemical Properties A fine fluffy white powder which decomposes if heated appreciably above 100°. Freely soluble in water; soluble in acetone; sparingly soluble in chloroform; relatively insoluble in organic solvents. Log *P* (octanol/water), −1.0.

Colour Test Nessler's reagent—brown-orange.

Infrared Spectrum Principal peaks at wavenumbers 1667, 1036, 1117, 1020, 810, 1239 cm^{-1} (Nujol mull).



Quantification See under Fluoroacetic Acid.

Disposition in the Body Rapidly absorbed after ingestion or through cuts and abrasions. It is converted to fluoroacetic acid and then to fluorocitric acid.

Toxicity Fluoroacetamide is extremely toxic to animals and humans but its lethal action is slower than sodium fluoroacetate.

Fluoroacetic Acid

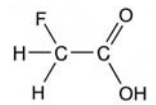
Rodenticide

$C_2H_3FO_2 = 78.0$

CAS—144-49-0

IUPAC Name 2-Fluoroacetic acid

Synonyms Fluoroethanoic acid; gifblaar poison; monofluoroacetic acid.



Chemical Properties Fluoroacetic acid is the toxic principle of the South African plant gifblaar, Dichapetalum cymosum (= Chailletia cymosa) (Dichapetalaceae). Crystals. Mp 33°. Bp 165°. Soluble in water and ethanol; practically insoluble in most common organic solvents. pK_a 2.6 (25°). Log *P* (octanol/water), 0.03.

Sodium Fluoroacetate

$C_2H_2FNaO_2 = 100.0$

CAS—62-74-8

Synonyms Compound 1080; sodium monofluoroacetate.

Chemical Properties A white hygroscopic powder which decomposes at 200° to 202°. Very soluble in water; relatively insoluble in organic solvents.

Quantification

Serum **GC-MS** Limit of detection, 0.02 mg/L [Sporkert *et al.* 2002].

Tissues **GC** ECD. Limit of detection, 100 ng/g [Okuno *et al.* 1982].

GC-MS Limits of detection, 700 ng/g for fluoroacetamide, 100 ng/g for fluoroacetic acid [Stevens *et al.* 1976].

Disposition in the Body Fluoroacetic acid and sodium fluoroacetate are absorbed rapidly through cuts and abrasions but less rapidly from intact skin. Sodium fluoroacetate is converted to fluorocitric acid which inhibits the citric acid cycle and leads to accumulation of citrate.

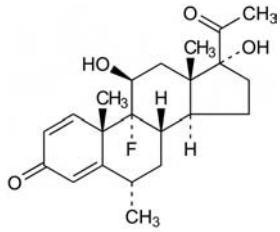
Toxicity Fluoroacetic acid and sodium fluoroacetate are extremely toxic to animals and humans; the minimum lethal dose is about 5 mg/kg. Toxic effects are delayed for several hours following ingestion or absorption through the skin. The maximum permissible atmospheric concentration is 50 $\mu g/m^3$.

Okuno I *et al.* (1982). Modified gas-liquid chromatographic method for determination of compound 1080 (sodium fluoroacetate). *J Assoc Off Anal Chem* 65: 1102–1105.
Sporkert F *et al.* (2002). Headspace solid-phase microextraction with 1-pyrenyldiazomethane on-fibre derivatisation for analysis of fluoroacetic acid in biological samples. *J Chromatogr B Anal Technol Biomed Life Sci* 772: 45–51.
Stevens HM *et al.* (1976). The recovery and identification of fluoroacetamide and fluoroacetic acid from tissues. *Forensic Sci* 8: 131–137.

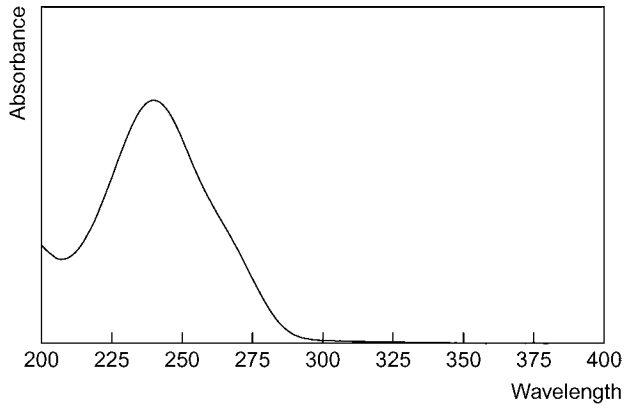
Fluorometholone

Corticosteroid
$C_{22}H_{29}FO_4 = 376.5$
CAS—426-13-1

IUPAC Name (6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-17-Acetyl-9-fluoro-11,17-dihydroxy-6,10,13-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-3-one
Synonym (6α,11β)-9-Fluoro-11,17-dihydroxy-6-methylpregna-1,4-diene-3,20-dione
Proprietary Names *Delmeson*; *Efflumidex*; *Eflone*; *Flarex*; *Flu Oph*; *Fluaton*; *Flucon*; *Fluforte*; *Flumetol*; *Fluor-Op*; *Fluoropos*; *FML*; *Oxylone*; *Semplice*.



Chemical Properties A white to yellowish-white crystalline powder. Mp about 280°, with decomposition. Practically insoluble in water; soluble 1 in 200 of ethanol and 1 in 2200 of chloroform; very slightly soluble in ether. Log *P* (octanol/water), 2.0.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.68; system TF—R_f 0.52; system TAE—R_f 0.91; system TAJ—R_f 0.46; system TAK—R_f 0.26; system TAL—R_f 0.90; system TAM—R_f 0.86.
High Performance Liquid Chromatography System HX—RI 470.
Ultraviolet Spectrum Methanol—241 nm (A^1_1=375a).

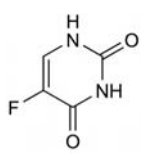


Use Usually applied as a 0.025% cream or ointment and is also available as a 0.1% ophthalmic suspension.

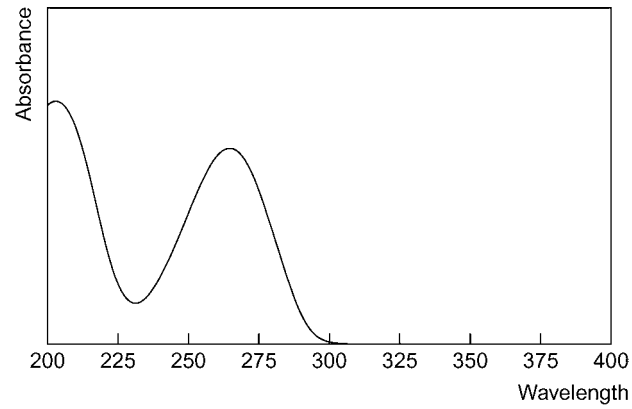
Fluorouracil

Antineoplastic
$C_4H_3FN_2O_2 = 130.1$
CAS—51-21-8

IUPAC Name 5-Fluoro-2,4(1*H*,3*H*)-pyrimidinedione
Synonyms 5-Fluorouracil; 5-FU.
Proprietary Names *AccuSite*; *Adrucil*; *Cinkef-U*; *Effluderm*; *Efudex*; *Efudix*; *Fiverocil*; *Fivoflu*; *Flurablastin*; *Fluracedyl*; *Fluroblastin(e)*; *Fluoroplex*; *Flurox*; *Ofluor*; *Rhonuracil*; *Ribofluor*.



Chemical Properties A white crystalline powder which decomposes at 282°. Sparingly soluble in water; slightly soluble in ethanol; practically insoluble in chloroform and ether. Solutions discolour on storage. pK_a 8.0, 13.0. Log *P* (octanol/pH 7.4), −1.0.
Caution Fluorouracil is an irritant; avoid contact with skin and mucous membranes.
Thin-layer Chromatography System TE—R_f 0.04; system TF—R_f 0.20.
Gas Chromatography System GA—RI 2090.
High Performance Liquid Chromatography System HX—RI 70; system HY—RI 96; system HAA—retention time 3.4 min.
Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1=552a).



Infrared Spectrum Principal peaks at wavenumbers 1653, 1242, 1718, 816, 1220, 1495 cm^{-1} (KBr disk).
Quantification
Blood **HPLC** Limit of detection, 10 µg/L [Wattanatorn *et al.* 1997].
Plasma **GC-MS** Fluorouracil and α-fluoro-β-alanine, limit of detection, <1 µg/L for fluorouracil [Anderson *et al.* 1997]. Limit of detection, <0.5 µg/L [Anderson *et al.* 1992]. Selected-ion monitoring. Limit of detection, 0.4 µg/L [Bates *et al.* 1991].
HPLC UV detection. Limit of detection, 5 µg/L [Nassim *et al.* 2002]. Diode-array detection. Fluorouracil and flucytosine [Torano *et al.* 2001]. Limit of detection, <2 µg/L [Escoriaza *et al.* 1999]. UV detection. Limit of detection, <26 µg/L [Loos *et al.* 1999]. UV detection. Fluorouracil and uracil [House 1998]. UV detection. Fluorouracil and dihydrofluorouracil, limit of detection, 26 µg/L [Ackland *et al.* 1997]. UV detection. Fluorouracil and its major metabolites, limit of detection, 10 µg/L [Joulia *et al.* 1997]. See Blood [Wattanatorn *et al.* 1997]. UV detection. Limit of detection, 25 µg/L [Compagnon *et al.* 1996; Coe *et al.* 1996]. Fluorouracil and folinic acid [Vandenbosch 1993].
HPLC-MS Limit of detection, <1 µg/L [Wang *et al.* 1998].
Tissue **HPLC** Fluorescence detection. Limit of detection, 3 ng/g [Jochheim *et al.* 1994].
Disposition in the Body
Therapeutic Concentration
Seventeen subjects received fluorouracil, either 1 mg/m^2 orally (combined with eniluracil) twice daily or 300 mg/m^2 by continuous IV infusion, for 7 days. The mean steady-state plasma concentration during IV infusion was 104 µg/L compared with 38.1 µg/L for oral fluorouracil [Adjei *et al.* 2002].
Dose Initially 12 mg/kg daily IV, to a maximum of 1 g daily, for 3 or 4 days.

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Adjei AA *et al.* (2002). Comparative pharmacokinetic study of continuous venous infusion fluorouracil and oral fluorouracil with eniluracil in patients with advanced solid tumors. *J Clin Oncol* 20: 1683–1691.
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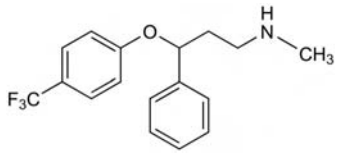
Anderson D *et al.* (1997). Simultaneous gas chromatographic-mass spectrophotometric determination of alpha-fluoro-beta-alanine and 5-fluorouracil in plasma. *J Chromatogr B Biomed Sci Appl* 688: 87–93.
Bates CD *et al.* (1991). The analysis of 5-fluorouracil in human plasma by gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICIMS) with stable isotope dilution. *J Pharm Biomed Anal* 9: 19–21.
Coe RA *et al.* (1996). Determination of 5-fluorouracil in human plasma by a simple and sensitive reversed-phase HPLC method. *J Pharm Biomed Anal* 14: 1733–1741.
Compagnon P *et al.* (1996). Simple high-performance liquid chromatographic method for the quantitation of 5-fluorouracil in human plasma. *J Chromatogr B Biomed Appl* 677: 380–383.
Escoriaza J *et al.* (1999). Simple and sensitive determination of 5-fluorouracil in plasma by high-performance liquid chromatography. Application to clinical pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 736: 97–102.
House LK *et al.* (1998). Simultaneous determination of 5-fluorouracil and uracil by high-performance liquid chromatography using four serial columns. *J Chromatogr B Biomed Sci Appl* 720: 245–250.
Jochheim C *et al.* (1994). A procedure for the determination of 5-fluorouracil in tissue using microbore HPLC and fluorescence detection. *Anal Biochem* 217: 285–291.
Joulia JM *et al.* (1997). Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography: application to a pharmacokinetic study. *J Chromatogr B Biomed Sci Appl* 692(2): 427–435.

Loos WJ *et al.* (1999). Determination of 5-fluorouracil in microvolumes of human plasma by solvent extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 735: 293–297.
Nassim MA *et al.* (2002). An HPLC method for the measurement of 5-fluorouracil in human plasma with a low detection limit and a high extraction yield. *Int J Mol Med* 10: 513–516.
Torano JS *et al.* (2001). Simultaneous determination of flucytosine and fluorouracil in human plasma by high-performance liquid chromatography. *Biomed Chromatogr* 15: 89–94.
Vandenbosch C *et al.* (1993). Determination of leucovorin and 5-fluorouracil in plasma by high-performance liquid chromatography. *J Chromatogr* 612: 77–85.
Wang K *et al.* (1998). Derivatization of 5-fluorouracil with 4-bromomethyl-7-methoxycoumarin for determination by liquid chromatography-mass spectrometry. *J Am Soc Mass Spectrom* 9: 970–976.
Wattanatorn W *et al.* (1997). High-performance liquid chromatographic assay of 5-fluorouracil in human erythrocytes, plasma and whole blood. *J Chromatogr B Biomed Sci Appl* 692: 233–237.

Fluoxetine

Antidepressant
$C_{17}H_{18}F_3NO$ = 309.3
CAS—54910-89-3

IUPAC Name *N*-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine
Synonym DB00472
Proprietary Name *Sarafem*



Chemical Properties Solubility in water 78.24 mg/L (25°). Log *P* (octanol/water), 4.05; (octanol/water pH 7.4), 1.82. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005].

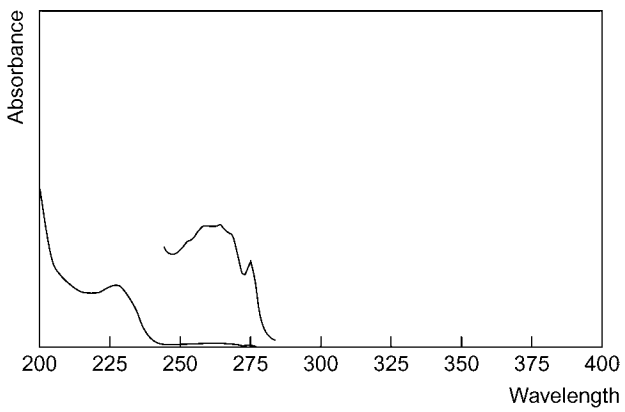
Fluoxetine Hydrochloride

$C_{17}H_{18}F_3NO,HCl$ = 345.8
CAS—59333-67-4
Synonym LY-110,140
Proprietary Names *Adofen*; *Docutrix*; *Erocap*; *Fluctin*; *Fluctine*; *Fluoxeren*; *Fontex*; *Foxetin*; *Lorien*; *Lovan*; *Mutan*; *Prozac*; *Prozyn*; *Reneuron*; *Sanzur*; *Zactin*.
Chemical Properties A white to off-white crystalline solid. Mp 138°. It is soluble in methanol, ethanol, acetonitrile, chloroform and acetone; slightly soluble in ethyl acetate, dichloromethane and water (maximum 14 g/L); insoluble in toluene, cyclohexane and hexane.

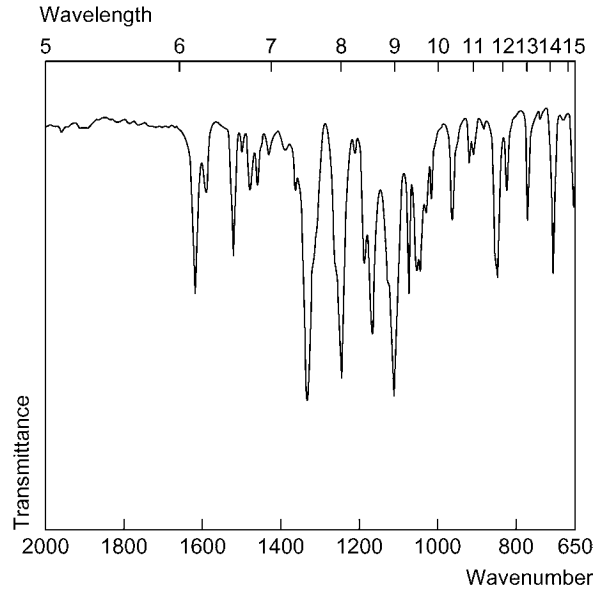
Fluoxetine Oxalate

$C_{17}H_{18}F_3NO,C_2H_4$ = 397.4
Chemical Properties Crystals from ethylacetate-methanol. Mp 179° to 182°.

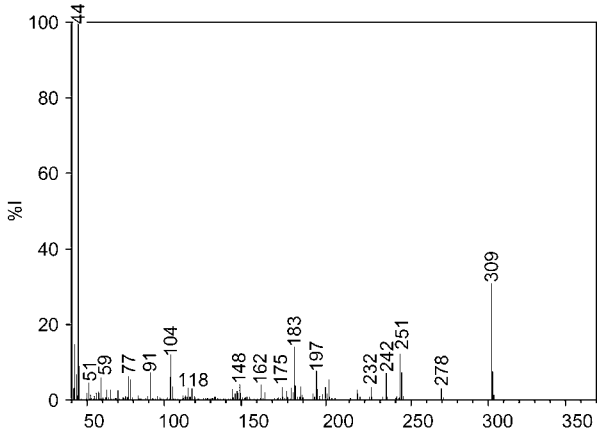
Thin-layer Chromatography System TB—R_f 0.13, M (nor) R_f 0.12; system TE—R_f 47, M (nor) R_f 0.47; System TAE—R_f 0.11; M (nor) R_f 0.11.
Gas Chromatography System GA—RI 1859, M (nor-) RI 1851, M (acetyl-) RI 2250, M (noracetyl-) RI 2190; system GB—RI 1903, M (nor-) RI 1888, M (acetyl-) RI 2319, M (noracetyl-) RI 2278; system GM—RRT 0.304, M (nor-) RI 0.284.
High Performance Liquid Chromatography System HY—RI 400; system HZ—RT 7.6 min, M (desmethyl-)RT 6.7 min; system HAA—RT 16.2 min; system HAX—RT 12.2 min; system HAY—RT 7.07 min
Ultraviolet Spectrum Aqueous acid (methanol)—227, 264, 268, 275 nm (fluoxetine); 0.025 mol/L sulfuric acid—226.5 nm (fluoxetine); (0.2 mol/L sulfuric acid)—226, 263, 275 nm (norfluoxetine); basic—264, 275 nm (norfluoxetine).



Infrared Spectrum Principal peaks at wave numbers 2986, 2772, 1614, 1329, 1257, 1122; 1335, 1240, 1110 cm^{-1} (norfluoxetine, KBr pellet).



Mass Spectrum Principal ions at *m/z* (fluoxetine) 44, 309, 183, 104, 251, 91, 77, 59, 148; (norfluoxetine) 134, 104, 191, 143, 162, 77.



Quantification

Blood **GC** Column: HP (25 m × 0.20 mm i.d., 0.11 µm). Carrier gas: He. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 220 µg/L, limit of detection, 66 µg/L [Martinez *et al.* 2003].

GC-MS Column: Varian FactorFour (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° to 290° at 30°/min for 2.67 min. SIM acquisition mode. Limit of detection not reported [Johnson *et al.* 2007]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 0.5 min at 10°/min, to 270° for 2.5 min. EI ionisation, SIM acquisition mode. Limit of detection not reported [Ferslew *et al.* 1998]. Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 mm). Carrier gas: He, 0.7 mL/min. Temperature programme: 100° for 2 min to 210°,at 15°/min to 280° at 20°/min for 5 min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 12.5 µg/L [Crifasi *et al.* 1997].

LC-MS Column: C_{18}. Mobile phase: acetonitrile : 0.1% trifluoroacetic acid (50 : 50), flow rate 0.4 mL/min. ESI ionisation, SIM acquisition mode [Pufal, Sykutera 2008]. Column: XTerra RP18 (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mol/L ammonium formate buffer (15 : 85 for 1 min to 35 : 65 over 12 min for 1 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of detection, 5 µg/L [Castaing *et al.* 2007].

Plasma **GC** Column: Rtx-1 (15 m × 0.25 mm i.d., 1.0 µm). Carrier gas: H2. Temperature programme: 170° for 7 min. NPD. Limit of detection, 1.5 µg/L [Ulrich 2003]. Column: OV-1. NPD. Limit of detection, 0.3 µg/L [Fontanille *et al.* 1997]. Column: DB-17 (30 m × 0.25 mm i.d.). ECD. Limit of quantification, 5 µg/L [Lantz *et al.* 1993]

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 15 psi. Temperature programme: 80° to 280° at 30°/min for 5 min. EI ionisation, full scan and SIM acquisition mode. Limit of quantification, 1.37 ng/L [Fernandes *et al.* 2008]. Column: J&W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min, to 180° at 50°/min for 10 min, to 300° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 12.5 µg/L [Wille *et al.* 2007]. Limit of quantification, 1 µg/L for (*R*)- and (*S*)-enantioners [Eap *et al.* 1996].

HPLC Column: LiChrospher 60 RP-Select B (125 × 4 mm i.d., 5 µm). Mobile phase: 0.005 mol/L sodium acetate buffer (pH 4.5) : acetonitrile (50 : 50), flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 290 nm). Limit of detection not reported [de Freitas *et al.* 2010]. Column: LiChrosphere 60 RP:Select B (250 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer : acetonitrile (57 : 43). Flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 25 µg/L [Chaves *et al.* 2009]. Column: Chiralcel OD-R. Mobile phase: 7.5 mmol/L potassium hexafluorophosphate with 0.25 mol/L sodium phosphate : acetonitrile (75 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 290 nm). Limit of quantification, 10 µg/L for (*R*)- and (*S*)-enantioners [Silva *et al.* 2009]. Fluorescence detection. Limit of quantification, 0.7 µg/L [Clausing *et al.* 1997]. Column: Symmetry C_{18} (250 × 4.6 mm i.d., 5 µm). DAD (λ = 226, 254, 400 nm). Limit of detection, 5 µg/L [Aymard *et al.* 1997]. See also Suckow *et al.* [1992], Thomare *et al.* [1992], Wong *et al.* [1990].

LC-MS Mobile phase: acetonitrile : 0.05 mol/L ammonium formate buffer, flow rate 200 µL/min. Limit of quantification, 5 µg/L, limit of detection, 3 µg/L [Saber 2009]. Column: Sunfire C_{18} IS (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 2 mol/L ammonium formate (pH 3, 5 : 85 for 0.5 min to 50 : 50 over 4 min to 70 : 30 over 1 min), flow rate 0.4 mL/min. Limit of quantification, 2 µg/L [De Castro *et al.* 2008]. Column: C_{18}. ESI. Limit of quantification, 1 µg/L [Santos-Neto *et al.* 2008]. Column: Zorbax Eclipse SB-C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (296 : 204), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection not reported [Guo *et al.* 2006].

Serum **GC** See Plasma [Ulrich 2003].

HPLC Column: Nova-Pak. Mobile phase: acetonitrile : TEA-acetic acid buffer (pH 5.5), flow rate 1.7 mL/min. UV detection (λ = 226 nm). Limit of detection, 15 µg/L [Orsulak *et al.* 1988].

LC-MS Column: Beckman C_{18} ODS (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water : formic acid with 2 mol/L ammonium acetate (68 : 32 : 0.1), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.17 µg/L, limit of detection 0.06 µg/L [Franceschi *et al.* 2009]. Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mol/L acetate buffer (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min, to 20 : 80), flow rate 1.0 mL/min. ESI, MRM acquisition mode. Limit of quantification, 2.17 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **GC-MS** See Blood [Johnson *et al.* 2007]. Carrier gas: He, 0.91 mL/min. Temperature programme: 100° for 0.8 min to 220° at 50°/min for 1 min. SIM acquisition mode. Limit of detection, 5.7 µg/L [Berzas Nevado *et al.* 2006]. Column: CP-SIL_8 CB (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 2 min, to 200° at 20°/min, to 280° over 5 min. Limit of detection, 0.25 µg/L [Salgado-Petinal *et al.* 2005]. See Blood [Crifasi *et al.* 1997; Ferslew *et al.* 1998].

HPLC Column: Extrasil ODS (250 × 4.0 mm i.d., 5 µm). Mobile phase: 0.4% tetramethylammonium chloride (pH 4.0) : acetonitrile (60 : 40), flow rate 1.0 mL/min. Fluorescence detection (λ = 228 nm). Limit of quantification, 1.0–10 µg/L [Unceta *et al.* 2010]. Column: Eclipse X-DB-C_8 (150 × 4.6 mm i.d.). Mobile phase: sodium dihydrogen phosphate buffer (pH 3.0) : acetonitrile : methanol (70 : 25 : 5). UV detection (λ = 254 nm). Limit of detection, 90.1 µg/L [Cruz-Vera *et al.* 2008]. Column: Spherisorb ODS2 (150 × 4.0 mm i.d., 5 µm). Mobile phase: 0.15% tetramethylammonium chloride (pH 4.0) : acetonitrile (50 : 50), flow rate 1.0 mL/min. DAD (λ = 230 nm). Limit of detection, 0.01 mg/L [Unceta *et al.* 2008].

Bile **GC** Column: DB-17 (15 m × 0.32 mm i.d., 0.15 µm). Carrier gas: He, 17.5 psi. Temperature programme: 130° to 300° at 15°/min for 1 min. NPD. Limit of detection not reported [Kincaid *et al.* 1990]

GC-MS See Blood [Johnson *et al.* 2007].

Milk **HPLC** UV detection [Hostetter *et al.* 2004].

Oral Fluid **LC-MS** Column: Zorbax Eclipse XDB-C_{18} (50 × 4.6 mm i.d., 1.8 µm). Mobile phase: 0.2% acetic acid : methanol (70 : 30 to 30 : 70 over 8 min), flow rate 1 mL/min. ESI. Limit of quantification, 5 µg/L [Coulter *et al.* 2010]. See Plasma [De Castro *et al.* 2008].

Stomach Contents **GC-MS** See Blood [Ferslew *et al.* 1998].

Vitreous Humour **GC** Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 50° to 275° at 35°/min over 4.5 min. NPD. Limit of detection not reported [Cantrell *et al.* 2009].

GC-MS See Blood [Crifasi *et al.* 1997; Johnson *et al.* 2007].

Brain **GC-MS** Column: J&W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min, to 180° at 50°/min for 10 min, to 300° at 10°/min. EI ionisation, SIM acquisition mode. Limit of detection not reported [Wille *et al.* 2009]. See Blood [Johnson *et al.* 2007].

HPLC See Urine. Limit of quantification, 2–20 ng/g [Unceta *et al.* 2010]. See Plasma [Clausing *et al.* 1997].

Hair **GC-MS** See Brain [Wille *et al.* 2009].

Kidney **GC-MS** See Blood [Johnson *et al.* 2007].

Liver **GC** See Vitreous Humour [Cantrell *et al.* 2009].

GC-MS See Blood [Crifasi *et al.* 1997; Johnson *et al.* 2007].

Lung **GC-MS** See Blood [Johnson *et al.* 2007].

Muscle **GC-MS** See Blood [Johnson *et al.* 2007].

Disposition in the Body Fluoxetine is readily absorbed after oral administration and is extensively metabolised in the liver by demethylation. The primary active metabolite is norfluoxetine, which is excreted via the kidneys. Further metabolism can occur by *O*-dealkylation producing *p*-trifluoromethylphenol and hippuric acid. Of a drug dose, 80% is excreted in urine, with <10% as the unchanged parent drug, and 15% is excreted in faeces. Fluoxetine is widely distributed throughout the body and is secreted into breast milk.

Therapeutic Concentration Therapeutic serum concentrations are 0.15–0.5 mg/L (fluoxetine) and 0.1–0.5 mg/L (norfluoxetine).

Single doses of fluoxetine 40 mg given to 6 subjects produced maximum plasma concentrations of fluoxetine of 0.03–0.055 mg/L after 4–8 h; maximum plasma norfluoxetine concentrations were 0.006–0.036 mg/L after 48–96 h [Aronoff *et al.* 1984].

Daily doses of 20–80 mg of fluoxetine were given to 12 patients. Average plasma concentrations were 48.7, 142, 199.2 and 208.7 µg/L from daily doses of 20, 40, 60 and 80 mg, respectively [de Freitas *et al.* 2010].

Toxicity Blood concentrations of 1.3–6.8 mg/L fluoxetine and 0.9–5.0 mg/L norfluoxetine have been associated with deaths. Serum levels of 1.96 mg/L fluoxetine (0.42 mg/L norfluoxetine) have been associated with seizures.

A 44-year-old man was found dead, and medication at the scene included clozapine (Clorazil) and fluoxetine (Prozac). Toxicological analysis showed 0.7 mg/L fluoxetine and 0.6 mg/L norfluoxetine in the man's blood, as well as 4.9 mg/L clozapine and 350 mg/L ethanol. Death resulted from an overdose of fluoxetine and clozapine, which resulted in a fatal drug interaction [Ferslew *et al.* 1998].

A 58-year-old woman with suicidal tendencies was found dead at home surrounded by empty fluoxetine prescription vials. Postmortem blood and bile concentrations for fluoxetine plus norfluoxetine were 6.0 and 5.0 mg/L, and 13.0 mg/L [Kincaid *et al.* 1990].

A 31-year-old woman who died despite resuscitation attempts had the following postmortem fluoxetine and norfluoxetine concentrations, respectively: 33 and 12 mg/L in central blood, 400 and 460 mg/kg in liver and 5.2 and 2.2 mg/L in vitreous humour [Cantrell *et al.* 2009].

Bioavailability ≈60%.

Half-life Fluoxetine 4–6 days; norfluoxetine 4–16 days.

Volume of Distribution 27 L/kg (between 20 and 42 L/kg for both fluoxetine and norfluoxetine).

Clearance Renal clearance, 0.6 L/kg/h; also reported as 20.8 L/h for fluoxetine and 8.7 L/h for norfluoxetine.

Protein Binding Fluoxetine is bound to serum proteins 94.5%.

Dose A usual dose of 20 mg daily is administered for depression and 60 mg daily for bulimia nervosa. A maximum dose of 80 mg may be given daily. Dose is reduced for patients with renal and hepatic impairment. Not recommended for children.

Aronoff GR *et al.* (1984). Fluoxetine kinetics and protein binding in normal and impaired renal function. *Clin Pharmacol Ther* 36: 138–144.

Aymard G *et al.* (1997). Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection. *J Chromatogr B Biomed Sci Appl* 700: 183–189.

Berzas Nevado JJ *et al.* (2006). Screening of citalopram, fluoxetine and their metabolites in human urine samples by gas chromatography–mass spectrometry. A global robustness/ruggedness study. *J Chromatogr A* 1123: 130–133.

Cantrell FL *et al.* (2009). Fatal fluoxetine intoxication with markedly elevated central blood, vitreous, and liver concentrations. *J Anal Toxicol* 33: 62–64.

Castaing N *et al.* (2007). Quantification of eight new antidepressants and five of their active metabolites in whole blood by high-performance liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 31: 334–341.

Chaves AR *et al.* (2009). Solid-phase microextraction using poly(pyrrole) film and liquid chromatography with UV detection for analysis of antidepressants in plasma samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 587–593.

Clausing P *et al.* (1997). Determination of D-fenfluramine, D-norfenfluramine and fluoxetine in plasma, brain tissue and brain microdialysate using high-performance liquid chromatography after precolumn derivatization with dansyl chloride. *J Chromatogr B Biomed Sci Appl* 692: 419–426.

Coulter C *et al.* (2010). Antidepressant drugs in oral fluid using liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 34: 64–72.

Crifasi JA *et al.* (1997). Simultaneous identification and quantitation of fluoxetine and its metabolite, norfluoxetine, in biological samples by GC-MS. *J Anal Toxicol* 21: 415–419.

Cruz-Vera M *et al.* (2008). Combined use of carbon nanotubes and ionic liquid to improve the determination of antidepressants in urine samples by liquid chromatography. *Anal Bioanal Chem* 391: 1139–1145.

DeCastro A *et al.* (2008). LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. *J Pharm Biomed Anal* 48: 183–193.

deFreitas DF *et al.* (2010). Three-phase, liquid-phase microextraction combined with high performance liquid chromatography–fluorescence detection for the simultaneous determination of fluoxetine and norfluoxetine in human plasma. *J Pharm Biomed Anal* 51: 170–177.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Eap CB *et al.* (1996). Simultaneous determination of plasma levels of fluvoxamine and of the enantiomers of fluoxetine and norfluoxetine by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 682: 265–272.

Fernandes C *et al.* (2008). Determination of fluoxetine in plasma by gas chromatography–mass spectrometry using stir bar sorptive extraction. *Anal Chim Acta* 614: 201–207.

Ferslew KE *et al.* (1998). A fatal drug interaction between clozapine and fluoxetine. *J Forensic Sci* 43: 1082–1085.

Fontanille P *et al.* (1997). Direct analysis of fluoxetine and norfluoxetine in plasma by gas chromatography with nitrogen–phosphorus detection. *J Chromatogr B Biomed Sci Appl* 692: 337–343.

Franceschi L *et al.* (2009). A simple method to monitor serum concentrations of fluoxetine and its major metabolite for pharmacokinetic studies. *J Pharm Biomed Anal* 49: 554–557.

Guo B *et al.* (2006). Rapid and direct measurement of free concentrations of highly protein-bound fluoxetine and its metabolite norfluoxetine in plasma. *Rapid Commun Mass Spectrom* 20: 39–47.

Hostetter AL *et al.* (2004). A novel system for the determination of antidepressant concentrations in human breast milk. *Ther Drug Monit* 26: 47–52.

Johnson RD *et al.* (2007). The distribution of fluoxetine in human fluids and tissues. *J Anal Toxicol* 31: 409–414.

Kincaid RL *et al.* (1990). Report of a fluoxetine fatality. *J Anal Toxicol* 14: 327–329.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Lantz RJ *et al.* (1993). Determination of fluoxetine and norfluoxetine in human plasma by capillary gas chromatography with electron-capture detection. *J Chromatogr* 614: 175–179.
Martinez MA *et al.* (2003). A comparative solid-phase extraction study for the simultaneous determination of fluoxetine, amitriptyline, nortriptyline, trimipramine, maprotiline, clomipramine, and trazodone in whole blood by capillary gas–liquid chromatography with nitrogen–phosphorus detection. *J Anal Toxicol* 27: 353–358.
Orsulak PJ *et al.* (1988). Determination of the antidepressant fluoxetine and its metabolite norfluoxetine in serum by reversed-phase HPLC with ultraviolet detection. *Clin Chem* 34: 1875–1878.
Pufal E, Sykutera M (2008). Application of liquid chromatography coupled with mass spectrometry (LC/MS) to determine antidepressants in blood samples. *Arch Med Sadowej Kryminol* 58: 171–176.
Saber AL (2009). On-line solid phase extraction coupled to capillary LC-ESI-MS for determination of fluoxetine in human blood plasma. *Talanta* 78: 295–299.
Salgado-Petinal C *et al.* (2005). Rapid screening of selective serotonin re-uptake inhibitors in urine samples using solid-phase microextraction gas chromatography–mass spectrometry. *Anal Bioanal Chem* 382: 1351–1359.
Santos-Neto AJ *et al.* (2008). Simultaneous analysis of five antidepressant drugs using direct injection of biofluids in a capillary restricted-access media-liquid chromatography–tandem mass spectrometry system. *J Chromatogr A* 1189: 514–522.
Silva BJ *et al.* (2009). Determination of fluoxetine and norfluoxetine enantiomers in human plasma by polypyrrole-coated capillary in-tube solid-phase microextraction coupled with liquid chromatography–fluorescence detection. *J Chromatogr A* 1216: 8590–8597.
Suckow RF *et al.* (1992). Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization. *Clin Chem* 38: 1756–1761.
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Ulrich S (2003). Direct stereoselective assay of fluoxetine and norfluoxetine enantiomers in human plasma or serum by two-dimensional gas–liquid chromatography with nitrogen–phosphorus selective detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 783: 481–490.
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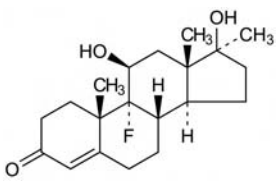
Unceta N *et al.* (2010). Development of a stir bar sorptive extraction based HPLC-FLD method for the quantification of serotonin reuptake inhibitors in plasma, urine and brain tissue samples. *J Pharm Biomed Anal* 51: 178–185.
Wille SM *et al.* (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic-mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *J Chromatogr A* 1176: 236–245.
Wille SM *et al.* (2009). Determination of antidepressants in human postmortem blood, brain tissue, and hair using gas chromatography–mass spectrometry. *Int J Legal Med* 123: 451–458.
Wong SH *et al.* (1990). Determination of fluoxetine and norfluoxetine by high-performance liquid chromatography. *J Chromatogr* 499: 601–608.

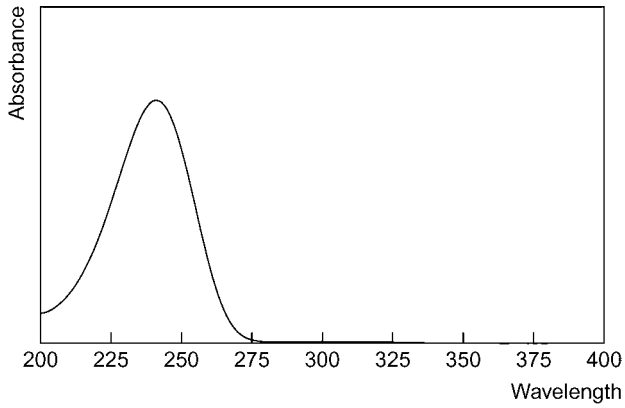
Fluoxymesterone

Androgen
$C_{20}H_{29}FO_3 = 336.4$
CAS—76-43-7

IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*S*)-9-Fluoro-11,17-dihydroxy-10,13,17-trimethyl-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[*a*]phenanthren-3-one
Synonyms (11β,17β)-9-Fluoro-11,17-dihydroxy-17-methylandrost-4-en-3-one; fluorohydroxymethyltestosterone.
Proprietary Names *Halotestin*; *Oratestin*; *Ora-Testryl*; *Stenox*; *Testoral*; *Ultandren*.
Note Testoral is also used as a proprietary name for testosterone.



Chemical Properties A white or creamy-white crystalline powder. Mp 270° with decomposition. Practically insoluble in water; soluble 1 in 70 of ethanol and 1 in 200 of chloroform; soluble in pyridine; slightly soluble in acetone; practically insoluble in ether, benzene, and hexanes. Log *P* (octanol/water), 2.4.
Colour Tests Antimony pentachloride—brown; naphthol–sulfuric acid—green-yellow/yellow; sulfuric acid—yellow (green fluorescence under UV light).
Thin-layer Chromatography System TP—R_f 0.51; system TQ—R_f 0.09; system TR—R_f 0.38; system TS—R_f 0.16, streaking may occur; system TAJ—R_f 0.41; system TAK—R_f 0.35; system TAL—R_f 0.91; system TAM—R_f 0.74.
Gas Chromatography System GA—RI 2835; system GAG—RRT 1.50(relative to testosterone); system GAI—RRT 1.155(relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAR—retention time 14.6 min.
High Performance Liquid Chromatography System HY—RI 427; system HATb—RRT 0.70 (relative to testosterone); system HAR—RRT 0.78 (relative to testosterone).
Ultraviolet Spectrum Dehydrated alcohol—240 nm (A_1^1=495a).



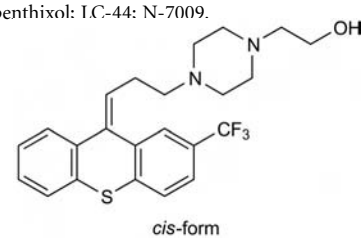
Infrared Spectrum Principal peaks at wavenumbers 1654, 867, 1036, 1247, 926, 1282 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 71, 55, 79, 91, 109, 123, 336.
Quantification
Serum **HPLC** UV detection [Capponi *et al.* 1985].
Hair **GC-MS** Fluoxymesterone and other anabolic steroids [Deng *et al.* 1999].
Dose 2 to 10 mg daily; up to 30 mg daily may be given.

Capponi VJ *et al.* (1985). Liquid chromatographic assay for fluoxymesterone in human serum with application to a preliminary bioavailability study. *J Pharm Sci* 74: 308–311.
Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.

Flupentixol

Tranquilliser
$C_{23}H_{25}F_3N_2OS = 434.5$
CAS—2709-56-0

IUPAC Name 4-[3-[2-(Trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]-1-piperazine ethanol
Synonyms Flupenthixol; LC-44; N-7009.



cis-form

Chemical Properties Log *P* (octanol/buffer pH 7.0), 3.0. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Flupentixol Decanoate

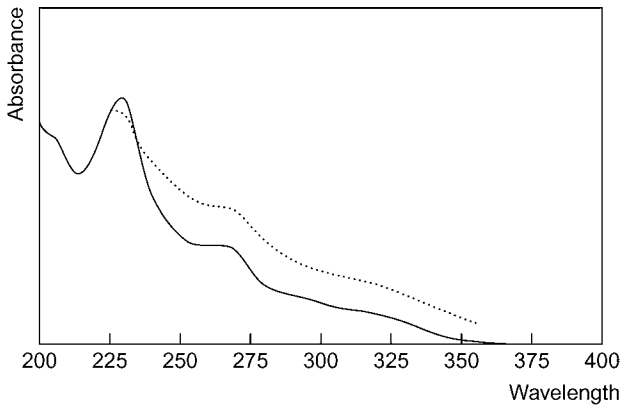
$C_{33}H_{43}F_3N_2O_2S = 588.8$
CAS—30909-51-4
Synonyms (*Z*)-Flupenthixol decanoate; *cis*-flupenthixol decanoate.
Proprietary Names *Depixol (injection)*; *Fluanxol Depot*.
Chemical Properties A yellow oil. Very slightly soluble in water; soluble in ethanol; freely soluble in chloroform and ether.

Flupentixol Hydrochloride

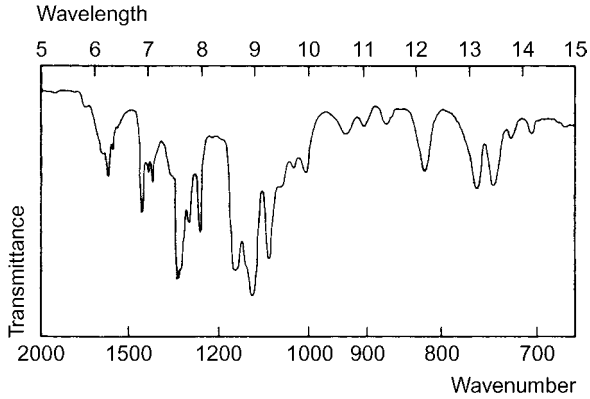
$C_{23}H_{25}F_3N_2OS,2HCl = 507.4$
CAS-2413-38-9
Synonyms Flupenthixol dihydrochloride; flupenthixol hydrochloride.
Proprietary Names *Depixol (tablets)*; *Fluanxol (tablets)*. It is an ingredient of *Deanxit*.
Chemical Properties A white or yellowish-white powder. Mp 230° to 240°. Soluble in water and ethanol; very slightly soluble in chloroform; practically insoluble in ether.

Colour Tests Formaldehyde–sulfuric acid—red (orange fluorescence under UV light); Liebermann's reagent—red; Mandelin's test—red; Marquis test—orange-red; sulfuric acid—orange (fluoresces under UV light).
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.06; system TC—R_f 0.33; system TE—R_f 0.46; system TAE—R_f 0.50 (acidified iodoplatinate solution, strong reaction).
Gas Chromatography System GA—flupentixol – *cis*-isomer RI 3058, M (ring) RI 2190, M (*N*-oxide) RI 2120, flupentixol-AC RI 3045, M (desalkyl-dihydro-)-AC RI 3055, M (dihydro-)-AC RI 3005; system GB—flupentixol – *cis*-isomer RI 3199, flupentixol – *trans*-isomer RI 3217, M (desalkyl-)-*cis*- RI 2832, M (desalkyl-)-*trans*- RI 2855.
High Performance Liquid Chromatography System HA—flupentixol *k* 1.2, flupentixol sulfoxide *k* 1.3; system HX—RI 475; system HY—RI 435; system HZ—retention time 10.7 min; system HAA—retention time 17.4 min; system HAX—retention time 13.7 min; system HAY—retention time 7.5 min.

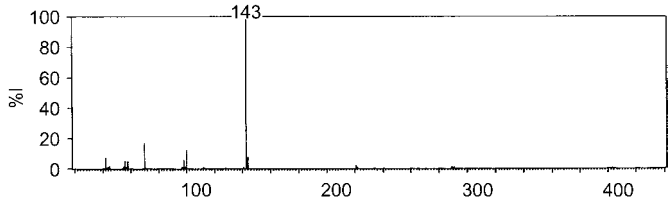
Ultraviolet Spectrum Aqueous acid—230 nm.



Infrared Spectrum Principal peaks at wavenumbers 1119, 1320, 1160, 1081, 1253, 1287 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 143, 70, 100, 144, 42, 98, 58, 56.



Quantification

Plasma **GC** NPD (comparison with radioimmunoassay). Limit of detection, <0.5 µg/L [Balant-Gorgia *et al.* 1985].

Serum **HPLC** UV detection. Flupentixol and haloperidol enantiomers, limit of detection for flupentixol, <0.5 µg/L [Walter *et al.* 1998].

Radioimmunoassay *cis*-flupentixol, limit of detection, 300 ng/L [Jørgensen 1978].

Disposition in the Body Readily absorbed after oral administration. Flupentixol decanoate is very slowly absorbed from the site of IM injection. Peak plasma concentrations are attained about 3 to 6 h after oral administration and 3 to 7 days after IM injection. The main metabolic reactions are sulfoxidation, side-chain *N*-dealkylation, and glucuronic acid conjugation. *N*-Desalkylflupentixol and flupentixol sulfoxide are the major metabolites found in plasma (both are inactive). Numerous metabolites are excreted in the urine and faeces and there is evidence of enterohepatic circulation. Flupentixol crosses the placenta and it is found in small amounts in breast milk.

Bioavailability About 55%.

Half-life Biological half-life of flupentixol hydrochloride, about 35 h.

Note For information on the pharmacokinetics of flupentixol, see Jørgensen [1980].

Dose For psychoses, the equivalent of 6 to 18 mg of flupentixol daily, orally.

Balant-Gorgia AE *et al.* (1985). Comparative determination of flupentixol in plasma by gas chromatography and radioimmunoassay in schizophrenic patients. *Ther Drug Monit* 7: 229–235.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Jørgensen A (1978). A sensitive and specific radioimmunoassay for cis (Z)-flupenthixol in human serum. *Life Sci* 23: 1533–1542.

Jørgensen A (1980). Pharmacokinetic studies in volunteers of intravenous and oral cis (Z)-flupentixol and intramuscular cis (Z)-flupentixol decanoate in Viscoleo. *Eur J Clin Pharmacol* 18: 355–360.

Walter S *et al.* (1998). Quantification of the antipsychotics flupentixol and haloperidol in human serum by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 720: 231–237.

Fluphenazine

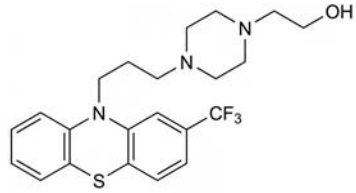
Tranquilliser

$C_{22}H_{26}F_3N_3OS$ = 437.5

CAS—69-23-8

IUPAC Name 4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol

Synonym Triflumethazine



Chemical Properties pK_a 3.9, 8.1. Log *P* (octanol/buffer pH 7.0), 3.5. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Fluphenazine Decanoate

$C_{32}H_{44}F_3N_3O_2S$ = 591.8

CAS—5002-47-1

Synonym Fluphenazini decanoas

Proprietary Names *Anatensol*; *Dapotum*; *Lyogen*; *Lyorodin*; *Modecate*; *Moditen Depot*; *Prolixin*; *Siqualone*.

Chemical Properties A pale yellow viscous liquid or a yellow crystalline oily solid. Mp 30° to 32°. Insoluble in water; very soluble in alcohol, chloroform, cyclohexane and ether.

Fluphenazine Enantate

$C_{29}H_{38}F_3N_3O_2S$ = 549.7

CAS—2746-81-8

Synonyms Fluphenazini enantas; fluphenazine enanthate; fluphenazine heptanoate.

Proprietary Names *Anatensol*; *Moditen (injection)*; *Prolixin*.

Chemical Properties A pale yellow to yellow-orange, clear to slightly turbid, viscous liquid or a yellow crystalline oily solid. Practically insoluble in water; soluble 1 in <1 of ethanol and of chloroform and 1 in 2 of ether.

Fluphenazine Hydrochloride

$C_{22}H_{26}F_3N_3OS,2HCl$ = 510.4

CAS—146-56-5

Synonym Fluphenazini hydrochloridum

Proprietary Names *Anatensol*; *Cenilene*; *Dapotum*; *Lyogen*; *Lyorodin*; *Moditen (tablets)*; *Omca*; *Pacinol*; *Permitil*; *Phenazin*; *Prolixin*; *Sevinol*; *Siqualone*. It is an ingredient of *Dominans*, *Motipress* and *Motival*.

Chemical Properties A white crystalline powder. Mp about 230°. Soluble 1 in 10 or less of water; slightly soluble in ethanol, acetone and chloroform; practically insoluble in ether.

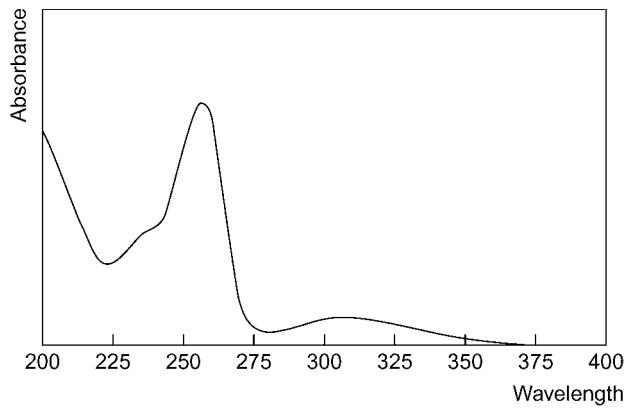
Colour Tests Formaldehyde–sulfuric acid—red; Forrest reagent—orange; FPN reagent—orange; Mandelin's test—brown; Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.05; system TC—R_f 0.23; system TE—R_f 0.45; system TL—R_f 0.10; system TAE—R_f 0.45; system TAF—R_f 0.49; system TAJ—R_f 0.06; system TAK—R_f 0.00; system TAL—R_f 0.41 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, brown; ninhydrin spray, positive).

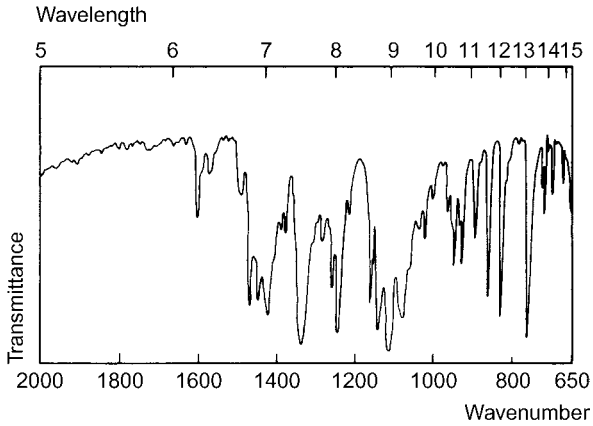
Gas Chromatography System GA—fluphenazine RI 3050, M (ring) RI 2190, fluphenazine-AC RI 3170, M (amino-)-AC RI 2765, M (desalkyl-)-AC RI 3145; system GB—fluphenazine RI 3194.

High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 462; system HY—RI 471; system HZ—retention time 10.1 min; system HAA—retention time 17.4 min; system HAX—retention time 13.6 min; system HAY—retention time 7.2 min.

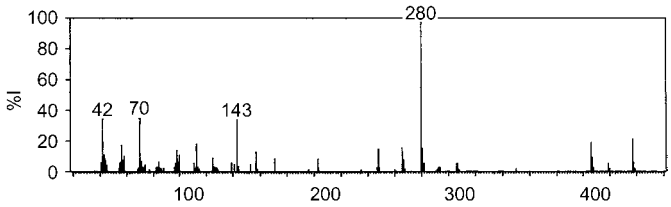
Ultraviolet Spectrum Aqueous acid—256 (A^1_1=690a), 306 nm.



Infrared Spectrum Principal peaks at wavenumbers 1116, 767, 1245, 1144, 1084, 836 cm^{-1} (fluphenazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 280, 143, 42, 70, 437, 406, 113, 56.



Quantification

Plasma **GC** AFID. Limit of detection, 500 ng/L [Javaid *et al.* 1981].

GC-MS Limit of detection, <50 ng/L [Jemal *et al.* 1987]. Limit of detection, 80 ng/L [McKay *et al.* 1983].

HPLC Coulometric detection. Fluphenazine and fluphenazine decanoate, limit of detection, 0.1 µg/L [Luo *et al.* 1997].

HPLC–radioimmunoassay Fluphenazine, related phenothiazines, and metabolites, limit of detection, 160 pg [Goldstein, Van Vunakis 1981].

Radioimmunoassay 7-Hydroxyfluphenazine, limit of detection, 0.1 µg/L [Aravagiri *et al.* 1994].

Serum **GC-MS** Fluphenazine decanoate [Glazer *et al.* 1992].

Urine **GC** AFID. Free and conjugated fluphenazine, limit of detection, 2 µg/L [Whelpton, Curry 1976].

Disposition in the Body The hydrochloride is well absorbed after oral administration; the decanoate and enantate are slowly absorbed from sites of injection. Fluphenazine is metabolised by sulfoxidation, hydroxylation and conjugation with glucuronic acid or sulfate. Fluphenazine sulfoxide and 7-hydroxyfluphenazine have been detected in urine and faeces. After an oral dose of the hydrochloride, 20% is excreted in the urine and 60% is eliminated in the faeces in 7 days; after an IM dose of the enantate, 26% is eliminated in the faeces and 14% excreted in the urine in 14 days; after an IM dose of the decanoate, 17% is eliminated in the faeces and 6% excreted in the urine in 30 days.

Therapeutic Concentration

Following a single oral dose of 5 mg of fluphenazine hydrochloride to 6 subjects, peak plasma concentrations of 0.3 to 1.0 µg/L (mean 0.6) were attained in 2.8 h [Midha *et al.* 1983].

Following daily oral doses of 20 mg of fluphenazine hydrochloride to 18 subjects, steady-state plasma concentrations of 0.2 to 4.0 µg/L were reported [Javaid *et al.* 1981].

In 24 subjects receiving continuous treatment with depot IM fluphenazine, steady-state plasma concentrations were less than 0.1 to 27.9 µg/L (median 0.5 µg/L) [Miller *et al.* 1995].

Of 12 subjects given a single oral dose of 12 mg fluphenazine hydrochloride, those given an immediate-release formulation had peak plasma concentrations of 2.3 µg/L at 2.8 h and those given a slow-release formulation had a peak plasma concentration of 1.2 µg/L at 4.6 h. The plasma level in the same subjects 10 min after being given an IV bolus dose of 2.5 mg was 0.261 mg/L [Koytchev *et al.* 1996].

Half-life Plasma half-life, fluphenazine hydrochloride about 15 h; fluphenazine decanoate about 5 to 12 days and fluphenazine enantate about 3 to 4 days, following IM injection.

Protein Binding About 99%.

Dose Usually 1 to 5 mg of fluphenazine hydrochloride daily; up to 20 mg daily has been given.

Aravagiri M *et al.* (1994). Radioimmunoassay for 7-hydroxy metabolite of fluphenazine and its application to plasma level monitoring in schizophrenic patients treated long term with oral and depot fluphenazine. *Ther Drug Monit* 16: 21–29.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Glazer WM *et al.* (1992). The determination of the steady-state pharmacokinetic profile of fluphenazine decanoate by gas chromatography/mass spectrometry detection. *Schizophr Res* 8: 111–117.

Goldstein SA, Van Vunakis H (1981). Determination of fluphenazine, related phenothiazine drugs and metabolites by combined high-performance liquid chromatography and radioimmunoassay. *J Pharmacol Exp Ther* 217: 36–43.

Javaid JI *et al.* (1981). Fluphenazine determination in human plasma by a sensitive gas chromatographic method using nitrogen detector. *J Chromatogr Sci* 19: 439–443.

Jemal M *et al.* (1987). Picogram level determination of fluphenazine in human plasma by automated gas chromatography/mass selective detection. *Biomed Environ Mass Spectrom* 14: 699–704.

Koytchev R *et al.* (1996). Absolute bioavailability of oral immediate and slow release fluphenazine in healthy volunteers. *Eur J Clin Pharmacol* 51: 183–187.

Luo JP *et al.* (1997). Sensitive method for the simultaneous measurement of fluphenazine decanoate and fluphenazine in plasma by high-performance liquid chromatography with coulometric detection. *J Chromatogr B Biomed Sci Appl* 688: 303–308.

McKay G *et al.* (1983). Subnanogram determination of fluphenazine in human plasma by gas chromatography mass spectrometry. *Biomed Mass Spectrom* 10: 550–555.

Midha KK *et al.* (1983). Kinetics of oral fluphenazine disposition in humans by GC-MS. *Eur J Clin Pharmacol* 25: 709–711.

Miller RS *et al.* (1995). Monitoring plasma levels of fluphenazine during chronic therapy with fluphenazine decanoate. *J Clin Pharm Ther* 20: 55–62.

Whelpton R, Curry SH (1976). Methods for study of fluphenazine kinetics in man. *J Pharm Pharmacol* 28: 869–873.

Flupirtine

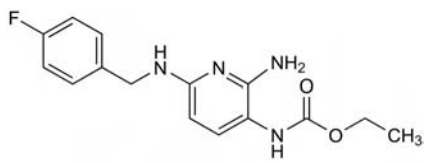
Analgesic

$C_{15}H_{17}FN_4O_2$ = 304.3

CAS—56995-20-1

IUPAC Name Ethyl *N*-[2-amino-6-[(4-fluorophenyl)methylamino]pyridin-3-yl]carbamate

Synonyms [2-Amino-6-[[(4-fluorophenyl)methyl]amino]-3-pyridinyl]carbamic acid ethyl ester; D-9998.



Chemical Properties A crystalline powder. Mp 115° to 116°. In 5% ethanol solution is colourless, turning green on exposure to air for 20 h. Log *P* (octanol/water), 2.66. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Flupirtine Hydrochloride

$C_{15}H_{17}FN_4O_2,HCl$ = 340.8

Chemical Properties A crystalline powder. Mp 214° to 215°.

Flupirtine Maleate

$C_{15}H_{17}FN_4O_2,C_4H_4O_4$ = 420.4

CAS—75507-68-5

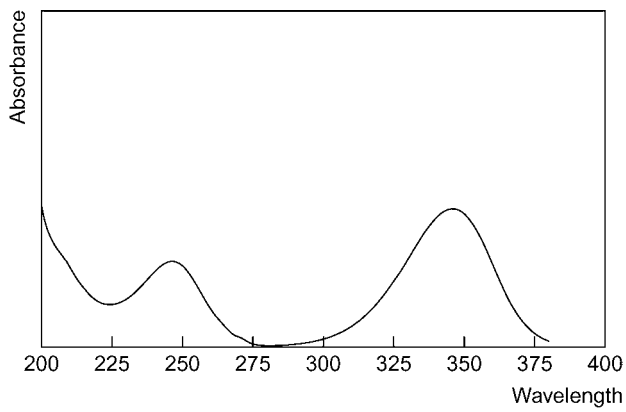
Synonym W-2964M

Proprietary Names *Katadolon*; *Trancopal Dolo*.

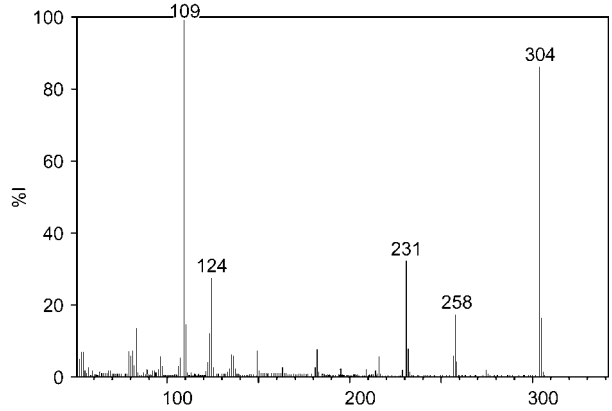
Chemical Properties A colourless, crystalline powder. Mp 175.5° to 176°. Freely soluble in water.

Gas Chromatography System GA—RI 2603.

Ultraviolet Spectrum Principal peaks at 247, 346 nm.



Mass Spectrum Principal ions at *m/z* 109, 304, 231, 124, 258, 110, 83, 123.



Quantification

Blood **HPLC** Column: ODS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phases: (A) 10 mmol/L phosphate buffer (pH 3), (B) acetonitrile : methanol (50:50). Elution programme: (A:B) (40:60) to (30:70) over 8 min, held 3 min, flow rate 1.4 mL/min. Internal standard (IS): D-09925. Fluorescence detection (λ_{ex}=323 nm, λ_{em}=380 nm). Retention time: flupirtine, 6.0 min; flupirtine acetylated metabolite, 4.4 min; IS, 9.38 min. Limit of quantification, 0.025 mg/L [Niebch *et al.* 1992].

Plasma **HPLC** Column: ODS Ultrasphere (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 5 mmol/L phosphate buffer (pH 6.7, 32:32:36), flow rate 1.4 mL/min. IS: flupirtine dimethyl derivative. Fluorescence detection (λ_{ex}=323 nm, λ_{em}=370 nm). Retention time: flupirtine, 3.73 min; IS, 6.08 min. Limit of detection, flupirtine 0.03 mg/L and acetylated metabolite 0.5 mg/L [Narang *et al.* 1984].

Urine **HPLC** See Plasma [Narang *et al.* 1984].

Disposition in the Body Flupirtine is almost completely absorbed and undergoes oxidative degradation and acetylation of the hydrolysis product, in the liver, to two major metabolites; *p*-fluorohippuric acid and an acetylated metabolite, respectively. 72% of a dose is excreted in urine as the parent drug and as metabolites, and 18% in faeces. It is known to cross the blood–brain barrier and appears quickly in CSF. <0.005% of a dose is secreted in breast milk.

Therapeutic Concentration

Healthy subjects aged between 18 and 35 years, and 66 and 83 years and patients with renal impairment (creatine clearance 44 to 99 mL/min) were administered a single 100 mg dose of flupirtine. Peak plasma concentrations of 0.77, 1.12 and 0.72 mg/L were reached within 1.6 to 1.8 h [Abrams *et al.* 1988].

Toxicity Moderately toxic by ingestion and subcutaneous routes.

Half-life 8.5 to 10.7 h, depending on type of administration.

Bioavailability Approximately 90% (oral administration); 73% (suppositories).

Volume of Distribution 1.15 to 1.16 L/kg.

Clearance 16.5 L/h.

Protein Binding 80 to 90% bound to albumin proteins.

Dose Usual oral dose of 100 mg three or four times a day is administered in the maleate form with doses up to 600 or 900 mg daily.

Abrams SM *et al.* (1988). Pharmacokinetics of flupirtine in elderly volunteers and in patients with moderate renal impairment. *Postgrad Med J* 64: 361–363.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Narang PK *et al.* (1984). Quantitation of flupirtine and its active acetylated metabolite by reversed-phase high-performance liquid chromatography using fluorometric detection. *J Chromatogr* 305: 135–143.

Niebch G *et al.* (1992). Dose-proportional plasma levels of the analgesic flupirtine maleate in man. Application of a new HPLC assay. *Arzneimittelforshung* 42: 1343–1345.

Fluquinconazole

Insecticide

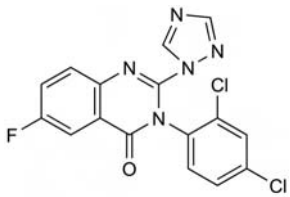
$C_{16}H_8Cl_2FN_5O$ = 376.2

CAS—136426-54-5

IUPAC Name 3-(2,4-Dichlorophenyl)-6-fluoro-2-(1*H*-1,2,4-triazol-1-yl)-4-(3*H*)-quinazolinone

Synonym SN-597265

Proprietary Names *Aventis*; *Castellan*; *Diablo*; *Flamenco*; *Jockey F*; *Jockey Flexi*; *Vista*.



Chemical Properties An off-white particulate solid. Mp 191.5° to 193°. Its solubility in water is 1 mg/L at 20°. Also soluble in dimethyl sulfoxide (150 g/L at 20°), acetone (44 g/L at 20°), xylene (10 g/L at 20°) and ethanol (3 g/L at 20°). Log *P* (octanol/water), 3.2.

Flurazepam

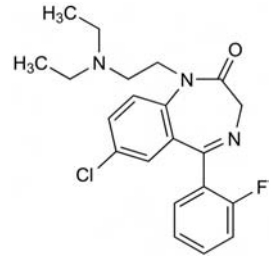
Benzodiazepine, Hypnotic

$C_{21}H_{23}ClFN_3O$ = 387.9

CAS—17617-23-1

IUPAC Name 7-Chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-3*H*-1,4-benzodiazepin-2-one

Synonym 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one



Chemical Properties White crystals. Mp 77° to 82°. Soluble in chloroform. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Flurazepam Hydrochloride

$C_{21}H_{23}ClFN_3O,2HCl$ = 460.8

CAS—1172-18-5

Synonyms Flurazepam dihydrochloride; NSC-78559; Ro-5-6901.

Proprietary Names *Dalmadorm*; *Dalmane*; *Dormodor*; *Felison*; *Felmane*; *Flunox*; *Morfex*; *Remdue*; *Somnol*; *Staurodorm*; *Valdorm*. Includes proprietary names of flurazepam monohydrochloride.

Chemical Properties An off-white to yellow crystalline powder. Mp 190° to 220°. Soluble 1 in 2 of water, 1 in 4 of ethanol, 1 in 90 of chloroform and 1 in 3 of methanol; very slightly soluble in ether.

Flurazepam Monohydrochloride

$C_{21}H_{23}ClFN_3O,HCl$ = 424.3

CAS—36105-20-1

Synonym Aminazine

Proprietary Names See above under flurazepam hydrochloride.

Chemical Properties A white crystalline powder. Very soluble in water; freely soluble in ethanol; practically insoluble in ether. pK_{a1} 1.9, pK_{a2} 8.2. Log *P* (octanol/water pH 7.4), 2.3.

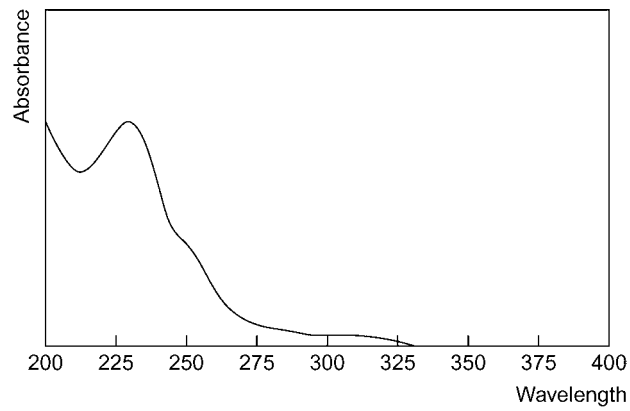
Colour Test Formaldehyde-sulfuric acid—pink.

Thin-layer Chromatography System TA—R_f 62; system TB—R_f 30; system TC—R_f 48; system TD—R_f 03; system TE—R_f 71; system TF—R_f 03; system TL—R_f 40; system TAD—R_f 41; system TAE—R_f 52; system TAF—R_f 45 (acidified iodoplatinate solution—positive).

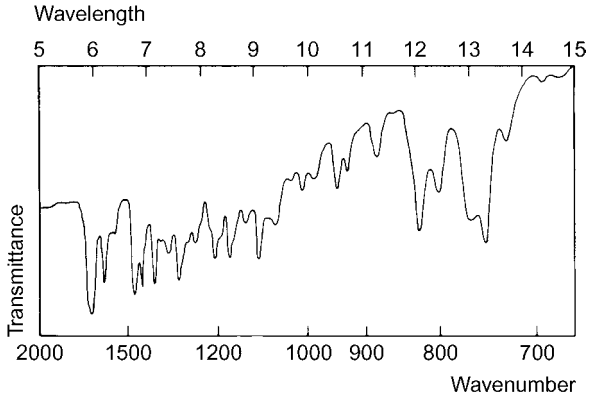
Gas Chromatography System GA—flurazepam RI 2780, M (2-OH-ethyl-) RI 2675, M (desalkyl-) RI 2470, M (desalkyl-OH-) RI 2255, M (didesethyl-) RI 2694; system GB—flurazepam RI 2896, M (desalkyl-) RI 2559, M (desalkyl-)-TMS RI 2350, M (2-OH-ethyl-) RI 2805, M (2-OH-ethyl-)-TMS RI 2778, M (bis-desethyl-) RI 2739, M (desalkyl-OH-) RI 2373; system GF—RI 3210; system GG—RI 3220.

High Performance Liquid Chromatography System HA—flurazepam *k* 1.3, M (desalkyl-) *k* 0.1; system HI—M (desalkyl-) *k* 5.19, M (OH-ethyl-) *k* 4.27; system HJ—*k* 3.19; system HK—flurazepam *k* 6.50, M (desalkyl-) *k* 1.52, M (2-OH-ethyl-) *k* 1.43; system HX—RI 397; system HY—RI 305; system HZ—RT 4.2 min; system HAI—RRT 12.03 (relative to nitrazepam); system HAX—RT 10.5 min; system HAY—RT 5.5 min; system HAZ—*k* 0.73; system HBH—*k* 12.98; system HBI—*k* 3.12.

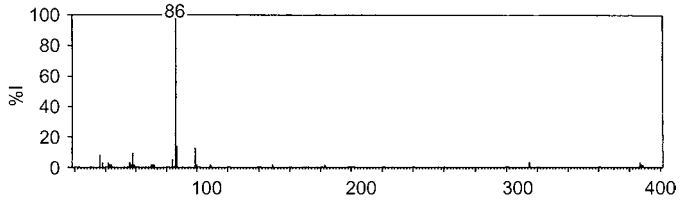
Ultraviolet Spectrum Aqueous acid—236 (A^1_1= 620b), 284 nm; aqueous alkali—231 (A^1_1= 856b), 312 nm (A^1_1= 53b).



Infrared Spectrum Principal peaks at wavenumbers 1672, 1613, 1316, 1211, 1171, 1100 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 87, 99, 58, 84, 387, 315, 56 (flurazepam); 260, 259, 288, 287, 261, 289, 262, 290 (N^1-desalkylflurazepam); 30, 313, 246, 211, 273, 274, 302, 183 (didesethylflurazepam); 288, 273, 331, 287, 304, 290, 289, 275 (N^1-[2-hydroxyethyl]flurazepam).



Quantification

Blood **GC** Columns: DB-1 and DB-1701 (15 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 28° at 30°/min to 230° at 2°/min to 300° at 30°/min for 1 min or 120° for 1 min to 230° at 40°/min to 280° at 8°/min for 9 min. ECD. Limit of detection, 0.033 μmol/L (DB-1701) [Gjerde *et al.* 1992]. Column: SE-54 5% phenylmethylsiloxane (25 m × 0.31 mm i.d., 0.17 μm). Carrier gas: He, 2 to 3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 7.65 min. Limit of detection not reported [Lillsunde, Seppälä 1990]. ECD. Limit of detection, 0.5 μg/L [Hasegawa, Matsubara 1975].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1.96 μg/L, limit of detection, 0.65 μg/L [Papoutsis *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 10 μg/L, limit of detection, 10 μg/L for desalkylflurazepam [Tiscione *et al.* 2008]. Column: DB-35 (300 × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15°/min for 4 min. EI ionisation, SIM acquisition mode. Limit of quantification not reported [Gunnar *et al.* 2004]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 100 μg/L [Pirnay *et al.* 2002].

LC-MS Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5 to 50 : 25 : 25 at 7 min to 10 : 45 : 45 at 27 min for 3 min to 95 : 5 : 5 at 31 min), flow rate 0.45 mL/min. APCI. Limit of quantification, 2 μg/L [Dussy *et al.* 2006]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.006 mol/L formic acid (pH 3.0) : methanol (70 : 30 to 60 : 40 at 5 min to 50 : 50 in 25 min to 40 : 60 at 30 min for 5 min to 70 : 30 at 36 min for 9 min), flow rate 0.2 mL/min. Limit of quantification, 5.5 μg/L, limit of detection, 1.6 μg/L [Smink *et al.* 2004].

Plasma **GC** Column: 10% OV-101 on 80/100 Chromosorb WHP (1.22 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 50 mL/min. Temperature: 310°. Limit of detection, 1 μg/L [Burstein *et al.* 1988]. Column: DB-1701 (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: H_2, 1.0 or 1.2 bar. Temperature programme: 150° for 0.5 min to 260° at 80°/min for 8 min or 140° for 0.5 min to 220° at 10°/min to 260° at 5°/min for 2 min. ECD. Limit of quantification, 1 μg/L [Salama *et al.* 1988]. Column: 3% OV-17 on 100/120 Gas-Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 22 mL/min. Temperature programme: 255°. ECD. Retention time: 2.02 min. Limit of detection, 3 μg/L [Cooper, Drolet 1982].

GC-MS See Blood [Gunnar *et al.* 2004].

LC-MS Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.0025 mg/L, limit of detection, 0.0025 mg/L [Kratzsch *et al.* 2004].

Serum **GC-MS** See Blood [Gunnar *et al.* 2004].

HPLCSee Blood [Dussy *et al.* 2006]

LC-MS Unison UK-C18 RP ODS (150 × 2 mm i.d., 3 μm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 over 20 min to 20 : 80 over 5 min), flow rate 0.25 mL/min. MRM acquisition mode. Limit of quantification, 1.3 μg/L, limit of detection, 0.4 μg/L [Nakamura *et al.* 2009].

Urine **GC** See Blood [Hasegawa, Matsubara 1975].

GC-MS Column: SGE BP1 capillary (300 × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° to 250° at 15°/min for 8 min, to 300° at 10°/min for 2 min. Limit of detection, 1.1 μg/L [Borrey *et al.* 2001].

Oral Fluid **GC-MS** Column: methyl silicone capillary (165 × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70°. EI ionisation mode, SIM acquisition mode. Limit of quantification, 10.9 μg/L, limit of detection, 3.6 μg/L [Pujadas *et al.* 2007].

LC-MS Zorbax Eclipse XDB C_{18} (50 × 4.6 mm i.d., 1.8 μm). Mobile phase: 20 mmol/L ammonium formate : acetonitrile (50 : 50), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Moore *et al.* 2007].

Disposition in the Body Flurazepam is readily absorbed after oral administration; ~70% of a dose is metabolised during the first pass through the liver. The major metabolites in blood are N^1-desalkylflurazepam and N^1-(2-hydroxyethyl) flurazepam, which are both pharmacologically active and accumulate on daily administration; 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2-oxo-2*H*-1,4-benzodiazepin-1-acetaldehyde has also been detected in plasma. Other active metabolites found in urine include monodesethyl- and didesethylflurazepam and N^1-desalkyl-3-hydroxyflurazepam, but these are not detectable in blood. Up to 60% of a dose may be excreted in the urine in 48 h, with ~40% of the dose as a glucuronide/sulfate conjugate of N^1-(2-hydroxyethyl)flurazepam and ~4% as conjugated N^1-desalkyl-3-hydroxyflurazepam and conjugated flurazepam-N^1-ylacetic acid; only trace amounts of unchanged flurazepam and the unconjugated metabolites are present. N^1-(Hydroxyethyl)-3-hydroxyflurazepam has also been detected. Approximately 9% of a dose is eliminated in the faeces.

Therapeutic Concentration Flurazepam is rapidly metabolised and hence blood concentrations of the unchanged drug are low and quickly decrease; for normal doses, the peak concentration is usually in the range 0.0005–0.03 mg/L. The blood concentration of N^1-desalkylflurazepam is usually in the range 0.04–0.06 mg/L.

Following a single oral dose of 30 mg flurazepam hydrochloride to 2 subjects, peak blood concentrations were attained as follows: flurazepam 0.001 and 0.005 mg/L at 3 h, N^1-(2-hydroxyethyl)flurazepam 0.005 and 0.01 mg/L at 3 h and N^1-desalkylflurazepam 0.010 and 0.012 mg/L at 24 h [De Silva *et al.* 1974].

Following daily oral doses of 15 mg to 18 subjects, steady-state plasma concentrations of N^1-desalkylflurazepam of 0.03 0.15 mg/L (mean, 0.08) were reported [Greenblatt *et al.* 1981].

Toxicity Blood concentrations >0.2 mg/L flurazepam or 0.5 mg/L N^1-desalkylflurazepam may be toxic, and blood concentrations >0.5 mg/L flurazepam may be fatal.

In a fatality attributed to flurazepam overdose, the following postmortem concentrations were reported for flurazepam, N^1-desalkylflurazepam, and N^1-(2-hydroxyethyl)flurazepam, respectively: blood 0.51, 0.14, 9 mg/L; urine 7, 3.9, 98 mg/L. The amount ingested was estimated to be more than 2.4 g [Aderjan, Mattern 1979].

In a 5-year-old child who died following the ingestion of flurazepam and phenobarbital, the following postmortem tissue concentrations were reported for flurazepam, N^1-desalkylflurazepam and N^1-(2-hydroxyethyl)flurazepam, respectively: blood 3.2, 1.8 and 2.5 mg/L; brain 0.8, 0.7 and 0.7 μg/g; kidney 0.9, 0.6 and 1.1 μg/g; and liver 2.7, 3.1 and 3.5 μg/g. Phenobarbital concentrations were consistent with a therapeutic dose and low concentrations of phenytoin were also detected [Ferrara *et al.* 1979].

In a 52-year-old female whose death was attributed to suicidal ingestion of up to 2.2 g flurazepam, the following postmortem tissue concentrations were reported: femoral blood 5.5 mg/L, liver 130 μg/g, bile 33 mg/L, vitreous humour 1.3 mg/L, urine 3.3 mg/L, gastric contents 600 mg (total); desalkylflurazepam was also detected in blood, liver, bile and vitreous humour, but at concentrations much lower than the parent compound [McIntyre *et al.* 1994].

A 68-year-old woman was found dead at home with the following flurazepam concentrations: heart blood 2.8 mg/L, bile 323 mg/L and urine 172 mg/L [Martello *et al.* 2006].

Half-life Plasma : flurazepam 2–3 h, N^1-desalkylflurazepam 2–5 days, N^1-(2-hydroxyethyl)flurazepam 10–20 h.

Protein Binding Flurazepam ~97%, N^1-desalkylflurazepam ~98% and N^1-(2-hydroxyethyl)flurazepam ~90%.

Dose The equivalent of 15 to 30 mg of flurazepam, as a hypnotic.

Aderjan R, Mattern R (1979). [A fatal monointoxication by flurazepam (Dalmadorm). Problems of the toxicological interpretation (author's trans.)]. *Arch Toxicol* 43: 69–75.

Borrey D *et al.* (2001). Simultaneous determination of fifteen low-dosed benzodiazepines in human urine by solid-phase extraction and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765: 187–197.

Burstein ES *et al.* (1988). Quantitation of flurazepam and three metabolites by electron capture gas liquid chromatography. *J Anal Toxicol* 12: 122–125.

Cooper SF, Drolet D (1982). Gas–liquid chromatographic determination of flurazepam and its major metabolites in plasma with electron-capture detection. *J Chromatogr* 231: 321–331.

De Silva JA *et al.* (1974). Spectrofluorodensitometric determination of flurazepam and its major metabolites in blood. *J Pharm Sci* 63: 1837–1841.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

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Ferrara SD *et al.* (1979). Concentrations of phenobarbital, flurazepam, and flurazepam metabolites in autopsy cases. *J Forensic Sci* 24: 61–69.

Gjerde H *et al.* (1992). Simultaneous determination of common benzodiazepines in blood using capillary gas chromatography. *J Pharm Biomed Anal* 10: 317–322.
Greenblatt DJ *et al.* (1981). Kinetics and clinical effects of flurazepam in young and elderly noninsomniacs. *Clin Pharmacol Ther* 30: 475–486.
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Hasegawa M, Matsubara I (1975). Metabolic fates of flurazepam. I. Gas chromatographic determination of flurazepam and its metabolites in human urine and blood using electron capture detector. *Chem Pharm Bull (Tokyo)* 23: 1826–1833.
Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.
Lillsunde P, Seppälä T (1990). Simultaneous screening and quantitative analysis of benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen–phosphorus detection. *J Chromatogr* 533: 97–110.
Martello S *et al.* (2006). Acute flurazepam intoxication: a case report. *Am J Forensic Med Pathol* 27: 55–57.
McIntyre IM *et al.* (1994). A fatality due to flurazepam. *J Forensic Sci* 39: 1571–1574.
Moore C *et al.* (2007). Determination of benzodiazepines in oral fluid using LC-MS-MS. *J Anal Toxicol* 31: 596–600.
Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography–tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.
Papoutsis II *et al.* (2010). Development and validation of an EI-GC-MS method for the determination of benzodiazepine drugs and their metabolites in blood: applications in clinical and forensic toxicology. *J Pharm Biomed Anal* 52: 609–614.
Pirnay S *et al.* (2002). Sensitive method for the detection of 22 benzodiazepines by gas chromatography–ion trap tandem mass spectrometry. *J Chromatogr A* 954: 235–245.
Pujadas M *et al.* (2007). A simple and reliable procedure for the determination of psychoactive drugs in oral fluid by gas chromatography–mass spectrometry. *J Pharm Biomed Anal* 44: 594–601.
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Smink BE *et al.* (2004). Quantitative analysis of 33 benzodiazepines, metabolites and benzodiazepine-like substances in whole blood by liquid chromatography–(tandem) mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 13–20.
Tiscione NB *et al.* (2008). Quantitation of benzodiazepines in whole blood by electron impact-gas chromatography–mass spectrometry. *J Anal Toxicol* 32: 644–652.

Flurbiprofen

Analgesic

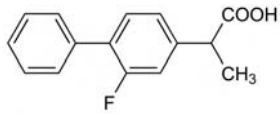
$C_{15}H_{13}FO_2 = 244.3$

CAS—5104-49-4

IUPAC Name 2-(3-Fluoro-4-phenylphenyl)propanoic acid

Synonym 2-Fluoro-α-methyl-[1,1′-biphenyl]-4-acetic acid

Proprietary Names *Ansaid*; *Antadys*; *Benactiv*; *Cebutid*; *Edolfene*; *Evril*; *Fenomel*; *Flurofen*; *Froben*; *Ocufen*; *Ocuflur*; *Reupax*; *Strefen*; *Strepfen*; *Transact*.



Chemical Properties A colourless crystalline solid. Mp about 110°. Slightly soluble in water; freely soluble in most organic solvents. Log *P* (octanol/water), 4.2.

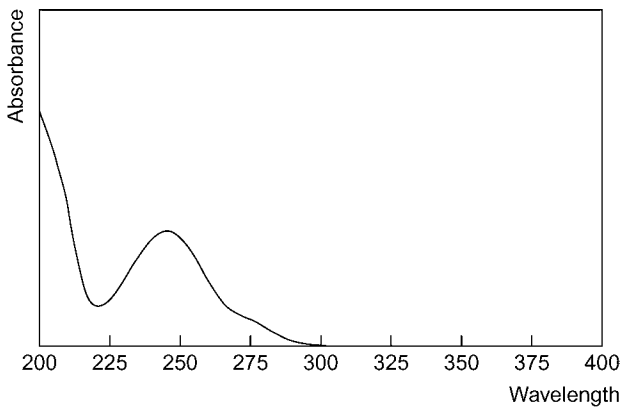
Colour Tests Liebermann's reagent—brown; Marquis test—red.

Thin-layer Chromatography System TD—R_f 0.30; system TE—R_f 0.06; system TF—R_f 0.30; system TG—R_f 0.16; system TAD—R_f 0.45; system TAJ—R_f 0.47; system TAK—R_f 0.69; system TAL—R_f 0.91 (Ludy Tenger reagent, orange).

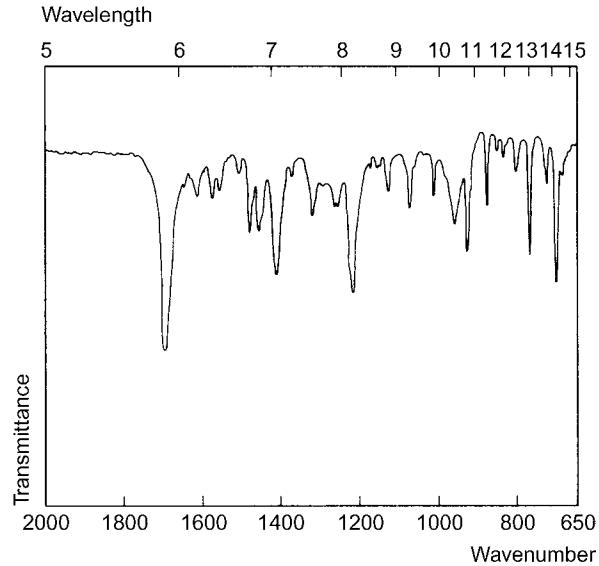
Gas Chromatography System GA—flurbiprofen RI 1900, flurbiprofen-Me RI 1885, M (OH-)-Me_2 RI 2180, M (OH-methoxy)-Me_2 RI 2310; system GD—methyl derivative RRT 1.30 (relative to n-$C_{16}H_{34}$); system GL—flurbiprofen-Me RI 1880, M (OH-)-Me_2 RI 2180.

High Performance Liquid Chromatography System HV—RRT 0.89 (relative to meclofenamic acid); system HX—RI 585; system HZ—retention time 11.8 min; system HAA—retention time 21.3 min; system HAX—retention time 8.0 min; system HAY—retention time 8.9 min.

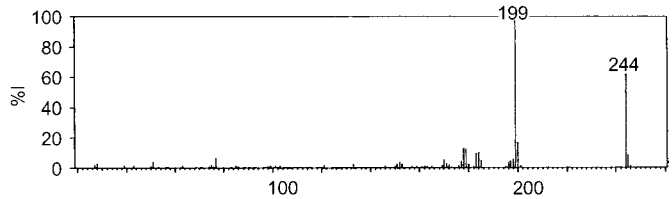
Ultraviolet Spectrum Aqueous acid—247 nm (A_1^1=787b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1695, 1220, 707, 930, 773, 960 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 199, 244, 200, 178, 179, 184, 183, 245.



Quantification

Plasma **GC** ECD. Limit of detection, 50 µg/L [Kaiser *et al.* 1974].

GC-MS Limit of detection, <0.5 mg/L [Satomoto *et al.* 2002]. Limit of detection, 1 µg/L [Kawahara *et al.* 1981].

HPLC Fluorescence detection. Flurbiprofen and 4′-hydroxyflurbiprofen, limit of detection, <0.25 mg/L for flurbiprofen [Hutzler *et al.* 2000]. UV detection. Flurbiprofen enantiomers, limit of detection, <50 µg/L [Geisslinger *et al.* 1992]. Fluorescence detection. Flurbiprofen enantiomers and metabolites [Knadler, Hall 1989]. UV detection. Flurbiprofen and ibuprofen, limit of detection, 200 µg/L [Askholt, Nielsen-Kudsk 1986].

Serum **HPLC** UV detection. Limit of detection, 40 µg/L [Snider *et al.* 1981].

Urine **HPLC** See Plasma [Hutzler *et al.* 2000]. UV detection. Flurbiprofen and other NSAIDs, limit of detection, 0.05 mg/L [Hirai *et al.* 1997]. See Plasma [Knadler, Hall 1989].

Crevicular Fluid **HPLC** [Heasman *et al.* 1990].

Ocular Fluids **HPLC** UV detection. Flurbiprofen and diclofenac, limit of detection, 3 to 4 µg/L [Riegel, Ellis 1994].

Disposition in the Body Readily absorbed after oral administration. About 95% of a dose is excreted in the urine in 24 h, mainly as the 4′-hydroxy, 3′,4′-dihydroxy and 4′-methoxy metabolites, which are excreted partly as conjugates; about 25% of a dose is excreted as unchanged drug.

Therapeutic Concentration

After a single oral dose of 100 mg, administered to 6 subjects, peak serum concentrations of 9.1 to 16.6 mg/L (mean 12) were attained in about 1.5 h [Cardoe *et al.* 1977].

A single oral dose of 200 mg administered as a sustained-release capsule produced peak plasma concentrations of 4 to 10 mg/L and 4 to 8 mg/L, respectively, in 10 young and 9 elderly subjects at ≈6 and 9 h. On continuing the same dosage once daily, a mean steady-state level of ≈6 mg/L was achieved in all subjects [Hamdy *et al.* 1990].

Half-life Plasma half-life, 2 to 6 h (mean 3.5).

Volume of Distribution About 0.1 L/kg.

Clearance Plasma clearance, about 0.3 mL/min/kg.

Protein Binding About 99%.

Note For a review of the pharmacokinetics of flurbiprofen, see Brogden *et al.* [1979] and Davies [1995].

Dose 150 to 300 mg daily.

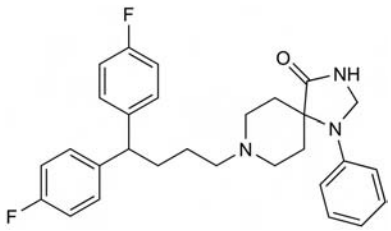
Askholt J, Nielsen-Kudsk F (1986). Rapid HPLC-determination of ibuprofen and flurbiprofen in plasma for therapeutic drug control and pharmacokinetic applications. *Acta Pharmacol Toxicol (Copenh)* 59(5): 382–386.
Brogden RN *et al.* (1979). Flurbiprofen: a review of its pharmacological properties and therapeutic use in rheumatic diseases. *Drugs* 18: 417–438.
Cardoe N *et al.* (1977). Serum concentrations of flurbiprofen in rheumatoid patients receiving flurbiprofen over long periods of time. *Curr Med Res Opin* 5: 21–25.

Davies NM (1995). Clinical pharmacokinetics of flurbiprofen and its enantiomers. *Clin Pharmacokinet* 28: 100–114.
Geisslinger G *et al.* (1992). Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma. *J Chromatogr* 573: 163–167.
Hamdy RC *et al.* (1990). A multiple dose pharmacokinetic and tolerance study of once daily 200 mg sustained-release flurbiprofen capsules in young and very elderly patients. *Eur J Clin Pharmacol* 39: 267–270.
Heasman PA *et al.* (1990). Flurbiprofen in human crevicular fluid analyzed by high-performance liquid chromatography. *J Periodontal Res* 25: 88–92.
Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692: 375–388.
Hutzler JM *et al.* (2000). Sensitive and specific high-performance liquid chromatographic assay for 4′-hydroxyflurbiprofen and flurbiprofen in human urine and plasma. *J Chromatogr B Biomed Sci Appl* 749: 119–125.
Kaiser DG *et al.* (1974). GLC determination of dl-2-(2-fluoro-4-biphenylyl)propionic acid (flurbiprofen) in plasma. *J Pharm Sci* 63: 567–570.
Kawahara K *et al.* (1981). Determination of flurbiprofen in human plasma using gas chromatography-mass spectrometry with selected ion monitoring. *J Chromatogr* 223(1): 202–207.
Knadler MP, Hall SD (1989). High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine. *J Chromatogr* 494: 173–182.
Riegel M, Ellis PP (1994). High-performance liquid chromatographic assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids. *J Chromatogr B Biomed Appl* 654: 140–145.
Satomoto M *et al.* (2002). [A simple method for determination of flurbiprofen in human plasma by gas chromatography-mass spectrometry]. *Masui* 51: 431–434.
Snider BG *et al.* (1981). Determination of flurbiprofen and ibuprofen in dog serum with automated sample preparation. *J Pharm Sci* 70: 1347–1349.

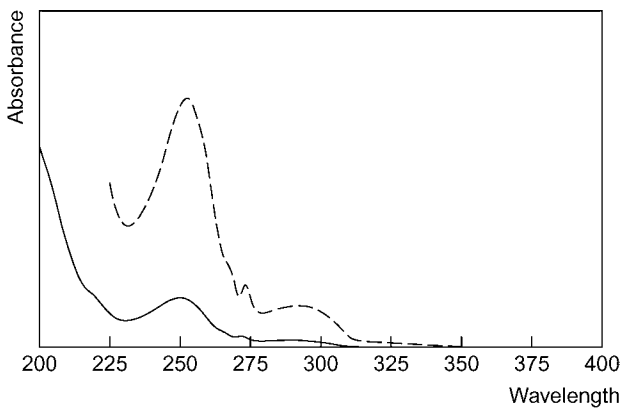
Fluspirilene

Tranquilliser
$C_{29}H_{31}F_2N_3O = 475.6$
CAS—1841-19-6

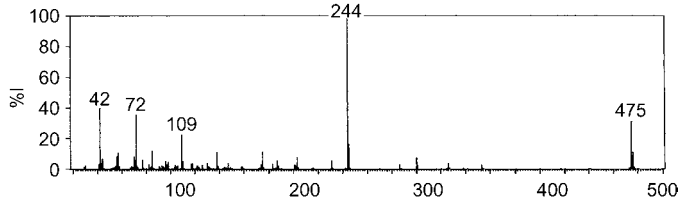
IUPAC Name 8-[4,4-bis(4-Fluorophenyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one
Proprietary Names *Fluspi*; *Imap*; *Kivat*; *Redeptin*.



Chemical Properties A white to yellowish, amorphous or crystalline powder. Mp 187.5° to 190°. Practically insoluble in water; slightly soluble in ethanol and ether; sparingly soluble in acetone; freely soluble in chloroform. Log *P* (octanol/water), 5.9. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.04; system TC—R_f 0.59; system TE—R_f 0.71; system TL—R_f 0.49; system TAE—R_f 0.63; system TAF—R_f 0.78.
Gas Chromatography System GA—fluspirilene RI 1017, M (*N*-desalkyl-oxo-) RI 2405, M (desamino-OH-) RI 2120, M (desamino-carboxy-) RI 2230.
High Performance Liquid Chromatography System HX—RI 538; system HAX—retention time 18.3 min; system HAY—retention time 9.8 min.
Ultraviolet Spectrum Methanol—252 (A^1_1=295a), 273, 293 nm.



Infrared Spectrum Principal peaks at wavenumbers 1710, 1505, 1596, 1220, 743, 1230 cm^{-1} (KBr disk). Polymorphism may occur.
Mass Spectrum Principal ions at *m/z* 244, 42, 72, 475, 109, 245, 85, 476.



Quantification
Plasma **HPLC-MS** Limit of detection, <21 ng/L [Swart *et al.* 1998].
Dose Usually 2 to 8 mg weekly, by deep IM injection; maximum of 20 mg weekly.

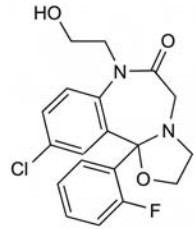
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Poster presentation at TIAFT*, Seoul: 481–486.
Swart KJ *et al.* (1998). Determination of fluspirilene in human plasma by liquid chromatography-tandem mass spectrometry with electrospray ionisation. *J Chromatogr A* 828: 219–227.

F

Flutazolam

Anxiolytic
$C_{19}H_{18}ClFN_2O_3 = 376.8$
CAS—27060-91-9

IUPAC Name 10-Chloro-11b-(2-fluorophenyl)-2,3-7,11b-tetrahydro-7-(2-hydroxyethyl)oxazolo[3,2-*d*][1,4]benzodiazepin-6-(5*H*)-one
Synonyms MS-4101; Ro-7-6102.
Proprietary Name *Coreminal*



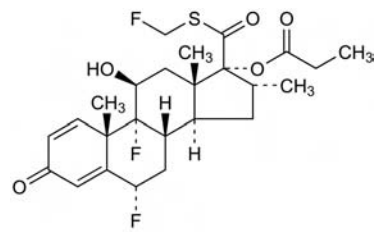
Chemical Properties White prisms from toluene. Mp 142° to 147°. Freely soluble in chloroform and ethanol; moderately soluble in acetone, benzene and methanol; practically insoluble in water. pK_a 5.4.
Thin-layer Chromatography System TE—R_f 0.68; system TAE—R_f 0.84.
Gas Chromatography System GA—RI 2460.
High Performance Liquid Chromatography System HAF—retention time 32.2 min.
Mass Spectrum Principal ions at *m/z* 289, 245, 246, 210, 259, 211, 183, 291.
Quantification
Serum **HPLC** Limit of quantification, 0.01 mg/L [Tanaka *et al.* 1996].

Tanaka E *et al.* (1996). Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-microns porous microspherical silica gel. *J Chromatogr B Biomed Appl* 682: 173–178.

Fluticasone Propionate

Antiinflammatory
$C_{25}H_{31}F_3O_5S = 500.6$
CAS—80474-14-2

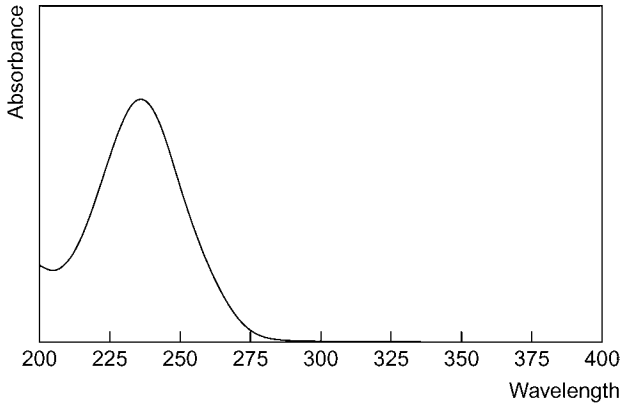
IUPAC Name (6α,11β,16α,17α)-6,9-Difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxo-propoxy)androsta-1,4-diene-17-carbothioic acid *S*-(fluoromethyl) ester
Synonym CCI-18781
Proprietary Names *Atemur*; *Axotide*; *Cutivate*; *Flixonase*; *Flixotide*; *Flonase*; *Flovent*; *Flunase*; *Flutide*; *Flutivate*.



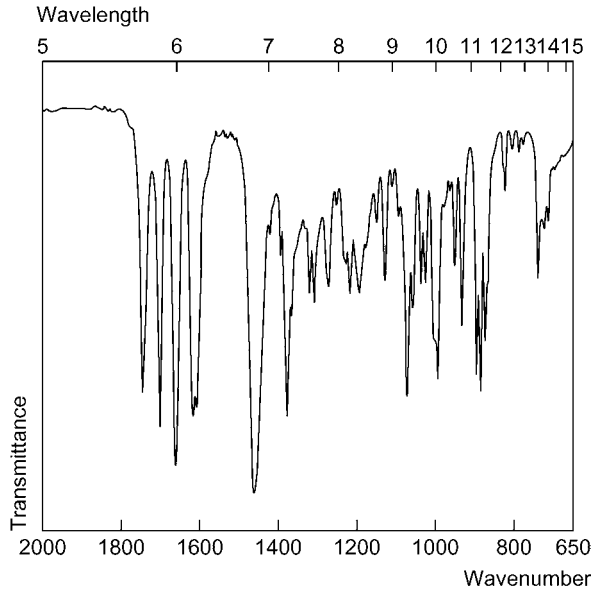
Chemical Properties A white to almost white crystalline powder. Mp 272° to 273°. Practically insoluble in water; slightly soluble in methanol and ethanol (95%); freely soluble in diemethylformamide and dimethylsulfoxide; sparingly soluble in acetone and dichloromethane.
High Performance Liquid Chromatography Column: ODS Hypersil (100 × 2.1 mm i.d., 5 µm). Mobile phase: ethanol : water (50:50), flow rate

0.45 mL/min. MS detection (APCI, SIM at *m/z* 501.2 for fluticasone propionate and 473.2 for internal standard (IS)). IS: 22*R* epimer of budesonide acetate. Retention time: fluticasone propionate, 3.09 min; IS, 3.35 min [Li *et al.* 1997].

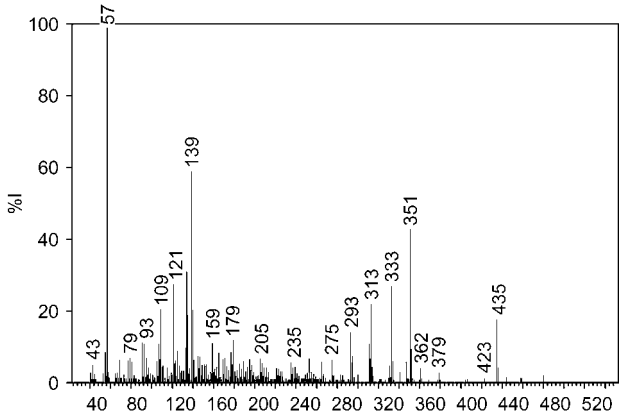
Ultraviolet Spectrum



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 57, 139, 351, 134, 121, 333, 313, 109.



Quantification

Plasma **HPLC** Limit of detection, 0.01 µg/L [Krishnaswami *et al.* 2000]. MS–MS detection. Limit of quantification, 0.025 µg/L [Laugher *et al.* 1999].

Disposition in the Body Fluticasone is poorly absorbed and undergoes extensive first-pass metabolism. The only known metabolite in humans is a 17β-carboxylic acid metabolite. Less than 5% of a dose is excreted in urine as the metabolite with the remainder being excreted in faeces as the parent drug (up to 75%) and the metabolite. Oral bioavailability is ≈1%. After IV administration, the drug is extensively administered in the body.

Therapeutic Concentration

Six healthy subjects were administered single doses of 0.25, 0.5, 1.0 and 3.0 mg fluticasone propionate. Maximum serum concentrations of 0.09 µg/L for the 0.25 mg dose and 0.40 µg/L for the 3.0 mg dose were reached within 0.5 to 1.0 h [Möllmann *et al.* 1998].

Twelve healthy males, aged between 21 and 38 years, were administered 250, 500 and 1000 µg fluticasone propionate intravenously over 3 min. Peak plasma concentrations were rapidly reached after administration was completed, 12.8, 32.8 and 63.7 µg/L, respectively [Mackie *et al.* 1996].

Toxicity Acute overdosing in excess of the recommended dose and chronic overdosing of >2 mg, over a prolonged period of time, leads to temporary adrenal suppression.

Half-life 7.8 h.

Volume of Distribution 4.2 L/kg (range 2.3 to 16.7 L/kg), also reported as 318 L.

Clearance Total blood clearance, 0.0182 L/h (range 0.0103 to 0.0284 L/h). Plasma clearance, 1.1 L/min.

Protein Binding 91%.

Dose Adults (by inhalation): usual dose of 100 to 250 µg twice a day up to 500 to 1000 µg twice a day. Children >4 years old: 50 to 100 µg twice a day. Oral dose: 5 mg four times a day.

Krishnaswami S *et al.* (2000). A sensitive LC-MS/MS method for the quantification of fluticasone propionate in human plasma. *J Pharm Biomed Anal* 22: 123–129.

Laugher L *et al.* (1999). An improved method for the determination of fluticasone propionate in human plasma. *J Pharm Biomed Anal* 21(4): 749–758.

Li YN *et al.* (1997). A sensitive method for the quantification of fluticasone propionate in human plasma by high-performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry. *J Pharm Biomed Anal* 16: 447–452.

Mackie AE *et al.* (1996). Pharmacokinetics of intravenous fluticasone propionate in healthy subjects. *Br J Clin Pharmacol* 41: 539–542.

Möllmann H *et al.* (1998). Pharmacokinetic and pharmacodynamic evaluation of fluticasone propionate after inhaled administration. *Eur J Clin Pharmacol* 53(6): 459–467.

Flutoprazepam

Anxiolytic

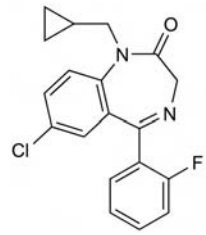
$C_{19}H_{16}ClFN_2O$ = 342.8

CAS—25967-29-7

IUPAC Name 7-Chloro-1-(cyclopropylmethyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

Synonyms KB-509; ID-1937.

Proprietary Names *Restar*; *Restas*.



Chemical Properties Crystals. Mp 118° to 122°.

Quantification

Plasma **HPLC** Column: C_{18} µBondapak (10 µm, 300 × 3.9 mm i.d.). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate : acetonitrile (pH 4.5, 50:50), flow rate 1.2 mL/min. UV detection (λ=235 nm). Retention time: flutoprazepam, 16 min; norflutoprazepam, 6 min; 3-hydroxynorflutoprazepam, 4 min; 3-hydroxyflutoprazepam, 12 min. Limit of detection of flutoprazepam 25 µg/L and 10 µg/L for norflutoprazepam [Conti *et al.* 1991].

Serum **GC-MS** Column: cross-linked methylsilicone (12 × 0.2 m i.d.). Temperature programme: 170° (1 min), increased to 270° at 8°/min, held for 10 min. Carrier gas: He, 14.4 psi (99.3 kPa). Detection: mass spectrometer (SIM, *m/z* 313 for flutoprazepam and 256 for internal standard (IS)). IS: diazepam. Retention time: flutoprazepam, 10.6 min; IS, 8.6 min. Limit of detection, 1 µg/L [Barzaghi *et al.* 1989].

HPLC UV detection (λ=235 nm). Limit of detection of norflutoprazepam, 3-hydroxyflutoprazepam and *N*-desalkyl-3-hydroxyflutoprazepam, 2 µg/L [Barzaghi *et al.* 1989].

Disposition in the Body Flutoprazepam is well absorbed after oral administration and undergoes extensive hepatic metabolism, by *N*-dealkylation, to an active metabolite, norflutoprazepam. Other possible metabolites include 3-hydroxyflutoprazepam and *N*-desalkyl-3-hydroxyflutoprazepam (both found in urine), and glucuronic acid conjugates of these. Extremely low bioavailability.

Therapeutic Concentration

Eight young, healthy males between 20 and 28 years were administered a single oral dose of flutoprazepam (2 mg), after overnight fasting. Peak plasma concentrations of norflutoprazepam ranged between 10.6 and 32.4 µg/L and were reached within 2 to 12 h [Barzaghi *et al.* 1989].

Toxicity Flutoprazepam is moderately toxic by ingestion and intraperitoneal routes.

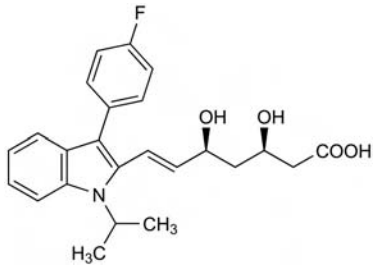
Half-life Norflutoprazepam, ≈87 ± 22 h.
Dose Usual dose of 2 to 4 mg flutoprazepam once daily.

Barzaghi N *et al.* (1989). Pharmacokinetics of flutoprazepam, a novel benzodiazepine drug, in normal subjects. *Eur J Drug Metab Pharmacokinet* 14: 293–298.
Conti I *et al.* (1991). Propranolol does not alter flutoprazepam kinetics and metabolism in the rat. *Eur J Drug Metab Pharmacokinet* 16: 53–58.

Fluvastatin

Antihyperlipoproteinaemic
$C_{24}H_{26}FNO_4$ = 411.5
CAS—93957-54-1

IUPAC Name (3*R*,5*S*,6*E*)-*rel*-7-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid

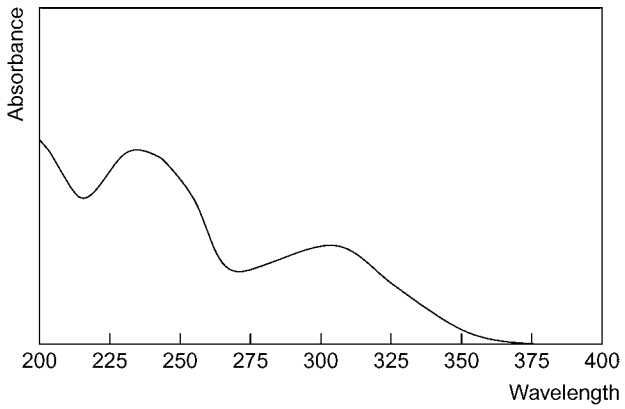


Chemical Properties pK_a 4.6. Log *P* (octanol/water), 4.85.

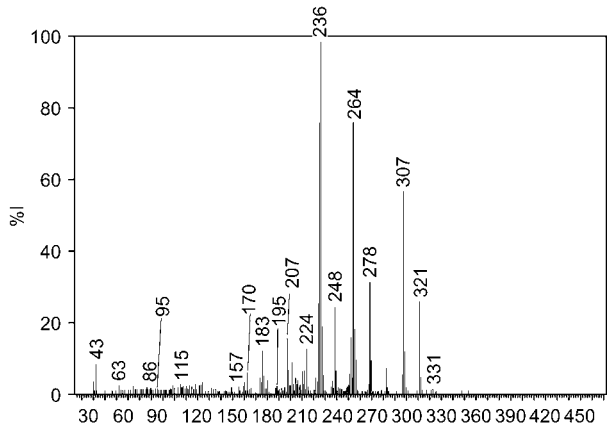
Fluvastatin Sodium

$C_{24}H_{25}FNNaO_4$ = 433.5
CAS—93957-55-2
Synonyms XU-62-320; Fluidostatin.
Proprietary Names *Canef*; *Cranoc*; *Fractal*; *Lescol*; *Lipaxan*; *LOCOL*; *Lymetel*; *Primesin*; *Vastin*.
Chemical Properties A white to pale yellow, hygroscopic powder. Mp 194° to 197°. Soluble in water, ethanol and methanol.

High Performance Liquid Chromatography Column: Rx-C8 Zorbax (150 × 4.6 mm i.d., 5 µm). Temperature: 40°. Mobile phase: aqueous TBAF (tetrabutylammonium fluoride) : 0.1 mol/L phosphate buffer (pH 6.0) : methanol (15:25:60), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=305 nm, λ_{em}=390 nm). Retention time: 15 min [Toreson, Eriksson 1996].
Ultraviolet Spectrum Aqueous acid—235, 305 nm.



Mass Spectrum Principal ions at *m/z* 236, 235, 264, 235, 307, 278, 321, 248, 207.



Quantification

Plasma **HPLC** Fluorescence detection (λ_{ex}=305 nm, λ_{em}=390 nm). Limit of detection, 1.0 µg/L [Kalafsky, Smith 1993].
Disposition in the Body Fluvastatin is rapidly and completely absorbed after oral administration and undergoes extensive first pass metabolism in the liver, the primary site of action. Hydroxylation of the indole rings at the 5- and 6- positions as well as *N*-dealkylation and β-oxidation of the side chains occurs. The main metabolite, *N*-desisopropylpropionic acid, is inactive. The active metabolite, fluvastatin lactone, is rapidly eliminated and not in significant amounts in plasma. Excretion is mainly via faeces, ≈90% (<2% unchanged drug) and 6% in urine. Fluvastatin is secreted in breast milk.

Therapeutic Concentration

Twelve healthy males, aged between 18 and 32 years, were administered a single 2 or 10 mg dose of fluvastatin sodium. Peak plasma concentrations of 5.6 ± 1.8 and 23 ± 4.5 µg/L, respectively were reached within 0.5 h. 6 healthy males, between 22 and 44 years, were administered a daily dose of 40 mg for 6 days in a multiple dose study. Peak concentrations of 438 ± 92 µg/L were observed [Tse *et al.* 1992].

Toxicity There are no clinically significant adverse reactions with a 60 mg dose of fluvastatin sodium (the maximum dose used in healthy volunteers). Potential hazards to the fetus.
Bioavailability Approximately 24%.
Half-life 0.5 to 1.0 h, also reported as 2.3 ± 0.9 h.
Volume of Distribution Steady state, 0.35 to 0.42 L/kg, also reported as 34.4 L.
Clearance Plasma clearance, 36.6 L/h; body clearance, 0.97 L/h/kg.
Distribution in Blood Plasma : blood ratio is 1.8.
Protein Binding >98% bound.
Dose A usual dose of 20 to 40 mg fluvastatin sodium is taken once daily and can be increased at intervals of up to 4 weeks, to a maximum of 40 mg twice daily.

Tse FL *et al.* (1992). Pharmacokinetics of fluvastatin after single and multiple doses in normal volunteers. *J Clin Pharmacol* 32: 630–638.
Kalafsky G, Smith HT (1993). High-performance liquid chromatographic method for the determination of fluvastatin in human plasma. *J Chromatogr* 614: 307–313.
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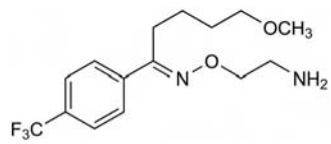
Fluvoxamine

Antidepressant, Selective Serotonin Reuptake Inhibitor (SSRI)
$C_{15}H_{21}F_3N_2O_2$ = 318.3
CAS—54739-18-3

IUPAC Name 2-[(*E*)-[5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]amino]oxyethanamine
Synonym (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime



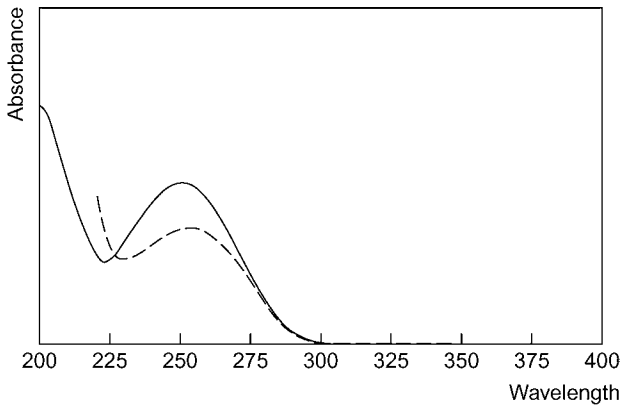
Chemical Properties pK_a 8.7. Log *P* (*n*-heptane/water), 0.04, (octanol/pH 7.2), 1.34 [Kristensen *et al.* 2002]. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005]. Stable in plasma and oral fluid after 3 freeze-thaw cycles [De Castro *et al.* 2008]. Stock solutions were stable for 15 days when stored at 4°. Stable in plasma and urine stored at −20° after 3 freeze-thaw cycles or at room temperature for 24 h. Stable in plasma and urine for 3 and 2 months, respectively [Ulu 2007].

Fluvoxamine Maleate

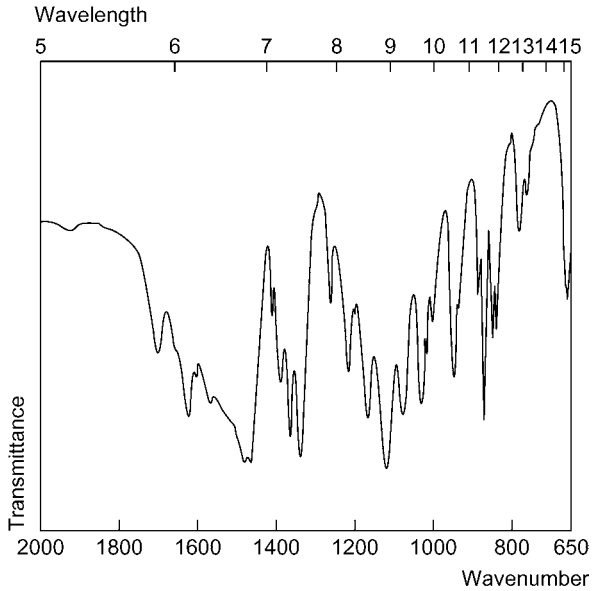
$C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ = 434.4
CAS—61718-82-9
Synonyms DU-23000; MK-264.
Proprietary Names *Avoxin*; *Depromel*; *Dumirox*; *Dumyrox*; *Faverin*; *Fevarin*; *Floxyfral*; *Luvoxe*; *Maveral*; *Servox*.
Chemical Properties White or off-white odourless crystalline powder. Mp 120° to 121.5°. Sparingly soluble in water; freely soluble in ethanol, methanol and in chloroform; practically insoluble in diethyl ether.

Thin-layer Chromatography System TAE—R_f 0.18; system TB—R_f 0.12; system TE—R_f 0.46.
Gas Chromatography System GA—RI 1885; system GB—RI 1911; system GM—RRT 0.295.
High Performance Liquid Chromatography System HAA—retention time 15.3 min; system HAX—retention time 10.0 min; system HAY—retention time 5.9 min; system HX—RI 430; system HY—RI 363; system HZ—retention time 5.6 min.
Liquid Chromatography-Mass Spectrometry Column: Phenomenex Synergi Fusion (20 × 2 mm i.d., 2.5 µm). Mobile phase: water-acetonitrile-formic acid (95 : 5 : 0.1) : acetonitrile with 0.1% formic acid (97 : 3 to 10 : 90 at 0.6 min), flow rate 1 mL/min. MRM acquisition mode [Youdim *et al.* 2008].

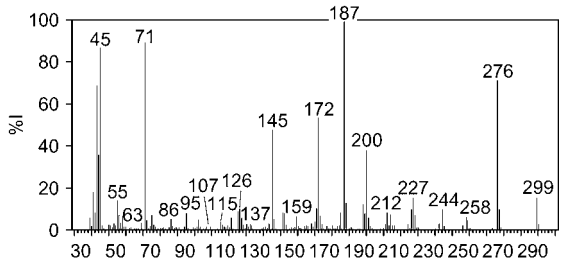
Ultraviolet Spectrum Aqueous acid—254 nm ($A_1^1 = 0.310$).



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 187, 71, 45, 276, 43, 172, 145, 200.



Quantification

Blood **GC** Column: HP cross-linked methylsilicone (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 90 µg/L, limit of detection, 27 µg/L [Martinez *et al.* 2004].

GC-MS Column: HP-1 cross linked methylsilicone (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 125° to 290° at 20°/min for 12.7 min. FID, MSD, EI ionisation, full scan mode. Limit of quantification, 0.125 mg/L [Kunsman *et al.* 1999].

LC-MS Column: XTerra RP18 (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2; 15 : 85 for 1 min to 35 : 65 over 12 min for 1 min), flow rate 1mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 µg/L [Castaing *et al.* 2007].

Plasma For a TLC method see Schweitzer *et al.* [1986].

GC-MS Column: J & W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min for 2.5 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 5 µg/L [Wille *et al.* 2007]. Column: Varian factor FOUR VF-5ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 0.5 min to 180° at 50°/min for 10 min to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection not reported [Wille *et al.* 2005]. Column: Optima 5 fused silica capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 50 mL/min. Temperature programme: 145° for 0.5 min to 207° at 30°/min for 7 min to 221° at 1°/min to 290° at 30°/min. Injector temperature: 250°. EI, SIM acquisition mode. Retention time: 20.0 min. Limit of quantification, 2 µg/L [Eap *et al.* 1996].

HPLC Column: Phenomenex C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20), flow rate 1 mL/min. UV detection (λ = 450 nm). Retention time: 4.8 min. Limit of quantification, 5 µg/L [Ulu 2007]. Column: Varian ResElut C_8 reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10.5 mmol/L phosphate buffer containing 0.12% triethylamine (pH 3.5; 30 : 70), flow rate 1.2 mL/min. UV detection (λ = 245 nm). Limit of quantification, 15 µg/L, limit of detection, 5.0 µg/L [Saracino *et al.* 2006]. Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8; 25 : 75 to 40 : 60 over 10 min to 44 : 56 in 8 min), flow rate 1.0 mL/min. DAD (λ = 240 nm). Retention time: 17.3 min. Limit of quantification 25 µg/L, limit of detection, 5 µg/L [Duverneuil *et al.* 2003]. Column: Grand Pack C_4-5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% potassium dihydrogen phosphate : acetonitrile (pH 2.5; 75 : 25), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 21.7 min. Limit of detection, 5 µg/L [Gerstenberg *et al.* 2003]. Column: Grand Pack C_4-5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% potassium dihydrogen phosphate (pH 2.5) : acetonitrile (75 : 25), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 21.7 min. Limit of detection, 10 µg/L [Ohkubo *et al.* 2003].

See also Titier *et al.* [2003], Spigset *et al.* [2001], Palego *et al.* [2000], Lucca *et al.* [2000], Härtter *et al.* [1992],Pullen & Fatmi [1992], van der Meersch-Mougeot and Diquet [1991], Foglia *et al.* [1989], and Schweitzer *et al.* [1986].

LC-MS Column: Sunfire C_{18} (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: 2 mmol/L ammonium formate buffer (pH 3.0) : acetonitrile (85 : 15 for 0.5 min to 50 : 50 at 4 min to 30 : 70 at 5 min for 3 min), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3.8 min. Limit of quantification, 10 µg/L [De Castro *et al.* 2008]. Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.2 mg/L, limit of detection, 0.13 mg/L [Shinozuka *et al.* 2006].

Serum **HPLC** Column: Shimpack CLC-ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.05 mol/L sodium phosphate buffer (pH 2.8; 72 : 28) containing 1 mL/L triethylamine, flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 470 nm, λ_{em} = 537 nm). Retention time: 4.1 min. Limit of quantification, 0.5 µg/L, limit of detection, 0.2 µg/L [Bahrami, Mohammadi 2007]. Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogenphosphate (pH 7.0) : acetonitrile (60 : 40), flow rate, 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 11.6 min. Limit of detection not reported [Frahnert *et al.* 2003]. Column: ODS C_{18} (25 cm × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L sodium phosphate buffer (pH 3.8; 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 22.4 nm). Retention time: 9.51 min. Limit of detection, 50 ng [Tournel *et al.* 2001].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.17 µg/L [Kirchherr, Kühn-Velten 2006]. Column: Uptisphere (12.5 cm × 2 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0)-acetonitrile: acetonitrile (100 : 0), flow rate 200 µL/min for 5 min to 300 µL/min in 1 min for 3 min to 200 µL/minin 0.5 min for 0.5 min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 19 nmol/L [Gutteck, Rentsch 2003].

Urine **HPLC** See Plasma. Limit of quantification, 2 µg/L [Ulu 2007].

GC-MS Column: CP-SIL 8 CB (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 2 min to 200° at 20°/min to 280° at 5°/min. EI ionisation at 70 eV. Limit of detection, 0.38 µg/lL [Salgado-Petinal *et al.* 2005]. Column: HP cross-linked methylsilicane (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of detection, 100 µg/L [Maurer, Bickeboeller-Friedrich 2000].

Milk **HPLC** Column: MOS- Hypersil C_8 reversed phase (100 × 2 mm i.d., 3 µm). Mobile phase: 0.02 mol/L monobasic potassium phosphate : *N, N*-dimethyloctylamine (pH 6.0) : acetonitrile (64 : 0.11 : 30), flow rate 0.5 mL/min. UV detection (λ = 225 nm). Retention time: 6.74 min. Limit of quantification, 2.0 µg/L, limit of detection, 1.25 µg/L [Hostetter *et al.* 2004]. Column: Select B C_{18} (250 × 4.6 mm i.d.). Mobile phase: 45 mmol/L phosphate buffer (pH 3) : acetonitrile (55 : 45), flow rate 1.5 mL/min. UV detection (λ = 210 nm) Limit of detection, 2 µg/L [Kristensen *et al.* 2002].

Oral Fluid **LC-MS** See Plasma. Limit of quantification, 2 µg/L [De Castro *et al.* 2008].

Disposition in the Body

Fluvoxamine is readily and completely absorbed after oral administration with peak plasma concentrations occurring after about 2 to 8 h (prolonged with enteric-coated tablets to 4 to 12 h). However, it undergoes significant first-pass metabolism and the bioavailability is about 53%. It is widely distributed. Fluvoxamine undergoes extensive metabolism in the liver, mainly by oxidative deamination, oxidative demethylation and *N*-acetylation to produce several inactive metabolites. The parent and all 11 metabolites have been recovered in urine; <4% as the parent drug [Benfield, Ward 1986; Perucca *et al.* 1994]. Fluvoxamine is metabolised primarily by CYP2D6 and, to a lesser, extent

CYP1A2 [Spigset *et al.* 2001]. Fluvoxamine appears to inhibit the following: CYP1A2, 2C9, and 3A4. It is distributed into breast milk [Arnold *et al.* 2000].

Therapeutic Concentration The therapeutic serum concentration is 50 to 250 μg/L.

Twenty-four healthy volunteers were administered a single oral dose of 100 mg fluvoxamine as 2 separate preparations. Mean maximum plasma concentrations were 46.2 and 48.5 μg/L, and were reached at 5.3 and 5.6 h for each formulation [Bahrami, Mohammadi 2007].

Seven healthy female volunteers were administered 50 mg fluvoxamine. The mean maximum plasma concentration was 58 μg/L at 5.2 h [Ulu 2007].

A 34-year-old female was treated with 100 mg fluvoxamine a day for severe anxiety and obsessive thinking. At 40-weeks' gestation she gave birth to a baby girl. The maternal serum concentration was 7 μg/L and the concentration in the cord blood was 5 μg/L [Hostetter *et al.* 2000]

Six healthy, young volunteers, 25 to 31 years' old, and 13 elderly individuals, aged 63 to 77 years, were administered single and multiple doses (twice daily) of 50 mg fluvoxamine, after an overnight fast. The mean plasma concentration after the single dose was 30 μg/L (range, 18 to 48 μg/L) for the younger individuals and a concentration of 93 (range 32 to 300) μg/L was observed for the multiple dosing regimen. These concentrations were observed at 6 (4 to 8) h and 5 (0 to 12) h, respectively. For the elderly individuals, after multiple dosing for 31 days, the mean concentration was 62 (range 32 to 225) μg/L at 5 h (range 4 to 12) [de Vries *et al.* 1992].

Note Enoxacin slightly inhibits the metabolism of fluvoxamine so the 2 should be combined with caution [Kunii *et al.* 2005].

Toxicity 12 g of fluvoxamine maleate has been ingested with no sequelae.

A 25-year-old female ingested 9.6 g fluvoxamine. Approximately 4 h after she presented at hospital she developed generalised tonic-clonic seizures that did not respond to lorazepam or midazolam. Her serum fluvoxamine concentration on arrival at hospital was 1970 μg/L, but she was discharged after 72 h after 24 h seizure-free [Wood *et al.* 2007].

A 32-year-old female attempted suicide with an unknown quantity of fluvoxamine and alimemazine. At 24 h after drug ingestion her serum fluvoxamine concentration was ≈500 μg/L, which decreased steadily with a half-life of 38 h to ≈150 μg/L at 96 h. After 150 μg/L the half life was ≈19 h with serum concentrations again declining to ≈5 μg/L at 168 h after drug intake [Spigset, Ohman 1996].

A 58-year-old female ingested 5.5 g of fluvoxamine in an attempted suicide. Several hours later she had severe sinus bradycardia that returned to normal over the next couple of days [Amital *et al.* 1994].

A 31-year-old female ingested 4.8 g of fluvoxamine together with 0.75 g of amitriptylline and 7 g of naproxen in an attempted suicide. She suffered no sequelae, although 1 month later she had a manic episode that was thought to have been triggered by the fluvoaxmine overdose [Lebegue 1990].

A 74-year-old female who had ingested 3 g fluvoxamine and 250 mg of temazepam was admitted to hospital unconscious. She remained unconscious for 5 days. On admission the serum fluvoxamine concentration was 160 μg/L. This increased to >1400 μg/L on day 3 [Banerjee 1988].

Note For a review of the pharmacology and toxicology of 168 cases of SSRI-related deaths, see Goeringer *et al.* [2000], and for 221 cases of overdose with fluvoxamine, see Garnier *et al.* [1993]. For a case of overdose in a 4-year-old child following the ingestion of 400 mg fluvoxamine, see Fraser and South [1999]. For cases of serotonin syndrome following fluvoxamine ingestion, see Gill *et al.* [1999], Mullins and Horowitz [1999], Ebert *et al.* [1997] or Bastani *et al.* [1996]. For a review of the safety of fluvoxamine in overdose, see Henry [1991]. There is a possible extrapyramidal interacton with clindamycin [Jakob and Wolf [2007]. Fluvoxamine is safe during pregnancy and while breastfeeding, see Gentile [2006], Kristensen *et al.* [2002] and Piontek *et al.* [2001]. Fluvoxamine may cause suicidal ideation [Chong, Cheong 1999]. For 2 cases of psychotic symptoms in schizophrenics during fluvoxamine therapy, see Silver *et al.* [1995]. For a case of neurotoxic malignant syndrome following fluvoxamine, see Reeves *et al.* [2002] or for a case of hand-foot syndrome, see Ke *et al.* [2006].

Half-life Plasma half-life, 17 to 22 h, after repeated dosing.

Volume of Distribution 25 L/kg [Goeringer *et al.* 2000].

Clearance Plasma clearance, 3 L/min (oral); 1.5 L/min (IV).

Protein Binding 77%, mostly to albumin [Perucca *et al.* 1994].

Note For a review of fluvoxamine, see Benfield and Ward [1986]. For a review of the pharmacokinetics of fluvoxamine, see Perucca *et al.* [1994] and van Harten [1995]. For reviews of the pharmacokinetics of selective serotonin reuptake inhibitors, see Catterson and Preskorn [1996], Preskorn [1997] and Baumann [1996].

Dose 100 to 300 mg daily of fluvoxamine maleate.

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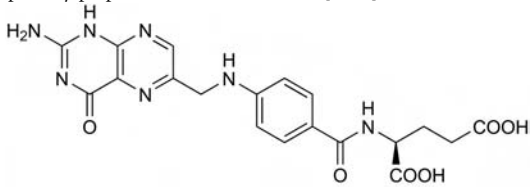
Ulu ST (2007). HPLC method for the determination of fluvoxamine in human plasma and urine for application to pharmacokinetic studies. *J Pharm Biomed Anal* 43: 1444–1451.
van der Meersch-Mougeot V, Diquet B (1991). Sensitive one-step extraction procedure for column liquid chromatographic determination of fluvoxamine in human and rat plasma. *J Chromatogr* 567: 441–449.
van Harten J (1995). Overview of the pharmacokinetics of fluvoxamine. *Clin Pharmacokinet* 29 (Suppl1): 1–9.
Wille SM *et al.* (2005). Development of a solid phase extraction for 13 'new' generation antidepressants and their active metabolites for gas chromatographic-mass spectrometric analysis. *J Chromatogr A* 1098: 19–29.
Wille SM *et al.* (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic-mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *J Chromatogr A* 1176: 236–245.
Wood DM *et al.* (2007). Status epilepticus following intentional overdose of fluvoxamine: a case report with serum fluvoxamine concentration. *Clin Toxicol (Phila)* 45: 791.
Youdim KA *et al.* (2008). An automated, high-throughput, 384 well Cytochrome P450 cocktail IC50 assay using a rapid resolution LC-MS/MS end-point. *J Pharm Biomed Anal* 48: 92–99.

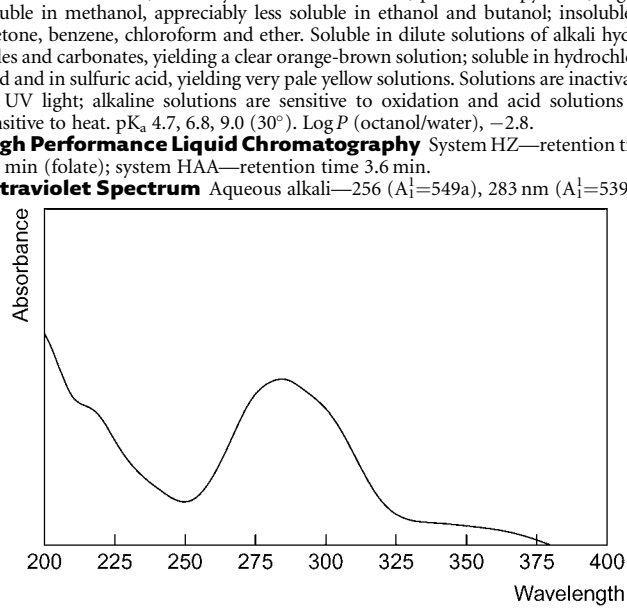
Folic Acid

Vitamin
$C_{19}H_{19}N_7O_6 = 441.4$
CAS—59-30-3

IUPAC Name (2*S*)-2-[[4-[(2-Amino-4-oxo-1*H*-pteridin-6-yl)methylamino] benzoyl]amino] pentanedioic acid
Synonyms *N*-[4-[[(2-Amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino] benzoyl]-L-glutamic acid; folacin; folinsyre; pteroylglutamic acid; pteroylmonoglutamic acid.
Proprietary Names *Acfol*; *Endofolin*; *Folacin*; *Folarell*; *Folasic*; *Fol-Asmedic*; *Folatine*; *Folavit*; *Folicare*; *Foldine*; *Folettes*; *Folsan*; *Folverlan*; *Folvite*; *Lafol*; *Lexpec*; *Megafol*; *Novo-Folacid*; *Preconceive*; *RubieFol*; *Specifoldine*. It is an ingredient of many proprietary preparations—see Sweetman [2009].



Chemical Properties A yellow to orange microcrystalline powder. Very slightly soluble in cold water; relatively soluble in acetic acid, phenol and pyridine; slightly soluble in methanol, appreciably less soluble in ethanol and butanol; insoluble in acetone, benzene, chloroform and ether. Soluble in dilute solutions of alkali hydroxides and carbonates, yielding a clear orange-brown solution; soluble in hydrochloric acid and in sulfuric acid, yielding very pale yellow solutions. Solutions are inactivated by UV light; alkaline solutions are sensitive to oxidation and acid solutions are sensitive to heat. pK_a 4.7, 6.8, 9.0 (30°). Log *P* (octanol/water), −2.8.
High Performance Liquid Chromatography System HZ—retention time 1.5 min (folate); system HAA—retention time 3.6 min.
Ultraviolet Spectrum Aqueous alkali—256 (A_1^1=549a), 283 nm (A_1^1=539a).

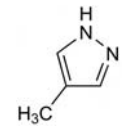


Infrared Spectrum Principal peaks at wavenumbers 1686, 1602, 1636, 1191, 1567, 1225 cm^{-1} (KBr disk).
Dose 2.5 to 20 mg daily.

Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.

Fomepizole

Alcohol Dehydrogenase Inhibitor
$C_4H_6N_2 = 82.1$
CAS—7554-65-6
IUPAC Name 4-Methyl-1*H*-pyrazole
Synonym 4-MP
Proprietary Name *Antizol*



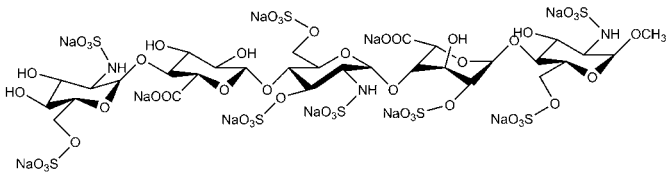
Chemical Properties Mp 15.5° to 18.5°. Soluble in water and alcohol. pK_a 2.91. Log *P* (octanol/water), 0.61.
Ultraviolet Spectrum Ethanol—220 nm; acid (6 mol/L hydrochloric acid)—226 nm.
Mass Spectrum Principal ions at *m/z* 82, 81, 54, 28, 52, 55, 53, 51.
Quantification
Plasma **GC** Column: 5% carbowax (20 mol/L) and 2% potassium hydroxide on 80 to 100 mesh Chromosorb G (HP-5) (1.22 m × 2 mm i.d.). Column, injector and detector temperature: 135°, 250° and 300°, respectively. Carrier gas: He, flow rate 30 mL/min. Detection: nitrogen–phosphorus. Internal standard (IS): 3-methylpyrazole. Retention time: fomepizole, 10 min; IS, 8 min. Limit of quantification, 0.025 mg/L, limit of detection, 0.1 mg/L [Achari, Mayersohn 1984].
HPLC Column: 100 RP-18 Lichrospher (125 × 4 mm i.d., 5 µm). Temperature: 40°. Mobile phase: acetonitrile : 5 mmol/L potassium phosphate buffer (pH 6, 7.5:92.5), flow rate 1.5 mL/min. UV detection (λ=220 nm). IS: 3-methylpyrazole. Retention time: fomepizole, 4.9 min; IS, 4.1 min. Limit of quantification, 0.3 mg/L [Jobard *et al.* 1997].
Urine **GC** See Plasma [Achari, Mayersohn 1984].
Disposition in the Body Fomepizole is rapidly distributed to total body water. It is metabolised to the primary metabolite, 4-carboxypyrazole (80 to 85% of the dose), 4-hydroxymethylpyrazole and the *N*-glucuronide conjugates of these. Fomepizole is mainly eliminated via urine with 1 to 3.5% as the unchanged drug.
Therapeutic Concentration
Fifteen healthy male volunteers, aged between 20 and 40 years, were administered 10, 20, 50 and 100 mg/kg body weight doses. Mean peak plasma concentrations of 132, 326, 759 and 1425 µmol/L were reached for the doses, respectively. All peaks were observed between 0.5 and 2 h. Each mg/kg increase in dose resulted in an increase of 13 to 16 mg/L to the peak plasma concentration [Jacobsen *et al.* 1989].
Half-life Approximately 5.2 h.
Volume of Distribution 0.6 to 0.7 L/kg.
Protein Binding Negligible.
Dose The usual dose is 5 to 15 mg/kg body weight every 12 h.

Achari R, Mayersohn M (1984). Analysis of 4-methylpyrazole in plasma and urine by gas chromatography with nitrogen-selective detection. *J Pharm Sci* 73(5): 690–692.
Jacobsen D *et al.* (1989). Non-linear kinetics of 4-methylpyrazole in healthy human subjects. *Eur J Clin Pharmacol* 37(6): 599–604.
Jobard E *et al.* (1997). High-performance liquid chromatographic determination of 4-methylpyrazole in plasma and in dialysate. *J Chromatogr B Biomed Sci Appl* 695: 444–447.

Fondaparinux Sodium

Antithrombotic, Inhibitor of Activated Factor Xa, Pentasaccharide
$C_{31}H_{43}N_3Na_{10}O_{49}S_8 = 1728.1$
CAS—114870-03-0
IUPAC Name Decasodium (2*R*,3*S*,4*S*,5*R*,6*R*)-3-[(2*R*,3*R*,4*R*,5*R*,6*R*)-5-[(2*R*,3*R*,4*R*,5*S*,6*S*)-6-carboxylato-5-[(2*R*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-3-(sulfonatoamino)-6-(sulfonatooxymethyl)oxan-2-yl]oxy-3,4-dihydroxyoxan-2-yl]oxy-3-(sulfonatoamino)-4-sulfonatooxy-6-(sulfonatooxymethyl)oxan-2-yl]oxy-4-hydroxy-6-[(2*R*,3*S*,4*R*,5*R*,6*S*)-4-hydroxy-6-methoxy-5-(sulfonatoamino)-2-(sulfonatooxymethyl) oxan-3-yl]oxy-5-sulfonatooxyoxane-2-carboxylate
Synonyms Fondaparin sodium; IC-851589; methyl *O*-2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranuronosyl-(1→4)-*O*-2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-2-*O*-sulfo-

α-L-idopyranuronysl-(1→4)-2-deoxy-2-(sulfoamino)-α-D-glucopyranoside 6-(hydrogen sulfate) decasodium salt; Org-31540; *SR*-90107A.
Proprietary Name *Arixtra*



Chemical Properties White powder [O'Neil *et al.* 2006].
Quantification
Other **HPLC** Mammalian Liver Fractions. Column: HR 5/5 mono-Q. Mobile phase: 0.5 mol/L sodium chloride : 2 mol/L sodium chloride (100 : 0 to 0 : 100 over 25 min). Radioactivity detection (^{35}S). Retention time: 18 min. Limit of quantification not reported [Lieu *et al.* 2002].
Disposition in the Body Rapidly and completely absorbed after administration by SC injection, reaching peak plasma concentrations after 1.7 h. It distributes mainly in blood and to a minor extent in extravascular fluid. Fondaparinux is eliminated unchanged in urine (up to 77% in 72 h), and elimination is prolonged in patients with renal impairment, in the elderly, and in those weighing less than 50 kg. Although an *in vitro* study reported that fondaparinux does not cross the placenta, a small study in pregnant women who had received fondaparinux found that anti-factor Xa activity was elevated in umbilical cord blood, suggesting that a small amount of placental transfer had taken place.
Therapeutic Concentration
Sixty-six healthy male and female elderly subjects (aged 60 to 85 years) were administered single IV doses of 2, 4, 5.5, 12, 16, 18 or 20 mg fondaparinux sodium or 2, 4 and 8 mg SC. Mean peak plasma concentrations were as follows:

Dose (mg)	*C_{max} (mg/L)*	*Time (h)*	*Half-life (h)*
IV			
2	0.60	—	16.9
4	1.11	—	18.4
5.5	1.16	—	18.3
12	2.49	—	16.6
16	3.17	—	16.4
18	3.49	—	17.0
20	3.84	—	16.5
SC			
2	0.28	2.2	20.7
4	0.48	2.6	19.2
8	0.91	2.3	18.8

In another study, a group of healthy male subjects (aged 18 to 40 years) was administered single doses of 2.5 and 10 mg fondaparinux sodium SC as well as 4 and 10 mg in a repeat dose regimen. The mean C_{max} values were 0.34 and 1.26 mg/L for the single doses, respectively, observed at 1.7 and 1.9 h, respectively. In the repeated dosing regimen, mean C_{max} values for the 4 and 10 mg doses were 0.56 mg/L and 1.36 to 1.52 mg/L, respectively, attained at 2.0 h and 1.8 to 2.3 h, respectively [Donat *et al.* 2002].
Bioavailability 100%.
Half-life Elimination, 17 to 21 h.
Volume of Distribution 7 to 11 L.
Clearance Plasma, 0.31 ± 0.08 to 0.51 ± 0.07 L/h.
Protein Binding Extensively bound (>97%), predominantly to antithrombin III (>94%).
Note For a review of the use of fondaparinux sodium, see Reynolds *et al.* [2004].
Dose Once daily 2.5 mg as SC injection for deep vein thrombosis prophylaxis following hip fracture, hip or knee replacement surgery, and abdominal surgery. Once daily 5 mg (bodyweight <50 kg), 7.5 mg (bodyweight 50 to 100 kg) or 10 mg (bodyweight >100 kg) SC for the treatment of deep vein thrombosis and pulmonary embolism.

Donat F *et al.* (2002). The pharmacokinetics of fondaparinux sodium in healthy volunteers. *Clin Pharmacokinet* 41: 21–9.
Lieu C *et al.* (2002). Fondaparinux sodium is not metabolised in mammalian liver fractions and does not inhibit cytochrome P450-mediated metabolism of concomitant drugs. *Clin Pharmacokinet* 41: 219–26.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.
Reynolds NA *et al.* (2004). Fondaparinux sodium: a review of its use in the prevention of venous thromboembolism following major orthopaedic surgery. *Drugs* 64: 1575–1596.

Formaldehyde

Disinfectant
CH_2O = 30.03
IUPAC Name Formaldehyde
Synonyms Formic aldehyde; methanal; methylene oxide; oxomethane.
Chemical Properties A colourless inflammable gas. Very soluble in water; soluble in ethanol and ether.

Formaldehyde Solution

CAS—50-00-0
Synonyms Formalin; formol.
Proprietary Names *Diformil*; *Emoform*; *Formadon*; *Formitrol*; *Lazerformaldehyde*; *Lysoform*; *Veracur.*
Chemical Properties An aqueous solution containing 34–38% w/w formaldehyde (CH_2O), with methanol as a stabilising agent. A colourless liquid. Approximate weight, 1.08 g/mL. Miscible with water, ethanol and acetone; immiscible with chloroform and ether. pK_a 13.3 (25°). Log *P* (octanol/water), 0.35 [Hansch *et al.* 1995].

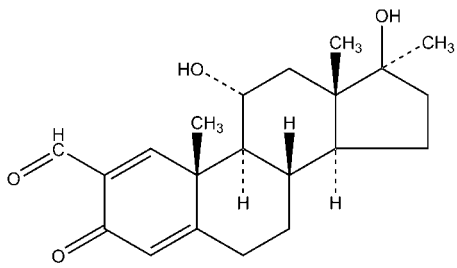
Colour Test Chromotropic acid—red.
A little resorcinol is added to a solution containing formaldehyde; after shaking, sulfuric acid is then carefully added to form a layer under the solution; a red–violet ring forms at the junction of the 2 layers.
Mass Spectrum Principal ions at *m/z* 29, 30, 28, 31, 32.
Quantification
Plasma **HPLC** Column: HP Zorbax StableBond SB-C_{18} (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : water (25 : 75), flow rate 1 mL/min. Fluorescence detection. Limit of quantification, 0.87 mg/L, limit of detection, 0.46 mg/L [Luo *et al.* 2001].
Urine **GC** Column: DB-5 (30 m × 0.53 mm, 1.5 µm). Carrier gas: He, 5.0 mL/min. Temperature: 80°. ECD. Limit of detection, 108 µg/L [Takeuchi *et al.* 2007].
Disposition in the Body Formaldehyde is rapidly metabolised in the body tissues to formic acid and methanol, especially in the liver and erythrocytes; the formic acid may then be excreted in the urine as formates or metabolised to labile methyl groups. Formaldehyde is a metabolite of methanol.
Toxicity Formaldehyde is very irritable to mucous membranes; inhalation of the vapour causes intense irritation of the respiratory tract, which may lead to bronchitis and pneumonia. Atmospheric concentrations of 2–3 ppm may cause mild irritation of the mucous membranes and exposure to concentrations of 10–20 ppm for only a few minutes may cause moderate to severe irritation. Adverse effects have been reported as a result of release of formaldehyde fumes from synthetic foam insulation. Few fatalities have been recorded but 30 mL formaldehyde solution may be fatal in an adult. The maximum permissible atmospheric concentration is 2 ppm or 3 mg/m^3.
In a fatality resulting from ingestion of 120 mL of formaldehyde solution, the following blood concentrations were reported 0.5 h after ingestion: formic acid 500 mg/L, formaldehyde 4.8 mg/L and methanol 420 mg/L; after 17 h, these had declined to 300, 2 and 70 mg/L, respectively. Death occurred ≈28 h after ingestion [Eells *et al.* 1981].
In 2 deaths occurring 3 and 8 weeks after the ingestion of an unknown amount of formaldehyde, formic acid plasma levels of 280 and 210 mg/L were reported on admission to hospital. In the second case, formic acid levels declined to 70 mg/L at 6 h after admission [Köppel *et al.* 1990].
A 58-year-old man died 12 h after ingesting approximately 4 ounces of a formaldehyde solution in a suicide attempt. The plasma levels of formaldehyde, formate and methanol 1.5 h after ingestion were 0.16, 16.0 and 5.8 mmol/L, respectively; at 5.5 h after ingestion, these levels were 0.32, 29.6 and 7.4 mmol/L, respectively. After this time, formaldehyde levels declined and methanol levels continued to increase, reaching 0.14 and 10.6 mmol/L at 12 h after ingestion [Burkhart *et al.* 1990].

Burkhart KK *et al.* (1990). Formate levels following a formalin ingestion. *Vet Hum Toxicol* 32: 135–137.
Eells JT *et al.* (1981). Formaldehyde poisoning. Rapid metabolism to formic acid. *JAMA* 246: 1237–1238.
Hansch C *et al.* (1995). The expanding role of quantitative structure–activity relationships (QSAR) in toxicology. *Toxicol Lett* 79: 45–53.
Köppel C *et al.* (1990). Suicidal ingestion of formalin with fatal complications. *Intensive Care Med* 16: 212–214.
Luo W *et al.* (2001). Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 753: 253–257.
Takeuchi A *et al.* (2007). Determination of formaldehyde in urine by headspace gas chromatography. *Bull Environ Contam Toxicol* 79: 1–4.

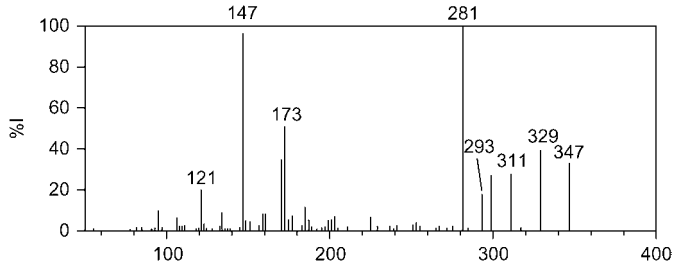
Formebolone

Anabolic Steroid
$C_{21}H_{28}O_4$ = 344.4
CAS—2454-11-7
IUPAC Name (8*S*,9*S*,10*R*,11*R*,13*S*,14*S*,17*S*)-11,17-Dihydroxy-10,13,17-trimethyl-3-oxo-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthrene-2-carbaldehyde

Synonyms 11α,17β-Dihydroxy-17-methyl-3-oxoandrosta-1,4-diene-2-carboxaldehyde; formyldienolone.
Proprietary Name *Esiclene*



Chemical Properties Crystals. Mp 209° to 212°. Soluble in water. Log *P* (octanol/water), 1.43 [Meylan, Howard 1995].
Mass Spectrum



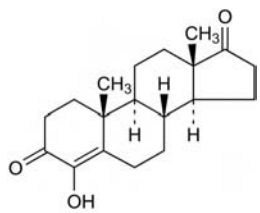
Quantification
Urine **LC-MS** Column: LiChroCART Purospher RP C_{18} (125 × 3 mm, 5 µm). Mobile phase: 5 mmol/L ammonium acetate–0.01% acetic acid : 90% methanol (50 : 50 to 0 : 100 at 15 min for 3 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.4 µg/L for formebolone metabolite 2-hydroxymethyl-17α-methyl-androsta-1,4-diene-11α,17β-diol-3-one [Leinonen *et al.* 2004].
Therapeutic Concentration
In a double-blind between-patient controlled study in 50 patients (31 males, 19 females, aged 15 to 81 years), participants were given a standard diet for 21 days. For the first 6 days there was no treatment and then 25 patients were given 20 mg/day formebolone while the rest were given placebo for the successive 15 days. There was a significant decrease in urinary nitrogen excretion in all who received the drug. Plasma concentrations of formebolone were not reported [Gelli, Vignati 1976].

Gelli D, Vignati E (1976). Metabolic studies with formebolone (2-formyl-17 (alpha)-methyl-androsta-1,4-diene-11 (alpha), 17 (beta)-diol-3-one) in humans. *J Int Med Res* 4,96–105.
Leinonen A *et al.* (2004). Screening of free 17-alkyl-substituted anabolic steroids in human urine by liquid chromatography–electrospray ionization tandem mass spectrometry. *Steroids* 69: 101–109.
Meylan W, Howard MPH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

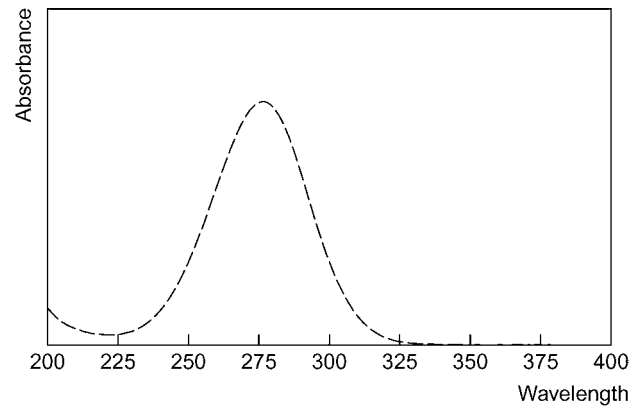
Formestane

Antineoplastic Aromatase Inhibitor
$C_{19}H_{26}O_3$ = 302.4
CAS—566-48-3

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*)-4-Hydroxy-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,17-dione
Synonyms BRN 1889793; CCRIS 7483; CGP-32349: 4-OHA; 4-hydroxyandrost-4-ene-3,17-dione; 4-OHAD.
Proprietary Names *Lentare*; *Lentaron*.



Chemical Properties A white to slightly yellow lyophilised solid. Poorly soluble in aqueous media. Needles from aqueous methanol, Mp 199° to 202°; also reported as crystals from ethyl acetate, Mp 203.5° to 206°.
Thin-layer Chromatography Plate: kiesel gel 60 F_{254} Merck 5714 (0.2 mm layer). Mobile phase (A): chloroform : methanol : water (6:4:1); (B): chloroform : methanol (19:1). R_f (formestane glucuronide metabolite) 0.5 (mobile phase A) and 0.71 (mobile phase B) [Goss *et al.* 1986].
High Performance Liquid Chromatography Column: C_{18} Supelco (3 µm, 250 × 2.1 mm i.d.). Mobile phase: acetonitrile (20%) in 10 mmol/L NH_4OAc, 5 min, up to 100% acetonitrile in 15 min, flow rate 0.2 mL/min. MS–MS detection. Retention time: formestane glucuronide, 10.6 min; sulfate, 12.0 min [Poon *et al.* 1992a].
Column: ODS APEX II (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (60:40) containing formic acid (0.1%), flow rate 1.2 mL/min. MS detection. Retention time: formestane, 8.1 min [Poon *et al.* 1992b].
Ultraviolet Spectrum Ethanol—278 nm.



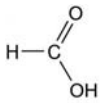
Quantification
Plasma **GC** Column: 3% OV-225 on Supelcoport, 80 to 100 mesh (1.5 m × 2 mm), Pyrex glass. Temperature: 275°. Injector and detector temperatures: 250° and 350°, respectively. Carrier gas: N_2, flow rate 40 mL/min. IS: 17α-ethinylestradiol. Detection: electron capture, ^{63}Ni. Retention time: 4-OHA PFB-derivative, 3 min; IS, 2 min. Limit of detection, 0.02 nmol per sample (6 ng) [Degen, Schneider 1991].
GC-MS Limit of detection, 65 pg [Guarna *et al.* 1989].
Disposition in the Body After IM injection, there is a slow release of the active drug into systemic circulation, with systemic uptake of 20 to 25% of the dose, taking place over 14 days. Formestane is metabolised in the liver by conjugation into an inactive glucuronide metabolite and, also, by sulfation. Metabolites include 4-hydroxytestosterone (4-OHT), 3-β-hydroxy-5-α-androstan-4,17-dione (3-α-OHA)-sulfate, 4-hydroxyandrosta-4,6-diene-3,17-dione and 3-α-17-dihydroxy-5-β-androstan-4-one (3,17-OHA)-sulfate. <1% of the dose is detected in urine unchanged.
Therapeutic Concentration
Healthy volunteers were administered with single oral doses of formestane, 250 and 500 mg. Peak plasma concentrations were reached within 0.5 to 4 h and the drug was detected in serum 5 min after administration. Plasma levels peaked at 79.2 and 119.7 µg/L, respectively [Dowsett *et al.* 1992].
Toxicity Clinical trials with doses up to 1000 mg/week only showed an increase in the frequency of the adverse reactions, for example rash, hot flushes, nausea, sore throat and vomiting.
Half-life 5 to 6 days.
Protein Binding About 85%.
Dose Usual dose, 250 mg every two weeks.

Degen PH, Schneider W (1991). Determination of 4-hydroxyandrostenedione in plasma and urine by extractive alkylation and electron-capture gas chromatography. *J Chromatogr* 565: 67–73.
Dowsett M *et al.* (1992). An endocrine and pharmacokinetic study of four oral doses of formestane in postmenopausal breast cancer patients. *Eur J Cancer* 28: 415–420.
Goss PE *et al.* (1986). Metabolism of the aromatase inhibitor 4-hydroxyandrostenedione in vivo. Identification of the glucuronide as a major urinary metabolite in patients and biliary metabolite in the rat. *J Steroid Biochem* 24(2): 619–622.
Guarna A *et al.* (1989). Quantitative determination of 4-hydroxy-4-androstene-3,17-dione (4-OHA), a potent aromatase inhibitor, in human plasma, using isotope dilution mass spectrometry. *J Steroid Biochem* 32(5): 699–702.
Poon GK *et al.* (1992a). Investigation of conjugated metabolites of 4-hydroxyandrost-4-ene-3,17-dione in patient urine by liquid chromatography-atmospheric pressure ionization mass spectrometry. *Drug Metab Dispos* 20: 941–947.
Poon GK *et al.* (1992b). Identification of 4-hydroxyandrost-4-ene-3,17-dione metabolites in prostatic cancer patients by liquid chromatography-mass spectrometry. *J Chromatogr* 576: 235–244.

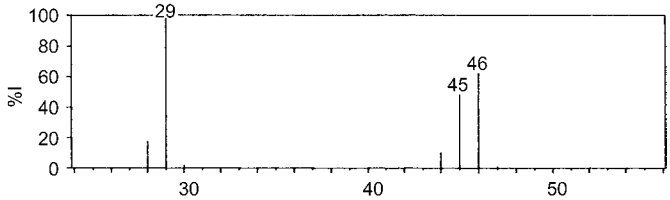
Formic Acid

Acid
HCOOH = 46.03
CAS—64-18-6

IUPAC Name Formic acid
Synonym Aminic acid
Proprietary Names It is an ingredient of *Aciforin*; *Berggeist*; *Discmigon*; *Euphon*; *Fortalis*; *Rubistenol*; *Rubjovit*; *Step 2*.



Chemical Properties A colourless liquid containing ~25% w/w of CH_2O_2. Bp 101°. Miscible with water, acetone, ethyl acetate, ethanol, and ether; partially soluble in benzene, toluene and xylenes. pK_a 3.8 (25°). Log *P* (octanol/water), −0.54 [Hansch *et al* 1995].
Colour Test Warm with sulfuric acid in a tube—carbon monoxide is evolved; the gas can be ignited at the mouth of the tube and burns with a blue flame.
Mass Spectrum Principal ions at *m/z* 29, 46, 45, 28, 44, 30, 47.



Quantification

Blood **GC** Column: Porapak Q (1.8 m × 3 mm i.d.). Carrier gas: N_2, 24 mL/min. Temperature: 124°. FID. Limit of detection, 5 mg/L [Abolin *et al.* 1980]. Column: fused silica (30 m × 0.32 mm i.d., 0.5 µm). Carrier gas: He, 2.8 mL/min. Limit of quantification not reported [Westphal *et al.* 2001].
Urine **GC** See Blood [Abolin *et al.* 1980]. Column: DB-WAX (60 m × 0.53 mm i. d., 1.0 µm). Carrier gas: He, 59 g/cm^2. Temperature: 60°. FID. Limit of detection, 1 mg/L [Yasugi *et al.* 1992].
Bile **GC** See Blood [Westphal *et al.* 2001].
Gastric Contents **GC** See Blood [Westphal *et al.* 2001].
Kidney **GC** See Blood [Westphal *et al.* 2001].
Liver **GC** See Blood [Westphal *et al.* 2001].
Disposition in the Body Formic acid is an intermediate in normal metabolism; when administered it probably takes part in the metabolism of 1-carbon compounds to produce methyl groups; it is excreted in the urine and also undergoes oxidation to carbon dioxide. Together with formaldehyde, it is a metabolite of methanol and the 2 are probably mainly responsible for the effect of methanol on vision.
Therapeutic Concentration Formic acid concentrations of 2–30 mg/L (mean, 13) were reported in the urine of normal subjects [Triebig, Schaller 1980].
Toxicity The minimum lethal dose is ~30 mL. Formic acid is dangerously caustic to the skin. The maximum permissible atmospheric concentration is 5 ppm or 9 mg/m^3; the estimated acceptable daily intake is up to 3 mg/kg.

In a fatality involving ingestion of formic acid, the blood concentration on admission was 348 mg/L [Verstraete *et al.* 1989].

Two men were fatally intoxicated with methanol and the formic acid levels in their blood, urine and organs were as follows: 320 and 230 mg/L in blood, 227 and 47 mg/L in urine, 0.11 and 1.17 mg/g in brain, 0.54 and 0.51 mg/g in liver, and 0.13 and 1.19 mg/g in kidneys. The total amounts of formic acid in the gastric contents were 108 and 23.2 mg [Tanaka *et al.* 1991].

In a death caused by the intentional ingestion of a decalcifying agent containing formic acid, the blood concentration on admission to hospital was 370.3 mg/L and declined to 13.9 mg/L after 6.5 h of haemodialysis. Postmortem concentrations were heart blood 855.4 mg/L, gastric contents 2712 mg/L, haemorrhagic fluid from abdominal cavity 1128 mg/L, bile 3051 mg/L, contents of small intestine 2664 mg/L, liver 442.7 µg/g and kidney 542.3 g/g [Westphal *et al.* 2001].

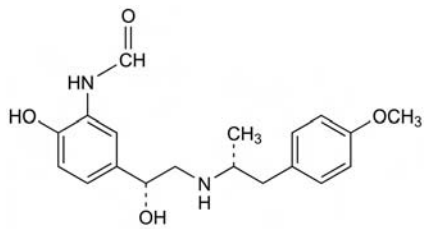
Note Formic acid is the principal ingredient (~60%) of several proprietary preparations used for descaling kettles.

Abolin C *et al.* (1980). Gas chromatographic head-space assay of formic acid as methyl formate in biologic fluids: potential application to methanol poisoning. *Biochem Med* 23: 209–218.
Hansch C *et al.* (1995). The expanding role of quantitative structure-activity relationships (QSAR) in toxicology. *Toxicol Lett* 79: 45–53.
Tanaka E *et al.* (1991). Postmortem determination of the biological distribution of formic acid in methanol intoxication. *J Forensic Sci* 36: 936–938.
Triebig G, Schaller KH (1980). A simple and reliable enzymatic assay for the determination of formic acid in urine. *Clin Chim Acta* 108: 355–360.
Verstraete AG *et al.* (1989). Formic acid poisoning: case report and in vitro study of the hemolytic activity. *Am J Emerg Med* 7: 286–290.
Westphal F *et al.* (2001). Fatal intoxication with a decalcifying agent containing formic acid. *Int J Legal Med* 114: 181–185.
Yasugi T *et al.* (1992). Formic acid excretion in comparison with methanol excretion in urine of workers occupationally exposed to methanol. *Int Arch Occup Environ Health* 64: 329–337.

Formoterol

Antiasthmatic
$C_{19}H_{24}N_2O_4$ = 344.4
CAS—73573-87-2

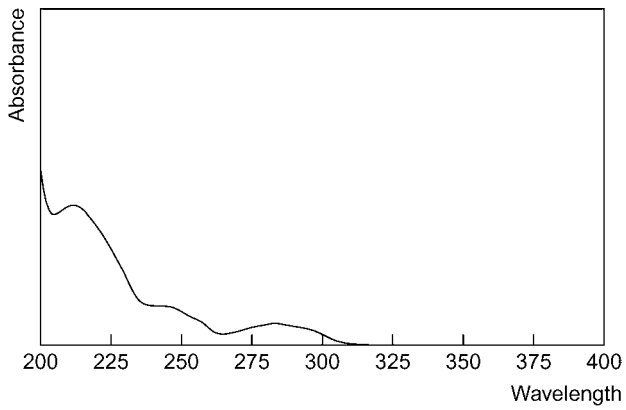
IUPAC Name *rel-N*-[2-Hydroxy-5-[(1*R*)-1-hydroxy-2-[[(1*R*)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide
Synonym Eformoterol



Chemical Properties pKa, 7.82; 8.53. Log *P* (octanol/pH 7), 0.10; (octanol/pH7.4), 0.4.

Formoterol Fumarate Dihydrate

$(C_{19}H_{24}N_2O_4)_2.C_4H_4O_4.2H_2O$ = 804.9
CAS—43229-80-7
Synonyms Eformoterol fumerate; BD-40A; CGP-25827A.
Proprietary Names *Atock*; *Eolus*; *Foradil*; *Foradile*; *Neblik*; *Oxis*; *Oxeze*.
Chemical Properties Crystals from 95% isopropyl alcohol. Mp 138° to 140°.
Ultraviolet Spectrum Aqueous acid—212, 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 1690, 1515, 1370, 1250 cm^{-1}.
Mass Spectrum Principal ions at *m/z* (formoterol glucuronide) 122, 119, 77, 78, 91, 107, 65, 63.

Quantification

Plasma **HPLC** Electrochemical detection. Limit of detection, 0.004 µg/L [Campestrini *et al.* 1997]. Column: C_8 Alltech (100 × 4.6 mm i.d., 3 µm). Mobile phase: citrate buffer (0.031 mol/L sodium acetate, 0.012 mol/L citric acid, 0.033 mol/L sodium hydroxide, pH 6.0) : methanol (61.5:38.5), flow rate 1 mL/min. Internal standard (IS): bromoformoterol. Electrochemical detection. Retention time: formoterol, 5.6 min; IS, 10.0 min. Limit of detection, 0.02 µg/L [van der Berg *et al.* 1994].
Urine **GC-MS** Limit of detection, 5 µg/L [Kamimura *et al.* 1982].
Disposition in the Body After inhalation of formoterol, there is rapid absorption. Metabolism is mainly via direct glucuronidation and *O*-demethylation, and this is the main route of elimination. Formoterol is also sulfated to some extent to a sulfate conjugate. 8 to 13% of an oral dose is excreted unmetabolised in urine (20% for IV administration and 24% for an inhaled dose). 62% of a total dose is recovered in urine with 24% as faeces.
Therapeutic Concentration

Twelve healthy volunteers (8 male and 4 female) were administered a single 120 µg dose of formoterol fumarate (by inhalation). In 9 of the volunteers, peak plasma concentrations were reached within 5 min after inhalation, with a mean peak of 266 pmol/L [Lecaillon *et al.* 1999].

Toxicity Possible side effects include tremors, headaches, palpitations, tachycardia, hypotension and hyperglycaemia. It is a poison by intraperitoneal and IV routes and moderately toxic by ingestion and subcutaneous routes.
Half-life Elimination half-life ≈10 h.
Protein Binding Approximately 50%.
Dose A usual dose of formoterol fumarate is 12 or 24 µg twice daily (inhalation or aerosol), or 80 µg twice daily orally.

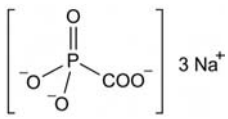
Campestrini J *et al.* (1997). Automated and sensitive method for the determination of formoterol in human plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Sci Appl* 704: 221–229.

Kamimura H *et al.* (1982). Quantitative determination of the beta-adrenoceptor stimulant formoterol in urine by gas chromatography mass spectrometry. *J Chromatogr* 229: 337–345.
Lecaillon JB *et al.* (1999). Pharmacokinetics and tolerability of formoterol in healthy volunteers after a single high dose of Foradil dry powder inhalation via Aerolizer. *Eur J Clin Pharmacol* 55: 131–138.
van den Berg BT *et al.* (1994). First high-performance liquid chromatography assay of formoterol concentrations in the low-picogram-per-milliliter range. *Ther Drug Monit* 16: 196–199.

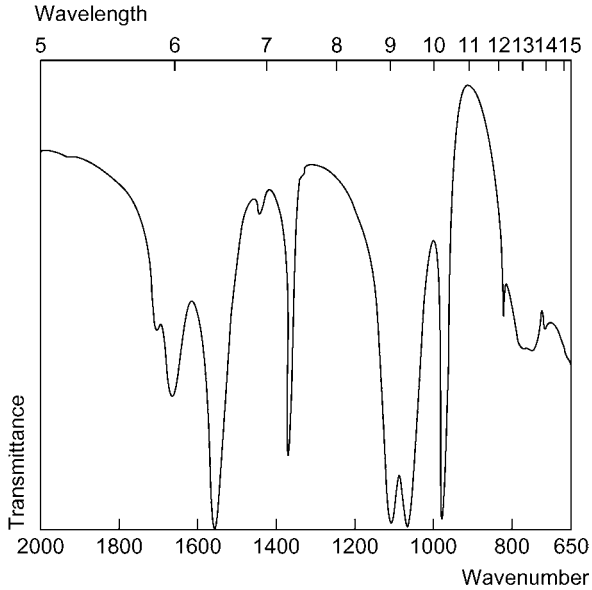
Foscarnet Sodium

Antiviral
$CNa_3O_5P = 192.0$
CAS—63585-09-1; 34156-56-4 (hexahydrate)

IUPAC Name Trisodium phosphonatoformate
Synonyms A-29622; dihydroxyphosphinecarboxylic acid oxide trisodium salt; EHB-776; phosphonatoformate trisodium; phosphonoformate trisodium; trisodium carboxyphosphate.
Proprietary Names *Foscavir*; *Triapten*; *Virudin*.



Chemical Properties A white crystalline powder. Mp 25°. Soluble in water; practically insoluble in alcohol. pK_a 0.5, 3.4, 7.3.
Ultraviolet Spectrum Aqueous acid—236 nm.
Infrared Spectrum



Quantification

Plasma **HPLC** Column: C_{18} Novapak (4 µm). Mobile phase: methanol containing 0.001 mol/L $THAHO_4$ (ion pairing agent) : 60 mmol/L phosphate buffer (pH 5.8) (30:70), also 0.0002 mol/L pyrophosphoric acid was added and final pH adjusted to 5.8, flow rate 0.7 mL/min. Electrochemical detection. Internal standard (IS): hydrochlorothiazide. Retention time: foscarnet sodium, 12.0 min; IS, 18.9 min. Limit of quantification, 0.033 mg/L [Hassanzadeh *et al.* 1990].
Serum **HPLC** Column: Kromasil 100 C_{18} (150 × 4.6 mm i.d., 5 µm), polyether ether ketone (PEEK) column. Mobile phase: methanol : 40 mmol/L disodium phosphate buffer (pH 7.6, containing 0.25 mmol/L tetrahexylammonium sulfate) ($THAHO_4$) (25:75), flow rate 1.0 mL/min. Amperometric detection (glassy carbon at +1.125 V). Retention time: 6.06 min. Electrochemical detection. Limit of quantification, 0.015 mg/L [Ba *et al.* 1999].
Urine **HPLC** See Plasma [Hassanzadeh *et al.* 1990].
Note For a review of chromatographic methods for the determination of antivirals in biological fluids, see Riley *et al.* [1990].
Disposition in the Body The pharmacokinetics of foscarnet are complicated by the high incidence of renal function impairment during therapy and by the deposition and subsequent gradual release of foscarnet from bone. The estimation of half-life thus depends on duration of foscarnet therapy. Foscarnet is poorly absorbed after oral administration and not metabolised to a great extent. It crosses the blood–brain barrier in variable amounts; CSF concentrations of between 0 and 300% of plasma concentrations have been reported. The drug is excreted mostly unchanged in the urine by glomerular filtration and tubular secretion, and is also cleared by uptake into bones (14 to 22%). It is removed by haemodialysis.

Therapeutic Concentration
Administration of 90 mg/kg (infused over 2 h) every 12 h to 10 patients with AIDS and disease of the GI tract (aged between 34 and 45 years) produced mean peak plasma concentrations of 0.621 mg/L on day 1 and 0.687 mg/L at steady state (after 17 to 21 days) at the end of the infusion [Dieterich *et al.* 1997].

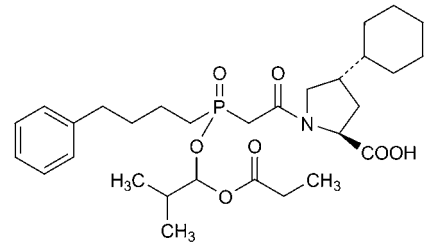
Administration of 60 mg/kg (infused over 2 h) every 8 h to 8 AIDS patients (aged between 25 and 43 years, mean 34) for 14 days, produced mean peak and trough concentrations of 0.509 mg/L (range 0.306 to 0.876) and 0.098 mg/L (0.062 to 0.147 mg/L), respectively, on day 3 and 0.495 (range 0.272 to 0.699) mg/L and 0.126 (0.057 to 0.225) mg/L, respectively, on day 14 [Aweeka *et al.* 1989].
Toxicity Renal impairment is the major toxicity. Seizures related to alterations in plasma minerals and electrolytes have also been associated with foscarnet therapy.
Overdosing has been reported in 33 patients with the highest ≈10 times the prescribed dose. Of these, 28 experienced adverse effects, 5 had no ill effects connected with overdose and 4 died owing to respiratory and cardiac arrest with organ failure [Astra Pharmaceuticals Ltd].
Half-life Plasma half-life, 4.5 h.
Volume of Distribution 0.3 L to 0.74 L/kg.
Clearance Plasma clearance, 6 to 13 L/h/1.73 m^2; 1.7 to 1.9 mL/min/kg (101 to 106 mL/min/1.73 m^2).
Protein Binding 14 to 17%.
Note For reviews of foscarnet, see Chrisp and Clissold [1991] and Wagstaff and Bryson [1994].
Dose The usual dose is 60 mg/kg intravenously (over 1 to 2 h) every 8 h for 2 to 3 weeks, followed by 60 to 120 mg/kg daily.

Astra Pharmaceuticals Limited data
Aweeka F *et al.* (1989). Pharmacokinetics of intermittently administered intravenous foscarnet in the treatment of acquired immunodeficiency syndrome patients with serious cytomegalovirus retinitis. *Antimicrob Agents Chemother* 33: 742–745.
Ba BB *et al.* (1999). Determination of phosphonoformate (foscarnet) in calf and human serum by automated solid-phase extraction and high-performance liquid chromatography with amperometric detection. *J Chromatogr B Biomed Sci Appl* 724: 127–136.
Chrisp P, Clissold SP (1991). Foscarnet. A review of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with cytomegalovirus retinitis. *Drugs* 41: 104–129.
Dieterich DT *et al.* (1997). Treatment of gastrointestinal cytomegalovirus infection with twice-daily foscarnet: a pilot study of safety, efficacy, and pharmacokinetics in patients with AIDS. *Antimicrob Agents Chemother* 41: 1226–1230.
Hassanzadeh MK *et al.* (1990). Determination of phosphonoformic acid in human plasma and urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 525: 133–140.
Riley CM *et al.* (1990). Chromatographic methods for the bioanalysis of antiviral agents. *J Chromatogr* 531: 295–368.
Wagstaff AJ, Bryson HM (1994). Foscarnet. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with viral infections. *Drugs* 48: 199–226.

Fosinopril

ACE Inhibitor, Antihypertensive
$C_{30}H_{46}NO_7P = 563.7$
CAS—98048-97-6

IUPAC Name (2*S*,4*S*)-4-Cyclohexyl-1-[2-[[(1*S*)-2-methyl-1-propanoyloxypropoxy]-(4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid
Synonyms (4*S*)-4-Cyclohexyl-1-[[(*RS*)-2-methyl-1-(propionyloxy)propoxy](4-phenylbutyl)phosphinoylacetyl]-L-proline; fosenopril.



Chemical Properties Log *P* (octanol/water), 6.61 [Meylan, Howard 1995]. There were no stability-related problems: working solutions were stable at room temperature and stock solutions of fosinopril and fosinoprilat were stable for 2 weeks [Cui *et al.* 2007]. Following acidification with sodium acetate buffer (pH 4), fosinopril and fosinoprilat were stable at room temperature for at least 24 h. Fosinoprilat was stable in serum after 3 freeze-thaw cycles, in reconstituted samples at room temperature for at least 48 h, and at –20° for 181 days [Jemal, Mulvana 2000].

Fosinopril Sodium

$C_{30}H_{45}NNaO_7P = 585.6$
CAS—88889-14-9
Synonyms Fosenopril sodium; SQ-28555.

Proprietary names *Acecor*; *Dynacil*; *Eliten*; *Fosinorm*; *Fosipres*; *Fositens*; *Fozitec*; *Hiperlix*; *Monopril*; *New Ace*; *Secorvas*; *Staril*; *Tensogard*; *Tenso Stop*; *Tensozide*.
Chemical Properties White to off-white crystalline powder. It is soluble in water (100 g/L), methanol and ethanol; slightly soluble in hexane.

Fosinopril Diacid

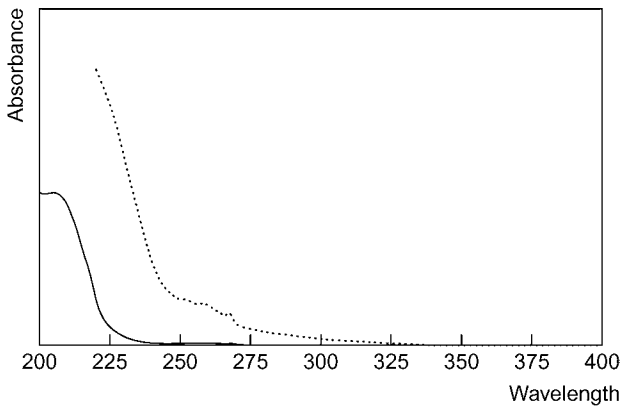
$C_{23}H_{34}NO_5P = 435.5$
Synonym SQ-27519
Chemical Properties Mp 149° to 153°.

High Performance Liquid Chromatography Column: alkylphenyl (300 × 4.0 mm i.d., 10 µm). Mobile phase: methanol : 0.2% phosphoric acid (72 : 28), flow rate 2 mL/min. UV detection (λ = 215 nm). Retention time: 10.5 min. Limit of quantification, 100 mg/L [Lozano *et al.* 1995].

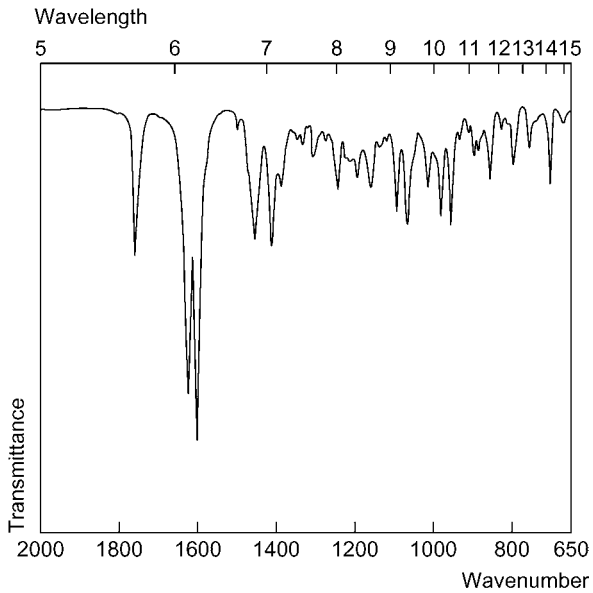
Column: Resolve (Waters, 150 × 3.9 mm, 5 µm). Temperature: 32°. Mobile phase: acetonitrile : water : orthophosphoric acid (4000 : 15 : 2), flow rate 1 mL/min. UV detection (λ = 205 nm). Retention time: 5.13 min. Limit of detecetion, 0.02 µg/L [Kirschbaum *et al.* 1990].

Capillary Electrophoresis Column: fused silica capillary (50/57 cm effective/total length × 75 µm i.d.). Buffer: 50 mmol/L sodium tetraborate (ph 8.3). UV detection (λ = 200 nm). Limit of quantification, 0.26 mg/L [Lozano *et al.* 1995].

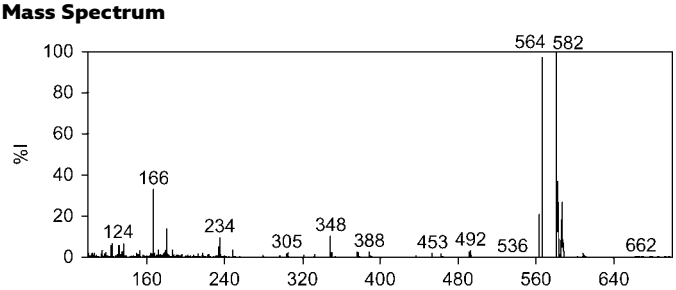
Ultraviolet Spectrum Basic—252, 258, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1759, 1409, 1064 cm^{-1} (KBr pellet).



Mass Spectrum



Quantification

Serum **GC** Column: fused silica capillary (5 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 34 kPa. Temperature programme: 210° for 2 min to 260° at 10°/min for 1 min. NPD. Limit of detection 10 µg/L. [Jemal *et al.* 1985].

LC-MS Column: LiChrospher-C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (60 : 40 for 2 min to 85 : 15 at 10 min to 60 : 40 at 12 min), flow rate 1.0 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 8.7 min. Limit of quantification, 0.1 µg/L [Cui *et al.* 2007]. Column: ODP PVA C_{18} (50 × 2 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate in water : methanol (pH 4; 75 : 25) : 10 mmol/L ammonium acetate in methanol (70 : 30 to 5 : 95 in 3 minto 70 : 30 at 5 min), flow rate 200 µL/min. SRM acquisition mode. Retention time: 1.4 min. Limit of quantification,1.17 µg/L. [Jemal, Mulvana 2000].

Disposition in the Body Thirty-six percent of an orally administered dose of fosinopril is absorbed. It is rapidly and completely hydrolysed to fosinoprilat, a *p*-hydroxy metabolite and a glucuronide conjugate of fosinoprilat. Metabolism occurs by esterases in the gastrointestinal mucosa and in the liver. The metabolites are excreted in urine and faeces via bile. During 96-h post-IV administration, 90% of a dose is recovered, 44% in urine and 46% in faeces [Kelly, O'Malley 1990]. For oral administration, however, 13 to 16% is recovered in urine and 73 to 78% in faeces. The drug has also been detected in breast milk.

Therapeutic Concentration

Twenty healthy Chinese volunteers (18–25 years) were administered 20 mg fosinopril sodium. The mean maximum concentration of fosinopril sodium and fosinoprilat were 4.6 ± 2.34 and 409.43 ± 136 µg/L at 1.2 ± 0.42 and 3.74 ± 0.87 h, respectively. The mean plasma elimination half-life of fosinopril was 2.72 ± 1.75 h and for fosinoprilat was 7.25 ± 0.81 h [Cui *et al.* 2007].

Thirteen patients with renal impairment (mean creatine clearance, 55.7 mL/min) and 13 healthy volunteers (mean creatine clearance, 102.5 mL/min) were administered 20 mg fosinopril sodium once daily for 5 days. On day 1: the renally impaired patients reached peak plasma concentrations of 387 ± 0.2 µg/L within 3.5 h, and the healthy volunteers 324 ± 0.3 µg/L in 3 h. On day 5: the patients with renal impairment had peak plasma concentrations of 517 ± 0.4 µg/L and the healthy volunteers 357 ± 0.2 µg/L, both reached within 3 h. [O'Grady *et al.* 1999].

After an oral dose of 10 mg fosinipril to patients with mild, moderate and severe renal failure, mean maximum plasma concentrations of 165 ± 11, 136 ± 23, 127 ± 22 µg/L were reached at ≈4 h [Hui *et al.* 1991].

Toxicity Hypotension. For a case of hepatotoxicity induced by fosinopril, see Romero-Gómez *et al.* [2001].

Half-life ≈11.5 h in healthy volunteers; 14 h in patients with renal impairment.

Volume of Distribution Steady state, 1.525 L/kg (Caucasian); 0.074 L/kg (Chinese).

Clearance Fosinopril, body clearance, 1.55 to 2.35 L/h; fosinoprilat, 26 to 39 mL/min.

Protein Binding Fosinopril is bound >95% and fosinoprilat 89 to 99.8%.

Dose An initial dose of 10 mg daily is administered. Maintenance dose: 10–40 mg daily.

Cui S *et al.* (2007). Development and validation of liquid chromatography-tandem mass spectrometric method for simultaneous determination of fosinopril and its active metabolite fosinoprilat in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 143–151.

Hui KK *et al.* (1991). Pharmacokinetics of fosinopril in patients with various degrees of renal function. *Clin Pharmacol Ther* 49: 457–467.

Jemal M, Mulvana DE (2000). Liquid chromatographic-electrospray tandem mass spectrometric method for the simultaneous quantitation of the prodrug fosinopril and the active drug fosinoprilat in human serum. *J Chromatogr B Biomed Sci Appl* 739: 255–271.

Jemal M *et al.* (1985). Determination of SQ 27,519, the active phosphinic acid-carboxylic acid of the prodrug SQ 28,555, in human serum by capillary gas chromatography with nitrogen-phosphorus detection after a two-step derivatization. *J Chromatogr* 345: 299–307.

Kelly JG, O'Malley K (1990). Clinical pharmacokinetics of the newer ACE inhibitors. A review. *Clin Pharmacokinet* 19: 177–196.

Kirschbaum J *et al.* (1990). High-performance liquid chromatography of the drug fosenopril. *J Chromatogr* 507: 165–170.

Lozano R *et al.* (1995). Quantitative analysis of fosinopril sodium by capillary zone electrophoresis and liquid chromatography. *J Pharm Biomed Anal* 13: 139–148.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

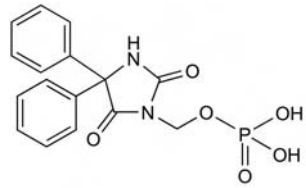
O'Grady P *et al.* (1999). Fosinopril/hydrochlorothiazide: single dose and steady-state pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 48: 375–381.

Romero-Gómez M *et al.* (2001). Hepatotoxicity induced by fosinopril. *J Hepatol* 35: 309–310.

Fosphenytoin

Anticonvulsant
$C_{16}H_{15}N_2O_6P = 362.3$
CAS—93390-81-9

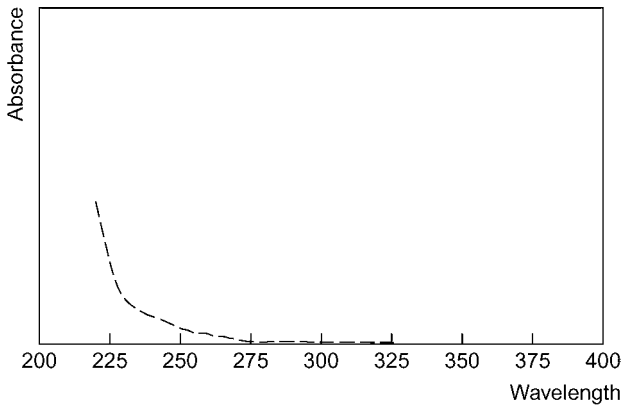
IUPAC Name (2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)methyl dihydrogen phosphate
Synonym 5,5-Diphenyl-3-[(phosphonooxy)methyl]-2,4-imidazolidinedione



Fosphenytoin Sodium

$C_{16}H_{13}N_2Na_2O_6P = 406.2$
CAS—92134-98-0
Synonyms ACC-9653; Acc-9653-010; CI-982; PD-135711-15B.
Proprietary Names *Cerebyx*; *Pro-Epanutin*.
Chemical Properties Freely soluble in aqueous solutions.

High Performance Liquid Chromatography Column: C_{18} μBondapak (150 × 3.9 mm i.d., 10 μm). Mobile phase: 0.01 mol/L tetrabutylammonium dihydrogen phosphate : methanol (53:43%), flow rate 1.7 mL/min. Retention time: fosphenytoin sodium, 4.5 min [Gerber *et al.* 1988].
Ultraviolet Spectrum Ethanol—258 nm.



Quantification

Plasma **HPLC** Column: C_{18} (150 × 3.9 mm i.d., 5 μm). Mobile phase: acetonitrile (20%) in deionised water containing 0.005 mol/L tetrabutylammonium sulfate (TBA), (pH 2.5), flow rate 2.0 mL/min. Retention time: 8.8 min. Limit of quantification, 0.4 mg/L, limit of detection, 0.1 mg/L [Cwik *et al.* 1997].
Formulations **HPLC** Column: C_{18} Resolve (150 × 3.9 mm i.d., 5 μm). Mobile phase: acetonitrile : water : phosphoric acid (85%) (57:75:0.09) containing 5 mmol/L TBA, flow rate 2 mL/min. Retention time: fosphenytoin, 9.1 min. Limit of quantification, 15 mg/L [Fischer *et al.* 1997].
Disposition in the Body Fosphenytoin sodium is rapidly and completely metabolised to phenytoin and other metabolites including phosphate and formaldehyde which are subsequently converted to formate and further metabolites. Phenytoin is metabolised to 5-(*p*-hydroxyphenyl)-5-phenylhydantoin. The metabolites are excreted in urine. It is distributed in CSF, saliva, semen, gastrointestinal fluids, bile and breast milk.
Therapeutic Concentration

Twenty healthy, male volunteers (Caucasian, Black, Hispanic and Asian) were administered with a single IV dose of 150, 300, 600 and 1200 mg fosphenytoin sodium over 30 min. Peak plasma concentrations of fosphenytoin were observed half an hour after the beginning of the infusion and reached mean levels of 20, 36, 75 and 129 mg/L, respectively. Peak phenytoin plasma concentrations were reached at 2.2, 4.0, 7.4 and 17.2 mg/L, respectively, ≈51 to 109 min from the end of infusion [Gerber *et al.* 1988].

Toxicity The blood toxic range is 30 to 50 mg/L and a concentration of >100 mg/L has been associated with fatalities. Acute hepatotoxicity and hypersensitivity reactions have been observed and phenytoin and the other metabolites may result in toxicity after overdosing, including hypotension, coma and respiratory or circularity depression which may be fatal.
Bioavailability About 90%.
Half-life (IM administration) 33 min, (IV administration) 8 min.
Volume of Distribution Adults and children: 0.6 to 0.7 L/kg, infants: 0.7 to 0.7 L/kg, full term neonates: 0.8 to 0.9 L/kg and premature neonates: 1 to 1.2 L/kg.
Clearance 19.8 L/h.
Protein Binding About 90 to 99%.
Dose An initial dose of 10 to 15 mg phenytoin sodium equivalent (PSE)/kg body weight is administered at a rate of 50 to 150 mg/min. A daily maintenance dose of 4 to 5 mg/kg body weight is then administered. The dose is reduced in the elderly and patients with renal or hepatic impairment. Up to 450 mg has been administered intramuscularly in adults and IV doses of up to 3000 mg (38 mg/kg body weight).

Cwik MJ *et al.* (1997). Simultaneous rapid high-performance liquid chromatographic determination of phenytoin and its prodrug, fosphenytoin in human plasma and ultrafiltrate. *J Chromatogr B Biomed Sci Appl* 693: 407–414.
Fischer JH *et al.* (1997). Stability of fosphenytoin sodium with intravenous solutions in glass bottles, polyvinyl chloride bags, and polypropylene syringes. *Ann Pharmacother* 31: 553–559.
Gerber N *et al.* (1988). Safety, tolerance and pharmacokinetics of intravenous doses of the phosphate ester of 3-hydroxymethyl-5,5-diphenylhydantoin: a new prodrug of phenytoin. *J Clin Pharmacol* 28: 1023–1032.

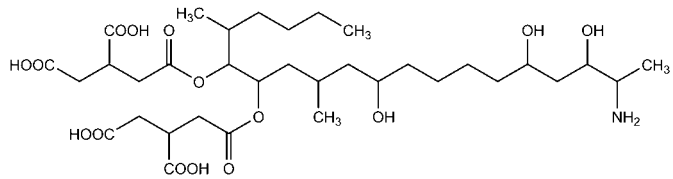
Fumonisins

Polyhydroxyl Alkylamine, Mycotoxin

Chemical Properties Toxic secondary metabolites produced by several species of the fungus *Fusarium*, first reported in South Africa in 1988. Among the most common and highest-yielding fumonisin species are *Fusarium verticillioides* (Sacc.) Nirenberg (formerly *Fusarium moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg, both of which are ubiquitous on corn/maize and rice. There are at least 15 characterised fumonisins: FB_1, FB_2, FB_3, FB_4, FB_5 with a free amine function; FA_1, FA_2, FA_3, which are amides (i.e. the *N*-acetyl derivatives of FB_1, FB_2 and FB_3, respectively); FC_1, FC_2, FC_3, FC_4, which lack the C-1 terminal methyl group; and FP_1, FP_2 and FP_3. They are heat-stable compounds, structurally analogous to the sphingoid bases sphinganine and sphingosine. Minor losses occur after heating aqueous solutions of FB_1 and FB_2 at temperatures <150° (~20 to 30%). Temperatures >175° (for 1 h) are necessary to cause substantial fumonisin reduction (>80%) in maize-based foods [Jackson *et al.* 1996]. *o*-Phthaldialdehyde derivatives of fumonisins are unstable at 24° but the stability is much improved at 4° [Williams *et al.* 2004]. Decomposition of FB_1 and FB_2 occurs in methanolic solutions (percentage decreases after 6-week storage at 4°, 25° and 40° are 5%, 35% and 60%, respectively), whereas they are stable in acetonitrile : water (1 : 1) for up to 6 months at –18°, 4° and 25° [Visconti *et al.* 1994]. Gamma irradiation of maize flour at 15 kGy was shown to reduce FB_1 and FB_2 content by ~ 20% [Visconti *et al.* 1996]. The extraction efficiency of fumonisins from corn-based products is dependent on solvent composition and temperature and is most efficient with an ethanol: water (3 : 7) extraction solvent at 80° [Lawrence *et al.* 2000]. Studies have shown that some essential oils (eugenol, cinnamic aldehyde, thymol, carvacol and myristin) are effective in inhibiting the growth of *F. moniliforme* [Juglal *et al.* 2002] and that reaction with D-glucose may detoxify FB_1 [Lu *et al.* 2002].

Fumonisin B_1

$C_{34}H_{59}NO_{15} = 721.8$
CAS—116355-83-0
IUPAC Name 1,2,3-Propanetricarboxylic acid 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester
Synonyms FB1; macrofusine.



Chemical Properties Most prevalent toxin produced from *F. moniliforme*, a common mould associated with maize. The maximum yield of FB_1 produced by *F. moniliforme* in maize cultures is at temperatures of 20° to 25° and incubation periods of between 7 and 13 weeks [Alberts *et al.* 1990]. Powder, very hygroscopic.

Fumonisin B_2

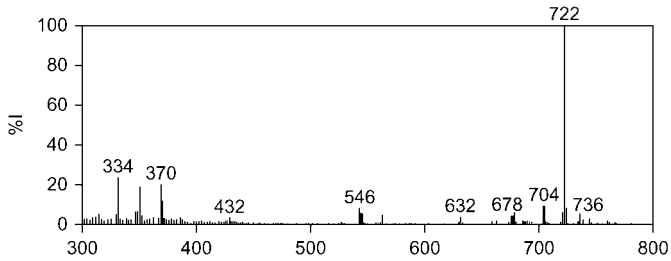
$C_{34}H_{59}NO_{14} = 691$
CAS—116355-84-1
IUPAC Name 1,2,3-Propanetricarboxylic acid 1,1′-[1-(12-amino-9,11-dihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester
Chemical Properties Powder, very hygroscopic.

Thin-layer Chromatography Plates: C_{18}. Solvent system: methanol : 1% aqueous potassium chloride (3 : 2). Locating reagent fluorescamine solution, buffered acetonitrile and UV detection. R_f values: FB_1 0.10, FB_2 0.50. Limit of quantification not reported [Dutton 1996] Plates: method 1, normal-phase silica; method 2, C_{18} silica (activated at 110° for 10 min). Solvent systems: method 1, chloroform : methanol : acetic acid (60 : 35 : 10); method 2, methanol : water (80 : 20). Locating reagent, acidic anisaldehyde. R_f values: method 1, FB_1 0.32, FB_2 0.52; method 2, FB_1 0.61, FB_2 0.47. Limit of quantification not reported [Ackermann 1991].
High Performance Liquid Chromatography Column: C_{18} (250 x 4.0 mm i.d., 5 μm). Mobile phase: methanol-0.05 mol/L sodium dihydrogen phosphate (pH 5.0; 50 : 50) : acetonitrile-water (80 : 20; 100 : 0 for 5 min to 50 : 50 for 15 min), flow rate 1.0 mL/min. Fluorescence detection (*o*-phthalaldehyde [OPA] derivatives $\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm; 4-fluoro-7-nitro-2,1,3-benzoxadiazole [NBD-F] derivatives $\lambda_{ex} = 440$ nm, $\lambda_{em} = 535$ nm). Limit of detection, 0.01 mg/kg for FB_1 and FB_2 (OPA derivatives), 0.08 mg/kg (NBD-F derivatives) [Hinojo *et al.* 2006]. Column: C_8 (250 x 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile-water-trifluoroacetic acid (pH 2.7; 5 : 95 : 0.025) : acetonitrile-water-trifluoroacetic acid (90 : 10 : 0.025; 80 : 20 to 40 : 60 over 30 min to 20 : 80 over 10 min to 0 : 100 over 2 min), flow rate 1.0 mL/min. ELS detection. Retention times: FB_1 16.3 min, FB_2

21.4 min, FB_3 19.1 min, FB_4 24.4 min (underivatised samples). Limit of quantification not reported [Wilkes *et al.* 1995]. Column: C_{18} (250 x 3.2 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 3.35; 75 : 25), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention times: FB_1 ~5 min, FB_2 ~ 12 min (OPA derivatives). Limit of quantification not reported [Visconti *et al.* 1994].

Liquid Chromatography-Mass Spectrometry Column: C_{18} (150 x 4.6 mm i.d., 4 µm). Mobile phase: 0.2% formic acid : acetonitrile containing 0.2% formic acid (75 : 25 for 5 min to 60 : 40 over 30 min for 2 min to 40 : 60 over 3 min for 5 min), flow rate 1.0 mL/min. ESI, positive ion mode. Limit of quantification not reported [Josephs 1996].

Mass Spectrum



Quantification

Urine **LC-MS** Column: C_{18} (50 x 4.6 mm i.d., 5 µm). Mobile phase: water-acetonitrile-formic acid (90 : 10 : 0.1) : water-acetonitrile-formic acid (10 : 90 : 0.1; 75 : 25 to 25 : 75 over 11 min), flow rate 1.0 mL/min. ESI, positive ion mode. Retention time: FB_1 8.0 min. Limit of quantification, 20 ng/L [Gong *et al.* 2008].

Hair **LC-MS** Column: C_{18} (150 x 4.6 mm i.d., 5 µm). Mobile phase: water-acetonitrile-formic acid (90 : 10 : 0.1) : water-acetonitrile-formic acid (10 : 90 : 0.1; 80 : 20 to 72 : 28 over 24 min for 1 min to 80 : 20 over 2 min for 8 min), flow rate 0.7 mL/min. ESI, positive ion mode. Retention times: FB_1 11.5 min, FB_2 26.5 min, FB_3 20.5 min. Limit of quantification, 60 pg on-column; limit of detection, 25 pg on-column [Sewram *et al.* 2003].

Other **TLC** Maize Samples. Plates: C_{18}. Solvent system: methanol : 4% aqueous potassium chloride (70 : 30). Locating reagent or derivatisation reagent, fluorescamine. UV detection. R_f value: FB_1 0.35. Limit of quantification, 0.5 mg/kg [Shephard, Sewram 2004]. Plates: C_{18}. Solvent system: 4% aqueous potassium chloride : methanol (2 : 3). Derivatisation spray agents (sequentially): 0.1 mol/L sodium tetraborate, 0.40 g/L fluorescamine in acetonitrile; dry for 1 to 5 min and then spray with 0.01 mol/L boric acid : acetonitrile (2 : 3); dry plate at 55° to 60° for 15 min. UV detection ($\lambda = 366$ nm). R_f values: FB_1 0.5, FB_2 0.25. Limit of detection, $FB_1$0.1 mg/kg [Preis, Vargas 2000].

HPLC Maize Samples and Laboratory Cultures. Column: C_{18} (250 x 4.6 mm, 5 µm). Mobile phase: acetonitrile-acetic acid (99 : 1) : water-acetic acid (99 : 1; 60 : 40 for 8 min to 80 : 20 over 16 min for 4 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 420$ nm, $\lambda_{em} = 500$ nm). Limit of quantification, FB_1 and FB_2 (2,3-naphthalene dicarboxaldehyde derivatives) 25 µg/kg [Arino *et al.* 2007]. Maize Samples. C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : acetic acid (61 : 38 : 1), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 420$ nm, $\lambda_{em} = 500$ nm). Retention times: FB_1 7.4 min, FB_2 13.3 min (naphthalene-2,3-dicarboxaldehyde derivatives). Limit of detection, FB_1 20 µg/kg, FB_2 15 µg/kg [Lino *et al.* 2006]. Swine Liver Samples. Column: phenylhexyl (250 x 4.6 mm i.d., 5 µm). Mobile phase: aqueous buffer (pH 3.4)-2% glacial acetic acid-0.1% TEA : acetonitrile (30 : 70 to 50 : 50 over 50 min), flow rate 1.0 mL/min. Pre-column derivatisation with OPA. Fluorescence detection ($\lambda_{ex} = 334$ nm, $\lambda_{em} = 440$ nm). Retention times: FB_1 ~ 45 min, aminopentol-1 ~ 41 min. Limit of quantification, FB_1 75 µg/kg, aminopentol-1 42 µg/kg; limit of detection, FB_1 20 µg/kg, aminopentol-1 10 µg/kg [Pagliuca *et al.* 2005]. Herbal Tea and Medicinal Plant Samples. Column: C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 3.3; 8 : 2), flow rate 0.7 mL/min. Fluorescence detection ($\lambda_{ex} = 338$ nm, $\lambda_{em} = 455$ nm). Retention times: FB_1 10.9 min, FB_2 25.6 min (OPA derivatives). Limit of quantification, FB_1 103 µg/kg, FB_2 1562 µg/kg; limit of detection, FB_1 31 µg/kg, FB_2 468 µg/kg [Martins *et al.* 2001; Omurtag, Yazicioglu 2004]. Maize Kernels, Tortillas, Masa samples. Column: C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 3.35; 77 : 23), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Limit of detection, 25 µg/kg [De La Campa *et al.* 2004; Dilkin *et al.* 2001; Duncan *et al.* 1998; Sydenham *et al.* 1996; Visconti *et al.* 2001]. Maize Silage Samples. Column: C_{18} (250 x 4.0 mm i.d., 5 µm). Mobile phase: methanol-acetonitrile-water-propan-2-ol-acetic acid (100 : 52 : 73 : 20 : 4) : propan-2-ol (100 : 0 for 30 min to 70 : 30 over 30 min), flow rate 1.0 mL/min. Retention times: FB_1 20.9 min, FB_3 40.7 min, FB_2 43.3 min (naphthalene dicarboxaldehyde derivatives). Fluorescence detection ($\lambda_{ex} = 268$ nm, $\lambda_{em} = 470$ nm). Limit of detection, FB_1 50 µg/kg, FB_2 and FB_3 <50 µg/kg [Kim *et al.* 2004]. Maize-based Products. Column: C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : acetic acid (60 : 40 : 1), flow rate 0.5 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Limit of detection, FB_1, hydrolysed FB_1, and FB_2 8 to 10 µg/kg [Park *et al.* 2004; Stack 1998]. Column: C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 3.35; 80 : 20), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Limit of detection, 2.5 ppb (OPA derivative). Limit of detection, FB_1 2.5 ppb [Ho, Durst 2003]. Column: Fluofix 120E (30 x 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.1% trifluoroacetic acid (1 : 1), flow rate 0.8 mL/min. Postcolumn derivatisation with OPA. Fluorescence detection ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 450$ nm). Limit of detection, FB_1 and FB_2 0.01 mg/kg [Akiyama *et al.* 1997]. *Fusarium* cultures. Column: C_{18} (250 x 4.0 mm i.d., 5 µm). Mobile phase: methanol-0.05 mol/L sodium dihydrogen phosphate (pH 5.0; 50 : 50) : acetonitrile-water (80 : 20; 100 : 0 to 50 : 50 at 5 min for 15 min), flow rate 1.0 mL/min. Fluorescence detection. Limit of detection, FB_1, FB_2 5 µg/kg (OPA derivatives), 40 µg/kg (NBD-F derivatives) [Mateo *et al.* 2002]. Beer Samples. Column: C_{18} (33 x 4.6 mm i.d., 3 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate-acetonitrile (pH 3.3; 60 : 40) : acetonitrile : water (80 : 20; 100 : 0 for 6 min to 70 : 30 over 5 min for 2 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention times: FB_1 ~8 min, FB_2 ~14 min (OPA derivatives). Limit of quantification, FB_1 and FB_2 0.3 µg/L [Hlywka, Bullerman 1999]. Bovine Urine and Faeces. Column: C_{18} (250 x 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-water-acetic acid (40 : 59 : 1) : acetonitrile-water-acetic acid (60 : 39 : 1; 100 : 0 to 0 : 100 over 9 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 229$ nm, $\lambda_{em} = 442$ nm). Retention times: FB_1 ~11 min, FB_2 ~16 min (OPA derivatives). Limit of quantification not reported [Smith, Thakur 1996]. Maize, Poultry Feed and *Fusarium* spp. Maize Culture Samples. Column: C_{18} (33 x 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 50 mmol/L potassium dihydrogen phosphate (pH 3.3; 40 : 60), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention times: FB_1 ~4.5 min, hydrolysed FB_1 ~5.1 min, FB_2 ~16 min, FB_3 ~17.5 min (OPA derivatives). Limit of quantification, FB_1 0.1 mg/kg, FB_2 and FB_3 0.2 mg/kg [Rice *et al.* 1995]. Frozen or Canned Maize Samples. Column: C_{18} (300 x 3.9 mm i.d.). Mobile phase: water : acetonitrile : acetic acid (50 : 50 : 1), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention time: FB_1 10.5 min (OPA derivative). Limit of quantification, 0.1 µg/L [Trucksess *et al.* 1995]. Rat Plasma, Urine and Faecal Samples. Column: C_{18} (50 x 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 3.4; 72 : 28), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention time: FB_2 ~10 min (OPA derivative). Limit of quantification not reported [Shephard *et al.* 1995]. Milk Samples. Column: C_{18} (125 x 4.0 mm i.d., 4 µm). Mobile phase: 0.1 mol/L sodium dihydrogen phosphate : methanol (pH 6.0; 33 : 67), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Limit of quantification, FB_1 50 µg/L; limit of detection, 10 µg/L (OPA derivative) [Spotti *et al.* 2001]. Column: C_{18} (220 x 4.6 mm i.d., 5 µm). Mobile phase: methanol-water-acetic acid (75 : 24 : 1) : acetonitrile-water-acetic acid (75 : 24 : 1; 70 : 30 to 55 : 45 at 2.6 min to 0 : 100 at 10.1 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 250$ nm, $\lambda_{em} = 470$ nm). Retention times: FB_1 ~10 min, FB_2 ~ 17 min (naphthalene-2,3-dicarboxaldehyde derivatives). Limit of detection, FB_1 and FB_2 5 µg/L [Maragos, Richard 1994]. Monkey Faeces. Column: C_{18} (100 x 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 6.0; 67 : 33), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention times: FB_1 ~ 4.5 min, partially hydrolysed FB_1 metabolites 6.5 min and 7.5 min, aminopentol 14 min (OPA derivatives). Limit of detection, 0.4 mg/kg for FB_1 in dry faeces [Shephard *et al.* 1994]. Rat Urine and Plasma Samples. Column: C_8 (125 x 4.0 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L sodium dihydrogen phosphate (pH 3.4; 66 : 34), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Retention time: FB_1 8 min (OPA derivative). Limit of detection, 50 µg/L in urine and in plasma [Shephard *et al.* 1992].

LC-MS Maize Silage Samples. Column: C_{18} (150 x 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water-0.5% acetic acid (pH 3; 5 : 95 to 50 : 50 over 15 min to 80 : 20 over 10 min), flow rate 0.3 mL/min. ESI, positive and negative ion modes, SIM acquisition mode. Retention times: deoxynivalenol 6.9 min, aflatoxin B_1 15.4 min, citrinin 16.7 min, FB_1 15.1 min, gliotoxin 14.2 min, ochratoxin A 20.3 min, zearalenone 20.4 min. Limit of quantification, deoxynivalenol 5 ppb; gliotoxin 6.5 ppb; zearalenone 20 ppb; aflatoxin B_1, citrinin, FB_1, ochratoxin A 1.5 ppb [Richard *et al.* 2007]. Maize Samples. Column: C_{18} (150 ′ 2.0 mm i.d., 5 µm). Mobile phase: water : methanol-0.5% acetic acid-1 mmol/L ammonium acetate (80 : 20 for 3 min to 60 : 40 to 37 : 63 over 35 min for 11 min), flow rate 0.2 mL/min. ESI, negative and positive ion modes, MRM acquisition mode. Retention times: FB_1 30 min, FB_2 45 min, deoxynivalenol 7.5 min, aflatoxin G_2 15.5 min, aflatoxin G_1 17.0 min, aflatoxin B_2 18.5 min, aflatoxin B_1 20.5 min, HT-2 29 min, T-2 36 min, zearalenone 42 min, ochratroxin A 48.5 min. Limit of detection, FB_1 1.1 µg/kg, FB_2 0.4 µg/kg, other toxins 0.3 to 4.2 µg/kg [Lattanzio *et al.* 2007]. Rice Samples. Column: C_{18} (250 x 2.1 mm i.d., 5 µm). Mobile phase: water : acetonitrile-0.1% formic acid (75 : 25 to 60 : 40 over 22 min, to 0 : 100 over 5 min, for 3 min), flow rate 0.3 mL/min. ESI, positive ion mode. Retention times: FB_1 8.2 min, FB_2 15.6 min, FB_3 12.9 min, FB_4 20.8 min, FB_5 7.1 min, FA_1 12.4 min, FA_2 27.9 min, FA_3 19.6 min, FC_1 7.6 min, FC_2 15.0 min, FC_3 11.8 min, FC_4 19.5 min (method presents retention times for over 35 fumonisin-like compounds). Limit of quantification not reported [Bartok *et al.* 2006]. Maize-based Products. Column: C_{18} (250 x 2.1 mm i.d., 5 µm). Mobile phase: water : methanol both containing 25 mmol/L formic acid (40 : 60 for 3 min, to 10 : 90 over 5 min, to 0 : 100 for 10 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: FB_1 8.2 min, FB_2 9.6 min, FB_3 8.9 min, FB_4 10.2 min. Limit of detection, FB_1 2 µg/kg, FB_2 1 µg/kg

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[Faberi *et al.* 2005]. Column: Synergi Polar PR (150 x 2.0 mm i.d., 4 μm). Mobile phase: acetonitrile-methanol (50 : 50) : 0.02% aqueous formic acid (35 : 65 for 1 min to 60 : 40 over 9 min for 6 min), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, FB_1, hydrolysed FB_1 0.5 μg/kg [Park *et al.* 2004]. Column: C_{18} (150 x 2.1 mm i.d., 5 μm). Mobile phase: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile (70 : 30 to 55 : 45 over 15 min to 1 : 99 over 2 min), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 10 μg/kg for FB_1, hydrolysed FB_1, and *N*-(carboxymethyl)-FB_1 [Hartl, Humpf 1999; Seefelder *et al.* 2001]. Maize Meal Samples. Column: C_{18} (250 ′ 2.1 mm i.d., 5 μm). Mobile phase: water : methanol-10 mmol/L formic acid (pH 3.8; 80 : 20 to 50 : 50 over 10 min to 20 : 80 over 15 min to 0 : 100 for 10 min), flow rate 0.2 mL/min. ESI, positive and negative ion modes, MRM acquisition mode. Retention times: FB_1 19.2 min, FB_2 23.3 min, FB_3 21.4 min, nivalenol 5.6 min, deoxynivalenol 7.6 min, fusarium X 9.6 min, neosolaniol 10.1 min, 3- and 15-acetyldeoxynivalenol 12.5 min, monoacetoxyscirpenol 14.2 min, diacetoxyscirpenol 16.7 min, HT-2 20.2 min, T-2 22.5 min, α-zearalenol 24.0 min, zearalanone 24.1 min, zearalenone 24.7 min. Limit of detection, FB_1 5 μg/kg, FB_2 3 μg/kg, other analytes 2–40 ng/g [Cavaliere *et al.* 2005]. Cornflakes Samples. Column: C_{18} (150 ′ 3.2 mm i.d., 5 μm). Mobile phase: acetonitrile : water-0.3% formic acid (60 : 40), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: FB_1 3.5 min, FB_2 5.3 min, FB_3 7.1 min. Limit of quantification, 40, 15 and 25 μg/kg for FB_1, FB_2 and FB_3, respectively, limit of detection, 20, 7.5 and 12.5 μg/kg for FB_1, FB_2 and FB_3, respectively [Paepens *et al.* 2005]. Milk Samples. Column: C_{18} (150 x 4.6 mm i.d., 5 μm). Mobile phase: water-methanol (90 : 10) : water-methanol (10 : 90) both containing 0.02% acetic acid (30 : 70 for 2.2 min to 18 : 82 over 0.5 min for 11 min), flow rate 0.6 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: FB_1 6 min, FB_2 9.5 min. Limit of quantification, FB_1 50 ng/L, FB_2 40 ng/L; other toxins also detected [Sorensen, Elbaek 2005]. Soil Leachate Samples. Column: C_{18} (150 x 3.0 mm i.d., 5 μm). Mobile phase: methanol : water-0.3% acetic acid (60 : 40 to 90 : 10 over 20 min), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times: FB_1 4.1 min, FB_2 9.8 min, FB_3 5.6 min (OPA derivatives). Limit of quantification not reported [Williams *et al.* 2003]. Porcine Tissue, Body Fluid Samples. Column: C_{18} (150 x 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : water : water–formic acid (95 : 5; 20 : 70 : 10 for 1 min to 80 : 10 : 10 over 18 min for 2 min), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 5 to 10 μg/kg for FB_1 depending on sample [Meyer *et al.* 2003]. Monkey and Rat Hair Samples. Column: C_{18} (150 x 4.6 mm i.d., 5 μm). Mobile phase: water-acetonitrile-formic acid (90 : 10 : 0.1): water-acetonitrile-formic acid (10 : 90 : 0.1; 80 : 20 to 72 : 28 over 24 min for 1 min), flow rate 0.7 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, FB_1, FB_2, FB_3 60 pg on-column, aminopentol-1 and aminopentol-2 80 pg. Limit of detection, FB_1, FB_2, FB_3 25 pg on-column; aminopentol-1 and aminopentol-2 35 pg [Sewram *et al.* 2001]. Maize Samples. Column: Fluofix 120E (30 x 4.6 mm i.d., 5 μm). Mobile phase: methanol : 0.1% trifluoroacetic acid (1 : 1), flow rate 0.8 mL/min. ESI, positive ion mode. Limit of quantification not reported [Akiyama *et al.* 1997].

CE Rodent Feed Samples. Column: uncoated capillary (total/effective length: 75/70 cm, 75 μm). Running buffer: 10% acetonitrile in 25 mmol/L sodium borate buffer (pH 9). Fluorescence detection ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm). Migration times: FB_1 ~16 min, FB_2 ~17 min (9-fluorenylmethylchloroformate derivatives). Limit of detection, 0.2 ppm [Holcomb, Thompson 1996]. Maize Samples. Column: capillary (50 cm, 75 μm i.d.). Running buffer: 60 mmol/L borate buffer (pH 9.5). Fluorescence detection. Migration time: 20 min (fluorescein isothiocyanate derivative). Limit of detection, 50 ppb [Maragos *et al.* 1996].

Note For a review of LC-MS methods for the detection of mycotoxins, see Zollner and Mayer-Helm [2006] and Sforza *et al.* [2006]. For a stable isotope dilution assay of fumonisins, see Rychlik and Asam [2008]. For a review of TLC systems and reagents and their applicability to FB_1 analysis, see Shephard and Sewram [2004]. For the analysis of sphinganine and sphingosine in blood and urine, see Ribar *et al.* [2001] and van der Westhuizen *et al.* [1999]; in serum, see Shephard and van der Westhuizen [1998]; in urine and tissues, see Castegnaro *et al.* [1996], Castegnaro *et al.* [1998]and Solfrizzo *et al.* [1997]. For an LC-ELISA method for the quantification of FB_1, FB_2, FB_3 and AAL toxins, see Yu and Chu [1998].

Disposition in the Body In animal studies, fumonisins are poorly absorbed from the gastrointestinal tract, have a low bioavailability (<3.5%), and are rapidly distributed and eliminated. Liver and kidney retain most of the absorbed material. Fumonisins do not appear to cross the placental barrier and are not metabolised *in vitro* or *in vivo*. Studies in rats administered FB_2 via IP injection have shown that it is rapidly absorbed from the peritoneum, reaching a peak plasma concentration within 20 min. It is rapidly eliminated from plasma, with a half-life of 26 min, and is not detected after 24 h. Elimination is mainly via faeces, and FB_2 appears to be excreted unmetabolised. Fumonisins, structurally similar to the sphingoid bases sphinganine and sphingosine, cause the inhibition of a key sphingolipid biosynthetic enzyme, ceramide synthase, which, in turn, can alter the activity of CYP enzymes. The inhibition of ceramide synthase leads to an elevation of sphinganine levels in cells, effectively killing the cells by the induction of apoptosis. The elevated sphinganine/sphingosine ratio resulting from the accumulation of sphinganine in blood and urine has been investigated as a possible biomarker for fumonisin exposure. The sphinganine/sphingosine ratio is a useful biomarker in animal studies (where exposures or doses are high), although exposures in human populations may be too low to produce definitive changes in these parameters to be conclusive.

Toxicity Fumonisins have been associated with several diseases in animals, including leukoencephalomalacia in horses, pulmonary oedema in pigs, and hepatocarcinoma in rats. Consumption of maize that is highly contaminated with fumonisins has been associated with increased risk of human oesophageal and liver cancer in South Africa, China, and northern Italy [Chu, Li 1994; Sun *et al.* 2007; Yoshizawa *et al.* 1994]. Furthermore, there is a possibility that fumonisins are connected with infant neural tube defects in southern Texas and Mexico [Hendricks 1999; Marasas *et al.* 2004; Missmer *et al.* 2006]. FB_1 is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans (group 2B, [IARC 2002]). FDA recommended maximum levels for total fumonisins (FB_1, FB_2, FB_3) in human foods are 2 ppm for degermed dry-milled maize products, 4 ppm for whole or partially degermed dry-milled maizeproducts and dry-milled maize bran, and 3 ppm for cleaned maize intended for popcorn. FB_1 and FB_2 are also phytotoxic [Martins *et al.* 2001]. For a review on the biomarkers of fumonisin exposure, see Shephard *et al.* [2007].

In a study investigating tortilla consumption and human urinary FB_1 levels, 75 women were selected from a cohort of 996 volunteers according to their consumption of maize-based food in the previous year, as derived from a questionnaire. This group of volunteers were further split into three categories according to their level of maize-based food intake: high intake ($n = 26$), medium intake ($n = 25$) and low intake ($n = 24$). Urine samples were collected and analysed for FB_1 levels. Results were repoted as follows:

	Maize Consumption group		
	Low	***Medium***	***High***
Tortillas consumed each meal (median, range)	2 (1 to 5)	3 (3 to 3)	10 (6 to 16)
Urinary FB_1 levels (ng/L)	35.0	63.1	147.4

Although a correlation was demonstrated, a great inter-individual variation was observed in the urine levels, which reflects numerous factors (from variations in food preparation to the fact that other sources of fumonisin exposure were not considered in the study). An estimated daily exposure of 368 ng/kg bodyweight was calculated for the high-intake group (WHO and FDA daily recommendation 2 μg/kg). It is worth noting that the nixtamalisation process involved in the production of tortillas is known to reduce the fumonisin level up to 80% [Voss *et al.* 2001]. Therefore, FB_1 levels can be expected to be much higher when maize is consumed in forms other than tortillas [Gong *et al.* 2008].

In a study investigating the use of hair to assess exposure to fumonisins, monkeys receiving control, low-dose and high-dose fumonisin-contaminated diets had hair FB_1concentrations of 5.98, 33.77 and 65.93 μg/g, respectively. Similar results were observed in rats, thus indicating that hair may be an appropriate matrix for the assessment of chronic exposure to fumonisins [Sewram *et al.* 2001].

Half-life Estimated at 128 min.

Note For reviews and conference proceedings on fumonisins, see *Food Addit Contam* [Vol. 24, Issue 10, 2007; Vol. 18, Issue 3, 2001]. For a review on the toxicology of fumonisins, see *Environmental Health Perspectives* [Vol. 109, Suppl.2, 2001] and *Advances in Experimental and Medical Biology* [Vol. 392, 1996]. For general overviews of mycotoxins, see [de Vries *et al.* 2002] and [Trucksess and Pohland 2001]. For a review of the toxic effects of mycotoxins in humans, see [Peraica *et al.* 1999].

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Furaltadone

Antibacterial (Veterinary)

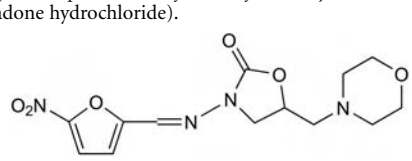
$C_{13}H_{16}N_4O_6$ = 324.3

CAS—139-91-3; 59302-14-6 (±)

IUPAC Name 5-(4-Morpholinylmethyl)-3-[[(5-nitro-2-furanyl)methylene] amino]-2-oxazolidinone

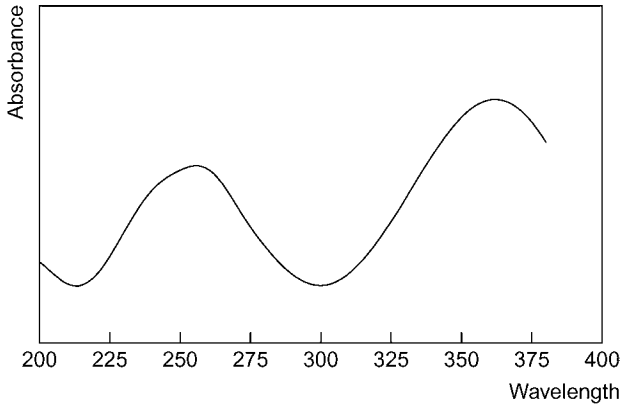
Synonyms Furmethonol; nitrofurmethonum.

Proprietary Names *Altafur*; *Altabactina*; *Furazolin*; *Ibifur*; *Medifuran*; *Nitraldone*; *Otifuril*; *Sepsinol*; *Ultrafur*; *Unifur*; *Valsyn*. It is an ingredient of *Panotile* (furaltadone hydrochloride).

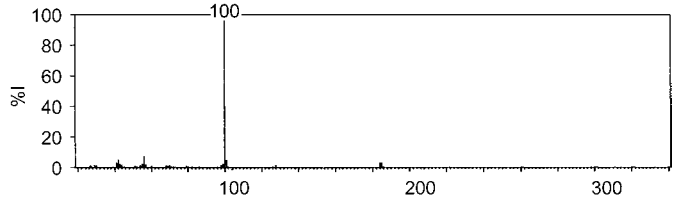


Chemical Properties A yellow crystalline powder. Mp about 205°. Soluble 1 in 2000 of water, 1 in 1000 of ethanol and 1 in 300 of chloroform; practically insoluble in ether. Log *P* (octanol/water), 0.2.

Colour Test Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.43; system TB—R_f 0.00; system TC—R_f 0.40; system TL—R_f 0.40; system TAJ—R_f 0.35; system TAK—R_f 0.00; system TAL—R_f 0.62 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HAA—retention time 8.9 min.
Ultraviolet Spectrum Aqueous acid—255, 362 nm.



Infrared Spectrum Principal peaks at wavenumbers 1755, 1226, 1250, 1110, 1020, 1315 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 100, 56, 101, 42, 185, 184, 41, 128.

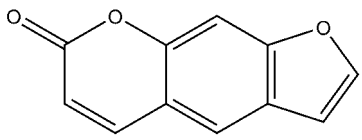


Disposition in the Body
Toxicity It was formerly administered orally, but preparations were withdrawn owing to its toxic effects. It is used in preparations for ear disorders.

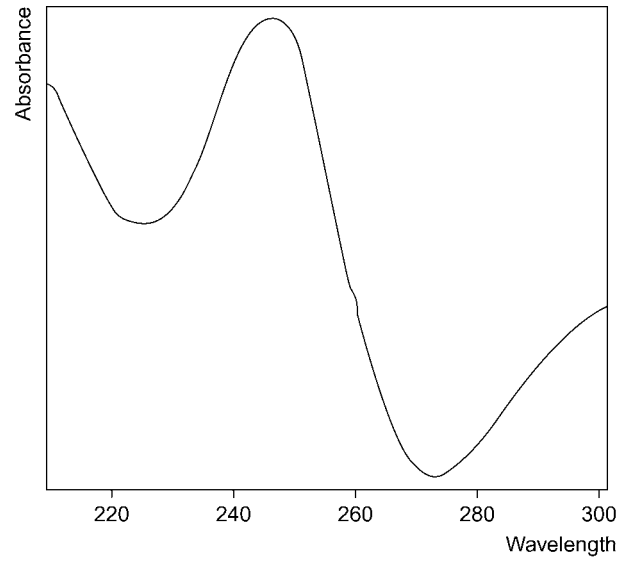
Furanocoumarins

Photosensitiser, Phytoalexin, Psoralens
$C_{11}H_6O_3$ = 186.2
CAS—66-97-7

IUPAC Name Furo[3,2-*g*]chromen-7-one
Synonyms Ficusin; furanocoumarin; 7*H*-Furo[3,2-*g*][1]benzopyran-7-one; furo[3,2-*g*]coumarin; furocoumarin; 6-hydroxy-5-benzofuranacrylic acid δ-lactone.



Chemical Properties Furanocoumarins (or psoralens) are potent photosensitising toxins that also act as phytoalexins, predominantly in plants of the Umbelliferae (parsley, celery, parsnips, carrots), Rutaceae (grapefruit, lime, bitter orange), and Moraceae (fig) families and several other plant sources. Furanocoumarins have been increasingly used for photochemotherapeutic treatment of dermatological diseases. Psoralen is one of the major linear furanocoumarins together with 8-methoxypsoralen (xanthotoxin), 5-methoxypsoralen (bergapten), and 5,8-dimethoxypsoralen (pimpinellin). White powder. Mp 158° to 161° [Valenciennes *et al.* 1999]; also reported as crystals from ether. Mp 163° to 164°, also as 169° to 179° [O'Neil *et al.* 2006]. Log *P* (octanol/water), 1.97 [Caffieri 2001]. Standard solutions of psoralen and bergapten were found to be stable for up to 24 h at 22°, 2 months at 4°, and 6 months at −20° [Cardoso *et al.* 2006].
Note For partition studies of psoralen and isopsoralen in different media, see Liu *et al.* [2004]. For the partition coefficient of psoralen derivatives and angelicin derivatives, see Caffieri [2001].
Ultraviolet Spectrum Principal peak at 250 nm



Infrared Spectrum Principal peaks at wavenumbers 1140, 1600–1650, 1725, 3100 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 186, 158, 102, 51, 130, 50, 76, 75 [Valenciennes *et al.* 1999].
Quantification
Serum **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (60 : 40), flow rate 1.0 mL/min. UV detection. *k* values: psoralen 2.2, bergapten 3.2. Limit of detection, 6 µg/L [Stolk *et al.* 1987].
Urine **HPLC** Column: C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : water (0 : 30 : 70 for 10 min, to 25 : 25 : 50 over 12 min), flow rate 1.0 mL/min. UV detection (λ = 312 nm). Retention times: psoralen ~7.8 min, 7-hydroxycoumarin ~4.2 min, coumarin ~5.6 min, 7-methoxycoumarin ~6.6 min, 8-methoxypsoralen (xanthotoxin) ~8.4 min, 5,7-dimethoxycoumarin (citropten) ~9.6 min, 5,8-dimethoxypsoralen (isopimpinellin) ~10.6 min, 7-ethoxy-4-methylcoumarin ~13.2 min, 9-isopenteneoxypsoralen (imperatorin) ~20.8 min, 5-methoxypsoralen (bergapten) not reported. Limit of quantification, psoralen and isopimpinellin 2.5 mg/L; coumarin, 7-methoxycoumarin, citropten, bergapten and imperatorin 2.0 mg/L; 7-hydroxycoumarin and xanthotoxin 1.0 mg/L; 7-ethoxy-4-methylcoumarin 3.0 mg/L, limits of detection also reported [Wang, Jiang 2006].
Other **TLC** Plant Extracts (*Aglae marmelos*, *Trachyspermum ammi*, *Foeniculum vulgare*). Plates: precoated silica gel $60F_{254}$ (20 × 10 cm, 0.2 mm). UV detection (λ = 304 nm for psoralen, 331 nm for umbelliferone and 280 nm for eugenol). Solvent system: toluene : methanol (9.5 : 0.5). R_f values: psoralen 0.58, umbelliferone 0.3, eugenol 0.7. Limit of quantification, psoralen 16 ng/spot, umbelliferone 1.2 ng/spot, eugenol 150 ng/spot; limit of detection, psoralen 8.0 ng/spot, umbelliferone 0.8 ng/spot, eugenol 60 ng/spot [Dhalwal *et al.* 2007].
GC Cream and Pomade Phytochemical Formulations. Column: LM-5 fused silica capillary (15 m × 0.2 mm i.d., 0.2 µm). Carrier gas: H_2, 0.8 mL/min. Temperature programme: 150° to 240° at 10°/min to 280° at 5°/min for 15 min. FID. Limit of quantification, psoralen 4.3 mg/L, bergapten 2.0 mg/L, pimpinellin 9.7 mg/L, isopimpinellin 3.0 mg/L; limit of detection, psoralen 1.3 mg/L, bergapten 0.6 mg/L, pimpinellin 2.9 mg/L, isopimpinellin 1.0 mg/L [Cardoso *et al.* 2000].
GC-MS Capsules and Tablets of Phytochemical Preparations (*Dorstenia multiformes, Plumeria lancifolia*, *Cereus jamacaru*, *Erythrina mulungu*). Column: LM-5 fused silica capillary (15 m × 0.2 mm i.d., 0.2 µm). Temperature programme: 150° to 240° at 10°/min for 20 min. EI ionisation at 70 eV. Retention times: psoralen 4.3 min, bergapten 6.3 min, 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one (DT) 16.5 min. Limit of quantification, psoralen 0.33 mg/L, bergapten 0.30 mg/L, DT 0.8 mg/L, limit of detection, psoralen 0.1 mg/L, bergapten 0.09 mg/L, DT 0.24 mg/L [Cardoso *et al.* 2006].
HPLC Traditional Chinese Medicine Samples (*Psoralea corylifolia*). Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water-0.1% acetic acid (10 : 90 to 82 : 18 over 40 min), flow rate 1.0 mL/min. UV detection (λ = 310 nm). Retention times: psoralen 17.4 min, isopsoralen 17.9 min, psoralenoside 7.5 min, isopsoralenoside 7.9 min, bavachromene 23.5 min, corylifolin 24.7 min, corylin 25.8 min, psoralidin 26.3 min, isobavachalcone 29.1 min, bavachinin 30.1 min, corylifol A 30.7 min, bakuchiol 37.7 min. Limit of quantification, (on-column) psoralen 2.42 ng, isopsoralen 2.35 ng, limit of detection, (on-column) psoralen 0.85 ng, isopsoralen 0.77 ng [Qiao *et al.* 2007]. Traditional Chinese Medicine Samples (*Umbelliferae* spp.). Column: C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : water (0 : 30 : 70 for 10 min to 25 : 25 : 50 over 12 min), flow rate 1.0 mL/min. UV detection (λ = 312 nm). Retention times: psoralen ~7.8 min, 7-hydroxycoumarin ~4.2 min, coumarin ~5.6 min, 7-methoxycoumarin ~6.6 min, xanthotoxin ~8.4 min, citropten ~9.6 min, isopimpinellin ~10.6 min, 7-ethoxy-4-methylcoumarin ~13.2 min, imperatorin ~20.8 min, bergapten not reported. Limit

of quantification not reported [Wang, Jiang 2006]. Capsules, Tablets and Topical Preparations of Phytochemicals (*D. multiformes, P. lancifolia*, *C. jamacaru*, *E. mulungu*). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (55 : 45), flow rate 1.0 mL/min. UV detection (λ = 223 nm). Retention times: psoralen 6.2 min, bergapten 7.5 min, DT 11.4 min. Limit of quantification, psoralen 0.1 mg/L, bergapten 0.23 mg/L, DT 0.5 mg/L, limit of detection, psoralen 0.03 mg/L, bergapten 0.07 mg/L, DT 0.15 mg/L [Cardoso *et al.* 2002a, 2002b, 2006; Pires *et al.* 2004]. Plant Extracts (*Zanthoxylum americanum*). Column: C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: water : sodium dihydrogen phosphate buffer : acetonitrile (80 : 10 : 20 to 50 : 10 : 50 over 20 min to 45 : 10 : 55 over 10 min), flow rate 1.0 mL/min. UV detection (λ = 225 nm). Limit of quantification not reported [Bafi-Yeboa *et al.* 2005]. Traditional Chinese Medicine Samples (*P. corylifolia*). Column: C_{18} (200 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : water (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: psoralen ~26 min, isopsoralen ~30 min. Limit of quantification not reported [Liu *et al.* 2004]. Celery and Parsnip Samples. Column: C_{18} (200 × 2.1 mm i.d., 4 µm). Mobile phase: water : methanol (45 : 55), flow rate 0.2 mL/min. UV detection (λ = 304, 270, 246 nm). Limit of detection, 0.5 mg/L [Ostertag *et al.* 2002]. Column: diphenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-methanol-water (20 : 180 : 800) : acetonitrile-methanol (20 : 180; 100 : 0 for 10 min to 70 : 30 over 20 min to 25 : 75 over 15 min), flow rate 1.5 mL/min. DAD. Retention times: psoralen 18 min, angelicin 20 min, xanthotoxin 23 min, bergapten 26 min, trioxsalen 36 min. Limit of quantification, 0.03 mg/kg (DAD), 0.1 mg/kg (UV λ = 254 nm) [Lombaert *et al.* 2001].

LC-MS Traditional Chinese Medicine Samples (*P. corylifolia*). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: water-acetic acid (100 : 0.1) : acetonitrile (60 : 40 to 50 : 50 over 15 min to 40 : 60 over 20 min to 30 : 70 over 10 min to 20 : 80 over 10 min), flow rate 1.0 mL/min. ESI, positive ion mode and UV detection (λ = 245 nm). Retention times: psoralen ~12 min, isopsoralen ~13 min, neobavaisoflavone ~20 min, bavachin ~24 min, corylin ~26 min, bavachromene ~28 min, psoralidin ~31 min, isobavachalcone ~37 min, bavachinin ~39 min, bavachalcone ~52 min. Limit of quantification not reported [Zhao *et al.* 2005].

CE Plant Samples (*P. corylifolia* L.) and Traditional Chinese Medicine Samples. Column: fused silica capillary (total/effective length: 50/42.2 cm, 75 µm i.d.). Running buffer: 30% microemulsion (ethyl acetate : SDS : butan-1-ol : water [3.2 : 3.5 : 0.8 : 92.5]) with 25 mmol/L borate (pH 8.5). UV detection (λ = 254 nm). Limit of detection, psoralen 0.42 mg/L, isopsoralen 0.32 mg/L [Zhang *et al.* 2007]. Column: fused silica capillary (total/effective length: 59/51 cm, 75 µm i.d.). Running buffer: 18 mmol/L borate buffer, 12 mmol/L phosphate buffer, 25 mmol/L SDS (pH 9.2). UV detection (λ = 254 nm). Limit of quantification, psoralen and isopsoralen 0.05 mg/L [Wang *et al.* 1999].

Disposition in the Body Furanocoumarins can intercalate into DNA in the presence of UV light. These molecules absorb energy and can react with epidermal DNA producing a covalent bond between the furancoumarin and nucleic acid. Depending on the dose administered, this bond can lead to either cell death or DNA repair, synthesis and replication.

Toxicity Furanocoumarins are capable of inducing photodermatitis and high-dose exposure may result in swelling, redness and lesion formation on the epidermis, or even cutaneous carcinoma in extreme cases. The storage of furanocoumarin-containing foodstuff can dramatically affect levels of furanocoumarins present and should be monitored [Ostertag *et al.* 2002].

Note For a case study of a severe bullous reaction in a 6-year-old boy resulting from soaking both hands in a lime-based drink, see Wagner *et al.* [2002] (no furanocoumarin levels measured in human samples).

Protein Binding Approximately 89% to serum albumin.

Note For an overview of psoralens in the treatment of psoriasis, see McEvoy and Stern [1987].

Dose Used as a photochemotherapy in conjunction with high-intensity UV for the treatment of skin diseases.

Bafi-Yeboa NF *et al.* (2005). Antifungal constituents of northern prickly ash, *Zanthoxylum americanum* Mill. *Phytomedicine* 12: 370–377.

Caffieri S (2001). Reversed-phase high-performance liquid chromatography (RP-HPLC) determination of lipophilicity of furocoumarins: relationship with DNA interaction. *J Pharm Sci* 90: 732–739.

Cardoso CA *et al.* (2000). Rapid determination of furanocoumarins in creams and pomades using SPE and GC. *J Pharm Biomed Anal* 22: 203–214.

Cardoso CA *et al.* (2002). Simple and rapid determination of psoralens in topic solutions using liquid chromatography. *J Pharm Biomed Anal* 27: 217–224.

Cardoso CA *et al.* (2002). Simultaneous determination of furanocoumarins in infusions and decoctions from 'Carapia' (*Dorstenia* species) by high-performance liquid chromatography. *J Agric Food Chem* 50: 1465–1469.

Cardoso CA *et al.* (2006). A method for quantitative determination of furanocoumarins in capsules and tablets of phytochemical preparations. *Chem Pharm Bull (Tokyo)* 54: 442–447.

Dhalwal K *et al.* (2007). Rapid densitometric method for simultaneous analysis of umbelliferone, psoralen, and eugenol in herbal raw materials using HPTLC. *J Sep Sci* 30: 2053–2058.

Liu R *et al.* (2004). Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. *J Chromatogr A* 1057: 225–228.

Lombaert GA *et al.* (2001). Furanocoumarins in celery and parsnips: method and multiyear Canadian survey. *J AOAC Int* 84: 1135–1143.

McEvoy M, Stern TRS (1987). Psoralens and related compounds in the treatment of psoriasis. *Pharmacol Ther* 34: 75–97.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Ostertag E *et al.* (2002). Effects of storage conditions on furocoumarin levels in intact, chopped, or homogenized parsnips. *J Agric Food Chem* 50: 2565–2570.

Pires AE *et al.* (2004). A method for fast determination of psoralens in oral solutions of phytomedicines using liquid chromatography. *J Pharm Biomed Anal* 36: 415–420.

Qiao CF *et al.* (2007). Chemical fingerprint and quantitative analysis of fructus psoraleae by high-performance liquid chromatography. *J Sep Sci* 30: 813–818.

Stolk LM *et al.* (1987). Determination of psoralen in serum by reversed-phase high-performance liquid chromatography. *J Chromatogr* 423: 383–386.

Valenciennes E *et al.* (1999). Screening for biological activity and chemical composition of *Euodia borbonica* var. *borbonica* (Rutaceae), a medicinal plant in Reunion Island. *J Ethnopharmacol* 64: 283–288.

Wagner AM *et al.* (2002). Bullous phytophotodermatitis associated with high natural concentrations of furanocoumarins in limes. *Am J Contact Dermat* 13: 10–14.

Wang L, Jiang HSY (2006). Simultaneous determination of urinary metabolites of methoxypsoralens in human and Umbelliferae medicines by high-performance liquid chromatography. *J Chromatogr Sci* 44: 473–478.

Wang D *et al.* (1999). Micellar electrokinetic capillary chromatography of psoralen and isopsoralen. *Electrophoresis* 20: 1895–1899.

Zhang H *et al.* (2007). Separation and determination of psoralen and isopsoralen by microemulsion electrokinetic chromatography. *Biomed Chromatogr* 21: 1083–1087.

Zhao L *et al.* (2005). Fingerprint analysis of *Psoralea corylifolia* L. by HPLC and LC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 821: 67–74.

Furazolidone

F

Antimicrobial

$C_8H_7N_3O_5$ = 225.2

CAS—67-45-8

IUPAC Name 3-[[(5-Nitro-2-furanyl)methylene]amino]-2-oxazolidinone

Synonym Nifurazolidonum

Proprietary Names *Coryzium* (vet.); *Exofur*; *Furasian*; *Furion*; *Furovag*; *Furoxane*; *Furoxona*; *Furoxone*; *Fuxol*; *Giardil*; *Giarlam*; *Medaron*; *Neftin* (vet.); *Nicolen*; *Nifulidone*; *Nifuran*; *Novafur*; *Ortazol*; *Quimefuran*; *Roptazol*; *Salmocide*; *Seforman*; *Tikofuran*; *Topazone*; *Tricofuron*.

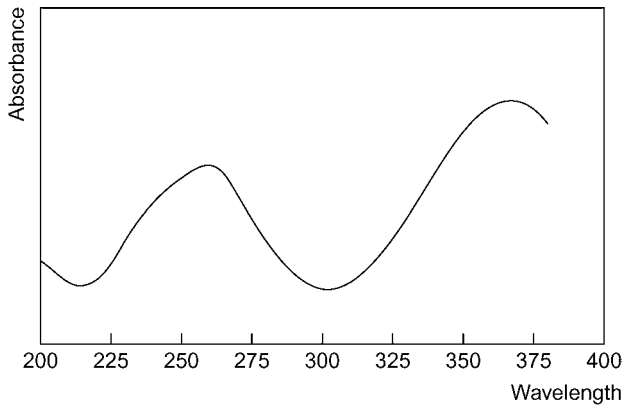
Chemical Properties A yellow crystalline powder. Mp about 259°, with decomposition. Very slightly soluble in water and ethanol; slightly soluble in chloroform; practically insoluble in ether. Log *P* (octanol/water), −0.04.

Colour Test Dissolve 1 mg in 1 mL of dimethylformamide with 0.05 mL of 1 mol/L ethanolic potassium hydroxide—deep blue.

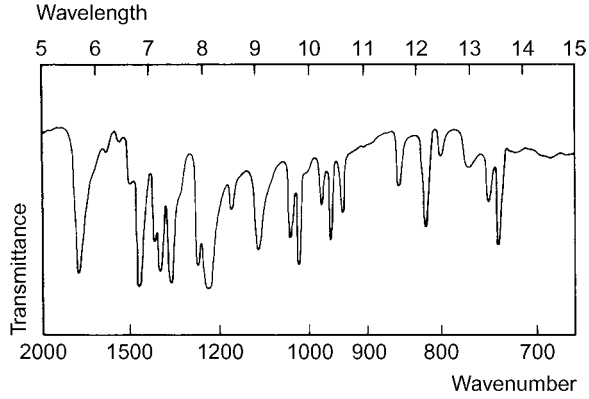
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.00; system TC—R_f 0.47; system TE—R_f 0.46; system TF—R_f 0.18; system TL—R_f 0.59; system TAE—R_f 0.56.

High Performance Liquid Chromatography System HAA—retention time 12.2 min; system HX—RI 336.

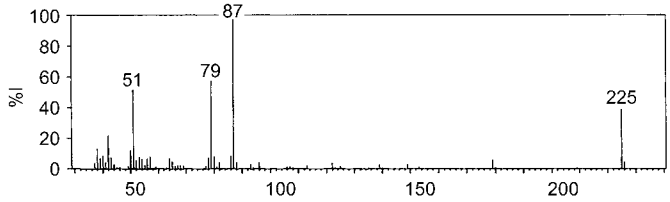
Ultraviolet Spectrum After solution in dimethylformamide and dilution with water—259, 367 nm (A^1_1=754a).



Infrared Spectrum Principal peaks at wavenumbers 1227, 1739, 1015, 1250, 1101, 738 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 87, 79, 51, 225, 42, 50, 86, 80.



Quantification
Biological Fluids **HPLC** Limit of detection, 50 µg/L [Valadez-Salazar *et al.* 1989].
Dose 400 mg daily.

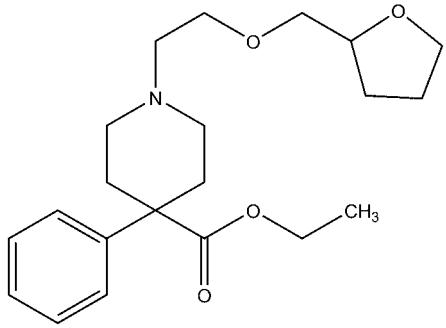
Valadez-Salazar A *et al.* (1989). Detection of furazolidone in human biological fluids by high performance liquid chromatography. *J Antimicrob Chemother* 23: 589–595.

F

Furethidine

Narcotic
$C_{21}H_{31}NO_4$ = 361.5
CAS—2385-81-1

IUPAC Name Ethyl 1-[2-(oxolan-2-ylmethoxy)ethyl]-4-phenylpiperidine-4-carboxylate
Synonyms Ethyl 4-phenyl-1-(2-tetrahydrofurfuryloxyethyl)piperidine-4-carboxylate; ethyl 1-(tetrahydrofurfuryloxyethyl)-4-phenylpiperidine-4-carboxylate; 4-phenyl-1-[2-(tetrahydro-2-furanyl)methoxy]ethyl]-4-piperidinecarboxylic acid ethyl ester; 4-phenyl-1-[2-(tetrahydrofurfuryloxy)ethyl]isonipecotic acid ethyl ester; TA 48; 1-(2′-tetrahydrofurfuryloxyethyl)norpethidine.



Chemical Properties A yellow oil. Mp ≈28°. Soluble in dilute hydrochloric acid. pKa 7.48 [O'Neil *et al.* 2006]. Furethidine is extracted by organic solvents from aqueous alkaline solutions.

Furethidine Methiodide
$C_{22}H_{34}INO_4$ = 503.4

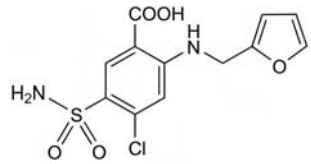
Chemical Properties Crystals from ethyl acetate. Mp 174°.
Colour Test Sulfuric acid-formaldehyde test—orange (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.66 (location reagent acidified iodoplatinate spray, positive reaction).

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

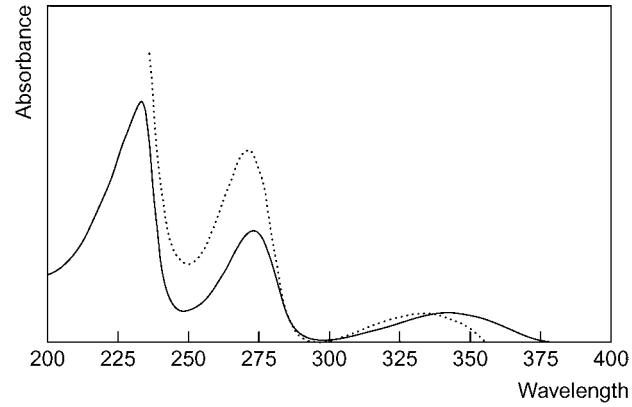
Furosemide

Diuretic
$C_{12}H_{11}ClN_2O_5S$ = 330.7
CAS—54-31-9

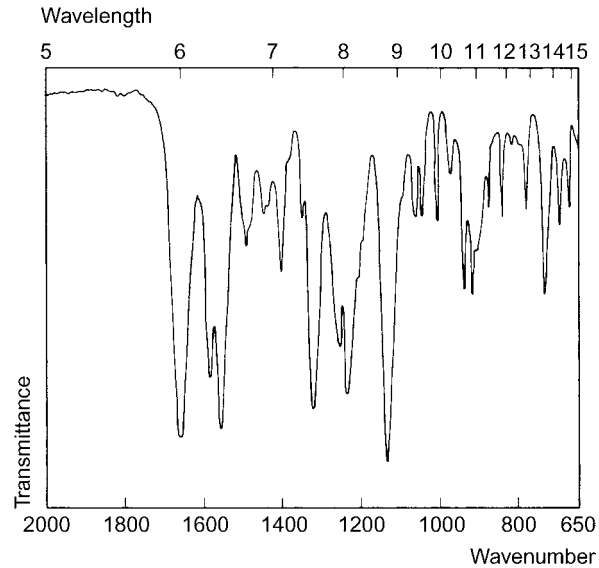
IUPAC Name 4-Chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid
Synonyms 5-(Aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; furosemidum; fursemide; frusemide; LB-502.
Proprietary Names *Dryptal*; *Froop*; *Frumax*; *Frusid*; *Frusol*; *Lasix*; *Rusyde*; *Tenkafruse*. See Sweetman [2009] for further proprietary names. It is an ingredient of *Aridil*, *Diumide-K*, *Frumil*, *Frusemek*, *Frusene*, *Lasikal*, *Lasilactone* and *Lasoride*.



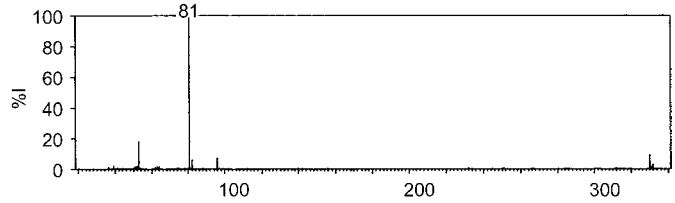
Chemical Properties A white or slightly yellow crystalline powder. Mp about 206°, with decomposition. Slightly soluble in water and chloroform; soluble 1 in 75 of ethanol, 1 in 15 of acetone and 1 in 850 of ether; soluble in dimethylformamide. pK_a 3.9 (20°). Log *P* (octanol/water), 2.0. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Tests Koppanyi-Zwikker test—violet; Liebermann's reagent—black; sulfuric acid—yellow.
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.01; system TE—R_f 0.06; system TF—R_f 0.07; system TG—R_f 0.19; system TAD—R_f 0.07; system TAE—R_f 0.86; system TAJ—R_f 0.10; system TAK—R_f 0.25; system TAL—R_f 0.70 (mercurous nitrate spray, positive; acidified potassium permanganate solution, positive; Van Urk reagent, pink-brown).
Gas Chromatography System GA—furosemide not eluted, furosemide-Me RI 2890, furosemide-Me_2 RI 2850, furosemide-Me_3 RI 2800, art (-SO_2NH) RI 2040, art (-SO_2NH)-Me RI 2020, art (-SO_2NH)-Me_2 RI 2050, M (*N*-desalkyl-)-Me RI 2750, M (*N*-desalkyl-)-Me_2 RI 2450; system GD—methyl derivative RRT 2.64 (relative to n-$C_{16}H_{34}$); system GX—furosemide-Me_3 retention time 7.0 min; system GY—furosemide-Me_3 retention time 4.7 min.
High Performance Liquid Chromatography System HV—RRT 0.45 (relative to meclofenamic acid); system HX—RI 435; system HY—RI 380; system HZ—retention time 3.5 min; system HAA—retention time 15.2 min; system HAX—retention time 5.8 min; system HAY—retention time 5.0 min.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1=1333a), 274 (A^1_1=600a), 342 nm; aqueous alkali—271 (A^1_1=580a), 333 nm.



Infrared Spectrum Principal peaks at wavenumbers 1143, 1668, 1565, 1240, 1590, 1260 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 81, 53, 330, 96, 82, 332, 64, 63.



Quantification
Plasma **GC** ECD. Limit of detection, 20 µg/L [Keller *et al.* 1981].
GC-MS Selected ion monitoring [Ptacek *et al.* 1996].
HPLC Limit of detection, 5 µg/L [Abou-Auda *et al.* 1998]. Fluorescence detection. Furosemide and its acyl glucuronide [Vree *et al.* 1994]. Fluorescence detection.

Limit of detection, 1.8 µg/L [Farthing *et al.* 1992]. Fluorescence detection. Furosemide and amiloride. Limit of detection 0.3 µg/L for furosemide [Reeuwijk *et al.* 1992]. Fluorescence detection. Limit of detection, 5 µg/L [Saugy *et al.* 1991]. Fluorescence detection. Limit of detection, 100 µg/L [Kerremans *et al.* 1982]. Fluorescence detection. Furosemide and its glucuronide metabolite. Limit of detection, 8 µg/L [Smith *et al.* 1980].

HPLC-MS Limit of detection, 10 µg/L [Abdel-Hamid 2000].

Serum **HPLC** [Okuda *et al.* 1996].

Urine **GC** See Plasma [Keller *et al.* 1981].

GC-MS See Plasma [Ptacek *et al.* 1996].

HPLC UV detection [Nava-Ocampo *et al.* 1999]. See Plasma [Abou-Auda *et al.* 1998]. Amperometric detection. Furosemide and piretanide, [Barroso *et al.* 1996]. See Plasma [Vree *et al.* 1994]; [Saugy *et al.* 1991]; limit of detection, 500 µg/L [Kerremans *et al.* 1982]; [Smith *et al.* 1980].

Other **HPLC** Neonatal plasma. Fluorescence detection [Sidhu, Charles 1993].

Disposition in the Body Rapidly but incompletely absorbed after oral administration. Up to 90% of an IV dose is excreted in the urine, mainly as unchanged drug with up to 14% of the dose as a glucuronide conjugate. 2-Amino-4-chloro-5-sulfamoylanthranilic acid has been reported as a metabolite in several studies, but in other cases it has not been detected and it has been suggested that it is an analytical artefact produced during acid extraction procedures. In normal subjects, about 6 to 18% of a dose is eliminated in the faeces after IV administration; this may be increased to about 60% in renal failure. It crosses the placenta and is found in breast milk.

Therapeutic Concentration

Following a single oral dose of 80 mg to 8 fasting subjects, peak serum concentrations of 1.8 to 4.9 mg/L (mean 2.3) were attained in 60 to 70 min [Kelly *et al.* 1974].

Plasma concentrations averaging 7.5 mg/L were reported 10 min after an IV dose of 40 mg to 9 subjects [Smith *et al.* 1980].

Bioavailability About 60 to 70%.

Half-life Plasma half-life, about 1 to 3 h, increased in subjects with renal failure, congestive heart failure, liver disease and in neonates (up to about 46 h).

Volume of Distribution About 0.1 to 0.2 L/kg, increased in subjects with liver disease, nephrotic syndrome and in neonates.

Clearance Plasma clearance, about 1 to 3 mL/min/kg, decreased in uraemia, heart failure, the elderly and neonates.

Protein Binding About 99%, decreased in patients with cirrhosis, the nephrotic syndrome and uraemia.

Note For reviews of furosemide pharmacokinetics, see Benet [1979] and Cutler and Blair [1979].

Dose Usually 20 to 80 mg daily; in oliguria, up to maximum single dose of 2 g.

Abdel-Hamid ME (2000). High-performance liquid chromatography-mass spectrometric analysis of furosemide in plasma and its use in pharmacokinetic studies. *Farmaco* 55: 448–454.

Abou-Auda HS *et al.* (1998). High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies. *J Chromatogr B Biomed Sci Appl* 710: 121–128.

Barroso MB *et al.* (1996). Determination of piretanide and furosemide in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection. *J Chromatogr B Biomed Appl* 675: 303–312.

Benet LZ (1979). Pharmacokinetics/pharmacodynamics of furosemide in man: a review. *J Pharmacokinet Biopharm* 7: 1–27.

Cutler RE, Blair AD (1979). Clinical pharmacokinetics of frusemide. *Clin Pharmacokinet* 4: 279–296.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Farthing D *et al.* (1992). External-standard high-performance liquid chromatographic method for quantitative determination of furosemide in plasma by using solid-phase extraction and on-line elution. *J Pharm Sci* 81: 569–571.

Keller E *et al.* (1981). Influence of hepatic cirrhosis and end-stage renal disease on pharmacokinetics and pharmacodynamics of furosemide. *Eur J Clin Pharmacol* 20: 27–33.

Kelly MR *et al.* (1974). Pharmacokinetics of orally administered furosemide. *Clin Pharmacol Ther* 15: 178–186.

Kerremans AL *et al.* (1982). Specimen handling and high-performance liquid chromatographic determination of furosemide. *J Chromatogr* 229: 129–139.

Nava-Ocampo AA *et al.* (1999). Simplified method to quantify furosemide in urine by high-performance liquid chromatography and ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 730: 49–54.

Okuda T *et al.* (1996). High-performance liquid chromatography using on-line solid-phase extraction: determination of furosemide in human serum. *J Chromatogr B Biomed Appl* 682: 343–348.

Ptacek P *et al.* (1996). Determination of furosemide in plasma and urine by gas chromatography/mass spectrometry. *Arzneimittelforschung* 46: 277–283.

Reeuwijk HJ *et al.* (1992). Simultaneous determination of furosemide and amiloride in plasma using high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 575(2): 269–274.

Saugy M *et al.* (1991). Rapid high-performance liquid chromatographic determination with fluorescence detection of furosemide in human body fluids and its confirmation by gas chromatography-mass spectrometry. *J Chromatogr* 564: 567–578.

Sidhu JS, Charles BG (1993). Simple microscale high-performance liquid chromatographic method for determination of furosemide in neonatal plasma. *J Chromatogr* 612: 161–165.

Smith DE *et al.* (1980). Absorption and disposition of furosemide in healthy volunteers, measured with a metabolite-specific assay. *Drug Metab Dispos* 8: 337–342.

Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn, London: Pharmaceutical Press.

Vree TB *et al.* (1994). Determination of furosemide with its acyl glucuronide in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis with fluorescence detection. Preliminary pharmacokinetics and effect of probenecid. *J Chromatogr B Biomed Appl* 655: 53–62.

F

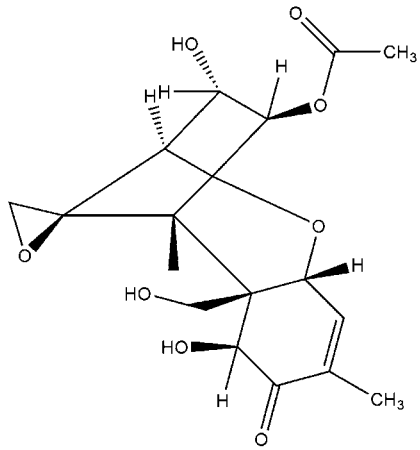
Fusarenone-X

12,13-Epoxytrichothecene, Mycotoxin

$C_{17}H_{22}O_8 = 354.4$

IUPAC Name 4β-Acetoxy-3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one

Synonyms Fusarenon-X, fusarenon, nivalenol monoacetate, monoacetylnivalenol.



Ultraviolet Spectrum Principal peak at 220 nm [Sydenham *et al.* 1996].

Mass Spectrum Principal peaks at *m/z* 179, 159, 191, 247, 217, 229 [Sydenham *et al.* 1996].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported on the table as followe:

	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Deoxynivalenol	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyldeoxynivalenol	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	**65 : 35**	**0.63**	**0.73**	**65 : 35**	**0.76**	**0.75**	**N/A**	**N/A**	**N/A**
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Nivalenol	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989]

Quantification **LC-MS** Food (peanuts, pistachios, wheat, maize, cornflakes, raisins, figs). Column: Alltima C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (90 : 10 to 30 : 70 at 12 min for 4 min to 10 : 90 at 17.5 min for 2.5 min to 90 : 10 at 21 min for 4 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode, positive ion mode. Limit of detection, 50 µg/kg [Spanjer *et al.* 2008].

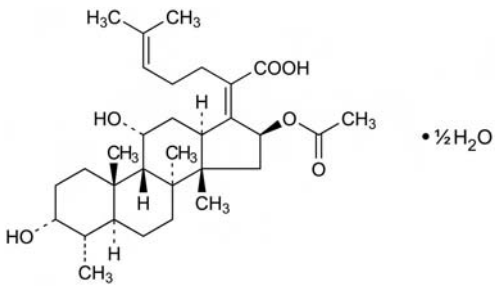
Abramson D *et al.* (1989). Chromatography of mycotoxins on precoated reverse-phase thin-layer plates. *Arch Environ Contam Toxicol* 18: 327–330.
Spanjer MC *et al.* (2008). LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Addit Contam* 25: 472–489.
Sydenham EW *et al.* (1996). Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in corn: AOAC–IUPAC Collaborative Study. *J AOAC Int* 79: 688–696.

Fusidic Acid

F

Antibiotic
$C_{31}H_{48}O_6, {}^1/_2H_2O$ = 525.7
CAS—6990-06-3 (anhydrous)

IUPAC Name (2*Z*)-2-[(3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-Acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-ylidene]-6-methylhept-5-enoic acid
Synonym (3α,4α,8α,9β,11α,13α,14β,16β,17*Z*)-16-(Acetyloxy)-3,11-dihydroxy-29-nordammara-17(20),24-dien-21-oic acid hemihydrate
Proprietary Names *Fucidin (suspension)*; *Fucithalmic*; *Futasole*.

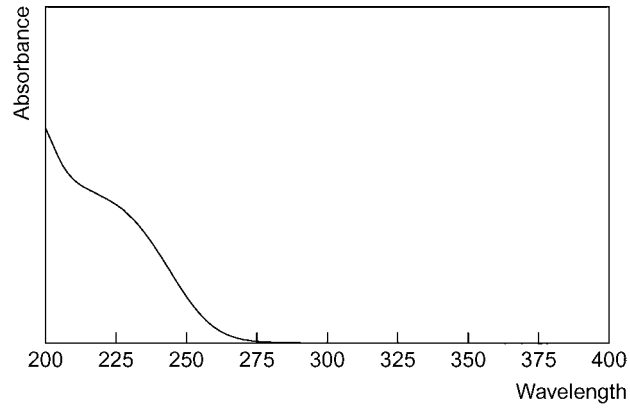


Chemical Properties A white crystalline powder. Mp about 193°. Sparingly soluble in water and hexane; soluble 1 in 5 of ethanol, 1 in 4 of chloroform and 1 in 60 of ether; soluble in acetone, pyridine, and dioxane. pK_a 5.4. Log *P* (octanol/water), 6.8.

Sodium Fusidate

$C_{31}H_{47}NaO_6$ = 538.7
CAS—751-94-0
Synonym Fusidate sodium
Proprietary Names *Dermomycin*; *Fucidin(e) (tablets)*.
Chemical Properties A white, slightly hygroscopic, crystalline powder. Soluble 1 in 1 of water, 1 in 1 of ethanol and 1 in 350 of chloroform; practically insoluble in ether.

High Performance Liquid Chromatography System HAA—retention time 24.9 min.
Ultraviolet Spectrum

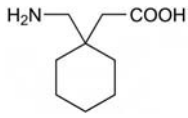


Infrared Spectrum Principal peaks at wavenumbers 1267, 1547, 1706, 1238, 1020, 966 cm^{-1} (sodium fusidate, KBr disk).
Quantification
Plasma **HPLC** UV detection. Limit of detection, 200 µg/L [Rahman, Hoffman 1988]. UV detection. Limit of detection, 500 µg/L [Hikal 1983].
Dose 1.5 to 3 g of sodium fusidate daily.

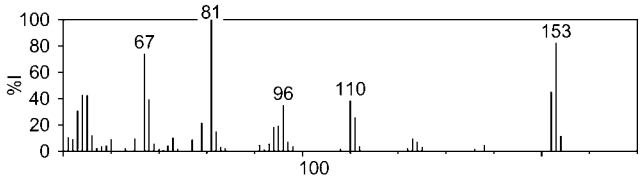
Hikal AH (1983). *Int J Pharm* 13: 297–301.
Rahman A, Hoffman NE (1988). High-performance liquid chromatographic determination of fusidic acid in plasma. *J Chromatogr* 433: 159–166.

Gabapentin

Anticonvulsant
$C_9H_{17}NO_2$ = 171.2
CAS—60142-96-3
IUPAC Name 1-(Aminomethyl)cyclohexaneacetic acid
Synonyms CI-945; GOE-3450.
Proprietary Names *Aclonium*; *Neurontin*.



Chemical Properties A white to off-white crystalline solid. Mp 162° to 166°, also reported as 165° to 167°. Feely soluble in water (4491 mg/L at 25°), basic and acidic aqueous solutions. pK_a 3.68, 10.7. Log *P* (octanol/water), −1.10. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Gas Chromatography System GA—gabapentin-H_2O RI 1750, gabapentin-H_2O-Me RI 1560; system GB—gabapentin-H_2O RI 1633.
Mass Spectrum Principal ions at *m/z* 81, 153, 67, 152, 55, 54, 68, 96 (gabapentin-H_2O).



Quantification

Plasma **GC** Column: capillary Megabore DB-1 (30 m × 0.53 mm, i.d., 1.5 µm). Temperature programme: held at 160° for 4 min, to 260° at 15°/min. Injector and detector temperature: 285°. Carrier gas: He, flow rate 8 mL/min. IS: Go-3609A. Detection: FID. Retention time: gabapentin, 7.24 min; IS, 9.0 min. Limit of quantification, 0.2 mg/L [Hooper *et al.* 1990].
Serum **HPLC** Column: C_{18} Hypersil BDS (125 × 3 mm i.d., 3 µm). Temperature: 35°. Mobile phase: 250 mmol/L phosphate buffer-acetonitrile-water (32:9.5:58.5) : acetonitrile-methanol-water (28 : 24 : 48; 42:58 for 7 min, to 35:65 for 6 min to 25:74 for 3 min, then to 0:100 for 7 min, flow rate 0.45 mL/min. Fluorescence detection (λ_{ex}=340 nm, λ_{em}=440 nm). Retention time: 16.7 min. Limit of quantification, 0.9 mg/L, limit of detection, 0.2 mg/L [Ratnaraj, Patsalos 1998]. Column: C_{18} Ultrasphere (250 × 4.6 mm i.d.). Mobile phase: water : acetonitrile : acetic acid : *n*-butylamine, (pH <4.5, 480:520:1.0:0.1), flow rate 1.2 mL/min. IS: 1-(aminomethyl)cycloheptaneacetic acid. UV detection (λ= 340 nm). Retention time: gabapentin, 9.8 min; IS, 13.3 min. Limit of detection, 0.03–0.05 mg/L [Lensmeyer *et al.* 1995].
Urine **GC** Limit of quantification, 5 mg/L, see Plasma [Hooper *et al.* 1990].
Disposition in the Body Gabapentin is well absorbed after oral administration but is not metabolised within the body. 80% of a single dose is excreted unchanged in urine and the rest in faeces. It is widely distributed throughout the body and easily crosses the blood–brain barrier.
Therapeutic Concentration

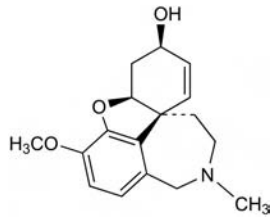
Healthy male volunteers were orally administered with single doses of 300, 600, 900, 1200, 1800, 2400, 3600 and 4800 mg gabapentin. Peak plasma concentrations increased with dose with 1.91, 3.29, 4.02, 5.50, 8.46, 7.65, 11.9 and 12.4 mg/L, respectively and were all reached between 1.8 and 2.8 h [*Eur J Clin Pharmacol* 1989.]

Toxicity Up to 49 g of gabapentin has been reported in a single overdose with drowsiness, mild diarrhoea, dizziness, double vision, slurred speech and lethargy as symptoms. Full recovery was made.
Bioavailability Absolute bioavailability is ≈60% over the dose range 300–600 mg, but outside this, as dose increases, bioavailability decreases.
Half-life 5–7 h.
Volume of Distribution 0.6–0.8 L/kg.
Distribution in Blood Drug penetrates red blood cells. Extravascular distribution.
Protein Binding <3%.
Dose Initial adult dose is 300 mg/day, 300 mg twice a day (2nd day of treatment), 300 mg three times a day (third day), with a maximum of 2.4 g a day. Usual range is between 0.9 and 1.8 g a day. Reduced for elderly patients and those with reduced renal function.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Eur J Clin Pharmacol 36: A310.
Hooper WD *et al.* (1990). Determination of gabapentin in plasma and urine by capillary column gas chromatography. *J Chromatogr* 529: 167–174.
Lensmeyer GL *et al.* (1995). Optimized method for determination of gabapentin in serum by high-performance liquid chromatography. *Ther Drug Monit* 17: 251–258.
Ratnaraj N, Patsalos PN (1998). A high-performance liquid chromatography micromethod for the simultaneous determination of vigabatrin and gabapentin in serum. *Ther Drug Monit* 20: 430–434.

Galantamine

Treatment of Alzheimer's Disease, Anticholinesterase
$C_{17}H_{21}NO_3$ = 287.4
CAS—357-70-0
Synonyms Galanthamine; (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-e,f][2]benzazepin-6-ol; jilkon; lycoremin; lycoremine; NSC-100058.



Chemical Properties Crystals from benzene. Mp 126° to 127°. Fairly soluble in hot water; freely soluble in alcohol, acetone and chloroform; less soluble in benzene and ether. Solubility in water is 31 g/L (pH 6). pK_a 8.2. Log *P* (octanol/pH 12), 1.09.

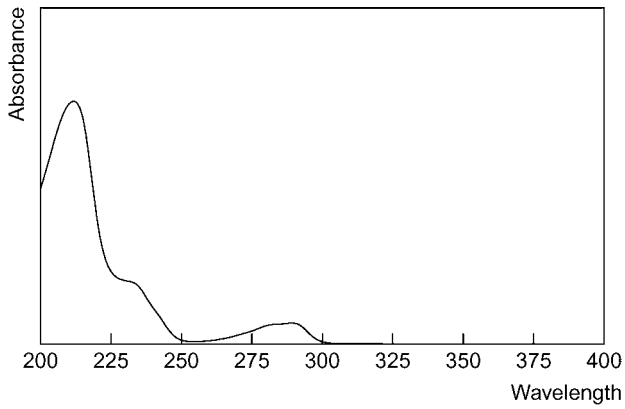
Galantamine Hydrochloride

$C_{17}H_{21}NO_3$,HCl = 323.8
Chemical Properties Crystals from water. Mp 256° to 257° (dec.). Sparingly soluble in cold water; more soluble in hot water; very sparingly soluble in alcohol and acetone.

Galantamine Hydrobromide

$C_{17}H_{21}NO_3$,HBr = 368.3
CAS—1953-04-4
Proprietary Names *Nivalin*; *Reminyl*.
Chemical Properties Crystals from water. Mp 246° to 247° (dec.).

Gas Chromatography System GA—RI 2285.
Ultraviolet Spectrum Principal peaks at 213, 233, 289 nm.



Quantification

Plasma **HPLC** Fluorescence detection (λ_{ex}=290, λ_{em}=320 nm). Limit of detection, 1 µg/L for galantamine and epigalantamine and 15 µg/L for galantaminone [Bickel *et al.* 1991a].
Disposition in the Body The presence of food delays the rate of absorption of galantamine but does not affect the extent. Patients with Alzheimer's disease tend to produce plasma drug concentrations 30 to 40% higher than healthy, young subjects. Galantamine is metabolised in the liver by the cytochrome P450 isoenzymes CYP2D6 and CYP3A4, and ≈75% of the dose is metabolised. Metabolites include norgalantamine (active), *N*-oxide-galantamine, *O*-desmethylgalantamine (active), *O*-desmethylnorgalantamine (active), epigalantamine and galantaminone. No active metabolites have been detected in the unconjugated forms in plasma. The majority of the dose is excreted in urine; 18 to 22% as the unchanged drug during the 24 h period after dosing. A minor amount is excreted in faeces. The drug does not appear to accumulate after multiple dosing.

Therapeutic Concentration

Eight healthy male volunteers, aged 21 to 35 years (mean age, 29.5 years), were fasted overnight and administered with 5 mg and 10 mg galantamine intravenously as a constant-rate infusion over 30 min. The same subjects were also administered with a 10 mg solution and tablet form orally. The peak plasma concentrations for the IV doses were 24.2 to 42.9 µg/L for the 5 mg dose and 50.7 to 97.4 µg/L for the 10 mg dose. For the oral doses, the peak concentrations were 28.7 to 57.9 µg/L for the tablet, observed at 0.5 to 2.0 h, and 28.9 to 56.5 µg/L for the oral solution at 0.5 to 1.25 h [Bickel *et al.* 1991b].

Eight female patients, aged 38 to 79 years, undergoing minor gynaecological operations were treated with 0.3 mg/kg galantamine hydrobromide (equivalent to 0.234 mg free base) intravenously by rapid infusion. The patients were already anaesthetically induced with althesin, fentanyl and nitrous oxide, and the galantamine dose was administered at the end of surgery. The peak serum concentrations obtained were 543 µg/L at 2 min and 128 µg/L after 30 min [Westra *et al.* 1986].

Toxicity

Significant overdosing of galantamine can lead to signs and symptoms similar to those observed with overdosing of other cholinomimetics; generally involving the central nervous system, the parasympathetic nervous system and the neuromuscular junction. Symptoms include muscle weakness or fasciculations, severe nausea, vomiting, gastro-intestinal cramping, salivation, lacrimation, urination, defecation, sweating, bradycardia, hypotension, collapse and convulsions. Increasing muscle weakness along with tracheal hypersecretions and bronchospasm, may result in vital airway compromise [Janssen-Cilag Ltd.].

Bioavailability Absolute, 88.5%.

Half-life 7 to 8 h.

Volume of Distribution 175 L; steady state, 2.64 L/kg (194.8 L).

Clearance Plasma, 0.34 L/h/kg; serum, 5.37 mL/min. Clearance is approximately 20% lower in females than males and 25% lower in poor metabolisers of galantamine than extensive metabolisers.

Protein Binding 18%.

Dose Initially 4 mg is administered twice daily for 4 weeks and can be increased to 8 mg twice daily for 4 weeks. The maintenance dose is 8 to 12 mg twice daily. For patients with moderate hepatic impairment, the dose is 4 mg once daily and should not exceed 8 mg.

Bickel U *et al.* (1991a). Galanthamine: pharmacokinetics, tissue distribution and cholinesterase inhibition in brain of mice. *Neuropharmacology* 30: 447–454.

Bickel U *et al.* (1991b). Pharmacokinetics of galanthamine in humans and corresponding cholinesterase inhibition. *Clin Pharmacol Ther* 50: 420–428.

Janssen-Cilag Limited. Patient information leaflet.

Westra P *et al.* (1986). Pharmacokinetics of galanthamine (a long-acting anticholinesterase drug) in anaesthetized patients. *Br J Anaesth* 58: 1303–1307.

Gallamine Triethiodide

Muscle Relaxant

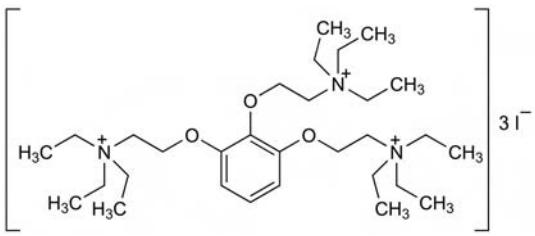
$C_{30}H_{60}I_3N_3O_3 = 891.5$

CAS—153-76-4 (gallamine); 65-29-2 (triethiodide)

IUPAC Name 2,2′,2″[1,2,3-Benzenetriyltris(oxy)]tris[*N*,*N*,*N*-triethylethanaminium] triiodide

Synonym Bencurine iodide

Proprietary Names *Flaxedil*; *Miowas G*; *Relaxan*.



Chemical Properties A white, or faintly cream-coloured, hygroscopic powder. Mp about 235°, with decomposition. Soluble 1 in 0.6 of water, 1 in 115 of ethanol and 1 in 1500 of chloroform; freely soluble in dilute acetone; sparingly soluble in anhydrous acetone, ether and benzene. Log *P* (octanol/water), −6.4.

Thin-layer Chromatography System TA—R_f 0.00; system TN—R_f 0.34; system TO—R_f 0.05 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2625.

Ultraviolet Spectrum Aqueous acid—225 (A^1_1=525a), 266 nm (A^1_1=9a).

Infrared Spectrum Principal peaks at wavenumbers 1100, 1120, 1260, 1000, 780, 1600 cm^{-1} (KBr disk).

Quantification

Plasma **Spectrofluorimetry** Limit of detection, 50 ng [Ramzan *et al.* 1980].

Disposition in the Body Slowly and incompletely absorbed after oral administration; absorbed after IM administration but is generally given by the IV route. Small amounts enter the CSF. Almost 100% of a dose is excreted unchanged in the urine in 24 to 30 h. Negligible amounts are excreted in the bile.

Half-life Plasma half-life, 2 to 3 h; greatly increased in renal failure.

Volume of Distribution About 0.3 L/kg.

Clearance Plasma clearance, about 1.4 mL/min/kg.

Dose 40 to 120 mg IV.

Ramzan MI *et al.* (1980). Pharmacokinetic studies in man with gallamine triethiodide. I. Single and multiple clinical doses. *Eur J Clin Pharmacol* 17: 135–143.

Gallium Nitrate

Antihypercalcaemic

$GaN_3O_9 = 255.7$

CAS—135886-70-3 (nonahydrate); 13494-90-1 (anhydrous)

IUPAC Name Gallium nitrate

Synonyms Gallium trinitrate; gallium (III) nitrate; gallium salt : nitric acid; NSC-15200; WR-135675.

Proprietary Name *Ganite*

Chemical Properties A white crystalline powder. Mp 110°. Very soluble in water; soluble in warm and cold aqueous solutions and absolute alcohol; insoluble in ether.

Disposition in the Body Up to 70% of a dose is excreted by the kidneys with ≈50% of a dose excreted in urine within the first 48 h, mostly in the first 6 h.

Therapeutic Concentration

Twenty-five patients (male and female) with non-small cell lung cancer, aged between 39 and 88 years, were administered 700 mg/m^2 IV doses of gallium nitrate over 30 min. The mean peak plasma concentration was 15.2 mg/L, with a range from 9.5–21.2 mg/L. Most of the gallium remained ultrafilterable for the first 10 h but by 48 h, only 11% of the dose was present as ultrafilterable gallium. Total gallium present reached a peak at 1.9 mg/L within 8–12 h. Maximum urine concentration of gallium was 40–196 mg/L [Webster *et al.* 2000].

Toxicity Gallium nitrate is a poison by intraperitoneal, IV and SC routes, and moderately toxic by ingestion. An IV dose of 7 mg/kg can result in gastrointestinal tract toxicity and 144 mg/kg in blood toxicity. It can also cause severe skin and eye irritations, at doses >500 mg. Serious nephrotoxicity can also occur.

Half-life 25–111 h.

Dose The usual dose is 100 to 200 mg/m^2 body surface administered once daily.

Webster LK *et al.* (2000). A pharmacokinetic and phase II study of gallium nitrate in patients with non-small cell lung cancer. *Cancer Chemother Pharmacol* 45(1): 55–58.

γ-Butyrolactone

Depressant

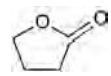
$C_4H_6O_2 = 86.1$

CAS—96-48-0

IUPAC Name Oxolan-2-one

Synonyms γBL; 1,2-Butanolide; butyrylactone; dihydro-2(3H)-furanone; gamma hydroxybutyric acid lactone; gamma lactone; 2-Oxanolone; tetrahydro-2-furanone.

Proprietary Names *Blue Nitro*; *Blue Nitro Vitality*; *Firewater*; *Gamma G*; *GH Revitalizer*; *Insom-X*; *Invigorate*; *Longevity*; *Remforce*; *Renewtrient*; *Revivarant*; *Revivarant G*.



Chemical Properties Colourless liquid. Mp −43°; Bp 204°. It is miscible with water, alcohol, ketones, esters and aromatic hydrocarbons; limited solubility in aliphatic and cycloaliphatic hydrocarbons. Log *P* (octanol/water), −0.64 [Hansch *et al.* 1995]. No significant instability [Wood *et al.* 2004]. Stock standard solutions, internal standard solutions and prepared solutions were stable for 3 months at 4°. Stable in plasma for 3 months [Fukui *et al.* 2003].

(S)-3-Hydroxy-γ Butyrolactone

$C_4H_6O_3 = 102.0$

CAS—7331-52-4

Synonym HGB

Chemical Properties Pale-yellow liquid. Bp 98° to 100°.

Colour Test Specific tests for GBL using mixtures of several common reagents are given in the Colour Tests chapter.

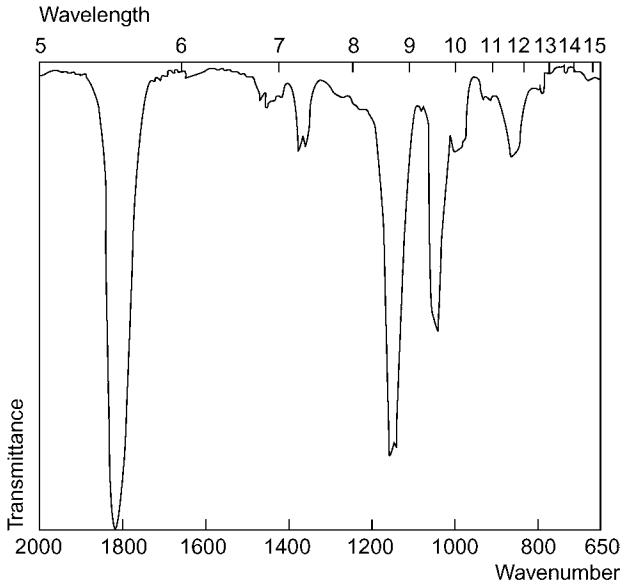
Gas Chromatography System GAO—GBL RT 4.0 min;GHB-TMS_2 RT 5.6 min.

Gas Chromatography-Mass Spectrometry Column: HP Ultra 1 bonded phase capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.2 kg/cm^2. Temperature programme: 50° for 0.6 min to 275° at 15°/min. SIM acquisition mode (*m/z*: 41, 42, 56, 86, 100). Retention time: 2.6 min [Ferrara *et al.* 1993].

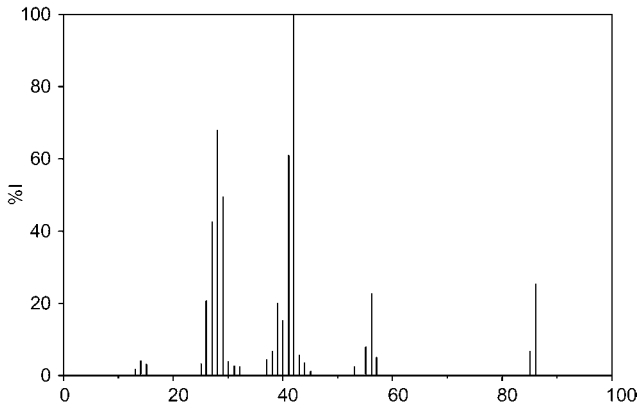
High Performance Liquid Chromatography System HBG—GBL RT 4.0 min; GHB RT 3.5 min.

Column: C_{18} µBondapak (300 × 3.9 mm i.d., 10 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate buffer (pH 3.0): methanol (70 : 30), flow rate 1.0 mL/min. DAD (λ = 215 nm). Retention time: 3.96 min for GBL; 3.45 min for GHB [Mesmer, Satzger 1998].

Infrared Spectrum Principal peaks at wavenumbers 1820, 1163, 1047, 2993, 2909, 874 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 42, 28, 41, 29, 27, 86, 56, 39.



Quantification

Blood **GC** Column: DB-624 capillary (30 m × 0.25 mm i.d., 1.4 μm). Temperature programme: 50° for 3 min to 150° at 20°/min for 7 min. Carrier gas: N_2, 34 cm/s. IS: γ butyrolactone-d_6. FID, SIM acquisition mode (*m/z*: 86 for GBL and 92 for IS). Retention time: 11.2 min for GBL; 12.7 min for IS. Limit of detection, 0.5 mg/L for GHB [LeBeau *et al.* 2000].

GC-MS Column: ZB-FFAP (30 m). Carrier gas: He. EI ionisation. Limit of detection not reported [Strickland *et al.* 2005]. Column: DB5-MS (30 m × 0.25 mm i.d., 5 μm).Carrier gas: He, 15 psi. Temperature programme: 60° for 1 min to 85° for 1 min to 300° for 15 min all at 30°/min. MID. Limit of detection, 10 mg/L [Duer *et al.* 2001]. Column: 5% phenylmethylsilicone (30 m × 0.25 mm i.d., 0.33 μm). Carrier gas: He. Temperature programme: 60° for 2 min to 180° at 20°/min to 250° at 35°/min for 4 min. SIM acquisition mode. Limit of quantification, 1 mg/L, limit of detection, 0.5 mg/L [Couper, Logan 2000]. EI ionisation, full scan mode. Retention time: 10.9 min. Limit of detection, 0.5 mg/L for GHB [LeBeau *et al.* 2000].

Plasma **GC-MS** Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 60° to 115 at 10°/min up to 300° at 30°/min. Retention time: 4.54 min. Limit of quantification, 8 mg/L [Jones *et al.* 2008]. Column: Varian Factor IV (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 60° for 2 min to 180° at 20°/min to 250° at 50°/min for 9.4 min. EI ionisation, MRM acquisition mode. Limit of quantification, 100 mg/L [Paul *et al.* 2006]. Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 38 cm/s. Temperature programme: 60° for 1 min to 90° at 10°/min to 270° at 35/°min for 1 min. SIM acquisition mode. Retention time: 4.35 min. Limit of quantification, 0.5 mg/L, limit of detection, 0.2 mg/L [Brenneisen *et al.* 2004]. Column: DB-WAX capillary (30 m × 0.32 mm i.d., 0.25 μm). Temperature programme: 50° for 1 min to 190° at 20°/min to 250° at 40°/min for 2 min. NICI at 70 eV, SIM acquisition mode. Retention time: 5.89 min. Limit of detection, 10 μg/L [Fukui *et al.* 2003]. Column: FFAP (acid-modified polyethylene glycol) capillary (25 m × 0.2 mm i.d., 0.3 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 50° for 1.5 min to 240° at 30°/min for 2 min. IS: γ-butyrolactone-d_6. PICI, SIM acquisition mode (*m/z*: 86, 87, 88 for GBL and 92,93,94 for IS). Retention time: 6.54 min for GBL; 6.53 min, IS. Limit of detection, 0.05 mg/L [Frison *et al.* 2000].

Serum **GC-MS** Macherey-Nagel Optima 5MS 5% phenyl 95% methylsiloxane (30 m × 0.25 mm i.d., 1 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 35° for 1 min to 100° at 10°/min to 200° at 50°/min for 1 min. EI ionisation at 70 eV or PICI. Limit of quantification, 0.2 mg/L, limit of detection, 0.16 mg/L [Lenz *et al.* 2009]. Column: Macherey-Nagel Optima 5MS GC capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 35° for 1 min to 100° at 10°/min to 200° at 50°/min for 1 min. PICI, full scan mode. Limit of quantification, 2 mg/L, limit of detection, 0.5 mg/L [Lenz *et al.* 2008].

Urine **GC** See Blood [Duer *et al.* 2001]. See Blood [LeBeau *et al.* 2000].

GC-MS See Serum. Limit of detection, 0.17 mg/L [Lenz *et al.* 2009]. See Serum. Limit of quantification, 4 mg/L [Lenz *et al.* 2008]. See Blood. Limit of quantification, 0.2 mg/L, limit of detection, 0.1 mg/L [Brenneisen *et al.* 2004]. Column: DB5-MS. Carrier gas: He, 12 psi. Temperature programme: 60° to 270° at 9°/min for 23.3 min in total. EI ionisation, SIM acquisition mode. Limit of detection, 2 mg/L [Blair *et al.* 2001]. See Blood [LeBeau *et al.* 2000]. See Plasma. Limit of detection, 0.1 mg/L [Frison *et al.* 2000]. Column: HP-1MS cross-linked methylsiloxane capillary (12 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.6 mL/min. Temperature programme: 65° for 0.5 min to 105° at 15°/min to 300° at 25°/min. SIM acquisition mode. Limit of detection, 5 mg/L [McCusker *et al.* 1999].

LC-MS Column: Atlantis dC_{18} (100 × 3 mm i.d., 5 μm). Mobile phase: 0.1% aqueous formic acid: methanol (90 : 10), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 5.1 min for β-hydroxybutyric acid. Limit of quantification, 1.0 mg/L [Wood *et al.* 2004].

Ocular Fluid **GC** See Blood [Duer *et al.* 2001].

Brain **GC** See Blood [Duer *et al.* 2001].

Other **GC-MS** Commercial Beverages. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 45° for 2 min to 110° at 5 K/min to 300° at 30 K/min. Full scan mode. Limit of quantification, 150 ppb, limit of detection, 50 ppb [Sabucedo, Furton 2004]. Wine. Column: DB-5 (20 m × 0.18 mm). Carrier gas: He, 10.2 psi. Temperature programme: 60° for 1 min to 300° at 15°/min. Retention time: 3.6 min. Limit of detection, 5 mg/L [Vose *et al.* 2001].

CE Beverages. Column: Polymicro capillary (total/effective length 80/72 cm, 50 μm). Mobile phase: 20 mmol/L SDS (pH 9.2) with 7.0% acetonitrile. DAD. Limit of detection, 152 mg/L [Bishop *et al.* 2004].

Note For the positive detection of GBL in vermouth, sherry, port, and red and white wine see Elliott, Burgess [2005].

Disposition in the Body γ-butyrolactone (GBL) is well absorbed after administration and is biotransformed in the body to γ- hydroxybutyrate (GHB), which is a potent CNS depressant. This conversion can occur within minutes. GBL has a greater bioavailability than GHB.

Toxicity Signs and symptoms of toxicity can include prolonged unconsciousness and coma, respiratory depression (including respiratory arrest and very dangerously slow breathing), nausea/vomiting, confusion, anxiety/nervousness, cardiac arrest and seizures.

A 44-year-old male tourist collapsed after consuming 'Furamax Revitaliser' – 2.5 g of 2(3H) furanone dihydro (GBL) per fluid ounce (8 g/100 mL). He subsequently made a full recovery [Dupont, Thornton 2001].

A 19-year-old female was offered a drink from a plastic container at a party. After consuming some of the drink, she had no recollection of the rest of the evening. Her friends described her as being 'drunker than she should have been'. A sexual assault examination indicated that intercourse had occurred. A urine sample had a concentration of GBL of 4.4 mg/L, 9 h after the drink was consumed. GHB was detected at concentrations consistent with endogenous levels. A 25-year-old male was found sleeping behind the wheel of his car in the middle of a busy street. In the car there was a container of RenewTrient, a GBL-containing product. Blood analysis showed a concentration of GHB of 157 mg/L but GBL was not detected. [LeBeau *et al.* 2000].

Note See Lenz *et al.* [2008] for more case studies.

Half-life 20–30 min.

Bishop SC *et al.* (2004). Micellar electrokinetic chromatographic screening method for common sexual assault drugs administered in beverages. *Forensic Sci Int* 141: 7–15.

Blair S *et al.* (2001). Determination of gamma-hydroxybutyrate in water and human urine by solid phase microextraction-gas chromatography/quadrupole ion trap spectrometry. *J Forensic Sci* 46: 688–693.

Brenneisen R *et al.* (2004). Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J Anal Toxicol* 28: 625–630.

Couper FJ, Logan BK (2000). Determination of gamma-hydroxybutyrate (GHB) in biological specimens by gas chromatography–mass spectrometry. *J Anal Toxicol* 24: 1–7.

Duer WC *et al.* (2001). Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. *J Anal Toxicol* 25: 576–582.

Dupont P, Thornton J (2001). Near-fatal gamma-butyrolactone intoxication–first report in the UK. *Hum Exp Toxicol* 20: 19–22.

Elliott SP, Burgess V (2005). Clinical urinalysis of drugs and alcohol in instances of suspected surreptitious administration ("spiked drinks"). *Sci Justice* 45: 129–134.

Ferrara SD *et al.* (1993). Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 11: 483–487.

Frison G *et al.* (2000). Determination of gamma-hydroxybutyric acid (GHB) in plasma and urine by headspace solid-phase microextraction and gas chromatography/positive ion chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom* 14: 2401–2407.

Fukui Y *et al.* (2003). Validation of a simple gas chromatographic-mass spectrometric method for the determination of gamma-butyrolactone in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 785: 73–80.

Hansch C, *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Jones AW *et al.* (2008). Driving under the influence of gamma-hydroxybutyrate (GHB). *Forensic Sci Med Pathol* 4: 205–211.

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LeBeau MA *et al.* (2000). Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. *J Anal Toxicol* 24: 421–428.
Lenz D *et al.* (2009). Determination of gamma-hydroxybutyric acid in serum and urine by headspace solid-phase dynamic extraction combined with gas chromatography-positive chemical ionization mass spectrometry. *J Chromatogr A* 1216: 4090–4096.
Lenz D *et al.* (2008). Intoxications due to ingestion of gamma-butyrolactone: organ distribution of gamma-hydroxybutyric acid and gamma-butyrolactone. *Ther Drug Monit* 30: 755–761.
McCusker RR *et al.* (1999). Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. *J Anal Toxicol* 23: 301–305.
Mesmer MZ, Satzger RD (1998). Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spetrometry. *J Forensic Sci* 43: 489–492.
Paul R *et al.* (2006). GC-MS-MS determination of gamma-hydroxybutyrate in blood and urine. *J Anal Toxicol* 30: 375–379.
Sabucedo AJ, Furton KG (2004). Extractionless GC/MS analysis of gamma-hydroxybutyrate and gamma-butyrolactone with trifluoroacetic anhydride and heptafluoro-1-butanol from aqueous samples. *J Sep Sci* 27: 703–709.
Strickland RM *et al.* (2005). Survival of massive gamma-hydroxybutyrate/1,4-butanediol overdose. *Emerg Med Australas* 17: 281–283.
Vose J *et al.* (2001). Detection of gamma-butyrolactone (GBL) as a natural component in wine. *J Forensic Sci* 46: 1164–1167.
Wood M *et al.* (2004). Simultaneous analysis of gamma-hydroxybutyric acid and its precursors in urine using liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1056: 83–90.

γ-Hydroxybutyrate

Anaesthetic

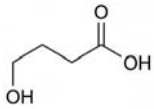
$C_4H_8O_3$ = 104.1

CAS—591-81-1

IUPAC Name 4-Hydroxybutanoic

Synonyms BRN-1720582; gamma hydroxybutyric acid; GHB; 4-hydroxybutyric acid.

Street Names Georgia home boy; grievous bodily harm; liquid ecstasy; scoop; fantasy.



Chemical Properties Crystals. It is water soluble and produces solutions that are mildly saline. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Sodium Gamma Hydroxybutyrate

Synonyms NSC-84223; sodium gamma hydroxybutyric acid; sodium oxybate; sodium oxybutyrate; Wy3478.

Proprietary Names *Alcover*; *Gamma-OH*; *Somatomax PM*; *Somsanit*; *Xyrem*.

Sodium-4-hydroxybutyrate

$C_4H_8NaO_3$ = 127.1

CAS—502-85-2

Chemical Properties A colourless, odourless powder, capsules or liquid.

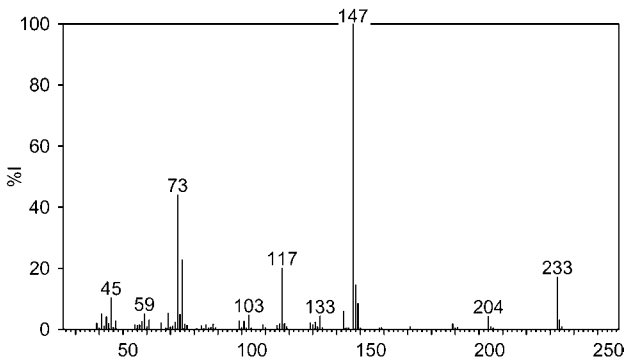
Colour Test Specific tests using mixtures of several common reagents are given in the Colour Tests chapter.

Gas Chromatography System GAO—γ-hydroxybutyrate (GHB)-TMS_2 RT 5.6 min, γ-butyrolactone (GBL) RT 4.0 min.

High Performance Liquid Chromatography System HBG—GHB-TMS_2 RT 3.5 min, GBL RT 4.0 min.

Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate buffer (pH 3.0) : methanol (70 : 30), flow rate 1.0 mL/min. Retention time: GHB 3.45 min, GBL 3.96 min [Mesmer, Satzger 1998].

Mass Spectrum Principal ions at *m/z* 147, 73, 75, 233, 117, 148, 77, 59 (GHB-TMS_2).



Quantification

Blood **GC** Column: DB-624 (30 m × 0.25 mm i.d., 1.4 μm). Carrier gas: N_2, 34 cm/s. Temperature programme: 50° for 3 min to 150° at 20°/min for 7 min. FID. Limit of detection, 0.5 mg/L [LeBeau *et al.* 2000].

GC-MS See GC. EI ionisation, full scan mode. Retention time: 10.9 min. Limit of detection, 0.5 mg/L [LeBeau *et al.* 2000].

Plasma **GC-MS** Column: ULTRA-1 (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 0.2 kg/cm^2. Temperature programme: 50° for 0.6 min to 275° at 15°/min. EI ionisation, SIM acquisition mode. Limit of detection, 0.2 mg/L [Ferrara *et al.* 1993]. Column: HP ULTRA 1 bonded phase (12 m × 0.2 mm i.d., 0.3 μm). EI ionisation, SIM acquisition mode. Limit of detection, 1 mg/L [Ferrara *et al.* 1992].

Urine **GC** See Blood [LeBeau *et al.* 2000].

GC-MS Column: HP-1 (25 m × 0.32 mm i.d., 0.5 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 1 min to 300° at 10°/min. Limit of detection, 2 mg/L [Kavanagh *et al.* 2001]. Column: HP-1 100% polydimethylsiloxane (12 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 60° for 2 min to 260° at 30°/min for 1 min. Carrier gas: He, 0.5 mL/min. I.S.: GHB-d_6. SIM acquisition mode, *m/z* 233, 234, 235 for GHB and 239, 240, 241 for IS. Retention time: GHB 4.43 min, IS 4.41 min. Limit of detection, 2 mg/L [Elian 2000]. See Blood [LeBeau *et al.* 2000]. Column: HP-1MS cross-linked methylsiloxane (12 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 65° for 0.5 min to 105° at 15°/min to 300° at 25°/min. Carrier gas: He, 1.6 mL/min. SIM acquisition mode, *m/z* as above. I.S.: GHB-d_6. Retention time: GHB 3.7 min. Limit of detection, 5 mg/L [McCusker *et al.* 1999]. See Plasma. Limit of detection, 0.1 mg/L [Ferrara *et al.* 1993]. See Plasma. Limit of detection, 0.2 mg/L [Ferrara *et al.* 1992]

Hair **LC-MS** Limit of quantitation 0.4 ng/mL, limit of detection, 0.2 ng/mL [Stout *et al.* 2010].

Disposition in the Body GHB is readily absorbed after oral administration and rapidly metabolised in the liver by oxidative enzymes. Conversion to GBL can occur and elimination is rapid, via the kidneys, with urine recovery virtually complete within 8 h of administration. Only negligible amounts of the parent drug are recovered unchanged in urine (<5%); the drug is not detectable after 12 h. GHB crosses the blood–brain barrier and the placental barrier. Endogenous concentrations of GHB are thought to be up to 10 mg/L in urine and 4 mg/L in antemortem plasma of individuals not suffering from the genetic disorder gamma hydroxybutyric aciduria. Endogenous concentrations appear to be increased postmortem, particularly in blood.

Therapeutic Concentration

Eight healthy non-smoker males, aged between 22 and 26 years, were administered with 12.5, 25 and 50 mg/kg single doses of GHB after an overnight fast. Peak plasma concentrations reached 23 ± 9, 23 ± 11 and 20 ± 7 mg/L, respectively, achieved within 25 ± 20, 30 ± 20, 45 ± 30 min, respectively [Palatini *et al.* 1993].

Toxicity Doses greater than 50 mg/kg can lead to a decrease in cardiac output, respiratory depression/arrest, seizure-like activity and, possibly, death. An intoxicating dose is 15 mg/kg and a dose of 4 g has been associated with death. It has also been reported that doses between 5 and 10 g can be toxic or fatal. Effects usually occur within 20 min of taking a dose and are also associated with a steep dose–response curve that may result in accidental overdosage.

An 18-year-old male was admitted to hospital unresponsive and in respiratory failure, he died 30 min later. Toxicological postmortem analysis showed a 309.4 mg/L blood concentration of GHB along with alcohol, cocaine, cocaethylene and benzoylecgonine [Mozayani *et al.* 1998].

A 47-year-old Caucasian male was found dead on his living room floor. At postmortem GHB concentrations in his blood, vitreous and urine were 538, 652, and 2927 mg/L, respectively [Win, Baselt 2000].

A 22-year-old female who had taken a suspected single dose of liquid GHB revealed a postmortem femoral blood concentration of 330 mg/L and a frontal cortex brain tissue concentration of 221 ng/mg. GHB was also detected in her hair. No other drugs were detected [Kalasinsky *et al.* 2001].

A 24-year-old woman ingested 4 mL fantasy – the South Australian street name for GHB. Her blood concentration was 1200 mg/L [Strickland *et al.* 2005].

Half-life Terminal half-life, 0.3–1.0 h.

Volume of Distribution 0.5 L/kg.

Clearance Oral, 9.0 mL/min/kg (range, 4.7–15.3).

Protein Binding None.

Dose Usual dose required for general anaesthesia is 50 mg/kg, for treatment of amnesia; as an analgesic dose is 10 to 20 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Elian AA (2000). A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults. *Forensic Sci Int* 109: 183–187.
Ferrara SD *et al.* (1992). Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br J Clin Pharmacol* 34: 231–235.
Ferrara SD *et al.* (1993). Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography–mass spectrometry. *J Pharm Biomed Anal* 11: 483–487.
Kalasinsky KS *et al.* (2001). Blood, brain, and hair GHB concentrations following fatal ingestion. *J Forensic Sci* 46: 728–730.
Kavanagh PV *et al.* (2001). The urinary excretion of gamma-hydroxybutyric acid in man. *J Pharm Pharmacol* 53: 399–402.
LeBeau MA *et al.* (2000). Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. *J Anal Toxicol* 24: 421–428.
McCusker RR *et al.* (1999). Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography–mass spectrometry. *J Anal Toxicol* 23: 301–305.
Mesmer MZ, Satzger RD (1998). Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spectrometry. *J Forensic Sci* 43: 489–492.
Mozayani A *et al.* (1998). A fatality involving GHB. In: *Proceedings of a Joint Meeting of the Society of Forensic Toxicologists and the International Association of Forensic Toxicologists*, Albuquerque, NM.
Palatini P *et al.* (1993). Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. *Eur J Clin Pharmacol* 45: 353–356.
Stout PA *et al.* (2010). Quantitative analysis of gamma-hydroxybutyrate at endogenous concentrations in hair using liquid chromatography tandem mass spectrometry. *J Forensic Sci* 55: 531–537.

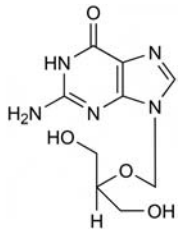
Strickland RM *et al.* (2005). Survival of massive gamma-hydroxybutyrate/1,4-butanediol overdose. *Emerg Med Australas* 17: 281–283.
Win BH, Baselt RC (2000). Apparent suicide with Renewtrient. *J Toxicol Clin Toxicol* 38: 809.

Ganciclovir

Antiviral
$C_9H_{13}N_5O_4 = 255.2$
CAS—82410-32-0

IUPAC Name 2-Amino-1,9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-6*H*-purin-6-one

Synonyms BIOLF-62; BW-759; BW-B759U; BW-759U; DHPG; dihydroxypropoxymethylguanine; 2′-NDG; 2′-nor-2′-deoxyguanosine; RS-21592.



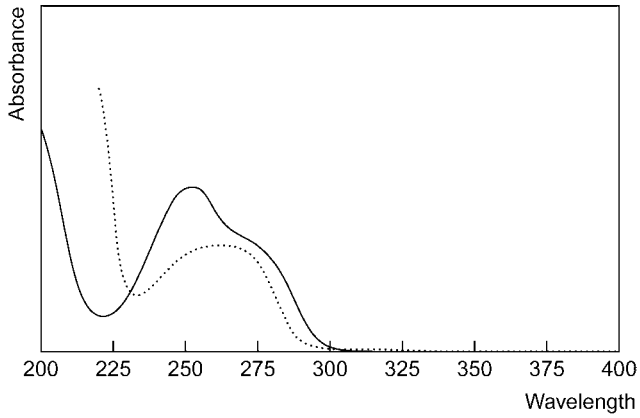
Chemical Properties A white to off-white crystalline powder. Mp 250° (decomposes). Soluble 2.6 g/L in water at 25°; 4.3 g/L in solution at pH 7. pK_a 2.2, 9.4. Log *P* (octanol/water), −1.66.

Ganciclovir Sodium

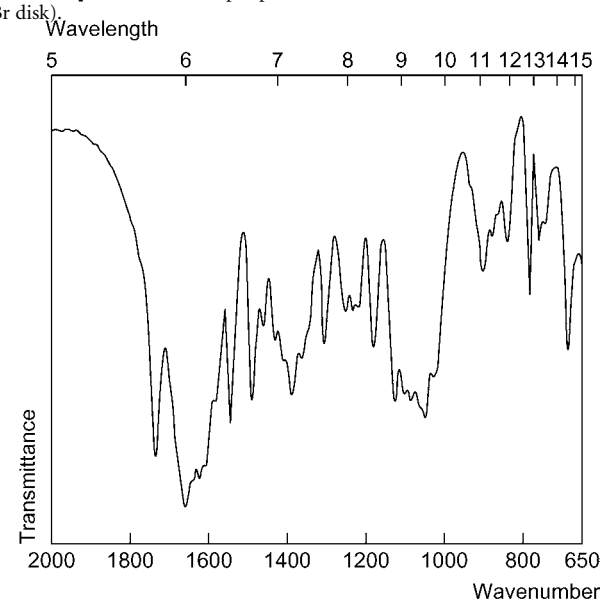
$C_9H_{12}N_5NaO_4 = 277.2$
CAS—107910-75-8

Proprietary Names *Citovirax*; *Cymevan*; *Cymeven*; *Cymevene*; *Cytovene*; *Denosine*; *Virgan*; *Vitrasert*.

Ultraviolet Spectrum Aqueous acid—255 nm; methanol—254 nm; aqueous base—264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1647, 1379, 1041 cm^{-1} (KBr disk).



Quantification

***Plasma* HPLC** Column: RP Hypersil ODS (100 × 4.6 mm, 3 µm). Temperature: 40°. Mobile phase: 0.1 mol/L sodium hydrogen phosphate monohydrate : 0.04 mol/L triethylamine, pH 6.6, flow rate 1.0 mL/min. IS: acyclovir. UV detection (λ=254 nm). Retention time: ganciclovir, 4.0 min; IS, 5.1 min. Limit of quantification, 0.05 mg/L, limit of detection, 0.01 mg/L [Campanero *et al.* 1998]. UV detection (λ=254 nm). Limit of quantification, 0.01 mg/L, limit of detection, 0.003 mg/L [Cociglio *et al.* 1998]. UV detection (λ=254 nm). Limit of detection, 0.5 µg/L [Boulieu *et al.* 1991]. UV detection. Limit of detection, 0.25 mg/L [Fletcher *et al.* 1986].

***Serum* HPLC** Column: Lichrospher RP8e (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L 1-octanesulfonic acid in 0.1 mol/L phosphate buffer (pH 3), flow rate 1.0 mL/min. Internal standard (IS): guanosine. Fluorescence detection (λ_{ex}=285 nm, λ_{em}=380 nm). Retention time: ganciclovir, 4.71 min; IS, 7.35 min. UV detection. Limit of detection, 50 µg/L. Fluorescence detection. Limit of detection, 0.01 mg/L [Koel, Nebinger 1994]. UV detection (λ=254 nm). Limit of quantification, 0.05 mg/L [Page *et al.* 1996].

***Urine* HPLC** See Serum [Page *et al.* 1996].

***CSF* HPLC** See Plasma [Fletcher *et al.* 1986].

Review For a review of chromatographic methods for the determination of antiviral drugs in biological fluids, see Riley *et al.* [1990].

Disposition in the Body Ganciclovir is poorly absorbed after oral administration; peak plasma concentrations are reached after about 2 to 4 h. Following IV administration, it is widely distributed to body tissues and fluids including intraocular fluid and CSF. It crosses the placenta. The drug is excreted unchanged in the urine by glomerular filtration and active tubular secretion; ≈90% of an oral dose is excreted in urine within 24 h of administration. With an IV dose, on the other hand, the majority is excreted in faeces. Haemodialysis reduces plasma concentrations by about 50%. Ganciclovir does not accumulate in plasma after repeated dosing.

Therapeutic Concentration The trough serum therapeutic concentration is 0.2 to 1.0 mg/L and peak, 5.0 to 12.5 mg/L.

Ninety-seven patients with AIDS and life-threatening or sight-threatening cytomegalovirus infections, mean age 36.2 years, were administered intravenously with doses of 1, 2.5 and 5 mg/kg (infused over 1 h) every 8 h. Mean peak steady-state plasma concentrations of 0.0094, 0.0203 and 0.0445 mg/L, respectively, were measured after the last dose [Laskin *et al.* 1987].

Six subjects (bone marrow recipients aged between 13 and 53 years) were administered intravenously with 2.5 mg/kg (infused over 1 h) every 8 h, for 10 days. Plasma concentrations between 3.93 and 4.45 mg/L 15 min after the end of the infusion were observed. The mean CSF concentration measured 2.75 h after IV administration was 0.51 mg/L [Fletcher *et al.* 1986].

20 mg/kg was orally administered, every 6 h, to 4 subjects with AIDS and CMV retinitis, after an overnight fast which was continued for 1 h after administration. 4 to 6 months prior to this treatment, the patients were also administered 2.5 mg/kg intravenously every 8 h, for 10 days, and 5 to 7.5 mg/kg daily 5 days a week. The 20 mg/kg dose produced mean peak and trough steady-state plasma concentrations of 2.96 µg/L and 1.05 µg/L, respectively, on day 3. The 2.5 mg/kg intravenous dose produced a peak concentration of 18.0 µg/L; the 5 mg/kg dose, 40.0 µg/L and a 10 mg/kg oral dose every 6 h produced a peak concentration of 2.01 µg/L. These were observed after ≈1 h [Jacobson *et al.* 1987].

Toxicity The trough serum toxic concentration is 3 to 5 mg/L and peak, 20 mg/L.

Bioavailability 5 to 8%; increases when administered with food.

Half-life Plasma half-life, 2.5 to 4 h.

Volume of Distribution 0.5 to 2.0 L/kg; quoted as 0.74 L/kg. Also reported as 33 to 45 L.

Clearance Plasma clearance, 200 mL/min/1.73 m^2; 0.1 L/h/kg.

Protein Binding 1 to 2%.

Note For reviews of ganciclovir, see Faulds and Heel [1990], Markham and Faulds [1994], Crumpacker [1996] and Noble and Faulds [1998]. For a review of the pharmacokinetics of antiviral nucleosides, see Morse *et al.* [1993].

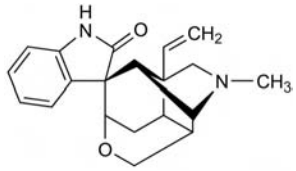
Dose Initially, 5 mg/kg (of ganciclovir) intravenously over 1 h every 12 h for 14 to 21 days. Orally, 1 g (of ganciclovir) three times daily.

Boulieu R *et al.* (1991). Modified high-performance liquid chromatographic method for the determination of ganciclovir in plasma from patients with severe renal impairment. *J Chromatogr* 571: 331–333.
Campanero MA *et al.* (1998). Development and validation of a sensitive method for the determination of ganciclovir in human plasma samples by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 706: 311–317.
Cociglio M *et al.* (1998). Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma. *J Chromatogr B Biomed Sci Appl* 705: 79–85.
Crumpacker CS (1996). Ganciclovir. *N Engl J Med* 335: 721–729.
Faulds D, Heel RC (1990). Ganciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. *Drugs* 39: 597–638.
Fletcher C *et al.* (1986). Human pharmacokinetics of the antiviral drug DHPG. *Clin Pharmacol Ther* 40: 281–286.
Jacobson MA *et al.* (1987). Human pharmacokinetics and tolerance of oral ganciclovir. *Antimicrob Agents Chemother* 31: 1251–1254.
Koel M, Nebinger P (1994). HPLC determination of serum ganciclovir using ultrafiltration, ultraviolet and fluorescence detection. *J Pharm Biomed Anal* 12(3): 429–432.
Laskin OL *et al.* (1987). Ganciclovir for the treatment and suppression of serious infections caused by cytomegalovirus. *Am J Med* 83: 201–207.
Markham A, Faulds D (1994). Ganciclovir. An update of its therapeutic use in cytomegalovirus infection. *Drugs* 48: 455–484.

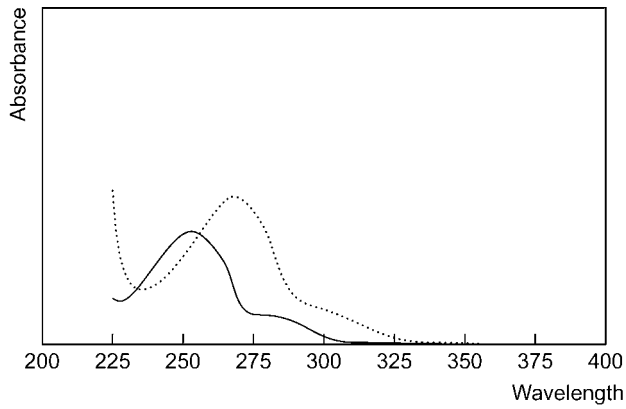
Morse GD *et al.* (1993). Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin Pharmacokinet* 24: 101–123.
Noble S, Faulds D (1998). Ganciclovir. An update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. *Drugs* 56: 115–146.
Page T *et al.* (1996). Simple reversed-phase high-performance liquid chromatography quantitation of ganciclovir in human serum and urine. *J Chromatogr Biomed Appl* 675: 342–346.
Riley CM *et al.* (1990). Chromatographic methods for the bioanalysis of antiviral agents. *J Chromatogr* 531: 295–368.

Gelsemine

CNS Depressant
$C_{20}H_{22}N_2O_2$ = 322.4
CAS—509-15-9



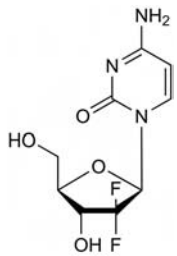
Chemical Properties An alkaloid present in gelsemium, the dried rhizome and roots of *Gelsemium sempervirens* (Loganiaceae). Crystals. Mp 178°. Slightly soluble in water; soluble in ethanol, chloroform, benzene, acetone, dilute acids and ether. Log *P* (octanol/water), 1.4.
Colour Test Mandelin's test—red→green.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.09; system TE—R_f 0.52; system TAE—R_f 0.25 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2850.
High Performance Liquid Chromatography System HX—RI 290.
Ultraviolet Spectrum Aqueous acid—252 nm (A^{1}_{1}=220b); aqueous alkali—268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1715, 1620, 1100, 747, 759, 1237 cm^{-1}.
Disposition in the Body
Toxicity Death has been caused by the ingestion of 4 mL of a 1:1 fluid extract.

Gemcitabine

Antineoplastic
$C_9H_{11}F_2N_3O_4$ = 263.2
CAS—95058-81-4
IUPAC Name 4-Amino-1-[(2*R*,4*R*,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)-oxolan-2-yl]pyrimidin-2-one
Synonyms dFdC; dFdCyd; 2′-deoxy-2′,2′-difluorocytidine; LY-188011.

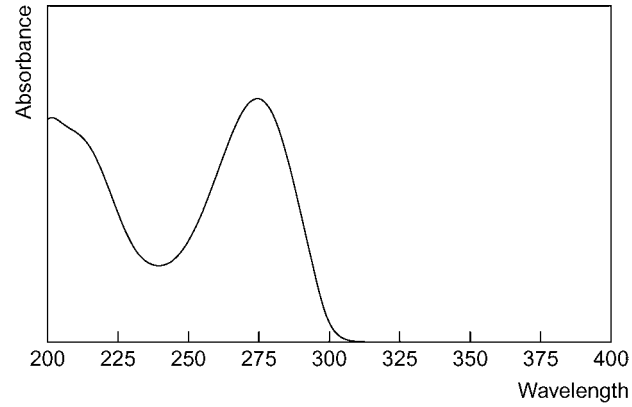


Chemical Properties pK_a 3.6.

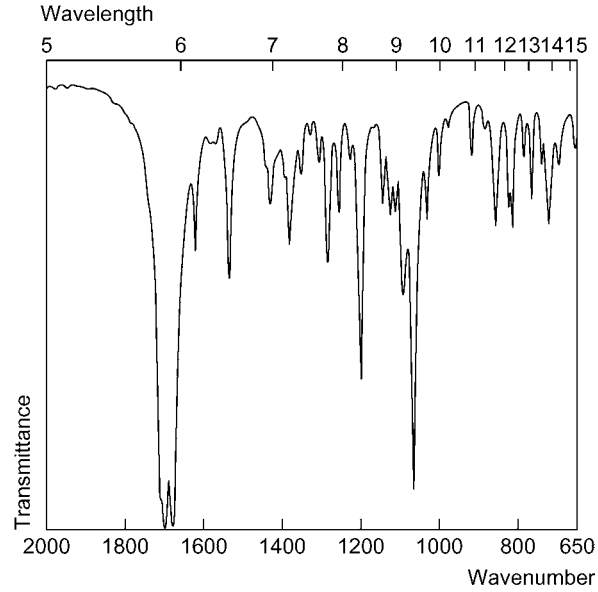
Gemcitabine Hydrochloride

$C_9H_{11}F_2N_3O_4$,HCl = 299.7
CAS—122111-03-9

Proprietary Name *Gemzar*
Chemical Properties A white to off-white solid. Mp 287° to 292°. Soluble in water; slightly soluble in methanol; practically insoluble in ethanol and in polar organic solvents.
Ultraviolet Spectrum Aqueous acid—204, 274 nm.



Infrared Spectrum



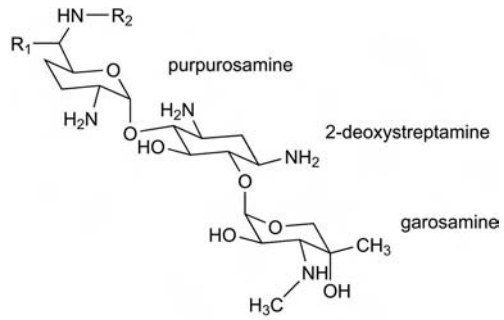
Quantification
Plasma **HPLC** UV detection (λ=272 nm). Limit of quantification, 0.05 mg/L [Freeman *et al.* 1995].
Urine **HPLC** Limit of quantification, 20 mg/L, see Plasma [Freeman *et al.* 1995].
Disposition in the Body Following IV administration, gemcitabine is rapidly cleared from blood and is metabolised by cytidine deaminase in the liver, kidney, blood and other tissues. The pharmacokinetics of gemcitabine are significantly affected by gender and age of patient and length of time over which the drug is infused. Clearance is ≈30% lower in women compared to men. It is not distributed extensively into tissues. Less than 10% of the drug is excreted in urine as the unchanged drug and the rest as metabolites. Approximately 1% of a dose is excreted in faeces. The primary, inactive metabolite is dFdU (2′-deoxy-2′,2′-difluorouridine) which has been observed in both plasma and urine. Other metabolites detected in plasma and urine include the gemcitabine mono, di and triphosphates, the latter are considered active.
Therapeutic Concentration
In a total of seven studies, 121 women and 232 men, aged between 29 and 79 years, diagnosed with non-small cell lung cancer or pancreatic cancer, were administered doses of 500 to 1592 mg/m^2 over an infusion period of 0.4 to 1.2 h. Peak plasma concentrations of 3.2 to 45.5 mg/L were observed within 5 min of the end of the infusion. The peak plasma concentration for the metabolite, dFdU, is 28 to 52 mg/L observed 3 to 15 min after the end of a 30-min infusion of 1 g/m^2 [Eli Lilly & Ltd.].
Toxicity The most severe toxicity is possible life-threatening oesophagitis and pneumonitis observed in patients receiving radical radiotherapy to thorax in association with gemcitabine.
Half-life Plasma half-life, 0.7 to 1.6 h, depending on age and gender; 0.18 to 0.43 (mean 0.28) h for gemcitabine triphosphate.
Volume of Distribution Steady state, 17 (range 9 to 30) L/m^2 (gemcitabine); 150 L/m^2 (range 96 to 228 L/m^2) (metabolite, dFdU).

Clearance Systemic, ranges between 29.2 and 92.2 L/h/m^2 depending on age and gender. Total body, 90 (range 40 to 130) L/h/m^2.
Protein Binding Negligible.
Note For reviews of gemcitabine, see Plunkett *et al.* [1995] and Noble and Goa [1997].
Dose Usually 1 g/m^2 body surface is administered intravenously, over an hour, once a week; adjusted up to 1250 or 1500 mg/m^2 according to response and toxicity.

Eli Lilly & Company Ltd. Supplied data.
Freeman KB *et al.* (1995). Validated assays for the determination of gemcitabine in human plasma and urine using high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Appl* 665: 171–181.
Noble S, Goa KL (1997). Gemcitabine. A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. *Drugs* 54: 447–472.
Plunkett W *et al.* (1995). Gemcitabine: metabolism, mechanisms of action, and self-potentiation. *Semin Oncol* 22(Suppl. 11): 3–10.

Gentamicin

Antibiotic
CAS—1403-66-3



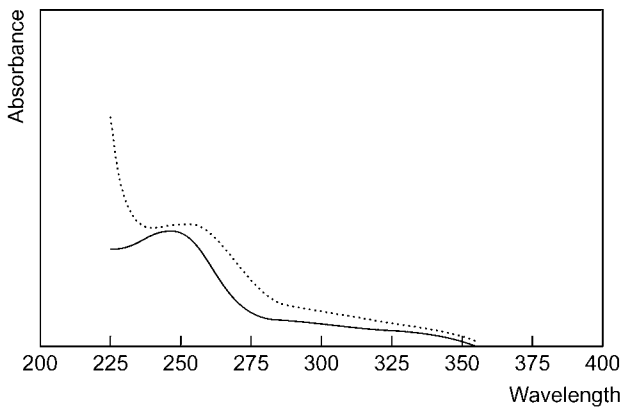
Gentamicin C_1 $R_1 = R_2 = CH_3$
Gentamicin C_2 $R_1 = CH_3$, $R_2 = H$
Gentamicin C_{1a} $R_1 = R_2 = H$

Chemical Properties A mixture of isomeric aminoglycoside antibiotics (gentamicin C_1, gentamicin $C_{1A}$and gentamicin C_2) produced by *Micromonospora purpurea* or *M. echinospora*. A white amorphous powder. Mp 102° to 108°. Freely soluble in water; soluble in pyridine; moderately soluble in methanol, ethanol and acetone; sparingly soluble in chloroform; practically insoluble in benzene and halogenated hydrocarbons. Log *P* (octanol/water), −1.9.

Gentamicin Sulfate

CAS—1405-41-0
Proprietary Names *Alcomicin*; *Cidomycin*; *Garamycin*; *Genoptic*; *Gentacidin*; *Gentak*; *Gentalline*; *Gentasol*; *Genticin*; *Gentigan*; *G-Myticin*; *Jenamicin*; *Lugacin*; *Ocu-Mycin*; *Refobacin*; *Sulmycin*. It is an ingredient of many proprietary preparations—see Sweetman [2009].
Chemical Properties A white to buff-coloured powder. Mp 218° to 237°. Soluble in water, ethylene glycol and formamide; practically insoluble in ethanol, chloroform and ether.

Colour Tests Nessler's reagent (100°)—black; ninhydrin (heat for 4 min)—violet.
High Performance Liquid Chromatography System HAA—retention time 14.0 min.
Ultraviolet Spectrum Aqueous acid—247 nm; aqueous alkali—251 nm.



Infrared Spectrum Principal peaks at wavenumbers 1120, 1060, 1625, 1525, 1290, 880 cm^{-1} (KBr disk).
Quantification See also under Amikacin.
Plasma **HPLC** UV detection. Gentamicin isomers (C_1, $C_{1A}$and C_2) [Isoherranen, Soback 2000]. Fluorescence detection (comparison with fluorescence polarisation immunoassay) [Yusuf *et al.* 1999]. Fluorescence detection. Gentamicin components C_1, C_{1A} and C_2, limit of detection, 500 µg/L [D'Souza, Ogilvie 1982].
Serum **Immunoassay** Fluoroimmunoassay [Munro *et al.* 1982]. Enzyme immunoassay, limit of detection, 100 µg/L [Wills, Wise 1979].
Urine **HPLC** Fluorescence detection. Limit of detection, 75 µg/L [Al-Amoud *et al.* 2002]. See Plasma [Isoherranen, Soback 2000]; [D'Souza, Ogilvie 1982].
Reviews For a review of methods for the determination of gentamicin in serum, see Hospes *et al.* [1982]. For a review of HPLC methods for the determination of gentamicin and other aminoglycosides in biological fluids, see Soltes [1999].
Disposition in the Body Poorly absorbed after oral administration but rapidly absorbed after IM injection; it is also absorbed systemically following topical application to wounds. In normal subjects it is rapidly excreted in the urine as unchanged drug, up to 80% of a dose being excreted in 24 h. It accumulates in the tissues and there may be considerable intersubject variation in the pharmacokinetics. Gentamicin may be detected in serum and urine for several days after cessation of treatment.
Therapeutic Concentration During treatment the serum concentration should be in the range 4 to 12 mg/L and should be monitored regularly, especially in patients who have renal insufficiency. During multiple dosing, the trough concentration immediately preceding a dose should not exceed 2 mg/L.

A single IM dose of 1 mg/kg given to 10 subjects produced a mean peak serum concentration of 5.8 mg/L in 0.5 to 1 h [Chung *et al.* 1980].

In 11 subjects, a 30-min IV infusion of 6 or 2 mg/kg produced respective mean plasma concentrations of 35.0 and 10.1 mg/L at the end of the infusion, 17.0 and 5.4 mg/L 30 min after the end of the infusion, and 0.45 and 0.69 mg/L at the end of the dosing interval [Sangha *et al.* 1995].

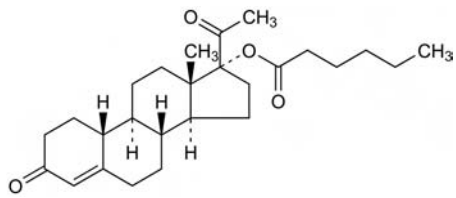
Toxicity Toxic effects may be produced at serum concentrations of 12 mg/L or more, or during chronic treatment, if the trough serum concentration exceeds 2 mg/L.
Half-life Plasma half-life, about 2 to 4 h, increased in renal failure; a very long terminal elimination phase of several days has also been reported.
Volume of Distribution About 0.2 L/kg.
Clearance Plasma clearance, about 1 mL/min/kg.
Protein Binding Less than 30%.
Dose The equivalent of 3 to 5 mg/kg of gentamicin daily, given parenterally.

Al-Amoud AI *et al.* (2002). Determination of gentamicin in urine samples after inhalation by reversed-phase high-performance liquid chromatography using pre-column derivatisation with o-phthalaldehyde. *J Chromatogr B Analyt Technol Biomed Life Sci* 769: 89–95.
Chung M *et al.* (1980). Comparison of netilmicin and gentamicin pharmacokinetics in humans. *Antimicrob Agents Chemother* 17: 184–187.
D'Souza J, Ogilvie RI (1982). Determination of gentamicin components C1a, C2 and C1 in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 232: 212–218.
Hospes W *et al.* (1982). Comparison of an HPLC method with a RIA, EMIT and FIA method for the assay of serum gentamicin with extensive statistical evaluation. *Pharm Weekbl Sci Edn* 4: 32–37.
Isoherranen N, Soback S (2000). Determination of gentamicins C(1), C(1a), and C(2) in plasma and urine by HPLC. *Clin Chem* 46: 837–842.
Munro AJ *et al.* (1982). A simplified quenching fluoroimmunoassay for gentamicin and the effects of some potential interfering factors. *J Antimicrob Chemother* 9: 47–51.
Sangha KS *et al.* (1995). Pharmacokinetics of once-daily dosing of gentamicin in surgical intensive care unit patients with open fractures. *Ann Pharmacother* 29: 117–119.
Soltes L (1999). Aminoglycoside antibiotics–two decades of their HPLC bioanalysis. *Biomed Chromatogr* 13: 3–10.
Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.
Wills PJ, Wise R (1979). Rapid, simple enzyme immunoassay for gentamicin. *Antimicrob Agents Chemother* 16: 40–42.
Yusuf A *et al.* (1999). Simplified high-performance liquid chromatographic method for the determination of gentamicin sulfate in a microsample of plasma: comparison with fluorescence polarization immunoassay. *Ther Drug Monit* 21: 647–652.

Gestonorone Caproate

Progestational Steroid
$C_{26}H_{38}O_4$ = 414.6
CAS—1253-28-7

IUPAC Name 17-[(1-Oxohexyl)oxy]-19-norpregn-4-ene-3,20-dione
Synonym Gestronol hexanoate
Proprietary Names *Depostat*; *Primostat*.

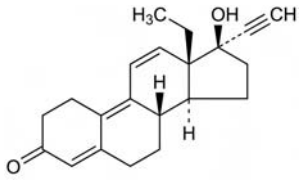


Chemical Properties A white to creamy-white crystalline powder. Mp 123° to 124°; a polymorph melting at 119° to 123° may also occur. Soluble in ethanol and methanol; freely soluble in acetone and chloroform; sparingly soluble in ether. Log *P* (octanol/water), 5.2.
Thin-layer Chromatography System TB—R_f 0.31; system TE—R_f 0.83; system TF—R_f 0.59.
Gas Chromatography System GA—RI 3440.
Ultraviolet Spectrum Methanol—240 nm (A^1_1=415b).
Infrared Spectrum Principal peaks at wavenumbers 1733, 1667, 1715, 1266, 1250, 1618 cm^{-1} (KBr disk).
Dose 200 to 400 mg IM every 5 to 7 days.

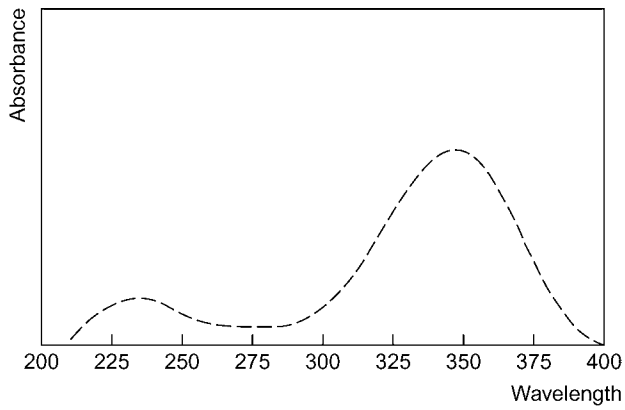
Gestrinone

Anabolic Steroid, β_2-Adrenoceptor Agonist
$C_{21}H_{24}O_2$ = 308.4
CAS—16320-04-0; 40542-65-2

IUPAC Name (8*S*,13*S*,14*S*,17*R*)-13-Ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,14,15,16-octahydrocyclopenta[*a*]phenanthren-3-one
Synonyms A46745; (17α)-13-ethyl-17-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one; ethylnorgestrienone; R2323; RU2323.
Proprietary Names *Dimetriose*; *Dimetrose*; *Nemestran*; *Trodomose*.



Chemical Properties A white to slightly yellow powder. Mp 154°.
High Performance Liquid Chromatography Column: C_{18} UG120, Capcell pack (150 × 2.0 mm i.d., 5 µm). Temperature: 40°. Mobile phase: acetonitrile : water (A:B). Elution programme: (5:95) to (30:70) in 3 min, to (52.5:47.5) in 14 min, to (100:0) in 3 min, held for 5 min. UV detection (λ=345 nm). Retention time: gestrinone, 16.3 min; metabolite 1, 11.3 min; metabolite 2, 12.2 min [Kim *et al.* 2000].
Ultraviolet Spectrum Neutral—235, 347 nm.



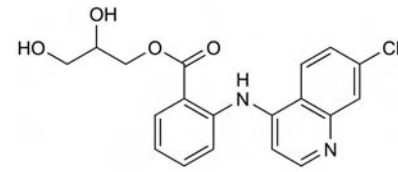
Disposition in the Body Gestrinone is well absorbed after oral administration with negligible first-pass metabolism. It is metabolised in the liver by hydroxylation to two unknown metabolites and the conjugate forms. It is excreted as the latter.
Therapeutic Concentration Peak plasma concentrations are observed about 3 h after administration.
Half-life Approximately 24 h.
Dose A usual dose of 2.5 mg is administered twice weekly.

Kim Y *et al.* (2000). Determination of the metabolites of gestrinone in human urine by high performance liquid chromatography, liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 14: 1717–1726.

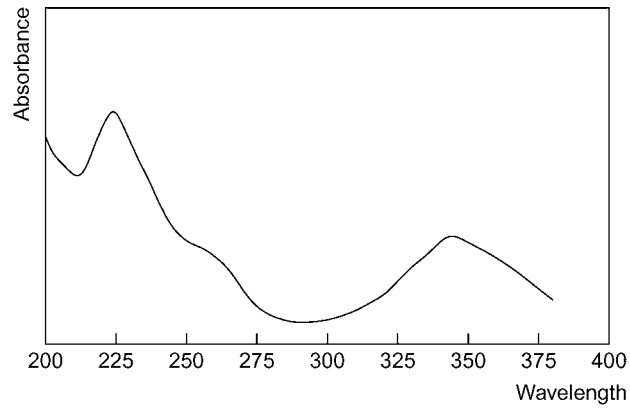
Glafenine

Analgesic
$C_{19}H_{17}ClN_2O_4$ = 372.8
CAS—3820-67-5

IUPAC Name 2,3-Dihydroxypropyl 2-[(7-chloro-4-quinolinyl)amino]benzoate
Synonym Glaphenine
Proprietary Names *Glifan*; *Glifanan*; *Privadol*.



Chemical Properties A white or slightly yellow crystalline powder. Mp 169° to 170°. Practically insoluble in water; slightly soluble in absolute alcohol, acetone, ether, benzene and chloroform; soluble in dilute aqueous alkalis and acids. Log *P* (octanol/water), 3.7.
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.01; system TC—R_f 0.38; system TE—R_f 0.46; system TF—R_f 0.03; system TL—R_f 0.40; system TAE—R_f 0.81; system TAF—R_f 0.78.
Gas Chromatography System GA—glafenine, not eluted; glafenine-Me RI 2770.
High Performance Liquid Chromatography System HX—RI 372; system HY—RI 276; system HZ—retention time 2.3 min.
Ultraviolet Spectrum Aqueous acid—345 nm (A^1_1=467b).



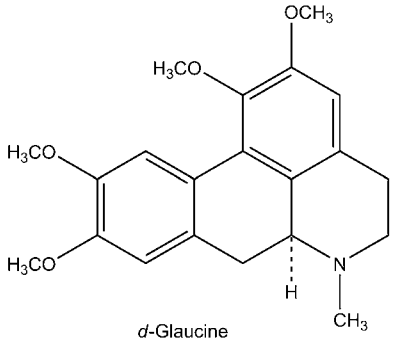
Quantification
Plasma **HPLC** UV detection. Limits of detection, 50 µg/L for glafenine , 250 µg/L for glafenic acid [Ennachachibi *et al.* 1988]. Glafenine and floctafenine. Limit of detection, about 2 µmol/L for glafenine [Tracqui *et al.* 1988]. UV detection. Glafenine and metabolites. Limits of detection, 500 µg/L for glafenine and hydroxyglafenic acid, 200 µg/L for glafenic acid [Tournet *et al.* 1981].
Disposition in the Body Absorbed after oral administration with peak plasma concentrations occurring about 1 to 2 h after ingestion. It is hydrolysed to glafenic acid which is excreted in the urine as an acylglucuronide conjugate; glafenic acid is found in plasma at concentrations greater than those of unchanged drug. Small amounts of unconjugated *N*-oxide of glafenic acid, and hydroxyglafenic acid which is mainly conjugated, have also been found in urine.
Therapeutic Concentration
After a single 400-mg oral dose of glafenine, a mean peak plasma concentration of 0.7 mg/L was achieved in 1.5 h in 12 healthy subjects and 2.2 mg/L in 2.8 h in 12 cirrhotic subjects; the large hepatic first-pass effect observed in healthy subjects was markedly reduced in cirrhotic subjects (ratio of C_{max} glafenic acid to C_{max} glafenine=18.9 in healthy subjects and 3.6 in cirrhosis) [Vermerie *et al.* 1992].
Toxicity Plasma concentrations >75 mg/L may be associated with toxic effects. It has a high incidence of anaphylactic reactions and has been withdrawn from the market in most countries.
Half-life About 1 to 2 h.
Dose Up to 1.2 g daily.

Ennachachibi A *et al.* (1988). Effective high-performance liquid chromatographic determination of glafenine in plasma: pharmacokinetic application. *J Chromatogr* 427: 307–314.
Tournet MC *et al.* (1981). *J Chromatogr* 224: 348–352.
Tracqui A *et al.* (1988). Simultaneous determination of glafenine and floctafenine in human plasma using high-performance liquid chromatography. *Ann Biol Clin (Paris)* 46: 665–667.
Vermerie N *et al.* (1992). Pharmacokinetics of glafenine and glafenic acid in patients with cirrhosis, compared to healthy volunteers. *Fundam Clin Pharmacol* 6: 197–203.

Glaucine

Cough Suppressant, Drug of Abuse, Quinoline
$C_{21}H_{25}NO_4$ = 355.4
CAS—475-81-0 (*d*-glaucine); 5630-11-5 (*dl*-glaucine).

Synonyms Boldine dimethyl ether; (6a*S*)-5,6,6a-7-tetrahydro-1,2,9,10-tetramethoxy-6-methyl-4*H*-dibenzo[*de*,*g*]quinoline; 1,2,9,10-tetramethoxyaporphine.
Proprietary Name *Glauvent*. It is also an ingredient in *Bronchitussin*; *Bronchocin*; *Broncholytin*.



d-Glaucine

Chemical Properties Found in *Glaucium flavum* Crantz (*G. luteum* Scop.), Papaveraceae, in *Dicentra* and *Corydalis* species, Fumariaceae and several more species. The *d*-form is prevalent in nature. Orthorhombic plates, prisms from ethyl acetate or ether. Mp 120°. Soluble in acetone, alcohol, chloroform, ethyl acetate; moderately soluble in ether, petroleum ether; practically insoluble in water and benzene. Dimethoxy analogue of boldine.

Glaucine Hydrochloride Trihydrate

$C_{21}H_{25}NO_4$,HCl, $3H_2O$ = 445.9
Chemical Properties Mp 232° (anhydrous). Soluble in water, alcohol and chloroform.

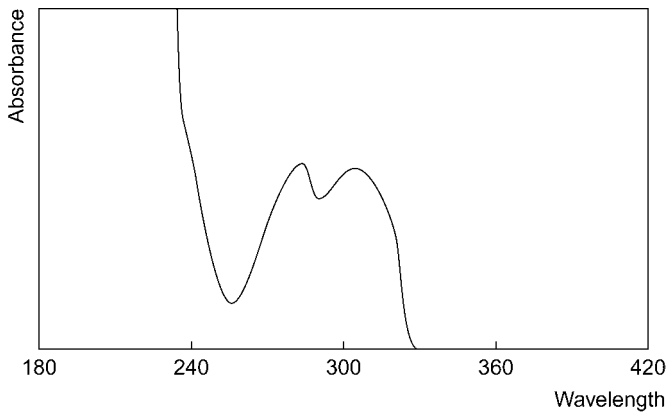
Glaucine Hydrobromide

$C_{21}H_{25}NO_4$,HBr = 436.1
CAS—5996-06-5 (*d*-form)
Chemical Properties Mp 235°. Less soluble than the hydrochloride.

Glaucine Phosphate

$(C_{21}H_{25}NO_4)_2$,$3H_3PO_4$ = 1004.8
CAS—73239-87-9 (*dl*-form)
Synonym DL-832
Chemical Properties Crystalline powder.

Ultraviolet Spectrum Principal peaks at 290, 310 nm.



Mass Spectrum Principal ions at *m/z* 354, 355, 80.

Quantification

Plasma **HPLC** Column: LiChrosorb Si 60 (125 × 4.0 mm i.d., 5 µm). Mobile phase: *n*-hexane : methanol : tetrahydrofuran : diethylamine (88.5 : 7.5 : 4 : 0.15), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 310 nm, λ_{em} = 340 nm). Retention time: 3.4 min. Limit of quantification, 5 µg/L [Fels *et al.* 1984].

Serum **GC-MS** Column: (5%-phenyl)-methylpolysiloxane capillary (30 m × 0.25 mm, 0.5 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 4 min to 290° at 25°/min for 9.6 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 19.1 min (methyl *tert*-butyl ether derivative). Limit of quantification not reported [Dargan *et al.* 2008].

Urine **HPLC** Column: LiChrosorb Si 60 (125 × 4.0 mm i.d., 5 µm). Mobile phase: *n*-hexane : methanol : tetrahydrofuran : diethylamine (88.5 : 7.5 : 4 : 0.15), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 310 nm, λ_{em} = 340 nm). Retention time: 3.4 min. Limit of quantification, 2 µg/L [Fels *et al.* 1984].

GC-MS Column: (5%-phenyl)-methylpolysiloxane capillary (30 m × 0.25 mm, 0.5 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 4 min to 290° at 25°/min for 9.6 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 19.1 min (methyl *tert*-butyl ether derivative). Limit of quantification not reported [Dargan *et al.* 2008].

Other **TLC** Plant Samples (*Thalictrum flavum*). Plates: silica gel GF_{254}. Solvent system: petroleum ether : chloroform : acetone : methanol (4 : 4 : 1 : 1). Location reagent Dragendorff's reagent. R_f values: glaucine 0.71, thalidazine 0.38, thaliglucine 0.35. Limit of quantification not reported [Velcheva *et al.* 1992].

GC-MS Plant Samples (members of the genus *Sarcocapnos*). Column: HP-1 fused silica capillary (12 m × 0.2 mm i.d., 0.33 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 200° for 4 min to 250° at 10°/min for 15 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention times: glaucine 8.0 min, sarcophylline 6.2 min, sarcocapnine 6.5 min, cularicine 6.6 min, *O*-methylcularicine 6.8 min, cularidine 7.2 min, celtisine 7.3 min, cularine 7.5 min, sarcocapnidine 7.5 min, celtine 7.6 min, protopine 8.8 min, ribasine 9.5 min, dihydrosanguinarine 10.9 min, chelidonine 11.7 min. Limit of quantification, not reported [Suau *et al.* 2005].

HPLC Plant MaterialSamples (*Critesion lechleri*). Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: (leaf samples) acetonitrile : 0.1 mol/L phosphate (pH 2.5)-10 mmol/L SDS-0.08% TEA (43 : 57); (latex samples) acetonitrile : 0.1 mol/L phosphate (pH 2.5)-10 mmol/L SDS-0.1% TEA (45 : 55), flow rate 2 mL/min. UV detection (λ = 254 nm). Retention times: (leaf samples) glaucine 9.8 min, norisoboldine 4.2 min, isoboldine 4.6 min, magnoflorine 5.2 min, thaliporphine 6.5 min, taspine 9.0 min. Limit of quantification not reported [Milanowski *et al.* 2002].

Therapeutic Concentration

Three healthy volunteers were administered a single 60 mg oral dose of *d*-glaucine. Peak plasma concentrations were reported as follows:

	Subject 1	*Subject 2*	*Subject 3*
C_{max} (µg/L)	200	285	255
Time (h)	1.5	2	0.75
Urinary excretion 0–72 h (% of dose)	0.10	0.27	0.13

[Fels *et al.* 1984].

Toxicity LD_{50} in mice 98 mg/kg (IV), 401 mg/kg (oral).

A 23-year-old woman presented to an emergency department following the ingestion of 2 tablets of 'head candy', marketed as a 1-benzylpiperazine (BZP)-free 'herbal high'. She developed nausea and vomiting within 30 min of ingestion. Serum and urine samples were collected at admission and both contained glaucine. The serum glaucine concentration was ~0.7 mg/L. She was discharged the following day her symptoms had resolved [Dargan *et al.* 2008].

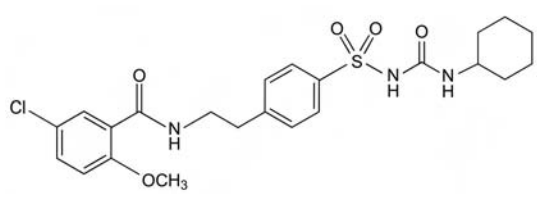
Note For a study of the mechanism of action of glaucine in the rat, see Orallo *et al.* [1993]; for a study of the abuse potential of glaucine in monkeys, see Schuster *et al.* [1982].

Dargan PI *et al.* (2008). Detection of the pharmaceutical agent glaucine as a recreational drug. *Eur J Clin Pharmacol* 64: 553–554.
Fels JP *et al.* (1984). Determination of glaucine in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 308: 273–281.
Milanowski DJ *et al.* (2002). Geographic distribution of three alkaloid chemotypes of *Croton lechleri*. *J Nat Prod* 65: 814–819.
Orallo F *et al.* (1993). Study of the mechanism of the relaxant action of (+)-glaucine in rat vas deferens. *Br J Pharmacol* 110: 943–948.
Schuster CR *et al.* (1982). Experimental studies of the abuse potential of *d*,*l*-glaucine 1,5-phosphate in rhesus monkeys. *Pharmacol Biochem Behav* 16: 851–854.
Suau R *et al.* (2005). Identification and quantification of isoquinoline alkaloids in the genus *Sarcocapnos* by GC-MS. *Phytochem Anal* 16: 322–327.
Velcheva M *et al.* (1992). The alkaloids of the roots of *Thalictrum flavum* L. *Acta Pharm Nord* 4: 57–58.

Glibenclamide

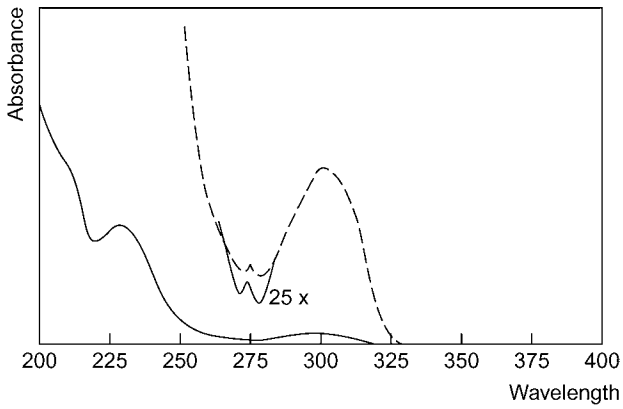
Antidiabetic
$C_{23}H_{28}ClN_3O_5S$ = 494.0
CAS—10238-21-8

IUPAC Name 5-Chloro-*N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]-phenyl]ethyl]-2-methoxybenzamide
Synonyms Glybenclamide; glyburide; glycbenzcyclamide.
Proprietary Names *Azuglucon*; *Basstiverit*; *Calabren*; *Daonil*; *DiaBeta*; *Diabetamide*; *Diacon*; *Euglucan*; *Euglucon*; *Glib*; *Gliben*; *Glibenbeta*; *Gluconorm*; *Glucoremed*; *Glynase*; *Hemi-Doanil*; *Libanil*; *Malix*; *Maninil*; *Micronase*; *Miglucan*; *Semi-Daonil*; *Semi-Euglucon*.

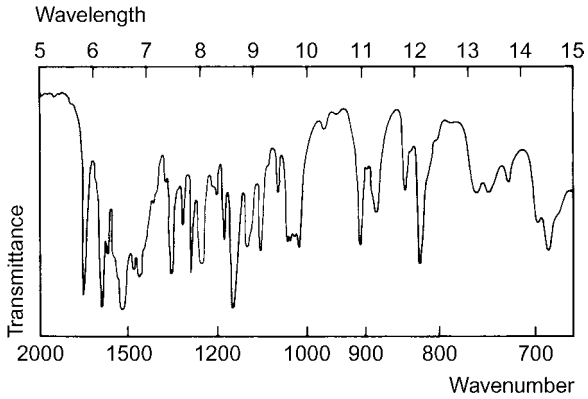


Chemical Properties A white crystalline powder. Mp 172° to 174°. Sparingly soluble in water; practically insoluble in ether; soluble 1 in 330 of ethanol, 1 in 36 of chloroform and 1 in 250 of methanol. pK_a 5.3. Log *P* (octanol/water), 4.8. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].
Colour Tests Aromaticity (method 2)—yellow/orange; Liebermann's reagent (100°)—orange (15 s).

Thin-layer Chromatography System TA—R_f 0.80; system TB—R_f 0.00; system TD—R_f 0.30; system TE—R_f 0.11; system TF—R_f 0.30; system TAD—R_f 0.57; system TAE—R_f 0.90 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—glibenclamide-Me RI 3800, glibenclamide-Me_2 RI 3840.
High Performance Liquid Chromatography System HX—RI 637; system HY—RI 571; system HZ—retention time 14.4 min; system HAA—retention time 22.0 min; system HAX—retention time 8.5 min; system HAY—retention time 9.8 min.
Ultraviolet Spectrum Methanol—275, 300 nm (A_1^1=63a).



Infrared Spectrum Principal peaks at wavenumbers 1524, 1160, 1623, 1718, 1276, 823 cm^{-1} (KBr disk).



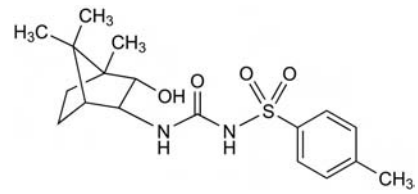
Quantification
Plasma **GC** ECD. Limit of detection, 5 µg/L [Castoldi, Tofanetti 1979].
HPLC Limit of detection, <10 µg/L [Niopas, Daftsios 2002]. Fluorescence detection. Limit of detection, 5 µg/L [Khatri *et al.* 2001]. UV detection. Limit of detection, 5 µg/L [Valdes Santurio, Gonzalez Porto 1996]. Glibenclamide and other sulfonylureas. Limit of detection, 10 to 40 µg/L [Sener *et al.* 1995]. Limit of detection, 5 to 10 µg/L [Emilsson *et al.* 1986].
Serum **GC** UV detection. Glibenclamide and its major metabolites. Limit of detection, 1 µg/L for glibenclamide [Rydberg *et al.* 1991].
HPLC UV detection. Limit of detection, 20 µg/L [Abdel-Hamid *et al.* 1989]. Fluorescence detection. Limit of detection, 10 µg/L [Adams *et al.* 1982].
Urine **GC** See Serum [Rydberg *et al.* 1991].
HPLC See Plasma [Emilsson *et al.* 1986].
Disposition in the Body Readily absorbed after oral administration; widely distributed throughout the body and metabolised mainly by 4-*trans*- and 3-*cis*-hydroxylation of the cyclohexyl ring. About 20 to 50% of a dose is excreted in the urine in 24 h, mainly as metabolites; about 45 to 75% of a dose is eliminated in the faeces over a period of 5 days.
Therapeutic Concentration
After a single oral dose of 5 mg to 2 subjects, peak plasma concentrations of 0.17 to 0.36 mg/L were attained in 3 h [Balant *et al.* 1975].
Toxicity
In a suicide attempt, a concentration of 0.6 mg/L was found in the serum initially. After 84 h the concentration had declined to less than 0.1 mg/L [Berger *et al.* 1977].
Half-life Plasma half-life, 5 to 16 h.
Volume of Distribution About 0.3 L/kg.
Distribution in Blood Plasma : whole blood ratio, 2.
Protein Binding About 99%.
Note For reviews of the pharmacokinetics of hypoglycaemic drugs, see Balant [1981] and Jackson and Bressler [1981].

Dose 5 to 15 mg daily.

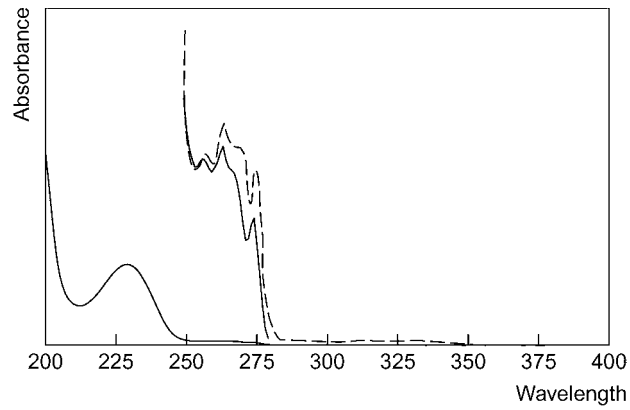
Abdel-Hamid ME *et al.* (1989). A rapid high-performance liquid chromatography assay of glibenclamide in serum. *J Clin Pharm Ther* 14: 181–188.
Adams WJ *et al.* (1982). Determination of glyburide in human serum by liquid chromatography with fluorescence detection. *Anal Chem* 54: 1287–1291.
Balant L (1981). Clinical pharmacokinetics of sulphonylurea hypoglycaemic drugs. *Clin Pharmacokinet* 6: 215–241.
Balant L *et al.* (1975). Comparison of the pharmacokinetics of glipizide and glibenclamide in man. *Eur J Clin Pharmacol* 8: 63–69.
Berger M *et al.* (1977). [Attempted suicide using glibenclamide:course of glucose, insulin, glibenclamide and C-peptide blood levels]. *Dtsch Med Wochenschr* 102: 586–587.
Castoldi D, Tofanetti O (1979). Gas chromatographic determination of glibenclamide in plasma. *Clin Chim Acta* 93(2): 195–198.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Emilsson H *et al.* (1986). High-performance liquid chromatographic determination of glibenclamide in human plasma and urine. *J Chromatogr* 383: 93–102.
Jackson JE, Bressler R (1981). Clinical pharmacology of sulphonylurea hypoglycaemic agents: part 1. *Drugs* 22: 211–245.
Khatri J *et al.* (2001). A novel extractionless hplc fluorescence method for the determination of glyburide in the human plasma: application to a bioequivalence study. *J Pharm Pharm Sci* 4: 201–206.
Niopas I, Daftsios AC (2002). A validated high-performance liquid chromatographic method for the determination of glibenclamide in human plasma and its application to pharmacokinetic studies. *J Pharm Biomed Anal* 28: 653–657.
Rydberg T *et al.* (1991). Determination of glibenclamide and its two major metabolites in human serum and urine by column liquid chromatography. *J Chromatogr* 564: 223–233.
Sener A *et al.* (1995). Standardized procedure for the assay and identification of hypoglycemic sulfonylureas in human plasma. *Acta Diabetol* 32: 64–68.
Valdes Santurio JR, Gonzalez Porto E (1996). Determination of glibenclamide in human plasma by solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 682: 364–370.

Glibornuride

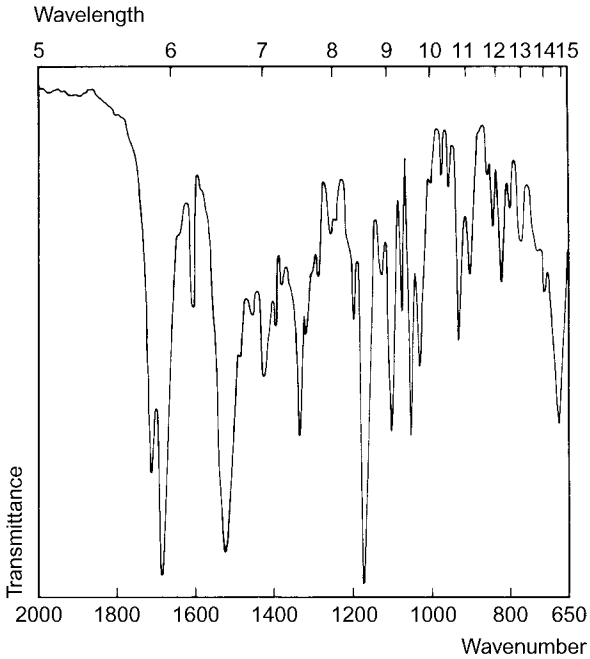
Antidiabetic
$C_{18}H_{26}N_2O_4S$ = 366.5
CAS—26944-48-9
IUPAC Name 1-(3-Hydroxy-4,7, 7-trimethyl-2-bicyclo[2.2.1]heptanyl)-3-(4-methylphenyl)sulfonylurea
Synonym [1*S*-(*endo*,*endo*)]-*N*-[[(3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]hept-2-yl)amino]carbonyl]-4-methylbenzenesulfonamide
Proprietary Names *Gluborid*; *Glutril*.



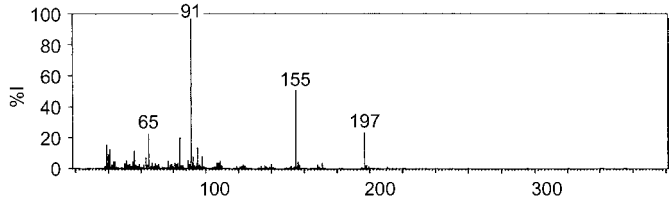
Chemical Properties Crystals. Mp 192° to 195°.
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.40; system TE—R_f 0.05; system TF—R_f 0.60; system TAD—R_f 0.54; system TAE—R_f 0.92.
Gas Chromatography System GA—art (methylsulfonamide) RI 1730, art (methylsulfonamide)-Me RI 1740, art (amide) RI 1620, M (OH-)-art (sulfonamide)-Me RI 2265, M (OH-)-art (sulfonamide)-Me_2 RI 2030, M (COOH-)-art (sulfonamide)-Me_3 RI 1955; system GB—glibornuride, not eluted, art (methylsulfonamide) RI 1660, art (amide) RI 1695.
Ultraviolet Spectrum Ethanol—257, 264 (A_1^1=17a), 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1170, 1682, 1520, 1710, 1050, 1100 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 155, 197, 65, 84, 39, 95, 41.



Quantification

Plasma **Spectrofluorimetry** Limit of detection, 40 µg/L [Becker 1977].

Serum **Spectrofluorimetry** See Plasma [Becker 1977].

HPLC UV detection. Limit of detection, 500 µg/L [Harzer 1980].

Disposition in the Body Readily absorbed after oral administration. Extensively metabolised by hydroxylation to a series of inactive or almost inactive compounds and by oxidation to a *p*-carboxy derivative. About 60 to 70% of a dose is excreted in the urine as metabolites; four hydroxylated derivatives account for about 75% of the urinary material, *p*-hydroxyglibornuride accounts for about 6% and *p*-carboxyglibornuride for about 7%; the remainder of a dose is eliminated in the faeces.

Therapeutic Concentration

After an oral dose of 50 mg to 7 subjects, peak plasma concentrations of 1.6 to 3.4 mg/L were attained in about 3 to 4 h [Rentsch *et al.* 1972].

Half-life Plasma half-life, 5 to 11 h (mean 8).

Volume of Distribution 0.15 to 0.35 L/kg.

Protein Binding About 95%.

Note For reviews of the pharmacokinetics of sulfonylurea hypoglycaemic drugs, see Balant [1981] and Jackson and Bressler [1981].

Dose 12.5 to 75 mg daily.

Balant L (1981). Clinical pharmacokinetics of sulphonylurea hypoglycaemic drugs. *Clin Pharmacokinet* 6: 215–241.

Becker R (1977). [Fluorometric determination of glibornuride in plasma and serum using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole]. *Arzneimittelforschung* 27: 102–104.

Harzer K (1980). [Analysis of glibornuride in serum in reverse phase high performance chromatography]. *J Chromatogr* 183: 115–117.

Jackson JE, Bressler R (1981). Clinical pharmacology of sulphonylurea hypoglycaemic agents: part 1. *Drugs* 22: 211–245.

Rentsch G *et al.* (1972). [Pharmacokinetics of glibornuride]. *Arzneimittelforschung* 22: 2209–2212.

Gliclazide

Antidiabetic

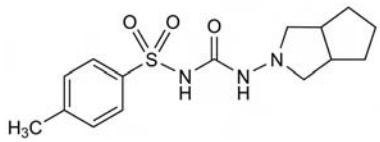
$C_{15}H_{21}N_3O_3S = 323.4$

CAS—21187-98-4

IUPAC Name 1-(3,3*a*,4,5,6,6*a*-Hexahydro-1*H*-cyclopenta[*c*]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea

Synonym *N*-[[(Hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)amino]carbonyl]-4-methylbenzenesulfonamide

Proprietary Names *Cadicon*; *Diabeside*; *Diabrezide*; *Diaclaron*; *Diaglyk*; *Dialoc*; *Diamexon*; *Diamicron*; *Dramion*; *Glicron*; *Glimicron*; *Glucozide*; *Glyade*; *Glycemirex*; *Glycon*; *Glycron*; *Medoclazide*; *Serviclazide*; *Sun-Glizide*; *Ziclin*.



Chemical Properties A crystalline solid. Mp about 181°. pK_a 5.8. Log *P* (octanol/water), 2.1.

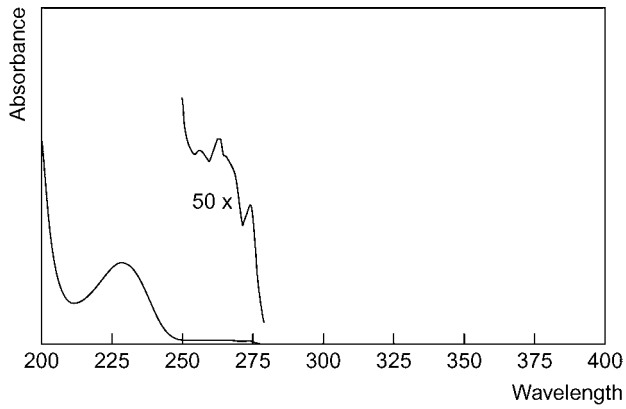
Colour Tests Liebermann's reagent—yellow; mercurous nitrate—black.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.09; system TAE—R_f 0.84.

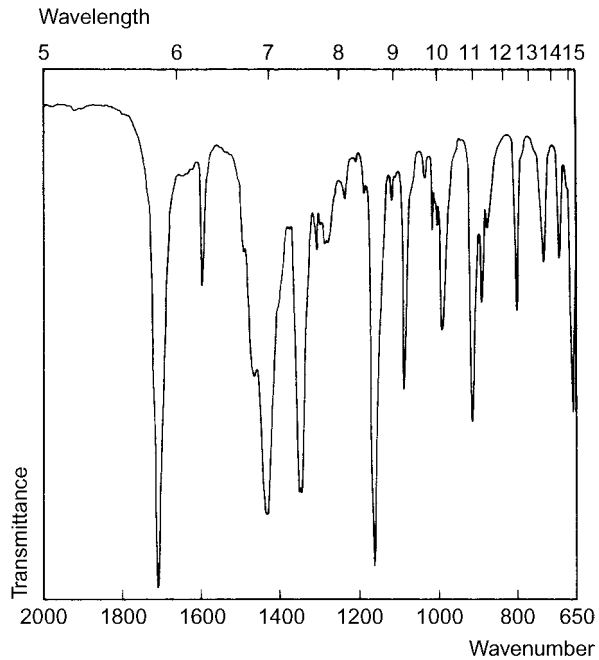
Gas Chromatography System GA—art 1-Me RI 1545; art (amide) RI 1620; art 3 RI 1670; art (methylsulfonamide) RI 1730; art (methylsulfonamide)-Me RI 1740; M art (OH-)(sulfonamide)-Me RI 2265; M art (OH-)(sulfonamide)-Me_2 RI 2030; M art (HOOC-)(sulfonamide)-Me_3 RI 1955; system GB—art (methylsulfonamide) RI 1660.

High Performance Liquid Chromatography System HX—RI 536; system HY—RI 483; system HZ—retention time 8.8 min; system HAA—retention time 20.5 min.

Ultraviolet Spectrum Aqueous acid—230 nm (A^1_1=440b); aqueous alkali—263 nm (A^1_1=20b).



Infrared Spectrum Principal peaks at wavenumbers 1707, 1162, 920, 667, 1089, 997 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** Gliclazide and other sulfonylureas. Limit of detection, 10–40 µg/L [Sener *et al.* 1995]. UV detection. Limit of detection, 300 µg/L [Kimura *et al.* 1980a].

Serum **HPLC** UV detection. Limit of detection, 20 µg/L [Noguchi *et al.* 1992]. UV detection. Limit of detection, 200 µg/L [Kimura *et al.* 1980b].

Disposition in the Body Absorbed after oral administration. It is extensively metabolised by hydroxylation, *N*-oxidation and oxidation to several inactive metabolites; the *p*-carboxy metabolite, which accounts for about 1% of the plasma concentration, has no hypoglycaemic activity but has some antithrombotic activity.

About 60–70% of a dose is excreted in the urine with less than 5% as unchanged drug. The *p*-carboxy and *N*-oxide metabolites account for about 40% of the dose. About 10–20% of the dose is eliminated in the faeces as metabolites.

Therapeutic Concentration

After a single oral dose of 80 mg to 23 subjects, peak plasma concentrations of 0.7–4.9 mg/L were attained in about 4 h. Following daily oral administration of 80 mg to 144 subjects, steady-state plasma concentrations of 0.3 to 8.2 mg/L (mean 2.5) were reported [Campbell *et al.* 1980].

Half-life Plasma half-life, 6 to 14 h (mean 10).

Protein Binding 85–95%.

Dose 40 to 320 mg daily.

Campbell DB *et al.* [1980]. Pharmacokinetics and metabolism of gliclazide. In Keen H *et al.* (eds). *Gliclazide and the Treatment of Diabetes*. London: Royal Society of Medicine, pp. 71–82.

Kimura M *et al.* (1980a). Reversed-phase high-performance liquid chromatographic determination of gliclazide in human plasma. *Chem Pharm Bull* 28: 344–346.

Kimura M *et al.* (1980b). Determination of gliclazide in human serum by high-performance liquid chromatography using an anion-exchange resin. *J Chromatogr B Biomed Appl* 183: 467–473.

Noguchi H *et al.* (1992). Determination of gliclazide in serum by high-performance liquid chromatography using solid-phase extraction. *J Chromatogr* 583: 266–269.

Sener A *et al.* (1995). Standardized procedure for the assay and identification of hypoglycemic sulfonylureas in human plasma. *Acta Diabetol* 32: 64–68.

Glimepiride

Antidiabetic

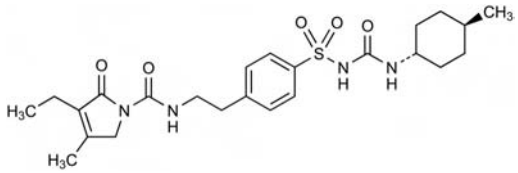
$C_{24}H_{34}N_4O_5S = 490.6$

CAS—93479-97-1

IUPAC Name 3-Ethyl-2,5-dihydro-4-methyl-*N*-[2-[4-[[[[(*trans*-4-methylcyclohexyl)-amino]carbonyl]amino]sulfonyl]phenyl]ethyl]2-oxo-1*H*-pyrrole-1-carboxamide

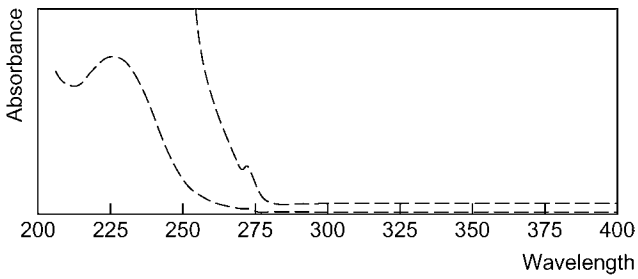
Synonym HOE-490

Proprietary Name *Amaryl*

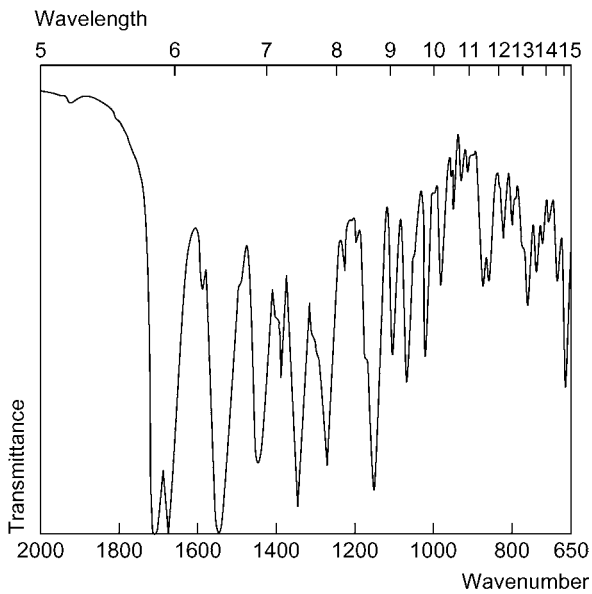


Chemical Properties A white to yellowish-white crystalline, almost odourless powder. Mp 207°. Practically insoluble in water.

Ultraviolet Spectrum Methanol—226, 273 nm.



Infrared Spectrum



Quantification

Serum **HPLC** In serum—column: ODS Spherisorb (125 × 4.6 mm i.d., 5 µm). Mobile phase: (A) 0.05 mol/L perchloric acid : acetonitrile (60:40); after 6 min, switched to (B) 0.05 mol/L perchloric acid : acetonitrile (42:58); after 8 min, (A), flow rate 2 mL/min. Retention time: (derivatives) glimepiride, 11.3 min; metabolites, 4.3 and 5.2 min. In urine—column: ODS Spherisorb (125 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L perchloric acid : acetonitrile (60:40), flow rate 2 mL/min. UV detection (λ=350 nm). Retention time: (derivatives) metabolites, 4.8 and 5.6 min. Limit of detection, 5 µg/L [Lehr, Damm 1990].

Urine **HPLC** See Serum [Lehr, Damm 1990].

Disposition in the Body Glimepiride is completely absorbed after oral administration. Peak plasma concentrations are attained within about 2 to 3 h. It is completely metabolised principally in the liver, mainly to the cyclohexyl hydroxy methyl derivative that has about a third the pharmacological activity of glimepiride, and the carboxyl derivative. These metabolites appear rapidly after administration of the parent drug. Cytochrome P450 isoenzyme 2C9 is involved in the formation of the cyclohexyl hydroxy methyl derivative. Glimepiride is distributed into breast milk and crosses the placenta. About 40% of a dose is eliminated in faeces as metabolites and about 60% of a dose is eliminated in urine as metabolites.

Therapeutic Concentration

Forty-two healthy volunteers, with a mean age of 27 years, were administered a single dose of 0.25 to 1.5 mg after an overnight, 10 to 12 h fast. The serum concentration measured 30 min after administration was 54 to 247 µg/L and after 180 min, 17 to 72 µg/L [Ratheiser *et al.* 1993].

Twelve healthy volunteers were administered single doses of 1, 2, 4 or 8 mg glimepiride. Mean drug concentrations were 103.2, 176.8, 307.8 and 550.8 µg/L for the doses at 2.3, 2.4, 2.1 and 2.8 h, respectively [Malerczyk *et al.* 1994].

Half-life Plasma half-life, 5 h after single dose; 9 h after multiple doses.

Volume of Distribution Approximately 113 mL/kg; 8.8 L.

Clearance Total body, 2.7 to 3.4 L/h.

Protein Binding >99.5%.

Note For a review of glimepiride, see Langtry and Balfour [1998].

Dose Usually up to 4 mg daily.

Langtry HD, Balfour JA (1998). Glimepiride. A review of its use in the management of type 2 diabetes mellitus. *Drugs* 55: 563–584.

Lehr KH, Damm P (1990). Simultaneous determination of the sulphonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after precolumn derivatization. *J Chromatogr* 526: 497–505.

Malerczyk V *et al.* (1994). Dose linearity assessment of glimepiride (Amaryl) tablets in healthy volunteers. *Drug Metab Drug Interact* 11(4): 341–357.

Ratheiser K *et al.* (1993). Dose relationship of stimulated insulin production following intravenous application of glimepiride in healthy man. *Arzneimittelforschung* 43: 856–858.

Glipizide

Antidiabetic

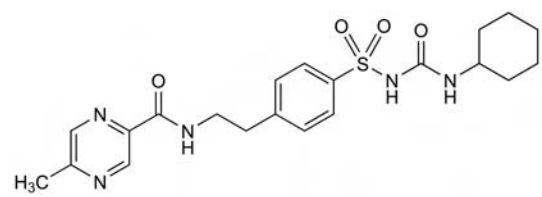
$C_{21}H_{27}N_5O_4S = 445.5$

CAS—29094-61-9

IUPAC Name *N*-[2-[4-[[[(Cyclohexylamino)carbonyl]amino]sulfonyl]phenyl] ethyl]-5-methylpyrazinecarboxamide

Synonyms Glipizidum; glydiazinamide; K-4024; CP-28720.

Proprietary Names *Apamid*; *Diasef*; *Dipazide*; *Glibenese*; *Glidiab*; *Glipid*; *Glipiscand*; *Gluco-Rite*; *Glucotrol*; *Glupital*; *Glygen*; *Melizid(e)*; *Mindiab*; *Minidiab*; *Minodiab*; *Ozidia*.



Chemical Properties A white powder. Mp about 205°. Practically insoluble in water and ethanol; soluble in chloroform, dimethylformamide and dilute solutions of alkali hydroxides; sparingly soluble in acetone. pK_a 5.9. Log *P* (octanol/water), 1.9.

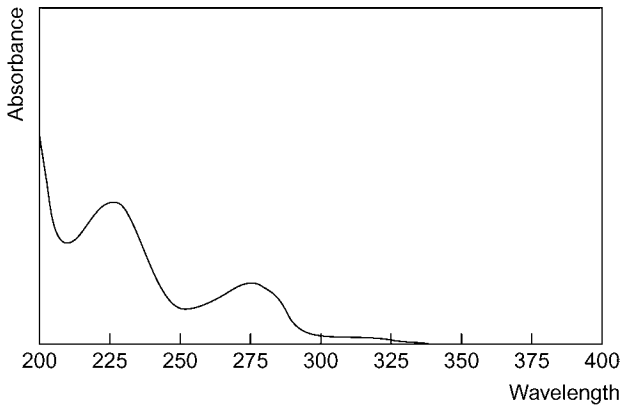
Colour Test Mercurous nitrate—black.

Thin-layer Chromatography System TA—R_f 0.87; system TB—R_f 0.00; system TC—R_f 0.41; system TE—R_f 0.07; system TL—R_f 0.05; system TAE—R_f 0.86.

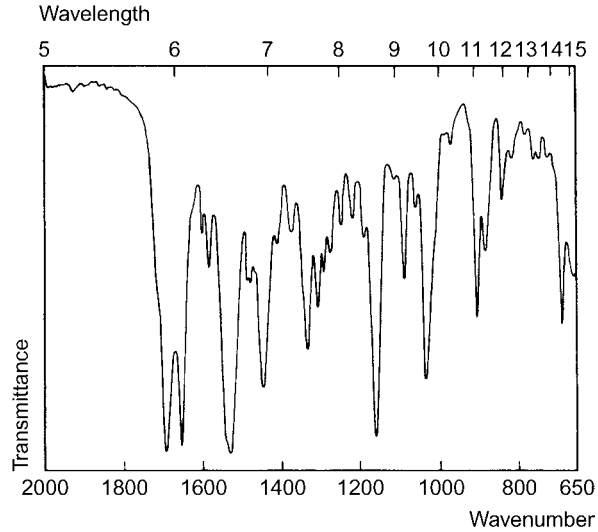
Gas Chromatography System GA—glipizide-Me RI 3420, glipizide-Me_2 RI 3455.

High Performance Liquid Chromatography System HX—RI 478; system HY—RI 423; system HZ—retention time 4.5 min; system HAA—retention time 17.6 min.

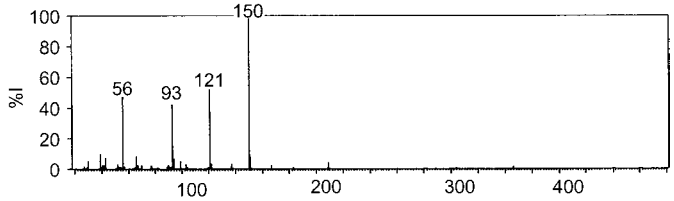
Ultraviolet Spectrum Aqueous acid—276 nm (A^1_1=231a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1528, 1690, 1650, 1159, 1032, 900 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 150, 121, 56, 93, 39, 151, 66, 94.



Quantification

Plasma **HPLC** Glipizide and other sulfonylureas. Limit of detection, 10 to 40 µg/L [Sener *et al.* 1995]. UV detection. Limit of detection, 5 to 10 µg/L [Emilsson 1987].

Radioimmunoassay Limit of detection, 1 µg/L [Maggi *et al.* 1981].

Serum **HPLC** UV detection. Limit of detection, 20 µg/L [Wåhlin-Boll, Melander 1979].

Urine **HPLC** See Plasma [Emilsson 1987].

Disposition in the Body Readily absorbed after oral administration. Metabolised by hydroxylation to form a number of inactive metabolites, principally the 4-*trans*-hydroxycyclohexyl and 3-*cis*-hydroxycyclohexyl derivatives. About 65 to 85% of a dose is excreted in the urine in 24 h, with about 3 to 10% as unchanged drug, up to 80% as hydroxylated metabolites, mainly the 4-*trans*-hydroxycyclohexyl derivative, and about 1 to 2% as an *N*-acetamido metabolite; about 11% of a dose is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 5 mg given to 6 subjects, peak serum concentrations of 0.11 to 0.49 mg/L (mean 0.33) were attained in about 1.6 h [Wåhlin-Boll *et al.* 1982].

Bioavailability Almost 100%.

Half-life Plasma half-life, 2 to 4 h.

Volume of Distribution About 0.2 L/kg.

Clearance Plasma clearance, about 0.6 mL/min/kg.

Protein Binding About 98%.

Note For a review of the pharmacokinetics of glipizide, see Brogden *et al.* [1979].

Dose Usually 2.5 to 20 mg daily; up to 40 mg have been used.

Brogden RN *et al.* (1979). Glipizide: a review of its pharmacological properties and therapeutic use. *Drugs* 18: 329–353.

Emilsson H (1987). High-performance liquid chromatographic determination of glipizide in human plasma and urine. *J Chromatogr* 421: 319–326.

Maggi E *et al.* (1981). Radioimmunoassay of glipizide in human plasma. *Eur J Clin Pharmacol* 21: 251–255.

Sener A *et al.* (1995). Standardized procedure for the assay and identification of hypoglycemic sulfonylureas in human plasma. *Acta Diabetol* 32: 64–68.

Wåhlin-Boll E, Melander A (1979). High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum. *J Chromatogr* 164: 541–546.

Wåhlin-Boll E *et al.* (1982). Bioavailability, pharmacokinetics and effects of glipizide in type 2 diabetics. *Clin Pharmacokinet* 7: 363–372.

Gliquidone

Antidiabetic

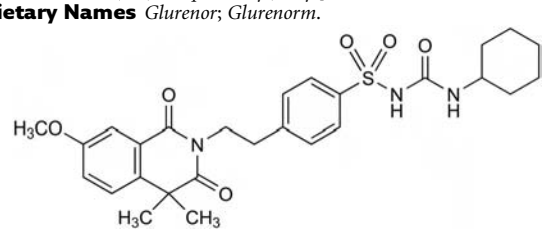
$C_{27}H_{33}N_3O_6S$ = 527.6

CAS—33342-05-1

IUPAC Name 1-Cyclohexyl-3-[4-[2-(7-methoxy-4,4-dimethyl-1,3-dioxoisoquinolin-2-yl)ethyl]phenyl]sulfonylurea

Synonym *N*-[(Cyclohexylamino)carbonyl]-4-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]benzenesulfonamide

Proprietary Names *Glurenor*; *Glurenorm*.



Chemical Properties A white or slightly yellow crystalline substance. Mp about 178°. Practically insoluble in water; slightly soluble in ethanol and methanol; soluble in acetone and chloroform. Log *P* (octanol/water), 4.6.

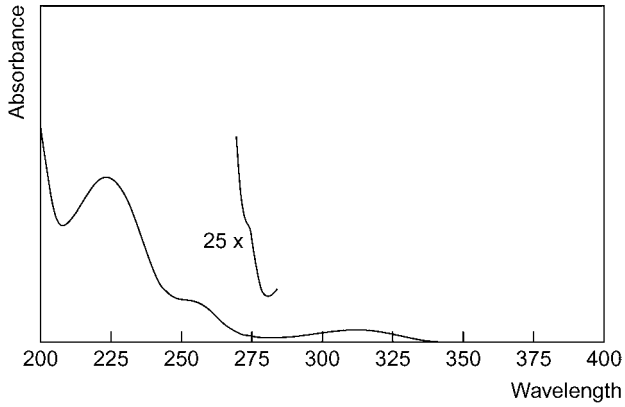
Colour Test Koppanyi–Zwikker test—violet.

Thin-layer Chromatography System TAE—R_f 0.93.

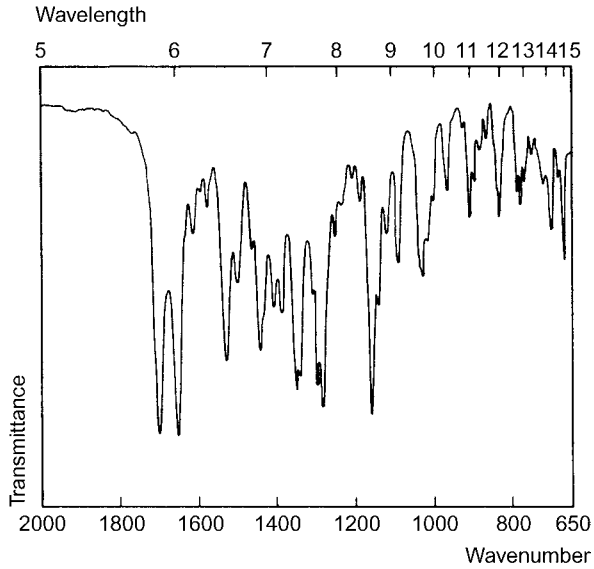
Gas Chromatography System GA—gliquidone RI 2024, gliquidone-Me RI 3850.

High Performance Liquid Chromatography System HY—RI 667.

Ultraviolet Spectrum Methanol—311 nm (A^1_1=50a); aqueous alkali—276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1700, 1652, 1160, 1285, 1295, 1530 cm^{-1} (KBr disk).



Quantification
Plasma **HPLC** Gliquidone and other sulfonylureas. Limit of detection, 10 to 40 µg/L [Sener *et al.* 1995]. UV detection. Limit of detection, 30 µg/L [Guo *et al.* 1992].

Radioimmunoassay Limit of detection, 1 µg/L [Kopitar, Kompa 1975].

Disposition in the Body Readily absorbed after oral administration. Extensively metabolised by hydroxylation and demethylation to inactive metabolites. Less than 5% of a dose is excreted in the urine and about 95% is eliminated in the faeces, via the bile.

Therapeutic Concentration
After a single oral dose of 15 mg to 10 subjects, peak plasma concentrations of about 0.7 mg/L, and peak blood concentrations of about 0.37 mg/L were reported [Kopitar 1975].

In 20 subjects, a single 30-mg oral dose produced peak plasma concentrations of 0.12 to 2.14 mg/L (mean 0.65) at 1.25 to 4.75 h (2.25) [von Nicolai *et al.* 1997].

Half-life Plasma half-life, about 1.5 h.

Distribution in Blood Plasma:whole blood ratio, about 1.9.

Protein Binding About 99%.

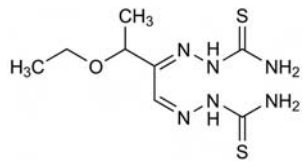
Dose 15 to 180 mg daily.

Guo P *et al.* (1992). [Direct injection of plasma to determine gliquidone in plasma using HPLC column switching technique]. *Yao Xue Xue Bao* 27: 452–455.
Kopitar Z (1975). [Human pharmacokinetics and metabolism of 14C-labeled gliquidone (AR-DF 26)]. *Arzneimittelforschung* 25: 1455–1460.
Kopitar Z, Kompa HE (1975). [Radioimmuno assay of the sulfonylurea AR-DF 26 (author's transl)]. *Arzneimittelforschung* 25: 1469–1472.
Sener A *et al.* (1995). Standardized procedure for the assay and identification of hypoglycemic sulfonylureas in human plasma. *Acta Diabetol* 32: 64–68.
von Nicholai H *et al.* (1997). Duration of action and pharmacokinetics of the oral antidiabetic drug gliquidone in patients with non-insulin-dependent (type 2) diabetes mellitus. *Arzneimittelforschung* 47: 247–252.

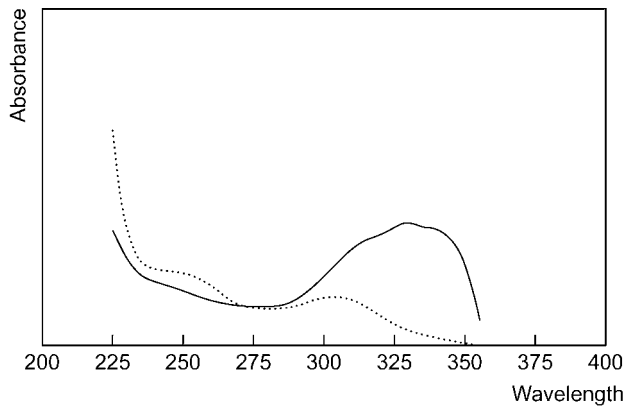
Gloxazone

Anaplasmodastat (Veterinary)
$C_8H_{16}N_6OS_2$ = 276.4
CAS—2507-91-7

IUPAC Name [(*E*)-[(1*E*)-1-(Carbamothioylhydrazinylidene)-3-ethoxybutan-2-ylidene] amino]thiourea
Synonym 2,2′-[1-(1-Ethoxyethyl)-1,2-ethanediylidene] bishydrazinecarbothioamide



Chemical Properties A yellow powder. Practically insoluble in water and ether; slightly soluble in chloroform.
Colour Test Palladium chloride—red.
Thin-layer Chromatography System TA—R_f 0.77 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—329 nm; aqueous alkali—303 nm.

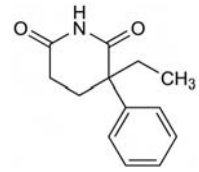


Infrared Spectrum Principal peaks at wavenumbers 1587, 1079, 1228, 1259, 1575, 1022 cm^{-1} (KBr disk).

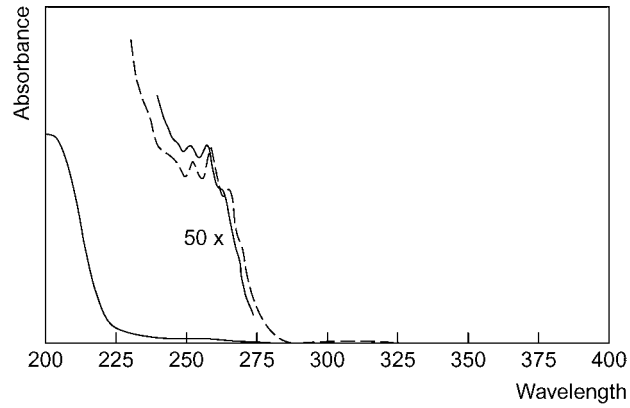
Glutethimide

Hypnotic
$C_{13}H_{15}NO_2$ = 217.3
CAS—77-21-4

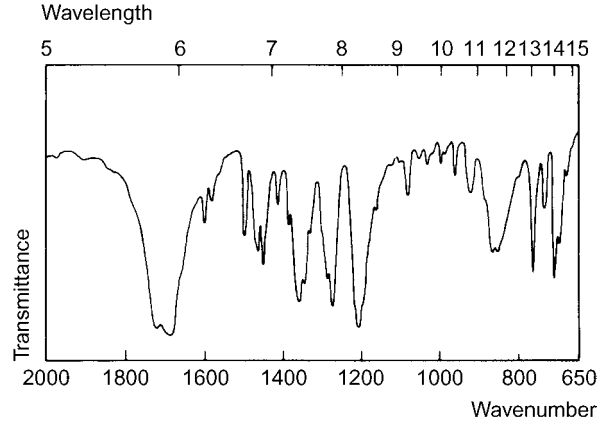
IUPAC Name 3-Ethyl-3-phenyl-2,6-piperidinedione
Synonyms Glutethimidum; glutetimide.
Proprietary Name *Doriden(e)*



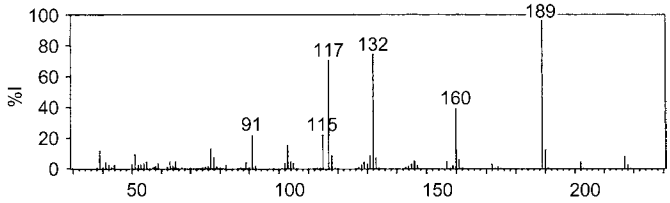
Chemical Properties Colourless crystals or white crystalline powder. Mp 85° to 89°. Practically insoluble in water; soluble 1 in 5 of ethanol, 1 in less than 1 of chloroform and 1 in 12 of ether; freely soluble in acetone. pK_a 9.2. Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.31; system TD—R_f 0.63; system TE—R_f 0.80; system TF—R_f 0.62; system TAD—R_f 0.70; system TAE—R_f 0.86; system TAF—R_f 0.89 (Dragendorff spray, weak reaction; acidified iodoplatinate solution, positive; mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black).
Gas Chromatography System GA—glutethimide RI 1830, M (OH-ethyl-) RI 1865, M (OH-phenyl-) RI 1875, M (OH-ethyl-)-AC RI 2060, M (OH-phenyl-)-AC RI 2250; system GB—glutethimide RI 1910, M (OH-ethyl-) RI 1958, M (OH-phenyl-) RI 2040; system GF—RI 2315.
High Performance Liquid Chromatography System HE—*k* 7.97; system HX—RI 436; system HY—RI 401; system HZ—retention time 4.8 min; system HAX—retention time 6.6 min; system HAY—retention time 6.2 min.
Ultraviolet Spectrum Ethanol—252, 258 ($A^{1\%}_{1cm}$=18a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1686, 1710, 1200, 1270, 1281, 704 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 189, 132, 117, 160, 91, 115, 103, 77; 4-hydroxyglutethimide 146, 233, 103, 133, 91, 117, 115, 77; 2-phenylglutarimide 104, 189, 103, 117, 78, 91, 51, 146.



Quantification

Plasma **GC** FID. Glutethimide and 4-hydroxyglutethimide. Limit of detection, 200 µg/L for glutethimide and 500 µg/L for 4-hydroxyglutethimide [Hansen, Fischer 1974].

GC-MS Glutethimide and six metabolites. Limit of detection, 50 µg/L [Kennedy *et al.* 1978].

Serum **GC** FID. Limit of detection, 250 µg/L [Kadar, Kalow 1972].

Urine **GC** See Plasma [Hansen, Fischer 1974].

GC-MS See Plasma [Kennedy *et al.* 1978].

Tissues **GC** See Plasma [Hansen, Fischer 1974].

Disposition in the Body Irregularly absorbed from the gastrointestinal tract; absorption is enhanced if alcohol is taken concomitantly. Widely distributed in body tissues and fat. Glutethimide is a racemate; the (+)-isomer is metabolised in the liver to 4-hydroxyglutethimide and the (−)-isomer to 2-(1-hydroxyethyl)-2-phenylglutarimide. 4-Hydroxyglutethimide is twice as active as glutethimide in animals but does not appear to contribute to the effects of single therapeutic doses in humans; however it tends to accumulate in the plasma during intoxication and may contribute to the central nervous depression in overdose cases. Both hydroxylated metabolites are converted to glucuronides and undergo enterohepatic circulation; they are still being excreted in the urine after >48 h. 2-Ethyl-2-(4-hydroxyphenyl)glutarimide is a major metabolite during chronic administration. Other metabolites include 2-phenylglutarimide, α-phenyl-γ-butyrolactone and 2-ethyl-2-phenylglutaconimide, which are pharmacologically active; numerous other mono- and dihydroxyphenyl metabolites have been isolated from human urine. <2% of a dose is excreted unchanged in the urine in 24 h. 2-Phenylglutarimide and 2-ethyl-2-phenylglutaconimide each account for about 2 to 4% of the dose as urinary excretion products and the remainder consists largely of glucuronide conjugates of the hydroxylated metabolites. It crosses the placenta and traces are found in breast milk.

Therapeutic Concentration

Following single oral doses of 500 mg to 6 subjects, peak plasma concentrations of 2.8 to 7.1 mg/L (mean 4.3) were attained in 1 to 6 h (mean 2) [Curry *et al.* 1971].

Toxicity The estimated minimum lethal dose is 5 g, although recoveries have occurred after the ingestion of up to 10 g. Blood concentrations of 3.4 to 27 to 48 mg/L of glutethimide have been associated with toxic effects and concentrations of 25 to 48 to 90 mg/L have been associated with fatalities. Postmortem blood concentrations of about 10 mg/L may be indicative of poisoning if the survival time has been prolonged.

In a review of 11 fatalities, blood concentrations of glutethimide were reported to range from 10 to 97 mg/L (mean 45) and liver concentrations from 63 to 141 µg/g (mean 89) [Baselt, Cravey 1977].

In a suicide attempt involving the ingestion of a glutethimide preparation that had been stored for 20 years, a blood concentration of 5 mg/L and a urinary concentration of 11 mg/L were reported. [Mankowski *et al.* 2002].

Half-life Plasma half-life, biphasic with a half-life of up to 22 h for the terminal phase; in acute intoxication the plasma half-life has an average value of about 40 h but may exceed 100 h.

Volume of Distribution About 3 L/kg.

Protein Binding About 50%.

Dose Usually 250 to 500 mg has been given as a hypnotic.

Baselt RC, Cravey RH (1977). *J Anal Toxicol* 1: 81–103.

Curry S *et al.* (1971). Disposition of glutethimide in man. *Clin Pharmacol Ther* 12: 849–857.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Hansen AR, Fischer LJ (1974). Gas-chromatographic simultaneous analysis for glutethimide and an active hydroxylated metabolite in tissues, plasma, and urine. *Clin Chem* 20: 236–242.

Kadar D, Kalow W (1972). A method for measuring glutethimide (Doriden) in human serum after intake of therapeutic doses. *J Chromatogr* 72: 21–27.

Kennedy KA *et al.* (1978). A selected ion monitoring method for glutethimide and six metabolites: application to blood and urine from humans intoxicated with glutethimide. *Biomed Mass Spectrom* 5: 679–685.

Mankowski W *et al.* (2002). [Suicidal attempts with old (currently unused) drug]. *Przegl Lek* 59: 390–391.

Glyceryl Trinitrate

Antianginal Vasodilator

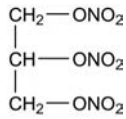
$C_3H_5N_3O_9$ = 227.1

CAS—55-63-0

IUPAC Name 1,2,3-Propanetriol trinitrate

Synonyms Glonoin; nitroglycerin; nitroglycerol; trinitrin; trinitroglycerin.

Proprietary Names *Coro-Nitro*; *Deponit*; *Glytrin*; *Minitran*; *Nitrek*; *Nitro-Bid*; *Nitrocine*; *Nitro-Derm*; *Nitrodisc*; *Nitro-Dur*; *Nitrogard*; *Nitroglyn*; *Nitrol*; *Nitrolingual*; *Nitromin*; *Nitrotime*; *Nitronal*; *Nitrong*; *NitroQuick*; *Nitrostat*; *NitroTab*; *NTS*; *Percutol*; *Suscard*; *Sustac*; *Transdermal-NTG*; *Transderm-Nitro*; *Transiderm-Nitro*; *Tridil*.



Chemical Properties A colourless, slightly volatile, oily liquid. It explodes on rapid heating or on percussion. Mass per mL about 1.6 g. Soluble 1 in 800 of water and 1 in 4 of ethanol; miscible with acetone, glacial acetic acid, ethyl acetate, benzene, pyridine, chloroform and ether; sparingly soluble in petroleum ether, liquid petrolatum and glycerol. Log *P* (octanol/water), 1.6.

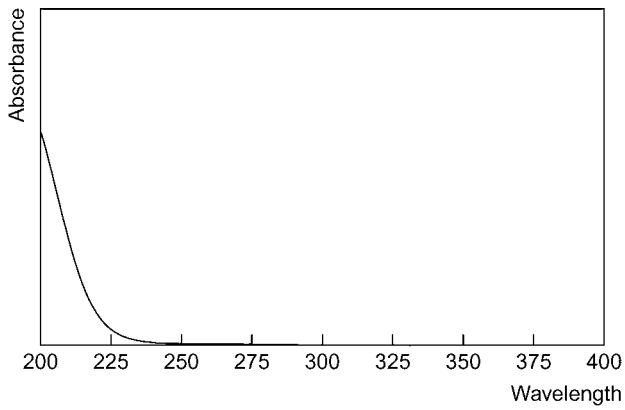
Caution In fatty or oily solution it is safe and stable but in alcoholic solution the substance must be handled with the utmost caution.

Colour Test Ferrous sulfate—red.

Thin-layer Chromatography System TD—R_f 0.71; system TE—R_f 0.86; system TF—R_f 0.72.

High Performance Liquid Chromatography System HY—RI 499.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1647, 1279, 843, 1013, 903, 754 cm^{-1}.

Quantification

Plasma **GC** ECD. Glyceryl trinitrate and its dinitrate metabolites. Limit of detection, about 0.2 nmol [Jorgensen *et al.* 1992]. Glyceryl trinitrate, isosorbide dinitrate and their metabolites. Limit of detection, for glyceryl trinitrate 0.75 nmol [Booth *et al.* 1990]. ECD. Glyceryl trinitrate and its dinitrate metabolites. Limits of detection, 0.025 µg/L for glyceryl trinitrate and 0.1 µg/L for dinitrate metabolites [Lee *et al.* 1988]. ECD. Limit of detection, 0.1 µg/L [Hennig, Benecke 1987]. ECD. 1,2- and 1,3-glyceryl dinitrates, limit of detection 250 ng/L [Sioufi *et al.* 1987]. ECD. Limit of detection, 25 ng/L [Noonan *et al.* 1984]. ECD. Glyceryl trinitrate and its mono- and di-nitrate metabolites. Limit of detection, <0.4 µg/L [Han *et al.* 1992].

HPLC Thermal energy analyser detection. Glyceryl trinitrate and its dinitrate metabolites. Limit of detection, 0.05 µg/L [Woodward *et al.* 1984]. Limit of detection, 500 ng/L [Spanggord, Keck 1980].

Serum **GC** ECD. Glyceryl trinitrate and dinitrate metabolites. Limit of detection, 50 ng/L for glyceryl trinitrate [Janssens *et al.* 1989].

Biological Samples **GC** ECD. Glycerol 1-nitrate and glycerol 2-nitrate [Scharpf *et al.* 1987].

Disposition in the Body Readily absorbed from the skin and mucous membranes; less readily absorbed after oral administration. It is rapidly metabolised in the body to dinitrates, which are active, and to inactive mononitrates. About 20% of a sublingual dose is excreted in the urine in 24 h, mainly as the mononitrate.

Therapeutic Concentration

After sublingual administration of 0.3 mg to 1 subject, a blood concentration of about 0.001 mg/L was attained in 3 min; following an oral dose of 6.5 mg or application of an ointment containing 16 mg of glyceryl trinitrate to the same subject, peak blood concentrations of about 0.0002 to 0.0003 mg/L were reported, 20 to 60 min after the dose [Blumenthal *et al.* 1977].

Following sublingual administration of 0.5 mg to 6 subjects, a mean peak plasma concentration of 0.0014 mg/L was attained in 3 min; after oral administration of 6.4 mg, a peak plasma concentration of 0.0026 mg/L was reported at 2 to 4 h, and after topical administration of 35 mg, a mean peak plasma concentration of 0.0025 mg/L was attained in 1 h. Plasma nitrate and nitrite concentrations were undetectable after sublingual administration; after oral administration peak plasma concentrations of about 0.5 mg/L of nitrate and nitrite were attained in 5 and 2 h respectively, and following topical administration, peak concentrations of about 0.7 mg/L were reported at 5 h and 1 h respectively [Bashir *et al.* 1982].

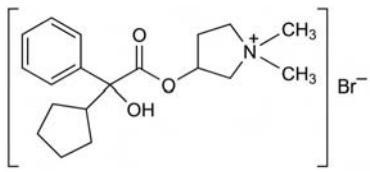
Toxicity The estimated minimum oral lethal dose is 2 g and the maximum permissible atmospheric concentration is 0.2 ppm.
Half-life Plasma half-life, after IV administration about 2 to 5 min; after sublingual administration about 5 min.
Volume of Distribution About 3 L/kg.
Note For a review of the pharmacokinetics of organic nitrates, see Bogaert [1983]. For a review of IV glyceryl trinitrate, see Sorkin *et al.* [1984].
Dose Usually the equivalent of 0.5 to 1 mg of glyceryl trinitrate sublingually, repeated as required; doses of 5.2 to 38.4 mg daily are given orally as sustained-release tablets.

Bashir A *et al.* (1982). Pharmacokinetic studies of various preparations of glyceryl trinitrate. *Br J Clin Pharmacol* 14: 779–784.
Blumenthal HP *et al.* (1977). Plasma nitroglycerin levels after sublingual, oral and topical administration. *Br J Clin Pharmacol* 4: 241–242.
Bogaert MG (1983). Clinical pharmacokinetics of organic nitrates. *Clin Pharmacokinet* 8: 410–421.
Booth BP *et al.* (1990). Assay of glyceryl trinitrate, isosorbide dinitrate, and their metabolites in plasma by large-bore capillary column gas-liquid chromatography. *Biopharm Drug Dispos* 11: 663–677.
Han C *et al.* (1992). Improved gas chromatographic assay for the simultaneous determination of nitroglycerin and its mono- and dinitrate metabolites. *J Chromatogr* 579: 237–245.
Hennig B, Benecke R (1987). [Gas chromatographic determination of nitroglycerin in human plasma]. *Pharmazie* 42: 507–510.
Janssens JJ *et al.* (1989). Quantitative determination of nitroglycerin by capillary gas chromatography-electron capture detection. *J Pharm Biomed Anal* 7: 1631–1634.
Jorgensen M *et al.* (1992). Simultaneous determination of nitroglycerin and its dinitrate metabolites by capillary gas chromatography with electron-capture detection. *J Chromatogr* 577: 167–170.
Lee FW *et al.* (1988). Simultaneous determination of nitroglycerin and its dinitrate metabolites by capillary gas chromatography with electron-capture detection. *J Chromatogr* 426: 259–266.
Noonan PK *et al.* (1984). Determination of picogram nitroglycerin plasma concentrations using capillary gas chromatography with on-column injection. *J Pharm Sci* 73: 923–927.
Scharpf F *et al.* (1987). Gas chromatographic assay of glycerol mononitrates in biological samples. *J Chromatogr* 413: 91–99.
Sioufi A *et al.* (1987). Determination of the two dinitrate metabolites of nitroglycerin in human plasma by capillary gas chromatography with electron-capture detection. *J Chromatogr* 413: 101–108.
Sorkin EM *et al.* (1984). Intravenous glyceryl trinitrate (nitroglycerin). A review of its pharmacological properties and therapeutic efficacy. *Drugs* 27: 45–80.
Spanggord RJ, Keck RG (1980). Application of high-pressure liquid chromatography and thermal energy analyzer to analysis of trinitroglycerin and its metabolites in blood. *J Pharm Sci* 69: 444–446.
Woodward AJ *et al.* (1984). Determination of nitroglycerin and its dinitrate metabolites in human plasma by high-performance liquid chromatography with thermal energy analyzer detection. *J Pharm Sci* 73: 1838–1840.

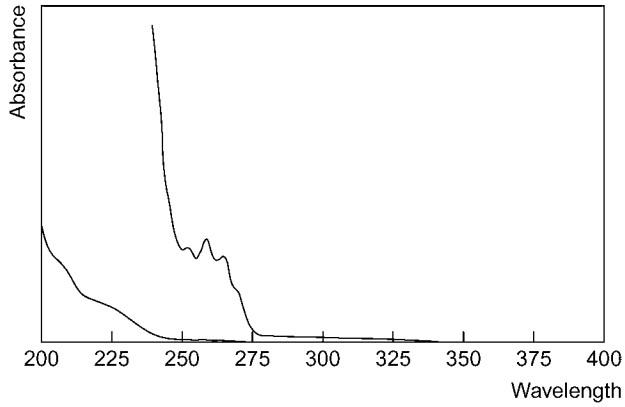
Glycopyrronium Bromide

Anticholinergic
$C_{19}H_{28}BrNO_3$ = 398.3
CAS—596-51-0

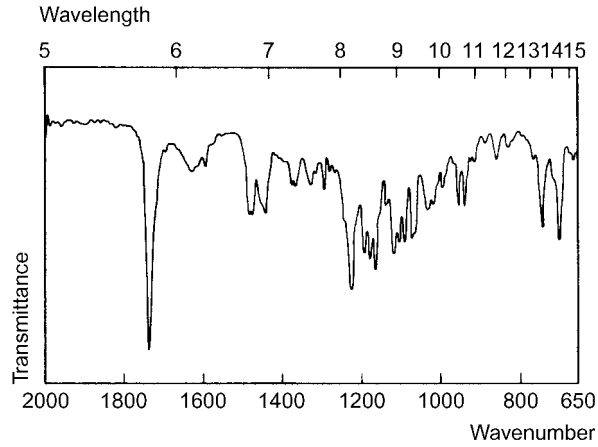
IUPAC Name 3-[(Cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethylpyrrolidinium bromide
Synonym Glycopyrrolate
Proprietary Names *Gastrodyn*; *Nodapton*; *Ronanul*; *Robinul*; *Tarodyl*; *Tarodyn*.



Chemical Properties A white crystalline powder. Mp 193° to 198°. Soluble 1 in about 5 of water and 1 in 10 of ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), −1.0.
Colour Test The following test is performed on glycopyrronium nitrate: Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.03; system TE—R_f 0.01; system TAE—R_f 0.03 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2120.
High Performance Liquid Chromatography System HA—*k* 3.2 (tailing peak).
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=7.1a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1738, 1225, 1164, 1178, 1115, 1192 cm^{-1}.



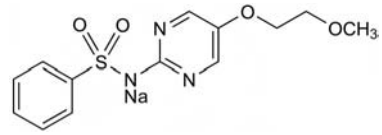
Quantification
Plasma **GC** Nitrogen-sensitive detection [Murray *et al.* 1984].
Disposition in the Body
Therapeutic Concentration
Following a single IM injection of glycopyrronium at a dose of 8 μg/kg to 9 subjects, peak plasma concentrations of 5.03 to 26.55 μg/L (mean 15.79) were achieved in 16.11 min [Ali-Melkkila *et al.* 1990].
Dose 2 to 12 mg daily.

Ali-Melkkila TM *et al.* (1990). Pharmacokinetics of i.m. glycopyrronium. *Br J Anaesth* 64: 667–669.
Murray GR *et al.* (1984). Quantitative capillary column gas chromatographic method for the determination of glycopyrronium in human plasma. *J Chromatogr* 308: 143–151.

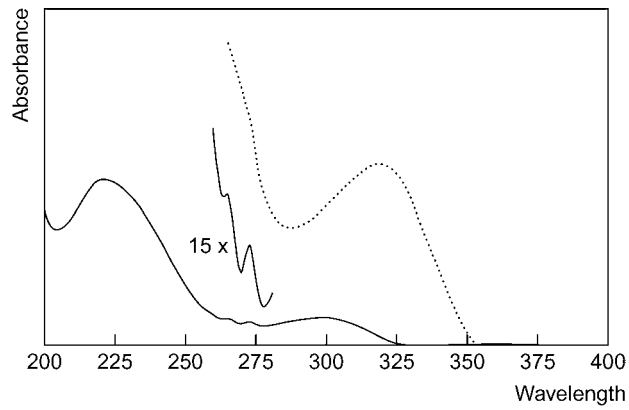
Glymidine Sodium

Antidiabetic
$C_{13}H_{14}N_3NaO_4S$ = 331.3
CAS—3459-20-9

IUPAC Name Sodium benzenesulfonyl-[5-(2-methoxyethoxy)pyrimidin-2-yl] azanide
Synonyms Glidiazine sodique; glycodiazine sodium; sodium glymidine.
Proprietary Names *Glyconormal*; *Gondafon*; *Lycanol*; *Redul*.
Note The sodium salt of *N*-[5-(2-methoxyethoxy)-2-pyrimidinyl]-benzenesulfonamide.

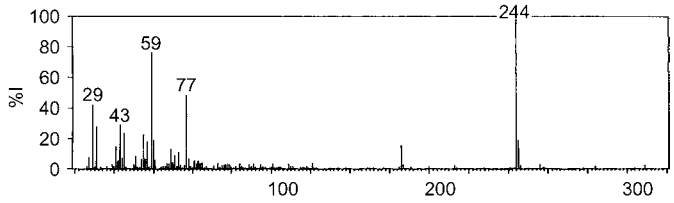


Chemical Properties A white crystalline powder. Mp 221° to 226°. Soluble in water; sparingly soluble in ethanol.
Colour Tests Koppanyi–Zwikker test—pink-violet; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.00; system TC—R_f 0.65; system TE—R_f 0.05.
Gas Chromatography System GA—RI 1632; system GB—RI 2750; system GJ—methyl derivative RRT 0.53 (relative to griseofulvin).
Ultraviolet Spectrum Aqueous acid—273, 299 nm; aqueous alkali—243, 317 nm.



Infrared Spectrum Principal peaks at wavenumbers 1130, 1092, 1267, 1247, 691, 1220 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 244, 59, 77, 29, 43, 31, 45, 55.



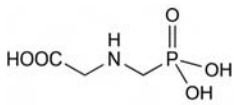
Quantification
Blood **GC** ECD. Limit of detection, 1.5 mg/L [Schlicht *et al.* 1978].
Plasma **Spectrofluorimetry** Limit of detection, 1 mg/L [Held *et al.* 1970].
TLC Glymidine sodium and metabolites. Limit of detection, 2.5 mg/L [Soyfer *et al.* 1969].
Urine **Spectrofluorimetry** Desmethyl and carboxy metabolites, see Plasma [Held *et al.* 1970].
Disposition in the Body Almost completely absorbed after oral administration. Metabolised by demethylation to an active metabolite and oxidation to an inactive carboxy derivative. About 85 to 95% of a dose is excreted in the urine in 48 h, with <1% of the urinary material as unchanged drug, 20 to 40% as the desmethyl metabolite and 60 to 80% as the carboxy metabolite; about 6% of a dose is eliminated in the faeces.
Half-life Plasma half-life, about 2 to 6 h, increased in subjects with hepatic disease.
Protein Binding About 80%.
Dose 0.5 to 2 g daily.

Held H *et al.* (1970). [Spectrofluorometric method for determination of glycodiazine in plasma and of the both glycodiazine metabolites in urine]. *Arzneimittelforschung* 20: 1927–1929.
Schlicht HJ *et al.* (1978). Gas chromatographic procedure for the simultaneous determination of five common antidiabetic drugs in blood. *J Chromatogr* 155: 178–181.
Soyfer JC *et al.* (1969). *Chim Ther* 4: 131–135.

Glyphosate

Herbicide
$C_3H_8NO_5P$ = 169.1
CAS—1071-83-6

IUPAC Name 2-(Phosphonomethylamino)acetic acid
Synonym *N*-(Phosphoromethyl)glycine
Proprietary Names *Acrion*; *Agriguard*; *Bronco*; *Gallup*; *Gliakla*; *Landmaster*; *MON 0573*; *MON 2139*; *MON 6000*; *Pondmaster*; *Ranger*; *Rattler 4AS*; *Sonic*; *Spasor*; *Sting*; *Tumbleweed*.



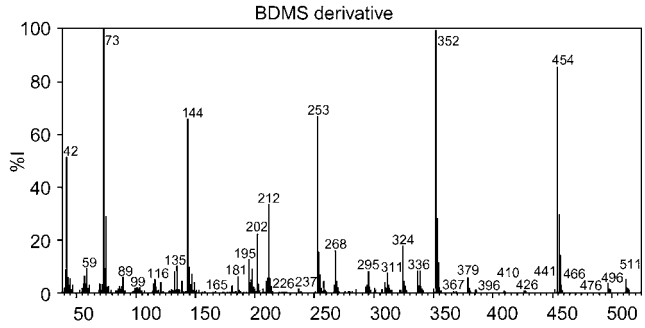
Chemical Properties A white solid/ colourless crystals (pure state) or light amber to brown liquid (technical state). Mp 230°. Soluble in water (12 g/L at 25°); insoluble in most common organic solvents. pK_a 0.8 (also reported as 5.6). Log *P* (octanol/water), −4.00.

Glyphosate Isopropylammonium
$C_6H_{17}N_2O_5P$ = 228.2
CAS—38641-94-0
Synonym MON-2139
Proprietary Names *Drat*; *Glifonox*; *Glycel*; *Liphadione*; *Quick*; *Revoke*; *Rodeo*; *Rondo*; *Roundup*.
Chemical Properties Very soluble in water.

Glyphosate Sesquisodium Salt
$C_6H_{13}N_2Na_3O_{10}P_2$ = 404.1
CAS—70393-85-0
Synonym Sodium glyphosate
Proprietary Names *MON-8000*; *Polado*.

Glyphosate Trimesium
$C_{12}H_{32}NO_5PS_3$ = 245.2
CAS—81591-81-3
Synonyms SC-0224; sulfosate.
Proprietary Name *Touchdown*

Thin-layer Chromatography System TAB—R_f 0.00(glyphosate acid); system TAC—R_f 0.00(glyphosate acid).
Gas Chromatography System GA—glyphosate-(Me_3) RI 1410, glyphosate-(Me_4) RI 1390.
Ultraviolet Spectrum Acetonitrile—257 nm.
Mass Spectrum Principal ions at *m/z* (*tert*-butyldimethylsilyl (BDMS) derivative) 73, 352, 454, 253, 144, 42, 212, 455.



Quantification
Serum **HPLC** Limit of detection, 0.3 mg/L for glyphosate, 0.2 mg/L for the metabolite AMPA. [Tomita *et al.* 1991].
Biological Specimens **GC** Limit of detection, 100 mg/L [Tsunoda 1993].
GC-MS Limit of detection, 10 mg/L [Tsunoda 1993].
Disposition in the Body Glyphosate is poorly absorbed through the skin and from the digestive tract after ingestion. It is mostly excreted unchanged but one metabolite identified is (aminomethyl)phosphonic acid (AMPA). There is no significant evidence that glyphosate accumulates in tissue.
Toxicity Glyphosate is practically non-toxic by ingestion. The allowed daily intake is 0.3 mg/kg body weight. Minimum lethal dose is 60 mL (84-year-old man). Individuals over 40 years old who ingest over 150 mL of glyphosate are at a higher risk of death than those who are younger.

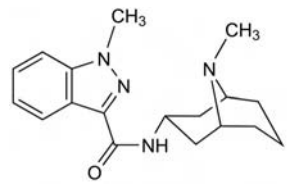
A 58-year-old man was found collapsed by his wife at home but was pronounced dead by the emergency services on arrival. Postmortem analysis discovered a dark greenish-brown fluid material in his stomach. Toxicological analysis determined that there were glyphosate concentrations of 0.30 g/L, 1.03 g/L, and 92.6 g/L in his blood, urine, and stomach contents, respectively. It was concluded that the man had consumed the Touchdown found by the side of his body [Sato *et al.* 1997].

Sato Y *et al.* (1997). An autopsy case of glyphosate herbicide: Touchdown poisoning and detection of glyphosate using HPLC. Poster abstract at *XXXV TIAFT Annual Meeting*, abstract 145.
Tomita M *et al.* (1991). High-performance liquid chromatographic determination of glyphosate and (aminomethyl)phosphonic acid in human serum after conversion into p-toluenesulphonyl derivatives. *J Chromatogr* 566(1): 239–243.
Tsunoda N (1993). Simultaneous determination of the herbicides glyphosate, glufosinate and bialaphos and their metabolites by capillary gas chromatography-ion-trap mass spectrometry. *J Chromatogr* 637(2): 167–173.

Granisetron

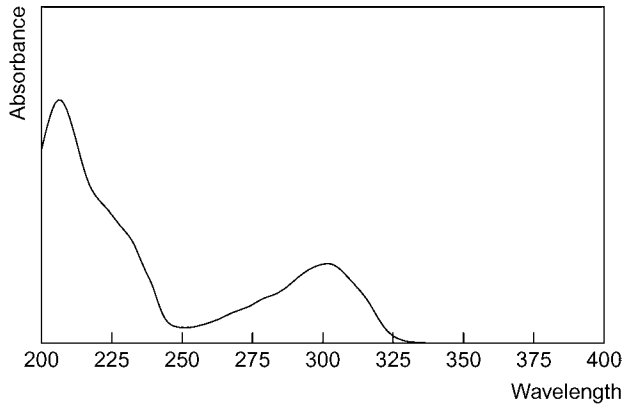
Antiemetic
$C_{18}H_{24}N_4O$ = 312.4
CAS—109889-09-0

IUPAC Name 1-Methyl-*N*-[(3-*endo*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide
Synonym BRL-43694

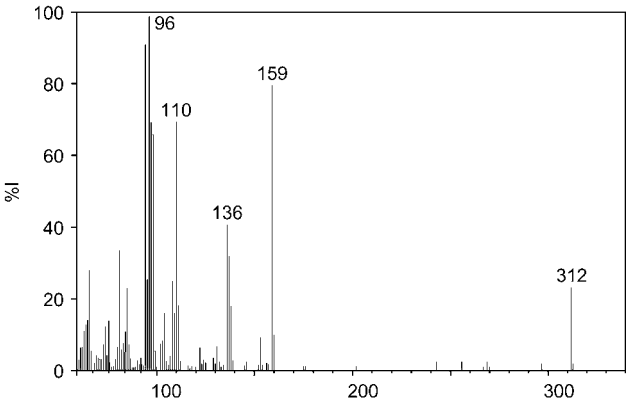


Granisetron Hydrochloride
$C_{18}H_{24}N_4O.HCl$ = 348.9
CAS—107007-99-8
Synonym BRL-43694A
Proprietary Name *Kytril*
Chemical Properties A white to off-white solid. Mp 290° to 292°. Readily soluble in water and normal saline at 20°.

Thin-layer Chromatography System TB—R_f 0.18; system TE—R_f 0.51; system TAE—R_f 0.14.
High Performance Liquid Chromatography System HX—RI 359.
Column: Apex CN (250 × 4.5 mm i.d., 10 µm). Mobile phase: 0.05 mol/L sodium acetate buffer (3%) (pH 6.0). Internal standard (IS): BRL-43704. Fluorescence detection (λ_{ex}=305 nm, λ_{em}=360 nm). Retention time: granisetron, 13.5 min; IS, 19.2 min [Clarkson *et al.* 1987].
Ultraviolet Spectrum Principal peaks at 207, 303 nm.



Mass Spectrum Principal ions at *m/z* 96, 94, 159, 110, 97, 98, 136, 137.



Quantification

Plasma **HPLC** Fluorescence detection. Limit of quantification 0.1 µg/L for granisetron and 0.25 µg/L for 7-hydroxygranisteron [Boppana 1995]. UV detection (λ=305 nm). Limit of detection, 0.019 µg/L [Capacio *et al.* 1993].

Disposition in the Body Granisetron is rapidly and completely absorbed after oral administration and is metabolised in the liver by 7-hydroxylation, *N*-demethylation, aromatic ring oxidation and conjugation. Its active metabolite is 7-hydroxygranisetron. <20% of a dose is recovered in urine unchanged and the rest is excreted in urine and faeces as its metabolites.

Therapeutic Concentration

In clinical trials, 27 cancer patients and 39 healthy volunteers were administered a single dose of 1.0 mg. Mean peak plasma concentrations of 3.63 µg/L (range of 0.27 to 9.14 µg/L) were reached. Receiving 1.0 mg doses daily for 7 days, peak concentrations were 5.99 µg/L (range 0.63 to 30.9 µg/L). Peak plasma concentrations can increase up to 30% in fasted states [SmithKline Beecham].

Toxicity During clinical trials, one patient received over 10 times the recommended dose (total of 38.5 mg) and complained of a headache only. Acute overdose: somnolence and myalgia.

Bioavailability Oral, 60%.

Half-life In healthy volunteers: 3 to 4 h; in patients with cancer: 9 to 12 h.

Volume of Distribution 2 to 3 L/kg, also reported as 200 L.

Clearance 0.2 to 0.5 L/h/kg.

Distribution in Blood Distributed freely between plasma and red blood cells.

Protein Binding 65% bound.

Dose Adults: intravenously: a single 3 mg dose of granisetron is administered, repeated if necessary with a maximum daily dose of 9 mg. Oral dose of 2 mg daily can be administered. Children: the usual dose is 10 to 40 µg/kg body weight intravenously with a maximum of 3 mg daily.

Boppana VK (1995). Simultaneous determination of granisetron and its 7-hydroxy metabolite in human plasma by reversed-phase high-performance liquid chromatography utilizing fluorescence and electrochemical detection. *J Chromatogr A* 692(1-2): 195–202.

Capacio BR *et al.* (1993). An HPLC method for the determination of granisetron in guinea pig plasma. *J Anal Toxicol* 17(3): 151–155.

Clarkson A *et al.* (1987). *Br J Pharmacol* 90136P.

SmithKline Beecham, data on file

Griseofulvin

Antifungal

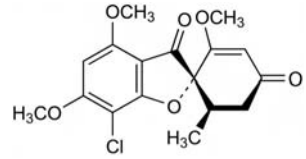
$C_{17}H_{17}ClO_6 = 352.8$

CAS—126-07-8

IUPAC Name (1′*S*,6′*R*)-7-Chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]-cyclohexene]-3,4′-dione

Synonym Curling factor

Proprietary Names *Erlivin*; *Fulcin*; *Fulvicin*; *Fulvina*; *Grifulvin V*; *Grisactin*; *Grisaltin*; *Grisefuline*; *Griseo*; *Griseomed*; *Griseostatin*; *Grisovin*; *Gris-PEG*; *Grivin*; *Krisovin*; *Lamoryl*; *Likuden M*; *Medofulvin*; *Microcidal*; *Neo-Fulcin*; *neofulvin*; *Polygris*; *Polcyl-FP*; *Spirofulvin*; *Sporostatin*; *Trivanex*.



Chemical Properties A white to pale cream-coloured powder. Mp 217° to 224°. Practically insoluble in water and petroleum ether; soluble 1 in 300 of dehydrated alcohol, 1 in 20 of acetone and 1 in 25 of chloroform; soluble in dimethylformamide; slightly soluble in benzene, ethyl acetate and acetic acid. Log *P* (octanol/water), 2.2.

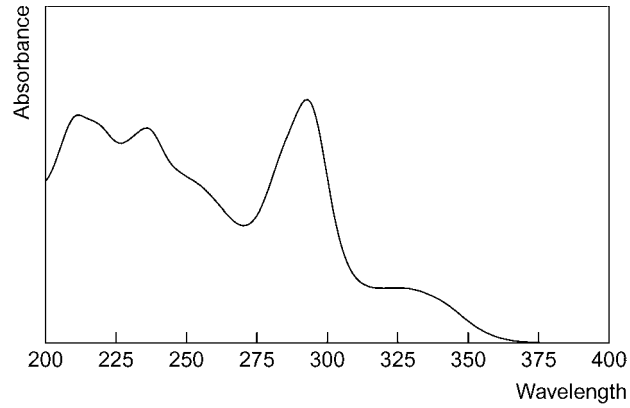
Colour Test Dissolve 5 mg in 1 mL of sulfuric acid and add 5 mg of powdered potassium dichromate—red.

Thin-layer Chromatography System TD—R_f 0.52; system TE—R_f 0.69; system TF—R_f 0.37; system TAD—R_f 0.68; system TAE—R_f 0.78; system TAJ—R_f 0.68; system TAK—R_f 0.48; system TAL—R_f 0.96.

Gas Chromatography System GA—RI 2700.

High Performance Liquid Chromatography System HY—RI 488; system HAA—retention time 18.4 min.

Ultraviolet Spectrum Dehydrated alcohol—236, 291 nm (A_1^1=686a).



Infrared Spectrum Principal peaks at wavenumbers 1220, 1611, 1210, 1583, 1135, 800 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 138, 352, 215, 310, 214, 69, 321, 354.

Dose 0.5 to 1 g daily.

Guaifenesin

Expectorant

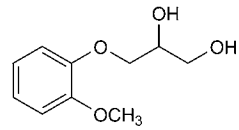
$C_{10}H_{14}O_4 = 198.2$

CAS—93-14-1

IUPAC Name 3-(2-Methoxyphenoxy)-propane-1.2-diol

Synonyms Glyceryl guaiacolate; glycerylguayacolum; guaiacol glycerol ether; guaiacyl glyceryl ether; guaiphenesin; guajacolum glycerolatum; 3-(2-methoxyphenoxy)-1,2-propanediol.

Proprietary Names *Anti-Tuss*; *Benylin Children's Chesty Coughs*; *Breonesin*; *Duratuss G*; *Expulin Chesty Cough*; *Famel Expectorant*; *Fenesin*; *Genatuss*; *Glyate*; *Glycotuss*; *Glytuss*; *Hill's Balsam Chesty Cough*; *Hytuss*; *Jackson's All Fours*; *Jacksons Bronchial Balsam*; *Owbridges for Chesty Coughs*; *Robitussin for Chesty Coughs*; *Tixylix Chesty Cough*; *Vicks Vaposyrup for Chesty Coughs*. It is an ingredient of many proprietary preparations [Sweetman 2007].



Chemical Properties White or slightly-grey crystals or crystalline aggregates. Mp 78° to 82°. Soluble 1 in 33 of water, 1 in 11 of ethanol, 1 in 11 of chloroform, and 1 in 100 of ether; soluble in glycerol and propylene glycol; moderately soluble in benzene; practically insoluble in petroleum ether. Log *P* octanol/water), 1.4.Samples in plasma stored at −70° were stable for up to a year. Stable for 3 freeze-thaw cycles and for 6 h on the benchtop [Eichhold *et al.* 2007]. Samples were stable following

freeze-thaw cycles, after bench top for 2 h, after reinjection and after −20° for 30 days [Chen *et al.* 2005]. Plasma samples are stable at −20° [Jancic *et al.* 2005].

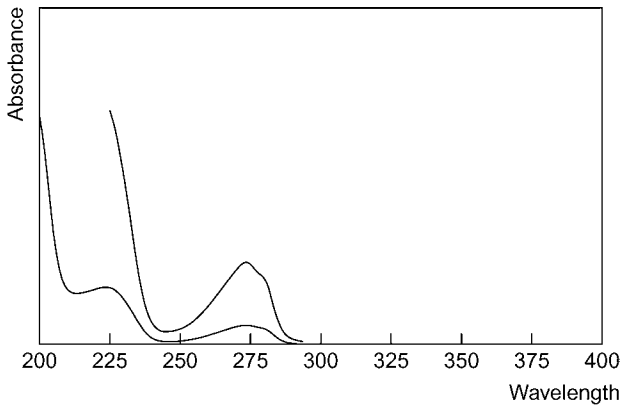
Colour Tests Liebermann's reagent—black; Mandelin's test—grey-green; Marquis test—violet.

Thin-layer Chromatography System TAD—R_f 0.40; system TAE—R_f 0.81; system TB—R_f 0.02; system TD—R_f 0.11; system TE—R_f 0.39; system TF—R_f 0.17.

Gas Chromatography System GA—guaifenesin RI 1650, (M *O*-desmethyl-) RI 1700, guaifenesin-AC_2 RI 1865, M (OH-methoxy-)-AC_2 RI 2290.

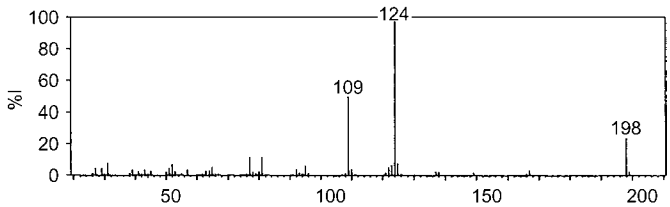
High Performance Liquid Chromatography System HAA—RT 11.4 min; system HX—RI 328; system HY—RI 262.

Ultraviolet Spectrum Aqueous acid—273 nm ($A^1_1 = 125a$). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1255, 1510, 740, 1230, 1125, 1020 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 124, 109, 198, 81, 77, 125, 52, 31.



Quantification

Blood **GC** Column: 3% XE-60 on dichromatous earth (1.21 m). Carrier gas: N_2, 40 mL/min. Temperature: 144°. ECD. Retention time: 6 min 10 s. Limit of detection not reported [Maynard, Bruce 1970].

Plasma **GC** Column: 3% OV-17 on Gas-Chron Q 100/120 mesh (1.5 m × 0.2 cm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 240°. ECD. Limit of detection, 15 µg/L [Singhawangcha *et al.* 1980].

HPLC Column: µBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: methanol : acetonitrile: 0.95 Mol/L potassium hydrogen phosphate buffer (11 : 11 : 78), containing 4 mmol/L heptane sulfonic acid and 1% glacial acetic acid, flow rate 1.0 mL/min. UV detection (λ = 272 nm). Retention time: 7.9 min. Limit of detection, 25 µg/L [Aluri, Stavchansky 1993].

LC-MS Column: Xterra MSC_{18} (30 × 2.1 mm i.d.). Mobile phase: 0.1% formic acid : 0.1% formic acid in methanol (80 : 20 for 2.25 min to 30 : 70 in 0.75 mn for 0.25 min), flow rate 0.35 mL/min. TIS, positive ion mode. Limit of quantification, 1.0 µg/L [Eichhold *et al.* 2007]. Column: Zorbax SB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (80 : 20 : 0.5), flow rate 0.6 mL/min. APCI, positive ion mode, SRM acquisition mode. Limit of quantification, 10 µg/L [Chen *et al.* 2005]. Column: Bakerbond ENV (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 g diisopropyl ether, 20 g of SDS, 60 g of *n*-propanol in 910 mL of 25 mmol/L disodium hydrogen phosphate (pH 2.8), flow rate 1.0 mL/min. Retention time: 21.0 min. Limit of detection, 0.4 mg/L [Jancic *et al.* 2005]. Column: XTerrra MS C_{18} (30 × 2.1 mm i.d., 3.5 µm). Mobile phase: 0.1% formic acid : 0.1% formic acid in methanol (80 : 20 to 30 : 70 for 0.25 min at 1.26 min 80 : 20), flow rate 0.35 mL/min. TIS. SRM acquisition mode. Retention time: 2.0 min. Limit of quantification, 5.0 µg/L [Kuhlenbeck *et al.* 2005]. Column: XTerra MS C_{18} (50 × 3.0 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water (50 : 50), flow rate 0.25 mL/min. CID, ESI. Limit of detection, 0.05 µg/L [Sheen, Her 2004].

Serum **GC-MS** Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 280° at 10°/min for 20 min. EI ionisation at 70 eV. Retention time: 16.3 min. Limit of detection, 0.025 mg/L, limit of detection, <0.025 mg/L [Maresova *et al.* 2008].

Urine **GC-MS** Column: HP cross-linked methylsilicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, scan mode. RI: 1865. Limit of detection, 10–20 µg/L [Maurer 1990].

CE Column: fused silica capillary (50.2 cm × 50 µm i.d. effective length, 10 cm). Electrolyte: 35 mmol/L phosphate buffer: acetonitrile (75 : 25) containing 120 mmol/L sodium dodecyl sulfate. UV detection (λ = 200 nm). Limit of detection, 3.0 µmol/L [Lin *et al.* 2008].

Disposition in the Body Readily absorbed after oral administration. It is rapidly metabolised by oxidation to β-(2-methoxyphenoxy)lactic acid; about 40% of a dose is excreted as this metabolite in the urine in 3 h.

Therapeutic Concentration

Twenty extensive metabolisers and 8 poor metabolisers were administered 20 mg dextromethorphan hydrobromide and 200 mg guaifenesin. Mean peak plasma concentration was 1200 µg/L in the extensive metaboliser compared with 1000 µg/L in the poor metabolisers, at 1 h in both groups [Eichhold *et al.* 2007].

After a single oral dose of 600 mg given to 3 subjects, a mean peak blood concentration of ≈1.4 mg/L was attained in 15 min [Maynard, Jr., Bruce 1970].

Toxicity

In a fatality due to ingestion of cough syrup containing guaifenesin and hydrocodone, postmortem blood concentrations of 14 and 3 mg/L, were reported respectively [Baselt 2008].

In a suicide involving the ingestion of guaifenesin, diphenhydramine, and chlorphenamine by a 48-year-old female, the following postmortem tissue concentrations (mg/L) were reported, respectively:

Heart blood 27.4, 8.8, and 0.2
Urine 21, 29, and 3.6
Bile 222, 89, and 3
Gastric contents 7700, 635, and 56
Vitreous humour 7, 1, and 0.1
CSF 13, 2.7, and 0.32
[Wogoman *et al.* 1999].

Half-life Blood half-life, ≈1 h.

Volume of Distribution ≈1 L/kg.

Dose 100 to 200 mg every 2 to 4 h.

Aluri JB, Stavchansky S (1993). Determination of guaifenesin in human plasma by liquid chromatography in the presence of pseudoephedrine. *J Pharm Biomed Anal* 11: 803–808.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, California: Biomedical Publications.

Chen X *et al.* (2005). Sensitive liquid chromatography-tandem mass spectrometry method for the simultaneous determination of paracetamol and guaifenesin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 817: 263–269.

Eichhold TH *et al.* (2007). Simultaneous determination of dextromethorphan, dextrorphan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 43: 586–600.

Jancic B *et al.* (2005). Development of liquid chromatographic method for fosinoprilat determination in human plasma using microemulsion as eluent. *J Chromatogr A* 1088: 187–192.

Kuhlenbeck DL *et al.* (2005). On-line solid phase extraction using the Prospekt-2 coupled with a liquid chromatography/tandem mass spectrometer for the determination of dextromethorphan, dextrorphan and guaifenesin in human plasma. *Eur J Mass Spectrom (Chichester, Eng)* 11: 199–208.

Lin YT *et al.* (2008). A simple micellar electrokinetic capillary chromatographic method for the quantitative analysis of organic expectorants. *Electrophoresis* 29: 3524–3530.

Maresova V *et al.* (2008). Screening and semiquantitative analysis of drugs and drugs of abuse in human serum samples using gas chromatography-mass spectrometry. *Neuro Endocrinol Lett* 29: 749–754.

Maurer HH (1990). Identification and differentiation of barbiturates, other sedative-hypnotics and their metabolites in urine integrated in a general screening procedure using computerized gas chromatography-mass spectrometry. *J Chromatogr* 530: 307–326.

Maynard WR *Jr.* Bruce RB (1970). GLC determination of guaiacol glyceryl ether in blood. *J Pharm Sci* 59: 1346–1348.

Sheen JF, Her GR (2004). Analysis of neutral drugs in human plasma by fluoride attachment in liquid chromatography/negative ion electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 18: 1911–1918.

Singhawangcha S *et al.* (1980). The determination of bifunctional compounds. IX. A selective reaction for the determination of guaifenesin in plasma by gas chromatography. *J Chromatogr* 183: 433–439.

Sweetman S, ed. (2007). *Martindale, The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Wogoman H *et al.* (1999). Acute intoxication with guaifenesin, diphenhydramine, and chlorpheniramine. *Am J Forensic Med Pathol* 20: 199–202.

Guanethidine

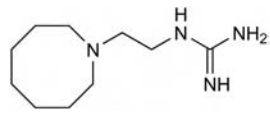
Antihypertensive

$C_{10}H_{22}N_4 = 198.3$

CAS—55-65-2

IUPAC Name 2-[2-(Azocan-1-yl)ethyl]guanidine

Synonym [2-(Hexahydro-1(2*H*)-azocinyl)ethyl]guanidine

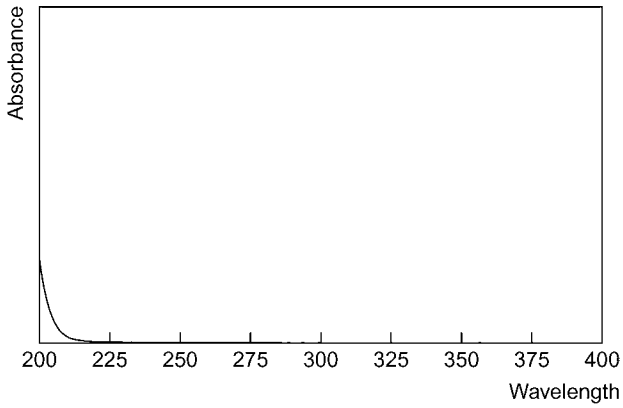


Chemical Properties pK_a 8.3, 11.4 (20°). Log *P* (octanol/water), 0.9.

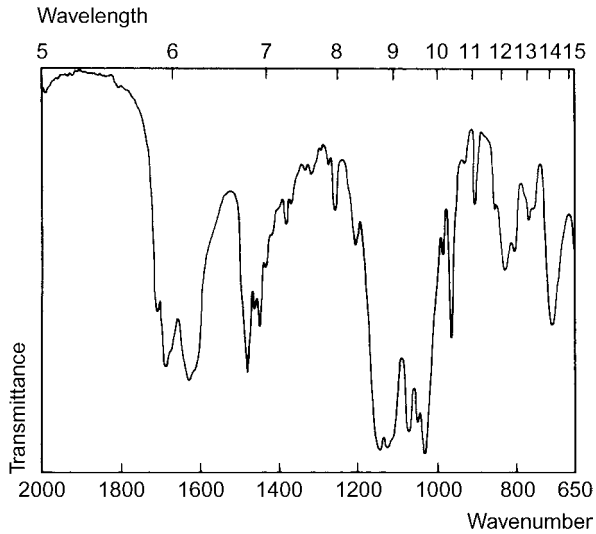
Guanethidine Monosulfate

$C_{10}H_{22}N_4,H_2SO_4 = 296.4$
CAS—645-43-2
Synonym Guanethidine sulfate
Proprietary Names *Ismelin(e)*; *Sanotensin*; *Vitutensil*. It is an ingredient of *Esimil*, *Ganda* and *Suprexon*.
Chemical Properties A colourless crystalline powder. Mp about 250°, with decomposition. Soluble 1 in 1.5 of water; very slightly soluble in alcohol; practically insoluble in chloroform and ether.

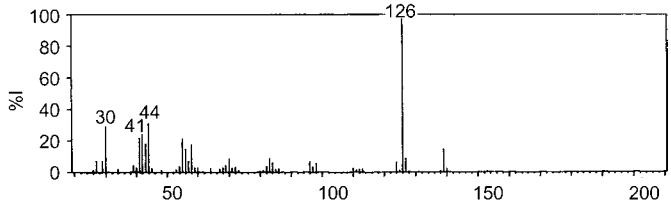
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.02; system TE—R_f 0.01; system TL—R_f 0.00; system TN—R_f 0.56; system TO—R_f 0.50; system TAE—R_f 0.03; system TAF—R_f 0.30; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.16 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1030, 1143, 1127, 1070, 1050, 1630 cm^{-1} (guanethidine sulfate, KBr disk).



Mass Spectrum Principal ions at *m/z* 126, 44, 30, 42, 41, 55, 43, 58.



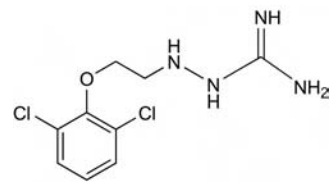
Quantification
Plasma **GC-MS** Guanethidine and other guanido-containing drugs. Limit of detection, 100 µg/L using FID and 1 µg/L using MID [Hengstmann *et al.* 1974].
Radioimmunoassay Limit of detection, 4 µg/L [Loeffler, Pittman 1979].
Urine **GC-MS** See Plasma [Hengstmann *et al.* 1974].
Disposition in the Body Incompletely absorbed after oral administration. Up to about 25% of an oral dose is excreted in the urine in 24 h as unchanged drug and two metabolites, guanethidine *N*-oxide and 2-(6-carboxyhexylamino)ethylguanidine; the proportion of metabolites to unchanged drug is variable. A total of about 40% of an oral dose is excreted in the urine in 9 days and about 40 to 47% is eliminated in the faeces as unchanged drug in 6 days.
Therapeutic Concentration In plasma, usually in the range 0.008 to 0.017 mg/L. After a single oral ^{3}H-labelled dose of 50 mg to 1 subject, a peak plasma-guanethidine concentration of 0.009 mg/L was attained in 3 h and was almost constant over the period 1 to 12 h, declining slowly to about 0.003 mg/L at 24 h; a peak total metabolite concentration of about 0.04 mg/L was reported at 2 to 4 h [McMartin *et al.* 1970].

Following daily oral doses of 25 mg to 8 subjects, steady-state plasma-guanethidine concentrations of 0.003 to 0.016 mg/L (mean 0.009) were reported; after oral doses of 37.5 mg daily to 8 subjects, the steady-state plasma-guanethidine concentrations ranged from 0.008 to 0.015 mg/L (mean 0.011) [Walter *et al.* 1975].
Bioavailability About 20%.
Half-life Plasma half-life, about 4 to 8 days.
Protein Binding Not significantly bound.
Note For a review of guanethidine disposition, see Lukas [1973].
Dose Usually 10 to 100 mg of guanethidine monosulfate daily; up to 300 mg daily may be given.

Hengstmann JH *et al.* (1974). Quantitative determination of guanethidine and other guanido-containing drugs in biological fluids by gas chromatography with flame ionization detection and multiple ion detection. *Anal Chem* 46: 34–39.
Loeffler LJ, Pittman AW (1979). Development of radioimmunoassay for guanethidine. *J Pharm Sci* 68: 1419–1423.
Lukas G (1973). Metabolism and biochemical pharmacology of guanethidine and related compounds. *Drug Metab Rev* 2: 101–116.
McMartin C *et al.* (1970). The fate of guanethidine in two hypertensive patients. *Clin Pharmacol Ther* 11: 423–431.
Walter IE *et al.* (1975). The relationship of plasma guanethidine levels to adrenergic blockade. *Clin Pharmacol Ther* 18: 571–580.

Guanoclor

Antihypertensive
$C_9H_{12}Cl_2N_4O = 263.1$
CAS—5001-32-1
IUPAC Name 1-[2-(2,6-Dichlorophenoxy)ethylamino]guanidine

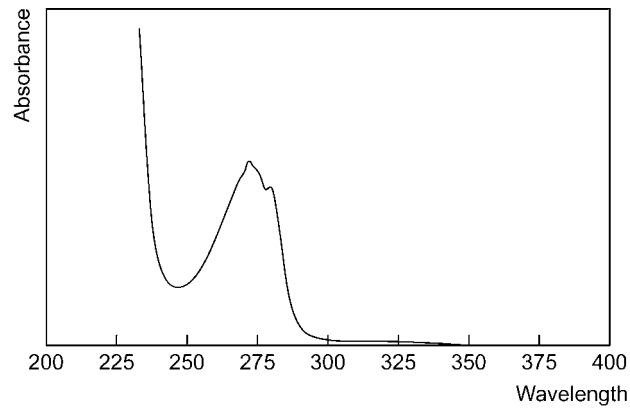


Chemical Properties Log *P* (octanol/water), 1.5.

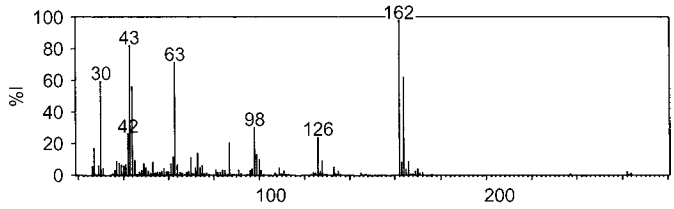
Guanoclor Sulfate

$(C_9H_{12}Cl_2N_4O)_2,H_2SO_4 = 624.3$
CAS—551-48-4
Proprietary Name *Vatensol*
Chemical Properties A white crystalline powder. Mp about 208°. Soluble 1 in about 400 of water.

Colour Test Mandelin's test—violet→orange→brown-yellow.
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—272 (A^1_1=14b), 278 nm.



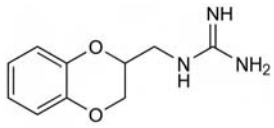
Infrared Spectrum Principal peaks at wavenumbers 1672, 1134, 1652, 1050, 765, 1243 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 162, 43, 63, 164, 30, 44, 98, 42.



Dose 10 to 120 mg of guanoclor sulfate daily; doses of 200 mg or more daily have been given.

Guanoxan

Antihypertensive
$C_{10}H_{13}N_3O_2$ = 207.2
CAS—2165-19-7
IUPAC Name [(2,3-Dihydro-1,4-benzodioxin-2-yl)methyl]guanidine



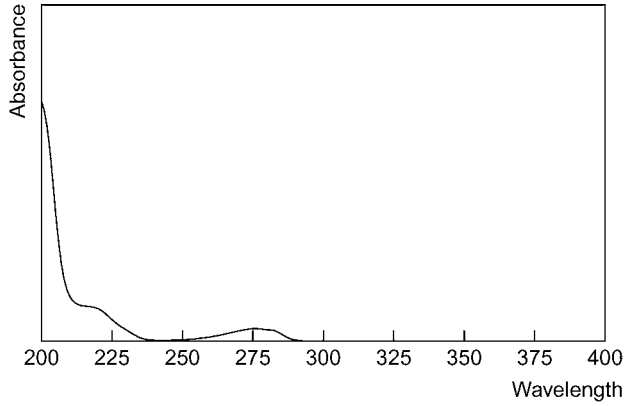
Chemical Properties Crystals. Mp 164° to 165°. pK_a 12.3.

Guanoxan Sulfate

$(C_{10}H_{13}N_3O_2)_2,H_2SO_4$ = 512.5
CAS—5714-04-5
Proprietary Name *Envacar*

Chemical Properties A white crystalline powder. Mp about 206°. Soluble 1 in 50 of water.

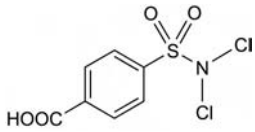
Colour Tests Mandelin's test—green, sometimes violet, depending on relative proportions of test material and reagent; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.03; system TAF—R_f 0.76 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HX—RI 295.
Ultraviolet Spectrum Aqueous acid—275 nm (A^1_1=104b).



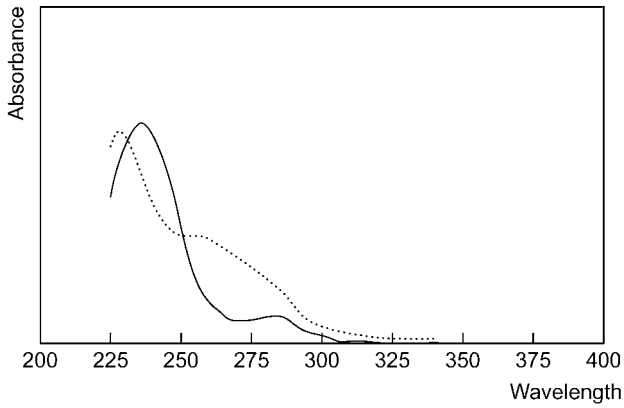
Infrared Spectrum Principal peaks at wavenumbers 1115, 1495, 1264, 1665, 1592, 748 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 30, 72, 43, 135, 121, 148, 52, 56.
Dose 10 to 50 mg of guanoxan sulfate daily; doses of 120 mg or more daily have been given.

Halazone

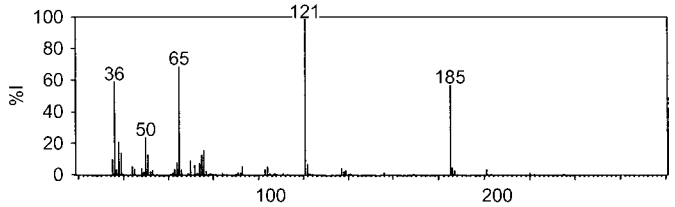
Disinfectant
$C_7H_5Cl_2NO_4S$ = 270.1
CAS—80-13-7
IUPAC Name 4-[(Dichloroamino)sulfonyl]benzoic acid
Synonym Pantocide
Proprietary Names *Clordispenser*; *Speton*; *Steridrolo a rapida idrolisi*.



Chemical Properties A white crystalline powder containing about 52% of 'available chlorine'. Mp about 195°, with decomposition. Slightly soluble in water, chloroform and ether; soluble 1 in 140 of ethanol; soluble in glacial acetic acid, and in aqueous solutions of alkali hydroxides and carbonates. Log *P* (octanol/water), −0.2.
Ultraviolet Spectrum Aqueous acid—237 nm; aqueous alkali—229 nm (A^1_1=382b).

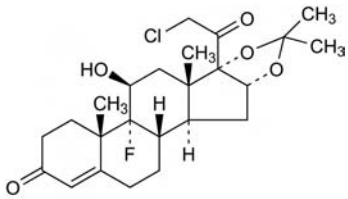


Mass Spectrum Principal ions at *m/z* 121, 65, 36, 185, 50, 38, 76, 39.

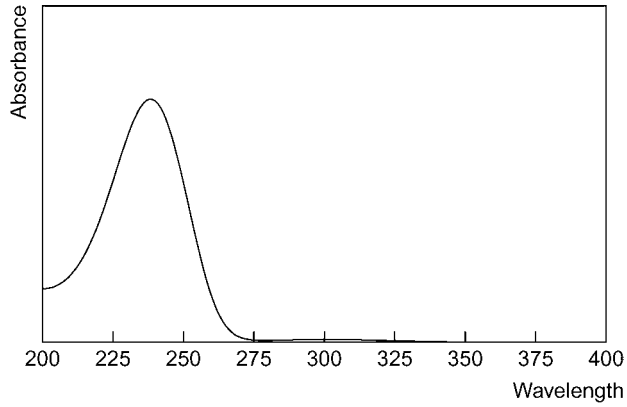


Halcinonide

Corticosteroid
$C_{24}H_{32}ClFO_5$ = 455.0
CAS—3093-35-4
Synonym (11β,16α)-21-Chloro-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)-bis(oxy)]pregn-4-ene-3,20-dione
Proprietary Names *Betacortone*; *Dermalog*; *Halciderm*; *Halcimat*; *Halog*.



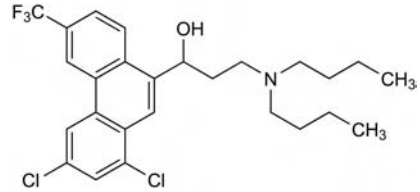
Chemical Properties Crystals. Mp 264° to 265°, with decomposition. Insoluble in water and hexanes; soluble in acetone and chloroform; slightly soluble in benzene, ethanol, ethyl ether and methanol. Log *P* (octanol/water), 2.9.
Thin-layer Chromatography System TAJ—R_f 0.62; system TAK—R_f 0.58; system TAL—R_f 0.91
Ultraviolet Spectrum Methanolic acid—238 nm (A^1_1=370b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1060, 1730, 1080, 1250, 1175 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 377, 43, 55, 246, 278, 59, 378, 41.
Use Topically in concentrations of 0.025 to 0.1%.

Halofantrine

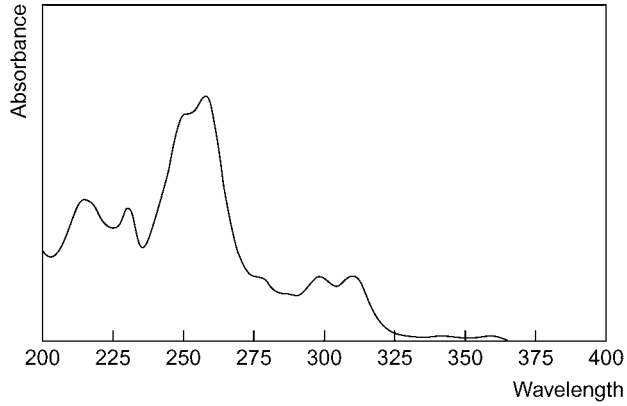
Antimalarial
$C_{26}H_{30}Cl_2F_3NO$ = 500.4
CAS—69756-53-2 (halofantrine); 66051-63-6 (racemic halofantrine)
IUPAC Name (1*S*)-3-(Dibutylamino)-1-[1,3-dichloro-6-(trifluoromethyl)phenanthren-9-yl]propan-1-ol
Synonym 1,3-Dichloro-α-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol



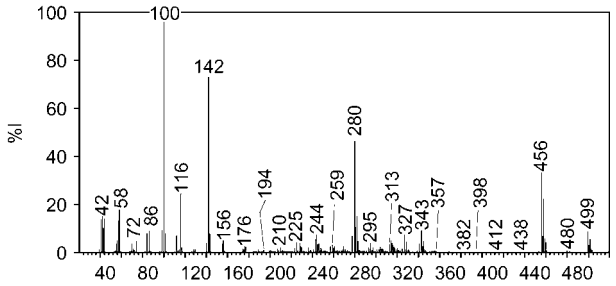
Halofantrine Hydrochloride
$C_{26}H_{30}Cl_2F_3NO$, HCl = 536.9
CAS—36167-63-2
Synonyms SKF-102886; WR-171669.
Proprietary Name *Halfan*
Chemical Properties Two crystalline forms of the hydrochloride with Mp 93° to 96° and 203° to 204°.

Halofantrine β-Glycerophosphate
$C_{29}H_{39}Cl_2F_3NO_7P$ = 672.5
Chemical Properties White crystals. Mp 60° to 65°.

Thin-layer Chromatography System TB—R_f 0.50(halofantrine); R_f 0.12 (desbutylhalofantrine); system TE—R_f 0.88(halofantrine); R_f 0.61(desbutylhalofantrine); system TAE—R_f 0.56(halofantrine); R_f 0.19(desbutylhalofantrine).
High Performance Liquid Chromatography System HX—RI 800(halofantrine); RI 618(desbutylhalofantrine); system HAA—retention time 23.0 min (halofantrine); retention time 20.5 (desbutylhalofantrine) min.
Ultraviolet Spectrum Aqueous acid—258, 215, 230, 298 nm.



Mass Spectrum Principal ions at *m/z* 100, 142, 280, 456, 116, 58, 458, 42.



Quantification

Blood **HPLC** Fluorescence detection. Halofantrine and metabolite. Limit of detection, 0.9 µg/L [Gawienowski *et al.* 1988]. Column: Hypersil 5 ODS (250 × 4.6 mm i.d.), stainless steel. Mobile phase: water : acetonitrile (35:65) containing triethylamine (1%),(pH 4), flow rate 2.0 mL/min. Internal standard (IS): BL-22312. UV detection (λ=254 nm). Retention time: halofantrine, 8.5 min; *N*-desbutylhalofantrine, 4.5 min; IS, 11.0 min. Halofantrine and metabolite. Limit of quantification, 10 µg/L [Mberu *et al.* 1992]. UV detection (λ=254 nm). Halofantrine and metabolite. Limit of detection, 2 µg/L in red blood cells [Milton *et al.* 1988].

Plasma **HPLC** Column: Lichrosorb RP-18 (200 × 4.6 mm i.d., 10 µm). Mobile phase: methanol:0.05 mol/L potassium dihydrogen phosphate (70:30) with 55 mmol/L perchloric acid, pH 3.1, flow rate 1.3 mL/min. UV detection (λ=254 nm). Retention time: halofantrine, 7.5 min; desbutylhalofantrine, 5.3 min. Halofantrine and metabolite. Limit of detection, 2.5 µg/L [Onyeji, Aideloje 1997]. Fluorescence detection (λ_{ex}=300 nm, λ_{em}=380 nm). Halofantrine and metabolite. Limit of quantification, 6 µg/L [Terefe, Blaschke 1994]. See Blood [Gawienowski *et al.* 1988; Mberu *et al.* 1992]. See Blood. Limit of detection 1 µg/L [Milton *et al.* 1988].

Serum **HPLC** Column: Ultrasphere C_8 bonded-phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (75:25) with 0.2% SDS (sodium dodecyl sulfate) and 0.2% glacial acetic acid, flow rate 1.5 mL/min. IS: BL-22312. UV detection (λ=257 nm). Retention time: halofantrine, 8.3 min; desbutylhalofantrine, 5.5 min; IS, 11.5 min. Limit of detection, 5 µg/L, for halofantrine, and 3 µg/L, for metabolite [Keeratithakul *et al.* 1991].

Urine **HPLC** See Blood [Milton *et al.* 1988].

Disposition in the Body After oral administration, halofantrine is slowly and erratically absorbed and the drug enters systemic circulation within 1 h. The rate and extent of absorption are increased in the presence of food. Peak concentrations are observed 3–6 h after administration. There is wide and unpredictable interindividual variability of peak plasma concentrations. The major metabolite is *N*-desbutylhalofantrine which is active and has a longer half-life than halofantrine. Peak concentrations of the metabolite are observed after 12–20 h and are 2–4 times lower than the drug levels. The main route of elimination is in faeces as the unchanged drug.

Therapeutic Concentration

Six adult volunteers, aged between 23 and 54 years, were administered 250 mg at 6-h intervals and after the last of 3 doses, the drug concentrations were measured. Mean maximum plasma concentrations were halofantrine 621.4 µg/L at 16.2 h and *N*-desbutylhalofantrine 114.9 µg/L at 43.2 h [Gimenez *et al.* 1994].

Six male adult patients of Melanesian origin with uncomplicated falciparum malaria (most severe form), mean age 22.7 years, were treated with 500 mg of halofantrine hydrochloride every 6 h for a total of 1.5 g. The mean concentration for halofantrine was 896 µg/L after 15 h and 491 µg/L after 56 h for the metabolite, *N*-desbutylhalofantrine [Veenendaal *et al.* 1991].

Half-life Plasma half-life, 38 h in healthy volunteers and 91–113 h in patients with malaria (halofantrine); 103 h in healthy individuals and 79–118 h in those with malaria (*N*-desbutylhalofantrine).

Volume of Distribution 73 L/kg (patients with malaria); 8572 L (healthy individuals).

Clearance Plasma clearance, 0.58 L/h/kg.

Note For a review of halofantrine, see Bryson and Goa [1992]. For a review of the pharmacokinetics of halofantrine, see Karbwang and Na Bangchang [1994].

Dose Three doses of 500 mg at 6-h intervals.

Bryson HM, Goa KL (1992). Halofantrine. A review of its antimalarial activity, pharmacokinetic properties and therapeutic potential. *Drugs* 43: 236–258.

Gawienowski M *et al.* (1988). Ion-paired liquid chromatographic method for the analysis of blood and plasma for the antimalarial drug halofantrine and its putative mono-debutylated metabolite. *J Chromatogr* 430: 412–419.

Gimenez F *et al.* (1994). Plasma concentrations of the enantiomers of halofantrine and its main metabolite in malaria patients. *Eur J Clin Pharmacol* 46: 561–562.

Karbwang J, Na Bangchang K (1994). Clinical pharmacokinetics of halofantrine. *Clin Pharmacokinet* 27: 104–119.

Keeratithakul D *et al.* (1991). An improved high-performance liquid chromatographic method for the simultaneous measurement of halofantrine and desbutylhalofantrine in human serum. *Ther Drug Monit* 13: 64–68.

Mberu EK *et al.* (1992). Measurement of halofantrine and its major metabolite desbutylhalofantrine in plasma and blood by high-performance liquid chromatography: a new methodology. *J Chromatogr* 581(1): 156–160.

Milton KA *et al.* (1988). Determination of halofantrine and its principal metabolite desbutylhalofantrine in biological fluids by reversed-phase high-performance liquid chromatography. *J Chromatogr* 433: 339–344.

Onyeji CO, Aideloje SO (1997). Ion-pair reversed-phase high-performance liquid chromatographic analysis of halofantrine and desbutylhalofantrine in human plasma. *Ther Drug Monit* 19: 682–687.

Terefe H, Blaschke G (1994). Direct determination of the enantiomers of the antimalarial drug halofantrine and its active metabolite N-desbutylhalofantrine in human plasma. *J Chromatogr* 657: 238–242.

Veenendaal JR *et al.* (1991). Pharmacokinetics of halofantrine and n-desbutylhalofantrine in patients with falciparum malaria following a multiple dose regimen of halofantrine. *Eur J Clin Pharmacol* 41: 161–164.

Haloperidol

Antipsychotic, Butyrophenone

$C_{21}H_{23}ClFNO_2$ = 375.9

CAS—52-86-8

IUPAC Name 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one

Synonyms 4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone; 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone; 4′-fluoro-4-(4-hydroxy-4-*p*-chlorophenylpiperidino)butyrophenone; 1-(3-*p*-fluorobenzoylpropyl)-4-*p*-chlorophenyl-4-hydroxypiperidine; *R*-1625.

Proprietary Names *Dozic*; *Haldol*; *Serenace*.

Chemical Properties Crystals. Mp 148.0° to 149.4°. Solubility in water (1.4 mg/100 mL); freely soluble in chloroform, methanol, acetone, benzene and dilute acids. pK_a 8.3. Log *P* (octanol/water) 3.23. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Haloperidol Decanoate

$C_{31}H_{41}ClFNO_3$ = 530.1

CAS—74090-97-8

IUPAC Name 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one decanoate

Synonym KD-136

Proprietary Name *Haldol*

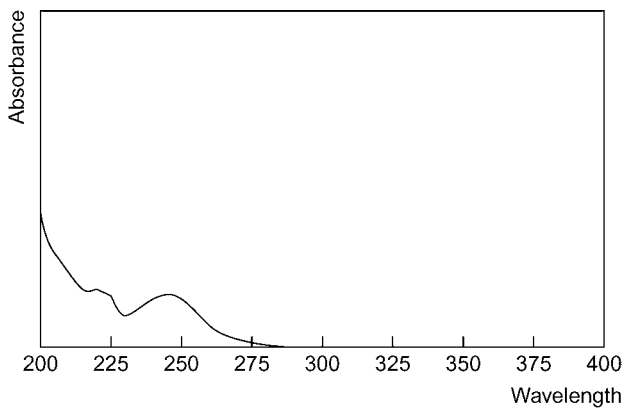
Colour Tests Aromaticity (method 2)—yellow/orange-red; Liebermann's reagent—brown.

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.11; system TC—R_f 0.27; system TE—R_f 0.76; system TL—R_f 0.33; system TAE—R_f 0.51; system TAF—R_f 0.75 (acidified iodoplatinate solution—positive).

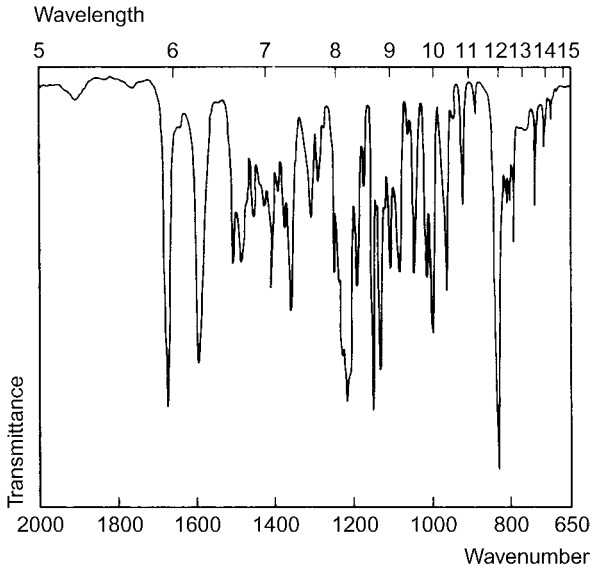
Gas Chromatography System GA—RI 2930, haloperidol-H_2O I 2965, M (reduced) RI 3152, M (*N*-desalkyl-oxo-)-$2H_2O$ RI 1650, M (*N*-desalkyl-) RI 1800; system GB—RI 3094, M (reduced) RI 3152, M (*N*-desalkyl-oxo-)-$2H_2O$ RI 1707; system GV—RT 23.3 min.

High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 421; system HY—RI 316; system HZ—RT 5.8 min; system HAA—RT 14.4 min; system HAX—RT 11.1 min; system HAY—RT 6.2 min; system HAZ—*k* 0.72.

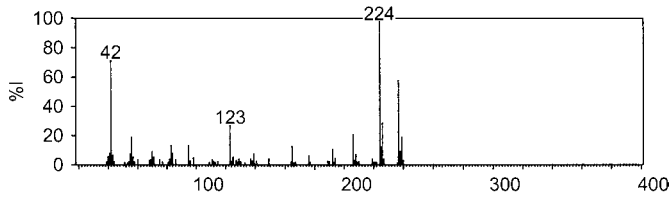
Ultraviolet Spectrum Aqueous acid (methanol)—245 nm (A_1^1 = 340a). No alkaline shift



Infrared Spectrum Principal peaks at wavenumbers 832, 1151, 1673, 1217, 1136, 1598 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 224, 42, 237, 226, 123, 206, 239, 56 (none above 240).



Quantification

Blood **GC** NPD. Limit of quantification, 515 µg/L, limit of detection, 156 µg/L [Sanchez de la Torre *et al.* 2005]. SID. Limit of detection, 0.1 pmol [Seno *et al.* 1993].

HPLC Column: Waters Nova-Pak C_{18} (150 × 3.9 mm i.d., 3 µm). Mobile phase: phosphate buffer (pH 7.25) : acetonitrile (65 : 25), flow rate 1.3 mL/min. Limit of detection, 0.1 µg/L [Eyles *et al.* 1992].

LC-MS Limit of detection, 0.1 µg/L [Seno *et al.* 2000].

Plasma **GC** NPD. Limit of detection, 0.4 ng/mL [Ulrich *et al.* 1995]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (1.5 m × 2 mm i.d.). Carrier gas: N_2, 55 mL/min. Temperature: 300°. NPD. Retention time: 2.9 min. Limit of detection, 0.5 µg/L [Franklin 1980].

GC-MS Column: fused silica bonded phase. NICI, SIM acquisition mode. Limit of detection, 0.1 µg/L [Häring *et al.* 1987].

HPLC Column: Chromsep C_8 reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer (pH 3.0) containing 0.5% TEA (30 : 70), flow rate 1.0 mL/min. UV detection (λ = 238 nm). Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Mercolini *et al.* 2007]. Column: RP-18e (100 × 4.6 mm i.d.). Mobile phase: 100 mmol/L phosphate buffer (pH 3.5) : acetonitrile (80 : 20), flow rate 2 mL/min. UV detection (λ = 230 nm). Limit of detection, 3 ng [Aboul-Enein *et al.* 2006]. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% phosphoric acid (240 : 760), flow rate 0.5 mL/min to 1.5 mL/min until 8 min to 1.65 mL/min until 20 min. UV detection (λ = 250 nm). Limit of detection, 7.5 µg/L [Higashi *et al.* 2006]. Haloperidol and its reduced metabolite. Limit of quantification, 2 µg/L with UV detection, 0.5 µg/L with electrochemical detection [Pan *et al.* 1998]. See Blood [Eyles *et al.* 1992]. Column: Waters µBondapak CN (30 × 3.9 cm i.d., 10 µm). Mobile phase: acetonitrile : phosphate buffer (pH 6.8, 45 : 55). Electrochemical detection. Limit of detection, 0.5 ng [Korpi *et al.* 1983].

LC-MS Column: Bondapak C_{18} (150 × 3.9 mm i.d.). Mobile phase: 0.4 mol/L ammonium acetate : methanol (40 : 60 at 0 min to 20 : 80 at 15 min to 0 : 100 at 25 min until 32 min). ESI. Limit of detection, 0.1 µg/L [Yun *et al.* 2005]. Limit of detection, 5 µg/L [Arinobu *et al.* 2002].

CE Buffer: Tris phosphate with dimethyl-β-cyclodextrin and PEG 6000. Limit of detection, 15 µg/L for haloperidol and 30 µg/L for both enantimers of its chiral metabolites [Wu *et al.* 1999].

Serum **GC** SID. Limit of detection, 1.1 µg/L [Fujii *et al.* 1996]. See Plasma [Ulrich *et al.* 1995]. NPD or AFID. Limit of detection, 0.5 µg/L [Abernethy *et al.* 1984].

GC-MS CI. SIM acquisition mode [Szczepanik-van Leeuwen 1985].

HPLC Column: Lichrosorb Si60 (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (90 : 10) containing 0.01% TEA and 0.02% acetic acid. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 420 nm). Limit of detection, 0.22 µg/L [Kishikawa *et al.* 2006]. UV detection. Limit of quantification, 4 µg/L [Angelo, Petersen 2001]. UV detection. Limit of quantification, 0.3 µg/L [Walter *et al.* 1998]. Limit of detection, 0.5 µg/L [Hoffman, Edkins 1994].

Urine **GC** See Blood [Seno *et al.* 1993].

GC-MS SIM acquisition mode (*m/z* 206, 237, 224). Limit of detection, 10 µg/L [Olmos-Carmona, Hernandez-Carrasquilla 1999].

HPLC Pyridinium metabolites of haloperidol, Fluorescence detection [Eyles *et al.* 1994].

LC-MS See Plasma [Arinobu *et al.* 2002].

Breast Milk **HPLC** UV detection. Limit of detection, 5 µg/L T[Ohkubo *et al.* 1992].

CSF **GC** See Serum [Abernethy *et al.* 1984].

Oral Fluid **GC-MS** Column: methyl silicone. SIM acquisition mode. Limit of quantification, 0.9–44.2 µg/L [Pujadas *et al.* 2007].

Hair **HPLC** Column: TSK-Gel 80-TM ODS (150 × 4.6 mm i.d.). Mobile phase: 50 mmol/L potassium phosphate buffer (pH 6.8) : acetonitrile : methanol (3 : 2 : 1), flow rate 0.8 mL/min. Electrochemical detection. Limit of detection, 0.5 µg/L [Matsuno *et al.* 1990].

Note For an ELISA in serum, limit of quantification, 0.3 µg/L see Terauchi *et al.* [1990]. See also Poland, Rubin [1981], Rubin *et al.* [1982] and Smith *et al.* [1984], Smith *et al.* [1985].

Other **GC-MS** Microsomes [Tyndale, Inaba 1990].

CE-MS Mouse and guinea-pig Liposomes. ESI [Tomlinson *et al.* 1993a, 1993b].

Disposition in the Body Haloperidol is readily absorbed after oral administration. It is localised in the tissues and rapidly taken up by the brain. It is slowly excreted in the urine, ≈40% of a dose being eliminated in 5 days with only ≈1% of the dose as unchanged drug; ≈15% of a dose is excreted in the bile. It is metabolised by oxidative *N*-dealkylation. Metabolites that have been identified in urine are 4-fluorobenzoylpropionic acid and 4-fluorophenylaceturic acid (the glycine conjugate of 4-fluorophenylacetic acid), both of which are inactive. Haloperidol is also metabolised by reduction of the ketone group to a secondary alcohol (reduced haloperidol). Haloperidol is excreted in breast milk.

Therapeutic Concentration There is considerable inter-subject variation in steady-state blood concentrations. However, for normal doses (up to 15 mg per day), the serum concentration is usually below 0.05 mg/L.

There has been much discussion regarding the relationship between serum concentrations and the therapeutic effect of haloperidol in schizophrenia and related disorders. A review of ≈50 clinical studies concluded that the therapeutic window for haloperidol was in the range 0.0056–0.0169 mg/L [Ulrich *et al.* 1998].

Steady-state serum concentrations of 0.0008–0.033 mg/L (mean, 0.0065) were attained after daily oral administration of 1–14 mg (mean, 6) to 34 patients [Forsman, Ohman 1977].

In a study of the effect of CYP2D6 polymorphism on haloperidol pharmacokinetics in 172 patients, the mean haloperidol and reduced haloperidol serum trough concentrations were 0.007 and 0.0072 mg/L, respectively, in patients with ultrafast CYP2D activity (daily dose 14 mg), 0.0073 and 0.0020 mg/L in those with extensive CYP2D6 activity (daily dose 12 mg), 0.0086 and 0.0042 mg/L in those with intermediate CYP2D6 activity (daily dose 12 mg), and 0.0069 and 0.0095 mg/L in those with poor CYP2D6 activity (daily dose 13 mg) [Brockmöller *et al.* 2002].

Toxicity The minimum lethal dose is probably in excess of 3 g. Toxic effects may appear with blood concentrations greater than approx. 0.05 mg/L but there is considerable inter-subject variation.

A psychiatric patient who committed suicide by running into traffic had haloperidol concentrations of 1.2 mg/L in heart blood, 2.7 mg/L in brain and 10.8 mg/L in liver [Johnson 1988].

In a death attributed to a suicidal overdose of haloperidol, the following postmortem tissue concentrations were reported for haloperidol and reduced haloperidol, respectively: blood 1.9 and 1.4 mg/L, bile 3.4 and 1.6 mg/L, liver 44 and 43 µg/g, and urine 6.6 and 5.7 mg/L [Levine *et al.* 1991].

In a case believed to be a natural cardiac death, the postmortem tissue concentrations of haloperidol and reduced haloperidol were blood 0.6 mg/L and not reported, bile 0.4 and 0.5 mg/L, kidney 0.7 and 2.3 µg/g, liver 5.0 and 13 µg/g, and urine 0.4 and 2.3 mg/L [Levine *et al.* 1991].

In 24 children who received an accidental overdosage of haloperidol at the same hospital, the maximum blood level measured within 4 days of the final administration was 0.0289 mg/L (dosage unknown); symptoms of acute toxicity were evident (disturbances in consciousness, tremor, oculogyric crisis, dysarthria, drooling, akathisia, hyperreflexia, opisthotonos, elevated liver enzyme values, abnormal electrocardiograph) [Yoshida *et al.* 1993].

Bioavailability 38–86% (mean, 58%).

Half-life Plasma half-life, 10–40 h (mean, 20).

Volume of Distribution 10–30 L/kg (mean, 18).

Clearance Plasma clearance, 8–17 mL/min/kg (mean, 12).

Distribution in Blood Plasma : whole blood ratio, ≈0.93.

Protein Binding ≈92%.

Dose Haloperidol 1 to 30 mg daily by mouth. Up to 100 mg daily may be required; rarely, higher doses have been used.

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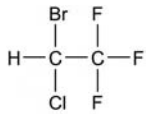
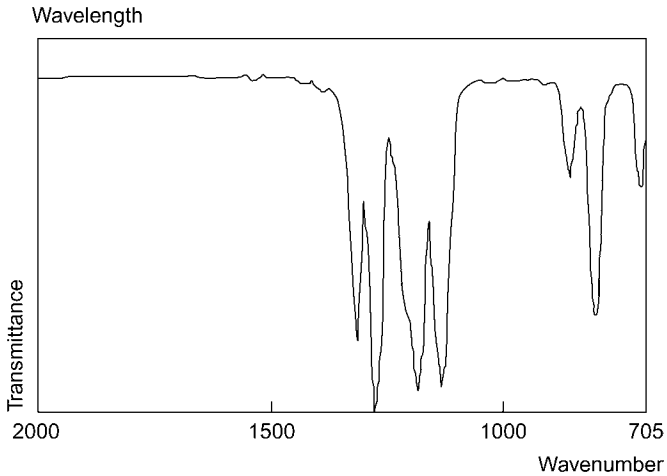
Halothane

Anaesthetic (General)
$CF_3CHBrCl = 197.4$
CAS—151-67-7

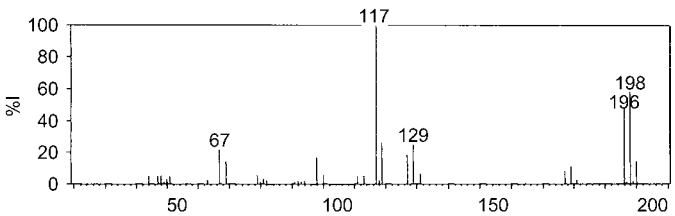
IUPAC Name 2-Bromo-2-chloro-1,1,1-trifluoroethane
Synonyms Alotano; phthorothanum.
Proprietary Names *Fluothane*; *Rhodialothan*.



Chemical Properties A colourless, mobile, heavy, non-inflammable liquid. Bp 49° to 51°. Refractive index at 20°, 1.3695 to 1.3705. Halothane contains thymol 0.01% w/w as a preservative. Soluble 1 in 400 of water; miscible with dehydrated alcohol, chloroform, and ether. Log *P* (octanol/water), 2.32 [Cole *et al.* 1975].
Gas Chromatography System GA—RI 533; system GI—RT 8.5 min; system GR—RT 4.2 min; system GAA—RI 543.
Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 117, 198, 196, 119, 129, 67, 127, 98.



Quantification
Blood **GC** Column: Rtx-Volatiles (30 m × 0.32 mm i.d.,m 1.5 µm). Carrier gas: He, 2 mL/min. Temperature programme: 40° for 1 min to 70° at 10°/min to 250° at 20°/min for 4 min. FID. Limit of detection, 20 µg/L [Kojima *et al.* 2001]. Halothane and other fluorinated inhalation anaesthetics [Yang *et al.* 2001]. 15% Apiezon L on 80/100 mesh Chromosorb W (2 m × 4 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 130°. FID. Limit of detection not reported [Flynn *et al.* 1989a, 1989b].Column: Porapak Q 100/120 mesh (1.8 m × 2 mm i.d.). Carier gas: N_2, 300 mL/min. FID. Limit of detection, 2.0 nmol/L for 2-chloro-1,1-difluoroethylene and and 2.5 nmol/L for 2-chloro-1,1,1-trifluoroethane [Maiorino *et al.* 1979]. Column: 15% FFAP (275 × 0.4 cm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 100°. FID. Limit of detection, 50 mg/L [Cole *et al.* 1975]. See also Fink, Mikawa [1970], Tham *et al.* [1972], Wagner *et al.* [1974] and Yokota *et al.* [1967].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 2 min to 280° at 30°/min. EI ionisation. SIM acquisition mode. Limit of detection, 0.004 mg/kg [Musshoff *et al.* 2000]. Column: J&W (30 m × 0.53 mm i.d.). Carrier gas: He: 15 mL/min. Temperature: 60°. EI ionisation, positive ion mode, SIM acquisition mode. Retention time: 4.2 min. Limit of detection, 0.2 mg/L [Saito *et al.* 1995].
Note For a continuous *in vivo* blood gas device see Richman *et al.* [1980].
Plasma **GC** Column: Porapak Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 110°. FID. Limit of detection, 300 µg/L for trifluoroacetic acid [Maiorino *et al.* 1980].

HPLC Column: Dionex IonPac AS11 (250 × 4 mm i.d.). Mobile phase: sodium hydroxide (0.5 mmol/L for 5 min to 3.0 mmol/L over 10.5 min to 80 mmol/L at 25 mmol/min for 5 min to 0.5 mol/L at 14.4 mmol/min for 7 min), flow rate 2.0 mL/

min. Limit of quantification, 10 µmol/L for trifluroacetic acid, 2 µmol/L for bromide [Hankins, Kharasch 1997].

Serum **GC** See Plasma [Maiorino *et al.* 1980].

Urine **GC** See Plasma. Limit of detection, 1 mg/L [Maiorino *et al.* 1980].

GC-MS See Blood [Musshoff *et al.* 2000]. Column: RT-QPLOT (30 m × 0.32 mm i.d.). Carrier gas: He. Temperature programme: 40° for 3 min to 130° at 15°/min for 1 min to 180° at 10°/min for 2 min. SIM acquisition mode. Limit of detection, <30 ng/L [Poli *et al.* 1999].

HPLC See Plasma [Hankins, Kharasch 1997].

Bile **GC-MS** See Blood [Musshoff *et al.* 2000].

Stomach Contents **GC-MS** See Blood [Musshoff *et al.* 2000].

Brain **GC-MS** See Blood [Musshoff *et al.* 2000].

Kidney **GC-MS** See Blood [Musshoff *et al.* 2000].

Liver **GC-MS** See Blood [Musshoff *et al.* 2000].

Tissues **GC** See Blood [Maiorino *et al.* 1979].

Disposition in the Body Halothane is rapidly absorbed upon inhalation; blood : gas partition coefficient is ≈2.4. It accumulates in adipose tissue. Approximately 60–80% of an absorbed dose is exhaled unchanged from the lungs in 24 h and smaller amounts continue to be exhaled for several days or weeks. A variable amount is metabolised in the liver by debromination and dechlorination; replacement of a fluorine atom by a methoxy group followed by glucuronic acid conjugation occurs to a limited extent. Other metabolites detected in expired air and in blood are 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethylene. Up to ≈20% of a dose may be excreted in the urine as trifluoroacetic acid and its salts. Bromide ion is slowly excreted in the urine.

Therapeutic Concentration During surgical anaesthesia, concentrations in blood are usually in the range 22–84 mg/L. Venous blood concentrations lag behind arterial concentrations during induction and decline less rapidly during recovery.

Blood concentrations ranging from 50.5–106.5 mg/L were reported in 8 patients following anaesthesia with 1.5% halothane for 20 min. At recovery 10 min later, the range was 22–30 mg/L; traces were still detectable after 44 h; bromide concentrations increased during the post-anaesthetic period [Atallah, Geddes 1973].

Peak plasma-bromide concentrations occurred in surgical patients 2–3 days after anaesthesia and ranged from 52–180 mg/L [Tinker *et al.* 1976].

Toxicity The minimum lethal dose by ingestion or inhalation is ≈10 mL although 1 case of recovery after the ingestion of 250 mL has been reported. Blood concentrations of 7–310 mg/L (mean, 40 mg/L) have been associated with fatalities.

In 1 fatality involving the ingestion of 35 mL halothane, the following postmortem tissue concentrations were reported: blood 650 mg/L, brain 1560 µg/g, lung 500 µg/g, liver 880 µg/g, spleen 230 µg/g and urine 20 mg/L; salicylate and an unidentified barbiturate were also present [Spencer, Green 1968].

Dose For induction of anaesthesia, 2–3% of the vapour by inhalation; maintenance, 0.5 to 1.5%.

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Tham MK *et al.* (1972). A gas-chromatographic method using surfactants for analysis of volatile anesthetics in blood. *Anesthesiology* 37: 647–649.

Tinker JH *et al.* (1976). Elevation of plasma bromide levels in patients following halothane anesthesia: time correlation with total halothane dosage. *Anesthesiology* 44: 194–196.

Wagner PD *et al.* (1974). Simultaneous measurement of eight foreign gases in blood by gas chromatography. *J Appl Physiol* 36: 600–605.

Yang NC *et al.* (2001). Simultaneous determination of fluorinated inhalation anesthetics in blood by gas chromatography–mass spectrometry combined with a headspace autosampler. *J Chromatogr B Biomed Sci Appl* 759: 307–318.

Yokota T *et al.* (1967). Direct injection method for gas chromatographic measurement of inhalation anesthetics in whole blood and tissues. *Anesthesiology* 28: 1064–1073.

Haloxon

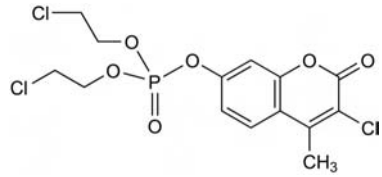
Anthelmintic (Veterinary)

$C_{14}H_{14}Cl_3O_6P$ = 415.6

CAS—321-55-1

IUPAC Name Bis(2-chloroethyl) 3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphate

Proprietary Names *Galloxon*; *Loxon*; *Luxon*; *Ruby Horse Wormer.*



Chemical Properties A white powder. Mp 88° to 93°. Practically insoluble in water; soluble 1 in 9 of ethanol, 1 in 4 of acetone and 1 in 2 of chloroform. Log *P* (octanol/water), 3.2.

Ultraviolet Spectrum After solution in dioxan and dilution with acid methanol—290, 312 nm.

Infrared Spectrum Principal peaks at wavenumbers 1738, 1010, 1070, 1036, 1277, 1605 cm^{-1} (KBr disk).

Halquinol

Antimicrobial

CAS—8067-69-4

IUPAC Name 5-Chloroquinolin-8-ol; 7-chloroquinolin-8-ol; 5,7-dichloroquinolin-8-ol

Synonyms Chlorhydroxyquinoline; chlorquinol.

Proprietary Names *Capitrol (chloroxine)*; *Valpeda*.

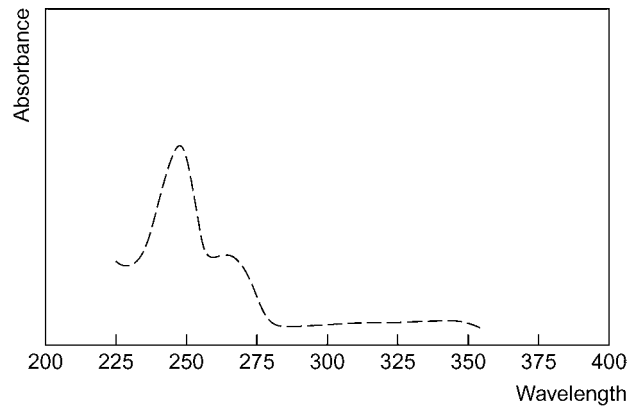
Chemical Properties A mixture of the chlorinated products of quinolin-8-ol containing 57 to 74% of 5,7-dichloroquinolin-8-ol (chloroxine), 23 to 40% of 5-chloroquinolin-8-ol and not more than 4% of 7-chloroquinolin-8-ol. A yellowish-white to yellowish-grey, voluminous powder. Practically insoluble in water; soluble 1 in 250 of ethanol, 1 in 50 of chloroform and 1 in 130 of ether; soluble in acids.

Colour Tests Mandelin's test—yellow; Marquis test—yellow; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.19, streaking (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1743.

Ultraviolet Spectrum Aqueous acid—258 nm (A^1_1=2187a); aqueous alkali—259, 343 nm; methanol—248, 263, 334 nm.



Infrared Spectrum Principal peaks at wavenumbers 1512, 1282, 812, 1200, 950, 785 cm^{-1} (KBr disk).

Dose 3 to 4 g daily for 5 days.

Harmaline

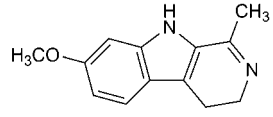
Hallucinogen

$C_{13}H_{14}N_2O$ = 214.3

CAS—304-21-2

IUPAC Name 7-Methoxy-1-methyl-3,4-dihydro-2*H*-pyrido[3,4-*b*]indole

Synonym 4,9-Dihydro-7-methoxy-1-methyl-3*H*-pyrido[3,4-*b*]indole



Chemical Properties Alkaloid obtained from peganum, the dried seeds of *Peganum harmala* (Zygophyllaceae). It is also found (together with harmine) in the South American hallucinogenic drink *caapi*, also known as *Yagé* and *ayahuasca*. Mp 249° to 250°, with decomposition. Slightly soluble in water, ethanol, and ether. Log *P* octanol/water), 4.64 [Meylan, Howard 1995]. Harmaline solutions were unstable with a 30% loss of activity [Yritia *et al.* 2002]. Standard solutions in drug-free urine were stable for at least 9 months at −2° [Björnstad *et al.* 2009].
Colour Tests Mandelin's test—green-brown; Marquis test—brown-green.
Thin-layer Chromatography System TA—R_f 0.38 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—harmaline RI 2430, harmaline-2H (harmine) RI 2291, M (*O*-desmethyl-)-2H- RI 2550, art (dihydro-) RI 2375.
Ultraviolet Spectrum Aqueous acid—256 nm; aqueous alkali—330 nm; methanol—260 ($A^1_1 = 288$b), 344 nm ($A^1_1 = 468$b).
Mass Spectrum Principal ions at *m/z* 213, 214, 170, 198, 169, 115, 63, 143.
Disposition in the Body The P450 isoenzymes that *O*-demethylate exogenous harmaline are CYP1A2 and polymorphic CYP2D6 [Yu *et al.* 2003].
Quantification
Blood **LC-MS** Column: XTerra MS-C_{18} (100 × 3.0 mm, 3.5 µm). Mobile phase: 0.02 mol/L ammonium formate : acetonitrile (75 : 25), flow rate 0.4 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Sklerov *et al.* 2005].
Plasma **HPLC** Column: Kromasil 100 C_{18} (150 × 4 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate buffer (pH 8.0)-acetonitrile : methanol (20 : 30) (63 : 37) : acetonitrile-methanol (20 : 30; 100 : 0 for 6.5 min to 68.3 : 31.7 at 8.5 min), flow rate 0.8 mL/min for 6.5 min to 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 495$ nm). Limit of quantification, 0.3 µg/L, limit of detection, 0.1 µg/L [Yritia *et al.* 2002]. Column: Supelcosil LC-DB-8 (15.0 × 4.6 mm i.d., 5 mm). Mobile phase: methanol : acetonitrile : 0.1 mmol/L ammonium acetate (pH 6.9; 20 : 20 : 60), flow rate 2.0 mL/min. Fluorescence detection ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 495$ nm). Limit of quantification, 1 µg/L [Callaway *et al.* 1996].
Urine **GC-MS** Column: HP-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: 1 mL/min. Temperature programme: 50° for 2 min to 220° at 15°/min to 300° at 30°/min. TIC, full scan mode. Retention time: 15.7 min. Limit of detection, 1 mg/L [Frison *et al.* 2008].
LC-MS Column: Hypersil GOLD (100 × 2.1 mm i.d., 5 µm). Mobile phase: 1% acetonitrile in 10 mmol/L formic acid : 60% acetonitrile in 10 mmol/L formic acid (100 : 0 to 0 : 100 over 10 min to 100 : 0 for 4 min), flow rate 200 µL/min. SRM acquisition mode. Limit of detection, 3 µg/L [Björnstad *et al.* 2009].
Bile **LC-MS** See Blood [Sklerov *et al.* 2005].
Stomach Contents **LC-MS** See Blood [Sklerov *et al.* 2005].
Brain **LC-MS** See Blood [Sklerov *et al.* 2005].
Kidney **LC-MS** See Blood [Sklerov *et al.* 2005].
Liver **LC-MS** See Blood [Sklerov *et al.* 2005].
Other **HPLC** Column: Regis REXCHROM phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% trifluoroacetic acid : 40% acetonitrile (40 : 60), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 250$ nm, $\lambda_{em} = 480$ nm). Limit of detection not reported [Yu *et al.* 2003].
LC-MS Column: Luna C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.1% formic acid (50 : 50) or methanol : 0.1% formic acid (70 : 30), flow rate 0.2 mL/min. TIS, positive ion mode. Limit of detection, 0.2 µmol/L [Yu *et al.* 2003].
Note For a review of the analysis of toxic alkaloids in plants, see Beyer *et al.* [2009].
Toxicity

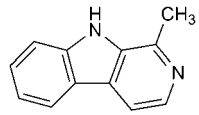
Following the ingestion of *P. harmala*, an 18-year-old was admitted to hospital suffering psychomotor agitation, visual hallucinations, diffuse tremours, ataxia and vomiting. His urine concentration of harmaline was 0.45 mg/mL. He recovered with no further sequelae [Frison *et al.* 2008].

A 25-year-old white male ingested a preparation from a South American tree bark, 'ooasca' and 4 h later he ingested tryptamines. He went to sleep and was found dead the following morning. The table below shows the toxicological findings. The medical examiner ruled cause of death was 'hallucinogenic amine intoxication' and manner of death was 'undetermined'.

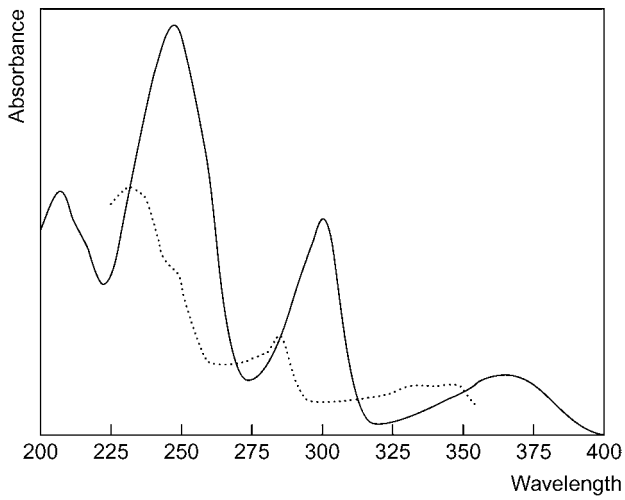
Beyer J *et al.* (2009). Analysis of toxic alkaloids in body samples. *Forensic Sci Int* 185: 1–9.
Björnstad K *et al.* (2009). A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1162–1168.
Callaway JC *et al.* (1996). Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *J Anal Toxicol* 20: 492–497.
Frison G *et al.* (2008). A case of beta-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. *Forensic Sci Int* 179: e37–e43.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Sklerov J *et al.* (2005). A fatal intoxication following the ingestion of 5-methoxy-*N*,N-dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 29: 838–841.
Yritia M *et al.* (2002). Determination of *N*,*N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of Ayahuasca. *J Chromatogr B Analyt Technol Biomed Life Sci* 779: 271–281.
Yu AM *et al.* (2003). Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic beta-carboline alkaloids harmaline and harmine. *J Pharmacol Exp Ther* 305: 315–322.

Harman

β-*Carboline, Alkaloid*
$C_{12}H_{10}N_2 = 182.2$
CAS—486-84-0
IUPAC Name 1-Methyl-9*H*-pyrido[3,4-*b*]indole
Synonyms Aribine; harmane; loturine.



Chemical Properties Alkaloid present in passion flower, the dried flowering and fruiting tops of *Passiflora incarnata* (Passifloraceae). Crystals. Mp about 238°. Sparingly soluble in hot water; soluble in ethanol, chloroform, dilute acids, and ether. Log *P* (octanol/water), 3.1.
Note Harman may be found in tobacco smoke and in homemade wine. It may also be found as a putrefactive base, particularly in cases where embalming has taken place. It is a condensation product of tryptophan and acetaldehyde.
Colour Tests Mandelin's test—green; Marquis test—green.
Thin-layer Chromatography System TA—R_f 0.70; system TAE—R_f 0.72; system TAF—R_f 0.76; system TL—R_f 0.33; system TB—R_f 0.02; system TC—R_f 0.40; system TE—R_f 0.57 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—harman RI 1952; M (1-OH-) RI 1920; M (1-OH-) RI 2015; M (1-OH-) RI 2290.
High Performance Liquid Chromatography System HX—RI 319.
Ultraviolet Spectrum Aqueous acid—245 ($A^1_1 = 1987$a), 300 nm; aqueous alkali—232, 284 nm.



Infrared Spectrum Principal peaks at wavenumbers 751, 1317, 1235, 1504, 1245, 1567 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 182, 57, 43, 55, 40, 69, 41, 181.
Quantification
Plasma **HPLC** Column: Econosphere C_{18} ODS2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 17.5 mmol/L potassium phosphate buffer (pH 6.5) : methanol

Substance	*Heart blood (mg/L)*	*Peripheral blood (mg/L)*	*Gastric (mg/L)*	*Bile (mg/L)*	*Brain (mg/kg)*	*Kidney (mg/kg)*	*Liver (mg/kg)*	*Urine (mg/L)*
DMT	0.02	0.01	3.3	0.57	ND	ND	ND	0.89
5-MeO-DMT	1.88	1.20	202	9.81	0.15	22.8	16.4	9.59
Tetrahydroharmine	0.38	0.24	12.5	4.78	0.43	6.89	13.24	6.02
Harmaline	0.07	0.04	6.4	0.41	0.04	2.24	3.6	2.26
Harmine	0.17	0.08	122	1.64	0.16	0.74	2.31	1.15

ND: None detected [Sklerov *et al.* 2005]

(30 : 70), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 435$ nm). Retention time: 7–8 min. Limit of detection, 206 ng/L [Zheng *et al.* 2000]. Column: Nucleosil C8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L potassium hydrogen phosphate (pH 3) : methanol (65 : 35), flow rate 0.9 mL/min. Fluorescence detection ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 433$ nm). Limit of quantification, 4 pg [Breyer-Pfaff *et al.* 1996]. Column: Zorbax BP C_8 Chrompak (250 × 4.6 mm i.d., 7 to 8 µm). Mobile phase: 25 mmol/L diammonium phosphate with 35% 2-propanol (pH 6.0). Fluorometric detection ($\lambda_{ex} = 375$ nm, $\lambda_{em} = 434$ nm). Limit of detection not reported [Schouten, Bruinvels 1985].

Other **LC-MS** Meat-Based Infant Foods. Column: TSK-gel ODS-80TS (250 × 2.0 mm i.d., 5 µm). Mobile phase: ammonium formate-formic acid buffer (pH 2.8): acetonitrile (95 : 5 to 45.5 : 54.5 in 20 min for 3 min to 95 : 5 in 3 min for 10 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 1.0 ng/g, limit of detection, 0.5 ng/g [Calbiani *et al.* 2007]. Coffee. Column: Zorbax SB-C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.5% formic acid: 0.5% formic acid in acetonitrile (100 : 0 to 20 : 80 in 30 min), flow rate 0.25 mL/min. Fluorescence detection ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 433$ nm). ESI, positive ion mode. Limit of detection, 0.4 ± 0.1 µg/g [Herraiz, Chaparro 2006].

Breyer-Pfaff U *et al.* (1996). Elevated norharman plasma levels in alcoholic patients and controls resulting from tobacco smoking. *Life Sci* 58: 1425–1432.

Calbiani F *et al.* (2007). Validation of an ion-pair liquid chromatography-electrospray-tandem mass spectrometry method for the determination of heterocyclic aromatic amines in meat-based infant foods. *Food Addit Contam* 24: 833–841.

Herraiz T, Chaparro C (2006). Human monoamine oxidase enzyme inhibition by coffee and beta-carbolines norharman and harman isolated from coffee. *Life Sci* 78: 795–802.

Schouten MJ, Bruinvels J (1985). High-performance liquid chromatography of tetrahydro-beta-carbolines extracted from plasma and platelets. *Anal Biochem* 147: 401–409.

Zheng W *et al.* (2000). Determination of harmane and harmine in human blood using reversed-phased high-performance liquid chromatography and fluorescence detection. *Anal Biochem* 279: 125–129.

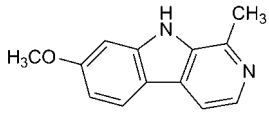
Harmine

Hallucinogen

$C_{13}H_{12}N_2O$ = 212.3

CAS—442-51-3

IUPAC Name 7-Methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole



Chemical Properties An alkaloid obtained from peganum, the dried seeds of *Peganum harmala* (Zygophyllaceae). Harmine is identical to an alkaloid known as banisterine or telepathine obtained from *Banisteria caapi* (Malpighiaceae) and with the alkaloid, yageine, from *Haemadictyon amazonicum* (Apocynaceae). It is also found (together with harmaline) in the South American hallucinogenic drink *caapi*, also known as *Yagé* and *ayahuasca*. Crystals. Mp 261°, with decomposition or sublimation. Slightly soluble in water, ethanol, chloroform and ether. pK_a 7.6 (20°). Log *P* (octanol/water), 3.56 [Sangster 1997]. Standard solutions in drug-free urine were stable for at least 9 months at –2° [Björnstad *et al.* 2009].

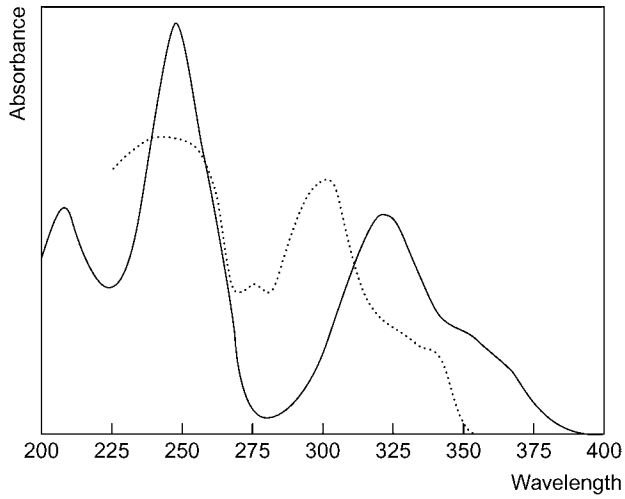
Colour Tests *p*-Dimethylaminobenzaldehyde—red; Mandelin's test—blue→green; Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.63; system TAE—R_f 0.65; system TAF—R_f 0.68; system TL—R_f 0.28; system TB—R_f 0.00; system TC—R_f 0.22; system TE—R_f 0.52 (acidified iodoplatinate solution, positive).

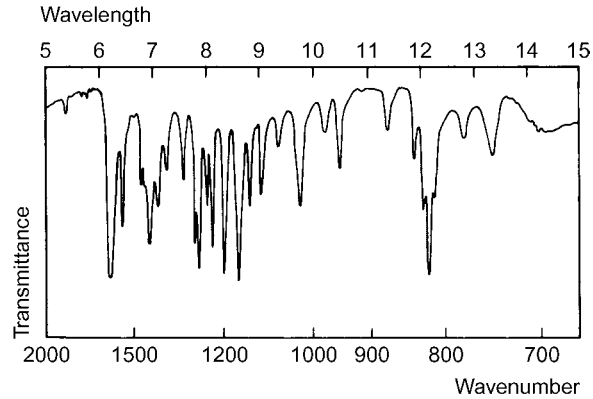
Gas Chromatography System GA—harmine RI 2291, harmine-AC RI 2545, M (*O*-desmethyl-) RI 2550, M (*O*-desmethyl-)-AC RI 2600; system GB—harmine RI 2322.

High Performance Liquid Chromatography System HA—*k* 0.8; system HX—RI 339; system HY—RI 271.

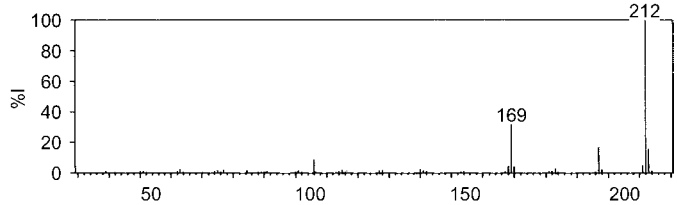
Ultraviolet Spectrum Aqueous acid—247 ($A_1^1 = 1927$a), 319 nm; aqueous alkali—243, 275, 300 nm.



Infrared Spectrum Principal peaks at wavenumbers 1165, 817, 1629, 1200, 1282, 1239 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 212, 169, 197, 213, 106, 211, 170, 168.



Disposition in the Body The P450 isoenzymes that *O*-demethylate exogenous harmine are CYP1A2 and polymorphic CYP2D6 [Yu *et al.* 2003].

Quantification

Blood **HPLC** Column: Econosphere C_{18} ODS2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 17.5 mmol/L potassium phosphate buffer (pH 6.5) : methanol (30 : 70), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 435$ nm). Retention time: 10 to 11 min. Limit of detection, 81 ng/L [Zheng *et al.* 2000].

LC-MS Column: XTerra MS-C_{18} (100 × 3.0 mm, 3.5 µm). Mobile phase: 0.02 mol/L ammonium formate : acetonitrile (75 : 25), flow rate 0.4 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Sklerov *et al.* 2005].

Plasma **HPLC** Column: Kromasil 100 C_{18} (150 × 4 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate buffer (pH 8.0)-acetonitrile : methanol (20 : 30) (63 : 37) : acetonitrile-methanol (20 : 30; 100 : 0 for 6.5 min to 68.3 : 31.7 at 8.5 min), flow rate 0.8 mL/min for 6.5 min to 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 370$ nm). Limit of quantification, 0.5 µg/L, limit of detection, 0.1 µg/L [Yritia *et al.* 2002]. Column: Supelcosil LC-DB-8 (15.0 × 4.6 mm i.d., 5 mm). Mobile phase: methanol : acetonitrile : 0.1 mmol/L ammonium acetate (pH 6.9; 20 : 20 : 60), flow rate 2.0 mL/min. Fluorescence detection ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 495$ nm). Limit of quantification, 2 µg/L [Callaway *et al.* 1996].

Urine **GC-MS** Column: HP-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: 1 mL/min. Temperature programme: 50° for 2 min to 220° at 15°/min to 300° at 30°/min. TIC, full scan mode. Retention time: 15.9 min. Limit of detection, 1 mg/L [Frison *et al.* 2008].

LC-MS Column: Hypersil GOLD (100 × 2.1 mm i.d., 5 µm). Mobile phase: 1% acetonitrile in 10 mmol/L formic acid: 60% acetonitrile in 10 mmol/L formic acid (100 : 0 to 0 : 100 over 10 min to 100 : 0 for 4 min), flow rate 200 µL/min. SRM acquisition mode. Limit of detection, 3 µg/L [Björnstad *et al.* 2009].

Bile **LC-MS** See Blood [Sklerov *et al.* 2005].

Stomach Contents **LC-MS** See Blood [Sklerov *et al.* 2005].

Brain **LC-MS** See Blood [Sklerov *et al.* 2005].

Kidney **LC-MS** See Blood [Sklerov *et al.* 2005].

Liver **LC-MS** See Blood [Sklerov *et al.* 2005].

Other **HPLC** Column: Regis REXCHROM phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% trifluoroacetic acid : 40% acetonitrile (35 : 65), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 350$ nm, $\lambda_{em} = 490$ nm). Limit of detection not reported.

LC-MS Column: Luna C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.1% formic acid (50 : 50) or methanol : 0.1% formic acid (70 : 30), flow rate 0.2 mL/min. TIS, positive ion mode. Limit of detection, 0.2 µmol/L [Yu *et al.* 2003].

Note For a review of the analysis of toxic alkaloids in plants, see Beyer *et al.* [2009].

Toxicity

Following the ingestion of *P. harmala*, an 18-year-old was admitted to hospital suffering psychomotor agitation, visual hallucinations, diffuse tremours, ataxia and vomiting. His urine concentration of harmine was 0.06 mg/mL. He recovered with no further sequelae [Frison *et al.* 2008].

A 25-year-old white male ingested a preparation from a South American tree bark, 'ooasca' and 4 h later he ingested tryptamines. He went to sleep and was found dead the following morning. The table overleaf shows the toxicological findings. The medical examiner ruled cause of death to be 'hallucinogenic amine intoxication' and the manner of death to be 'undetermined'.

Substance	Heart blood (mg/L)	Peripheral blood (mg/L)	Gastric (mg/L)	Bile (mg/L)	Brain (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	Urine (mg/L)
DMT	0.02	0.01	3.3	0.57	ND	ND	ND	0.89
5-MeO-DMT	1.88	1.20	202	9.81	0.15	22.8	16.4	9.59
Tetrahydroharmine	0.38	0.24	12.5	4.78	0.43	6.89	13.24	6.02
Harmaline	0.07	0.04	6.4	0.41	0.04	2.24	3.6	2.26
Harmine	0.17	0.08	122	1.64	0.16	0.74	2.31	1.15

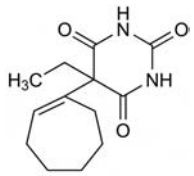
ND: None detected [Sklerov *et al.* 2005]

Beyer J *et al.* (2009). Analysis of toxic alkaloids in body samples. *Forensic Sci Int* 185: 1–9.
Björnstad K *et al.* (2009). A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1162–1168.
Callaway JC *et al.* (1996). Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *J Anal Toxicol* 20: 492–497.
Frison G *et al.* (2008). A case of beta-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. *Forensic Sci Int* 179: e37–e43.
Sangster J (1997). *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*. London: John Wiley and Sons.
Sklerov J *et al.* (2005). A fatal intoxication following the ingestion of 5-methoxy-*N*,*N*-dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 29: 838–841.
Yritia M *et al.* (2002). Determination of N,N-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of Ayahuasca. *J Chromatogr B Analyt Technol Biomed Life Sci* 779: 271–281.
Yu AM *et al.* (2003). Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic beta-carboline alkaloids harmaline and harmine. *J Pharmacol Exp Ther* 305: 315–322.
Zheng W *et al.* (2000). Determination of harmane and harmine in human blood using reversed-phased high-performance liquid chromatography and fluorescence detection. *Anal Biochem* 279: 125–129.

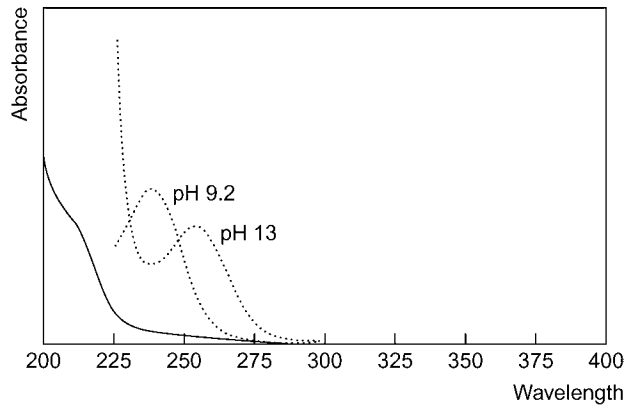
Heptabarb

Sedative, Barbiturate
$C_{13}H_{18}N_2O_3 = 250.3$
CAS—509-86-4

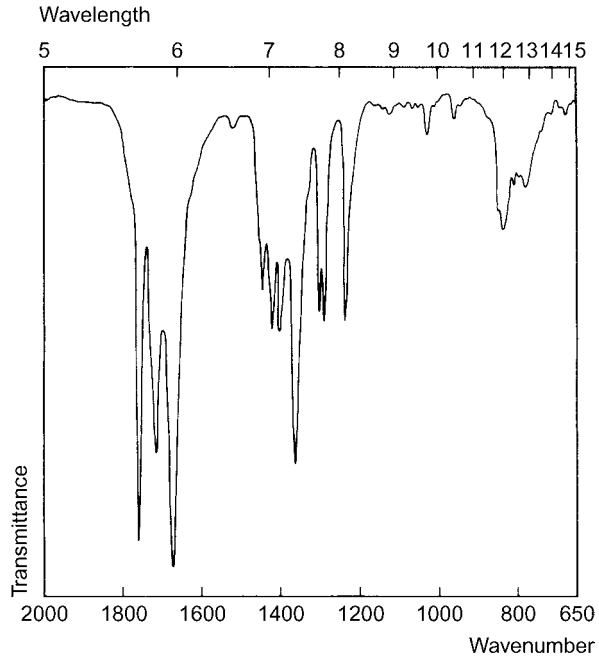
IUPAC Name 5-(1-Cyclohepten-1-yl)-5-ethyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Heptabarbital (distinguish from heptobarbitalum); heptabarbitone.
Proprietary Names *Heptadorm*; *Medomin(e)*.



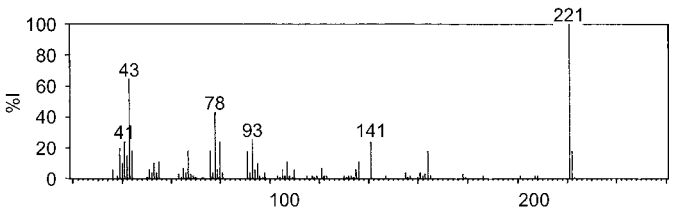
Chemical Properties A white crystalline powder. Mp 174°. Very sparingly soluble in water; soluble 1 in 30 of ethanol, 1 in 20 of acetone and 1 in 75 of chloroform; soluble in solutions of alkali hydroxides. pK_a 7.5 (20°). Log *P* (octanol/pH 7.4), 2.2.
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—violet-red/colourless.
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.38; system TF—R_f 0.64; system TH—R_f 0.62; system TAD—R_f 0.59; system TAE—R_f 0.88 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).
Gas Chromatography System GA—heptabarb RI 2055, 3′-hydroxyheptabarbital RI 2275, 3′-oxoheptabarbitone RI 2320; system GB—heptabarb RI 2110; system GF—RI 2940; system GAJ—heptabarb RRT 1.282 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 9.90; system HH—*k* 4.93; system HX—RI 416; system HY—RI 377; system HZ—retention time 3.9 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=413a); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=326b).



Infrared Spectrum Principal peaks at wavenumbers 1673, 1761, 1718, 1237, 1292, 1303 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 221, 43, 78, 93, 80, 41, 141, 39.



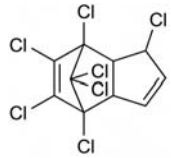
Quantification See under Amobarbital.
Disposition in the Body Readily absorbed after oral administration. Less than 1% of a dose is excreted unchanged in the urine. Major metabolites which have been identified in the urine are 3′-oxoheptabarbitone and 3′-hydroxyheptabarbitone.
Therapeutic Concentration In plasma, usually in the range 1 to 4 mg/L. After a single oral dose of 200 mg administered to 7 subjects, peak plasma concentrations of 1.3 to 2.4 mg/L (mean 1.9) were attained in 1.5 to 4 h [Breimer *et al.* 1975].
Toxicity The estimated minimum lethal dose is 2 g. Plasma concentrations greater than about 8 mg/L may produce toxic effects.
Half-life Plasma half-life, 6 to 11 h.
Volume of Distribution About 1 L/kg.
Note For a review of the clinical pharmacokinetics of barbiturates, see Breimer [1977].
Dose 150 to 400 mg daily.

Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.
Breimer DD *et al.* (1975). Pharmacokinetics and relative bioavailability of heptabarbital and heptabarbital sodium after oral administration to man. *Eur J Clin Pharmacol* 9: 169–178.

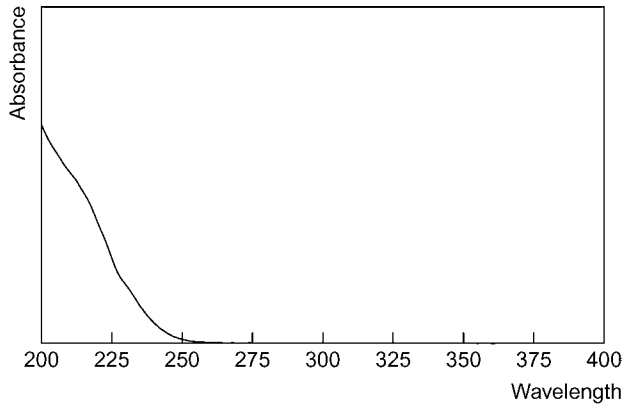
Heptachlor

Insecticide
$C_{10}H_5Cl_7 = 373.3$
CAS—76-44-8

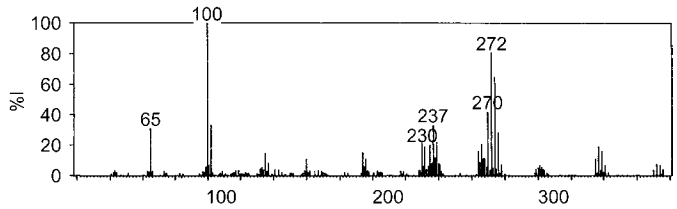
Synonym 1*H*-1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Proprietary Names *Drinox*; *Heptamul*; *Velsicol 104*.



Chemical Properties The pure substance is a white crystalline solid and the technical grade is a soft, waxy solid which contains about 72% of heptachlor and 28% of related compounds. Heptachlor may be found as an impurity in chlordane. Mp 95° to 96° (pure substance), 46° to 74° (technical grade). Practically insoluble in water; soluble 1 in 22 of ethanol. Log *P* (octanol/water), 6.1.
Thin-layer Chromatography System TB—R_f 0.74; system TE—R_f 0.89; system TF—R_f 0.75; system TX—R_f 0.84; system TY—R_f 0.97; system TAB—R_f 0.69; system TAC—R_f 0.51.
Gas Chromatography System GA—RI 1880; system GK—RRT 0.85(relative to caffeine).
High Performance Liquid Chromatography System HX—RI 1072; system HAO—*k* 0.00; system HAP—*k* 20.8.
Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 100, 272, 274, 270, 237, 102, 65, 276.

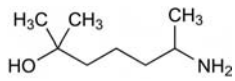


Quantification
Serum **GC** ECD. Heptachlor and other chlorinated hydrocarbon pesticides [Saady, Poklis 1990].
GC-MS Selected ion monitoring. Heptachlor and other polychlorinated biphenyl congeners [Dmitrovic *et al.* 2002].

Dmitrovic J *et al.* (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicol Lett* 134: 253–258.
Saady JJ, Poklis A (1990). Determination of chlorinated hydrocarbon pesticides by solid-phase extraction and capillary GC with electron capture detection. *J Anal Toxicol* 14(5): 301–304.

Heptaminol

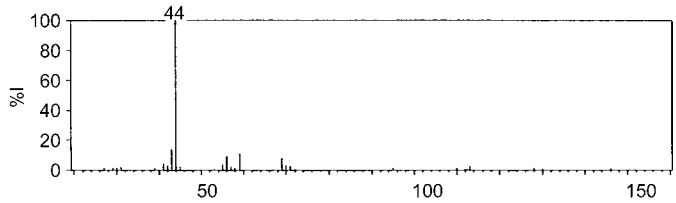
Cardiac Stimulant, Vasodilator
$C_8H_{19}NO$ = 145.2
CAS—372-66-7
IUPAC Name 6-Amino-2-methyl-2-heptanol



Chemical Properties Log *P* (octanol/water), 1.2.

Heptaminol Hydrochloride
$C_8H_{19}NO$, HCl = 181.7
CAS—543-15-7
Proprietary Names *Ampecyclal*; *Coreptil*; *Cortensor*; *Eoden*; *Hept-a-myl*; *Heptylon*.
Chemical Properties A white crystalline powder. Mp 150°. Freely soluble in water; soluble in ethanol; practically insoluble in acetone, benzene and ether.

Thin-layer Chromatography System TA—R_f 0.23; system TB—R_f 0.01; system TC—R_f 0.02; system TE—R_f 0.22; system TL—R_f 0.05; system TAE—R_f 0.14 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—heptaminol RI 1120, heptaminol-AC_2 RI 1530.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1529, 1537, 896, 1622, 1153, 1614 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 44, 43, 59, 56, 69, 55, 41, 113.

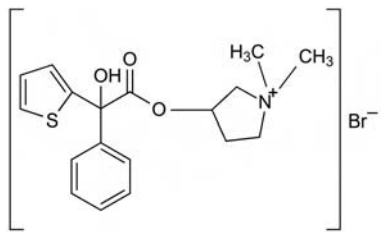


Quantification
Plasma **HPLC** Fluorescence detection. Limit of detection, 100 µg/L [Brodie *et al.* 1983].
Urine **HPLC** See Plasma [Brodie *et al.* 1983].
Dose Heptaminol hydrochloride has been given in doses of 0.3 to 1.6 g daily.

Brodie RR *et al.* (1983). Determination of heptaminol in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 274: 179–186.

Heteronium Bromide

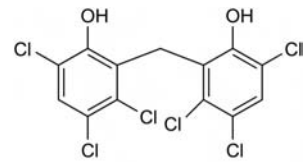
Anticholinergic
$C_{18}H_{22}BrNO_3S$ = 412.3
CAS—7247-57-6
IUPAC Name 1,1-Dimethyl-3-(α-2-thienylmandeloyloxy)pyrrolidinium bromide



Chemical Properties A white crystalline powder. Mp 182° to 184°. Very soluble in water and ethanol. Log *P* (octanol/water), −1.5.
Thin-layer Chromatography System TA—R_f 0.03 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—234 nm ($A^{1\%}_1$=235b).
Infrared Spectrum Principal peaks at wavenumbers 1735, 1219, 1085, 1234, 1063, 700 cm^{-1}.

Hexachlorophene

Disinfectant
$C_{13}H_6Cl_6O_2$ = 406.9
CAS—70-30-4
IUPAC Name 3,4,6-Trichloro-2-[(2,3,5-trichloro-6-hydroxyphenyl)methyl] phenol
Synonyms Hexachlorophane; 2,2′-methylenebis[3,4,6-trichlorophenol].
Proprietary Names *Aknefug simplex*; *Dermalex*; *Hexaphenyl*; *Lotocreme*; *Phaisohex*; *pHisoHex*; *Sapoderm*; *Ster-Zac*. It is an ingredient of *Anacal*.



Chemical Properties A white or pale buff, crystalline powder. Mp 161° to 167°. Practically insoluble in water; soluble 1 in 3.5 of ethanol, 1 in less than 1 of

acetone and 1 in less than 1 of ether; soluble in chloroform (possibly with turbidity) and propylene glycol. pK_a 5.7. Log *P* (octanol/water), 7.5.

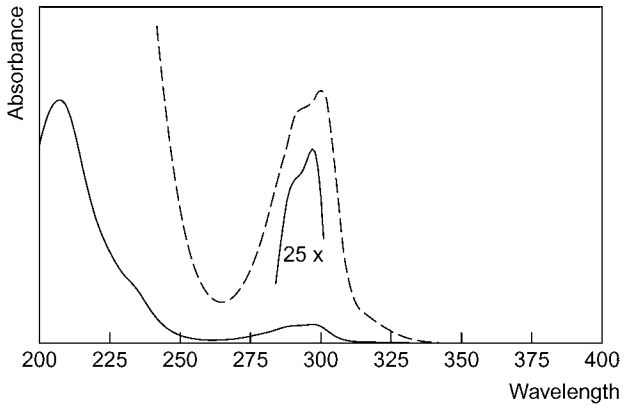
Colour Tests Ferric chloride—violet (transient); nitric acid, fuming—orange-red/orange/orange-brown.

Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.32; system TE—R_f 0.30; system TF—R_f 0.34; system TAD—R_f 0.44; system TAE—R_f 0.94; system TAF—R_f 0.98; system TAJ—R_f 0.56; system TAK—R_f 0.71; system TAL—R_f 0.91.

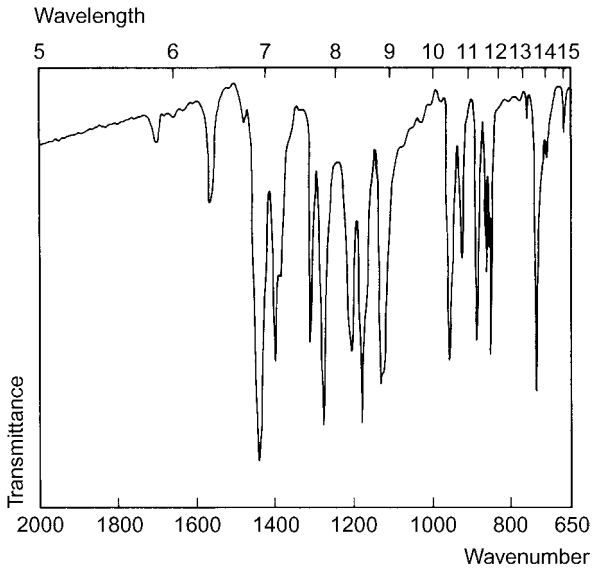
Gas Chromatography System GA—RI 2795.

High Performance Liquid Chromatography System HX—RI 936.

Ultraviolet Spectrum Ethanol—299 nm (A_1^1=155a); aqueous alkali—249 (A_1^1=400b), 320 nm (A_1^1=312a).



Infrared Spectrum Principal peaks at wavenumbers 1276, 1182, 737, 1139, 960, 1213 cm^{-1} (KBr disk).



Quantification

Blood **GC** ECD [Dodson *et al.* 1977]; [Browning *et al.* 1968].

Urine **HPLC** See Blood [Browning *et al.* 1968].

Disposition in the Body Absorbed after oral administration and through the skin. Percutaneous absorption may be significant in premature infants and through damaged skin. After topical application, up to about 10% may be excreted in the urine over a period of 4 to 5 days.

Blood Concentration

Cord-blood concentrations of 0.003 to 0.18 mg/L (mean 0.02) were reported in 50 neonates at birth. After washing once daily for 1 to 11 days with a 3% solution of hexachlorophene diluted with 50 to 100 mL of water, whole blood concentrations of 0.009 to 0.65 mg/L (mean 0.11) were attained [Curley *et al.* 1971].

After whole-body washing with 30 mL of a 3% skin-cleansing product once daily, blood concentrations in 36 adults reached a plateau of about 0.6 mg/L after 3 to 5 weeks and decreased to about 0.3 to 0.4 mg/L after 7 to 8 weeks [Calesnick *et al.* 1975].

Toxicity The estimated minimum lethal dose is 5 g. A number of deaths have occurred after accidental ingestion and also after chronic application for the treatment of burns. Repeated exposure of neonates and infants to high concentrations of hexachlorophene has been associated with spongy lesions of the brain. Fatalities have been associated with blood concentrations >2 mg/L, although recovery has occurred after development of plasma concentrations up to 90 mg/L.

In an epidemic of percutaneous poisoning which occurred in 1972 in infants and children due to exposure to a talcum powder accidentally contaminated with 6% of hexachlorophene, 36 children died. An antemortem serum concentration of 1.15 mg/L was reported in 1 child and the following postmortem tissue concentrations were reported: brain 1 to 149 µg/g (mean 21, 23 cases), kidney 17 to 43 µg/g (mean 26, 4 cases), liver 12.5 to 1080 µg/g (mean 133, 25 cases), lung 10 to 67 µg/g (mean 30, 17 cases), and skin 1 to 392 µg/g (mean 52, 30 cases) [Martin-Bouyer *et al.* 1982].

Use In soaps and creams at a concentration of 0.25 to 3%.

Browning RS *et al.* (1968). Gas chromatographic determination of hexachlorophene in blood and urine. *J Pharm Sci* 57: 2165–2166.

Calesnick B *et al.* (1975). Percutaneous absorption of hexachlorophene following daily whole body washings. *Toxicol Appl Pharmacol* 32: 204–211.

Curley A *et al.* (1971). Dermal absorption of hexochlorophane in infants. *Lancet* 2: 296–297.

Dodson W *et al.* (1977). Micromethod for measuring hexachlorophene in whole blood by gas-liquid chromatography. *Clin Chem* 23: 944–947.

Martin-Bouyer G *et al.* (1982). Outbreak of accidental hexachlorophene poisoning in France. *Lancet* 1: 91–95.

Hexaconazole

Fungicide

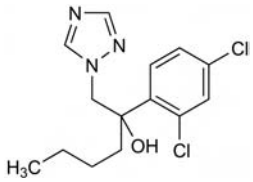
$C_{14}H_{17}Cl_2N_3O$ = 314.2

CAS—79983-71-4

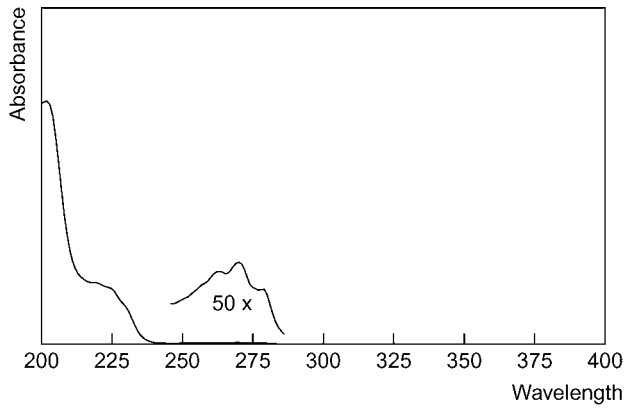
IUPAC Name 2-(2,4-Dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol

Synonyms α-Butyl-α-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-1-ethanol; R154523; PP 523; ICIA-0523.

Proprietary Names *Anvil*; *Planete*.



Chemical Properties White crystalline solid. Mp 111°. Soluble in water at 17 mg/L (20°), methanol (246 g/L at 20°), acetone (164 g/L at 20°), toluene (59 g/L at 20°) and hexane (0.8 g/L at 20°). Log *P* (octanol/water), 3.90.



Disposition in the Body Hexaconazole is rapidly excreted from the body with no significant retention of the substance in the body.

Toxicity Hexaconazole is moderately toxic by ingestion and via skin exposure.

Hexamethonium Bromide

Antihypertensive

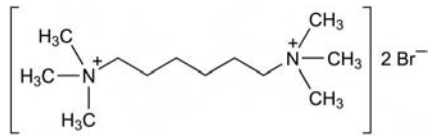
$C_{12}H_{30}Br_2N_2$ = 362.2

CAS—60-26-4 (hexamethonium); 55-97-0 (dibromide)

IUPAC Name *N*,*N*,*N*,*N*′,*N*′,*N*′-Hexamethyl-1,6-hexanediaminium dibromide

Synonyms Hexamethone bromide; hexonium bromide.

Proprietary Names *Esametina*; *Gangliostat*; *Simpatoblock*; *Vegolysen*; *Vegolysin*.



Chemical Properties A white or creamy-white hygroscopic powder. Mp 274° to 276°. Soluble 1 in less than 1 of water and 1 in 60 of ethanol; insoluble in acetone, chloroform and ether.

Hexamethonium Iodide

$C_{12}H_{30}I_2N_2$ = 456.2
CAS—870-62-2
Synonym Hexonium iodide
Proprietary Name *Hexathide*
Chemical Properties A white, slightly hygroscopic, crystalline powder. Soluble 1 in 2 of water; practically insoluble in ethanol.

Thin-layer Chromatography System TA—R_f 0.00; system TN—R_f 0.36; system TO—R_f 0.10 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 913, 970, 1630, 945, 1063, 1000 cm^{-1} (KBr disk).
Disposition in the Body
Toxicity

A healthy subject taking part in a pharmacological trial died after receiving about 1 g of hexamethonium by inhalation (a non-standard route of administration) [Ramsay 2001].

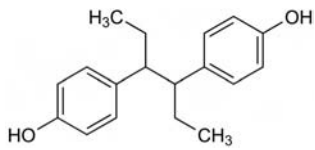
Dose Hexamethonium bromide has been given parenterally in doses of up to 500 mg daily.

Ramsay S (2001). Johns Hopkins takes responsibility for volunteer's death. *Lancet* 358: 213.

H

Hexestrol

Oestrogen
$C_{18}H_{22}O_2$ = 270.4
CAS—5635-50-7; 84-16-2 (meso)
IUPAC Name 4-[4-(4-Hydroxyphenyl)hexan-3-yl]phenol
Synonyms Dihydrostilboestrol; 4,4′-(1,2-diethyl-1,2-ethanediyl)bisphenol; hexanoestrol; hexoestrol; synestrol; synoestrol.
Proprietary Names *Cycloestrol*; *Hormoestrol* (tablets); *Synthovo*; *Syntrogene*.

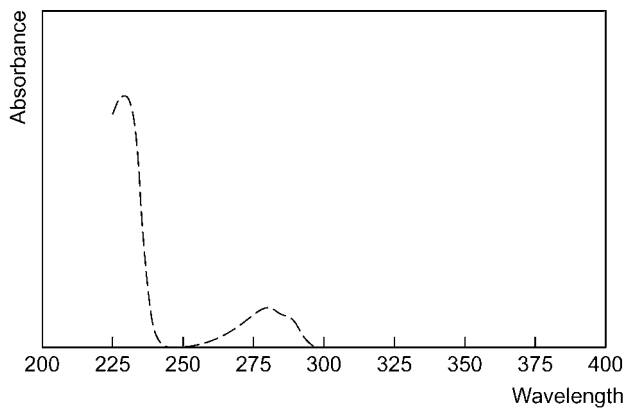


Chemical Properties Colourless crystals or white crystalline powder. Mp 185° to 188°. Practically insoluble in water and dilute mineral acids; freely soluble in ether; soluble in ethanol and acetone; slightly soluble in benzene and chloroform. Log *P* (octanol/water), 5.6.

Hexestrol Dipropionate

$C_{24}H_{30}O_4$ = 382.5
CAS—4825-53-0
Proprietary Names *Hormoestrol* (injection); *Retalon Oleosum*.
Chemical Properties A white crystalline powder. Mp 127° to 128°. Sparingly soluble in water; soluble in warm ethanol and ether.

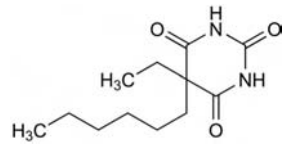
Thin-layer Chromatography System TB—R_f 0.02; system TE—R_f 0.73; system TF—R_f 0.70; system TAE—R_f 0.88.
Gas Chromatography System GA—RI 2402.
High Performance Liquid Chromatography System HX—RI 618.
Ultraviolet Spectrum Ethanol—230 (A^1_1=775b), 280 nm (A^1_1=135a); aqueous alkali—242 (A^1_1=965b), 297 nm (A^1_1=175b).



Infrared Spectrum Principal peaks at wavenumbers 1175, 1523, 1220, 840, 857, 1615 cm^{-1} (KBr disk).
Dose Hexestrol has been given in doses of 1 to 5 mg daily.

Hexethal

Barbiturate
$C_{12}H_{20}N_2O_3$ = 240.3
CAS—77-30-5
IUPAC Name 5-Ethyl-5-hexyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione

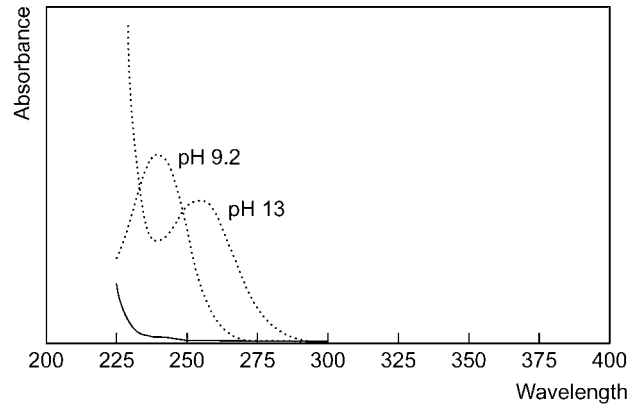


Chemical Properties pK_a 8.0. Log *P* (octanol/water), 2.5.

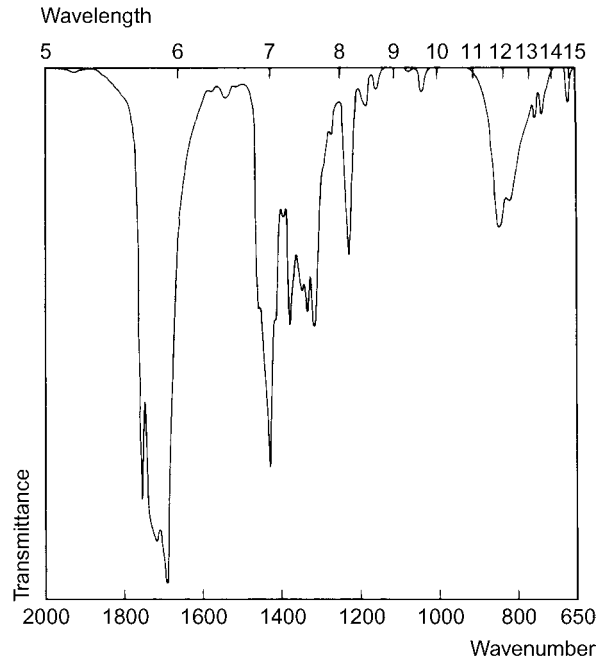
Hexethal Sodium

$C_{12}H_{19}N_2NaO_3$ = 262.3
CAS—144-00-3
Proprietary Names *Hebarel*; *Ortal Sodium*.
Chemical Properties A white or slightly yellowish powder. Mp 126°. Very soluble in water; soluble in ethanol; insoluble in ether and benzene. Aqueous solutions are unstable and decompose on standing.

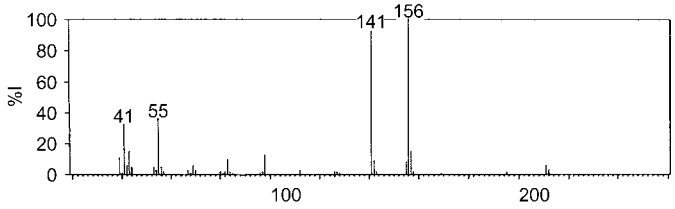
Thin-layer Chromatography System TD—R_f 0.53; system TE—R_f 0.44; system TF—R_f 0.67; system TH—R_f 0.74; system TAD—R_f 0.60.
Gas Chromatography System GA—hexethal RI 1850, hexethal-Me_2 RI 1745.
High Performance Liquid Chromatography System HG—*k* 34.28; system HH—*k* 20.39; system HY—RI 451.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—238 (A^1_1=423b); 1 mol/L sodium hydroxide (pH 13)—253 nm (A^1_1=323b).



Infrared Spectrum Principal peaks at wavenumbers 1698, 1720, 1757, 1316, 1230, 850 cm^{-1} (hexethal sodium).



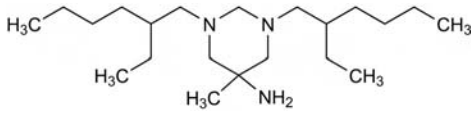
Mass Spectrum Principal ions at *m/z* 156, 141, 55, 41, 157, 43, 98, 39.



Hexetidine

Antimicrobial
$C_{21}H_{45}N_3$ = 339.6
CAS—141-94-6

IUPAC Name 1,3-Bis(2-ethylhexyl)hexahydro-5-methyl-5-pyrimidinamine
Proprietary Names *Bactidol*; *Collu-Hextril*; *Doreperol N*; *Drossadin*; *Gurfix*; *Hexatin*; *Hexifluor*; *Hexigel*; *Hexoral*; *Hextril*; *Isozid-H*; *Kleenosept*; *Oraldene*; *Oraldine*; *Oraseptic*; *Oralspray*; *Steri/Sol*; *Vagi-Hex*.



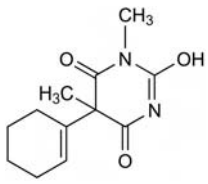
Chemical Properties A viscous oil. Mass per mL about 0.87 g. Refractive index 1.466. Practically insoluble in water; soluble in ethanol, acetone, chloroform, petroleum ether and benzene. pK_a 8.3. Log *P* (octanol/water), 5.3.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.48; system TC—R_f 0.40; system TE—R_f 0.79; system TL—R_f 0.20; system TAE—R_f 0.30; system TAF—R_f 0.91 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2093.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1093, 854, 1300, 910, 1176, 1234 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 142, 57, 42, 197, 185, 339, 240, 226.
Quantification
Saliva **HPLC** [McCoy *et al.* 2000].
Use As a 0.1% solution.

McCoy CP *et al.* (2000). Determination of the salivary retention of hexetidine in-vivo by high-performance liquid chromatography. *J Pharm Pharmacol* 52: 1355–1359.

Hexobarbital

Sedative, Barbiturate
$C_{12}H_{16}N_2O_3$ = 236.3
CAS—56-29-1

IUPAC Name 5-(Cyclohexen-1-yl)-1,5-dimethyl-1,3-diazinane-2,4,6-trione
Synonyms Ciclobarbital; 5-(1-cyclohexen-1-yl)-1,5-dimethyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; enhexymalum; enimal; hexobarbitalum; hexobarbitone; methexenyl; methyl-cyclohexenylmethyl-barbitursäure; methylhexabarbital.
Proprietary Names *Noctivane*; *Sombulex*.

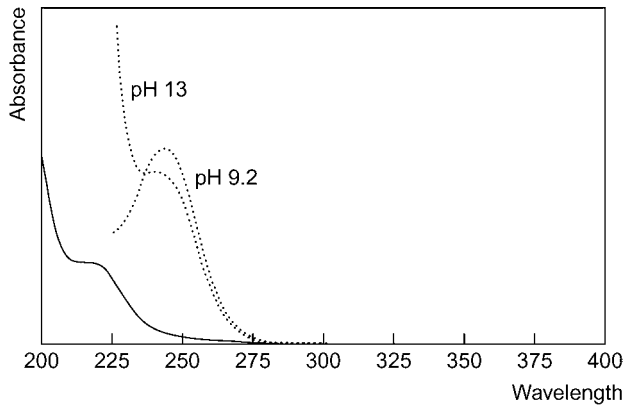


Chemical Properties Colourless crystals or a white crystalline powder. Mp 145° to 147°. Practically insoluble in water; soluble 1 in 45 of ethanol, 1 in 4 of chloroform and 1 in 80 of ether; soluble in acetone, benzene and methanol. pK_a 8.2 (20°). Log *P* (octanol/water), 2.0.

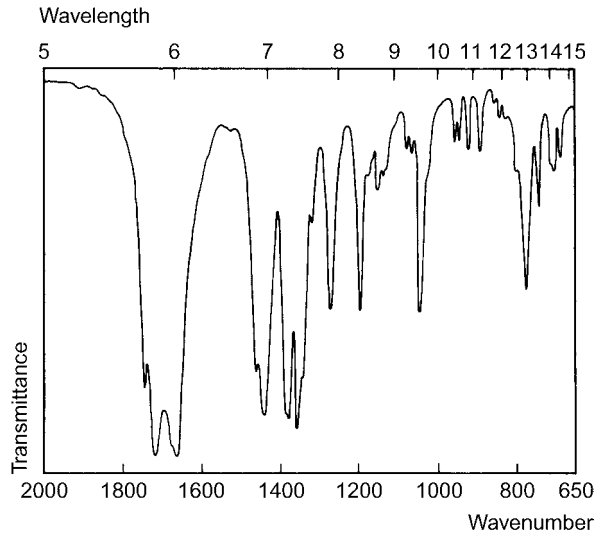
Hexobarbital Sodium

$C_{12}H_{15}N_2NaO_3$ = 258.3
CAS—50-09-9
Synonyms Enhexymalnatrium; hexenalum; sodium hexobarbitone; soluble hexobarbital.
Proprietary Names *Evipan-Natrium*; *Noctivane Sodium*.
Chemical Properties A white, very hygroscopic powder. Discolours on exposure to air. Very soluble in water; freely soluble in ethanol, acetone and methanol; practically insoluble in chloroform, ether and benzene. A solution in water slowly decomposes.

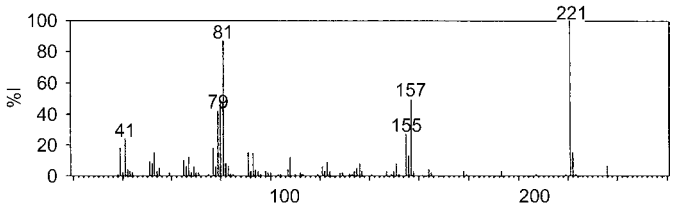
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown/violet.
Thin-layer Chromatography System TD—R_f 0.65; system TE—R_f 0.53; system TF—R_f 0.65; system TH—R_f 0.85; system TAD—R_f 0.69; system TAE—R_f 0.85 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown on violet).
Gas Chromatography System GA—hexobarbital RI 1855, M (1,5-dimethyl-5-(3-oxo-1-cyclohexen-1-yl)barbituric acid) RI 2050, M (nor-) RI 1980, M (3′-oxo-) RI 2055, M (OH-)-H_2O RI 1970, hexobarbital-Me RI 1800, M (oxo-)-Me RI 2020; system GF—RI 2380; system GAJ—hexobarbital RRT 0.940 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 7.37; system HH—*k* 5.67; system HX—RI 419; system HY—RI 242; system HZ—retention time 4.3 min; system HAL—retention time 2.4 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—243 nm (A_1^1=331a); 1 mol/L sodium hydroxide (pH 13) —243 nm (A_1^1=301b).



Infrared Spectrum Principal peaks at wavenumbers 1720, 1665, 1748, 1200, 1275, 1045 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 221, 81, 157, 80, 79, 155, 41, 77; 3′-oxohexobarbital 250, 95, 39, 235, 66, 207, 41, 193.



Quantification See also under Amobarbital.
Plasma **GC-MS** Hexobarbital enantiomers and metabolites [Prakash *et al.* 1991].
Urine **GC-MS** See Plasma [Prakash *et al.* 1991].
Disposition in the Body Readily absorbed after oral administration. The sodium salt has a very short duration of action and is usually administered intravenously. Hexobarbital is inactivated in the liver by *N*-demethylation and oxidation. About 32% of a dose is excreted in the urine in 24 h as 3′-oxohexobarbital, 5% as 3′-hydroxyhexobarbital and 18% as 1,5-dimethylbarbituric acid; <1% of a dose is excreted unchanged in the urine in 24 h.
Therapeutic Concentration In plasma, usually in the range 1 to 5 mg/L.
Following a single oral dose of 500 mg to 6 subjects, peak plasma concentrations of 4.9 to 10.9 mg/L (mean 7) were attained in about 1 h [Vermeulen *et al.* 1983].

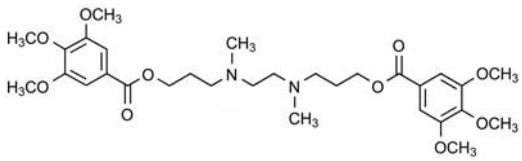
Toxicity The estimated minimum lethal dose is 2 g. Plasma concentrations greater than about 8 mg/L may produce toxic effects.
Half-life Plasma half-life, 3 to 7 h.
Volume of Distribution About 1 L/kg.
Clearance Plasma clearance, about 3.5 mL/min/kg.
Protein Binding 42 to 52%.
Note For a review of the pharmacokinetics of barbiturates, see Breimer [1977].
Dose 250 to 500 mg, as a hypnotic; up to 750 mg daily in divided doses, as a sedative.

Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.
Prakash C *et al.* (1991). Enantiospecific quantification of hexobarbital and its metabolites in biological fluids by gas chromatography/electron capture negative ion chemical ionization mass spectrometry. *Biol Mass Spectrom* 20: 559–564.
Vermeulen NP *et al.* (1983). Disposition of hexobarbitone in healthy man: kinetics of parent drug and metabolites following oral administration. *Br J Clin Pharmacol* 15: 459–464.

Hexobendine

Antianginal Vasodilator
$C_{30}H_{44}N_2O_{10}$ = 592.7
CAS—54-03-5

IUPAC Name 3-[Methyl-[2-[methyl-[3-(3,4,5-trimethoxybenzoyl)oxypropyl]amino]ethyl]amino]propyl 3,4,5-trimethoxybenzoate
Synonym 1,2-Ethanediylbis[(methylimino)-3,1-propanediyl]-3,4,5-trimethoxybenzoate

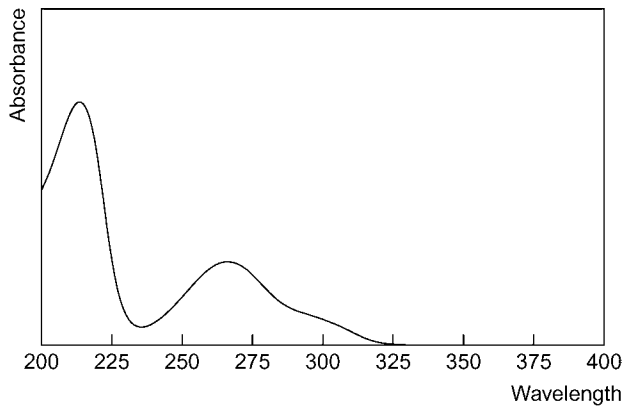


Chemical Properties Mp 75° to 77°. Log *P* (octanol/water), 2.7.

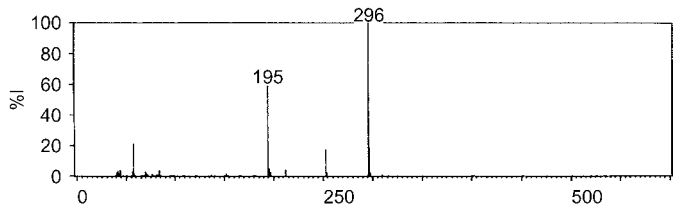
Hexobendine Hydrochloride

$C_{30}H_{44}N_2O_{10}$, 2HCl = 665.6
CAS—50-62-4
Proprietary Names *Reoxyl*; *Ustimon*.
Chemical Properties A white crystalline powder. Mp 170° to 174°. Freely soluble in water; soluble in chloroform; slightly soluble in ethanol; practically insoluble in ether.

Colour Tests Liebermann's reagent—black; Mandelin's test—violet.
Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.10; system TC—R_f 0.44; system TE—R_f 0.16; system TL—R_f 0.06; system TAE—R_f 0.12 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—265 nm (A^1_1=311a).



Infrared Spectrum Principal peaks at wavenumbers 1123, 1219, 1703, 1585, 1497, 1171 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 296, 195, 58, 297, 253, 196, 212, 84.

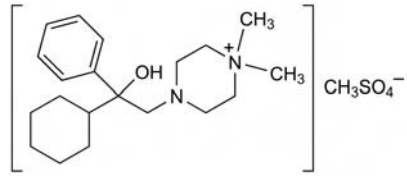


Dose Hexobendine hydrochloride has been given in doses of 60 to 180 mg daily.

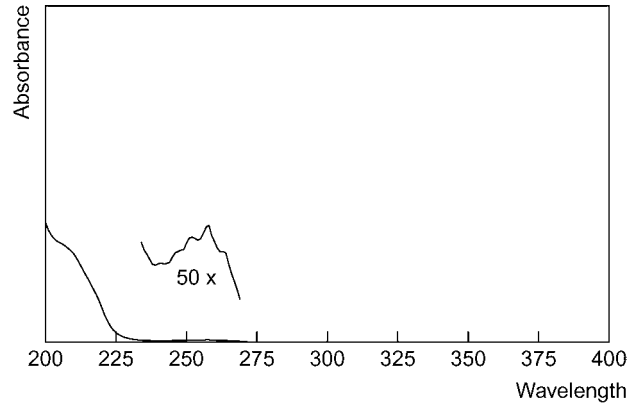
Hexocyclium Metilsulfate

Anticholinergic, Antispasmodic
$C_{20}H_{33}N_2O$, CH_3SO_4 = 428.6
CAS—6004-98-4 (hexocyclium); 115-63-9 (metilsulfate)

IUPAC Name 4-(2-Cyclohexyl-2-hydroxy-2-phenylethyl)-1,1-dimethylpiperazinium methylsulfate
Proprietary Names *Tral*; *Tralin*.



Chemical Properties A white powder. Mp 200° to 210°. Soluble 1 in 2 of water; slightly soluble in chloroform; practically insoluble in ether. Log *P* (octanol/water), 1.0.
Colour Tests Mandelin's test—blue-violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.02; system TE—R_f 0.01; system TAE—R_f 0.03 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—252, 257 (A^1_1=5.3b), 264 nm.

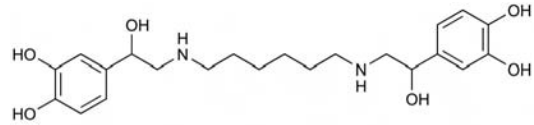


Infrared Spectrum Principal peaks at wavenumbers 1224, 1005, 748, 1052, 700, 770 cm^{-1} (KBr disk).
Dose Initially, 100 mg daily.

Hexoprenaline

Sympathomimetic
$C_{22}H_{32}N_2O_6$ = 420.5
CAS—3215-70-1

IUPAC Name 4,4′-[1,6-Hexanediylbis[imino(1-hydroxy-2,1-ethanediyl)]]bis-1,2-benzenediol
Proprietary Name *Ipradol*



Chemical Properties Crystals. Mp 162° to 165° (hemihydrate).

Hexoprenaline Hydrochloride

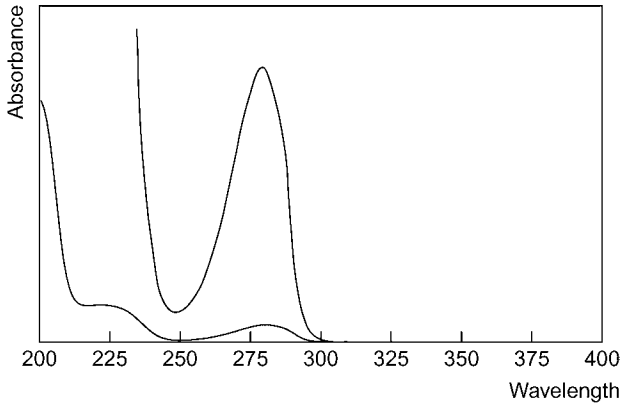
$C_{22}H_{32}N_2O_6$, 2HCl = 493.4
CAS—4323-43-7
Proprietary Name *Ipradol*
Chemical Properties Crystals. Mp 197° to 198°.

Hexoprenaline Sulfate

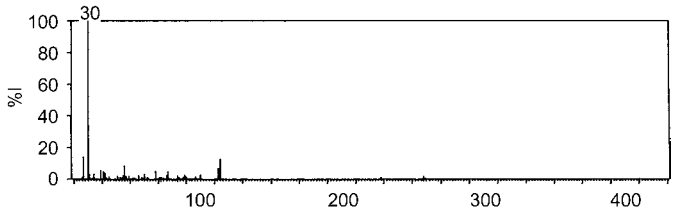
$C_{22}H_{32}N_2O_6$, H_2SO_4 = 518.6
CAS—32266-10-7
Proprietary Names *Bronalin*; *Delaprem*; *Etoscol*; *Gynipral*; *Ipradol*; *Leanol*.
Chemical Properties A white crystalline powder. Mp 214°, with decomposition. Soluble in water and dilute hydrochloric acid; practically insoluble in ethanol.

Colour Tests Ammoniacal silver nitrate—red→brown→black; ferric chloride—orange; Folin-Ciocalteu reagent—blue; Mandelin's test—orange-yellow; Marquis test—red; methanolic potassium hydroxide—orange→brown; Nessler's reagent—black; potassium dichromate (method 1)—green→brown (30 s); sulfuric acid—orange-yellow.
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.01; system TC—R_f 0.00; system TE—R_f 0.71; system TL—R_f 0.01; system TAE—R_f 0.12 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=129a).



Infrared Spectrum Principal peaks at wavenumbers 1102, 1242, 1190, 1605, 1517, 1280 cm^{-1} (hexoprenaline sulfate, KBr disk).
Mass Spectrum Principal ions at *m/z* 30, 27, 124, 56, 123, 87, 78, 41.

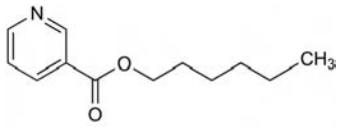


Dose Hexoprenaline sulfate has been given in doses of 0.75 to 1.5 mg daily.

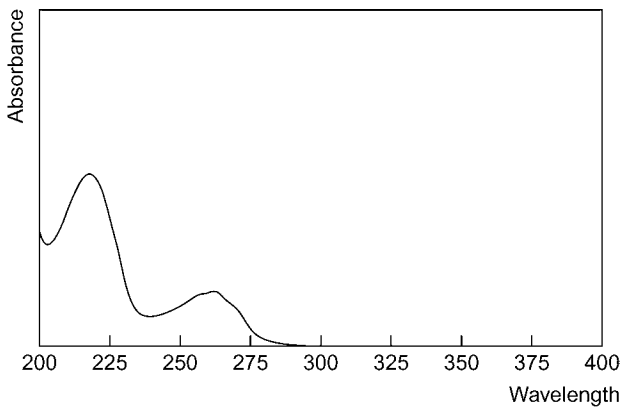
Hexyl Nicotinate

Vasodilator (Topical)
$C_{12}H_{17}NO_2$ = 207.3
CAS—23597-82-2

IUPAC Name Hexyl pyridine-3-carboxylate
Synonym *n*-Hexyl nicotinate
Proprietary Names It is an ingredient of *Hipodor*, *Transvane* and *Transvasin*.



Chemical Properties A pale yellow liquid. Practically insoluble in water; soluble in ethanol, chloroform, and methanol. Log *P* (octanol/water), 3.5.
Thin-layer Chromatography System TA—R_f 0.70 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—260 nm (A_1^1=268b).

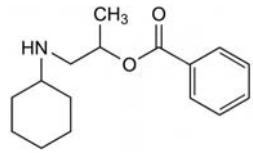


Infrared Spectrum Principal peaks at wavenumbers 1724, 1276, 1111, 741, 1022, 1590 cm^{-1} (KBr disk).
Use Topically in a concentration of 2%.

Hexylcaine

Anaesthetic (Local)
$C_{16}H_{23}NO_2$ = 261.4
CAS—532-77-4

IUPAC Name 1-(Cyclohexylamino)-2-propanol benzoate



Chemical Properties pK_a 9.1. Log *P* (octanol/water), 0.9 (hydrochloride).

Hexylcaine Hydrochloride

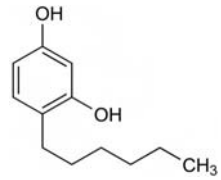
$C_{16}H_{23}NO_2$, HCl = 297.8
CAS—532-76-3
Proprietary Name *Cyclaine*
Chemical Properties A white powder. Mp 182° to 184°. Soluble 1 in 17 of water; freely soluble in ethanol and chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.01; system TE—R_f 0.80; system TAJ—R_f 0.29; system TAK—R_f 0.15; system TAL—R_f 0.70 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1965.
Ultraviolet Spectrum Aqueous acid—232 (A_1^1=485a), 275 nm (A_1^1=43a).
Infrared Spectrum Principal peaks at wavenumbers 1270, 1718, 1105, 710, 1068, 1022 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 112, 77, 105, 139, 55, 41, 96, 56.
Use Hexylcaine hydrochloride has been used in concentrations of up to 5%.

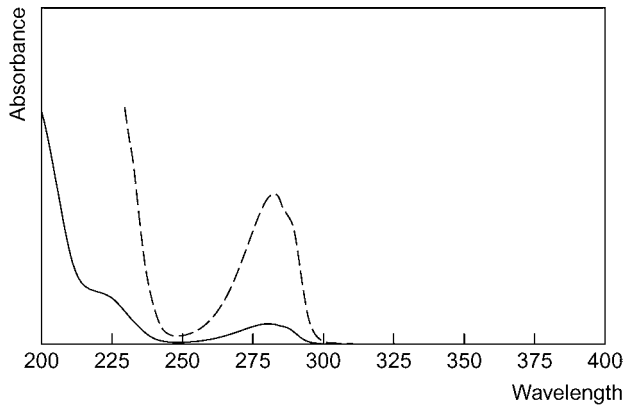
Hexylresorcinol

Anthelmintic
$C_{12}H_{18}O_2$ = 194.3
CAS—136-77-6

IUPAC Name 4-Hexyl-1,3-benzenediol
Proprietary Names *Ascaryl*; *Benylin Sore Throat Lozenge*; *Caprokol*; *Crystoids*; *Gelovermin*; *Lemsip Sore Throat*; *Mac Dual Action*; *Strepsils Extra*; *Sucrets*; *Worm-Agen*.



Chemical Properties White or yellowish-white acicular crystals, crystalline plates or crystalline powder. Acquires a brownish-pink tint on exposure to light and air. Mp 62° to 68°. Soluble 1 in 2000 of water; soluble in acetone, ethanol, chloroform and ether; slightly soluble in petroleum ether. Log *P* (octanol/water), 3.4.
Caution Hexylresorcinol is irritating to the oral mucosa, to the respiratory tract and to the skin; ethanolic solutions have vesicant properties.
Colour Test Ferric chloride—green.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.60; system TF—R_f 0.65; system TAE—R_f 0.88.
Gas Chromatography System GA—RI 1830.
High Performance Liquid Chromatography System HX—RI 579; system HY—RI 528.
Ultraviolet Spectrum Methanol—282 nm (A_1^1=160c).



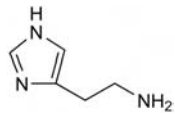
H

Infrared Spectrum Principal peaks at wavenumbers 970, 980, 1185, 1616, 1150, 1205 cm^{-1} (KBr disk).
Disposition in the Body Partly absorbed after oral administration. About 20 to 30% of a dose is rapidly excreted unchanged in the urine and the remainder is eliminated in the faeces.
Dose 1 g as a single dose.

Histamine

Diagnostic Agent (Gastric Secretion)
$C_5H_9N_3$ = 111.1
CAS—51-45-6

IUPAC Name 1*H*-Imidazole-4-ethanamine
Synonym Ergotidine
Proprietary Name *Destamin*



Chemical Properties Deliquescent acicular crystals. Mp 83° to 84°. Freely soluble in water, ethanol and hot chloroform; practically insoluble in ether. pK_a 5.9, 9.7 (25°). Log *P* (octanol/water), −0.7.

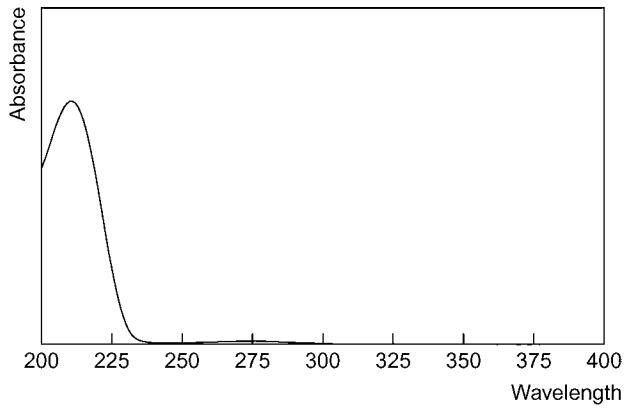
Histamine Acid Phosphate

$C_5H_9N_3$, $2H_3PO_4$ = 307.1
CAS—51-74-1
Synonyms Histamine diphosphate; histamine phosphate.
Chemical Properties Colourless crystals. Mp 130° to 133°. Soluble 1 in 4 of water; slightly soluble in ethanol; practically insoluble in ether.

Histamine Hydrochloride

$C_5H_9N_3$, 2HCl = 184.1
CAS—56-92-8
Synonym Histamine dihydrochloride
Proprietary Name *Maxamine*. It is an ingredient of *Histadestil*, *Histaglobin*, *Midalgan* and *Radalgin*.
Chemical Properties Hygroscopic colourless crystals or white crystalline powder. Mp about 245°, with decomposition. Freely soluble in water and methanol; soluble in ethanol and acetone; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.07; system TL—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.03 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1497.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



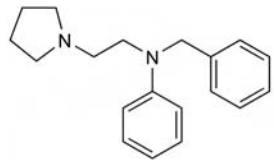
Infrared Spectrum Principal peaks at wavenumbers 840, 1622, 1522, 1084, 802, 1577 cm^{-1} (histamine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 82, 30, 81, 54, 28, 55, 83, 41.
Quantification
Biological Fluids **HPLC** Fluorescence detection [Tsikas *et al.* 1993]. Electrochemical detection. Histamine and *N*-methylhistamine [Houdi *et al.* 1987].
Tissues **HPLC** See Biological Fluids [Tsikas *et al.* 1993].
Disposition in the Body
Toxicity
Harbour workers experienced allergy-like symptoms of toxicity after handling bags containing fish flour contaminated with histamine (510 mg/100 g) [Macan *et al.* 2000].
Dose Histamine acid phosphate is given subcutaneously, in a dose of 40 µg/kg, following administration of a large dose of an antihistamine.

Houdi AA *et al.* (1987). A simple and sensitive determination of histamine and N tau-methylhistamine in biological fluids by high-performance liquid chromatography with electrochemical detection. *J Pharm Sci* 76: 398–401.
Macan J *et al.* (2000). Occupational histamine poisoning by fish flour: a case report. *Occup Med (Lond.)* 50: 22–24.
Tsikas D *et al.* (1993). Ion-pair extraction of histamine from biological fluids and tissues for its determination by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 614: 37–41.

Histapyrrodine

Antihistamine
$C_{19}H_{24}N_2$ = 280.4
CAS—493-80-1

IUPAC Name *N*-Benzyl-*N*-(2-pyrrolidin-1-ylethyl)aniline
Synonym *N*-Phenyl-*N*-(phenylmethyl)-1-pyrrolidineethanamine

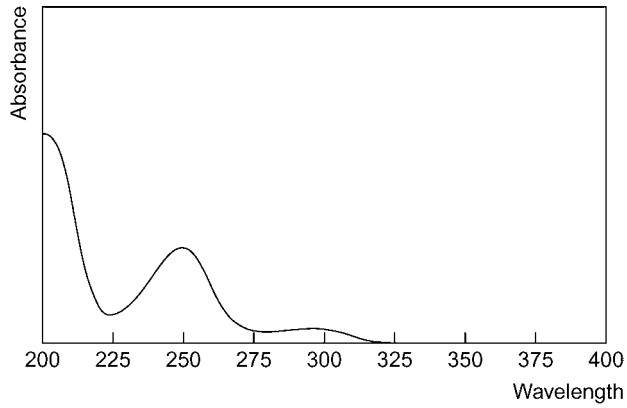


Chemical Properties An oil. Log *P* (octanol/water), 4.2.

Histapyrrodine Hydrochloride

$C_{19}H_{24}N_2$, HCl = 316.9
CAS—6113-17-3
Proprietary Names *Calcistin*; *Domistan*; *Luvistin*.
Chemical Properties A white powder. Mp 196°. Solubility in water (at 18°), 2 g in 100 mL.

Colour Test Mandelin's test—red-violet.
Thin-layer Chromatography System TA—R_f 0.60; system TE—R_f 0.75; system TAE—R_f 0.32 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—histapyrrodine RI 2240, M (OH-) RI 2500, M (oxo-) RI 2570, M (*N*-desbenzyl-) RI 1800, M (*N*-desbenzyl-oxo-) RI 2120, M (*N*-desphenyl-oxo-)-H_2O RI 2100.
High Performance Liquid Chromatography System HA—*k* 3.0.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1=141a), 295 nm.

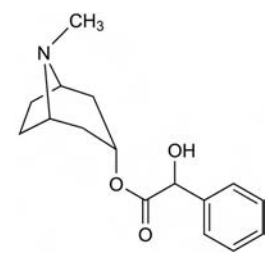


Infrared Spectrum Principal peaks at wavenumbers 1513, 1604, 750, 698, 1500, 732 cm^{-1}.
Mass Spectrum Principal ions at *m/z* M (OH-) 84, 91, 212, 296, 213, 297; M (oxo-) 91, 196, 209, 197, 275, 294; M (*N*-desbenzyl-)84, 190, 106, 111, 122, 77; M (*N*-desbenzyl-oxo-) 106, 119, 118, 98, 77, 204; M (*N*-desphenyl-oxo--H_2O 91, 159, 216, 160, 215), 84.
Dose Histapyrrodine hydrochloride has been given in doses of 50 to 150 mg daily.

Homatropine

Anticholinergic
$C_{16}H_{21}NO_3$ = 275.3
CAS—87-00-3

IUPAC Name 8-Methyl-8-azabicyclo[3.2.1]oct-3-yl-*endo*-α-hydroxybenzeneacetate
Proprietary Names *Homasedin*; *Homatropil*; *Homo*; *Pasmolit*.

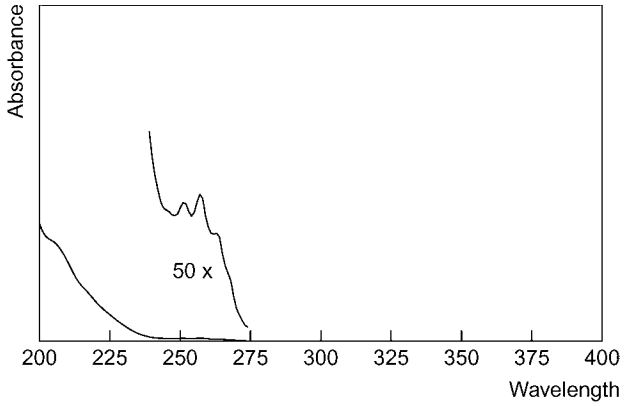


Chemical Properties Small white prismatic crystals or coarse crystalline powder. Mp 99° to 100°. Slightly soluble in water; soluble in ethanol, benzene, acetone, dilute acids, chloroform and ether. pK_a 9.9 (20°). Log *P* (octanol/water), 1.4.

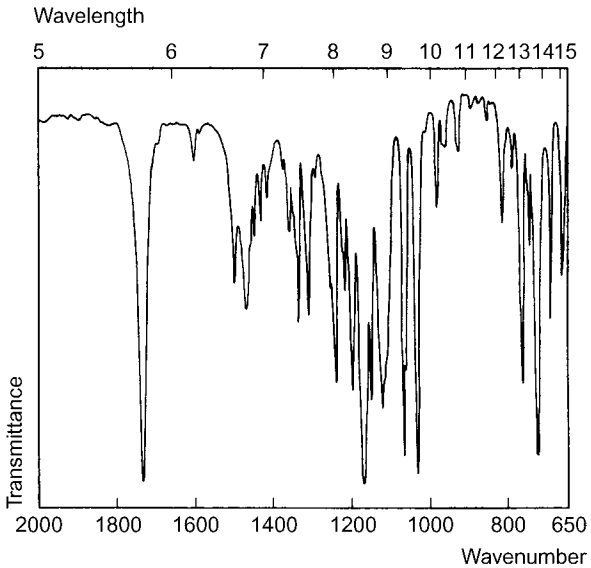
Homatropine Hydrobromide

$C_{16}H_{21}NO_3$, HBr = 356.3
CAS—51-56-9
Synonym Tropyl mandelate hydrobromide
Proprietary Names *Bufopto Homatrocel*; *Homat*; *Homatrisol*; *Homatrocil*; *Homatrop*.
Chemical Properties Colourless crystals or a white crystalline powder. Mp 214° to 217°, with decomposition. Soluble 1 in 6 of water, 1 in 60 of ethanol and 1 in 420 of chloroform; practically insoluble in ether.

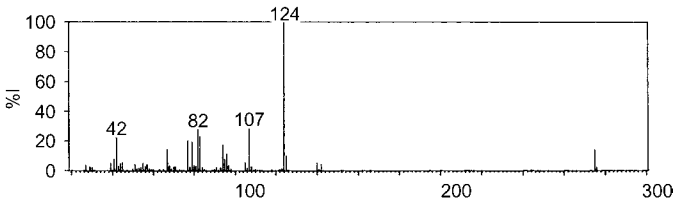
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.05; system TC—R_f 0.01; system TE—R_f 0.23; system TL—R_f 0.01; system TAE—R_f 0.07; system TAF—R_f 0.27; system TAJ—R_f 0.00; system TAK—R_f 0.02; system TAL—R_f 0.34 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2072; system GB—RI 2165.
High Performance Liquid Chromatography System HA—*k* 4.2 (tailing peak); system HX—RI 272; system HY—RI 223; system HAX—retention time 6.8 min; system HAY—retention time 3.6 min.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=7.3a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1172, 1030, 735, 1063, 1125 cm^{-1} (KBr disk).



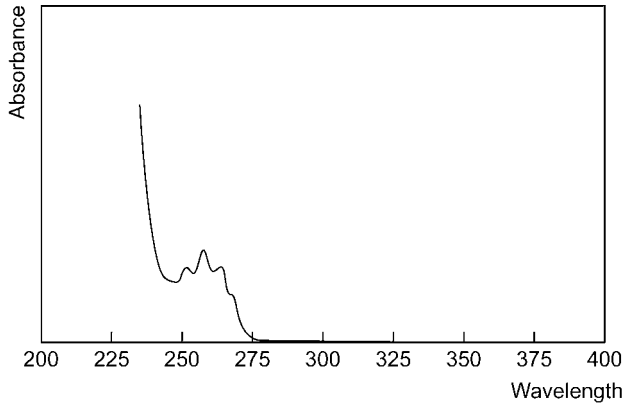
Mass Spectrum Principal ions at *m/z* 124, 107, 82, 83, 42, 77, 79, 94.



Use Homatropine hydrobromide is used as a 2% ophthalmic solution.

Homatropine Methylbromide

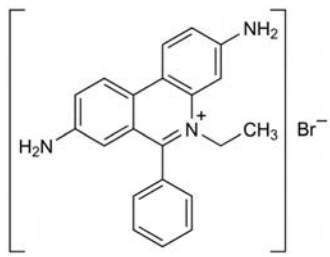
Anticholinergic
$C_{16}H_{21}NO_3$, CH_3Br = 370.3
CAS—80-49-9
IUPAC Name (8,8-Dimethyl-8-azoniabicyclo[3.2.1]octan-3-yl) 2-hydroxy-2-phenylacetate bromide
Synonyms Homatropine methobromide; 8-methyl-8-azabicyclo[3.2.1]oct-3-yl-*endo*-α-hydroxybenzeneacetate methylbromide; methylhomatropinium bromide.
Proprietary Names *Arkitropin*; *Homapin*; *Malcotran*; *Mesopin*; *Novatrin*; *Novatropina*; *Sethyl*.
Chemical Properties A white powder. Mp about 190°. Freely soluble in water and ethanol; practically insoluble in acetone and ether.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.12 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 262.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=5.8b), 264 nm.



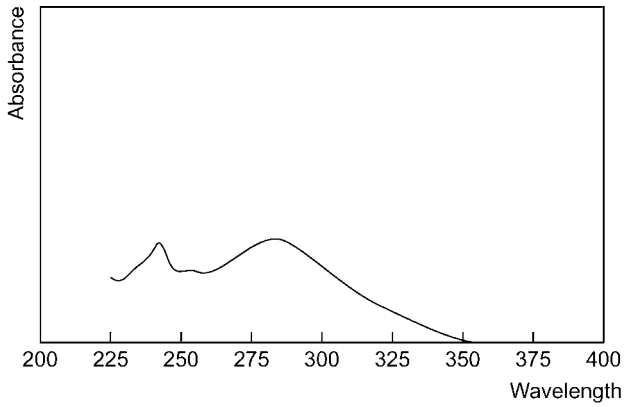
Infrared Spectrum Principal peaks at wavenumbers 1724, 1179, 1160, 1195, 1044, 935 cm^{-1} (KBr disk).
Dose Usually 12 to 40 mg daily.

Homidium Bromide

Trypanocide (Veterinary)
$C_{21}H_{20}BrN_3$ = 394.3
CAS—1239-45-8
IUPAC Name 3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide
Synonym Ethidium bromide
Proprietary Name *Dromilac*



Chemical Properties A dark purple crystalline or amorphous powder. Mp 245°, with decomposition. Soluble 1 in 20 of water and 1 in 750 of chloroform.
Colour Test The test is performed on homidium nitrate: Mandelin's test—yellow.
Thin-layer Chromatography System TA—R_f 0.55 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—242, 283 nm (A_1^1=785a).

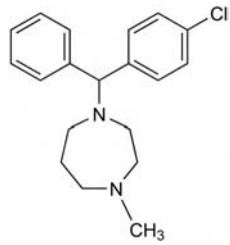


Infrared Spectrum Principal peaks at wavenumbers 1628, 1492, 1260, 1312, 836, 1077 cm^{-1} (KBr disk).

Homochlorcyclizine

Antihistamine
$C_{19}H_{23}ClN_2 = 314.9$
CAS—848-53-3

IUPAC Name 1-[(4-Chlorophenyl)phenylmethyl]hexahydro-4-methyl-1*H*-1,4-diazepine
Proprietary Name *Homoclomin (hydrochloride)*



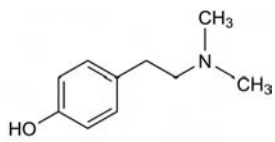
Chemical Properties A white crystalline powder. Soluble in dilute acetic acid. Log *P* (octanol/water), 4.1.
Thin-layer Chromatography System TA—R_f 0.28 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—233 (A_1^1=457b), 258, 263, 270 nm.
Infrared Spectrum Principal peaks at wavenumbers 1135, 1010, 1600, 1618, 1630, 720 cm^{-1} (KBr disk).
Quantification
Urine **HPLC** UV detection. Homochlorcyclizine enantiomers. Limit of detection, less than 50 µg/L [Nishikata *et al.* 1993].
Dose Homochlorcyclizine has been given in doses of 30 to 60 mg daily.

Nishikata M *et al.* (1993). Enantioselective pharmacokinetics of homochlorcyclizine. III. Simultaneous determination of (+)- and (–)- homochlorcyclizine in human urine by high-performance liquid chromatography. *J Chromatogr* 612: 239–244.

Hordenine

Sympathomimetic
$C_{10}H_{15}NO = 165.2$
CAS—539-15-1

IUPAC Name 4-[2-(Dimethylamino)ethyl]phenol



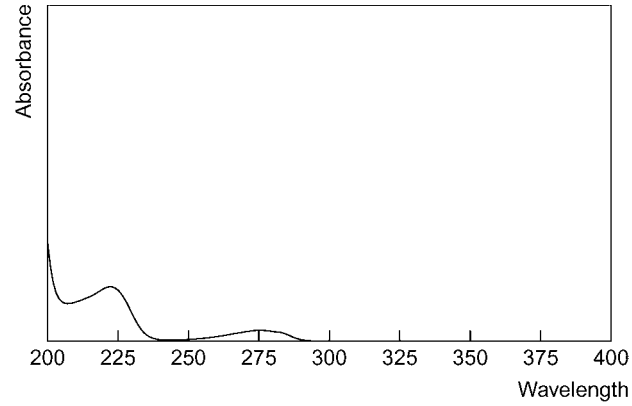
Chemical Properties Occurs naturally in germinating barley and other Gramineae. Crystals. Mp 117° to 118°. Slightly soluble in water; very soluble in ethanol, chloroform and ether; sparingly soluble in benzene, toluene and xylene; practically insoluble in petroleum ether. Log *P* (octanol/water), 1.5.

Hordenine Sulfate

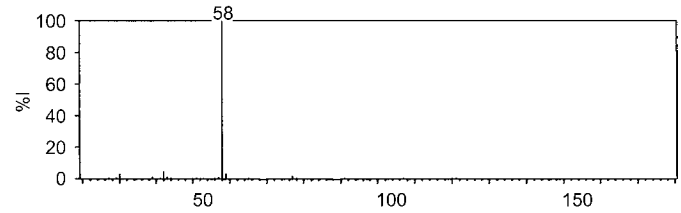
$(C_{10}H_{15}NO)_2, H_2SO_4, 2H_2O = 464.6$
CAS—622-64-0 (anhydrous); 6202-17-1 (dihydrate)

Chemical Properties Crystals. Mp 197°. Soluble in water; slightly soluble in ethanol; practically insoluble in ether.

Colour Tests Mandelin's test—grey-green; Marquis test—brown→green.
Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.05; system TC—R_f 0.06; system TE—R_f 0.52; system TL—R_f 0.05; system TAJ—R_f 0.03; system TAK—R_f 0.00; system TAL—R_f 0.20 (Acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1432.
High Performance Liquid Chromatography System HB—*k* 2.00.
Ultraviolet Spectrum Aqueous acid—274 nm.



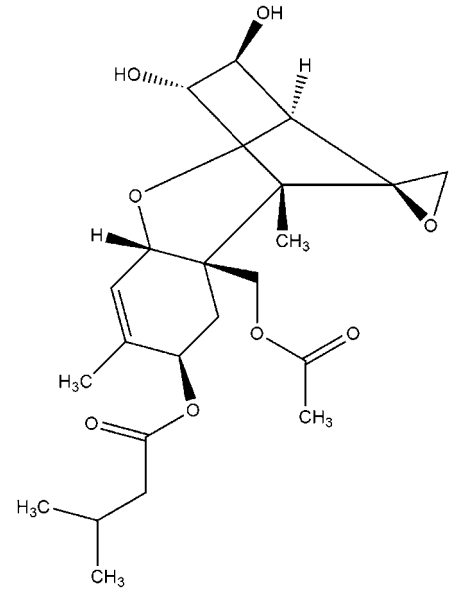
Infrared Spectrum Principal peaks at wavenumbers 1252, 1512, 820, 1612, 1270, 869 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 42, 59, 30, 77, 107, 57, 51.



HT-2 Toxin

Trichothecene Mycotoxin
$C_{22}H_{32}O_8 = 424.5$
CAS—26934-87-2

IUPAC Name 15-Acetoxy-3α,4β-dihydroxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene
Synonyms 12,13-Epoxytrichothec-9-ene-3,4,8,15-tetraol 4,15-diacetate 8-isovalerate; mycotoxin HT 2; NSC 278571; Toxin HT 2.



	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Deoxynivalenol	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyldeoxynivalenol	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	**65 : 35**	**0.23**	**0.32**	**65 : 35**	**0.65**	**0.58**	**N/A**	**N/A**	**N/A**
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Nivalenol	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989].

H

Chemical Properties HT-2 toxin is a trichothecene mycotoxin produced by a saprophyte *Fusarium sporotrichoides* and other *Fusarium* spp. It is not normally found in grains at harvest but results from water damage to grain, which provides favourable conditions for the growth of *Fusarium* spp. [WHO/JECFA 2002]. White solid. Mp 151° to 152°. Soluble in dichloromethane, ethanol, ethyl acetate and DMSO; slightly soluble in petroleum ether; very slightly soluble in water. For a study of the stability of tricothecenes in calibrant samples, see [Widestrand, Pettersson 2001].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ = 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as in the table above.

High Performance Liquid Chromatography Column: C_{18} (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water (70 : 30), flow rate 3 mL/min. UV detection (λ = 278 nm). Retention time: 9.6 min. Limit of detection, 30–50 ng for HT-2 toxin and other trichothecene mycotoxins [Yagen *et al.* 1986].

Ultraviolet Spectrum No UV absorption [Rejakylä *et al.* 1987; Sydenham *et al.* 1996].

Quantification

Blood **GC-MS** MRM acquisition mode. Limit of detection, 0.5 ppb for HT-2 and T-2 toxins [Pawlosky *et al.* 1989]. Column: BP-5 (12 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 8 psi. Temperature programme: 90° for 2 min to 180° at 20°/min to 240° at 5°/min for 2 min. CI, negative ion mode, SIM acquisition mode. Limit of detection, 2–7 ppb for HT-2, deoxynivalenol, nivalenol, T-2, T-2 tetraol, diacetoxyscirpenol, scirpentriol, 15-acetylscirpenol (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986]. Column: Varian 3700 J&W DB-5 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 35 cm/s. Temperature programme: 50° for 2 min to 300° at 10°/min for 8 min. CI, SIM acquisition mode. Limit of detection, 0.7 ng/g [D'Agostino *et al.* 1986].

Plasma **GC** Column: OV-17. ECD. Limit of detection, 5 µg/L for HT-2 and 30 µg/L for T-2 [Yagen *et al.* 1986].

Urine **GC-MS** See [Pawlosky *et al.* 1989]. Column: BP-1 methyl silicone (25 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 15 psi. Temperature programme: 160° for 1 min to 275° at 10°/min, for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of detection, 2 to 7 ppb for HT-2, deoxynivalenol, nivalenol, T-2 toxin, T-2-tetraol, diacetoxyscirpenol, scirpentriol and 15-acetylscirpenol (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986].

Other **TLC** Liquid Culture. Fluorometric detection [Kotsonis, Ellison 1975].

GC Cereals. ECD. Limits of quantification, 40 to 200 µg/kg for HT-2 and other trichothecenes [Kotal *et al.* 1999]. Bananas. Fluorescence detection. HT-2 toxin and other mycotoxins [Jimenez, Mateo 1997]. *In vitro* Bovine Ruminal Fluid. Column: DB-1701 capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 250° to 275° at 5°/min for 5 min for TMS or 225° to 275° at 5°/min for 5 min for trifluroacetic acid. Retention time: 8.26 min [Swanson *et al.* 1987]. Cereal Samples. Column: SE-52 (12 m × 0.25 mm i.d.). Carrier gas: H_2, 40 kPa. Temperature programme: 180° to 260° at 4°/min. FID. Limit of detection, for HT-2 and other fusariotoxins, 100 ppb [Bata *et al.* 1983].

GC-MS Wheat Samples. Column: HP-5 capillary (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: N_2, 1 mL/min. Temperature programme: 80° for 1 min to 160° at 30°/min to 183° at 1°/min to 280° at 12°/min for 5 min. ECD. Limit of quantification, 20 µg/kg for HT-2 toxin and other trichothecenes, limit of detection, 10 µg/kg [González *et al.* 2008]. Soy Sauce Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 140° for 2 min to 275° at 7°/min for 2 min to 290° at 30°/min for 5 min. Limit of detection, 1–19 µg/kg for HT-2 toxin and other *Fusarium* toxins [Schollenberger *et al.* 2007]. Food Samples. Column: Rtx-200 (60 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 115° for 5 min to 125° at 50°/min to 300° at 5°/min for 10 min. EI ionisation at 70 eV, positive ion mode. Retention time: 24.6 min for deoxynivalenol, 28.8 min for 3-acetyldeoxynivalenol, 28.3 min for 15-acetyldeoxynivalenol, 26.9 min for nivalenol, 26.4 min for fusarenone-X, 29.4 min for diacetoxyscirpenol, 30.1 min for neosolaniol, 33.7 min for HT-2 and 34.5 min for T-2. Limit of quantification, 0.30 µg/kg for deoxynivalenol, 0.26 µg/kg for 3-acetyldeoxynivalenol, 0.19 µg/kg for 15-acetyldeoxynivalenol, 0.28 µg/kg for nivalenol, 0.16 µg/kg for fusarenone-X, 0.36 µg/kg for diacetoxyscirpenol, 0.37 µg/kg for neosolaniol, 0.26 µg/kg for HT-2 and 0.18 µg/kg for T-2, limit of detection, 0.16 µg/kg [Schothorst *et al.* 2005]. Grain Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 80° to 245° at 60°/min for 3 min to 260° at 3°/min to 270° at 10°/min for 7 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of quantification, 30 µg/kg for deoxynivalenol, 3-acetyldeoxynivalenol, fusarenone-X and diacetoxyscirpenol; 20 µg/kg for HT-2 and T-2; 30 µg/kg for nivalenol (*N*,*O*-bis (trimethylsilyl)acetamide: trimethylchlorosilane: *N*-trimethylsilylimidazole derivatives) [Jestoi *et al.* 2004]. Food and Food Products. Column: fused silica capillary. EI and NCI or PCI mode. Limit of quantification, 50–5000 pg, limit of detection, 10–50 pg for HT-2 and T-2 toxins [Melchert, Pabel 2004]. *In vitro* Bacterial Suspensions. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 80° to 245° at 60°/min for 3 min to 260° at 4°/min to 270° at 10°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported for HT-2 toxin and other *Fusarium* toxins [el Nezami *et al.* 2002]. Fungal Extracts. Column: HP-5 (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 40 cm/s. Temperature programme: 80° for 1 min to 160° at 40°/min to 205° at 4°/min to 240° at 8°/min to 300° at 40°/min for 3 min. EI ionisation, positive ion mode, CID or NICI mode. Limit of detection, 30–70 pg or 50–120 pg (NICI) for HT-2 toxin and other trichothecenes [Nielsen, Thrane 2001]. Spiked Barley. Column: HP-5 5% phenylmethylsiloxane capillary (25 m × 0.2 mm i.d., 0.3 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 333K for 1 min to 473K at 20K/min to 553K at 10K/min for 10 min. EI ionisation at 70 eV. Limit of detection, 0.005–0.05 mg/kg for HT-2 toxin and other trichothecenes [Kostiainen, Nokelainen 1990]. 'Yellow Rain' Powder Sample. Column: glass packed with 3% OV-17 on 100/120 mesh Chromosorb (1.0 m × 2.0 mm i.d.). Carrier gas: He, 25 mL/min. Temperature programme: 180° to 260° at 12°/min for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 2.3 min for deoxynivalenol, 6.2 min for T-2, 5.5 min for HT-2, 6.5 min for zearalenone and 4.0 min for diacetoxyscirpenol (trimethylsilyl derivatives). Limit of quantification not reported [Rosen, Rosen 1982].

HPLC Oat-based Media. DAD. Limit of detection, 0.459 mg/kg for HT-2 toxin and 0.508 mg/kg for T-2 toxin [Medina *et al.* 2010]. Cereal Grains. Fluorescence detection. Limit of detection, 2.3–6.3 ng for HT-2 toxin and 2–10 ng for T-2 toxin [Lippolis *et al.* 2008]. Cereals. Fluorescence detection. Limit of quantification, 8 µg/kg for HT-2 and T-2 toxins [Trebstein *et al.* 2008]. Eggs (whole with shells removed). Fluorescence detection. Limit of detection, 5 µg/L for HT-2 and T-2 toxins [Maragos 2006]. Cereal Grains. Column: reversed phase. Fluorimetric detection (λ_{ex} = 381 nm, λ_{em} = 470 nm). Limit of detection, 3 µg/kg for HT-2 toxin and 5 µg/kg for T-2 toxin [Visconti *et al.* 2005]. Grain Samples. Column: C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (65 : 35) containing 0.75% acetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 292 nm, λ_{em} = 425 nm). Limit of detection, 0.4 µg/kg for deoxynivalenol and HT-2, 0.2 µg/kg for nivalenol and diacetoxyscirpenol, 1.0 µg/kg for 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol, and 0.6 µg/kg for fusarenone-X and T-2 (coumarin-3-carbonyl derivatives) [Dall'Asta *et al.* 2004; Mateo *et al.* 2001]. Column: LiChrospher 100 C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (65 : 35) containing 0.75% acetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 292 nm,

λ_{em}= 425 nm). Retention time: ≈8.5 min. Limit of detection, 10 ng/g for T-2 toxin) and 15 ng/g for HT-2 toxin and other trichothecenes [Jimenez *et al.* 2000]. Cereal Grains. Limit of detection, 0.02–0.15 mg/kg for HT-2 toxin and other mycotoxins [Stratton *et al.* 1993].

LC-MS Wheat Flour. Modified QuEChers method. H-2 toxin and other trichothecenes [Sospedra *et al.* 2010]. Cereals. QuEChers-like method and accelerated solvent extraction. ESI. Limit of quantification, 5–100 μg/kg for HT-2 toxin and T-2 toxin [Desmarchelier *et al.* 2010]. Food Grains. Limit of detection, 0.005 mg/kg for HT-2 toxin and 0.002 mg/kg for T-2 toxin [Sedova *et al.* 2009]. Food Supplements (soy isoflavones, St John's Wort, garlic, *Ginkgo biloba*, Black radish). Acquity UPLC system coupled to a Micromass Quattro triple quadropole MS. Limit of quantification, 1–100 μg/kg, limit of detection, 0.3–30 μg/kg, for HT-2 toxin and other mycotoxins [Diana Di Mavungu *et al.* 2009]. Cereals (maize, wheat, oats). Total sum of HT-2 and T-2 toxins for food (peanuts, pistachios, wheat, maize, cornflakes, raisins, figs). Column: Alltima C_{18} (150 × 3.2 mm i.d., 5 μm). Mobile phase: 0.1% formic acid : 0.1% formic acid in acetonitrile (90 : 10 to 30 : 70 at 12 min for 4 min to 10 : 90 at 17.5 min for 2.5 min to 90 : 10 at 21 min for 4 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode, positive ion mode. Limit of detection, 50 μg/kg [Lattanzio *et al.* 2009; Spanjer *et al.* 2008]. Cereals and Cereal-based Products (infant foods, snacks, biscuits, wafers). APCI. Limit of detection, 0.4–1.7 μg/kg for HT-2 toxin and 0.4–1.0 μg/kg for T-2 toxin [Lattanzio *et al.* 2008]. Maize. Column: reversed phase. Mobile phase: methanol : water containing 0.5% acetic acid and 1 mmol/L ammonium acetate. ESI. Limit of detection, 0.3–4.2 μg/kg for H-2 toxin and other mycotoxins [Lattanzio *et al.* 2007]. Corn, Wheat, Cornflakes and Biscuits. Column: Zorbax Eclipse XDB-C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : methanol (90 : 10 to 0 : 100 in 40 min), flow rate 200 μL/min. APCI. Limit of detection 0.1–6.1 ng/g for HT-2 toxin and other mycotoxins [Tanaka *et al.* 2006]. Cereals and Cereal-based Foods. ESI. Limit of detection, 0.3–5 ng/g for HT-2 toxin and other trchothecenes [Klötzel *et al.* 2006]. Maize. APCI. Limit of detection, 0.3–3.8 μg/kg [Berthiller *et al.* 2005]. Wheat Flour. Column: LC-18 Supelcosil (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol (80 : 20 to 35 : 65 in 9 min for 8 min to 10 : 90 for 5 min to 80 : 20 for 7 min), flow rate 1.0 mL/min. ESI, positive ion mode. Limits of quantification, 10–20 ng/g for HT-2 toxin and other trichothecenes [Biancardi *et al.* 2005]. Column: SB-RP18-Zorbax (150 × 3.0 mm i.d., 3.5 μm). Mobile phase: methanol : water (30 : 70 to 100 : 0 in 8 min), flow rate 250 μL/min. ESI, negative and positive ion mode, MRM acquisition mode. Retention time: 13.6 min. Limit of quantification, 0.02–10 ppb for HT-2 toxin and other mycotoxins [Biselli, Hummert 2005]. Wheat and Oat Samples. ESI. Limit of detection, 0.18–5.0 ng/g [Klötzel *et al.* 2005]. Bovine Milk. Column: Hypersil ENV or Luna C_{18}. Positive ion mode. Limit of detection, 0.020–0.15 μg/L for HT-2 toxin and 17 other mycotoxins [Sorensen, Elbaek 2005]. Barley Tea and Beer. Column: ODS. Mobile phase: water: methanol : acetonitrile, flow rate 0.2 mL/min. HT-2 toxin and other trichothecenes [Suga *et al.* 2004]. Spiked Oats and Naturally Contaminated Oats, Maize, Wheat and Barley. Column: ODS-2 C_{18} (200 × 2.1 mm i.d., 5 μm). Mobile phase: 1 mmol/L ammonium acetate : acetonitrile (80 : 20 to 0 : 100 in 5 min for 3 min to 80 : 20 for 10 min), flow rate 0.3 mL/min. APCI, positive ion mode, SIM acquisition mode. Limit of detection, 50–80 ng/g for HT-2 toxin and other type A trichothecenes [Razzazi-Fazeli *et al.* 2002]. Wheat Samples. Column: C_{18} (125 × 2.0 mm i.d., 3 μm). Mobile phase: methanol : water (25 : 75 to 2 : 98 over 12 min), flow rate 0.25 mL/min. APCI, positive ion mode, full-scan mode. Limit of quantification, 50 ppb for deoxynivalenol and neosolaniol, 100 ppb for nivalenol and 15-acetyldeoxynivalenol, 40 ppb for fusarenone-X, 25 ppb for3-acetyldeoxynivalenol, 20 ppb for diacetoxyscirpenol, 10 ppb for HT-2, 60 ppb for T-2, limit of detection, 3 ppb [Berger *et al.* 1999].

Note For a screening immunoassay for HT-2 and T-2 toxins in cereal and baby foods, see Meneely *et al.* [2010]; for ELISA assays for screening cereals and other food or fodder for mycotoxins, see Nikulin *et al.* [1996], Stratton *et al.* [1993] and Yoshizawa *et al.* [2004]. For an assessment of immunochemical methods for the analysis of trichothecene mycotoxins, see Park, Chu [1996]. For an assessment of methods for the analysis of trichothecene, see Koch [2004]. For an HPLC screening method for mycotoxins, see Kuronen [1989]. For the determination of 39 mycotoxins in wheat and maize by LC-MS, see Sulyok *et al.* [2006]. For a review of LC-MS methods of mycotoxin analysis in biological and food matrices, see Zollner, Mayer-Helm [2006]. For a purity assessment study of mycotoxin standards comparing UV, HPLC-UV, GC-ECD, GC-FID, GC-MS, LC-MS, DSC, NMR and FT-IR methods, see Krska *et al.* [2005]. For the stability of HT-2, T-2 and T-2 tetraol in blood and urine, see Pace, Matson [1988].

Disposition in the Body In humans, T-2 toxin undergoes rapid metabolism to its main metabolite HT-2 toxin. In animals, the major metabolic pathways of T-2 toxin are hydrolysis, hydroxylation, de-epoxidation and conjugation. HT-2 toxin formed from T-2 toxin then undergoes further hydroxylation to yield 3′-hydroxy-HT-2 toxin which is considered an activation pathway. T-2 toxin also undergoes metabolism to T-2 tetraol, which is an inactivation pathway [Ueno 1986; Wu *et al.* 2010]. See also T-2 toxin.

Toxicity LD_{50} in chickens (oral) 7.22 mg/kg; in guinea pigs (oral) 0.5 mg/kg; in mice (oral) 3.8 mg/kg, (IP) 6.5 mg/kg, (SC) 6.7 mg/kg; in rats: (SC) 1 mg/kg, (intracerebral) 0.052 mg/kg.

Note For toxicity data on HT-2 toxin and other mycotoxins, see Schlatter [2004], and Wu *et al.* [2010]; for reviews of trichothecene toxicology and potential effects on humans, see Pestka, Smolinski [2005], Rotter *et al.* [1996], Sudakin [2003] and Wu *et al.* [2010].

Half-life 19.6 min (after IV administration in dogs) [Sintov *et al.* 1986].

Volume of Distribution 4.47 L/kg (after IV administration in dogs) [Sintov *et al.* 1986].

Clearance 0.167 L/min/kg (after IV administration in dogs) [Sintov *et al.* 1986].

Dose For a report of the mycotoxin content of UK organic and conventional oats, see Edwards [2009]. The tolerable daily intake for the sum of HT-2 and T-2 toxins has been established as 0.06 μg/kg [Schlatter 2004]. The average daily intake of HT-2 toxin has been estimated to be 9 ng/kg [WHO/JECFA 2002]. It has been reported that the trichothecenes have been used as an agent of biological/chemical warfare ('yellow rain') [Mirocha *et al.* 1983; Rosen, Rosen 1982; Stark 2005].

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Hydralazine

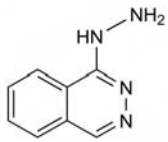
Antihypertensive

$C_8H_8N_4$ = 160.2

CAS—86-54-4

IUPAC Name 1(2*H*)-Phthalazinone hydrazone

Synonym Hydrallazine



Chemical Properties Yellow crystals. Mp 172° to 173°. Soluble 1 in 12 of warm methanol. pK_a 0.5, 7.1. Log *P* (octanol/water), 1.0.

Hydralazine Hydrochloride

$C_8H_8N_4$, HCl = 196.6

CAS—304-20-1

Synonym Apressinum

Proprietary Names *Alphapress*; *Apresolina*; *Apresolin(e)*; *Cesoline*; *Hydrapres*; *Hyperphen*; *Novo-Hylazin*; *Nu-Hydral*; *Rolazine*.

Chemical Properties A white crystalline powder. Mp 273°, with decomposition. Soluble 1 in 25 of water and 1 in 500 of ethanol; very slightly soluble in ether; practically insoluble in chloroform.

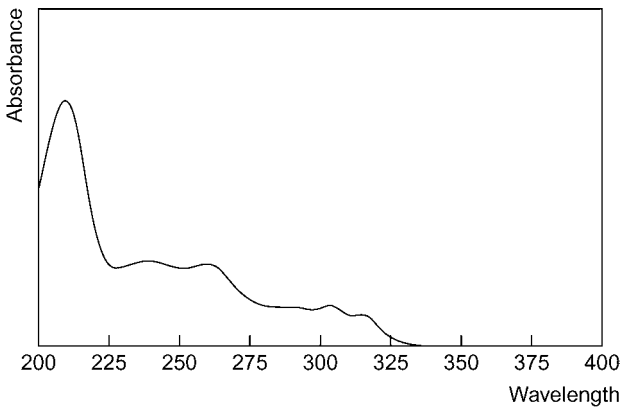
Colour Test Nessler's reagent—black.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.41; system TC—R_f 0.11; system TE—R_f 0.80; system TL—R_f 0.64; system TAE—R_f 0.73; system TAJ—R_f 0.01; system TAK—R_f 0.01; system TAL—R_f 0.25 (acidified iodoplatinate solution, positive).

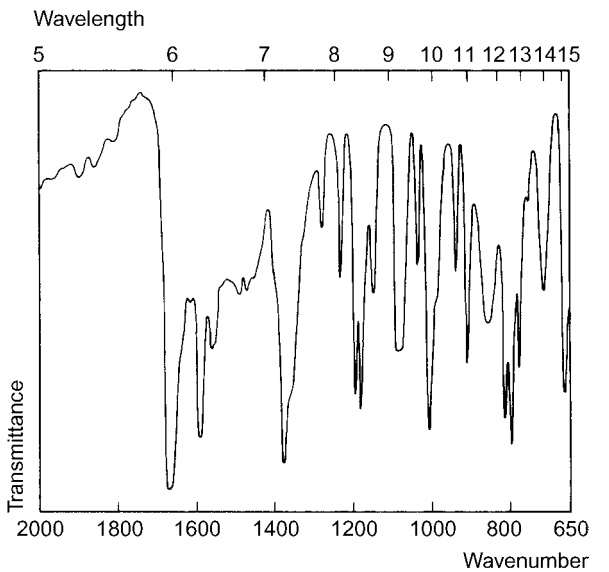
Gas Chromatography System GA—RI 1528; system GB—RI 1914.

High Performance Liquid Chromatography System HX—RI 193; system HY—RI 132; system HZ—retention time 1.9 min; system HAX—retention time 6.5 min; system HAY—retention time 3.5 min.

Ultraviolet Spectrum Water—240 (A^1_1=675a), 260 (A^1_1=675a), 304, 315 nm.

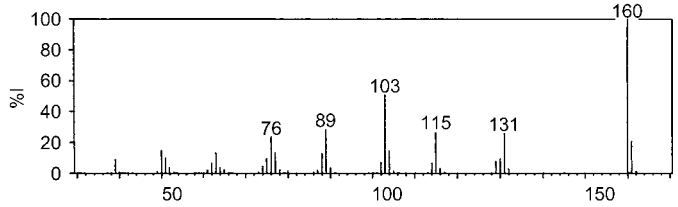


Infrared Spectrum Principal peaks at wavenumbers 1665, 790, 1582, 1000, 810, 1175 cm^{-1} (hydralazine hydrochloride, KBr disk).



H

Mass Spectrum Principal ions at *m/z* 160, 103, 89, 131, 115, 76, 161, 104.



Quantification

Blood **HPLC** Limit of detection, 300 ng/L [Semple *et al.* 1988]. UV detection. Limit of detection, 1 µg/L [Ludden *et al.* 1983].

Plasma **HPLC** Electrochemical detection [Wong *et al.* 1987]. Fluorescence detection. Limit of detection, 1 µg/L for hydralazine and 200 ng/L for hydralazine pyruvic acid hydrazone [Reece *et al.* 1980].

Note Spectrophotometric and gas chromatographic assays have been shown to be non-selective owing to interference from acid-labile hydrazones—see Reece *et al.* [1978] and Ludden *et al.* [1982].

Disposition in the Body Readily absorbed after oral administration. It undergoes first-pass acetylation, the extent of which is genetically determined. The major metabolites are: 3-methyl-1,2,4-triazolo[3,4-*a*]phthalazine (MTP—the acetylation product); hydralazine pyruvic acid hydrazone (HPH) which is the major plasma metabolite; 4-(2-acetylhydrazino)phthalazin-1-one (*N*-AcHPZ) which is the major urinary metabolite; 3-hydroxymethyl-1,2,4-triazolo[3,4-*a*]phthalazine (3-OHMTP). About 65% of a dose is excreted in the urine in 24 h. In rapid acetylators, about 30% is excreted as *N*-AcHPZ and 10 to 30% as conjugated 3-OHMTP; in slow acetylators, about 15 to 20% is excreted as *N*-AcHPZ and up to 10% as conjugated 3-OHMTP. Other metabolites include phthalazin-1-one (PZ), 1,2,4-triazolo[3,4-*a*] phthalazine (TP), 9-hydroxy-MTP, phthalazine, tetrazolo[5,1-*a*]phthalazine and hydrazones of hydralazine formed with acetone and α-ketoglutaric acid. About 10% of a dose is eliminated in the faeces.

Therapeutic Concentration

Following a single oral dose of 1 mg/kg to 4 rapid acetylators, a mean peak plasma hydralazine concentration of 0.05 mg/L was attained in 0.4 h and a mean peak plasma HPH concentration of 0.26 mg/L was attained in 0.6 h; after the same dose was given to 4 slow acetylators the corresponding peak plasma concentrations were 0.17 mg/L of hydralazine and 0.54 mg/L of HPH in 0.3 h and 1 h, respectively. [Shepherd *et al.* 1980].

Bioavailability About 30 to 35% in slow acetylators, 10 to 16% in rapid acetylators.

Half-life Plasma half-life, hydralazine 0.4 to 2 h, HPH about 4 h, MTP about 1.5 to 2 h.

Volume of Distribution About 3 to 8 L/kg.

Note For a review of the pharmacokinetics of hydralazine, see Ludden *et al.* [1982].

Dose 50 to 200 mg of hydralazine hydrochloride daily.

Ludden TM *et al.* (1982). Clinical pharmacokinetics of hydralazine. *Clin Pharmacokinet* 7: 185–205.
Ludden TM *et al.* (1983). Determination of hydralazine in human whole blood. *J Pharm Sci* 72: 693–695.
Reece PA *et al.* (1978). Interference in assays for hydralazine in humans by a major plasma metabolite, hydralazine pyruvic acid hydrazone. *J Pharm Sci* 67: 1150–1153.
Reece PA *et al.* (1980). Selective high-performance liquid chromatographic assays for hydralazine and its metabolites in plasma of man. *J Chromatogr* 181: 427–440.
Semple HA *et al.* (1988). Assay for hydralazine as its stable p-nitrobenzaldehyde hydrazone. *Pharm Res* 5: 383–386.
Shepherd A *et al.* (1980). Hydralazine kinetics after single and repeated oral doses. *Clin Pharmacol Ther* 28: 804–811.
Wong JK *et al.* (1987). Determination of hydralazine in human plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 385: 261–266.

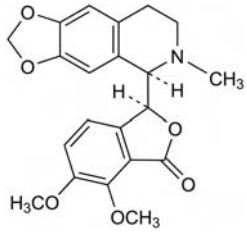
Hydrastine

Alkaloid

$C_{21}H_{21}NO_6$ = 383.4

CAS—118-08-1

IUPAC Name (3*S*)-6,7-Dimethoxy-3-[(5*R*)-5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]-1(3*H*)-isobenzofuranone



Chemical Properties An alkaloid obtained from hydrastis, the dried rhizome and roots of golden seal, *Hydrastis canadensis* (Ranunculaceae). Crystals. Mp 132°. Insoluble in water; soluble 1 in 210 of ethanol, 1 in 1 of chloroform and 1 in 245 of ether; freely soluble in acetone and benzene. pK_a 6.2 (25°). Log *P* (octanol/water), 1.9.

Hydrastine Hydrochloride

$C_{21}H_{21}NO_6$, HCl = 419.9

CAS—5936-28-7

Chemical Properties A white or creamy-white hygroscopic powder. Mp 116°. Very soluble in water and ethanol; slightly soluble in chloroform; very slightly soluble in ether.

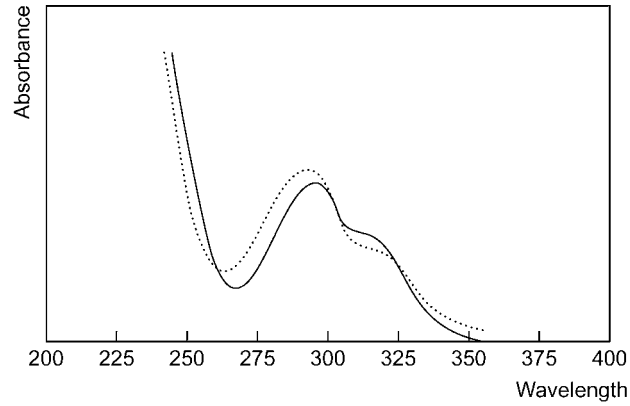
Colour Tests Liebermann's reagent—green; Mandelin's test—red-brown→red.

Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.15; system TC—R_f 0.64; system TE—R_f 0.71; system TL—R_f 0.52; system TAE—R_f 0.59; system TAF—R_f 0.57 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2988.

High Performance Liquid Chromatography System HY—RI 280.

Ultraviolet Spectrum Aqueous acid—294 nm (A^{1}_{1}=195b); aqueous alkali—292 nm.



Infrared Spectrum Principal peaks at wavenumbers 1760, 1501, 1037, 1260, 1020, 1111 cm^{-1} (KBr disk).

Dose Hydrastine hydrochloride was formerly given in doses of 15 to 60 mg.

Hydrastinine

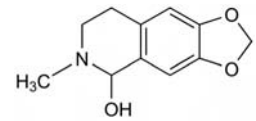
Alkaloid

$C_{11}H_{13}NO_3$ = 207.2

CAS—6592-85-4

IUPAC Name 6-Methyl-7,8-dihydro-5*H*-[1,3]dioxolo[4,5-g]isoquinolin-5-ol

Synonym 5,6,7,8-Tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-ol



Chemical Properties An alkaloid produced by oxidation of hydrastine. A white crystalline substance. Mp 117°. Moderately soluble in hot water; freely soluble in ethanol, chloroform, dilute acids and ether. Log *P* (octanol/water), 1.9.

Hydrastinine Hydrochloride

$C_{11}H_{11}NO_2$, HCl = 225.7

CAS—4884-68-8

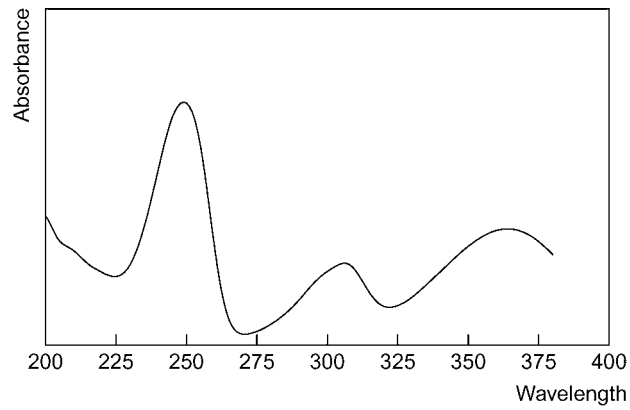
Chemical Properties Pale yellow crystals or crystalline powder. Mp 212°, with decomposition. Very soluble in water and ethanol; sparingly soluble in chloroform and ether. Solutions in water show a blue fluorescence.

Colour Test Mandelin's test—orange→green.

Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.46; system TC—R_f 0.00; system TE—R_f 0.37; system TL—R_f 0.00; system TAE—R_f 0.01; system TAF—R_f 0.15 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1590.

Ultraviolet Spectrum Aqueous acid—249, 306, 363 nm.

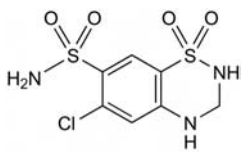


Infrared Spectrum Principal peaks at wavenumbers 1235, 1032, 1499, 925, 1075, 934 cm^{-1} (KBr disk).
Dose Hydrastinine hydrochloride was formerly given in doses of 15 to 60 mg as a cardiotonic.

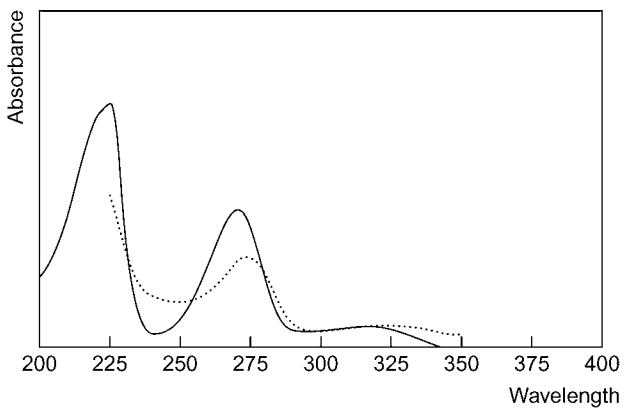
Hydrochlorothiazide

Diuretic
$C_7H_8ClN_3O_4S_2 = 297.7$
CAS—58-93-5

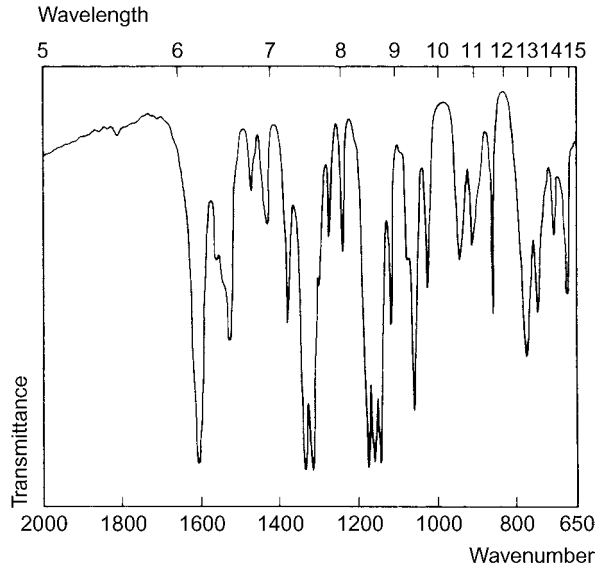
IUPAC Name 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
Synonyms Chlorosulthiadil; hidroclorotiazida; hydrochlorothiazidum.
Proprietary Names *Acuretic*; *Apo-Hydro*; *Clorana*; *Dichlotride*; *Diclotride*; *Didralin*; *Di-Ertride*; *Direma*; *Disalunil*; *Disothiazide*; *Dithiazid*; *Diuret-P*; *Diurezin*; *Drenol*; *Esidrex*; *Esidrix*; *Hidrosaluretil*; *Hydrex*; *Hydro-Aquil*; *HydroDiuril*; *HydroSaluric*; *Hydro-Z*; *Hydrozide*; *Novo-Hydrazide*; *Microzide*; *Mictrin*; *Oretic*; *Servithiazid*; *Thiuretic*.



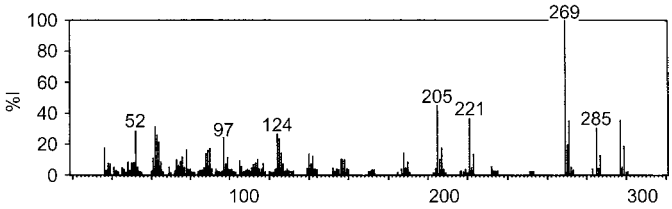
Chemical Properties Hydrochlorothiazide is an ingredient of many proprietary preparations—see Sweetman [2009]. A white crystalline powder. Mp 273° to 275°. Practically insoluble in water, chloroform and ether; soluble 1 in 200 of ethanol and 1 in 20 of acetone; freely soluble in dimethylformamide and solutions of alkali hydroxides. pK_a 7.0, 9.2. Log *P* (octanol/water), −0.1. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Tests Chromotropic acid—violet, after dilution; Koppanyi–Zwikker test—violet; Liebermann's reagent—blue-green.
Thin-layer Chromatography System TD—R_f 0.04; system TE—R_f 0.34; system TF—R_f 0.34; system TAD—R_f 0.11; system TAE—R_f 0.78; system TAJ—R_f 0.09; system TAK—R_f 0.00; system TAL—R_f 0.40 (mercuric chloride–diphenylcarbazone reagent, positive).
Gas Chromatography System GA—hydrochlorothiazide, not eluted; hydrochlorothiazide-Me_4 RI 2966; system GX—hydrochlorothiazide-Me_4 retention time 9.0 min; system GY—hydrochlorothiazide-Me_4 retention time 5.0 min.
High Performance Liquid Chromatography System HN—*k* 0.70; system HX—RI 294; system HY—RI 255; system HZ—retention time 2.2 min; system HAX—retention time 5.1 min; system HAY—retention time 4.0 min.
Ultraviolet Spectrum Aqueous acid—272 (A_1^1=644a), 318 nm; aqueous alkali—274 (A_1^1=520a), 324 nm.



Infrared Spectrum Principal peaks at wavenumbers 1318, 1180, 1150, 1168, 1602, 1060 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 269, 205, 221, 297, 271, 62, 285, 124.



Quantification

Blood **GC** ECD. Limit of detection, 5 µg/L [Redalieu *et al.* 1978]. ECD and FID [Lindström *et al.* 1975].
Plasma **GC** See Blood [Redalieu *et al.* 1978]. Limit of detection, 10 µg/L, see Blood [Lindström *et al.* 1975].
HPLC [Medvedovici *et al.* 2000]. Limit of detection, 10 µg/L [de Vries, Voss 1993]. [Kuo *et al.* 1990]. UV detection. Limit of detection, 10 µg/L for hydrochlorothiazide and chlorothiazide [Barbhaiya *et al.* 1981].
Serum **HPLC** Electrochemical detection. Limit of detection, <5 µg/L [Richter *et al.* 1996].
Urine **GC** See Blood [Lindström *et al.* 1975].
GC-MS Hydrochlorothiazide and other diuretics [Carreras *et al.* 1994].
HPLC UV detection. Limit of detection, 1 mg/L [Farthing *et al.* 1998]. See Plasma. Limit of detection, 200 µg/L [de Vries, Voss 1993]. See Plasma. Limit of detection, 2 mg/L [Barbhaiya *et al.* 1981].
Disposition in the Body Rapidly but incompletely absorbed after oral administration. >95% of an IV dose is excreted unchanged in the urine. About 65% of an oral dose is excreted in the urine unchanged in 24 h. It crosses the placenta and is distributed into breast milk.
Therapeutic Concentration
Peak plasma concentrations of 0.18–0.43 mg/L (mean 0.26) were attained in 2– 4 h, following a single oral dose of 50 mg given to 8 subjects [Beermann, Groschinsky–Grind 1977].
Steady-state plasma concentrations of 0.05–0.16 mg/L (mean 0.1) were achieved after daily oral doses of 75 mg to 8 subjects. [Beermann, Groschinsky-Grind 1978].
Bioavailability About 65–70%.
Half-life Plasma half-life, 5–15 h.
Volume of Distribution About 0.8 L/kg.
Distribution in Blood Plasma : whole blood ratio, 0.41.
Protein Binding About 60%.
Note For a review of the pharmacokinetics of diuretics, see Beermann and Groschinsky-Grind [1980].
Dose 25 to 200 mg daily.

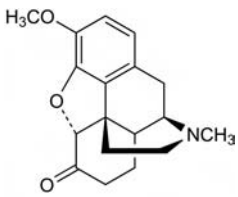
Barbhaiya RH *et al.* (1981). High-pressure liquid chromatographic determination of chlorothiazide and hydrochlorothiazide in plasma and urine: preliminary results of clinical studies. *J Pharm Sci* 70: 291–295.
Beermann B, Groschinsky-Grind M (1977). Pharmacokinetics of hydrochlorothiazide in man. *Eur J Clin Pharmacol* 12: 297–303.

Beermann B, Groschinsky-Grind M (1978). Antihypertensive effect of various doses of hydrochlorothiazide and its relation to the plasma level of the drug. *Eur J Clin Pharmacol* 13: 195–201.
Beermann B, Groschinsky-Grind M (1980). Clinical pharmacokinetics of diuretics. *Clin Pharmacokinet* 5: 221–245.
Carreras D *et al.* (1994). Comparison of derivatization procedures for the determination of diuretics in urine by gas chromatography-mass spectrometry. *J Chromatogr A* 683: 195–202.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
de Vries JX, Voss A (1993). Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography. *Biomed Chromatogr* 7: 12–14.
Farthing D *et al.* (1998). Simple method for determination of hydrochlorothiazide in human urine by high performance liquid chromatography utilizing narrowbore chromatography. *J Pharm Biomed Anal* 17: 1455–1459.
Kuo BS *et al.* (1990). Column-switching high-performance liquid chromatographic (HPLC) determination of hydrochlorothiazide in rat, dog, and human plasma. *Pharm Res* 7: 1257–1261.
Lindström B *et al.* (1975). Gas chromatographic determination of hydrochlorothiazide in plasma, blood corpuscles and urine using an extractive alkylation technique. *J Chromatogr* 114(2): 458–462.
Medvedovici A *et al.* (2000). Liquid extraction and HPLC-DAD assay of hydrochlorothiazide from plasma for a bioequivalence study at the lowest therapeutic dose. *Eur J Drug Metab Pharmacokinet* 25(2): 91–96.
Redalieu E *et al.* (1978). Determination of plasma hydrochlorothiazide levels in humans. *J Pharm Sci* 67: 726–728.
Richter K *et al.* (1996). New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection. *J Chromatogr A* 729: 293–296.
Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.

Hydrocodone

Antitussive, Narcotic Analgesic
$C_{18}H_{21}NO_3$ = 299.4
CAS—125-29-1

IUPAC Name 4,5-Epoxy-3-methoxy-17-methylmorphinan-6-one
Synonym Dihydrocodeinone



Chemical Properties Mp 198°. Insoluble in water; soluble in ethanol and dilute acids. pK_a 8.3 (20°). Log *P* (octanol/water), 2.2.

Hydrocodone Hydrochloride

$C_{18}H_{21}NO_3$, HCl,$2^1/_2H_2O$ = 380.9
CAS—25968-91-6 (anhydrous)
Synonym Dihydrocodeinone hydrochloride
Proprietary Name *Dicodid* (injection)
Chemical Properties A white crystalline powder. Soluble 1 in 2 of water; soluble in ethanol.

Hydrocodone Phosphate

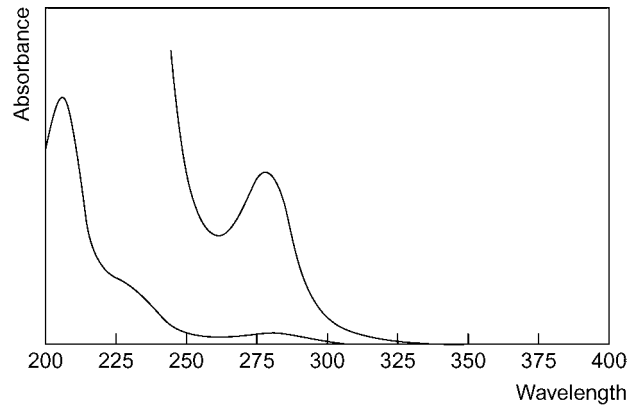
$C_{18}H_{21}NO_3$, $1^1/_2H_3PO_4$ = 446.4
CAS—34366-67-1
Chemical Properties A white or yellowish-white crystalline powder. Soluble in water; practically insoluble in ethanol, chloroform, and ether.

Hydrocodone Tartrate

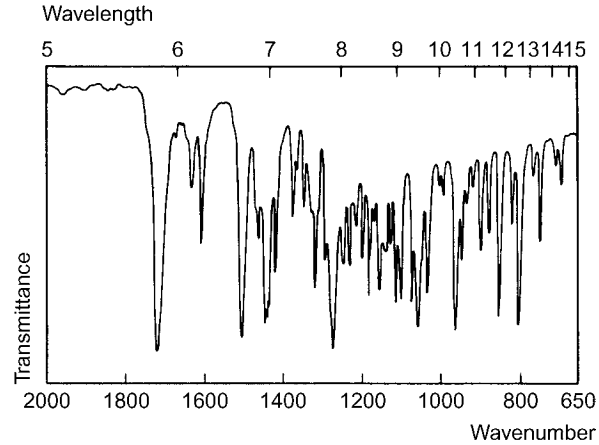
$C_{18}H_{21}NO_3$, $C_4H_6O_6$,$2^1/_2H_2O$ = 494.5
CAS—143-71-5 (anhydrous); 34195-34-1 (hemipentahydrate)
Synonyms Dihydrocodeinone acid tartrate; hydrocodone acid tartrate; hydrocodone bitartrate; hydrocone bitartrate.
Proprietary Names *Biocodone*; *Dicodid* (tablets); *Hycodan*; *Robidone*. It is an ingredient of many preparations [Sweetman *et al.* 2007].
Chemical Properties White crystals or crystalline powder. Mp 118° to 128°. Soluble 1 in 16 of water and 1 in 150 of ethanol; practically insoluble in chloroform and ether.

Colour Test Marquis test—yellow→brown→violet.
Thin-layer Chromatography System TA—R_f 0.25; system TB—R_f 0.04; system TC—R_f 0.20; system TE—R_f 0.33; system TL—R_f 0.04; system TAE—R_f 0.11; system TAF—R_f 0.13 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; Marquis test, violet).
Gas Chromatography System GA—hydrocodoneRI 2440, M (dihydromorphine) RI 2400, M (nor-)-AC RI 2760, M (nor-dihydro-)-AC RI 2700; system GB—hydrocodoneRI 2580, M (-TMS) RI 2674, M (nor-) RI 2599; system GC—RI 3028; system GF—RI 2930.
High Performance Liquid Chromatography System HA—*k* 7.1 (tailing peak); system HC—*k* 2.17; system HX—RI 286; system HY—RI 231; system HAX—RT 6.9 min; system HAY—RT 3.7 min.

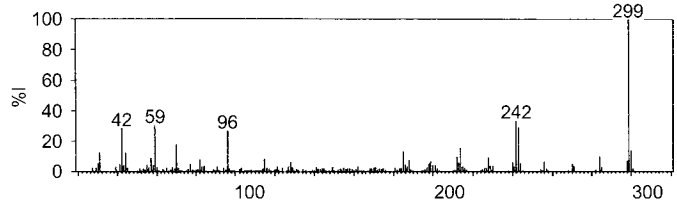
Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1 = 41a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1720, 1270, 1500, 959, 1055, 800 cm^{-1}(KBr disk).



Mass Spectrum Principal ions at m/z 299, 242, 59, 243, 42, 96, 70, 214.



Quantification

Blood **GC** Column: 3% OV-17 on 100/120 mesh Chromosorb W HP (1.2 m × 3 mm i.d.) or 2% FFAP on 100/120 mesh Chromosorb W (0.6 m × 3 mm i.d.). Temperature: 240° or 220°, respectively. AFID. Limit of detection, 100 µg/L [Cimbura, Koves 1981].

GC-MS [Goldberger *et al.* 2010]. Hydrocodone and hydromorphone [Balikova *et al.* 2000].

LC-MS [Dahn *et al.* 2010]. Column: Nova-Pak CN HP (100 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate buffer (pH 3.0), flow rate 0.525 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 µg/L, limit of detection, 1 µg/L [Coles *et al.* 2007].

Plasma **LC-MS** See Blood [Dahn *et al.* 2010]. Column: $C_{18.}$ Mobile phase: acetonitrile : water (78 : 22, 0.1% acetic acid). ESI. Limit of detection, 0.1 µg/L [Zhang *et al.* 2009]. See Blood [Coles *et al.* 2007].

Note For a radioimmunoassay, see Honigberg, Stewart [1980].

Serum **GC** ECD. Limit of detection, 1 ng/mL [Barnhart, Caldwell 1977].

LC-MS See Blood [Dahn *et al.* 2010]. See Blood [Coles *et al.* 2007].

Urine **GC-MS** See Blood [Balikova *et al.* 2000; Goldberger *et al.* 2010]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 290° at 10°/min for 0.5 min. EI ionisation. Retention time: 291 s. Limit of quantification, 25 µg/L [Meatherall 1999]. Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 150° for 1.5 min to 250° at 20°/min. MSD. Retention time: 7.46 min. Limit of quantification, 75 µg/L [Broussard *et al.* 1997]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (1.52 m × 2 mm i.d.). EI ionisation at 80 eV. Carrier gas: CH_4. Temperature: 210°. Limit of detection, 10 µg/L [Cone, Darwin 1978].

LC-MS See Blood [Coles *et al.* 2007; Dahn *et al.* 2010].

Oral Fluid **GC-MS** Column: DB-5 5% phenyl 95% methyl silicone (25 m × 0.2 mm i.d., 0.33 mm). Temperature programme: 150° for 1 min to 245° at 20°/min for

8 min to 290° at 50°/min. MSD in EI ionisation mode. Limit of quantification, 10 g/L, limit of detection, 3 µg/L [Jones *et al.* 2002].

***Hair* GC-MS** See Oral Fluid. Limit of quantification, 0.06 ng/mg, limit of detection, 0.06 ng/mg [Jones *et al.* 2002].

Disposition in the Body Hydrocodone is absorbed after oral administration. It is metabolised by demethylation and reduction of the 6-keto group. Approximately 26% of a dose is excreted in the urine in 72 h, with 12% of the dose as unchanged drug, 5% as norhydrocodone, 4% as conjugated hydromorphone, 3% as 6-hydrocodol and 0.1% as conjugated 6-hydromorphol. Hydrocodol and hydromorphol exist as stereoisomers. The unconjugated metabolites are thought to be active. Hydrocodone is a metabolite of codeine.

Therapeutic Concentration

After a single oral dose of 10 mg of hydrocodone tartrate to 5 subjects, peak serum concentrations of 0.018–0.032 mg/L (mean 0.023) were attained in 1.5 h [Barnhart, Caldwell 1977].

Toxicity The estimated lethal dose is 200 mg. Fatalities have occurred at blood concentrations >0.1 mg/L.

In 2 deaths caused by the ingestion of hydrocodone and phenyltoloxamine, the following postmortem concentrations were reported for the 2 cases, respectively: hydrocodone 0.3 mg/L in blood (both cases); hydrocodone 14.3 mg/L and none in bile, hydromorphone 98 and 48 mg/L in bile; in the first case, phenyltoloxamine was present in bile at a concentration of 0.4 mg/L [Park *et al.* 1982].

Half-life Plasma half-life, ~4 h.

Dose Usually 5 to 10 mg hydrocodone tartrate by mouth every 4 to 6 h.

Balikova M *et al.* (2000). [Evaluation of methods of trace analysis of various opiates including hydrocodone and hydromorphone in the blood and urine using gas chromatography–mass spectrometry]. *Soud Lek* 45: 11–16.

Barnhart JW, Caldwell WJ (1977). Gas chromatographic determination of hydrocodone in serum. *J Chromatogr* 130: 243–249.

Broussard LA *et al.* (1997). Simultaneous identification and quantitation of codeine, morphine, hydrocodone, and hydromorphone in urine as trimethylsilyl and oxime derivatives by gas chromatography–mass spectrometry. *Clin Chem* 43: 1029–1032.

Cimbura G, Koves E (1981). Radioimmunological screening and gas chromatographic determination of morphine and related narcotic analgesics in post mortem blood. *J Anal Toxicol* 5: 296–299.

Coles R *et al.* (2007). Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC-MS-MS. *J Anal Toxicol* 31: 1–14.

Cone EJ, Darwin WD (1978). Simultaneous determination of hydromorphone, hydrocodone and their 6alpha- and 6beta-hydroxy metabolites in urine using selected ion recording with methane chemical ionization. *Biomed Mass Spectrom* 5: 291.

Dahn T *et al.* (2010). Quantitation of morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and 6-monoacetylmorphine (6-MAM) in urine, blood, serum, or plasma using liquid chromatography with tandem mass spectrometry detection. *Methods Mol Biol* 603: 411–422.

Goldberger BA *et al.* (2010). Quantitation of opioids in blood and urine using gas chromatography–mass spectrometry (GC-MS). *Methods Mol Biol* 603: 399–410.

Honigberg IL, Stewart JT (1980). Radioimmunoassay of hydromorphone and hydrocodone in human plasma. *J Pharm Sci* 69: 1171–1173.

Jones J *et al.* (2002). The simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, 6-acetylmorphine, and oxycodone in hair and oral fluid. *J Anal Toxicol* 26: 171–175.

Meatherall R (1999). GC-MS confirmation of codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone in urine. *J Anal Toxicol* 23: 177–186.

Park JI *et al.* (1982). Hydromorphone detected in bile following hydrocodone ingestion. *J Forensic Sci* 27: 223–224.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35 edn. London: Pharmaceutical Press.

Zhang R *et al.* (2009). Determination and pharmacokinetic study of hydrocodone in human plasma by liquid chromatography coupled with tandem mass spectrometry. *Artif Cells Blood Substit Immobil Biotechnol* 37: 203–207.

Hydrocortisone

Corticosteroid

$C_{21}H_{30}O_5$ = 362.5

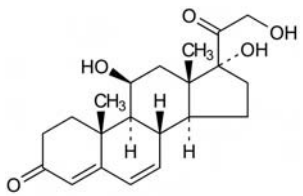
CAS—50-23-7

IUPAC Name (8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15, 16-decahydro-1*H*-cyclopenta[a]phenanthren-3-one

Synonyms Compound F; cortisol; 17-hydroxycorticosterone; (11β)-11,17,21-trihydroxypregn-4-ene-3,20-dione.

Note Cortisol is also used as a proprietary name for cortisone acetate.

Proprietary Names Hydrocortisone and its esters are ingredients of many proprietary preparations—see Sweetman [2009].



Chemical Properties A white crystalline powder. Mp about 214°, with decomposition. A solution in dioxan is dextrorotatory. Practically insoluble in water and ether; soluble 1 in 40 of ethanol; slightly soluble in chloroform; very soluble in dioxan. Hydrocortisone sodium succinate, pK_a 5.1. Log *P* (octanol/water), 1.6.

Hydrocortisone Acetate

$C_{23}H_{32}O_6$ = 404.5

CAS—50-03-3

Synonym Hydrocortisone 21-acetate

Chemical Properties A white crystalline powder. Mp about 220°, with decomposition. Practically insoluble in water and ether; soluble 1 in 230 of ethanol; slightly soluble in chloroform; soluble in dioxane.

Hydrocortisone Butyrate

$C_{25}H_{36}O_6$ = 432.6

CAS—13609-67-1

Synonym Hydrocortisone 17-butyrate

Hydrocortisone Cipionate

$C_{29}H_{42}O_6$ = 486.6

CAS—508-99-6

Synonyms Hydrocortisone cypionate; hydrocortisone cyclopentylpropionate.

Chemical Properties A white crystalline powder. Practically insoluble in water; soluble in ethanol; very soluble in chloroform; slightly soluble in ether.

Hydrocortisone Hydrogen Succinate

$C_{25}H_{34}O_8$ = 462.5

CAS—2203-97-6

Synonyms Cortisol hemisuccinate; hydrocortisone hemisuccinate.

Chemical Properties A white crystalline powder. Mp 170° to 173° or 210° to 214°. Practically insoluble in water; soluble 1 in 40 of ethanol and 1 in 7 of dehydrated alcohol.

Hydrocortisone Sodium Phosphate

$C_{21}H_{29}Na_2O_8P$ = 486.4

CAS—6000-74-4

Chemical Properties A white or light yellow hygroscopic powder. Soluble 1 in 4 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Hydrocortisone Sodium Succinate

$C_{25}H_{35}NaO_8$ = 484.5

CAS—125-04-2

Synonym Hydrocortisone 21-sodium succinate

Chemical Properties A white, hygroscopic, crystalline powder or amorphous solid. Mp 169.0° to 171.2°. Soluble 1 in 3 of water and 1 in 34 of ethanol; practically insoluble in chloroform and ether. It is unstable in aqueous solution.

Hydrocortisone Valerate

$C_{26}H_{38}O_6$ = 446.6

CAS—57524-89-7

Synonym Hydrocortisone 17-valerate

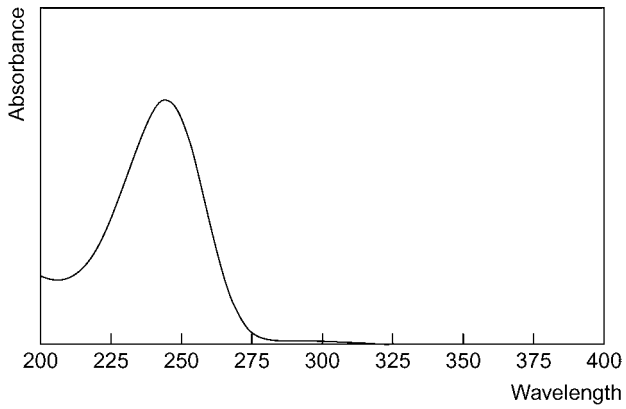
Colour Tests Antimony pentachloride—orange; naphthol–sulfuric acid—yellow-brown/yellow-brown; sulfuric acid—green, orange dichroism (green fluorescence under ultraviolet light).

Thin-layer Chromatography Hydrocortisone: system TA—R_f 0.96; system TB—R_f 0.00; system TE—R_f 0.45; system TF—R_f 0.28; system TP—R_f 0.27; system TQ—R_f 0.02; system TR—R_f 0.08; system TS—R_f 0.00; system TAE—R_f 0.86; system TAJ—R_f 0.36; system TAK—R_f 0.05; system TAL—R_f 0.74; system TAM—R_f 0.58. Hydrocortisone acetate: system TP—R_f 0.51; system TQ—R_f 0.11; system TR—R_f 0.38; system TS—R_f 0.00. Hydrocortisone hydrogen succinate: system TP—R_f 0.08; system TQ—R_f 0.00; system TR—R_f 0.00; system TS—R_f 0.00. Hydrocortisone sodium phosphate: system TP—R_f 0.00; system TQ—R_f 0.00; system TR—R_f 0.00; system TS—R_f 0.00 (DPST solution).

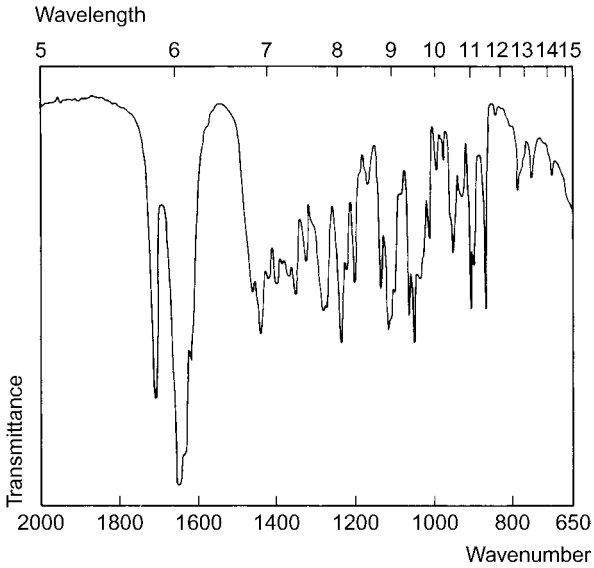
Gas Chromatography System GA—RI 2740.

High Performance Liquid Chromatography System HT—*k* 5.8; system HX—RI 403; system HY—RI 349; system HAA—retention time 17.7 min.

Ultraviolet Spectrum Ethanol—240 nm (A^1_1=435a).



Infrared Spectrum Principal peaks at wavenumbers 1640, 1702, 1610, 1232, 1042, 1115 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** Fluorescence. Hydrocortisone and other glucocorticoids. Limit of detection, 0.1 µg/L for hydrocortisone [Shibata *et al.* 1998]. UV detection. Hydrocortisone, prednisone and prednisolone. Limit of detection, <5 µg/L [Jusko *et al.* 1994]. UV detection. Limit of detection, 300 ng/L [Hariharan *et al.* 1992]. UV detection. Hydrocortisone and hydrocortisone succinate. Limits of detection, 0.2 mg/L for hydrocortisone, and 0.5 mg/L for hydrocortisone succinate [Iwasaki 1987]. UV detection. Limit of detection, 5 µg/L [Toothaker *et al.* 1982].

Urine **HPLC** See Plasma [Shibata *et al.* 1998].

Disposition in the Body Hydrocortisone (cortisol) is the main glucocorticoid secreted by the adrenal cortex. It is administered parenterally as the sodium phosphate or sodium succinate ester in emergencies. Hydrocortisone is readily absorbed after oral administration and through the skin; hydrocortisone acetate is less well absorbed. It is metabolised in the liver and other tissues by reduction, hydroxylation, side-chain cleavage and conjugation with glucuronic acid. About 90% of a dose is excreted in the urine in 24 h; <1% is excreted unchanged, 40% is the 5α- and 5β-forms of tetrahydrocortisol and the 5β-form of tetrahydrocortisone, 17% is a mixture of the 5α- and 5β- and 20α- and 20β-cortols and cortolones, and 6% is 11-hydroxy- and 11-keto-etiocholanolone; most of the metabolites are excreted as glucuronide conjugates; at plasma concentrations of >0.2 mg/L, the amount of unchanged hydrocortisone excreted in the urine is increased.

Blood Concentration Endogenous hydrocortisone exhibits a diurnal variation in plasma concentrations; in the morning, concentrations are in the approximate range 0.08 to 0.20 mg/L and, in the evening, in the range 0.04 to 0.10 mg/L.

Following a single intravenous injection of 40 mg to 6 subjects, a mean plasma concentration of 1.85 mg/L was reported at 10 min [Toothaker, Welling 1982].

Half-life Plasma half-life, about 1.5 h.

Volume of Distribution About 0.3 L/kg.

Protein Binding More than 90%.

Dose 10 to 30 mg daily, by mouth. In the treatment of certain medical emergencies, the equivalent of 100 to 500 mg of hydrocortisone by IV injection, 3 or 4 times daily.

Hariharan M *et al.* (1992). Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography. *Clin Chem* 38: 346–352.

Iwasaki E (1987). Hydrocortisone succinate and hydrocortisone simultaneously determined in plasma by reversed-phase liquid chromatography, and their pharmacokinetics in asthmatic children. *Clin Chem* 33: 1412–1415.

Jusko WJ *et al.* (1994). Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma. *J Chromatogr* 658: 47–54.

Shibata N *et al.* (1998). Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile. *J Chromatogr B Biomed Sci Appl* 706: 191–199.

Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.

Toothaker RD *et al.* (1982). Oral hydrocortisone pharmacokinetics: a comparison of fluorescence and ultraviolet high-pressure liquid chromatographic assays for hydrocortisone in plasma. *J Pharm Sci* 71: 573–576.

Toothaker RD, Welling PG (1982). Effect of dose size on the pharmacokinetics of intravenous hydrocortisone during endogenous hydrocortisone suppression. *J Pharmacokinet Biopharm* 10: 147–156.

Hydroflumethiazide

Diuretic

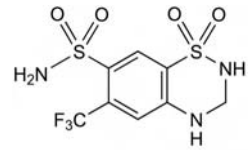
$C_8H_8F_3N_3O_4S_2$ = 331.3

CAS—135-09-1

IUPAC Name 3,4-Dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Synonym Trifluoromethylhydrothiazide

Proprietary Names *Diucardin*; *Elodrine*; *Finuret*; *Hydol*; *Hydrenox*; *Leodrine*; *Rodiuran*; *Rontyl*; *Saluron*; *Sisuril*. It is an ingredient of *Aldactide*, *Protensin-M*, *Rautrax*, *Salutensin* and *Spio-Co*.



Chemical Properties White or cream-coloured glistening crystals or crystalline powder. Mp 272° to 273°. Soluble 1 in 3000 of water, 1 in about 50 of ethanol and 1 in 4 of acetone; practically insoluble in chloroform and ether. pK_a 8.5, 10.0 (20°). Log *P* (octanol/water), 0.4.

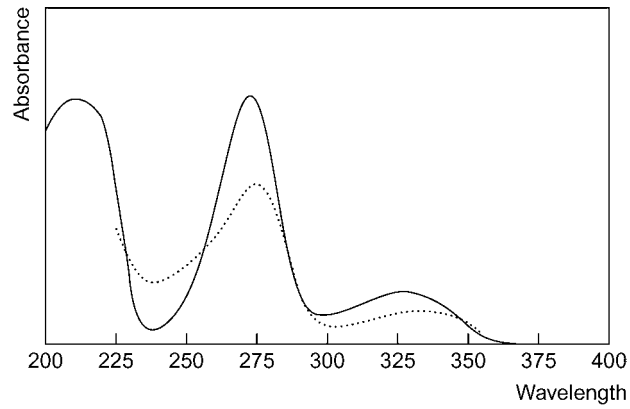
Colour Tests Chromotropic acid—violet, after dilution; Koppanyi–Zwikker test—violet; Liebermann's reagent—blue-green.

Thin-layer Chromatography System TA—R_f 0.86; system TD—R_f 0.07; system TE—R_f 0.36; system TF—R_f 0.47; system TAD—R_f 0.13; system TAE—R_f 0.87; system TAJ—R_f 0.09; system TAK—R_f 0.00; system TAL—R_f 0.43 (location under ultraviolet light, violet fluorescence; acidified potassium permanganate solution, faint reaction).

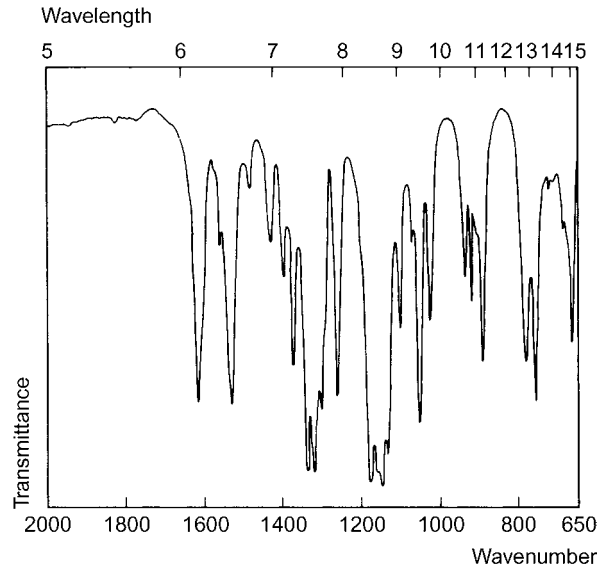
Gas Chromatography System GA—hydroflumethiazide, not eluted; hydroflumethiazide-Me_4 RI 2653; system GX—hydroflumethiazide-Me_4 retention time 6.3; hydroflumethiazide-Me_4 retention time 4.4 min.

High Performance Liquid Chromatography System HN—*k* 1.30.

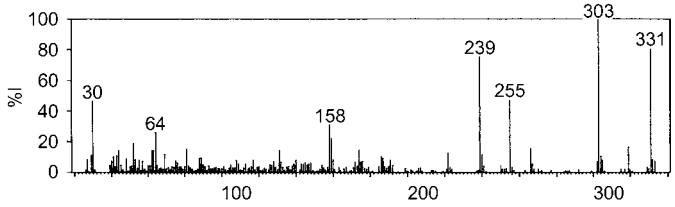
Ultraviolet Spectrum Aqueous acid—273 (A_1^1=578a), 325 nm; aqueous alkali—274, 333 nm.



Infrared Spectrum Principal peaks at wavenumbers 1151, 1180, 1125, 1052, 1300, 1525 (KBr disk).



Mass Spectrum Principal ions at *m/z* 303, 331, 239, 255, 30, 158, 64, 159.



Quantification

Plasma **Spectrofluorimetry** Limit of detection, 10 µg/L in plasma [Brørs *et al.* 1977].

TLC Limit of detection, 10 µg/L [Garceau *et al.* 1974].

Urine **Spectrofluorimetry** Limit of detection, 100 µg/L, see Plasma [Brørs *et al.* 1977].

TLC Limit of detection, 500 µg/L, see Plasma [Garceau *et al.* 1974].

Disposition in the Body Incompletely but fairly rapidly absorbed after oral administration. During daily dosing, 50 to 70% of a dose is excreted in the 24-h urine as unchanged drug and about 2% as 2,4-disulfamyl-5-trifluoromethylaniline (DTA).

Therapeutic Concentration

Following single oral doses of 100 mg to 12 subjects, peak plasma concentrations of 0.17 to 0.6 mg/L (mean 0.4) were attained in 2 to 4 h [Yakatan *et al.* 1977].

Half-life Derived from urinary excretion data, 5 to 18 h (dose-dependent).

Distribution in Blood Plasma : whole blood ratio, about 0.7.

Protein Binding About 75%.

Dose 25 to 200 mg daily.

Brørs O *et al.* (1977). Fluorometric determination of hydroflumethiazide in human plasma and urine after its oral administration. *Eur J Clin Pharmacol* 11: 149–154.

Garceau Y *et al.* (1974). Quantitative fluorometric TLC procedure for determination of hydroflumethiazide in biological fluids. *J Pharm Sci* 63: 1793–1795.

Yakatan G *et al.* (1977). Pharmacokinetics of orally administered hydroflumethiazide in man. *J Clin Pharmacol* 17: 37–47.

Hydrogen Cyanide

Rodenticide, Insect Fumigant

HCN = 27.0

CAS—74-90-8

Synonyms CHN; hydrocyanic acid; prussic acid.

Chemical Properties Colourless gas or liquid with a characteristic odour. Burns in air with a blue flame. Flammable. Intensely poisonous even when mixed with air. Mp −13.4°. Bp 25.6°. Miscible in water and alcohol, slightly soluble in ether. Aqueous solutions of alkalis decompose it to alkali cyanide and alkali bromide. pK_a 9.2 [USEPA 1999]. Log *P* (octanol/water) −0.25 [Hansch *et al.* 1995].

Capillary Electrophoresis Capillary: Fused silica (50 cm × 75 µm i.d.). Buffer: 10 mmol/L borate buffer (pH 9.5). Fluorescence detection (λ= 388 nm). Limit of detection, 9.3 µg/L [Copper, Collins 2004].

Quantification

Blood **GC** Column: CP Sil 8B methylsilicone (50 m × 0.23 mm i.d., 1.2 µm). Carrier gas: He, 2 mL/min. Temperature: 60°. ECD. Limit of quantification, 100 µg/L, limit of detection, 5 µg/L [Odoul *et al.* 1994]. Column: Halcomid-M-18 (3 m × 3 mm i.d.). ECD. Limit of detection, ≈50 µg/L [Shiono *et al.* 1991]. Column: 7% Halcomid-M-18 (3 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. ECD. Limit of detection, 50 µg/L [Maseda *et al.* 1989].

GC-MS Column: 7% Halcomid-M-18 (9ft × $^1/_4$ in i.d.). Carrier gas: He, 30 mL/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 µmol/L [Thomson, Anderson 1980].

Other **GC** Cyanogenic Plants. Column: 100% methylsilicone capillary (15 m × 0.32 mm i.d., 12 µm). Carrier gas: N_2. Temperature: 60°. ECD. Limit of detection, 69 µg/L [Curtis *et al.* 2002].

Disposition in the Body

Toxicity

A 32-year-old woman was admitted to hospital with a lack of co-ordination and fixed, dilated pupils. Her Glasgow Coma Score was fluctuating between 6 and 11. She was hypothermic and tachycardic, but was breathing spontaneously. She had documented breast cancer with liver metastases. Conventional treatment having failed, she took only 'vitamin supplements' bought via the Internet. Over the course of the next 6 h, she required mechanical ventilation and increasing doses of inotropes. Diabetes insipidus developed. Upon inspection of her medications, a bottle labelled 'vitamin B17' was discovered. While not a vitamin, it contains amygdalin, a drug linked to cyanide toxicity. A blood sample showed levels of thiocyanate of 445 µg/L. The patient made a full recovery over the next 8 h [O'Brien *et al.* 2005].

The concentrations (µg/mL) of cyanide in the hearts of 18 people who died in house fires were reported as shown in the following table. The left ventricular concentration was twice the right ventricular concentration in half of the victims.

Victim number	*Left ventricle*	*Right ventricle*
1	2.23	1.07
2	0.49	0.20
3	1.69	0.91
4	1.32	1.24
5	0.66	0.64
6	1.83	0.54
7	2.70	1.96
8	18.12	9.44
9	1.05	0.12
10	3.46	1.50
11	2.42	1.14
12	6.98	4.18
13	0.87	0.46
14	4.36	2.47
15	3.98	1.65
16	2.07	0.11
17	0.11	0.09
18	0.82	0.54

[Shiono *et al.* 1991].

Copper CL, Collins GE (2004). Separation of thiol and cyanide hydrolysis products of chemical warfare agents by capillary electrophoresis. *Electrophoresis* 25: 897–902.

Curtis AJ *et al.* (2002). Simultaneous determination of cyanide and carbonyls in cyanogenic plants by gas chromatography-electron capture/photoionization detection. *Analyst* 127: 1446–1449.

Hansch C *et al.* (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Maseda C *et al.* (1989). Improved gas chromatography with electron-capture detection using a reaction pre-column for the determination of blood cyanide: a higher content in the left ventricle of fire victims. *J Chromatogr* 490: 319–327.

O'Brien B *et al.* (2005). Severe cyanide toxicity from 'vitamin supplements'. *Eur J Emerg Med* 12: 257–258.

Odoul M *et al.* (1994). Specific determination of cyanide in blood by headspace gas chromatography. *J Anal Toxicol* 18: 205–207.

Shiono H *et al.* (1991). Rapid and sensitive quantitation of cyanide in blood and its application to fire victims. *Am J Forens Med Pathol* 12: 50–53.

Thomson I, Anderson RA (1980). Determination of cyanide and thiocyanate in biological fluids by gas chromatography-mass spectrometry. *J Chromatogr* 188: 357–362.

USEPA (1999). Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Hydrogen Cyanide. *United States Environmental Protection Agency.*

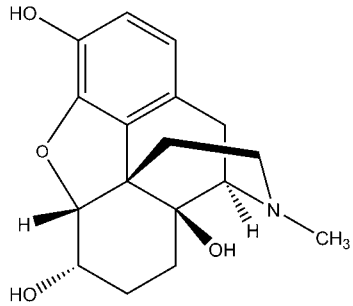
Hydromorphinol

Narcotic Analgesic

$C_{17}H_{21}NO_4$ = 303.4

Synonym 4,5-Epoxy-3,6,14-trihydroxy-*N*-methyl-morphinan

Proprietary Name *Numorphan Oral*



Chemical Properties A creamy-white crystalline powder. Insoluble in water. Hydromorphinol is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve the yield.

Colour Tests Ammonium molybdate test—purple→blue→yellow (limit of detection, 0.1 µg); ammonium vanadate test—grey (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—purple (limit of detection, 0.1 µg); Vitali's test—yellow/yellow/orange.

Thin-layer Chromatography System T1—R_f 0.48 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum 0.1 N sulphuric acid—284 nm (E1%, 1 cm 54) and an inflexion at about 278 nm.

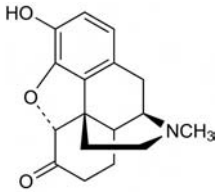
Infrared Spectrum Principal peaks at wavenumbers 1086, 1111, 1318, 1327 cm^{-1} (KBr disk).

Dose Usually 15 mg.

Hydromorphone

Narcotic Analgesic
$C_{17}H_{19}NO_3 = 285.3$
CAS—466-99-9

Synonyms Dihydromorphinone; dimorphone; 4,5-epoxy-3-hydroxy-17-methylmorphinan-6-one.

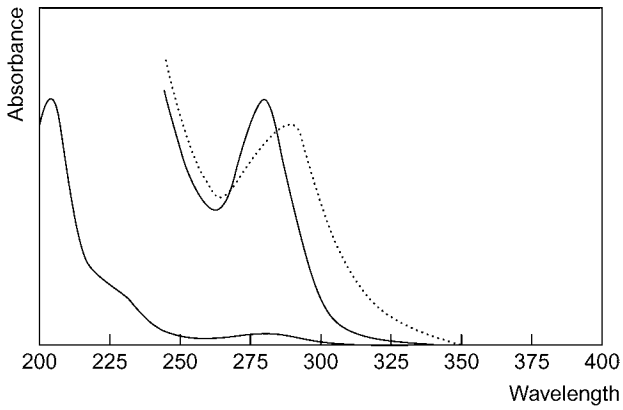


Chemical Properties Crystals. A fine, white, crystalline powder. Mp 266° to 267° (crystals from ethanol). Slightly soluble in water; freely soluble in ethanol; very soluble in chloroform. pK_a 8.2 (20°). Log *P* (heptane/pH 7.4), −4.0. Extraction yield (chlorobutane), 0.1 [Demme *et al.* 2005].

Hydromorphone Hydrochloride

$C_{17}H_{19}NO_3$, HCl = 321.8
CAS—71-68-1
Synonym Dihydromorphinone hydrochloride
Proprietary Names *Dilaudid*; *Hydal*; *Hydromorph*; *Opidol*; *Palladon(e)*; *Sophidone*.
Chemical Properties A white crystalline powder that is affected by light. Mp 305° to 315°, with decomposition. Soluble 1 in 3 of water and 1 in 100 of ethanol (90%); practically insoluble in chloroform and ether.

Colour Test Mandelin's test—violet→orange; Marquis test—yellow→red→violet.
Thin-layer Chromatography System TA—R_f 0.23; system TB—R_f 0.03; system TC—R_f 0.09; system TE—R_f 0.18; system TL—R_f 0.02; system TAE—R_f 0.12; system TAF—R_f 0.14 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—violet).
Gas Chromatography System GA—hydromorphone RI 2445, hydromorphone-AC RI 2595, hydromorphone-enol-AC_2 RI 2625, hydromorphone-PFP RI 2250, hydromorphone-enol-PFP_2 RI 2320, hydromorphone-enol-TFA_2 RI 2230, M (dihydromorphine) RI 2451; system GB—hydromorphone RI 2598, hydromorphone-TMS RI 2621, hydromorphone-enol-TMS_2 RI 2595, hydromorphone oxime-TMS_2 RI 2678.
High Performance Liquid Chromatography System HA—*k* 7.9 (tailing peak); system HX—RI 240; system HY—RI 187; system HAX—RT 5.8 min; system HAY—RT 3.4 min.
Ultraviolet Spectrum Aqueous acid—280 nm ($A_1^1 = 50a$); aqueous alkali—290 nm.



Infrared Spectrum Principal peaks at wavenumbers 1727, 1247, 1279, 1034, 757, 1500 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 285, 96, 229, 228, 70, 214, 115, 200.
Quantification
Blood **GC** Column: 3% OV-17 on 100/120 mesh Chromosorb W HP (1.2 m × 3 mm i.d.) or 2% FFAP (0.6 m × 3 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 240° and 220°, respectively. AFID. Limit of detection, 100 µg/L [Cimbura, Koves 1981].

GC-MS [Balikova *et al.* 2000]. Column: 3% OV-101 100/120 mesh (0.9 m × 2 mm i.d.). Carrier gas: He, 35 mL/min. Temperature programme: 205° for 1 min to 240° at 12°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 2.4 min Limit of detection, 80 µg/L [Saady *et al.* 1982].

HPLC Column: Supelcosil LC-18-DB (5 µm). Mobile phase: 0.1 mol/L sodium dihydrogen phosphate : methanol (67.5 : 32.5, pH 7.3), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 4.4 min. Limit of detection, 62 µg/L [Sawyer *et al.* 1988].

LC-MS Column: Waters HSS T3 (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 1% formic acid in water : 1% formic acid in acetonitrile (97 : 3 for 0.1 min to 80 : 20 at 2.5 min to 1 : 99 at 2.55 min for 0.45 min to 97 : 3 at 3.01), flow rate 0.6 mL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L [Dahn *et al.* 2010]. Column: Phenomenex Synergi reversed-phase. Mobile phase: 10 mmol/L ammonium formate : acetonitrile. Limit of quantitiation, 0.5–4.09 µg/L, limit of detection, 0.16–1.2 µg/L [Al Asmari, Anderson 2007].
Plasma **HPLC** Column: Spherisorb C_8 (100 × 3.2 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L disodium hydrogen phosphate (1.5 : 8.5) with 3 mmol/L orthophosphoric acid, flow rate 0.8 mL/min. Electrochemical detection. Retention time: 8.0 min. Limit of detection, 2.5 µg/L [Bouquillon *et al.* 1992].
LC-MS See Blood [Dahn *et al.* 2010].
Note For a radioimmunoassay for hydromorphone and morphine, see Lee *et al.* [1991] or Honigberg, Stewart [1980].
Serum **GC-MS** See Blood [Saady *et al.* 1982].
LC-MS See Blood [Dahn *et al.* 2010].
Urine **GC** FID [Cone *et al.* 1977].
GC-MS See Blood [Balikova *et al.* 2000]. Hydromorphone and other opioids, see Broussard *et al.* [1997].
HPLC See Blood [Sawyer *et al.* 1988].
LC-MS See Blood [Dahn *et al.* 2010].
Oral Fluid **GC-MS** See Hair. Limit of quantification, 2 ng/mg, limit of detection, 3 ng/mg [Jones *et al.* 2002].
Tissue Homogenates **GC-MS** See Blood [Saady *et al.* 1982].
Vitreous Humour **HPLC** See Blood [Sawyer *et al.* 1988].
Hair **GC-MS** Column: DB-5 5% phenyl 95% methyl silicone (25 m × 0.2 mm i.d., 0.33 mm). Carrier gas: 1.5 mL/min. Temperature programme: 150° for 1.0 min to 245° at 20°/min for 8 min to 290° at 50°/min. Limit of quantification, 0.15 ng/mg, limit of detection, 0.01 ng/mg [Jones *et al.* 2002].
Postmortem Tissues **HPLC** See Blood [Sawyer *et al.* 1988].
Disposition in the Body Hydromorphone is rapidly but incompletely absorbed after oral administration. Approximately 6% of a dose is excreted in the urine in 24 h as free hydromorphone, with ~30% as conjugated hydromorphone and only traces of the active 6α- and 6β-hydroxy metabolites. It crosses the placenta. Hydromorphone is a metabolite of hydrocodone.
Therapeutic Concentration

Following a single oral dose of 8, 16 or 32 mg hydromorphone as a sustained-release formulation or 8 mg as an immediate-release formulation to 12 subjects, the plasma concentration peaked later after the sustained-release oral preparation (9–13.5 h versus 0.8–1.0 h for the 8 mg dose). Peak plasma concentrations were 0.00474 ± 0.00176 mg/L for the 8 mg immediate-release formulation, 0.00077 ± 0.00033 mg/L for the 8 mg sustained-release formulation, 0.00145 ± 0.00043 mg/L for the 16 mg sustained-release formulation and 0.00241 ± 0.00085 mg/L for the 32 mg sustained-release formulation [Angst *et al.* 2001].

In 43 patients with chronic severe pain receiving hydromorphone therapy, 7 achieved good pain control with doses of 24–96 mg daily (median, 48) and had serum levels of ≥0.004 mg/L [Reidenberg *et al.* 1988].

Following a single oral dose of 4 mg to 6 subjects, peak plasma concentrations of 0.018–0.027 mg/L (mean, 0.022) were attained in ~1 h [Vallner *et al.* 1981].

Toxicity The estimated minimum lethal dose is 200 mg. Plasma concentrations >0.1 mg/L may be toxic.

In a fatality attributed to hydromorphone overdose, the following tissue concentrations were reported: blood 1.2 mg/L, brain a trace, kidney 1.2 µg/g, liver 0.4 µg/g and urine 1.1 mg/L; diazepam was also detected [Baselt 1978].

The following postmortem tissue concentrations were reported in a fatality caused by an injected overdose of hydromorphone: blood 0.17 mg/L, bile 8.6 mg/L, kidney 0.13 µg/g and liver 0.07 µg/g [Walls 1976].

Half-life Plasma half-life, ~2.5 h.
Bioavailability Approximately 50% but there is considerable intersubject variation.
Volume of Distribution ~3 L/kg.
Protein Binding ~7%.
Dose Hydromorphone hydrochloride 1.3 to 4 mg orally every 4 to 6 h.

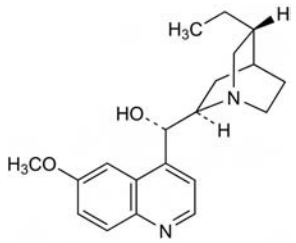
Al Asmari AI, Anderson RA (2007). Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 31: 394–408.
Angst MS *et al.* (2001). Pharmacodynamics of orally administered sustained- release hydromorphone in humans. *Anesthesiology* 94: 63–73.
Balikova M *et al.* (2000). [Evaluation of methods of trace analysis of various opiates including hydrocodone and hydromorphone in the blood and urine using gas chromatography–mass spectrometry]. *Soud Lek* 45: 11–16.
Baselt RC (1978). *TIAFT Bull* 1420.
Bouquillon AI *et al.* (1992). Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 577: 354–357.
Broussard LA *et al.* (1997). Simultaneous identification and quantitation of codeine, morphine, hydrocodone, and hydromorphone in urine as trimethylsilyl and oxime derivatives by gas chromatography–mass spectrometry. *Clin Chem* 43: 1029–1032.
Cimbura G, Koves E (1981). Radioimmunological screening and gas chromatographic determination of morphine and related narcotic analgesics in post mortem blood. *J Anal Toxicol* 5: 296–299.

Cone EJ *et al.* (1977). Urinary excretion of hydromorphone and metabolites in humans, rats, dogs, guinea pigs, and rabbits. *J Pharm Sci* 66: 1709–1713.
Dahn T *et al.* (2010). Quantitation of morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and 6-monoacetylmorphine (6-MAM) in urine, blood, serum, or plasma using liquid chromatography with tandem mass spectrometry detection. *Meth Mol Biol* 603: 411–422.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Honigberg IL, Stewart JT (1980). Radioimmunoassay of hydromorphone and hydrocodone in human plasma. *J Pharm Sci* 69: 1171–1173.
Jones J *et al.* (2002). The simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, 6-acetylmorphine, and oxycodone in hair and oral fluid. *J Anal Toxicol* 26: 171–175.
Lee JW *et al.* (1991). Sensitive and specific radioimmunoassays for opiates using commercially available materials. I: Methods for the determinations of morphine and hydromorphone. *J Pharm Sci* 80: 284–288.
Reidenberg MM *et al.* (1988). Hydromorphone levels and pain control in patients with severe chronic pain. *Clin Pharmacol Ther* 44: 376–382.
Saady JJ *et al.* (1982). Rapid, simultaneous quantification of morphine, codeine, and hydromorphone by GC/MS. *J Anal Toxicol* 6: 235–237.
Sawyer WR *et al.* (1988). Heroin, morphine, and hydromorphone determination in postmortem material by high performance liquid chromatography. *J Forensic Sci* 33: 1146–1155.
Vallner JJ *et al.* (1981). Pharmacokinetics and bioavailability of hydromorphone following IV and oral administration to human subjects. *J Clin Pharmacol* 21: 152–156.
Walls HC (1976). *Bull TIAFT* 12: 7–8.

Hydroquinidine

Antiarrhythmic
$C_{20}H_{26}N_2O_2$ = 326.4
CAS—1435-55-8

IUPAC Name (*S*)-[(2*R*, 5R)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl) methanol
Synonyms Dihydrochinidin; (9*S*)-10,11-dihydro-6′-methoxycinchonan-9-ol; dihydroquinidine; hydroconchinine.

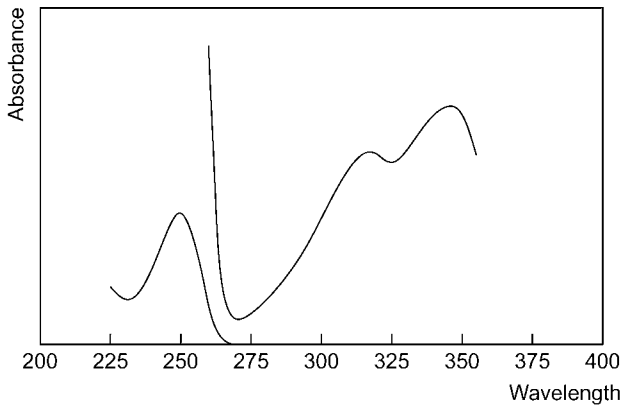


Chemical Properties An alkaloid present in the bark of species of *Cinchona* (Rubiaceae). Crystals. Mp about 169°. Slightly soluble in water and ether; readily soluble in hot alcohol. Log *P* (octanol/water), 3.4.

Hydroquinidine Hydrochloride

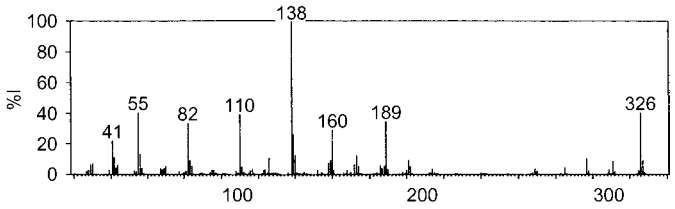
$C_{20}H_{26}N_2O_2$, HCl = 362.9
CAS—1476-98-8
Proprietary Names *Lentoquine*; *Serecor.*
Chemical Properties Colourless crystals. Mp 273° to 274°. Freely soluble in chloroform and methanol; less readily soluble in water and ethanol.

Colour Tests Sulfuric acid—yellow (fluoresces under ultraviolet light); thalleioquin test—green.
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.03; system TC—R_f 0.08; system TE—R_f 0.43; system TL—R_f 0.05; system TAE—R_f 0.20; system TAF—R_f 0.70 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2810.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1=1240b), 316, 345 nm (A^1_1=180b).



Infrared Spectrum Principal peaks at wavenumbers 1509, 1261, 1620, 1234, 854, 1030 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 138, 326, 55, 110, 189, 82, 160, 139.



Quantification

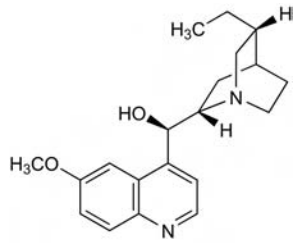
Plasma **HPLC** Fluorescence detection. Hydroquinidine, quinidine, hydroxyquinidine, and quinidine *N*-oxide. Limit of detection, <10 nmol/L [Nielsen *et al.* 1994].
Serum **HPLC** Limit of detection, 0.2 mg/L [Camsonne *et al.* 1984].
Urine **HPLC** Limit of detection, <25 nmol/L (urine), see Plasma [Nielsen *et al.* 1994].
Dose Usually 450 to 600 mg of hydroquinidine hydrochloride daily.

Camsonne R *et al.* (1984). A simple fluorescence high-performance liquid chromatographic assay for dihydroquinidine in serum. *Ther Drug Monit* 6: 471–473.
Nielsen F *et al.* (1994). Determination of quinidine, dihydroquinidine, (3S)-3-hydroxyquinidine and quinidine N-oxide in plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 660: 103–110.

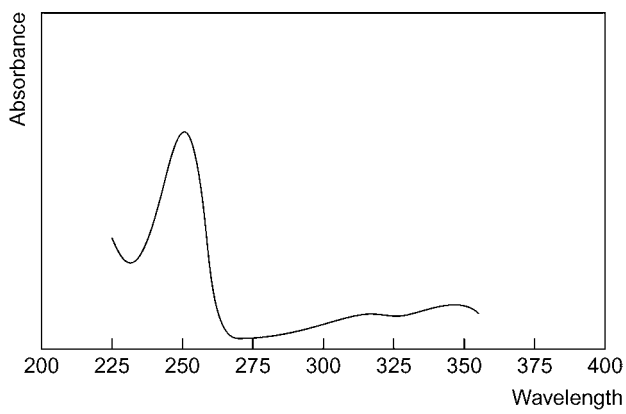
Hydroquinine

Alkaloid
$C_{20}H_{26}N_2O_2$ = 326.4
CAS—522-66-7

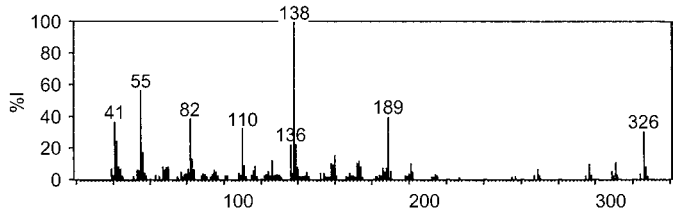
IUPAC Name (8α,9*R*)-10,11-Dihydro-6′-methoxycinchonan-9-ol
Synonym Dihydroquinine



Chemical Properties An alkaloid present in the bark of species of *Cinchona* (Rubiaceae). Crystals. Mp 172°. Almost insoluble in water; freely soluble in acetone, ethanol, chloroform, ether, and petroleum ether. pK_a, 5.33. Log *P* (octanol/water), 3.4.
Colour Tests Sulfuric acid—yellow (fluoresces under ultraviolet light); thalleioquin test—green
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.02; system TC—R_f 0.07; system TE—R_f 0.40; system TL—R_f 0.03; system TAE—R_f 0.21; system TAF—R_f 0.69 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1450.
High Performance Liquid Chromatography System HZ—retention time 2.8 min.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1=915b), 316, 345 nm (A^1_1=167b).



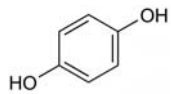
Infrared Spectrum Principal peaks at wavenumbers 1240, 1225, 1507, 1623, 1026, 1132 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 138, 55, 189, 82, 41, 110, 326, 42.



Hydroquinone

Depigmenting Agent, Antioxidant
$C_6H_6O_2$ = 110.1
CAS—123-31-9

IUPAC Name 1,4-Benzenediol
Synonyms Hydroquinol; quinol.
Proprietary Names *African Gold*; *Aida*; *Banishing Cream*; *Claripel*; *Discromil*; *Eldopaque*; *Eldoquin*; *Epocler*; *Esoteric*; *Hidroquin*; *Jouvence*; *Lustra*; *Melanex*; *Melanox*; *Melpaque HP*; *Melquin HP*; *Nadinola*; *Neostrata AHA*; *Porcelana*; *Solaquin*; *Tecquinol*.



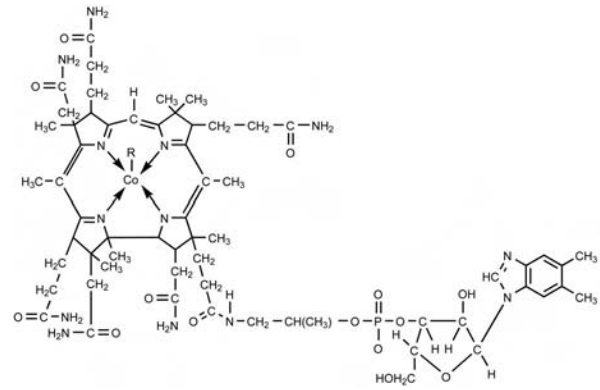
Chemical Properties Fine white crystals, or white crystalline powder, which darken on exposure to light and air. Mp 170° to 171°. Soluble 1 in 14 of water, 1 in 4 of ethanol, 1 in 51 of chloroform and 1 in 16 of ether; slightly soluble in benzene. pK_a 10.9 (25°). Log *P* (octanol/pH 7.4), 0.6.
Colour Tests Ammoniacal silver nitrate—grey-yellow/brown; Benedict's reagent—red; ferric chloride—green
Gas Chromatography System GA—hydroquinone RI 1240; M (2-OH-) RI 1460; hydroquinone-AC_2 RI 1395.
Ultraviolet Spectrum Ethanol—295 nm (A^1_1=282b).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1192, 1520, 760, 1240, 813 cm^{-1} (KBr disk).
Quantification
Urine **GC-MS** [Saito *et al.* 1994].
HPLC Electrochemical detection [Wittig *et al.* 2001]. Fluorescence detection. Hydroquinone, catechol and phenol [Lee *et al.* 1993].
Kidney **GC-MS** See Urine [Saito *et al.* 1994].
Liver **GC-MS** See Urine [Saito *et al.* 1994].
Disposition in the Body
Toxicity
In a fatality involving the ingestion of photographic developer solution containing hydroquinone, tissue concentrations were: urine 3.4 mg/L, liver 0.5 µg/g, kidney 0.2 µg/g [Saito *et al.* 1994].
Use As a 2 to 5% ointment for depigmentation of the skin.

Lee BL *et al.* (1993). Simultaneous determination of hydroquinone, catechol and phenol in urine using high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 619: 259–266.
Saito T *et al.* (1994). Detection of hydroquinone in a poisoning case. *J Forensic Sci* 39: 266–270.
Wittig J *et al.* (2001). Validated method for the determination of hydroquinone in human urine by high-performance liquid chromatography-coulometric-array detection. *J Chromatogr B Biomed Sci Appl* 761: 125–132.

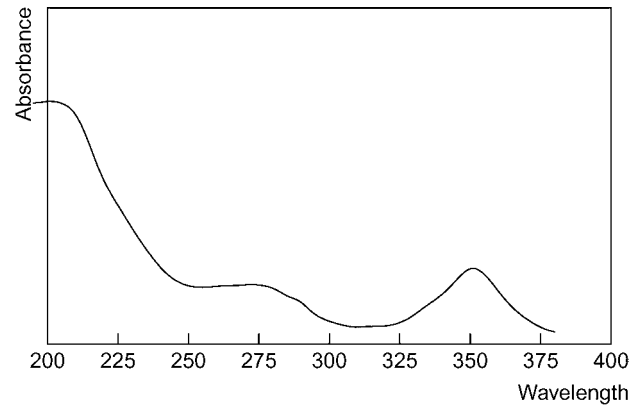
Hydroxocobalamin

Haemopoietic Vitamin
$C_{62}H_{89}CoN_{13}O_{15}P$ = 1346.4
CAS—13422-51-0

IUPAC Name Cobalt(2+); [(2*R*,3*S*,4*R*,5*S*)-5-(5, 6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] [(2*R*)-1-[3-[(2*R*,3*R*,4*Z*,7*S*,9*Z*,12*S*,13*S*,14*Z*,17*S*,18*S*,19*R*)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1*H*-corrin-21-id-3-yl] propanoylamino]propan-2-yl] phosphate; hydrate
Synonyms Coα-[α-(5,6-Dimethylbenzimidazol-1-yl)]-Coβ-hydroxocobamide; vitamin B_{12a}; vitamin B_{12b}.
Proprietary Names *Alpha Cobione*; *Alpha Redisol*; *Alpha-Ruvite*; *Axlon*; *Cobalin-H*; *Codroxomin*; *Droxomin*; *Duradoc*; *Duralta-12*; *Hydroxo B_{12}*; *Neo-Betalin 12*; *Neo-Cytamen*; *Novobedouze*; *Redisol H*; *Rubesol-LA*; *Sytobex-H*.



Chemical Properties Hydroxocobalamin occurs either as aquocobalamin chloride [α-(5,6-dimethylbenzimidazol-1-yl)aquocobamide chloride], $C_{62}H_{90}ClCoN_{13}O_{15}P$, or as aquocobalamin sulfate, $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$. Dark red crystals or crystalline powder. Soluble 1 in 50 of water and 1 in 100 of ethanol; practically insoluble in acetone, benzene, chloroform, ether, and petroleum ether.
Note In acid solutions, hydroxocobalamin takes up a hydrogen ion which converts the hydroxyl group to a co-ordinated water molecule, and in this form it is known as aquocobalamin, which is basic and forms salts with acids. In solution, aquocobalamin exists in equilibrium with hydroxocobalamin and, since it is more stable in acid solution, it usually occurs commercially in the form of aquocobalamin [Smith *et al.* 1962].
Ultraviolet Spectrum Acetic acid—274, 351 nm.



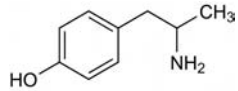
Quantification
Plasma **HPLC** UV detection. Hydroxocobalamin and cyanocobalamin [Astier, Baud 1995].
Dose Initially 0.25 to 1 mg, by IM injection, daily or on alternate days.

Astier A, Baud FJ (1995). Simultaneous determination of hydroxocobalamin and its cyanide complex cyanocobalamin in human plasma by high-performance liquid chromatography. Application to pharmacokinetic studies after high-dose hydroxocobalamin as an antidote for severe cyanide poisoning. *J Chromatogr B Biomed Appl* 667: 129–135.
Smith EL *et al.* (1962). *Analyst* 87: 183–186.

Hydroxyamfetamine

Sympathomimetic
$C_9H_{13}NO$ = 151.2
CAS—103-86-6; 1518-86-1 (±)

IUPAC Name 4-(2-Aminopropyl)phenol
Synonyms Oxamfetamine; oxamphetamine; PHA.

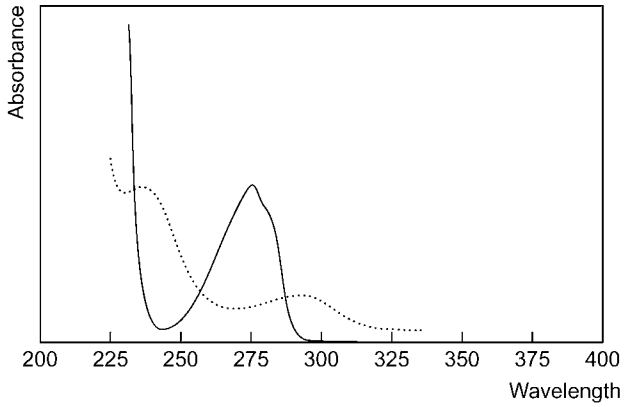


Chemical Properties Crystals. Mp 125° to 126°. Soluble in water, ethanol, chloroform and ethyl acetate. pK_a 9.3 (25°). Log *P* (octanol/water), 1.3.

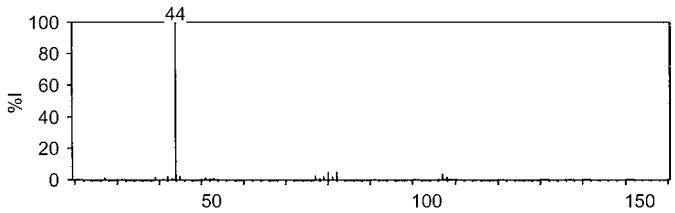
Hydroxyamfetamine Hydrobromide

$C_9H_{13}NO$, HBr = 232.1
CAS—306-21-8; 140-36-3 (±)
Proprietary Name *Paredrine*
Chemical Properties A white crystalline powder. Mp 189° to 192°. Soluble 1 in 1 of water and 1 in 2.5 of ethanol; freely soluble in acetone; slightly soluble in chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.35; system TB—R_f 0.02; system TC—R_f 0.02; system TL—R_f 0.11 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—hydroxyamfetamineRI 1480, M (methoxyamfetamine) RI 1465, M (-AC) RI 1890, M (-AC_2) RI 1900.
High Performance Liquid Chromatography System HB—*k* 2.24; system HC—*k* 1.11; system HY—RI 166.
Ultraviolet Spectrum Aqueous acid—275 nm ($A_1^1 = 103a$); aqueous alkali—238 ($A_1^1 = 672b$), 294 nm.



Infrared Spectrum Principal peaks at wavenumbers 1259, 1517, 1599, 1102, 813, 1111 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 44, 82, 80, 107, 77, 108, 81, 79.



Quantification

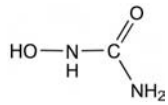
Plasma **GC-MS** Column: HP-5MS capillary (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 µg/L [Peters *et al.* 2003].
Urine **HPLC**Column: Spherisorb C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : ammonium acetate buffer with 0.1% TEA (pH 3.9, 23 : 77 for 19 min to 35 : 65 for 16 min), flow rate 0.7 mL/min for 8 min to 1.2 mL/min at 9 min for 10 min to 1.4 mL/min at 21 min for 14 min. UV detection (λ= 210 nm). Limit of detection, 5.3 ng [Soares *et al.* 2004]. Column: Cosmosil 5C18 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L potassium dihydrogen phosphate with dipotassium hydrogen phosphate (pH 6.0) : acetonitrile : tetrahydrofuran : EDTA (x : 2 : 0.2 : 0.005), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, ~50 pg [Shimosato *et al.* 1986a; Shimosato *et al.* 1986b].
Body Fluids **GC-MS** Column: 5% OV-17 on Chromosorb W HP 80/100 mesh (2 m × 3 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 190° to 220° at 7°/min. Limit of detection, 0.05 µg [Hara *et al.* 1988].
Disposition in the Body Readily absorbed after oral administration. Approximately 90% of a dose is excreted in the urine in 24 h as free and conjugated hydroxyamfetamine, with ~4% of the dose as free and conjugated 4′-hydroxynorephedrine.
Toxicity The estimated minimum lethal dose, intranasally, in children up to 2 years of age is 200 mg, and in adults ~2 g.
Dose Hydroxyamfetamine hydrobromide has been given in doses of 60 to 240 mg daily.

Hara K *et al.* (1988). Simultaneous quantitative analysis of methamphetamine and 4-hydroxymethamphetamine in body fluids by gas chromatography/mass spectrometry. *Z Rechtsmed* 100: 231–236.
Peters FT *et al.* (2003). Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom* 38: 659–676.
Shimosato K *et al.* (1986a). Rapid determination of *p*-hydroxylated methamphetamine metabolites by column liquid chromatography–electrochemistry. *J Chromatogr* 377: 279–286.
Shimosato K *et al.* (1986b). Urinary excretion of *p*-hydroxylated methamphetamine metabolites in man. I. A method for determination by high-performance liquid chromatography–electrochemistry. *Arch Toxicol* 59: 135–140.
Soares ME *et al.* (2004). Simultaneous determination of amphetamine derivatives in human urine after SPE extraction and HPLC-UV analysis. *Biomed Chromatogr* 18: 125–131.

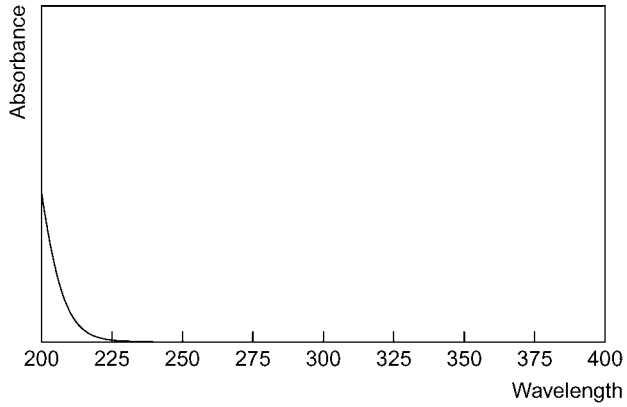
Hydroxycarbamide

Antineoplastic
$CH_4N_2O_2 = 76.1$
CAS—127-07-1

Synonym Hydroxyurea
Proprietary Names *Droxia*; *Hydrea*; *Litalir*; *Mylocel*; *Onco-Carbide*; *Oxeron*; *Syrea*.



Chemical Properties A white crystalline powder. Hygroscopic and decomposes in the presence of moisture. Mp 133°, with decomposition. Freely soluble in water and hot ethanol; slightly soluble in ethanol. Log *P* (octanol/water), −1.8.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1639, 1592, 1111, 1492, 812, 758 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection [Iyamu *et al.* 1998]. Electrochemical detection. Limit of detection, 0.02 mmol/L [Havard *et al.* 1992].
Serum **HPLC** UV–Vis detection [Manouilov *et al.* 1998].

Disposition in the Body

Therapeutic Concentration

In 30 subjects, the maximum tolerated dose of intravenous hydroxycarbamide given as a 24-h continuous infusion was 13.52 g/m^2 following a bolus of 1.69 g/m^2 and for a 48-h infusion the maximum tolerated dose was 17.576 g/m^2 following a bolus of 2.197 g/m^2; mean plasma steady-state concentrations were 146.8 mg/L and 108.8 mg/L, respectively [Smith *et al.* 1993].

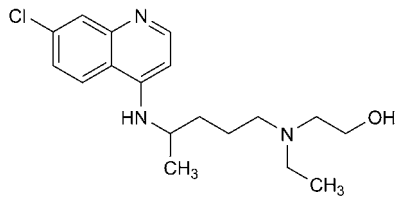
Note For a review of the pharmacokinetics and pharmacodynamics of hydroxycarbamide, see Gwilt and Tracewell [1998].
Dose 20 to 30 mg/kg daily or 80 mg/kg every third day, orally.

Gwilt PR, Tracewell WG (1998). Pharmacokinetics and pharmacodynamics of hydroxyurea. *Clin Pharmacokinet* 34: 347–358.
Havard J *et al.* (1992). Determination by high-performance liquid chromatography of hydroxyurea in human plasma. *J Chromatogr* 584: 270–274.
Iyamu EW *et al.* (1998). New isocratic high-performance liquid chromatographic procedure to assay the anti-sickling compound hydroxyurea in plasma with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 709: 119–126.
Manouilov KK *et al.* (1998). Colorimetric determination of hydroxyurea in human serum using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 708: 321–324.
Smith DC *et al.* (1993). A phase I trial of high-dose continuous-infusion hydroxyurea. *Cancer Chemother Pharmacol* 33: 139–143.

Hydroxychloroquine

Antimalarial
$C_{18}H_{26}ClN_3O = 335.9$
CAS—118-42-3

IUPAC Name 2-[[4-[(7-Chloro-4-quinolinyl)amino]pentyl]ethylamino]ethanol
Synonym Oxichlorochin

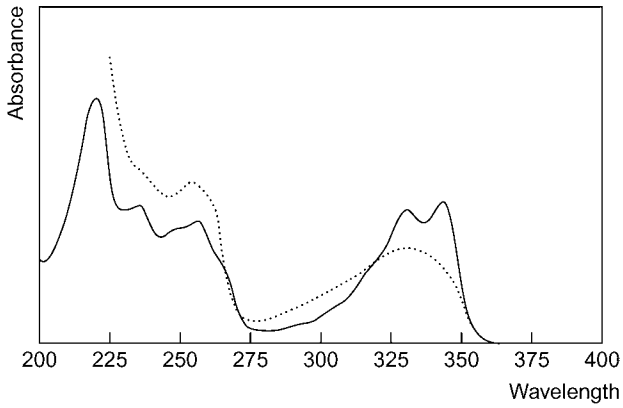


Chemical Properties Crystals. Mp 89° to 91°.

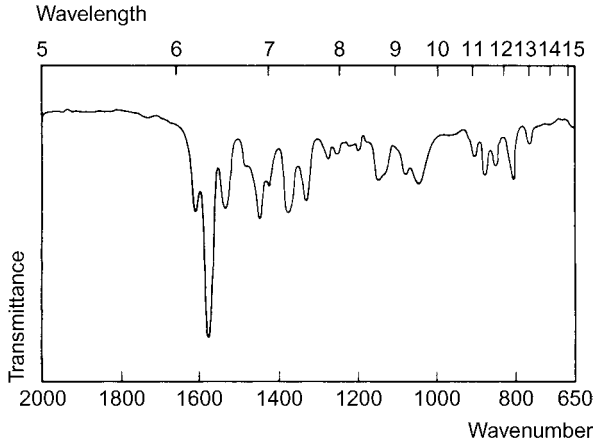
Hydroxychloroquine Sulfate

$C_{18}H_{26}ClN_3O$, H_2SO_4 = 433.0
CAS—747-36-4
Proprietary Names *Ercoquin*; *Plaquenil*; *Quensyl*.
Chemical Properties White crystalline powder. There are 2 forms: one melting at about 198° and the other at about 240°. Soluble 1 in 5 of water; practically insoluble in ethanol, chloroform and ether. Log *P* (octanol/water), 3.0.

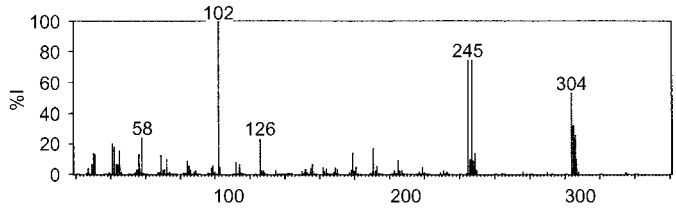
Thin-layer Chromatography System TA—R_f 0.45; system TAE—R_f 0.07; system TL—R_f 0.03; system TB—R_f 0.02; system TC—R_f 0.02; system TE—R_f 0.37 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2872.
High Performance Liquid Chromatography System HX—RI 280; system HZ—RT 1.9 min.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1 = 560a), 256, 329, 343 nm; aqueous alkali—253, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1579, 1608, 1530, 1050, 1150, 810 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 102, 245, 247, 304, 305, 306, 58, 126.



Quantification

Blood **HPLC** Column: PRP-1 polystyrene divinyllbenzene (150 × 4.1 mm i.d., 2 µm). Mobile phase: methanol : water (80 : 20) with 100 mmol/L triethylamine (pH 11), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 337 nm, λ_{em} = 370 nm). Limit of detection, 1 µg/L [Tett *et al.* 1985]. Column: Radial-PAK silica (100 × 8 mm, 5 µm). Fluorescence detection. Limit of detection, 10 µg/mL for hydroxychloroquine [Williams *et al.* 1988]. Hydroxychloroquine enantiomers, UV detection [Brocks *et al.* 1992]. Column: Spherisorb S5SCX sulfophenylpropyl-modified silica (150 × 5 mm i.d., 5 µm). Mobile phase: 4.705 g ammonium perchlorate in 500 mL methanol : water (98.5 : 1.5), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 215 nm). Hydroxychloroquine, chloroquine, quinine and desethylchloroquine, limit of detection <5 ng/mL [Croes *et al.* 1994].

Plasma **HPLC** See Blood [Tett *et al.* 1985]; see Blood [Brocks *et al.* 1992].
Serum **HPLC** See Blood [Brocks *et al.* 1992]; [Croes *et al.* 1994]; Column: Nova-Pak C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: 58 mmol/L monobasic sodium phosphate buffer with 6 mmol/L heptanesulfonic acid sodium salt (pH 3.1) with hydroxychloroquine, chloroquine and corticosteroids, DAD [Volin 1995].
Urine **HPLC** See Blood [Williams *et al.* 1988]. Column: Partisil 5 C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate (pH 3.5) : methanol : ethanol : tetraethylammonium (78 : 22 : 1 : 0008), flow rate 0.75 to 8 mL/min. Limit of detection, 12.5 µg/L [Brocks *et al.* 1992]. Column: Ultremex Cyano. Mobile phase: 0.03 mol/L sodium phosphate buffer (pH 7.0) : ethanol : acetonitrile (79 : 20 : 1) containing 0.05 mol/L *N,N*-DMOA phosphate, flow rate 0.7 mL/min. Limit of quantification, 5 µg/L for hydroxychloroquine enantiomers and metabolites [Fieger *et al.* 1993].
Note For an UV spectrophotometric method for the detection of hydroxychloroquine in blood and urine, see [Dalley and Hainsworth 1965]. For a spectrofluorimetric method for the detection of hydroxychloroquine in plasma, see McChesney *et al.* [1962].
Disposition in the Body Rapidly and almost completely absorbed after oral administration. It is metabolised by de-ethylation. About 6% of a single dose is excreted in the urine over 10 days and about 25% of a dose is eliminated in the faeces in 3 days as unchanged drug plus the de-ethylated metabolites. During daily therapy, <13% of a dose is excreted in the 24 h urine with 8% of the dose as unchanged drug, 2% as desethylchloroquine, 2% as desethylhydroxychloroquine, and 0.5% as didesethylchloroquine.

Therapeutic Concentration

After an oral dose of hydroxychloroquine sulfate equivalent to 310 mg of the base to 6 subjects, peak plasma concentrations of 0.003 to 0.20 mg/L (mean 0.08) were attained in 3 h. Following oral administration of hydroxychloroquine diphosphate equivalent to 1264 mg of the base to 3 subjects, peak plasma concentrations of 0.29 to 1.0 mg/L (mean 0.64) were attained in 3 h and therapeutic concentrations greater than 0.01 mg/L were maintained for 240 h [McChesney *et al.* 1962].

In 23 subjects receiving 200 or 400 mg hydroxychloroquine sulfate orally daily, mean steady-state hydroxychloroquine concentrations at 6 months were 450.6 and 870.3 µg/L, respectively [Tett *et al.* 2000].

Toxicity

In a fatality due to the ingestion of at least 12 g, the following postmortem tissue concentrations were reported: blood 48 mg/L, heart blood 61 mg/L, liver 71 µg/g, urine 970 mg/L [Dalley, Hainsworth 1965].

Postmortem tissue concentrations in a $2^1/_2$-year-old boy who died after ingesting an estimated 1800 mg of hydroxychloroquine were as follows: blood 104 mg/L, stomach 1.2 mg (total), small intestine 75 mg (total), liver 500 µg/g [Kemmenoe 1990].

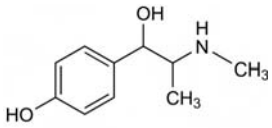
An 18-year-old female who survived after ingesting 20 g of hydroxychloroquine had a plasma concentration of 9.87 mg/L 2 h after ingestion [Jordan *et al.* 1999].

Half-life Plasma half-life, about 3 days.
Dose For an acute attack of malaria, 2 g of hydroxychloroquine sulfate given over three days (1.2 g on the first day). In rheumatoid arthritis, initially 400 to 800 mg daily.

Brocks DR *et al.* (1992). Analytical and semi-preparative high-performance liquid chromatographic separation and assay of hydroxychloroquine enantiomers. *J Chromatogr* 581: 83–92.
Croes K *et al.* (1994). Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood. *J Anal Toxicol* 18: 255–260.
Dalley RA, Hainsworth D (1965). Fatal Plaquenil poisoning. *J Forensic Sci Soc* 12: 99–101.
Fieger H *et al.* (1993). Enantioselective determination of hydroxychloroquine and its major metabolites in urine and the observation of a reversal in the (+)/(-)-hydroxychloroquine ratio. *Chirality* 5: 65–70.
Jordan P *et al.* (1999). Hydroxychloroquine overdose: toxicokinetics and management. *J Toxicol Clin Toxicol* 37: 861–864.
Kemmenoe AV (1990). An infant fatality due to hydroxychloroquine poisoning. *J Anal Toxicol* 14: 186–188.
McChesney EW *et al.* (1962). Laboratory studies on the 4-aminoquinoline antimalarials: II. Plasma levels of chloroquine and hydroxychloroquine in man after various oral dosage regimens. *Antibiot Chemother* 12: 583–594.
Tett SE *et al.* (1985). High-performance liquid chromatographic assay for hydroxychloroquine and metabolites in blood and plasma, using a stationary phase of poly(styrene divinylbenzene) and a mobile phase at pH 11, with fluorimetric detection. *J Chromatogr* 344: 241–248.
Tett SE *et al.* (2000). Concentration-effect relationship of hydroxychloroquine in patients with rheumatoid arthritis—a prospective, dose ranging study. *J Rheumatol* 27: 1656–1660.
Volin P (1995). Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J Chromatogr B Biomed Appl* 666: 347–353.
Williams SB *et al.* (1988). Analysis of blood and urine samples for hydroxychloroquine and three major metabolites by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 433: 197–206.

Hydroxyephedrine

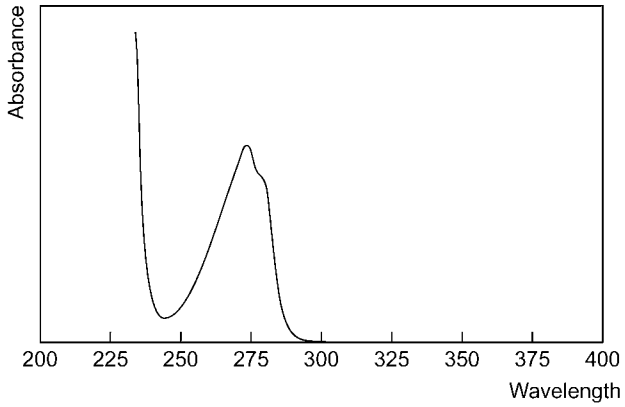
Sympathomimetic
$C_{10}H_{15}NO_2$ = 181.2
CAS—365-26-4
IUPAC Name 4-Hydroxy-α-[1-(methylamino)ethyl]benzenemethanol
Synonyms *p*-Hydroxyephedrine; methylsynephrine; oxyephedrine.



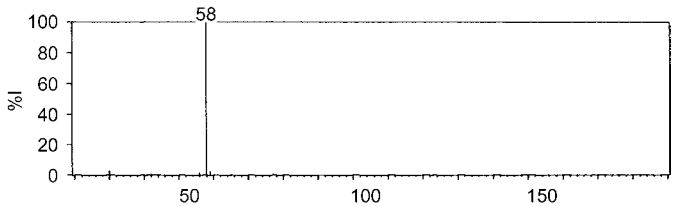
Chemical Properties A crystalline powder. Mp 152° to 154°. Sparingly soluble in water, ethanol and ether; readily soluble in dilute acids and sodium hydroxide solution. Log *P* (octanol/water), 0.2.

Hydroxyephedrine Hydrochloride
$C_{10}H_{15}NO_2$, HCl = 217.7
CAS—942-51-8
Proprietary Name *Carnigen*
Chemical Properties A crystalline powder. Mp 209° to 211°. Soluble 1 in 3 of water, 1 in 5 of glycerol and 1 in 10 of ethanol; sparingly soluble in acetone.

Colour Tests Liebermann's reagent—black; Mandelin's test—green; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.35, streaking (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1682.
High Performance Liquid Chromatography System HB—*k* 0.73.
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1=75b); aqueous alkali—242 (A_1^1=760b), 290 nm (A_1^1=131b).



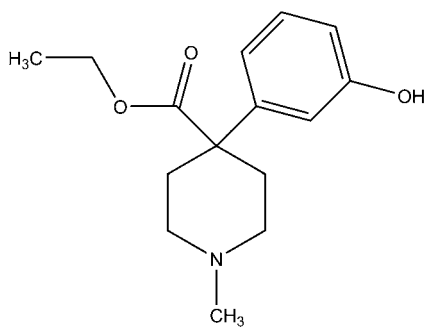
Infrared Spectrum Principal peaks at wavenumbers 980, 1238, 835, 1157, 1129, 792 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 30, 59, 56, 77.



Dose Hydroxyephedrine hydrochloride has been given in doses of 10 mg daily.

Hydroxypethidine

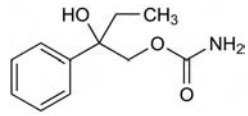
Narcotic
$C_{15}H_{21}NO_3$ = 263.3
IUPAC Name Ethyl 4-(3-hydroxyphenyl)-1-methylpiperidine-4-carboxylate
Synonyms 446; 10446; Hoechst 10446; oxypetidin; Win 771.



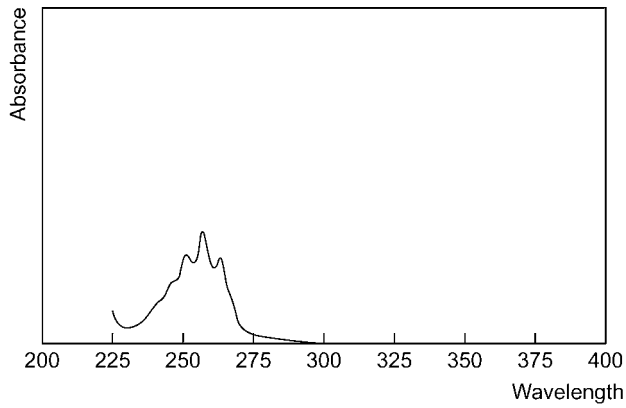
Chemical Properties Hydoxypethidine is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve the yield.
Colour Tests Ammonium molybdate test—bright blue, fading (limit of detection, 0.25 µg); ammonium vanadate test—dark green (limit of detection, 0.5 µg); sulfuric acid-formaldehyde test—dull orange (limit of detection, 0.1 µg); Vitali's test—yellow (limit of detection, 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.47 (location reagent potassium permanganate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.75 (relative to diphenhydramine); system G4—retention time 0.56 (relative to codeine).

Hydroxyphenamate

Tranquilliser
$C_{11}H_{15}NO_3$ = 209.2
CAS—50-19-1
IUPAC Name 2-Phenyl-1,2-butanediol 1-carbamate
Synonym Oxyfenamate
Proprietary Name *Listica*



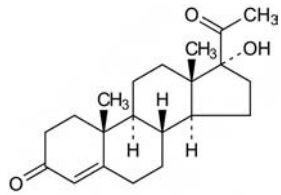
Chemical Properties A white crystalline powder. Mp 55° to 56.5°. Soluble 1 in 40 of water; soluble in ethanol and chloroform. Log *P* (octanol/water), 1.1.
Thin-layer Chromatography System TA—R_f 0.74 (van Urk reagent, positive).
Gas Chromatography System GA—RI 1724.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=9.0a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1715, 1072, 700, 1603, 1110, 760 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 135, 57, 91, 77, 43, 119, 105, 180.
Dose Hydroxyphenamate has been given in doses of 600 mg daily.

Hydroxyprogesterone

Progestational Steroid
$C_{21}H_{30}O_3$ = 330.5
CAS—68-96-2
IUPAC Name 17-Hydroxypregn-4-ene-3,20-dione

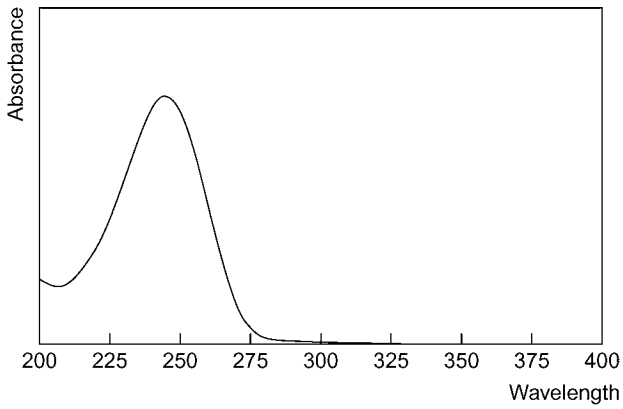


Chemical Properties A crystalline powder. Mp 222° to 223° with rapid heating; with slow heating it undergoes molecular rearrangement accompanied by partial resolidification and melts at 276°. Log *P* (octanol/water), 3.2.

Hydroxyprogesterone Caproate
$C_{27}H_{40}O_4$ = 428.6
CAS—630-56-8
Synonyms 17 AHPC; hydroxyprogesterone hexanoate.

Proprietary Names *Caposten*; *Delalutin*; *Hylutin*; *Hyprogest*; *Hyproval PA*; *Lentogest*; *Primolut Depot*; *Proluton Depot*; *Relutin*.
Chemical Properties A white or creamy-white crystalline powder. Mp 120° to 124°. Practically insoluble in water; soluble 1 in 10 of ethanol, 1 in 0.4 of chloroform, and 1 in 10 of ether.

Colour Tests Antimony pentachloride—orange; naphthol-sulfuric acid—orange-red/blue, violet dichroism; sulfuric acid—yellow (green fluorescence under ultraviolet light).
Thin-layer Chromatography Hydroxyprogesterone: system TB—R_f 0.38; system TE—R_f 0.85; system TF—R_f 0.63; system TAE—R_f 0.86. Hydroxyprogesterone caproate: system TP—R_f 0.81; system TQ—R_f 0.55; system TR—R_f 0.99; system TS—R_f 0.90 (*p*-toluenesulfonic acid solution, positive.)
High Performance Liquid Chromatography Hydroxyprogesterone: system HX—RI 1054.
Ultraviolet Spectrum Hydroxyprogesterone caproate: dehydrated alcohol—240 nm (A^1_1=395a).

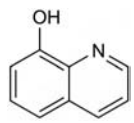


Infrared Spectrum Principal peaks at wavenumbers 1728, 1670, 1177, 1188, 1716, 1224 cm^{-1} (hydroxyprogesterone caproate, KBr disk).
Quantification
Blood **GC–MS** Limit of detection, 20 µg/L [Lai *et al.* 2002].
Plasma **GC–MS** For method for quantification of hydroxyprogesterone, 4-androstenedione, and testosterone, see Wudy *et al.* [1995]. Limit of detection, <1 pg [Shimizu 1988].
Dose Usually 250 to 500 mg of hydroxyprogesterone caproate weekly, by IM injection.

Lai CC *et al.* (2002). Rapid screening assay of congenital adrenal hyperplasia by measuring 17 alpha-hydroxyprogesterone with high-performance liquid chromatography/electrospray ionization tandem mass spectrometry from dried blood spots. *J Clin Lab Anal* 16: 20–25.
Shimizu K *et al.* (1988). Determination of 17-hydroxyprogesterone in plasma by gas chromatography-mass spectrometry with high-resolution selected-ion monitoring. *J Chromatogr* 432: 21–28.
Wudy SA *et al.* (1995). 17 alpha-hydroxyprogesterone, 4-androstenedione, and testosterone profiled by routine stable isotope dilution/gas chromatography-mass spectrometry in plasma of children. *Pediatr Res* 38: 76–80.

Hydroxyquinoline

Antibacterial, Antifungal
C_9H_7NO = 145.2
CAS—148-24-3
IUPAC Name 8-Quinolinol
Synonyms Oxine; oxyquinoline; 8-quinolinol.

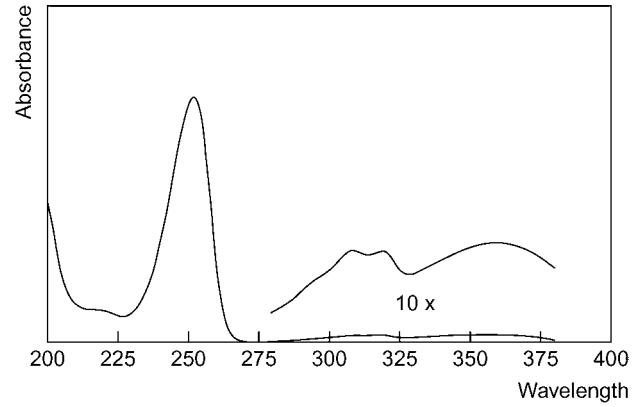


Chemical Properties A white or faintly yellow crystalline powder. Mp 76°. Soluble 1 in 1500 of water; freely soluble in ethanol, acetone, benzene, aqueous mineral acids and chloroform; almost insoluble in ether. pK_a 5.0, 9.9 (20°). Log *P* (octanol/water), 2.0.

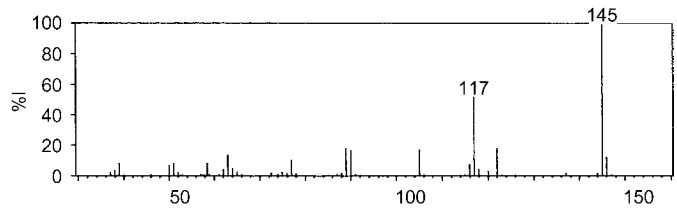
Hydroxyquinoline Sulfate
$(C_9H_7NO)_2, H_2SO_4$ = 388.4
CAS—134-31-6
Synonyms Chinosol; chinosolum; oxyquinol; oxyquinoline sulfate.
Proprietary Name *Sérorhinol*
Chemical Properties A light-yellow powder. Mp 175° to 178°. Soluble 1 in 1 of water, 1 in 100 of ethanol and 1 in 100 of glycerol; practically insoluble in chloroform and ether.

Potassium Hydroxyquinoline Sulfate
Synonyms Oxyquinol potassium; potassium oxyquinoline sulfate.
Proprietary Names It is an ingredient of *Auralgicin*, *Quinocaort*, *Quinoderm*, *Quinoped* and *Valderma*.
Chemical Properties An equimolecular mixture of potassium sulfate and 8-quinolinol sulfate, containing the equivalent of 50% of 8-quinolinol. A pale-yellow microcrystalline powder. Mp 172° to 184°. Soluble 1 in 2 of water; partly soluble in ethanol; practically insoluble in ether.

Colour Test Ferric chloride—green.
Thin-layer Chromatography System TA—R_f 0.32, streaking (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—251 (A^1_1=3930a), 308 (A^1_1=105a), 318, 356 nm.



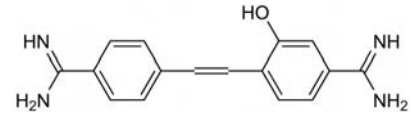
Infrared Spectrum Principal peaks at wavenumbers 1509, 779, 709, 1282, 1219, 1190 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 145, 117, 122, 89, 105, 90, 63, 146.



Use Potassium hydroxyquinoline sulfate is used topically in concentrations of 0.05 to 0.5%.

Hydroxystilbamidine

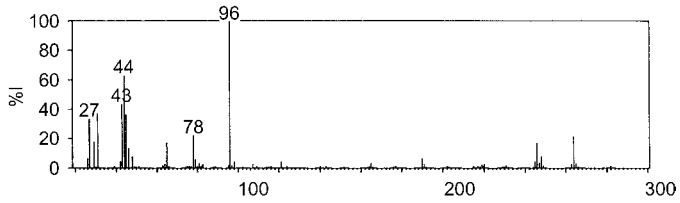
Antifungal, Antiprotozoal
$C_{16}H_{16}N_4O$ = 280.3
CAS—495-99-8
IUPAC Name 4-[2-[4-(Aminoiminomethyl)phenyl]ethenyl]-3-hydroxybenzene carboximidamide
Synonym Oxistilbamidine



Chemical Properties Yellow crystals. Mp 235°. Log *P* (octanol/water), 1.6.

Hydroxystilbamidine Isetionate
$C_{16}H_{16}N_4O, 2C_2H_6O_4S$ = 532.6
CAS—533-22-2
Chemical Properties A fine, yellow, crystalline powder, which decomposes on exposure to light. Mp 286°. Freely soluble in water; slightly soluble in ethanol; practically insoluble in ether.

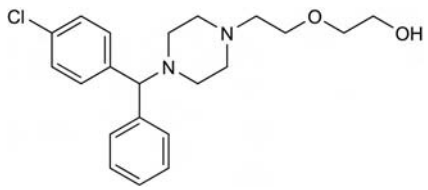
Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—blue-green.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—344 nm (A^1_1=1093a); aqueous alkali—310 nm (A^1_1=811b).
Infrared Spectrum Principal peaks at wavenumbers 1196, 1028, 1667, 1597, 742, 1053 cm^{-1} (hydroxystilbamidine isetionate, KBr disk).
Mass Spectrum Principal ions at *m/z* 96, 44, 43, 31, 45, 27, 78, 264.



Dose 225 or 250 mg of hydroxystilbamidine isetionate daily or on alternate days, by IV infusion.

Hydroxyzine

Antihistamine, Tranquilliser
$C_{21}H_{27}ClN_2O_2$ = 374.9
CAS—68-88-2
IUPAC Name 2-[2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy] ethanol



Chemical Properties pK_a 2.1, 7.1. Log *P* (octanol/water), 2.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

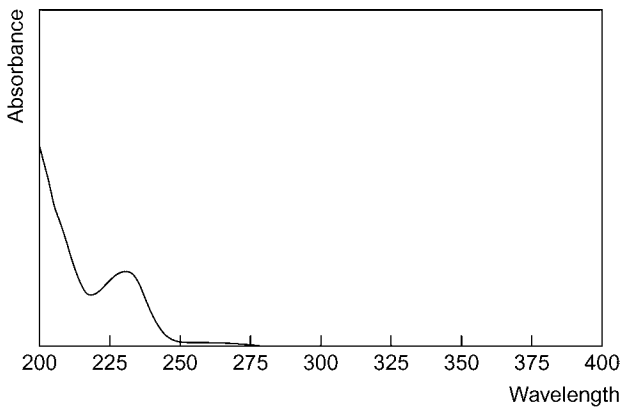
Hydroxyzine Embonate

$C_{21}H_{27}ClN_2O_2$, $C_{23}H_{16}O_6$ = 763.3
CAS—10246-75-0
Synonym Hydroxyzine pamoate
Proprietary Names *Equipose*; *Masmoran*; *Paxistil*; *Vistaril* (capsules and oral suspension).
Chemical Properties A pale-yellow powder. Practically insoluble in water, chloroform, ether, and methanol; soluble 1 in 700 of ethanol and 1 in 10 of dimethylformamide.

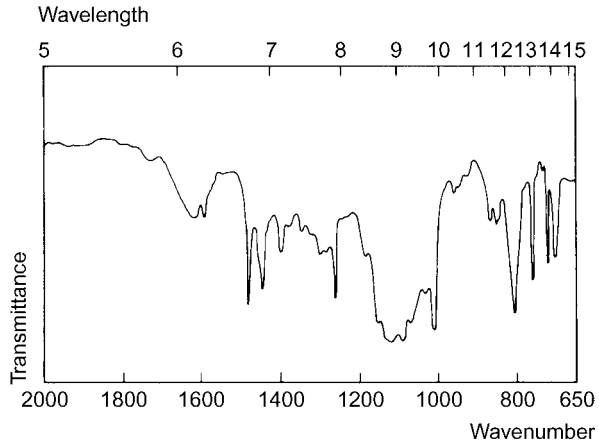
Hydroxyzine Hydrochloride

$C_{21}H_{27}ClN_2O_2$, 2HCl = 447.8
CAS—2192-20-3
Proprietary Names *Alamon*; *Atarax*; *Atazina*; *Aterax*; *Durrax*; *Orgatrax*; *Quiess*; *Sedaril*; *Vistaril* (injection).
Chemical Properties A white crystalline powder. Mp 193°. Soluble 1 in 1 of water, 1 in 4.5 of ethanol, and 1 in 13 of chloroform; practically insoluble in ether.

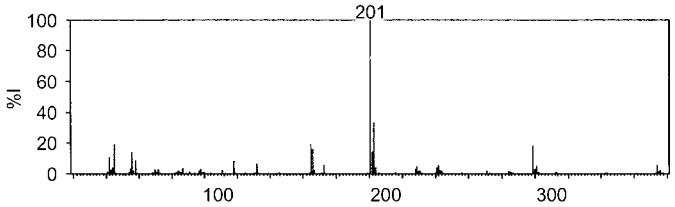
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.10; system TC—R_f 0.54; system TE—R_f 0.54; system TL—R_f 0.19; system TAE—R_f 0.57; system TAF—R_f 0.65; system TAJ—R_f 0.26; system TAK—R_f 0.00; system TAL—R_f 0.34 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, green).
Gas Chromatography System GA—hydroxyzine RI 2880; M (4-chlorobenzophenone) RI 1850; M (4-chloromethylbiphenyl) RI 1600; M (OH-chlorobenzophenone) RI 2300; M (desalkyl-) RI 2520; system GB—hydroxyzine RI 3000; M (norchlorcyclizine) RI 2355; M (4-chlorobenzophenone) RI 1862; M (4-chloromethylbiphenyl) RI 1688; M (OH-chlorobenzophenone) RI 2230.
High Performance Liquid Chromatography System HA—*k* 1.4; system HX—RI 437; system HY—RI 326; system HZ—retention time 5.7 min; system HAA—retention time 15.3 min; system HAX—retention time 11.4 min; system HAY—retention time 6.3 min.
Ultraviolet Spectrum Aqueous acid—232 (A_1^1=416a), 258, 263, 270 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1082, 1130, 1005, 1149, 1063, 800 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 201, 203, 165, 45, 299, 166, 202, 56.



Quantification

Plasma **GC** NPD. Limit of detection, 0.8 µg/L [Kintz *et al.* 1990].
GC–MS Limit of detection, 2 µg/L [Fouda *et al.* 1979].
Biological Fluids **GC** NPD. For method of quantification see Kintz *et al.* [1990].

Disposition in the Body

Therapeutic Concentration

Following a single oral dose of 100 mg of the hydrochloride given to 4 subjects, peak plasma concentrations of 74 to 89 µg/L (mean, 83) were attained in 2 to 4 h [Fouda *et al.* 1979].

Toxicity

A plasma concentration of 102.7 mg/L was reported 8.5 h after the ingestion of about 500 mg of hydroxyzine by a 13-month-old child; the child recovered within 72 h [Magera 1981].

In a fatality due to hydroxyzine, the following postmortem tissue concentrations were reported: blood 39 mg/L, brain 163 µg/g, bile 122 mg/L, liver 414 µg/g, urine 19 mg/L [Johnson 1982].

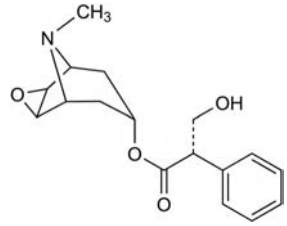
In a fatal self-poisoning attributed to hydroxyzine, postmortem tissue concentrations were: blood 4.18 mg/L, urine 1.43 mg/L, bile 23.24 mg/L, stomach content 78.23 mg/L [Kintz *et al.* 1990].

Half-life Plasma half-life, about 3 h.
Dose 75 to 400 mg of hydroxyzine hydrochloride daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Fouda HG *et al.* (1979). Sensitive assay for determination of hydroxyzine in plasma and its human pharmacokinetics. *J Pharm Sci* 68: 1456–1458.
Johnson GR (1982). A fatal case involving hydroxyzine. *J Anal Toxicol* 6: 69–70.
Kintz P *et al.* (1990). Gas chromatographic identification and quantification of hydroxyzine: application in a fatal self-poisoning. *Forensic Sci Int* 48: 139–143.
Magera BE (1981). Hydroxyzine intoxication in a 13-month-old child. *Pediatrics* 67: 280–283.

Hyoscine

Anticholinergic
$C_{17}H_{21}NO_4$ = 303.4
CAS—51-34-3
IUPAC Name (1α,2β,4β,5α,7β)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]non-7-yl-(α-*S*)-α-(hydroxymethyl)benzene acetate
Synonym Scopolamine
Proprietary Names *Scopoderm*; *Transcop*; *Transderm Scop*.

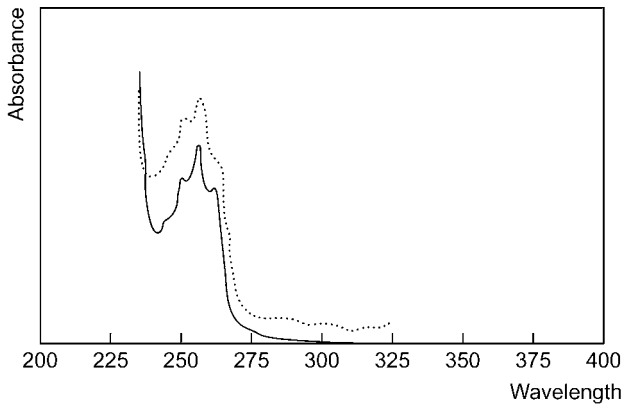


Chemical Properties Crystals. An alkaloid found in various solanaceous plants, particularly species of *Datura*, *Scopolia* and *Duboisia*. A viscous liquid which forms a crystalline monohydrate with a Mp 59°. Soluble 1 in 9.5 of water at 15°, freely soluble in hot water; freely soluble in ethanol, chloroform, acetone and ether; sparingly soluble in benzene and petroleum ether. pK_a 7.6 (23°). Log *P* (octanol/pH 7.4), 1.2.

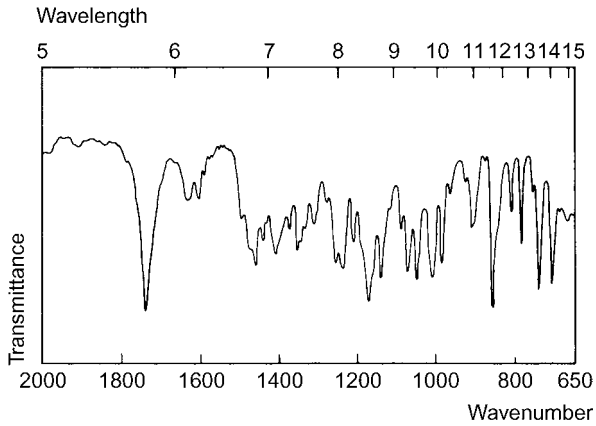
Hyoscine Hydrobromide

$C_{17}H_{21}NO_4$, $HBr,3H_2O$ = 438.3
CAS—114-49-8 (anhydrous); 6533-68-2 (trihydrate)
Synonyms Scopolamine bromhydrate; scopolamine hydrobromide.
Proprietary Names *Joy-Rides*; *Kwells*; *Scopace*; *Travacalm HO*; *Travel Calm*; *Vorigeno*.
Chemical Properties Colourless transparent crystals or white crystalline powder; slightly efflorescent in dry air. Mp 195°. Soluble 1 in 1.5 of water and 1 in 20 of ethanol; practically insoluble in chloroform and ether.

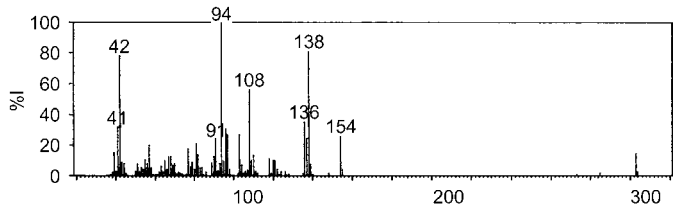
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.06; system TC—R_f 0.37; system TE—R_f 0.48; system TL—R_f 0.18; system TAE—R_f 0.49; system TAF—R_f 0.47; system TAJ—R_f 0.61; system TAK—R_f 0.49; system TAL—R_f 0.93 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; Marquis test, pink).
Gas Chromatography System GA—hyoscine RI 2300, M (hydrate) RI 2230, M (art desacyl-) RI 1210; system GB—hyoscine RI 2427, M (hydrate) RI 2255; system GF—RI 2885.
High Performance Liquid Chromatography System HA—*k* 1.1; system HX—RI 270; system HY—RI 253; system HAA—RT 7.4 min; system HAX—RT 7.0 min; system HAY—RT 3.7 min.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1 = 6.3a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1730, 853, 1166, 736, 705, 1047 cm^{-1} (hyoscine hydrobromide, KBr disk).



Mass Spectrum Principal ions at *m/z* 94, 138, 42, 108, 136, 41, 96, 97.



Quantification

Plasma **GC-MS** Column: 3% OV-17 on Gas Chrom Q 100/120 (1.8 m × 2 mm i.d.). Carrier gas: 30 mL/min. Temperature: 95°. Limit of detection, 50 ng/L [Bayne *et al.* 1975].
Serum **GC-MS** Column: Durabond 5 fused silica capillary (15 m × 0.25 mm i.d., 0.1 μm). Carrier gas: H_2. Temperature: 290°. Limit of detection, 50 ng/L [Oertel *et al.* 1996].
Note For an immunoassay see Hagemann *et al.* [1992].
Urine **HPLC** Column: Spherisorb S5W (250 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : 0.1 moL/L ammonium nitrate buffer (pH 9.3, 20 : 70 : 10), flow rate 0.8 mL/min. Electrochemical detection. Limit of detection, 5 ng [Whelpton *et al.* 1992].
GC-MS See Plasma [Bayne *et al.* 1975].
Disposition in the Body Hyoscine is readily absorbed after oral administration of the hydrobromide and extensively metabolised. Approximately 5% of an oral dose is excreted in the urine as unchanged drug. Hyoscine is well absorbed following application to the skin. It crosses the blood–brain barrier and may cross the placenta.
Therapeutic Concentration

Administration of hyoscine hydrobromide nasally at a dose of 0.2 mg/0.05 mL or 0.4 mg/0.10 mL in formulations of varying pH to 18 subjects produced average peak plasma concentrations of 0.262, 0.419 and 0.488 μg/L after the 0.2–mg dose at pH 4, 7 and 9, respectively, and 0.503, 0.933 and 1.308 μg/L after the 0.4–mg dose at the same pH values. At both doses, the average time to reach peak plasma concentration decreased linearly with a decrease in formulation pH (e.g. at the 0.4–mg dose, the time to peak concentration was 26.7, 15.0 and 8.8 min at pH 4, 7 and 9, respectively) [Ahmed *et al.* 2000].

Following administration of a single dose of 0.4 mg hyoscine bromide by the intranasal or oral route in 12 subjects (at least 2 weeks separated the doses), peak plasma concentrations of 0.00168 and 0.000164 mg/L, respectively, were attained within 1 h of dosing (0.37 h after intranasal dose and 0.78 h after oral dose) [Putcha *et al.* 1996].

A peak plasma concentration of 0.55 ± 0.06 mg/L was attained within 15 min in all but 2 of 8 subjects given 0.25% hyoscine eye drops unilaterally [Lahdes *et al.* 1990].

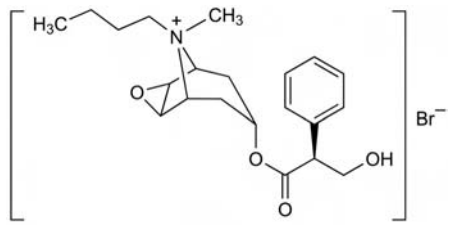
Following a single oral dose equivalent to 415 μg hyoscine to 10 subjects, a mean peak plasma concentration of 0.3 μg/L was attained in 0.5–1 h, decreasing to 50% of the peak concentration in 2– h [Muir, Metcalfe 1983].

Toxicity The lethal dose in children may be as low as 10 mg but fatalities after hyoscine poisoning are rare.
Protein Binding Binding occurs.
Dose Hyoscine hydrobromide, 0.9 mg orally over 24 h; 1 mg hyoscine over 72 h transdermally.

Ahmed S *et al.* (2000). Effects of pH and dose on nasal absorption of scopolamine hydrobromide in human subjects. *Pharm Res* 17: 974–977.
Bayne WF *et al.* (1975). Submicrogram assay for scopolamine in plasma and urine. *J Pharm Sci* 64: 288–291.
Hagemann K *et al.* (1992). Monoclonal antibody-based enzyme immunoassay for the quantitative determination of the tropane alkaloid, scopolamine. *Planta Med* 58: 68–72.
Lahdes K *et al.* (1990). Systemic absorption of ocular scopolamine in patients. *J Ocul Pharmacol* 6: 61–66.
Muir C, Metcalfe R (1983). A comparison of plasma levels of hyoscine after oral and transdermal administration. *J Pharm Biomed Anal* 1: 363–367.
Oertel R *et al.* (1996). Determination of scopolamine in human serum by gas chromatography–ion trap tandem mass spectrometry. *J Chromatogr B Biomed Appl* 682: 259–264.
Putcha L *et al.* (1996). Bioavailability of intranasal scopolamine in normal subjects. *J Pharm Sci* 85: 899–902.
Whelpton R *et al.* (1992). Liquid chromatographic determination of hyoscine (scopolamine) in urine using solid phase extraction. *Biomed Chromatogr* 6: 198–204.

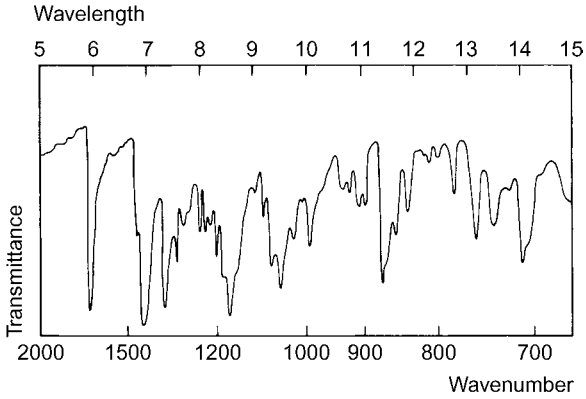
Hyoscine Butylbromide

Anticholinergic
$C_{21}H_{30}BrNO_4$ = 440.4
CAS—149-64-4
IUPAC Name [7(*S*)-(1α,2β,4β,5α,7β)]-9-Butyl-7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane bromide
Synonyms Butylscopolamonii bromidum; hyoscine-*N*-butyl bromide; scopolamine butylbromide.
Proprietary Name *Buscopan*



Chemical Properties A white crystalline powder. Mp 140° to 144°. Soluble 1 in 1 of water, 1 in 50 of ethanol and 1 in 5 of chloroform; practically insoluble in ether. Log *P* (octanol/water), −1.1.
Colour Tests Aromaticity (method 2)—colourless/yellow; Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.08 (acidified iodoplatinate solution, positive).

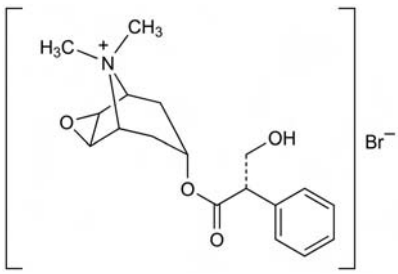
High Performance Liquid Chromatography System HY—RI 294.
Ultraviolet Spectrum Aqueous acid—252 (A^1_1=3.7a), 258 (A^1_1=4.6a), 264 nm (A^1_1=3.6a).
Infrared Spectrum Principal peaks at wavenumbers 1175, 1721, 1052, 874, 1072, 709 cm^{-1} (Nujol mull).



Disposition in the Body Poorly absorbed after oral administration. About 90% of an oral dose is eliminated in the faeces and <10% is excreted in the urine. After IV administration, about 40% of a dose is excreted in the urine.
Half-life Plasma half-life, about 8 h.
Protein Binding About 10%.
Dose 20 mg given parenterally, repeated if necessary; 80 mg daily, orally.

Hyoscine Methobromide

Anticholinergic
$C_{18}H_{24}BrNO_4$ = 398.3
CAS—155-41-9
IUPAC Name (1α,2β,4β,5α,7β)-7-[(2*S*)-3-Hydroxy-1-oxo-2-phenylpropoxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane bromide
Synonyms Epoxymethamine bromide; hyoscine methylbromide; methoscopolamine bromide; scopolamine methylbromide.
Proprietary Names *Holopon*; *Pamine*.



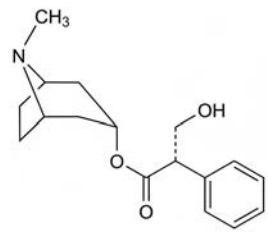
Chemical Properties White crystals or crystalline powder. Mp about 225°, with decomposition. Soluble 1 in 3 of water, 1 in 100 of ethanol and 1 in 40 of methanol; practically insoluble in chloroform. Log *P* (octanol/water), −2.6.
Colour Test Aromaticity (method 2)—colourless/yellow.
Ultraviolet Spectrum Aqueous acid—253 (A^1_1=3.7b), 258 (A^1_1=4.4a), 264 nm (A^1_1=3.4b).
Infrared Spectrum Principal peaks at wavenumbers 1718, 1180, 1174, 1042, 923, 858 cm^{-1} (KBr disk).
Dose 10 to 12.5 mg daily.

Hyoscine Methonitrate

Anticholinergic
$C_{18}H_{24}N_2O_7$ = 380.4
CAS—6106-46-3
IUPAC Name (1α,2β,4β,5α,7β-7-[(2*S*)-3-Hydroxy-1-oxo-2-phenylpropoxyl]-9,9-dimethyl-3-oxo-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane nitrate
Synonyms Hyoscine methylnitrate; methscopolamine nitrate; methylhyoscini nitras; methylscopolamini nitras; scopolamine methylnitrate.
Proprietary Name *Skopyl*
Chemical Properties Colourless hygroscopic crystals or a white crystalline powder. Mp 194° to 199°. Soluble 1 in 1.5 of water and 1 in 40 of ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), 0.4.
Colour Tests Aromaticity (method 2)—colourless/violet (transient); Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—251 (A^1_1=4.3a), 257 (A^1_1=5.1a), 263 nm (A^1_1=3.8a).
Infrared Spectrum Principal peaks at wavenumbers 1735, 1175, 1185, 1317, 860, 923 cm^{-1} (KBr disk).
Dose Up to 12 mg daily.

Hyoscyamine

Anticholinergic
$C_{17}H_{23}NO_3$ = 289.4
CAS—101-31-5
IUPAC Name 8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [3(*S*)-*endo*]-α-(hydroxymethyl)-benzeneacetate
Synonym L-Hyoscyamine
Proprietary Name *Cystospaz*



Chemical Properties An alkaloid obtained from various solanaceous plants, *Hyoscyamus muticus* and *Duboisia myoporoides* being the best sources. It is the levo-isomer of atropine. A white crystalline powder. Mp 106° to 109°. Soluble 1 in 280 of water, 1 in 1 of chloroform, 1 in 69 of ether, 1 in 150 of benzene; freely soluble in ethanol and dilute acids. pK_a 9.7 (21°). Log *P* (octanol/water), 1.9.

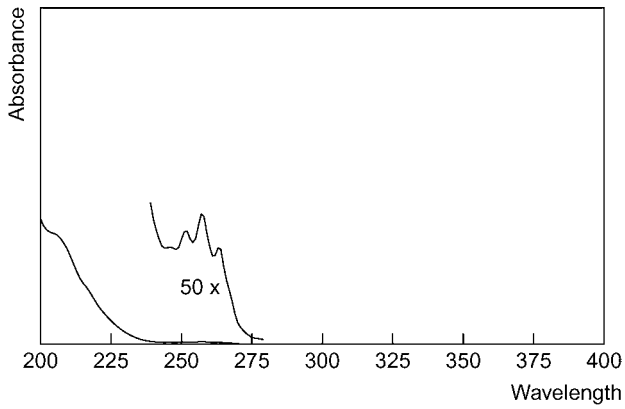
Hyoscyamine Hydrobromide

$C_{17}H_{23}NO_3$, HBr = 370.3
CAS—306-03-6
Synonym Hyoscyamine bromhydrate
Chemical Properties White prismatic crystals or crystalline powder. Mp 152°. Very soluble in water; soluble 1 in 3 of ethanol and 1 in 1.2 of chloroform; very slightly soluble in ether.

Hyoscyamine Sulfate

$(C_{17}H_{23}NO_3)_2$, H_2SO_4, $2H_2O$ = 712.9
CAS—620-61-1 (anhydrous); 6835-16-1 (dihydrate)
Proprietary Names *Anaspaz*; *Cystospaz-M*; *Egacen(e)*; *Egazil*; *Levsin*; *Levsinex*; *Peptard*.
Chemical Properties Colourless needles or a white deliquescent crystalline powder. Mp about 203°, with decomposition. Soluble 1 in 0.5 of water and 1 in 5 of ethanol; sparingly soluble in dehydrated alcohol; very slightly soluble in chloroform and ether.

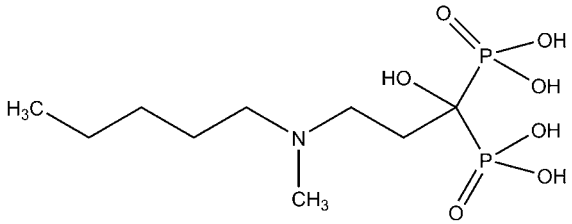
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.18; system TE—R_f 0.26; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.34 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2190; system GB—RI 2293.
High Performance Liquid Chromatography System HA—*k* 3.7 (tailing peak); system HAA—retention time 9.7 min.
Ultraviolet Spectrum Aqueous acid—252 (A^1_1=6.2a), 258 (A^1_1=8.2a), 264 nm (A^1_1=6.2a).



Infrared Spectrum Principal peaks at wavenumbers 1738, 1160, 1025, 1145, 1225, 1050 cm^{-1} (hyoscyamine hydrobromide, KBr disk).
Quantification See under Atropine.
Dose 0.6 to 1.2 mg daily.

Ibandronic Acid

Aminobiphosphonate, Bone Resorption Inhibitor
$C_9H_{23}NO_7P_2 = 319.2$
CAS—114084-78-5
IUPAC Name [1-Hydroxy-3-(methyl-pentylamino)-1-phosphonopropyl]phosphonic acid
Synonyms BM-21.0955; [1-hydroxy-3-(methylpentylamino)propylidene] biphosphonic acid.



Ibandronate Sodium

$C_9H_{22}NNaO_7P_2, H_2O = 359.2$
CAS—138926-19-9
Proprietary Names *Bandrobon*; *Bondronat*; *Boniva*; *Bonviva*; *Elasterin*; *Femorel*; *Idena*; *Modifical.*
Chemical Properties White to off-white powder. Freely soluble in water, practically insoluble in organic solvents [Hoffmann-La Roche 2006]. pK_{a1} 2.0, pK_{a2} 6.3, pK_{a3} 10.5 [Barrett *et al.* 2004]. Log *P* (octanol/water), −1.04 [Wishart 2006].

Capillary Electrophoresis Column: uncoated fused silica capillary (total/effective length: 50/40, 50 µm i.d.). Buffer: 10 mmol/L potassium chromate : tetradecyltrimethylammonium bromide : water (pH 10.0; 10 : 4 : 6). UV detection (λ = 254 nm). Retention time: ibandronate 2.8 min, phosphyte 3.1 min, phosphate 3.2 min. Limit of quantification not reported [Rodriguez *et al.* 2007].
Quantification
Plasma **GC-MS** Column: DB-WAX fused silica (10 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.5 mL/min. Temperature programme: 80° for 1 min to 220° at 40° for 1 min. CI, SIM acquisition mode. Retention time: 2.2 min (*N*-pentyl, *N*-methyl-β-alanine methyl ester derivative). Limit of quantification, 1 µg/L [Endele *et al.* 2005].
Urine **GC-MS** See Plasma. Limit of quantification, 2 µg/L [Endele *et al.* 2005].
Bone **GC-MS** See Plasma. Limit of quantification, 20 ng/kg [Endele *et al.* 2005].
Disposition in the Body Like other bisphosphonates, ibandronate is poorly absorbed after oral doses. Absorption is decreased by food, especially by products containing calcium or other polyvalent cations, so it is usually administered with a 1 h post-dose fast. Absorption is thought to occur in the upper gastrointestinal tract, with peak plasma concentrations reached within 1 h, declining rapidly (down to 10% of peak plasma concentrations within 3 to 8 h) as ibandronate binds to bone (40 to 50% of a dose, with <2% retained in soft tissue) or is excreted unchanged in urine. Subsequent falls in plasma levels are much slower as ibandronate is redistributed back into the blood from bone and cleared through the kidneys. Elimination is dependent on the rate of bone turnover. Bisphosphonates do not appear to be metabolised, and the unabsorbed fraction of ibandronate is excreted unchanged in urine. Biliary excretion of the drug is virtually zero. It is removed by dialysis.
Therapeutic Concentration

A group of 40 Caucasian patients with varying degrees of renal impairment were administered a 6 mg IV dose of ibandronate over 30 min. Peak plasma concentrations were reported as follows:

Renal function	C_{max} *(µg/L)*	*Time (min)*
Normal	327	37.5
Mild impairment	352	32.2
Moderate impairment	332	31.5
Severe impairment	310	31.7

[Bergner *et al.* 2007].

Toxicity Ibandronate does not inhibit or interact with CYP450 enzymes; it therefore has little potential for drug-drug interactions.
Bioavailability Less than 1%. Compared with administration in the fasted state, bioavailability is reduced by ~90% when taken with a standard breakfast, by ~30% when taken 30 min before a meal, and by 75% when administered 2 h after a meal.
Half-life In healthy post-menopausal women, 10 to 23 h; also reported as 10 to 72 h.
Volume of Distribution In the range 90 to 175 L.
Clearance Total, 98 to 130 mL/min; renal, 60 mL/min (dependent on renal function and directly related to creatinine clearance).
Distribution in Blood Blood : plasma ratio, ≈0.7.
Protein Binding Approximately 87%; also reported as 90.9 to 99.5%. Binding to erythrocytes and platelets is very low.

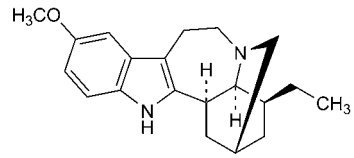
Dose Ibandronate sodium is given by IV infusion or orally, the dose being expressed in terms of ibandronic acid: ibandronate sodium 1.13 mg is equivalent to ≈1 mg ibandronic acid. For hypercalcaemia of malignancy, a single IV dose of the equivalent of 2 to 4 mg ibandronic acid is given, up to a maximum of 6 mg. For the prevention of skeletal events in patients with breast cancer and bone metastases, the equivalent of 6 mg ibandronic acid is given IV. The dose is repeated every 3 to 4 weeks. Alternatively, ibandronic acid 50 mg daily may be given by mouth.

For the prevention and treatment of postmenopausal osteoporosis, ibandronate is given orally in a usual dose equivalent to 150 mg of ibandronic acid once monthly on the same date each month; alternatively, 2.5 mg daily by mouth may be given. Alternatively, treatment may be given IV, in a dose equivalent to 3 mg of ibandronic acid once every 3 months.

Barrett J *et al.* (2004). Ibandronate: a clinical pharmacological and pharmacokinetic update. *J Clin Pharmacol* 44: 951–965.
Bergner R *et al.* (2007). Renal safety and pharmacokinetics of ibandronate in multiple myeloma patients with or without impaired renal function. *J Clin Pharmacol* 47: 942–950.
Endele R *et al.* (2005). Anall methods for the quantification of ibandronate in body fluids and bone. *J Pharm Biomed Anal* 39: 246–256.
Hoffmann-La Roche (2006). *Boniva (Ibandronate Sodium) Product Information*. Nutley, NJ: Hoffmann-La Roche. http://www.rocheexchange.com/osteoporosis/productinformation/boniva. (accessed 21 November 2009).
Rodriguez JA *et al.* (2007). Validation of a capillary electrophoresis method for the analysis of ibandronate related impurities. *J Pharm Biomed Anal* 44: 305–308.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Ibogaine

Alkaloid
$C_{20}H_{26}N_2O = 310.4$
CAS—83-74-9
Synonym 12-Methoxyibogamine



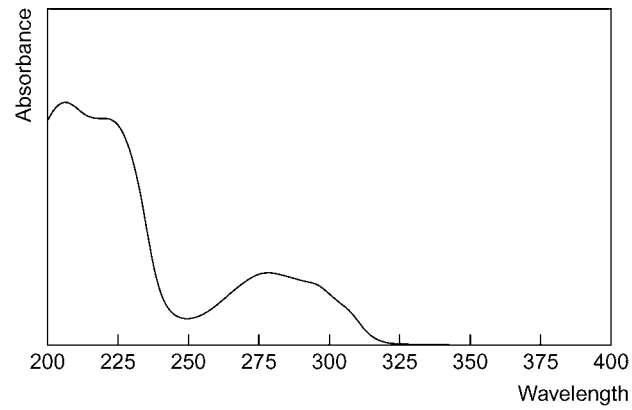
Chemical Properties Alkaloid obtained from *Tabernanthe iboga* (Apocynaceae). Crystals. Mp 152° to 153°. Practically insoluble in water; soluble in acetone, benzene, ethanol, chloroform and ether. Ibogaine was stable in plasma stored at –20° [Alburges *et al.* 1995]. Stock solutions can be stored for up to 7 months at 10° [Gallagher *et al.* 1995]. Freezing and thawing had no effect when plasma samples were taken and stored at –20° [Hearn *et al.* 1995].

Ibogaine Hydrochloride

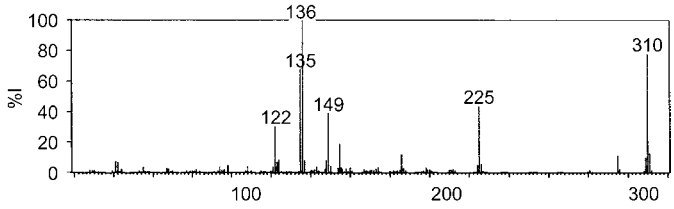
$C_{20}H_{26}N_2O, HCl = 346.9$
CAS—5934-55-4
Chemical Properties Crystals. Mp 299° to 300°, with decomposition. Soluble in water, ethanol and methanol; slightly soluble in acetone and chloroform; practically insoluble in ether. Log *P* (octanol/water), 3.6.

Colour Tests Liebermann's reagent—black; Mandelin's test—grey→violet; Marquis test—grey→orange.

Thin-layer Chromatography System TA—R_f 0.65; system TAE—R_f 0.39; system TAF—R_f 0.72; system TL—R_f 0.62; system TB—R_f 0.28; system TC—R_f 0.50; system TE—R_f 0.86 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2872.
High Performance Liquid Chromatography System HA—*k* 2.1; system HY—RI 321.
Ultraviolet Spectrum Aqueous acid—278 nm ($A^1_1 = 212c$).



Infrared Spectrum Principal peaks at wavenumbers 1490, 1210, 1140, 810, 1020, 835 (Ibogaine hydrochloride) (KBr disk).
Mass Spectrum Principal ions at *m/z* 136, 310, 135, 225, 149, 122, 155, 311.



Quantification

Plasma **GC-MS** Column: DB-1 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 125° for 0.5 min to 250° at 40°/min for 1 min to 300° at 10°/min for 3 min. SIM acquisition mode. Retention time: 10 min. Limit of detection, 3 µg/L [Ley *et al.* 1996]. Column: DB-1 dimethylpolysiloxane fused silica capillary (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: H_2, 80 cm/s. Temperature programme: 160° for 1.5 min to 310° at 20°/min for 1 min. PICI. Limit of detection, 10 µg/L for ibogaine and 12-hydroxyibogamine [Alburges *et al.* 1995]. Column: DB-5 phenylmethylsilicone FSOT (15 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 1 mL/min. Temperature programme: 50° for 1 min to 230° at 25°/min for 30 s to 300° at 5°/min for 5 min. Limit of detection, 5 µg/mL for ibogaine and 12-hydroxyibogamine. Column: DB-5 phenylmethylsilicone FSOT (15 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 1 mL/min. Temperature programme: 50° for 1 min to 230° at 25°/min, after 30 s to 230° at 5°/min for 7 min. Limit of detection, 5 µg/L [Hearn *et al.* 1995].

Urine **GC** Column: 1% SE-30 Chromosorb W 80/100 mesh (1.8 × 0.3 m i.d.). Carrier gas: N_2. Temperature programme: 180 to 225°. AFID. Limit of detection, 1 µg [Cartoni, Giarusso 1972].

GC-MS Column: DB-5MS (30 m × 0.25 mm i.d., 0.1 µm). Temperature: 90° to 250° at 70°/min. EI ionisation at -70 eV. Multiple ion monitoring. Retention time: 9.9 min. Limit of detection, 20 µg/mL [Gallagher *et al.* 1995]. See Plasma [Hearn *et al.* 1995].

Brain **GC-MS** See Plasma [Hearn *et al.* 1995].

Alburges ME *et al.* (1995). Determination of ibogaine and 12-hydroxy-ibogamine in plasma by gas chromatography-positive ion chemical ionization-mass spectrometry. *J Anal Toxicol* 19: 381–386.

Cartoni GP, Giarusso A (1972). Gas chromatographic determination of ibogaine in biological fluids. *J Chromatogr* 71: 154–158.

Gallagher CA *et al.* (1995). Identification and quantification of the indole alkaloid ibogaine in biological samples by gas chromatography-mass spectrometry. *Biochem Pharmacol* 49: 73–79.

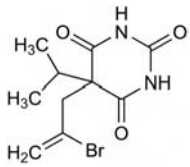
Hearn WL *et al.* (1995). Identification and quantitation of ibogaine and an *o*-demethylated metabolite in brain and biological fluids using gas chromatography-mass spectrometry. *J Anal Toxicol* 19: 427–434.

Ley FR *et al.* (1996). Determination of ibogaine in plasma by gas chromatography–chemical ionization mass spectrometry. *J Chromatogr A* 723: 101–109.

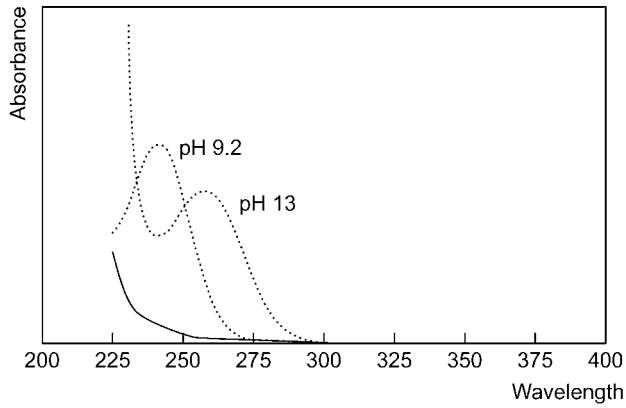
Ibomal

Hypnotic, Barbiturate
$C_{10}H_{13}BrN_2O_3$ = 289.1
CAS—545-93-7

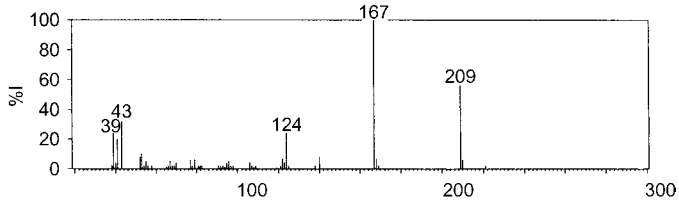
IUPAC Name 5-(2-Bromo-2-propenyl)-5-(1-methylethyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Bromoaprobarbitone; isopropyl-bromallyl-barbitursäure; propallylonal.
Proprietary Name *Noctal*



Chemical Properties A white crystalline powder. Mp 177° to 179°. Slightly soluble in water; freely soluble in acetone, ethanol and glacial acetic acid; sparingly soluble in benzene, chloroform and ether. pK_a 7.7 (20°). Log *P* (octanol/water), 1.5.
Colour Test Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.32; system TF—R_f 0.66; system TH—R_f 0.61; system TAD—R_f 0.56; system TAE—R_f 0.91.
Gas Chromatography System GA—ibomal RI 1880, M (desbromo-OH-) RI 1770; ibomal-Me_2 RI 1745; M (desbromo-oxo-)-Me_2 RI 1720; M (desbromo-OH-)-Me_2 RI 1730. Also metabolised to aprobarbital RI 1618.
High Performance Liquid Chromatography System HG—*k* 4.01; system HH—*k* 2.58; system HX—RI 379; system HY—RI 352.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—240 nm (A_1^1=307a); 1 mol/L sodium hydroxide (pH 13)—257 nm (A_1^1=240b).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1722, 1210, 1300, 830, 1622 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 167, 209, 43, 124, 39, 41, 53, 140.

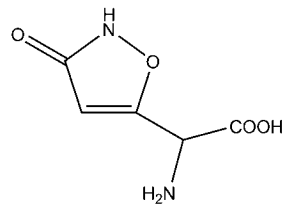


Disposition in the Body Metabolised by side-chain oxidation to 5-(2-acetonyl)-5-isopropylbarbituric acid. About 6 to 16% of a dose is slowly excreted in the urine as the metabolite with about 1 to 3% as unchanged drug; the metabolite is still detectable in urine 9 days after a single dose.
Therapeutic Concentration In plasma, usually 0.3 to 10 mg/L.
Toxicity Blood concentrations greater than about 10 mg/L may be toxic or lethal.
Protein Binding About 34%.
Dose 100 to 400 mg, as a hypnotic.

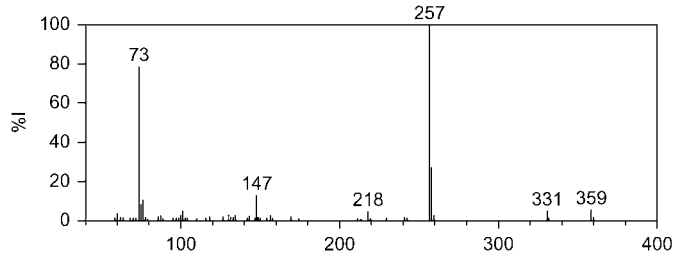
Ibotenic Acid

Isoxazole, Neurotoxin
$C_5H_6N_2O_4$ = 158.1
CAS—2552-55-8

IUPAC Name 2-Amino-2-(3-oxo-1,2-oxazol-5-yl) acetic acid
Synonyms α-Amino-2,3-dihydro-3-oxo-5-isoxazoleacetic acid; α-amino-3-hydroxy-5-isoxazoleacetic acid; amino-(3-hydroxy-5-isoxazolyl)acetic acid.



Chemical Properties Crystals. Mp 151° to 152°. Readily soluble in water. Log *P* (octanol/water), −1.073 [ACD 2007]. Fly-killing and narcosis-potentiating amino acid structurally similar to kainic acid [q.v.] extracted from poisonous mushroom species [O'Neil *et al.* 2006].
Mass Spectrum Principal ions at *m/z* 257, 73, 147, 359, 331, 218(*tri*-TMS derivative) [Tsujikawa *et al.* 2006].



Quantification

Other **GC-MS** Dried Mushrooms. Column: DB-5 MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 53.0 mL/min. Temperature programme: 100° for

1 min to 300° at 15°/min. EIionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 µg/L [Tsujikawa *et al.* 2006].

LC-MS Dried Mushrooms. Column: Symmetry C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (70 : 30 for 1 min to 10 : 90 at 25 min for 5 min to 70 : 30 at 31 min for 15 min), flow rate 0.2 mL/min. DAD (λ = 256 nm). Limit of quantification, 25.9 µg/L; limit of detection, 7.8 µg/L [Tsujikawa *et al.* 2007].

Note For a GLC-mass spectral analysis of fungal metabolites, see Repke *et al.* [1978].

Disposition in the Body After ingestion, ibotenic acid hydrolysed to muscimol in the low pH of the gastric fluid; muscimol proceeds to the brain or is eliminated via the systemic circulation [Michelot, Melendez-Howell 2003]. Both ibotenic acid and muscimol are found in urine 1 h after consumption [Satora *et al.* 2006].

Toxicity The LD_{50} in mice is 15 mg/kg (IV) and 38 mg/kg (oral); in rats it is 42 mg/kg (IV) and 129 mg/kg (oral) [O'Neil *et al.* 2006]. For an overview of the features of various types of mushroom poisoning, see DiPalma [1981].

A 47-year-old mother and 27-year-old daughter ingested 5 fried mushroom caps thinking that they were parasol mushrooms *Macrolepiota procera*. Approximately 2 h later, the women suffered from nausea, stomach ache, and diarrhoea and had vomited several times. After several days in hospital, both patients were discharged [Satora *et al.* 2006].

A 48-year-old man consumed a plate of what he thought were *A. caesarea* mushrooms. Half an hour later he became nauseous, vomited and fell asleep. His wife found him 3 h later comatose and having a seizure-like episode. Examination of the mushrooms he had picked revealed *A. muscaria* among them. Ten hours after ingestion he awoke and was completely oriented. Eight hours later, however, his condition deteriorated and he became confused and uncooperative. He developed visual and auditory hallucinations as well as paranoia. He refused treatment and was diagnosed with paranoid psychosis. He was transferred to a psychiatric hospital 3 days later. On the fifth day he started to drink and take tablets and on the sixth day all symptoms of psychosis disappeared and he was discharged [Brvar *et al.* 2006].

Note For a study investigating the ibotenic acid-muscimol content of various *Amanita* species from several continents, see Chilton and Ott [1976].

Dose Used as a neurobiological tool.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Brvar M *et al.* (2006). Prolonged psychosis after *Amanita muscaria* ingestion. *Wien Klin Wochenschr* 118: 294–297.

Chilton W, Ott SJ (1976). Toxic metabolites of *Amanita pantherina*, *A. cothurnata*, *A. muscaria* and other *Amanita* species. *Lloydia* 39: 150–157.

DiPalma JR (1981). Mushroom poisoning. *Am Fam Physician* 23: 169–172.

Michelot D, Melendez-Howell LM (2003). *Amanita muscaria*: chemistry, biology, toxicology, and ethnomycology. *Mycol Res* 107: 131–146.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Repke DB *et al.* (1978). GLC–mass spectral analysis of fungal metabolites. *J Pharm Sci* 67: 485–487.

Satora L *et al.* (2006). Panther cap *Amanita pantherina* poisoning case report and review. *Toxicon* 47: 605–607.

Tsujikawa K *et al.* (2006). Analysis of hallucinogenic constituents in *Amanita* mushrooms circulated in Japan. *Forensic Sci Int* 164: 172–178.

Tsujikawa K *et al.* (2007). Determination of muscimol and ibotenic acid in *Amanita* mushrooms by high-performance liquid chromatography and liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 430–435.

Ibuprofen

Analgesic, COX Inhibitor, NSAID

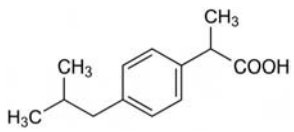
$C_{13}H_{18}O_2$ = 206.3

CAS—15687-27-1

IUPAC Name 2-[4-(2-Methylpropyl)phenyl]propanoic acid

Synonyms Ibuprofenum; RD-13621; U-18573.

Proprietary Names *Advil*; *Apsifen*; *Arthrofen*; *Brufen*; *Cuprofen*; *Ebufac*; *Fenbid*; *Galprofen*; *Genpril*; *Haltran*; *Ibrufhalal*; *Ibu*; *Ibufac*; *Ibufem*; *Ibugel*; *Ibular*; *Ibuleve*; *Ibumousse*; *Ibuspray*; *Inoven*; *Isisfen*; *Junifen*; *Librofem*; *Lidifen*; *Manorfen*; *Menadol*; *Migrafen*; *Motrin*; *Novaprin*; *Nuprin*; *Nurofen*; *Obifen*; *Orbifen*; *Pacifene*; *PhorPain*; *Proflex*; *Relcofen*; *Rimafen*; *Saleto-200*; *Seclodin*; *Uniprofen*. Ibuprofen is an ingredient of many proprietary preparations [Sweetman 2007].



Chemical Properties A white powder or crystals. Mp 75° to 77°. Practically insoluble in water; soluble 1 in 1.5 of ethanol, 1 in 1 of chloroform and 1 in 2 of ether. pK_{a1} 4.4, pK_{a2} 5.2. Log *P* (octanol/water), 4.0. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

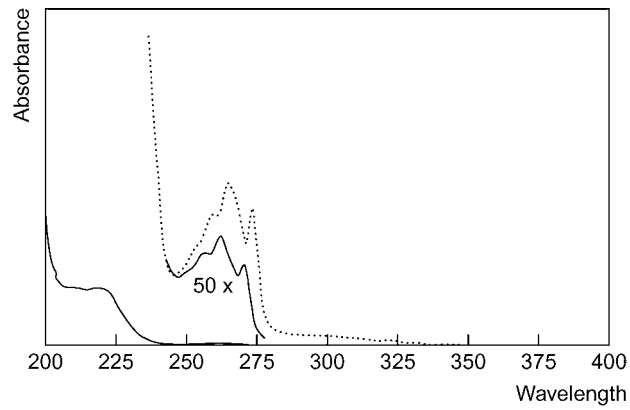
Colour Tests Liebermann's reagent—brown-orange; Marquis test—brown (→orange at 100°).

Thin-layer Chromatography System TD—R_f 0.46; system TE—R_f 0.06; system TF—R_f 0.57; system TG—R_f 0.18; system TAD—R_f 0.54; system TAE—R_f 0.75; system TAJ—R_f 0.59; system TAK—R_f 0.76; system TAL—R_f 0.93.

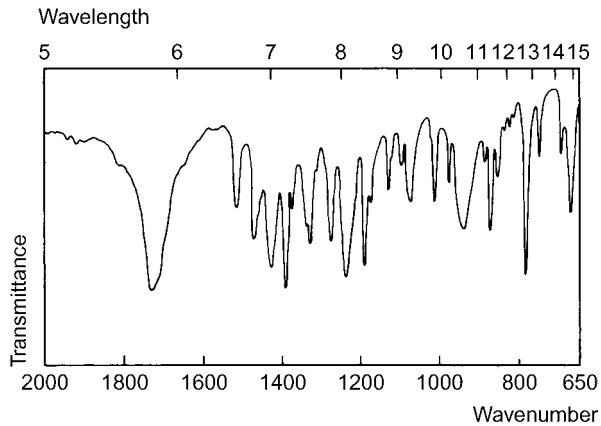
Gas Chromatography System GA—ibuprofen RI 1615, ibuprofen-Me RI 1510, M (3-OH-)-Me RI 1630, M (OH-)-Me RI 1750, M (COOH-)-Me_2 RI 1765; system GB—ibuprofen RI 1637, M (2-OH-) RI 2096; system GD—ibuprofen-Me RRT 0.89 (relative to *n*-hexadecane); system GL—ibuprofen-Me RI 1505, M (3-OH-)-Me RI 1680.

High Performance Liquid Chromatography System HD—*k* 15.1; system HX—RI 616; system HY—RI 598; system HZ—RT 16.5 min; system HAA—RT 23.8 min; system HAX—RT 8.1 min; system HAY—RT 10.5 min.

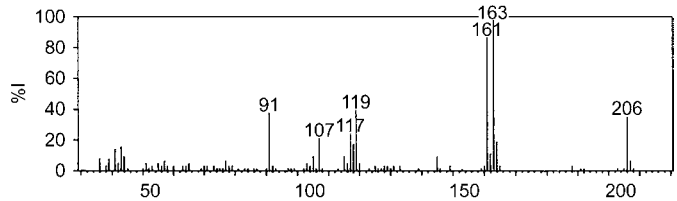
Ultraviolet Spectrum Aqueous alkali—265 (A_1^{1} = 18.5a), 273 nm.



Infrared Spectrum Principal peaks at wavenumbers 1721, 1232, 779, 1185, 1273, 870 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 163, 161, 119, 91, 206, 117, 107, 164.



Quantification

Blood **GC-MS** Quantification of ibuprofen [Huber, Garg 2010]. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (2.0 m × 4 mm i.d.). Carrier gas: N_2, 70 mL/min. Temperature: 275°. ECD. Limit of detection, ~20 pg [Montagna, Groppi 1980].

Plasma **HPLC** Column: Waters Symmetry C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (pH 2.6, 60 : 40), flow rate 2 mL/min. UV detection (λ = 220 nm). Limit of quantification, 1.56 mg/L [Farrar *et al.* 2002]. Column: Waters Symmetry C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : acetic acid : triethylamine (pH 5.0, 60 : 40 : 0.1 : 0.02 to 100 : 0 : 0: at 20 min for 5 min), flow rate 1.6 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 320 nm). Limit of quantification, 0.1 mg/L [Canaparo *et al.* 2000]. UV detection (λ = 254 nm). Limit of detection, 50 ng [Save *et al.* 1997]. Column: C_{18} (33 × 4.6 mm i.d., 3 µm). Mobile phase: concentrated phosphoric acid : water : acetonitrile (4 : 600 : 400), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Retention time: 3.9 min. Limit of detection, 25 mg/L [Rifai *et al.* 1996]. Column: C_{18} Econosil ODS (250 × 10 mm i.d., 10 µm). Mobile phase: methanol : 10 mmol/L acetic acid (pH 3.0, 65 : 35), flow rate 3.5 mL/min, UV detection (λ = 225 or 214 nm). Retention time: 24.8 min. Limit of detection, 0.5 mg/L [Castillo, Smith 1993]. C_{18}

reversed phase (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : triethylamine (60 : 40 : 0.1 : 0.02), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 320 nm) [Lemko *et al.* 1993] Spherisorb ODS (125 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (pH 3.3, 65 : 35), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention time: 8 min. Limit of detection, 5 mg/L [Blagbrough *et al.* 1992]. Column: C_{18} reversed phase. Mobile phase: acetonitrile : phosphoric acid (pH 2.2, 1 : 1). UV detection (λ = 220 nm). Limit of detection, 25 µg/L [Rustum 1991].

GC-MS Column: DB-1 (15 m × 0.22 mm i.d.). Temperature programme: 100° to 280° at 12°/min. Retention time: 17 min. Limit of detection, 5 mg/L [Way *et al.* 1997]. Column: 3% phenylmethylsiloxane on Chromosorb WHP 100/120 mesh (2.0 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 320°. CI at 100 eV, SIM acquisition mode. Retention time: 2.15 min for (*S*)-(+)-ibuprofen and 2.55 min for (*R*)-(−)-ibuprofen. Limit of detection, 5 µg/L [Zhao *et al.* 1994]. Column: cross-linked methyl silicone HP-1 (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 55 cm/s. Temperature programme: 60° to 200° at 30°/min to 240° at 8°/min to 290° at 30°/min. EI ionisation, SIM acquisition mode. Retention time: 8.86 min for (*S*)-ibuprofen and 8.96 min for (*R*)-ibuprofen. Limit of quantification, 3 µg/L, limit of detection, <1 µg/L [Jack *et al.* 1992].

Serum **HPLC** Column: YMC ODS (70 × 4.6 mm i.d., 5 µm) or TSK ODS 80 TM (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 3.5) : acetonitrile (60 : 40), containing 5 mmol/L tetra-*n*-butylammonium bromide or 20 mmol/L phosphate buffer (pH 7.0) : acetonitrile (70 : 30) containing 5 mmol/L tetra-*n*-butylammonium bromide or 20 mmol/L phosphate buffer (pH 7.0) : acetonitrile (70 : 30), flow rate 1.0 mL/min. UV detection (λ = 221 nm). Limit of detection, 0.5 µg/L [Yamashita *et al.* 1991].

GC Column: 10% 3-cyanopropyl silicone on 100/120 mesh Gas Chrom Q (1.83 m × 3 mm i.d.). Carrier gas: CH_4 : Ar (5 : 95), 10 mL/min. Temperature: 190°. ECD. Retention time: 9.1 min. Limit of detection, 100 µg/L [Kaiser, Martin 1978]. Column: 5% FFAP on Gas Chrom W (HP) 80/100 mesh (1.83 m × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 220°. FID. Retention time: 4.3 min. Limit of detection, 500 µg/L [Hoffman 1977].

Urine **HPLC** Column: Inertsil ODS-2 (150 × 4.6 mm, i.d., 5 µm). Mobile phase: 50 mmol/L phosphate buffer (58 : 42, pH 5.0), flow rate 0.9 mL/min. Limit of detection, 50 µg/L [Hirai *et al.* 1997]. Column: Polygosi 60-5 (120 × 4.6 mm, i.d., 5 µm). Mobile phase: acetonitrile : 0.2 mol/L potassium phosphate (pH 7.4) : water (18 : 15 : 67), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of quantification, 4–23.3 mg/L, limit of detection, 1–12 mg/L for phase I and II metabolites [Kepp *et al.* 1997]. Column: Partisil silica column (250 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : ethanol (98.2 : 1.8) containing 0.05% trifluroacetic acid, flow rate 2 mL/min. UV detection (λ = 220 nm). Limit of quantification, 10 mg/L [Tan *et al.* 1997].

GC-MS See Blood [Montagna, Groppi 1980].

Biological Fluids, Plasma and Urine **HPLC** Column: C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : acetic acid : TEA (46.5 : 53.5 : 0.1 : 0.03), flow rate 1.6 mL/min. UV detection (λ = 225 nm). Retention time: 17.7 min for (*S*)-ibuprofen and 15.7 min for (*R*)-ibuprofen. Limit of detection, 0.25 mg/L [Wright *et al.* 1992]. Column: Partisil 10 ODS-3 (250 × 4.5 mm i.d.). Mobile phase: acetonitrile-water (28 : 72) : acetonitrile-0.05 mol/L monobasic potassium phosphate (50 : 50), flow rate 2.0 mL/min. UV detection (λ = 220 nm). Limit of detection, 1 mg/L for ibuprofen in plasma, 5 mg/L for ibuprofen or the hydroxy or carboxy metabolites in urine [Lockwood, Wagner 1982].

GC-MS Column: 3% OV-17 on Chromosorb W AW DMCS 120/140 mesh (0.91 m × 2 mm i.d.). Carrier gas: CH_4, 20 mL/min. Temperature programme: 150°. CI at 110 eV. Limit of detection, 50 pg [Whitlam, Vine 1980].

Synovial Fluid **GC-MS** See Plasma [Jack *et al.* 1992].

HPLC See Plasma [Blagbrough *et al.* 1992].

Disposition in the Body Ibuprofen is readily and almost completely absorbed after oral administration. >60% of a dose is excreted in the urine in 24 h, including ~9% of the dose as the 2-hydroxy metabolite, 2-[4-(2-hydroxy-2-methylpropyl) phenyl]propionic acid; ~17% as the conjugated hydroxy metabolite; ~16% as the 2-carboxy metabolite, 2-[4-(carboxypropyl)phenyl]propionic acid; and ~19% as the conjugated carboxy metabolite (both metabolites are inactive); <10% of a dose is excreted unchanged. The remainder of the dose is probably eliminated in the faeces after excretion in the bile; excretion is virtually complete within 24 h. It is found in breast milk in very low concentrations. Ibuprofen's disposition is stereoselective and there is some metabolic conversion of the inactive (*R*)-(−)-enantiomer to the active (*S*)-(+)-form (dexibuprofen).

Therapeutic Concentration In plasma, usually in the range 20–30 mg/L.

In 98 patients receiving high doses of ibuprofen (20–30 mg/kg IV as a single dose), peak plasma concentrations of 21–150 mg/L (mean, 83) were attained in 1–3 h (mean, 1.77) [Murry *et al.* 1999].

Ibuprofen lysine (10 mg/kg) given as an IV bolus within the first 3 h after birth to 21 premature neonates produced an ibuprofen plasma concentration of 180.6 mg/L (±11.1) after 1 h. In 5 of the neonates, maintenance doses of 5 mg/kg once daily on days 2 and 3 resulted in mean plasma concentrations of 116.6 mg/L (±54.5) and 113.6 mg/L (±58.2), respectively [Aranda *et al.* 1997].

Percutaneous application of ibuprofen 500 mg (10 g of a 5% gel under occlusion for 2 h, on the back 20 × 20 cm) produced a peak plasma concentration of 7.1 mg/L (±4.4) at 2.4 h (±0.8) in 18 subjects. In the same subjects, a single oral dose of 400 mg produced a peak plasma concentration of 36.7 mg/L (±7.5) at 1.1 h (±0.8) [Kleinbloesem *et al.* 1995].

After a single oral dose of 200 mg to 2 subjects, peak plasma concentrations of 18 and 24 mg/L ibuprofen were attained in 1.5 h; peak plasma concentrations of 0.6 and 1 mg/L for the hydroxy metabolite and 1.7 and 2.1 mg/L for the carboxy metabolite were attained in 3 h; plasma concentrations after oral doses of 200 mg three times daily for 14 days were of the same order [Mills *et al.* 1973].

Toxicity

A 26-year-old male with a history of ibuprofen overdose was found dead after having recently been issued with a prescription for ibuprofen. The following postmortem tissue concentrations were reported: heart blood 518.0 mg/L, femoral blood 348.3 mg/L, liver 942 µg/g, brain 283.9 µg/g, gastric contents 131 mg total [Kunsman, Rohrig 1993].

In 2 overdoses caused by ibuprofen, plasma concentrations of 400 and 711 mg/L were reported ~1.5 h after ingestion. In the first case, the estimated dose was 14–16 g; both subjects recovered [Court *et al.* 1981].

In an attempted suicide involving the ingestion of 12 g ibuprofen, an initial serum concentration of 840 mg/L was reported; this declined to 220 mg/L at 3 h; chlorpheniramine (chlorphenamine) was also detected in the urine. The subject was comatose but recovered within 24 h [Hunt, Leigh 1980].

Half-life Plasma half-life, ~2 h.

Volume of Distribution ~0.1 L/kg.

Protein Binding ~99%.

Note For a review of the clinical pharmacokinetics of ibuprofen, see Davies [1998].

Dose 0.6 to 2.4 g daily.

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Kepp DR *et al.* (1997). Simultaneous quantitative determination of the major phase I and II metabolites of ibuprofen in biological fluids by high-performance liquid chromatography on dynamically modified silica. *J Chromatogr B Biomed Sci Appl* 696: 235–241.

Kleinbloesem CH *et al.* (1995). Pharmacokinetics and bioavailability of percutaneous ibuprofen. *Arzneimittelforschung* 45: 1117–1121.

Kunsman GW, Rohrig TP (1993). Tissue distribution of ibuprofen in a fatal overdose. *Am J Forensic Med Pathol* 14: 48–50.

Lemko CH *et al.* (1993). Stereospecific high-performance liquid chromatographic assay of ibuprofen: improved sensitivity and sample processing efficiency. *J Chromatogr* 619: 330–335.

Lockwood GF, Wagner JG (1982). High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids. *J Chromatogr* 232: 335–343.

Mills RF *et al.* (1973). The metabolism of ibuprofen. *Xenobiotica* 3: 589–598.

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Murry KR *et al.* (1999). Pharmacodynamic characterization of nephrotoxicity associated with once-daily aminoglycoside. *Pharmacotherapy* 19: 1252–1260.

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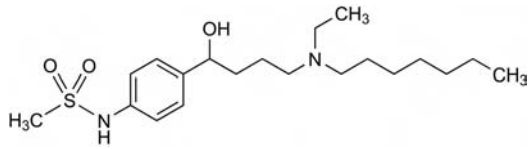
Yamashita K *et al.* (1991). Column-switching techniques for high-performance liquid chromatography of ibuprofen and mefenamic acid in human serum with short-wavelength ultraviolet detection. *J Chromatogr* 570: 329–338.

Zhao MJ *et al.* (1994). Gas chromatographic-mass spectrometric determination of ibuprofen enantiomers in human plasma using *R*(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol as derivatizing reagent. *J Chromatogr B Biomed Appl* 656: 441–446.

Ibutilide

Antiarrhythmic
$C_{20}H_{36}N_2O_3S = 384.6$
CAS—122647-31-8

IUPAC Name *N*-[4-[4-(Ethylheptylamino)-1-hydroxybutyl]phenyl]methanesulfonamide



Chemical Properties pK_a 10.5 (pH <6); 8.36 (pH >6).

Ibutilide Fumarate

$(C_{20}H_{36}N_2O_3S)_2, C_4H_4O_4 = 885.2$
CAS—122647-32-9
Synonym U-70226E
Proprietary Name *Corvert*
Chemical Properties A white to off-white powder. Mp 117° to 119°. Soluble (>100 g/L) in solutions at pH 7 or less.

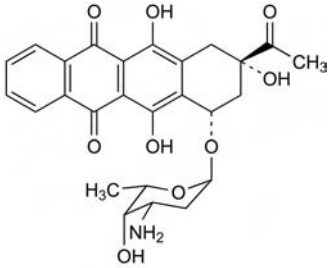
Ultraviolet Spectrum Aqueous acid (ethanol)—228 , 267 nm.
Quantification
Plasma **HPLC** Fluorescence detection (λ_{ex}=224 nm, λ_{em}=340 nm). Limit of detection, <0.018 µg/L [Hsu, Walters 1995].
Disposition in the Body After IV administration, ibutilide is widely distributed in the body and extensively metabolised in the liver, to produce eight metabolites, by *O*-oxidation followed by sequential β-oxidation of the heptyl side chain. Approximately 82% of a dose may be excreted in urine with 7% as the unchanged drug, and up to 19% in excreted in faeces.
Therapeutic Concentration The peak serum concentration, after a 0.01 mg/kg infusion, is ~10 µg/L. Ibutilide has a low therapeutic dosage of 0.01 to 0.03 mg/kg, with peak plasma concentrations not reaching >20 µg/L.
Toxicity Acute toxicity can cause potentially fatal arrhythmias, also tachycardia, hypotension, congestive heart failure, myocardial depression, third degree atrioventricular (AV) block and renal failure. Poisonous by the IV route. Moderately toxic by ingestion.
Half-life 2 to 12 h, with an average of 6 h.
Volume of Distribution 9 to 13 L/kg.
Clearance Plasma clearance, 29 mL/min/kg.
Protein Binding Approximately 40%.
Dose In patients with a body weight >60 kg, a 1 mg dose of ibutilide fumarate is suggested. For those weighing <60 kg, 0.01 mg/kg body weight is administered. Usual dose between 0.01 and 0.10 mg/kg body weight.

Hsu CL, Walters RR (1995). Assay of the enantiomers of ibutilide and artilide using solid-phase extraction, derivatization, and achiral-chiral column-switching high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 667(1): 115–128.

Idarubicin

Antineoplastic
$C_{26}H_{27}NO_9 = 497.5$
CAS—58957-92-9

IUPAC Name (7*S*,9*S*)-9-Acetyl-7-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenedione
Synonyms 4-Demethoxydaunomycin; 4-DMDR; IMI 30; NSC-256439.



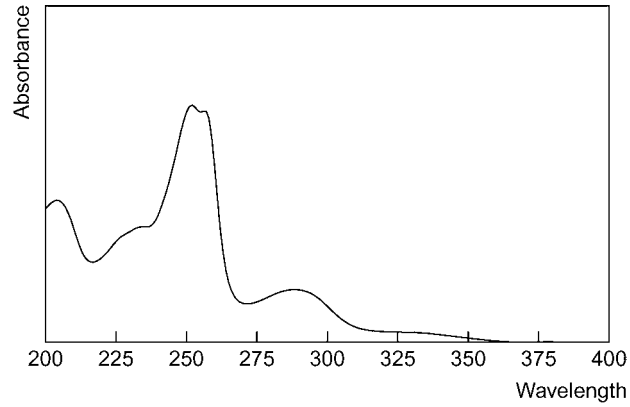
Chemical Properties Log *P* (octanol/water), 2.10.

Idarubicin Hydrochloride

$C_{26}H_{27}NO_9$, HCl = 534.0
CAS—57852-57-0
Proprietary Names *Idamycin*; *Zavedos*.
Chemical Properties An orange crystalline powder. Mp 183° to 185°, also reported as Mp 172° to 174°.

Thin-layer Chromatography System TE—R_f 0.06; system TF—R_f 0.00.
High Performance Liquid Chromatography System HX—RI 410.

Ultraviolet Spectrum Principal peaks at 253, 289 nm.



Quantification
Plasma **HPLC** Column: LC-CN5 Supelcosil (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L monobasic sodium phosphate : acetonitrile (65:35), (pH 4.0), flow rate 1 mL/min. Fluorescence detection (λ_{ex}=480 nm, λ_{em}=560 nm). Retention time: idarubicin, 11.3 min; idarubicinol, 8.0 min. Limit of detection and quantification, 0.4 µg/L [Fogli *et al.* 1999].
Serum **LC-MS** Limit of quantification, 5 µg/L [Lachatre *et al.* 2000].
Disposition in the Body After IV administration, idarubicin is rapidly distributed into body tissues and extensively tissue bound. After oral administration, idarubicin is rapidly absorbed. It is mostly metabolised in the liver, by aldoketoreductase, which reduces the ketone function at the C_{13} position to idarubicinol (1,3-dihydroidarubicin), the principal metabolite. The unchanged drug and metabolite are excreted mainly in bile but also in urine. Oral bioavailability is between 24 and 39% after oral administration.
Therapeutic Concentration
Thirteen male and 7 female patients with advanced malignant disease, for example, non-Hodgkins lymphoma, aged between 22 and 76 years, were administered 15 mg/m^2 over 1 min (IV). 11 patients were also administered 40 and 50 mg/m^2 oral doses of idarubicin. Peak plasma levels of idarubicin and idarubicinol ranged between 7.4 and 89.4 nmol/L and reached 1.5 to 8 h after administration [Gillies *et al.* 1987].
Toxicity Acute/high doses of idarubicin (>600 mg/m^2) can lead to myocardial toxicity, with potentially fatal congestive heart failure (CHF), life-threatening arrhythmias and serious gastrointestinal events, for example, bleeding and prefarations.
Half-life 12 to 35 h (idarubicin); 50 to 70 h (idarubicinol).
Volume of Distribution Steady state, 1500 L/m^2.
Clearance Plasma clearance, 60 L/h/m^2.
Protein Binding Idarubicin is 97% bound to plasma proteins and idarubicinol, 94%.
Dose In adults: dose ranges from 8 to 12 mg/m^2/daily for 3 to 5 days (IV) and 15 to 45 mg/m^2/daily, alone or in combination, in a single dose or for 3 days (orally).
In children: 10 mg/m^2/daily for 3 days (IV). Maximum cumulative dose for all indications is 400 mg/m^2. Dose reduced if patient has impaired hepatic or renal function.

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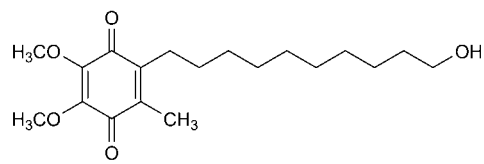
Gillies HC *et al.* (1987). Pharmacokinetics of idarubicin (4-demethoxydaunorubicin; IMI-30; NSC 256439) following intravenous and oral administration in patients with advanced cancer. *Br J Clin Pharmacol* 23: 303–310.
Lachatre F *et al.* (2000). Simultaneous determination of four anthracyclines and three metabolites in human serum by liquid chromatography-electrospray mass spectrometry. *J Chromatogr B Biomed Sci Appl* 738: 281–291.

Idebenone

Nootropic
$C_{19}H_{30}O_5 = 338.4$
CAS—58186-27-9

IUPAC Name 2-(10-Hydroxydecyl)-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1,4-dione
Synonyms CV-2619; 2-(10-hydroxydecyl)-5,6-dimethoxy-3methyl-2,5-cyclohexadiene-1,4-dione; ide,6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-*p*-benzoquinone.
Proprietary Names *Avan*; *Daruma*; *Mneisis*.

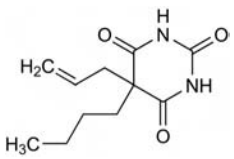


Chemical Properties Orange needles from ligroin. Mp 46° to 50°. Also reported to be crystals from hexane and ethylacetate. Mp 52° to 53°. Soluble in organic solvents; practically insoluble in water.
High Performance Liquid Chromatography Column: μBondapak C_{18}. Mobile phase: isopropanol : dioxane : water (15 : 15 : 70) containing 0.05% acetic acid. UV detection (λ = 280 nm). Retention time: 6.85 min [Barkworth *et al.* 1985].
Quantification
***Plasma* LC-MS** Column: Supelco C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (80 : 20), 1 mL/min. APCI, SIM acquisition mode. Limit of quantification, 20 μg/L [Hu *et al.* 2000].
***Serum* HPLC** Column: Nucleosil C_{18} (25 × 0.4 cm i.d., 5 μm). Mobile phase: methanol : water containing 20 mmol/L 95% lithium perchlorate, flow rate 1.2 mL/min. ECD. Limit of detection, 500 pmol/L [Artuch *et al.* 2002].
Disposition in the Body Idebenone is metabolised by oxidation followed by β-oxidation, reduction and subsequent conjugation (sulfation and glucuronidation). Two of the main metabolites are QS-4 and QS-6. It is widely distributed in tissues and is excreted as the metabolites in urine and faeces. The ratio of idebenone: QS-4 : QS-6 in urine is 2 : 89 : 9.
Therapeutic Concentration
A peak plasma concentration of 316 ± 85 μg/L was achieved within 96 ± 34 min of the administration of an oral dose of 30 mg to 10 volunteers [Hu *et al.* 2000].
Ten healthy males aged between 21 and 30 years were administered a single oral dose of 100 mg idebenone on days 1 and 35; in between doses of 300 mg daily were received. Peak serum concentrations of 0.65 and 0.85 mg/L were reached on days 1 and 35 within 4 to 5 h, respectively for the parent drug. Peak concentrations for the 2 metabolites, QS-4 and QS-6 were 0.35 and 0.40 mg/L (days 1 and 35) and 0.035 and 0.045 mg/L, respectively, which were reached within 3 h for both metabolites [Barkworth *et al.* 1985].
Half-life 18 h.
Protein Binding ≈90%.
Dose 90 mg daily.

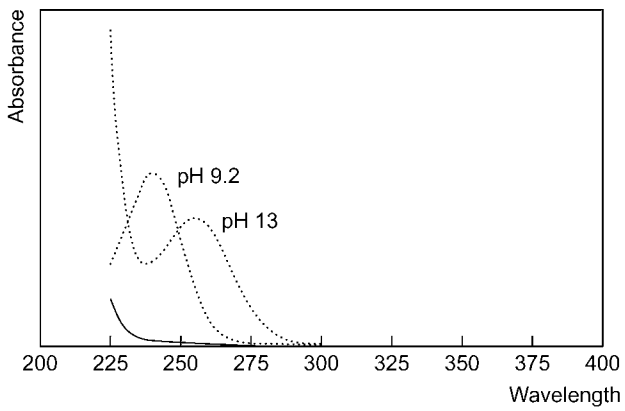
Artuch R *et al.* (2002). Monitoring of idebenone treatment in patients with Friedreich's ataxia by high-pressure liquid chromatography with electrochemical detection. *J Neurosci Methods* 115: 63–66.
Barkworth MF *et al.* (1985). An early phase I study to determine the tolerance, safety and pharmacokinetics of idebenone following multiple oral doses. *Arzneimittelforschung* 35: 1704–1707.
Hu P *et al.* (2000). Determination of idebenone in plasma by HPLC/MS. *Acta Pharmacol Sin* 21: 306–308.

Idobutal

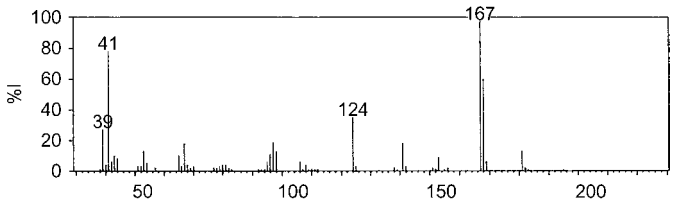
Hypnotic, Barbiturate
$C_{11}H_{16}N_2O_3$ = 224.3
CAS—3146-66-5
IUPAC Name 5-Butyl-5-prop-2-enyl-2,4,6(1H,3H,5H)-pyrimidinetrione
Synonym *n*-Butylallylbarbituric acid



Chemical Properties Crystals. Mp 128°. Slightly soluble in water; freely soluble in ethanol and ether. Log *P* (octanol/water), 2.3.
Thin-layer Chromatography System TD—R_f 0.55; system TE—R_f 0.41; system TF—R_f 0.69; system TH—R_f 0.71; system TAD—R_f 0.59.
Gas Chromatography System GA—idobutal RI 1700; idobutal-Me_2 RI 1610.
High Performance Liquid Chromatography System HG—*k* 8.12; system HH—*k* 4.77; system HY—RI 357.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=434b); 1 mol/L sodium hydroxide (pH 13)—254 nm (A^1_1=328b).

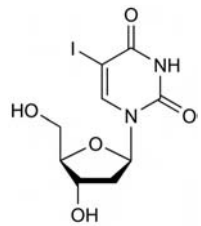


Infrared Spectrum Principal peaks at wavenumbers 1696, 1728, 1755, 835, 1290, 1207 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 167, 41, 168, 124, 39, 97, 141, 67.

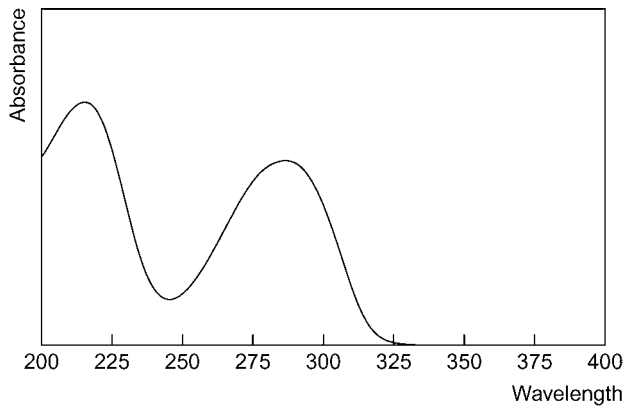


Idoxuridine

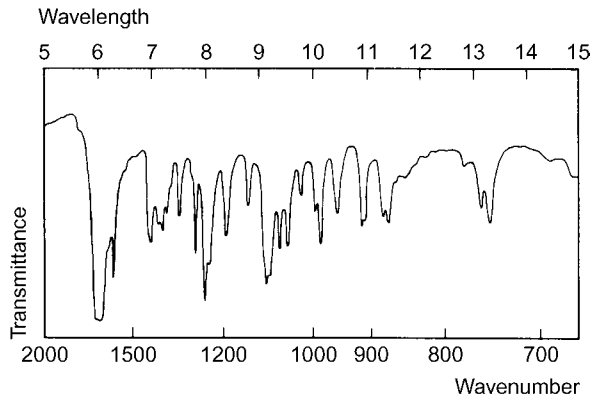
Antiviral
$C_9H_{11}IN_2O_5$ = 354.1
CAS—54-42-2
IUPAC Name 2′-Deoxy-5-iodouridine
Synonyms IDU; 5 IDUR.
Proprietary Names *Herpid*; *Herpidu*; *Herplex*; *Iderpes*; *Idina*; *Idoxene*; *Iducher*; *Idulea*; *Iduridin*; *Idustatin*; *Iduviran*; *Kerecid*; *Ophthalmadine*; *Stoxil*; *Virexen*; *Virudox*; *Virunguent*; *Zostrum*. It is an ingredient of *Herpesine*, *Iducol* and *Virasolve*.



Chemical Properties Colourless crystals or a white crystalline powder. It decomposes on heating with the liberation of iodine vapour. Soluble 1 in 500 of water, 1 in 400 of ethanol and 1 in 230 of methanol; practically insoluble in chloroform and ether. pK_a 8.3. Log *P* (octanol/water), −1.0.
Colour Test Iodine test—positive.
Ultraviolet Spectrum Aqueous acid—288 nm (A^1_1=220a); aqueous alkali—279 nm (A^1_1=162a).



Infrared Spectrum Principal peaks at wavenumbers 1667, 1259, 1096, 1605, 1289, 1070 cm^{-1} (KBr disk).



Quantification

Serum **HPLC** UV detection. For method of quantification for idoxuridine and its metabolite 5-iodouracil, see Belotto *et al.* [1991].

Use Solutions of idoxuridine in dimethyl sulfoxide are used topically in concentrations of 5 to 40%.

Belotto N *et al.* (1991). Determination of 2′-deoxy-5-iodouridine and its metabolite 5-iodouracil by high-performance liquid chromatography with ultraviolet absorbance detection in human serum. *J Chromatogr* 572: 327–332.

Ilodecakin

Antiinflammatory

CAS—149824-15-7

Synonyms Sch-52000; recombinant human interleukin-10.

Dose It is under investigation for its antiinflammatory properties in several diseases including psoriasis, inflammatory bowel disease and hepatitis C. It is postulated that interleukin-10 has immunostimulant as well as immunosuppressive properties.

Imidacloprid

Insecticide

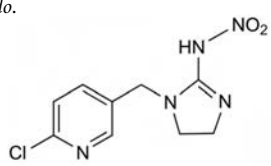
$C_9H_{10}ClN_5O_2$ = 255.7

CAS—138261-41-3; 105827-78-9

IUPAC Name 1-[(6-Chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1*H*-imidazol-2-amine

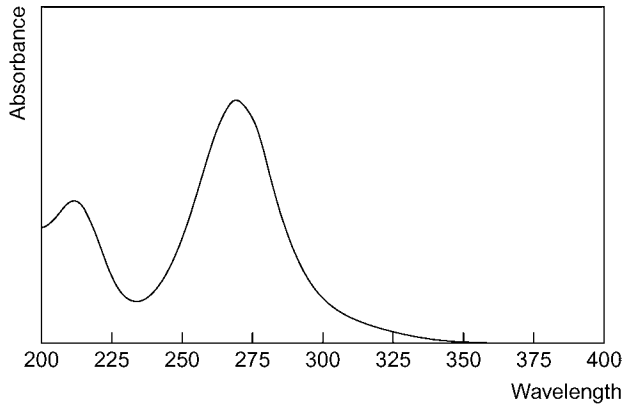
Synonym BAY-NTN 33893

Proprietary Names *Admire*; *Confidor*; *Gaucho*; *Imazethepyr*; *Marathon*; *Merit*; *Premier*; *Premise*; *Provado*.



Chemical Properties A colourless crystalline solid. Mp 136° to 144°. It is soluble in water (0.51 to 0.61 g/L), dichloromethane (50 to 100 g/L at 20°), isopropanol (1 to 2 g/L at 20°), toluene (0.5 to 1 g/L at 20°) and *n*-hexane (<0.1 g/L at 20°). Log *P* (octanol/water), 0.57 (22°).

Ultraviolet Spectrum Principal peaks at 220, 270 nm.



Disposition in the Body Imidacloprid is quickly and almost completely absorbed after ingestion and degraded to 6-chloronicotinic acid, which may be conjugated with glycine and then eliminated or reduced to guanidine. Of an administered dose, 70 to 80% may be excreted in urine, with 20 to 30% in faeces.

Imidapril

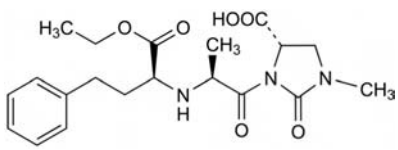
Antihypertensive

$C_{20}H_{27}N_3O_6$ = 405.4

CAS—89371-37-9

IUPAC Name (*4S*)-3-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1-methyl-2-oxo-4-imidazolidinecarboxylic acid

Proprietary Names *Novarok*; *Tanatril*.



Chemical Properties Colourless crystals (from ethyl acetate and *n*-hexane). Mp 139° to 140°.

Imidaprilat

$C_{18}H_{23}N_3O_6$ = 377.4

CAS—89371-44-8

Chemical Properties Crystals. Mp 239° to 241°.

Imidapril Monohydrochloride

$C_{20}H_{27}N_3O_6$, HCl = 441.9

CAS—89396-94-1

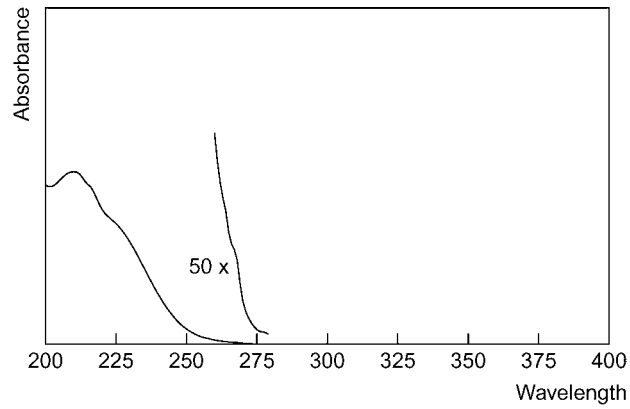
Synonym TA-6366

Proprietary Names *Novaloc*; *Tanatril*.

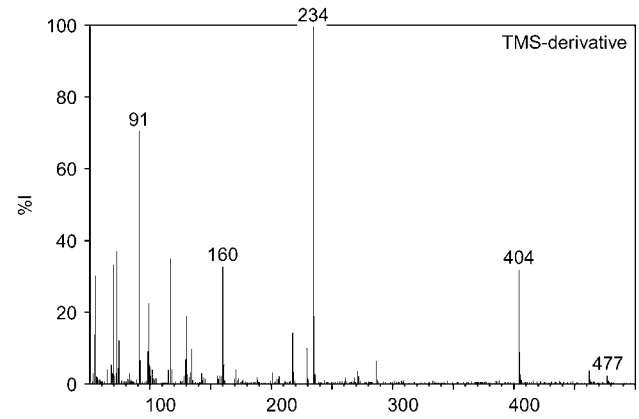
Chemical Properties Colourless crystals. Mp 214° to 216° (dec).

High Performance Liquid Chromatography Column: Symmetry C_{18}(100 × 2.1 mm i.d., 3.5 µm). Temperature: 40°. Mobile phase: acetonitrile : 0.05% formic acid (1:3), flow rate 0.2 mL/min. MS–MS detection (ESI, *m/z*: 406→234 for imidapril and 378→206 for imidaprilat). Retention time: imidapril, 3.06 min, imidaprilat, 4.39 min [Mabuchi *et al.* 1999].

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 234, 91, 74, 107, 70, 160, 55, 404 (TMS derivative).



Quantification

Plasma **GC–MS** Limit of detection, 1 µg/L for the metabolites [Matsuoka *et al.* 1992].

HPLC Limit of determination, 0.2 µg/L for imidapril and 0.5 µg/L for imidaprilat [Horimoto *et al.* 1993]. Limit of detection, 0.2 µg/L and 10 µg/L, for imidapril and its active metabolite, imidaprilat, respectively [Tagawa *et al.* 1993].

Urine **GC–MS** Limit of detection, 5 µg/L for the metabolites [Matsuoka *et al.* 1992].

HPLC See Plasma [Tagawa *et al.* 1993].

Disposition in the Body Imidapril is rapidly absorbed and extensively metabolised in the liver, by de-esterification, to its pharmacologically active diacid metabolite, imidaprilat and a number of minor metabolites. Peak concentrations of the drug are seen after 2 h and the active metabolite, 7 to 8 h after administration. It is excreted in urine mainly as the metabolites.

Therapeutic Concentration

Ten hypertensive patients without renal or hepatic disease or diabetes, aged 37 to 72 years, 3 males and 7 females, were administered a 10 mg single dose of imidapril and 10 mg daily for 4 weeks in a multiple dose study. Peak concentrations of 39.3 µg/L imidapril and 15.7 µg/L imidaprilat were observed 1 to 2 h and 5 to 10 h, respectively, after administration. In the multiple dosing study, levels reached 34.7 and 20.4 µg/L for the parent drug and its metabolite, respectively in 1 to 2 h and 3 to 6 h [Harder *et al.* 1998].

Eight patients with normal liver function, with a mean age of 42 years, and 8 patients with liver dysfunction, mean age 55 years, were administered a 10 mg dose once daily for 7 days. The mean peak plasma concentration of imidapril was 33.9 μg/L, observed 1.1 to 2.5 h after administration, for the healthy individuals and 38.0 μg/L after 1.5 to 3.0 h for those with liver dysfunction. Mean concentrations for the metabolite, imidaprilat, were 12.0 μg/L and 5.2 μg/L, respectively for the two groups of patients observed after 6.0 to 8.1 h, and 4.2 to 12.1 h [Hoogkamer *et al.* 1997].

Group 1: 8 volunteers with normal renal function, mean age 53 years. Group 2: 8 patients with moderate renal failure, mean age 58 years. Group 3: 8 patients with severe renal failure. All groups were administered 10 mg once daily for 7 days. The mean peak plasma concentrations of imidapril were 25.9, 37.8 and 75.8 μg/L, for the three groups, observed 1.0 to 3.5 h after administration. Mean concentrations for the metabolite, imidaprilat, were 8.6, 17.1 and 51.7 μg/L for groups 1, 2 and 3, respectively. These concentrations were seen 3.0 to 12.4 h after administration [Hoogkamer *et al.* 1998].

Bioavailability About 20%.
Half-life 2 h (imidapril); 8 h (imidaprilat).
Protein Binding 85%.
Dose 5 to 10 mg daily, as the hydrochloride.

Harder S *et al.* (1998). Single dose and steady state pharmacokinetics and pharmacodynamics of the ACE-inhibitor imidapril in hypertensive patients. *Br J Clin Pharmacol* 45(4): 377–380.

Hoogkamer JF *et al.* (1997). Pharmacokinetics of imidapril and its active metabolite imidaprilat following single dose and during steady state in patients with impaired liver function. *Eur J Clin Pharmacol* 51(6): 489–491.

Hoogkamer JF *et al.* (1998). Pharmacokinetics of imidapril and its active metabolite imidaprilat following single dose and during steady state in patients with chronic renal failure. *Eur J Clin Pharmacol* 54(1): 59–61.

Horimoto S *et al.* (1993). Rapid determination of a new angiotensin-converting enzyme inhibitor, imidapril, and its active metabolite in human plasma by negative-ion desorption chemical ionization-tandem mass spectrometry (MS/MS). *Chem Pharm Bull* 41: 699–702.

Mabuchi M *et al.* (1999). Determination of imidapril and imidaprilat in human plasma by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 734: 145–153.

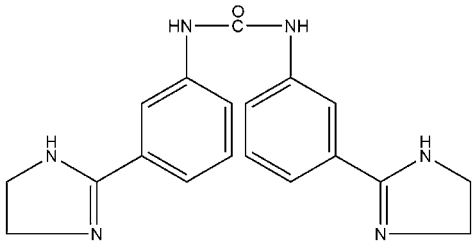
Matsuoka M *et al.* (1992). Determination of three metabolites of a new angiotensin-converting enzyme inhibitor, imidapril, in plasma and urine by gas chromatography-mass spectrometry using multiple ion detection. *J Chromatogr* 581(1): 65–73.

Tagawa K *et al.* (1993). Highly sensitive determination of imidapril, a new angiotensin I-converting enzyme inhibitor, and its active metabolite in human plasma and urine using high-performance liquid chromatography with fluorescent labelling reagent. *J Chromatogr* 617(1): 95–103.

Imidocarb

Antiprotozoal (Veterinary)
$C_{19}H_{20}N_{60}$ = 348.4
CAS—27885-92-3

IUPAC Name 1,3-Bis[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]urea
Synonym 3,3′-Di(2-imidazolin-2-yl)carbanilide



Chemical Properties White crystalline powder, Mp 370°, with decomposition. Soluble in dilute acetic acid.

Imidocarb Dihydrochloride

Synonym 4A65
Chemical Properties Imidocarb is extracted by ether from aqueous alkaline solutions.

Colour Test Vitali's test—pale yellow/orange (limit of detection, 1.0 μg).
Thin-layer Chromatography System T1—R_f 0.03 (location reagent acidified iodoplatinate spray, strong reaction).
Gas Chromatography System G2/225—no peak within 30 min; system G4/225—no peak within 30 min.
High Performance Liquid Chromatography Column: C_{18} RP, ABZ+ Plus (150 × 4.6mm, 5 μm). Mobile phase: acetonitrile : 0.005 mol/L 1-pentansulfonic acid sodium salt in water containing 0.1% triethylamine with glacial acetic acid (pH 3.2, 10 : 90 for 2 min to 20 : 80 at 8 min for 4 min to 10 : 90 at 12 min for 3 min), flow rate 1 mL/min. UV detection (λ= 250 nm). Retention time: 9.18 ± 0.10 min. Limit of detection, 0.025 mg/L. [Lai *et al.* 2002].

Column: C_{18} RP, XBridge™ (150 × 4.6 mm, 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : acetonitrile (89 : 11), flow rate 1.0 mL/min. UV detection. Retention time: 10.0 min. Limit of quantification, 0.01 mg/L, limit of detection, 0.005 mg/L [Su *et al.* 2007].

Column: C_{18} (100 × 4.6 mm, 50 μm). Mobile phase: 0.01 mol/L sodium acetate with 0.001 mol/L sodium trifluoroacetate (pH 7)-acetonitrile (85 : 15) : 0.01 mol/L sodium acetate with 0.01 mol/L trifluoroacetic acid and 0.01 mol/L tetramethylammonium chloride (pH 2)-acetonitrile (90 : 10, 85 : 15 for 5 min to 90 : 10 at 5 min for 15 min, flow rate 1.0 mL/min. UV detection (λ= 260 nm) [Tarbin, Shearer 1992].

Disposition in the Body
Intramuscularly injected imidocarb was administered to 7 sheep (4.5 mg/kg), the peak plasma concentration after 4 h was 7.9 mg/L, rapidly decreasing to 4.6 mg/L in the next 2 h. Trace amounts of imidocarb were still present 4 weeks after administration. Twenty-four hours after IM administration the highest concentrations of imidocarb were found in the kidney, liver and brain. Within 24 h 11–17% was excreted in the urine. High concentrations were also found in bile and in the milk from lactating ewes. [Aliu *et al.* 1977].
Toxicity LD_{50} (SC) in mice 107 mg/kg and in rats 150 mg/kg.
Dose 3 mg/kg body weight in cattle (red water disease) [Tarbin, Shearer 1992].

Aliu YO *et al.* (1977). Absorption, distribution, and excretion of imidocarb dipropionate in sheep. *Am J Vet Res* 38: 2001–2007.

Lai O *et al.* (2002). Depletion and bioavailability of imidocarb residues in sheep and goat tissues. *Vet Hum Toxicol* 44: 79–83.

Su D *et al.* (2007). Pharmacokinetics and bioavailability of imidocarb dipropionate in swine. *J Vet Pharmacol Ther* 30: 366–370.

Tarbin JA, Shearer G (1992). High-performance liquid chromatographic determination of imidocarb in cattle kidney with cation-exchange clean-up. *J Chromatogr* 577: 376–381.

Iminodimethylphenylthiazolidine

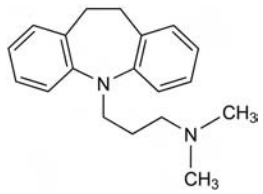
Sympathomimetic
$C_{11}H_{14}N_2S$ = 206.3

Synonym (—)-2-Imino-3,4-dimethyl-5-phenylthiazolidine
Chemical Properties Insoluble in water; soluble in chloroform.
Colour Test Ammonium vanadate test—(green) orange (limit of detection, 0.1 μg).
Thin-layer Chromatography System T1—R_f 0.48 (location reagent acidified iodoplatinate spray, positive reaction).

Imipramine

Antidepressant
$C_{19}H_{24}N_2$ = 280.4
CAS—50-49-7

IUPAC Name 10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenz[*b*,*f*]azepine-5-propanamine
Synonyms 5-(3-Dimethylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine; *N*-(γ-dimethylaminopropyl)iminodibenzyl; imizin; G-22355.
Proprietary Name *Topramine*



Chemical Properties pK_a 9.53 (24°) [Esrafili *et al.* 2007]. Log *P* (octanol/water), 4.47 [Esrafili *et al.* 2007], (octanol/water pH 7.4), 2.5. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Imipramine Embonate

$(C_{19}H_{24}N_2)2$, $C_{23}H_{16}O_6$ = 949.2
CAS—10075-24-8
Synonym Imipramine pamoate
Proprietary Name *Tofranil-PM*
Chemical Properties A yellow powder. Practically insoluble in water; soluble in ethanol, chloroform and ether.

Imipramine Hydrochloride

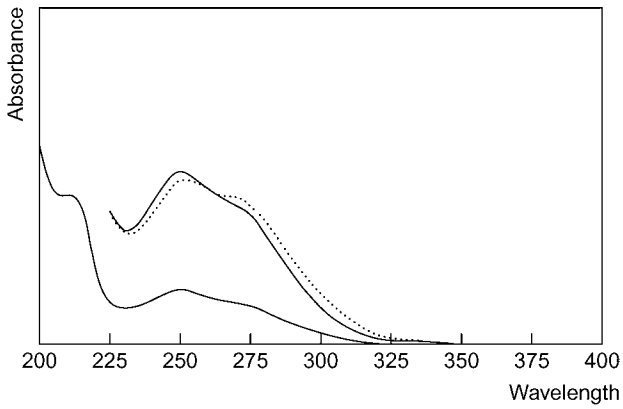
$C_{19}H_{24}N_2$, HCl = 316.9
CAS—113-52-0
Synonym Imizine
Proprietary Names *Antidep*; *Berkomine*; *Celamine*; *Ethipramine*; *Imavate*; *Impril*; *Iramil*; *Janimine*; *Medipramine*; *Melipramine*; *Mipralin*; *Novo-Pramine*; *Presamine*; *Primonil*; *Pryleugan*; *Sermonil*; *Talpramin*; *Tofranil*.
Chemical Proprieties A white or slightly yellow crystalline powder. Mp 174° to 175° (crystals from acetone). Soluble 1 in 2 of water, 1 in 1.5 of ethanol and 1 in 1.5 of chloroform; sparingly soluble in acetone; practically insoluble in ether.

Colour Test Forrest reagent—blue; FPN reagent—blue; Liebermann's reagent—blue; Mandelin's test—blue (add water).
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.48; system TC—R_f 0.23; system TE—R_f 0.67; system TL—R_f 0.13; system TAE—R_f 0.21; system TAF—R_f 0.47; system TAJ—R_f 0.07; system TAK—R_f 0.02; system TAL—R_f 0.52 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; FPN reagent, blue; Marquis test, blue).

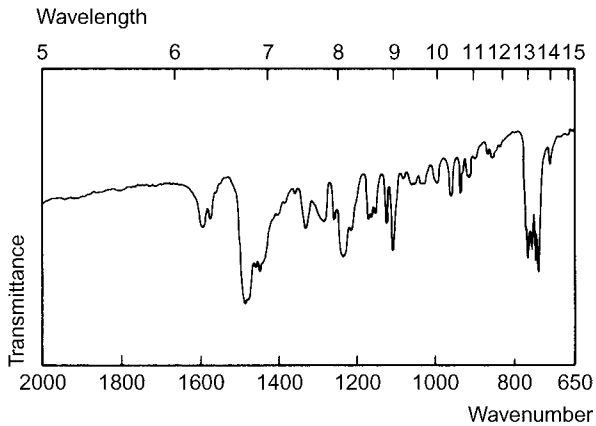
Gas Chromatography System GA—imipramine RI 2230, desipramine RI 2235, M (OH-) RI 2565, M (OH-methoxy-ring) RI 2390, M (OH-ring) RI 2240, M (di-OH-ring) RI 2600, M (ring) RI 1930, M (OH-)-AC RI 2610, M (OH-methoxy-ring)-AC RI 2370, M (OH-ring)-AC RI 2535, M (bis-nor-)-AC RI 2640, M (OH-)-Me RI 2480; system GB—imipramine RI 2314, M (2-OH-) RI 2636, M (10-OH-) RI 2494, M (di-OH-) RI 2962, M (OH-methoxy-) RI 2715; system GF—RI 2540; system GM—RRT 0.784 (relative to iprindole); system GS—RT 17.1 min.

High Performance Liquid Chromatography System HA—imipramine *k* 4.2, desipramine *k* 2.1, 2-hydroxydesipramine *k* 1.2, 2-hydroxyimipramine *k* 3.1; system HF—imipramine *k* 4.17, desipramine *k* 3.60; system HX—RI 437; system HY—RI 335; system HZ—RT 6.7 min; system HAA—RT 15.1 min; system HAM—RT 9.0 min; system HAV—*k* 6.4; system HAX—RT 14.7 min; system HAY—RT 6.8 min; system HAZ—imipramine *k* 1.62, M (10-OH-) *k* 0.39, M (2-OH-) *k* 0.39, M (*N*-oxide) *k* 1.85.

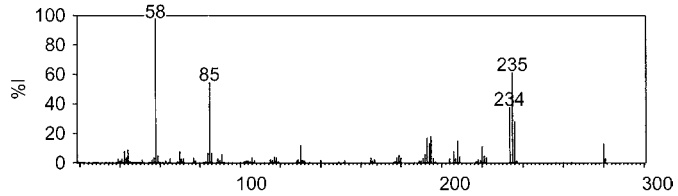
Ultraviolet Spectrum Aqueous acid—251 nm ($A_1^1 = 298a$); aqueous alkali—252 nm (see below).



Infrared Spectrum Principal peaks at wavenumbers 740, 747, 765, 1230, 1110, 756 cm^{-1} (imipramine hydrochloride, KBr disk, see below).



Mass Spectrum Principal ions at *m/z* 58, 235, 85, 234, 236, 195, 193, 208; desipramine 235, 195, 208, 44, 234, 193, 194, 71; 2-hydroxyimipramine 58, 251, 250, 211, 85, 42, 209, 296; 2-hydroxydesipramine 44, 209, 211, 250, 210, 224, 42, 251.



Quantification

Blood **GC-MS** Column: Chem Elut and Bond Elut Certify. Limit of quantification, 128–504 µg/L and 70–330 µg/L, respectively; limit of detection, 39–153 µg/L and 21–100 µg/L, respectively [Martinez *et al.* 2002].

LC-MS Column: XTerra RP-18. Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2). MRM acquisition mode. Limit of quantification, 2 µg/L [Titier *et al.* 2007].

Plasma **GC** Limit of quantification, 25 µg/L [Way *et al.* 1998]. Column: DB-17 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2 0.7 mL/min. Temperature programme: 260° for 1 min. NPD. Limit of quantification, 90 µg/L [Ulrich, Martens 1997].

GC-MS EI ionisation, SIM acquisition mode, positive ion mode. Limit of quantification, 2–5 µg/L, limit of detection, 0.5–2 µg/L [Lee *et al.* 2008]. MSD, 234 *m/z* [Pommier *et al.* 1997]. Column: 3% OV-17 on 80/100 mesh Chromosorb WHP or 1.5% Poly*S*-179 on 80/100 mesh Chromosorb W AW DMCS (1.5 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 230° to 250°. EI ionisation at 37 eV. Retention time: 2.4–2.7 min. Limit of detection, 0.2 µg/L [Alkalay *et al.* 1979].

HPLC Column: A Kromasil C_8 (250 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (45 : 55), flow rate 1.5 mL/min [Samanidou *et al.* 2007]. Column: Zorbax Extend-C_{18} (100 × 2.1 mm i.d., 3.5 µm i.d.). Mobile phase: 0.02 mol/L acetic acid (pH 4.0) : methanol (54 : 46), flow rate 0.25 mL/min. UV detection (λ = 215 nm). Limit of detection, 0.5–0.7 µg/L [Esrafili *et al.* 2007]. Column: Nucleosil 100 C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : phosphate buffer (pH 3.0, 60 : 40), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 3.6 min. Limit of quantification, 2 µg/L, limit of detection, 1.0 µg/L [Chmielewska *et al.* 2006], Column: C_{18}. Mobile phase: methanol : acetate buffer (70 : 30). UV detection (λ = 215 nm). Limit of detection, 2 µg/L [Bakkali *et al.* 1999]. Mobile phase: acetonitrile : 0.1 mol/L dipotassium hydrogen phosphate (pH 6.0, 30 : 70) flow rate 2 mL/min. Limit of quantification, 3 µg/L [Chen *et al.* 1997]. Column: Eicompak C_{18} reversed phase (250 × 4.6 mm i.d., 7 µm). Mobile phase: 0.05 mol/L potassium monophosphate buffer (pH 4.0) : acetonitrile : methanol (670 : 330 : 5) with 2.5 mmol/L sodium 1-octane sulfonate, flow rate 1.3 mL/min. Electrochemical detection. Retention time: 24.5 min. Limit of quantification, 0.5 µg/L [Koyama *et al.* 1993]. See also Foglia *et al.* [1991], Kabra *et al.* [1981], Nielsen, Brosen [1993] and Suckow, Cooper [1981].

LC-MS Column: Inertsil C_8. Mobile phase: methanol : 10 µmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. Limit of detection, 30 and 630 µg/L [Shinozuka *et al.* 2006].

Serum **GC-MS** See Plasma [Alkalay *et al.* 1979].

HPLC See Plasma [Kabra *et al.* 1981].

Urine **HPLC** See Plasma [Chen *et al.* 1997; Esrafili *et al.* 2007; Koyama *et al.* 1993; Nielsen, Brosen 1993].

Disposition in the Body Imipramine is readily absorbed after oral administration and widely distributed throughout the tissues; bioavailability is ~50%, but there is considerable intersubject variation. Imipramine undergoes considerable first-pass metabolism, mainly by demethylation to the primary active metabolite desipramine. Other major metabolic reactions include hydroxylation at the 2- or 10-positions followed by conjugation. Both pathways are genetically determined. 2-Hydroxyimipramine and 2-hydroxydesipramine appear to be active. A large number of metabolites have been identified in the urine and <10% of a dose is excreted unchanged. A total of ~40% of a dose is excreted in the urine in 24 h and ~70% in 72 h. Of the urinary material, up to 40% consists of free and conjugated 2-hydroxydesipramine, up to ~25% is free and conjugated 2-hydroxyimipramine, and ~15% is free and conjugated 2-hydroxyiminodibenzyl; small amounts of didesmethylimipramine, free iminodibenzyl and 10-hydroxydesipramine are also found. The excretion of unchanged drug and unconjugated metabolites is pH dependent and is increased in acid urine. Approximately 20% of a dose is eliminated in the faeces. Imipramine and desipramine cross the blood–brain barrier and placenta. They are excreted in breast milk in concentrations similar to those found in plasma.

Therapeutic Concentration Plasma concentrations vary considerably between individual subjects; therapeutic effect has been correlated with plasma concentrations of imipramine plus desipramine greater than 0.1 mg/L.

Following a single oral dose of 50 mg to 8 subjects, peak plasma concentrations of 0.010–0.083 mg/L (mean 0.03) imipramine and 0.004–0.014 mg/L (mean 0.008) desipramine were attained in ~3.4 h and 4.8 h, respectively [Sistovaris *et al.* 1983].

After daily oral doses of 50 to 300 mg (mean 100) to 24 subjects, the following steady-state plasma concentrations were reported: imipramine 0.01–0.11 mg/L (mean 0.05), desipramine 0.02–0.33 mg/L (mean 0.09), 2-hydroxyimipramine 0–0.02 mg/L (mean 0.01), 2-hydroxydesipramine 0–0.06 mg/L (mean 0.03) and didesmethylimipramine 0–0.04 mg/L (mean 0.007) [Gram *et al.* 1983].

Toxicity In adults the estimated minimum lethal dose is 1 g, although fatalities have occurred with less and patients have survived the ingestion of as much as 5 g. In children, as little as 350 mg may be fatal. Blood concentrations greater than 0.5 mg/L (imipramine plus desipramine) may cause toxic effects and imipramine concentrations of 0.8–13 mg/L (mean 4.5) have been associated with fatalities.

In 5 deaths attributed to overdosage with imipramine, the following postmortem concentrations were found: blood 0.3–4.1 mg/L (mean 2.7), bile 19.2–71.7 mg/L (mean 46, 4 cases), liver blood 0.2–9.6 mg/L (mean 4.6), urine 0.8–12.7 mg/L (mean 5.3, 3 cases) [Robinson, Holder 1979].

A 49-year-old female was found dead after having ingested 33 tablets, equivalent to 925 mg imipramine; blood and tissue concentrations were as follows: blood 14 mg/L, bile 88 mg/L, brain 25 µg/g, liver 75 µg/g [Singh *et al.* 1978].

In 2 deaths caused by imipramine overdose, the following postmortem tissue concentrations were reported:

	Imipramine	*Desipramine*
Blood (mg/L)	10, 3	3, trace
Kidney (µg/g)	9, —	6, -
Liver (µg/g)	16, 46	10, 13
Urine (mg/L)	-, 64	-, 13

[Hodda 1974]

In a death involving the ingestion of imipramine, paracetamol and ethanol, the following concentrations of imipramine, desipramine, 2-hydroxyimipramine and paracetamol, respectively, were reported: blood 9.0, 1.1, 3.9 and 11 mg/L; urine 92, 14, 42, – mg/L. Ethanol concentration in the blood and urine was <100 mg/L and 1050 mg/L, respectively [Fraser *et al.* 1987].

In 2 unrelated cases, a 7-year-old boy and a 21-year-old woman died suddenly while receiving chronic imipramine therapy; the evidence indicated that neither had ingested an acute overdose. The following postmortem tissue concentrations were reported for imipramine and desipramine, respectively, in the boy: left femoral blood 0.5 and 6.7 mg/L, right femoral blood 1.2 and 9.9 mg/L, aorta blood 1.0 and 8.7 mg/L, liver 68 and 400 µg/g. In the female, the imipramine and desipramine concentrations, respectively, were 0.6 and 3.74 mg/L in the femoral blood and 37 and 261 µg/g in the liver [Swanson *et al.* 1997]

Half-life Plasma half-life, imipramine 8–20 h, increased in children, elderly subjects and after overdose; desipramine 10–35 h.

Volume of Distribution 10–20 L/kg.

Clearance Plasma clearance, ~15 mL/min/kg, decreased in elderly subjects.

Distribution in Blood Plasma : whole blood ratio, 0.98.

Protein Binding 85–95%.

Note For a review of the pharmacokinetics of tricyclic antidepressants see Molnar, Gupta [1980].

Dose In the treatment of depression, 25 to 200 mg imipramine hydrochloride daily; up to 300 mg daily has been given.

Alkalay D *et al.* (1979). A sensitive method for the simultaneous determination in biological fluids of imipramine and desipramine or clomipramine and *N*-desmethylclomipramine by gas chromatography mass spectrometry. *Biomed Mass Spectrom* 6: 200–204.

Bakkali A *et al.* (1999). Solid-phase extraction with liquid chromatography and ultraviolet detection for the assay of antidepressant drugs in human plasma. *Talanta* 49: 773–783.

Chen AG *et al.* (1997). Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 693: 153–158.

Chmielewska A *et al.* (2006). Sensitive quantification of chosen drugs by reversed-phase chromatography with electrochemical detection at a glassy carbon electrode. *J Chromatogr B Analyt Technol Biomed Life Sci* 839: 102–111.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

Esrafili A *et al.* (2007). Hollow fiber-based liquid phase microextraction combined with high-performance liquid chromatography for extraction and determination of some antidepressant drugs in biological fluids. *Anal Chim Acta* 604: 127–133.

Foglia JP *et al.* (1991). Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection. *J Chromatogr* 572: 247–258.

Fraser AD *et al.* (1987). Analysis of 2-hydroxyimipramine in an imipramine-related fatality. *J Forensic Sci* 32: 543–549.

Gram LF *et al.* (1983). Imipramine metabolites in blood of patients during therapy and after overdose. *Clin Pharmacol Ther* 33: 335–342.

Hodda AE (1974). *TIAFT Bull* 10: 8–9.

Kabra PM *et al.* (1981). Simultaneous liquid chromatographic analysis of amitriptyline, nortriptyline, imipramine, desipramine, doxepin, and nordoxepin. *Clin Chim Acta* 111: 123–132.

Koyama E *et al.* (1993). Simultaneous high-performance liquid chromatography–electrochemical detection determination of imipramine, desipramine, their 2-hydroxylated metabolites, and imipramine *N*-oxide in human plasma and urine: preliminary application to oxidation pharmacogenetics. *Ther Drug Monit* 15: 224–235.

Lee XP *et al.* (2008). Determination of tricyclic antidepressants in human plasma using pipette tip solid-phase extraction and gas chromatography–mass spectrometry. *J Sep Sci* 31: 2265–2271.

Martinez MA *et al.* (2002). Simultaneous determination of viloxazine, venlafaxine, imipramine, desipramine, sertraline, and amoxapine in whole blood: comparison of two extraction/cleanup procedures for capillary gas chromatography with nitrogen–phosphorus detection. *J Anal Toxicol* 26: 296–302.

Molnar G, Gupta RN (1980). Plasma levels and tricyclic antidepressant therapy. Part 2. Pharmacokinetic, clinical and toxicologic aspects. *Biopharm Drug Dispos* 1: 283–305.

Nielsen KK, Brosen K (1993). High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine. *J Chromatogr* 612: 87–94.

Pommier F *et al.* (1997). Simultaneous determination of imipramine and its metabolite desipramine in human plasma by capillary gas chromatography with mass-selective detection. *J Chromatogr B Biomed Sci Appl* 703: 147–158.

Robinson AE, Holder AT (1979). Chemical evaluation of 'drug cocktails' in autopsy specimens. *J Anal Toxicol* 3: 3–13.

Samanidou VF *et al.* (2007). Development of an HPLC method for the monitoring of tricyclic antidepressants in biofluids. *J Sep Sci* 30: 2391–2400.

Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.

Singh KD *et al.* (1978). *TIAFT Bull* 1421.

Sistovaris N *et al.* (1983). Thin-layer chromatographic determination of imipramine and desipramine in human plasma and urine at single-dose levels. *J Chromatogr* 277: 273–281.

Suckow RF, Cooper TB (1981). Simultaneous determination of imipramine, desipramine, and their 2-hydroxy metabolites in plasma by ion-pair reversed-phase high-performance liquid chromatography with amperometric detection. *J Pharm Sci* 70: 257–261.

Swanson JR *et al.* (1997). Death of two subjects due to imipramine and desipramine metabolite accumulation during chronic therapy: a review of the literature and possible mechanisms. *J Forensic Sci* 42: 335–339.

Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.

Ulrich S, Martens J (1997). Solid-phase microextraction with capillary gas–liquid chromatography and nitrogen–phosphorus selective detection for the assay of antidepressant drugs in human plasma. *J Chromatogr B Biomed Sci Appl* 696: 217–234.

Way BA *et al.* (1998). Isotope dilution gas chromatographic-mass spectrometric measurement of tricyclic antidepressant drugs. Utility of the 4-carbethoxyhexafluorobutyryl derivatives of secondary amines. *J Anal Toxicol* 22: 374–382.

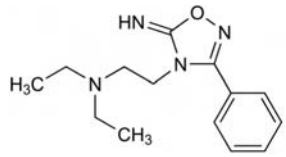
Imolamine

Antianginal Vasodilator

$C_{14}H_{20}N_4O$ = 260.3

CAS—318-23-0

IUPAC Name 4-[2-(Diethylamino)ethyl]-5-imino-3-phenyl-Δ^2-1,2,4-oxadiazoline



Chemical Properties Log *P* (octanol/water), 3.6.

Imolamine Hydrochloride

$C_{14}H_{20}N_4O$, HCl = 296.8

CAS—15823-89-9

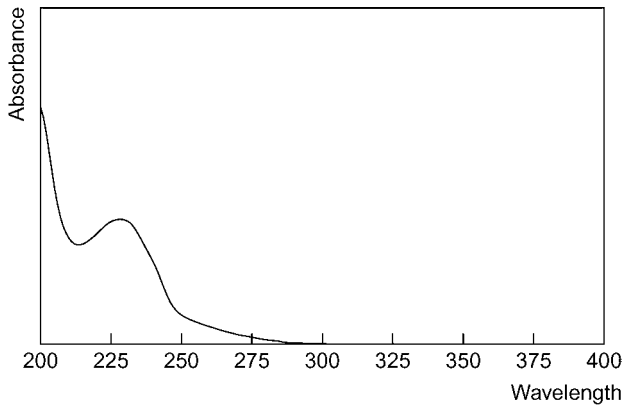
Proprietary Name *Irrigor*

Chemical Properties A white powder. Mp 154° to 155°. Soluble 1 in 1 of water and 1 in 500 of ethanol; slightly soluble in chloroform.

Thin-layer Chromatography System TA—R_f 0.56 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2177.

Ultraviolet Spectrum Principal peak at 230 nm.



Infrared Spectrum Principal peaks at wavenumbers 1638, 698, 752, 1610, 1500, 1070 cm^{-1} (KBr disk).

Dose Imolamine hydrochloride has been given in doses of 30 to 90 mg daily.

Indapamide

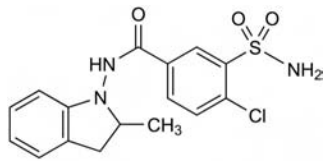
Diuretic

$C_{16}H_{16}ClN_3O_3S$ = 365.8

CAS—26807-65-8

IUPAC Name 3-(Aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide

Proprietary Names *Agelan*; *Clonilix*; *Dapamax*; *Dapa-Tabs*; *Daptril*; *Extur*; *Fludapamide*; *Fludex*; *Inamide*; *Indaflex*; *Indamol*; *Indanorm*; *Indolin*; *Lozol*; *Napamide*; *Naplin*; *Natramid*; *Natrilix*; *Nindaxa*; *Opumide*; *Pressural*; *Tandix*; *Tertensif*.



Chemical Properties Log *P* (octanol/water), 2.7.

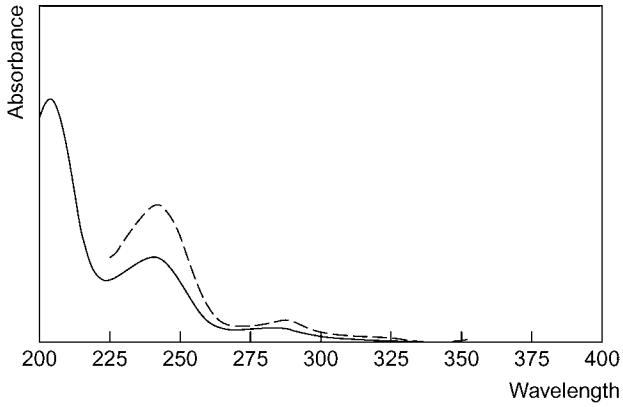
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—violet-red; Mandelin's test—red; Marquis test—orange→violet; mercurous nitrate—black; sulfuric acid—pink (slow).

Thin-layer Chromatography System TD—R_f 0.38; system TE—R_f 0.66; system TF—R_f 0.61; system TAD—R_f 0.46; system TAE—R_f 0.89; system TAJ—R_f 0.56; system TAK—R_f 0.22; system TAL—R_f 0.92.

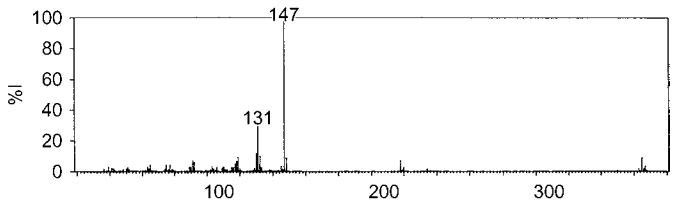
Gas Chromatography System GA—indapamide-Me_3 RI 3035; system GX—indapamide-Me_3 retention time 9.0 min.

High Performance Liquid Chromatography System HA—*k* 0.1.

Ultraviolet Spectrum Ethanol—242 (A^{1}_{1}=627b), 288 nm.



Mass Spectrum Principal ions at *m/z* 147, 131, 130, 132, 119, 148, 365, 218.

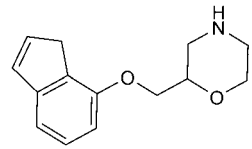


Quantification
Blood **HPLC** UV detection. For method of quantification, see Miller *et al.* [1993]. UV detection. Limit of detection, 50 µg/L [Choi *et al.* 1982].
Plasma **HPLC** UV detection. Limit of detection, 25 µg/L [Choi *et al.* 1982].
Urine **HPLC** Amperometric detection. Limit of detection, <1 µg/L [Legorburu *et al.* 1999]. UV detection. Limit of detection, 25 µg/L for indapamide and 4-chloro-3-sulfamoylbenzoic acid [Pietta *et al.* 1982]. See Blood [Choi *et al.* 1982].
Disposition in the Body Rapidly absorbed after oral administration. It is extensively metabolised and about 60% of a dose is slowly excreted in the urine over a period of 8 days with <5% as unchanged drug. About 20% of a dose is slowly eliminated in the faeces.
Therapeutic Concentration
Following a single oral dose of 5 mg to 22 subjects, a mean peak plasma concentration of 0.26 mg/L was attained in about 2 to 3 h [Grebow *et al.* 1982].
Following daily oral doses of 2.5 mg to 6 subjects, steady-state plasma concentrations of 0.02 to 0.05 mg/L (mean 0.03) were attained in 3 days [Campbell *et al.* 1977].
In 12 subjects, a single oral dose of indapamide as a 2.5 mg immediate-release preparation or as a 1.5 mg sustained-release formulation produced a dose-normalised mean peak plasma concentration of 39.3 µg/L at 0.8 h and 17.6 µg/L at 12.3 h, respectively [Schiavi *et al.* 2000].
Half-life Plasma half-life, about 15 h.
Protein Binding About 80%.
Note For a review of indapamide, see Chaffman *et al.* [1984].
Dose 2.5 to 5 mg daily.

Campbell DB *et al.* (1977). *Curr Med Res Opin* 5: 13–24.
Chaffman M *et al.* (1984). Indapamide. A review of its pharmacodynamic properties and therapeutic efficacy in hypertension. *Drugs* 28: 189–235.
Choi RL *et al.* (1982). High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood. *J Chromatogr* 230(1): 181–187.
Grebow PE *et al.* (1982). Pharmacokinetics and bioavailability of indapamide–a new antihypertensive drug. *Eur J Clin Pharmacol* 22: 295–299.
Legorburu MJ *et al.* (1999). Quantitative determination of indapamide in pharmaceuticals and urine by high-performance liquid chromatography with amperometric detection. *J Chromatogr Sci* 37: 283–287.
Miller RB *et al.* (1993). High-performance liquid chromatographic method for the determination of indapamide in human whole blood. *J Chromatogr* 614: 293–298.
Pietta P *et al.* (1982). High-performance liquid chromatographic assay for monitoring indapamide and its major metabolite in urine. *J Chromatogr* 228: 377–381.
Schiavi P *et al.* (2000). Pharmacokinetics of sustained and immediate release formulations of indapamide after single and repeated oral administration in healthy volunteers. *Fundam Clin Pharmacol* 14: 139–146.

Indeloxazine

Nootropic
$C_{14}H_{17}NO_2$ = 231.3
CAS—60929-23-9
IUPAC Name (±)-2-[(Inden-7-yl-oxy)methyl]morpholine
Synonym Indeloxacine



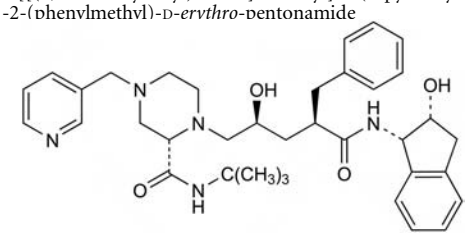
Indeloxazine Hydrochloride
$C_{14}H_{17}NO_2$, HCl = 267.8
CAS—65043-22-3
Synonyms CI-874; YM-08054-1.
Proprietary Names *Elen*; *Noin*.

Chemical Properties (±)-Form: pale-yellow needles from methanol, Mp 169° to 170°. Also reported as colourless acicular crystals from acetone, Mp 155° to 156°. (+)-Form: crystals from ethanol, Mp 112° to 113°. (−)-Form: crystals form isopropanol, Mp 142° to 142.5°.
Mass Spectrum Principal ions at *m/z* 100, 56, 231, 132, 103, 115, 57, 70.
Quantification
Plasma **GC-MS** Column: 3% OV-1 on Chromosorb W 80/100 mesh (50 cm × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 210°. Limit of detection, 2 µg/L [Kamimura *et al.* 1985].
HPLC Column: LiChrosorb SI-60 (15 cm × 4 mm i.d., 5 µm). Mobile phase: *n*-heptane : ethyl acetate (20 : 3), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 350 nm, λ_{em} = 505 nm). Retention time: 3.4 min. Limit of detection 5 µg/L [Kamimura *et al.* 1985].
Disposition in the Body Indeloxazine is metabolised in the liver by glucuronidation and metabolites include *trans*-4-(2-morpholinylmethoxy)-1,2-indenediol and *trans*-6-[[(1,2-dihydroxy-4-indanyl)oxy]methyl]-3-morpholine.
Toxicity Poison by SC and IV routes. Moderately toxic by ingestion.

Kamimura H *et al.* (1985). Determination of indeloxazine in plasma by liquid chromatography and gas chromatography-mass spectrometry. *J Pharm Sci* 74: 559–561.

Indinavir

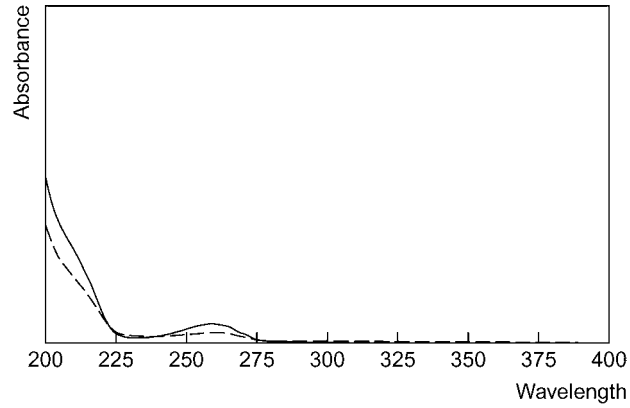
Protease Inhibitor, Antiviral
$C_{36}H_{47}N_5O_4$ = 613.8
CAS—150378-17-9
IUPAC Name 2,3,5-Trideoxy-*N*-[(1*S*,2*R*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl]-5-[(2*S*)-2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-*erythro*-pentonamide



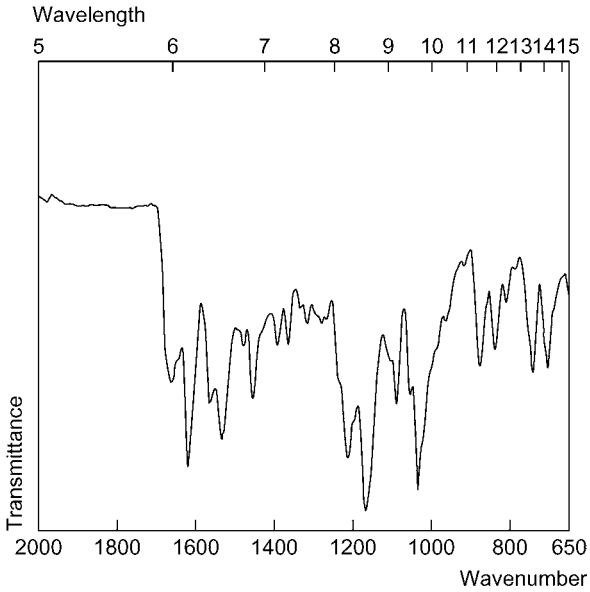
Chemical Properties Crystalline two anhydrous forms, Mp 153° to 154° (first anhydrous form), 167.5° to 168° (second anhydrous form). It is soluble in water: 0.015 g/L (unbuffered) and > 1.5 g/L (pH 4).

Indinavir Sulfate
$C_{36}H_{47}N_5O_4$, H_2SO_4 = 711.9
CAS—157810-81-6
Synonyms L-735524; MK-639; MK-0639.
Proprietary Name *Crixivan*
Chemical Properties Crystals from absolute ethanol. Mp 150° to 153° (softens at 135°).

High Performance Liquid Chromatography System HAB—retention time 4.2 min; system HAC—*k* 2.0.
Ultraviolet Spectrum Aqueous acid—210 (major band), 259 nm (minor).



Infrared Spectrum Principal peaks at wavenumbers 3295, 1619, 1535, 1215, 1169, 1035 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 596, 513, 465, 364, 338.

Quantification

***Plasma* HPLC** UV detection (λ=261 nm). Limit of quantification, 0.05 mg/L [Aymard *et al.* 2000]. UV detection (λ=210 nm). Limit of quantification, 0.005 mg/L [Woolf *et al.* 1995].

***Serum* HPLC** UV detection (λ=250 nm). Limit of detection, 0.21 mg/L [Simon *et al.* 2001].

***Urine* HPLC** See Plasma [Woolf *et al.* 1995].

Disposition in the Body Indinavir is rapidly absorbed after oral administration and peak plasma concentrations are reached within 0.8 h. Absorption is reduced when administration occurs with a high fat meal. The drug is metabolised by oxidation by the CYP3A4 isoenzyme in the liver and also glucuronidation, *para*-hydroxylation, 3′-hydroxylation and *N*-depyridomethylation. Seven metabolites have been isolated and identified; minor product identified as a 2′,3′-*trans*-dihydroxyindan analogue. <20% of the absorbed dose is excreted in urine, with about half being the unchanged drug. The rest is excreted in faeces.

Therapeutic Concentration

Twelve HIV-positive male patients, aged 40 to 49 years, were fasted overnight and administered indinavir as three dosing regimens. Group 1: 800 mg indinavir twice daily and 100 mg ritonavir twice daily. Group 2: 1200 mg indinavir twice daily. Group 3: 1200 mg indinavir twice daily and 100 mg ritonavir twice daily. The peak plasma indinavir concentration for group 1 was 8.7 mg/L (range, 7.5 to 10.0 mg/L); group 2, 13.8 (range, 9.5 to 18.0) mg/L; group 3, 15.4 mg/L. These levels were observed at 2.3 (0.5 to 4.0), 1.0 (0.5 to 1.5) and 1.0 to 1.5 h for the three groups, respectively [van Heeswijk *et al.* 1999].

Eleven children, aged between 9.0 and 13.6 years (mean, 11.0 years), infected with HIV-1 and previously treated with a nucleoside analogue reverse transcriptase inihibitor (zidovudine alone or in combination with dideoxyinosine) were included in the study. Indinavir was administered at 500 mg/m^2 every 8 h in combination with lamivudine and stavudine. The mean maximum plasma level observed was 12.3 mg/L (range 3.2 to 28.8 mg/L) at 1.9 h (1.0 to 4.0 h). The mean minimum level was 0.14 (0.05 to 0.13) mg/L [Gatti *et al.* 2000].

Toxicity

In a study of 11 children undergoing treatment with 500 mg/m^2 indinavir every 8 h (with stavudine or lamivudine), toxicity was observed as renal side effects ranging from mild to severe. This included kidney stones, renal cholic and acute renal failure (serum creatinine level greater than 5 mg/L above the base-line) [Gatti *et al.* 2000].

Bioavailability 65%.

Half-life 1.8 h; 0.77 h (children).

Volume of Distribution 195 L.

Clearance Apparent, 110 L/h.

Protein Binding 61%.

Dose A dose of 800 mg daily is administered as the sulfate. The dose is reduced to 600 mg in those with mild to moderate hepatic impairment.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

Gatti G *et al.* (2000). Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. *Antimicrob Agents Chemother* 44(3): 752–755.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

van Heeswijk RP *et al.* (1999). The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. *AIDS* 13(14): F95–F99.

Woolf E *et al.* (1995). Determination of L-735 524, an human immunodeficiency virus protease inhibitor, in human plasma and urine via high-performance liquid chromatography with column switching. *J Chromatogr A* 692(1-2): 45–52.

Indometacin

Analgesic

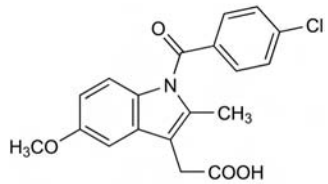
$C_{19}H_{16}ClNO_4$ = 357.8

CAS—53-86-1

IUPAC Name 2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid

Synonym 1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid, indomethacin

Proprietary Names *Amuno*; *Artracin*; *Confortid*; *Flexin Continus*; *Imbrilon*; *Indochron*; *Indocid*; *Indocin*; *Indoflex*; *Indolar*; *Indomax*; *Indotard*; *Maximet SR*; *Mobilan*; *Pardelprin*; *Rheumacin*; *Rimacid*; *Slo-Indo.*



Chemical Properties A white to yellow-tan, crystalline powder. Mp about 155° to 162°. It exhibits polymorphism. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in 30 of chloroform and 1 in about 40 of ether; soluble in acetone. pK_a 4.5. Log *P* (octanol/pH 7.4), −1.0. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

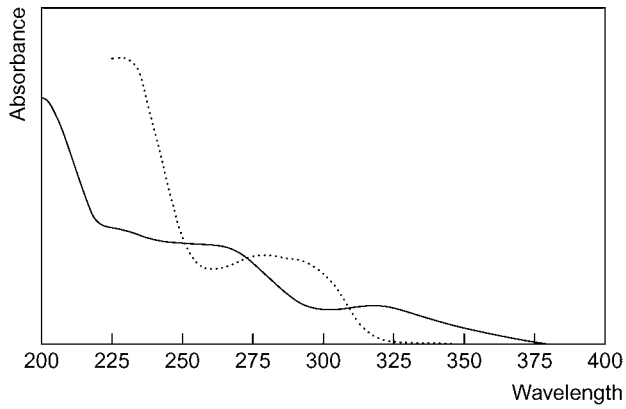
Colour Tests Liebermann's reagent—black; Mandelin's test—grey; Marquis test—orange; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.94; system TD—R_f 0.16; system TE—R_f 0.05; system TF—R_f 0.13; system TG—R_f 0.20; system TAD—R_f 0.38; system TAE—R_f 0.83; system TAF—R_f 0.63; system TAJ—R_f 0.46; system TAK—R_f 0.90; system TAL—R_f 0.90 (chromic acid solution, grey-brown; acidified potassium permanganate solution, positive).

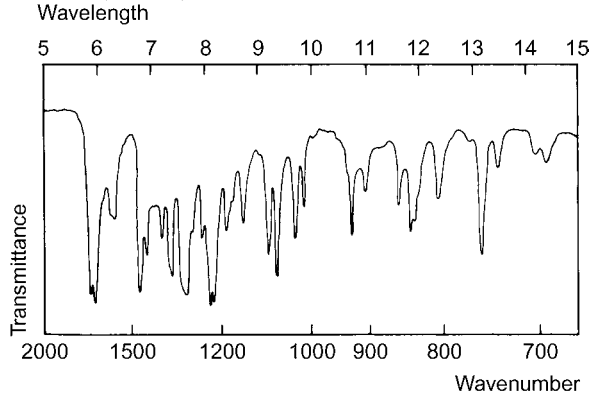
Gas Chromatography System GA—indometacinRI 2550 (capillary), RI 2685 (packed); system GD—RRT 1.55 (methyl derivative) andRRT 0.49 (methyl derivative) (relative to n-$C_{16}H_{34}$); system GL—indometacin-Me RI 2770, M (OH-)-Me_2 RI 2880.

High Performance Liquid Chromatography System HD—*k* 6.95; system HV—RRT 0.87 (relative to meclofenamic acid); system HX—RI 607; system HY—RI 590; system HZ—retention time 14.4 min; system HAA—retention time 21.7 min; system HAX—retention time 8.5 min; system HAY—retention time 9.2 min.

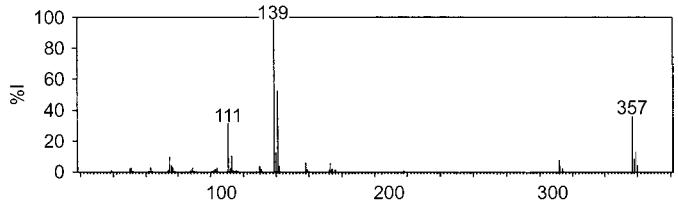
Ultraviolet Spectrum Methanolic acid—318 nm (A_1^1=180a); aqueous alkali—230, 279 nm (A_1^1=213a).



Infrared Spectrum Principal peaks at wavenumbers 1681, 1228, 1218, 1706, 1299, 1065 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 139, 141, 357, 111, 359, 140, 113, 75; M (OH-)-Me_2 239, 298, 135, 191, 107, 103.



Quantification

Plasma **TLC** Limit of detection, 30 µg/L [Søndergaard, Steiness 1979].

GC ECD. Limit of detection, 10 µg/L for indometacin and desmethylindometacin [Guissou *et al.* 1983].

GC-MS For method of quantification for indometacin and other NSAIDs, see Singh *et al.* [1991]. Limit of detection, <0.1 µg/L [Dawson *et al.* 1990].

HPLC UV detection. Limit of detection, about 50 µg/L [Sato *et al.* 1997]. UV detection. Indometacin and mefanamic acid. Limit of detection, 60 µg/L for indometacin [Niopas, Mamzoridi 1994]. Indometacin and its metabolites. Limit of detection, <60 µg/L for indometacin [Vree *et al.* 1993]. Limit of detection, <50 µg/L [Johnson, Ray 1992]. Diode-array, UV, and fluorescence detection. Limit of detection, 50–500 µg/L for indometacin and other NSAIDs [Singh *et al.* 1991]. Spectrophotometric detection. Limit of detection, 10 µg/L [Hubert *et al.* 1989]. Fluorescence detection. Indometacin and desmethylindometacin. Limit of detection, 25 µg/L for indometacin [Bernstein, Evans 1982].

LC-MS For method for quantification, see Taylor *et al.* [1998].

Radioimmunoassay Limit of detection, 50 µg/L [Hare *et al.* 1977].

Serum **HPLC** Limit of detection, 20 µg/L [Roberts, Smith 1987].

Urine **TLC** See Plasma [Søndergaard, Steiness 1979].

GC See Plasma [Guissou *et al.* 1983].

GC-MS See Plasma [Singh *et al.* 1991].

HPLC See Plasma [Vree *et al.* 1993]. See Plasma [Singh *et al.* 1991]. See Plasma. For method of quantification for indometacin and desmethylindometacin, see Bernstein and Evans [1982].

Radioimmunoassay See Plasma [Hare *et al.* 1977].

Synovial fluid **GC-MS** See Plasma [Dawson *et al.* 1990].

Disposition in the Body Readily and almost completely absorbed after oral administration. Indometacin is subject to considerable enterohepatic circulation. Metabolic reactions include *O*-demethylation, *N*-deacetylation and glucuronic acid conjugation, the major metabolites being desmethylindometacin (DMI), deschlorobenzoylindometacin (DBI), and desmethyldeschlorobenzoylindometacin (DMBI) and their glucuronides. These substances, together with unchanged indometacin and its glucuronide, are excreted in the urine (up to about 60% of the dose in 48 h) and the faeces (up to about 30% of the dose in 96 h) in variable amounts. Expressed as a percentage of the dose, the average amounts excreted in the urine in 48 h are: unchanged indometacin 5 to 20% (dependent on urinary pH), indometacin glucuronide 6 to 26%, DMI and its glucuronide 8 to 23%, DBI and its glucuronide 4 to 20%, DMBI and its glucuronide <3%; in the faeces the major metabolites found are DMBI (up to about 16%) and DMI (up to about 12%), with only small amounts of unchanged indometacin and DBI.

Therapeutic Concentration In plasma, usually in the range 0.5–3 mg/L.

After a single oral dose of 50 mg given to 20 subjects, peak plasma concentrations of 1.0–3.0 mg/L (mean 1.9) were attained in 1–4 [Kwan *et al.* 1976].

Steady-state plasma concentrations of 0.31–0.63 mg/L (mean 0.49) were attained in about 6 days after oral doses of 25 mg three times a day to 5 subjects [Alván *et al.* 1975].

Toxicity Toxic effects may be produced by plasma concentrations >5 mg/L.

Half-life Plasma half-life, 3–15 h.

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, 1–2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.9.

Protein Binding 90–99%.

Note For a review of the pharmacokinetics of indometacin, see Helleberg [1981].

Dose 50 to 200 mg daily.

Alván G *et al.* (1975). Pharmacokinetics of indomethacin. *Clin Pharmacol Ther* 18(3): 364–373.

Bernstein MS, Evans MA (1982). High-performance liquid chromatography-fluorescence analysis for indomethacin and metabolites in biological fluids. *J Chromatogr* 229(1): 179–187.

Dawson M *et al.* (1990). Gas chromatography/negative ion chemical ionization/tandem mass spectrometric quantification of indomethacin in plasma and synovial fluid. *Biomed Environ Mass Spectrom* 19: 453–458.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Guissou P *et al.* (1983). Gas chromatographic determination of indomethacin and its O-desmethylated metabolite in human plasma and urine. *J Chromatogr* 277: 368–373.

Hare LE *et al.* (1977). Radioimmunoassay of indomethacin in biological fluids. *J Pharm Sci* 66: 486–489.

Helleberg L (1981). Clinical Pharmacokinetics of indomethacin. *Clin Pharmacokinet* 6: 245–258.

Hubert P *et al.* (1989). A fully automated high-performance liquid chromatographic method for the determination of indomethacin in plasma. *J Pharm Biomed Anal* 7: 1819–1827.

Johnson AG, Ray JE (1992). Improved high-performance liquid chromatographic method for the determination of indomethacin in plasma. *Ther Drug Monit* 14: 61–65.

Kwan KC *et al.* (1976). Kinetics of indomethacin absorption, elimination, and enterohepatic circulation in man. *J Pharmacokinet Biopharm* 4: 255–280.

Niopas I, Mamzoridi K (1994). Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 656: 447–450.

Roberts I, Smith IM (1987). A high performance liquid chromatography method for the analysis of total and free indomethacin in serum. *Ann Clin Biochem* 24: 167–171.

Sato J *et al.* (1997). Simple, rapid and sensitive method for the determination of indomethacin in plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 692: 241–244.

Singh AK *et al.* (1991). Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicylic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry. *J Chromatogr* 568: 351–361.

Søndergaard I, Steiness E (1979). Determination of indomethacin in plasma and urine by direct quantitative thin-layer chromatography. *J Chromatogr* 162: 485–488.

Taylor PJ *et al.* (1998). Plasma indomethacin assay using high-performance liquid chromatography-electrospray-tandem mass spectrometry: application to therapeutic drug monitoring and pharmacokinetic studies. *Ther Drug Monit* 20: 691–696.

Vree TB *et al.* (1993). Determination of indomethacin, its metabolites and their glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis. Preliminary pharmacokinetics and effect of probenecid. *J Chromatogr* 616: 271–282.

Indoprofen

Analgesic

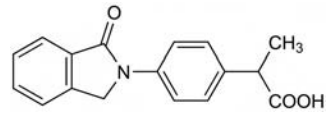
$C_{17}H_{15}NO_3 = 281.3$

CAS—31842-01-0

IUPAC Name 2-[4-(3-Oxo-1*H*-isoindol-2-yl)phenyl]propanoic acid

Synonyms 4-(1,3-Dihydro-1-oxo-2*H*-isoindol-2-yl)-α-methylbenzeneacetic acid; isindone.

Proprietary Names *Bor-Ind*; *Flosin*; *Flosint*; *Isindone*; *Praxis*; *Reumofene*.



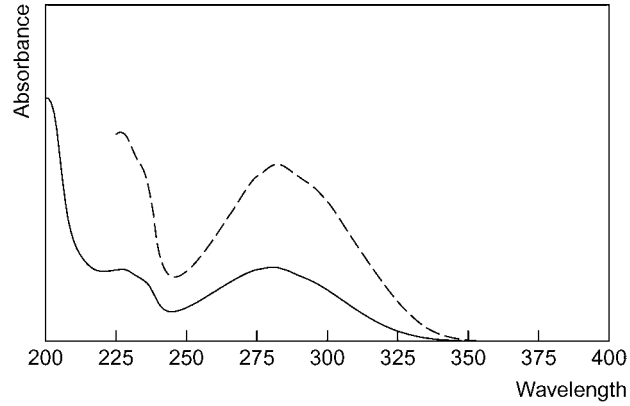
Chemical Properties A white crystalline powder. Mp 212°. Practically insoluble in water; very slightly soluble in chloroform; freely soluble in dimethylformamide; slightly soluble in methanol; sparingly soluble in 0.1 mol/L sodium hydroxide. pK_a 5.8. Log *P* (octanol/water), 2.8.

Thin-layer Chromatography System TG—R_f 0.08 (Ludy Tenger reagent, brown-black).

Gas Chromatography System GA— RI 2708 indoprofen-Me; system GD—RRT 2.27 methyl derivative and RRT 2.07 methyl derivative (relative to n-$C_{16}H_{34}$).

High Performance Liquid Chromatography System HD—*k* 1.2; system HV—RRT 0.52 (relative to meclofenamic acid); system HY—RI 406.

Ultraviolet Spectrum Methanol—282 nm (A^1_1=505a).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1510, 730, 1300, 1220, 1605 cm^{-1} (KBr disk).

Quantification

Plasma **GC** FID. Limit of detection, 400 µg/L [Smith *et al.* 1977].

HPLC For method for quantification of indoprofen enantiomers, see Bjorkman [1985]. UV detection. Limit of detection, 500 µg/L [Lanbeck *et al.* 1980].

Urine **HPLC** See Plasma [Lanbeck *et al.* 1980].

Disposition in the Body Rapidly and almost completely absorbed after oral administration. About 80 to 90% of a dose is excreted in the urine in 24 h as the glucuronide conjugate together with small amounts of unchanged drug and metabolites.

Therapeutic Concentration

After a single oral dose of 100 mg given to 4 subjects, peak plasma concentrations of 6.9 to 14.3 mg/L were attained in 1 to 1.5 h; following a single oral dose of 200 mg, peak plasma concentrations of 14.2 to 26.1 mg/L were reported at 0.5 to 2 h [Tamassia *et al.* 1976].

Toxicity Indoprofen was suspended from use in the United Kingdom in December 1983 because of reports of side-effects and fatalities.

Half-life Plasma half-life, about 2 h.

Volume of Distribution About 0.1 L/kg.

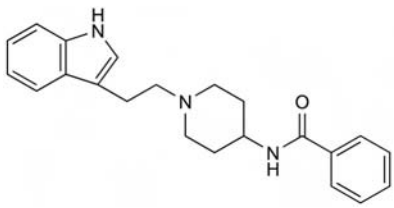
Clearance Plasma clearance, about 0.7 mL/min/kg.

Protein Binding About 99%.
Dose Indoprofen has been given in doses of 200 to 800 mg daily.

Bjorkman S (1985). Determination of the enantiomers of indoprofen in blood plasma by high-performance liquid chromatography after rapid derivatization by means of ethyl chloroformate. *J Chromatogr* 339: 339–346.
Lanbeck K *et al.* (1980). High-performance liquid chromatographic determination of indoprofen in plasma and urine. *J Chromatogr* 182(2): 262–266.
Smith RV *et al.* (1977). GLC determination of indoprofen in plasma. *J Pharm Sci* 66: 132–134.
Tamassia V *et al.* (1976). *Eur J Clin Pharmacol* 10: 257–262.

Indoramin

Antihypertensive
$C_{22}H_{25}N_3O = 347.5$
CAS—26844-12-2
IUPAC Name *N*-[1-[2-(1*H*-Indol-3-yl)ethyl]-4-piperidinyl]benzamide

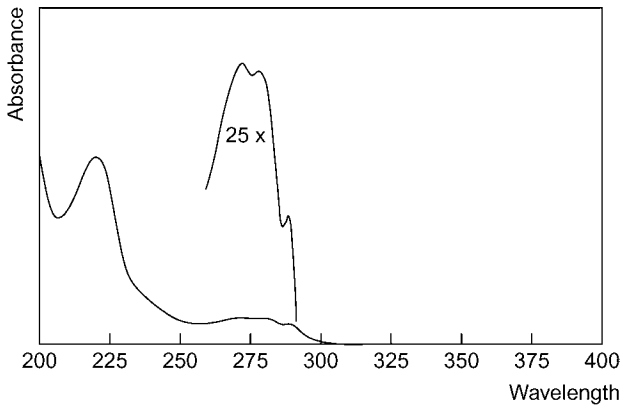


Chemical Properties pK_a 7.7. Log *P* (octanol/water), 3.6.

Indoramin Hydrochloride

$C_{22}H_{25}N_3O$, HCl = 383.9
CAS—33124-53-7 (xHCl)
Proprietary Names *Baratol*; *Doralese*; *Orfidora*; *Vidora*; *Wypresin*.
Chemical Properties Crystals. Mp 230° to 232°. Slightly soluble in water and chloroform; sparingly soluble in ethanol; soluble in methanol.

Thin-layer Chromatography System TA—R_f 0.84; system TE—R_f 0.74; system TAJ—R_f 0.13; system TAK—R_f 0.10; system TAL—R_f 0.77.
High Performance Liquid Chromatography System HAA—retention time 12.5 min.
Ultraviolet Spectrum Ethanol—273 (A^1_1=187b), 279 (A^1_1=190b), 289 nm (A^1_1=159b).



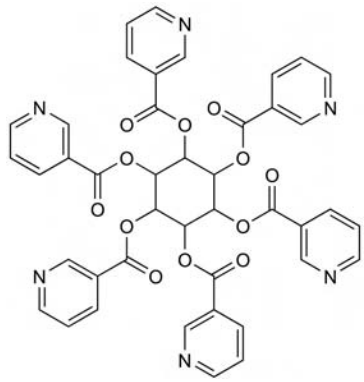
Infrared Spectrum Two major polymorphic forms exist. Principal peaks at wavenumbers 1640, 1535, 1316, 745, 1575, 715 cm^{-1} (indoramin hydrochloride form I (Nujol mull)); 1620, 1630, 1540, 1560, 740, 1317 cm^{-1} (indoramin hydrochloride form II (Nujol mull)).
Quantification
Plasma **HPLC** Electrochemical detection. Limit of detection, 0.5 µg/L [Leelavathi *et al.* 1986]. Fluorescence detection. Limit of detection, 1 µg/L [Swaisland 1981].
Disposition in the Body Readily absorbed after oral administration, but undergoes extensive first-pass metabolism to active metabolites. About 35% of a dose is excreted in the urine in 5 days, mainly as metabolites; <10% of a dose is excreted in the urine as unchanged drug; about 47% of a dose is eliminated in the faeces, mainly as metabolites; 6-hydroxyindoramin has been identified as a major metabolite. Other metabolites include acid-labile conjugates of indoramin and 6-hydroxyindoramin.
Therapeutic Concentration
Following a single oral dose of 50 mg to 6 middle-aged female subjects, peak plasma concentrations of 4 to 90 µg/L (mean, 20) were attained in about 3 h. After the same dose given to 5 elderly female subjects, peak plasma concentrations of 11 to 90 µg/L (mean 40) were attained after about 4 h [Norbury *et al.* 1984].

Following daily oral doses of 150 mg given to 5 subjects, steady-state plasma concentrations of 0.08 to 0.22 mg/L (mean, 0.13) were reported [Draffan *et al.* 1976].
Toxicity
In a fatality attributed to indoramin overdose, an antemortem plasma concentration of 6.4 mg/L was reported together with an alcohol concentration of 2.25 g/L [Hunter 1982].
Half-life Plasma half-life, 2 to 8 h (mean, 5), increased in elderly subjects.
Volume of Distribution About 7 L/kg.
Clearance Plasma clearance, about 20 mL/min/kg.
Protein Binding About 70 to 90% (concentration-dependent).
Dose The equivalent of 50 to 200 mg of indoramin daily.

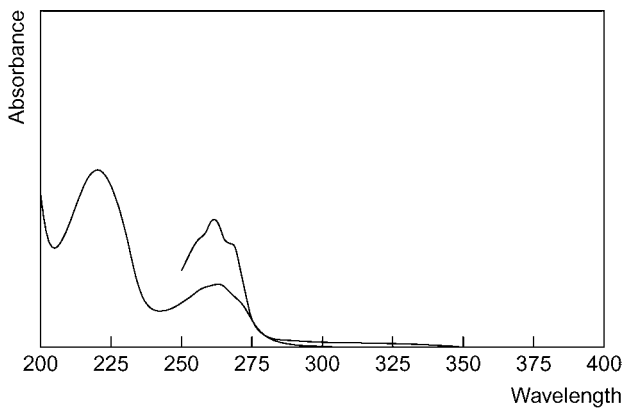
Draffan GH *et al.* (1976). Pharmacokinetics of indoramin in man. *Br J Clin Pharmacol* 3: 489–495.
Hunter R (1982). Death due to overdose of indoramin. *Br Med J (Clin Res Ed)* 285: 1011.
Leelavathi DE *et al.* (1986). Liquid chromatographic assay using electrochemical detection for the quantitation of indoramin in human plasma. *J Pharm Sci* 75: 421–423.
Norbury HM *et al.* (1984). Pharmacokinetics of oral indoramin in elderly and middle-aged female volunteers. *Eur J Clin Pharmacol* 27: 247–249.
Swaisland AJ (1981). Determination of therapeutic concentrations of indoramin by liquid chromatography with fluorimetric detection. *Analyst* 106: 717–719.

Inositol Nicotinate

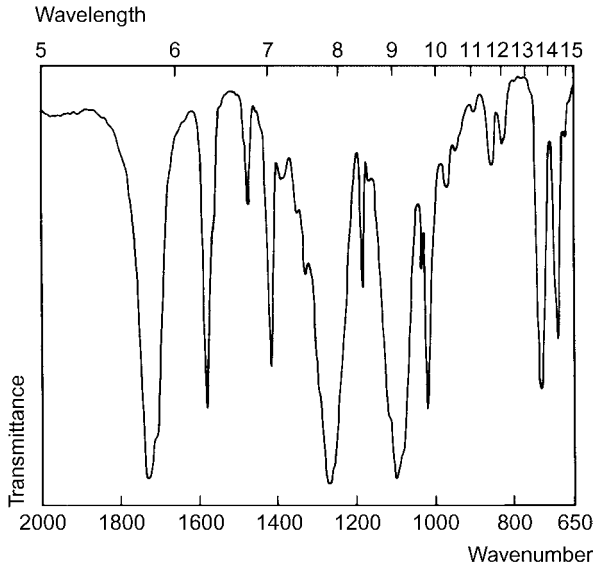
Vasodilator
$C_{42}H_{30}N_6O_{12} = 810.7$
CAS—6556-11-2
IUPAC Name [2,3,4,5,6-Pentakis(pyridine-3-carbonyloxy)cyclohexyl] pyridine-3-carboxylate
Synonyms Hexanicotinoyl inositol; *myo*-inositol hexa-3-pyridinecarboxylate; inositol niacinate.
Proprietary Names *Dilcit*; *Dilexpal*; *Hamovannad*; *Hexanicit*; *Hexogen*; *Hexopal*; *Linodil*; *Mesonex*; *Mesotal*; *Nicolip*; *Palohex*; *Vasodil*.



Chemical Properties A white crystalline powder. Mp 254.3° to 254.9°. Practically insoluble in water and most organic solvents; soluble 1 in 100 of chloroform; soluble in dilute acids. Log *P* (octanol/water), 1.6.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.01; system TC—R_f 0.43; system TL—R_f 0.16 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—261 nm (A^1_1=352b).



Infrared Spectrum Principal peaks at wavenumbers 1272, 1734, 1102, 1593, 1025, 718 cm^{-1} (KBr disk).



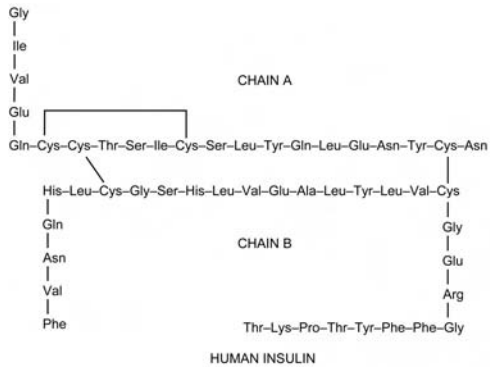
Dose 1.5 to 4 g daily.

I

Insulin

Antidiabetic

CAS—9004-10-8; 11061-68-0 (human)

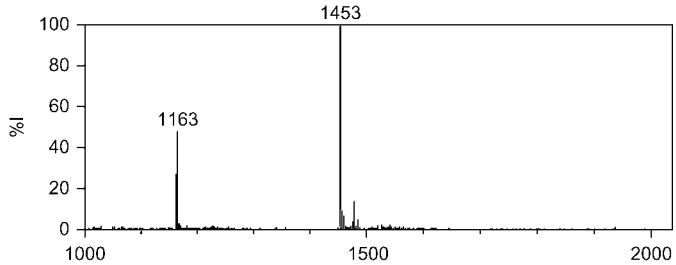


Chemical Properties Insulin is an amphoteric pancreatic protein that is extracted from beef or pork pancreas and purified by crystallisation. Insulin that is structurally identical with human insulin may be produced by chemical manipulation of animal insulin or by recombinant DNA technology.

A white crystalline powder. Slightly soluble in water; practically insoluble in ethanol, chloroform and ether; soluble in dilute solutions of mineral acids and, with degradation, in solutions of alkali hydroxides.

Ultraviolet Spectrum Aqueous acid—276 nm ($A_1^1 = 10.3$a).

Mass Spectrum



Quantification

***Plasma* HPLC** Column: RP-C_{18}. Mobile phase: 0.2 mol/L sodium sulfate anhydrous (pH 2.3) : acetonitrile (74 : 26), flow rate 1.2 mL/min. UV detection (λ= 214 nm). Limit of detection, 50 IU/L [Khaksa *et al.* 1998].

***Serum* LC-MS** Column: XBridge C_{18} (10 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile-water (10 : 90) : acetonitrile-water (70 : 30, 10 : 90 to 40 : 60 in 3 min to 70 : 30 in 0.1 min for 3 min to 10 : 90 for 5 min), flow rate 0.25 mL/min. ESI, positive ion mode, CID. Limit of quantification, 8.6 pmol/L, limit of detection, 1.5 fmol [Van Uytfanghe *et al.* 2007].

***Other* HPLC** Innovative Formulations. Column: Reversed phase C_8. Mobile phase: 40 µmol/L sodium sulfate solution (pH 3.7) : acetonitrile (24 : 76,), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 276 nm, λ_{em}= 306 nm). Limit of quantification, 100 µg/L, limit of detection, 30 µg/L [Mercolini *et al.* 2008].

Note For enzyme immunoassay for the quantification of insulin see Yoshioka *et al.* [1979]. For radioimmunoassay for the quantification of insulin see Dickson *et al.* [1977] and Gennaro, Van Norman [1975].

Disposition in the Body Insulin is an endogenous hormone secreted by the β-cells of the islets of Langerhans of the pancreas; it consists of 2 linked polypeptide chains. It is ineffective after oral administration, owing to rapid inactivation by proteolytic enzymes, but is well absorbed after SC or IM injection. The rate of absorption from different sites depends on local blood flow, absorption from the abdomen being faster than that from the arm, and that from the arm faster than from either the thigh or buttock. Exercise may increase absorption. It circulates in the blood as a polymer with a molecular weight of 17 000–43 000 and is rapidly taken up by the tissues. Approximately 50% of endogenous insulin in the portal vein is metabolised in a single passage through the liver. It is reduced to 2 separate chains by glutathione insulin transhydrogenase. The intact molecule or reduced chains may be metabolised by insulin-specific protease to peptides and amino acids. Insulin is filtered at the glomeruli but is reabsorbed and metabolised in the renal tubules, and only small amounts are excreted unchanged in the urine. It is also excreted in the bile.

Therapeutic Concentration In normal subjects, endogenous blood insulin concentrations vary throughout the day. The fasting or basal concentration in the plasma is usually in the range 5–40 mU/L (mean 17), with 50–100 mU/L in the portal vein. During absorption of food the peripheral blood concentration may increase by 10–15 times.

Serum insulin concentrations were measured in 21 diabetics; the daily dose varied from 3–80 units (mean 36) and the treatment period from 1 month to 35 years. The concentration of free insulin in the serum was in the range 10–440 mU/L (mean 47) and total insulin (free and antibody bound) varied from 67–17 020 mU/L (mean 2676) [Gennaro, Van Norman 1975].

After SC administration of 0.4 units/kg neutral protamine insulin or neutral protamine lispro insulin to 8 subjects, plasma concentrations of 1.86 µg/L (± 0.47) and 2.02 µg/L (± 0.69) were attained in 394 min (± 166) and 371 min (± 185), respectively [Rave *et al.* 1999] as reported by Roach, Woodworth [2002].

In 10 patients receiving two 240–unit doses of inhaled regular insulin via an inhaler and two 24–unit doses of SC regular insulin on 4 separate study days, a mean peak concentration of 96 mU/L occurred 76 min after the inhaled dose and 47 mU/L at 193 min after the SC dose [Perera *et al.* 2002].

Toxicity

In a 49-year-old diabetic man who intentionally injected himself IV with 150–250 units of NPH insulin (beef-pork species), a total insulin level of 1389 mU/L was reported, with a free insulin level of 378 mU/L; these levels declined to 1105 and 123 mU/L, respectively, after 18 h [Hsi, Werner 1987].

In 12 deaths caused by insulin injection, the following postmortem tissue concentrations were reported 1 day to 6 weeks after death: blood 1–155 mU/L (mean 49), CSF 62–64 mU/L (mean 63). In 1 case, selected as an example, in which a 43-year-old male was discovered next to empty insulin vials (1200 units in total), postmortem insulin levels were heart blood 155 mU/L and femoral vein blood 78 mU/L [Kernbach-Wighton, Puschel 1998].

A 51-year-old non-diabetic woman who was found dead next to 2 empty insulin syringes (300 units of intermediate type human insulin) and 1 empty ampoule of 10 mg diazepam. Analysis of heart blood revealed an insulin concentration of 382 mU/L, a diazepam concentration of 210 µg/L and a C-peptide concentration of 0.58 µg/L [Logemann *et al.* 1993].

The body of a 68-year-old non-diabetic physician was discovered next to 3 used insulin syringes; a suicide note was found. At postmortem, the concentration of insulin was 1848.8 mU/L in serum and 0.4 mU/L in aqueous humour; C-peptide (0.5 mU/L), metoprolol (0.4 mg/L) and alcohol (1.22 g/L) were also present in the blood [Junge *et al.* 2000].

Half-life Plasma half-life, ~5–6 min in normal subjects but may be longer in diabetics who develop anti-insulin antibodies.

Protein Binding Approximately 5% in normal subjects, but in diabetics the binding capacity may be extremely high owing to formation of insulin-binding antibodies.

Note For a review of the biochemistry and forensic aspects of insulin, see Fletcher [1983].

Dose Usually 10 to 80 units daily, SC; a dose of more than 80 units would be unusual and may indicate insulin resistance.

Dickson SJ *et al.* (1977). The isolation and quantitation of insulin in post-mortem specimens: a case report. *Forensic Sci* 9: 37–42.

Fletcher SM (1983). Insulin: a forensic primer. *J Forensic Sci Soc* 23: 5–17.

Gennaro WD, VanNorman JD (1975). Quantitation of free, total, and antibody-bound insulin in insulin-treated diabetics. *Clin Chem* 21: 873–879.

Hsi E, Werner PL (1987). An unusual case of insulin overdose. *Diabetes Care* 10: 255–256.

Junge M *et al.* (2000). Suicide by insulin injection in combination with beta-blocker application. *Forensic Sci Int* 113: 457–460.

Kernbach-Wighton G, Puschel K (1998). On the phenomenology of lethal applications of insulin. *Forensic Sci Int* 93: 61–73.

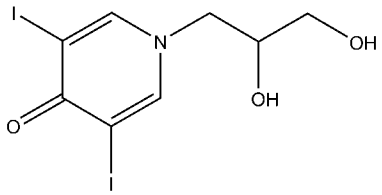
Khaksa G *et al.* (1998). High-performance liquid chromatographic determination of insulin in rat and human plasma. *Anal Biochem* 260: 92–95.

Logemann E *et al.* (1993). [Postmortem diagnosis of exogenous insulin administration]. *Arch Kriminol* 191: 28–36.
Mercolini L *et al.* (2008). Determination of insulin in innovative formulations by means of LC coupled to fluorescence detection. *J Pharm Biomed Anal* 48: 1303–1309.
Perera AD *et al.* (2002). Absorption and metabolic effect of inhaled insulin: intrapatient variability after inhalation via the Aerodose insulin inhaler in patients with type 2 diabetes. *Diabetes Care* 25: 2276–2281.
Rave K *et al.* (1999). Premixed formulations of insulin lispro: activity profiles in type 1 diabetic patients. *Diabetes Care* 22: 865–866.
Roach P, Woodworth JR (2002). Clinical pharmacokinetics and pharmacodynamics of insulin lispro mixtures. *Clin Pharmacokinet* 41: 1043–1057.
VanUytfanghe K *et al.* (2007). New liquid chromatography/electrospray ionisation tandem mass spectrometry measurement procedure for quantitative analysis of human insulin in serum. *Rapid Commun Mass Spectrom* 21: 819–821.
Yoshioka M *et al.* (1979). Evaluation of a commercial enzyme immunoassay for insulin in human serum, and its clinical application. *Clin Chem* 25: 35–38.

Iopydol

Radio-opaque Substance
$C_8H_9I_2NO_3 = 421.0$

IUPAC Name 1-(2,3-Dihydroxypropyl)-3,5-diiodopyridin-4-one
Synonyms 1-(2,3-Dihydroxypropyl)-1,4-dihydo-3,5-di-iodo-4-oxopyridine; NPP.
Proprietary Name It is an ingredient of *Hytrast*, together with iopydone.

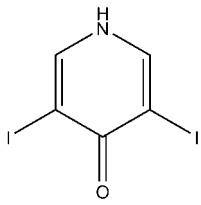


Chemical Properties A white crystalline powder. Mp 183° to 190°. Soluble 1 in 500 of water; soluble in methanol and pyridine.
Disposition in the Body After the bronchial administration in dogs of 20 mL of *Hytrast*, 60% was recovered in the faeces and 0.5% in the urine in 24 h. In cats given 15 mL of *Hytrast* orally, 0.06 g of iodine was detected in the urine in 24 h. This was equivalent to 1.3% of the ingested dose. A crystalline precipitate of iopydol was detected in the urine.
Toxicity An oral dose of 80 mL/kg of *Hytrast* proved to be non-toxic to mice.
Dose Iopydol is an X-ray contrast medium used in conjunction with iopydone as the proprietary preparation, *Hytrast*, for examination of the bronchial tract. *Hytrast* is a mixture of 46% iopydol and 30.5% iopydone in aqueous suspension; it is given by cannula or catheter into the lungs. Maximum dose: 25 mL.

Iopydone

Radio-opaque Substance
$C_5H_3I_2NO = 346.9$
CAS—5579-93-1

IUPAC Name 3,5-Diiodo-1*H*-pyridin-4-one
Synonym DJP
Proprietary Name Combined with iopydol, it is an ingredient of *Hytrast*.



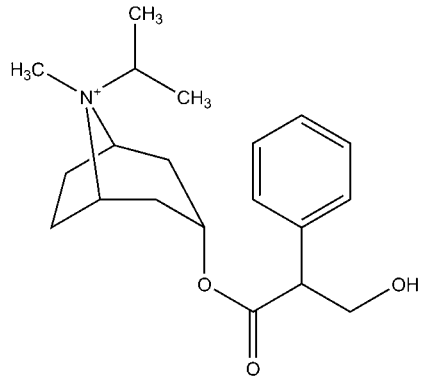
Chemical Properties A white crystalline powder. Mp 318° to326° with decomposition. Insoluble in water; soluble in aqueous alkaline solutions and methanol.
Thin-layer Chromatography System T1—R_f 0.87 (location reagent potassium permanganate spray, positive reaction).
Disposition in the Body After the bronchial administration of 20 mL of *Hytrast* to dogs, 60% was recovered in the faeces and 0.5% in the urine in 24 h. In cats given 15 mL of *Hytrast* orally, 0.06 g of iodine was detected in the urine after 24 h. This was equivalent to 1.3% of the ingested dose. A crystalline precipitate of iopydol was detected in the urine.
Toxicity An oral dose of 80 mL/kg of *Hytrast* proved non-toxic to mice, although a group of rats (264–295 g) receiving 1.5 mL *Hytrast* all died and 50% of a group receiving 0.75 mL *Hytrast* also died [Ginai *et al.* 1986].
Dose Iopydone is an X-ray contrast medium used in conjunction with iopydol in the proprietary preparation, *Hytrast*, for examination of the bronchial tract. *Hytrast* is a mixture of 46% iopydol and 30.5% iopydone in aqueous suspension, and is given by cannula or catheter into the lungs. The maximum dose is 25 mL.

Ginai AZ *et al.* (1986). Intraperitoneal toxicity of *Hytrast*: an experimental study. *Br J Radiol* 59: 1079–1082.

Ipratropium

Antiarrhythmic, Antimuscarinic, Bronchodilator, Quaternary Ammonium
$C_{20}H_{30}NO_3 = 332.5$
CAS—60205-81-4

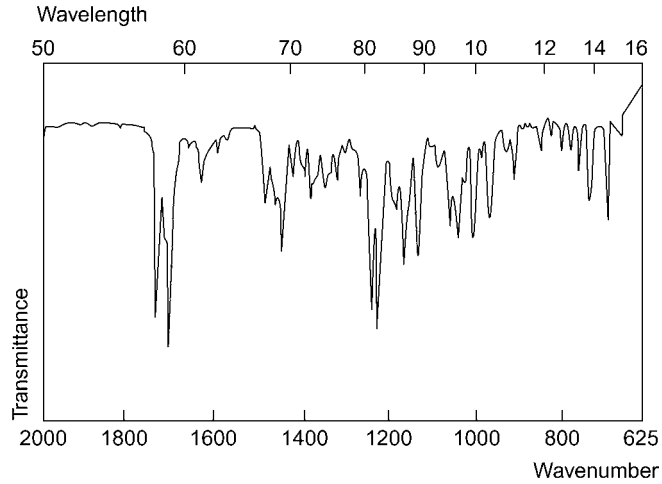
IUPAC Name [(1*R*,5*R*)-8-Methyl-8-propan-2-yl-8-azoniabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate
Synonym (1*R*,3*R*,5*S*,8*R*)-8-Isopropyl-3-[(±)-tropoyloxy]tropanium
Chemical Properties Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].



Ipratropium Bromide

$C_{20}H_{30}BrNO_3, H_2O = 430.4$
CAS—22254-24-6 (anhydrous ipratropium bromide); 66985-17-9 (ipratropium bromide monohydrate)
IUPAC Name [(1*S*,5*R*)-8-Methyl-8-propan-2-yl-8-azoniabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate bromide hydrate
Synonyms 3α-Hydroxy-8-isopropyl-1α*H*,5α*H*-tropanium bromide (±)-tropate; (3-endo,8-syn)-3(3-hydroxy-1-oxo-2-phenylproxy)-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane bromide; 8-isopropylnoratropine methobromide; *N*-isopropylnoratropinium bromomethylate; Sch-1000; Sch-1000-Br-monohydrate.
Proprietary Names *Aerotrop*; *Alvent*; *Apo-Ipravent*; *Apoven*; *Atem*; *Atronase*; *Atrovent*; *Cyclovent*; *Iprabron*; *Ipranase*; *Ipraneo*; *Ipratrin*; *Ipravent*; *Ipraxa*; *Ipvent*; *Itrop*; *Novo-Ipramide*; *Respontin*; *Rhinovent*; *Rinovagos*; *Rinatec*; *Ritanec*. It is also an ingredient in several preparations: see Sweetman [2009] for further details.
Chemical Properties White, crystalline substance with a bitter taste. Mp 230° to 232°. Freely soluble in water and lower alcohols; insoluble in ether, chloroform, or fluorocarbons [Deckers 1975]. Log *P* (octanol/buffer pH 7.4) −1.42 [Wood *et al.* 1995].

Infrared Spectrum [Deckers 1975].



Quantification
Blood **LC-MS** Column: C_{18} (100 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : 15 mmol/L heptafluorobutyric acid : 20 mmol/L ammonium formate buffer (pH 3.3; 5 : 95 to 90 : 10 over 18 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 13.6 min. Limit of quantification, 19.5 µg/L, limit of detection, 5.9 µg/L [Ariffin, Anderson 2006].
Disposition in the Body Ipratropium bromide has poor systemic absorption after oral and inhaled administration. The absolute amount of ipratropium bromide in the blood after oral administration is ~0.5% of the administered dose.

Blood concentrations after inhalation of the drug are ~1000 times lower than concentrations achieved after oral administration of an equibronchodilatory dose. Approximately 90% of the inhaled drug remains in the upper airways and the mouth. The drug diffuses rapidly into tissues after IV or IM administration. It is partially metabolised to 8 metabolites, which have little anticholinergic activity. The main metabolites are tropic acid (formed by enzymatic hydrolysis of the ester) and *N*-isopropylmethylnortropanium. After inhalation, the parent drug and metabolites are eliminated in the urine and faeces, with ~3.2% recovered in the urine and 69% recovered in faeces after 6 days. Renal excretion occurs at ~6 times the glomerular filtration rate. It does not cross the blood–brain barrier and it is not known whether placental transfer or excretion to human milk occurs.

Therapeutic Concentration

Ten patients with severe vasomotor rhinitis and 10 healthy volunteers were administered 20 µg/dose ipratropium intranasally. All participants were administered doses 3 times into each nostril at time 0 and thereafter: 2 series of 3 doses into each nostril were repeated twice at 15 min intervals. The total amount of ipratropium bromide administered was 360 µg in 30 min. Mean peak plasma concentrations in the patient group and in the healthy volunteers group were 380 and 245 ng/L, respectively, reached after 39.5 and 34.5 min, respectively. All concentrations were measured by radioimmunoassay [Kaila *et al.* 1990].

Seven healthy volunteers were administered a single 30 mg oral dose of ipratropium bromide. The mean peak plasma concentration was 25 µg/L, reached at 3 h. Plasma concentrations remained above 20 µg/L for a further 3 h before declining gradually over the next 6 h. In another group of 7 healthy volunteers administered an IV dose of 1 mg ipratropium bromide, the mean plasma concentration 1 h after the end of infusion was 12 µg/L, and after 5 h, 3.3 µg/L. Following inhalation of a very high dose (555 µg) by 5 healthy volunteers, ipratropium appeared in plasma within 2 min. At 3 h post-dose, the plasma concentration was 0.06 µg/L, representing 0.03% of the inhaled dose. All concentrations were measured by RIA [Adlung *et al.* 1976].

Bioavailability Approximately 3.3% (range 0.9 to 6.1%) [Ensing *et al.* 1989].

Half-life Approximately 3.5 h.

Clearance 2325 mL/min [Ensing *et al.* 1989].

Protein Binding Minimal, ~0 to 9% *in vitro*.

Dose Usual UK dosage by inhalation from a metred-dose aerosol is 20 or 40 µg three or four times daily. In children aged 6 to 12 years, the usual dosage is 20 or 40 µg three times daily, and below 6 years the usual dosage is 20 µg three times daily. Dry powder inhalation capsules are also available for use in adults, the usual dosage being 40 µg three or four times daily. Ipratropium bromide may also be given by inhalation as a nebulised solution in doses of 250 to 500 µg up to four times daily. In children aged 6 to 12 years, the usual dose by nebuliser is 250 µg, which may be repeated up to a total daily dose of 1 mg. In the management of acute asthma in children under 6 years old, doses of 125 to 250 µg may be repeated at intervals of at least 6 h, up to a total daily dose of 1 mg.

Ipratropium bromide, given intranasally, is also used in the management of rhinorrhoea associated with rhinitis. A dose of 42 µg is given into each nostril by metred-dose nasal spray two or three times daily.

Adlung J *et al.* (1976). Studies on pharmacokinetics and biotransformation of ipratropiumbromide in man [author's translation]. *Arzneimittelforschung* 26: 1005–1010.

Ariffin M *et al.* (2006). LC/MS/MS analysis of quaternary ammonium drugs and herbicides in whole blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 842: 91–97.

Deckers W (1975). The chemistry of new derivatives of tropane alkaloids and the pharmacokinetics of a new quarternary compound. *Postgrad Med J* 51: 76–81.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Ensing K *et al.* (1989). Pharmacokinetics of ipratropium bromide after single dose inhalation and oral and intravenous administration. *Eur J Clin Pharmacol* 36: 189–194.

Kaila T *et al.* (1990). Vasomotor rhinitis and the systemic absorption of ipratropium bromide. *Rhinology* 28: 83–89.

Sweetman SC, ed. (2009). *Martindale: The complete drug reference*, 39 edn. London: Pharmaceutical Press.

Wood CC *et al.* (1995). Product characteristics and pharmacokinetics of intranasal ipratropium bromide. *J Allergy Clin Immunol* 95: 1111–1116.

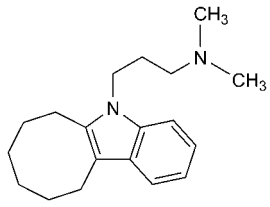
Ipridole

Antidepressant

$C_{19}H_{28}N_2$ = 284.4CAS—5560-72-5

IUPAC Name 3-(6,7,8,9,10,11-hexahydrocycloocta[*b*]indol-5-yl)-*N*,*N*-dimethylpropan-1-amine

Synonyms 6,7,8,9,10,11-Hexahydro-*N*,*N*-dimethyl-5*H*-cyclo-oct[*b*]indole-5-propanamine; pramindole.



Chemical Properties pK_a 8.2. Log *P* (octanol/water), 5.74 [Meylan, Howard 1995].

Ipridole Hydrochloride

$C_{19}H_{28}N_2$, HCl = 320.9

CAS—20432-64-8

Proprietary Names *Galatur*; *Prondol*.

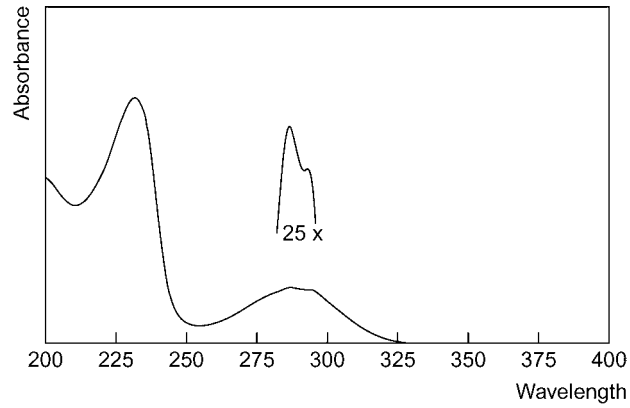
Chemical Properties White powder. Mp about 144°. Soluble in water, ethanol and chloroform.

Thin-layer Chromatography System TA—R_f 0.47; system TL—R_f 0.16; system TB—R_f 0.49; system TC—R_f 0.34 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2335; system GB—RI 2437; system GM—RRT 1.000 (relative to ipridole).

High Performance Liquid Chromatography System HA—*k* 4.1; system HF—*k* 10.83.

Ultraviolet Spectrum Aqueous acid—287 (A^1_1 = 248a), 293 nm; aqueous alkali—302 nm.



Infrared Spectrum Principal peaks at wavenumbers 738, 1650, 1612, 1145, 1173, 1040 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 170, 284, 213, 145, 212, 159, 144.

Quantification

Plasma GC Column: Apiezon L plus 10% KOH Anakrom ABS 100/200 mesh (1.91 m × 2 mm i.d.). Carrier gas: N_2, 29 mL/min. Temperature: 285°. AFID, Retention time: 8.4 min. Limit of detection, 5 µg/L.[Caillé *et al.* 1982].

Disposition in the Body Well absorbed after oral administration. It is slowly excreted in the urine, about 50% of a dose being eliminated in 3 days, and it is still detectable one week after administration; only about 5% of a dose is excreted in the urine as unchanged drug.

Therapeutic Concentration

After a single oral dose of 60 mg given to 5 subjects, peak plasma concentrations of 0.05 to 0.09 mg/L (mean 0.07) were attained in 2 to 4 h. Following oral administration of 30 mg three times daily to 4 subjects, plasma concentrations of 0.03 to 0.08 mg/L (mean 0.06) were reported 10 h after the final daily dose on day 21 [Caillé *et al.* 1982].

Half-life Plasma half-life, ≈35 to 70 h (mean 52).

Dose The equivalent of 45 to 180 mg of iprindole daily.

Caillé G *et al.* (1982). Quantitation of iprindole in plasma by GLC. *Biopharm Drug Dispos* 3: 11–17.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Iproniazid

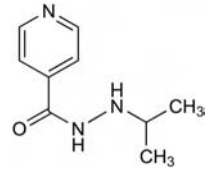
Antidepressant

$C_9H_{13}N_3O$ = 179.2

CAS—54-92-2

IUPAC Name *N*′-Propan-2-ylpyridine-4-carbohydrazide

Synonym 4-Pyridinecarboxylic acid 2-(1-methylethyl)hydrazide



Chemical Properties Crystals. Mp 113°. Freely soluble in water and ethanol. Log *P* (chloroform/pH 7.4), 0.

Iproniazid Phosphate

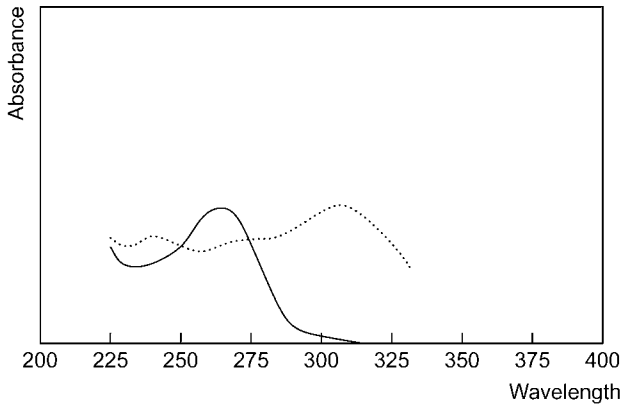
$C_9H_{13}N_3O$, H_3PO_4 = 277.2

CAS—305-33-9

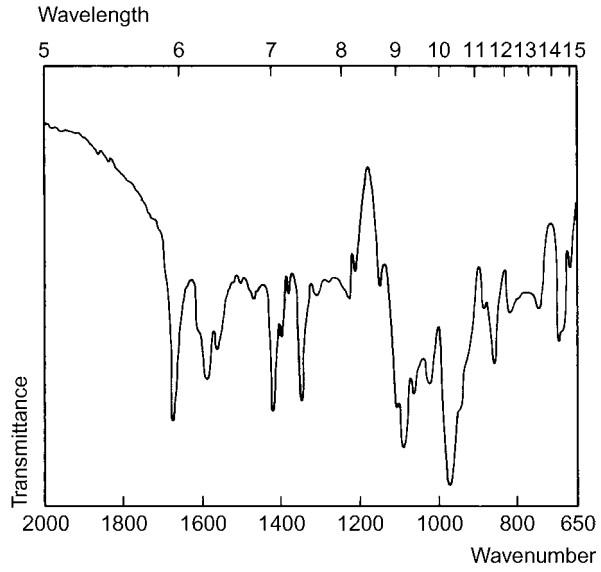
Proprietary Name *Marsilid*

Chemical Properties A white crystalline powder. Mp 175° to 184°. Soluble 1 in 5 of water and 1 in 90 of ethanol; practically insoluble in chloroform and ether.

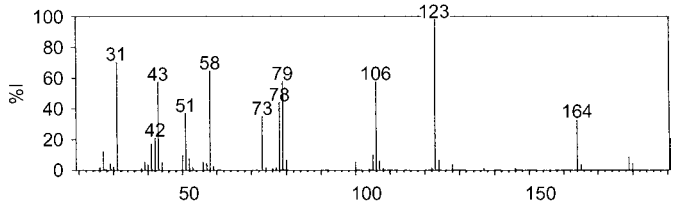
Colour Tests Cyanogen bromide—orange-pink; Nessler's reagent—black.
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.01; system TC—R_f 0.23; system TE—R_f 0.41; system TL—R_f 0.17; system TAE—R_f 0.70; system TAF—R_f 0.69 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1593; system GB—RI 1609.
High Performance Liquid Chromatography System HX—RI 249.
Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1=235c); aqueous alkali—242, 307 nm.



Infrared Spectrum Principal peaks at wavenumbers 975, 1090, 1672, 1110, 1065, 1025 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 123, 31, 58, 106, 79, 43, 78, 51; isonicotinic acid 123, 51, 78, 106, 50, 52, 105, 39.

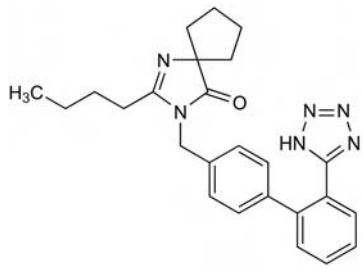


Quantification
Urine **GC** FID. For method for quantification, see de Sagher *et al.* [1976].
Disposition in the Body Readily absorbed after oral administration. It is excreted in the urine mainly as metabolites, principally isonicotinic acid, with up to 15% as unchanged drug.
Half-life Plasma half-life, about 10 h.
Dose The equivalent of 25 to 150 mg of iproniazid daily.

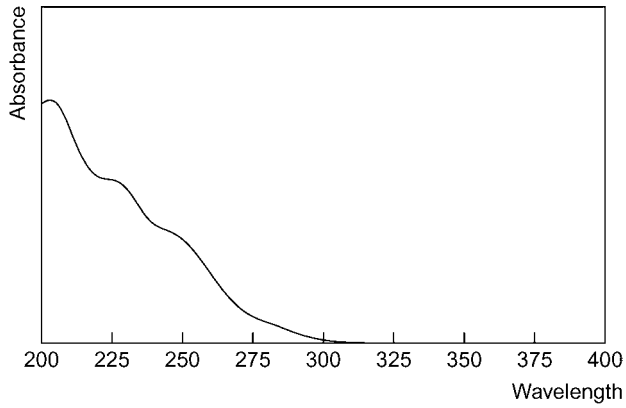
de Sagher RM *et al.* (1976). Identification and quantitative GLC determination of iproniazid in human urine. *J Pharm Sci* 65: 878–882.

Irbesartan

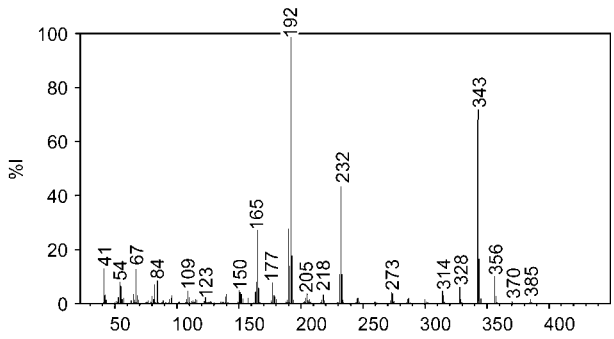
Angiotensin II Receptor Antagonist
$C_{25}H_{28}N_6O$ = 428.5
CAS—138402-11-6
IUPAC Name 2-Butyl-3-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one
Synonyms BMS-186295; SR-47436.
Proprietary Names *Aprovel*, *Avapro*.



Chemical Properties White to off-white crystals (from ethanol, 96%). Mp 180° to 181°. It is slightly soluble in alcohol and methylene chloride. It is practically insoluble in water. pK_a 4.5. Log *P* (octanol/water), 10.1.
Ultraviolet Spectrum Aqueous acid (0.2 mol/L HCl)—224, 246 nm.



Mass Spectrum Principal ions at *m/z* 192, 343, 342, 232, 190, 165, 193, 67.



Quantification
Plasma **HPLC** Fluorescence detection (λ_{ex}=250 nm, λ_{em}=371 nm). Limit of quantification, 1.0 µg/L [Chang *et al.* 1997].
Urine **HPLC** See Plasma [Chang *et al.* 1997].
Disposition in the Body Irbesartan is almost completely absorbed after administration and steady state concentrations are reached within 3 to 4 days. No significant accumulation has been observed. Eight metabolites have been identified and are formed by glucuronidation or oxidation, primarily by the cytochrome P450 CYP2C9. Excretion is mainly through faeces with <2% of an administered dose recovered unchanged in urine.
Therapeutic Concentration
Twelve healthy male volunteers were administered a single oral dose of 50 mg. Peak plasma concentrations of 0.942 mg/L were reached within ≈3 h [Chang *et al.* 1997].
Forty-eight healthy men, aged between 18 and 45 years, were administered a single oral dose of 150, 300, 600 or 900 mg irbesartan (9 in each group and 12 received placebo). Peak plasma concentrations after single doses were 1.9, 2.9, 4.9 and 5.3 mg/L, respectively, which were reached within 1.5 to 2 h. Peak concentrations were very similar after multiple dosing [Marino *et al.* 1998].
Bioavailability 60 to 80%.

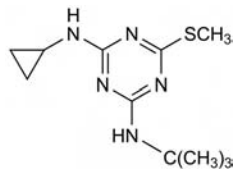
Half-life 11 to 15 h.
Volume of Distribution 53 to 93 L.
Clearance Plasma, 0.07 L/h/kg (healthy) and 0.12 L/h/kg (hepatic cirrhosis).
Protein Binding 90%.
Dose 150 mg once daily which may be increased to 300 mg if necessary. In the elderly or those undergoing haemodialysis, the dose may be reduced to 75 mg.

Chang SY *et al.* (1997). High-performance liquid chromatographic assay for the quantitation of irbesartan (SR 47436/BMS-186295) in human plasma and urine. *J Chromatogr B Biomed Sci Appl* 702: 149–155.
Marino MR *et al.* (1998). Pharmacokinetics and pharmacodynamics of irbesartan in healthy subjects. *J Clin Pharmacol* 38: 246–255.

Irgarol

Insecticide
$C_{11}H_{19}N_5S = 253.4$
CAS—28159-98-0

IUPAC Name 2-*N*-tert-Butyl-4-N-cyclopropyl-6-methylsulfanyl-1,3,5-triazine-2, 4-diamine
Synonym *N*-Cyclopropyl-*N*′-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
Proprietary Names *Irgarol 1051*; *Irgarol 1071*.

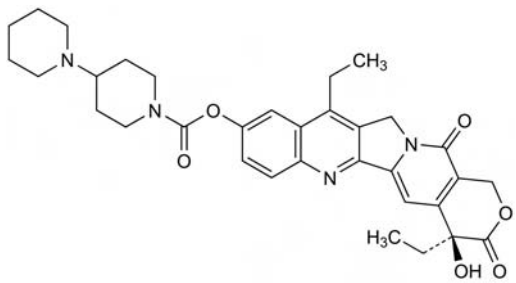


Chemical Properties Crystals. Mp 128° to 133°. It is practically insoluble in water. Log *P* (octanol/water), 3.95.

Irinotecan

Antineoplastic
$C_{33}H_{38}N_4O_6 = 586.7$
CAS—97682-44-5

Synonyms [1,4′-Bipiperidine]-1′-carboxylic acid (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl ester; CPT-11.



Chemical Properties Pale-yellow powder. Mp 222° to 223°.

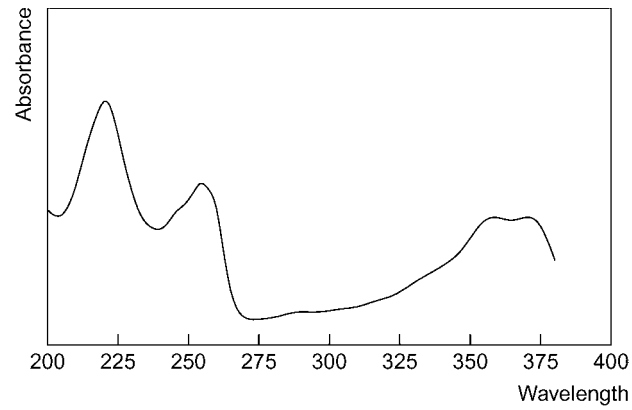
Irinotecan Hydrochloride
$C_{33}H_{39}ClN_4O_6 = 623.2$
CAS—100286-90-6

Irinotecan Hydrochloride Trihydrate
$C_{33}H_{38}N_4P_6$, HCl, $3H_2O = 677.2$
CAS—136572-09-3
Synonyms DQ-2805; U-101440E.
Proprietary Names *Campto*; *Camptosar*; *Topotectin*.
Chemical Properties A pale-yellow to yellow crystalline powder. Mp 256.5°. It is soluble in water and glacial acetic acid; partially soluble in chloroform; slightly soluble in methanol.

High Performance Liquid Chromatography Columns: TSK gel ODS-80Ts (150 × 4.6 mm i.d., 5 µm). Temperature: 30°. Mobile phase: acetonitrile : 50 mmol/L sodium hydrogen phosphate (28:72) containing sodium 1-heptanesulfonate, (pH 3.0), flow rate, 1 mL/min. IS: camptothecin. Fluorescence detection (λ_{ex}=380 nm, λ_{em}=556 nm). Retention time: irinotecan, 5.4 min; IS, 8.8 min [Sumiyoshi *et al.* 1995].

Column: C_{18} Nova-Pak (100 × 5 mm, 4 µm). Mobile phase: acetonitrile : 75 mmol/L ammonium acetate (pH 6.4) (22:78) containing 5 mmo/L tetrabutylammonium phosphate. Fluorescence detection (λ_{ex}=355 nm, λ_{em}=515 nm). Retention time: irinotecan (carboxylate form), 4.2 min; (lactone form), 8.2 min [Rivory, Robert 1994].

Ultraviolet Spectrum Aqueous acid—221, 254, 359, 372 nm.



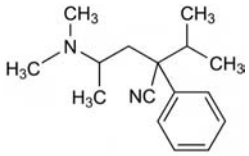
Quantification
Plasma **HPLC** Columns: C_{18} Zorbax SB (150 × 4.6 mm, 3.5 µm). Mobile phase: acetonitrile : 100 mmol/L ammonium acetate (pH 6.4) : triethylamine (15.6:80:0.1) containing 5 mmol/L tetrabutylammonium phosphate, flow rate, 1.5 mL/min. Fluorescence detection (λ_{ex}=375 nm, λ_{em}=460 nm). Retention time: irinotecan (carboxylate form), 5.7 min; (lactone form), 11.2 min. Limit of quantification,1.0 µg/L for irinotecan, 0.5 µg/L for the metabolite, SN-38 [Herben *et al.* 1998].
Urine **HPLC**Columns: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Temperature: 50°. Mobile phase: (A) methanol : 100 mmol/L ammonium acetate containing 10 mmol/L tetrabutylammonium sulfate (35:65), (pH 5.5); (B) (30:70), (pH 5.3), flow rate, 1 mL/min. Fluorescence detection (λ_{ex}=355 nm, λ_{em}=515 nm). Retention time: 8.3 min (A); 16.1 min (B) [de Bruijn *et al.* 1997]; [Sparreboom *et al.* 1998]. Limit of quantification, 100 µg/L [Sparreboom *et al.* 1998].
Faeces **HPLC** See Urine [Sparreboom *et al.* 1998].
Disposition in the Body After IV administration, irinotecan is metabolised, by carboxyesterase, in the liver to the active metabolite 7-ethyl-10-hydroxycamptothecin, SN-38 and carboxylic acid. SN-38 then undergoes conjugation, by UDP-glucuronyltransferase, to SN-38 glucuronide. Another metabolite 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamphothecin (APC) is produced by oxidative attack at the piperidine group. About 20% of the dose is excreted in urine within 24 h, <1% as SN-38 and ≈3 to 6% as SN-38 glucuronide. Further excretion is via bile.
Therapeutic Concentration
In phase I clinical studies, three patients were administered a dose of 100 mg/m^2, over 30 min, and peak plasma concentrations reached ≈2800 µg/L by the end of infusion. Peak concentrations of the metabolite, SN-38, reached 35.5 ± 10.9 µg/L [Barilero *et al.* 1992].
Toxicity Diarrhoea if prolonged and over 24 h can be life threatening.
Half-life 6 to 12 h; 10 to 20 h for the metabolite SN-38.
Volume of Distribution About 157 L/m^2.
Clearance Body clearance, 15 L/m^2/h.
Protein Binding Irinotecan is ~65% (range between 30 and 68%) bound, SN-38 metabolite 95%.
Dose The usual dosage range is 40 mg/m^2 body surface to 250 mg/m^2 administered three times a week, once a week to once every 3 weeks. One suggested regimen is 125 mg/m^2 infused over 90 min, once a week for 4 weeks, followed by 2-week rest period. Dose is modified according to toxicity. The maximum dose is 750 mg/m^2/day.

Barilero I *et al.* (1992). Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in cancer patients. *J Chromatogr* 575(2): 275–280.
de Bruijn P *et al.* (1997). Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 698: 277–285.
Herben VMM *et al.* (1998). *J Liq Chromatogr Relat Technol* 21: 1541–1558.
Rivory LP, Robert J (1994). Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. *J Chromatogr B Biomed Appl* 661 (1): 133–141.
Sparreboom A *et al.* (1998). Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces. *J Chromatogr B Biomed Sci Appl* 712: 225–235.
Sumiyoshi H *et al.* (1995). High-performance liquid chromatographic determination of irinotecan (CPT-11) and its active metabolite (SN-38) in human plasma. *J Chromatogr B Biomed Appl* 670 (2): 309–316.

Isoaminile

Cough Suppressant
$C_{16}H_{24}N_2 = 244.4$
CAS—77-51-0

IUPAC Name 4-(Dimethylamino)-2-phenyl-2-propan-2-ylpentanenitrile
Synonym α-[2-(Dimethylamino)propyl]-α-(1-methylethyl)benzeneacetonitrile

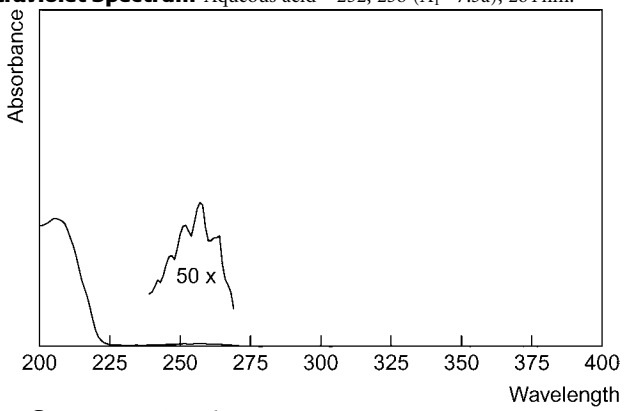


Chemical Properties A liquid. Log *P* (octanol/water), 3.5.

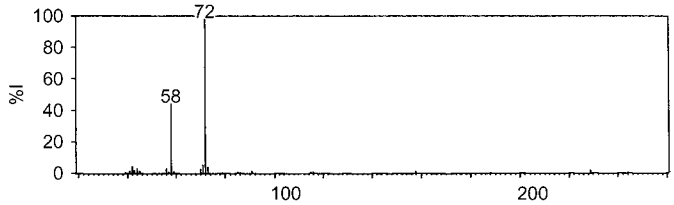
Isoaminile Citrate

$C_{16}H_{24}N_2$, $C_6H_8O_7$ = 436.5
CAS—28416-66-2
Proprietary Names *Dimyril*; *Perocan* (citrate or cyclamate).
Chemical Properties Crystals. Mp 63° to 64°. Soluble in water.

Colour Tests Liebermann's reagent—red-orange; sodium picrate (Steyn test)—orange.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.58; system TC—R_f 0.54; system TE—R_f 0.81; system TL—R_f 0.55; system TAE—R_f 0.45.
Gas Chromatography System GA—RI 1830.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=7.3a), 264 nm.



Mass Spectrum Principal ions at *m/z* 72, 58, 71, 42, 73, 70, 56, 44.

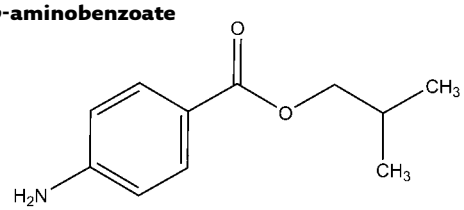


Dose 40 mg of isoaminile citrate 3 to 5 times daily.

Isobutyl Aminobenzoate

Anaesthetic (Local)
$C_{11}H_{15}NO_2$ = 193.2
IUPAC Name 2-Methylpropyl 4-aminobenzoate
Synonym Isobutylcaine
Proprietary Names *Cyclocaine*; *Cycloform*; *Cyclogesin*.

Isobutyl *p*-aminobenzoate

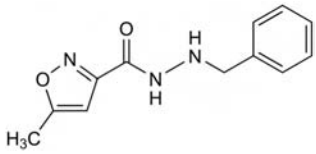


Chemical Properties White scales or a white crystalline powder. Mp 65°. Slightly soluble in water; soluble in ethanol and ether. Isobutyl aminobenzoate is extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Diazotization test—red (limit of detection, 1.0 µg); *p*-dimethylaminobenzaldehyde test—bright yellow (limit of detection, 0.1 µg); Vitali's test—yellow-brown/bright yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.77 (location reagents: diazotisation followed by alkaline betanaphthol spray, red; p-dimethylaminobenzaldehyde spray, positive reaction).
Ultraviolet Spectrum Ethanol—maxima at 223 nm (A_1^1 371) and 294 nm (A_1^1 1150).
Infrared Spectrum Principal peaks at wavenumbers A 1278, B 1172, C 1109 cm^{-1}; or A 1607, B 1683, C 1313 cm^{-1} (KBr disk).

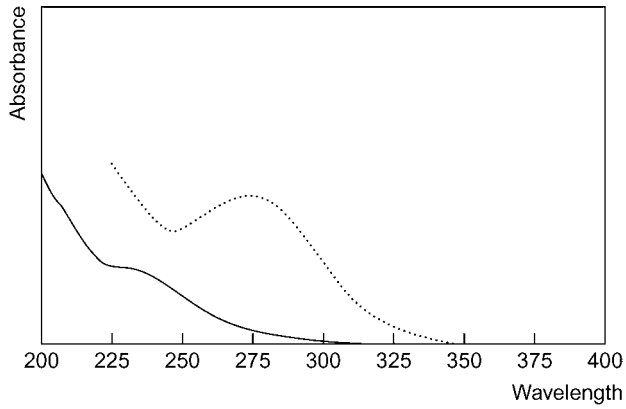
Isocarboxazid

Antidepressant
$C_{12}H_{13}N_3O_2$ = 231.3
CAS—59-63-2

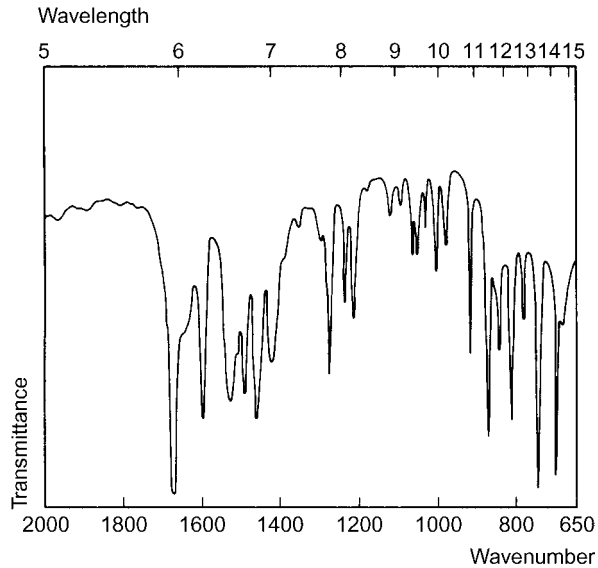
IUPAC Name *N′*-Benzyl-5-methyl-1,2-oxazole-3-carbohydrazide
Synonym 5-Methyl-3-isoxazolecarboxylic acid 2-benzylhydrazide
Proprietary Name *Marplan*



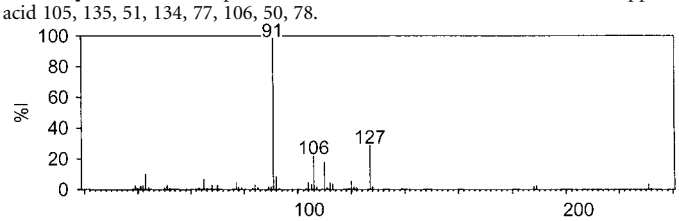
Chemical Properties A white or creamy-white crystalline powder. Mp 105° to 108°. Very sparingly soluble in hot water; soluble 1 in 150 of ethanol, 1 in 3 of chloroform, and 1 in 50 of ether. pK_a 10.4. Log *P* (octanol/pH 7.4), 1.5.
Colour Tests Liebermann's reagent—red-orange; Nessler's reagent—black
Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.20; system TC—R_f 0.74; system TE—R_f 0.75; system TL—R_f 0.61; system TAE—R_f 0.84; system TAF—R_f 0.86; system TAJ—R_f 0.67; system TAK—R_f 0.67; system TAL—R_f 0.92 (Dragendorff spray, positive; FPN reagent, pink-yellow; acidified iodoplatinate solution, positive; Marquis reagent, brown; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1949.
High Performance Liquid Chromatography System HX—RI 392; system HY—RI 353.
Ultraviolet Spectrum Aqueous alkali—274 nm (A_1^1=240b).



Infrared Spectrum Principal peaks at wavenumbers 1670, 750, 705, 870, 815, 1595 cm^{-1} (KBr disk).



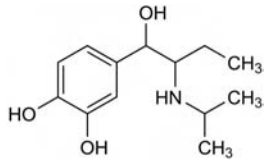
Mass Spectrum Principal ions at *m/z* 91, 127, 106, 110, 43, 92, 65, 120; hippuric acid 105, 135, 51, 134, 77, 106, 50, 78.



Disposition in the Body Rapidly absorbed after oral administration. Metabolised by oxidation to benzoic acid followed by glycine conjugation to form hippuric acid; an active metabolite, benzhydrazine, has been detected in animal studies, but its formation in humans has not been confirmed. About 60% of a dose is excreted in the urine in 24 h and about 70% in 8 days; 90% of the excreted material is hippuric acid and about 2% is unchanged drug.
Therapeutic Concentration Peak plasma concentrations are attained about 4 h after oral administration. Maximal therapeutic effects are attained within 5 to 10 days after beginning treatment.
Toxicity Toxic reactions from overdosage may occur in hours despite the long delay in onset of a therapeutic response. A fatality has been reported 24 h after ingestion of 400 mg.
Half-life Plasma half-life, about 36 h.
Dose 10 to 30 mg daily.

Isoetarine

Sympathomimetic
$C_{13}H_{21}NO_3 = 239.3$
CAS—530-08-5
IUPAC Name 4-[1-Hydroxy-2-[(1-methylethyl)amino]butyl]-1,2-benzenediol
Synonyms *N*-Isopropylethylnoradrenaline; isoetharine; Win-3046.
Proprietary Names *Dilabron*; *Neoisoprel*.

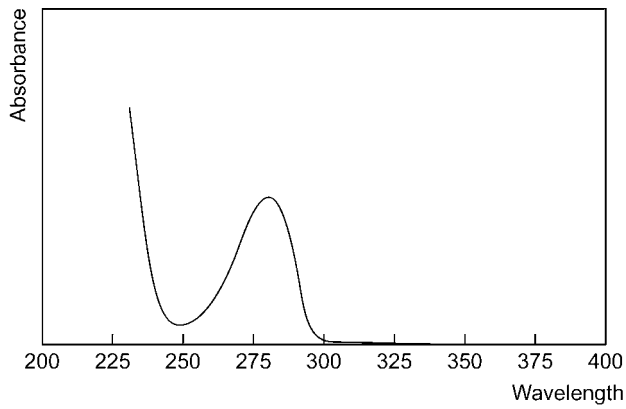


Chemical Properties Log *P* (octanol/water), 1.4.

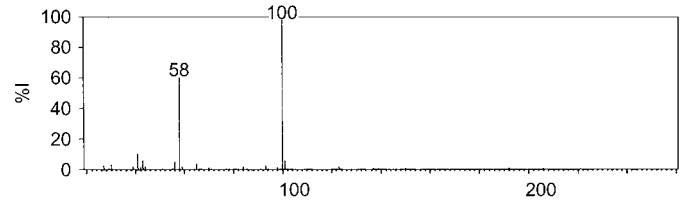
Isoetarine Hydrochloride
$C_{13}H_{21}NO_3$, HCl = 275.8
CAS—2576-92-3
Synonym Etyprenalinum hydrochloridum
Proprietary Names *Asthmalitan Depot*; *Beta-2*; *Bronkosol*; *Numotac*.
Chemical Properties A white crystalline solid. Mp 196° to 208°, with decomposition. Soluble in water; sparingly soluble in ethanol; practically insoluble in ether.

Isoetarine Mesilate
$C_{13}H_{21}NO_3$, $CH_4O_3S = 335.4$
CAS—7279-75-6
Synonym Isoetarine methanesulfonate
Proprietary Name *Bronkometer*
Chemical Properties White crystals. Mp 162° to 168°. Freely soluble in water; soluble in ethanol; practically insoluble in acetone and ether.

Colour Tests Ammoniacal silver nitrate—red/brown-orange; ferric chloride—green; Folin–Ciocalteu reagent—blue; Mandelin's test—brown; Marquis test—yellow→orange; methanolic potassium hydroxide—red→orange→yellow; Nessler's reagent—black; palladium chloride—orange→brown; potassium dichromate (method 1)—green→brown (30 s).
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.36; system TL—R_f 0.00; system TAE—R_f 0.73; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.26 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HX—RI 222.
Ultraviolet Spectrum Aqueous acid—278 nm (A^1_1=132a).



Infrared Spectrum Principal peaks at wavenumbers 1200, 1287, 1075, 1114, 1518, 1160 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 100, 58, 41, 101, 43, 56, 65, 30.

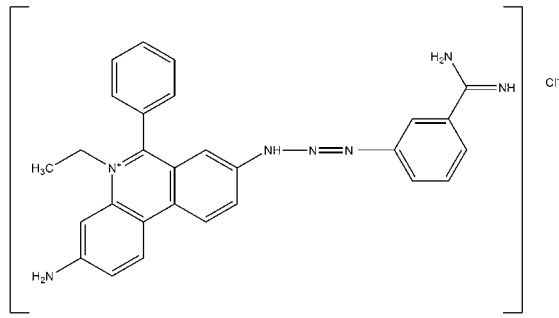


Quantification
Plasma **HPLC** Electrochemical detection. Limit of detection, 500 µg/L [Park *et al.* 1982].
Dose 30 to 80 mg of isoetarine hydrochloride daily.

Park GB *et al.* (1982). Determination of isoetharine in plasma by reversed-phase chromatography with amperometric detection. *J Pharm Sci* 71: 932–934.

Isometamidium

Trypanocide (Veterinary)
$C_{28}H_{26}ClN_7 = 496.0$
IUPAC Name 3-[2-(3-Amino-5-ethyl-6-phenylphenanthridin-5-ium-8-yl)iminohydrazinyl] benzenecarboximidamide
Synonym 8-(3-*m*-Amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridinium chloride [Also contains 2 impurities, (A) 3,8-di(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride and (B) 2-,4-,7-, or 9-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride].

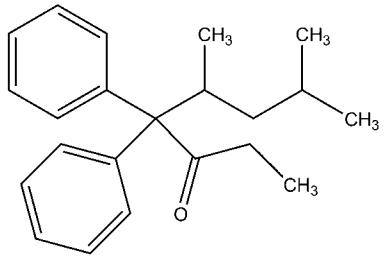


Isometamidium Hydrochloride
Synonyms B-4180A; M-4180A.
Proprietary Name *Samorin*
Chemical Properties Dark-red crystals. Mp 244° to 245°. Soluble in water and dilute acetic acid. Isometamidium is a quaternary ammonium compound, but can be extracted by ether from aqueous alkaline solutions.

Colour Tests Ammonium molybdate test—(dark brown) yellow-brown→purple (limit of detection, 0.1 µg); ammonium vanadate test—(dark brown) yellow-brown→grey-green, developing slowly (limit of detection, 0.1 µg); sulfuric acid test—(dark brown) yellow-brown→red-purple (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—(dark brown) orange→red-purple (limit of detection, 0.1 µg); Vitali's test—(dark brown) purple-brown/brown/brown (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.05, visible brown streak (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.
Ultraviolet Spectrum Methanol—284 (A^1_1 470) and 317 nm (A^1_1 510), minima at 262, 295 and 348 nm; 0.1 N hydrochloric acid—243 (A^1_1 550), 252 (A^1_1 520) and 280 nm (A^1_1 450), minima at 231, 248 and 270 nm.

Isomethadone

Narcotic
$C_{21}H_{27}NO = 309.5$
CAS—466-40-01
IUPAC Name 6-Dimethylamino-5-methyl-4,4-di(phenyl)hexan-3-one
Synonyms 6-Dimethylamino-4,4-diphenyl-5-methyl-3-hexanone; 1-dimethylamino-2-methyl-3,3-diphenyl-4-hexanone; 6-(dimethylamino)-5-methyl-4,4-diphenyl-3-hexanone; isoamidone.
Proprietary Name Combined with iopydol, it is an ingredient of *Hytrast*.



Chemical Properties Isomethadone occurs as an oil. Isomethadone is extracted by organic solvents from aqueous alkaline solutions.

Isomethadone (*dl*-Form)
CAS—116836-09-0
Chemical Properties Slightly yellow, very viscous liquid. Bp 215°.

Isomethadone Hydrobromide (*dl*-Form)
$C_{21}H_{27}NO$, HBr = 390.4
Chemical Properties Crystals from water. Mp 149° to 150°.

Isomethadone (*d*-Form)
CAS—26594-41-2
Chemical Properties Oil.

Isomethadone Hydrochloride (*d*-Form)
$C_{21}H_{27}NO$, HCl = 345.9
CAS—63814-06-2
Chemical Properties Mp 231° to 232°.

Isomethadone Hydrochloride Monohydrate (*d*-Form)
$C_{21}H_{27}NO$, HCl, H_2O = 363.9
Chemical Properties Mp 176° to 177°.

Isomethadone (*l*-Form)
CAS—561-10-4
Chemical Properties Oily liquid. Bp 162° to 165°.

Isomethadone Hydrobromide (*l*-Form)
Chemical Properties Crystals. Mp 217° to 218°.

Isomethadone Hydrochloride (*l*-Form)
CAS—7487-81-2
Chemical Properties Mp 231° to 233°. Soluble in water and alcohol. Solutions and tablets are stable.

Isomethadone Hydrochloride Monohydrate (*l*-Form)
Chemical Properties Mp 173° to 174°.

Colour Tests Ammonium vandate test—brown-purple→violet-blue (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.61 (location reagent acidified iodoplatinate spray, positive reaction).

Plates: Silica Gel G (20 × 20 cm, 0.25 mm). Solvent system A: chloroform : acetone : diethylamine (88 : 2 : 10). Solvent system B: benzene : methanol : diethylamine (75 : 15 : 10). Solvent system C: methanol : benzene : *n*-butanol : ammonia (0.88) : water (60 : 10 : 15 : 5 : 10). R_f values were reported as follows:

Solvent system	*Isomethadone*	*Isomethadone cyclic metabolite*	*Isomethadone N-oxide*
A	0.70	0.68	0.07
B	0.71	0.68	0.35
C	0.91	0.17	0.79

[a][Beckett *et al.* 1971].

Gas-Liquid Chromatography Column: Gas Chrom Q OV17 (A; 2 m × 1/4 in o.d.) or Chromosorb G (B; 1 m × 1/8 in o.d.). Carrier gas: N_2, 65 mL/min for column A, 36 mL/min for column B. Temperature programme: 195° for column A and 180° for column B. Retention times were reported as follows:

Column	*Unchanged drug*	*Cyclic metabolite*
A	12.2	10.4
B	11.4	9.2

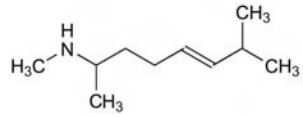
[a][Beckett *et al.* 1971].

Ultraviolet Spectrum Ethanol—259, 265, 298 nm.

Beckett AH *et al.* (1971). Identification and quantitative determination of some metabolites of methadone, isomethadone and normethadone. *J Pharm Pharmacol* 23: 347–352.

Isometheptene

Sympathomimetic
$C_9H_{19}N$ = 141.3
CAS—503-01-5
IUPAC Name *N*,6-Dimethyl-5-hepten-2-amine
Synonym Methyloctenylamine
Proprietary Names *Octin*; *Octon*; *Octanil*.

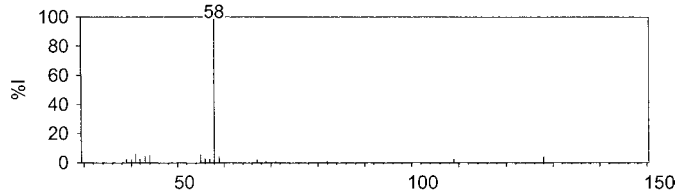


Chemical Properties A colourless or slightly yellow oily liquid. Bp 176° to 178°. Practically insoluble in water; freely soluble in acetone, ethanol, chloroform and ether. Log *P* (octanol/water), 3.0.

Isometheptene Hydrochloride
$C_9H_{19}N$, HCl = 177.7
CAS—6168-86-1
Proprietary Name *Octinum (injection)*
Chemical Properties An almost white, very hygroscopic, crystalline powder. Mp 68° to 69°. Soluble in water and ethanol.

Isometheptene Mucate
$(C_9H_{19}N)_2$, $C_6H_{10}O_8$ = 492.7
CAS—7492-31-1
Synonym Isometheptene galactarate
Proprietary Names *Octinum (tablets)*. It is an ingredient of *Midrid*.
Chemical Properties A white crystalline powder. Mp about 152°. Freely soluble in water; soluble in ethanol; almost insoluble in chloroform and ether.

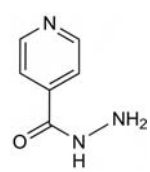
Colour Test Mandelin's test—brown.
Thin-layer Chromatography System TA—R_f 0.24; system TAJ—R_f 0.00; system TAK—R_f 0.04; system TAL—R_f 0.43 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1052; system GB—RI 994.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Mass Spectrum Principal ions at *m/z* 58, 55, 41, 44, 43, 128, 59, 56.



Dose Isometheptene mucate has been given in doses of up to 520 mg daily.

Isoniazid

Tuberculostatic
$C_6H_7N_3O$ = 137.1
CAS—54-85-3
IUPAC Name 4-Pyridinecarboxylic acid hydrazide
Synonyms FSR-3; INAH; INH; isonicotinic acid hydrazide; isonicotinoylhydrazine; isonicotinylhydrazide; isonicotinylhydrazine; RP-5015; Tubazid.
Proprietary Names *Cemidon*; *Isotamine*; *Isotinyl*; *Laniazid*; *Neoteben*; *Nicizina*; *Niconyl*; *Nicotibine*; *Nicozid*; *Nydrazid*; *Panazid*; *Rimifon*; *Tebesium*; *Teebaconin*; *Trisofort*; *Tubilysin*. It is an ingredient of *Mynah*, *Rifinah*, *Rimactazid*, *Rimafate* and *Rifater*.

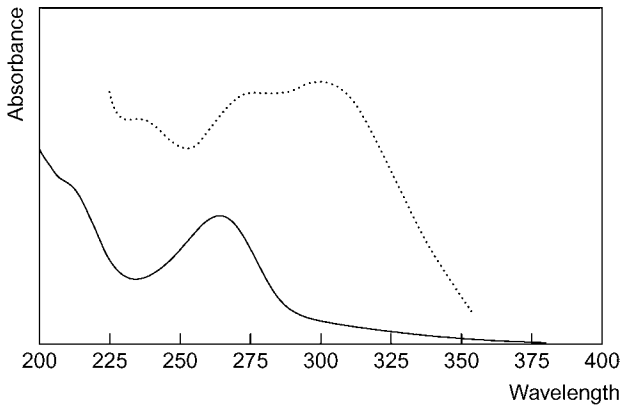


Chemical Properties Colourless crystals or white crystalline powder. Mp 170° to 174°. Soluble 1 in 8 of water, 1 in about 45 of ethanol and 1 in 1000 of chloroform; practically insoluble in benzene and ether. pK_a 1.8, 3.5, 10.8 (20°). Log *P* (octanol/pH 7.4), −1.1.

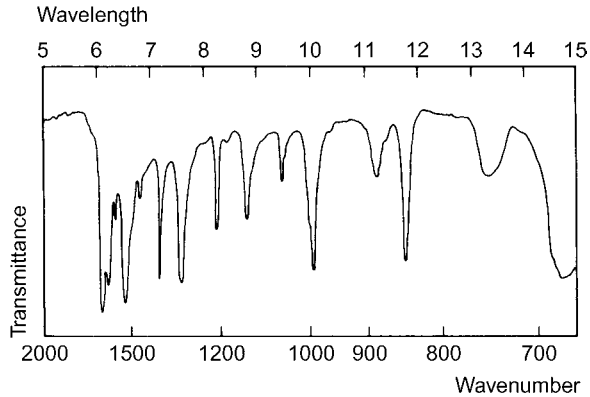
Isoniazid Aminosalicylate
$C_6H_7N_3O$, $C_7H_7NO_3$ = 290.3
CAS—2066-89-9

Synonyms GEWO-399; pasiniazid.
Proprietary Names *Dipasic*; *Paraniazide*.
Chemical Properties Yellow crystals. Mp 140° to 142°. Sparingly soluble in water.

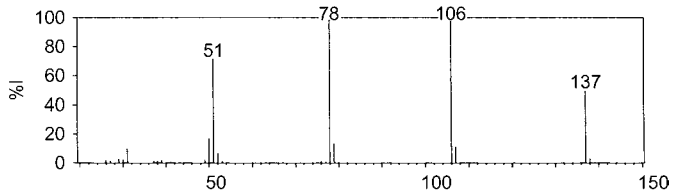
Colour Tests Cyanogen bromide—orange; Nessler's reagent—black.
Thin-layer Chromatography System TA—R_f 0.47; system TAD—R_f 0.18; system TAE—R_f 0.55; system TAF—R_f 0.49; system TL—R_f 0.20; system TB—R_f 0.01; system TC—R_f 0.11; system TE—R_f 0.29; system TAJ—R_f 0.09; system TAK—R_f 0.03; system TAL—R_f 0.47 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1670.
High Performance Liquid Chromatography System HY—RI 246; system HZ—retention time 1.6 min.
Ultraviolet Spectrum Aqueous acid—266 nm (A^1_1=390a); aqueous alkali—298 nm.



Infrared Spectrum Principal peaks at wavenumbers 1653, 1541, 1621, 676, 992, 845 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 106, 78, 51, 137, 50, 79, 107, 31; isonicotinic acid 123, 51, 78, 106, 50, 52, 105, 39.



Quantification

Blood **HPLC** For method for quantification of isoniazid and other antitubercular drugs, see Khuhawar and Rind [2002].

Plasma **GC–MS** Limit of detection, 100 µg/L for isoniazid and acetylisoniazid, 50 µg/L for hydrazine metabolites [Lauterburg *et al.* 1981].

HPLC UV detection. For method for quantification of isoniazid and acetylisoniazid, see Moussa *et al.* [2002]. For method for quantification of isoniazid, rifampicin and pyrazinamide, see Smith *et al.* [1999]. Coulometric detection. For method, see Delahunty *et al.* [1998]. For method for quantification of isoniazid and other antitubercular drugs, see Walubo *et al.* [1994]. UV detection. Limit of detection, 100 µg/L for isoniazid and acetylisoniazid [Holdiness 1982].

Spectrofluorimetry For method for quantification of isoniazid and acetylisoniazid, see Olson *et al.* [1977].

Serum **HPLC** Limit of detection, <82 µg/L for isoniazid and other antitubercular drugs [Gennaro *et al.* 2001]. UV detection. For method, see Sadeg *et al.* [1996].

Urine **GC** AFID. Limit of detection, 2 mg/L for isoniazid and acetylisoniazid, 400 µg/L for hydrazine, monoacetylhydrazine and diacetylhydrazine [Timbrell *et al.* 1977].

HPLC Fluorimetric detection. Limit of detection, 0.2 mg/L [Kohno *et al.* 1991].

Biological Fluids **HPLC** UV detection. Isoniazid, acetylisoniazid, and hydrazine. Limit of detection, <1 mg/L for isoniazid [Seifart *et al.* 1995].

Bronchoalveolar Lavage **HPLC** See Plasma [Delahunty *et al.* 1998].

Alveolar Cells **HPLC** See Plasma [Delahunty *et al.* 1998].

Disposition in the Body Isoniazid is readily absorbed after oral administration. The main metabolic reaction is acetylation and the rate at which this occurs shows genetic variation, ≈40% of the population being rapid acetylators and the remainder slow acetylators. Other metabolic reactions which occur are hydrolysis, glycine conjugation, hydrazone formation and *N*-methylation. Metabolites include acetylisoniazid, isonicotinic acid, isonicotinylglycine and mono- and diacetylhydrazine. All the metabolites are inactive with the exception of monoacetylhydrazine, which is a reactive metabolite and is hepatotoxic. Up to about 70% of a dose is excreted in the urine in 24 h, most being excreted in the first 12 h. In slow acetylators, about 30% of the dose is excreted in the urine as unchanged drug, up to about 25% as acetylisoniazid, about 10% as isonicotinic acid and about 8% as diacetylhydrazine. In rapid acetylators, about 10% of a dose is excreted as unchanged drug, up to 45% as acetylisoniazid, about 20% as isonicotinic acid and about 25% as diacetylhydrazine. Small amounts of monoacetylhydrazine are also excreted in the urine. Less than 10% of a dose is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 3 to 10 mg/L.

Following a single oral dose of 300 mg to 6 fasting subjects classified as slow acetylators, mean peak serum concentrations of 7.2 mg/L of isoniazid and 1.7 mg/L of acetylisoniazid were attained in 0.6 and 3.7 h, respectively. Following a single oral dose of 300 mg given to 4 rapid acetylators, mean peak serum concentrations of 7.2 mg/L of isoniazid and 4.5 mg/L of acetylisoniazid were attained in 0.4 h and 2.6 h, respectively [Männistö *et al.* 1982].

In 94 subjects, aged 1 to 13 years, with tuberculosis given rifampicin and isoniazid at a dose of 12 and 10 mg/kg/day, respectively, for 10 to 12 days, mean serum-rifampicin concentrations of 3.38 to 3.88 mg/L were achieved in 2 h and mean serum-isoniazid concentrations of 4.38 to 8.17 mg/L were achieved in 1 h; concentrations of both drugs were still much above those required for therapeutic efficacy at 7 to 8 h [Seth *et al.* 1993].

Toxicity Serious toxic symptoms may occur with doses of 3 g or more. In children, toxic effects have occurred after ingestion of 0.9 g or more but a child has recovered after taking as much as 20 g. Toxic effects have been associated with plasma concentrations >20 mg/L.

A 16-year-old girl who had ingested an unknown quantity of isoniazid had a serum concentration of 127 mg/L on admission to hospital and died 7 h later. Two children aged $1^1/_2$ and $2^1/_2$ years had serum concentrations of 103 mg/L and 35 mg/L, respectively, on admission to hospital following ingestion of isoniazid and subsequently recovered [Miller *et al.* 1980].

In a fatality due to the ingestion of isoniazid, the tissue distribution was as follows: heart blood 43 mg/L, subclavian blood 94 mg/L, urine 470 mg/L, bile 900 mg/L, liver 650 µg/g, kidney 110 µg/g, stomach contents 4 mg [LoDico *et al.* 1992].

A 7-year-old boy who ingested 3 g (125 mg/kg) of isoniazid had blood levels of 250 mg/L ~6 h later (gastric content 450 mg/L and urine 760 mg/L); haemodialysis resulted in recovery [Orlowski *et al.* 1988].

Bioavailability About 80%.

Half-life Plasma half-life, about 1 h in rapid acetylators and 3 to 5 h in slow acetylators.

Volume of Distribution About 0.8 L/kg.

Clearance Plasma clearance, about 2.5 and about 7 mL/min/kg in slow and rapid acetylators, respectively.

Protein Binding Not significantly bound.

Note For a review of the pharmacokinetics of isoniazid, see Weber and Hein [1979]. For a review of isoniazid overdose, see Romero and Kuczler [1998].

Dose Usually 4 to 5 mg/kg daily, orally, to a maximum of 300 mg daily.

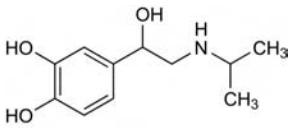
Delahunty T *et al.* (1998). Sensitive liquid chromatographic technique to measure isoniazid in alveolar cells, bronchoalveolar lavage and plasma in HIV-infected patients. *J Chromatogr B Biomed Sci Appl* 705: 323–329.
Gennaro MC *et al.* (2001). Ion interaction reagent reversed-phase high-performance liquid chromatography determination of anti-tuberculosis drugs and metabolites in biological fluids. *J Chromatogr B Biomed Sci Appl* 754: 477–486.
Holdiness MR (1982). *J Liq Chromatogr* 5: 707–714.
Khuhawar MY, Rind FM (2002). Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 357–363.
Kohno H *et al.* (1991). Fluorometric determination of isoniazid and its metabolites in urine by high-performance liquid chromatography using in-line derivatization. *Ther Drug Monit* 13: 428–432.
Lauterburg BH *et al.* (1981). *J Chromatogr* 224: 431–438.
LoDico CP *et al.* (1992). Distribution of isoniazid in an overdose death. *J Anal Toxicol* 16(1): 57–59.
Männistö P *et al.* (1982). Influence of various diets on the bioavailability of isoniazid. *J Antimicrob Chemother* 10: 427–434.
Miller J *et al.* (1980). *Am J Dis Childh* 134: 290–292.
Moussa LA *et al.* (2002). Therapeutic isoniazid monitoring using a simple high-performance liquid chromatographic method with ultraviolet detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 181–187.
Olson WA *et al.* (1977). Spectrophotofluorometric assay for isoniazid and acetyl isoniazid in plasma adapted to pediatric studies. *Clin Chem* 23: 745–748.
Orlowski JP *et al.* (1988). Treatment of a potentially lethal dose isoniazid ingestion. *Ann Emerg Med* 17: 73–76.
Romero JA, Kuczler FJ (1998). Isoniazid overdose: recognition and management. *Am Fam Physician* 57: 749–752.
Sadeg N *et al.* (1996). Rapid, specific and sensitive method for isoniazid determination in serum. *J Chromatogr B Biomed Appl* 675: 113–117.
Seifart HI *et al.* (1995). High-performance liquid chromatographic determination of isoniazid, acetylisoniazid and hydrazine in biological fluids. *J Chromatogr B Biomed Appl* 674: 269–275.

Seth V *et al.* (1993). Serum concentrations of rifampicin and isoniazid in tuberculosis. *Indian Pediatr* 30: 1091–1098.
Smith PJ *et al.* (1999). Determination of rifampicin, isoniazid and pyrazinamide by high performance liquid chromatography after their simultaneous extraction from plasma. *Int J Tuberc Lung Dis* 3(11): 3S325–S328,S351–S352.
Timbrell JA *et al.* (1977). Determination of hydrazine metabolites of isoniazid in human urine by gas chromatography. *J Chromatogr* 138: 165–172.
Walubo A *et al.* (1994). Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography. *J Chromatogr B Biomed Appl* 658: 391–396.
Weber WW, Hein DW (1979). Clinical pharmacokinetics of isoniazid. *Clin Pharmacokinet* 4: 401–422.

Isoprenaline

Sympathomimetic
$C_{11}H_{17}NO_3 = 211.3$
CAS—7683-59-2

IUPAC Name 4-[1-Hydroxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol
Synonyms A-21; isopropylarterenol; isopropylnoradrenaline; isoproterenol.
Proprietary Names *Aludrine*; *Asmalar*; *Bellasthman*; *Isoprel*; *Novodrin*; *Respifral*; *Saventrine*.



Chemical Properties Crystals. Mp 155°. pK_a 8.6, 10.1, 12.0 (20°). Log *P* (octanol/water), 0.2.

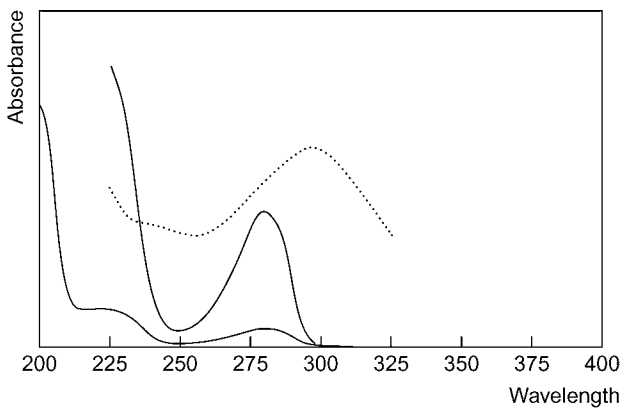
Isoprenaline Hydrochloride

$C_{11}H_{17}NO_3$, HCl = 247.7
CAS—51-30-9
Synonym Isoproterenol hydrochloride
Proprietary Names *Aerolone*; *Imuprel*; *Isuprel*; *Lenoprel*; *Proternol*; *Saventrine*.
Chemical Properties A white crystalline powder. Gradually darkens on exposure to air and light. Mp 165° to 170°, with decomposition. Soluble 1 in less than 1 of water and 1 in about 50 of ethanol; practically insoluble in chloroform and ether. Aqueous solutions become pink to brownish-pink on standing exposed to air, and almost immediately so when made alkaline.

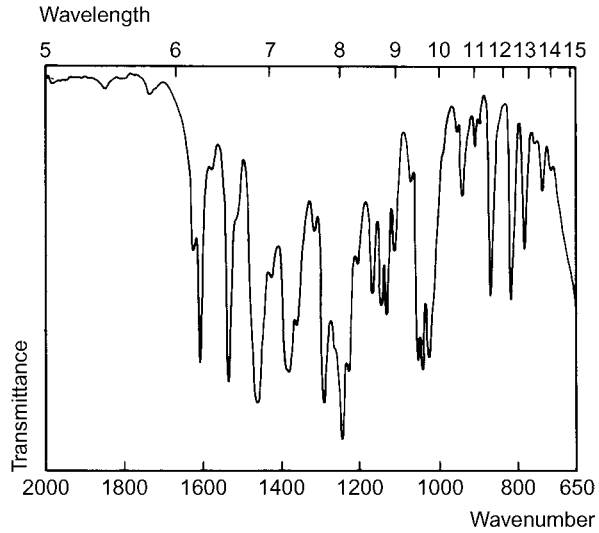
Isoprenaline Sulfate

$(C_{11}H_{17}NO_3)_2$, H_2SO_4, $2H_2O$ = 556.6
CAS—299-95-6 (anhydrous); 6700-39-6 (dihydrate)
Synonym Isoproterenol sulfate
Proprietary Names *Aleudrina*; *Ingelan*; *Kattwilon N*; *Medihaler Iso*; *Norisodrine*.
Chemical Properties A white crystalline powder. Gradually darkens on exposure to light and air. Mp about 128°, with decomposition. Soluble 1 in 4 of water; slightly soluble in ethanol; practically insoluble in benzene, chloroform and ether. Aqueous solutions become pink to brownish-pink on standing exposed to air, and almost immediately so when made alkaline.

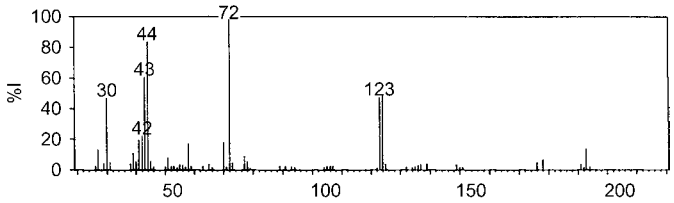
Colour Tests Ammoniacal silver nitrate—red→red-brown/brown; ferric chloride—green; Folin–Ciocalteu reagent—blue; Mandelin's test—brown; Marquis test—brown→violet; methanolic potassium hydroxide—orange→yellow; Nessler's reagent—black; potassium dichromate—green→brown (30 s).
Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.21; system TL—R_f 0.03; system TAE—R_f 0.14; system TAF—R_f 0.69 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1730.
High Performance Liquid Chromatography System HX—RI 79.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=134a); aqueous alkali—297 nm.



Infrared Spectrum Principal peaks at wavenumbers 1246, 1293, 1535, 1230, 1040, 1607 cm^{-1} (isoprenaline hydrochloride, KCl disk).



Mass Spectrum Principal ions at *m/z* 72, 44, 43, 124, 123, 30, 42, 41.



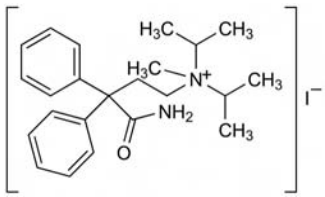
Quantification
***Plasma* HPLC** Electrochemical detection. Limit of detection, 1 µg/L [Causon *et al.* 1984]. Fluorescence detection. Limit of detection, 40 pg [Kishimoto *et al.* 1982].
***Urine* HPLC** See Plasma [Causon *et al.* 1984]. See Plasma [Kishimoto *et al.* 1982].
Disposition in the Body Isoprenaline is irregularly absorbed after oral or sublingual administration with extensive metabolism and conjugation occurring in the gut. Only about 10% of an inhaled dose reaches the lungs. It is rapidly metabolised by 3-*O*-methylation and sulfate conjugation; 3-*O*-methylisoprenaline exhibits weak activity. After IV administration, about 90% of a dose is excreted in the urine in 24 h, mostly as the conjugated 3-*O*-methyl metabolite, with up to about 15% of the dose as unchanged drug; after inhalation or oral administration, 68 to 94% of a dose is excreted as conjugated isoprenaline, with about 2 to 8% of a dose as the 3-*O*-methyl conjugate and <5% as unchanged drug or free 3-*O*-methylisoprenaline; small amounts of a dose are excreted in the bile, mainly as metabolites.
Therapeutic Concentration
After a dose of 500 µg administered by aerosol inhalation, plasma concentrations of about 0.0003 mg/L were attained in 5 min in 2 subjects [Blackwell *et al.* 1970].
After an oral dose of 0.2 mg/kg, plasma concentrations of about 0.5 mg/L of isoprenaline plus metabolites were attained within 90 min; in blood the major component was the sulfate ester together with small amounts of the methylated metabolite [Conolly *et al.* 1972].
Toxicity Estimated minimum lethal dose for children, applied to mucous membranes, 100 mg.
Half-life Plasma half-life, 3 to 7 h.
Volume of Distribution About 0.5 L/kg.
Protein Binding About 68%.
Dose 90 to 840 mg of isoprenaline hydrochloride daily, orally, as sustained-release tablets.

Blackwell EW *et al.* (1970). The fate of isoprenaline administered by pressurized aerosols. *Br J Pharmacol* 39: 194P–195P.
Causon RC *et al.* (1984). Determination of d-isoproterenol sulphate by high-performance liquid chromatography with amperometric detection. *J Chromatogr* 306: 257–268.
Conolly ME *et al.* (1972). Metabolism of isoprenaline in dog and man. *Br J Pharmacol* 46: 458–472.
Kishimoto Y *et al.* (1982). Method for the simplified analysis of deproteinized plasma and urinary isoproterenol by high-performance liquid chromatography. *J Chromatogr* 231: 121–127.

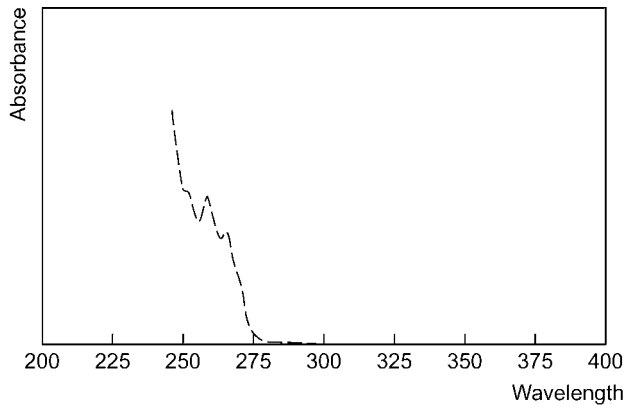
Isopropamide Iodide

Anticholinergic
$C_{23}H_{33}IN_2O = 480.4$
CAS—7492-32-2 (isopropamide); 71-81-8 (iodide)

IUPAC Name (4-Amino-4-oxo-3,3-diphenylbutyl)-methyl-di(propan-2-yl)azanium iodide
Synonym γ-(Aminocarbonyl)-*N*-methyl-*N*,*N*-bis(1-methylethyl)-γ-phenylbenzenepropanaminium iodide
Proprietary Names *Priamide*. It is an ingredient of *Combid*, *Ornade*, and *Stelabid*.



Chemical Properties A white to pale-yellow crystalline powder. Mp 198° to 201°, with decomposition. Soluble 1 in 50 of water, 1 in 10 of ethanol and 1 in 5 of chloroform; practically insoluble in ether. Log *P* (octanol/water), 1.2.
Colour Tests Aromaticity (method 2)—colourless/brown; Liebermann's reagent—grey.
Thin-layer Chromatography System TA—R_f 0.05; system TB—R_f 0.00; system TC—R_f 0.05; system TE—R_f 0.03; system TL—R_f 0.00; system TAE—R_f 0.03; system TAF—R_f 0.41; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.19 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2022 and RI 2670.
High Performance Liquid Chromatography System HA—*k* 2.4 (tailing peak); system HX—RI 379.
Ultraviolet Spectrum Methanol—259 (A^1_1=9.1a), 265 nm.

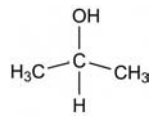


Infrared Spectrum Principal peaks at wavenumbers 1650, 1576, 705, 769, 757, 1500 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 114, 100, 44, 238, 115, 56, 72.
Dose The equivalent of 5 to 10 mg of isopropamide every 12 h.

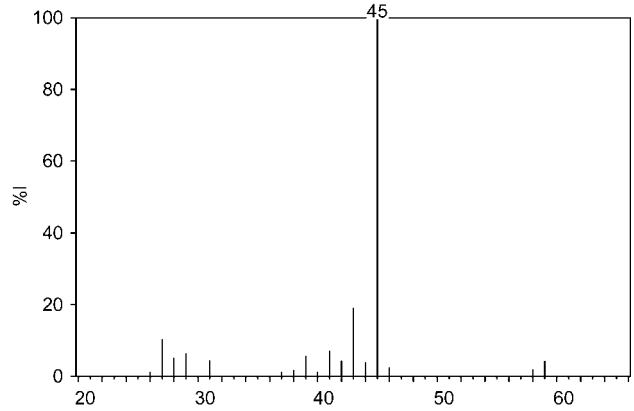
Isopropyl Alcohol

Solvent
C_3H_8O = 60.10
CAS—67-63-0

IUPAC Name 2-Propanol
Synonyms Alcohol isopropylicus; dimethyl carbinol; isopropanol; 2-propanol; secondary propyl alcohol.
Proprietary Names *Alcojel*; *Alcowipe*; *Alko Isol*; *Avitracid*; *Clearasil Daily Face*; *Duonale*; *Medi-Swab*; *Mundisept*; *Sterets*; *Steriwipe*.



Chemical Properties A clear, colourless, mobile, volatile, inflammable liquid. Macs per mL 0.784 to 0.786 g. Bp 81° to 83°. Refractive index, at 20°, 1.377 to 1.380. Miscible with water, ethanol, chloroform and ether. pK_a 17.1. Log *P* (octanol/water), 0.
Colour Test Potassium dichromate (method 2)—green.
Gas Chromatography System GA—RI 530; system GI—retention time 4.0 min.
Infrared Spectrum Principal peaks at wavenumbers 952, 1123, 1162, 819, 1111, 1298 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 45, 43, 27, 41, 39, 29, 59, 44; acetone 43, 58, 59, 27, 42, 26, 39, 29.



Quantification
Blood **GC** For method, see Daniel *et al.* [1981].
Serum **GC** Limits of detection, 10 mg/L to 25 mg/L, for isopropyl alcohol and other alcohols and glycols [Williams *et al.* 2000].
Disposition in the Body Isopropyl alcohol is readily absorbed after oral administration and slowly absorbed through intact skin. It is metabolised more slowly than ethanol; it is largely converted to acetone, which is slowly excreted from the lungs and in the urine; acetone may be further metabolised to acetate, formate and carbon dioxide; some unchanged isopropyl alcohol may be excreted in the urine together with its glucuronide conjugate, particularly after large doses.
Toxicity The estimated minimum lethal dose is 240 mL. Isopropyl alcohol is about twice as toxic as ethanol and the symptoms of intoxication are similar. Fatalities have been associated with blood concentrations >1000 mg/L. The maximum permissible atmospheric concentration is 400 ppm. A dose of 16 mL has been ingested daily for 3 days without discomfort, but marked depression has been observed following a dose of 23 mL. Recovery has followed treatment by haemodialysis in subjects with initial blood concentrations of up to 4400 mg/L of isopropyl alcohol.

In a review of 31 fatalities attributed solely to isopropyl alcohol poisoning, postmortem blood concentrations ranged from 100 to 2500 (mean, 1400) mg/L for isopropyl alcohol and 400 to 3000 (mean, 1700) mg/L for acetone [Alexander *et al.* 1982].

A woman who died about 3 h after ingesting isopropyl alcohol was reported to have the following postmortem tissue concentrations: isopropyl alcohol, blood 3300 mg/L, brain 1800 µg/g, urine 2000 mg/L; acetone, blood 1200 mg/L, brain 600 µg/g, urine 700 mg/L [Cravey 2000].

In 4 fatalities due to the ingestion of isopropyl alcohol, the following antemortem or postmortem tissue concentrations were reported: blood 200 to 2000 mg/L (mean 1300 mg/L, 4 cases), brain 1000 µg/g (1 case), liver 1000 µg/g (1 case), spleen 1300 µg/g (1 case), urine 1500 and 1800 mg/L (2 cases) [Adelson 1962].

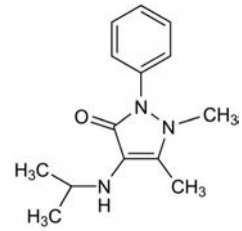
Half-life Blood half-life, about 3 h in subjects with acute intoxication.

Adelson L (1962). Fatal intoxication with isopropyl alcohol (rubbing alcohol). *Am J Clin Pathol* 38: 144–151.
Alexander CB *et al.* (1982). Isopropanol and isopropanol deaths-ten years' experience. *J Forensic Sci* 27: 541–548.
Cravey RH. Baselt RC, ed. (2000). *Disposition of Toxic Drugs and Chemicals in Man*, 5th edn. California: Biomedical Publications, 449–450.
Daniel DR *et al.* (1981). Isopropyl alcohol metabolism after acute intoxication in humans. *J Anal Toxicol* 5: 110–112.
Williams RH *et al.* (2000). Simultaneous detection and quantitation of diethylene glycol, ethylene glycol, and the toxic alcohols in serum using capillary column gas chromatography. *J Anal Toxicol* 24: 621–626.

Isopropylaminophenazone

Analgesic (Veterinary)
$C_{14}H_{19}N_3O$ = 245.3
CAS—3615-24-5

IUPAC Name 1,5-Dimethyl-2-phenyl-4-(propan-2-ylamino)pyrazol-3-one
Synonyms 1,2-Dihydro-1,5-dimethyl-4-[(1-methylethyl)amino]-2-phenyl-3*H*-pyrazol-3-one; isopyrin; ramifenazone.
Note The name Isopyrin has also been applied to isoniazid.
Proprietary Names It is an ingredient of *Tomanol*.



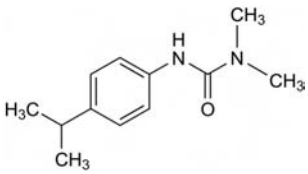
Chemical Properties Crystals. Mp 80°. Log *P* (octanol/water), 1.3.

Thin-layer Chromatography System TE—R_f 0.63; system TAE—R_f 0.78.
Gas Chromatography System GA—RI 2033.
Ultraviolet Spectrum Aqueous acid—260 nm (A_1^1=377b); aqueous alkali—271 nm (A_1^1=334b); methanol—243 (A_1^1=310b), 269 nm (A_1^1=381b).

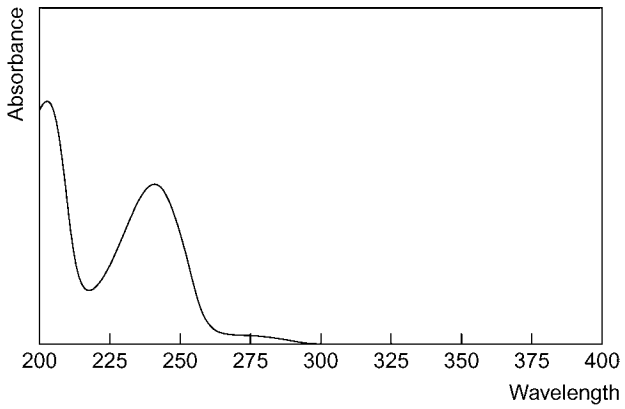
Isoproturon

Herbicide
$C_{12}H_{18}N_2O$ = 206.3
CAS—34123-59-6

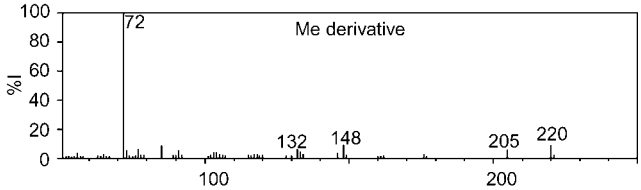
IUPAC Name *N*,*N*-dimethyl-*N*′-[4-(1-methylethyl)phenyl]urea
Synonyms CGA-18731; DPX 6774; HOE-16410; IP 50; IP Flo; IPU.
Proprietary Names *Alon*; *Arelon*; *Augur*; *Avanon*; *Belgran*; *Graminon*; *Hytane*; *Isotop*; *Nocilon*; *Protugan*; *Sabre*; *Swing*; *Tolkan*.



Chemical Properties A colourless to white crystalline solid. Mp 158°. Soluble in water (72 mg/L at 20°); readily soluble in common organic solvents, e.g. alcohol, ketones, esters, aromatic hydrocarbons, chlorinated hydrocarbons, methanol (56 g/L at 20°), dichloromethane (63 g/L at 20°), benzene (5 g/L at 20°) and hexane (0.1 g/L at 20°). Log *P* (octanol/water), 2.87.
Gas Chromatography System GA—isoproturon-Me RI 1685.
Ultraviolet Spectrum Principal peaks at 205, 240 nm.



Mass Spectrum Principal ions at *m/z* 72, 220, 148, 132, 205 (methyl derivative).

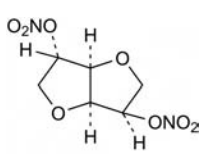


Disposition in the Body Isoproturon is excreted in urine with ≈50% of an administered dose being excreted within the first 8 h.

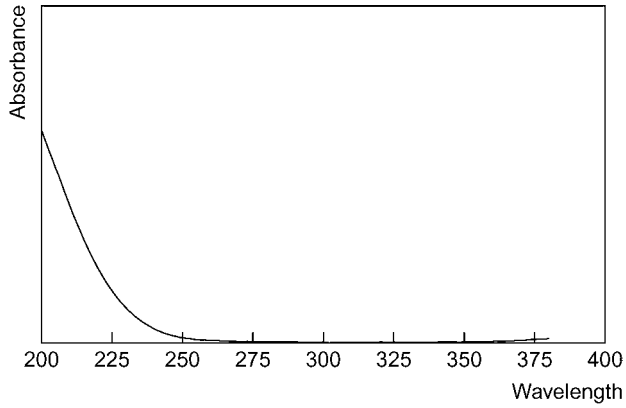
Isosorbide Dinitrate

Antianginal Vasodilator
$C_6H_8N_2O_8$ = 236.1
CAS—87-33-2

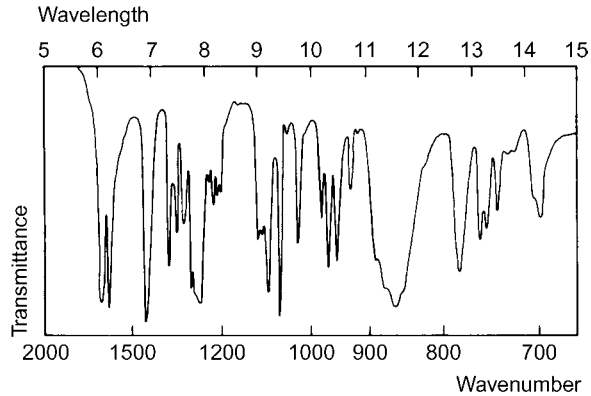
IUPAC Name [(3*S*,3a*S*,6*R*,6a*S*)-3-Nitrooxy-2,3,3*a*,5,6,6*a*-hexahydrofuro[3,2-*b*]furan-6-yl] nitrate
Synonyms ,4:3,6-Dianhydro-D-glucitol dinitrate; dinitrosorbide; sorbide nitrate.
Proprietary Names *Angitak*; *Cedocard*; *Corovliss*; *Dilatrate*; *Dinit*; *Disorlon*; *Imtack*; *Iso Mack*; *Isocard*; *Isodinit*; *Isoket*; *Isordil*; *Isotrate*; *Jeridin*; *Langoran*; *Nitrosid*; *Risordan*; *Soni-Slo*; *Sorbichew*; *Sorbid*; *Sorbidilat*; *Sorbitrate*; *Sorquad*; *Vascardin*; *Vasorbate*; *Vasotrate*.



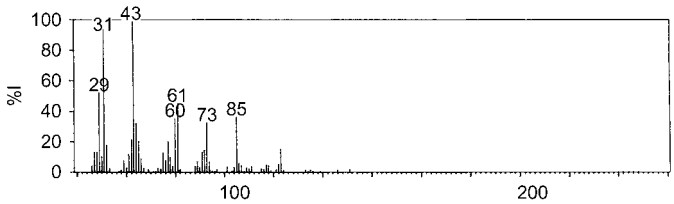
Chemical Properties A white crystalline powder. Mp 70°. Sparingly soluble in water; freely soluble in acetone, ethanol and chloroform; soluble in methanol. Log *P* (octanol/water), 1.3.
Caution Isosorbide dinitrate may explode if subjected to percussion or excessive heat.
High Performance Liquid Chromatography System HY—RI 396.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1062, 1618, 862, 1266, 1653, 1089 cm^{-1} (isosorbide (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 43, 31, 29, 61, 60, 85, 73, 44.



Quantification

Plasma **GC** ECD. Limit of detection, 2.5 µg/L for isosorbide dinitrate and its metabolites [Pommier *et al.* 1996]. Isosorbide dinitrate, glyceryl trinitrate and their metabolites. Limit of detection, 20 µg/L for isosorbide dinitrate [Booth *et al.* 1990]. ECD. Limit of detection, 0.5 µg/L for isosorbide dinitrate, 2 µg/L for isosorbide 2-mononitrate and 10 µg/L for isosorbide 5-mononitrate [Santoni *et al.* 1984]. ECD. Limit of detection, 0.5 µg/L [Sioufi, Pommier 1982].

HPLC Limit of detection, 200 pg [Yu, Goff 1983].

Urine **GC** See Plasma [Sioufi, Pommier 1982].

Disposition in the Body Isosorbide dinitrate is readily absorbed after sublingual or oral administration. It is metabolised by enzymatic denitration followed by glucuronide conjugation. Isosorbide 2-mononitrate and isosorbide 5-mononitrate have some pharmacological activity. About 80% of a dose is excreted in the urine in 24 h, of which about 50% is isosorbide glucuronide, up to 15% is free and conjugated isosorbide 5-mononitrate, about 1% is free isosorbide 2-mononitrate, and <1% is unchanged drug.

Therapeutic Concentration Isosorbide 5-mononitrate accumulates in the plasma during chronic treatment.

Following a single oral dose of 20 mg of isosorbide dinitrate to 8 subjects, peak plasma concentrations of 0.02 to 0.09 (mean, 0.05) mg/L of isosorbide dinitrate, 0.02 to 0.06 (mean, 0.04) mg/L of isosorbide 2-mononitrate, and 0.10 to 0.22 (mean, 0.14) mg/L of isosorbide 5-mononitrate, were attained in 0.25, 0.7, and 1 h, respectively [Laufen *et al.* 1983].

After a single sublingual dose of 5 mg to 6 subjects, peak plasma concentrations of 0.007 to 0.015 (mean, 0.009) mg/L were attained in 10 to 15 min [Assinder *et al.* 1977].

I

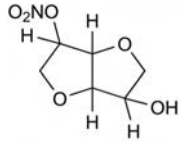
Bioavailability About 60% after sublingual administration and about 20% orally, but there is considerable intersubject variation.
Half-life Plasma half-life, isosorbide dinitrate about 0.5 to 1.5 h, isosorbide 2-mononitrate about 2 h, isosorbide5-mononitrate 3 to 7 h. A longer terminal elimination half-life of about 8 h has been reported for isosorbide dinitrate.
Saliva Plasma : saliva ratio, about 1.5.
Protein Binding About 30%.
Note For a review of the pharmacokinetics of organic nitrates, see Bogaert [1983].
Dose 10 to 240 mg daily, orally.

Assinder DF *et al.* (1977). Plasma isosorbide dinitrate concentrations in human subjects after administration of standard and sustained-release formulations. *J Pharm Sci* 66: 775–778.
Bogaert MG (1983). Clinical pharmacokinetics of organic nitrates. *Clin Pharmacokinet* 8: 410–421.
Booth BP *et al.* (1990). Assay of glyceryl trinitrate, isosorbide dinitrate, and their metabolites in plasma by large-bore capillary column gas-liquid chromatography. *Biopharm Drug Dispos* 11: 663–677.
Laufen H *et al.* (1983). Oral absorption and disposition of isosorbide dinitrate and isosorbide mononitrates in man. *Arzneimittelforschung* 33: 980–984.
Pommier F *et al.* (1996). Simultaneous determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by capillary gas chromatography with electron-capture detection. *J Chromatogr B Biomed Appl* 678: 354–359.
Santoni Y *et al.* (1984). Determination of isosorbide dinitrate and its mononitrate metabolites in human plasma using Extrelut purification and capillary column gas-liquid chromatography. *J Chromatogr* 306: 165–172.
Sioufi A, Pommier F (1982). Gas chromatographic determination of isosorbide dinitrate in human plasma and urine. *J Chromatogr* 229: 347–353.
Yu WC, Goff EU (1983). Determination of vasodilators and their metabolites in plasma by liquid chromatography with a nitrosyl-specific detector. *Anal Chem* 55: 29–32.

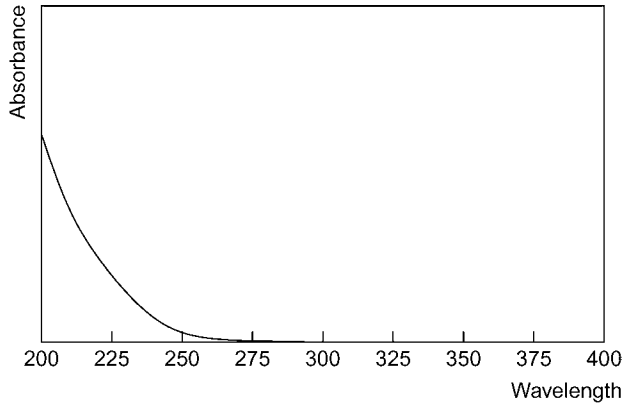
Isosorbide Mononitrate

Antianginal Vasodilator
$C_6H_9NO_6$ = 191.1
CAS—16051-77-7

IUPAC Name [(3*S*,3*aR*,6*R*,6*aS*)-3-Hydroxy-2,3,3*a*,5,6,6*a*-hexahydrofuro[3,2-*b*]furan-6-yl] nitrate
Synonyms 1,4:3,6-Dianhydro-D-glucitol 5-nitrate; isosorbide 5-mononitrate.
Proprietary Names *Angeze*; *Chemydur*; *Coleb*; *Conpin*; *Corangin*; *Duride*; *Dynamin*; *Elantan*; *Imdur*; *Imtrate*; *Isib*; *Ismo*; *Isodur*; *Isomonit*; *Isotard*; *Isotrate*; *MCR-50*; *Monicor*; *Modisal*; *Monit*; *Mono-Cedocard*; *Monoket*; *Monomax*; *Monosorb*; *Xismox*.



Chemical Properties Colourless prismatic crystals. Mp 89° to 91°. Soluble in water, ethanol, acetone and methanol; slightly soluble in chloroform and ether. Log *P* (octanol/water), −0.2.
Caution Isosorbide mononitrate may explode if subjected to percussion or excessive heat.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1280, 1650, 1635, 852, 1090, 968 cm^{-1} (KBr disk).
Quantification
Plasma **GC** ECD. Limit of detection, 2 µg/L [Yang *et al.* 1997]. ECD. Limit of detection, 1 µg/L [Straehl, Galeazzi 1984].
GC–MS For method, see Lauro-Marty *et al.* [1995].
Disposition in the Body Isosorbide mononitrate is rapidly and almost completely absorbed after oral or sublingual administration. It is metabolised by enzymatic denitration and by glucuronide conjugation. About 80% of a dose is excreted in the urine in 24 h.
Therapeutic Concentration Following a single oral dose of 20 mg given to 19 subjects, peak plasma concentrations of 0.34 to 0.68 (mean, 0.48) mg/L were attained in 1 h [Abshagen *et al.* 1981].

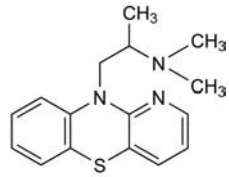
Toxicity
A 15-year-old girl survived after ingesting 1.6 g of isosorbide mononitrate and 20 mg of glyceryl trinitrate in a suicide attempt. Plasma levels of isosorbide mononitrate measured 4 and 6 h after gastric lavage (performed 2 h after ingestion) were 2.993 and 3.140 mg/L, respectively; glyceryl trinitrate was not detected [Sobrino *et al.* 1992].
Bioavailability Almost 100%.
Half-life Plasma half-life, 3 to 7 h.
Volume of Distribution About 0.6 L/kg.
Clearance Plasma clearance, about 1 to 2 mL/min/kg.
Saliva Plasma : saliva ratio, about 0.8.
Protein Binding <5%.
Dose 20 to 120 mg daily.

Abshagen U *et al.* (1981). Pharmacokinetics of intravenous and oral isosorbide-5-mononitrate. *Eur J Clin Pharmacol* 20: 269–275.
Lauro-Marty C *et al.* (1995). Gas chromatographic-mass spectrometric determination of isosorbide 5-mononitrate in human plasma. *J Chromatogr B Biomed Appl* 663: 153–159.
Sobrino JM *et al.* (1992). Massive ingestion of isosorbide-5-mononitrate and nitroglycerin: suicide attempt by an adolescent girl without previous heart disease. *Eur Heart J* 13: 145.
Straehl P, Galeazzi RL (1984). Determination of isosorbide 5-mononitrate in human plasma by capillary column gas chromatography. *J Pharm Sci* 73: 1317–1319.
Yang LL *et al.* (1997). Determination of isosorbide-5-mononitrate in plasma by GC-ECD and study on its pharmacokinetics in ten volunteers. *Yao Xue Xue Bao* 32: 773–776.

Isothipendyl

Antihistamine
$C_{16}H_{19}N_3S$ = 285.4
CAS—482-15-5

IUPAC Name *N*,*N*-Dimethyl-1-pyrido[3,2-*b*][1,4]benzothiazin-10-ylpropan-2-amine
Synonyms D-201; *N*,*N*,α-trimethyl-10*H*-pyrido[3,2-*b*][1,4]benzothiazine-10-ethanamine.

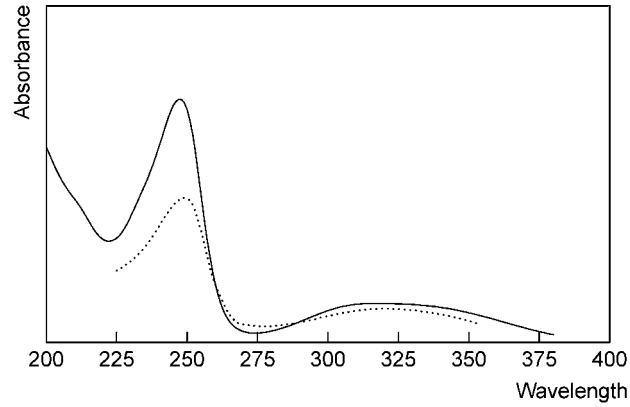


Chemical Properties Log *P* (octanol/water), 3.9.

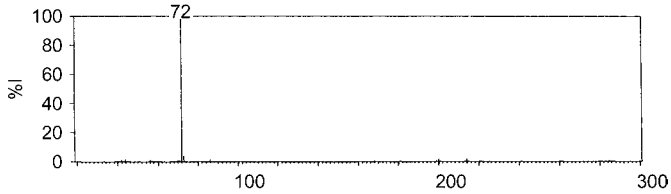
Isothipendyl Hydrochloride

$C_{16}H_{19}N_3S$, HCl = 321.9
CAS—1225-60-1
Proprietary Names *Andantol*; *Apaisyl*; *Calmogel*; *Istamyl*; *Nilergex*; *Sedermyl*; *Thiodantyl*.
Chemical Properties A fine white crystalline powder. Mp about 212°, with decomposition. Soluble 1 in 5 of water, 1 in 60 of ethanol and 1 in 10 of chloroform; practically insoluble in ether.

Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.41; system TC—R_f 0.30; system TE—R_f 0.64; system TL—R_f 0.14; system TAE—R_f 0.22; system TAF—R_f 0.35 (Dragendorff spray, positive; FPN reagent, yellow-pink; acidified iodoplatinate solution, positive; Marquis reagent, pink; ninhydrin spray, positive).
Gas Chromatography System GA—isothipendyl RI 2225, M (nor-) RI 2220, M (OH-) RI 2450, M (bis-nor-) RI 2230.
High Performance Liquid Chromatography System HA—*k* 3.8; system HX—RI 390; system HAA—retention time 13.5 min.
Ultraviolet Spectrum Aqueous acid—245 nm (A^{1}_{1}=860a); aqueous alkali—250 nm.



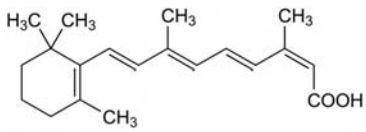
Infrared Spectrum Principal peaks at wavenumbers 1017, 1030, 1070, 987, 1090, 1080 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 72, 73, 214, 200, 44, 285, 86, 56.



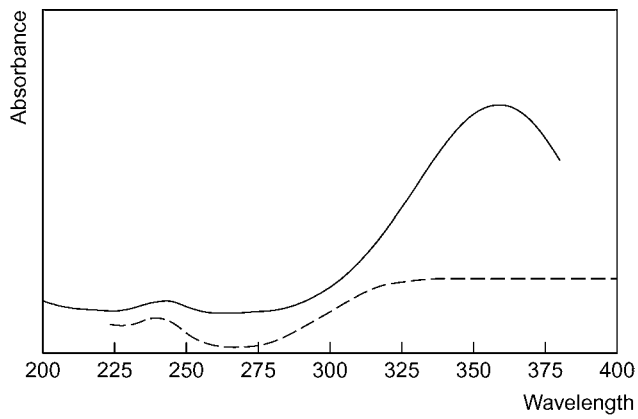
Dose 12 to 32 mg of isothipendyl hydrochloride daily.

Isotretinoin

Dermatological Agent
$C_{20}H_{28}O_2$ = 300.4
CAS—4759-48-2
IUPAC Name (2*Z*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid
Synonyms (13*Z*)-15-Apo-β-caroten-15-oic acid; 13-*cis*-retinoic acid; 13-RA; 2-*cis*-vitamin A acid; neovitamin A acid; Ro-4-3780.
Proprietary Names *Accure*; *Accutane*; *Isotrex*; *Isotrexin (multi-ingredient)*; *Roaccutan*; *Roaccutane*.



Chemical Properties Reddish-orange plates from isopropyl alcohol. Mp 174° to 175°. Practically insoluble in water; sparingly soluble to slightly soluble in alcohol; sparingly soluble in ether and isopropyl alcohol; soluble in chloroform and dichloromethane. Log *P* (octanol/water), 2.8; also reported as 6.0.
Thin-layer Chromatography System TF—R_f 0.45.
High Performance Liquid Chromatography System HX—RI 939.
Column: μC_{18} RP (300 × 4.6 mm). Mobile phase: methanol : water (80:20), flow rate 1.0 mL/min. IS: Ro-11-5036. UV detection (λ=350 nm). Retention time: isotretinoin, 6.3 min; IS, 5.6 min [Khan *et al.* 1996].
Ultraviolet Spectrum Aqueous acid—243, 359 nm.



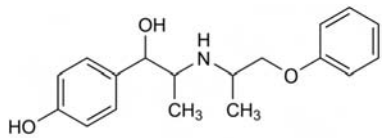
Quantification

Plasma **HPLC** UV detection (λ=360 nm). Limit of quantification, 0.3 µg/L [Wyss, Bucheli 1997]. Column: RP C_{18} Nova-Pak (150 × 3.9 mm i.d.) and RP C_{18} Nova-Pak (75 × 3.9 mm). Temperature: 40°. Mobile phase: acetonitrile; 0.1 mol/L ammonium acetate, (pH 5). (80:20 for 10 min, increased to 90:10). Internal standard (IS): acitretin. MS detection, SIM mode (m/z 414). Retention time: isotretinoin, 25.5 min; IS, 16.2 min. Limit of detection, 25 pg [Lehman, Franz 1996]. MS detection. Limit of quantification, 0.3 µg/L [Ranalder *et al.* 1993].
Serum **HPLC** UV detection (λ=360 nm). Limit of quantification, 6.17 µg/L for isotretinoin and 3.48 µg/L for 4-oxo-isotretinoin, limit of detection, 1.85 µg/L for isotretinoin and 1.05 µg/L for 4-oxo-isotretinoin [Gundersen *et al.* 1997]. UV detection (λ=365 nm). Limit of detection, 12 µg/L [Gadde, Burton 1992].
Disposition in the Body Isotretinoin is rapidly absorbed after oral administration; the extent of which is increased in the presence of food. Bioavailability of the drug is low because first pass metabolism occurs in the liver and also metabolism occurs in the gut wall. The major metabolite identified in blood is 4-oxo-isotretinoin; tretinoin and 4-oxo-tretinoin can also be detected. Both the drug and metabolites undergo enterohepatic recycling. Equal amounts of the absorbed drug are excreted in faeces, mainly as the unchanged drug, and in urine, as the metabolites. Isotretinoin crosses the placenta.
Therapeutic Concentration
Thirty-eight patients with high-risk neuroblastoma, aged 2 to 12 years, were administered 100, 125, 160 or 200 mg/m^2 daily for 2 weeks, followed by a 2-week rest period. The peak concentrations were 4.9, 5.7, 7.2 and 8.9 µg/L for the 4 doses observed at 3.7, 3.8, 4.1 and 4.0 h, respectively. The minimum concentrations observed at 2.8, 4.2, 4.1 and 3.9 µg/L for the 100, 125, 160 and 200 mg doses [Khan *et al.* 1996].
Toxicity Overdosing with isotretinoin has been associated with vomiting, facial flushing, abdominal pain, headache, dizziness and problems with muscle co-ordination/regularity.
A 29-year-old male ingested 900 mg isotretinoin (equivalent to 12.5 mg (/kg/day); 30 times the prescribed dose) and 1 day later he complained of a mild headache which was easily controlled with aspirin. Five days after the overdose he was admitted to hospital with a persistent headache and eczema. The serum isotretinoin concentrations were 12, 5, 3 and <2 µg/L at 4, 5, 6 and 11 days after ingestion, respectively. The corresponding 4-oxo-isotretinoin concentrations were 562, 290, 114 and 22 µg/L, respectively [Aubin *et al.* 1995].
Half-life 10 to 20 h.
Clearance Total body, mean 5.64 L/m^2/h (children aged 2 to 12 years old).
Volume of Distribution Apparent, 29.45 L/m^2.
Protein Binding 99.9%.
Dose The initial dose is 0.5 to 1.0 mg/kg daily with a maximum dose up to 2.0 mg/kg daily for 15 to 20 weeks. The dose may be adjusted according to the response of the disease and any clinical toxic effects observed.

Aubin S *et al.* (1995). Massive isotretinoin intoxication. *Clin Exp Dermatol* 20(4): 348–350.
Gadde RR, Burton FW (1992). Simple reversed-phase high-performance liquid chromatographic method for 13-cis-retinoic acid in serum. *J Chromatogr* 593(1-2): 41–46.
Gundersen TE *et al.* (1997). Quantitative high-performance liquid chromatographic determination of retinoids in human serum using on-line solid-phase extraction and column switching. Determination of 9-cis-retinoic acid, 13-cis-retinoic acid, all-trans-retinoic acid, 4-oxo-all-trans-retinoicacid and 4-oxo-13-cis-retinoic acid. *J Chromatogr B Biomed Sci Appl* 691(1): 43–58.
Khan AA *et al.* (1996). Pharmacokinetic studies of 13-cis-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation. *Cancer Chemother Pharmacol* 39: 34–41.
Lehman PA, Franz TJ (1996). A sensitive high-pressure liquid chromatography/particle beam/mass spectrometry assay for the determination of all-trans-retinoic acid and 13-cis-retinoic acid in human plasma. *J Pharm Sci* 85(3): 287–290.
Ranalder UB *et al.* (1993). Micro liquid chromatography-mass spectrometry with direct liquid introduction used for separation and quantitation of all-trans- and 13-cis-retinoic acids and their 4-oxo metabolites in human plasma. *J Chromatogr* 617: 129–135.
Wyss R, Bucheli F (1997). Determination of endogenous levels of 13-cis-retinoic acid (isotretinoin), all-trans-retinoic acid (tretinoin) and their 4-oxo metabolites in human and animal plasma by high-performance liquid chromatography with automated column switching and ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 700(1-2): 31–47.

Isoxsuprine

Vasodilator
$C_{18}H_{23}NO_3$ = 301.4
CAS—395-28-8
IUPAC Name 4–Hydroxy-α-[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]benzenemethanol



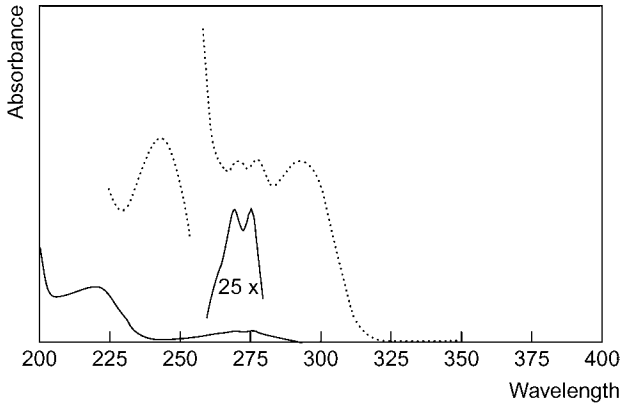
Chemical Properties Crystals. Mp 102.5° to 103.5°. pK_a 8.0, 9.8. Log *P* (octanol/water), 2.4.

Isoxsuprine Hydrochloride

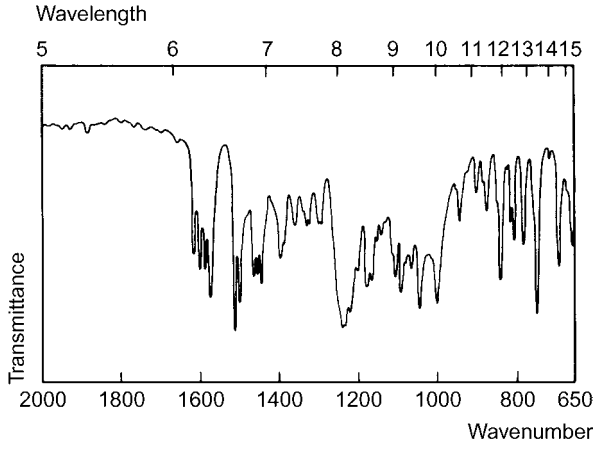
$C_{18}H_{23}NO_3$, HCl = 337.8
CAS—579-56-6
Synonym Phenoxyisopropylnorsuprifen
Proprietary Names *Dilavase*; *Dilum*; *Duvadilan*; *Duviculine*; *Fenam*; *Inibina*; *Vadosilan*; *Vasodilan*; *Vasolan*; *Vasoplex*; *Vasotran*; *Voxsuprine*; *Xuptin*.
Chemical Properties A white crystalline powder. Mp about 200°, with decomposition. Soluble 1 in 500 of water, 1 in 100 of ethanol and dilute sodium hydroxide solution, and 1 in 2500 of dilute hydrochloric acid; practically insoluble in chloroform and ether.

Colour Tests Mandelin's test—green; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.78; system TB—R_f 0.03; system TC—R_f 32; system TE—R_f 0.62; system TL—R_f 0.53; system TAE—R_f 0.62; system TAF—R_f 0.81; system TAJ—R_f 0.13; system TAK—R_f 0.05; system TAL—R_f 0.60 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2300.

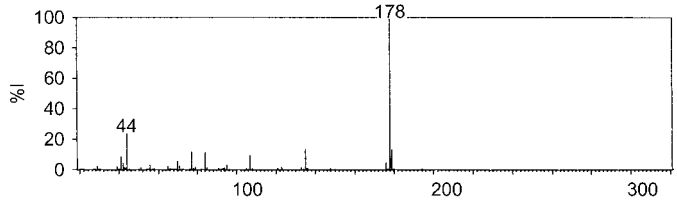
High Performance Liquid Chromatography System HA—*k* 0.8; system HX—RI 353; system HY—RI 301.
Ultraviolet Spectrum Aqueous acid—269, 274 nm (A_1^1=82a); aqueous alkali—243 (A_1^1=450a), 270, 277, 292 nm (A_1^1=78b).



Infrared Spectrum Principal peaks at wavenumbers 1514, 1240, 745, 1220, 1043, 1500 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 178, 44, 135, 179, 77, 84, 107, 41.



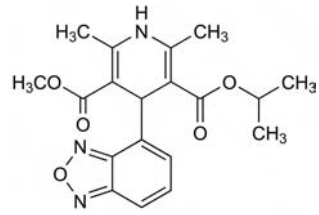
Quantification
Plasma **GC** ECD. Limit of detection, 0.5 µg/L [Cova *et al.* 1983].
Disposition in the Body Isoxsuprine is absorbed after oral administration; peak blood concentrations are attained within 1 h of a dose. It is excreted in the urine partly unchanged and partly as a conjugate.
Dose Up to 80 mg of isoxsuprine hydrochloride daily.

Cova D *et al.* (1983). Determination of isoxsuprine in human plasma by gas chromatography with electron capture detection. *Pharmacology* 27: 117–124.

Isradipine

Calcium Antagonist
$C_{19}H_{21}N_3O_5$ = 371.4
CAS—75695-93-1
IUPAC Name 3-*O*-Methyl 5-*O*-propan-2-yl 4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3, 5-dicarboxylate
Synonyms 4-(4-Benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid methyl 1-methylethyl ester; isrodipine; PN-200-110.

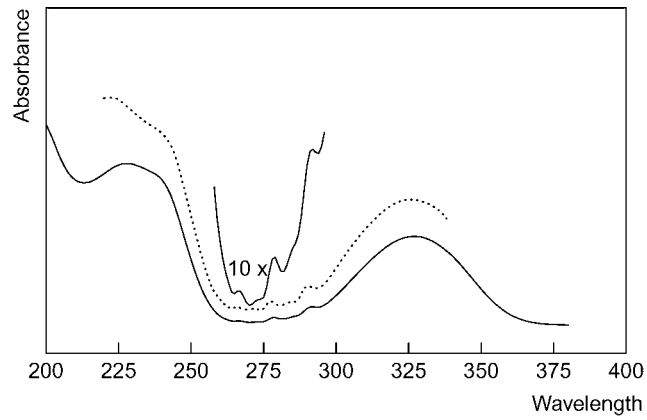
Proprietary Names *Clivoten*; *DynaCirc*; *Esradin*; *Icaz*; *Lomir*; *Prescal*; *Rebriden*; *Vascal*; *Vaslan*.



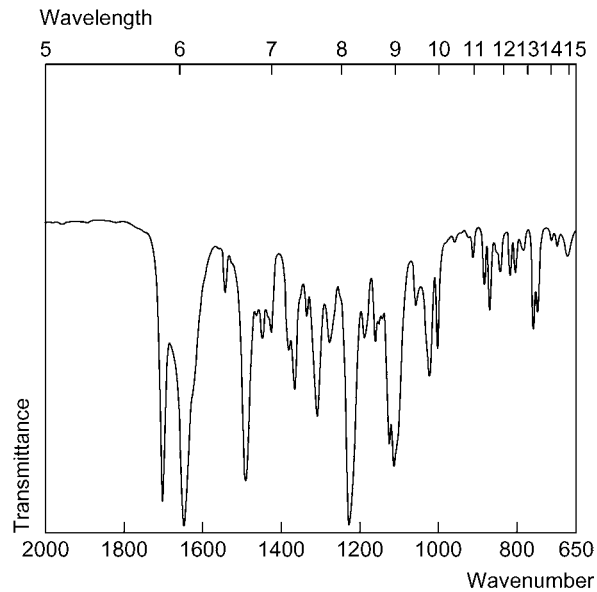
Chemical Properties A yellow crystalline powder with Mp 168° to 170°. Freely soluble in acetone, chloroform, methylene chloride; soluble in ethanol; practically insoluble in water (<10 mg/L at 37°). Log *P* (octanol/water), 4.28.
Thin-layer Chromatography System TB—R_f 0.04; system TE—R_f 0.74; system TF—R_f 0.59; system TAE—R_f 0.88.
Gas Chromatography System GP—RI 2270M (dehydro-deisopropyl)-ME; RI 2360M (dehydro-).

Gas Chromatography-Mass Spectrometry. Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. Detection: mass spectrometer. Retention index: 2619 [Mills, Roberson 1993a].
High Performance Liquid Chromatography System HAA—RT 22.4 min; system HX—RI 632; system HZ—RT 14.9 min.

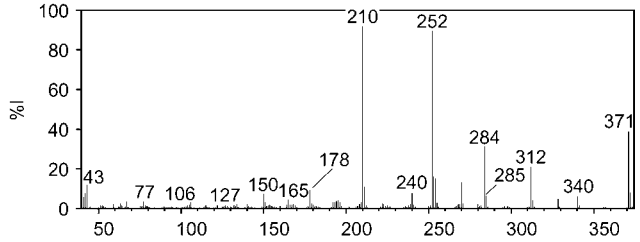
Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, 0.5 mL/min flow rate. UV diode array detection. Retention time: 3.7 min [Mills, Roberson 1993b].
Ultraviolet Spectrum Aqueous base—266, 278, 291, 326 nm.



Infrared Spectrum Principal peaks at wavenumbers 1648, 1225, 1702, 1491 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 210, 252, 371, 284, 312, 43, 178, 240.



Quantification

Plasma **GC** ECD. Limit of detection, 0.2 µg/L [Chellingsworth *et al.* 1988].

GC–MS Column: cross-linked methylsilicone (10 m × 0.53 mm i.d., 2.65 µm). Column and injector temperature: 230° and 350°, respectively. Carrier gas: methane, flow rate 10 mL/min. Detection: GM-MS. (CI, SIM at *m/z* 311). Retention time: isradipine, 2.5 min; pyridinic metabolite, 1.3 min. Limit of detection, 0.04 µg/L [Jean, Laplanche 1988].

HPLC UV detection (λ=325 nm). Limit of detection, 0.1 to 0.6 µg/L [Boutagy *et al.* 1989].

Disposition in the Body Isradipine is rapidly and almost completely absorbed (90 to 95%) after oral administration with peak plasma concentrations after 2 h. It undergoes rapid and extensive first-pass hepatic metabolism to produce inactive pyridine and carboxylic acid derivatives. Four major metabolites have been isolated, but only one has considerable pharmacological activity. The drug is excreted as metabolites in urine (70% of the dose) and the remainder in faeces (mostly as the unchanged drug). No unchanged drug is detected in urine. Excretion is virtually complete within 120 h. The pharmacokinetics of isradipine, especially the peak concentrations, half-lives and bioavailability, are increased in the elderly and those with hepatic dysfunction.

Therapeutic Concentration

Nine patients with hypertension (6 males and 3 females), aged between 38 and 68 years, were administered a single dose of 5 mg isradipine after an overnight fast. The mean peak concentration was 6 µg/L (range, 1.6 to 18.4) which was observed after 1.5 h (range, 0.5 to 4.0). Under steady state conditions, a peak plasma concentration of 3.2 (1.2 to 6.1) µg/L was observed after 1.2 h (range, 0.5 to 2.0) [Shenfield *et al.* 1990].

Twelve healthy males, aged 20 to 39 years, were administered a single 5 or 20 mg dose or a 5 mg multiple dose after an 8-h fast. The mean peak plasma concentration was 2.1 µg/L after the 5 mg single isradipine dose and 9.1 µg/L after the 20 mg dose, and both were observed between 2 and 3 h. A peak concentration of 2.2 µg/L was detected after about 1 h for the multiple dosing study [Tse, Jaffe 1987].

Bioavailability 15 to 24% (varies between poor and extensive metabolisers).

Half-life 8 h (<4 h also reported).

Volume of Distribution 2.9 L/kg (283 L).

Clearance Plasma, 40 L/h.

Distribution in Blood Erythrocyte : plasma ratio 0.24.

Protein Binding Approximately 97%, mainly to α-1-glycoprotein acids.

For a general review of isradipine, see Grimm *et al.* [1990].

Dose 2.5 to 20 mg daily.

Boutagy J *et al.* (1989). Determination of isradipine and the oxidative pyridine metabolite in human plasma by high-performance liquid chromatography. *J Chromatogr* 487: 483–488.
Chellingsworth MC *et al.* (1988). *Am J Med* 84: 3B72–79.
Grimm RH *et al.* (1990). *Drugs* 40: 231–74.
Mills T, Roberson JC (1993a). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 6: 20–21.
Mills T, Roberson JC (1993b). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 6: 126–127.
Jean C, Laplanche R (1988). Assay of isradipine and of its major metabolites in biological fluids by capillary gas chromatography and chemical ionization mass spectrometry. *J Chromatogr* 428: 61–69.
Shenfield GM *et al.* (1990). The pharmokinetics of isradipine in hypertensive subjects. *Eur J Clin Pharmacol* 38: 209–11.
Tse FL, Jaffe JM (1987). Pharmacokinetics of PN 200-110 (isradipine), a new calcium antagonist, after oral administration in man. *Eur J Clin Pharmacol* 32(4): 361–365.

Itraconazole

Triazole Antifungal

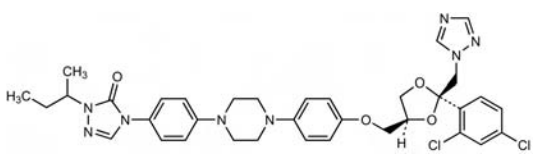
$C_{35}H_{38}Cl_2N_8O_4$ = 705.6

CAS—84625-61-6

IUPAC Name 4-[4-[4-[4-[[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3*H*-1,2,4-triazol-3-one

Synonyms Oriconazole; R-51211.

Proprietary Names *Canadiol*; *Hongoseril*; *Itrizole*; *Sempera*; *Siros*; *Sporanox*; *Triasporin*.

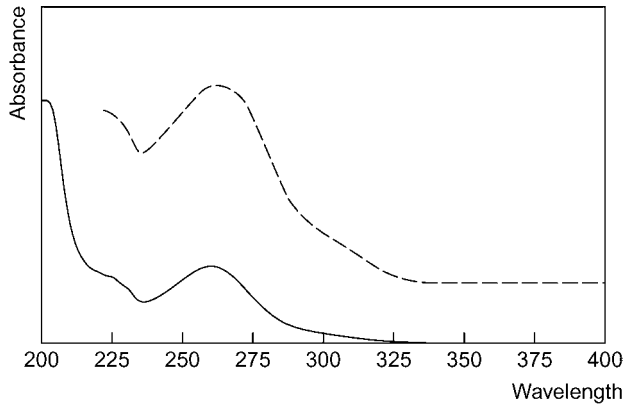


Chemical Properties A white to off-white powder; crystals from toluene. Mp 166.2°. It is soluble in dichloromethane; sparingly soluble in tetrahydrofuran; practically insoluble in water and dilute acidic solutions. pK_a 3.7. Log *P* (*n*-octanol/buffer pH 8.1), 5.66.

Thin-layer Chromatography System TB—R_f 0.01; system TE—R_f 0.79; system TF—R_f 0.11; system TAE—R_f 0.87.

High Performance Liquid Chromatography System HX—RI 725.

Ultraviolet Spectrum Aqueous acid—263 nm.



Quantification

Plasma **HPLC** UV detection (λ=254 nm). Lower limit of detection, 0.001 mg/L [Woestenborghs *et al.* 1987].

Serum **HPLC** Column: RP C_{18} RsiL C18HL (150 × 2.1 mm i.d., 5 µm). Mobile phase: water : acetonitrile (40:60), flow rate 0.5 mL/min. Internal standard (IS): R-51012. UV detection (λ=254 nm). Retention time: itraconazole, 4.3 min; IS, 5.8 min. Limit of detection, 0.01 mg/L [Gubbins *et al.* 1998]. Column: RP C_{18} Hypersil (100 × 4.5 mm i.d., 3 µm). Mobile phase: water : acetonitrile (40:60) containing 0.03% diethylamine (pH 7.8), flow rate 1 mL/min. IS: R-51012. UV detection (λ=254 nm). *k* value: itraconazole, 7.4; hydroxyitraconazole, 2.7; IS, 10.8. Limit of quantification, 0.02 mg/L for the drug and its metabolites [Law *et al.* 1994].

Bioassay For method, see Law *et al.* [1994].

Disposition in the Body Itraconazole is slowly but well absorbed after oral administration with peak concentrations reached in ~4 h. Absorption is enhanced in the presence of food and in an acidic intragastric environment. It is metabolised, mainly via oxidative pathways, to inactive metabolites which are excreted via bile and in urine. Over 30 metabolites have been isolated and most are biologically inactive, including a hydroxy metabolite (bioactive) and hydroxyitraconazole. Both the drug and hydroxyitraconazole are inhibitors of the CYP3A4 enzyme system. The drug is widely distributed throughout the body with small amounts detected in most body fluids and accumulation occurring in tissues. Itraconazole has also been detected in skin, hair and nail, but it does not easily redistribute in these tissues. 3 to 18% is excreted in the faeces as unchanged drug.

Therapeutic Concentration The trough serum therapeutic concentration is >0.25 mg/L and for the sum of the parent drug and hydroxyitraconazole metabolite, 1 to 4 g/L.

Seven healthy males and 5 females with a mean age of 34 years (range, 22 to 51) were administered a 100 mg dose of itraconazole either after a 12-h fast (group 1), after a light breakfast (group 2) or after a full breakfast (group 3). Group 1: a mean peak plasma concentration of 0.11 mg/L was observed 3.33 h after ingestion. Group 2: 0.21 mg/L at 3.67 h. Group 3: peak concentration of 0.23 mg/L after 4.17 h [Zimmermann *et al.* 1994].

Toxicity The serum toxic concentration is 6 mg/L (sum of itraconazole and its hydroxy metabolite).

Bioavailability Dose dependent, increases with increasing dosage; 55% after a 100 mg dose (as solution).

Half-life 20 h (following a 100 mg single dose); increases to 30 h on multiple dosing.

Volume of Distribution 10.7 L/kg; also reported as 561 L.

Clearance Plasma, 22.9 L/h; also reported as 18.7 L/h.

Protein Binding Approximately 99.8%, albumin.

Dose 100 to 400 mg daily depending on indications and severity.

Gubbins PO *et al.* (1998). Rapid and sensitive high performance liquid chromatographic method for the determination of itraconazole and its hydroxy-metabolite in human serum. *J Pharm Biomed Anal* 16: 1005–1012.
Law D *et al.* (1994). Bioassay for serum itraconazole concentrations using hydroxyitraconazole standards. *Antimicrob Agents Chemother* 38(7): 1561–1566.
Woestenborghs R *et al.* (1987). Determination of itraconazole in plasma and animal tissues by high-performance liquid chromatography. *J Chromatogr* 413: 332–337.
Zimmermann T *et al.* (1994). Influence of concomitant food intake on the oral absorption of two triazole antifungal agents, itraconazole and fluconazole. *Eur J Clin Pharmacol* 46: 147–150.

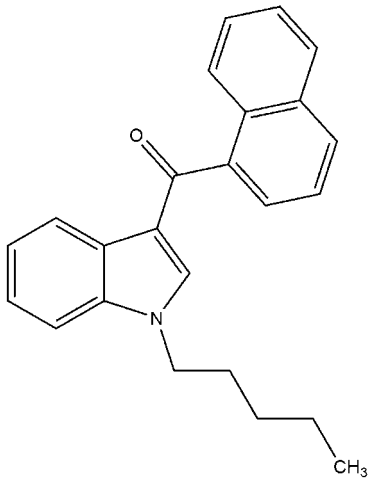
JWH-018

Analgesic, CB1 and CB2 Agonist

$C_{24}H_{23}NO = 341.5$

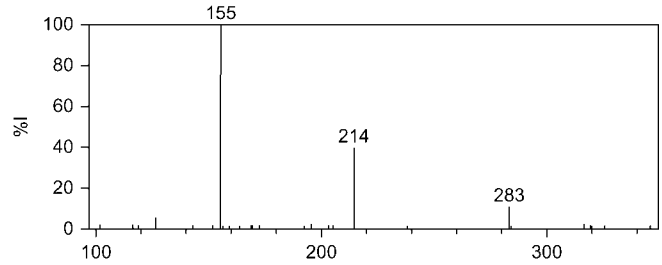
IUPAC Name Naphthalen-1-yl-(1-pentylindol-3-yl)methanone

Synonyms 1-Pentyl-3-(1-naphthoyl), herbal incense.



Chemical Properties Aminoalkylindole, which produces effects similar to tetrahydrocannabinol, is one of the active ingredients of the herbal blend 'Spice'. It is illegal in Austria, Germany and France.

Mass Spectrum Principal peaks at *m/z* 155, 214, 283.



Quantification

Blood **LC-MS** MRM acquisition mode. Limit of quantification, 0.6 μg/L, limit of detection, 0.2 μg/L [Neukamm *et al.* 2009].

Urine **LC-MS** Enhanced product ion scan. Limit of detection not reported [Kraemer *et al.* 2009].

GC-MS EI ionisation. Limit of detection not reported [Kraemer *et al.* 2009].

Hair **LC-MS** See Blood [Neukamm *et al.* 2009].

Other **LC-MS** Herbal Products. Column: UPLC HSS T3 (100 × 2.1 mm i.d., 1.8 μm). Mobile phase: 0.1% formic acid in acetonitrile : water, flow rate 0.3 mL/min. Limit of detection not reported [Uchiyama *et al.* 2009].

Kraemer, T. *et al.* (2009). Studies on the metabolism of JWH-018 and of a homologue of CP 47,497, pharmacologically active ingredients of different misused incense ('Spice') using GC-MS and LC-MS techniques. *Ann Toxicol Anal* 21: S1-21–S1-22.

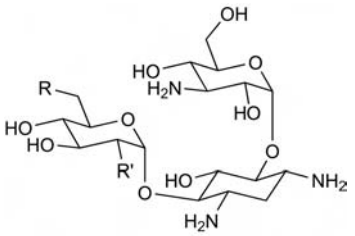
Neukamm, MA. *et al.* (2009). Quantitative determination of the active 'spice' ingredient JWH-018 in blood and hair by liquid chromatography–tandem mass spectrometry. *Ann Toxicol Anal* 21S1-21.

Uchiyama, N. *et al.* (2009). Identification of cannabinoid analogs as new type of designer drugs in herbal products. *Ann Toxicol Anal* 21: S1-53–S1-54.

Kanamycin

Antibiotic
$C_{18}H_{36}N_4O_{11}$ = 484.5
CAS—59-01-8

IUPAC Name *O*-3-Amino-3-deoxy-α-D–glucopyranosyl-(1→6)-*O*–[6-amino-6-deoxy-α-D–glucopyranosyl-(1→4)]-2-deoxy-D-streptamine
Synonym Kanamycin A
Proprietary Names *Kanapat*; *Kanibel*.



	R	R'
Kanamycin A	NH_2	OH
Kanamycin B	NH_2	NH_2
Kanamycin C	OH	NH_2

Chemical Properties An antimicrobial substance produced by the growth of *Streptomyces kanamyceticus*. A whitish powder. pK_a 7.2. Log *P* (octanol/water), −6.7.

Kanamycin Acid Sulfate

$C_{18}H_{36}N_4O_{11}$, $1.7H_2SO_4$ = 651.2
Proprietary Names *Kangen*; *Kannasyn*.
Chemical Properties A white hygroscopic powder. Soluble 1 in 1 of water; practically insoluble in ethanol, acetone, chloroform and ether.

Kanamycin Sulfate

$C_{18}H_{36}N_4O_{11}$, H_2SO_4 = 582.6
CAS—25389-94-0
Synonym Kanamycin A sulfate
Proprietary Names *Kamycine*; *Kanacolirio*; *Kanamytrex*; *Kanasig*; *Kana-Stulln*; *Kancin*; *Kanescin*; *Kan-Ophtal*; *Kantrex*.
Chemical Properties A white crystalline powder. Soluble 1 in 8 of water; practically insoluble in ethanol, acetone, chloroform and ether.

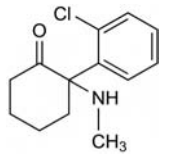
Thin-layer Chromatography System TA—R_f 0.01; system TAE—R_f 0.00 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—249 (A^1_1 = about 3b), 306 nm.
Infrared Spectrum Principal peaks at wavenumbers 1033, 1068, 1143, 1123, 980, 1516 cm^{-1} (kanamycin sulfate, KBr disk).
Quantification
Serum **HPLC** Fluorescence detection. For method for quantification of kanamycin and dibekacin, see Kubo *et al.* [1985].
Immunoassay Fluorescence detection DeCastro *et al.* [1986].
Spectrofluorimetry Limit of detection, 50 µg/L [Csiba 1979].
Bioassay For method of quantification of aminoglycoside antibiotics in biological fluids, see Broughall [1978].
Urine **Spectrofluorimetry** See Serum [Csiba 1979].
Disposition in the Body Kanamycin is poorly absorbed after oral administration, but rapidly and completely absorbed after IM injection. Up to 95% of a dose is excreted unchanged in the urine in 24 h, most being excreted within the first 6 h. A small amount is excreted in the bile.
Therapeutic Concentration During treatment, the serum concentration should be in the range 20–25 mg/L and should be monitored regularly, especially in patients who have renal insufficiency. During multiple dosing, the trough concentration immediately preceding a dose should not exceed 10 mg/L.
Toxicity Toxic effects may be produced at serum concentrations of 30 mg/L or more, or during chronic treatment if the trough serum concentration exceeds 10 mg/L.
Half-life Plasma half-life, about 2 to 4 h; increased to 4–5 days in renal failure.
Volume of Distribution About 0.3 L/kg.
Clearance Plasma clearance, about 1.4 mL/min/kg.
Protein Binding Not significantly bound.
Dose The equivalent of up to 1.5 g of kanamycin daily, by IM injection.

Broughall JM (1978). Aminoglycosides. In: Reeves DS *et al.*, eds.*Laboratory Methods in Antimicrobial Chemotherapy.* Edinburgh: Churchill Livingstone, 194–207.
Csiba A (1979). Spectrofluorimetric method for aminoglycoside antibiotics. *J Pharm Pharmacol* 31: 115–116.
DeCastro AF *et al.* (1986). Determination of kanamycin concentration in serum by substrate-labeled fluorescent immunoassay. *Antimicrob Agents Chemother* 29: 961–964.
Kubo H *et al.* (1985). Rapid method for determination of kanamycin and dibekacin in serum by use of high-pressure liquid chromatography. *Antimicrob Agents Chemother* 28: 521–523.

Ketamine

Anaesthetic (General)
$C_{13}H_{16}ClNO$ = 237.7
CAS—6740-88-1

IUPAC Name 2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone

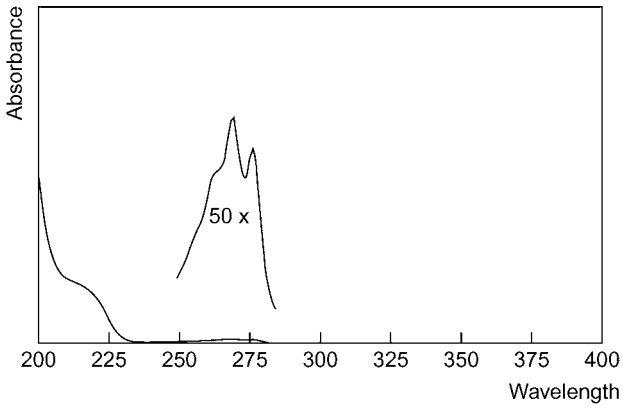


Chemical Properties Crystals Mp 92° to 93°. pK_a 7.5. Log *P* (octanol/water), 3.1. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

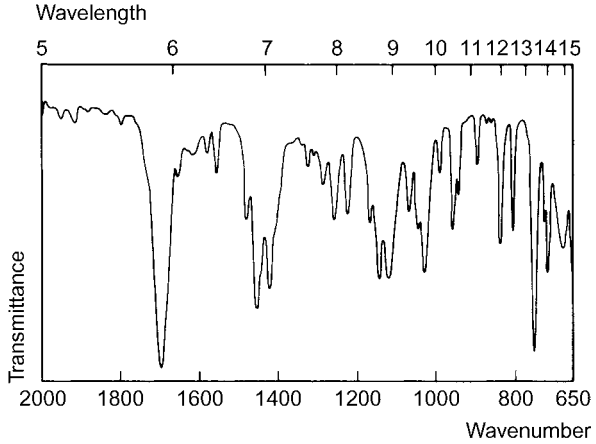
Ketamine Hydrochloride

$C_{13}H_{16}ClNO$, HCl = 274.2
CAS—1867-66-9

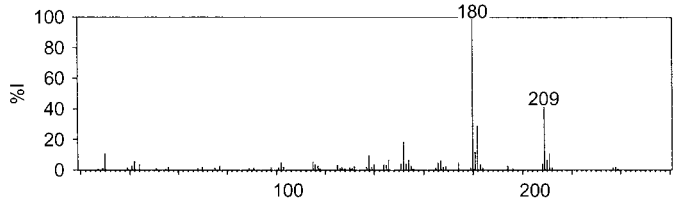
Chemical Properties A white crystalline powder. Mp 262° to 263°. Soluble 1 in 4 of water, 1 in 14 of ethanol and 1 in 6 of methanol; sparingly soluble in chloroform; practically insoluble in ether.
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.37; system TC—R_f 0.63; system TE—R_f 0.79; system TL—R_f 0.64; system TAE—R_f 0.68; system TAF—R_f 0.72; system TAJ—R_f 0.47; system TAK—R_f 0.04; system TAL—R_f 0.43 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1840, M (nor-) RI 1810, M (nor-OH-)-H_2O RI 1960, M (nor-OH-)-NH_3 RI 1740, M (nor-di-OH-)-$2H_2O$ RI 1920; system GB—RI 1939, M (nor-) RI 1907, M (nor-OH-)-H_2O RI 2058, M (nor-OH-)-NH_3 RI 1840, M (nor-di-OH-)-$2H_2O$ RI 2009, M (nor-)-H_2O RI 1931; system GM—RRT 0.427, M (nor-) RRT 0.423 (both relative to iprindole); system GV—RRT 10.4 min.
High Performance Liquid Chromatography System HX—RI 311; system HY—RI 262; system HZ—RT 2.4 min; system HAA—RT 9.6 min.
Ultraviolet Spectrum Aqueous acid—269 (A^1_1 = 25a), 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1696, 747, 1142, 1120, 712, 1027 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 180, 209, 182, 152, 181, 30, 211, 138.



Quantification

Plasma **GC** Column: SE-30 or OV-17 (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 180° for 8 min to 225° at 15°/min for 3 min. NSD. Limit of detection, 80 µg/L [Stiller *et al.* 1982].

GC-MS Column: DB-5 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 90° to 260° at 25°/min for 10 min. EI ionisation, SIM acquisition mode. Limit of detection, 10 µg/L [Feng *et al.* 1995].

HPLC Column: Chiralcel OD. Mobile phase: *n*-hexane : propan-2-ol (98 : 2). UV detection (λ = 215 nm). Limit of detection, 5 µg/L [Yanagihara *et al.* 2000]. Column: Cyano CN (250 × 4.6 mm, 5 µm). Mobile phase: methanol : acetonitrile : orthophosphoric acid : 0.01 mol/L sodium dihydrogen phosphate (200 : 80 : 2 : 718). UV detection (λ = 215 nm) [Gross *et al.* 1999]. Column: Purospher RP-18. Mobile phase: acetonitrile : 0.03 mol/L phosphate buffer (23 : 77, pH 7.2). Limit of quantification, 5 µg/L [Bolze, Boulieu 1998]. Column: Chiral AGP. Mobile phase: 16% methanol and a 10 µmol/L phosphate buffer (pH 7.0). UV detection (λ = 220 nm). Limit of detection, 5 µg/L [Svensson, Gustafsson 1996]. Column: C_{18}. Mobile phase: acetonitrile : 0.05 mol/L sodium phosphate buffer (pH 4.45, 30 : 70), flow rate 1 mL/min. UV detection (λ = 210 nm). Limit of detection, 5 µg/L [Adams *et al.* 1992]. See also Geisslinger *et al.* [1991].

Serum **GC** FID. Limit of detection, 25 µg/L [Wieber *et al.* 1975].

Urine **GC** See Plasma [Stiller *et al.* 1982]. See Serum [Wieber *et al.* 1975].

GC-MS Column: DB 5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 90° for 2 min to 160° at 30°/min to 275° at 10°/min for 15 min. MSD, EI ionisation, SIM acquisition mode. Retention time: 10.4 min. Limit of detection, 5 µg/L [Olmos-Carmona, Hernandez-Carrasquilla 1999].

Disposition in the Body Ketamine rapidly distributes into tissues including the brain following parenteral administration. It crosses the placenta. It has a bi- or triexponential elimination pattern. In the first phase, ketamine exerts its anaesthetic effects in the CNS, and it is then redistributed into peripheral tissues and metabolised in the liver. Approximately 90% of a dose is excreted in the urine in 72 h, with ~2% of the dose as unchanged drug, 2% as norketamine, 16% as dehydronorketamine and 80% as conjugates of hydroxylated metabolites. Norketamine (which has ~17% of the potency of ketamine) and dehydronorketamine are found in the serum in concentrations similar to those of ketamine. It has been suggested that dehydronorketamine may be an analytical artefact rather than a metabolite.

Therapeutic Concentration

Following single IV injections of 2.5 mg/kg to 5 subjects, serum concentrations of ~1 mg/L were reported after 15 min [Wieber *et al.* 1975].

During continuous IV infusion at an average rate of 41 µg/kg/min to 31 subjects, a mean steady-state plasma concentration of 2.2 mg/L was reported; norketamine and dehydronorketamine had mean peak plasma concentrations of 1.1 mg/L and 0.7 mg/L, respectively, attained at ~3 h. The subjects awoke at an average plasma concentration of 0.64 mg/L [Idvall *et al.* 1979].

Following an IM injection of 0.5 mg/kg in 6 subjects, peak plasma concentrations of 0.10–0.43 mg/L (mean 0.24) were attained in ~0.3 h; norketamine attained a mean peak plasma concentration of 0.09 mg/L at ~1 h [Clements *et al.* 1982].

Toxicity Ketamine produces hallucinogenic effects similar to those of phencyclidine and may be subject to abuse.

The following postmortem tissue concentrations were reported in a fatality involving ketamine: blood 1.8 mg/L, urine 2.0 mg/L, brain 4.3 µg/g, spleen 6.1 mg/g, liver 4.9 µg/g, kidney 3.6 µg/g; ethanol was also detected at a blood concentration of 1700 mg/L [Moore *et al.* 1997].

In a fatality caused by homicidal overdose of ketamine, tissue concentrations at postmortem were as follows: blood 27.4 mg/L, urine 8.51 mg/L, bile 15.2 mg/L, brain 3.24 mg/L, liver 6.6 mg/L, kidney 3.38 mg/L; norketamine was also detected in all samples but not quantified [Licata *et al.* 1994].

Half-life Plasma half-life, ~2–3 h.

Volume of Distribution ~4 L/kg.

Clearance Plasma clearance, ~17 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~0.6.

Protein Binding ~20–50%.

Note For a study of the stability of ketamine and its metabolites in human biological samples, see Hijazi *et al.* [2001]. For a review of the recent trend of ketamine misuse in the USA, see Moore *et al.* [2001].

Dose The equivalent of 1 to 4.5 mg/kg ketamine, by slow IV injection or 6.5 to 13 mg/kg, IM.

Adams HA *et al.* (1992). [The simultaneous determination of ketamine and midazolam using high pressure liquid chromatography and UV detection (HPLC/UV)]. *Anaesthesist* 41: 619–624.
Bolze S, Boulieu R (1998). HPLC determination of ketamine, norketamine, and dehydronorketamine in plasma with a high-purity reversed-phase sorbent. *Clin Chem* 44: 560–564.
Clements JA *et al.* (1982). Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. *J Pharm Sci* 71: 539–542.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.
Feng N *et al.* (1995). Development of a gas chromatography–mass spectrometry method for determination of ketamine in plasma and its application to human samples. *Ther Drug Monit* 17: 95–100.
Geisslinger G *et al.* (1991). Stereoselective high-performance liquid chromatographic determination of the enantiomers of ketamine and norketamine in plasma. *J Chromatogr* 568: 165–176.
Gross AS *et al.* (1999). Simultaneous analysis of ketamine and bupivacaine in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 728: 107–115.
Hijazi Y *et al.* (2001). Stability of ketamine and its metabolites norketamine and dehydronorketamine in human biological samples. *Clin Chem* 47: 1713–1715.
Idvall J *et al.* (1979). Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth* 51: 1167–1173.
Licata M *et al.* (1994). A fatal ketamine poisoning. *J Forensic Sci* 39: 1314–1320.
Moore KA *et al.* (1997). Tissue distribution of ketamine in a mixed drug fatality. *J Forensic Sci* 42: 1183–1185.
Moore KA *et al.* (2001). Urine concentrations of ketamine and norketamine following illegal consumption. *J Anal Toxicol* 25: 583–588.
Olmos-Carmona ML, Hernandez-Carrasquilla M (1999). Gas chromatographic-mass spectrometric analysis of veterinary tranquillizers in urine: evaluation of method performance. *J Chromatogr B Biomed Sci Appl* 734: 113–120.
Stiller RL *et al.* (1982). Gas chromatographic analysis of ketamine and norketamine in plasma and urine: nitrogen-sensitive detection. *J Chromatogr* 232: 305–314.
Svensson JO, Gustafsson LL (1996). Determination of ketamine and norketamine enantiomers in plasma by solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 678: 373–376.
Wieber J *et al.* (1975). Pharmacokinetics of ketamine in man. *Anaesthesist* 24: 260–263.
Yanagihara Y *et al.* (2000). Stereoselective high-performance liquid chromatographic determination of ketamine and its active metabolite, norketamine, in human plasma. *J Chromatogr B Biomed Sci Appl* 746: 227–231.

Ketanserin

Antihypertensive, 5-HT_{2A} Receptor Antagonist

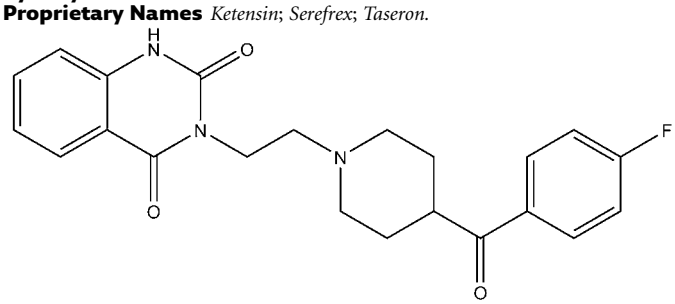
$C_{22}H_{22}FN_3O_3$ = 395.4

CAS—74050-98-9

IUPAC Name 3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,4[1*H*,3*H*]-quinazolinedione

Synonym R-41468

Proprietary Names *Ketensin*; *Serefrex*; *Taseron.*



Chemical Properties Crystals. Mp 227° to 235°. Solubility 0.001 g/100 mL water, 0.038 g/100 mL ethanol, and 2.34 g/100 mL DMF. pK_a 7.5 [O'Neil MJ *et al.* 2006]. Log *P* (octanol/water), 3.21 [ACD 2007]. Stability of standard solutions was verified at 6° for 3 months [Kurowski 1985]. Ketanserin was stable in plasma at −20° for 3 months and there was no effect of freeze-thaw [Okonkwo *et al.* 1983; Yassen *et al.* 2003].

Ketanserin Tartrate

$C_{22}H_{22}FN_3O_3$, $C_4H_6O_6$ = 545.5

CAS—83846-83-7

Synonyms R-49945; Ket.

Proprietary Names *Perketan*; *Serepress*; *Sufrexal.*

High Performance Liquid Chromatography Column: Waters µBondapak phenyl reversed phase (300 × 3.9 mm i.d., 10 µm). Mobile phase: 5.44 g potassium dihydrogen phosphate in approx. 1200 mL water with 608 mL acetonitrile, pH 2.3 made up to 2 L, flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 50 µg/L [Simon, Somani 1982].

Quantification

Blood **HPLC** Column: Macherey Nagel Nucleosil reversed phase (250 × 2 mm i.d., 10 µm). Mobile phase: water : acetonitrile (70 : 30) with 0.2% diethylamine, flow rate 1.0 mL/min. Fluorometric detection (λ_{ex} = 270 nm, λ_{em} = 410 nm). Limit of detection, 0.5 µg/L [Okonkwo *et al.* 1983].

Plasma **HPLC** Column: Hypersil BDS (100 × 4.5 mm i.d., 3 µm). Mobile phase: 0.01 mol/L acetate buffer (pH 4.9) : methanol : acetonitrile (52 : 40 : 8), flow rate 0.9 mL/min. Fluorescence detection (λ_{ex} = 332 nm, λ_{em} = 410 nm). Limit of quantification, 2 µg/L for ketanserin, 1.7 µg/L for ketanserinol [Yassen *et al.* 2003]. Column: RP-18 Chromosorb LC-7 (30 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium acetate (pH 6.8) : 1.0 mol/L ammonium acetate (pH 6.8)-methanol–acetonitrile (10 : 45 : 45; 95 : 5 to 30 : 70 at 50 min), flow rate 1.5 mL/min. UV detection (λ = 230 or 240 nm). Limit of detection, 1 µg/L [Meuldermans *et al.* 1988]. Column: Macherey Nagel Nucleosil (250 × 4 mm i.d., 5 µm). Mobile phase: 2% acetic acid: 0.17 mol/L ammonium acetate : acetonitrile (50 : 10 : 40), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 310 nm, λ_{em} = 370 nm). Limit of detection, 200 ng/L for ketanserin, 100 ng/L for ketanserinol [Kurowski 1985]. Column: Chrompack CP Spher C_8 (250 × 4.6 mm i.d., 8 µm).

Mobile phase: acetonitrile : water (55 : 45) with buffer (pH 5.8) containing 30% acetic acid and 100% diethylamine (10 : 4.25). UV detection ($\lambda = 214$ nm). Limit of detection, 10 µg/L [Lindelauf 1983]. Column: LiChrosorb ODS reversed phase RP_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 0.02 mol/L sodium acetate (pH 4.5, 1 : 4 : 6), flow rate 1.0 mL/min. Limit of detection, 7.9 µg/L [Kacprowicz *et al.* 1983]. Column: Macherey Nagel Nucleosil reversed phase (250 × 2 mm i.d., 10 µm). Mobile phase: water : acetonitrile (70 : 30) with 0.2% diethylamine, flow rate 1.0 mL/min. Fluorometric detection ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 410$ nm). Limit of detection, 0.5 µg/L [Okonkwo *et al.* 1983].

***Urine* HPLC** Column: RP-18 Chromosorb LC-7 (300 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium acetate (pH 6.8) : 1.0 mol/L ammonium acetate (pH 6.8)–methanol–acetonitrile (10 : 45 : 45; 95 : 5 to 30 : 70 at 50 min), flow rate 1.5 mL/min. UV detection ($\lambda = 230$ or 240 nm). Limit of detection, 1 µg/L [Meuldermans *et al.* 1988]. Column: Macherey Nagel Nucleosil (250 × 4 mm i.d., 5 µm). Mobile phase: 2% acetic acid : 0.17 mol/L ammonium acetate : acetonitrile (50 : 10 : 40), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 310$ nm, $\lambda_{em} = 370$ nm). Limit of detection, 200 µg/L for ketanserin, 100 µg/L for ketanserinol [Kurowski 1985]. Column: Macherey Nagel Nucleosil reversed phase (250 × 2 mm i.d., 10 µm). Mobile phase: water : acetonitrile (70 : 30) with 0.2% diethylamine, flow rate 1.0 mL/min. Fluorometric detection ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 410$ nm). Limit of detection, 0.5 µg/L [Okonkwo *et al.* 1983].

Disposition in the Body After oral administration, ketanserin is almost completely absorbed (90%) and peak plasma concentrations are reached within 0.5 to 2 h. Less than 2% of the parent compound is excreted in the urine. The most common mechanism of metabolism of ketanserin is through ketone reduction (29%), leading to the formation of ketanserinol, which is mainly excreted in the urine. Oxidative *N*-dealkylation at the piperidine nitrogen with the formation of the urinary metabolite 1,4-dihydro-2,4-dioxo-3(2*H*)-quinazolineacetic acid accounts for approx. 20% of ketanserin metabolism, while aromatic hydroxylation at the quinazolinedione moiety and the formation of ether glucuronides accounts for the rest.

Therapeutic Concentration Following IV administration, ketanserin concentrations decay triexponentially with sequential half-lives of 0.13, 2 and 14.3 h. Following long-term oral dosing (20 or 40 mg twice daily) steady-state concentrations are reached within 4 days [Persson *et al.* 1991].

Ten healthy male volunteers were administered a 10 mg IV injection, or a 10 mg IM injection, or an oral dose of 20 mg, 40 mg or 60 mg ketanserin. The values were observed as indicated in the table below.

Pharmacokinetics and bioavailability of ketanserin in elderly subjects were in good agreement with those in young volunteers. There is a small tendency towards a longer half-life in the elderly patients, which may be explained by the physiological reduction in elimination processes in the elderly [Kurowski 1985].

Pharmacokinetic parameters in pre-eclamptic women are comparable with those in healthy non-pregnant volunteers [Yassen *et al.* 2003].

Note For a pharmacokinetic study of ketanserin in patients with renal failure, see Barendregt *et al.* [1990].

Bioavailability Approximately 50%.

Half-life Triexponentially decay of 0.13, 2 and 14.3 h after IV administration; approx. 17 h after IM or oral administration.

Volume of Distribution 3 to 6 L/kg.

Clearance Approximately 25 L/h in healthy volunteers; 38 L/h in pre-eclamptic women.

Distribution in Blood Blood : plasma concentration 0.70.

Protein Binding Approximately 95%, mainly to albumin.

Dose 40 mg twice daily.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Barendregt JN *et al.* (1990). Ketanserin pharmacokinetics in patients with renal failure. *Br J Clin Pharmacol* 29: 715–723.

Heykants J *et al.* (1986). Pharmacokinetics of ketanserin and its metabolite ketanserinol in man after intravenous, intramuscular and oral administration. *Eur J Clin Pharmacol* 31: 343–350.

Kacprowicz AT *et al.* (1983). Determination of ketanserin in human plasma by high-performance liquid chromatography. *J Chromatogr* 272: 417–420.

Kurowski M (1985). Bioavailability and pharmacokinetics of ketanserin in elderly subjects. *Eur J Clin Pharmacol* 28: 411–417.

Lindelauf F (1983). Determination of ketanserin and its major metabolite (reduced ketanserin) in human plasma by high-performance liquid chromatography. *J Chromatogr* 277: 396–400.

Meuldermans W *et al.* (1988). Absorption, metabolism and excretion of ketanserin in man after oral administration. *Arzneimittelforschung* 38: 789–794.

Okonkwo PO *et al.* (1983). High-performance liquid chromatographic assay with fluorometric detection of ketanserin, a new antihypertensive agent and serotonin S_2 antagonist in human plasma, blood and urine. *J Chromatogr* 272: 411–416.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Persson B *et al.* (1991). Clinical pharmacokinetics of ketanserin. *Clin Pharmacokinet* 20: 263–279.

Simon V, Somani P (1982). Rapid method for determination of ketanserin, a novel antiserotonin drug, by high-performance liquid chromatography. *J Chromatogr* 232: 186–191.

Yassen A *et al.* (2003). Simultaneous quantitative analysis of ketanserin and ketanserinol in plasma by RP–HPLC with fluorescence detection. *Biomed Chromatogr* 17: 517–521.

Ketazolam

Tranquilliser

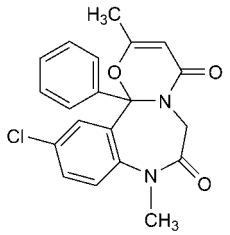
$C_{20}H_{17}ClN_2O_3 = 368.8$

CAS—27223-35-4

IUPAC Name 11-Chloro-2,8-dimethyl-12b-phenyl-6*H*-[1,3]oxazino[3,2-*d*][1,4]benzodiazepine-4,7-dione

Synonyms 11-Chloro-8,12b-dihydro-2,8-dimethyl-12b-phenyl-4*H*-[1,3]oxazino[3,2-*d*][1,4]benzodiazepine-4,7(6*H*)-dione; U-28774.

Proprietary Names *Anseren*; *Contamex*; *Loftran*; *Marcen*; *Sedotime*; *Solatran*; *Unakalm*.



Chemical Properties White crystalline powder. Mp 183°. Slightly soluble in water.

Colour Test Formaldehyde–sulfuric acid—orange.

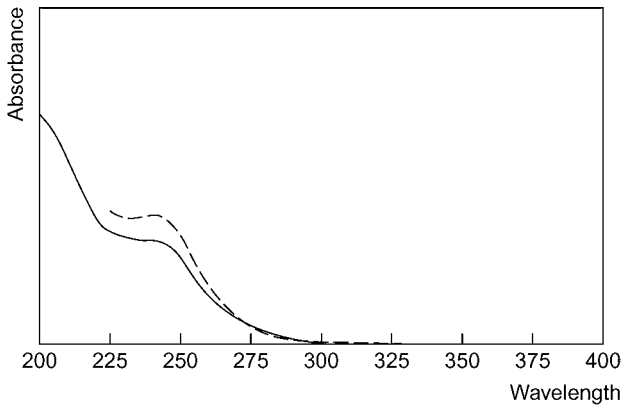
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.14; system TC—R_f 0.64; system TD—R_f 0.45; system TE—R_f 0.74; system TF—R_f 0.45; system TL—R_f 0.66; system TAD—R_f 0.62; system TAE—R_f 0.83; system TAF—R_f 0.80 (Dragendorff spray, positive; furfuraldehyde reagent, dark grey; acidified iodoplatinate solution, positive; mercuric chloride–diphenylcarbazone reagent, pale blue; ninhydrin spray, pale yellow; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—ketazolam RI 2444, diazepam RI 2428, oxazepam RI 2325; system GB—ketazolam RI 2552, diazepam RI 2556, oxazepam RI 2438; system GG—diazepam RI 2940, oxazepam RI 2803 (rearrangement can occur on the column to form diazepam).

Gas Chromatography-Mass Spectrometry Column: SGE BP1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° for 1 min to 250° at 15°/min for 8 min to 300° at 10°/min for 2 min. EI ionisation, scan or SIM acquisition mode. Retention time: 14.7 min. Limit of quantification, 2.5 to 5 mg/L and 0.1 to 0.5 mg/L for scan and SIM modes, respectively [Borrey *et al.* 2001a].

High Performance Liquid Chromatography System HI—*k* 12.81; system HJ—*k* 2.45; system HK—*k* 0.04; system HX—RI 577; system HY—RI 522; system HZ—retention time 10.3 min; system HAX—retention time 7.7 min; system HAY—retention time 9.2 min; system HBH—*k* 10.78; system HBI—*k* 2.22.

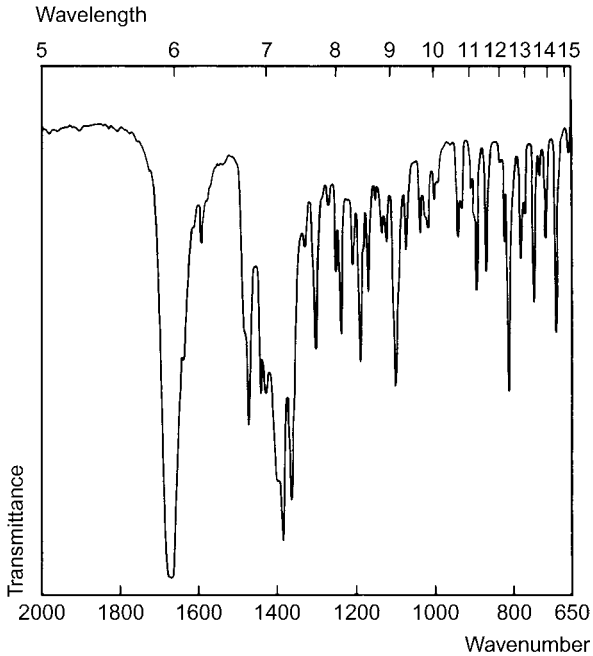
Ultraviolet Spectrum Ethanol—242 nm ($A^{1}_{1} = 492$a).



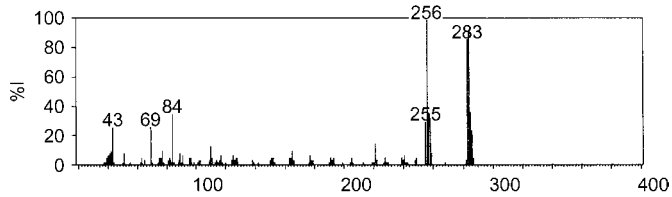
	10 mg IV	*10 mg IM*	*20 mg PO*	*40 mg PO*	*60 mg PO*
C_{max} (ng/mL)	—	196 ± 108	71.4 ± 26.2	198 ± 53.0	287 ± 129
t_{max} (h)	—	9.30 ± 4.20	0.70 ± 0.20	0.60 ± 0.24	0.53 ± 0.22
$T_{1/2\beta}$(h)	14.3 ± 4.40	17.2 ± 4.50	16.5 ± 3.30	17.8 ± 3.80	16.9 ± 3.20
AUC (ng·h/mL)	298 ± 136	333 ± 68.0	279 ± 59.0	625 ± 100	992 ± 183

[Heykants *et al.* 1986].

Infrared Spectrum Principal peaks at wavenumbers 1675, 820, 1105, 1195, 1308, 1242 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 256, 284, 283, 285, 84, 257, 258, 255; oxazepam 257, 77, 268, 239, 205, 267, 233, 259.



Quantification

Urine **GC-MS** Column: SGE BP1 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° for 1 min to 250° at 15°/min for 8 min to 300° at 10°/min for 2 min. EI ionisation, scan or SIM acquisition mode. Limit of detection, 16 and 1.1 µg/mL for scan and SIM mode, respectively [Borrey *et al.* 2001b].

Disposition in the Body Readily absorbed after oral administration. Rapidly metabolised by *N*-demethylation to desmethylketazolam and desmethyldiazepam (nordazepam). About 80% of a dose is excreted in the urine as metabolites with only traces of unchanged drug. Of the material excreted in the urine, about 56% is oxazepam and the remainder is a mixture of minor metabolites, including diazepam, desmethyldiazepam, temazepam, and other unidentified metabolites. About 20% of a dose is eliminated in the faeces, mainly as metabolites.

Therapeutic Concentration

Following a single oral dose of 30 mg of ^{14}C-labelled ketazolam, peak plasma concentrations of about 0.004 mg/L of ketazolam, 0.017 µg/L of diazepam, and 0.127 mg/L of *N*-demethylated metabolites were attained in 2, 10 and 14 h, respectively [Eberts, Jr. 1977].

Toxicity Overdoses of up to 0.5 g have been ingested without serious toxic effects.

Half-life Plasma half-life, ketazolam 1–3 h [Borrey *et al.* 2001a]

Dose 15 to 60 mg daily.

Borrey D *et al.* (2001a). Sensitive gas chromatographic–mass spectrometric screening of acetylated benzodiazepines. *J Chromatogr A* 910: 105–118.

Borrey D *et al.* (2001b). Simultaneous determination of fifteen low-dosed benzodiazepines in human urine by solid-phase extraction and gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765: 187–197.

Eberts FSJr (1977). Disposition of ketazolam, a new anxiolytic agent, in man. *Pharmacologist* 19165.

Ketobemidone

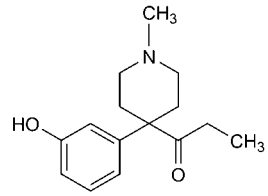
Narcotic Analgesic

$C_{15}H_{21}NO_2$ = 247.3

CAS—469-79-4

IUPAC Name 1-[4-(3-Hydroxyphenyl)-1-methylpiperidin-4-yl]propan-1-one

Synonyms Cetobemidone; 1-[4-(3-hydroxyphenyl)-1-methyl-4-piperidyl]-1-propanone.



Chemical Properties Crystals. Mp 156° to 157°.

Ketobemidone Hydrochloride

$C_{15}H_{21}NO_2$, HCl = 283.8CAS—5965-49-1

Synonyms Hoechst 10720; Win-1539.

Proprietary Names *Cliradon*; *Cymidon*; *Ketodur*; *Ketogan*; *Ketogin*; *Ketorax*.

Chemical Properties Crystals. Mp 201° to 202°. Soluble in water, slightly soluble in alcohol. pK_a 8.7 (20°). Log *P* (heptane/pH 7.4), −3.0.

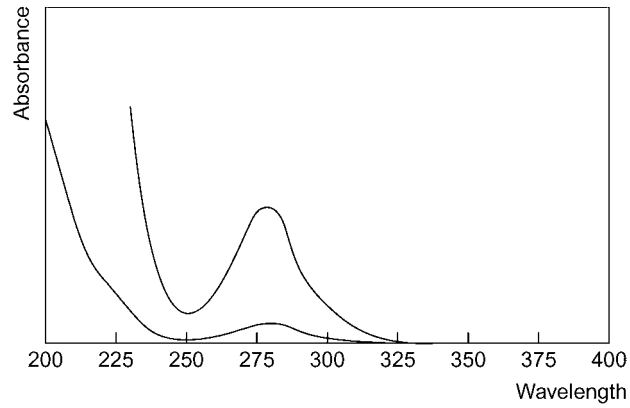
Colour Tests Mandelin's test—blue-green; Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.02; system TC—R_f 0.09; system TE—R_f 0.37; system TL—R_f 0.06; system TAE—R_f 0.26; system TAJ—R_f 0.03; system TAK—R_f 0.01; system TAL—R_f 0.40 (acidified iodoplatinate solution, positive).

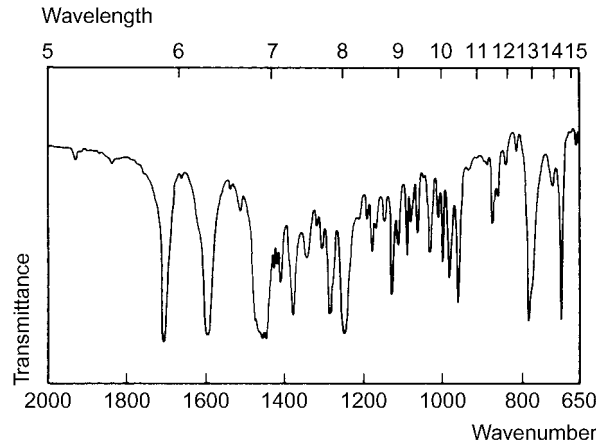
Gas Chromatography System GA—RI 2040.

High Performance Liquid Chromatography System HA—*k* 2.8 (tailing peak); system HX—RI 294; system HY—RI 245.

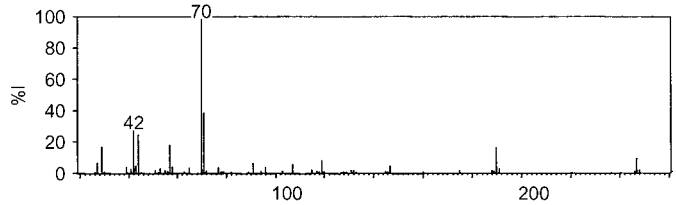
Ultraviolet Spectrum Aqueous acid—280 nm (A^{1}_{1} = 81a); aqueous alkali—300 nm (A^{1}_{1} = 126b).



Infrared Spectrum Principal peaks at wavenumbers 1705, 1597, 1248, 777, 699, 1285 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 70, 71, 42, 44, 57, 190, 29, 247.



Quantification

Blood **GC** Column: HP5 (25 m × 0.32 mm i.d., 0.52 µm). Temperature programme: 160° for 5 min to 220° at 5°/min for 8.5 min to 250° at 20°/min for 3 min. NPD. Limit of detection not reported [Steentoft, Worm 1994].

HPLC Column: RP-18. Mobile phase: acetonitrile : methanol : 10 mmol/L phosphate buffer (pH 7.0; 45 : 30 : 15), flow rate 0.9 mL/min. UV detection ($\lambda = 215$ nm) or electrochemical detection. Limit of detection not reported [Steentoft, Worm 1994].

***Plasma* GC-MS** Column: SE-30 Ultrasep capillary (25 m × 0.3 mm i.d.). Temperature: 220°. EI ionisation at 35 eV. Limit of detection, 5 µg/L [Bondesson *et al.* 1983].

LC-MS Column: Luna C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 25 mmol/L formic acid solution containing 2% acetonitrile : 25 mmol/L formic acid solution containing 70% acetonitrile (95 : 5 to 72 : 28 at 5 min to 0 : 100 for 0.4 min to 95 : 5 for 4.6 min), flow rate 0.3 mL/min. API, ESI, positive ion mode, SIM acquisition mode. Limit of quantification, ≈3 nmol/L. [Svensson *et al.* 2001].

***Urine* GC-MS** See Plasma [Svensson *et al.* 2001].

HPLC Column: ChromSep SS Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: acetonitrile-25 mmol/L potassium phosphate buffer (pH 3.2; 10 : 90) : acetonitrile (100 : 0 to 94 : 6 at 6 min to 70 : 30 at 16 min for 5 min to 40 : 60 at 25 min), flow rate 0.4 mL/min for first 6 min, 0.6 mL/min otherwise. UV detection ($\lambda = 220$ nm). Retention time: 8.39 min. Limit of detection, 5 mg/L [Klinke, Linnet 2007].

LC-MS Column: Luna C_{18}-2 (150 × 2.0 mm i.d., 5 µm). Mobile phase: 20% methanol in 0.1% acetic acid, flow rate 0.2 mL/min. ESI-TOF. Limit of detection not reported [Sundström *et al.* 2001; Sundström *et al.* 2002]. Column: Zorbax Eclipse XDB-C_8 (150 × 3.0 mm i.d., 5 µm). Mobile phase: 4 mmol/L formic acid in water : 4 mmol/L formic acid in acetonitrile (90 : 10 for 1 min to 20 : 80 in 14 min for 2 min to 90 : 10 in 2 min), flow rate 0.5 mL/min. API, positive ion mode, SIM acquisition mode. Limit of detection, 0.025 mg/L [Breindahl, Andreasen 1999].

***Liver* GC** See Blood [Steentoft, Worm 1994].

HPLC See Blood [Steentoft, Worm 1994].

***Muscle* GC** See Blood [Steentoft, Worm 1994].

HPLC See Blood [Steentoft, Worm 1994].

Disposition in the Body Ketobemidone is extensively metabolised by humans. A mean of 72% of an oral dose was recovered as ketobemidone and metabolites in urine, 34–56% being excreted as free and conjugated ketobemidone. The fraction excreted in urine as the *N*-demethylated metabolite norketobemidone amounted 10–31% of an oral dose [Bondesson *et al.* 1983]. Poorly absorbed after oral or rectal administration. Metabolised by *N*-demethylation.

Therapeutic Concentration

Seventeen critically ill patients, many with signs of hepatic dysfunction but no liver failure, many with signs of sepsis, and 5 with renal failure, were administered 5 mg ketobemidone via a gastric tube. The mean maximum plasma concentration was 38 nmol/L at 48 min [Al-Shurbaji, Tokics 2002].

Toxicity

In fatal intoxications in Sweden in 1992–2002, 84 were positive for ketobemidone: 40 were suicides, 12 were accidents and 32 were uncertain [Jönsson *et al.* 2004].

In 76 fatalities attributed to ketobemidone (23 also involving alcohol and 26 also involving other drugs), tissue concentrations were as follows: blood 0.2–3.2 µg/g (median 0.5) after oral administration and 0.1–1.5 µg/g (median 0.3) after injection; liver 0.2– 6.2 µg/g (median 1.3) after oral administration and 0.2–1.3 µg/g (median 0.5) after injection; muscle 0.2–0.5 µg/g (median 0.4) after oral administration and 0.1–1.1 µg/g (median 0.2) after injection. If the blood alcohol concentration was >1 mg/g, the median ketobemidone concentrations were: blood 0.1 µg/g; liver 0.4 µg/g (after both oral and IV injection) [Steentoft, Worm 1994].

Note For fatal intoxications in the Nordic population in 1984 and 1985, see Steentoft *et al.* [1989b]and Steentoft *et al.* [1989a].

Bioavailability ≈40%.

Half-life ≈2 h.

Volume of Distribution ≈2 to 3 L/kg.

Clearance ≈10 mL/min/kg.

Dose Ketobemidone has been given in doses of 5 to 10 mg.

Al-Shurbaji A, Tokics L (2002). The pharmacokinetics of ketobemidone in critically ill patients. *Br J Clin Pharmacol* 54: 583–586.

Bondesson U *et al.* (1983). Simultaneous determination of ketobemidone and its *N*-demethylated metabolite in patient plasma samples by gas chromatography mass spectrometry with selected ion monitoring. *Biomed Mass Spectrom* 10: 283–286.

Breindahl T, Andreasen K (1999). Validation of urine drug-of-abuse testing methods for ketobemidone using thin-layer chromatography and liquid chromatography-electrospray mass spectrometry. *J Chromatogr B Biomed Sci Appl* 736: 103–113.

Jönsson A *et al.* (2004). Fatal intoxications in a Swedish forensic autopsy material during 1992-2002. *Forensic Sci Int* 143: 53–59.

Klinke HB, Linnet K (2007). Performance of four mixed-mode solid-phase extraction columns applied to basic drugs in urine. *Scand J Clin Lab Invest* 67: 778–782.

Steentoft A, Worm K (1994). Cases of fatal intoxication with Ketogan. *J Forensic Sci Soc* 34: 181–185.

Steentoft A *et al.* (1989a). Fatal intoxications in the age group 15-34 years in Denmark in 1984 and 1985. A forensic study with special reference to drug addicts. *Z Rechtsmed* 103: 93–100.

Steentoft A *et al.* (1989b). Fatal intoxications in the Nordic countries. A forensic toxicological study with special reference to young drug addicts. *Z Rechtsmed* 102: 355–365.

Sundström I *et al.* (2001). Identification of phase I and phase II metabolites of ketobemidone in patient urine using liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 763: 121–131.

Sundström I *et al.* (2002). Identification of glucuronide conjugates of ketobemidone and its phase I metabolites in human urine utilizing accurate mass and tandem time-of-flight mass spectrometry. *J Mass Spectrom* 37: 414–420.

Svensson JO *et al.* (2001). Determination of ketobemidone and its metabolites in plasma and urine using solid-phase extraction and liquid chromatography-mass spectrometry. *Ther Drug Monit* 23: 399–405.

Ketoconazole

Antifungal

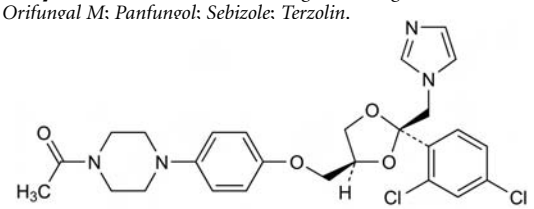
$C_{26}H_{28}Cl_2N_4O_4 = 531.4$

CAS—65277-42-1

IUPAC Name *cis*-1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine

Synonym R-41400

Proprietary Names *Daktarin Gold*; *Fungarest*; *Fungoral*; *Ketoderm*; *Ketoisdin*; *Nizoral*; *Orifungal M*: *Panfungol*: *Sebizole*: *Terzolin*.

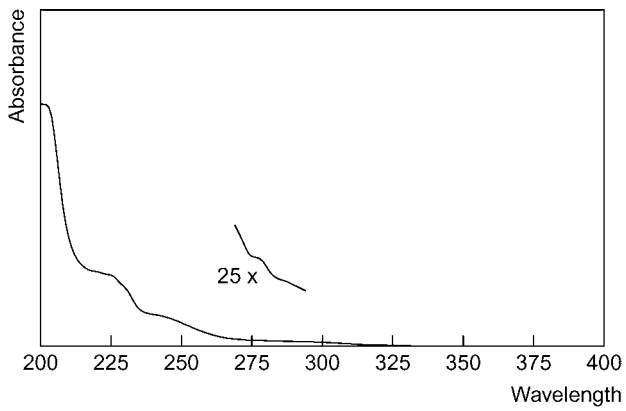


Chemical Properties Crystals. Mp 146°. Practically insoluble in water; soluble 1 in 54 of ethanol, 1 in about 2 of chloroform and 1 in 9 of methanol; very slightly soluble in ether. Log *P* (octanol/water), 4.4.

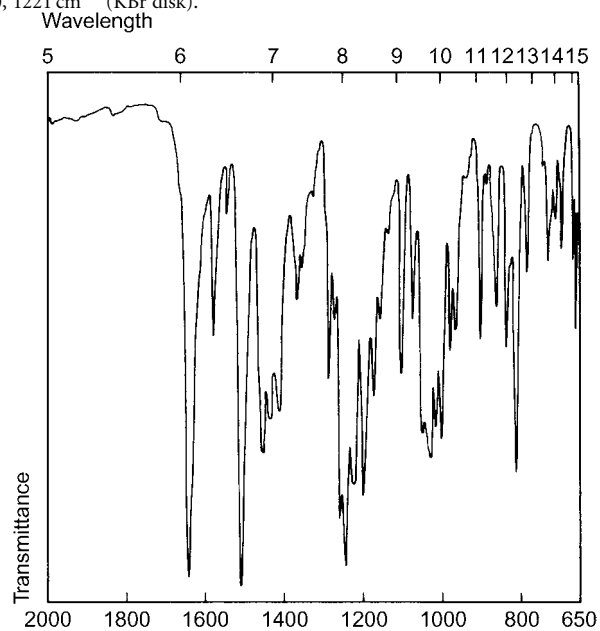
Thin-layer Chromatography System TB—R_f 00; system TE—R_f 0.50; system TAE—R_f 0.68.

High Performance Liquid Chromatography System HX—RI 439; system HY—RI 464; system HZ—retention time 5.2 min; system HAA—retention time 15.7 min.

Ultraviolet Spectrum Aqueous acid—269 nm ($A^1_1 = 26$a); aqueous alkali—287 nm ($A^1_1 = 29$b); methanol—244 ($A^1_1 = 280$b), 296 nm ($A^1_1 = 32$b).



Infrared Spectrum Principal peaks at wavenumbers 1507, 1640, 1240, 1258, 1200, 1221 cm^{-1} (KBr disk).



Quantification

***Plasma* HPLC** Fluorescence detection. Limit of detection, 40 µg/L [Yuen, Peh 1998]. Electrochemical detection. For method, see Hoffman *et al.* [1988]. Fluorescence detection. Limit of detection, 40 µg/L [Pascucci *et al.* 1983]. UV detection. Limit of detection, <100 µg/L [Alton 1980].

HPLC–MS Limit of detection, <20 µg/L [Chen *et al.* 2002].

Serum **HPLC** See Plasma [Pascucci *et al.* 1983].
Cerebrospinal Fluid **HPLC** See Plasma [Pascucci *et al.* 1983].
Saliva **HPLC** See Plasma [Hoffman *et al.* 1988].
Synovial Fluid **HPLC** See Plasma [Pascucci *et al.* 1983].
Disposition in the Body Ketoconazole is readily but incompletely absorbed after oral administration and extensively metabolised to inactive metabolites. About 13% of a dose is excreted in the urine in 4 days and 57% is eliminated in the faeces in the same period.
Therapeutic Concentration
Following single oral doses of 200 and 400 mg to 6 subjects, mean peak serum concentrations of 3.6 and 6.5 mg/L were attained in 2 and 3 h, respectively [Daneshmend *et al.* 1981].
Half-life Plasma half-life, about 6 to 10 h.
Distribution in Blood Plasma : whole blood ratio, 1.6.
Protein Binding About 99%.
Note For a review of the pharmacokinetics of systemic antifungal drugs, see Daneshmend and Warnock [1983].
Dose 200 to 400 mg daily.

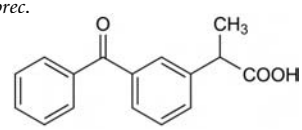
Alton KB (1980). Determination of the antifungal agent, ketoconazole, in human plasma by high-performance liquid chromatography. *J Chromatogr* 221: 337–344.
Chen YL *et al.* (2002). Determination of ketoconazole in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 774: 67–78.
Daneshmend TK *et al.* (1981). Pharmacokinetics of ketoconazole in normal subjects. *J Antimicrob Chemother* 8: 299–304.
Daneshmend TK, Warnock DW (1983). Clinical pharmacokinetics of systemic antifungal drugs. *Clin Pharmacokinet* 8: 17–42.
Hoffman DW *et al.* (1988). Electrochemical detection for high-performance liquid chromatography of ketoconazole in plasma and saliva. *Anal Biochem* 172: 495–498.
Pascucci VL *et al.* (1983). Quantitation of ketoconazole in biological fluids using high-performance liquid chromatography. *J Pharm Sci* 72: 1467–1469.
Yuen KH, Peh KK (1998). Simple high-performance liquid chromatographic method for determination of ketoconazole in human plasma. *J Chromatogr B Biomed Sci Appl* 715: 436–440.

K

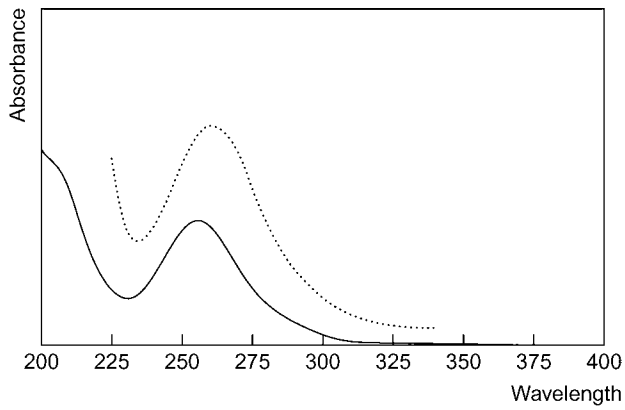
Ketoprofen

Analgesic
$C_{16}H_{14}O_3 = 254.3$
CAS—22071-15-4

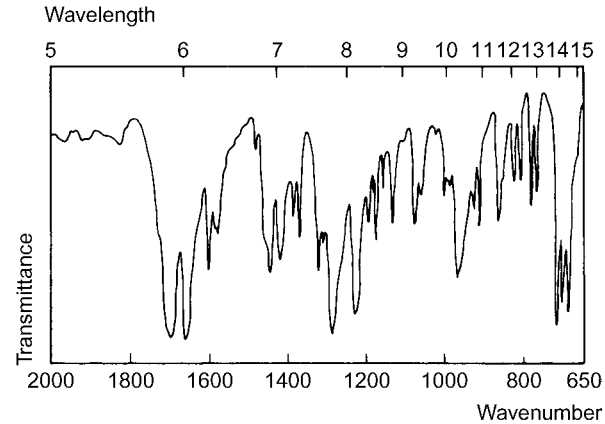
IUPAC Name 3-Benzoyl-α-methylbenzeneacetic acid
Synonym RP-19583
Proprietary Names *Actron*; *Actroneffix*; *Alrheumat*; *Alrheumun*; *Apo-Keto*; *Fastum*; *Fenoket*; *Gabrilen*; *Jomethid*; *Keral*; *Ketil*; *Ketocid*; *Ketofen*; *Ketolist*; *Ketotard*; *Ketovail*; *Ketozip*; *Ketum*; *Larafen*; *Orudis*; *Oruvail*; *Powergel*; *Profenid*; *Solpaflex*; *Topfena*; *Toprec*.



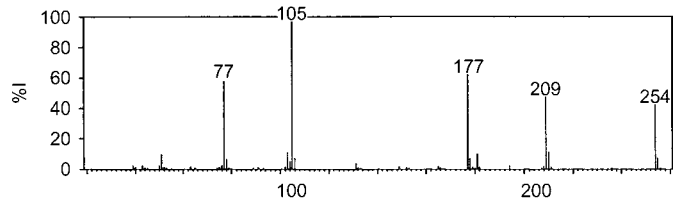
Chemical Properties A white crystalline powder. Mp 93° to 96°. Slightly soluble in water; soluble in acetone, ethyl acetate, ethanol, chloroform and ether. pK_a 4.5. Log *P* (octanol/buffer pH 7.4), 0.
Colour Tests Aromaticity (method 2)—colourless/yellow; Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TD—R_f 0.27; system TE—R_f 0.06; system TF—R_f 0.25; system TG—R_f 0.14; system TAD—R_f 0.41; system TAE—R_f 0.85; system TAJ—R_f 0.54; system TAK—R_f 0.82; system TAL—R_f 0.98 (Ludy Tenger reagent, orange).
Gas Chromatography System GA—ketoprofen RI 2245, ketoprofen-Me RI 2090; system GD—(methyl derivative) retention time 1.45 relative to n-$C_{16}H_{34}$; system GL—ketoprofen-Me RI 2090, M (OH-)-Me_2 RI 2250.
High Performance Liquid Chromatography System HAA—retention time 19.6 min; system HD—*k* 2.4; system HV—retention time 0.66 relative to meclofenamic acid; system HX—RI 495; system HZ—retention time 6.4 min.
Ultraviolet Spectrum Aqueous acid—260 nm ($A^1_1 = 665$a); aqueous alkali—262 nm ($A^1_1 = 647$a).



Infrared Spectrum Principal peaks at wavenumbers 1656, 1693, 1284, 714, 690, 1226 cm^{-1} (KBr disk). Two polymorphic forms may occur.



Mass Spectrum Principal ions at *m/z* 105, 177, 77, 209, 254, 210, 103, 181.



Quantification

Plasma **GC** ECD. Limit of detection, 130 µg/L [Stenberg *et al.* 1979].
GC–MS SIM acquisition mode. Ketoprofen and ibuprofen. Limit of detection, <2 µg/L for ketoprofen [Jack *et al.* 1992].
HPLC Limit of detection, 20 µg/L [Kokki *et al.* 2002]. Limit of detection, 1 mg/L [Rifai *et al.* 1997]. For method for quantification of ketoprofen and its acyl glucuronides, see Grubb *et al.* [1996]. UV detection. For method, see Carr *et al.* [1995]. For method for quantification of ketoprofen enantiomers, see Oda *et al.* [1992]; Shibukawa *et al.* [1992]; Lovlin *et al.* [1996]; Boisvert *et al.* [1997]. Limit of detection, 150 µg/L for ketoprofen enantiomers [Hayball *et al.* 1991]. UV detection. Enantiomers of ketoprofen, ibuprofen and fenoprofen. Limit of detection, 0.1 mg/L for ketoprofen [Menzel-Soglowek *et al.* 1990]. UV detection. Limit of detection, 20 µg/L for ketoprofen enantiomers [Yagi *et al.* 1990]. UV detection. For method for quantification of ketoprofen and other NSAIDs, see Streete [1989]. UV detection. Limit of detection, 50 µg/L [Kaye *et al.* 1981].
Serum **HPLC** See Plasma [Streete 1989].
Urine **HPLC** See Plasma [Carr *et al.* 1995]. See Plasma [Kaye *et al.* 1981].
Cerebrospinal Fluid **HPLC** See Plasma [Kokki *et al.* 2002].
Dialysate **HPLC** See Plasma [Grubb *et al.* 1996].
Synovial Fluid **GC–MS** See Plasma [Jack *et al.* 1992].
Disposition in the Body Ketoprofen is readily absorbed after oral, rectal, or IM administration. About 75% of a single oral dose is excreted in the urine in 24 h, mostly in the first 6 h, about 90% of which is the glucuronide conjugate; hydroxylation may also occur.
Therapeutic Concentration
After oral administration of 100 mg as capsules, to 7 subjects, peak plasma concentrations of 6.0 to 14.3 (mean 10) mg/L were attained in 0.45 to 2.5 h; a single rectal dose of 100 mg produced peak plasma concentrations of 4.7 to 10.5 (mean, 7.5) mg/L in 0.75 to 1.5 h, and an IM dose of 100 mg produced peak concentrations of 8.3 to 13.2 (mean, 10.4) mg/L in 0.33 to 0.5 h. After oral doses of 50 mg four times a day to 7 subjects, mean maximum steady-state concentrations of 5.6 mg/L were reported [Ishizaki *et al.* 1980].
After administration of a single oral dose of 200 mg (as sustained-release granules) to 12 subjects, mean peak plasma ketoprofen levels of 4.51 mg/L were attained in 2 h and remained practically constant for at least 12 h. The same dose of a conventional sustained-release capsule resulted in a mean peak concentration of 5.91 mg/L at 4.17 h and administration of 100 mg ketoprofen, twice at a 12-h interval, as a prompt-release capsule produced mean peak plasma concentrations of 10.52 mg/L 1.38 h after the first dose and 12.80 mg/L 1.46 h after the second dose [Roda *et al.* 2002].
Half-life Plasma half-life, 1 to 4 h.
Volume of Distribution About 0.1 to 0.2 L/kg.
Clearance Plasma clearance, about 1 to 2 mL/min/kg.
Protein Binding About 95%.
Note For a review of the pharmacokinetics of ketoprofen, see Jamali and Brocks [1990].
Dose 100 to 200 mg daily.

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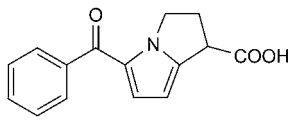
Ketorolac

Analgesic, COX Inhibitor, NSAID
$C_{15}H_{13}NO_3$ = 255.3
CAS—74103-06-3

IUPAC Name 5-Benzoyl-2,3-dehydro-1*H*-pyrrolizine-1-carboxylic acid
Synonym RS-37619



Chemical Properties Crystals from ethyl acetate/ether. Mp 160° to 161°.

Ketorolac Tromethamine

$C_{19}H_{24}N_2O_6$ = 376.4
CAS—74103-07-4
Synonyms (±)-5-Benzoyl-2,3-dehydro-1*H*-pyrrolizine-1-carboxylic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1 : 1); RS-37619-00-31-3.
Proprietary Names *Acular*; *Dolac*; *Droal*; *Lixidol*; *Tarasyn*; *Tonum*; *Toradol*; *Toratex*.
Chemical Properties (+)-isomer: crystals from ethyl acetate/hexane, Mp 174° (Mp 154° to 156° also reported). (−)-isomer: crystals from ethyl acetate/hexane, Mp 169° to 170° (Mp 153° to 155° also reported). pK_a 3.49. Log *P* (octanol/water), 0.26 [Logan *et al.* 1995], 2.32. Stable through 3 freeze-thaw cycles, during storage for 6 months, during storage in an autosampler for 48 h, and in stock solutions for 48 h. Ketorolac tromethamine was also stable on plates stored for 48 h [López-Bojórquez *et al.* 2008]. Samples were stable for up to 18 months when stored at –20°, for 1 month when stored refrigerated (1–4°) and for 1 week when stored at room temperature (20–23°) [Tsina *et al.* 1996].

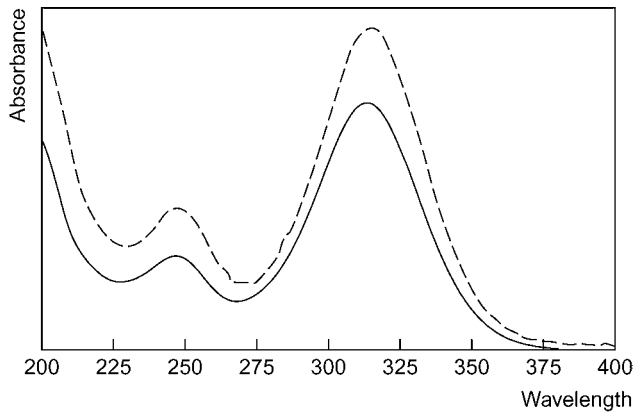
High Performance Liquid Chromatography System HAX—retention time 6.3 min; system HAY—retention time 5.6 min; system HZ—retention time 4.1 min.

Column: μBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: acetonitrile : water : acetic acid (40 : 60 : 0.2), flow rate 1.3 mL/min. UV detection (λ = 313 nm). Limit of detection not reported [Yang *et al.* 2007].

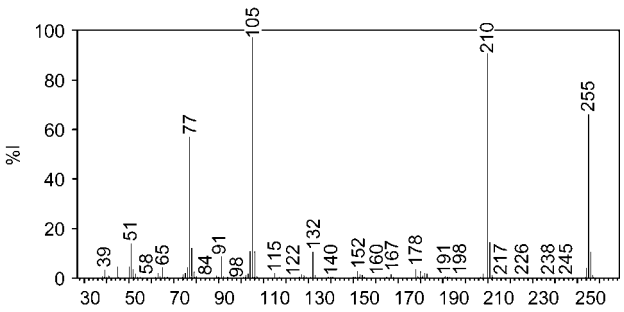
Column: Radialpak, RCM 100 radial compression module with C_{18} (100 × 8 mm i.d., 10 μm). Mobile phase: water : acetonitrile : dibutylamine phosphate (pH 2.5; 30 : 20 : 1), flow rate, 1.8 mL/min tolmetin sodium. UV detection (λ = 313 nm). Retention time: 6.5 min for ketorolac; 9.4 min for IS [Wang *et al.* 2001].

Column: RP C_{18} Nucleosil (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (with 10 mmol/L sodium dihydrogen orthophosphate, and 1 mmol/L sodium lauryl sulfate, pH 2.8; 35 : 65), flow rate 1.5 mL/min. UV detection (λ = 355 nm). Retention time: 10.3 min [Mason, Hobbs 1995].

Ultraviolet Spectrum Methanol—245, 312 nm [Logan *et al.* 1995].



Mass Spectrum Principal ions at *m/z* 105, 210, 255, 77, 51, 132, 91, 65.



Quantification

Blood **GC-MS** Column: CP-SIL fused silica WCOT (8.15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 7 psi. Temperature programme: 100° for 2.5 min to 290° at 25°/min for 3 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [Campobasso *et al.* 2008]. Column: Econocap BP-5 (30 m, 2.3 μm) 5% phenylmethyl silicone. Temperature programme: 100° to 295° over 15 min for 5 min. MSD. Limit of detection not reported [Logan *et al.* 1995].

HPLC Column: Spherisorb ODS II (150 × 4.6 mm i.d., 5 μm), ProntoSIL 120-5-C_{18}-AQ (250 × 2 mm i.d., 5 μm), or Nucleosil 120-5 C_{18} (250 × 3 mm i.d., 5 μm). Mobile phase: 20 mmol/L ammonium acetate (pH 3.7) : acetonitrile (95 : 5 to 20 : 80 at 10 min for 2 min to 95 : 5 at 12 min in 2 min for 8 min), flow rate 1 mL/min. UV detection (λ = 270 nm). Limit of detection, 5 ng [Sultan *et al.* 2005]. Column: Lichrospher RP-8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L potassium hydrogen phosphate-hydrogen phosphate buffer (pH 3; 36 : 64), flow rate 1.5 mL/min. DAD (λ = 312 nm). Limit of detection not reported [Logan *et al.* 1995]. Column: Novapak C_{18} (150 × 3.9 mm i.d., 4 μm). Mobile phase: acetonitrile : 1 mmol/L aqueous *o*-phosphoric acid (pH 3.0; 32 : 68), flow rate 1 mL/min. UV detection (λ = 313 nm). Limit of detection, 3 μg/L [Flores-Murrieta *et al.* 1994].

LC-MS Column: Hypersil BDS C_{18} (100 × 1 mm i.d., 3 μm). Mobile phase: 20 mmol/L ammonium acetate (pH 3.7) : acetonitrile (99 : 1 to 35 : 65 in 10 min for 2 min to 99 : 1 in 2 min for 6 min), flow rate 150 μL/min. APCI, negative ion mode, full scan mode. Retention time: 9.64 min. Limit of detection, 3 mg/L [Sultan *et al.* 2005].

Plasma **HPTLC** Plates: Macherey-Nagel silica gel 60 (20 × 10 cm or 10 × 10 cm, 0.2 mm). Mobile phase: *n*-butanol: chloroform : acetic acid : ammonium hydroxide : water (9 : 3 : 5 : 1 : 2). UV detection (λ = 323 nm). R_f 0.90. Limit of quantification, 200 μg/L, limit of detection, 100 μg/L [López-Bojórquez *et al.* 2008].

HPLC Column: Zorbax Eclipse XDB-C18 (15 cm × 4.6 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium acetate buffer (pH 3.0) : acetonitrile (56 : 44), flow rate 1.0 mL/min. UV detection (λ = 313 nm). Retention time: 3.9 min. Limit of quantification, 0.1 mg/L, limit of detection, 0.03 mg/L [Galán-Herrera *et al.* 2008]. Column: Zorbax eclipse XDB-C_{18} (15 cm × 4.6 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium acetate buffer (pH 3.0) : acetonitrile (56 : 44), flow rate 1.0 mL/min. UV detection (λ = 313 nm). Limit of quantification, 200 μg/L; limit of detection, 100 μg/L [López-Bojórquez *et al.* 2008]. Column: Radial-Pak C_{18} (10 cm × 8 mm i.d., 10 μm). Mobile phase: water : acetonitrile : 1.0 mol/L dibutylamine phosphate (DBAP, pH 2.5, 30 : 20 : 1), flow rate 1.8 mL/min. UV detection (λ = 313 nm). Retention time: 6.5 min. Limit of detection, 0.05 mg/L [Wang *et al.* 2001]. Column: chiral AGP (100 × 4 mm i.d., 5 μm). Mobile phase: isopropanol : 0.05 mol/L phosphate buffer (pH 5.5; 5 : 95). UV detection (λ = 317 nm). Limit of quantification, 0.02 mg/L [Hamunen *et al.* 1999]. Column: μBondapak C_{18} (5 μm). Mobile phase: acetonitrile : 0.05 mol/L sodium acetate buffer (pH 6.0; 35 : 65), flow rate 1–2 mL/min. UV detection (λ = 313 nm). Limit of quantification, 5 μg/L [Kauffman *et al.* 1999].

See also Vakily *et al.* [1995], Tsina *et al.* [1996], Mason, Hobbs [1995], Jones, Bjorksten [1994], Mills *et al.* [1994], Flores-Murrieta *et al.* [1994], Mroszczak *et al.* [1987].

Serum **HPLC** Column: LiChrospher 100 RP-18 (12.5 × 4 mm i.d., 5 mm). Mobile phase: acetonitrile : acetate buffer (pH 3.0; 30 : 70). UV detection (λ = 320 nm). Retention time: 4.2 min. Limit of detection, 0.05 mg/L [González-Martin *et al.* 1997]. Column: Chiral-HSA (100 × 4 mm i.d.). Mobile phase: propan-2-ol : 20 mmol/L potassium phosphate buffer (pH 5.5; 10 : 90), flow rate 0.8 mL/min. UV detection (λ = 310 nm). Limit of detection, 2 mg/L [Hayball *et al.* 1994a]. Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile : water (pH 2.8; 40 : 60), flow rate 1.4 mL/min. UV detection (λ = 313 nm). Limit of detection, 0.01 mg/L [Chaudhary *et al.* 1993].

Urine **HPLC** See Plasma [Kauffman *et al.* 1999].

Oral Fluid **HPTLC** Plates: 60F254 silica gel (10 × 10 cm, 0.2 mm). Solvent system: chloroform : ethyl acetate : glacial acetic acid (3 : 8 : 0.1). UV detection (λ = 323 nm). R_f 0.62. Limit of quantification, 40 ng, limit of detection, 20 ng [Devarajan *et al.* 2000].

Other **HPLC** *In-Vitro* Samples. Column: Novapak (100 × 8 mm i.d., 4 μm) or Chiral-AGP (100 × 4 mm i.d.). Mobile phase: acetonitrile : 20 mmol/L sodium acetate buffer (pH 3.0; 40 : 60), flow rate 1.5 mL/min. Retention time: ≈6.5 min. Scintillation counting. Limit of detection not reported [Hayball *et al.* 1994b].

Disposition in the Body Ketorolac tromethamine is more hydrophilic than ketorolac, and is rapidly absorbed after oral and IM administration. Peak plasma concentrations are reached between 30 and 60 min. The presence of food reduces the rate of absorption but not the extent. It is metabolised via conjugation and hydroxylation, but does not undergo a significant degree of pre-systemic metabolism. The major metabolites are the acyl glucuronide and *p*-hydroxyketorolac, which represent 77% and 12%, respectively, of an oral dose as urinary excretion products. However, the glucuronic acid conjugates are not detected in plasma. *p*-hydroxyketorolac has about 20% of the anti-inflammatory activity and 1% of the analgesic activity of ketorolac. A small amount is distributed in breast milk. It does not readily penetrate the blood–brain barrier but crosses the placenta.

Therapeutic Concentration The serum therapeutic concentration is 0.22–3.50 mg/L.

A study in 27 healthy Mexican adults showed that a test formulation of 30 mg sublingual ketorolac was equivalent to the reference with mean maximum plasma concentrations of 3.61 and 3.44 mg/L reached at 0.66 and 0.94 h, respectively [Galán-Herrera *et al.* 2008].

Six healthy subjects were administered a single sublingual dose of 30 mg ketorolac. The mean peak plasma concentration was ≈3.0 mg/L at ≈1 h [López-Bojórquez *et al.* 2008].

Eighteen children (6–11 years), 18 adolescents (12–17 years) and 18 adults (18–44 years) were administered 0.5 mg/kg racemic ketorolac tromethamine IV at the end of eye surgery. The pharmacokinetic parameters shown below were measured in each group:

	S(−)-Ketorolac	*R(+)-Ketorolac*
Children		
CL (mL/h/kg)	66.8 ± 13.8	21.1 ± 4.9
V_{ss} (L/kg)	0.715 ± 0.415	0.155 ± 0.034
AUC $_{(0-\infty)}$ (μg·min/mL)	2.63 ± 0.48	8.50 ± 2.18
$t_{1/2}$ (min)	13.2 ± 6.8	6.8 ± 1.4
Adolescents		
CL (mL/h/kg)	66.2 ± 17.5	21.4 ± 5.9
V_{ss} (L/kg)	0.442 ± 0.305	0.141 ± 0.030
AUC $_{(0-\infty)}$ (μg·min/mL)	2.73 ± 0.67	8.51 ± 2.3
$t_{1/2}$ (min)	9.5 ± 5.8	6.5 ± 1.0
Adults		
CL (mL/h/kg)	57.7 ± 11.0	22.2 ± 4.3
V_{ss} (L/kg)	0.282 ± 0.14	0.129 ± 0.022
AUC $_{(0-\infty)}$ (μg·min/mL)	3.04 ± 0.55	7.92 ± 1.6
$t_{1/2}$ (min)	7.6 ± 4.8	6.2 ± 1.5

[Hamunen *et al.* 1999]

Fifty children with a median age of 11.6 years (range, 3–18 years) received 0.6 mg/kg racemic ketorolac IV. The mean maximum plasma concentration was 4968 ± 1641 μg/L. The pharmacokinetic parameters shown below were measured for the ketorolac enantiomers.

Parameter	*S(−)-Ketorolac*	*R(+)-Ketorolac*
C_{max} (μg/L)	1609 ± 751	2845 ± 1007
V_d (L/kg)	0.82 ± 0.38	0.5 ± 0.34
$t_{1/2}\alpha$ (min)	17 ± 21	32 ± 29
$t_{1/2}\lambda$ (min)	107 ± 59	259 ± 131
AUC $_{(0-\infty)}$ (μg·min/mL)	126 ± 63	508 ± 208
Plasma CL (mL/min/kg)	6.2 ± 3.3	1.4 ± 0.5

[Kauffman *et al.* 1999]

Fourteen children (2–8 years) were administered a single IV dose of ketorolac 0.5 or 0.9 mg/kg. The mean maximum plasma concentration was 4.8 ± 0.59 and 9.1 ± 2 mg/L following the low and high dose, respectively [González-Martin *et al.* 1997].

Twenty adult male volunteers received either 30 mg ketorolac tromethamine IV 4 times daily (13) or placebo (7) for 5 days. Following the first dose the mean maximum plasma concentration was 5.01 mg/L at 3.2 min. Following the last dose the C_{max} was 5.42 mg/L at 4.2 min, an increase of 8% [Lucker *et al.* 1994].

Thirteen healthy men and women aged between 65 and 78 years (mean, 72 years), and 16 young male volunteers aged between 20 and 39 years (mean, 30 years) were administered an oral dose of 10 mg ketorolac or 30 mg IM. Mean peak plasma levels of 2.99 mg/L (range, 1.60 to 4.78) and 2.52 mg/L (1.45 to 3.82) were obtained for the young and elderly, respectively, after the IM dose. The times to peak concentrations were 0.75 and 0.97 h (range, 0.33 to 2.0 h). Mean peak plasma concentrations of 0.86 mg/L (range 0.55 to 1.10) and 0.90 mg/L (0.57 to 1.44) were reached with the oral dose in the elderly and young, respectively. The times to peak concentrations were 0.33 and 0.72 h (range 0.33 to 1.0), respectively [Jallad *et al.* 1990].

Note For a study on the transdermal delivery of ketorolac, see Roy *et al.* [1995].

Toxicity Adverse effects include nausea, rash oedema, headache and hypertension, occurring at ketorolac plasma concentration above 5 mg/L.

A 45-year-old Caucasian female died after acute asthmatic episodes. The postmortem blood concentration of ketorolac was 1.9 mg/L [Campobasso *et al.* 2008].

A 53-year-old female was found dead near a syringe and 3 empty vials of Toradol. The postmortem blood concentration was 8 mg/L [Oliva *et al.* 2007].

A 30-year-old Caucasian female was administered with an IM injection of 60 mg of ketorolac tromethamine after complaining of acute lower back pain. Shortly afterwards she suffered an apparent cardiac arrest. Blood postmortem examination revealed the presence of phenytoin, 31.1 mg/L, diazepam, 0.1 mg/L, lidocaine, 4.0 mg/L, procainamide, 9.8 mg/L and *N*-acetyl procainamide, 11.3 mg/L. All these drugs were administered during attempts at resuscitation. The ketorolac concentration was 2.9 mg/L and, although this is within the therapeutic range and consistent with the administered dose of 60 mg, the cause of death was ruled to be an acute anaphylactic drug reaction to ketorolac [Logan *et al.* 1995].

Bioavailability 81–100% after administration of an oral solution.

Half-life 3–6 h; 4–7 h (elderly); 9–10 h (renal impairment). 4 h in children [Kauffman *et al.* 1999].

Volume of Distribution 0.15–0.33 L/kg [Baselt 2008], 0.35 ± 0.2 L/kg in children [Kauffman *et al.* 1999], 113 ± 33 mL/kg in children [Dsida *et al.* 2002].

Clearance Oral dose: 0.39 mL/min/kg (elderly); 0.55 mL/min/kg (young). IM dose: 0.21 to 0.57 mL/min/kg (mean 0.32); 0.28 to 0.76 mL/min/kg (mean 0.44). 1.1 ± 0.5 mL/min/kg in children [Kauffman *et al.* 1999]. 0.57 ± 0.17 mL/min/kg in children [Dsida *et al.* 2002].

Protein Binding >99% [Mroszczak *et al.* 1987].

Dose Oral: 10 mg every 4 to 6 h (6 to 8 h for the elderly) with a maximum of 40 mg daily. IV: initial dose of 10 mg followed by 10 to 30 mg every 4 to 6 h. Maximum daily dose is 90 mg and 60 mg for the elderly and patients weighing <50 kg.

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Vakily M *et al.* (1995). The problem of racemization in the stereospecific assay and pharmacokinetic evaluation of ketorolac in human and rats. *Pharm Res* 12: 1652–1657.
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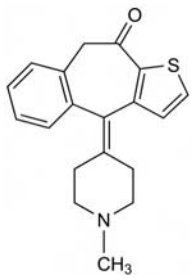
Yang JH *et al.* (2007). Preparation and evaluation of ketorolac tromethamine gel containing genipin for periodontal diseases. *Arch Pharm Res* 30: 871–875.

Ketotifen

Antiallergic
$C_{19}H_{19}NOS$ = 309.4
CAS—34580-13-7

IUPAC Name 4,9-Dihydro-4-(1-methyl-4-piperidinylidene)-10*H*-benzo[4,5]cyclohepta-[1,2-*b*]thiophen-10-one



Chemical Properties Crystals. Mp 151° to 153°.

Ketotifen Fumarate

$C_{19}H_{19}NOS, C_4H_4O_4$ = 425.5
CAS—34580-14-8

Proprietary Names *Airvitess*; *Asdron*; *Asmafen*; *Asmalergin*; *Asmax*; *Asmen*; *Astifat*; *Broncoten*; *Cipanfeno*; *Ketasma*; *Ketof*; *Nemesil*; *Padiatifen*; *Profilasmin*; *Profiten*; *Quefeno*; *Zaditen*; *Zaditor*; *Zatofug*.

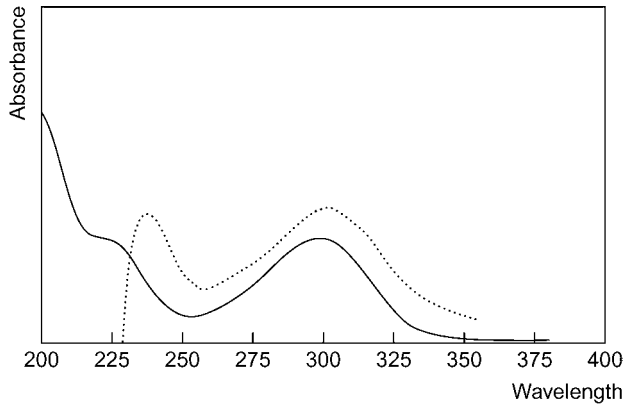
Chemical Properties A white crystalline powder. Mp 192°, with decomposition. Slightly soluble in water; soluble in ethanol; sparingly soluble in chloroform.

Thin-layer Chromatography System TB—R_f 0.24; system TE—R_f 0.52; system TAE—R_f 0.24.

Gas Chromatography System GA—RI 2607.

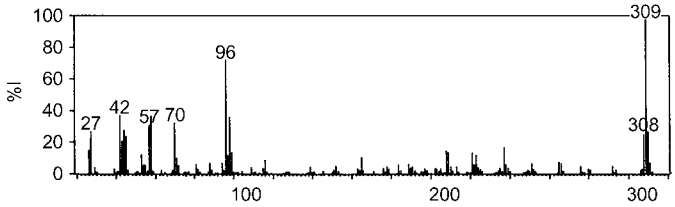
High Performance Liquid Chromatography System HX—RI 373; system HY—RI 315.

Ultraviolet Spectrum Aqueous acid—297 nm (A^1_1 = 475a); aqueous alkali—237, 302 nm.



Infrared Spectrum Principal peaks at wavenumbers 1650, 1280, 1255, 1302, 1720, 1180 cm^{-1} (ketotifen fumarate, KBr disk).

Mass Spectrum Principal ions at *m/z* 309, 96, 42, 58, 98, 70, 57, 44.



Quantification

***Plasma* GC–MS** Limit of detection, 0.01 µg/L [Tzvetanov *et al.* 1999]. Limit of detection, 0.05 µg/L for ketotifen and the desmethyl metabolite, 0.3 µg/L for 10-hydroxyketotifen [Julien-Larose *et al.* 1983].

Disposition in the Body Ketotifen is absorbed after oral administration. It is metabolised by 10-hydroxylation, *N*-demethylation, and glucuronic acid conjugation. It is excreted in the urine and faeces as unchanged drug and metabolites.

Therapeutic Concentration

Following a single oral dose of 2 mg to 3 subjects, a peak plasma concentration of about 0.0006 mg/L was attained in 2 h; a peak plasma concentration of about 0.015 mg/L of ketotifen glucuronide was attained in about 4 h [Julien-Larose *et al.* 1983].

Toxicity

In 8 cases of overdose involving ingestion of up to 120 mg, drowsiness and other toxic effects were reported, but in each case full recovery occurred within 12 h. In 3 cases involving the ingestion of 10, 50 and 120 mg, plasma concentrations were 0.016, 0.054 and 0.122 mg/L, respectively, at 5, 3 and 20 h after ingestion [Jeffreys, Volans 1981].

In a fatality involving ketotifen, a postmortem blood concentration of 1.2 mg/L was reported [Stead AH, Moffat AC, personal communication].

Dose The equivalent of 2 to 4 mg of ketotifen daily.

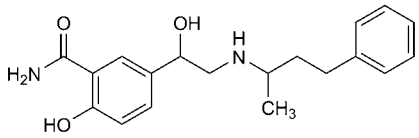
Jeffreys DB, Volans GN (1981). Ketotifen overdose: surveillance of the toxicity of a new drug. *Br Med J (Clin Res Ed)* 282: 1755–1756.
Julien-Larose C *et al.* (1983). Quantification of ketotifen and its metabolites in human plasma by gas chromatography mass spectrometry. *Biomed Mass Spectrom* 10: 136–142.
Tzvetanov S *et al.* (1999). Gas chromatographic-mass spectrometric method for quantitative determination of ketotifen in human plasma after enzyme hydrolysis of conjugated ketotifen. *J Chromatogr B Biomed Sci Appl* 732: 251–256.

Labetalol

Antihypertensive, α-Adrenoceptor Antagonist, β-Adrenoceptor Antagonist
$C_{19}H_{24}N_2O_3 = 328.4$
CAS—36894-69-6

IUPAC Name 2-hydroxy-5-[1-hydroxy-2-(4-phenylbutan-2-ylamino)ethyl] benzamide

Synonyms 2-Hydroxy-5-[1-hydroxy-2-[(1-methyl-3–phenylpropyl)amino] ethyl]benzamide; ibidomide.



Chemical Properties pK_a 7.4 (phenol), 8.7 (-NH_2) [Baselt 2008; Cheymol *et al.* 1997]. Log *P* (octanol/water), 3.09 [Hansch *et al.* 1995; Rapado Martínez *et al.* 1999]. Labetalol is stable in plasma for at least 48 h at 4° and for at least 8 weeks at –80°. Stock standard solutions were stable for at least 3 months when kept at 4° [Umezawa *et al.* 2008].

Labetalol Hydrochloride

$C_{19}H_{24}N_2O_3$,HCl = 364.9
CAS—32780-64-6

Proprietary Names *Abetol*; *Albetol*; *Alfabetal*; *Amipress*; *Hybloc*; *Ipolab*; *Labrocol*; *Normodyne*; *Presolol*; *Pressalolo*; *Trandate*.

Chemical Properties White crystalline solid. Mp ≈180°. Soluble in water and ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), 2.41 [Meylan, Howard 1995]. Labetalol hydrochloride 40 mg/mL in 3 oral liquids was stable for up to 60 days when stored in the dark at 5° and 25° [Allen, Erickson 1996]

Colour Tests Ferric chloride—violet; Folin–Ciocalteu reagent—blue; Liebermann's reagent—brown-orange; Mandelin's test—green-blue; Marquis test—red→brown-red; Nessler's reagent (100°)—black.

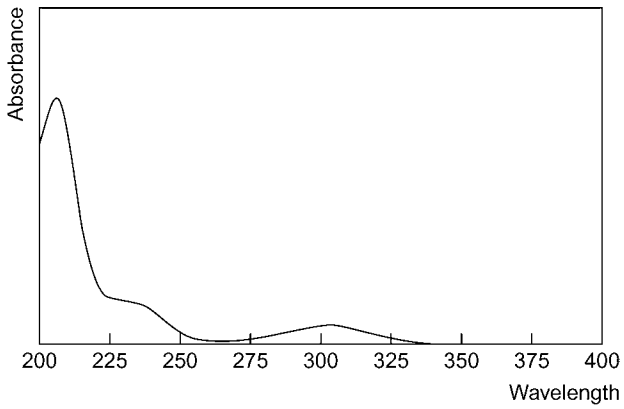
Thin-layer Chromatography System TAE—R_f 0.32; system TB—R_f 0.00; system TE—R_f 0.29; system TF—R_f 0.10.

Gas Chromatography System GA—RI 1320; system GB—RI 1270 (both for labetalol Art).

Gas Chromatography-Mass Spectrometry Column: DB-5 methylphenyl silicone (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 150° for 1 min to 260° at 10°/min. Relative retention time: 1.608 or 1.719 min for MBA, 4.191 or 4.371 min for BBA [Zamecnik 1990].

High Performance Liquid Chromatography System HA—*k* 1.7 (tailing peak); system HX—RI 365; system HY—RI 290; system HZ—retention time 3.0 min.

Ultraviolet Spectrum Aqueous acid—302 nm (A_1^1 = 95a); aqueous alkali—246 (A_1^1 = 267a), 333 nm (A_1^1 = 161a).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1643, 1502, 1270, 820, 1250 cm^{-1} (labetalol hydrochloride, Nujol mull).

Quantification

Blood **HPLC** Column: Ultrasphere octyl (150 × 4.6 mm i.d., 5 um). Mobile phase: 0.01 mol/L ammonium phosphate : methanol (1 : 1, pH 3.0). UV detection (λ = 216 nm). Retention time: 5 min. Limit of detection, 10 µg/L [Hidalgo, Muir 1984]. Column: µBondapak C_{18} (30 cm). Mobile phase: acetonitrile : 0.1% phosphoric acid (28 : 72), flow rate 1.7 mL/min. UV detection (λ = 313 nm). Limit of detection not reported [Pannell *et al.* 1982].

Plasma **HPLC** Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8; 10 : 90 to 35 : 65 at 25 min for 1 min), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 21.8 min. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Delamoye *et al.* 2004]. Column: PRP-1. Mobile phase: 0.05 mol/L: ammonium carbonate buffer (pH 9.5) : acetonitrile (72 : 28). Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 418 nm) Limit of quantification, 5 µg/L, limit of detection, 1 µg/L [Johnson *et al.* 2000]. Column: Chirex 3022 (250 × 4.0 mm i.d.). Mobile phase: hexane : 1,2-dichloroethane : ethanol : TFA (55.75 : 35 : 9 : 0.25), flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 220 nm, λ_{em} = 412 nm). Retention time: 33, 37, 43 and 51 min for (*S,R*)-, (*S,S*)-, (*R,S*)- and (*R,R*)-labetalol, respectively. Limit of detection, 1.5–1.8 µg/mL [Dakers *et al.* 1997]. Column: ChromTech Chiral AGP (4 × 100 mm i.d., 10 µm). Mobile phase: 0.05 mol/L ammonium carbonate buffer (pH 9.5) : acetonitrile (72 : 28) or Column: LiChrosorb cyanopropyl (12.5 × 0.4 cm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.0). UV (λ = 220 or 305 nm) or fluorescence detection (λ_{ex} = 335 nm, λ_{em} = 370 nm). Limit of detection, 100 µg/L [Musch *et al.* 1989]. Column: PRP1 (150 × 5 mm i.d.). Mobile phase: ammonium carbonate solution : acetonitrile (73 : 27), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 334 nm, λ_{em} = 412 nm). Limit of detection, 4 µg/L [Awni *et al.* 1988]. See also Ostrovská *et al.* [1988], Bates *et al.* [1987], Chung *et al.* [1986], Luke *et al.* [1987], Alton *et al.* [1984], Hidalgo and Muir [1984], Wood *et al.* [1982], Oosterhuis *et al.* [1981], Meredith *et al.* [1981], Woodman and Johnson [1981], Dusci and Hackett [1979].

LC-MS Column: Shodex MSpak GF-310 (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 at 4 min for 5.5 min to 100 : 0 at 9.5 min for 5.5 min), flow rate 0.55 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 6.6 min. Limit of detection, 1 µg/L [Umezawa *et al.* 2008]. Column: Spherisorb ODS-2 (50 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 0.1 mol/L ammonium acetate (60 : 40) containing 1% formic acid, flow rate 1.0 mL/min. TSP, SIR. Limit of detection, 5 µg/L [Lant *et al.* 1987].

Note For a spectrofluorometric method for the determination of labetalol in plasma, see Abdine *et al.* [2005].

Note For a radioimmunoassay in plasma, see Kelly *et al.* [1981].

Serum **HPLC** See Blood [Hidalgo, Muir 1984]. See Plasma [Woodman, Johnson 1981].

Urine **GC-MS** Column: DB-1 fused silica (10 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 30 cm/s. Temperature programme: 100° to 200° at 15°/min to 204° at 2°/min to 300° at 30°/min. NICI, SIM acquisition mode. Limit of detection, 0.1 µg/L for enantiomers of labetalol metabolite 3-amino-1-phenylbutane [Changchit *et al.* 1991].

HPLC Column: ODS-2 (120 × 4.6 mm i.d., 5 µm). Mobile phase: 0.11 mol/L SDS : 8% propanol : 0.01 mol/L sodium dihydrogen phosphate (pH 3.0), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 210 nm, λ_{em} = 434 nm). Limit of detection, 28.3 µg/L [Carda-Broch *et al.* 1999]. Column: Spherisorb ODS-2 (120 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L SDS : 15% propanol : 1% triethylamine : 0.02 mol/L phosphate buffer, flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 440 nm). Limit of detection, 20 µg/L [Rapado Martínez *et al.* 1999]. Column: Supelcosil ABZ + Plus (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (30 : 70) containing 5 mmol/L acetate buffer, flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 10 mg/L [Ceniceros *et al.* 1998].

LC-MS Column: Radial-Pak CN-HP cyano spherical (10 cm, 4 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 3.0) : methanol (60 : 40), flow rate, 1.2 mL/min. Full scan mode or MID. Relative retention: -4.38. Limit of detection, 2 and 0.2 ng for full scan and MID, respectively [Leloux *et al.* 1991].

Hair **GC-MS** Column: HP5-MS (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.2 mL/min. Temperature programme: 110° for 1 min to 170° at 20°/min to 225° at 7°/min to 295° at 24°/min for 10 min. EI ionisation. Retention time: 17.9 min. Limit of detection, 8 pg/mg [Kintz *et al.* 2000].

Kidney **HPLC** See Blood [Pannell *et al.* 1982].

Liver **HPLC** See Blood [Pannell *et al.* 1982].

Other **HPLC** Oral Liquids. Column: Bakerbond C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L monobasic sodium phosphate : methanol (55 : 45), flow rate 1.3 mL/min. UV detection (λ = 230 nm). Retention time: 7.5 min. Limit of detection, 100 mg/L [Allen, Erickson 1996].

Disposition in the Body Well absorbed after oral administration but undergoes extensive first-pass metabolism. The major metabolites are glucuronides of labetalol. Up to ~60% of a dose is excreted in the urine in 24 h as conjugates, 1 of which has been identified as the *O*-phenylglucuronide; ~5% of a dose is excreted as unchanged drug. 3-Amino-1-phenylbutane (APB) has been found in the urine of patients receiving labetalol at concentrations of 0.5–15 mg/L [Gal *et al.* 1988].

Therapeutic Concentration

After a single oral dose of 100 mg given to 6 subjects, peak plasma concentrations of 0.09 to 0.25 mg/L (mean 0.16) were attained in about 0.5 h; following a single oral dose of 200 mg to the same subjects, peak plasma concentrations of 0.09 to 0.27 mg/L (mean 0.19) were reported at ≈1 h [McNeil *et al.* 1979].

Two normotensive volunteers were administered labetalol as an oral dose of 2 × 200 mg tablets or a dose of 1 mg/kg over 2 min IV. Following oral administration the maximum concentration was ≈600 µg/L reached at ≈50 min. The elimination half-life was 3.4 and 3.0 h [Meredith *et al.* 1981].

Following oral administration of 200 mg twice daily to 9 subjects, average steady-state plasma concentrations of 0.04 to 0.18 mg/L (mean 0.09) were reported [McNeil *et al.* 1982].

A 28-year-old anephric male was administered 1 mg/kg labetalol between haemodialyses. The terminal plasma half-life was 5 h [Wood *et al.* 1982].

Continuous infusion of labetolol (1.5 mg/kg over 5 min, followed 30-min later by 0.2 mg/kg/h for 5.5 h) administered to 6 subjects resulted in a mean steady-state plasma concentration of 264 µg/L being achieved within 2 h [Chauvin *et al.* 1987].

Sixteen healthy volunteers (mean age, 27 years) received 5 different oral formulations of labetalol on 5 different occasions every 12 hours for 5 doses. The mean V, β/F, V_{dss}/F, TBC/F, $t_{1/2\beta}$ and $AUC_{\Pi-0}$ ranged from 18.1 and 161.9 L/kg, 7.1 and 53.9 L/kg, 1.3 and 5.72 L/h/kg, 6.9 and 11.0 h and 154 and 520 µg.h/L [Awni *et al.* 1988].

When 19 hypertensive patients were administered 693 ± 465 and 680 ± 482 mg/day for men and women, respectively, the pharmacokinetic parameters were 80% higher in the females [Johnson *et al.* 2000].

Toxicity

A 44-year-old female was found dead. Postmortem concentrations of labetalol were 31.1, 14.2, 7.8, 5.4, and 5.2 µg/g in gastric contents, liver, heart, kidney, and lung, respectively [Grassin Delyle *et al.* 2008].

A 79-year-old female who overdosed and died within a short period of time had a postmortem heart blood labetalol concentration of 15 mg/L [Baselt 2008].

A 68-year-old subject suffering from chronic respiratory disease died about 1 h after inadvertently being given 200 mg of labetalol. Postmortem kidney and liver concentrations of 10.6 and 20.5 µg/g, respectively, were reported and were considered to be consistent with a therapeutic dose; death was attributed to respiratory disease [Pannell *et al.* 1982].

A 25-year-old male suffered acute renal failure about 3 days after deliberately ingesting 6 g of labetalol plus 7 pints of lager; haemodialysis for 16 days was necessary to bring about recovery [Korzets *et al.* 1990].

Note For a case of labetalol-induced hepatitis, see Marinella [2002] or Stronkhorst *et al.* [1992] and for a case of hepatocellular necrosis associated with labetalol, see Long *et al.* [2007]. For a case of acute renal failure following labetalol ingestion, see Smit *et al.* [1986]. For a case of cutaneous rash in response to labetalol, see Branford *et al.* [1978] or Finlay *et al.* [1978]. A case of iatrogenic overdose was successfully treated with amrinone Kollef [1994].

Bioavailability 10 to 80% (mean 30).

Half-life Plasma half-life, 3–6 h.

Volume of Distribution ≈3–10 L/kg (mean 7).

Clearance Plasma clearance, 10–40 mL/min/kg (mean, 25).

Protein Binding ≈50% [Martin *et al.* 1976].

Note For a review of the pharmacokinetics of labetalol, see McNeil and Louis [1984] and in hypertensive patients, see McNeil *et al.* [1982] or McNeil *et al.* [1979].

Dose 200 to 800 mg of labetalol hydrochloride daily; doses of 2.4 g daily have been given.

Abdine H *et al.* (2005). Spectrofluorometric determination of some beta-blockers in tablets and human plasma using 9 10-dimethoxyanthracene-2-sodium sulfonate. *Pharmazie* 60: 265–268.

Allen LV,Jr Erickson MAIII (1996). Stability of labetalol hydrochloride, metoprolol tartrate, verapamil hydrochloride, and spironolactone with hydrochlorothiazide in extemporaneously compounded oral liquids. *Am J Health Syst Pharm* 53: 2304–2309.

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Gal J *et al.* (1988). Labetalol is metabolized oxidatively in humans. *Res Commun Chem Pathol Pharmacol* 62: 3–17.

Grassin Delyle S *et al.* (2008). Fatal intoxication with labetalol (Trandate). *Forensic Sci Int* 178: e19–e21.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

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Lant MS *et al.* (1987). Automated sample preparation on-line with thermospray high-performance liquid chromatography-mass spectrometry for the determination of drugs in plasma. *J Chromatogr* 394: 223–230.

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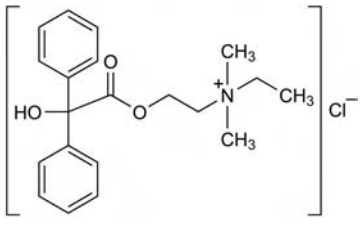
Lachesine Chloride

Anticholinergic

$C_{20}H_{26}ClNO_3 = 363.9$

CAS—1164-38-1

IUPAC Name *N*-Ethyl-2-[(hydroxydiphenylacetyl)oxy]-*N*,*N*-dimethylethanaminium chloride



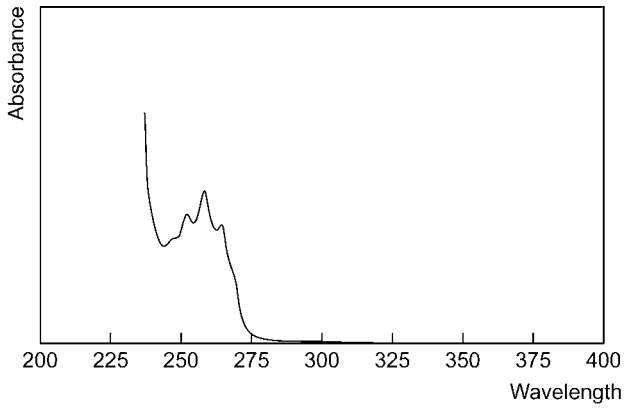
Chemical Properties A white amorphous powder. Mp 212° to 214°. Soluble 1 in 3 of water and 1 in 10 of ethanol (90%); very slightly soluble in chloroform and ether. Log *P* (octanol/water), −1.1.

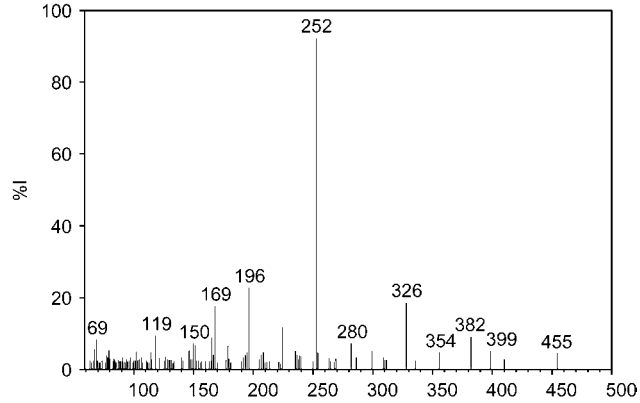
Colour Tests Mandelin's test—orange→green; Marquis test—orange→green→blue.

Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1852.

Ultraviolet Spectrum Aqueous acid—252, 258 (A^{1}_{1}=12b), 264 nm. No alkaline shift.



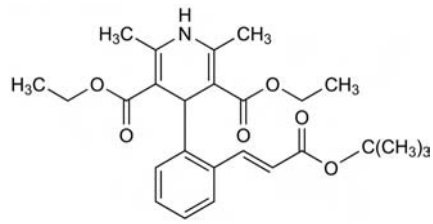


Infrared Spectrum Principal peaks at wavenumbers 1739, 1241, 699, 743, 1176, 1191 cm^{-1} (KBr disk).
Use As a 1% ophthalmic solution.

Lacidipine

Calcium Antagonist
$C_{26}H_{33}NO_6$ = 455.5
CAS—103890-78-4

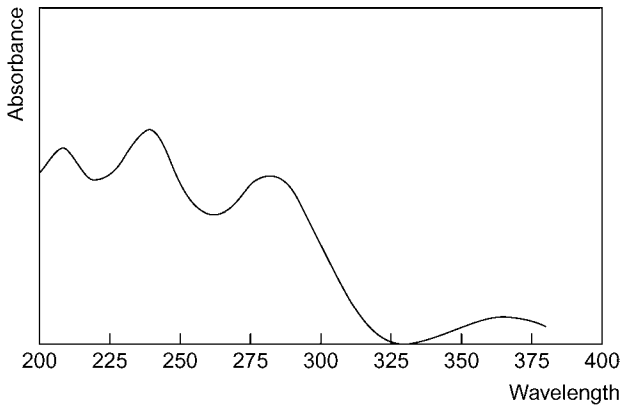
IUPAC Name (*E*)-4-[2-[3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl]phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid diethyl ester
Synonyms GR-43659X; GX-1048.
Proprietary Names *Aponil*; *Caldine*; *Lacimen*; *Lacipil*; *Lacirex*; *Midotens*; *Motens*; *Viapres*; *Zascal*.



Chemical Properties A crystalline solid with Mp 174° to 175°. Log *P* (octanol/water), 5.20.
Thin-layer Chromatography System TE—R_f 0.81; system TF—R_f 0.65.
High Performance Liquid Chromatography System HX—RI 900; system HAA—retention time 27.2 min.

Column: RP C_{18} (250 × 4.6 mm i.d., 5 µm). Temperature: 30°. Mobile phase: methanol : water (70:30), containing 2 mmol/L acetate buffer (pH 5.0), flow rate 1 mL/min. Detection: ECD. Retention time: 15.7 min [Lopez *et al.* 2000].

Column: RP C_{18} Novapak (100 × 4.6 mm i.d.). Temperature: 50°. Mobile phase: methanol : acetonitrile : water (35:10:55 for 7 min to 60:10:30 in 23 min), containing 0.05 mol/L ammonium acetate (pH 5.0), flow rate 1.2 mL/min. (35:10:55 for 7 min to 60:10:30 in 23 min, to 80:10:10) in 5 min for 10 min. UV detection (λ=282 nm). Retention time not specified [Scandola 1993].
Ultraviolet Spectrum Aqueous acid—239, 209, 282, 366 nm.



Mass Spectrum Principal ions at *m/z* 252, 196, 326, 169, 119, 382, 69, 150.

Quantification
***Plasma* HPLC** In conjunction with radioimmunoassay. Limit of detection, 0.02 µg/L [Pellegatti *et al.* 1992]. Limit of detection, 0.5 µg/L [Evans *et al.* 1990].
Disposition in the Body Lacidipine is rapidly but poorly absorbed after oral administration and undergoes extensive first-pass metabolism. Two main metabolites have been identified: a pyridine analogue and a carboxylic acid analogue. It is eliminated by metabolism in the liver and the metabolites are excreted mainly via bile. Approximately 70% of an oral dose is eliminated in faeces and the remainder in urine. No parent drug has been detected in urine or faeces.
Therapeutic Concentration
Two healthy volunteers were administered 4 mg lacidipine orally after an overnight fast. The peak plasma concentrations observed were 1.918 µg/L and 3.392 µg/L, 1 h after administration of the dose [Pellegatti *et al.* 1992].

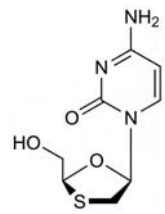
Seventeen healthy young volunteers were administered with oral doses of 4, 6 and 8 mg lacidipine. Peak concentrations of 2.8, 6.1 and 8.6 µg/L were observed about 1 h after administration [Squassante *et al.* 1994].
Bioavailability 18.5% (range 4 to 52%).
Half-life 13 to 19 h.
Volume of Distribution 0.9 to 2.3 L/kg also reported as 537 L with high inter-subject variation.
Protein Binding >95% (mainly albumin).
Dose The usual initial dose is 2 mg once daily which may be increased to 4 or 6 mg if necessary after 3 to 4 weeks.

Evans GL *et al.* (1990). *Methodol Surv Biochem Anal* 20: 285–290.
Lopez JA *et al.* (2000). High-performance liquid chromatography with amperometric detection applied to the screening of 1,4-dihydropyridines in human plasma. *J Chromatogr A* 870: 105–114.
Pellegatti M *et al.* (1992). Validation of a high-performance liquid chromatographic-radioimmunoassay method for the determination of lacidipine in plasma. *J Chromatogr*,105–111.
Scandola M (1993). *J Chromatogr* 647: 155–166.
Squassante L *et al.* (1994). A study of plasma disposition kinetics of lacidipine after single oral ascending doses. *J Cardiovasc Pharmacol* 23: 5S94–S97.

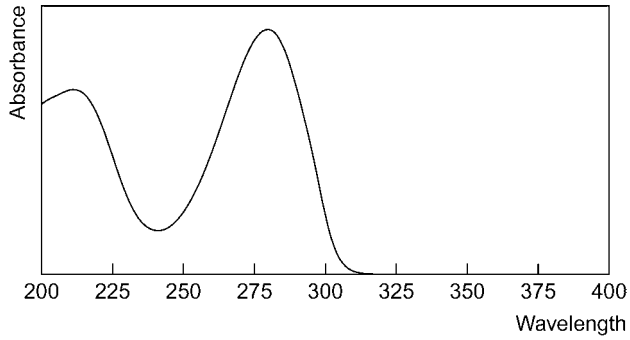
Lamivudine

Nucleoside Reverse Transcriptase Inhibitor, Antiviral
$C_8H_{11}N_3O_3S$ = 229.3
CAS—134678-17-4

IUPAC Name (2*R-cis*)-4-Amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1*H*)-pyrimidinone
Synonyms 3TC; GR-109714X; (−)-BCH-189.
Proprietary Names *Epivir*; *Zeffix*.



Chemical Properties White crystals from boiling ethanol or white solid from methanol/ethyl acetate. Mp 160° to 162°. Solubility in water at 20°, 70 mg/mL.
High Performance Liquid Chromatography System HAD—*k* 2.70.
Ultraviolet Spectrum Aqueous acid—212, 279 nm.



Quantification

Plasma **HPLC** UV detection (λ=260 nm). Limit of quantification, 0.02 mg/L [Aymard *et al.* 2000].

Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 0.26 mg/L [Simon *et al.* 2001]. Column: C_{18} Keystone Aquasil (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (15:85), 0.3 mL/min flow rate. MS–MS detection (m/z 230 to 112). Retention time: 2.5 min. Limit of quantification, 3 µg/L [Kenney *et al.* 2000].

Urine **HPLC** Limit of quantification, 0.5 mg/L [Morris, Selinger 1994].

Disposition in the Body In healthy volunteers lamivudine is rapidly absorbed following an oral administration, reaching peak plasma concentrations within 1 to 1.5 h. About 70% of the total dose is excreted unchanged in urine and between 5 and 10% undergoes hepatic metabolism to form a *trans*-sulfoxide metabolite.

Therapeutic Concentration

Group 1: 6 HIV-infected males, aged between 29 and 41 years, with creatinine clearance ≥60 mL/min. Group 2: 4 HIV-infected males and females, 29 to 68 years, with creatinine clearance between 10 and 40 mL/min. Group 3: 6 males and females (31 to 51 years old), creatinine clearance <10 mL/min. Each group was administered a single oral dose of 300 mg after an overnight fast. The mean peak plasma concentration for group 1 was 2.524 mg/L, group 2, 3.538 mg/L and group 3, 5.684 mg/L and these levels were reached within 1, 1 and 2 h, respectively [Heald *et al.* 1996].

Bioavailability 80 to 87%.

Half-life 5 to 7 h (after a single dose) and 11 h (when sampling for 48 h), which may increase in presence of renal impairment.

Volume of Distribution Approximately 100 L.

Protein Binding <36%.

Dose Adults: 100 mg daily to 150 mg every 12 h. Children (3 months to 12 years): 4 mg/kg every 12 h with a maximum of 300 mg daily.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

Heald AE *et al.* (1996). Pharmacokinetics of lamivudine in human immunodeficiency virus-infected patients with renal dysfunction. *Antimicrob Agents Chemother* 40: 1514–1519.

Kenney KB *et al.* (2000). Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry. *J Pharm Biomed Anal* 22(6): 967–983.

Kenney KB *et al.* (2000). Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 742: 173–183.

Morris DM, Selinger K (1994). Determination of 2′-deoxy-3′-thiacytidine (3TC) in human urine by liquid chromatography: direct injection with column switching. *J Pharm Biomed Anal* 12: 255.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

Lamotrigine

Antiepileptic, Anticonvulsant

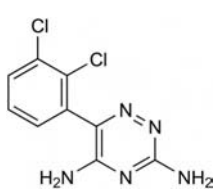
$C_9H_7Cl_2N_5$ = 256.1

CAS—84057-84-1

IUPAC Name 6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine

Synonyms BW-430C; 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine; LTG.

Proprietary Name *Lamictal*.

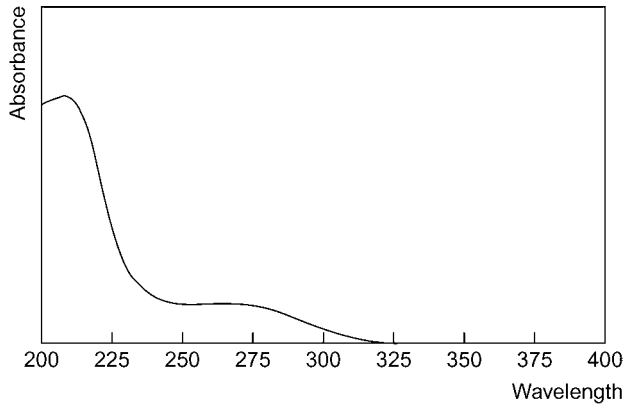


Chemical Properties White to pale cream crystals from propan-2-ol, Mp 216° to 218°. Solubility at 25°: 0.17 mg/mL in water, 4.1 mg/mL in hydrochloric acid (0.1 mol/L); practically insoluble in ethanol. pK_a 5.7. Extraction yield (chlorobutane), 0.17 [Demme *et al.* 2005].

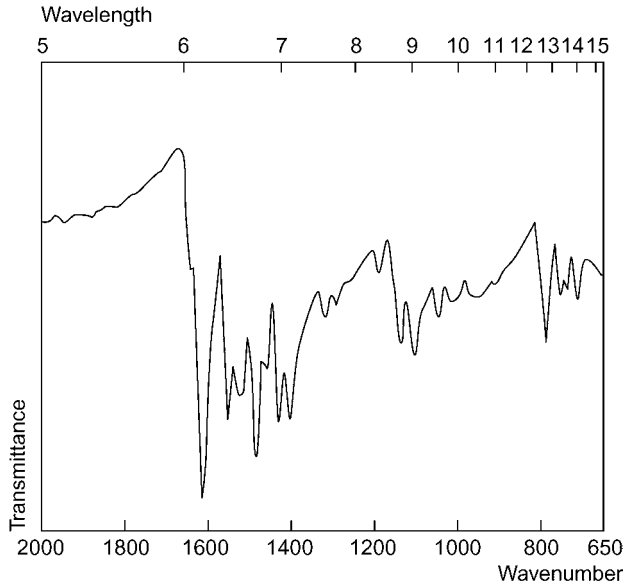
Gas Chromatography System GB—RI 2562; system GAJ—RRT 1.941 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HY—RI 272; system HZ—RT 2.3 min.

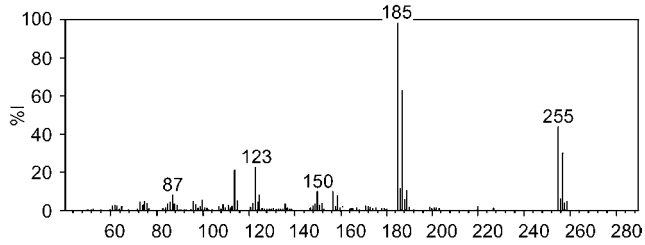
Ultraviolet Spectrum Aqueous acid (0.025 mol/L sulfuric acid)—208, 264.5 nm; (0.1 mol/L ammonium formate pH 3)—267 nm; ethanol—307.5 nm.



Infrared Spectrum Principal peaks at wavenumbers 1614, 1488, 1429, 1404 cm^{-1} KBr disk.



Mass Spectrum Principal ions at *m/z* 185, 187, 255, 257, 123, 117, 157, 150.



Quantification

Plasma **HPLC** Column: Reversed phase monolithic. Mobile phase: 0.1 mol/L (pH 6.5) phosphate buffer : methanol : acetonitrile (77 : 20 : 3). UV detection (λ= 210 nm) [Heideloff *et al.* 2010]. Column: Synergi Hydro-RP (250 × 4.6 mm i.d., 4 µm). Mobile phase: potassium dihydrogen phosphate buffer (50 µmol/L, pH 4.5) : acetonitrile : methanol (65 : 26.2 : 8.8), flow rate of 0.8 mL/min. UV detection (λ= 210 nm). Limit of quantification, 2 µg/L [Contin *et al.* 2010]. DAD [Zufia *et al.* 2009]. Column: C_8 RP. Mobile phase: methanol : 0.45 µmol/L (pH 3.5) phosphate buffer containing 0.17% TEA (24 : 76). DAD (λ= 220 nm) [Saracino *et al.* 2007a]. Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50.0 mmol/L phosphate buffer (pH 3.5, 27 : 73). UV detection (λ= 220 nm) [Saracino *et al.* 2007b]. See also Angelis-Stoforidis *et al.* [1999], Barbosa, Midio [2000], Beck *et al.* [2006], Cheng *et al.* [2005], Contin *et al.* [2005], Posner *et al.* [1989], Ren *et al.* [1998] and Sallustio, Morris [1997].

LC-MS Column: Shimdazu Shimpack XR-ODS (50 × 4.6 mm, i.d., 2.2 μm). Mobile phase: acetate buffer : methanol: acetonitrile : tetrahydrofuran. APCI, SIM acquisition mode [Subramanian *et al.* 2008].

CE CZE based [Theurillat *et al.* 2002].

Serum **HPLC** Column: Chromolith RP-18e (50 × 4.6 mm i.d.). Mobile phase: acetonitrile : 15 mmol/L phosphate buffer (pH 7.0, 20 : 80), flow rate 2.0 mL/min. UV detection (λ = 215 nm). Limit of detection, 2 μg/L [Brunetto *et al.* 2009]. See Plasma [Heideloff *et al.* 2010].

CE See Plasma [Theurillat *et al.* 2002].

Urine **HPLC** Column: Reversed phase C_{18} (250 × 4.6 mm). Mobile phase: potassium phosphate buffer : acetonitrile : methanol (70 : 16 : 14). Limit of detection, 30 μg/L [Ren *et al.* 1998]. Limit of detection, 100 ng [Doig, Clare 1991].

Brain **HPLC** See Urine [Ren *et al.* 1998].

Disposition in the Body Lamotrigine is readily and virtually completely absorbed, reaching peak concentrations in ~1.5–5 h. The presence of food does not affect the drug's absorption. A second peak may be seen 4–6 h after administration. Elimination is mainly via the kidneys (70% of dose in urine) with 10% as the unchanged drug: ~75–90% as glucuronide conjugate, trace amounts as the 2-*N*-methyl metabolite and <5% as the other metabolites. Approximately 2% is eliminated in faeces. The amount of unchanged drug excreted is 30% greater in Gilbert's syndrome.

Therapeutic Concentration The serum therapeutic concentration is 4–10 mg/L, with trough of 2–5 mg/L.

A group of 5 healthy males, aged between 27 and 49 years, were administered with a weekly doubling dose from 7.5 to 240 mg. A second group of 10 healthy male and females, 19 to 61 years of age, received a single dose of 120 mg lamotrigine. The mean peak plasma concentrations for group 1 were 0.40, 0.80, 1.60 and 3.16 mg/L for a 30- 60- 120- and 240- mg dose, respectively, observed ~1.9, 1.7, 2.1 and 3.1 h after administration, respectively. Group 2 showed a mean peak concentration of 1.56 mg/L (range 1.19–1.93), which was reached at 0.5–4 h (mean, 2.8 h) after dosing. [Kilpatrick *et al.* 1996].

Toxicity

No serious toxicity was seen in a patient who took 1.35 g lamotrogine and was subsequently treated with gastric lavage and activated charcoal [Buckley *et al.* 1993]. Similarly a patient who ingested between 4 and 5 g was admitted to hospital with coma, which lasted 8–12 h, followed by recovery over the next 2–3 days. Another patient who ingested 5.6 g was found unconscious and recovered after treatment with activated charcoal and sleeping for 16 h.

Bioavailability Oral formulation, 98%.

Half-life Mean range (administration with no other medication) is 25 ± 10 h. After a single dose in healthy volunteers, half-life is 24.1 ± 5.7 h; after multiple doses over 7 days in healthy volunteers, it is 25.5 ± 10.2 h. In the elderly (mean age 71 years, healthy volunteers), half-life is 31.2 ± 5.4 h. In adults with epilepsy, half-life is 13.5 h. After a single dose co-administration with carbamazepine and phenytoin, the mean half-life is 15 h (range 7.8–33.3) and after a single dose co-administration with valproic acid, the mean is 59 h (range 30.5–88.8). Plasma elimination half-life is lower (37%) in those with Gilbert's syndrome. Half-life in chronic renal failure (creatinine clearance ~18 mL/min) is 36 h.

Volume of Distribution 1–1.4 L/kg for healthy volunteers administered with a single 120–mg dose and 0.77–1.04 L/kg for healthy males administered with a weekly doubling dose of 30 to 240 mg. The mean apparent volume of distribution is 12% lower in the elderly (mean age 71 years, healthy volunteers).

Clearance 20.7–52.3 (mean, 41.7) mL/min for healthy volunteers receiving a 120–mg single dose and 22.4–37.7 (mean 29.6) mL/min for those administered with a weekly doubling dose between 30 and 240 mg. The mean apparent clearance is 37% lower in the elderly (mean age 71 years, healthy volunteers). The mean oral clearance is 32% lower in Gilbert's syndrome and reduced by 9-fold in chronic renal failure.

Protein Binding 56%.

Dose 50 to 400 mg per day.

Angelis-Stoforidis P *et al.* (1999). Determination of lamotrigine in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 727: 113–118.

Barbosa NR, Midio AF (2000). Validated high-performance liquid chromatographic method for the determination of lamotrigine in human plasma. *J Chromatogr B Biomed Sci Appl* 741: 289–293.

Beck O *et al.* (2006). Determination of lamotrigine and its metabolites in human plasma by liquid chromatography–mass spectrometry. *Ther Drug Monit* 28: 603–607.

Brunetto MR *et al.* (2009). Development and validation of a rapid column-switching high-performance liquid chromatographic method for the determination of lamotrigine in human serum. *J Chromatogr Sci* 47: 478–484.

Buckley NA *et al.* (1993). Self-poisoning with lamotrigine. *Lancet* 342: 1552–1553.

Cheng CL *et al.* (2005). Determination of lamotrigine in small volumes of plasma by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 817: 199–206.

Contin M *et al.* (2005). Simultaneous liquid chromatographic determination of lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in plasma of patients with epilepsy. *J Chromatogr B Analyt Technol Biomed Life Sci* 828: 113–117.

Contin M *et al.* (2010). Simultaneous HPLC-UV analysis of rufinamide, zonisamide, lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in deproteinized plasma of patients with epilepsy. *J Chromatogr B Analyt Technol Biomed Life Sci* 878: 461–465.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Doig MV, Clare RA (1991). Use of thermospray liquid chromatography–mass spectrometry to aid in the identification of urinary metabolites of a novel antiepileptic drug, lamotrigine. *J Chromatogr* 554: 181–189.

Heideloff C *et al.* (2010). A novel HPLC method for quantification of 10 antiepileptic drugs or metabolites in serum/plasma using a monolithic column. *Ther Drug Monit* 32: 102–106.

Kilpatrick ES *et al.* (1996). Concentration–effect and concentration–toxicity relations with lamotrigine: a prospective study. *Epilepsia* 37: 534–538.

Posner J *et al.* (1989). The pharmacokinetics of lamotrigine (BW430C) in healthy subjects with unconjugated hyperbilirubinaemia (Gilbert's syndrome). *Br J Clin Pharmacol* 28: 117–120.

Ren S *et al.* (1998). Determination of lamotrigine in biologic materials by a simple and rapid liquid chromatographic method. *Ther Drug Monit* 20: 209–214.

Sallustio BC, Morris RG (1997). High-performance liquid chromatography quantitation of plasma lamotrigine concentrations: application measuring trough concentrations in patients with epilepsy. *Ther Drug Monit* 19: 688–693.

Saracino MA *et al.* (2007a). Rapid HPLC analysis of the antiepileptic lamotrigine and its metabolites in human plasma. *J Sep Sci* 30: 2249–2255.

Saracino MA *et al.* (2007b). Simultaneous high-performance liquid chromatographic determination of olanzapine and lamotrigine in plasma of bipolar patients. *Ther Drug Monit* 29: 773–780.

Subramanian M *et al.* (2008). High-speed simultaneous determination of nine antiepileptic drugs using liquid chromatography–mass spectrometry. *Ther Drug Monit* 30: 347–356.

Theurillat R *et al.* (2002). Therapeutic drug monitoring of lamotrigine using capillary electrophoresis: evaluation of assay performance and quality assurance over a 4-year period in the routine arena. *J Chromatogr A* 979: 353–368.

Zufia L *et al.* (2009). LC method for the therapeutic drug monitoring of lamotrigine: evaluation of the assay performance and validation of its application in the routine area. *J Pharm Biomed Anal* 49: 547–553.

Lanatoside C

Cardiac Glycoside

$C_{49}H_{76}O_{20}$ = 985.1

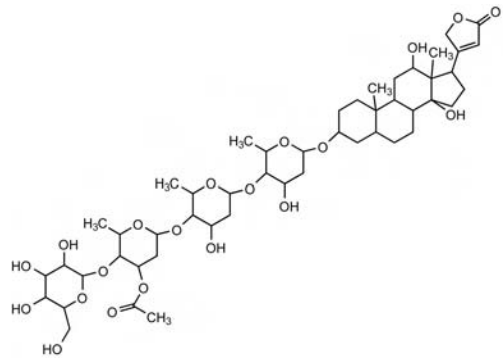
CAS—17575-22-3

IUPAC Name (3β,5β,12β)-3-[(*O*-β-D-Glucopyranosyl-(1→4)-*O*-3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxycard-20(22)-enolide

Synonym Celanide

Proprietary Names *Allocor*; *Cedilanid*; *Ceglunat*; *Celadigal*; *Cetosanol*; *Lanimerck*.

Note The name *Cedilanid* is also applied to a preparation of deslanoside.

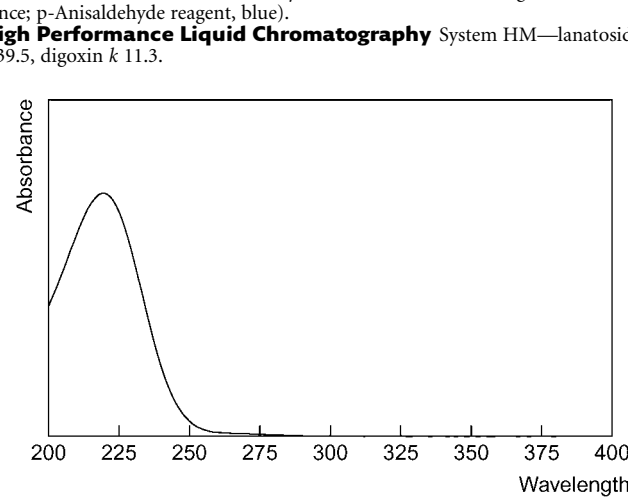


Chemical Properties A glycoside obtained from the leaves of the woolly foxglove, *Digitalis lanata* (Scrophulariaceae). Colourless or white hygroscopic crystals or white crystalline powder. Mp 240° with decomposition. Practically insoluble in water and ether; sparingly soluble in ethanol; soluble 1 in 2000 of chloroform and 1 in 20,000 of methanol; freely soluble in dioxane and pyridine. Log *P* (octanol/water), 0.1.

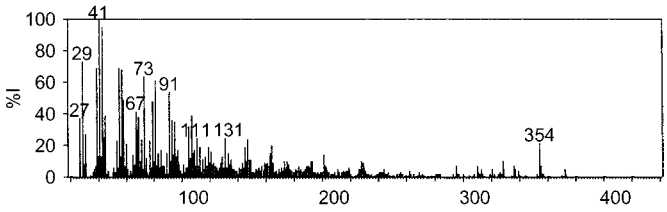
Colour Tests Antimony pentachloride—yellow→brown→black-violet; dissolve 2 to 3 mg in 5 mL of a solution containing 0.5 mL of 9% ferric chloride solution and 100 mL of acetic acid; underlay with 5 mL of sulfuric acid—an intense blue colour forms in the acetic acid layer and a brown ring free from red at the junction of the two liquids (see also Deslanoside).

Thin-layer Chromatography System TE—R_f 0.06; system TAE—R_f 0.89 (perchloric acid solution, followed by examination under UV light, blue fluorescence; p-Anisaldehyde reagent, blue).

High Performance Liquid Chromatography System HM—lanatoside C *k* 39.5, digoxin *k* 11.3.



Mass Spectrum Principal ions at *m/z* 41, 43, 29, 55, 39, 57, 73, 81; digoxin 73, 58, 57, 43, 41, 39, 29, 45 (no peaks above 360).



Quantification

Plasma **HPLC–MS** Lanatocide C and other cardiac glycosides. Limit of detection, 0.6 µg/L for lanatocide C [Tracqui *et al.* 1997].

Serum **Radioimmunoassay** For method, see Moffat [1974].

Disposition in the Body Lanatoside C is poorly absorbed after oral administration; it is converted to digoxin in the gastrointestinal tract and very little unchanged drug is found in the plasma or urine after oral administration. Hydrolysis to derivatives of digoxigenin and deacetylation to deslanoside also occur. After an oral dose, about 18% is excreted in the urine in 24 h, mostly as digoxin and metabolites of digoxigenin; after IV administration, about 30% of a dose is excreted in the urine in 24 h with about 70% of the urinary material consisting of unchanged drug and the remainder being digoxin and deslanoside. A total of about 60% of an IV dose is excreted in the urine in 5 days.

Therapeutic Concentration In serum, usually in the range 0.0004 to 0.001 mg/L. Following a single oral dose of 0.5 mg of ^{3}H-lanatoside C to 9 subjects, peak plasma concentrations of unchanged drug plus metabolites of about 0.002 mg/L were attained after about 1 h and a second peak of approximately the same concentration was reported in each subject after 5 to 8 h [Aldous *et al.* 1972].

Toxicity

A postmortem blood concentration of 0.047 mg/L was reported in a fatality involving an overdose of lanatoside C; death occurred 48 h after ingestion [Moffat 1974].

Distribution in Blood Plasma : whole blood ratio, 1.2.

Protein Binding About 25%.

Dose For slow digitalisation, 1.5 to 2 mg daily for 3 to 5 days; maintenance, 0.25 to 1.5 mg daily.

Aldous S *et al.* (1972). *Aust J Pharm Sci* 1: 35–41.

Moffat AC (1974). Interpretation of post mortem serum levels of cardiac glycosides after suspected overdosage. *Acta Pharmacol Toxicol (Copenh)* 35(5): 386–394.

Tracqui A *et al.* (1997). High-performance liquid chromatography-ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. *J Chromatogr B Biomed Sci Appl* 692(1): 101–109.

Landiolol

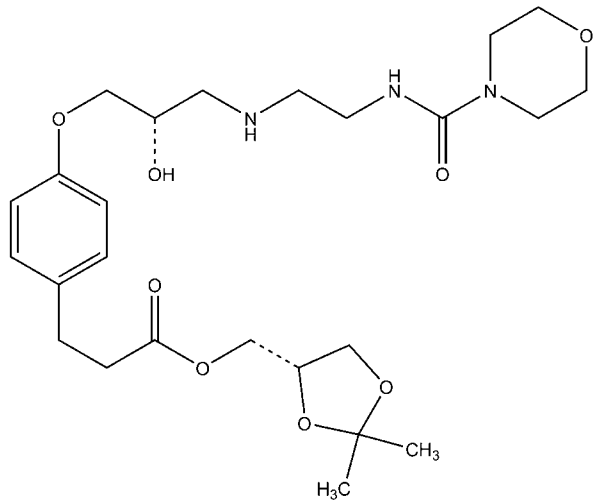
β_1-Adrenoceptor Antagonist, Antiarrhythmic

$C_{25}H_{39}N_3O_8$ = 509.6

CAS—133242-30-5

IUPAC Name [(4*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]methyl 3-[4-[(2*S*)-2-hydroxy-3-[2-(morpholine-4-carbonylamino)ethylamino]propoxy]phenyl]propanoate

Synonyms (−)-2,2-Dimethyl-1,3-dioxolan-4*S*-ylmethyl-3-[4-[3-[2-(morpholinocarbonylamino) ethyl]amino-2*S*-hydroxypropoxy]phenyl]propionate; 4-[(*S*)-2-hydroxy-3-[[2-[(4-morpholinylcarbonyl)amino]ethyl]amino]propoxy]benzenepropanoic acid [(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl ester.



Landiolol Hydrochloride

$C_{25}H_{39}N_3O_8$.HCl = 546.1

CAS—144481-98-1

IUPAC Name (−)-[(*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]methyl *p*-(*S*)-2-hydroxy-3-{[2-(4-morpholinecarboxamido)ethyl]amino}propoxy)hydrocinnamate hydrochloride

Synonym ONO-1101

Proprietary Name *Onoact*

Chemical Properties Mp 125.4°.

Thin-layer Chromatography Plates: Kiesel gel 60F_{254}, (0.25 mm). Solvent system: ethyl acetate : methanol (4 : 1). R_f 0.163 [Iguchi *et al.* 1992].

Disposition in the Body Rapidly metabolised by serum pseudocholinesterase and carboxyesterase in the liver to an inactive metabolite, which is subsequently excreted in urine.

Therapeutic Concentration

A group of 19 patients with various tachyarrhythmias was administered landiolol hydrochloride IV at 10, 20, 40 or 80 µg/kg/min for 5 min. Blood landiolol concentrations at the end of the infusion were reported as follows:

Dose (µg/kg/min)	*Blood landiolol at 5 min (µg/L)*	*Elimination Half-life (min)*
10	243	4.02
20	475	3.10
40	969	2.34
80	1861	2.40

[Atarashi *et al.* 2000]

In a separate study, patients with ventricular premature contraction were administered 20, 40 or 80 µg/kg/min IV over 15 min. Blood landiolol concentrations were as follows:

Dose (µg/kg/min)	*Blood landiolol (µg/L)*		*Elimination half-life (min)*
	5 min	*15 min*	
20	342	549	3.07
40	693	900	3.05
80	1570	1451	3.34

[Atarashi *et al.* 2000]

Six healthy volunteers and 6 patients with hepatic impairment were administered a 1 min loading infusion of 60 µg/kg/min landiolol hydrochloride, followed by another 60 min infusion of 20 µg/kg/min. Peak plasma concentrations and concentrations at 61 min (C_{61}) for landiolol hydrochloride and its metabolite (M1) were reported as follows:

Parameter	*Hepatic impairment*	*Healthy volunteers*
Landiolol HCl		
C_{max} (µg/L)	942	665
C_{61} (µg/L)	866	641
Half-life (min)	4.0	4.0
M1		
C_{max} (µg/L)	1380	1340
Half-life (min)	180	140

A small fraction of landiolol was detected unchanged in urine (6.2 and 4.7% for patients and healthy volunteers, respectively) with the remainder identified as M1 (71.3 and 57.7% for patients and healthy volunteers, respectively) [Takahata *et al.* 2005].

Half-life Approximately 4 min.

Volume of Distribution In healthy volunteers, 0.20 L/kg; in patients with hepatic impairment, 0.14 L//kg.

Clearance In healthy volunteers, 0.035 L/min/kg; in patients with hepatic impairment, 0.024 mL/min/kg.

Protein Binding Approximately 1.5 to 7.0%.

Note May be restricted in certain sports, as it is considered to be a member of a prohibited group (β-blockers).

Dose Given IV as the hydrochloride in the management of intra-operative cardiac arrhythmias.

Atarashi H *et al.* (2000). Pharmacokinetics of landiolol hydrochloride, a new ultra-short-acting beta-blocker, in patients with cardiac arrhythmias. *Clin Pharmacol Ther* 68: 143–150.

Iguchi S *et al.* (1992). Development of a highly cardioselective ultra short-acting beta-blocker. ONO-1101. *Chem Pharm Bull (Tokyo)* 40: 1462–1469.

Takahata T *et al.* (2005). Influence of hepatic impairment on the pharmacokinetics and pharmacodynamics of landiolol hydrochloride, an ultra-short-acting beta1-blocker. *Drugs R.D* 6: 385–394.

Lanreotide

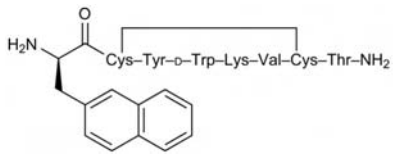
Antineoplastic

$C_{54}H_{69}N_{11}O_{10}S_2 = 1096.3$

CAS—108736-35-2

IUPAC Name 3-(2-Naphthalenyl)-D-alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinyl-L-threoninamide cyclic (2→7)-disulfide

Synonyms Angiopeptin; BIM-23014; DC 13-116.



Lanreotide Acetate

$C_{54}H_{69}N_{11}O_{10}S_2,xC_2H_4O_2$

CAS—127984-74-1

Proprietary Name *Somatuline LP*

Disposition in the Body Following the IM administration of sustained-release lanreotide, an initial rapid liberation of the drug is followed by more prolonged release.

Therapeutic Concentration

Eight healthy males were administered 30 mg lanreotide IM. Peak plasma concentration rose to 38.8 µg/L after 2 h, then decreasing progressively to 1.5 µg/L 11 days later and finally reaching 0.92 µg/L 2 weeks after injection [Kuhn *et al.* 1994].

Bioavailability About 50%.

Half-life Approximately 5 days.

Dose Given as a long-acting IM depot in the treatment of acromegaly.

Kuhn JM *et al.* (1994). Pharmacokinetic and pharmacodynamic properties of a long-acting formulation of the new somatostatin analogue, lanreotide, in normal healthy volunteers. *Br J Clin Pharmacol* 38: 213–219.

Lansoprazole

Proton Pump Inhibitor

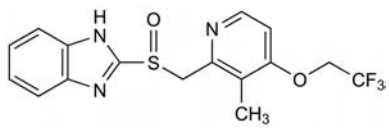
$C_{16}H_{14}F_3N_3O_2S = 369.4$

CAS—103577-45-3

IUPAC Name 2-[[[3-Methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridinyl]methyl] sulfinyl]-1*H*-benzimidazole

Synonyms A-65006; AG-1749; lanzoprazole.

Proprietary Names *Agopton*; *Bamalite*; *Dakar*; *Lansox*; *Lanzor*; *Limpidex*; *Ogast*; *Opiren*; *Prevacid*; *Takepron*; *Zoton*. It is also an ingredient of *Prevpac*.



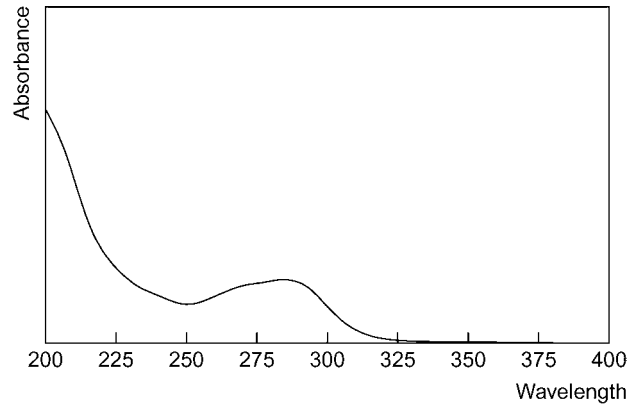
Chemical Properties White to brownish-white odourless crystalline powder. Mp 166°. Freely soluble in dimethylformamide; soluble in methanol; sparingly soluble in ethanol; slightly soluble in ethylacetate, dichloromethane and acetonitrile; very slightly soluble in ether and practically insoluble in water and hexane.

High Performance Liquid Chromatography System HAA—retention time 16.6 min.

Column: Chiralcel OD-R (250 × 4.6 mm i.d., 5 µm). Temperature 30°. Mobile phase: methanol : water (75:25), flow rate 0.5 mL/min. Internal standard (IS) : isobutyl 4-hydroxybenzoate. UV detection (λ=285 nm). Retention time: (+)-lansoprazole, 15.7 min; (−)-lansoprazole, 18.1 min; 5-hydroxylansoprazole, 10.7 min; lansoprazole sulfone, 13.6 min; IS, 12.3 min [Katsuki *et al.* 2001].

Column: C_{18} ODS (250 × 4.6 mm i.d., 5 µm). Temperature: 40° to 43°. Mobile phase (premix): 35% aqueous acetonitrile solution with 1 mL/L n-octylamine and 5 mmol/L *N*-acetohydroxamic acid, (pH 7), flow rate, 1.0 mL/min for 15 min then 2.5 mL/min. IS: omeprazole. UV detection (λ=285 nm). Retention time: lansoprazole, 13.2 min; metabolites: sulfide, 22.4 min, hydroxylated sulfide, 12.0 min, hydroxylated sulfinyl, 6.5 min, sulfone, 16.4 min; IS, 7.6 min [Karol *et al.* 1995].

Ultraviolet Spectrum Aqueous acid—284 nm.



Quantification

Plasma **HPLC** UV detection (λ=285 and 303 nm). Limit of quantification, 2 µg/L for lansoprazole, 3 to 5 µg/L for its metabolites [Landes *et al.* 1992].

Serum **HPLC** UV detection (λ=285 nm). Limit of quantification, 5 µg/L [Aoki *et al.* 1991].

Urine **HPLC** See Serum [Aoki *et al.* 1991].

Disposition in the Body Lansoprazole is rapidly absorbed after oral administration and peak plasma concentrations are reached within ~1.5 h. The drug is extensively metabolised in the liver to its sulfide and sulfone metabolites, and the 5-hydroxy metabolites of these. The drug and its metabolites, in the form of glucuronides, are excreted mainly in faeces via bile with 15 to 30% of an administered dose in urine. No unchanged drug has been detected. Clearance is reduced in elderly patients and in those with liver disease.

Therapeutic Concentration

Ten healthy male volunteers were administered an oral 30 mg dose of the enteric coated formulation in a fasted state. The maximum serum concentration was ~0.8 mg/L and was reached within 2 to 3 h [Aoki *et al.* 1991].

Group 1: 6 patients with mild renal dysfunction (creatinine clearance 40 to 60 mL/min). Group 2: 6 patients with moderate dysfunction (creatinine clearance 20 to 40 mL/min). Group 3: 5 patients with severe dysfunction (creatinine clearance <20 mL/min). Group 4: 8 patients with hepatitis. Group 5: 8 patients with compensated cirrhosis. Group 6: 8 patients uncompensated cirrhosis. All patients were aged between 34 and 74 years, male and female, and administered a 30 mg dose of lansoprazole, as were a control group of 18 healthy volunteers aged between 20 and 32 years. Peak plasma concentrations for the groups of individuals were 1.279, 0.94, 0.942, 1.08, 1.44 and 1.14 mg/L, respectively, which were reached in 1.6, 1.7, 1.4, 1.4, 2.1 and 2.1 h, respectively. The healthy individuals reached peak concentrations of 1.033 mg/L in 1.5 h [Delhotal-Landes *et al.* 1993].

Bioavailability 80 to 90%; may be reduced by antacids and sucralfate if taken within one hour of taking lansoprazole.

Half-life 1.4 to 2 h.

Volume of Distribution 0.45 L/kg.

Clearance Oral, 0.26 L/h/kg.

Protein Binding 97%.

Note For an update of the pharmacological properties of lansoprazole, see Langtry and Wilde [1997].

Dose 15 to 60 mg daily.

Aoki I *et al.* (1991). High-performance liquid chromatographic determination of lansoprazole and its metabolites in human serum and urine. *J Chromatogr* 571: 283–290.

Delhotal-Landes B *et al.* (1993). Pharmacokinetics of lansoprazole in patients with renal or liver disease of varying severity. *Eur J Clin Pharmacol* 45: 367–371.

Karol MD *et al.* (1995). Determination of lansoprazole and five metabolites in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 668(1): 182–186.

Katsuki H *et al.* (2001). High-performance liquid chromatographic assay for the simultaneous determination of lansoprazole enantiomers and metabolites in human liver microsomes. *J Chromatogr B, Biomed Sci Appl* 757: 127–133.

Landes BD *et al.* (1992). Determination of lansoprazole and its metabolites in plasma by high-performance liquid chromatography using a loop column. *J Chromatogr* 577: 117–122.

Langtry HD, Wilde MI (1997). Lansoprazole. An update of its pharmacological properties and clinical efficacy in the management of acid-related disorders. *Drugs* 54: 473–500.

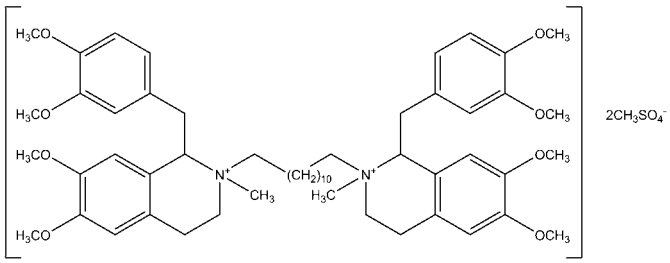
Laudexium Methyl Sulfate

Quaternary Ammonium, Muscle Relaxant

$C_{52}H_{74}N_2O_8,C_2H_6O_8S_2 = 1077.4$

IUPAC Name 1-[(3,4-Dimethoxyphenyl)methyl]-2-[10-[1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium-2-yl] decyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-2-ium methyl sulfate

Synonyms Compd 20; curarexium methylsulfate; decamethylene-α-ω-bis[1-(3′,4′-dimethoxybenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolinium methosulfate]; 2-decamethylenebis[1,2,3,4-tetrahydro-6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2-methylisoquinolinium methyl sulfate]; 2′-decamethylenebis[1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium methyl sulfate]; 2,2′-(1,10-decanediyl)bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolium] bis(methyl sulfate).
Proprietary Name *Laudolissin*



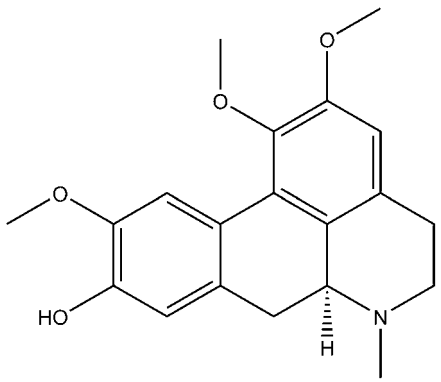
Chemical Properties Cream-coloured granules from alcohol and ether. Mp 172° to 174° [O'Neil *et al.* 2006].
Colour Tests Ammonium molybdate test—blue→purple→brown (limit of detection, 0.1 µg); ammonium vanadate test—blue→purple→brown (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—dull purple (limit of detection, 1.0 µg); Vitali's test—yellow/brown/brown (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.01 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—231, 280 nm.
Disposition in the Body
Toxicity In mice the LD_{50} (SC) is 2.95 mg/kg [Collier, Macauley 1954].
Note Laudexium does not inhibit cholinesterases *in vitro* [Brown 1954].

Brown LH (1954). Failure to detect anticholinesterase activity in laudexium methylsulphate. *Br J Anaesth* 26: 244–245.
Collier HO, Macauley B (1954). The effects on animals of large doses of laudexium methylsulphate. *Br J Anaesth* 26: 237–244.
O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Lauroscholtzine

Anxiolytic, Isoquinoline Alkaloid
$C_{20}H_{23}NO_4$ = 341.4
CAS—2169-44-0

Synonyms *N*-Methyllaurotetanine; NSC 247506; NSC 247564; rogersine; (*S*)-5,6,6a,7-tetrahydro-1,2,10-trimethoxy-6-methyl-4*H*-dibenzo[d,e,g]quinolin-9-ol; 1,2,10-trimethoxy-6a-α-aporphin-9-ol.
Proprietary Name It is an ingredient of the phytopharmaceutical *Sympathyl* as a component of *Eschscholtzia californica*.



Chemical Properties Aporphine (isoquinoline) alkaloid present in the leaves of the South American shrub *Peumus boldus* Molina (Monimiaceae, 'boldo leaves'), from leaves of *Guatteria dumetorum,* and from *Eschscholtzia californica* (Papaveraceae, 'California poppy' or 'Yellow poppy') [Correa *et al.* 2006; Hanus *et al.* 2004; Hughes *et al.* 1968]. Yellow needles from chloroform. Mp 237° to 238° [Chang *et al.* 1998].
High Performance Liquid Chromatography Column: C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.02 mol/L phosphoric acid (pH 4.3) : acetonitrile : methanol (45 : 12 : 1), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention times: lauroscholtzine 13.0 min, glaziovine 4.2 min, laurolitsine 5.6 min, boldine 6.0 min, isoboldine 8.0 min, isocorydine 10.4 min, laurotetanine 11.8 min, isodomesticine 14.0 min. Limit of quantification not reported [Sun *et al.* 1996].
Quantification
Other **HPLC** Leaf Samples (*G. dumetorum*). Column: C_{18} (250 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : water : TEA (70 : 30 : 0.1), flow rate 2.0 mL/min. UV and MS (Chemical ionisation) detection. Retention time: 32 min. Limit of quantification not reported [Correa *et al.* 2006]. Leaf Samples (*E. californica*). Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water containing 1 mmol/L SDS and 10 mmol/L TEA (pH 2.5; 80 : 20 to 60 : 40 over 40 min for 5 min to 0 : 100 over 5 min for 5 min), flow rate 1.0 mL/min. UV detection (λ = 280 nm) and MS detection (ESI). Retention times: lauroscholtzine 34.8 min, protopine 37.7 min, californidine 38.7 min (11 further alkaloids detected). Limit of quantification not reported [Fabre *et al.* 2000].
Disposition in the Body
Toxicity *Eschscholtzia* has been described as a substitute drug for marijuana, causing similar mild euphoria after ingestion or smoking, which lasts for 20 to 30 min. Gradual increase of the dose is recommended until the desired effect occurs. Regular and prolonged use is not thought to be habit-forming.
Dose Plant sources of lauroscholtzine are used in phytotherapy preparations for the treatment of anxiety disorders. They are also misused as herbal drugs of abuse (marijuana substitutes).

Chang FR *et al.* (1998). Two new 7-dehydroaporphine alkaloids and antiplatelet action aporphines from the leaves of *Annona purpurea*. *Phytochemistry* 49: 2015–2018.
Correa JE *et al.* (2006). Minor alkaloids from *Guatteria dumetorum* with antileishmanial activity. *Planta Med* 72: 270–272.
Fabre N *et al.* (2000). Direct characterization of isoquinoline alkaloids in a crude plant extract by ion-pair liquid chromatography–electrospray ionization tandem mass spectrometry: example of *Eschscholtzia californica*. *J Chromatogr A* 904: 35–46.
Hanus M *et al.* (2004). Double-blind, randomised, placebo-controlled study to evaluate the efficacy and safety of a fixed combination containing two plant extracts (*Crataegus oxyacantha* and *Eschscholtzia californica*) and magnesium in mild-to-moderate anxiety disorders. *Curr Med Res Opin* 20: 63–71.
Hughes DW *et al.* (1968). Alkaloids of *Peumus boldus.* Isolation of laurotetanine and laurolitsine. *J Pharm Sci* 57: 1619–1620.
Sun SW *et al.* (1996). Determination of lauraceous aporphine alkaloids by high-performance liquid chromatography. *J Pharm Biomed Anal* 14: 1383–1387.

Lead

Metal
Pb = 207.2
CAS—7439-92-1

Synonyms Olow; pigment metal; plumbum.
Proprietary Name *CI77575*
Chemical Properties Bluish-grey solid. Mp 327.4°. Bp 1740°. Insoluble in water, organic solvents; soluble in nitric acid, hot concentrated sulfuric acid. Valencies: Pb(0), Pb(+2), Pb(+4). Naturally occurring metal found in small amounts in the earth's crust. Used in alloys; lead–acid batteries; as construction material for tank linings and piping; in the metallurgy of steel and other metals; for X-ray and radiation protection; in ceramics, plastics and electronic devices.

Lead Acetate
$PbC_4H_6O_4$ = 325.3
CAS—301-04-2
Synonyms Lead (+2) acetic acid; plumbous acetate.
Proprietary Names *Salt of Saturn*; *Sugar of Lead*; *Unichem PBA*.
Chemical Properties White solid with a slightly acetic odour. Mp 280°. Very soluble in water; soluble in glycerol; very slightly soluble in alcohol. Used as a mordant in cotton dyes; drier in paints, varnishes and pigment inks; colorant in hair dyes; astringent; in lead coating for metals.

Lead Azide
PbN_6 = 291.3
CAS—13424-46-9
Synonym Initiating explosive (lead azide, dextrinated type only)
Chemical Properties White needles or powder. Explodes at 350°. Slightly soluble in water; soluble in acetic acid. Used as a primer in explosives.

Lead Chloride
$PbCl_2$ = 278.1
CAS—7758-95-4
Synonyms Lead (+2) chloride; lead (II) chloride; plumbous chloride.
Chemical Properties White crystalline powder. Mp 501°. Bp 950°. Soluble in water; insoluble in alcohol. Used in the manufacture of Pattison's white lead, Verona yellow, Turner's Patent Yellow, lead oxychloride; as solder and flux.

Lead Chromate
$PbCrO_4$ = 323.2
CAS—7758-97-6
Synonyms Lead chromate (VI); phoenicochroite.
Proprietary Names *40-2250*; *Canary Chrome Yellow*; *Cologne Yellow*; *King's Yellow*.
Chemical Properties Orange-yellow solid. Mp 844°. Slightly soluble in water; soluble in dilute acid; insoluble in acetic acid. Used as pigment for paints and inks, in oil and water colours, in printing fabrics, in decorating china and porcelain, in traffic paints.

Lead Iodide
PbI_2 = 461.0
CAS—10101-63-0
Synonyms Lead diiodide; lead (II) iodide; plumbous iodide.
Chemical Properties Bright or golden yellow hexagonal crystals. Slightly soluble in water; soluble in concentrated solutions of alkali iodides; freely soluble in solution of sodium thiosulfate; soluble in hot aniline; insoluble in alcohol or cold HCl. Used in bronzing, gold pencils, mosaic gold, printing, photography.

Lead Nitrate
PbN_2O_6 = 331.2
CAS—10099-74-8
Synonyms Lead dinitrate; lead (II) nitrate; nitric acid lead (+2) salt; plumbous nitrate.
Chemical Properties White or colourless translucent crystals. Very soluble in water; soluble in methanol; very sparingly soluble in alcohol, insoluble in concentrated nitric acid. Used in the manufacture of matches and special explosives; as mordant in dyeing and printing on textiles, for staining horn, and for mother-of-pearl; as an oxidiser in dye industry; as sensitiser in photography; for process engraving.

Lead Oxide
PbO = 223.2
CAS—1317-36-8
Synonyms Lead monoxide; lead protoxide; litharge; massicot; plumbous oxide.
Proprietary Names *CI77577*; *CI Pigment Yellow 46*.
Chemical Properties Reddish-yellow crystals at ordinary temperature, yellow crystals above 489°. Mp 886° (litharge). Slightly soluble in water; soluble in nitric acid, alkali chlorides; insoluble in alcohol. Used in ointments, plasters, glazing pottery, lead glass, varnishes, pigment for rubber, assay of gold and silver ores.

Lead Sulfate
$PbSO_4$ = 303.3
CAS—7446-14-2
Synonyms Lead (II) sulfate; sulfuric acid (+2) salt; occurs as the minerals anglesite and lanarkite.
Proprietary Names *CI77630*; *Fast White*; *Lead Bottoms*; *Mulhouse White*.
Chemical Properties White solid. Mp 1170°. Slightly soluble in water, nitric acid, hot concentrated sulfuric acid; insoluble in alcohol. Used as a pigment instead of white lead; in manufacture of batteries; in lithography, oil varnishes, weighting fabrics.

Lead Sulfide
PbS = 239.3
CAS—1314-87-0
Synonyms Lead monosulfide; lead (+2) sulfide; natural galena; plumbous sulfide.
Chemical Properties Black, blue or silvery cubic or metallic crystals with a pleasant, musty sweet odour. Mp 1114°. Bp 1281°. Very sparingly soluble in water; soluble in nitric acid, hot concentrated sulfuric acid; insoluble in alcohol. Used on glazing earthenware.

Colour Test Applicable to gastric contents and scene residues. Add 0.1 mL of sodium tartrate buffer (pH 2.8; prepared by mixing sodium bitartrate [19 g/L] and tartaric acid [15 g/L] in purified water) to 0.1 mL of test solution and vortex for 5 s. Apply 50 µL of the solution to a phase-separating filter paper and add 50 µL of sodium rhodizonate solution (10 g/L)—Lead salts give a purple colour. Coloured complexes are also formed by a number of other metals. Limit of detection, 2 mg/L.

Quantification Specimen collection: Blood—5 mL K-EDTA tube (must be lead free); urine—20 mL plastic universal container.

Note For reference values for lead in the German population, see Wilhelm *et al.* [2004].

Blood **DPASV** Limit of detection, 0.5 µg/L [Moreno *et al.* 1999].

AAS Perkin-Elmer 5000 Zeeman. Limit of detection, 1.0 µg/L [Palminger Hallén *et al.* 1995].

FAAS Perkin-Elmer 303 (λ = 283.3 nm). Air: acetylene flame, flow rate 4.0 and 22.8 L/min. Limit of detection, 11.9 µg/L [Delves 1970].

ETAAS Perkin-Elmer 1200. Perkin-Elmer 4100ZL. Limit of detection, 0.1 pg [Manton *et al.* 2001]. Perkin-Elmer 5100. Limit of detection, 4.0 µg/L [White 1999]. Dry cycle: 110° to 120°. Char cycle: 350°. Atomisation cycle: 2000° (λ = 283.3 nm). Limit of detection not reported [Baranowska 1995]. Carrier gas: Ar, 300 L/min. Dry cycle: 120° at 5 s for 15 s. Char cycle: 260° in 1 s for 5 s to 800° in 5 s for 27 s. Atomisation cycle: 1600° for 5 s, gas stop. Lead hollow cathode lamp (λ = 283.3 nm). Limit of detection, ~2.5 µg/L, [Bannon *et al.* 1994]. Carrier gas: Ar, 300 mL/min. Dry cycle: 150° at 1 s for 30 s. Char cycle: 550° in 1 s for 30 s to 950° in 10 s for 40 s. Atomisation cycle: 1900° for 2 s, gas stop. Perkin-Elmer/Zeeman 5000 (283.3 nm). Limit of quantification, 0.25 µmol/L, limit of detection, 0.08 µmol/L [Shuttler, Delves 1986].

ICP-MS Plasma gas: 15 L/min. Auxiliary gas: 1.13 L/min. Nebuliser gas: 1.0 mL/min. Limit of detection, not reported [Rainska *et al.* 2007]. Plasma gas: Ar, 14.8 L/min. Auxiliary gas: Ar, 0.9 L/min. Carrier gas: 1.1 L/min. Babington nebuliser (*m/z* 27). Limit of detection not reported [Botta *et al.* 2006]. Plasma gas: 16 L/min. Auxiliary gas: 1 L/min. Nebuliser gas: 1.15 or 1.33 mL/min. Limit of detection, 2 µg/L [De Boer *et al.* 2004]. Perkin-Elmer Sciex Elan 5000. Nebuliser gas: 1.0 mL/min. Limit of detection, 0.1 µg/L [White 1999]. Limit of detection, 3 µg/L [Bergdahl *et al.* 1998]. See also Schutz *et al.* [1996] and Delves, Campbell [1988].

Plasma **ICP-MS** Limit of detection, 0.1 µg/L [Bergdahl *et al.* 1998]. Plasma gas: 1.0 mL/min. Nebuliser gas: 0.9 L/min. Intermediate gas: 0.8 L/min. Outer gas: 13 L/min. Limit of detection, 0.012 to 0.015 ng/L [Schutz *et al.* 1996]. Outer gas: Ar, 12 L/min. Nebuliser gas: Ar, 0.75 L/min. Limit of quantification, 3.8 nmol/L; limit of detection, 0.23 nmol/L [Mauras *et al.* 1993].

Serum **ETAAS** Perkin-Elmer 4100ZL. Limit of detection, 0.1 pg [Manton *et al.* 2001].

ICP-AES External gas: 14.0 L/min. Indirect gas: 0.5 L/min. Bearing gas: 1.0 L/min. Meinhard nebuliser (λ = 165 to 460 nm). Limit of detection not reported [Olszewski *et al.* 2006].

ICP-MS Plasma gas: Ar, 11.0 L/min. Auxiliary gas: Ar, 1.4 L/min. Nebuliser gas: Ar, 0.9 to 1.0 L/min. Limit of detection, 0.04 µg/L [Gercken, Barnes 1991].

Urine **ETAAS** See Blood [White 1999]. Dry cycle: 110° for 20 s. Char cycle: 700° for 20 s. Atomisation cycle: 2000° for 2 s. Carbon furnace mode (λ = 283.3 nm). Limit of detection not reported [Smith, Griffiths 1982].

ICP-AES Perkin-Elmer 40 (λ = 220 nm). Plasma gas: Ar, 10 to 12 L/min. Auxiliary gas: Ar, 0.8 to 1.0 L/min. Nebuliser gas: 2.9 L/min. Limit of detection, 6.2 µg/L [Lopez-Artiguez *et al.* 1993].

ETV-ID-ICP-MS Outer gas: 15.0 L/min. Intermediate gas: 0.74 L/min. Carrier gas: 1.08 L/min. Dry cycle: 90° at 30 s for 10 s to 130° in 10 s for 10 s. Char cycle: 300° in 10 s for 20 s. Atomisation cycle: 1000° in 1 s for 20 s. Limit of detection, 5 ng/L [Lee *et al.* 1998].

ICP-MS See Blood [Rainska *et al.* 2007; Botta *et al.* 2006]. Limit of detection, 1 µg/L [De Boer *et al.* 2004]. Plasma gas: 15 L/min. Nebuliser gas: 0.825 L/min. Auxillary gas: 0.8 L/min. Limit of detection, 0.03 µg/L [Schramel *et al.* 1997].

Colostrum **ETAAS** Dry cycle: 50° to 120° at 20 s for 20 s. Char cycle: 120° to 900° in 30 s for 30 s. Atomisation cycle: 1600° for 7 s. Limit of detection, 0.92 µg/L [Turan *et al.* 2001].

Milk **ETAAS** Perkin-Elmer 5100 PC/HGA 600 with Zeeman background correction. Limit of detection, 1.0 µg/L [Palminger Hallén *et al.* 1995].

Ocular Fluid **ICP-MS** Perkin-Elmer Sciex Elan 6100. Limit of detection, 5 µg/L [Erie *et al.* 2005].

Oral Fluid **ICP-MS** Plasma gas: 13 L/min. Auxillary gas: 0.55 L/min. Nebuliser gas: 0.1 L/min. Limit of detection, 0.05 µg/L [Menegario *et al.* 2001].

Placenta **ETAAS** Perkin-Elmer 5100 (λ = 283.3 nm). Limit of detection not reported [Zagrodzki *et al.* 2003]. See Blood [Baranowska 1995].

Synovial Fluid **ICP-MS** Coolant gas: Ar, 15.0 L/min. Auxiliary gas: 0.99 L/min. Nebuliser gas: 1.0 L/min. Limit of detection, low ng/L [Krachler *et al.* 2000].

Bone **ETAAS** Dry cycle: 110° to 120° in 30 s. Char cycle: 300° to 350° in 30 s. Atomisation cycle: 2000° for 20 s. Carrier gas: Ar, 200 mL/min (λ = 283.3 nm). Limit of detection, 0.025 mg/L [Baranowska *et al.* 1995].

Hair **ETAAS** Dry cycle: 80° to 120° in 10 s. Char cycle: 300° to 400° at 10 s. Atomisation cycle: 2700° to 2800° in 5 s. Carrier gas: 200 mL/min. Hitachi model 180-50, S.N.5721-2 (λ = 193.8 nm). Limit of detection not reported [Kazi *et al.* 2006].

ICP-MS Limit of detection, 0.03 mg/kg [Nadal *et al.* 2005]. Plasma gas: 15 L/min. Auxiliary gas: 0.8 L/min. Nebuliser gas: 0.8 L/min. Limit of detection not reported [Samanta *et al.* 2004].

Note For a study following the trace element hair analysis of one man over 2 decades, see Klevay *et al.* [2004].

Iliac Crest **ETAAS** Dry cycle: 60° to 90° in 10 s for 5 s to 100° in 10 s for 5 s to 150° in 10 s, 200 mL/min. Char cycle: 150° to 1000° in 10 s for 20 s, 100 mL/min. Atomisation cycle: 2700° for 4 s. Lamp current: 10.0 mA (λ = 283.3 nm). Limit of detection, 1.0 µg/L [Scancar *et al.* 2000].

Liver **ICP-MS** Perkin-Elmer Elan 6000. Nebuliser gas: Ar, 1.04 L/min. Limit of detection, 171 ng/L [Patriarca *et al.* 1999].

Nail **ICP-MS** See Hair [Samanta *et al.* 2004].

Other **DPASV** Yemeni Khat. Limit of detection, 0.3 µg/kg [Matloob 2003].

ETAAS Eggs and Chicken Feed. Dry cycle: 120° at 1 s for 50 s. Char cycle: 1400° in 1 s for 30 s to 20° in 1 s for 5 s. Carrier gas: Ar, 300 mL/min. Atomisation cycle: 2300° for 5 s, gas stop. Limit of detection, 0.52 mg/kg [Fakayode, Olu-Owolabi 2003]. Cocaine Samples. Char cycle: 1000°. Atomisation cycle: 2400° (λ = 283.3 nm). Limit of detection not reported [Bermejo-Barrera *et al.* 1999].

ICP-AES Argentine Wine. Outer gas: 8.5 L/min. Auxiliary gas: 1.0 L/min. Nebuliser gas: 1.0 L/min. (λ = 220.4 nm). Limit of detection, 0.28 µg/L [Lara *et al.* 2005].

ICP-MS Solid and Liquid Food Samples. Limit of quantification, solid samples 2 µg/kg, liquid samples,0.2 µg/L; limit of detection, solid samples 0.6 µg/kg, liquid samples 0.06 µg/L [Cheung Chung *et al.* 2008]. Seafood. Perkin-Elmer Elan 6000. Limit of detection, 0.02 mg/kg [Falcó *et al.* 2006]. Gunshot residue. Coolant gas: 15 L/min. Auxiliary gas: 1.1 L/min. Sample gas: 0.97 L/min. Limit of detection, 0.117 µg/L [Reis *et al.* 2003]. Food. Varian-Vista with an ultrasonic nebuliser. Limit of detection, 0.04 mg/kg [Llobet *et al.* 2003]. Meals from Catering Eestablishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 26 µg/kg [Noel *et al.* 2003]. Beverages. Limit of detection, 0.2 µg/kg [MacIntosh *et al.* 2000].

Disposition in the Body Only 5 to 10% of lead orally ingested from the diet is absorbed. Lead accumulates in the body from birth and the total body burden (in adults) ranges from 100 to 400 mg, with 90% deposited in the bones as insoluble lead phosphate. Minor amounts are excreted in hair, nails and urine. Normal output via the kidneys is less than 10 µg/day (50 µmol/day). In blood, 99% of lead is contained in the erythrocytes.

Normal concentrations in blood: Urban exposure (adults and children), <100 µg/L (0.5 µmol/L). UK guidelines for occupational exposure: women of child-bearing age, <250 µg/L (1.2 µmol/L); men, <500 µg/L (2.4 µmol/L). Concentrations in the body: brain—0.02 to 0.78 mg/kg, hair—1.0 to 20 mg/kg, kidney—0.15 to 1.9 mg/kg, liver—0.18 to 3.1 mg/kg, nails—0.65 to 15 mg/kg, urine—0.01 to 0.19 mg/kg.

Toxicity Prior to 1970, blood concentrations greater than 60 µg/L signified lead poisoning. In 1971 the threshold was reduced to 40 µg/L. This was subsequently reduced to 30 µg/L in 1975 and 25 µg/L in 1985. In 1991 the Centers for Disease Control and Prevention in the USA redefined elevated blood lead concentrations in children as those ≥10 µg/L and recommended a new set of guidelines for treatment of lead concentrations ≥15 µg/L [Papanikolaou *et al.* 2005]. Ongoing studies are investigating whether the regulatory limit for children should be reduced to 5 µg/L [McQuirter *et al.* 2001]. Lead damages many enzyme systems, notably those involved in haem synthesis. Chronic exposure leads to gastrointestinal colic, anaemia, weight loss, muscle weakness and kidney damage. Blood lead measurements in children of industrially exposed fathers showed that 42% had concentrations above 0.3 mg/L with 11% having concentrations higher than 0.8 mg/L [Baker *et al.* 1977].

Three employees exposed to lead had blood lead concentrations of 159, 114 and 108 µg/L [Coyle *et al.* 2005].

The hair of autistic children had lead concentrations of 6.75 mg/kg compared with 3.20 mg/kg in controls [Fido, Al Saad 2005].

A 45-year-old woman presented with gastrointestinal problems. Her blood lead concentration was 100 µg/L and she was treated with chelation therapy with dimercaptosuccinic acid (DMSA). Ten years later her blood lead concentration was 210 µg/L, at which point DMSA therapy was started again. Four months later her blood lead concentration was 550 µg/L. An X-ray of her abdomen revealed a dense metal object 6 mm in diameter. During a bout of gastroenteritis, the pellet was excreted and it was identified as a lead shot pellet used for game hunting [Gustavsson, Gerhardsson 2005].

A 4-year-old boy presented with intermittent abdominal pain. An X-ray revealed a metallic foreign body in his stomach. His blood lead concentration was 123 µg/L. After 48 h dimercaprol and 4 days of EDTA his blood lead concentration fell to 57 µg/L. The patient was switched to oral DMSA but EDTA was resumed when his blood lead concentration rebounded to 69 µg/L. Following three 19-day courses of DMSA his blood concentration was <40 µg/L [VanArsdale *et al.* 2004].

A 66-year-old man worked for 20 years as a repairer in a glassworks. The hard tissue lead content of one of his teeth was 14.0 ppm [Bachanek *et al.* 2000]

A 12-month-old girl chewed the layers of paint from the top of her cot over a 2 month period. On admission to hospital, her blood lead concentration was 122.9 µmol/L. She died of acute lead encephalopathy before her lead concentrations could be reduced [Holmes 1994].

A 37-year-old woman who survived the ingestion of 7 g of a lead salt had a blood lead concentration of 2.28 mg/L [Karpatkin 1961].

Three children with histories of pica died from lead poisoning with blood lead concentrations of 1.11 to 3.50 mg/L [Alexander, Delves 1972].

A 32-year-old man sustained a gunshot wound to his elbow 6 years before presenting to a hospital. His serum lead concentration was 143 µg/L. A synovial biopsy was performed and the lead concentrations were in excess of 1000 µg/L [Sokolowski, Sisson 2005].

A 45-year-old man who had been shot in the wrist 16 months previously had a serum lead concentration of 84 µg/L and a urine lead concentration of 178 µg/L [Viegas, Calhoun 1986].

A 54-year-old woman who died 5 months after being shot in the leg and who absorbed lead from the retained bullet had a postmortem blood lead concentration of 5.3 mg/L [DiMaio *et al.* 1983].

Three patients with lead toxicity following gunshot wounds had blood concentrations of 525, 140 and 67 µg/L [Linden *et al.* 1982].

Fire-arms instructors exposed to lead fumes in an indoor pistol range complained of abdominal pain and had blood lead concentrations of 1.09 to 1.39 mg/L [Landrigan *et al.* 1975].

In a fatal case of lead poisoning in a child, tissue lead concentrations of 5.8 mg/kg (brain), 40 mg/kg (liver), 8.8 mg/L (kidney), 268 mg/kg (flat bone), and 132 mg/kg (long bone) were reported [Baselt 2005].

Five cases of toxicity following the self-injection of lead and opium resulted in blood lead concentrations of 4.56 to 2.02 µg/L. Postmortem concentrations in the 2 patients who died were as follows:

	Patient 1	*Patient 2*
Liver (mg/kg)	7.07	157
Kidney (µg/kg)	225	–
Skeletal muscle (µg/kg)	136	–
Heart (µg/kg)	124	–
Brain (µg/kg)	118	–
Blood (mg/kg)	–	2.5

[Beattie *et al.* 1975].

Traditional herbal medicines can contain up to 8 mg of lead per dose unit.

Two patients with lead poisoning following the ingestion of Indian herbal remedies had blood concentrations of 46 and 65 µg/L and urinary concentrations of 2489 and 1711 µg/24 h following IV EDTA [Muzi *et al.* 2005].

A lead-containing aphrodisiac from Bangladesh caused gastrointestinal symptoms in a 24-year-old man, who had a blood lead level of 1.36 mg/L [Waldron 1979].

A 4-month-old infant who was given herbal medicine treatment became unconscious and developed a blood lead concentration of 1.37 mg/L, but recovered with treatment [Chan *et al.* 1977].

A 59-year-old woman who was treated with a Chinese herbal medicine had joint pain and insomnia and a blood lead concentration of 0.9 mg/L [Kalman 1977].

Note For an evaluation of metal levels in welders, see Iarmarcovai *et al.* [2005]; for a study on the environmental influences on the trace element content of teeth, see Brown *et al.* [2004]; for a comparison of trace metal profiles in hair samples from children in urban or rural areas, see Hasan *et al.* [2004]; for a study of lead in the blood, serum, and red blood cells of patients with motor neuron disease, see Pamphlett *et al.* [2001]. Rahil-Khazen *et al.* [2002] have studied trace element levels in the postmortem tissue of 30 Norwegians. For biological monitoring of lead in workers in hazardous-waste incineration, see Schuhmacher *et al.* [2002]; for workers in glass manufacturing, see Arai *et al.* [1994] or Ludersdorf *et al.* [1987]. For a review of lead poisoning, see Markowitz [2000]. For blood lead concentrations in copper smelter workers in Japan, see Karita *et al.* [2000].

Half-life 0.4–3.6 years.

Distribution in Blood Erythrocyte : serum ratio, 19.

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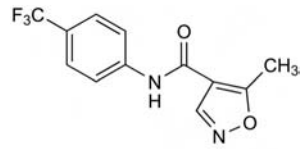
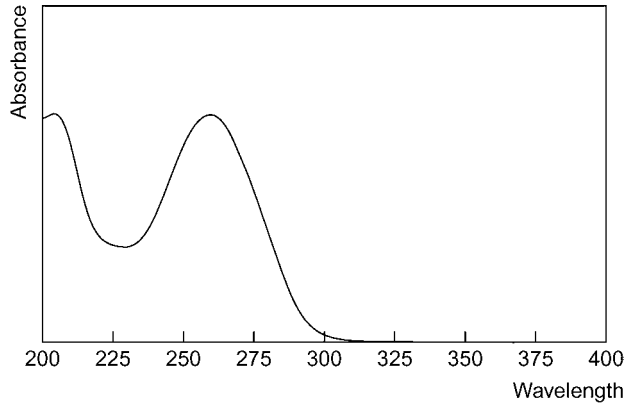
Leflunomide

Antirheumatic
$C_{12}H_9F_3N_2O_2 = 270.2$
CAS—75706-12-6

IUPAC Name 5-Methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide
Synonym HWA-486
Proprietary Name *Arava*



Chemical Properties White crystalline powder (two polymorphic forms). Mp 165° to 167°. pK_a (23°) 10.8.
Ultraviolet Spectrum Principal peaks at 206, 261 nm.



Quantification

Blood **HPLC** UV detection (λ=280 nm). Limit of detection, 400 µg/L (0.25 mL sample), 40 µg/L (1.0 mL sample) [Dias *et al.* 1995].
Plasma **HPLC** See Blood [Dias *et al.* 1995].
Disposition in the Body Leflunomide is rapidly and extensively metabolised, after absorption, to an active metabolite, A771726, responsible for the majority of the drug's immunosuppressive activity. The exact site of metabolism is not known, but is possibly the gut wall, plasma and the liver. The metabolite undergoes enterohepatic circulation and biliary recycling. The parent drug is not detected in plasma, only the metabolite. Peak plasma concentrations of the metabolite are observed 6 to 12 weeks after administration and bioavailability is not affected by the presence of food. Elimination of the metabolite is very slow (6 to 40 days) and approx. Ninety percent of the dose is recovered in faeces and urine. In faeces, leflunomide is primarily excreted as the metabolite, and in urine as leflunomide glucuronides and the oxanilic acid derivative of the metabolite. Small amounts are distributed into breast milk. Steady state concentrations are reached after ~20 weeks of leflunomide dosing.
Therapeutic Concentration
Fifty-four patients with rheumatoid arthiritis were administered 10 or 25 mg leflunomide daily. Steady state metabolite concentrations were 18 and 63 mg/L, respectively, after 24 days [Hoechst Marion Roussel Ltd. 1998].
Bioavailability About 80%.
Half-life 7 to 8 days (after a single dose); average, 11.1 days (range, 3.7 to 28.4 days) (after multiple dosing for 14 days).
Volume of Distribution 0.13 L/kg (metabolite).
Clearance 0.031 L/h (metabolite).
Protein Binding 99.4%, mainly to albumin (metabolite).
Note For a review of leflunomide, see Prakash and Jarvis [1999].
Dose 100 mg once daily for the first 3 days and then a maintenance dose of 10 to 20 mg once daily.

Dias VC *et al.* (1995). Measurement of the active leflunomide metabolite (A77 1726) by reverse-phase high-performance liquid chromatography. *Ther Drug Monit* 17(1): 84–88.
Hoechst Marion Roussel Ltd. (1998). Data on file. Kansas City.
Prakash A, Jarvis B (1999). Leflunomide: a review of its use in active rheumatoid arthritis. *Drugs* 58: 1137–1164.

Lepirudin

Anticoagulant, Thrombolytic
$C_{287}H_{440}N_{80}O_{111}S_6 = 6979.4$
CAS—138068-37-8

IUPAC Name 1-L-Leucine-2-L-threonine-63-desulfohirudin (Hirudo medicinalis isoform HV1)
Synonyms HBW-0.23; recombinant hirudin.
Proprietary Name *Refludan*
Chemical Properties A white powder.
Disposition in the Body Lepirudin is rapidly distributed and essentially confined to extracellular fluids. Metabolism and excretion mainly take place in the

kidneys with little in the liver. Approximately 48% of an administered dose is detected in urine with 35% as the unchanged drug. Metabolic pathways are not fully established. It is thought that catabolic hydrolysis of the parent drug causes a release of amino acids. Plasma concentrations increase proportionally with the dose and there is no evidence of accumulation.

Therapeutic Concentration The maximum plasma concentration after a single IV dose is 0.6 to 1 mg/L.

Half-life 1.3 h.

Volume of Distribution 12.2 L (young and healthy); 18.7 L (healthy elderly); 18.0 L (renally impaired); 32.1 L (patients with heparin induced thrombocytopenia).

Clearance Mean, 164 mL/min (young and healthy); 139 mL/min (elderly and healthy); 61 mL/min (patients with renal impairment); 114 mL/min (patients with heparin induced thrombocytopenia). In female patients, systemic clearance is ~25% lower compared with male patients.

Protein Binding <10%.

Dose The initial dose administered is 0.4 mg/kg (maximum 44 mg) with a maintenance dose of 0.15 mg/kg/h (maximum 16.5 mg).

Letrozole

Antineoplastic

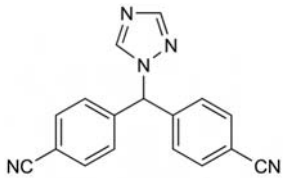
$C_{17}H_{11}N_5$ = 285.3

CAS—112809-51-5

IUPAC Name 4, 4′-(1*H*-1,2,4-Triazol-1-ylmethylene)bisbenzonitrile

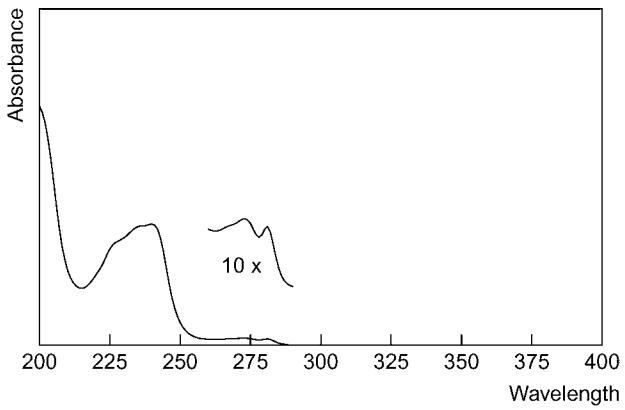
Synonym CGS-20267

Proprietary Names *Femar*; *Femara*.

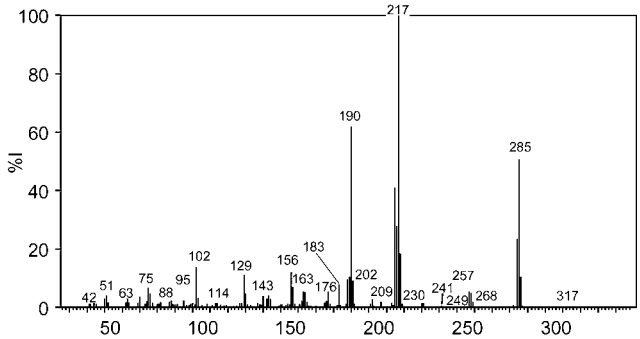


Chemical Properties A white to yellowish crystalline powder. Mp 181° to 183°, also reported as 184° to 185°. Freely soluble in dichloromethane; slightly soluble in ethanol; practically insoluble in water.

Ultraviolet Spectrum Aqueous acid—240, 273, 281 nm.



Mass Spectrum Principal ions at *m/z* 217, 190, 285, 215, 216, 284, 102, 218.



Quantification

Plasma **HPLC** UV detection (λ=234 nm). Limit of quantification, 8.9 nmol/L [Sioufi *et al.* 1997]. Column: ODS Hypersil C_{18} (200 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L phosphate buffer (pH 7) : acetonitrile (70:30), flow rate 1.5 mL/min. Internal standard (IS): CGP-47645. Fluorescence detection (λ_{ex}=230 nm, λ_{em}=295 nm). Retention time: letrozole, 11.3 min; metabolite 1 (CGP-44645), 13.3 min; IS, 16.7 min. Limit of quantification 0.4 µg/L [Marfil *et al.* 1996]. Column: RP-8 Lichrospher 100 (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 60 mmol/L phosphate buffer, (pH 7 39:6:55), flow rate 0.8 mL/min. IS: CGS-18320. UV detection (λ=234 nm). Retention time: letrozole, 8.9 min; IS, 10.7 min. Limit of quantification, 8 µg/L [Pfister *et al.* 1994].

Bioassay Limit of detection, 0.1 mg [Pfister *et al.* 1994].

Urine **HPLC** Fluorescence detection (λ_{ex}=230 nm, λ_{em}=295 nm). Limit of quantification 0.8 µg/L [Marfil *et al.* 1996]. See Plasma. Limit of quantification, 52 µg/L [Pfister *et al.* 1994].

Bioassay Limit of detection, 0.1 mg [Pfister *et al.* 1994].

Disposition in the Body Letrozole is rapidly and completely absorbed after oral administration, which can be affected by the presence of food. Peak concentrations tend to be larger when the drug is administered without food and these concentrations are reached in about half the time compared with individuals who are administered letrozole with food. It is rapidly and extensively distributed to cells. It is metabolised slowly in the liver to an inactive carbinol metabolite, 4,4′-methanol-bisbenzonitrile, which undergoes subsequent glucuronidation, and the conjugates are excreted by the renal route. Approximately 90% of the dose is excreted in urine; 75% as the glucuronide of the metabolite, 9% unidentified metabolites and 6% unchanged drug. Steady state plasma concentrations are reached within 2 to 6 weeks and remain constant over time without accumulation of the drug.

Therapeutic Concentration

Twelve healthy male volunteers, 28 to 44 years old, were fasted overnight and administered 2.5 mg letrozole with or without a standard high-fat breakfast. Those administered the drug in the fasting state were fasted for an additional hour after dosing. The peak plasma concentrations for the fasted volunteers were 129 (range, 94 to 164) nmol/L observed at 0.5 to 1.5 h and for the fed volunteers, 98.7 (74.5 to 146.0) nmol/L at 0.5 to 3.5 h [Sioufi *et al.* 1997].

Twelve healthy, post-menopausal women and 22 patients with breast cancer were administered 2.5 mg letrozole. Peak concentrations reached 115 nmol/L at ~1.0 h. The trough steady state concentration observed was ~400 nmol/L [Lamb, Adkins 1998].

Bioavailability 99.9%.

Half-life Approximately 2 days.

Volume of Distribution 1.9 L/kg.

Clearance Body clearance, 2.21 L/h.

Distribution in Blood The concentration of letrozole in erythrocytes is ~80% of that found in plasma.

Protein Binding 60% (mainly albumin).

Note For a review of letrozole, see Lamb and Adkins [1998]. For a general review of aromatase inhibitors, see Njar and Brodie [1999].

Dose 2.5 mg daily until tumour progression is evident.

Lamb HM, Adkins JC (1998). Letrozole. A review of its use in postmenopausal women with advanced breast cancer. *Drugs* 56(6): 1125–1140.

Marfil F *et al.* (1996). High-performance liquid chromatography of the aromatase inhibitor, letrozole, and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection. *J Chromatogr B Biomed Appl* 683(2): 251–258.

Njar VC, Brodie AMH (1999). Comprehensive pharmacology and clinical efficacy of aromatase inhibitors. *Drugs* 58: 233–255.

Pfister CU *et al.* (1994). Development, application and comparison of an enzyme immunoassay and a high-performance liquid chromatography method for the determination of the aromatase inhibitor CGS 20,267 in biological fluids. *J Pharm Sci* 83(4): 520–524.

Sioufi A *et al.* (1997). Comparative bioavailability of letrozole under fed and fasting conditions in 12 healthy subjects after a 2.5 mg single oral administration. *Biopharm Drug Dispos* 18(6): 489–497.

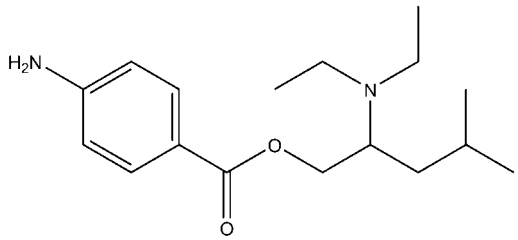
Leucinocaine

Anaesthetic (Local)

$C_{17}H_{28}N_2O_2$ = 292.4

IUPAC Name (2-Diethylamino-4-methylpentyl) 4-aminobenzoate

Synonym 2-Diethylamino-5-methylpentyl *p*-aminobenzoate



Chemical Properties Leucinocaine is extracted by organic solvents from aqueous alkaline solutions.

Leucinocaine Mesylate

$C_{17}H_{28}N_2O_2,CH_3SO_3H$ = 388.5

CAS—135-44-4

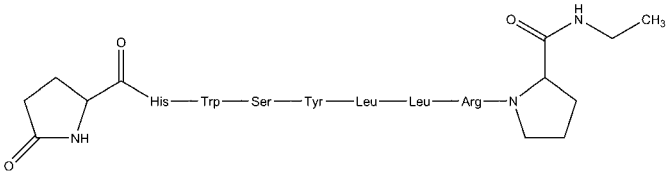
Synonyms *p*-Aminobenzoic acid β-diethylaminoisohexyl ester methanesulfonate; *p*-aminobenzoic acid *N,N*-diethylleucinol ester; *p*-aminobenzoyl-*N*-1-diethylamino-1-isobutylethanol methanesulfonate; 2-(diethylamino)-4-methyl-1-pentanol 4-aminobenzoate (ester) monomethanesulfonate (salt); 2-diethylamino-4-methylpentyl *p*-aminobenzoate methanesulfonate; methanesulfonate; 2-methyl-4-diethylaminopentan-5-ol *p*-aminobenzoate.
Proprietary Name *Panthesin*. It is an ingredient of *Hypacom*.
Chemical Properties Powder. Mp 157° to 159°. Soluble 1 in 3 of water; soluble in ethanol.

Colour Tests *p*-Dimethylaminobenzaldehde test—yellow; Vitali's test—yellow/bright yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.62 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—279 nm with infexions at 273 and 290 nm.

Leuprorelin

Antineoplastic (Hormonal), Gonadorelin Analogue, LH-RH Agonist
$C_{59}H_{84}N_{16}O_{12}$ = 1209.4
CAS—53714-56-0

IUPAC Name *N*-[1-[[1-[[1-[[1-[[1-[[1-[[5-(Diaminomethylideneamino)-1-[2-(ethylcarbamoyl)pyrrolidin-1-yl]-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino]-3-hydroxy-1-oxopropan-2-yl]amino]-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-3-(1*H*-imidazol-5-yl)-1- oxopropan-2-yl]-5-oxopyrrolidine-2-carboxamide
Synonyms (D-Leu6)-des-Gly10-LH–RH-ethylamide; 6-DL-leucine-9-(*N*-ethyl-L-prolinamide)-10-deglycinamide luteinizing hormone-releasing factor (pig); leuprolide; 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.



Chemical Properties A white or almost white hygroscopic powder. Store in airtight containers at a temperature not exceeding 30°. Protect from light.

Leuprorelin Acetate

$C_{59}H_{84}N_{16}O_{12}.C_2H_4O_2$ = 1269.5
CAS—74381-53-6
Synonyms Abbott-43818; leuprolide acetate; TAP-144.
Proprietary Names *Carcinil*; *Daronda*; *Depo-Eligard*; *Eligard*; *Enantone*; *Lectrum*; *Leuplin*; *Lorelin*; *Lucrin*; *Luprolex*; *Lupron*; *Procren*; *Prostap*; *Reliser*; *Trenantone*; *Viadur*.

Quantification

Serum **HPLC** Column: ODS (150 × 4.6 mm i.d.). Mobile phase: acetonitrile–water (10 : 90) with 0.1% trifluoroacetic acid : acetonitrile–water (40 : 60) with 0.1% trifluoroacetic acid (100 : 0 to 60 : 40 over 4 min to 40 : 60 over 20 min to 0 : 100 over 0.1 min for 3.9 min), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: leuprorelin 23.0 min, M1 15.3 min, M2 21.0 min, M3 8.9 min, M4 1.8 min. Limit of detection, 0.05 µg/L for leuprorelin and M1 [Ueno, Matsuo 1991].
Urine **HPLC** See Serum [Ueno, Matsuo 1991].
Disposition in the Body Leuprorelin has poor membrane permeability and undergoes significant deactivation by intestinal proteolytic enzymes. Therefore, it is not active when given orally but is well absorbed on SC or IM injection. Metabolism and excretion of leuprorelin have not been extensively studied in humans, although the pentapeptide 5–9 has been detected in serum and urine and is thought to be the major metabolite, formed by peptidase cleavage of the Ser^4–Tyr^5 bond. Other metabolites detected in animal studies include the tripeptides 1–3 and 5–7, and dipeptide 1–2.
Therapeutic Concentration

A group of 23 healthy male volunteers was administered 3 different formulations of leuprorelin acetate given as aerosol sprays. Formulation A consisted of a solution containing 0.5 mg leuprorelin acetate together with a number of solvents and excipients; formulations B and C were suspensions of 0.5 mg and 1.0 mg leuprorelin acetate, respectively, in 0.5% sorbitan triolate. Mean peak plasma concentrations of leuprorelin acetate for the three formulations and the IV control were reported as:

Formulation	*Dose (mg)*	*C_{max} (µg/L)*	*Time (h)*
A	1	0.970	2.3
B	1	4.380	1.6
C	2	11.37	1.1
IV control	1	133.2	N/A

The absolute bioavailabilities of the three aerosol formulations A, B and C were calculated as 4.3, 18.3 and 14.3%, respectively. The extent of pulmonary absorption of leuprorelin acetate was approx. 50% (range 43 to 55%) [Adjei, Garren 1990].

A group of 117 patients diagnosed with prostate cancer were administered a SC depot dose of 7.5 mg of leuprorelin acetate at monthly (28-day) intervals for 6 months. The mean peak serum leuprorelin acetate concentration was 26.3 µg/L reached after 4.7 h. Serum leuprorelin acetate was detectable for a mean of 37 days (range 28 to 49 days) [Perez-Marreno *et al.* 2002].

A group of 26 patients diagnosed with prostate cancer were administered a SC depot dose of 45 mg leuprorelin acetate at 6-monthly intervals (at day 1 and day 168). Mean peak serum concentrations of leuprorelin acetate were 82.0 µg/L at 4.4 h after the first injection and 102.4 µg/L at 4.8 h after the second. Serum leuprorelin acetate decreased slowly from day 3 to day 168 (plateau phase). During the plateau phase, mean serum leuprorelin acetate generally remained between 0.2 and 2.0 µg/L [Crawford *et al.* 2006].

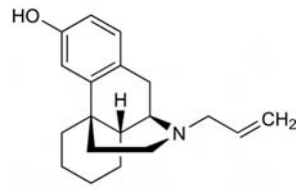
Bioavailability Less than 1%.
Half-life After parenteral administration, approx. 3 h.
Volume of Distribution Approximately 27.4 to 37.1 L.
Clearance Approximately 9.1 L/h.
Note For a review of the pharmacokinetics of depot leuprorelin, see Periti *et al.* [2002].
Dose In the management of advanced prostate cancer, leuprorelin acetate may be given by SC injection in a usual single daily dose of 1 mg. It is also given SC or IM as depot preparations (3.75 mg once a month, by SC or IM injection, or 11.25 mg SC every 3 months). A depot preparation of 45 mg given SC once every 6 months is also used in the USA. For the management of endometriosis and uterine fibroids, leuprorelin acetate 3.75 mg monthly may be given as a single depot injection, IM or SC. Alternatively, 11.25 mg may be given as an IM depot every 3 months. In the management of central precocious puberty leuprorelin acetate has been given by IM depot injection in a dose of 300 µg/kg every 4 weeks, adjusted according to response. Doses of 50 µg/kg daily by SC injection, adjusted according to response, have also been used.

Leuprorelin acetate has also been given in other sex-hormone-related disorders and has been tried in some gastrointestinal disorders such as irritable bowel syndrome.

Adjei A, Garren J (1990). Pulmonary delivery of peptide drugs: effect of particle size on bioavailability of leuprolide acetate in healthy male volunteers. *Pharm Res* 7: 565–569.
Crawford ED *et al.* (2006). A 12-month clinical study of LA-2585 (45.0 mg): a new 6-month subcutaneous delivery system for leuprolide acetate for the treatment of prostate cancer. *J Urol* 175: 533–536.
Perez-Marreno R *et al.* (2002). A six-month, open-label study assessing a new formulation of leuprolide 7.5 mg for suppression of testosterone in patients with prostate cancer. *Clin Ther* 24: 1902–1914.
Periti P *et al.* (2002). Clinical pharmacokinetics of depot leuprorelin. *Clin Pharmacokinet* 41: 485–504.
Ueno H, Matsuo S (1991). High-performance liquid chromatography followed by radioimmunoassay for the determination of a luteinizing hormone-releasing hormone analogue, leuprorelin, and its metabolite. *J Chromatogr* 566: 57–66.

Levallorphan

Narcotic Antagonist
$C_{19}H_{25}NO$ = 283.4
CAS—152-02-3
IUPAC Name 17-(2-Propenyl)morphinan-3-ol



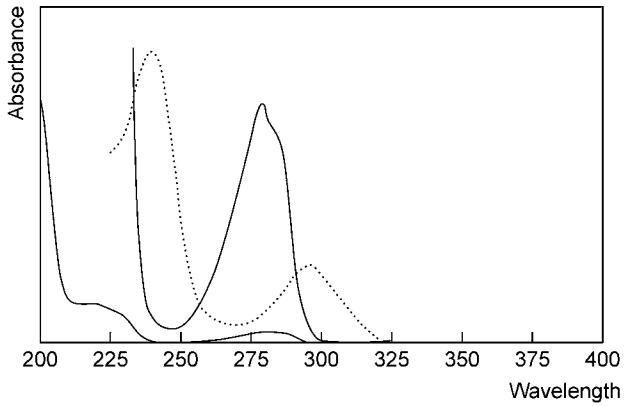
Chemical Properties White crystals. Mp 180° to 182°. pK_a 4.5, 6.9. Log *P* (octanol/buffer pH 7.4), 2.3.

Levallorphan Tartrate

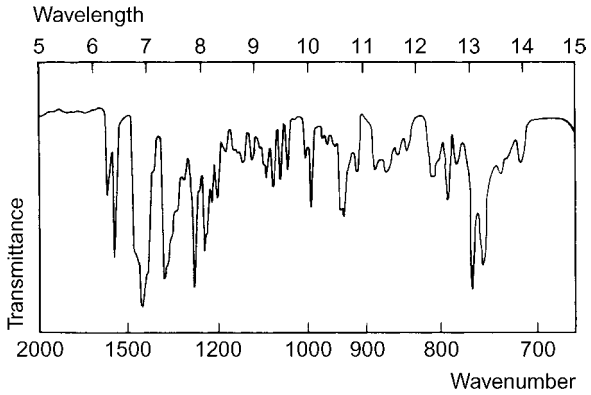
$C_{19}H_{25}NO.C_4H_6O_6$ = 433.5
CAS—71-82-9
Proprietary Names *Lorfan*. It is an ingredient of *Pethilorfan*.
Chemical Properties A white crystalline powder. Mp 174° to 177°. Soluble 1 in about 20 of water, 1 in 100 of ethanol, 1 in 3300 of chloroform, 1 in 5000 of ether and 1 in 13 of methanol.

Colour Tests Aromaticity (method 2)—yellow/orange; Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.19; system TC—R_f 0.24; system TE—R_f 0.74; system TL—R_f 0.45; system TAE—R_f 0.42; system TAF—R_f 0.73; system TAJ—R_f 0.10; system TAK—R_f 0.06; system TAL—R_f 0.66 (Dragendorff spray, positive; FPN reagent, yellow; acidified iodoplatinate solution, positive; Marquis reagent, grey).

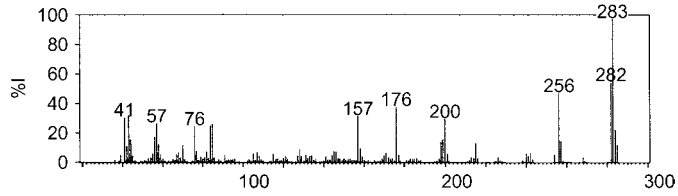
Gas Chromatography System GA—levallorphan RI 2355, levallorphan-AC RI 2390; system GB—levallorphan RI 2460.
High Performance Liquid Chromatography System HA—*k* 1.9 (tailing peak); system HC—*k* 1.46; system HX—RI 356; system HY—RI 291.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=71a); aqueous alkali—240 (A^1_1=312a), 299 nm.



Infrared Spectrum Principal peaks at wavenumbers 759, 1271, 747, 1575, 1242, 932 cm^{-1} (Nujol mull).



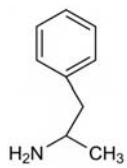
Mass Spectrum Principal ions at *m/z* 283, 282, 256, 176, 157, 43, 41, 57.



Disposition in the Body Levallorphan is effective within 1 min of IV injection and the effects may last up to 4 h. It is metabolised by *N*-dealkylation and glucuronic acid conjugation.
Toxicity The estimated minimum lethal dose is 0.2 g or 2 g for an addict.
Dose 0.5 to 1 mg of levallorphan tartrate intravenously, repeated if necessary.

Levamfetamine

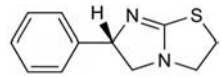
Anorectic, CNS Stimulant
$C_9H_{13}N$ = 135.2
CAS—156-34-3
IUPAC Name (−)-α-Methylbenzeneethanamine
Synonyms (−)-Amfetamine; laevo-amfetamine; levamphetamine.



Chemical Properties pK_a 10.1 (20°). Log *P* (octanol/water), 1.8.
Note For analytical data see under Amfetamine.

Levamisole

Anthelmintic
$C_{11}H_{12}N_2S$ = 204.3
CAS—14769-73-4
IUPAC Name (6*S*)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole
Synonym l-Tetramisole
Proprietary Names *Anthelpor* (vet.); *Levadin* (vet.).

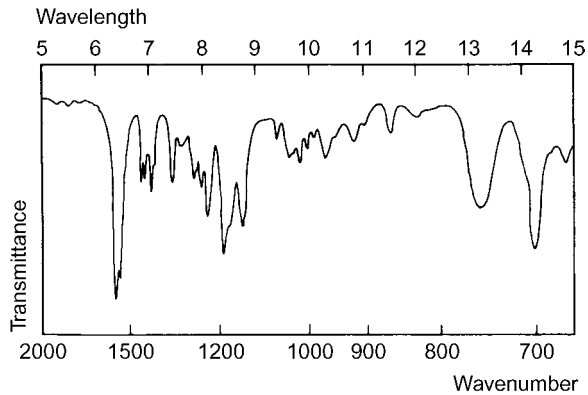


Chemical Properties A white crystalline powder. Mp 60° to 61.5°. Soluble in dilute acetic acid and in chloroform. pK_a 8.0. Log *P* (octanol/water), 1.8.

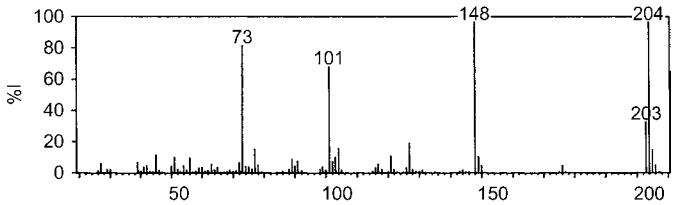
Levamisole Hydrochloride

$C_{11}H_{12}N_2S,HCl$ = 240.8
CAS—16595-80-5
Proprietary Names *Armadose* (vet.); *Ascaridil*; *Chanaverm* (vet.); *Decaris*; *Decazole* (vet.); *Ergamisol*; *Ketrax*; *Levacide* (vet.); *Levacur* (vet.); *Levasole* (vet.); *Nemicide* (vet.); *Nilverm* (vet.); *Ripercol* (vet.); *Solaskil*; *Spartakon* (vet.); *Sure* (vet.); *Tramisole* (vet.); *Vermisole* (vet.); *Wormaway* (vet.).
Chemical Properties A white to pale cream-coloured crystalline powder. Mp 227° to 229°. Soluble 1 in 2 of water and 1 in 5 of methanol; practically insoluble in ether.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—orange→grey-green.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.18; system TC—R_f 0.48; system TE—R_f 0.65; system TL—R_f 0.42; system TAE—R_f 0.53; system TAF—R_f 0.52 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1928.
High Performance Liquid Chromatography System HY—RI 238; system HAA—retention time 7.0 min.
Infrared Spectrum Principal peaks at wavenumbers 1587, 1575, 1197, 699, 1150, 1248 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 204, 148, 73, 101, 203, 127, 104, 205.



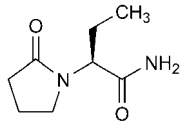
Quantification
Plasma **GC** FID. Limit of detection, 2 µg/L [Kouassi *et al.* 1986]. AFID. Limit of detection, 4 µg/L [Rousseau *et al.* 1981].
HPLC UV detection. Limit of detection, 21 µg/L [Vandamme *et al.* 1995].
Urine **GC** FID. For method, see Kouassi *et al.* [1986].
HPLC UV detection. Limit of detection, 0.5 mg/L for *p*-hydroxylevamisole [Kouassi *et al.* 1986].
Other UV detection (in sheep or cattle plasma). Limit of detection 20 µg/L, UV detection [Marriner *et al.* 1980].
Disposition in the Body Levamisole is readily absorbed after oral administration, but extensively metabolised. It is almost completely excreted in the urine and faeces within 48 h.
Therapeutic Concentration
Following single oral doses of 2.5 mg/kg and 5 mg/kg to 11 subjects, mean peak plasma concentrations of 0.7 and 1.5 mg/L, respectively, were attained within 2 h [Luyckx *et al.* 1982].
Half-life Plasma half-life, about 4 h.
Dose The equivalent of 2.5 to 5 mg/kg of levamisole daily, for 2 or 3 days.

Kouassi E *et al.* (1986). Novel assay and pharmacokinetics of levamisole and p-hydroxylevamisole in human plasma and urine. *Biopharm Drug Dispos* 7: 71–89.
Luyckx M *et al.* (1982). Pharmacokinetics of levamisole in healthy subjects and cancer patients. *Eur J Drug Metab Pharmacokinet* 7: 247–254.
Marriner S *et al.* (1980). Determination of the anthelmintic levamisole in plasma and gastrointestinal fluids by high-performance liquid chromatography. *Analyst, Lond* 105: 993–996.
Rousseau F *et al.* (1981). Gas-chromatographic determination of levamisole in human plasma-normalization and reliability of the method. *Eur J Drug Metab Pharmacokinet* 6: 281–288.
Vandamme TF *et al.* (1995). Quantitation of levamisole in plasma using high performance liquid chromatography. *Eur J Drug Metab Pharmacokinet* 20(2): 145–149.

Levetiracetam

Antiepileptic
$C_8H_{14}N_2O_2 = 170.2$
CAS—102767-28-2

IUPAC Name (2*R*)-2-(2-Oxopyrrolidin-1-yl)butanamide
Synonyms (α*S*)-α-Ethyl-2-oxo-1-pyrrolidineacetamide; (*S*)-α-Ethyl-2-oxo-1-pyrrolidine acetamide; SIB-S1; UCB-L059.
Proprietary Name *Keppra*



Chemical Properties White to off-white crystalline powder with a faint odour and bitter taste. Crystals from ethyl acetate. Mp 117°. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005]. It is very soluble in water (104 g/100 mL); freely soluble in chloroform (65.3 g/100 mL) and methanol (53.6 g/100 mL); soluble in ethanol (16.5 g/100 mL); sparingly soluble in acetonitrile (5.7 g/100 mL); practically insoluble in *n*-hexane. Stable in plasma in the autosampler at 25° for 24 h, after 5 freeze–thaw cycles and for 30 days when kept frozen at –80° [Matar 2008]. Stock solutions were stable at room temperature for 24 h and at 2 to 8° for 17 days. Leviracetam in control human plasma was stable at room temperature for at least 6 h. Leviracetam was stable for at least 3 freeze-thaw cycles. Leviracetam-spiked plasma samples stored at –70° were stable for 66 days [Jain *et al.* 2006].

Gas Chromatography System GB—RI 1629.

Column: (analytical) DB-FFAP megabore (30 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 30 mL/min. Temperature programme: 70° to 190° at 40°/min to 250° at 5°/min for 2 min. IS: UCB -G025. NPD. Retention time: levetiracetam, 14.0 min; IS, 11.5 min [Vermeij, Edelbroek 1994].

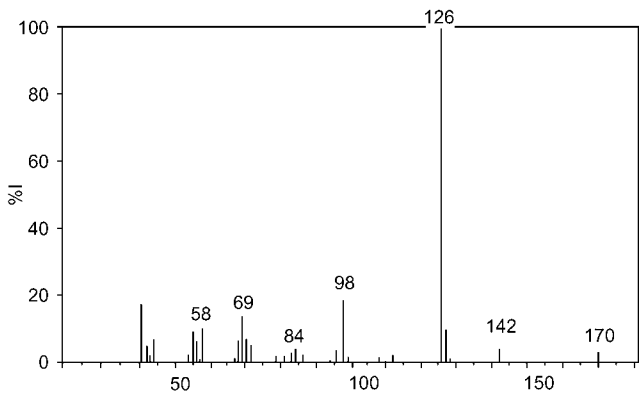
Gas Chromatography-Mass Spectrometry Column: 6-TBDMS-2,3-perme-β-cyclodextrin (20% w/w) in SE52 fused-silica capillary (10 m × 0.25 mm i.d., 0.5 µm). Carrier gas: He, 40 cm/s. Temperature programme: 110° for 2 min to 170° at 10°/min for 5 min. IS: *N*-dimethylvalproyl glycinamide. SIM acquisition mode (*m/z*: 69, 98, 126 for levetiracetam; *m/z*: 129, 157, 186 for IS). Retention time: (*S*)-levetiracetam, 8.6 min; (*R*)-levetiracetam, 8.8 min; IS, 10.3 min. [Isoherranen *et al.* 2000].

High Performance Liquid Chromatography Column: LiChrospher, 60 RP-select B (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 5.6; 15 : 85), flow rate 0.8 mL/min. IS: UCB-17025. UV detection (λ = 220 nm). Retention time: levetiracetam, 5.4 min; IS, 6.8 min [Ratnaraj *et al.* 1996].

Column: Spherisorb, RP 3ODS2 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (6 : 94 to 20 : 80 in 5 min to 40 : 60 in 1 min for 10 min to 6 : 94 in 1 min), flow rate 1.0 mL/min. IS: UCB-G025. DAD (λ = 205 nm). Retention time: levetiracetam 6.4 min; IS 7.8 min [Vermeij, Edelbroek 1994].

Capillary Electrophoresis Column: uncoated fused silica (total/effective length 50/41 cm, 50 µm i.d.). Rinse: 1.0 mol/L sodium hydroxide for 10 min, 0.1 mol/L sodium hydroxide for 10 min, distilled water for 10 min and background electrolyte (BGE) for 10 min. Microemulsions: with 1-propanol and 1-butanol as cosurfactants: 10 mmol/L aqueous borate buffer (pH 9.2) : *n*-octane : SDS : alcohol (93.76 : 0.48 : 1.80 : 3.96); with 1-pentanol as cosurfactant: aqueous borate buffer: *n*-octane : SDS : alcohol (96.41 : 0.50 : 2.07 : 1.02); with 1-hexanol as cosurfactant : aqueous borate buffer : *n*-octane : SDS : alcohol (96.87 : 0.51 : 2.10 : 0.52). Limit of quantification, ≈10 ppm [Ivanova *et al.* 2003].

Mass Spectrum Principal ions at *m/z* 126, 98, 41, 69, 58, 55, 127, 44.



Quantification

Plasma **GC** Column: HP-FFAP fused silica capillary (25 m × 0.32 mm i.d.). NPD. Limit of quantification, 4.0 mg/L, limit of detection, 0.5 mg/L [Fay *et al.* 2005]. Column: fused silica capillary FFAP (15 m × 0.32 mm i.d.). Carrier gas: 20 mL/min. Temperature: 230°. NPD. Retention time: 7 min. Limit of quantification, 0.5 mg/L [Perucca *et al.* 2003].

GC-MS Column: 6-TBDMS-2,3-perme-β-cyclodextrin (10%) in SE52 (10 m × 0.25 mm i.d., 0.5 µm). Carrier gas: He, 40 cm/s. Temperature programme: 110° for 2 min to 170° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 2.35 pg/L, limit of detection, 0.94 pg/L [Isoherranen *et al.* 2000].

HPLC Column: Synergi Hydro-RP (150 × 4.6 mm, 4 µm). Mobile phase: 50 mmol/L potassium dihydrogen phosphate buffer (pH 4.5) : acetonitrile (94 : 6), flow rate 1.5 mL/min. UV detection (λ = 205 nm). Retention time: 7.2 min. Limit of quantification, 2.0 mg/L, limit of detection, 1.0 mg/L [Contin *et al.* 2008]. Column: XTerra MS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium buffer (pH 10; 30 : 70), flow rate 0.8 mL/min. DAD (λ = 200 nm). Retention time: 6 min. Limit of quantification, 0.5 mg/L [Lancelin *et al.* 2007]. Column: Luna phenyl hexyl (150 × 2.0 mm i.d., 5 µm). Mobile phase: water : dibutylammonium phosphate : methanol (100 : 1 vial : 100), flow rate 0.5 mL/min. UV detection (λ = 220 nm). Retention time: 1.33 min. Limit of quantification, 5 mg/L [Juenke *et al.* 2006]. Column: ResElut reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 3 mmol/L phosphate buffer containing 0.5 mL triethylamine (6 : 5 : 89), flow rate 1.0 mL/min. DAD (λ = 205 nm). Retention time: 7.3 min. Limit of quantification, 500 µg/L, limit of detection, 150 µg/L [Pucci *et al.* 2004]. Column: LiChrospher 60 RP-select B (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 5.6; 15 : 85), flow rate 0.8 mL/min. UV detection (λ = 220 nm). Retention time: 5.4 min. Limit of quantification, 5 µmol/L, limit of detection, 1 µmol/L [Ratnaraj *et al.* 1996].

LC-MS Column: Symmetry C_{18} (50 × 3.9 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (97 : 0.3 : 0.25), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 1.0 mg/L [Matar 2008]. Column: Supelco LC-18-DB (3.3 cm × 3.0 mm i.d., 3 µm). Mobile phase: 15 mmol/L ammonium acetate-methanol (98 : 2) : 16 mmol/L ammonium acetate-methanol (3 : 97) both containing 0.1% acetic acid (100 : 0 for 2.5 min to 0 : 100 at 2.51 min for 2.5 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.9 min. Limit of detection, 0.1 mg/L [Guo *et al.* 2007]. Column: Alltech C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 2 mmol/L ammonium acetate : acetonitrile : 0.1% formic acid (60 : 30 : 30). Limit of detection, 0.1 mg/L [Zhao *et al.* 2007]. Column: Betasil C_{18} (100 × 3.0 mm i.d., 3 µm). Mobile phase: buffer (pH 3.2) : acetonitrile (20 : 80), flow rate 500 µL/min. TIS, positive ion mode, SRM acquisition mode. Retention time: 0.87 min. Limit of quantification, 0.5 mg/L [Jain *et al.* 2006].

CE Capillary: fused silica (total/effective length 32/23.6 cm, 50 um i.d.). Running buffer: 100 mmol/L phosphate buffer (pH 8.0). UV detection (λ = 215 nm). Retention time: <8 min [Mertzman, Foley 2004]. Capillary: uncoated fused silica (total/effective length 50/41 cm, 50 µm i.d., 360 µm o.d.). Running buffer: 30 mmol/L borate buffer (pH 9.2) with 50 mmol/L SDS. UV detection (λ = 214 nm). Limit of quantification, ≈10 ppm [Ivanova *et al.* 2003].

Serum **GC** Column: DB-FFAP (30 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 30 mL/min. Temperature programme: 70° to 190° at 40°/min to 250° at 5°/min for 2 min. NPD. Retention time: 14 min. Limit of quantification, 0.29 mg/L [Vermeij, Edelbroek 1994].

HPLC Column: Thermo Hypercarb (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.423% hydrogen phosphate : acetonitrile (95 : 5 to 70 : 30 at 8 min to 0 : 100 for 4 min), flow rate 1 mL/min. UV detection (λ = 205 nm). Retention time: 6.8 min. Limit of detection, 0.1 mg/L [Martens-Lobenhoffer, Bode-Böger 2005]. See Plasma [Ratnaraj *et al.* 1996]. Column: Spherisorb 3ODS2 (15 × 0.46 cm i.d.). Mobile phase: acetonitrile : water (6 : 94 to 20 : 80 at 5 min to 40 : 60 at 6 min for 4 min to 6 : 94 at 11 min for 4 min. DAD (λ = 205 nm). Retention time: 6.4 min. Limit of quantification, 0.36 mg/L [Vermeij, Edelbroek 1994].

LC-MS See Plasma [Guo *et al.* 2007].

CE Column: untreated capillary (35 cm × 50 µm). Running buffer: 140 mmol/L boric acid (pH 8.9) containing 40 g (13.8 mmol/L) SDS and 250 mL methanol/L. UV detection (λ = 214 nm). Retention time: 6.5 min. Limit of detection, ≈3 mg/L [Shihabi *et al.* 2003].

Urine **GC-MS** See Plasma. Limit of quantification 15 pg/L [Isoherranen *et al.* 2000].

Oral Fluid **LC-MS** See Plasma [Guo *et al.* 2007].

Disposition in the Body Levetiracetam is rapidly and almost completely absorbed after oral administration. The extent of absorption is not affected by food [Fay *et al.* 2005] but peak plasma concentrations may be reduced by ≈20%, and time to reach these concentrations may be prolonged by ≈1.5 h. Peak plasma concentrations are reached, generally, within 1 h and steady state achieved after two days of multiple twice-daily dosing. The major metabolic pathway is enzymatic hydrolysis, which is not extensive and occurs in several tissues (including blood cells). A carboxylic acid metabolite, ucb LO57, is produced. Two other minor metabolites have been detected; they are products of hydroxylation. Levitaracetam is not metabolised by the hepatic CYP450 system and thus avoids interactions with other antiepileptic drugs. The drug and its metabolites are excreted renally, with 66% of a dose excreted unchanged in urine, whereas 27% as an inactive hydrolysis product (on the acetamido group) [Neels *et al.* 2004; Radtke 2001].

Therapeutic Concentration In serum, 10–37 mg/L [Neels *et al.* 2004].

Sixty-nine patients (39 males and 30 females) aged 18–78 years were treated with 500–3000 mg/day levetiracetam associated with one or more

antiepileptic drug. In patients treated with 500, 1000, 1500, 2000 and 3000 mg/day the mean ± SD concentrations were 3.1 ± 0.9, 6.5 ± 2.4, 10.7 ± 5.1, 12.4 ± 4.5, and 16.8 ± 5.9 mg/L, respectively [Lancelin *et al.* 2007].

Twenty-six healthy male Chinese subjects were administered 500 or 1500 mg levetiracetam as a single dose. Mean maximum plasma concentrations were 13.6 ± 3.2 and 47.1 ± 12.1 mg/L at 0.5 h for each dose, respectively [Zhao *et al.* 2007].

Seventeen healthy subjects were administered a single 1500-mg dose of levetiracetam as a 15-min IV infusion or as an oral dose. The mean maximum plasma concentration following the IV infusion was 50.5 mg/L compared with 47.7 mg/L after the oral dose [Ramael *et al.* 2006].

Following a twice daily dose of 1 mg/kg levetiracetam to 590 epilepsy patients concentrations were 2.1 and 0.8 mg/L at 1 and 12 h, respectively [Perucca *et al.* 2003].

Seven male patients with intractable epilepsy, mean age 41 years (range, 24 to 71 years) and having epilepsy for 2 days to 19 years (mean, 9.7 years) with 1 or more seizures a week participated in the study. Patients were administered with ascending doses of levetiracetam between 0.5 and 2.0 g daily; increased by 0.5 g every 4 weeks according to its tolerability and safety as determined by an initial trial period. The doses administered varied between 5.4 mg/kg and 32.2 mg/kg (mean, 15.96 mg/kg) and these produced peak plasma concentrations of 6.29 and 38.17 mg/L (mean, 22.25 mg/L) [Sharief *et al.* 1996].

Toxicity Overdose causes sedation and coma as well as respiratory depression. A 12-year-old female with idiopathic partial epilepsy developed acute psychosis 10 days after the administration of leviracetam. The psychotic behaviour resolved soon after discontinuation of the drug [Youroukos *et al.* 2003]. Four other cases in children have been reported by Kossoff *et al.* [2001] whereas a psychotic episode following levitacetam consumption in a 30-year-old female has been reported [Bayerlein *et al.* 2004].

A 38-year-old female was admitted into hospital 6 h after ingesting 60 tablets of 500 mg levetiracetam. Her serum concentrations were 400, 72, 60 mg/L at 6, 18, and 20.5 h, respectively. No other drugs were detected and she recovered the next day without sequelae [Barrueto, Jr. *et al.* 2002].

Note For 2 cases of overdose in children, see Awaad [2007].

Bioavailability Oral, almost 100% [Franco Spinola *et al.* 2008].

Half-life 6–8 h; increased in the elderly [Patsalos 2000] and in subjects with renal impairment.

Volume of Distribution 0.5–0.7 L/kg [Neels *et al.* 2004; Radtke 2001].

Clearance Total body, 0.96 mL/min/kg; reduced in the elderly and those with renal impairment – 40% in patients with mild impairment, 50% moderate impairment and 60% for those with severe impairment; increased in paediatric patients by ≈40%.

Protein Binding <10% [Grim *et al.* 2003; Neels *et al.* 2004; Radtke 2001].

Note For a review of the pharmacokinetic profile of levetiracetam, see Patsalos [2000] and for a safety profile of levetiracetam, see Harden [2001].

Dose The usual dose is 500 mg twice daily which may be increased in increments of 1 g daily every 2 weeks; maximum 3 g daily. The dose may be adjusted for patients with renal impairment.

Awaad Y (2007). Accidental overdosage of levetiracetam in two children caused no side effects. *Epilepsy Behav* 11: 247.

Barrueto FJr *et al.* (2002). A case of levetiracetam (Keppra) poisoning with clinical and toxicokinetic data. *J Toxicol Clin Toxicol* 40: 881–884.

Bayerlein K *et al.* (2004). Drug-induced psychosis after long-term treatment with levetiracetam. *Can J Psychiatry* 49: 868.

Contin M *et al.* (2008). Simple and validated HPLC-UV analysis of levetiracetam in deproteinized plasma of patients with epilepsy. *J Chromatogr B Analyt Technol Biomed Life Sci* 873: 129–132.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fay MA *et al.* (2005). Oral absorption kinetics of levetiracetam: the effect of mixing with food or enteral nutrition formulas. *Clin Ther* 27: 594–598.

Franco Spinola AC *et al.* (2008). Bioequivalence of two formulations of levetiracetam. *Int J Clin Pharmacol Ther* 46: 591–596.

Grim SA *et al.* (2003). Correlation of levetiracetam concentrations between serum and saliva. *Ther Drug Monit* 25: 61–66.

Guo T *et al.* (2007). Determination of levetiracetam in human plasma/serum/saliva by liquid chromatography-electrospray tandem mass spectrometry. *Clin Chim Acta* 375: 115–118.

Harden C (2001). Safety profile of levetiracetam. *Epilepsia* 42(suppl4): 36–39.

Isoherranen N *et al.* (2000). Enantioselective analysis of levetiracetam and its enantiomer *R*-alpha-ethyl-2-oxo-pyrrolidine acetamide using gas chromatography and ion trap mass spectrometric detection. *J Chromatogr B Biomed Sci Appl* 745: 325–332.

Ivanova M *et al.* (2003). Microemulsion electrokinetic chromatography applied for separation of levetiracetam from other antiepileptic drugs in polypharmacy. *Electrophoresis* 24: 992–998.

Jain DS *et al.* (2006). Determination of levetiracetam in human plasma by liquid chromatography/electrospray tandem mass spectrometry and its application to bioequivalence studies. *Rapid Commun Mass Spectrom* 20: 2539–2547.

Juenke J *et al.* (2006). Drug monitoring and toxicology: a procedure for the monitoring of levetiracetam and zonisamide by HPLC-UV. *J Anal Toxicol* 30: 27–30.

Kossoff EH *et al.* (2001). Levetiracetam psychosis in children with epilepsy. *Epilepsia* 42: 1611–1613.

Lancelin F *et al.* (2007). Therapeutic drug monitoring of levetiracetam by high-performance liquid chromatography with photodiode array ultraviolet detection: preliminary observations on correlation between plasma concentration and clinical response in patients with refractory epilepsy. *Ther Drug Monit* 29: 576–583.

Martens-Lobenhoffer J, Bode-Böger SM (2005). Determination of levetiracetam in human plasma with minimal sample pretreatment. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 197–200.

Matar KM (2008). Quantification of levetiracetam in human plasma by liquid chromatography-tandem mass spectrometry: application to therapeutic drug monitoring. *J Pharm Biomed Anal* 48: 822–828.

Mertzman MD, Foley JP (2004). Chiral cyclodextrin-modified microemulsion electrokinetic chromatography. *Electrophoresis* 25: 1188–1200.

Neels HM *et al.* (2004). Therapeutic drug monitoring of old and newer anti-epileptic drugs. *Clin Chem Lab Med* 42: 1228–1255.

Patsalos PN (2000). Pharmacokinetic profile of levetiracetam: toward ideal characteristics. *Pharmacol Ther* 85: 77–85.

Perucca E *et al.* (2003). Effects of antiepileptic comedication on levetiracetam pharmacokinetics: a pooled analysis of data from randomized adjunctive therapy trials. *Epilepsy Res* 53: 47–56.

Pucci V *et al.* (2004). High-performance liquid chromatographic determination of levetiracetam in human plasma: comparison of different sample clean-up procedures. *Biomed Chromatogr* 18: 37–44.

Radtke RA (2001). Pharmacokinetics of levetiracetam. *Epilepsia* 42(suppl4): 24–27.

Ramael S *et al.* (2006). Single-dose bioavailability of levetiracetam intravenous infusion relative to oral tablets and multiple-dose pharmacokinetics and tolerability of levetiracetam intravenous infusion compared with placebo in healthy subjects. *Clin Ther* 28: 734–744.

Ratnaraj N *et al.* (1996). A micromethod for the determination of the new antiepileptic drug levetiracetam (ucb LO59) in serum or plasma by high performance liquid chromatography. *Ther Drug Monit* 18: 154–157.

Sharief MK *et al.* (1996). Efficacy and tolerability study of ucb LO59 in patients with refractory epilepsy. *J Epilepsy* 9: 106–112.

Shihabi ZK *et al.* (2003). Analysis of the antiepileptic drug Keppra by capillary electrophoresis. *J Chromatogr A* 1004: 9–12.

Vermeij TA, Edelbroek PM (1994). High-performance liquid chromatographic and megabore gas-liquid chromatographic determination of levetiracetam (ucb L059) in human serum after solid-phase extraction. *J Chromatogr B Biomed Appl* 662: 134–139.

Youroukos S *et al.* (2003). Acute psychosis associated with levetiracetam. *Epileptic Disord* 5: 117–119.

Zhao Q *et al.* (2007). Single-dose pharmacokinetics of levetiracetam in healthy Chinese male subjects. *Br J Clin Pharmacol* 63: 614–617.

Levobunolol

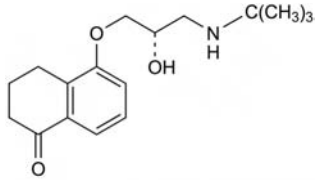
β-*Blocker*

$C_{17}H_{25}NO_3$ = 291.4

CAS—47141-42-4

IUPAC Name 5-[(2*S*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone

Synonyms *l*-Bunolol; W-6421A.



Chemical Properties pK_a 9.4. Log *P* (octanol/water), 2.40.

Levobunolol Hydrochloride

$C_{17}H_{25}NO_3$,HCl = 327.9

CAS—27912-14-7

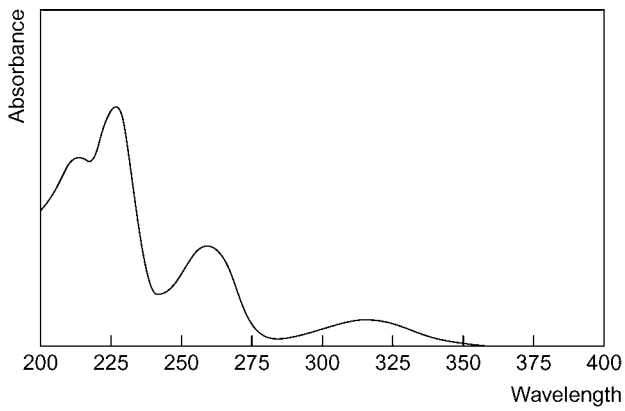
Synonyms (−)-Bunolol hydrochloride; *l*-bunolol hydrochloride; W-7000A;

Proprietary Names *Betagan*; *Gotensian*; *Vistagan*.

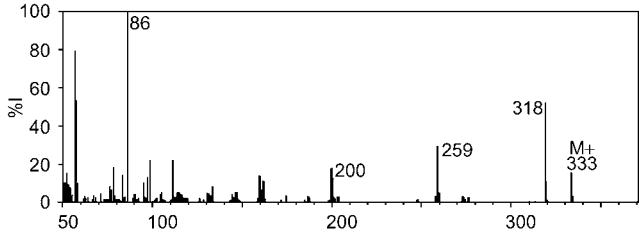
Chemical Properties A white or pinkish-white, odourless crystalline powder. Mp 209° to 211°. Soluble to freely soluble in water; slightly to sparingly soluble in alcohol; soluble in methyl alcohol; slightly soluble in chloroform.

Gas Chromatography Mass Spectrometry. Column: HP capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 100° to 310° at 30°/min. Carrier gas: He, 2 mL/min. Retention index: 2460 [Maurer, Pfleger 1986].

Ultraviolet Spectrum Aqueous acid—223, 209, 256, 313 nm.



Mass Spectrum Principal ions at *m/z* 86, 57, 318, 259, 99, 112, 200, 333 (levobunolol acetylated derivative).



Quantification

Blood **HPLC** Column: μBondapak C_{18} (250 × 4.6 mm i.d., 10 μm). Mobile phase: methanol : water (48:52), flow rate 2 mL/min. Internal standard (IS): metoprolol. Fluorescence detection (λ_{ex}=225 nm, λ_{em}=295 nm). Retention time: dihydrolevobunolol, 3.8 min; IS, 4.6 min. Limit of detection, 0.5 to 1 μg/L [Hengy *et al.* 1985].

Urine **HPLC** See Blood [Hengy *et al.* 1985].

Disposition in the Body Some systemic absorption occurs following topical application, but after oral administration levobunolol is rapidly and almost completely absorbed from the gastrointestinal tract. It is extensively metabolised in the liver and the principal metabolite is dihydrolevobunolol, which possesses β-blocking activity, ~65% of that of the parent drug. Other metabolites include bunolol glucuronide, bunolol sulfate and dihydrobunolol glucuronide. Levobunolol and its metabolites are subsequently glucuronidated. Metabolites and some of the unchanged drug are excreted in urine (78%) and in faeces (3%), in 4 days. The metabolism of levobunolol following ophthalmic application has not been clearly determined. Peak levels of the drug and metabolite are reached within 1 h and the duration of action is 12 to 14 h. Distribution has not been determined.

Therapeutic Concentration

After oral administration to 6 healthy volunteers, the peak plasma concentration reached was 22.9 μg/L after ~3 h [Kolle *et al.* 1983].

Toxicity Respiratory and cardiac reactions have been reported, rarely resulting in death caused by bronchospasm or associated with cardiac failure. No data available on human overdose.

Bioavailability 75%.

Half-life Mean (levobunolol) 6.1 h; (metabolite, dihydrolevobunolol) between 5.7 and 7.1 h.

Volume of Distribution 5.5 L/kg.

Clearance Total plasma clearance, 0.66 L/h/kg.

Dose Eye drops applied once or twice daily.

Hengy H *et al.* (1985). Determination of levobunolol and dihydrolevobunolol in blood and urine by high-performance liquid chromatography using fluorescence detection. *J Chromatogr* 338: 444–449.

Kolle EU *et al.* (1983). *Naunyn Schmiedebergs Arch Pharmacol* 322 R9/34.

Maurer H, Pfleger K (1986). Identification and differentiation of beta-blockers and their metabolites in urine by computerized gas chromatography-mass spectrometry. *J Chromatogr* 382: 147–165.

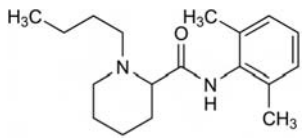
Levobupivacaine

Anaesthetic

$C_{18}H_{28}N_2O$ = 288.4

CAS—27262-47-1

IUPAC Name (*S*)-1-Butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide



Chemical Properties Crystals from isopropanol. Mp 135° to 137°. pKa 8.09. Log *P* (oleyl alcohol/water), 1624.

Levobupivacaine Hydrochloride

$C_{18}H_{28}N_2O$,HCl = 324.9

CAS—27262-48-2

Proprietary Name *Chirocaine*

Chemical Properties Mp 255° to 257°. Solubility in water (20°): 100 mg/mL.

Quantification

Plasma **HPLC** UV detection (λ=210 nm). Limit of quantification, 0.3 mg/L [Bardsley *et al.* 1998].

Disposition in the Body Levobupivacaine is extensively metabolised (NADPH dependent) in the liver, by the cytochrome P450 system. The major metabolite is 3-hydroxylevobupivacaine, which may be subsequently converted to glucuronic acid and sulfate ester conjugates. Approximately 70% of a dose is excreted in urine as its metabolites and a little in faeces. No parent drug has been detected in urine or faeces. Approximately 95% of a dose is recovered within 48 h of dosing. Levobupivacaine crosses the placenta.

Therapeutic Concentration

Fourteen healthy male volunteers, aged 19 to 40 years (mean 29.5 years) were fasted overnight and IV administered a maximum of 150 mg at an infusion rate of 10 mg/min for 2 to 15 min. The mean dose administered was 56.1 mg which produced a mean maximum plasma concentration of 2.62 mg/L shortly after the end of infusion. At 5 and 10 min after infusion, the mean maximum concentration was 1.93 mg/L before decreasing to a range of 0.3 to 0.6 mg/L, 45 to 120 min after infusion [Bardsley *et al.* 1998].

Ten patients, aged 18 to 80 years, were scheduled to undergo elective lower abdominal surgery, with epidural anaesthesia. They were premedicated with midazolam and 1% lidocaine, and subsequently administered an epidural injection of 0.75% concentration (total dose 150 mg) levobupivacaine. The peak plasma concentration was 0.84 mg/L at 0.04 h. The concentration of the (*S*)-isomer was larger than that observed for the (*R*)-isomer [Kopacz *et al.* 2000].

Eighteen patients scheduled for varicose vein surgery, under extradural anaesthesia, were administered 15 mL 0.5% levobupivacaine (total dose of 75 mg) and 0.75% (total dose 112.5 mg) as an epidural injection. The peak plasma concentrations for the two doses were 0.58 and 0.81 mg/L observed at 0.37 and 0.29 h, respectively [Faccenda *et al.* 1998].

Toxicity IV administration of levobupivacaine can be associated with cardiac arrest.

Half-life 1.3 h.

Volume of Distribution 66.9 L.

Clearance Plasma, 39.1 L/h.

Protein Binding >97%.

Dose For surgical anaesthesia, the maximum dose is 150 mg depending on the type of surgery undertaken. For acute pain, 15 to 25 mg (as a 2.5 mg/mL solution).

Bardsley H *et al.* (1998). A comparison of the cardiovascular effects of levobupivacaine and racbupivacaine following intravenous administration to healthy volunteers. *Br J Clin Pharmacol* 46 (3): 245–249.

Faccenda KA *et al.* (1998). *Reg Anesth Pain Med* 23(3): 52.

Kopacz DJ *et al.* (2000). A comparison of epidural levobupivacaine 0.75% with racemic bupivacaine for lower abdominal surgery. *Anesth Analg* 90: 642–648.

Levocabastine

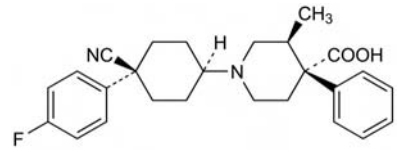
Antihistamine (Topical)

$C_{26}H_{29}FN_2O_2$ = 420.5

CAS—79516-68-0

IUPAC Name [3*S*-[1(*cis*),3α,4β]]-1-[4-Cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenyl-4-piperidinecaboxylic acid

Synonym (−)-Cabastine



Cabastine (racemate of Levocabastine)

CAS—79449-98-2

Levocabastine Hydrochloride

$C_{26}H_{29}FN_2O_2$,HCl = 457.0

CAS—79547-78-7

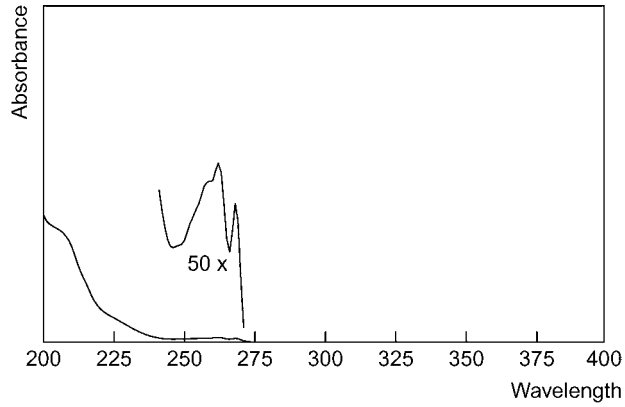
Synonym R-50547

Proprietary Names *Levophta*; *Livostin*.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.12; system TF—R_f 0.02; system TAE—R_f 0.76.

High Performance Liquid Chromatography System HX—RI 431.

Ultraviolet Spectrum Aqueous acid—258, 263, 269 nm.



Disposition in the Body Levocabastine is rapidly and completely absorbed after intranasal, ocular and oral administration, but peak plasma concentrations are low. First-pass metabolism is negligible and it is excreted in urine as the unchanged

drug (70%) and as an acetylglucuronide metabolite which is biologically inactive (10%). The remaining proportion of the unchanged drug is excreted in faeces (20%). Trace amounts of the drug are found in breast milk.

Therapeutic Concentration

Healthy volunteers were administered single doses of 0.5, 1.0 and 2.0 mg. Peak plasma concentrations of 7.3, 12.1 and 22.2 μg/L, respectively, were observed within 2 h of administration. Volunteers administered with a 0.2 mg dose of levocabastine by nasal administration gave peak concentrations between 1.4 and 2.2 μg/L within 1 to 2 h. A 0.04 mg dose, administered as eye drops, produced a concentration of 0.26 to 0.29 μg/L within 1 to 2 h [Heykents J. *et al* 1984].

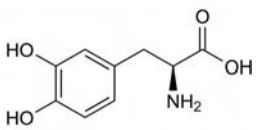
Bioavailability 60 to 80% (intranasal); 30 to 60% (ocular); 100% (oral).
Half-life 33 to 40 h.
Volume of Distribution 1.14 L/kg (82 L).
Clearance Plasma, 1.8 L/h.
Protein Binding 55%
Note For a review of levocabastine, see Noble and McTavish [1995].
Dose 0.5 mg/mL suspension: two sprays per nostril or one drop per eye twice daily. Three to four times daily if necessary.

Heykents J *et al.* (1984). Unpublished reports. *Janssen Research Foundation, Beerse.*
Noble S, McTavish D (1995). Levocabastine. An update of its pharmacology, clinical efficacy and tolerability in the topical treatment of allergic rhinitis and conjunctivitis. *Drugs* 50: 1032–1049.

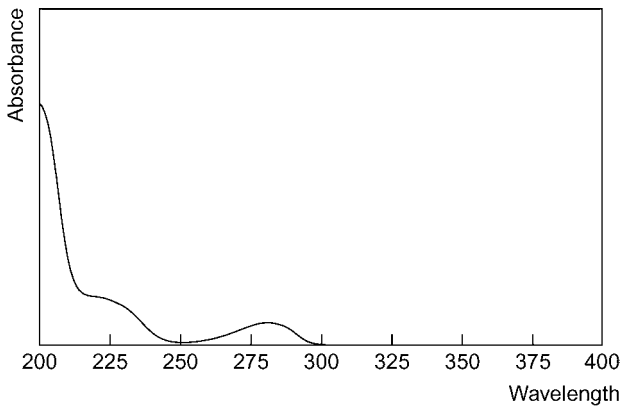
Levodopa

Antiparkinsonian
$C_9H_{11}NO_4 = 197.2$
CAS—59-92-7

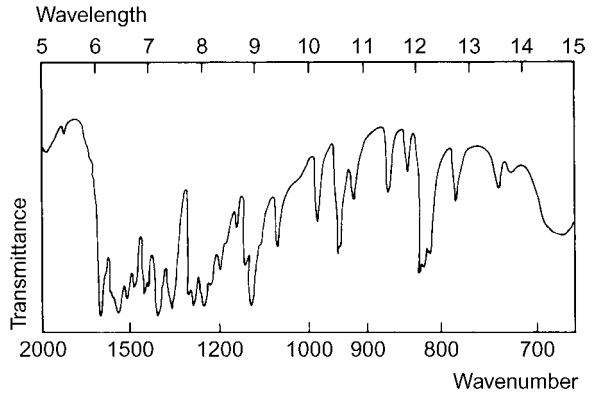
IUPAC Name (2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid
Synonyms Dihydroxyphenylalanine; dopa; L-dopa; 3-hydroxy-L-tyrosine; laevodopa.
Proprietary Names *Bendopa*; *Cidanopa*; *Deadopa*, *Dopaflex*; *Dopar*; *Doparl*; *Dopasol*; *Dopaston*; *Dopastral*; *Doprin*; *Eldopal*; *Eurodopa*; *Larodopa*; *Ledopa*; *Levopa*; *Levomet*; *Syndopa*; *Veldopa*. It is an ingredient of Atamet, *Madopar*, *Prolopa* and *Sinemet*.



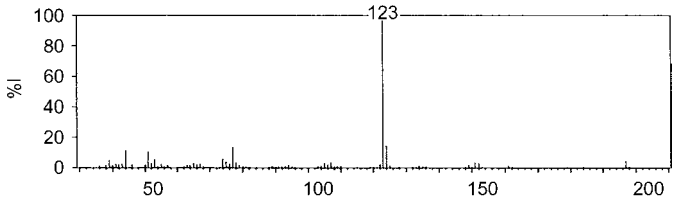
Chemical Properties A white or slightly cream-coloured crystalline powder, which darkens on exposure to air and light. Mp about 275°, with decomposition. Soluble 1 in 300 of water; practically insoluble in benzene, ethyl acetate, ethanol, chloroform and ether; soluble in aqueous solutions of mineral acids and alkali carbonates. pK_a 2.3, 8.7, 9.7, 13.4 (25°). Log *P* (octanol/water), −2.4. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Tests Ammoniacal silver nitrate—yellow→brown/black; ferric chloride—green; Folin–Ciocalteu reagent—blue; methanolic potassium hydroxide—pink→red-brown; Millon's reagent (cold)—brown-red; Nessler's reagent—black; palladium chloride—orange (→brown); potassium dichromate (method 1)—green→brown.
Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.00; system TAE—R_f 0.11; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.00.
High Performance Liquid Chromatography System HX—RI 65; system HAA—retention time 3.6 min.
Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=141a).



Infrared Spectrum Principal peaks at wavenumbers 1647, 1562, 1117, 1241, 1274, 1520 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 123, 124, 77, 44, 51, 74, 53, 39.



Quantification

Plasma **GC** ECD. For method of quantification for levodopa and dopamine, see Mizuno [1977].

HPLC Amperometric detection. Levodopa, carbidopa and their metabolites. Limit of detection, 5 μg/L for levodopa [Sagar, Smyth 2000]. Electrochemical detection. Limit of detection, 1 μg/L for levodopa, 3 μg/L for its metabolite 3-*O*-methyldopa [Blandini *et al.* 1997]. For method for quantification of levodopa and its metabolites, see Dethy *et al.* [1997]. Electrochemical detection (also L-tyrosine with fluorescence detection). For method, see Letellier *et al.* [1997]. Amperometric detection. For method for quantification of levodopa, its main metabolites and carbidopa, see Wikberg [1991]. Coulometric detection. For method for quantification of levodopa, its metabolites and carbidopa, see Lucarelli *et al.* [1990]. Electrochemical detection. For method for quantification of levodopa, carbidopa, 3-*O*-methyldopa and dopamine, see Titus *et al.* [1990]. For method, see Tsuchiya and Hayashi [1989]. Electrochemical detection. Limit of detection, 1 μg/L for levodopa and 3,4-dihydroxyphenylacetic acid [Nissinen, Taskinen 1982].

Urine **HPLC** See Plasma [Sagar, Smyth 2000]. See Plasma [Titus *et al.* 1990]. UV and fluorescence detection. For method for quantification of levodopa and metabolites, see Seki *et al.* [1981].

Platelets **HPLC** See Plasma [Blandini *et al.* 1997].

Disposition in the Body Levodopa is rapidly absorbed from the small bowel after oral administration and widely distributed in the tissues; <1% of a dose reaches the brain. It is extensively metabolised, mainly by decarboxylation to dopamine which is further metabolised, and also by methylation to 3-*O*-methyldopa which accumulates in the central nervous system; most of a dose is decarboxylated by the gastric mucosa before entering the systemic circulation; the decarboxylase activity is inhibited by carbidopa and benserazide. Dopamine is further metabolised to noradrenaline [norepinephrine], 3-methoxytyramine, and to the two major excretory metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA). During prolonged therapy, the rate of levodopa metabolism appears to increase, possibly due to enzyme induction. About 70 to 80% of a dose is excreted in the urine in 24 h. Of the material excreted in the urine, about 50% is DOPAC and HVA, 10% is dopamine, up to 30% is 3-*O*-methyldopa, and <1% is unchanged drug. Less than 1% of a dose is eliminated in the faeces. During prolonged therapy the ratio of the amount of DOPAC produced to that of HVA may be increased.

Therapeutic Concentration There is considerable inter-subject variation in plasma concentrations and there appears to be no significant correlation between plasma concentrations of levodopa or its metabolites and therapeutic effect.

Following single oral doses of 1.5 g to 42 subjects, peak plasma-levodopa concentrations averaged about 1 mg/L at 1 to 2 h but there was a tenfold variation in peak concentrations. Peak plasma concentrations of 3-*O*-methyldopa of about 1.5 mg/L were attained in about 4 h and concentrations of HVA in the cerebrospinal fluid reached a maximum of about 0.15 mg/L in 8 h [Bergmann *et al.* 1974].

Ten subjects receiving 300 to 1500 mg levodopa orally daily (treatment duration 3 to 25 years) were studied during daily activity at home and work. Steady-state plasma concentrations varied widely (0.5 to 7.1 (mean, 2.0) mg/L for levodopa and 2.4 to 20.8 (mean, 7.1) mg/L for 3-*O*-methyldopa) [Nyholm *et al.* 2002].

Bioavailability About 33%.
Half-life Plasma half-life, about 1 h which may be increased by concomitant administration of peripheral decarboxylase inhibitors; 3-*O*-methyldopa about 13 h.

Clearance Plasma clearance, about 23 mL/min/kg.
Dose Initially, 0.25 to 1 g daily; maintenance, up to 8 g daily.

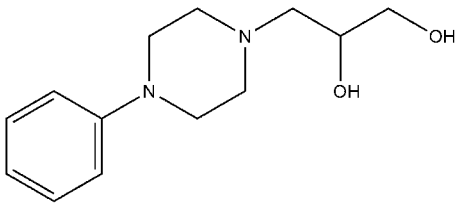
Bergmann S *et al.* (1974). *Br J Clin Pharmacol* 1: 417–424.
Blandini F *et al.* (1997). Simultaneous determination of L-dopa and 3-O-methyldopa in human platelets and plasma using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B, Biomed Sci Appl* 700: 278–282.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Dethy S *et al.* (1997). Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients. *Clin Chem* 43: 740–744.
Letellier S *et al.* (1997). Determination of the L-DOPA/L-tyrosine ratio in human plasma by high-performance liquid chromatography. Usefulness as a marker in metastatic malignant melanoma. *J Chromatogr B Biomed Sci Appl* 696: 9–17.
Lucarelli C *et al.* (1990). Simultaneous measurement of L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients by improved sample pretreatment and high-performance liquid chromatographic determination. *J Chromatogr* 511: 167–176.
Mizuno Y (1977). Simple gas chromatographic analysis of plasma dopa and dopamine. *Clin Chim Acta* 74(1): 11–19.
Nissinen E, Taskinen J (1982). Simultaneous determination of carbidopa, levodopa and 3,4-dihydroxyphenyl-acetic acid using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 231: 459–462.
Nyholm D *et al.* (2002). Levodopa pharmacokinetics and motor performance during activities of daily living in patients with Parkinson's disease on individual drug combinations. *Clin Neuropharmacol* 25: 89–96.
Sagar KA, Smyth MR (2000). Simultaneous determination of levodopa, carbidopa and their metabolites in human plasma and urine samples using LC-EC. *J Pharm Biomed Anal* 22(3): 613–624.
Seki J *et al.* (1981). High-performance liquid chromatographic determination of L-3,4-dihydroxyphenylalanine (L-DOPA) and its metabolites in the urine of patients with Parkinson's disease, control patients and normal subjects after oral administration of L-DOPA. *Chem Pharm Bull* 29: 789–795.
Titus DC *et al.* (1990). Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection. *J Chromatogr* 534: 87–100.
Tsuchiya H, Hayashi T (1989). Determination of L-3,4-dihydroxyphenylalanine in blood by high-performance liquid chromatography after solvent extraction. *J Chromatogr* 491: 291–298.
Wikberg T (1991). Simultaneous determination of levodopa, its main metabolites and carbidopa in plasma by liquid chromatography. *J Pharm Biomed Anal* 9: 167–176.

L

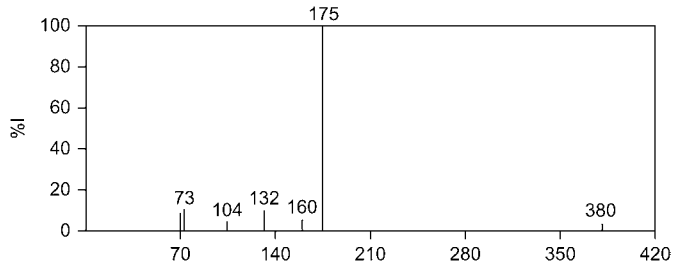
Levodropropizine

Cough Suppressant
$C_{13}H_{20}N_2O_2$ = 236.3
CAS—99291-24-4

IUPAC Name (2*S*)-3-(4-Phenylpiperazin-1-yl)propane-1,2-diol
Proprietary Names *Danka (Mediolanum)*; *Levotuss (Dompé)*; *Rapitux (De Angeli)*.



Chemical Properties Levo isomer of dropropizine. White solid from acetone. Mp 104° to 105°.
Mass Spectrum Principal peaks at *m/z* 175, 73, 70, 132, 160, 104 [Zaratin *et al.* 1988].



Quantification
Plasma GC-MS Column: SPB 5 Supelco (30 m × 0.32 mm i.d.). Temperature: 245°. EI ionisation at 70 eV. Limit of detection, 5 µg/L [Zaratin *et al.* 1988].

HPLC Column: Bio-Gel PRP 70-5 poly(styrene–divinylbenzene) (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L potassium monobasic phosphate buffer (pH 3): methanol(70 : 30), containing 0.5% tetrahydrofuran, flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 240 nm, λ_{em}= 350 nm). Limit of detection, 3 µg/L [Tagliaro *et al.* 1996].

LC-MS Column: Nucleosil C18 (100 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate with 1% formic acid: methanol (55 : 45), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.25 µg/L [Tang *et al.* 2005].

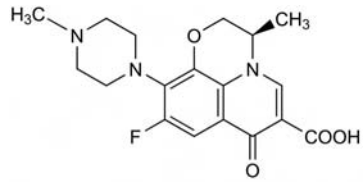
Protein Binding 11–14%.

Tagliaro F *et al.* (1996). High-performance liquid chromatographic determination of levodropropizine in human plasma with fluorometric detection. *J Chromatogr B Biomed Appl* 685: 165–170.
Tang Y *et al.* (2005). Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of levodropropizine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 185–189.
Zaratin P *et al.* (1988). Gas chromatographic-mass spectrometric determination of levodropropizine plasma levels in healthy volunteers. *Arzneimittelforschung* 38: 1156–1158.

Levofloxacin

Antibacterial
$C_{18}H_{20}FN_3O_4$ = 361.4
CAS—100986-85-4

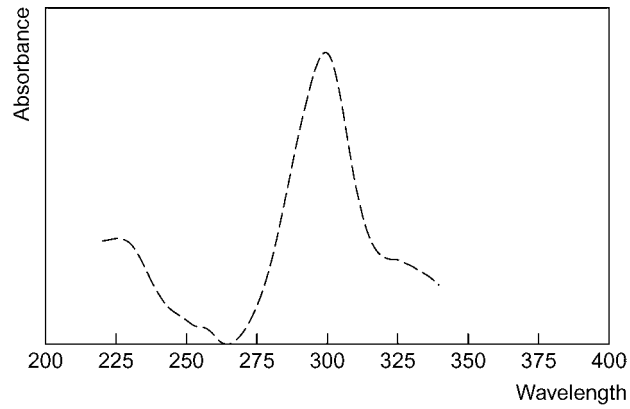
Synonyms DR-3355; (−)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid; HR-355; RWJ-25213; *S*-(−)-ofloxacin.
Proprietary Names *Cravit*; *Levaquin*; *Quixin*; *Tavanic*.



Chemical Properties Light-yellow to yellow-white crystals. Freely soluble in glacial acetic acid and chloroform; sparingly soluble in water.

Levofloxacin Hemihydrate
$C_{18}H_{20}FN_3O_4, {}^1/_2H_2O$ = 370.4
CAS—138199-71-0
Chemical Properties Needle-like crystals from ethanol/ethyl ether with Mp 225° to 227° (dec).

Ultraviolet Spectrum Ethanol—226, 300 nm.



Quantification
Plasma **HPLC** Column: RP ODS-2 Inertsil (250 × 4.6 mm i.d., 5 µm). Temperature: 35°. Mobile phase: methanol : 5 mmol/L copper (II) sulfate pentahydrate (containing 10 mmol/L L-isoleucine) (12.5:87.5), flow rate 1 mL/min. Internal standard (IS): ciprofloxacin. UV detection (λ=330 nm). Retention time: levofloxacin, 8 min; IS, 13 min. Limit of quantification, 80 µg/L [Wong *et al.* 1997]. Fluorescence detection (λ_{ex}=298 nm, λ_{em}=458 nm). Limit of detection 3 µg/L [Lehr, Damm 1988].
Urine **HPLC** See Plasma [Wong *et al.* 1997]. See Plasma. Limit of detection 80 µg/L [Lehr, Damm 1988].
Disposition in the Body Levofloxacin is rapidly absorbed after oral administration with maximum plasma concentrations being reached ~1 h after a dose. The absorption of levofloxacin is little affected by the presence of food in the gastrointestinal tract but administration with a high-fat meal may slightly prolong the time to peak concentration. It is widely distributed throughout the body, crosses the placenta and has been detected in breast milk. Levofloxacin undergoes limited metabolism and is excreted mainly as the unchanged drug in urine (80 to 85%) and faeces (2%) within 24 h. The major urinary metabolites are desmethyllevofloxacin and levofloxacin *N*-oxide, each accounting for only 2% of a dose.
Therapeutic Concentration
Five non-fasting, healthy male volunteers, aged between 27 and 36 years, were administered a 100 mg dose of levofloxacin. The mean peak plasma concentration was 1.35 mg/L and was observed about 1.8 h after ingestion [Okazaki *et al.* 1991].
Bioavailability 100%.
Half-life 6 to 8 h; may be prolonged in renal impairment.
Volume of Distribution 1.0 to 1.25 L/kg (single doses, healthy subjects). Also reported as 89 to 112 L.

Clearance 8.51 L/h/1.73 m^2 or 144 to 226 mL/min (mean body clearance); 7.14 L/h/1.73 m^2 or 96 to 142 mL/min (mean renal clearance).
Protein Binding 30 to 40% (mainly to albumin); a figure of 47 to 52% has been reported (assessed by measuring the *S*(−)-enantiomer of ofloxacin at a drug concentration of 1 mg/L).
Dose The usual dose is 250 to 500 mg daily.

Lehr K, Damm P (1988). Quantification of the enantiomers of ofloxacin in biological fluids by high-performance liquid chromatography. *J Chromatogr* 425: 153–161.
Okazaki O *et al.* (1991). Enantioselective disposition of ofloxacin in humans. *Antimicrob Agents Chemother* 35: 2106–2109.
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Levomepromazine

Phenothiazine, Tranquilliser
$C_{19}H_{24}N_2OS$ = 328.5
CAS—60-99-1

IUPAC Name 3-(2-Methoxyphenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine
Synonyms CL-36467; CL-39743; methotrimeprazine; (βR)-2-methoxy-*N*,*N*,β-trimethyl-10*H*-phenothiazine-10-propanamine; RP-7044; SKF-5116.
Proprietary Names *Levium*; *Levocina*; *Levoprome*; *Levozin(e)*; *Methozane*; *Minozinan*; *Neozine*; *Neurocil*; *Novo-Meprazine*; *Nozinan*; *Sinogan*. It is an ingredient of *Immobilon* (for small animals). (Includes proprietary names of salts of levomepromazine).
Chemical Properties Fine, white, crystalline powder. Mp ≈126°. Practically insoluble in water; sparingly soluble in ethanol; soluble 1 in 2 of chloroform and 1 in 10 of methanol; freely soluble in ether. pK_a 9.19 [Sangster 1997]. Log *P* (octanol/water), 4.68 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Plasma samples were stable at –20° for 2 months [Mercolini *et al.* 2007]. Serum samples were stable for up to 8 h at room temperature and up to 2 weeks when stored at 4° or –20° [Tanaka *et al.* 2007].

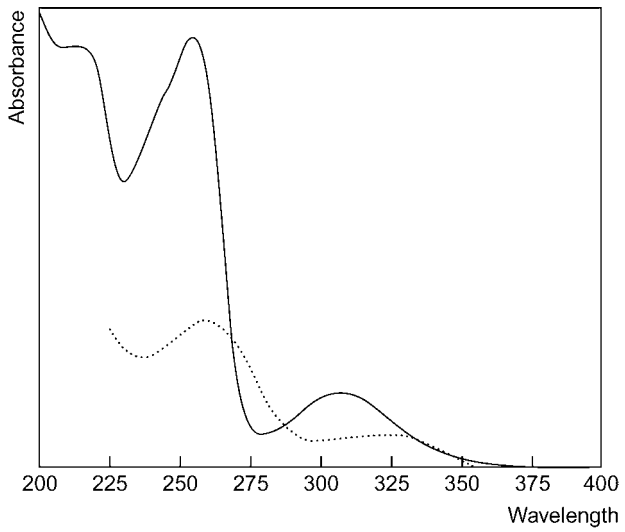
Levomepromazine Hydrochloride

$C_{19}H_{24}N_2OS,HCl$ = 364.9
CAS—4185-80-2
Chemical Properties Very soluble in water and ethanol.

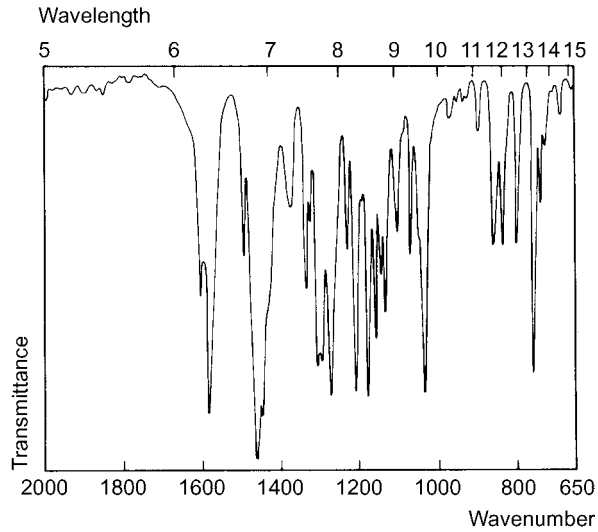
Levomepromazine Maleate

$C_{19}H_{24}N_2OS,C_4H_4O_4$ = 444.5
CAS—7104-38-3; 17086-29-2
Synonym Levomepromazine hydrogen maleate
Chemical Properties White crystalline powder. Mp ≈190°, with decomposition. Sparingly soluble in water and ethanol.

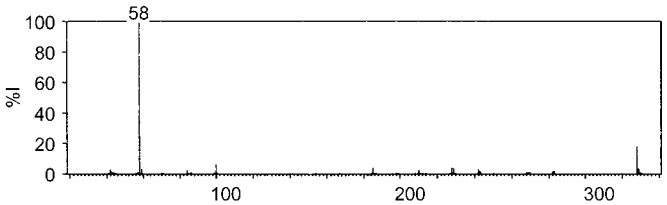
Colour Tests Formaldehyde–sulfuric acid—blue; Forrest reagent—violet; FPN reagent—violet; Mandelin's test—blue-violet; Marquis test—blue-violet.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.47; system TC—R_f 0.38; system TE—R_f 0.76; system TL—R_f 0.46; system TAE—R_f 0.32; system TAF—R_f 0.49; system TAJ—R_f 0.27; system TAK—R_f 0.19; system TAL—R_f 0.81 (Dragendorff spray, positive; FPN reagent, violet; acidified iodoplatinate solution, positive; Marquis reagent, violet).
Gas Chromatography System GA—RI 2514; system GB—levomepromazine RI 2641, M (norsulfoxide) RI 3088, M (sulfoxide) RI 3114; system GF—RI 2965; system GW—retention time 23.6 min.
High Performance Liquid Chromatography System HA—*k* 3.2; system HX—RI 435; system HY—RI 381; system HZ—retention time 7.5 min; system HAX—retention time 15.2 min; system HAY—retention time 7.2 min; system HAM—RT not detected; system HAZ—*k* 1.82.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1 = 783a), 302 nm; aqueous alkali—259, 323 nm.



Infrared Spectrum Principal peaks at wavenumbers 1580, 1270, 1175, 1030, 1205, 752 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 328, 100, 228, 185, 329, 242, 229.



Quantification

Blood **GC** Column: cross-linked methylsilicone (25 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min. NPD. Retention time: 8.52 min. Limit of quantification, 172 μg/L, limit of detection, 52 μg/L. [Sánchez de la Torre *et al.* 2005]. Column: DB-1 capillary (0.32 mm i.d., 0.25 μm). Carrier gas: He, I mL/min. Temperature programme: 110° to 320° at 8°/min. NPD. Limit of detection not reported [Avis, Holzbecher 1996]. Column: Shimadzu CBP-1 bonded methylsilicone (12 m × 0.53 mm i.d., 1.0 μm). Carrier gas: He, 10 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min for 2 min. NPD. Limit of detection not reported [Jitsufuchi *et al.* 1995]. Column: DB-1 fused silica (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 22 cm/s. Temperature programme: 120° to 280° at 6°/min. SID. Retention time: 23.6 min. Limit of detection, 250–500 ng/L [Hattori *et al.* 1992]. Column: SPB-1 (30 m × 0.75 mm i.d., 1.0 μm). Carrier gas: He, 8 mL/min. Temperature programme: 100° for 2 min to 200° at 20°/min to 280° at at 7.5°/min for 4 min. FID. Limit of detection not reported [Klys, Brandys 1988]. Column: 3% OV-17 on 80/100 mesh Supelcoport (100 cm × 2 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature programme: 255° for 4 min to 280° at 32°/min for 7 min. AFID. Limit of detection, 25 μg/L [Dahl *et al.* 1982].

GC-MS Column: 3% OV-17 on 80/100 mesh Supelcoport (100 cm × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature: 250°. EI ionisation at 70 eV. Retention time: 2.5 min. Limit of detection not reported [Dahl *et al.* 1982].

Plasma **GC** Column: SGE BPX-5 capillary (30 m × 0.53 mm i.d., 1 μm). Carrier gas: N_2, 20 mL/min. Temperature programme: 160° to 260° at 40°/min to 274° at 4°/min for 1 min NPD. Retention time: 4.5 min. Limit of quantification, 5 μg/L, limit of detection, 2 μg/L [Kruggel, Ulrich 2000]. Column: Shimadzu CBP1-bonded methyl silicone (12 m × 0.53 mm i.d., 1 μm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. NPD. Limit of detection, 50–100 μg/L [Tokunaga *et al.* 1996; Tokunaga *et al.* 1997]. See Blood [Dahl *et al.* 1982].

GC-MS Column: 3% OV-17 on 80/100 mesh Gas Chrom Q (120 cm × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 242°. EI ionisation at 70 eV. Limit of detection not reported [Dahl, Garle 1977].

HPLC Column: Discovery reversed phase pentafluorophenylpropyl (150 × 4.6 mm i.d., 5 μm). Mobile phase: 3.8 mmol/L phosphate buffer (pH 1.9) containing 0.18% triethylamine : acetonitrile (68 : 32), flow rate 1.5 mL/min. Electrochemical detection. Retention time: 20.3 min. Limit of quantification, 0.5 μg/L, limit of detection, 0.17 μg/L [Saracino *et al.* 2008]. Column: C_8 reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 34 mmol/L phosphate buffer (pH 2.0) containing 0.3% triethylamine (29 : 71), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of quantification, 9 μg/L, limit of detection, 3 μg/L [Mercolini *et al.* 2007]. Column: Symmetry C_8 (150 × 3.9 mm i.d., 5 μm). Mobile phase: 0.1 mol/L potassium dihydrogen phosphate (pH 3.5) with 17% phosphoric acid : acetonitrile (70 : 30), flow rate 1.3 mL/min. DAD (λ = 250 nm). Retention time: 8.61 min. Limit of quantification, 4.1 μg/L, limit of detection, 3.7 μg/L [ter

Horst *et al.* 2003]. Column: Supelcosil C_{18}-DB (250 nm × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L SDS in 500 mmol/L ammonium acetate buffer (pH 5.0) : acetonitrile (50 : 50), flow rate 1.5 mL/min. Retention time: 24.5 min. Limit of detection not reported [Loennechen, Dahl 1990]. Column: Spherisorb S5 ODS (15 cm × 4.6 mm, 5 µm). Mobile phase: 0.01 mol/L potassium phosphate buffer (pH 5.5) : methanol : acetonitrile (20 : 30 : 50). UV detection (λ = 254 nm). Limit of detection, 2 µg/L [Holt *et al.* 1982].

See also [Murakami *et al.* 1982]

Serum **HPLC** Column: Inertsil ODS-SP (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6; 300 : 200 : 500), flow rate 0.9 mL/min. UV detection (λ = 250 nm). Limit of quantification, 4.5 µg/L [Tanaka *et al.* 2007]. Column: Supelcosil-C_{18}-DB reversed phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L SDS in 0.5 mol/L ammonium acetate buffer (pH 5) : 5% tetrahydrofuran in acetonitrile (50 : 50), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection, <0.028 µmol/L [Loennechen *et al.* 1990]. Column: Develosil C_8-5 and Develosil CN-5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% potassium dihydrogen phosphate (pH 6.0) : acetonitrile (65 : 35). UV detection (λ = 254 nm). Limit of detection, 5 µg/L [Ohkubo *et al.* 1993]. Column: Nucleosil 100 CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : pyridine : 0.14 mol/L sodium acetate (pH 3.1; 698 : 2 : 300), flow rate 0.9 mL/min. Electrochemical detection. Limit of detection, 0.2 µg/L [Bagli *et al.* 1994].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.47 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **GC** See Blood [Avis, Holzbecher 1996]. Column: 5% OV-1 80/100 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 70 mL/min. Temperature: 230°. FID. Retention time: 4 min 39 s [De Leenheer, Heyndrickx 1972].

GC-MS Column: SPB-5 Supelco (30 m × 0.2 mm i.d., 0.2 µm). Carrier gas: He. Temperature programme: 45° for 2 min to 250° at 20°/min. EI ionisation at 70 eV, positive ion mode, full scan acquisition mode. Limit of detection not reported [Hals, Dahl 1995]. Column: 3% OV-17 on 80/100 Supelcoport. Carrier gas: N_2, 30 mL/min. Temperature: 260°. NPD. Retention time: 2.5 min. Limit of detection not reported [Johnsen, Dahl 1982]. See Plasma [Dahl, Garle 1977].

HPLC Column: LiChrosorb C_8 (150 × 4.6 mm i.d.)-LiChrosorb C_{18} (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : triethylamine (40 : 40 : 20 : 2), flow rate 0.5 mL/min. UV detection (λ = 250 nm). Limit of quantification, 200 µg/L, limit of detection, 60 µg/L [Cruz-Vera *et al.* 2009]. See Serum. Limit of detection, 15 nmol/L [Loennechen *et al.* 1990]. Column: Nucleosil C_{18} (150 × 4 mm i.d., 5 µm). Mobile phase: pyridine : tetrhydrofuran : 0.1 mol/L acetate buffer (pH 3.5) : acetonitrile (0.1 : 1.0 : 30.0 : 68.9) containing 20 mmol/L sodium perchlorate, flow rate 0.7 mL/min. Electrochemical detection. Retention time: 7.0 min. Limit of detection, 0.5 µg/L [Murakami *et al.* 1982].

Bile **GC** See Blood [Avis, Holzbecher 1996].

Breast Milk **HPLC** See Serum. Mobile phase: 0.5% potassium dihydrogen phosphate (pH 4.5) : acetonitrile (65 : 35). [Ohkubo *et al.* 1993].

Vitreous Humour **GC** See Blood [Avis, Holzbecher 1996].

[Maresova *et al.* 2008; Shinmen *et al.* 2008].

Brain **GC** See Blood [Jitsufuchi *et al.* 1995].

Kidney **GC** See Blood [Jitsufuchi *et al.* 1995].

Liver GC See Blood [Jitsufuchi *et al.* 1995].

Muscle **GC** See Blood [Jitsufuchi *et al.* 1995].

Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the body. Approximately 50% of an oral dose may reach the systemic circulation. Extensively metabolised to numerous phase-I and -II metabolites, it is not metabolised to a great extent by CYP2D6 [Bagli *et al.* 1995]. The major metabolites are the sulfoxide and *N*-monodesmethyl derivative (active); hydroxylation also occurs. Less than 1% of a dose is excreted in the urine as unchanged drug in 24 h [Afifi, Way 1968]. Approximately 10% of a dose is excreted in the urine as the sulfoxide.

Therapeutic Concentration 0.03–0.15 mg/L.

After a single oral dose of 50 mg to 5 male psychiatric subjects, peak plasma concentrations of 0.016–0.04 mg/L were attained in 1–4 h; concentrations of the sulfoxide metabolite exceeded those of unchanged drug in each case [Dahl 1976].

Following daily oral doses of 300–400 mg to 4 subjects, steady-state plasma concentrations of 0.05–0.14 mg/L (mean 0.08) were reported; steady-state plasma concentrations of the sulfoxide metabolite ranged from 0.26–0.39 mg/L (mean 0.33); the *N*-monodesmethyl metabolite was also detected [Dahl, Garle 1977].

The concentration of levomepromazine in human breast milk from a patient receiving 10 mg/day levomepromazine was 7.5 µg/L and the concentration in the serum of patients receiving 200–700 mg/day was 14–99 µg/L [Ohkubo *et al.* 1993].

Toxicity 0.5 mg/L.

In 3 postmortem examinations of a 40-, a 19- and a 35-year-old female the serum concentrations of levomepromazine were 36 µg/L, 1119 µg/L, and 772 µg/L, respectively [Tanaka *et al.* 2007].

In a 42-year-old male who committed suicide by ingesting methotrimeprazine, the following postmortem body-fluid concentrations of methotrimeprazine, desmethylmethotrimeprazine, and methotrimeprazine sulfoxide were reported, respectively: blood 4.1, 2.0, and 1.8 mg/L, urine 0.80, 1.2, and 8.9 mg/L, bile 70, 35, and 26 mg/L, vitreous humour 0.25, 0.20, and 0.90 mg/L. No other drugs were detected [Avis, Holzbecher 1996].

A 46-year-old schizophrenic male died in hospital. The concentration of levomepromazine, amongst other antipsychotcs, in his blood, brain, kidney, liver and muscle was 750, 1284, 4128, 11570, and 1173 ng/g respectively. Cause of death was asphyxia [Jitsufuchi *et al.* 1995].

A 30-year-old female took an unknown amount of psychotropic drugs. At postmortem the concentration of levomepromazine in the blood, liver and kidney was 5.0, 4.1 and 2.1 mg/kg, respectively [Klys, Brandys 1988].

The following postmortem concentrations were reported in a fatality attributed to levomepromazine overdose: blood 8 mg/L, liver 160 µg/g [Bonnichsen *et al.* 1970].

Half-life Plasma half-life, methotrimeprazine 16–78 h (mean 27), sulfoxide metabolite 10–30 h (mean 20).

Volume of Distribution ≈30 L/kg.

Distribution in Blood Plasma : whole blood ratio, ≈1.2 but there is considerable inter-subject variation.

Dose Usually 25 to 50 mg of methotrimeprazine maleate daily; up to 1 g daily has been given.

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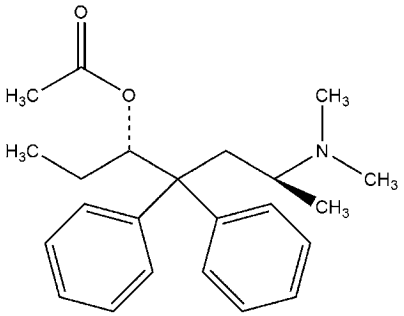
Levomethadyl Acetate

Diphenylheptane Derivative, μ *Opioid Receptor Agonist, Narcotic Analgesic*
$C_{23}H_{31}NO_2 = 353.5$
CAS—1477-40-3

IUPAC Name [(3*S*,6*S*)-6-Dimethylamino-4,4-di(phenyl)heptan-3-yl] acetate
Synonyms α-$_L$-Acetylmethadol; (−)-6-(dimethylamino)-4,4-diphenyl-3-heptanol acetate (ester); (α*S*)-β-[(2*S*)-2-(dimethylamino)propyl]-α-ethyl-β-phenylbenzeneethanol acetate (ester); LAAM; levo-α-acetylmethadol.



Chemical Properties pK_a 8.3 [Wilkins *et al.* 1998]. Log *P* (octanol/water) 6.14 [Wishart 2006]. Analytes were stable after 3 freeze-thaw cycles, room temperature storage for 20 h, and extracts were stable both at −20° for 6 days and in the autosampler (10°) for 4 days [Huang *et al.* 2003].

Levomethadyl Acetate Hydrochloride

$C_{23}H_{31}NO_2$,HCl = 390
CAS—43033-72-3
Synonym LAM
Proprietary Name *ORLAAM*
Chemical Properties Crystals. Mp 215°. Soluble in water. Log *P* (octanol/water) 5.82 [Sigma-Aldrich 2007].

Mass Spectrum Principal ions at *m/z* 72, 171, 294, 105, 207, 354, 46.

Quantification

Plasma **GC-MS** Column: Supelco SPB-5 5% diphenylsiloxane 95% dimethylsiloxane fused silica (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 50 mL/min. Temperature programme: 160° to 260° at 30°/min. SIM acquisition mode. Limit of quantification, 4 μg/L [Eap *et al.* 2004]. Column: 5% phenylmethylsiloxane fused capillary (15–20 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 80 cm/s. Temperature programme: 140° to 285° at 15°/min. CI, positive ion mode. Limit of quantification, 5 μg/L [Moody *et al.* 1995]. Column: DB-1701 capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 90° for 1.5 min to 260° at 50°/min for 2 min to 300° at 25°/min. CI, SIM acquisition mode. Retention time: 6.0 min for levomethadyl acetate (LAAM), 7.0 min for dinor-LAAM and 7.3 min for nor-LAAM. Limit of quantification, 1.0 μg/kg, limit of detection, 0.5 μg/kg [Thomas *et al.* 1994].

LC-MS Column: Zorbax Eclipse XDB-C_{18} (50 × 2.1 mm i.d., 5 μm). Mobile phase: 0.05% trifluoroacetic acid in methanol : 0.05% trifluoroacetic acid in water (55 : 45 for 1 min to 90 : 10 over 3 min for 2 min to 55 : 45 for 4 min), flow rate 0.25 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times 4.7 min for LAAM, 4.9 min for nor-LAAM and 5.1 min for dinor-LAAM. Limit of quantification, 2.0 μg/L for LAAM, 1.0 μg/L for nor-LAAM and dinor-LAAM [Kharasch *et al.* 2005]. Column: InertSil ODS3 (100 × 2.1 mm i.d., 5 μm). Mobile phase: methanol : 0.1% formic acid (45 : 55), flow rate 0.2 mL/min. ESI. Limit of quantification, 0.25 μg/L for LAAM, nor-LAAM and dinor-LAAM [Huang *et al.* 2003].

Serum **HPLC** Column: OV-1 open tubular fused silica capillary (25 m × 0.2 mm i.d.). Carrier gas: He, 0.5 mL/min. Temperature programme: 190° for 35 s to 220° at 30°/min for 5.8 min to 240° at 18°/min for 2 min. NPD. Retention times, 5.59 min for LAAM, 10.10 min for nor-LAAM and 9.13 min for dinor-LAAM. Limit of detection, 5 μg/L for for LAAM, nor-LAAM and dinor-LAAM [Verebey *et al.* 1985].

Urine **GC-MS** Column: 5% phenyl methyl silicone fused capillary (15–20 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 80 cm/s. Temperature programme: 140° to 285° at 15°/min. CI, positive ion mode. Limit of quantification, 10 μg/L [Moody *et al.* 1995].

HPLC Column: Varian Micro-Pak LiChrosorb Si-60 (300 × 4 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile-0.015% ammonium hydroxide (70 : 30), flow rate 1.5 mL/min. UV detection (λ = 218 nm). Limit of detection, 10 μg/L [Kiang *et al.* 1981].

LC-MS Column: Zorbax Eclipse XDB-C_{18} (50 × 2.1 mm i.d., 5 μm). Mobile phase: 0.05% trifluoroacetic acid in acetonitrile : 0.05% trifluoroacetic acid in water (40 : 60 to 55 : 45 over 3 min to 90 : 10 over 0.5 min for 0.5 min to 40 : 60 over 0.5 min for 4 min), flow rate 0.25 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 2.1 min for LAAM, 2.4 min for nor-LAAM and 2.7 min for dinor-LAAM. Limit of quantification, 8 μg/L for LAAM, 40 μg/L for nor-LAAM and dinor-LAAM [Kharasch *et al.* 2005]. Column: InertSil ODS3 (100 × 2.1 mm i.d., 5 μm). Mobile phase: methanol : 0.1% formic acid (45 : 55), flow rate 0.2 mL/min. ESI. Limit of quantification, 0.25 μg/L for LAAM, nor-LAAM and dinor-LAAM [Huang *et al.* 2003]

Placenta **HPLC** Column: Phenomenex Luna C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 3.5 g/L ammonium carbonate in methanol : water (80 : 20), flow rate 1 mL/min. UV detection (λ = 218 nm). Limit of detection, 25 μg/L for nor-LAAM and 50 μg/L for dinor-LAAM [Deshmukh *et al.* 2004].

Hair **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 165° for 0.5 min to 310° at 25°/min for 0.5 min. CI, positive ion mode. Limit of quantification, 0.5 μg/kg for LAAM and 0.3 μg/kg for nor-LAAM and dinor-LAAM [Wilkins *et al.* 1997].

Other **GC-MS** Rat Plasma, Liver and Brain. Column: 5% phenyl methyl silicone fused capillary (15–20 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 80 cm/s. Temperature programme: 140° to 285° at 15°/min. CI, positive ion mode. Limit of quantification, 10 μg/L [Moody *et al.* 1995].

LC-MS Microsomes. Column: InertSil ODS3 (100 × 2.1 mm i.d., 5 μm). Mobile phase: methanol : 0.1% formic acid (45 : 55), flow rate 0.2 mL/min. ESI. Limit of quantification, 0.25 μg/L for LAAM, nor-LAAM and dinor-LAAM [Huang *et al.* 2003].

Disposition in the Body LAAM undergoes extensive first-pass metabolism by CYP3A4 (and to a lesser extent CYP2B6 and CYP2C18) to the active demethylated metabolite nor-LAAM, which is further demethylated to a second active metabolite, dinor-LAAM. These metabolites are more potent than the parent drug. Other metabolic pathways of LAAM involve hydrolytic cleavage of the acetyl–ester bond.
Note For a study on the metabolism of LAAM in human placenta, see Deshmukh *et al.* [2004].

Therapeutic Concentration Six opioid-experienced male volunteers (age 28 to 46 years) who were not physically dependent on opioids were administered 20 or 40 mg/70 kg LAAM either IV or orally. Pharmacokinetic parameters for LAAM, nor-LAAM and dinor-LAAM were as follows:

	20 mg IV	*40 mg IV*	*20 mg oral*	*40 mg oral*
LAAM				
C_{max} (μg/L)	212 ± 32	756 ± 229	39 ± 7	63 ± 8
t_{max} (h)	0.06 ± 0.01	0.06 ± 0.01	2.5 ± 0.4	2.6 ± 0.2
$t_{1/2}$ (h)	14.3 ± 1.7	20.9 ± 3.6	7.9 ± 1.2	18.5 ± 4.9
Nor-LAAM				
C_{max} (μg/L)	13 ± 1	26 ± 2	26 ± 3	44 ± 4
t_{max} (h)	4.5 ± 1.7	8.0 ± 3.4	3.1 ± 0.5	3.9 ± 0.7
$t_{1/2}$ (h)	30.0 ± 5.2	37.9 ± 5.4	33.6 ± 4.2	23.9 ± 3.2
Dinor-LAAM				
C_{max} (μg/L)	9 ± 0	15 ± 1	12 ± 1	19 ± 1
t_{max} (h)	40.8 ± 7.4	48.0 ± 6.2	17.9 ± 7.3	31.0 ± 9.6
$t_{1/2}$ (h)	88.9 ± 39.9	80.5 ± 14.7	75.6 ± 15.4	65.8 ± 10.1

[Walsh *et al.* 1998].

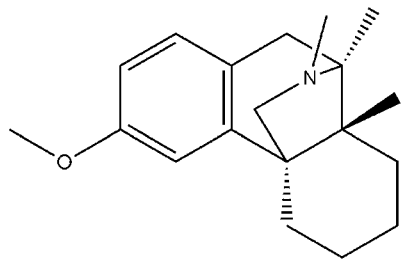
Toxicity As a derivative of methadone, LAAM was used in the treatment of opioid dependence. However, its proarrhythmic effects have led to its withdrawal from markets in the European Union and the USA.
Bioavailability ≈47–48%.
Half-life 2.6 days.
Clearance LAAM IV: 352 ± 25 and 295 ± 35 mL/h/kg for 20 and 40 mg, respectively; LAAM oral dose: 357 ± 25 and 296 ± 35 mL/h/kg for 20 and 40 mg, respectively.
Protein Binding ≈80%.
Dose 20 to 40 mg a day or higher but no more than 120 mg.

Deshmukh SV *et al.* (2004). *N*-Demethylation of levo-alpha-acetylmethadol by human placental aromatase. *Biochem Pharmacol* 67: 885–892.
Eap CB *et al.* (2004). Determination of human plasma levels of levo-alpha-acetylmethadol and its metabolites by gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 805: 141–146.
Huang W *et al.* (2003). Determination of *l*-alpha-acetylmethadol (LAAM), norLAAM, and dinorLAAM in clinical and in vitro samples using liquid chromatography with electrospray ionization and tandem mass spectrometry. *J Pharm Sci* 92: 10–20.
Kharasch ED *et al.* (2005). Paradoxical role of cytochrome P450 3A in the bioactivation and clinical effects of levo-alpha-acetylmethadol: importance of clinical investigations to validate in vitro drug metabolism studies. *Clin Pharmacokinet* 44: 731–751.
Kiang CH *et al.* (1981). Determination of acetylmethadol and metabolites by use of high-performance liquid chromatography. *J Chromatogr* 222: 81–93.

Moody DE *et al.* (1995). A gas chromatographic-positive ion chemical ionization-mass spectrometric method for the determination of *l*-alpha-acetylmethadol (LAAM), norLAAM, and dinorLAAM in plasma, urine, and tissue. *J Anal Toxicol* 19: 343–351.
Sigma-Aldrich (2007). *Material Safety Data Sheet: LAAM hydrochloride DEA schedule II*. St Louis, MO: Sigma-Aldrich.
Thomas BF *et al.* (1994). Determination of *l*-alpha-acetylmethadol, *l*-alpha-noracetylmethadol and *l*-alpha-dinoracetylmethadol in plasma by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 655: 201–211.
Verebey K *et al.* (1985). Quantitation of *l*-alpha-acetylmethadol and its metabolites in human serum by capillary gas–liquid chromatography and nitrogen detection. *J Chromatogr* 343: 339–348.
Walsh SL *et al.* (1998). Intravenous and oral *l*-alpha-acetylmethadol: pharmacodynamics and pharmacokinetics in humans. *J Pharmacol Exp Ther* 285: 71–82.
Wilkins DG *et al.* (1997). Quantitative analysis of *l*-alpha-acetylmethadol, *l*-alpha-acetyl-*N*-normethadol, and *l*-alpha-acetyl-*N,N*-dinormethadol in human hair by positive ion chemical ionization mass spectrometry. *J Anal Toxicol* 21: 420–426.
Wilkins DG *et al.* (1998). Incorporation of drugs for the treatment of substance abuse into pigmented and nonpigmented hair. *J Pharm Sci* 87: 435–440.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucl Acids Res* 134.

Levomethorphan

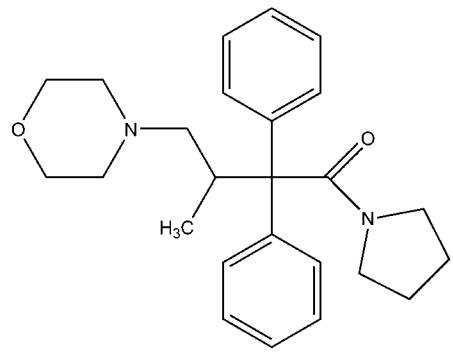
Antitussive, Cough Suppressant, NMDA Antagonist, Narcotic Analgesic
$C_{18}H_{25}NO = 271.4$
Synonyms *l*-Methorphan; (−)-3-methoxy-*N*-methylmorphinan.



Chemical Properties Methyl analogue of dextrorphan. A white crystalline powder. Soluble 1 in 65 of water and 1 in 10 of ethanol; freely soluble in chloroform; insoluble in ether.
Colour Test Ammonium molybdate test—blue→green (limit of detection, 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.24 (acidified iodoplatinate spray—positive).
Gas Chromatography System G2/225—RRT 0.70 (relative to codeine).
UV Spectrum Acidic (0.05 mol/L sulfuric acid)—maximum at 278 nm (A^{1}_{1} = 78) with an inflexion at 283 nm (A^{1}_{1} = 71.3) nm.

Levomoramide

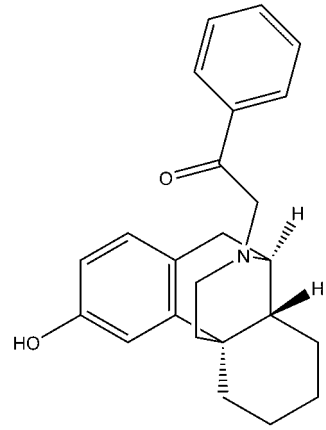
Narcotic Analgesic
$C_{25}H_{32}N_2O_2 = 392.5$
IUPAC Name 3-Methyl-4-morpholin-4-yl-2,2-di(phenyl)-1-pyrrolidin-1-yl-butan-1-one
Synonym (–)-1-(3-Methyl-4-morpholino-2,2-diphenylbutyrl)pyrrolidine



Chemical Properties Mp 190°. Levomoramide is extracted by organic solvents from aqueous alkaline solutions.
Thin-layer Chromatography System T1—R_f 0.70 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—254, 259, 265 nm.

Levophenacylmorphan

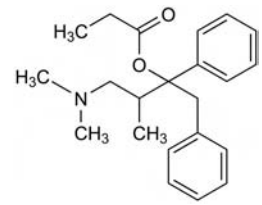
Narcotic
$C_{24}H_{27}NO_2 = 361.5$
CAS—10061-32-2
Synonyms (–)-3-Hydroxy-*N*-phenacylmorphinan; NIH 7525.



Chemical Properties Levophenacylmorphan is extracted by organic solvents from aqueous ammoniacal solutions.
Colour Tests Ammonium molybdate test—blue, fading (limit of detection, 1.0 µg); ammonium vanadate test—faint grey (limit of detection, 1.0 µg); sulfuric acid-formaldehyde test—faint purple→grey (limit of detection, 1.0 µg); Vitali's test—yellow/faint yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.15, streaking (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulphuric acid—251 nm (E1%, 1 cm 317) and inflexions at 275 and 286 nm.

Levopropoxyphene

Cough Suppressant
$C_{22}H_{29}NO_2 = 339.5$
CAS—2338-37-6
IUPAC Name α-[2-(Dimethylamino)-1-methylethyl]-α-phenylbenzeneethanol propanoate



Chemical Properties Crystals. Mp 75° to 76°. Log *P* (octanol/water), 4.2. For analytical data see under Dextropropoxyphene.

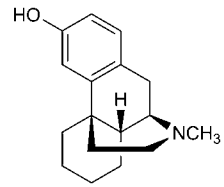
Levopropoxyphene Napsilate

$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O = 565.7$
CAS—5714-90-9 (anhydrous); 55557-30-7 (monohydrate)
Synonym Levopropoxyphene naphthalene-2-sulfonate
Proprietary Names *Contratuss*; *Letusin*; *Novrad*.
Chemical Properties A white powder. Mp 158° to 165°. Very slightly soluble in water; soluble 1 in 17 of ethanol and 1 in 2 of chloroform; soluble in acetone and methanol.

Dose The equivalent of 50 to 100 mg of levopropoxyphene every 4 h.

Levorphanol

Narcotic Analgesic
$C_{17}H_{23}NO = 257.4$
CAS—77-07-6
Synonyms (-)-3-Hydroxy-*N*-methylmorphinan; levorphan; methorphinan; 17-methylmorphinan-3-ol.

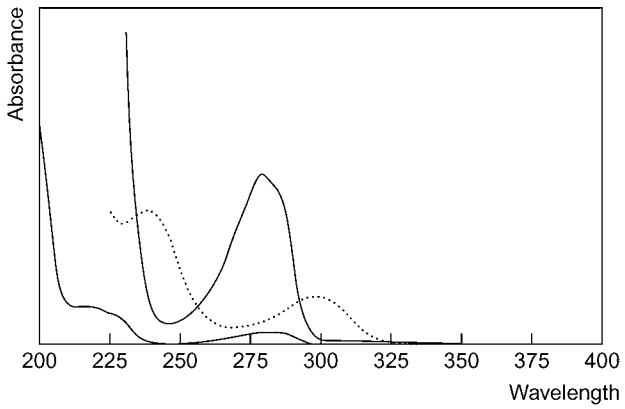


Chemical Properties Crystals. Mp 198° to 199°. pK_a 8.2 (20°). Log *P* (octanol/pH 7.4), 1.1.

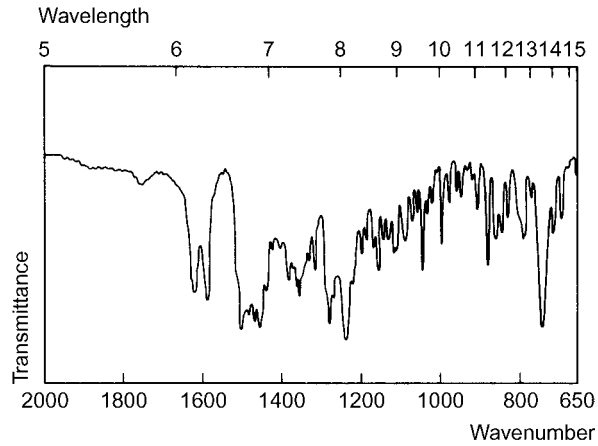
Levorphanol Tartrate

$C_{17}H_{23}NO,C_4H_6O_6,2H_2O = 443.5$
CAS—125-72-4 (anhydrous); 5985-38-6 (dihydrate)
Synonym Levorphanol bitartrate
Proprietary Names *Dromoran*; *Levo-Dromoran*.
Chemical Properties White crystalline powder. Mp 113° to 115° (dihydrate), Mp 206° to 208° (anhydrous). Soluble 1 in about 45 of water, 1 in about 110 of ethanol, and 1 in 50 of ether; practically insoluble in chloroform.

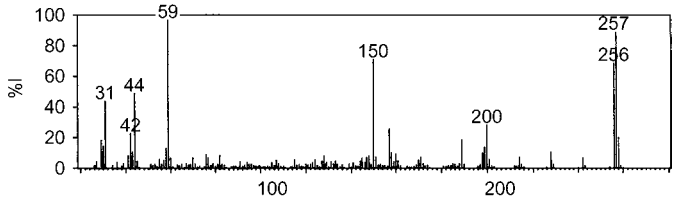
Thin-layer Chromatography System TA—R_f 0.35; system TB—R_f 0.13; system TC—R_f 0.07 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2230; system GC—RI 2230 (metabolised to dextrorphan).
High Performance Liquid Chromatography System HA—*k* 4.4 (tailing peak); system HC—*k* 3.20; system HY—RI 265.
Ultraviolet Spectrum Aqueous acid—279 nm ($A_1^1 = 79a$); aqueous alkali—240 ($A_1^1 = 339a$), 299 nm ($A_1^1 = 119a$).



Infrared Spectrum Principal peaks at wavenumbers 1238, 1495, 753, 1278, 1578, 1608 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 59, 257, 150, 256, 44, 31, 200, 157.



Quantification

Plasma **GC** Column: Restek Rtx-65TG fused silica (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 2.25 mL/min. Temperature programme: 150° for 1 min to 310° at 10°/min. ECD. Retention time: 13.7 min (pentaflurobenzoyl derivative). Limit of detection, <0.25 µg/L [Everhart *et al.* 1999].

GC-MS Column: 3% poly S-176 80/100 mesh on Chromosorb W (120 × 2 cm). Carrier gas: CH_4, 101 kPa. Temperature: 280°. SIM acquisition mode. Retention time: 2 min (pentaflurobenzoyl derivative). Limit of detection, 1 µg/L [Min *et al.* 1982].

HPLC Column: µBondapak C_{18} (300 × 4 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.01 mol/L sodium chloride (pH 4.8; 30 : 70), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 6.4 min. Limit of detection, 1.25 µg/L [Lucek, Dixon 1985].

Note For a radioimmunoassay method, see Dixon *et al.* [1981].

Disposition in the Body Absorbed after oral administration. Metabolised by 3-glucuronidation.

Therapeutic Concentration

A patient who had received 16 mg of levorphanol PO twice-daily had steady-state concentrations of 65–87 µg/L up to 3 h after drug administration. When the same patient was switched to 8 mg given IM with the same dosing schedule similar concentrations of 58–76 µg/L were observed [Lucek, Dixon 1985].

Toxicity

Postmortem concentrations in a 41-year-old female found dead after having ingested an unknown quantity of levorphanol were: blood 2.7 µg/mL, bile 24 µg/mL, brain 1.8 µg/g, kidney 3.4 µg/g, liver 5.4 µg/g, lung 17 µg/g, urine 2.3 µg/mL [Turner, Richards 1977].

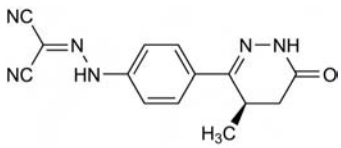
A 61-year-old male ingested 30 mg of levorphanol and died about 18 h later. The antemortem blood concentration was 0.8 mg/mL. Concentrations in other fluids and tissues (samples taken after the body had been embalmed) were: bile 15 mg/mL, kidney 1 µg/g, liver 11 µg/g [Bednarczyk 1978].

Half-life Plasma half-life, ≈13 h.
Dose 1.5 to 9 mg of levorphanol tartrate daily.

Bednarczyk LR (1978). A death due to levorphanol. *J Anal Toxicol* 3: 217–219.
Dixon R *et al.* (1981). Levorphanol: a simplified radioimmunoassay for clinical use. *Res Commun Chem Pathol Pharmacol* 32: 545–548.
Everhart ET *et al.* (1999). Quantitation of levorphanol in human plasma at subnanogram per milliliter levels using capillary gas chromatography with electron-capture detection. *J Chromatogr B Biomed Sci Appl* 729: 173–181.
Lucek R, Dixon R (1985). Quantitation of levorphanol in plasma using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 341: 239–243.
Min BH *et al.* (1982). Determination of levorphanol (Levo-Dromoran) in human plasma by combined gas chromatography–negative ion chemical ionization mass spectrometry. *J Chromatogr* 231: 194–199.
Turner JE, Richards RG (1977). A fatal case involving levorphanol. *J Anal Toxicol* 1: 103–104.

Levosimendan

Cardiotonic
$C_{14}H_{12}N_6O = 280.3$
CAS—141505-33-1
IUPAC Name [[4-[(4*R*)-1,4,5,6-Tetrahydro-4-methyl-6-oxo-3-pyridazinyl]phenyl]-hydrazono]propanedinitrile
Synonyms (*R*)-Simendan; OR-1259.
Proprietary Name *Simdax*



Chemical Properties Yellow crystalline powder. Mp 210° to 214°. Soluble in water. pK_a 6.3.

Quantification

Plasma **HPLC** Column: RP-18 LiChrosorb (250 × 4 mm i.d., 10 µm). Mobile phase: 32 mmol/L monosodium dihydrogen phosphate buffer : methanol : tetrahydrofuran (45:65:1), (pH 3.5). flow rate 1.0 mL/min. Internal standard (IS): OR-1097. UV detection (λ=380 nm). Retention time: levosimendan, 6.4 min; IS, 5.4 min. Limit of quantification and limit of detection, 5 µg/L [Karlsson *et al.* 1997]. UV detection (λ=380 nm). Limit of detection, 10 µg/L [Wikberg *et al.* 1996].

Disposition in the Body Levosimendan is rapidly absorbed after administration and distributed quickly around the body. It is completely metabolised with negligible amounts of the unchanged drug being detected in urine and faeces. The main metabolites are conjugates of the glutathione pathway and cyclic, *N*-acetylated cysteine or cysteinylglycine derivatives. These metabolites are biologically inactive and are slowly formed.

Therapeutic Concentration

Ten healthy subjects, aged 21 to 27 years (mean 24 years), were administered a single dose of levosimendan (2 mg) as an IV infusion (5 min infusion) and orally in the form of a conventional tablet; conventional capsule and a slow-release tablet. Doses were administered after an overnight fast and a washout period of 1 week was allowed between treatments. After the IV dose, the maximum plasma concentration was 180 µg/L at the end of the infusion. For oral administration, the conventional tablet and capsule gave maxima of 72 and 76 µg/L at 0.9 and 1.2 h, respectively; the slow-release formulation produced lower concentrations of 14 µg/L at 2.8 h. Metabolites were detected up to 24 h after the slow-release tablet but only up to 8 h for the other formulations. At 8 h, the metabolite concentrations were 93, 79, 53 and 1074 ng/L for the conventional tablet, conventional capsule, after the IV dose and after the slow-release tablet, respectively [Sundberg *et al.* 1998].

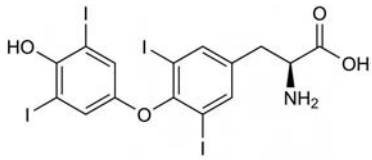
Bioavailability Oral, 85%.
Half-life 0.96 h (healthy individuals); 1.03 h (patients with mild congestive heart failure).
Volume of Distribution Steady state, 21.9 L (healthy); 19.5 L (patients with mild congestive heart failure). Also reported as 14.7 L.
Clearance 359 mL/min (healthy); 296 mL/min (patients with mild congestive heart failure). Also reported as 246 mL/min.
Protein Binding 97 to 98%.
Dose The usual dose (used in clinical trials) is a 6 to 12 μg/kg loading dose over 10 min followed by 0.05 to 0.2 μg/kg/min as a continuous IV infusion.

Karlsson M *et al.* (1997). Automated analysis of levosimendan in human plasma by on-line dialysis and liquid chromatography. *Biomed Chromatogr* 11(1): 54–58.
Sundberg S *et al.* (1998). Integrated pharmacokinetics and pharmacodynamics of the novel calcium sensitizer levosimendan as assessed by systolic time intervals. *Int J Clin Pharmacol Ther* 36(12): 629–635.
Wikberg T *et al.* (1996). Enantiomeric bioanalysis of simendan and levosimendan by chiral high-performance liquid chromatography. *Chirality* 8(7): 511–517.

Levothyroxine

Thyroid Agent
$C_{15}H_{11}I_4NO_4$ = 776.9
CAS—51-48-9

IUPAC Name (−)-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]alanine
Synonyms Thyroxine; L-thyroxine. The abbreviation T_4 is often used for thyroxine in medical and biochemical reports.



Chemical Properties Crystals. Mp 235° to 236°, with decomposition. pK_a 2.2, 6.7, 10.1. Log *P* (octanol/water), 4.12.

Levothyroxine Sodium

$C_{15}H_{10}I_4NNaO_4,xH_2O$ = 798.9 (anhydrous)
CAS—55-03-8 (anhydrous); 25416-65-3 (hydrate)
Synonyms Levothyroxinnatrium; lexothyroxinum natricum; thyroxine sodium; thyroxinum natricum; tirossina; tiroxina sodica.
Proprietary Names *Dexnon*; *Elthyrone*; *Eltroxin*; *Euthyrox*; *Eutirox*; *Levaxin*; *Levotec*; *Levo-T*; *Levothroid*; *Levothyrox*; *Levoxyl*; *Oroxine*; *Synthroid*; *Thyrax*; *Thyrex*; *Tiracrin*; *Tirosint*; *Unithroid*. It is an ingredient of *Thyrolar*.
Chemical Properties A white to pale-brownish-yellow, hygroscopic, amorphous, or crystalline powder. It may assume a slight pink colour on exposure to light. Soluble 1 in 700 of water and 1 in 300 of ethanol; practically insoluble in acetone, chloroform and ether; soluble in solutions of alkali hydroxides. Alkaline solutions are unstable.

Ultraviolet Spectrum Aqueous alkali—325 nm (A_1^1=78a).
Infrared Spectrum Principal peaks at wavenumbers 1628, 1585, 1308, 1185, 1240, 1148 cm^{-1} (KBr disk).
Quantification
Serum **GC** ECD. Limit of detection, 10 μg/L for levothyroxine, 2.7 μg/L for liothyronine [Nihei *et al.* 1971].

HPLC–MS Tandem MS detection (SIM). Limit of detection, 6 pg [De Brabandere *et al.* 1998].

Enzyme immunoassay Limit of detection, 4 nmol/L [Akman *et al.* 1995].
Hair **HPLC–radioimmunoassay** For method, see Tagliaro *et al.* [1998].
Disposition in the Body Incompletely and variably absorbed after oral administration. 48 to 80% of an administered dose may be absorbed. The extent of absorption increases in the fasted state and the degree of absorption may be affected by the formulation of the drug used. It is metabolised, in the liver and kidney, by de-iodination to liothyronine (tri-iodothyronine), which is the principal active form of levothyroxine; further de-iodination to thyroacetic acid (4-*p*-hydroxyphenoxy-phenylacetic acid), and conjugation with glucuronic acid and sulfate also occur. Deamination and decarboxylation of levothyroxine to the tetrone is a further metabolic pathway. Elimination from the body is slow. About 30 to 55% of a dose is excreted in the urine and 20 to 40% is eliminated in the faeces; of the urinary material about 40% is thyroacetic acid and 20% is liothyronine.

Endogenous serum-levothyroxine concentrations range from 50 to 120 μg/L in normal subjects. A minimum amount of the drug crosses the placenta and can be detected in breast milk.
Therapeutic Concentration

Twenty-four patients with hypothyroidism, receiving treatment with levothyroxine 100 μg daily in the form of a test or a reference preparation, had a mean peak plasma concentration of 98.4 and 108 μg/L at 3 and 2 h, respectively [Vaisman *et al.* 2001].

Fourteen euthyroid goitrous patients were orally administered 150 μg levothyroxine after an overnight fast, as a tablet or in solution. The mean levothyroxine basal concentration was 90.7 μg/L for the solution administration day and 89.0 μg/L for the tablet day. The maximum serum levothyroxine concentration was reached at the second hour after administration for the solution, at 111.5 μg/L and at the third hour for the tablet formulation, maximum 118.1 μg/L [Carpi *et al.* 1993].

Thirty healthy females (mean age, 34 years) were administered a single dose of 600 μg levothyroxine as two different formulations, Synthroid (A) or Levoxine (B), after an overnight fast. Fasting continued for 5 h after dosing and a wash-out period of 35 days was allowed between doses. The mean maximum plasma concentrations of levothyroxine were 59.1 and 71.2 μg/L for the two formulations, respectively, at 3.67 and 3.03 h [Berg and Mayor 1992].
Toxicity

A 34-year-old male ingested ~900 tablets (a total of 720 mg) levothyroxine. He became lethargic 2 to 3 days after administration, had vomiting and insomnia on day 4; was agitated, aggressive, and incoherent on day 5 and was confused when he returned to hospital on day 6. While in hospital for 6 days, he was tachycardic. Free levothyroxine concentrations were >130 μg/L on day 6 and 12 μg/L on day 12. By day 15 he had lost 20 kg in weight [Hack *et al.* 1999].

The following peak serum concentrations were reported in a thyroidectomised patient who had attempted suicide by taking 2000 μg levothyroxine: levothyroxine (on second day) 315 μg/L, FT_4 0.048 μg/L, reverse triiodothyronine 0.80 μg/L, triiodothyronine (on third day) 1.92 μg/L; levothyroxine and reverse triiodothyronine levels returned to their normal range 13 to 17 days after the suicide attempt [Ishihara *et al.* 1998].

Over a one-year period, 15 cases of acute levothyroxine overdose were studied; all patients were <5-years-old and the majority were male. The estimated dose ingested by 10 patients was 1.5 to 8.8 mg (0.1 to 0.73 mg/kg) and all were examined 1 to 6 h after administration. Three patients had initial serum levothyroxine concentrations >750 μg/L and developed signs of toxicity within 12 to 48 h, including tachycardia, hypertension and agitation, which had disappeared within 24 to 60 h [Lewander *et al.* 1989].
Half-life Plasma half-life, 6 to 7 days which may be increased in pregnancy or myxoedema and decreased in hyperthyroidism.
Protein Binding >99.9%.
Dose Adult, 50 to 100 μg of anhydrous levothyroxine sodium daily; increased by increments of 50 μg until desired result (usually, 100 to 200 μg daily). Children, (up to 1 month old) 5 to 10 μg/kg daily (>1 month old); 5 μg/kg daily initially and then increased by 25 μg every 2 to 4 weeks until desired result.

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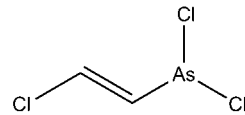
Berg JA, Mayor GH (1992). A study in normal human volunteers to compare the rate and extent of levothyroxine absorption from Synthroid and Levoxine. *J Clin Pharmacol* 32(12): 1135–1140.
Carpi A *et al.* (1993). Comparison of the effect of a single oral L-thyroxine dose (150 micrograms) in tablet and in solution on serum thyroxine and TSH concentrations. *Thyroidology* 5(1): 9–12.
De Brabandere VI *et al.* (1998). Isotope dilution-liquid chromatography/electrospray ionization-tandem mass spectrometry for the determination of serum thyroxine as a potential reference method. *Rapid Commun Mass Spectrom* 12(16): 1099–1103.
Hack JB *et al.* (1999). Severe symptoms following a massive intentional L-thyroxine ingestion. *Vet Hum Toxicol* 41(5): 323–326.
Ishihara T *et al.* (1998). Thyroxine (T4) metabolism in an athyreotic patient who had taken a large amount of T4 at one time. *Endocr J* 45: 371–375.
Lewander WJ *et al.* (1989). Acute thyroxine ingestion in pediatric patients. *Pediatrics* 84(2): 262–265.
Nihei NN *et al.* (1971). Measurements of triiodothyronine and thyroxine in human serum by gas-liquid chromatography. *Anal Biochem* 43: 433–445.
Tagliaro F *et al.* (1998). Determination of thyroxine in the hair of newborns by radioimmunoassay with high-performance liquid chromatographic confirmation. *J Chromatogr B Biomed Sci Appl* 716: 77–82.
Vaisman M *et al.* (2001). Comparative bioavailability of two oral L-thyroxine formulations after multiple dose administration in patients with hypothyroidism and its relation with therapeutic endpoints and dissolution profiles. *Arzneimittelforschung* 51: 246–252.

Lewisite

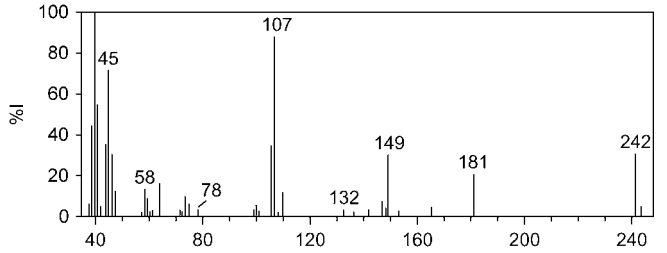
Organic Arsenical, Vesicant
$C_2H_2AsCl_3$ = 207.3
CAS—541-25-3

IUPAC Name 2-Chloroethenyldichloroarsine
Synonyms (2-Chloroethenyl)arsenous dichloride; 2-chlorovinyldichloroarsine; dew of death; dichloro(2-chlorovinyl)arsine.



Chemical Properties Colourless and odourless when pure. Oily, brown liquid with an odour of geraniums when industrially produced. Mp −18°. For a description of the stability of lewisite derivatives under various conditions, see Muir *et al.* [2005].

Mass Spectrum Principal ions at *m/z* 107, 45, 149, 242, 181, 58 Lewisite oxide (2-chlorovinylarsonous oxide (CVAO)) (1,3-propanedithiol (PDT) derivative) [Tomkins *et al.* 2001].



Quantification

Urine **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 45° for 5 min to 165° at 20°/min to 210° at 8°/min to 300° at 50°/min for 5 min. SIM acquisition mode. Limit of detection, 7.4 ng/L [Wooten *et al.* 2002].

Other **GC** Soil. Column: Rtx-5 fused silica capillary (30 m × 0.53 mm i.d., 1.0 µm). Carrier gas: He, 30 mL/min. Temperature programme: 100° for 2 min to 175° at 20°/min to 200° at 4°/min to 250° at 50°/min for 5 min. FPD. Limit of detection, 0.30 and 0.09 mg/kg for CVAO and phenylarsine oxide (PhAsO), respectively [Tomkins *et al.* 2001].

GC-MS Chemical Weapons. Column: DB-5 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 100° for 5 min to 280° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Hanaoka *et al.* 2006]. Soil. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 40° for 1 min to 280° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, tripropanethiol arsine and Clarke I propanethiol derivative 6 µg/kg, phenyldipropanethiol arsine 3 µg/kg [Tornes *et al.* 2006]. Air. Column: DB5-MS capillary (25 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 40° for 2 min to 160° at 20°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of detection, 260 µg/m^3 [Muir *et al.* 2005]. Complex Matrices: Column: DB5-MS capillary (30 m × 0.25 mm i.d., 0.33 µm). Temperature programme: 40° for 1 min to 300° at 20°/min. Electron ionisation, SIM acquisition mode. Limit of detection, butanethiol derivative of Lewisite I 0.12 ng [Muir *et al.* 2004]. Soil. Column: HP-5MS (30 m × 0.25 mm i. d., 0.25 µm). Carrier gas: He. Temperature programme: 100° for 2 min to 175° at 20°/min to 200° at 4°/min to 250° at 50°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, CVAO 0.066 mg/kg, PhAsO 0.023 mg/kg [Tomkins *et al.* 2001]. Soil and Water. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 28 cm/s. Temperature programme: 45° for 1.5 or 5 min to 165° at 20°/min to 210° at 8°/min to 300° at 50°/min for 5 min. Scan mode. Limit of detection, 2-chlorovinylarsonous acid (CVAA)–1,2-ethanedithiol 2.0 µg/L, CVAA-PDT derivative 1.0 µg/L [Szostek, Aldstadt 1998].

Note For a multicapillary chromatographic method using ion mobility increment spectrometry for the analysis of explosives, chemical warfare agents and drugs, see Buryakov [2004].

Disposition in the Body In the environment, Lewisite rapidly hydrolyses to CVAA, which is also toxic and which, in turn, slowly degrades to CVAO (CAS—3088-37-7). This is also the case in an aqueous environment, such as blood plasma [Noort *et al.* 2002].

Toxicity For a review article on the pharmacology and toxicology of lewisite, see Goldman and Dacre [1989].

Note British anti-Lewisite (2,3-dimercapto-1-propanol) was developed just before the end of World War II for the treatment of lewisite gas exposure and is still used clinically as a chelating agent in acute heavy metal poisoning, especially with arsenic, gold, mercury and lead. For a GC-MS method for the determination of British anti-Lewisite in plasma, see Byers *et al.* [2004].

Protein Binding Approximately 25 to 50% of a dose associates with globin.

Buryakov IA (2004). Express analysis of explosives, chemical warfare agents and drugs with multi-capillary column gas chromatography and ion mobility increment spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 800: 75–82.

Byers CE *et al.* (2004). Gas chromatographic–mass spectrometric determination of British anti-lewisite in plasma. *J Anal Toxicol* 28: 384–389.

Goldman M, Dacre JC (1989). Lewisite: its chemistry, toxicology, and biological effects. *Rev Environ Contam Toxicol* 110: 75–115.

Hanaoka S *et al.* (2006). Determination of mustard and lewisite related compounds in abandoned chemical weapons (Yellow shells) from sources in China and Japan. *J Chromatogr A* 1101: 268–277.

Muir B *et al.* (2004). Analysis of chemical warfare agents. I. Use of aliphatic thiols in the trace level determination of Lewisite compounds in complex matrices. *J Chromatogr A* 1028: 313–320.

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Noort D *et al.* (2002). Biomonitoring of exposure to chemical warfare agents: a review. *Toxicol Appl Pharmacol* 184: 116–126.

Szostek B, Aldstadt JH (1998). Determination of organoarsenicals in the environment by solid-phase microextraction–gas chromatography–mass spectrometry. *J Chromatogr A* 807: 253–263.

Tomkins BA *et al.* (2001). Determination of lewisite oxide in soil using solid-phase microextraction followed by gas chromatography with flame photometric or mass spectrometric detection. *J Chromatogr A* 909: 13–28.

Tornes JA *et al.* (2006). Determination of organoarsenic warfare agents in sediment samples from Skagerrak by gas chromatography–mass spectrometry. *Sci Total Environ* 356: 235–246.

Wooten JV *et al.* (2002). Quantitation of 2-chlorovinylarsonous acid in human urine by automated solid-phase microextraction–gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 147–153.

Lidocaine

Antiarrhythmic, Anaesthetic (Local)

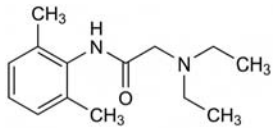
$C_{14}H_{22}N_2O$ = 234.3

CAS—137-58-6

IUPAC Name 2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide

Synonyms 2-Diethylaminoaceto-2′,6′-xylidide; lidokain; lignocaine.

Proprietary Names *Cuivasil*; *Dentipatch*; *ELA-Max*; *Lidoderm*; *LidoPosterine*; *Vagisil*; *Zilactin-L*. Lidocaine and lidocaine hydrochloride are ingredients of many proprietary preparations [Sweetman 2009].



Chemical Properties A white to slightly yellow crystalline powder. Mp 68° to 69° (needles from benzene or alcohol). Insoluble in water; soluble in ethanol, chloroform, benzene and ether. pK_a 7.95 (25°) [Lau *et al.* 1991], 8.01 [Sangster 1994]. Log *P* (*n*-hexane/buffer), 0.32, (octanol/buffer), 1.70, (ether/buffer) 1.77 [Lau *et al.* 1991], (octanol/water), 2.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Lidocaine Hydrochloride

$C_{14}H_{22}N_2O,HCl,H_2O$ = 288.8

CAS—73-78-9 (anhydrous); 6108-05-0 (monohydrate).

IUPAC Name 2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide hydrochloride

Synonym Lignocaine hydrochloride

Proprietary Names *Anestacon*; *Basicaina*; *Batixim*; *Dilocaine*; *Duo-Trach Kit*; *Dynexan*; *Heweneural*; *Laryng-O-Jet*; *Licain*; *Lidesthesin*; *Lidocard*; *Lidocation*; *Lidocaton*; *Lidocord*; *Lidofast*; *Lidoject*; *LidoPen*; *Lidosen*; *Lidrian*; *Lignostab-A*; *Nervocaine*; *Octocaine*; *Odontalg*; *Rinstead* (contact pastilles); *Sedagul*; *Ultracaine*; *Uro-Jet*; *Xylocaine*; *Xylocard*; *Xylocitin*; *Xyloneural*; *Xylotox*.

Chemical Properties A white crystalline powder. Mp 77° to 78°. Soluble 1 in 0.7 of water, 1 in 1.5 of ethanol, and 1 in 40 of chloroform; practically insoluble in ether.

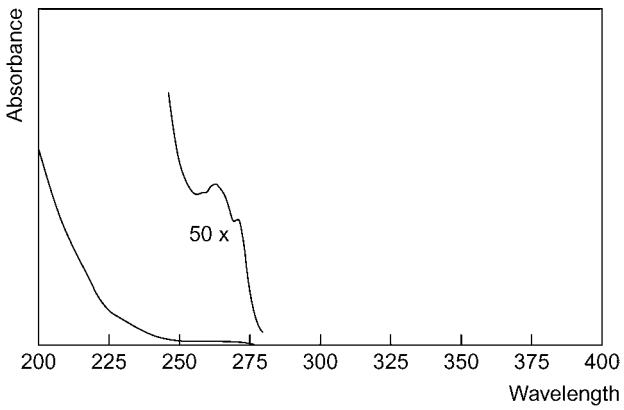
Colour Test To a 2% solution in water, add 1 mL of dilute nitric acid and 3 mL of mercuric nitrate solution and heat to boiling—yellow or yellow-green.

Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.35; system TC—R_f 0.71; system TE—R_f 0.80; system TL—R_f 0.63; system TAE—R_f 0.72; system TAF—R_f 0.69; system TAJ—R_f 0.55; system TAK—R_f 0.00; system TAL—R_f 0.28 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—pink).

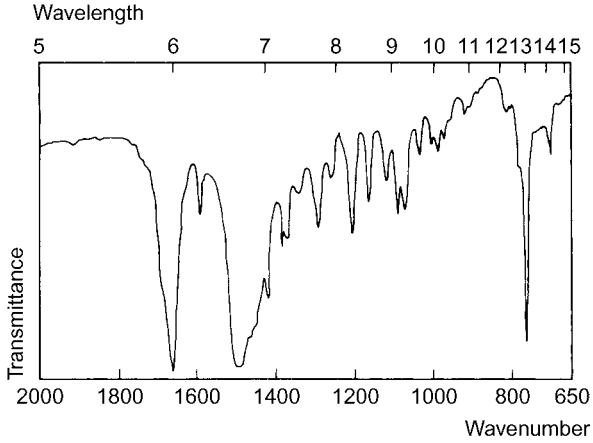
Gas Chromatography System GA—lidocaine RI 1870, M (2,6-dimethylaniline) RI 1180, M (OH-) RI 2350, M (monoethylglycinexylidide) RI 1800, M (desethyl-) RI 1790, M (dimethylhydroxyaniline) RI 1460; system GF—RI 2240; system GQ—lidocaine RT 6.0 min, M (monoethylglycinexylidide) RT 5.5 min.

High Performance Liquid Chromatography System HA—lidocaine *k* 0.6, M (monoethylglycinexylidide) *k* 1.2; system HQ—*k* 0.79; system HX—RI 288; system HY—RI 258; system HZ—RT 2.6 min; system HAA—RT 9.9 min; system HAM—lidocaine RT not detected; system HAX—RT 8.0 min; system HAY—RT 4.5 min.

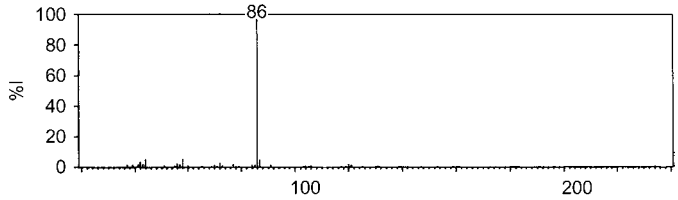
Ultraviolet Spectrum Aqueous acid—263 (A_1^1 = 19a), 272 nm.



Infrared Spectrum Principal peaks at wavenumbers 1662, 1495, 762, 1204, 1290, 1086 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 87, 58, 44, 72, 42, 120, 85.



Quantification

Blood **GC** Column: Ulbon HR-1 capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22.5 cm/s. Temperature programme: 100° to 280° at 10°/min. SID. Limit of detection, 0.5–10 µg/L [Hattori *et al.* 1991]. Column: 3% OV-101 on 80/100 Supelcoport (2 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 200°. NPD. Limit of detection, 0.1 mg/L [Levine *et al.* 1983].

GC-MS [Teatino, Barbaro 2004]. Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 100° for 5 min to 280° at 20°/min. SIM acquisition mode. Limit of quantification, 0.1 µg/g, limit of detection, 0.05 µg/g [Watanabe *et al.* 1998].

Plasma **GC** Column: HP-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: N_2, 1 mL/min. Temperature programme: 80° for 3 min to 215° at 20°/min to 230° at 5°/min to 290° at 25°/min. FID. Limit of detection, 5 µg/L [Koster *et al.* 2000]. Column: HP-5 (30 m × 0.32 mm i.d.). Carrier gas: N_2, 5 mL/min. Temperature programme: 80° for 1 min to 210° at 40°/min for 3.5 min. NPD. Limit of quantification, 2.5 µg/L, limit of detection, 1 µg/L [Laroche *et al.* 1998]. UV detection [Rodovnichenko *et al.* 1998]. Column: Restek RTX-200 cross-linked trifluoropropylmethylpropylmethylpolysiloxane (30 m × 0.53 mm i.d., 0.25 µm). Carrier gas: He, 70 kPa. Temperature programme: 65° to 225° at 8°/min. NPD. Limit of quantification, 3 µg/L, limit of detection, 2 µg/L [Grouls *et al.* 1995]. Column: 2% Carbowax 20M and 0.5% potassium hydroxide on 100/120 Gas Chrom Q (0.92 m × 2.0 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 190°. NPD. Limit of detection, 300 µg/L [Hawkins *et al.* 1982]. See Blood [Levine *et al.* 1983].

GC-MS Column: DB-1 (15 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 15 mL/min. Temperature programme: 140° to 300° at 16°/min for 10 min. Limit of quantification, 50 µg/L (lidocaine and mepivacaine) and 100 µg/L (monoethylglycinexylidide and other local anaesthetics) [Ohshima, Takayasu 1999].

HPLC Column: Zorbax Dikema C_{18} (150 × 4.6 mm, 5 µm). Mobile phase: methanol : diethylamine-acetic acid buffer (pH 4.0, 22 : 78). Limit of detection, 160 µg/L [Chen *et al.* 2007]. Column: ODS TSkgel (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.05 mol/L phosphate buffer (pH 4.0, 10 : 30 : 60), flow rate 0.6 mL/min. UV detection (λ = 210 nm). Retention time: 10 min. Limit of detection, 20 µg/L for lidocaine and metabolites [Kakiuchi *et al.* 2002]. Column: α_1-acid glycoprotein (150 × 4 mm i.d., 5 µm). Mobile phase: 8 mmol/L sodium dihydrogen phosphate and 0.1 mol/L sodium chloride containing 4% propan-2-ol and 0.6% diethylamine (pH 7.05), flow rate 0.9 mL/min. UV detection (λ = 214 nm). Limit of detection, 10 µg/L for lidocaine, 4 µg/L for bupivacaine enantiomers [Abraham *et al.* 1997]. Column: Nucleosil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L sodium dihydrogen phosphate buffer (pH 2.8, 23 : 77), flow rate 1.4 mL/min. UV detection (λ = 210 nm). Retention time: 3.6 min. Limit of quantification, 10 µg/L [Lotfi *et al.* 1997]. Column: LiChroCART Superspher 60 RP-select B (125 × 4 mm i.d., 4 µm). Mobile phase: phosphate buffer (pH 5.8) : acetonitrile (70 : 30). UV detection. Limit of quantification, 2.5 mg/L [Sattler *et al.* 1995]. See Serum [Chen *et al.* 1992a, b]. See Urine [Tam *et al.* 1987].

LC-MS Column: Supelcosil LC-Si (100 × 4.6 mm i.d.). Mobile phase: 2% formic acid in acetonitrile : water (50 : 50), flow rate 1 mL/min. TIS. Retention time: 5.1 min. Limit of quantification, 0.5 µg/L [Chik *et al.* 2006]. Mobile phase: methanol: 0.1% formic acid (1 : 1), flow rate 0.2 mL/min. ESI, positive ion mode. MRM acquisition mode. Limit of quantification, 2 nmol/L [Altun *et al.* 2004]. Column: YMC (150 × 3.0 mm i.d., 3 µm). Mobile phase: 0.1% formic acid in acetonitrile-water (0.5 : 99.5) : 0.1% formic acid in acetonitrile-water (80 : 20, 99.5 : 0.5 to 20 : 80 at 8 min for 2 min to 99.5 : 0.5 at 10.1 min), flow rate 0.35 mL/min. ESI, positive ion mode. Limit of quantification, 1.6–5 nmol/L for lidocaine and metabolites [Abdel-Rehim *et al.* 2000]. Column: C_{18}. Mobile phase : acetonitrile : 26 mmol/L ammonium acetate (pH 4.5, 70 : 30), flow rate 1 mL/min. Positive ion mode. Limit of quantification, 0.2 µg/L [Bo *et al.* 1999].

Serum **GC** Column: HP-Innowax (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 2 mL/min. Temperature programme: 50° for 1 min to 150° at 25°/min to 240° at 5°/min for 12 min. FID. Limit of quantification, ~50 µg/L [Baniceru *et al.* 2004]. Column: capillary. NPD. Limit of detection, for lidocaine, bupivacaine and their metabolites, 15 µg/L[Lorec *et al.* 1994]. See Plasma [Hawkins *et al.* 1982].

HPLC Column: Ultracarb ODS-30(5) (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : PBS (pH 5.0, 50 : 50), flow rate 0.8 mL/min. UV detection (λ = 238 nm). Retention time: 6.9 min. Limit of quantification, 1.5 mg/L [Perrotti *et al.* 2006]. Column: Supelcosil LC-8-DB. Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen orthophosphate (pH 3.0, 12 : 78). UV detection (λ = 205 nm). Limit of quantification, 200 µg/L for lidocaine and 10 µg/L for monoethylglycinexylidide [Piwowarska *et al.* 2004]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.05 mol/L potassium dihydrogen phosphate buffer (pH 4.0, 14 : 86), flow rate 1.3 mL/min. UV detection (λ = 205 nm). Retention time: 11.3 min. Limit of detection, 10 µg/L [Chen *et al.* 1992a, b]. UV detection. Limit of detection, 100 µg/L for lidocaine and metabolites [Lindberg *et al.* 1983].

Urine **GC** Column: Alltech EC-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 110° for 1 min to 260° at 32°/min for 5 min. NPD. Limit of detection, 0.01 µg/L [Raikos *et al.* 2009]. See Plasma [Rodovnichenko *et al.* 1998].

GC-MS See Blood [Teatino, Barbaro 2004]. See Plasma [Ohshima, Takayasu 1999].

HPLC Column: Johnsson Spherigel C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile and 11 mmol/L TEA-0.1% phosphoric acid (10 : 90) : acetonitrile and 20 mmol/L TEA-0.1% phosphoric acid (50 : 50, 100 : 0 to 30 : 70 at 15 min to 0 : 100 at 30 min), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, <0.05 mg/L for lidocaine, bupivacaine and tetracaine [Ma *et al.* 2006]. Column: C_{18} Novopak (115 × 8 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% phosphoric acid with 0.15% TEA (12 : 88, pH 3.0), flow rate 2 mL/min. UV detection (λ = 200 nm). Limit of detection, 0.1 mg/L [Tam *et al.* 1987].

LC-MS See Plasma [Abdel-Rehim *et al.* 2000].

Aqueous Humour **HPLC** Column: C_8. Mobile phase: methanol : 30 mmol/L sodium dihydrogen phosphate containing 10 mmol/L sodium pentane sulfonate (pH 2.5, 50 : 50). UV detection (λ = 225 nm). Limit of quantification, 2.5 mg/L, limit of detection, 0.2 mg/L [Manna *et al.* 2002].

CSF **GC** See Blood [Hattori *et al.* 1991].

HPLC Column: Zorbax Dikema C_{18} (150 × 4.6 mm, 5 µm). Mobile phase: methanol : diethylamine–acetic acid buffer (pH 4.0, 22 : 78). Limit of detection, 140 µg/L [Chen *et al.* 2007].

Milk **GC-MS** Column: 5% diphenylsiloxane 95% dimethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 20 cm/s. Temperature programme: 60° for 1 min to 140° at 10°/min to 250° at 25°/min for 1 min. MSD, SIM acquisition mode. Limit of detection, 0.2 ppb for 2,6-dimethylaniline [Puente, Josephy 2001].

Oral Fluid **GC** See Plasma [Rodovnichenko *et al.* 1998].

Hair **GC-MS** See Blood [Teatino, Barbaro 2004]. Column: Supelcowax 10 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 2 min to 250° at 20°/min to 250° at 20°/min for 7 min. MSD, SIM acquisition mode. Limit of quantification, 0.4 µg/g; limit of detection, 0.1 µg/g [Sporkert, Pragst 2000].

Tissues **GC** See Blood [Levine *et al.* 1983].

Other **GC** Biological Samples. Column: 3% W/W SP 2250 on Chromosorb W 80/100 mesh (2 m × 2 mm), Temperature programme: 210° for 5 min to 280° at 10°/min. NSD. Limit of detection, 0.1 mg/L [Lau *et al.* 1991].

Note For a review of the identification and application of up-to-date chromatographic methods for assay of local anaesthetics in blood and urine, see Stoliarov *et al.* [2009].

Disposition in the Body

Lidocaine is readily absorbed from the gastrointestinal tract, mucous membranes, damaged skin (poor absorption from intact skin) and after IM injection; oral bioavailability is low (~35%) as a result of first-pass metabolism. Metabolism in the liver is rapid, with ~90% of a dose being dealkylated to form monoethylglycinexylidide, which is 60–80% as potent as lidocaine, and glycinexylidide, which is also active. Both have longer half-lives than lidocaine. Further metabolism occurs and metabolic reactions also include hydrolysis, and ring hydroxylation. <10% of a dose is excreted in the urine as unchanged drug in 24 h, 40–70% as 4-hydroxy-2,6-xylidine, and ~4% as the active monoethylglycinexylidide; excretion of unchanged drug is increased if the urine is acid. Other metabolites include 2,6-xylidine, 3′-hydroxylignocaine, and 3′-hydroxymonoethylglycinexylidide. Glycinexylidide and the hydroxy metabolites are excreted as acid-hydrolysable conjugates. Glycinexylidide has also been detected in plasma after prolonged IV infusion. Lidocaine crosses the placenta and the blood–brain barrier and it is excreted in breast milk.

Therapeutic Concentration In plasma, usually in the range 2–5 mg/L.

Following IV infusions at rates varying between 20 and 50 µg/min/kg to 24 subjects, the following steady-state serum concentrations were reported: lidocaine 1.7–11.4 mg/L (mean, 5.7), monoethylglycinexylidide 0.2–5.2 mg/L (mean, 2.0) and glycinexylidide 0–1.4 mg/L (mean, 0.5) [Drayer *et al.* 1983].

A mean peak plasma concentration of 0.4 mg/L lidocaine was attained at 3 h in 24 subjects who received instillation of 200 mg lidocaine with adrenaline 1 : 500 000 into the intraperitoneal cavity after total abdominal hysterectomy. The highest concentration in any patient was 0.87 mg/L [Williamson *et al.* 1997].

In 15 patients with mild asthma, inhalation of lidocaine 10% (total dose: 5.0 mg/kg) produced a mean peak plasma concentration of 1.4 mg/L at ~13 min [Groeben *et al.* 2000].

In a study in 10 patients, 5 to 10 g of a cream containing lidocaine–prilocaine (EMLA) was given as a single 24 h application to leg ulcers (50–100 cm^2). Peak plasma lidocaine and prilocaine levels of 185–705 µg/L and 62–277 µg/L, respectively, occurred after 2–4 h (except in 1 patient, where they occurred at 6–8 h) [Stymne, Lillieborg 2001].

Following topical administration of lidocaine patches (four 5% patches) to 20 subjects for 18 h on each of 3 consecutive days, mean peak plasma concentrations of 0.1451, 0.153 and 0.1538 mg/L (occurring at 18, 16.5 and 16.5 h, respectively) were reported on days 1, 2 and 3, respectively [Gammaitoni, Davis 2002].

In another similar study, topical application of 4 patches every 12 or 24 h for 3 days resulted in steady-state maximum plasma levels of 0.186 mg/L and 225 mg/L, respectively [Gammaitoni *et al.* 2002].

In a study in 10 patients, 11 mL of 2% lidocaine gel (Instillagel) was inserted into the uterine cavity after thermal ablation. Plasma levels were 40.3, 66.3, 64.9 and 75 µg/L after 5, 15, 30 and 60 min, respectively [Rousseau *et al.* 2002].

Levels of free lidocaine following epidural injection of a 1.5% solution with adrenaline was increased in elderly patients [Fukuda *et al.* 2003].

In pregnant women at term given peridural anaesthesia with 200 mg lidocaine as a 2% solution, peak plasma levels of lidocaine were 1.146 and 0.879 mg/L in women with and without gestational diabetes mellitus, respectively. Peak levels of the main metabolite, monoethylglycinexylidide, were 141.4 and 82.7 µg/L, respectively. Clearance of lidocaine appears to be reduced in diabetes mellitus [Moisés *et al.* 2008].

Toxicity Toxic effects are associated with plasma concentrations >6 mg/L, and fatalities with blood concentrations >14 mg/L.

In 3 deaths involving accidental ingestion of 25 g, the following postmortem tissue concentrations were reported: blood 44, 92 and 11 mg/L; brain 17, 32 and 7 µg/g; kidney 66, – and 68 µg/g; liver 70, 96 and 20 µg/g; lung 94, 130 and 49 µg/g; and urine 59, – and – mg/L; in the first 2 cases, death occurred within a few minutes of ingestion, whereas the third subject survived for 24 h [Borkowski, Dluzneiwska, 1976].

In 33 patients with cardiac disorder and given lidocaine infusions for more than 1 day, serum levels above 8 mg/L occurred in 6 of 27 patients who had no toxicity; 5 of 6 patients with toxicity had levels below 8 mg/L [Drayer *et al.* 1983].

In a death cause by accidental IV injection of 2 g lidocaine, the following postmortem tissue concentrations were reported: blood 30 mg/L, brain 135 µg/g, heart 106 µg/g, kidney 204 µg/g, lung 87 µg/g and skeletal muscle 20 µg/g [Poklis *et al.* 1984].

In 2 deaths from deliberate self-poisoning with lidocaine, the first by oral ingestion and the second by IV injection, postmortem blood analysis revealed concentrations of 40 and 53 mg/L, respectively; urine contained 49 mg/L lidocaine in the first victim [Dawling *et al.* 1989].

In the case of a 1-month-old infant who inadvertently received an IV injection of 50 mg lidocaine, the calculated maximum level was 5.39 mg/L [Jonville *et al.* 1990].

In a 32-year-old hospital patient whose death was attributed to an overdose of lidocaine (~1500 mg) administered with homicidal intent, postmortem revealed the following lidocaine concentrations: blood, 22.2 mg/L, liver 43.6 µg/g, kidney 28.3 µg/g, brain 23.1 µg/g and heart 13.1 µg/g [Kalin, Brissie 2002].

In a fatality in a patient with paroxysmal ventricular arrhythmia given an injection of 5 mL of 10% lidocaine hydrochloride (500 mg) instead of 2.5 mL of 2% (50 mg), the following concentrations were reported in postmortem tissues that had been fixed in formalin for 40 days: parietal lobe 308 ng/g, occipital lobe 208.7 ng/g, temporal lobe 318 ng/g, frontal lobe, 223.2 ng/g, cerebellum 200.9 ng/g, pons 285.7 ng/g, liver 109.5 ng/g, kidney 109.5 ng/g and skeletal muscle 127 ng/g; formalin solution contained 8.4 µg/L. Animal studies found levels in these tissues decreased to 25–33% of the original value on storage in formalin [Kudo *et al.* 2004].

In the case of suicide by a 31-year-old woman through oral ingestion of lidocaine, the following postmortem levels were reported: blood 31 mg/L, gastric contents 2.5 g, liver 10 µg/g, kidney 12 µg/g, brain 9 µg/g, spleen 24 µg/g, lung 84 µg/g, heart 9 µg/g, urine 9 mg/L and bile 6 mg/L [Centini *et al.* 2007].

Half-life Plasma half-life, lidocaine 1–2 h, increased in subjects with liver disease or after acute myocardial infarction; monoethylglycinexylidide ~1–2 h, glycinexylidide ~10 h.

Volume of Distribution Approximately 1–2 L/kg.

Clearance Plasma clearance, ~5–20 mL/min/kg.

Protein Binding Approximately 70% at therapeutic concentrations but there is considerable intersubject variation and binding appears to be concentration-dependent

Note For a review of the pharmacokinetics of lidocaine, see Benowitz, Meister [1978]; for a review of the pharmacokinetics and pharmacodynamics of IV agents for ventricular arrhythmias, see Nolan [1997]; for a study of the pharmacokinetics of epidural lidocaine and bupivacaine during caesarean section, see Downing *et al.* [1997].

Dose For local anaesthesia, the maximum single dose of lidocaine hydrochloride by injection should not exceed 300 mg (4.5 mg/kg), unless administered with adrenaline. For ventricular arrhythmias, initially 50–100 mg IV, followed by an infusion.

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Lidoflazine

Antianginal

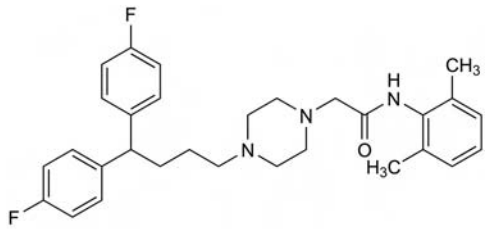
$C_{30}H_{35}F_2N_3O = 491.6$

CAS—3416-26-0

IUPAC Name 4-[4,4-Bis(4-fluorophenyl)butyl]-*N*-(2,6-dimethylphenyl)-1-piperazineacetamide

Synonyms Ordiflazine; R-7904.

Proprietary Names *Clinium*; *Corflazine*; *Klinium*.



Chemical Properties A white or slightly-yellow amorphous powder. Mp 159° to 161°. Almost insoluble in water; soluble 1 in 90 of ethanol and 1 in 220 of ether; very soluble in chloroform. Log *P* (octanol/water), 5.6.

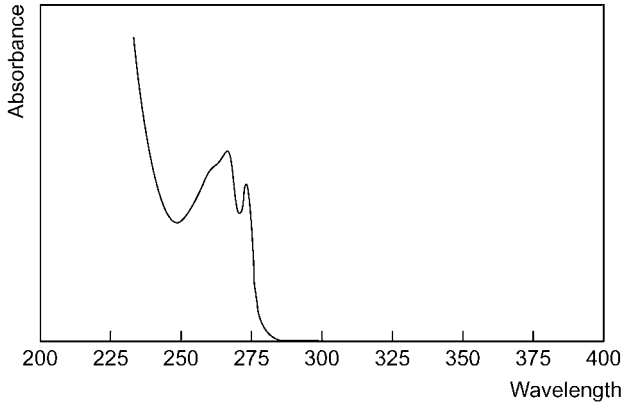
Colour Tests Mandelin's test—yellow; Marquis test—yellow.

Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.11; system TC—R_f 0.63; system TE—R_f 0.70; system TL—R_f 0.36; system TAE—R_f 0.70; system TAF—R_f 0.77 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—lidoflazine RI 3870, M (desaminocarboxy-) RI 2230, M (desaminocarboxy-)-Me RI 2125; system GB—lidoflazine, not eluted.

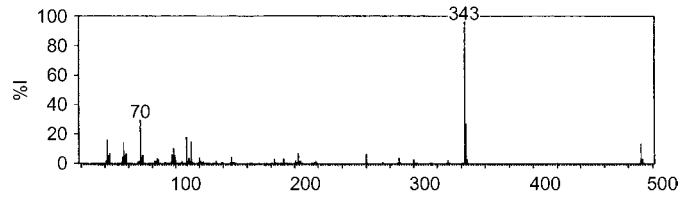
High Performance Liquid Chromatography System HA—*k* 0.6; system HX—RI 530.

Ultraviolet Spectrum Acid isopropyl alcohol—266 (A_1^1=47b), 272 nm.



Infrared Spectrum Principal peaks at wavenumbers 1505, 1645, 1220, 1155, 835, 775 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 343, 70, 344, 109, 42, 113, 491, 56.



Disposition in the Body Lidoflazine is well absorbed after oral administration. It is excreted mainly as metabolites, the major metabolites being bis(4-fluorophenyl)butyric acid and the glucuronide conjugate of bis(4-fluorophenyl)butan-1-ol. In 7 days, about 40% of an oral dose is excreted in the urine and about 40% is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 120 mg given with food, peak plasma concentrations of about 0.06 mg/L were attained in about 1 h. Following repeated oral administration of 120 mg three times a day, a mean steady-state plasma concentration of 0.12 mg/L was reported [Vanhoutte, Van Nueten 1973].

Half-life Plasma half-life, about 1 day.

Dose 120 to 360 mg daily.

Vanhoutte PM, Van Nueten JM (1973). Lidoflazine. In: Scriabine A, ed. *New Drugs Annual: Cardiovascular Drugs*. New York: Raven Press, 203–226.

Limaprost

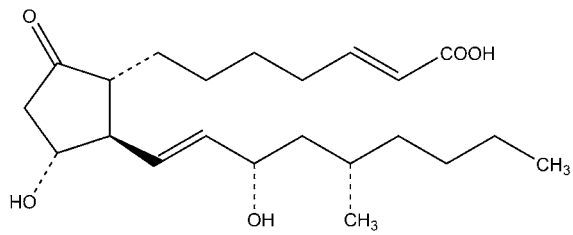
Antianginal, Prostaglandin

$C_{22}H_{36}O_5 = 380.5$

CAS—74397-12-9

IUPAC Name (*E*)-7-[(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(*E*,3*S*,5*S*)-3-hydroxy-5-methylnon-1-enyl]-5-oxocyclopentyl]hept-2-enoic acid

Synonyms (2*E*,11α,13*E*,15*S*,17*S*)-11,15-Dihydroxy-17,20-dimethyl-9-oxoprosta-2,13-dien-1-oic acid; 17*S*,20-dimethyl-*trans*-2,3-didehydro-PGE1; (*E*)-7-{(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-nonenyl]-5-oxocyclopentyl}-2-heptenoic acid; 17*S*-methyl-ω-homo-*trans*-Δ2-PGE1; ONO-1206; OP-1206; 9-oxo-11α,15α-dihydroxy-17S,20-dimethylprosta-*trans*-2,*trans*-13-deenoic acid.



Chemical Properties White crystals. Mp 97° to 100°. Under high humidity conditions, limaprost degrades rapidly; aqueous solutions of limaprost are most stable at pH 3 to 4 [Moribe *et al.* 2007]. Limaprost is stable in plasma for up to 73 days at –80°, after three freeze–thaw cycles, up to 4 h at room temperature, in whole blood for 4 h at 4°, and in processed samples for 96 h at 4° [Komaba *et al.*, 2007].

Limaprost Alfadex

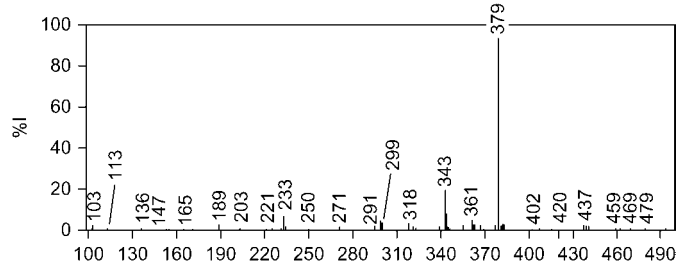
CAS—88852-12-4

Proprietary Names *Opalmon*; *Prorenal*.

Chemical Properties α-Cyclodextrin inclusion compound of limaprost.

High Performance Liquid Chromatography Column: Inertsil ODS-2 (150 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile (linear gradient, not described), flow rate 1.0 mL/min. Samples derivatised with 4-(*N*,*N*-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoadiazole. Fluorescence detection (λ_{ex} = 440 nm, λ_{em} = 569 nm). Limit of detection, 1.7 to 5.0 fmol/L [Toyo'oka *et al.* 1992]. Column: ODS (150 × 4.6 mm i.d.). Mobile phase: 0.02 mol/L potassium dihydrogen phosphate : acetonitrile : isopropyl alcohol (9 : 5 : 2), flow rate 0.8 mL/min. UV detection (λ = 215 nm). Limit of detection not reported [Moribe *et al.* 2007].

Mass Spectrum

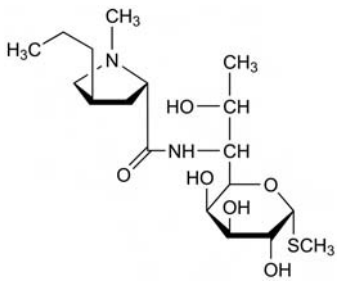


Quantification
Plasma **LC-MS** Column: Capcell PAK phenyl UG120 (150 × 2.0 mm, 5 μm). ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 0.1 ng/L [Komaba *et al.*, 2007]
Disposition in the Body Rapidly absorbed following oral administration, with peak plasma concentrations reached within 1 h. Studies in rats have shown that 70% of an orally administered radiolabelled dose is excreted in the faeces, with the majority of the remainder excreted in urine over a 96-h post-dose period. At the time of writing, the extent or site of metabolism and route of excretion has not yet been investigated in humans.
Therapeutic Concentration
Twenty-four healthy volunteers were administered either 5 or 10 μg limaprost orally and monitored for 4 h. Mean peak plasma concentrations for the 2 doses were 1.02 and 1.93 ng/L, respectively, reached at 1.0 h [Komaba *et al.*, 2007].
Twelve healthy adult volunteers were administered either 5 or 10 μg limaprost orally. Mean peak plasma concentrations for the 2 doses were 1.18 and 2.06 ng/L reached at 0.75 h and 0.5 h, respectively [Tsuboshima *et al.*, 1992].
Half-life Approximately 1 h.
Clearance Approximately 3110 L/h.
Dose Given by mouth as limaprost alfadex, in a dose equivalent to limaprost 15 to 30 μg daily in three divided doses.

Komaba J *et al.* (2007). Ultra sensitive determination of limaprost, a prostaglandin E_1 analogue, in human plasma using on-line two-dimensional reversed-phase liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 590–597.
Moribe K *et al.* (2007). Stabilization mechanism of limaprost in solid dosage form. *Int J Pharm* 338: 1–6.
Toyo'oka T *et al.* (1992). Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N*,*N*-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ). *Biomed Chromatogr* 6: 143–148.
Tsuboshima M *et al.* (1992). Prostaglandins: synthetic and pharmacological studies and development. *Yakugaku Zasshi* 112: 447–469.

Lincomycin

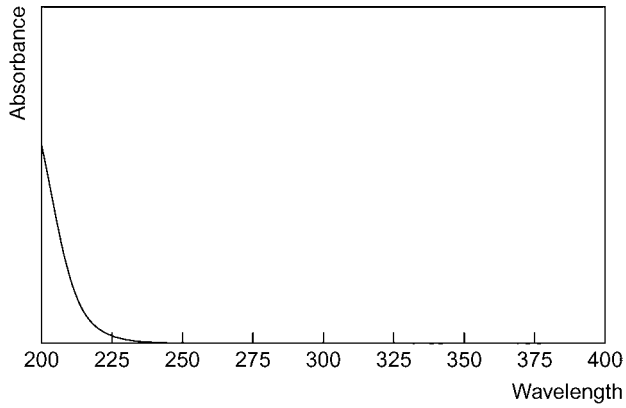
Antibiotic
$C_{18}H_{34}N_2O_6S$ = 406.5
CAS—154-21-2
IUPAC Name (2*S*,4*R*)-*N*-[(1*R*,2*R*)-2-Hydroxy-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide
Synonym (2*S*-*trans*)-Methyl 6,8–dideoxy-6-[[(1-methyl-4–propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside; NSC-70731.



Chemical Properties Slightly soluble in water; soluble in methanol, lower alcohols, ethyl acetate, acetone and chloroform. pK_a 7.5. Log *P* (octanol/water), 0.6.

Lincomycin Hydrochloride
$C_{18}H_{34}N_2O_6S,HCl,H_2O$ = 461.0
CAS—859-18-7 (anhydrous); 7179-49-9 (monohydrate)
Proprietary Names *Albiotic*; *Anbycin*; *Cillimicina*; *Frademicina*; *Fredcina*; *Linco*; *Lincocin(e)*; *Lincocina*; *Lincogin*; *Lincolan*; *Lincomy*; *Linco-Plus*; *Lincorex*; *Lingo*; *Linmycin*; *Macrolin*; *Mycivin*; *Princol*; *Rimsalin*.
Chemical Properties A white crystalline powder. Soluble 1 in 1 of water, 1 in 40 of ethanol and 1 in 20 of dimethylformamide; soluble in methanol; practically insoluble in chloroform and ether.

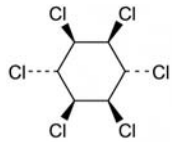
Colour Test Sodium nitroprusside (method 3)—violet.
Thin-layer Chromatography System TA—R_f 0.67; system TAE—R_f 0.75 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



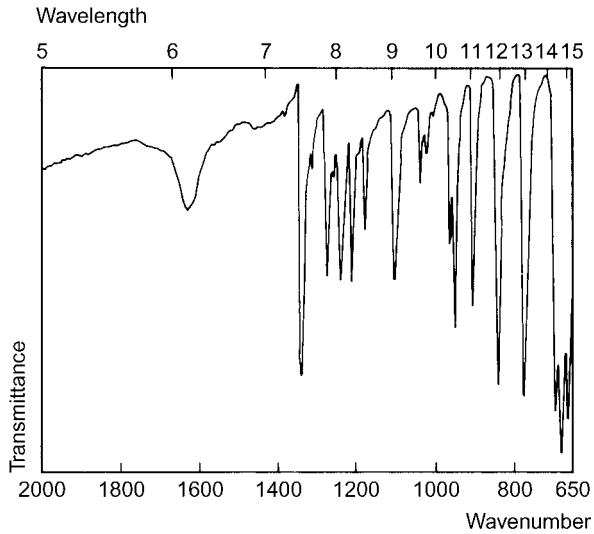
Infrared Spectrum Principal peaks at wavenumbers 1655, 1104, 1075, 1564, 1040, 1262 cm^{-1} (lincomycin hydrochloride, KBr disk).
Dose The equivalent of 1.5 to 2 g of lincomycin daily.

Lindane

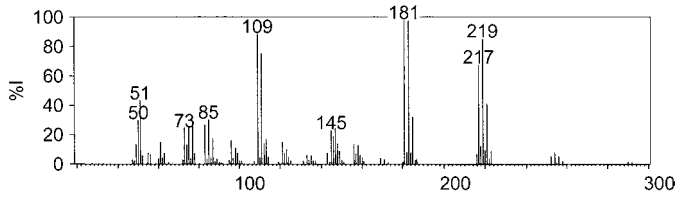
Insecticide
$C_6H_6Cl_6$ = 290.8
CAS—58-89-9
IUPAC Name (1α,2α,3β,4α,5α,6β)-1,2,3,4,5,6-Hexachlorocyclohexane
Synonyms 666; Benhexachlor; gamma benzene hexachloride; gamma-BHC; gamma-HCH; HCH; hexicide.
Proprietary Names *Aphtiria*; *Delitex N*; *Elentol*; *Escabin*; *Esoderm*; *Gambex*; *Gamene*; *GBH*; *G-Well*; *Hexicid*; *Hexit*; *Jacutin*; *Kwell*; *Kwellada*; *Lendianon*; *Lindanoxil*; *Lorexane*; *Pediletan*; *Pilensar*; *Piodrex*; *Pioletal*; *Pionax*; *Plurisan*; *Pruritrat*; *Quellada*; *Sarnapin*; *Sarpiol*; *Scabecid*; *Scabene*.



Chemical Properties A white crystalline powder. Mp 112.5°. Practically insoluble in water; soluble 1 in 19 of dehydrated alcohol, 1 in 2 of acetone, 1 in 3.5 of chloroform and 1 in 5.5 of ether. Log *P* (octanol/water), 3.7.
Thin-layer Chromatography System TE—R_f 0.86; system TF—R_f 0.75; system TX—R_f 0.51; system TY—R_f 0.92.
Gas Chromatography System GA—RI 1745; system GK—RRT 0.76(relative to caffeine).
Infrared Spectrum Principal peaks at wavenumbers 680, 665, 695, 778, 840, 950 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 181, 183, 109, 219, 111, 217, 51, 221.



Quantification

Blood **GC** ECD. Limit of detection, 1 µg/L [Radomski, Ray 1970]. ECD. For method, see Jain *et al.* [1965].

Serum **GC** ECD. Limit of detection, <7 µg/L [Saady, Poklis 1990].

Tissues **GC** See Blood [Radomski, Ray 1970].

Disposition in the Body Readily absorbed after ingestion, inhalation, or through the skin. It is stored in the body fat and adrenal glands. Metabolised by oxidation and dehydrohalogenation to a series of chlorinated phenols which are excreted mainly in the urine in free and conjugated form.

After IV administration, about 25% of the dose is excreted in the urine; after topical administration about 10% of the dose is recovered in the urine. [Feldmann, Maibach 1974.]

Blood Concentration

Blood-lindane concentrations in subjects with low occupational exposure ranged from 1 to 9 µg/L (mean, 4), and concentrations in subjects with high dermal occupational exposure ranged from 6 to 93 µg/L (mean, 31) [Milby *et al.* 1968].

Average serum concentrations of 0.07, 0.19 and 0.04 mg/L of α-benzene hexachloride (α-BHC), β-BHC and γ-BHC, respectively, were reported in 57 subjects who worked in a factory manufacturing lindane; β-BHC was the only isomer observed to accumulate on chronic exposure. A mean fat concentration of 45.6 µg/g of β-BHC was reported in 8 subjects [Baumann *et al.* 1980].

Fifteen subjects with long-term (5 to 17 years) exposure to wood-preserving chemicals containing lindane and pentachlorophenol had mean serum levels of 0.085 and 43.6 µg/L, respectively [Peper *et al.* 1999].

Toxicity Lindane is not highly toxic when applied externally in the concentrations usually employed (0.1 to 1%), but when ingested it may cause convulsions; dusts may irritate the nose and throat when used in a confined space. Blood concentrations >0.02 mg/L have been associated with toxic effects. The estimated minimum oral lethal dose is 200 mg/kg and the maximum permissible atmospheric concentration is 0.5 mg/m^3. Toxic doses or long-term exposure may cause liver necrosis. The maximum acceptable daily intake is 10 µg/kg.

A young girl who ingested about 1.6 g of lindane was found to have a serum concentration of 0.84 mg/L after 2 h, following convulsions; the concentration decreased to 0.49 mg/L after 4 h; urinary concentrations of individual free phenolic metabolites determined 5.5 h after ingestion ranged from 0.04 to 0.74 mg/L [Starr, Clifford 1972].

A fat concentration of 343 µg/g was reported in a fatality caused by lindane [Hayes, Vaughn 1977].

Half-life Derived from urinary excretion data, about 26 h.

Use Topically in concentrations of 0.1 to 1%.

Baumann K *et al.* (1980). Occupational exposure to hexachlorocyclohexane. I. Body burden of HCH-isomers. *Int Arch Occup Environ Health* 47: 119–127.

Feldmann RJ, Maibach HJ (1974). Percutaneous penetration of some pesticides and herbicides in man. *Toxicol Appl Pharmacol* 28: 126–132.

Hayes WJ, Vaughn WK (1977). Mortality from pesticides in the United States in 1973 and 1974. *Toxicol Appl Pharmacol* 42: 235–252.

Jain N *et al.* (1965). Simplified gas chromatographic analysis of pesticides from blood. *J Pharm Pharmacol* 17: 362–367.

Milby TH *et al.* (1968). Humane exposure to lindane; blood lindane levels as a function of exposure. *J Occup Med* 10: 584–587.

Peper M *et al.* (1999). Long-term exposure to wood-preserving chemicals containing pentachlorophenol and lindane is related to neurobehavioral performance in women. *Am J Ind Med* 35: 632–641.

Radomski JL, Ray A (1970). *J Chromatogr Sci* 8: 108–114.

Saady JJ, Poklis A (1990). Determination of chlorinated hydrocarbon pesticides by solid-phase extraction and capillary GC with electron capture detection. *J Anal Toxicol* 14: 301–401.

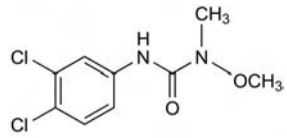
Starr HG, Clifford NJ (1972). Acute lindane intoxication: a case study. *Arch Environ Health* 25: 374–375.

Linuron

Herbicide

$C_9H_{10}Cl_2N_2O_2$ = 249.1

CAS—330-55-2



IUPAC Name *N′*-(3,4-Dichlorophenyl)-*N*-methoxy-*N*-methylurea

Synonyms Du Pont herbicide 326; HOE-2810.

Proprietary Names *Afalon*; *Garnitan*; *Linex*; *Linorox*; *Linurex*; *Lorox*; *Premalin*; *Rotalin*; *Sarclex*; *Sinuron*; *Siolcid*.

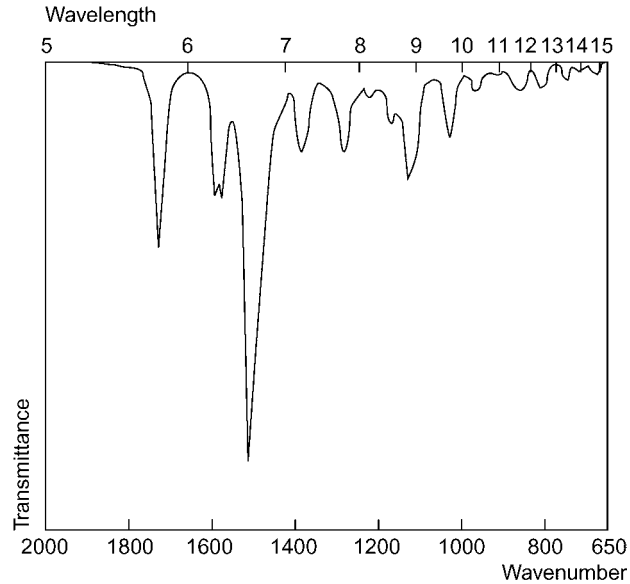
Chemical Properties A white crystalline solid (fine flakes or coarse powder). Mp 93° to 94°. Soluble in water (81 mg/L at 25°) and soluble in acetone (500 g/kg at 25°), ethanol (150 g/kg at 25°), benzene (150 g/kg at 25°), toluene, xylene (130 g/kg at 25°), and heptane (15 g/kg at 25°); readily soluble in dimethylformamide, chloroform and diethyl ether; moderately soluble in aromatic hydrocarbons; sparingly soluble in aliphatic hydrocarbons. Log *P* (octanol/water), 3.00.

Thin-layer Chromatography System TX—R_f 0.22; system TY—R_f 0.31.

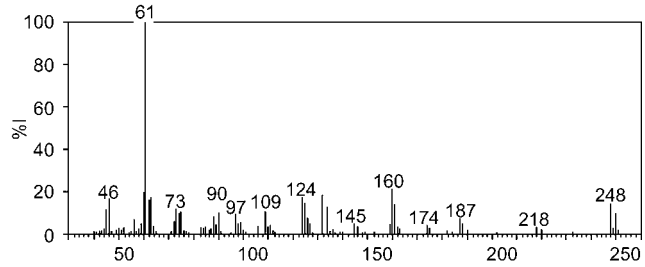
Gas Chromatography System GA—linuron RI 1927, linuron-Me RI 1785, 3,4-dichloroaniline RI 1323; system GK—linuron RRT 0.97, 3,4-dichloroaniline RRT 0.36, 3,4-dichlorophenyl-isocyanateRRT 0.13 (all relative to caffeine).

High Performance Liquid Chromatography System HY—RI 506; system HAA—retention time 21.3 min.

Infrared Spectrum Principal peaks at wavenumber 2269, 1507, 1729, 1594, 1132, 1282 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 61, 160, 132, 124, 46, 248, 73, 109.



Disposition in the Body Linuron is broken down completely after passing through the liver. It is unlikely to bioaccumulate. Metabolites include 3,4-dichloroaniline and 3,4-dichlorophenylisocyanate.

Liothyronine

Thyroid Agent

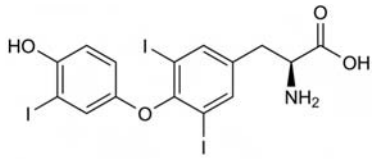
$C_{15}H_{12}I_3NO_4$ = 651.0

CAS—6893-02-3

IUPAC Name (2*S*)-2-Amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoic acid

Synonyms *O*-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine; L-tri-iodothyronine.

Note The abbreviation T_3 is often used for tri-iodothyronine.



Chemical Properties Crystals. Mp 236° to 237°, with decomposition. Insoluble in water, alcohol and propylene glycol; soluble in dilute alkalis. pK_a 8.5. Log *P* (octanol/water), 3.0.

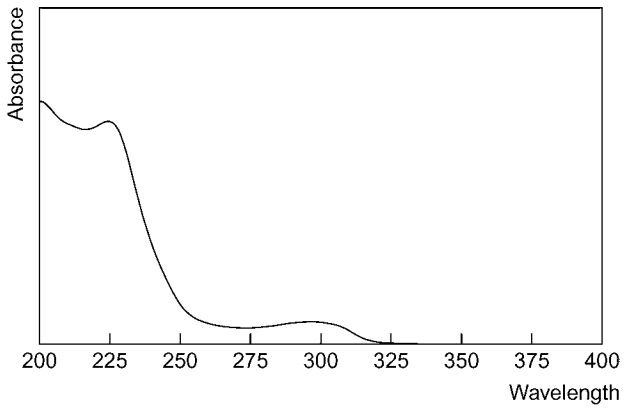
Liothyronine Hydrochloride

$C_{15}H_{12}I_3NO_4,HCl = 687.4$
CAS—6138-47-2
Proprietary Name *Thybon*
Chemical Properties Long birefringent needles. Mp 202° to 203° with decomposition.

Liothyronine Sodium

$C_{15}H_{11}I_3NNaO_4 = 673.0$
CAS—55-06-1
Synonym Sodium liothyronine
Proprietary Names *Cynomel*; *Cytomel*; *Dispon*; *Neo-Tiroimade*; *Ro-Thyronine*; *Tertroxin*; *Thybon*; *Thyrotardin*; *Ti-Tre*; *Triostat*; *Triyodisan*; *Triyotex*. It is an ingredient of *Euthroid*, *Novothyral*, and *Thyrolar*.
Chemical Properties A white to buff-coloured solid or crystalline powder. Practically insoluble in water, chloroform, ether and most other organic solvents; soluble 1 in 500 of ethanol; soluble in solutions of alkali hydroxides.

Ultraviolet Spectrum Liothyronine sodium: aqueous alkali—319 nm (A^1_1=65a).

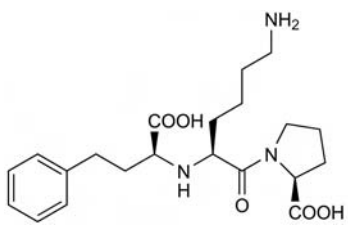


Infrared Spectrum Principal peaks at wavenumbers 1608, 1180, 1493, 1250, 1535, 1320 cm^{-1} (KBr disk).
Quantification See under Levothyroxine.
Disposition in the Body Liothyronine is absorbed after oral or IM administration. It is metabolised by conjugation with glucuronic acid or sulfate, de-iodination oxidative deamination and decarboxylation. It is excreted mainly in the bile and faeces, and is subject to enterohepatic circulation; some iodide is excreted in the urine.

Endogenous serum concentrations range from 0.0010 to 0.0016 mg/L, in normal subjects.
Half-life Plasma half-life, about 2 days.
Protein Binding >99%.
Dose Up to 100 µg of liothyronine sodium daily.

Lisinopril

Antihypertensive
$C_{21}H_{31}N_3O_5 = 405.5$
CAS—76547-98-3
IUPAC Name (2*S*)-1-[(2*S*)-6-Amino-2-[[(2*S*)-1-hydroxy-1-oxo-4-phenylbutan-2-yl]amino] hexanoyl]pyrrolidine-2-carboxylic acid
Synonyms (*S*)-1-[N^2-(1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline; L-154,826-000T; lisinoprilum; MK-521.



Chemical Properties A white to off-white crystalline powder. It is soluble in water (1 in 10), methyl alcohol (1 in 70); practically insoluble in alcohol, acetone, chloroform and in ether. pK_a 2.5, 4.0, 6.7, 10.1 (25°). Log *P* (octanol/water), −1.22; log *P* (phosphate buffer (0.1 mol/L, pH 7)/octanol), 10.2 ± 0.5 (room temperature). Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Lisinopril Dihydrate

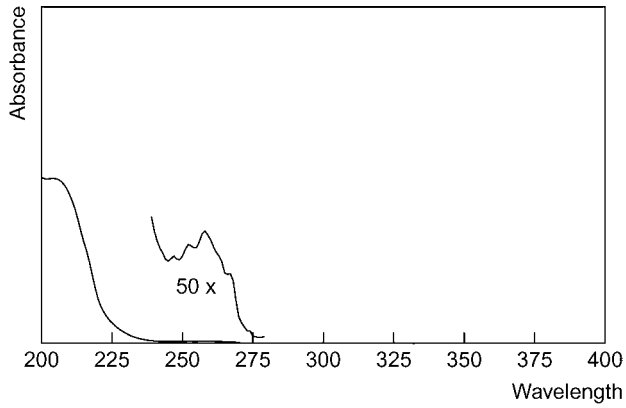
$C_{21}H_{31}N_3O_3,2H_2O = 441.5$
CAS—83915-83-7
Proprietary Names *Acemin*; *Acerbon*; *Alapril*; *Carace*; *Coric*; *Doneka*; *Prinil*; *Prinivil*; *Novatec*; *Tensopril*; *Vivatec*; *Zestril*. Also an ingredient of *Novazyd*; *Prinzide*; *Zestoretic*.
Chemical Properties An off-white crystalline powder. Solubility: water (97 g/L), methanol (14 g/L), ethanol, acetone, acetonitrile, chloroform and *N*,*N*-dimethylformamide (<0.1 g/L).

Thin-layer Liquid Chromatography System TB—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.27.
High Performance Liquid Chromatography System HX—RI 271; system HY—RI 250; system HZ—retention time 1.5 min.

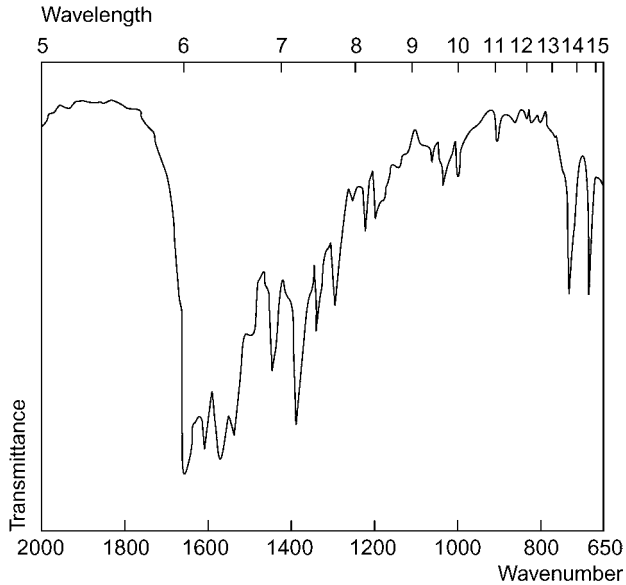
Column: ILS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : TEA (triethylamine) (pH 2.6, 50:50:0.1), flow rate 1 mL/min. UV detection (λ=210 nm); fluorescence detection (λ_{ex}=340 nm, λ_{em}=455 nm). Retention time: 2.9 min [El-Gindy *et al.* 2001].

Column: ODS Hypersil (250 × 4.5 mm i.d., 5 µm). Mobile phase: 20 mmol/L sodium heptanesulfonate (pH 2.5) : ACN (acetonitrile) (with 5% THF, tetrahydrofuran) (63:37), flow rate 1 mL/min. UV detection (λ= 215 nm). Retention time: 5.0 min [Bonazzi *et al.* 1997].

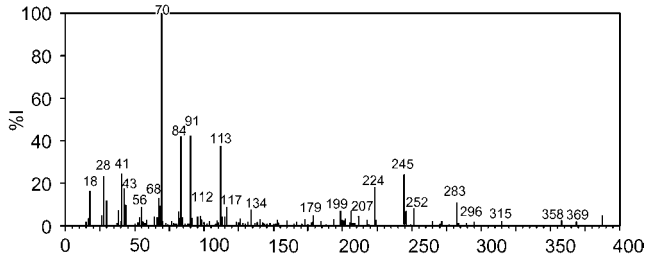
Column: C_{18} µBondapak (300 × 3.9 i.d., 10 µm). Temperature: 40°. Mobile phase: 15 mmol/L potassium dihydrogen phosphate (pH 2.9) : ACN : methanol : THF (92:6:1:1), flow rate 1.5 mL/min. Internal standard (IS): enalaprilat. UV detection (λ=206 nm). Retention time: lisinopril, 7.5 min; IS, 10.5 min [Wong, Charles 1995].
Ultraviolet Spectrum Aqueous alkali (0.1 mol/L sodium hydroxide)—246, 254, 258, 261, 267 nm; aqueous acid (0.1 mol/L hydrochloric acid)—246, 253, 258, 264, 267 nm.



Infrared Spectrum Principal peaks at wavenumbers 1655, 1570, 1388, 741, 732 cm^{-1} (broad peak around 3000 cm^{-1}) (KBr).



Mass Spectrum Principal peaks at *m/z* 70, 91, 84, 113, 245, 41, 28, 224.



Quantification

Plasma **GC–MS** Limit of detection 0.5 µg/L [Shioya *et al.* 1989].

Urine **GC–MS** Limit of detection 5 µg/L [Shioya *et al.* 1989].

Disposition in the Body Lisinopril is slowly and incompletely absorbed after oral administration. Absorption varies between 6 and 60% depending on the individual, but on average is 25% of the dose. Metabolism does not occur as it is already an active diacid (a lysine derivative of enalaprilat). The absorbed drug is excreted unchanged in urine via the kidneys and the unabsorbed drug is excreted in faeces.

Therapeutic Concentration The serum therapeutic concentration is 20 to 70 µg/L.

Twelve healthy male volunteers were administered with an oral dose of 10 mg lisinopril. Peak serum concentrations of 0.095 ± 0.055 µg/L were observed in 7 ± 1 h [Ulm *et al.* 1982].

Bioavailability 25 to 50%.

Half-life 12 h.

Volume of Distribution Mean 124 L.

Clearance 6.36 L/h (following a 10 mg oral dose in healthy subjects); reduced in renal impairment and the elderly. Clearance reduced in impaired renal function (glomerular filtration rate <30 mL/min).

Protein Binding Negligible.

Note For a review of lisinopril, see Simpson and Jarvis [2000]. For a general review of the pharmacokinetics of ACE inhibitors, see Kelly and O'Malley [1990].

Dose 2.5 to 20 mg daily with a maximum of 40 mg daily.

Bonazzi D *et al.* (1997). Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). *J Pharm Biomed Anal* 16(3): 431–438.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

El-Gindy A *et al.* (2001). Spectrophotometric, septrofluorimetric and LC determination of lisinopril. *J Pharm Biomed Anal* 25: 913–922.

Kelly JG, O'Malley K (1990). Clinical pharmacokinetics of the newer ACE inhibitors. A review. *Clin Pharmacokinet* 19: 177–196.

Shioya H *et al.* (1989). Determination of a new angiotensin-converting enzyme inhibitor (CS-622) and its active metabolite in plasma and urine by gas chromatography-mass spectrometry using negative ion chemical ionization. *J Chromatogr* 496: 129–135.

Simpson K, Jarvis B (2000). Lisinopril: a review of its use in congestive heart failure. *Drugs* 59: 1149–1167.

Ulm EH *et al.* (1982). Enalapril maleate and a lysine analogue (MK-521): disposition in man. *Br J Clin Pharmacol* 14: 357–362.

Wong YC, Charles BG (1995). Determination of the angiotensin-converting enzyme inhibitor lisinopril in urine using solid-phase extraction and reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 673(2): 306–310.

Lithium Carbonate

Tranquilliser

$Li_2CO_3 = 3.89$

CAS—302-27-2

IUPAC Name Dilithium carbonate

Synonyms CP-15467-61; lithii carbonas; lithium carb; NSC-16895.

Proprietary Names *Camcolit*; *Carbolit*; *Carbolith*; *Carbolithium*; *Eskalith*; *Hypnorex*; *Leukominerase*; *Li 450*; *Liskonum*; *Lithane*; *Litheum*; *Lithicarb*; *Lithizine*; *Lithobid*; *Lithonate*; *Lithosun*; *Lithotabs*; *Litiocar*; *Lito*; *Neurolepsin*; *Phasal*; *Plenur*; *Priadel*; *Quilonorm*; *Quilonum Retard*; *Teralithe*.

Chemical Properties A white granular powder. Mp ~720°. Soluble 1 in 78 of cold water and 1 in 140 of boiling water; practically insoluble in ethanol; soluble, with effervescence, in dilute mineral acids. Log *P* (octanol/water), −6.2.

Lithium Citrate

$C_6H_5Li_3O_7,4H_2O = 282.0$

CAS—919-16-4 (anhydrous); 6080-58-6 (tetrahydrate).

IUPAC Name Trilithium 2-hydroxypropane-1,2,3-tricarboxylate tetrahydrate

Synonym Lithii citras

Proprietary Names *Li-Liquid*; *Litarex*; *Lithonate-S*.

Chemical Properties A white, somewhat deliquescent, crystalline powder. Soluble 1 in 2 of water; practically insoluble in ethanol and ether.

Quantification

Blood **AAS** Varian hollow cathode lamp ($\lambda = 670.8$ nm). Limit of detection, 1 mg/L [Scott 1982].

ETAAS ($\lambda = 670.8$ nm). Limit of detection, 2 nmol/L [Robertson *et al.* 1973].

Serum **ETAAS** Lithium hollow cathode lamp ($\lambda = 668$ nm). Limit of detection, 50 nmol/L [Levy, Katz 1970].

Urine **ETAAS** See Blood. Limit of detection, 30 nmol/L. [Robertson *et al.* 1973].

Disposition in the Body Readily absorbed after oral administration. Approximately 97% of a dose is excreted unchanged in the urine in 10 days, with 50–60% in the first 24 h, and only small amounts being found in the faeces, saliva, and sweat. The urinary excretion of lithium appears to be slower in the elderly than the young and also appears to occur more slowly at night, resulting in a diurnal rhythm in excretion rate. Its excretion is also markedly influenced by sodium and, possibly, potassium status; in subjects where the sodium intake is reduced, lithium is reabsorbed by the renal tubules and in subjects who have a high sodium intake the excretion of lithium is increased. Lithium is widely distributed throughout the body and higher concentrations may be found in bones, the thyroid gland, and parts of the brain than in plasma. It crosses the placenta and is excreted in breast milk.

Therapeutic Concentration Lithium plasma concentrations should be monitored 12 h after a dose to ensure that they are within the range 0.5–1.2 mmol/L.

Administration of lithium 600 mg once daily as a slow-release capsule formulation to 18 subjects resulted in mean plasma concentrations (over 96 h) of 0.252 mmol/L, compared with 0.214 mmol/L following twice daily dosing with 300 mg of a standard-release preparation. Corresponding values for the first 24 h were 0.303 and 0.264 mmol/L, respectively [Castrogiovanni 2002].

Following a single oral dose of 600 mg to 10 subjects, peak plasma concentrations of 0.45–0.80 mmol/L (mean 0.64) were attained in ~2 h [Meinhold *et al.* 1979].

After daily oral doses of 1.5–1.7 g to 16 subjects, steady-state serum concentrations were in the range 0.7–0.9 mmol/L [Marini, Sheard 1976].

Toxicity Toxic effects may be produced by serum concentrations greater than 2 mmol/L. Blood concentrations of ~5 mmol/L or more are usually fatal, although in one instance of survival after the ingestion of 22.5 g lithium carbonate, the maximum serum concentration was 8.2 mmol/L. A fatality after acute ingestion of lithium carbonate has also been reported in which the serum concentration was 2.43 mmol/L on admission to hospital and 1.93 mmol/L at the time of death.

Acute lithium intoxication followed a suicide attempt in a 51-year-old woman on chronic lithium therapy, in which she ingested 50 slow-release 450 mg lithium carbonate tablets. Serum levels were reported to be 10.6 mmol/L 13 h after the ingestion and 5.8 mmol/L at 24 h [Nagappan *et al.* 2002].

A 29-year-old male who ingested 8 g lithium in the form of a sustained-release preparation had a plasma concentration of 2.38 mmol/L 15 h after the ingestion and 3.12 mmol/L at 25 h. Following 4 h of haemodialysis, the lithium level was 2.16 mmol/L [Astruc *et al.* 1999].

A 48-year-old woman was admitted to hospital having ingested 97 tablets of immediate-release lithium carbonate (300 mg each) 60 min earlier. Her serum lithium concentration was 4.3 mmol/L. After stabilisation and transfer to the intensive care unit, the patient's serum lithium level was 3.1 mmol/L and decreased to 1.5 mmol/L after haemodialysis. However, 9 h later the level had risen to 2.1 mmol/L. After the patient complained of a vaginal burning sensation, she admitted to having inserted 80 lithium carbonate tablets (300 mg) intravaginally. Vaginal irrigation with 3 L normal saline resulted in the lithium level dropping to 1.3 mmol/L by the next morning and to 0.5 mmol/L after 24 h [Temte *et al.* 1994].

In 3 fatalities from lithium intoxication, the following postmortem tissue concentrations were reported: blood 1.9, 3.0, and 4.6 mmol/L, brain 1.4, 5.2, and 4.2 mmol/kg, kidney 1.9, 5.9, and 9.6 mmol/kg, liver 1.9, 2.9, and 6.7 mmol/kg, urine 4.9, –, and 6.7 mmol/L [Baselt 2008].

Half-life Plasma half-life is ~20–24 h after a single dose but appears to be dependent on the duration of treatment. In patients on their first course of treatment, a plasma half-life averaging 31 h has been reported; this increased to 40 h in subjects receiving lithium for less than 1 year and 58 h in those taking the drug for longer than 1 year. Half-life is increased in the elderly and in renal impairment.

Bioavailability 80–100%.

Volume of Distribution Approximately 0.7 L/kg.

Clearance Apparent plasma clearance after a single dose is ~0.4 mL/min/kg, decreased in uraemia and in the elderly.

Distribution in Blood Lithium is taken up by erythrocytes to a variable extent, which appears to be partly genetically determined. The erythrocyte:plasma ratio was reported to range from 0.19–1.0 (mean 0.5), in 42 subjects [Smith *et al.* 1979]. The plasma : saliva ratio is ~0.4 but there is considerable intersubject variation.

Protein Binding Lithium is not bound to plasma proteins.

Note For a review of the pharmacokinetics of lithium, see Ward *et al.* [1994]; for a discussion on target serum lithium concentrations, see Sproule [2002]; for a study of the effect of acute alcohol consumption on lithium kinetics, see Anton *et al.* [1985].

Dose The dose of lithium depends on the preparation given since different preparations vary widely in bioavailability. Dosage is usually adjusted to provide a serum concentration of 0.4 to 1.0 mmol/L, with maintenance doses at lower end of the range.

Anton RF *et al.* (1985). Effect of acute alcohol consumption on lithium kinetics. *Clin Pharmacol Ther* 38: 52–55.

Astruc B *et al.* (1999). Overdose with sustained-release lithium preparations. *Eur Psychiatry* 14: 172–174.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn. Foster City, CA: Biomedical Publications.

Castrogiovanni P (2002). A novel slow-release formulation of lithium carbonate (Carbolithium Once-A-Day) vs. standard Carbolithium: a comparative pharmacokinetic study. *Clin Ter* 153: 107–115.

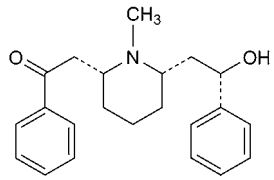
Levy AL, Katz EM (1970). Comparison of serum lithium determinations by flame photometry and atomic absorption spectrophotometry. *Clin Chem* 16: 840–842.

Marini JL, Sheard MH (1976). Sustained-release lithium carbonate in double-blind study: serum lithium levels, side effects, and placebo response. *J Clin Pharmacol* 16: 276–283.
Meinhold JM *et al.* (1979). Bioavailability of lithium carbonate: *in vivo* comparison of two products. *J Clin Pharmacol* 19: 701–703.
Nagappan R *et al.* (2002). Acute lithium intoxication. *Anaesth Intensive Care* 30: 90–92.
Robertson R *et al.* (1973). On the determination of lithium in blood and urine. *Clin ChimActa* 45: 25–31.
Scott IM (1982). The determination of lithium in blood serum by atomic absorption spectrophotometry. *J Forensic Sci Soc* 22: 41–42.
Smith JA *et al.* (1979). The red blood cell lithium/plasma lithium ratio. Significance in recurrent affective illness. *Med J Aust* 1: 631–632.
Sproule B (2002). Lithium in bipolar disorder: can drug concentrations predict therapeutic effect? *Clin Pharmacokinet* 41: 639–660.
Temte JL *et al.* (1994). Intentional overdose by intravaginal insertion of lithium carbonate. *JAMA* 272: 1723–1724.
Ward ME *et al.* (1994). Clinical pharmacokinetics of lithium. *J Clin Pharmacol* 34: 280–285.

Lobeline

Respiratory Stimulant
$C_{22}H_{27}NO_2 = 37.5$
CAS—90-69-7

IUPAC Name 2-[6-(2-Hydroxy-2-phenylethyl)-1-methylpiperidin-2-yl]-1-phenylethanone
Synonyms Alpha-lobeline; [2*R*-[2α,6α(*S*)]]-2-[6-(2-hydroxy-2-phenylethyl)-1-methyl-2-piperidinyl]-1-phenylethanone.
Proprietary Name *Butt-Out*



Chemical Properties An alkaloid obtained from *Lobelia inflata* (Lobeliaceae). Crystals. Mp 130° to 131°. Very slightly soluble in water and petroleum ether; soluble in hot ethanol, benzene, chloroform and ether. Log *P* (octanol/water), 3.7.

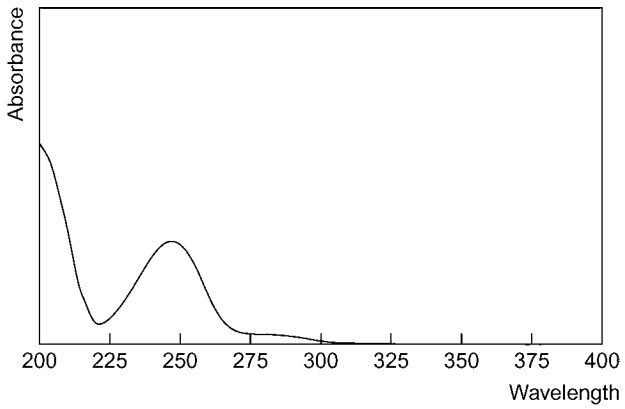
Lobeline Hydrochloride

$C_{22}H_{27}NO_2,HCl = 373.9$
CAS—134-63-4
Proprietary Names *Cig-Ridettes*; *Lobron*; *Zoolobelin*.
Chemical Properties A white crystalline or granular powder. Mp not lower than 180°. Soluble 1 in 40 of water and 1 in 12 of ethanol; very soluble in chloroform; very slightly soluble in ether.

Lobeline Sulfate

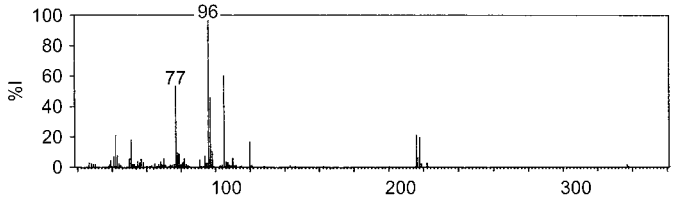
$(C_{22}H_{27}NO_2)_2,H_2SO_4 = 73.0$
CAS—134-64-5
Proprietary Names *Bantron*; *Lobatox*; *Lobeton*; *Lobidan*; *Nikoban*; *Nofum*; *Smokeless*; *Toban*; *Unilobin*.
Chemical Properties Soluble 1 in ~30 of water; slightly soluble in alcohol.

Colour Tests Mandelin's test—grey; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.17; system TC—R_f 0.35; system TE—R_f 0.75 (acidified iodoplatinate solution, positive); system TAE—R_f 0.23; system TAG—R_f 0.29.
Gas Chromatography System GA—RI 1820.
High Performance Liquid Chromatography System HAA—RT 14.6 min.
Ultraviolet Spectrum Aqueous acid—248 nm ($A^1_1 = 416$a).



Infrared Spectrum Principal peaks at wavenumbers 1687, 700, 1211, 1115, 1052, 769 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z*96, 105, 77, 97, 216, 42, 218, .

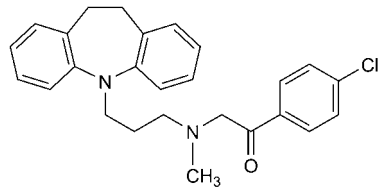


Dose Lobeline hydrochloride was formerly given IM in doses of 3 to 10 mg.

Lofepramine

Tricyclic Antidepressant
$C_{26}H_{27}ClN_2O = 419.0$
CAS—23047-25-8

IUPAC Name 1-(4-Chlorophenyl)-2-[3-(5,6-dihydrobenzo[*b*][1]benzazepin-11-yl)propyl-methylamino]ethanone
Synonyms 1-(4-Chlorophenyl)-2-[[3-(10,11-dihydro-5*H*-dibenz[*b*,*f*]azepin-5-yl)propyl]methylamino]ethanone; lopramine.

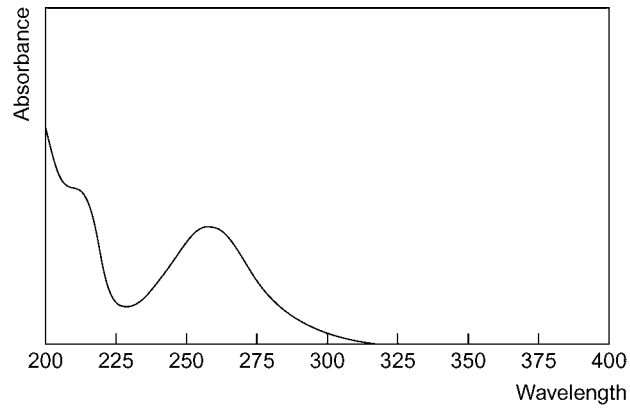


Chemical Properties Colourless crystals, which become discoloured in air. Mp 106°. Practically insoluble in water; slightly soluble in ethanol; soluble in chloroform. Log *P* (octanol/water), 7.3. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

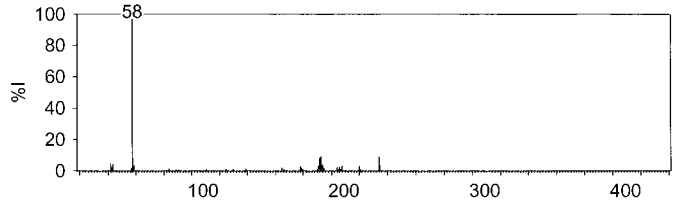
Lofepramine Hydrochloride

$C_{26}H_{27}ClN_2O,HCl = 455.4$
CAS—26786-32-3
Proprietary Names *Deprimil*; *Feprapax*; *Gamanil*; *Gamonil*; *Lomont*; *Timelit*; *Tymelyt*.
Chemical Properties Colourless crystals or yellowish-white microcrystalline powder. Mp 152° to 154°. Practically insoluble in water; soluble in ethanol, methanol and chloroform.

Thin-layer Chromatography System TE—R_f 0.90; system TAE—R_f 0.82.
Gas Chromatography System GA—lofepramine, not eluted (metabolised to desipramine).
High Performance Liquid Chromatography System HA—iofepramine *k* 0.6, desipramine *k* 2.1, 2-hydroxydesipramine *k* 1.2.
Ultraviolet Spectrum Methanol—255 nm ($A^1_1 = 596$a).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1235, 1495, 1590, 1100, 763 cm^{-1} (lofepramine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 234, 193, 192, 42, 235, 194, 59; desipramine235, 195, 208, 44, 234, 193, 194, 71; 2-hydroxydesipramine44, 209, 211, 250, 210, 224, 42, 251.



Quantification

Plasma **GC** Column: 3% Hi-Eff 8BP (5′ × 1/8" i.d. [150 cm × 3 mm i.d.]). Carrier gas: N_2, 3.9×10^5 N/m^2. FID. Limit of detection, 1 µg/mL [Lundgren *et al.* 1977].

GC-MS Column: 2% PEG-20M on Gas Chrom Q 80/100 mesh (2 m × 3 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 220°. Multiple ion detection. Limit of detection, lofepramine and desipramine 2 µg/mL, 2-hydroxydesipramine 20 µg/mL [Matsubayashi *et al.* 1977].

HPLC Column: Supelcosil LC-PCN (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.015 mol/L phosphate buffer (120 : 35 : 100), flow rate 2.5 mL/min. UV detection (λ = 254 nm). Retention time: ~8 min. Limit of quantification, 5 nmol/L, limit of detection, 3 nmol/L [Elm, Hansen 1995].

Urine **GC-MS** See Plasma [Matsubayashi *et al.* 1977].

Disposition in the Body Readily absorbed after oral administration but undergoes extensive first-pass demethylation to desipramine, which is the principal active metabolite. It is also metabolised by *N*-oxidation, hydroxylation, and conjugation and is excreted in the urine mainly as metabolites; a second active metabolite, 2-hydroxydesipramine, has been reported to be the major unconjugated urinary metabolite.

Therapeutic Concentration After a single oral dose of 2 mg/kg given to six subjects, peak plasma lofepramine concentrations of 0.04 to 0.14 mg/L (mean 0.09) were attained in ~1 h; peak plasma desipramine concentrations of 0.01–0.02 mg/L were attained in 3–8 h in 4 subjects. When a similar dose was given to the same subjects on a second occasion some weeks later, peak plasma lofepramine concentrations of 0.05–0.27 mg/L (mean 0.13) were reported. Following oral doses of 70 mg three times a day for 10 days to 3 subjects, plasma concentrations of ~0.003 mg/L of lofepramine and 0.01–0.05 mg/L (mean 0.025) of desipramine were reported, determined immediately prior to a dose [Forshell *et al.* 1976].

Protein Binding In plasma, approximately 99%.

Note For a review of the pharmacokinetics of lofepramine and its efficacy in depression, see Lancaster and Gonzalez [1989].

Dose 70 to 210 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings in the 12th TIAFT*, Seoul: 481–486.

Elm T, Hansen EL (1995). Simultaneous determination of lofepramine and desipramine by a high-performance liquid chromatographic method used for therapeutic drug monitoring. *J Chromatogr B Biomed Appl* 665: 355–361.

Forshell GP *et al.* (1976). Pharmacokinetics of lofepramine in man: relationship to inhibition of noradrenaline uptake. *Eur J Clin Pharmacol* 9: 291–298.

Lancaster SG, Gonzalez JP (1989). Lofepramine A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs* 37: 123–140.

Lundgren R *et al.* (1977). Gas chromatographic determination of lofepramine and desmethylimipramine in plasma. *Acta Pharm Suec* 14: 81–94.

Matsubayashi K *et al.* (1977). Mass fragmentographic determination of lofepramine and its metabolites in human plasma and urine using deuterated internal standards. *J Chromatogr* 143: 571–580.

Lofexidine

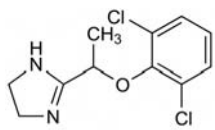
Antihypertensive

$C_{11}H_{12}Cl_2N_2O$ = 259.1

CAS—31036-80-3

IUPAC Name 2-[1-(2,6-Dichlorophenoxy)ethyl]-4,5-dihydro-1*H*-imidazole

Proprietary Names *Almide*; *BritLofex*.



Chemical Properties A colourless, crystalline substance with mp 126° to 128°. Log *P* (water/octanol), 3.58.

Lofexidine Hydrochloride

$C_{11}H_{12}Cl_2N_2O$,HCl = 295.6

CAS—21498-08-8

Synonyms Ba-168; MDL-14042A; RMI-14042A.

Proprietary Names *BritLofex*; *Lofetensin*; *Loxacor*.

Chemical Properties Crystals from ethanol/ether or propan-2-ol, mp 221° to 223°; also reported as 230° to 232°. Very soluble in water and ethanol; slightly soluble in 2-propanol; poorly soluble in chloroform; practically insoluble in ether.

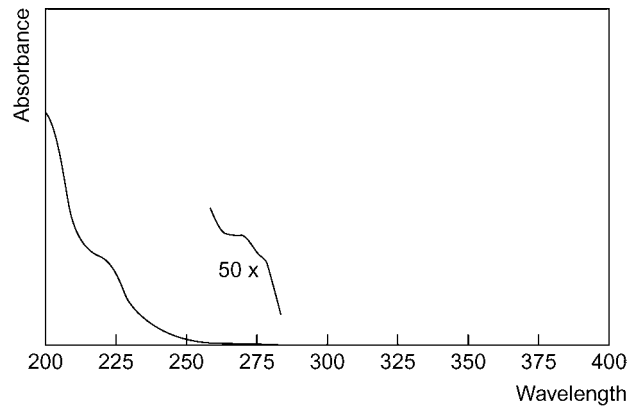
Thin-layer Chromatography System TE—R_f 0.53; system TAE—R_f 0.17.

Plate: silica gel plates 60 F_{254}. Mobile phase: chloroform : methanol : ammonia (25%) (70:26:4). Spray reagent bromocresol green (0.05%). Rf 0.85.

Plate: silica gel plates 60 F_{254}. Mobile phase: butan-1-ol:acetic acid : water (80:20:20). Spray reagent bromocresol green (0.05%). Rf 0.49 [Betzing *et al.* 1982].

High Performance Liquid Chromatography Column: C_{18} µBondapak (300 × 4 mm i.d., 10 µm). Mobile phase: (A:B) methanol : 0.05 mol/L aqueous potassium dihydrogen phosphate (pH 2.5). Elution programme: (A:B) (30:70) to (70:30) in 20 min and back to initial conditions over 5 min, flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: lofexidine, 11.2 min; glucuronide conjugate metabolites, 3.5 and 6.3 min [Midgley *et al.* 1983].

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1610, 1445, 1240, 1095, 780 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 258, 260, 262.

Disposition in the Body Lofexidine is rapidly absorbed after administration, extensively biotransformed and excreted mainly in urine (87%) and faeces (5%). The principal metabolite is 2,6-dichlorophenol, and minor metabolites have been detected including glucuronic acid conjugates. Renal excretion is rapid and is the most important route of elimination. 73 to 94.4% (mean, 85%) of the drug is excreted in urine and 0.4 to 4.2% (mean, 1.5%) in faeces. Peak plasma concentration occurs 2 to 5 h after administration. The rate of bioavailability is rapid and subject dependent.

Toxicity Overdosage may cause hypotension, bradycardia and sedation.

Half-life Lofexidine undergoes biphasic elimination with half-lives 1.3 to 3.7 h and 9.0 to 18.3 h; also reported as 11 h.

Protein Binding Approximately 40% at nominal concentration (2 and 4 µg/L) and 80 to 90% at peak concentration.

Dose An initial oral dose of 200 µg is administered twice daily which may increase in steps of 200 to 400 µg daily to a maximum of 2.4 mg daily for a duration of 7 to 10 days.

Betzing H *et al.* (1982). Chemistry of lofexidine. *Arzneimittelforschung* 32(8a): 916–918.

Midgley I *et al.* (1983). Biotransformation of lofexidine in humans. *Xenobiotica* 13(2): 87–95.

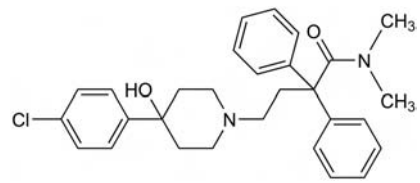
Loperamide

Antidiarrhoeal

$C_{29}H_{33}ClN_2O_2$ = 477.0

CAS—53179-11-6

IUPAC Name 4-(4-Chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutanamide



Chemical Properties Log *P* (octanol/water), 5.2.

Loperamide Hydrochloride

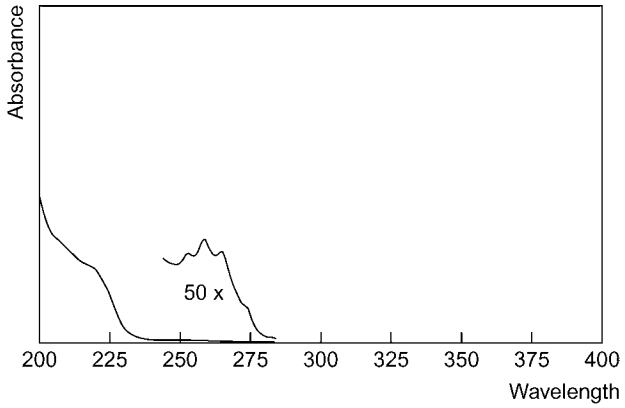
$C_{29}H_{33}ClN_2O_2$,HCl = 513.5

CAS—34552-83-5

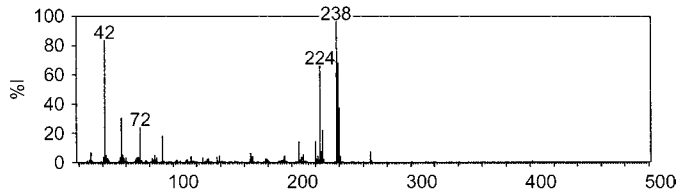
Proprietary Names *Altocel*; *Arestal*; *Arret*; *Blox*; *Celkalm*; *Diar-Limit*; *Diareze*; *Diasorb*; *Diocalm Ultra*; *Diocaps*; *Endiaron*; *Entrocalm*; *Imodium*; *Kaopectate II*; *Lodiar*; *Lopemid*; *LoperaGen*; *Maalox Anti-Diarrheal*; *Neo-Diarel*; *Norimode*; *Normaloe*; *Pepto Diarrhea Control*.

Chemical Properties A white or yellowish-white, amorphous or microcrystalline powder. Mp about 225°, with some decomposition. Slightly soluble in water; freely soluble in chloroform and methanol.

Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.09; system TC—R_f 0.32; system TE—R_f 0.74; system TL—R_f 0.22; system TAE—R_f 0.52; system TAF—R_f 0.81 (Dragendorff spray).
High Performance Liquid Chromatography System HX—RI 510; system HZ—retention time 14.0 min.
Ultraviolet Spectrum Acid isopropyl alcohol—254, 260 (A_1^1=14b), 266 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 700, 830, 765, 964, 986 cm^{-1} (loperamide hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 238, 42, 239, 224, 240, 56, 72, 226.



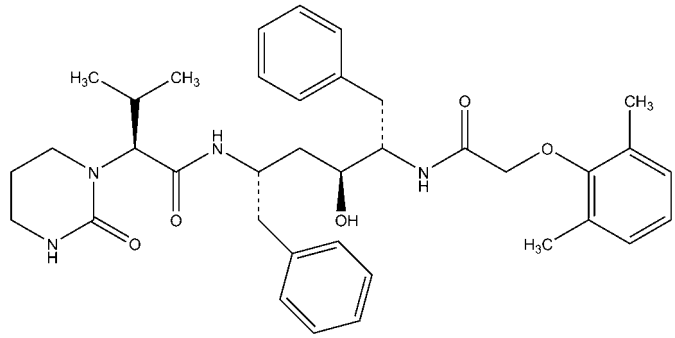
Quantification
Plasma **HPLC–MS** Selected-ion monitoring. Limit of detection, <0.12 µg/L for loperamide and *N*-demethylloperamide [He *et al.* 2000].
Serum **Radioimmunoassay** For method, see Weintraub *et al.* [1977].
Urine **Radioimmunoassay** For method, see Weintraub *et al.* [1977].
Disposition in the Body Loperamide is poorly absorbed after oral administration. About 1 to 2% of a dose is excreted in the urine as free or conjugated loperamide in 48 h. Up to 10% of a dose may be excreted in the urine in 8 days and about 40% is eliminated in the faeces over the same period.
Therapeutic Concentration
Following oral administration of capsules containing 8 mg of loperamide hydrochloride to 6 subjects, peak serum concentrations of about 0.002 mg/L were attained in about 4 to 5 h [Killinger *et al.* 1979].
Twenty–four subjects in a cross-over study received a single 16 mg dose of loperamide, either as tablets or in capsules, on two days. A mean peak plasma concentration of 3.35 µg/L occurred at 4.08 h after the tablets and 3.98 µg/L at 4.38 h after the capsules [Doser *et al.* 1995].
Half-life Plasma half-life, 7 to 15 (mean 11) h.
Protein Binding About 97%.
Note For a review of the pharmacokinetics of loperamide, see Heel *et al.* [1978].
Dose Up to 16 mg of loperamide hydrochloride daily.

Doser K *et al.* (1995). Bioequivalence evaluation of two different oral formulations of loperamide (Diarex Lactab vs Imodium capsules). *Int J Clin Pharmacol Ther* 33: 431–436.
He H *et al.* (2000). Quantitation of loperamide and N-demethyl-loperamide in human plasma using electrospray ionization with selected reaction ion monitoring liquid chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 744: 323–331.
Heel RC *et al.* (1978). Loperamide: a review of its pharmacological properties and therapeutic efficacy in diarrhoea. *Drugs* 15: 33–52.
Killinger JM *et al.* (1979). Human pharmacokinetics and comparative bioavailability of loperamide hydrochloride. *J Clin Pharmacol* 19: 211–218.
Weintraub HS *et al.* (1977). *Curr Ther Res* 21: 867–876.

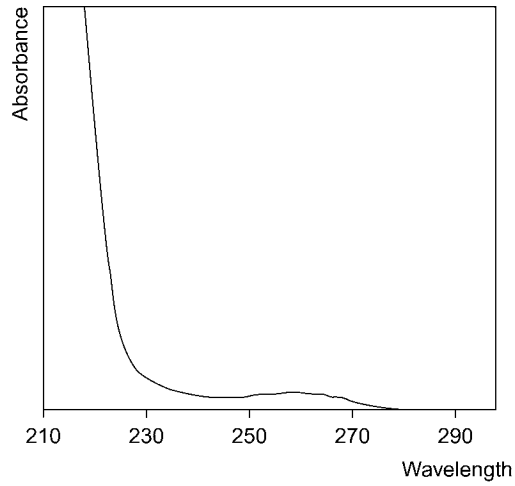
Lopinavir

Antiretroviral, Protease Inhibitor
$C_{37}H_{48}N_4O_5$ = 628.8
CAS—192725-17-0

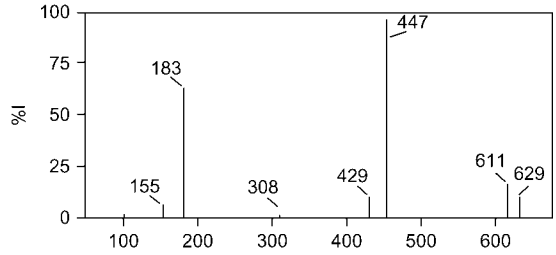
IUPAC Name (2*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[[2-(2,6-Dimethylphenoxy)acetyl]amino]-4-hydroxy-1,6-di(phenyl)hexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide
Synonyms A-157378; ABT-378; (α*S*)-*N*-[1*S*,3*S*,4*S*)-4-[[(2,6-dimethylphenoxy) acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide; [1*S*-[1*R*,(*R*),3*R*,4*R*]]-*N*-[4-[[(2,6-dimethyl phenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyramidine acetamide; (2*S*,3*S*,5*S*)-2-(2,6-dimethylphenoxyacetyl)amino-3-hydroxy-5-[2*S*-(1-tetrahydro-pyrimid-2-only)-3-methylbutanoyl]amino-1,6-diphenylhexane; (α*S*)-tetrahydro-*N*-[(α*S*)-α-[(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xylyloxy)acetamido]butyl]phenethyl]-α-isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide.
Proprietary Names *Aluvia*; *Kaletra* (co-formulation with ritonavir).



Chemical Properties White to light tan solid. Mp 124° to 127°. Freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water. Stability was established in plasma after 3 freeze–thaw cycles [Gehrig *et al.* 2007]. Sample stability was verified in plasma at room temperature for 24 h, after 3 freeze–thaw cycles and at 4° for 7 days [Rezk *et al.* 2006]. Lopinavir was stable in human plasma for at least 184 days at –20° [Wang *et al.* 2006]. Stability of standard solutions was established up to 72 h and there was no significant loss of drug after 3 freeze–thaw cycles [Colombo *et al.* 2005].
Ultraviolet Spectrum 260 nm Rebiere *et al.* [2007].



Mass Spectrum Principal ions at *m/z* 447, 183, 611, 429, 629, 155 and 308 .



Quantification
Blood **LC-MS** Column: SymmetryShield RP_{18} (30 × 2.1 mm i.d., 3.5 µm). Mobile phase: 2 mmol/L ammonium acetate containing 0.1% formic acid : acetonitrile containing 0.1% formic acid (98 : 2 to 75 : 25 at 2 min to 0 : 100 at 10 min), flow rate 0.3 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, <0.5 µg/L [Colombo *et al.* 2005]. Column: Supelcosil ABZ (150 × 4.6 mm i.d., 3 µm). Mobile phase: monobasic potassium phosphate : methanol : acetonitrile with 0.02 mol/L heptane sulfonic acid (44.5 : 32.5 : 23), flow rate 1 mL/min. UV detection (λ = 240 nm). Limit of quantification, 42 µg/L [Antoniou *et al.* 2005]. Column: X-Terra MS C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.4% formic acid (pH 3; 50 : 50), flow rate 1.0 mL/min. APCI, positive ion mode, SIM acquisition mode. Limit of quantification, 2.0 ng/3 × 10^6 mononuclear cells; limit of detection, 0.49 ng/3 × 10^6 mononuclear cells [Rouzes *et al.* 2004].
Plasma **HPLC** Column: Allsphere hexyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 15 mmol/L sodium dihydrogen phosphate buffer (pH 4.5,

35 : 20 : 45), flow rate 1.0 mL/min. UV detection ($\lambda = 215$ nm). Fluorescence detection ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 340$ nm). Limit of quantification, 25 μg/L [Verbesselt *et al.* 2007]. Column: reversed phase S-3 (150 × 3.0 mm i.d., 0.5 μm). Mobile phase: 25 mmol/L monobasic potassium (pH 4.9) : acetonitrile (52 : 48), flow rate 0.4mL/min. UV detection ($\lambda = 212$ nm). Limit of detection, 50 μg/L [Weller *et al.* 2007]. Column: YMC C_8 reversed phase (100 × 4.6 mm i.d., 3 μm). Mobile phase: 20 mmol/L sodium acetate : acetonitrile (55 : 45). UV detection ($\lambda = 212$ nm). Limit of detection, 25 μg/L [Ofotokun *et al.* 2007]. Column: Symmetry C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate : acetonitrile (94 : 6 to 0 : 100 in 35 min), flow rate 1.0 mL/min. UV detection ($\lambda = 240$ and 260 nm). Limit of detection not reported [Notari *et al.* 2006]. Column: Develosil Ph-UG-3 (150 × 2.0 mm i.d., 3 μm). Mobile phase: acetonitrile–25 mmol/L sodium dihydrogen phosphate containing 6 mmol/L sodium 1-hexanesulfonate (pH 5.1, 34 : 66) : acetonitrile–25 mmol/L sodium dihydrogen phosphate containing 6 mmol/L sodium 1-hexanesulfonate (pH 5.3; 64 : 36; 100 : 0 for 14.6 min to 70 : 30 at 16.6 min until 35.5 min), flow rate 200 μL/min. UV detection ($\lambda = 212$ nm). Limit of quantification, 50 μg/L [Hirabayashi *et al.* 2006]. See also Rezk *et al.* [2006], Takahashi *et al.* [2005].

LC-MS Column: Nucleosil C_{18}-100 Nautilus (125 × 3.0 mm, 3 μm). Mobile phase: 20 mmol/L ammonium acetate containing 1% aqueous acetic acid : acetonitrile (95 : 5 for 0.5 min to 5 : 95 at 5 min for 10 min), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 10 μg/L [Gehrig *et al.* 2007]. Column: Waters Symmetry C_{18} (150 × 3.9 mm i.d., 5 μm). Mobile phase: acetonitrile : 2mmol/L ammonium acetate containing 0.01% formic acid (70 : 30), flow rate 1.0 mL/min. APCI, MRM acquisition mode. Limit of quantification, 19 μg/L [Wang *et al.* 2006]. Column: Vydac C_{18} (250 × 1 mm i.d., 3.0 μm). Mobile phase: acetonitrile with 0.2% formic acid : water with 0.2% formic acid (5 : 95 to 100 : 0 in 16 min), flow rate 70 μL/min. MALDI-TOF, positive ion mode. Limit of detection, 1.57 μg/L [Notari *et al.* 2006].

***CSF* LC-MS** Column: Nucleosil C_{18}-100 Nautilus. Mobile phase: 20 mmol/L ammonium acetate containing 1% aqueous acetic acid : acetonitrile. ESI, positive ion mode. Limit of detection, 10 μg/L [Gehrig *et al.* 2007].

***Liver* HPLC** Column: Beckman Ultrasphere C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 25 mmol/L ammonium acetate (pH 4.8; 25 : 75 to 55 : 45 at 57 min), flow rate 1 mL/min. DAD ($\lambda = 220$ nm). Limit of detection, not reported [Kumar *et al.* 1999].

LC-MS Column: Beckman Ultrasphere C_{18} (250 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 4.8; 30 : 70 to 80 : 20 at 15 min for 5 min), flow rate 200 μL/min. ESI at 90 eV, positive ion mode. Limit of detection, not reported [Kumar *et al.* 1999].

***Other* HPLC** Pharmaceutical Products. Column: Symmetry C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 50 mmol/L potassium phosphate buffer (pH 5.65) : acetonitrile (60 : 40 for 5 min to 40 : 60 in 35 min for 5 min to 60 : 40 in 1 min), flow rate 1.5 mL/min. DAD ($\lambda = 260$ nm). Limit of detection, 10 mg/L [Rebiere *et al.* 2007].

Disposition in the Body Lopinavir is metabolised by the hepatic enzymes CYP3A4 and CYP3A5 to 12 metabolites in liver microsomes, of which M1, M3 and M4 are predominant. M3 and M4 are the epimeric C-4 hydroxy products of oxidation in the cyclic urea moiety and M1 is the C-4 oxo product.

Therapeutic Concentration

A group of 9 men and 11 women were administered lopinavir/ritonavir 400/100 mg twice daily for 15 days followed by 800/200 mg once daily for the next 15 days. There were no sex differences in the pharmacokinetic profiles. The mean maximum plasma concentrations for men and women were 12.4 and 12.3 mg/L, respectively, attained at 4.00 and 4.03 h, respectively [Ofotokun *et al.* 2007].

Pharmacokinetics of lopinavir/ritonavir in patients infected with HIV and hepatitis C has been investigated; all pharmacokinetic parameters were comparable apart from the volume of distribution of lopinavir, which was 125% higher in hepatitis C-positive patients with hepatic fibrosis [Molto *et al.* 2007].

A study including 542 HIV-infected patients showed that the minimum plasma concentrations of ritonavir were significantly lower when it was given in combination with lopinavir [Guiard-Schmid *et al.* 2003].

Five severely immunocompromised HIV-infected patients were treated with lopinavir or amprenavir and nevirapine. Lopinavir concentrations varied widely, with a mean peak plasma concentrations ranging from 2.71 to 12.6 mg/L, attained at 1.9 to 12.8 h [Fatkenheuer *et al.* 2001].

Note For a study of the weight, age and sex differences in the pharmacokinetics of lopinavir in children from birth to 18 years old, see Jullien *et al.* [2006]; for a study of the effect of pregnancy on lopinavir pharmacokinetics, see Stek *et al.* [2006].

Volume of Distribution 24.6 L in children; 44.0 L in hepatitis C virus-negative adults.

Clearance Men, 4.37 L/h, women 4.76 L/h, children 2.58 L/h.

Protein Binding More than 98% bound, mainly to α_1-acid-glycoprotein and albumin.

Dose 400 mg (with ritonavir 100 mg) twice daily with food.

Antoniou T *et al.* (2005). Steady-state pharmacokinetics and tolerability of indinavir–lopinavir/r combination therapy in antiretroviral-experienced patients. *Ther Drug Monit* 27: 779–781.

Colombo S *et al.* (2005). Intracellular measurements of anti-HIV drugs indinavir, amprenavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, efavirenz and nevirapine in peripheral blood mononuclear cells by liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 259–276.

Fatkenheuer G *et al.* (2001). Pharmacokinetics of amprenavir and lopinavir in combination with nevirapine in highly pretreated HIV-infected patients. *AIDS* 15: 2334–2335.

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Hirabayashi Y *et al.* (2006). Simultaneous determination of six HIV protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir), the active metabolite of nelfinavir (M8) and non-nucleoside reverse transcriptase inhibitor (efavirenz) in human plasma by high-performance liquid chromatography. *Biomed Chromatogr* 20: 28–36.

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Takahashi M *et al.* (2005). Conventional HPLC method used for simultaneous determination of the seven HIV protease inhibitors and nonnucleoside reverse transcription inhibitor efavirenz in human plasma. *Biol Pharm Bull* 28: 1286–1290.

Verbesselt R *et al.* (2007). Simultaneous determination of 8 HIV protease inhibitors in human plasma by isocratic high-performance liquid chromatography with combined use of UV and fluorescence detection: amprenavir, indinavir, atazanavir, ritonavir, lopinavir, saquinavir, nelfinavir and M8-nelfinavir metabolite. *J Chromatogr B Analyt Technol Biomed Life Sci* 845: 51–60.

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Weller DR *et al.* (2007). An isocratic liquid chromatography method for determining HIV non-nucleoside reverse transcriptase inhibitor and protease inhibitor concentrations in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 848: 369–373.

Loprazolam

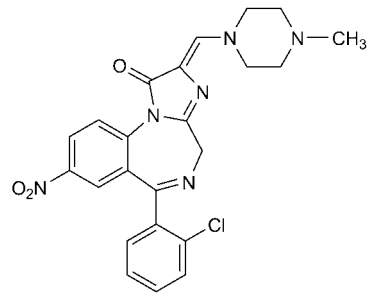
Benzodiazepine, Hypnotic, Sedative

$C_{23}H_{21}ClN_6O_3 = 64.9$

CAS—61197-73-7

IUPAC Name (2*Z*)-6-(2-Chlorophenyl)-2-[(4-methylpiperazin-1-yl)methylidene]-8-nitro-4*H*-imidazo[1,2*a*][1,4]benzodiazepin-1-one

Synonym (*Z*)-6-(2-Chlorophenyl)-2,4-dihydro-2-[(4-methyl-1-piperazinyl)methylene]-8-nitro-1*H*-imidazo[1,2-*a*][1,4]benzodiazepin-1-one



Chemical Properties Crystals. Mp 214° to 215°. pK_a 6.0 (24°). Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Limited stability at room temperature in buffer, urine or bile; consequently samples are stored at –2 0°. [^{14}C]-Loprazolam degrades rapidly (95% within 1 h) at room temperature in 0.1 mol/L HCl or NaOH but is relatively stable between pH 6 and 9 [Illing *et al.* 1983].

Loprazolam Mesilate

$C_{23}H_{21}ClN_6O_3,CH_4SO_3,H_2O = 579.0$

CAS—70111-54-5 (anhydrous)

Proprietary Names *Dormonoct*; *Havlane*; *Somnovit*; *Sonin*.

Chemical Properties A yellow powder. Mp 242° to 245°, with decomposition. Soluble 1 in ~100 water, 1 in ~200 ethanol and 1 in ~500 chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.1; system TC—R_f 0.48; system TD—R_f 0.03; system TE—R_f 0.40; system TF—R_f 0.01; system TAD—R_f 0.36; system TAE—R_f 0.26; system TAF—R_f 0.15; system TAG—R_f 0.05.

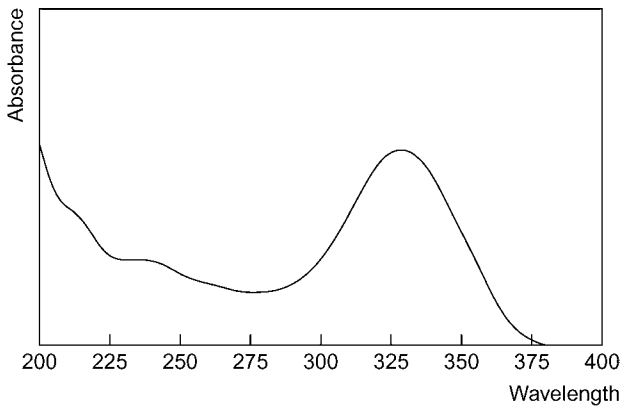
Gas Chromatography System GA—not eluted; system GB—not eluted.

High Performance Liquid Chromatography System HAA—RT 13.4 min; system HX—RI 388.

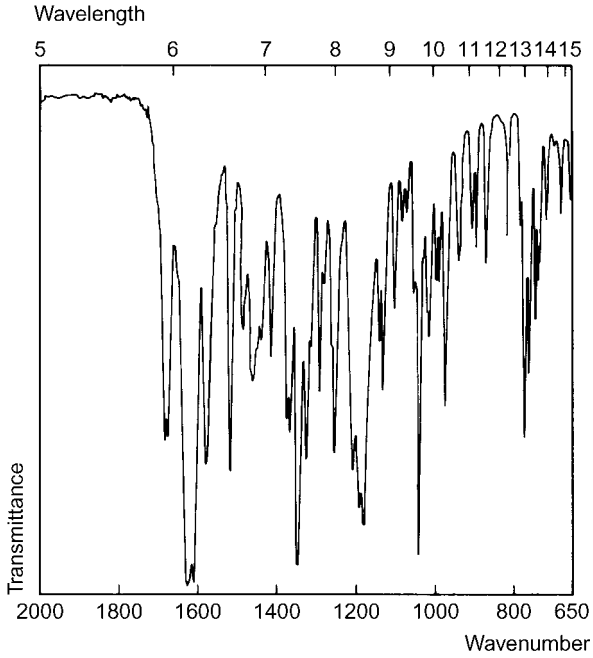
	0.5 mg dose		*1.0 mg dose*	
	Young (n = 10)	*Elderly (n = 9)*	*Young (n = 10)*	*Elderly (n = 10)*
C_{max} (μg/L [range])				
Loprazolam	2.27 (1.58–3.28)	2.37 (1.31–3.8)	5.28 (3.44–7.61)	5.15 (3.6–8.1)
N-oxide	1.26 (0.85–2.07)	1.32 (0.69–1.98)	2.59 (1.81–3.57)	2.73 (1.28–4.24)
t_{max} (h [range])				
Loprazolam	2.75 (1.5–6.0)	2.67 (1.0–8.0)	1.56 (1.0–2.0)	2.19 (0.5–4.0)
N-oxide	4.5 (3.0–6.0)	6.44 (4.0–12.0)	3.55 (1.5–6.0)	5.13 (1.0–8.0)

[Ford *et al.* 1987].

Ultraviolet Spectrum Ethanol—330 nm ($A_1^1 = 884$b).



Infrared Spectrum Principal peaks at wavenumbers 1628, 1610, 1045, 1182, 1192, 1518 cm^{-1} (loprazolam mesilate, KBr disk).



Quantification

Plasma **GC** Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh. Carrier gas: Ar : CH_4 (95 : 5), 60 mL/min. Temperature programme: 260°. ECD. Retention time: 9.0 min. Limit of detection, 1 μg/L [Levasseur *et al.* 1985].

Urine **LC-MS** Column: 5% OV-101 Chromosorb G HP 100/120 mesh (600 × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Disposition in the Body A single oral dose of loprazolam is eliminated in urine (39%) and faeces (53%) over a 2 week period. The drug is extensively biotransformed. Urinary elimination products include unchanged drug (<1%), loprazolam *N*-oxide (17%), acetamidoloprazolam (4%), hydroxyloprazolam (3%), and unidentified polar metabolites (25%). Loprazolam *N*-oxide is thought to be pharmacologically active [Baselt 2008; Illing *et al.* 1983].

Therapeutic Concentration

The pharmacokinetics of loprazolam and its *N*-oxide were investigated in young subjects (aged 21 to 25 years) and elderly patients (aged 63 to 86 years) following a single oral evening dose of 0.5 or 1 mg loprazolam. The pharmacokinetic parameters were measured: As per the table above.

Nine healthy male volunteers (aged 22 to 38 years) were administered a single oral dose of 2 mg loprazolam. A mean maximum plasma concentration of 6.10 ± 0.6 μg/L was reached between 1.5 and 2 h [Levasseur *et al.* 1985].

Following single and repeated doses of loprazolam 1 mg to 6 healthy subjects, mean peak plasma concentrations were 4.1 ± 2.19 and 4.6 ± 2.07 μg/L, respectively, reached at 5.0 ± 3.63 and 5.5 ± 2.66 h, respectively [McInnes *et al.* 1985].

Following a single oral dose of 2 mg to 8 subjects, a mean peak serum concentration of 0.0097 mg/L was attained in 2.4 h. Following repeated oral doses of 2 mg daily for 8 days, a mean peak serum concentration of 0.012 mg/L was reported 2.2 h after the last dose [Stevens *et al.* 1983].

Toxicity Adverse effects include drowsiness, lethargy, confusion and ataxia [Baselt 2008].

Volume of Distribution 3.2–6.3 L/kg [Baselt 2008].

Protein Binding Approximately 80% [Illing *et al.* 1983].

Half-life Plasma half-life, 6–23 h [Baselt 2008].

Note For a review of the pharmacokinetics of the newer benzodiazepines, see Garzone and Kroboth [1989].

Dose The equivalent of 1 to 2 mg loprazolam, as a hypnotic.

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Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

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Levasseur FA *et al.* (1985). Electron-capture gas chromatographic determination of loprazolam in plasma and a pharmacokinetic application. *Biopharm Drug Dispos* 6: 381–387.

Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography–mass spectrometry. *J Chromatogr* 422: 85–101.

McInnes GT *et al.* (1985). Pharmacokinetics and pharmacodynamics following single and repeated nightly administrations of loprazolam, a new benzodiazepine hypnotic. *Br J Clin Pharmacol* 19: 649–656.

Stevens LA *et al.* (1983). Single and repeated dose kinetics of the hypnotic agent loprazolam in healthy volunteers. *Eur J Clin Pharmacol* 25: 651–655.

Loratadine

Antihistamine (Non-Sedating)

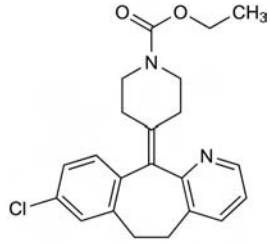
$C_{22}H_{23}ClN_2O_2 = 382.9$

CAS—79794-75-5

IUPAC Name 4-(8-Chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)-1-piperidinecarboxylic acid ethyl ester

Synonym SCH-29851

Proprietary Names *Allerfre*; *Civeran*; *Claritin*; *Clarityn*; *Fristamin*; *Lisino*; *Lorastyne*; *Optimin*; *Polaratyne*; *Sanelor*; *Velodan*; *Versal*; *Viatin*. It is also an ingredient of *Loractin*.



Chemical Properties Crystals from acetonitrile. Mp 134° to 136°. Soluble in alcohol (1 in 10); insoluble in water. pK_a 5.0. Log *P* (octanol/water), 5.20.

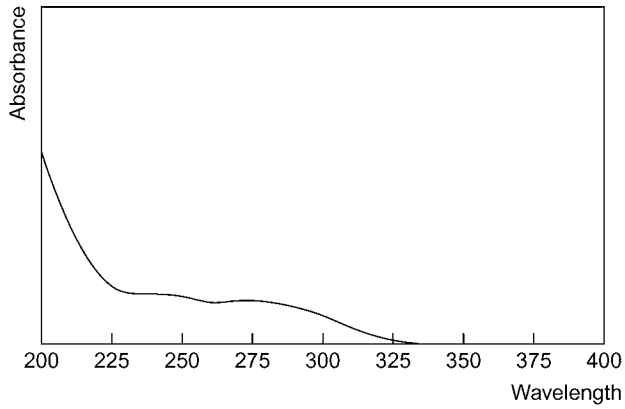
Thin-layer Chromatography System TB—R_f 0.20; system TE—R_f 0.78; system TF—R_f 0.29; system TAE—R_f 0.86.

Gas Chromatography System GB—RI 3236.

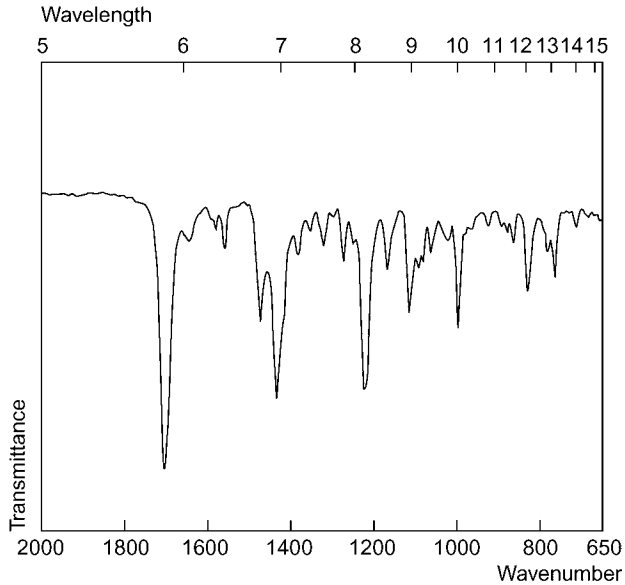
High Performance Liquid Chromatography System HX—RI 523; system HY—RI 362; system HZ—retention time 14.6 min; system HAA—retention time 22.9 min; system HAX—retention time 10.9 min; system HAY—retention time 13.3 min.

Column: LC-18-DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 0.5 M potassium dihydrogen phosphate : orthophosphoric acid (pH 3.0, 440:480:80:1), flow rate 1.8 mL/min. IS: diazepam. UV detection (λ=200 nm). Retention time: loratadine, 4.1 min; IS, 4.7 min [Kunicki 2001].

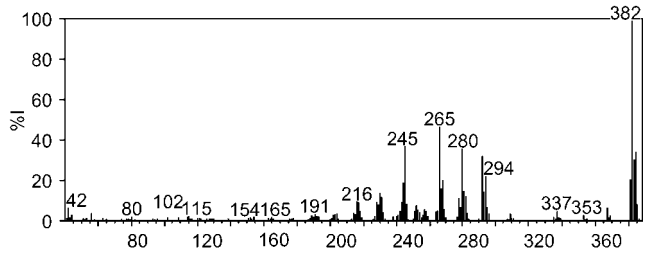
Ultraviolet Spectrum Aqueous acid—280 nm; aqueous base—252 nm.



Infrared Spectrum Principal peaks at wavenumbers 1709, 1435, 1226, 997 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 382, 266, 245, 280, 292, 384, 383, 294.



Quantification

Plasma **GC** NPD (nitrogen–phosphorus detection). Limit of quantification, 0.1 µg/L for loratadine and its metabolite [Johnson *et al.* 1994].

HPLC MS detection (ESI, MRM (multiple reaction monitoring) mode). Limit of detection, 0.1 µg/L for loratadine and metabolite [Sutherland *et al.* 2001]. Limit of quantification, 0.6 µg/L [Radwanski *et al.* 1987].

Serum **GC–MS** Column: HP-Ultra 1 capillary (25 m × 0.2 mm i.d.). Temperature programme: held at 100° for 2 min, increased to 310° at 15°/min, held for 2.5 min. Carrier gas: He, flow rate 1 mL/min. Internal standard (IS): etoloxamine. Detection: mass spectrometer (EI, SIM mode, *m/z*: 382.1 for loratadine; *m/z*: 86.2, 283.2 for IS). Retention time: loratadine, 17.2 min; IS, 11.9 min. Limit of quantification, 1.5 µg/L, limit of detection, 0.5 µg/L [Martens 1995].

Disposition in the Body Loratadine is rapidly absorbed after oral administration and undergoes extensive metabolism. Peak plasma concentrations are observed 1 to 1.5 h after administration. Steady-state concentrations are achieved after 5 days. The major metabolite desloratadine (descarboethoxyloratadine) is rapidly formed and has antihistamine activity. It undergoes further oxidation and hydroxylation to several products. Excretion, in the form of metabolites, is via urine and faeces. Twenty-seven percent of a dose is excreted in urine within 24 h and 40% is excreted in urine and 42% in faeces over 10 days. Loratadine and its metabolites are also distributed in breast milk but do not appear to cross the blood–brain barrier to a significant extent.

Therapeutic Concentration The serum therapeutic concentration is 15 to 27 µg/L and descarboethoxyloratadine, 7 to 28 µg/L.

Eighteen men and women, aged between 29 and 60 years (mean, 22-years-old), selected with a creatinine clearance >80 mL/min (group 1), ≤30 mL/min (group 2) and with end-stage renal disease (group 3) (6 volunteers in each group) were administered a single 40 mg dose in the fasted state. Mean peak plasma concentrations of 24.3 (range, 6.2 to 44.7) µg/L, 35.8 (10.7 to 61.9) µg/L and 42.2 (12.6 to 81.7) µg/L were reached for the three groups, respectively, between 1 and 2 h. Mean peak plasma concentrations of the metabolite desloratidine were 14.8 (11.3 to 19.0), 32.2 (19.7 to 56.6) and 26.3 (18.6 to 43.4) µg/L for groups 1, 2 and 3, respectively. These levels were observed between 1 and 10 h with a mean of 2.6 h for each group [Matzke *et al.* 1990].

Half-life Loratadine, 8 to 11 h; desloratadine, 17 to 28 h (37 h for patients with chronic alcohol liver disease).

Volume of Distribution 119 L/kg (range, 32 to 261) for patients with creatinine clearance >80 mL/min; 50 (27 to 64) L/kg for patients with ≤30 mL/min creatinine clearance; 92 L/kg (range 36 to 190) for patients with end stage renal disease.

Clearance 239 mL/kg/min (range 88 to 585) for patients with creatinine clearance >80 mL/min; 109 (36 to 164) mL/kg/min for patients with ≤30 mL/min creatinine clearance; 137 mL/kg/min (range, 48 to 339) for patients with end-stage renal disease.

Protein Binding Loratadine, 97 to 99%; desloratadine, 73 to 76%.

Dose 10 mg daily. Children (2 to 5 years): 5 mg daily.

Sutherland F *et al.* (2001). Sensitive liquid chromatography-tandem mass spectrometry method for the determination of loratadine and its major active metabolite descarboethoxyloratadine in human plasma. *J Chromatogr A* 914: 37–43.

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Radwanski E *et al.* (1987). Loratadine: multiple-dose pharmacokinetics. *J Clin Pharmacol* 27: 530–533.

Lorazepam

Tranquilliser

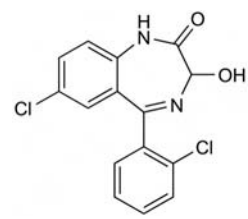
$C_{15}H_{10}Cl_2N_2O_2$ = 321.2

CAS—846-49-1

IUPAC Name 7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one

Synonyms 7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one; lorazepamum; Wy-4036.

Proprietary Names *Almazine*; *Ansilor*; *Ativan*; *Control*; *Emotival*; *Lorans*; *Lorax*; *Loraz*; *Lorenin*; *Lorsedal*; *Lorsilan*; *Lorzem*; *Merlit*; *ProDorm*; *Psicopax*; *Punktyl*; *Quait*; *Sedatival*; *Sedazin*; *Serenase*; *Somagerol*; *Tavor*; *Temesta*; *Tolid*; *Wypax*.



Chemical Properties A white powder. Mp 166° to 168°. Solubility: water 0.08 mg/mL, chloroform 3 mg/mL, alcohol 14 mg/mL, propylene glycol 16 mg/mL, ethyl acetate 30 mg/mL. pK_a 1.3, 11.5 (20°). Log *P* (octanol/pH 7.4), 2.4. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

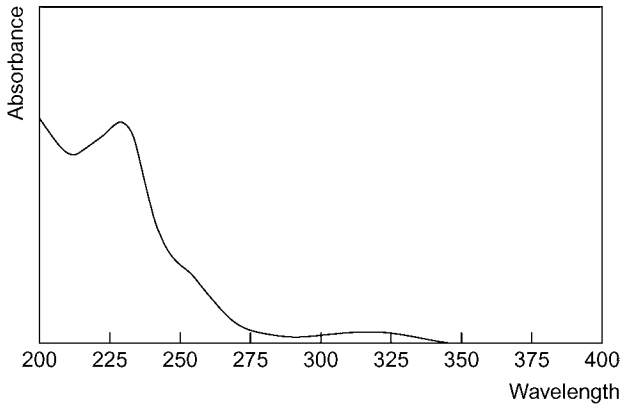
Colour Tests Formaldehyde-sulfuric acid—orange; Marquis test—yellow; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.01; system TC—R_f 0.36; system TD—R_f 0.23; system TE—R_f 0.43; system TF—R_f 0.41; system TL—R_f 0.28; system TAD—R_f 0.42; system TAE—R_f 0.82; system TAF—R_f 0.82; system TAJ—R_f 0.46; system TAK—R_f 0.42; system TAL—R_f 0.86 (acidified iodoplatinate solution—positive; acidified potassium permanganate—positive).

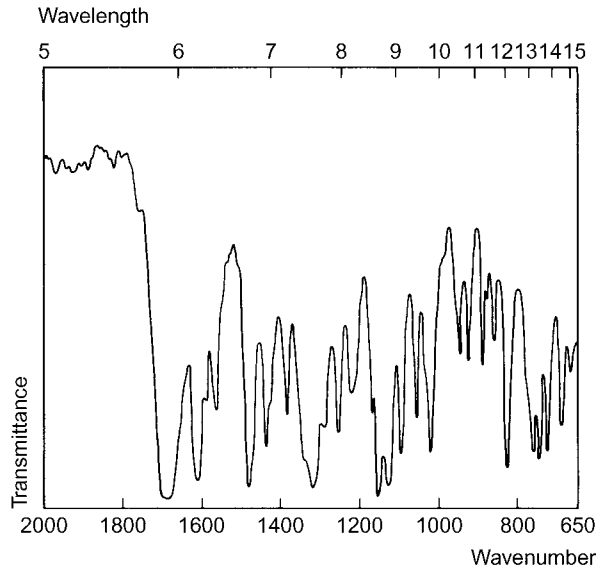
Gas Chromatography System GA—RI 2410; system GB—lorazepam RI 2528, lorazepam-TMS_2 RI 2566; system GG—RI 2910.

High Performance Liquid Chromatography System HA—*k* 0.1; system HI—*k* 4.60; system HK—*k* 0.14; system HX—RI 444; system HY—RI 400; system HZ—RT 4.1 min; system HAA—RT 17.2 min; system HAF—RT 15.0 min; system HAL—RT 5.1 min; system HAM—RT 4.2 min; system HAX—RT 6.1 min; system HAY—RT 5.8 min; system HBH—*k* 5.16; system HBI—*k* 1.14.

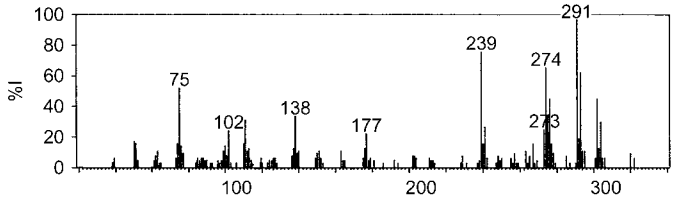
Ultraviolet Spectrum Ethanol—230 (A_1^1= 1100b), 316 nm.



Infrared Spectrum Principal peaks at wavenumbers 1685, 1149, 1317, 1120, 1605, 826 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 291, 239, 274, 293, 75, 302, 276, 138.



Quantification

Blood **GC** Column: fused silica SE-54. ECD and NPD. Lorazepam and other benzodiazepines [Lillsunde, Seppälä 1990].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 295° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 21.4 min. Limit of quantification, 11.8 µg/L, limit of detection, 3.89 µg/L [Papoutsis *et al.* 2010]. Column: 100% methylsiloxane or 5% phenylsiloxane-95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 25°/min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 50 µg/L, limit of detection, 5 µg/L [Tiscione *et al.* 2008].

HPLC Column: Chromolith Performance P-18e (100 × 4.0 mm). Mobile phase: 35 mmol/L phosphate buffer (pH 2.1) : acetonitrile (70 : 30), flow rate 2 mL/min. Lorazepam and other benzodiazepines [Bugey, Staub 2004].

LC-MS Column: Acquity BEH Phenyl (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (80 : 20 for 0.25 min to 65 : 35 over 2.25 min for 2.5 min to 20 : 80 over 1 min to 80 : 20 over 0.01 min for 1.4 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode [Gunn *et al.* 2010]. Lorazepam and related drugs [Deveaux *et al.* 2008]. Limit of quantification, 2–3 µg/L for lorazepam and some other benzodiazepines, 5 µg/L for lormetazepam and 6 µg/L for bromazepam [Dussy *et al.* 2006].

Plasma **GC** Column: C_8 reversed-phase sorbent. NP and ECD. Lorazepam and related drugs [Gaillard *et al.* 1993]. Column: 3% OV-17 on 80/100 mesh Chromosorb W HP (1.8 m (6 ft) × 4 mm i.d.). Carrier gas: He, 50 mL/min. Temperature: 280°. ECD. Limit of detection, 1–3 µg/L for lorazepam and lorazepam glucuronide [Greenblatt *et al.* 1978]. See Blood [Lillsunde, Seppälä 1990].

GC-MS Column: Restek Rtx-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 295° at 30°/min for 5 min. CID. Limit of quantification, 0.1 µg/L [Pichini *et al.* 1999]. Column: 3% OV-1 on Chromosorb W AW DMCS 80/100 mesh (1 m × 3 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 210°. Limit of detection, 2 µg/L for lorazepam and lorazepam glucuronide [Higuchi *et al.* 1979].

HPLC Column: LC-18DB (250 × 4.6 mm, 5 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate buffer (pH 6.0) : methanol : diethyl ether (55 : 40 : 5), flow rate 0.8 mL/min. UV detection (245 nm). Limit of quantification, 30 to 50 µg/L for lorazepam and other benzodiazepines [Borges *et al.* 2009]. Column: Kromasil C(8) (250 × 5 mm, 5 µm). Mobile phase: methanol, acetonitrile and 0.05 mol/L ammonium acetate. Limit of quantification, 0.07–1.57 mg/L for lorazepam and other benzodiazepines; limit of detection, 0.02–0.47 mg/L for lorazepam and other benzodiazepines [Uddin *et al.* 2008]. Column: Synergi Max RP (150 × 4.6 mm, 4 µm). Mobile phase: 10 mM potassium dihydrogen phosphate buffer (pH 2.4) : acetonitrile (65 : 35), flow rate 2.5 mL/min. UV detection. Limit of quantification, 10 µg/L, limit of detection, 2.5 µg/L [Muchohi *et al.* 2005]. Column: Hisep. Mobile phase: acetonitrile: 0.18 mol/L ammonium acetate (pH 2.5). Limit of quantification, 0.5 mg/L for lorazepam, clobazam, nitrazepam and oxazepam, limit of detection, 0.16 mg/L for lorazepam, clobazam, nitrazepam and oxazepam [Pistos, Stewart 2003]. Column: octadecyl or select B. Limits of detection, 5–10 µg/L for lorazepam glucuronide and other benzodiazepine glucuronides [Franzelius, Besserer 1993]. See also Kondo *et al.* [1993].

LC-MS Column: Xterra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Reteention time: 5.03 min. Limit of quantification, 20 µg/L, limit of detection, 10 µg/L [Marin, McMillin 2010; Marin *et al.* 2008]. Column: Chiralpak OD-R (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water : acetic acid (80 : 20 : 0.1), flow rate 1.0 mL/min. ESI. Limit of quantification, 1 µg/L [Papini *et al.* 2006a, 2006b]. Column: Zorbax C_{18}(100 × 2.1 mm). Mobile phase: acetonitrile : 10 mmol/L aqueous formic acid (65 : 35). SIM mode. Limit of detection, 0.10 µg/L [Zhu, Luo 2005]. Column: Merck LiChroCART with Superspher 60 RP Select B stationary phase. Mobile phase: aqueous ammonium formate and acetonitrile. SIM acquisition mode. Lorazepam and related compounds [Kratzsch *et al.* 2004]. Column: CAPCELL PAK ODS. UG 120 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L phosphate buffer (3 : 7), flow rate 1.0 mL/min. SIM acquisition mode [Kanazawa *et al.* 1998].

Serum **HPLC** Column: reversed phase with stationary phase with low silanol activity. Limit of quantification, 2.5 µg/L [Kazemifard *et al.* 2006]. Column C_{18} reversed phase: condition 1 (100 × 4.6 mm i.d., 2 µm), TS gel Super-ODS; condition 2 (100 × 4.6 mm i.d., 5 µm), Hypersil ODS-C_{18}). Mobile phase: methanol : 5 mmol/L sodium dihydrogen phosphate (pH 6, 45 : 55), flow rate 0.65 mL/min. UV detection (λ= 254 nm). Limits of quantification, 4–10 times better under condition 1 than condition 2 for lorazepam and other benzodiazepines [Tanaka *et al.* 1996, 1998].

LC-MS Column: Unison UK-C_{18} ODS (150 × 2 mm i.d., 3 µm) or Cadenza CD-C_{18} ODS (150 × 2.0 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 to 20 : 80 at 20 min for 5 min), flow rate 0.25 mL/min. ESI. Limit of detection, 0.3–11.4 µg/L [Nakamura *et al.* 2009]. See Plasma [Marin, McMillin 2010; Marin *et al.* 2008]. See Blood [Dussy *et al.* 2006].

Urine **GC** Column: HP-5 capillary. NPD. Limit of detection, 5 µg/L [Jiang *et al.* 2001]. See Plasma [Greenblatt *et al.* 1978].

GC-MS Column: polydimethylsiloxane. Scan and SIM acquisition mode. Limit of quantification, 2.5–5 µg/L (scan mode), 0.1–0.5 µg/L (SIM) for lorazepam and other benzodiazepines [Borrey *et al.* 2001a]. Column: polydimethylsiloxane. Scan mode and SIM. Limits of detection, 13–30 µg/L (scan mode) and 1.0–1.7 µg/L (SIM) for lorazepam and other benzodiazepines [Borrey *et al.* 2001b]. Column: 5% phenylmethylsiloxane (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.5 mL/min. Temperature programme: 240° to 260° at 25°/min to 300° at 30°/min. SIM acquisition mode. Limit of detection, 0.1 mg/L [Black *et al.* 1994; Hagan 1995]. Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature

programme: 160° for 1 min to 280° at 20°/min for 3 min. NCI. Limit of quantification, <10 µg/L [Fitzgerald *et al.* 1993]. Column: DB-5 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 66 cm/s. Temperature programme: 210° to 300° at 20°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification <10 µg/L, limit of detection, <10 µg/L [Dickson *et al.* 1992]. See Blood [Goldberger *et al.* 2010; Higuchi *et al.* 1979; Pichini *et al.* 1999].

HPLC See Plasma [Uddin *et al.* 2008] and [Franzelius, Besserer 1993].

LC-MS Limit of detection, <0.5 µg/L for lorazepam and related drugs [Deveaux *et al.* 2008]. See Plasma [Marin, McMillin 2010; Marin *et al.* 2008; Papini *et al.* 2006a; Papini *et al.* 2006b]. Column: XTerra C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium acetate (pH 3.6, 5 : 95 to 80 : 20 at 10 min), flow rate 200 µL/min. ESI, MRM acquisition mode. Limit of detection, 0.02 µg/L [Kintz *et al.* 2004].

CE-MS Running buffer: methanol : water (50 : 50) containing 0.1% formic acid, flow rate 0.5 mL/min. ESI, TOF. Limit of quantification, 1 µg/L, limit of detection, 50 µg/L [Blas, McCord 2008].

Meconium **LC-MS** See Plasma [Marin, McMillin 2010; Marin *et al.* 2008].

Oral Fluid **HPLC** See Plasma [Uddin *et al.* 2008].

LC-MS See Urine. Limit of detection, 0.05 µg/L [Kintz *et al.* 2004].

Hair **GC-MS** Column: XTerra C_{18}. Limit of detection, 0.5 ng/g [Kintz *et al.* 2004]. Column: HP-Ultra 2 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 70° for 2 min to 220° at 25°/min to 255° at 5°/min to 300° for 7 min. EI ionisation at 70 eV. Retention time: 10.4 min. Limit of detection, 1 ng/mg. [Yegles *et al.* 1997]. Column: HP-5MS 5% phenyl-95% methylsiloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 295° at 30°/min for 5 min. NCI, SIM acquisition mode. Retention time: 9.96 min. Limit of detection, 2 pg/mg [Cirimele *et al.* 1996].

Note For an ELISA for the detection of lorazepam, see Miller *et al.* [2006].

Disposition in the Body Lorazepam is readily absorbed after oral administration; bioavailability is ~95%. Lorazepam crosses the blood–brain barrier and the placenta. It is found in breast milk. Approximately 75% of a dose is excreted in the urine as the inactive glucuronide conjugate within 5 days (up to ~50% in the first 24 h) and 14% is excreted as conjugates of minor metabolites, which include ring-hydroxylation products and quinazoline derivatives; only negligible amounts are excreted as free lorazepam; ~7% of a dose is eliminated in the faeces. Lorazepam is a metabolite of lormetazepam.

Therapeutic Concentration In plasma, the usual therapeutic range is 0.05–0.24 mg/L. Lorazepam glucuronide accumulates in plasma, achieving concentrations greater than those of unchanged drug.

Peak plasma levels of 16.9 µg/L lorazepam were reported 2 h after single 2 mg oral doses of lorazepam. The apparent elimination half-life was 12 h [Greenblatt *et al.* 1976].

Following daily oral doses of 6 mg in 8 subjects, mean steady-state plasma concentrations of 0.09 mg/L lorazepam and 0.17 mg/L lorazepam glucuronide were reported; in 7 subjects receiving 10 mg daily, the mean steady-state plasma concentrations were 0.16 and 0.27 mg/L, respectively [Greenblatt *et al.* 1977a].

After a single oral dose of 0.05 mg/kg given to 9 subjects, peak plasma concentrations of 0.04–0.06 mg/L (mean, 0.05) were attained in ~0.5–1.5 h; the maximum therapeutic effect was associated with plasma concentrations of 0.03–0.05 mg/L and concentrations below 0.01 mg/L were ineffective [Bradshaw *et al.* 1981].

Administration of lorazepam 288 mg as continuous IV infusions in 2 patients or as 55 mg IV injections in 11 patients resulted in plasma levels of 629 and 49 µg/L, respectively. The median plasma level was 59 µg/L and clearance was maintained at 92 mL/min [de Wit *et al.* 2006].

For the pharmacokinetics of lorazepam after IV and IM injections, see Greenblatt *et al.* [1977b], Greenblatt *et al.* [1977c].

Toxicity

Plasma concentrations of 0.3–0.6 mg/L were reported in 3 subjects suffering toxic effects after the ingestion of overdoses of lorazepam; the estimated amounts ingested were 100 and 120 mg in 2 of these cases. The subjects recovered within 24–30 h [Allen *et al.* 1980].

In a review of 170 drug-impaired driving cases involving lorazepam and submitted to Washington DC State Toxicological Laboratory between 1998 and 2003, the mean blood level of lorazepam was 0.048 mg/L (range, <0.005–0.39 mg/L). In 86% of these drivers other drugs were found that may have contributed to driving impairment. In 23 cases where lorazepam was the only drug detected, mean blood levels were 0.051 mg/L (range, <0.01–0.38 mg/L) [Clarkson *et al.* 2004].

Half-life Plasma half-life: lorazepam 9–24 h (mean, 14), lorazepam glucuronide ~16 h.

Volume of Distribution ~1–2 L/kg.

Clearance Plasma clearance, ~1 mL/min/kg.

Protein Binding ~90%; decreased in patients with cirrhosis.

Note For reviews of the clinical pharmacokinetics of lorazepam, see Kyriakopoulos *et al.* [1978], and Greenblatt [1981].

Dose For anxiety disorders, 1 to 10 mg in divided doses, orally daily.

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Papini O *et al.* (2006a). Quantitative assay of lorazepam and its metabolite glucuronide by reverse-phase liquid chromatography–tandem mass spectrometry in human plasma and urine samples. *J Pharm Biomed Anal* 40: 389–396.

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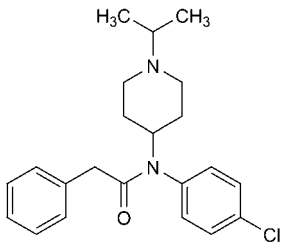
Lorcainide

Antiarrhythmic
$C_{22}H_{27}ClN_2O = 370.9$
CAS—59729-31-6

IUPAC Name *N*-(4-Chlorophenyl)-*N*-(1-propan-2-ylpiperidin-4-yl)acetamide
Synonyms *N*-(4-Chlorophenyl)-*N*-[1-(1-methylethyl)-4-piperidinyl]benzeneacetamide; isocainide; socainide.



Lorcainide Hydrochloride

$C_{22}H_{27}ClN_2O,HCl = 407.4$
CAS—58934-46-6
Proprietary Name *Remivox*
Chemical Properties Crystals. Mp 263°. Freely soluble in water, ethanol, chloroform, and methanol. Log *P* (octanol/water), 4.9.

Thin-layer Chromatography System TB—R_f 0.48; system TE—R_f 0.80; system TAE—R_f 0.41.
Gas Chromatography System GA—lorcainide RI 2810, M (nor-) RI 2660, M (OH-)-AC RI 2880, M (OH-dimethoxy-)-AC RI 3010, M (OH-methoxy-)-AC RI 2940, M (*N*-desalkyl-desacyl-)-AC_2 RI 2490, M (desacyl-) RI 2100, M (desacyl-)-AC RI 2200; system GB—lorcainide RI 2923, M (nor-) RI 2789.
High Performance Liquid Chromatography System HA—*k* 1.8; system HX—RI 425; system HZ—RT 6.6 min.
Ultraviolet Spectrum Water—257 ($A_1^1 = 14.3$b), 263 nm ($A_1^1 = 12.1$b).
Infrared Spectrum Principal peaks at wavenumbers 1649, 716, 1094, 1298, 1153, 1016 cm^{-1} (lorcainide hydrochloride, KBr disk).
Quantification
Plasma **GC** Column: 3% OV-22 on 80/100 Supelcoport (200 × 0.2 cm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 260°. ECD. Limit of detection, lorcainide 5 µg/L, norlorcainide 10 µg/L, hydroxylated metabolites 10 to 15 µg/L [Woestenborghs *et al.* 1979]. Column: 3% OV-17 glass (2 m). Carrier gas: N_2, 60 mL/min. ECD. Limit of detection, lorcainide and norlorcainide 10 µg/L [Klotz *et al.* 1978]

HPLC Column: µBondapak phenyl reversed phase (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : phosphate buffer (2 : 3), flow rate 80 mL/h. UV detection (λ = 196 nm). Retention time: 6.0 min. Limit of detection, lorcainide and norlorcainide 5 µg/L [Yee, Kates 1981]. Column: Spherisorb hexyl (150 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate (pH 3.0; 60 : 40), flow rate 1.5 mL/min. UV detection (λ = 230 nm). Limit of detection, 0.025 mg/L [Verbesselt *et al.* 1991]. Column: µBondapak phenyl reversed phase (300 × 3.9 mm i.d., 10 µm). Mobile phase: potassium dihydrogen phosphate : acetonitrile (pH 2.3; 750 : 1250), flow rate 1 mL/min. UV detection (λ = 254 nm). Retention time: 9.8 min. Limit of detection, 0.06 mg/L [Simon, Somani 1982].
Serum **HPLC** Column: YWG C_{18} H37 (200 × 5.0 mm i.d., 5 µm). Mobile phase: methanol : water: 0.625 mol/L ammonium acetate (pH 8.0; 86 : 13 : 1), flow rate 1.0 mL/min. UV detection (λ = 226 nm). Limit of detection, 5 µg/L [Luo, Zeng 1995].
Urine **GC** See Plasma [Klotz *et al.* 1978; Woestenborghs *et al.* 1979].

GC-MS Column: HP cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. FID. Limit of detection not reported [Maurer 1990].
Tissues **GC** See Plasma [Woestenborghs *et al.* 1979].

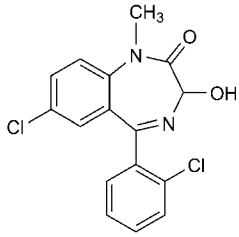
Disposition in the Body Well absorbed after oral administration but undergoes extensive, saturable first-pass metabolism. The principal active metabolite, norlorcainide, is found in high concentrations in plasma during chronic oral dosing. Other major metabolites include a 4-hydroxyphenyl derivative and a 4-hydroxy-3-methoxyphenyl compound. After IV administration, less than 2% of a dose is excreted in the urine as unchanged drug in 48 h; a total of ~60% of a dose is excreted in the urine as metabolites in 4 days and 35% is eliminated in the faeces [Lauwers *et al.* 1983].
Therapeutic Concentration
Following oral doses of 100 mg twice a day to eight subjects for 2 weeks, mean steady-state plasma concentrations were 0.05–0.50 mg/L (mean 0.19) for lorcainide and 0.16–0.68 mg/L (mean 0.32) for norlorcainide [Kates *et al.* 1983].
Toxicity
A 15-year-old girl died 3 h after ingesting at least 2.5 g lorcainide; the lorcainide concentration in a blood sample taken 30 to 60 min post-ingestion was 1.82 mg/L [Evers, Buttner-Belz 1995].
Bioavailability Approximately 30% after a single dose of 150 mg but almost 100% during chronic oral dosing.
Half-life Plasma half-life, lorcainide 3–15 h (mean 8), increased in subjects with cirrhosis of the liver and during chronic oral dosing; norlorcainide 20–40 h (mean 27).
Volume of Distribution Approximately 8 L/kg but there is considerable inter-subject variation.
Clearance Plasma clearance, 10–25 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, approximately 1.3.
Protein Binding 80–85% [Klotz *et al.* 1978].
Note For reviews of lorcainide, see Eiriksson and Brogden [1984]; Klotz *et al.* [1978].
Dose The equivalent of 200 to 300 mg of lorcainide daily.

Eiriksson C, Brogden RN (1984). Lorcainide A preliminary review of its pharmacodynamic properties and therapeutic efficacy. *Drugs* 27: 279–300.
Evers J, Buttner-Belz U (1995). Fatal lorcainide poisoning. *J Toxicol Clin Toxicol* 33: 157–159.
Kates RE *et al.* (1983). Lorcainide disposition kinetics in arrhythmia patients. *Clin Pharmacol Ther* 33: 28–34.
Klotz U *et al.* (1978). Pharmacokinetics of lorcainide in man: a new antiarrhythmic agent. *Clin Pharmacokinet* 3: 407–418.
Lauwers WF *et al.* (1983). Mass spectral investigation of the metabolites of lorcainide in man. *Eur J Drug Metab Pharmacokinet* 8: 351–362.
Luo X, Zeng FD (1995). [HPLC method for determination of lorcainide hydrochloride in human serum]. *Yao Xue Xue Bao* 30: 605–609.
Maurer HH (1990). Identification of antiarrhythmic drugs and their metabolites in urine. *Arch Toxicol* 64: 218–230.
Simon V, Somani P (1982). Rapid and simple method for determination of lorcainide, a new antiarrhythmic drug, and its major metabolite, norlorcainide, by high-performance liquid chromatography. *J Chromatogr* 231: 478–484.
Verbesselt R *et al.* (1991). High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Ther Drug Monit* 13: 157–165.
Woestenborghs R *et al.* (1979). Simultaneous gas chromatographic determination of lorcainide hydrochloride and three of its principal metabolites in biological samples. *J Chromatogr* 164: 169–176.
Yee YG, Kates RE (1981). High-performance liquid chromatographic analysis of lorcainide and its active metabolite, norlorcainide, in human plasma. *J Chromatogr* 223: 454–459.

Lormetazepam

Benzodiazepine, Hypnotic
$C_{16}H_{12}Cl_2N_2O_2 = 35.2$
CAS—848-75-9

IUPAC Name 7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one
Synonym Wy-4082
Proprietary Names *Aldosomnil*; *Ergocalm*; *Loramet*; *Loretam*; *Minias*; *Noctamid(e)*; *Octonox*; *Pronoctan*; *Stilaze*.



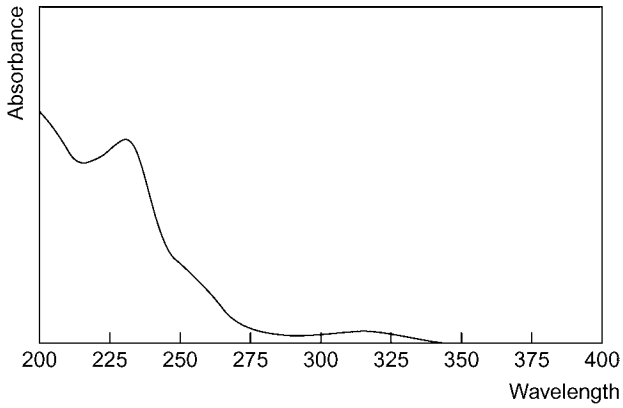
Chemical Properties A white crystalline powder. Mp 209° to 211°, with decomposition. Practically insoluble in water; slightly soluble in ethanol and methanol; freely soluble in chloroform. Log *P* (octanol/water), 2.2. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Test Formaldehyde–sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.06; system TC—R_f 0.61; system TD—R_f 0.46; system TE—R_f 0.59; system TF—R_f 0.45; system TL—R_f 0.50; system TAD—R_f 0.60; system TAE—R_f 0.82; system TAF—R_f

0.82 (acidified potassium permanganate—positive; Dragendorff spray—positive; FPN reagent—pale yellow; Marquis reagent—very weak pale yellow; mercuric chloride–diphenylcarbazone reagent—blue, on heating turns pale pink).

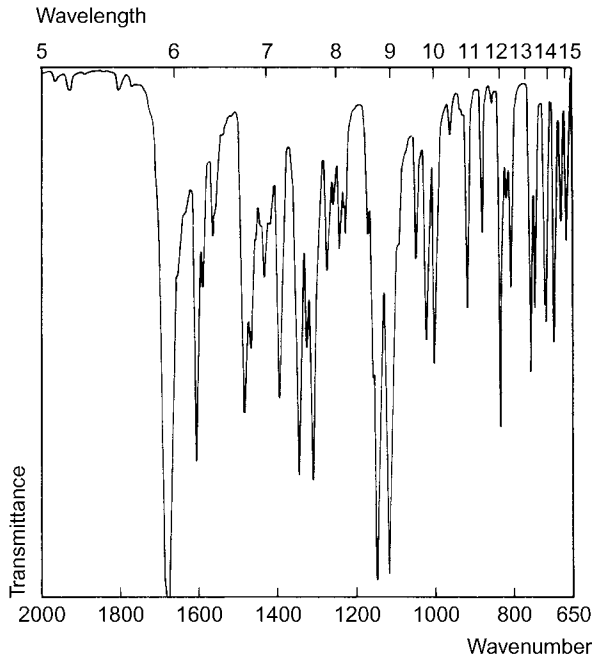
Gas Chromatography System GA—lormetazepam RI 2660, lorazepam RI 2410, decomposition product RI 2727; system GB—lormetazepam RI 2770; lorazepam RI 2528, lormetazepam-TMS_2 RI 2799; lorazepam-TMS_2 RI 2566.

High Performance Liquid Chromatography System HI—*k* 6.32; system HK—*k* 0.08; system HX—RI 487; system HY—RI 463; system HZ—RT 6.2 min; system HBH—*k* 7.19; system HBI—*k* 1.46.

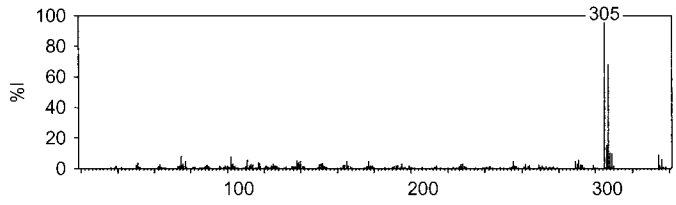
Ultraviolet Spectrum Aqueous acid—231 ($A_1^1 = 1030$b), 311 nm ($A_1^1 = 59$b).



Infrared Spectrum Principal peaks at wavenumbers 1682, 1153, 1121, 1315, 1610, 843 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 305, 307, 306, 309, 308, 334, 102, 75; lorazepam 291, 239, 274, 293, 75, 302, 276, 138.



Quantification

Plasma **GC** Column: 3% OV-17 on Chromsorb W HP 100/120 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature programme: 280°. ECD. Retention time: 4.2 min. Limit of detection, 200 ng/L [Pierce *et al.* 1984].

Note For a radioimmunoassay method, see Hümpel *et al.* [1980].

Urine **GC-MS** Column: SGE BP1 capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° for 1 min to 250° at 15°/min for 8 min to 300° at 10°/min for 2 min. EI ionisation, scan or SIM acquisition mode. Limit of detection, 18 µg/L (scan mode) and 1.1 µg/L (SIM) [Borrey *et al.* 2001].

LC-MS Column: 5% OV-101 Chromosorb G HP 100/120 mesh (600 × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Disposition in the Body Well absorbed after oral administration. Metabolised to some extent by *N*-demethylation to lorazepam. Approximately 80% of a dose is excreted in the urine as lormetazepam glucuronide in 72 h, and ~6% as lorazepam glucuronide.

Therapeutic Concentration

Six subjects were given single oral doses of 1 mg and 3 mg. Mean peak plasma concentrations were 0.006 and 0.016 mg/L, respectively, attained at 2–3 h [Hümpel *et al.* 1980].

Bioavailability Approximately 80%

Half-life Plasma half-life, lormetazepam ~10 h, appears to be increased in elderly subjects; lormetazepam glucuronide ~13 h.

Volume of Distribution Approximately 5 L/kg.

Clearance Plasma clearance, approximately 4 mL/min/kg.

Protein Binding Approximately 90%.

Dose 0.5 to 1.5 mg, as a hypnotic.

Borrey D *et al.* (2001). Simultaneous determination of fifteen low-dosed benzodiazepines in human urine by solid-phase extraction and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765: 187–197.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Hümpel M *et al.* (1980). Kinetics and biotransformation of lormetazepam. II. Radioimmunologic determinations in plasma and urine of young and elderly subjects: first-pass effect. *Clin Pharmacol Ther* 28: 673–679.

Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography–mass spectrometry. *J Chromatogr* 422: 85–101.

Pierce DM *et al.* (1984). Pharmacodynamic correlates of modified absorption: studies with lormetazepam. *Br J Clin Pharmacol* 18: 31–35.

Lornoxicam

Analgesic, COX Inhibitor, NSAID

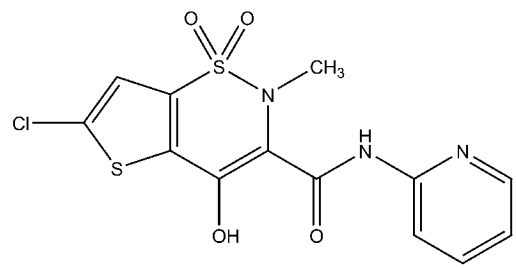
$C_{13}H_{10}ClN_3O_4S_2 = 371.8$

CAS—70374-39-9

IUPAC Name (3*E*)-6-Chloro-3-[hydroxy-(pyridin-2-ylamino)methylidene]-2-methyl-1,1-dioxothieno[2,3-e]thiazin-4-one

Synonyms 6-Chloro-4-hydroxy-2-methyl-3-(2-pyridylcarbamoyl)-2*H*-thieno[2,3-e]-1,2-thiazine-1,1-dioxide; 6-chloro-4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-thieno[2,3-e][1,2]-thiazine-3-carboxamide 1,1-dioxide; chlortenoxican; CTX; Ro-13-9297; TS-110.

Proprietary Names *Acabel*; *Artok*; *Bosporon*; *Hypodol*; *Lorcam*; *Lornox*; *Noxon*; *Taigalor*; *Telos*; *Xefo*.

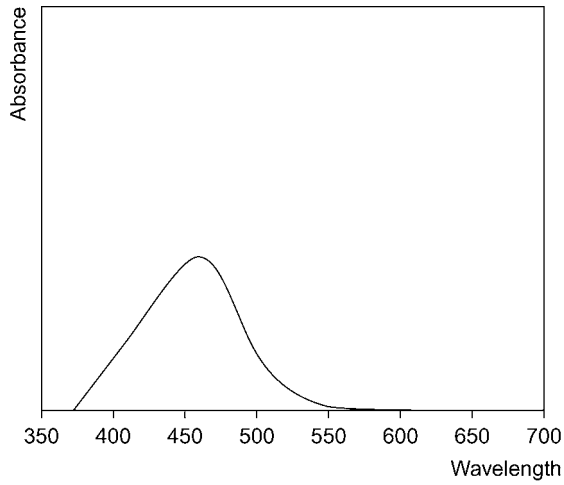


Chemical Properties Orange to yellow crystals. Mp 225° to 230°. pK_a 4.7 [O'Neil *et al.* 2006]. Log *P* (octanol/pH 7.4 buffer), 1.8 [O'Neil *et al.* 2006]. Stability of blank plasma was established after storage at –20° for 5 months [Radhofer-Welte, Dittrich 1998].

Thin-layer Chromatography Plates: aluminium precoated with silica gel F_{254} (20 × 20 cm, 0.25 mm). Solvent system: ethyl acetate : methanol : 26% ammonia (17 : 3 : 0.35). Limit of quantification, 0.26 µg/spot; limit of detection, 0.08 µg/spot [Taha *et al.* 2004].

High Performance Liquid Chromatography Column: Nova-Pak reversed phase C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : acetonitrile : acetate buffer (pH 4.6, 45 : 5 : 50), flow rate 0.8 mL/min. UV detection (λ = 280 nm). Limit of quantification, 0.04 mg/L; limit of detection, 0.01 mg/L [Taha *et al.* 2004].

Ultraviolet Spectrum 371 nm [Nemutlu *et al.* 2005].



Quantification

Plasma **HPLC** Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L sodium dihydrogen phosphate buffer (pH 6) : methanol (50 : 50), flow rate 1.5 mL/min. UV detection (λ = 372 nm). Limit of detection, 10 μg/L [Radhofer-Welte, Dittrich 1998]. Column: Hypersil ODS (250 × 4.6 mm i.d., 5 μm). Mobile phase: 50 mmol/L phosphate buffer (pH 6) : methanol (50 : 50), flow rate 1.3 mL/min. UV detection (λ = 371 nm). Limit of detection, 10 μg/L [Bareggi *et al.* 1997]. Column: Sumipax ODS A-212 C_{18} (150 × 6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate : acetonitrile : methanol (60 : 23 : 17), flow rate 1.5 mL/min. Electrochemical detection. Limit of detection, 5 and 10 μg/L for lornoxicam and 5′-hydroxylornoxicam, respectively [Suwa *et al.* 1993]. Column: Perkin Elmer. Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 6; 55 : 45), flow rate 1.0 mL/min. UV-vis detection (λ = 370 nm). Limit of detection, 3 μg/L [Dittrich *et al.* 1990].

LC-MS Column: Sunfire C_{18}. Mobile phase: methanol : 10 mmol/L ammonium formate (70 : 30). ESI, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Kim *et al.* 2007]. Column: Zorbax XDB-C_8. Mobile phase: methanol : water : formic acid (80 : 20 : 0.5), flow rate 0.7 mL/min. APCI, SRM acquisition mode. Limit of quantification, 2 μg/L [Zeng *et al.* 2004].

Synovial Fluid **HPLC** Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L sodium dihydrogen phosphate buffer (pH 6) : methanol (50 : 50), flow rate 1.5 mL/min. UV detection (λ = 372 nm). Limit of detection, 10 μg/L [Radhofer-Welte, Dittrich 1998].

Note For a UV spectrophotometric method for the detection of lornoxicam, see Nemutlu *et al.* [2005].

Disposition in the Body Following oral administration, lornoxicam is completely absorbed. Maximum plasma concentrations are reached within an average of 1 to 3 h. The major inactive metabolite, 5′-hydroxylornoxicam, is formed in the liver by CYP2C9. This pathway accounts for 95% of lornoxicam clearance, with no unchanged drug being found in urine. The presence of food reduces the peak plasma concentration by approx. 30% and delays time to the maximum slightly.

Therapeutic Concentration

A study in 18 healthy Chinese volunteers revealed that the pharmacokinetics of lornoxicam are dependent on CYP2C9 polymorphism, with the presence of the *CYP2C9*3* allele impairing oral clearance [Zhang *et al.* 2005]. An allele designated *CYP2C9*13* occurs in 2% of the Chinese population and is also associated with decreased clearance of lornoxicam. After a single oral dose of lornoxicam in healthy volunteers with *CYPC9*1/*1*, *CYP2C9*1/*3,* and *CYP2C9*1/*13* genotypes, mean peak plasma concentrations were 1.28, 1.62, and 1.83 mg/L, attained at 2.2, 7.98 and 7.97 h, respectively [Guo *et al.* 2005].

A study in 18 healthy volunteers compared the pharmacokinetics of a granular formulation of lornoxicam with standard tablets. The study showed that the granular formation had a faster absorption rate. Mean peak plasma concentrations were 783 ± 56.5 and 682 ± 33.3 μg/L, reached at 1.4 ± 0.2 and 1.8 ± 0.1 h, for granules and tablets, respectively [Bareggi *et al.* 1997]. Mean peak plasma concentration was 299 ± 63 μg/L at 3.0 h in young volunteers after a single oral dose of 4 mg lornoxicam compared with 353 ± 127 μg/L at 2.0 h in healthy elderly volunteers [Turner, Johnston 1990].

Note For an overview of the pharmacokinetics of lornoxicam, see Skjodt and Davies [1998]. For studies on the effects of antacids on the pharmacokinetics of lornoxicam, see Ravic *et al.* [1993] or Dittrich *et al.* [1990].

Bioavailability Approximately 100%.

Half-Life 3 to 5 h.

Volume of Distribution 0.1 to 0.2 L/kg.

Clearance 1.5 to 3.4 L/h in healthy volunteers.

Protein Binding 99.7% bound, mainly to albumin.

Dose For osteoarthritis and rheumatoid arthritis, an oral daily dose of 12 mg in two or three separate doses. For pain 8 to 16 mg orally.

Bareggi SR *et al.* (1997). Absorption of oral lornoxicam in healthy volunteers using a granular formulation in comparison with standard tablets. *Arzneimittelforschung* 47: 755–757.

Dittrich P *et al.* (1990). The effect of concomitantly administered antacids on the bioavailability of lornoxicam, a novel highly potent NSAID. *Drugs Exp Clin Res* 16: 57–62.

Guo Y *et al.* (2005). Role of CYP2C9 and its variants (CYP2C9*3 and CYP2C9*13) in the metabolism of lornoxicam in humans. *Drug Metab Dispos* 33: 749–753.

Kim YH *et al.* (2007). Liquid chromatography–electrospray ionization tandem mass spectrometric determination of lornoxicam in human plasma. *Arch Pharm Res* 30: 905–910.

Nemutlu E *et al.* (2005). Determination of lornoxicam in pharmaceutical preparations by zero and first order derivative UV spectrophotometric methods. *Pharmazie* 60: 421–425.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Radhofer-Welte S, Dittrich P (1998). Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid. *J Chromatogr B Biomed Sci Appl* 707: 151–159.

Ravic M *et al.* (1993). A pharmacokinetic interaction between cimetidine or ranitidine and lornoxicam. *Postgrad Med J* 69: 865–866.

Skjodt N, Davies MNM (1998). Clinical pharmacokinetics of lornoxicam. A short half-life oxicam. *Clin Pharmacokinet* 34: 421–428.

Suwa T *et al.* (1993). Simultaneous high-performance liquid chromatographic determination of lornoxicam and its 5′-hydroxy metabolite in human plasma using electrochemical detection. *J Chromatogr* 617: 105–110.

Taha EA *et al.* (2004). Stability-indicating chromatographic methods for the determination of some oxicams. *J AOAC Int* 87: 366–373.

Turner P, Johnston A (1990). Clinical pharmacokinetic studies with lornoxicam. *Postgrad Med J* 66 (Suppl4): S28–S29.

Zeng YL *et al.* (2004). Determination of lornoxicam in human plasma by LC/MS/MS. *Yao Xue Xue Bao* 39: 132–135.

Zhang Y *et al.* (2005). Lornoxicam pharmacokinetics in relation to cytochrome P450 2C9 genotype. *Br J Clin Pharmacol* 59: 14–17.

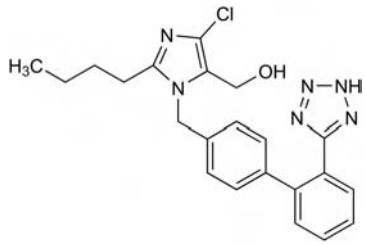
Losartan

Angiotensin II Receptor Antagonist

$C_{22}H_{23}ClN_6O$ = 422.9

CAS—114798-26-4

IUPAC Name 2-Butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl] methyl]-1*H*-imidazole-5-methanol



Chemical Properties A light-yellow solid. Mp 183.5° to 184.5°. pK_a 5 to 6. Log *P* (octanol/water), 4.01.

Losartan Potassium

$C_{22}H_{22}ClKN_6O$ = 461.0

CAS—124750-99-8

Synonyms DUP-753; E-3340; MK-954.

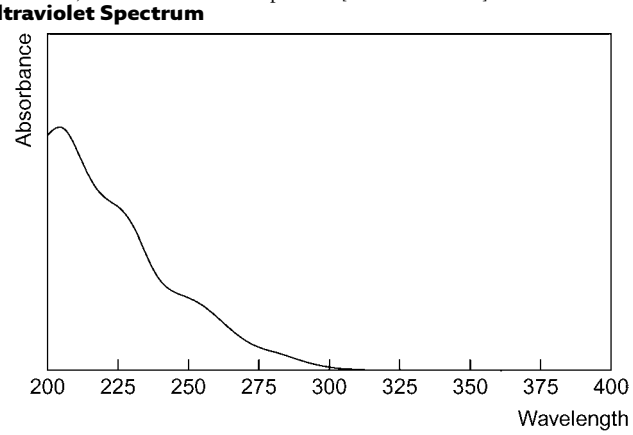
Proprietary Names *Cozaar*; *Lortaan*; *Losaprex*; *Neo-Lotan*; *Oscaar*. It is also an ingredient of *Hyzaar* and *Losazid*.

Gas Chromatography System GP—RI 3555(losartan-2ME).

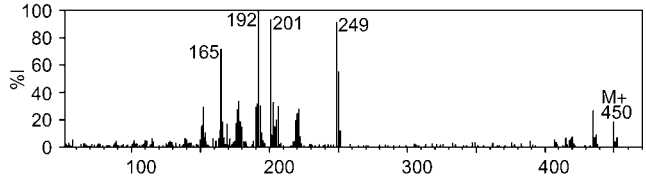
High Performance Liquid Chromatography Column: RP C_{18} (250 × 2.0 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium phosphate (with 0.02% sodium azide) : acetonitrile : methanol : TEA (pH 3.2, 60:30:10:0.04), flow rate 0.3 mL/min. Internal standard (IS): L-158809. UV detection (λ=254 nm). Retention time: losartan, 12.0 min; E-3174, 20.0 min; IS, 8.4 min [Yeung *et al.* 2000].

Column: RP C_8 Spherisorb (250 × 4.6 mm i.d.). Temperature: 40°. Mobile phase: (A:B) acetonitrile : 1 mmol/L phosphate buffer (pH 2.3). Elution programme: (40:60) to (75:25) in 25 min, hold for 5 min, flow rate 1.5 mL/min. UV detection (λ=230 nm). Retention time: not specified [Zhao *et al.* 1999].

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 192, 201, 249, 165, 450 (losartan-2ME).



Quantification

***Plasma* HPLC** Fluorescence detection (λ_{ex}=250 nm, λ_{em}=375 nm). Limit of detection, 1 µg/L [Farthing *et al.* 1997]. UV detection (λ=254 nm). Limit of quantification, 0.01 mg/L for losartan, 0.02 mg/L, for the metabolite [Ohtawa *et al.* 1993]. UV detection (λ=254 nm). Limit of quantification, 0.02 mg/L for losartan, 0.017 mg/L for the metabolite, E-3174 [Munafo *et al.* 1992].

***Urine* HPLC** Fluorescence detection (λ_{ex}=250 nm, λ_{em}=375 nm). Limit of detection, 1 µg/L [Farthing *et al.* 1997]. UV detection (λ=254 nm). Limit of quantification, 0.01 mg/L for losartan, 0.02 mg/L, for the metabolite [Ohtawa *et al.* 1993].

***Dialysate* HPLC** Fluorescence detection (λ_{ex}=250 nm, λ_{em}=375 nm). Limit of detection, 1 µg/L [Farthing *et al.* 1997].

Disposition in the Body Losartan is readily absorbed after oral administration. The drug undergoes extensive first-pass metabolism to form the active carboxylic acid metabolite, E-3174, as well as a number of inactive metabolites. After administration, peak plasma concentrations of losartan and E-3174 occur at about 1 h and 3 to 4 h, respectively. It is excreted in urine (35%) and in faeces (60%) via bile as the unchanged drug and as its metabolites. There is no evidence for accumulation of the drug.

Therapeutic Concentration

Six young, healthy male volunteers, aged 20 to 30 years (mean, 26 years) were administered single doses of 40, 80 and 120 mg. The peak plasma concentrations of the parent drug, losartan, were 0.104, 0.244 and 0.544 mg/L, respectively, and were reached about 1 h after administration. The peak concentrations for the metabolite, E-3174, were 0.164, 0.504 and 0.821 mg/L for the doses, respectively, and were observed at 3.7, 3.0 and 2.3 h after dosing [Munafo *et al.* 1992].

Healthy males (23 to 48 years old) were administered either 25, 50, 100 or 200 mg after an overnight fast. Mean peak plasma concentrations were 84.5, 197.6, 800.5 and 1394.9 µg/L for losartan and 188.9, 462.5, 1210.8 and 2219.0 µg/L for the metabolite, respectively. These concentrations were observed 0.7 and 1.3 h for the drug and 2 to 4 h for the metabolite [Ohtawa *et al.* 1993].

Toxicity Generally well tolerated, but fetal toxicity has been observed.

Bioavailability Around 33%.

Half-life Losartan: 1.5 to 2.5 h; active metabolite: 3 to 9 h.

Volume of Distribution 34 L; active metabolite: 12 L.

Clearance Plasma clearance: losartan, 600 mL/min; active metabolite, 50 mL/min.

Protein Binding ≥98%, mainly to albumin (both losartan and active metabolite).

Dose 50 to100 mg daily.

Farthing D *et al.* (1997). Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate. *J Chromatogr B Biomed Sci Appl* 704: 374–378.

Munafo A *et al.* (1992). Drug concentration response relationships in normal volunteers after oral administration of losartan, an angiotensin II receptor antagonist. *Clin Pharmacol Ther* 51: 513–521.

Ohtawa M *et al.* (1993). Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans. *Br J Clin Pharmacol* 35: 290–297.

Yeung P *et al.* (2000). Determination of plasma concentrations of losartan in patients by HPLC using solid phase extraction and UV detection. *Int J Pharm* 204: 17–22.

Zhao Z *et al.* (1999). Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS. *J Pharm Biomed Anal* 20: 129–136.

Losigamone

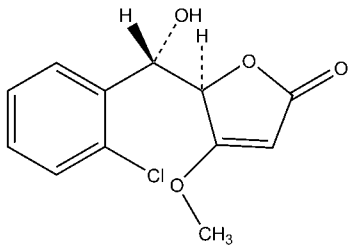
Antiepileptic, β-Methoxybutenolide

$C_{12}H_{11}ClO_4$ = 254.7

CAS—112856-44-7

IUPAC Name (5*R*)-5-[(*S*)-(2-Chlorophenyl)-hydroxymethyl]-4-methoxy-5*H*-furan-2-one

Synonyms ADD137022; AO-33; (5*R**)-5-[(α*S**)-*o*-chloro-α-hydroxybenzyl]-4-methoxy-2-(5*H*)-furanone; LSG.



Quantification

***Plasma* HPLC** Column: LiChroCart (250 × 4.0 mm i.d., 5 µm). Mobile phase: 0.01 mol/L sodium dihydrogen phosphate (pH 4.2) : methanol : acetonitrile (89 : 7 : 4), flow rate 0.7 mL/min. UV detection (λ = 230 nm). Retention times: (+)-losigamone, 8.5 min; (−)-losigamone, 9.5 min. Limit of quantification, 30 µg/L; limit of detection, 7.8 µg/L [Torchin *et al.* 1999]. Column: LiChrospher 60 RP select-B (125 × 4.6 mm i.d., 5 µm). Mobile phase: 18 mmol/L ammonium dihydrogen phosphate (pH 5.5) : acetonitrile (70 : 30), flow rate 0.8 mL/min. UV detection (λ = 220 nm). Limit of quantification, 100 µg/L. Column: Chiraspher (250 × 4.0 mm i.d., 5 µm). Mobile phase: hexane : methanol : propan-2-ol (850 : 300 : 20), flow rate 1 mL/min. UV detection (λ = 220 nm). Limit of detection not reported [Peeters *et al.* 1998]. Column: Nucleosil C_{18} (150 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 8 mmol/L ammonium dihydrogen phosphate (30 : 70), flow rate 1.2 mL/min. UV detection (λ = 220 nm). Limit of quantification, 50 µg/L [Biber, Dienel 1996].

Disposition in the Body Rapidly absorbed with peak plasma concentrations achieved within 3 h. It is primarily eliminated by oxidation and conjugation via CYP2A6, with less than 1% of the administered dose being found unchanged in urine and ~15% being excreted as the glucuronide conjugate. Stereoselective metabolism has been demonstrated with (+)-losigamone being primarily metabolised to a single hydroxyl metabolite whereas the (−)-form is metabolised to 3 distinctly different metabolites. It appears that (−)-losigamone has considerably greater affinity for CYP2A6 than does (+)-losigamone. There is evidence that losigamone elimination is accelerated by concomitant treatment with phenytoin.

Therapeutic Concentration

Five healthy volunteers was administered 100 mg of either (+)- or (−)-losigamone after a 10 h fast. Mean peak concentrations were 0.44 mg/L after 0.35 h for the (−)-enantiomer and 1.69 mg/L after 0.55 h for the (+)-enantiomer. It is thought that these differences are caused by a different affinity of the 2 enantiomers for CYP2A6. In a separate study, 5 healthy volunteers were administered 200 mg [^{14}C]losigamone. A mean peak plasma concentration of 1.99 mg/L was reached after 0.7 h. Radioactivity recovered in urine and faeces was 84.8 and 12.3%, respectively [Peeters *et al.* 1998].

Nine healthy male subjects were administered single 100, 300 and 700 mg doses of fast-releasing losigamone after breakfast. Mean peak plasma concentrations of 0.70, 1.69 and 4.36 mg/L were reached within 3.21, 2.34 and 2.56 h, respectively [Biber, Dienel 1996].

Nine healthy volunteers were administered a fast-release formulation of 500 mg losigamone 3 times daily on days 3 and 10. The peak concentration on day 3 was 3.36 mg/L after 1.69 h and on day 10 the steady-state concentration was 3.86 mg/L after 1.92 h [Biber, Dienel 1996].

Twenty healthy men were administered 500 mg (film-coated formulation) losigamone on days 1 and 8 after an 8 h fast and on the remaining days after a 2 h fast. The mean peak concentration on day 3 was 3.39 mg/L after 2.03 h; on day 10, the steady-state concentration was 3.94 mg/L after 1.67 h [Biber, Dienel 1996].

Twenty-four volunteers were administered 200, 400 and 600 mg losigamone 3 times daily for 28 days. Steady-state concentrations were 1.44, 3.02 and 5.31 mg/L for the 3 doses, respectively, and observed at 1.42, 2.25 and 0.87 h, respectively [Biber, Dienel 1996].

Note For a study of the stereoselective metabolism of losigamone, see Torchin *et al.* [1996].

Half-life Between 3.2 to 4.5 h.

Clearance Approximately 0.35 L/min.

Protein Binding Approximately 40 to 55%.

Dose Has been investigated in different doses as adjunctive therapy in the treatment of partial seizures.

Biber A, Dienel A (1996). Pharmacokinetics of losigamone, a new antiepileptic drug, in healthy male volunteers. *Int J Clin Pharmacol Ther* 34: 6–11.

Peeters PA *et al.* (1998). Pharmacokinetics of [^{14}C]-labelled losigamone and enantiomers after oral administration to healthy subjects. *Eur J Drug Metab Pharmacokinet* 23: 45–53.

Torchin CD *et al.* (1996). Stereoselective metabolism of a new anticonvulsant drug candidate, losigamone, by human liver microsomes. *Drug Metab Dispos* 24: 1002–1008.

Torchin CD *et al.* (1999). Chiral high-performance liquid chromatographic analysis of enantiomers of losigamone, a new candidate antiepileptic drug. *J Chromatogr B Biomed Sci Appl* 724: 101–108.

Lovastatin

HMG-CoA Reductase Inhibitor, Antihyperlipoproteinaemic

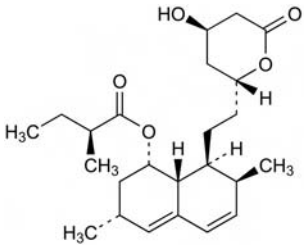
$C_{24}H_{36}O_5$ = 404.5

CAS—75330-75-5

IUPAC Name (2*S*)-2-Methylbutanoic acid (1*S*,3*R*,7*S*,8*S*,8a*R*)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl]ethyl]-1-naphthalenyl ester

Synonyms L-154803; MB-530B; MK-803; MSD-803; monacolin K; 6α-methylcompactin; mevinolin.

Proprietary Names *Lipofren*; *Lovalip*; *Mevacor*; *Mevinacor*; *Mevlor*; *Nergadan*; *Sivlor*; *Taucor.*

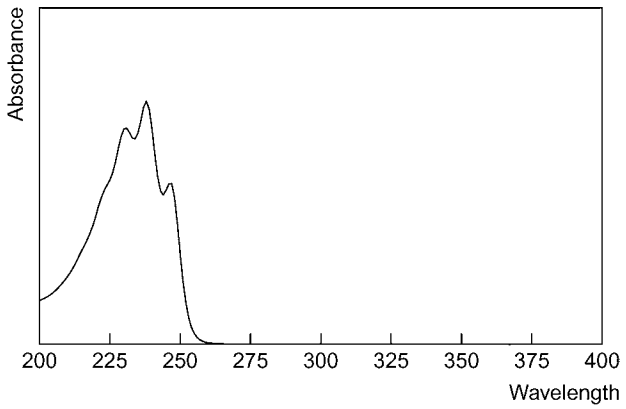


Chemical Properties A white crystalline powder. Mp 174.5°. It is practically insoluble in water (0.4×10^{-3}g/L) and petroleum spirit; sparingly soluble in low molecular weight alcohols (methanol, 28 g/L, ethanol, 16 g/L, isopropanol 20 g/L); soluble in acetone and acetonitrile; freely soluble in chloroform. Log *P* (octanol/water), 4.26; hydroxyacid derivative, 1.2×10^4; Log *P* (octanol/phosphate buffer pH 7.4), hydroxyacid derivative, 14.1.

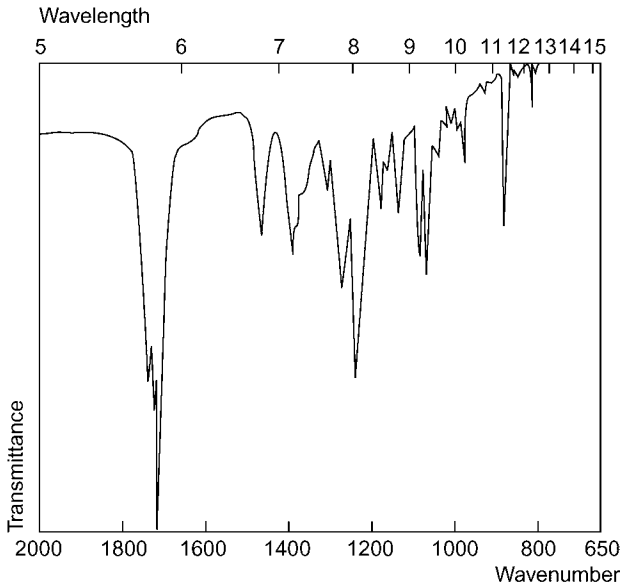
High Performance Liquid Chromatography System HAX—retention time 13.8 min; system HAY—retention time 14.8 min.

Column: RP C_{18} (250 × 4.6 i.d., 5 µm). Mobile phase: methanol : 25 mmol/L sodium dihydrogen phosphate (pH 4.5, 82:18), flow rate 1 mL/min. IS: Bay W 62230. UV detection (λ=236 nm). Retention time: lovastatin, 8.9 min; lovastatin acid, 6.4 min; IS, 5.6 min [Ye *et al.* 2000].

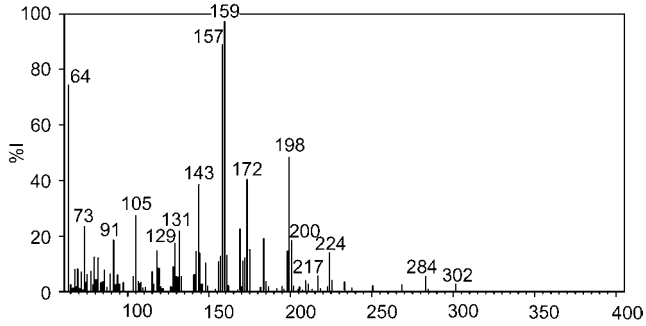
Ultraviolet Spectrum Aqueous acid—231, 238, 247 nm.



Infrared Spectrum Principal peaks at wavenumbers 1725, 1260, 1072, 1460 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 159, 157, 64, 198, 172, 143, 105, 73.



Quantification

***Plasma* HPLC** UV detection (λ=238 nm). Limit of detection, 25 µg/L [Stubbs *et al.* 1986].

***Serum* GC–MS** Column: DB-1701 (10 m × 0.25 mm i.d., 0.15 µm). Temperature programme: 210° for 0.75 min, to 290° at 30°/min, for 2 min. Carrier gas: He. Internal standard (IS): compactin. (NICI, SIM acquisition mode at *m/z* 565 for lovastatin and m/z 551 for IS). Retention time: lovastatin, 3.3 min; IS, 3.2 min. Limit of detection, 0.5 µg/L [Wang-Iverson *et al.* 1989].

Disposition in the Body Lovastatin is absorbed after oral administration (30%) and is hydrolysed in the liver to the active β-hydroxyacid form. Three other metabolites have also been detected: 3-hydroxy, 3-hydroxymethyl and 3-exomethylene derivatives. The drug undergoes extensive first-pass metabolism with <5% of a dose reaching circulation. Peak plasma concentrations are reached within 2 to 4 h and steady state within 2 to 3 days. Lovastatin is mainly excreted in faeces (85% of an administered dose) and 10% in urine over 72 h. It crosses the blood–brain and placental barriers.

Therapeutic Concentration

Seventeen healthy male volunteers, aged between 19 and 27 years, were administered a 20 mg single dose of lovastatin. The peak plasma concentration of the active inhibitors was 14.5 µg (equivalent)/L which was reached in 2.4 h. The peak concentration for the total inhibitors was 27.1 µg (equivalent)/L in 2.1 h [Pan *et al.* 1991].

Twelve healthy males, aged between 22 and 29 years, were administered a single 40 mg dose. The peak plasma concentration of the active inhibitors was 9.5 µg (equivalent)/L and total inhibitors 19.9 µg (equivalent)/L, reached in 2.9 and 2.6 h, respectively [Pentikainen *et al.* 1992].

Half-life 1 to 2 h (active metabolite).

Clearance Has been quoted as 30 to 1248 mL/min.

Protein Binding Both the parent drug and metabolite are >95% bound to plasma proteins.

Note For a general review of statins, see Hsu [1995].

Dose An initial dose of 10 to 20 mg daily is administered and increased to 80 mg daily if necessary over a period of 4 weeks. In patients taking immunosuppressants the initial daily dose is 10 mg and the overall dose should not exceed 20 mg daily.

Hsu I (1995). Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. *Ann Pharmacother* 29: 743–759.

Pan HY *et al.* (1991). Pharmacokinetic interaction between propranolol and the HMG-CoA reductase inhibitors pravastatin and lovastatin. *Br J Clin Pharmacol* 31: 665–670.

Pentikainen PJ *et al.* (1992). Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J Clin Pharmacol* 32: 136–140.

Stubbs RJ *et al.* (1986). Determination of mevinolin and mevinolinic acid in plasma and bile by reversed-phase high-performance liquid chromatography. *J Chromatogr* 383: 438–443.

Wang-Iverson D *et al.* (1989). Determination of lovastatin acid in serum by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 3(5): 132–134.

Ye LY *et al.* (2000). Determination of lovastatin in human plasma using reverse-phase high-performance liquid chromatography with UV detection. *Ther Drug Monit* 22: 737–741.

Loxapine

Antipsychotic, Tranquilliser, Tricyclic Dibenzoxazepine

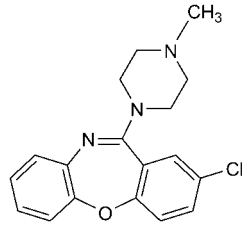
$C_{18}H_{18}ClN_3O = 327.8$

CAS—1977-10-2

IUPAC Name 2-Chloro-11-(4-methyl-1-piperazinyl)dibenz[*b,f*][1,4]oxazepine

Synonym Oxilapine

Proprietary Name *Loxapac* (solution)



Chemical Properties Pale yellowish crystals. Mp 109° to 110°. pK_a 6.6 [Mazzola *et al.* 2000]. Log *P* (octanol/water), 3.6. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Samples were stable for 4 weeks at –20°, for 48 h at 4°, and for 24 h at >20° [Garay Garcia *et al.* 2003].

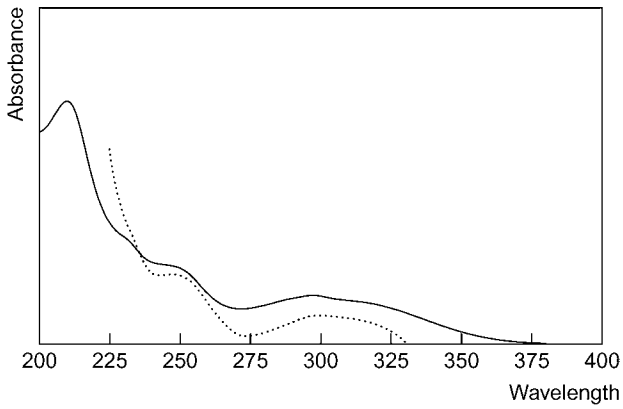
Loxapine Succinate

$C_{18}H_{18}ClN_3O,C_4H_6O_4 = 45.9$
CAS—27833-64-3
Proprietary Names *Loxapac* (capsules); *Loxitane* (capsules).
Chemical Properties A white crystalline solid.

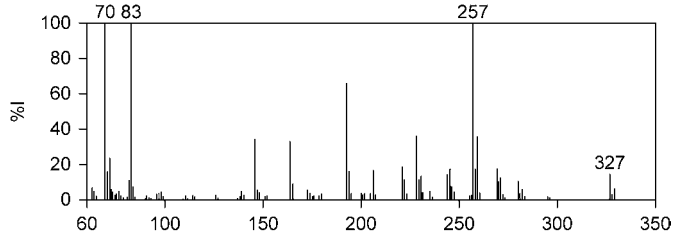
Loxapine Hydrochloride

$C_{18}H_{18}ClN_3O,HCl = 364.3$
Proprietary Names *Laxapac*; *Loxitane (oral solution; injection)*.

Colour Test Liebermann's reagent (50 to 60°)—orange; heat with nitric acid for 3 min at 100° and then dilute tenfold with water—yellow precipitate.
Thin-layer Chromatography System TB—R_f 0.36; system TE—R_f 0.54; system TAE—R_f 0.49.
Gas Chromatography System GA—loxapine RI 2555, M (8-OH-) RI 2931, M (amoxapine) RI 2638, M (7-OH-amoxapine) RI 2951, M (8-OH-amoxapine) RI 2959; system GB—loxapine RI 2717, M (8-OH-) RI 3077, M (7-OH-) RI 3068; M (amoxapine) RI 2746, M (7-OH-amoxapine) RI 3525, M (8-OH-amoxapine) RI 3546.
High Performance Liquid Chromatography System HA—*k* 1.1; system HX—RI 407; system HY—RI 336; system HAA—RT 14.6 min.
Ultraviolet Spectrum Aqueous acid—251 ($A_1^1 = 346b$), 292 nm; aqueous alkali—248 ($A_1^1 = 374b$), 298 nm.



Infrared Spectrum Principal peaks at wavenumbers 1590, 1603, 1564, 1109, 1188, 1248 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 70, 83, 42, 257, 193, 56, 228, 164; amoxapine 245, 257, 247, 193, 56, 246, 228, 259.



Quantification

Blood **GC** Column: Rtx-50 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 130° for 3 min to 270° at 10°/min for 10 min to 295° at 10°/min for 5 min. NPD. Relative retention time: 1.15. Limit of quantification, 0.05 mg/L [Mazzola *et al.* 2000]. OV-1 3% on Supelcoport 100/120 mesh (2 m × 2 mm i.d.). Carrier gas: Ar:CH_4 (95 : 5), 25 mL/min. Temperature: 255°. Or Carrier gas: He, 25 mL/min. Temperature programme: 260° for 4 min to 270° at 4°/min for 4 min. ECD. Limit of detection not reported [Cooper *et al.* 1981]
Plasma **GC** Column: 3% OV-17 100/120 mesh (6′ × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 170° to 230° at 2°/min for 8 min. Limit of detection not reported [Hepler *et al.* 1982]. Column: 3% OV-7 on 80/100 mesh Gas Chrom Q (1.8 m × 4 mm i.d.,). Carrier gas: Ar:CH_4 (95 : 5), 50 mL/min. Temperature programme: 225°. Relative retention time: 1.8. ECD. Limit of detection, 140 ng/L [Cooper *et al.* 1979].

HPLC Column: XTerra MS C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 62.4 mmol/L phosphate buffer (pH 4.2; 38 : 62), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Relative retention time: 2.5 min. Limit of quantification, 15 µg/L, limit of detection, 4 µg/L [Garay Garcia *et al.* 2003]. Column: Lichrospher reversed phase CN (20 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.5 mol/L acetic acid (30 : 70) containing 0.05% hexylamine, flow rate 0.8 mL/min. UV detection (λ = 310 nm). Limit of detection, loxapine 5.3 µg/L, amoxapine 5.9 µg/L, hydroxylated metabolites 3.5–6.3 µg/L [Hue *et al.* 1998]. Column: Spherisorb C_6 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L potassium dihydrogen phosphate containing 14 mmol/L orthophosphoric acid : acetonitrile containing 105 µmol/L nonylamine (77 : 23), flow rate 2.2 mL/min. UV detection (λ = 210 nm). Relative retention time: 1.39 min. Limit of detection, loxapine and amoxapine 2 ng, hydroxylated metabolites 1 ng [Cheung *et al.* 1991]. Column: Zorbax ODS (25 cm × 4.6 mm i.d.). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate containing 0.6% *n*-nonylamine (pH 3.0, 30 : 70), flow rate 1 mL/min. UV detection (λ = 210 nm). Limit of detection, 12.5 µg/L [Yufu *et al.* 1984].
Serum **GC** Column: 3% OV-17 on 100/120 Gas-Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 235° for 6 min to 280° at 32°/min for 4 min. AFID. Retention time: 9.5 min. Limit of detection, 2 µg/L [Vasiliades *et al.* 1979].

GC Column: Supelcoport 3% SP 2100 on 100/120 mesh (1.8 m × 2 or 4 mm i.d.). Carrier gas: Ar:CH_4 (95 : 5). Temperature: 255°. ECD. Limit of detection, 20 µg/L [Cooper, Kelly 1979].
Urine **GC** See Plasma [Cooper *et al.* 1979].

GC FID. See Serum. Carrier gas: He. FID. Limit of detection, 5 µg/sample [Cooper, Kelly 1979].
Bile **GC** See Blood [Mazzola *et al.* 2000].
Stomach Contents **GC** See Blood [Mazzola *et al.* 2000].
Vitreous Humour **GC** See Blood [Mazzola *et al.* 2000].
Brain **GC** See Blood [Cooper *et al.* 1981].
Liver **GC** See Blood [Cooper *et al.* 1981].
Lung **GC** See Blood [Cooper *et al.* 1981].
Spleen **GC** See Blood [Cooper *et al.* 1981].
Disposition in the Body Readily absorbed after oral administration. The major urinary metabolite appears to be 8-hydroxyloxapine; other metabolites that have been identified include 7-hydroxyloxapine, desmethylloxapine (amoxapine) and its 7-hydroxy and 8-hydroxy derivatives, loxapine *N*-oxide and 8-methoxyloxapine. Some unchanged drug is also excreted in the urine. Loxapine metabolism *in vitro* is inhibited by alimemazine, cyamemazine, daunorubicin, fluvoxamine, ketoconozole, levomepromazine, miconazole, nefopam, pirarubicin, prazepam, quercetin and verapamil [Bun *et al.* 2003].
Therapeutic Concentration There is considerable intersubject variation in plasma concentrations.

After a single oral dose of 25 mg to 25 subjects, mean peak serum concentrations were 0.02 mg/L loxapine, 0.05 mg/L 8-hydroxyloxapine, and 0.006 mg/L 8-hydroxyamoxapine, attained in 1–2 h [Khan *et al.* 1980].

Two subjects receiving 100 mg daily were found to have serum concentrations, respectively, of 0.031 and 0.006 mg/L loxapine, 0.072 and 0.041 mg/L 8-hydroxyloxapine, and 0.064 and 0.031 mg/L 8-hydroxyamoxapine immediately prior to a morning dose [Cooper, Kelly 1979].

Toxicity

In a fatality attributed to loxapine overdose, the following postmortem tissue concentrations (mg/L or µg/g) were reported:

	Blood	*Brain*	*Liver*	*Lung*	*Spleen*
Loxapine	1.22	4.46	11.88	4.98	3.96
8-Hydroxyloxapine (conjugated)	1.51	–	7.98	1.59	0.93
8-Hydroxyloxapine (unconjugated)	0.76	3.80	4.38	3.28	3.26
Amoxapine	1.01	4.84	10.00	3.80	6.30
8-Hydroxyamoxapine (conjugated)	1.92	–	1.89	1.32	–
8-Hydroxyamoxapine (unconjugated)	1.98	3.71	25.47	9.44	14.17

[Cooper *et al.* 1981].

A 22-year-old female ingested approximately 2.5 g loxapine and died after 12 days in a coma. Concentrations on admission to hospital were: 1.9 mg/L in blood and 0.4 mg/L in urine; 24 h later, the urine concentration was 8 mg/L [Reynolds *et al.* 1979].

In a second fatality, postmortem blood and liver concentrations of 7.7 mg/L and 150 µg/g, respectively, were reported [Reynolds *et al.* 1979].

A 69-year-old female died after ingesting loxapine tablets; loxapine and amoxapine concentrations, respectively, in postmortem tissues were 9.5 and 0.6 mg/L in heart blood, 28.8 and 4.7 mg/L in bile, 278 mg/L and none in gastric contents, and 1.5 mg/L and negative in vitreous humour [Mazzola *et al.* 2000].

A 19-year-old man ingested 20 mg fluoxetine and 10 mg loxapine in a suicide attempt. On admission, his serum loxapine concentration was 40 µg/L. He developed atrial flutter shortly after ingestion. A repeat serum

loxapine 16 h after ingestion contained less than 20 μg/L. Concentrations of metabolites were 28 μg/L for 8-hydroxyloxapine, less than 20 μg/L for amoxapine, and less than 10 μg/L for 8-hydroxyamoxapine. He recovered with no sequelae [Roberge, Martin 1994].

A 20-month-old girl ingested an unknown quantity of loxapine succinate tablets. Her blood concentration of loxapine was 0.72 mg/L. She made a full recovery [Hepler *et al.* 1982].

Note

For overdose in an 8-year-old boy, see Tarricone [1998]; for neuroleptic malignant syndrome secondary to loxapine, see Chong and Abbott [1991]; for seizures induced by overdose, see Peterson [1981]; for rhabdomyelosis and renal failure secondary to loxapine, see Tam *et al.* [1980].

Half-life Plasma half-life, loxapine 1–4 h; 8-hydroxyloxapine ~8 h; 8-hydroxyamoxapine ~36–48 h.

Dose Usually the equivalent of 20 to 100 mg loxapine daily; maximum 250 mg daily.

Bun SS *et al.* (2003). Interspecies variability and drug interactions of loxapine metabolism in liver microsomes. *Eur J Drug Metab Pharmacokinet* 28: 295–300.

Cheung SW *et al.* (1991). Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high-performance liquid chromatography. *J Chromatogr* 564: 213–221.

Chong LS, Abbott PM (1991). Neuroleptic malignant syndrome secondary to loxapine. *Br J Psychiatry* 159: 572–573.

Cooper TB, Kelly RG (1979). GLC analysis of loxapine, amoxapine, and their metabolites in serum and urine. *J Pharm Sci* 68: 216–219.

Cooper SF *et al.* (1979). Determination of loxapine in human plasma and urine and identification of three urinary metabolites. *Xenobiotica* 9: 405–414.

Cooper TB *et al.* (1981). Postmortem blood and tissue levels of loxapine and its metabolites. *J Anal Toxicol* 5: 99–100.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Garay Garcia L *et al.* (2003). Simultaneous determination of four antipsychotic drugs in plasma by high-performance liquid chromatography. Application to management of acute intoxications. *J Chromatogr B Analyt Technol Biomed Life Sci* 795: 257–264.

Hepler BR *et al.* (1982). Acute loxapine intoxication in a child. *J Anal Toxicol* 6: 258–259.

Hue B *et al.* (1998). Concurrent high-performance liquid chromatographic measurement of loxapine and amoxapine and of their hydroxylated metabolites in plasma. *Ther Drug Monit* 20: 335–339.

Khan MA *et al.* (1980). Loxapine: A study of bioequivalency and bioavailability. *Curr Ther Res* 28: 277–283.

Mazzola CD *et al.* (2000). Loxapine intoxication: case report and literature review. *J Anal Toxicol* 24: 638–641.

Peterson CD (1981). Seizures induced by acute loxapine overdose. *Am J Psychiatry* 138: 1089–1091.

Reynolds PC *et al.* (1979). Loxapine fatalities. *Clin Toxicol* 14: 181–185.

Roberge RJ, Martin TG (1994). Mixed fluoxetine/loxapine overdose and atrial flutter. *Ann Emerg Med* 23: 586–590.

Tam CW *et al.* (1980). Loxapine-associated rhabdomyolysis and acute renal failure. *Arch Intern Med* 140: 975–976.

Tarricone NW (1998). Loxitane overdose. *Pediatrics* 101: 496.

Vasiliades J *et al.* (1979). Determination of therapeutic and toxic concentrations of doxepin and loxapine using gas–liquid chromatography with a nitrogen-sensitive detector, and gas chromatography–mass spectrometry of loxapine. *J Chromatogr* 164: 457–470.

Yufu N *et al.* (1984). Simultaneous measurement of various antidepressants in the plasma of depressed patients by high performance liquid chromatography. *Folia Psychiatr Neurol Jpn* 38: 57–64.

Loxoprofen

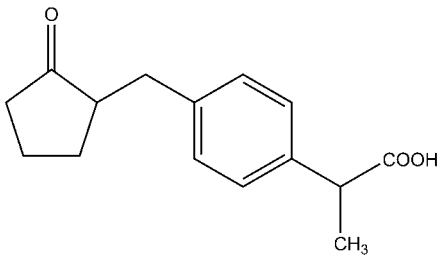
Analgesic, NSAID

$C_{15}H_{18}O_3 = 246.3$

CAS—68767-14-6

IUPAC Name 2-[4-[(2-Oxocyclopentyl)methyl]phenyl]propanoic acid

Synonyms α-Methyl-4-[(2-oxocyclopentyl)methyl]benzeneacetic acid; (±)-*p*-[(2-oxocyclopentyl)methyl]hydratropic acid.



Chemical Properties Colourless oil. Mp 108.5° to 111°. Bp 190° to 195°. Stock solutions were stable for at least a month when stored at –20° [Hirai *et al.* 1997].

Sodium Loxoprofen

$C_{15}H_{17}NaO_3,2H_2O = 304.3$

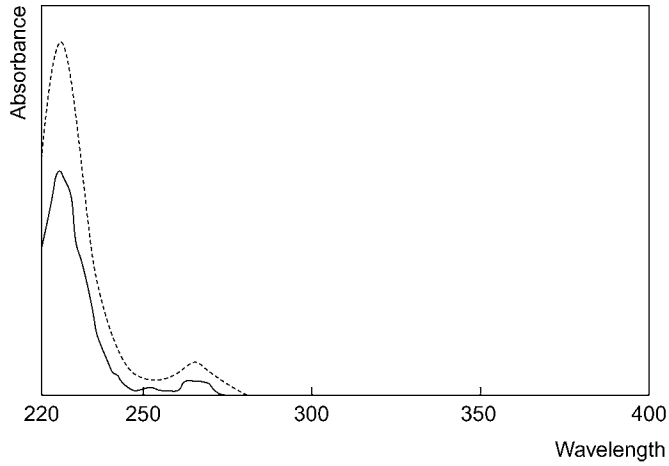
CAS—80382-23-6

IUPAC Name Sodium 2-[4-[(2-oxocyclopentyl)methyl]phenyl]propanoate

Synonyms CS-600; Sodium (±)-*p*-[(2-oxocyclopentyl)methyl]hydratropate dihydrate.

Proprietary Names *Loxonin*; *Oxeno*.

Ultraviolet Spectrum Methanol—226 nm [Kanazawa *et al.* 2002].



Quantification

Plasma **HPLC** Column: Chiralcel OJ (250 × 4.6 mm i.d.). Mobile phase: propan-2-ol : hexane : trifluoroacetic acid (5 : 95 : 0.1), flow rate 1.0 mL/min. UV and CD detection (λ = 225 nm). Retention time: 22.6, 25.6, 30.1 and 35.5 min for (1′*R*,2*R*)-, (1′*S*,2*R*)-, (1′*R*,2*S*), and (1′*S*,2*S*)-loxoprofen, respectively. Limit of detection not reported [Kanazawa *et al.* 2002]. Column: ODS (250 × 4.5 mm i.d., 5 μm). Mobile phase: acetonitrile : water (pH 3.0; 35 : 65), flow rate 1.2 mL/min. Spectrophotometric detection (λ = 220 nm). Limit of detection, 200 μg/L [Kim *et al.* 2002]. Column: Luna Phenomenex ODS (250 × 4.5 mm i.d., 5 μm). Mobile phase: acetonitrile : water (pH 3.0; 35 : 65), flow rate 1.2 mL/min. UV detection (λ = 220 nm). Limit of detection, 200 μg/L [Choo *et al.* 2001]. Column: Zorbax ODS (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid (55 : 45 : 1), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 355 nm, λ_{em} = 435 nm). Limit of quantification, 10 μg/L [Naganuma, Kawahara 1990].

Urine **HPLC** Column: Luna Phenomenex ODS (250 × 4.5 mm i.d., 5 μm). Mobile phase: acetonitrile : water (pH 3.0, 35 : 65), flow rate 1.2 mL/min. UV detection (λ = 220 nm). Limit of detection, 500 μg/L [Choo *et al.* 2001]. Column: Inertsil reversed phase ODS-2 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 50 mmol/L phosphate buffer: acetonitrile (pH 5.0; 58 : 42), flow rate 0.9 mL/min. UV detection (λ= 230 nm). Limit of detection, 50 μg/L [Hirai *et al.* 1997]. Column: μPorasil (30 cm × 3.9 mm i.d., 10 μm). Mobile phase: *n*-hexane : ethylacetate (68 : 32), flow rate 1.7 mL/min. Fluorescence detection (λ_{ex} = 313 nm, λ_{em} = 420 nm). Limit of quantification, ~5 ng on the column [Nagashima *et al.* 1985]. Column: Zorbax ODS (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid (55 : 45 : 1), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 355 nm, λ_{em} = 435 nm). Limit of quantification, 0.05 mg/L [Naganuma, Kawahara 1990].

Disposition in the Body An aldehyde–ketone reductase metabolises the parent compound to the reactive metabolites, followed by subsequent phase II metabolism (i.e. conjugation with glucuoronic acid or taurine). The alcohol metabolites and their conjugates comprise >95% of the metabolites excreted by dogs [Kim *et al.* 2002].

Therapeutic Concentration

Six healthy Korean volunteers (aged 24 to 28 years; weight 58 to 70 kg) were administered 60 mg loxoprofen as the anhydrous form daily for 3 days. On day 10, the volunteers were given an additional dose. The oral bioavailability was decreased in some of the patients on the second administration [Kim *et al.* 2002].

Sixteen healthy male volunteers (aged 46.8 ± 3.7 years; weight 63.9 ± 3.7 kg) were administered single oral doses of 60 mg loxoprofen. The drug was rapidly absorbed and a mean maximum plasma concentration of 4.92 mg/L was achieved after 31 min. [Naganuma, Kawahara 1990].

Half-life 1.15 h.

Dose Anhydrous form: 60 mg orally three times daily.

Choo KS *et al.* (2001). Simultaneous determination of loxoprofen and its diastereomeric alcohol metabolites in human plasma and urine by a simple HPLC–UV detection method. *J Pharm Biomed Anal* 25: 639–650.

Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692: 375–388.

Kanazawa H *et al.* (2002). Stereospecific analysis of loxoprofen in plasma by chiral column liquid chromatography with a circular dichroism-based detector. *J Chromatogr A* 948: 303–308.

Kim IW *et al.* (2002). Altered metabolism of orally administered loxoprofen in human subjects after an oral administration of loxoprofen for three consecutive days followed by a seven-day washout. *J Pharm Sci* 91: 973–979.

Naganuma H, Kawahara Y (1990). High-performance liquid chromatographic determination of loxoprofen and its diastereomeric alcohol metabolites in biological fluids by fluorescence labelling with 4-bromomethyl-6, 7-methylenedioxycoumarin. *J Chromatogr* 530: 387–396.

Nagashima H *et al.* (1985). Column liquid chromatography for the simultaneous determination of the enantiomers of loxoprofen sodium and its metabolites in human urine. *J Chromatogr* 345: 373–379.

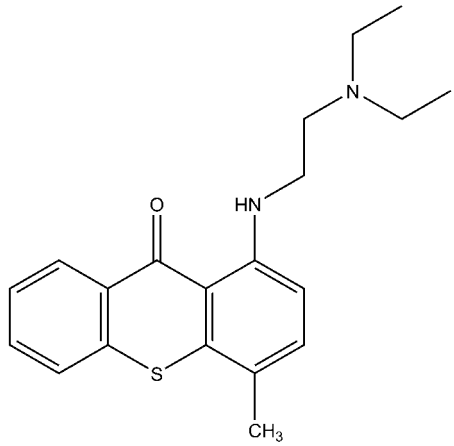
Lucanthone

Filaricide

$C_{20}H_{24}N_2OS = 340.5$

IUPAC Name 1-(2-Diethylaminoethylamino)-4-methyl-thiaxanthen-9-one

Synonyms BW 57-233; NSC 14574.



Chemical Properties Lucanthone melts at 66°. Lucanthone is extracted by organic solvents from aqueous alkaline solutions.

Lucanthone Hydrochloride

Proprietary Names *Miracil D*; *Nilodin*.

Chemical Properties A yellowish-orange crystalline powder. Mp 195° to 198°. Soluble 1 in 110 of water, 1 in 85 of ethanol, and 1 in 20 of chloroform; readily soluble in warm water and warm ethanol; insoluble in ether.

Colour Tests Sulfuric acid–formaldehyde test—(yellow)orange→purple→blue (limit of detection, 0.1 µg); Vitali's test—(yellow) yellow/yellow/red (limit of detection, 1 in 1000).

Thin-layer Chromatography System T1—R_f 0.53 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Lucanthone in 0.1 N sulfuric acid, maxima at 223 (E1%, 1 cm 566), 257 (E1%, 1 cm 1325) and 331 nm (E1%, 1 cm 236) and an inflexion at 280 nm.

Infrared Spectrum Principal peaks at A 1616, B 1224, 1512 or 1591 (base) (KBr disk).

Disposition in the Body

Metabolism Lucanthone hydrochloride is readily absorbed from the gastrointestinal tract. Most of it is metabolised by the body, only 7% to 10% appearing unchanged in the urine.

Toxicity Lucanthone is more toxic when given by injection than when given by mouth. The skin may turn yellow or orange and remain so for about 4 weeks. Heptic or renal damage has resulted from chronic poisoning.

LD_{50} (oral): mice 140 mg/kg.

Dose Usually 1 g twice daily for 3 days.

Lymecycline

Antibacterial

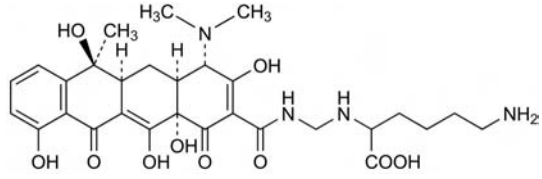
$C_{29}H_{38}N_4O_{10} = 602.6$

CAS—992-21-2

IUPAC Name [4S-(4a,4aα,5aα,6β,12aα)]-N^6-[[[[4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenyl]-carbonyl]amino]methyl]-L-lysine

Synonyms Limeciclina; Tetracycline-L-methylenelysine.

Proprietary Names *Armyl*; *Ciclolysal*; *Mucomycin*; *Tetralysal*; *Tetramyl*.

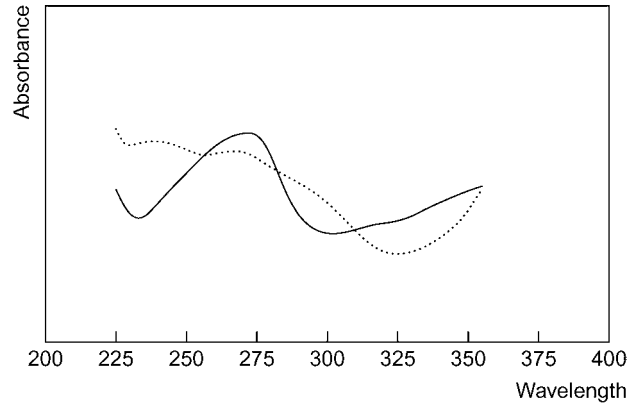


Chemical Properties A water-soluble combination of tetracycline, lysine and formaldehyde with a molecular weight of ~603. A yellow, very hygroscopic powder which darkens on exposure to light and air. Mp above 200°, with slow decomposition. Soluble 1 in <1 of water; slightly soluble in ethanol and methanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), −3.2.

Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—brown-red; Mandelin's test—violet-brown→yellow; Marquis test—orange; sulfuric acid—red-brown.

Thin-layer Chromatography System TA—R_f 0.05, streaking (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—269 (A_1^1=292a), 356 nm; aqueous alkali—239, 267 nm.



Dose Usually 816 mg (the equivalent of 600 mg of tetracycline) daily.

Lynestrenol

Progestational Steroid

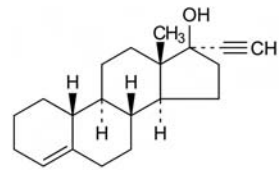
$C_{20}H_{28}O = 284.4$

CAS—52-76-6

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Ethynyl-13-methyl-2,3,6,7,8,9,10,11,12,14,15,16-dodecahydro-1*H*-cyclopenta[a]phenanthren-17-ol

Synonyms Lynenol; lynoestrenol; (17α)-19-norpregn-4-en-20-yn-17-ol.

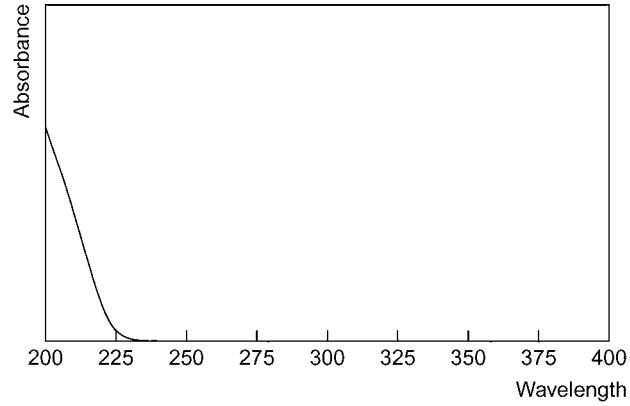
Proprietary Names *Exlutena*; *Exluton(a)*; *Orgametil*; *Orgametril*.



Chemical Properties It is an ingredient of many oral contraceptives, see Sweetman [2009]. A white crystalline powder. Mp 158° to 160°. Practically insoluble in water; soluble 1 in 15 of ethanol, 1 in 8 of chloroform and 1 in 12 of ether. Log *P* (octanol/water), 4.8.

Thin-layer Chromatography System TP—R_f 0.77; system TQ—R_f 0.55; system TR—R_f 0.99; system TS—R_f 0.97.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 683, 1040, 1014, 1053, 813, 1266 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 91, 79, 67, 201, 77, 105, 93, 120.

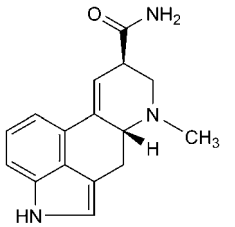
Dose 2.5 mg daily, for courses of 22 days.

Sweetman SC (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.

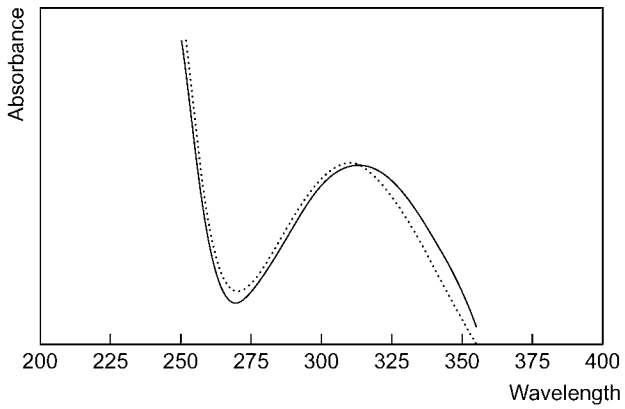
Lysergamide

Hallucinogen
$C_{16}H_{17}N_3O$=267.3
CAS—478-94-4

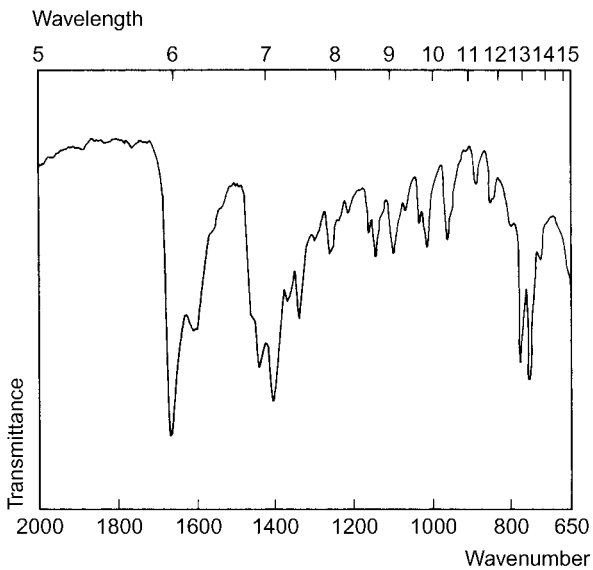
Synonyms 9,10-Didehydro-6-methylergoline-8β-carboxamide; ergine; lysergic acid amide.



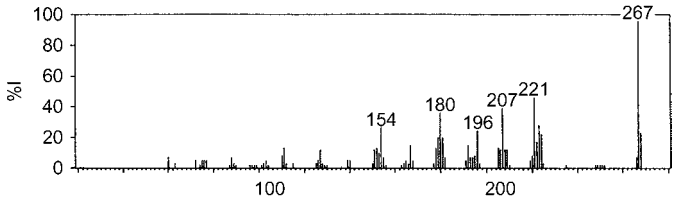
Chemical Properties Lysergamide is found in *Rivea corymbosa*, *Ipomoea* spp. (Convolvulaceae), and ergot. Crystals. It decomposes at 242°.
Colour Test Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.00; system TC—R_f 0.19; system TE—R_f 0.36; system TL—R_f 0.06; system TM—R_f 0.27; system TAE—R_f 0.57; system TAF—R_f 0.51; system TAG—R_f 0.07 (Van Urk reagent, blue).
High Performance Liquid Chromatography System HA—*k* 0.5; system HP—*k* 0.33.
Ultraviolet Spectrum Aqueous acid—313 nm; aqueous alkali—309 nm.



Infrared Spectrum Principal peaks at wavenumbers 1670, 760, 783, 1618, 1158, 1270 cm^{-1} (KBr disk).



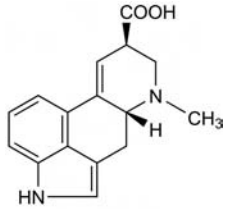
Mass Spectrum Principal ions at *m/z* 267, 221, 207, 180, 223, 154, 196, 268.



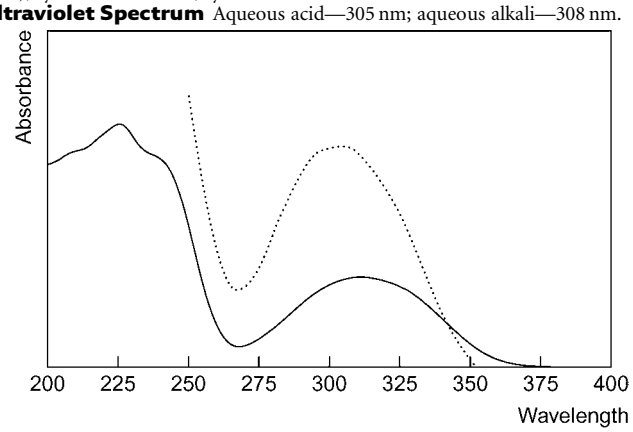
Lysergic Acid

Hallucinogen
$C_{16}H_{16}N_2O_2$ = 268.3
CAS—82-58-6

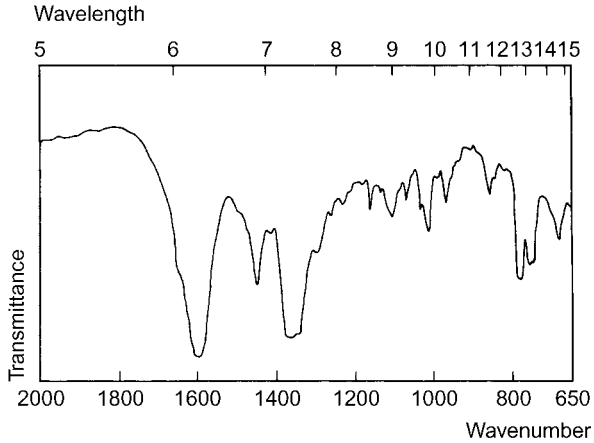
IUPAC Name 7-Methyl-4,6,6*a*,7,8,9-hexahydro-indolo [4,3-*fg*] quinoline-9-carboxylic acid
Synonym 9,10-Didehydro-6-methylergoline-8-carboxylic acid



Chemical Properties A white crystalline powder. Mp 240°, with decomposition. Sparingly soluble in water; soluble in dilute acids and alkalis; moderately soluble in pyridine. pK_a 3.4, 6.3.
Colour Tests Mandelin's test—green-brown; Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.00; system TC—R_f 0.00; system TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TL—R_f 0.00; system TM—R_f 0.00; system TAD—R_f 0.00; system TAE—R_f 0.70; system TAF—R_f 0.16; system TAJ—R_f 0.48; system TAK—R_f 0.07; system TAL—R_f 0.79 (acidified potassium permanganate solution, positive; location under UV light, violet fluorescence; mercuric chloride–diphenylcarbazone reagent, blue; Van Urk reagent, blue).
High Performance Liquid Chromatography System HA—*k* 0.8 (tailing peak); system HP—*k* 0.00; system HY—RI 236.
Ultraviolet Spectrum Aqueous acid—305 nm; aqueous alkali—308 nm.

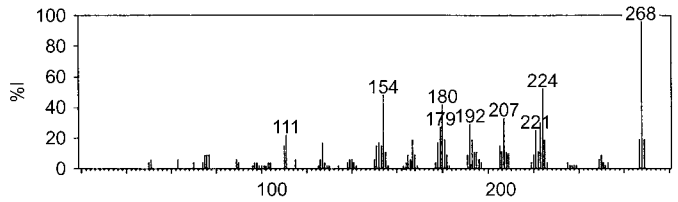


Infrared Spectrum Principal peaks at wavenumbers 1592, 787, 758, 1308, 691, 1015 cm^{-1} (KBr disk).



L

Mass Spectrum Principal ions at *m/z* 268, 224, 154, 180, 207, 223, 192, 179.



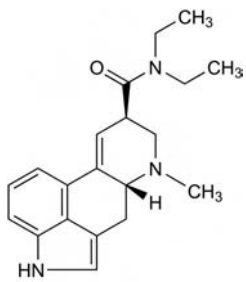
Lysergide

Hallucinogen

$C_{20}H_{25}N_3O$ = 323.4

CAS—50-37-3

Synonyms 9,10-Didehydro-*N*,*N*-diethyl-6-methylergoline-8β-carboxamide; *N*,*N*-diethyl-*d*-lysergamide; LSD; LSD-25; lysergic acid diethylamide.



Chemical Properties A colourless crystalline substance. Mp 80° to 85°. Soluble in water. pK_a 7.5. Log *P* (octanol/water), 2.9. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005]. There is evidence that LSD degrades readily in biological specimens when exposed to light or elevated temperatures. It may also bind to glass containers in acidic solutions.

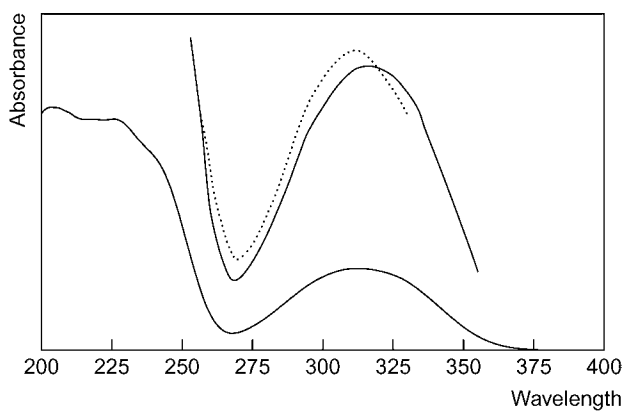
Colour Tests *p*-Dimethylaminobenzaldehyde—violet; Mandelin's test—grey; Marquis test—grey.

Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.03; system TC—R_f 0.39; system TD—R_f 0.30; system TE—R_f 0.56; system TL—R_f 0.24; system TM—R_f 0.70; system TAE—R_f 0.60; system TAF—R_f 0.59; system TAJ—R_f 0.33; system TAK—R_f 0.02; system TAL—R_f 0.59 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—grey; van Urk reagent—blue).

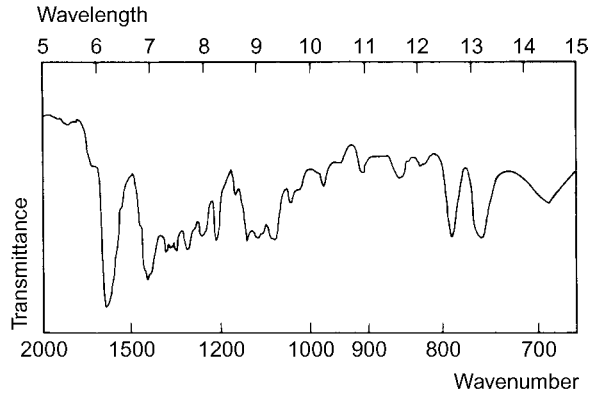
Gas Chromatography System GA—LSD RI 3445, LSD-TMS RI 3595, iso-LSD-TMS RI 3515, M (nor-)-TMS RI 3705, M (nor-)-TMS_2 RI 3515, M (2-oxo-3-OH-)-TMS_2 RI 3430; system GB—RI 3332; system GAL—RT 7.3 min.

High Performance Liquid Chromatography System HA—*k* 0.7; system HP—LSD *k* 1.83, M (2-oxy-) *k* 0.92; system HX—RI 362; system HAA—RT 12.0; system HBE—RT 5.3 min.

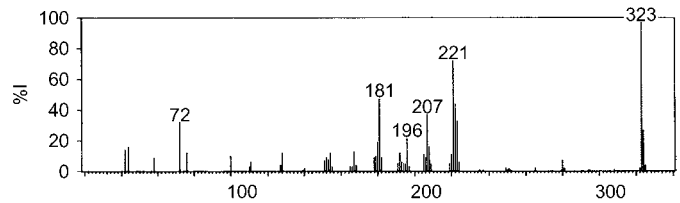
Ultraviolet Spectrum Aqueous acid—315 nm (A_1^1 = 225a); aqueous alkali—310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1626, 1307, 1136, 1066, 1212, 749 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 323, 221, 181, 222, 207, 72, 223, 324.



Quantification

Blood **GC-MS** Column: CP-Sil 8CB-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.4 mL/min. Temperature programme: 200° for 1.5 min to 220° at 30°/min to 280° at 20°/min to 300° at 2°/min for 4.83 min. CI and EI modes. Limit of detection, 0.02 μg/L [Libong *et al.* 2003]. Column: Ultra-2 (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: H_2, 67 cm/s. Temperature programme: 175° for 0.8 min to 298° at 19°/min for 3–5 min. Desorption CI. Limit of quantification, 20 ng/L [Nelson, Foltz 1992].

HPLC Column: LiChroSphere 60 RP-Select B (5 μm). Fluorescence detection (λ_{ex}=320 nm, λ_{em}= 420 nm). Limit of detection, 0.05 μg/L [Bergemann *et al.* 1999].

LC-MS Column: BEH C_{18} (50 × 2.5 mm i.d., 1.7 μm). Mobile phase: 20 mmol/L ammonium acetate buffer (pH 4.0) : acetonitrile (90 : 10 for 1 min to 75 : 25 in 10 min for 1 min), flow rate 0.2 mL/min. API, ESI, MRM acquisition mode. Limit of quantification, 20 ng/L for LSD and iso-LSD, and 50 ng/L for nor-LSD and 2-oxo-3-OH-LSD, limit of detection, 5 ng/L for LSD and iso-LSD, and 10 ng/L for nor-LSD and 2-oxo-3OH-LSD [Chung *et al.* 2009]. Column: Luna CN (150 × 2 mm i.d., 5 μm). Mobile phase: water-0.1% formic acid-2 mmol/L ammonium formate (pH 3.0): acetonitrile-0.1% formic acid-2 mmol/L ammonium formate (80 : 20 to 20 : 80 in 8 min for 4 min), flow rate 0.3 mL/min. ESI. Limit of quantification, 20 ng/L for LSD and nor-LSD, 10 ng/L for iso-LSD, limit of detection, 10 ng/L for LSD and nor-LSD [Favretto *et al.* 2007]. Column: Zorbax SB-C_{18} (30 × 2.1 mm i.d., 3.5 μm). Mobile phase: 5% acetonitrile in 0.05% formic acid : 100% acetonitrile with 0.05% formic acid (5 : 95 for 2 min to 40 : 60 over 10 min), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.01 μg/kg for LD and iso-LSD [Johansen, Jensen 2005]. Column: Spherisorb 5 RP 8S (100 × 2.1 mm i.d., 5 μm). Mobile phase: water : acetonitrile (40 : 60) containing 0.1% formic acid and 2 mmol/L ammonium formate, flow rate 400 μL/min. ESI, MRM acquisition mode. Retention time: 4.3 min. Limit of quantification, 0.02 μg/L for LSD and iso-LSD [Canezin *et al.* 2001]. Column: Eclipse XDB-C_{18} (75 × 4.6 mm i.d., 3.5 μm). Mobile phase: 20 mmol/L ammonium formate (pH 4.3) : methanol (70 : 30 for 5 min to 66 : 44 over 5.5 min for 11 min in total) flow rate 0.7 mL/min. ESI. SIM acquisition mode. Limit of detection, 100 ng/L for LSD and 400 ng/L for OH-LSD [Sklerov *et al.* 2000]. See also de Kanel *et al.* [1998] and Bogusz *et al.* [1998].

Plasma **GC-MS** Column: Ultra-1 dimethylsilicone (12.5 m × 0.2 mm i.d., 0.33 μm) or DB-5 (10 m × 0.25 mm i.d., 1.0 μm). Carrier gas: H_2, 90 cm/s. Temperature programme: 180° for 1 min to 300° at 20°/min for 1 min. ECD. Limit of detection, 0.1 μg/L [Papac, Foltz 1990].

LC-MS Column: Zorbax SB-phenyl (75 × 4.6 mm i.d., 3.5 μm). Mobile phase: ammonium acetate buffer (pH 4.0) : acetonitrile-propan-1-ol (35 : 65), flow rate 250 μL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.05 μg/L for LSD, limit of detection, 0.025 μg/L for LSD and *N*-demethyl-LSD [de Kanel *et al.* 1998].

Serum **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 150° for 2 min to 290° at 30°/min for 25 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.1 μg/L [Musshoff, Daldrup 1997].

HPLC Column: LiChrospher 60 RP Select B HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Mobile phase: 0.05 mol/L phosphate buffer (pH 2.3) : acetonitrile (75 : 25), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 231 nm, λ_{em}= 420 nm). Retention time: 14.4 min for LSD, 15.3 min for LAMPA. Limit of detection, 50 pg [Röhrich *et al.* 2000]. Comparison with radioimmunoassay, limit of detection, 0.5 μg/L [McCarron *et al.* 1990].

LC-MS Column: Superspher RP-18 (125 × 3 mm i.d., 4 μm) Mobile phase: acetonitrile : 50 mmol/L ammonium formate (pH 3.0, 25 : 75), flow rate 0.5 mL/min.

APCI, SIM acquisition mode. Limit of detection, 0.5 μg/L [Bogusz *et al.* 1998]. See Plasma [de Kanel *et al.* 1998].

Urine **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 200° for 1 min to 260° at 50°/min to 290° at 10°/min to 320° at 50°/min for 5 min. SIM acquisition mode. Limit of quantification, 1.0 μg/L for 2-oxo-3-OH-LSD; limit of detection, 0.5 μg/L for 2-oxo-3-OH-LSD) [Burnley, George 2003]. Column: Eclipse XDB-C_{18} (150 × 4.6 mm i.d., 3.5 μm). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 8.0) : acetonitrile (80 : 20) with 0.02% TEA. APCI, positive ion mode. Limit of quantification, 400 ng/L for OH-LSD, limit of detection, 200 ng/L for OH-LSD [Poch *et al.* 1999]. Column: ZB-5 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 20 psi (137.9 kPa). Temperature programme: 175° for 0.8 min to 298° at 20°/min. PICI, SRM acquisition mode. Limit of detection, 10 ng/L for LSD and 2-oxo-3-OH-LSD [Reuschel *et al.* 1999a]. Column: ZB-5 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.4 mL/min. Temperature programme: 150° for 1.5 min to 220° at 30°/min to 280° at 20°/min to 300° at 10°/min for 1.5 min. EI ionisation, CID. Limit of quantification, 80 ng/L, limit of detection, 20 ng/L, [Sklerov *et al.* 1999]. See Blood [Nelson, Foltz 1992]. See also Clarkson *et al.* [1998], Francom *et al.* [1988], Lim *et al.* [1988], Paul *et al.* [1990].

HPLC See Blood [Bergemann *et al.* 1999]. Limit of detection, <1 μg/L [Blum *et al.* 1990]. See Serum [McCarron *et al.* 1990].

LC-MS See Blood. Limit of quantification, 20 ng/L for LSD and iso-LSD, and 50 ng/L for nor-LSD, 2-oxo-3-OH-LSD; limit of detection, 10 ng/L for LSD, iso-LSD, nor-LSD and 2-oxo-3OH-LSD [Chung *et al.* 2009]. Column: RP C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: water : acetonitrile (80 : 20) with 0.1% formic acid, flow rate 200 μL/min. ESi. Limit of quantification, 0.2 ng/L [Chapuis-Hugon *et al.* 2009]. See Blood [Favretto *et al.* 2007]. Column: Atlantis dC_{18} (100 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile : ammonium formate buffer (pH 3.0, 0 : 100 for 1 min to 50 : 50 in 10 min for 2.5 min to 0 : 100 in 0.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 10.4 min. Limit of quantification, 0.2 μg/L, limit of detection, 0.1 μg/L, [Concheiro *et al.* 2007]. Column; Zorbax Eclipse XDB C_8 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 2 mmol/L ammonium acetate in acetonitrile : water (70 : 30), flow rate 0.8 mL/min. ESI, SRM acquisition mode. Limit of quantification, 1 μg/L [Cui *et al.* 2004]. Mobile phase: buffer : acetonitrile (79 : 21 for 2 min to 63 : 37 over 9 min), flow rate 0.8 mL/min. SIM acquisition mode. Limit of quantification, 250 ng/L for LSD and 2-oxo-3-OH-LSD, limit of detection, 250 ng/L for LSD and 2-oxo-3-OH-LSD [Horn *et al.* 2003]. Column; Luna C_{18} (100 × 2.0 mm i.d., 3 μm). Mobile phase: 20 mmol/L acetic acid-5 mmol/L ammonia : acetonitrile (pH 4.5, 80 : 20), flow rate 0.3 mL/min. API, ESI. Limit of detection, 0.02 μg/L [Bodin, Svensson 2001]. See Blood [Canezin *et al.* 2001; Johansen, Jensen 2005; Sklerov *et al.* 2000]. See Plasma [de Kanel *et al.* 1998]. See Serum [Bogusz *et al.* 1998]. See also Hoja *et al.* [1997], Klette *et al.* [2002], Poch *et al.* [2000], Webb *et al.* [1996], White *et al.* [1997] and White *et al.* [1999].

CE Capillary: fused silica (65/60 cm total/effective length, 50 μm i.d.). Running buffer: acetonitrile : methanol : water (5 : 35 : 60) containing 100 mmol/L SDS, 3 mmol/L Brij-30 and 50 mmol/L hydrogen phosphate. LTFS detection. Limit of detection, 20 ppt (with sweeping-MEKC) and 60 ppt (with CSEI-sweep-MEKC) [Fang *et al.* 2002].

Note For a comparison of EMIT and CEDIA photometric immunoassays with DPC radioimmunoassay, see Wiegand *et al.* [2002].

Body Fluids **HPLC-radioimmunoassay** Fluorescence detection. Limit of detection, 0.5 μg/L [Twitchett *et al.* 1978].

Fluoroimmunoassay Limit of detection, 4 μg/L [Hubbard *et al.* 1983].

Vitreous Humour **LC-MS** See Blood [Favretto *et al.* 2007].

Hair **HPLC** See Serum [Röhrich *et al.* 2000].

LC-MS See Urine. Limit of detection, 0.1 μg/g [Chapuis-Hugon *et al.* 2009].

Other **HPTLC** Illicit dosage forms. Limit of detection, 2 ng [Kraus *et al.* 1980].

Note For reviews of methods for determination of LSD and other drugs of abuse for investigation of drug-related driving, see Moeller, Kraemer [2002]; for methods for the determination of LSD in body fluids, see Reuschel *et al.* [1999b], Schneider *et al.* [1998] and Smith, Robinson [1985].

Note Known interferences to LSD detection include ergometrine, ergonovine, methylergometrine, dihydroergotamine, ergocornine, ergocristine, methysergide and ergotamine. Several of these agents are used clinically for various medical conditions.

Disposition in the Body LSD is readily absorbed from all mucous membranes. It can be administered through inhalation and topical application, although oral ingestion is more common. LSD-paper can be placed on skin or in the conjunctival sac for absorption of the drug. It is believed to undergo extensive biotransformation in the liver (hydroxylation and glucuronide conjugation) to inactive metabolites. A very small percentage crosses the blood–brain barrier and it is extensively bound to plasma proteins. Approximately 1% of a dose is excreted unchanged in the urine in 24 h. 2-Oxy-LSD is a major metabolite although 13-OH-LSD and 14-OH-LSD also appear.

As little as 0.5–1.5 μg/kg bodyweight is known to produce hallucinations. LSD is thought to produce its effects by binding to and activating the 5-hydroxytryptamine receptor subtype 2 (5-HT_2).

Single doses of 200–400 μg were administered to 8 subjects; the urinary concentration of LSD or a closely related metabolite ranged from 0.001–0.055 mg/L in the 24 h after ingestion [Taunton-Rigby *et al.* 1973].

Blood Concentration

A single oral dose of 160 μg was administered to 13 subjects; plasma concentrations measured at intervals over a period of 2.5 h varied considerably but were in the range 0–0.009 mg/L [Upshall, Wailling 1972].

A peak plasma concentration of 1.9 μg/L LSD was found 3 h after the administration of an oral dose of 1 μg/kg to a healthy subject. Apparent plasma half-life was 5.1 h [Papac, Foltz 1990].

Toxicity Hallucinatory effects are produced with doses of 200–400 μg but recovery has occurred after much larger doses (up to 10 mg). Plasma concentrations >0.001 mg/L have been associated with toxic effects.

Following a dinner party, 4 men and 4 women (age range 19–39 years) were admitted to hospital after 'snorting' 2 lines of white powder believed to be cocaine. Analysis of the white powder identified it as almost pure (80–90%) LSD tartrate. Emesis and collapse occurred along with sign of sympathetic overactivity, hyperthermia, coma and respiratory arrest. Concentrations of LSD tartrate ranged from 2.1–26 μg/L in serum and 1000–7000 μg/100 mL in gastric fluid. All patients recovered with supportive care [Klock *et al.* 1975].

A 25-year-old man died 16 h after being admitted to hospital. Postmortem toxicological analysis by radioimmunoassay (RIA) and HPLC revealed the following LSD concentrations (μg/L):

Sample	*RIA*	*HPLC*
Antemortem serum	14.4	–
Antemortem plasma	14.8	8
Postmortem blood	4.8	–
Stomach contents	55.2	60
Liver blood	7.2	–

[Fysh *et al.* 1985].

An 8-month-old baby was brought to the emergency department by his father after the baby had ingested 5 blotters of LSD. Charcoal and magnesium sulfate were administered. Both urine and gastric acid tested positive for LSD. The baby was admitted to the paediatric intensive care unit for observation but no further treatment was necessary [Maslanka, Scott 1992].

For a report of the clinical findings in 5 patients with suspected LSD intoxication, see [Blaho *et al.* 1997].

Half-life Plasma half-life, ~2.5 h.

Volume of Distribution ~0.3 L/kg.

Protein Binding Approximately 90%, decreasing to ~65% in overdose subjects.

Dose In psychotherapy, doses of 100 to 750 μg have been given.

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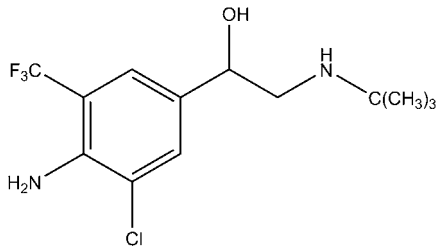
Mabuterol

β_2-*Adrenoceptor Agonist, Bronchodilator, Sympathomimetic*
$C_{13}H_{18}ClF_3N_2O = 310.7$
CAS—56341-08-3

IUPAC Name 1-(4′-Amino-3′-chloro-5′-trifluoromethylphenyl)-2-*tert*-butylaminoethanol

Synonyms Ambuterol; 4-amino-3-chloro-α-[[(1,1-dimethylethyl)amino]methyl]-5-(trifluoromethyl)benzene-methanol; 4-amino-α-[(*tert*-butylamino)methyl]-3-chloro-5-(trifluoromethyl)benzyl alcohol; 1-(4′-amino-3′-chloro-5′-trifluoromethylphenyl)-2-tert-butylaminoethanol.



Chemical Properties Mabuterol is stable in plasma after 3 freeze–thaw cycles and after storage at 4° [Lu *et al.* 2005].

dl-Mabuterol Hydrochloride

$C_{13}H_{18}ClF_3N_2O,HCl = 347.2$
CAS—95656-48-7
Synonyms KF-868; PB-868Cl.
Proprietary Name *Broncholin*
Chemical Properties Crystals. Mp 205° to 206°. Fairly soluble in water.

d-Mabuterol Hydrochloride

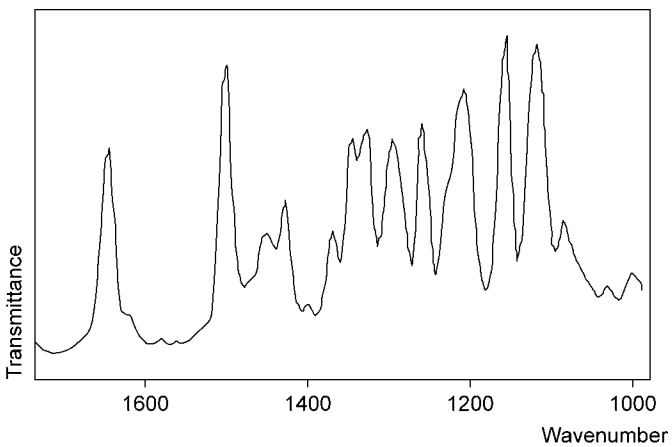
CAS—95656-54-5
Chemical Properties Mp >194°.

l-Mabuterol Hydrochloride

CAS—95656-55-6
Chemical Properties Mp >194°.

Gas Chromatography Column: CpSil-5 CB (TMS derivative; 25 m × 0.25 mm i.d.) DB-5 capillary (MBA derivative, 30 m × 0.25 mm i.d.). Carrier gas: He, 0.9 mL/min. Temperature programme: 80° for 1 min to 180° at 20°/min to 290° at 10°/min for 5 min. FTIR interface. Retention time: 13.5 min. Limit of detection, 1 mg/L [Visser *et al.* 1994].

Infrared Spectrum Principal peaks at wavenumbers 1637, 1494, 1422, 1341, 1323, 1287 cm^{-1} (MBA derivative) [Visser *et al.* 1994].



Quantification

Other **TLC** Rat Urine. Plates: silica gel 60 F_{254} (2 mm). Solent system: (1) benzene : isopropanol : ammonia (80 : 20 : 1); (2) chloroform : ethylacetate : formic acid (50 : 50 : 1); (3) chloroform : ethylacetate : methanol : acetic acid : water (50 : 50 : 35 : 1 : 1). Autoradiographic detection. Limit of detection not reported [Horiba *et al.* 1984].

GC-MS Bovine Urine. Column: HP-5 fused silica (24 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He. Temperature programme: 120° to 300° at 11°/min for 5 min. EI ionisation at 70 eV. Limit of quantification, 1.54 μg/L; limit of detection, 0.5 μg/L [Spisso *et al.* 2000]. Bovine hair. Column: CP-Sil 5CB-MS capillary (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 90° for 2 min to 210° at 12.5°/min for 5 min to 300° at 15°/min. EI ionisation or chemical ionisation. Limit of quantification, 5.0 and 1.7 μg/kg for black and white hair, respectively; limit of detection, 2.9 and 1.1 μg/kg for black and white hair, respectively [Haasnoot *et al.* 1998]. Bovine Urine. Column: HP 1 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 0.1 min to 245° at 15°/min to 300° at 30°/min for 4.0 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.7 and 0.3 μg/L for the MBA and BBA derivatives, respectively [Ramos *et al.* 1998]. Bovine Urine and Lliver. Column: DB-5 Permabond SE-52 fused silica (25 m × 0.32 mm i.d., 0.5 μm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 310° at 10°/min. EI ionisation at 70 eV, positive ion mode, MRM acquisition mode. Limit of detection, 0.05 and 0.1 μg/kg for urine and liver, respectively [van Vyncht *et al.* 1996].

HPLC Rat Plasma. Column: Agilent C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: 20 mmol/L sodium dihydrogen phosphate (pH 6.6) : acetonitrile (80 : 20), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of quantification, 10 μg/L [Lu *et al.* 2005]. Equine Plasma. Column: LiChrosorb (select B) C_{18} (125 × 4.0 mm i.d., 7.0 μm). Mobile phase: phosphate buffer (pH 4.0 to 4.1) : acetonitrile with 0.2 mmol/L sodium 1-heptanesulfonate (77 : 23). Electrochemical detection. Limit of detection, 2 μg/L [Qureshi, Eriksson 1988].

LC-MS Bovine Urine. Column: Inertsil C_8 (150 × 2.1 mm i.d., 5 μm). Mobile phase: 0.06 mol/L ammonium formate buffer (pH 3.2) : acetonitrile (93 : 7 to 40 : 60 at 20 min to 93 : 7 at 30 min for 5 min), flow rate 0.3 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of detection, 0.73 μg/L [Dickson *et al.* 2005]. Bovine Liver and Retina. Column: Inertsil C_8 (150 × 2.1 mm i.d., 5 μm). Mobile phase: ammonium formate buffer (pH 3.2) : acetonitrile (93 : 7 to 50 : 50 at 16 min to 93 : 7 at 17 min for 12 min), flow rate 0.3 mL/min. APCI, positive ion mode. Limit of detection, 0.2 and 2 μg/kg for liver and retina, respectively [Fesser *et al.* 2005]. Bovine Liver. Column: Symmetry C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: methanol : 0.032% trifluoroacetic acid (22 : 78), flow rate 0.3 mL/min. ESI, positive ion mode, full scan mode. Limit of detection, 0.1 μg/kg [De Wasch *et al.* 1998]. Bovine Urine and Liver. Column: α-chrom C_{18} (25 cm × 3 mm i.d., 5 μm). Mobile phase: acetonitrile–10 mmol/L ammonium acetate (pH 7.0)–water (77 : 12 : 11) : acetonitrile–25 mmol/L ammonium acetate (pH 3.5, 65 : 36; 100 : 0 for 5.5 min to 0 : 100 at 5.6 min to 15 min), flow rate 0.5 mL/min. EI ionisation at 70 eV, positive ion mode, MRM acquisition mode. Limit of detection, 0.05 μg/kg [van Vyncht *et al.* 1996].

Disposition in the Body Mainly metabolised by oxidative deamination to 6 metabolites.

Therapeutic Concentration

Six healthy male volunteers were administered a single oral dose of 40 μg radiolabelled mabuterol. Peak concentrations of 96 to 160 ng/L were reached between 1.5 and 3.2 h [Guentert *et al.* 1984].

Toxicity Oral LD_{50} values were between 200 and 319 mg/kg when malbuterol was studied in mouse, rat, rabbit and dog [Amemiya *et al.* 1984].

Volume of Distribution 5.8 L/kg.

Clearance 3.0 mL/min/kg.

Distribution in Blood Blood : plasma ratio 0.92 to 1.14.

Dose 50 μg twice a day.

Amemiya K *et al.* (1984). Toxicology of mabuterol. *Arzneimittelforschung* 34: 1680–1684.

De Wasch K *et al.* (1998). LC-MS-MS to detect and identify four beta-agonists and quantify clenbuterol in liver. *Analyst* 123: 2701–2705.

Dickson LC *et al.* (2005). Determination of beta-agonist residues in bovine urine using liquid chromatography-tandem mass spectrometry. *J AOAC Int* 88: 46–56.

Fesser AC *et al.* (2005). Determination of beta-agonists in liver and retina by liquid chromatography–tandem mass spectrometry. *J AOAC Int* 88: 61–69.

Guentert TW *et al.* (1984). Single dose pharmacokinetics of mabuterol in man. *Arzneimittelforschung* 34: 1691–1696.

Haasnoot W *et al.* (1998). A fast immunoassay for the screening of beta-agonists in hair. *Analyst* 123: 2707–2710.

Horiba M *et al.* (1984). Pharmacokinetic studies of mabuterol, a new selective beta 2-stimulant II: Urinary metabolites of mabuterol in rats and their pharmacological effects. *Arzneimittelforschung* 34: 1668–1679.

Lu X *et al.* (2005). Enantioselective pharmacokinetics of mabuterol in rats studied using sequential achiral and chiral HPLC. *Biomed Chromatogr* 19: 703–708.

Qureshi G *et al.* (1988). Determination of clenbuterol and mabuterol in equine plasma by ion-pair liquid chromatography with electrochemical detection. Chromatographic and electrochemical characteristics. *J Chromatogr* 441: 197–205.

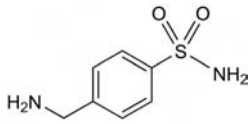
Ramos F *et al.* (1998). Beta2-adrenergic agonist residues: simultaneous methyl- and butylboronic derivatization for confirmatory analysis by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 716: 366–370.

Spisso BF *et al.* (2000). Determination of beta2-agonists in bovine urine: comparison of two extraction/clean-up procedures for high-resolution gas chromatography-mass spectrometry analysis. *J Anal Toxicol* 24: 146–152.
vanVyncht G *et al.* (1996). Gas and liquid chromatography coupled to tandem mass spectrometry for the multiresidue analysis of beta-agonists in biological matrices. *J Chromatogr A* 750: 43–49.
Visser T *et al.* (1994). Confirmational analysis of beta-agonists by cryotrapping gas chromatography–Fourier transform infra-red spectrometry. *Analyst* 119: 2681–2685.

Mafenide

Sulfonamide, Antibacterial
$C_7H_{10}N_2O_2S$ = 186.2
CAS—138-39-6

IUPAC Name 4-(Aminomethyl)benzenesulfonamide
Synonyms Bensulfamide; benzamsulfonamide; 4-homosulfanilamide; sulfa benzamine.



Chemical Properties A white powder. Mp 151° to 154°. Sparingly soluble in water; soluble in methanol and in dilute acids and alkalis. Log *P* (octanol/water), −0.6.

Mafenide Acetate

$C_7H_{10}N_2O_2S,C_2H_4O_2$ = 246.3
CAS—13009-99-9
Proprietary Names *Mafatate*; *Sulfamylon*.
Chemical Properties A white crystalline powder. Mp 151° to 152°. Freely soluble in water and methanol; sparingly soluble in ethanol.

Mafenide Hydrochloride

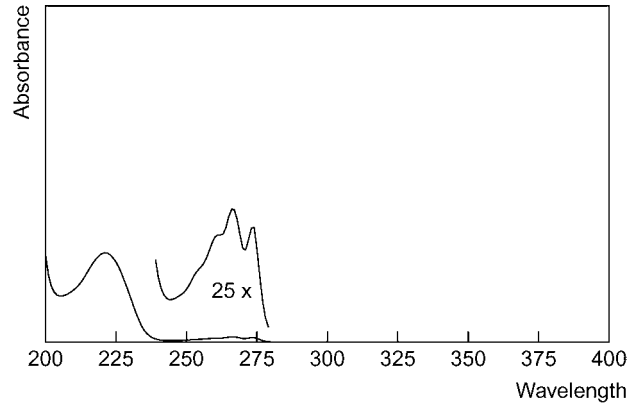
$C_7H_{10}N_2O_2S$,HCl = 222.7
CAS—138-37-4
Synonyms Homosulfaminum; maphenide.
Chemical Properties A colourless or white crystalline powder. Mp about 260°. Soluble 1 in 1.7 of water and 1 in 100 of ethanol; practically insoluble in chloroform and ether.

Mafenide Propionate

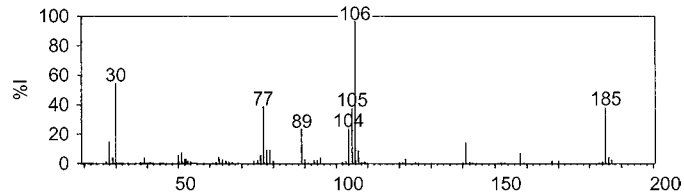
$C_7H_{10}N_2O_2S,C_3H_6O_2$ = 260.3
CAS—12001-72-8
Proprietary Name *Sulfomyl*
Chemical Properties Colourless crystals. Mp 158°. Readily soluble in water.

Colour Test Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.49; system TD—R_f 0.01; system TE—R_f 0.27; system TF—R_f 0.01; system TAD—R_f 0.02; system TAE—R_f 0.22; system TAJ—R_f 0.00; system TAK—R_f 0.72; system TAL—R_f 0.93 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—mafenide RI 2340, mafenide-Me_2 RI 1920, mafenide-Me_3 RI 1900, mafenide-Me_4 RI 1870, M (acetyl-) RI 2425, M (acetyl-)-Me RI 2300.
Ultraviolet Spectrum Aqueous acid—267 (A_1^1=41a), 274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1151, 1316, 1299, 897, 1089, 1592 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 106, 30, 77, 185, 105, 104, 89, 141.

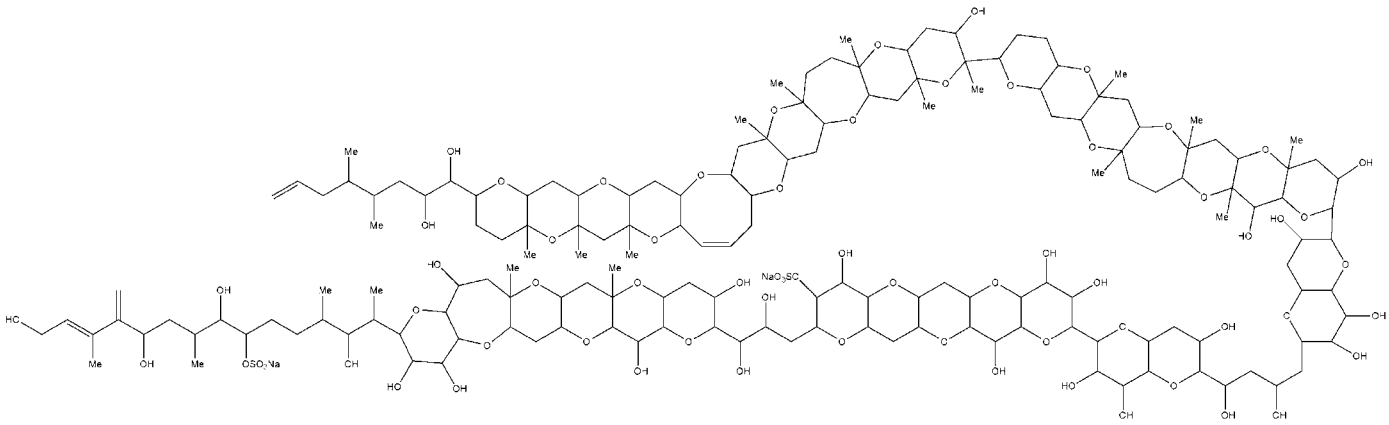


Quantification
Serum **TLC** Limit of detection, 1 mg/L [Steyn 1977].
Disposition in the Body Mafenide is absorbed from wounds into the circulation and metabolised to *p*-carboxybenzenesulfonamide which is excreted in the urine.
Use Topically as a cream containing 8.5% mafenide (as the acetate).

Steyn JM (1977). Thin-layer chromatographic determination of mafenide [(p-aminomethyl) benzenesulphonamide] in human serum. *J Chromatogr* 143: 210–213.

Maitotoxin

Marine Neurotoxin
Synonym MTX



Structure of maitotoxin

Chemical Properties Family of lipid-soluble polyether toxins responsible for ciguatera food poisoning common in tropical and subtropical regions. Maitotoxin was first detected in the gut of the surgeon fish *Ctenochaetus striatus* ('maito' is the Tahitian name for surgeon fish) and later in the dinoflagellate *Gambierdiscus toxicus* [Terao *et al.* 1988]. It is the largest secondary metabolite as yet isolated from any living creature [Nicolaou *et al.* 2008]. Three strains can be purified to homogeneity: maitotoxin-1 (M_r 3422 for the sodium salt), maitotoxin-2 (M_r 3298 for the sodium salt) and maitotoxin-3 (M_r 1060 for the sodium salt) [Holmes, Lewis 1994].

Maitotoxin-1

$C_{164}H_{256}O_{68}S_2Na_2 = 3422$
CAS—59392-53-9
Synonym MTX-1
Chemical Properties Colourless amorphous solid [Escobar *et al.* 1998].

Maitotoxin-2

MW=3298
Synonym MTX-2
Chemical Properties White powder.

Maitotoxin-3

MW=1060
Synonym MTX-3
Chemical Properties White amorphous solid.

Capillary Electrophoresis Column: Polyvinylalcohol-coated fused silica capillary (50.5/42 cm total/effective length, 50 µm i.d.). Running buffer: 40 mmol/L phosphate buffer (pH 6.8). DAD (λ= 195 nm). Retention time: 10.4 min. Limit of detection, 50 pg [Bouaicha *et al.* 1997].
Ultraviolet Spectrum Methanol—230 nm [Holmes, Lewis 1994; Yokoyama *et al.* 1988].
Toxicity The primary action of MTX in the BC_3H_1 muscle line was a pore- or channel-forming activity of a non-classical type, resulting in a massive uptake of Ca^{2+} [Sladeczek *et al.* 1988]. Ingestion of toxic surgeon fish causes neurological disturbances, gastrointestinal afflictions, and cardiovascular disorders in humans [Bagnis 1968].

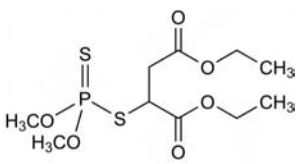
LD_{50} in mice (IP), 0.05 µg/kg for MTX-1, 0.08 µg/kg for MTX-2 [Holmes, Lewis 1994]. LD_{50} in mice (IP), 0.06 µg/kg for monodesulfo-MTX, 15 µg/kg for didesulfo-MTX, 0.13 µg/kg for MTX [Murata *et al.* 1991].
Use It is used as a pharmacological research tool [Gusovsky, Daly 1990].

Bagnis R (1968). Clinical aspects of ciguatera (fish poisoning) in French Polynesia. *Hawaii Med J* 28: 25–28.
Bouaicha N *et al.* (1997). A new method for determination of maitotoxin by capillary zone electrophoresis with ultraviolet detection. *Toxicon* 35: 955–962.
Escobar LI *et al.* (1998). Maitotoxin, a cationic channel activator. *Neurobiology (Bp)* 6: 59–74.
Gusovsky F, Daly JW (1990). Maitotoxin: a unique pharmacological tool for research on calcium-dependent mechanisms. *Biochem Pharmacol* 39: 1633–1639.
Holmes MJ, Lewis RJ (1994). Purification and characterisation of large and small maitotoxins from cultured Gambierdiscus toxicus. *Nat Toxins* 2: 64–72.
Murata M *et al.* (1991). Effect of maitotoxin analogues on calcium influx and phosphoinositide breakdown in cultured cells. *Toxicon* 29: 1085–1096.
Nicolaou KC *et al.* (2008). Chemical synthesis of the GHIJKLMNO ring system of maitotoxin. *J Am Chem Soc* 130: 7466–7476.
Sladeczek F *et al.* (1988). New insights into maitotoxin action. *Eur J Biochem* 174: 663–670.
Terao K *et al.* (1988). Histopathological studies of experimental marine toxin poisoning. II. The acute effects of maitotoxin on the stomach, heart and lymphoid tissues in mice and rats. *Toxicon* 26: 395–402.
Yokoyama A *et al.* (1988). Some chemical properties of maitotoxin, a putative calcium channel agonist isolated from a marine dinoflagellate. *J Biochem* 104: 184–187.

Malathion

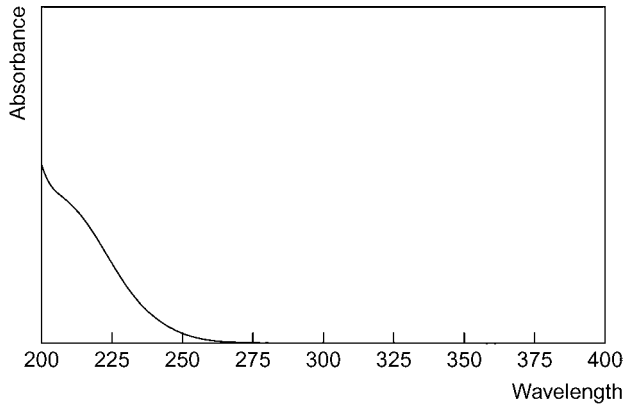
Insecticide, Organophosphate
$C_{10}H_{19}O_6PS_2 = 330.4$
CAS—121-75-5

IUPAC Name Diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate
Synonyms Carbofos; carbophos; compound 4047; diethyl[(dimethoxyphosphinothioyl)thio]butanedioate; OMS-1.
Proprietary Names *Derbac-M*; *Ovide*; *Prioderm*; *Quellada M*; *Suleo-M*.

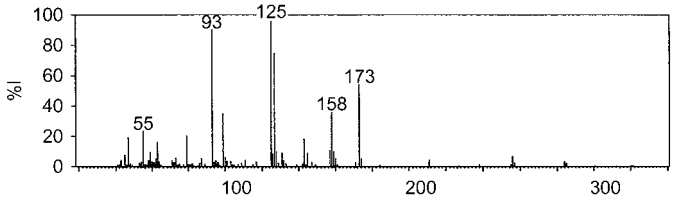


Chemical Properties A colourless to light amber liquid that decomposes in strong acid or high humidity. Although stable in light, it decomposes at high temperatures. Fp 2.85°. Refractive index 1.4985. Slightly soluble in water; miscible with many organic solvents. Log *P* (octanol/water), 2.4.
Colour Tests Palladium chloride—brown; phosphorus test—yellow; sodium nitroprusside (method 2)—violet.

Thin-layer Chromatography System TE—R_f 0.99; system TW—R_f 0.74; system TX—R_f 0.31; system TY—R_f 0.53; system TZ—R_f 0.82; system TAA—R_f 0.33; system TAB—R_f 0.24; system TAC—R_f 0.14; system TAE—R_f 0.84.
Gas Chromatography System GA—malathion RI 1925, M (malaoxon) RI 1890; system GK—RRT 1.03 (relative to caffeine).
High Performance Liquid Chromatography System HZ—RT 5.4 min.
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1018, 1748, 1174, 1209, 824, 1250 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 125, 93, 127, 173, 158, 99, 55, 79.



Quantification

Blood **Colorimetry** [Faragó 1967].

GC Column: Alltech EC-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 120° for 3 min to 230° at 10°/min for 4 min. NPD. Limit of detection, 45 µg/L [Tsoukali *et al.* 2005]. NPD and ECD. Limit of detection, malathion and other pesticides [Garcia-Repetto *et al.* 2001].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 290° at 20°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.17 mg/L, limit of detection, 0.09 mg/L [Park *et al.* 2009]. Limit of detection, malathion and other pesticides and their metabolites [Ostrea *et al.* 2009]. Column: Rtx-CL (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 100 kPa. EI ionisation, SIM acquisition mode. Retention time: 26.9 min. Limit of detection, 2–5 µg/L [Liu, Pleil 2002]. Column: HP-5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 1 min to 290° at 10°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.05 µg/g [Musshoff *et al.* 2002]. Column: Ultra-1 (25 m × 0.33 mm i.d., 200 µm). Carrier gas: He, 1.7 mL/min. Temperature programme: 60° for 3 min to 290° at 12°/min for 10 min. EI ionisation. Limit of quantification, 1.52 µg/L, limit of detection, 0.45 µg/L [Garcia-Repetto *et al.* 2001]. See also [Namera *et al.* 1997]

HPLC Limit of detection, for malathion and other pesticides, 100 ng [Sharma *et al.* 1990].

Plasma **GC** See Blood. [Tsoukali *et al.* 2005].

Serum **TLC** High performance. UV detection. Limit of detection, 0.12 mg/L [Futagami *et al.* 1997].

HPLC DAD (λ= 230 nm). Limit of detection, for malathion and other pesticides, 0.05–6.8 mg/L [Cho *et al.* 1997].

LC-MS Column: C(18). Mobile phase: 10 mmol/L ammonium formate-methanol. Limit of quantification, 0.25–1.25 mg/L for malathion and other pesticides, limit of detection, 0.125–1 mg/L for malathion and other pesticides [Inoue *et al.* 2007].

Urine **GC** FID. Limit of detection, 40–130 µg/L (dialkyl phosphate residues) [Reid, Watts 1981]. FID. Limit of detection, 2 µg/L for malathion dicarboxylic acid metabolite and 5 µg/L for malathion α-monocarboxylic acid metabolite [Bradway, Shafik 1977]. See Blood [Tsoukali *et al.* 2005].

GC-MS Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 60° for 1.75 min to 270° at 20°/min for 20 min. EI ionisation at 70 eV. Limit of quantification, 0.24 ng/L, limit of detection, 0.07 ng/L [Cruz-Márquez *et al.* 2001].

HPLC See Serum [Cho *et al.* 1997].

LC-MS ESI. Limit of quantification, 0.1–1 µg/L for malathion and other pesticides [Olsson *et al.* 2003]. APCI. Limit of detection, 20–500 ng/L for malathion and metabolites [Baker *et al.* 2000]. Column: Partisil 5 ODS-3 (250 × 4.6 mm i.d.).

M

Mobile phase: acetonitrile : 0.2% glacial acetic acid (60 : 40), flow rate 1.0 mL/min. APCI, positive ion mode. Limit of detection, 0.5 μg/L (malathion metabolites) [Beeson *et al.* 1999].

CSF **GC** See Blood. Limit of detection, 40 μg/L [Tsoukali *et al.* 2005].

Meconium **GC-MS** See Blood [Ostrea *et al.* 2009].

Hair **GC-MS** See Blood [Ostrea *et al.* 2009].

Kidney **GC** See Blood. Limit of detection, 50 μg/L [Tsoukali *et al.* 2005].

Liver **GC** See Blood. Limit of detection, 50 μg/L [Tsoukali *et al.* 2005].

HPLC See Blood [Sharma *et al.* 1990].

Lung **HPLC** See Blood [Sharma *et al.* 1990].

Tissues **Colorimetry** See Blood [Faragó 1967].

Other **GC-MS** Environmental Media (soil, dog hair). See Blood [Liu, Pleil 2002].

Disposition in the Body Malathion is absorbed after oral ingestion but absorbed only slowly and to a small extent through intact skin. It is metabolised by conversion to malaoxon, the toxic keto analogue, and by hydrolysis to malathion α-mono- and dicarboxylic acids, which are the major metabolites. Other hydrolysis products include dimethylthiophosphoric acid and dimethyldithiophosphoric acid, which have been detected in the urine of subjects exposed to malathion. After ingestion, up to 25% is excreted in the 24 h urine as ether-extractable phosphates, mostly in the first 8 h.

Toxicity Malathion is less toxic than most other organophosphorus pesticides; the estimated minimum lethal dose is 25 g; the maximum permissible atmospheric concentration is 10 mg/m^3 and the maximum acceptable daily intake is 20 μg/kg.

In 6 cases of suspected poisoning caused by malathion, postmortem tissue concentrations were as follows: blood 175–517 mg/L, lungs 77–330 μg/g, liver 198–383 μg/g, kidneys 280–616 μg/g, spleen 175–475 μg/g, brain 84–387 μg/g, heart 160–315 μg/g, muscles 8–40 μg/g, urine 33–189 mg/L and gastric contents 452–989 mg/L [Jadhav *et al.* 1992].

An 80-year-old woman who committed suicide by ingesting malathion mixed with a fruit drink had an antemortem blood concentration of 23.9 mg/L [Zivot *et al.* 1993].

In a fatality involving a 40-year-old woman who ingested a malathion pesticide (350–400 mL), postmortem tissue concentrations were as follows: blood 1.7–1.9 mg/L, gastric contents 975–981 mg/L and liver not detected [Thompson *et al.* 1998].

Half-life Derived from urinary excretion data, ~3 h.

Dose Topically in concentrations of 0.5 or 1% in the treatment of pediculosis.

Baker SE *et al.* (2000). Quantification of selected pesticide metabolites in human urine using isotope dilution high-performance liquid chromatography/tandem mass spectrometry. *J Expo Anal Environ Epidemiol* 10: 789–798.

Beeson MD *et al.* (1999). Isotope dilution high-performance liquid chromatography/tandem mass spectrometry method for quantifying urinary metabolites of atrazine, malathion, and 2,4-dichlorophenoxyacetic acid. *Anal Chem* 71: 3526–3530.

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Mandelic Acid

Antibacterial (Urinary)

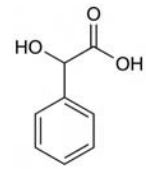
$C_8H_8O_3$ = 152.1

CAS—90-64-2; 611-72-3 (±)

IUPAC Name α-Hydroxybenzeneacetic acid

Synonyms Amygdalic acid; phenylglycollic acid; racemic mandelic acid.

Proprietary Name *Uromaline*



Chemical Properties White crystals, which turn yellow on exposure to light. Mp 119° to 121°. Soluble 1 in 6 of water, 1 in 1 of ethanol, 1 in 45 of chloroform and 1 in 6 of ether. pK_a 3.4 (25°). Log *P* (octanol/water), 0.6.

Ammonium Mandelate

$C_8H_7O_3NH_4$ = 169.2

CAS—530-31-4

Chemical Properties A white, very hygroscopic, crystalline powder, which discolours on exposure to light. Very soluble in water; sparingly soluble in ethanol.

Calcium Mandelate

$(C_8H_7O_3)_2Ca$ = 342.4

CAS—134-95-2

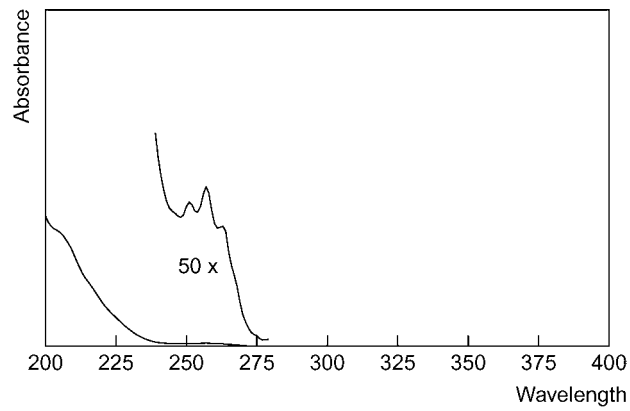
Synonym Calcium amygdalate

Chemical Properties A white crystalline powder. Soluble 1 in 100 of water and 1 in 4500 of ethanol (90%).

Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

Gas Chromatography System GA—RI 1487.

Ultraviolet Spectrum Aqueous acid—251 (A^1_1=10.9b), 257 (A^1_1=13.7b), 262 nm (A^1_1=11.2b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1710, 1060, 1245, 1185, 690, 720 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 107, 79, 77, 51, 152, 105, 50, 78.

Quantification

Urine **GC** ECD or FID. Limit of detection, 1 mg/L and 5 to 8 mg/L, respectively, for mandelic acid enantiomers [Kezic *et al.* 2000]. Limit of detection, 30 mg/L for mandelic and phenylglycoxylic acids [Lanchote *et al.* 1994]. Limit of detection, 1 mg/L for mandelic and phenylglycoxylic acids [Dills *et al.* 1991].

HPLC For method of quantification for mandelic acid and other aromatic hydrocarbon metabolites, see Burrini [1998]. UV detection. Limit of detection, 5 mg/L for mandelic and phenylglycoxylic acids [Chua *et al.* 1993].

Disposition in the Body Mandelic acid is a urinary metabolite of several drugs and toxic chemicals, and is also found endogenously in normal urine at concentrations of up to 5 mg/L.

Dose Mandelic acid has been given in doses of 12 g daily, usually as the calcium or ammonium salt.

Burrini C (1998). The simultaneous determination of hippuric acid, o-, m-, p-methylhippuric acids, mandelic acid and phenylglyoxylic acid in urine by HPLC. *Med Lav* 89: 404–411.

Chua SC *et al.* (1993). Determination of mandelic acid and phenylglyoxylic acid in the urine and its use in monitoring of styrene exposure. *J Anal Toxicol* 17: 129–132.

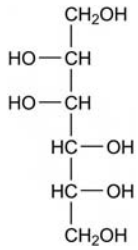
Dills RL *et al.* (1991). Capillary gas chromatographic method for mandelic and phenylglyoxylic acids in urine. *Int Arch Occup Environ Health* 62: 603–606.

Kezic S *et al.* (2000). Determination of mandelic acid enantiomers in urine by gas chromatography and electron-capture or flame ionisation detection. *J Chromatogr B Biomed Sci Appl* 738: 39–46.
Lanchote VL *et al.* (1994). An improved method for the simultaneous determination of mandelic and phenylglyoxylic acids by gas chromatography. *J Anal Toxicol* 18: 143–146.

Mannitol

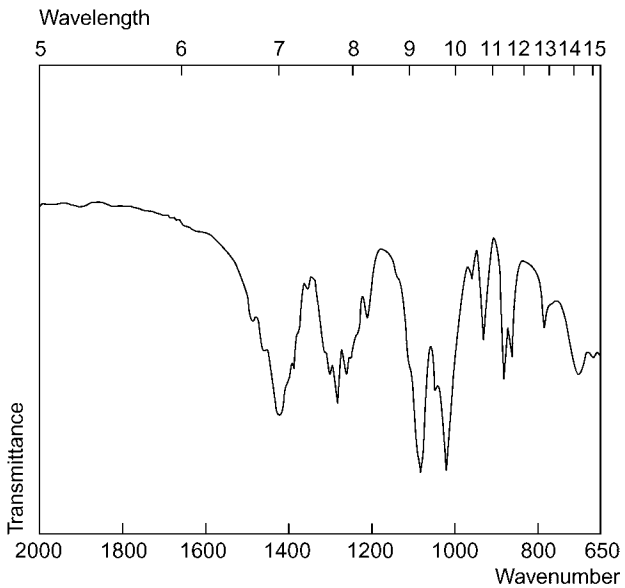
Diuretic
$C_6H_{14}O_6$ = 182.2
CAS—69-65-8

IUPAC Name 1,2,3,4,5,6-Hexanehexol
Synonyms Cordycepic acid; E421; manita; manitol; manna sugar; mannite; D-mannitol; mannitolum; NCI-C50362.
Proprietary Names *Diosmol*; *Manicol*; *Mannidex*; *Osmitrol*; *Osmosal*; *Resectisol*.

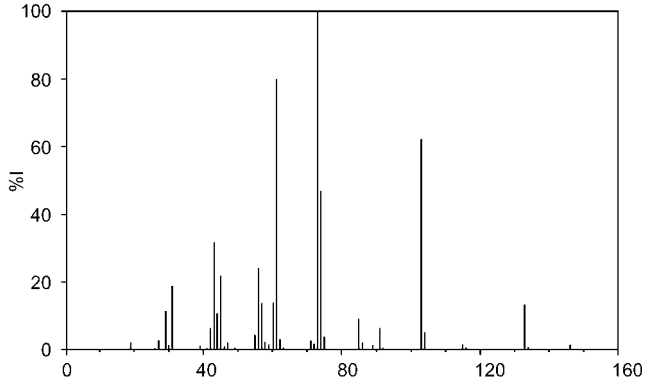


Chemical Properties A white odourless crystalline powder or granules with a sweet taste. Freely soluble in water (182 g/L at 25°); slightly soluble in pyridine, aniline, glycerol (1 g in 18 mL); very slightly soluble in alcohol; practically insoluble in ether. Mp 166° to 168°. Bp 290° to 295° at 3 to 5 mmHg. pK_a 13.5 (25°). Log *P* (octanol/water), −3.10.
Colour Test 3 mL of 100 g/L solution of pyrocatechol with 6 mL sulfuric acid. Add 0.3 mL of a 5 g of carbon dioxide free water diluted to 50 mL with solvent. Heat for 30 s.—Pink colour.
High Performance Liquid Chromatography Column: $LiNH_2$ Lichrosorb NH_2 (130 × 4 mm i.d.). Mobile phase: acetonitrile : deionised water (70 : 30), flow rate 2 mL/min. Detection: refractive index. Retention time: 6.8 min [Mills, Roberson 1993].

Column: sulfonated styrene–divinylbenzene copolymer with zinc ions Gelpack, GL-C64Z (150 × 6 mm i.d., 10 µm). Temperature: 80°. Mobile phase: acetonitrile : water (80 : 20), flow rate 1 mL/min. MS detection (APCI (atmospheric pressure chemical ionisation), SIM at *m/z* 217 for chloride ion). Retention time: 12.5 min [Niwa *et al.* 1993].
Infrared Spectrum Principal peaks at wavenumbers 1019, 1083, 1421, 1280 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 73, 61, 103, 74, 43, 56, 45, 31 (mannitol); *m/z* 115, 187, 139, 145, 157, 170, 259, 289 (mannitol hexaacetate).



Quantification
Blood **HPLC** UV detection (λ=228 nm). Limit of detection, 1 to 5 pg/L (benzoyl derivatives) [Oehlke *et al.* 1994].
Urine **GC** FID. Limit of detection, 0.9 µg/L [Shippee *et al.* 1992].

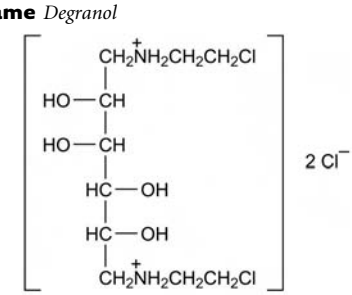
HPLC Column: carbohydrate Alltech 700 CH (300 × 6.5 mm, 10 µm). Temperature: 85°. Mobile phase: water, flow rate 0.5 mL/min. IS: cellbiose. ELS detection. Retention time: mannitol, 13.3 min; IS, 7.6 min. Limit of detection, 0.65 mg/L [Marsilio *et al.* 1998]. Column: HPIC-AS6 Dionex (250 × 40 mm). Mobile phase: 150 mmol/L sodium hydroxide, flow rate 1.0 mL/min. Electrochemical detection. Retention time: 3 min. Amperometric detection. Limit of detection, 0.3 mg/L [Fleming *et al.* 1990].
Disposition in the Body Only a small amount of mannitol is absorbed from the gastrointestinal tract after ingestion and is eliminated unchanged in urine (80% within 3 h). It is metabolised only very slightly to glycogen in the liver. Following IV injection it is excreted rapidly by the kidneys before metabolism can take place in the liver. It is freely filtered by the glomeruli and undergoes tubular reabsorption. It does not cross the blood–brain barrier, unless in very high concentrations, or penetrate the eye. Distributed in the extracellular fluid.
Toxicity Mannitol is mildly toxic by ingestion, IP and IV routes. It may cause blood pressure elevation, bladder tubule changes, nausea or vomiting. When heated to decomposition it emits acrid smoke and fumes.
Bioavailability Negligible oral bioavailability.
Half-life In healthy volunteers the plasma half-life is 0.25 to 1.7 h and in those with renal failure, 6 to 36 h.
Clearance Approximately 125 mL/h/kg in men and 116 mL/h/kg in women.
Dose Adult: range from 50 to 100 g by IV infusion of a 5 to 25% solution. Children: 0.25 to 2 g/kg body weight.

Fleming SC *et al.* (1990). Rapid and simultaneous determination of lactulose and mannitol in urine, by HPLC with pulsed amperometric detection, for use in studies of intestinal permeability. *Clin Chem* 36(5): 797–799.
Marsilio R *et al.* (1998). Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics. *Clin Chem* 44: 1685–1691.
Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 2: 1304–1305.
Niwa T *et al.* (1993). Analysis of polyols in uremic serum by liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr* 613: 9–14.
Oehlke J *et al.* (1994). Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis. *J Chromatogr B Biomed Appl* 665(1): 105–111.
Shippee R *et al.* (1992). Simultaneous determination of lactulose and mannitol in urine of burn patients by gas-liquid chromatography. *Clin Chem* 38(3): 343–345.

Mannomustine

Antineoplastic
$C_{10}H_{22}Cl_2N_2O_4,2HCl$ = 378.1
CAS—551-74-6

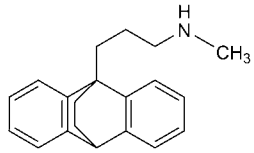
IUPAC Name 1,6-Bis(2-chloroethylamino)-1,6-dideoxy-D-mannitol dihydro chloride
Synonyms BCM; mannitol mustard.
Proprietary Name *Degranol*



Chemical Properties A white crystalline powder. Mp 241°, with decomposition. Soluble 1 in 2 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether. Log *P* (octanol/water), −1.8.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1065, 1013, 1298, 1284, 1102, 1581 cm^{-1} (mannomustine hydrochloride, KBr disk).
Dose Mannomustine hydrochloride has been given in doses of 50 to 100 mg daily, by IV injection.

Maprotiline

Tetracyclic Antidepressant
$C_{20}H_{23}N = 277.4$
CAS—10262-69-8
IUPAC Name *N*-Methyl-9,10-ethanoanthracene-9(10*H*)-propanamine

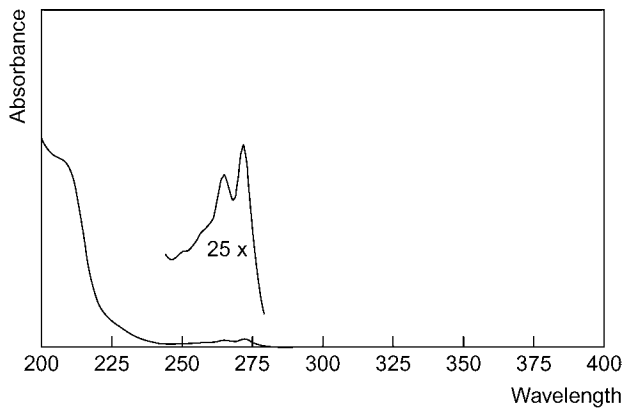


Chemical Properties Mp 92° to 94°. pKa 10.5. Log *P* (octanol/water), 4.52 [Meylan, Howard 1995; Wille *et al.* 2005]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

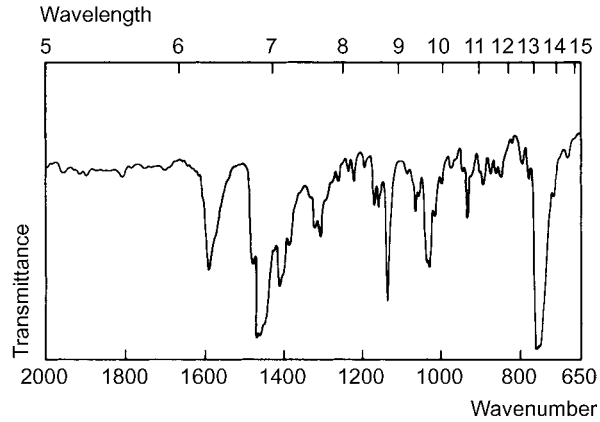
Maprotiline Hydrochloride

$C_{20}H_{23}N,HCl = 313.9$
CAS—10347-81-6
Synonyms Ba-34276; maprotilini hydrochloridum.
Proprietary Names *Aneural*; *Deprilept*; *Ludiomil*; *Maludil*; *Maprolu*; *Melodil*; *Mirpan*; *Psymion*.
Chemical Properties A white crystalline powder. Mp 230° to 232°. Slightly soluble in water; freely soluble in methanol and chloroform; practically insoluble in isooctane. Stable in human plasma for more than 3 months when stored at –2 2° [Aymard *et al.* 1997]. Plasma standards stored frozen at –2 0° are stable for 6 months [Salonen, Scheinin 1983]. Samples in whole blood stored at –4° are stable for 9 months [Alkalay *et al.* 1979].

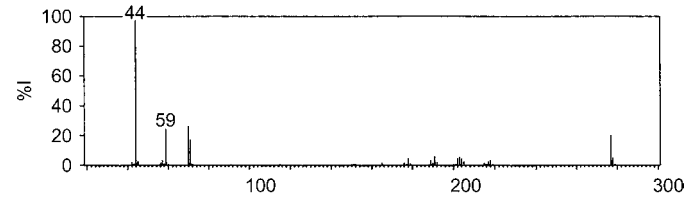
Colour Test Liebermann's reagent—orange-brown; Mandelin's test—blue; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.15; system TB—R_f 0.18; system TC—R_f 0.05; system TE—R_f 0.36; system TL—R_f 0.02; system TAE—R_f 0.06; system TAF—R_f 0.71; system TAL—R_f 0.50.
Gas Chromatography System GA—maprotiline RI 2390, M (nor-) RI 2293, M (desamino-di-OH-) RI 2570; system GB—maprotiline RI 2440, M (nor-) RI 2404, M (desamino-di-OH-) RI 2603; system GM—maprotiline RRT 1.0861, M (nor-) RRT 1.107 (both relative to iprindole); system GS—maprotiline RT 21.0 min.
High Performance Liquid Chromatography System HA—maprotiline *k* 2.2, desmethylmaprotiline *k* 1.1; system HF—maprotiline *k* 4.92; system HX—RI 438; system HY—RI 389; system HZ—RT 6.6 min; system HAA—RT 15.5 min; system HAZ—maprotiline *k* 1.44.
Ultraviolet Spectrum Aqueous acid—265, 272 nm ($A_1^1 = 52b$).



Infrared Spectrum Principal peaks at wavenumbers 759, 1140, 1592, 1032, 1308, 935 cm^{-1} (maprotiline hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 70, 59, 277, 71, 191, 278, 203.



Quantification

Blood **GC** Column: 5% phenyl 95% methyl silicone (12.7 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 15 mL/min. Temperature programme: 180° for 2 min to 280° at 15°/min for 3.33 min. NPD. Retention time: 6.6 min. Limit of detection not reported [Keller, Zollinger 1997]. Column: 1% OV-225 on 80/100 mesh Supelcoport (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30–40 mL/min. Temperature programme: 200 to 230°. ECD. Retention time: 3.65 min. Limit of detection, 10 µg/L [Alkalay 1982]. Column: 3% SP-2250 on 100/120 Supelcoport (6′ [1.8 m]). Carrier gas: He, 40 mL/min. Temperature: 270°. NPD. Retention time: 58 s. Limit of detection not reported [Rejent, Doyle 1982]. Column: 3% HI-EFF-8BP on Chromosorb W HP 100/120 mesh (300 × 3.0 mm i.d.). Carrier gas: 20 mL/min. Temperature: 245°. NPD. Limit of detection, maprotiline 20 µg/L, desmethylmaprotiline 50 µg/L [Sioufi, Richard 1980]. Column: 3% methylsilicone on Gas-Chrom Q (4′ × 3 mm i.d.). Carrier gas: N_2, 45 mL/min. Temperature programme: 230°. Limit of detection, <10 ng/sample [Geiger *et al.* 1975].

GC-MS Column: Supelco SPB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 5 min to 280° at 20°/min. SIM acquisition mode. Limit of detection, 25 ng/g [Namera *et al.* 1998]. Column: HP-5 Ultra 2 cross-linked 5% phenylmethyl silicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 2.25 min to 180° at 40°/min to 290° at 10°/min for 15 min. EI ionisation at 70 eV, continuous scan mode. Limit of detection, 0.5 mg/L [Polettini 1996]. Column: 1.5% Poly S-179 on 80/100 mesh (1.0 m × 2 mm i.d.). Carrier gas: CH_4, 20 mL/min. Temperature programme: 240 to 250°. CI. Retention time: 1.3–1.6 min. Limit of detection, 0.5 µg/L [Alkalay *et al.* 1979].

HPLC Column: C_8 reversed phase TSK gel Super-Octyl (100 × 4.6 mm i.d., 2 µm) or Hypersil MOS-C_8 (100 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L potassium dihydrogen phosphate (pH 7.0) : methanol (40 : 60), flow rate 0.6 mL/min. UV detection (λ = 254 nm). Retention time, maprotiline 5.6, desmethylmaprotiline 7.8 min. Limit of quantification, 0.05 mg/L [Tanaka *et al.* 1997]. Column: Spherisorb S5CN (250 × 3.2 mm i.d., 6.2 mm o.d.). Mobile phase: acetonitrile : methanol : 5 mmol/L sodium hydrogen phosphate (pH 7; 35 : 35 : 30), flow rate 1.5 mL/min. UV detection (λ = 215 nm). Retention time: ~10 min. Limit of detection not reported [Rejent, Doyle 1982].

Plasma **GC** Column: DB-17 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 140° to 220° at 20°/min to 270° at 2°/min. NPD. Retention time: 20.96 min. Limit of quantification, 200 µg/L [Ulrich, Martens 1997]. Column: fused silica capillary 5% phenylmethyl silicone (25 m × 0.31 mm i.d., 0.5 µm). Carrier gas: He, 30 mL/min. Temperature programme: 105° for 0.5 min to 295° at 25°/min. NPD. Retention time: 11.11 min. Limit of quantification, <10 ng [Drebit *et al.* 1988]. Column: 5% OV-17 on Gas-Chrom Q 100/120 mesh (1.83 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 255°. FID. Retention time: 11.5 min. Limit of detection, 2 µg/L [Charette *et al.* 1981]. See Blood [Geiger *et al.* 1975].

GC-MS Column: J & W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min for 2.5 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 2 µg/L [Wille *et al.* 2007]. Column: Varian factorFOUR VF-5ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature

programme: 90° for 0.5 min to 180° at 50°/min for 10 min to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection not reported [Wille *et al.* 2005]. Column: 1% OV-17 on 100/120 mesh Gas Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 225°. Multiple ion detection, 70 eV. Retention time: 3.0 min. Limit of detection, maprotiline and desmethylmaprotiline 2 µg/L [Jindal *et al.* 1980].

HPLC Column: Symmetry C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.067 mol/L potassium dihydrogen phosphate (pH 3.0) : acetonitrile (65 : 35), flow rate 1.2 mL/min. UV detection ($\lambda = 226$ or 400 nm). Retention time: 10.92 min, k' 0.82. Limit of quantification, 5 µg/L [Aymard *et al.* 1997]. Column: TSkgel ODS-80 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 100 mmol/L acetate buffer (pH 3.2; 2 : 3), flow rate 1.0 mL/min. Chemiluminescence detection. Retention time: 39.5 min. Limit of detection, 0.1 µg/L [Ishida *et al.* 1995]. Column: Nucleosil 50-7 (300 × 4.5 mm i.d., 7 µm). Mobile phase: hexane : absolute ethanol (95 : 5), flow rate 1.8 mL/min. Fluorometric detection ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 420$ nm). Retention time: 3.8 min. Limit of detection, ~10 µg/L [Breyer-Pfaff *et al.* 1984]. Column: Zorbax Sil (250 × 4.6 mm i.d.). Mobile phase: ammonium hydroxide : methanol (2 : 998), flow rate 1.5 mL/min. UV detection ($\lambda = 214$ and 254 nm). Limit of detection, 10 µg/L [Sutfin *et al.* 1984]. Column: µBondapak C_{18}(300 × 3.9 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L potassium phosphate buffer (pH 2.5; 30 : 70), flow rate 2.0 mL/min. UV detection ($\lambda = 205$ nm). Limit of detection, 11 nmol/L [Salonen, Scheinin 1983]. Column: µBondapak C_{18} (300 × 4.6 mm i.d., 10 µm) or MC-18 (300 × 4.6 mm i.d., 5 µm). Mobile phase: 0.2 mol/L phosphate buffer (pH 4.7) : acetonitrile (80 : 20), flow rate 1.5 mL/min. UV detection ($\lambda = 214$ nm). Limit of detection, 3 ng [Wong, Waugh 1983]. See also Kuss, Feistenauer [1981].

LC-MS Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.2 mg/L, limit of detection, 0.15 mg/L [Shinozuka *et al.* 2006].

TLC For a TLC method, see Prinoth and Mutschler [1984].

Serum **GC** Column: 1.4% Carbowax 20M plus 1.4% KOH on GasChrom Q 60/80 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 210°. FID. Limit of detection, 40 nmol/L [Kärkkäinen, Seppälä 1980]. Column: SPB-1 100% polymethylsiloxane (60 m × 0.75 mm i.d., 1.0 µm). Carrier gas: He, 8 mL/min. Temperature programme: 260°. NSD. Retention time: approx. 8.5 min. Limit of detection, 800 µg/L [Rifai *et al.* 1988]. Column: 3% HI-EFF-8BP on Gas Chrom Q 80/100 mesh (1.2 m × 2 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature programme: 240°. AFID. Limit of quantification, 10 µg/L [Gupta *et al.* 1977].

GC-MS Column: 3% OV-17 80/100 mesh (1.2 m × 2 mm). Carrier gas: CH_4, 0.25 to 0.35 mmHg. Temperature programme: 270°. Multiple ion detection, CI. Limit of detection, 50 µg/L [Skrinska *et al.* 1984].

HPLC Column: LiChroCART Superspher 60 RP-select B (250 cm × 4 mm i.d., 4 µm). Mobile phase: water–TEA (pH 2.0) : acetonitrile (70 : 30), flow rate 1.5 mL/min. Fluorescence detection ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 300$ nm). Limit of quantification, 10.0 µg/L, limit of detection, 3.3 µg/L [Waschgler *et al.* 2002]. Column: CLC-ODS reversed phase (150 × 6 mm i.d.). Mobile phase: 0.1 mol/L phosphate buffer (pH 3.0) containing perchlorate (3 : 2), flow rate 1.2 mL/min. UV detection ($\lambda = 220$ nm). Limit of detection, 5 µg/L [Fukuchi *et al.* 1990]. Column: TSkgel ODS-80TM (150 × 4.0 mm i.d.). Mobile phase: acetonitrile : 100 mmol/L potassium phosphate buffer (pH 2.7; 32.5 : 67.5) containing 0.2 g/L sodium 1-heptane sulfonate, flow rate 1.0 mL/min. UV detection ($\lambda = 210$ nm). Limit of detection, ~10 µg/L [Matsumoto *et al.* 1989]. Column: Supelcosil-CN (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 7.0) : methanol (60 : 25 : 15), flow rate 1.7 mL/min. UV detection ($\lambda = 211$ nm). Retention time: 10.8 min. Limit of detection, 10 µg/L [Ketchum *et al.* 1983]. See also Koteel *et al.* [1982], Kristinsson [1981].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 6.7 µg/L [Kirchherr, Kühn-Velten 2006]. Column: Xterra MS C_{18} (50 × 2.1 mm i.d., 5 µm). ESI, positive ion mode, MRM acquisition mode. Retention time: 4.75 min. Limit of detection, 10 µg/L [Sauvage *et al.* 2006]. Column: µPorasil (300 × 3.9 mm i.d., 10 µm). Mobile phase: absolute ethanol : acetonitrile : *tert*-butylamine (98 : 2 : 0.05), flow rate 2.0 mL/min. UV detection ($\lambda = 214$ nm). Limit of detection, 15 µg/L [Beierle, Hubbard 1983].

Urine **GC** See Plasma [Drebit *et al.* 1988]. See Blood [Geiger *et al.* 1975; Rejent, Doyle 1982].

HPLC See Blood [Rejent, Doyle 1982; Tanaka *et al.* 1997].

Bile **GC** See Blood [Keller, Zollinger 1997].

Stomach Contents **GC** See Blood [Rejent, Doyle 1982; Keller, Zollinger 1997].

HPLC See Blood [Rejent, Doyle 1982].

Brain **GC** See Blood [Rejent, Doyle 1982].

HPLC See Blood [Rejent, Doyle 1982; Tanaka *et al.* 1997].

Hair **GC-MS** Column: HP-1 100% methyl silicone. Temperature programme: 100° for 2 min to 300° at 15 K/min for 10 min. SIM acquisition mode. Limit of detection, 5–50 ng/sample [Pragst *et al.* 1997].

LC-MS Column: RP-C_8-select B (125 × 2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate-0.1% formic acid (pH 3) : acetonitrile-0.1% formic acid (pH 3.0; 90 : 10 to 70 : 30 at 6.6 min to 30 : 70 at 26.6 min to 10 : 90 at 33.3 min). Positive ion mode, full scan mode or MRM acquisition mode. Limit of detection, 0.1 ng/mg [Müller *et al.* 2000].

Liver **GC** See Blood [Rejent, Doyle 1982].

HPLC See Blood [Rejent, Doyle 1982; Tanaka *et al.* 1997]. See also Rana *et al.* [2008], Yufu *et al.* [1984].

Skull **GC** Column: WCOT SE-30 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 44 cm/s. Temperature programme: 180° to 260° at 10°/min. Retention time: 21.07 min. Limit of detection not reported [Fernandez *et al.* 1985].

Disposition in the Body Slowly but completely absorbed following oral administration. Concentrations in the CSF are 2–13% of those in serum. Maprotiline is found in breast milk. It appears to undergo significant first-pass metabolism; bioavailability ~70%. After oral administration, ~66% of a dose is excreted in the urine and 30% in the faeces over a period of 21 days; <10% of the dose is excreted as unchanged drug. The principal metabolite is the desmethyl derivative, which has been shown to be active in animals, but hydroxylation also occurs to form phenolic derivatives, which may be further converted to aromatic methoxy ethers or excreted as glucuronide conjugates. *N*-Oxidation also occurs and maprotiline *N*-oxide has been reported to be active; numerous minor metabolites have been identified in urine.

Therapeutic Concentration Blood concentrations vary considerably between subjects and there appears to be no clear correlation between clinical response, dose, side-effects, and blood or plasma concentrations. Peak blood concentrations are usually attained 3–8 h after a single dose.

After a single oral dose of 75 mg to 6 subjects, peak blood concentrations of 0.012–0.037 mg/L (mean 0.023) and peak plasma concentrations of 0.016–0.031 mg/L (mean 0.024) were attained in 4–8 h [Maguire *et al.* 1980].

After daily oral doses of 50, 100, and 150 mg to 78 subjects, steady-state blood concentrations were: 0.02–0.15 mg/L (mean 0.07), 0.07–0.25 mg/L (mean 0.14), and 0.14–0.32 mg/L (mean 0.22) [Riess *et al.* 1975].

Following daily oral doses of 150 mg to 4 subjects for 22 days, steady-state serum concentrations were 0.05–0.24 mg/L for maprotiline and 0.02–0.13 mg/L for desmethylmaprotiline [Gupta *et al.* 1977].

Five inpatients were administered a single oral dose of 75 mg maprotiline. The following morning, mean serum concentration was 107 µg/L with inter-individual variations ranging from 75–158 µg/L [Kärkkäinen, Seppälä 1980].

Plasma concentrations of maprotiline in 20 patients ingesting 125 mg per day varied from 34–225 µg/L (128 ± 56 µg/L [461 ± 202 nmol/L] [Breyer-Pfaff *et al.* 1984].

Toxicity

The following postmortem tissue distribution was reported in a suicide case: axillary blood 6.2 mg/L, cardiac blood 2.0 mg/L, bile 161 mg/L, kidney 34.0 µg/g, liver 82.0 µg/g [Robinson *et al.* 1977].

In a fatality resulting from the ingestion of 4.5–6 g maprotiline, the following postmortem tissue concentrations were reported: blood 44.5 mg/L, brain 158 µg/g, liver 605 µg/g, urine 12.6 mg/L [Rejent, Doyle 1982].

A 47-year-old depressed female prescribed Ludiomil (maprotiline hydrochloride) and Mellaril (thioridazine hydrochloride) went missing. Her remains were found approximately a month later. The concentration of maprotiline was 20.4 µg/g in the skull [Fernandez *et al.* 1985].

A 52-year-old woman took an unknown quantity of Ludiomil coated tablets (75 mg). At postmortem, the following concentrations were found:

	Maprotiline (mg/L)	*N-Desmethylmaprotiline (mg/L)*
Peripheral blood	8.6	1.6
Heart blood	7.3	ND
Duodenal contents	518	23
Small intestine contents	491	26
Stomach contents	2771	77
Bile	137	6

ND, not determined.
[Keller, Zollinger 1997].

Following an overdose with maprotiline, hair concentration was 3.1 ng/mg [Müller *et al.* 2000].

Half-life Plasma half-life, 20–70 h (mean 45).

Volume of Distribution 23–70 L/kg (mean 52).

Clearance Plasma clearance, 6–20 mL/min/kg (mean 15).

Distribution in Blood Blood : plasma ratio, 1.7. However, the plasma : whole blood ratio has been reported to vary between individuals and may also vary between single and multiple dosing [Drebit *et al.* 1988].

Protein Binding Approximately 90%.

Note For a review of the pharmacokinetics of maprotiline, see Pinder *et al.* [1977]; for a review of the metabolism of some 'second'- and 'fourth'-generation antidepressants, see Rotzinger *et al.* [1999].

Dose Maprotiline hydrochloride 30 to 150 mg daily; doses of up to 225 mg daily may be required in severely depressed hospitalised patients.

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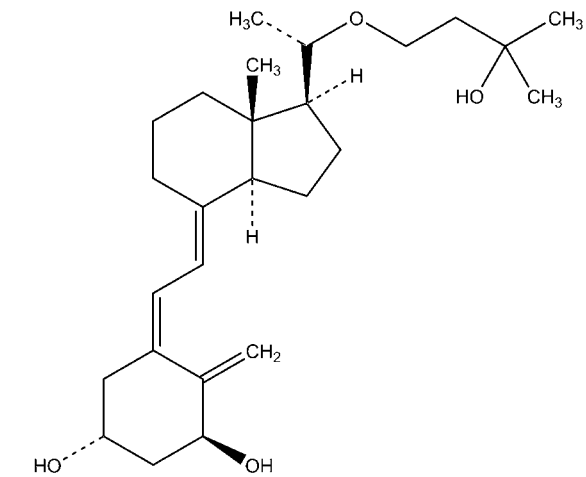
Maxacalcitol

Antihyperparathyroid, Antipsoriatic, Vitamin D Activity
$C_{26}H_{42}O_4$ = 418.6
CAS—103909-75-7

IUPAC Name (1*R*,3*S*,5*Z*)-5-[(2*E*)-2-[(1*S*,3a*S*,7a*S*)-1-[(1*S*)-1-(3-Hydroxy-3-methylbutoxy)ethyl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol

Synonyms 1α,25-Dihydroxy-22-oxavitamin D3; (+)-(5*Z*,7*E*,20*S*)-20-(3-hydroxy-3-methylbutoxy)-9,10-secopregna-5,7,10(19)-triene-1α,3β-diol; MC-1275; (1*R*,3*S*,5*Z*)-4-methylene-5-[(2*E*)-[(1*S*,3a*S*,7a*S*)-octahydro-1-[(1*S*)-1-(3-hydroxy-3-methylbutoxy)ethyl]-7a-methyl-4*H*-inden-4-ylidene]ethylidene]-1,3-cyclohexanediol; OCT; 22-oxacalcitriol; 22-oxa-1α,25-dihydroxyvitamin D_3; 22-oxa-1,25(OH)2D_3; Sch-209579.

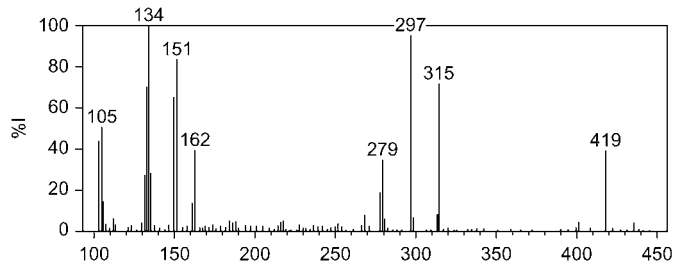
Proprietary Names *Oxarol*; *Prezios*.



Chemical Properties Colourless crystals. Mp 122°.

Ultraviolet Spectrum Ethanol—263 nm.

Mass Spectrum Principal ions at *m/z* 78, 131, 103, 503, 454, 80, 217, 544 (tri-TMS, pyro form), 75 eV [Ishigai *et al.* 1998].



Quantification

Plasma **GC-MS** Column: cross-linked methylsilicon fused silica (30 m × 0.1 mm i.d.). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 280° at 16°/min to 320° at 4°/min for 5 min. EI ionisation at 75 eV. Retention times (TMS derivatives): 12.2 min (pyro form), 13.0 min (isopyro form); 20-oxo-hexanor metabolite 8.7 min (pyro form), 9.3 min (isopyro form); 24*R*- hydroxylated metabolite 13.2 min, and 24*S*-hydroxylated metabolite 13.3 min (pyro forms); 25*R*-hydroxylated metabolite 13.8 min and 25*S*-hydroxylated metabolite 13.9 min (pyro forms). Limit of detection, 50 µg/L [Ishigai *et al.* 1997].

LC-MS Column: Capcell Pak C_{18} SG120 (250 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : water (40 : 60), flow rate 1 mL/min. APCI, positive ion mode. Retention time: 25.8 min. Limit of detection, 5 µg/L [Ishigai *et al.* 1997].

Serum **LC-MS** Column: Capcell Pak C_{18} UG120 (250 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (90 : 10), flow rate

0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 4.5 min. Limit of quantification, 20 ng/L [Ishigai *et al.* 1998].

***Other* HPLC** Human Keratinocytes. Column: Zorbax-SIL (80 × 6.2 mm i.d., 3 μm). Mobile phase: hexane : isopropanol : methanol (91 : 7 : 2), flow rate 1.0 mL/min. UV detection (λ = 265 nm). Retention time: approx. 11.0 min. Limit of quantification not reported [Masuda *et al.* 1996]. Ointment. Column: YMC-Pack SIL A-004 (300 × 4.6 mm i.d.). Mobile phase: *n*-hexane : isopropanol : methanol (135 : 8 : 2), flow rate 2.0 mL/min. UV detection (λ = 265 nm). Limit of quantification not reported [Yamaguchi *et al.* 2006].

LC-MS Rat Skin. Column: C_{18} (150 × 2.0 mm i.d.). Mobile phase: methanol : 10 mmol/L ammonium acetate (85 : 15), flow rate 0.2 mL/min. API, positive ion mode, SIM acquisition mode. Limit of quantification not reported [Yamaguchi *et al.* 2006].

Disposition in the Body Readily absorbed from the gastrointestinal tract. The presence of bile is essential for adequate intestinal absorption; absorption may be decreased in patients with decreased fat absorption. *In vitro* metabolism studies of maxacalcitol in human keratinocytes have shown that it is extensively metabolised into a variety of hydroxylated and side-chain-truncated compounds. These include the polar compounds hexanor-1α,20-dihydroxyvitamin D_3, the 24- and 26-hydroxylated forms of maxacalcitol, and less polar compounds such as 3-*epi*-maxacalcitol and 2 further dehydrates (at the 24 and 25 positions, two pairs of epimers), all of which exhibit different degrees of activity. Vitamin D analogues and metabolites circulate in the blood bound to a specific α-globulin. Vitamin D analogues can be stored in adipose and muscle tissue for long periods of time. They are slowly released from such storage sites and from the skin where they are formed in the presence of sunlight or ultraviolet light. Vitamin D compounds and their metabolites are excreted mainly in the bile and faeces, with only small amounts appearing in urine; there is some enterohepatic recycling but it is considered to have a negligible contribution to vitamin D status.

Dose Given IV at a dose of 2.5 to 10 μg three times weekly; the dose may be gradually increased if necessary to a maximum of 20 μg three times weekly.

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Mazindol

Anorectic

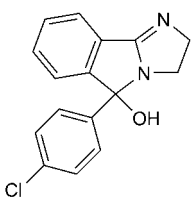
$C_{16}H_{13}ClN_2O$=284.7

CAS—22232-71-9

IUPAC Name 5-(4-Chlorophenyl)-2,3-dihydroimidazo[2,1-*a*]isoindol-5-ol

Synonym 5-(4-Chlorophenyl)-2,5-dihydro-3*H*-imidazo[2,1-*a*]isoindol-5-ol

Proprietary Names *Absten S*; *Dasten*; *Diestet*; *Fagolipo*; *Liofindol*; *Magrilon*; *Magrinex*; *Mazanor*; *Mazildene*; *Sanorex*; *Teronac*.



Chemical Properties Crystals. Mp 198° to 199°. Insoluble in water; soluble in ethanol. pK_a 8.6. Log *P* (octanol/water), 4.09 [Meylan, Howard 1995]. Mazindol is stable in plasma at room temperature for short-term (24 h) and long-term (30 day) periods. The short-term (24 h) stability of mazindol in human plasma at –70°, –20°, and 4° was acceptable although the sample at room temperature rapidly degraded. Stability in plasma was unaffected by 3 freeze-thaw cycles [Kim *et al.* 2009]. Mazindol was unstable in plasma left at room temperature and at 4° [Kaddoumi *et al.* 2001].

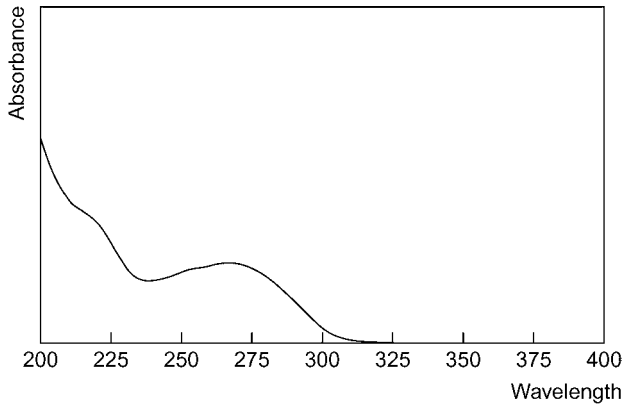
Colour Test Sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.07; system TC—R_f 0.13; system TE—R_f 0.53; system TL—R_f 0.13; system TAE—R_f 0.46; system TAF—R_f 0.65; system TAJ—R_f 0.04; system TAL—R_f 0.24.

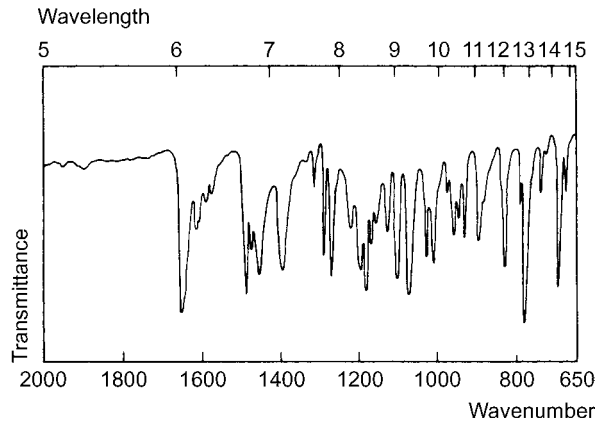
Gas Chromatography System GA—mazindol RI 2325, mazindol-AC RI 2705; system GB—mazindol RI 2504.

High Performance Liquid Chromatography System HA—*k* 1.8; system HC—*k* 0.20; system HX—RI 357; system HY—RI 286; system HZ—RT 3.6 min.

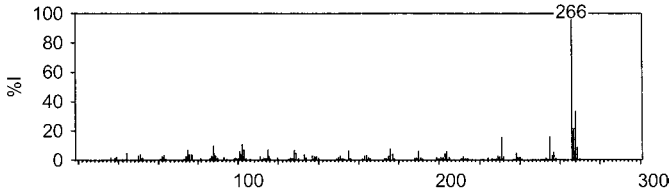
Ultraviolet Spectrum Aqueous acid—271 nm (A^1_1 = 509a); aqueous alkali—269 (A^1_1 = 170b), 275 nm (A^1_1 = 173b).



Infrared Spectrum Principal peaks at wavenumbers 763, 1656, 1063, 1175, 674, 1093 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 266, 268, 267, 255, 231, 102, 88, 176.



Quantification

***Plasma* HPLC** Column: Daisopak SP-120-5-ODS-BP (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile: 0.067 mol/L phosphate buffer (pH 3.5; 24 : 76), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: 17 min. Limit of quantification, 0.1 μg/L, limit of detection, 0.07 μg/L [Kaddoumi *et al.* 2001].

LC-MS Column: Capcell Pak MGII C_{18} (50 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 20 mmol/L ammonium formate (50 : 50, pH 3.5), flow rate 0.2 mL/min. ESI. Limit of quantification, 0.1 μg/L [Kim *et al.* 2009].

***Urine* GC-MS** Column: HP-1 cross-liked methyl silicone (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 1.15 mL/min. Temperature programme: 80° for 2 min to 280° at 40°/min for 1 min. SIM or full scan acquisition mode. Retention time: 7.23 min. Limit of detection, 100 μg/L [Valentine, Middleton 2000].

Disposition in the Body Absorbed after oral administration and slowly excreted in the urine, partly unchanged and partly as metabolites.

Therapeutic Concentration

Twenty-four healthy male volunteers were administered 2 mg mazindol orally. The mean maximum plasma concentration was 5.07 ± 1.7 μg/L, reached at 3.27 ± 1.1 h [Kim *et al.* 2009].

Half-life Approximately 12 to 24 h.

Dose 2 to 3 mg daily.

Kaddoumi A *et al.* (2001). High performance liquid chromatographic determination of mazindol in human plasma. *Analyst* 126: 1963–1968.

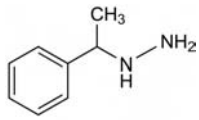
Kim SS *et al.* (2009). Validated method for determination of mazindol in human plasma by liquid chromatography/tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1011–1016.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Valentine JL, Middleton R (2000). GC-MS identification of sympathomimetic amine drugs in urine: rapid methodology applicable for emergency clinical toxicology. *J Anal Toxicol* 24: 211–222.

Mebanazine

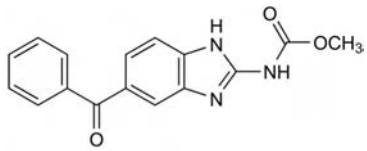
Antidepressant
$C_8H_{12}N_2$ = 136.2
CAS—65-64-5
IUPAC Name α-Methylbenzylhydrazine



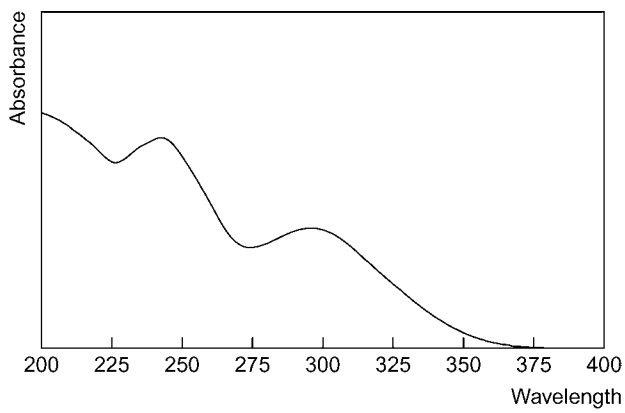
Chemical Properties Practically insoluble in water, ethanol and ether; soluble in chloroform.
Colour Tests Nessler's reagent—black; palladium chloride—black; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.48; system TC—R_f 0.69; system TL—R_f 0.63.
Gas Chromatography System GA—RI 1240.
High Performance Liquid Chromatography System HA—*k* 0.2.
Ultraviolet Spectrum Aqueous acid—251, 257, 261, 267 nm.
Infrared Spectrum Principal peaks at wavenumbers 699, 1175, 760, 1199, 1292, 1070 cm^{-1} (Nujol mull).
Dose Mebanazine has been given in doses of 5 to 30 mg daily.

Mebendazole

Anthelmintic
$C_{16}H_{13}N_3O_3$ = 295.3
CAS—31431-39-7
IUPAC Name Methyl-(5-benzoyl-1*H*-benzimidazol-2-yl)carbamate
Synonym R-17635
Proprietary Names *Anthex*; *Bantenol*; *Chanazole* (vet.); *Cipex*; *D-Worm*; *Lomper*; *Madicure*; *Menbandan*; *Mindol*; *Ovex*; *Ovitelmin* (vet.); *Oxitover*; *Pantelmin*; *Pharmamin* (vet.); *Pripsen*; *Sufil*; *Surfont*; *Telmin* (vet.); *Toloxim*; *Vermox*; *Wormgo*; *Wormstop*.

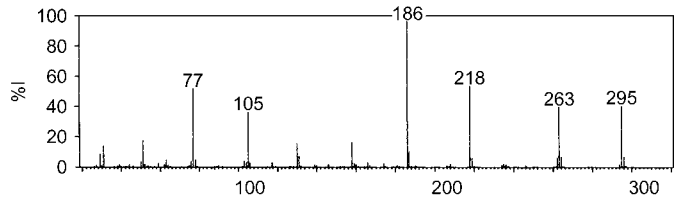


Chemical Properties A white to slightly-yellow amorphous powder. Mp about 290°. Practically insoluble in water, ethanol, chloroform, ether and dilute mineral acids; soluble in formic acid. Log *P* (octanol/water), 2.8.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.00; system TC—R_f 0.59; system TE—R_f 0.60; system TL—R_f 0.49; system TAE—R_f 0.80; system TAF—R_f 0.84; system TAJ—R_f 0.58; system TAK—R_f 0.57; system TAL—R_f 0.93 (Dragendorff spray).
High Performance Liquid Chromatography System HX—RI 438; system HY—RI 322; system HAA—retention time 16.1 min.
Ultraviolet Spectrum Acid isopropyl alcohol—234 (A^1_1=1000b), 288 nm (A^1_1=524b); alkaline isopropyl alcohol—270 (A^1_1=802b), 355 nm (A^1_1=653b).



Infrared Spectrum Principal peaks at wavenumbers 1635, 1260, 1590, 1730, 1230, 705 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 186, 218, 77, 295, 263, 105, 51, 158.

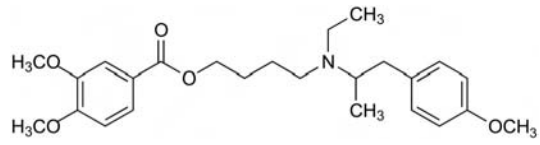


Quantification
Plasma **HPLC** UV detection. Limit of detection, 10 µg/L for mebendazole, 60 µg/L for the 5-(α-hydroxy) metabolite, 30 µg/L for the 2-amino metabolite [Allan *et al.* 1980].
Serum **HPLC** Coulometric detection. For method for quantification of mebendazole and its metabolites, see Betto *et al.* [1991].
Disposition in the Body Mebendazole is poorly absorbed after oral administration. It is metabolised to the 5-(α-hydroxy) derivative and by decarboxylation to the 2-amino metabolite, both of which are detectable in plasma at concentrations higher than those of unchanged mebendazole. <10% of a dose is excreted in the urine; the major urinary metabolite is the 2-amino-5-(α-hydroxy) derivative. Biliary excretion and enterohepatic circulation have been reported.
Therapeutic Concentration
Following single oral doses of 10 mg/kg to 5 subjects, peak plasma concentrations of 0.018 to 0.116 (mean 0.07) mg/L were attained in 2.5 to 7 (mean 5) h [Braithwaite *et al.* 1982].
Half-life Plasma half-life, 1.5 to 9 h.
Volume of Distribution About 2 L/kg.
Distribution in Blood Plasma : whole blood ratio, about 1.2.
Protein Binding About 95%.
Dose Usually 200 mg daily for 3 days.

Allan RJ *et al.* (1980). Two high-performance liquid chromatographic determinations for mebendazole and its metabolites in human plasma using a rapid Sep Pak C18 extraction. *J Chromatogr* 183: 311–319.
Betto P *et al.* (1991). Application of a high-performance liquid chromatography coulometric method for the estimation of mebendazole and its metabolites in human sera. *J Chromatogr* 563: 115–123.
Braithwaite PA *et al.* (1982). Clinical pharmacokinetics of high dose mebendazole in patients treated for cystic hydatid disease. *Eur J Clin Pharmacol* 22: 161–169.

Mebeverine

Antispasmodic
$C_{25}H_{35}NO_5$ = 429.6
CAS—3625-06-7
IUPAC Name 3,4-Dimethoxybenzoic acid 4-[ethyl[2-(4-methoxyphenyl)-1-methylethyl]amino]-butyl ester

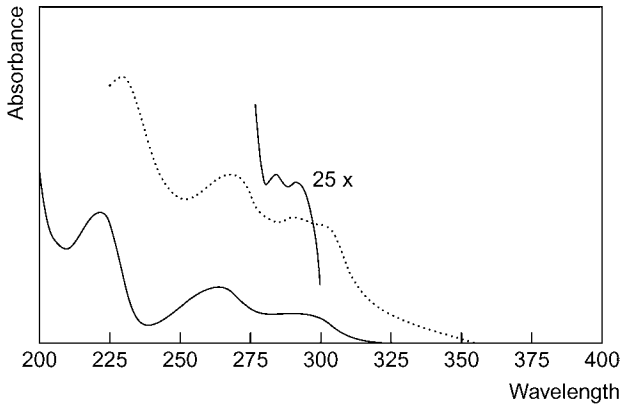


Chemical Properties Soluble in chloroform. Log *P* (octanol/water), 5.1.

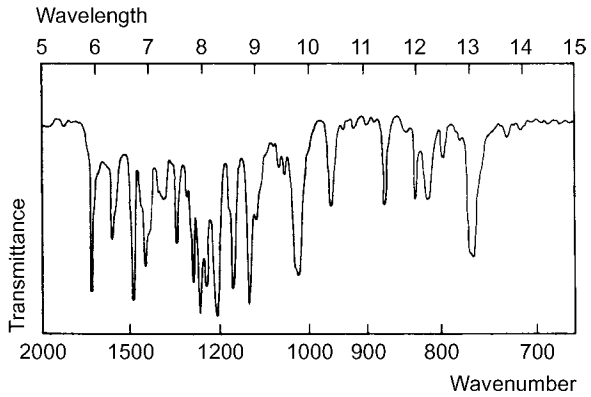
Mebeverine Hydrochloride

$C_{25}H_{35}NO_5$,HCl = 466.0
CAS-2753-45-9
Synonym CSAG 144
Proprietary Names *Bevispas*; *Colese*; *Colofac*; *Colopriv*; *Colotal*; *Duspatal*; *Duspatalin*; *Equilon*; *Mebemerck*; *Mebetin*; *Monosor*; *Spasmonal*; *Spasmopriv*.
Chemical Properties A white crystalline powder. Mp 131° to 136°. Soluble in water and ethanol; sparingly soluble in ether.

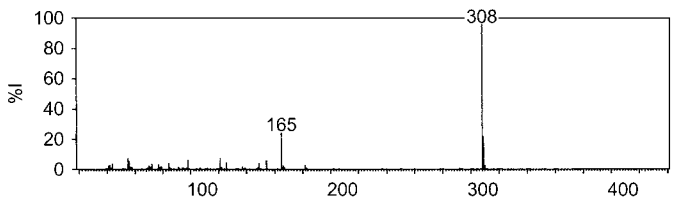
Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.40; system TC—R_f 0.53; system TE—R_f 0.86; system TL—R_f 0.49; system TAE—R_f 0.32 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3045.
High Performance Liquid Chromatography System HA—*k* 1.9; system HX—RI 448; system HZ—retention time 7.1 min.
Ultraviolet Spectrum Aqueous acid—262 nm (A^1_1=307b); aqueous alkali—269, 290 nm.



Infrared Spectrum Principal peaks at wavenumbers 1216, 1266, 1132, 1510, 1715, 1174 cm^{-1} (mebeverine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 308, 165, 309, 121, 55, 154, 98, 56.



Quantification
Plasma **GC–MS** SIM. Limit of detection, 0.5 µg/L for mebeverine alcohol and desmethylmebeverine alcohol [Tulich *et al.* 1996].
Disposition in the Body Mebeverine is metabolised to 4-methoxyethylamfetamine.
Toxicity

In a fatality attributed to the ingestion of mebeverine and thioridazine, the following postmortem concentrations were reported: mebeverine, blood 36 mg/L, liver 15 µg/g, urine 24 mg/L; thioridazine, not detected in blood and urine, liver 2 µg/g [del Villar 1977].

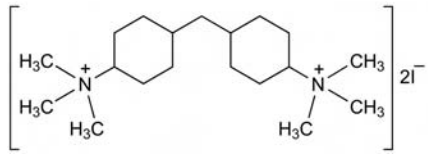
Dose 405 mg of mebeverine hydrochloride daily.

del Villar G (1977). *Bull Int Assoc Forensic Toxicol* 13(1&2): 23–24.
Tulich LJ *et al.* (1996). Determination of two mebeverine metabolites, mebeverine alcohol and desmethylmebeverine alcohol, in human plasma by a dual stable isotope-based gas chromatographic-mass spectrometric method. *J Chromatogr B Biomed Appl* 682(2): 273–281.

Mebezonium Iodide

Muscle Relaxant
$C_{19}H_{40}I_2N_2$ = 550.3
CAS—7681-78-9
IUPAC Name 4,4′-Methylenebis(cyclohexyltrimethylammonium)diiodide

Proprietary Name It is an ingredient of *Tanax* (vet.).



Chemical Properties A white crystalline powder. Mp 260°, with decomposition. Soluble in water.
Thin-layer Chromatography System TA—R_f 0.00 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 961, 888, 831, 1621, 985, 1020 cm^{-1} (KBr disk).
Quantification
Blood **TLC–UV spectrophotometry** For method, see Bertol *et al.* [1983].
Urine **TLC–UV spectrophotometry** For method, see Bertol *et al.* [1983].
Disposition in the Body
Toxicity

In 3 fatalities due to the injection of a preparation containing mebezonium iodide and embutramide, the following postmortem concentrations (mg/L) were reported:

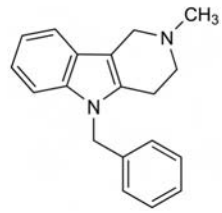
	Mebezonium iodide	*Embutramide*
Blood	4.5, 6.0, 7.5	3.0, 15.5, 12.1
Urine	0.8, 2.0, 1.8	2.0, 6.3, 4.5

[Bertol *et al.* 1983]

Bertol E *et al.* (1983). Analytical toxicological studies in cases of suicide by injection of Tanax, a veterinary euthanasia agent. *J Pharm Biomed Anal* 1: 373–377.

Mebhydrolin

Antihistamine
$C_{19}H_{20}N_2$ = 276.4
CAS—524-81-2
IUPAC Name 2,3,4,5-Tetrahydro-2-methyl-5-(phenylmethyl)-1*H*-pyrido[4,3-*b*]indole

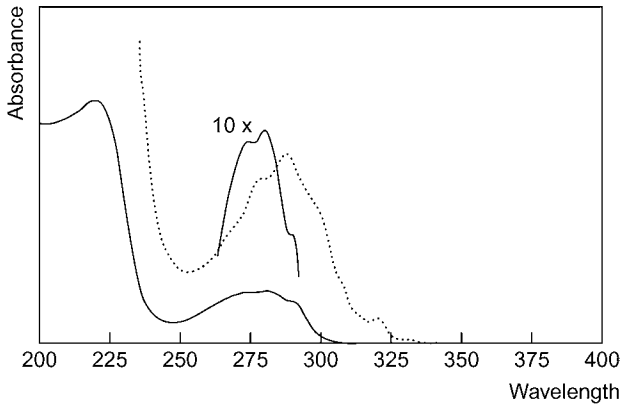


Chemical Properties A white crystalline powder. Mp 95°. Practically insoluble in water; freely soluble in ethanol, methanol, chloroform and acetone; slightly soluble in ether. pK_a 6.7. Log *P* (octanol/water), 3.8.

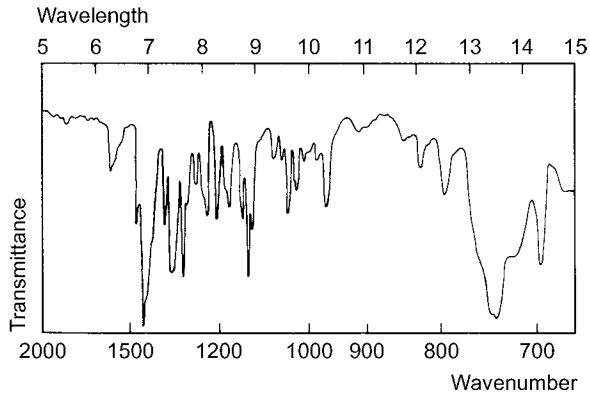
Mebhydrolin Napadisilate

$(C_{19}H_{20}N_2)_2,C_{10}H_8O_6S_2$ = 841.1
CAS—6153-33-9
Synonyms Diazolinum; mebhydrolin naphthalenedisulphonate.
Proprietary Names *Dayhist*; *Fabahistin*; *Incidal*; *Omeril*; *Posidol*.
Chemical Properties A white powder. Mp 280°, with decomposition. Practically insoluble in water; sparingly soluble in hot glacial acetic acid; soluble in hot formamide.

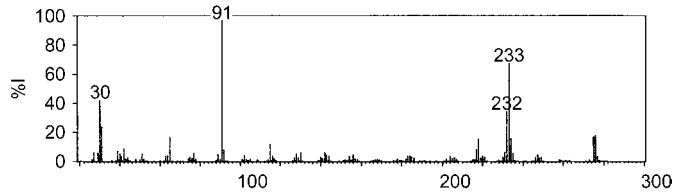
Colour Tests Mandelin's test—blue; Marquis test—grey-blue.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.27; system TC—R_f 0.45; system TE—R_f 0.65; system TL—R_f 0.20; system TAE—R_f 0.36; system TAF—R_f 0.46 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2450; system GB—RI 2575; system GC—RI 2739; system GF—RI 2920.
High Performance Liquid Chromatography System HA—*k* 3.0 (tailing peak); system HX—RI 411; system HZ—retention time 5.3 min.
Ultraviolet Spectrum Aqueous acid—286 ($A^{\frac{1}{1}}$=409b), 320 nm.



Infrared Spectrum Principal peaks at wavenumbers 737, 1134, 1316, 695, 1122, 1212 cm^{-1} (thin film).



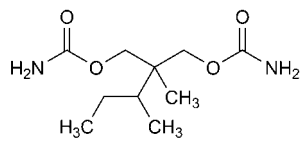
Mass Spectrum Principal ions at *m/z* 91, 233, 30, 232, 31, 276, 275, 65.



Dose 150 to 300 mg daily.

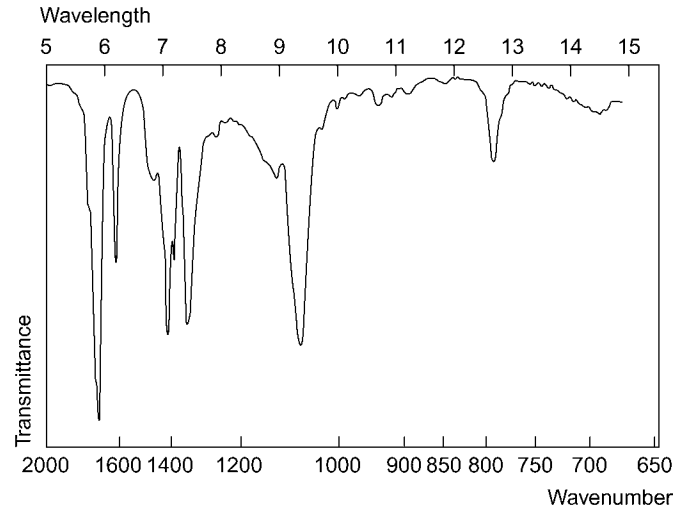
Mebutamate

Tranquilliser
$C_{10}H_{20}N_2O_4$ = 32.3
CAS—64-55-1
IUPAC Name [2-(Carbamoyloxymethyl)-2,3-dimethylpentyl] carbamate
Synonym 2-Methyl-2-(1-methylpropyl)-1,3-propanediol dicarbamate
Proprietary Names *Axiten*; *Butatensin*; *Capla*; *Carbuten*; *Dormate*; *Ipotensivo*; *Mebutina*; *No-Press*; *Prean*; *Sigmafon*; *Vallene*.

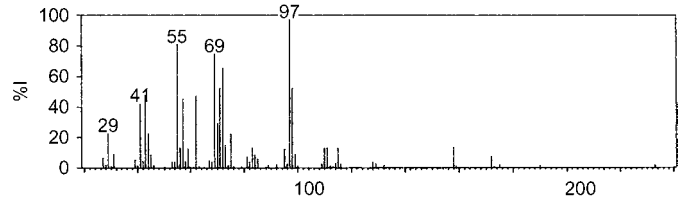


Chemical Properties A white crystalline powder. Mp 77° to 79°. Slightly soluble in water; soluble in most organic solvents. Log *P* (octanol/water), 1.4.
Colour Tests Furfuraldehyde—black; Nessler's reagent—brown (slow).
Thin-layer Chromatography System TB—R_f 0.00; system TC—R_f 0.33; system TD—R_f 0.10; system TE—R_f 0.60; system TF—R_f 0.35; system TAD—R_f 0.35; system TAE—R_f 0.82; system TAF—R_f 0.85; system TAG—R_f 0.56; system TAJ—R_f 0.35; system TAK—R_f 0.47; system TAL—R_f 0.87.

Gas Chromatography System GA—RI 1889.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1682, 1070, 1710, 1601, 1140, 788 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 97, 55, 69, 72, 71, 98, 43, 62.

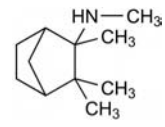


Quantification
Plasma **GC** Column: 3.8% UC-W98 methyl silicone on 80/100 mesh (121.9 cm). Carrier gas: He, 65 mL/min. Temperature programme: 180°. FID. Retention time: 2.9 min. Limit of detection, 1 mg/L [Douglas *et al.* 1969].
Urine **GC** See Plasma [Douglas *et al.* 1969].
Dose 0.9 to 1.2 g daily.

Douglas JF *et al.* (1969). Gas chromatographic determination of mebutamate, carisoprodol, and tybamate in plasma and urine. *J Pharm Sci* 58: 145–146.

Mecamylamine

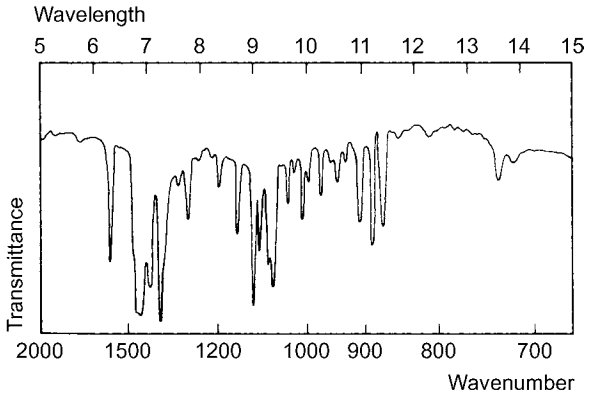
Antihypertensive
$C_{11}H_{21}N$ = 167.3
CAS—60-40-2
IUPAC Name *N*-2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine



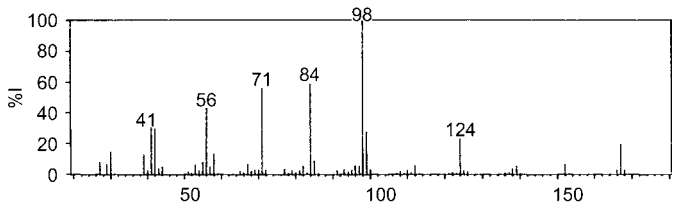
Chemical Properties An oily liquid. Slightly soluble in water. pK_a 11.3. Log *P* (octanol/water), 3.3.

Mecamylamine Hydrochloride
$C_{11}H_{21}N,HCl$ = 203.8
CAS—826-39-1
Proprietary Names *Inversine*; *Mevasine*.
Chemical Properties A white crystalline powder. Mp about 245°, with decomposition. Soluble 1 in 5 of water and 1 in 12 of ethanol; freely soluble in chloroform; practically insoluble in ether.
Thin-layer Chromatography System TA—R_f 0.16; system TB—R_f 0.51; system TC—R_f 0.02; system TL—R_f 0.04; system TAJ—R_f 0.00; system TAK—R_f 0.16; system TAL—R_f 0.58.
High Performance Liquid Chromatography System HA—*k* 1.7.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1111, 1068, 1078, 1592, 1098, 890 cm^{-1} (mecamylamine hydrochloride (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 98, 84, 71, 56, 41, 42, 99, 124.

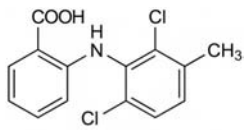


Quantification
Plasma **GC–MS** Mecamylamine, nicotine and cotinine. Limit of detection, <2 µg/L for mecamylamine [Jacob *et al.* 2000].
Disposition in the Body Mecamylamine is almost completely absorbed after oral administration; high concentrations are found in the liver and kidney. >50% of a dose is excreted in 24 h as unchanged drug in acid urine; the excretion is reduced if the urine is alkaline.
Dose 5 to 30 mg of mecamylamine hydrochloride daily.

Jacob P *et al.* (2000). Simultaneous determination of mecamylamine, nicotine, and cotinine in plasma by gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 23: 653–661.

Meclofenamic Acid

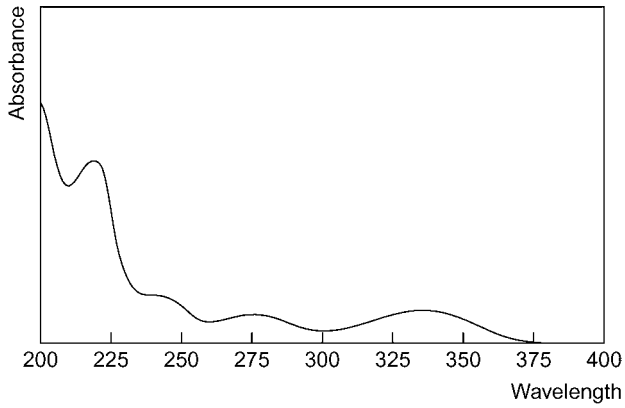
Analgesic
$C_{14}H_{11}Cl_2NO_2$ = 296.2
CAS—644-62-2
IUPAC Name 2-[(2,6-Dichloro-3-methylphenyl)amino]benzoic acid
Synonyms CI-583; INF-4668.
Proprietary Names *Arquel* (vet.); *Lenidolor*; *Movens.*



Chemical Properties White crystals. Mp 257° to 259° also reported as 248° to 250°. Practically insoluble in water. Log *P* (octanol/water), 6.0.

Meclofenamate Sodium
$C_{14}H_{10}Cl_2NNaO_2$ = 318.1
CAS—6385-02-0
Proprietary Names *Lenidolor*; *Meclodol*; *Meclomen*; *Movens.*
Chemical Properties Mp 289° to 291°. Sparingly soluble in water.

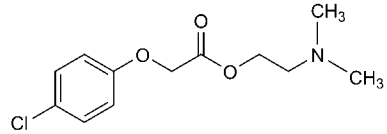
Thin-layer Chromatography System TE—R_f 0.12; system TF—R_f 0.43; system TG—R_f 0.38; system TAJ—R_f 0.59; system TAK—R_f 0.77; system TAL—R_f 0.92 (chromic acid solution, violet).
Gas Chromatography System GA—RI 2420; system GD—methyl derivati- veretention time 1.62 relative to n-$C_{16}H_{34}$; system GL—meclofenamic acid-Me RI 2240.
High Performance Liquid Chromatography Meclofenamic acid is used as a reference substance in System HV. System HX—RI 653; system HY—RI 690.
Ultraviolet Spectrum Aqueous alkali—275 (A^1_1= 240b), 319 nm (A^1_1=180b); methanol—242 (A^1_1=342b), 282 (A^1_1=235b), 331 nm (A^1_1=235b).



Dose The equivalent of 200 to 400 mg of meclofenamic acid daily.

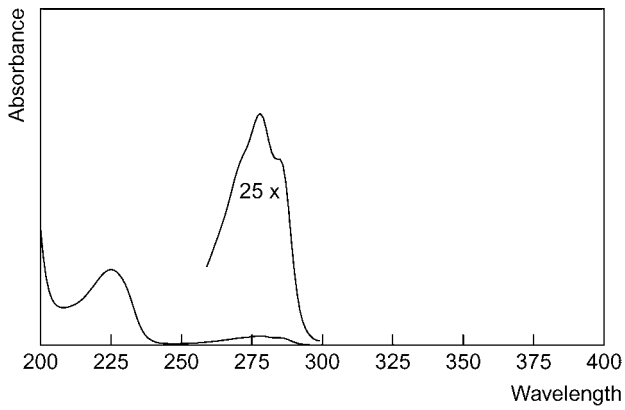
Meclofenoxate

CNS Stimulant
$C_{12}H_{16}ClNO_3$ = 57.7
CAS—51-68-3
IUPAC Name 2-Dimethylaminoethyl 2-(4-chlorophenoxy)acetate
Synonyms Centrophenoxine; clofenoxine; clophenoxate; deanol 4-chlorophenoxyacetate; 2-(dimethylamino)ethyl-(4-chlorophenoxy)acetate; meclofenoxane.

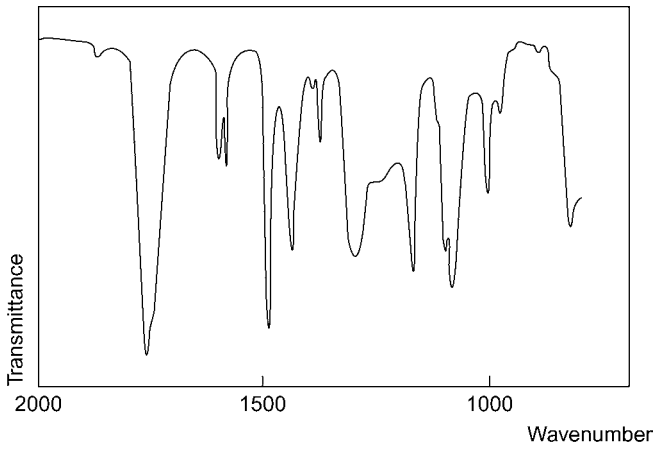


Meclofenoxate Hydrochloride
$C_{12}H_{16}ClNO_3$,HCl = 294.2
CAS—3685-84-5
Proprietary Names *Cerutil*; *Helfergin*; *Lucidril.*
Chemical Properties A white powder. Mp 136°. Soluble in cold water; sparingly soluble in cold isopropyl alcohol and acetone; practically insoluble in benzene, ether, and chloroform. It rapidly hydrolyses in aqueous solution. Log *P* (octanol/water), 1.96 [Meylan, Howard 1995].

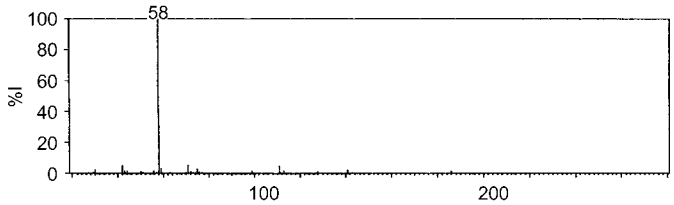
Thin-layer Chromatography System TA—R_f 0.77; system TB—R_f 0.26; system TC—R_f 0.42; system TE—R_f 0.67; system TL—R_f 0.22; system TAE—R_f 0.46; system TAG—R_f 0.22 (acidified potassium permanganate solution, positive).
Plates: Silicagel HF_{254}. Solvent system: dichloromethane : methanol : water (6 : 4 : 3). Reagent: methyl yellow (1% in ethanol). R_f 0.65 [Araman *et al.* 1992].
Gas Chromatography System GA—RI 1770; system GB—RI 1804; system GC—RI 2200.
High Performance Liquid Chromatography System HA—*k* 1.7.
Column: ZORBAX ODS (250 × 4.5 mm i.d., 5 µm). Mobile phase: 0.2% TEA in 0.01 mol/L ammonium carbonate : acetonitrile (70 : 30), flow rate 1.0 mL/min. UV detection (λ = 277 nm). Retention time: 5.39 min. Limit of quantification, 15 mg/L [El Bardicy *et al.* 2007]. Column: µBondapak C_{18}PN27324 (30 × 3.9 cm i.d.). Mobile phase: methanol : 1% ammonium carbonate (pH 3.2). Retention time: 8.03 min [Araman *et al.* 1992]. Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: methanol : 0.1% ammonium carbonate (pH 3.1; 55 : 45), flow rate 2.0 mL/min. UV detection (λ = 227 nm). Retention time: 2.2 min. Limit of detection, 25 mg/L [Tatsuhara, Tabuchi 1980].
Ultraviolet Spectrum Aqueous acid—277 nm (A^1_1 = 44a).



Infrared Spectrum Principal peaks at wavenumbers 1167, 1183, 1742, 1080, 833, 1768 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 111, 71, 42, 75, 59, 141, 113.



Quantification

Plasma **HPLC** Column: C_{18} (150 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 1.0% acetic acid (70 : 30), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.047 mg/L [Zou *et al.* 2008]. Column: TSK 410 (150 × 4 mm i.d.). Mobile phase: 50 mmol/L phosphate buffer (pH 2.5) : methanol (1 : 1), flow rate 1 mL/min. UV detection (λ = 280 nm). Limit of detection not reported [Yoshioka *et al.* 1987].

Disposition the Body

Therapeutic Concentration Twenty-four healthy male Chinese volunteers were administered a single dose of 200 mg meclofenoxate hydrochloride as a capsule or a tablet. There was a 1 week wash-out period between formulations. Mean maximum plasma concentrations for the capsule and the tablet formulation were 12.6 and 12.8 mg/L attained at 2.1 and 2.0 h, respectively [Zou *et al.* 2008]

Dose Meclofenoxate hydrochloride 0.9 to 1.5 g daily.

Araman A *et al.* (1992). Stability of meclofenoxate hydrochloride in artificial gastric and intestinal media. *Pharmazie* 47: 147.

El Bardicy MG *et al.* (2007). Kinetic study on the degradation of meclophenoxate hydrochloride in alkaline aqueous solutions by high performance liquid chromatography. *Yakugaku Zasshi* 127: 193–199.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Tatsuhara T, Tabuchi F (1980). Analysis of meclofenoxate and its degradation products by high performance liquid chromatography. *Chem Pharm Bull (Tokyo)* 28: 779–782.

Yoshioka S *et al.* (1987). Kinetics of hydrolysis of meclofenoxate hydrochloride in human plasma. *J Pharm Pharmacol* 39: 215–218.

Zou JJ *et al.* (2008). Bioequivalence and pharmacokinetic comparison of a single 200-mg dose of meclofenoxate hydrochloride capsule and tablet formulations in healthy Chinese adult male volunteers: a randomized sequence, open-label, two-period crossover study. *Clin Ther* 30: 1651–1657.

Mecloqualone

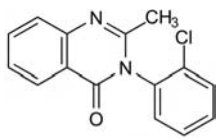
Hypnotic

$C_{15}H_{11}ClN_2O = 270.7$

CAS—340-57-8

IUPAC Name 3-(2-Chlorophenyl)-2-methyl-4(3*H*)-quinazolinone

Proprietary Name *Nubarene*



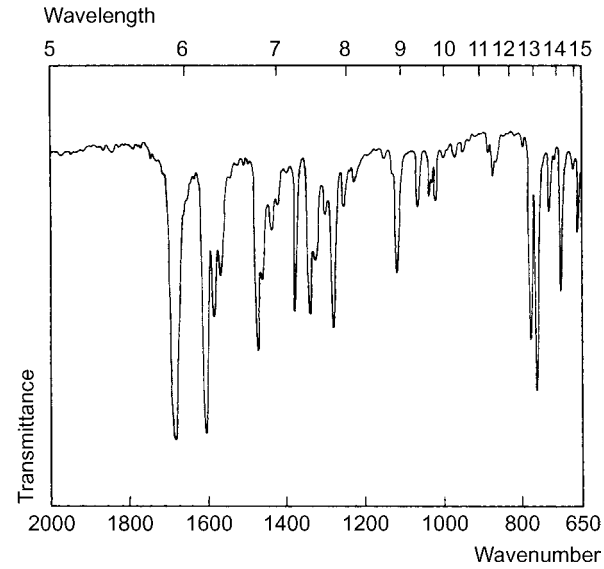
Chemical Properties Crystals. Mp 126° to 128°. Log *P* (octanol/water), 4.4.

Thin-layer Chromatography System TB—R_f 0.25; system TE—R_f 0.76; system TAE—R_f 0.80; system TAJ—R_f 0.77; system TAK—R_f 0.68; system TAL—R_f 0.96.

Gas Chromatography System GA—RI 2255.

High Performance Liquid Chromatography System HX—RI 482.

Infrared Spectrum Principal peaks at wavenumbers 1682, 1605, 768, 782, 1282, 1583 cm^{-1}.



Dose 150 to 300 mg, as a hypnotic.

Meclozine

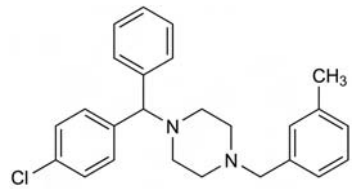
Antihistamine

$C_{25}H_{27}ClN_2 = 391.0$

CAS—569-65-3

IUPAC Name 1-[(4-Chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl] piperazine

Synonyms Histamethizine; meclizine; parachloramine.



Chemical Properties pK_a 3.1, 6.2 (25°). Log *P* (octanol/water), 5.9.

Meclozine Hydrochloride

$C_{25}H_{27}ClN_2,2HCl, H_2O = 481.9$

CAS—1104-22-9 (anhydrous); 31884-77-2 (monohydrate)

Synonyms Meclizine hydrochloride; meclizinium chloride; parachloramine hydrochloride.

Proprietary Names *Agyrax*; *Ancolan*; *Antivert*; *Antrizine*; *Bonamine*; *Bonine*; *Calmonal*; *Chiclida*; *Dizmiss*; *Dramamine II*; *Dramine*; *Duremesan*; *Marevit*; *Meni-D*; *Navicalm*; *Peremesin*; *Postafen(e)*; *Sea-legs*; *Suprimal*; *Vergon*; *Vertin*.

Chemical Properties A white or slightly-yellowish crystalline powder. Mp 217° to 224°, with decomposition. Soluble 1 in 1000 of water, 1 in 25 of ethanol and 1 in 5 of chloroform; practically insoluble in ether; freely soluble in pyridine.

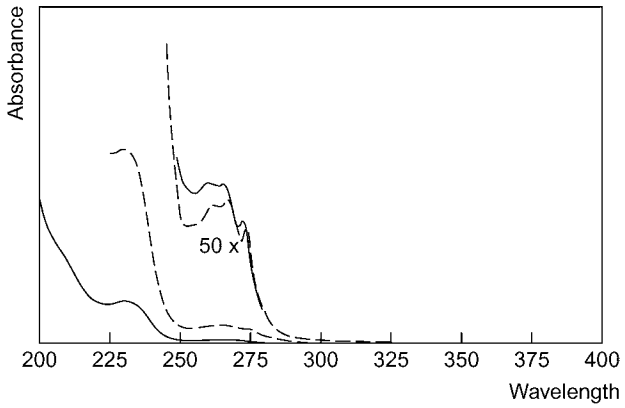
Colour Test Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.61; system TC—R_f 0.79; system TE—R_f 0.87; system TL—R_f 0.70; system TAE—R_f 0.80; system TAF—R_f 0.88; system TAJ—R_f 0.65; system TAK—R_f 0.24; system TAL—R_f 0.95 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown-yellow; ninhydrin spray, positive).

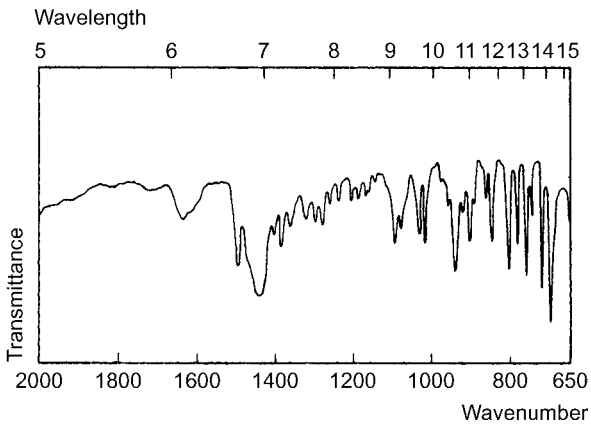
Gas Chromatography System GA—meclozine RI 3035, M (N-desalkyl-) RI 2520; system GB—RI 3193.

High Performance Liquid Chromatography System HA—*k* 0.7; system HX—RI 587; system HY—RI 398; system HAA—retention time 20.0 min.

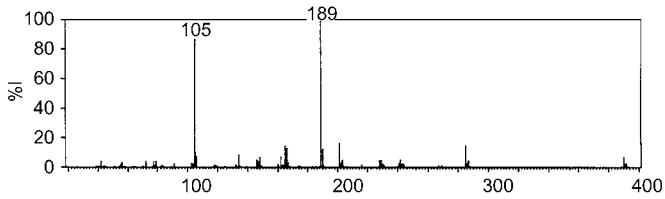
Ultraviolet Spectrum Ethanol—230 (A^1_1=391a), 266 (A^1_1=27b), 273 nm.



Infrared Spectrum Principal peaks at wavenumbers 700, 720, 760, 940, 805, 1491 cm^{-1} (meclozine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 189, 105, 201, 285, 165, 166, 190, 134.



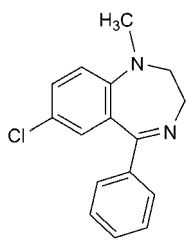
Quantification
Plasma **GC–MS** Limit of detection, 5 µg/L [Fouda *et al.* 1978].
Dose Usually 25 to 100 mg of meclozine hydrochloride daily.

Fouda HG *et al.* (1978). Selected ion monitoring assay for meclizine in human plasma. *Biomed Mass Spectrom* 5: 491–494.

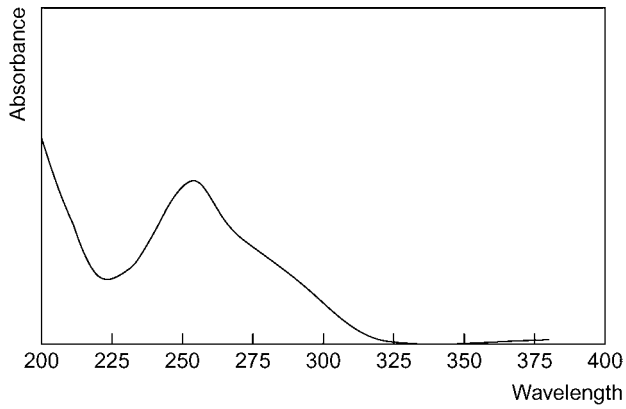
Medazepam

Benzodiazepine, Tranquilliser
$C_{16}H_{15}ClN_2 = 70.8$
CAS—2898-12-6

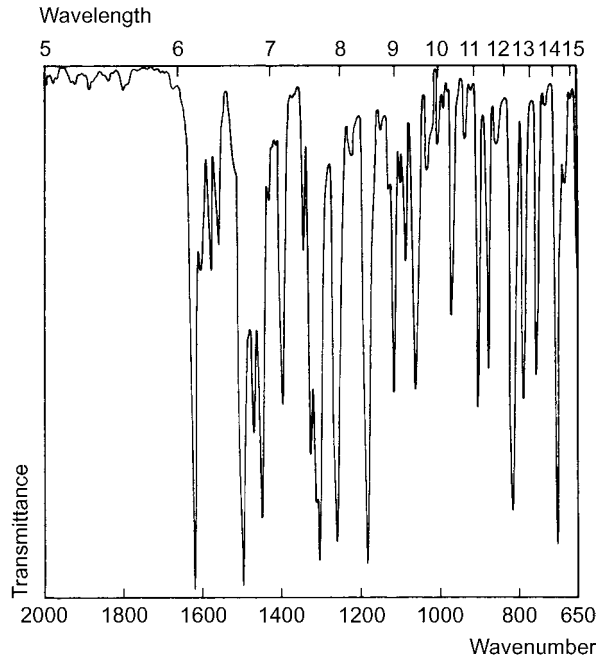
IUPAC Name 7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1,4-benzodiazepine
Proprietary Names *Nobrium*; *Rudotel*.
Synonym 7-Chloro-2,3-dihydro-1-methyl-5-phenyl-1*H*-1,4-benzodiazepine



Chemical Properties A white to greenish-yellow crystalline powder. Mp 95° to 97° (crystals from ether and petroleum ether). Practically insoluble in water; soluble 1 in 8 of ethanol, 1 in 1 of chloroform, and 1 in 5 of ether. pKa 6.2 (37°). Log *P* (octanol/water), 4.41 [Capella-Peiro *et al.* 2002], (octanol/pH 7.4), 4.0. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Nitric acid, cold—red; formaldehyde–sulfuric acid—orange (add water).
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.41; system TC—R_f 0.74; system TD—R_f 0.54; system TE—R_f 0.78; system TF—R_f 0.40; system TL—R_f 0.62; system TAD—R_f 0.73; system TAE—R_f 0.79; system TAF—R_f 0.83; system TAJ—R_f 0.70; system TAK—R_f 0.12; system TAL—R_f 0.95 (acidified iodoplatinate solution, positive). See also Sawada *et al.* 1976].
Gas Chromatography System GA—medazepam RI 2235, M (nor-) RI 2280, nordazepam RI 2490, diazepam RI 2428; system GB—medazepam RI 2340, nordazepam RI 2625, diazepam RI 2556; system GF—medazepam RI 2640, diazepam RI 3045; system GG—medazepam RI 2620, nordazepam RI 3041, diazepam RI 2940.
High Performance Liquid Chromatography System HA—medazepam *k* 0.2; system HJ—*k* 7.05; system HK—*k* 4.44; system HX—RI 405; system HY—RI 334; system HZ—RT 5.4 min; system HAA—RT 15.8 min; system HAL—RT 8.2 min; system HAM—not detected; system HBH—k 41.46; system HBI—*k* 6.31.
Ultraviolet Spectrum Aqueous acid—253 nm ($A_1^1 = 860$a).

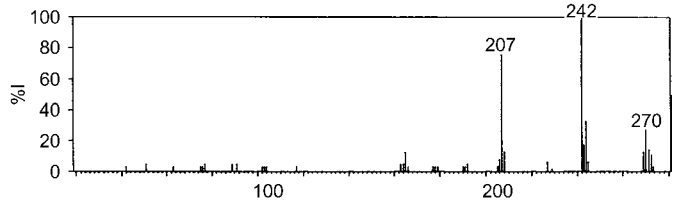


Infrared Spectrum Principal peaks at wavenumbers 1610, 1178, 1298, 700, 1255, 815 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 242, 207, 244, 270, 243, 271, 269, 165; nordazepam 242, 269, 270, 241, 243, 271, 244, 272; desmethylmedazepam 193, 255, 228, 256, 257, 165, 230, 258; diazepam 256, 283, 284, 285, 257, 255, 258, 286.

M



Quantification

Blood **GC** Column: 1% OV-17 or 2% OV-1 (213 × 0.4 cm i.d.). Carrier gas: Ar, 42 or 43 mL/min respectively. Temperature programme: 235° and 260°, respectively. FID. Retention time: 3.8 and 2.6 min, respectively. Limit of detection, 80 µg/L [Greaves 1974].

Plasma **GC** Column: SE-54 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 48 kPa. Temperature programme: 110° for 8 min to 300° at 10°/min for 10 min. FID or NPD. Limit of detection, 10 µg/L [Herráez-Hernández *et al.* 1996]. Column: 3% OV-17 on 60/80 mesh Gas Chrom Q (120 × 4 mm i.d.). Carrier gas: Ar: CH_4 (90 : 10), 100 to 110 mL/min. Temperature programme: 230°. ECD. Retention time: 4.2 min. Limit of detection, 40 µg/L [De Silva, Puglisi 1970].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.5 µg/L (SIM), limit of detection, 0.5 µg/L (scan) [Kratzsch *et al.* 2004].

Serum **HPLC** Column: Eclipse XDBC-8 (150 × 4.6 mm i.d., 5 µm) or Kromasil C_{18} (120 × 4.6 mm i.d., 5 µm). Mobile phase: 0.06 mol/L SDS : butanol (pH 7.0, 95 : 5), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 11 µg/L [Capella-Peiro *et al.* 2002].

LC-MS Column: LiChrospher 60-RP select B (100 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : water : acetonitrile (1 : 1 : 1, pH 6.0), flow rate 100 µL/min. ESI, SRM acquisition mode, positive ion mode. Limit of quantification, 2 µg/L [Kleinschnitz *et al.* 1996].

Urine **GC** See Plasma [De Silva, Puglisi 1970].

HPLC See Serum [Kleinschnitz *et al.* 1996].

LC-MS Column: 5% OV-101 Chromosorb G HP 100/120 mesh (600 × 2 mm i. d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Disposition in the Body Readily absorbed after oral administration. At least 5 metabolites have been identified [Schwartz, Carbone 1970]. The active metabolites desmethyldiazepam (nordazepam) and diazepam may be detected in the blood shortly after dosing; desmethyldiazepam accumulates during chronic treatment. Desmethylmedazepam has also been detected in plasma. A total of up to approx. 75% of a dose is excreted in the urine and ~20% is eliminated in the faeces. The major urinary metabolite is oxazepam glucuronide, 2– 3% of a dose being excreted in this form in 72 h; other urinary metabolites include desmethyldiazepam and temazepam.

Therapeutic Concentration

Following single oral doses of 10 mg given to four subjects, peak plasma concentrations of 0.14 to 0.26 g/L (mean 0.21) of medazepam were attained in ~1 h [Hailey, Baird 1979].

Doses of 10 to 50 mg given daily to 20 subjects, resulted in the following steady-state plasma concentrations: medazepam 0.01–0.16 g/L (mean 0.06), diazepam 0–0.12 g/L (mean 0.03), desmethyldiazepam 0.2–1.7 g/L (mean 0.7) [Bond *et al.* 1977].

Half-life Plasma half-lives, medazepam 1–2 h, desmethyldiazepam ~40–100 h; however, there is considerable intersubject variation (see Nordazepam).

Distribution in Blood Plasma : whole blood ratio, 1.9.

Protein Binding Almost completely bound.

Dose Usually 10 to 20 mg daily, but doses of up to 60 mg daily have been given.

Bond AJ *et al.* (1977). Plasma concentrations of benzodiazepines. *Br J Clin Pharmacol* 4: 51–56.

Capella-Peiro ME *et al.* (2002). Direct injection micellar liquid chromatographic determination of benzodiazepines in serum. *J Chromatogr B Analyt Technol Biomed Life Sci* 780: 241–249.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

De Silva JA, Puglisi CV (1970). Determination of medazepam (nobrium), diazepam (Valium) and their major biotransformation products in blood and urine by electron capture s-liquid chromatography. *Anal Chem* 42: 1725–1736.

Greaves MS (1974). Quantitative determination of medazepam, diazepam, and nitrazepam in whole blood by flame-ionization gas–liquid chromatography. *Clin Chem* 20: 141–147.

Hailey DM, Baird ES (1979). Plasma concentrations of medazepam and its metabolites after oral administration. *Br J Anaesth* 51: 493–496.

Herráez-Hernández R *et al.* (1996). Automated on-line dialysis for sample preparation for gas chromatography: determination of benzodiazepines in human plasma. *J Pharm Biomed Anal* 14: 1077–1087.

Kleinschnitz M *et al.* (1996). Determination of 1,4-benzodiazepines by high-performance liquid chromatography–electrospray tandem mass spectrometry. *J Chromatogr B Biomed Appl* 676: 61–67.

Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.

Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography–mass spectrometry. *J Chromatogr* 422: 85–101.

Sawada H *et al.* (1976). Isolation and identification of benzodiazepine drugs and their metabolites in urine by use of Amberlite XAD-2 resin and thin-layer chromatography. *Clin Chem* 22: 1596–1603.

Schwartz MA, Carbone JJ (1970). Metabolism of ^{14}C-medazepam hydrochloride in dog, rat and man. *Biochem Pharmacol* 19: 343–361.

Medroxyprogesterone Acetate

Progestational Steroid

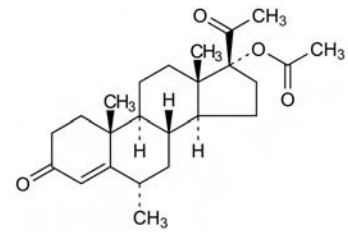
$C_{24}H_{34}O_4$ = 386.5

CAS—520-85-4 (medroxyprogesterone); 71-58-9 (acetate)

IUPAC Name 17α-Acetoxy-6α-methylprogesterone

Synonyms Methylacetoxyprogesterone; metipregnone.

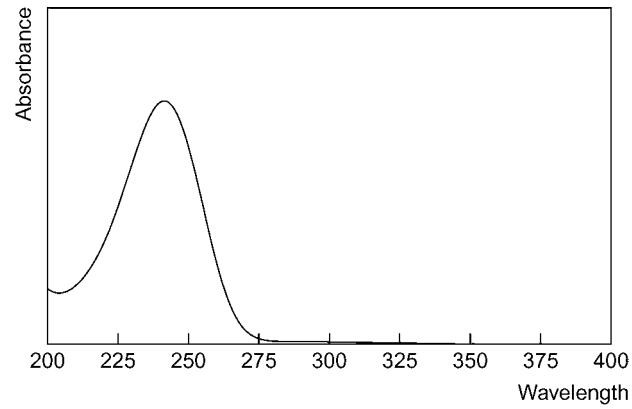
Proprietary Names *Adgyn Medro*; *Alti-MPA*; *Amen*; *Cycrin*; *Cykrina*; *Depocon*; *Depo-Prodasone*; *Depo-Provera*; *Depo-Ralovera*; *Farlutal*; *Gen-Medroxy*; *Gestoral*; *Novo-Medrone*; *Perlutex*; *Proclim*; *Prodafem*; *Prodasone*; *Progevera*; *Provera*; *Ralovera*.



Chemical Properties A white crystalline powder. Mp about 204°. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 800 of ethanol, 1 in 50 of acetone and 1 in 10 of chloroform; slightly soluble in ether. Log *P* (octanol/water), 3.5 (medroxyprogesterone).

Thin-layer Chromatography System TP—R_f 0.80; system TQ—R_f 0.50; system TR—R_f 0.98; system TS—R_f 0.85, streaking may occur.

Ultraviolet Spectrum Ethanol—241 nm (A^1_1=426 a).



Infrared Spectrum Principal peaks at wavenumbers 1727, 1669, 1258, 1608, 967, 1188 cm^{-1} (medroxyprogesterone, KBr disk).

Quantification

Plasma **GC** ECD. Limit of detection, 5 µg/L [Rossi *et al.* 1979].

GC–MS Limit of detection, 1 ng [Phillipou, Frith 1980].

HPLC UV detection. Limit of detection, 4 µg/L [Milano *et al.* 1982].

HPLC–MS Limit of detection, <0.05 µg/L [Kim, Kim 2001].

Serum **GC–MS** Limit of detection, <0.5 µg/L [Jarvinen *et al.* 1989].

HPLC Chemiluminescence detection. Limit of detection, 9 µg/L [Uzu *et al.* 1992].

Dose 2.5 to 10 mg daily, orally; up to 1 g daily may be given.

Jarvinen T *et al.* (1989). Specific and sensitive quantitation of medroxyprogesterone acetate in human serum by gas chromatography-mass spectrometry. *J Chromatogr* 495: 13–20.

Kim SM, Kim DH (2001). Quantitative determination of medroxyprogesterone acetate in plasma by liquid chromatography/electrospray ion trap mass spectrometry. *Rapid Commun Mass Spectrom* 15: 2041–2045.

Milano G *et al.* (1982). Determination of medroxyprogesterone acetate in plasma by high-performance liquid chromatography. *J Chromatogr* 232: 413–417.

Phillipou G, Frith RG (1980). Specific quantitation of plasma medroxyprogesterone acetate by gas chromatography/mass spectrometry. *Clin Chim Acta* 103: 129–133.

Rossi E *et al.* (1979). Quantitative gas-liquid chromatographic determination of medroxyprogesterone acetate in human plasma. *J Chromatogr* 169: 416–421.

Uzu S *et al.* (1992). Determination of medroxyprogesterone acetate in serum by HPLC with peroxyoxalate chemiluminescence detection using a fluorogenic reagent, 4-(N,N-dimethylaminosulphonyl)-7-hydrazino-2,1,3-benzoxadiazole. *J Pharm Biomed Anal* 10: 979–984.

Mefenamic Acid

Analgesic

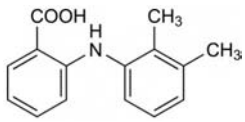
$C_{15}H_{15}NO_2 = 241.3$

CAS—61-68-7

IUPAC Name 2-[(2,3-Dimethylphenyl)amino]benzoic acid

Synonyms Acidum mefenamicum; CI-473; CN-35355; INF-3355.

Proprietary Names *Artriden*; *Contraflam*; *Coslan*; *Dysman*; *Dyspen*; *Fenamin*; *Lysalgo*; *Medicap*; *Mefacap*; *Mefalgic*; *Mefanacide*; *Mefenix*; *Mefic*; *Meflam*; *Namifen*; *Napan*; *Opustan*; *Parkemed*; *Pinalgesic*; *Ponac*; *Ponalar*; *Ponalgic*; *Ponmel*; *Ponstan*; *Ponstel*; *Ponstyl*; *Pontyl*; *Spiralgin*.



Chemical Properties A white to greyish-white microcrystalline powder. Mp 230° to 231°, with effervescence. Practically insoluble in water; soluble 1 in 185 of ethanol, 1 in 150 of chloroform, and 1 in 80 of ether; soluble in solutions of alkali hydroxides. pK_a 4.2. Log *P* (octanol/water), 5.1.

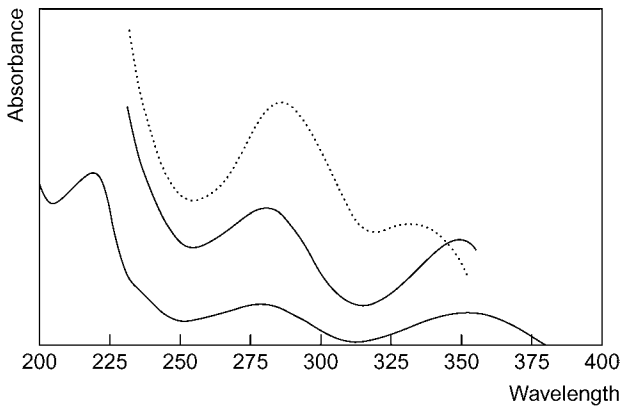
Colour Test Liebermann's reagent—blue.

Thin-layer Chromatography System TA—R_f 0.96; system TD—R_f 0.41; system TE—R_f 0.11; system TF—R_f 0.48; system TG—R_f 0.32; system TAD—R_f 0.54; system TAE—R_f 0.87; system TAJ—R_f 0.68; system TAK—R_f 0.86; system TAL—R_f 0.95 (chromic acid solution, green).

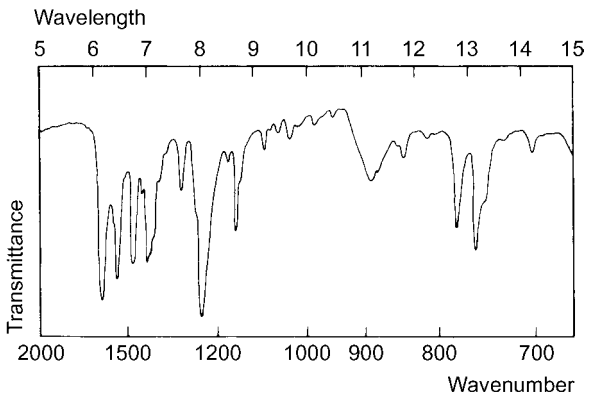
Gas Chromatography System GA—mefenamic acid RI 2201, mefenamic acid-Me RI 2069; system GB—RI 2370; system GD—mefenamic acid-Me RRT 1.45 (relative to n-$C_{16}H_{34}$).

High Performance Liquid Chromatography System HD—*k* 21.1; system HV—RRT 0.95 (relative to meclofenamic acid); system HX—RI 661; system HY—RI 686; system HAY—retention time 9.8 min; system HAX—retention time 13.1 min.

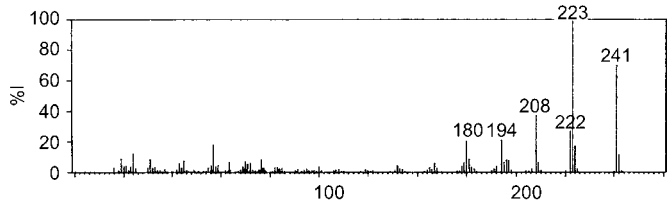
Ultraviolet Spectrum Acid methanol—279 (A_1^1=357a), 350 nm; aqueous alkali—285 (A_1^1=420a), 322 nm.



Infrared Spectrum Principal peaks at wavenumbers 1255, 1647, 1572, 1504, 757, 1163 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 223, 241, 208, 222, 194, 180, 77, 224; 3′-carboxymefenamic acid 44, 180, 209, 227, 223, 77, 208, 179; 3′-hydroxymethylmefenamic acid 209, 257, 180, 208, 210, 77, 44, 194.



Quantification

Plasma **HPLC** UV detection. Mefenamic acid and indometacin. Limit of detection, 80 µg/L for mefenamic acid [Niopas, Mamzoridi 1994]. UV detection. For method for quantification of mefenamic acid and other NSAIDs, see Streete [1989]. UV detection. For method for quantification of mefenamic acid and flufenamic acid, see Lin *et al.* [1980].

Serum **GC** FID. Limit of detection, 1 g/L [Dusci, Hackett 1978].

HPLC UV detection. For method for quantification of mefenamic acid and other NSAIDs, see Streete [1989].

Urine **HPLC** UV detection. Mefenamic acid and other anthranilic acid derivatives. Limit of detection, about 2 µg/L for mefenamic acid [Mikami *et al.* 2000]. UV detection. Mefenamic acid and other NSAIDs. Limit of quantification, 50 µg/L for mefenamic acid [Hirai *et al.* 1997].

Disposition in the Body Readily absorbed after oral administration. Metabolised by hydroxylation of the 3′-methyl group followed by oxidation to produce the 3′-carboxy metabolite; glucuronide conjugation also occurs. About 52% of a dose is excreted in the urine in 48 h, 6% as mefenamic acid and the other 46% as conjugated metabolites; about 20% of a dose is eliminated in the faeces, mostly as unconjugated 3′-carboxymefenamic acid. Trace amounts of mefenamic acid may be present in breast milk.

Therapeutic Concentration

Following a single oral dose of 1 g to 6 subjects, a mean peak plasma concentration of 10 g/L was attained in about 2 h; the free and conjugated 3′-hydroxymethyl and 3′-carboxy metabolites attained similar concentrations after about 3 h, and 6 to 8 h respectively [Glazko 1966].

After oral administration of 250 mg 3 times a day for four days to 10 subjects, peak plasma concentrations of 0.3 to 2.4 g/L (mean 0.9) were reported 2 h after the morning dose [Buchanan *et al.* 1968].

Administration of mefenamic acid at an oral dose of 2 mg/kg at 24-h intervals to 17 preterm infants with patent *ductus arteriosus* resulted in a peak plasma concentration of 1.2 to 6.1 mg/L (mean 3.8) at 2 to 18 h after the first dose. For closure of ductus to take place, plasma concentrations above 2 mg/L needed to be maintained for at least 12 h [Ito *et al.* 1994].

Toxicity Toxic effects are usually associated with plasma concentrations greater than 10 g/L.

In a survey of 29 cases of mefenamic acid overdose, plasma concentrations were in the range 11 to 148 g/L; in 11 of the cases convulsions occurred (mean plasma concentration 73 g/L). All the patients recovered [Balali-Mood *et al.* 1981].

Half-life Plasma half-life, about 2 to 4 h.

Protein Binding About 99%.

Dose Up to 1.5 g daily.

Balali-Mood M *et al.* (1981). Mefenamic acid overdosage. *Lancet* 1: 1354–1356.

Buchanan RA *et al.* (1968). The breast milk excretion of mefenamic acid. *Curr Ther Res* 10: 592–596.

Dusci LJ, Hackett LP (1978). Gas-liquid chromatographic determination of mefenamic acid in human serum. *J Chromatogr* 161: 340–342.

Glazko AJ (1966). Experimental observations on flufenamic, mefenamic and meclofenamic acids. 3. Metabolic disposition. *Ann Phys Med* 9: 23–26.

Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692: 375–388.

Ito K *et al.* (1994). Pharmacokinetics of mefenamic acid in preterm infants with patent ductus arteriosus. *Acta Paediatr Jpn* 36(4): 387–391.

Lin CK *et al.* (1980). Determination of two fenamates in plasma by high-performance liquid chromatography. *J Pharm Sci* 69: 95–97.

Mikami E *et al.* (2000). Simultaneous analysis of anthranilic acid derivatives in pharmaceuticals and human urine by high-performance liquid chromatography with isocratic elution. *J Chromatogr B Biomed Sci Appl* 744: 81–89.

Niopas I, Mamzoridi K (1994). Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 656: 447–450.

Streete PJ (1989). Rapid high-performance liquid chromatographic methods for the determination of overdose concentrations of some non-steroidal anti-inflammatory drugs in plasma or serum. *J Chromatogr* 495: 179–193.

Yamashita K *et al.* (1991). Column-switching techniques for high-performance liquid chromatography of ibuprofen and mefenamic acid in human serum with short-wavelength ultraviolet detection. *J Chromatogr* 570: 329–338.

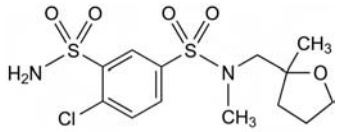
Mefruside

Diuretic

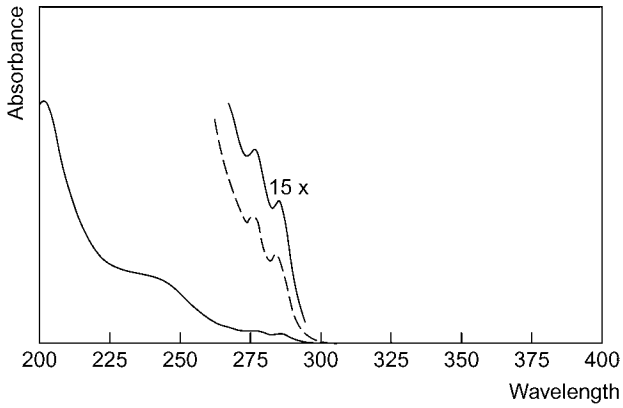
$C_{13}H_{19}ClN_2O_5S_2 = 382.9$

CAS—7195-27-9

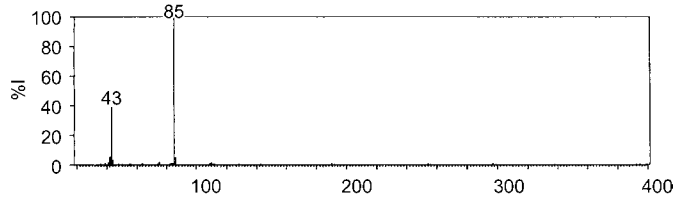
IUPAC Name 4-Chloro-N^1-methyl-N^1-[(tetrahydro-2-methyl-2-furanyl)methyl]-1,3-benzenedisulfonamide
Proprietary Name *Baycaron*



Chemical Properties A white powder. Mp 148° to 149°. Practically insoluble in water; soluble in dilute solutions of sodium hydroxide. Log *P* (octanol/water), 1.5.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—blue; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.45; system TE—R_f 0.67; system TF—R_f 0.58; system TAD—R_f 0.55.
Gas Chromatography System GA—mefruside-Me_2 RI 2860; system GX—mefruside-Me_2 retention time 7.4; M (5-oxo-)-Me_2 retention time 9.8 min; system GY—mefruside-Me_2 retention time 4.7; M (5-oxo-)-Me_2 retention time 5.1 min.
High Performance Liquid Chromatography System HN—*k* 8.67; system HY—RI 417.
Ultraviolet Spectrum Methanol—276 (A_1^1=44a), 284 nm.



Infrared Spectrum Principal peaks at wavenumbers 1162, 1176, 819, 1041, 746, 909 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 85, 43, 42, 86, 44, 41, 75, 110.

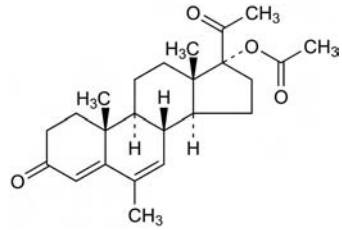


Quantification
Blood **GC** AFID. For method, see Fleuren *et al.* [1979].
Plasma **GC** AFID. Limit of detection, 25 ng for 5-oxomefruside and its hydroxycarboxylic acid analogue [Fleuren *et al.* 1980]. AFID. Limit of detection, 5 µg/L [Fleuren *et al.* 1979].
Urine **GC** AFID. For method, see Fleuren *et al.* [1979].
HPLC UV detection. For method for quantification of mefruside and its metabolites, see Little *et al.* [1977].
Erythrocytes **GC** AFID. Limit of detection, 25 ng for 5-oxomefruside and its hydroxycarboxylic acid analogue [Fleuren *et al.* 1980]. AFID. For method, see Fleuren *et al.* [1979].
Disposition in the Body Readily absorbed after oral administration. About 13% of a dose is excreted in the urine as 5-oxomefruside, about 35 to 55% as the hydroxycarboxylic acid analogue of 5-oxomefruside and up to about 15% as a conjugate of this acid analogue. Both of these metabolites appear to be active. <1% of the dose is excreted as unchanged drug.
Therapeutic Concentration There is considerable intersubject variation in plasma concentrations after therapeutic doses.
Peak plasma concentrations of 0.07 to 0.13 mg/L were attained 2 to 5 h after a single oral dose of 50 mg to 6 subjects [Fleuren *et al.* 1980].
Half-life Plasma half-life, mefruside 3 to 12 h (mean 7), 5-oxomefruside 10 to 14 h, hydroxycarboxylic acid analogue 8 to 12 h.
Volume of Distribution About 6 L/kg.
Clearance Plasma clearance, 5 to 30 mL/min/kg (mean 13).
Distribution in Blood Plasma : whole blood ratio, about 0.03.
Dose 25 to 100 mg daily.

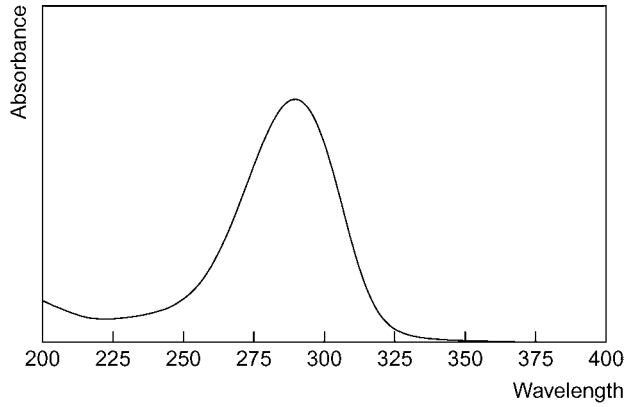
Fleuren HL *et al.* (1979). Quantitative gas chromatographic determination of mefruside in body fluids by extractive methylation and nitrogen detection. *Arzneimittelforschung* 29: 1041–1047.
Fleuren HL *et al.* (1980). Quantitative gas chromatographic determination of two oxidized metabolites of the diuretic mefruside in human urine, plasma and red blood cells. *J Chromatogr* 182: 179–190.
Fleuren HL *et al.* (1980). Pharmacokinetics of mefruside and two active metabolites in man. *Eur J Clin Pharmacol* 17: 59–69.
Little CJ *et al.* (1977). Determination of mefruside and its metabolites in urine by high performance liquid chromatography. *Anal Chem* 49: 1311–1313.

Megestrol Acetate

Progestational Steroid
$C_{24}H_{32}O_4$ = 384.5
CAS—3562-63-8 (megestrol); 595-33-5 (acetate)
IUPAC Name 17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate
Proprietary Names *Acestrol*; *Borea*; *Maygace*; *Megace*; *Megefren*; *Megestat*; *Megestil*; *Megestin*; *Megostat*; *Nia*; *Niagestin*; *Prazoken*.



Chemical Properties A white to creamy-white crystalline powder. Mp about 217°. A solution in chloroform is dextrorotatory. Practically insoluble in water; soluble 1 in 55 of ethanol, 1 in 0.8 of chloroform and 1 in 130 of ether; soluble in acetone. Log *P* (octanol/water), 4.0.
Thin-layer Chromatography System TP—R_f 0.80; system TQ—R_f 0.50; system TR—R_f 0.98; system TS—R_f 0.85, streaking may occur.
Ultraviolet Spectrum Dehydrated alcohol—287 nm (A_1^1=630a).



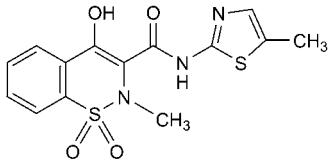
Infrared Spectrum Principal peaks at wavenumbers 1263, 1249, 1733, 1662, 1712, 1630 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 281, 43, 282, 187, 107, 91, 55, 105.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 5 µg/L [Gaver *et al.* 1985].
Serum **HPLC** UV detection. Limit of detection, 5 µg/L for megestrol acetate and cyproterone acetate [Dikkeschei *et al.* 1990].
Dose 40 to 320 mg daily.

Dikkeschei LD *et al.* (1990). Determination of megestrol acetate and cyproterone acetate in serum of patients with advanced breast cancer by high-performance liquid chromatography. *J Chromatogr* 529: 145–154.
Gaver RC *et al.* (1985). Liquid chromatographic procedure for the quantitative analysis of megestrol acetate in human plasma. *J Pharm Sci* 74: 664–667.

Meloxicam

COX-2 Inhibitor, NSAID
$C_{14}H_{13}N_3O_4S_2$ = 51.4
CAS—71125-38-7
IUPAC Name 3-[Hydroxy-[(5-methyl-1,3-thiazol-2-yl)amino]methylidene]-2-methyl-1,1-dioxobenzo[*e*]thiazin-4-one
Synonyms 4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide; UH-AC-62.

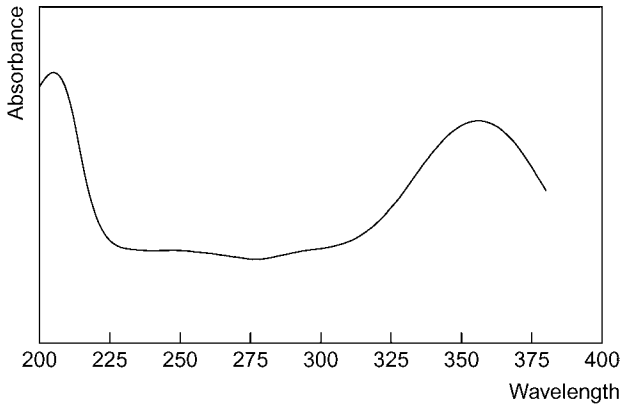
Proprietary Names *Metacam*; *Mobic*.



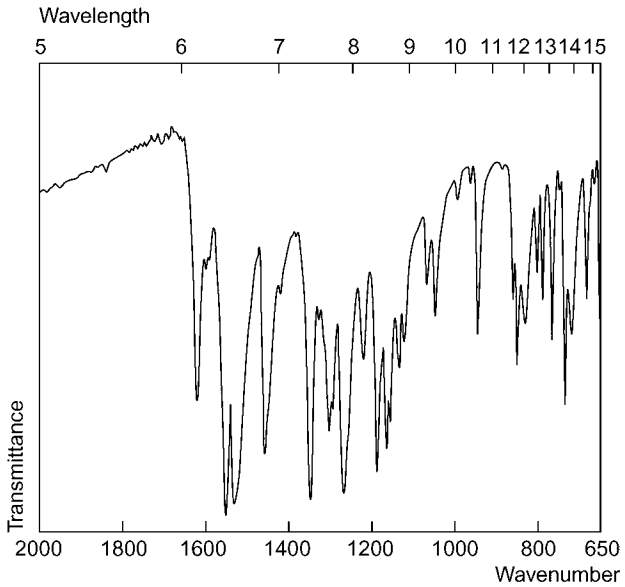
Chemical Properties Crystals from ethylene chloride with Mp 254°. pK_{a1} 1.1; pK_{a2} 4.2. Log *P* (octanol/water), 3.43. Stock solutions at –2 0° were stable for at least 30 days. Applying freeze-thaw did not appear to affect the samples [Zhang, Choi 2008]. Plasma samples were unaffected by 3 freeze-thaw cycles and were stable for at least 1 month when stored at –7 0°; defrosting samples and keeping them at room temperature for 6 h had no effect.[Bae *et al.* 2007].

High Performance Liquid Chromatography System HZ—retention time 6.8 min.

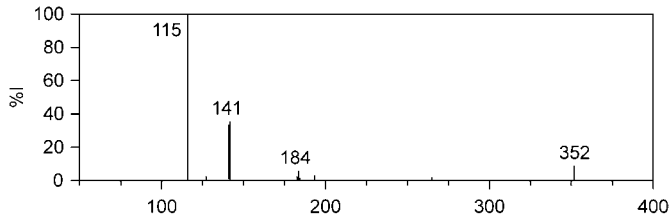
Ultraviolet Spectrum



Infrared Spectrum



Mass Spectrum



Quantification

Plasma **HPLC** Column: Superspher 60 RP-Select B (125 × 3.0 mm i.d., 5 µm). Mobile phase: 2.5 g pentanesulfonic acid, 3.0 g potassium dihydrogen phosphate, 750 g acetonitrile, 300 g methanol (pH 3.75) in 1.5 L, flow rate 1.0 mL/min. UV detection (λ = 355 nm). Retention time: 2.6 min. Limit of quantification, 25 µg/L [Gschwend *et al.* 2007]. Column: Sunfire C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L potassium monophosphate : acetonitrile (60 : 40, pH 3.6), flow rate 1.2 mL/min. UV detection (λ = 355 nm). Retention time: 11.6 min. Limit of quantification, 8.96 µg/L [Bae *et al.* 2007]. Column: RP-18e (100 × 4.6 mm i.d.). Mobile phase: 20 mmol/L disodium hydrogen phosphate (pH 6) : methanol (9 : 1 for 2 min to 6 : 4 in 0.01 min to 6 : 4 until 9 min), flow rate 2 mL/min. DAD (λ = 356 nm). Limit of detection, 30 µg/L [Medvedovici *et al.* 2005]. Column: Lichrospher C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: 170 mmol/L sodium acetate buffer (pH 3.3) : acetonitrile (62 : 38), flow rate 1.0 mL/min. UV detection (λ = 355 nm). Retention time: 6.0 min. Limit of quantification, 50 µg/L; limit of detection, 10 µg/L [Dasandi *et al.* 2002]. Column: Lichrocart RP C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: 50 mmol/L diammonium hydrogen phosphate : methanol : acetonitrile (50 : 40 : 10), flow rate 1 mL/min. UV detection (λ = 364 nm). Retention time: 2.7 min. Limit of quantification, 0.1 mg/L, limit of detection, 0.029 mg/L [Velpandian *et al.* 2000]. Column: ODS Hypersil (125 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : acetonitrile : acetic acid (600 : 500 : 50 : 20) with 1 g 1-heptanesulfonic acid, flow rate 1.5 mL/min. Fluorometric detection (λ_{ex} = 355 nm, λ_{em} = 500 nm). Limit of detection, 70 µg/L [Schmid *et al.* 1995]. See also Türck *et al.* [1995].

LC-MS Column: Prevail C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (70 : 30), flow rate 100 µL/min. TIS, positive ion mode, MRM acquisition mode. Retention time: 1.8 min. Limit of quantification, 20 µg/L [Rigato *et al.* 2006]. Column: Sunfire (100 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : 15 mmol/L ammonium formate (pH 3.0; 60 : 40), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 µg/L [Ji *et al.* 2005]. Column: Phenomenex Luna C_{18} (150 × 2 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.2% aqueous formic acid (65 : 35, pH 3.1), flow rate 0.3 mL/min. ESI. Retention time: ~2.5 min. Limit of quantification, 8.96 µg/L [Wiesner *et al.* 2003].

Serum **HPLC** Column: µBondapak C_{18} (250 × 4.6 mm i.d., 10 µm). Mobile phase: 1% acetic acid : acetonitrile (54 : 46), flow rate 1.0 mL/min. UV detection (λ = 360 nm). Limit of quantification, 45 µg/L, limit of detection, 10 µg/L [Zhang, Choi 2008].

Disposition in the Body Meloxicam is well absorbed after oral administration, reaching maximum concentration 5 to 6 h after once daily dose of 15 mg. After a single 15 mg intramuscular dose, the mean maximum plasma concentration is only approximately twice that observed after oral administration. Intravenous administration is associated with a markedly higher initial concentration (ratio IV/PO 2.99/0.93) followed by a rapid decline. Meloxicam is extensively metabolised via oxidation and the metabolites (4 isolated in total; ~97% of the dose) are excreted via urine and faeces in equal proportions. Less than 0.5% appears as the unchanged drug in urine. The pharmacokinetics of meloxicam alter in renal impairment, with lower total plasma meloxicam concentrations and higher free fractions being observed at creatinine clearances of 20–40 mL/min. However, there is no apparent risk of increased toxicity.

Therapeutic Concentration

Twenty-four healthy male subjects were administered 15 mg meloxicam as two different tablet formulations in a bioequivalence assessment. The mean maximum plasma concentrations were 1147 ± 192 and 1065 ± 181 µg/L, reached at 5 h [Gschwend *et al.* 2007.

A separate study of 24 healthy volunteers who received a 15 mg dose of meloxicam as one of two different tablet formulations showed mean maximum plasma concentrations of 1120 and 1210 µg/L reached at 4 h [Rigato *et al.* 2006].

In another similar study, the 24 healthy volunteers received a 15 mg dose of meloxicam as one of two different tablet formulations. Mean maximum plasma concentrations were 1303.26 ± 364.8 and 1345.41 ± 407.44 µg/L, reached at 3.4 and 5.6 h, respectively [Medvedovici *et al.* 2005].See also [Marcelín-Jiménez *et al.* 2005].

A single oral dose of 15 mg meloxicam was administered to 11 healthy Korean volunteers. A mean maximum plasma concentration of 1446 µg/L was reached at 4.1 h. The elimination half-life was ~22 h [Bae *et al.* 2007].

A single oral dose of 15 mg meloxicam was administered to 26 healthy volunteers. The mean maximum plasma concentration was 1538 µg/L reached at ~4.5 h. The elimination half-life was ~24 h [Wiesner *et al.* 2003].

Six healthy volunteers (aged 27 to 49 years) were administered a single oral 30 mg dose after an overnight fast or 10–20 min after a continental (light) breakfast. The fasting individuals reached peak plasma concentrations of 1.51 mg/L at ~10.7 h compared with the fed volunteers, who showed peak concentrations of 1.43 mg/L at 9.7 h. In a second study, 16 healthy volunteers (aged 24 to 47 years) were administered a 15 mg dose after a 12 h fast or immediately after a high fat breakfast. Those given the dose after the fast showed peak concentrations of 0.928 mg/L at 8.8 h and those with the dose coadministered with a high fat meal reached 1.14 mg/L approximately 6.1 h after ingestion [Türck *et al.* 1995].

Four healthy males were administered an intravenous 30 mg dose (over 15 min) or a single oral 30 mg dose, 2 h after a light breakfast. Peak plasma concentrations were 5.35 mg/L and 3.09 mg/L for the IV and oral doses, respectively, attained 2 h after administration [Schmid *et al.* 1995].

Bioavailability Approximately 89%.

Half-life 25–20 h.

Volume of Distribution 11.5–16.5 L (decreased by coadministration with a high fat meal); also reported as 10 L.

Clearance Plasma, 7.25–8.5 mL/min.

Distribution in Blood <10% in red blood cells.
Protein Binding 99.4%, mainly to albumin.
Dose Orally and via suppositories: the usual dose is 7.5 to 15 mg daily. A lower daily dose of 7.5 mg is recommended in the elderly and those on renal dialysis.

Bae JW *et al.* (2007). Determination of meloxicam in human plasma using a HPLC method with UV detection and its application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 859: 69–73.
Dasandi B *et al.* (2002). LC determination and pharmacokinetics of meloxicam. *J Pharm Biomed Anal* 28: 999–1004.
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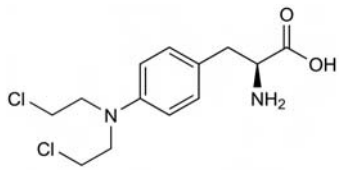
Marcelín-Jiménez G *et al.* (2005). Bioequivalence evaluation of two brands of meloxicam tablets (Promotion and Mobicox): pharmacokinetics in a healthy female Mexican population. *Biopharm Drug Dispos* 26: 167–171.
Medvedovici A *et al.* (2005). A non-extracting procedure for the determination of meloxicam in plasma samples by HPLC-diode array detection. *Arzneimittelforschung* 55: 326–331.
Rigato HM *et al.* (2006). Meloxicam determination in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) in Brazilian bioequivalence studies. *Int J Clin Pharmacol Ther* 44: 489–498.
Schmid J *et al.* (1995). Pharmacokinetics and metabolic pattern after intravenous infusion and oral administration to healthy subjects. *Drug Metab Dispos* 23: 1206–1213.
Türck D *et al.* (1995). Effect of food on the pharmacokinetics of meloxicam after oral administration. *Clin Drug Invest* 9: 270–276.
Velpandian T *et al.* (2000). Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples. *J Chromatogr B Biomed Sci Appl* 738: 431–436.
Wiesner JL *et al.* (2003). Sensitive and rapid liquid chromatography–tandem mass spectrometry method for the determination of meloxicam in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 785: 115–121.
Zhang H, Choi HK (2008). Analysis of meloxicam by high-performance liquid chromatography with cloud-point extraction. *Anal BioAnal Chem* 392: 947–953.

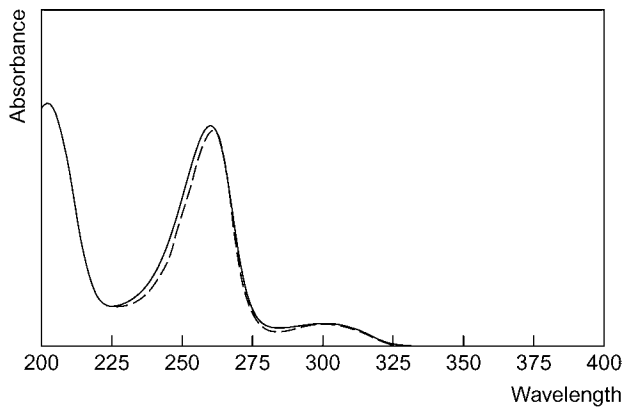
Melphalan

Antineoplastic
$C_{13}H_{18}Cl_2N_2O_2 = 305.2$
CAS—148-82-3

IUPAC Name 4-[Bis(2-chloroethyl)amino]-L-phenylalanine
Synonyms PAM; phenylalanine nitrogen mustard.
Note Merphalan is the racemic form of melphalan; medphalan is the D-isomer of melphalan.
Proprietary Name *Alkeran*



Chemical Properties A white to buff-coloured powder. Mp about 177° to 180°, with decomposition. Practically insoluble in water, chloroform and ether; soluble in ethanol and propylene glycol; soluble in dilute mineral acids. Log *P* (octanol/water), −0.5.
High Performance Liquid Chromatography System HAA—retention time 12.9 min.
Ultraviolet Spectrum Methanol—260 (A^{1}_{1}=707a), 301 nm.



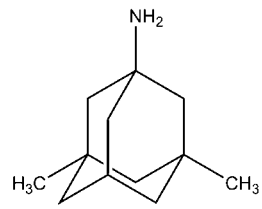
Infrared Spectrum Principal peaks at wavenumbers 1621, 1513, 1186, 1160, 1250, 1205 cm^{-1} (KBr disk).
Quantification
Blood **HPLC** Fluorescence detection. Limit of detection, <30 µg/L [Osterheld *et al.* 1988].
Plasma **HPLC** UV detection. For method, see Pinguet *et al.* [1996]. Melphalan and metabolites. Limit of detection, <1.4 ng for melphalan [Wu *et al.* 1995]. Limit of detection, 10 µg/L [Kato *et al.* 1992]. Melphalan and other nitrogen mustard anticancer drugs. Limit of detection, 1 ng for melphalan [Cummings *et al.* 1991]. Electrochemical detection. For method, see Silvestro *et al.* [1991]. See Blood [Osterheld *et al.* 1988]. Fluorescence detection. Limit of detection, 10 µg/L [Woodhouse, Henderson 1982]. UV detection. Limit of detection, 50 µg/L [Chang *et al.* 1978].
Perfusate **HPLC** See Plasma [Wu *et al.* 1995].
Tissue **HPLC** See Plasma. Limit of detection, <7.2 ng for melphalan [Wu *et al.* 1995].
Therapeutic Concentration
When administered as a 24 h continuous IV infusion to 21 subjects at a dose of 20, 30 and 40 mg/m^2, steady-state melphalan concentrations of 0.080, 0.121 and 0.206 mg/L were obtained; the maximum tolerated dose was 30 mg/m^2 [Pinguet *et al.* 2000].
Dose 200 to 300 µg/kg daily, orally, for 4 to 6 days.

Chang SY *et al.* (1978). High-pressure liquid chromatographic analysis of melphalan in plasma. *J Pharm Sci* 67: 679–682.
Cummings J *et al.* (1991). Determination of reactive nitrogen mustard anticancer drugs in plasma by high-performance liquid chromatography using derivatization. *Anal Chem* 63: 1514–1519.
Kato Y *et al.* (1992). Direct injection analysis of melphalan in plasma using column-switching high-performance liquid chromatography. *Ther Drug Monit* 14: 66–71.
Osterheld HK *et al.* (1988). A sensitive high-performance liquid chromatographic assay for melphalan and its hydrolysis products in blood and plasma. *Cancer Chemother Pharmacol* 21: 156–162.
Pinguet F *et al.* (1996). High-performance liquid chromatographic assay for melphalan in human plasma. Application to pharmacokinetic studies. *J Chromatogr B Biomed Appl* 686: 43–49.
Pinguet F *et al.* (2000). A phase I and pharmacokinetic study of melphalan using a 24-hour continuous infusion in patients with advanced malignancies. *Clin Cancer Res* 6: 57–63.
Silvestro L *et al.* (1991). Quantitation of melphalan in plasma of patients by reversed-phase high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 563: 443–450.
Woodhouse KW, Henderson DB (1982). *Br J Clin Pharmacol* 13605P.
Wu ZY *et al.* (1995). High-performance liquid chromatographic assay for the measurement of melphalan and its hydrolysis products in perfusate and plasma and melphalan in tissues from human and rat isolated limb perfusions. *J Chromatogr B Biomed Appl* 673: 267–279.

Memantine

Amine, NMDA Antagonist, Treatment of Alzheimer's Disease
$C_{12}H_{21}N = 179.3$
CAS—19982-08-2

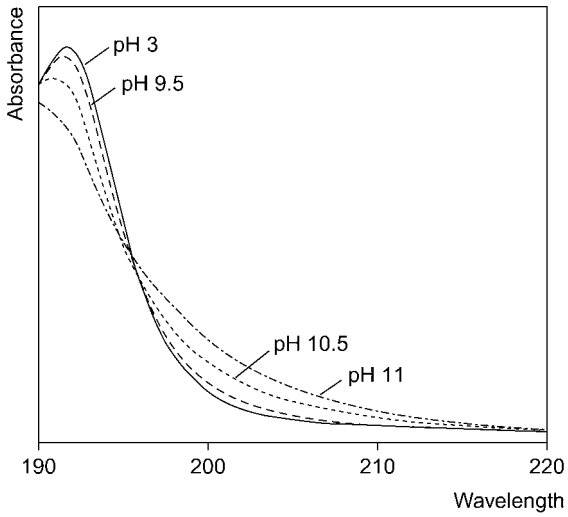
IUPAC Name 3,5-Dimethyladamantan-1-amine
Synonyms 1-Amino-3,5-dimethyladamantane; D-145; 3,5-dimethyl-1-adamantanaamine; 3,5-dimethyltricyclo[3.3.1.1.3,7]decan-1-amine; DMAA.



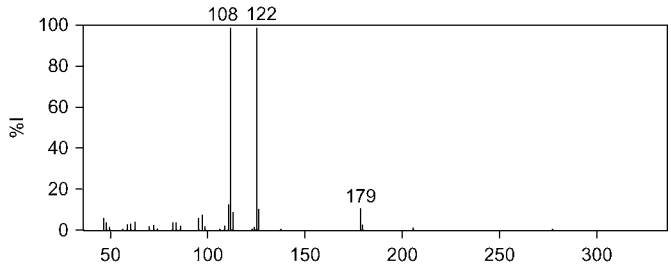
Chemical Properties Oil. Stable in plasma samples for at least 150 days at –20° [Almeida *et al.* 2007].

Memantine Hydrochloride

$C_{12}H_{21}N,HCl = 215.8$
CAS—41100-52-1
Proprietary Names *Abixa*; *Akatinol*; *Axura*; *Carrier*; *Conxine*; *Ebixa*; *Eutebrol*; *Fentina*; *Lucidex*; *Memax*; *Memox*; *Merital*; *Mimetix*; *Namenda*; *Neuroplus*; *Pronervon*.
Chemical Properties Fine white to off-white powder. Mp 258°. Soluble in water. pK_a 10.4. Log *P* (octanol/water), 3.28 [Koeberle *et al.* 2003].
High Performance Liquid Chromatography Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : ethanol : water : trifluroacetic acid (40 : 20 : 40 : 0.1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 470 nm, λ_{em} = 540 nm). Retention time: ~35 min (4-fluoro-7-nitro-2,1,3-benzoxadiazole derivative). Limit of quantification, 25 µg/L [Higashi *et al.* 2006]. Column: C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : tetrahydrofuran : water: triethylamine (75 : 10 : 15 : 0.02), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 348 nm). Retention time: 8.5 min ([2-naphthoxy]acetic acid derivative). Limit of quantification, 0.5 µmol/L, limit of detection, 0.15 µmol/L [Duh *et al.* 2003].
Capillary Electrophoresis Capillary: uncoated fused silica (430 cm × 75 µm i.d., 36.5 cm to detector). Buffer: 5 mmol/L 4-methylbenzylamine in ethanol : water (1 : 4, pH 9.0). UV detection (λ = 210 nm). Limit of quantification, 2 mg/L; limit of detection, 0.35 mg/L [Reichova *et al.* 2002].
Ultraviolet Spectrum Principal peak at 192 nm[Reichova *et al.* 2002].



Mass Spectrum Principal ions at *m/z* 108, 122, 179, 164 [Wesemann *et al.* 1982].



Quantification

Blood **GC-MS** Column: DB-5MS capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 8.3 mL/min. Temperature programme: 80° to 300° at 20°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 2.3 min. Limit of quantification, 0.1 mg/L; limit of detection, 0.01 mg/L [Bynum *et al.* 2007].

Plasma **GC-MS** Column: DB-5MS capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 100° for 1 min to 300° at 40°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 4.8 min (pentafluorobenzyl chloride derivative). Limit of quantification, 117 ng/L; limit of detection, 10 ng/L [Leis *et al.* 2002].

HPLC Column: Lichrospher C_{18} (150 × 6.0 mm i.d., 5 µm). Mobile phase: methanol : water (85 : 15), flow rate 1.0 mL/min. UV detection (λ = 256 nm). Retention time: 21 min (anthraquinone-2-sulfonyl chloride derivative). Limit of quantification, 50 µg/L; limit of detection, 20 µg/L [Shuangjin *et al.* 2007]. Column: C_{18} (125 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : tetrahydrofuran : water : triethylamine (75 : 10 : 15 : 0.02), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 348 nm). Retention time: 8.5 min ([2-naphthoxy]acetic acid derivative). Limit of quantification, 0.5 µmol/L; limit of detection, 0.15 µmol/L [Duh *et al.* 2003]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.025 mol/L monobasic potassium phosphate : acetonitrile (27 : 73) with 0.5 mL *o*-phosphoric acid and 0.6 mL *n*-butylamine, flow rate 1.8 mL/min. Fluorescence detection (λ_{ex} = 235 nm, λ_{em} = 470 nm). Retention time: 12.1 min (dansyl chloride derivative). Limit of quantification, 3 µg/L [Suckow *et al.* 1999].

LC-MS Column: C_{18} (50 × 3.0 mm i.d., 3 µm). Mobile phase: methanol : water : formic acid (80 : 20 : 0.1), flow rate 0.15 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.1 µg/L [Almeida *et al.* 2007]. Column: Zorbax SB-C_8 (150 × 4.6 mm i.d., 3.5 µm). APCI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.5 µg/L [Rao *et al.* 2005].

Urine **GC-MS** Column: DB-5MS capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 8.3 mL/min. Temperature programme: 80° to 300° at 20°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 2.3 min. Limit of quantification, 0.1 mg/L; limit of detection, 0.01 mg/L [Bynum *et al.* 2007].

HPLC Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : tetrahydrofuran : water : triethylamine (75 : 10 : 15 : 0.02), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 348 nm). Derivatisation agent: (2-naphthoxy)acetyl chloride. Limit of detection, 0.2 µmol/L [Duh *et al.* 2005].

Vitreous Humour **GC-MS** See Urine [Bynum *et al.* 2007].

Stomach Contents **GC-MS** See Urine [Bynum *et al.* 2007].

Liver **GC-MS** See Urine [Bynum *et al.* 2007].

Other **GC** Rat Tissue. Column: glass column with 2% Emulphor OU on GasChrom P 80-100 mesh (1 m × 3.4 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 180°. FID. Retention time: 2.5 min. Limit of detection, 60 ng/g [Wesemann *et al.* 1982].

HPLC Melanin. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.2% acetic acid (pH 7.0, 45 : 55), flow rate 0.3 mL/min for 18 min to 1.5 mL/min for 11 min. Fluorescence detection (λ_{ex} = 370 nm, λ_{em} = 506 nm). Retention time: 15.2 min (dansyl chloride derivative). Limit of detection, 0.012 µmol/L [Higashi, Fujii 2005].

LC-MS Melanin. Column: ODS-3 (100 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in methanol (50 : 50 to 30 : 70 over 3 min for 2 min), flow rate 0.8 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 3.6 min. Limit of quantification, 0.10 nmol/L when sample prepared in deionised water [Koeberle *et al.* 2003].

Disposition in the Body Well absorbed after oral doses with peak plasma concentrations achieved in approx. 3 to 8 h. It undergoes partial hepatic metabolism; the main metabolites include *N*-3,5-dimethyl-gludantan and 1-nitroso-3,5-dimethyl-adamantane. The majority of a dose is excreted unchanged via the kidney (50%); some active renal tubular secretion and re-absorption occurs. The remainder of memantine that is not excreted as unchanged drug in urine is converted to the polar metabolites memantine glucuronide conjugate (approx. 25%), 6-hydroxy-memantine and 1-nitroso-deaminated memantine, which are minimally active. Memantine rapidly crosses the blood–brain barrier and is detectable in CSF approx. 30 min after infusion.

Therapeutic Concentration

A group of 32 volunteers with different degrees of renal impairment was administered a single oral dose of 20 mg memantine hydrochloride. Mean peak plasma concentrations for the different groups were reported as follows:

Parameter	*Healthy subjects*	*Renal impairment*		
		Mild	*Moderate*	*Severe*
CL_{CR} (mL/min)	93.5	60.9	41.6	20.1
C_{max} (µg/L)	22.1	19.3	24.3	25.8
Time (h)	7.6	7.0	9.0	8.3

The cumulative amount of drug excreted (Cum.Ae) in urine was also measured and reported as:

Parameter	*Healthy subjects*	*Renal impairment*		
		Mild	*Moderate*	*Severe*
Memantine				
Cum.Ae (mg)	7.95	5.44	6.43	3.82
% Dose	47.9	32.8	38.7	23.0
Memantine plus glucuronic acid conjugate				
Cum.Ae (mg)	12.35	11.80	10.62	7.41
% Dose	74.3	71.0	63.9	44.6

[Periclou *et al.* 2006].

A single oral dose of 20 mg memantine was given to 21 healthy young volunteers (mean age 26.1 years). The mean peak plasma concentration was 29.3 µg/L after 4.8 h. In another study where glyburide (1.25 mg) and metformin (250 mg) were co-administered with 20 mg memantine, the mean peak plasma concentration of memantine was 27.9 µg/L after 6 h, thus demonstrating a lack of interaction between the drugs [Rao *et al.* 2005].

Nineteen healthy young volunteers (mean age 27.4 years) were administered 10 mg memantine as a single dose on day 1. Following a 14-day washout period, donepezil was administered once daily in the morning starting at 5 mg/day for 7 days and escalated to 10 mg/day for 22 days thereafter. On the final morning of donepezil treatment (day 43), subjects received both memantine and donepezil 10 mg. The mean peak plasma concentration of memantine without donepezil (day 1) was 12.8 µg/L after 6.5 h. On day 43, the mean peak plasma concentration of memantine (with co-administered donepezil) was 13.0 µg/L after 6.5 h, thus demonstrating a lack of interaction between the drugs [Periclou *et al.* 2004].

Toxicity

A 62-year-old man with a history of early-onset Alzheimer's disease and hypertension was found dead in bed. He had prescriptions for donepezil, clonazepam, propoxyphene with paracetamol, venlafaxine, and memantine. Postmortem memantine concentrations in blood and tissues were reported as follows:

Matrix	*Memantine concentration*
Blood (aorta, mg/L)	1.80
Blood (iliac, mg/L)	0.65
Urine (mg/L)	6.20
Gastric (mg/kg)	6.20
Vitreous humour (mg/L)	0.39
Liver (mg/kg)	6.10

The memantine levels were not considered to be toxic and concentrations of the other medicines were within therapeutic ranges. However, chloroform was detected at 10 mg/L in aortic and iliac blood and 12 mg/kg in liver. The cause of death was therefore attributed to chloroform poisoning [Bynum *et al.* 2007].

Bioavailability 100%.

Half-life Ranges from 60 to 100 h, although under alkaline conditions the rate of elimination is reduced.

Volume of Distribution Approximately 9 to 10 L/kg.

Clearance In subjects with normal renal function 170 mL/min/1.73 m^2.

Protein Binding Approximately 45%.

Dose In the treatment of Alzheimer's disease, the initial dose of memantine hydrochloride is 5 mg daily in the morning for the first week; this should be increased in weekly increments of 5 mg to a maximum dose of 20 mg daily. Doses of 10 mg daily and over should be taken in two divided doses. Reduced doses are recommended in patients with renal impairment.

Almeida AA *et al.* (2007). Determination of memantine in human plasma by liquid chromatography–electrospray tandem mass spectrometry: application to a bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci* 848: 311–316.

Bynum N *et al.* (2007). Postmortem memantine concentrations. *J Anal Toxicol* 31: 233–236.

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Higashi Y, Fujii Y (2005). Simultaneous determination of the binding of amantadine and its analogues to synthetic melanin by liquid chromatography after precolumn derivatization with dansyl chloride. *J Chromatogr Sci* 43: 213–217.

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Leis HJ *et al.* (2002). Quantitative analysis of memantine in human plasma by gas chromatography/negative ion chemical ionization/mass spectrometry. *J Mass Spectrom* 37: 477–480.

Periclou AP *et al.* (2002). Lack of pharmacokinetic or pharmacodynamic interaction between memantine and donepezil. *Ann Pharmacother* 38: 1389–1394.

Periclou A *et al.* (2006). Pharmacokinetic study of memantine in healthy and renally impaired subjects. *Clin Pharmacol Ther* 79: 134–143.

Rao RN *et al.* (2005). Development and validation of a reversed-phase liquid chromatographic method for separation and simultaneous determination of COX-2 inhibitors in pharmaceuticals and its application to biological fluids. *Biomed Chromatogr* 19: 362–368.

Reichova N *et al.* (2002). Electrophoretic behavior of adamantane derivatives possessing antiviral activity and their determination by capillary zone electrophoresis with indirect detection. *Electrophoresis* 23: 259–262.

Shuangjin C *et al.* (2007). New method for high-performance liquid chromatographic determination of amantadine and its analogues in rat plasma. *J Pharm Biomed Anal* 44: 1100–1105.

Suckow RF *et al.* (1999). Sensitive and selective liquid chromatographic assay of memantine in plasma with fluorescence detection after pre-column derivatization. *J Chromatogr B Biomed Sci Appl* 729: 217–224.

Wesemann W *et al.* (1982). Distribution of memantine in brain, liver, and blood of the rat. *Arzneimittelforschung* 32: 1243–1245.

Menadione

Vitamin K Analogue

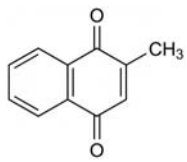
$C_{11}H_8O_2$ = 172.2

CAS—58-27-5

IUPAC Name 2-Methyl-1,4-naphthalenedione

Synonyms Menaphthene; menaphthone; methylnaphthochinonum; vitamin K_3.

Proprietary Names *Kappaxin*; *Kayquinone*; *Thyloquinone*. It is an ingredient of *Bilkaby*.



Chemical Properties A bright-yellow crystalline powder. On exposure to light it decomposes and darkens to light brown. Mp 105° to 107°. Insoluble in water; soluble 1 in 60 of ethanol and 1 in 10 of benzene; moderately soluble in chloroform and carbon tetrachloride; soluble in ether. Log *P* (octanol/water), 2.2.

Caution The powder is irritating to the respiratory tract and to the skin. The ethanolic solution has vesicant properties.

Menadione Sodium Bisulfite

$C_{11}H_8O_2NaHSO_3,3H_2O$ = 330.3

CAS—130-37-0 (anhydrous); 6147-37-1 (trihydrate)

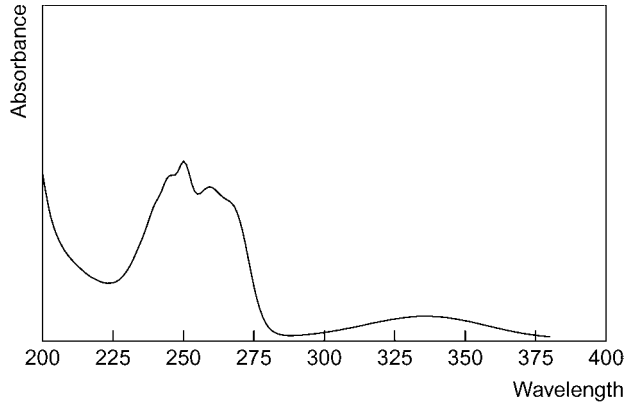
Synonyms Kavitanum; vikasolum.

Proprietary Names *Hykinone*; *Klotogen*; *K-Thrombin*.

Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in about 2 of water; slightly soluble in ethanol; almost insoluble in ether and benzene.

Colour Test Dissolve about 0.5 mg in 5 mL of ethanol and add 2 mL of strong ammonia solution and a few drops of ethyl cyanoacetate—violet; add 5 mL of sodium hydroxide solution—brown-yellow. The violet colour is destroyed on the addition of acid or on exposure to sunlight.

Ultraviolet Spectrum Aqueous acid—244 (A^1_1=1052b), 249 (A^1_1=1133b), 261 (A^1_1=990b), 340 nm (A^1_1=159b).



Quantification

Plasma **HPLC** Limit of detection, <10 µg/L [Hu *et al.* 1995].

Dose Menadione is usually administered by IM injection as a 0.5% solution in oil.

Hu OY *et al.* (1995). Determination of anticancer drug vitamin K3 in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 666: 299–305.

Menthol

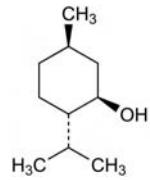
Decongestant, Antipruritic

$C_{10}H_{20}O$ = 156.3

CAS—89-78-1; 1490-04-6; 2216-51-5 (–); 15356-70-4 (±)

IUPAC Name 5-Methyl-2-propan-2-ylcyclohexan-1-ol

Synonyms Mentol; (1α,2β,5α)-5-methyl-2-(1-methylethyl)cyclohexanol.



Chemical Properties Menthol is natural laevo-menthol, obtained from the volatile oils of various species of *Mentha* (Labiatae), or synthetic laevo-menthol, or racemic menthol. Colourless crystals or crystalline powder. Mp of 41° to 43° and of (±)-menthol,27° to 28°(rising on prolonged stirring to 30° to 32°). Slightly soluble in water; soluble 1 in 0.2 of ethanol, 1 in 0.25 of chloroform and 1 in 0.4 of ether; freely soluble in glacial acetic acid and liquid petrolatum. Log *P* (octanol/water), 3.4.

Gas Chromatography System GA—RI 1206; system GB—RI 1194.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1046, 1026, 995, 1102, 1078, 979 cm^{-1} (Nujol mull).

Mass Spectrum Principal ions at *m/z* 81, 95, 71, 41, 67, 55, 138, 123.

Quantification

Plasma **GC** FID. Limit of detection, 1 µg/L for menthol, camphor and methylsalicylate [Valdez *et al.* 1999].

Urine **GC** FID. Limit of detection, 250 µg/L for menthol and its glucuronide [Kaffenberger, Doyle 1990].

Disposition in the Body Menthol is excreted in the bile and urine as a glucuronide conjugate; the various stereoisomers differ quantitatively in the extent to which they conjugate with glucuronic acid. Menthol may occur in the conjugated form as a metabolite of pulagone which is a constituent of pulegium oil.

Toxicity The minimum lethal dose has been estimated to be about 2 g. The application of drops or ointments containing menthol to the nostrils of infants is dangerous and may cause instant collapse. Ingestion of excess menthol may cause severe abdominal pain and coma.

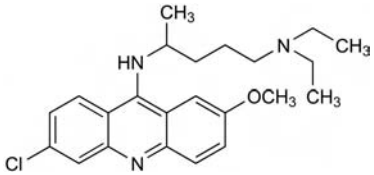
Kaffenberger RM, Doyle MJ (1990). Determination of menthol and menthol glucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection. *J Chromatogr* 527: 59–66.

Valdez JS *et al.* (1999). Sensitive and selective gas chromatographic methods for the quantitation of camphor, menthol and methyl salicylate from human plasma. *J Chromatogr B Biomed Sci Appl* 729: 163–171.

Mepacrine

Antimalarial, Antiprotozoal
$C_{23}H_{30}ClN_3O = 400.0$
CAS—83-89-6

IUPAC Name N^4-(6-Chloro-2-methoxy-9-acridinyl)-N^1,N^1-diethyl-1,4-pentanediamine
Synonyms Acrichinum; acrinamine; atebrine; chinacrina; quinacrine.



Chemical Properties pK_a 7.7, 10.3 (20°). Log *P* (octanol/water), 5.8.

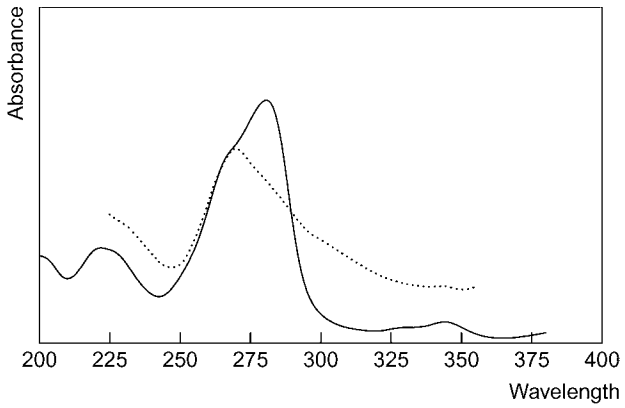
Mepacrine Hydrochloride

$C_{23}H_{30}ClN_3O,2HCl,\ 2H_2O = 508.9$
CAS—69-05-6 (anhydrous); 6151-30-0 (dihydrate)
Synonyms Antimalarinae chlorhydras; erion; quinacrine hydrochloride.
Proprietary Name *Atabrine*
Chemical Properties A bright yellow crystalline powder. Mp 248° to 250°, with decomposition. Soluble 1 in about 35 of water, more soluble in hot water; slightly soluble in ethanol; very slightly soluble in chloroform; insoluble in ether, benzene and acetone.

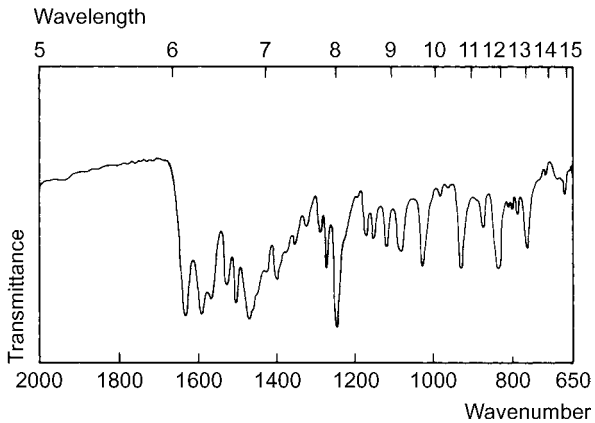
Mepacrine Mesilate

$C_{23}H_{30}ClN_3O,2CH_3SO_3H,\ H_2O = 610.2$
CAS—316-05-2 (anhydrous)
Synonym Mepacrine methanesulphonate
Chemical Properties Bright yellow crystals. Soluble 1 in 3 of water and 1 in 36 of ethanol.

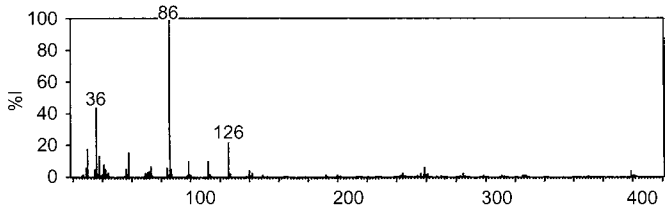
Colour Tests Mandelin's test—violet→yellow; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.43; system TB—R_f 0.15; system TC—R_f 0.05; system TL—R_f 0.09 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HX—RI 345; system HY—RI 299.
Ultraviolet Spectrum Aqueous acid—279 (A^1_1=1706a), 343 nm; aqueous alkali—270, 345 nm.



Infrared Spectrum Principal peaks at wavenumbers 1245, 1627, 1587, 1500, 1560, 1522 cm^{-1} (mepacrine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 36, 126, 30, 58, 38, 112, 99.



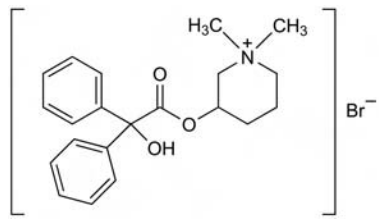
Quantification
***Plasma* HPLC** Fluorescence detection [Bjorkman, Elisson 1987].
Disposition in the Body Readily absorbed after oral administration; it appears in the blood within 2 h, peak concentrations being attained in 8 h. Mepacrine accumulates in the body during chronic administration and is widely distributed, high concentrations being found in the liver, lungs and spleen; it also persists in the skin and finger-nails. The urine and skin may become yellow. It is excreted very slowly in the urine and bile with significant amounts detectable in the urine for at least 2 months after the discontinuation of therapy. Several metabolites are excreted but these account for not more than 5% of the dose.
Toxicity Large doses may give rise to nausea and vomiting and occasionally to transient mental disturbances. Attempts at suicide with massive doses have not been successful.
Half-life About 5 days.
Distribution in Blood Concentrated in the erythrocytes.
Protein Binding About 90%.
Dose 300 to 900 mg daily.

Bjorkman S, Elisson LO (1987). Determination of quinacrine (mepacrine) in plasma by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 420: 341–348.

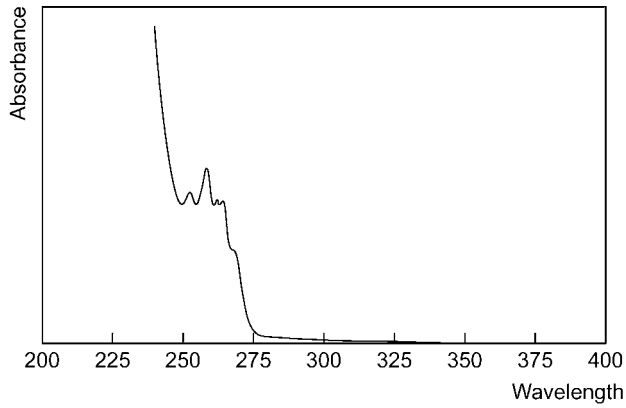
Mepenzolate Bromide

Anticholinergic
$C_{21}H_{26}BrNO_3 = 420.3$
CAS—25990-43-6 (mepenzolate); 76-90-4 (bromide)

IUPAC Name 3-[(Hydroxydiphenylacetyl)oxy]-1,1-dimethylpiperidinium bromide
Proprietary Names *Cantil*; *Cantril*; *Gastropidil*; *Trancolan*.



Chemical Properties A white or light cream-coloured powder. Mp 228° to 229°, with decomposition. Soluble 1 in 110 of water, 1 in 120 of dehydrated alcohol, 1 in about 630 of chloroform and 1 in about 8 of methanol; practically insoluble in ether. Log *P* (octanol/water), −0.8.
Colour Tests The following tests are performed on mepenzolate nitrate: Liebermann's reagent—brown; Marquis test—orange (transient).
Thin-layer Chromatography System TA—R_f 0.01; system TAJ—R_f 0.42; system TAK—R_f 0.04; system TAL—R_f 0.52 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2327.
High Performance Liquid Chromatography System HA—*k* 4.1 (tailing peak).
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=11b), 261, 263 nm.

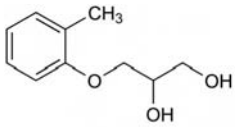


M

Infrared Spectrum Principal peaks at wavenumbers 1220, 1727, 1163, 763, 1062, 1093 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 97, 105, 77, 96, 42, 183, 98, 82.
Dose 75 to 200 mg daily.

Mephenesin

Muscle Relaxant
$C_{10}H_{14}O_3$ = 182.2
CAS—59-47-2
IUPAC Name 3-(2-Methylphenoxy)-1,2-propanediol
Synonyms Cresoxydiol; glykresin.
Proprietary Names *Decontractyl*; *DoloVisano M*.



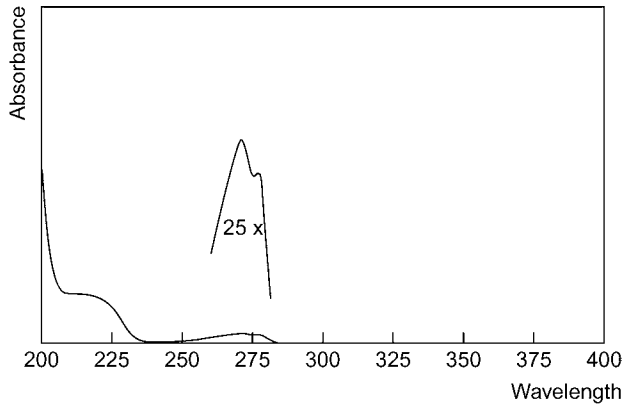
Chemical Properties White crystals or crystalline aggregates. Mp 70° to 71°. Soluble 1 in 100 of water, 1 in 8 of ethanol and 1 in 12 of chloroform; soluble in ether; freely soluble in propylene glycol. Log *P* (octanol/water), 1.4.

Mephenesin Carbamate

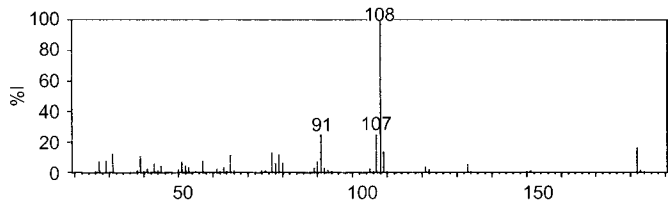
$C_{11}H_{15}NO_4$ = 225.2
CAS—533-06-2
Chemical Properties White crystals. Mp 93°. Slightly soluble in water; freely soluble in ethanol; soluble 1 in 50 of chloroform.

Colour Tests Mephenesin carbamate: Mandelin's test—grey-brown; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.02; system TC—R_f 0.43; system TL—R_f 0.57; system TAE—R_f 0.87; system TAF—R_f 0.89; system TAJ—R_f 0.45; system TAK—R_f 0.29; system TAL—R_f 0.78 (Marquis reagent, positive).

System TD—Mephenesin carbamate R_f 0.14; system TE—Mephenesin carbamate R_f 0.55; system TF—Mephenesin carbamate R_f 0.36; system TAD—Mephenesin carbamate R_f 0.35 (furfuraldehyde reagent, positive).
Gas Chromatography System GA—RI 1568.
High Performance Liquid Chromatography System HA—*k* 0.2; system HX—RI 364; system HY—RI 322.
Ultraviolet Spectrum Aqueous acid—270 (A_1^1=87a), 276 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1047, 1248, 1124, 1493, 756, 748 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 108, 107, 91, 182, 109, 77, 79, 31; mephenesin carbamate 118, 108, 182, 91, 225, 107, 57, 75.

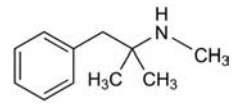


Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the tissues. It is rapidly metabolised to the inactive metabolites, β-(*o*-methylbenzoyloxy)lactic acid and β-(2-methyl-4-hydroxyphenoxy)lactic acid. <2% of a dose is excreted in the urine as unchanged drug.
Therapeutic Concentration
Following a single oral dose of 3 g to 4 subjects, a mean peak plasma concentration of about 10 mg/L was attained in 0.5 h [London, Poet 1957].
Toxicity
A 43-year-old woman, who ingested between 5.5 and 11 g of mephenesin recovered within about 10 h following treatment [Barron, Milliken 1960].
Dose 0.5 to 6 g of mephenesin daily.

Barron DW, Milliken TG (1960). Mephenesin poisoning. *Lancet* 1: 262.
London I, Poet RB (1957). Comparative plasma levels of mephenesin and its carbamic acid ester. *Proc Soc Exp Biol Med* 94: 191.

Mephentermine

Sympathomimetic
$C_{11}H_{17}N$ = 163.3
CAS—100-92-5
IUPAC Name *N*,2-Dimethyl-1-phenylpropan-2-amine
Synonyms Mephenterdrine; mephetedrine; *N*,α,α-trimethylbenzeneethanamine.

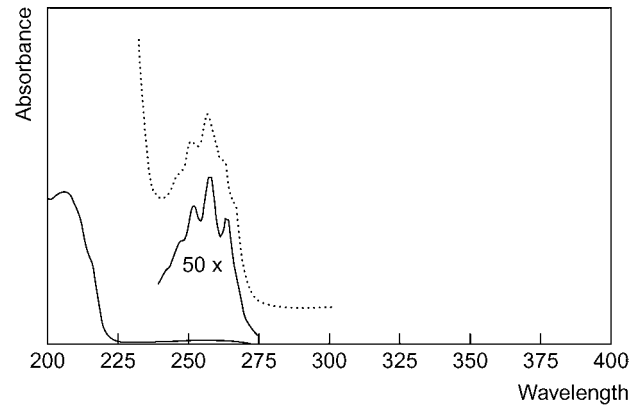


Chemical Properties A clear, colourless to pale yellow liquid. Practically insoluble in water; freely soluble in ethanol; soluble in ether. pK_a 10.4. Log *P* (octanol/water), 2.7.

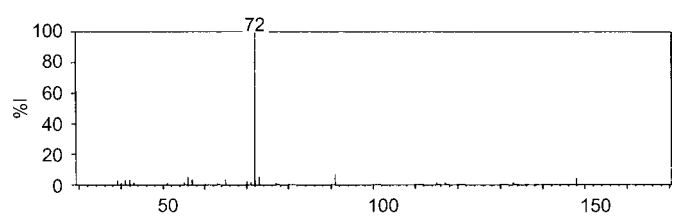
Mephentermine Sulfate

$(C_{11}H_{17}N)_2,H_2SO_4,\ 2H_2O$ = 460.6
CAS-1212-72-2 (anhydrous); 6190-60-9 (dihydrate)
Proprietary Names *Wyamine*. It is an ingredient of *Emergent-Ez*.
Chemical Properties Colourless crystals or white crystalline powder. Mp 215° to 217°, with decomposition. Soluble 1 in about 20 of water and 1 in 150 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Liebermann's reagent—red-orange; Marquis test—orange→brown.
Thin-layer Chromatography System TA—R_f 0.25; system TB—R_f 0.34; system TC—R_f 0.08; system TE—R_f 0.40; system TL—R_f 0.02; system TAE—R_f 0.06; system TAJ—R_f 0.00; system TAK—R_f 0.03; system TAL—R_f 0.36 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown).
Gas Chromatography System GA—mephentermine RI 1240, mephentermine-TFA RI 1335, mephentermine-AC RI 1501, phentermine RI 1155; system GB—mephentermine RI 1250, phentermine RI 1191; system GC—mephentermine RI 1668, phentermine RI 1450.
High Performance Liquid Chromatography System HA—mephentermine *k* 1.5, phentermine *k* 0.6; system HC—*k* 2.48; system HX—RI 294; system HY—RI 246.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=10c), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1111, 711, 1162, 696, 763, 1587 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 72, 91, 73, 56, 148, 65, 57, 42; phentermine 58, 91, 42, 41, 134, 65, 59, 40.



Quantification

Urine GC FID. Mephentermine, chlorphentermine and phentermine [Beckett, Brookes 1971].
Disposition in the Body Readily absorbed after oral or parenteral administration and rapidly metabolised by demethylation and hydroxylation. About 50 to

85% of a dose is excreted in the urine unchanged, together with 15 to 20% as phentermine. Conjugated *N*-hydroxymephentermine has also been detected in the urine in small quantities.

Toxicity The estimated minimum lethal dose in children up to 2 years of age is 200 mg, and in adults is 2 g.

Half-life Derived from urinary excretion data, about 6 to 20 h.

Dose The equivalent of 15 to 80 mg of mephentermine has been given parenterally.

Beckett AH, Brookes LG (1971). The metabolism and urinary excretion in man of phentermine, and the influence of N-methyl and p-chloro-substitution. *J Pharm Pharmacol* 23: 288–294.

Mephenytoin

Anticonvulsant

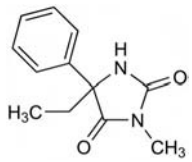
$C_{12}H_{14}N_2O_2 = 218.3$

CAS—50-12-4

IUPAC Name 5-Ethyl-3-methyl-5-phenyl-2,4-imidazolidinedione

Synonyms Mephenetoin; methoin; methantoin; phenantoin.

Proprietary Names *Epilan*; *Insulton*; *Mesantoin*; *Phenantoin*; *Sacerno*.



Chemical Properties Colourless lustrous crystals or white crystalline powder. Mp 136° to 137°. Soluble 1 in 1400 of water, 1 in 15 of ethanol, 1 in 3 of chloroform and 1 in 90 of ether. pK_a 8.1. Log *P* (octanol/water), 1.7.

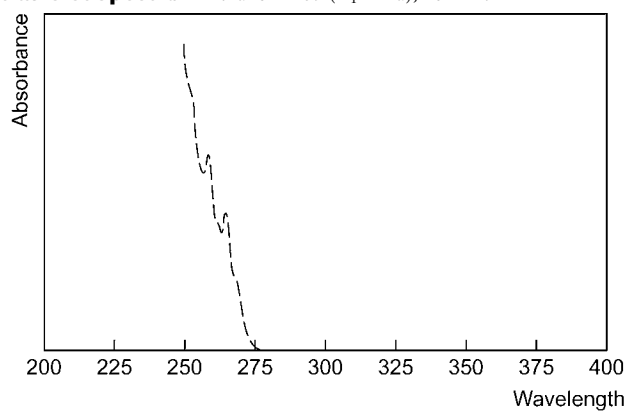
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.62; system TE—R_f 0.74; system TF—R_f 0.58; system TAD—R_f 0.66; system TAJ—R_f 0.64; system TAK—R_f 0.70; system TAL—R_f 0.91.

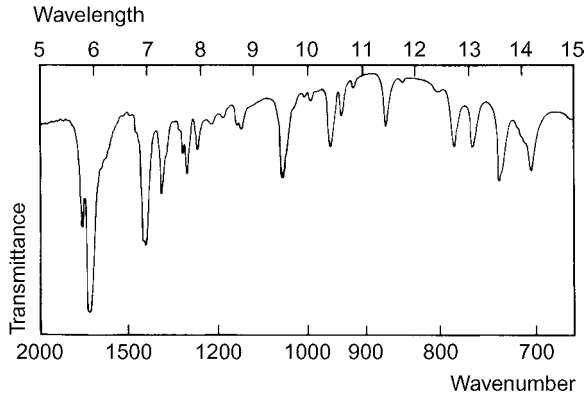
Gas Chromatography System GA—mephenytoin RI 1785, M (4-OH-) RI 2400, M (OH-methoxy-) RI 2380, M (nor-) RI 1950; system GE—mephenytoin RRT 0.55 and RRT 0.73 for 5-ethyl-5-phenylhydantoin (both relative to phenytoin); system GAJ—RRT 0.918 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HY—RI 366; system HZ—retention time 3.7 min; system HAX—retention time 6.0 min; system HAY—retention time 5.4 min.

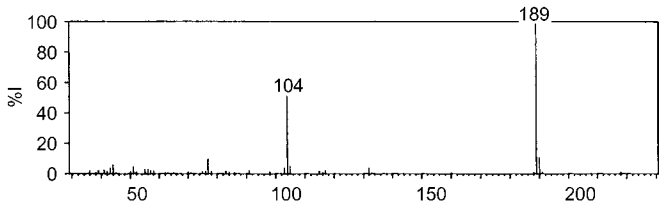
Ultraviolet Spectrum Ethanol—257 (A_1^1 = 11a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1704, 1754, 733, 1052, 1302, 702 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 189, 104, 190, 77, 44, 105, 132, 103.



Quantification

Plasma **GC-MS** Limit of detection, 10 µg/L for mephenytoin, 50 µg/L for 5-ethyl-5-phenylhydantoin [Yonekawa, Kupferberg 1979].

Serum **GC** FID. Limit of detection, 5 mg/L for mephenytoin, 10 mg/L for 5-ethyl-5-phenylhydantoin [Raisys *et al.* 1979].

HPLC UV detection. Limit of detection, 1 mg/L for mephenytoin and 5-ethyl-5-phenylhydantoin [Kabra *et al.* 1978].

Urine **HPLC** UV detection (comparison with GC). Limit of detection, 12.5 µg/L for mephenytoin enantiomers [Huang *et al.* 1998]. Electrochemical detection. Limit of detection, <0.76 mg/L for 4-Hydroxymephenytoin [Tanaka *et al.* 1996]. UV detection. 4′-Hydroxymephenytoin [Xie *et al.* 1995]. UV detection. Limit of detection, 25 µg/L for mephenytoin, 50 µg/L for 4′-hydroxymephenytoin [Ruan *et al.* 1994]. UV detection. Mephenytoin and metabolites [Küpfer *et al.* 1982].

Disposition in the Body Rapidly absorbed after oral administration. During chronic administration the major metabolite is 5-ethyl-5-phenylhydantoin (nirvanol) which is a more active anticonvulsant than mephenytoin and accounts for at least 22% of the daily dose in the 24-h urine. After a single dose, the major metabolite is 4-hydroxymephenytoin which is excreted in the urine as a glucuronide conjugate and accounts for 43% of a dose in 24 h; only about 1% of a single dose is excreted in the urine as 5-ethyl-5-phenylhydantoin; 5-ethyl-5-(4-hydroxyphenyl)-hydantoin has also been detected in the urine in both free and conjugated forms, together with minor amounts of other conjugated hydroxy metabolites; very little unchanged drug is excreted in the urine.

Therapeutic Concentration In plasma, mephenytoin plus metabolites, usually in the range 15–40 mg/L.

Following a single oral dose of 7 mg/kg to 5 subjects, peak serum concentrations of about 3 mg/L each of mephenytoin and 5-ethyl-5-phenylhydantoin were attained in about 1 h and 27 h respectively [Troupin 1979].

A mean steady-state serum concentration of 26 mg/L of total hydantoin was reported for 173 subjects receiving daily oral doses averaging 6.8 mg/kg; mephenytoin concentrations averaged 8% of the total hydantoin concentration [Troupin *et al.* 1976].

Toxicity The estimated minimum lethal dose is 5 g. Mephenytoin gives rise to toxic symptoms more frequently than phenytoin and cases of fatal aplastic anaemia have been reported; the toxicity of the drug is attributed to the proven toxicity of 5-ethyl-5-phenylhydantoin. Acute overdosage may result in coma.

Half-life Plasma half-life, after a single dose, mephenytoin about 7 h, 5-ethyl-5-phenylhydantoin about 95 h; the half-life of the metabolite decreases to about 72 h during chronic treatment.

Saliva Plasma : saliva ratio, about 1.6.

Protein Binding About 40%.

Dose Initially, 50 to 100 mg daily; maintenance, 200 to 600 mg daily.

Huang SL *et al.* (1998). Determination of S/R ratio of mephenytoin in human urine by chiral HPLC and ultraviolet detection and its comparison with gas chromatography. *Zhongguo Yao Li Xue Bao* 19: 548–550.

Kabra PM *et al.* (1978). *J Anal Toxicol* 2: 127–133.

Küpfer A *et al.* (1982). Analysis of hydroxylated and demethylated metabolites of mephenytoin in man and laboratory animals using gas-liquid chromatography and high-performance liquid chromatography. *J Chromatogr* 232: 93–100.

Raisys VA *et al.* (1979). Gas-chromatographic determination of mephenytoin and desmethylmephenytoin, after off-column alkylation. *Clin Chem* 25: 172–175.

Ruan ZR *et al.* (1994). [Determination of mephenytoin and 4′-hydroxymephenytoin in urine by high performance liquid chromatography]. *Yao Xue Xue Bao* 29: 624–628.

Tanaka M *et al.* (1996). Simple and selective assay of 4-hydroxymephenytoin in human urine using solid-phase extraction and high-performance liquid chromatography with electrochemical detection and its preliminary application to phenotyping test. *J Chromatogr B Biomed Appl* 676: 87–94.

Troupin AS (1979). Clinical pharmacology of mephenytoin and ethotoin. *Ann Neurol* 6: 410–414.

Troupin AS *et al.* (1976). Mephenytoin: a reappraisal. *Epilepsia* 17: 403–414.

Xie HG *et al.* (1995). High-performance liquid chromatographic determination of urinary 4′-hydroxymephenytoin, a metabolic marker for the hepatic enzyme CYP2C19, in humans. *J Chromatogr B Biomed Appl* 668: 125–131.

Yonekawa W, Kupferberg HJ (1979). Measurement of mephenytoin (3-methyl-5-ethyl-5-phenylhydantoin) and its demethylated metabolite by selective ion monitoring. *J Chromatogr* 163: 161–167.

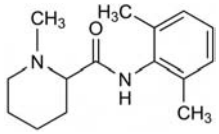
Mepivacaine

Anaesthetic (Local)

$C_{15}H_{22}N_2O = 246.4$

CAS—96-88-8

IUPAC Name *N*-(2,6-Dimethylphenyl)-1-methyl-2-piperidinecarboxamide



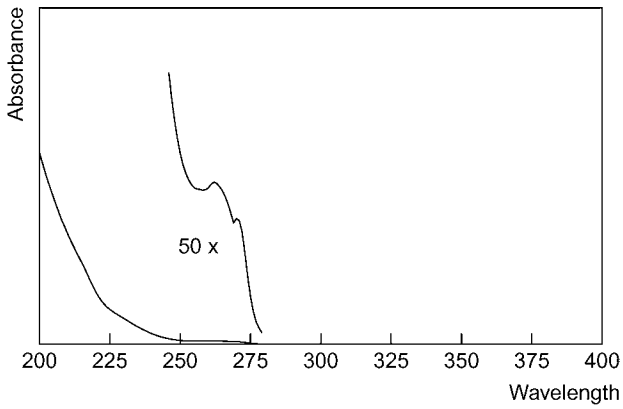
Chemical Properties Yellow-white crystals. Mp 150° to 151° (crystals from ether). pK_a 7.7 (20°). Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Mepivacaine Hydrochloride

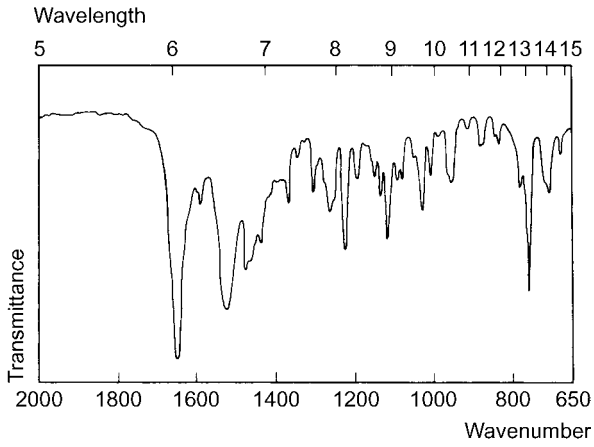
$C_{15}H_{22}N_2O,HCl = 282.8$
CAS—1722-62-9

Synonyms Mepivacaini chloridum; mepivacaini hydrochloridum.
Proprietary Names *Carbocain(e)*; *Chlorocain*; *Isocaine*; *Isogaine*; *Mecain*; *Meaverin*; *Mepi-Mynol*; *Mepicain*; *Mepident*; *Mepiforan*; *Mepyl*; *Optocain*; *Pericaina*; *Polocaine*; *Scandicain(e)*; *Scandinibsa*; *Scandonest*; *Tevacaine*.
Chemical Properties A white crystalline powder. Mp 262° to 264°. Freely soluble in water and methanol; soluble 1 in 10 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

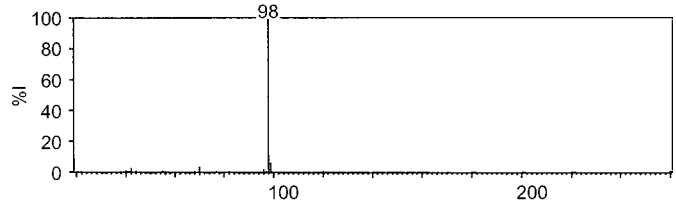
Colour Test Liebermann's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.31; system TC—R_f 0.62; system TE—R_f 0.66; system TL—R_f 0.48; system TAE—R_f 0.63; system TAF—R_f 0.60; system TAJ—R_f 0.28; system TAK—R_f 0.02; system TAL—R_f 0.40 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown; ninhydrin spray, positive).
Gas Chromatography System GA—mepivacaine RI 2065, M (OH-) RI 2410, M (oxo-) RI 2400; system GF—RI 2345; system GQ—retention time 7.2 min.
High Performance Liquid Chromatography System HA—*k* 0.9; system HQ—*k* 1.09; system HX—RI 296; system HY—RI 260; system HZ—retention time 2.6 min; system HAX—retention time 7.7 min; system HAY—retention time 4.2 min.
Ultraviolet Spectrum Aqueous acid—263 (A_1^1=18a), 271 nm.



Infrared Spectrum Principal peaks at wavenumbers 1650, 1523, 760, 1220, 1123, 1265 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 98, 99, 70, 42, 96, 55, 41, 40 (no peaks above 100).



Quantification

Blood **GC** FID. Limit of detection, 40 ng [Ashling *et al.* 1969].
GC-MS Mepivacaine and other local anaesthetics [Watanabe *et al.* 1998].
Plasma **GC-MS**. SIM acquisition mode, *m/z* 82. Limit of quantification, 40 µg/L, limit of detection, 50 µg/L for mepivacaine and other local anaesthetics [Ohshima, Takayasu 1999].
HPLC UV detection. Limit of detection, 100 µg/L [Murtaza *et al.* 2001]. UV detection. Limit of detection, 3 µg/L for mepivacaine enantiomers [Vletter *et al.* 1996].
Serum **HPLC** UV detection. Limit of detection, 100–150 µg/L for mepivacaine enantiomers [Siluveru, Stewart 1997a; Siluveru, Stewart 1997b].
Urine **GC** FID. Mepivacaine and 3 metabolites [Thomas, Meffin 1972].
GC-MS See Plasma [Ohshima, Takayasu 1999].
Body Fluids **GC** SID. Limit of detection, 5–10 pg for mepivacaine and other local anaesthetics [Hattori *et al.* 1991].
Biological Samples **GC** NSD. Mepivacaine and other local anaesthetics [Lau *et al.* 1991].

Disposition in the Body Rapidly absorbed into the circulation following epidural and paracervical injection. Mepivacaine crosses the placenta and the ratio of fetal to maternal concentrations has been reported to be about 0.7. Rapidly metabolised by hydroxylation to the 3′- and 4′-hydroxy metabolites and by *N*-demethylation to 2′,6′-pipecoloxylidide (PPX). Over 50% is excreted as metabolites into the bile but only small amounts are eliminated in the faeces; this may be due to enterohepatic circulation. Less than 10% of a dose is excreted in the urine as unchanged drug in 24 h; about 16% of a dose is excreted in the 24-h urine as 3′-hydroxymepivacaine, 12% as 4′-hydroxymepivacaine and 2% as 2′,6′-pipecoloxylidide; the excretion of unchanged drug is increased in acid urine and in neonates.
Therapeutic Concentration In plasma, usually in the range 2–5 mg/L.
Following epidural administration of 250–580 mg to 8 subjects, plasma concentrations at delivery were 3.7–5.5 mg/L (mean 4.7) for mepivacaine and <0.1–0.38 mg/L for 2′,6′-pipecoloxylidide [Meffin *et al.* 1973].
Following paracervical injection of 200 mg to 5 subjects, peak plasma concentrations of 1.4–4.0 mg/L (mean 2.3) were attained in about 30 min [Teramo, Rajamaki 1971].
Toxicity
Mepivacaine had been given as a local anaesthetic (between 75 and 150 mL of a 2% solution) to a young woman who died while undergoing cosmetic surgery. Postmortem concentrations were: blood 50 mg/L, bile 50 mg/L, brain 51 µg/g, kidney 51 µg/g, liver 75 µg/g, spleen 72 µg/g. Salicylate and glutethimide were also found in the blood and urine [Sunshine, Fike 1964].
Half-life Plasma half-life, about 2 to 3 h.
Volume of Distribution About 1 L/kg.
Clearance Plasma clearance, about 5 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.1.
Protein Binding About 77%.
Note For a review of the pharmacokinetics of local anaesthetics, see Tucker and Mather [1979].
Dose Mepivacaine hydrochloride is given by injection in doses of up to 400 mg; maximum of 1 g in 24 h.

Ashling JH *et al.* (1969). Gas chromatographic determination of mepivacaine in capillary blood. *Anesthesiology* 31: 458–461.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Hattori H *et al.* (1991). Determination of local anaesthetics in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 564: 278–282.
Lau OW *et al.* (1991). Gas-liquid chromatographic determination and pharmacological studies of six clinically-used local anesthetics. *Methods Find Exp Clin Pharmacol* 13: 475–481.
Meffin P *et al.* (1973). Clearance and metabolism of mepivacaine in the human neonate. *Clin Pharmacol Ther* 14: 218–225.
Murtaza R *et al.* (2001). Simultaneous determination of mepivacaine, tetracaine, and p-butylaminobenzoic acid by high-performance liquid chromatography. *J Pharmacol Toxicol Methods* 46: 131–136.
Ohshima T, Takayasu T (1999). Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography-mass spectrometry with solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 726(1–2): 185–194.
Siluveru M, Stewart JT (1997a). Stereoselective determination of mepivacaine in human serum using a brush-type chiral stationary phase and solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 690: 359–362.
Siluveru M, Stewart JT (1997b). HPCE determination of R(+) and S(-) mepivacaine in human serum using a derivatized cyclodextrin and ultraviolet detection. *J Pharm Biomed Anal* 15: 1751–1756.
Sunshine I, Fike WW (1964). Value of thin-layer chromatography in two fatal cases of intoxication due to lidocaine and mepivacaine. *N Engl J Med* 271: 487–490.
Teramo K, Rajamaki A (1971). Foetal and maternal plasma levels of mepivacaine and foetal acid-base balance and heart rate after paracervical block during labour. *Br J Anaesth* 43: 300–312.

Thomas J, Meffin P (1972). Aromatic hydroxylation of lidocaine and mepivacaine in rats and humans. *J Med Chem* 15: 1046–1049.
Tucker GT, Mather LE (1979). Clinical pharmacokinetics of local anaesthetics. *Clin Pharmacokinet* 4: 241–278.
Vletter AA *et al.* (1996). High-performance liquid chromatographic assay of mepivacaine enantiomers in human plasma in the nanogram per milliliter range. *J Chromatogr B Biomed Appl* 678: 369–372.
Watanabe T *et al.* (1998). Simple analysis of local anaesthetics in human blood using headspace solid-phase microextraction and gas chromatography-mass spectrometry-electron impact ionization selected ion monitoring. *J Chromatogr B Biomed Sci Appl* 709: 225–232.

Meprobamate

Tranquilliser

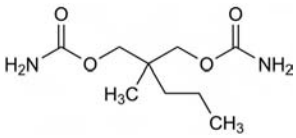
$C_9H_{18}N_2O_4 = 218.3$

CAS—57-53-4

IUPAC Name 2-Methyl-2-propyl-1,3-propanediol dicarbamate

Synonyms Meprobamatum; meprotanum.

Proprietary Names *Cyrpon*; *Dapaz*; *Epikur*; *Equanil*; *Meprate*; *Mepro*; *Meprodil*; *Meprospan*; *Miltaun*; *Miltown*; *Neuramate*; *Norgagil*; *Novalm*; *Novo-Mepro*; *Pertranquil*; *Procalmadiol*; *Quanil*; *Reposo-Mono*; *Restenil*; *Anobamat*; *Visano N*; *Visano-mini*. It is an ingredient of *Deprol*; *Epromate*; *Equagesic*; *Equazine M*; *Micrainin*; *Paxidal*.



Chemical Properties Colourless crystals or white crystalline powder. Mp 104° to 106° (crystals from hot water). Soluble 1 in 240 of water, 1 in 7 of ethanol, 1 in 80 of chloroform and 1 in 70 of ether; freely soluble in acetone. Log *P* (octanol/water), 0.7. Extraction yield (chlorobutane), 0.1 [Demme *et al.* 2005].

Colour Test Furfuraldehyde—black; Nessler's reagent (100°)—grey-black.

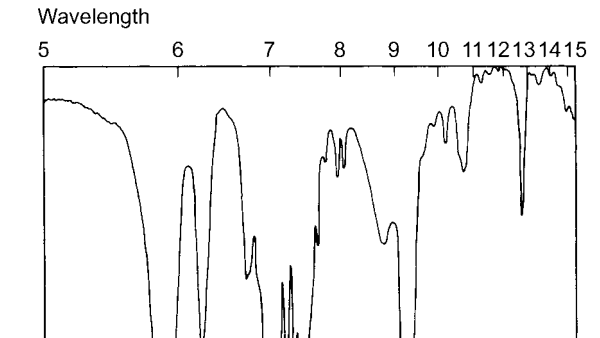
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.00; system TC—R_f 0.32; system TD—R_f 0.09; system TE—R_f 0.56; system TF—R_f 0.36; system TL—R_f 0.58; system TAD—R_f 0.32; system TAE—R_f 0.63; system TAF—R_f 0.87; system TAJ—R_f 0.35; system TAK—R_f 0.29; system TAL—R_f 0.78 (furfuraldehyde reagent, positive; Van Urk reagent, yellow).

Gas Chromatography System GA—RI 1785; system GB—RI 1854; system GF—RI 2460.

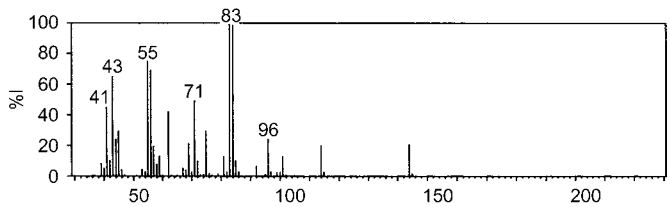
High Performance Liquid Chromatography System HZ—RT 2.4 min.

Ultraviolet Spectrum No significant absorption, 230–360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1688, 1069, 1090, 1590, 1310, 1140 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 83, 84, 55, 56, 43, 71, 41, 62 (no peaks above 160).



Quantification

Blood **GC-MS** Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.3 mL/min. Temperature programme: 140° for 0.5 min to 290° at 30°/min for 2.5 min. SIM acquisition mode. Limit of quantification, 0.4 mg/L, limit of detection, 0.4 mg/L [Downey *et al.* 2009].

Plasma **GC** Column: CP SIL 5 CB (25 m × 0.25 mm i.d., 0.12 μm). Temperature: 190°. FID. Retention time: 3 min. Limit of detection, 1 mg/L [Trenque *et al.* 1993]. Column: 3% OV 17 on 100/120 mesh Chromosorb W HP (1.80 m × 2.0 mm i.d.). Carrier gas: N_2, 0.95 bar. Temperature: 190°. FID. Retention time: 6.6 min. Limit of detection, 1 mg/L [Gaillard *et al.* 1992] Column: 3% SE-30 on Chromosorb W 80/100 mesh (1.5 m × 6.3 mm i.d.). Carrier gas: He, 50 mL/min. Temperature: 115°. FID. [Martis, Levy 1974].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 190° for 5 min to 245° at 30°/min to 255° at 2°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 4.33 min. Limit of quantification, 1.93 mg/L, limit of detection, 0.58 mg/L. [Daval *et al.* 2006]. Column: HP-1 methylmethylsiloxane capillary (12 m × 0.2 mm i.d.). Carrier gas: He, 1.8 mL/min. Temperature programme: 100° for 3 min to 280° at 30°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 25 μg/L [Kintz, Mangin 1993].

HPLC UV detection [Gupta, Eng 1980].

Serum **GC** See Plasma [Gaillard *et al.* 1992].

Urine **GC** See Plasma [Martis, Levy 1974].

GC-MS See Plasma. Limit of detection, 20 μg/L [Kintz, Mangin 1993].

Hair **GC-MS** Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° to 240° at 25°/min to 300° at 10°/min for 0.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.4 ng/mg, limit of detection, 0.12 ng/mg [Kim *et al.* 2005]. See Plasma. Limit of detection, 0.2 ng/mg [Kintz, Mangin 1993].

Disposition in the Body Meprobamate is readily absorbed after oral administration. It is widely distributed and mainly metabolised in the liver. Approximately 90% of a dose is excreted in the urine in 24 h, with ~10–20% of the dose as unchanged drug and the remainder as metabolites, mainly 2-hydroxypropylmeprobamate and meprobamate *N*-glucuronide. Approximately 10% of the dose is eliminated in the faeces. Meprobamate crosses the placenta. Levels in breast milk may be up to 4 times higher than those found in plasma.

Meprobamate is a minor metabolite of tybamate and is a metabolite of carisoprodol.

Therapeutic Concentration In plasma, usually in the range 5–20 g/L.

Following 2 oral doses of 800 mg, given to 5 subjects over a period of 1.5 h, peak blood concentrations of 18.6–26.6 g/L (mean 23.8) were attained in ~3.5 h [Parker *et al.* 1970].

Toxicity The estimated minimum lethal dose is 12 g, although recovery has occurred after much larger doses. Blood concentrations greater than ~50 g/L may cause coma, and fatalities have usually been associated with blood concentrations greater than ~70 g/L.

In 19 fatalities involving meprobamate overdose, postmortem blood concentrations of 41–397 mg/L (mean 182) were reported [Gaillard *et al.* 1997].

A postmortem blood concentration of 204.6 mg/L was reported in a fatality resulting from the deliberate ingestion of 90 tablets (400 mg tablets) of meprobamate; the highest tissue concentration was found in the heart (708 μg/g) [Kintz *et al.* 1988].

In 4 cases of severe meprobamate intoxication, maximum plasma concentrations of 176, 180, 190, and 203 mg/L were reported; all patients survived (1 was resuscitated from cardiac arrest), with 3 being teated with charcoal haemoperfusion [Jacobsen *et al.* 1987].

The following postmortem tissue concentrations were reported in 16 deaths caused solely by meprobamate ingestion: blood 35–240 g/L (mean 95), liver 58–360 mg/g (mean 148) [Baselt, Cravey 1977].

Half-life Plasma half-life, 6–17 h (mean 11); it may be much longer after chronic administration and may be dose dependent.

Volume of Distribution ~0.7 L/kg.

Distribution in Blood Plasma : whole blood ratio, ~0.2–0.3.

Protein Binding ~20%.

Dose 1.2 to 2.4 g daily.

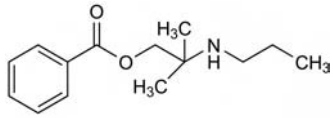
Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–103.
Daval S *et al.* (2006). A one-step and sensitive GC-MS assay for meprobamate determination in emergency situations. *J Anal Toxicol* 30: 302–305.
Demme U *et al.* (2005). Systemic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.
Downey D *et al.* (2009). Quantitative analysis of carisoprodol and meprobamate in whole blood using benzylcarbamate and deuterated meprobamate as internal standards. *J Anal Toxicol* 33: 278–282.
Gaillard Y *et al.* (1992). Gas chromatographic determination of meprobamate in serum or plasma after solid-phase extraction. *J Chromatogr* 577: 171–173.
Gaillard Y *et al.* (1997). Meprobamate overdosage: a continuing problem. Sensitive GC-MS quantitation after solid phase extraction in 19 fatal cases. *Forensic Sci Int* 86: 173–180.
Gupta RN, Eng F (1980). GC and HPLC determination of meprobamate in plasma. *J High Resolut Chromatogr Chromatogr Commun* 3: 419–420.
Jacobsen D *et al.* (1987). Meprobamate kinetics during and after terminated hemoperfusion in acute intoxications. *J Toxicol Clin Toxicol* 25: 317–331.
Kim JY *et al.* (2005). Simultaneous determination of carisoprodol and meprobamate in human hair using solid-phase extraction and gas chromatography/mass spectrometry of the trimethylsilyl derivatives. *Rapid Commun Mass Spectrom* 19: 3056–3062.
Kintz P, Mangin P (1993). Determination of meprobamate in human plasma, urine, and hair by gas chromatography and electron impact mass spectrometry. *J Anal Toxicol* 17: 408–410.

Kintz P *et al.* (1988). Fatal meprobamate self-poisoning. *Am J Forensic Med Pathol* 9: 139–140.
Martis L, Levy RH (1974). GLC determination of meprobamate in water, plasma, and urine. *J Pharm Sci* 63: 834–837.
Parker KD *et al.* (1970). Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide, or diphenylhydantoin. *Clin Toxicol* 3: 131–145.
Trenque T *et al.* (1993). Gas chromatographic determination of meprobamate in human plasma. *J Chromatogr* 615: 343–346.

Meprylcaine

Anaesthetic (Local)
$C_{14}H_{21}NO_2$ = 235.3
CAS—495-70-5

IUPAC Name 2-Methyl-2-propylaminopropyl benzoate



Chemical Properties An oil. Practically insoluble in water; soluble in ethanol and ether.

Meprylcaine Hydrochloride

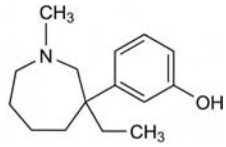
$C_{14}H_{21}NO_2$,HCl = 271.8
CAS-956-03-6
Chemical Properties A white crystalline powder. Mp 150° to 153°. Soluble 1 in 6 of water, 1 in 5 of ethanol, 1 in 3 of chloroform and 1 in 12 of ether.

Ultraviolet Spectrum Aqueous acid—232 nm (A^1_1=422b).
Mass Spectrum Principal ions at *m/z* 100, 58, 105, 77, 56, 101, 41, 70.

Meptazinol

Narcotic Analgesic
$C_{15}H_{23}NO$ = 233.4
CAS—54340-58-8

IUPAC Name 3-(3-Ethylhexahydro-1-methyl-1*H*-azepin-3-yl)phenol
Proprietary Name *Meptid* (tablets)

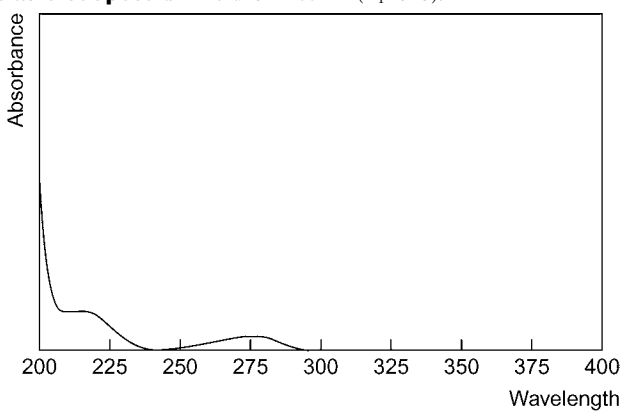


Chemical Properties Crystals. Mp 127° to 133°. Very slightly soluble in water; soluble in ethanol; sparingly soluble in chloroform and ether. pK_a 8.7 (-NH_2), 11.9 (phenol). Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Meptazinol Hydrochloride

$C_{15}H_{23}NO$,HCl = 269.8
CAS—59263-76-234154-59-1 (±)
Proprietary Names *Meptid* (injection); *Meptidol*.
Chemical Properties A white crystalline powder. Mp 183° to 187°. Soluble 1 in about 4 of water and 1 in 12 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

Gas Chromatography System GA—meptazinol RI 1920, M (nor-) RI 1995, M (oxo-) RI 2410; system GB—meptazinol RI 1980, M (nor-) RI 2069, M (oxo-) RI 2600; system GM—meptazinol RRT 0.429, M (nor-) RRT 0.428 (all relative to iprindole).
High Performance Liquid Chromatography System HA—*k* 3.1; system HY—RI 269.
Ultraviolet Spectrum Ethanol—277 nm (A^1_1=91b).



Infrared Spectrum Principal peaks at wavenumbers 1580, 708, 795, 1225, 1205, 1300 cm^{-1} (meptazinol hydrochloride, KBr disk).

Quantification

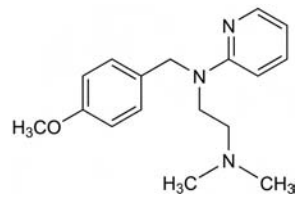
Plasma **HPLC** Fluorescence detection. Limit of detection, 3 µg/L [Frost 1981].
Biological Samples **HPLC** [Rudolphi, Blaschke 1995].
Disposition in the Body Readily and almost completely absorbed after oral administration. Rapidly absorbed after intramuscular or rectal administration. It is excreted rapidly in the urine, mainly as the glucuronide conjugate, with <5% of a dose as unchanged drug, and about 5 to 10% as 7-oxometazinol. Both metabolites are inactive. Over 50% of a dose is excreted in the urine in 9 h and over 60% in 24 h. <10% of an oral dose is eliminated in the faeces.
Therapeutic Concentration
Following a single oral dose of 200 mg to 9 subjects, peak plasma concentrations of 0.01 to 0.11 mg/L (mean 0.06) were attained in 0.25 to 2 h; accumulation in plasma did not occur after repeated oral administration [Norbury *et al.* 1983].
After a single IM dose of 50 mg to 4 subjects, peak plasma concentrations of 0.19 to 0.26 mg/L (mean 0.22) were attained in 10 min [Davies *et al.* 1982].
Toxicity
A 61-year-old woman who recovered after intentionally ingesting 200 mg meptazinol with whisky had serum meptazinol and alcohol levels of 20.1 and 210 mg/L, respectively, approx. $2^1/_2$ h after the overdose [Davison *et al.* 1987].
Bioavailability 2 to 19% (mean 9) owing to extensive first-pass metabolism.
Half-life Plasma half-life, about 2 h.
Volume of Distribution About 5 L/kg.
Clearance Plasma clearance, about 30 mL/min/kg.
Protein Binding About 27%.
Dose 200 mg, orally, every 4 h; the equivalent of 50 to 100 mg of meptazinol parenterally.

Davies G *et al.* (1982). Pharmacokinetics of meptazinol in man following repeated intramuscular administration. *Eur J Clin Pharmacol* 23: 535–538.
Davison AG *et al.* (1987). Meptazinol overdose producing near fatal respiratory depression. *Hum Toxicol* 6: 331.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Frost T (1981). Determination of meptazinol in plasma by high-performance liquid chromatography with fluorescence detection. *Analyst, Lond* 106: 999–1001.
Norbury HM *et al.* (1983). Pharmacokinetics of the new analgesic, meptazinol, after oral and intravenous administration to volunteers. *Eur J Clin Pharmacol* 25: 77–80.
Rudolphi C, Blaschke G (1995). Determination of the stereoselective aspects in in-vitro and in-vivo metabolism of the analgesic meptazinol by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 663: 315–326.

Mepyramine

Antihistamine
$C_{17}H_{23}N_3O$ = 285.4
CAS—91-84-9

IUPAC Name *N*-[(4-Methoxyphenyl)methyl]-*N*′,*N*′-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine
Synonyms Pyranisamine; pyrilamine; RP-2786.

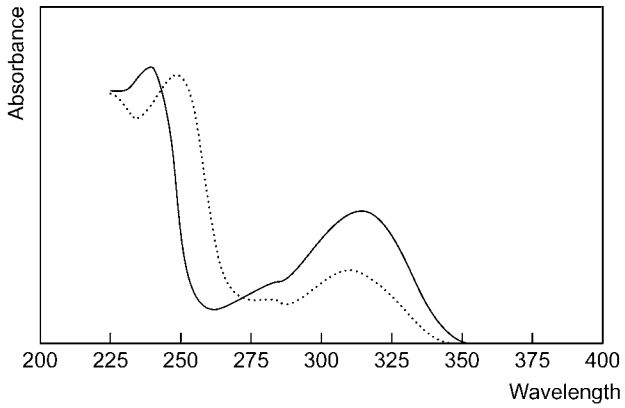


Chemical Properties An oily liquid. pK_a 4.0, 8.9 (25°). Log *P* (octanol/water), 2.8.

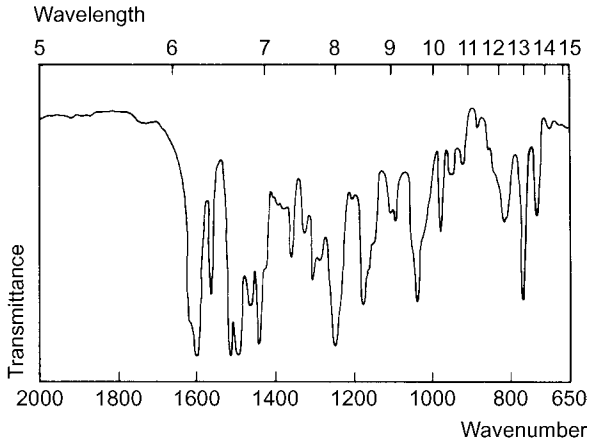
Mepyramine Maleate

$C_{17}H_{23}N_3O$,$C_4H_4O_4$ = 401.5
CAS-59-33-6
Proprietary Names *Anthisan*. It is an ingredient of many proprietary preparations—see Sweetman [2009].
Chemical Properties A white or creamy-white crystalline powder. Mp 100° to 101°. Soluble 1 in 0.4 of water, 1 in 2.5 of ethanol and 1 in 1.5 of chloroform; slightly soluble in ether and benzene.

Colour Tests Cyanogen bromide—yellow; Mandelin's test—violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.39; system TC—R_f 0.25; system TE—R_f 0.58; system TL—R_f 0.14; system TAE—R_f 0.22; system TAF—R_f 0.33 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, red).
Gas Chromatography System GA—mepyramine RI 2220, M (*N*-desalkyl-)RI 2120, M (*N*-desmethoxybenzyl-) RI 1580; system GB—RI 2328; system GF—RI 2560.
High Performance Liquid Chromatography System HA—*k* 3.9; system HX—RI 448; system HY—RI 257.
Ultraviolet Spectrum Aqueous acid—239 (A^1_1=633a), 316 nm; aqueous alkali—248, 312 nm.



Infrared Spectrum Principal peaks at wavenumbers 1492, 1512, 1598, 1247, 1175, 1035 cm^{-1} (KBr disk).

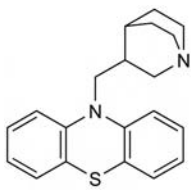


Mass Spectrum Principal ions at *m/z* 121, 58, 72, 71, 214, 122, 215, 78.
Quantification
Urine **HPLC** Fluorescence detection. Limit of detection, 1 ng/g [Thompson, Holder 1984].
Postmortem Tissues **GC** FID [Johnson 1981].
Disposition in the Body Absorbed after oral administration.
Toxicity
In a fatality due to mepyramine ingestion, the following postmortem tissue concentrations were reported: blood 11 mg/L, brain 71 µg/g, liver 18 µg/g [Johnson 1981].
Dose 0.3 to 1 g of mepyramine maleate daily.

Johnson GR (1981). A fatal case involving pyrilamine. *Clin Toxicol* 18: 907–909.
Sweetman SC, ed. (2009). *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.
Thompson HC, Holder CL (1984). Trace analysis of the antihistamines methapyrilene hydrochloride, pyrilamine maleate and triprolidine hydrochloride monohydrate in animal feed, human urine and wastewater by high-performance liquid chromatography and gas chromatography with nitrogen-phosphorus detection. *J Chromatogr* 283: 251–264.

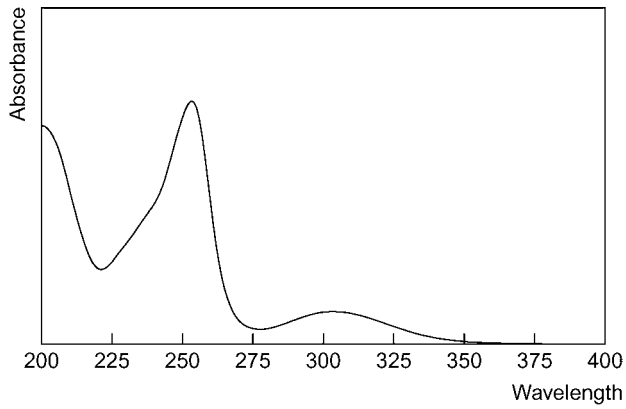
Mequitazine

Antihistamine
$C_{20}H_{22}N_2S$ = 322.5
CAS—29216-28-2
IUPAC Name 10-(1-Azabicyclo[2.2.2]oct-3-ylmethyl)-10*H*-phenothiazine
Synonym LM-209
Proprietary Names *Metaplexan*; *Mircol*; *Primalan*; *Primasone*; *Quitadrill*.

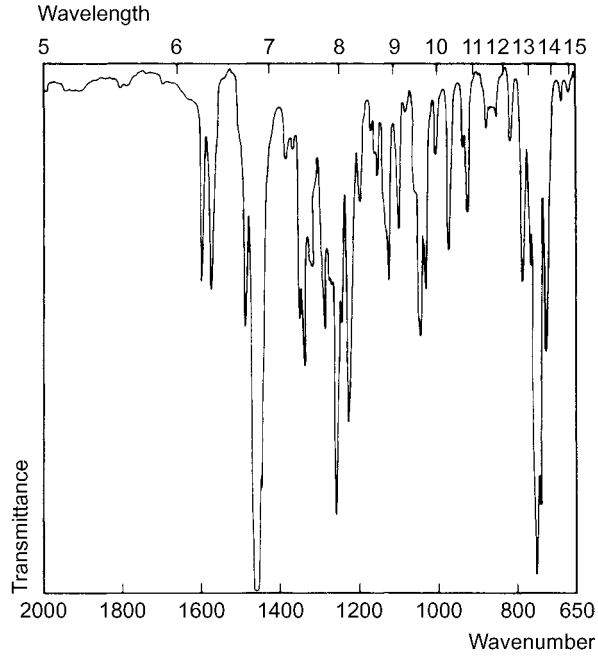


Chemical Properties Mp about 143°. Practically insoluble in water; soluble in ethanol and chloroform. Log *P* (octanol/water), 5.7.

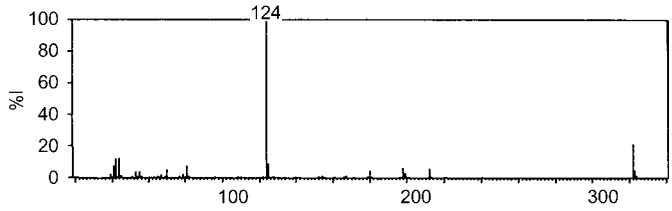
Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—red; FPN reagent—orange-red; Liebermann's reagent—green; Mandelin's test—red; Marquis test—red (slow); sulfuric acid (100°)—red (slow).
Thin-layer Chromatography System TA—R_f 0.10; system TB—R_f 0.06; system TC—R_f 0.06; system TE—R_f 0.27; system TL—R_f 0.00; system TAE—R_f 0.03.
Gas Chromatography System GA—mequitazine RI 2780, M (phenothiazine) RI 2020, M (sulfoxide) RI 3120, M (sulfone) RI 3250; system GB—mequitazine RI 2939, M (phenothiazine) RI 2130.
High Performance Liquid Chromatography System HA—*k* 8.3 (tailing peak).
Ultraviolet Spectrum Aqueous acid—256 (A_1^1=1060a), 307 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 751, 1250, 742, 1220, 729, 1042 cm^{-1} (KBr disk).



Mass Spectrum

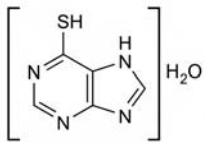


Dose 10 mg daily.

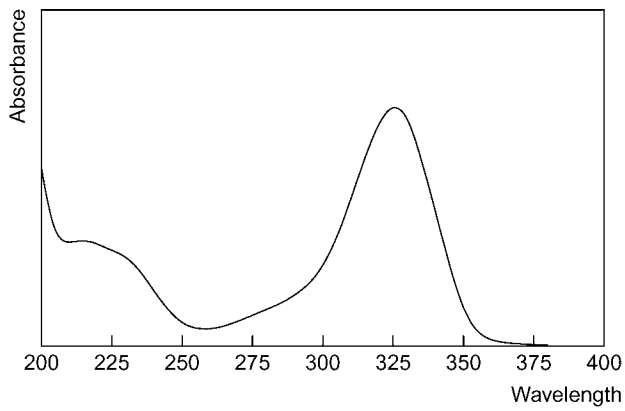
Mercaptopurine

Antineoplastic
$C_5H_4N_4S,H_2O$ = 170.2
CAS—50-44-2 (anhydrous); 6112-76-1 (monohydrate)

IUPAC Name 3,7-Dihydropurine-6-thione hydrate
Synonyms 1,7-Dihydro-6*H*-purine-6-thione monohydrate; 6MP.
Proprietary Names *Ismipur*; *Mercaptina*; *Puri-Nethol*.



Chemical Properties A yellow crystalline powder which darkens on exposure to air and light. Mp 308° with decomposition. Insoluble in water, acetone, chloroform and ether; soluble in hot ethanol; soluble in solutions of alkali hydroxides. pK_a 7.7, 11.0 (20°). Log *P* (octanol/water), 0.0.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.02; system TF—R_f 0.00; system TAE—R_f 0.77.
Gas Chromatography System GA—RI 1517.
High Performance Liquid Chromatography System HX—RI 187.
Ultraviolet Spectrum Mercaptopurine monohydrate: aqueous acid—325 nm (A^1_1=1165a); aqueous alkali—231 (A^1_1=831a), 310 nm (A^1_1=1160a).



Infrared Spectrum Principal peaks at wavenumbers 1610, 1220, 1570, 1010, 865, 780 cm^{-1} (KBr disk).
Quantification
Plasma **GC-MS** Limit of detection, 2 µg/L [Floberg *et al.* 1981].
HPLC UV detection. Mercaptopurine and metabolites. Limit of detection, 3.5 µg/L for mercaptopurine [Bruunshuus, Schmiegelow 1989]. Fluorescence detection. Limit of detection, <2 µg/L [Jonkers *et al.* 1982].
Urine **HPLC** Limit of detection, 1.7 µg/L for mercaptopurine, see Plasma [Bruunshuus, Schmiegelow 1989].
Disposition in the Body Readily absorbed after oral administration. It is distributed throughout the body water and diffuses into the cerebrospinal fluid. Mercaptopurine is activated in the body by intracellular conversion to nucleotide forms including the ribonucleotide thioinosinic acid. It is metabolised by xanthine oxidase to inactive 6-thiouric acid which is excreted in the urine; inorganic sulfate may also be present. About 50% of an oral dose is excreted in the urine in 24 h, up to 8% as unchanged drug. Small amounts are excreted for up to 17 days. Mercaptopurine is a metabolite of azathioprine.
Therapeutic Concentration
After a single oral dose of 75 mg/m^2 to 14 children (mean age 13 years) with acute lymphoblastic leukaemia, peak plasma concentrations of 0.04 to 0.28 mg/L (mean 0.14) were attained in 0.5 to 4 h (mean 2) [Zimm *et al.* 1983].
Daily oral doses of 75 mg/m^2 to 89 children with acute lymphoblastic leukaemia produced mean peak plasma concentrations of 0.10 (range 0.02 to 0.39) mg/L occurring at 0.5 to 3 h [Balis *et al.* 1998].
Five children with acute lymphoblastic leukaemia or malignant lymphoma were treated with 50 mg/m^2 oral mercaptopurine daily and 5 were treated with the same dose administered by rapid IV injection. The plasma concentrations were 0.23 to 0.42 mg/L (median 0.26) 5 min after IV infusion and 0.005 to 0.18 mg/L (median 0.06) 1 h after oral administration; the time to peak plasma concentration following oral administration varied (1 h in 4 children, 2 h in 4 children and 3 h in 2 children) [Mawatari *et al.* 2001].
Toxicity
A 64-year-old woman took 200 mg of mercaptopurine every 8 h for about 2 weeks, because of an error in dispensing. She died 3 days later and the following postmortem concentrations were reported: blood 110 mg/L, bile 16 mg/L, brain 10 µg/g, kidney 33 µg/g [Lin *et al.* 1982].
Bioavailability About 16% and very variable.
Half-life Plasma half-life, about 0.5 to 1.5 h.
Protein Binding About 20%.
Dose Initially 2.5 mg/kg daily, orally.

Balis FM *et al.* (1998). Pharmacokinetics and pharmacodynamics of oral methotrexate and mercaptopurine in children with lower risk acute lymphoblastic leukemia: a joint children's cancer group and pediatric oncology branch study. *Blood* 92: 3569–3577.
Bruunshuus I, Schmiegelow K (1989). Analysis of 6-mercaptopurine, 6-thioguanine nucleotides, and 6-thiourie acid in biological fluids by high-performance liquid chromatography: Technical Note. *Scand J Lab Invest* 49: 779–784.
Floberg S *et al.* (1981). Extractive alkylation of 6-mercaptopurine and determination in plasma by gas chromatography-mass spectrometry. *J Chromatogr* 225: 73–81.
Jonkers RE *et al.* (1982). Analysis of 6-mercaptopurine in human plasma with a high-performance liquid chromatographic method including post-column derivatization and fluorimetric detection. *J Chromatogr* 233: 249–255.
Lin RL *et al.* (1982). A Purinethol (6-mercaptopurine) fatality in a case of prescription negligence: a gas chromatographic determination of 6-mercaptopurine. *J Forensic Sci* 27: 454–460.
Mawatari H *et al.* (2001). Comparative pharmacokinetics of oral 6-mercaptopurine and intravenous 6-mercaptopurine riboside in children. *Pediatr Int* 43: 673–677.
Zimm S *et al.* (1983). Variable bioavailability of oral mercaptopurine. Is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered? *N Engl J Med* 308: 1005–1009.

Mercury

Metal
Hg = 200.6
CAS—7439-97-6

Synonyms Colloidal mercury; hydrargyrum; liquid silver; metallic mercury; quicksilver.
Chemical Properties Silver-white heavy, mobile, liquid metal. Solid mercury is tin-white, ductile and malleable. Mp −38.87°. Bp 356.72°. Practically insoluble in water; soluble in sulfuric acid upon boiling, in lipids; readily soluble in nitric acid; insoluble in hydrochloric acid; slightly soluble in pentane. Valencies: Hg(+1) and Hg(+2). Used in manufacture of scientific instruments, all mercury salts, pharmaceuticals, agricultural chemicals and anti-fouling paints; in dentistry, electroanalysis.

Mercuric (II) Chloride
$HgCl_2$ = 271.5
CAS—7487-94-7
Synonyms Bichloride of mercury; corrosive sublimate; dichloromercury; mercury chloride; mercury dichloride; mercury perchloride.
Proprietary Names *Calochlor*; *Fungchex*; *TL 898*.
Chemical Properties Crystals, granules or powder. Mp 277°. Bp 302°. Soluble in water; very soluble in alcohol, benzene, ether, glycerol, acetone, ethyl acetate; slightly soluble in carbon disulfide, pyridine. Used for preserving wood and anatomical specimens, disinfecting, browning and etching steel and iron, intensifier in photography, white reserve in fabric printing, tanning leather, electroplating aluminium, depolariser for dry batteries, freeing gold from lead, staining wood, manufacture of inks, and treating seed potatoes. Important reagent in analytical chemistry.

Mercuric (II) Sulfide
HgS = 232.7
CAS—1344-48-5
Synonyms Artificial cinnabar; Chinese red; Chinese vermillion; CI pigment red 106; CI 77766; etiops mineral; quicksilver vermillion; red mercury sulfide; vermillion.
Chemical Properties Two forms exist: black or greyish black amorphous powder (mercuric chloride, black) or bright scarlet-red powder (mercuric sulfide, red). Transition temperature (red to black) 386°.

Mercurous (I) Chloride
Hg_2Cl_2 = 472.1
CAS—10112-91-1
Synonyms Mercury monochloride; mercury protochloride; mercury subchloride; mild mercury chloride.
Proprietary Names *Calogren*; *Calomel*; *Calotab*; *Cyclosan*.
Chemical Properties White heavy powder. Sublimes at 400° to 500° without melting. Practically insoluble in water; insoluble in alcohol, ether. Used as cathartic, diuretic, antiseptic, antisyphilitic, in dark green Bengal lights, in fungicides, in pesticides, for calomel electrodes.

Mercuric Acetate
$HgC_4H_6O_4$ = 318.7
CAS—1600-27-7
Synonyms Bis(acetyloxy) mercury; diacetocymercury; mercuriacetate.
Chemical Properties White crystalline powder with a slight acetic odour. Mp 178° to 180°. Very soluble in water; soluble in alcohol, acetic acid. Used for mercuration of organic compounds; for the absorption of ethylene.

Methylmercuric Chloride
$HgCH_3Cl$ = 251.1
CAS—115-09-3
Synonyms Chloromethylmercury; methylmercury chloride; methylmercury monochloride; monomethyl mercury chloride.
Proprietary Name *Caspan*

Chemical Properties White crystals. Mp 170°. Slightly soluble in water; soluble in DMSO, ethanol, acetone. Formerly used as disinfectant for grain seeds.

Dimethylmercury

C_2H_6Hg = 230.7
CAS—593-74-8
Chemical Properties Colourless liquid. Bp 92°. Insoluble in water; soluble in ether, alcohol. Used in the preparation of NMR standards and MS calibration standards.

Phenylmercuric Acetate

$HgC_8H_8O_2$ = 336.8
CAS—62-38-4
Synonyms (Acetato)phenylmercury; acetoxyphenylmercury; phenylmercuriacetate.
Proprietary Names *Cerasan Slaked Lime*; *Gollitox*; *Liquiphene*; *Mersolite*; *Nylmerate*; *PMA*; *PMAC*; *Pmacetate*; *PMAS*; *Riogen*; *Scutl*; *Tag Fungicide*; *Tag HL-331*.
Chemical Properties Small white to cream lustrous prisms. Mp 149°. Soluble in water, alcohol, benzene, acetone, trichloromethane, ether. Used in inks, adhesives, caulking compounds, interior and exterior paints, as fungicide in seed dressings, as a catalyst in polyurethane manufacture.

Colour Tests Reinsch test. Applicable to urine, gastric contents and scene residues. Clean a 5 mm × 10 mm square of copper foil (2–3 cm copper wire) with aqueous nitric acid (500 mL/L) until shiny. Rinse the copper with purified water and add 10 mL of concentrated hydrochloric acid and 20 mL of test sample in a 100 mL conical flask. Heat on a boiling water bath in a fume cupboard for 1 h. Cool and gently wash the copper with purified water.—A silvery deposit is imparted to the copper if mercuric compounds are present (antimony, arsenic, bismuth, selenium and tellurium give black deposits).

Confirmatory Test Applied to the silver stained foil. Add 0.1 mL of copper (I) iodide suspension to a filter paper. Lay the foil onto the suspension, cover and leave for 1–12 h.—The presence of mercury is indicated by the development of a salmon-pink colour, which may take up to 12 h to appear with low concentrations. Limit of detection, 5 mg/L. (The copper (I) iodide suspension is prepared by dissolving 5 g of copper (II) sulfate and 3 g of ferrous sulfate in 10 mL of purified water with continuous stirring and adding 5 g of potassium iodide in 50 mL of water. The copper (I) iodide precipitate that forms is filtered, washed with water and transferred to a brown glass bottle using a small volume of water (the suspension is stable).

Quantification Specimen collection: Blood—10 mL K-EDTA tube; urine—20 mL plastic universal container (must be hard plastic without preservative; stored specimens require acidification and freezing to avoid loss of mercury).
Note For reference values for mercury in the German population, see Wilhelm *et al.* [2004].
Blood **GC** Column: DB-5 (30 m × 0.24 mm i.d., 0.25 µm). Temperature programme: 85° for 2 min to 200° at 20°/min for 1 min. Carrier gas: He, 1 kg/cm^3. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.5 µg/L [Brunmark *et al.* 1992]. Column: 1.5% OV-17 and 1.95% QF-1 Chromosob W-HP on 80/100 mesh (1.22 m × 4 mm i.d.). Carrier gas: N_2, 120 mL/min. Temperature: 110° for methyl- and ethylmercury, 180° for phenylmercury. Limit of detection, 1.36, 1.30 and 1.12 ppb for methyl-, ethyl- and phenylmercury, respectively [Cappon, Smith 1977]

AAS Perkin-Elmer model 107 (λ = 253.7 nm). Limit of detection not reported [Ngim *et al.* 1988].

ICP-MS Perkin-Elmer Sciex Elan 5000. Nebuliser gas: 1.0 mL/min. Limit of detection, 60 ng/L [White 1999].
Urine **AAS** Pye Unicam mercury hollow cathode lamp (λ = 253.7 nm). Limit of detection, 0.82 µg/L for inorganic mercury [Littlejohn *et al.* 1976].

GC-ICP-MS Limit of detection, 2–12 pg/L [Kresimon *et al.* 2001].

ICP-MS Plasma gas: 15 L/min. Nebuliser gas: 0.825 L/min. Auxillary gas: 0.8 L/min. Limit of detection, 0.03 µg/L [Schramel *et al.* 1997].
Ocular Fluid **ICP-MS** Perkin-Elmer Sciex Elan 6100. Limit of detection, 1 µg/L [Erie *et al.* 2005].
Hair **AAS** Model 3390A integrator (λ = 253.7 nm). Limit of detection, 0.1 µg/L for mineral mercury, 0.44 µg/L for organic mercury [Pineau *et al.* 1990].

ICP-MS Limit of detection, 0.07 mg/kg [Nadal *et al.* 2005]. Plasma gas: 15 L/min. Auxiliary gas: 0.8 L/min. Nebuliser gas: 0.8 L/min. Limit of detection not reported [Samanta *et al.* 2004].
Nail **ICP-MS** See Hair [Samanta *et al.* 2004].
Other **ICP-MS** Solid and Liquid Food Samples. Limit of quantification, 2 µg/kg for solid samples, 0.2 µg/L for liquid samples; limit of detection, 0.6 µg/kg for solid samples, 0.06 µg/L for liquid samples [Cheung Chung *et al.* 2008]. Seafood. Perkin-Elmer Elan 6000. Limit of detection, 0.05 mg/kg [Falcó *et al.* 2006]. Food. Varian-Vista with ultrasonic nebuliser. Limit of detection, 0.04 mg/kg [Llobet *et al.* 2003]. Meals from Catering Establishments. Plasma gas: 15 L/min. Auxiliary gas: 0.9 L/min. Nebuliser gas: 0.73 L/min. Limit of quantification, 2 µg/kg [Noel *et al.* 2003].
Note For the direct determination of organic mercury in water, urine and blood by AAS following sodium borohydride reduction, see Margel and Hirsh [1984].

Disposition in the Body When metallic mercury vapour is inhaled, ≈75% is absorbed efficiently by the lung and passes into the circulation. The metal is lipid soluble and reaches the brain fairly rapidly. Slow oxidation to divalent mercury occurs and 2% of the absorbed dose is excreted in the urine and 9% in the faeces over a 7 day period. Mercuric salts are more soluble than the mercurous form and, as a result, are more rapidly and extensively absorbed from the gut. Significant amounts are deposited in the liver and kidneys. Methyl mercury is lipid soluble and accumulates in fatty tissues such as the brain. Approximately 50% of an oral dose of methylmercury is sequestered, initially in the liver. It is slowly converted to inorganic divalent mercury, most likely by the intestinal flora. The whole-body half-life is ~70 days and over 49 days ~35% of an oral dose is excreted in the faeces and 3% in the urine. Hair concentrations are thought to correlate well with blood concentrations during exposure to methylmercury.

Normal concentrations: Blood—<4 µg/L (20 nmol/L), urine—<5 µg/L (25 nmol/L), brain—0.04 to 0.23 mg/kg, hair—1.4 to 15 mg/kg, kidney—0.2 to 2.6 mg/kg, liver—0.16 to 1.3 mg/kg, lung—0.02 to 0.30 mg/kg. Occupational exposure: urine (pre-shift) total mercury—<35 µg/g creatinine, blood (end of shift)—<15 µg/L (75 nmol/L).
Note For concentrations in the brain regions of 8 postmortem subjects, see Glomski *et al.* [1971].
Toxicity Inhalation of metallic mercury can lead to an acute pneumonitis (mercury fume fever) accompanied by gastrointestinal disorders and damage to the CNS. Chronic exposure to metallic mercury vapour causes mainly psychological disturbances, fatigue, weight loss and tremor. Five exposed victims who developed these symptoms had blood mercury concentrations of 0.4 to 0.9 mg/L and urine concentrations of 2.4 to 8.3 mg/L [Sexton *et al.* 1978].

A 24-year-old man committed suicide by injecting himself with heroin and cutting his left wrist. Postmortem examination of his heart revealed metallic droplets consistent with mercury. Tissue concentrations of mercury were as follows:

Tissue	*Concentration*
Blood	0.04 mg/L
Bile	0.7 mg/L
Urine	0.17 mg/L
Brain	0.1 mg/kg
Hair	0.43 mg/kg
Heart	106 g/kg
Kidney	180 mg/kg
Liver	7.5 mg/kg
Lung	543 mg/kg
Stomach	0.02 mg/kg

The deceased had injected himself with mercury 5 months previously in a separate suicide attempt [Kedziora, Duflou 1995].

A 33-year-old man presented with densities on his chest radiograph. His serum mercury concentration was 67.0 µg/L. Three months later the concentration was 65.2 µg/L and the concentration in his hair was 5.7 mg/kg. The patient finally admitted to injecting mercury that he had obtained from thermometers. The granulomas were excised and 3 months later his serum mercury concentration had fallen to 11.4 µg/L and at 5 months to 8.7 µg/L [Netscher *et al.* 1991].

A 79-year-old Cree Indian whose main diet was fish had postmortem mercury concentrations of 0.32, 0.40, 2.5, 5.1, 0.43 and 0.15 mg/kg in his cerebrum, cerebellum, liver, kidney, heart and spleen, respectively [Wheatley *et al.* 1979].

A 3-year-old child who died after playing with metallic mercury had mercury concentrations of 1.3 mg/kg (brain), 3.7 mg/kg (lung), 3.9 mg/kg (liver) and 14 to 30 mg/kg (kidney). Antemortem urine concentrations ranged from 0.16 to 0.86 mg/L [Johnson *et al.* 1978].

A 16-year-old boy presented with a firm, erythematous, subcutaneous mass that contained metallic mercury. He admitted deliberate injection of mercury into the skin; his urine concentration of mercury was 2.3 mg/L [Lupton *et al.* 1985].

Chronic use of a skin-lightening cream caused kidney damage in several patients and urine mercury concentrations of 0.09 to 0.25 mg/L were measured [Gerstner, Huff 1977].

Some Chinese herbal medicines contain mercuric sulfide or mercurous chloride, and chronic use has led to toxicity with urine levels of up to 2.8 mg/L [Kang-Yum, Oransky 1992].

The hair of autistic children had mercury concentrations of 4.5 mg/kg compared with 0.3 mg/kg in controls [Fido, Al Saad 2005].

The lethal dose of a soluble inorganic mercury salt is ~1 g.

Two adults who absorbed lethal amounts of mercury after receiving peritoneal lavage following surgery had postmortem blood concentrations of 1.7 and 2.1 mg/L [Cross *et al.* 1979].

Mercury concentrations at postmortem in an adult who died 2 h following the ingestion of a large dose of mercuric chloride were 22 mg/L (blood), 3 mg/kg (brain), 56 mg/kg (liver) and 136 mg/kg (kidney) [Klendshoj, Rejent 1966].

Chronic poisoning is produced in adults by a daily intake of 0.3 mg of methylmercury and at steady state, approximate concentrations are 0.2 mg/L (blood) and 60 mg/kg (hair), with a body mercury burden of 20 to 30 mg [National Academy of

Sciences Panel on Mercury 1978]. In moderate poisoning, hair methylmercury concentrations have reached 200 to 800 mg/kg [Gerstner, Huff 1977] and in severe intoxications have been as high as 2436 mg/kg [Pierce *et al.* 1972]. Following large-scale incidents of methylmercury poisoning in Iraq and Japan (Minimata), liver concentrations in 57 Iraqi victims were 1.4 to 76 mg/kg and ranged from 22 to 71 mg/kg in Minimata victims [Magos *et al.* 1976]. Suspected exposure to mercury is also suggestive of decreased brain weights [Takeuchi *et al.* 1996].

An outbreak of inorganic mercury poisoning resulted in blood concentrations ranging from 183 to 620 µg/L and hair concentrations ranging from 0.7 to 114 mg/kg [Sexton *et al.* 1978].

Total mercury concentrations in 8 fatal cases of accidental methylmercury poisoning were 0.6 to 6.0 mg/L (blood), 18 to 35 mg/kg (brain), 4.2 to 78 mg/kg (liver) and 2.4 to 41 mg/kg (kidney) [Al Saleem 1976].

Note For a review of the methylmercury epidemic in Iraq in 1971–1972, see Greenwood [1985] and the Supplement to volume 53 of the *Bulletin of the World Health Organization*. For a study of mortality and survival of Minamata disease following the repeated ingestion of methylmercury-contaminated fish, see Tamashiro *et al.* [1985]; for a study of the causes of death in Minamata disease, see Tamashiro *et al.* [1984]; for biological monitoring of mercury in workers in hazardous-waste incineration, see Schuhmacher *et al.* [2002]. For a study of mercury in the blood, serum and red blood cells of patients with motor neuron disease, see Pamphlett *et al.* [2001]. Mercury toxicity has been implicated in the deaths of Mozart [Fluker [1972] and Robert Burns [Buchanan, Kean 1982].

Half-life Inorganic mercury, 24 days, methylmercury, 52 days.

Distribution in Blood Erythrocyte : serum ratio 19.

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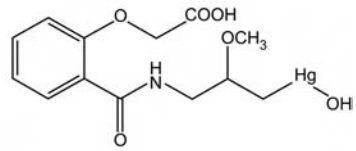
Mersalyl Acid

Diuretic

CAS—486-67-9

IUPAC Name Hydron; mercury(2+); 2-[2-(2-methoxypropylcarbamoyl)phenoxy]acetate; hydroxide



Chemical Properties A mixture of {3-[2-(carboxymethoxy)benzamido]-2-methoxypropyl}-hydroxymercury ($C_{13}H_{17}HgNO_6 = 483.9$) and its anhydrides. A white, slightly hygroscopic powder. Slightly soluble in water and dilute mineral acids; soluble in solutions of alkali hydroxides. Log *P* (octanol/water), −1.5 (mersalyl sodium).

Mersalyl Sodium

$C_{13}H_{16}HgNNaO_6 = 505.9$

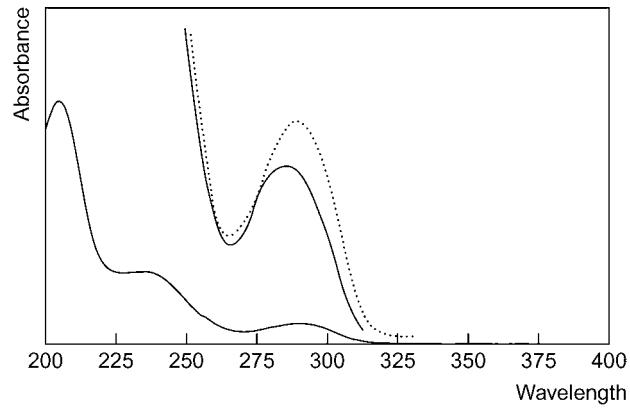
CAS—492-18-2

Synonym Mersalyl

Chemical Properties A white deliquescent powder. Soluble 1 in 1 of water, 1 in 3 of ethanol and 1 in 2 of methanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.24.

Ultraviolet Spectrum Aqueous acid—286 nm; aqueous alkali—290 nm (A^1_1=63b).



Dose 50 to 200 mg of mersalyl sodium, intramuscularly.

Mescaline

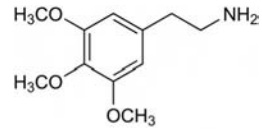
Hallucinogen

$C_{11}H_{17}NO_3 = 211.3$

CAS—54-04-6

IUPAC Name 3,4,5-Trimethoxybenzeneethanamine

Synonym Mezcaline



Chemical Properties An alkaloid obtained from the cactus *Lophophora williamsii* (= *Anhalonium williamsii* = *A. lewinii*) (Cactaceae), which grows in the northern regions of Mexico. The cactus is also known as 'peyote' or 'peyotl' and dried slices of the cactus are called 'mescal buttons'. Crystals that take up carbon dioxide from air. Mp 35° to 36°. Moderately soluble in water; soluble in ethanol, chloroform and benzene; practically insoluble in ether and petroleum ether. pK_a 9.6. Log *P* (octanol/water), 0.8.

Mescaline Hydrochloride

$C_{11}H_{17}NO_3$,HCl = 247.8
Chemical Properties Needles. Mp 181°. Soluble in water and alcohol.

Mescaline Sulfate Dihydrate

$(C_{11}H_{17}NO_3)_2$,H_2SO_4, $2H_2O$ = 540.7
Chemical Properties Prisms. Mp 183° to 186°. Soluble in hot water and methanol; sparingly soluble in cold water and ethanol.

Mescaline Acid Sulfate

$C_{11}H_{17}NO_3$,H_2SO_4 = 309.4
Chemical Properties Crystals. Mp 158°.

N-Benzoylmescaline

$C_{17}H_{21}NO_3$ = 287.4
Chemical Properties Needles. Mp 121°. Very soluble in alcohol and ether.

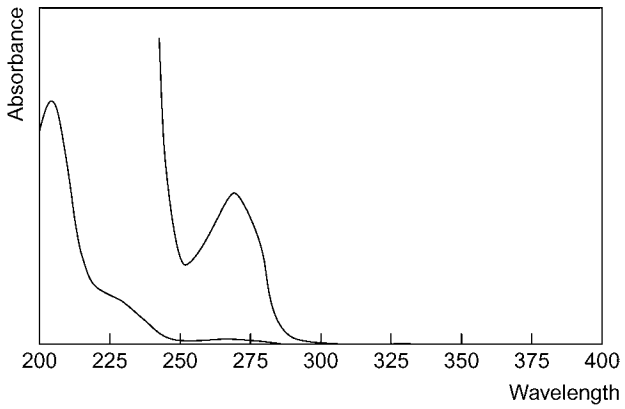
N-Methylmescaline

$C_{12}H_{19}NO_3$ = 225.3
Chemical Properties Occurs naturally. Bp 130° to 140°.

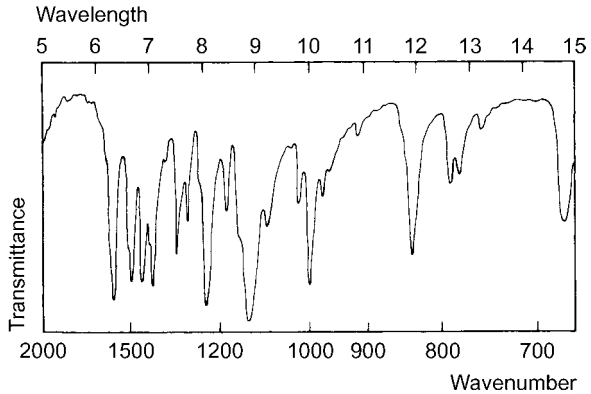
N-Acetylmescaline

$C_{13}H_{19}NO_4$ = 253.3
Chemical Properties Occurs naturally. Mp 94°.

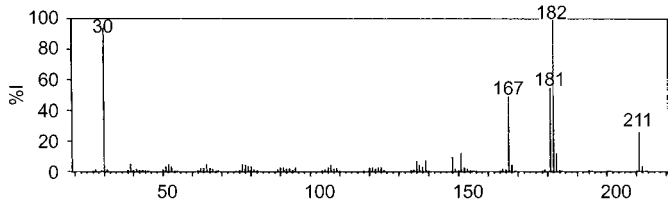
Colour Test Liebermann's reagent—black; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.20; system TB—R_f 0.03; system TC—R_f 0.10; system TE—R_f 0.24; system TL—R_f 0.12; system TAE—R_f 0.06; system TAF—R_f 0.63; system TAJ—R_f 0.02; system TAK—R_f 0.09; system TAL—R_f 0.51 (acidified iodoplatinate solution, positive; Dragendorff spray, positive; FPN reagent, positive; Marquis test, yellow; ninhydrin spray, positive).
Gas Chromatography System GA—mescaline RI 1680, mescaline-AC RI 2160, mescaline precursor (trimethoxyphenylacetonitrile) RI 1610.
High Performance Liquid Chromatography System HA—*k* 1.3; system HB—*k* 16.82; system HC—*k* 2.17; system HX—RI 272; system HY—RI 243.
Ultraviolet Spectrum Aqueous acid—268 nm (A^1_1 = 34c). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1127, 1242, 1592, 996, 1513, 834 cm^{-1} (mescaline hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 182, 30, 181, 167, 211, 183, 151, 148.



Quantification

Plasma **GC-MS** Limit of quantification, 5 µg/L [Habrdova *et al.* 2005].
LC-MS Column: strong cation exchange separation. ESI, MRM acquisition mode. Limit of quantification, 10 µg/L [Beyer *et al.* 2007].
Urine **HPLC** Column: ODS (3 µm). Mobile phase: acetonitrile : water : phosphoric acid : hexylamine. DAD (λ= 198 or 220 nm). Limit of detection, 26–56 µg/L and 0.04 µg/mg [Helmlin, Brenneisen 1992].
LC-MS Column: HyPurity C_{18} (5 µm). Mobile phase: methanol : ammonium acetate buffer. Limit of detection, 3–5 µg/L [Björnstad *et al.* 2008].
Hair **GC-MS** Limit of quantification, <0.08 ng/mg [Kim *et al.* 2007].
Other **GC-MS** MFD. Limit of detection, 5 µg/L [Van Peteghem *et al.* 1980].
Disposition in the Body Mescaline is readily absorbed after oral administration. It is concentrated in the kidneys, liver and spleen. Approximately 90% of a dose is excreted in the urine in 24 h, with ~30% as 3,4,5-trimethoxyphenylacetic acid, which is inactive; the remainder is mostly unchanged drug, together with 3,4-dihydroxy-5-methoxyphenylacetic acid, which is excreted as a glutamine conjugate. Other metabolites include *N*-acetylmescaline and *N*-acetyl-3,4-dimethoxy-5-hydroxyphenethylamine.
Toxicity Mescaline induces psychotic changes similar to those produced by lysergide, but it is less potent. The effects of a single dose may persist for ~12 h.

In a mescaline-related fatality, where the individual ran off a cliff and plunged 600 feet to his death, the concentrations were reported to be 9.7 mg/L, 70.8 µg/g and 1163 mg/L in the blood, liver, and urine, respectively [Reynolds, Jindrich 1985].

Note For a report of a fatal peyote ingestion, see Nolte, Zumwalt [1999].
Protein Binding Partly bound.
Note For a historical article on mescaline use, see Bruhn *et al.* [2002].

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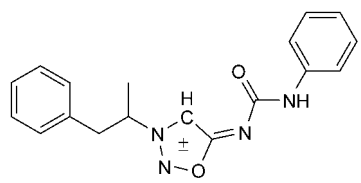
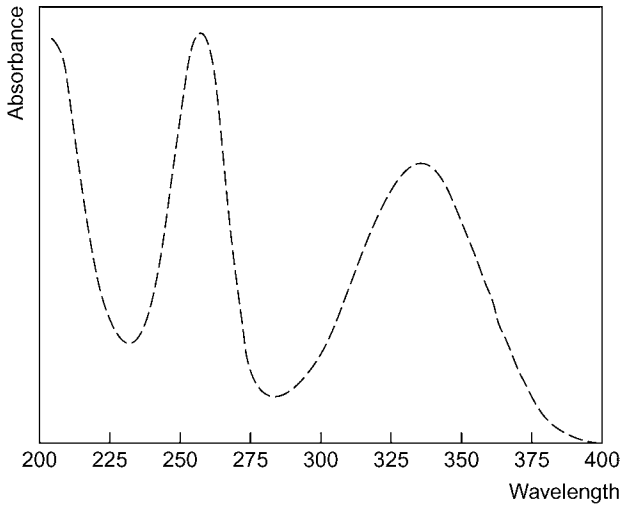
Van Peteghem C *et al.* (1980). GLC–mass spectral determination of mescaline in plasma of rabbits after IV injection. *J Pharm Sci* 69: 118–120.

Mescarb

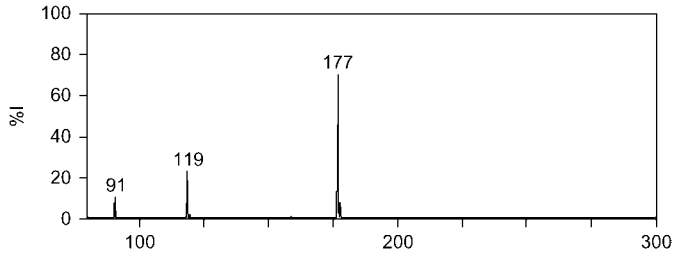
CNS Stimulant
$C_{18}H_{18}N_4O_2$=22.4
CAS—34262-84-5
IUPAC Name *N*-Phenyl-*N*′-[3-(1-phenylpropan-2-yl)oxadiazol-3-ium-5-yl] carbamimidate
Synonyms 3-(α-Methylphenethyl)-*N*-(phenylcarbamoyl)sydnone imine; sidnocarb; sydnocarbum; synocarb.



Chemical Properties Plasma and urine extracts are stable over time [Shpak *et al.* 2005].
Ultraviolet Spectrum



Mass Spectrum



Quantification

Plasma **LC-MS** Column: Zorbax SB-C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.2 mmol/L ammonium acetate (pH 6.7) : methanol (80 : 20 to 40 : 60 over 20 min), flow rate 0.2 mL/min. ESI, positive ion mode. Retention time: 29.9 min. Limit of quantification, 0.6 µg/L, limit of detection, 0.1 µg/L [Shpak *et al.* 2005].

Urine **HPLC** Column: Ultrasphere, C_{18} ODS (75 × 4.6 mm i.d., 3 µm). Mobile phase: 0.1 mol/L ammonium acetate (pH 3) : acetonitrile (90 : 10 to 85 : 15 in 2 min to 55 : 45 in 3 min to 40 : 60 in 3 min for 1 min), flow rate 1 mL/min. DAD (λ = 200 to 400 nm). Retention time: 7.8 min. Limit of detection not reported [Ventura *et al.* 1993].

LC-MS Column: Thermo Gold C_{18} (50 × 2.1 mm i.d., 3 µm). Mobile phase: 15 mmol/L ammonium formate containing 0.1% formic acid : acetonitrile (100 : 0 to 20 : 80 at 6.0 min for 2.5 min to 100 : 0 at 9 min for 4 min), flow rate 200 µL/min for 8 min to 400 µL/min for 1 min to 200 µL/min. ESI, positive ion mode, SRM acquisition mode. Limit of detection, 5.0 µg/L [Kang *et al.* 2007]. Column: Discovery-Supelco C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% acetic acid : acetonitrile with 0.1% acetic acid (85 : 15 to 40 : 60 in 7 min to 0 : 100 at 14 min), flow rate 0.25 mL/min. ESI, MRM acquisition mode, CID. Limit of detection, 100 µg/L [Mazzarino, Botré 2006]. Column: Nucleosil C_{18} (100 × 3.0 mm i.d., 5 µm). Mobile phase: 1% acetic acid : acetonitrile (85 : 15 for 2 min to 45 : 55 in 10 min to 35 : 65 in 8 min to 85 : 15 for 10 min. ESI. Limit of detection not reported [Deventer *et al.* 2005]. See Plasma. Limit of quantification, 0.05 µg/L, limit of detection, 0.001 µg/L [Shpak *et al.* 2005]. Column: Zorbax SB-C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.2 mmol/L ammonium acetate : methanol (80 : 20 to 40 : 60 at 20 min for 15 min), flow rate 0.2 mL/min for 20 min, 0.3 mL/min for 15 min. API-ES, MRM acquisition mode. Retention time: 29.9 min. Limit of quantification, 1 µg/L [Appolonova *et al.* 2004].

Note Mesocarb is a psychostimulant in clinical practice in Russia as a primary and adjunct therapy for several psychiatric disorders. It is a stimulant with an addiction liability and toxicity less than that of amfetamines. For a literature review of the toxicokinetics of amfetamines and their *N*-alkyl derivatives, see Kraemer and Maurer [2002].

Appolonova SA *et al.* (2004). Liquid chromatography–electrospray ionization ion trap mass spectrometry for analysis of mesocarb and its metabolites in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 800: 281–289.

Deventer K *et al.* (2005). Simultaneous determination of beta-blocking agents and diuretics in doping analysis by liquid chromatography/mass spectrometry with scan-to-scan polarity switching. *Rapid Commun Mass Spectrom* 19: 90–98.

Kang MJ *et al.* (2007). Validation and application of a screening method for beta2-agonists, anti-estrogenic substances and mesocarb in human urine using liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 21: 252–264.

Kraemer T, Maurer HH (2002). Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their *N*-alkyl derivatives. *Ther Drug Monit* 24: 277–289.

Mazzarino M, Botré F (2006). A fast liquid chromatographic/mass spectrometric screening method for the simultaneous detection of synthetic glucocorticoids, some stimulants, anti-oestrogen drugs and synthetic anabolic steroids. *Rapid Commun Mass Spectrom* 20: 3465–3476.

Shpak AV *et al.* (2005). Validation of liquid chromatography–electrospray ionization ion trap mass spectrometry method for the determination of mesocarb in human plasma and urine. *J Chromatogr Sci* 43: 11–21.

Ventura R *et al.* (1993). Determination of mesocarb metabolites by high-performance liquid chromatography with UV detection and with mass spectrometry using a particle-beam interface. *J Chromatogr* 647: 203–210.

Mesoridazine

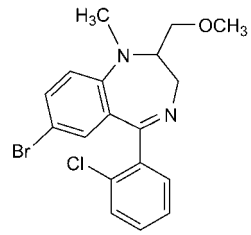
Tranquilliser

$C_{21}H_{26}N_2OS_2$=86.6

CAS—5588-33-0

IUPAC Name 10-[2-(1-Methylpiperidin-2-yl)ethyl]-2-methylsulfinylphenothiazine

Synonyms Mesuridazine; 10-[2-(1-methyl-2-piperidinyl)ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine.



Chemical Properties Soluble in chloroform. Log *P* (octanol/water), 5.6.

Mesoridazine Benzenesulfonate

$C_{21}H_{26}N_2OS_2,C_6H_6O_3S$ = 544.7

CAS—32672-69-8

Synonym Mesoridazine besilate

Proprietary Name *Serentil*

Chemical Properties A white to pale yellow, crystalline powder. Mp ~ 178° with decomposition. Soluble 1 in 1 of water, 1 in 11 of ethanol, 1 in 3 of chloroform and 1 in 6300 of ether; freely soluble in methanol.

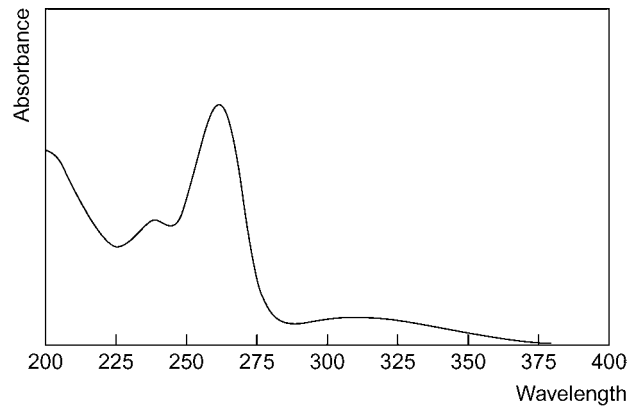
Colour Tests Formaldehyde–sulfuric acid—violet; Forrest reagent—red; FPN reagent—red; Mandelin's test—green-brown; Marquis test—red→violet; sulfuric acid—violet→blue.

Thin-layer Chromatography System TA—R_f 0.38; system TB—R_f 0.03; system TC—R_f 0.06; system TE—R_f 0.30; system TAE—R_f 0.11; system TAG—R_f 0.01 (Acidified iodoplatinate solution, positive).

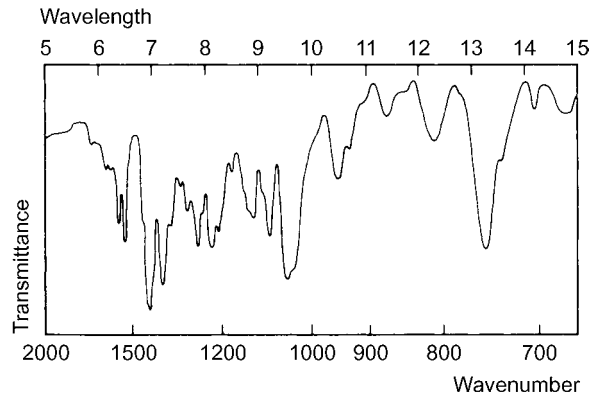
Gas Chromatography System GA—RI 3380; system GB—RI 3629.

High Performance Liquid Chromatography System HA—*k* 5.0; system HY—RI 337; system HZ—RT 3.4 min; system HAX—RT 10.1 min; system HAY—RT 5.0 min.

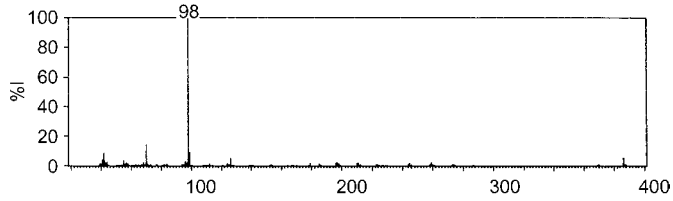
Ultraviolet Spectrum Aqueous acid—238, 262 (A_1^1 = 773b), 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1050, 752, 1239, 1282, 1562, 1091 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 98, 70, 99, 42, 386, 126, 55, 41.



Quantification

Blood **HPLC** Column: μBondapak C_{18}. Mobile phase: methanol : water (66 : 34) containing 1% acetic acid and 0.005 mol/L 1-heptane sulfonic acid sodium salt, flow rate 2 mL/min. UV detection (λ = 263 nm). Limit of detection, 0.25 mg/L [McCutcheon 1979].

Plasma **GC** Column: 3% OV-17 on Chromosorb Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: He, 100 mL/min. Temperature programme: 275°. FID. Retention time: ~8.9 min. Limit of detection, 50 μg/L [Dinovo *et al.* 1974; Dinovo *et al.* 1976].

Note For a radioimmunoassay in plasma, see Chakraborty *et al.* [1987].

Disposition in the Body Readily absorbed after oral administration. The major metabolite is sulforidazine which is pharmacologically active. Mesoridazine is a major metabolite of thioridazine [Lin *et al.* 1993].

Therapeutic Concentration Following a single IM injection of 2 mg/kg to 6 subjects, peak plasma concentrations of 0.1–1.1 mg/L (mean 0.5) for mesoridazine, and 0.1–0.6 mg/L (mean 0.3) for sulforidazine, were attained in ~4 h [Gottschalk *et al.* 1976].

Toxicity

In a fatality attributed to the ingestion of 2.5 g mesoridazine, the following postmortem tissue concentrations were reported: blood 3 mg/L, kidney 17 μg/g, and liver 114 μg/g. In a second case in which 8 g had been ingested, the postmortem blood concentration was 4 mg/L [Donlon, Tupin 1977].

A 23-year-old female died approximately 6–6.5 h after ingesting an unknown quantity of mesoridazine. Her antemortem blood level of mesoridazine was 16 mg/L [Vertrees, Siebel 1987].

Half-life Plasma half-life, mesoridazine 2–9 h (mean 5), sulforidazine 6–25 h (mean 13).

Dose The equivalent of 150 mg mesoridazine daily; up to 400 mg daily has been given.

Chakraborty BS *et al.* (1987). Development of a radioimmunoassay procedure for mesoridazine and its comparison with a high-performance liquid chromatographic method. *Ther Drug Monit* 9: 464–471.

Dinovo EC *et al.* (1974). Isolation of a possible new metabolite of thioridazine and mesoridazine from human plasma. *Res Commun Chem Pathol Pharmacol* 7: 489–496.

Dinovo EC *et al.* (1976). GLC analysis of thioridazine, mesoridazine, and their metabolites. *J Pharm Sci* 65: 667–669.

Donlon PT, Tupin JP (1977). Successful suicides with thioridazine and mesoridazine: a result of probable cardiotoxicity. *Arch Gen Psychiatry* 34: 955–957.

Gottschalk LA *et al.* (1976). Plasma levels of mesoridazine and its metabolites. In: Gottschalk LS, ed. *Pharmacokinetics of Psychoactive Drugs.* New York: Spectrum Publications, pp. 171-189.

Lin G *et al.* (1993). The metabolism of piperidine-type phenothiazine antipsychotic agents.III. Mesoridazine in dog, human and rat. *Xenobiotica* 23: 37–52.

McCutcheon JR (1979). Reverse-phase HPLC determination of thioridazine and mesoridazine in whole blood. *J Anal Toxicol* 3: 105–107.

Vertrees JE, Siebel G (1987). Rapid death resulting from mesoridazine overdose. *Vet Hum Toxicol* 29: 65–67.

Mestanolone

Anabolic Steroid

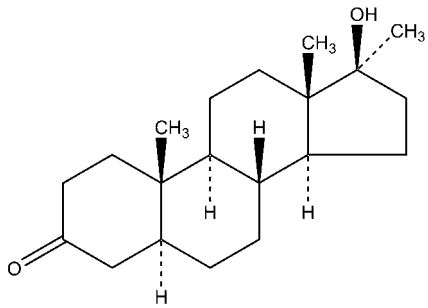
$C_{20}H_{32}O_2$ = 304.5

CAS—521-11-9

IUPAC Name (5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13,17-trimethyl-2,4,5,6,7,8,9,11,12,14,15,16-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-one

Synonyms 17β-Hydroxy-17α-methyl-3-androstanone; (5α,17β)-17-hydroxy-17-methylandrostan-3-one; 17α-methylandrostan-17β-ol-3-one; 17α-methylandrostan-3-on-17β-ol.

Proprietary Names *Anabo*; *Antalone*; *Duramin*; *Mechiaron*; *Prohormo*; *Protenolon*; *Tantarone*.



Chemical Properties Crystals. Mp 192° to 193°. Insoluble in water. Soluble in acetone, alcohol, ether and ethyl acetate. Log *P* (octanol/water), 3.52 [Meylan, Howard 1995], 4.29 [ACD 2007].

Mass Spectrum Principal ions at *m/z* 360, 375, 390, 405 (MO-TMS derivative).

Quantification

Urine **GC-MS** Column 1: HP5 SE54 fused silica capillary cross-linked 5% phenyl methylsilicone (17 m × 0.2 mm i.d., 0.3 μm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. Column 2: OV1 fused silica capillary cross-linked methylsilicone (20 m × 0.25 mm i.d., 0.33 μm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. EI ionisation at 70 eV. Limit of detection not reported [Schanzer *et al.* 1992].

HPLC Column: Merck Lichrosorb (R) RP_{18} (100 × 4.0 mm i.d., 5.0 μm). Mobile phase: acetonitrile : water (30 : 70 to 80 : 20 after 20 min), flow rate 1.0 mL/min. Limit of detection not reported [Schanzer *et al.* 1992].

Hair **GC-MS** Column: CP-SIL 5 CB fused silica (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min. EI ionisation at 70 eV. Limit of quantification, 0.1 ng/mg; limit of detection, 0.05 ng/mg [Deng *et al.* 1999].

Disposition in the Body A healthy male volunteer was administered 20 mg mestanolone orally. No mestanolone or its epimer were detected in urine over 72 h after dosing. The metabolite 17α-methyl-5α-androstane-3α,17β-diol and its 17β-methyl epimer were detected and reported to be 2.26% and 0.02% of the applied dose of mestanolone, respectively [Schanzer *et al.* 1992]. Another healthy male volunteer was administered 10 mg mestanolone orally. No trace of the 17-epimer and dehydration products of the administered steroid were detected in urine over 72 h after dosing [Bi, Masse 1992].

Toxicity Anabolic 17β-hydroxy-17α-methyl steroids are well known for their potent liver toxicities and carcinogenicities [Lovisetto *et al.* 1979].

ACD (2007). *ACD/LogP DB.* Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Bi H, Masse R (1992). Studies on anabolic steroids: 12. Epimerization and degradation of anabolic 17beta-sulfate-17alpha-methyl steroids in human: qualitative and quantitative GC/MS analysis. *J Steroid Biochem Mol Biol* 42: 533–546.

Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.

Lovisetto P *et al.* (1979). Features of liver damage caused by 17-alpha-alkyl-substituted anabolic steroids. *Minerva Med* 70: 769–790.

Meylan W *et al.* (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Schanzer W *et al.* (1992). 17-Epimerization of 17alpha-methyl anabolic steroids in humans: metabolism and synthesis of 17alpha-hydroxy-17beta-methyl steroids. *Steroids* 57: 537–550.

Mesterolone

Androgen

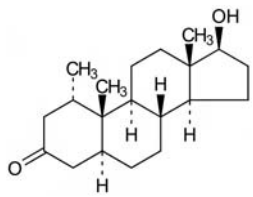
$C_{20}H_{32}O_2$ = 304.5

CAS—1424-00-6

IUPAC Name (1*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-1,10,13-trimethyl-1,2,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[*a*]phenanthren-3-one

Synonym (1α,5α,17β)-17-Hydroxy-1-methylandrostan-3-one

Proprietary Names *Mestoranum*; *Pro-Viron*.



Chemical Properties A white crystalline powder. Mp about 210°. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in 6 of chloroform and 1 in 150 of ether. Log *P* (octanol/water), 3.5.

High Performance Liquid Chromatography System HATb—RRT 1.52 (relative to testosterone).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1706, 1068, 1055, 1252, 1238, 1032 cm^{-1} (KBr disk).

Quantification

Urine **GC-MS** Mesterolone and other anabolic steroids. Limits of detection, 5 to 20 ppb [Choi *et al.* 1999].

Hair **GC-MS** Mesterolone and other anabolic steroids. Limits of detection, 0.002 to 0.05 μg/L [Deng *et al.* 1999].

Dose 50 to 100 mg daily.

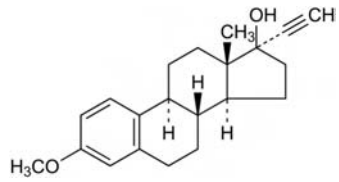
Choi MH *et al.* (1999). Determination of anabolic steroids by gas chromatography/negative-ion chemical ionization mass spectrometry and gas chromatography/negative-ion chemical ionization tandem mass spectrometry with heptafluorobutyric anhydride derivatization. *Rapid Commun Mass Spectrom* 13: 376–380.

Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.

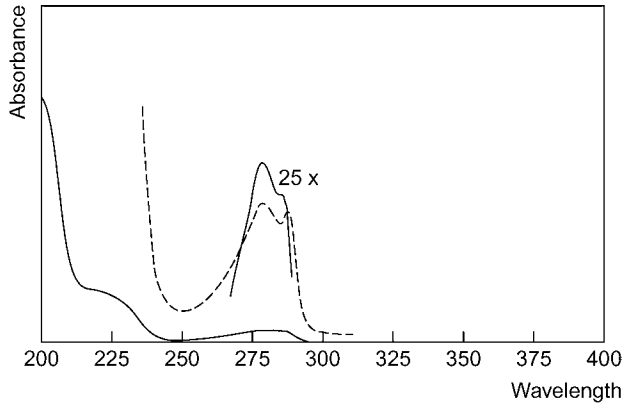
Mestranol

Oestrogen
$C_{21}H_{26}O_2$ = 310.4
CAS—72-33-3

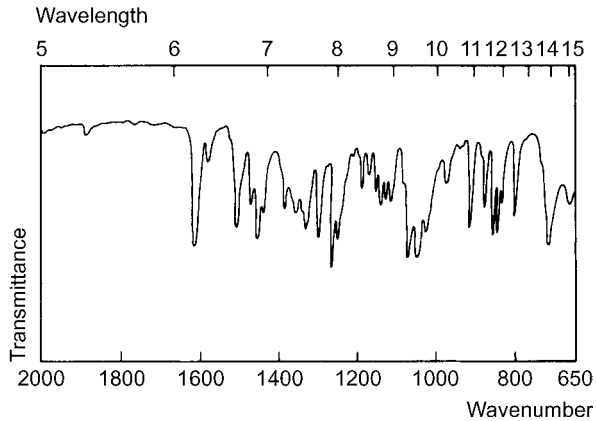
IUPAC Name (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-3-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-ol
Synonyms EE3ME; ethinyloestradiol-3-methyl ether; (17α)-3-methoxy-19-nor-pregna-1,3,5(10)-trien-20-yn-17-ol.
Proprietary Names It is an ingredient of *Enovid*, *Genora*, *Menophase*, *Necon*, *Nelova*, *Norethin*, *Norinyl* and *Ortho-Novin*.



Chemical Properties A white to creamy-white, crystalline powder. Mp 146° to 154°. Practically insoluble in water; soluble 1 in 44 of ethanol, 1 in 4.5 of chloroform and 1 in 23 of ether; soluble in dioxane and acetone; slightly soluble in methanol. Log *P* (octanol/water), 4.7.
Caution Mestranol is a powerful oestrogen. Contact with the skin or inhalation should be avoided.
Colour Tests Antimony pentachloride—green; Liebermann's reagent—red; naphthol-sulfuric acid—red/red; sulfuric acid—orange-red (yellow fluorescence under ultraviolet light).
Thin-layer Chromatography System TP—R_f 0.86; system TQ—R_f 0.52; system TR—R_f 0.90; system TS—R_f 0.90.
Gas Chromatography System GA—RI 2612.
Ultraviolet Spectrum Methanol—278 (A^1_1=61a), 287 nm.



Infrared Spectrum Principal peaks at wavenumbers 1255, 1060, 1035, 1612, 1291, 1241 cm^{-1} (KBr disk).

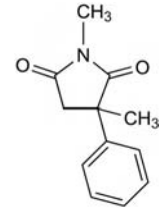


Mass Spectrum Principal ions at *m/z* 227, 310, 174, 284, 147, 160, 173, 199.
Dose Up to 100 µg daily.

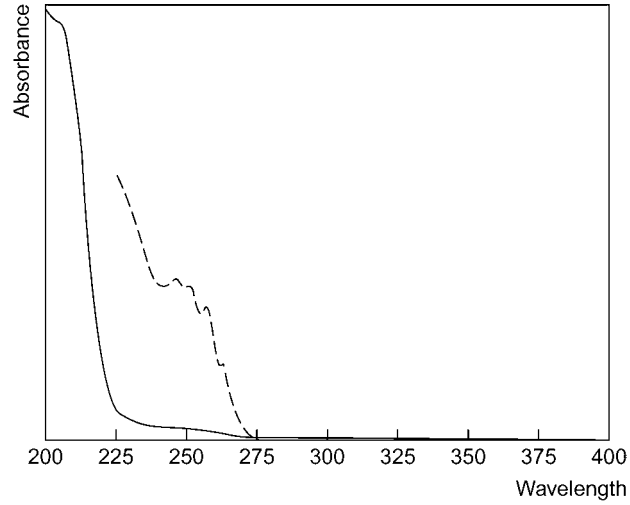
Mesuximide

Anticonvulsant
$C_{12}H_{13}NO_2$ = 203.2
CAS—77-41-8

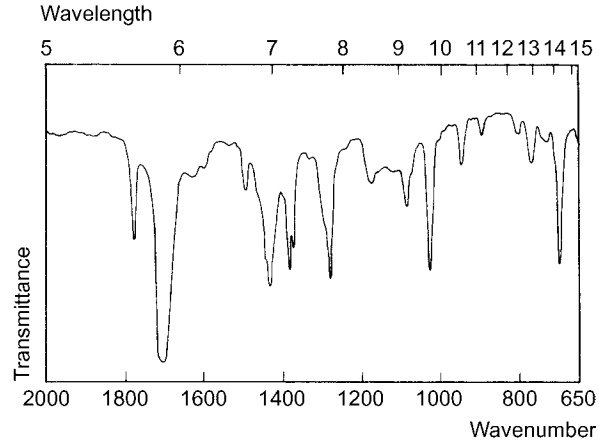
IUPAC Name 1,3-Dimethyl-3-phenyl-2,5-pyrrolidinedione
Synonym Methsuximide
Proprietary Names *Celontin*; *Petinutin*.



Chemical Properties A white to greyish-white crystalline powder. Mp 50° to 56°. Soluble 1 in 350 of water, 1 in 3 of ethanol, 1 in <1 of chloroform and 1 in 2 of ether. Log *P* (octanol/water), 1.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Thin-layer Chromatography System TA—R_f 0.76; system TE—R_f 0.86; system TAE—R_f 0.90; system TAJ—R_f 0.85; system TAK—R_f 0.70; system TAL—R_f 0.98.
Gas Chromatography System GA—mesuximide RI 1705; M (nor-) RI 1750; M (OH-) RI 2220; M (nor-OH-) RI 2300; system GE—RRT 0.35; system GAJ—RRT 0.689.
High Performance Liquid Chromatography System HE—*k* 6.02; system HY—RI 387; system HZ—retention time 4.8 min.
Ultraviolet Spectrum Methanol—247 (A^1_1=21a), 252, 258, 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1705, 1282, 1030, 699, 1775, 1086 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 118, 117, 203, 103, 77, 78, 119, 91.
Quantification
Plasma **GC** ECD. *N*-desmethylmesuximide, limit of detection, 500 µg/L [Wallace *et al.* 1979].
Serum **GC** See Plasma [Wallace *et al.* 1979]. FID. Limit of detection, 1 mg/L [Bonitati 1976].
HPLC UV detection. Limit of detection, 1 mg/L [Kabra *et al.* 1978].
Body Fluids **GC** FID. Limit of detection, 0.5 mg/L for mesuximide and 5 mg/L for *N*-desmethylsuximide [Cardella *et al.* 1988].

Disposition in the Body Rapidly absorbed after oral administration and metabolised to the active *N*-desmethyl derivative. <1% of a dose is excreted in the urine as unchanged drug. The major urinary metabolites are conjugated 4-hydroxyphenyl derivatives; minor metabolites include the 3-hydroxy, 2-hydroxymethyl and 2-dihydrodihydroxyphenyl derivatives. Mesuximide increases its own rate of metabolism on chronic dosing.

Therapeutic Concentration In plasma, *N*-desmethylmesuximide, usually in the range 10 to 40 mg/L.

Following daily oral doses of 600 to 1200 mg to 8 subjects experiencing good seizure control, steady-state plasma concentrations of 0.01 to 0.11 mg/L (mean 0.04) and 16 to 37 mg/L (mean 25) for mesuximide and the *N*-desmethyl metabolite, respectively, were reported [Strong *et al.* 1974].

Toxicity The estimated minimum lethal dose is 5 g. Toxic effects are usually associated with plasma *N*-desmethylmesuximide concentrations of >40 mg/L.

In a case of severe coma, which began several hours after the ingestion of nearly 10 g mesuximide in a suicide attempt, blood concentrations of 18 mg/L of mesuximide and 44 mg/L of the *N*-desmethyl metabolite were reported 14 h after ingestion; the coma persisted for about 80 h and correlated with the presence of the *N*-desmethyl metabolite in the blood [Karch 1973].

Half-life Plasma half-life, mesuximide about 3 h, but may reduce to one quarter of this value on chronic dosing; *N*-desmethylmesuximide about 30 to 40 h.

Protein Binding Not significantly bound.

Dose 0.3 to 1.2 g daily; up to 3.6 g daily has been given.

Bonitati J (1976). Gas-chromatographic analysis for succinimide anticonvulsants in serum: macro- and micro-scale methods. *Clin Chem* 22: 341–345.

Cardella DS *et al.* (1988). Measurement of methsuximide and N-desmethylmethsuximide using solid-phase extraction and wide-bore capillary gas chromatography. *Clin Biochem* 21: 329–331.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings at the 12th TIAFT*, Seoul: 481–486.

Kabra PM *et al.* (1978). *J Anal Toxicol* 2: 127–133.

Karch SB (1973). Methsuximide overdose. Delayed onset of profound coma. *JAMA* 223: 1463–1465.

Strong JM *et al.* (1974). Plasma levels of methsuximide and N-desmethylmethsuximide during methsuximide therapy. *Neurology* 24: 250–255.

Wallace JE *et al.* (1979). Electron-capture gas-liquid chromatographic determination of ethosuximide and desmethylmethsuximide in plasma or serum. *Clin Chem* 25: 252–255.

Metabutethamine

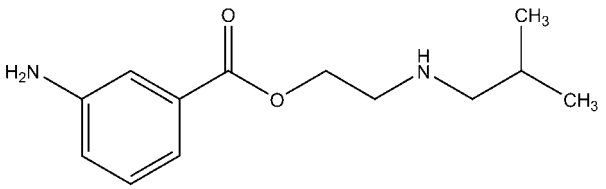
Anaesthetic (Local)

$C_{13}H_{20}N_2O_2$ = 236.3

CAS—4439-25-2

IUPAC Name 2-(2-Methylpropylamino)ethyl 3-aminobenzoate

Synonym 2-Isobutyaminoethyl *m*-aminobenzoate



Chemical Properties Metabutethamine is an isomer of butethamine. Metabutethamine is extracted by organic solvents from aqueous alkaline solutions.

Metabutethamine Hydrochloride

$C_{13}H_{20}N_2O_2$,HCl = 272.8

CAS—550-01-6

Synonym Primacaine hydrochloride

Proprietary Name *Unacaine Hydrochloride*

Chemical Properties A white crystalline solid or powder. Mp 181° to 184°. Soluble in water; slightly soluble in ethanol and chloroform.

Colour Tests Diazotisation test—red (limit of detection, 0.1 µg); Vitali's test—yellow/yellow (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.60 (location reagent acidified iodoplatinate spray, positive reaction; *p*-dimethylaminobenzaldehyde spray, yellow).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—228 and 275 nm with an inflexion at 280 nm.

Disposition in the Body

Toxicity Metabutethamine is used by injection in dentistry, usually as a 3.8% solution with adrenaline. It provides a relatively short duration of anaesthesia. It is considerably less toxic than procaine hydrochloride when injected SC or IP, but has about the same toxicity when injected IV [Cahill *et al.* 1965].

Cahill JF *et al.* (1965). The relation between acute toxicity and critical rate of disposal of several local anaesthetics. *Can J Physiol Pharmacol* 43: 343–349.

Metabutoxycaine

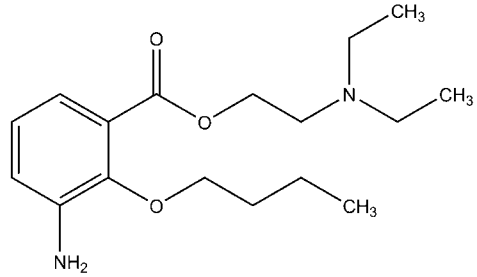
Anaesthetic (Local)

$C_{17}H_{28}N_2O_3$ = 308.4

CAS—3624-87-1

IUPAC Name 2-Diethylaminoethyl 3-amino-2-butoxybenzoate

Synonyms 3-Amino-2-butoxybenzoic acid 2-(diethylamino)ethyl ester; 2-butoxy-3-aminobenzoic acid β-diethylaminoethyl ester; 2′-diethylaminoethyl 3-amino-2-butoxybenzoate; β-diethylaminoethyl 2-butoxy-3-aminobenzoate.



Chemical Properties Metabutoxycaine is extracted by organic solvents from aqueous alkaline solutions.

Metabutoxycaine Hydrochloride

$C_{17}H_{28}N_2O_3$,HCl = 344.9

CAS—550-01-6

Synonym Primacaine hydrochloride

Chemical Properties A white crystalline powder. Mp 117° to 120°. Very soluble in water and ethanol; very slightly soluble in ether; sparingly soluble in ether.

Thin-layer Chromatography Sunshine and Fike [1964] described 3 systems which separate metabutoxycaine and other local anaesthetics.

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—313 nm.

Disposition in the Body

Toxicity Metabutoxycaine is used in dentistry as a 1.5% solution with adrenaline. Its potency is greater than that of procaine hydrochloride in the same concentration [Harris, Worley 1958].

Harris SC, Worley RC (1958). Comparative local anesthetic potencies of lidocaine and metabutoxycaine. *J Am Dent Assoc* 57: 66–70.

Sunshine I, Fike WW (1964). Value of thin-layer chromatography in two fatal cases of intoxication due to lidocaine and mepivacaine. *N Engl J Med* 271: 487–490.

Metaclazepam

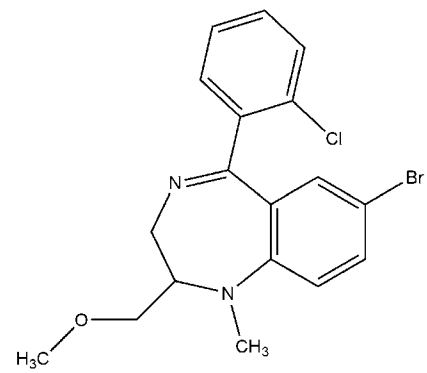
Anxiolytic, Benzodiazepine

$C_{18}H_{18}BrClN_2O$=393.7

CAS—84031-17-4

IUPAC Name 7-Bromo-5-(2-chlorophenyl)-2-(methoxymethyl)-1-methyl-2,3-dihydro-1,4-benzodiazepine

Synonyms a-2547; brometazepam; 7-bromo-5-(2-chlorophenyl)-2,3-dihydro-2-(methoxymethyl)-1-methyl-1*H*-1,4-benzodiazepine; KC-2547; metuclazepam.



Chemical Properties Yellow, odourless crystals. Mp 88° to 90°. pK_a 5.5 [Fernandez-Arciniega, Hernandez 1985], 5.9 [Althaus *et al.* 1986] Log *P* (octanol/buffer pH 7), 2000; (octanol/buffer pH 2), 3.

Metaclazepam Hydrochloride

$C_{18}H_{18}BrClN_2O$,HCl = 430.2

CAS—61802-93-5

Synonym Brometazepam hydrochloride

Proprietary Name *Talis*

Chemical Properties A bright orange, crystalline powder with a slightly bitter taste. Mp 193° to 196°. Solubility: 43 g/100 mL water; 73 g/100 mL methanol; 14 g/100 mL ethanol; 29 g/100 mL chloroform; 0.02 g/100 mL benzene.

Thin-layer Chromatography System TA—R_f 0.77 (metaclazepam), R_f 0.76 (M nor); system TB—R_f 0.40 (metaclazepam), R_f 0 (M dinor), R_f 0.15 (M nor); system TC—R_f 0.73 (metaclazepam), R_f 0.68 (M nor); system TD—R_f 0.47 (metaclazepam), R_f 0.33 (M nor); system TE—R_f 0.79 (metaclazepam), R_f 0.46 (M dinor)

0.09, R_f 0.73 (M nor); system TF—R_f 0.35 (metaclazepam), R_f 0.9 (M dinor), R_f 0.25 (M nor); system TAD—R_f 0.71 (metaclazepam), R_f 0.65 (M nor); system TAE—R_f 0.82 (metaclazepam), R_f 0.81 (M dinor), R_f 0.81 (M nor); system TAF—R_f 0.84 (metaclazepam), R_f 0.84 (M nor); system TAG—R_f 0.62 (metaclazepam), R_f 0.58 (M nor).

Plates: silica gel $60F_{254}$. Mobile phase: toluene : acetone (85 : 15). Detection fluorescence quenching at 254 nm. R_f 0.48 [Althaus *et al.* 1986].

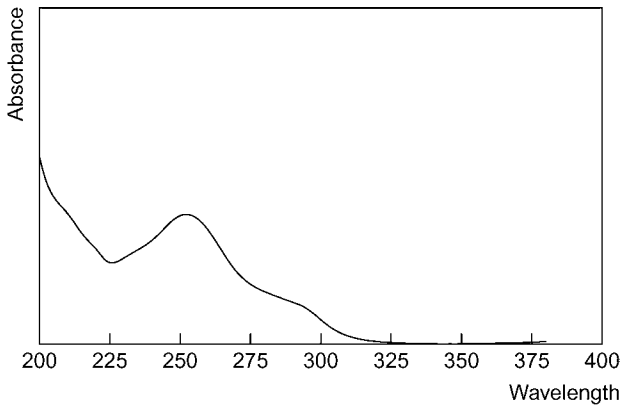
Gas Chromatography System GA—metaclazepam RI 2640, M *O*-desmethyl, RI 2730, M nor, RI 2690.

Column: Chromopack WCOT fused silica CP-Sil 5CB (25 m × 0.32 mm i.d.). Temperature programme: 200° to 300° at 4°/min. Carrier gas: He, 30 cm/s. NSD. Retention time: ~15 min [Althaus *et al.* 1986].

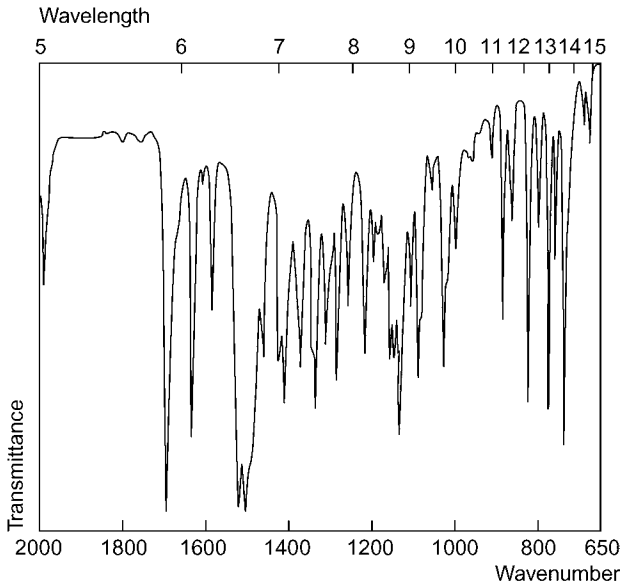
High Performance Liquid Chromatography System HX—RI 451 (metaclazepam), RI 393 (M dinor), RI 425 (M nor).

Column: ODS Hypersil (30 + 100 × 4.0 mm i.d., 5 µm). Mobile phase: water : methanol (350 : 650) plus 1 mL 40% methylamine, flow rate 1.2 mL/min. UV detection (λ = 230 nm). Retention time: 11.6 min [Althaus *et al.* 1986]

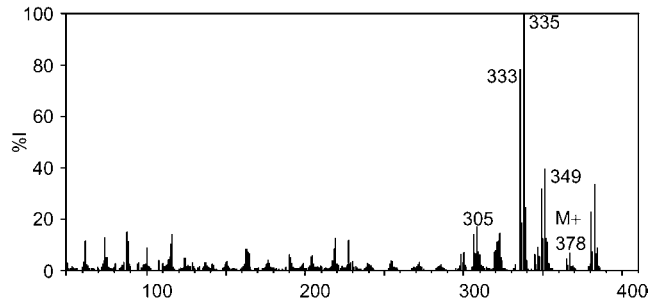
Ultraviolet Spectrum Aqueous methanol—464, 380, 242 nm; aqueous methanolic hydrochloride (0.1 mol/L)—464 (A^1_1 = 151), 255 (A^1_1 = 561) nm; aqueous methanolic sodium hydroxide—377 (A^1_1 = 58), 274 (A^1_1 = 196), 236 (A^1_1 = 535) nm.



Infrared Spectrum Principal peaks at wavenumbers 1646, 1482, 772, 735, 2365, 819 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 335, 333, 349, 305, 378 (M nor).



Quantification

Note For a thin-layer chromatography method, see Althaus *et al.* [1986].

Plasma **LC-MS** Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 0.025 mg/L (SIM), limit of detection, 0.025 mg/L (scan) [Kratzsch *et al.* 2004].

Urine **LC-MS** Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Disposition in the Body Metaclazepam is rapidly metabolised yielding *N*-1 desmethyl and *O*-desmethyl benzapines as the main active products [Borchers *et al.* 1984]. No accumulation after repetitive administration is observed. Small amounts are distributed in breast milk.

Dose The oral daily dose is 15 mg.

Althaus W *et al.* (1986). Analytical profile of metaclazepam. *Arzneimittelforschung* 36: 1302–1306.

Borchers F *et al.* (1984). Metabolism and pharmacokinetics of metaclazepam (Talis), Part III: Determination of the chemical structure of metabolites in dogs, rabbits and men. *Eur J Drug Metab Pharmacokinet* 9: 325–346.

Fernandez-Arciniega MA, Hernandez L (1985). Analytical properties of metaclazepam, a new 1,4-benzodiazepine. *Farmaco Prat* 40: 81–86.

Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.

Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography–mass spectrometry. *J Chromatogr* 422: 85–101.

Metadoxine

Treatment of Alcohol Poisoning

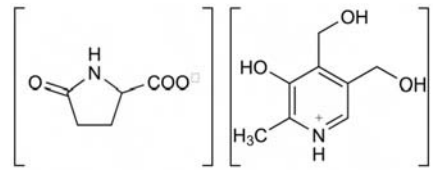
$C_{13}H_{18}N_2O_6$ = 298.3

CAS—74536-44-0

IUPAC Name 3-Hydroxy-4,5-di(hydroxymethyl)-2-methylpyridine-5-oxo-2-pyrrolidinecarboxylaten

Synonyms Pyridoxine pyrrolidonecarboxylate; pyridoxol L, 2-pyrrolidone-5-carboxlate.

Proprietary Name *Metadoxil*



Chemical Properties A white, odourless, crystalline powder with acidic taste. Mp 96° to 98°. Soluble in water.

Dose Two 500 mg tablets daily; 300 to 600 mg daily by IM or IV administration.

Metadrenaline

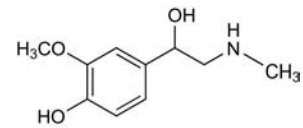
Catecholamine Metabolite

$C_{10}H_{15}NO_3$ = 197.2

CAS—5001-33-2

IUPAC Name 4-[1-Hydroxy-2-(methylamino)ethyl]-2-methoxyphenol

Synonyms 4-Hydroxy-3-methoxy-α-(methylaminomethyl)benzenemethanol; metanephrine.

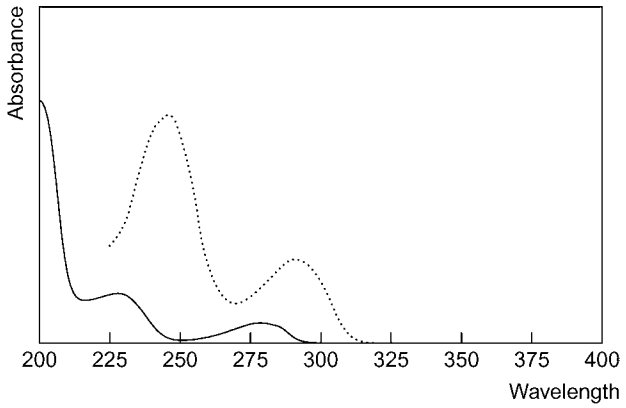


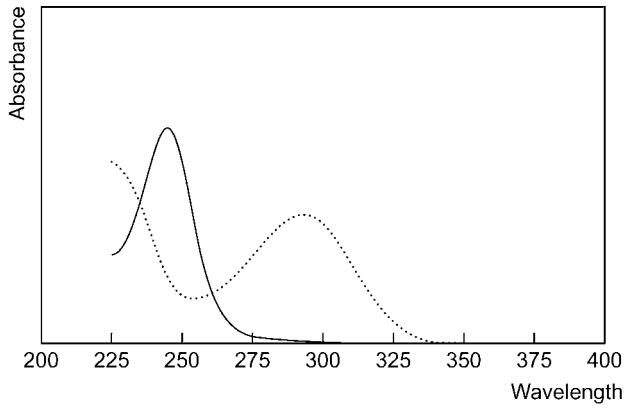
Chemical Properties Log *P* (octanol/water), −0.6.

Colour Tests Mandelin's test—green; Marquis test—orange→violet-brown.

Thin-layer Chromatography System TA—R_f 0.21 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—277 nm; aqueous alkali—245, 291 nm.





Quantification See Normetadrenaline.
Disposition in the Body Metadrenaline is a metabolite of adrenaline.

Metaldehyde

Molluscicide
$(C_2H_4O)_x$
CAS—9002-91-9

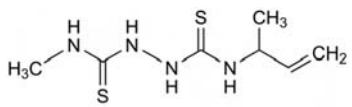
IUPAC Name 2,4,6,8-Tetramethyl-1,3,5,7-tetraoxocane
Proprietary Names It is an ingredient of many proprietary preparations used for the destruction of slugs. As a compressed fuel, it is known as *Meta*.
Chemical Properties A polymer of acetaldehyde. A white crystalline solid which burns readily with a non-luminous carbon-free flame and sublimes at 112°. In the presence of acids it decomposes slowly to acetaldehyde. Practically insoluble in water; soluble in benzene and chloroform; sparingly soluble in alcohol and ether.
Colour Test Add 1 drop of sulfuric acid to a small amount of solid, then add a trace of pyrocatechol—violet-red.
Gas Chromatography System GA—RI 1020.
Infrared Spectrum Principal peaks at wavenumbers 1094, 1113, 1163, 1074, 975, 1205 cm^{-1} (KBr disk).
Quantification
Plasma **GC** [Booze, Oehme 1985].
Urine **GC** [Booze, Oehme 1985].
Disposition in the Body
Toxicity Metaldehyde probably decomposes slowly in the body to acetaldehyde which is further oxidised. Fatalities have occurred after the ingestion of about 3 g.

Booze TF, Oehme FW (1985). Gas chromatographic analysis of metaldehyde in urine and plasma. *J Anal Toxicol* 9: 172–173.

Metallibure

Hypothalamus and Pituitary Suppressant
$C_7H_{14}N_4S_2 = 218.3$
CAS—926-93-2

IUPAC Name 1-But-3-en-2-yl-3-(methylcarbamothioylamino)thiourea
Synonym *N*-Methyl-*N*′-(1-methyl-2-propenyl)-1,2-hydrazinedicarbothioamide

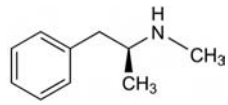


Chemical Properties A white powder. Mp 198° to 200°, with decomposition. Practically insoluble in water and chloroform; soluble 1 in 100 of acetone and 1 in 200 of methanol; soluble in pyridine. Log *P* (octanol/water), 0.3.
Caution Protective gloves should be worn when handling metallibure.
Colour Tests Nessler's reagent (100°)—black; palladium chloride—orange.
Thin-layer Chromatography System TA—R_f 0.79 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—243 nm (A_1^1=1340b); aqueous alkali—293 nm.

Metamfetamine

Central Stimulant, Phenethylamine
$C_{10}H_{15}N = 149.2$
CAS—537-46-2

IUPAC Name (2*S*)-*N*-Methyl-1-phenylpropan-2-amine
Synonyms *d*-Deoxyephedrine; desoxyephedrine; (α*S*)-*N*,α-dimethylbenzeneethanamine; methamphetamine; methylamfetamine; methylamphetamine; phenylmethylaminopropane.
Note Metamfetamine in a smokeable and snortable form: crank; crystal; crystal meth; ice; meth; speed.
Proprietary Name *Norodin*

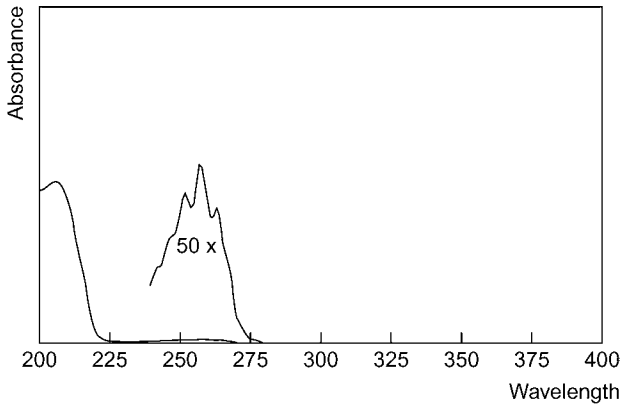


Chemical Properties A clear, colourless, slowly volatile, mobile liquid. Bp ~214°. Slightly soluble in water; miscible with ethanol, chloroform and ether. pK_{a1} 10.1 [Huestis, Cone 2007], pK_{a2} 9.87 [Fan *et al.* 2005]. Log *P* (octanol/water), 2.07 [Fan *et al.* 2005]. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].

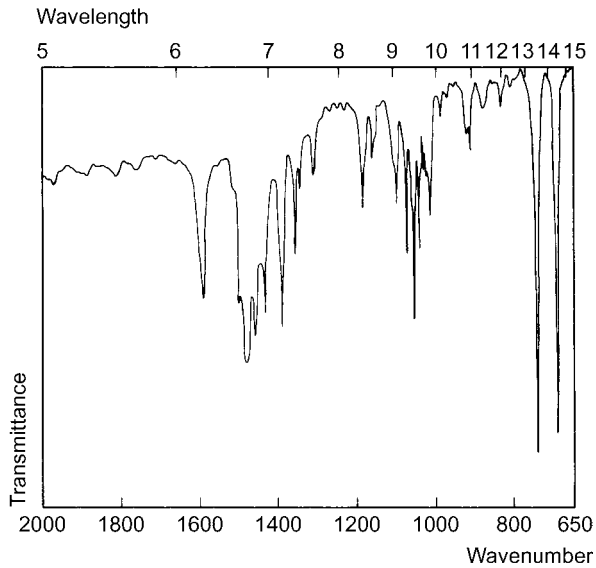
Metamfetamine Hydrochloride

$C_{10}H_{15}N,HCl = 185.7$
CAS—51-57-0
IUPAC Name (2*S*)-*N*-methyl-1-phenylpropan-2-amine hydrochloride
Proprietary Names *Amphedroxyn*; *Desfedrin*; *Desoxyfed*; *Desoxyn*; *Destim*; *Doxephrin*; *Drinalfa*; *Gerobit*; *Hiropon*; *Isophen*; *Madrine*; *Methampex*; *Methedrine*; *Methylisomyn*; *Pervitin*; *Soxysympamine*; *Syndrox*; *Tonedron*.
Chemical Properties White crystals or crystalline powder. Mp 170° to 175°. Soluble 1 in 2 of water, 1 in 4 of ethanol and 1 in 5 of chloroform; practically insoluble in ether. Log *P* (octanol/water), −0.66 [Meylan, Howard 1995].

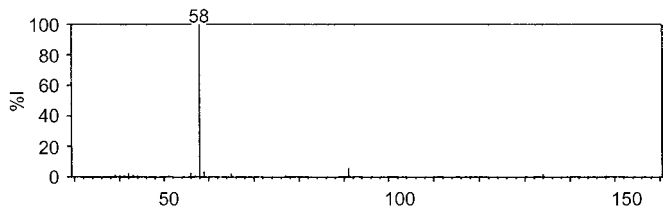
Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.31; system TB—R_f 0.28; system TC—R_f 0.13; system TE—R_f 0.42; system TL—R_f 0.05; system TAE—R_f 0.09; system TAF—R_f 0.63; system TAJ—R_f 0.00; system TAK—R_f 0.03; system TAL—R_f 0.45 (acidified iodoplatinate solution—positive; acidified potassium permanganate—positive; Dragendorff spray—positive; Marquis test—brown; ninhydrin (spray)—positive).
Gas Chromatography System GA—metamfetamine RI 1175, amfetamine RI 1125, M (4-OH-)(pholedrine) RI 1885, M (OH-methoxy-) RI 1810; system GB—metamfetamine RI 1200, amfetamine RI 1150; system GC—metamfetamine RI 1722, amfetamine RI 1536; system GF—metamfetamine RI 1335, amfetamine RI 1315; system GAD—metamfetamine RT 8.5 min, amfetamine RT 7.5 min.
High Performance Liquid Chromatography System HA—metamfetamine *k* 2.0, amfetamine *k* 0.9; system HB—metamfetamine *k* 10.52, amfetamine *k* 8.48; system HC—metamfetamine *k* 2.07, amfetamine *k* 0.98; system HX—RI 262; system HY—RI 216; system HZ—RT 2.4 min; system HAA—RT 8.4 min.
Ultraviolet Spectrum Aqueous acid—252, 257 (A_1^1= 12.1a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 747, 698, 1060, 1491, 1590, 1085 cm^{-1} (metamfetamine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 91, 59, 134, 65, 56, 42, 57 (metamfetamine); 44, 91, 40, 42, 65, 45, 39, 43 (amfetamine).



Quantification

Blood **GC** Limit of detection, 220 µg/L [Sun *et al.* 2007].

GC-MS Column: HP-5MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 60° for 1 min to 220° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 5.3 min for metamfetamine, 4.66 min for amfetamine, 7.14 min for 3,4-methylenedioxymethamfetamine (MDMA) [Gunn *et al.* 2010]. Column: DB-5HT (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2.5 to 3.5 mL/min. Temperature programme: 130° for 0.1 min to 230° at 70°/min for 0.17 min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L for metamfetamine and amfetamine [Gunnar *et al.* 2007]. EI ionisation, positive ion mode, SIM acquisition mode. Limit of detection, 1.5 µg/L for metamfetamine and 1.1 µg/L for amfetamine [Hasegawa *et al.* 2007]. Column: HP-5MS. SIM acquisition mode [Rasmussen *et al.* 2006]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 1 min to 300° at 10°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5.0 ng/g for metamfetamine and amfetamine [Nishida *et al.* 2002; Nishida *et al.* 2003] Column: PTE-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.4 mL/min. Temperature programme: 60° for 3 min to 280° at 20°/min for 3 min. EI ionisation, SIM acquisition mode. Limit of detection, 0.5 ng/g for metamfetamine and amfetamine [Okajima *et al.* 2001]. See also Nagasawa *et al.* [1996], Namera *et al.* [2000] and Sato and Mitsui [1997].

LC-MS Column: Agilent HPLC 1100 with a Varian Pursuit 3 C_{18} column (3 × 100 mm, 3 µm). Mobile phase: methanol-ammonium formate. Limit of quantification, 0.0005–0.01 mg/kg for metamfetamine and other drugs of abuse [Bjork *et al.* 2010]. Column: Nucleodur Sphinx RP. Mobile phase: 10 mmol/L ammonium formate buffer and acetonitrile. Limit of quantification, 2.5 µg/L for metamfetamine and related drugs, limit of detection, 0.05–0.5 µg/L for metamfetamine and related drugs, [Fernández Mdel *et al.* 2009]. Column: BEH C_{18} (50 × 2.1 mm i.d.). Mobile phase: aqueous pyrrolidine : methanol (52 : 48), flow rate 0.4 mL/min. ESI, positive ion mode, SIR acquisition mode. Retention time: 1.81 min for metamfetamine, 1.2 min for amfetamine [Apollonio *et al.* 2006]. Column: Uptispher ODB C_{18} (150 × 2.1 mm, 5 µm). Mobile phase: acetonitrile and 2 mmol/L formate buffer (pH 3.0). Limit of quantification, 0.1 µg/L for metamfetamine and other amfetamines [Chèze *et al.* 2007].

Plasma **GC** Column: HP-5 5% phenylmethyl (25 × 0.32 m, 0.52 µm). Carrier gas: He, 2.5 mL/min. Temperature programme: 70° to 120° at 30°/min to 185° at 5°/min to 300° at 70°/min for 1.5 min. NPD. Limit of detection, 1 µg/L [Cheung *et al.* 1997]. Column: HP Ultra-1 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 110° for 0.5 min to 150° at 5°/min for 7.25 min to 300° at 50°/min for 3 min. Retention time: 11.72 min for metamfetamine, 9.76 min for amfetamine. Limit of detection, <10 µg/L for metamfetamine and amfetamine [Jacob *et al.* 1995].

GC-MS See Blood [Gunn *et al.* 2010]. Limit of quantification, 2.5 µg/L for metamfetamine and metabolites [Huestis, Cone 2007]. Column: 5% phenylmethylsiloxane HP-5MS (30 m × 0.25 mm i.d., 250 nm. Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 180° at 30°/min to 230° at 5°/min to 310° at 30°/min. NICI, SIM acquisition mode. Limit of detection, <1 µg/L [Peters *et al.* 2003a]. Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 µg/L for metamfetamine and related drugs [Peters *et al.* 2003b]. Column: HP-5MS (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 180° at 30°/min to 230° at 5°/min to 310° at 30°/min. NICI, SIM acquisition mode. Limit of quantification, 5 µg/L for metamfetamine and amfetamine, limit of detection, 1 µg/L for metamfetamine and amfetamine [Peters *et al.* 2002].

HPLC Column: Daisopak SP-120-5-ODS-BP (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L citrate buffer (pH 4.0, 55 : 45), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 325 nm, λ_{em} = 430 nm). Limit of detection, 0.87 µg/L for metamfetamine and 0.46 µg/L for amfetamine [Nakashima *et al.* 2003].

LC-MS Column: Alltima C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L formic acid (10 : 90 for 2 min to 25 : 75 in 16 min to 35 : 65 in 0.1 min to 100 : 0 in 12 min. TIS, positive ion mode, MRM acquisition mode. Retention time: 14 min. Limit of quantification, 1.2 µg/L; limit of detection, 0.2 µg/L [Sergi *et al.* 2009]. Column: CAPCELL PAK SCX UG 80 (250 × 1.5 mm i.d.). Mobile phase: 25 mmol/L ammonium acetate (pH 4.0) : acetonitrile (3 : 7), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection, 50 µg/L for metamfetamine and related drugs and metabolites [Kuwayama *et al.* 2008]. Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (75 : 25), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.2 µg/L [Wood *et al.* 2003].

Serum **GC-MS** See Blood [Gunn *et al.* 2010]. See Plasma [Peters *et al.* 2002]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° to 120° at 30°/min to 180° at 20°/min to 250° at 30°/min for 0.5 min. Limit of detection, 50 ng/L for metamfetamine and 80 ng/L for amfetamine [Lee *et al.* 2000]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 200° at 20°/min to 240° at 5°/min to 310° at 30°/min for 6 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 6.1 min for metamfetamine, 5.3 min for amfetamine. Limit of quantification, 1.8 µg/L for metamfetamine, 4.9 µg/L for amfetamine, limit of detection, 1.4 µg/L for metamfetamine, 1.5 µg/L for amfetamine [Weinmann *et al.* 2000].

Note For a fluoroimmunoassay, see Kimura *et al.* [2005].

Urine **TLC** Limit of detection, for *p*-hydroxymetamfetamine, 10 ng [Kato *et al.* 2005].

GC Limit of detection, 10 µg/L [Wang *et al.* 2008]. Column: HP-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° for 1.5 min to 300° at 12.5 min for 7 min. FID. Limit of quantification, 0.1 mg/L, limit of detection, 0.05 mg/L [Shakleya *et al.* 2006]. Column: AT-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 1 min to 280° at 20°/min for 5 min. FID. Limit of detection, 30 µg/L for metamfetamine and amfetamine [Raikos *et al.* 2003]. Column: CB-5 methylphenylpolysiloxane (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 110° to 140° at 10°/min to 20° at 30°/min for 3 min. NPD. Limit of detection, ~35 µg/L for metamfetamine and amfetamine [Taylor *et al.* 1989]. Column: 2% Thermon-3000 on Chromosorb W (AW-DMCS) 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 200°. ECD. Retention time: 3.3 min for metamfetamine and 6.9 min for amfetamine. Limit of detection, 10 µg/L for metamfetamine and amfetamine [Terada *et al.* 1982]. See Plasma [Cheung *et al.* 1997; Jacob *et al.* 1995].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 127 kPa. Temperature programme: 70° for 1 min to 300° at 20°/min for 2 min. EI ionisation at 70 eV, full scan mode. Limit of detection, 5 µg/L for metamfetamine and MDMA, 10 µg/L for amfetamine and methylenedioxiamfetamine (MDA) [Nakamoto *et al.* 2010]. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 160° at 60°/min for 0.5 min to 210° at 50°/min for 0.5 min to 250° at 40°/min for 0.5 min to

280° at 30°/min for 2 min. NCI. Limit of quantification, 43.5 ng/L for metamfetamine and 4 ng/L for amfetamine, limit of detection, 13.04 ng/L for metamfetamine and 1.20 ng/L for amfetamine [Chung *et al.* 2009]. Column: DB-1MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 2 min to 140° at 20°/min to 190° at 10°/min to 250° at 20°/min for 1 min. SIM acquisition mode. Limit of detection, 0.1 μg/L for metamfetamine and 0.5 μg/L for amfetamine [He *et al.* 2009]. Column: HP-5MS (20 m × 250 μm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 1 min to 190° at 20°/min for 2 min to 250° at 5°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, ≤7.7 μg/L, limit of detection, ≤2.3 μg/L [Aasim *et al.* 2008]. See Plasma [Huestis, Cone 2007]. Column: DB-5MS (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2 mL/min. Temperature programme: 60° for 1 min to 200° at 20°/min to 300° at 40°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.08 μg/L for metamfetamine and 0.1 μg/L for amfetamine [Kumazawa *et al.* 2007]. See also Miranda *et al.* [2007], Saito *et al.* [2007], Fuh *et al.* [2006], Fujii *et al.* [2006], Lua *et al.* [2006], Maresová *et al.* [2006], Holler *et al.* [2005], Klette *et al.* [2005], Wang *et al.* [2005], Dasgupta, Spies [1998], Paul *et al.* [2004], Huang *et al.* [2002], Namera *et al.* [2002], Stout *et al.* [2002], Yamada *et al.* [2002], Wu *et al.* [2001], Jurado *et al.* [2000], Hensley, Cody [1999], Myung *et al.* [1998], Dallakian *et al.* [1996], Meatherall [1995], Platoff *et al.* [1992], Gan *et al.* [1991], Taylor *et al.* [1989]. See Blood. Limit of detection, 5.0 μg/L for metamfetamine and amfetamine [Nishida *et al.* 2003; Nishida *et al.* 2002].

HPLC Column: CAPCELL PAK SXC. Mobile phase: 0.1 mol/L borate buffer (pH 9.4). UV detection (λ = 210 nm). Limit of quantification, 0.005 mg/L [Kumihashi *et al.* 2007]. Column: Symmetryshield RP8 (150 × 4.6 mm i.d., 3.5 μm). Mobile phase: water : acetonitrile (90 : 10) with 25 mmol/L phosphate (pH 3.0), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 0.3 μg/L for metamfetamine and amfetamine [He *et al.* 2007]. Column: C_8 Genesis (150 × 4.6 mm, 5 μm). Mobile phase: acetonitrile : phosphate buffer (pH 2.5) containing 0.3% TEA (12 : 88). Metamfetamine and related drugs [Bugamelli *et al.* 2006]. Column: Kromasil ODS (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.02 mol/L disodium hydrogen phosphate-0.02 mol/L methanesulfonic acid (pH 2.9, 12.5 : 87.5), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 1.4–4.0 μg/L for metamfetamine and related drugs [Fan *et al.* 2005]. Column: CAPCELL PAK C_{18} UG 120 S5 (250 × 1.5 mm i.d., 5 μm). Mobile phase: 0.1 mol/L Tris-HCl buffer (pH = 7.0) : acetonitrile (45 : 55), flow rate 0.2 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 440 nm). Limit of detection, 0.67 fmol/injection for metamfetamine and 0.14 fmol/injection for amfetamine [Al Dirbashi *et al.* 2000a]. See also al Dirbashi *et al.* [1999a]; al Dirbashi *et al.* [1998a]; al Dirbashi *et al.* [1998b]; al Dirbashi *et al.* [1997]; Makino *et al.* [1999], Foster *et al.* [1998], Campins-Falcó *et al.* [1996],Campins Falcó [1995], Molins Legua *et al.* [1995], Hayakawa *et al.* [1993].

LC-MS Column: Nucleodur Sphinx RP. Mobile phase: 10 mmol/L ammonium formate buffer : acetonitrile. Limit of quantification, 25 μg/L, limit of detection, 0.25–2.5 μg/L, for metamfetamine and related drugs [Fernández Mdel *et al.* 2009]. Column: C_{18} (100 × 2 mm i.d., 3 μm). Mobile phase: 25 mmol/L formic acid-1% acetonitrile : 25 mmol/L formic acid-90% acetonitrile (91 : 9 to 80 : 20 in 4 min to 0 : 100 in 3 min to 91 : 9 for 5 min), flow rate 0.3 mL/min. ESI, SRM acquisition mode. k' 3.1 for metamfetamine, 2.8 for amfetamine. Limit of quantification, 28 μg/L for metamfetamine, 143 μg/L for amfetamine, limit of detection, 8 μg/L for metamfetamine, 43 μg/L for amfetamine [Andersson *et al.* 2008]. Column CAPCELL PAK C_{18} MG II (150 × 2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium formate (pH 4) acetonitrile, flow rate 230 μL/min. Limit of detection, <1.95 μg/L for metamfetamine and related drugs [Kim *et al.* 2008]. See Plasma [Kuwayama *et al.* 2008]. Column: Atlantis dC_{18} (100 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile : ammonium formate buffer (pH 3.0, 0 : 100 for 1 min to 50 : 50 in 10 min for 2.5 min to 0 : 100 in 0.5 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 8.5 min. Limit of quantification, 0.5 μg/L for metamfetamine and 1–2 μg/L for amfetamine, limit of detection, 0.5 μg/L for metamfetamine and amfetamine [Concheiro *et al.* 2007a; Concheiro *et al.* 2007b]. Column: Alltech Platinum EPS C_{18} (30 × 2.1 mm i.d., 1.5 μm). Mobile phase: 0.01 mol/L ammonium formate (pH 3) : acetonitrile (77 : 23), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 0.05 mg/L for metamfetamine and amfetamine [Cheng *et al.* 2007]. See Blood [Chèze *et al.* 2007]. See also Katagi *et al.* [1996], Katagi *et al.* [2000] and Wu, Fuh [2005].

CE Column: fused silica capillary. Mobile phase: 50 mmol/L phosphate buffer (pH 3) : acetonitrile (80 : 20) and 100 mmol/L SDS. Limit of detection, 20 μg/L for metamfetamine and 15 μg/L for amfetamine and *p*-hydroxymetamfetamine [Cheng *et al.* 2006]. Column: fused silica capillary. Mobile phase: 25 mmol/L phosphate buffer (pH 2.5) : methanol (80 : 20) and 100 mmol/L SDS. Limit of detection, 15 μg/L [Lin *et al.* 2006].

Note For a comparison of 6 immunoassays for detection of metamfetamine and other amfetamines, see Verstraete, Heyden [2005]; for a fluoroimmunoassay in serum, see Kimura *et al.* [2005]; for a radioimmunoassay see Inayama *et al.* [1980].

Bile **LC-MS** See Plasma [Kuwayama *et al.* 2008].

Oral Fluid **GC-MS** Comparison with immunoassay [Crooks, Brown 2010]. See Plasma [Huestis, Cone 2007]. SIM acquisition mode. Amfetamine and related drugs [Scheidweiler, Huestis 2006].

LC-MS See Plasma. Limit of quantification, 0.4 μg/L, limit of detection, 0.2 μg/L [Sergi *et al.* 2009]. See Plasma [Wood *et al.* 2003].

Sweat **GC-MS** Column: DB-1 (10 m × 0.18 mm i.d.). Temperature programme: 100° to 240° at 20°/min to 300° at 50°/min. SIM acquisition mode. Retention time: 5.2 min for metamfetamine and 4.8 min for amfetamine. Limit of detection, 3.69 μg/L [Fay *et al.* 1996].

Hair **GC** Column: CBJ-17. NPD. Limit of detection, 0.4 μg/g for metamfetamine and 0.1 μg/g for amfetamine [Koide *et al.* 1998].

GC-MS Limit of detection, <0.028 μg/g for metamfetamine and other amfetamines [Kim *et al.* 2010]. Limit of quantification, 0.24–0.46 μg/g for metamfetamine and other amfetamines, limit of detection, 0.07–0.14 μg/g for metamfetamine and other amfetamines [Johansen, Jornil 2009]. SIM acquisition mode. Limit of detection, ~50 ng/g for metamfetamine and other amfetamines [Meng *et al.* 2009]. PTV. Limit of quantification, 0.20 μg/g for metamfetamine and amfetamine [Miyaguchi *et al.* 2009]. Column: Uptispher ODB C_{18} (150 × 2.1 mm, 5 μm). Mobile phase: acetonitrile : 2 mmol/L formate buffer (pH 3.0). Limit of quantification, 5 ng/g for metamfetamine, *N*-methyl-*p*-methoxyamfetamine (MMA), methylenedioxyethylamfetamine (MDEA) and methylbenzodioxolylbutanamine (MBDB), and 14.7 ng/g for amfetamine [Chèze *et al.* 2007]. See also Ando *et al.* [2004], Gottardo *et al.* [2007], Kim *et al.* [2005], Kintz *et al.* [1995], Martins *et al.* [2006], Miki *et al.* [2008], Pujadas *et al.* [2003], Skender *et al.* [2002], Villamor *et al.* [2005], Wu *et al.* [2001] Wu *et al.* [2008] and Zhu *et al.* [2007]. See Blood. Limit of detection, 0.25 ng/mg for metamfetamine and amfetamine [Nishida *et al.* 2003].

HPLC See Plasma. Mobile phase: methanol : acetonitrile: 0.01 mol/L citrate buffer (pH 4.0), flow rate 1.1 mL/min. Limit of detection, 0.12 μg/g for metamfetamine and 0.08 μg/g for amfetamine [Nakashima *et al.* 2003]. Fluorescence detection [al Dirbashi *et al.* 1999b; Al Dirbashi *et al.* 2000b].

LC-MS Limit of quantification, 0.5 μg/g for metamfetamine and other amfetamines, limit of detection, 0.1 μg/g for metamfetamine and other amfetamines [**Tabernero *et al.* 2009**]. ESI. Metamfetamine and amfetamine enantiomers and selegiline and metabolites [Nishida *et al.* 2006].

Note For a review of methods for the analysis of metamfetamine and its metabolites in hair, see Takayama *et al.* [2003].

Other **HPLC** Clothing. UV detection (λ = 330 nm) or fluorescence detection (λ_{ex} = 440 nm). Limit of detection, 37.3 pg (fluorescence) and 0.4 pg (UV) for metamfetamine and amfetamine [al Dirbashi *et al.* 2001].

Note For a review of the determination of metamfetamine enantiomers, see Jirovský *et al.* [1998].

Disposition in the Body Metamfetamine is readily absorbed after oral administration. Approximately 70% of a dose is excreted in the urine in 24 h. Under normal conditions, up to 43% of a dose is excreted as unchanged drug, up to 15% as 4-hydroxymetamfetamine, and ~5% as amfetamine, the major active metabolite. A number of other metabolites have been identified. Excretion of unchanged drug is dependent on the urinary pH, being increased in acidic urine and greatly reduced (to ~2% of a dose) if the urine is alkaline.

Metamfetamine is a metabolite of benzfetamine and selegiline.

Therapeutic Concentration In plasma, usually in the range 0.01–0.05 mg/L.

Following a single oral dose of 12.5 mg metamfetamine hydrochloride to 10 subjects, a mean peak blood concentration of ~0.02 mg/L was attained in ~2 h [Driscoll *et al.* 1971].

Average concentrations of 111 and 281 μg/L (metamfetamine) and 4 and 15 μg/L (amfetamine) were found in breast milk 24 h after IV administration of amfetamines to 2 breastfeeding mothers [Bartu *et al.* 2009].

Toxicity The estimated minimum lethal dose is 1 g, but fatalities attributed to metamfetamine are rare.

The following postmortem tissue concentrations, mg/L or μg/g, were reported in a 25-year-old female found dead after the IV abuse of metamfetamine followed by ingestion of ~1.5 g:

	Metamfetamine	Amfetamine
Blood	43	0.35
Brain	101.8	0.86
Kidney	75.5	0.72
Liver	174.6	1.30
Urine	277.5	10.0

[Kojima *et al.* 1984].

A 37-year-old man who died following mixed drug abuse had the following postmortem metamfetamine concentrations: blood 0.7 mg/L, bile 21.7 mg/L, urine 32.0 mg/L, gastric contents 2.9 mg/L, liver 2.2 μg/g and brain 2.7 μg/g [Moore *et al.* 1996].

Of 146 deaths in which metamfetamine was detected in the blood, 52 were directly attributed to the drug and 92 were classified as drug-related; a large proportion of the deaths resulted from homicidal (27%) or suicidal (15%) violence. The metamfetamine blood concentration in the 92 drug-related deaths was reported to be in the range 0.05–9.30 mg/L (median, 0.42), with 90% of the population having concentrations <2.20 mg/L. In 17 metamfetamine-related traffic deaths, the blood concentration was reported to be 0.05–2.60 mg/L, with most deaths occurring at concentrations >0.5 mg/L [Logan *et al.* 1998].

In 413 deaths where metamfetamine was detected, blood levels of metamfetamine and amfetamine were 2.08 mg/L and 0.217 mg/L, respectively, in cases where metamfetamine was related to the cause of

death. These levels were not significantly different from those where metamfetamine use was not related to the cause of death (1.78 and 0.19 mg/L, respectively) [Karch *et al.* 1999].

A pilot who died in a civil aircraft accident had postmortem blood levels of metamfetamine (1.13 mg/L) and amfetamine (0.022 mg/L). Urine levels of metamfetamine and amfetamine were 59.2 and 1.50 mg/L, respectively. Metamfetamine was distributed throughout the body, including the brain. Metamfetamine levels in gastric contents were 575-fold higher than amfetamine levels [Chaturvedi *et al.* 2004].

A 20-year-old woman who had drugs enclosed in plastic bags in her vagina, developed metamfetamine toxicity. Serum levels of metamfetamine and amfetamine were 3.1 and 0.11 mg/L, respectively [Kashani, Ruha 2004].

In another case, a man who died within 1 h of an IV injection of metamfetamine had a femoral blood level of 1.5 mg/L. Levels of 132 µg/g were found in the first three 2 cm hair samples and overall the levels in hair provided evidence of chronic metamfetamine use for >8 months [Beránková *et al.* 2005].

A man who collapsed shortly after an IV injection of metamfetamine was in a coma for 9 days before he died. More than 9 days after administration, metamfetamine was not detected in postmortem blood, urine or liver, but levels in hair were 1.1 µg/g [Ago *et al.* 2006].

Another case of 'body stuffing' in which a 25-year-old man had delayed onset toxicity after ingesting metamfetamine wrapped in plastic has been reported [Hendrickson *et al.* 2006].

There is a further report of 3 men in their thirties who attempted to smuggle metamfetamine 498, 292, and 73 g by 'body packing' the drug as packages each containing ~14 g wrapped in plastic film and Scotch tape. One man died from acute metamfetamine poisoning from ~20 g metafetamine that had leaked from the packages into his stomach. The plasma level of metamfetamine was 8.6 mg/L ~17 h before he died. Postmortem levels of metamfetamine and amfetamine in cardiac blood were 63.5 and 1.2 mg/L, respectively. Urine levels were 4518 and 72.4 mg/L, respectively; gastric contents were 8490 and 16.9 mg/L, respectively [Takekawa *et al.* 2007].

In a retrospective review of metamfetamine-related deaths, the median blood level was 0.2 mg/L (range, 0.02–15); other drugs, mainly bezodiazepines and morphine, were identified in 89% of cases [Kaye *et al.* 2008].

A woman in her late thirties who was hospitalised for 13 days following a road traffic accident suffered a cardiopulmonary arrest and died. At postmortem it was concluded that her injuries were not the cause of death. Postmortem cardiac blood levels of metamfetamine and amfetamine were 0.178 and 0.051 mg/L, respectively. Metamfetamine was also found in hair samples indicating metamfetamine use for at least 3 months before death [Ago *et al.* 2009].

A 49-year-old man who believed he was being followed by the police swallowed an '8-ball of meth' (one-eighth of an ounce of metamfetamine, equivalent to ~3 g). Levels of 3 mg/L metamfetamine were found in blood samples taken in hospital; postmortem femoral blood levels were 30 mg/L [Kiely *et al.* 2009].

There is another report of a fatality in a 39-year-old man who at postmortem was found to have 158 columned-shaped packages wrapped in tinfoil and plastic containing a total of 390 g metamfetamine in his alimentary tract. One package in his stomach had ruptured and was empty. Postmortem levels of metamfetamine were cardiac blood 24.5 mg/L, urine 191 mg/L, liver 116 mg/L and gastric contents 1045 mg/L [Li *et al.* 2009].

For other reports of fatalites associated with metamfetamine, see Logan *et al.* [1996], Molina, Jejurikar [1999], Sribanditmongkol *et al.* [2000] and Yamamoto *et al.* [1991]. For a report of recovery after overdose with ~2.3 g metamfetamine administered IV, see Buffum, Shulgin [2001].

Half-life Plasma half-life, ~9 h.

Dose 2.5 to 25 mg of metamfetamine hydrochloride daily, orally; 15 to 20 mg IM, or 10 to 15 mg IV.

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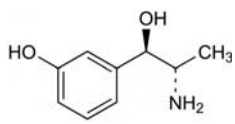
Metaraminol

Sympathomimetic
$C_9H_{13}NO_2 = 167.2$
CAS—54-49-9

IUPAC Name 3-[(1*R*,2*S*)-2-Amino-1-hydroxypropyl]phenol
Synonyms (α*R*)-α-[(1*S*)-1-Aminoethyl]-3-hydroxybenzenemethanol; hydroxynorephedrine.



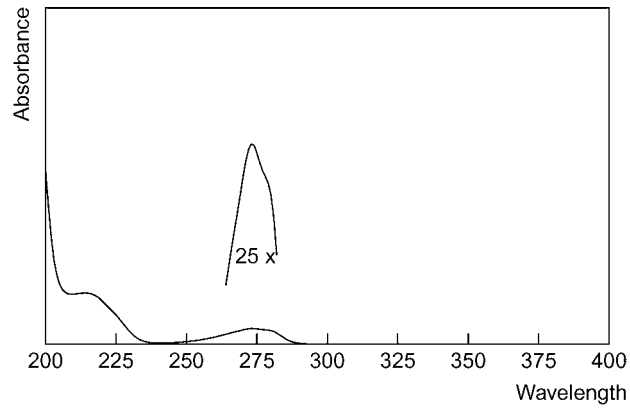
Chemical Properties pK_a 8.6. Log *P* (octanol/water), −0.3.

Metaraminol Tartrate

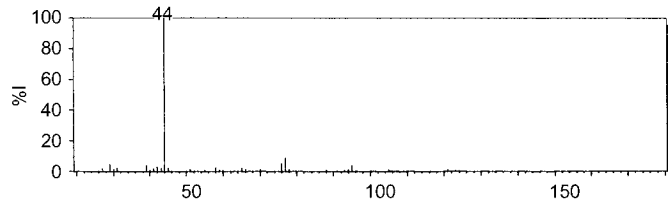
$C_9H_{13}NO_2,C_4H_6O_6 = 317.3$
CAS—33402-03-8

Synonyms Metaradrini bitartras; metaraminol acid tartrate; metaraminol bitartrate.
Proprietary Names *Aramin(e)*; *Levicor.*
Chemical Properties A white crystalline powder. Mp 176° to 177°. Soluble 1 in 3 of water and 1 in 100 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Folin-Ciocalteu reagent—blue; Mandelin's test—blue.
Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.18; system TL—R_f 0.24; system TAE—R_f 0.13; system TAF—R_f 0.76; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.20 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—metaraminol-AC_3 RI 2065.
High Performance Liquid Chromatography System HA—*k* 0.9; system HX—RI 84.
Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=110a); aqueous alkali—238 (A^1_1=533b), 292 nm (A^1_1=180b).



Infrared Spectrum Principal peaks at wavenumbers 1263, 1304, 1216, 1591, 1136, 1063 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 44, 77, 76, 29, 95, 39, 58, 42.

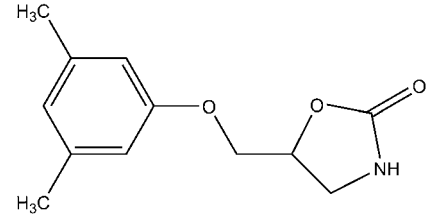


Dose The equivalent of 2 to 10 mg of metaraminol, subcutaneously or intramuscularly.

Metaxalone

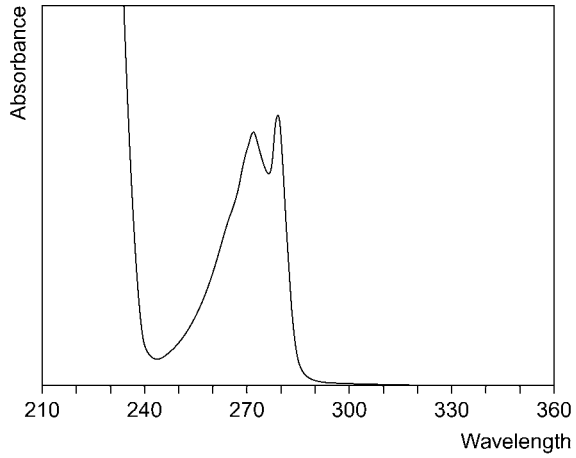
Oxazolidine, Skeletal Muscle Relaxant
$C_{12}H_{15}NO_3 = 221.3$
CAS—1665-48-1

IUPAC Name 5-[(3,5-Dimethylphenoxy)methyl]-1,3-oxazolidin-2-one
Synonyms AHR-438; 5-(3,5-xylyloxymethyl)oxazolidin-2-one.
Proprietary Name *Skelaxin*

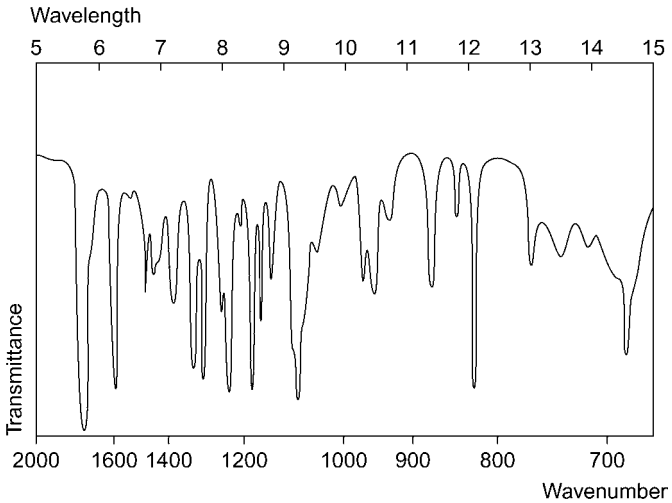


Chemical Properties White, odourless, bitter crystalline powder. Mp 121° to 125°. Freely soluble in chloroform, soluble in alcohol and propylene glycol, very slightly soluble in water [Kleber *et al.* 1964].
Colour Test Add 2 mL of a 1 in 1000 solution of vanillin in sulfuric acid solution (65 in 100) to 1 mL of a 1 mg/mL metaxalone solution in sulfuric acid solution—A cherry-red solution develops in approx. 10 min [Kleber *et al.* 1964].

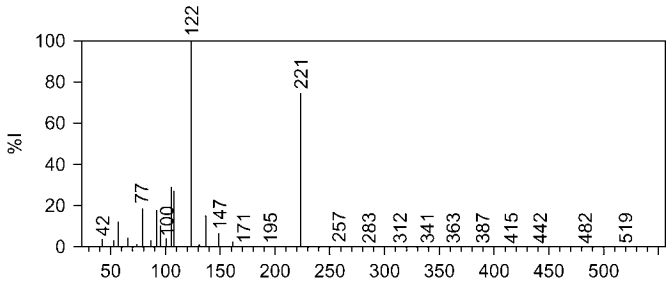
Ultraviolet Spectrum Alcohol—280, 272 nm [Kleber *et al.* 1964].



Infrared Spectrum See Kleber *et al.* [1964].



Mass Spectrum Principal ions at *m/z* 122, 221, 105, 91 [Poklis *et al.* 2004].



Quantification

Blood **GC** Column: DB-5 5% phenylmethyl silicone (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 1 min to 260° at 25°/min for 6 min. NPD. Limit of quantification, 25 µg/L [Moore *et al.* 2005]. Column: DB-5 (15 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 18 mL/min. Temperature programme: 120° to 300° at 20°/min. FID. Retention time: 6.4 min. Limit of quantification not reported [Poklis *et al.* 2004].

GC-MS Column: HP-5 (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 30°/min to 260° at 10°/min to 300° at 20°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of quantification, 2.5 mg/L [Moore *et al.* 2005]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 2 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, full scan mode. Limit of quantification not reported [Poklis *et al.* 2004]. Column: HP-1 methyl silicone (12.5 m × 0.2 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° to 280° at 20°/min. Full scan mode. Limit of quantification, 5 mg/L [Gruszecki *et al.* 2003].

Plasma **LC-MS** Column: C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: 0.5% glacial acetic acid : acetonitrile (20 : 80), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification not reported [Mistri *et al.* 2007]. Column: C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: 0.03% formic acid : acetonitrile (20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 50 µg/L [Nirogi *et al.* 2006].

Urine **GC** See Blood [Poklis *et al.* 2004].

GC-MS Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 2 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, full scan mode. Limit of quantification not reported [Poklis *et al.* 2004].

Vitreous Humour **GC** See Blood [Poklis *et al.* 2004].

GC-MS See Blood [Poklis *et al.* 2004].

Stomach Contents **GC** See Blood [Poklis *et al.* 2004].

GC-MS See Blood [Gruszecki *et al.* 2003; Poklis *et al.* 2004].

Bile **GC** See Blood [Poklis *et al.* 2004].

GC-MS See Blood [Poklis *et al.* 2004].

Brain **GC-MS** See Blood [Gruszecki *et al.* 2003].

Liver **GC** Column: DB-5 5% phenylmethyl silicone (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 1 min to 260° at 25°/min for 6 min. NPD. Limit of quantification, 5.0 mg/L [Moore *et al.* 2005]. See Blood [Poklis *et al.* 2004].

GC-MS See Blood. Limit of quantification, 5.0 mg/L [Moore *et al.* 2005]. See Blood [Gruszecki *et al.* 2003; Poklis *et al.* 2004].

Disposition in the Body Metaxalone is absorbed from the gastrointestinal tract, metabolised in the liver, and excreted in urine as metabolites. One metabolite is formed by the oxidation of 1 of the methyl groups to the carboxy analogue and appears in the urine as the glucuronide conjugate. Approximately 27% of the dose is excreted unchanged in urine and between 15 and 30% recovered is in faeces as metaxalone and its acid metabolite.

Therapeutic Concentration

A group of 6 healthy volunteers were administered an oral dose of 800 mg metaxalone under both fasted and fed conditions. Mean peak plasma concentrations were approx. 4.0 and 1.8 mg/L in the fed and fasted studies, respectively, achieved within 5 to 6 h. Similar results were obtained in another study where, after a single 400 mg metaxalone oral dose was administered to healthy volunteers under fasted conditions, a peak plasma concentration of approx. 866 µg/L was reached after 3.3 h. When the same dose was administered after a high-fat meal, the peak plasma concentration increased by between 115 and 178% [Nirogi *et al.* 2006].

Toxicity

A 21-year-old female was found dead in her bedroom. She had been prescribed multiple medications including metaxalone. The following postmortem drug concentrations were found in aortic blood: 19 mg/L metaxalone, 190 mg/L paracetamol, 0.28 mg/L hydrocodone, and <0.1 mg/L diazepam, nordiazepam, amitriptyline and nortriptyline. The following metaxalone concentrations were reported in other specimens: 17 mg/L in femoral blood, 44 mg/L in bile, 70 mg/kg in liver, 7 mg/L in urine, 202 mg/kg in gastric contents and 14 mg/L in vitreous humour [Poklis *et al.* 2004].

A 29-year-old female with cartilage-hair hypoplasia (a syndrome of short-limbed dwarfism) was found dead alongside 2 prescription bottles for forty 400 mg tablets of metaxalone and an empty litre bottle of wine. One of the metaxalone bottles was empty and the other contained 21 tablets. Femoral and heart blood concentrations were 39 and 54 mg/L, respectively. Brain and liver concentrations were 163 and 195 mg/kg, respectively. Total gastric and duodenum contents were 75 and 164 mg, respectively. Citalopram, ethanol, chlorphenamine and caffeine were also detected but at levels below those consistent with fatalities for these substances [Gruszecki *et al.* 2003].

A 54-year-old female with a history of pancreatitis and lower limb myopathy was found dead alongside several bottles of medication (flurazepam, gabapentin, citalopram, paracetamol and metaxalone). The measured blood and liver metaxalone concentrations were 21 mg/L and 82 mg/kg, respectively. The following blood concentrations were also found: 97 mg/L paracetamol, 0.4 mg/L citalopram and 24 mg/L gabapentin [Moore *et al.* 2005].

Half-life After a high-fat meal, 2 to 3 h; under fasted conditions, 9.2 h.

Dose Oral 800 mg three or four times daily.

Gruszecki AC *et al.* (2003). Polydrug fatality involving metaxalone. *J Forensic Sci* 48: 432–434.
Kleber J *et al.* (1964). Qualitative and quantitative tests for metaxolone. *J Pharm Sci* 53: 1522–1523.
Mistri HN *et al.* (2007). High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 853: 320–332.
Moore KA *et al.* (2005). A fatality involving metaxalone. *Forensic Sci Int* 149: 249–251.
Nirogi RV *et al.* (2006). Quantification of metaxalone in human plasma by liquid chromatography coupled to tandem mass spectrometry. *J Anal Toxicol* 30: 245–251.
Poklis JL *et al.* (2004). Metaxalone (Skelaxin)-related death. *J Anal Toxicol* 28: 537–541.

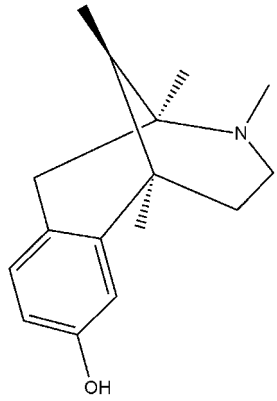
Metazocine

Narcotic

$C_{15}H_{21}NO$ = 231.3

CAS—114-91-0

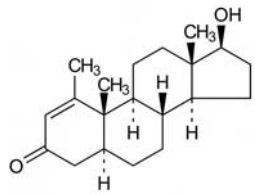
Synonyms 1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,7-methano-3-benzazocine; NIH 7410.



Chemical Properties Occurs as crystals from methanol. Mp 232° to 235°. Metazocine is extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—bright blue→green (limit of detection, 1.0 µg); ammonium vanadate test—greenish-brown (limit of detection, 1.0 µg); formaldehyde-sulfuric acid test—brown→greenish-grey→dull grey (limit of detection, 1.0 µg); Vitali's test—yellow/yellow/bright orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.21 (location reagents: acidified iodoplatinate spray, positive reaction; potassium permanganate spray, positive reaction).

Metenolone

Anabolic Steroid
$C_{20}H_{30}O_2$ = 302.5
CAS—153-00-4
IUPAC Name (5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-1,10,13-trimethyl-4,5,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonyms (5α,17β)-17-Hydroxy-1-methylandrost-1-en-3-one; methenolone.



Chemical Properties A crystalline powder. Mp 149.5° to 152°, also reported as 160° to 161°. Log *P* (octanol/water), 3.69.

Metenolone Acetate
$C_{22}H_{32}O_3$ = 344.5
CAS—434-05-9
Synonyms NSC-74226; SH-567; SQ-16496.
Proprietary Names *Primobolan*; *Primonabol*.
Chemical Properties Mp 138°. Soluble in methanol, ether and chloroform.

Metenolone Enantate
$C_{27}H_{42}O_3$ = 414.6
CAS—303-42-4
Synonyms Methenolone anantate; metenolone enanthate; methenolone oenanthate; NSC-64967; SH-601; SQ-16374.
Proprietary Names *Primobolan-Depot*; *Primonabol-Depot*.

Thin-layer Chromatography System TE—R_f 0.87; system TF—R_f 0.62; system TAE—R_f 0.92.

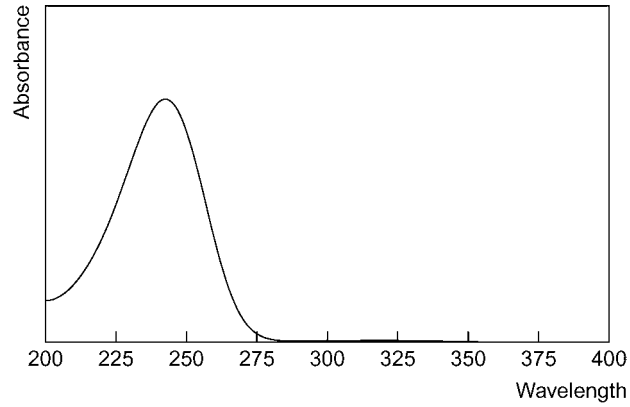
System 1: plate: silica gel IB2-F (Baker-flex with a fluorescent indicator). Mobile phase: *n*-hexane : ethylacetate (6:4). R_f 0.35.

System 2: plate: silica gel 60 F_{254} (silanised pre-coated). Mobile phase: acetone : water (3:1). R_f 0.62. UV detection (λ = 254 nm) or 2,4-dinitrophenylhydrazine reagent [Cavrini *et al.* 1983].
Gas Chromatography System GAI—metenolone RRT 0.993; 1-methylen-5α-androstan-3α-ol-17-one RRT 0.905 (both relative to 17α-methyl-5α-androstan-3β,17β-diol).

Column: 5% phenylmethylpolysiloxane cross-linked capillary (30 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 150° for 2 min, to 300° at 20°/min, held for 2 min. Injector temperature: 280°. IS: 1,2-d_2-testosterone. MS detection (NCI, precursor ion *m/z* 428). Retention time: metenolone metabolite (PFP derivative), 9.1 min [Choi *et al.* 1998].

High Performance Liquid Chromatography System HX—RI 809; system HATa—metenolone acetate RRT 1.26, metenolone enantate RRT 1.87(both relative to testosterone); system HATb—metenolone acetate RRT 3.54(relative to testosterone).

Column: RP monomeric C_{18} MicroPack MCH-10 (300 × 4.0 mm i.d., 10 µm). Mobile phase: acetonitrile : water (92:8), flow rate 2 mL/min. UV detection (λ=240 nm). Retention time: metenolone, 2.9 min; metenolone acetate, 3.5 min; metenolone enantate, 9.1 min [Cavrini *et al.* 1983].
Ultraviolet Spectrum Principal peak at 243 nm.

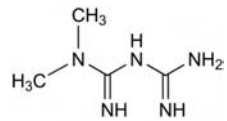


Infrared Spectrum Principal peaks at wavenumbers 1655, 1600, 1290, 1210, 1125, 1075 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 136, 123, 148, 83, 93, 107, 161, 67 (acetate); 136, 43, 123, 148, 161, 113, 83, 414.
Disposition in the Body Metenolone is metabolised and excreted in urine as the parent drug and its metabolites including, 1-methylen-5α-androstan-3α-ol-17-one.
Toxicity Moderately toxic by ingestion and IP routes.
Dose A usual dose of 10 to 20 mg daily is administered orally as the acetate and intramuscularly as the enanthate in doses of 100 mg every 2 to 4 weeks.

Cavrini V *et al.* (1983). *Int J Pharm* 13: 333–343.
Choi M *et al.* (1998). Determination of four anabolic steroid metabolites by gas chromatography/mass spectrometry with negative ion chemical ionization and tandem mass spectrometry. *Rapid Commun Mass Spectrom* 12: 1749–1755.

Metformin

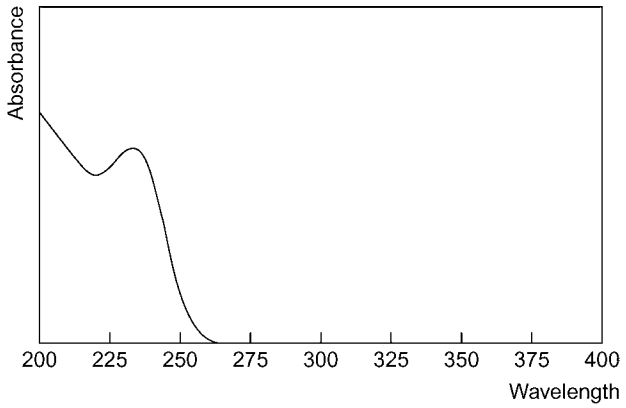
Antidiabetic
$C_4H_{11}N_5$ = 129.2
CAS—657-24-9
IUPAC Name *N,N*-Dimethylimidodicarbonimidic diamide
Synonym LA-6023



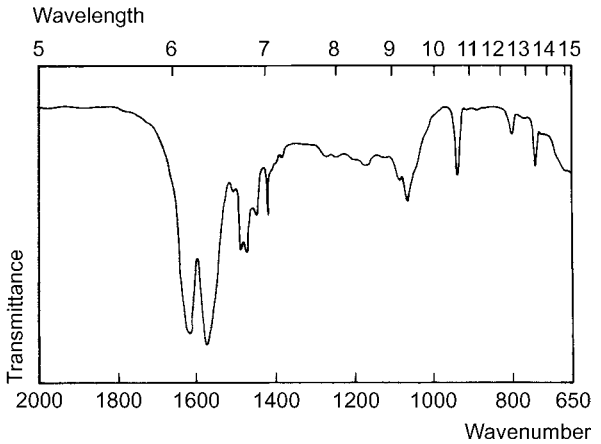
Chemical Properties pK_a 2.8, 11.5 (32°). Log *P* (octanol/water), −2.6.

Metformin Hydrochloride
$C_4H_{11}N_5$,HCl = 165.6
CAS—1115-70-4
Proprietary Names *Biocos*; *Diabesin*; *Diabetase*; *Diabetex*; *Diabex*; *Diaformin*; *Diformin*; *Glifage*; *Glucamet*; *Glucinan*; *Glucobon*; *Glucoformin*; *Glucohexal*; *Glucomet*; *Glucophage*; *Glycon*; *Glymax*; *Mediabet*; *Meglucon*; *Mescorit*; *Met*; *Metfirex*; *Metforem*; *Metiguanide*; *Novomet*; *Orabet*; *Stagid*.
Chemical Properties A white, hygroscopic, crystalline powder. Mp ~225°. Soluble 1 in 2 of water and 1 in 100 of ethanol; practically insoluble in chloroform and ether.

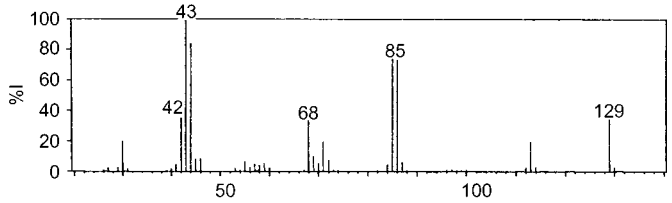
Thin-layer Chromatography System TA—R_f 0.01; system TAD—R_f 0.00; system TAE—R_f 0.03, R_f 0.93; system TAF—R_f 0.48; system TL—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00, R_f 0.80 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted; system GB—metformin, not eluted, metformin-nitrobenzoyltriazine RI 3050.
High Performance Liquid Chromatography System HX—RI 60; system HZ—RT 1.7 min; system HAA—RT 2.8 min.
Ultraviolet Spectrum Methanol—236 nm (A^1_1 = 1163b).



Infrared Spectrum Principal peaks at wavenumbers 1580, 1620, 1063, 1075, 935, 740 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 44, 85, 86, 42, 129, 68, 30.



Quantification

Plasma **GC** Column: 3% OV-17 on silanised Chromosorb W 80/100 mesh (2.0 m × 3 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 250°. AFID. Limit of detection, 25 µg/L [Brohon, Nöel 1978].

HPLC Column: reversed-phase phenyl type. Mobile phase: phosphate : acetonitrile, flow rate 1.0 mL/min. UV detection (λ= 236 nm). Retention time: 7.5 min. Limit of quantification, 30 µg/L [Porta *et al.* 2008]. Mobile phase: 10 µmol/L ammonium formate buffer : 0.1% formic acid in acetonitrile. SRM acquisition mode. Limit of quantification, 1–5 µg/L [Wang, Miksa 2007]. Column: Phenomenex Luna CN 100A (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 30 µmol/L ammonium acetate pH 5.0 (80 : 20), flow rate 0.2 mL/min. Limit of detection, 1 µg/L [Zhang *et al.* 2007]. Column: silica (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : 40 µmol/L aqueous sodium dihydrogen phosphate (pH 6, 25 : 75). Limit of quantification, 15.6 g/L [Amini *et al.* 2005]. UV detection (λ= 236 nm). Limit of quantification, <20 µg/L [Zhang *et al.* 2002]. See also Bonfigli *et al.* [1999], Cheng, Chou [2001], Charles *et al.* [1981], Huupponen *et al.* [1992], Kah, Kok [1998] and Vesterqvist *et al.* [1998],

LC-MS Column: HyPurity C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 3.0, 70 : 30), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.5 min. Limit of quantification, 20 µg/L [Mistri *et al.* 2007]. Column: Nucleosil C_{18} column (50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 10 µmol/L ammonium acetate pH 7.0 (20 : 20 : 60), flow rate 0.65 mL/min. APCI source, MRM acquisition mode. Limit of detection, 0.25 µg/L [Wang *et al.* 2004]. Column: C_8, (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : formic acid (70 : 30 : 1), flow rate 0.5 mL/min. APCI, positive ion mode, SRM acquisition mode. Limit of quantification, 2 µg/L [Chen *et al.* 2004]

Urine **GC** See Plasma [Brohon, Nöel 1978].

HPLC See Plasma [Charles *et al.* 1981].

Breast Milk **HPLC** See Plasma [Zhang *et al.* 2002].

Disposition in the Body Metformin is slowly absorbed after oral administration. Approximately 30–50% of an oral dose is excreted in the urine as unchanged drug in 24 h, and ~30% of the dose is eliminated unchanged in the faeces.

Therapeutic Concentration

Following single oral doses of 0.5 g and 1.5 g to 4 subjects, peak plasma concentrations of 0.59–1.3 mg/L (mean 1.0) and 1.8–4.0 mg/L (mean 3.1), respectively, were attained in ~2 h [Tucker *et al.* 1981].

Following daily oral doses of 0.5 g to 6 subjects, 1.7 g to 13 subjects and 2.5 g to 12 subjects, mean steady-state plasma concentrations of 0.16, 0.38 and 1.0 mg/L, respectively, were reported [Sirtori *et al.* 1978].

Toxicity

Plasma and urine concentrations of 85 and 389 mg/L, respectively, were reported in 1 subject, 12 h after the ingestion of 25.5 g; death occurred after 2 weeks [Larcan *et al.* 1979].

A 58-year-old man who died after ingesting ~20 g metformin plus 4.8 g diltiazem had an initial metformin level of 110 mg/L [Barrueto *et al.* 2002].

Bioavailability ~50–60%.

Half-life Plasma half-life after IV administration, ~1.5–4 h; half-life derived from urinary excretion data, ~16 h.

Volume of Distribution Variously reported as 1–4 L/kg.

Clearance Plasma clearance, ~7–10 mL/min/kg.

Protein Binding Not significantly bound.

Note For a review of the clinical pharmacokinetics of metformin, see Scheen [1996].

Dose Metformin hydrochloride 1.5 to 3 g daily.

Amini H *et al.* (2005). Determination of metformin in human plasma by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 824: 319–322.

Barrueto F *et al.* (2002). Clearance of metformin by hemofiltration in overdose. *J Toxicol Clin Toxicol* 40: 177–180.

Bonfigli AR *et al.* (1999). Determination of plasma metformin by a new cation-exchange HPLC technique. *Ther Drug Monit* 21: 330–334.

Brohon J, Nöel M (1978). Determination of metformin in plasma therapeutic levels by gas–liquid chromatography using a nitrogen detector. *J Chromatogr* 146: 148–151.

Charles BG *et al.* (1981). Rapid liquid-chromatographic determination of metformin in plasma and urine. *Clin Chem* 27: 434–436.

Chen X *et al.* (2004). Rapid determination of metformin in human plasma by liquid chromatography–tandem mass spectrometry method. *J Chromatogr B Analyt Technol Biomed Life Sci* 802: 377–381.

Cheng CL, Chou CH (2001). Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B Biomed Sci Appl* 762: 51–58.

Huupponen R *et al.* (1992). Determination of metformin in plasma by high-performance liquid chromatography. *J Chromatogr* 583: 270–273.

Kah HY, Kok KP (1998). Simple high-performance liquid chromatographic method for the determination of metformin in human plasma. *J Chromatogr B Biomed Sci Appl* 710: 243–246.

Larcan A *et al.* (1979). Acute intoxication by phenformine hyperlactatemia reversible with extrarenal purification. *Vet Hum Toxicol* 21(Suppl): 19–22.

Mistri HN *et al.* (2007). Liquid chromatography tandem mass spectrometry method for simultaneous determination of antidiabetic drugs metformin and glyburide in human plasma. *J Pharm Biomed Anal* 45: 97–106.

Porta V *et al.* (2008). HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies. *J Pharm Biomed Anal* 46: 143–147.

Scheen AJ (1996). Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 30: 359–371.

Sirtori CR *et al.* (1978). Disposition of metformin (*N,N*-dimethylbiguanide) in man. *Clin Pharmacol Ther* 24: 683–693.

Tucker GT *et al.* (1981). Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *Br J Clin Pharmacol* 12: 235–246.

Vesterqvist O *et al.* (1998). Determination of metformin in plasma by high-performance liquid chromatography after ultrafiltration. *J Chromatogr B Biomed Sci Appl* 716: 299–304.

Wang M, Miksa IR (2007). Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 856: 318–327.

Wang Y *et al.* (2004). Rapid and sensitive liquid chromatography–tandem mass spectrometric method for the quantitation of metformin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 808: 215–219.

Zhang M *et al.* (2002). Rapid and simple high-performance liquid chromatographic assay for the determination of metformin in human plasma and breast milk. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 175–179.

Zhang L *et al.* (2007). Simultaneous determination of metformin and rosiglitazone in human plasma by liquid chromatography/tandem mass spectrometry with electrospray ionization: application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 91–98.

Methacholine Chloride

Parasympathomimetic

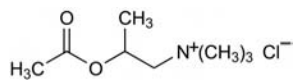
$C_8H_{18}ClNO_2$ = 195.7

CAS—55-92-5 (methacholine); 62-51-1 (chloride)

IUPAC Name 2-Acetyloxypropyl(trimethyl)azanium chloride

Synonyms Acetyl-β-methylcholine chloride; 2-(acetyloxy)-*N,N,N*-trimethyl-1-propanaminium chloride.

Proprietary Names *Amechol*; *Mecholyl*; *Provocholine*.



Chemical Properties Colourless, very hygroscopic crystals, or a white crystalline powder. Mp 170° to 173°. Soluble 1 in 0.4 of water, 1 in 1.2 of ethanol and 1 in 2.1 of chloroform; insoluble in ether. Log *P* (octanol/water), −3.0.

M

Methacholine Bromide

$C_8H_{18}BrNO_2$ = 240.1
CAS—333-31-3

Chemical Properties A white, very hygroscopic, crystalline powder. Mp about 148°. Soluble 1 in 0.3 of water, 1 in 0.8 of ethanol (90%) and 1 in 1.3 of chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).

Infrared Spectrum Principal peaks at wavenumbers 1739, 1248, 1066, 1632, 972, 1128 cm^{-1} (KBr disk).

Dose Methacholine has been given subcutaneously in doses of 10 to 25 mg (chloride), or orally in doses of 200 to 600 mg daily (bromide).

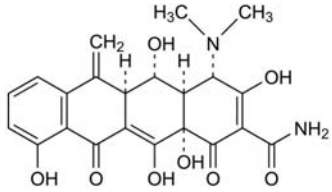
Methacycline

Antibiotic

$C_{22}H_{22}N_2O_8$ = 442.4
CAS—914-00-1

IUPAC Name (2*Z*,4*S*,4*aR*,5*S*,5*aR*,12*aS*)-2-[Amino(hydroxy)methylidene]-4-(dimethylamino)-5,10,11,12*a*-tetrahydroxy-6-methylidene-4,4*a*,5,5*a*-tetrahydrotetracene-1,3,12-trione

Synonyms [4*S*-(4α,4aα,5α,5aα,12aα)-4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide; méthylènecycline; 6-methyleneoxytetracycline.



Chemical Properties pK_a 3.1, 7.6, 9.5 (20°). Log *P* (octanol/pH 7.5), −0.4.

Methacycline Hydrochloride

$C_{22}H_{22}N_2O_8$,HCl = 478.9
CAS—3963-95-9

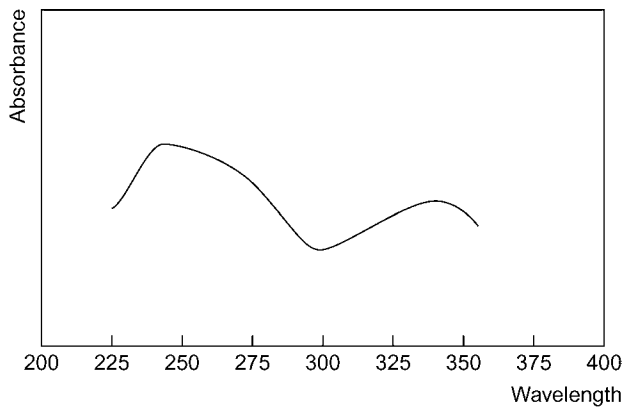
Proprietary Names *Esarondil*; *Lysocline*; *Optimycin*; *Physiomycine*; *Rondomycin (e)*; *Rotilen*; *Stafilon*.

Chemical Properties A yellow crystalline powder. Mp about 205°, with decomposition. Soluble 1 in 65 to 1 in 100 of water, 1 in 80 to 1 in 300 of ethanol and 1 in 30 of methanol; practically insoluble in chloroform and ether.

Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—red-orange; Mandelin's test—yellow→orange→violet; Marquis test—orange; sulfuric acid—red.

Thin-layer Chromatography System TA—R_f 0.05, streaking (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—243 (A^1_1=524a), 340 nm.



Infrared Spectrum Principal peaks at wavenumbers 1615, 1575, 1658, 1224, 1258, 1206 cm^{-1} (KBr disk).

Dose Usually 600 mg of methacycline hydrochloride daily.

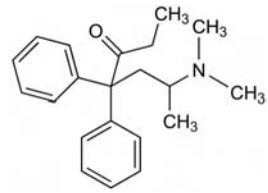
Methadone

Narcotic Analgesic

$C_{21}H_{27}NO$ = 309.5
CAS—76-99-3; 297-88-1 (±)

IUPAC Name 6-Dimethylamino-4,4-diphenyl-3-heptanone

Synonyms Amidine; amidone.



Chemical Properties Mp 78°. It is soluble in water at 48.5 mg/L (25°). pK_a 8.3 (20°); 8.94 (25°). Log *P* (octanol/pH 7.4), 2.1; (octanol/water), 3.93. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Methadone Hydrochloride

$C_{21}H_{27}NO$,HCl = 345.9
CAS—1095-90-5; 125-56-4 (±)

Synonyms Methadoni hydrochloridum; phenadone.

Proprietary Names *Adolan*; *Biodone*; *Dolmed*; *Dolophine*; *Eptadone*; *Heptadon*; *Ketalgine*; *Mephenon*; *Metasedin*; *Methaddict*; *Methadose*; *Methex*; *Pallidone*; *Phymet DTF*; *Physeptone*; *Pinadone DTF*; *Symoron*; *Synastone*.

Chemical Properties Colourless crystals or white crystalline powder. Mp 235° (platelets from alcohol and ether). Solubility (g/100 mL): water 12, alcohol 8, propan-2-ol 2.4; practically insoluble in ether and glycerol.

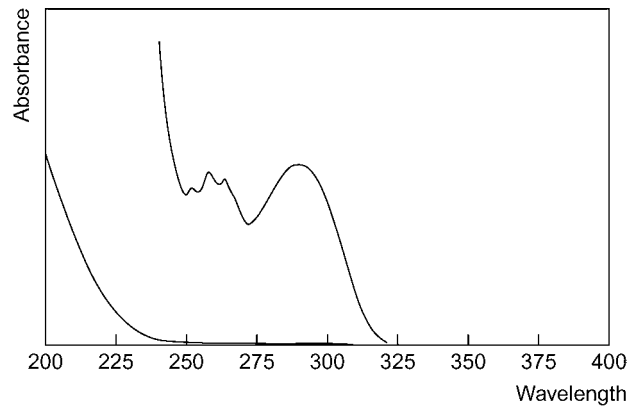
Colour Test Liebermann's reagent—brown-orange; Mandelin's test—green→blue

Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.59; system TC—R_f 0.20; system TE—R_f 0.77; system TL—R_f 0.27; system TAE—R_f 0.16; system TAF—R_f 0.60; system TAJ—R_f 0.08; system TAK—R_f 0.00; system TAL—R_f 0.45 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).

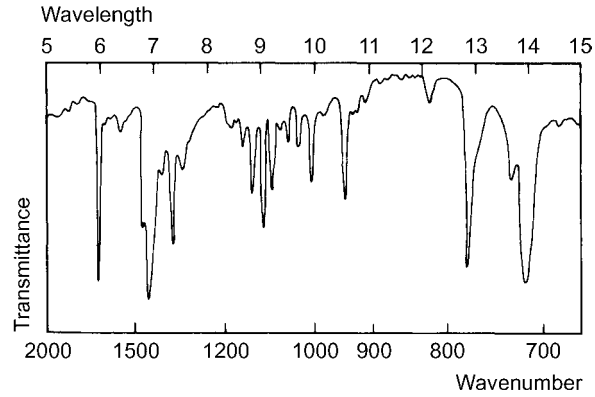
Gas Chromatography System GA—methadone RI 2145, M (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)) RI 2040, M (2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP)) RI 2021, M (nor-) RI 2095; system GB—methadone RI 2228, M (EDDP) RI 2120, M (EMDP) RI 2069; system GC—RI 2470; system GF—methadone RI 2370, M (EDDP) RI 2280, M (EMDP) RI 2190; system GM—methadone RRT 0.606, M (EDDP) RRT 0.520 (both relative to irpindole).

High Performance Liquid Chromatography System HA—methadone *k* 2.2, M (EDDP) *k* 2.8, M (EMDP) *k* 0.2; system HC—*k* 1.03; system HX—RI 440; system HY—RI 343; system HZ—RT 8.5 min; system HAA—RT 15.8 min; system HAX—RT 16.5 min; system HAY—RT 8.4 min.

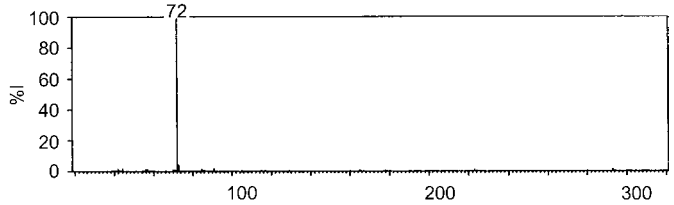
Ultraviolet Spectrum Aqueous acid—253, 259, 264, 292 nm (A^1_1= 18a).



Infrared Spectrum Principal peaks at wavenumbers 710, 1709, 769, 1107, 943, 1133 cm^{-1} (methadone hydrochloride, (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 72, 73, 91, 293, 223, 165, 85, 71 (methadone); 208, 193, 130, 115, 91, 165, 179, 207 (EMDP).



Quantification

Blood **GC** Column: 3% OV-101 plus 0.1% potassium hydroxide on Chromosorb W-HP 100/120 mesh (1.8 m (6 ft) × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 215°. AFID. Limit of detection, <0.005 mg/L [Jacob *et al.* 1981]. FID. Limit of detection, 0.02 mg/L [Thompson, Caplan 1977].

GC-MS Column: DB-35MS (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2.0 mL/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 25 μg/L, limit of detection, 10 μg/L [Gunnar *et al.* 2006].

LC-MS Column: Chiral-AGP (100 mm × 4.0-mm i.d., 5 μm). Mobile phase: acetonitrile : 10 μmol/L ammonium acetate buffer (pH 7.0, 22 : 78). ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.003 mg/kg, limit of detection, 0.001 mg/kg. [Johansen, Linnet 2008].

Plasma **GC** Column: Silica SE-52-CB (13 m × 0.25-mm i.d., 0.25 μm). Carrier gas: He, 50 psi. Temperature programme: 70° to 240° at 20°/min. NPD. Retention time: 11.9 min. Limit of quantification, 500 μg/L [Schmidt *et al.* 1993]. See Blood [Jacob *et al.* 1981].

GC-MS Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.3 mL/min. Temperature programme: 80° to 270° at 40°/min for 2 min to 300° at 40°/min for 4 min. EI ionisation. Limit of quantification, 2 μg/L, limit of detection, 0.6 μg/L. [Nikolaou *et al.* 2008]. Column: HP-5 phenylmethylsiloxane (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 1 min to 210° at 50°/min to 240° at 5°/min to 290° at 60°/min for 2 min. EI ionistaion at 70 eV, SIM acquisition mode. Limit of quantification, 20 μg/L, limit of detection, 6 μg/L [Bermejo *et al.* 2000]. Column: 3% OV-17 on 80/100 mesh Chromosorb W HP (1.6 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 200° to 270° at 10°/min. EI ionisation at 70 eV Limit of detection, 0.04 mg/L [Kang, Abbott 1982]. See also Alburges *et al.* [1996].

HPLC Column: reversed phase C_8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: phosphate buffer containing TEA : methanol : acetonitrile (pH 6.0, 40 : 50 : 10). Limit of quantification, 1 μg/L, limit of detection, 400 ng/L [Mercolini *et al.* 2007]. Column: X-Terra RP8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer (pH 6.53, 10 : 90 to 15 : 85 at 5 min to 20 : 80 at 7 min to 35 : 65 at 10 min to 50 : 50 at 15 min for 5 min to 10 : 90 at 22 min), flow rate 0.7 mL/min for 5 min to 0.8 mL/min until 20 min to 0.7 mL/min. DAD (λ= 292 nm). Retention time: 18.9 min. Limit of detection, 0.049 mg/L [Fernandez *et al.* 2006]. Column: reversed phase Zorbax (150 × 4.6 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium formate (pH 4.0) : acetonitrile (97 : 3 for 2 min to 87 : 13 at 2.6 min to 84.5 : 15.5 at 8.0 min to 20 : 80 at 8.1 min until 11 min to 97 : 3 at 11.1 min until 15 min), flow rate 1.0 mL/min. TIS, MRM acquisition mode. Retention time: 10.8 min. Limit of detection, 5 μg/L [Rook *et al.* 2005]. Column: Cyclobond Type I-Beta RSP. Mobile phase: acetonitroile : methanol; 1% TEA (pH 6.0, 200 : 50 : 750), flow rate 0.6 mL/min. UV detection (λ= 210 nm). Limit of quantification, 5 μg/L. [Norris *et al.* 1994]. See also Buice *et al.* [1982] and Schmidt *et al.* [1992].

LC-MS Column: chiral-AGP (100 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate buffer (pH 7.0, 18 : 82), flow rate 0.9 mL/min. MSD, SIM acquisition mode. [Rodriguez-Rosas *et al.* 2007].

Note For a fluorescence polarisation immunoassay for the quantification of methadone, see Beck *et al.* [1990] and Kell, Techman [1996].

Serum **GC** Column: 3% SP-2250 on Supelcoport 100/120 mesh (1.8 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. TID. Limit of detection, 1.5 μg/L [Chikhi-Chorfi *et al.* 1998].

HPLC Column: Cyclobond I-2000 RSP (250 × 4.6 mm i.d., 0.45 μm). Mobile phase: 0.1% TEA : 0.6% glacial acetic acid (pH 5.0), flow rate 0.3 mL/min. UV detection (λ= 220 nm). Limit of quantification, 2.5 μg/L [Boulton, DeVane 2000]. Column: Cyclobond 1-2000 RSP. UV detection (λ= 210 nm) [Pham-Huy *et al.* 1997]. Column: Chiral-AGP (100 × 4.0 mm i.d., 5 μm). Mobile phase: propan-2-ol : 10 mmol/L phosphate buffer: *N,N*-dimethyloctylamine (pH 6.6, 900 : 100 : 1), flow rate 0.6 mL/min. UV detection (λ= 205 nm). Limit of quantification, 25 μg/L, limit of detection, 10 μg/L [Rudaz, Veuthey 1996]. Column: CN and Chiral AGP. Mobile phase: acetonitrile : dimethyloctylamine : phosphate buffer. UV detection (λ= 200 nm). Limit of quantification, 5 nmol/L [Kristensen *et al.* 1994].

Urine **GC** Carrier gas: N_2, 1.0 mL/min. Temperature programme: 100° for 0.5 min to 300° at 20°/min for 3 min. NPD. Limit of detection, 0.001 mg/L [Myung *et al.* 1999]. See Serum [Chikhi-Chorfi *et al.* 1998]. See Plasma [Schmidt *et al.* 1993]. See Blood [Jacob *et al.* 1981; Thompson, Caplan 1977].

GC-MS Column: BPX5 (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 100° for 1 min to 310° at 20°/min for 3.5 min. PICI, full scan mode. Limit of quantification, 20 μg/L, limit of detection, 10 μg/L [Moore *et al.* 2001]. See Plasma [Alburges *et al.* 1996].

HPLC Column: Cyclobond I 2000 RSP (250 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : 1% TEA (pH 6.0, 9 : 11 : 80), flow rate 1.0 mL/min. UV detection (λ= 210 nm). Limit of quantification, 0.125 μmol/L [Foster *et al.* 2000]. See Serum [Boulton, DeVane 2000]. Column: SymmetryShield RP18 (150 × 3.9 mm i.d., 5 μm). Mobile phase: 0.1% trifluroacetic acid : methanol (60 : 40), flow rate 1.0 mL/min. UV detection (λ= 210 nm). Limit of detection, 20 μg/L [Cheng *et al.* 1999]. Column: LiChrospher RP8 coupled in series with a chiral AGP. UV detection (λ= 200 nm). Limit of quantification, 0.03 μmol [Angelo *et al.* 1999]. See Serum [Pham-Huy *et al.* 1997].

CSF **GC** See Plasma [Schmidt *et al.* 1993].

Oral Fluid **GC** See Serum [Chikhi-Chorfi *et al.* 1998].

GC-MS Column: HP-5 (12 m × 0.22 mm i.d., 0.33 μm). Temperature programme: 90° for 2 min to 200° at 30°/min for 5 min to 290° at 30°/min. EI ionisation at 70 eV. Limit of quantification, 45 μg/L, limit of detection, 4 μg/L [dos Santos Lucas *et al.* 2000]. See Plasma [Kang, Abbott 1982].

Meconium **GC-MS** Column: DB-5 MS (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 38 cm/s. Temperature programme; 150° for 0.5 min to 240° at 30°/min for 7.5 min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 ng/g, limit of detection, 10 ng/g [Elsohly *et al.* 2001].

HPLC DAD [Stolk *et al.* 1997].

Sweat **GC-MS** Column: chiral-AGP (100 × 4 mm i.d., 5 μm). Mobile phase: propan-2-ol : 2 mmol/L ammonium carbonate buffer (pH 5.8, 8 : 92 for 8 min to 20 : 80 at 9 min until 18 min to 8 : 92 at 18.5 min), flow rate 500 μL/min. ESI, positive ion mode. Limit of detection, 2 ng/patch [Kintz *et al.* 1998].

Hair **GC-MS** Column: DB-5MS (15 × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 80° for 1 min to 280° at 20°/min for 4 min. PICI. Limit of quantification, 0.05 ng/mg, limit of detection, 0.01 ng/mg [Girod, Staub 2001]. Column: HP-5 (12 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 1 min to 200° at 30°/min for 5 min to 290° at 30°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 1 ng/mg [Lucas *et al.* 2000]. Column: HP-5-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 2 min to 300° at 20°/min for 2 min. SIM acquisition mode. Limit of quantification, 0.1 ng/mg, limit of detection, 0.03 ng/mg [Sporkert, Pragst 2000a]. Column: HP 95% dimethylsiloxane 5% diphenylsiloxane (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 2 min to 205° at 30°/min to 240° at 2.5°/min to 290° at 30°/min. Retention time: 13.05 min. Limit of detection, 0.1 ng/mg [Sporkert, Pragst 2000b].

LC-MS Column: Zorbax SB-Phenyl (2.1 × 100 mm, 3.5 μm). Mobile phase: 25 mmol/L formic acid : acetonitrile (98 : 2 to 80 : 20 for 3 min to 10 : 90 in 4 min for 2 min). ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.0125 ng/mg [Hegstad *et al.* 2008].

Liver **GC-MS** See Plasma [Alburges *et al.* 1996].

Nail **GC-MS** [Lemos *et al.* 2000].

Note For an enzyme immunoassay for the quantification of methadone in serum, saliva and hair see Piekoszewski *et al.* [2001].

Disposition in the Body

Methadone is rapidly absorbed after oral administration and widely distributed in the tissues, with higher concentrations in the liver, lungs and kidneys than in the blood. It crosses the placental barrier and is also found in breast milk. Metabolism is mainly catalysed by the cytochrome P450 isozyme CYP3A4, but other isozymes may be involved. The main metabolic reaction is *N*-demethylation, resulting in a substance that spontaneously cyclises to form the major metabolites EDDP and EMDP, neither of which are active. Hydroxylation to methadol followed by *N*-demethylation to normethadol also occurs to some extent. Other metabolic reactions occur and there are at least eight known metabolites. In subjects on methadone maintenance, ~20–60% of a dose is excreted in the urine in 24 h, with up to ~33% of the dose as unchanged drug and up to ~43% as EDDP; EMDP accounts for ~5–10% of the dose. The ratio of EDDP to unchanged methadone is usually very much higher in the urine of patients on methadone maintenance treatment than in simple overdose cases. Urinary excretion of unchanged drug is pH dependent, being increased in acid urine. Up to 30% of a dose may be eliminated in the faeces, but this appears to decrease with increasing dosage. Approximately 75% of the total excreted material is unconjugated.

Therapeutic Concentration In plasma, usually in the range 0.05–1.0 mg/L. During methadone maintenance treatment, there are often considerable fluctuations in plasma concentrations from day to day, and there appears to be a decrease in concentration as dispositional tolerance develops.

Two regimens of epidural administration of methadone were compared in 90 patients requiring postoperative analgesia: 60 received initial doses of 3 to 6 mg followed by 6–12 mg every 24 h by continuous infusion for 72 h; a further 30 received repeated epidural boluses of 3–6 mg every 8 h for 72 h. The mean plasma methadone concentrations were as follows (mg/L):

	Continuous infusion	*Bolus infusion*
After 24 h	0.07769	0.11188
After 48 h	0.05029	0.07080
After 72 h	0.04905	0.07776

Both regimens provided satisfactory postoperative analgesia [Prieto-Alvarez *et al.* 2002].

In 180 patients receiving methadone maintenance therapy (10–350 mg daily), satisfactory abstinence from illicit opiates was obtained with an (*R*)-methadone plasma concentration of 0.25 mg/L and a racemic methadone concentration of 0.4 mg/L, with a higher specificity being calculated for

(*R*)-methadone; doses required to produce the required concentration could theoretically range from 55–921 mg daily in a 70 kg patient [Eap *et al.* 2000].

In 18 patients maintained on methadone 7.5–130 mg (0.12–1.9 mg/kg) daily for at least 2 months, opioid responses were strongly correlated with changes in plasma racemic methadone concentrations; peak plasma concentrations of 0.069–0.98 mg/L were achieved in ~3 h and pre-dose concentrations were 0.044–0.614 mg/L. Small changes in plasma concentrations had a relatively large effect on withdrawal severity [Dyer *et al.* 1999].

Twenty long-term opiate addicts were administered a mean oral dose of 60 mg methadone hydrochloride (range, 10–225). The peak plasma methadone concentrations varied from 0.124–1.255 mg/L and 0.01–0.301 mg/L and the steady-state concentrations from 0.065–0.63 mg/L and 0.005–0.055 mg/L [de Vos *et al.* 1995].

Toxicity The estimated minimum lethal dose is 50 mg, but addicts on maintenance treatment may tolerate doses of 200 mg or more. In non-addicted subjects, toxic reactions are associated with plasma concentrations in the region of 1–2 mg/L, and concentrations above 2 mg/L may be lethal. With serious overdose, respiratory depression, extreme somnolence including stupor or coma, skeletal muscle flaccidity, cold and clammy skin, bradycardia and hypotension may occur and with severe overdose, circulatory collapse, cardiac arrest and death can occur.

In 3 cases in which death was attributed to methadone overdose, liver concentrations of 1.9, 1.3 and 0.7 µg/g and urine concentrations of 13, 25 and 4 µg/L, respectively, were reported [Nelson, Selkirk 1975].

In 23 fatalities in which methadone was involved, the following postmortem tissue concentrations, mg/L or µg/g (mean, n), were reported:

	Methadone	*EDDP*
Blood	0–3 (1.2, 21)	0–0.4 (0.1, 10)
Bile	1.1–75 (13, 18)	0.2–315 (41, 18)
Brain	0.23–2.2 (0.9, 7)	—
Kidney	0.51–18.3 (3.5, 19)	0.5–3.1 (1.2, 14)
Liver	0.05–49.5 (6, 23)	0.02–2.7 (0.6, 16)
Lung	1.6–110 (16, 17)	0.01–0.98 (0.2, 10)
Spleen	1.6–20.9 (5.3, 7)	0–0.98 (0.7, 4)
Urine	0.52–76.2 (21, 22)	0.4–46.2 (11, 17)

[Garriott *et al.* 1973; Robinson, Holder 1973; Robinson, Holder 1974; Robinson, Williams 1971].

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Methadone was held to be the sole cause of death in 55 out of 111 fatalities involving methadone; 50 of these victims were aged between 17 and 51 years and 5 were under 14. For the adult deaths, the mean methadone concentration was 0.584 mg/L (range, 0.084–2.7). For those aged 15 to 49 years, death was from a combination of methadone and other drugs in 56 cases (mean methadone concentration 0.576 mg/L; range, 0.049–2.44) [Milroy, Forrest 2000].

From 1997–1998, methadone was detected in 38 out of 3317 reported deaths in San Francisco, USA, and 17 of these deaths were deemed as being caused by methadone toxicity. The mean blood methadone concentration was 0.957 mg/L and the mean EDDP concentration was 0.253 mg/L. The mean ratio of methadone to EDDP in blood was 13.6 : 1.0 [Karch, Stephens 2000].

Half-life Plasma half-life after a single dose, 10–25 h (mean, 15); increased during long-term maintenance therapy to 13–55 h (mean, 30).

Volume of Distribution ~4 L/kg.

Clearance Plasma clearance, ~2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.3.

Protein Binding Approximately 92%, but there is considerable intersubject variation.

Dose For pain, initially, 2.5 to 10 mg methadone hydrochloride every 3 to 8 h; doses of 30 mg have been given. In treatment of opioid dependence, initially 10 to 20 mg daily, increased to achieve stabilisation to a usual dose of 40 to 60 mg daily.

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Myung SW *et al.* (1999). Solid-phase microextraction for the determination of pethidine and methadone in human urine using gas chromatography with nitrogen–phosphorus detection. *Analyst* 124: 1283–1286.

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Schmidt N *et al.* (1992). Stereoselective determination of the enantiomers of methadone in plasma using high-performance liquid chromatography. *J Chromatogr* 583: 195–200.

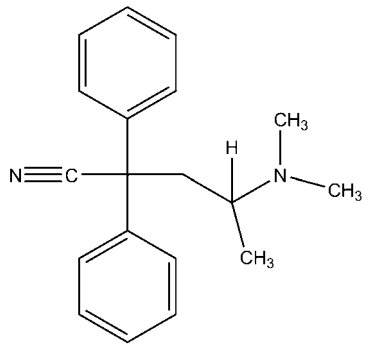
Schmidt N *et al.* (1993). Rapid determination of methadone in plasma, cerebrospinal fluid, and urine by gas chromatography and its application to routine drug monitoring. *Pharm Res* 10: 441–444.

Sporkert F, Pragst F (2000). Determination of methadone and its metabolites EDDP and EMDP in human hair by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 746: 255–264.

Sporkert F, Pragst F (2000). Use of headspace solid-phase microextraction (HS-SPME) in hair analysis for organic compounds. *Forensic Sci Int* 107: 129–148.
Stolk LM *et al.* (1997). Analysis of methadone and its primary metabolite in meconium. *J Anal Toxicol* 21: 154–159.
Thompson BC, Caplan YH (1977). A gas chromatographic method for the determination of methadone and its metabolites in biological fluids and tissues. *J Anal Toxicol* 1: 66–69.

Methadone Intermediate

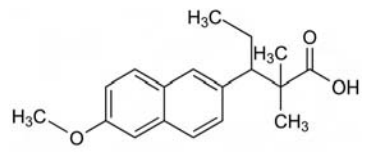
Narcotic Intermediate
$C_{19}H_{22}N_2$ = 278.4
Synonym 4-Cyano-2-(*N,N*-dimethylamino)-4,4-diphenylbutane



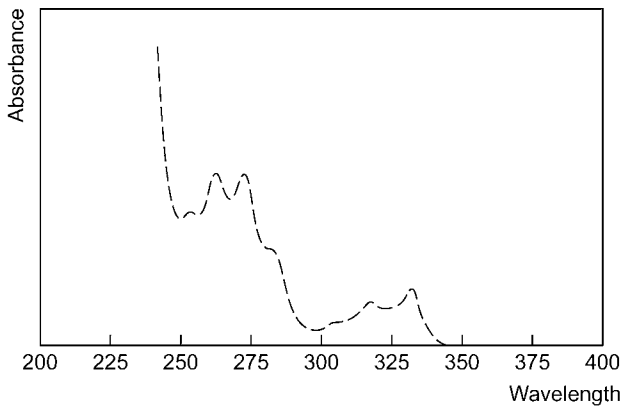
Chemical Properties Off-white powder. Slightly soluble in dilute acetic acid. Extracted by ether from aqueous alkaline solutions.
Thin-layer Chromatography System T1—R_f 0.65 and R_f 0.55 (location reagent acidified iodoplatinate spray, positive reaction).

Methallenestril

Oestrogen
$C_{18}H_{22}O_3$ = 286.4
CAS—517-18-0
IUPAC Name 3-(6-Methoxynaphthalen-2-yl)-2,2-dimethylpentanoic acid
Synonyms Methallenoestrol; 3-(6-methoxy-2-naphthyl)-2,2-dimethylvaleric acid.
Proprietary Name *Vallestril*



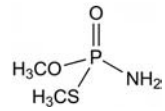
Chemical Properties A white crystalline powder. Mp about 138°. Very slightly soluble in water; soluble 1 in 10 of ethanol, 1 in 2 of chloroform and 1 in 8 of ether. Log *P* (octanol/water), 5.3.
Thin-layer Chromatography System TP—R_f 0.79; system TQ—R_f 0.18; system TR—R_f 0.70; system TS—R_f 0.54, streaking may occur.
Ultraviolet Spectrum Methanol—253, 264 (A_1^1=180a), 273 (A_1^1=180a), 317, 332 nm.



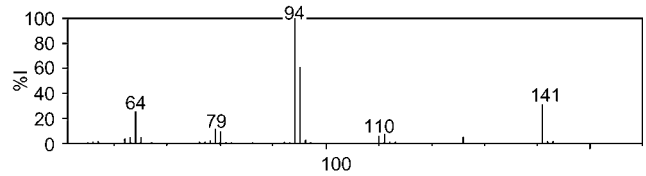
Infrared Spectrum Principal peaks at wavenumbers 1689, 1232, 1270, 857, 1606, 1149 cm^{-1} (KBr disk).
Dose 3 to 9 mg daily.

Methamidophos

Acaricide, Insecticide
$C_2H_8NO_2PS$ = 141.1
CAS—10265-92-6
IUPAC Name [Amino(methylsulfanyl)phosphoryl]oxymethane
Synonyms Acephate-met; Bayer 71628; ENT 27396; Ortho 9006; phosphoramidothioc acid *O,S*-dimethyl ester; SRA 5172.
Proprietary Names *Filitox*; *Metikar*; *Monitor*; *Nitofol*; *Nuratron*; *Patrole*; *Pillaron*; *Swipe*; *Tam*; *Tamanox*; *Tamaron*; *Vetaron*.



Chemical Properties A colourless, crystalline solid. Mp 54°. Readily soluble in water (90 g/L at 20°), ethanol, isopropanol (1400 g/L at 20°), xylene (<100 g/L at 20°), benzene (<100 g/L at 20°), dichloromethane (<25 g/L at 20°), toluene, diethyl ether (<25 g/L at 20°), kerosene (<10 g/L at 20°) and hexane (<10 g/L at 20°). Log *P* (octanol/water), −0.80.
Thin-layer Chromatography System TX—R_f 0.01; system TY—R_f 0.00.
Gas Chromatography System GA—RI 1190.
Column DB1 or DB1701 (30 m × 0.32 mm i.d., 0.25 µm), Temperature programmed from 130° (held 1 min) to 250° (held 2 min) at 10°/min. Carrier gas: He. TSD. Retention time 4 min (DB1), 7.1 min (DB1701) [Lewin *et al.* 1993].
Mass Spectrometry Principal ions at *m/z* 94, 95, 141, 64, 79, 80, 111, 110.

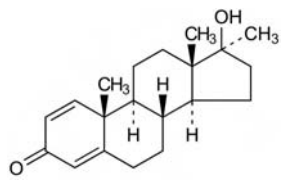


Quantification
Blood **GC** TSD. Limit of detection, 45 µg/L [Lewin *et al.* 1993].
Liver **GC** See Blood [Lewin *et al.* 1993].
Disposition in the Body Methamidophos is rapidly absorbed through the stomach, lungs and skin after exposure and is eliminated primarily via urine.
Toxicity Methamidophos is highly toxic via oral, dermal and inhalation administration. The allowed daily intake is 0.0006 mg/kg.
A 61-year-old male plantation worker collapsed and was admitted to hospital. A bottle of Nitofol (methamidophos) was found on the floor beside him. He died 11 h after ingestion. Toxicological analysis showed that there was a concentration of 130 mg/L methamidophos in his blood and 240 mg/L in his liver. The cause of death was due to an overdose of methamidophos [Lewin *et al.* 1993].

Lewin JF *et al.* (1993). *TIAFT Bulletin Case Notes* 23(1): .

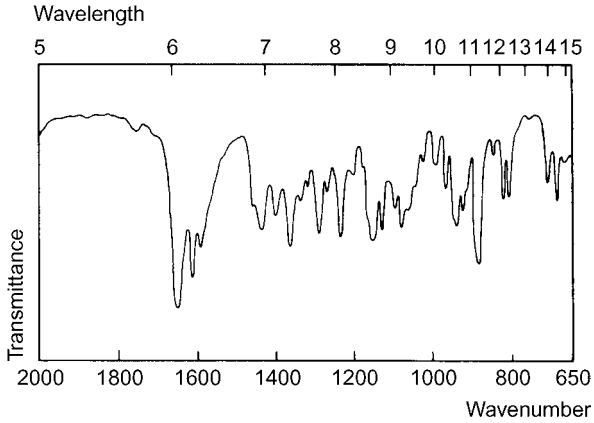
Methandienone

Anabolic Steroid
$C_{20}H_{28}O_2$ = 300.4
CAS—72-63-9
IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13,17-trimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-one
Synonym (17β)-17-Hydroxy-17-methylandrosta-1,4-dien-3-one; methandrostenolone.
Proprietary Names *Anabol*; *Danabol*; *Dianabol*; *Melic*; *Metastenol*; *Nerobol*; *Vetanabol* (vet.).



Chemical Properties A white or faintly yellowish-white, crystalline powder. Mp 163° to 167°. Practically insoluble in water; soluble 1 in 2 of ethanol, 1 in <1 of chloroform and 1 in 70 of ether. Log *P* (octanol/water), 3.5.
Thin-layer Chromatography System TA—R_f 0.86; system TE—R_f 0.80; system TP—R_f 0.65; system TQ—R_f 0.10; system TR—R_f 0.87; system TS—R_f 0.61; system TAJ—R_f 0.44; system TAK—R_f 0.61; system TAL—R_f 0.92; system TAM—R_f 0.88.

Gas Chromatography System GA—RI 2672; system GAG—RRT 1.2(relative to testosterone); system GAI—urinary metabolites: 17α-methyl-5β, (α)-androstan-3α,17β-diol RRT 0.925; 17α-methyl-5β-androst-1-en-3α,17β-diol RRT 0.921; 17α-methyl-1,4-androstadien-6β,17β-diol-3-one RRT 1.117 (relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAR—retention time 13.2 min.
High Performance Liquid Chromatography System HAR—RRT 0.86 (relative to testosterone); system HATb—RRT 0.87 (relative to testosterone).
Ultraviolet Spectrum Dehydrated alcohol—245 nm (A^{1}_{1}=516a).
Infrared Spectrum Principal peaks at wavenumbers 1660, 1620, 886, 1601, 1160, 1240 cm^{-1} (KBr disk).

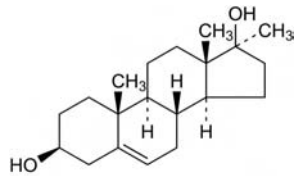


Quantification
Urine **GC** Limit of detection, 30 ng for methandienone, 10 ng for 17-epimethandienone and 6β-hydroxy-17-epimethandienone [Dürbeck *et al.* 1978].
GC-MS Methandienone metabolites [Kokkonen *et al.* 1999].
HPLC UV detection. Limit of detection, 5 ng [Frischkorn, Frischkorn 1978].
Dose Initially 2.5 to 5 mg daily.

Dürbeck HW *et al.* (1978). Gas chromatographic and capillary column gas chromatographic–mass spectrometric determination of synthetic anabolic steroids. I. Methandienone and its metabolites. *J Chromatogr* 167: 117–124.
Frischkorn CG, Frischkorn HE (1978). Investigations of anabolic drug abuse in athletics and cattle feed. II. Specific determination of methandienone (Dianabol) in urine in nanogram amounts. *J Chromatogr* 151: 331–338.
Kokkonen J *et al.* (1999). Comparison of sensitivity between gas chromatography-low-resolution mass spectrometry and gas chromatography-high-resolution mass spectrometry for determining metandienone metabolites in urine. *J Chromatogr B Biomed Sci Appl* 734: 179–189.

Methandriol

Anabolic Steroid
$C_{20}H_{32}O_2$ = 304.5
CAS—521-10-8
IUPAC Name (3*S*,8*R*,9*S*,10*R*,13*S*,14*S*,17S)-10,13,17-Trimethyl-1,2,3,4,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthrene-3,17-diol
Synonyms MAD; mestenediol; methylandrostenediol; (3β,17β)-17-methylandrost-5-ene-3,17-diol.
Proprietary Names *Androdiol*; *Androteston-M*; *Crestabolic*; *Diolostene*; *Masdiol*; *Megabion*; *Methanabol*; *Methandiol*; *Methosten*; *Metisione*; *Metocryst*; *Nabadial*; *Neostene*; *Neutrirmone*; *Neutrosteron*; *Notandron*; *Protandren*; *Sinasex*; *Troformone*.

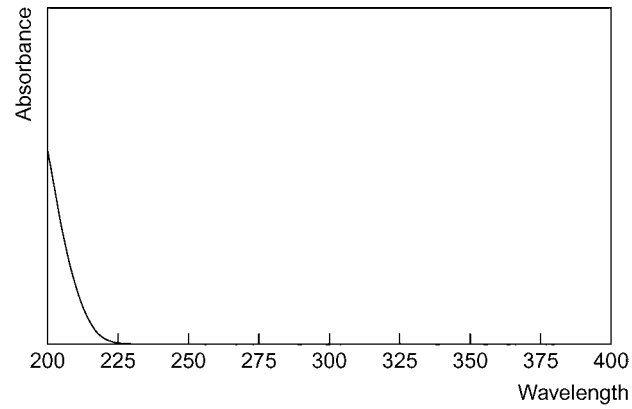


Chemical Properties A crystalline powder. Mp 205.5° to 206.5°. Insoluble in water; slightly soluble in some organic solvents. Log *P* (octanol/water), 4.35.

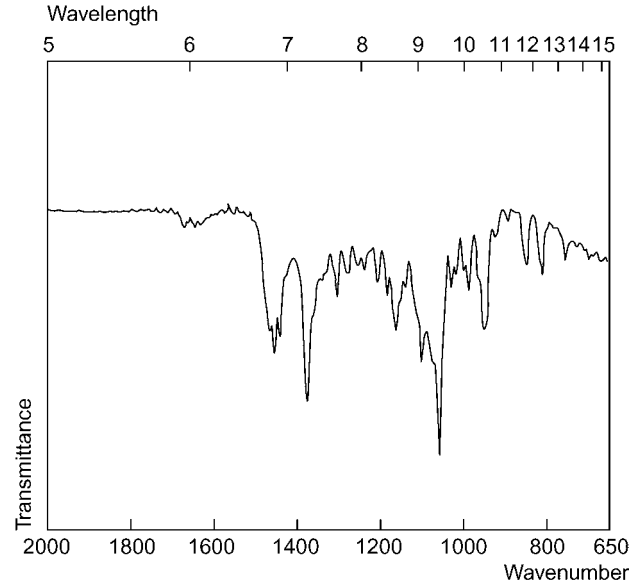
Methandriol Diacetate
$C_{24}H_{36}O_4$ = 388.5
CAS—2061-86-1
Chemical Properties Mp 145° to 146° (crystals from hexane).

Methandriol Dipropionate
$C_{26}H_{40}O_4$ = 416.6
CAS—3593-85-9
Synonym Methylandrostendiol dipropionate
Proprietary Name *Probolin*

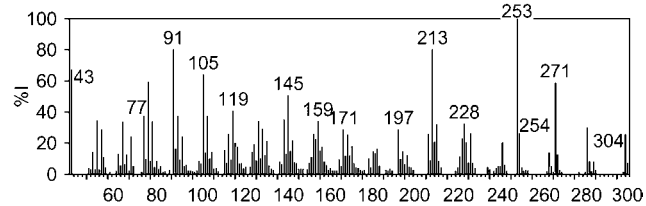
Gas Chromatography System GAG—methandriol RRT 0.89, methandriol dipropionateRRT 1.70 (both relative to testosterone); system GAI—urine metabolite: 17α-methyl-5β-androstan-3α,17β-diol RRT 0.925 (relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAR—retention time 12.3; (methandriol dipropionate) retention time 15.2 min.
Column: 3% OV-1 Chromosorb WHP 80/100 mesh (4′ × $^{1}/_{4}$″). Temperature: 280°. Carrier gas: N_2, flow rate 32 mL/min. Detection: flame ionisation. RI: 2637 [Mills, Roberson 1993].
High Performance Liquid Chromatography System HAR—methandriol RRT 1.25, methandriol dipropionate RRT 1.25 (both relative to testosterone); system HATb—methandriol RRT 1.29(relative to testosterone); system HATb—methandriol dipropionate RRT 2.75(relative to testosterone).
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1449, 1372, 1054, 942 cm^{-1}; (methandriol dipropionate) 1725, 1189, 1075 cm^{-1} (KBr disks).



Mass Spectrum Principal ions at *m/z* 253, 91, 213, 43, 105, 271, 79, 145; (dipropionate) *m/z* 57, 342, 253, 268, 145, 147, 81, 91 .



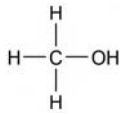
Disposition in the Body Methandriol is excreted in urine as metabolites, one of which is 17α-methyl-5β-androstan-3α,17β-diol.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 2: 1384–1385.

Methanol

Solvent
CH_3OH = 32.04
CAS—67-56-1

Synonym Methyl alcohol



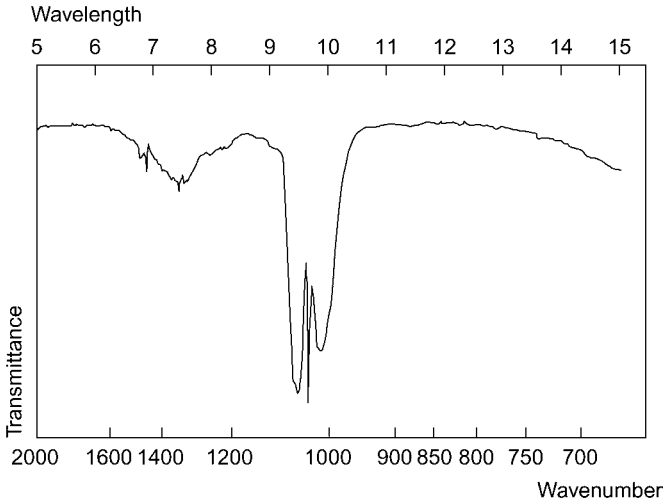
Chemical Properties A clear, colourless, highly flammable liquid. Bp 63.5° to 65.7°. Refractive index 1.328 to 1.329. Miscible with water, ethanol, benzene, chloroform, ether, ketones, and most other organic solvents. pK_a 15.3. Log *P* (octanol/water), 0.8.

Note The commercial substance known as 'wood naphtha', 'pyroxylic spirit', or 'wood spirit' contains 60–90% of methanol, together with acetone and other empyreumatic impurities. The variety used for denaturing ethanol (see under Ethanol) contains not less than 72% of methanol.

Colour Test Potassium dichromate (method 2)—green.

Gas Chromatography System GA—RI 491; system GI—retention time 0.7 min.

Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 31, 32, 29, 30, 28, 33, 34, 27; formic acid 29, 46, 45, 28, 44, 30, 47, –.

Quantification

Blood **GC** FID. Direct injection [Manno, Manno 1978].

Urine **GC** See Blood [Manno, Manno 1978].

Disposition in the Body Readily absorbed after oral administration and distributed in the body according to the water content of the tissues; it may also be absorbed by inhalation or through intact skin. It is metabolised, much more slowly than ethanol, by oxidation to formaldehyde, formic acid, and possibly other products. Oxidation to formaldehyde is probably accomplished by alcohol dehydrogenase since the metabolism is inhibited by ethanol. Maximum concentrations of formic acid in the blood and urine occur 2–3 days after ingestion. Small amounts of methanol are excreted unchanged in the urine and expired air and about 10% of a dose is excreted in the urine as formic acid.

Blood Concentration Endogenous blood-methanol concentrations are ~1.5 mg/L, and urinary formic acid concentrations are usually ~12–17 mg/L.

Toxicity The initial effects of methanol are much milder than those of ethanol and toxic effects are not usually seen until after a latent period of 8–36 h; the symptoms may include severe upper abdominal pain, visual disturbance often proceeding to incurable blindness, severe metabolic acidosis, and prolonged coma which may terminate in death from respiratory failure. The fatal dose varies greatly but is usually between 100 and 200 mL in adults, although ingestion of 30 mL is potentially lethal; permanent blindness has been caused by as little as 10 mL. Toxic effects are usually associated with blood concentrations >100 mg/L and blood concentrations >200 mg/L are indicative of severe poisoning and may be lethal. The maximum permissible atmospheric concentration is 200 ppm.

A 44-year-old man suffering from methanol intoxication was found comatose and had a serum concentration of 5.83 g/L on admission. The subject died 40 h later and the following postmortem concentrations were found (g/L or mg/g): blood 1.42, bile 1.75, brain 1.59, heart 0.93, kidney 1.30, liver, 1.07, lung 1.27, vitreous humour 1.73. [Wu Chen *et al.* 1985.]

The following postmortem tissue concentrations were reported in a 41-year-old man who died after ingesting a large quantity of methanol: kidney 5.13 mg/g, liver 4.18 mg/g, vitreous humour 3.96 g/L, heart 3.45 mg/g, urine 3.43 g/L, pericardial fluid 3.29 g/L, blood 2.84 g/L, stomach contents 2.21 g/L. [Pla *et al.* 1991.]

In 2 fatalities caused by ingestion of methanol (~100 g), femoral blood concentrations of methanol and formic acid were 2.19 and 0.41 g/L, respectively, in the first subject and 1.96 and 0.38 g/L in the second. [Kinoshita *et al.* 1998.]

Volume of Distribution ~0.6 L/kg.

Note For a review of methanol poisoning and its treatment, see Barceloux *et al.* 2002.

Protein Binding Not significantly bound.

Barceloux DG *et al.* (2002). American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. *J Toxicol Clin Toxicol* 40: 415–446.

Kinoshita H *et al.* (1998). Combined toxicity of methanol and formic acid: two cases of methanol poisoning. *Int J Legal Med* 111: 334–335.

Manno BR, Manno JE (1978). A simple approach to gas chromatographic microanalysis of alcohols in blood and urine by a direct-injection technique. *J Anal Toxicol* 2: 257–261.

Pla A *et al.* (1991). A fatal case of oral ingestion of methanol. Distribution in postmortem tissues and fluids including pericardial fluid and vitreous humor. *Forensic Sci Int* 49: 193–196.

Wu Chen NB *et al.* (1985). Methanol intoxication: distribution in postmortem tissues and fluids including vitreous humor. *J Forensic Sci* 30: 213–216.

Methanthelinium Bromide

Anticholinergic

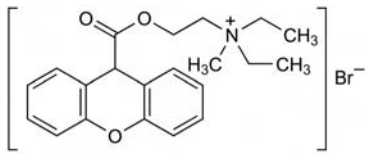
$C_{21}H_{26}BrNO_3$ = 420.3

CAS—5818-17-7 (methanthelinium); 53-46-3 (bromide)

IUPAC Name *N,N*-Diethyl-*N*-methyl-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]-ethanaminium bromide

Synonyms Bromuro de metantelina; dixamonum bromidum; methantheline bromide; methanthine bromide.

Proprietary Names *Asabaine*; *Avagal*; *Banthine*; *Bronerg*; *Doladene*; *Evogal*; *Gastron*; *Gastrosedan*; *Metanyl*; *Metaxan*; *Methanide*; *Uldumont*; *Vagamin*; *Vagantin*; *Xanteline*; *Xantenol.*



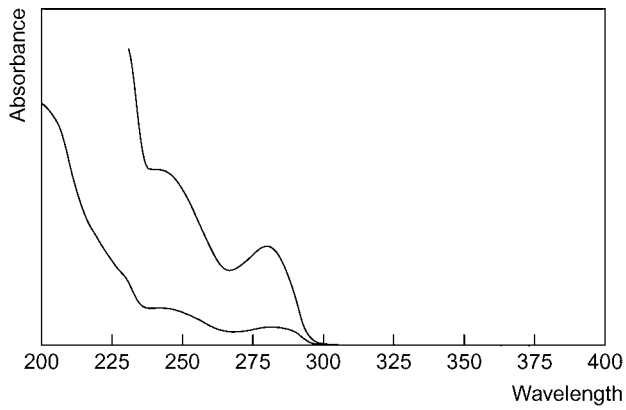
Chemical Properties A white crystalline powder. Mp 171° to 177°. Soluble 1 in less than 5 of water, ethanol and chloroform; practically insoluble in ether. Aqueous solutions decompose on standing. Log *P* (octanol/water), 1.7.

Colour Tests Mandelin's test—orange; Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.00; system TE—R_f 0.76; system TAE—R_f 0.03 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 418.

Ultraviolet Spectrum Aqueous acid—282 nm (A^1_1=65a).



Infrared Spectrum Principal peaks at wavenumbers 1155, 1248, 1724, 761, 1200, 755 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 72, 181, 85, 152, 42, 44, 58, 43.

Dose Methanthelinium bromide has been given in doses of 200 to 400 mg daily.

Methaphenilene

Antihistamine

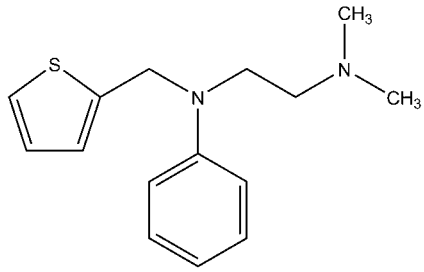
$C_{15}H_{20}N_2S$ = 260.4

CAS—493-78-7

IUPAC Name *N*′,*N*′-Dimethyl-*N*-phenyl-*N*-(thiophen-2-ylmethyl)ethane-1,2-diamine

Synonyms 00836; diatrin base; *N,N*-dimethyl-*N*′-phenyl-*N*′-(2-thenyl) ethylenediamine; *N,N*-dimethyl-*N*′-phenyl-*N*′-(2-thienylmethyl)-1,2-ethanediamine; RP-2740; W-50 base.

M



Chemical Properties Dark-yellow oil. Bp 183° to 185°. Methaphenilene is extracted by organic solvents from aqueous alkaline solutions.

Methaphenilene Hydrochloride

$C_{15}H_{20}N_2S,HCl = 296.9$
CAS—7084-07-3
Proprietary Names *Diatrine*; *Enstamine*; *Nilhistin*.
Chemical Properties A white-to-pale-yellow crystalline powder with a faint odour. Mp 184° to 189°. Soluble in water; sparingly soluble in ethanol and chloroform; almost insoluble in ether [Council on Pharmacy and Chemistry of the American Medical Association 1951].

Colour Tests Ammonium molybdate test—grey-blue (limit of detection, 1.0 µg); ammonium vandate test—orange→purple (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—dull purple (limit of detection, 1.0 µg); Vitali's test—red-brown/greenish-brown/brown (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.53 (location reagent acidified iodoplatinate solution, positive reaction).
Gas Chromatography System G2/225—retention time 1.30 (relative to diphenhydramine).
Disposition in the Body *N*-Oxide formation is a more important pathway in methaphenilene metabolism compared with methapyriline. This could explain the carcinogenicity of methapyrilene [Kammerer, Schmitz 1988] as well as the methylation of deoxycytosine [Hernandez *et al.* 1989].
Toxicity The estimated lethal dose in humans is 25 to 250 mg/kg. The LD_{50} (IP) in mice is 117 mg/kg. Methaphenilene (32 mmole) failed to induce tumours in F344 rats [Lijinsky, Kovatch 1986], although it was mutagenic in the Ames assay when tested with and without S9 activation [Kammerer *et al.* 1986], but it was non-mutagenic in Salmonella [Andrews *et al.* 1984]. Methaphenilene is a peroxisome proliferator [Reznik-Schuller, Lijinsky 1983].
Case Study

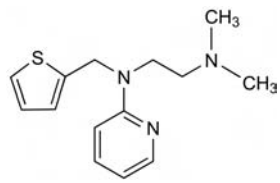
An 81-year-old man was taking methaphenilene for nasal congestion and cough. After 1 month of treatment he had a temperature of 101.4° and a pulse rate of 110 bpm. Three days later, he had a white blood cell count of 3600 with 99% lymphocytes and 1% monocytes. He was hospitalised immediately and started on a regimen of penicillin and blood transfusions. For 3 days his condition was critical, but improved on the 4th day. He was kept in hospital under observation for 19 days, then discharged [Drake 1950].

Dose Usually up to 200 mg daily.

Andrews AW *et al.* (1984). Mutagenicity of amine drugs and their products of nitrosation. *Mutat Res* 135: 105–108.
Council on Pharmacy and Chemistry of the American Medical Association NEW and nonofficial remedies: methaphenilene hydrochloride. *J Am Med Assoc* 147: 862.
Drake TG (1950). Agranulocytosis during therapy with the antihistaminic agent methaphenilene. *J Am Med Assoc* 142: 477.
Hernandez L *et al.* (1989). S-adenosylmethionine, S-adenosylhomocysteine and DNA methylation levels in the liver of rats fed methapyrilene and analogs. *Carcinogenesis* 10: 557–562.
Kammerer RC *et al.* (1986). Mutagenicity studies of selected antihistamines, their metabolites and products of nitrosation. *Food Chem Toxicol* 24: 981–985.
Kammerer RC, Schmitz DA (1988). A comparative in vitro metabolic study of methaphenilene and pyribenzamine. *Xenobiotica* 18: 1085–1096.
Lijinsky W, Kovatch RM (1986). Carcinogenicity studies of some analogs of the carcinogen methapyrilene in F344 rats. *J Cancer Res Clin Oncol* 112: 57–60.
Reznik-Schuller HM, Lijinsky W (1983). Methaphenilene, an analogue of the antihistaminic methapyrilene, is a "peroxisome proliferator". *Arch Toxicol* 52: 165–166.

Methapyrilene

Antihistamine
$C_{14}H_{19}N_3S = 261.4$
CAS—91-80-5
IUPAC Name *N*,*N*-Dimethyl-*N*′-2-pyridinyl-*N*′-(2-thienylmethyl)-1,2-ethanediamine
Synonym Thenylpyramine



Chemical Properties A liquid. pK_a 3.7, 8.9 (25°). Log *P* (octanol/water), 2.9.

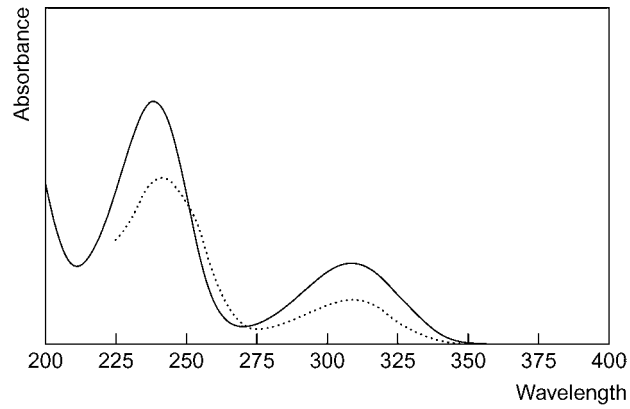
Methapyrilene Fumarate

$(C_{14}H_{19}N_3S)_2,3C_4H_4O_4 = 871.0$
CAS—33032-12-1
Chemical Properties A white crystalline powder. Mp 135° to 136°. Soluble 1 in 20 of water and 1 in 30 of ethanol.

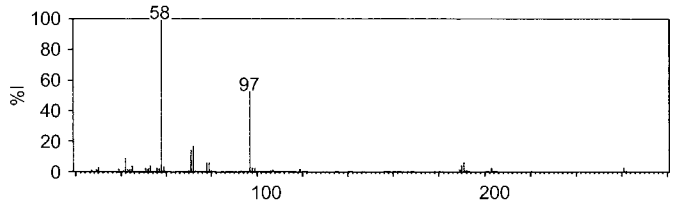
Methapyrilene Hydrochloride

$C_{14}H_{19}N_3S,HCl = 297.8$
CAS—135-23-9
Synonym Methapyrilenium chloride
Chemical Properties A white crystalline powder. Mp 162°. Soluble 1 in 0.5 of water, 1 in 5 of ethanol and 1 in 3 of chloroform; practically insoluble in ether and benzene.

Colour Tests Liebermann's reagent—red-brown; Mandelin's test—black-violet; Marquis test—black-violet; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.41; system TC—R_f 0.26; system TE—R_f 0.66; system TL—R_f 0.13; system TAE—R_f 0.21; system TAF—R_f 0.24; system TAJ—R_f 0.20; system TAK—R_f 0.00; system TAL—R_f 0.48 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, violet).
Gas Chromatography System GA—RI 1981; system GF—RI 2305.
High Performance Liquid Chromatography System HA—*k* 4.1; system HX—RI 342; system HY—RI 197.
Ultraviolet Spectrum Aqueous acid—237 (A^{1}_{1}=720a), 314 nm; aqueous alkali—241, 312 nm.



Infrared Spectrum Principal peaks at wavenumbers 1592, 765, 1558, 1250, 1158, 1316 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 97, 72, 71, 42, 191, 79, 78.



Quantification

Blood **GC** FID [Winek *et al.* 1977].
Plasma **GC** AFID. Limit of detection, 10 µg/L [Baselt, Franch 1980].
Urine **GC** Limit of detection, 50 µg/L, see Plasma [Baselt, Franch 1980]. See Blood [Winek *et al.* 1977].
Tissues **GC** See Blood [Winek *et al.* 1977].
Disposition in the Body Variably and incompletely absorbed after oral administration. <2% of a dose is excreted in the urine as unchanged drug in 24 h.
Therapeutic Concentration

After a single oral dose of 50 mg to 8 subjects, peak plasma concentrations of 0.006 to 0.05 mg/L (mean 0.024) were attained in about 1.5 h [Calandre *et al.* 1981].

Toxicity Methapyrilene has been shown to be carcinogenic in rats and is little used. Fatalities have occurred after ingestion of 7 g or more and toxic effects have been associated with plasma concentrations >30 mg/L. In 13 fatalities attributed to methapyrilene overdose, reported blood concentrations ranged from 2 to 380 mg/L (mean 50) (salicylamide and ethanol were also detected in several cases).

The following postmortem tissue concentrations were reported in one fatality: blood 9 mg/L, kidney 52 µg/g, liver 82 µg/g, lung 119 µg/g, urine 200 mg/L [Ainsworth, Biggs 1977].

Half-life Plasma half-life, about 1 to 2 h.
Volume of Distribution About 4 L/kg.
Clearance Plasma clearance, about 30 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, about 1.0.
Dose Methapyrilene hydrochloride has been given in doses of up to 200 mg daily.

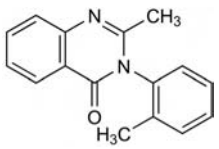
Ainsworth CA, Biggs JD (1977). A fatality involving methapyrilene. *Clin Toxicol* 11: 281–286.
Baselt RC, Franch S (1980). Plasma and urine concentrations of methapyrilene by nitrogen-phosphorus gas-liquid chromatography. *J Chromatogr* 183: 234–238.
Calandre EP *et al.* (1981). Methapyrilene kinetics and dynamics. *Clin Pharmacol Ther* 29: 527–532.
Winek CL *et al.* (1977). Methapyrilene toxicity. *Clin Toxicol* 11: 287–294.

Methaqualone

Hypnotic, Sedative
$C_{16}H_{14}N_2O = 250.3$
CAS—72-44-6

IUPAC Name 2-Methyl-3-(2-methylphenyl)-4(3*H*)-quinazolinone
Synonyms Methachalonum; methaqualonum; QZ-2; R-148; TR-495; CI-705; CN-38703.
Proprietary Names *Mequin*; *Normi-Nox*; *Pallidan*; *Quaalude*. It is an ingredient of *Toquilone Compositum*.

A preparation containing methaqualone and diphenhydramine hydrochloride was formerly marketed under the proprietary name *Mandrax*. Methaqualone has been withdrawn from the market in many countries because of problems with abuse.

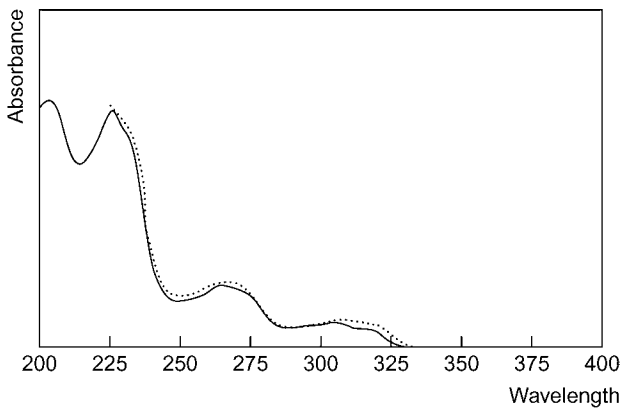


Chemical Properties A white crystalline powder. Mp 120° to 116°. Practically insoluble in water; soluble 1 in 12 of ethanol, 1 in 1 of chloroform and 1 in 50 of ether. pK_a 2.5. Log *P* (octanol/water), 4.3. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

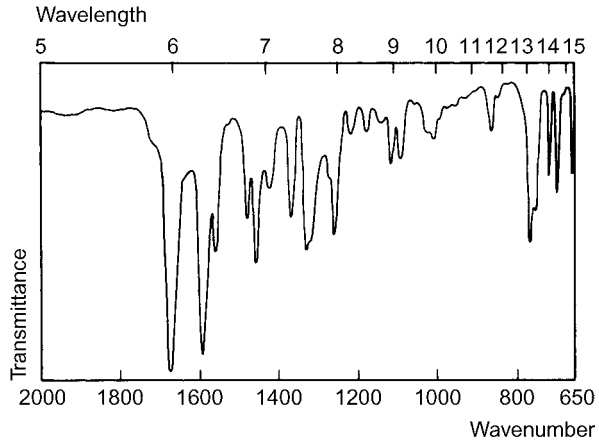
Methaqualone Hydrochloride

$C_{16}H_{14}N_2O,HCl = 286.8$
CAS—340-56-7
Chemical Properties A white crystalline powder. Mp 255° to 265°. Practically insoluble in water; soluble in ether and ethanol.

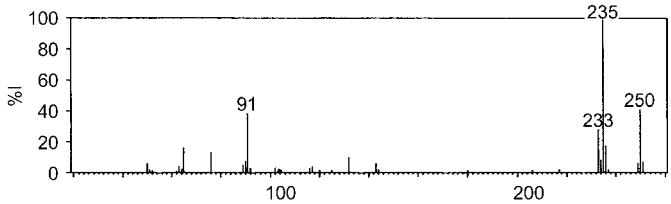
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.36; system TC—R_f 0.80; system TD—R_f 0.63; system TE—R_f 0.78; system TL—R_f 0.56; system TAE—R_f 0.79; system TAF—R_f 0.84 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—methaqualone RI 2135, M (2-formyl-) RI 2240, M (2-OH-methyl-) RI 2360, M (2-carboxy-) RI 2400, M (2′-OH-methyl-) RI 2410, M (3′-OH-) RI 2490, M (4′-OH-) RI 2510, M (6-OH-) RI 2525, M (OH-methoxy-) RI 2560; system GB—methaqualone RI 2256, M (2-formyl-)RI 2370, M (2-OH-methyl-) RI 2437, M (2′-OH-methyl-) RI 2500, M (OH-methoxy-) RI 2698; system GF—methaqualone RI 2580.
High Performance Liquid Chromatography System HA—*k* 0.2; system HX—RI 459; system HY—RI 400; system HZ—retention time 5.4 min; system HAX—retention time 6.8 min; system HAY—retention time 7.4 min.
Ultraviolet Spectrum Aqueous acid—234 (A_1^1=1320a), 269 nm; aqueous alkali—265 (A_1^1=347b), 306 nm.



Infrared Spectrum Principal peaks at wavenumbers 1682, 1599, 1565, 770, 1265, 697 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 235, 250, 91, 233, 236, 65, 76, 132; 4′-hydroxymethaqualone 251, 266, 249, 77, 143, 76, 252, 39.



Quantification

Blood **GC** UV detection or FID. Total amount of methaqualone and its metabolites. [Liu *et al.* 1994].
GC-MS [Plaut *et al.* 1998].
Plasma **GC** AFID. Methaqualone and the 2′-hydroxymethyl metabolite. Limit of detection, 20 µg/L [Peat, Finkle 1980].
HPLC UV detection. Limit of detection, 1 µg/L [Hux *et al.* 1982].
Urine **GC** See Blood [Liu *et al.* 1994].
GC-MS Limit of detection in urine, about 0.1 mg/L, see Blood [Plaut *et al.* 1998]. Comparison with immunoassays [Brenner *et al.* 1996]. Methaqualone and metabolites [Kazyak *et al.* 1977].
RIA–TLC Limit of detection, 1 mg/L for methaqualone and metabolites [Budd *et al.* 1980].
Gastric Contents **GC-MS** See Blood [Plaut *et al.* 1998].
Saliva **GC** See Plasma [Peat, Finkle 1980].
Hair **GC-MS** See Blood [Plaut *et al.* 1998].
Disposition in the Body Readily absorbed after oral administration and widely distributed throughout the body. The major metabolites are 4′-hydroxymethaqualone, 2′-hydroxymethylmethaqualone and their *O*-glucuronides or *O*-methyl ethers, and methaqualone *N*-oxide. A large number of metabolites are known and there is considerable intersubject variation in the relative amounts produced. About 40–50% of a dose is excreted in the urine in 72 h, mostly as conjugated metabolites, with <2% of a dose as unchanged drug. In 24 h, about 10% of a dose is excreted in the urine as the conjugated 4′-hydroxy metabolite, 7% as the *N*-oxide, 5% as the conjugated 2′-hydroxymethyl derivative and about 3% each as the conjugated 2-hydroxymethyl, 3′-hydroxy and 6-hydroxy metabolites. Up to 4% of a dose may be eliminated unchanged in the faeces.
Therapeutic Concentration In plasma, usually in the range 0.4–5 mg/L.
After a single oral dose of 250 mg of the base to 7 subjects, peak plasma concentrations of 1.0–4.0 mg/L (mean 2.2) were attained in 2 h; following an equivalent dose of the hydrochloride, peak plasma concentrations of 2.0–4.9 mg/L (mean 3.7) were attained in 1 h [Goenechea *et al.* 1973].
After daily oral doses of 300 mg to 8 subjects, a mean maximum steady-state plasma concentration of 3.7 mg/L was reported [Nayak *et al.* 1974].
Toxicity The estimated minimum lethal dose is 5 g in non-tolerant subjects. Drug accumulation is likely in chronic dosing because of the long half-life. Toxic effects may be associated with plasma concentrations >2 mg/L, and plasma concentrations greater than about 8 mg/L are likely to produce coma and may be lethal. The 2′-hydroxymethyl metabolite, which has been found unconjugated in both blood and urine in overdose cases, may contribute to the degree of intoxication.
Abuse of methaqualone, particularly when taken in conjunction with diphenhydramine, has been reported.

The following postmortem concentrations were reported in a 62-year-old man who died after ingesting methaqualone and diphenhydramine with suicidal intent: blood 800 mg/L and 20 mg/L, bile 30 and 13 mg/L, gastric contents 12 000 and 7 mg/L, respectively [Fucci 1996].

In 6 fatal cases, postmortem blood concentrations of 5–42 mg/L (mean 22) and liver concentrations of 26–89 μg/g (mean 55) were reported [Baselt, Cravey 1977].

In 19 mildly poisoned patients the plasma concentration on admission to hospital ranged from 2–20 mg/L (mean 9). In 9 more severe cases, a mean peak concentration of 27 mg/L was reached within 12 h of admission to hospital [Lawson, Brown 1966].

Half-life Plasma half-life, ≈20–60 h (mean 35); a slower terminal elimination half-life of up to ≈72 h has also been reported.
Volume of Distribution About 6 L/kg.
Distribution in Blood Plasma : whole blood ratio ≈1.1 at therapeutic concentrations decreasing to ≈0.5 at concentrations of 20 mg/L.
Saliva Plasma : saliva ratio, ≈9.
Protein Binding About 75–95% (concentration-dependent).
Note For a review of the pharmacokinetics of hypnotic drugs, see Breimer [1977].
Dose Usually up to 300 mg daily.

Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–103.

Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.

Brenner C *et al.* (1996). Comparison of methaqualone excretion patterns using Abuscreen ONLINE and EMIT II immunoassays and GC/MS. *Forensic Sci Int* 79: 31–41.

Budd RD *et al.* (1980). Mass screening and confirmation of methaqualone and its metabolites in urine by radioimmunoassay-thin-layer chromatography. *J Chromatogr* 190: 129–132.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fucci N (1996). A case of lethal intoxication after ingestion of toquilone compositum. *Am J Forensic Med Pathol* 17: 231–232.

Goenechea S *et al.* (1973). Gas chromatographic determination of methaqualone in small amounts of serum after ingestion of therapeutic doses (author's transl). *Arch Toxikol* 31 (1): 25–30.

Hux RA *et al.* (1982). Precolumns of amberlite XAD-2 for direct injection liquid chromatographic determination of methaqualone in blood plasma. *Anal Chem* 54: 113–117.

Kazyak L *et al.* (1977). Methaqualone metabolites in human urine after therapeutic doses. *Clin Chem* 23: 2001–2006.

Lawson AAH, Brown SS (1966). *Br Med J* 2: 1455–1456.

Liu F *et al.* (1994). Determination of methaqualone and its metabolites in urine and blood by UV, GC/FID and GC/MS. *Yao Xue Xue Bao* 29: 610–616.

Nayak RK *et al.* (1974). Methaqualone pharmacokinetics after single- and multiple-dose administration in man. *J Pharmacokinet Biopharm* 2: 107–121.

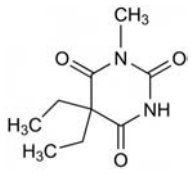
Peat MA, Finkle BS (1980). Determination of methaqualone and its major metabolite in plasma and saliva after single oral doses. *J Anal Toxicol* 4: 114–118.

Plaut O *et al.* (1998). Analysis of methaqualone in biological matrices by micellar electrokinetic capillary chromatography. Comparison with gas chromatography-mass spectrometry. *Forensic Sci Int* 92: 219–227.

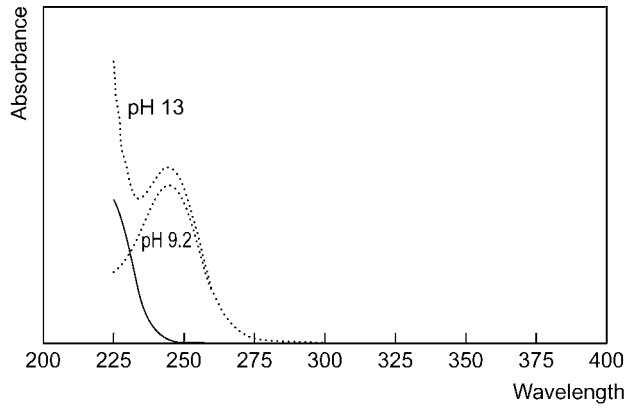
Metharbital

Anticonvulsant, Barbiturate
$C_9H_{14}N_2O_3$ = 198.2
CAS—50-11-3

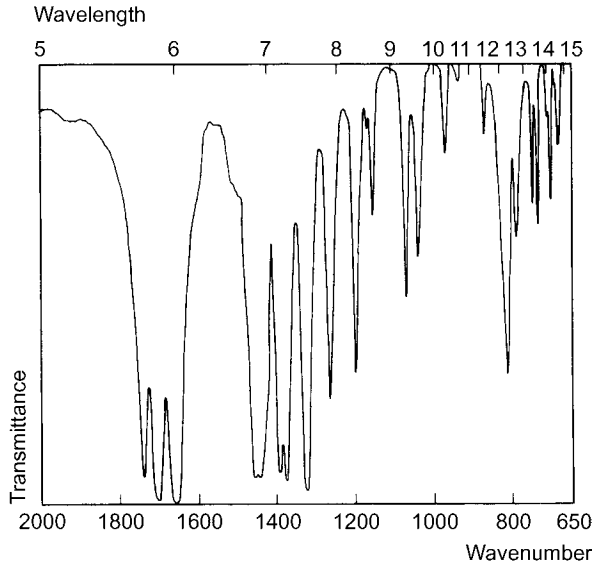
IUPAC Name 5,5-Diethyl-1-methyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Endiemal; metharbitone.
Proprietary Name *Gemonil*



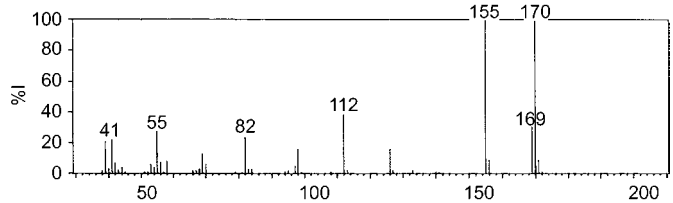
Chemical Properties A white crystalline powder. Mp 151° to 155°. Soluble 1 in 830 of water, 1 in 23 of ethanol and 1 in 40 of ether. pK_a 8.3 (20°). Log *P* (octanol/water), 1.2.
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.66; system TE—R_f 0.54; system TF—R_f 0.65; system TH—R_f 0.86; system TAD—R_f 0.69; system TAE—R_f 0.87 (mercurous nitrate spray, black; Zwikker's reagent, faint pink).
Gas Chromatography System GA—metharbital RI 1470.
High Performance Liquid Chromatography System HG—metharbital *k* 2.69, barbital*k* 1.11; system HH—metharbital*k* 1.99, barbital*k* 0.63; system HX—RI 435; system HY—RI 324.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—244 nm (A^1_1=433a); 1 mol/L sodium hydroxide (pH 13)—244 nm (A^1_1=458b).



Infrared Spectrum Principal peaks at wavenumbers 1655, 1699, 1755, 1275, 1205, 815 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 155, 170, 112, 169, 55, 82, 41, 39; barbital 156, 141, 55, 155, 98, 39, 82, 43.



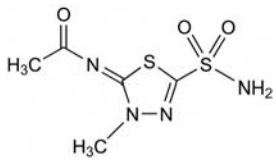
Quantification See under Amobarbital.
Disposition in the Body About 1% of a dose is excreted in the urine in 48 h as unchanged drug and about 10% as the active metabolite, barbital; the remainder is thought to be slowly excreted in the urine as barbital over a period of 2–3 weeks.
Therapeutic Concentration
Following daily oral doses of 300 mg for 14 days to 1 subject, metharbital reached a peak plasma concentration of 5 mg/L shortly after a dose, but was undetectable 8 h later when the plasma concentration of barbital was 26 mg/L [Butler, Waddell 1958].
Toxicity The estimated minimum lethal dose is 2 g.
Dose 100 to 300 mg daily.

Butler TC, Waddell WJ (1958). N-methylated derivatives of barbituric acid, hydantoin and oxazolidinedione used in the treatment of epilepsy. *Neurol Minneap* 8: 106–112.

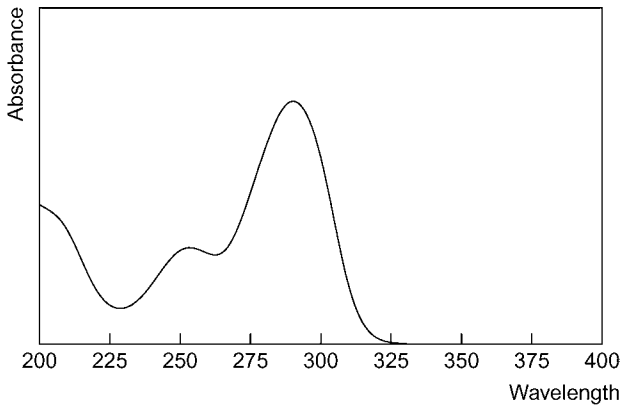
Methazolamide

Carbonic Anhydrase Inhibitor
$C_5H_8N_4O_3S_2$ = 236.3
CAS—554-57-4

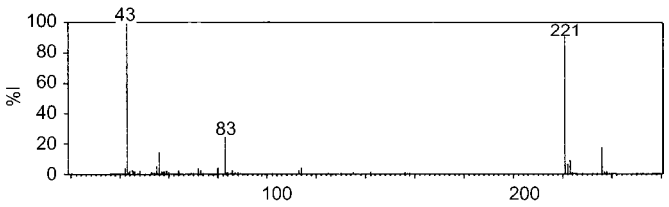
IUPAC Name *N*-(3-Methyl-5-sulfamoyl-1,3,4-thiadiazol-2-ylidene)acetamide
Synonym *N*-[5-(Aminosulfonyl)-3-methyl-1,3,4-thiadiazol-2(3*H*)-ylidene]-acetamide
Proprietary Names *GlaucTabs*; *MZM*; *Neptazane*.



Chemical Properties A white or faintly yellow, crystalline powder. Mp about 213°. Very slightly soluble in water and ethanol; slightly soluble in acetone; soluble in dimethylformamide. pK_a 7.3. Log *P* (ether/pH 7.4), −0.2.
Colour Test Koppanyi–Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.00; system TE—R_f 0.19; system TAE—R_f 0.84; system TAJ—R_f 0.44; system TAK—R_f 0.15; system TAL—R_f 0.78 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2187.
High Performance Liquid Chromatography System HX—RI 304; system HY—RI 265.
Ultraviolet Spectrum Aqueous acid—255, 290 nm (A^1_1=396a); aqueous alkali—288 nm (A^1_1=568a).



Mass Spectrum Principal ions at *m/z* 43, 221, 83, 236, 56, 223, 222, 55.



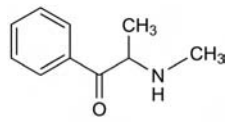
Quantification

Blood **GC-MS** UV detection [Iyer, Taft 1998]. See Bayne *et al.* [1981].
Plasma **GC-MS** See Blood [Iyer, Taft 1998]. See Blood. Limit of detection, <1 µg/L[Bayne *et al.* 1981].
Urine **GC-MS** See Blood [Iyer, Taft 1998; Bayne *et al.* 1981].
Disposition in the Body Well absorbed after oral administration. About 15–20% of a dose may be excreted in the urine in 24 h, of which about half is unchanged drug.
Therapeutic Concentration
After a single oral dose of 50 mg to 1 subject, peak concentrations of 19.1 and 0.08 mg/L in blood and plasma respectively were reported at 3 h; the corresponding concentrations, 3 h after a dose of 150 mg to a different subject were 36.1 and 1.4 mg/L. Following oral doses of 100 mg three times a day to 1 subject, steady-state concentrations of about 40 mg/L and about 9.5 mg/L were reported for blood and plasma, respectively [Bayne *et al.* 1981].
Distribution in Blood Plasma : erythrocyte ratio appears to be concentration-dependent, saturation of the carbonic anhydrase in the erythrocytes occurring at about 40 mg/L.
Protein Binding About 55%.
Dose In the treatment of glaucoma, 100 to 300 mg daily.

Bayne WF *et al.* (1981). Time course and disposition of methazolamide in human plasma and red blood cells. *J Pharm Sci* 70: 75–81.
Iyer GR, Taft DR (1998). Determination of methazolamide concentrations in human biological fluids using high performance liquid chromatography. *J Pharm Biomed Anal* 16: 1021–1027.

Methcathinone

Stimulant
$C_{10}H_{13}NO$ = 163.2
CAS—5650-44-2
IUPAC Name 2-(Methylamino)-1-phenylpropan-1-one
Synonyms 2-(Methylamino)-1-phenyl-1-propanone; α-methylaminopropiophenone; monomethylproprion.
Street Names Bathtub speed; cadillac express; catt; cosmos; ephedrone; gagers; gaggers; goob; jee cocktail; jeff; mulka; speed; The C; wild cat; wonder star.

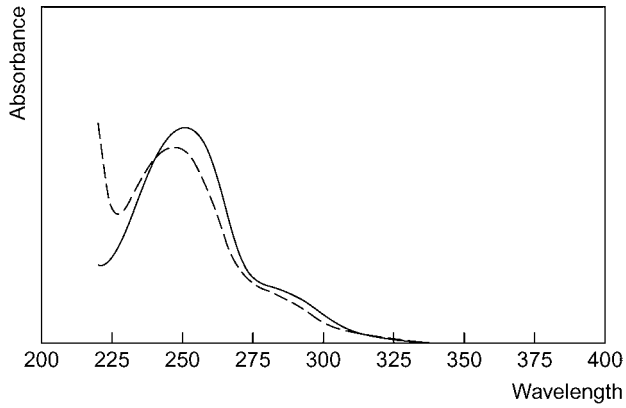


Chemical Properties Methcathinone is derived from (*S*)-(−)-cathinone, a natural compound found in young leaves of the khat shrub, *Catha edulis* (cathine). It is a white to off-white powder or colourless crystals. Mp 182° to 184°. Soluble in hydrochloric acid.

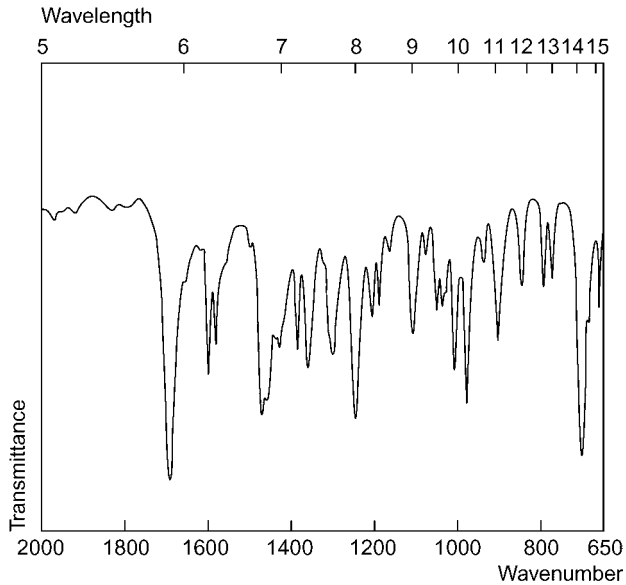
Methcathinone Hydrochloride

$C_{10}H_{13}NO,HCl$ = 199.7
CAS—49656-78-2
Synonym 2-(Methylamino)-1-phenyl-1-propanone hydrochloride
Chemical Properties Crystals from alcohol–acetone. Mp 176° to 177°. It is freely soluble in water and has a pungent taste.

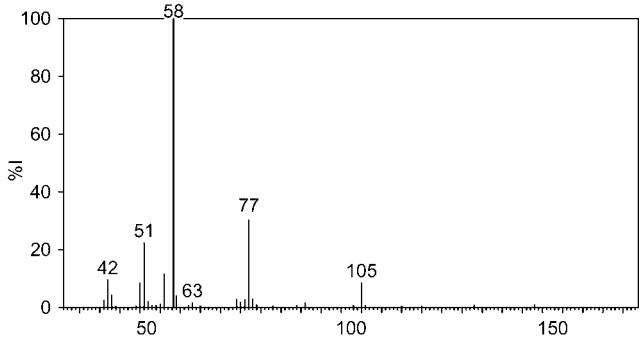
Colour Tests Chen's test—purple; cobalt thiocyanate (Scott's test)—faint blue; Lieberman's reagent—red/orange.
Gas Chromatography-Mass Spectrometry Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.9 mL/min. Temperature: 140°. Retention index: 1323 [Mills, Roberson 1993].
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid)—251 nm; basic—247 nm.



Infrared Spectrum Principal peaks at wavenumbers 1691, 705, 1245, 1469 cm^{-1} (KBr pellet).



Mass Spectrum Principal peaks at *m/z* 58, 77, 51, 56, 42, 105, 50, 43.



Quantification

Plasma **LC-MS** Column: SCX (150 × 2.1 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate (pH 3) : acetonitrile (95 : 5 for 17 min to 70 : 30 at 20 min for 1 min to 95 : 5 at 27 min), flow rate 1.5 mL/min. ESI, MRM acquisition mode. Retention time: ≈7 min. Limit of quantification, 11.8 µg/L [Beyer *et al.* 2007].

Urine **GC-MS** Column: DB-5 (15 m × 0.25 mm i.d.). Temperature programme: 140° for 1 min to 220° at 20°/min for 0.5 min to 250° at 30°/min for 2.5 min. IS: amphetamine-d_6. SIM acquisition mode at *m/z* 308, 280, 262 and 105 for methcathinone; *m/z* 294, 266, 248 for cathinone; *m/z*: 298, 270 for IS. Retention time (all as 4-carboethoxyhexafluorobutyryl derivatives): methcathinone 4.56 min, cathinone 4.60 min, IS 3.88 min. Limit of quantification, 12.5 µg/L [Paul, Cole 2001].

HPLC Column: Separon C_{18} (5 µm). Mobile phase: 0.2 mol/L orthophosphoric acid : methanol: diethylamine (75 : 20 : 1). UV detection (λ = 210 nm and 250 nm) [Bodrina *et al.* 1994].

Hair **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 90° for 3 min to 170° at 15°/min for 3 min to 210° at 25°/min for 1.5 min to 230° at 20°/min for 0.5 min to 300° at 35°/min for 0.5 min. EI ionisation mode at 70 eV, SIM acquisition mode. Limit of quantification, <80 pg/mg, limit of detection, 50 pg/mg [Kim *et al.* 2007]

LC-MS Column: LiChroCART Purospher 60 RP-18e (125 × 4 mm i.d., 5 mm i.d.). Mobile phase: acetonitrile with 0.1% formic acid : 0.1% formic acid (100 : 0 to 60 : 40 at 15 min to 100 : 0 at 15.2 min for 4.8 min), flow rate 1 mL/min. MSD, APCI, SIM acquisition mode. Retention time: 5.77 min. Limit of quantification, 0.3 ng/mg, limit of detection, 0.2 ng/mg [Stanaszek, Piekoszewski 2004].

Disposition in the Body The onset of effects occurs 10 min after intranasal administration of 80–250 mg methcathinone and lasts for 5–8 h. Both cathinone and methcathinone show activities similar to amfetamines.

Toxicity Dose-related neurotoxic and locomotor stimulant effects that are species and enantiomer dependent have been observed. Methcathinone shows a neurotoxic potential towards brain dopamine and/or serotonin neurones. Tolerance, craving and addiction have also been reported. The toxic dosage is estimated to be 80 to 250 mg by intranasal use.

Note For neurotoxic and pharmacologic studies of methcathinone in mice, see Sparago *et al.* [1996]; see also Glennon *et al.* [1987]. For occurrence of parkinsonism and dystonia following methcathinone, see Selikhova *et al.* [2008]. For occurrence of neurotoxicity following the ingestion of 'Russian cocktail', see Varlibas *et al.* [2009].

Dose Intranasal and IV routes, 500 to 1000 mg a day. Can be taken orally and by smoking.

Beyer J *et al.* (2007). Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. *J Mass Spectrom* 42: 150–160.

Bodrina DE *et al.* (1994). [The analysis of ephedrine and ephedrone in biological objects by chromatographic methods.]. *Sud Med Ekspert* 37: 23–26.

Glennon RA *et al.* (1987). Methcathinone: a new and potent amphetamine-like agent. *Pharmacol Biochem Behav* 26: 547–551.

Kim JY *et al.* (2007). Simultaneous determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 21: 1705–1720.

Mills TI, Roberson JC (1993). *Instrumental Data for Drug Analysis,* 2nd edn, Vol. 4-5. Boca Raton, FL: CRC Press.

Paul BD, Cole KA (2001). Cathinone (Khat) and methcathinone (CAT) in urine specimens: a gas chromatographic–mass spectrometric detection procedure. *J Anal Toxicol* 25: 525–530.

Selikhova M *et al.* (2008). Parkinsonism and dystonia caused by the illicit use of ephedrone: a longitudinal study. *Mov Disord* 23: 2224–2231.

Sparago M *et al.* (1996). Neurotoxic and pharmacologic studies on enantiomers of the *N*-methylated analog of cathinone (methcathinone): a new drug of abuse. *J Pharmacol Exp Ther* 279: 1043–1052.

Stanaszek R, Piekoszewski W (2004). Simultaneous determination of eight underivatized amphetamines in hair by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). *J Anal Toxicol* 28: 77–85.

Varlibas F *et al.* (2009). Neurotoxicity following chronic intravenous use of 'Russian cocktail'. *Clin Toxicol (Phila)* 47: 157–160.

Methdilazine

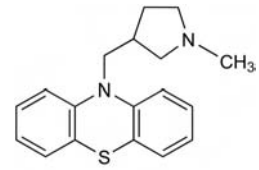
Antihistamine

$C_{18}H_{20}N_2S$ = 296.4

CAS—1982-37-2

IUPAC Name 10-[(1-Methyl-3-pyrrolidinyl)methyl]-10*H*-phenothiazine

Proprietary Names *Tacaryl* (chewable tablets); *Tacryl*.



Chemical Properties A light tan, crystalline powder. Mp 87° to 88°. Practically insoluble in water; soluble 1 in 2 of ethanol, 1 in 1 of chloroform and 1 in 8 of ether. pK_a 7.5. Log *P* (octanol/water), 5.2.

Methdilazine Hydrochloride

$C_{18}H_{20}N_2S$,HCl = 332.9

CAS—1229-35-2

Proprietary Names *Dilosyn*; *Tacaryl (syrup and tablets)*.

Chemical Properties A light tan, crystalline powder, which darkens on exposure to light. Mp 184° to 190°. Soluble 1 in 2 of water, 1 in 2 of ethanol and 1 in 6 of chloroform; practically insoluble in ether.

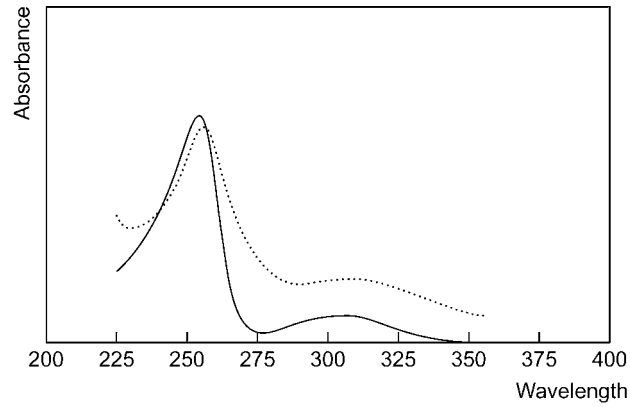
Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—red-orange; FPN reagent—red; Liebermann's reagent—green-brown; Mandelin's test—green→violet; Marquis test—red-violet.

Thin-layer Chromatography System TA—R_f 0.29; system TB—R_f 0.32; system TC—R_f 0.15; system TE—R_f 0.63; system TL—R_f 0.06; system TAJ—R_f 0.12; system TAK—R_f 0.19; system TAL—R_f 0.72 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2467; system GF—RI 2920.

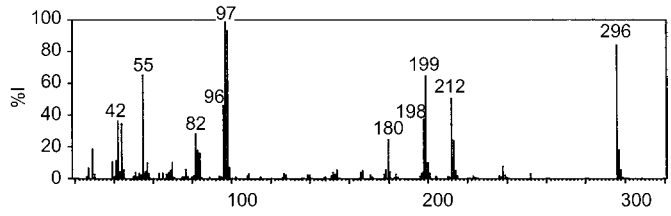
High Performance Liquid Chromatography System HA—*k* 6.0; system HAX—retention time 15.2 min; system HAY—retention time 6.7 min.

Ultraviolet Spectrum Aqueous acid—253 (A^1_1=982a), 302 nm; aqueous alkali—255, 308 nm.



Infrared Spectrum Principal peaks at wavenumbers 753, 1242, 1319, 1222, 1277, 1031 cm^{-1} (methdilazine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 97, 98, 296, 199, 55, 212, 96, 198.



Dose Usually 14.4 to 28.8 mg daily.

Methenamine

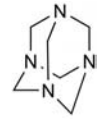
Antibacterial (Urinary)

$C_6H_{12}N_4$ = 140.2

CAS—100-97-0

Synonyms Aminoform; esammina; formine; hexamethylenamine; hexamethylenetetramine; hexamine; 1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}]decane; urotropine.

Proprietary Names *Antihydral*; *Uritone*; *Urotropina*.



Chemical Properties Colourless lustrous crystals or white crystalline powder. Mp 263°. Soluble 1 in 1.5 of water, 1 in 8 of ethanol (90%), 1 in 12 of chloroform and 1 in 320 of ether. Log *P* (octanol/water), −4.2.

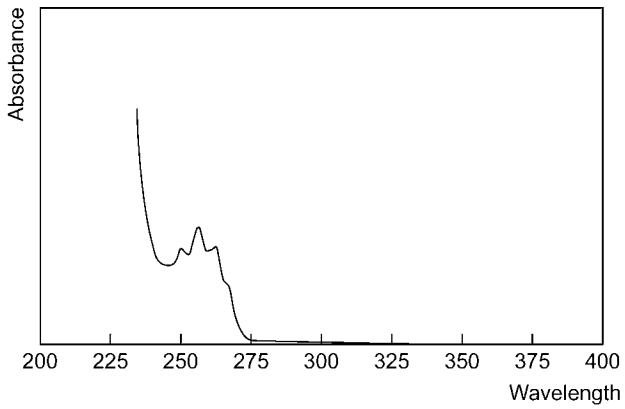
Methenamine Hippurate

$C_6H_{12}N_4,C_9H_9NO_3$ = 319.4
CAS—5714-73-8
Synonym Hexamine hippurate
Proprietary Names *Haiprex*; *Hiprex*; *Urex*; *Urotractan*; *Viapta*.
Chemical Properties A white crystalline powder. Mp 105° to 110°. Freely soluble in water and ethanol.

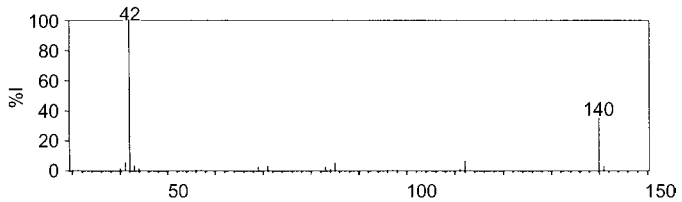
Methenamine Mandelate

$C_6H_{12}N_4,C_8H_8O_3$ = 292.3
CAS—587-23-5
Synonym Hexamine mandelate
Proprietary Names *Mandelamine*; *Purerin*; *Uromandelin*; *Uronamin*.
Chemical Properties A white crystalline powder. Mp 128° to 130°. Very soluble in water; soluble 1 in 10 of ethanol, 1 in 20 of chloroform and 1 in 350 of ether; slightly soluble in acetone.

Colour Test Mix 100 mg with an equal amount of salicylic acid and heat with 1 mL of sulfuric acid—a red colour is produced.
Thin-layer Chromatography System TA—R_f 0.30; system TB—R_f 0.04; system TC—R_f 0.13; system TL—R_f 0.03; system TAE—R_f 0.12; system TAF—R_f 0.12 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1210.
Ultraviolet Spectrum Aqueous acid—251, 257 (A^{1}_{1}=15.5b), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1232, 812, 671, 1010, 1044, 724 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 42, 140, 112, 41, 85, 43, 71, 141.



Quantification

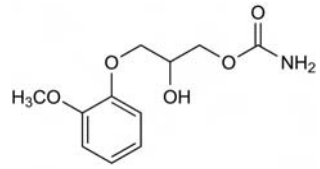
Serum **GC** Limit of detection, <5 mg/L [Nieminen *et al.* 1980].
Urine **Colorimetry** Methenamine and formaldehyde. Limit of detection, 25 mg/L for methenamine, 5 mg/L for formaldehyde [Gollamudi *et al.* 1979].
GC See Plasma [Nieminen *et al.* 1980].
Disposition in the Body Readily absorbed after oral administration and rapidly excreted unchanged in the urine. In acidic urine it is slowly hydrolysed, with the liberation of formaldehyde; this may also occur in the acid gastric secretions and 10 to 30% of a dose may be hydrolysed in the stomach.
Dose Usually 2 g of methenamine hippurate or 4 g of methenamine mandelate daily.

Gollamudi R *et al.* (1979). Simultaneous determination of methenamine and formaldehyde in the urine of humans after methenamine administration. *Biopharm Drug Dispos* 1: 27–36.
Nieminen AL *et al.* (1980). Determination of methenamine in biological samples by gas-liquid chromatography. *J Chromatogr* 181: 11–16.

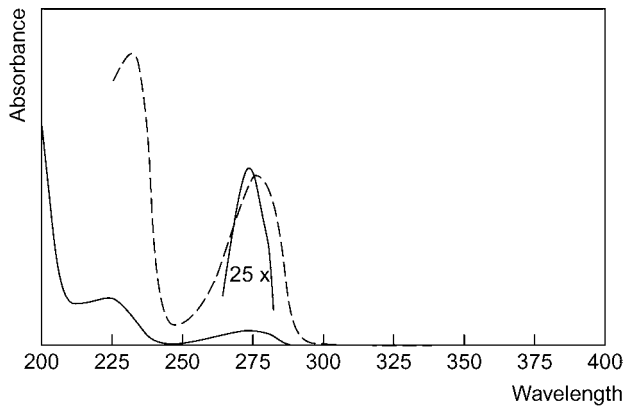
Methocarbamol

Muscle Relaxant
$C_{11}H_{15}NO_5$ = 241.2
CAS—532-03-6

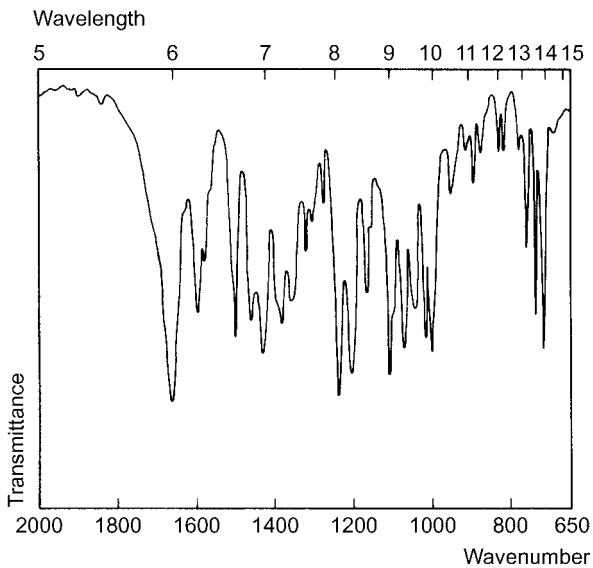
IUPAC Name [2-Hydroxy-3-(2-methoxyphenoxy)propyl] carbamate
Synonyms Guaifenesin carbamate; 3-(2-methoxyphenoxy)-1,2-propanediol 1-carbamate.
Proprietary Names *Delaxin*; *Labycarbol*; *Laxan*; *Lumirelax*; *Mebaxin*; *Musxan*; *Myosin*; *Myomethol*; *Ortoton*; *Robamol*; *Robaxin*; *Traumacut*. It is an ingredient of *Robaxisal*.



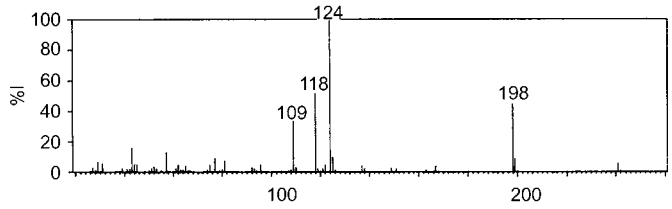
Chemical Properties A white powder. Mp about 94° to 90°. Soluble 1 in 40 of water; soluble in ethanol only with heating; sparingly soluble in chloroform; soluble in propylene glycol; practically insoluble in *n*-hexane. Log *P* (octanol/water), 0.6.
Colour Tests Liebermann's reagent—violet; Mandelin's test—green; Marquis test—blue-violet.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.00; system TD—R_f 0.07; system TE—R_f 0.47; system TF—R_f 0.23; system TAD—R_f 0.38; system TAE—R_f 0.83; system TAJ—R_f 0.41; system TAK—R_f 0.12; system TAL—R_f 0.72 (furfuraldehyde reagent, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—methocarbamol RI 2050, methocarbamol -AC RI 2145, M (guaifenesin) RI 1620.
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 345; system HY—RI 297; system HAX—retention time 5.0 min; system HAY—retention time 3.9 min.
Ultraviolet Spectrum Methanol—275 nm (A^{1}_{1}=101a).



Infrared Spectrum Principal peaks at wavenumbers 1667, 1248, 1119, 1215, 1010, 1080 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 124, 118, 198, 109, 43, 57, 125, 77.



Quantification

Blood **HPLC** UV detection [Sprague, Poklis 1980].
Plasma **HPLC** UV detection. Limit of detection, <1 mg/L [Weng *et al.* 1994].
Serum **Colorimetry** [Forist, Judy 1971].

Disposition in the Body Absorbed after oral administration. Metabolised by demethylation and *p*-hydroxylation followed by conjugation with glucuronic acid or sulfate. About 98% of a dose is excreted in the urine in 72 h, with <1% as unchanged drug and the remainder as free and conjugated metabolites; most of the excretion occurs in the first 8 h.

Therapeutic Concentration

Following a single oral dose of 2 g to 1 subject, a peak serum concentration of 25.8 mg/L was attained in 1 h [Forist, Judy 1971].

Toxicity

In a fatality due to ingestion of methocarbamol and alcohol, the following postmortem tissue concentrations were reported: blood, methocarbamol 525 mg/L, alcohol 1400 mg/L, salicylate 20 mg/L; urine, methocarbamol 575 mg/L [Kemal *et al.* 1982].

The following postmortem tissue concentrations of methocarbamol were reported in a fatality due to the ingestion of methocarbamol and ethanol: blood 257 mg/L, bile 927 mg/L. urine 255 mg/L, gastric content 3.7 g, liver 459 µg/g, kidney 83 µg/g; blood and urine ethanol concentrations were 1350 and 2490 mg/L respectively [Ferslew *et al.* 1990].

Half-life Plasma half-life, about 1 to 2 h.

Dose 4 to 6 g daily.

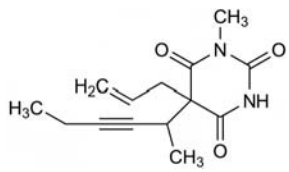
Ferslew KE *et al.* (1990). A fatal interaction of methocarbamol and ethanol in an accidental poisoning. *J Forensic Sci* 35: 477–482.
Forist AA, Judy RW (1971). Comparative pharmacokinetics of chlorphenesin carbamate and methocarbamol in man. *J Pharm Sci* 60: 1686–1688.
Kemal M *et al.* (1982). A fatal methocarbamol intoxication. *J Forensic Sci* 27: 217–222.
Sprague K, Poklis A (1980). *J Can Soc Forens Sci* 13: 31–36.
Weng N *et al.* (1994). Development and validation of a high-performance liquid chromatographic method for the determination of methocarbamol in human plasma. *J Chromatogr B Biomed Appl* 654: 287–292.

Methohexital

Anaesthetic, Barbiturate
$C_{14}H_{18}N_2O_3$ = 262.3
CAS—151-83-7; 18652-93-2

IUPAC Name 5-Hex-3-yn-2-yl-1-methyl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

Synonyms Methohexitone; 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione.



Chemical Properties A white to faintly yellowish-white, crystalline powder. Mp 92° to 96°. Very slightly soluble in water; slightly soluble in ethanol and chloroform. pK_a 8.3. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Methohexital Sodium

$C_{14}H_{17}N_2NaO_3$ = 284.3
CAS—309-36-4

Synonyms Enallynymalnatrium; sodium methohexitone.
Proprietary Names *Brevital Sodium*; *Brietal Sodium*.
Chemical Properties A white crystalline substance. Soluble in water.

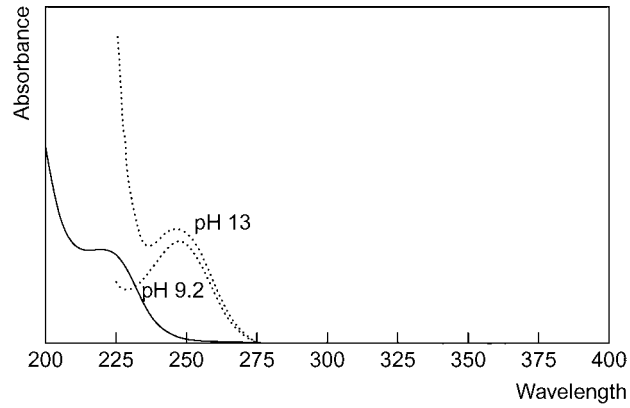
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—violet-brown/colourless.

Thin-layer Chromatography System TD—R_f 0.73; system TE—R_f 0.58; system TF—R_f 0.72; system TH—R_f 0.93; system TAD—R_f 0.71; system TAE—R_f 0.85 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, faint pink).

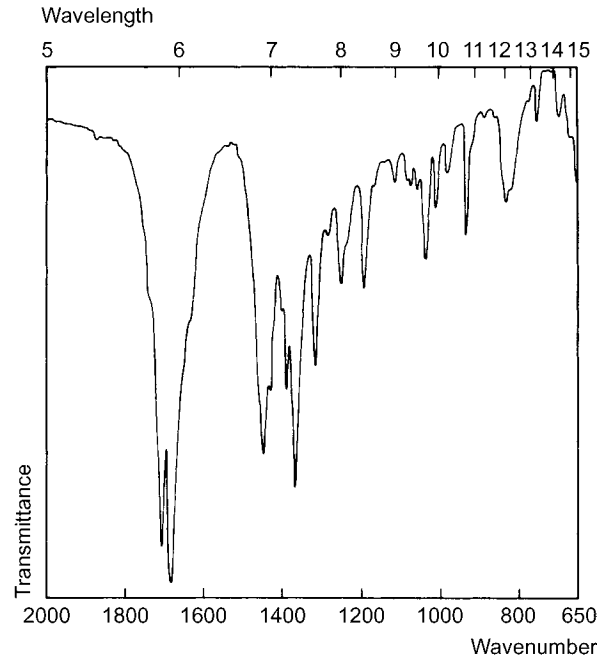
Gas Chromatography System GA—methohexital RI 1770, methohexital-Me RI 1735, M (OH-) RI 1880; system GB—methohexital RI 1827, methohexital-Me RI 1797; system GAJ—methohexital RRT 0.798 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HG—*k* 27.61; system HH—*k* 20.48; system HX—RI 503; system HY—RI 484.

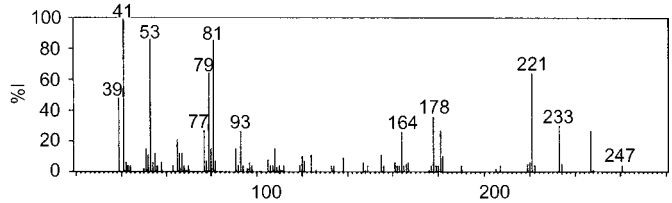
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—246 nm (A^1_1=276a); 1 mol/L sodium hydroxide (pH 13)—246 nm (A^1_1=308b).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1709, 1316, 1193, 1253, 1040 cm^{-1} (methohexital sodium).



Mass Spectrum Principal ions at *m/z* 41, 81, 53, 221, 79, 39, 178, 233.



Quantification

Blood **GC** AFID. Methohexital and 4′-hydroxymethohexital. Limit of detection, 100 µg/L in blood [Heusler *et al.* 1981].
Plasma **GC** Limit of detection, 50 µg/L in plasma, see Blood [Heusler *et al.* 1981].
Serum **GC** NSD. Limit of detection, 6 µg/L [Le Normand *et al.* 1988].
See also under Amobarbital.

Disposition in the Body Methohexital has a very short duration of action. It rapidly enters the brain and upon redistribution is localised in body fat but to a lesser extent than thiopental. <1% of a dose is excreted unchanged in the urine in 24 h; the major metabolite is 4′-hydroxymethohexital.

Therapeutic Concentration

Following an IV dose of 2 mg/kg of methohexital to 6 subjects, venous blood concentrations of 3.9 to 8.6 mg/L were reported at 1 to 3 min [Sunshine *et al.* 1966].

After a slow IV infusion of 3 mg/kg of methohexital sodium over a period of 60 min to 1 subject, a peak plasma concentration of about 3 mg/L was reported [Breimer 1976].

Following a rectal dose of 25 mg/kg methohexital as a 1% or 10% solution, the mean plasma concentration in 85 children was 6.5 and 4.7 μg/L, respectively, at 20 min and 4.9 and 4.1 μg/L at 30 min [Khalil *et al.* 1990].

Toxicity The estimated minimum lethal dose is 1 g.

The following postmortem tissue concentrations were reported in a fatal case involving the IV self-administration of methohexital: blood 103 mg/L, kidney 45 μg/g, liver 41 μg/g, spleen 41 μg/g; alcohol was also found in the blood [Finkle B, personal communication, 1967].

In a fatality due to the injection of about 300 mg of methohexital, postmortem blood and liver concentrations of 2 mg/L and 17 μg/g, respectively, were reported [Ryall 1981].

Half-life Plasma half-life, about 1 to 2 h.

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, about 12 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, about 1.5.

Protein Binding About 73%.

Dose For induction of anaesthesia, 50 to 120 mg of methohexital sodium, by slow IV injection.

Breimer DD (1976). Pharmacokinetics of methohexitone following intravenous infusion in humans. *Br J Anaesth* 48: 643–649.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Heusler H *et al.* (1981). Simultaneous determination of blood concentrations of methohexital and its hydroxy metabolite by gas chromatography and identification of 4′-hydroxymethohexital by combined gas–liquid chromatography–mass spectrometry. *J Chromatogr* 226: 403–412.

Khalil SN *et al.* (1990). Rectal methohexital: concentration and length of the rectal catheters. *Anesth Analg* 70: 645–649.

Le Normand Y *et al.* (1988). Quantitative analysis of serum methohexital by GLC using capillary column and nitrogen-selective detection. *Fundam Clin Pharmacol* 2: 551–558.

Ryall JE (1981). *Bull Int Assoc Forensic Toxicol* 16(2): 36–37.

Sunshine I *et al.* (1966). Distribution and excretion of methohexitone in man. A study using gas and thin layer chromatography. *Br J Anaesth* 38: 23–28.

Methoprotryne

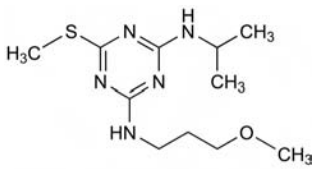
Herbicide

$C_{11}H_{21}N_5OS = 271.4$

CAS—841-06-5

IUPAC Name 4-*N*-(3-Methoxypropyl)-6-methylsulfanyl-2-*N*-propan-2-yl-1,3,5-triazine-2,4-diamine

Synonym 2-Isopropylamino-4-(3-methoxypropylamino)-6-methylthio-1,3,5-triazine

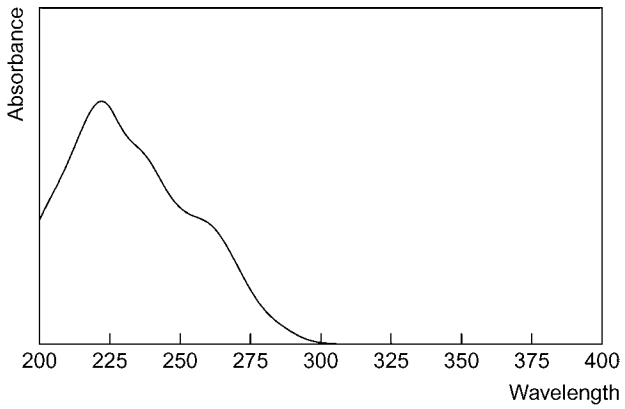


Chemical Properties A crystalline solid. Mp 68° to 70°. Soluble 1 in 3000 of water; soluble in most organic solvents. Log *P* (octanol/water), 2.8.

Thin-layer Chromatography System TA—R_f 0.74; system TX—R_f 0.19; system TY—R_f 0.11 (Dragendorff spray, positive).

Gas Chromatography System GA—RI 2098.

Ultraviolet Spectrum Principal peak at 225 nm.



Infrared Spectrum Principal peaks at wavenumbers 1538, 811, 1304, 1121, 1272, 1175 cm^{-1} (KBr disk).

Methoserpidine

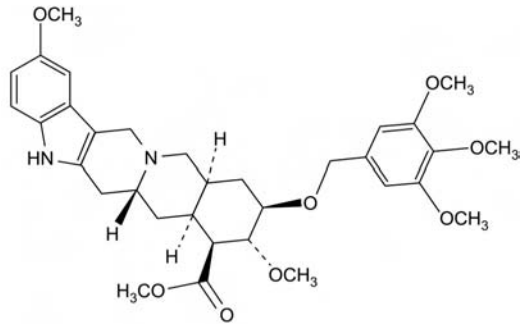
Antihypertensive

$C_{33}H_{40}N_2O_9 = 608.7$

CAS—865-04-3

Synonyms 10-Methoxydeserpidine; methyl 11-demethoxy-10-methoxy-18-*O*-(3,4,5-trimethoxybenzoyl)reserpate.

Proprietary Name *Decaserpyl*



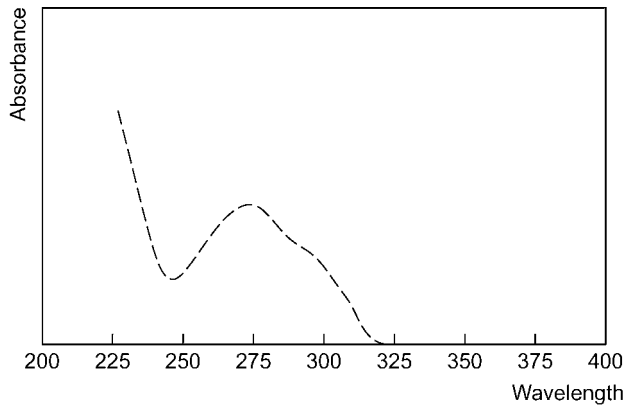
Chemical Properties A cream-coloured, hygroscopic, microcrystalline powder, which darkens on exposure to light. Mp about 171°, with decomposition. Practically insoluble in water; soluble 1 in 60 of ethanol, 1 in 5 of chloroform and 1 in 8 of dioxan.

Colour Tests Mandelin's test—grey-violet; Marquis test—grey.

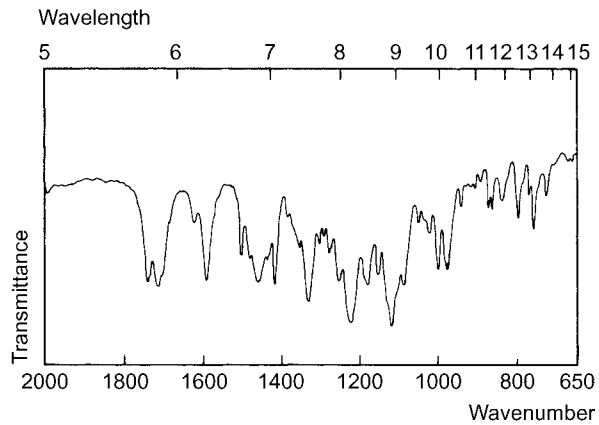
Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.04; system TC—R_f 0.77; system TL—R_f 0.64 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HA—*k* 0.5.

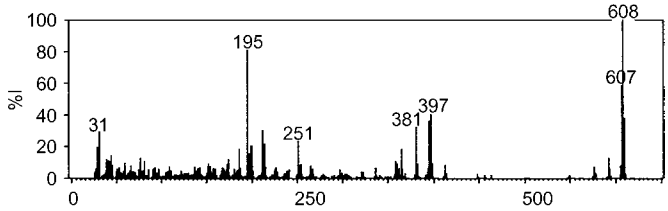
Ultraviolet Spectrum Dehydrated alcohol—273 nm (A_1^1=300a).



Infrared Spectrum Principal peaks at wavenumbers 1120, 1220, 1710, 1088, 1180, 1740 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 608, 195, 607, 397, 609, 395, 381, 396.



Dose 15 to 50 mg daily.

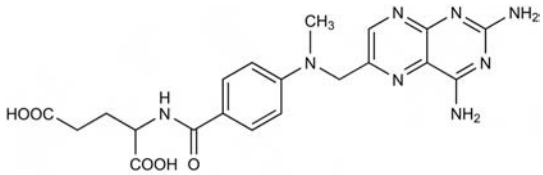
Methotrexate

Antineoplastic
$C_{20}H_{22}N_8O_5 = 454.4$
CAS—59-05-2

IUPAC Name *N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid

Synonyms Amethopterin; 4-amino-10-methylfolic acid; α-methopterin.

Proprietary Names *Abitrexate*; *Emthexat(e)*; *Farmitrexat*; *Folex*; *Lantarel*; *Ledertrexate*; *Maxtrex*; *Medsatrexate*; *Metex*; *Methoblastin(e)*; *Metrotex*; *Mexate*; *Novatrex*; *O-trexat*; *Rheumatrex*; *Rhodamer*; *Texate*; *Trexall*; *Trexan*; *Trexeron*; *Trixilem*; *Xaken*.



Chemical Properties A yellow to orange-brown, crystalline powder. Mp 182° to 189°. Practically insoluble in water, ethanol, chloroform and ether; very soluble in dilute solutions of alkali hydroxides and carbonates. pK_a 3.8, 4.8, 5.6. Log *P* (octanol/water), −1.8.

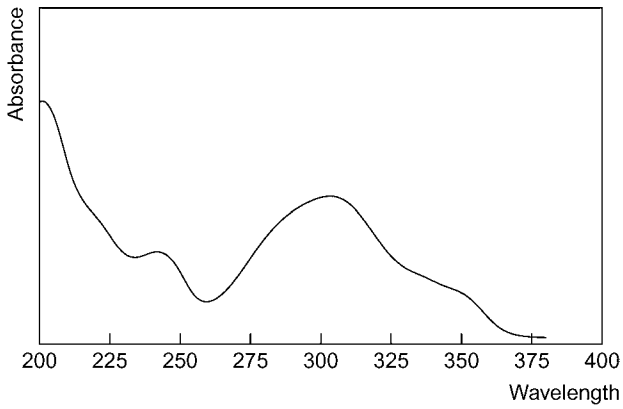
Caution Methotrexate is an irritant; avoid contact with skin and mucous membranes.

Colour Tests Mandelin's test—red; mercurous nitrate—black; Nessler's reagent—orange.

Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 292; system HAX—retention time 4.7 min; system HAY—retention time 2.9 min.

Ultraviolet Spectrum Aqueous acid—243 (A^1_1=372a), 307 nm (A^1_1=430a); aqueous alkali—258 (A^1_1=500a), 303 nm (A^1_1=500a).



Infrared Spectrum Principal peaks at wavenumbers 1600, 1511, 1634, 1305, 1200, 1538 cm^{-1} (Nujol mull).

Quantification

Plasma **HPLC** UV detection. Methotrexate and 7-hydroxymethotrexate. Limit of detection, 10 μg/L [Sparreboom *et al.* 1999]. Methotrexate and metabolites. Limit of detection, 0.1 nmol/L in plasma [Albertioni *et al.* 1995]. Comparison with fluorescence polarisation immunoassay [Najjar *et al.* 1992]. Methotrexate, 7-hydroxymethotrexate, folinic acid and N^5-methyltetrahydrofolate [van Tellingen *et al.* 1989]. Fluorescence detection. Limit of detection, 2×10^{-8} mol/L [Salamoun, Frantisek 1986]. UV detection. Methotrexate and 2 metabolites. Limit of detection, 50 μg/L [Breithaupt *et al.* 1982].

Serum **HPLC** UV detection. Methotrexate and 3 metabolites. Limit of detection, 12.5 μg/L for methotrexate [Farid *et al.* 1983]. UV detection. Methotrexate and 7-hydroxymethotrexate. Limit of detection, 100 μg/L for methotrexate [Lawson *et al.* 1981].

Radioimmunoassay Limit of detection, 450 ng/L [Aherne *et al.* 1977].

Urine **HPLC** Limit of detection, 10 nmol/L, see Plasma [Albertioni *et al.* 1995]. See Plasma [Salamoun, Frantisek 1986]; [Breithaupt *et al.* 1982]. See Serum [Farid *et al.* 1983].

HPLC Limit of detection, 0.2 μg/L [Turci *et al.* 2000].

Radioimmunoassay See Serum [Aherne *et al.* 1977].

Ascitic Fluid **HPLC** See Plasma [Salamoun, Frantisek 1986].

Biological Fluids **HPLC** See Plasma [van Tellingen *et al.* 1989].

CSF **HPLC** See Plasma [Breithaupt *et al.* 1982].

Radioimmunoassay See Serum [Aherne *et al.* 1977].

Saliva **HPLC** Limit of detection, 0.1 nmol/L, see Plasma [Albertioni *et al.* 1995].

Disposition in the Body When given in low doses, it is rapidly absorbed after oral administration to give plasma concentrations equivalent to those obtained by IV administration; higher doses may be less well absorbed. It is distributed mainly in the extracellular spaces but a proportion penetrates cell membranes and is strongly bound to dihydrofolate reductase. Small amounts of methotrexate diffuse into the CSF, higher concentrations being achieved with high doses. The major metabolite appears to be 4-amino-10-methylpteroic acid; 7-hydroxymethotrexate has been detected after high doses; 7-hydroxy-4-amino-10-methylpteroic acid has also been reported to be a metabolite. About 50 to 95% of a dose is excreted unchanged in the urine in 24 h (dose-dependent); after oral administration, about 30% of a dose may be excreted as metabolites as a result of the action of intestinal bacteria prior to absorption; after IV administration, up to about 10% of a dose is excreted in the urine as metabolites and up to about 15% of a dose may be excreted in the bile.

Therapeutic Concentration

After single oral doses of 15 mg/m^2 to 10 fasting subjects (children), peak serum concentrations of 0.18 to 0.73 mg/L (mean 0.41) were attained in 1 to 2 h [Pinkerton *et al.* 1980].

In 89 children receiving weekly oral methotrexate (20 mg/m^2) and daily oral mercaptopurine (75 mg/m^2), peak plasma concentrations ranged from 0.21 to 3.1 μmol/L (mean 1.2) for methotrexate and from 0.13 to 2.3 μmol/L (mean 0.56) for mercaptopurine [Balis *et al.* 1998].

Low-dose oral methotrexate (7.5 or 15 mg weekly, divided into 3 doses given at 12-hour intervals) was given to 24 subjects. In the first week, peak plasma concentrations were 94 to 137 nmol/L (mean 117) 1 to 2 h after the third dose of 2.5 mg and 161 to 215 nmol/L (mean 172) 1 to 2 h after the third dose of 5 mg. In week 13, peak plasma concentrations 1 to 2 h after the third dose of 2.5 mg or 5 mg were 88 to 152 nmol/L (mean 95) and 142 to 200 nmol/L (mean 157) [Chladek *et al.* 2002].

Toxicity Toxic effects are usually associated with plasma concentrations >4.5 mg/L, 24 h after a dose, or 0.45 mg/L, 48 h after a dose.

Half-life Plasma half-life, about 4 to 10 h; a longer terminal elimination phase of 10 to 70 h (mean 27) has also been reported.

Volume of Distribution About 0.8 L/kg.

Distribution in Blood Plasma : whole blood ratio, 0.9.

Protein Binding Variously reported as 50 to 95%.

Note For reviews of the clinical pharmacokinetics of methotrexate, see Shen and Azarnoff [1978]; Balis *et al.* [1983]; and Wang and Fujimoto [1984].

Dose 10 to 25 mg weekly, orally.

Aherne GW *et al.* (1977). Development and application of a radioimmunoassay for methotrexate. *Br J Cancer* 36: 608–617.

Albertioni F *et al.* (1995). Simultaneous quantitation of methotrexate and its two main metabolites in biological fluids by a novel solid-phase extraction procedure using high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 665: 163–170.

Balis FM *et al.* (1983). Clinical pharmacokinetics of commonly used anticancer drugs. *Clin Pharmacokinet* 8: 202–232.

Balis FM *et al.* (1998). Pharmacokinetics and pharmacodynamics of oral methotrexate and mercaptopurine in children with lower risk acute lymphoblastic leukemia: a joint children's cancer group and pediatric oncology branch study. *Blood* 92: 3569–3577.

Breithaupt H *et al.* (1982). Rapid high-pressure liquid chromatographic determination of methotrexate and its metabolites 7-hydroxymethotrexate and 2,4-diamino-N(10)-methylpteroic acid in biological fluids. *Anal Biochem* 121: 103–113.

Chladek J *et al.* (2002). Pharmacokinetics and pharmacodynamics of low-dose methotrexate in the treatment of psoriasis. *Br J Clin Pharmacol* 54: 147–156.

Farid YY *et al.* (1983). An assay for methotrexate and its metabolites in serum and urine by ion-pair high-performance liquid chromatography. *J Pharm Biomed Anal* 1: 55–63.

Lawson GJ *et al.* (1981). Rapid and simple method for the measurement of methotrexate and 7-hydroxymethotrexate in serum by high-performance liquid chromatography. *J Chromatogr* 223: 225–231.

Najjar TA *et al.* (1992). Comparison of a new high-performance liquid chromatography method with fluorescence polarization immunoassay for analysis of methotrexate. *Ther Drug Monit* 14: 142–146.

Pinkerton CR *et al.* (1980). Can food influence the absorption of methotrexate in children with acute lymphoblastic leukaemia? *Lancet* 2: 944–946.

Salamoun J, Frantisek J (1986). Determination of methotrexate and its metabolites 7-hydroxymethotrexate and 2,4-diamino-N10-methylpteroic acid in biological fluids by liquid chromatography with fluorimetric detection. *J Chromatogr* 378: 173–181.

Shen DD, Azarnoff DL (1978). Clinical pharmacokinetics of methotrexate. *Clin Pharmacokinet* 3: 1–13.

Sparreboom A *et al.* (1999). Liquid chromatographic analysis and preliminary pharmacokinetics of methotrexate in cancer patients co-treated with docetaxel. *J Chromatogr B Biomed Sci Appl* 735: 111–119.

Turci R *et al.* (2000). Determination of methotrexate in human urine at trace levels by solid phase extraction and high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 173–179.

van Tellingen O *et al.* (1989). Stable and sensitive method for the simultaneous determination of N5-methyltetrahydrofolate, leucovorin, methotrexate and 7-hydroxymethotrexate in biological fluids. *J Chromatogr* 488: 379–388.

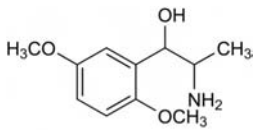
Wang YM, Fujimoto T (1984). Clinical pharmacokinetics of methotrexate in children. *Clin Pharmacokinet* 9: 335–348.

Methoxamine

Sympathomimetic
$C_{11}H_{17}NO_3 = 211.3$
CAS—390-28-3

IUPAC Name 2-Amino-1-(2,5-dimethoxyphenyl)propan-1-ol

Synonyms α-(1-Aminoethyl)-2,5-dimethoxybenzenemethanol; methoxamedrine.

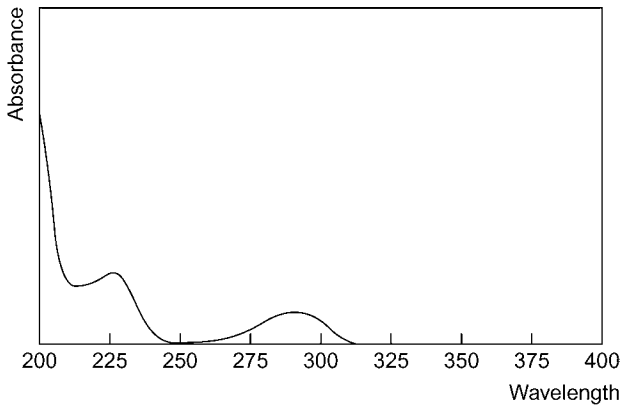


Chemical Properties pK_a 9.2 (25°). Log *P* (octanol/water), 0.4.

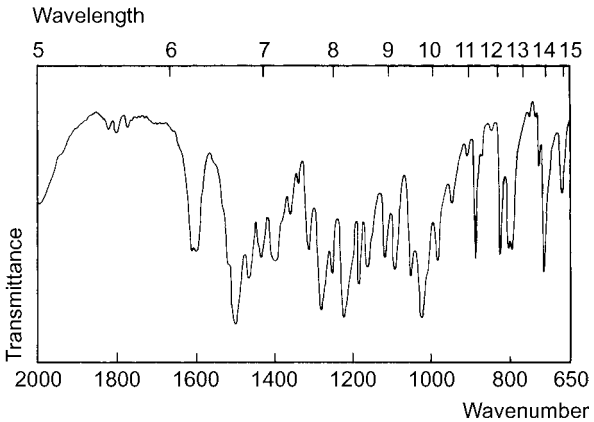
Methoxamine Hydrochloride

$C_{11}H_{17}NO_3$,HCl = 247.7
CAS—61-16-5
Proprietary Names *Vasoxine*; *Vasoxyl*.
Chemical Properties Colourless or white plate-like crystals or white crystalline powder. Mp 212° to 216°. Soluble 1 in 2.5 of water and 1 in 12 of ethanol; practically insoluble in benzene, chloroform and ether.

Colour Tests Mandelin's test—green-yellow; Marquis test—violet-brown→green.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.24; system TC—R_f 0.04; system TE—R_f 0.11; system TL—R_f 0.38; system TAE—R_f 0.12; system TAF—R_f 0.73; system TAJ—R_f 0.00; system TAK—R_f 0.12; system TAL—R_f 0.50 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1726.
High Performance Liquid Chromatography System HA—*k* 0.9; system HY—RI 228; system HAX—retention time 6.4 min; system HAY—retention time 3.6 min.
Ultraviolet Spectrum Aqueous acid—290 nm; water—290 nm (A_1^1=161a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1496, 1219, 1022, 1276, 1179, 1050 cm^{-1} (methoxamine hydrochloride, KBr disk).

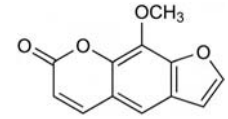


Mass Spectrum Principal ions at *m/z* 168, 137, 44, 139, 43, 152, 124, 167.
Dose 5 to 20 mg of methoxamine hydrochloride IM.

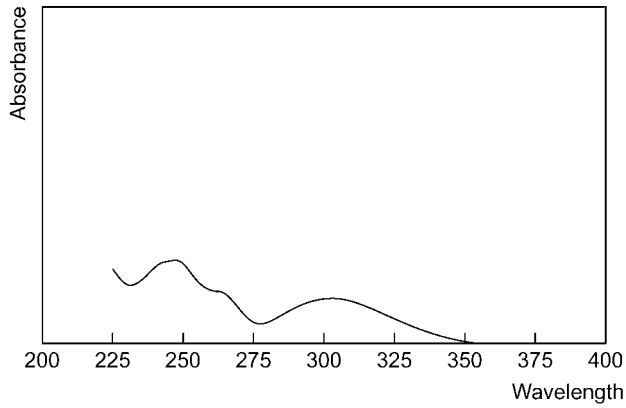
Methoxsalen

Pigmenting Agent
$C_{12}H_8O_4$ = 216.2
CAS—298-81-7

IUPAC Name 9-Methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one
Synonyms Ammoidin; 8-methoxypsoralen; metoxaleno; xanthotoxin.
Proprietary Names *Deltasoralen*; *Dermox*; *Geroxsalen*; *Meladinina*; *Meladinine*; *Mopsoralen*; *Oxsoralen*; *Puvasoralen*; *Ultramop*; *Uvadex*.



Chemical Properties A constituent of the fruits of *Ammi majus*. White to cream-coloured fluffy crystals. Mp 143° to 148°. Practically insoluble in cold water; sparingly soluble in boiling water and ether; soluble in boiling ethanol, acetic acid, propylene glycol, benzene and acetone; freely soluble in chloroform. Log *P* (octanol/water), 2.1.
Thin-layer Chromatography System TA—R_f 0.82; system TB—R_f 0.13; system TE—R_f 0.76; system TF—R_f 0.58; system TAE—R_f 0.82; system TAJ—R_f 0.82; system TAK—R_f 0.68; system TAL—R_f 0.96.
Gas Chromatography System GA—RI 1980.
High Performance Liquid Chromatography System HX—RI 457; system HZ—retention time 5.2 min.
Ultraviolet Spectrum Aqueous acid—247, 304 nm.



Infrared Spectrum Principal peaks at wavenumbers 1705, 1150, 1100, 1580, 1020, 1000 cm^{-1} (Nujol mull).

Quantification

Plasma **GC-MS** SIM [Cracco *et al.* 1992].

HPLC Fluorescence detection. Limit of detection, about 0.4 μg/L [Vielhauer *et al.* 1995]. UV detection. Limit of detection, 15 μg/L [Kucova *et al.* 1993]. Limit of detection, 1.5 μg/L [Ketchum *et al.* 1990]. Limit of detection, 10 μg/L [Gasparro *et al.* 1988].

Disposition in the Body

Therapeutic Concentration

After oral administration of 0.4 to 0.6 mg methoxsalen to 8 subjects, peak serum concentrations averaged 420 ± 80 μg/L within 40 min; during extracorporeal photophoresis, the concentration in the photoactivation chamber (so-called buffy coat fraction) was 134 μg/L [Shephard *et al.* 1999].

Plasma concentrations of methoxsalen after administration via various routes to 8 subjects were as follows: about 175 to 300 μg/L at 1 to 4 h after 0.6 or 1 mg/kg orally; about 5.5 μg/L 2 h after topical administration of a 0.1% cream; and about 1.2 μg/L 3 h after topical administration of a 3 mg/L bath [Tegeder *et al.* 2002].

In 10 subjects given 3 oral doses of 0.46 to 0.56 mg/kg methoxsalen in a liquid formulation, peak serum levels were about 650 μg/L in the fasted state, about 200 μg/L after a low-fat breakfast, and about 150 μg/L after a fat-rich breakfast; food delayed the time to reach peak plasma levels (30 min in the fasted state vs 1.5 to 3 h following a fat-rich meal) [Bech-Thomsen *et al.* 1992].

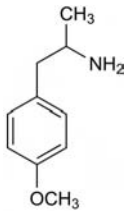
Dose 20 mg daily; doses of up to 50 mg have been given.

Bech-Thomsen N *et al.* (1992). The influence of food on 8-methoxypsoralen serum concentration and minimal phototoxic dose. *Br J Dermatol* 127: 620–624.
Cracco AT *et al.* (1992). Determination of 8-methoxypsoralen in plasma by gas chromatography-mass spectrometry using selected-ion monitoring. *J Chromatogr* 574: 156–160.
Gasparro FP *et al.* (1988). Rapid and sensitive analysis of 8-methoxypsoralen in plasma. *J Invest Derm* 90: 234–236.
Ketchum CH *et al.* (1990). Analysis of 8-methoxypsoralen by high-performance liquid chromatography. *Clin Chem* 36: 1956–1957.
Kucova D *et al.* (1993). High-performance liquid chromatographic determination of methoxsalen in plasma after liquid-solid extraction. *J Chromatogr* 614: 340–344.
Shephard SE *et al.* (1999). Pharmacokinetics of 8-methoxypsoralen during extracorporeal photopheresis. *Photodermatol Photoimmunol Photomed* 15: 64–74.
Tegeder I *et al.* (2002). Time course of 8-methoxypsoralen concentrations in skin and plasma after topical (bath and cream) and oral administration of 8-methoxypsoralen. *Clin Pharmacol Ther* 71: 153–161.
Vielhauer S *et al.* (1995). Evaluation and routine application of the novel restricted-access precolumn packing material Alkyl-Diol Silica: coupled-column high-performance liquid chromatographic analysis of the photoreactive drug 8-methoxypsoralen in plasma. *J Chromatogr B Biomed Appl* 666: 315–322.

Methoxyamfetamine

Hallucinogen, Phenethylamine
$C_{10}H_{15}NO = 165.2$
CAS—23239-32-9

IUPAC Name 1-(4-Methoxyphenyl)propan-2-amine
Synonyms 4-MA; *p*-methoxyamphetamine; 4-methoxyamphetamine; 4-methoxy-α-methylbenzeneethanamine; α-methyl-4-methoxyphenethylamine; PMA. Usually presented as 'Ecstasy'.



Chemical Properties A colourless oil. Practically insoluble in water; soluble in chloroform and dilute acids.

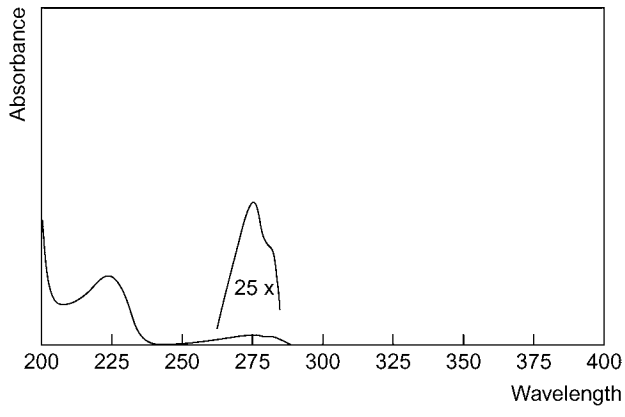
Methoxyamfetamine Hydrochloride

$C_{10}H_{15}NO,HCl = 201.7$
CAS—3706-26-1
IUPAC Name 1-(4-Methoxyphenyl)propan-2-amine hydrochloride
Chemical Properties White crystals. Soluble in water.

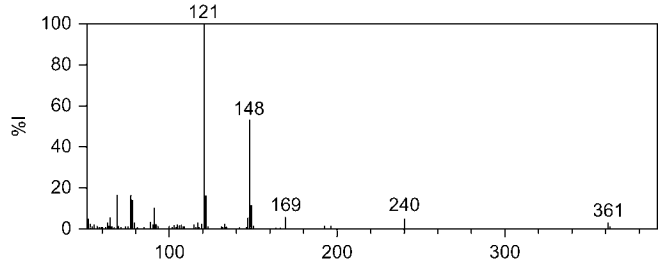
Colour Tests Mandelin's test—green; Marquis test—no colour change.
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.23; system TC—R_f 0.77; system TE—R_f 0.43; system TL—R_f 0.69; system TAE—R_f 0.09; system TAF—R_f 0.74 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1412; system GB—RI 1410.
Gas-Chromatography-Mass Spectrometry Column: HP-5MS (15 m × 0.25 mm i.d., 0.25 μm). Column temperature: 60° for 2 min to 280° at 15°/min for 3 min. Injector and detector temperatures: 250° and 300°, respectively. Pentafluoropropionic anhydride derivative RT 9.0 min. [Felgate *et al.* 1998].
High Performance Liquid Chromatography System HB—*k* 14.95; system HX—RI 274; system HY—RI 228.

Columns: μBondapack C_{18} (300 × 3.9 mm i.d.) or Ultracarb 5 C_{18} (150 × 4.6 mm i.d.) or Hypersil 5 Phenyl (150 × 4.6 mm i.d.). Mobile phase: 0.05 mol/L pH 3.0 phosphate buffer : acetonitrile (6 : 1), flow rate 1.5 mL/min for μBondapack and Ultracarb columns and 1.2 mL/min for Hypersil column (all separate analyses). UV detection (λ = 254 and 280 nm). Retention time: *p*-methoxyamfetamine (PAM) 6.5 min (μBondapack) and 4.5 min (Ultracarb and Hypersil). [Noggle *et al.* 1989].

Ultraviolet Spectrum Aqueous acid—274 nm (A^{1}_{1} = 80b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1512, 1259, 1033, 808, 1190, 1623 cm^{-1} (methoxyamfetamine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 121, 148, 122, 149, 169, 240, 361.



Quantification

Blood **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 60° for 2 min to 300° at 20°/min for 10 min. Scan mode. Limit of detection, 5–50 μg/L for PMA and other amfetamine-related drugs[Kudo *et al.* 2007].

LC-MS Column: Nucleodur Sphinx RP. Mobile phase: 10 mmol/L ammonium formate buffer : acetonitrile. PMA and other amfetamines. Limit of detection, 0.05–0.5 μg/L, limit of quantification, 2.5 μg/L [Fernández Mdel *et al.* 2009]. Column: phenyl type. Mobile phase: flow rate 0.3 mL/min. PMA and other amfetamine-related drugs [Mortier *et al.* 2002].

Urine **LC-MS** See Blood. Limit of quantification, 25 μg/L, limit of detection, 0.25–2.5 μg/L [Fernández Mdel *et al.* 2009]. See Blood [Mortier *et al.* 2002].

Hair **LC-MS** Column: LiChroCART Purospher 60 RP-18e (125 × 4 mm i.d., 5 mm). Mobile phase: acetonitrile : water both containing 0.1% formic acid (100 : 0 to 60 : 40 at 15 min to 100 : 0 at 15.2 min until 20 min), flow rate 1.0 mL/min. APCI, SIM acquisition mode. PMA and other amfetamines. Retention time: 7.35 min for PMA. Limit of detection, 0.20 μg/L for PMA [Stanaszek, Piekoszewski 2004].

Tissues **LC-MS** See Blood [Mortier *et al.* 2002].

Disposition in the Body Methoxyamfetamine is absorbed after oral administration. Approximately 80% of a dose is excreted in the urine in 24 h with up to 15% of the dose as unchanged drug, ~25% as free 4-hydroxyamfetamine, 50% as conjugated 4-hydroxyamfetamine, and 5% each as 4-hydroxynorephedrine and a conjugated *N*-oxidation product. The extent of *O*-demethylation appears to be genetically determined being decreased in poor metabolisers.

Toxicity Methoxyamfetamine is about 3 times as potent as methylenedioxyamfetamine and appears to be more toxic. At least 30 deaths worldwide have been reported caused by PMA toxicity, some involving other drugs. PMA can produce both CNS stimulation and hallucinogenic effects. Acute toxicity primarily results in hyperthermia but there may also be symptoms related to a 'serotonin syndrome' and some respiratory depression. Such symptoms may be produced at blood concentrations over 0.5 mg/L.

In 9 deaths from ingestion or IV administration of methoxyamfetamine, the following postmortem tissue concentrations were reported: blood 0.3–1.9 mg/L, bile 0.4–3 mg/L, liver 0.5–10.3 μg/g and urine 6–175 mg/L; alcohol was also detected in 4 cases [Cimbura 1974].

PMA concentrations in postmortem femoral blood ranged from 0.24–4.9 mg/L (mean 2.3 mg/L) in 6 deaths related to PMA poisoning. Corresponding liver concentrations ranged from 1.4–21 mg/kg (mean 8.9 mg/kg). Other amfetamines (including methamfetamine and MDMA) were detected in 5 of the 6 cases. All cases involved hyperthermia; in some cases rhabdomyolysis and haemorrhaging were also observed [Felgate *et al.* 1998]. See also Byard *et al.* [1998] and Ling *et al.* [2001].

In fatal PMA intoxication in Canada and the USA, PMA concentrations in postmortem blood were found to be 0.6 mg/L and 1.3 mg/L in 2 cases [Martin 2001] and 1.9 mg/L, 0.6 mg/L and 1.07 mg/L in 3 other cases [Kraner *et al.* 2001]. Other drugs of abuse were detected in 3 of these 5 cases.

In 3 deaths, levels of PMA were >0.3 mg/L and MDMA levels were >0.6 mg/L [Byard *et al.* 2002].

A 22-year-old man died after taking Ecstasy pills containing PMA and *p*-methoxymethylamfetamine (PMMA). Blood levels were 0.61 and 0.85 mg/L for PMA and PMMA, respectively [Becker *et al.* 2003].

Three cases of fatal intoxication with PMA and PMMA have been reported. Postmortem blood levels of PMA and PMMA, respectively, were 3.4 and 3.3 mg/kg (case 1) and 0.78 and 0.68 mg/kg (case 3). In case 2, death occurred 4 days after drug ingestion and only low levels of PMA were detected at postmortem [Johansen *et al.* 2003].

See also Byard *et al.* [1999], Caldicott *et al.* [2003], Chodorowski *et al.* [2002], Dams *et al.* [2003], Lamberth *et al.* [2008], Lora-Tamayo *et al.* [1997], Refstad [2003] and Voorspoels *et al.* [2002] for further reports of PMA-related deaths.

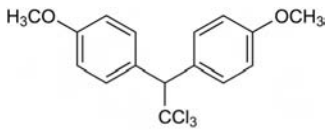
Becker J *et al.* (2003). A fatal paramethoxymethamphetamine intoxication. *Leg Med (Tokyo)* 5 (Suppl1): S138–S141.
Byard RW *et al.* (1998). Amphetamine derivative fatalities in South Australia: is 'Ecstasy' the culprit? *Am J Forensic Med Pathol* 19: 261–265.
Byard RW *et al.* (1999). Another PMA-related fatality in Adelaide. *Med J Aust* 170: 139–140.
Byard RW *et al.* (2002). Death and paramethoxyamphetamine: an evolving problem. *Med J Aust* 176: 496.
Caldicott DG *et al.* (2003). Dancing with 'death': *p*-methoxyamphetamine overdose and its acute management. *J Toxicol Clin Toxicol* 41: 143–154.
Chodorowski Z *et al.* (2002). [Fatal intoxication with paramethoxyamphetamine]. *Przegl Lek* 59: 379–380.
Cimbura G (1974). PMA deaths in Ontario. *CMAJ* 110: 1263–1267.
Dams R *et al.* (2003). Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. *J Anal Toxicol* 27: 318–322.
Felgate HE *et al.* (1998). Recent paramethoxyamphetamine deaths. *J Anal Toxicol* 22: 169–172.
Fernández Mdel M *et al.* (2009). High-throughput analysis of amphetamines in blood and urine with online solid-phase extraction-liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 33: 578–587.
Johansen SS *et al.* (2003). Three fatal cases of PMA and PMMA poisoning in Denmark. *J Anal Toxicol* 27: 253–256.
Kraner JC *et al.* (2001). Fatalities caused by the MDMA-related drug paramethoxyamphetamine (PMA). *J Anal Toxicol* 25: 645–648.
Kudo K *et al.* (2007). Simultaneous determination of 13 amphetamine related drugs in human whole blood using an enhanced polymer column and gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 855: 115–120.

Lamberth PG *et al.* (2008). Fatal paramethoxy-amphetamine (PMA) poisoning in the Australian Capital Territory. *Med J Aust* 188: 426.
Ling LH *et al.* (2001). Poisoning with the recreational drug paramethoxyamphetamine ('death'). *Med J Aust* 174: 453–455.
Lora-Tamayo C *et al.* (1997). Amphetamine derivative related deaths. *Forensic Sci Int* 85: 149–157.
Martin TL (2001). Case report: three cases of fatal paramethoxyamphetamine overdose. *J Anal Toxicol* 25: 649–651.
Mortier KA *et al.* (2002). Determination of paramethoxyamphetamine and other amphetamine-related designer drugs by liquid chromatography/sonic spray ionization mass spectrometry. *Rapid Commun Mass Spectrom* 16: 865–870.
Noggle FT *et al.* (1989). Liquid chromatographic and mass spectral analysis of 1-(3,4-methylenedioxyphenyl)-3-butanamines, homologues of 3,4-methylenedioxyamphetamines. *J Chromatogr Sci* 27: 607–611.
Refstad S (2003). Paramethoxyamphetamine (PMA) poisoning; a 'party drug' with lethal effects. *Acta Anaesthesiol Scand* 47: 1298–1299.
Stanaszek R, Piekoszewski W (2004). Simultaneous determination of eight underivatized amphetamines in hair by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). *J Anal Toxicol* 28: 77–85.
Voorspoels S *et al.* (2002). Resurgence of a lethal drug: paramethoxyamphetamine deaths in Belgium. *J Toxicol Clin Toxicol* 40: 203–204.

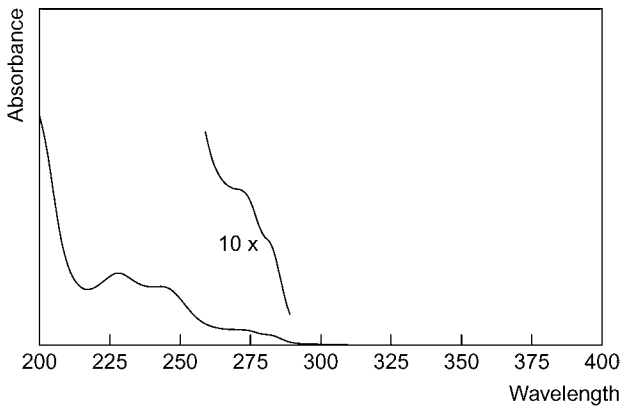
Methoxychlor

Insecticide
$C_{16}H_{15}Cl_3O_2$ = 345.7
CAS—72-43-5

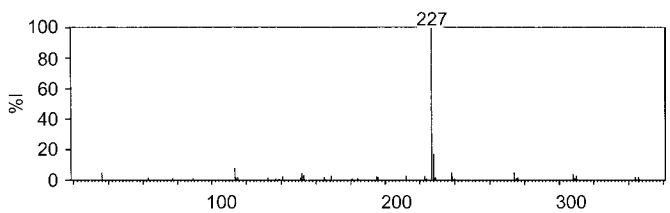
IUPAC Name 1-Methoxy-4-[2,2,2-trichloro-1-(4-methoxyphenyl)ethyl]benzene
Synonyms Dimethoxy-DT; DMDT; methoxy-DDT; 1,1′-(2,2,2-trichloroethylidene)bis[4-methoxybenzene].
Proprietary Name *Marlate*



Chemical Properties The technical product contains about 88% of methoxychlor and about 12% of related isomers. The pure *pp*′-isomer occurs as colourless crystals; the technical product is a grey powder. Mp 89°. Practically insoluble in water; soluble in ethanol and aromatic solvents; very soluble in ether. Log *P* (octanol/water), 5.1.
Colour Test Liebermann's reagent—black-violet.
Thin-layer Chromatography System TE—R_f 0.87; system TF—R_f 0.72; system TX—R_f 0.43; system TY—R_f 0.84; system TAA—R_f 0.65.
Gas Chromatography System GA—*op*′-methoxychlor RI 2417, methoxychlor RI 2445; system GK—*pp*′-methoxychlor RRT 1.46, *op*′-methoxychlor RRT 1.40 (both relative to caffeine).
High Performance Liquid Chromatography System HX—RI 900; system HAA—retention time 27.4 min.
Ultraviolet Spectrum Hexane—276 (A_1^1=100b), shoulder at 283 nm.



Mass Spectrum Principal ions at *m/z* 121, 227, 228, 114, 152, 122, 63, 165 (*op*′-methoxychlor); 227, 228, 114, 152, 212, 63, 169, 115 (*pp*′-methoxychlor).



Quantification
Serum **GC-MS** Methoxychlor and other pesticides [Dmitrovic *et al.* 2002].
Disposition in the Body Methoxychlor is less persistent than clofenotane. It is stored in the fat to a limited extent and disappears in 2 to 4 weeks after cessation of exposure. It is eliminated mainly in the faeces; small amounts are excreted in the urine.
Toxicity Methoxychlor is the least toxic of the chlorinated hydrocarbon insecticides. The maximum permissible atmospheric concentration is 10 mg/m^3 and the maximum permissible concentration in food is 14 µg/g.

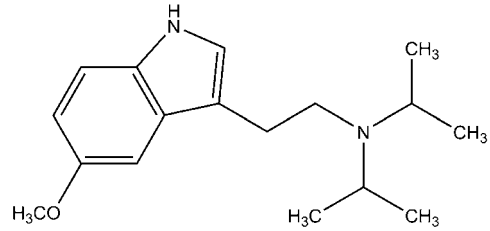
In a case of attempted suicide by ingestion of a product containing methoxychlor, a serum concentration of 0.67 mg/L was detected upon admission to hospital [Thompson, Vorster 2000].

Dmitrovic J *et al.* (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicol Lett* 134: 253–258.
Thompson TS, Vorster SJ (2000). Attempted suicide by ingestion of methoxychlor. *J Anal Toxicol* 24: 377–380.

5-Methoxy-*N*,*N*-diisopropyltryptamine

Hallucinogen
$C_{17}H_{26}N_2O$=274.4
CAS—4021-34-5

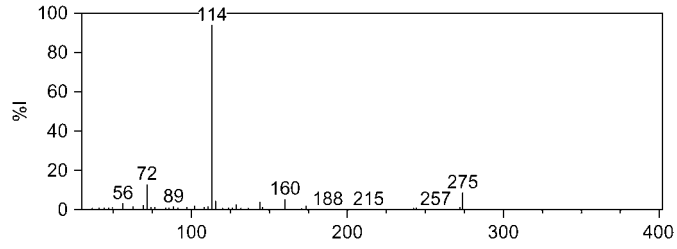
IUPAC Name *N*-[2-(5-Methoxy-1*H*-indol-3-yl)ethyl]-*N*-propan-2-ylpropan-2-amine
Synonyms 3-[2-(Diisopropylamino)ethyl]-5-methoxyindole; *N*,*N*-diisopropyl-5-methoxytryptamine; 5-MeO-DIPT; 5-methoxy-*N*,*N*-bis (1-methylethyl)-1*H*-indole-3-ethanamine.
Street Names Foxy Methoxy, Foxy.



5-Methoxy-*N*,*N*-diisopropyltryptamine Hydrochloride

$C_{17}H_{26}N_2O$,HCl = 310.9
CAS—2426-63-3
Chemical Properties White crystalline solid. Mp 180° to 181°. Log *P* (octanol/water), 3.73 [ACD 2007].

Colour Tests Ehrlich's reagent—purple→blue; Marquis test—yellow→black.
Infrared Spectrum Principal peaks at wavenumbers 731, 809, 826, 931, 1035, 1064 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 114, 72, 275, 160, 56, 89, 188, 215 [Meatherall, Sharma 2003].



Quantification
Blood **GC-MS** Column: HP-1MS fused silica capillary 100% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 2 min to 300° at 20°/min for 5 min. SIM acquisition mode. Limit of detection, 1 µg/L [Ishida *et al.* 2005].

LC-MS Column: Luna phenylhexyl (100 × 2.1 mm i.d., 3 µm). Mobile phase: 20 mmol/L ammonium formate (pH 4.3) : acetonitrile (75 : 25 for 1 min to 60 : 40 at 3 min until 7 min), flow rate 300 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 10 µg/L [Vorce, Sklerov 2004].
Plasma **GC-MS** Column: HP-5MS (19 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 1 min to 300° at 15°/min for 5 min. EI ionisation at 70 eV. Limit of quantification not reported [de Boer *et al.* 2001].
Urine **GC-MS** See Blood. Limit of detection, 5 µg/L [Ishida *et al.* 2005].

LC-MS Column: Semi-micro L-column ODS (150 × 1.5 mm i.d.). Mobile phase: methanol : 10 mmol/L ammonium formate (pH 3.5; 25 : 75 to 40 : 60 in 5 min), flow rate 0.1 mL/min. ESI, full scan mode, SIM acquisition mode. Limit of detection, 3 µg/L for 5-MeO-DIPT, 5-MeO-*N*-isopropyltryptamine (5-MeO-NIPT), 5-OH-

M

DIPT, and 30 μg/L for 6-OH-5-MeO-DIPT [Kamata *et al.* 2006]. See Blood. Limit of detection, 5 μg/L [Vorce, Sklerov 2004].

Other **GC-MS** Microsomes. Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: 0.6 mL/min. Temperature programme: 50° for 3 min to 310° at 40°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Theobald, Maurer 2007].

Disposition in the Body Metabolised via *O*-demethylation of the side chain to 5-OH-DIPT (major metabolite) followed by part conjugation to form its sulfate and glucuronide. 5-OH-DIPT undergoes further hydroxylation to form 5,6-$(OH)_2$-DIPT, which can then form 6-OH-5-MeO-DIPT (major metabolite) through methylation. This is also achieved by direct hydroxylation of 5-MeO-DIPT. 6-OH-5-MeO-DIPT is then conjugated to its sulfate and glucuronide. Another pathway is through *N*-deisopropylation to 5-MeO-*N*-isopropyltryptamine. Other possible metabolites include 5-methoxyindoleacetic acid and 5-hydroxyindoleacetic acid, formed by oxidative deamination. However, these may not be as useful for forensic or clinical purposes since they are known to be present at low concentrations in the urine of healthy humans [Kamata *et al.* 2006].

Toxicity 5-Methoxy-DIPT has CNS activity and can produce some euphoria, visual or auditory distortions, and hallucinations [Shulgin, Carter 1980].

A 5-MeO-DIPT urine level of 229 μg/L was found in an individual who had admitted to use of α-methyltryptamine (AMT) prior to submitting the sample [Vorce, Sklerov 2004].

A 23-year-old Caucasian male who had ingested a capsule containing 5-MeO-DIPT had serum and urine concentrations of 0.14 mg/L and 1.6 mg/L, respectively, for the drug and a urinary concentration of 0.17 mg/L for the 5-methoxyindoleacetic acid metabolite [Wilson *et al.* 2005].

A 21-year-old Caucasian male who had ingested a tablet containing 5-MeO-DIPT had a urine concentration of 1.7 mg/L for the drug and a concentration of 1.3 mg/L for the 5-methoxyidoleacetic acid metabolite [Meatherall, Sharma 2003].

In a fatal poisoning with 5-MeO-DIPT, the drug and 2 of its metabolites, 5-OH-DIPT and 5-MeO-NIPT, were identified by LC-MS. The concentrations of 5-MeO-DIPT, 5-OH-DIPT and 5-MeO-NIPT were 0.412, 0.327 and 0.02 mg/L, respectively, in blood and 1.67, 27.0 and 0.32 mg/L, respectively, in urine [Tanaka *et al.* 2006].

ACD (2007) ACD/LogP DB. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).
deBoer D *et al.* (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Sci Int* 121: 47–56.
Ishida T *et al.* (2005). Sensitive determination of alpha-methyltryptamine (AMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 823: 47–52.
Kamata T *et al.* (2006). Metabolism of the psychotomimetic tryptamine derivative 5-methoxy-*N*,*N*-diisopropyltryptamine in humans: identification and quantification of its urinary metabolites. *Drug Metab Dispos* 34: 281–287.
Meatherall R, Sharma P (2003). Foxy, a designer tryptamine hallucinogen. *J Anal Toxicol* 27: 313–317.
Shulgin A, Carter T, MF (1980). *N*, *N*-Diisopropyltryptamine (DIPT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT). Two orally active tryptamine analogs with CNS activity. *Commun Psychopharmacol* 4: 363–369.
Tanaka E *et al.* (2006). A fatal poisoning with 5-methoxy-*N*,*N*-diisopropyltryptamine. *Foxy. Forensic Sci Int* 163: 152–154.
Theobald D, Maurer S, HH (2007). Identification of monoamine oxidase and cytochrome P450 isoenzymes involved in the deamination of phenethylamine-derived designer drugs (2C-series). *Biochem Pharmacol* 73: 287–297.
Vorce S, Sklerov P, JH (2004). A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray-MS. *J Anal Toxicol* 28: 407–410.
Wilson JM *et al.* (2005). A foxy intoxication. *Forensic Sci Int* 148: 31–36.

5-Methoxy-*N*,*N*-dimethyltryptamine

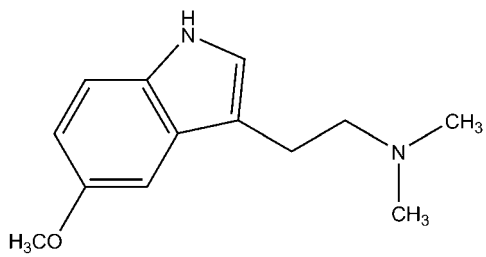
Hallucinogen

$C_{13}H_{18}N_2O$ = 218.3

CAS—1019-45-0

IUPAC Name 2-(5-Methoxy-1*H*-indol-3-yl)-*N*,*N*-dimethylethanamine

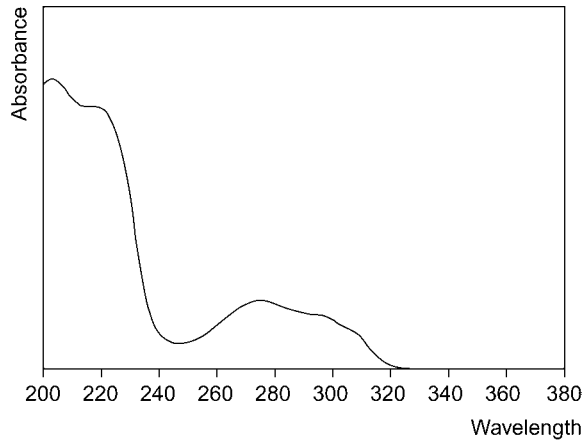
Synonyms CT 4334; 5-MeO-DMT; methoxybutofenin; methylbufotenine.



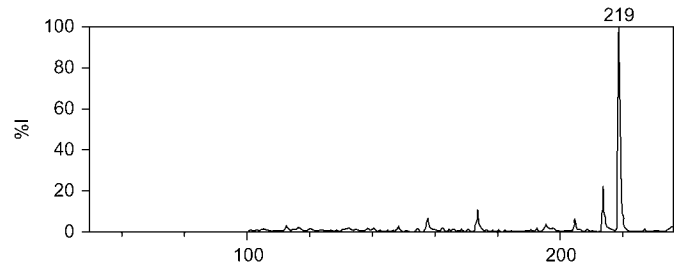
Chemical Properties Solid. Mp 69° to 70°. Log *P* (octanol/water), 2.02 [Meylan, Howard 1995]. Urine samples could be stored at 2 to 8° for up to 7 days or up to a month at -20° [Pomilio *et al.* 1999].

Colour Test Ehrlich's test—purple→blue; Marquis test—yellow→black.

Ultraviolet Spectrum Aqueous acid—203, 217, 275 nm.



Mass Spectrum Principal ions at *m/z* 58, 160, 218, 117, 154, 130, 42, 89.



Quantification

Blood **LC-MS** Column: XTerra MS-C_{18} (100 × 3.0 mm, 3.5 μm). Mobile phase: 0.02 mol/L ammonium formate : acetonitrile (75 : 25), flow rate 0.4 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 10 μg/L, limit of detection, 5 μg/L [Sklerov *et al.* 2005].

Urine **GC-MS** Column: SPB-1 fused silica capillary (30 m × 0.20 mm i.d.). Carrier gas: He. Temperature programme: 60° for 1 min to 290° at 10°/min for 5 min. Limit of detection not reported [Pomilio *et al.* 1999].

LC-MS See Blood [Sklerov *et al.* 2005]. Column: Brownlee O Spheri-5 RP-18 (100 × 1.0 mm i.d., 5 μm). Mobile phase: methanol : water with 0.2% formic acid (50 : 50), flow rate 40 μL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.1 μg/L [Forsstrom *et al.* 2001].

Bile **LC-MS** See Blood [Sklerov *et al.* 2005].

Gastric Contents **LC-MS** See Blood [Sklerov *et al.* 2005].

Brain **LC-MS** See Blood [Sklerov *et al.* 2005].

Kidney **LC-MS** See Blood [Sklerov *et al.* 2005].

Liver **LC-MS** See Blood [Sklerov *et al.* 2005].

Other **LC-MS** Frog Skin Secretions. Column: Luna C_{18} (2) (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05% trifluroacetic acid : water–acetonitrile–trifluroacetic acid (20 : 80 : 0.05; 100 : 0 to 0 : 100 over 240 min), flow rate 0.5 mL/min. ESI, positive ion mode. Limit of detection not reported [McClean *et al.* 2002].

Note For a study of the characterisation and determination of indole alkaloids in frog skin secretions, see McClean *et al.* [2002]. For information on the human intranasal, sublingual and oral pharmacology of 5-MeO-DMT, see Ott [2001].

Toxicity

A 25-year-old white male ingested a preparation from a South American tree bark, 'ooasca' and 4 h later he ingested tryptamines. He went to sleep and was found dead the following morning. The toxicological findings are in the table overleaf.

The medical examiner ruled cause of death was hallucinogenic amine intoxication and manner of death was undetermined [Sklerov *et al.* 2005].

Forsstrom T *et al.* (2001). Determination of potentially hallucinogenic *N*-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 61: 547–556.
McClean S *et al.* (2002). Characterisation and determination of indole alkaloids in frog-skin secretions by electrospray ionisation ion trap mass spectrometry. *Rapid Commun Mass Spectrom* 16: 346–354.
Meylan W, Howard M, PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Ott J (2001). Pharmepena-psychonautics: human intranasal, sublingual and oral pharmacology of 5-methoxy-*N*,*N*-dimethyl-tryptamine. *J Psychoactive Drugs* 33: 403–407.
Pomilio AB *et al.* (1999). Ayahoasca: an experimental psychosis that mirrors the transmethylation hypothesis of schizophrenia. *J Ethnopharmacol* 65: 29–51.
Sklerov J *et al.* (2005). A fatal intoxication following the ingestion of 5-methoxy-*N*,*N*-dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 29: 838–841.

Substance	Heart blood (mg/L)	Peripheral blood (mg/L)	Gastric (mg/L)	Bile (mg/L)	Brain (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	Urine (mg/L)
DMT	0.02	0.01	3.3	0.57	ND	ND	ND	0.89
5-MeO-DMT	1.88	1.20	202	9.81	0.15	22.8	16.4	9.59
Tetrahydroharmine	0.38	0.24	12.5	4.78	0.43	6.89	13.24	6.02
Harmaline	0.07	0.04	6.4	0.41	0.04	2.24	3.6	2.26
Harmine	0.17	0.08	122	1.64	0.16	0.74	2.31	1.15

ND, none detected

Methoxyflurane

Anaesthetic (General)
$C_3H_4Cl_2F_2O$ = 165.0
CAS—76-38-0

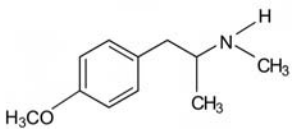
IUPAC Name 2,2-Dichloro-1,1-difluoro-1-methoxyethane
Proprietary Names *Metofane*; *Penthrane*; *Pentrane*.
Chemical Properties A clear, almost colourless, non-flammable mobile liquid. Mass per mL 1.423 to 1.427 g. Bp 103° to 108°. Methoxyflurane (B.P.) contains butylated hydroxytoluene 0.01% w/w. Soluble 1 in 500 of water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 2.2.
Gas Chromatography System GA—RI 701; system GI—retention time 17.6 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1315, 1063, 1136, 833, 1204, 1234 cm^{-1}.
Quantification
Blood **GC** FID [Cole *et al.* 1975]. FID. Limit of detection, 10 mg/L [Jones *et al.* 1972].
Dose For maintenance of anaesthesia, 0.2 to 0.5% of the vapour by inhalation.

Cole WJ *et al.* (1975). A method for the gas chromatographic analysis of inhalation anaesthetics in whole blood by direct injection into a simple precolumn device. *Br J Anaesth* 47: 1043–1047.
Jones PL *et al.* (1972). A technique for the analysis of methoxyflurane in blood by gas chromatography. *Br J Anaesth* 44: 124–130.

Methoxymetamfetamine

5-HT Receptor Releasing Agent
$C_{11}H_{17}NO$ = 179.3

IUPAC Name (α*S*)-*N*,α-Dimethyl-4-methoxy-benzeneethanamine
Synonyms 4-Methoxymethylamphetamine; 4-methoxy-*N*-methylamphetamine; *p*-methoxymethylamphetamine; 1-(4-methoxyphenyl)-2-aminopropane; 2-methylamino-1-(*p*-methoxyphenyl)propane; PMMA; 4-MMA. Usually presented as 'ecstasy'.



Chemical Properties A white powder. Mp 177° to 178°.
Colour Test Marquis test—no colour change.
Gas Chromatography Column: OV-1 (12 m × 0.31 mm i.d., 0.52 µm). Temperature programme: 70° to 150° at 15°/min then from 150° to 250° at 25°/min. MS detection. Retention time: within 7 min [Noggle *et al.* 1989].
High Performance Liquid Chromatography Columns: C_{18} µBondapak (300 × 3.9 mm i.d.); or C_{18} Ultracarb 5 (150 × 4.6 mm i.d.); or phenyl Hypersil 5 (150 × 4.6 mm i.d.). Mobile phase: 0.05 mol/L phosphate buffer (pH 3.0) : acetonitrile (6:1), flow rate 1.5 mL/min for µBondapak and Ultracarb columns and 1.2 mL/min for Hypersil column (all separate analyses). UV detection (λ=254 and 280 nm). Retention time: 8.0 min (µBondapak) and 5.0 min (Ultracarb and Hypersil) [Noggle *et al.* 1989].
Ultraviolet Spectrum Aqueous acid (0.1 mol/L sulfuric acid)—274 nm.
Mass Spectrum Principal ions at *m/z* 58, 121, 78, 91, 106, 148, 134, 164.
Disposition in the Body
Toxicity There are limited data regarding non-fatal and fatal PMMA intoxication. However, a few deaths related to PMMA toxicity have been reported in Europe, which also involved other amfetamine derivatives. PMMA can produce both CNS stimulation and hallucinogenic effects. Like PMA, acute PMMA toxicity can result in hyperthermia and possibly 'serotonin syndrome' related symptoms and some respiratory depression.

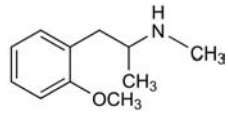
A PMMA concentration of 1.51 mg/L was measured in the postmortem blood of a 17-year-old male who felt ill when dancing. 3,4-Methylenedioxyethylamfetamine (MDEA) and 3,4-methylenedioxyamfetamine (MDA) were also detected in the blood (2.00 mg/L and 0.30 mg/L, respectively) along with ethanol (200 mg/L) [Lora-Tamayo *et al.* 1997].

Lora-Tamayo C *et al.* (1997). Amphetamine derivative related deaths. *Forensic Sci Int* 85: 149–157.
Noggle FT *et al.* (1989). *J Chromatogr Sci* 27: 607–611.

Methoxyphenamine

Sympathomimetic
$C_{11}H_{17}NO$ = 179.3
CAS—93-30-1

IUPAC Name 1-(2-Methoxyphenyl)-*N*-methylpropan-2-amine
Synonyms Methoxiphenadrin; 2-methoxy-*N*,α-dimethylbenzeneethanamine; mexyphamine.

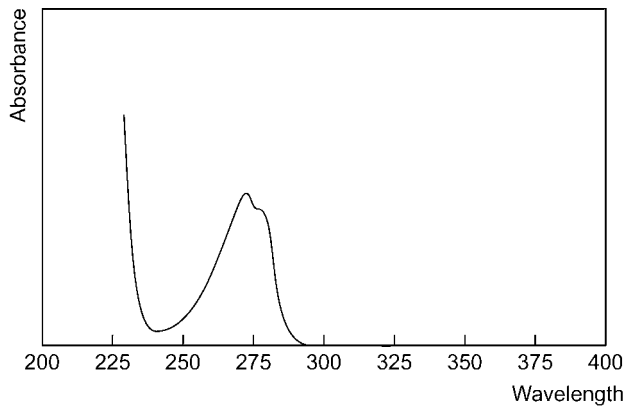


Chemical Properties An oil. pK_a 10.1. Log *P* (octanol/water), 2.3.

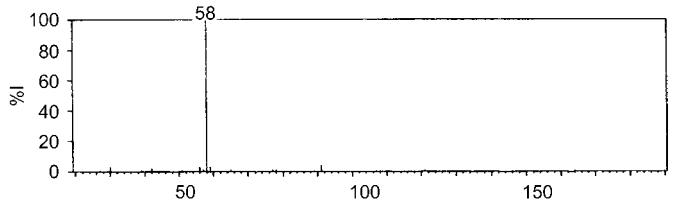
Methoxyphenamine Hydrochloride

$C_{11}H_{17}NO,HCl$ = 215.7
CAS—5588-10-3

Synonyms Methoxiphenadrin hydrochloride; mexyphamine hydrochloride.
Proprietary Names *Orthoxine*; *Proasma*. It is an ingredient of *Asmeton*, *Casacol*, *Cheracap*, *Orthoxicol* and *Sedagripe*.
Chemical Properties White crystals or powder. Mp 129° to 131° (crystals from ether and alcohol). Freely soluble in water, ethanol and chloroform; slightly soluble in ether and benzene.
Colour Tests Mandelin's test—blue-green; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.23; system TB—R_f 0.26; system TC—R_f 0.04; system TE—R_f 0.32; system TL—R_f 0.02; system TAE—R_f 0.07; system TAJ—R_f 0.05; system TAK—R_f 0.20; system TAL—R_f 0.64 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1361; system GB—RI 1416; system GC—RI 1670.
High Performance Liquid Chromatography System HA—*k* 1.7; system HB—*k* 32.17; system HX—RI 303.
Ultraviolet Spectrum Aqueous acid—271 nm (A^1_1=92a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1236, 749, 1047, 1125, 1026, 1175 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 58, 91, 59, 56, 30, 42, 121, 78.



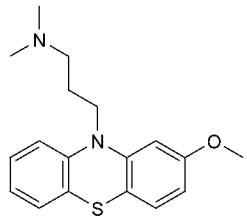
Quantification
Urine **GC** ECD. Methoxyphenamine enantiomers and metabolites [Srinivas *et al.* 1989].
Beard Hair **GC-MS** [Nakahara *et al.* 1993].
Disposition in the Body Readily absorbed after oral administration. Metabolites identified in urine include the *N*-desmethyl, *O*-desmethyl and 5-hydroxy derivatives and their glucuronide and/or sulfate conjugates. Unchanged drug is also excreted in the urine.
Toxicity The estimated minimum lethal dose for children up to 2 years of age is 200 mg and for adults is 2 g.
Dose 50 to 100 mg of methoxyphenamine hydrochloride every 4 h.

Nakahara Y *et al.* (1993). Hair analysis for drugs of abuse. VI. The excretion of methoxyphenamine and methamphetamine into beards of human subjects. *Forensic Sci Int* 63: 109–119.
Srinivas NR *et al.* (1989). Enantioselective gas chromatographic assays with electron-capture detection for methoxyphenamine and its three primary metabolites in human urine. *J Chromatogr* 487: 61–72.

Methoxypromazine

Tranquilliser
$C_{18}H_{22}N_2OS = 314.5$
CAS—61-01-8

IUPAC Name 3-(2-methoxyphenothiazin-10-yl)-*N,N*-dimethylpropan-1-amine
Synonyms 10-(3-Dimethylaminopropyl)-2-methoxyphenothiazine; methopromazine; RP 4632.



Methoxypromazine Maleate

Proprietary Name *Tentone*
Chemical Properties A crystalline powder. Soluble 1 in 330 of water: very slightly soluble in ethanol; insoluble in ether; soluble in chloroform. Methoxypromazine is extracted by organic solvents from aqueous alkaline solutions; hydrolysis of biological material is required in order to improve the yield.

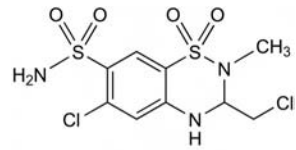
Colour Tests sulfuric acid–formaldehyde—purple (limit of detection, 0.1 µg); ammonium molybdate—purple (limit of detection, 0.1 µg); ammonium vanadate—purple (limit of detection: 0.5 µg); Vitali's test—purple flash→brown/brown/yellow (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.39; system T3—R_f 0.74; system T4—R_f 0.58; system T6—R_f 0.67 (location reagent acidified iodoplatinate spray, positive reaction for systems T1, T3, T4; for T6, the spot may be converted on the plate to the sulfoxide, Rf 0.35; location of both spots under ultraviolet light).
Ultraviolet Spectrum Aqueous acid (0.05 mol/L sulfuric acid)—250 (E1%, 1 cm 571), 278 nm (E1%, 1 cm 64) and 303 nm (E1%, 1 cm 95).
Infrared Spectrum Principle peaks at wavenumbers: A 1470, B 1356, C 748 or 1152 or 1582 (methoxypromazine maleate, KBr disc).
Disposition in the Body
Toxicity The acute lethal dose in humans of the phenothiazine derivatives appears to be in the range 15–150 mg/kg, although severe toxic symptoms have occurred with doses of less than 1 mg/kg.
Dose Up to 500 mg daily.

Methyclothiazide

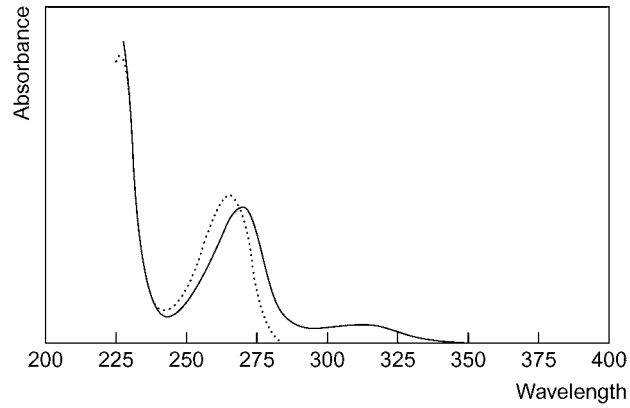
Diuretic
$C_9H_{11}Cl_2N_3O_4S_2 = 360.2$
CAS—135-07-9

IUPAC Name 6-Chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

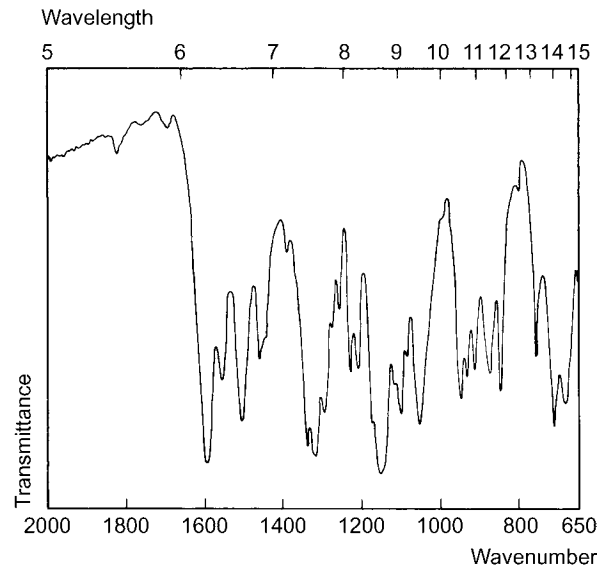
Proprietary Names *Aquatensen*; *Duretic*; *Enduron*; *Naturon*. It is an ingredient of *Enduronil*, *Enduronyl* and *Isobar*.



Chemical Properties A white crystalline powder. Mp 216°. Almost insoluble in water, chloroform and benzene; soluble 1 in about 90 of ethanol; very soluble in acetone and pyridine; soluble in solutions of alkali hydroxides and carbonates. pK_a 9.4. Log *P* (octanol/water), 1.4.
Colour Tests Koppanyi–Zwikker test—violet; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.87; system TD—R_f 0.19; system TE—R_f 0.53; system TF—R_f 0.50; system TAD—R_f 0.27; system TAJ—R_f 0.30; system TAK—R_f 0.08; system TAL—R_f 0.69.
Gas Chromatography System GA—methyclothiazide, not eluted; system GX—methyclothiazide-Me_3, retention time 9.9 min.
High Performance Liquid Chromatography System HN—*k* 3.82; system HY—RI 364; system HAA—retention time 15.4 min.
Ultraviolet Spectrum Aqueous acid—270 (A^1_1=525b), 314 nm; aqueous alkali—264, 300 nm.



Infrared Spectrum Principal peaks at wavenumbers 1158, 1595, 715, 1063, 1515, 1300 cm^{-1} (KBr disk).



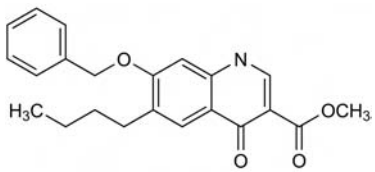
Mass Spectrum Principal ions at *m/z* 310, 64, 36, 312, 42, 43, 62, 63.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 1.5 µg/L [Hartman *et al.* 1981].
Dose 2.5 to 10 mg daily.

Hartman CA *et al.* (1981). Determination of methyclothiazide in human plasma by high-performance liquid chromatography. *J Chromatogr* 226: 510–513.

Methyl Benzoquate

Coccidiostat (Veterinary)
$C_{22}H_{23}NO_4$ = 365.4
CAS—13997-19-8

IUPAC Name Methyl 6-butyl-1,4-dihydro-4-oxo-7-(phenylmethoxy)-3-quinolinecarboxylate
Synonym Nequinate
Proprietary Name *Statyl*



Chemical Properties A white or creamy-white, amorphous powder. Mp 287° to 288°. Practically insoluble in water; very slightly soluble in ethanol, chloroform and methanol. Log *P* (octanol/water), 6.6.
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—brown; Marquis test—red-brown; sulfuric acid—yellow.
Ultraviolet Spectrum Methanolic acid—261 nm (A_1^1=1955a).
Infrared Spectrum Principal peaks at wavenumbers 1621, 1689, 1250, 1089, 1211, 1550 cm^{-1} (KBr disk).

Methyl Bromide

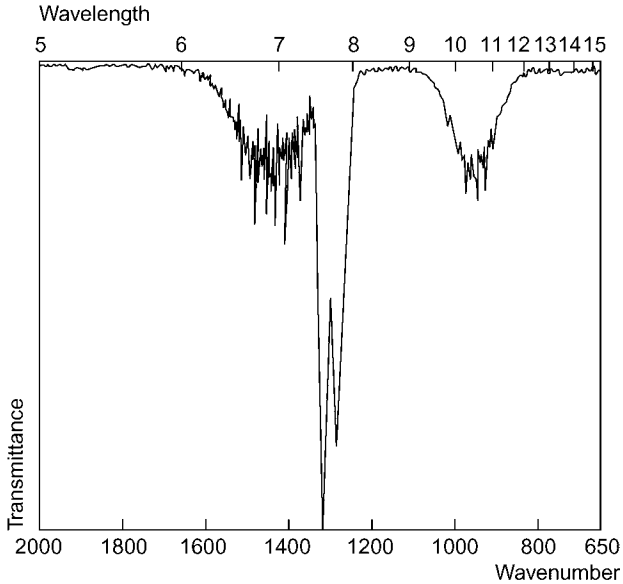
Insect Fumigant
CH_3Br = 94.9
CAS—74-83-9

IUPAC Name Bromomethane
Synonyms MB; MeBr.
Proprietary Names *Brom-o-Gas*; *Celfume*; *Embafume*; *Haltox*; *Methogas*; *Profume*; *Terr-o-Gas*; *Zytox*.

Chemical Properties A colourless gas or liquid. Mp −93.7° and Bp 3.56°. Soluble in water (1.75 g/100 g solution) and most organic solvents. Log *P* (octanol/water), 1.19.

Crystal Hydrate
$CH_3Br,2OH_2O$ = 375.3
Chemical Properties Freely soluble in alcohol, chloroform, ether, carbon disulfide, carbon tetrachloride and benzene.

Infrared Spectrum Principal peaks at wavenumbers 1319, 1295, 622, 594 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 94, 96, 15, 79, 81, 93, 95, 14.

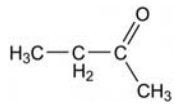
Quantification
Urine **GC** Limit of detection, 0.4 mg/L [Koga *et al.* 1991].
Disposition in the Body Methyl bromide is readily absorbed through the skin and lung alveoli. It is excreted through the lungs unchanged but a significant amount can also be excreted in urine and faeces, after metabolic decomposition. It is decomposed to a bromide ion and methanol which can be detected in blood, stomach fluids, mucus and tissue. Organic bromides have also been detected, when the bromide ion reacts with the molecular carbons of the biomolecules.
Toxicity
A 6 mg/L dose for 10 to 20 h or 30 mg/L for 1.5 h is associated with fatalities. The allowed daily intake is 1.0 mg/kg (as the bromide ion).
A newborn infant died 12 to 13 h after exposure to methyl bromide which was used as a fumigant in the neighbouring house and had leaked through the sewage. The concentration of bromide ions in the infant's blood was 170 mg/L. The parents had also suffered from clinical intoxication as a result of the leakage. Bromide ions were found in their blood at concentrations of 130 and 110 mg/L [Langard *et al.* 1996].
Half-life Approximately 12 days.

Koga M *et al.* (1991). Determination of bromide ion concentration in urine using a head-space gas chromatography and an ion chromatography–biological monitoring for methyl bromide exposure. *J UOEH* 13(1): 19–24.
Langard S *et al.* (1996). Fatal accident resulting from methyl bromide poisoning after fumigation of a neighbouring house; leakage through sewage pipes. *J Appl Toxicol* 16(5): 445–448.

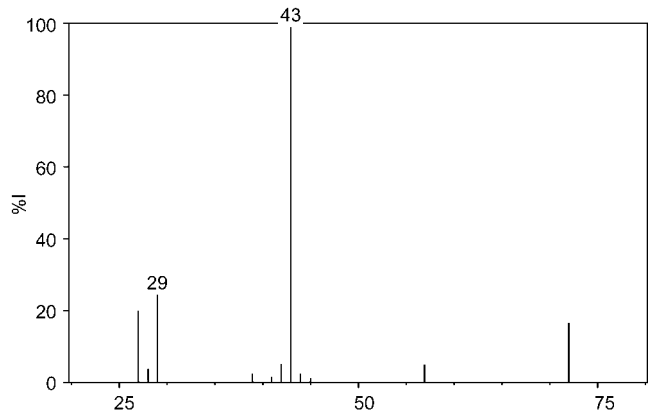
Methyl Ethyl Ketone

Solvent
C_4H_8O = 72.1
CAS—78-93-3

IUPAC Name 2-Butanone
Synonym Ethyl methyl ketone



Chemical Properties A flammable liquid. Bp 80°. Soluble 1 in about 4 of water, less soluble at higher temperatures; miscible with benzene, ethanol and ether. pK_a 14.7 (25°). Log *P* (octanol/water), 0.3.
Gas Chromatography System GA—RI 579; system GI—retention time 7.3 min.
Mass Spectrum Principal ions at *m/z* 43, 29, 27, 72, 57, 42, 44, 41.



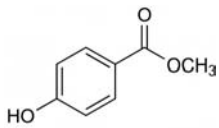
Quantification
Urine **GC** FID Limit of detection, about 4 µg/L [Chou *et al.* 1999]. Limit of detection, about 6 µg/L [Michitsuji *et al.* 1992]. Methyl ethyl ketone and its metabolites. Limits of detection, 0.1 to 1.4 mg/L [Kezic, Monster 1988].

Chou JS *et al.* (1999). Detection of methyl ethyl ketone in urine using headspace solid phase microextraction and gas chromatography. *J Occup Environ Med* 41: 1042–1047.
Kezic S, Monster AC (1988). Determination of methyl ethyl ketone and its metabolites in urine using capillary gas chromatography. *J Chromatogr* 428: 275–280.
Michitsuji H *et al.* (1992). [Determination of acetone, methanol, and methyl ethyl ketone in urine using head-space gas chromatography (HS.GC)]. *Sangyo Igaku* 34: 243–252.

Methyl Hydroxybenzoate

Preservative
$C_8H_8O_3$ = 152.1
CAS—99-76-3

IUPAC Name Methyl 4-hydroxybenzoate
Synonyms Metagin; methyl parahydroxybenzoate; methylis oxybenzoas; methylparaben.
Proprietary Names *Methyl Chemosept*; *Methyl Parasept*; *Nipagin M*; *Tegosept M*.

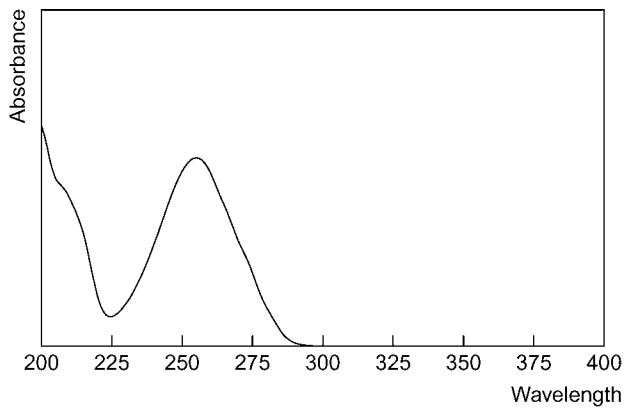


Chemical Properties Colourless crystals or a fine, white, crystalline powder. Mp 131°. Soluble 1 in 400 to 1 in 500 of water, 1 in 20 of boiling water, 1 in 3 to 1 in 3.5 of ethanol, 1 in 40 of chloroform and 1 in 10 of ether. pK_a 8.4 (22°). Log *P* (octanol/water), 2.0.

Sodium Methyl Hydroxybenzoate

$C_8H_7NaO_3$ = 174.1
CAS—5026-62-0
Synonyms Sodium methylparaben; soluble methyl hydroxybenzoate.
Proprietary Name *Nipagin M Sodium*
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 2 of water and 1 in 50 of ethanol.

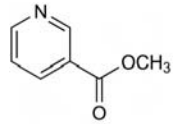
Thin-layer Chromatography System TA—R_f 0.95; system TD—R_f 0.62; system TE—R_f 0.44; system TF—R_f 0.65; system TAD—R_f 0.55; system TAE—R_f 0.81; system TAJ—R_f 0.61; system TAK—R_f 0.52; system TAL—R_f 0.91.
Gas Chromatography System GA—methyl hydroxybenzoate RI 1432; M (OH-)-AC RI 1570; M (methoxy-) RI 1480; M (*p*-hydroxyhippuric acid)-Me RI 1820.
High Performance Liquid Chromatography System HZ—retention time 3.2 min.
Ultraviolet Spectrum Ethanol—257 nm (A^1_1=1075a).



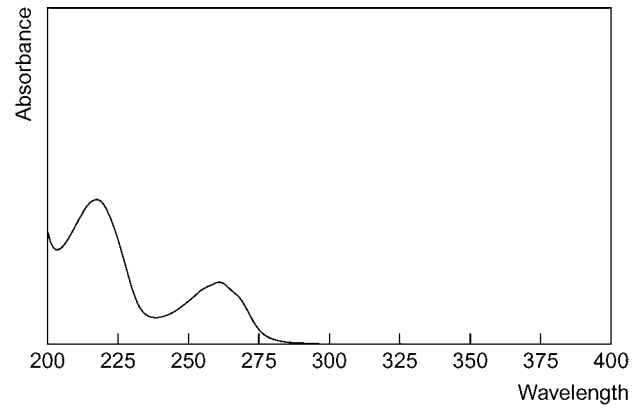
Mass Spectrum Principal ions at *m/z* 121, 152, 40, 93, 65.

Methyl Nicotinate

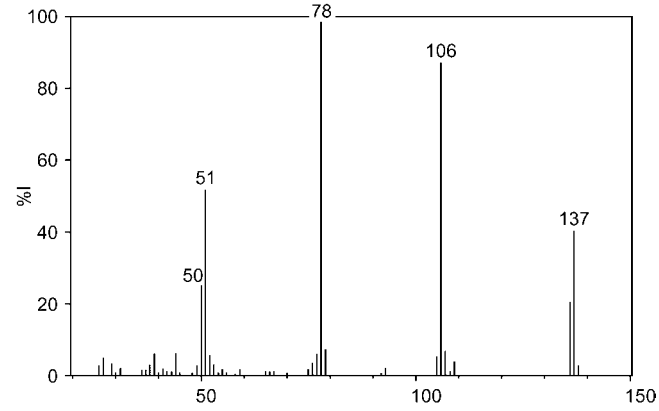
Vasodilator (Topical)
$C_7H_7NO_2$ = 137.1
CAS—93-60-7
IUPAC Name Methyl 3-pyridinecarboxylate
Proprietary Names *Pickles Chilblain Cream*; *Vas*. It is an ingredient of *Algipan*, *Arthricare*, *Cremalgin*, *Deep Heat Spray*, *Dubam*, *Fiery Jack*, *Musterole*, *Ralgex*, *Red Oil* and *Transvasin*.



Chemical Properties White crystals or crystalline powder; it darkens to a reddish colour on storage. Mp 39°. Soluble 1 in 0.7 of water and ethanol, 1 in 0.4 of chloroform and 1 in 1 of ether; soluble in benzene. pK_a 3.1 (22°). Log *P* (octanol/water), 0.8.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.37; system TC—R_f 0.66; system TE—R_f 0.72; system TL—R_f 0.59; system TAE—R_f 0.74; system TAF—R_f 0.77.
Gas Chromatography System GA—RI 1100.
Ultraviolet Spectrum Water—264 nm (A^1_1=230b).



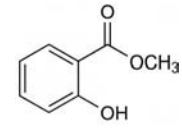
Infrared Spectrum Principal peaks at wavenumbers 1282, 1718, 1111, 741, 1022, 703 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 78, 106, 51, 137, 50, 136, 107, 79.



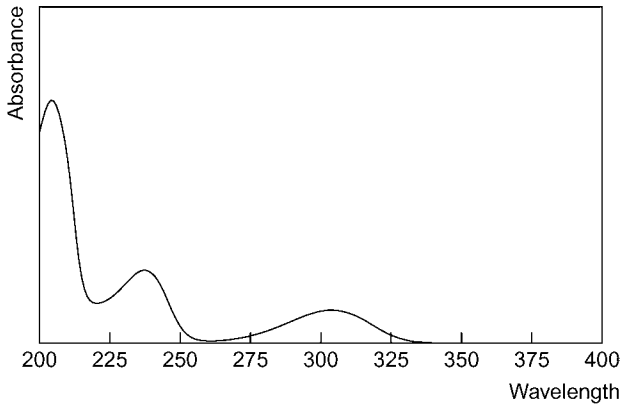
Use Topically in a concentration of 1%.

Methyl Salicylate

Analgesic
$C_8H_8O_3$ = 152.1
CAS—119-36-8
IUPAC Name Methyl 2-hydroxybenzoate
Synonym Wintergreen oil
Proprietary Names It is an ingredient of many proprietary preparations—see Sweetman [2009].



Chemical Properties A colourless or pale yellow liquid. Relative density 1.182 to 1.187. Bp about 221°, with some decomposition. Refractive index 1.535 to 1.538. Slightly soluble in water; soluble in chloroform and ether; miscible with ethanol (90%) and with most organic solvents. pK_a 9.9 (20°). Log *P* (octanol/water), 2.6.
Thin-layer Chromatography System TA—R_f 0.96; system TE—R_f 0.84; system TF—R_f 0.68; system TAF—R_f 0.90; system TAJ—R_f 0.95; system TAK—R_f 0.86.
Gas Chromatography System GA—methyl salicylate RI 1195, salicylic acid RI 1308; system GB—methyl salicylate RI 1228; system GL—methyl salicylate RI 1210.
High Performance Liquid Chromatography System HD—methyl salicylate *k* 3.9, salicylic acid *k* 0.7; system HX—RI 480; system HY—RI 449.
Ultraviolet Spectrum Ethanol—238 (A^1_1=581a), 306 nm (A^1_1=280a).

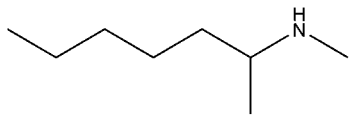


Infrared Spectrum Principal peaks at wavenumbers 1680, 705, 1310, 1220, 760, 1255 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 120, 92, 152, 121, 65, 64, 93, 63; salicylic acid 120, 92, 138, 64, 39, 63, 121, 65.
Disposition in the Body Poorly absorbed through intact skin; absorbed after oral ingestion. It is incompletely hydrolysed to salicylic acid.
Therapeutic Concentration
Following application of 5 g of an ointment containing 12.5% methyl salicylate twice daily for 4 days to 12 subjects, serum levels of 0.31 to 0.91 mg/L were reported within 1 h of the first application and peak levels of 2 to 6 mg/L were observed following the seventh application on day 4 [Morra *et al.* 1996.]
Toxicity Methyl salicylate is more toxic than salicylic acid. Deaths have occurred in children after ingestion of as little as 4 mL and doses of 30 mL are usually fatal in adults.
In a fatality due to the ingestion of about 120 mL of methyl salicylate, postmortem salicylic acid concentrations were: blood 615 mg/L and liver 455 µg/g [Ryall 1974].
Use Topically in liniments and ointments or in undiluted form.

Morra P *et al.* (1996). Serum concentrations of salicylic acid following topically applied salicylate derivatives. *Ann Pharmacother* 30: 935–940.
Ryall J (1974). *Bull Int Assoc Forensic Toxicol* 10(1): 10.
Sweetman SC (ed.) [2009]. *Martindale: The complete drug reference*, 36 edn. London: Pharmaceutical Press.

Methylaminoheptane

Sympathomimetic
$C_8H_{19}N = 129.2$
IUPAC Name *N*-Methylheptan-2-amine
Synonyms *N*,1-Dimethylhexylamine; 2-heptylmethylamine; 2-methylaminoheptane.



Chemical Properties Oily liquid. Bp 155°. Methylaminoheptane is extracted by organic solvents from aqueous alkaline solutions.

Methylaminoheptane Hydrochloride

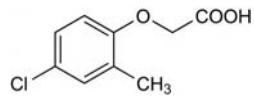
$C_8H_{19}N,HCl = 165.7$
Proprietary Names *Neosupranol*; *Oenethyl*; *Pacamine*.
Chemical Properties A crystalline solid. Readily soluble in water.

Quantification **LC** Column: Pirkle covalent (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: hexane : 2-propanol : acetonitrile (97 : 3 : 0.5) or carbon dioxide : 2-propanol (90 : 10), flow rate 2.0 mL/min. DAD (λ = 234 nm). Retention time: 1.20 and 0.89 min in each mobile phase, respectively. Limit of detection not reported [Macaudiere *et al.* 1986].
Disposition in the Body
Toxicity In rats the LD_{50} (oral) is 538 mg/kg.
Dose Usually up to 100 mg IM. Ruben [1950] concluded that *d*-desoxyephedrine was better than methylaminoheptane as a prophylactic versus hypotension in spinal anaesthesia.

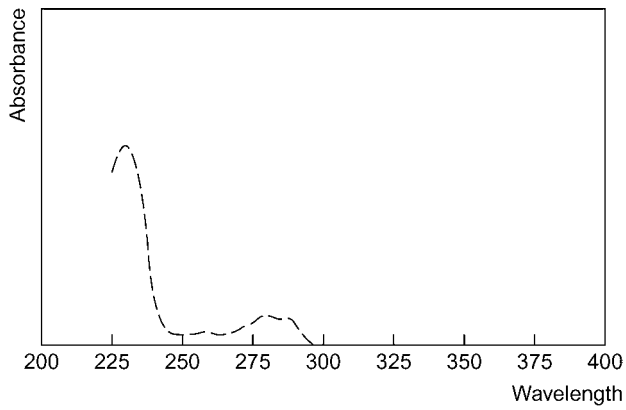
Macaudiere P *et al.* (1986). Resolution of enantiomeric amides on a Pirkle-type chiral stationary phase. A comparison of subcritical fluid and liquid chromatographic approaches. *J Chromatogr* 371: 177–193.
Ruben JE (1950). Evaluation of oenethyl (2-methyl-aminoheptane) as a vasopressor substance in spinal anesthesia. *Curr Res Anesth Analg* 29: 57–59.

Methylchlorophenoxyacetic Acid

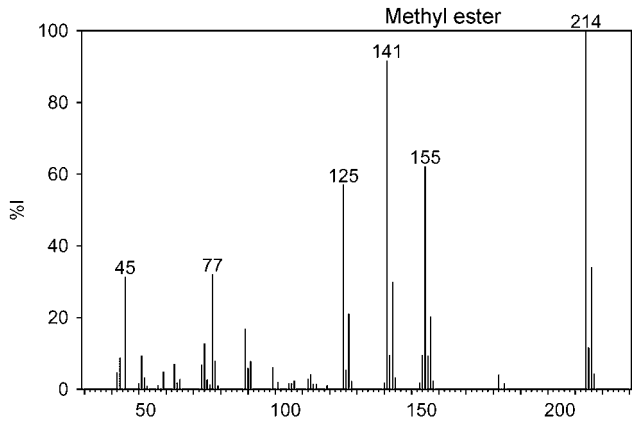
Herbicide
$C_9H_9ClO_3 = 200.6$
CAS—94-74-6
IUPAC Name (4-Chloro-2-methylphenoxy)acetic acid
Synonym MCPA



Chemical Properties A white crystalline solid. Mp pure compound, 118° to 119°; technical product, 100° to 115°. Practically insoluble in water; soluble 1 in 160 of ethanol and 1 in 80 of ether.
Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TD—R_f 0.09; system TE—R_f 0.05; system TF—R_f 0.11; system TX—R_f 0.00; system TY—R_f 0.00; system TAB—R_f 0.04; system TAC—R_f 0.02; system TAD—R_f 0.15.
Gas Chromatography Methylchlorophenoxyacetic acid methyl ester: system GK—RRT 0.54(relative to caffeine).
Ultraviolet Spectrum Methanol—280 (A_1^1=80a), 287 nm.



Infrared Spectrum Principal peaks at wavenumbers 1247, 1198, 1748, 1499, 1143, 806 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 214, 141, 155, 125, 46, 77, 89, 143 (methylchlorophenoxyacetic acid methyl ester).



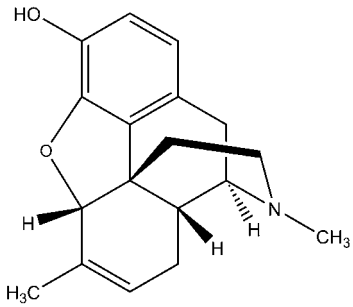
Disposition in the Body
Toxicity
After intentional ingestion of methylchlorophenoxyacetic acid, a man suffered burning in his mouth, spasmodic pain in the extremities and a severe hypotensive crisis; a plasma concentration of 546 mg/L was reported 2 h after ingestion, which rapidly declined to 6 mg/L following forced diuresis and alkalinisation of the urine on day 4 [Schmoldt *et al.* 1997].

Schmoldt A *et al.* (1997). Massive ingestion of the herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA). *J Toxicol Clin Toxicol* 35: 405–408.

Methyldesorphine

Narcotic
$C_{18}H_{20}NO_2 = 282.4$
CAS—16008-36-9

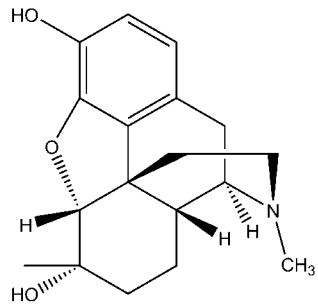
Synonyms 6,7-Dehydro-4,5-epoxy-3-hydroxy-*N*,6-dimethylmorphinan; MK 57.



Chemical Properties Methyldesorphine is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve the yield.
Colour Tests Ammonium molybdate test—black-purple→greenish-brown (limit of detection, 0.1 µg); ammonium vanadate test—brown (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—dull purple (limit of detection, 0.1 µg); Vitali's test—yellow/yellow/orange (limit of detection, 0.25 µg).
Gas Chromatography System G2/225—retention time 0.81 (relative to codeine).
Ultraviolet Spectrum 0.1 N sulfuric acid—283 nm (E1%, 1cm 42) and inflexion at about 278 nm (E1%, 1 cm 40).

Methyldihydromorphine

Narcotic
$C_{18}H_{23}NO_3 = 301.4$
Synonym Methydromorphine



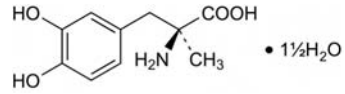
Chemical Properties Methyldihydromorphine is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve the yield.
Colour Tests Ammonium molybdate test—violet-blue→green (limit of detection, 0.1 µg); ammonium vandate test—grey-purple→brown (limit of detection, 0.5 µg); sulfuric acid-formaldehyde test—purple (limit of detection, 0.1 µg); Vitali's test—yellow/yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.28 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.00 (relative to codeine).
Disposition in the Body
Therapeutic Concentration
Methyldihydromorphine produces fewer adverse effects in morphine addicts than morphine. The intensity of abstinence after withdrawal is quite mild [Fraser, Isbell 1950].

Fraser HF, Isbell H (1950). Addiction liabilities of morphinan, 6-methyldihydromorphine and dihydrocodeinone. *J Pharmacol Exp Ther* 100: 128–135.

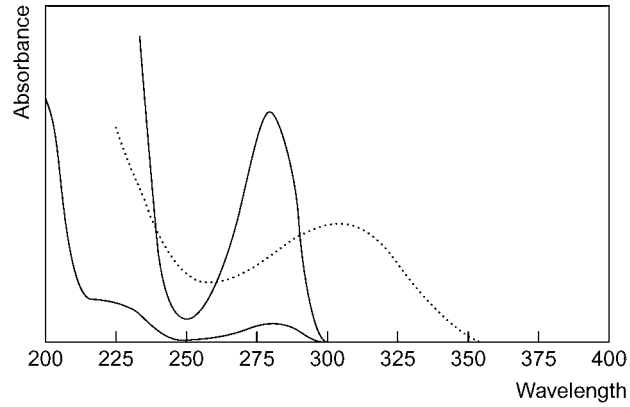
Methyldopa

Antihypertensive
$C_{10}H_{13}NO_4,1½H_2O = 238.2$
CAS—555-30-6 (anhydrous); 41372-08-1 (sesquihydrate)
IUPAC Name 2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid
Synonyms Alpha-methyldopa; 3-hydroxy-α-methyl-L-tyrosine sesquihydrate; methyldopum hydratum; metildopa.

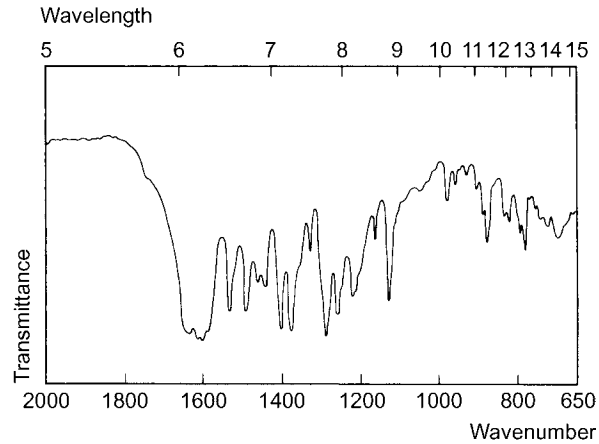
Proprietary Names *Aldomet*; *Aldometil*; *Aldomin*; *Aldopren*; *Amender*; *Cardin*; *Dopamet*; *Dopegyt*; *Hipten*; *Hydopa*; *Isomet*; *Medimet-250*; *Medomet*; *Medopren*; *Meldopa*; *MetalphaNovo-Medopa*; *Nudopa*; *Nu-Medopa*; *Presinol*; *Prodop*; *Pulsoton*; *Selm*; *Sembrina*; *Tenzone*.



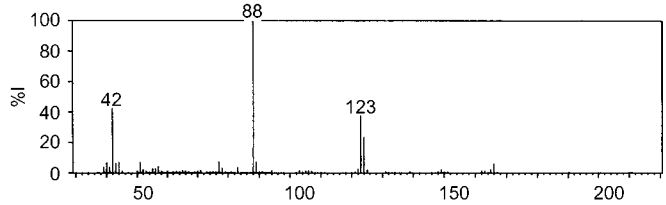
Chemical Properties Colourless crystals or a white to yellowish-white, fine powder. Mp about 310°. Soluble 1 in 100 of water, 1 in 400 of ethanol and 1 in 0.5 of dilute hydrochloric acid; practically insoluble in chloroform and ether. pK_a 2.2 (-COOH), 9.2 (-OH), 10.6 (-NH_2), 12.0 (-OH) (25°). Log *P* (octanol/water), −1.8.
Colour Tests Ammoniacal silver nitrate—red-brown/black; ferric chloride—green; Folin-Ciocalteu reagent—blue; Mandelin's test—orange; Marquis test—yellow→violet; methanolic potassium hydroxide—yellow→orange; Nessler's reagent—black; potassium dichromate (method 1)—green→brown (30 s).
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.02; system TL—R_f 0.01; system TAE—R_f 0.60; system TAF—R_f 0.75; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.06 (mercuric chloride–diphenylcarbazone reagent, white-blue; acidified potassium permanganate solution, positive; van Urk reagent, violet).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 69; system HZ—retention time 1.4 min; system HAA—retention time 3.0 min; system HAX—retention time 4.1 min; system HAY—retention time 2.8 min.
Ultraviolet Spectrum Aqueous acid—279 nm (A^1_1=130a); aqueous alkali—302 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1288, 1261, 1530, 1123, 1219 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 88, 42, 123, 124, 89, 77, 51, 44.



Quantification

Plasma **Spectrofluorimetry** Limit of detection, 100 µg/L [Kim, Koda 1977].

HPLC Fluorescence detection. Limit of detection, <10 µg/L [Rona *et al.* 1996]. Electrode coulometric detection. Methyldopa and its metabolite [Lucarelli *et al.* 1991]. Electrochemical detection. Limit of detection, 50 µg/L [Dilger *et al.* 1987]. Electrochemical detection [Hoskins, Holliday 1982].

HPLC-MS Limit of detection, <20 µg/L [Oliveira *et al.* 2002].

Urine **Spectrofluorimetry** See Plasma [Kim, Koda 1977].

HPLC See Plasma [Lucarelli *et al.* 1991]. Electrochemical detection. Limit of detection, 5 mg/L [Kochak, Mason 1981].

Breast Milk **HPLC** See Plasma [Hoskins, Holliday 1982].

Disposition in the Body Poorly absorbed after oral administration. It is mainly excreted as unchanged drug and as the mono-*O*-sulfate conjugate. After oral dosage, about 40% of the dose is excreted in the urine in 48 h, of which about 40% is the conjugate. A considerable amount of unchanged drug is eliminated in the faeces. After IV administration, the amount of conjugate excreted is much less than after oral dosage; a total of 52 to 82% of an IV dose is excreted in the urine in 36 h, only about 2% of which is the conjugate. Other metabolites and their conjugates which have been identified in the urine in small amounts (each <5% of the dose) include 3-*O*-methyl-α-methyldopa, α-methyldopamine, 3-*O*-methyl-α-methyldopamine and 3,4-dihydroxyphenylacetone.

Methyldopa is a metabolite of methyldopate.

Therapeutic Concentration In plasma, usually in the range 1 to 5 mg/L.

After a single oral dose of 750 mg administered to 12 subjects, mean peak plasma concentrations of 2.6 mg/L of unchanged drug and 1.3 mg/L of the conjugate were attained in 3 h. An IV infusion of 250 mg given over 90 min to 12 subjects produced, at the end of the infusion period, a mean peak plasma concentration of 7.5 mg/L; no conjugate was found in the plasma [Kwan *et al.* 1976].

Toxicity

In a fatality involving the ingestion of methyldopa, postmortem blood and urine concentrations of 9 and 1400 mg/L, respectively, were reported [Tamminen, Alha 1970].

Half-life Plasma half-life, about 2 h; a longer terminal elimination half-life has also been reported.

Volume of Distribution About 0.6 L/kg.

Clearance Plasma clearance, about 3 mL/min/kg.

Protein Binding <20%.

Note For a review of the pharmacokinetics of methyldopa, see Myhre *et al.* [1982].

Dose The equivalent of 0.5 to 3 g of anhydrous methyldopa daily.

Dilger C *et al.* (1987). Determination of methyldopa in plasma using high-performance liquid chromatography with electrochemical detection. Application to pharmacokinetic/bioavailability studies. *Arzneimittelforschung* 37: 1399–1401.

Hoskins JA, Holliday SB (1982). Determination of alpha-methyldopa and methyldopate in human breast milk and plasma by ion-exchange chromatography using electrochemical detection. *J Chromatogr* 230: 162–167.

Kim BK, Koda RT (1977). Fluorometric determination of methyldopa in biological fluids. *J Pharm Sci* 66: 1632–1634.

Kochak GM, Mason WD (1981). *Anal Lett (Part B)* 14: 439–449.

Kwan KC *et al.* (1976). Pharmacokinetics of methyldopa in man. *J Pharmacol Exp Ther* 198: 264–277.

Lucarelli C *et al.* (1991). High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (alpha-methyldopa) in human urine and plasma. *J Chromatogr* 541: 285–296.

Myhre E *et al.* (1982). Clinical pharmacokinetics of methyldopa. *Clin Pharmacokinet* 7: 221–233.

Oliveira CH *et al.* (2002). Quantification of methyldopa in human plasma by high-performance liquid chromatography-electrospray tandem mass spectrometry application to a bioequivalence study. *J Chromatogr B Anal Technol Biomed Life Sci* 768: 341–348.

Rona K *et al.* (1996). Determination of alpha-methyldopa in human plasma by validated high-performance liquid chromatography with fluorescence detection. *J Chromatogr A* 730: 125–131.

Tamminen V, Alha A (1970). *Bull Int Assoc Forensic Toxicol* 7(2): 2–3.

Methyldopate

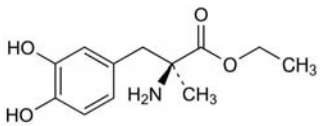
Antihypertensive

$C_{12}H_{17}NO_4$ = 239.3

CAS—2544-09-4

IUPAC Name Ethyl (2*S*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoate

Synonym 3-Hydroxy-α-methyl-L-tyrosine ethyl ester



Methyldopate Hydrochloride

$C_{12}H_{17}NO_4$,HCl = 275.7

CAS—2508-79-4

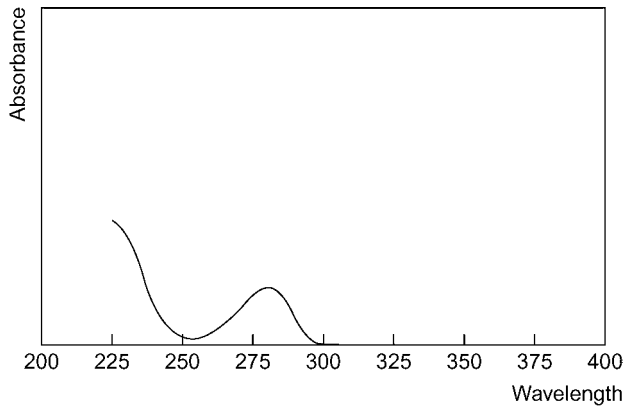
Proprietary Name *Aldomet (injection)*

Chemical Properties A white crystalline powder. Soluble 1 in 1 of water, 1 in 3 of ethanol and 1 in 2 of methanol; slightly soluble in chloroform; practically insoluble in ether.

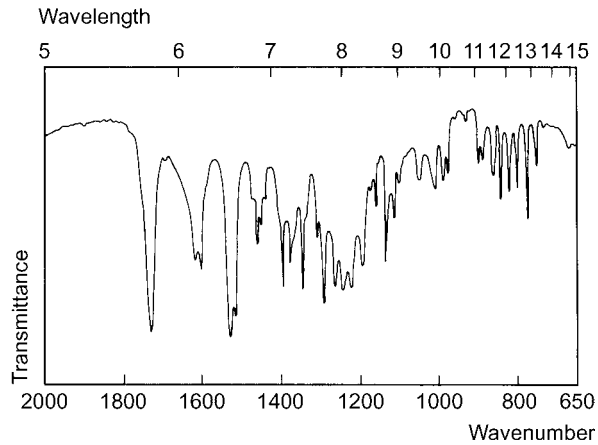
Colour Tests Ammoniacal silver nitrate—orange-brown/orange-brown; ferric chloride—green; Folin-Ciocalteu reagent—blue; Mandelin's test—orange; Marquis test—yellow→violet; methanolic potassium hydroxide—blue→orange; Nessler's reagent—black; potassium dichromate (method 1)—green→brown (30 s).

Thin-layer Chromatography System TA—R_f 0.65 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=115a).



Infrared Spectrum Principal peaks at wavenumbers 1527, 1726, 1516, 1290, 1240, 1215 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** Electrochemical detection [Hoskins, Holliday 1982].

Breast Milk **HPLC** See Plasma [Hoskins, Holliday 1982].

Disposition in the Body Metabolised by de-esterification to methyldopa; a small amount of sulfate conjugation also occurs.

Therapeutic Concentration

After an IV dose of 250 mg to 5 subjects, peak plasma concentrations of 0.8 to 2.2 mg/L (mean 1.6) of free and esterified methyldopa, and 1.0 to 2.2 mg/L (mean 1.5) of conjugated methyldopa were attained in about 1 h [Saavedra *et al.* 1975].

Half-life Plasma half-life, about 4 h.

Volume of Distribution About 2 L/kg.

Dose Usually 250 to 500 mg of methyldopate hydrochloride, by IV infusion, every 6 h.

Hoskins JA, Holliday SB (1982). Determination of alpha-methyldopa and methyldopate in human breast milk and plasma by ion-exchange chromatography using electrochemical detection. *J Chromatogr* 230: 162–167.

Saavedra JA *et al.* (1975). Plasma concentration of alpha-methyldopa and sulphate conjugate after oral administration of methyldopa and intravenous administration of methyldopa and methyldopa hydrochloride ethyl ester. *Eur J Clin Pharmacol* 8: 381–386.

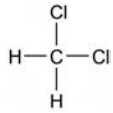
Methylene Chloride

Solvent

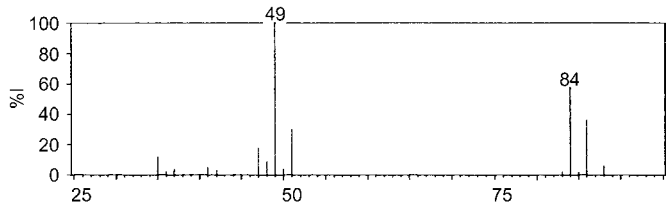
CH_2Cl_2 = 84.93

CAS—75-09-2

IUPAC Name Dichloromethane



Chemical Properties Dichloromethane A clear, colourless, volatile liquid. Mass per mL about 1.32 g. Bp 39° to 41°. Soluble 1 in 50 of water; miscible with ethanol and ether. Log *P* (octanol/water), 1.2.
Gas Chromatography System GA—RI 515; system GI—retention time 1.9 min.
Mass Spectrum Principal ions at *m/z* 49, 84, 86, 51, 47, 35, 88, 41.



Quantification
Blood **GC** FID. Methylene chloride and chloroform. Limit of detection, 0.3 mg/L in blood [Seno *et al.* 1999].
Urine **GC** Limit of detection, 0.2 mg/L in urine, see Blood [Seno *et al.* 1999].

Disposition in the Body
Toxicity Methylene chloride is widely used in paint strippers and several non-fatal and fatal cases of accidental inhalation have been reported. The maximum permissible atmospheric concentration is 200 ppm and the temporary estimated acceptable daily intake is up to 500 µg/kg.

The following tissue concentrations were reported in a fatality due to accidental methylene chloride poisoning: blood 252 mg/L, brain 75 µg/g, heart 30 µg/g [Kim *et al.* 1996].

Blood concentrations collected at autopsy in 2 cases of fatal methylene chloride poisoning were 572 and 601 mg/L; air concentrations a few hours after discovery of the bodies were up to 168 000 ppm [Manno *et al.* 1992].

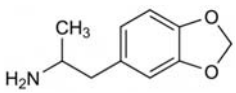
The following postmortem tissue concentrations were reported in a 47-year-old factory worker who died while making an inventory of the annual stock of methylene chloride contained in several tanks, one of which overflowed: blood 150 mg/L, urine 2.0 mg/L, gastric content 5.6 mg/L, brain 122 µg/g, fat 99 µg/g, liver 44 µg/g, lung 20 µg/g, kidney 15 µg/g [Goulle *et al.* 1999].

Goulle JP *et al.* (1999). Fatal case of dichloromethane poisoning. *J Anal Toxicol* 23: 380–383.
Kim NY *et al.* (1996). Two fatal cases of dichloromethane or chloroform poisoning. *J Forensic Sci* 41 (3): 527–529.
Manno M *et al.* (1992). Double fatal inhalation of dichloromethane. *Hum Exp Toxicol* 11: 540–545.
Seno H *et al.* (1999). Extraction of chloroform and methylene chloride in human whole blood and urine by headspace solid phase microextraction (SPME). *Med Sci Law* 39: 332–336.

Methylenedioxyamfetamine

Hallucinogen
$C_{10}H_{13}NO_2$ = 179.2
CAS—4764-17-4

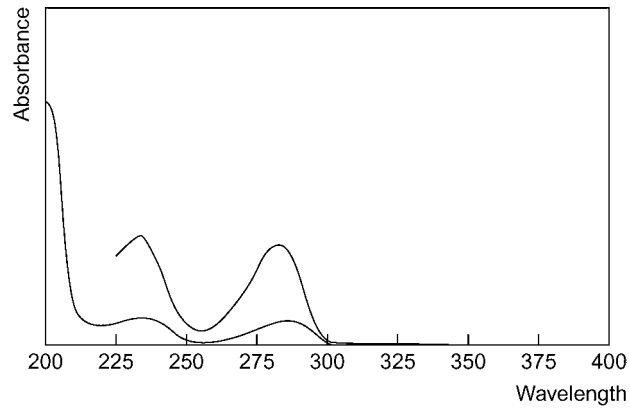
IUPAC Name 1-(1,3-Benzodioxol-5-yl)propan-2-amine
Synonyms MDA; α-methyl-1,3-benzodioxole-5-ethanamine; methylenedioxyamphetamine; *N*-methyl-3,4-methylenedioxyamfetamine; tenamfetamine; SKF-5.



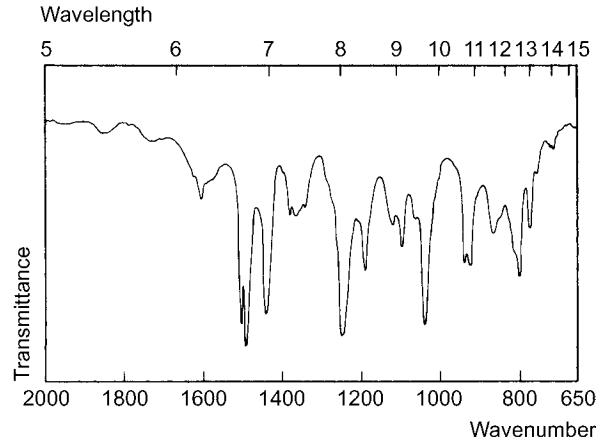
Chemical Properties Almost colourless oil. pK_a 9.67 (25°). Log *P* (octanol/water), 1.64.

Methylenedioxyamfetamine Hydrochloride
$C_{10}H_{13}NO_2$,HCl = 215.7
CAS—6292-91-7

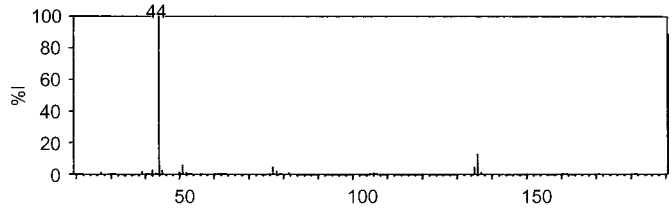
Colour Tests Liebermann's reagent—black; Mandelin's test—green→blue; Marquis test—blue-black; sulfuric acid—violet.
Thin-layer Chromatography System TA—R_f 0.39; system TB—R_f 0.18; system TC—R_f 0.12; system TE—R_f 0.42; system TL—R_f 0.17; system TAE—R_f 0.10; system TAF—R_f 0.76 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—MDA RI 1480, MDA-TFA RI 1615, MDA-PFP RI 1605, MDA-AC RI 1860, art (formyl) RI 1520; system GB—MDA RI 1512, art (formyl) RI 1689.
High Performance Liquid Chromatography System HC—*k* 0.98; system HX—RI 266; system HY—RI 248; system HZ—retention time 2.1 min; system HAA—retention time 8.1 min; system HAX—retention time 6.9 min; system HAY—retention time 3.8 min.
Ultraviolet Spectrum Aqueous acid—233 (A^1_1=216b), 285 nm.



Infrared Spectrum Principal peaks at wavenumbers 1490, 1257, 1038, 1504, 799, 1188 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 136, 51, 135, 77, 42, 78, 45.



Quantification
Blood **HPLC** Limit of quantification, 2 µg/L [Clauwaert *et al.* 2000]. Electrochemical detection. Limit of detection, 1 µg/L [Michel *et al.* 1993].
Plasma **GC** Limit of detection, <1.6 µg/L [Ortuno *et al.* 1999].
HPLC Limit of detection, 10 µg/L [Herraez-Hernandez *et al.* 2001].
Serum **GC-MS** Methylenedioxyamfetamine and other drugs of abuse [Weinmann *et al.* 2000].
HPLC See Blood [Clauwaert *et al.* 2000].
Urine **GC** See Plasma. Limit of detection, 47 µg/L[Ortuno *et al.* 1999]. NPD. Limit of detection, 15 µg/L [Ugland *et al.* 1999].
GC-MS Methylenedioxyamfetamine and related drugs. Limit of detection for MDA, ~31 µg/L [Stout *et al.* 2002]. Limit of quantification, 20 µg/L [Jurado *et al.* 2000]. Methylenedioxyamfetamine and other amfetamines. Limit of detection, ~10 µg/L [Hensley, Cody 1999]. Limit of detection, 15 µg/L [Ugland *et al.* 1999].
HPLC See Plasma [Herraez-Hernandez *et al.* 2001]. See Blood. Limit of quantification, 100 µg/L [Clauwaert *et al.* 2000].
Vitreous Humour **HPLC** See Blood [Clauwaert *et al.* 2000].

Hair **GC-MS** Methylenedioxyamfetamine and other drugs of abuse. Limits of detection, 0.05–0.3 μg/g [Skender *et al.* 2002]. Limit of detection, 0.01 μg/g [Rohrich, Kauert 1997]. Limit of detection, 0.05 μg/g [Kintz *et al.* 1995].

HPLC Limit of detection, <1 μg/L (0.1 μg/g) [Tagliaro *et al.* 1999].

Other **HPLC** Biological Tissues. See Blood [Michel *et al.* 1993].

Disposition in the Body

Toxicity The estimated lethal dose is 0.5 g.

A 21-year–old man was found dead in bed 7 h after being last seen in a 'normal' state. *Temazepam* tablets as well as 2 types of white tablets were found by his bedside. Toxicological analysis revealed the presence of the following drugs:

	Blood (mg/L)	*Stomach contents (mg/L)*	*Urine*
Amfetamine	0.256	<0.1	Present
MDA	8.5	299	Present
MDMA	2.1	96	Present
MDEA	3.5	324	Present

MDMA, methylenedioxymetamfetamine; MDEA, methylenedioxyethylamfetamine. [Forrest *et al.* 1994].

In 5 fatalities due to the ingestion of methylenedioxyamfetamine, the following postmortem tissue concentrations were reported: blood 6–26 mg/L (mean 14, 5 cases), bile 5–9 mg/L (mean 6, 3 cases), liver 8–17 μg/g (mean 11, 3 cases), urine 46–160 mg/L (mean 102, 3 cases) [Cimbura 1972].

In a fatality in which death occurred shortly after the ingestion of methylenedioxyamfetamine, the following postmortem concentrations were reported: blood 2.3 mg/L, bile 7 mg/L, liver 11 μg/g, urine 175 mg/L [Lukaszewski 1979].

Note For a review of the pharmacology of MDA, see Simpson and Rumack [1981]; for a distribution study of MDMA and MDA in a fatal overdose, see De Letter *et al.* [2002].

Cimbura G (1972). 3,4-methylenedioxyamphetamine (MDA): analytical and forensic aspects of fatal poisoning. *J Forensic Sci* 17: 329–333.

Clauwaert KM *et al.* (2000). Determination of the designer drugs 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxyamphetamine with HPLC and fluorescence detection in whole blood, serum, vitreous humor, and urine. *Clin Chem* 46: 1968–1977.

De Letter EA *et al.* (2002). Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. *J Anal Toxicol* 26: 113–118.

Forrest AR *et al.* (1994). A fatal overdose with 3,4-methylenedioxyamphetamine derivatives. *Forensic Sci Int* 64: 57–59.

Hensley D, Cody JT (1999). Simultaneous determination of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) enantiomers by GC-MS. *J Anal Toxicol* 23: 518–523.

Herraez-Hernandez R *et al.* (2001). Sensitive determination of methylenedioxylated amphetamines by liquid chromatography. *Analyst* 126(5): 581–586.

Jurado C *et al.* (2000). Rapid analysis of amphetamine, methamphetamine, MDA, and MDMA in urine using solid-phase microextraction, direct on-fiber derivatization, and analysis by GC-MS. *J Anal Toxicol* 24: 11–16.

Kintz P *et al.* (1995). Simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in human hair by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 670: 162–166.

Lukaszewski T (1979). 3,4-Methylenedioxyamphetamine overdose. *Clin Toxicol* 15: 405–409.

Michel RE *et al.* (1993). High-pressure liquid chromatography/electrochemical detection method for monitoring MDA and MDMA in whole blood and other biological tissues. *J Neurosci Methods* 50: 61–66.

Ortuno J *et al.* (1999). Quantification of 3,4-methylenedioxymetamphetamine and its metabolites in plasma and urine by gas chromatography with nitrogen-phosphorus detection. *J Chromatogr B Biomed Sci Appl* 723(1–2): 221–232.

Rohrich J, Kauert G (1997). Determination of amphetamine and methylenedioxy-amphetamine-derivatives in hair. *Forensic Sci Int* 84: 179–188.

Simpson DL, Rumack BH (1981). Methylenedioxyamphetamine. Clinical description of overdose, death, and review of pharmacology. *Arch Intern Med* 141: 1507–1509.

Skender L *et al.* (2002). Quantitative determination of amphetamines, cocaine, and opiates in human hair by gas chromatography/mass spectrometry. *Forensic Sci Int* 125: 120–126.

Stout PR *et al.* (2002). Rapid simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine in urine by solid-phase extraction and GC-MS: a method optimized for high-volume laboratories. *J Anal Toxicol* 26: 253–261.

Tagliaro F *et al.* (1999). High sensitivity simultaneous determination in hair of the major constituents of ecstasy (3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylene-dioxyethylamphetamine) by high-performance liquid chromatography with direct fluorescence detection. *J Chromatogr B Biomed Sci Appl* 723(1–2): 195–202.

Ugland HG *et al.* (1999). Automated determination of 'Ecstasy' and amphetamines in urine by SPME and capillary gas chromatography after propylchloroformate derivatisation. *J Pharm Biomed Anal* 19(3-4): 463–475.

Weinmann W *et al.* (2000). Automated solid-phase extraction and two-step derivatisation for simultaneous analysis of basic illicit drugs in serum by GC/MS. *Int, J Legal Med* 113: 229–235.

Methylenedioxymetamfetamine

Hallucinogen, Phenethylamine, Stimulant

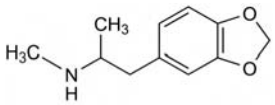
$C_{11}H_{15}NO_2 = 193.2$

CAS—42542-10-9

IUPAC Name 1-(1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amine

Synonyms (2-Benzol[1,3] dioxol-5-yl-1-methyl-ethyl)-methyl-amine; *N*,α-dimethyl-1,3,benzodioxole-5-ethanamine; MDMA; 3,4-methylenedioxymethamfetamine; 3,4-methylenedioxymethylamphetamine; *N*-methyl-3,4-methylenedioxyphenylisopropylamine.

Street Names A bean; Adam; Apples; Baby slits; Brownies; Burgers; Chocolate chips; Clarity; Crowns; Dennis the Menace; Diamond Whites; Disco biscuits; Doctor; Doves; E; Ecstasy; Elaine; Essence; Euphoria; Love doves; Lovers Speed; M, M; MDM; Mitsubishis; Mitsies; New Yorkers; Roll; Rhubarb, Custards; Stacy; Tangos; X; XTC.



Chemical Properties A viscous, colourless oil. Bp 100° to 110°. pK_a (pH 9.0) (benzene) 9.41, (hexane) 8.69, (ethyl acetate) 8.84. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].

Methylenedioxymetamfetamine Hydrochloride

$C_{11}H_{15}NO_2$,HCl = 229.75

CAS—64057-70-1

IUPAC Name 1-(1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amine hydrochloride

Chemical Properties A white to off-white crystalline powder with a bitter taste. Mp 147° to 148° for crystals from propan-2-ol/*n*-hexane, 152° to 153°for crystals from propan-2-ol/ether.

Colour Test Marquis reagent—black with dark purple halo.

Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.24; system TE—R_f 0.39; system TF—R_f 0.20; system TAE—R_f 0.08; system TAJ—R_f 0.03; system TAK—R_f 0.17; system TAL—R_f 0.57.

Gas Chromatography System GA—RI 1585, methylenedioxyamfetamine (MDA) RI 1480, MDMA-TFA RI 1720, MDMA-PFP RI 1830, MDMA-TMS RI 1710, MDMA-AC RI 2140; system GB—RI 1572, MDA RI 1512; system GAK—RT 8.7 min; system GAD—RT 10.9 min.

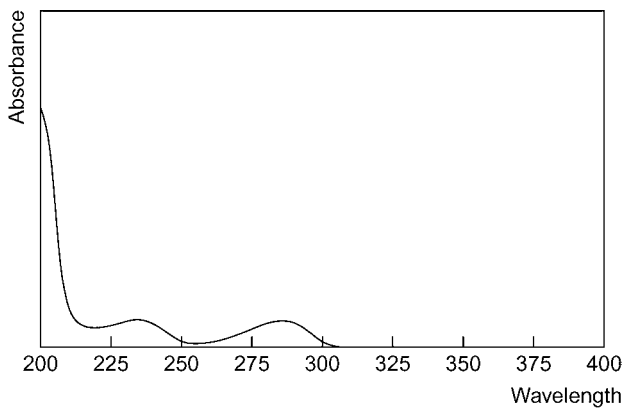
Column: 3% OV-1 Chromosorb WHP 80/100 mesh (1.2 m × 100 mm (4 ft × 4 in) i.d.). Column temperature: 200°. Carrier gas: N_2, 32 mL/min. FID. RI: 1559 [Mills, Roberson 1993].

High Performance Liquid Chromatography System HX—RI 278; system HY—RI 252; system HZ—RT 2.2 min; system HAA—RT 9.1 min; system HBC—RT 2.42 min; system HBD—RT 5.31 min.

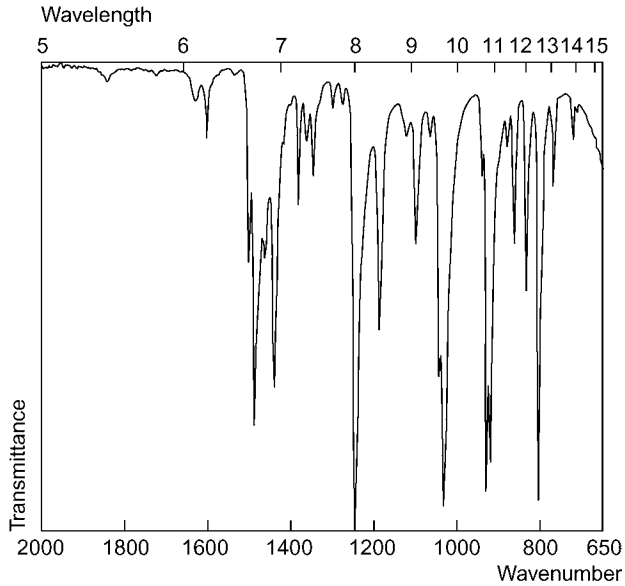
Column: ODS Hypersil Si-10 (300 × 4.0 mm i.d., 10 μm). Mobile phase: methanol with 1% ammonium hydroxide : methylene chloride (10 : 90), flow rate 2 mL/min. UV detection (λ = 254 nm). Retention time: 4.4 min [Mills, Roberson 1993].

Column: Zorbax CN (150 × 4.6 mm i.d., 5 m). Mobile phase: methanol: 0.1 mol/L acetate buffer (pH 4.56, 50 : 50). UV detection (λ = 280 and 284 nm). Retention time: 6.69 min for MDMA and 5.83 min for MDA [Garrett *et al.* 1991].

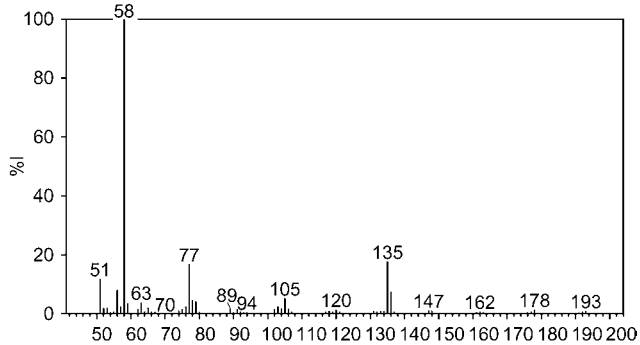
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid)—234, 285 nm; (0.025 mol/L sulfuric acid)—198, 234.5, 282.5 nm; (0.1 mol/L ammonium formate, pH 3)—285; basic—232, 285 nm.



Infrared Spectrum Principal peaks at wavenumber 1033, 930, 805, 1489, 1442, 1189.2 cm^{-1} (hydrochloride)



Mass Spectrum Principal peaks at *m/z* 58, 77, 135, 51, 105, 63, 89, 178.



Quantification

Blood **GC-MS** Column: HP-5MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 60° for 1 min to 220° at 20°/min. EI ionisation, SIM acquisition mode. Retention time: 7.14 min. Limit of detection not reported [Gunn *et al.* 2010]. SIM acquisition mode. MDMA, MDA and other amfetamines [Rasmussen *et al.* 2006]. Column: HP-5 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.7 mL/min. Temperature programme: 60° for 2 min to 250° at 20°/min for 5 min. SIM acquisition mode. Limit of quantification, 3.59 µg/g, limit of detection, 1.19 µg/g [Gentili *et al.* 2002]. Column: HP 5MS capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 50 kPa. Temperature programme: 60° for 1 min to 140° at 15°/min to 212° at 30°/min for 3 min to 285°. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.48 min. Limit of quantification, 10 µg/L for MDMA and 3,4-methylenedioxy-*N*-ethylamphetanime (MDEA) and 50 µg/L for MDA, limit of detection, 0.5–0.8 µg/L for MDMA, MDA and other amfetamines [Marquet *et al.* 1997].

HPLC Column: ODS. Mobile phase: 10 mmol/L citric acid: 20 mmol/L sodium hydrogen phosphate buffer (pH 4.0) : acetoxime (50 : 45 : 5). Fluorescence detection. Limit of detection, 0.36–0.83 µg/L for MDMA and MDA [Tomita *et al.* 2006]. Column: C_{18} BDS Hypersil (100 × 2.1 mm i.d., 3 µm). Mobile phase: 0.1 mol/L ammonium acetate-methanol-acetonitrile (90 : 10 : 10) : methanol-acetonitrile-water (45 : 45 : 10, 100 : 0 for 6 min to 30 : 70 in 14 min), flow rate 1 mL/min. I.S. 3,4 methylenedioxyphenyl-*N*-methyl propylamine (MDMPA). Fluorescence detection (λ_{ex} = 288 nm, λ_{em} = 324 nm). Retention time: 14.1 min for MDMA, 13.1 min for MDA, 15.1 min for MDEA and 17.1 min for IS Limit of quantification, 2 µg/L, limit of detection, 0.8 µg/L for MDMA, MDA and MDEA [Clauwaert *et al.* 2000]. Column: Whatman (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L sodium acetate (pH 4.25) : methanol (10 : 3), flow rate 0.8 mL/min. Electrochemical detection. Limit of detection, 1 µg/L for MDMA, MDA and MDE [Michel *et al.* 1993].

LC-MS Column: Agilent HPLC 1100 with Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: methanol : ammonium formate. MRM acquisition mode, positive ion mode. Limit of quantification, 0.0005–0.01 mg/kg for MDMA, MDA and other drugs of abuse [Bjork *et al.* 2010]. Column: Nucleodur Sphinx RP. Mobile phase: 10 mmol/L ammonium formate buffer : acetonitrile. Limit of quantification, 2.5 µg/L, limit of detection, 0.05–0.5 µg/L for MDMA, MDA and other amfetamines [Fernández Mdel *et al.* 2009]. Column: BEH C_{18} (50 × 2.1 mm i.d.). Mobile phase: aqueous pyrrolidine : methanol (52 : 48), flow rate 0.4 mL/min. ESI, positive ion mode. Retention time: 2.04 min. Limit of detection not reported [Apollonio *et al.* 2006].

Note For a review of methods published between 2002 and 2007 for the determination of MDMA, MDA, other amfetamines and drugs of abuse in blood, see Kraemer, Paul [2007].

Plasma **GC** Column: Ultra-2 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 2 min to 100° at 30°/min to 200° at 20°/min to 280° at 25°/min. NPD. Limit of quantification, 5.3 µg/L, limit of detection, 1.6 µg/L [Ortuño *et al.* 1999].

GC-MS Column: VF-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 300° at 15°/min for 10 min. EI ionisation, SIM acquisition mode. Retention time: 8.7 min. Limit of quantification, 5 µg/L, limit of detection, 2 µg/L for MDMA, MDA and related drugs [da Silva *et al.* 2010]. See Blood [Gunn *et al.* 2010]. Column: Zorbax 300-SCX (150 × 2.1 mm i.d., 5 µm). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (70 : 30). ESI, positive ion mode. Limit of detection not reported [Mueller *et al.* 2009]. Column: ZB-50 (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 70° to 150° at 35°/min for 0.5 min to 195° at 10°/min for 0.11 min to 100° at 50°/min to 275° at 800°/min to 190° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 2.5 µg/L, limit of detection, 0.5 µg/L [Kolbrich *et al.* 2008a]. Column: HP-5MS (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, 5 µg/L [Peters *et al.* 2003a; Peters *et al.* 2003b; Peters *et al.* 2005]. Column: 5% phenylmethylsiloxane (12 m × 0.22 mm i.d., 0.33 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 150° for 1 min to 290° at 20°/min for 7 min. EI ionisation, SIM acquisition mode. Limit of quantification, 7.3 µg/L for (*R*)-MDMA and 9.3 µg/L for (*S*)-MDMA, limit of detection, 2.4 µg/L for (*R*)-MDMA and 3.1 µg/L for (*S*)-MDMA [Pizarro *et al.* 2002; Pizarro *et al.* 2003].

HPLC See Blood [Tomita *et al.* 2006]. UV and fluorescence detection. Limit of detection, 25 µg/L for MDMA and MDE and 10 µg/L for MDA [Herráez-Hernández *et al.* 2001]. Column: ODS Hypersil C_{18} (130 mm). Mobile phase: methanol : 0.1 mol/L acetate buffer (pH 4.54 50 : 50), flow rate 0.7 mL/min. UV detection (λ = 280 nm). Limit of detection, 3.68 µg/L for MDMA and 2.0 µg/L for MDA [Garrett *et al.* 1991].

LC-MS Column: C_{18} Alltima (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L formic acid (10 : 90 for 2 min to 25 : 75 in 16 min to 35 : 65 in 0.1 min to 100 : 0 in 12 min), flow rate 1.0 mL/min. MRM acquisition mode, positive ion mode. Limit of quantification, 1.8 µg/L, limit of detection, 0.9 µg/L [Sergi *et al.* 2009]. Column: CAPCELL PAK SCX UG 80 (250 × 4.6 mm i.d.). Mobile phase: 25 mmol/L ammonium acetate (pH 4.0) : acetonitrile (3 : 7), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection, 50 µg/L [Kuwayama *et al.* 2008]. Column: Hypersil BDS-C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (75 : 25), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, <2 µg/L for MDMA, MDA and other amfetamines [Wood *et al.* 2003].

Serum **GC-MS** See Blood [Gunn *et al.* 2010]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 200° at 20°/min to 240° at 5°/min to 310° at 30°/min for 6 min. EI ionisation, SIM acquisition mode. Limit of quantification, 10.4 µg/L [Weinmann *et al.* 2000].

HPLC See Blood [Clauwaert *et al.* 2000].

Urine **TLC** Fluorescence detection (250 to 400 nm). Limit of detection, 50 ng for MDMA, MDA and other amfetamines [Kato *et al.* 2008].

GC Limit of detection, 20 µg/L for MDMA and MDA [Wang *et al.* 2008]. Column: AT-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 1 min to 280° at 20°/min for 5 min. FID. Limit of detection, 35 µg/L [Raikos *et al.* 2003]. NPD. Limit of detection, 47 µg/L for MDMA, MDA and other metabolites [Ortuño *et al.* 1999]. Column: SPB-1 polymethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature: 180°. Limit of detection, 5 µg/L for MDMA and MDEA and 15 µg/L for MDA [Ugland *et al.* 1999].

GC-MS SIM acquisition mode. Limit of quantification, 3.5 µg/L for MDMA, MDA and related drugs [da Silva *et al.* 2010]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 127 kPa. Temperature programme: 70° for 1 min to 300° at 20°/min for 2 min. EI ionisation at 70 eV. Limit of detection, 5 µg/L for MDMA and 10 µg/L for MDA [Nakamoto *et al.* 2010]. Column: Varian VF-5ht (30 m × 0.25 mm i.d., 0.10 µm). Temperature programme:150° to 270° at 10°/min. EI ionisation, full scan mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.1 mg/L [Brown *et al.* 2007]. Column: DB-35MS (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 psi. Temperature programme: 70° for 2 min to 160° at 20°/min for 2 min to 200° at 15°/min to 300° for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L for MDMA, MDA and other metabolites [Pirnay *et al.* 2006]. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L for MDMA, MDA and other amfetamines [Maresová *et al.* 2006]. See Blood [Gentili *et al.* 2002]. See Plasma [Pizarro *et al.* 2002; Pizarro *et al.* 2003]. See also Centini *et al.* [1996], Dallakian *et al.* [1996], de Boer *et al.* [1997], Gan *et al.* [1991], Hensley, Cody [1999], Jurado *et al.* [2000], Klette *et al.* [2005], Namera *et al.* [2002], Paul *et al.* [2004], Pellegrini *et al.* [2002] and Stout *et al.* [2002].

HPLC Column: Genesis C8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 2.5) containing 0.3% TEA (12 : 88). Fluorometric detection [Bugamelli *et al.* 2006]. UV detection. Limit of detection, 5.3–84 ng for MDMA, MDA and other amfetamines [Soares *et al.* 2004]. See Plasma [Herráez-Hernández *et al.* 2001]. See Blood. Limit of quantification, 0.1 mg/L, limit of detection, 2.5 µg/L for MDMA, MDA and MDEA [Clauwaert *et al.* 2000]

LC-MS See Blood. Limit of quantification, 25 µg/L, limit of detection, 0.25–2.5 µg/L for MDMA, MDA and other amfetamines [Fernández Mdel *et al.* 2009].

Column: C_{18} (100 × 2 mm i.d., 3 μm). Mobile phase: acetonitrile : 25 mmol/L formic acid (91 : 9 for 5 min to 80 : 20 in 4 min to 0 : 100 in 3 min to 91 : 9 for 5 min), flow rate 0.3 mL/min. ESI, SRM acquisition mode. Limit of quantification, 26 μg/L, limit of detection, 8 μg/L [Andersson *et al.* 2008]. Column: CAPCELL PAK C_{18} MG-II (150 × 2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium formate (pH 4.0) : acetonitrile. MRM acquisition mode. Limit of detection, <1.95 μg/L for MDMA, MDA and other amfetamines and metabolites [Kim *et al.* 2008]. Column: Atlantis dC_{18} (20 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile : ammonium formate buffer (pH 3.0, 0 : 100 to 10 : 90 in 2 min for 1.5 min to 100 : 0 in 1 min to 0 : 100 in 0.5 min). ESI, SRM acquisition mode. Limit of quantification, 1 μg/L for MDMA and 2 μg/L for MDA, limit of detection, 0.2 μg/L for MDMA and 1 μg/L for MDA [Concheiro *et al.* 2007a; Concheiro *et al.* 2007b]. Column: ODS (150 × 1.5 mm i.d., 5 μm). Mobile phase: methanol : 10 mmol/L ammonium formate buffer (pH 3.5, 15 : 85 to 35 : 65 over 25 min). ESI, CID. Limit of detection, 5 nmol/L [Shima *et al.* 2007]. See Plasma [Kuwayama *et al.* 2008]. See Hair [Wu *et al.* 2001]. See also Chèze *et al.* [2007], Jenkins *et al.* [2004] and Wu, Fuh [2005].

CE Column: fused silica (245/230 mm effective/paced length, 75 μm). Mobile phase: 20 mmol/L sodium phosphate buffer : acetonitrile (80 : 20). Limit of quantification, 12 μg/L, limit of detection, 5–15 μg/L for MDMA, MDA and other drugs of abuse [Aturki *et al.* 2009]. Column: monolithic capillary (245/336 mm effective/total length, 75 μm i.d.). Limit of quantification, 7.5 mg/L, limit of detection, 1 mg/L for MDMA and metabolites [Choodum *et al.* 2009].

Note For a comparison of a range of immunoassays for MDMA, MDA and other amfetamines, see Verstraete, Heyden [2005] or Hsu *et al.* [2003].

Bile **LC-MS** See Plasma [Kuwayama *et al.* 2008].

Oral Fluid **GC-MS** Column: Agilent HP-5MS (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 70° to 170° at 25°/min for 0.2 min to 195° at 5°/min for 0.5 min to 300° at 30°/min for 1 min. SIM acquisition mode. Limit of quantification, 5 μg/L, limit of detection, 2.5 μg/L [Scheidweiler, Huestis 2006]. See Blood [Gentili *et al.* 2002].

LC-MS See Sergi *et al.* [2009] and Wood *et al.* [2003]

Note For a review of the determination of MDMA, MDA and other 'Ecstasy' components in oral fluid, sweat and hair, see Kintz, Samyn [1999]. For the analysis of MDMA and its metabolites in oral fluid and correlation with plasma levels, see Navarro *et al.* [2001].

Vitreous Humour **HPLC** See Blood [Clauwaert *et al.* 2000]

Sweat See Oral Fluid [Kintz, Samyn 1999].

Hair **GC-MS** Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.1 mL/min. Temperature programme: 90° for 0.5 min to 150° at 25°/min to 230° at 15°/min for 1.0 min to 300° at 40°/min for 1.3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.05 ng/mg, limit of detection, 0.011 μg/g [Kim *et al.* 2010]. Limit of quantification, 0.24–0.46 μg/g, limit of detection, 0.07–0.14 μg/g for MDMA, MDA and other amfetamines [Johansen, Jornil 2009]. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 210° at 10°/min for 3 min. EI ionisation, SIM acquisition mode. Retention time: 11.7 min. Limit of quantification, 50 ng/g, limit of detection, 20 ng/g for MDMA, MDA and other amfetamines [Meng *et al.* 2009]. Column: HP-5MS 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 0.2 min to 280° at 20°/min for 3 min. EI ionisation, SIM acquisition mode. Retention time: 4.98 min. Limit of quantification, 0.05 ng/mg, limit of detection, 0.03 μg/g [Wu *et al.* 2008a; Wu *et al.* 2008b]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 2 min to 220° at 20°/min to 260° at 5°/min to 305° at 30°/min. NCI. Limit of detection, 0.002 ng/mg [Martins *et al.* 2007]. See Blood [Gentili *et al.* 2002]. See also Kim *et al.* [2007], Kintz *et al.* [1995], Martins *et al.* [2006], Pujadas *et al.* [2003], Röhrich, Kauert [1997], Skender *et al.* [2002], Villamor *et al.* [2005] and Zhu *et al.* [2007].

HPLC Column: CAPCELL PAK C_{18} UG120 (250 × 1.5 mm i.d., 5 μm). Mobile phase: 20 mmol/L imidazole-nitric acid buffer (pH 7.0) : acetonitrile : tetrahydrofuran (51.5 : 45 : 3.5), flow rate 0.1 mL/min. Electrochemical detection. Limit of quantification, 10 ng/mg, limit of detection, 3 ng/mg [Nakamura *et al.* 2007]. Column: Daisopak SP-120-5-ODS-BP (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : water (30 : 40 : 30), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 325 nm, λ_{em} = 430 nm). Limit of detection, 0.2 ng/mg [Kaddoumi *et al.* 2004]. Column: reversed phase (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1 mol/L potassium phosphate (pH 3) : acetonitrile (82 : 18). Fluorescence detection (λ_{ex} = 285 nm, λ_{em} = 320 nm). Limit of detection, <1 μg/L for MDMA, MDA and MDEA [Tagliaro *et al.* 1999].

LC-MS Column: Synergi Polar (150 × 2 mm i.d., 4 μm). Mobile phase: 0.01% acetic acid: methanol (50 : 50), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 μg/g, limit of detection, 0.1 μg/g for MDMA, MDA and other amfetamines [**Tabernero *et al.* 2009**]. Column: Supelcosil LC-CN (33 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : 50 mmol/L ammonium acetate (15 : 85), flow rate 0.4 mL/min. ESI, SIM acquisition mode. Limit of detection, 8–56 ng/L for MDMA and related drugs [Wu *et al.* 2001].

CE-MS Column: uncoated fused silica capillary (100 cm total length, 75 μm). ESI, SIM acquisition mode. Limit of quantification, 0.04 ng/mg, limit of detection, 002 ng/mg [Gottardo *et al.* 2007].

Note For a comparison of CE and GC-MS for the analysis of MDMA, see Fang *et al.* [2002].

Disposition in the Body MDMA is absorbed into the bloodstream after ingestion and excreted in urine, the majority of the dose being unchanged (65% within 3 days). Metabolism occurs by a number of routes: *N*-demethylation of the parent compound to MDA (7%) with further *O*-demethylation to 3,4-dihydroxymetamfetamine (HHMA) and 3,4-dihydroxyamfetamine (HHA). Both HHMA and HHA are subsequently *O*-methylated by catechol-*O*-methyltransferase (COMT) mainly to 4-hydroxy-3-methoxymetamfetamine (HMMA) and 4-hydroxy-3-methoxyamfetamine (HMA). These 4 metabolites are excreted in the urine as the conjugated glucuronide or sulfate metabolites.

Therapeutic Concentration

After the administration of a single oral dose of 1.5 mg/kg body weight MDMA to 2 patients, plasma and urine samples were collected over periods of 9 and 22 h, respectively. Peak plasma concentrations of MDMA and MDA were 331 μg/L after 2 h and 15 μg/L after 6.3 h, respectively. Peak concentrations of 28.1 μg/L MDMA in urine appeared after 21.5 h. Up to 2.3 μg/L MDA, 35.1 μg/L HMMA and 2.1 μg/L HMA were measured within 16 to 21.5 h, also in urine [Helmlin *et al.* 1996]

Eight healthy male volunteers, aged between 21 and 31 years, were given a 75 mg dose of MDMA. Mean peak plasma concentration was 0.13 mg/L after 1.8 h. Mean peak plasma concentrations of MDA were 7.8 μg/L approximately 5 h after administration [de la Torre *et al.* 2000].

In a double-blind, randomised study, 17 healthy subjects were given single oral doses of 1.0 mg/kg MDMA, 1.6 mg/kg MDMA or placebo. Peak plasma levels of MDMA were ≈163 and 292 μg/L after the low and high doses, respectively. Peak plasma levels of MDA were 8.4 and 13.8 μg/L following the low and high doses of MDMA, respectively. Mean half-lives of MDMA and MDA were 7–8 h and 10.5–12.5 h, respectively [Kolbrich *et al.* 2008b]

In a further study, 30.2–34.3% of urinary excretion was found to occur in the first 24 h following single 1.0 or 1.6 mg/kg doses of MDMA [Abraham *et al.* 2009].

Toxicity Fatalities with doses of 300 mg have been reported. Risk of adverse reaction is high but the pattern is varied. Severe acute toxic effects and morbidity are noted. MDMA is capable of causing severe toxicity and the pattern of acute toxicity reflects the circumstances in which it is misused. Circumstances associated with ingestion and direct effects of the drug are thought to influence toxicity. The amount of MDMA ingested is difficult to determine because the concentration in tablets is unknown and it may be mixed with other substances. A lethal toxic reference concentration of 0.4–0.8 mg/L has been noted, although some overdose cases report concentrations ten times this amount without fatality.

An 18-year-old man ingested 3 tablets and collapsed. He was unconscious on the floor for 60 min and died 2.5 h after admission to hospital. A plasma concentration of 0.36 mg/L MDMA was detected [Henry *et al.* 1992].

A 16-year-old woman ingested 1tablet and suffered from seizures and experienced hallucinations. The MDMA plasma concentration was 0.424 mg/L. She died 36 h after admission to hospital [Henry *et al.* 1992].

A 21-year-old woman ingested an unknown number of tablets and suffered from hyperactivity leading to convulsions and repeated seizures. Plasma concentration of MDMA was 0.11 mg/L. She underwent a liver transplant after 4 days but died after 18 days [Henry *et al.* 1992].

An 18-year-old man ingested 3 tablets and suffered from seizures and sweating. The MDMA concentration was 1.26 mg/L in plasma. He died 6.5 h after admission [Henry *et al.* 1992].

A 23-year-old man ingested 3 tablets and suffered from convulsions and agitation. The MDMA plasma concentration was 0.2 mg/L. He was hospitalised for 33 days [Henry *et al.* 1992].

A 19-year-old woman ingested 2 tablets and suffered from vomiting, watery diarrhoea and was in a coma. Plasma concentrations were MDMA 0.24 mg/L, MDA 0.02 mg/L and amfetamine 0.02 mg/L. She recovered [Henry *et al.* 1992].

A report of a traffic fatality in a 29-year-old man attributed the cause of death to the use of MDMA. Concentrations of MDMA were: blood 2.32 mg/L (clotted blood) and 2.14 mg/L (sodium fluoride–potassium oxalate antocoagulated blood), vitreous humour 1.11 mg/L and urine 118.8 mg/L. Levels of MDA were <0.25 mg/L in blood and vitreous humour and 3.86 mg/L in urine [Crifasi, Long 1996].

Following a fatal poisoning with MDMA, postmortem blood levels of (*S*)-MDMA and (*R*)-MDMA were 1.3 and 1.6 mg/L, respectively. Levels of (*S*)- and (*R*)-MDA were 0.8 mg/L [Moore *et al.* 1996].

A 30-year-old man was admitted to hospital after taking 50 ecstasy tablets, 10 oxazepam tablets (10 mg) and 5 units of alcohol over a period of 4 to 5 h. Presenting features were unconciousness, apnoea and convulsions. Toxicological analysis revealed the following MDMA and MDA concentrations:

Time after ingestion (h)	*Sample*	*MDMA (mg/L)*		*MDA (mg/L)*	
		R-(−)	*S-(+)*	*R-(−)*	*S-(+)*
4	Urine	44	42	nd	0.4
	Gastric lavage	70	72	nd	nd
36	Urine	36	17	3	7
60	Urine	18	10	5	4
	Serum	0.8	0.2	nd	nd

The individual recovered after a few days without sequelae [Ramcharan *et al.* 1998].

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Twelve hours after ingesting a maximum of 40 ecstasy tablets, a 19-year-old man was admitted to an intensive care unit. Toxicological analysis revealed the following MDMA concentrations:

Time after ingestion (h)	*Serum MDMA (mg/L)*	*Urine MDMA (mg/L)*
13	4.3	630
24	1.4	180
30	0.75	105

The individual recovered after a few days without sequelae [Regenthal *et al.* 1999].

A 28-year-old man was found unconscious in a bar. A postmortem examination was carried out roughly 28 h later, which revealed the following MDMA and MDA concentrations:

Sample	*MDMA*	*MDA*
Subclavian blood (mg/L)	3.5	0.09
Femoral blood (mg/L)	3.1	0.093
Serum (subclavian blood) (mg/L)	4.2	0.1
Vitreous humour (mg/L)	3.4	0.06
Urine (mg/L)	170.9	4
Bile (mg/L)	14.2	0.32
Liver (μg/g)	26.2	1.203
Stomach content (μg/g)	118.1	0.448
Spleen (μg/g)	10	0.264
Brain (frontal lobe) (μg/g)	17.4	0.296
Brain (parietal lobe) (μg/g)	17.1	0.362
Brainstem (μg/g)	13.2	0.22
Cerebellum (μg/g)	11.7	0.225

Cotinine, caffeine and trace amounts of benzoylecgonine were also detected [De Letter *et al.* 2002].

A 19-year-old woman died after taking ecstasy tablets. Blood levels of MDMA were 3.8 mg/L and traces of MDA were also found [Libiseller *et al.* 2005].

A 17-year-old man died after ingestion of ecstasy. Postmortem levels were highest in the stomach (835.97 μg/g) and kidney (801.14 μg/g). Lowest levels were found in the liver (22.26 μg/g) [Dordevic, Tomasevic 2007].

MDMA and MDA blood levels of 1.42 mg/L and 0.17 mg/L, respectively, were found at postmortem following fatal ecstasy poisoning in a 22-year-old man. Hair levels of 8.74–15.51 μg/g in 2 cm strands of hair confirmed chronic drug use [Klys *et al.* 2007].

A 14-month-old infant who swallowed a portion of an ecstasy tablet had a generalised convulsion 40 min after ingestion. He presented with hyperthermia, hypertension, ventricular extrasystoles, tachypnoea and mydriasis. Urine amfetamine levels were greater than 16 mg/L 5 h later and the serum level of MDMA was 0.591 mg/L at 8 h. He recovered after general supportive measures and IV benzodiazepines [Melian *et al.* 2004].

In another case of MDMA intoxication in an infant after accidental ingestion, MDMA and HMMA levels in the infant's hydrolysed urine were 11.7 and 34.4 mg/L, respectively [Garcia-Algar *et al.* 2005].

For other reports of ecstasy poisoning in young children, see Boucher *et al.* [2009] and Cassidy *et al.* [2009]. For other studies of MDMA and MDA distribution following fatalities associated with MDMA, see Dams *et al.* [2003], De Letter *et al.* [2004], [2007], Liu *et al.* [2006] and Valtier *et al.* [2007]. For an overview of ecstasy-related fatalities, see Schifano [2004]; for a review of MDMA deaths in New York City, see Gill *et al.* [2002]; for reviews of deaths in the UK associated with ecstasy use, see Schifano *et al.* [2003]; Schifano [2004]; and Schifano *et al.* [2006]; for a review of MDMA-related deaths in Taiwan, see Lin *et al.* [2009]; for a report on MDMA and driving impairment, see Logan, Couper [2001], see also Brookhuis *et al.* [2004], Kuypers *et al.* [2006], Lamers *et al.* [2003] and Moeller, Hartung [1997].

Half-life ≈6–7 h.

Clearance The mean total clearance of MDMA for a 75 mg dose is 86.9 L/h.

Protein Binding ≈65%

Note For methods of analysis and ecstasy tablet profiling, see Bonadio *et al.* [2009], Cheng *et al.* [2003], Choodum *et al.* [2009], Gimeno *et al.* [2002], Inoue *et al.* [2009], Marquis *et al.* [2008], Milliet *et al.* [2009], Parrott [2004], Rashed *et al.* [2000], Sherlock *et al.* [1999], Shetab Boushehri *et al.* [2009], Tanner-Smith [2006] and Vogels *et al.* [2009]. For a review of the history, neurochemistry and toxicology of ecstasy, see Rochester, Kirchner [1999]; see also Maurer [1996], Ricaurte *et al.* [2000] and Rogers *et al.* [2009].

Dose The usual dose is between 80 and 200 mg (more often 100 to 150 mg).

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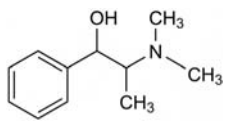
Methylephedrine

Sympathomimetic
$C_{11}H_{17}NO = 179.3$
CAS—552-79-4

IUPAC Name (1*R*,2*S*)-2-(Dimethylamino)-1-phenylpropan-1-ol
Synonyms α-[1-(Dimethylamino)ethyl]benzenemethanol; L-methylephedrine; L-*N*-methylephedrine.



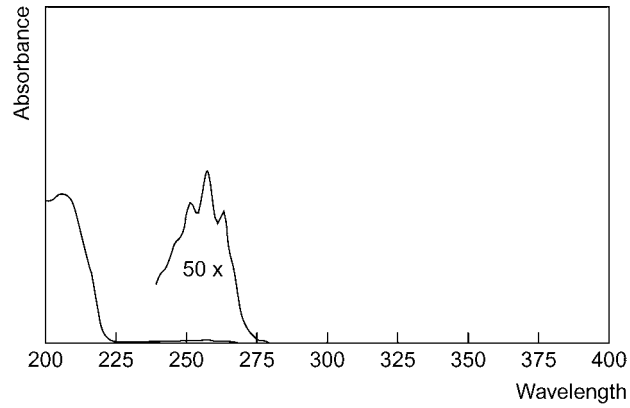
Chemical Properties Soluble in chloroform and ether. pK_a 9.3 (25°). Log *P* (octanol/water), 0.9.

Methylephedrine Hydrochloride

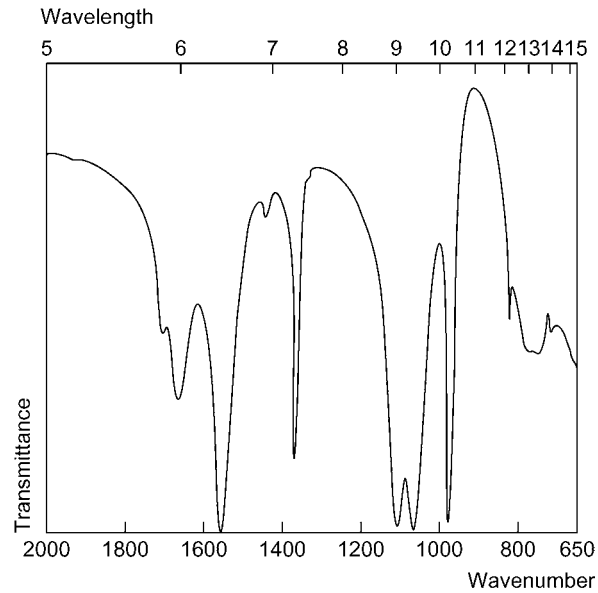
$C_{11}H_{17}NO,HCl = 215.7$
CAS—38455-90-2
Proprietary Names It is an ingredient of *Coughmin*, *Hustazol-C*, *Ilvico*, *Methorcon*, *Tossamine* plus *Tussoretardin*.
Chemical Properties A white crystalline powder. Mp 207° to 208° (crystals from acetone). Freely soluble in water; soluble in ethanol; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.32; system TE—R_f 0.35; system TAE—R_f 0.12 (acidified iodoplatinate solution, positive).

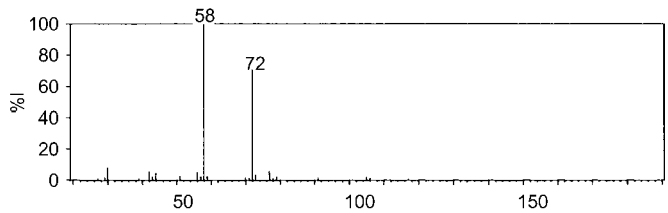
Gas Chromatography System GA—methylephedrine RI 1405; ephedrine RI 1365; system GB—methylephedrine RI 1451; ephedrine RI 1410; system GC—methylephedrine RI 1480; ephedrine RI 1467.
High Performance Liquid Chromatography System HA—methylephedrine *k* 2.3; ephedrine 1.0; system HC—methylephedrine *k* 1.83; ephedrine 1.79.
Ultraviolet Spectrum Aqueous acid—251, 257 ($A_1^1 = 11a$), 263 nm (see below).



Infrared Spectrum Principal peaks at wavenumbers 742, 1039, 702, 1052, 990, 1162 cm^{-1} (KBr disk, see below).



Mass Spectrum Principal ions at *m*/*z* (methylephedrine) 58, 72, 30, 77, 56, 44, 42, 73; (ephedrine) 58, 146, 56, 105, 77, 42, 106, 40.



Quantification

Blood **GC** Column: DB-5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. NPD. Retention time: 5 min. Limit of detection not reported [Kunsman *et al.* 1998]. Column: DB-5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 280° at 20°/min for 6 min. NPD. Limit of detection not reported [Levine *et al.* 1993].
Urine **GC** Column: Rtx-5 amine cross-linked 5% diphenylpolysiloxane-95% dimethylpolysiloxane (15 m × 0.25 mm i.d., 1.0 µm). Carrier gas: He. Temperature programme: 100° to 105° at 0.5°/min to 118° at 2°/min to 280° at

0°/min for 2 min. NPD. Retention time: 17.2 min. Limit of detection, 4 mg/L [Van Eenoo *et al.* 2001]. See Blood [Kunsman *et al.* 1998].

GC-MS Column: TC-1 cross-linked methyl silicone (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 4 psi. Temperature programme: 60° or 100° for 0.5 min to 280° at 20°/min or 10°/min, respectively. Limit of detection, 500 μg/L [Nakahara, Kikura 1997].

HPLC Column: Spherisorb ODS 1 (150 × 4.6 mm i.d., 5 μm). Mobile phase: tetraethylammonium phosphate : methanol (pH 2.8; 98 : 2), flow rate 1.0 mL/min. UV detection (λ = 214 nm). Retention time: 6.1 min. Limit of quantification, 2.5 mg/L, limit of detection, 0.5 mg/L [van der Merwe *et al.* 1994]. Column: LiChrospher 60 RP Select B (125 × 4 mm i.d., 5 μm). Mobile phase: 200 mmol/L phosphate buffer : 150 mmol/L TEA (pH 5.5), flow rate 1.3 mL/min. UV detection (λ = 215 nm). Limit of detection, 0.5 mg/L [Imaz *et al.* 1993];

***Hair* GC-MS** See Urine. Limit of detection, 0.1 ng/mg [Nakahara, Kikura 1997].

Disposition in the Body Methylephedrine is absorbed after oral administration. Approximately 32% of a dose is excreted in the urine unchanged and 8% is excreted as the demethylated metabolite ephedrine; the rate of excretion is dependent on the urinary pH [Inoue, Suzuki 1990].

Toxicity Although methylephedrine is not available in the USA, it was identified in 15 cases received by the Armed Forces Institute of Pathology, Rockville, Maryland. Blood methylephedrine concentrations ranged from <0.05 to 0.28 mg/L while the mean methylephedrine urine concentration was 1.6 mg/L (range 0.15–6.8 mg/L). As no evidence suggested that methylephedrine was present at toxic concentrations, it appears that blood methylephedrine concentrations of <0.3 mg/L are not associated with significant toxicity [Kunsman *et al.* 1998].

A 19-year-old white man was found dead in his family's home. Postmortem examination revealed the following drug concentrations (mg/L): methylephedrine 5.6, dihydrocodeine 4.7, chlorphenamine 2.6, salicylate 150.0 and verapamil 6.0. Concentrations in the liver were 6.7, 25, 8.9, –, and 130 mg/kg, respectively. Methylephedrine, dihydrocodeine and chlorphenamine appear in an over-the-counter cold medication in Japan under the trade name Bron. The cause of death was multiple drug intoxication [Levine *et al.* 1993].

Note For a study of the metabolism of methylephedrine in rat and man see Inoue, Suzuki [1990].

Dose Up to 200 mg methylephedrine hydrochloride has been given daily in divided doses.

Imaz C *et al.* (1993). Determination of ephedrines in urine by high-performance liquid chromatography. *J Chromatogr* 631: 201–205.

Inoue T, Suzuki S (1990). The metabolism of (±)-methylephedrine in rat and man. *Xenobiotica* 20: 99–106.

Kunsman GW *et al.* (1998). Methylephedrine concentrations in blood and urine specimens. *J Anal Toxicol* 22: 310–313.

Levine B *et al.* (1993). An intoxication involving Bron and verapamil. *J Anal Toxicol* 17: 381–383.

Nakahara Y, Kikura R (1997). Hair analysis for drugs of abuse. XIX. Determination of ephedrine and its homologs in rat hair and human hair. *J Chromatogr B Biomed Sci Appl* 700: 83–91.

van derMerwe PJ *et al.* (1994). Simultaneous quantification of ephedrines in urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 661: 357–361.

VanEenoo P *et al.* (2001). Simultaneous quantitation of ephedrines in urine by gas chromatography–nitrogen-phosphorus detection for doping control purposes. *J Chromatogr B Biomed Sci Appl* 760: 255–261.

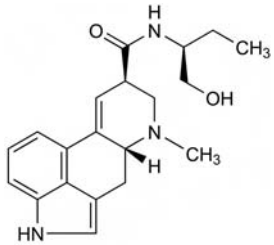
Methylergometrine

Uterine Stimulant

$C_{20}H_{25}N_3O_2$ = 339.4

CAS—113-42-8

Synonyms (8β)-9,10-Didehydro-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-6-methylergoline-8-carboxamide; methylergobasine; methylergonovine.



Chemical Properties Crystals. Mp 172°, with decomposition. Sparingly soluble in water; freely soluble in ethanol and acetone. pK_a 6.7 (24°). Log *P* (octanol/water), 1.0.

Methylergometrine Maleate

$C_{20}H_{25}N_3O_2,C_4H_4O_4$ = 455.5

CAS—57432-61-8

Proprietary Names *Basofortina*; *Ergotyl*; *Metenerin*; *Methergin(e)*; *Methylergobrevin*; *Metrine*; *Nathergen*; *Ryegonovin*; *Spametrin-M*.

Chemical Properties A white or pinkish-tan, crystalline powder, which darkens on exposure to light. Mp 185° to 195°, with decomposition. Soluble 1 in 200 of water and 1 in 140 of ethanol giving a blue fluorescence; very slightly soluble in chloroform and ether.

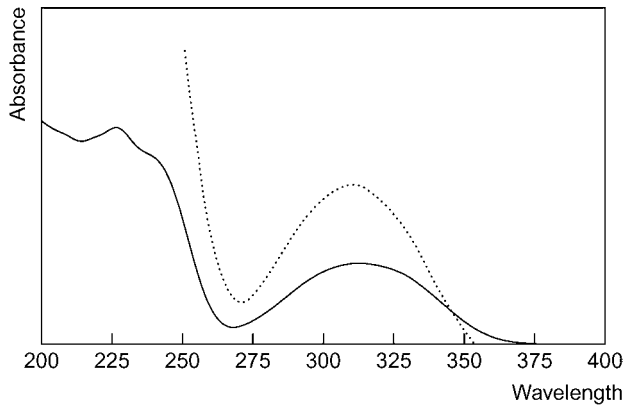
Colour Tests Mandelin's test—violet-brown; Marquis test—grey-brown.

Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.00; system TC—R_f 0.14; system TE—R_f 0.41; system TL—R_f 0.12; system TM—R_f 0.31; system TAE—R_f 0.69 (van Urk reagent, blue).

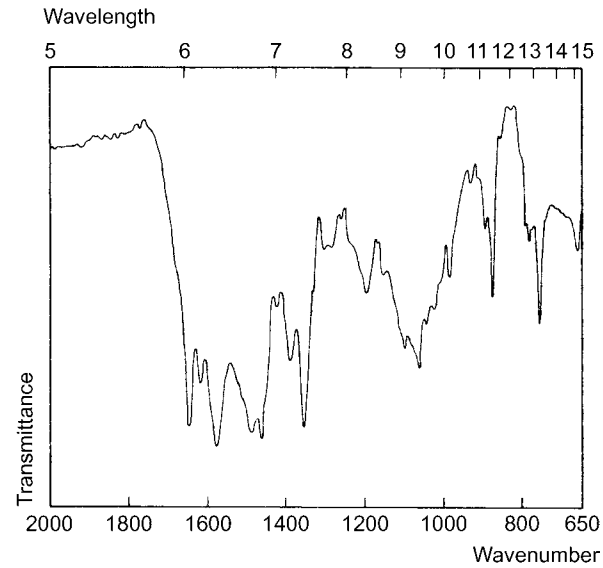
Gas Chromatography System GA—not eluted.

High Performance Liquid Chromatography System HA—*k* 0.4; system HP—*k* 0.83; system HX—RI 320; system HZ—retention time 2.1 min.

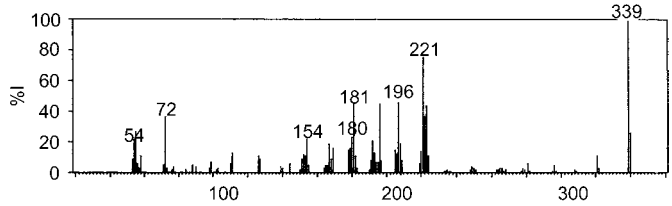
Ultraviolet Spectrum Aqueous acid—313 nm (A_1^1=255a); aqueous alkali—310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1580, 1490, 1650, 1620, 1065, 1103 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 339, 221, 196, 181, 207, 223, 222, 72.



Quantification

***Plasma* HPLC** Fluorescence detection. Methylergometrine and ergotamine. Limit of detection, 100 ng/L [Edlund 1981].

***Serum* HPLC** See Plasma [Edlund 1981].

Disposition in the Body Rapidly absorbed after oral administration. About 2 to 3% of a dose is excreted in the urine as unchanged drug in 24 h.

Therapeutic Concentration

After a single oral dose of 0.25 mg to 6 subjects, peak plasma concentrations of about 0.003 mg/L were attained in 0.5 h [Mantyla *et al.* 1978].

Half-life Plasma half-life, about 2 h.

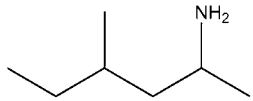
Volume of Distribution About 0.5 L/kg.
Protein Binding About 35%.
Dose 0.375 to 1 mg of methylergometrine maleate daily, for up to 7 days.

Edlund PO (1981). Determination of ergot alkaloids in plasma by high-performance liquid chromatography and fluorescence detection. *J Chromatogr* 226: 107–115.
Mantyla R *et al.* (1978). Methylergometrine (methylergonovine) concentrations in the human plasma and urine. *Int J Clin Pharmacol Biopharm* 16: 254–257.

Methylhexaneamine

α-Adrenoceptor Agonist
$C_7H_{17}N$ = 115.2
CAS—105-41-9

IUPAC Name 4-Methylhexan-2-amine
Synonyms 2-Amino-4-methylhexane; 1,3-dimethylamylamine; 1,3-dimethylpentylamine; methylhexamine; 4-methyl-2-hexaneamine.



Proprietary Names *Forthan*; *Forthane*.
Chemical Properties A colourless-to-pale yellow liquid with an amine odour. Bp 130° to 135° [Council on Pharmacy and Chemistry of the American Medical Association 1950]. Slightly soluble in water; very soluble in ethanol, ether and chloroform. Methylhexaneamine is volatile and may be isolated by distillation.
Disposition in the Body
Toxicity Excessive use of methylhexaneamine inhalers may produce headache, nervousness, mental overactivity, and tremors. The estimated minimum lethal dose in children up to 2 years is 200 mg.
Dose As the carbonate equivalent to 250 mg of base, it is used in inhalers for the temporary relief of nasal congestion [Council on Pharmacy and Chemistry of the American Medical Association 1950].

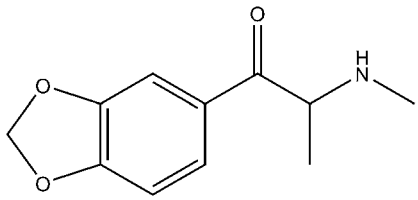
Council on Pharmacy and Chemistry of the American Medical Association NEW and nonofficial remedies: methylhexamine; forthane. *J Am Med Assoc* 143: 1156.

M

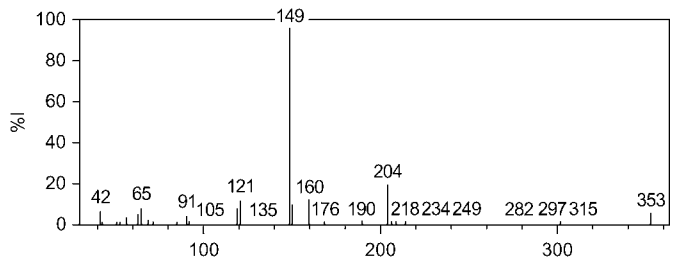
Methylone

Cathinone Derivative, Phenethylamine, Stimulant
$C_{11}H_{13}NO_3$ = 207.2
CAS—186028-79-5 (racemic); 191916-41-3 (*S*-form)

IUPAC Name 2-Methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one
Synonyms bk-MDMA; explosion; M1; MDMCAT; 3,4-methylenedioxymethcathinone.
Note The name Methylone is also a trademarked brand name for an injectable form of methylprednisolone.



Chemical Properties Cathinone analogue of MDMA (3,4-methylenedioxymethamphetamine). Mp 226° to 228° from diethyl ether [Dal Cason *et al.* 1997]; also reported as 225° with decomposition [Kikura-Hanajiri *et al.* 2007].
High-Performance Liquid Chromatography Column: C_{18} (100 × 4.0 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.01 mmol/L acetic acid buffer (pH 4.25). DAD. Retention times: methylone 5.4 min, 5-methoxy-*N*-methyl,*N*-isopropyl tryptamine 10.0 min. Limit of quantification not reported [Shimizu *et al.* 2007].
Mass Spectrum Principal ions at *m/z* 149, 204, 121, 160 (pentafluoropropionyl derivative).



Quantification
Urine **GC-MS** Column: DB-5MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 80° for 2 min to 320° at 15°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of quantification not reported [Kamata *et al.* 2006].

LC-MS Column: L-column C_{18} (150 × 1.5 mm i.d.). Mobile phase: methanol : 10 mmol/L ammonium formate buffer (pH 3.5, 5 : 95 to 40 : 60 over 30 min). ESI, positive ion mode, SIM acquisition mode. Limit of detection, methylone 2.5 µg/L, 3,4-methylenedioxycathinone (MDC) 25 µg/L, 4-hydroxy-3-methoxymethcathinone (HMMC) 10 µg/L, 3-hydroxy-4methoxymethcathinone (3-OH-4-MeO-MC) 5 µg/L [Kamata *et al.* 2006].
Other **GC-MS** Rat Hair and Plasma Samples. Column: DB35-MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 280° at 10°/min for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 13.2 min (pentafluoropropionyl derivative). Limit of quantification, 5 ng/mg in hair and 10 µg/L in plasma [Kikura-Hanajiri *et al.* 2007].
Disposition in the Body In humans and rats, the major metabolic pathways include *N*-demethylation to methylenedioxycathinone, and demethylation followed by *O*-methylation of the 3- or 4-OH group to HMMC or 3-OH-4-MeO-MC. Following administration of 5 mg/kg of methylone to rats, ~26% of the dose was excreted as HMMC within 48 h with <3% excreted unchanged.
Toxicity

A 27-year-old Japanese man was taken seriously ill 30 min after the ingestion of 200 mg of drug powder purchased from the Internet, thought to be pure methylone. Analysis of the remaining powder revealed a composition of 60% methylone and 38% 5-MeO-MIPT (5-methoxy-*N*-methyl,*N*-isopropyl tryptamine). Body fluids were not submitted for analysis. He was discharged the following day without any known sequelae [Shimizu *et al.* 2007].

A 19-year-old Japanese man was taken to the emergency department with strong dementia following the ingestion of methylone (dosage unclear). Urine levels of total methylone, HMMC and 3-OH-4-MeO-MC were reported as 48, 170, and 74 µg/L, respectively. 3,4-Methylenedioxycathinone (MDC) was also detected but not quantified [Kamata *et al.* 2006].

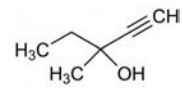
Note Studies on animals have found a methylone hair: plasma ratio of 0.55 [Kikura-Hanajiri *et al.* 2007].
Dose Reported as 150 to 200 mg orally, and 50 to 150 mg IV.

DalCason TA *et al.* (1997). Cathinone: an investigation of several *N*-alkyl and methylenedioxy-substituted analogs. *Pharmacol Biochem Behav* 58: 1109–1116.
Kamata HT *et al.* (2006). Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica* 36: 709–723.
Kikura-Hanajiri R *et al.* (2007). The disposition into hair of new designer drugs; methylone, MBDB and methcathinone. *J Chromatogr B Analyt Technol Biomed Life Sci* 855: 121–126.
Shimizu E *et al.* (2007). Combined intoxication with methylone and 5-MeO-MIPT. *Prog Neuropsychopharmacol Biol Psychiatry* 31: 288–291.

Methylpentynol

Hypnotic, Sedative
$C_6H_{10}O$ = 98.14
CAS—77-75-8

IUPAC Name 3-Methyl-1-pentyn-3-ol
Synonyms Meparfynol; methylparafynol.
Proprietary Names *Allotopral*; *Oblivon*.

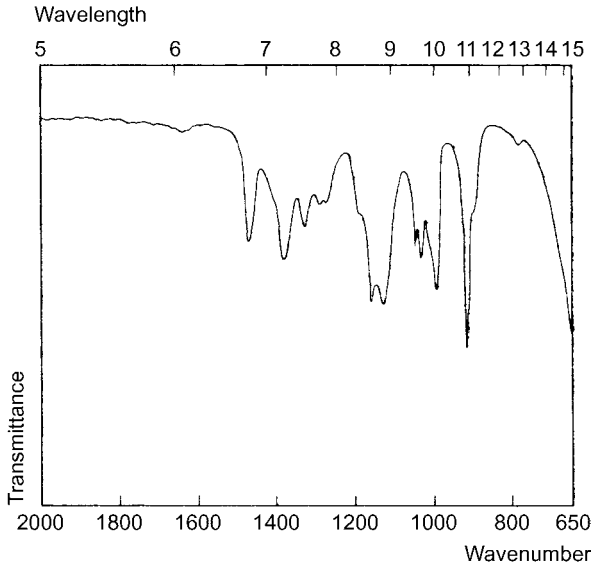


Chemical Properties A colourless or pale yellow liquid. Mass per mL 0.865 to 0.873 g. Bp about 120°. Refractive index, at 20°, 1.430 to 1.432. Soluble 1 in 10 of water; soluble in ether; miscible with organic solvents. Log *P* (octanol/water), 0.9.

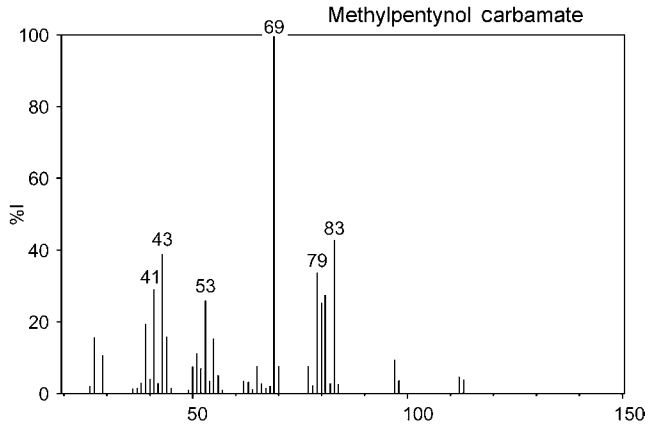
Methylpentynol Carbamate

$C_7H_{11}NO_2$ = 141.2
CAS—302-66-9
Synonym Mepentamate
Proprietary Names *Oblivon C*; *N-Oblivon*.
Chemical Properties A white powder. Mp 53° to 56°. Soluble 1 in <200 of water, 1 in 1 of ethanol and 1 in 2 of chloroform.

Thin-layer Chromatography Methylpentynol carbamate: system TD—R_f 0.49; system TE—R_f 0.74; system TF—R_f 0.62; system TAD—R_f 0.57 (furfuraldehyde reagent, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 715; system GI—retention time 20.1 min.
Infrared Spectrum Principal peaks at wavenumbers 917, 1126, 1156, 995, 1030, 1050 cm^{-1} (thin film).



Mass Spectrum Principal ions at *m/z* 69, 83, 43, 79, 41, 81, 53, 80 (methylpentynol carbamate).



Quantification
Serum **GC** FID. Methylpentynol [Grove, Martin 1977].
Urine **GC** 4-Hydroxymethylpentynol, see Serum [Grove, Martin 1977].
Disposition in the Body Absorbed after oral administration. It is almost completely metabolised with <1% being excreted in the urine as unchanged drug in 7 days; higher amounts of unchanged drug may be excreted after large doses. The major metabolite is the *N*-glucuronide which accounts for about 80% of the dose; the 4-hydroxy metabolite accounts for about 13% of a dose.
Toxicity A fatality has been reported after a dose of 5 g although recoveries have occurred after ingestion of up to 10 g. Repeated medication may cause toxic reactions in the liver and skin.

A liver concentration of 1100 µg/g and a urine concentration of 410 mg/L were reported in one fatal poisoning case [Jackson JV. 1967].

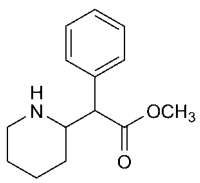
Dose 0.5 to 1 g daily.

Grove J, Martin BK (1977). Gas-liquid chromatography of methylpentynol carbamate and its metabolite 3-methylpentyne-3,4-diol. *J Chromatogr* 133: 267–272.
Jackson JV. (1967). Personal communication on file.

Methylphenidate

CNS Stimulant, Treatment of ADHD
$C_{14}H_{19}NO_2$=33.3
CAS—113-45-1

IUPAC Name Methyl 2-phenyl-2-piperidin-2-ylacetate
Synonyms Methylphenidan; methyl phenidate; methyl phenidylacetate; α-phenyl-2-piperidineacetic acid methyl ester.

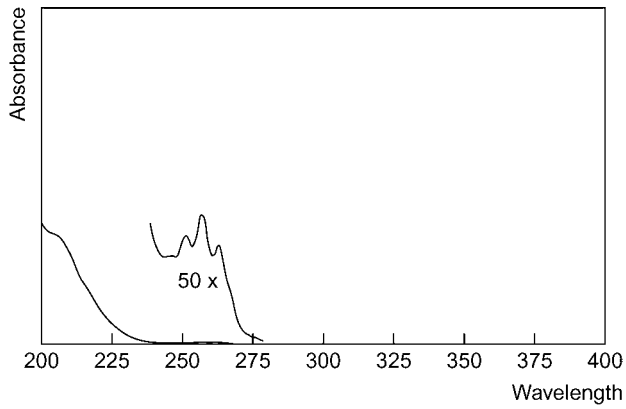


Chemical Properties Crystals. Mp 74° to 75°. Practically insoluble in water and petroleum ether; soluble in ethanol, ethyl acetate, and ether. pK_a 0.8 [Schubert 1970], 8.8. Log *P* (octanol/water), 0.2. Extraction yield (chlorobutane), 0.75 [Demme *et al.* 2005]. Stable in plasma through 3 freeze-thaw cycles [Modi *et al.* 2000]. Methylphenidate undergoes hydrolysis in pooled plasma and buffer solution. Methylphenidate is stable in processed samples at 4° for 10 days [Lin *et al.* 1999].

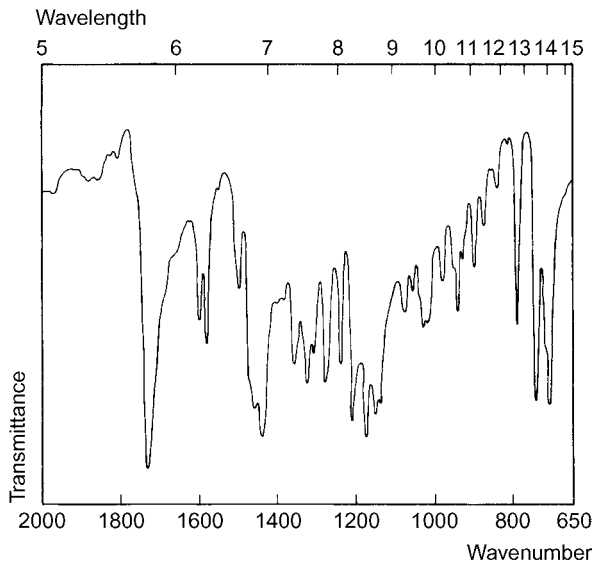
Methylphenidate Hydrochloride

$C_{14}H_{19}NO_2$,HCl = 269.8
CAS—298-59-9
Synonym Ciba 4311b
Proprietary Names *Attenta*; *Centedrin*; *Concerta*; *Equasym*; *Focalin*; *Metadate*; *Methidate*; *Methylin*; *Riphenidate*; *Ritalin(a)*; *Ritaline*; *Rubifen*.
Chemical Properties Fine, white, crystalline powder or acicular crystals. Mp 224° to 226°. Soluble in water, alcohol and chloroform.

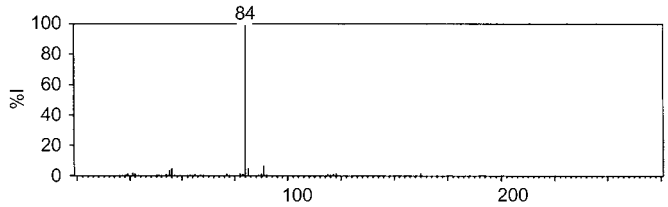
Colour Test Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.35; system TC—R_f 0.41; system TE—R_f 0.66; system TL—R_f 0.23; system TAE—R_f 0.40; system TAF—R_f 0.70; system TAJ—R_f 0.11; system TAK—R_f 0.04; system TAL—R_f 0.70 (acidified iodoplatinate solution—positive; acidified potassium permanganate—positive; Dragendorff spray—positive; ninhydrin spray—positive).
Gas Chromatography System GA—methylphenidate RI 1725, methylphenidate-AC RI 2085, methylphenidate-trifluroacetic acid RI 1730; system GB—methylphenidate RI 1793; system GC—methylphenidate RI 2200; system GF—methylphenidate RI 1935.
High Performance Liquid Chromatography System HA—*k* 1.7; system HC—*k* 0.36; system HY—RI 277; system HAX—retention time 8.6 min; system HAY—retention time 4.7 min.
Ultraviolet Spectrum Aqueous acid—251, 257 (A^1_1 = 9.0a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1735, 1175, 1210, 1150, 705, 740 cm^{-1} (methylphenidate hydrochloride, KCl disk).



Mass Spectrum Principal ions at *m/z* 84, 91, 85, 56, 55, 150, 41, 118 (no peaks above 150).



Quantification

Blood **GC** Column: 2% Hi Eff 3A on Gas Chrom Q 100/120 mesh (150 × 3 mm i.d.). Carrier gas: N_2, 75 mL/min. Temperature programme: 140°. FID. Limit of detection, 1 mg/L [Schubert 1970].

LC-MS Zorbax SB-Phenyl (75 × 4.6 mm i.d., 3.5 µm). ESI. Limit of quantification, 1.0 µg/L [Markowitz *et al.* 1999].

Plasma **GC** Column: OV-225 (30 m × 0.33 mm i.d.). Carrier gas: Ar:CH_4, 1 mL/min. Temperature programme: 175° for 1 min to 260° at 7°/min for 5 min. ECD. Limit of detection, 0.28 µg/L [Srinivas *et al.* 1990]. Column: 1.5% OV-7/1.5% OV-210 on 60/80 mesh Chromosorb AW-DMCS (1.83 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 220° for 1 min to 250° at 2°/min for 5 min. NPD or FID. Limit of detection, 0.43 µg/L [Lim *et al.* 1986]. Column: 3% SP-2250-DB on 100/120 Supelcoport (1.83 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 170°. NPD. Limit of detection, 1 µg/L [Potts *et al.* 1984]. Column: 3% OV-1 on 80/100 mesh Gas Chrom Q (2m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 190°. CI, SIM acquisition mode. Retention time: 2.5 min. Limit of detection, 0.05 µg/L [Iden, Hungund 1979]. Column: 3% OV-1 on Gas Chrom Q 100/120 mesh (1.83 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 180°. NPFID. Retention time: 3.72 min. Limit of detection, 1 µg/L [Hungund *et al.* 1978].

GC-MS Column: DB5 capillary. Carrier gas: CH_4, 0.5 Torr. Temperature programme: 100° for 0.2 min to 275° at 18°/min for 6 min. NCI. Limit of quantification, 0.75 µg/L [Lin *et al.* 1999]; limit of quantification, ~0.15 µg/L [Leis *et al.* 2000]. Column: 1.5% OV-7/1.5% OV-210 on Chromosorb W AW DMCS 80/100 mesh (1 m × 2.6 mm i.d.). Carrier gas: He, 37 mL/min. Temperature programme: 240°. CI, SIM acquisition mode. Limit of detection, 0.04 µg/L [Aoyama *et al.* 1990]. Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 50 cm/s. Temperature programme: 215°. EI ionisation at 30 eV. Retention time: 1.85 min. Limit of detection, 1 µg/L [Patrick, Jarvi 1990]. Column: 2% OV-17 on Chromosorb W AWDMCS 80/100 mesh (1 m× 2 mm i.d.). Carrier gas: He, 35 mL/min. Temperature programme: 220°. CI. Retention time: 1.5 min. Limit of detection, 0.5 ng [Nakajima *et al.* 1986]. Column: 3% SP-2100 on 80/100 mesh Supelcoport (2 m × 2 mm i.d.). Carrier gas: He, 15 mL/min. Temperature programme: 185°. EI ionisation at 70 eV. Limit of detection, 0.5 µg/L [Patrick *et al.* 1985]. Column: 3% OV-1 on Gas Chrom-Q 100/120 (6′ × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 165°. EI ionisation at 70 eV. Limit of detection, 1.5 µg/L [Milberg *et al.* 1975].

HPLC Column: Phenomenex Luna C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (73 : 27), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 460 nm). Retention time: 10.6 min. Limit of quantification, 1 µg/L [Zhu *et al.* 2007]. Column: chiral AGP (150 × 4.0 mm i.d.). Mobile phase: 0.4% acetic acid containing 0.1% DMOA (pH 3.4), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of quantification, 25 mg/L [Zhang *et al.* 2003].

LC-MS Column: Thermo Electron Hipersil Gold (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (80 : 20 for 3 min to 50 : 50 within 9 min to 80 : 20 within 6 min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 1.0 µg/L, limit of detection, 0.31 µg/LL [Marchei *et al.* 2009]. Column: Chirobiotic V (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05% trifluoroammonium acetate, flow rate 1.0 mL/min. Limit of quantification, 0.075 µg/L [Tuerck *et al.* 2007]. Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.2 mg/L, limit of detection, 0.12 mg/L [Shinozuka *et al.* 2006].

Serum **HPLC** Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: 20 mmol/L potassium phosphate buffer (pH 3.5) : acetonitrile (80 : 20), flow rate 1.6 mL/min. UV detection (λ = 192 nm). Limit of detection, 20 µg/L [Soldin *et al.* 1979a]. Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: 20 mmol/L potassium phosphate buffer (pH 3.8) : acetonitrile (93 : 7), flow rate 2.0 mL/min. UV detection (λ = 192 nm). Limit of detection, 25 µg/L for ritalinic acid [Soldin *et al.* 1979b].

GC-MS Column: 3% OV 22 on Chromosorb W 80/100 mesh (2.0 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 210°. SIM acquisition mode. Retention time: 3.4 min. Limit of detection, 1.0 µg/L [Chan *et al.* 1980].

Urine **GC** Column: CP-Sil 8 CB and CP-Sil 5 CB (25 m × 0.32 mm i.d.). Carrier gas: N_2, 1.3 mL/min. Temperature programme: 120° to 155° at 1.5° for 1 min to 280° at 30°/min for 10 min. NPD. Limit of detection, 50 µg/L for ritalin [Vu-Duc, Vernay 1992]. See Plasma. Limit of detection, 2.16 µg/L [Lim *et al.* 1986]. Column: 3% OV-17 Gas Chrom Q 100/120 (6′ × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 195°. FID. Retention time: 1.5 min. Limit of detection, ≥1.0 mg/L for ritalinic acic [Allen, Sedgwick 1984]. Column: 2.5% OV-225 Chromosorb W-HP 80/100 mesh (2 m × 2.0 mm i.d.). Carrier gas: N_2, 30 mL/min. NPD. Limit of detection not reported [Van Boven, Daenens 1979]. Column: 3% OV-17 on Gas-Chrom W 80/100 mesh (1.22 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 140° for 2 min to 270° at 16°/min for 4 min. AFID. Limit of detection, 30 µg/L [Dugal *et al.* 1978]. Column: glass (1.8 m × 2.0 mm [6′ × 0.08"] i.d.). Carrier gas: N_2, 25–30 mL/min. Temperature programme: 170°. FID. Limit of detection, 1.25 mg/L [Wells *et al.* 1974]. See Blood [Schubert 1970].

GC-MS Column: 5% phenylmethylpolysiloxane DB-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 50 cm/s. Temperature programme: 250°. EI ionisation at 70 eV. Limit of quantification, 10 µg/L [LeVasseur *et al.* 2008]. Column: J & W DB-17 MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 1 min to 200° at 10°/min to 280° at 25°/min for 5 min. EI ionisation, positive ion mode, SIR acquisition mode. Limit of detection, <0.5 nmol/L [Eichhorst *et al.* 2004]. Column: Ultra2 HP crosslinked 5% diphenylpolysiloxane 95% dimethylpolysiloxane (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° to 290° at 20°/min for 4 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [Solans *et al.* 1994]. See Plasma. Limit of detection, 1 µg/L [Aoyama *et al.* 1990]. See Plasma. Limit of detection, 2 µg/L [Milberg *et al.* 1975].

LC-MS See Plasma. Limit of quantification, 0.5 µg/L, limit of detection, 0.14 µg/L [Marchei *et al.* 2009].

Oral Fluid **LC-MS** See Plasma. Limit of quantification, 0.5 µg/L, limit of detection, 0.15 µg/L [Marchei *et al.* 2009].

Hair **LC-MS** Column: Thermo Electron-Hypersil Gold (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (80 : 20 for 3 min to 50 : 50 in 9 min to 80 : 20 in 6 min. ESI, SIM acquisition mode. Limit of quantification, 0.15 nm/mg, limit of detection, 0.05 ng/mg [Marchei *et al.* 2008].

Liver **LC-MS** See Blood [Markowitz *et al.* 1999].

Sweat **LC-MS** See Plasma. Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Marchei *et al.* 2009].

Disposition in the Body

Readily absorbed after oral administration. The rate but not the amount absorbed is increased by the presence of food in the stomach. Approximately 80% of a dose is excreted in the urine in 24 h, of which 60–80% is ritalinic acid [Faraj *et al.* 1974] and 5–12% is 6-oxo-α-phenylpiperidine-2-acetic acid; only a small amount is excreted as unchanged drug. There has been no evidence of methylphenidate as a substrate for CYP2D6 [DeVane *et al.* 2000].

Therapeutic Concentration

Following a single oral dose of 20 mg to 2 subjects, peak plasma concentrations of 0.013 and 0.058 mg/L were attained in 3 h; ritalinic acid concentrations averaged 0.22 mg/L at the same time [Milberg *et al.* 1975].

A healthy volunteer was administered 0.25 mg/kg methylphenidate. The maximal plasma concentrations of methylphenidate and ritalinic acid were 6.4 and 350 µg/L, respectively, reached within 1 h of administration. The concentration of methylphenidate decreased rapidly while ritalinic acid decreased slowly and was 21 µg/L at 24 h [Nakajima *et al.* 1986].

When a healthy adult volunteer was administered a single oral dose of 40 mg racemic methylphenidate hydrochloride, the following pharmacokinetic parameters were measured:

Compound	*Enantiomer*	t_{max} *(h)*	C_{max} *(µg/L)*
Methylphenidate	*d*	2.0	11.71
	l	2.0	1.97
Ritalinic acid	*d*	3.0	80.71
	l	3.0	178.6

[Srinivas *et al.* 1990].

Following oral doses of 10 to 30 mg daily, administered to four hyperkinetic children, peak plasma concentrations of 0.008 to 0.023 mg/L (mean 0.018) were reported [Hungund *et al.* 1979].

Thirty-five healthy subjects (22 male, 13 female) were given methylphenidate hydrochloride in single oral doses of 18, 36, or 54 mg, in the form of one, two, or three 18 mg OROS (oral, osmotic, controlled-release) systems. Mean peak plasma concentrations of (+)-methylphenidate and (−)-methylphenidate, respectively, were 3.87 and 0.095 µg/L (at 7.9 and 7.1 h) after the 18 mg dose, 7.28 and 0.17 µg/L (at 7.5 and 7.0 h) after the 36 mg dose, and 10.6 and 0.36 µg/L (at 7.2 and 6.1 h) after the 54 mg dose. Corresponding mean peak plasma concentrations of (+)-ritalinic acid and (+)-ritalinic acid were 53 and 69 µg/L (at 8.8 and 8.1 h) after the 18 mg dose, 105 and 132 µg/L (at 8.8 and 7.6 h) after the 36 mg dose, and 155 and 192 µg/L (at 8.5 and 7.8 h) after the 54 mg dose [Modi *et al.* 2000].

Nineteen subjects (10 male, 9 female) were administered either Ritalin LA 20 mg or Concerta 18 mg. The mean maximum plasma concentration for each formulation was 9.9 and 5.9 µg/L, reached at 5.5 and 6 h respectively [Markowitz *et al.* 2003].

Following the administration of 5, 10, 20, 30, and 40 mg (+)-methylphenidate to 25 healthy volunteers, the maximum plasma concentrations were 3.25, 6.05, 12.6, 18.5, and 25.2 µg/L, respectively [Tuerck *et al.* 2007].

Following a single oral dose of 0.3 mg/kg racemic methylphenidate to a healthy female volunteer, the plasma concentrations ranged from 2.04 to 15.72 µg/L over 10 h, with the maximum occurring at ~2 h [Zhu *et al.* 2007].

M

A poor metaboliser of methylphenidate excreted nearly 70 times more (+)-methylphenidate and 5 time more (+)-methylphenidate compared with the mean values for normal metabolisers [LeVasseur *et al.* 2008].

Note For a study comparing the pharmacokinetics of different formulations in children, see Quinn *et al.* [2007] and for the pharmacokinetics of the oral osmotic-controlled extended-release formulation, see Parasrampuria *et al.* [2007] or Reiz *et al.* [2008]. Food caused a significant increase in extent of absorption but had no effect on the rate of absorption of methylphenidate [Midha *et al.* 2001]. For a bioequivalence study of 2 methylphenidate preparations, see Meyer *et al.* [2000]

Toxicity The estimated minimum lethal dose for adults is 2 g but few instances of serious toxicity from overdosage have been reported. Abuse and dependence of the amfetamine type is not uncommon.

In 2 suicide victims who had overdosed on methylphenidate (co-ingested with ethanol), the following concentrations were reported at postmortem (mg/L or μg/g):

	Methylphenidate	*Ritalinic acid*	*Ethylphenidate*
CASE 1			
Peripheral blood	0.310	2.8	0.008
CASE 2			
Peripheral blood	1.6	2.0	0.001
Liver	0.310	1.2	0.0016

[Markowitz *et al.* 1999].

A 19-year-old white male snorted methylphenidate. At postmortem, however, methylphenidate was not detectable in the blood sample, although the concentration of ritalinic acid was 0.24 mg/L in the postmortem sample and 0.4 mg/L in the admission blood sample [Massello, Carpenter 1999].

The following postmortem tissue concentrations were reported in an accidental overdose fatality involving the intravenous injection of crushed methylphenidate tablets: blood 2.8 mg/L, bile 5.7 mg/L, kidney 3.0 μg/g, liver 2.1 μg/g, stomach contents 1.6 mg (total); other drugs were not detected [Levine *et al.* 1986].

Urine methylphenidate concentrations of 0.8–40 mg/L (mean 16) were reported in 6 arrested drivers who showed signs of drowsiness or hyperactivity [Schubert 1970].

Note For a case of fatal pulmonary hypertension from the IV injection of methylphenidate tablets, see Lewman [1972]; for a case of hepatotoxicity, see Goodman [1972]; for a case of methylphenidate interacting with risperidone, see Hollis and Thompson [2007]; for a case of orofacial and extremity dyskinesia in a 6.5-year-old child, see Balázs *et al.* [2007]. For a review of 113 methylphenidate exposures, see Foley *et al.* [2000], and in preschool children, see Bailey *et al.* [2005]. For a case of a 45-year-old abusing 700 mg methylphenidate over a 3-day period, see Coetzee *et al.* [2002].

Bioavailability Approximately 30% (11–51%) because it undergoes extensive first-pass metabolism.

Half-life Plasma half-life, ~2 h, ritalinic acid ~8 h.

Volume of Distribution Approximately 2.6 L/kg.

Clearance Plasma, ~6.5 mg/min/kg.

Protein Binding Approximately 15%.

Note For a review of the pharmacokinetics and clinical effectiveness of methylphenidate, see Kimko *et al.* [1999] and for a review of its toxicity and abuse, see Klein-Schwartz [2002] or Scharman *et al.* [2007].

Dose Usually up to 60 mg of methylphenidate hydrochloride daily.

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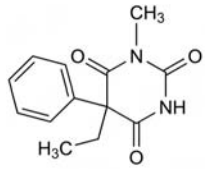
Methylphenobarbital

Sedative, Barbiturate

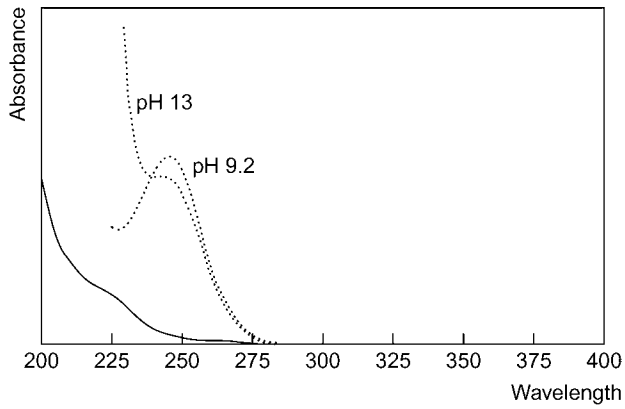
$C_{13}H_{14}N_2O_3$ = 246.3

CAS—115-38-8

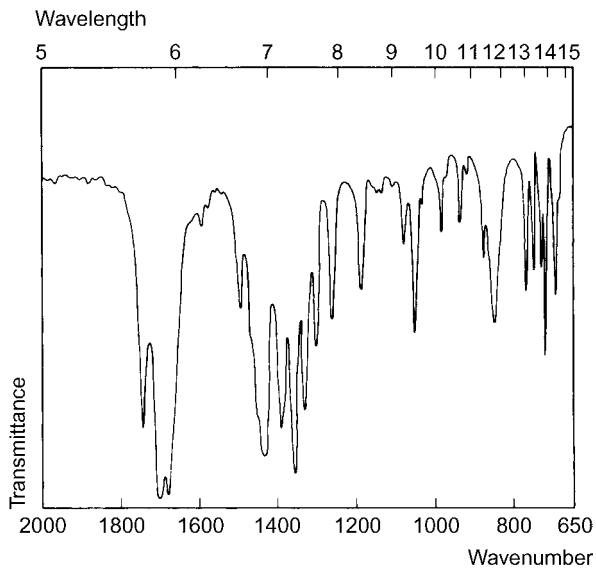
IUPAC Name 5-Ethyl-1-methyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
Synonyms Enphenemalum; mephobarbital; methylphenobarbitone; phemitone.
Proprietary Names *Mebaral*; *Prominal*.



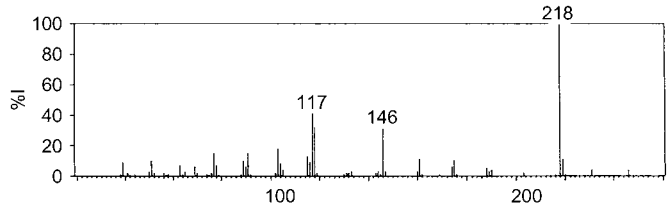
Chemical Properties Colourless crystals or white crystalline powder. Mp 176°. Slightly soluble in cold water, freely soluble in hot water; soluble 1 in 240 of ethanol, 1 in 40 of chloroform and 1 in 200 of ether; soluble in solutions of ammonia, alkali hydroxides and carbonates. pK_a 7.8 (20°). Log *P* (octanol/water), 1.8.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.70; system TE—R_f 0.41; system TF—R_f 0.67; system TH—R_f 0.72; system TAD—R_f 0.70; system TAE—R_f 0.86 (mercurous nitrate spray, black; Zwikker's reagent, faint pink).
Gas Chromatography System GA—methylphenobarbital RI 1890, phenobarbital RI 1953, methylphenobarbital-Me RI 1855, M (OH-) RI 2370, M (OH-methoxy-) RI 2310, M (nor-OH-) RI 2295; system GB—methylphenobarbital RI 2222; system GAJ—methylphenobarbital RRT 1.000.
High Performance Liquid Chromatography System HG—*k* 7.27; system HH—*k* 3.84; system HX—RI 435; system HY—RI 395; system HZ—retention time 4.6 min.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—244 nm (A_1^1=355c); 1 mol/L sodium hydroxide (pH 13)—243 nm (A_1^1=329b).



Infrared Spectrum Principal peaks at wavenumbers 1707, 1684, 1754, 720, 1298, 1050 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 218, 117, 118, 146, 103, 77, 91, 115; phenobarbital 204, 117, 146, 161, 77, 103, 115, 118.

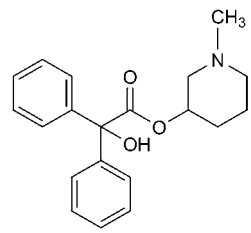


Quantification
Plasma **GC** FID. Methylphenobarbital and phenobarbital [Hooper *et al.* 1975].
GC-MS Methylphenobarbital and phenobarbital. Limit of detection, 20 µg/L for both compounds [Hooper *et al.* 1981].
Serum **GC** See Plasma [Hooper *et al.* 1975].
HPLC Methylphenobarbital, phenobarbital and other anticonvulsants. UV detection. Limit of detection, 500 µg/L [Kabra *et al.* 1978].
Urine **HPLC** UV detection. Phenobarbital, *p*-hydroxyphenobarbital and *p*-hydroxymethylphenobarbital. Limit of detection, 500 µg/L for each compound [Kunze *et al.* 1981].
See Amobarbital.
Disposition in the Body Incompletely absorbed after oral administration. Metabolised by *N*-demethylation to the active metabolite, phenobarbital, and by *p*-hydroxylation. Over a period of 10 days, <2% of a dose is excreted in the urine as unchanged drug, about 30 to 35% is excreted as *p*-hydroxymethylphenobarbital both free and conjugated, and up to 10% may be excreted as phenobarbital. Small amounts of *p*-hydroxyphenobarbital, 5-ethyl-5-(4-hydroxy-3-methoxyphenyl)barbituric acid and 5-ethyl-5-(4-hydroxy-3-methoxyphenyl)-1-methylbarbituric acid have also been detected in the urine.
Therapeutic Concentration
Following a single oral dose of 800 mg to 2 subjects, peak plasma concentrations of about 3 mg/L were attained in 3 to 6 h; peak phenobarbital plasma concentrations of 2 to 3 mg/L were attained in 4 to 6 days [Hooper *et al.* 1981].
Following daily oral doses of 60 to 600 mg to 11 subjects, steady-state plasma concentrations of 0.2 to 1.7 mg/L (mean 0.9) of methylphenobarbital and 4 to 32 mg/L (mean 15) of phenobarbital were reported [Kupferberg, Longacre-Shaw 1979].
Toxicity The estimated minimum lethal dose is 2 g.
Bioavailability About 70%.
Half-life Plasma half-life, methylphenobarbital about 50 to 60 h, phenobarbital about 100 h.
Volume of Distribution About 2 to 3 L/kg.
Clearance Plasma clearance, about 0.5 mL/min/kg.
Protein Binding About 40 to 60%.
Dose 90 to 600 mg daily.

Hooper WD *et al.* (1975). Simultaneous assay of methylphenobarbitone and phenobarbitone using gas-liquid chromatography with on-column butylation. *J Chromatogr* 110: 206–209.
Hooper WD *et al.* (1981). Simultaneous assay of methylphenobarbital and phenobarbital in plasma using gas chromatography–mass spectrometry with selected ion monitoring. *J Chromatogr* 223: 426–431.
Hooper WD *et al.* (1981). Pharmacokinetics and bioavailability of methylphenobarbital in man. *Ther Drug Monit* 3: 39–44.
Kabra PM *et al.* (1978). *J Anal Toxicol* 2: 127–133.
Kunze HE *et al.* (1981). High performance liquid chromatographic assay of methylphenobarbital metabolites in urine. *Ther Drug Monit* 3: 45–49.
Kupferberg HJ, Longacre-Shaw J (1979). Mephobarbital and phenobarbital plasma concentrations in epileptic patients treated with mephobarbital. *Ther Drug Monit* 1: 117–122.

Methylpiperidyl Benzilate

Hallucinogen
$C_{20}H_{23}NO_3$ = 25.4
CAS—3321-80-0
Synonym 1-Methyl-3-piperidyl benzilate



Chemical Properties A clear liquid. Practically insoluble in water; soluble in chloroform and most organic solvents.

Methylpiperidyl Benzilate Hydrochloride

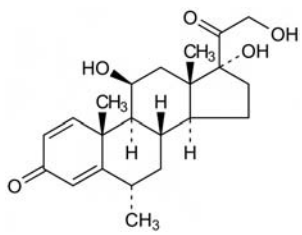
Chemical Properties A white powder. Mp 212° to 218°, with decomposition. Soluble in water; practically insoluble in chloroform.

Colour Tests Liebermann's reagent—brown; Mandelin's test—orange→brown→green; Marquis test—orange→green→blue; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.30; system TC—R_f 0.58; system TE—R_f 0.73; system TL R_f 0.33; system TAE—R_f 0.58; system TAF—R_f 0.50 (acidified iodoplatinate solution—positive).
High Performance Liquid Chromatography System HX—RI 377.
Ultraviolet Spectrum Aqueous acid—251 (a_1^1 = 18b), 257 nm (A_1^1 = 20b).
Infrared Spectrum Principal peaks at wavenumbers 1734, 1195, 965, 685, 1160, 720 cm^{-1} (methylpiperidyl benzilate hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 97, 105, 77, 183, 84, 36, 42, 51.

Methylprednisolone

Corticosteroid
$C_{22}H_{30}O_5$ = 374.5
CAS—83-43-2

IUPAC Name (6*S*,8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-17-(2-hydroxyacetyl)-6,10,13-trimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-one
Synonyms 6α-Methylprednisolone; (6α,11β)-11,17,21-trihydroxy-6-methylpregna-1,4-diene-3,20-dione.
Proprietary Names *Esametone*; *Firmacort*; *Medrol*; *Medrone*; *Metilpren*; *Metisona*; *Metypred*; *Metysolon*; *Radilem*; *Urbason*.



Chemical Properties A white crystalline powder. Mp about 228° to 237°. A solution in dioxan is dextrorotatory. Practically insoluble in water; soluble 1 in 100 of dehydrated alcohol; slightly soluble in acetone and chloroform; very slightly soluble in ether; sparingly soluble in dioxan and methanol. Log *P* (octanol/water), 1.8.

Methylprednisolone Acetate

$C_{24}H_{32}O_6$ = 416.5
CAS—53-36-1
Synonym 6α-Methylprednisolone 21-acetate
Proprietary Names *Adlone*; *Depoject*; *depMedalone*; *Depo Moderin*; *Depomedrate*; *Depo-Medrol*; *Depo-Medrone*; *Depo-Nisolone*; *Depopred*; *D-Med*; *Duralone*; *Medralone*; *Medrate*; *Medrone*; *Metypred*; *M-Prednisol*; *Solsolona*.
Chemical Properties A white crystalline powder. Mp 205° to 208°. Practically insoluble in water; sparingly soluble in ethanol and acetone; soluble 1 in 250 of chloroform and 1 in 1500 of ether; soluble in dioxan.

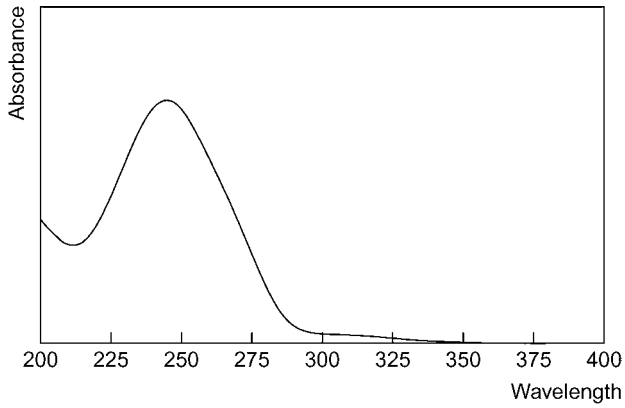
Methylprednisolone Hemisuccinate

$C_{26}H_{34}O_8$ = 474.5
CAS—2921-57-5
Synonym Methylprednisolone hydrogen succinate
Proprietary Name *Asmacortone*
Chemical Properties A white hygroscopic solid. Very slightly soluble in water; freely soluble in ethanol; soluble in acetone.

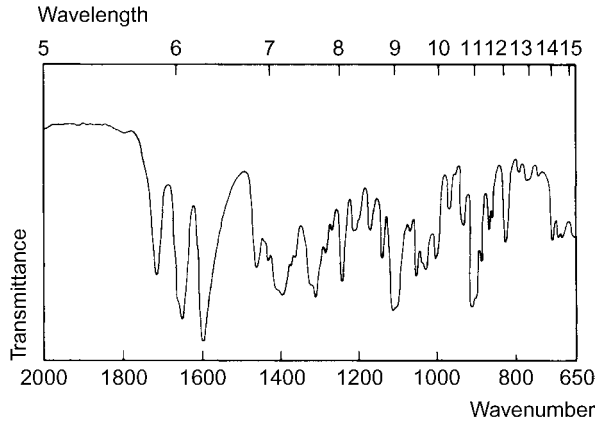
Methylprednisolone Sodium Succinate

$C_{26}H_{33}NaO_8$ = 496.5
CAS—2375-03-3
Synonym Sodium 6α-Methylprednisolone 21-Succinate
Proprietary Names *A-methapred*; *Emmetipi*; *Firmacort*; *Metypred*; *Metypresol*; *Solu-Medrol*; *Solu-Medrone*; *Solu-Moderin*; *Urbason*.
Chemical Properties A white, hygroscopic, amorphous powder. Soluble 1 in 1.5 of water and 1 in 12 of ethanol; practically insoluble in chloroform and ether; very slightly soluble in acetone.

Thin-layer Chromatography System TA—R_f 0.87; system TB—R_f 0.00; system TE—R_f 0.41; system TF—R_f 0.27; system TP—R_f 0.23; system TQ—R_f 0.80; system TR—R_f 0.03; system TS—R_f 0.00; system TAE—R_f 0.87; system TAJ—R_f 0.31; system TAK—R_f 0.13; system TAL—R_f 0.78; system TAM—R_f 0.56.
High Performance Liquid Chromatography System HT—*k* 7.5; system HX—RI 426; system HY—RI 390; system HAA—retention time 18.9 min.
Ultraviolet Spectrum Dehydrated alcohol—240 nm (A_1^1=400a).



Infrared Spectrum Principal peaks at wavenumbers 1595, 1650, 1114, 914, 1313, 1248 cm^{-1} (KBr disk).



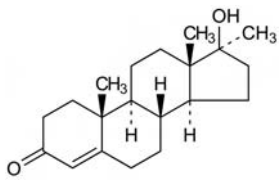
Quantification
Plasma **HPLC** UV detection. Limit of detection, about 7 µg/L [Shah *et al.* 1987].
Urine **GC-MS** Methylprednisolone and its metabolites [Rodchenkov *et al.* 1987].
HPLC UV detection. Limit of detection, about 8 µg/L [Shah, Weber 1989].
Dose Usually 4 to 48 mg daily.

Rodchenkov GM *et al.* (1987). Determination of methylprednisolone metabolites in human urine by gas chromatography-mass spectrometry. *J Chromatogr* 423: 15–22.
Shah JA, Weber DJ (1989). Simultaneous determination of methylprednisolone and methylprednisolone 21-[8-[methyl-(2-sulfoethyl)amino]-8-oxooctanoate] sodium salt in human urine by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 496: 245–254.
Shah JA *et al.* (1987). High-performance liquid chromatographic determination of methylprednisolone and methylprednisolone 21-[8-[methyl-(2-sulfoethyl)amino]-8-oxoctanoate] sodium salt in human plasma. *J Chromatogr* 414: 1–10.

Methyltestosterone

Androgen
$C_{20}H_{30}O_2$ = 302.5
CAS—58-18-4

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13,17-trimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-one
Synonym (17β)-17-Hydroxy-17-methylandrost-4-en-3-one
Proprietary Names *Android*; *Metandren*; *Neohombreol M*; *Oreton Methyl*; *Testotonic B*; *Testovis*; *Testred*; *Virilon*. It is an ingredient of *Prowess*, *Estratest*and *Menogen*.



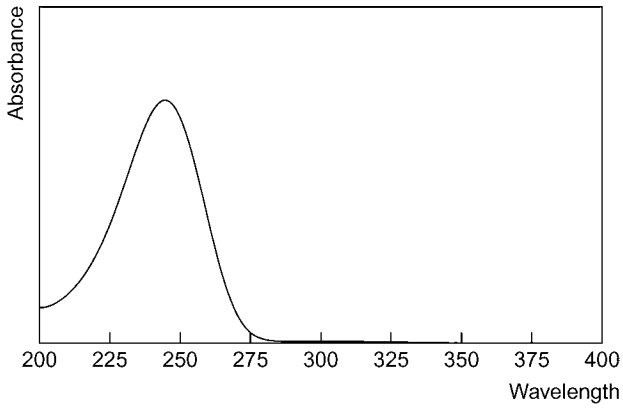
Chemical Properties A white or slightly yellowish-white, slightly hygroscopic, crystalline powder. Mp 162° to 168°. A solution in ethanol is dextrorotatory. Practically insoluble in water; soluble 1 in 5 of ethanol; freely soluble in chloroform; soluble in ether. Log *P* (octanol/water), 3.4.
Thin-layer Chromatography System TA—R_f 0.89; system TB—R_f 0.17; system TE—R_f 0.73; system TF—R_f 0.47; system TP—R_f 0.70; system TQ—R_f 0.16; system TR—R_f 0.91; system TS—R_f 0.71; system TAE—R_f 0.86; system TAF—R_f

0.86; system TAJ—R_f 0.60; system TAK—R_f 0.65; system TAL—R_f 0.92; system TAM—R_f 0.92.

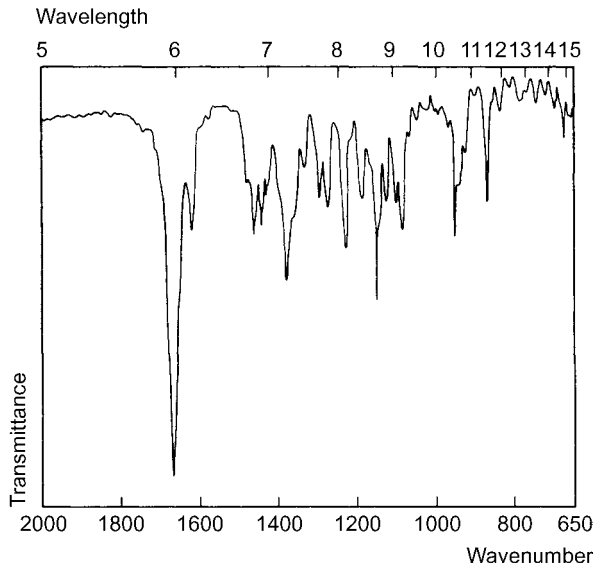
Gas Chromatography System GA—methyltestosterone RI 2645, methyltestosterone-AC RI 2770, methyltestosterone-TMS RI 2590, methyltestosterone enol-TMS_2 RI 2665; system GAG—RRT 1.05(relative to testosterone); system GAI—urinary metabolite: 17α-methyl-5β, (α)-androstan-3α,17β-diolRRT 0.925 (relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAR—methyltestosterone retention time 13.1 min.

High Performance Liquid Chromatography System HY—RI 587; system HATb—RRT 1.27 (relative to testosterone); system HAR—RRT 1.17 (relative to testosterone).

Ultraviolet Spectrum Ethanol—241 nm (A_1^1=540a).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1160, 1239, 950, 1612, 1090 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 302, 124, 43, 91, 79, 121, 105, 122.

Quantification

Plasma **Spectrofluorimetry** Limit of detection, 1 µg/L [Alkalay *et al.* 1972].

Serum **Spectrofluorimetry** See Plasma [Alkalay *et al.* 1972].

Dose 5 to 80 mg daily, orally; up to 200 mg daily has been given.

Alkalay D *et al.* (1972). Spectrophotofluorometric determination of methyltestosterone in plasma or serum. *J Pharm Sci* 61: 1746–1749.

Methylthioamfetamine

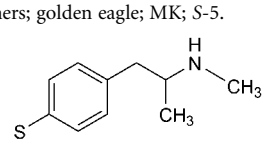
5-HT Receptor Releasing Agent

$C_{10}H_{15}NS$=181.3

CAS—14116-06-4

Synonyms *p*-Methylthioamphetamine; *p*-methylthioisopropylamine; MTA; 4-MTA.

Street Names Flatliners; golden eagle; MK; *S*-5.



Chemical Properties A white to off-white powder. Extraction yield (chlorobutane), 0.6 [Demme *et al.* 2005].

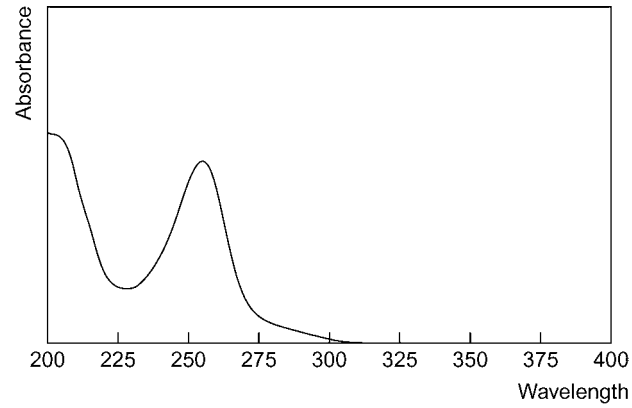
Colour Tests Marquis test—no colour change; cobalt thiocyanate (Scott's test)—blue.

Gas Chromatography Column: DB5 (25 m × 0.32 mm i.d., 0.52 µm). Temperature: 140°. NPD. Retention time: 4.26 min [Elliott 2000].

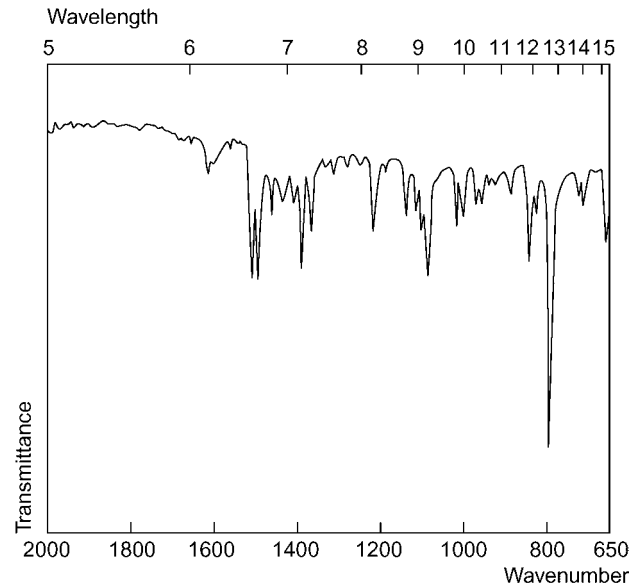
Gas Chromatography-Mass Spectrometry Column: HP Ultra-1 (12 m × 0.22 mm i.d., 0.25 µm). Temperature programme: 100° to 280° at 10°/min. MSD. Retention time: 5.11 min [Poortman, Lock 1999].

Column: DB-1 (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 150° for 1 min, to 250° at 8°/min, to 320° at 6°/min. Retention time: 4.96 min [Poortman, Lock 1999].

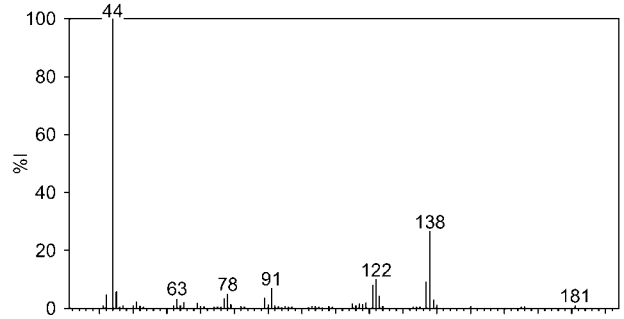
Ultraviolet Spectrum Aqueous acid (acetonitrile)—205, 255 nm; (0.025 mol/L sulfuric acid)—255 nm.



Infrared Spectrum Principal peaks at wavenumbers 793, 1080, 1492, 1508, 1385, 843 cm^{-1} (hydrochloride).



Mass Spectrum Principal ions at *m/z* 44, 138, 122, 137, 121, 91, 78, 45.



Quantification

Blood **HPLC** Column: Spherisorb S5OD/CN (150 × 4.6 mm i.d., 0.25 µm). Mobile phase: acetonitrile : 1.0 mol/L triethylammonium phosphate buffer

(pH 3.0; 20 : 80), flow rate 2 mL/min. I.S.: fenfluramine. DAD (λ = 205 and 595 nm). Retention time: MTA, 2.57 min; I.S., 5.37 min. Limit of detection, 0.5 mg/L [Elliott 2000].

Urine **HPLC** See Blood [Elliott 2000].

Disposition in the Body

Toxicity 4-MTA is thought to be responsible for at least 8 deaths worldwide (mainly in Europe) on its own or in association with other drugs. Toxic symptoms may include 'serotonin syndrome', convulsions, and respiratory depression. Such symptoms may be produced at plasma concentrations over 1 mg/L. A blood concentration over 4 mg/L could indicate an overdose or at least excessive usage.

The postmortem of a male (22 years old and in previously good health) that implicated 3,4-methylenedioxymethamfetamine (MDMA) poisoning actually revealed the presence of 4-MTA in femoral blood, perimortem blood, and urine at concentrations of 4.6 mg/L, 4.2 mg/L, and 87.2 mg/L, respectively. This was the first reported death involving 4-MTA in the United Kingdom and the first case involving 4-MTA alone [Elliott 2000].

A 27-year-old male died suddenly after a collapse and an intensive resuscitation attempt. Postmortem toxicological analysis revealed the following 4-MTA concentrations: femoral vein blood 5.23 mg/L, urine 95.5 mg/L, vitreous humour 1.31 mg/L, bile 36.4 mg/L, liver 30.8 mg/kg, spleen 4.10 mg/kg, and frontal lobe 31.7 mg/kg. MDMA was also found, but at much lower concentrations, and the cause of death was attributed to poisoning with 4-MTA [Decaestecker *et al.* 2001].

Note For a case report on the analytical profile of 4-MTA, see Poortman and Lock [1999].

Decaestecker T *et al.* (2001). Fatal 4-MTA intoxication: development of a liquid chromatographic-tandem mass spectrometric assay for multiple matrices. *J Anal Toxicol* 25: 705–710.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of 12th TIAFT*, Seoul: 481–486.

Elliott SP (2000). Fatal poisoning with a new phenylethylamine: 4-methylthioamphetamine (4-MTA). *J Anal Toxicol* 24: 85–89.

Poortman AJ, Lock E (1999). Analytical profile of 4-methylthioamphetamine (4-MTA), a new street drug. *Forensic Sci Int* 100: 221–233.

Methylthiouracil

Antithyroid Agent

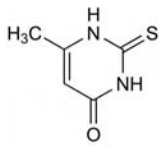
$C_5H_6N_2OS$ = 142.2

CAS—56-04-2

IUPAC Name 6-Methyl-2-sulfanylidene-1*H*-pyrimidin-4-one

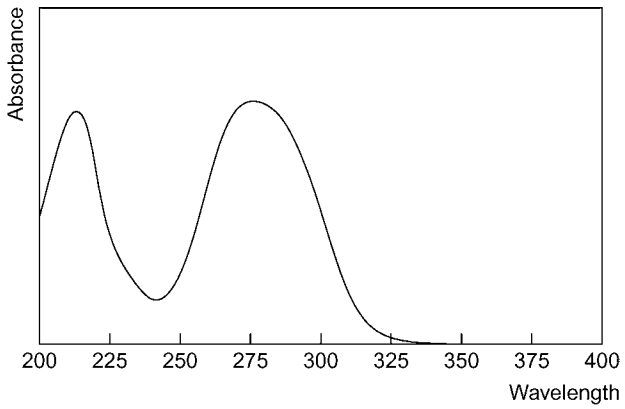
Synonym 2,3-Dihydro-6-methyl-2-thioxo-4(1*H*)-pyrimidinone

Proprietary Names *Alkiron*; *Antibason*; *Basecil*; *Basethyrin*; *Methiacil*; *Methicil*; *Methiocil*; *Muracil*; *Prostrumyl*; *Strumacil*; *Thimecil*; *Thyreostat*.



Chemical Properties A white crystalline powder. Mp 326° to 331°, with decomposition. Soluble 1 in 2000 of water, 1 in 150 of boiling water and 1 in 800 of ethanol; slightly soluble in acetone; very slightly soluble in ether; practically insoluble in benzene and chloroform; freely soluble in solutions of ammonium hydroxide and sodium hydroxide. pK_a 8.2 (20°). Log *P* (octanol/water), 1.4.

Ultraviolet Spectrum Aqueous acid—276 nm (A^1_1=1190b); aqueous alkali—256 nm (A^1_1=850b).



Infrared Spectrum Principal peaks at wavenumbers 1645, 1170, 1564, 1198, 850, 1240 cm^{-1}.

Dose Initially, 200 to 400 mg daily; maintenance, 50 to 150 mg daily.

5-Methyltryptamine

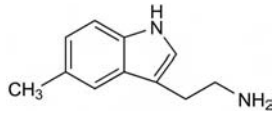
Hallucinogen

$C_{11}H_{14}N_2$ = 174.2

CAS—1821-47-2

IUPAC Name 2-(5-Methyl-1*H*-indol-3-yl)ethanamine

Synonyms 3-(2-Aminoethyl)-5-methylindole; 5-methyl-3-(aminoethyl)indole.



5-Methyltryptamine Hydrochloride

$C_{11}H_{14}N_2$,HCl = 210.7

CAS—1010-95-3

IUPAC Name 2-(5-Methyl-1*H*-indol-3-yl)ethanamine hydrochloride

Chemical Properties Light brown powder. Mp 289° to 292°.

Colour Tests Mandelin's test—green-orange; Marquis test—orange to brown; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.56 (acidified iodoplatinate solution—positive).

Gas Chromatography System GA—RI 1795.

Infrared Spectrum Principal peaks at wavenumbers 800, 1515, 1612, 1587, 1234, 1315 cm^{-1} (5-methyltryptamine hydrochloride, KBr disk).

α-Methyltryptamine

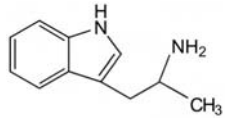
Hallucinogen

$C_{11}H_{14}N_2$ = 174.2

CAS—299-26-3

IUPAC Name 1-(1*H*-Indol-3-yl)propan-2-amine

Synonym 3-(2-Aminopropyl)indole



Chemical Properties White crystalline powder. Mp 98° to 99°. Soluble in chloroform and dilute acetic acid.

Colour Tests Mandelin's test—green→orange; Marquis test—orange→brown; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.58 (Acidified iodoplatinate solution, positive).

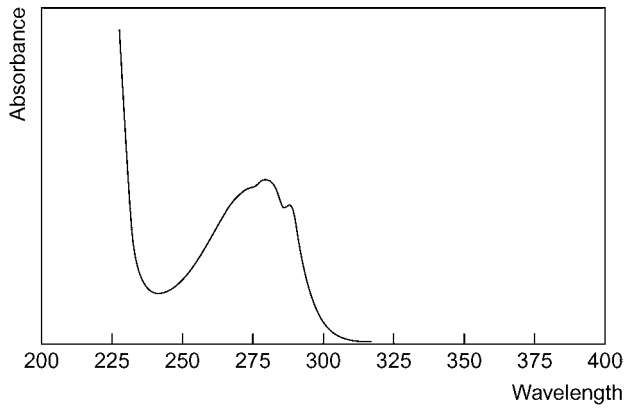
Gas Chromatography System GA—RI 1740.

Gas Chromatography-Mass Spectrometry Column: HP-5MS cross-linked 5% phenylpolysiloxane 95% dimethylpolysiloxane (30 m × 0.25 μm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 250° at 15°/min for 5 min. EI ionisation at 70 eV. Retention time: 11 min. Limit of detection, 12–15 mg/L [Wang *et al.* 2008].

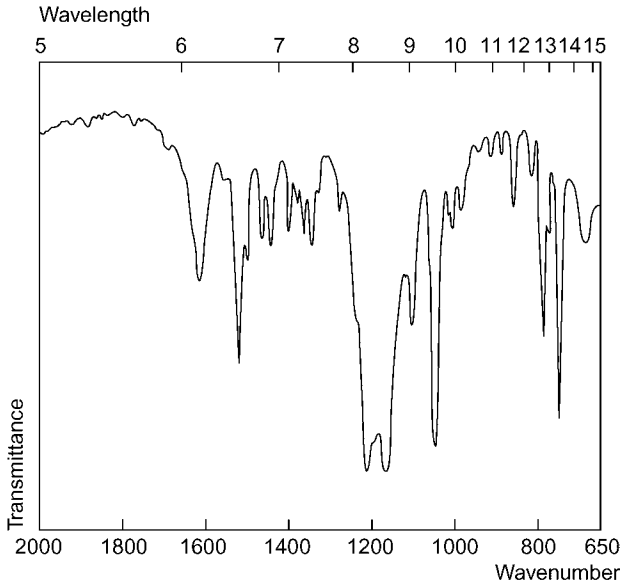
High Performance Liquid Chromatography Column: Cosmosil $5C_{18}$-MS (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1% formic acid (pH 2.5) : acetonitrile (90 : 10 to 62 : 38 at 20 min to 55 : 45 at 22 min to 40 : 60 at 32 min), flow rate 1.0 mL/miin. UV detection (λ= 280 nm). Retention time: ~19 min. Limit of detection, 0.4–0.5 mg/L [Wang *et al.* 2008].

Capillary Electrophoresis Capillary: total/effective length 67/80 cm, 50 μm). Running buffer: 75 mmol/L SDS and 50 mmol/L sodium dihydrogen phosphate in acetonitrile : methanol: water (5 : 30 : 65, pH 2.2). Sweeping-MEKC. Limit of detection, 5-8 μg/L [Wang *et al.* 2008].

Ultraviolet Spectrum Aqueous acid—279, 288 nm.



Infrared Spectrum Principal peaks at wavenumbers 1215, 1170, 1050, 750, 1520, 790 cm^{-1} (α-methyltryptamine esilate, KBr disk).



Quantification

Blood **GC** Column: DB-1 100% dimethylpolysiloxane or DB-17 50% phenylmethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 50° for 1 min to 190° at 20°/min for 1 min to 300° at 5°/min for 30 min. NPD. Limit of detection not reported [Boland *et al.* 2005].

GC-MS Column: capillary (16.5 m × 0.25 mm i.d., 0.3 µm). Temperature programme: 65° for 0.5 min to 290° at 15°/min. MSD, SIM acquisition mode. Limit of detection not reported [Boland *et al.* 2005]. Column: HP-1ms 100% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 2 min to 300° at 20°/min for 5 min. SIM acquisition mode. Retention time: 12.5 min. Limit of detection, 1 µg/L [Ishida *et al.* 2005]. Column: ZB-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 10°/min to 300° at 60°/min. SIM acquisition mode. Limit of detection, 25 µg/L [Vorce, Sklerov 2004].

Serum **GC** See Blood [Boland *et al.* 2005].

GC-MS See Blood [Boland *et al.* 2005].

Urine **GC-MS** See Blood. Limit of detection, 5 µg/L [Ishida *et al.* 2005].

LC-MS Column: Phenomenex (100 × 2.1 mm i.d., 3 µm). Mobile phase: 20 mmol/L ammonium formate (pH 4.3) : acetonitrile (75 : 25 for 1 min to 60 : 40 in 3 min for 7 min), flow rate 300 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 5 µg/L [Vorce, Sklerov 2004].

Stomach Contents **GC** See Blood [Boland *et al.* 2005].

GC-MS See Blood [Boland *et al.* 2005].

Brain **GC** See Blood [Boland *et al.* 2005].

GC-MS See Blood [Boland *et al.* 2005].

Liver **GC** See Blood [Boland *et al.* 2005].

GC-MS See Blood [Boland *et al.* 2005].

Other **GC-MS** Rat Urine. Column: J&W DB-5 ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 280° at 15°/min. EI ionisation at 70 eV. Limit of detection not reported [Kanamori *et al.* 2008].

Disposition in the Body

Toxicity

The following concentrations of α-methyltryptamine were measured at postmortem in 2 cases:

Specimen	*Case 1*	*Case 2*
Iliac vein blood	2.0 mg/L	
Gastric contents	9.6 mg total	
Liver	24.7 mg/kg	
Brain	7.8 mg/kg	
Serum	ND	1.5 mg/L

ND, not determined. [Boland *et al.* 2005]

Boland DM *et al.* (2005). Fatality due to acute alpha-methyltryptamine intoxication. *J Anal Toxicol* 29: 394–397.

Ishida T *et al.* (2005). Sensitive determination of alpha-methyltryptamine (AMT) and 5-methoxy-*N,N*-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 823: 47–52.

Kanamori T *et al.* (2008). In vivo metabolism of alpha-methyltryptamine in rats: identification of urinary metabolites. *Xenobiotica* 38: 1476–1486.

Vorce SP, Sklerov JH (2004). A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray-MS. *J Anal Toxicol* 28: 407–410.

Wang MJ *et al.* (2008). Comparison of the separation of nine tryptamine standards based on gas chromatography, high performance liquid chromatography and capillary electrophoresis methods. *J Chromatogr A* 1181: 131–136.

N-Methyltryptamine

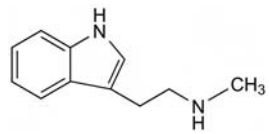
Hallucinogen

$C_{11}H_{14}N_2 = 174.2$

CAS—61-49-4

IUPAC Name 2-(1*H*-Indol-3-yl)-*N*-methylethanamine

Synonym 3-(2-Methylaminoethyl)indole

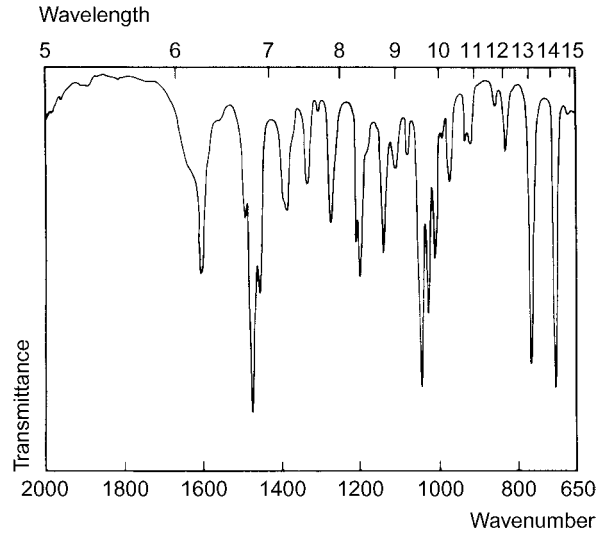


Colour Tests Marquis test—orange; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.18 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1770.

Infrared Spectrum Principal peaks at wavenumbers 700, 1040, 762, 1025, 1605, 1200 cm^{-1} (KBr disk).



Quantification

Urine **GC-MS** Column: SE-30 (36 m × 0.33 mm i.d.). Carrier gas: He, 2 mL/min. Temperature: 180°. CI, SIM acquisition mode. Limit of detection, 0.1 µg/L [Walker *et al.* 1984].

LC-MS Column: Brownlee O Spheri-5 RP-18 (100 × 1.0 mm i.d., 5 µm). Mobile phase: methanol : water (50 : 50) with 0.2% formic acid, flow rate 40 µL/min. ESI, MRM acquisition mode. Limit of detection, 0.05 µg/L [Forsström *et al.* 2001].

Forsström T *et al.* (2001). Determination of potentially hallucinogenic *N*-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 61: 547–556.

Walker RW *et al.* (1984). Capillary column gas–liquid chromatography selected ion monitoring assay for [^{13}C, ^{15}N]*N*-methyltryptamine in human urine: failure to detect conversion of [$^{13}C^{15}N$] tryptamine in schizophrenia patients. *J Chromatogr* 289: 223–229.

Methyprylon

Hypnotic

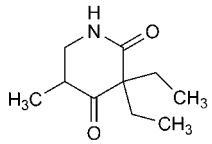
$C_{10}H_{17}NO_2 = 83.2$

CAS—125-64-4

IUPAC Name 3,3-Diethyl-5-methylpiperidine-2,4-dione

Synonym Methyprylone

Proprietary Names *3,3-Diethyl-5-methyl-2,4-piperidinedione*; *Dimerin*; *Noctan*; *Noludar*.



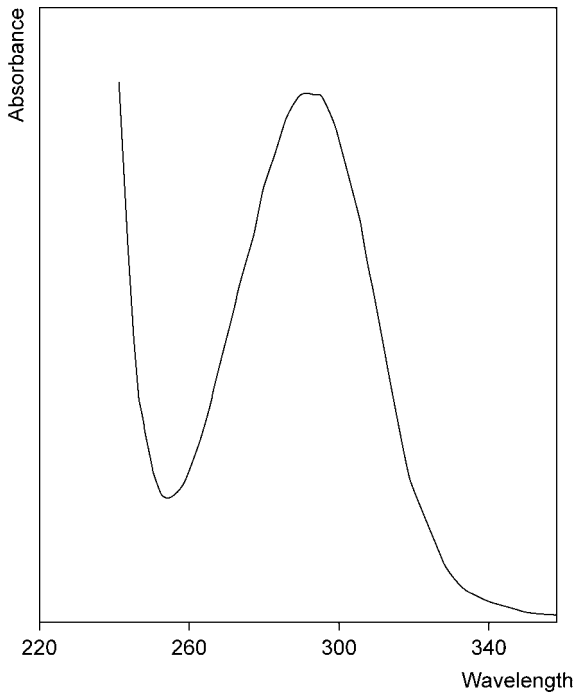
Chemical Properties A white crystalline powder. Mp 74° to 77°. Soluble 1 in 14 of water, 1 in 0.7 of ethanol, 1 in 0.6 of chloroform, and 1 in 3.5 of ether. pK_a 12.0. Log *P* (octanol/water), 0.8.

Thin-layer Chromatography System TA—R_f 0.58; system TD—R_f 0.31; system TE—R_f 0.63; system TF—R_f 0.25; system TAD—R_f 0.55; system TAE—R_f 0.78 (acidified potassium permanganate solution, positive).

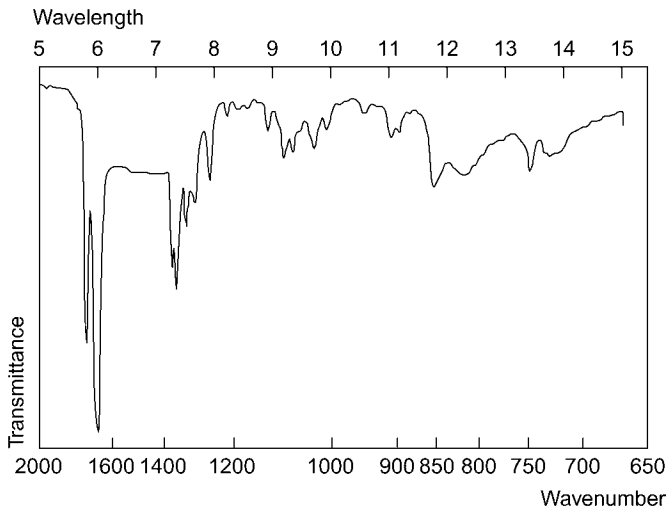
Gas Chromatography System GA—methyprylon RI 1527, M (OH-)-H_2O RI 1540, M (oxo-) RI 1870; methyprylon enol-AC RI 1610; system GB—methyprylon RI 1581, M (OH-)-H_2O RI 1601, M (oxo-) RI 1870; system GF—RI 2090.

High Performance Liquid Chromatography System HX—RI 347; system HY—RI 302.

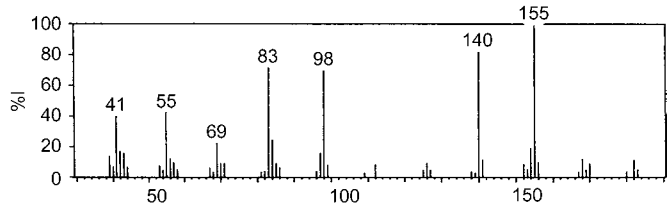
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1660, 1693, 1085, 1310, 750, 869 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 155, 140, 83, 98, 55, 41, 84, 69.



Quantification

Blood **GC** Column: 3% OV-17 on Gas Chrom Q 80/100 mesh. Carrier gas: N_2, 10 mL/min. FID. Limit of detection, 1 µg/mL [Bridges, Peat 1979]. Column: Superox 4 (50 m × 0.5 mm i.d.). Carrier gas: H_2, 5 mL/min. Temperature: 200°. FID. Limit of detection not reported [Van Boven, Sunshine 1979]. Column: 3% CHDMS (150 cm × 3 mm i.d.) on 60/80 mesh AW-DMCS Chromosorb W. Carrier gas: N_2, 25 mL/min. Temperature: 205° or Column: 2% OV-17 (1.5 m × 3 mm i.d.) on 60/80 mesh AW-DMCS Gas Chrom Q. Carrier gas: N_2, 25 mL/min. Temperature: 175°. FID. Retention time: 0.27 and 0.64 for each column, respectively. Limit of detection not reported [Dickson 1974].

Plasma **GC** See Blood [Bridges, Peat 1979].

HPLC Column: C_{18} (250 × 4.5 mm i.d., 5 µm). Mobile phase: water : acetonitrile : methanol (30 : 18 : 2), flow rate 1.2 mL/min. UV detection (λ = 306 nm for 8.5 min, 204 nm thereafter). Retention time: 10.0 min. Limit of detection, 0.1 mg/L [Contos *et al.* 1991]. Column: µPorasil (300 × 3.9 mm i.d., 10 µm). Mobile phase: hexane : tetrahydrofuran : methanol (72 : 6 : 2), flow rate 2.0 mL/min. UV detection (λ = 214 nm). Retention time: ~10 min. Limit of detection, 0.1 mg/L [Pankaskie, Brooks 1983].

Serum **GC** Column: 3% cyclohexane–dimethylpolyester of pyruvic acid on Chrom WHP 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 22 mL/min. Temperature programme: 180° to 235° at 10°/min. Retention time: 0.77. Limit of detection, 0.2 mg/L [Anweiler *et al.* 1976]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (900 × 2 mm i.d.). Carrier gas: N_2, 45 mL/min. Temperature programme: 140° to 240° at 20°/min. Limit of detection not reported [Shipe, Savory 1975].

HPLC Column: µBondapak C_{18} (300 × 4.0 mm i.d.). Mobile phase: acetonitrile : phosphate buffer (21.5 : 78.5), flow rate 3.0 mL/min. UV detection (λ = 195 nm). Retention time: 4.0 min. Limit of detection, 1 mg/L [Kabra *et al.* 1981].

Urine **GC** See Blood [Van Boven, Sunshine 1979]. See Blood [Bridges, Peat 1979; See Blood Dickson 1974]; see Serum [Shipe, Savory 1975].

Tissues **GC** See Blood [Van Boven, Sunshine 1979].

Disposition in the Body Absorbed after oral administration. The principal metabolites are 2,4-dioxo-3,3-diethyl-5-methyltetrahydropyridine (methylpersedon), which is active; 6-oxomethyprylon; 5-hydroxymethyprylon; and 5-carboxymethyprylon. Approximately 60% of a dose is excreted in the urine in 24 h, as free and conjugated metabolites together with 3% of the dose as unchanged drug.

Therapeutic Concentration In plasma, usually in the range 10–20 mg/L.

Following a single oral dose of 300 mg to 10 subjects, peak plasma concentrations of 3.51–8.38 mg/L (mean 5.62) were obtained in 1.03–2.17 h (mean 1.59) [Gwilt *et al.* 1985].

Following a single oral dose of 650 mg to 6 subjects, peak plasma concentrations averaging 10.2 mg/L were attained in 2 h [Randall *et al.* 1956].

Toxicity Prolonged use of methyprylon may lead to dependence of the barbiturate–alcohol type. Numerous cases of overdose have been reported but there have been few fatalities; death has occurred after the ingestion of 6 g but at least 2 recoveries following doses of 30 g have been reported. Plasma concentrations of 12 to 75 to 128 mg/L have been associated with toxic effects and concentrations >50 mg/L may be lethal.

In one fatality attributed to methyprylon overdose, the following postmortem tissue concentrations (mg/L or µg/g) were reported:

	Blood	*Brain*	*Kidney*	*Liver*	*Spleen*	*Urine*
Methyprylon	158	176	87	90	121	61
Methylpersedon	36	73	21	40	29	123
5-Hydroxymethyprylon	–	–	–	–	–	16
5-Carboxymethyprylon	2	–	10	7	16	80
6-Oxomethyprylon	16	18	9	8	3	68

[Van Boven, Sunshine 1979].

A 33-year-old woman was admitted to hospital following the ingestion of an unknown quantity of methyprylon. Her blood concentration of methyprylon was 142 mg/L 25 min after admission. The decline in concentration appeared to follow non-linear kinetics at concentrations >30 mg/L and first-order kinetics at concentrations below, with an apparent terminal half-life of 4.4 h. She made a full recovery [Contos *et al.* 1991].

In 4 cases of fatal overdose, the following tissue disposition was reported: blood 53–66 mg/L (mean 59), kidney 10–108 µg/g (mean 62), liver 62–260 µg/g (mean 118), urine 17–166 mg/L (mean 86) [Baselt 2008].

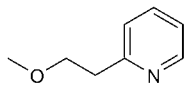
Half-life Plasma half-life following a single oral dose is ~4 h, increasing to ~50 h in acute intoxication.
Dose 200 to 400 mg, as a hypnotic.

Anweiler J *et al.* (1976). Simultaneous determination of glutethimide, methyprylon, and methaqualone in serum by gas liquid chromatography. *Arch Toxicol* 35: 187–193.
Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man,* 8th edn. Foster City, CA: Biomedical Publications.
Bridges RR, Peat MA (1979). Gas–liquid chromatographic analysis of methyprylon and its major metabolite (2,4-dioxo-3,3-diethyl-5-methyl-1,2,34-tetrahydropyridine) in an overdose case. *J Anal Toxicol* 3: 21–25.
Contos DA *et al.* (1991). Nonlinear elimination of methyprylon (noludar) in an overdosed patient: correlation of clinical effects with plasma concentration. *J Pharm Sci* 80: 768–771.
Dickson SJ (1974). The determination of methyprylon and its metabolites in biological fluids by gas chromatography. *Forensic Sci* 4: 177–182.
Gwilt PR *et al.* (1985). Pharmacokinetics of methyprylon following a single oral dose. *J Pharm Sci* 74: 1001–1003.
Kabra PM *et al.* (1981). Rapid method for screening toxic drugs in serum with liquid chromatography. *J Anal Toxicol* 5: 177–182.
Pankaskie MC, Brooks MA (1983). Determination of methyprylon and its dehydro metabolite, 5-methylpyrithyldione, in plasma by high-performance liquid chromatography. *J Chromatogr* 278: 458–463.
Randall LO *et al.* (1956). Metabolism of methyprylon. *Arch Int Pharmacodyn Ther* 106: 388–394.
Shipe JR, Savory J (1975). A comprehensive gas chromatography procedure for measurement of drugs in biological materials. *Ann Clin Lab Sci* 5: 57–64.
Van Boven M, Sunshine I (1979). Short communication: capillary gas chromatography for drug analysis. *J Anal Toxicol* 3: 174–176.

Methyridine

Anthelmintic (Veterinary)
$C_8H_{11}NO$ = 137.2
CAS—114-91-0

IUPAC Name 2-(2-Methoxyethyl)pyridine
Synonym Metyridine
Proprietary Names *Mintic*; *Promintic*.

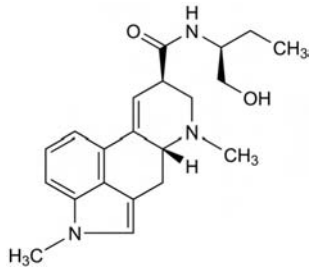


Chemical Properties A colourless or pale yellow to pale brown liquid which should be stored in airtight containers protected from light. Miscible with water and ethanol. Methyridine is extracted by organic solvents from aqueous alkaline solutions.
Thin-layer Chromatography System T1—R_f 0.65 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G5—retention time 1.09 (2.18 relative to barbital).
Ultraviolet Spectrum Cyclohexane—256 ($A_1^1$780) and 262 nm (A_1^1 770).
Disposition in the Body Methyridine is rapidly absorbed into the bloodstream and distributed throughout the tissues. It may be absorbed through the skin.
Toxicity Twice the therapeutic dose may cause death from respiratory depression, except in birds where the margin of safety is much greater. Because methyridine is absorbed from the skin, any spillage should be washed off at once with cold water.
Dose Usually 200 mg/kg. Maximum dose: cattle, 54 g; sheep and goats, 9 g.

Methysergide

Antimigraine
$C_{21}H_{27}N_3O_2$ = 353.5
CAS—361-37-5

IUPAC Name (8β)-9,10-Didehydro-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-1,6-dimethylergoline-8-carboxamide



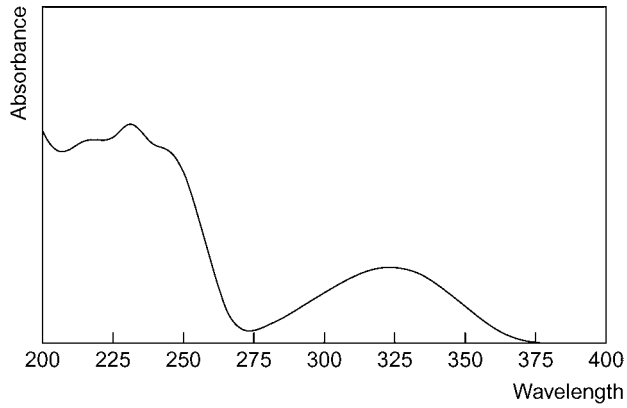
Chemical Properties Crystals. Mp 194° to 196°. pK_a 6.6 (24°). Log *P* (octanol/water), 1.6.

Methysergide Maleate

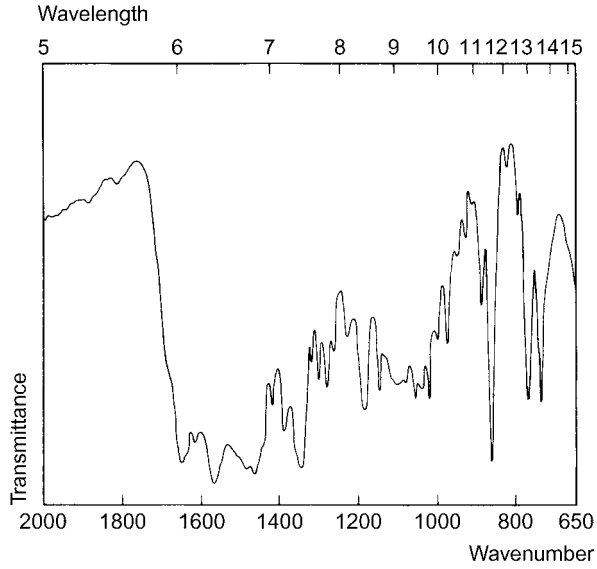
$C_{21}H_{27}N_3O_2,C_4H_4O_4$ = 469.5
CAS—129-49-7

Synonym 1-Methyl-D-lysergic acid butanolamide maleate
Proprietary Names *Deseril(a)*; *Désernil*; *Sansert*.
Chemical Properties A white to yellowish-white or reddish-white, crystalline powder. Soluble 1 in 500 of water, 1 in 165 of ethanol and 1 in 125 of methanol; practically insoluble in chloroform and ether.

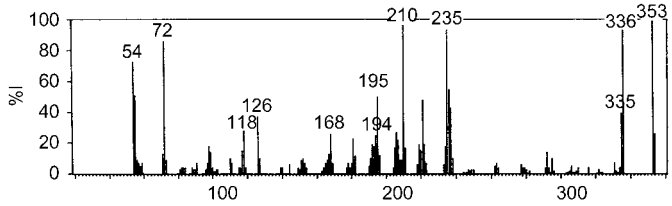
Colour Tests *p*-Dimethylaminobenzaldehyde—violet; Mandelin's test—brown; Marquis test—grey.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.01; system TC—R_f 0.21; system TE—R_f 0.45; system TL—R_f 0.15; system TM—R_f 0.33; system TAJ—R_f 0.23; system TAK—R_f 0.04; system TAL—R_f 0.66 (van Urk reagent, blue).
Gas Chromatography System GA—RI 3089.
High Performance Liquid Chromatography System HA—*k* 0.4; system HP—*k* 2.33; system HZ—retention time 2.4 min.
Ultraviolet Spectrum Aqueous acid—230, 322 nm (A_1^1=223a); aqueous alkali—243, 320 nm.



Infrared Spectrum Principal peaks at wavenumbers 1566, 1650, 863, 1615, 1190, 740 cm^{-1} (methysergide maleate, KBr disk).



Mass Spectrum Principal ions at *m/z* 353, 210, 235, 336, 72, 54, 236, 195.

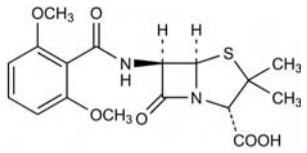


Dose The equivalent of 2 to 6 mg of methysergide daily.

Meticillin

Antibiotic
$C_{17}H_{20}N_2O_6S$ = 380.4
CAS—61-32-5

IUPAC Name (2*S*,5*R*,6*R*)-6-[(2,6-Dimethoxybenzoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Synonyms Dimethoxyphenecillin; dimethoxyphenyl penicillin.

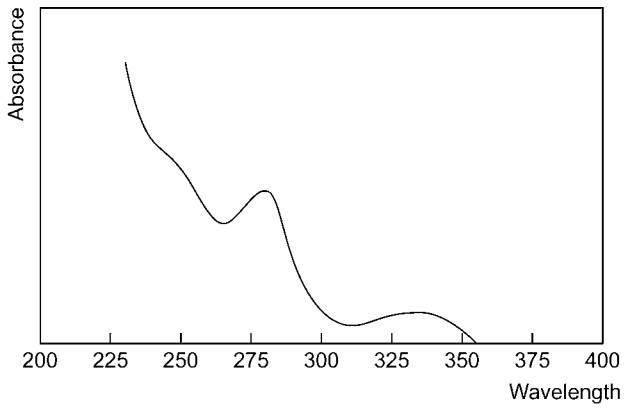


Chemical Properties pK_a 2.8 (25°). Log *P* (octanol/water), 1.2.

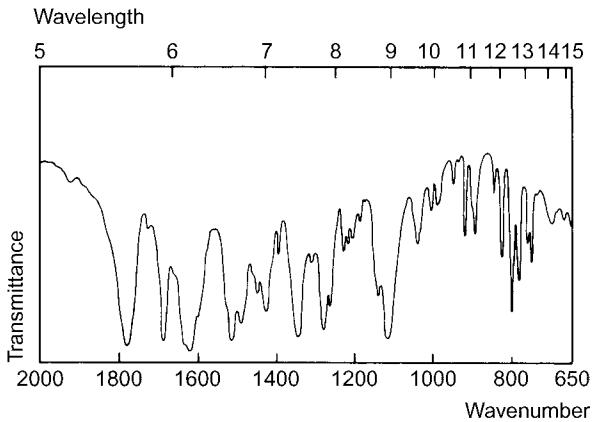
Meticillin Sodium

$C_{17}H_{19}N_2NaO_6S,H_2O$ = 420.4
CAS—132-92-3 (anhydrous); 7246-14-2 (monohydrate)
Synonym Meticillinum natricum
Proprietary Names *Azapen*; *Belfacillin*; *Celbenin*; *Celpillina*; *Cinopenil*; *Flabelline*; *Pénistaph*; *Staficyn*; *Staphcillin*.
Chemical Properties A fine white crystalline powder. Mp 196° to 197°, with decomposition. Soluble 1 in 0.6 of water and 1 in 35 of ethanol; slightly soluble in chloroform; practically insoluble in ether; freely soluble in methanol.

Ultraviolet Spectrum Water—280 nm (A_1^1=61a).



Infrared Spectrum Principal peaks at wavenumbers 1607, 1766, 1673, 1500, 1093, 1260 cm^{-1} (meticillin sodium, KBr disk).

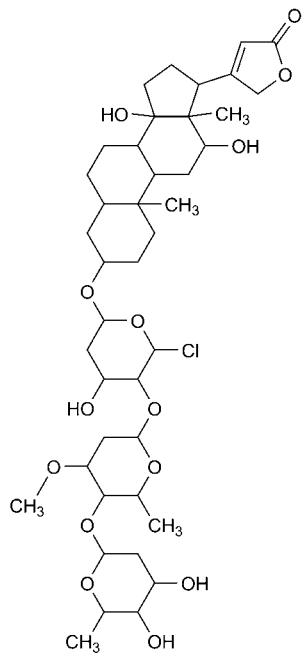


Dose Up to 12 g of meticillin sodium daily, by slow IV injection.

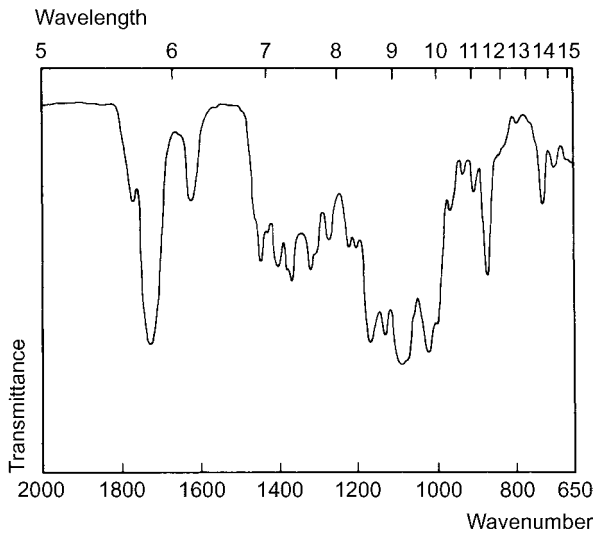
Metildigoxin

Cardiac Glycoside
$C_{42}H_{66}O_{14}$ = 95.0
CAS—30685-43-9

IUPAC Name 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*)-12,14-Dihydroxy-3-[(2*R*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-methoxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[*a*]phenanthren-17-yl]-2*H*-furan-5-one
Synonyms Medigoxin; β-methyl digoxin; β-methyldigoxin; 3β,12β,14β-trihydroxy-5β-card-20(22)-enolide-3-(4‴-*O*-methyltridigitoxoside).
Proprietary Names *Cardiolan*; *Lanirapid*; *Lanitop*; *Miopat*.



Chemical Properties A white crystalline powder. Mp 227° to 231°. Very slightly soluble in water; soluble in ethanol and chloroform. Log *P* (octanol/water), 1.8 [Hansch *et al.* 1995].
Infrared Spectrum Principal peaks at wavenumbers 1085, 1014, 1730, 1163, 1130, 864 cm^{-1} (KBr disk).



Quantification

Note For a radioimmunoassay in plasma, see Garrett and Hinderling [1977].
Disposition in the Body Rapidly and almost completely absorbed after oral administration. Metabolised by demethylation to digoxin and by hydrolysis to the bis- and monoglycosides. Approximately 75% of a dose is excreted in the urine over a period of several days, with ~25–30% being excreted in the first 24 h. Of the material excreted in the urine, ~30–50% is unchanged drug, 15% consists of conjugated bis- and monoglycosides, and the remainder is digoxin. Approximately 15–30% of a dose is eliminated in the faeces over a period of 7 days.
Therapeutic Concentration
Following a single oral dose of 0.4 mg to 17 subjects, a mean peak plasma concentration of 0.003 mg/L was attained in ~1 h. Following daily oral doses of

0.15–0.2 mg to 18 subjects, steady-state plasma concentrations of 0.6–2.0 μg/L (mean 1.0) were reported [Boerner *et al.* 1976].

Toxicity

In a fatality in which death occurred ~1 h after ingestion of a metildigoxin/medigoxin overdose, the following postmortem tissue concentrations of glycosides were reported: blood 0.075 mg/L, bile 2.4 mg/L, kidney 0.237 μg/g, liver 0.047 μg/g, left ventricular myocardium 0.14 μg/g, right ventricular myocardium 0.16 μg/g, urine 0.91 mg/L [Rietbrock *et al.* 1978].

Half-life Plasma half-life, 40 to 70 h.

Volume of Distribution Approximately 6 to 10 L/kg.

Clearance Plasma clearance, ~2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.1.

Protein Binding Approximately 10–30%.

Dose For rapid digitalisation, 600 μg daily for 2 to 4 days; maintenance, 200 to 300 μg daily.

Boerner D *et al.* (1976). Absorption of beta-methyl-digoxin determined after a single dose and under steady state conditions. *Eur J Clin Pharmacol* 9: 307–314.

Garrett ER, Hinderling PH (1977). Pharmacokinetics of beta-methyldigoxin in healthy humans IV: comparisons of radioimmunoassays, total radioactivity, and specific assays of beta-methyldigoxin and digoxin in plasma. *J Pharm Sci* 66: 806–810.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants.* Washington DC: American Chemical Society.

Rietbrock N *et al.* (1978). [Suicide with beta-methyldigoxin (author's transl)]. *Dtsch Med Wochenschr* 103: 1841–1844.

Metindizate

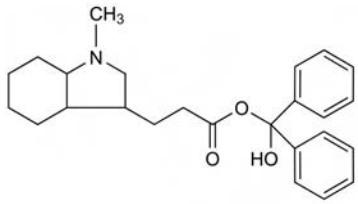
Antispasmodic (Veterinary)

$C_{25}H_{31}NO_3 = 393.5$

CAS—15687-33-9

IUPAC Name 2-(1-Methyl-2,3,3*a*,4,5,6,7,7*a*-octahydroindol-3-yl)ethyl 2-hydroxy-2,2-diphenylacetate

Synonym 2-(1-Methylperhydroindol-3-yl)ethyl benzilate



Metindizate Hydrochloride

$C_{25}H_{31}NO_3,HCl = 430.0$

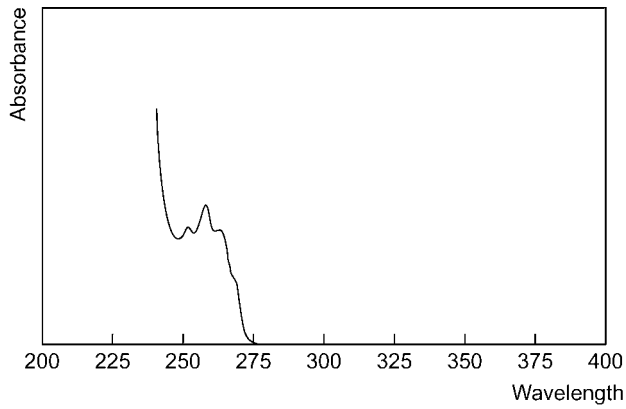
Proprietary Name It is an ingredient of *Isaverin*.

Chemical Properties A fine, white, crystalline powder. Mp 159° to 160°. Freely soluble in water and ethanol; practically insoluble in chloroform and ether.

Colour Tests Liebermann's reagent—orange→brown; Mandelin's test—brown; Marquis test—orange→green; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.36 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—251, 257 nm (A^{1}_{1}=10.3b).



Infrared Spectrum Principal peaks at wavenumbers 1739, 1238, 1227, 702, 1058, 1093 cm^{-1} (KBr disk).

Metipranolol

β-*Adrenoceptor Antagonist*

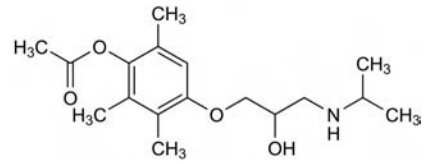
$C_{17}H_{27}NO_4 = 309.4$

CAS—22664-55-7

IUPAC Name 4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy]-2,3,6-trimethylphenol 1-acetate

Synonyms Methypranolum; trimepranol.

Proprietary Names *Betamann*; *Betanol*; *Beta-Ophtiole*; *Disorat*; *Glauline*; *Glausyn*; *OptiPranolol*; *Turoptin*.



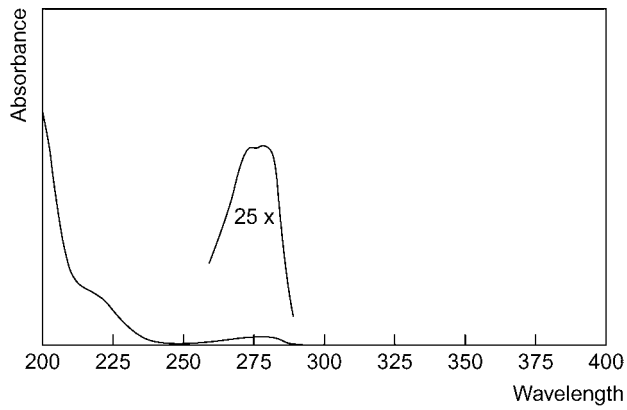
Chemical Properties A white crystalline powder. Mp 105° to 109°. Practically insoluble in water; freely soluble in ethanol, chloroform and benzene; slightly soluble in ether. Log *P* (octanol/water), 2.7.

Thin-layer Chromatography System TB—R_f 0.08; system TE—R_f 0.47; system TF—R_f 0.00; system TAE—R_f 0.21.

Gas Chromatography System GA—metipranolol RI 2241, metipranolol-AC RI 2260, M (desamino-OH-)-AC_2 RI 2240, M art (phenol)-AC RI 1610; system GB—metipranolol RI 2320.

High Performance Liquid Chromatography System HX—RI 379; system HZ—retention time 3.5 min.

Ultraviolet Spectrum Aqueous acid—278 nm (A^{1}_{1}=44b).



Quantification

Plasma **HPLC** Electrochemical detection. Desacetylmetipranolol. Limit of detection, 2 μg/L [Tkaczykova, Safarik 1989]. Metipranolol and other topically applied beta blockers. Limits of detection, 4 to 27 μg/L [Tracqui *et al.* 1988].

Urine **HPLC** See Plasma [Tracqui *et al.* 1988].

Disposition in the Body Readily absorbed after oral administration and rapidly metabolised to the active metabolite, desacetylmetipranolol. About 20% of a dose is excreted in the urine as unchanged drug.

Therapeutic Concentration

After a single oral dose of 40 mg to 13 subjects, peak plasma concentrations of the desacetyl metabolite of 0.034 to 0.214 mg/L (mean 0.14) were attained in about 1 h. Oral administration of 20 mg twice daily to 6 subjects, produced mean steady-state plasma concentrations (desacetyl derivative) of 0.06 to 0.08 mg/L determined on the 6th to 8th day; considerable intersubject variation was reported [Mayer 1980].

Following administration of metipranolol 40 mg to 6 subjects as either a conventional- or a controlled-release preparation, mean peak plasma concentrations of 22 μg/L (at 1 h) and 11 μg/L (at 3 h), respectively, were reported [Lapka *et al.* 1990].

Half-life Plasma half-life, desacetylmetipranolol about 2 to 4 h but there is considerable intersubject variation.

Dose Metipranolol has been given in doses of 10 to 120 mg daily.

Lapka R *et al.* (1990). Pharmacokinetics and pharmacodynamics of conventional and controlled-release formulations of metipranolol in man. *Eur J Clin Pharmacol* 38: 243–247.

Mayer O (1980). Beta-blocking drug metipranolol: plasma levels and pharmacodynamic action in man. *Int J Clin Pharmacol Ther Toxicol* 18: 113–120.

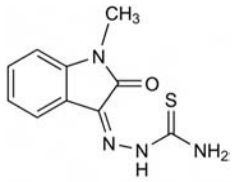
Tkaczykova M, Safarik L (1989). High-performance liquid chromatography of deacetylmetipranolol in plasma. *J Pharm Biomed Anal* 7: 1805–1810.

Tracqui A *et al.* (1988). A specific HPLC method for determination of beta-blockers topically used in ophthalmological diseases. *Forensic Sci Int* 38: 37–41.

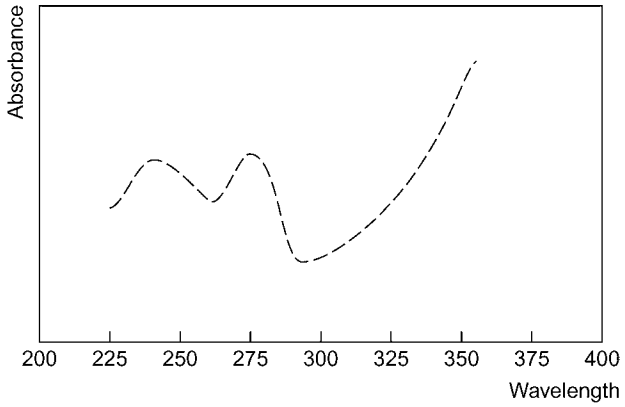
Metisazone

Antiviral
$C_{10}H_{10}N_4OS$ = 234.3
CAS—1910-68-5

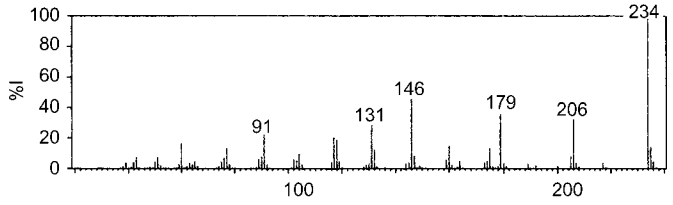
IUPAC Name [(*Z*)-(1-Methyl-2-oxoindol-3-ylidene)amino]thiourea
Synonyms 2-(1,2-Dihydro-1-methyl-2-oxo-3*H*-indol-3-ylidene)-hydrazinecarbothioamide; *N*-methylisatin β-thiosemicarbazone; methisazone.
Proprietary Names *Marboran*; *Viruzona*.



Chemical Properties A very fine, orange-yellow powder. Mp about 248°, with decomposition. Practically insoluble in water; soluble 1 in 25 of acetone and 1 in 800 of chloroform; soluble in warm dilute solutions of alkali hydroxides. Log *P* (octanol/water), 1.0.
Colour Tests Mandelin's test—violet→yellow; palladium chloride—yellow→orange→brown.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.03; system TC—R_f 0.68; system TL—R_f 0.69 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Ethanol—241, 274 nm (A^1_1=588b).



Infrared Spectrum Principal peaks at wavenumbers 1605, 1493, 1097, 1673, 1040, 826 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 234, 146, 179, 206, 131, 91, 117, 118.

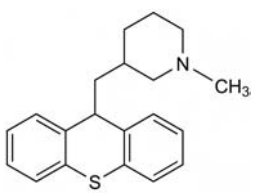


Dose Usually 6 g daily (prophylactic dose).

Metixene

Anticholinergic
$C_{20}H_{23}NS$ = 309.5
CAS—4969-02-2

IUPAC Name 1-Methyl-3-(9*H*-thioxanthen-9-ylmethyl)piperidine
Synonym Methixene

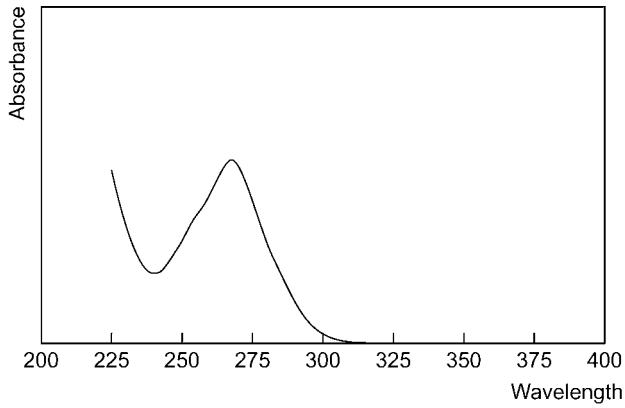


Chemical Properties A pale yellow viscous liquid. Insoluble in water. Log *P* (octanol/water), 5.7. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

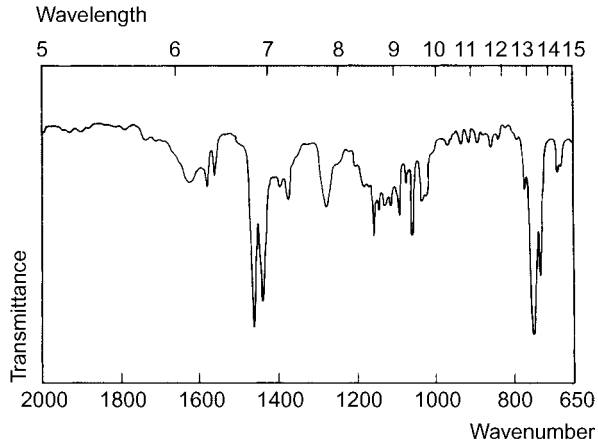
Metixene Hydrochloride

$C_{20}H_{23}NS,HCl$ = 345.9
CAS—1553-34-0 (anhydrous); 7081-40-5 (monohydrate)
Synonyms Methixene hydrochloride; metixeni hydrochloridum; NSC-78194; SJ-1977.
Proprietary Names *Metixen*; *Tremaril*; *Tremarit*; *Tremonil*; *Tremoquil*; *Trest*.
Chemical Properties A white crystalline powder. Mp about 216°. Soluble in water, ethanol and chloroform.

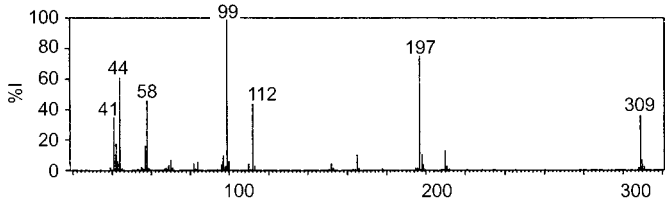
Colour Tests Formaldehyde-sulfuric acid—orange; Liebermann's reagent—red-orange; Mandelin's test—orange; sulfuric acid—orange (fluoresces under UV light).
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.45; system TC—R_f 0.25; system TE—R_f 0.61; system TL—R_f 0.12; system TAE—R_f 0.21; system TAJ—R_f 0.16; system TAK—R_f 0.22; system TAL—R_f 0.73 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2480; system GB—RI 2592; system GF—RI 2895.
High Performance Liquid Chromatography System HA—*k* 3.6; system HX—RI 451.
Ultraviolet Spectrum Aqueous acid—268 nm (A^1_1=324a).



Infrared Spectrum Principal peaks at wavenumbers 750, 729, 1052, 1162, 1086, 1280 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 99, 197, 44, 58, 112, 309, 41, 42.



Disposition in the Body Absorbed after oral administration. It is excreted in the urine, partly unchanged, partly as its two isomeric sulfoxides, and partly as the stereoisomeric sulfoxides of *N*-demethylated metixene.
Dose 7.5 to 60 mg of metixene hydrochloride daily in divided doses.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

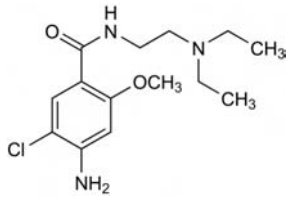
Metoclopramide

Antiemetic

$C_{14}H_{22}ClN_3O_2$ = 299.8

CAS—364-62-5

IUPAC Name 4-Amino-5-chloro-*N*-(2-diethylaminoethyl)-2-methoxybenzamide



Chemical Properties A white crystalline powder. Mp 146.5° to 148.0°. Practically insoluble in water; soluble 1 in 45 of ethanol and 1 in 15 of chloroform. pK_a 9.3 (25°). Log *P* (octanol/water), 2.6. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Metoclopramide Hydrochloride

$C_{14}H_{22}ClN_3O_2,HCl,H_2O$ = 354.3

CAS—7232-21-5 (anhydrous); 54143-57-6 (monohydrate)

IUPAC Name 4-Amino-5-chloro-*N*-(2-diethylaminoethyl)-2-methoxybenzamide dihydrochloride

Synonyms AHR-3070-C; DEL-1267; metoclopramidi hydrochloridum; MK-745.

Proprietary Names *Anausin*; *Clopra*; *Gastrobid Continus*; *Gastroflux*; *Gastromax*; *Intensol*; *Maxeran*; *Maxolon*; *Metalon*; *Metamide*; *Metopram*; *Mygdalon*; *Octamide*; *Parmid*; *Paspertin*; *Primperan*; *Reclomide*; *Reglan*. It is an ingredient of *Migramax*; *Migravess*; *Paramax*.

Chemical Properties A white crystalline powder. Mp 182.5° to 184.0°. Soluble 1 in 0.7 of water, 1 in 3 of ethanol, and 1 in 55 of chloroform; practically insoluble in ether.

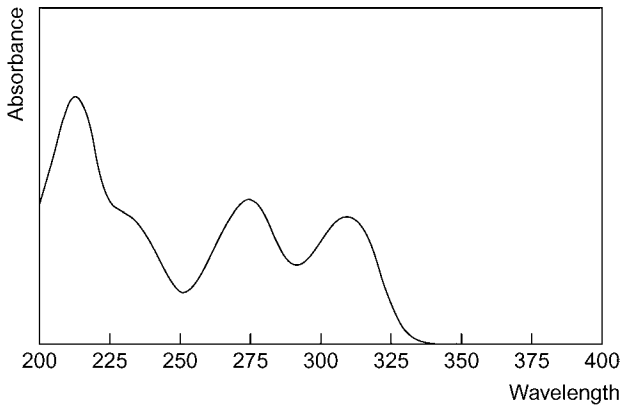
Colour Tests Coniferyl alcohol—orange; Liebermann's reagent—yellow; Mandelin's test—brown.

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.01; system TC—R_f 0.07; system TE—R_f 0.51; system TL—R_f 0.13; system TAE—R_f 0.17 (acidified iodoplatinate solution, positive).

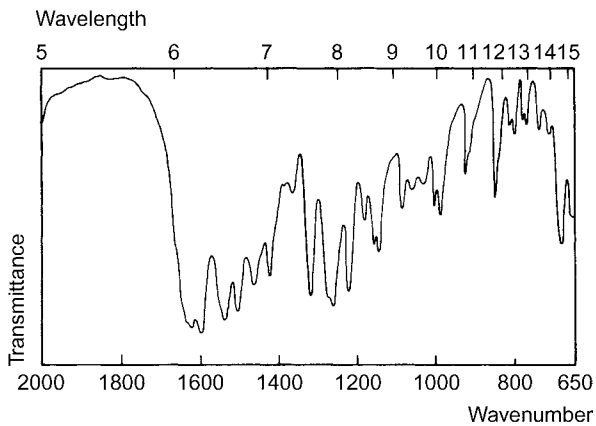
Gas Chromatography System GA—metoclopramide RI 2620, metoclopramide-AC RI 2735, M (desethyl-) RI 2095, M (desethyl-)-AC_2 RI 2900.

High Performance Liquid Chromatography System HA—*k* 5.0; system HX—RI 324; system HY—RI 263; system HZ—RT 2.6 min; system HAA—RT 9.9 min; system HAX—RT 7.9 min; system HAY—RT 4.3 min.

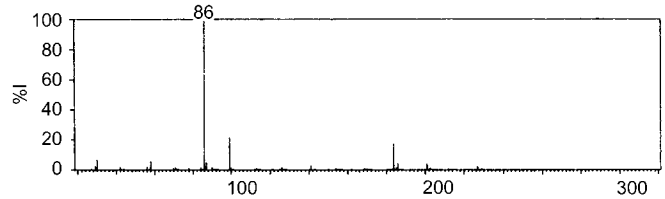
Ultraviolet Spectrum Aqueous acid—273 (A^1_1 = 467b), 309 nm.



Infrared Spectrum Principal peaks at wavenumbers 1590, 1614, 1530, 1496, 1254, 1311 cm^{-1} (metoclopramide hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 184, 58, 30, 87, 201, 186.



Quantification

Plasma **GC** Column: SE-54 fused silica capillary (25 m × 0.31 mm, 0.15 µm). Carrier gas: H_2, 1.0 mL/min. Temperature: 235°. ECD. Retention time: 3.2 min. Limit of detection, 4 µg/L [Riggs *et al.* 1983]. Column: 3% OV-101 on DMCS treated Gas Chrom Q 80/100 mesh (1.2 m × 2 mm i.d.). Carrier gas: N_2, 37 mL/min. Temperature: 200°. ECD. Limit of detection, 5 µg/L [Ross-Lee *et al.* 1980].

HLPC Column: C_{18}. Electrochemical detection [Chmielewska *et al.* 2006]. Column: Spherisorb CN (250 × 4.55 mm i.d., 5 µm). Mobile phase: 0.02 mol/L potassium dihydrogen phosphate (pH 3.0) : acetonitrile (60 : 40), flow rate 2.0 mL/min. UV detection (λ = 275 nm). Retention time: 4.6 min. Limit of quantification, 5 µg/L, limit of detection, 3 µg/L [Buss *et al.* 1990]. Column: Supelcosil LC-18-DB (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.03 mol/L acetic acid-acetonitrile (75 : 25) : TEA (1 L:500 µL), flow rate 1.3–1.5 mL/min. Fluorescence detection (λ_{ex} = 307 nm, λ_{em} = 380 nm). Limit of detection, 1.5 µg/L [Albani *et al.* 1987]. Column: cyanopropyl CN (100 × 8 mm i.d., 4 µm). Mobile phase: 35% acetonitrile : 1% SDS in 0.61 mol/L sodium acetate (pH 5.0), flow rate 1.0 mL/min. UV detection (λ = 275 nm). Retention time: 7.8 min. Limit of detection, 2 µg/L [Takahashi *et al.* 1987]. Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 1% acetic acid-acetonitrile (32 : 68) : methanol (3.7 : 1), flow rate 1.5 mL/min. UV detection (λ = 273 nm). Limit of detection, 8 µg/L [Bishop-Freudling, Vergin 1983].

LC-MS Column Atlantis HILIC silica. Mobile phase: acetonitrile : 100 mmol/L ammonium formate (pH 6.5, 85 : 15). ESI, SIM acquisition mode [Lee *et al.* 2009].

Serum **HPLC** Limit of detection, 1.2 µg/L [Javanbakht *et al.* 2009]. Column: TSkgel Super-ODS. Limit of detection, 0.5 µg/L [Chiba *et al.* 2003]. Column: C (18). Mobile phase: methanol : phosphate buffer (pH 3.0, 30 : 70). Electrochemical Detection. Limit of quantification, 2 µg/L [Lamparczyk *et al.* 2001]. Column: Spherisorb 5 ODS (100 × 3 mm i.d.). Mobile phase: acetonitrile : 2% TEA (pH 5.5), flow rate 0.7 mL/min. UV detection (λ = 310 nm). Limit of detection, 2 µg/L [Fairhead *et al.* 1989]. Column: Micro-Pak Si-5 (300 × 4 mm i.d.). Mobile phase: dichloromethane : methanol : 1 mol/L diethylamine (89 : 10 : 1), flow rate 1.0 mL/min. UV detection (λ = 308 nm). Limit of detection, 15 nmol/L [Popovic 1984]. See Plasma [Chmielewska *et al.* 2006].

Urine **HPLC** See Serum. Limit of detection, 3 µg/L [Javanbakht *et al.* 2009]. See Plasma [Albani *et al.* 1987; Takahashi *et al.* 1987]. See Serum. Limit of detection, 3 µmol/L [Popovic 1984].

Bile **HPLC** See Plasma [Takahashi *et al.* 1987].

Oral Fluid **HPLC** See Serum [Popovic 1984].

Disposition in the Body Metoclopramide is rapidly and almost completely absorbed after oral administration. Up to ~60% of an oral dose undergoes first-pass metabolism but there is considerable intersubject variation. Metoclopramide crosses the blood–brain barrier. It crosses the placenta and concentrations in fetal plasma may be 60–70% of those in maternal plasma. It is found in breast milk in concentrations that may be higher than those in plasma. Up to ~80% of a dose is excreted in the urine in 24 h, with ~10–20% as unchanged drug, up to 50% as conjugated metoclopramide (mainly the *N*-4-sulfate), and a small amount as 2-(4-amino-5-chloro-2-methoxybenzamido)acetic acid. Approximately 5% of a dose is excreted in the faeces via the bile.

Therapeutic Concentration

After a single oral dose of 10 mg given to 6 subjects, peak plasma concentrations of 0.04–0.06 mg/L (mean 0.05) were attained in 0.5–2 h; following IV administration of 10 mg to 6 subjects, plasma concentrations of 0.07–0.13 mg/L were reported after 5 min [Ross-Lee *et al.* 1981].

The mean plasma concentration at the end of an infusion of metoclopramide in 14 patients was 1.01 mg/L in those patients in whom the dose had been adjusted to individual pharmacokinetic parameters (mean loading dose 2.22 mg/kg, mean maintenance dose 1.96 mg/kg/4 h) and 0.54 mg/L in those patients in whom the dose had been adjusted as usual to body weight (mean loading dose 1.40 mg/kg, mean maintenance dose 0.52 mg/kg/4 h) [Brechot *et al.* 1991].

Following SC administration of metoclopamide 10 mg every 6 h for 3 days to 10 patients with gastroparesis, a peak plasma concentration of 0.0997 mg/L was attained 30 min after the last dose; at 60 min, the concentration was 0.0939 mg/L [McCallum *et al.* 1991].

In neonates, steady-state metoclopramide levels just before and 1 h after administration were 91.6 and 87.4 µg/L, respectively. There was a negative correlation between minimum plasma levels and gestational age [Vauzelle-Kervroedan *et al.* 1997].

Toxicity Ingestion by adults of up to 0.8 g without serious adverse effects has been reported.

In a 25-year-old female who died 4 days after ingesting metoclopramide and diltiazem with suicidal intent, the blood levels 1 h after admission to hospital were 4.4 mg/L metoclopramide and 8.49 mg/L diltiazem [Beno, Nemeth 1991].

For a report of metoclopramide poisoning in children, see Low, Goel [980].

Bioavailability Approximately 75%, with a wide intersubject variation of 30–100%.

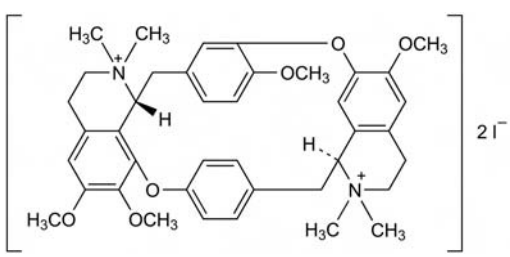
Half-life Plasma half-life, 4–6 h, increased in renal impairment.
Volume of Distribution Approximately 3.5 L/kg but there is considerable inter-subject variation.
Clearance Plasma clearance, 7–17 mL/min/kg (mean 11).
Distribution in Blood Plasma : whole blood ratio, 0.93.
Protein Binding ~40%.
Note For reviews of metoclopramide, see Bateman [1983], Harrington *et al.* [1983], Kearns *et al.* [1998], Lauritsen *et al.* [1990] and Rotmensch *et al.* [1997].
Dose The equivalent of 30 mg anhydrous metoclopramide hydrochloride daily.

Albani F *et al.* (1987). Liquid chromatographic analysis of metoclopramide with fluorescence detection in cirrhotic patients. *Biomed Chromatogr* 2: 135–136.
Bateman DN (1983). Clinical pharmacokinetics of metoclopramide. *Clin Pharmacokinet* 8: 523–529.
Beno JM, Nemeth DR (1991). Diltiazem and metoclopramide overdose. *J Anal Toxicol* 15: 285–287.
Bishop-Freudling GB, Vergin H (1983). Determination of metoclopramide in human plasma by high-performance liquid chromatography. *J Chromatogr* 273: 453–457.
Brechot JM *et al.* (1991). Continuous infusion of high-dose metoclopramide: comparison of pharmacokinetically adjusted and standard doses for the control of cisplatin-induced acute emesis. *Eur J Clin Pharmacol* 40: 283–286.
Buss DC *et al.* (1990). A rapid liquid chromatographic method for the determination of metoclopramide in human plasma. *Ther Drug Monit* 12: 293–296.
Chiba R *et al.* (2003). Direct determination of benzamides in serum by column-switching high-performance liquid chromatography. *Anal Sci* 19: 785–789.
Chmielewska A *et al.* (2006). Sensitive quantification of chosen drugs by reversed-phase chromatography with electrochemical detection at a glassy carbon electrode. *J Chromatogr B Analyt Technol Biomed Life Sci* 839: 102–111.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Fairhead AP *et al.* (1989). An automated high-performance liquid chromatographic trace enrichment method for the determination of metoclopramide in serum and its application to a bioequivalence human volunteer study. *Food Chem Toxicol* 27: 341–345.
Harrington RA *et al.* (1983). Metoclopramide: an updated review of its pharmacological properties and clinical use. *Drugs* 25: 451–494.
Javanbakht M *et al.* (2009). Novel molecularly imprinted polymers for the selective extraction and determination of metoclopramide in human serum and urine samples using high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 2537–2544.
Kearns GL *et al.* (1998). Pharmacokinetics of metoclopramide in neonates. *J Clin Pharmacol* 38: 122–128.
Lamparczyk H *et al.* (2001). RP-HPLC method with electrochemical detection for the determination of metoclopramide in serum and its use in pharmacokinetic studies. *Biomed Chromatogr* 15: 513–517.
Lauritsen K *et al.* (1990). Clinical pharmacokinetics of drugs used in the treatment of gastrointestinal diseases (Part I). *Clin Pharmacokinet* 19: 11–31.
Lee HW *et al.* (2009). Determination of metoclopramide in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1716–1720.
Low LC, Goel KM (1980). Metoclopramide poisoning in children. *Arch Dis Child* 55: 310–312.
McCallum RW *et al.* (1991). Subcutaneous metoclopramide in the treatment of symptomatic gastroparesis: clinical efficacy and pharmacokinetics. *J Pharmacol Exp Ther* 258: 136–142.
Popovic J (1984). High-pressure liquid chromatographic method for determination of metoclopramide in serum, urine, and saliva, with a pharmacokinetic study in patients. *Ther Drug Monit* 6: 77–82.
Riggs KW *et al.* (1983). Electron-capture determination of metoclopramide in biological fluids using fused silica capillary columns. Application to placental transport studies in sheep and humans. *J Chromatogr* 276: 319–328.
Ross-Lee LM *et al.* (1980). Electron-capture gas chromatographic assay for metoclopramide in plasma. *J Chromatogr* 183: 175–184.
Ross-Lee LM *et al.* (1981). Single-dose pharmacokinetics of metoclopramide. *Eur J Clin Pharmacol* 20: 465–471.
Rotmensch HH *et al.* (1997). Comparative central nervous system effects and pharmacokinetics of neu-metoclopramide and metoclopramide in healthy volunteers. *J Clin Pharmacol* 37: 222–228.
Takahashi H *et al.* (1987). Determination of metoclopramide and its glucuronide and sulfate conjugates in human biological fluids (plasma, urine and bile) by ion-pair high-performance liquid chromatography. *J Chromatogr* 419: 243–251.
Vauzelle-Kervroedan F *et al.* (1997). Metoclopramide plasma concentration in neonates. *Int J Clin Pharmacol Ther* 35: 519–521.

Metocurine Iodide

Muscle Relaxant
$C_{40}H_{48}I_2N_2O_6 = 906.6$
CAS—5152-30-7 (metocurine); 7601-55-0 (iodide)

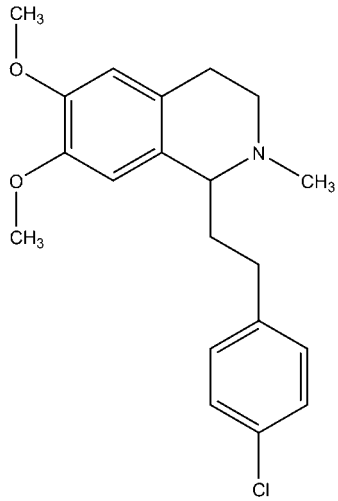
Synonyms Dimethyl tubocurarine iodide; dimethyltubocurarine iodide; 6,6′,7′,12′-tetramethoxy-2,2,2′,2′-tetramethyltubocuraranium diiodide.
Note The name dimethyltubocurarine iodide was based on the old empirical formula for tubocurarine.
Proprietary Name *Metubine Iodide*



Chemical Properties A white to pale yellow, crystalline powder. When heated to 257° to 267°, it decomposes with the evolution of gas. Soluble 1 in 400 of water; very slightly soluble in ethanol; practically insoluble in benzene, chloroform and ether; slightly soluble in dilute acids and alkalis.
Colour Tests Aromaticity (method 2)—yellow/orange; nitric acid, cold gives a brown colour.
Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=74b).
Infrared Spectrum Principal peaks at wavenumbers 1510, 1220, 1265, 1280, 1125, 1240 cm^{-1} (KBr disk).
Disposition in the Body About 50% of a dose is excreted in the urine unchanged in 48 h, and about 2% of the dose is excreted in the bile in the same period.
Half-life Plasma half-life, about 3 to 4 h.
Volume of Distribution About 0.4 L/kg.
Clearance Plasma clearance, about 1.3 mL/min/kg.
Protein Binding About 35%.
Dose Initially, 1.5 to 8 mg intravenously.

Metofoline

Analgesic
$C_{20}H_{24}ClNO_2 = 345.9$
IUPAC Name 1-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinoline
Synonyms 1-*p*-Chlorophenethyl-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinoline; methopholine; Ro 4-1778.



Chemical Properties Metofoline is extracted by organic solvents from aqueous alkaline solutions.

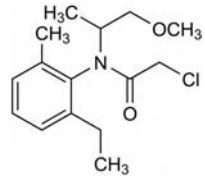
Metofoline Hydrochloride
Proprietary Name *Versidyne*

Colour Tests Ammonium molybdate test—dull green (limit of detection, 1.0 µg); ammonium vanadate test—pale green (limit of detection, 1.0 µg); sulphuric acid-formaldehyde test—pale yellow (limit of detection, 1.0 µg); Vitali's test—yellow/yellow/yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.57 (location reagent acidified iodoplatinate spray, positive reaction).
Disposition in the Body
Toxicity It has been reported to have a depressive effect on respiration. Tests in former opiate addicts have shown that metofoline has substantially fewer addiction-producing properties than morphine and codeine, and even fewer than dextropropoxyphene.
Dose Up to 240 mg daily.

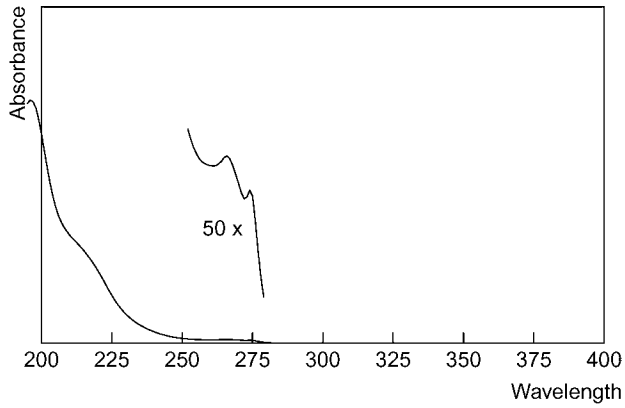
Metolachlor

Herbicide
$C_{15}H_{22}ClNO_2 = 283.8$
CAS—51218-45-2

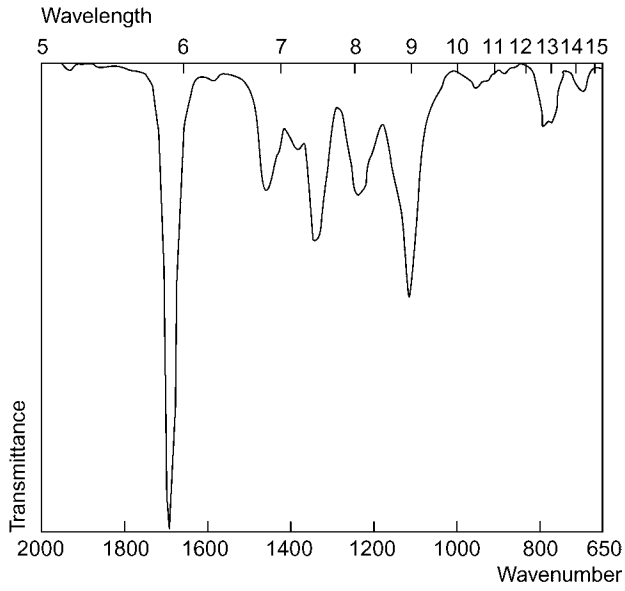
IUPAC Name 2-Chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide
Synonyms CGA 24705; matelilachlor; metolachlore.
Proprietary Names *Bicep*; *Codal*; *Cotoran multi*; *Dual*; *Ontrack*; *Pace*; *Pennant*; *Pimagram*; *Primextra*.



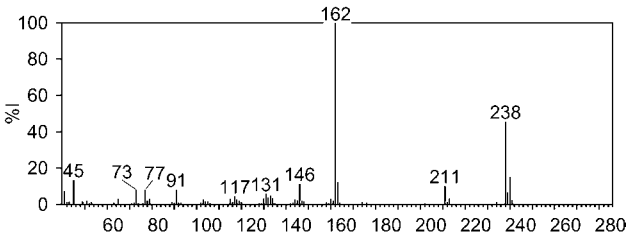
Chemical Properties An off-white to colourless liquid at room temperature. Bp 100°. Formulations are opaque white to tan in colour. It is soluble in water (530 ppm at 20°) and miscible with benzene, dichloromethane, hexane, methanol, octanol, xylene, toluene, dimethylformamide, ethylene dichloride and cyclohexane; insoluble in ethylene glycol and propylene glycol. Log *P* (octanol/water), 3.13.
High Performance Liquid Chromatography System HY—RI 562.
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1691, 1113, 1342, 1346, 1237, 1458 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 162, 238, 45, 146, 240, 163, 91, 77.

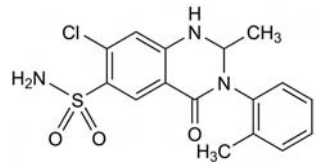


Disposition in the Body Metolachlor is quickly broken down into its metabolites after ingestion. It is totally eliminated via urine and faeces. Metolachlor is not detected in urine, faeces or tissues.

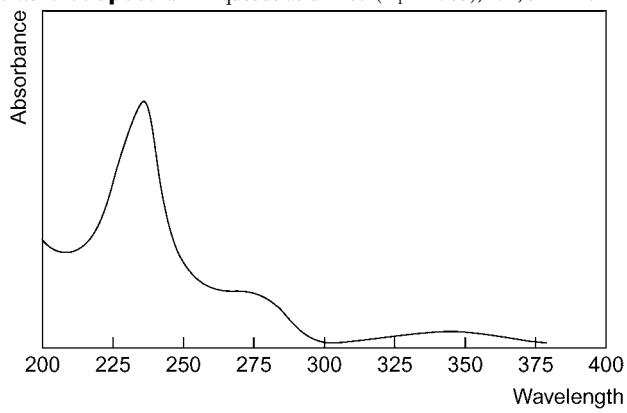
Toxicity Metolachlor is toxic by ingestion, although less so than other herbicides.

Metolazone

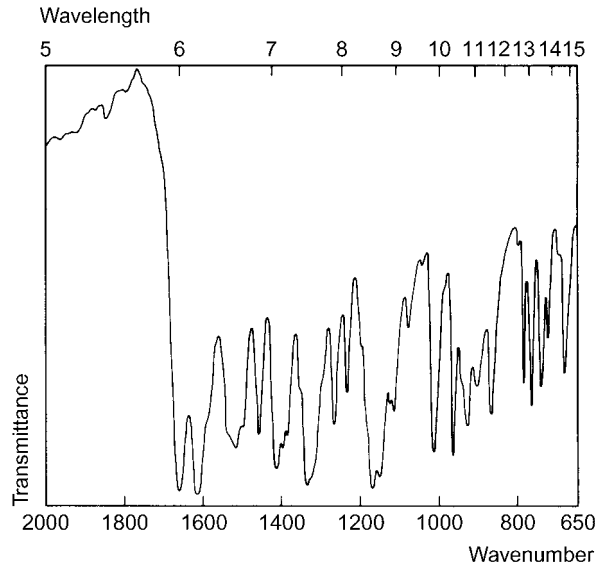
Diuretic
$C_{16}H_{16}ClN_3O_3S$ = 365.8
CAS—17560-51-9
IUPAC Name 7-Chloro-1,2,3,4-tetrahydro-2-methyl-3-(2-methylphenyl)-4-oxo-6-quinazoline sulfonamide
Proprietary Names *Birobin*; *Diondel*; *Diulo*; *Metenix*; *Mykrox*; *Xuret*; *Zaroxolyn(e)*.



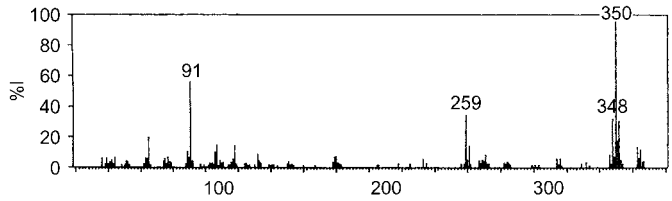
Chemical Properties Colourless crystals. It may occur in two polymorphic forms which melt at 227°. Sparingly soluble in water; more soluble in alkalis and organic solvents. pK_a 9.7. Log *P* (octanol/water), 1.8.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—orange→green-brown; methanolic potassium hydroxide—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TD—R_f 0.23; system TE—R_f 0.57; system TF—R_f 0.51; system TAD—R_f 0.33; system TAJ—R_f 0.34; system TAK—R_f 0.08; system TAL—R_f 0.75; system TAM—R_f 0.75.
Gas Chromatography System GA—metolazone, not eluted; metolazone-Me_3 RI 3910.
High Performance Liquid Chromatography System HN—*k* 4.89; system HY—RI 371.
Ultraviolet Spectrum Aqueous acid—237 (A^1_1=1470b), 271, 344 nm.



Infrared Spectrum Principal peaks at wavenumbers 1610, 1660, 1165, 1145, 962, 1012 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 350, 91, 259, 352, 348, 65, 351, 107.



Quantification

Blood **HPLC** UV detection. Limit of detection, 1 µg/L [Farthing *et al.* 1994].

Plasma **HPLC** See Blood [Farthing *et al.* 1994]. UV detection. Limit of detection, 2 µg/L [Brodie *et al.* 1981]. Fluorescence detection. Limit of detection, 1 µg/L [Vose *et al.* 1981].

Urine **GC-MS** Metolazone and other diuretics. Limits of detection, 10 to 50 µg/L [Lisi *et al.* 1992].

HPLC Fluorescence detection [Farthing *et al.* 1990]. Limit of detection, 5 µg/L, see Plasma [Vose *et al.* 1981].

Disposition in the Body Metolazone is incompletely but fairly readily absorbed after oral administration. About 80% of a dose is excreted in the urine as unchanged drug in 48 h; about 10% is excreted in the bile.

Therapeutic Concentration

After a single oral dose of 2.5 mg given to 3 subjects, a mean peak plasma concentration of about 0.01 mg/L was attained in 5 h [Tilstone *et al.* 1974].

Half-life Plasma half-life, about 18 h.

Volume of Distribution About 1.6 L/kg.

Clearance Plasma clearance, about 1.4 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.84.

Protein Binding 95%.

Dose Usually 2.5 to 10 mg daily; maximum of 80 mg daily.

Brodie RR *et al.* (1981). Determination of the diuretic agent metolazone in plasma by high-performance liquid chromatography. *J Chromatogr* 226: 526–532.

Farthing D *et al.* (1990). Quantitation of metolazone in urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 534: 228–232.

Farthing D *et al.* (1994). Novel high-performance liquid chromatographic method using solid-phase on-line elution for determination of metolazone in plasma and whole blood. *J Chromatogr B Biomed Appl* 653: 171–176.

Lisi AM *et al.* (1992). Diuretic screening in human urine by gas chromatography-mass spectrometry: use of a macroreticular acrylic copolymer for the efficient removal of the coextracted phase-transfer reagent after derivatization by direct extractive alkylation. *J Chromatogr* 581(1): 57–63.

Tilstone WJ *et al.* (1974). Pharmacokinetics of metolazone in normal subjects and in patients with cardiac or renal failure. *Clin Pharmacol Ther* 16: 322–329.

Vose CW *et al.* (1981). Quantitation of metolazone in plasma and urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 222: 311–315.

Metomidate

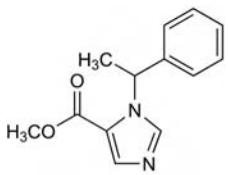
Sedative (Veterinary)

$C_{13}H_{14}N_2O_2 = 230.3$

CAS—5377-20-8

IUPAC Name Methyl 3-(1-phenylethyl)imidazole-4-carboxylate

Synonyms Methoxymol; methomidate; 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid methyl ester.



Chemical Properties Log *P* (octanol/water), 2.6.

Metomidate Hydrochloride

$C_{13}H_{14}N_2O_2$,HCl = 266.7

CAS—35944-74-2

Proprietary Name *Hypnodil*

Chemical Properties An off-white crystalline powder. Mp about 172°. Very soluble in water.

Colour Test Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.71; system TAE—R_f 0.77; system TAF—R_f 0.81; system TL—R_f 0.48; system TB—R_f 0.23; system TC—R_f 0.65; system TE—R_f 0.74 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 424.

Ultraviolet Spectrum Water—240 nm (A^1_1=408b).

Infrared Spectrum Principal peaks at wavenumbers 1730, 1230, 725, 770, 1288, 1200 cm^{-1} (metomidate hydrochloride, KBr disk).

Metopimazine

Antiemetic

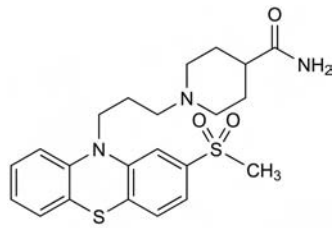
$C_{22}H_{27}N_3O_3S_2 = 445.6$

CAS—14008-44-7

IUPAC Name 1-[3-[2-(Methylsulfonyl)-10*H*-phenothiazin-10-yl]propyl]-4-piperidinecarboxamide

Synonyms Exp-999; RP-9965.

Proprietary Name *Vogalene*



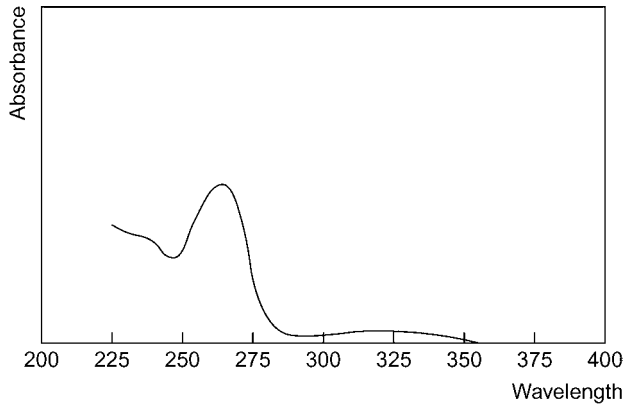
Chemical Properties A white crystalline powder. Mp 170° to 171°. Soluble in chloroform and dilute acetic acid. Log *P* (octanol/water), 2.4.

Colour Tests Formaldehyde-sulfuric acid—red; Forrest reagent—brown; FPN reagent—orange; Mandelin's test—green; Marquis test—red; sulfuric acid—red.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.00; system TC—R_f 0.11; system TL—R_f 0.12 (acidified iodoplatinate solution, positive).

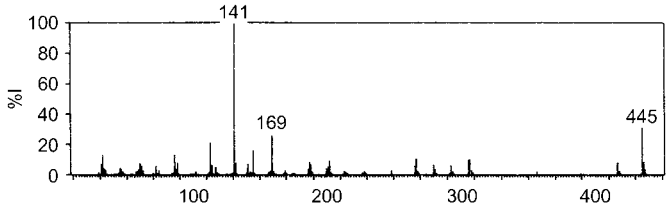
High Performance Liquid Chromatography System HA—*k* 1.4.

Ultraviolet Spectrum Aqueous acid—264 (A^1_1=922b), 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1150, 1310, 750, 1665, 1102, 1248 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 141, 445, 169, 123, 155, 96, 42, 317.



Quantification

Serum **HPLC** Fluorescence detection. Metopimazine and its acid metabolite [Angelo *et al.* 1989].

Disposition in the Body

Therapeutic Concentration

Administration of a single oral 20 or 50 mg dose of metopimazine before food in 6 subjects produced median peak plasma concentrations of 43 or 83 µg/L at 52 and 60 min, respectively. When the same doses were administered postprandially, the corresponding values were 22 or 51 µg/L, both at 90 min [Herrstedt *et al.* 1990].

Six subjects were given 40 mg of metopimazine either as a single microenema or as a single oral dose or were given 10 mg metopimazine as a 60 min continuous IV infusion. The median peak plasma concentration was 59 µg/L (range 28 to 182) 53 min (25 to 90) after oral administration, 128 µg/L (69 to 218) 10 min (10 to 10) after rectal administration and 108 µg/L (74 to 193) 60 min (45 to 60) after IV administration [Herrstedt *et al.* 1996].

Metopimazine 20, 30, 40, 50 or 60 mg was given orally every 4 h for 11 doses to 36 subjects. For the 50 mg dose, median peak plasma concentrations of 115, 108, 96, 183 and 109 µg/L were obtained at 1.5, 12, 24, 36 and 48 h after the first dose, respectively; the corresponding values for the 60 mg dose were 73, 52, 105, 96 and 70 µg/L. 6 doses of 30 mg were found to be safe [Herrstedt *et al.* 1997].

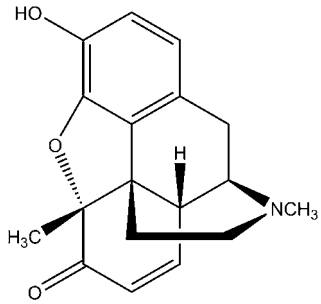
Dose 5 to 15 mg daily.

Angelo HR *et al.* (1989). High-performance liquid chromatographic method with fluorescence detection for the simultaneous determination of metopimazine and its acid metabolite in serum. *J Chromatogr* 496: 472–477.
Herrstedt J *et al.* (1990). The effect of food on serum concentrations of metopimazine. *Br J Clin Pharmacol* 30: 237–243.
Herrstedt J *et al.* (1996). Bioavailability of the antiemetic metopimazine given as a microenema. *Br J Clin Pharmacol* 41: 613–615.
Herrstedt J *et al.* (1997). Dose-finding study of oral metopimazine. *Support Care Cancer* 5: 38–43.

Metopon

Narcotic Analgesic
$C_{18}H_{21}NO_3 = 299.4$
CAS—143-52-2

Synonyms (5α)-4,5-Epoxy-3-hydroxy-5,17-dimethylmorphinan-6-one; 4,5-epoxy-3-hydroxy-*N*,5-dimethyl-6-oxomorphinan; methyldihydromorphinone.



Chemical Properties Needles from alcohol. Mp 243° to 245°. Slightly soluble in organic solvents. Metopon is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve yield.

Metopon Hydrochloride

$C_{18}H_{21}NO_3$,HCl = 335.8
CAS—124-92-5

Chemical Properties Crystals from alcohol. Mp 315° to 318°. Freely soluble in water, sparingly soluble in alcohol, slightly soluble in chloroform, very slightly soluble in ether, insoluble in benzene [Council on Pharmacy and Chemistry of the American Medical Association 1951; O'Neil *et al.* 2006].

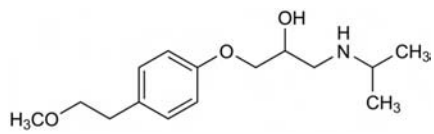
Colour Tests Ammonium molybdate test—blue-violet→blue→green (limit of detection, 0.1 µg); ammonium vanadate test—dull purple (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—purple (limit of detection, 0.5 µg); Vitali's test—yellow/yellow/orange (limit of detection, 0.25 µg).
Thin-layer Chromatography System T10—R_f 0.25 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.00 (relative to codeine).
Ultraviolet Spectrum Ethanol—285 nm; aqueous acid (0.1 N sulfuric acid)—280 nm with an inflexion at 225 nm.
Disposition in the Body
Toxicity Metopon is about 3 times as potent an analgesic as morphine after parenteral administration, and has a similar duration of action [Sargent, May 1970]. The estimated minimum lethal dose is 100 mg. In mice the LD_{50} (SC) is 25 mg/kg.
Dose Usually up to 6 mg.

Council on Pharmacy and Chemistry of the American Medical Association (1951). NEW and nonofficial remedies: metopon hydrochloride. *J Am Med Assoc* 145: 486.
O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.
Sargent LJ, May EL (1970). Agonists–antagonists derived from desomorphine and metopon. *J Med Chem* 13: 1061–1063.

Metoprolol

β-*Adrenoceptor Antagonist, Antianginal, Antiarrhythmic, Antihypertensive*
$C_{15}H_{25}NO_3 = 267.4$
CAS—37350-58-6

IUPAC Name (±)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol



Chemical Properties pK_a 9.7. Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005]. Plasma samples were stable for at least 8 h at ambient temperature (20–30°), at -20° for 1 month and in the autosampler at 10° for more than 24 h. Stable for at least 3 freeze-thaw cycles [Gowda *et al.* 2007].

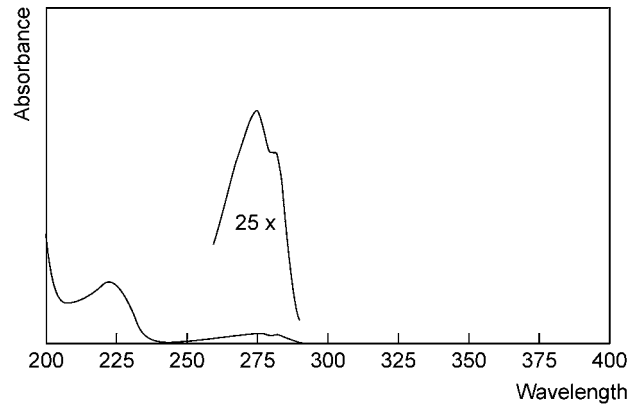
Metoprolol Succinate

$(C_{15}H_{25}NO_3)_2,C_4H_6O_4 = 652.8$
CAS—98418-47-4
Proprietary Names *Selo-Zok*; *Toprol-XL*.

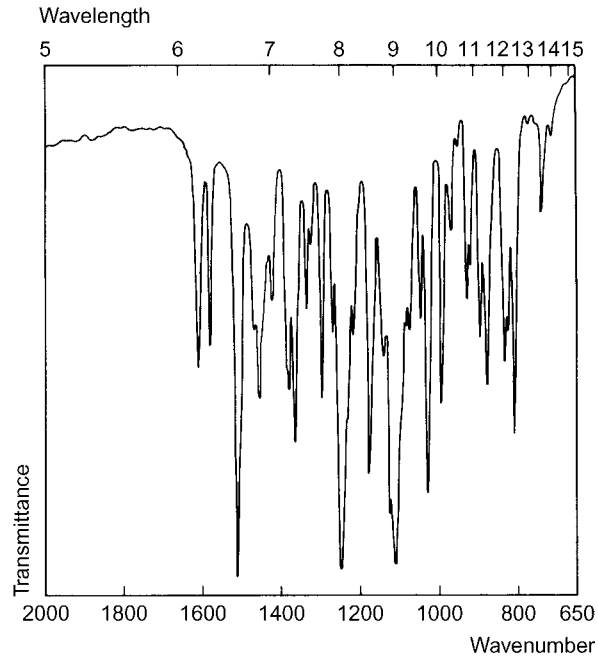
Metoprolol Tartrate

$(C_{15}H_{25}NO_3)_2,C_4H_6O_6 = 684.8$
CAS—56392-17-7
Synonyms CGP-2175E; H-93/26; metoprololi tartras.
Proprietary Names *Abralene*; *Azumetop*; *Beloc*; *Betaloc*; *Betazok*; *Jeprolol*; *Lanoc*; *Lopresor*; *Lopressor*; *Mepranix*; *Meprolol*; *Meto*; *Metocor*; *Metolol*; *Nu-Metop*; *Seloken*; *Toprol XL*. It is an ingredient of *Co-Betaloc*, *Logimax*, *Logroton*, *Lopresor HCT*.
Chemical Properties A white crystalline powder. Mp ~120°. Very soluble in water; soluble in ethanol and chloroform; practically insoluble in ether.

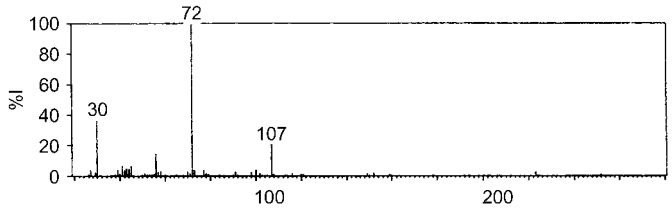
Colour Tests Liebermann's reagent—pink-brown; Mandelin's test—pink-brown; Marquis test—pink.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.10; system TC—R_f 0.08; system TE—R_f 0.44; system TL—R_f 0.09; system TAE—R_f 0.20; system TAF—R_f 0.74; system TAJ—R_f 0.02; system TAK—R_f 0.12; system TAL—R_f 0.70.
Gas Chromatography System GA—metoprolol RI 2035, M (OH-)(-AC_3) RI 2730, M (*O*-desmethyl-)(-AC_3) RI 2620; system GB—RI 2090.
High Performance Liquid Chromatography System HA—*k* 1.3; system HX—RI 326; system HY—RI 272; system HZ—RT 2.5 min; system HAA—RT 10.7 min; system HAX—RT 7.1 min; system HAY—RT 4.1 min.
Ultraviolet Spectrum Aqueous acid—274 (A^1_1= 52a), 280 nm (inflexion).



Infrared Spectrum Principal peaks at wavenumbers 1510, 1245, 1108, 1028, 1175, 810 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 30, 107, 56, 45, 41, 44, 43.



Quantification

Blood **GC-MS** Column: DB-1 (25 m × 0.2 mm i.d., 0.25 μm). Carrier gas: He, 0.6 mL/min. Temperature programme: 140° for 2.5 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.01 mg/L [Black *et al.* 1996].

Plasma **GC** Column: HP methyl silicone (12 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 60 mL/min. Temperature programme: 200° to 300° at 3°/min. FID. Limit of detection, 50 μg/L [Quaglio *et al.* 1993]. Column: OV7 phenyl methyl silicone with 20% phenyl silicone on HP Chrom W AW DMCS 100/120 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 190° for 3 min to 270° at 3°/min. FID. Limit of detection, 100 μg/L [Quaglio *et al.* 1992]. Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (0.5 m × 4 mm i.d.). Carrier gas: Ar, 230 mL/min. Temperature: 200°. ECD. Limit of detection, 3 μg/L [Kinney 1981]. Column; 3% OV-1 on Gas-Chrom Q 100/120 mesh (6 ft × 2 mm i.d.). Carrier gas: CH_4 : Ar (5 : 95). Temperature programme: 165° to 180°. ECD. Limit of detection, 6 μg/L [Quarterman *et al.* 1980].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. EI ionisation at 70 eV. Limit of quantification, 15 μg/L. Limit of detection, 5 μg/L [Yilmaz *et al.* 2009]. See GC. EI ionisation at 70 eV. Limit of detection, 10 μg/L [Quaglio *et al.* 1993]. Column: 3% OV-101 on Gas Chrom Q 120/140 mesh (2 m × 2 mm i.d.). Carrier gas: He, 15 mL/min. Temperature: 180°. EI ionisaton at 70 eV. Limit of detection, 300 μg/L [Ervik *et al.* 1981].

HPLC Column: MSpak GF-310 4B (50 × 4.6 mm i.d., 6 μm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 at 4 min for 5.5 min to 100 : 0 for 5.5 min), flow rate 0.55 mL/min. DAD (λ = 260 nm). Limit of quantification, 10 μg/L. Limit of detection, 1–3 μg/L [Umezawa *et al.* 2008]. Column: C4/E (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.02 mol/L phosphate buffer (pH 3.0) : acetonitrile: tetrahydrofuran (850 : 130 : 20) with 0.24 g 1-octane sulfonic acid, flow rate 1.9 mL/min. Fluorescence detection (λ_{ex} = 225 nm, λ_{em} = 310 nm). Limit of quantification, 1.0 μg/L [Mistry *et al.* 1998]. Column: Whatman silica (25 cm, 5 μm). Mobile phase: hexane : chloroform : methanol (85 : 14 : 1), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 220 nm, no emission filter). Limit of detection, 5 μg/L [Bhatti, Foster 1992]. Column: Chiralcel OD (250 × 4.6 mm i.d.). Mobile phase: 0.1% diethylamine, 200–1500 mg/L water, 4–10% of propan-1-ol and hexane, flow rate 0.25–1.75 mL/min. Limit of detection, 10 nmol/L [Balmer *et al.* 1991]. Column: Chiralcel OD (25 cm × 0.46 mm i.d.). Mobile phase: hexane : ethanol: *N,N*-diethylamine (95 : 5 : 0.1), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 220 nm, λ_{em} = 320 nm). Limit of detection, 4 μg/L [Herring *et al.* 1991].

See also Pautler, Jusko [1982].

LC-MS Column: Venusil MP-C_{18}.(100 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 10 mmol/L ammonium acetate : formic acid (pH 3.4, 50 : 50 : 0.05), flow rate of 0.8 mL/min. ESI, MRM acquisition mode. Limit of quantification, 3 μg/L [Gao *et al.* 2010]. Column: Chirobiotic T (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : acetic acid : ammonia (100/0.15/0.15). ESI, SRM acquisition mode. Limit of quantification, 0.5 μg/L [Jensen *et al.* 2008]. Column: C_8. (50 × 3 mm i.d., 3 μm). Mobile phase: 10 mmol/L ammonium formate : methanol (3 : 97), flow rate 1.0 mL/min. ESI, MRM acquisition mode. Limit of quantification, 5 μg/L, limit of detection, 1 μg/L [Gowda *et al.* 2007]. Column: CAPCELL PAK C_{18} (50 × 2.0 mm, 5 μm). Mobile phase: acetonitrile : water (5 : 95 to 50 : 50 over 3.5 min to 95: over 0.5 min to 5 : 95 over 0.5 min for 3 min), both containing 0.02% formic acid, flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3.2 min. Limit of quantification, 5 μg/L [Li *et al.* 2007].

Urine **GC** See Plasma [Kinney 1981]. See Plasma. Temperature: 185° [Quarterman *et al.* 1980].

GC-MS Column: HP-5 (14 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 30° for 2 min to 220° at 15°/min for 1 min to 260° at 5°/min to 320° at 15°/min for 3 min. Limit of detection, 30 μg/L [Hartonen, Riekkola 1996]. See Blood [Black *et al.* 1996]. See Plasma [Ervik *et al.* 1981].

HPLC Column: LiChroCART RP-18 (250 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile: methanol : 0.05% trifluoroacetic acid in water. DAD (λ = 200–450 nm). Limit of quantification, 0.24 mg/L, limit of detection, 0.08 mg/L [Baranowska *et al.* 2009]. Column: Reversed-phase LiChroCART Purospher C_{18}e (125 mm × 3 mm, 5 μm). Spectrophotometric and fluorometric detection [Baranowska, Wilczek 2009]. Column: Chiracel OD (250 × 4.6 mm i.d.). Mobile phase: *n*-hexane : ethanol : propan-2-ol : diethylamine (85 : 7.5 : 7.5 : 0.05), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 276 nm, λ_{em} = 309 nm). Limit of quantification, 25 μg/L [Kim *et al.* 2000]. Column: Reversed-phase Nova-Pak C_{18} (100 × 5 mm i.d., 4 μm). Mobile phase: acetonitrile : methanol : TEA : phosphate buffer (pH 3.0; 9 : 1 : 90), flow rate 1.4 mL/min. Fluorescence detection (λ_{ex} = 229 nm, λ_{em} = 309 nm). Limit of detection, 1 μg/L [Chiu *et al.* 1997]. Column: Bondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile : water (40 : 60), 5 μmol/L potassium dihydrogen phosphate/dipotassium hydrogen phosphate (pH 6.5), flow rate 1.3 mL/min. Electrochemical detection. Limit of quantification, 400 ppb [Maguregui *et al.* 1995]. See Plasma [Balmer *et al.* 1991]. See also Li *et al.* [1995] and Xie, Zhou [1995].

Note For a review of HPLC methods for enantioselective analysis of metoprolol and other β-blockers, see Egginger *et al.* [1993].

Disposition in the Body Metoprolol is well absorbed after oral administration; although the bioavailibity is low because of extensive first-pass metabolism. It crosses the blood–brain barrier and placental barrier; it is also excreted into breast milk. Approximately 95% of a dose is excreted in the urine within 48 h, with ~65% of the dose as the inactive metabolite, 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetic acid, and ~10% as a further inactive metabolite, 2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propionic acid. Two active metabolites, α-hydroxymetoprolol and *O*-desmethylmetoprolol, are also excreted in the urine in amounts equivalent to ~10% and <1% of the dose, respectively. Up to ~10% of a dose is excreted as unchanged drug.

Therapeutic Concentration

Following a single oral dose of 100 mg, given to 6 subjects, peak plasma concentrations of 0.03–0.28 mg/L (mean, 0.13) were attained in ~2.5 h [Kirch *et al.* 1983].

Following a single oral dose of 100 mg to 8 subjects, a mean peak plasma concentration of metoprolol of 0.12 mg/L was attained in 2–3 h; the α-hydroxy metabolite reached a mean peak of 0.07 mg/L in ~3 h. After repeated administration of 100 mg every 12 h, the mean peak plasma concentrations on the 8th day were 0.19 mg/L for metoprolol and 0.07 mg/for the α-hydroxy metabolite. The concentration of the metabolite appears to be increased in elderly subjects [Quarterman *et al.* 1981].

In 20 subjects receiving 100 mg metoprolol tartate twice daily for a total of 9 doses by mouth, the reported peak plasma concentration of (*S*)-metoprolol was 0.0855 mg/L in men and 0.146 mg/L in women, and that of (*R*)-metoprolol was 0.0672 mg/L in men and 0.124 mg/L in women; peak concentrations occurred at 1.3 h in men and 1.6 h in women [Luzier *et al.* 1999].

Toxicity

In a fatal overdose of metoprolol, the following postmortem tissue concentrations of the active (*S*)-isomer were reported: blood, 33 mg/L, liver 224 μg/g and stomach contents 56 mg/61 g. Concentrations of the (*R*)-isomer were blood 33 mg/L, liver 222 μg/g and stomach contents 55 mg/61 g [Mozayani *et al.* 1995].

The following postmortem concentrations were reported in a death from an intentional overdose of metoptolol: blood 19.8 mg/L, bile 83.1 mg/L, urine 1.6 mg/L and vitreous humour 15.1 mg/L; a blood ethanol concentration of 0.25 g/100 mL was also found [Rohrig *et al.* 1987].

In a fatality caused by the deliberate ingestion of metoprolol, the following postmortem tissue concentrations were reported: blood 4.7 mg/L, bile 254 mg/L, kidney 7.1 μg/g, liver 6.3 μg/g and urine 194 mg/L [Stajic *et al.* 1984].

In a fatality caused by metoprolol and salicylate, the following postmortem tissue concentrations were reported: metoprolol in blood 56 mg/L, bile 276 mg/L and liver 230 μg/g; salicylate in blood 220 mg/L [Holzbecher *et al.* 1982].

Bioavailability Approximately 40–50% (~70% after modified-release preparations).

Half-life Plasma half-life, metoprolol 3–4 h in fast hydroxylators and ~7 h in slow hydroxylators; α-hydroxymetoprolol 4 to 12 h (mean, 6).

Volume of Distribution ~4 L/kg.

Clearance Plasma clearance, ~15 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.77.

Protein Binding ~11%.

Note For a review of the clinical pharmacokinetics of metoprolol, see Regardh, Johnsson [1980] and for a review of controlled-release metoprolol, see Kendall *et al.* [1991].

Dose In hypertension 100 to 400 mg of metoprolol tartrate daily.

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Baranowska I *et al.* (2009). Development and validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples. *Anal Sci* 25: 1307–1313.

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Chiu FC *et al.* (1997). Efficient high-performance liquid chromatographic assay for the simultaneous determination of metoprolol and two main metabolites in human urine by solid-phase extraction and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 696: 69–74.

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Egginger G *et al.* (1993). Enantioselective bioanalysis of beta-blocking agents: focus on atenolol, betaxolol, carvedilol, metoprolol, pindolol, propranolol and sotalol. *Biomed Chromatogr* 7: 277–295.

Ervik M *et al.* (1981). Selected ion monitoring of metoprolol and two metabolites in plasma and urine using deuterated internal standards. *Biomed Mass Spectrom* 8: 322–326.

Gao F *et al.* (2010). Simultaneous quantitation of hydrochlorothiazide and metoprolol in human plasma by liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 52: 149–154.
Gowda KV *et al.* (2007). Liquid chromatography tandem mass spectrometry method for simultaneous determination of metoprolol tartrate and ramipril in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 858: 13–21.
Hartonen K, Riekkola ML (1996). Detection of beta-blockers in urine by solid-phase extraction–supercritical fluid extraction and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 676: 45–52.
Herring VL *et al.* (1991). Solid-phase extraction and direct high-performance liquid chromatographic determination of metoprolol enantiomers in plasma. *J Chromatogr* 567: 221–227.
Holzbecher M *et al.* (1982). Report of a metoprolol-associated death. *J Forensic Sci* 27: 715–717.
Jensen BP *et al.* (2008). Development and validation of a stereoselective liquid chromatography–tandem mass spectrometry assay for quantification of *S*- and *R*-metoprolol in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 865: 48–54.
Kendall MJ *et al.* (1991). Controlled release metoprolol. *Clinical pharmacokinetic and therapeutic implications, Clin Pharmacokinet* 21: 319–330.
Kim KH *et al.* (2000). Determination of metoprolol enantiomers in human urine by coupled achiral–chiral chromatography. *J Pharm Biomed Anal* 22: 377–384.
Kinney CD (1981). Determination of metoprolol in plasma and urine by gas–liquid chromatography with electron-capture detection. *J Chromatogr* 225: 213–218.
Kirch W *et al.* (1983). Influence of inflammatory disease on the clinical pharmacokinetics of atenolol and metoprolol. *Biopharm Drug Dispos* 4: 73–81.
Li F *et al.* (1995). Determination of the enantiomers of metoprolol and its major acidic metabolite in human urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Appl* 668: 67–75.
Li S *et al.* (2007). Simultaneous determination of ten antiarrhythic drugs and a metabolite in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 847: 174–181.
Luzier AB *et al.* (1999). Gender-related effects on metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. *Clin Pharmacol Ther* 66: 594–601.
Maguregui MI *et al.* (1995). High-performance liquid chromatography with amperometric detection applied to the screening of beta-blockers in human urine. *J Chromatogr B Biomed Appl* 674: 85–91.
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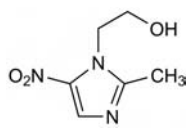
Mozayani A *et al.* (1995). Distribution of metoprolol enantiomers in a fatal overdose. *J Anal Toxicol* 19: 519–521.
Pautler DB, Jusko WJ (1982). Determination of metoprolol and alpha-hydroxymetoprolol in plasma by high-performance liquid chromatography. *J Chromatogr* 228: 215–222.
Quaglio MP *et al.* (1992). Simultaneous determination of propranolol or metoprolol in the presence of benzodiazepines in the plasma by gas chromatography. *Farmaco* 47: 799–809.
Quaglio MP *et al.* (1993). Simultaneous determination of propranolol or metoprolol in the presence of butyrophenones in human plasma by gas chromatography with mass spectrometry. *J Pharm Sci* 82: 87–90.
Quarterman CP *et al.* (1980). Determination of metoprolol metabolites in plasma and urine by electron-capture gas–liquid chromatography. *J Chromatogr* 183: 92–98.
Quarterman CP *et al.* (1981). The effect of age on the pharmacokinetics of metoprolol and its metabolites. *Br J Clin Pharmacol* 11: 287–294.
Regardh CG, Johnsson G (1980). Clinical pharmacokinetics of metoprolol. *Clin Pharmacokinet* 5: 557–569.
Rohrig TP *et al.* (1987). Fatality resulting from metoprolol overdose. *J Anal Toxicol* 11: 231–232.
Stajic M *et al.* (1984). Fatal metoprolol overdose. *J Anal Toxicol* 8: 228–230.
Umezawa H *et al.* (2008). Simultaneous determination of beta-blockers in human plasma using liquid chromatography–tandem mass spectrometry. *Biomed Chromatogr* 22: 702–711.
Xie HG, Zhou HH (1995). Assay of metoprolol and alpha-hydroxymetoprolol in human urine by reversed-phase liquid chromatography with direct-injection. *Zhongguo Yao Li Xue Bao* 16: 32–35.
Yilmaz B *et al.* (2009). Gas chromatography–mass spectrometry method for determination of metoprolol in the patients with hypertension. *Talanta* 80: 346–351.

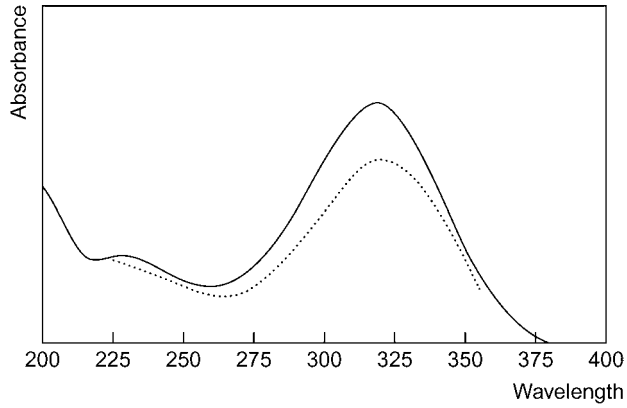
Metronidazole

Antiprotozoal, Antibacterial
$C_6H_9N_3O_3$ = 171.2
CAS—443-48-1

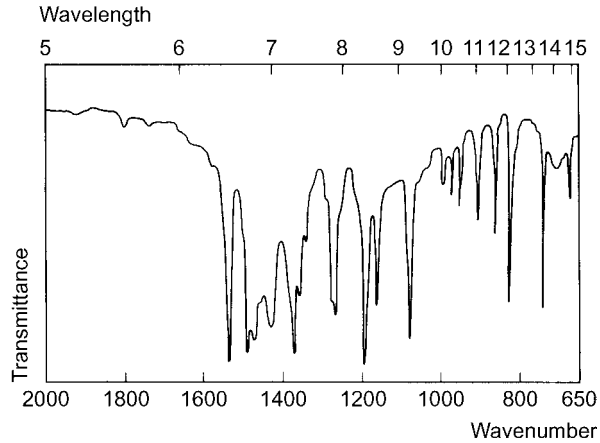
IUPAC Name 2-Methyl-5-nitroimidazole-1-ethanol
Synonyms Bayer 5630; RP-8823.
Proprietary Names *Anabact*; *Elyzol*; *Flagyl*; *Metizol*; *Metro*; *Metrocream*; *Metrogel*; *Metrogyl*; *Metrolyl*; *Metrotop*; *Metrozol(e)*; *Metryl*; *Norit(r)ate*; *Norzol*; *Protostat*; *Rozex*; *Trichozole*; *Vaginyl*; *Zadstat*; *Zidoval*; *Zyomet*. It is an ingredient of *Entamizole*, *Helidac* and *HeliMet*.



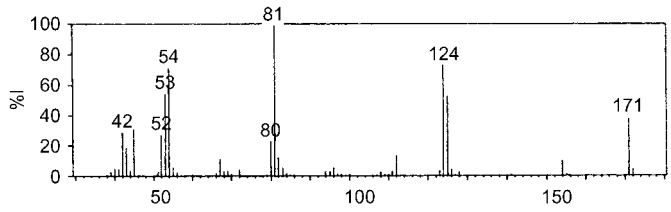
Chemical Properties A white to pale yellow crystalline powder or crystals. It darkens on exposure to light. Mp 158° to 160°. Soluble 1 in 100 of water, 1 in 200 of ethanol and 1 in 250 of chloroform; soluble in dilute acids; slightly soluble in ether. pK_a 2.5. Log *P* (octanol/buffer pH 7.4), −0.1.
Colour Test Methanolic potassium hydroxide—red.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.02; system TC—R_f 0.36; system TE—R_f 0.46; system TL—R_f 0.40; system TAD—R_f 0.32; system TAE—R_f 0.75; system TAF—R_f 0.70; system TAJ—R_f 0.32; system TAK—R_f 0.14; system TAL—R_f 0.75 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1592.
High Performance Liquid Chromatography System HX—RI 257; system HY—RI 226; system HAA—retention time 6.8 min.
Ultraviolet Spectrum Aqueous acid—277 nm (A^1_1=377a); aqueous alkali—319 nm (A^1_1=520b).



Infrared Spectrum Principal peaks at wavenumbers 1187, 1535, 1070, 1265, 745, 1160 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 81, 124, 54, 53, 125, 171, 45, 42.



Quantification

Blood **HPLC** UV detection. Metronidazole and metabolites [Kaye *et al.* 1980].
Plasma **HPLC** DAD. Limit of detection, 1 mg/L for metronidazole and amoxicillin [Menelaou *et al.* 1999]. UV detection. Limit of detection, 0.1 mg/L for metronidazole and omeprazole [Yeung *et al.* 1998]. Limit of detection, 0.25 mg/L for metronidazole, 0.2 mg/L for its hydroxy metabolite [Jessa *et al.* 1996]. UV detection. Metronidazole and chloroquine [Okonkwo, Eta 1988]. UV detection. Limit of detection, 25 µg/L [Gibson *et al.* 1984]. See Blood [Kaye *et al.* 1980].
Serum **HPLC** UV detection. Limit of detection, 10 µg/L [Galmier *et al.* 1998]. See Blood [Kaye *et al.* 1980].
Urine **HPLC** See Plasma [Okonkwo, Eta 1988]. See Blood [Kaye *et al.* 1980].
Gastric Contents **HPLC** See Plasma [Yeung *et al.* 1998]; [Jessa *et al.* 1996].
Saliva **HPLC** See Plasma [Jessa *et al.* 1996]. See Blood [Kaye *et al.* 1980].

Vaginal Tissue **HPLC** UV detection. Limit of detection, ~100 ng/g [Venkateshwaran, Stewart 1995].

Disposition in the Body Metronidazole is readily and almost completely absorbed after oral administration and is widely distributed in the body. It is metabolised by oxidation to 2-hydroxymethylmetronidazole and 2-methyl-5-nitroimidazol-1-acetic acid, and by conjugation with glucuronic acid. About 70–80% of a dose is excreted in the urine in 48 h with <10% of the dose as unchanged drug, up to 10% as conjugated metronidazole, about 27% as 2-hydroxymethylmetronidazole, 10% as the conjugated 2-hydroxymethyl metabolite, and 20% as the acid metabolite.

Therapeutic Concentration

Following a single oral dose of 400 mg to 7 subjects, peak plasma concentrations of 4.5–11.6 (mean 6.9) mg/L were attained in about 2 h; a mean peak plasma concentration of 1.6 mg/L of 2-hydroxymethylmetronidazole was attained in about 8 h [Jensen, Gugler 1983].

Fifty-four subjects given IV metronidazole, 500 mg every 8 h, had mean peak and trough plasma concentrations of 28.9 and 18.0 mg/L, respectively. Mean peak and trough concentrations of the hydroxy metabolite were 6.6 and 4.2 mg/L [Ti *et al.* 1996].

Toxicity

In a fatality attributed to an alcohol/metronidazole interaction, blood levels of 1620 mg/L alcohol, 46 mg/L acetaldehyde and 0.42 mg/L metronidazole were found [Cina *et al.* 1996].

Half-life Plasma half-life, about 8 h.

Volume of Distribution About 0.5–1 L/kg.

Clearance Plasma clearance, about 1 mL/min/kg.

Protein Binding <20%.

Note For a review of the pharmacokinetics of metronidazole, see Ralph [1983].

Dose Up to 2.4 g daily for 10 days.

Cina SJ *et al.* (1996). Sudden death due to metronidazole/ethanol interaction. *Am J Forensic Med Pathol* 17: 343–346.

Galmier MJ *et al.* (1998). Simple and sensitive method for determination of metronidazole in human serum by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 720: 239–243.

Gibson RA *et al.* (1984). Optimized liquid-chromatographic determination of metronidazole and its metabolites in plasma. *Clin Chem* 30: 784–787.

Jensen JC, Gugler R (1983). Single- and multiple-dose metronidazole kinetics. *Clin Pharmacol Ther* 34: 481–487.

Jessa MJ *et al.* (1996). Rapid and selective high-performance liquid chromatographic method for the determination of metronidazole and its active metabolite in human plasma, saliva and gastric juice. *J Chromatogr B Biomed Appl* 677: 374–379.

Kaye CM *et al.* (1980). A rapid and specific semi-micro method involving high-pressure liquid chromatography for the assay of metronidazole in plasma, saliva, serum, urine and whole blood. *Br J Clin Pharmacol* 9: 528–529.

Menelaou A *et al.* (1999). Simultaneous quantification of amoxycillin and metronidazole in plasma using high-performance liquid chromatography with photodiode array detection. *J Chromatogr B Biomed Sci Appl* 731: 261–266.

Okonkwo PO, Eta EI (1988). Simultaneous determination of chloroquine and metronidazole in human biological fluid by high pressure liquid chromatography. *Life Sci* 42: 539–545.

Ralph ED (1983). Clinical pharmacokinetics of metronidazole. *Clin Pharmacokinet* 8: 43–62.

Ti TY *et al.* (1996). Disposition of intravenous metronidazole in Asian surgical patients. *Antimicrob Agents Chemother* 40: 2248–2251.

Venkateshwaran TG, Stewart JT (1995). Determination of metronidazole in vaginal tissue by high-performance liquid chromatography using solid-phase extraction. *J Chromatogr* 672: 300–304.

Yeung PK *et al.* (1998). A simple high performance liquid chromatography assay for simultaneous determination of omeprazole and metronidazole in human plasma and gastric fluid. *J Pharm Biomed Anal* 17: 1393–1398.

Metyrapone

Diagnostic Agent (Pituitary Function)

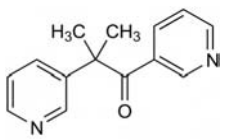
$C_{14}H_{14}N_2O$ = 226.3

CAS—54-36-4

IUPAC Name 2-Methyl-1,2-di-3-pyridyl-1-propanone

Synonym Methopyrapone; Su-4885.

Proprietary Name *Metopiron(e)*



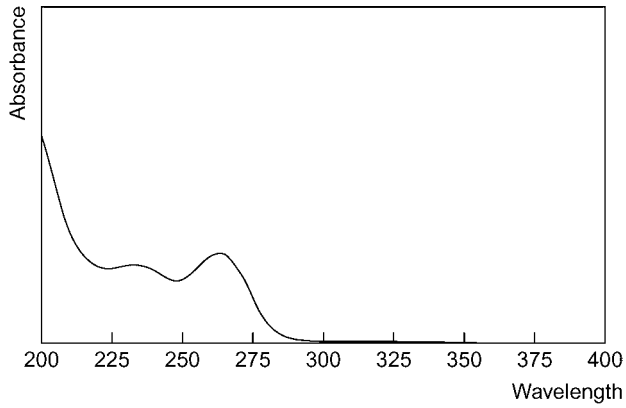
Chemical Properties A white to light amber, fine, crystalline powder which darkens on exposure to light. Mp 50° to 53°. Soluble 1 in 100 of water, 1 in 3 of ethanol and chloroform; soluble in methanol. Log *P* (octanol/water), 1.9.

Colour Test Cyanogen bromide—orange.

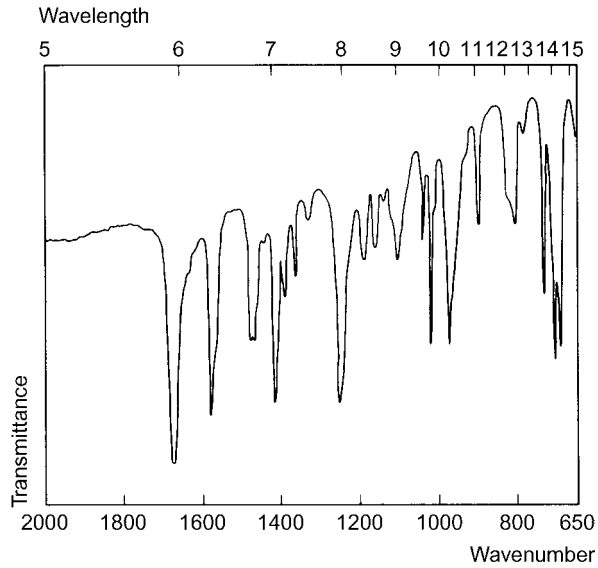
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.16; system TC—R_f 0.58; system TE—R_f 0.64; system TL—R_f 0.41; system TAE—R_f 0.66 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1860.

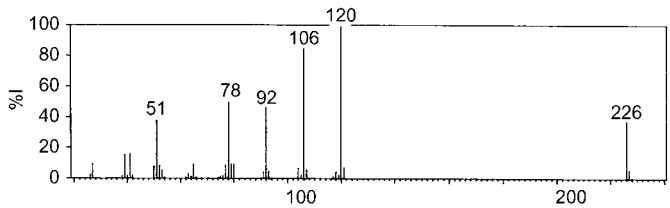
Ultraviolet Spectrum Aqueous acid—260 (A^{1}_{1}=500a), 233 nm.



Infrared Spectrum Principal peaks at wavenumbers 1672, 1578, 1255, 714, 699, 970 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 120, 106, 78, 92, 51, 226, 41, 39.



Quantification

Plasma **Colorimetry** Limit of detection, 5 mg/L for metyrapone, 2 mg/L for 1-hydroxymetyrapone [Szeberenyi *et al.* 1969].

Spectrofluorimetry [Meikle *et al.* 1969].

HPLC UV detection. Metyrapone and its enantiomeric metyrapol metabolites. Limit of detection, 0.045 mg/L [Cassiano *et al.* 2002].

Urine **GC** FID. Metyrapone and 1-hydroxymetyrapone [Hannah, Sprunt 1969].

Tissues **Colorimetry** See Plasma [Szeberenyi *et al.* 1969].

Disposition in the Body Metyrapone is metabolised by reduction to the 1-hydroxy derivative which is active. It is excreted in the urine mainly as the glucuronide conjugates of 1-hydroxymetyrapone and metyrapone. About 50% of a dose is excreted in the urine in 3 days.

Dose Up to 4.5 g daily.

Cassiano NM *et al.* (2002). Determination of the plasma levels of metyrapone and its enantiomeric metyrapol metabolites by direct plasma injection and multidimensional achiral-chiral chromatography. *Chirality* 14: 731–735.

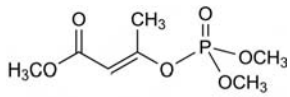
Hannah DM, Sprunt JG (1969). The quantitation of metyrapone and its reduced derivative in urine. *J Pharm Pharmacol* 21: 877–878.

Meikle AW *et al.* (1969). A simple fluorometric method for assay of plasma metyrapone. *J Lab Clin Med* 74: 515–520.
Szeberenyi S *et al.* (1969). A new method for the determination of metyrapone in plasma and tissues. *J Chromatogr* 40: 417–421.

Mevinphos

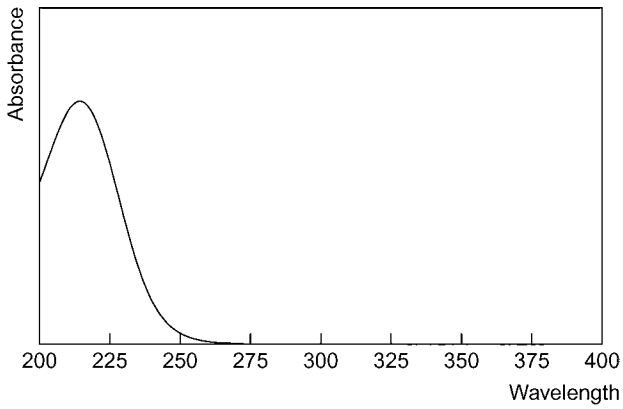
Insecticide
$C_7H_{13}O_6P$ = 224.1
CAS—7786-34-7

IUPAC Name Methyl (*E*)-3-dimethoxyphosphoryloxybut-2-enoate
Synonym 3-[(Dimethoxyphosphinyl)oxy]-2-butenoic acid methyl ester
Proprietary Name *Phosdrin*

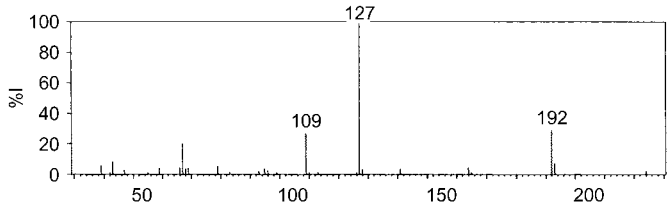


(*E*)- isomer

Chemical Properties The technical product is a pale yellow to orange liquid. Miscible with water, ethanol, acetone, benzene, carbon tetrachloride, toluene, xylene and chloroform; soluble 1 in 20 of carbon disulfide; practically insoluble in hexane. Log *P* (octanol/water), 0.1. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].
Colour Test Phosphorus test—yellow.
Thin-layer Chromatography System TE—R_f 0.75; system TW—R_f 0.23; system TX—R_f 0.12; system TY—R_f 0.10; system TAB—R_f 0.03; system TAC—R_f 0.02; system TAE—R_f 0.86.
Gas Chromatography System GA—RI 1450; system GK—RRT 0.50(relative to caffeine).
High Performance Liquid Chromatography System HAO—*k* 1.37; system HAP—*k* 0.45.
Ultraviolet Spectrum Principal peak at 215 nm.



Mass Spectrum Principal ions at *m/z* 127, 192, 109, 67, 43, 193, 39, 79.



Quantification
Blood **GC-MS** Mevinphos and other organophosphorus pesticides. Limits of detection, 0.01 to 0.3 µg/g [Musshoff *et al.* 2002].
Fluids **GC** AFID [Lewin, Love 1974].
Postmortem Tissues **GC** See Fluids [Lewin, Love 1974].

Disposition in the Body
Toxicity Mevinphos is extremely toxic by inhalation, ingestion or percutaneous absorption and the estimated lethal dose is <500 mg. The maximum permissible atmospheric concentration is 0.1 mg/m^3.

The following postmortem tissue concentrations were reported in a fatality due to the ingestion of mevinphos: blood 360 mg/L, brain 3 µg/g, kidney 20 µg/g, liver 240 µg/g, skeletal muscle 86 µg/g, urine 8 mg/L; alcohol was detected in blood and urine at concentrations of 1250 mg/L and 1550 mg/L, respectively; death occurred within 45 min of ingestion [Lewin, Love 1974].

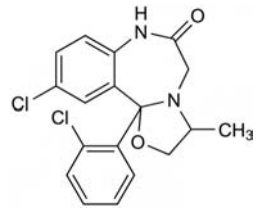
A 66-year-old man died about 1 min after ingesting 28 g of mevinphos [Lokan, James 1983].

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Lewin JF, Love JL (1974). A death caused by the ingestion of mevinphos. *Forensic Sci* 4: 253–255.
Lokan R, James R (1983). Rapid death by mevinphos poisoning while under observation. *Forensic Sci Int* 22: 179–182.
Musshoff F *et al.* (2002). Simple determination of 22 organophosphorous pesticides in human blood using headspace solid-phase microextraction and gas chromatography with mass spectrometric detection. *J Chromatogr Sci* 40: 29–34.

Mexazolam

Anxiolytic
$C_{18}H_{16}Cl_2N_2O_2$ = 363.2
CAS—31868-18-5

IUPAC Name 10-Chloro-11b-(2-chlorophenyl)-2,3,7,11b-tetrahydro-3-methyloxazolo-[3,2-d][1,4]-benzodiazepin-6(5*H*)-one
Synonyms CS-386; methylcloxazolam.
Proprietary Name *Melex*

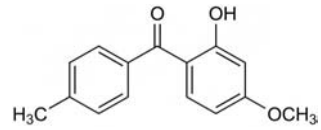


Chemical Properties Crystalline powder. Mp 172° to 175°. pK_a 6.7.
Thin-layer Chromatography System TE—R_f 0.78; system TF—R_f 0.55; system TAE—R_f 0.86.
Gas Chromatography System GA—RI 2600.
High Performance Liquid Chromatography System HX—RI 345.
Ultraviolet Spectrum Aqueous acid—241, 290, 373 nm; ethanol—244 nm; aqueous alkali—244 nm.
Infrared Spectrum Principal peaks at wavenumbers 1661, 1400, 1204, 1470, 1300, 1078 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 251, 70, 253, 28, 41, 42, 139, 18.

Mexenone

Sunscreen Agent
$C_{15}H_{14}O_3$ = 242.3
CAS—1641-17-4

IUPAC Name (2-Hydroxy-4-methoxyphenyl)(4-methylphenyl)methanone
Synonym Benzophenone-10
Proprietary Name It is an ingredient of *Uvistat*.



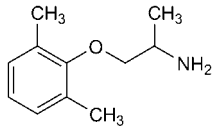
Chemical Properties A pale yellow crystalline powder. Mp 99° to 102°. Practically insoluble in water; soluble 1 in 70 of ethanol and 1 in 7 of acetone. Log *P* (octanol/water), 4.1.
Ultraviolet Spectrum Methanol—243 (A^1_1=353b), 287 (A^1_1=640a), 325 nm (A^1_1=440b).
Infrared Spectrum Principal peaks at wavenumbers 1260, 1598, 1637, 1211, 1613, 920 cm^{-1} (KBr disk).
Use Topically in a concentration of 4% in a cream or a solid basis.

Mexiletine

Antiarrhythmic
$C_{11}H_{17}NO$=179.3
CAS—31828-71-4

IUPAC Name 1-(2,6-Dimethylphenoxy)propan-2-amine

Synonym 1-(2,6-Dimethylphenoxy)-2-propanamine

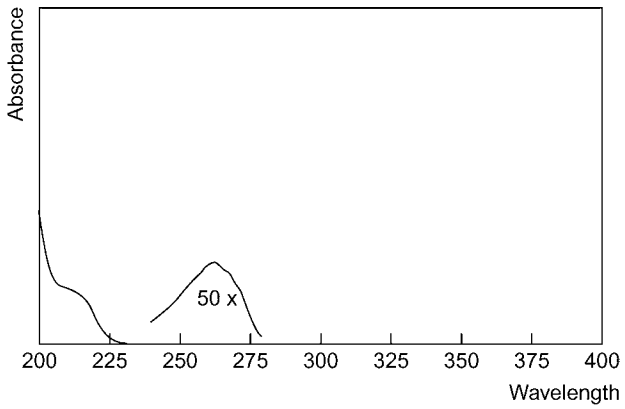


Chemical Properties Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

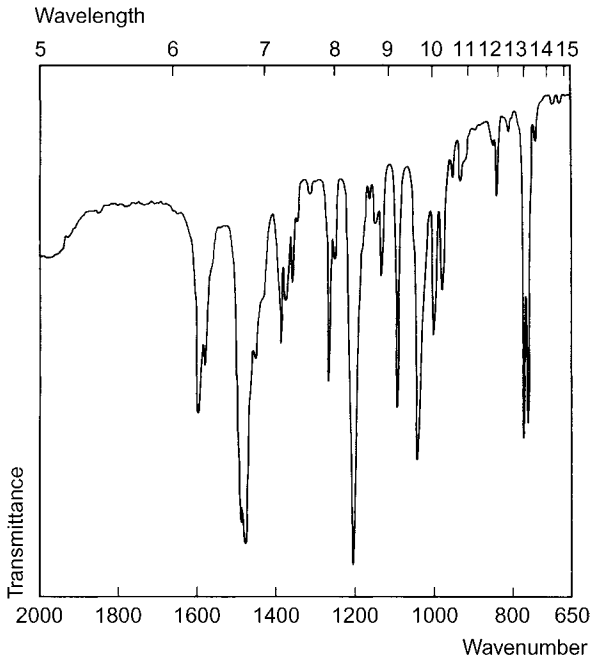
Mexiletine Hydrochloride

$C_{11}H_{17}NO,HCl$=215.7
CAS—5370-01-4
Proprietary Name *Mexitil*
Chemical Properties A white crystalline powder. Mp 198° to 204°. Soluble 1 in 2 of water, 1 in 3 of ethanol and 1 in 30 of chloroform; practically insoluble in ether. pK_a 9.06 [Katagiri *et al.* 1991], 8.5 [Labbé, Turgeon 1999], 8.4 [Kelly *et al.* 1981]. Log *P* (octanol/water), 2.2. Stock solutions in methanol were stable at –2 0° for 4 weeks. Plasma samples were stable at 4° for 24 h, at room temperature for 24 h, and after 3 freeze-thaw cycles [Li *et al.* 2007]. Serum samples were stable for 10 months at –2 5° [Paczkowski *et al.* 1992].

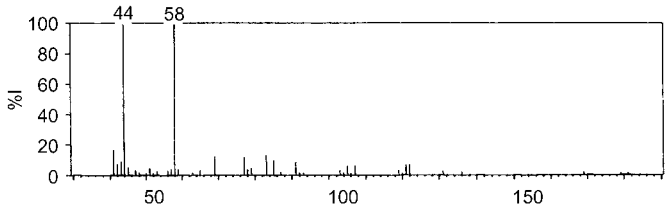
Colour Tests Liebermann's reagent—brown; Mandelin's test—brown-orange; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.17; system TC—R_f 0.04; system TE—R_f 0.55; system TAE—R_f 0.25; system TAF—R_f 0.78; system TAG—R_f 0.09.
Gas Chromatography System GA—mexiletene RI 1400, M (desamino-oxo-) RI 1350; system GB—mexiletene RI 1431, M (desamino-oxo-) RI 1395.
High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 329; system HY—RI 278; system HAA—RT 11.5 min.
Ultraviolet Spectrum Aqueous acid—260 nm (A_1^1 = 14a).



Infrared Spectrum Principal peaks at wavenumbers 1200, 1040, 770, 760, 1595, 1090 cm^{-1} (mexiletine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 44, 41, 83, 77, 69, 85, 43.



Quantification

Blood **GC** Column: HP-5 cross-linked 5% phenylmethyl silicone fused silica (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 200° at 30°/min to 260° at 10°/min to 300° at 20°/min for 8 min. Limit of detection not reported [Kempton *et al.* 1994]. Column: Restek RTX-50 (15 m). Temperature programme: 100° to 300° at 20°/min. FID. Limit of detection, 3.4 mg/L [Rohrig, Harty 1994]. Column: Spherisorb S5W silica (125 × 5 mm i.d.). Mobile phase: methanol : 2,2,4-trimethylpentane (80 : 20) containing 1 mmol/L (+)-10-camphorsulfonic acid, flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 200 nm). Retention time: 0.61. Limit of detection, 50 µg/L [Bhamra *et al.* 1984]. Column: 1.5% Carbowax 20M and 5% potassium hydroxide on Supelcoport 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 130°. AFID. Limit of detection, 5 µg/L [Smith, Meffin 1980].

Plasma **GC** Column: 1.5% OV-1 on 80/100 mesh Shimalite W 201D (1.5 m × 3 mm i.d). Carrier gas: N_2, 40 mL/min. Temperature: 180°. FID. Limit of detection, 20 ng/mL [Ji *et al.* 1993]. Column: 3% OV-17 on 80/100 mesh Gas-Chrom Q (200 × 2.5 mm i.d.). Carrier gas: Ar : CH_4 (90 : 10), 25 mL/min. Temperature: 150°. ECD. Limit of detection, 20 µg/L for mexiletine, 7 µg/L for 2′-hydroxymethyl metabolite[Pachecus *et al.* 1982]. Column: 3% OV-17 on Gas Chrom Q 80/100 mesh (1.68 m × 4 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 160°. NPD. Retention time: 4.0 min. Limit of detection, 5.0 µg/L [Pilling *et al.* 1982]. Column: 3% OV-17 on Gas Chrom Q 80/100 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 150°. FID. Limit of detection, 50 µg/L [Chan *et al.* 1980]. See Blood [Smith, Meffin 1980]. See also Bradbrook *et al.* [1977], Frydman *et al.* [1978], Holt *et al.* [1979], Kelly *et al.* [1973], Willox, Singh [1976].

GC-MS Column: Supelco SPB-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 30 mL/min. Temperature programme: 100° for 0.5 min to 280° at 25°/min for 2.0 min. SIM acquisition mode. Retention time: 6.3 min. Limit of quantification, 0.1 µg/L [Minnigh *et al.* 1994]. Column: 5% Carbowax 20 M/5% KOH on Chromosorb WHP 100/120 mesh (2 m × 2 mm i.d.). Temperature: 155°. FID. Retention time: 4.3 min. Limit of detection, 0.03 mg/L [Kacprowicz 1982].

HPLC Column: Lichrospher RP-18 (125 × 4.0 mm i.d., 5 µm). Mobile phase: propan-2-ol : acetonitrile: 0.05 N acetate buffer (pH 5.5; 22 : 10 : 68 or 25 : 10 : 65), flow rate 1 or 1.5 mL/min. Fluorescence detection (λ_{ex} = 350 nm, λ_{em} = 455 nm). Retention time: 17.7 and 19.2 for (*S*)- and (*R*)-enantiomers, respectively. Limit of quantification, 10 µg/L for *p*-hydroxymexiletine enantiomers [Lanchote *et al.* 1997]. Column: Chiralcel OJ (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : ethanol (7.1 : 2.9), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 340 nm). Limit of detection, 1 µg/L [Lanchote *et al.* 1996]. Column: Chiralpak AD (250 × 4.6 mm i.d.). Mobile phase: hexane : ethanol (94 : 6 for 15 min to 75 : 25 at 15.1 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 350 nm, λ_{em} = 444 nm). Limit of detection not reported [Fieger, Wainer 1993]. Column: Apex C_{18} ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L sodium acetate (pH 7.3; 65 : 35), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 350 nm, λ_{em} = 445 nm). Limit of detection, 1.5 µg/L [Abolfathi *et al.* 1992]. Column: Spherisorb hexyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium dihydrogen phosphate (pH 4.0; 40 : 60), flow rate 1.5mL/min. UV detection (λ = 210 nm). Limit of quantification, 0.05 mg/L [Verbesselt *et al.* 1991]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : acetonitrile : water (50 : 20 : 30), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 338 nm, λ_{em} = 455 nm). Limit of quantification, 0.05 mg/L [Contreras de Condado *et al.* 1990]. Column: C_8 RAC II (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 0.5 mol/L dipotassium hydrogen phosphate (380 : 610 : 10), flow rate 70 mL/h. UV detection (λ = 214 nm). Retention time: 6.1 min. Limit of detection, 0.02 ng/L [Paczkowski *et al.* 1989].

See also Breithaupt, Wilfling [1982], Dusci, Hackett [1985], Grech-Bélanger *et al.* [1984], Gupta, Lew [1985], Kelly *et al.* [1981], Mastropaolo *et al.* [1984] and McErlane *et al.* [1987]

LC-MS Column: Capcell Pak C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water both containing 0.02% formic acid (5 : 95 to 50 : 50 at 3.5 min to 95 : 5 in 0.5 min to 5 : 95 in 0.5 min for 3 min), flow rate 0.3 mL/min. TIS, positive ion mode. Retention time: 4.1 min. Limit of quantification, 10 µg/L [Li *et al.* 2007].

Serum **GC** Column: 5% OV-1 on Chromosorb W A W DMCS 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature programme: 150°. FID. Limit of detection, 25 µg/L [Marko 1987]. Column: 2% OV-101 Chromosorb W-HP 100/120 mesh (0.6 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 130° for 1 min to 170° at 30°/min for 1 min to 240° at 30°/min for

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4 min. NPD. Limit of detection, 0.25 mg/L [Vasiliades *et al.* 1984]. Column: 10% Apiezon L-2% potassium hydroxide on 80/100 mesh (2.1 m × 4.0 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 195°. FID. Retention time: 0.73. Limit of detection, 0.1 mg/L [Holt *et al.* 1979].

GC-MS Column: Ultra-1 (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 195° for 5 min to 300° at 20°/min for 3 min. CI, SIM acquisition mode. Limit of detection, 0.1 mg/L [Dasgupta *et al.* 1998; Dasgupta, Yousef 1998].

HPLC Column: Pirkle 1-A phenylglycine (250 × 4.6 mm i.d., 5 µm). Mobile phase: propan-2-ol : chloroform : hexane (7 : 15 : 78), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 270 nm, λ_{em} = 420 nm). Limit of quantification, 2.5 µg/L; limit of detection, 0.5 µg/L [Kowk *et al.* 1994]. Column: Supelcosil LC8-DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol: 0.5 mol/L potassium dihydrogen phosphate : water (120 : 40 : 6 : 334), flow rate 1.2 mL/min. UV detection (λ = 203 nm). Limit of detection, 2 µg/L [Paczkowski *et al.* 1992]. Column: Shim-Pack CLC-ODS M (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L tris(hydroxymethyl)aminomethane (pH 9.0) : ethanol : propan-2-ol (55 : 35 : 10), flow rate 1.0 mL/min. Fluorometric detection (λ_{ex} = 397 nm, λ_{em} = 479 nm). Retention time: 14.0 min. Limit of detection, 0.005 mg/L [Shibata *et al.* 1991]. Column: NovaPak cyano HP (100 × 5 mm i.d.). Mobile phase: 5 mmol/L acetate buffer containing 0.05% TEA: acetonitrile (pH 6.0; 90 : 10), flow rate 1.5 mL/min. UV detection (λ = 210 nm). Retention time: ~1.1. Limit of detection, 0.7 mg/L [vasBinder, Annesley 1991].Column: Hypersil CPS (CN) reversed phase (250 × 4.6 mm i.d., 5 µm). Mobile phase: 973.5 mL water with 25 mL PIC B-8 low UV reagent, 1 mL butylamine and 0.5 mL PIC D-4 reagent : acetonitrile (95 : 5), flow rate 2.0 mL/min. UV detection (λ = 215 nm). Limit of quantification, 0.05 mg/L [Krämer *et al.* 1989]. See also Elfving *et al.* [1981], Farid and White [1983], Filipek *et al.* [1988], Grech-Bélanger *et al.* [1984], and Paczkowski *et al.* [1989].

Urine **GC** Column: heptakis (6-*O*-*tert*-butyl-dimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin 50% in polysiloxan OV1701 (25 m × 0.25 mm i.d., 0.125 µm). Carrier gas: He, 40 mL/min. Temperature programme: 120° for 1 min to 175° at 1°/min. FID. Retention time: 32 min. Limit of detection, 0.1 mg/L [Knoche *et al.* 1996]. See Plasma. Limit of detection, 1.0 mg/L [Pilling *et al.* 1982]. See Plasma [Chan *et al.* 1980]. Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (2 m × 0.64 cm o.d.). Carrier gas: N_2, 60 cm^3/min. Temperature: 160°. Limit of detection, 5 µg/L [Beckett, Chidomere 1977]. Column: 1% cyclohexane dimethanol succinate on 100/120 mesh Gas-Chrom Q (3 m × 4 mm i.d.). Carrier gas: CH_4:Ar (5 : 95), 45 mL/min. Temperature programme: 180°. Retention time, 3.3 min. Limit of detection, 2 mg/L [Willox, Singh 1976]. Column: 3% cyclohexane dimethanol succinate 100/120 Gas-Chrom Q (120 × 0.62 cm [4′ × 0.25"] o.d.). Carrier gas: He, 70 mL/min. Temperature programme: 220°. FID. Retention time: 3.1 min. Limit of detection, <10 µg/L [Kelly *et al.* 1973].

GC-MS Column: HP capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, scan mode. Limit of detection not reported [Maurer 1990].

HPLC See Plasma [Fieger, Wainer 1993]. Column: Pirkle type 1A (25 × 0.46 mm i.d., 5 µm). Mobile phase: propan-2-ol : *n*-hexane (5.5 : 94.5), flow rate 1.4 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 340 nm). Limit of detection, 5 µg/L [McErlane *et al.* 1987]. See Plasma [Breithaupt, Wilfling 1982].

Bile **GC** See Urine [Willox, Singh 1976].

CSF **HPLC** See Plasma [Breithaupt, Wilfling 1982].

Liver Microsomes **HPLC** Column: XL ODS (75 × 4.6 mm i.d., 3 µm). Mobile phase: 10 mmol/L potassium phosphate buffer (pH 4.2) : methanol : acetonitrile (90 : 5 : 5), flow rate 0.75 mL/min to 7 min to 1.5 mL/min at 9 min decreased from 20 min to 0.75 mL/min at 22 min. Fluorescence detection (λ_{ex} = 270 nm, λ_{em} = 312 nm). Limit of detection, 0.35 nmol/L for hydroxymethylmexilitene and 0.08 nmol/mL for *p*-hydroxymexilitene [Broly *et al.* 1988].

Disposition in the Body Readily absorbed after oral administration; bioavailability 80–90%. It is extensively metabolised; the major metabolites are the 4′-hydroxy and 2′-hydroxymethyl derivatives, which may be further metabolised by deamination to the corresponding alcohols. The rate and extent of renal excretion is dependent on the urinary pH. Under normal conditions, up to ~20% of a dose may be excreted in the urine as unchanged drug in 24 h, but this may be increased to ~50% if the urinary pH is maintained at ~5. CYP1A2 seems to be implicated in the *N*-oxidation of mexiletine [Labbé, Turgeon 1999] while non-CYP2D6-dependent metabolic pathways seem to be responsible for the metabolism of mexilitene in humans [Abolfathi *et al.* 1993]. See also [Senda *et al.* 2001].

Therapeutic Concentration In plasma, usually in the range 0.5–2 mg/L.

With an oral dose of 200 mg, peak plasma concentrations of 1–2 µg/mL occur 30–60 min following ingestion and decline to ~100 µg/L after 24 h [Willox, Singh 1976].

A 45-year-old was administered a 250 mg IV dose (3.5 mg/kg) of mexiletine hydrochloride. The half-life was 7.9 h, the elimination constant was $-0.087\ h^{-1}$, and the central volume of distribution was 386.1. Following administration of a 300 mg oral dose (4.0 mg/kg) of mexiletine hydrochloride, the half-life was 14.9 h and the elimination constant was $-0.046\ h^{-1}$ [Frydman *et al.* 1978].

Following a single oral dose of 400 mg to 6 subjects, peak plasma concentrations of 0.65–0.94 mg/L (mean 0.77) were attained in ~2 h [Haselbarth *et al.* 1981].

Following daily oral doses of 450 mg to 10 subjects, steady-state plasma concentrations of 0.75–2.18 mg/L (mean 1.6) were reported [Ohashi *et al.* 1984].

A healthy young volunteer was administered a single oral dose of 200 mg racemic mexilitene hydrochloride. The peak plasma concentrations of ~250 and 290 µg/L were reached after 4 h for *S*(+) and *R*(−)-mexilitene, respectively [Grech-Bélanger *et al.* 1985].

Ten healthy subjects were administered a single oral dose of 400 mg mexilitene. Peak mexilitene serum concentrations were observed at 3 h and ranged from 0.552–0.925 mg/L (mean 0.686 mg/L). Disappearance of the drug was realatively slow from all subjects and the drug was still detectable at 24 h with concentrations ranging from 0.082–0.174 mg/L. The first detectable levels for hydroxymethylmexiletine and *p*-hydroxymexiletine were observed 1 h after the dose. The peak serum concentrations for both metabolites were observed between 4 and 6 h and ranged from 0.385–0.690 mg/L for hydroxymethylmexiletine and from 0.06–0.225 mg/L for *p*-hydroxymexiletine [Paczkowski *et al.* 1990].

A 62-year-old female was administered chronic oral therapy with 200 mg racemic-mexiletine 3 times a day. The mean pharmacokinetic parameters were as follows:

Parameter	*p-Hydroxymexiletine*		*Hydroxymethylmexiletine*	
	(S)	*(R)*	*(S)*	*(R)*
C_{max} (µg/L)	63.5	73.0	29.0	50.3
t_{max} (h)	5	5	4	4

[Lanchote *et al.* 1997].

In 5 subjects with painful alcoholic neuropathy, the minimum effective oral dose of mexiletine was 300 mg daily, with an effective plasma concentration of 0.66 mg/L (± 0.15) [Nishiyama, Sakuta 1995].

Note For a study of the pharmacokinetics of mexiletine in patients with acute myocardial infarction, see Pentikäinen *et al.* [1984]; in renal failure, see Wang *et al.* [1985]. For pharmacokinetic parameters in combination with cimetidine and ranitidine, see Brockmeyer *et al.* [1989].

Toxicity

In a fatality from mexiletine overdose, postmortem blood and liver concentrations of 44.3 mg/L and 636 µg/g, respectively, were reported [Blackmore, Osselton 1982].

A 26-year-old male was found dead. Postmortem mexiletine concentrations were as follows:

Specimen	*Concentration (mg/L or mg/kg)*
Heart blood	38
Subclavian blood	14
Urine	370
Bile	440
Vitreous humour	17
Liver	190
Kidney	170
Stomach contents	2.9

[Kempton *et al.* 1994].

In a fatality involving mexiletine, tissue distribution was as follows: heart blood 44.8 mg/L, femoral blood 10.0 mg/L, brain 84 µg/g, vitreous humour 8.6 mg/L, liver 171.6 µg/g, gastric contents 1464 mg [Rohrig, Harty 1994].

A 41-year-old woman survived after ingesting up to 1.8 g mexiletine in a suicide attempt; her serum level was 20 mg/L [Nelson, Hoffman 1994].

Bioavailability 88% [Wulf 1983].

Half-life There is considerable intersubject variation and the plasma half-life appears to vary with the urinary pH; it is usually in the range 7–25 h (mean 11 h in normal subjects, increased in subjects with arrhythmias).

Volume of Distribution Approximately 8 L/kg, but there is considerable intersubject variation.

Clearance Plasma clearance, ~7 mL/min/kg with considerable intersubject variation. Milk : plasma ratio of 0.8 to 1.9, with a mean of 1.45 ± 0.3 [Wulf 1983].

Protein Binding Approximately 70% [Labbé, Turgeon 1999]

Note For a review of mexiletine, see Wulf [1983] and Labbé and Turgeon [1999].

Dose 0.6 to 1 g of mexiletine hydrochloride daily.

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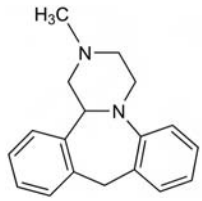
Mianserin

Antidepressant

$C_{18}H_{20}N_2 = 264.4$

CAS—24219-97-4

IUPAC Name 1,2,3,4,10,14*b*-Hexahydro-2-methyldibenzo[*c*,*f*]-pyrazino[1,2-*a*] azepine



Chemical Properties Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Mianserin Hydrochloride

$C_{18}H_{20}N_2,HCl = 300.8$

CAS—21535-47-7

Synonyms Mianserini hydrochloridum; Org-GB-94.

Proprietary Names *Athymil*; *Bolvidon*; *Bonserin*; *Hopacem*; *Lantanon*; *Lerivon*; *Lumin*; *Miabene*; *Mianeurin*; *Miaxan*; *Norval*; *Prisma*; *Tolmin*; *Tolvin*; *Tolvon*. The name Norval was formerly applied to a preparation containing docusate sodium.

Chemical Properties White crystals or crystalline powder. Mp 282° to 284°. Soluble 1 in 50 of water, 1 in 100 of ethanol, and 1 in 20 of chloroform.

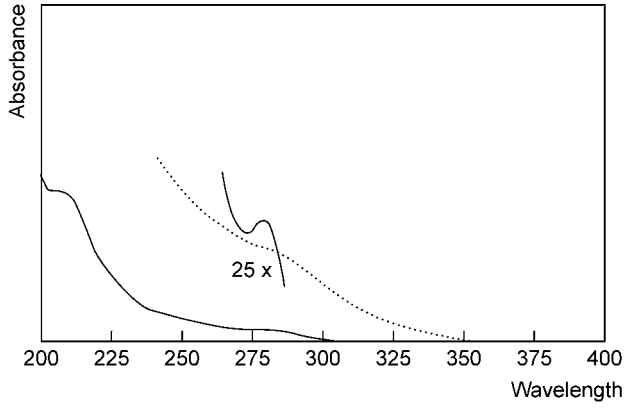
Colour Test Liebermann's reagent—violet; Mandelin's test—violet

Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.39; system TC—R_f 0.58; system TE—R_f 0.68; system TL—R_f 0.23; system TAE—R_f 0.48; system TAF—R_f 0.50 (acidified iodoplatinate solution, blue; Mandelin's reagent, violet).

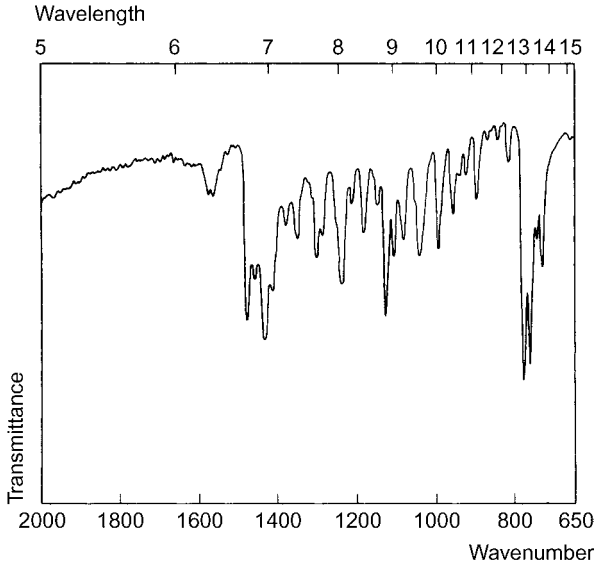
Gas Chromatography System GA—mianserin RI 2210, M (nor-) RI 2235, M (8-OH-) RI 2495, M (*N*-oxide) RI 2202, M (OH-methoxy-) RI 2530; system GB—mianserin RI 2302, M (nor-) RI 2348, M (8-OH-) RI 2628; system GF—RI 2595; system GM—mianserin RRT 0.879, M (nor-) RRT 1.105 (both relative to iprindole); system GS—RT 18.1 min.

High Performance Liquid Chromatography System HA—mianserin *k* 1.8, M (*N*-desmethyl-) *k* 2.4; system HX—RI 391; system HY—RI 342; system HZ—RT 4.6 min; system HAA—RT 13.8 min; system HAZ—mianserin *k* 1.18, M (nor-) *k* 0.88, M (*N*-oxide) *k* 0.53, M (8-OH-) *k* 0.19.

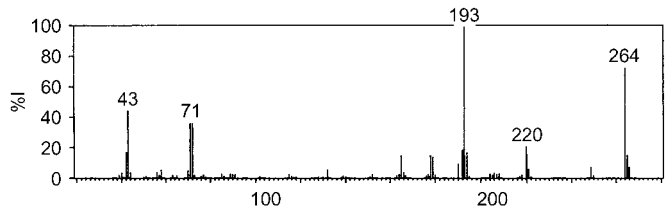
Ultraviolet Spectrum Aqueous acid—279 nm (A_1^1 = 75a).



Infrared Spectrum Principal peaks at wavenumbers 787, 772, 1138, 1254, 1314, 742 cm^{-1} (mianserin hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 193, 264, 43, 72, 71, 220, 192, 194; *N*-desmethylmianserin 193, 208, 194, 250, 178, 192, 29, 179.



M

Quantification

Blood **GC** Column: Rtx-5MS fused silica (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 3 mL/min. Temperature programme: 100° for 1 min to 250° at 20°/min to 300° at 5°/min. FID. Limit of detection, ~1 μg/L [Ishii *et al.* 2000].

GC-MS [Wille *et al.* 2009]. Column: SPB-1 (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 5 min to 280° at 20°/min. SIM acquisition mode. Retention time: 13.7 min. Limit of detection, 5.0 ng/g [Namera *et al.* 1998].

HPLC Column: XTerra RP-18. Mobile phase: acetonitrile : ammonium formate buffer 4 mmol/L (pH 3.2). Limit of quantification, 2 μg/L [Titier *et al.* 2007].

Plasma **GC** Column: HP 5890, DB-17 capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: N_2, 0.7 mL/min. Temperature programme, 260° for 1 min. NPD. Limit of quantification, 125 μg/L [Ulrich, Martens 1997]. Column: 3% OV-1 on 100/120 Gas-Chrom Q. Carrier gas: N_2, 40 mL/min. Temperature: 210°. NSD. Retention time: 2.3 min. Limit of detection, 2.5 μg/L [Lachâtre *et al.* 1982].

GC-MS Column: J, W-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 10 μg/L [Wille *et al.* 2007]. Column: 1.5% OV-1 on Gas Chrom Q 100/120 mesh (1.8 m × 2 mm i.d.). Temperature: 205°. SIM acquisition mode. Retention time: 3 min. Limit of detection, 5 μg/L [Jindal *et al.* 1982].

HPLC Column: Thermo Hypersil-HyPurity C_{18} (150 × 2.1 mm, 5 μm). Mobile phase: 10 μmol/L ammonium acetate (pH 3.4) : methanol : acetonitrile (35 : 50 : 15), flow rate 0.22 mL/min. Retention time 3.4 min. Limit of quantification, 1 μg/L [Xu *et al.* 2008]. Column: Supelcosil LC-SI (150 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : methanol : 25% ammonium hydroxide (345 : 65 : 1.6), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, <10 nmol [Tybring *et al.* 1995]. Limits of quantification, 4 μg/L and 2.5 μg/L [Eap *et al.* 1994]. Column: reversed-phase C_{18} (150 × 3.9 mm i.d.). Mobile phase: phosphate buffer : acetonitrile : methanol . Electrochemical detection. Limit of quantification, 1.67 μg/L [Brown *et al.* 1992]. Column: reversed-phase trimethylsilyl-packed. Mobile phase: acetate : acetonitrile . Electrochemical detection. Limit of detection, 5 μg/L [Suckow *et al.* 1982].

Serum **HPLC** Column: Hichrom RPB (250 × 4.6 mm, 5 mm). Mobile phase: 0.05 mol/L phosphoric acid (pH 3.0). UV detection (λ= 214 nm). Limit of quantification, 2 μg/L [Lukaszkiewicz *et al.* 2007].

Urine **GC** See Blood [Ishii *et al.* 2000].

HPLC See Plasma. Limit of quantification, 2.5 μg/L and 5 μg/L [Eap *et al.* 1994].

Brain **GC-MS** See Blood [Wille *et al.* 2009].

Hair **GC-MS** See Blood [Wille *et al.* 2009]. Column: BP-5 fused silica (12 m × 0.53 mm i.d., 1.0 μm). Carrier gas: He, 3 mL/min. Temperature programme: 100° for 2 min to 310° at 7.5°/min for 10 min. NPD. Limit of detection not reported [Couper *et al.* 1995].

Disposition in the Body Mianserin is readily absorbed after oral administration; bioavailability is reduced by first-pass metabolism. The major metabolites are *N*-desmethylmianserin and 8-hydroxymianserin, which are both active, and mianserin *N*-oxide. Approximately 30–40% of a dose is excreted in the urine in 24 h, and a total of ~70% is excreted over a period of several days, mainly as conjugated metabolites. Approximately 5% of a dose is excreted in the urine unchanged. It crosses the blood–brain barrier and the placenta; it is found in breast milk.

Therapeutic Concentration In plasma, usually in the range 0.015–0.07 mg/L. *N*-Desmethylmianserin is detectable in plasma at concentrations ~one-third of those of mianserin.

Following a single oral dose of 60 mg given to eight subjects, peak plasma concentrations of 0.05–0.14 mg/L (mean 0.10) and peak blood concentrations of 0.04–0.09 mg/L (mean 0.06) were attained in ~3 h [Maguire *et al.* 1982].

Following oral doses of 20 mg three times a day to 10 subjects, minimum steady-state plasma concentrations of 0.03–0.09 mg/L (mean 0.06) were reported [Lachâtre *et al.* 1982].

Toxicity Overdosage does not appear to cause the complications associated with overdose of tricyclic antidepressants. Toxic effects have been associated with plasma concentrations >0.5 mg/L.

Repeated episodes of complete heart block following overdose reported in a case study [Hla, Boyd 1987].

Plasma concentrations of 0.11 μg/mL mianserin and 0.5 mg/L lorazepam were reported on admission to hospital in a comatose subject who had ingested 600 mg mianserin plus an unknown amount of lorazepam; the patient died the following day [Crome, Newman 1977].

Half-life Plasma half-life, 6–40 h (mean 16), increased in elderly subjects.

Volume of Distribution ~13 L/kg.

Protein Binding ~90%.

Note For a review of metabolism of some 'second'- and 'fourth'-generation antidepressants, see Rotzinger *et al.* [1999]; for a review of mianserin, see Brogden *et al.* [1978].

Dose 30 to 90 mg of mianserin hydrochloride daily; maximum of 200 mg daily.

Brogden RN *et al.* (1978). Mianserin: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs* 16: 273–301.

Brown LW *et al.* (1992). Automated high-performance liquid chromatographic method for the determination of mianserin in plasma using electrochemical detection. *J Chromatogr* 582: 268–272.

Couper FJ *et al.* (1995). Detection of antidepressant and antipsychotic drugs in postmortem human scalp hair. *J Forensic Sci* 40: 87–90.

Crome P, Newman B (1977). Poisoning with maprotiline and mianserin. *Br Med J* 2: 260.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

Eap CB *et al.* (1994). Determination of the enantiomers of mianserin, desmethylmianserin, and 8-hydroxymianserin in the plasma and urine of mianserin-treated patients. *Chirality* 6: 555–563.

Hla KK, Boyd O (1987). Mianserin and complete heart block. *Hum Toxicol* 6: 401–402.

Ishii A *et al.* (2000). Sensitive determination of mianserin and setiptiline in body fluids by gas chromatography with surface ionization detection (GC-SID). *Leg Med (Tokyo)* 2: 115–118.

Jindal SP *et al.* (1982). Selected ion monitoring assay for the antidepressant mianserin in human plasma with stable isotope labeled analog as internal standard. *J Anal Toxicol* 6: 34–37.

Lachâtre GF *et al.* (1982). Determination of mianserin in human plasma by gas–liquid chromatography. *Ther Drug Monit* 4: 359–364.

Lukaszkiewicz J *et al.* (2007). Development, validation and application of the HPLC method for determination of mianserin in human serum. *Acta Pol Pharm* 64: 103–107.

Maguire KP *et al.* (1982). A pharmacokinetic study of mianserin. *Eur J Clin Pharmacol* 21: 517–520.

Namera A *et al.* (1998). Simple analysis of tetracyclic antidepressants in blood using headspace-solid-phase microextraction and GC-MS. *J Anal Toxicol* 22: 396–400.

Rotzinger S *et al.* (1999). Metabolism of some 'second'- and 'fourth'-generation antidepressants: iprindole, viloxazine, bupropion, mianserin, maprotiline, trazodone, nefazodone, and venlafaxine. *Cell Mol Neurobiol* 19: 427–442.

Suckow RF *et al.* (1982). Determination of mianserin and metabolites in plasma by liquid chromatography with electrochemical detection. *J Pharm Sci* 71: 889–892.

Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.

Tybring G *et al.* (1995). Enantioselective determination of mianserin and its desmethyl metabolite in plasma during treatment of depressed Japanese patients. *Ther Drug Monit* 17: 516–521.

Ulrich S, Martens J (1997). Solid-phase microextraction with capillary gas–liquid chromatography and nitrogen–phosphorus selective detection for the assay of antidepressant drugs in human plasma. *J Chromatogr B Biomed Sci Appl* 696: 217–234.

Wille SM *et al.* (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic-mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *J Chromatogr A* 1176: 236–245.

Wille SM *et al.* (2009). Determination of antidepressants in human postmortem blood, brain tissue, and hair using gas chromatography–mass spectrometry. *Int J Legal Med* 123: 451–458.

Xu P *et al.* (2008). Determination of mianserin in human plasma by high performance liquid chromatography–electrospray ionization mass spectrometry (HPLC-ESI/MS): application to a bioequivalence study in Chinese volunteers. *J Pharm Biomed Anal* 47: 994–999.

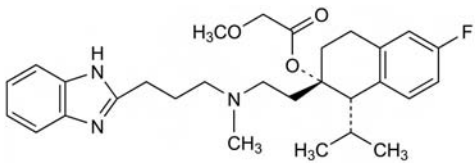
Mibefradil

Calcium Antagonist

$C_{29}H_{38}FN_3O_3$ = 495.6

CAS—116644-53-2

IUPAC Name [(1*S*,2*S*)-2-[2-[3-(1*H*-Benzimidazol-2-yl)propyl-methylamino] ethyl]-6-fluoro-1-propan-2-yl-3,4-dihydro-1*H*-naphthalen-2-yl] 2-methoxyacetate
Synonyms Methoxyacetic acid (1*S*,2*S*)-2-[2-[[3-(1*H*-benzimidazol-2-yl)-propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester; mibefradilum; Ro-40-5967.

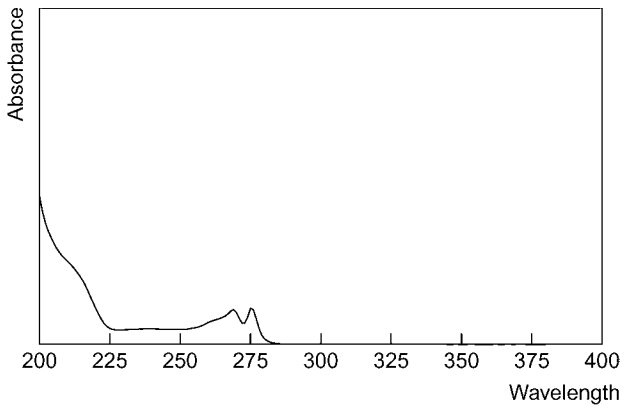


Chemical Properties pK_a 4.8; 5.5 (dihydrochloride).

Mibefradil Dihydrochloride

$C_{29}H_{38}FN_3O_3,2HCl = 568.6$
CAS—116666-63-8
Synonyms Miberfradil hydrochloride; Ro-40-5967/001.
Proprietary Name *Posicor*
Chemical Properties A white crystalline powder. Mp 128°. Soluble in water.

Ultraviolet Spectrum



Quantification
***Plasma* HPLC** Column: RP C_{18} LiChrospher, RP Select-B. Mobile phase: acetonitrile : aqueous solution of 39.3 mmol/L potassium dihydrogen phosphate and 8.2 mmol/L sodium pentanesulfonic acid (38:62), flow rate 2 mL/min. Fluorescence detection (λ_{ex}=270 nm, λ_{em}=300 nm). Retention time: 10.7 min. Limit of detection, 0.5 µg/L [Skerjanec *et al.* 1995].
***Urine* HPLC** See Plasma [Skerjanec *et al.* 1995].
Disposition in the Body Mibefradil is completely and rapidly absorbed with absorption unaffected by food. There is extensive distribution into peripheral tissues and it undergoes first-pass metabolism. The drug is metabolised via parallel pathways: esterase-catalysed hydrolysis and CYP3A4-mediated oxidation. The esterase pathway yields an alcohol metabolite, which has 10% of the pharmacological effect. After chronic dosing, the oxidative pathway becomes less important. The oxidation pathway leads to *O*-demethylation, *N*-demethylation and ring hydroxylation, yielding many identified metabolites. Elimination is via biliary (75%) and renal (25%) routes. 3% of the dose is excreted into urine as the unchanged drug. The drug displays zero order kinetics. Peak plasma level is reached within 1 to 2 h; steady state occurs within 3 to 4 days. Renal and mild-to-moderate hepatic dysfunction causes no change in pharmacokinetics.
Therapeutic Concentration
In a study of 42 healthy males, aged 18 to 35 years, peak plasma concentrations were 26.7, 67.8, 115.0, 61.0, 495.0, 777.0 and 1490.0 µg/L following oral administration of mibefradil at doses of 10, 20, 40, 80, 120, 160 and 320 mg, respectively, after an overnight fast. These were observed at 1.00, 0.96, 0.86, 0.87, 1.38, 1.50 and 2.00 h, respectively.

Three to 5 healthy males, with a mean age of 32.4 years, were administered orally 100, 150 or 250 mg mibefradil once daily, after an overnight fast, for 28 days. Peak plasma concentrations on day 1 were 520, 739 and 1322 µg/L for the three doses observed at 2.33, 1.17 and 2.33 h, respectively. On day 28, peak concentrations reached 718, 914 and 1506 µg/L in the same time period.

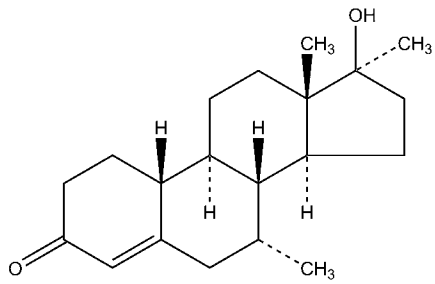
Twelve hypertensive patients were administered for 8 days with doses of 50, 100, 150 or 200 mg daily, after an overnight fast. On day 1, plasma concentrations of 220, 478, 952 and 1116 µg/L were observed at 0.94, 1.09, 1.54 and 1.67 h, respectively. On day 8, however, peak concentrations were 402, 929, 1373 and 1538 µg/L for the four doses and were seen at 1.72, 1.62, 2.14 and 1.33 h post-dosing [Welker 1998].

Bioavailability Approximately 80%.
Half-life 17 to 25 h.
Volume of Distribution Steady state, 176 to 255 L; also reported as 3.5 to 5.7 L/kg.
Clearance Plasma, 14.46 to 19.26 L/h; blood systemic, 26.16 L/h.
Distribution in Blood The blood : plasma ratio is 0.64.
Protein Binding >99%, mainly to α_1-acid glycoproteins.
Dose 50 to 100 mg/day.

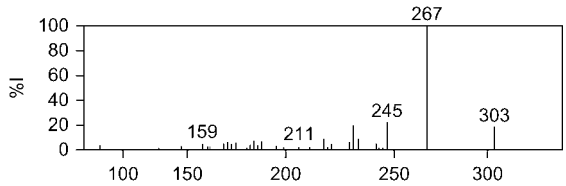
Skerjanec A *et al.* (1995). High-performance liquid chromatographic analysis of mibefradil in dog plasma and urine. *J Chromatogr* 669: 377–382.
Welker HA (1998). Single- and multiple-dose mibefradil pharmacokinetics in normal and hypertensive subjects. *J Pharm Pharmacol* 50(9): 983–987.

Mibolerone

Anabolic Steroid, Antigonadotropic (Veterinary)
$C_{20}H_{30}O_2 = 302.5$
CAS—3704-09-4
IUPAC Name (7*R*,8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-7,13,17-trimethyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one
Synonyms 7α,17α-Dimethyl-19-nortestosterone; (7α,17β)-17-hydroxy-7,17-dimethylestr-4-en-3-one; U-10997.
Proprietary Names *Cheque*; *Matenon*.



Chemical Properties Crystalline solid. Soluble in deionised water. Log *P* (octanol/water), 3.19 [Sangster 1997]. Stability was at a maximum between pH 5.5 and 6.4 in aqueous solution [Amin *et al.* 1976].
Mass Spectrum Principal ions at *m/z* 303, 121, 285, 177, 95, 245, 227, 163.



Quantification
***Other* GC** Aqueous Solutions. Column: 1% QF-1 Gas Chrom 3 (460 × 3 mm i.d.). Carrier gas: He 70 mL/min. Temperature: 260°. FID . Limit of detection not reported [Amin *et al.* 1976].

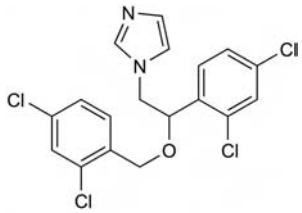
LC-MS Canine Urine. Column: SB-C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: water : acetonitrile (75 : 25 to 65 : 35 in 30 min to 35 : 65 in 5 min for 5 min), flow rate 0.208 mL/min. DAD (λ = 246 nm). ESI, positive ion mode, full scan and SIM acquisition mode. Limit of detection not reported [Williams *et al.* 2000a]. Microsomes. Column: SB-C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 0.75% trifluroacetic acid in water : acetonitrile (1 : 1). DAD (λ = 244 nm). ESI. Limit of quantification not reported [Williams *et al.* 2000b].

Amin MI *et al.* (1976). Stability of aqueous solutions of mibolerone. *J Pharm Sci* 65: 1777–1779.
Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* Chichester, UK: Wiley.
Williams TM *et al.* (2000a). Characterization of urinary metabolites of testosterone, methyltestosterone, mibolerone and boldebone in greyhound dogs. *J Vet Pharmacol Ther* 23: 121–129.
Williams TM *et al.* (2000b). Drug metabolism: in vitro biotransformation of anabolic steroids in canines. *J Vet Pharmacol Ther* 23: 57–66.

Miconazole

Antifungal
$C_{18}H_{14}Cl_4N_2O = 416.1$
CAS—22916-47-8
IUPAC Name 1-[2-(2,4-Dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy] ethyl]-1-*H*-imidazole

Proprietary Name *Daktarin*

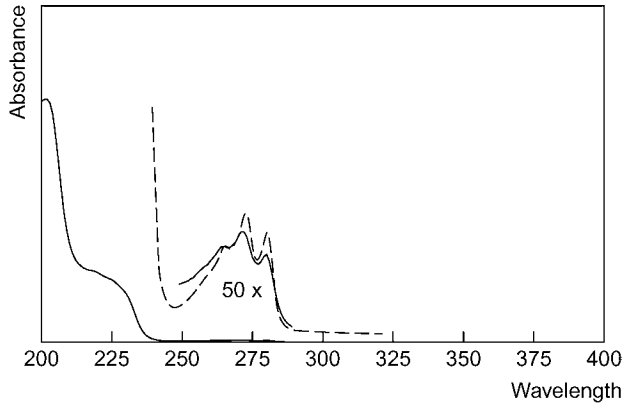


Chemical Properties A white crystalline powder. Very slightly soluble in water; very soluble in chloroform; soluble in most other organic solvents. pK_a 6.7.

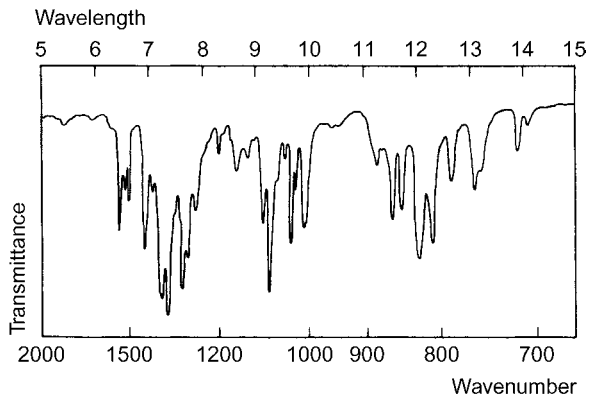
Miconazole Nitrate

$C_{18}H_{14}Cl_4N_2O,HNO_3 = 479.1$
CAS—22832-87-7
Proprietary Names *Albistat*; *Daktarin* (cream); *Dermonistat*; *Gyno-Daktarin*; *Micatin*; *Monistat*.
Chemical Properties A white crystalline powder. Mp about 182°. Very slightly soluble in water and ether; soluble 1 in 140 of ethanol; slightly soluble in chloroform.

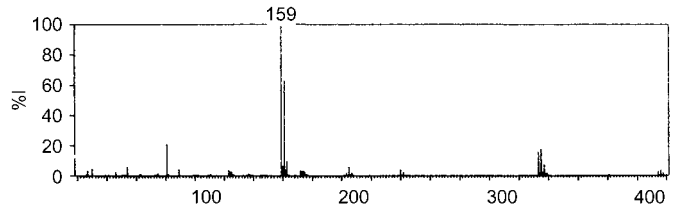
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.11; system TC—R_f 0.67; system TE—R_f 0.80; system TF—R_f 0.07; system TL—R_f 0.37; system TAD—R_f 0.56; system TAE—R_f 0.77; system TAF—R_f 0.80 (Dragendorff spray).
Gas Chromatography System GA—RI 2965.
Ultraviolet Spectrum Methanol—264, 272 (A^1_1=17a), 280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1085, 1319, 827, 1302, 1038, 812 cm^{-1} (miconazole nitrate, KBr disk).



Mass Spectrum Principal peaks at *m/z* 159, 161, 81, 335, 333, 163, 337, 205.



Quantification
Plasma **GC** [Szathmary, Luhmann 1988].
HPLC UV detection (λ=230 nm). Limit of detection, 5 µg/L [Kobylinska *et al.* 1996].
Serum **GC** ECD. Limit of detection, 1 µg/L [Männistö *et al.* 1982].
Bioassay [Espinel-Ingroff *et al.* 1977].
Saliva **HPLC** UV detection. Limit of detection, 500 µg/L [Turner, Warnock 1982].
Vaginal Fluid **HPLC** [Selinger *et al.* 1988].
Note For a rapid HPLC screen of antifungal agents in serum, see Ng *et al.* [1996].
Disposition in the Body Incompletely absorbed after oral administration and poorly absorbed after topical administration. Metabolised by oxidation to 2,4-dichloromandelic acid and 2-(2,4-dichlorobenzyloxy)-2-(2,4-dichlorophenyl) acetic acid which are both inactive. About 10 to 20% of an oral or IV dose is excreted in the urine in 6 days, with only about 1% of the dose as unchanged drug. About 50% of an oral dose is eliminated in the faeces, mostly unchanged.
Therapeutic Concentration
Following an IV infusion of 522 mg to 4 subjects, plasma concentrations of 2.0 to 9.1 µg/mL (mean 6.2) were reported at the end of the infusion [Lewi *et al.* 1976].
Toxicity For a report of convulsions after miconazole overdose, see Coulthard *et al.* [1987].
Half-life Plasma half-life, about 24 h.
Volume of Distribution About 20 L/kg.
Protein Binding About 92%.
Note For a review of the pharmacokinetics of systemic antifungal drugs, see Daneshmend and Warnock [1983].
Dose 0.6 to 3.6 g daily by IV infusion; 1 g daily orally.

Coulthard K *et al.* (1987). Convulsions after miconazole overdose. *Med J Aust* 146: 57–58.
Daneshmend TK, Warnock DW (1983). Clinical pharmacokinetics of systemic antifungal drugs. *Clin Pharmacokinet* 8: 17–42.
Espinel-Ingroff A *et al.* (1977). Bioassay for miconazole. *Antimicrob Agents Chemother* 11: 365–368.
Kobylinska M *et al.* (1996). High-performance liquid chromatographic analysis for the determination of miconazole in human plasma using solid-phase extraction. *J Chromatogr B Biomed Appl* 685: 191–195.
Lewi PJ *et al.* (1976). *Eur J Clin Pharmacol* 10: 49–54.
Männistö PT *et al.* (1982). Impairing effect of food on ketoconazole absorption. *Antimicrob Agents Chemother* 21: 730–733.
Ng TK *et al.* (1996). Rapid high performance liquid chromatographic assay for antifungal agents in human sera. *J Antimicrob Chemother* 37: 465–472.
Selinger K *et al.* (1988). High-performance liquid chromatographic method for the determination of miconazole in vaginal fluid. *J Chromatogr* 434: 259–264.
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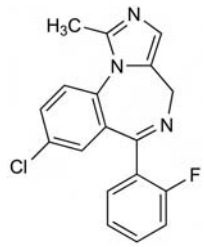
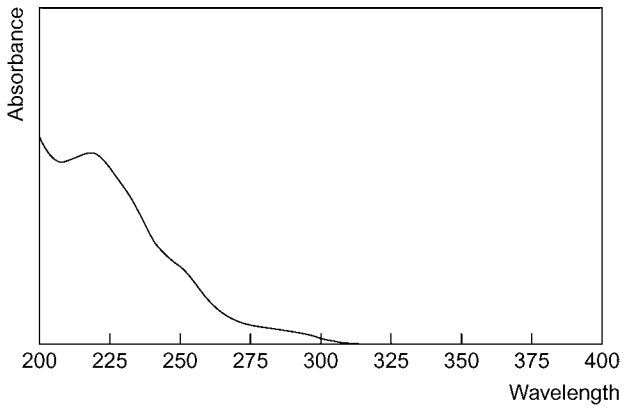
Midazolam

Benzodiazepine, Hypnotic
$C_{18}H_{13}ClFN_3 = 325.8$
CAS—59467-70-8
IUPAC Name 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine
Synonyms Midazolamum; Ro-21-3971.
Proprietary Names *Dormicum*; *Dormonid*; *Hypnovel*; *Ipnovel*; *Midolam*; *Versed* (includes proprietary names of salts of midazolam).

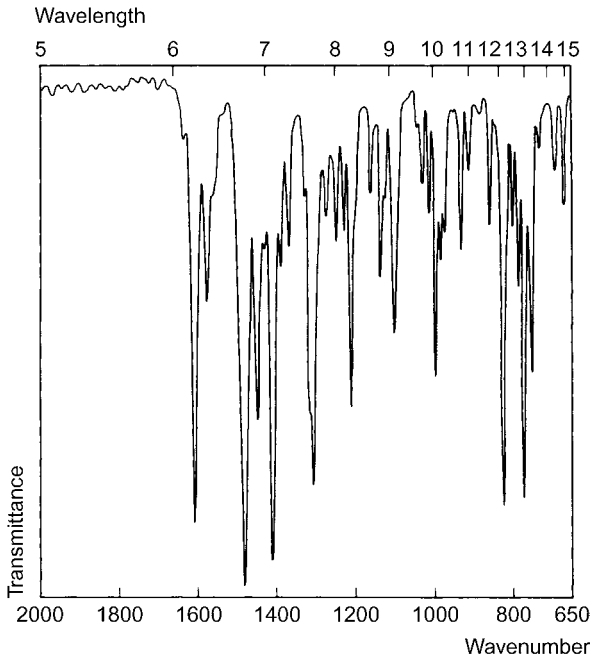


Chemical Properties Colourless crystals. Mp 158° to 160°. Soluble in water. pKa 6.2. Log P (octanol/water), 4.3. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].
Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.06; system TC—R_f 0.60; system TD—R_f 0.13; system TE—R_f 0.60; system TF—R_f 0.05; system TL—R_f 0.19; system TAD—R_f 0.53; system TAE—R_f 0.69; system TAF—R_f 0.70.
Gas Chromatography System GA—midazolam RI 2575, M (α-OH-) RI 2830; system GB—midazolam RI 2722, M (α-OH-) RI 2901, M (α-OH-)-TMS RI 2866, M (4-OH-)-TMS RI 2775.
High Performance Liquid Chromatography System HI— *k* 9.75; system HJ—*k* 2.10; system HK— *k* 5.90; system HX—RI 399; system HY—RI 306; system HZ—RT 4.2 min; system HAA—RT 14.9 min; system HAX—RT 10.2 min; system HAY—RT 6.3 min.

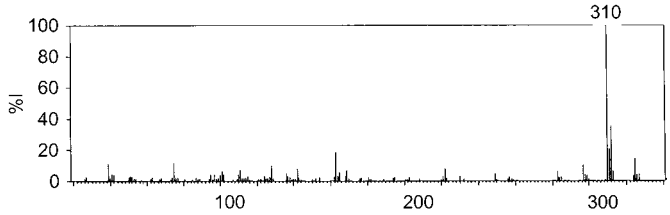
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1608, 820, 767, 1310, 1210, 995 cm^{-1} (KBr disk, see below).



Mass Spectrum Principal ions at *m/z* 310, 312, 311, 163, 325, 75, 39, 297.



Quantification

Blood **HPLC** Column: C_8 semi-microcolumn (Lichrospher select B, 125 × 3 mm). Mobile phase: 20 µmol/L phosphate buffer (pH 2.2) : acetonitrile (70 : 30, v/v). DAD (λ= 254 nm). [El Mahjoub, Staub 2001a]. Column: Lichrospher select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 2.1) : acetonitrile (65 : 35), flow rate 0.3 mL/min. DAD (λ= 220 nm). Limit of quantification, 10 µg/L, limit of detection, 3.5 µg/L [El Mahjoub, Staub 2000b].

Plasma **GC-MS** Column: OV-1 capillary (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 85° for 1 min to to 200° at 30°/min to 310° at 10°/min for 1 min. MID, NCI, SIM acquisition mode. Limit of quantification, 10 ng/L [Eap *et al.* 2004]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 150° for 0.5 min to 300° at 20°/min for 5 min. SIM acquisition mode. Limit of detection, 1 µg/L [Frison *et al.* 2001]. ECD, Limit of detection, 500 ng/L [Smith *et al.* 1981].

HPLC Column: LiChrospher Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer (pH 7.2, 6 : 94). UV detection (λ= 254 nm). Limit of detection, 15 µg/L [El Mahjoub, Staub 2000a]. Column: Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.02% perchloric acid (30 : 70). UV detection (λ= 200 nm). Limit of quantification, 10 µg/L [Portier *et al.* 1999]. Column: C_{18} capillary (150 mm × 0.8 mm i.d., 3 µm). Mobile phase: phosphate buffer : acetonitrile (65 : 35) for 16 min, phosphate buffer : acetonitrile (40 : 60) for 1 min, flow rate 16 µL/min. UV detection. Limit of quantification, 1 µg/L [Eeckhoudt *et al.* 1998]. Column: Ultrasphere ODS (75 × 4.6 mm, 3 µm). Mobile phase: 35.2% acetonitrile : 4.8% methanol : 60% 0.1 mol/L buffer acetate (pH 4.7), flow rate 1 mL/min. UV detection (λ= 254 nm). Limit of detection, 8 nmol [Carrillo *et al.* 1998]. Column: C_{18} Nova-Pak (150 × 3.9 mm, 4 µm). Mobile phase: acetonitrile : 0.05 mol/L phosphate buffer, pH 4.5, flow rate 1.2 mL/min. Retention time, 13.5 min. UV detection (λ= 207 nm) [Van Brandt *et al.* 1997].

See also Adams *et al.* [1992], Bourget *et al.* [1996], Chan, Jones [1993], Ha *et al.* [1993], Lauber *et al.* [1994], Lee, Charles [1996], Lehmann, Boulieu [1995], Mastey *et al.* [1994], Sautou *et al.* [1991] and Vasiliades, Sahawneh [1981].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5). ESI, positive ion mode, MRM acquisition mode. Retention time: 4.03 min. Limit of detection, <10 µg/L [Marin *et al.* 2008]. Column: Reversed phase. Mobile phase: acetonitrile : 0.1 mol/L phosphate buffer [Kanazawa *et al.* 1998].

Serum **HPLC** See Blood [[El Mahjoub, Staub 2000b]. See Plasma [El Mahjoub, Staub 2000a]. Column: LiChrospher 100 (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : 0.05 mol/L HEPES buffer and 15 mmol/L dodecylammonium chloride (pH 5.25, 65 : 35), flow rate 1.0 mL/min. DAD (λ= 254 nm). Limit of quantification, 0.1 mg/L [van Rij *et al.* 1999]. Column: cyanopropyl type (150 × 4.6 mm). Mobile phase: acetonitrile 0.02 mol/L : sodium acetate at pH 3.0 (80 : 20), flow rate 1.2 mL/min. UV detection (λ = 240 nm) [Odou *et al.* 1997].

LC-MS See Plasma [Marin *et al.* 2008].

Urine **HPLC** See Plasma [Chan, Jones 1993; Ha *et al.* 1993].

LC-MS See Plasma [Marin *et al.* 2008].

Note For polarography for the quantification of midazolam, see Puglisi *et al.* [1978].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008].

Note For a comparison of various chromatographic methods, see Vasiliades, Sahawneh [1982].

Hair **HPLC** Column: C_8 semi micro, Lichrospher select B (125 × 3 mm). DAD (λ= 254 nm) [El Mahjoub, Staub 2001b].

LC-MS Limit of quantification, 0.5–5 pg/mg [Villain *et al.* 2005].

Disposition in the Body Midazolam is rapidly absorbed after oral administration and undergoes first-pass metabolism. It crosses the placenta. Small quantities are found in breast milk. It is metabolised by hydroxylation to 1-hydroxymethylmidazolam (which is active), 4-hydroxymidazolam and 4-hydroxy-1-hydroxymethylmidazolam; the hydroxy metabolites are then conjugated with glucuronic acid. Up to 90% of a dose is excreted in the urine in 24 h as the conjugated metabolites, mainly as conjugated 1-hydroxymethylmidazolam (60–70% of a dose). Less than 1% of a dose is excreted in the urine as unchanged drug.

Therapeutic Concentration

Midazolam administered orally as a single dose (0.25–1.0 mg/kg) to 85 children, aged 6 months to 15 years, provided maximal sedation at plasma concentrations of 0.13796 mg/L (range 0.020–0.0.472) midazolam and 0.08958 mg/L (range 0.0048–0.180) α-hydroxymidazolam 30 min after dosing [Marshall *et al.* 2000].

Blood concentrations of 0.019–0.583 mg/L (mean 0.121) were attained 5–120 min after dosing in 60 mechanically ventilated pre-term infants administered midazolam 100 µg/kg (IV over 2 min) every 4–6 h [Lee *et al.* 1999].

The steady-state plasma concentration range was 0.010–0.147 mg/L (median 0.030) in 11 subjects receiving midazolam by continuous SC infusion at a rate 10–60 mg daily (median 20) [Bleasel *et al.* 1994].

In children given midazolam 0.2 mg/kg intranasally, 0.5 mg/kg orally or 0.3 mg/kg rectally, blood concentrations of 0.146 mg/L were attained in 11.5 min by the intranasal route, 0.104 mg/L in 21 min by the rectal route, and 0.093 mg/L in 23.1 min by the oral route [Malinovsky *et al.* 1995].

Toxicity

A 63-year-old man developed cardiorespiratory depression ~5 min after receiving 10 mg midazolam during endoscopic retrograde cholangiopancreatography. Blood and urine levels of midazolam of 2.8 and 0.18 mg/L, respectively, were reported ~40 min after the dose. Death occurred after 2 days and the postmortem midazolam blood concentration was 2.4 mg/L [Michalodimitrakis *et al.* 1999].

In a death caused by self-injection of midazolam and sufentanil, the following postmortem tissue concentrations were reported for midazolam and sufentanil, respectively: blood 0.05 and 0.0011 mg/L, urine 0.3 and 0.0013 mg/L, liver 930 and 1.75 ng/g, kidney 290 and 5.5 ng/g and vitreous humour 0.0012 mg/L (sufentanil only) [Ferslew *et al.* 1989].

Bioavailability After IM administration, more than 90%. Reduced to 30% from ~70% by first-pass metabolism.

Half-life Plasma half-life, ~2 h; increased in elderly subjects, in obesity, and in cirrhosis.

Volume of Distribution 1–3 L/kg.

Clearance Plasma clearance, 3–8 mL/min/kg (mean 5).

Protein Binding ~95–98%.

Dose As a sedative, the equivalent of 70 µg/kg midazolam by slow IV injection, usual dose range 2.5 to 7.5 mg; for induction of anaesthesia, 200 µg/kg by slow IV injection.

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Carrillo JA *et al.* (1998). Analysis of midazolam and metabolites in plasma by high-performance liquid chromatography: probe of CYP3A. *Ther Drug Monit* 20: 319–324.
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Ferslew KE *et al.* (1989). Postmortem determination of the biological distribution of sufentanil and midazolam after an acute intoxication. *J Forensic Sci* 34: 249–257.
Frison G *et al.* (2001). Determination of midazolam in human plasma by solid-phase microextraction and gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 15: 2497–2501.
Ha HR *et al.* (1993). Determination of midazolam and its alpha-hydroxy metabolite in human plasma and urine by high-performance liquid chromatography. *Ther Drug Monit* 15: 338–343.
Kanazawa H *et al.* (1998). Determination of sedatives and anesthetics in plasma by liquid chromatography–mass spectrometry with a desalting system. *J Chromatogr A* 797: 227–236.
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Lee TC, Charles B (1996). Measurement by HPLC of midazolam and its major metabolite, 1-hydroxymidazolam in plasma of very premature neonates. *Biomed Chromatogr* 10: 65–68.
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Marin SJ *et al.* (2008). Quantitation of benzodiazepines in urine, serum, plasma, and meconium by LC-MS-MS. *J Anal Toxicol* 32: 491–498.
Marshall J *et al.* (2000). Pediatric pharmacodynamics of midazolam oral syrup. Pediatric Pharmacology Research Unit Network. *J Clin Pharmacol* 40: 578–589.
Mastey V *et al.* (1994). Determination of midazolam and two of its metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 655: 305–310.
Michalodimitrakis M *et al.* (1999). Death related to midazolam overdose during endoscopic retrograde cholangiopancreatography. *Am J Forensic Med Pathol* 20: 93–97.
Odou P *et al.* (1997). A routine HPLC method for monitoring midazolam in serum. *Biomed Chromatogr* 11: 19–21.
Portier EJ *et al.* (1999). Simultaneous determination of fentanyl and midazolam using high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 723: 313–318.
Puglisi CV *et al.* (1978). Determination of water soluble imidazo-1,4-benzodiazepines in blood by electron- capture gas–liquid chromatography and in urine by differential pulse polaragraphy. *J Chromatogr* 145: 81–96.
Sautou V *et al.* (1991). Solid-phase extraction of midazolam and two of its metabolites from plasma for high-performance liquid chromatographic analysis. *J Chromatogr* 571: 298–304.
Smith MT *et al.* (1981). The pharmacokinetics of midazolam in man. *Eur J Clin Pharmacol* 19: 271–278.
Van Brandt N *et al.* (1997). A rapid high-performance liquid chromatographic method for the measurement of midazolam plasma concentrations during long-term infusion in ICU patients. *Ther Drug Monit* 19: 352–357.
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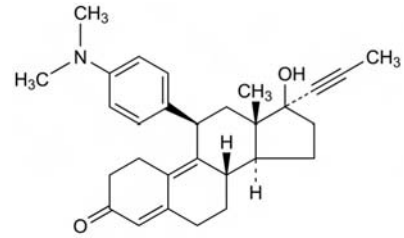
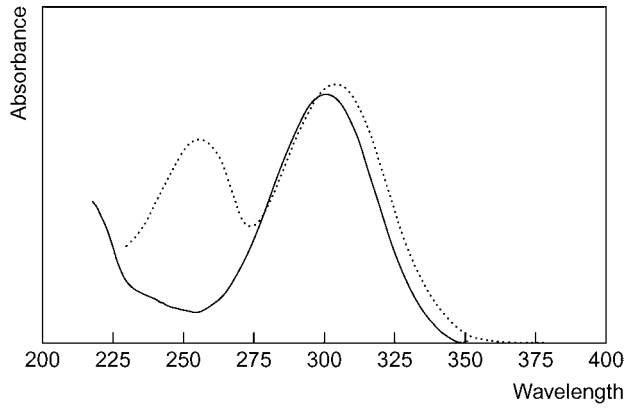
Mifepristone

Antiprogestogenic Steroid
$C_{29}H_{35}NO_2$ = 429.6
CAS—84371-65-3

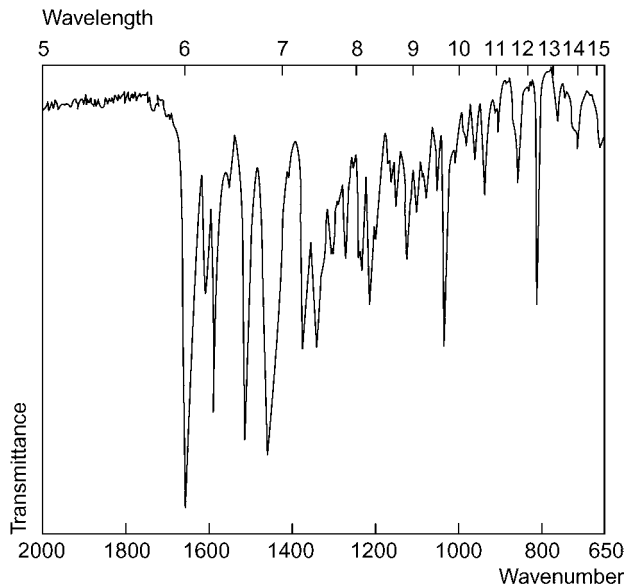
IUPAC Name (11β,17β)-11-[4-(Dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one
Synonyms RU-486; RU-38486.
Proprietary Name *Mifegyne*



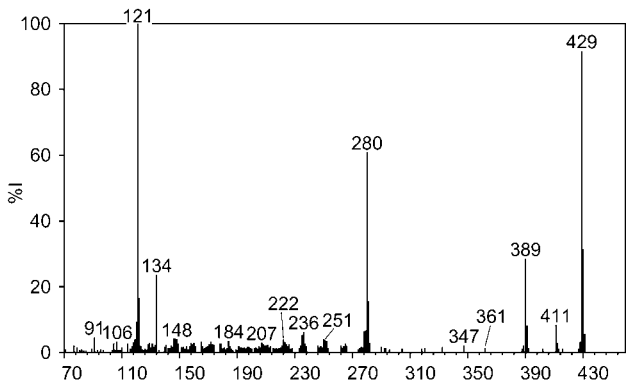
Chemical Properties Mp 150°.
Ultraviolet Spectrum Acid (0.1 mol/L HCl in 95% ethanol)—302 nm; base (0.1 mol/L NaOH in 95% ethanol)—256, 305 nm.



Infrared Spectrum Principal peaks at wavenumbers 1657, 1517, 1592, 1378, 1348, 1038 cm^{-1} (Nujol).



Mass Spectrum Principal ions at *m/z* 121, 429, 280, 430, 389, 134, 122, 281.



Quantification

Plasma **HPLC** Column: ODS RP (200 × 3.5 mm i.d.). Mobile phase: methanol : methylcyanide : water (42:28:30), flow rate 1 mL/min. Detection: not specified. Retention time: 10.37 min; monodemethylated metabolite, 6.92 min; didemethylated metabolite, 5.04 min; hydroxylated metabolite, 6.14 min. Limit of detection, 0.01 mg/L [Shi *et al.* 1993].

Disposition in the Body Mifepristone is rapidly absorbed after oral administration with peak plasma concentrations reached within 1 to 2 h. It undergoes metabolism in the liver, by rapid demethylation and hydroxylation, and its metabolites are excreted via the bile and eliminated in faeces. Metabolites include monodemethylated, didemethylated and hydroxylated derivatives which may undergo further metabolism before excretion. Only a small proportion of the parent drug is detected in urine.

Therapeutic Concentration

Healthy female volunteers were administered with 12.5, 25, 50 or 100 mg mifepristone twice daily for 4 days. The peak plasma concentrations ranged between 1.4 and 1.7 mg/L for a dose ≥50 mg twice daily [Heikinheimo *et al.* 1989].

Nine non-pregnant and 36 pregnant healthy women, mean age 28.9 years, were administered doses of 25, 100, 400 and 600 mg mifepristone. For the non-pregnant women, mean peak plasma concentrations were 0.82, 1.66, 2.09 and 2.29 mg/L for the four doses observed at 1.7, 1.7, 1.4 and 1.6 h, respectively. The pregnant women produced concentrations of 0.75, 1.32, 1.73 and 1.91 mg/L, respectively, at 1.8, 2.1, 1.6 and 1.5 h [Shi *et al.* 1993].

Bioavailability 70% (also reported as 40% after a 100 mg oral dose).

Half-life 18 h; also reported as 54 to 90 h, possibly due to cross-reacting of the metabolites.

Volume of Distribution Steady state, 25.7 L (8 L after 280 mg IV dose).

Clearance Plasma, 3 L/h.

Protein Binding 98%.

Dose The usual dose is 600 mg.

Heikinheimo O *et al.* (1989). Pharmacokinetics of the antiprogesterone RU 486 in women during multiple dose administration. *J Steroid Biochem* 32: 21–25.

Shi Y *et al.* (1993). Pharmacokinetic study of RU 486 and its metabolites after oral administration of single doses to pregnant and non-pregnant women. *Contraception* 48: 133–149.

Miglitol

α-*Glucosidase Inhibitor, Antidiabetic, Desoxynojirimycin Derivative*

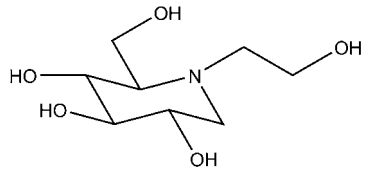
$C_8H_{17}NO_5$ = 207.2

CAS—72432-03-2

IUPAC Name (2*R*,3*R*,4*R*,5*S*)-1-(2-Hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol

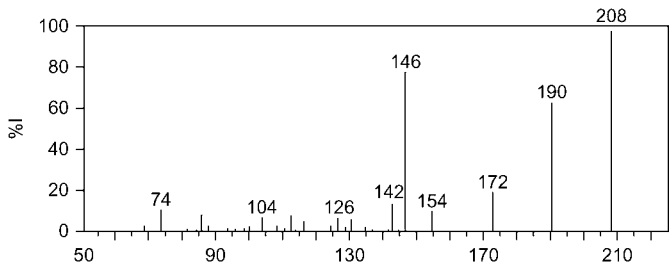
Synonyms Bay m 1099; 1,5-dideoxy-1,5-[(2-hydroxyethyl)imino]-D-glucitol; *N*-(β-hydroxyethyl)-1-deoxynojirimycin; *N*-(2-hydroxyethyl)moranoline.

Proprietary Names *Diastabol*; *Glyset*.



Chemical Properties Crystals. Mp 114°. Soluble in water. pK_a 5.9 [O'Neil *et al.* 2006]. Log *P* (octanol/water), −3.21 [Wishart *et al.* 2006]; distribution in blood (erythrocyte/plasma) log *P* 0.22 [Ahr *et al.* 1997]. Stock solutions showed no significant degradation when stored at room temperature for 12 h and they were stable for more than 2 months at −20° after 3 freeze–thaw cycles [Li *et al.* 2007]. Stable in human plasma for 3 freeze–thaw cycles, when stored below –50° and thawed to room temperature [Nirogi *et al.* 2006].

Mass Spectrum Principal ions at *m/z* 208, 190, 146, 172, 142, 112 .



Quantification

Plasma **LC-MS** Column: Nucleosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate, flow rate 1.0 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 1.0 µg/L [Li *et al.* 2007]. Column: YMC-Pack Ph reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium formate buffer : acetonitrile (40 : 60), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 mg/L [Nirogi *et al.* 2006].

Disposition in the Body Absorption of miglitol is saturable at high doses, with 25 mg being completely absorbed and 100 mg only 50 to 70% absorbed. Miglitilol is not metabolised in humans or any other species studied. After IV administration, miglitol is excreted rapidly and completely via the renal route.

Therapeutic Concentration After the IV administration of 1.4 mg/kg radiolabelled miglitol, the first elimination phase was characterised by a half-life of 1.8 h. After oral administration of 1.4 mg/kg, plasma concentrations reached 1.13 ± 1.35 kg/L at 2.3 ± 1.35 h [Ahr *et al.* 1997].

Toxicity Miglitol is unlikely to produce hypoglycaemia in overdose but abdominal discomfort and diarrhoea may occur. There are no published cases of overdose [Spiller 1998].

Half-life Approximately 0.4 to 1.8 h.

Volume of Distribution 0.3 to 0.8 L/kg.

Clearance Range of the glomerular filtration rate.

Distribution in Blood Log *P* (erythrocyte/plasma) 0.22.

Protein Binding Negligible (<4%).

Dose Oral dose 25 mg three times daily, increasing if necessary to a maximum of 100 mg three times a day.

Ahr HJ *et al.* (1997). Pharmacokinetics of miglitol. Absorption, distribution, metabolism, and excretion following administration to rats, dogs, and man. *Arzneimittelforschung* 47: 734–745.

Li X *et al.* (2007). Determination of miglitol in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 21: 247–251.

Nirogi RV *et al.* (2006). Liquid chromatographic tandem mass spectrometry method for the quantification of miglitol in human plasma. *Arzneimittelforschung* 56: 328–336.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Spiller HA (1998). Management of antidiabetic medications in overdose. *Drug Saf* 19: 411–424.

Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Milnacipran

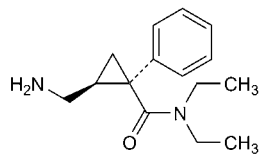
Antidepressant

$C_{15}H_{22}N_2O$ = 246.4

CAS—92623-85-3

IUPAC Name (1*R*,2*S*)-*rel*-2-(Aminomethyl)-*N*,*N*-diethyl-1-phenylcyclopropanecarboxamide

Synonym Mildacipran



Chemical Properties Plasma samples were unaffected by 3 freeze-thaw processes or by storage at room temperature for 24 h [Puozzo *et al.* 2004].

Milnacipran Hydrochloride

$C_{15}H_{22}N_2O$,HCl = 282.8

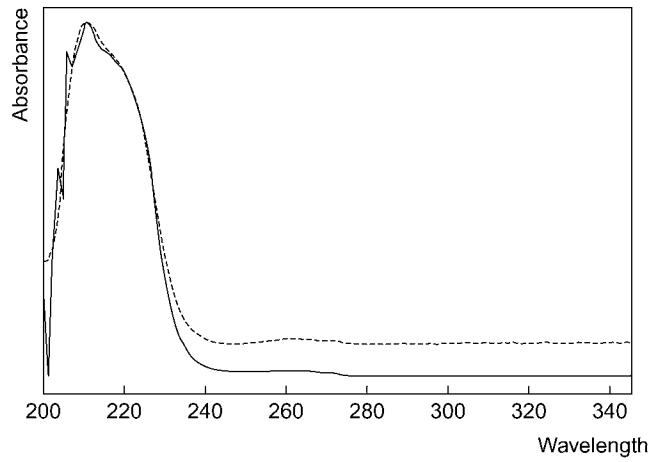
CAS—101152-94-7

Synonym F-2207

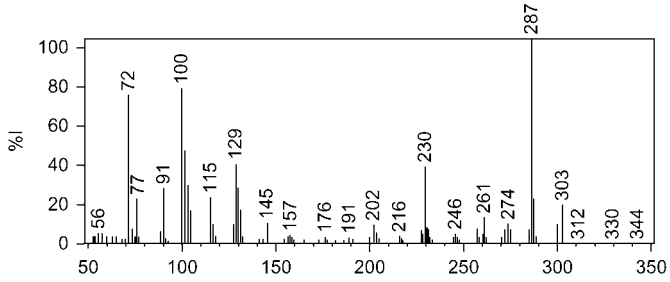
Proprietary Names *Ixel*; *Toledomin*.

Chemical Properties A white crystalline powder. Mp 180°.

Ultraviolet Spectrum



Mass Spectrum



Quantification

Blood **HPLC** Column: Symmetry (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.1 mol/L ammonium acetate (30 : 30 : 40), flow rate 1.0 mL/min. DAD (λ = 200 to 350 nm). Retention time: 4.64 min. Limit of detection not reported [Rop *et al.* 2002].

Plasma **HPLC** Column: YMC-Pack ODS-AQ (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L potassium dihydrogen phosphate : acetonitrile (20 : 80 for 10 min to 30 : 70 at 20 min to 20 : 80 at 25 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 340 nm, λ_{em} = 440 nm). Limit of detection not reported [Higuchi *et al.* 2003].

LC-MS Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 6.5 min. Limit of quantification, 0.15 mg/L, limit of detection, 0.08 mg/L [Shinozuka *et al.* 2006]. Column: Zorbax C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 6.67 mmol/L phosphate buffer (pH 7.0) : methanol (37 : 63), flow rate 0.5 mL/min. CI. Limit of quantification, 5 µg/L [Puozzo *et al.* 2004].

Serum **HPLC** Column: Hypersil ODS (150 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 5 mmol/L sodium acetate (pH 4.5; 70 : 30), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 340 nm, λ_{em} = 520 nm). Retention time: 11.1 min. Limit of quantification, 5 mg/L, limit of detection, 2.5 mg/L [Lacassie *et al.* 2000].

Disposition in the Body Absorption of milnacipran is rapid and at least 90% is absorbed. First-pass metabolism is low. The drug shows linear pharmacokinetics and peak plasma concentrations are reached within 3 h of dosing. Steady-state concentrations are rapidly achieved within 2 to 3 days of administration and the drug does not accumulate after multiple doses. The major metabolite is the glucuronide conjugate of the parent drug and no active metabolites has been identified: 50 to 60% of the drug is excreted unchanged, 20–30% as the glucurono-conjugated drug, and <20% as the glucurono-conjugated phase I metabolites including *N*-dealkylated milnacipran. Urine is the main route of elimination. More than 90% of the dose recovered in urine, with 5% in faeces over 96 h. Elimination is rapid and over 85% of the initial dose is recovered within the first 24 h. The metabolites are not pharmacologically active at clinically relevant doses. The liver and the kidney are both involved in the elimination of the drug. Milnacipran oxidative metabolism is not mediated through CYP2D6 or CYP2C19 polymorphic pathways, nor does it significantly interact with CYP1A2, CYP2C19, CYP2D6 or CYP3A4 [Puozzo *et al.* 2005]

Therapeutic Concentration

Six healthy male and female volunteers (aged 37–64 years; mean, 51.2 years) and 11 patients with moderate to severe liver impairment (aged 38–63 years; mean 53.4 years) were administered a 50 mg single dose with a light breakfast after a 10 h overnight fast. The peak plasma concentrations of unchanged milnacipran were 0.135 and 0.170 mg/L for the healthy individuals and patients observed at 2.0 and 2.7 h, respectively. The total drug concentrations were 0.323 and 0.242 mg/L attained at 2.5 and 3.6 h, respectively [Puozzo *et al.* 1998a].

Six healthy volunteers (aged 25–36 years; mean 30 years) with creatinine clearance 93–147 mL/min and 8 patients with renal impairment (aged 27–65 years; mean 51.5 years) and creatinine clearance 9–55.9 mL/min were administered 50 mg milnacipran orally after an overnight fast and were fed a light breakfast 2 h after dosing. The peak plasma concentrations were 0.147 mg/L and 0.190 mg/L for the unchanged drug and 0.269 and 0.530 mg/L for total drug concentrations for the healthy individuals and patients, respectively. These concentrations were observed at 1.9 h (unchanged concentration) and 1.7 h (total) for the healthy individuals and 1.9 h (unchanged) and 4.9 h (total) for the patients. [Puozzo *et al.* 1998b].

Note For pharmacokinetic parameters in depressed patients, see Higuchi *et al.* [2003].

Toxicity

A 44-year-old woman died in a road traffic accident. Her blood milnacipran concentration was 3.15 mg/L, 10.5 times the therapeutic concentration [Rop *et al.* 2002].

Half-life 6.1–8.1 h

Bioavailability 85%.

Volume of Distribution 5.5 L/kg (healthy); 6.3 L/kg (patients with liver impairment).

Clearance Total apparent clearance in healthy individuals, 40.0 L/h with oral administration and 34.3 L/h with IV administration; in patients with liver impairment, 27.6 L/h with oral administration and 29.6 L/h with IV administration.

Protein Binding Low, non-saturable binding to plasma protein is ~13%.

Note For a general review of milnacipran, see Spencer and Wilde [1998].

Dose The usual oral dose is 100 mg daily.

Higuchi H *et al.* (2003). Milnacipran plasma levels and antidepressant response in Japanese major depressive patients. *Hum PsychoPharmacol* 18: 255–259.

Lacassie E *et al.* (2000). Methods for the determination of seven selective serotonin reuptake inhibitors and three active metabolites in human serum using high-performance liquid chromatography and gas chromatography. *J Chromatogr B Biomed Sci Appl* 742: 229–238.

Puozzo C *et al.* (1998a). Pharmacokinetics of milnacipran in liver impairment. *Eur J Drug Metab Pharmacokinet* 23: 273–279.

Puozzo C *et al.* (1998b). Pharmacokinetics of milnacipran in renal impairment. *Eur J Drug Metab Pharmacokinet* 23: 280–286.

Puozzo C *et al.* (2004). Determination of milnacipran, a serotonin and noradrenaline reuptake inhibitor, in human plasma using liquid chromatography with spectrofluorimetric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 806: 221–228.

Puozzo C *et al.* (2005). Lack of interaction of milnacipran with the cytochrome p450 isoenzymes frequently involved in the metabolism of antidepressants. *Clin Pharmacokinet* 44: 977–988.

Rop PP *et al.* (2002). Blood concentration of milnacipran in a case of a fatal automobile accident. *J Anal Toxicol* 26: 123–126.

Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.

Spencer CM, Wilde MI (1998). Milnacipran. A review of its use in depression. *Drugs* 56: 405–427.

Milrinone

Cardiotonic, Phosphodiesterase Inhibitor

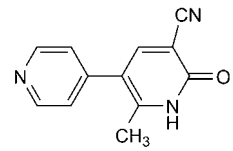
$C_{12}H_9N_3O$ = 211.2

CAS—78415-72-2

IUPAC Name 1-(2,6-Dimethylphenoxy)propan-2-amine

Synonyms 1,6-Dihydro-2-methyl-6-oxo-(3,4′-bipyridine)-5-carbonitrile; Win-47203-2.

Proprietary Names *Corotrop*; *Corotrope*; *Milrila*.



Chemical Properties Off-white crystals from ethanol or dimethylformamide (DMF) and water. Mp <300°. Very slightly soluble in water and chloroform, slightly soluble in methanol. Log *P* (octanol/water), 1.17.

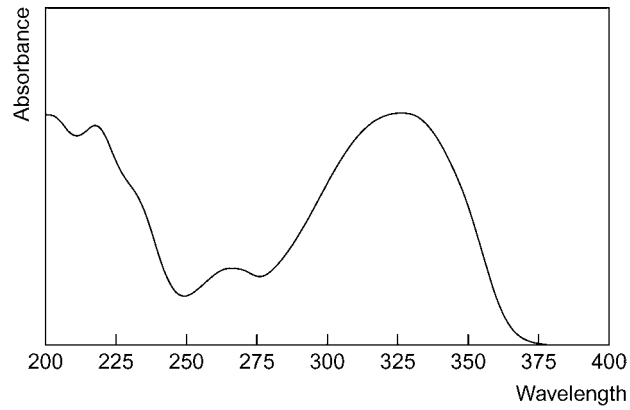
Milrinone Lactate

$C_{12}H_9N_3O,C_3H_6O_3$ = 301.3

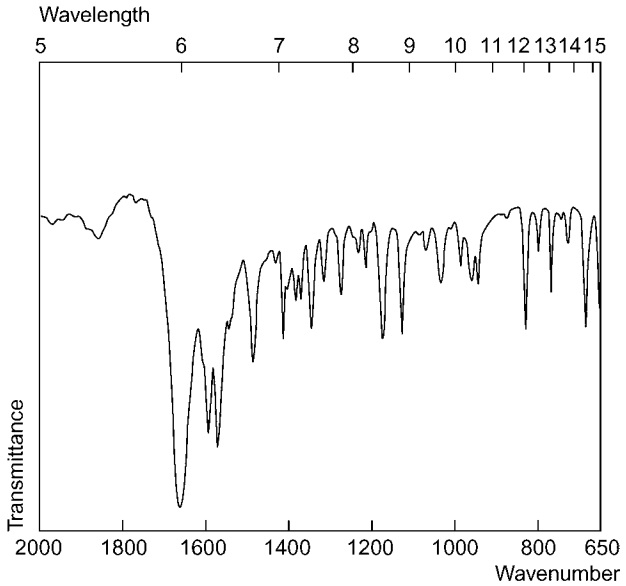
CAS—100286-97-3

Proprietary Name *Primacor*

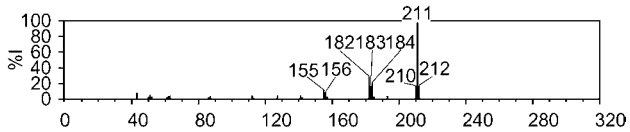
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—266, 322 nm; basic—282, 331 nm.



Infrared Spectrum Principal peaks at wavenumbers 1666, 1574, 590 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 211, 182, 184, 210, 212, 183, 155, 156.



Quantification

Plasma **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogen phosphate-3 mmol/L sulfuric acid-3.6 mmol/L TEA : methanol : acetonitrile (90 : 7 : 3), flow rate 1.0 mL/min. UV detection (λ = 326 nm). Retention time: 4.8 min. Limit of detection, 10 µg/L [Brocks *et al.* 2005]. Column: Partisil ODS-3 (250 × 4.6 mm i.d., 10 µm). Mobile phase: 0.1 mol/L sodium phosphate : acetonitrile (4 : 1), flow rate 2.5 mL/min. UV detection (λ = 340 nm). Limit of quantification, 5 µg/L [Woolfrey *et al.* 1995]. Column: RP Select B RT 250-4. Mobile phase: methanol : 25 mmol/L ammonium acetate buffer (pH 3.75; 25 : 75), flow rate 0.75 mL/min. UV detection (λ = 331 nm). *k* value: 2.3. Limit of detection, 5 µg/L [Verrijk *et al.* 1989]. Column: ODS (Spherisorb, 250 × 4.0 mm i.d., 5 µm). Mobile phase: 0.1 mol/L phosphate buffer (pH 6) : tetrahydrofuran:acetonitrile (73 : 2 : 25), flow rate 1 mL/min. UV detection (λ = 340 nm). Retention time: 4.3 min. Limit of detection, 5 µg/L. [Oddie *et al.* 1986]. Column: Partisil 10/25 ODS-3 (10 µm). Mobile phase: tetrahydrofuran : acetonitrile : 0.1 mol/L phosphate buffer (pH 6.0; 28 : 260 : 1000), flow rate 1.2 mL/min. UV detection (λ = 340 nm). Retention time: ~5 min. Limit of quantification, 2.3 µg/L [Edelson *et al.* 1983].

Urine **HPLC** Column: Partisil ODS-3 (250 × 4.6 mm i.d., 10 µm). Mobile phase: 0.1 mol/L sodium phosphate buffer : acetonitrile : tetrahydrofuran (250 : 65 : 7), flow rate 1.5 mL/min. UV detection (λ = 340 nm). Limit of quantification, 100 µg/L [Woolfrey *et al.* 1995]. See Plasma. Flow rate 1.5 mL/min. Limit of quantification, 0.003 mg/L [Edelson *et al.* 1983].

Disposition in the Body Milrinone is rapidly and almost completely absorbed after oral administration but in practice it is given IV because of the association of a higher mortality rate with oral administration. It is eliminated mainly in urine, with 80–85% of a dose detected as the unchanged drug and the remainder as its *O*-glucuronide metabolite. There are 5 metabolites but the *O*-glucuronide represents the major pathway of biotransformation.

Therapeutic Concentration The serum therapeutic concentration is 150–250 µg/L.

A group of 7 patients (aged 19–65 years) with stable chronic renal impairment (creatinine clearance 30–63 mL/min/1.73 m^2), a group of 7 patients (aged 51–70 years; mean 63 years) with creatinine clearance 9–29 mL/min/1.73 m^2, and 7 healthy men and women (aged 41–58 years; mean 48 years) were administered a single oral dose of 5 mg milrinone. A mean peak plasma concentration of 162 µg/L was reached for the healthy individuals in 0.64 h, and of 210 µg/L in 1.19 h for both groups with renal impairment [Larsson *et al.* 1986].

Seven adult patients (3 male) who needed inotropic support after cardiac surgery were enrolled and given an IV loading dose of milrinone 50 µg/kg over 10 min followed by an IV infusion of 0.5 µg/kg/min for a minimum of 5 h. Plasma concentrations of milrinone exceeded 100 µg/L within 2 min of starting the loading dose and were maintained above this concentration for the duration of the infusion [Das *et al.* 1994].

Twenty five patients just before or immediately after separation from cardiopulmonary bypass were administered a 50 µg/kg dose of milrinone plus 0.5 µg/kg/min milrinone. This resulted in plasma concentrations above 100 µg/L. The central compartment volume was 102 mL/kg; volume of distribution was 1698 mL/kg and elimination clearance was 1.88 mL/kg/min [Bailey *et al.* 1994].

Note For pharmacokinetics of milrinone in patients with CHF and moderate and severe renal failure, see Woolfrey *et al.* [1995].

Toxicity The toxic serum concentration is 0.3 mg/L.

In a study involving 1088 patients with severe chronic liver failure, 561 were treated with 40 mg milrinone and 527 received 40 mg placebo daily for 9 months. The study showed a 28% increase in mortality for those treated with milrinone compared with the placebo. An increase of 34% in cardiac mortality was also observed [Packer *et al.* 1991].

Bioavailability Healthy volunteers 92%; patients with congestive heart failure 76% (also reported as 80%).

Half-life Healthy individuals following oral and IV doses: 50 to 55 min. Patients with congestive heart failure: 1.7 h (IV dose) and 2.1 h (oral dose). Patients with renal impairment: 1.8 h (moderate impairment) and 3.2 h (severe impairment). Also reported as 2.3 h.

Volume of Distribution After doses of 10–125 µg/kg administered to healthy individuals: 0.32 L/kg. After IV doses of 12.5 to 75 µg/kg administered to patients with CHF: 0.3 to 0.42 L/kg.

Clearance 0.13 L/kg/h.

Protein Binding 70–80%.

Note For a general review of phosphodiesterase inhibitors in heart failure, see Arnold [1993].

Dose An initial loading dose of 50 µg/kg bodyweight is administered over 10 min followed by a maintenance dose titrated between 0.375 and 0.75 µg/kg/min. The total daily dose should not exceed 1.13 mg/kg. Dosage may be reduced in patients with renal impairment.

Arnold JM (1993). The role of phosphodiesterase inhibitors in heart failure. *Pharmacol Ther* 57: 161–170.

Bailey JM *et al.* (1994). Pharmacokinetics of intravenous milrinone in patients undergoing cardiac surgery. *Anesthesiology* 81: 616–622.

Brocks DR *et al.* (2005). A sensitive and specific high performance liquid chromatographic assay for milrinone in rat and human plasma using a commercially available internal standard and low sample volume. *J Pharm Pharm Sci* 8: 124–131.

Das PA *et al.* (1994). Disposition of milrinone in patients after cardiac surgery. *Br J Anaesth* 72: 426–429.

Edelson J *et al.* (1983). High-performance liquid chromatographic analysis of milrinone in plasma and urine. Intravenous pharmacokinetics in the dog. *J Chromatogr* 276: 456–462.

Larsson R *et al.* (1986). Pharmacokinetics and effects on blood pressure of a single oral dose of milrinone in healthy subjects and in patients with renal impairment. *Eur J Clin Pharmacol* 29: 549–553.

Oddie CJ *et al.* (1986). Analysis of milrinone in plasma using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr* 374: 209–214.

Packer M *et al.* (1991). Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. *N Engl J Med* 325: 1468–1475.

Verrijk R *et al.* (1989). High-performance liquid chromatographic determination of milrinone in biological tissues and fluids. *J Chromatogr* 491: 265–268.

Woolfrey SG *et al.* (1995). Dose regimen adjustment for milrinone in congestive heart failure patients with moderate and severe renal failure. *J Pharm Pharmacol* 47: 651–655.

Minocycline

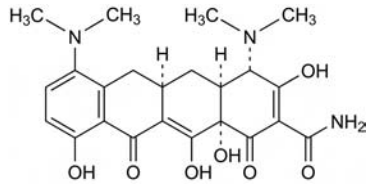
Antibacterial

$C_{23}H_{27}N_3O_7$ = 457.5

CAS—10118-90-8

IUPAC Name (2*Z*,4*S*,4*aS*,5*aR*,12*aS*)-2-[Amino(hydroxy)methylidene]-4,7-bis(dimethylamino)-10,11,12*a*-trihydroxy-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-1,3,12-trione

Synonym [4*S*-(4α,4aα,5aα,12aα)]-4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide



Chemical Properties A bright yellow-orange amorphous solid. pK_a 2.8, 5.0, 7.8, 9.5. Log *P* (octanol/water), 0.1.

Minocycline Hydrochloride

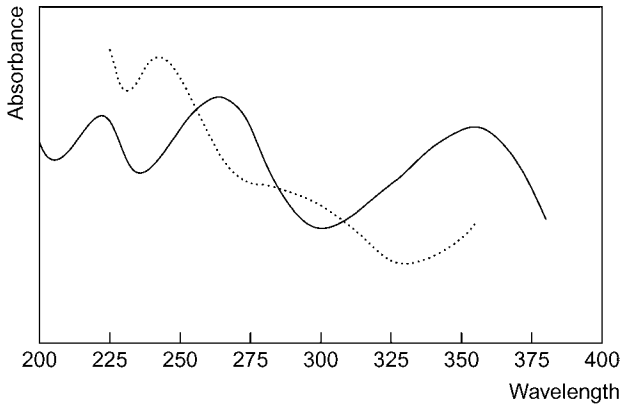
$C_{23}H_{27}N_3O_7$,HCl = 493.9

CAS—13614-98-7

Proprietary Names *Akamin*; *Aknemin*; *Aknin-N*; *Aknosan*; *Arestin*; *Blemix*; *Cyclomin*; *Dentomycin(e)*; *Dermirex*; *Dynacin*; *Klinomycin*; *Klinotab*; *Logryx*; *Mino(x)*; *Minocin*; *Minoclin*; *Minogal*; *Minomycin*; *Minotab(s)*; *Mynocine*; *Udima*; *Ultramycin*; *Vectrin*; *Zacnan*.

Chemical Properties A yellow crystalline powder. Soluble in water and in solutions of alkali hydroxides and carbonates; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Colour Test Sulfuric acid—yellow.
Thin-layer Chromatography System TAE—R_f 0.88; System TAJ—R_f 0.02; System TAK—R_f 0.00; System TAL—R_f 0.08.
High Performance Liquid Chromatography System HY—RI 240; system HAA—retention time 22.6 min.
Ultraviolet Spectrum Aqueous acid—265 (A_1^1=400b), 354 nm; aqueous alkali—243 nm (A_1^1=414b).

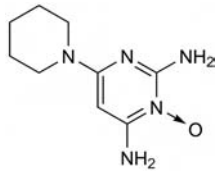


Infrared Spectrum Principal peaks at wavenumbers 1600, 1645, 1525, 1225, 1310, 1050 cm^{-1} (minocycline hydrochloride, KBr disk).
Quantitative
Plasma **HPLC** UV detection. Limit of detection, 100 µg/L [Orti *et al.* 2000]. UV detection. Limit of detection, 30 µg/L [Mascher 1998].
HPLC-MS Limit of detection, 5 µg/L [Araujo *et al.* 2001].
Parotid Saliva **HPLC** See Plasma [Orti *et al.* 2000].
Disposition in the Body
Therapeutic Concentration
In 223 acne patients receiving minocycline at the recommended dose (100 mg daily) or a high dose (200 mg daily) in the form of a standard preparation or a modified-release formulation, mean serum levels were as follows: 0.15 to 3.51 mg/L (mean 1.20 mg/L for males and 1.68 mg/L for females) for 100 mg daily of the standard preparation; 0.42 to 5.15 mg/L (mean 2.18 for males and 2.58 for females) for 200 mg daily of the standard preparation; 0.16 to 3.77 mg/L (mean 1.38 for males, 1.56 for females) for 100 mg daily of the modified-release formulation; 0.36 to 6.81 mg/L (mean 2.62 for males, 2.83 for females) for 200 mg daily of the modified-release formulation (plasma concentrations were measured, at steady state, within 6 h of the previous dose) [Gardner *et al.* 1997].
Dose The equivalent of 100 to 200 mg of minocycline daily.

Araujo MV *et al.* (2001). Determination of minocycline in human plasma by high-performance liquid chromatography coupled to tandem mass spectrometry: application to bioequivalence study. *J Chromatogr B Biomed Sci Appl* 755: 1–7.
Gardner KJ *et al.* (1997). Comparison of serum antibiotic levels in acne patients receiving the standard or a modified release formulation of minocycline hydrochloride. *Clin Exp Dermatol* 22: 72–76.
Mascher HJ (1998). Determination of minocycline in human plasma by high-performance liquid chromatography with UV detection after liquid-liquid extraction. *J Chromatogr A* 812: 339–342.
Orti V *et al.* (2000). High-performance liquid chromatographic assay for minocycline in human plasma and parotid saliva. *J Chromatogr B Biomed Sci Appl* 738: 357–365.

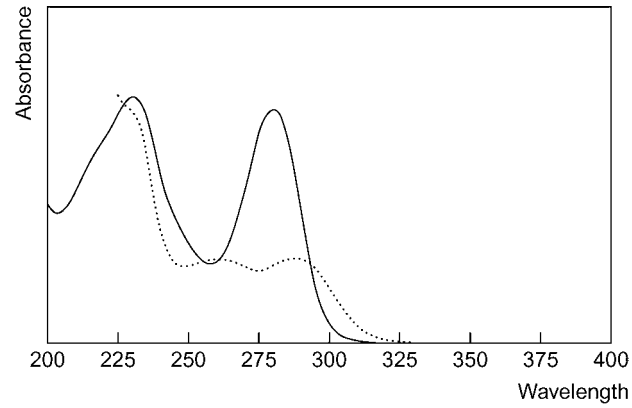
Minoxidil

Antihypertensive
$C_9H_{15}N_5O$ = 209.3
CAS—38304-91-5
IUPAC Name 3-Hydroxy-2-imino-6-piperidin-1-ylpyrimidin-4-amine
Synonyms PDP; 6-(1-piperidinyl)-2,4-pyrimidinediamine 3-oxide; U-10858.
Proprietary Names *Apo-Gain*; *Apohair*; *Alopexy*; *Alostil*; *Aloxidil*; *Folcress*; *Hairgain*; *Headway*; *Loniten*; *Lonnoten*; *Lonolox*; *Minona*; *Minovital*; *Minoxidine*; *Minox(i)*; *Minoxigaine*; *Minoximen*; *Moxiral*; *Neoxidil*; *Normoxidil*; *Ralogaine*; *Regaine*; *Regro*; *Rogaine*; *Tricoxidil*; *Unipexil*.

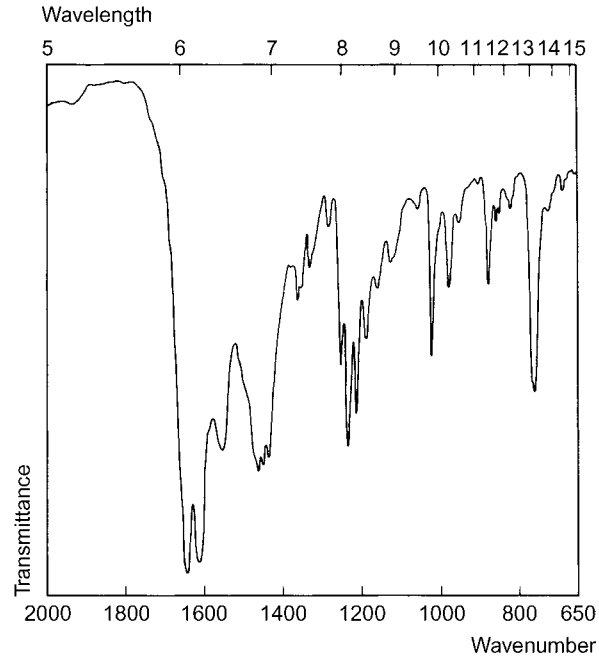


Chemical Properties A white crystalline solid. Mp about 225°, with decomposition. Soluble 1 in about 500 of water; readily soluble in ethanol and propylene glycol; practically insoluble in chloroform. pK_a 4.6. Log *P* (octanol/water), 1.2.

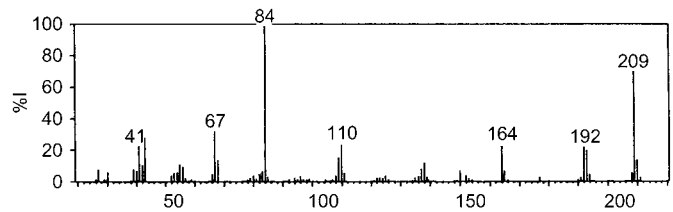
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.00; system TC—R_f 0.03; system TE—R_f 0.18; system TL—R_f 0.00; system TAE—R_f 0.44.
High Performance Liquid Chromatography System HX—RI 297; system HZ—retention time 2.4 min; system HAA—retention time 9.8 min.
Ultraviolet Spectrum Aqueous acid—230, 281 nm (A_1^1=547b); aqueous alkali—262, 288 nm.



Infrared Spectrum Principal peaks at wavenumbers 1640, 1610, 1550, 1231, 1210, 758 (KBr disk).



Mass Spectrum Principal ions at *m/z* 84, 209, 67, 43, 110, 41, 192, 164.



Quantification
Plasma **HPLC** Electrochemical detection. Limit of detection, 500 ng/L [Carrum *et al.* 1986].
Serum **Radioimmunoassay** Limit of detection, 3 µg/L [Royer *et al.* 1977].
Disposition in the Body Minoxidil is readily absorbed after oral administration. >80% of a dose is excreted in the urine in 24 h, mainly as glucuronide conjugates with <10% as unchanged drug; about 3% of a dose is eliminated in the faeces.
Therapeutic Concentration
Following daily oral administration of 27 to 30 mg to 6 subjects, serum concentrations of 0.04 to 0.25 (mean 0.10) mg/L were reported 1 to 2 h after a dose [Lowenthal *et al.* 1978].

Toxicity

The total serum-minoxidil concentration in a 20-year-old female who survived after ingesting an unknown quantity of minoxidil tablets in a suicide attempt was 3.14 mg/L [Poff, Rose 1992].

Half-life Plasma half-life, about 3 to 4 h.

Protein Binding Not significantly bound.

Note For a review of minoxidil, see Campese [1981].

Dose 5 to 50 mg daily; up to 100 mg daily has been given.

Campese VM (1981). Minoxidil: a review of its pharmacological properties and therapeutic use. *Drugs* 22: 257–278.

Carrum G *et al.* (1986). Minoxidil analysis in human plasma using high-performance liquid chromatography with electrochemical detection. Application to pharmacokinetic studies. *J Chromatogr* 381: 127–135.

Lowenthal DT *et al.* (1978). Long-term clinical effects, bioavailability, and kinetics of minoxidil in relation to renal function. *J Clin Pharmacol* 18: 500–508.

Poff SW, Rose SR (1992). Minoxidil overdose with ECG changes: case report and review. *J Emerg Med* 10: 53–57.

Royer ME *et al.* (1977). Radioimmunoassay of minoxidil in human serum. *J Pharm Sci* 66: 1266–1269.

Mirtazapine

Antidepressant, Piperazinoazepine

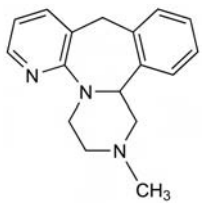
$C_{17}H_{19}N_3$ = 265.4

CAS—61337-67-5

IUPAC Name 1,2,3,4,10,14*b*-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine

Synonyms 6-Azamianserin; mirtazepine; mepirzepine; Org-3770.

Proprietary Names *Remergil*; *Remeron*; *Zispin*.



Chemical Properties A white to creamy white crystalline powder. Mp 114° to 116°. It is slightly soluble in water. pK_a, 7.1. Log *P* (octanol/water), 3.3 [Kelder *et al.* 1997]. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

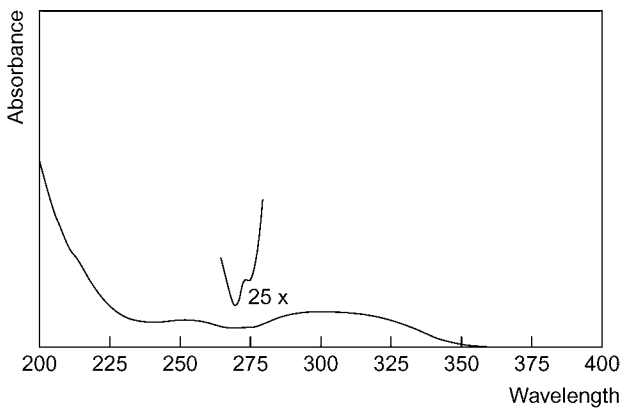
Gas Chromatography System GA—RI 2250; system GB—mirtazapine RI 2361; M (nor-) RI 2414; M (oxo-) RI 2665.

Column: HP-5 (15 m × 0.25 mm i.d., 0.25 µm) and HP-35 (15 m × 0.32 mm i.d., 0.32 µm). Temperature programme: 140° for 0.50 min to 300° at 10°/min for 10.5 min. IS: carbinoxamide maleate. NPD. Retention time: mirtazapine 9.76 min, IS 8.2 min (HP-5 column); mirtazapine 11.68 min, IS 9.6 min (HP-35) [Anderson *et al.* 1999].

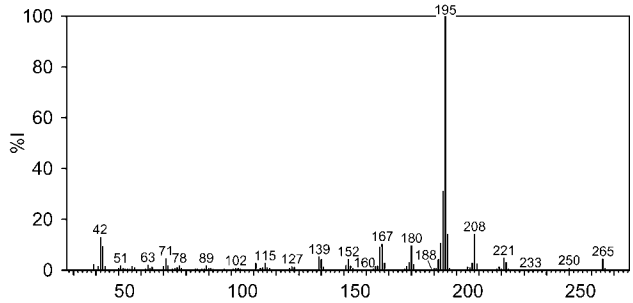
High Performance Liquid Chromatography System HZ—mirtazapine RT 2.8 min; desmethylmirtazapine RT 2.3 min.

Column: Chiralpak AD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : ethanol : propan-2-ol (98 : 1 : 1), flow rate 1.5 mL/min. IS: imipramine. UV detection (λ= 290 nm). Retention time: (+)-mirtazapine 12.5 min, (−)-mirtazapine 15.0 min [Dodd *et al.* 2000].

Ultraviolet Spectrum Aqueous acid (0.025 mol/L sulfuric acid)—314.5, 252.5 nm.



Mass Spectrum Principal ions at *m/z* 195, 208, 42, 180, 167, 71, 111, 265 (mirtazapine); *m/z* 195, 194, 196, 209, 180, 167 (desmethylmirtazapine).



Quantification

Blood **GC** Column: HP-50+ (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 50° for 0.5 min to 295° at 35° for 5.5 min. NPD. Limit of detection, 0.01 mg/L [Kirkton, McIntyre 2006]. NPD [Sánchez de la Torre *et al.* 2005]. Column: HP-5 (15 m × 0.25 mm i.d., 0.25 µm) or HP-35 (15 m × 0.32 mm i.d., 0.32 µm). Temperature programme: 140° for 0.5 min to 300° at 10°/min for 10.5 min. NPD. Retention times: 9.76 min (HP-5) and 11.8 min (HP-35). Limit of detection, 25 µg/L [Anderson *et al.* 1999]. Column: DB-5MS 5% phenylmethyl silicone (20 m × 0.18 mm i.d., 0.18 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 110° for 1.0 min to 200° at 20°/min to 290° at 10°/min for 7 min. Limit of detection, 0.01 mg/L [Moore *et al.* 1999].

GC-MS Column: DB-5 cross-linked 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 2 min to 180° at 30°/min to 280° at 5°/min for 19 min. Full scan mode. Retention time: 18.0 min. Limit of quantification, 0.05 mg/L [Paterson *et al.* 2004].

LC-MS Column: XTerra RP_{18} (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2, 15 : 85 for 1 min to 35 : 65 over 12 min for 1 min), flow rate 1 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 µg/L [Castaing *et al.* 2007]. Column: Zorbax Eclipse XDB-C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 4 mmol/L ammonium acetate (pH 3.2, 20 : 20 : 60), flow rate 0.28 mL/min. TIS, MRM acquisition mode. Limit of detection, 1 µg/L [Wenzel *et al.* 2006].

Plasma **GC** Column: 5% phenylmethyl silicone cross-linked (25 m × 0.3 mm i.d., 0.54 µm). Carrier gas: He, 6 mL/min. Temperature programme: 80° for 1 min to 190° at 30°/min for 5 min to 270° at 10°/min for 10 min. NPD. Retention time: 9.6 min. Limit of detection, 0.5 µg/L [Paanakker, van Hal 1987].

GC-MS Column: J &W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min for 2.5 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 7 µg/L [Wille *et al.* 2007]. Column: Varian factor FOUR VF-5ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 0.5 min to 180° at 50°/min for 10 min to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection not reported [Wille *et al.* 2005].

HPLC Column: LiChrospher 60 RP-select B C_{18}. (250 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer (pH 3.8) : acetonitrile (53 : 47), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Retention time: 4.88 min. Limit of quantification, 50 µg/L [Silva *et al.* 2008]. Column: RP18 LicroCART (125 × 4 mm i.d., 5 µm). Mobile phase: 0.25 mol/L acetate buffer (pH 4.5) : acetonitrile : methanol (60 : 37 : 3), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Retention time: 8.9 min. Limit of quantification, 40 µg/L [Chaves *et al.* 2007]. Column: LiChrospher 60 RP-select B (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile-methanol : 0.25 mol/L sodium acetate buffer (pH 4.5, 35 : 65), low rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of quantification, 10 µg/L [Malfará *et al.* 2007]. Column: LiChrospher 60 RP-select B C_{18}. Mobile phase: 0.25 mol/L acetate buffer solution (pH 4.5) : acetonitrile : methanol (60 : 37 : 3), flow rate 1.0 mL/min. DAD (λ= 230 nm). Limit of quantification, 50 µg/L, limit of detection, 25 µg/L [Gonçalves Silva *et al.* 2007]. Column: Chirobiotic V (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L ammonium acetate (pH 5.0) : methanol : ethanol (60 : 25 : 15), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 390 nm). Limit of detection, 0.25 µg/L [Meineke *et al.* 2006].

See also de Santana *et al.* [2004], Dodd *et al.* [2000], Duverneuil *et al.* [2003], Maris *et al.* [1999], Meineke *et al.* [2004], Ptácek *et al.* [2003], Titier *et al.* [2003].

LC-MS Column: Chiralpak AD-RH (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : ethanol (98 : 1 : 1), flow rate 1.0 mL/min. ESI, positive ion mode. Retention time: 3.52 min. Limit of quantification, 1.25 µg/L [de Santana, Bonato 2008]. Column: Chirobiotic V (250 × 4.6 mm i.d.). Mobile phase: methanol : water : acetic acid (80 : 20 : 0.01), flow rate 0.5 mL/min. ESI, SIM acquisition mode. Limit of quantification, 0.5 µg/L [Paus *et al.* 2004].

Serum **GC-MS** Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1 min to 280° at 10°/min for 20 min. EI ionisation at 70 eV. Retention time: 21.3 min. Limit of detection, 0.025 mg/L, limit of detection, <0.025 mg/L [Maresova *et al.* 2008].

HPLC Column: Spherisorb Phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L potassium dihydrogen phosphate (pH 3.5, 20 : 80), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 290 nm, λ_{em}= 380 nm). Limit of

quantification, 10 nmol/L, limit of detection, 4 nmol/L [Reis *et al.* 2005]. Column: Lichrospher CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.15% TEA (pH 2.5; 15 : 85), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 290 nm, λ_{em} = 350 nm). Retention time: 15.07 min. Limit of quantification, 10 µg/L for mirtazapine, 5 µg/L for *N*-desmethylmirtazapine [Shams *et al.* 2004]. Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogen phosphate (pH 7.0) : acetonitrile (60 : 40), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 16.6 min. Limit of detection not reported [Frahnert *et al.* 2003].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 3.1 µg/L. [Kirchherr, Kühn-Velten 2006].

Urine **GC** See Blood [Anderson *et al.* 1999; Moore *et al.* 1999].

GC-MS Column: CP-SIL 8 CB (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 2 min to 200° at 20°/min to 280° at 5°/min. EI ionisation at 70 eV. Limit of detection, 0.23 µg/L [Salgado-Petinal *et al.* 2005]. Column: HP-1 capillary (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

HPLC See Plasma [Meineke *et al.* 2006].

LC-MS See Blood [Wenzel *et al.* 2006].

Bile **GC** See Blood [Anderson *et al.* 1999; Moore *et al.* 1999].

LC-MS See Blood [Wenzel *et al.* 2006].

Gastric Contents **GC** See Blood [Anderson *et al.* 1999; Kirkton, McIntyre 2006].

LC-MS See Blood [Wenzel *et al.* 2006].

Vitreous Humour **GC** See Blood [Anderson *et al.* 1999; Kirkton, McIntyre 2006].

Brain **LC-MS** See Blood [Wenzel *et al.* 2006].

Hair **LC-MS** Column: Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol-water (20 : 80) with 0.1% formic acid : methanol-water (90 : 10) with 0.1% formic acid (100 : 0 to 0 : 100 over 25 min), flow rate 0.5 mL/min. ESI. Retention time: 9.41 min. Limit of detection not reported [Doherty *et al.* 2007]. Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: methanol-water-formic acid-trifluoroacetic acid (20 : 80 : 0.1 : 0.1) : methanol-water-formic acid-trifluoroacetic acid (90 : 10 : 0.1,0.1, 100 : 0 to 0 : 100 over 25 min), flow rate 0.5 mL/min. API, ESI. Limit of detection, 39 nmol/L [Smyth *et al.* 2006].

Kidney **GC** See Blood [Anderson *et al.* 1999; Moore *et al.* 1999].

LC-MS See Blood [Wenzel *et al.* 2006].

Liver **GC** See Blood [Anderson *et al.* 1999; Kirkton, McIntyre 2006; Moore *et al.* 1999].

LC-MS See Blood [Wenzel *et al.* 2006].

Lung **LC-MS** See Blood [Wenzel *et al.* 2006].

Muscle **LC-MS** See Blood [Wenzel *et al.* 2006].

Spleen **GC** See Blood [Anderson *et al.* 1999].

Other **CE** Formulations. Capillary: fused silica (48.5/40 cm total/effective length, 50 µm i.d.). Running buffer: 70 mmol/L phosphate in water : methanol (75 : 25). UV detection (λ = 210 nm). Limit of quantification, 0.07–0.13%, limit of detection, 0.02–0.4% [Wynia *et al.* 1997].

Disposition in the Body Mirtazapine is rapidly and completely absorbed after oral administration from the gastrointestinal tract. Peak plasma levels are reached after approximately 2 h. Steady state is reached after 3–4 days. Linear pharmacokinetics occur with the recommended dose range. Food intake has no influence on the pharmacokinetics. It is extensively metabolised in the liver and eliminated in urine (75%) and faeces (15%) within a few days. Metabolism occurs by the P450 cytochrome oxidase pathway into 4 metabolites via demethylation and hydroxylation followed by glucuronide conjugation. CYP2D6 and CYP1A2 are involved in the formation of 8-hydroxy metabolites, while the CYP3A isoform is involved in forming the *N*-desmethyl (active) and *N*-oxide metabolites [Anttila *et al.* 2001]. Elimination of the parent compound is mainly via hepatic metabolism, with demethylation and oxidation with subsequent conjugation of the metabolites [Grasmäder *et al.* 2005]. Clearance in CYP2D6 intermediate metabolisers is reduced by 26% compared with extensive metabolisers [Grasmäder *et al.* 2004].

Therapeutic Concentration The serum therapeutic concentration is 20–100 µg/L and the sum of the parent drug and desmethylmirtazepine is 50–300 µg/L.

Serum concentrations in patients receiving 15 mg mirtazapine daily were a median of 37 µg/L mirtazapine and 20 µg/L desmethylmirtazapine. Patients given a 30 mg dose showed higher median concentrations: 48 µg/L mirtazapine and 31 µg/L desmethylmirtazapine. After 45 mg mirtazapine daily, 62 µg/L mirtazapine and 52 µg/L desmethylmirtazapine were observed. The highest dose of 60 mg daily gave rise to concentrations of 83 µg/L mirtazapine and 65 µg/L desmethylmirtazapine [Meineke *et al.* 2004].

After a 15 mg daily dose, peak plasma concentrations (steady state) of 27–51 µg/L were reached in ≈5 days. After a 75 mg daily dose, peak plasma concentrations in the range 137–225 µg/L were achieved [Lavins *et al.* 1998].

Twenty healthy male volunteers were either administered 30 mg mirtazapine at 21.00 h for 7 days (NOCTE) or 2 doses of 15 mg mirtazapine administered at 21.00 h or 0.900 h for 7 days (BID). Pharmacokinetic parameters were as follows:

Parameter	*NOCTE*	*BID (evening)*	*BID (morning)*
C_{max} (ng/mL)	76 ± 33	47 ± 17	53 ± 19
t_{max}	1.99 ± 1.65	1.78 ± 0.84	1.79 ± 0.92
$C_{ss.min}$ (ng/mL)	11.4 ± 3.4	15.7 ± 4.1	16.1 ± 4.0
t_{ss} (days)	6	4	4
$t_{1/2}$ (h)	19.7 ± 3.0	–	20.8 ± 2.7

ss, steady state
[Timmer *et al.* 1997].

Note For pharmacokinetics of mirtazapine in patients with major depressive disorder see Reis *et al.* [2005].

Toxicity The toxic serum concentration (sum of the parent drug and metabolite) is 1 mg/L. For a review of cases see Waring *et al.* [2007].

A 76-year-old man was found dead near five empty packages of Zoloft. Postmortem concentrations (µg/L or ng/g) were as follows:

Tissue	*Mirtazapine*	*Desmethylmirtazapine*
Femoral vein blood	1030	NA
Heart blood	380	460
Gastric contents	1890	810
Bile	2950	19830
Urine	590	2410
Muscle	320	2060
Brain	560	4950
Lung	1190	8050
Kidney	1770	4890
Liver	2160	9940

NA, not available
[Wenzel *et al.* 2006]

Thirteen cases from the Los Angeles County Coroner's Office have been reported with postmortem concentrations (mg/L or mg/kg) in table at top of next page.

The following postmortem concentrations (mg/L or mg/kg) were reported in 8 cases:

Case	*Heart blood*	*Periph blood*	*Bile*	*Liver*	*Kidney*	*Urine*
1	0.33	ND	-0.11	ND	ND	0.5
2	0.07	ND	ND	ND	ND	0.14
3	0.13	0.14	0.9	0.52	0.48	ND
4	0.01	0.01	0.15	0.09	0.03	0.01
5	0.05	0.05	0.42	0.24	0.12	0.06
6	<0.01	<0.01	ND	0.04	0.02	0.06
7	0.12	ND	0.51	3.6	ND	0.33
8	0.13	0.14	1.6	0.77	0.4	3.2

ND, not determined
[Moore *et al.* 1999].

Six cases were reported from the San Diego County Medical Examiner's Office with the following concentrations (mg/L or mg/kg):

Case	*Peripheral blood*	*Central blood*	*Liver*	*Vitreous humour*	*Gastric contents*	*Mode of death*
1	2.1	2.3	15	1.0	120	Suicide
2	3.4	2.0	14	1.2	11	Suicide
3	0.45	0.38	1.7	0.14	9	Accident
4	0.44	0.36	1.5	0.30	ND	Natural
5	0.08	0.08	0.49	0.04	–	Suicide
6	0.03	0.04	0.2	0.01	–	Suicide

ND, not determined
Mirtazapine was identified as the cause of death in cases 1, 2 and 3 [Kirkton, McIntyre 2006].

For the analysis of samples from 1997 and 1998, see Robertson [1999].

Bioavailability ≈50%.

Half-life 20–40 h (the mean half-life for women is 37 h and men 26 h).

Protein Binding ≈85% over a concentration range of 0.01–10 mg/L.

Note For a general review of mitarzapine, see Holm, Markham [1999].

Dose The usual dose is 15 to 45 mg daily.

Anderson DT *et al.* (1999). Distribution of mirtazapine (Remeron) in thirteen postmortem cases. *J Anal Toxicol* 23: 544–548.

Anttila AK *et al.* (2001). Fluvoxamine augmentation increases serum mirtazapine concentrations three- to fourfold. *Ann Pharmacother* 35: 1221–1223.

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Case	Heart blood	Femoral blood	Vitreous humour	Liver	Bile	Urine	Gastric contents	Other	Mode of death
1	0.27	0.18	–	2.1	6.3	1.1	–	–	Suicide
2	0.21	–	0.06	1.1	6.6	2.5	–	–	Natural
3	0.32	0.24	0.10	0.83	1.4	0.23	2.7 mg	–	Natural
4	0.13	0.13	–	0.53	–	1.7	0.012 mg	–	Suicide
5	0.10	QNS	–	0.85	1.2	0.43	–	–	Suicide
6	0.06	0.10	–	0.32	–	0.37	0.06 mg	–	Undetermined
7	0.57	QNS	–	–	–	–	–	–	Suicide
8	0.12	0.07	QNS	0.59	–	–	0.58 mg	Kidney 0.23	Natural
9	0.31	0.22	0.09	1.6	2.8	0.63	0.06 mg	Red top Bld 0.20	Accident
10	0.03	0.04	–	0.34	0.63	0.12	0.001 mg	Spleen 0.17	Homicide
11	0.24	0.22	–	0.85	–	0.65	0.15 mg	–	Accident
12	0.07	–	–	0.48	0.40	0.22	0.003 mg	–	Natural
13	0.15	0.11	–	0.52	–	0.32	0.17 mg	–	Accident

–, Specimen not submitted; QNS, quantity not sufficient.
Mirtazapine was not implicated in the cause of death of any of the cases studied [Anderson *et al.* 1999].

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Kirkton C, McIntyre IM (2006). Therapeutic and toxic concentrations of mirtazapine. *J Anal Toxicol* 30: 687–691.

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Ptácek P *et al.* (2003). Determination of mirtazapine in human plasma by liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 794: 323–328.

Reis M *et al.* (2005). Inter- and intraindividual pharmacokinetic variations of mirtazapine and its *N*-demethyl metabolite in patients treated for major depressive disorder: a 6-month therapeutic drug monitoring study. *Ther Drug Monit* 27: 469–477.

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Sánchez de la Torre C *et al.* (2005). Determination of several psychiatric drugs in whole blood using capillary gas–liquid chromatography with nitrogen phosphorus detection: comparison of two solid phase extraction procedures. *Forensic Sci Int* 155: 193–204.

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Wille SM *et al.* (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic-mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *J Chromatogr A* 1176: 236–245.

Wynia GS *et al.* (1997). Development and validation of a capillary electrophoresis method within a pharmaceutical quality control environment and comparison with high-performance liquid chromatography. *J Chromatogr A* 773: 339–350.

Misoprostol

Prostaglandin Analogue, Proton Pump Inhibitor

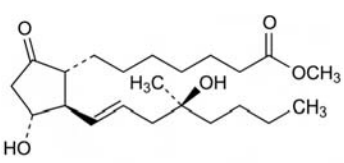
$C_{22}H_{38}O_5$ = 382.5

CAS—59122-46-2

IUPAC Name Methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate

Synonyms (11α,13*E*)-11,16-Dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester; SC-29333.

Proprietary Names *Cytotec*; *Arthrotec* (with *Diclofenac*); *Napratec* (with *Naproxen*).



(11*R*, 16*S*)-Form

Chemical Properties Light yellow oil. It is soluble in water. Log *P* (octanol/water), 4.96.

High Performance Liquid Chromatography Column: ODS Supelco (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : water (45:20:35), flow

rate 1.5 mL/min. UV detection (λ=280 nm). Retention time: 7.5 min [Roston *et al.* 1995].

Quantification

Plasma **HPLC** Column: C_8 Zorbax (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L potassium phosphate buffer (pH 3) : acetonitrile : methanol (46:30:24), flow rate 1.7 mL/min. UV detection (λ=210 nm). Retention time: 18.5 min. Limit of quantification, 0.6 µg [Womack *et al.* 1996].

Disposition in the Body Misoprostol is rapidly absorbed after oral administration and metabolised to the active form, misoprostol acid (SC-30695), which is further metabolised by oxidation and excreted mainly via urine as its dimer and tetramer (65%) and some in faeces (15%). Approximately 88% of a dose is absorbed. No unchanged drug has been detected in plasma or urine. Excretion is mainly in the form of metabolites but little as the unchanged drug. The rate of absorption from the gastrointestinal tract is reduced by the presence of food but the extent of absorption appears to be unaffected. Peak plasma concentrations of the active metabolite have been observed after about 30 min. No accumulation of the metabolite occurs in plasma after repeated dosing of 400 µg twice daily.

Therapeutic Concentration

Six healthy volunteers, with a mean age of 26 years, were administered a mean oral dose of 386 µg radiolabelled misoprostol. The mean peak plasma concentrations, observed at 30 and 12 min, were 6.08 µg/L for misoprostol.

Twenty-four healthy subjects (mean age 27 years) were administered a single oral dose of 200 or 400 µg misoprostol after an overnight fast. Peak plasma concentrations were 0.397 and 0.835 µg/L, respectively [Information supplied by Pharmacia Limited, UK].

Half-life Misoprostol, <30 min; misoprostol acid, 20 min.

Volume of Distribution Approximately 6.6 L/kg.

Protein Binding 85% (the free acid of misoprostol).

Dose The usual daily dose is between 400 and 800 µg.

Pharmacia Limited, UK. Information on file.

Roston DA *et al.* (1995). Supercritical fluid extraction-liquid chromatography method development for a polymeric controlled-release drug formulation. *J Pharm Biomed Anal* 13: 1513–1520.

Womack IM *et al.* (1996). A high performance liquid radiochromatographic assay for the simultaneous analysis of iloprost and misoprostol. *Prostaglandins* 52: 249–259.

M

Mitoxantrone

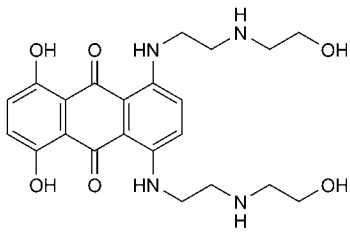
Antineoplastic

$C_{22}H_{28}N_4O_6$ = 44.5

CAS—65271-80-9

IUPAC Name 1,4-Dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]anthracene-9,10-dione

Synonyms DHAQ; 1,4-dihydroxy-5,8-bis{[2-[(2-hydroxyethyl)amino]ethyl]amino}-9,10-anthracenedione; mitozantrone; NSC-279836.



Chemical Properties Crystals. Mp 160° to 162°. It is sparingly soluble in water; slightly soluble in methanol; practically insoluble in acetone, acetonitrile and chloroform. pK_{a1} 5.99, pK_{a2} 8.13 [Rentsch *et al.* 1996]. Mitoxantrone reacts with sodium metabisulfite and EDTA although solutions containing metoxantrone are stabilised by 0.5% ascorbic acid. In the absence of ascorbic acid mitoxantrone was found to undergo rapid, biphasic degradation in plasma at 24° and 37°, with terminal half-lives of ~70 h [Priston, Sewell 1994]. Stable in spiked whole blood for 3–6 h provided the samples are kept on ice. Stable in plasma and deproteinised plasma samples for at least 24 h [Slordal *et al.* 1993].

Mitoxantrone Hydrochloride

$C_{22}H_{28}N_4O_6,2HCl$ =517.4

CAS—70476-82-3

IUPAC Name 1,4-Dihydroxy-5,8-bis{[2-[(2-hydroxyethyl)amino]ethyl]amino}-9,10-anthracenedione dihydrochloride

Synonyms CL-232315; DHAD; dihydroxyanthracenedione dihydrochloride; mitoxantrani hydrochloridum; NSC-301739.

Proprietary Names *Novantron*; *Novantrone*; *Onkotrone*; *Pralifan*.

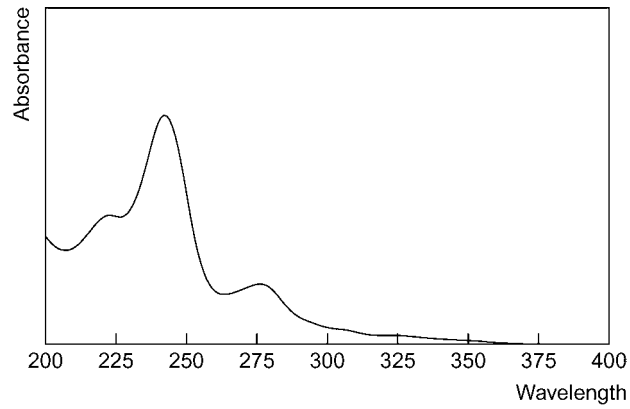
Chemical Properties A dark blue electrostatic, hygroscopic powder with Mp 203° to 205°. It is sparingly soluble in water; slightly soluble in methanol; practically insoluble in acetone, acetonitrile and chloroform. pK_a 5.99 and 8.13.

Thin-layer Chromatography All these systems refer to mitoxantrone hydrochloride:

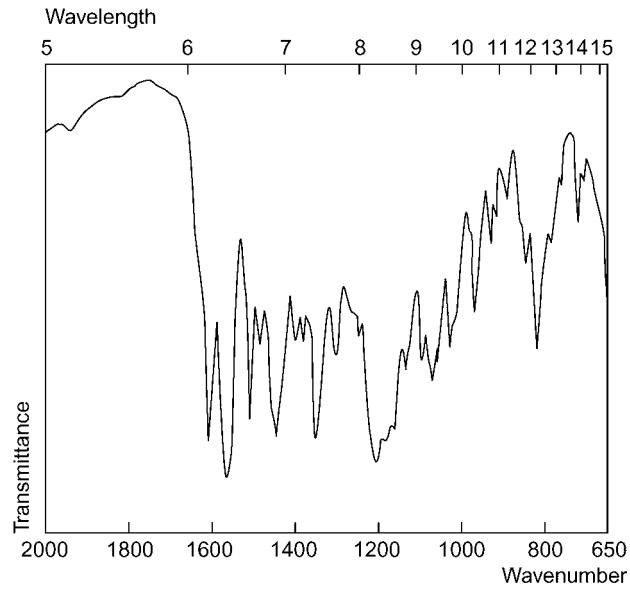
Plate: silica gel. Mobile phase: (A) methanol : ammonium solution (100 : 1.5); (B) chloroform : methanol : ammonia (9 : 3 : 1). R_f 0.04 (A); 0.77 (B). Plate: cellulose. Mobile phase: formic acid : water (4 : 96). R_f 0.33. Plate: micropolyamide. Mobile phase: (A) methanol : strong ammonia solution (100 : 1.5); (B) formic acid : water (4 : 96). R_f 0.53 (A); 0.92 (B). Plate: reversed phase KC-18F. Mobile phase: dimethylformamide : 0.5 mol/L sodium chloride solution: 1 mol/L ammonia : acetic acid (8 : 8 : 1 : 2). R_f 0.44. [Beijnen *et al.* 1991].

High Performance Liquid Chromatography Column: Nova-Pak ODS (75 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : TEA phosphate : 1 mol/L tetramethylammonium chloride : water (30 : 1 : 10 : 2 : 57), flow rate 1 mL/min. UV detection (λ = 658 nm). Retention time: 3.5 min. [Lin *et al.* 1989].

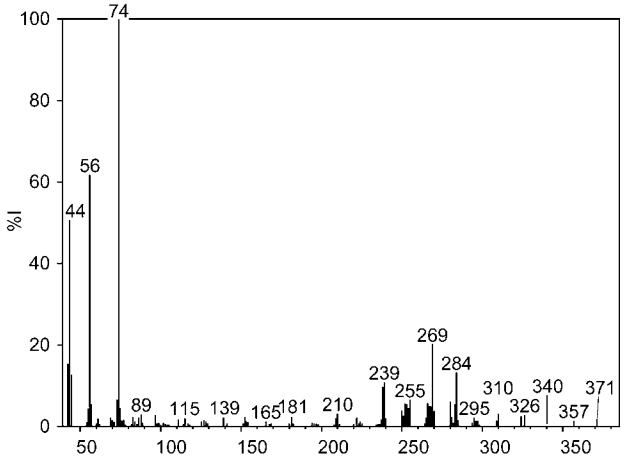
Ultraviolet Spectrum Aqueous acid (ethanol)—244, 279, 525, 620, 660 nm; 241, 273, 608, 658 nm (hydrochloride).



Infrared Spectrum Principal peaks at wavenumbers 1615, 1570, 1450, 1210, 825 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 74, 56, 44, 269, 284, 239, 255, 210.



Quantification

Plasma **HPLC** Column: HIRPB (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L ammonium formate buffer (pH 3.6; 22 : 78), flow rate 1.2 mL/min. UV detection (λ = 612 nm). Retention time: 5 min. Limit of detection not reported [Loadman, Calabrese 2001]. Column: Techopak 10 C_{18} (150 × 3.8 mm i.d., 10 µm). Mobile phase: 0.5 mol/L ammonium formate (pH 3.0) : acetonitrile (73 : 27), flow rate 1.1 mL/min. UV detection (658 nm). Limit of detection, 2 µg/L [Priston, Sewell 1994]. Column: Supelcosil C_{18} ODS (150 × 4.6 mm i.d., 3 µm). Mobile phase: triethylammonium formate buffer (pH 3.5) : acetone mixture (75 : 25), flow rate 1.0 mL/min. UV detection (λ = 658 nm). Limit of detection, 2.5 nmol/L (1.1 µg/L) [Slordal *et al.* 1993]. Column: Novapak ODS (75 × 3.9 mm i.d., 4 µm). Mobile phase: 1 mol/L TEA phosphate (pH 3.0) : tetrahydrofuran : methanol : 1 mol/L tetramethylammonium chloride : water (10 : 1 : 30 : 2 : 57) flow rate 1.0 mL/min. UV detection (λ = 658 nm). Limit of quantification, 1 µg/L [Lin *et al.* 1989]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.55 mol/L ammonium formate (pH 4.3) : acetonitrile (73 : 27), flow rate 0.5 mL/min. UV detection (λ = 658 nm). Limit of detection, 1 µg/L [Smyth *et al.* 1986].

Serum **HPLC** Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : water (25 : 75) containing 5 mmol/L 1-pentanesulfonic acid, flow rate 1 mL/min. UV detection (λ = 658 nm). Limit of detection, <1 µg/L [Ehninger *et al.* 1985].

Note For an ELISA, see Flavell and Flavell [1988] or Nicolau *et al.* [1985].

Urine **HPLC** See Plasma [Smyth *et al.* 1986]. See Serum. Limit of detection, 0.2 µg/L [Ehninger *et al.* 1985].

Ascitic Fluid **HPLC** Column: Alltech C_{18} (150 × 2.1 mm, 5 µm). Mobile phase: acetonitrile : 0.16 mol/L ammonium formate (pH 2.5; 27 : 73) with 8 mmol/L heptanesulfonic acid, flow rate 0.35 mL/min. UV detection (λ = 658 nm). Limit of detection not reported [Nagel *et al.* 1992].

Other **HPLC** Mouse Whole Blood and Tissues. Column: Nucleosil C_{18} (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.16 mol/L ammonium formate buffer (pH 2.7; 33 : 67) containing 0.25 mol/L hexanesulfonic acid, flow rate 1.0 mL/min. UV detection (λ = 658 nm). Retention time: 6.0 min. Limit of detection, 2 µg/L [Rentsch *et al.* 1996].

Disposition in the Body Mitoxantrone is rapidly and extensively distributed in the body after IV administration. It is extensively tissue bound and undergoes very slow redistribution back into the plasma. It is slowly excreted in urine and bile as the unchanged drug and its metabolites, monocarboxylic and dicarboxylic acid derivatives; 20–32% of the dose is excreted within 5 days. Between 6 and 11% of the dose is recovered from urine and between 13 and 25% in faeces; 65% of the unchanged drug is found in urine and 35% of the 2 inactive metabolites and their glucuronide conjugates. Two thirds of excretion occurs during the first day. Tissue concentrations exceed those in blood during the terminal elimination phase. Mitoxantrone does not appear to cross the blood–brain barrier. Hepatic metabolism is the major route of elimination of mitoxantrone so caution should be exercised when using the drug in patients with hepatic dysfunction [Smyth *et al.* 1986].

Therapeutic Concentration

Five patients with cancer of the peritoneal cavity were administered doses between 20 and 50 mg/m^2 via the IP route, as a 15 min infusion. The median peak plasma concentration was 1 µg/L and the peak peritoneal dialysate concentration was 490 µg/L [Nagel *et al.* 1992].

Patients with liver metastasis were administered 12 mg/m^2 doses by intra-arterial infusions of 2 and 24 h. The peak plasma concentrations were 305 µg/L at 0.9 h during the 2 h infusion, and 244 µg/L at 5.5 h during the 24 h infusion [Czejka *et al.* 1990].

Toxicity Fatalities have occurred on rare occasions as a result of severe leucopenia with infection after accidental administration of a single bolus injection of mitozantrone at over 10 times the recommended dose. Four patients received 140–180 mg/m^2 by single bolus injection and died from severe leucopenia [*APBI*].

Half-life 5–18 days. Initial phase 3–10 min, intermediate phase 0.3–3.1 h, terminal phase 23–215 h (mean 75 h). Terminal phase also reported as 37.4 h.

Volume of Distribution 13.8–14.4 L/kg or >2248 L/m^2 (Steady state >1000 L/m^2).

Clearance The mean body clearance is 238.7 mL/h/kg and is known to be reduced in hepatic impairment. Triphasic plasma clearance occurs.

Protein Binding Approximately 95% and 78% (albumin).

Note For a review of the pharmacokinetics of mitoxantrone, see Ehninger *et al.* [1990]; for a general review, see Faulds *et al.* [1991] or Smyth *et al.* [1986].

Dose The usual dose is 14 mg/m^2 body surface.

APBI Compendium of data sheets and summaries of product characteristics 1999–2000. *Epsom, Surrey: Datapharm Publications Ltd.*

Beijnen JH *et al.* (1991). In: Florey K, ed. *Analytical Profiles of Drug Substances and Excipients,* Vol. 17. San Diego, CA: Academic Press, pp. 221-258.

Czejka MJ *et al.* (1990). Pharmacokinetics of mitoxantrone in patients after 2 h and 24 h intra-arterial administration. *Eur J Drug Metab Pharmacokinet* 15: 219–222.

Ehninger G *et al.* (1985). Detection and separation of mitoxantrone and its metabolites in plasma and urine by high-performance liquid chromatography. *JChromatogr* 342: 119–127.

Ehninger G *et al.* (1990). Pharmacokinetics and metabolism of mitoxantrone, a review. *Clin Pharmacokinet* 18: 365–380.

Faulds D *et al.* (1991). Mitoxantrone A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the chemotherapy of cancer. *Drugs* 41: 400–449.

Flavell SU, Flavell DJ (1988). Development of a sensitive monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for mitozantrone. *J Immunol Methods* 115: 179–185.

Lin KT *et al.* (1989). High-performance liquid chromatographic determination of mitoxantrone in plasma utilizing non-bonded silica gel for solid-phase isolation to reduce adsorptive losses on glass during sample preparation. *JChromatogr* 465: 75–86.

Loadman PM, Calabrese CR (2001). Separation methods for anthraquinone related anti-cancer drugs. *JChromatogr B Biomed Sci Appl* 764: 193–206.

Nagel JD *et al.* (1992). Clinical pharmacokinetics of mitoxantrone after intraperitoneal administration. *Cancer ChemoTher Pharmacol* 29: 480–484.

Nicolau G *et al.* (1985). Radioimmunoassay for mitoxantrone, a new antitumor agent. *Invest New Drugs* 3: 51–56.

Priston MJ, Sewell GJ (1994). Improved LC assay for the determination of mitozantrone in plasma: analytical considerations. *J Pharm Biomed Anal* 12: 1153–1162.

Rentsch KM *et al.* (1996). Determination of mitoxantrone in mouse whole blood and different tissues by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 679: 185–192.

Slordal L *et al.* (1993). A sensitive and simple high-performance liquid chromatographic method for the determination of mitoxantrone in plasma. *Ther Drug Monit* 15: 328–333.

Smyth JF *et al.* (1986). The clinical pharmacology of mitozantrone. *Cancer Chemother Pharmacol* 17: 149–152.

M

Mivacurium Chloride

Muscle Relaxant

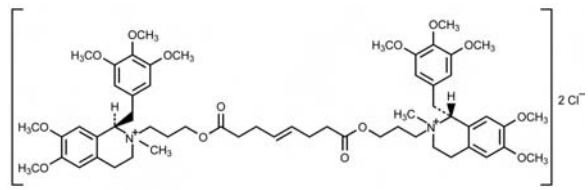
$C_{58}H_{80}Cl_2N_2O_{14}$ = 1100.2

CAS—106861-44-3

IUPAC Name (1*R*,1′*R*)-2,2′-[[(4*E*)-1,8-Dioxo-4-octene-1,8-diyl]bis(oxy-3,1-propane-diyl)]bis[1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]]isoquinolinium dichloride

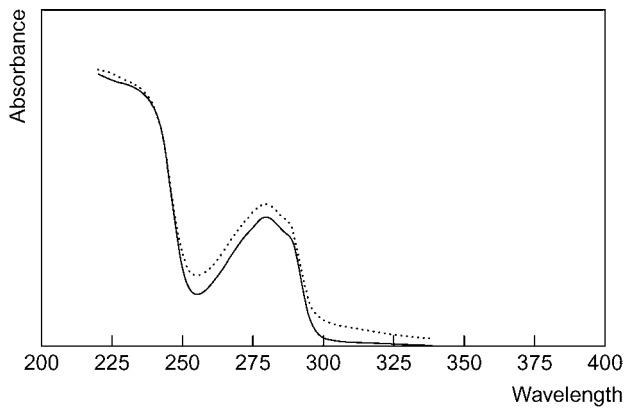
Synonym BW-B1090U

Proprietary Name *Mivacron*

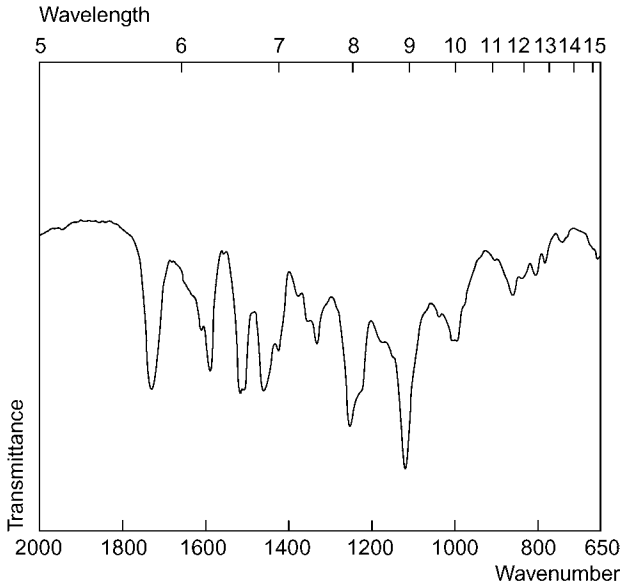


Chemical Properties An amorphous solid. Log *P* (octanol/water), 0.015.

Ultraviolet Spectrum Aqueous acid—280 nm; aqueous alkali—280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1123, 1520, 1732, 1337 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** Column: C_1 Spherisorb (150 × 4.6 mm i.d., 5 µm). Mobile phase: (A) acetonitrile : 10 mmol/L potassium dihydrogen phosphate (pH 3, containing 10% methanol) (70:30); (B) acetonitrile : 50 mmol/L potassium dihydrogen phosphate (pH 3, containing 10% methanol) (70:30), flow rate 1 mL/min. Elution programme: (A:B) (100:0) for 3 min; to (0:100) in 2 min; hold for 4 min; to (100:0) in 2 min; hold for 2 min. Internal standard (IS): D-tubocurarine. Fluorescence detection (λ_{ex}=220 nm, λ_{em}=320 nm). Retention time: mivacurium chloride, 9.7 min; IS, 8.6 min. Column: SCX Spherisorb (250 × 4.6 mm, 10 µm). Mobile phase: acetonitrile : 70 mmol/L disodium sulfate (in 5 mmol/L sulfuric acid) (60:40), flow rate 1 mL/min. IS: laudanosine. Retention time: mivacurium chloride: *cis–cis* isomer, 6.5 min; *cis–trans* isomer, 6.8 min; *trans–trans* isomer, 7.0 min; IS, 4.8 min. Limit of detection, 1 µg/L [Weindlmayr-Goettel *et al.* 1996]. Fluorescence detection (λ_{ex}=202 nm, λ_{em}=290 nm). Limit of quantification, 3.9, 4.1, 10.4 and 15.6 µg/L for the mivacurium isomers, *cis* monoester, *trans* monoester and the alcohol metabolites [Lacroix *et al.* 1995]. Fluorescence detection (λ_{ex}=202 nm, λ_{em} = 320 nm). Limit of quantification, 5 µg/L [Brown *et al.* 1992].

Disposition in the Body Mivacurium undergoes enzymatic hydrolysis by plasma cholinesterases to inactivate the drug. Quaternary alcohol and monoester metabolites of the different isomers are produced. Peak concentrations of these can be observed ~25 s after an injection. Elimination of mivacurium occurs both in the kidneys and the liver with excretion in urine and bile, mostly as the metabolites (32 to 82% as the monoesters, 16 to 53% as the quaternary alcohols) and little unchanged drug (4 to 15% of a dose).

Therapeutic Concentration

Eight healthy patients (men and women) with a mean age of 33 years, were administered an IV dose of 0.15 mg/kg mivacurium chloride. Peak plasma concentrations of 4486, 2198 and 504 µg/L for the *trans–trans, cis–trans* and *cis–cis* isomers, respectively, were observed after 25 and 35 s. Plasma concentrations of the *trans–trans* and *cis–trans* isomers declined rapidly and could not be detected 10 to 20 min after administration. Peak concentrations of the *cis–cis* isomer declined less rapidly and it could still be detected up to 45 min after injection [Lacroix *et al.* 1997].

Toxicity Prolonged muscle paralysis and its consequences are the main signs of overdosing.

Half-life *Trans–trans* isomer, 2.4 min; *cis–trans* isomer, 2.0 min; *cis–cis* isomer, 28.5 min (mean values).

Volume of Distribution Drug in general, 0.2 L/kg. *Trans–trans* isomer, 0.047 L/kg; *cis–trans* isomer, 0.054 L/kg; *cis–cis* isomer, 0.189 L/kg.

Clearance Plasma: *trans–trans* isomer, 29.2 mL/min/kg; *cis–trans* isomer, 45.7 mL/min/kg; *cis–cis* isomer, 6.7 mL/min/kg.

Note For a general review of the pharmacology of mivacurium chloride, see Frampton and McTavish [1993].

Dose Initial dose is 70 to 250 µg/kg body weight IV injection and the maintenance dose is 100 µg/kg. Reduced doses may be necessary in the elderly. Continuous treatment may be at 8 to 10 µg/kg/min which can be increased in 1 µg/kg/min steps. Children 2 months to 12 years: 11 to 14 µg/kg/min.

Brown AR *et al.* (1992). Stereoselective high-performance liquid chromatographic assay with fluorometric detection for the isomers of mivacurium in human plasma. *J Chromatogr* 578: 302–308.

Frampton JE, McTavish D (1993). Mivacurium. A review of its pharmacology and therapeutic potential in general anaesthesia. *Drugs* 45: 1066–1089.

Lacroix M *et al.* (1995). High-performance liquid chromatographic assays with fluorometric detection for mivacurium isomers and their metabolites in human plasma. *J Chromatogr B Biomed Appl* 663: 297–307.

Lacroix M *et al.* (1997). Pharmacokinetics of mivacurium isomers and their metabolites in healthy volunteers after intravenous bolus administration. *Anesthesiology* 86(2): 322–330.

Weindlmayr-Goettel M *et al.* (1996). Determination of mivacurium in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 685: 123–127.

Mizolastine

Antihistamine

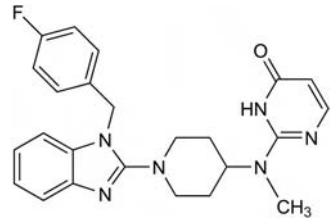
$C_{24}H_{25}FN_6O$ = 432.5

CAS—108612-45-9

IUPAC Name 2-[[1-[1-[(4-Fluorophenyl)methyl]-1*H*-benzimidazol-2-yl]-4-piperidinyl]-methylamino]-4(1*H*)-pyrimidinone

Synonym SL85-0324

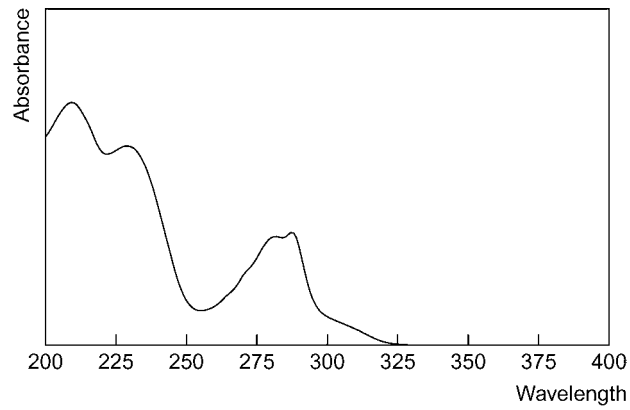
Proprietary Names *Mistamine*; *Mizollen*; *Zolim*.



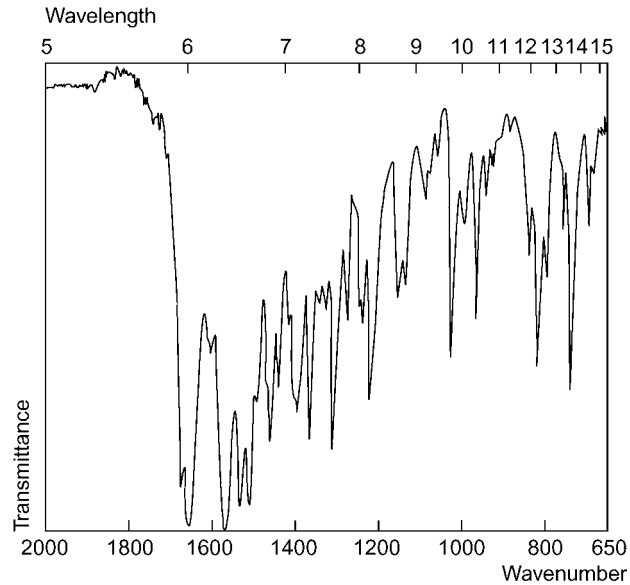
Chemical Properties Crystals. Mp 217°. Soluble in methanol.

High Performance Liquid Chromatography Column: C_8Ultra-base B5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogen phosphate (pH 2.5) : acetonitrile (72:28), flow rate 1 mL/min. UV detection (λ=285 nm). Retention time: not specified [Chosidow *et al.* 1996].

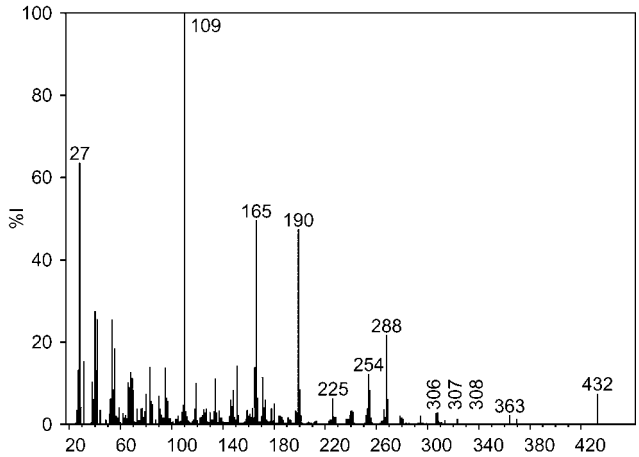
Ultraviolet Spectrum Aqueous acid—208, 229, 281, 288 nm; ethanol—214, 287 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 109, 27, 165, 198, 40, 55, 57, 288.



Quantification

Plasma **HPLC** Limit of detection, 0.5 µg/L [Ascalone, Rouchouse 1993].

Disposition in the Body Mizolastine is rapidly absorbed from the gastrointestinal tract and extensively metabolised. 0.5% of an administered dose is recovered from urine as the unchanged drug and 84 to 95% of the drug is excreted in faeces. 66% of the administered dose is metabolised by glucuronidation, and other metabolic pathways are also involved, including metabolism by cytochrome P450 isoenzyme CYP3A4 with the formation of inactive metabolites. No active metabolites have been identified. Linear kinetics are observed. Steady state is reached on the third day of administration and accumulation does not occur.

Therapeutic Concentration Peak plasma concentrations of 276, 408 and 595 µg/L have been observed 1.5 h after oral doses of 5, 10 and 15 mg, respectively.

Bioavailability Tablets: 65%. Capsules: 70%. Not influenced by food or alcohol.

Half-life Approximately 13 h. In patients with hepatic impairment, 15.1 ± 1.7 h and renal impairment, 19.2 ± 1.7 h.

Volume of Distribution 1.4 L/kg.

Clearance A total clearance of 1.15 mL/min/kg and systemic plasma clearance of 0.69 L/h/kg.

Protein Binding Approximately 98.4%.

Dose 10 mg daily.

Ascalone V, Rouchouse A (1993). Determination of mizolastine, a new antihistaminic drug, in human plasma by liquid-liquid extraction, solid-phase extraction and column-switching techniques in combination with high-performance liquid chromatography. *J Chromatogr* 619: 275–284.

Chosidow O *et al.* (1996). Plasma and skin suction-blister-fluid pharmacokinetics and time course of the effects of oral mizolastine. *Eur J Clin Pharmacol* 50: 327–333.

Moclobemide

Antidepressant

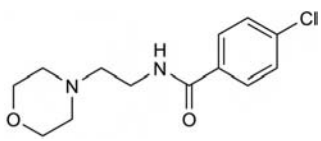
$C_{13}H_{17}ClN_2O_2$ = 268.7

CAS—71320-77-9

IUPAC Name 4-Chloro-*N*-[2-(4 morpholinyl)ethyl]benzamide

Synonyms Ro-11-1163; Ro-11-1163/000.

Proprietary Names *Aurorix*; *Manerix*; *Moclamine*.



Chemical Properties Crystals from propan-2-ol. Mp 137°. pK_a 6.2. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Moclobemide Hydrochloride

$C_{13}H_{17}ClN_2O_2$,HCl = 305.2

Chemical Properties Crystals from propan-2-ol with Mp 208°.

Thin-layer Chromatography System TB—R_f 0.1; system TE—R_f 0.52; system TAE—R_f 0.65.

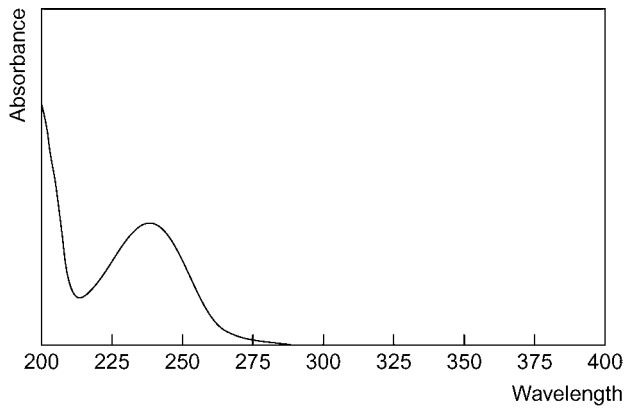
Gas Chromatography System GB—RI 2333; system GM—RRT 0.967; system GT—RI 2210.

Column: HP5 (0.32 mm i.d.). Temperature: 140° for 0.5 min to 300° at 20°/min for 2 min. NPD. Retention time: 3.0 min [Singer, Jones 1997].

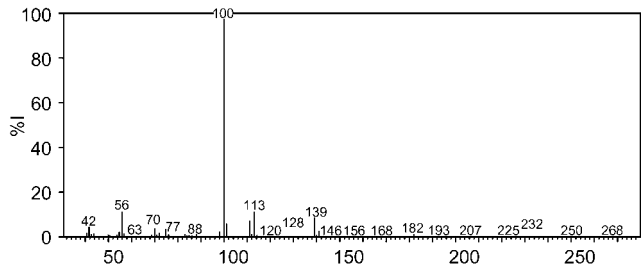
High Performance Liquid Chromatography System HX—RI 295; system HZ—RT 2.4 min; system HAA—RT 10.2 min; system HAX—RT 6.9 min; system HAY—RT 3.9 min.

Column: C_{18} (Kromasil, 150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol-water (90 : 10) : water-methanol (90 : 10) both containing 0.0125 mol/L sodium hydroxide (40 : 60 to 25 : 75 in 5 min for 8 min to 0 : 100 in 10 min, for 5 min. Internal standard (IS): Ro-11-9900. UV detection (λ = 237 nm). Retention time: 4.5 min for moclobemide, 5.5 min for IS [Isbister *et al.* 2001]. Column: Spherisorb S5 C_6 (125 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 67 mmol/L potassium phosphate (pH 3.9, 30 : 320) flow rate 1.3 mL/min. IS: Ro-11-9900. UV detection (λ = 240 nm). Retention time: 7.2 min for moclobemide, 5.3 min for metabolite, 8.6 min for *N*-oxide metabolite, 15.1 min for lactam derivative metabolite, 12.7 min for IS [Geschke *et al.* 1987].

Ultraviolet Spectrum Aqueous acid (pH 2.8)—196, 238 nm.



Mass Spectrum Principal ions at *m/z* 100, 182, 225, 250, 268, 113, 56, 42.



Quantification

Blood **HPLC** Column: XTerra RP-18. Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2). Limit of quantification, 2 µg/L [Titier *et al.* 2007]. UV detection (λ = 238 nm). Limit of detection, 0.012 mg/L [Gaillard, Pepin 1997].

Plasma **HPLC** Column: C_{18}. Mobile phase: acetonitrile : water (pH 2, 725 : 75). Spectrophotometric detection (λ = 239 nm). Limit of quantification, 10 µg/L [Plenis *et al.* 2007]. Column: Waters XTerra RP-18 (150 × 4.6 mm, 5 µm). Mobile phase: 10 µmol/L potassium dihydrogen phosphate with 1% TEA (pH 3.9) : acetonitrile (83 : 17), flow-rate, 1.2 mL/min. UV detection (λ = 240 nm). Limit of quantification, 0.02 mg/L [Rakic *et al.* 2007]. Column: ODS (250 × 4.6 mm, 5 µm). Mobile phase: 5 µmol/L sodium dihydrogen phosphate : acetonitrile : TEA (pH 3.4, 1000 : 350 : 10), flow-rate 1.0 mL/min. UV detection (λ = 240 nm). Limit of quantification, 15.6 µg/L, limit of detection, 5 µg/L [Amini *et al.* 2004]. Column: HyPurity C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : phosphate buffer (pH 3.8), flow rate 1.0 mL/min. DAD (λ = 220, 240, 290 nm). Limit of detection, 2.5–5 µg/L [Duverneuil *et al.* 2003]. Column: Waters Nova-Pak Phenyl (150 × 2 mm i.d., 4 µm). Mobile phase: 0.2 mol/L ammonium formate buffer (pH 3.57) : acetonitrile. Limit of quantification, 1 µg/L [Hoskins *et al.* 2001]. See also Geschke *et al.* [1987].

Serum **HPLC** Column: Nucleosil 100-Protect 1. Mobile phase: acetonitrile : potassium dihydrogen phosphate buffer [Frahnert *et al.* 2003].

Urine **GC-MS** Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

HPLC See Plasma [Geschke *et al.* 1987].

Disposition in the Body Moclobemide is readily absorbed after oral administration, with peak plasma concentrations observed after 1 to 2 h. The drug is almost completely absorbed and undergoes extensive and rapid hepatic metabolism to 19 metabolites, mostly by oxidation, deamination and aromatic hydroxylation.

Absorption is delayed in the presence of food. The drug is almost entirely metabolised before it is eliminated from the body. Slow metabolisers and extensive metabolisers both exist. The main metabolite is the lactam derivative, which is pharmacologically inactive, but the other metabolites retain some activity, in particular the *N*-oxide of the drug. It is widely distributed throughout the body including breast milk, and accumulation can occur. Moclobemide is excreted in urine mainly as the metabolites and a small amount as the unchanged drug (<1%), <5% in faeces.

Therapeutic Concentration The trough serum therapeutic concentration is 0.4–1.0 mg/L and peak, 1.5–4.0 mg/L.

Eight male volunteers, 29 to 37 years of age (mean, 33), were administered a single dose of 100 mg moclobemide. The mean peak plasma concentration was 575 µg/L (range, 333–1076), which was observed at 0.9 h (0.3–1.5 h) after administration [Schoerlin *et al.* 1991].

Toxicity Blood concentrations of 5–8 mg/L can be toxic and blood concentrations of 16 mg/L are sometimes associated with fatalities.

A 39-year-old woman was admitted to hospital with convulsions and a raised body temperature of 42.6°. The highest blood concentration measure was 36.5 mg/L and it was estimated that she probably ingested 10 g moclobemide. Her blood concentration was several fold higher than the peak concentrations observed after a single oral dose of 1.2 g observed in tolerance studies (11–12 mg/L, 0.25–0.5 h after administration). The patient recovered after receiving anticonvulsants and artificial respiration [Schoerlin *et al.* 1987].

A 48-year-old woman with a history of depression and repeated suicide attempts was found dead at home. Postmortem toxicological analysis showed a concentration of moclobemide in blood of 137 mg/L and in liver of 432 mg/kg. Low levels of diazepam, nordiazepam and trifluoperazine were also detected. It was concluded that death was caused by acute poisoning by moclobemide (the first case report of death caused by the toxic effects of this drug on its own) [Camaris, Little 1997].

A 41-year-old Caucasian man was found dead at his home; he had a history of depression and suicide attempts. Surrounding the man was a number of medication bottles and a bottle of whiskey. Toxicological analysis showed that the man had ethanol, lormetazepam, cotinine, caffeine, moclobemide and citalopram in his urine and blood. Moclobemide was detected in blood at a concentration of 5.62 mg/L and in urine at 204.0 mg/L. The metabolite 3-ketomoclobemide was found at concentrations of 2.26 mg/L and 49.7 mg/L in blood and urine, respectively, and moclobemide *N*-oxide at 424 mg/L in urine (this metabolite was not detected in blood). The cause of death was determined as the synergistic toxicity of moclobemide and citalopram; death was ruled as multiple drug intoxication [Dams *et al.* 2001].

Bioavailability 55–95% (single dose); 80% (multiple dosing).

Half-life 1–4 h.

Volume of Distribution 1.2 L/kg.

Clearance Blood, 20–50 L/h.

Protein Binding 50%.

Note For an update of the pharmacological properties and therapeutic use of moclobemide, see Fulton, Benfield [1996].

Dose The initial dose is 300 mg daily, divided in three doses; this may be adjusted if necessary. During therapy, the usual range is 150 to 600 mg daily.

Amini H *et al.* (2004). Determination of moclobemide in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 807: 271–275.

Bickeboeller-Friedrich J, Maurer HH (2001). Screening for detection of new antidepressants, neuroleptics, hypnotics, and their metabolites in urine by GC-MS developed using rat liver microsomes. *Ther Drug Monit* 23: 61–70.

Camaris C, Little D (1997). A fatality due to moclobemide. *J Forensic Sci* 42: 954–955.

Dams R *et al.* (2001). A fatal case of serotonin syndrome after combined moclobemide-citalopram intoxication. *J Anal Toxicol* 25: 147–151.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.

Duverneuil C *et al.* (2003). A high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. *Ther Drug Monit* 25: 565–573.

Frahnert C *et al.* (2003). Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. *J Chromatogr B Analyt Technol Biomed Life Sci* 794: 35–47.

Fulton B, Benfield P (1996). Moclobemide: an update of its pharmacological properties and therapeutic use. *Drugs* 52: 450–474.

Gaillard Y, Pepin G (1997). Moclobemide fatalities: report of two cases and analytical determinations by GC-MS and HPLC-PDA after solid-phase extraction. *Forensic Sci Int* 87: 239–248.

Geschke R *et al.* (1987). Determination of the new monoamine oxidase inhibitor moclobemide and three of its metabolites in biological fluids by high-performance liquid chromatography. *J Chromatogr* 420: 111–120.

Hoskins JM *et al.* (2001). High-performance liquid chromatography–electrospray ionization mass spectrometry method for the measurement of moclobemide and two metabolites in plasma. *J Chromatogr B Biomed Sci Appl* 754: 319–326.

Isbister GK *et al.* (2001). A fatal case of moclobemide–citalopram intoxication. *J Anal Toxicol* 25: 716–717.

Plenis A *et al.* (2007). A validated high-performance liquid chromatographic method for the determination of moclobemide and its two metabolites in human plasma and application to pharmacokinetic studies. *Biomed Chromatogr* 21: 958–966.

Rakic A *et al.* (2007). High-performance liquid chromatographic method for the determination of moclobemide and its two major metabolites in human plasma. *J Pharm Biomed Anal* 43: 1416–1422.

Schoerlin MP *et al.* (1987). Data on File. Welwyn Garden City, UK: Hoffman-Roche.

Schoerlin MP *et al.* (1991). Cimetidine alters the disposition kinetics of the monoamine oxidase-A inhibitor moclobemide. *Clin Pharmacol Ther* 49: 32–38.

Singer PP, Jones GR (1997). An uncommon fatality due to moclobemide and paroxetine. *J Anal Toxicol* 21: 518–520.

Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.

Modafinil

α_1-Adrenoceptor Agonist, CNS Stimulant

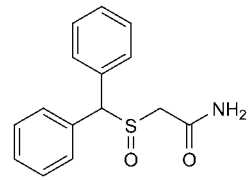
$C_{15}H_{15}NO_2S = 273.4$

CAS—68693-11-8

IUPAC Name 2-Benzhydrylsulfinylacetamide

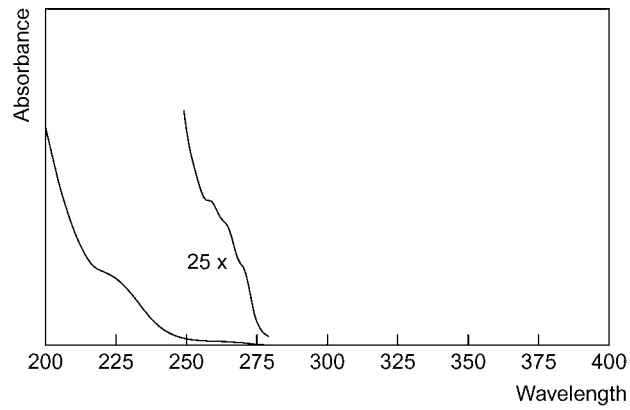
Synonyms CEP-1538; CRL-40476; 2-[(diphenylmethyl)sulfinyl]acetamide.

Proprietary Names *Modiodal*; *Provigil*.

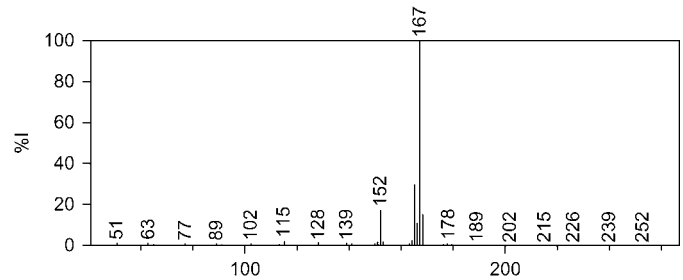


Chemical Properties A white to off-white crystalline powder with Mp 164° to 166°. Practically insoluble in water and cyclohexane; sparingly to slightly soluble in methanol and acetone. Extraction yield (chlorobutane), 0.35 [Demme *et al.* 2005]. Stability in human plasma has been demonstrated at –20° after 166 days, at –9 0° for 383 days and after 5 freeze-thaw cycles [Gorman 2002].

Ultraviolet Spectrum



Mass Spectrum



Quantification

Plasma **HPLC** Column: APS-Nucleosil (150 × 4.6 mm i.d., 7 µm) followed by octyl BSA (10 × 46 mm i.d., 10 µm). UV detection (λ = 240 or 228 nm). Limit of quantification, 0.1 mg/L for (+)-modafinil; limit of detection, 0.05 mg/L for (+)-modafinil [Cass, Ferreira 2008]. Column: Symmetry C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol: water: acetic acid (500 : 500 : 1), flow rate, 1.0 mL/min. UV detection (λ = 220 or 233 nm). Retention time: 11.5 min. Limit of detection, 0.1 mg/L [Schwertner, Kong 2005]. Column: Cyclobond I-2000 RSP (250 × 4.6 mm i.d.). Mobile phase: 20 mmol/L sodium phosphate (pH 3.0) : acetonitrile-20 mmol/L sodium phosphate (pH 3.0; 30 : 70, 30 : 70 to 60 : 40 over 30 min to 30 : 70 for 5 min), flow rate 1.0 mL/min. UV detection (λ = 225 nm). Retention time: 22.1 min

for (+)-modafinil, 23.8 min for (−)-modafinil. Limit of quantification, 0.5 mg/L, limit of detection, 0.01 mg/L [Donovan *et al.* 2003]. Column: Hypersil BDS phenyl (150 × 2.0 mm i.d., 5 µm). Mobile phase: 0.02 mol/L phosphate buffer (pH 2.5) : methanol (64 : 36), flow rate 0.3 mL/min. UV detection (λ = 235 nm). Limit of quantification, 0.1 mg/L [Gorman 2002]. Column: APS-Nucleosil (150 × 4.6 mm i.d., 7 µm). Mobile phase: acetonitrile : water (25 : 75), flow rate 0.5 mL/min. UV detection (λ = 228 nm). Limit of quantification, 0.02 mg/L [Cass *et al.* 2001]. Column: β-cyclodextrin (250 × 4.0 mm i.d., 5 µm). Temperature programme: 12°. Mobile phase: 0.02 mol/L phosphate buffer (pH 3.0) : acetonitrile (84 : 16), flow rate 0.6 mL/min. I.S.: 3,3-diphenylpropylamine. DAD (λ = 225 nm). Retention time: (*R*)-modafinil, 19.0 min; (*S*)-modafinil, 22.0 min; I.S., 9 min. Limit of quantification, 0.1 mg/L [Gorman 1999]. Column: ChiraDex β-cyclodextrin (250 × 4.0 mm i.d.). UV detection (λ = 233 nm). Limit of detection, 0.1 mg/L [Wong *et al.* 1999a]. Column: Symmetry C_8 (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L orthophosphoric acid (26 : 74), flow rate 1.1 mL/min. UV detection (λ = 225 nm). Limit of quantification, 0.10 mg/L, limit of detection, 0.01 mg/L [Burnat *et al.* 1998]. See also [Moachon, Matinier 1994].

Urine **GC-MS** Column: 5% phenylmethyl silicone (17 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 18 psi. Temperature programme: 85° for 1.5 min to 270° at 15°/min to 290° at 50°/min for 2.5 min. EI ionisation at 70 eV, SIM and scan acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [Strano-Rossi *et al.* 2008]. Column: HP-5MS cross-linked 5% diphenylpolysiloxane 95% dimethylpolysiloxane (25 m × 0.25 mm i.d., 0.33 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 90° to 240° at 15°/min to 300° at 10°/min for 5 min. EI ionisation at 70 eV, full scan mode. Limit of quantification, 0.363 mg/L; limit of detection, 0.109 mg/L [Tseng *et al.* 2005].

HPLC See Plasma [Schwertner, Kong 2005].

Saliva **GC-MS** See Urine [Strano-Rossi *et al.* 2008].

Disposition in the Body Modafinil is a racemic compound whose enantiomers have different pharmacokinetics. It is rapidly and well absorbed from the gastrointestinal tract following oral administration. It is well distributed in tissues and metabolised in the liver by hydrolytic deamination, *S*-oxidation, aromatic ring hydroxylation, and glucuronide conjugation. Modafinil shows a possible induction effect on its own metabolism after chronic administration of dosages greater than 400 mg/day. The major metabolite is modafinil acid but there are at least 6 other metabolites present in much lower concentrations. The major route of elimination (~90%) is metabolism, primarily by the liver, with subsequent renal elimination; <10% of the dose is eliminated unchanged. Renal impairment does not affect pharmacokinetics. In hepatic impairment, oral clearance is decreased by ~60% and steady-state concentration is doubled. Steady state is achieved within 8 days.

Therapeutic Concentration

Twelve healthy young males (aged 22–37 years; mean 29 years), 12 young females (19–40 years; mean 26 years), and 12 elderly males (aged 53–72 years; mean 63 years) were administered a single oral dose of 200 mg modafinil after an overnight fast. The peak plasma modafinil concentration was 4.21 mg/L for the young males, which was observed at 2.0 h; 5.2 mg/L for the young females at 1.7 h, and 4.81 mg/L at 1.7 h for the elderly males. For the metabolite modafinil acid, concentrations were 2.06, 2.65, and 2.65 mg/L for the young males, females, and elderly males, respectively, observed at 2.8, 2.9 and 3.5 h, respectively [Wong *et al.* 1999a].

Thirty-two healthy male volunteers (aged 20 to 39 years; mean 26.2 years) were administered a 200, 400, 600, or 800 mg dose of modafinil after breakfast for 7 consecutive days. Peak plasma concentrations of the parent drug were 4.8 and 6.4 mg/L for the 200 mg dose on days 1 and 7, respectively. For the 400 mg dose, concentrations reached 8.7 mg/L at 2.3 h on day 1 and 11.0 mg/L at 1.7 h on day 7. For the 600 mg dose, concentrations were 13.0 and 17.0 mg/L on days 1 and 7, respectively, observed at 2.5 and 2.3 h, respectively. On day 1, the 800 mg dose of modafinil produced concentrations of 15.0 mg/L at 2.3 h [Wong *et al.* 1999b].

Note For pharmacokinetic parameters in cocaine-dependent subjects, see Donovan *et al.* [2003]; for a pharmacokinetic study of the effect of modafinil on warfarin, see Robertson *et al.* [2002a] and on ethinylestradiol and triazolam, see Robertson *et al.* [2002b].

Toxicity Doses of 4 g led to limited, expected, and not life-threatening adverse experiences, with patients making full recovery.

Half-life 10–15 h.

Volume of Distribution Approximately 0.9 L/kg or 80 L.

Clearance 3–5 L/h.

Protein Binding Approximately 62% (albumin).

Dose 200 to 400 mg.

Burnat P *et al.* (1998). High-performance liquid chromatographic determination of modafinil and its two metabolites in human plasma using solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 706: 295–304.

Cass QB, Ferreira GT (2008). A method for determination of the plasma levels of modafinil enantiomers, (+/−)-modafinic acid and modafinil sulphone by direct human plasma injection and bidimensional achiral-chiral chromatography. *J Pharm Biomed Anal* 46: 937–944.

Cass QB *et al.* (2001). An enantioselective assay for (+/−)-modafinil. *J Pharm Biomed Anal* 26: 123–130.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Donovan JL *et al.* (2003). Chiral analysis of *d*- and *l*-modafinil in human serum: application to human pharmacokinetic studies. *Ther Drug Monit* 25: 197–202.

Gorman SH (1999). Determination of the D- and L-enantiomers of modafinil in human plasma utilizing liquid–liquid extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 730: 1–7.

Gorman SH (2002). Determination of modafinil, modafinil acid and modafinil sulfone in human plasma utilizing liquid–liquid extraction and high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 269–276.

Moachon G, Matinier D (1994). Simultaneous determination of modafinil and its acid metabolite by high-performance liquid chromatography in human plasma. *J Chromatogr B Biomed Appl* 654: 91–96.

Robertson PJr *et al.* (2002a). Effect of modafinil at steady state on the single-dose pharmacokinetic profile of warfarin in healthy volunteers. *J Clin Pharmacol* 42: 205–214.

Robertson PJr *et al.* (2002b). Effect of modafinil on the pharmacokinetics of ethinyl estradiol and triazolam in healthy volunteers. *Clin Pharmacol Ther* 71: 46–56.

Schwertner HA, Kong SB (2005). Determination of modafinil in plasma and urine by reversed phase high-performance liquid-chromatography. *J Pharm Biomed Anal* 37: 475–479.

Strano-Rossi S *et al.* (2008). Parallel analysis of stimulants in saliva and urine by gas chromatography/mass spectrometry: perspectives for "in competition" anti-doping analysis. *Anal ChimActa* 606: 217–222.

Tseng YL *et al.* (2005). Detection of modafinil in human urine by gas chromatography–mass spectrometry. *J Pharm Biomed Anal* 39: 1042–1045.

Wong YN *et al.* (1999a). Open-label, single-dose pharmacokinetic study of modafinil tablets: influence of age and gender in normal subjects. *J Clin Pharmacol* 39: 281–288.

Wong YN *et al.* (1999b). A double-blind, placebo-controlled, ascending-dose evaluation of the pharmacokinetics and tolerability of modafinil tablets in healthy male volunteers. *JClin Pharmacol* 39: 30–40.

Moexipril

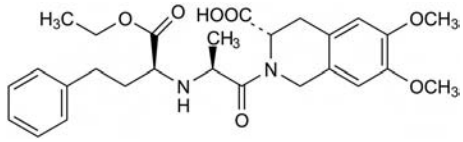
Antihypertensive

$C_{27}H_{34}N_2O_7$ = 498.6

CAS—103775-10-6

IUPAC Name (3*S*)-2-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid

Synonym RS-10085



Chemical Properties pK_a 3.05 (25°); 5.40 (25°). Log *P* (pH 7.5), 0.25; (pH 1.0), 2.34.

Moexipril Diacid Hydrochloride

$C_{27}H_{30}N_2O_7$,HCl = 531.0

CAS—82586-57-0

Synonym Moexiprilat hydrochloride

Chemical Properties Crystals from tetrahydrofuran and ethanol. Mp 145° to 170°.

Moexipril Hydrochloride

$C_{27}H_{34}N_2O_7$,HCl = 535.0

CAS—82586-52-5

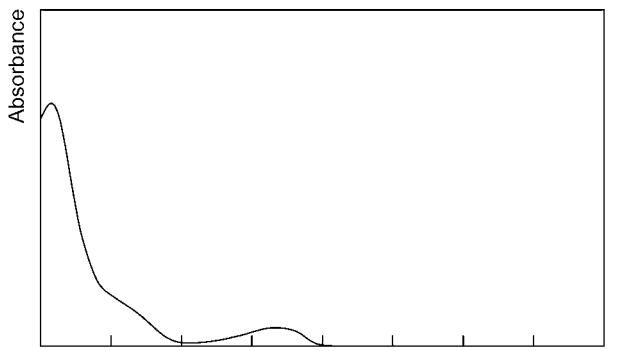
Synonyms CI-925; RS-10085-197; SPM-925.

Proprietary Names *Femipres*; *Perdix*; *Univasc*. It is also an ingredient of *Uniretic*.

Chemical Properties A fine-white to off-white powder. Mp 141° to 161°. Soluble in distilled water at room temperature.

Gas Chromatography System GP—RI 3575 moexipril-ME, RI 3580 M (moexiprilate)-ME3, RI 3775 M (moexiprilate-H_2O)-ME3.

Ultraviolet Spectrum Aqueous solution—283 nm (unaffected by pH).



Infrared Spectrum Principal peaks at wavenumbers 702, 753, 858, 1115, 1215, 1256 cm^{-1}.

Quantification
Plasma **GC-MS** Column: DB-1 fused-silica capillary (10 m × 0.32 mm i.d., 0.25 μm). Temperature programme: held at 150° for 0.1 min, to 300° at 25°/min, held for 1.5 min. Injector temperature: 70° to 300° at 10°/s, held for 5 min. Carrier gas: He, inlet pressure 13.8 kPa. Internal standard (IS): quinapril hydrochloride. MS detection (NICI, SIM at *m/z*: 302 and 288 for moexipril and moexiprilat, respectively). Retention time: moexipril, 7.32 min; moexiprilat, 7.25 min; IS, 6.2 min. Limit of quantification for moexipril and moexiprilat, 0.5 μg/L [Hammes *et al.* 1995].

Disposition in the Body Moexipril is rapidly but incompletely absorbed after oral administration and de-esterified to its active metabolite, moexiprilat. Absorption is reduced with the presence of food, and peak plasma concentrations of the metabolite are reached within 1.5 h. Both the parent drug and the active metabolite are mainly eliminated in urine along with other minor metabolites including diketopiperazine, and some moexiprilat is excreted in faeces (20% of an IV dose and 52% of an oral dose). 40% of an IV dose is excreted as moexiprilat in urine (7% oral dose) and 26% as the unchanged drug (1% oral). The pharmacokinetics of moexipril are significantly altered in patients with severe renal impairment (creatinine clearance 40 mL/min) and those with liver cirrhosis.

Therapeutic Concentration
Healthy volunteers were administered a 15 mg single dose of moexipril and peak plasma concentrations of 50 μg/L and 5 μg/L were respectively reached for the drug and its metabolite moexiprilat within 2 h [Stimpel *et al.* 1995].

Bioavailability 22%.
Half-life Approximately 10 h.
Volume of Distribution 183 L (moexipril and moexiprilat).
Clearance Plasma, moexipril, 441 mL/min; moexiprilat, 232 mL/min.
Protein binding Moexipril, 50%; moexiprilat, 72%.
Note For a review of the use of moexipril in the management of essential hypertension, see Brogden and Wiseman [1998].
Dose The usual initial dose is 7.5 mg and a maintenance dose of 7.5 to 15 mg daily is administered. Doses up to 30 mg may also be given. In the elderly, patients with renal failure or hepatic cirrhosis, and those being treated with a diuretic or nifedipine, the initial dose should be reduced by half.

Brogden RN, Wiseman LR (1998). Moexipril. A review of its use in the management of essential hypertension. *Drugs* 55: 845–860.
Hammes W *et al.* (1995). Simultaneous determination of moexipril and moexiprilat, its active metabolite, in human plasma by gas chromatography-negative-ion chemical ionization mass spectrometry. *J Chromatogr B Biomed Appl* 670: 81–89.
Stimpel M *et al.* (1995). *Cardiovasc Drug Rev* 13(3): 211–229.

Molgramostim

Antineoplastic, Antineutropenic
$C_{639}H_{1007}N_{171}O_{196}S_{18}$ = 14798.03
CAS—99283-10-0

Synonym Sch-39300
Proprietary Names *Leucomax*; *Mielogen*.
Chemical Properties Colony-stimulating factor 2 (human clone pHG25, protein moiety reduced). Soluble in water.
Quantification
Note For an ELISA for the quantification of molgramostim, see Cebon *et al.* [1988].

Disposition in the Body Maximum serum concentrations increase with administered dose but appear larger with IV doses compared with subcutaneous administration. Concentrations reached with SC treatment >1 μg/L, are maintained longer than those with IV administration. Molgramostim has been detected in urine, but only in very small amounts, suggesting non-renal elimination.

Therapeutic Concentration
Thirty-three male and female patients with advanced malignancy or neutropenia, aged between 30 and 74 years, were administered 0.3–30.0 μg/kg molgramostim per day as a SC bolus injection. Patients were also administered 0.3–20.0 μg/kg daily IV as a bolus injection or as a 2-h infusion. Molgramostim was detected within 30 min of the SC injection for those receiving 1.0 μg/kg and 5–15 min for the higher doses. The mean peak serum concentration was 22.1 μg/L for the patients receiving doses of 15.0 μg/kg and above. Serum concentrations were >1.0 μg/L for those receiving 1.0 μg/kg molgramostim and above but not for doses below this. After the IV bolus injection, peak concentrations ranged from 6.9–95.7 μg/L as the dose increased. After the 2-h IV infusion, molgramostim was detected in serum after 15 min. The peak concentration ranged from 18.2–235.0 μg/L; not correlated to dose [Cebon *et al.* 1990].

Half-life 0.24 to 1.18 h (after IV bolus dosing); mean 1.66 (range, 1.33–1.88) h (IV infusion); mean 3.16 h (after SC dosing).
Clearance Serum, 18.6–25.1 L/h (1.0 μg/kg SC dose of molgramostim); 33.3 L/h (3.0 μg/kg dose); 8.3 L/h at higher doses (10.0–30.0 μg/kg); and for doses 0.3–3.0 μg/kg, the clearance increased. After an IV bolus dose, 0.3 μg/kg, 16.0 L/h; IV infusion, 5.7 L/h.
Dose For antineoplastic therapy: SC injection, 5 to 10 μg/kg body weight daily starting 24 h after the last dose of antineoplastic. Treatment for 7 to 10 days.

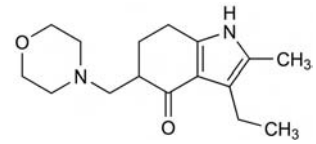
For bone marrow transplantation: IV infusion, 10 μg/kg body weight over 4 to 6 h. Up to 30 days' treatment starting the day after the transplant.

Cebon J *et al.* (1988). Pharmacokinetics of human granulocyte-macrophage colony-stimulating factor using a sensitive immunoassay. *Blood* 72: 1340–1347.
Cebon J *et al.* (1990). The effects of dose and route of administration on the pharmacokinetics of granulocyte-macrophage colony-stimulating factor. *Eur J Cancer* 26: 1064–1069.

Molindone

Tranquilliser
$C_{16}H_{24}N_2O_2$ = 276.4
CAS—7416-34-4

IUPAC Name 3-Ethyl–1,5,6,7-tetrahydro-2-methyl-5-(4-morpholinylmethyl)-4*H*-indol-4-one



Chemical Properties Crystals. Mp 180° to 181°. pK_a 6.9 (25°). Log *P* (octanol/water), 2.3.

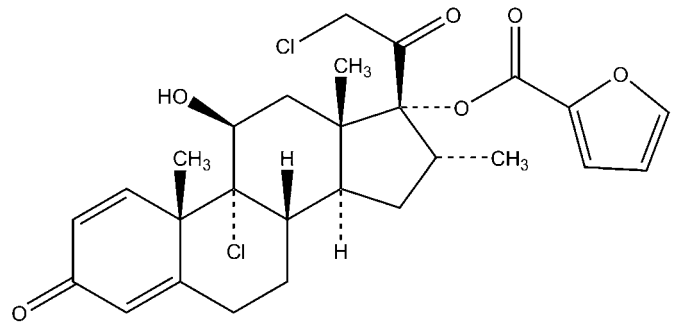
Molindone Hydrochloride
$C_{16}H_{24}N_2O_2$,HCl = 312.8
CAS—15622-65-8
Synonym EN-1733 A
Proprietary Names *Lidone*; *Moban*.
Chemical Properties A white crystalline powder. Freely soluble in water and ethanol.

Thin-layer Chromatography System TAJ—R_f 0.24; system TAK—R_f 0.00; system TAL—R_f 0.37.
Gas Chromatography System GA—RI 2465.
Ultraviolet Spectrum Aqueous acid—255 (A^1_1=41b), 299 nm.
Mass Spectrum Principal ions at *m/z* 100, 56, 42, 176, 98, 120, 70, 189.
Disposition in the Body Molindone is readily absorbed after oral administration; peak plasma concentrations of unchanged drug are attained in 1 to 2 h. It is extensively metabolised and excreted in the urine and faeces almost entirely as metabolites.
Dose 15 to 225 mg of molindone hydrochloride daily.

Mometasone Furoate

Antiinflammatory, Corticosteroid
$C_{27}H_{30}Cl_2O_6$ = 521.4
CAS—83919-23-7

IUPAC Name [(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Chloro-17-(2-chloroacetyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-17-yl] furan-2-carboxylate
Synonyms 9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17(2-furoate); (11β,16α)-9,21-dichloro-17-[(2-furanylcarbonyl)oxy]-11-hydroxy-16-methylpregna-1,4-diene-3,20-dione; Sch-32088.
Proprietary Names *Asmanex*; *Dermosona*; *Ecural*; *Elocon*; *Elovent*; *Flogocort*; *Lisoder*; *Metason*; *Metaspray*; *Movesan*; *Nasonex*; *Novasone*; *Rinelon*; *Topcort*; *Uniclar*. It is also an ingredient of *Elisalic*.



Chemical Properties Crystals. Mp 218° to 220°. Practically insoluble in water; slightly soluble in octanol; moderately soluble in ethyl alcohol. Degrades with increasing solution pH to several products [Sahasranaman *et al.* 2004].
Ultraviolet Spectrum Methanol—247 nm.
Quantification
Plasma **HPLC** Column: C_8 (150 × 2.0 mm i.d., 5 μm). Mobile phase: methanol : water (59 : 41), flow rate 0.28 mL/min. UV detection (λ= 248 nm). Limit of quantification, 0.2 mg/L [Teng *et al.* 2001].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (85 : 15), flow rate 1 mL/min. APCI, negative ion mode, MRM acquisition mode. Limit of quantification, 15 ng/L [Sahasranaman *et al.* 2005]. Column: C_{18} (33 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : 25 mmol/L ammonium acetate (80 : 20), flow rate 1.0 mL/min. API, positive ion mode. Limit of quantification, 49.7 ng/L [Affrime *et al.* 2000].

Urine **HPLC** See Plasma [Teng *et al.* 2003].

Other **HPLC** Simulated Lung Fluid. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (63 : 37), flow rate 1 mL/min. UV detection (λ = 254 nm). Limit of quantification, 0.125 mg/L [Sahasranaman *et al.* 2004].

LC-MS Simulated Lung Fluid. Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (70 : 30), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification not reported [Sahasranaman *et al.* 2004].

Disposition in the Body Following inhalation or intranasal administration, only a small amount of each dose (<20% from a conventional inhaler and 30% of an intranasal dose) reaches the lung or nose, while the majority is deposited in the oropharynx and is subsequently swallowed, resulting in very low measurable plasma levels. It is primarily metabolised in the liver, in part by CYP3A4. It also undergoes 6β-hydroxylation, hydrolysis of the furoate ester and substitution of the C-21 chloride with a hydroxyl group. At least 3 degradation products, including 9,11-epoxide mometasone furoate, have been identified in human lung tissue. Approximately 74% of a dose is recovered in the faeces, mostly derived from the proportion of the dose deposited in the oropharynx and swallowed. Mean urinary recovery is approx. 8% of a dose while 0 to 14% is exhaled.

Therapeutic Concentration

A group of 24 volunteers were administered 400 µg mometasone furoate as an IV infusion over 1 min. The plasma concentration was measured as 6.8 µg/L. In another study, the same group was administered the same dose of mometasone furoate via a dry-powder inhaler. The mean peak plasma concentration was 49.8 ng/L after 2 h (very close to the lower limit of quantification of the LC-MS method used) [Affrime *et al.* 2000].

Bioavailability Less than 1% (range 0.00–4.85).

Half-life 4.5 h.

Volume of Distribution Approximately 332 L.

Clearance 53.5 L/h

Dose Used as a cream, ointment or lotion containing 0.1% in skin disorders. A nasal suspension of mometasone furoate 0.05%, as the monohydrate, is given in the treatment and prophylaxis for allergic rhinitis. For mild to moderate asthma, an initial dose of 400 µg is given once daily.

Affrime MB *et al.* (2000). Bioavailability and metabolism of mometasone furoate following administration by metered-dose and dry-powder inhalers in healthy human volunteers. *J Clin Pharmacol* 40: 1227–1236.

Sahasranaman S *et al.* (2004). Characterization of degradation products of mometasone furoate. *Pharmazie* 59: 367–373.

Sahasranaman S *et al.* (2005). A sensitive liquid chromatography–tandem mass spectrometry method for the quantification of mometasone furoate in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 175–179.

Teng XW *et al.* (2001). High-performance liquid chromatographic analysis of mometasone furoate and its degradation products: application to in vitro degradation studies. *J Pharm Biomed Anal* 26: 313–319.

Teng XW *et al.* (2003). Mometasone furoate degradation and metabolism in human biological fluids and tissues. *Biopharm Drug Dispos* 24: 321–333.

6-Monoacetylmorphine

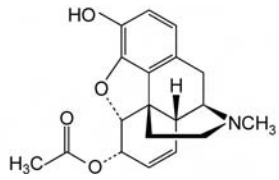
Opioid Derivative

$C_{19}H_{21}NO_4$ = 327.4

CAS—2784-73-8

IUPAC Name 6-*O*-Acetylmorphine

Synonym 6-MAM



6-Monoacetylmorphine Hydrochloride

$C_{19}H_{21}NO_4$,HCl = 363.8

CAS—36418-22-1

Chemical Properties Crystals. Soluble in water.

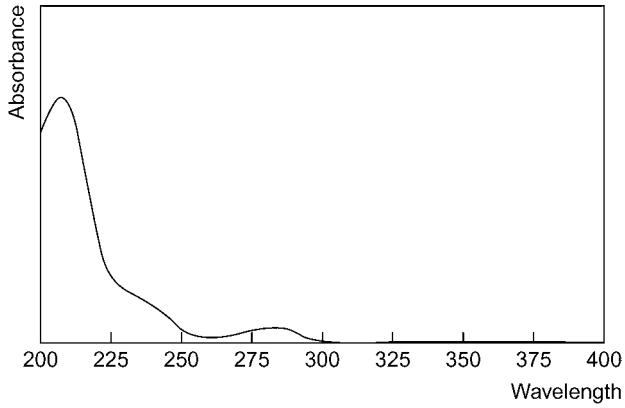
Colour Test Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.06; system TC—R_f 0.19; system TAJ—R_f 0.13; system TAK—R_f 0.02; system TAL—R_f 0.51 (acidified iodoplatinate solution—positive).

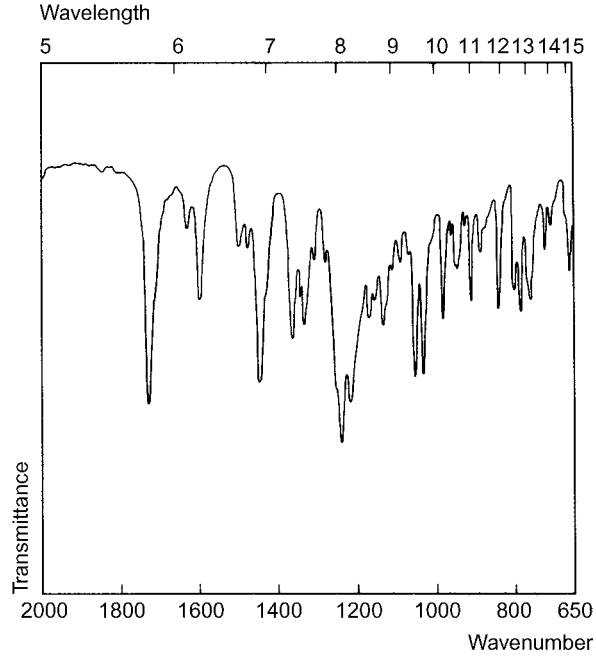
Gas Chromatography System GA—M (6-monoacetylmorphine) RI 2525, M (6-monoacetylmorphine-PFP) RI 2650, M (6-monoacetylmorphine-TMS) RI 2590, diamorphine RI 2615; system GB—M (6-monoacetylmorphine) RI 2646, M (6-monoacetylmorphine-TMS) RI 2688, diamorphine RI 2769.

High Performance Liquid Chromatography System HA—*k* 3.6 (tailing peak); system HC—*k* 0.80; system HS—*k* 1.00; system HAA—RT 7.3 min.

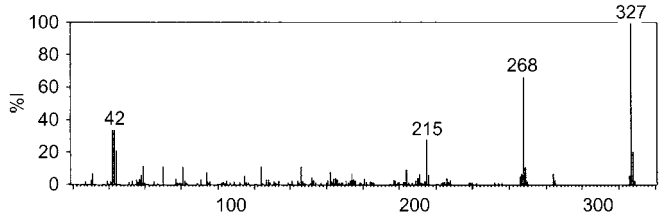
Ultraviolet Spectrum Aqueous acid—287 nm (A^1_1 = 45b).



Infrared Spectrum Principal peaks at wave numbers 1238, 1730, 1213, 1050, 1030, 1131 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 327, 268, 46, 43, 215, 44, 328, 269.



Quantification

Blood **GC** EI ionisation. Limit of quantification, 0.01 µg/mL [Mykkänen *et al.* 2000].

GC-MS Temperature programme: 150° for 0.5 min to 320° at 25°/min for 3 min. SIM acquisition mode [Goldberger *et al.* 2010]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 305° at 10°/min for 0.5 min. EI ionisation [Meatherall 2005] Column: Chromabond DB-1 C_{18} (30 m × 25 mm i.d., 0.25 µm). Carrier gas: He, 30 mL/min. Temperature programme: 100° to 300° at 20°/min. TIC, SIM acquisition mode. Limit of detection, <5 µg/L [Geier *et al.* 1996]. Column: OV1 (12 m × 0.2 mm i.d). Temperature programme: 150° for 2 min to 220° at 40°/min for 6 min. MSD. Retention time: 8.2 min. Limit of detection, <1 µg/L [Musshoff, Daldrup 1993]. Column: 95% dimethyl polysiloxane, 5% diphenylpolysiloxane Rtx (15 m × 25 mm i.d., 0.1 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 150° for 1 min to 200° at 12.5°/min for 15 s to 290° at 30°/min for 4 min. SIM acquisition mode. Limit of quantification, 1 µg/L [Goldberger *et al.* 1993].

M

Column: HP Ultra1 cross-linked methyl silicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 120° for 30 s to 220° at 40°/min to 244° at 4°/min to 300° at 40°/min. EI ionisation at 70 eV. Limit of detection, 0.05 µmol/L [Krogh *et al.* 1993].

LC-MS Column: Waters HSS T3 (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 1% formic acid in water : 1% formic acid in acetonitrile (97 : 3 for 0.1 min to 80 : 20 at 2.5 min to 1 : 99 at 2.55 min for 0.45 min to 97 : 3 at 3.01 min), flow rate 0.6 mL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L [Dahn *et al.* 2010]. Column: Nova-Pak CN (100 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate buffer (pH 3.0, 15 : 85). ESI, MRM acquisition mode [Coles *et al.* 2007]. SIM acquisition mode. Limit of quantification, 0.5 µg/L [Dienes-Nagy *et al.* 1999]. Column: ODS. Mobile phase: acetonitrile : 50 mmol/L ammonium formate buffer (pH 3.0, 5 : 95), flow rate 0.6–1.1 mL/min. SIM acquisition mode, *m/z* 334. Limit of detection, 2 µg/mL [Bogusz *et al.* 1997].

Plasma **GC-MS** See Blood [Geier *et al.* 1996; Goldberger *et al.* 1993; Krogh *et al.* 1993].

HPLC Electrochemical detection. Limit of detection, 4 µg/L [Rop *et al.* 1994]. Fluorescence detection. Limit of quantification, 25 µg/L [Barrett *et al.* 1991].

LC-MS ESI. Limit of quantification, 0.28–1.22 µg/L, limit of detection, 0.08–0.37 µg/L [Al Asmari *et al.* 2010]. See Blood [Coles *et al.* 2007; Dahn *et al.* 2010]. Limit of quantification, 2 ng/mL, limit of detection, 0.5–1 µg/L [Concheiro *et al.* 2006]. Limit of detection, 50 µg/L [Edinboro *et al.* 2005].

Serum **GC-MS** Comparison with radioimmunoassay [Moeller, Mueller 1995]. See Blood [Musshoff, Daldrup 1993].

HPLC See Blood [Bogusz *et al.* 1997].

LC-MS See Blood [Coles *et al.* 2007; Dahn *et al.* 2010]. Column: Supelcosil LC-Si (250 × 2.1 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile : formic acid (59.8 : 5.2 : 34.65 : 0.35), flow rate 230 µL/min. SIM acquisition mode (*m/z* 328). Retention time: 17.8 min. Limit of detection, 4 µg/L [Zuccaro *et al.* 1997].

Urine **GC** Column: fused silica capillary (25 m × 0.32 mm i.d., 0.12 µm). Temperature programme: 120° to 200° at 10°/min to 250° at 2°/min (purged by increasing to 280° at 30°/min, for 10 min, before cooling). Carrier gas: N_2, 1.3 mL/min. NSD or FID. Retention time: 22.4 min (NSD), 21.6 min (FID). Limit of detection, 2 µg/L (NPD) and 4 µg/L (FID) [Vu-Duc, Vernay 1990].

GC-MS See Blood [Goldberger *et al.* 2010]. Column: HP5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 200° for 0.5 min to 300° at 50°/min for 1.2 min [Meadway *et al.* 2002]. EI ionisation, SIM acquisition mode. Limit of detection, 3–12 µg/L [Karacic, Skender 2000]. Column: HP-5 capillary (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: dimethylsiloxane : diphenylsiloxane (95 : 5), 1 mL/min. Temperature programme: 120°; for 2 min; to 280° at 20°/min for 10 min. SIM acquisition mode (*m/z* 364, 423). Retention time: 13.54 min Limit of quantification, 1 µg/L [Pragst *et al.* 1999]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 290° at 10°/min for 0.5 min. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Meatherall 1999]. Column: DB-5MS 5% phenyl 95% methylsiloxane (15 m × 0.25 mm i.d.). Carrier gas: H_2. Limit of detection, 0.5 µg/L [Paul *et al.* 1999]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Temperature: 280°. SIM acquisition mode (*m/z* 383, 324, 268). Limit of quantification, 2 µg/L, limit of detection, 2 µg/L [O'Neal, Poklis 1997]. See Serum [Moeller, Mueller 1995]. See Blood [Goldberger *et al.* 1993]. See also Cone *et al.* [1991], Fuller, Anderson [1992], Low, Taylor [1995] and Vu-Duc, Vernay [1990].

HPLC See Blood [Bogusz *et al.* 1997]. Column: Hypersil (200 × 2 mm i.d., 3 µm). Mobile phase: dichloromethane : pentane (10 : 90), flow rate 0.4 mL/min. Limit of detection, 4–20 µg/L [Low, Taylor 1995]. Electrochemical detection. Limit of detection, 2 µg/L [Hanisch, Meyer 1993]. Column: Nova-Pak phenyl (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L sodium dihydrogen phosphate (pH 6.6, 10 : 90), flow rate 1.2 mL/min. UV detection (λ = 210 nm) or ECD. IS nalorphine. Retention time: 30.5 min for 6-monoacetylmorphine, 25.2 min for I.S. Limit of detection, 40 µg/L [Gerostamoulos *et al.* 1993].

LC-MS See Blood [Dahn *et al.* 2010]. 5 mmol/L ammonium bicarbonate (pH 10.2) : methanol. ESI, positive ion mode. Limit of quantification, 0.003–0.06 mg/L, limit of detection, 0.001–0.02 µg/mL [Berg *et al.* 2009]. See Blood [Coles *et al.* 2007].

Biological Fluids **GC-MS** 6-Monoacetylmorphine, codeine and morphine [Wasels, Belleville 1994].

GC-MS See Urine [Pragst *et al.* 1999]. Limit of detection, 10 µg/L [Lin *et al.* 1997].

CSF **HPLC** See Blood [Bogusz *et al.* 1997].

GC-MS See Urine [Pragst *et al.* 1999].

Meconium **LC-MS** See Blood [Coles *et al.* 2007].

Oral Fluid **GC-MS** Column: methylsilicone capillary. SIM acquisition mode. Limit of detection, 0.9–44.2 µg/L [Pujadas *et al.* 2007]. Limit of detection, 0.6 µg/L [Campora *et al.* 2006]. See Hair [Jones *et al.* 2002]. See Blood [Goldberger *et al.* 1993].

LC-MS Column: Atlantis dC_{18} (2.1 × 50 mm i.d., 3 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (10 : 90 to 40 : 60 at 4 min to 90 : 10 at 4.1 min to 8 min to 10 : 90 at 8.1 min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.82 µg/L [Oiestad *et al.* 2007].

Sweat **GC-MS** Column: HP-5MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.1 mL/min. Temperature programme: 100° for 0.5 min to 245° at 25°/min to 255° at 2°/min to 300° at 30°/min for 7 min. Limit of quantification, 10 ng/patch, limit of detection, 2.5 ng/patch [Brunet *et al.* 2008].

Vitreous Humour **HPLC** See Blood [Bogusz *et al.* 1997].

Hair **GC-MS** Limit of quantification, 0.01 ng/mg [Moller *et al.* 2010]. SIM acquisition mode. Limit of quantification, 0.05 ng/mg [Barroso *et al.* 2010]. Column: J&W DB-5ms (30 m × 0.25 mm i.d., 0.25 µm) and SGE BPX50 (2 m × 0.1 mm i.d., 0.1 µm). Carrier gas: H_2, flow rate 1.0 mL/min. Temperature 250° [Guthery *et al.* 2010]. EI ionisation. 6-monoacetylmorphine and other opioids [Jones *et al.* 2002]. Column: Restek Rtx-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 260° at 15°/min to 290° at 7°/min for 6 min. Limit of quantification, 0.02 ng/mg [Hong *et al.* 1998]. See Serum [Moeller, Mueller 1995]. See also Hold *et al.* [1998], Kauert, Rohrich [1996], Kintz, Mangin [1995], Moeller *et al.* [1993], Nakahara *et al.* [1994] and Polettini *et al.* [1993].

LC-MS Column: XBridge phenyl (150 × 4.6 mm i.d., 3.5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 4.0, 95 : 5), flow rate 500 µL/min. Limit of detection, 10 pg/mg [Huang *et al.* 2009]. ESI. Limit of detection, 0.02–0.09 ng/mg [Miller *et al.* 2008].

Nails **GC-MS** Column: DB-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier Gas: H_2, flow rate 1.0 mL/min. Temperature: 200°. Limit of quantification, 0.3 ng [Engelhart *et al.* 1998].

Note For a study of monitoring opiate use in substance abuse treatment patients with sweat and urine drug testing, see Huestis *et al.* [2000].

Disposition in the Body 6-Monoacetylmorphine is the first hydrolysis product of diamorphine. It is further metabolised to morphine.

Al Asmari A *et al.* (2010). Method for the quantification of diamorphine and its metabolites in pediatric plasma samples by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 34: 177–195.

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Campora P *et al.* (2006). Use of gas chromatography/mass spectrometry with positive chemical ionization for the determination of opiates in human oral fluid. *Rapid Commun Mass Spectrom* 20: 1288–1292.

Coles R *et al.* (2007). Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC-MS-MS. *J Anal Toxicol* 31: 1–14.

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Dahn T *et al.* (2010). Quantitation of morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and 6-monoacetylmorphine (6-MAM) in urine, blood, serum, or plasma using liquid chromatography with tandem mass spectrometry detection. *Methods Mol Biol* 603: 411–422.

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Edinboro LE *et al.* (2005). Direct analysis of opiates in urine by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 29: 704–710.

Engelhart DA *et al.* (1998). Detection of drugs of abuse in nails. *J Anal Toxicol* 22: 314–318.

Fuller DC, Anderson WH (1992). A simplified procedure for the determination of free codeine, free morphine, and 6-acetylmorphine in urine. *J Anal Toxicol* 16: 315–318.

Geier A *et al.* (1996). Evaluation of a solid-phase extraction procedure for the simultaneous determination of morphine, 6-monoacetylmorphine, codeine and dihydrocodeine in plasma and whole blood by GC/MS. *Int J Legal Med* 109: 80–83.

Gerostamoulos J *et al.* (1993). Simultaneous determination of 6-monoacetylmorphine, morphine and codeine in urine using high-performance liquid chromatography with combined ultraviolet and electrochemical detection. *J Chromatogr* 617: 152–156.

Goldberger BA *et al.* (1993). Measurement of heroin and its metabolites by isotope-dilution electron-impact mass spectrometry. *Clin Chem* 39: 670–675.

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Guthery B *et al.* (2010). Qualitative drug analysis of hair extracts by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *J Chromatogr A* 1217: 4402–4410.

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Hong Z *et al.* (1998). [Detection of heroin metabolites: 6-monoacetylmorphine and morphine in human hair by GC/MS]. *Yao Xue Xue Bao* 33: 616–620.

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Miller EI *et al.* (2008). Simultaneous detection and quantification of amphetamines, diazepam and its metabolites, cocaine and its metabolites, and opiates in hair by LC-ESI-MS-MS using a single extraction method. *J Anal Toxicol* 32: 457–469.
Moeller MR, Mueller C (1995). The detection of 6-monoacetylmorphine in urine, serum and hair by GC/MS and RIA. *Forensic Sci Int* 70: 125–133.
Moeller MR *et al.* (1993). Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. *Forensic Sci Int* 63: 185–206.
Moller M *et al.* (2010). Solid-phase microextraction for the detection of codeine, morphine and 6-monoacetylmorphine in human hair by gas chromatography–mass spectrometry. *Forensic Sci Int* 196: 64–69.
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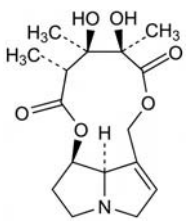
Monocrotaline

Alkaloid

$C_{16}H_{23}NO_6 = 325.4$

CAS—315-22-0

Synonyms Crotaline; (13α,14α)-14,19-dihydro-12,13-dihydroxy-20-norcrotolanan-11,15-dione; NSC-28693; NCI-C56462.



Chemical Properties An alkaloid obtained from *Crotalaria spectabilis* and other species of *Crotalaria* (Leguminosae). A white crystalline powder. Mp 197° to 198°, with decomposition. Soluble 1 in 80 of water; soluble in chloroform and dilute acetic acid. Log *P* (octanol/water), −1.2.

Colour Test Mandelin's test—green.

Thin-layer Chromatography System TA—R_f 0.36 (acidified iodoplatinate solution, positive).

Infrared Spectrum Principal peaks at wavenumbers 1736, 1064, 1110, 1183, 1242, 1174 cm^{-1} (KBr disk).

Monocrotophos

Acaricide, Insecticide

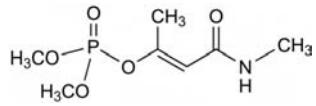
$C_7H_{14}NO_5P = 223.2$

CAS—6923-22-4

IUPAC Name Dimethyl [(*E*)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate

Synonyms C-1414; ENT-27129; (*E*)-phosphoric acid dimethyl [1-methyl-3-(methylamino)-3-oxo-1-propenyl]ester; SD-9129.

Proprietary Names *Azodrin*; *Bilobran*; *Crisodrin*; *Monocil*; *Monocron*; *Nuvacron*; *Pillardin*; *Plantdrin*; *Susvin*.

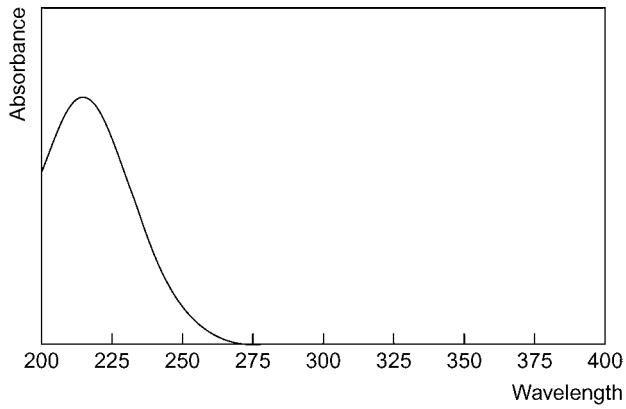


Chemical Properties A reddish-brown crystalline solid (commercial product). Mp 25° to 30° or Mp 54° to 55° (pure state). It is miscible with water (1 kg/kg at 20°) and soluble in acetone (700 g/kg at 20°), ethanol, diesel oils, kerosene, dichloromethane (800 g/kg at 20°), methanol (1000 g/kg at 20°), octan-1-ol (250 g/kg at 20°) and toluene (60 g/kg at 20°). Log *P* (octanol/water), −0.22.

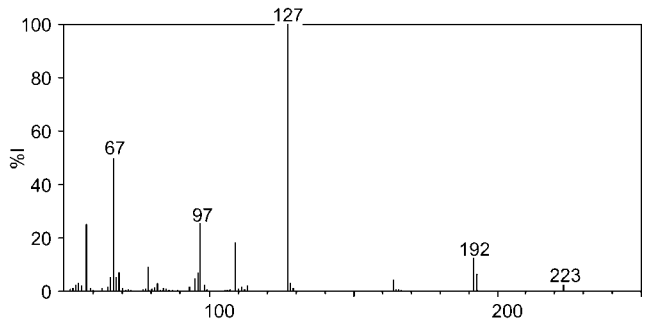
Thin-layer Chromatography System TX—R_f 0.01; system TY—R_f 0.01.

Gas Chromatography System GA—monocrotophos RI 1665; monocrotophos-TFA RI 1540; system GK—monocrotophos RRT 0.9 (relative to caffeine).

Ultraviolet Spectrum Principal peak at 215 nm.



Mass Spectrometry Principal ions at *m/z* 127, 67, 58, 97, 109, 192, 79, 223.



Quantification

Serum **GC** NPD. Limit of detection, 0.03 mg/L [Papa *et al.* 1998].

GC-MS Limit of detection, 0.06 mg/L for dimethylphosphate [Tarbah *et al.* 1998]. NPD. Limit of detection, 0.03 mg/L [Papa *et al.* 1998].

Disposition in the Body Monocrotophos is quickly metabolised by *N*-demethylation, *O*-demethylation and cleavage of the vinyl phosphate bond and excreted primarily in urine (70 to 90%). <10% can be found in faeces. One metabolite identified is dimethylphosphate (DMP). Some of the dose may also be excreted unchanged owing to its solubility in water, and is evenly distributed between tissues and organs. It does not appear to accumulate in the body and 60 to 65% of a dose is eliminated within the first 24 h. It is also readily absorbed through the skin.

Toxicity The allowed daily intake is 0.6 μg/kg.

An adult female was found dead after ingesting a plant protective containing monocrotophos, dodine and dinocap. Monocrotophos could be detected in

M

all tissues and in blood (at a concentration of 12 µg/g). Monocrotophos was found at concentrations of 13 µg/g in the lungs and brain, 11 µg/g in the kidneys, 1.8 µg/g in the liver and a total of 52 mg in the stomach. Small amounts of dodine and dinocap were only detected in the stomach [Gelbke, Schlicht 1978].

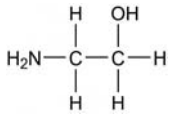
A 55-year-old male floriculturist was found unconscious in his greenhouse and died 7 days later despite hospital treatment. The first blood sample received was 44 h after intoxication and toxicological analysis showed a serum monocrotophos concentration of 24.0 mg/L. The serum levels decreased with time and were less than 0.03 mg/L at the time of death but cholinesterase levels never recovered. It is believed that 1200 mg of the pesticide was ingested [Papa *et al.* 1998].

Gelbke HP, Schlicht HJ (1978). Fatal poisoning with a plant protective containing monocrotophos, dodine and dinocap. *Toxicol Eur Res* 1(3): 181–184.
Papa P *et al.* (1998). *TIAFT Bulletin Case Notes* 28(2).
Tarbah FA *et al.* (1998). *TIAFT Poster Session* 4.

Monoethanolamine

Sclerosing Agent
C_2H_7NO = 61.1
CAS—141-43-5

IUPAC Name 2-Aminoethanol
Synonyms Aethanolaminum; ethanolamine; hydroxyethylamine; olamine.
Proprietary Names *Ethamolin (Oleate)*; *Oldamin (Oleate)*.



Chemical Properties A clear, colourless or pale-yellow, viscous liquid. Mass per mL 1.014 to 1.023 g. Bp about 170°. Miscible with water, acetone, ethanol and chloroform; slightly soluble in ether. pK_a 9.4 (25°). Log *P* (octanol/water), −1.3.

Monoethanolamine Oleate

$C_2H_7NO,C_{18}H_{34}O_2$ = 343.5
CAS—2272-11-9
Synonyms Aethanolaminum; monoethanolamine oleate; 2-hydroxyethylamine compound with oleic acid; 2-aminoethanol compound with oleic acid.
Proprietary Names *Antivariz*; *Esclerosina*; *Ethamolin*; *Oldamin*.
Chemical Properties Miscible with water, alcohol, acetone, chloroform and glycerol. Immiscible with petroleum spirit and fixed oils but will dissolve many essential oils.

Gas Chromatography System GA—monoethanolamine RI 780.
Mass Spectrum Principal ions at *m/z* 42, 31, 61, 43, 29, 27, 44, 41.
Disposition in the Body
Toxicity Overexposure to monoethanolamine oleate can lead to respiratory problems, skin and eye irritation, and lethargy.

Monoethanolamine oleate has been associated with acute renal failure (which did clear spontaneously within 3 weeks of treatment) in 2 obese women treated with injections of ethanolamine oleate. Injections of 15 to 20 mL containing 5% oleate and 2% benzyl alcohol were administered [Maling, Cretney 1975].

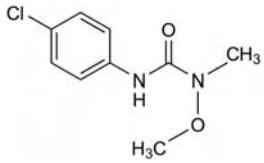
Dose Varicose veins, 2 to 5 mL 5% oleate solution; oesophageal varices, 1.5 to 5 mL 5% oleate solution per varix, maximum total of 20 mL per treatment.

Maling TJ, Cretney MJ (1975). Ethanolamine oleate and acute renal failure. *N Z Med J* 82: 269–270.

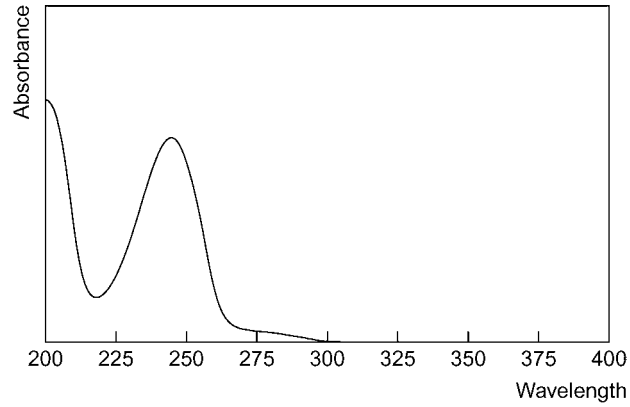
Monolinuron

Herbicide
$C_9H_{11}ClN_2O_2$ = 214.6
CAS—1746-81-2

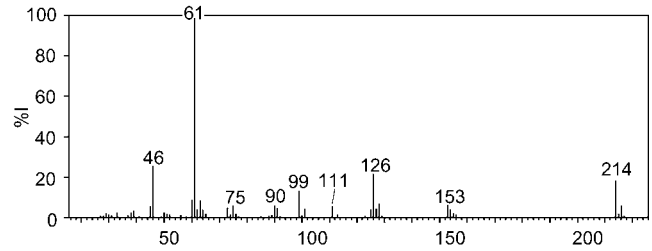
IUPAC Name 3-(4-Chlorophenyl)-1-methoxy-1-methylurea
Synonyms Chlorophenyl-*N*′-methoxy-*N*′-methylurea; HOE 2747.
Proprietary Names *Aresin*; *Aresine*; *Arresin*; *Monorotox*; *Premalin*.



Chemical Properties Solid. Mp 80° to 83°. Soluble in water (930 mg/L at 20°) and many organic solvents including alcohol, acetone, dioxane, xylene, chloroform and diethyl ether. Log *P* (octanol/water) 2.20.
Thin-layer Chromatography System TX—R_f 0.23; system TY—R_f 0.30.
High Performance Liquid Chromatography System HY—RI 411.
Gas Chromatography System GA—monolinuron RI 1910; monolinuron-Me RI 1675; M art (HOOC-)-Me RI 1690; system GK—monolinuron RRT 0.78 (relative to caffeine).
Ultraviolet Spectrum Principal peak at 244 nm.



Mass Spectrometry Principal ions at *m/z* 61, 46, 126, 214, 99, 60, 63, 153.



Disposition in the Body Monolinuron is metabolised to methylated monolinuron.
Toxicity

The allowed daily intake is 0.014 mg/kg. No toxic effect has been observed with levels of 250 mg/kg.

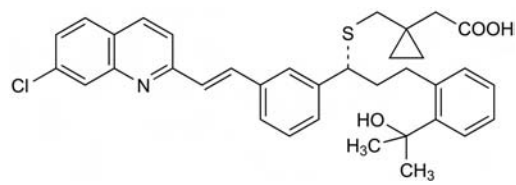
A 59-year-old male farmer attempted to commit suicide by ingesting Gramonol, containing paraquat and monolinuron. The plasma paraquat level was 1.1 mg/L (a fatal level) 8 h after ingestion but the man did not die until 10 days later due to renal, respiratory and liver failure. Methaemoglobinaemia also occurred 8 h after ingestion and this was a result of ingesting 40 mg/kg of monolinuron [Casey *et al.* 1994].

Casey PB *et al.* (1994). Methemoglobinemia following ingestion of a monolinuron/paraquat herbicide (Gramonol). *J Toxicol Clin Toxicol* 32(2): 185–189.

Montelukast

Antiasthmatic
$C_{35}H_{36}ClNO_3S$ = 586.2
CAS—158966-92-8

IUPAC Name 1-[[[(1*R*)-1-[3-[(1*E*)-2-(7-Chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid
Synonym MK-0476



Chemical Properties A white to off-white powder.

Montelukast Sodium

$C_{35}H_{35}ClNNaO_3S$ = 608.2
CAS—151767-02-1
Synonym MK-476
Proprietary Name *Singulair*
Chemical Properties A white to off-white powder. Freely soluble in ethanol, methanol and water; practically insoluble in acetonitrile.

High Performance Liquid Chromatography Column: C_8 Novapak (100 × 8.0 mm i.d., 4 µm). Temperature: 40°. Mobile phase: 25 mmol/L sodium acetate (pH 4) : acetonitrile (20:80), with 50 µL triethylamine, flow rate 1 mL/

min. Internal standard (IS): quinine bisulfate. Fluorescence detection (λ_{ex}=350 nm, λ_{em}=400 nm). Retention time: montelukast sodium, 6.1 min; IS, 4.2 min [Al-Rawithi *et al.* 2001].

Quantification

Blood **HPLC** Fluorescence detection (λ_{ex}=350 nm, λ_{em}=400 nm). Limit of detection, 9.6 µg/L [Liu *et al.* 1997].

Plasma **HPLC** Column: Apex octadecyl (50 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.05 mol/L ammonium phosphate buffer (pH 3.5, 62:38). Fluorescence detection (λ_{ex}=350 nm, λ_{em}=400 nm). Retention time: 4.0 min. Limit of quantification, 30 µg/L, limit of detection, 5 µg/L [Amin *et al.* 1995].

Disposition in the Body Montelukast is rapidly absorbed following oral administration. It is extensively metabolised by cytochromes P450 3A4, 2A6, 2C9. Montelukast and its metabolites are excreted almost exclusively via bile with <0.2% of the drug excreted in urine. Renal impairment and gender do not affect the pharmacokinetics of montelukast.

Therapeutic Concentration After a 10 mg tablet of montelukast, the mean peak plasma concentration is achieved within 3 h. After a 5 mg chewable tablet, mean peak plasma concentration is achieved within 2 h. At therapeutic doses, the plasma concentration of metabolites is undetectable at steady state in adults and children.

Bioavailability With a 10 mg tablet, 64%; 5 mg chewable tablet, 73%, which is reduced to 63% by food.

Half-life 2.7 to 5.5 h. In patients with mild–moderate hepatic insufficiency and clinical evidence of cirrhosis, the mean elimination half-life is prolonged to roughly 7.4 h.

Volume of Distribution 8 to 11 L at steady state.

Clearance 45 mL/min in healthy adults. Montelukast clearance is only slightly decreased in the elderly compared to younger adults.

Protein Binding 99%.

Note For a review of montelukast sodium and its therapeutic potential in persistent asthma, see Jarvis and Markham [2000].

Dose Adults, 10 mg; children, 5 mg.

Al-Rawithi S *et al.* (2001). Expedient liquid chromatographic method with fluorescence detection for montelukast sodium in micro-samples of plasma. *J Chromatogr B, Biomed Sci Appl* 754: 527–531.

Amin R *et al.* (1995). Determination of MK-0476 in human plasma by liquid chromatography. *J Pharm Biomed Anal* 13: 155–158.

Jarvis B, Markham A (2000). Montelukast: a review of its therapeutic potential in persistent asthma. *Drugs* 59: 891–928.

Liu L *et al.* (1997). Determination of montelukast (MK-0476) and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column-switching. *J Pharm Biomed Anal* 15: 631–638.

Moracizine

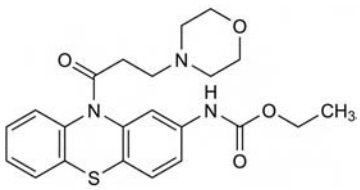
Antiarrhythmic

$C_{22}H_{25}N_3O_4S$ = 427.5

CAS—31883-05-3

IUPAC Name Ethyl *N*-[10-(3-morpholin-4-ylpropanoyl)phenothiazin-2-yl] carbamate

Synonyms EN-313; moricizine; [10-[3-(4-morpholinyl)-1-oxopropyl]-10*H*-phenothiazin-2-yl]carbamic acid ethyl ester.



Chemical Properties Crystals. Mp 156° to 157°. pK_a 6.4. Log *P* (octanol/water), 2.98.

Moracizine Hydrochloride

$C_{22}H_{25}N_3O_4S,HCl$ = 464.0

CAS—29560-58-5

Proprietary Names *Ethmozin*; *Ethmozine*.

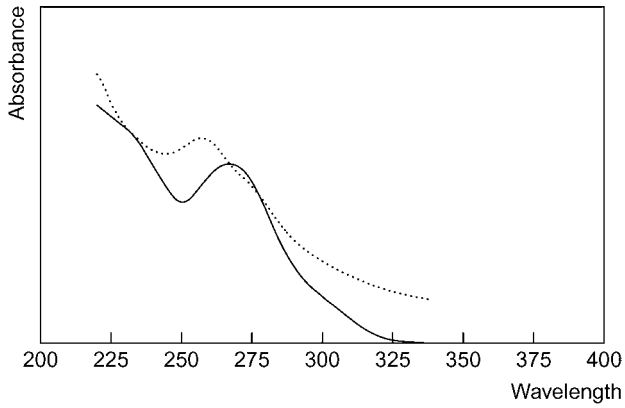
Chemical Properties A white to tan crystalline powder or crystals from dichloroethane. Mp 189°. Freely soluble in water and in alcohol.

Gas Chromatography Column: methyl silicone (HP1, 0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. MS detection. Retention index: 3526 [Mills, Roberson 1993].

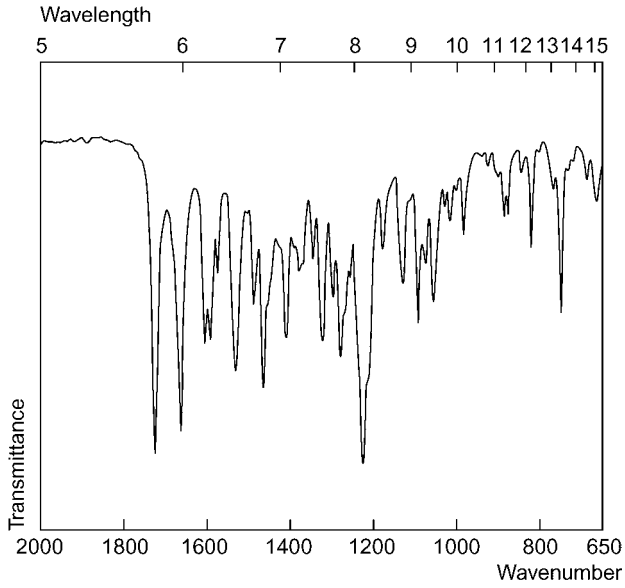
High Performance Liquid Chromatography Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. UV diode array detection. Retention time: 5.6 min [Mills, Roberson 1993].

Column: C_{18}µBondapak (100 × 8.0 mm i.d., 10 µm). Mobile phase: methanol : water : triethylamine (65:35:0.5), flow rate 2.0 mL/min. Internal standard (IS): clozapine. UV detection (λ=254 nm). Retention time: moracizine, 6.2 min; moracizine sulfoxide, 3.5 min; moracizine sulfone, 2.7 min; IS, 9.7 min [Yang, Chan 1995].

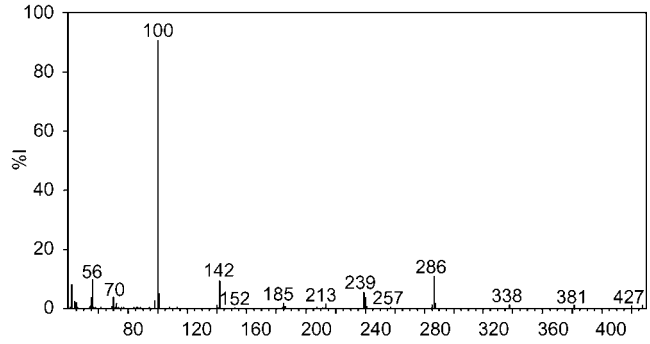
Ultraviolet Spectrum Aqueous acid—267 nm; basic—259 nm.



Infrared Spectrum Principal peaks at wavenumbers 1231, 1725, 1664, 1466 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 100, 286, 142, 56, 42, 239, 70, 185.



Quantification

Plasma **HPLC** UV detection (λ=268 nm). Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Poirier 1985]. UV detection (λ=268 nm). Limit of detection, 10 µg/L [Whitney *et al.* 1981].

Disposition in the Body Moracizine is readily and virtually completely absorbed after oral administration, with peak plasma concentrations being reached 0.5 to 2.0 h after administration. It undergoes hepatic metabolism to form many metabolites (~26, 9 of which have been identified) at a concentration of <1% of the dose. Two metabolites, moracizine sulfoxide and phenothiazine-2-carbamic acid ethyl ester sulfoxide, have anti-arrhythmic activity but represent <0.6% of the dose with a half-life of ~3 h. Moracizine is excreted mainly in faeces (56%) and urine (39%) with <1% as the unchanged drug. The drug appears in urine mainly as unconjugated metabolites and glucuronide-conjugated metabolites.

Therapeutic Concentration Therapeutic plasma concentration is 0.25 to 3.0 mg/L. Twelve healthy males, aged between 19 and 36 years, non-smokers, were administered either 250 mg every 8 h for 14 days or 250 mg every 8 h for 7

days followed by placebo for 7 days. The mean peak concentrations were 0.64 mg/L after a single dose, 0.64 mg/L after 6 days and 0.55 mg/L after 13 days. These concentrations were observed after ~1 h (range, 0.75 to 1.5 h) for both treatments [Benedek *et al.* 1994].

Toxicity In total 2 deaths have been attributed to moracizine overdose; 2.25 and 10 g [DuPont Pharmaceuticals, data on file].

Bioavailability 38%.

Half-life 2 to 4 h (healthy individuals); 6 to 13 h (patients with arrhythmias).

Volume of Distribution 8.3 to 11.1 L/kg (oral administration); 185 to 210 L (IV administration).

Clearance Plasma, 2.2 to 2.65 L/min (healthy, oral); 1.3 L/min (patients, IV).

Distribution in Blood 57% of a dose is associated with erythrocytes.

Protein Binding 81 to 95%.

Dose The usual dose is 600 to 900 mg daily (in the hydrochloride form). Reduction may be required in patients with renal and hepatic impairment.

Benedek IH *et al.* (1994). Enzyme induction by moricizine: time course and extent in healthy subjects. *J Clin Pharmacol* 34: 167–175.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton: CRC Press, 6: 158–159.

Poirier JM (1985). Sensitive high performance liquid chromatographic analysis of ethmozin in plasma. *Ther Drug Monit* 7(4): 439–441.

Whitney CC *et al.* (1981). High-performance liquid chromatographic determination of ethmozin in plasma. *J Pharm Sci* 70: 462–463.

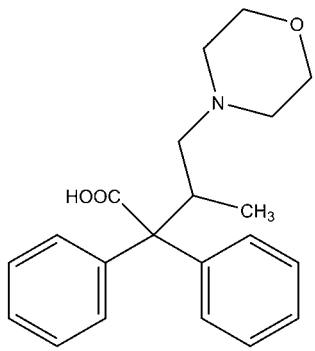
Yang JM, Chan K (1995). Simultaneous determination of moricizine and its sulphoxidation metabolites in biological fluids by high-performance liquid chromatography. *J Chromatogr* 663; Biomed Appl.: 172–176.

Moramide Intermediate

Narcotic Intermediate

$C_{21}H_{25}NO_3 = 339.4$

Synonym 2-Methyl-3-morpholino-1,1-diphenylpropane-carboxylic acid



Chemical Properties White powder. Soluble in ether and dilute acetic acid. Moramide intermediate is extracted by ether from aqueous alkaline solutions.

Colour Tests Ammonium molybdate test—faint blue-grey (limit of detection, 1.0 µg); ammonium vanadate test—dull purple (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.68 (location reagent iodoplatinate spray, positive reaction).

Morantel

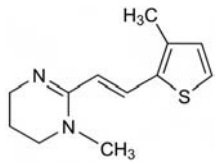
Anthelmintic (Veterinary)

$C_{12}H_{16}N_2S = 220.3$

CAS—20574-50-9

IUPAC Name 1-Methyl-2-[(*E*)-2-(3-methylthiophen-2-yl)ethenyl]-5,6-dihydro-4*H*-pyrimidine

Synonym (*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)ethenyl]-pyrimidine



Chemical Properties Crystals. Mp 239° to 241°. Log *P* (octanol/water), 3.7.

Morantel Citrate

$C_{12}H_{16}N_2S,C_6H_8O_7,\ H_2O = 430.5$

CAS—69525-81-1

Proprietary Names *Exhelm*; *Wormtec*.

Morantel Tartrate

$C_{12}H_{16}N_2S,C_4H_6O_6 = 370.4$

CAS—26155-31-7

Synonym CP-12009-18

Proprietary Names *Equiban*; *Paratect*; *Rumatel*.

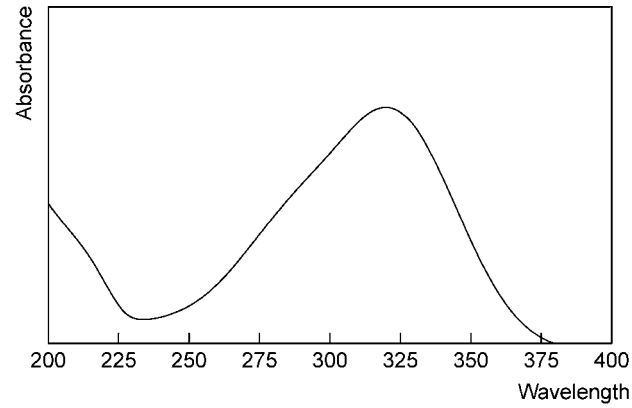
Chemical Properties Pale yellow to greenish-yellow crystals. Mp 167° to 170°. Readily soluble in water.

Colour Tests Liebermann's reagent—black; Mandelin's test—violet; Marquis test—blue-violet; sulfuric acid—violet-red.

Thin-layer Chromatography System TA—R_f 0.60 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—not eluted.

Ultraviolet Spectrum Methanol—322 nm (A^1_1=864b).



Infrared Spectrum Principal peaks at wavenumbers 1626, 1577, 1319, 1305, 1148, 955 cm^{-1} (KBr disk).

Morazone

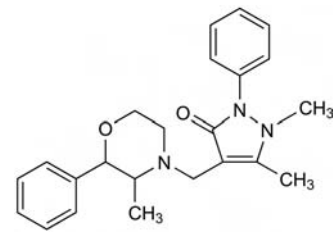
Analgesic

$C_{23}H_{27}N_3O_2 = 377.5$

CAS—6536-18-1

IUPAC Name 1,5-Dimethyl-4-[(3-methyl-2-phenylmorpholin-4-yl)methyl]-2-phenylpyrazol-3-one

Synonyms 1,2-Dihydro-1,5-dimethyl-4–[(3-methyl-2–phenyl-4-morpholinyl) methyl]-2-phenyl-3*H*-pyrazol-3-one; R-445.



Chemical Properties A white crystalline powder. Mp 149° to 150°. Freely soluble in chloroform; soluble in methanol and acetone; slightly soluble in ether. Log *P* (octanol/water), 2.0.

Morazone Hydrochloride

$C_{23}H_{27}N_3O_2,HCl = 413.9$

CAS—50321-35-2

Proprietary Names *Rosimon-Neu*; *Tarugan*.

Chemical Properties Crystals. Mp 171° to 172°, with decomposition. Soluble in water.

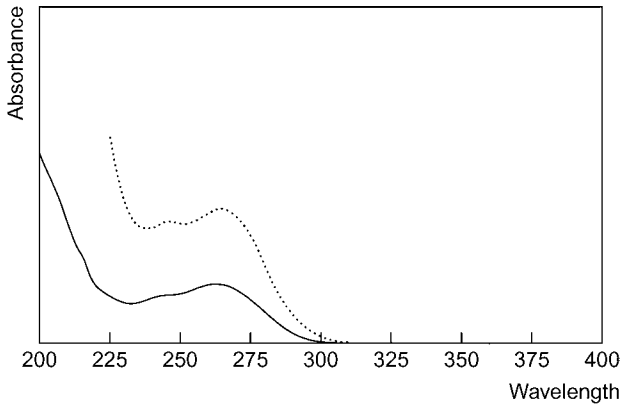
Colour Test Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.08; system TC—R_f 0.46; system TE—R_f 0.58; system TL—R_f 0.31; system TAE—R_f 0.61 (acidified iodoplatinate solution, positive).

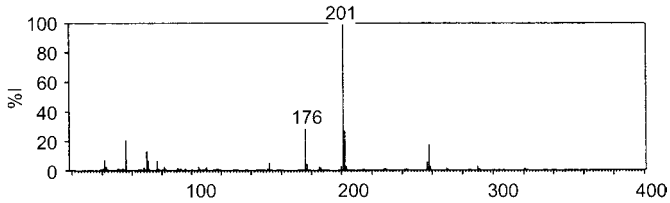
Gas Chromatography System GA—Morazone RI 3130, M (phenmetrazine) RI 1432, M (OH-phenmetrazine isomer 1) RI 1830, M (OH-phenmetrazine isomer 2) RI 1865, M (OH-MeO-phenmetrazine) RI 1900; system GB—M (phenmetrazine) RI 1483. Morazone is also metabolised to phenazone.

High Performance Liquid Chromatography System HA—*k* 0.7; system HD—*k* 0.4; system HW—*k* 2.05; system HY—RI 294.

Ultraviolet Spectrum Aqueous acid—258 nm (A^1_1=212a); aqueous alkali—244, 263 nm (A^1_1=201b).



Infrared Spectrum Principal peaks at wavenumbers 754, 1656, 700, 1492, 1590, 1117 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 201, 176, 202, 56, 258, 70, 71, 42.

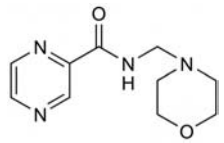


Dose Morazone hydrochloride has been given in doses of 75 to 150 mg.

Morinamide

Tuberculostatic
$C_{10}H_{14}N_4O_2$ = 222.2
CAS—952-54-5

IUPAC Name *N*-(4-Morpholinylmethyl)pyrazinecarboxamide
Synonyms Morphazinamide; B-2310.

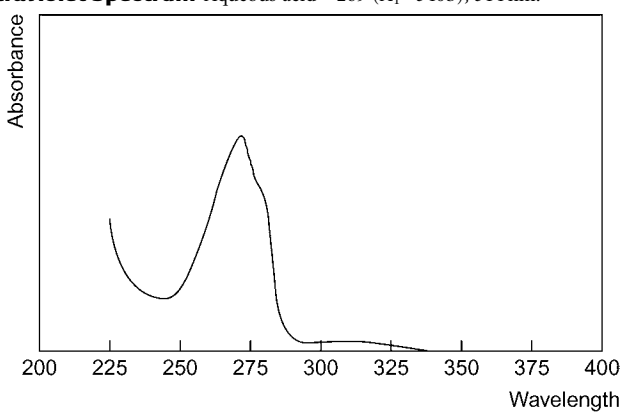


Chemical Properties Crystals. Mp 118.5° to 119.5°. Soluble 1 in 3 of water, 1 in 30 of ethanol, 1 in 30 of benzene and 1 in 2.5 of chloroform. Log *P* (octanol/water), −1.2.

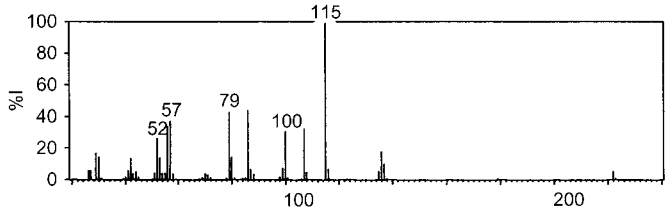
Morinamide Hydrochloride

$C_{10}H_{14}N_4O_2$,HCl = 258.7
CAS—1473-73-0
Synonym B-2311.
Proprietary Name *Piazofolina*
Chemical Properties A white crystalline powder. Mp 196°. Soluble 1 in 2 of water, 1 in 350 of ethanol and 1 in 2000 of chloroform.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.08; system TC—R_f 0.49; system TL—R_f 0.32 (acidified iodoplatinate solution, weak reaction; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1906.
Ultraviolet Spectrum Aqueous acid—269 (A_1^1=340b), 314 nm.



Infrared Spectrum Principal peaks at wavenumbers 1667, 1101, 1499, 1125, 1002, 1019 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 115, 86, 79, 57, 56, 107, 100, 52.

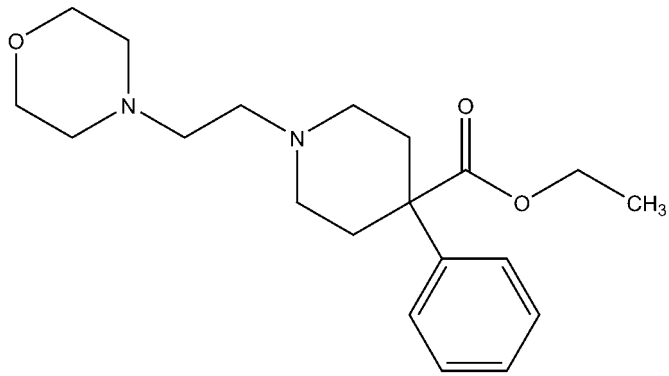


Dose Morinamide has been given in doses of 3 g daily.

Morpheridine

Narcotic
$C_{20}H_{30}N_2O_3$ = 346.5
CAS—469-81-8

IUPAC Name Ethyl 1-(2-morpholin-4-ylethyl)-4-phenylpiperidine-4-carboxylate
Synonyms Ethyl 1-(2-morpholinoethyl)-4-phenylpiperidine-4-carboxylate; morpholinoethylnorpethidine; 1-[2-(morpholinyl)ethyl]-4-phenyl-4-piperidinecarboxylic acid ethyl ester.



Chemical Properties Liquid. Bp 188° to 192° Morpheridine is extracted by organic solvents from aqueous alkaline solutions; hydrolysis of biological material is required to improve the yield.

Morpheridine Dihydrochloride

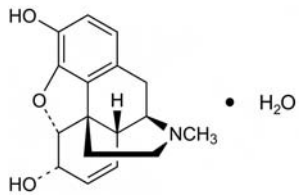
$C_{20}H_{30}N_2O_3$,2HCl = 419.4
Synonym TA-1
Chemical Properties Crystals from dilute ethanol. Mp 264° to 266° with decomposition.

Colour Test Sulfuric acid-formaldehyde test—dull orange (limit of detection, 1.0 μg).
Thin-layer Chromatography System T1—R_f 0.56 (location reagent iodoplatinate spray, positive reaction).
Gas Chromatagraphy System G2/225—retention time 1.54 (relative to codeine).
Disposition in the Body
Toxicity LD_{50} in mice 45 mg/kg (IV), 118 mg/kg (IP).

Morphine

Narcotic Analgesic
$C_{17}H_{19}NO_3$,H_2O = 303.4
CAS—57-27-2 (anhydrous); 6009-81-0 (monohydrate)

IUPAC Name (5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol monohydrate
Synonym Morphia



Chemical Properties The principal alkaloid of opium. A white crystalline powder or colourless or white acicular crystals. Mp 254° to 256°, with decomposition. Soluble 1 in 5000 of water, 1 in 210 of ethanol, 1 in 1220 of chloroform and 1 in 125 of glycerol; practically insoluble in ether. Solubility can vary according to the

method of preparation and the crystalline state. pK_{a1} 8.0, pK_{a2} 9.9 (20°). Log *P* (octanol/water pH 7.4), −0.1. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Morphine Acetate

$C_{17}H_{19}NO_3,C_2H_4O_2,3H_2O = 399.4$
CAS—596-15-6 (anhydrous); 5974-11-8 (trihydrate)
Chemical Properties A white amorphous or crystalline powder. Soluble 1 in 2.25 of water, 1 in 22 of ethanol, 1 in 4.75 of chloroform and 1 in 4.5 of glycerol; practically insoluble in ether.

Morphine Hydrochloride

$C_{17}H_{19}NO_3,HCl,3H_2O = 375.8$
CAS—52-26-6 (anhydrous); 6055-06-7 (trihydrate)
Chemical Properties Colourless silky crystals or crystalline powder, or cubical white masses. A solution in water is laevorotatory. Soluble 1 in 17.5 of water and 1 in 52 of ethanol; slowly soluble in glycerol; practically insoluble in chloroform and ether.

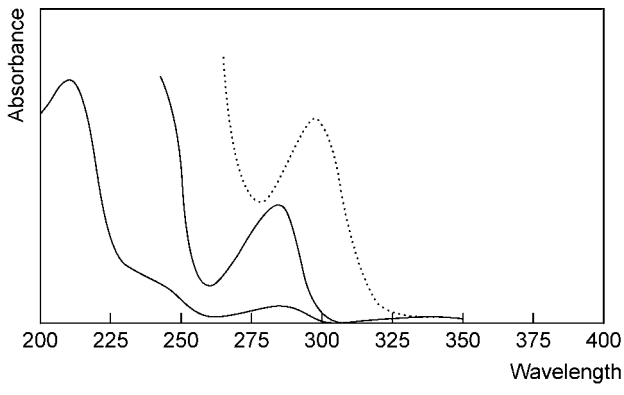
Morphine Sulfate

$(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O = 758.8$
CAS—64-31-3 (anhydrous); 6211-15-0 (pentahydrate)
Proprietary Names *Astramorph*; *Duramorph*; *Infumorph*; *Kadian*; *Moraxen*; *Morcap*; *MS Contin(us)*; *MSIR*; *MST-Continus*; *MXL*; *Oramorph*; *RMS*; *Roxanol*; *Sevredol*; *Zomorph*.
Chemical Properties White, acicular crystals, cubical masses or crystalline powder. When exposed to air, it gradually loses water of crystallisation. It darkens on prolonged exposure to light. Mp 250°, with decomposition (anhydrous form). A solution in water is laevorotatory. Soluble 1 in 15.5 of water and 1 in 565 of ethanol; practically insoluble in chloroform and ether.

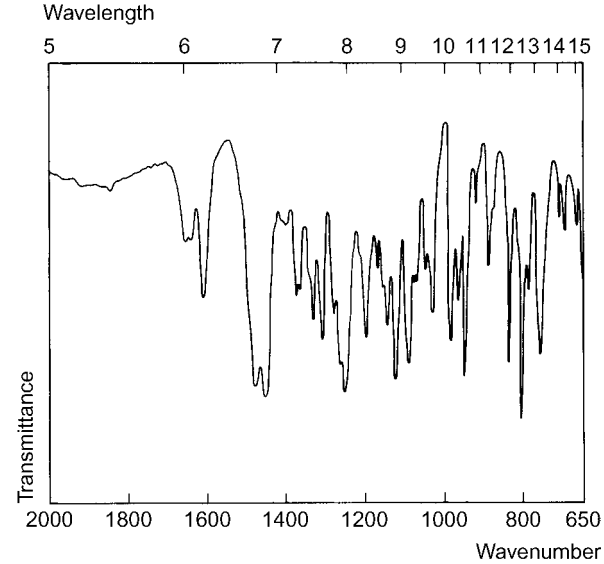
Morphine Tartrate

$(C_{17}H_{19}NO_3)_2,C_4H_6O_6,3H_2O = 491.4$
CAS—302-31-8 (anhydrous); 6032-59-3 (trihydrate)
Proprietary Names It is an ingredient of *Cyclimorph*.
Chemical Properties Minute, colourless, acicular, efflorescent crystals.Soluble 1 in 11 of water and 1 in 1000 of ethanol; practically insoluble in chloroform,ether, and carbon disulfide.

Colour Test Ferric chloride—blue; Liebermann's reagent—black; Mandelin's test—blue-grey; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.37; system TB—R_f 0.00; system TC—R_f 0.09; system TE—R_f 0.20; system TL—R_f 0.01; system TAE—R_f 0.18; system TAF—R_f 0.23; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.15. (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis reagent—violet).
Gas Chromatography System GA—RI 2445, RI 2615 morphine-AC_2 (diamorphine), RI 2360 morphine-PFP_2, RI 2560 morphine-TMS_2, RI 2250 morphine-TFA_2, RI 2459, M (nor-)-AC_3 RI 2955, RI 2440 M (nor-)-PFP_2, RI 2405 M (nor-)PFP_3, RI 2605 M (nor-)-TMS_3, RI 2375 codeine, RI 2388 norcodeine; system GB—RI 2564, RI 2769 diamorphine, RI 2602 morphine-TMS_2, RI 2511 codeine, RI 2535 norcodeine; system GC—RI 2542, RI 2681 codeine; system GM—not eluted.
High Performance Liquid Chromatography System HA—*k* 3.8 (tailing peak), *k* 4.8 (tailing peak) codeine, *k* 3.2 (tailing peak) morphine *N*-oxide, *k* 3.1 (tailing peak) norcodeine, *k* 2.9 (tailing peak) normorphine; system HC—*k* 1.30, *k* 1.21 codeine, *k* 1.56 M (3-glucuronide), *k* 3.51 norcodeine, *k* 3.92 normorphine; system HS—*k* 5.16; system HX—RI 200, RI 266 codeine; system HY—RI 182, RI 133 normorphine, codeine RI 237; system HZ—RT 1.8 min; system HAA—RT 3.3 min; system HAX—RT 5.6 min; system HAY—RT 3.2 min.
Ultraviolet Spectrum Aqueous acid—285 nm (A_1^1 = 52a); aqueous alkali—298 nm (A_1^1 = 92a).



Infrared Spectrum Principal peaks at wave numbers 805, 1243, 1118, 945, 1086, 833 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 285, 162, 42, 215, 286, 124, 44, 284 (morphine); 299, 42, 162, 124, 229, 59, 300, 69 (codeine); 285, 81, 215, 148, 286, 164, 110, 115 (norcodeine); 271, 81, 150, 201, 148, 110, 272, 82 (normorphine)
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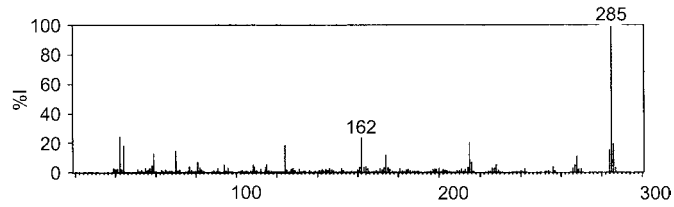

Quantification

Blood **GC** EI ionisation. Limit of quantification, 0.01 mg/L [Mykkänen *et al.* 2000]. ECD. Limit of detection, 1 µg/L [Felby 1979]. FID. Limit of detection, 50 µg/L [Nakamura, Way 1975].

GC-MS Limit of detection, 5 µg/L [Geier *et al.* 1996].

HPLC Varian Pursuit C_{18} (100 × 3 mm i.d., 3 µm). Limit of quantification, 0.0005–0.01 mg/kg [Bjork *et al.* 2010]. Column: LiChrospher 60 RP-select B (250 × 4 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate-2 mmol/L heptanosulfonic acid (pH 2.5) : acetonitrile (92.5 : 7.5). Fluorescence detection. Limit of detection, 3 ng/g [Beike *et al.* 1999]. Morphine and its glucuronides, fluorescence detection [Beike *et al.* 1997]. UV and fluorescence detection. Limit of quantification, 0.1 mg/L [Crump *et al.* 1994].

LC-MS Column: Waters HSS T3 (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 1% formic acid in water : 1% formic acid in acetonitrile (97 : 3 for 0.1 min to 80 : 20 at 2.5 min to 1 : 99 at 2.55 min for 0.45 min to 97 : 3 at 3.01), flow rate 0.6 mL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L [Dahn *et al.* 2010]. Column: Inertsil ODS-3 (150 × 3 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium formate (pH 3) : acetonitrile (95 : 5 for 5 min to 80 : 20 in 5 min for 5 min), flow rate 0.4 mL/min. SIM acquisition mode (*m/z* 286.1). Retention time: 2.7 min for morphine, 2.2 min for morphine 3-glucuronide and 4.3 min for morphine 6-glucuronide [Dienes-Nagy *et al.* 1999]. Limit of quantification, 0.0007–0.02 g/L [Karinen *et al.* 2009]. Screening for opioid drugs [Gergov *et al.* 2009]. Column: Phenomenex Synergi. Mobile phase : 10 mmol/L ammonium formate (pH 3) : acetonitrile. Limit of quantification, 0.5–4.09 µg/L, limit of detection, 0.16–1.2 µg/L [Al Asmari, Anderson 2007]. Column: Superspher RP 18 (125 × 3 mm i.d., 4 µm). Mobile phase acetonitrile : 50 mmol/L ammonium formate buffer (pH 3, 5 : 95), flow rate 0.6 mL/min for 4 min then 1.1 mL/min. SIM acquisition mode (*m/z* 334). Limit of detection, 1 µg/L for morphine, 5 µg/L for the 3-glucuronide and the 6-glucuronide metabolite [Bogusz *et al.* 1997].

Plasma **GC-MS** Limit of quantification, 1.25 nmol/L [Leis *et al.* 2002a]. Morphine glucuronides [Leis *et al.* 2002b]. Column: DB-5MS fused silica capillary (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 5 mL/min. Temperature: 280°. Limit of quantification, ~0.8 µg/L [Leis *et al.* 2000]. Column: 5% phenylmethylsiloxane capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min Temperature programme: 150° for 1 min to 250° at 40°/min for 21.5 min..SIM acquisition mode (*m/z* 414.3). Retention time: 5.84 min. Limit of quantification, 0.75 µg/L, estimated limit of detection, 0.2 µg/L [Fryirs *et al.* 1997]. See Blood [Geier *et al.* 1996].

HPLC UV detection. Limit of detection, 0.5 ng/mL for morphine and 10 µg/L for its glucuronides [Ary, Rona 2001]. Electrochemical and fluorometric detection. Limit of detection, 0.1 µg/L [Meng *et al.* 2000]. Electrochemical detection [Wright, Smith 1998]. Column: µPorasil (300 × 3.9 mm i.d., 10 µm). Mobile phase:

5 mmol/L sodium acetate buffer (pH 3.75) : acetonitrile (25 : 75), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 10.45 min (*k*, 5.04). Limit of detection, 0.1 µg/L [Liaw *et al.* 1998]. Fluorescence detection [Emara 1998]. Electrochemical detection [Liu *et al.* 1997]. UV detection (λ= 240 nm). Limit of quantification, 50 µg/L [Freiermuth, Plasse 1997]. Fluorescence detection. Limit of detection, 1 µg/L [Huwyler *et al.* 1995; Rotshteyn, Weingarten 1996]. Electrochemical detection. Limit of detection, 0.02 µmol/L [Wright *et al.* 1994; Wright, Smith 1998] Electrochemical detection. Limit of detection, 1 µg/L [Rop *et al.* 1994]. Fluorimetric detection. Limit of detection, ~5–10 µg/L [Hartley *et al.* 1993]. Fluorescence detection. Limit of quantification, 10 µg/L [Barrett *et al.* 1991].

LC-MS See Blood [Dahn *et al.* 2010]. Column: YMC ODS-AQ (150 × 2.0 mm i.d., 3 µm). Mobile phase: water-1% formic acid : acetonitrile (95 : 5), flow rate 0.2 mL/min [Slawson *et al.* 1999].

Serum **GC-MS** Morphine and other opioids [Piekoszewski *et al.* 2001]. Column: DB-5 (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2. Temperature: 280°. SIM acquisition mode (*m/z* 557). Limit of quantification, 5.7 ng/L for morphine and 0.71 ng/L for its glucuronides [Hofmann *et al.* 1999].

HPLC Column: Nucleosil C_{18} (250 × 8.4 mm i.d., 5 µm). Mobile phase: acetonitrile : TEA phosphate buffer (1 : 99 to 3 : 97 in 8 min until 23 min. flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 245 nm, λ_{em}= 345 nm) Retention time: 6.97 min for morphine, 4.91 min morphine 3-glucuronide and 8.71 min for morphine 6-glucuronide. Limit of detection, 5 µg/L [Aderjan *et al.* 1995].

LC-MS See Blood [Dahn *et al.* 2010]. Limit of detection, 0.025 mg/L [Maresova *et al.* 2008]. Limit of quantification, 1.3 pmol/mL [Schanzle *et al.* 1999]. Column: LiChropher 30 × 4 mm i.d.). Mobile phase: acetonitrile : 0.001 mol/L ammonium formate (6 : 94)-1% formic acid, flow rate 0.3 mL/min. Limit of quantification, 1 µg/L [Blanchet *et al.* 1999]. Column: Supelcosil LC-Si (25 m × 2.1 mm I.d. 5 µm). Mobile phase: methanol : acetonitrile: water : formic acid (59.8 : 5.2 : 34.65 : 0.35), flow rate 230 µL/min to 460 µL/min. SIM acquisition mode (*m/z* 286). Limit of quantification, 4.0 µg/L [Zuccaro *et al.* 1997]. Morphine and its glucuronides. Limit of quantification, ~0.8 µg/L [Tyrefors *et al.* 1996]. Column: Supelcosil ABZ column (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (85 : 15 to 40 : 60), flow rate 0.8 mL/min. SIM acquisition mode (*m/z* 286). Limit of quantification, 10 µg/L [Pacifici *et al.* 1995].

Urine **GC** NPD, FID [Vu-Duc, Vernay 1990]. See Blood. Limit of detection, 500 µg/L [Nakamura, Way 1975].

GC-MS Morphine and other opioids [Meadway *et al.* 2002]. Morphine and other drugs of abuse [Vorce *et al.* 2000]. Morphine and other drugs of abuse. Limit of detection, 3–12 µg/L [Karacic, Skender 2000]. SIM acquisition mode (*m/z* 364, 477). Limit of quantification, 1 µg/L [Pragst *et al.* 1999]. See Serum [Hofmann *et al.* 1999]. SIM acquisition mode (*m/z* 341). Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Meatherall 1999]. SIM acquisition mode (*m/z* 341, 268, 397). Limit of quantification, 10.0 µg/L, limit of detection, 2.0 µg/L [O'Neal, Poklis 1997]. Morphine and other opioids [Broussard *et al.* 1997; Cremese *et al.* 1998].

HPLC Quantification of morphine and metabolites [Ahsman *et al.* 2010]. Electrochemical detection [Rashid *et al.* 1998]. See Blood [Bogusz *et al.* 1997]. UV and Electrochemical detection. Limit of detection, 50 µg/L [Gerostamoulos *et al.* 1993].

LC-MS Quantification of morphine and other opiates [Shakleya *et al.* 2010]. See Blood [Gergov *et al.* 2009]. See Serum. Limit of quantification, 10 nmol/L [Schanzle *et al.* 1999].

Bile **HPLC** See Blood [Crump *et al.* 1994].

CSF **GC-MS** See Urine [Pragst *et al.* 1999].

HPLC See Plasma [Wright *et al.* 1994; Wright, Smith 1998]. See Blood [Bogusz *et al.* 1997].

LC-MS See Serum [Schanzle *et al.* 1999].

Oral Fluid **GC-MS** See Serum [Piekoszewski *et al.* 2001]. See Hair [Jones *et al.* 2002].

Vitreous Humour **HPLC** See Blood [Bogusz *et al.* 1997].

GC-MS See Urine [Pragst *et al.* 1999].

Bone and Bone Marrow **GC** Column: AT-5 (30 m × 0.25 mm i.d., 0.25 µm). Temperature: 250°. FID [Raikos *et al.* 2001].

Brain **LC-MS** See Blood. Limit of quantification, 0.002–0.06 µg/g [Karinen *et al.* 2009].

Hair **GC-MS** Quantitation of morphine and metabolites. Limit of quantification, 0.08 ng/mg, limit of detection, 0.05 ng/mg [Wu *et al.* 2008]. Morphine and other opioids [Jones *et al.* 2002]. Column: HP ultra 2 5% phenyl silicone, methyl silicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: H_2, flow rate 1 mL/min. Temperature: 250°. SIM acquisition mode (*m/z* 399) [Montagna *et al.* 2002]. See Serum [Piekoszewski *et al.* 2001]. Morphine, codeine and cocaine [Brewer *et al.* 2001]. Column: Restek Rtx-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 1 mL/min. Temperature: 250°. SIM acquisition mode (*m/z* 429.20) [Pichini *et al.* 1999]. SIM acquisition mode (*m/z* 430 and 433 (morphine), 629 (normorphine)). Limit of quantification, 0.5 mg/g [Hold *et al.* 1998]. Morphine and other opioids. Limit of detection, 0.12–0.28 µg/L [Gaillard, Pepin 1997].

Kidney **GC** See Blood. Limit of detection, 300 ng/g [Nakamura, Way 1975].

Liver **GC** See Blood. Limit of detection, 300 ng/g [Nakamura, Way 1975].

Disposition in the Body Morphine is rapidly absorbed after SC, IV or IM administration. Absorption after oral administration is variable and there is considerable first-pass metabolism; bioavailability ~20–30%. Enterohepatic circulation may occur. It is widely distributed, mainly in kidneys, liver, lungs and spleen, with lower concentrations in the brain and muscles, but it does not accumulate in the tissues. Morphine crosses the blood–brain barrier and the placenta; it is found in breast milk and sweat. Approximately 90% of a dose is metabolised after administration. The major metabolic reaction is conjugation to form morphine 3- and 6-glucuronides; morphine 6-glucuronide probably contributes to the analgesic effect, but the 3-glucuronide may antagonise analgesia. Other reactions include *N*-demethylation, *O*-methylation, and *N*-oxide formation, and other active metabolites include normorphine, codeine and morphine ethereal sulfate. After a parenteral dose, up to 90% is excreted in the urine in 24 h, including ~10% of the dose as free morphine, 65–70% as conjugated morphine, up to 10% as morphine 3-ethereal sulfate, 1% as normorphine, and 3% as normorphine glucuronide. After an oral dose, ~60% is excreted in the urine in 24 h, and ~3% of the dose is excreted as free morphine in 48 h. The urinary excretion of morphine appears to be pH dependent to some extent; as the urine becomes more acid, excretion of free morphine rises, and as the urine becomes more alkaline, excretion of the glucuronide conjugate rises. Up to ~10% of a dose may be excreted in the bile.

Morphine is the major active metabolite of diamorphine, and is also a metabolite of codeine.

Note Morphine may be slowly converted to pseudomorphine in cadavers and can be found as such on exhumation.

Therapeutic Concentration In plasma, usually in the range 0.01–0.07 mg/L.

In 10 patients receiving oral morphine as either a once-a-day extended-release formulation or a twice-a-day extended-release formulation for 7 days, plasma concentrations 30 min after a dose were reported as follows (normalised to a total daily dose of 100 mg):

	Twice daily	*Once daily*
Morphine (µg/L)	11.0	17.1
Morphine 3-glucuronide (µg/L)	592	709
Morphine 6-glucuronide (µg/L)	85.8	91.8

[Portenoy *et al.* 2002]

Thirty-one newborn infants (gestational age, 24–41 weeks) on ventilation were administered 140 µg/kg morphine for 1 h followed by 20 µg/kg/h for 2–4 days. The mean serum morphine concentration during the constant infusion was 105 µg/L, observed at 2 h. The mean steady-state concentration was 167 µg/L, which was achieved between 24 and 48 h after starting the infusion. Steady-state concentrations of morphine 6-glucuronide and morphine 3-glucuronide were not reached within 60 h [Saarenmaa *et al.* 2000].

In 8 patients receiving morphine, 60–3000 mg daily via chronic (8–160 days) SC infusion for severe cancer pain, the normalised plasma concentrations of morphine, morphine 6-glucuronide, and morphine 3-glucuronide were 0.3 to 0.8 µg/L, 1.0 to 3.1 µg/L, and 6.8 to 24.3 µg/L, respectively [Vermeire *et al.* 1998].

Twelve patients with post-hepatic cirrhosis (mean age, 40.3 years; range, 35–45) and 10 healthy volunteers (mean age, 39.3 years) were administered 30 mg controlled-release morphine. The peak serum concentration was 35.2 µg/L in the cirrhotic patients and 12.8 µg/L in the healthy volunteers, observed at 174.0 and 142.5 min, respectively [Kotb *et al.* 1997].

Four healthy volunteers, aged 19–34 years, were administered 3 consecutive doses of 2.2, 4.4 and 8.8 mg morphine sulfate pentahydrate at 40-min intervals via an aerosol delivery system. Plasma morphine concentrations were proportional to the dose in this study. Additionally, 6 volunteers were administered 4.4 mg aerosolised morphine sulfate over a 2.1-min period on 3 occasions and IV infusions of 2 and 4 mg morphine sulfate over 3 min. The mean peak plasma morphine concentration for the aerosol dose was 109 µg/L (range, 39–398), attained at 2.7 min; it was 165 µg/L for the 2 mg IV dose, attained at 3.0 min, and 273 µg/L for the 4 mg IV dose, attained at 3.2 min [Ward *et al.* 1997].

Toxicity The estimated minimum lethal dose for adults is 200 mg, but addicts may be able to tolerate up to 10 times as much. Morphine is initially eliminated from the blood fairly quickly and blood concentrations are difficult to interpret, especially as toxic effects depend on the degree of tolerance that has been acquired.

A 46-year-old woman who was admitted to hospital in a coma with severe respiratory failure later developed cardiovascular instability and convulsions but recovered sufficiently 2 days later to communicate in writing that she had ingested a large quantity of controlled-release morphine tablets. At 60 h after the presumed intake, the plasma concentrations of morphine, morphine 3-glucuronide, and morphine 6-glucuronide were 2160, 13100 and 2330 nmol/L, respectively; urinary recovery of morphine and its metabolites amounted to 6.8 mmol, equivalent to an oral intake of at least 2500 mg [Westerling *et al.* 1998].

An 8-year-old girl accidentally ingested morphine before going to bed and was found dead the next morning. Following a tonsillectomy, she was prescribed meperidine syrup at a dose of 100 mg every 4 h but the pharmacist accidentally dispensed Roxanol containing 20 g/L morphine sulfate. The little girl took 1–2 teaspoons as prescribed. The morphine concentration in blood was 0.128 mg/L, in bile 135 mg/L and in stomach

contents 16 mg/L (a total amount of 2.3 mg). The cause of death was determined as morphine poisoning, which resulted in respiratory depression [Poklis *et al.* 1995].

A 90-year-old woman apparently died of natural causes in a nursing home. Subsequent investigation indicated that she had received an unauthorised bolus of morphine just before her death. Following exhumation of the embalmed body, toxicological analysis of the liver revealed a free morphine concentration of 1.5 µg/g. This level was deemed consistent with a lethal concentration. The manner of death was homicide [Levine *et al.* 1994].

Bioavailability About 20–30%

Half-life Plasma half-life, ~2–3 h.

Volume of Distribution ~3–5 L/kg.

Clearance Plasma clearance, ~15–20 mL/min/kg; mean total body clearance for newborn infants (24–41 weeks of gestation), 2.4 mL/min/kg (range, 0.8–6.4).

Protein Binding 20–35%.

Note For a review of the metabolism of morphine, see Christrup [1997]; for a review of the pharmacokinetics of sustained-release morphine, see Gourlay [1998].

Dose Morphine hydrochloride, sulfate, or tartrate, 5 to 20 mg by mouth or parenterally, every 4 h.

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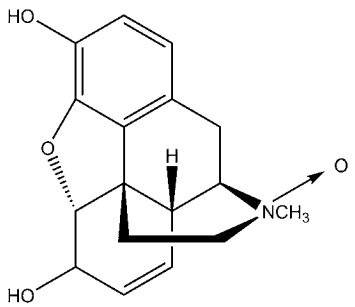
Westerling D *et al.* (1998). Near fatal intoxication with controlled-release morphine tablets in a depressed woman. *Acta Anaesthesiol Scand* 42: 586–589.
Wright AW, Smith MT (1998). Improved one-step solid-phase extraction method for morphine, morphine-3-glucuronide, and morphine-6-glucuronide from plasma and quantitation using high-performance liquid chromatography with electrochemical detection. *Ther Drug Monit* 20: 215–218.
Wright AW *et al.* (1994). Quantitation of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in plasma and cerebrospinal fluid using solid-phase extraction and high-performance liquid chromatography with electrochemical detection. *Ther Drug Monit* 16: 200–208.
Wu YH *et al.* (2008). Simultaneous quantitative determination of amphetamines, ketamine, opiates and metabolites in human hair by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 22: 887–897.
Zuccaro P *et al.* (1997). Simultaneous determination of heroin 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography–atmospheric pressure ionspray-mass spectrometry. *J Anal Toxicol* 21: 268–277.

Morphine N-oxide

Narcotic
$C_{17}H_{19}NO_4 = 301.3$
Synonyms Genomorphine; morphine aminoxide; *N*-oxymorphine.



Chemical Properties Mp 275°. It is stated to be bimolecular. Slightly soluble in water, insoluble in chloroform.
Colour Tests Ammonium molybdate test—deep purple→blue→green (limit of detection, 0.1 µg); ammonium vandate test—dull purple→brown (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—purple (limit of detection., 0.1 µg).
Thin-layer Chromatography System T10—R_f 0.23 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—284.5 nm with an inflexion at 279 nm.
Quantification
Other **TLC** Guinea-Pig Urine. Plates: silica gel. Solvent system A: 1-butanol : acetic acid : water (35 : 3 : 10). Solvent system B: ethyl acetate : methanol : ammonium hydroxide (17 : 2 : 1). Visualisation with iodoplatinate. R_f 0.8 and 0.4 for system A and B, respectively [Yeh *et al.* 1979].

GC Guinea-Pig Urine. Column: Gas Chrom Q 3% OV-17 (0.9 m × 2 mm). Temperature: 220°. Retention time: 5.6 min. Limit of detection not reported [Yeh *et al.* 1979].

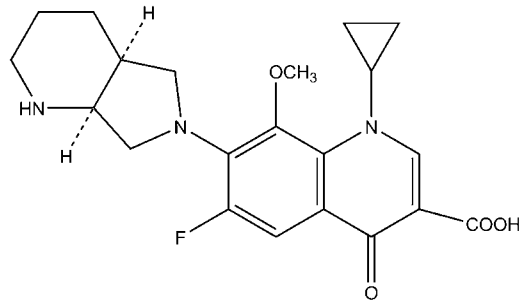
GC-MS Guinea-Pig Urine. Column: Gas Chrom Q 3% OV-17 (0.9 m × 2 mm). Carrier gas: CH_3. Temperature: 210°. CI at 80 eV, full scan mode. Limit of detection not reported [Yeh *et al.* 1979].

Disposition in the Body After the administration of morphine *N*-oxide to rats, morphine appeared in the urine equivalent to 61% of the amount of the dose. Reduction of *N*-oxides to the corresponding tertiary base is an established metabolic pathway. Morphine *N*-oxide inhibits demethylation *in vivo*, a finding which could explain the lack of normorphine in the urine of rats. Reduction is the only demonstrable metabolic pathway for morphine *N*-oxide *in vivo* [Heimans *et al.* 1971].
Toxicity The IV and SC acute toxicities of morphine *N*-oxide in mice are 3.2- and 8-times less than that of morphine, respectively [Fennessy, Fearn 1969].

Fennessy MR, Fearn HJ (1969). Some observations on the toxicology of morphine-*N*-oxide. *J Pharm Pharmacol* 21: 668–673.
Heimans RL *et al.* (1971). Some aspects of the metabolism of morphine-*N*-oxide. *J Pharm Pharmacol* 23: 831–836.
Yeh SY *et al.* (1979). Isolation and identification of morphine *N*-oxide alpha- and beta-dihydromorphines, beta- or gamma-isomorphine, and hydroxylated morphine as morphine metabolites in several mammalian species. *J Pharm Sci* 68: 133–140.

Moxifloxacin

Antibacterial, Fluoroquinolone
$C_{21}H_{24}FN_3O_4 = 401.4$
CAS—151096-09-2
IUPAC Name 7-[(4a*S*,7a*S*)-1,2,3,4,4a,5,7,7a-Octahydropyrrolo[3,4b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid
Synonym 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid



Chemical Properties Mp 238° to 242°. Log *P* (octanol/water), 2.03 [Wishart 2006].

Moxifloxacin Hydrochloride

$C_{21}H_{24}FN_3O_4,HCl = 437.9$
CAS—186826-86-8
Synonym Bay-12-8039
Proprietary Names *Actimax*; *Actira*; *Avalox*; *Avelon*; *Avelox*; *Flovacil*; *Izilox*; *Megaxin*; *Moxicip*; *Octegra*; *Proflox*; *Vigamox*; *Vigamoxi*.
Chemical Properties Slightly yellow to yellow crystalline powder. Mp 324° to 325° with decomposition. Stable in serum samples at room temperature over 24 h [Nguyen *et al.* 2004].
Quantification
Plasma **HPLC** Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L orthophosphoric acid (pH 2.5, 80 : 20), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 464$ nm, $\lambda_{em} = 537$ nm). Retention time: 4.8 min. Limit of quantification, 15 µg/L; limit of detection, 6 µg/L [Tatar Ulu 2007]. Column: Supelco LC-Hisep (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.25 mol/L trisodium phosphate (pH 3.0, 5 : 95) with 10 mmol/L sodium dodecyl sulfate, flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 500$ nm). Retention time: 6.2 min. Limit of quantification, 3.0 µg/L, limit of detection, 1.0 µg/L [Laban-Djurdjevi *et al.* 2006]. Column: Adsorbosphere HS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium dodecyl sulfate–10 mmol/L tetrabutylammonium acetate–25 mmol/L citric acid : acetonitrile (57 : 43, pH 3.5), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 293$ nm, $\lambda_{em} = 500$ nm) or UV detection ($\lambda = 293$ nm). Retention time: 7.0 min. Limit of quantification, 20 µg/L (fluorescence detection), 100 µg/L (UV detection) [Liang *et al.* 2002]. Column: Supelcosil ABZ + (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium dihydrogen phosphate (pH 4.0, 18 : 82), flow rate 1.25 mL/min. UV detection ($\lambda = 296$ nm). Retention time: 6.1 min. Limit of quantification, 30 µg/L, limit of detection, 6.5 µg/L [Lemoine *et al.* 2000]. Column: Nucleosil 100 C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L tetrabutylammonium sulfate–0.05 mol/L sodium dihydrogen phosphate (pH 3.0) : acetonitrile (95 : 5 to 75 : 25 at 1 min for 4 min to 30 : 70 at 5.1 min for 1.4 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 296$ nm, $\lambda_{em} = 504$ nm). Retention time: 6.6 min. Limit of quantification, 2.5 µg/L [Stass, Dalhoff 1997].

CE Column: fused silica capillary (270 × 0.05 mm i.d.). Fluorescence detection ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 520$ nm). Retention time: 4.1 min. Limit of quantification; 2.5 µg/L, limit of detection, 0.5 µg/L [Moller *et al.* 1998].
Serum **HPLC** Column: Supelcosil ABZ + (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 2.5) with 2 mmol/L tetrabutylammonium bromide : acetonitrile (88 : 12), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 296$ nm, $\lambda_{em} = 504$ nm). Retention time: 5.2 min. Limit of quantification, 125 µg/L, limit of detection, 35 µg/L [Nguyen *et al.* 2004].
Oral Fluid **HPLC** See Plasma. Retention time: 7.6 min. Limit of quantification, 10 µg/L [Stass and Dalhoff 1997].
Urine **HPLC** See Plasma. Retention time: 9.0 min. Limit of quantification, 50 µg/L [Stass, Dalhoff 1997].
Aqueous Humour **HPLC** Column: Waters AccQ Tag amino acid (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile–methanol–0.05 mol/L tetrabutylammonium chloride–trifluoroacetic acid (pH 3.0, 37.5 : 12.5 : 949 : 1) : acetonitrile–methanol–0.05 mol/L tetrabutylammonium chloride–trifluoroacetic acid (pH 3.0, 75 : 25 : 899 : 1; 100 : 0 for 3 min to 0 : 100 at 12 min to 100 : 0 at 16 min for 5 min), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 500$ nm). Retention time: 16.7 min. Limit of quantification, 10 µg/L [Chan *et al.* 2006].
Vitreous Humour **HPLC** See Aqueous Humour [Chan *et al.* 2006].
Other **HPLC** Lung Tissue. Column: Supelcosil ABZ + (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium dihydrogen phosphate (pH 4.0; 18 : 82), flow rate 1.25 mL/min. UV detection ($\lambda = 296$ nm). Retention time:

M

6.1 min. Limit of quantification, 0.4 µg/g; limit of detection, 0.05 µg/L [Lemoine *et al.* 2000].

CE Microdialysate. Column: fused silica capillary (27 cm × 50 µm). Fluorescence detection ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 520$ nm). Migration time: 4.1 min. Limit of quantification, 5 µg/L; limit of detection, 0.5 µg/L [Moller *et al.* 1998].

Disposition in the Body Readily absorbed from the gastrointestinal tract after oral doses. It is widely distributed throughout the body tissues. It does not appear to be metabolised by the CYP450 pathways but is metabolised mainly via sulfate and glucuronide conjugation; it is excreted in the urine and the faeces as unchanged drug and as metabolites. The sulfate conjugate is excreted primarily in the faeces and the glucuronide is excreted exclusively in the urine. Distribution into milk has been found in animals.

Therapeutic Concentration

A group of 27 adult patients undergoing elective pars plana vitrectromy was administered a single tablet of 400 mg of moxifloxacin 1 to 2 h before surgery. The mean sampling times after oral administration of the drug for serum and aqueous and vitreous humours were 2.0, 1.5 and 1.5 h, respectively. Moxifloxacin mean concentrations measured in the serum and aqueous and vitreous humours were 1.34, 0.21 and 0.09 mg/L, respectively. Some values obtained were considered outliers as one patient had serum levels approx. 67-fold the mean value calculated and 2 other patients had vitreous levels 45 times below the mean values of the rest of the group [Vedantham *et al.* 2006].

A group of 35 patients scheduled for cataract surgery was administered a single 400 mg tablet of moxifloxacin. The mean peak aqueous humour moxifloxacin concentration was 1.17 mg/L after 10 h and the mean peak value in serum was 3.16 mg/L at 4 h [Walter *et al.* 2007].

A total of 45 healthy volunteers were administered single ascending doses of moxifloxacin (range 50 to 800 mg) in a dose-finding study. Mean peak plasma concentrations were as follows:

Dose (mg)	*C_{max} (mg/L)*	*Time (h)*
50	0.29	1.75
100	0.59	2.0
200	1.16	2.5
400	2.5	1.5
600	3.19	2.5
800	4.73	3.0

Plasma concentrations decreased between 30 and 55% of the peak concentrations within 4 to 5 h. Pharmacokinetics in saliva were investigated over the dose range 50 to 200 mg. Following administration of the drug, peak concentrations in saliva were reached approximately 1 to 4 h after administration and were 0.14 to 0.52 mg/L after the 50 mg dose, 0.41 to 1.80 mg/L after the 100 mg dose, and 1.12 to 2.83 mg/L after the 200 mg dose. The renal clearance data indicated partial tubular reabsorption of the drug [Stass *et al.* 1998].

A group of 49 patients undergoing lung surgery was administered 400 mg moxifloxacin daily either orally or as an IV infusion for 5 days. Plasma and lung concentrations in the IV groups were as follows:

Group	*Time (h)*	*Plasma (mg/L)*	*Lung (µg/g)*
A	1	5.9	8.8
B	2	2.5	12.4
C	3.4	3.3	10.4
D	8.9	2.0	8.7
E	23.8	0.7	2.1
F	33.4	0.4	1.3

In the orally treated groups, values were:

Group	*Time (h)*	*Plasma (mg/L)*	*Lung (µg/g)*
G	2.4	2.5	16.2
H	3.1	3.2	15.6
I	8.3	2.5	9.3
J	12.0	1.9	8.3
K	24.1	0.7	2.5
L	36.3	0.8	1.7

[Breilh *et al.* 2003].

A group of 97 patients scheduled to undergo cataract surgery was administered 0.5% moxifloxacin as eye drops. The patients were split into 4 groups according to administration method and dose. All patients received the drops four times a day starting 2 days before surgery. Patients in subgroup A did not receive any drops on the day of surgery. Those in subgroup B received the drops 3 times at 15 min intervals starting approx. 2 h before surgery. In subgroup C, eyes were dilated with a wick soaked in a dilating mixture containing moxifloxacin that was placed in the lower fornix for 10 min approx. 2 h before surgery. The final subgroup (D) had the wick and also received drops at the time of preparation for surgery. Aqueous humour concentrations of moxifloxacin in the 4 groups were:

Group	*Moxifloxacin (mg/L)*
A	0.38
B	2.16
C	0.88
D	0.97

[Ong-Tone 2007].

Topical application of moxifloxacin 0.5% (4 times a day for 3 days before surgery and every 15 min for 3 doses beginning 1 h before surgery) in eyes of 14 patients (average age, 71 years) resulted in a mean aqueous humour concentration of 1.31 mg/L [Solomon *et al.* 2005].

Toxicity Absorption of moxifloxacin is impaired by concomitant administration of aluminium- and magnesium-containing antacids, and administration of these agents should be staggered. An interval of 2 h before or 4 h after taking the antacid ensures that the effect of the interaction is not clinically relevant [Stass *et al.* 2001].

Bioavailability 90%.

Half-life Approximately 11 to 14 h.

Volume of Distribution 216 to 308 L.

Clearance 202 to 297 mL/min.

Protein Binding Approximately 48%.

Dose Moxifloxacin is given by mouth, or by IV infusion over 60 min, for the treatment of susceptible infections, including respiratory, skin and skin structure, and intra-abdominal infections. Moxifloxacin is given as the hydrochloride but doses are expressed in terms of the base; moxifloxacin hydrochloride 436.3 mg is equivalent to approx. 400 mg moxifloxacin. The usual dose is 400 mg once daily.

Moxifloxacin is also used topically as the hydrochloride in eye drops containing the equivalent of 0.5% of moxifloxacin for the treatment of bacterial conjunctivitis.

Breilh D *et al.* (2003). Diffusion of oral and intravenous 400 mg once-daily moxifloxacin into lung tissue at pharmacokinetic steady-state. *J Chemother* 15: 558–562.

Chan KP *et al.* (2006). Determination of ofloxacin and moxifloxacin and their penetration in human aqueous and vitreous humor by using high-performance liquid chromatography fluorescence detection. *Anal Biochem* 353: 30–36.

Laban-Djurdjevic A *et al.* (2006). Optimization and validation of the direct HPLC method for the determination of moxifloxacin in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 844: 104–111.

Lemoine T *et al.* (2000). Determination of moxifloxacin (BAY 12-8039) in plasma and lung tissue by high-performance liquid chromatography with ultraviolet detection using a fully automated extraction method with a new polymeric cartridge. *J Chromatogr B Biomed Sci Appl* 742: 247–254.

Liang H *et al.* (2002). Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 53–63.

Moller JG *et al.* (1998). Capillary electrophoresis with laser-induced fluorescence: a routine method to determine moxifloxacin in human body fluids in very small sample volumes. *J Chromatogr B Biomed Sci Appl* 716: 325–334.

Nguyen HA *et al.* (2004). Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. *J Chromatogr B Analyt Technol Biomed Life Sci* 810: 77–83.

Ong-Tone L (2007). Aqueous humor penetration of gatifloxacin and moxifloxacin eyedrops given by different methods before cataract surgery. *J Cataract Refract Surg* 33: 59–62.

Solomon R *et al.* (2005). Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. *Ophthalmology* 112: 466–469.

Stass H, Dalhoff A (1997). Determination of BAY 12-8039, a new 8-methoxyquinolone, in human body fluids by high-performance liquid chromatography with fluorescence detection using on-column focusing. *J Chromatogr B Biomed Sci Appl* 702: 163–174.

Stass H *et al.* (1998). Pharmacokinetics, safety, and tolerability of ascending single doses of moxifloxacin, a new 8-methoxy quinolone, administered to healthy subjects. *Antimicrob Agents Chemother* 42: 2060–2065.

Stass H *et al.* (2001). Evaluation of the influence of antacids and H_2 antagonists on the absorption of moxifloxacin after oral administration of a 400 mg dose to healthy volunteers. *Clin Pharmacokinet* 40(Suppl1): 39–48.

Tatar Ulu S (2007). High-performance liquid chromatography assay for moxifloxacin: pharmacokinetics in human plasma. *J Pharm Biomed Anal* 43: 320–324.

Vedantham V *et al.* (2006). Vitreous and aqueous penetration of orally administered moxifloxacin in humans. *Eye* 20: 1273–1278.

Walter S *et al.* (2007). Concentration of moxifloxacin in serum and human aqueous humor following a single 400 mg oral dose. *J Cataract Refract Surg* 33: 553–555.

Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

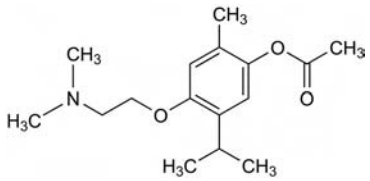
Moxisylyte

Vasodilator

$C_{16}H_{25}NO_3 = 279.4$

CAS—54-32-0

IUPAC Name [4-(2-(Dimethylaminoethoxy)-2-methyl-5-propan-2-ylphenyl]acetate
Synonyms 4-[2-(Dimethylamion)ethoxy]-2-methyl-5-(1-methylethyl)phenol] acetate; thymoxamine

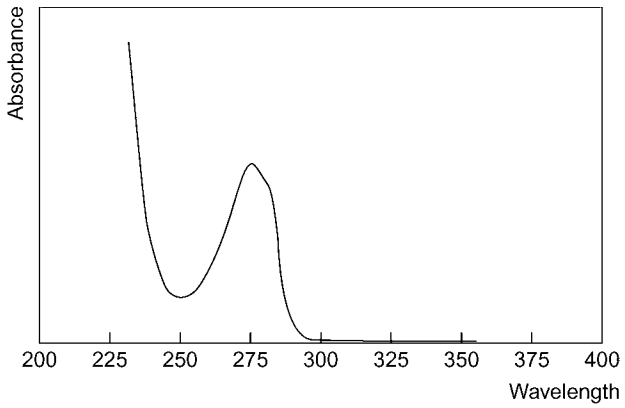


Chemical Properties Practically insoluble in water; soluble in chloroform. pK_a 8.7. Log *P* (octanol/water), 3.2.

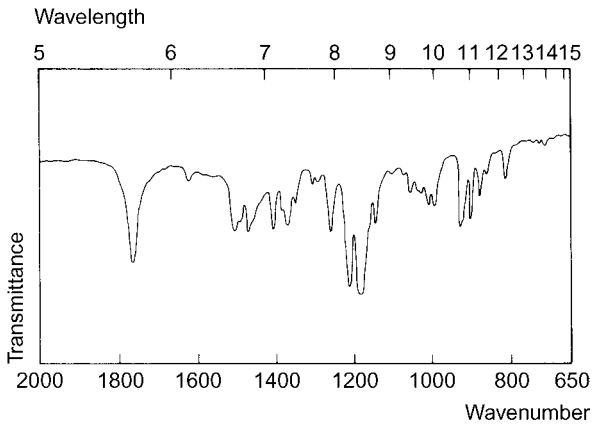
Moxisylyte Hydrochloride

$C_{16}H_{25}NO_3$,HCl = 315.8
CAS—964-52-3
Proprietary Names *Arlitene*; *Carlytène*; *Icavex*; *Opilon*; *Vasoklin*.
Chemical Properties A white crystalline powder. Mp 208° to 210°. Soluble 1 in 2.5 of water, 1 in 11 of ethanol and 1 in 3 of chloroform; practically insoluble in ether.

Colour Test Marquis test—yellow-brown.
Thin-layer Chromatography System TA—R_f 0.52; system TL—R_f 0.19; system TB—R_f 0.31; system TC—R_f 0.44 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1832.
High Performance Liquid Chromatography System HA—Moxisylyte *k* 2.9, desacetylmoxisylyte *k* 2.3.
Ultraviolet Spectrum Aqueous acid—275 nm (A_1^1=80a); aqueous alkali—235, 301 nm (A_1^1=146b).



Infrared Spectrum Principal peaks at wavenumbers 1184, 1212, 1761, 1261, 1504, 931 cm^{-1} (moxisylyte hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 72, 279, 234, 151, 192, 166, 165.
Quantification
Plasma **HPLC** Fluorescence detection. Metabolites of moxisylyte. Limit of detection, 2.5 to 2.8 µg/L for desacetyl and sulfate metabolites [Marquer, Bressolle 1997]. Fluorimetric detection. For method of quantification for metabolites of moxisylyte, see Costa *et al.* [1993].

Spectrofluorimetry For method, see Arbab and Turner [1971].
Urine **HPLC** See Plasma. Limit of detection, 40 to 200 µg/L for desacetyl and sulfate metabolites [Marquer, Bressolle 1997]. See Plasma [Costa *et al.* 1993].
Other (Monkey Plasma) **HPLC** Fluorescence detection. Metabolites of moxisylyte. Limit, of detection, 2 ng for desacetylmoxisylyte and 4 ng for desmethyldesacetylmoxisylyte [Geahchan, Chambon 1980].
Disposition in the Body Moxisylyte is poorly absorbed after oral administration. Deacetylation to give desacetylmoxisylyte and subsequent demethylation to give desmethyldesacetylmoxisylyte may occur.
Therapeutic Concentration

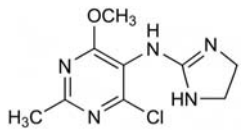
After single oral doses of 150 mg to 4 female subjects, plasma concentrations of about 0.06 mg/L were achieved after 30 to 60 min in 2 subjects; the drug was not detected in the other 2 subjects. After an IV injection of 0.2 mg/kg over 2 min to 2 male subjects, plasma concentrations at 2, 7 and 15 min were 0.14, 0.08 and 0.05 mg/L in the first subject and 0.1, 0.04 and 0.03 mg/L in the other [Arbab, Turner 1971].

Dose The equivalent of 160 mg of moxisylyte daily.

Arbab AG, Turner P (1971). The fluorimetric determination of thymoxamine in plasma. *J Pharm Pharmacol* 23: 719–721.
Costa P *et al.* (1993). Multiple-dose pharmacokinetics of moxisylyte after oral administration to healthy volunteers. *J Pharm Sci* 82: 968–971.
Geahchan AE, Chambon PL (1980). Determination of metabolites of thymoxamine in plasma by high performance liquid chromatography. *Anal Chem* 52: 999–1001.
Marquer C, Bressolle F (1997). High-performance liquid chromatographic determination of the conjugate metabolites of moxisylyte in human plasma and urine. *J Chromatogr B Biomed Sci Appl* 691: 389–396.

Moxonidine

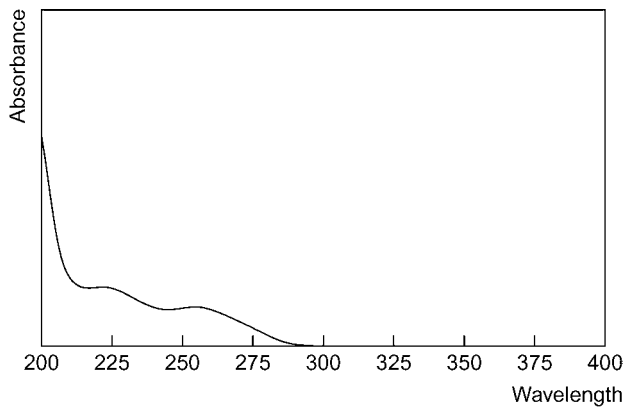
Antihypertensive
$C_9H_{12}ClN_5O$ = 241.7
CAS—75438-57-2
IUPAC Name 4-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-methoxy-2-methyl-5-pyrimidinamine
Synonyms BDF-5895, BE-5895.
Proprietary Names *Cynt*; *Physiotens*.



Chemical Properties Mp 217° to 219°.

Moxonidine Hydrochloride

$C_9H_{12}ClN_5O.xHCl$
CAS—75438-58-3
Chemical Properties Crystals from isopropanol/ether. Mp 189°.
Ultraviolet Spectrum Principal peaks at 222, 258 nm.



Quantification
Plasma **GC–MS** MS detection (NICI, SIM at *m/z* 721). Limit of quantification, 0.1 µg/L, limit of detection, 0.05 µg/L [Kirch 1990].

Disposition in the Body Moxonidine is well absorbed after oral administration (peak plasma concentrations are reached between 0.5 and 3.0 h). It is not subject to first-pass metabolism and food does not interfere with its pharmacokinetics. It is metabolised mainly to 4,5-dehydromoxonidine and a guanine derivative. It is excreted in urine mainly as the unchanged drug (50 to 75%) and as its metabolites. Only 1% of a dose is excreted via faeces. The drug is detectable in breast milk.

Therapeutic Concentration

Eight hypertensive patients previously treated with antihypertensive therapy, aged between 32 and 69 years, were administered a single oral dose of 0.25 mg moxonidine. A mean peak concentration of 1.96 µg/L (range, 1.16 to 3.06 µg/L) was observed at 1.07 h (0.29 to 2.55 h) after administration [Kirch 1990].

Group 1: 8 individuals with normal renal function (creatinine clearance, 6.4 L/h), group 2: 8 patients with moderately impaired renal function (creatinine clearance, 3 L/h), group 3: 8 patients with severe renal dysfunction (creatinine clearance, 1.4 L/h). Each group was administered 0.3 mg daily for 7 days. The peak plasma concentrations were 1.5, 2.1 and 2.3 µg/L for groups 1, 2 and 3, respectively, observed at 1.0, 1.3 and 1.1 h [Kirch *et al.* 1988].

Toxicity

Three cases of overdose have been reported, two of which involved the children (2 to 3 years old) of 2 patients accidentally ingesting moxonidine. Full recoveries were made without any signs of cardiovascular disorder. A third case involved a 23-year-old man who intentionally ingested moxonidine, ranitidine and alcohol in a suicide attempt. Plasma concentrations were 70-fold higher than would be expected after ingestion of the recommended dose. Bradycardia, somnolence and hypotension were observed and treated, and the patient made a full recovery [Schachter *et al.* 1998].

Bioavailability 88%.

Half-life 2 to 3 h (prolonged in renal impairment).

Volume of Distribution 1.39 L/kg (0.85 to 1.75 L/kg).

Clearance Plasma, 0.77 L/h/kg (43.6 to 69 L/h/kg; 32.9 L/h/kg for those with moderate renal impairment; 22.1 L/h/kg, severe renal impairment).

Protein Binding 5.8 to 7.9%.

Note For a review of moxonidine, see Prichard *et al.* [1999].

Dose The usual initial dose is 200 µg daily which may be increased to a maximum of 600 µg over a number of weeks. The dose may be reduced in those with renal impairment.

Kirch W (1990). Pharmacodynamic action and pharmacokinetics of moxonidine after single oral administration in hypertension patients. *J Clin Pharmacol* 30: 1088–1095.

Kirch W *et al.* (1988). The influence of renal function on clinical pharmacokinetics of moxonidine. *Clin Pharmacokinet* 15: 245–253.

Prichard BN *et al.* (1999). Moxonidine: a new antiadrenergic antihypertensive agent. *J Hypertens Suppl* 17(3): S41–S54.

Schachter M *et al.* (1998). Safety and tolerability of moxonidine in the treatment of hypertension. *Drug Safety* 19: 191–203.

Mupirocin

Antibacterial (Topical)

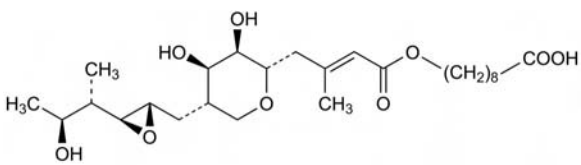
$C_{26}H_{44}O_9 = 500.6$

CAS—12650-69-0

IUPAC Name 9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxy-nonanoic acid

Synonyms (2*E*)-5,9-Anhydro-2,3,4,8-tetradeoxy-8-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]-3-methyl-L-*talo*-non-2-enonic acid, 8-carboxyoctyl ester; BRL-4910A, pseudomonic acid A; *trans*-pseudomonic acid.

Proprietary Names *Bactoderm*; *Bactroban Ointment*; *Celefer*; *Plasimine*; *Eismycin*; *Turixin*.



Chemical Properties An off-white crystalline solid. Mp 77° to 78°. Soluble in dehydrated alcohol, acetone and chloroform; slightly soluble in water and ether. pKa (22°), 5.

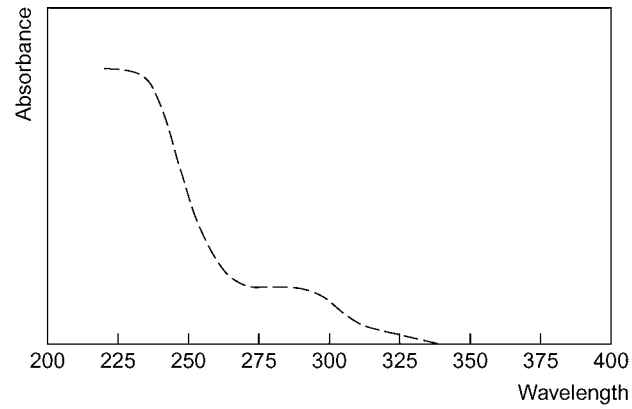
Mupirocin Calcium

Proprietary Name *Bactroban Nasal*

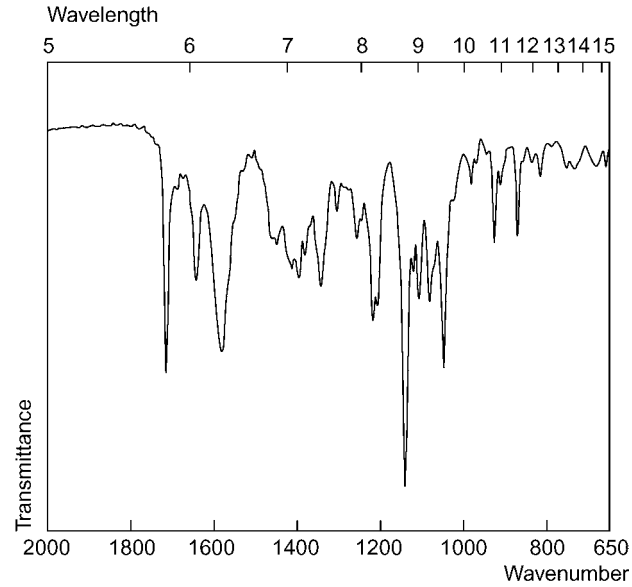
Thin-layer Chromatography System TE—R_f 0.01; system TF—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 459.

Ultraviolet Spectrum Ethanol—222 nm; basic—287 nm.



Infrared Spectrum Principal peaks at wavenumbers 1718, 1146, 1052 cm^{-1}.



Disposition in the Body Systemic absorption after topical application is very small. Mupirocin is rapidly metabolised to monic acid (microbiologically inactive) by de-esterification. Following IV administration of a single 125 mg mupirocin sodium salt dose, ~0.5% is excreted unchanged in urine, whereas 72% is excreted as monic acid within 12 h. Mupirocin does not appear to be inactivated by non-specific esterases present in blood.

Therapeutic Concentration

Following IV infusions over 25 min, in healthy adults, of 31.3, 61.2, 125 and 252 mg mupirocin sodium salt, peak plasma concentrations reached were 1.9, 1.9, 7.1 and 14.1 µg/mL, respectively. Serum concentrations declined rapidly and mupirocin was undetectable within 3 h [GlaxoSmithKline].

Half-life After IV administration, mupirocin, 15 to 30 min; monic acid, 32 to 78 min.

Protein Binding 95 to 97%.

Dose Applied topically as a 2% ointment in macrogol bases for the treatment of various bacterial skin infections up to 3 times a day for up to 10 days.

GlaxoSmithKline, Patient Information Leaflet, data on file.

Muscimol

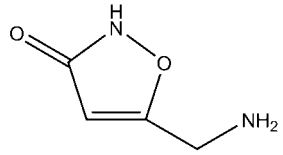
CNS Depressant, $GABA_A$ Receptor Agonist

$C_4H_6N_2O_2 = 114.1$

CAS—2763-96-4

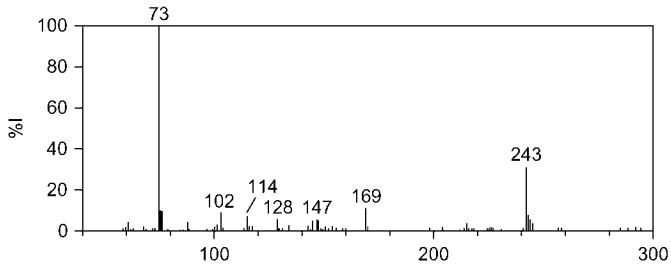
IUPAC Name 5-(Aminomethyl)-1,2-oxazol-3-one

Synonyms Agarin; 5-aminomethyl-3-hydroxyisoxazole; 5-(aminomethyl)-3-isoxazolol; 5-(aminomethyl)-3(2*H*)-isoxazolone; 3-hydroxy-5-aminomethylisoxazole; pantherine.



Chemical Properties Crystals. Mp 175°. Log *P* (octanol/water), −2.39 [Hansch *et al.* 1995]. Muscimol is the major psychoactive alkaloid of the mushroom *Amanita muscaria* (Fly Agaric).

Mass Spectrum Principal ions at *m/z* 73, 243, 169, 102, 114, 147, 128 (di-TMS derivative) [Tsujikawa *et al.* 2006].



Quantification

Other **TLC** Rat Brain and Urine. Plates: Whatman PK5F. Solvent system: methanol : dichloromethane : 0.1 mmol/L hydrochloric acid (50 : 40 : 10). Scintillation counting. Limit of detection not reported [Matthews *et al.* 1981].

GC-MS Dried Mushrooms. Column: DB-5 MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 53.0 mL/min. Temperature programme: 100° for 1 min to 300° at 15°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 25 μg/L [Tsujikawa *et al.* 2006].

LC-MS Dried Mushrooms. Column: Symmetry C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (70 : 30 for 1 min to 10 : 90 at 25 min for 5 min to 70 : 30 at 31 min for 15 min), flow rate 0.2 mL/min. DAD (λ = 256 nm). Limit of quantification, 4.6 μg/L; limit of detection, 1.4 μg/L [Tsujikawa *et al.* 2007].

Note For a GLC-mass spectral analysis of fungal metabolites, see Repke *et al.* [1978].

Disposition in the Body After ingestion, muscimol proceeds to the brain or is eliminated via the systemic circulation [Michelot, Melendez-Howell 2003].

Toxicity The lethal dose of muscimol is estimated to be 12 mg and symptoms of toxicity appear within 6 h of ingestion. For an overview of the features of various types of mushroom poisoning, see DiPalma [1981]. The LD_{50} in mice is 3.8 mg/kg (SC) and 2.5 mg/kp (IP); in rats it is 4.5 mg/kg (IV) and 45 mg/kg (oral) [O'Neil *et al.* 2006].

A 72-year-old woman ingested *Amanita pantherina* and developed diarrhoea and severe transient neurological disorders followed by hallucinations and deep coma. The patient presented with skeletal muscle flaccidity with hyporeflexia. A CT scan showed a small ischaemic focus in capsula interna. Over the following 6 h, the neurological symptoms resolved. It was concluded that the patient's symptoms were a result of panther cap poisoning complicated by transient ischaemia of the brain [Magdalan, Antonczyk 2007].

A 47-year-old mother and her 27-year-old daughter ingested 5 fried mushroom caps thinking that they were parasol mushrooms *Macrolepiota procera*. Approximately 2 h after ingestion, the women suffered from nausea, stomach ache, and diarrhoea and had vomited several times. After several days in hospital, both patients were discharged [Satora *et al.* 2006].

A 48-year-old man consumed a plate of what he thought were *Amanita caesarea* mushrooms. Half an hour later he became nauseous, vomited, and fell asleep. His wife found him 3 h later comatose and having a seizure-like episode. Examination of the mushrooms he had picked revealed *A. muscaria* among them. Ten hours after ingestion he awoke and was completely oriented. Eight hours later, however, his condition deteriorated and he became confused and uncooperative. He developed visual and auditory hallucinations as well as paranoia. He refused treatment and was diagnosed with paranoid psychosis. He was transferred to a psychiatric hospital 3 days later. On the fifth day he started to drink and take tablets and on the sixth day all symptoms of psychosis disappeared and he was discharged [Brvar *et al.* 2006].

Four men (aged 18 to 21 years) and an 18-year-old woman picked, skinned, shredded and dried 6 large *A. muscaria* mushrooms. That evening the mushrooms were served at a party. After ~20 min, each person experienced auditory and visual hallucinations. The girl, however, experienced severe hallucinations followed by a loss of consciousness. Her companions induced her to vomit and drink fluids. The following morning she was admitted to hospital. Urine samples taken 5 h after ingestion were negative for tetrahydrocannabinol, amfetamines, opiates and amanitin. Because not all of the active compounds in this species of mushroom are known, she was kept in for observation. She was discharged 4 days later [Satora *et al.* 2005].

Nine patients aged 1 to 6 years were admitted to hospital between April 6 and May 23, 1992. They presented with CNS depression, ataxia, waxing and waning obtundation, hallucinations, intermittent hysteria, or hyperkinetic activity. Seizures occurred in 4 of the 9 patients but this was controlled with standard anticonvulsant therapy. Recovery was rapid (within 12 h) and complete in all cases [Benjamin 1992].

Note For a study investigating the ibotenic acid-muscimol content of various *Amanita* species from several continents, see Chilton and Ott [1976].

Dose Muscimol is used as a neuropharmacological tool to investigate GABA receptor function.

Benjamin DR (1992). Mushroom poisoning in infants and children: the *Amanita pantherina/muscaria* group. *J Toxicol Clin Toxicol* 30: 13–22.

Brvar M *et al.* (2006). Prolonged psychosis after *Amanita muscaria* ingestion. *Wien Klin Wochenschr* 118: 294–297.

Chilton WS, Ott J (1976). Toxic metabolites of *Amanita pantherina, A. cothurnata*, *A. muscaria* and other *Amanita* species. *Lloydia* 39: 150–157.

DiPalma JR (1981). Mushroom poisoning. *Am Fam Physician* 23: 169–172.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society.

Magdalan J, Antonczyk A (2007). *Amanita pantherina* poisoning or brain stroke? *Przegl Lek* 64: 341–343.

Matthews WD *et al.* (1981). Correlation of [^{14}C]muscimol concentration in rat brain with anticonvulsant activity. *Eur J Pharmacol* 69: 249–254.

Michelot D, Melendez-Howell LM (2003). *Amanita muscaria*: chemistry, biology, toxicology, and ethnomycology. *Mycol Res* 107: 131–146.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Repke DB *et al.* (1978). GLC–mass spectral analysis of fungal metabolites. *J Pharm Sci* 67: 485–487.

Satora L *et al.* (2005). Fly agaric (*Amanita muscaria*) poisoning, case report and review. *Toxicon* 45: 941–943.

Satora L *et al.* (2006). Panther cap *Amanita pantherina* poisoning case report and review. *Toxicon* 47: 605–607.

Tsujikawa K *et al.* (2006). Analysis of hallucinogenic constituents in *Amanita* mushrooms circulated in Japan. *Forensic Sci Int* 164: 172–178.

Tsujikawa K *et al.* (2007). Determination of muscimol and ibotenic acid in *Amanita* mushrooms by high-performance liquid chromatography and liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 430–435.

Mustine

Antineoplastic

$C_5H_{11}Cl_2N$ = 156.1

IUPAC Name 2-Chloro-*N*-(2-chloroethyl)-*N*-methylethanamine

Synonyms Chlorethazine; chlormethine; 2,2′-dichloro-*N*-methyldiethylamine; HN 2; mechlorethamine; methchlorethamine; nitrogen mustard.

Chemical Properties Oily substance. Relatively insoluble in water.

Mustine Hydrochloride

Proprietary Name *Mustargen hydrochloride*

Chemical Properties White, hygroscopic, vesicant, crystalline powder or mass. Mp about 108°. Very soluble in water.

Caution Mustine hydrochloride is highly toxic; it is a strong vesicant and a strong nasal irritant.

Disposition in the Body In neutral or alkaline solutions mustine rapidly undergoes intramolecular transformation with release of a chloride ion to form a cyclic ethyleneimmonium derivative which is highly chemically reactive. Some of the physiological effects of mustine may be due to deactivation of crucial enzymes, but it is more likely that mustine acts mainly on proliferating cells by alkylating the nucleic acids of the chromosomes. Mustine is active only if given parenterally, and it is rapidly removed from the blood-stream. Metabolism is so rapid that <0.01% of the drug may be recovered in the urine.

Toxicity Severe haemopoietic depression may follow administration of even therapeutic doses. Bone-marrow changes may lead to severe anaemia and thrombocytopenic purpura. Skin reactions may also occur.

LD_{50} (oral): in rats 10 mg/kg.

Dose The usual dose is 0.4 mg/kg IV.

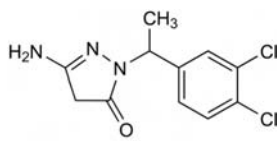
Muzolimine

Diuretic

$C_{11}H_{11}Cl_2N_3O$ = 272.1

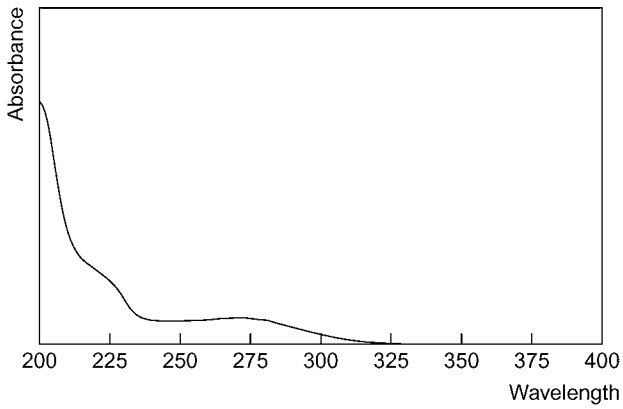
CAS—55294-15-0

IUPAC Name 5-Amino-2-[1-(3,4-dichlorophenyl)ethyl]-2,4-dihydro-3*H*-pyrazol-3-one



Chemical Properties Crystals. Mp 134° to 137°. Practically insoluble in water; soluble 1 in 4 of ethanol, 1 in 9 of acetone and 1 in 3 of chloroform. pK_a 9.3.

Ultraviolet Spectrum Methanolic acid—240 nm (A_1^1=489b).



Infrared Spectrum Principal peaks at wavenumbers 1690, 1640, 1602, 835, 665, 880 cm^{-1} (KBr disk).

Quantification

Plasma **TLC** Limit of detection, 2 μg/L [Ritter 1977].

Urine **TLC** See Plasma [Ritter 1977].

Disposition in the Body Muzolimine is readily absorbed following oral administration. About 5% of a dose is excreted unchanged in the urine in 10 h.

Therapeutic Concentration

After a single oral dose of 30 mg given to 6 subjects, peak plasma concentrations of 0.29 to 0.51 (mean 0.44) mg/L were attained in 1 to 3 h [Loew *et al.* 1977].

Half-life Plasma half-life, 10 to 17 h.

Dose 40 to 80 mg daily.

Loew D *et al.* (1977). Comparison of the pharmacodynamic effects of furosemide and BAY g 2821 and correlation of the pharmacodynamics and pharmacokinetics of BAY g 2821 (muzolimine). *Eur J Clin Pharmacol* 12: 341–344.

Ritter W (1977). Thin-layer densitometric determination of muzolimine (BAY g 2821), a structurally new diuretic drug, at the nanogram level in biological fluids. *J Chromatogr* 142: 431–440.

M

Mycophenolate Mofetil

Immunosuppressant

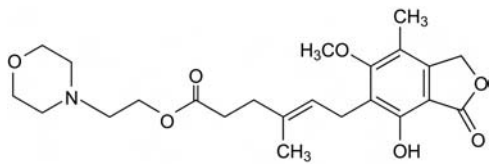
$C_{23}H_{31}NO_7$ = 433.5

CAS—115007-34-6; 128794-94-5

IUPAC Name 2-Morpholinoethyl-(*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate

Synonyms Morpholinoethyl; mycophenolate; RS-61443.

Proprietary Name *CellCept*



Chemical Properties White to off-white crystalline powder. Mp 93° to 94°. Slightly soluble in water; soluble in acetone and methanol; sparingly soluble in ethanol. pK_a 5.6 (mycophenolate mofetil), 4.5 (acid). Log *P* (octanol/phosphate buffer pH 2), 0.0085; (octanol/phosphate buffer pH 7.4), 238 (mycophenolate mofetil); log *P* (octanol/phosphate buffer pH 2), 570; (octanol/phosphate buffer pH 7.4), 1.6 (acid).

Mycophenolic Acid

$C_{17}H_{20}O_6$ = 320.3

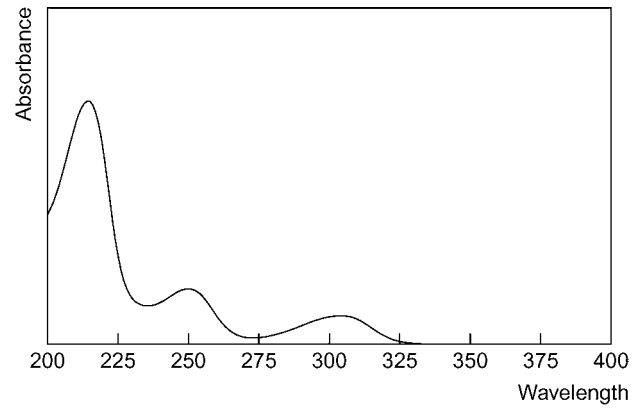
CAS—24280-93-1

IUPAC Name 6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoic acid

Chemical Properties Needles from hot water. Mp 141°. It is soluble in alcohol; moderately soluble in ether and chloroform; sparingly soluble in toluene; almost insoluble in cold water.

High Performance Liquid Chromatography Column: ODS (150 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : trifluoroacetic acid (48:52), flow rate 1.5 mL/min. IS: suprofen. UV detection (λ=250 nm). Retention time: mycophenolic acid, 14.6 min; mycophenolate phenol glucuronide, 3.6 min; IS, 9.2 min [Wiwattanawongsa *et al.* 2001].

Ultraviolet Spectrum Principal peaks at 214, 251, 303 nm.



Quantification

Plasma **HPLC** UV detection (λ=254 nm). Limit of quantification, 0.1 mg/L for mycophenolic acid and 4.0 mg/L for its glucuronic conjugate [Tsina *et al.* 1996].

Body Fluids **HPLC** UV detection (λ=215 nm and 304 nm). Limit of detection, 0.17 mg/L [Sugioka *et al.* 1994].

Skin **HPLC** MS detection. Limit of detection, 0.85 μg/L for mycophenolate mofetil, 1 μg/L for mycophenolic acid [Platzer *et al.* 2001].

Disposition in the Body Mycophenolate mofetil is an ethyl ester prodrug. It is rapidly absorbed after oral administration and completely hydrolysed to form the active drug mycophenolic acid (MPA). Mycophenolic acid is further metabolised through glucuronidation to form an inactive metabolite (MPAG), which is excreted in urine. About 6% of the dose is excreted in faeces. The pharmacokinetics of MPA and MPAG are altered if mycophenolate mofetil is administered with a high fat content meal or antacids containing aluminium and magnesium hydroxides. Food has little effect on the bioavailability of mycophenolate mofetil, but antacids reduce the peak plasma concentration by 40%.

Therapeutic Concentration

Ten patients with rheumatoid arthiritis, 6 females and 4 males, with a mean age of 55.6 years, were administered 2 g mycophenolate mofetil after an overnight fast. It was either administered on its own, with food, or with an antacid. The mean peak plasma concentrations of MPA were 23.8, 18 and 14.8 mg/L, respectively, and were observed between 0.5 and 3 h post-administration. Mean concentrations of MPAG at 52.7, 68.4 and 38.8 mg/L were reached in individuals in the fasting state, fed state and for those administered with an antacid, respectively. These levels were seen 2 to 4 h after administration [Bullingham *et al.* 1996].

Bioavailability 94%.

Half-life Mycophenolic acid, 18 h.

Volume of Distribution Mycophenolic acid, 3.6 L/kg.

Clearance 11.6 L/h (193 mL/min).

Protein Binding Mycophenolic acid, 97%; MPAG (glucuronide metabolite), 82%.

Note For a review of the pharmacokinetics of mycophenolate mofetil, see Fulton and Markham [1996].

Dose The initial dose is 2 g daily which may be increased to 3 g if necessary.

Bullingham R *et al.* (1996). Effects of food and antacid on the pharmacokinetics of single doses of mycophenolate mofetil in rheumatoid arthritis patients. *Br J Clin Pharmacol* 41: 513–516.

Fulton B, Markham A (1996). Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. *Drugs* 51: 278–298.

Platzer M *et al.* (2001). Quantification of mycophenolate mofetil in human skin extracts using high-performance liquid chromatography-electrospray mass spectrometry. *J Chromatogr B Biomed Sci Appl* 755: 355–359.

Sugioka N *et al.* (1994). Determination of a new immunosuppressant, mycophenolate mofetil, and its active metabolite, mycophenolic acid, in rat and human body fluids by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 654: 249–256.

Tsina I *et al.* (1996). Manual and automated (robotic) high-performance liquid chromatography methods for the determination of mycophenolic acid and its glucuronide conjugate in human plasma. *J Chromatogr B Biomed Appl* 675: 119–129.

Wiwattanawongsa K *et al.* (2001). Determination of mycophenolic acid and its phenol glucuronide metabolite in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 763: 35–45.

Myristicin

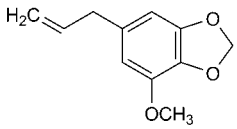
Hallucinogen

$C_{11}H_{12}O_3$ = 92.2

CAS—607-91-0

IUPAC Name 4-Methoxy-6-prop-2-enyl-1,3-benzodioxole

Synonym 4-Methoxy-6-(2-propenyl)-1,3-benzodioxole



Chemical Properties The most toxic principle of nutmeg and nutmeg oil. Mp below −20°. Bp 276° to 277°. Refractive index 1.5403. Slightly soluble in ethanol; soluble in ether.

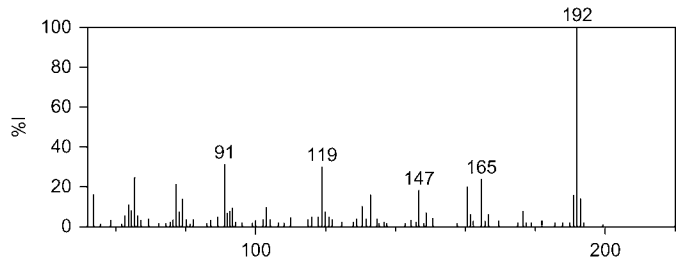
Nutmeg

Synonyms Muscade; myristica; nux moschata.
Chemical Properties The dried kernels of the seeds of *Myristica fragrans* (Myristicaceae), containing not less than 5% of volatile oil.

Nutmeg Oil

CAS—8008-45-5
Synonyms Essence de muscade; myristica oil; oleum myristicae.
Chemical Properties A volatile oil obtained by distillation from nutmeg. East Indian oil contains ~15% myristicin and West Indian ~2%. A colourless, pale yellow, or pale green liquid. Wt per mL: East Indian oil 0.885–0.915 g; West Indian oil 0.86–0.88 g. Refractive index 1.472–1.488. Freely soluble in ethanol (90%); soluble in glacial acetic acid. Log *P* (octanol/water), 3.5.

Mass Spectrum



Quantification

Urine **GC-MS** Column: HP-1MS (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV. Limit of detection not reported [Beyer *et al.* 2006].

Disposition in the Body

Toxicity Severe symptoms of poisoning have been caused by the ingestion of 1–1.5 nutmegs, and doses of 5 g or more of nutmeg or nutmeg powder will produce a marked depressive action on the CNS.

A 28-year-old woman ingested 18.3 g of powdered nutmeg. Following a period of disorientation, delirium, and excitement, she became semi-stuporous for 12 h. For some days she complained of numbness, dizziness, and nausea and there were further periods of excitement. No specific therapy was given and she was discharged after 7 days [Green 1959].

Two young women consumed 15 and 25 g, respectively, of ground nutmeg in order to experience its hallucinogenic action. Both became intoxicated but recovered [Åkesson, Walinder 1965].

The cause of death in a 55-year-old woman was attributed to the ingestion of nutmeg. The concentration of myristicin in the postmortem blood was 4.0 mg/L (it was speculated that the ingested dose of nutmeg had been 560 to 840 mg/kg); flunitrazepam was also present (0.072 mg/L). In a 2-year period in which 7 cases of nutmeg poisoning had been recorded by the Erfurt Poison Information Centre, even where higher doses (20–80 g of powder; 280–1100 mg/kg) had been ingested, a life-threatening situation had never arisen; in 1 case a myristicin blood level of 2 mg/L was measured 8 h after ingestion of ~14–21 g (280–420 mg/kg) of nutmeg powder [Stein *et al.* 2001].

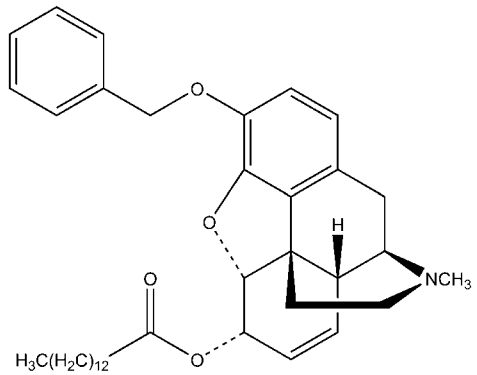
Note For a review of the toxic effects and uses of nutmeg, see Weil [1966].

Åkesson HO, Walinder J (1965). Nutmeg intoxication. *Lancet*,1271–1272.
Beyer J *et al.* (2006). Abuse of nutmeg (*Myristica fragrans* Houtt.): studies on the metabolism and the toxicologic detection of its ingredients elemicin, myristicin, and safrole in rat and human urine using gas chromatography/mass spectrometry. *Ther Drug Monit* 28: 568–575.
Green RC Jr (1959). Nutmeg poisoning. *JAMA* 171: 1342–1344.
Stein U *et al.* (2001). Nutmeg (myristicin) poisoning: report on a fatal case and a series of cases recorded by a poison information centre. *Forensic Sci Int* 118: 87–90.
Weil AT (1966). The use of nutmeg as a psychotropic agent. *Bull Narc* 18: 15–23.

Myrophine

Narcotic
$C_{38}H_{51}NO_4$ = 585.8
CAS—467-18-5

Synonyms 3-Benzyloxy-7,8-dehydro-4,5-epoxy-*N*-methyl-6-myristoyloxymorphinan; 3-benzylmorphine 6-myristate; benzylmorphine myristic acid ester; benzylmorphinyl myristate; 3-benzyloxy-6-myristoyloxy-*N*-methyl-4,5-epoxy-7-morphinene; 3-benzyloxy-6-myristoyloxymorphine; C 5; 7,8-didehydro-4,5-epoxy-17-methyl-3-(phenylmethoxy)morphinan-6-ol tetradecanoate (ester); leucodinine; myricodine; myristylbenzylmorphine; myrocodine; myrophinium; NIH 5986; peronine myristate.



Chemical Properties Solid. Mp 41°. Myrophine is extracted by organic solvents from aqueous alkaline solutions.

Myrophine Hydrochloride

$C_{38}H_{51}NO_4$,HCl = 622.3
Chemical Properties Solid Mp 198° to 199°.

Colour Tests Ammonium molybdate test—blue-violet (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—brown-purple (limit of detection, 0.25 µg); Vitali's test—yellow/yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.52 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum 0.1 N hydrochloric acid—283 nm (E1%, 1 cm 29), minimum at 261 nm.

Nabilone

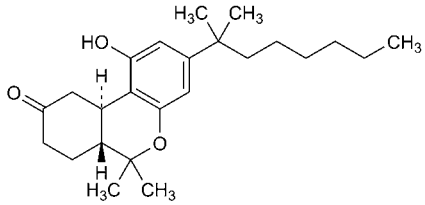
Antiemetic

$C_{24}H_{36}O_3$ = 72.5

CAS—51022-71-0

IUPAC Name 6a*R*,10a*R*)-*rel*-3-(1,1-Dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9*H*-dibenzo[*b*,*d*]pyran-9-one

Proprietary Name *Cesamet*

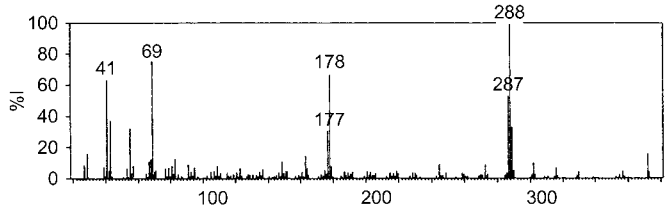


Chemical Properties Nabilone is a synthetic cannabinoid.

Ultraviolet Spectrum Methanol—275 (A^1_1 = 35b), 282 nm (A^1_1 = 35b).

Infrared Spectrum Principal peaks at wavenumbers 1696, 1619, 1574, 1260, 1100, 1038 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 288, 69, 178, 41, 287, 43, 289, 55.



Disposition in the Body Readily absorbed after oral administration; rapidly distributed into the tissues and metabolised by reduction of the 9-keto group forming a mixture of isomeric alcohols (carbinols) together with several other unidentified metabolites. Approximately 65% of a dose is eliminated in the faeces, and ~20% is excreted in the urine as polar acidic metabolites [Rubin *et al.* 1977].

Half-life Plasma half-life, ~2 h.

Dose 2 to 4 mg daily; maximum of 6 mg daily.

Rubin A *et al.* (1977). Physiologic disposition of nabilone, a cannabinol derivative, in man. *Clin Pharmacol Ther 22*,85–91.

Nabumetone

NSAID

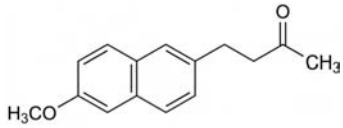
$C_{15}H_{16}O_2$ = 228.3

CAS—42924-53-8

IUPAC Name 4-(6-Methoxy-2-naphthalenyl)-2-butanone

Synonyms BRL-14777, nabumetonum.

Proprietary Names *Arthaxan*; *Balmox*; *Consolan*; *Diosmal*; *Nabuser*; *Relafen*; *Relifen*; *Relifex*.



Chemical Properties A white crystalline powder which is soluble in acetone and slightly soluble in methyl alcohol. Mp 80°. Practically insoluble in water. Log *P* (octanol/phosphate buffer [pH 7.4]), 2400 for nabumetone; log *P* (octanol/water), 0.5 for 6-methoxy-2-naphthylacetic acid (6-MNA).

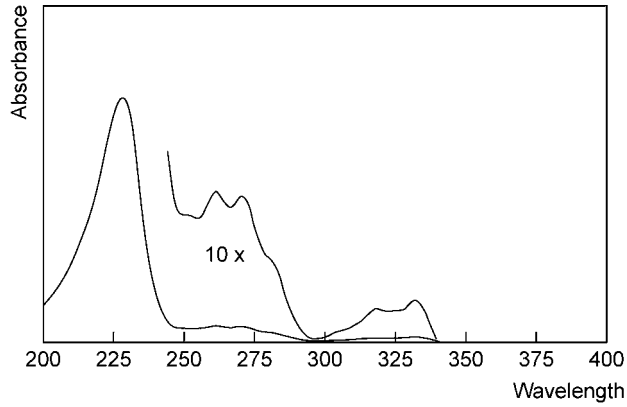
Gas Chromatography System GB—nabumatone RI 2084 , M (6-MNA) RI 2322 , M (O-desmethyl) RI 2385.

Gas Chromatography-Mass Spectrometry Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, 0.9 mL/min. Retention index: 2082 [Mills, Roberson 1993].

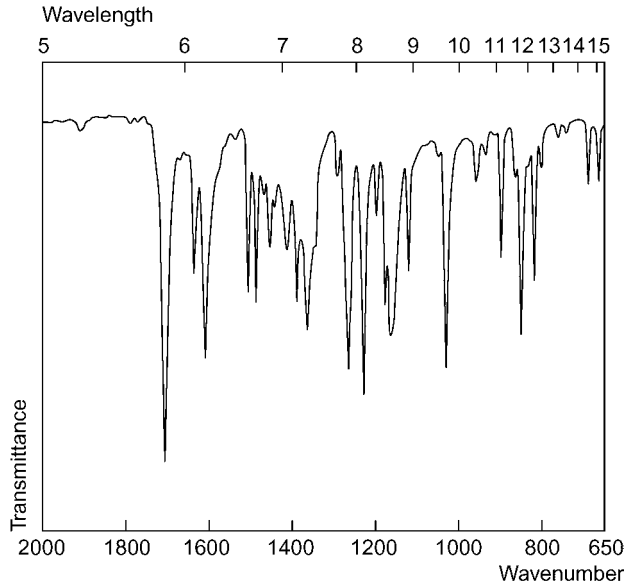
High Performance Liquid Chromatography System HZ—retention time 13.6 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : aqueous 0.01 mol/L dihydrogen potassium phosphate buffer (pH 3.5), flow rate 1 mL/min. DAD. Retention time: 3.4 min [Mills, Roberson 1993].

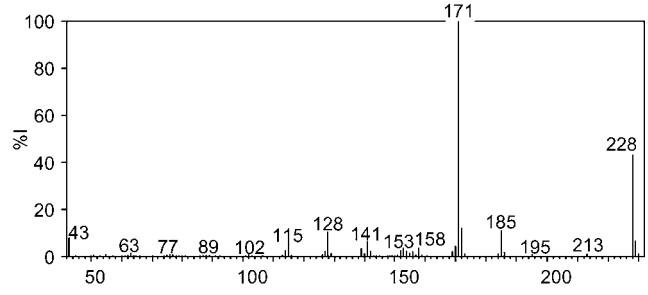
Ultraviolet Spectrum Aqueous acid—230, 261, 270, 333 nm.



Infrared Spectrum Principal peaks at wavenumbers 1696, 1260, 1020 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 171, 228, 172, 185, 128, 43, 115, 141, 158.



Quantification

Plasma **HPLC** UV detection (λ=280 nm). Limit of quantification, 70 µg/L [Jager *et al.* 2000].

Urine **HPLC** Column: 5-C_{18} Wakosil (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : triethylamine (500 : 500 : 1), containing 0.5 g/L 1-heptane sulfonic acid, flow rate 1.0 mL/min. IS: methyl *p*-toluate. UV detection (λ=270 nm); fluorescence detection (λ_{ex}=280 nm; λ_{em}=350 nm). *k* values: nabumetone, 6.3; 6-methoxy-2-naphthylacetic acid, 1.9; IS, 4.6. Limit of detection, 120 µg for nabumetone, 16 µg for 6-methoxy-2-naphthylacetic acid [Mikami *et al.* 2000].

Other **HPLC** Pharmaceuticals. See Urine [Mikami *et al.* 2000].

Disposition in the Body Nabumetone is well absorbed from the gastrointestinal tract and rapidly converted to the active metabolite 6-methoxy-2-

naphthylacetic acid (6-MNA; BRL-10720) via hepatic metabolism. Peak plasma concentrations of 6-MNA are observed 2–8 h after administration with a possible secondary peak at 28–36 h. The active metabolite 6-MNA undergoes further metabolism via *O*-methylation and conjugation to 6-hydroxy-2-naphthylacetic acid, which may also be conjugated. Alternative metabolic pathways are *O*-demethylation and reduction of the ketone to alcohol and esters. Other metabolites include 4-(6-hydroxy-2-naphthyl)-butan-2-one, 4-(6-hydroxy-2-naphthyl)-butan-2-ol and 6-hydroxy-2-naphthylacetic acid, which may undergo further conjugation resulting in many metabolites detected in urine. About 80% of the dose is excreted in urine as inactive metabolites with ~1% as unchanged 6-MNA. 10% is excreted in faeces. 6-MNA diffuses in synovial fluids.

Therapeutic Concentration

Twelve healthy young male and female volunteers, aged between 18 and 21 years, were administered a 1 g single dose of nabumetone. Twelve male and female elderly volunteers, 63–83 years, were also administered with the same dose. This single dose study was followed by multiple dosing of 1 g daily. Mean peak plasma concentrations of 21.9 mg/L (range, 11.3–34.0 mg/L) and 33.7 (range, 6.7–64.1) mg/L were observed for the young and elderly individuals, respectively, for the single 1 g dose, and were observed between 3 and 24 h (mean, 13 and 12.2 h, respectively). After multiple dosing, the plasma levels reached 52.3 (range, 32.0–71.9) mg/L and 66.7 (range, 15.1–103.0) mg/L for the young and elderly, respectively, ~5 h after administration (1–10 h) [Kendall *et al.* 1989].

Bioavailability The absolute mean bioavailability is 38%.

Half-life 6-MNA, 22–27 h (young healthy subjects), 25–34 h (elderly).

Volume of Distribution 7.5 L (steady state); ranges between 5 and 10 L (0.1–0.2 L/kg).

Clearance The apparent clearance is 0.7 L/h (creatinine clearance, 40 to >80 mL/min); 0.93 (creatinine clearance, 10–39 mL/min); 1.41 (creatinine clearance, <10 mL/min) after single oral doses.

Protein Binding 6-MNA, >99%.

Note For a review of nabumetone, see Friedel *et al.* [1993].

Dose 1 g administered at night with 0.5 to 1 g extra in the morning if necessary. The elderly are administered 0.5 to 1 g.

Friedel HA *et al.* (1993). Nabumetone. A reappraisal of its pharmacology and therapeutic use in rheumatic diseases. *Drugs* 45: 131–156.

Jager AD *et al.* (2000). Extractionless determination of 6-methoxy-2-naphthylacetic acid, a major metabolite of nabumetone, in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 740: 247–251.

Kendall MJ *et al.* (1989). A pharmacokinetic study of the active metabolite of nabumetone in young healthy subjects and older arthritis patients. *Eur J Clin Pharmacol* 36: 299–305.

Mikami E *et al.* (2000). Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography. *J Pharm Biomed Anal* 23(5): 917–925.

Mills T, Roberson JC (1993) *Instrumental Data for Drug Analysis*, 2nd edn., Vol. 5. Boca Raton: CRC Press, 294–295

Nadolol

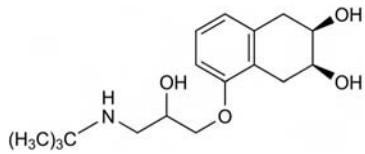
β-*Blocker*

$C_7H_{27}NO_4 = 309.4$

CAS—42200-33-9

IUPAC Name 5-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol

Proprietary Names *Apo-nadol*; *Corgard*; *Solgol*. It is an ingredient of *Corgaretic*, *Corzide* and *Solgeretik*.



Chemical Properties A white crystalline powder. Mp 124° to 136°. Soluble in water; freely soluble in ethanol and propylene glycol; slightly soluble in chloroform; insoluble in acetone, benzene, ether and hexane. Log *P* (octanol/water), 0.8. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

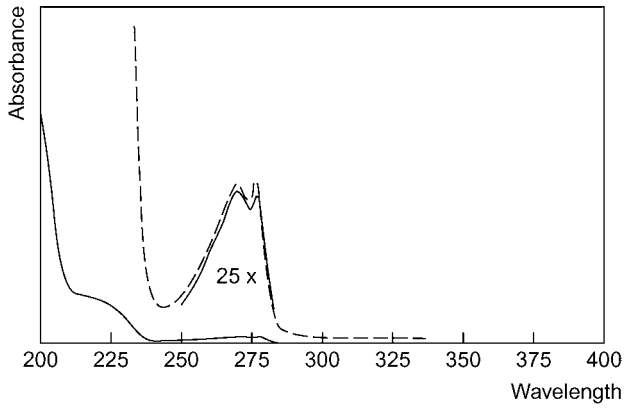
Colour Tests Liebermann's reagent—brown; Mandelin's test—brown-red; Marquis test—red; Nessler's reagent—brown.

Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.20; system TL—R_f 0.01; system TAE—R_f 0.14.

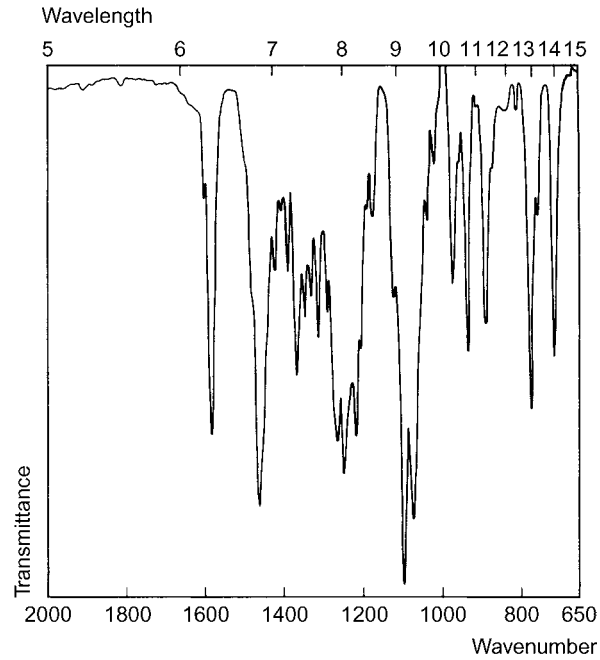
Gas Chromatography System GA—nadolol RI 2540, art RI 2560; system GB—nadolol RI 2658, art RI 2670.

High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 288; system HY—RI 249; system HAA—retention time 6.8 min.

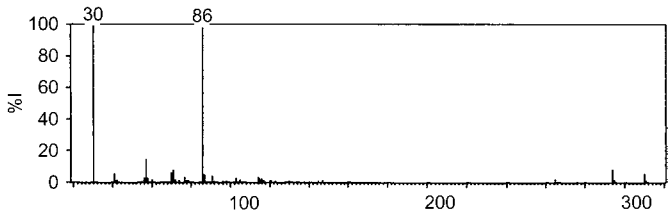
Ultraviolet Spectrum Aqueous acid—269, 276 nm; methanol—270, 278 nm (A^1_1=38a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1092, 1070, 1248, 1262, 1582, 1217 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 30, 86, 57, 294, 71, 310, 70, 87.



Quantification

Plasma **HPLC** Fluorescence detection. Limit of detection, 2.5 µg/L for nadolol enantiomers [Belas *et al.* 1995]. Fluorescence detection. Limit of detection, 5 µg/L [Srinivas *et al.* 1995a]. Fluorescence detection. Limit of detection, 2 µg/L for nadolol enantiomers [Srinivas *et al.* 1995b]. UV or fluorescence detection. For method for quantification of nadolol and other beta-blockers, see Musch *et al.* [1989].

Serum **GC–MS** Limit of detection, 7 µg/L [Funke *et al.* 1978].

HPLC Fluorescence detection. Limit of detection, 1 µg/L [Noguchi *et al.* 1992]. Electrochemical detection. For method, see Surmann [1980].

Spectrofluorimetry Limit of detection, <10 µg/L [Ivashkiv 1977].

Urine **HPLC** Amperometric detection. Limit of detection, 15 ppb to 500 ppb for nadolol and other beta-blockers [Maguregui *et al.* 1995].

Spectrofluorimetry See Serum [Ivashkiv 1977].

Disposition in the Body Nadolol is absorbed after oral administration. It is excreted almost entirely as unchanged drug; about 15 to 21% of the dose is excreted in the urine and 68 to 85% is eliminated in the faeces in 4 days, after oral administration. After IV administration, about 75% of a dose is excreted in the urine unchanged.

Therapeutic Concentration

Following single oral doses of 60 and 120 mg to 7 subjects, peak plasma concentrations of 0.02 to 0.14 (mean, 0.07) mg/L and 0.05 to 0.25 (mean, 0.13) mg/L were attained in 1 to 4 h [Schäfer-Korting *et al.* 1984].

Single oral doses of 80 mg to 4 subjects resulted in peak plasma concentrations of 0.04 to 0.21 (mean, 0.14) mg/L in 1 to 4 h. During daily oral dosing of 4 subjects with 80 mg a day, peak plasma concentrations of 0.11 to 0.24 (mean, 0.22) mg/L were observed 2 to 4 h after a morning dose [Dreyfuss *et al.* 1979].

Toxicity

Following intentional overdose, a 57-year-old woman presented in a poorly responsive state with haemodynamic compromise and an elevated nadolol serum concentrations of 0.39 mg/L; other drugs were present within their normal therapeutic range (diltiazem, paracetamol, salicylate) [Ehgartner, Zelinka 1988].

Bioavailability About 30%.
Half-life Plasma half-life, 6 to 24 h.
Volume of Distribution About 2 L/kg.
Clearance Plasma clearance, about 3 mL/min/kg.
Protein Binding About 4 to 30% (mean, 14%).
Note For a review of the pharmacokinetics of nadolol, see Heel *et al.* [1980].
Dose 40 to 240 mg daily.

Belas FJ *et al.* (1995). Simultaneous determination of nadolol enantiomers in human plasma by high-performance liquid chromatography using fluorescence detection. *Biomed Chromatogr* 9: 140–145.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dreyfuss J *et al.* (1979). Pharmacokinetics of nadolol, a beta-receptor antagonist: administration of therapeutic single- and multiple-dosage regimens to hypertensive patients. *J Clin Pharmacol* 19: 712–720.

Ehgartner GR, Zelinka MS (1988). Hemodynamic instability following intentional nadolol overdose. *Arch Intern Med* 148: 801–802.

Funke PT *et al.* (1978). Determination of serum nadolol levels by GLC–selected ion monitoring mass spectrometry: comparison with a spectrofluorometric method. *J Pharm Sci* 67: 653–657.

Heel RC *et al.* (1980). Nadolol: a review of its pharmacological properties and therapeutic efficacy in hypertension and angina pectoris. *Drugs* 20: 1–23.

Ivashkiv E (1977). Fluorometric determination of nadolol in human serum and urine. *J Pharm Sci* 66: 1168–1172.

Maguregui MI *et al.* (1995). High-performance liquid chromatography with amperometric detection applied to the screening of beta-blockers in human urine. *J Chromatogr B Biomed Appl* 674: 85–91.

Musch G *et al.* (1989). A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography. *J Pharm Biomed Anal* 7: 483–497.

Noguchi H *et al.* (1992). Determination of nadolol in serum by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 573: 336–338.

Schäfer-Korting M *et al.* (1984). Pharmacokinetics of nadolol in healthy subjects. *Eur J Clin Pharmacol* 26: 125–127.

Srinivas NR *et al.* (1995a). High-performance liquid chromatographic assay for the quantitation of nadolol in human plasma using fluorescence detection. *Biomed Chromatogr* 9: 75–79.

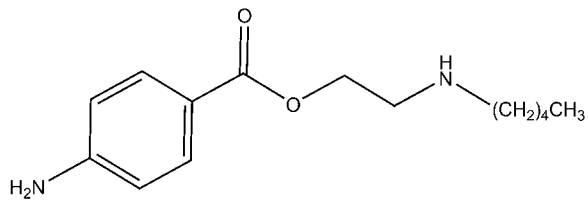
Srinivas NR *et al.* (1995b). Stereoselective analysis of nadolol in human plasma. *Biomed Chromatogr* 9: 226–228.

Surmann P (1980). Feasibility of determining nadolol in serum. *Arch Pharm (Weinheim)* 313: 1052–1054.

Naepaine

Anaesthetic (Local)
$C_{14}H_{22}N_2O_2$ = 250.3
CAS—2188-67-2

IUPAC Name 2-(Pentylamino)ethyl 4-aminobenzoate
Synonyms Amalcaine; 2-pentylaminoethyl *p*-aminobenzoate.



Chemical Properties Naepaine is extracted by organic solvents from aqueous alkaline solutions. Mp 64° to 66°.

Naepaine Hydrochloride

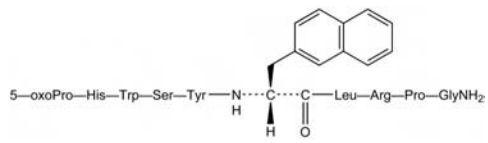
$C_{14}H_{22}N_2O_2$,HCl = 286.8
Proprietary Name *Amylsine*
Chemical Properties Fine white crystals, or a fine powder. Mp of one form 153° and of the other form 175° to 177°. Soluble in water; sparingly soluble in ethanol; insoluble in ether and chloroform.

Colour Test Vitali's test—yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.63 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid—273 (E1%, 1 cm 83), 279 (E1%, 1 cm 84) and about 290 nm (E1%, 1 cm 61).
Infrared Spectrum Principal peaks at wavenumbers 1605, 1277, 1170 or 1685 cm^{-1} (KBr disk, naepaine base).
Disposition in the Body
Toxicity Naepaine hydrochloride is about 1.5-times as toxic as procaine hydrochloride systemically. The maximum amount which is safe for surface use is 100 mg of the hydrochloride. The minimum IV lethal dose in guinea-pigs is 10 to 40, in rabbits 28.5, and in rats 25 to 30 mg/kg.

Nafarelin

Gonadorelin Analogue, Treatment of Endometriosis
$C_{66}H_{83}N_{17}O_{13}$ = 1322.5
CAS—76932-56-4

IUPAC Name *N*-[1-[[1-[[1-[[1-[[1-[[1-[[1-[[1-[[2-[(2-Amino-2-oxoethyl)carbamoyl] pyrrolidin-1-yl]-5-(diaminomethylideneamino)-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-naphthalen-2-yl-1-oxopropan-2-yl] amino]-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino]-3-hydroxy-1-oxopropan-2-yl] amino]-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-3-(1*H*-imidazol-5-yl)-1-oxopropan-2-yl]-5-oxopyrrolidine-2-carboxamide
Synonyms NAG; 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-3-(2-naphthyl)-D-alanyl-L-leucyl-L-arginyl-L-prolylglycinamide.



Nafarelin Monoacetate

$C_{66}H_{83}N_{17}O_{13}C_2H_4O_2$ = 1382.6
Chemical Properties Mp 188° to 190°.

Nafarelin Acetate Hydrate

$C_{66}H_{83}N_{17}O_{13}$, $xC_2H_4O_2$, yH_2O
CAS—86220-42-0
Synonyms D-Nal(2)^6-LHRH acetate hydrate; RS-94991-298.
Proprietary Names *Nacenyl*; *Nasanyl*; *Synarel*; *Synarela*; *Synrelina*.

High Performance Liquid Chromatography Column: silica TSK 2000 SW (300 × 7.5 mm i.d.). Mobile phase: acetonitrile (initially 100%; stepped to 30% in 0.15 mmol/L phosphate buffer) : 13 mmol/L $NaClO_4$ (85:15), flow rate 1.0 mL/min. UV detection (λ=225 nm). Retention time: 22 min [Kenley *et al.* 1987].
Disposition in the Body Nafarelin is rapidly absorbed after intranasal administration, and peak plasma concentrations are reached within ~20 min. It is metabolised by peptidases in the body and is excreted in urine as the metabolites and a small amount as the unchanged drug. The most common metabolites are the (5–10) hexapeptide amine (the key intermediate for the other metabolites) and the naphthylalanine. Metabolites are excreted in urine (44 to 55%) and faeces (18.5 to 44.2%). Only about 3% of unchanged nafarelin is excreted in urine. The drug is rapidly distributed throughout the body and undergoes extensive extravascular distribution.

Therapeutic Concentration

Ten women, aged 32 to 59 years, were intranasally administered total doses of 84, 206 and 391 µg (4 women for each dosage). Peak plasma concentrations of 0.10, 0.35 and 1.59 µg/L were reached within 0.34, 0.46 and 0.28 h, respectively. Twenty-three men, 20 to 54 years old, were administered a 400 µg dose of nafarelin SC and 304 µg intranasally. Mean peak concentrations of 13.66 µg/L were observed for the SC dose and 4.02 µg/L for the nasally administered dose. These levels were reached at 0.72 and 0.20 h, respectively [Chan *et al.* 1988].

Bioavailability 4 to 21% (intranasal administration).
Half-Life 3 to 4 h (intranasal administration). Also reported as 2.0 to 2.7 h after SC and intranasal administration.
Protein Binding 78 to 84%, mostly albumin.
Note For a review of nafarelin, see Chrisp and Goa [1990].
Dose The usual dose is 200 to 800 µg twice daily intranasally.

Chan RL *et al.* (1988). Absorption and metabolism of nafarelin, a potent agonist of gonadotropin-releasing hormone. *Clin Pharmacol Ther* 44: 275–282.

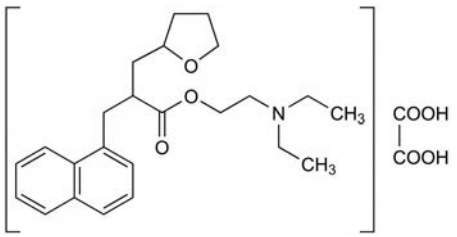
Chrisp P, Goa KL (1990). Nafarelin. A review of its pharmacodynamic and pharmacokinetic properties, and clinical potential in sex hormone-related conditions. *Drugs* 39: 523–551.

Kenley RA *et al.* (1987). Silica-based size exclusion chromatography to characterize the decapeptide nafarelin in a controlled-release pharmaceutical formulation. *Anal Chem* 59: 2050–2054.

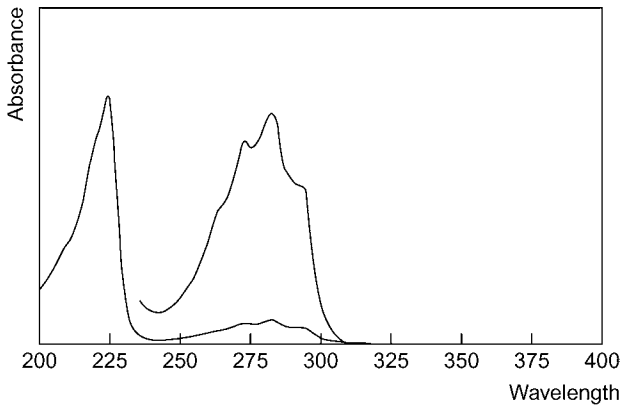
Naftidrofuryl Oxalate

Vasodilator
$C_{24}H_{33}NO_3$,$C_2H_2O_4$ = 473.6
CAS—31329-57-4 (naftidrofuryl); 3200-06-4 (oxalate)

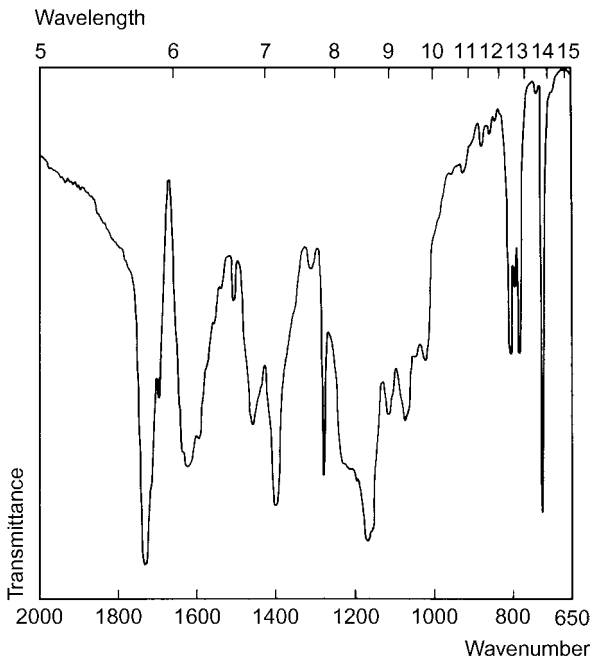
IUPAC Name Tetrahydro-α-(1-naphthalenylmethyl)-2-furanpropanoic acid 2-(diethylamino)ethyl ester acid oxalate
Synonym Nafronyl oxalate
Proprietary Names *Artocoron*; *Azunaftil*; *Di-Actane*; *Dusodril*; *Esedril*; *Gevatran*; *Luctor*; *Nafti*; *Naftilong*; *Naftilux*; *Naftodril*; *Praxilene*; *Sodipryl retard*; *StimLor*.



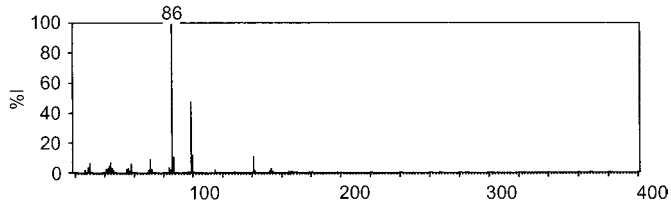
Chemical Properties A white powder. Mp about 108°. Soluble in water. pK_a 8.2 (30°). Log *P* (octanol/water), 5.4 (naftidrofuryl).
Thin-layer Chromatography Naftidrofuryl: system TA—R_f 0.64; system TB—R_f 0.52; system TC—R_f 0.41; system TE—R_f 0.78; system TL—R_f 0.35; system TAE—R_f 0.43.
Gas Chromatography System GA—RI 2748.
High Performance Liquid Chromatography Naftidrofuryl: system HY—RI 409; system HAA—retention time 15.8 min.
Ultraviolet Spectrum Aqueous acid—273, 283 nm (A^1_1=141a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1165, 722, 1280, 1620, 1070 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 100, 141, 87, 71, 44, 58 (naftidrofuryl).

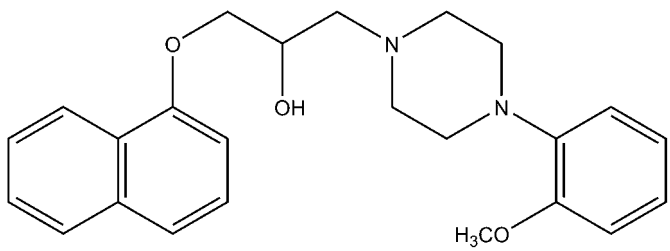


Quantification
***Plasma* HPLC** Fluorescence detection. Naftidrofuryl and its metabolite naftidrofuryl acid. Limit of detection, 4 µg/L for naftidrofuryl [Stehlik, Houbova 1990]. UV detection. Limit of detection, 20 µg/L [Brodie *et al.* 1979].
Disposition in the Body Naftidrofuryl oxalate is absorbed after oral administration. It is metabolised by hydrolysis to the free acid, 3-(1-naphthyl)-2-tetrahydrofurfuryl propionic acid. <1% of a dose is excreted in the urine in 48 h as free or conjugated naftidrofuryl. Most of a dose appears to be excreted in the bile.
Therapeutic Concentration
After oral administration of 100 mg to 2 subjects, peak plasma concentrations averaging 0.21 mg/L were attained in 0.5 to 0.75 h [Brodie *et al.* 1979].
Dose 300 to 600 mg daily.

Brodie RR *et al.* (1979). Determination of naftidrofuryl in the plasma of humans by high-performance liquid chromatography. *J Chromatogr* 164: 534–540.
Stehlik P, Houbova H (1990). Determination of naphthidrofuryl and naphthidrofurylic acid in human plasma using RP-HPLC and fluorimetric detection. *Cesk Farm* 39: 394–399.

Naftopidil

α_1-Adrenoceptor Antagonist, Antihypertensive, 5-HT_1 Receptor Agonist, Treatment of BPH
$C_{24}H_{28}N_2O_3$ = 392.5
CAS—57149-07-2
IUPAC Name 1-[4-(2-Methoxyphenyl)piperazin-1-yl]-3-naphthalen-1-yloxypropan-2-ol
Synonyms BM-15275; KT-611; (±)-4-(*o*-methoxyphenyl)-α-[(1-naphthyloxy)methyl]-1-piperazineethanol; 1-(2-methoxyphenyl)-4-[3-(naphth-1-yloxy)-2-hydroxypropyl]-piperazine; (*RS*)-1-[4-(2-methoxyphenyl)-1-piperazinyl]-3-(1-naphthoxy)-propan-2-ol.
Proprietary Names *Avishot*; *Flivas*.



Chemical Properties Colourless crystals. Mp 125° to 129°. Insoluble in water. Log *P* (octanol/water), 75 [O'Neil *et al.* 2006]. Plasma samples were stable at –18° for up to 1 year [Niebch *et al.* 1990].

Naftopidil Dihydrochloride
$C_{24}H_{28}N_2O_3$,2HCl = 465.4
CAS—57149-08-3
Chemical Properties Crystals. Mp 212° to 213°. Log *P* (octanol/water), 4.81 [ACD 2007].

Quantification
***Plasma* HPLC** Column: RP-C_{18}. Mobile phase: methanol : acetonitrile: 0.02 mol/L potassium dihydrogen phosphate. UV detection (λ = 240 nm). Retention time: 10.5 min. Limit of detection, 5 µg/L [Yuan *et al.* 1998]. Column: Hibar RP-LiChrosorb Select B (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L potassium dihydrogen phosphate (pH 1.8) : acetonitrile–methanol (1 : 1; 55 : 45), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 215 nm, λ_{em} = 320 nm). Limit of quantification, 2 µg/L [Niebch *et al.* 1990].
***Urine* TLC** Plates: Silicagel SIF/Riedel. Solvent systems: (1) chloroform : methanol : concentrated ammonia (80 : 30 : 8); (2) chloroform : methanol : 0.01 mol/L ammonia (90 : 5 : 5). Liquid scintillation counting. Limit of detection not reported [Niebch *et al.* 1991].
***Bile* TLC** See Urine [Niebch *et al.* 1991].
***Faeces* TLC** See Urine [Niebch *et al.* 1991].

Note For the determination of naftopidil in biological fluids by a non-protected fluid phosphorimetric procedure at room temperature, see Murillo Pulgarin *et al.* [2001a] or Murillo Pulgarin *et al.* [2001b]. For a flow injection method for the determination of naftopidil based on oxidation by potassium permanganate in a sulfuric acid medium, see Townshend *et al.* [2005].

Disposition in the Body Metabolic products include (naphthyl)hydroxyl-naftopidil, methoxyphenyl-piperazinpropyleneglycol, (phenyl)hydroxyl-naftopidil and *o*-desmethyl-naftopidil. Hydroxylation and cleavage of naptofidil to the propylene glycol metabolite are the predominant reactions in humans. Demethylation only occurs to a minor extent.

Therapeutic Concentration

Ten patients (aged 48 to 69 years; weight 55 to 92 kg) with hepatic impairment were administered naftopidil either as an IV infusion or as a 50 mg tablet. After the IV infusion, the pharmacokinetic parameters were comparable in hepatic impairment and controls: $t_{1/2}$ 3.6 ± 3.4 h and 3.3 ± 2.1 h, respectively; clearance 11.9 ± 4.7 mL/min/kg and 11.0 ± 1.6 mL/min/kg, respectively. The exception was C_{max}, where mean values were 180 and 65 µg/L for patients and controls, respectively, attained at 0.75 and 0.5 h, respectively. After oral administration, plasma levels and half-lives were significantly increased in liver impairment [Farthing *et al.* 1994].

Bioavailability Healthy subjects, 17%; patients with hepatic dysfunction, 75%.

Half-life Healthy subjects, 5.4 ± 3.2 h; after an oral dose in patients with hepatic dysfunction, 16.6 ± 19.3 h.

Protein Binding 82% to 97% in rats.

Dose 50 to 75 mg daily.

ACD (2007). *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).

Farthing MJ *et al.* (1994). Pharmacokinetics of naftopidil, a novel anti-hypertensive drug, in patients with hepatic dysfunction. *Postgrad Med J* 70: 363–366.

Murillo Pulgarin JA *et al.* (2001). Direct determination of naftopidil by non-protected fluid room temperature phosphorescence. *Analyst* 126: 234–238.

Murillo Pulgarin JA *et al.* (2001). Non-protected fluid room-temperature phosphorimetric procedure for the direct determination of naftopidil in biological fluids. *Fresenius J Anal Chem* 371: 903–908.

Niebch G *et al.* (1990). High-performance liquid chromatography of naftopidil, a novel antihypertensive drug, and two metabolites in human plasma. *J Chromatogr* 534: 247–252.

Niebch G *et al.* (1991). Metabolic fate of the novel antihypertensive drug naftopidil. *Arzneimittelforschung* 41: 1027–1032.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Townshend A *et al.* (2005). Flow injection chemiluminescence determination of naftopidil based on potassium permanganate oxidation in the presence of formaldehyde or formic acid. *Anal Bioanal Chem* 381: 925–931.

Yuan M *et al.* (1998). Determination of naftopidil and its main metabolite in plasma by high-performance liquid chromatography. *Yao Xue Xue Bao* 33: 768–771.

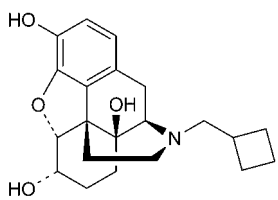
Nalbuphine

Narcotic Analgesic

$C_{21}H_{27}NO_4 = 57.4$

CAS—20594-83-6

IUPAC Name (5α,6α)-17-(Cyclobutylmethyl)-4,5-epoxymorphinan-3,6,14-triol



Chemical Properties Crystals Mp ≈231°. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005]. Stock and working solutions were stable for at least 4 weeks and 12 h, respectively. Stable in plasma for 6 h after storage at 20° and 4° [de Cazanove *et al.* 1997]. Nalbuphine was stable in mobile phase for 24 h, in plasma at 37° for 4 h, and in plasma at -20° for 6 weeks [Ho *et al.* 1996] and 7 weeks [Dubé *et al.* 1988]. Stable in plasma stored for 18 weeks at –15° to –20° [Lo *et al.* 1984].

Nalbuphine Hydrochloride

$C_{21}H_{27}NO_4$,HCl = 393.9

CAS—23277-43-2

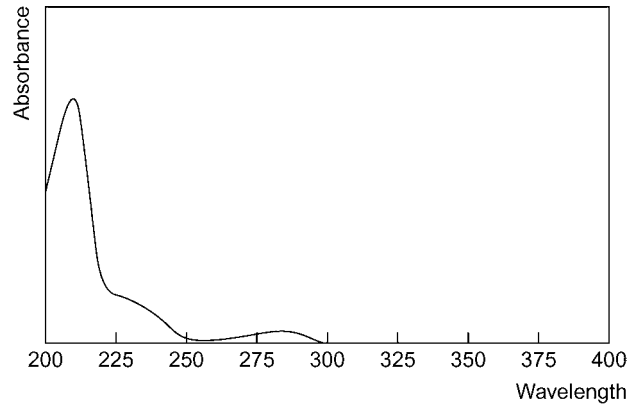
Proprietary Names *Azerty*; *Bufigen*; *Bufilem*; *Intapan*; *Nalbu*; *Nubain*.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.34; system TAE—R_f 0.58.

Gas Chromatography System GA—RI 2960; system GB—not eluted.

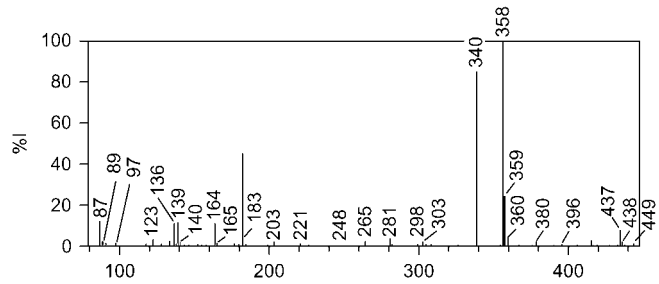
High Performance Liquid Chromatography System HX—RI 295.

Ultraviolet Spectrum Aqueous acid—284 nm ($A_1^1 = 46b$); aqueous alkali—297 nm.



Infrared Spectrum Principal peaks at wavenumbers 970, 1080, 1033, 1060, 1115, 1495 cm^{-1}.

Mass Spectrum



Quantification

Plasma **GC-MS** Column: BP 5 SGE (12 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 1.8 mL/min. Temperature programme: 60° to 280° at 30°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 7.42 min. Limit of detection, 2 µg/L [Kintz *et al.* 1992].

GC Column: 3.8% methyl vinyl silicone gum rubber (1.2 m × 4 mm i.d.). Carrier gas: He, 75 mL/min. Temperature: 235°. ECD. Retention time: 3.7 min. Limit of detection, 0.5 µg/L [Weinstein *et al.* 1978].

HPLC Column: Supelcosil LC_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium acetate (pH 6) : acetonitrile (70 : 30). Fluorescence detection ($\lambda_{ex} = 210$ nm, $\lambda_{em} = 346$ nm). Limit of quantification, 20 µg/L [Jacqz-Aigrain *et al.* 2007]. Column: Lichrosphere C_{18} (150 × 4.6 mm i.d., 5 mm). Mobile phase: methanol : 24 mmol/L potassium dihydrogen phosphate plus 0.06 mmol/L EDTA (20 : 80), flow rate 0.8 mL/min. Electrochemical detection. Limit of quantification, 0.1 µg/L [Bessard *et al.* 1997]. Column: Ultrasphere ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : phosphate buffer (20 : 80), flow rate 1.0 mL/min. Electrochemical detection. Limit of quantification, 0.3 µg/L, limit of detection, 0.1 µg/L [de Cazanove *et al.* 1997]. Column: µPorasil (300 × 3.9 mm i.d., 10 µm). Mobile phase: 5 mmol/L sodium acetate buffer (pH 6.0) : acetonitrile (40 : 60), flow rate 1.2 mL/min. UV detection (λ = 210 nm). Retention time: 13.9 min. Limit of quantification, 0.75 µg/L [Ho *et al.* 1996]. Column: Lichrospher (150 × 4.6 mm i.d.,m 5 µm). Mobile phase: methanol : 24 mmol/L potassium dihydrogen phosphate plus 0.06 mmol/L EDTA (20 : 80), flow rate 0.8 mL/min. Electrochemical detection. Limit of quantification, 0.1 µg/L, limit of detection, 0.02 µg/L [Nicolle *et al.* 1997]. Limit of quantification, 0.5 µg/L, limit of detection, 0.1 µg/L [Nicolle *et al.* 1996]. See also Aitkenhead *et al.* [1988]; Dubé *et al.* [1988]; Keegan and Kay [1984]; Kintz *et al.* [1992]; Lake *et al.* [1982]; Lo *et al.* [1984]; Nicolle *et al.* [1995]; and Wetzelsberger *et al.* [1988].

Urine **GC-MS** Column: SE-54 (15 m × 0.25 mm i.d.). Carrier gas: He, 7 mL/min. Temperature programme: 200° for 1 min to 280° at 10°/min for 10 min. EI ionisation at 70 eV. Limit of detection, 0.05 mg/L [Yoo *et al.* 1995]. See Plasma. Limit of detection, 3 µg/L [Kintz *et al.* 1992].

HPLC See Plasma [Kintz *et al.* 1992].

Milk **HPLC** See Plasma [Jacqz-Aigrain *et al.* 2007].

Hair **LC-MS** Column: XTerra MSC_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 50 mmol/L ammonium formate (pH 3.0) : acetonitrile containing 0.05% formic acid (95 : 5 for 2 min to 10 : 90 over 16 min to 95 : 5 over 4 min for 6 min), flow rate, 0.2 mL/min. ESI, positive ion mode, full scan mode. Limit of quantification, 0.008 ng/mg, limit of detection, 0.0024 ng/mg [Klinzig *et al.* 2007].

Disposition in the Body Absorbed after oral administration, but there is thought to be considerable first-pass metabolism; rapidly absorbed after IM or SC injection. The major metabolic reaction is conjugation to form nalbuphine glucuronide (inactive); oxidation to 6-oxonalbuphine occurs, and the desalkyl derivative, 7,8-dihydro-14-hydroxynormorphine, has also been identified as a metabolite. Unchanged nalbuphine, its conjugates, and the 2 metabolites have been detected in the urine.

Therapeutic Concentration

A nurse had hair concentrations of nalbuphine of 5.07, 7.06, and 5.7 ng/mg [Klinzig *et al.* 2007].

Ten children undergoing general anaesthesia were administered 0.3 mg/kg by the rectal route. The mean peak plasma concentration was 24 ± 15 µg/L reached at 25 ± 11 min. The elimination half-life was 2.7 ± 0.7 h [Bessard *et al.* 1997].

A study with 28 participants reported a fetomaternal ratio of 0.74. Plasma concentrations ranged from 5.0 ± 79.2 µg/L in the maternal samples and from 3.0 ± 46.6 µg/L in umbilical cord samples [Nicolle *et al.* 1996].

Patients receiving 25 µg/kg/h IV nalbuphine had a steady-state concentration of 17.7 µg/L at ~4 h. After the infusion stopped, the plasma concentrations decline monoexponentially with a terminal half-life of 2 h [Dubé *et al.* 1988].

Following the oral administration of 60 mg nalbuphine to 10 healthy volunteers, a mean maximum plasma concentration of 21.4 µg/L was reached at 46.6 min [Aitkenhead *et al.* 1988].

Twenty-four healthy male volunteers were administered nalbuphine 10 mg IV over 2 min, 45 mg orally as a solution, or 45 mg as 2 separate tablet formulations. The following pharmacokinetic parameters were measured:

	C_{max} (µg/L)	t_{max} (h)	$t_{1/2}$ (h)
45 mg IV			2.3
45 mg as solution	14.4	0.94	7.4
45 mg formulation A	15.1	1.23	7.7
45 mg formulation B	15.5	1.11	6.9

[Lo *et al.* 1987].

A single subject administered 45 mg nalbuphine hydrochloride achieved a plasma concentration of 9.1µg/L at 1 h. The terminal half-life was 7.6 h [Lo *et al.* 1984].

Following a single oral dose of 30 mg to 3 subjects, a peak plasma concentration of ~0.015 mg/L was attained in 1 h. Following a single intramuscular dose of 10 mg to 6 subjects, peak plasma concentrations of 0.038–0.059 mg/L (mean 0.052) were attained in 15–30 min (Bullingham 1984).

Note For pharmacokinetic parameters in cardiac patients, see Lake *et al.* [1982].

Bioavailability 16.4–17.4% [Lo *et al.* 1987], 7.3 % [Lo *et al.* 1984], 11.8% [Aitkenhead *et al.* 1988].

Half-life Plasma half-life, approximately 5 h.

Clearance 1.42 L/min [Lo *et al.* 1984].

Note For a review of nalbuphine, see Errick and Heel [1983].

Dose 10 to 20 mg of nalbuphine hydrochloride parenterally, every 3 to 6 h.

Aitkenhead AR *et al.* (1988). The pharmacokinetics of oral and intravenous nalbuphine in healthy volunteers. *Br J Clin Pharmacol* 25: 264–268.

Bessard G *et al.* (1997). Pharmacokinetics of intrarectal nalbuphine in children undergoing general anaesthesia. *Fundam Clin Pharmacol* 11: 133–137.

Bullingham RES (1984). Pharmacokinetics of nalbuphine. In: Nimmo WS, Smith G, eds. *Opioid Agonist/Antagonist Drugs in Clinical Practice*. Oxford: Excerpta Medica, pp. 115-122.

de Cazanove F *et al.* (1997). Determination of nalbuphine in human plasma by high-performance liquid chromatography with electrochemical detection. Application to a pharmacokinetic study. *J Chromatogr B Biomed Sci Appl* 690: 203–210.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dubé LM *et al.* (1988). High-performance liquid chromatographic determination of diltiazem and four of its metabolites in plasma: evaluation of their stability. *J Chromatogr* 430: 103–111.

Errick JK, Heel RC (1983). Nalbuphine A preliminary review of its pharmacological properties and therapeutic efficacy. *Drugs* 26: 191–211.

Ho ST *et al.* (1996). Determination of nalbuphine by high-performance liquid chromatography with ultraviolet detection: application to human and rabbit pharmacokinetic studies. *J Chromatogr B Biomed Appl* 678: 289–296.

Jacqz-Aigrain E *et al.* (2007). Excretion of ketoprofen and nalbuphine in human milk during treatment of maternal pain after delivery. *Ther Drug Monit* 29: 815–818.

Keegan M, Kay B (1984). Detection of nalbuphine in plasma: an improved high-performance liquid chromatographic assay. *J Chromatogr* 311: 223–226.

Kintz P *et al.* (1992). Determination of nalbuphine using high-performance liquid chromatography coupled to photodiode-array detection and gas chromatography coupled to mass spectrometry. *J Chromatogr* 579: 172–176.

Klinzig F *et al.* (2007). Hair analysis by LC-MS as evidence of nalbuphine abuse by a nurse. *J Anal Toxicol* 31: 62–65.

Lake CL *et al.* (1982). High-performance liquid chromatographic analysis of plasma levels of nalbuphine in cardiac surgical patients. *J Chromatogr* 233: 410–416.

Lo MW *et al.* (1984). Determination of nalbuphine in human plasma by automated high-performance liquid chromatography with electrochemical detection. *Res Commun Chem Pathol Pharmacol* 43: 159–168.

Lo MW *et al.* (1987). The disposition and bioavailability of intravenous and oral nalbuphine in healthy volunteers. *J Clin Pharmacol* 27: 866–873.

Nicolle E *et al.* (1995). Rapid and sensitive high-performance liquid chromatographic assay for nalbuphine in plasma. *JChromatogr B Biomed Appl* 663: 111–117.

Nicolle E *et al.* (1996). Therapeutic monitoring of nalbuphine: transplacental transfer and estimated pharmacokinetics in the neonate. *Eur J Clin Pharmacol* 49: 485–489.

Nicolle E *et al.* (1997). Modified method of nalbuphine determination in plasma: validation and application to pharmacokinetics of the rectal route. *JChromatogr B Biomed Sci Appl* 690: 89–97.

Weinstein SH *et al.* (1978). Quantitative determination of nalbuphine in plasma using electron-capture detection. *JPharm Sci* 67: 547–548.

Wetzelsberger N *et al.* (1988). Internally standardized method for the determination of nalbuphine in human plasma by means of high performance liquid chromatography with electrochemical coulometric detection. *Arzneimittelforschung* 38: 1768–1771.

Yoo YC *et al.* (1995). Determination of nalbuphine in drug abusers' urine. *J Anal Toxicol* 19: 120–123.

Naled

Acaricide, Insecticide, Organophosphate

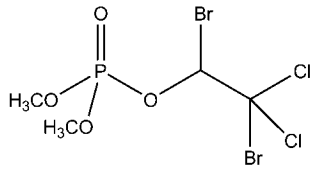
$C_4H_7Br_2Cl_2O_4P = 380.8$

CAS—300-76-5

IUPAC Name (1,2-Dibromo-2,2-dichloroethyl) dimethyl phosphate

Synonyms Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate; ENT-24988; OMS-75; phosphoric acid 1,2-dibromo-2,2-dichloroethyl dimethyl ester; RE-4355.

Proprietary Names *Alvora*; *Bromchlorphos*; *Bromochlorphos*; *Bromex*; *Dibrom*; *Dibromfos*; *Hibrom*; *Fly-Killer-D*; *Lucanal*; *Ortho-Dibrom*.



Chemical Properties A colourless solid or a straw-coloured liquid with a slightly pungent odour. Mp 26.5° to 27.5°. Bp 110° at 0.5 mmHg. It is soluble in aromatic hydrocarbons, chlorinated hydrocarbons, alcohols and ketones; sparingly soluble in mineral oils and petroleum solvents; practically insoluble in water (<1 mg/L at 20°) [O' Neil *et al.* 2006]. Log *P* (octanol/water), 1.38 [Hansch *et al.* 1995]. Unstable in the field environment but stable at 4° and 23° for at least 7 days when stored in hexane [Zhong, Latham 2001].

Quantification

***Other* GC** Environmental Samples. Column: DB-5 fused silica capillary (30 m × 0.25 mm i.d., 0.1 µm). Temperature programme: 120° for 0.5 min to 260° at 25°/min for 1 min. TSD. Limit of quantification, 10 µg/L, limit of detection, 1 µg/L [Zhong, Latham 2001].

LC-MS Fruit and Vegetables. Column: ODS. Mobile phase: acetic acid : ammonium acetate : methanol. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 1 µg/kg [Ibuki *et al.* 2007].

Disposition in the Body Naled is readily absorbed through the skin, lungs and intestines into the bloodstream [EXTOXNET 1996].

Toxicity

Naled is moderately to highly toxic if ingested orally, with reported oral LD_{50} values of 91 to 430 mg/kg in rats and 330 to 375 mg/kg in mice. It is moderately toxic through contact with the skin, with reported dermal LD_{50} values of 1100 and 800 mg/kg in rabbits and rats, respectively. Acute exposure to naled may lead to the following symptoms: numbness, tingling sensations, incoordination, headache, dizziness, tremour, nausea, abdominal cramps, sweating, blurred vision, respiratory depression and slow heartbeat. Chronic exposure to naled may also cause the neurological and neuromuscular effects associated with cholinesterase inhibition [EXTOXNET 1996].

EXTOXNET (1996). Naled. In: *Extension Toxicology Network Pesticide Information Profiles.* http://extoxnet.orst.edu/pips/naled.htm (accessed 12 November 2009).

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society.

Ibuki S *et al.* (2007). Simultaneous determination of dichlorvos, trichlorfon and naled in fruits and vegetables by liquid chromatography with tandem mass spectrometry. *Shokuhin Eiseigaku Zasshi* 48: 139–143.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station. NJ: Merck Research Laboratories.

Zhong H, Latham M (2001). Development and validation of a rapid analytical method to quantify naled residue. *J Am Mosq Control Assoc* 17: 225–230.

Nalidixic Acid

Antibacterial (Urinary)

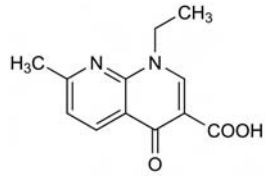
$C_{12}H_{12}N_2O_3 = 232.2$

CAS—389-08-2

IUPAC Name 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

Synonyms Nalidixinic acid; Win-18320.

Proprietary Names *Acidex*; *Acinal*; *Betaxina*; *Fardixon*; *Labydon*; *Lidinal*; *Lidixin*; *Nadiwil*; *Nalidixan*; *Nalidixin*; *Nalidoid*; *Naligram*; *Nalissina*; *Nalix*; *NegGram*; *Negram*; *Puromylon*; *Seltomylon*; *Unidixina*; *Uralgin*; *Uriben*; *Uri-Flor*; *Urigram*; *Urlix*; *Urogram*; *Wintomilon*; *Winlomylon*; *Wintomylon*. It is an ingredient of *Mictral*.

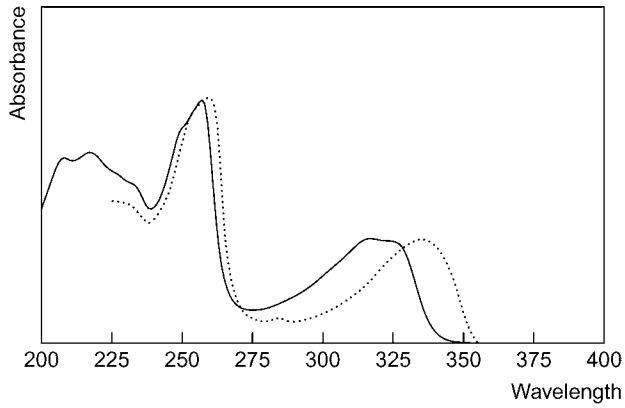


Chemical Properties An almost white or very pale yellow crystalline powder. Mp 229° to 230°. Practically insoluble in water; soluble 1 in 910 of ethanol and 1 in 35 of chloroform; very slightly soluble in ether; soluble in solutions of alkali hydroxides and carbonates. pK_a 6.0. Log *P* (octanol/water), 1.6.

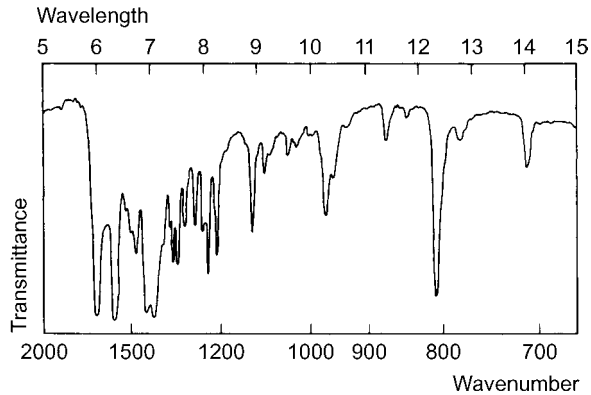
Thin-layer Chromatography System TD—R_f 0.39; system TE—R_f 0.02; system TF—R_f 0.31; system TAD—R_f 0.62; system TAE—R_f 0.63.

High Performance Liquid Chromatography System HY—RI 380; system HAA—retention time 16.0 min.

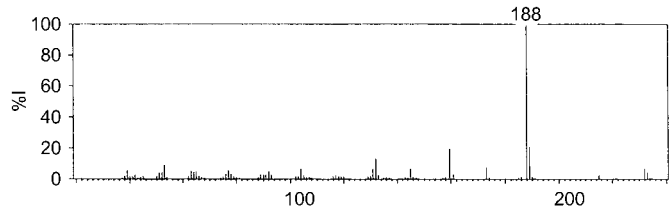
Ultraviolet Spectrum Aqueous acid—257 (A^1_1=1233b), 315 nm; aqueous alkali—258 (A^1_1=1120a), 334 nm.



Infrared Spectrum Principal peaks at wavenumbers 1613, 1704, 809, 1250, 1225, 1515 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 188, 189, 159, 132, 53, 173, 131, 145.



Quantification

Plasma **TLC** Fluorescence detection. Limit of detection, 200 µg/L [Hundt, Barlow 1981].

GC FID. Limit of detection, 5 mg/L [Roseboom *et al.* 1979].

HPLC For method of quantification for nalidixic acid and its metabolites, see Vree *et al.* [1993]. UV detection. Limit of detection, 500 µg/L for nalidixic acid, 7-hydroxynalidixic acid and 7-carboxynalidixic acid [Cuisinaud *et al.* 1980].

Urine **GC** FID. For method of quantification for nalidixic acid and 3,7-dicarboxynalidixic acid, see Wu *et al.* [1989].

HPLC UV and fluorimetric detection. For method of quantification for nalidixic acid and other quinolone antibacterials, see Duran Meras *et al.* [1997]. See Plasma [Vree *et al.* 1993]. See Plasma [Cuisinaud *et al.* 1980].

Disposition in the Body Nalidixic acid is readily absorbed after oral administration. The major metabolite, 7-hydroxynalidixic acid, is active; other metabolites include glucuronide conjugates of nalidixic acid and 7-hydroxynalidixic acid, and a 7-carboxy metabolite, all of which are inactive. About 80% of a dose is excreted in the urine in 8 h, mainly as glucuronide conjugates with about 2 to 3% as unchanged drug.

Therapeutic Concentration

Following a single oral dose of 1 g to 11 female subjects, a mean peak plasma concentration of nalidixic acid of 27.3 mg/L was attained in 1.5 h; concentrations of 7-hydroxynalidixic acid reached a maximum of about 10 mg/L within 1 h. After repeated oral administration of 1 g twice daily to the same subjects, a peak plasma concentration of 33 mg/L of nalidixic acid was reported on the seventh day. Urinary concentrations of nalidixic acid plus 7-hydroxynalidixic acid following single or multiple dose administration were in excess of about 200 mg/L for 8 h in all subjects, and in some cases >50 mg/L for 12 h [Ferry *et al.* 1981].

Toxicity Toxic effects have been associated with plasma concentrations >40 mg/L.

Half-life Plasma half-life, nalidixic acid and the 7-hydroxy metabolite about 7 h; increased in renal failure to about 20 h.

Volume of Distribution About 0.4 L/kg.

Protein Binding Nalidixic acid about 93%, 7-hydroxynalidixic acid about 60%.

Dose 2 to 4 g daily.

Cuisinaud G *et al.* (1980). Determination of nalidixic acid and its two major metabolites in human plasma and urine by reversed-phase high-performance liquid chromatography. *J Chromatogr* 181: 399–406.

Duran Meras I *et al.* (1997). Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series. *J Chromatogr A* 787: 119–127.

Ferry N *et al.* (1981). Nalidixic acid kinetics after single and repeated oral doses. *Clin Pharmacol Ther* 29: 695–698.

Hundt HK, Barlow EC (1981). Thin-layer chromatographic method for the quantitative analysis of nalidixic acid in human plasma. *J Chromatogr* 223: 165–172.

Roseboom H *et al.* (1979). Rapid gas chromatographic method for the determination of nalidixic acid in plasma. *J Chromatogr* 163: 92–95.

Vree TB *et al.* (1993). Direct gradient reversed-phase HPLC analysis and preliminary pharmacokinetics of nalidixic acid, 7-hydroxymethylnalidixic acid, 7-carboxynalidixic acid, and their corresponding glucuronide conjugates in humans. *Pharm World Sci* 15: 98–104.

Wu SM *et al.* (1989). A preliminary report on the derivatization-gas chromatographic determination of nalidixic acid and 3,7-dicarboxynalidixic acid in urine. *Gaoxiong Yi Xue Ke Xue Za Zhi* 5: 630–636.

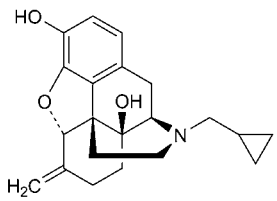
Nalmefene

Narcotic Antagonist

$C_{21}H_{25}NO_3$ = 339.4

CAS—55096-26-9

Synonyms (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-6-methylenemorphinan-3,14-diol; JF-1; ORF-11676.



Chemical Properties A white to off white crystalline powder with Mp 188° to 190°. Soluble in water (140 mg/L at 25°). Log *P* (octanol/water), 2.66 [Meylan, Howard 1995]. Stable in plasma for at least 1 month at −25° [Xie *et al.* 2002].

Nalmefene Hydrochloride

$C_{21}H_{25}NO_3$,HCl = 375.9

CAS—58895-64-0

Synonyms Nalmetrene hydrochloride; 6-desoxy-6-methylene-naltrexone hydrochloride.

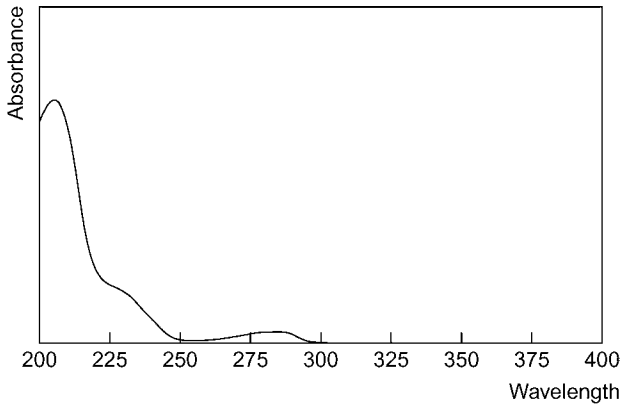
Proprietary Name *Revex*

Chemical Properties A white to off white crystalline powder with Mp 180° to 185° to a clear liquid. Very soluble in water and methanol; soluble in ethanol (86.2 g/L), acetonitrile (1.07 g/L), acetone (0.23 g/L), and chloroform (0.13 g/L). pK_a 7.63. Log *P* (octanol/water), −1.125. Revex was uniformly stable in injectable solutions for up to 72 h when kept at 4, 21 or 40° [Murthy, Brittain 1996].

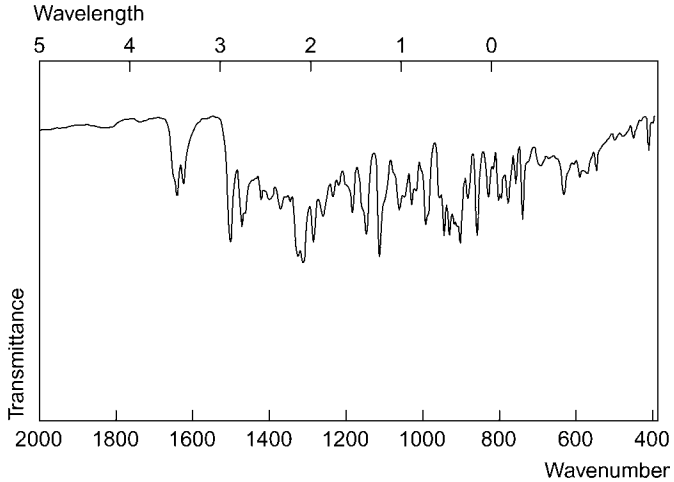
Thin-layer Chromatography Plates: silica gel 60 F_{254}. Solvent system: cyclohexane : chloroform : diethylamine (10 : 75 : 15). UV detection (λ = 254 nm). Reference compound: nalmefen, R_f 0.41; bisnalmefene, R_f 0.07 [Brittain 1996].

Gas Chromatography Column: HP1 (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, 0.9 mL/min. FID. RI: 2700 [Mills, Roberson 1993].

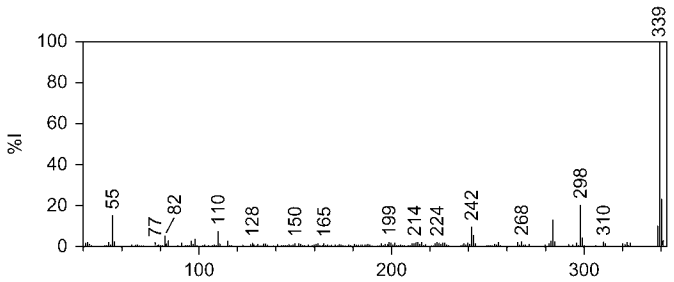
Ultraviolet Spectrum Methanol—211, 230, 285 nm (hydrochloride); aqueous acid (0.2 mol/L H_2SO_4)—284 nm; basic—298 nm.



Infrared Spectrum Principal peaks at wavenumber 1170, 904, 814, 1322, 1619, 1638 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 55, 339, 110, 36, 298, 82, 242, 96.



Quantification

Plasma **GC-MS** Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: CH_4:NH_3 (95 : 5). Temperature programme: 80° for 1 min to 280° at 30°/min. NCI, SIM acquisition mode. Retention time: 7.27 min. Limit of quantification, 0.5 µg/L [Xie *et al.* 2002].

HPLC Column: NovaPak phenyl (100 × 8 mm i.d., 4 µm). Mobile phase: acetonitrile : 5 mmol/L sodium pentanesulfonic acid monohydrate-0.0045% orthophosphoric acid (pH 3.1; 30 : 70), flow rate 1.2 mL/min. Electrochemical detection. Retention time: ~12 min. Limit of detection, 1 µg/L [Chou *et al.* 1993]. Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: methanol : 0.05 mol/L tetramethylammonium hydroxide (25 : 75), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 3 µg/L [Hsiao, Dixon 1983].

LC-MS Column: Zorbax Eclipse XDB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L ammonium acetate (75 : 26), flow rate 0.5 mL/min. TIS, MRM acquisition mode. Limit of quantification, 10 ng/L [Li *et al.* 2007].

Note For a radioimmunoassay for nalmefene in plasma, see Dixon *et al.* [1984].

Urine **HPLC** Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: methanol : 0.05 mol/L tetramethylammonium hydroxide (25 : 75), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 80 µg/L [Dixon *et al.* 1987].

Disposition in the Body Nalmefene is rapidly and almost completely absorbed following oral administration, but absorption is delayed after intramuscular and subcutaneous injection. It is metabolised in the liver, mainly to the inactive glucuronide conjugate; trace amounts of *N*-dealkylated metabolite have been detected. It is mainly excreted in urine (83%) with some of the dose excreted in the faeces. It may undergo enterohepatic recycling. Less than 5% of a dose is excreted unchanged in urine and 17% in faeces.

Therapeutic Concentration

Twenty-four healthy males (aged 19–46 years; mean, 29 years) were administered 2, 6, 12 or 24 mg doses of nalmefene hydrochloride after an overnight fast that continued for 4 h after administration. The peak plasma concentrations were ~12, 36, 85 and 110 µg/L for the 4 doses, respectively, observed by the end of infusion [Dixon *et al.* 1986].

Young, healthy individuals (mean age, 23.8 years) and elderly individuals (mean age, 70.7 years) were administered a 1 mg IV dose of nalmefene. Young (mean age 28.1 years) and elderly (mean age 68.3 years) individuals were also given a 2 mg IV dose. In both dose groups in the elderly there was a 30–40% smaller volume of distribution and a significantly shorter half-life [Frye *et al.* 1996].

Nine healthy male Chinese volunteers (aged 19–24 years) were administered a single dose of 30 µg nalmefene IV over a period of 5 min. There was considerable inter-individual variation, with the AUC_{0-t} ranging from 192.7–900.5 pg·h/mL [Li *et al.* 2007].

Half-life Oral: 11 h, IV: 8–9 h [Wang *et al.* 1998].

Volume of Distribution 8.5 L/kg.

Clearance 1.37 L/h/kg, decreased by as much as 30% in hepatic dysfunction [Wang *et al.* 1998].

Protein Binding 45%.

Dose The usual IV dose is 0.5 to 1 mg every 2 min up to 2 mg and orally 1 to 2 mg.

Brittain HG (1996). *Analytical Profiles of Drug Substances and Excipients*. London: Academic Press.

Chou JZ *et al.* (1993). Determination of nalmefene in plasma by high-performance liquid chromatography with electrochemical detection and its application in pharmacokinetic studies. *J Chromatogr* 613: 359–364.

Dixon R *et al.* (1984). Nalmefene: radioimmunoassay for a new opioid antagonist. *J Pharm Sci* 73: 1645–1646.

Dixon R *et al.* (1986). Nalmefene: intravenous safety and kinetics of a new opioid antagonist. *Clin Pharmacol Ther* 39: 49–53.

Dixon R *et al.* (1987). Nalmefene: safety and kinetics after single and multiple oral doses of a new opioid antagonist. *JClin Pharmacol* 27: 233–239.

Frye RF *et al.* (1996). The effect of age on the pharmacokinetics of the opioid antagonist nalmefene. *Br JClin Pharmacol* 42: 301–306.

Hsiao J, Dixon R (1983). Nalmefene: quantitation of a new narcotic antagonist in human plasma using high performance-liquid chromatography with electrochemical detection. *Res Commun ChemPathol Pharmacol* 42: 449–454.

Li P *et al.* (2007). Application of a sensitive liquid chromatographic/tandem mass spectrometric method to pharmacokinetic study of nalmefene in humans. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 479–484.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *JPharm Sci* 84: 83–92.

Mills TI, Roberson JC (1993). *Instrumental Data for Drug Analysis,* 2nd edn, Vol. 4–5. Boca Raton, FL: CRC Press.

Murthy SS, Brittain HG (1996). Stability of Revex, nalmefene hydrochloride injection, in injectable solutions. *J Pharm Biomed Anal* 15: 221–226.

Wang DS *et al.* (1998). Nalmefene: a long-acting opioid antagonist. Clinical applications in emergency medicine. *J Emerg Med* 16: 471–475.

Xie S *et al.* (2002). Rapid and sensitive determination of nalmefene in human plasma by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 773: 143–149.

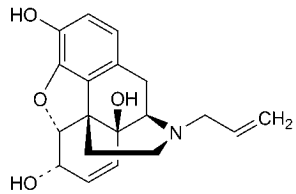
Nalorphine

Narcotic Antagonist

$C_{19}H_{21}NO_3$=11.4

CAS—62-67-9

Synonym (5α,6α)-7,8-Didehydro-4,5-epoxy-17-(2-propenyl)morphinan-3,6-diol



Chemical Properties Crystals. Mp 208°. Sparingly soluble in water and ether; soluble in ethanol, acetone, dilute alkalis and chloroform. pK_a 7.8 (20°). Log *P* (octanol/pH 7.4), 1.5. Extraction yield (chlorobutane), 0.3 [Demme *et al.* 2005].

Nalorphine Hydrobromide

$C_{19}H_{21}NO_3$, HBr = 392.3

CAS—1041-90-3

Proprietary Names *Lethidrone*; *Norfin*.

Chemical Properties A white to creamy-white crystalline powder. Mp ≈206°, with decomposition. Soluble 1 in 24 of water and 1 in 35 of ethanol. Aqueous solutions may deposit crystals of the dihydrate; the dihydrate is readily soluble in dehydrated alcohol but the solution rapidly yields a deposit of the anhydrous salt.

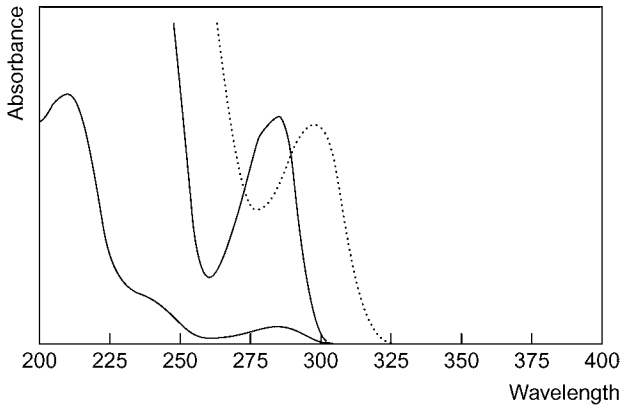
Nalorphine Hydrochloride

$C_{19}H_{21}NO_3$,HCl = 347.8

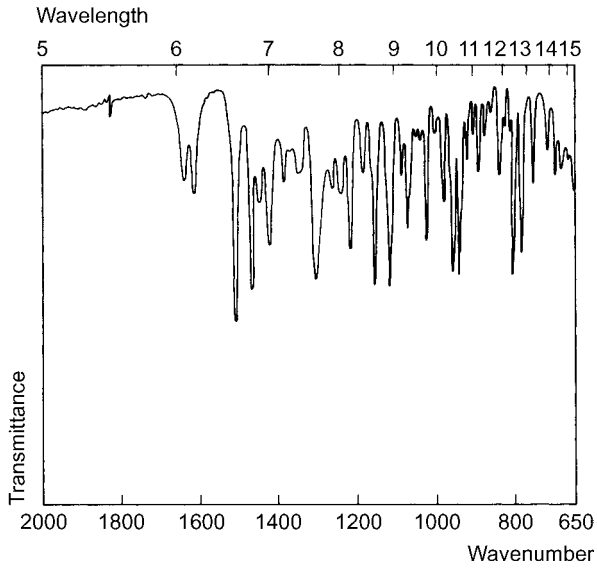
CAS—57-29-4

Proprietary Name *Nalline*
Chemical Properties A white crystalline powder that slowly darkens on exposure to air and light. Mp 260° to 263°. Soluble 1 in 8 of water and 1 in 35 of ethanol; practically insoluble in chloroform and ether; soluble in dilute solutions of alkali hydroxides.

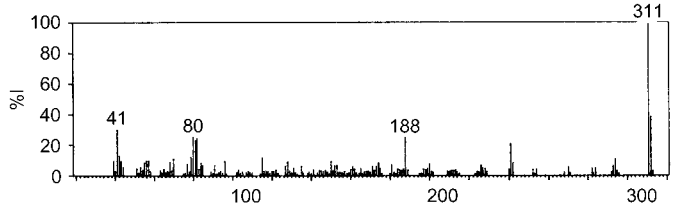
Colour Test Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.01; system TC—R_f 0.23; system TE—R_f 0.32; system TAE—R_f 0.57; system TAF—R_f 0.59; system TAG—R_f 0.29 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—RI 2620; system GB—not eluted.
High Performance Liquid Chromatography System HA—*k* 1.0; system HC—*k* 0.29; system HX—RI 260; system HY—RI 237; system HAA—retention time 4.8 min.
Ultraviolet Spectrum Aqueous acid—285 nm ($A^1_1 = 49a$); aqueous alkali—251 ($A^1_1 = 190a$), 298 nm ($A^1_1 = 81a$).



Infrared Spectrum Principal peaks at wavenumbers 1505, 1121, 1155, 1304, 805, 945 cm^{-1} (nalorphine hydrobromide , KBr disk).



Mass Spectrum Principal ions at *m/z* 311, 312, 41, 188, 80, 82, 81, 241.

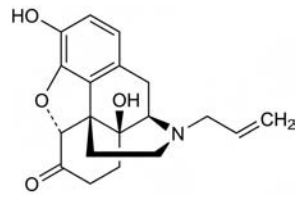


Disposition in the Body Absorbed after oral administration but undergoes extensive first-pass metabolism. It is excreted in the urine mainly as metabolites and glucuronide conjugates, with ~2–6% of a dose as unchanged drug.
Toxicity The estimated minimum lethal dose is 200 mg. The administration of nalorphine to morphine addicts may be followed by a typical withdrawal syndrome.
Dose 5 to 10 mg of nalorphine hydrobromide IV, repeated if necessary.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Naloxone

Narcotic Antagonist
$C_{19}H_{21}NO_4 = 327.4$
CAS—465-65-6
IUPAC Name (5α)-4,5-Epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one
Synonym Allylnoroxymorphone

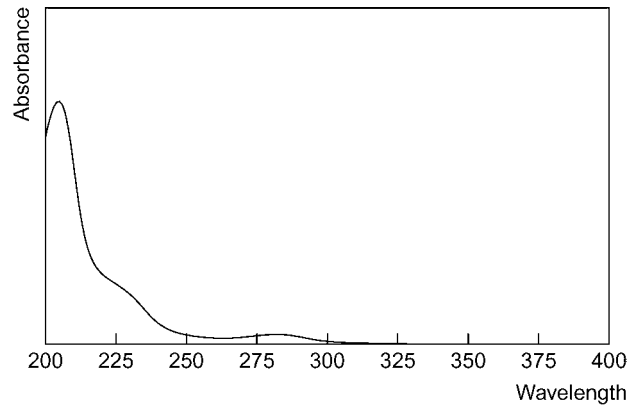


Chemical Properties Crystals. Mp 177° to 180°. Soluble in chloroform; practically insoluble in petroleum ether. pK_a 7.9. Log *P* (octanol/pH 7.4), 1.5. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

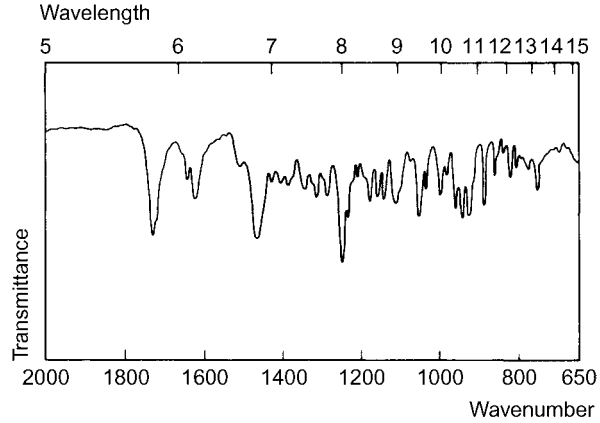
Naloxone Hydrochloride

$C_{19}H_{21}NO_4$, HCl = 363.8
CAS—357-08-4 (anhydrous); 51481-60-8 (dihydrate)
Proprietary Names *Nalone*; *Narcan*; *Narcanti*; *Naxolan*; *Zynox*.
Chemical Properties A white powder. Mp 200° to 205°. Soluble in water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

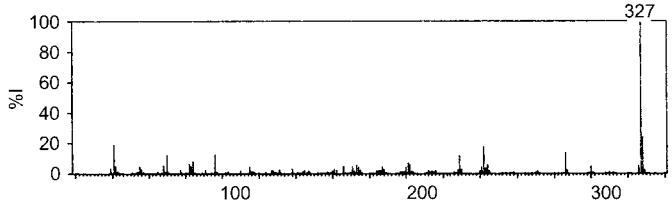
Colour Test Folin-Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—violet→brown; Marquis test—brown→violet.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.09; system TC—R_f 0.66; system TE—R_f 0.47; system TAE—R_f 0.74; system TAG—R_f 0.63 (acidified Iodoplatinate Solution—positive).
Gas Chromatography System GA—RI 2715; system GB—not eluted.
High Performance Liquid Chromatography System HA—*k* 1.4; system HC—*k* 0.17; system HY—RI 238; system HZ—RT 2.0 min; system HAA—RT 14.0 min.
Ultraviolet Spectrum Aqueous acid—281 nm.



Infrared Spectrum Principal peaks at wavenumbers 1244, 1728, 940, 1230, 922, 1050 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 327, 328, 41, 242, 286, 96, 229, 70.



Quantification

Plasma **GC** [Meffin, Smith 1980].

HPLC Column: Nova-Pak phenyl (100 × 8 mm i.d., 4 µm). Mobile phase: acetonitrile : 5 mmol/L sodium pentanesulfonic acid (18 : 82). Electrochemical detection. Limit of detection, 3 µg/L [Albeck *et al.* 1989]. Electrochemical detection. Limit of detection, 2–5 µg/L [Derendorf *et al.* 1984].

Urine **GC** See Plasma [Meffin, Smith 1980].

HPLC See Plasma [Derendorf *et al.* 1984].

Note For a radioimmunoassay for the quantification of naloxone, see Hahn *et al.* [1983].

Disposition in the Body Naloxone is rapidly absorbed after oral administration but undergoes extensive first-pass metabolism. It is rapidly but incompletely excreted in the urine, mainly as conjugated metabolites, with ~30% of a dose being excreted in 6 h but only 60% being excreted in 72 h. Metabolites that have been identified include naloxone 3-glucuronide and the *N*-desalkyl and 6-hydroxy derivatives, together with their glucuronide conjugates.

Therapeutic Concentration

Following IV administration of 0.4 mg to 9 subjects, mean serum concentrations of 0.01 mg/L were reported after 2 min [Ngai *et al.* 1976].

During constant IV infusion of 0.02 mg/min in 3 subjects, steady-state plasma concentrations of 0.014, 0.015 and 0.030 mg/L were reported [Hahn *et al.* 1983].

Three subjects were given naloxone orally once daily. Peak plasma levels of 3.35 and 6.1 mg/L were achieved in the first subject with a dose of 12 and 16 mg, respectively; peak plasma levels for the second subject were: 7.3 and 8.0 mg/L after doses of 12 and 16 mg, respectively, and <5 mg/L after a dose of 16 mg for the third subject [Culpepper-Morgan *et al.* 1992].

Half-life Plasma half-life, ~1–2 h.

Volume of Distribution ~3 L/kg.

Clearance Plasma clearance, ~20–30 mL/min/kg.

Protein Binding ~40%.

Dose Naloxone hydrochloride 0.4 to 2 mg parenterally, repeated as necessary.

Albeck H *et al.* (1989). Quantitative and pharmacokinetic analysis of naloxone in plasma using high-performance liquid chromatography with electrochemical detection and solid-phase extraction. *J Chromatogr* 488: 435–445.

Culpepper-Morgan JA *et al.* (1992). Treatment of opioid-induced constipation with oral naloxone: a pilot study. *Clin Pharmacol Ther* 52: 90–95.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Derendorf H *et al.* (1984). Electrochemical chromatographic determinations of morphine antagonists in biological fluids, with applications. *J Pharm Sci* 73: 621–624.

Hahn EF *et al.* (1983). Naloxone radioimmunoassay: an improved antiserum. *J Pharm Pharmacol* 35: 833–836.

Meffin PJ, Smith KJ (1980). Gas chromatographic analysis of naloxone in biological fluids. *J Chromatogr* 183: 352–356.

Ngai SH *et al.* (1976). Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action. *Anesthesiology* 44: 398–401.

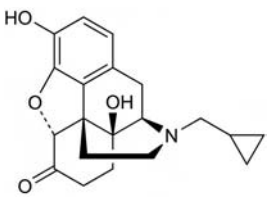
Naltrexone

Narcotic Antagonist

$C_{20}H_{23}NO_4$ = 341.4

CAS—16590-41-3

Synonyms (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one; UM-792.



Chemical Properties Crystals. Mp 167° to 174°. Log *P* (octanol/water), 1.92 [Hansch *et al.* 1995]. Stable in human plasma at room temperature for at least 12 h. Extracted samples were unstable when stored at –20°, 4°, 25° and 37° for 48 h. Extracted samples were stable in an autosampler at 4° for 24 h, but not for 48 h [Clavijo *et al.* 2008]. Stable in human plasma at room temperature for up to 24 h. Extracted sample residues were stable for at least 5 days when stored at –20° or at least 2 days at ambient temperature in an autosampler. Stock solutions were stable for at least 22 h at room temperature and at least 115 days when stored at –20° [Slawson *et al.* 2007]. Stable in human plasma for 24 h at room temperature and following 2 freeze-thaw cycles [Huang *et al.* 1997].

Naltrexone Hydrochloride

$C_{20}H_{23}NO_4$,HCl = 377.9

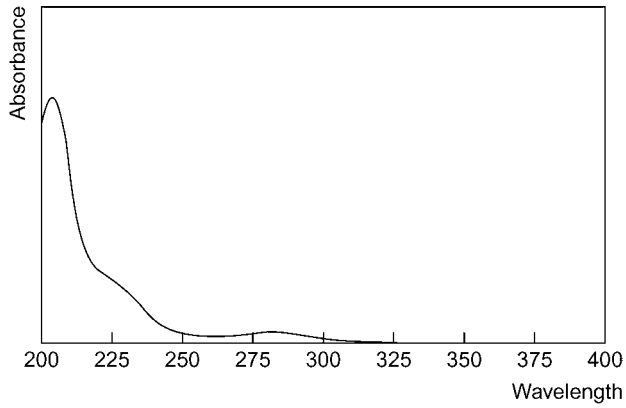
CAS—16676-29-2

Proprietary Names *Antaxone*; *Basinal*; *Celupan*; *Depade*; *Nalorex*; *Narcoral*; *Nemexin*; *Revia*; *Trexan*.

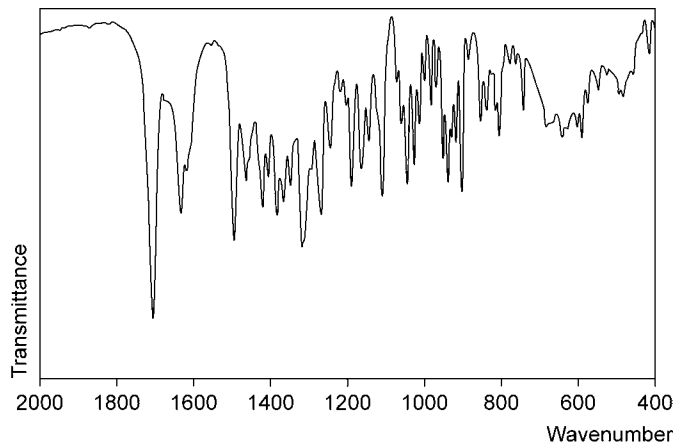
Chemical Properties White powder. Mp 274° to 276°. Soluble in water, dilute acids and alkali.

Gas Chromatography System GA—RI 2880; system GB—not eluted.

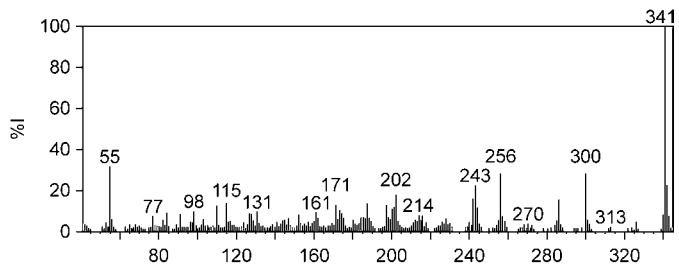
Ultraviolet Spectrum Aqueous acid—281 nm (A_1^1 = 34b); aqueous alkali—293 nm.



Infrared Spectrum Principal peaks at wavenumbers 1716, 1505, 1280, 1639, 1115, 909 cm^{-1} (naltrexone hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 341, 55, 256, 300, 243, 202, 342, 115.



Quantification

Blood **GC** Column: 3% OV-17 80/100 mesh on Gas Chrom Q (2 m × 2 mm i.d.). Carrier gas: H_2, 30 mL/min. Temperature: 230°. ECD and FID. Retention time: 7.4 min. Limit of detection not reported [Verebey *et al.* 1980]. Column: 3% OV-17 on Gas-Chrom Q (6′). Carrier gas: N_2, 60 mL/min. Temperature: 275°. Retention time: 9.6 or 10.75 min for the bis- or mono-TMS derivative. Limit of detection not reported [Verebely *et al.* 1975a].

LC-MS Column: Phenomenex Gemini C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile containing 0.1% formic acid : ammonium acetate buffer (pH 3.2, 15 : 85 for 9 min to 30 : 70 in 13 min to 80 : 20 in 10 min to 95 : 5 in 1 min), flow rate 150 µL/min. TIS, positive ion mode, MRM acquisition mode. Retention time: 2.8 min. Limit of detection, 0.0002 mg/L [Gergov *et al.* 2009].

Plasma **GC** Column: BP 10 (SGE, 25 m × 0.22 mm i.d.). Carrier gas: He, 3.2 mL/min. Temperature: 240°. NPD. Retention time: 18.06 min. Limit of detection, 6.0 µg/L [Kintz *et al.* 1989]. Column: 3% OV-7 on 100/120 mesh Gas Chrom Q (2.4 m × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 28 mL/min. Temperature: 203°. ECD. Limit of detection, <1 µg/L [Reuning *et al.* 1981]. See Blood [Verebey *et al.* 1980]. Column: 3% OV-22 on Supelcoport 80/100 mesh (6′ × 2 mm i.d.). Carrier gas: CH_4 : Ar (10 : 90), 35 mL/min. Temperature: 215°. ECD. Retention time: 7.27 min. Limit of detection, 5–10 pg [Verebey *et al.* 1976a].

GC-MS Column: Chrompack CP-Sil 5 CB (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 100° to 310° at 40°/min for 6 min. SIM acquisition mode. Limit of detection, 0.1 µg/L [Toennes *et al.* 2004]. Limit of quantification, 2 µg/L, limit of detection, 1 µg/L [Chan *et al.* 2001;Chan *et al.* 2004]. Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 130° to 285° at 20°/min. ECD, NICI. Limit of quantification, 0.1 µg/L [Huang *et al.* 1997]. Column: 5% phenylmethylsilicone (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: H_2, 10 psi. Temperature programme: 160° to 260° at 22°/min for 5 min. NICI. Limit of quantification, 0.1 µg/L [Nelson *et al.* 1993]. Column: DB-1 (20 m × 0.32 mm i.d., 1.0 µm). Carrier gas: H_2, 60–100 cm/s. Temperature programme: 130° for 0.5 min to 235° at 15°/min. NICI. Limit of quantification, 0.1 µg/L [Monti *et al.* 1991].

See also Wall *et al.* [1981a].

HPLC Column: Symmetry C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: 0.06% triethylamine (pH 2.8, 8 : 92), flow rate 1.0 mL/min. UV detection (λ= 204 nm). Retention time: 3.95 min. k': 2.3. Limit of detection, 8 µg/L [Kambia *et al.* 2000]. Column: Perisorb RP-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L disodium hydrogen orthophosphate (pH 3.5) : acetonitrile (85.5 : 14.5), flow rate 0.8 mL/min. Electrochemical detection. Retention time: 9.42 min. Limit of detection, 1 µg/L [Peh *et al.* 1997]. Column: YMC phenyl (100 × 4.6 mm i.d., 3 µm). Mobile phase: methanol: 50 mmol/L phosphoric acid (pH 3.2, 20 : 80), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 5.0 min. Limit of quantification, 0.25 µg/L [Davidson *et al.* 1996]. Column: Supelcosil LC-18 DB (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium phosphate (pH 3.0) : acetonitrile (85 : 15), flow rate 0.8 mL/min. Electrochemical detection. Retention time: 7.40 min. Limit of quantification, 5 µg/L [Zuccaro *et al.* 1991]. Column: Ultrex ODS silica (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.1 mol/L ammonium dihydrogenphosphate and 0.9 mmol/L octanesulfonic acid (pH 4.5). Electrochemical detection. Limit of detection, 50 µg/L [O'Connor *et al.* 1989].

See also Derendorf *et al.* [1984], Wall *et al.* [1984].

For an HPLC method for the detection of methylnaltrexone in plasma, see Osinski *et al.* [2002].

LC-MS Column: Luna Phenomenex (30 × 4.6 mm i.d., 5 µm). Mobile phase: methanol containing 0.1% acetic acid : 2 mmol/L ammonium acetate containing 0.1% acetic acid (40 : 60 to 95 : 5 at 4.5 min), flow rate 1.0 mL/min. APCI, MRM acquisition mode. Limit of quantification, 5 ng/L [Clavijo *et al.* 2008]. Column: Inertsil ODS3 or Betasil (50 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile: water: formic acid (10 : 90 : 1 to 70 : 30 : 1 at 2 min to 10 : 90 : 1 at 2.1 min, or 90 : 10 : 1 to 50 : 50 : 1 at 1.0 min for 1 min to 90 : 10 : 1 at 2.1 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquision mode. Retention time: 1.32 or ≈2.2 min, respectively. Limit of detection, 200 ng/L [Naidong *et al.* 2002]. Column: DS-AQ (100 × 2 mm i.d., 5 µm). Mobile phase: 0.1% formic acid : methanol (85 : 15), flow rate 0.2 mL/min. ESI, SRM acquisition mode, CID. Limit of quantification, 0.1 µg/L [Slawson *et al.* 2007].

Serum **HPLC** Column: ODS-5-80 C_{18} (150 × 4.6 mm i.d.). Mobile phase: methanol: water: 85% phosphoric acid with 0.03 mmol/L octanesulfonic acid (50 : 50 : 0.1), flow rate 0.75 mL/min. UV detection (λ= 210 nm). Retention time: 6.7 min. Limit of detection not reported [Hurst *et al.* 1999].

Urine **GC** See Blood. Limit of detection, 10–20 µg/L [Verebey *et al.* 1980]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (8′ × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 24 mL/min. Temperature: 203°. ECD. Retention time: 10.0 min. Limit of detection not reported [Malspeis *et al.* 1975]. See Blood [Verebely *et al.* 1975a]. Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (6′ × 2 mm i.d.). Carrier gas: He, 60 mL/min. Temperature: 270°. FID. Limit of detection, 0.02 mg/L [Verebely *et al.* 1975b]. Column: 3% OV-17 on Gas Chrom Q (6′ × 2 mm i.d.). Carrier gas: N_2, 24 mL/min. Temperature programme: 200° to 270° at 8°/min. FID. Limit of detection not reported [Cone *et al.* 1974].

GC-MS See Plasma [Monti *et al.* 1991]. Column: 2% OV-17 on 100/120 mesh Supelcoport (1.8 × 4 mm i.d.). EI ionisation at 70 eV. Limit of detection not reported [Wall *et al.* 1981a]. Column: 3% OV-7 on 100/120 mesh Gas Chrom Q (2.4 m × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 28 mL/min. Temperature: 203°. Limit of detection, 2 µg/L [Reuning *et al.* 1981]. Column: 2% OV-17 on 80/100 mesh Supelcoport (1.8 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 240°. EI ionisation at 70 eV. Retention time: 13.7 min. Limit of detection, 1 µg/L [Wall, Brine 1981].

HPLC See Plasma. Limit of detection, 10 µg/L [Derendorf *et al.* 1984]. See Plasma [Wall *et al.* 1984].

LC-MS See Blood [Gergov *et al.* 2009].

Note For a TLC method for the measurement of naltrexone in urine, see Verebey *et al.* [1986]. For the measurement of naltrexone metabolites, see Cone *et al.* [1978].

Milk **GC-MS** See Plasma [Chan *et al.* 2001; Chan *et al.* 2004].

Saliva **GC** See Blood [Verebey *et al.* 1980].

Other **GC** Rabbit, Monkey and Rat Urine. Column: 3% QF-1 on 100/120 mesh Gas-Chrom Q (2 m × 2 mm i.d.). Carrier gas: He, 27 mL/min. Temperature: 300°. FID. Retention time: 4.9 min. Limit of detection, 0.025 mg/L [Dayton, Inturrisi 1976].

Disposition in the Body Rapidly and almost completely absorbed after oral administration. It is metabolised by reduction to 6β-naltrexol, which has weak activity [Dayton, Inturrisi 1976]; naltrexone and 6β-naltrexol are conjugated with glucuronic acid. About 40% of an oral dose is excreted in the urine in 24 h, with about 8% of the dose as naltrexone (mainly conjugated), 26% as 6β-naltrexol (mainly unconjugated), and about 4% as free 2-hydroxy-3-methoxy-6β-naltrexol. About 2% of a dose is eliminated in the faeces in 24 h, mainly as 6β-naltrexol.

Therapeutic Concentration

After a single oral dose of naltrexone to a single subject, a peak concentration of 15 µg/L naltrexone and 84 µg/L β-natraxol were reached at 2 and 4 h, respectively [Verebey *et al.* 1976a].

After a single oral dose of 100 mg to 4 subjects, peak plasma concentrations of 0.01–0.06 mg/L (mean 0.04) of naltrexone and 0.08–0.13 mg/L (mean 0.10) of 6β-naltrexol were attained in 1–2 h and 1–4 h, respectively [Verebey *et al.* 1976b].

Note For pharmacokinetic values for haemodialysis patients receiving naltrexone see Kambia *et al.* [2000]. Naltrexone in combination with brief motivational counselling may be effective to reduce heavy drinking among young adults [Leeman *et al.* 2008].

Toxicity Five patients have overdosed fatally on opioids while they had naltrexone implants, see Gibson *et al.* [2007]. See also Tait *et al.* [2008], Oliver *et al.* [2005], Miotto *et al.* [1997] or Schecter, Kauders [1975].

Half-life Plasma half-life, after oral administration, about 10 h for naltrexone and about 12 h for 6β-naltrexol; after IV administration, naltrexone about 3 h.

Volume of Distribution ≈20 L/kg.

Clearance Plasma clearance, ≈70 mL/min/kg.

Protein Binding ≈27% [Derendorf *et al.* 1984], 20.7 [Wall *et al.* 1981a].

Note For a review of the pharmacokinetics of naltrexone see Crabtree [1984] and for a review of the metabolism of naltrexone see Wall *et al.* [1981b].

Dose Usually 50 mg of naltrexone hydrochloride daily.

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Cone EJ *et al.* (1974). The urinary excretion profile of naltrexone and metabolites in man. *Drug Metab Dispos* 2: 506–512.

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Davidson AF *et al.* (1996). Determination of naltrexone and its major metabolite, 6-beta-naltrexol, in human plasma using liquid chromatography with electrochemical detection. *J Pharm Biomed Anal* 14: 1717–1725.

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Huang W *et al.* (1997). Determination of naltrexone and 6-beta-naltrexol in plasma by solid-phase extraction and gas chromatography-negative ion chemical ionization-mass spectrometry. *J Anal Toxicol* 21: 252–257.

Hurst WJ *et al.* (1999). A rapid sample preparation method for the HPLC determination of the opioid antagonist naltrexone in serum. *Pharmazie* 54: 595–596.

Kambia K *et al.* (2000). High-performance liquid chromatographic determination of naltrexone in plasma of hemodialysis patients. *Biomed Chromatogr* 14: 151–155.

Kintz P *et al.* (1989). Simultaneous identification and quantification of several opiates and derivatives by capillary gas chromatography and nitrogen selective detection. *Z Rechtsmed* 103: 57–62.

Leeman RF *et al.* (2008). A pilot study of naltrexone and BASICS for heavy drinking young adults. *Addict Behav* 33: 1048–1054.

Malspeis L *et al.* (1975). Metabolic reduction of naltrexone. I. Synthesis, separation and characterization of naloxone and naltrexone reduction products and qualitative assay of urine and bile following administration of naltrexone, alpha-naltrexol, or beta-naltrexol. *Res Commun Chem Pathol Pharmacol* 12: 43–65.

Miotto K *et al.* (1997). Overdose, suicide attempts and death among a cohort of naltrexone-treated opioid addicts. *Drug Alcohol Depend* 45: 131–134.

Monti KM *et al.* (1991). Analysis of naltrexone and 6-beta-naltrexol in plasma and urine by gas chromatography/negative ion chemical ionization mass spectrometry. *J Anal Toxicol* 15: 136–140.

Naidong W *et al.* (2002). Simultaneous development of six LC-MS-MS methods for the determination of multiple analytes in human plasma. *J Pharm Biomed Anal* 28: 1115–1126.

Nelson CC *et al.* (1993). Gas chromatography/tandem mass spectrometry measurement of delta 9-tetrahydrocannabinol, naltrexone, and their active metabolites in plasma. *Ther Drug Monit* 15: 557–562.

O'Connor EF *et al.* (1989). Simultaneous extraction and chromatographic analysis of morphine, dilaudid, naltrexone and naloxone in biological fluids by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 491: 240–247.

Oliver P *et al.* (2005). Fatal opiate overdose following regimen changes in naltrexone treatment. *Addiction* 100: 560–561.

Osinski J *et al.* (2002). Determination of methylnaltrexone in clinical samples by solid-phase extraction and high-performance liquid chromatography for a pharmacokinetics study. *J Chromatogr B Analyt Technol Biomed Life Sci* 780: 251–259.

Peh KK *et al.* (1997). Simple liquid chromatographic method for the determination of naltrexone in human plasma using amperometric detection. *J Chromatogr B Biomed Sci Appl* 701: 140–145.
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Schecter A, Kauders F (1975). Patient deaths in a narcotic antagonist (naltrexone) and l-alpha-acetylmethadol program. *Am J Drug Alcohol Abuse* 2: 443–449.
Slawson MH *et al.* (2007). Quantitative analysis of naltrexone and 6beta-naltrexol in human, rat, and rabbit plasma by liquid chromatography-electrospray ionization tandem mass spectrometry with application to the pharmacokinetics of Depotrex in rabbits. *J Anal Toxicol* 31: 453–461.
Tait RJ *et al.* (2008). Mortality in heroin users 3 years after naltrexone implant or methadone maintenance treatment. *J Subst Abuse Treat* 35: 116–124.
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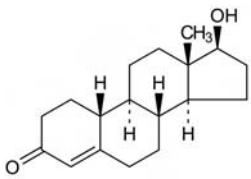
Verebey K *et al.* (1986). Determination of 6-beta-naltrexol and naltrexone by bonded-phase adsorption thin-layer chromatography. *J Chromatogr* 378: 261–266.
Wall ME, Brine DR (1981). Analytical methods for quantitative and qualitative analysis of naltrexone and metabolites in biological fluids. *NIDA Res Monogr* 28: 52–65.
Wall ME *et al.* (1981a). Metabolism and disposition of naltrexone in man after oral and intravenous administration. *Drug Metab Dispos* 9: 369–375.
Wall ME *et al.* (1981b). The metabolism of naltrexone in man. *NIDA Res Monogr* 28: 105–131.
Wall ME *et al.* (1984). Naltrexone disposition in man after subcutaneous administration. *Drug Metab Dispos* 12: 677–682.
Zuccaro P *et al.* (1991). Determination of naltrexone and 6 beta-naltrexol in plasma by high-performance liquid chromatography with coulometric detection. *J Chromatogr* 567: 485–490.

Nandrolone

Anabolic Steroid
$C_{18}H_{26}O_2 = 274.4$
CAS—434-22-0

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-13-methyl-2,6,7,8,9,10,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-one
Synonyms (17β)-17-Hydroxyestr-4-en-3-one; nortestosterone.



Chemical Properties Crystals. Two polymorphic forms occur; Mp 112° and 124°. Soluble in ethanol, chloroform and ether. Log *P* (octanol/water), 2.6.

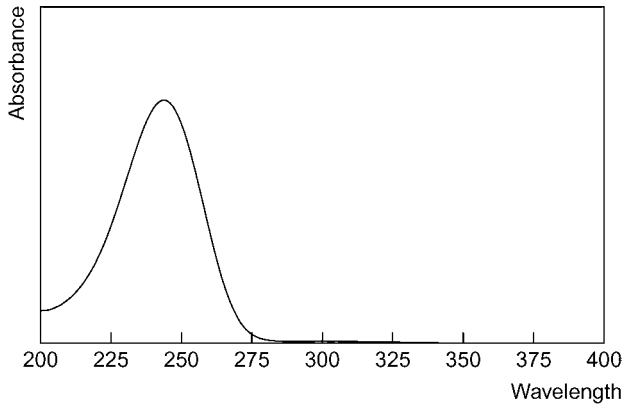
Nandrolone Decanoate

$C_{28}H_{44}O_3 = 428.7$
CAS—360-70-3
Synonym Nortestosterone decylate
Proprietary Names *Androlone-D*; *Deca-Durabol*; *Deca-Durabolin*; *Deca-Noralone*; *Hybolin Decanoate*; *Kabolin*; *Neo-Durabolic*; *Retabolil*.
Chemical Properties A white to creamy-white crystalline powder. Mp 32° to 35°. Practically insoluble in water; soluble 1 in 1 of ethanol; freely soluble in acetone, chloroform and ether.

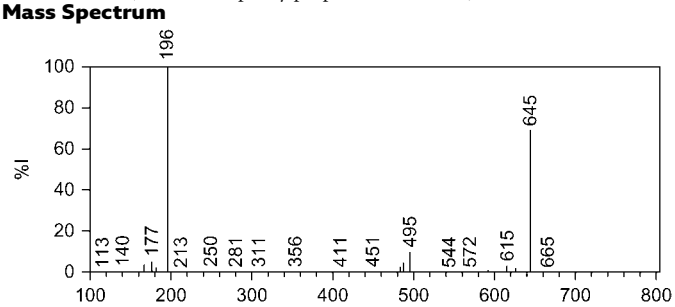
Nandrolone Phenylpropionate

$C_{27}H_{34}O_3 = 406.6$
CAS—62-90-8
Synonyms Nandrolone phenpropionate; 19-norandrostenolone phenylpropionate; nortestosteronum phenylpropionicum.
Proprietary Names *Activin*; *Androlone*; *Durabolin*; *Nandrolin*; *Noralone*; *Stenabolin*; *Superanabolon*.
Chemical Properties A white crystalline powder. Mp 95° to 96°. Practically insoluble in water; soluble 1 in 20 of ethanol; soluble in chloroform.

Thin-layer Chromatography system TP—decanoate R_f 0.88, phenylpropionate R_f 0.87; system TQ—decanoate R_f 0.49, phenylpropionate R_f 0.48; system TR—decanoate R_f 0.97, phenylpropionate R_f 0.97; system TS—decanoate R_f 0.95, phenylpropionate R_f 0.95.
Gas Chromatography System GA—RI 2395, nandrolone-TMS RI 2760; system GAR—RT 12.5 min.
Ultraviolet Spectrum Dehydrated alcohol—240 nm ($A_1^1 = 407$a) (nandrolone decanoate), 240 nm ($A_1^1 = 430$a) (nandrolone phenylpropionate).



Infrared Spectrum Principal peaks at wavenumbers 1679, 1733, 1205, 1178, 1255, 692 cm^{-1} (nandrolone phenylpropionate, KBr disk).
Mass Spectrum



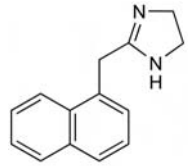
Quantification
***Hair* GC-MS** Column: CP SIL 8 CB (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 2 min to 310° at 15°/min for 2 min. MSD, positive ion mode [Gaillard *et al.* 2000]. Column: CP SIL 8 CB (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.1 mL/min. Temperature programme: 80° for 2 min to 310° at 15°/min for 14 min. Retention time: 17.3 min. Limit of detection, 0.08 pg/mg [Gaillard *et al.* 1999]. Column: HP Ultra 1 cross-linked methylsiloxane (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 10 psi. Temperature programme: 180° for 0.1 min to 320° at 20°/min for 5.4 min. Limit of detection, 50 ng/g [Höld *et al.* 1999]. Column: HP5-MS capillary 5% phenylsiloxane 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 295° at 30°/min for 6 min. EI ionisation. Retention time: 7.43 min. Limit of detection, 3 pg/mg [Kintz *et al.* 1999]. For a review of testing for anabolic steroids in hair, see Kintz [2003].
Disposition in the Body In humans, nandrolone is excreted in the urine mainly as the 2 metabolites 19-norandrosterone and 19-noretiocholanolone. In the horse, the major metabolites are 5α-estrane-3β,17α-diol, excreted mainly as the glucuronide conjugate, and its 17 β-epimer, which is excreted as the sulfate conjugate.
Dose By IM injection: 25 to 100 mg of nandrolone decanoate every 3 weeks; 25 to 50 mg of nandrolone phenylpropionate weekly.

Gaillard Y *et al.* (1999). Gas chromatographic-tandem mass spectrometric determination of anabolic steroids and their esters in hair: application in doping control and meat quality control. *J Chromatogr B Biomed Sci Appl* 735: 189–205.
Gaillard Y *et al.* (2000). Compared interest between hair analysis and urinalysis in doping controls: results for amphetamines, corticosteroids and anabolic steroids in racing cyclists. *Forensic Sci Int* 107: 361–379.
Höld KM *et al.* (1999). Detection of nandrolone, testosterone, and their esters in rat and human hair samples. *J Anal Toxicol* 23: 416–423.
Kintz P (2003). Testing for anabolic steroids in hair: a review. *Leg Med (Tokyo)* 5(Suppl1): S29–S33.
Kintz P *et al.* (1999). Testing for anabolic steroids in hair from two bodybuilders. *Forensic Sci Int* 101: 209–216.

Naphazoline

Sympathomimetic
$C_{14}H_{14}N_2 = 210.3$
CAS—835-31-4

IUPAC Name 2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole
Synonym 4,5-Dihydro-2-(1-naphthalenylmethyl)-1*H*-imidazole



Chemical Properties Mp about 120°. pK_a 10.9 (20°). Log *P* (chloroform/pH 7.4), −0.3.

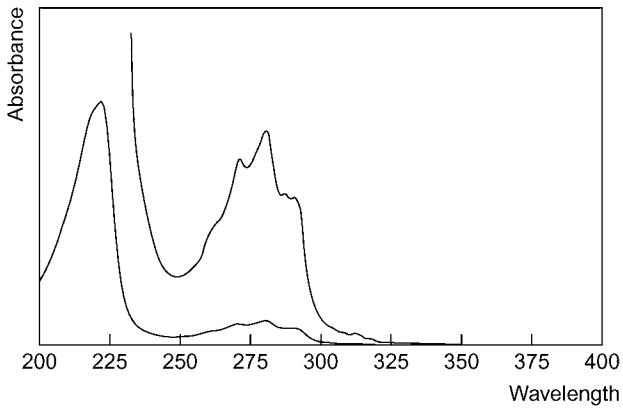
Naphazoline Hydrochloride

$C_{14}H_{14}N_2,HCl = 246.7$
CAS—550-99-2
Proprietary Names *Aconex*; *Ak-Con*; *Albalon*; *All Clear*; *Allerest*; *Allersol*; *Clear Eyes*; *Clera*; *Coldan*; *Degest*; *Isoftal*; *Murine*; *Nafazair*; *Napha Forte*; *Naphcon*; *Optazine*; *Privine*; *Rhino-Mex-N*; *Rhinon*; *Rhinoperd*; *Vasocon*.
Chemical Properties A white crystalline powder. Mp about 255° to 260°. Soluble 1 in 6 of water and 1 in 15 of ethanol; slightly soluble in chloroform; insoluble in benzene and ether.

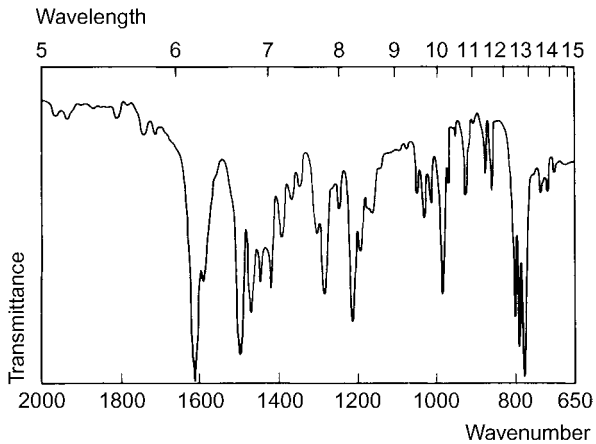
Naphazoline Nitrate

$C_{14}H_{14}N_2,HNO_3 = 273.3$
CAS—5144-52-5
Proprietary Names *Deltarhinol-Mono*; *Imidazyl*; *Privin*; *Rinazina*. It is an ingredient of *Antistin-Privine*.
Chemical Properties A white crystalline powder. Mp about 168°. Soluble 1 in 36 of water and 1 in 16 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

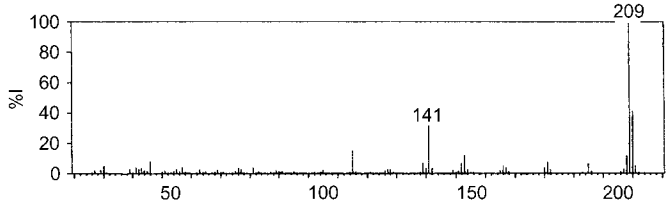
Colour Tests Mandelin's test—brown-violet; Marquis test—grey-green.
Thin-layer Chromatography System TA—R_f 0.14; system TB—R_f 0.03; system TC—R_f 0.06; system TE—R_f 0.27; system TAE—R_f 0.03; system TAF—R_f 0.52 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, green).
Gas Chromatography System GA—RI 2100; system GC—RI 2457.
High Performance Liquid Chromatography System HA—*k* 2.4; system HX—RI 320; system HY—RI 263; system HAX—Retention time 9.1 min; system HAY—Retention time 4.8 min.
Ultraviolet Spectrum Aqueous acid—271, 281 (A_1^1=321a), 288, 291 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1615, 780, 1499, 791, 1211, 800 cm^{-1} (KBr disk).



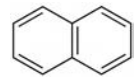
Mass Spectrum Principal ions at *m/z* 209, 210, 141, 115, 153, 208, 46, 181.



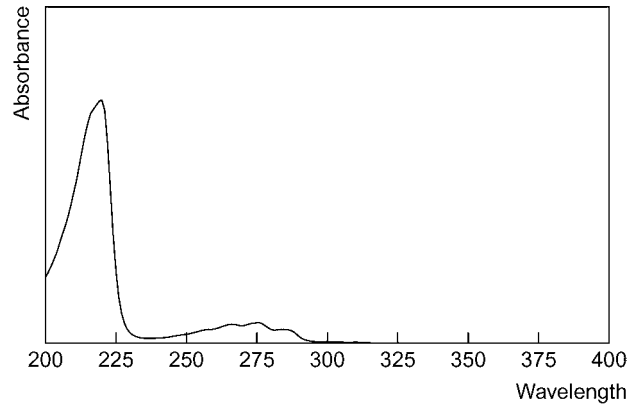
Disposition in the Body Systemic absorption has been reported following topical application of solutions of naphazoline. It is not used systemically but is readily absorbed from the gastrointestinal tract.
Toxicity The estimated minimum lethal dose is 10 mg, intranasally.
Use Naphazoline hydrochloride and nitrate are used as 0.05 to 0.1% aqueous solutions.

Naphthalene

Antiseptic, Anthelmintic
$C_{10}H_8 = 128.2$
CAS—91-20-3
Synonym Naphthalin

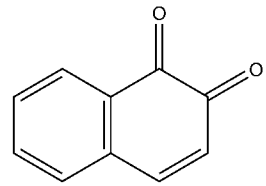


Chemical Properties Colourless transparent scales. Mp 80.2°. Bp 218°. Insoluble in water; soluble 1 in 13 of ethanol, 1 in 3.5 of benzene or toluene, 1 in 1.2 of carbon disulfide and 1 in 2 of chloroform or carbon tetrachloride; very soluble in ether. Log *P* (octanol/water), 3.3.
Gas Chromatography System GA—RI 1186.
High Performance Liquid Chromatography System HY—RI 558; system HAA—retention time 23.3 min.
Ultraviolet Spectrum Ethanol—266 (A_1^1=411b), 275 (A_1^1=454b), 286 nm (A_1^1=307b).



1,2-Naphthoquinone

Haemostatic
$C_{10}H_6O_2 = 158.2$
CAS—542-42-5
Synonyms 1,2-Naphthalenedione; β-naphthoquinone.

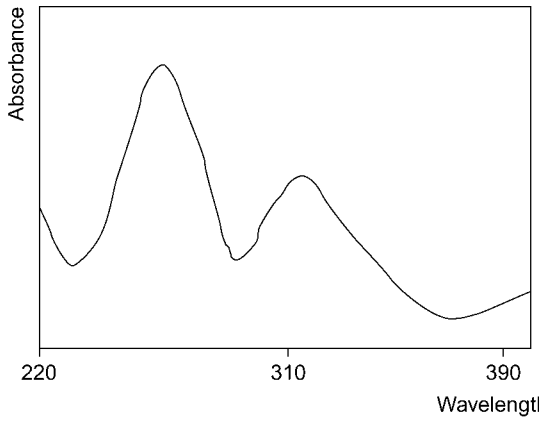


Chemical Properties Golden-yellow needles. Soluble in alcohol, benzene, ether, 5% NaOH, 5% $NaHCO_3$, and concentrated H_2SO_4 with a green colour; practically insoluble in water [O'Neil *et al.* 2006].

1,2-Naphthoquinone 2-Semicarbazone

$C_{10}H_6O_2, CH_3N_3I = 215.2$
CAS—31853-38-0
Synonym SCBN
Proprietary Names *Haemostop injection*; *Karbinon*; *Mediaven*; *Naftazone*.
Chemical Properties An orange-red microcrystalline powder. Mp 182° to 187° with decomposition. 1,2-Napthoquinone 2–semicarbazone is extracted by chloroform from aqueous alkaline solutions.

Colour Tests Ammonium molybdate test—grey-purple (limit of detection, 1.0 µg); sulfuric acid test—dull purple (limit of detection, 1.0 µg); sulfuric acid-formaldehyde test—dull purple (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.63 (located as a visible orange-brown spot; location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 0.32 relative to diphenhydramine.
Ultraviolet Spectrum Methanol—272, 319 nm [Herber *et al.* 1995].



Mass Spectrum Principal ions at *m/z* 369, 394, 315, 370, 333, 395 (glucuronide) [Herber *et al.* 1995]

Disposition in the Body Up to 99.5% of an IV dose is excreted in the urine as sulfo- and glucuroconjugates. The oxo group is reduced first in the presence of NADPH or NADH, followed readily by glucuronidation of the phenolic moiety, leading to a 1β-*O*-glucuronide [Herber *et al.* 1995]

Toxicity IV administration of 2.5 g/kg naftazone to mice did not produce toxic symptoms.

Dose Up to 300 μg daily.

Herber R *et al.* (1995). Reduction and glucuronidation of naftazone by human and rat liver microsomes. *Drug Metab Dispos* 23: 1305–1314.

O'Neil MJ *et al.* (2006). *The Merck Index: an Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co. Inc.

Naproxen

Analgesic

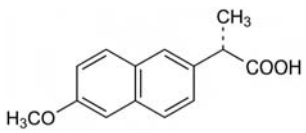
$C_{14}H_{14}O_3 = 230.3$

CAS—22204-53-1

IUPAC Name (2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid

Synonyms (α*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid; naproxenum; RS-3540.

Proprietary Names *Arthrosin*; *Arthroxen*; *Condrotex*; *Laraflex*; *Laser*; *Napratec*; *Naprel*; *Naprex*; *Naprius*; *Naprobene*; *Naprocoat*; *Naprodil*; *Naprometin*; *Napromex*; *Napronet*; *Naproscript*; *Naproaian*; *Naprosyn(e)*; *Naproval*; *Naproxi*; *Napxen*; *Nycopren*; *Proxen*; *Rimoxyn*; *Timpron*; *Xenar*.



Chemical Properties A white crystalline powder. Mp 152° to 154° (crystals from acetone-hexane). Practically insoluble in water; soluble 1 in 25 of ethanol, 1 in 15 of chloroform, and 1 in 40 of ether. pK_a 4.2 (25°). Log *P* (octanol/water), 3.2.

Naproxen Sodium

$C_{14}H_{13}NaO_3 = 252.2$

CAS—26159-34-2

Synonym RS-3650

Proprietary Names *Aleve*; *Anaprox*; *Naprelan*; *Naprodil*; *Naprogesic*; *Naprorex*; *Naproso*; *Naprosyn*; *Naprovite*; *Synflex*.

Chemical Properties Mp 244° to 246° (crystals from acetone).

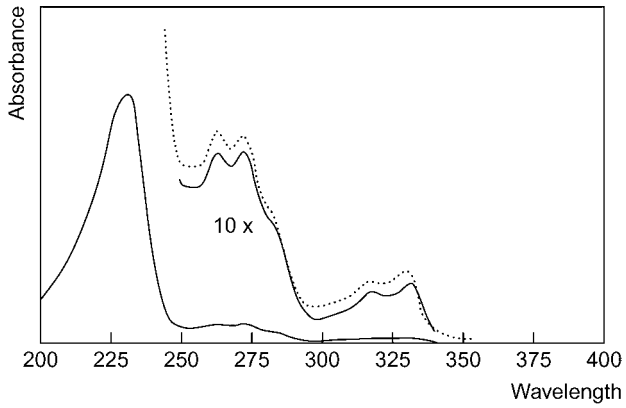
Colour Tests Liebermann's reagent—black-green; Marquis test—brown; sulfuric acid—orange.

Thin-layer Chromatography System TD—R_f 0.33; system TE—R_f 0.06; system TF—R_f 0.38; system TG—R_f 0.14; system TAD—R_f 0.44; system TAE—R_f 0.82; system TAJ—R_f 0.60; system TAK—R_f 0.75; system TAL—R_f 0.93 (Ludy Tenger reagent, orange).

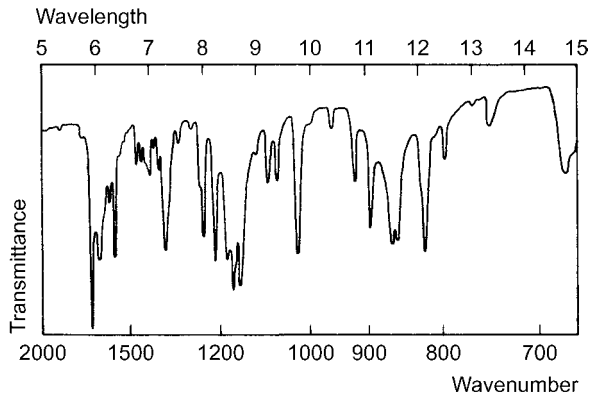
Gas Chromatography System GA—naproxen RI 2045, naproxen-Me RI 1980, M (O-desmethyl-)-Me_2 RI 1980, M (OH-)-Me_2 RI 1800; system GB—naproxen RI 2337, M (O-desmethyl-) RI 2396; system GD—naproxen-Me RRT 1.37 and RRT 1.18 (relative to *n*-$C_{16}H_{34}$); system GL—naproxen-Me RI 2120,M (O-desmethyl-)-Me_2 RI 2120, M (OH-)-Me_2 RI 1800.

High Performance Liquid Chromatography System HD—*k* 3.3; system HF—RRT 0.67 (relative to meclofenamic acid); system HX—RI 501; system HY—RI 468; system HZ—RT 6.8 min; system HAX—RT 7.0 min; system HAY—RT 7.2 min.

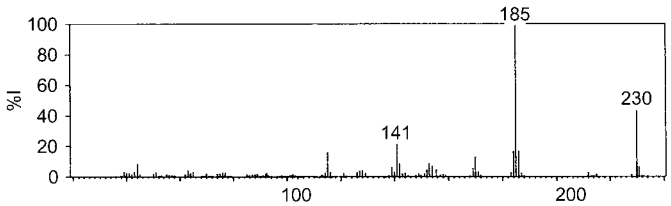
Ultraviolet Spectrum Aqueous acid—262 (A^1_1=208b), 272 (A^1_1=215b), 315 (A^1_1=52b), 328 nm (A^1_1=63b); aqueous alkali—261 (A^1_1=218b), 271 (A^1_1=218b), 316, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1724, 1174, 1155, 1223, 1190, 1681 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 185, 230, 141, 186, 184, 115, 170, 153.



Quantification

Plasma **HPLC** For method of quantification for naproxen and 6-*O*-desmethylnaproxen and conjugates, see Andersen and Hansen [1992]. For method of quantification for naproxen, ibuprofen and diclofenac, see Blagbrough *et al.* [1992]. Limit of quantification, 1.5 mg/L for naproxen and 6-*O*-desmethylnaproxen and conjugates [Vree *et al.* 1992]. Fluorescence detection. Limit of detection, 100 μg/L for naproxen and 6-*O*-desmethylnaproxen [van Loenhout *et al.* 1982].

Serum **Spectrofluorimetry** Limit of detection, 500 μg/L [Anttila 1977].

UV spectrophotometry See Holzbecher *et al.* [1979].

GC FID. Limit of detection, 2 mg/L [Weber *et al.* 1981].

Urine **GC** FID. For method of quantification for naproxen and metabolites, see Wan and Matin [1979].

HPLC UV detection. Limit of detection, 3 ng for naproxen naproxen and nabumetone and its metabolite [Mikami *et al.* 2000]. UV detection. Limit of detection, 5 μg/L for naproxen and other NSAIDs [Hirai *et al.* 1997]. See Plasma [Andersen, Hansen 1992]. See Plasma [Vree *et al.* 1992]. See Plasma [van Loenhout *et al.* 1982].

Synovial Fluid **HPLC** For method of quantification for naproxen, ibuprofen and diclofenac, see Blagbrough *et al.* [1992].

Disposition in the Body Readily and almost completely absorbed after oral or rectal administration. Naproxen crosses the placenta and small amounts are found in breast milk; it also diffuses into synovial fluid. About 50% of a dose is excreted in the urine in 24 h and ~94% in 5 days together with ~1–2% in the faeces. Of the material excreted in the urine, ~60% is conjugated naproxen, 5% is 6-*O*-desmethylnaproxen, and 20% is conjugated desmethylnaproxen. <10% of the excreted material is unchanged drug.

Hydrolysis of conjugated naproxen has been reported during storage of urine samples, and assays carried out immediately after collection suggested that only very small amounts of unchanged drug were excreted [Upton *et al.* 1980.]

Therapeutic Concentration Plasma concentrations are dose-dependent at doses of up to 500 mg twice daily; at larger doses the increase in concentration is non-linear and renal clearance is accelerated.

Following single oral doses of 250 mg to 6 subjects, peak plasma concentrations of 26–51 mg/L (mean, 38) were attained in 2–4 h; peak plasma concentrations of 49–69 mg/L (mean, 60) were attained in 3 subjects, 2 h after a single oral dose of 500 mg; peak concentrations of the desmethyl metabolite were about 0.1–0.2 mg/L [Tomson *et al.* 1981.]

Toxicity

A serum concentration of 414 mg/L was reported in 1 subject 15 h after the ingestion of 25 g of naproxen; the only toxic effect was mild gastrointestinal distress [Fredell, Strand 1977].

A 15-year-old girl who had rapidly developed severe metabolic acidosis and seizures after ingesting ~50 × 275 mg naproxen sodium tablets (each containing 250 mg naproxen and 25 mg sodium) had a serum naproxen concentration of 12.9 g/L 14.5 h after the ingestion; serum bicarbonate levels returned to normal after 12.5 h [Martinez *et al.* 1989.]

Half-life Plasma half-life, 10–20 h (mean, 14).

Volume of Distribution About 0.16 L/kg.

Clearance Plasma clearance, 0.13 mL/min/kg.

Protein Binding >99% at normal therapeutic concentrations; decreased at higher plasma concentrations and in subjects with liver disease.

Note For a review of naproxen, see Brogden *et al.* [1979] and for a review of its pharmacokinetics, see Davies and Anderson [1997].

Dose Usually 0.5 to 1 g daily.

Andersen JV, Hansen SH (1992). Simultaneous quantitative determination of naproxen, its metabolite 6-O-desmethylnaproxen and their five conjugates in plasma and urine samples by high-performance liquid chromatography on dynamically modified silica. *J Chromatogr* 577: 325–333.

Anttila M (1977). Fluorometric determination of naproxen in serum. *J Pharm Sci* 66: 433–434.

Blagbrough IS *et al.* (1992). High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man. *J Chromatogr* 578: 251–257.

Brogden RN *et al.* (1979). Naproxen up to date: a review of its pharmacological properties and therapeutic efficacy and use in rheumatic diseases and pain states. *Drugs* 18: 241–277.

Davies NM, Anderson KE (1997). Clinical pharmacokinetics of naproxen. *Clin Pharmacokinet* 32: 268–293.

Fredell EW, Strand LJ (1977). Naproxen overdose. *JAMA* 238: 938.

Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692: 375–388.

Holzbecher M *et al.* (1979). An ultraviolet spectrophotometric procedure for the routine determination of naproxen. *Clin Biochem* 12: 66–67.

Martinez R *et al.* (1989). Severe metabolic acidosis after acute naproxen sodium ingestion. *Ann Emerg Med* 18: 1102–1104.

Mikami E *et al.* (2000). Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography. *J Pharm Biomed Anal* 23(5): 917–925.

Tomson G *et al.* (1981). Relation of naproxen kinetics to effect on platelet prostaglandin release in men and dysmenorrheic women. *Clin Pharmacol Ther* 29: 168–173.

Upton RA *et al.* (1980). Negligible excretion of unchanged ketoprofen, naproxen, and probenecid in urine. *J Pharm Sci* 69: 1254–1257.

van Loenhout JWA *et al.* (1982). *J Liq Chromatogr* 5: 549–561.

Vree TB *et al.* (1992). Determination of naproxen and its metabolite O-desmethylnaproxen with their acyl glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatography. *J Chromatogr* 578: 239–249.

Wan SH, Matin SB (1979). Quantitative gas-liquid chromatographic analysis of naproxen, 6-O-desmethyl-naproxen and their conjugates in urine. *J Chromatogr* 170: 473–478.

Weber SS *et al.* (1981). Effect of Mylanta on naproxen bioavailability. *Ther Drug Monit* 3: 75–83.

Naratriptan

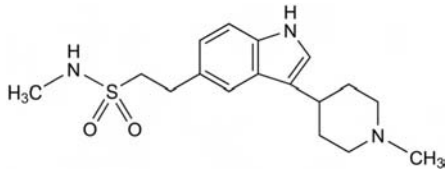
Antimigraine

$C_{17}H_{25}N_3O_2S = 335.4$

CAS—121679-13-8

IUPAC Name *N*-Methyl-3-(1-methyl-4-piperidinyl)-1*H*-indole-5-ethanesulfonamide

Synonym GR-85548X



Chemical Properties Crystals from ethyl acetate. Mp 170° to 171°.

Naratriptan Hydrochloride

$C_{17}H_{25}N_3O_2S,HCl = 371.9$

CAS—121679-19-4; 143388-64-1

Synonym GR-85548A

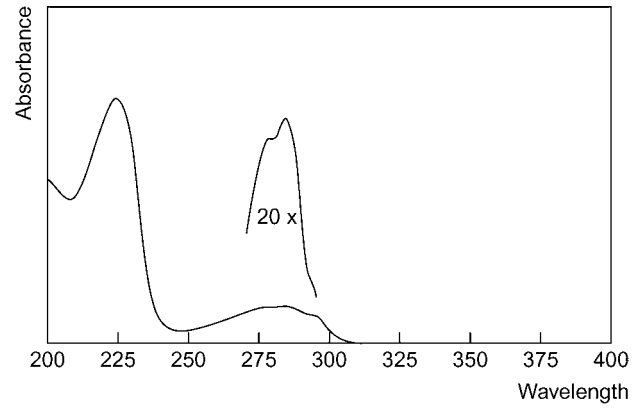
Proprietary Names *Amerge*; *Naramig*.

Chemical Properties Microcrystals. Mp 237° to 239°.

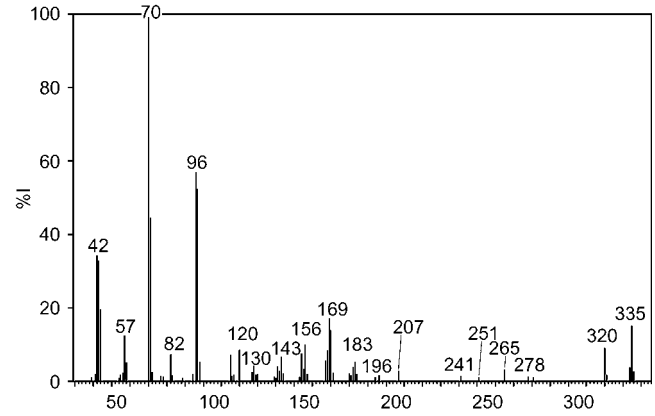
High Performance Liquid Chromatography System HZ—retention time 2.1 min.

Column: silica Alltech (150 × 2.1 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 4 to pH 2.7) : methanol : acetonitrile (80 : 10 : 10), flow rate 300 µL/min. IS: bufotemine. MS–MS detection (ESI, MRM with *m/z*: 336 to 98 for naratriptan and 205 to 160 for IS). Retention time: naratriptan, 2.71 min; IS, 2.35 min [Vishwanathan *et al.* 2000].

Ultraviolet Spectrum Aqueous acid (pH 2.38)—224, 278, 284, 295 nm; ethanol—285, 296 nm.



Mass Spectrum Principal ions at *m/z* 70, 96, 97, 71, 42, 43, 44, 169.



Quantification

Serum **HPLC–MS** MS–MS detection (ESI). Limit of detection, 0.1 µg/L [Vishwanathan *et al.* 2000].

Disposition in the Body Naratriptan is rapidly and well absorbed after administration, and undergoes some hepatic metabolism by a wide range of cytochrome P450 isoenzymes. Renal excretion is the main route of elimination and the drug is predominately excreted in urine, with 50% of the dose being recovered as the unchanged drug and 30% as the inactive metabolite. It displays linear pharmacokinetics over the therapeutic range and repeat administration does not lead to accumulation of the drug. Food does not alter the absorption.

Therapeutic Concentration

Seven adolescent migraine patients, aged between 12 and 16 years, were administered a single dose of 2.5 mg naratriptan outside a migraine attack. The mean naratriptan plasma concentration was 8.0 µg/L (range, 5.9 to 10.7) observed between 1.5 and 4 h after dosing [Christensen *et al.* 2001].

Bioavailability Men, 63%; women, 74%.

Half-life Approximately 4.5 to 5.5 h; prolonged in patients with renal and hepatic impairment.

Volume of Distribution 170 L.

Clearance Systemic, 6.6 mL/min/kg; reduced by 26% in the elderly and by 50% in renal impaired individuals.

Protein Binding 28 to 31% (concentration range, 50 to 1000 µg/L).

Note For a review of naratriptan use in migraine treatment, see Dulli [1999].

Dose Oral administration: initial dose is 2.5 mg in the UK; 1 to 2.5 mg in the US and a second dose if required. The maximum dose is 5 mg daily.

Christensen ML *et al.* (2001). Pharmacokinetics of naratriptan in adolescent subjects with a history of migraine. *J Clin Pharmacol* 41(2): 170–175.

Dulli DA (1999). Naratriptan: an alternative for migraine. *Ann Pharmacother* 33: 704–711.

Vishwanathan K *et al.* (2000). Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14(3): 168–172.

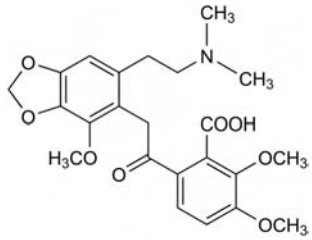
Narceine

Alkaloid

$C_{23}H_{27}NO_8 = 445.5$

CAS—131-28-2 (anhydrous)

IUPAC Name 6-[[6-[2-(Dimethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]-acetyl]-2,3-dimethoxybenzoic acid



Chemical Properties An alkaloid present in opium. White silky crystals. Mp 176°. The anhydrous substance melts at about 138° and is very hygroscopic. Slightly soluble in water; soluble in ethanol; soluble in alkali hydroxide solutions and dilute mineral acids; practically insoluble in benzene, chloroform, ether and petroleum ether. pK_a 3.8 (20°). Log *P* (octanol/water), 2.0.

Narceine Hydrochloride

$C_{23}H_{27}NO_8$, HCl = 481.9
CAS—4901-03-5

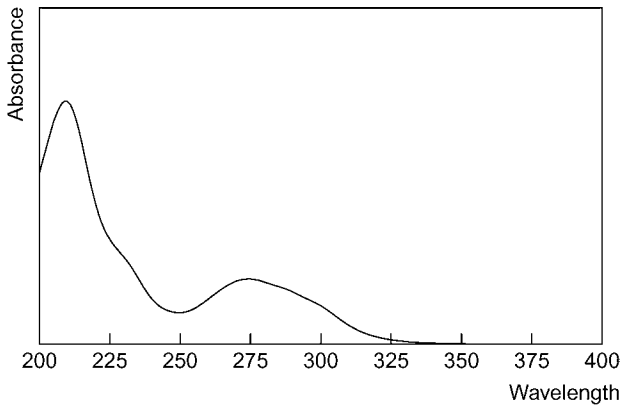
Chemical Properties Prismatic crystals. Mp 192° to 193°. Slightly soluble in hot water and ether; soluble in hot ethanol.

Colour Tests Liebermann's reagent—black; Mandelin's test—green-brown; Marquis test—brown→green.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.00; system TC—R_f 0.03; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.34; system TAF—R_f 0.14; system TAJ—R_f 0.02; system TAK—R_f 0.01; system TAL—R_f 0.36 (acidified iodoplatinate solution, positive).

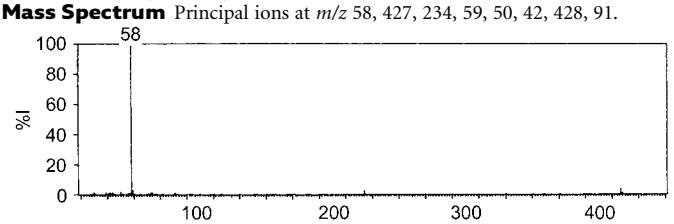
High Performance Liquid Chromatography System HA—*k* 0.7; system HX—RI 360.

Ultraviolet Spectrum Aqueous acid—277 nm (A_1^1=306a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1253, 1582, 1060, 1090, 1000, 1047 cm^{-1} (KCl disk).

Mass Spectrum Principal ions at *m/z* 58, 427, 234, 59, 50, 42, 428, 91.



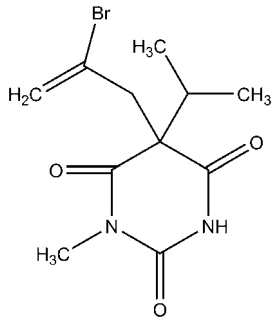
Narcobarbital

Barbiturate
$C_{11}H_{15}BrN_2O_3$ = 303.2

IUPAC Name 5-(2-Bromoprop-2-enyl)-1-methyl-5-propan-2-yl-1,3-diazinane-2,4,6-trione

Synonyms 5-(2-Bromoallyl)-5-isopropyl-1-methyl-barbituric acid; enibomal.

Proprietary Names *Eunarcon*; *Narcodorm*; *Narkotal*; *Venopan*.



Chemical Properties White powder. Slightly soluble in water; soluble 1 in 20 of ethanol; soluble in ether and chloroform. Narcobarbital may be isolated in the A2 fraction.

Disposition in the Body Narcobarbital is a very short-acting barbiturate.

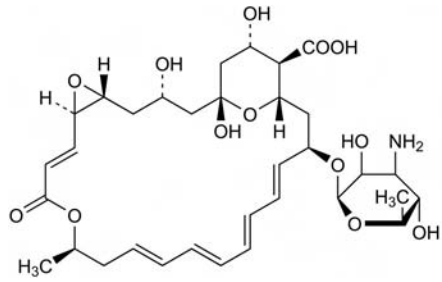
Dose Up to 1 g IV.

Natamycin

Antifungal
$C_{33}H_{47}NO_{13}$ = 665.7
CAS—7681-93-8

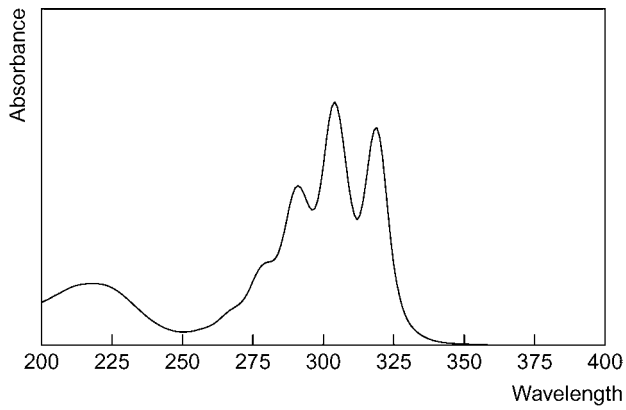
Synonyms Antibiotic A 5283; CL-12625; pimaricin; tennecetin.

Proprietary Names *Deronga Heilpaste*; *Natacyn*; *Natafucin*; *Pima Biciron N*; *Pimafucin(e)*.



Chemical Properties An antibiotic produced by the growth of *Streptomyces natalensis*. A white crystalline powder, which may discolour slightly on storage. Very slightly soluble in water and ethanol; practically insoluble in chloroform and ether; readily soluble in dilute acids and alkalis, forming salts. Log *P* (octanol/water), −3.7.

Ultraviolet Spectrum Acid methanol—290 (A_1^1=795b), 303 (A_1^1= 1250b), 318 nm (A_1^1=1145b).



Infrared Spectrum Principal peaks at wavenumbers 1062, 1002, 1563, 1709, 1101, 1258 cm^{-1} (KBr disk).

Uses Topically in concentrations of 2 to 5%; by inhalation in a dose of 7.5 mg daily.

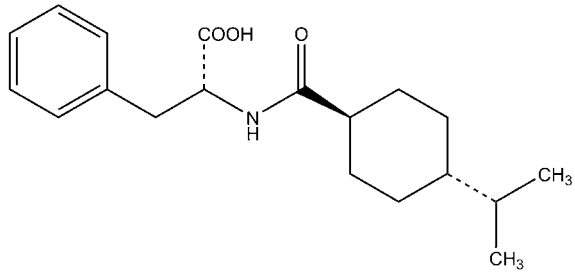
Nateglinide

Antidiabetic, Meglitinide
$C_{19}H_{27}NO_3$ = 317.4
CAS—105816-04-4

IUPAC Name (2*R*)-3-Phenyl-2-[(4-propan-2-ylcyclohexanecarbonyl)amino] propanoic acid

Synonyms A-4166; AY-4166; (−)-*N*-(*trans*-4-isopropylcyclohexyl-1-carbonyl)-D-phenylalanine; *N*-[[*trans*-4-(1-methylethyl)cyclohexyl]carbonyl]-D-phenylalanine; 3-phenyl-2-(4-propan-2-ylcyclohexyl)carbonylamino-propanoic acid; SDZ-DJN-608; YM-026.

Proprietary Names *Fastic*; *Starlix*; *Starsis*.



Chemical Properties White powder. Mp 129° to 130°. Freely soluble in methanol, ethanol and chloroform; soluble in ether; sparingly soluble in acetonitrile and octanol; practically insoluble in water. Log *P* (octanol/water), 3.82 [Wishart 2006].

Quantification

Plasma **HPLC** Column: C_{18} (250 × 3 mm i.d., 5 µm). Mobile phase: 0.1 mol/L potassium hydrogen phosphate (pH 4.0) : methanol : acetonitrile (700 : 80 : 300), flow rate 1.0 mL/min. Internal standard: carbamazepine. UV detection (λ = 210 nm). Retention times: nateglinide, 14.1 (internal standard, 6.7 min). Limit of quantification, 0.1 mg/L, limit of detection, 1 µg/L [Bauer *et al.* 2003]. Column: ODS-2 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L ammonium acetate (pH 6.0) : acetonitrile (100 : 0 for 5 min to 80 : 20 at 10 min for 15 min to 65 : 35 at 35 min for 5 min to 55 : 45 at 55 min for 5 min to 0 : 100 at 70 min), flow rate 1.0 mL/min; UV detection (λ = 210 nm). Limit of quantification, 50 µg/L [Weaver *et al.* 2001].

Other **LC-MS** Rabbit Serum. Column: Zorbax Extend C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: water: formic acid (80 : 20 : 1), flow rate 0.8 mL/min. Positive ion mode, SRM acquisition mode. Limit of quantification, 0.25 mg/L [He *et al.* 2004].

Disposition in the Body Nateglinide is rapidly absorbed after oral administration, taken prior to a meal, with peak plasma concentrations reached within 1 h. Administration of the drug with food results in delayed absorption and a decrease in the peak plasma concentration. It is metabolised to 9 known metabolites by hydroxylation and glucuronide conjugation, via CYP2C9 and CYP3A4. The major metabolites have less antidiabetic activity than nateglinide, but the isoprene minor metabolite has antidiabetic activity comparable to that of nateglinide. The drug and metabolites are rapidly eliminated within 6 h of dosing. Approximately 75% of the drug is recovered in urine, with 16% as the parent drug, and a small amount in faeces.

Therapeutic Concentration

Twenty male and female patients suffering from type 1 or type 2 diabetes (aged 21 to 70 years) and 20 healthy volunteers were administered a single 120 mg nateglinide dose after a 10 h fast. Peak plasma concentrations for the healthy volunteers reached 9 to 10 mg/L after 0.78 h and for the diabetic patients, 4.7 mg/L (on dialysis) and 7.39 mg/L (non-dialysis), attained after 0.95 and 0.8 h, respectively [Devineni *et al.* 2003].

Six healthy male subjects (mean age 30.5 years) were administered a radiolabelled nateglinide solution either as an oral dose of 120 mg or a 60 mg IV dose infused over 10 min. After a 3-week washout period, subjects received the radiolabelled dose by the alternative route of administration. The mean peak plasma concentration (5.69 mg/L) was reached 1 h after oral dosing [Weaver *et al.* 2001].

Eight healthy male subjects and eight patients suffering from cirrhosis (43 to 65 years old) were administered a single oral dose of 120 mg nateglinide. Mean peak plasma concentrations of 5.62 and 7.70 mg/L were reached 0.72 and 0.59 h after dosing, respectively [Choudhury *et al.* 2000].

Bioavailability Approximately 73%.

Half-life 1.5 h.

Volume of Distribution Approximately 10 L.

Clearance In healthy individuals, 7.29 L/h; diabetic patients, 7.38 L/h.

Distribution in Blood Blood : plasma ratio, 1 : 2.

Protein Binding Approximately 98%.

Dose 120 mg three times a day.

Bauer S *et al.* (2003). Rapid and simple method for the analysis of nateglinide in human plasma using HPLC analysis with UV detection. *J Pharm Biomed Anal* 31: 551–555.

Choudhury S *et al.* (2000). Single-dose pharmacokinetics of nateglinide in subjects with hepatic cirrhosis. *J Clin Pharmacol* 40: 634–640.

Devineni D *et al.* (2003). Pharmacokinetics of nateglinide in renally impaired diabetic patients. *J Clin Pharmacol* 43: 163–170.

He Z *et al.* (2004). Study on the bioavailability of nateglinide–hydroxypropyl-beta-cyclodextrin complex capsule in rabbits by liquid chromatographic–tandem mass spectrometry. *Biomed Chromatogr* 18: 532–537.

Weaver ML *et al.* (2001). Pharmacokinetics and metabolism of nateglinide in humans. *Drug Metab Dispos* 29: 415–421.

Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Nateplase

Thrombolytic

CAS—159445-63-3

IUPAC Name *N*-[N^2-(*N*-glycyl-L-alanyl)-L-arginyl]plasminogen activator (human tissue-type 1-chain form, protein moiety), glycoform β (major component) and plasminogen activator (human tissue-type 1-chain form, protein moiety), glycoform β mixture

Proprietary Name *Milyser*

Dose Used in acute myocardial infarction.

Nealbarbital

Barbiturate, Sedative

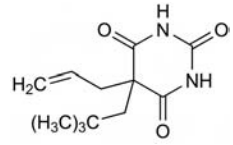
$C_{12}H_{18}N_2O_3$ = 238.3

CAS—561-83-1

IUPAC Name 5-(2-Methylbutan-2-yl)-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

Synonyms Alneobarbital; 5-(2,2-dimethylpropyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; nealbarbitone; neallymalum.

Proprietary Names *Censedal*; *Nevental*.



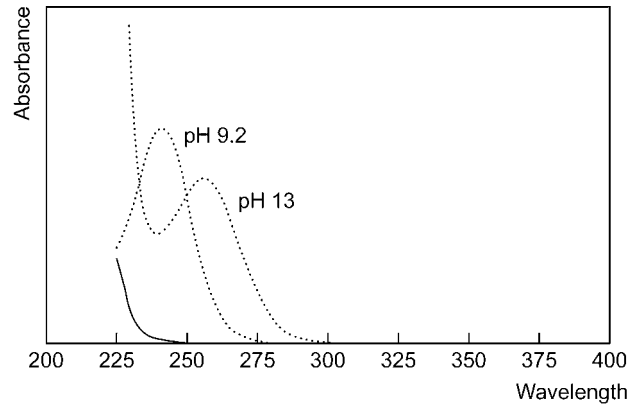
Chemical Properties A white or slightly cream-coloured powder. Mp 155° to 157°. Soluble 1 in 5000 of water, 1 in 4 of ethanol, 1 in 80 of chloroform and 1 in 5 of ether; freely soluble in acetone; soluble in aqueous solutions of alkalis; practically insoluble in petroleum ether. pK_a 7.2 (20°). Log *P* (octanol/water), 2.1.

Thin-layer Chromatography System TD—R_f 0.58; system TE—R_f 0.44; system TF—R_f 0.68; system TH—R_f 0.78; system TAD—R_f 0.60; system TAE—R_f 0.92 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).

Gas Chromatography System GA—nealbarbital RI 1720; nealbarbital-Me_2 RI 1620; system GAJ—RRT 0.789 (relative to methylphenobarbital); system GF—RI 2460.

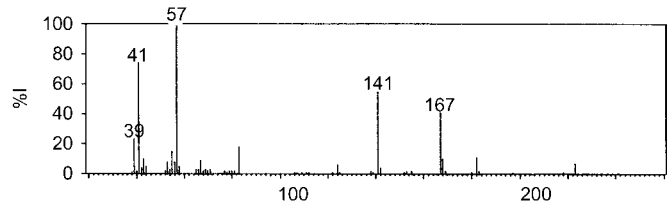
High Performance Liquid Chromatography System HG—*k* 10.22; system HH—*k* 6.19; system HX—RI 417; system HY—RI 382.

Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—240 nm (A^1_1=396b); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=303b).



Infrared Spectrum Principal peaks at wavenumbers 1695, 1752, 1265, 833, 1205, 775 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 57, 41, 141, 167, 39, 83, 55, 182.



Dose Nealbarbital has been given in doses of up to 200 mg as a hypnotic.

Nebivolol

β-*Blocker*

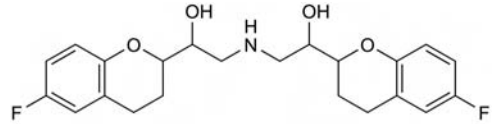
$C_{22}H_{25}F_2NO_4$ = 405.4

CAS—99200-09-6

IUPAC Name 1-(6-Fluoro-3,4-dihydro-2*H*-chromen-2-yl)-2-[[2-(6-fluoro-3,4-dihydro-2*H*-chromen-2-yl)-2-hydroxyethyl]amino]ethanol

Synonyms α,α′-[Iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol]; narbivolol; R-65824; R-67555.

Proprietary Name *Nebilet*



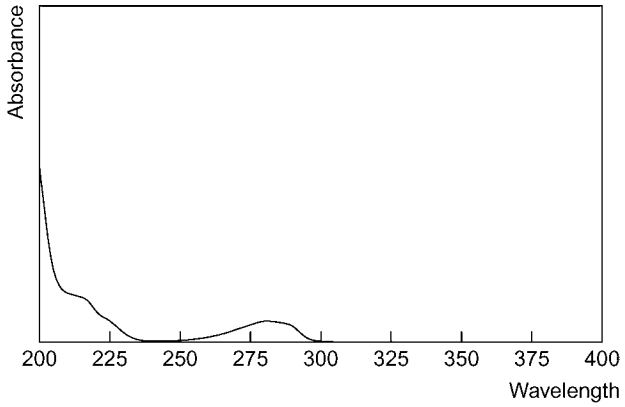
Chemical Properties Crystals. Mp 140.7°. pK_a (base), 8.22. Log *P* (octanol/water), 3.23; 4.03 (pH 11.8, 23°).

Nebivolol Hydrochloride

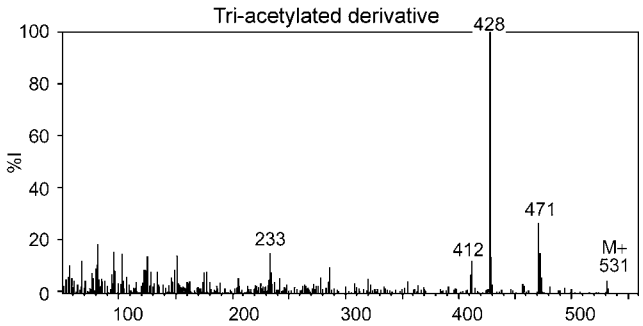
$C_{22}H_{26}ClF_2NO_4$ = 441.9

High Performance Liquid Chromatography Column: Chiralpak AD-RH (150 × 4.6 mm i.d., 5 μm). Mobile phase: 1-propanol, flow rate 0.5 mL/min. UV detection (λ=220 nm). Retention time: (+)-nebivolol, 13.17 min; (−)-nebivolol, 17.45 min [Aboul-Enein, Ali 2001].

Ultraviolet Spectrum Principal peaks at 217, 283 nm.



Mass Spectrum Principal ions at *m/z* 428, 471, 82, 103, 233, 151, 472, 412 (tri-acetylated derivative).



Quantification

Plasma **HPLC** Fluorescence detection. Limit of detection, 0.1 μg/L [Woestenborgh *et al* 1988].

Disposition in the Body The metabolism of nebivolol is complex with formation of numerous metabolites, and varies between individuals; they may be fast or slow metabolisers. Aromatic hydroxylation is the major pathway and also involved are alicyclic hydroxylation and glucuronidation pathways. Urinary and faecal excretion of unconjugated parent drug are <0.5% of a dose.

Therapeutic Concentration Peak plasma levels of 1 μg/L in fast metabolisers and 3 μg/L in slow metabolisers following a 5 mg oral dose.

Bioavailability 12% (fast metabolisers) and 96% (slow metabolisers).

Half-life 8 (fast metabolisers) to 27 (slow metabolisers) h.

Volume of Distribution 10.1 to 39.4 L/kg.

Clearance Total body, 51.6 L/h (healthy); 71.6 L/h (obese individuals).

Protein Binding 98%.

Dose A daily oral dose of 5 mg.

Aboul-Enein HY, Ali I (2001). HPLC enantiomeric resolution of nebivolol on normal and reversed amylose based chiral phases. *Pharmazie* 56: 214–216.

Woestenborgh R *et al.* (1988). In *Methodological Surveys in Biochemistry and Analysis*. US: Plenum Press, vol. 18, pp. 215–21.

Nedocromil

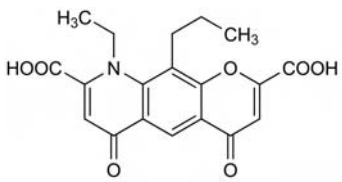
Antiasthmatic

$C_{19}H_{15}NO_7$ = 371.3

CAS—69049-73-6

IUPAC Name 9-Ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4*H*-pyranol[3,2-g] quinoline-2,8-dicarboxylic acid

Synonym FPL-59002



Chemical Properties A yellow powder. Mp 298° to 300° (dec). Log *P* (octanol/water), 2.22.

Nedocromil Calcium

$C_{19}H_{15}CaNO_7$ = 411.5

CAS—101626-68-0

Synonym FPL-59002KC

Nedocromil Sodium

$C_{19}H_{15}NO_7,Na_2$ = 415.3

CAS—69049-74-7

Synonym FPL-59002KP

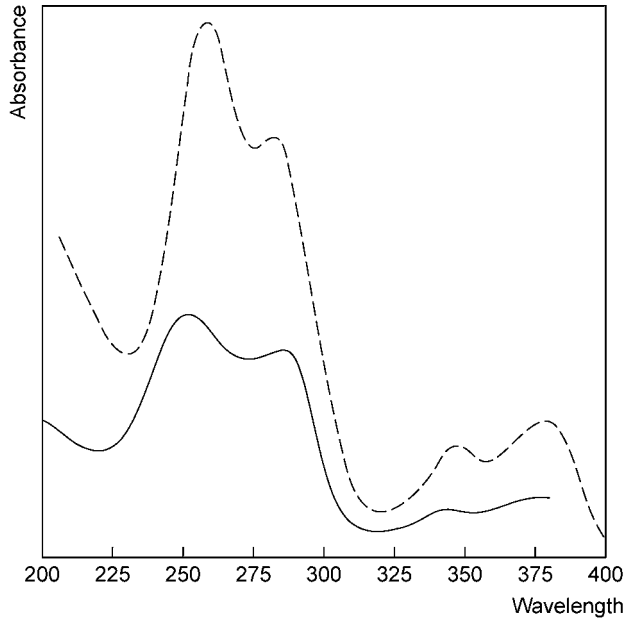
Proprietary Names *Alocril*; *Brionil*; *Cetimil*; *Halamid*; *Ildor*; *Irtan*; *Kovilen*; *Kovinal*; *Tilade*; *Tilarin*; *Tilavist*; *Rapitil*.

Chemical Properties A yellow powder. Soluble in water.

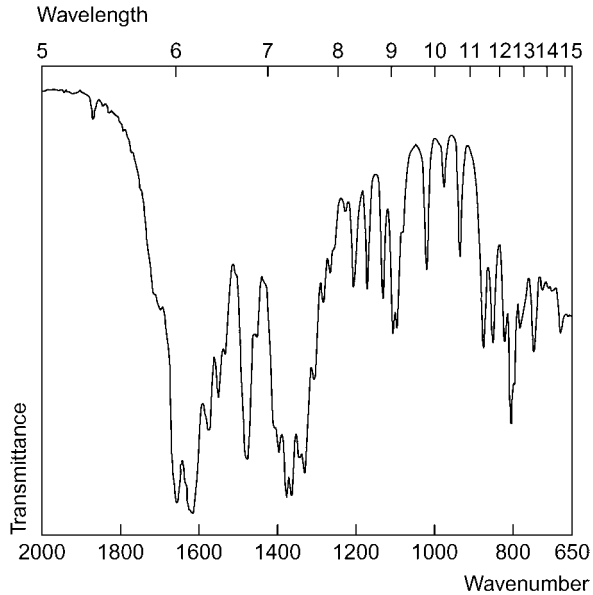
Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

High Performance Liquid Chromatography System HX—RI 318.

Ultraviolet Spectrum Water—255, 286, 345, 378 nm.



Infrared Spectrum



Quantification

Urine **HPLC** Column: C_8 Spherisorb (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 45 mmol/L phosphate buffer : 0.5 mol/L dodecyl triethyl ammonium phosphate (550 : 447.6 : 2.4), (pH 2.3), flow rate 0.85 mL/min. Internal standard: sodium cromoglycate. UV detection (λ=256 nm). *k* value: 13.3 (retention time,

N

43.1 min); IS, *k* 8.5. Limit of quantification, 75 µg/L, limit of detection, 40 µg/L [Aswania *et al.* 1998]. UV detection (λ=253 nm). Limit of quantification, 20 µg/L [Baker *et al.* 1995].

Disposition in the Body A small amount of nedocromil (5% of dose) is absorbed after inhalation and excreted unchanged in urine and bile. Absorption is through the nasolacrimal duct rather than through the conjuctiva. It is reversibly bound to plasma proteins and therefore not metabolised. Accumulation does not occur. Approximately 80% of an IV dose is recovered in urine within 90 min. In healthy individuals, <10% of a dose is excreted in urine within 72 h.

Therapeutic Concentration

Healthy volunteers (6 men and 7 women, aged between 22 and 54 years) and those suffering from asthma (4 men and 8 women, 22 to 59 years old) were administered with 4 mg nedocromil sodium (inhaled). The mean peak plasma concentrations were 3.3 µg/L and 2.8 µg/L, respectively, and were reached after ~20 and 40 min, respectively [Neale *et al.* 1987].

Bioavailability 7 to 9% (2 to 3% oral; 5 to 6% respiratory).

Half-life 1.5 to 3.3 h.

Volume of Distribution 0.43 L/kg (after a 6 µg/kg, 30 min IV dose).

Clearance 10.2 ± 1.3 mL/min/kg (after a 6 µg/kg, 30 min IV dose).

Protein Binding About 89%.

Dose The usual dose is 4 mg inhaled four times daily and may be decreased to twice daily.

Aswania OA *et al.* (1998). Validation of a high-performance liquid chromatography assay for urinary nedocromil sodium following oral and inhaled administration. *J Chromatogr Biomed Sci Appl* 718: 290–295.

Baker PR *et al.* (1995). Automated high-performance liquid chromatographic method for the determination of nedocromil sodium in human urine using bimodal column switching. *J Chromatogr B Biomed Appl* 668: 59–65.

Neale MG *et al.* (1987). The pharmacokinetics of nedocromil sodium, a new drug for the treatment of reversible obstructive airways disease, in human volunteers and patients with reversible obstructive airways disease. *Br J Clin Pharmacol* 24: 493–501.

Nefazodone

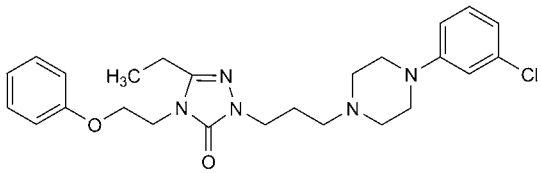
5-HT$_2$ Receptor Antagonist, Antidepressant

$C_{25}H_{32}ClN_5O_2$ = 470.0

CAS—83366-66-9

IUPAC Name 2-[3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl]-5-ethyl-4-(2-phenoxyethyl)-1,2,4-triazol-3-one

Synonym 2-[3-[4-(3-Chlorophenyl)-1-piperazinyl]propyl]-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3*H*-1,2,4-triazol-3-one



Chemical Properties Non-hygroscopic white crystalline solid. Mp 83° to 84°. Freely soluble in chloroform; soluble in propylene glycol; slightly soluble in polyethylene glycol and water. pK_a 6.6. Log *P* (octanol/water), 5.0 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Samples were stable on the laboratory bench and in the refrigerator for 60 days [Sreenivas Rao *et al.* 2001]

Nefazodone Hydrochloride

$C_{25}H_{32}ClN_5O_2$,HCl = 506.5

CAS—82752-99-6

Synonyms BMY-13754; 2-[3-[4-[(3-chlorophenyl)-1-piperazinyl]propyl]-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3*H*-1,2,4-triazol-3-one hydrochloride; MJ-13754-1.

Proprietary Names *Dutonin*; *Serzone*.

Chemical Properties Crystals from ethanol. Mp 175° to 177°.

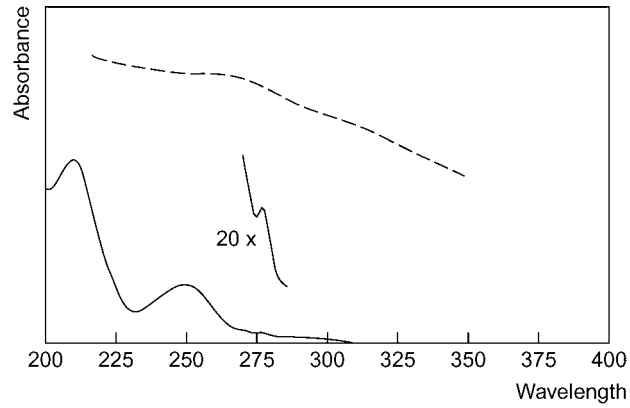
Gas Chromatography System GB—*m*-chlorophenylpiperazine (*m*CPP) RI 1806; system GT—RI 4510.

High Performance Liquid Chromatography System HZ—retention time 7.7 min.

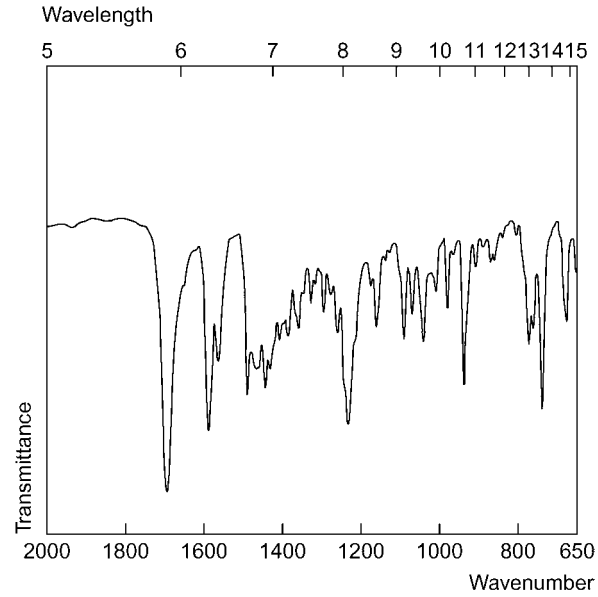
Column: Inertsil, ODS-3V (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L potassium dihydrogen phosphate (pH 3.0) : acetonitrile : methanol (50 : 40 : 10), flow rate 1 mL/min. UV detection (λ = 220 nm). Retention time: 8.5 min [Sreenivas Rao *et al.* 2001].

Liquid Chromatography-Mass Spectrometry Column: Luna Phenylhexyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate (pH 3.0) : acetonitrile (95 : 5 for 5 min to 40 : 60 in 30 min to 10 : 90 in 2 min to 95 : 5 in 1 min), flow rate 1.0 mL/min. ESI. Retention time: 30.44 min. Limit of detection not reported [Li *et al.* 2007].

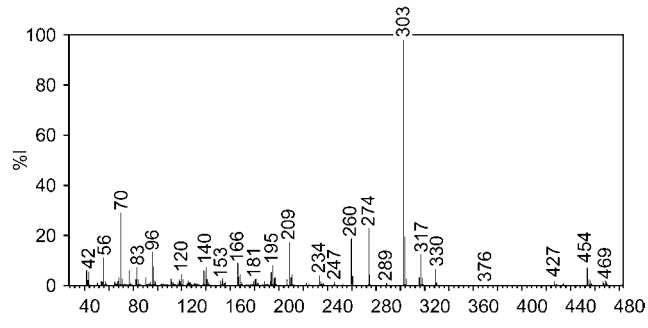
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid)—245, 275 nm.



Infrared spectrum Principal peaks at wavenumbers: 1699, 1594, 1242 cm^{-1} (KBr disk).



Mass spectrum Principal ions at *m*/*z* 303, 274, 260, 69, 454, 209, 318, 469.



Quantification

***Plasma* HPLC** Column: Alphabond C_{18} (150 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 50 mmol/L potassium dihydrogen phosphate (40 : 60), flow rate 1.0 mL/min. UV detection (λ = 205 nm). Retention time: 16.0 min. Limit of quantification, 200 µg/L [Dodd *et al.* 1999]. Column: Cyanopropyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.04 mol/L potassium phosphate buffer (pH 6.6) : acetonitrile : methanol (600 : 225 : 175), flow rate 1.4 mL/min. Electrochemical detection. Retention time: 3.2, 4.0 and 5.0 for nefazodone, hydroxynefazodone and *m*CPP, respectively. Limit of quantification, 0.9, 0.6 and 0.2 µg/L for nefazodone, hydroxynefazodone and *m*CPP, respectively [Franklin 1993]. Column: Zorbax phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.01 mol/L tetramethylammonium hydroxide with 0.01 mol/L dibasic ammonium phosphate (45 : 10 : 45), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of quantification, nefazodone and hydroxynefazodone 10 µg/L, *p*-hydroxynefazodone 20 µg/L, and *m*CPP 2.5 µg/L. Limit of detection, nefazodone and hydroxynefazodone 5 µg/L, *p*-hydroxynefazodone 10 µg/L, and *m*CPP 1 µg/L [Franc *et al.* 1991].

LC-MS Column: Luna C_8 (50 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium formate : 0.05% trifluoroacetic acid : acetonitrile-methanol (50 : 50, 47 : 0.05 : 53), flow rate 1.35 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 2 µg/L [Mao *et al.* 2007]. Column: Hypersil, C_{18} BDS (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 4; 45 : 55), flow rate 0.3 mL/min. I.S.: trazodone. APCI, positive ion mode, SIM acquisition mode (*m/z*: 470.4 [nefazodone], 486.2 [hydroxynefazodone], 458.1 [triazoledione], 197.0 [*m*CPP], and 372.0 [I.S.]). Retention time: nefazodone 4.47 min, hydroxynefazodone 2.80 min, triazoledione 2.34 min, *m*CPP 1.48 min, I.S. 1.77 min. Limit of quantification, 2.0 µg/L [Yao, Srinivas 2000]. Column: BDS Hypersil ODS (100 × 2 mm i.d., 3 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 4; 45 : 55), flow rate 0.3 mL/min. API, positive ion mode. Limit of quantification, 2 µg/L [Yao *et al.* 1998].

Urine **GC-MS** Column: HP-1 capillary (12 m × 0.2 mm, i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

Breast Milk **HPLC** See Plasma [Dodd *et al.* 1999].

Disposition in the Body Rapidly and completely absorbed after oral administration, with peak plasma concentration reached within 0.5–2 h. Nefazodone undergoes extensive first-pass hepatic metabolism, with only 20% of a dose reaching the systemic circulation. It is extensively metabolised via *N*-dealkylation and aromatic hydroxylation and it is excreted via urine (55%) and faeces (25%) after additional glucuronidation. Less than 10% of the drug is present unchanged in the urine. Three active metabolites, hydroxynefazodone (the major metabolite), *m*CPP, and desethylhydroxynefazodone, have been identified. Nefazodone is metabolised by CYP3A4 but it is also an inhibitor of the enzyme [Stewart 2002].

Therapeutic Concentration The therapeutic concentration is variable and dose dependent. There is large intersubject variability [Barbhaiya *et al.* 1996a] with exposure at steady state being ~50% higher in elderly females (all extensive metabolisers) [Barbhaiya *et al.* 1996b].

Eighteen healthy males (aged 19 to 35 years) were administered a single oral dose of 50, 100 or 200 mg nefazodone hydrochloride after an overnight fast. Mean peak plasma concentrations of 84, 196 and 392 µg/L were observed for the single oral 50, 100 and 200 mg doses, respectively, attained at 0.9, 1.0 and 1.3 h, respectively, after dosing. A steady-state study was also carried out where a dose of 50, 100 or 200 mg was administered twice daily for 7 days after meals (final dose followed an overnight fast). The steady-state values were 72, 732 and 2047 µg/L for the 50, 100 and 200 mg doses reached 0.7, 0.9 and 1.2 h, respectively, after administration [Kaul *et al.* 1995].

Note For a pharmacokinetic study of a possible interaction of nefazodone with triazolam, see Barbhaiya *et al.* [1995]; with alprazolam, see Greene *et al.* [1995a]; and with lorazepam, see Greene *et al.* [1995b]. Nefazodone does not appear to interact with lithium [Laroudie *et al.* 1999].

Toxicity Instances of overdosage with up to 11 g of the drug have been reported without fatalities. Victims have manifested nausea, vomiting and somnolence.

A 27-year-old woman ingested 30 nefazodone 100 mg tablets. Her serum nefazodone concentration was 5.5 mg/L [Gaffney *et al.* 1998].

A 16-year-old girl took an overdose of 24 100 mg tablets. On admission to hospital, her plasma nefazodone concentration was 7.5 mg/L [Isbister, Hackett 2003].

Nefazodone is safe and well tolerated at oral doses of up to 450 mg three times daily [D'Amico *et al.* 1990]. Clinical overdoses of 3400 and 3600 mg produced no significant clinical/life-threatening symptoms [Fontaine 1992]. An overdose of 16 800 mg nefazodone resulted in no serious sequelae [Catalano *et al.* 1999]. See also Benson *et al.* [2000].However, caution is required when prescribing nefadozone in combination with other drugs that are meabolised by CYP3A4 or to patients with preexisting liver disease [Stewart 2002]. Some serious cases of hepatocellular injury have been described [Carvajal Garcia-Pando *et al.* 2002; Eloubeidi *et al.* 2000]. See also Schwetz [2002] and Lucena *et al.* [2003].

A 27-year-old developed hepatitis following nefazodone treatment [Schrader, Roberts-Thompson 1999].

In Canada in November 2003 the sale of Serzone (nefazodone) was discontinued by the manufacturer because of concerns of hepatotoxicity [Choi 2003].

Note For a case of serotonin syndrome in a 50-year-old man associated with fluoxetine in combination with nefazodone, see Smith and Wenegrat [2000]; in a 21-year-old woman associated with valproic acid and nefazodone, see Brazelton *et al.* [1997]. For a case of a 47-year-old man and a 48-year-old woman experiencing a selective deficit in the ability to perceive motion, see Horton and Trobe [1999].

Bioavailability 15–23% oral, regular release [Barbhaiya *et al.* 1996a; Barbhaiya *et al.* 1996c]; decreases by 18% when administered with food.

Half-life Dose dependent; nefazodone, 2–5 h (at dose of 200 mg twice daily); hydroxynefazodone, 2–4 h; desethylhydroxynefazodone, 18–33 h; *m*CPP, 4–9 h.

Volume of Distribution 0.22–0.87 L/kg.

Protein Binding Nefazodone and hydroxynefazodone, 98.4–99.8%; *m*CPP, 66–74%.

Note For a review of nefazodone, see Davis *et al.* [1997].

Dose Initially 50 to 100 mg twice daily, which may be increased to a maximum of 300 mg twice daily. In the elderly, the maximum dose is 100 to 200 mg twice daily.

Barbhaiya RH *et al.* (1995). Coadministration of nefazodone and benzodiazepines: II. A pharmacokinetic interaction study with triazolam. *J Clin Psychopharmacol* 15: 320–326.

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Barbhaiya RH *et al.* (1996b). A study of the effect of age and gender on the pharmacokinetics of nefazodone after single and multiple doses. *J Clin Psychopharmacol* 16: 19–25.

Barbhaiya RH *et al.* (1996c). Pharmacokinetics, absolute bioavailability, and disposition of [^{14}C] nefazodone in humans. *Drug Metab Dispos* 24: 91–95.

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D'Amico MF *et al.* (1990). Placebo-controlled dose-ranging trial designs in phase II development of nefazodone. *Psychopharmacol Bull* 26: 147–150.

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Franklin M (1993). Determination of nefazodone and its metabolites in plasma by high-performance liquid chromatography with coulometric detection. *J Pharm Biomed Anal* 11: 1109–1113.

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Greene DS *et al.* (1995). Coadministration of nefazodone and benzodiazepines: IV. A pharmacokinetic interaction study with lorazepam. *J Clin Psychopharmacol* 15: 409–416.

Horton JC, Trobe JD (1999). Akinetopsia from nefazodone toxicity. *Am J Ophthalmol* 128: 530–531.

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Schrader GD, Roberts-Thompson IC (1999). Adverse effect of nefazodone: hepatitis. *Med J Aust* 170: 452.

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Smith DL, Wenegrat BG (2000). A case report of serotonin syndrome associated with combined nefazodone and fluoxetine. *J Clin Psychiatry* 61: 146.

Sreenivas Rao D *et al.* (2001). LC determination and purity evaluation of nefazodone HCl in bulk drug and pharmaceutical formulations. *J Pharm Biomed Anal* 26: 629–636.

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Yao M *et al.* (1998). Sensitive liquid chromatographic-mass spectrometric assay for the simultaneous quantitation of nefazodone and its metabolites hydroxynefazodone *m*-chlorophenylpiperazine and triazole-dione in human plasma using single-ion monitoring. *J Chromatogr B Biomed Sci Appl* 718: 77–85.

Nefopam

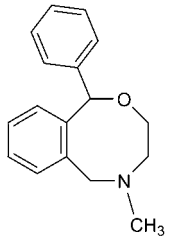
Analgesic, Benzoxazocine

$C_{17}H_{19}NO$=253.3

CAS—13669-70-0

IUPAC Name 5-Methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine

Synonym 3,4,5,6-Tetrahydro-5-methyl-1-phenyl-1*H*-2,5-benzoxazocine



Chemical Properties pK_a 9.2 [Sweetman 2007], 9.36 [Liu *et al.* 1987], 8.24 [Burton *et al.* 1990]. Log*P* (octanol/water), 3.05 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

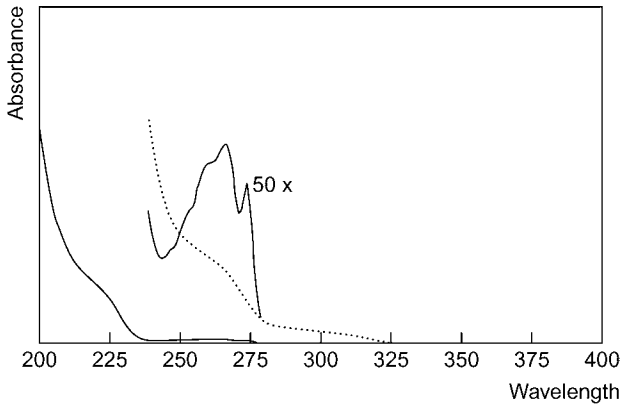
Nefopam Hydrochloride

$C_{17}H_{19}NO,HCl$ = 289.8

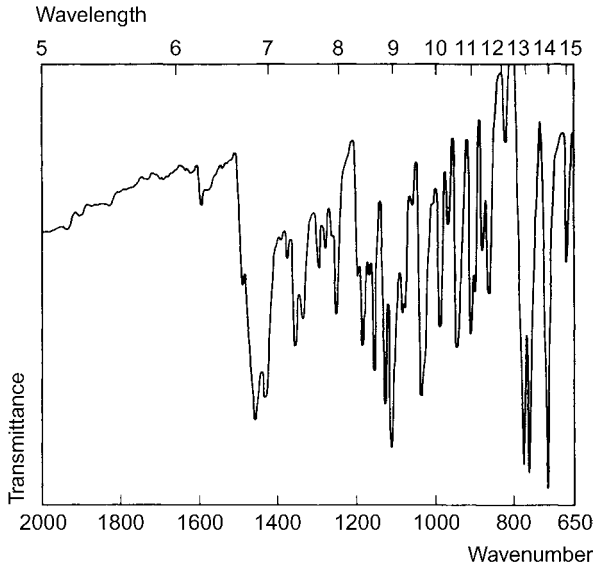
CAS—23327-57-3

IUPAC Name 5-Methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine hydrochloride
Proprietary Names *Acupan*; *Ajan*; *Nefadol*; *Nefam*; *Oxadol*; *Silentan*.
Chemical Properties A white crystalline powder. Mp ≈238° to 242°. Soluble in water, chloroform and methanol.

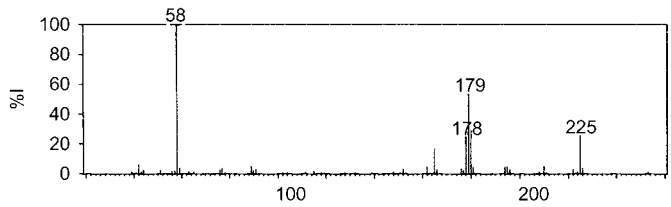
Colour Tests Liebermann's reagent—brown-orange→brown; Marquis test—orange→brown; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.33; system TC—R_f 0.32; system TE—R_f 0.59; system TAE—R_f 0.30; system TAG—R_f 0.17.
Gas Chromatography System GA—nefopam RI 2035; system GB—nefopam RI 2106, M (nor-) RI 2116, M (OH-) RI 2266, M (nor-di-OH-) RI 2649; system GF—RI 2380.
High Performance Liquid Chromatography System HA—*k* 3.0; system HY—RI 313; system HAA—Retention time 12.7 min.
Ultraviolet Spectrum Aqueous acid—267 (A_1^1 = 29b), 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 720, 760, 775, 1112, 1130, 1038 cm^{-1} (nefopam hydrochloride).



Mass Spectrum Principal ions at *m/z* 58, 179, 180, 225, 178, 165, 42, 210.



Quantification
Plasma **GC** Column: 3% OV-17 80/100 mesh Chromosorb W HP (1.80 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 190° for 8.5 min to 240° at 30°/min for 3 min. Retention time: 7.6 min. NPFID. Limit of detection, 5 µg/L [Chang *et al.* 1981]. Column: 3% 100/120 mesh cyclohexanedimethyl succinate on Gas Chrom Q (1.82 m × 2 mm i.d.). Carrier gas: He, 40 mL/min. Temperature: 221°. FID. Limit of detection, 20 µg/L [Schuppan *et al.* 1978]. Column: 3% OV-225 on Gas-Chrom Q 100/120 mesh (0.90 m × 2 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature: 170°. FID. Limit of detection, 5 µg/L [Ehrsson, Eksborg 1977].

HPLC Column: Symmetry C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.015 mol/L potassium dihydrogen phosphate-0.005 mol/L octane sulfonic acid (pH 3.7) : acetonitrile (77 : 33), flow rate 1.5 mL/min. UV detection (λ = 210 nm). Limit of detection, 1 µg/L [Aymard *et al.* 2002]. Column: Spherisorb S5W (150 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L ammonium nitrate (pH 9.1) : acetonitrile (7 : 93), flow rate 1 mL/min. Electrochemical detection. Limit of quantification, 1 µg/L [Burton *et al.* 1990].

LC-MS Column: Hypurity C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (50 : 50), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.781 µg/L, limit of detection, 0.195 µg/L [Hoizey *et al.* 2006].
Urine **HPLC** See Plasma. Limit of detection, 5 µg/L [Aymard *et al.* 2002].
CSF **GC** See Plasma [Chang *et al.* 1981].
Milk **HPLC** Column: Zorbax CN (250 × 4.6 mm i.d., 7 µm). Mobile phase: acetonitrile : 0.01 mol/L sodium pentanesulfonic acid (pH 3.15; 50 : 55), flow rate 1.5 mL/min. UV detection (λ = 215 nm). Limit of detection, 1.6 µg/L [Liu *et al.* 1987].
Oral Fluid **GC** See Plasma [Chang *et al.* 1981].
Disposition in the Body Rapidly absorbed after oral administration. It is extensively metabolized with <5% of a dose being excreted unchanged in the urine; metabolites include desmethylnefopam and the *N*-glucuronide, and nefopam *N*-oxide. Approximately 87% of a dose is excreted in the urine and ~8% in the faeces in 5 days.
Therapeutic Concentration
Following a single oral dose of 90 mg, peak blood concentrations of 0.07–0.15 mg/L were reported at 1–3 h [Heel *et al.* 1980].
Toxicity
A postmortem blood concentration of 11.9 mg/L was reported in a suicide with nefopam overdose; in a second non-fatal overdose case, a plasma concentration of 3.8 mg/L was reported 3 h after ingestion, declining to 0.9 mg/L at 19 h [Piercy *et al.* 1981].

In a fatality involving the intentional ingestion of an unknown quantity of nefopam and dihydrocodeine, the plasma nefopam concentration was 4.3 mg/L 1 h after ingestion; the postmortem plasma concentration was 0.6 mg/L (corresponding dihydrocodeine concentrations were 5.9 and 1.7 mg/L) [Urwin, Smith 1999].

A 37-year-old woman died after self-administering a massive IV dose of nefopam (300 mg). Postmortem tissue concentrations, measured ~7–10 days after death, were as follows: heart blood 4.38 mg/L, liver 59.12 µg/g, kidney 14.57 µg/g, lung 44.07 µg/g, and myocardium 14.87 µg/g [Tracqui *et al.* 2002].
Half-life Plasma half-life, 3–8 h (mean 4).
Protein Binding Approximately 75%.
Milk : plasma 1.2 ± 0.7 [Liu *et al.* 1987].
Note For a review of nefopam, see Heel *et al.* [1980].
Dose 90 to 270 mg of nefopam hydrochloride daily.

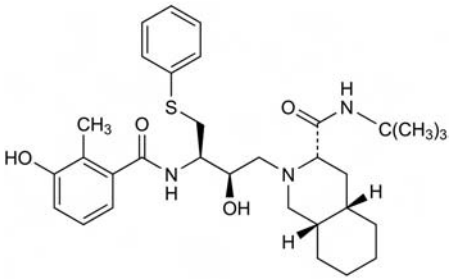
Aymard G *et al.* (2002). Sensitive determination of nefopam and its metabolite desmethyl-nefopam in human biological fluids by HPLC. *J Pharm Biomed Anal* 30: 1013–1021.
Burton LC *et al.* (1990). Determination of plasma nefopam by liquid chromatography and electrochemical detection. *J Chromatogr* 526: 159–168.
Chang SF *et al.* (1981). Quantitative determination of nefopam in human plasma, saliva and cerebrospinal fluid by gas–liquid chromatography using a nitrogen-selective detector. *J Chromatogr* 226: 79–89.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Ehrsson H, Eksborg S (1977). Quantitative gas chromatographic determination of nefopam in plasma. *J Chromatogr* 136: 154–158.
Heel RC *et al.* (1980). Nefopam: a review of its pharmacological properties and therapeutic efficacy. *Drugs* 19: 249–267.
Hoizey G *et al.* (2006). Specific and sensitive analysis of nefopam and its main metabolite desmethyl-nefopam in human plasma by liquid chromatography–ion trap tandem mass spectrometry. *J Pharm Biomed Anal* 42: 593–600.
Liu DT *et al.* (1987). Nefopam excretion in human milk. *Br J Clin Pharmacol* 23: 99–101.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *JPharm Sci* 84: 83–92.
Piercy DM *et al.* (1981). Death due to overdose of nefopam. *BMJ* 283: 1508–1509.
Schuppan D *et al.* (1978). GLC determination of nanogram quantities of a new analgesic, nefopam, in human plasma. *J Pharm Sci* 67: 1720–1723.
Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.
Tracqui A *et al.* (2002). Fatal overdosage with nefopam (Acupan). *J Anal Toxicol* 26: 239–243.
Urwin SC, Smith HS (1999). Fatal nefopam overdose. *Br J Anaesth* 83: 501–502.

Nelfinavir

Protease Inhibitor, Antiretroviral
$C_{32}H_{45}N_3O_4S$ = 567.8
CAS—159989-64-7

IUPAC Name (3*S*,4*aS*,8*aS*)-*N*-Tert-butyl-2-[(2*R*,3*R*)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-phenylsulfanylbutyl]-3,4,4*a*,5,6,7,8,8*a*-octahydro-1*H*-isoquinoline-3-carboxamide

Synonyms AG-1346; (3*S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)decahydro-2-[(2*R*,3*R*)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinolinecarboxamide.

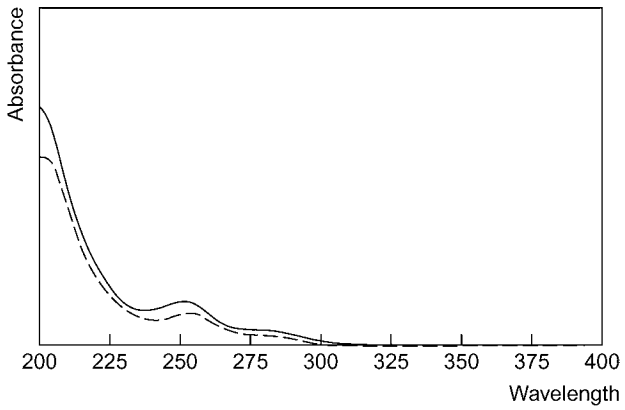


Chemical Properties White foam. Practically insoluble in water. pK_{a1} 6.00; pK_{a2} 11.06; pK_a −1.20 (mesilate). Log *P* (octanol/water), 4.1.

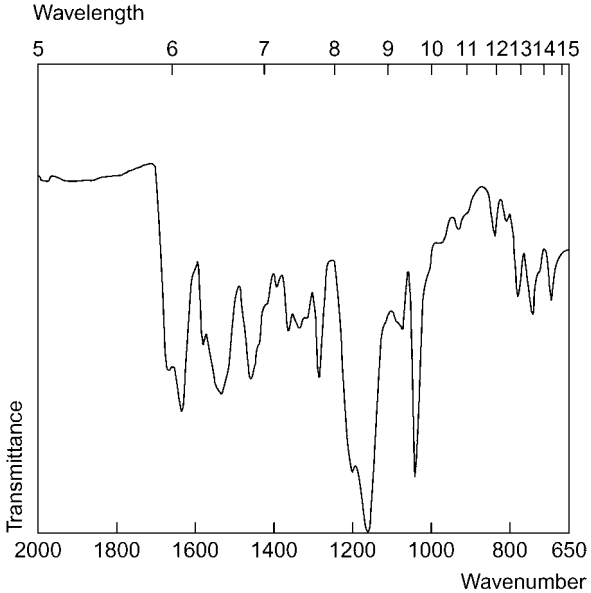
Nelfinavir Mesilate

$C_{32}H_{45}N_3O_4S$, CH_3SO_3H = 663.9
CAS—159989-65-8
Synonym AG-1343
Proprietary Name *Viracept*
Chemical Properties White powder. Solubility: 4.5 mg/mL in water, 2.6 mg/mL in 0.1 N HCl, 70 mg/g in glycerine, >100 mg/g in propylene glycol, >200 mg/g in PEG. Very soluble in methanol, ethanol, acetonitrile; practically insoluble in soybean oil and mineral oil.

High Performance Liquid Chromatography System HAC—*k* 17.3; system HAE—retention time 8.00 min.
Ultraviolet Spectrum Aqueous acid 252 nm.



Infrared Spectrum Principal peaks at wavenumbers 1639, 1536, 1460, 1285, 1158, 1040 cm^{-1}.

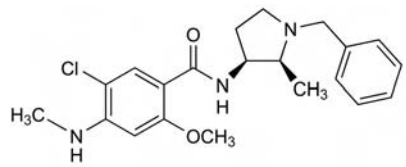


Quantification
Plasma **HPLC** UV detection (λ=254 nm). Limit of quantification, 0.05 mg/L [Aymard *et al.* 2000]. Column: ABZ-plus Supelcosil (250 × 4.6 mm i.d., 3 µm). Temperature: 45°. Mobile phase: 20 mmol/L potassium dihydrogen phosphate (pH 4) : methanol : acetonitrile (45 : 30 : 25), flow rate 1.0 mL/min. Internal standard: ritonavir. UV detection (λ=240 nm). Retention time: nelfinavir, 16.7 min; hydroxy-tert-butylamide metabolite, 7.2 min; IS, 12.7 min. Limit of quantification, 0.03 mg/L [Khaliq *et al.* 2000]. UV detection (λ=260 nm). Limit of quantification, 0.15 mg/L [Proust *et al.* 2000]. UV detection (λ=215 nm). Limit of quantification, 0.05 mg/L [Yamada *et al.* 2001].
Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 0.4 mg/L [Simon *et al.* 2001].
Disposition in the Body Nelfinavir is well absorbed after oral administration, and this is enhanced by administration with food. It is oxidised by the cytochrome P450 isoenzymes CYP2C19 and CYP2D6, to a hydroxy-*tert*-butylamide metabolite with *in vitro* antiviral activity. Approximately 50% of the drug is eliminated by this means, but the activity of CYP2C19 may be impaired in patients with reduced liver function or disease. Nelfinavir may induce its own metabolism. Elimination of the metabolite is predominantly controlled by the isoenzyme CYP3A4. Eighty–seven percent of a 750 mg dose is recovered in faeces, 22% as nelfinavir and 78% as the oxidative metabolites. It is distributed in breast milk.
Therapeutic Concentration
Eight Caucasian HIV-positive patients with liver disease, a mean age of 35 years (range, 29 to 42 years), participated in the study. Two of the patients had already been receiving nelfinavir for >8 weeks (750 mg every 8 h) and three others had been taking stavudine (40 mg) and lamivudine (150 mg) every 12 h for 12 weeks or longer. For the new patients starting treatment with nelfinavir, they were administered a single dose of 500 mg or 750 mg and then twice or three times daily for multiple dosing. During the multiple dosing, nelfinavir was co-administered with lamivudine (150 mg every 12 h) and either zidovudine (300 mg every 12 h) or stavudine (40 mg every 12 h). One patient also received 1000 mg saquinavir every 12 h. Steady-state plasma concentrations were measured after 10 and 19 days of multiple dosing. After the 500 mg single dose, the mean peak plasma concentration was 1.72 mg/L; after the 750 mg single dose, 2.70 mg/L; 250 mg twice daily, 1.7 mg/L; 500 mg twice daily, 4.1 mg/L; 750 mg twice daily, 3.1 mg/L; 250 mg three times daily, 2.5 mg/L and 750 mg three times daily, 4.73 mg/L [Khaliq *et al.* 2000].
Half-life 3.5 to 5 h.
Volume of Distribution 2 to 7 L/kg.
Clearance Plasma, mean, 24 L/h. Also reported as 24 to 33 L/h after a single dose and 26 to 61 L/h after multiple doses.
Protein Binding >98%.
Note For an update on the use of Nelfinavir in HIV infection, see Bardsley-Elliot and Plosker [2000].
Dose Adult: 2.25 g daily in three divided doses. Children (2 to 13 years): 20 to 30 mg/kg body weight three times daily.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 744: 227–240.
Bardsley-Elliot A, Plosker GL (2000). Nelfinavir: an update on its use in HIV infection. *Drugs* 59: 581–620.
Khaliq Y *et al.* (2000). Single and multiple dose pharmacokinetics of nelfinavir and CYP2C19 activity in human immunodeficiency virus-infected patients with chronic liver disease. *Br J Clin Pharmacol* 50: 108–115.
Proust V *et al.* (2000). Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma. *J Chromatogr Biomed Sci Appl* 742: 453–458.
Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1–2): 447–453.
Yamada H *et al.* (2001). Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir and nelfinavir in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 755: 85–89.

Nemonapride

Antipsychotic
$C_{21}H_{26}ClN_3O_2$ = 387.9
CAS—93664-94-9; 75272-39-8
IUPAC Name *cis*-*N*-(1-Benzyl-2-methyl-3-pyrrolidinyl)-5-chloro-4-(methylamine)-*o*-anisamide
Synonyms Emonapride; YM-09151-2.
Proprietary Name *Emilace*



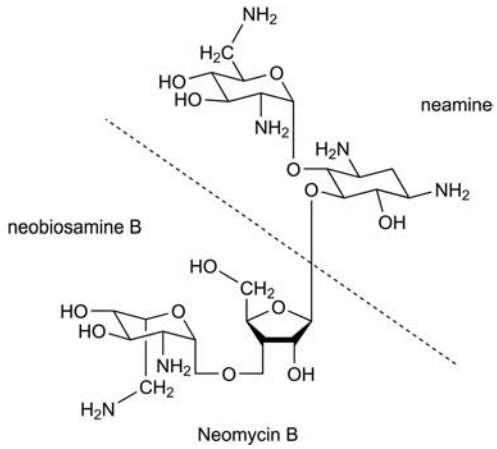
Chemical Properties Odourless, non-hygroscopic white crystals or crystalline powder. Mp 152° to 153°, also reported as 150°. Practically insoluble in water; freely soluble in glacial acetic acid and chloroform; sparingly soluble in methanol and ethanol; slightly soluble in ether. pK_a 0.40; 8.18. Log *P* (octanol/buffer pH 7.0), 3.20.
High Performance Liquid Chromatography System HAA—retention time 15.0 min.

Ultraviolet Spectrum Aqueous acid—285, 314 nm.
Infrared Spectrum Principal peaks at wavenumber 1624, 1602, 1284, 1244 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 388, 310, 215, 198, 173, 91.
Quantification
Plasma **HPLC** Electrochemical detection. Limit of quantification, 0.25 μg/L for nemonapride and its active metabolite, desmethylnemonapride [Nagasaki *et al.* 1998].
Disposition in the Body The plasma concentration of the unchanged drug reaches its peak 2 to 3 h after oral administration. No unchanged drug is detected in urine during the 24 h after oral administration. The major urinary metabolites are produced by *N*-demethylation, debenzylation and oxidation of the α-position of the pyrrolidine ring.
Half-life 2.5 to 4.5 h.
Dose Orally 9 to 36 mg daily. Maximum dose is 60 mg.

Nagasaki T *et al.* (1998). High-performance liquid chromatographic determination of nemonapride and desmethylnemonapride in human plasma using an electrochemical detection. *J Chromatogr Biomed Sci Appl* 714: 293–298.

Neomycin

Antibiotic
$C_{23}H_{46}N_6O_{13}$ = 614.6 (neomycin B)
CAS—1404-04-2 (neomycin); 3947-65-7 (neomycin A); 119-04-0 (neomycin B); 66-86-4 (neomycin C)



Chemical Properties A mixture of the two isomers, neomycins B and C, with neomycin A (neamine), an inactive component and degradation product of neomycins B and C. Neomycin is obtained from certain selected strains of *Streptomyces fradiae*.

Neomycin Sulfate
CAS—1405-10-3
Synonyms Fradiomycin sulfate; neomycin.
Proprietary Names It is an ingredient of many proprietary preparations—see Sweetman [2009].
Chemical Properties A white to yellowish-white hygroscopic powder. Slowly soluble 1 in 1 of water; very slightly soluble in ethanol; practically insoluble in chloroform and ether.

Neomycin Undecylenate
CAS—1406-04-8
Synonym Neomycin undecenoate
Proprietary Name It is an ingredient of *Audicort*.
Chemical Properties A yellowish-white to pale yellow, waxy, unctuous powder. Practically insoluble in water; very soluble in ethanol; soluble in chloroform.

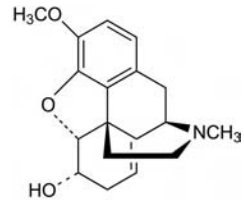
Thin-layer Chromatography System TA—R_f 0.00; system TAE—R_f 0.00 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Dose As an intestinal antiseptic, up to 12 g of neomycin sulfate daily, orally; it is also applied topically.

Sweetman SC, ed. (2009). *Martindale: The Complete Drug Reference*, 36 edn. London: Pharmaceutical Press.

Neopine

Alkaloid
$C_{18}H_{21}NO_3$ = 299.4
CAS—467-14-1

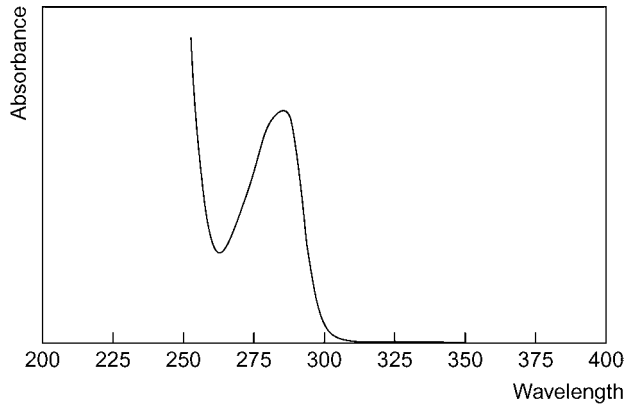
Synonym β-Codeine; (5α,6α)-8,14-didehydro-4,5-epoxy-3-methoxy-17-methyl morphinan-6-ol.
Note It has been incorrectly called hydroxycodeine.



Chemical Properties An alkaloid obtained from opium. Crystals. Mp about 127°. Slightly soluble in water; soluble in chloroform. Log *P* (octanol/water), 1.4.

Neopine Hydrobromide
$C_{18}H_{21}NO_3$,HBr = 380.3
Chemical Properties Crystals which darken at about 240° and decompose at 283°. Relatively insoluble in water, hence ease of separation from other opium alkaloids.

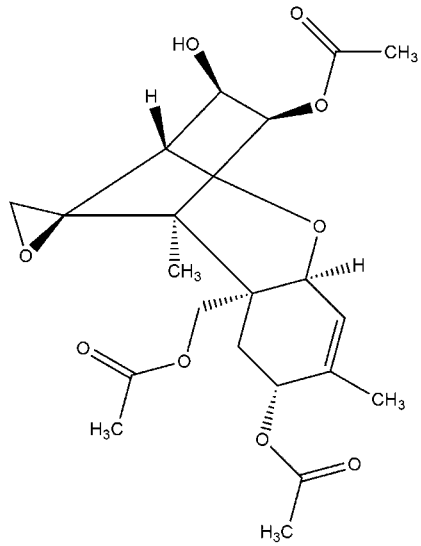
Colour Test Marquis test—blue-violet.
Thin-layer Chromatography System TA—R_f 0.35; system TB—R_f 0.05; system TC—R_f 0.12; system TL—R_f 0.04 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2395.
Ultraviolet Spectrum Aqueous acid—285 nm (A_1^1=39b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1275, 1500, 1118, 1050, 1250, 782 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 299, 162, 229, 123, 59, 42, 44, 300.

Neosolaniol

Mycotoxin
$C_{21}H_{28}O_9$ = 424.4
Synonyms 8-Acetylneosolaniol; 3-hydroxy-4β,8-α,15-triacetoxy-12,13-epoxy-trichothec-9-ene; neosolaniol monoacetate; trichothecene analogue.



Quantification

Other **GC-MS** Foodstuffs Marketed in Germany. Limit of detection, 1–19 µg/kg [Schollenberger *et al.* 2005].

LC-MS Nigerian Maize. Limit of detection, 20–200 µg/kg [Adejumo *et al.* 2007]. Wheat. APCI, positive ion mode. Limit of quantification, 10–100 ppb [Berger *et al.* 1999].

Adejumo TO *et al.* (2007). Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. *Int J Food Microbiol* 116: 350–357.

Berger U *et al.* (1999). Quantitative determination and structure elucidation of type A- and B-trichothecenes by HPLC/ion trap multiple mass spectrometry. *J Agric Food Chem* 47: 4240–4245.

Schollenberger M *et al.* (2005). Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany. *Int J Food Microbiol* 97: 317–326.

Neostigmine Bromide

Anticholinesterase

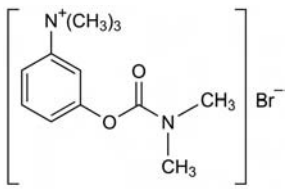
$C_{12}H_{19}BrN_2O_2$ = 303.2

CAS—59-99-4 (neostigmine); 114-80-7 (bromide)

IUPAC Name 3-[[(Dimethylamino)carbonyl]oxy]-*N*,*N*,*N*-trimethylbenzenaminium bromide

Synonyms Proserine bromide; synstigmine bromide.

Proprietary Names *Neoeserin*; *Normastigmin*; *Prostigmin(e)* (tablets).



Chemical Properties Colourless crystals or a white crystalline, slightly hygroscopic powder. Mp 171° to 176°, with decomposition. Soluble 1 in 0.5 of water, 1 in 8 of ethanol and 1 in 5 of chloroform; practically insoluble in ether. pK_a 12.0.

Neostigmine Methylsulfate

$C_{13}H_{22}N_2O_6S$ = 334.4

CAS—51-60-5

Synonym Proserinum

Proprietary Name *Glycostigmin*; *Intrastigmina*; *Normastigmin*; *Prostigmin(e)*; *Prostigmina*; *Robinul-Neostigmin(e)* (injection).

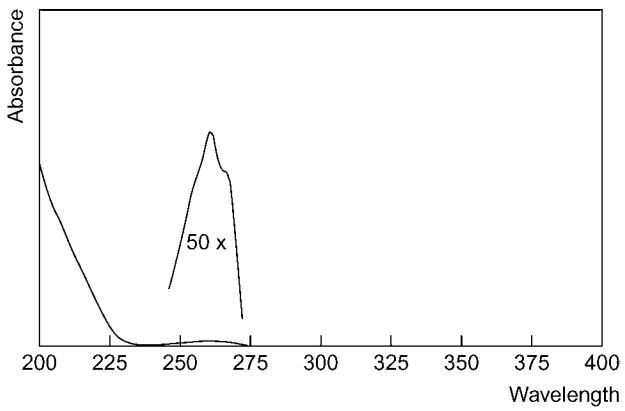
Chemical Properties Colourless crystals or a white crystalline powder. Mp 144° to 149°. Soluble 1 in 0.5 of water and 1 in 6 of ethanol.

Thin-layer Chromatography Neostigmine bromide: system TA—R_f 0.02. Neostigmine methylsulfate: system TB—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.00 (acidified iodoplatinate solution, positive).

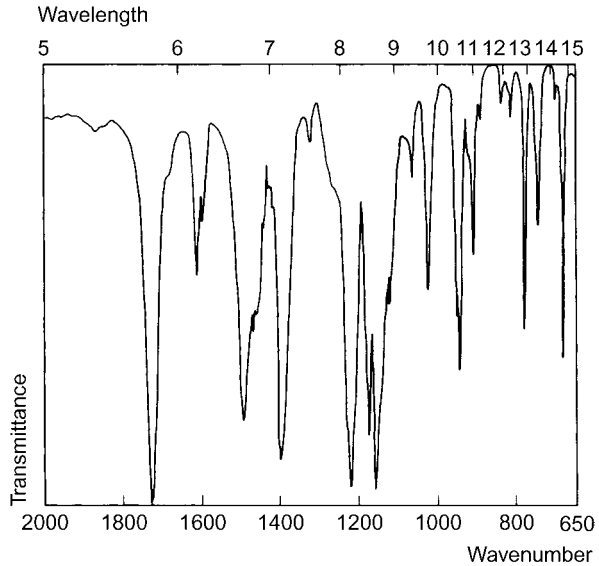
Gas Chromatography System GA—RI 1770.

High Performance Liquid Chromatography System HA—*k* 4.7 (tailing peak).

Ultraviolet Spectrum Aqueous acid—260 (A_1^1=16a), 266 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1711, 1215, 1154, 1176, 948, 690 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 42, 208, 108, 65, 73, 66, 39.

Quantification

Plasma **GC** AFID. Limit of detection, 50 µg/L [Chan *et al.* 1976].

GC–MS Limit of detection, 1 µg/L [Aquilonius *et al.* 1979].

HPLC UV detection. Limit of detection, <2.6 µg/L [Varin *et al.* 1999].

Urine **MS** Limit of detection, 20 to 150 µg/L for neostigmine bromide and other quaternary ammonium compounds [Nisikawa *et al.* 1991].

Cerebrospinal Fluid **HPLC** See Plasma [Varin *et al.* 1999].

Disposition in the Body Neostigmine bromide is poorly absorbed after oral administration. It is metabolised by ester hydrolysis to form 3-hydroxytrimethylanilinium bromide, which is active. About 20% of an oral dose is excreted in the urine with <5% as unchanged drug; about 50% of an oral dose is eliminated in the faeces. After IM administration, about 80% of a dose is excreted in the urine in 24 h, with ~50% consisting of unchanged drug and 15% as the 3-hydroxytrimethylanilinium metabolite.

Therapeutic Concentration

After a single oral dose of 30 mg to 3 subjects, peak plasma concentrations of 0.004 to 0.009 mg/L were attained in 1 to 2 h [Aquilonius *et al.* 1979].

Following IV administration of 5 mg to 5 subjects, plasma concentrations of 0.84 to 6.25 mg/L were reported at 2 min [Williams *et al.* 1978].

Half-life Plasma half-life, about 1 h.

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, about 11 mL/min/kg.

Dose Usually 75 to 300 mg of neostigmine bromide daily, orally; 1 to 2.5 mg of neostigmine methylsulfate daily, given parenterally.

Aquilonius SM *et al.* (1979). A pharmacokinetic study of neostigmine in man using gas chromatography-mass spectrometry. *Eur J Clin Pharmacol* 15: 367–371.

Chan K *et al.* (1976). A quantitative gas-liquid chromatographic method for the determination of neostigmine and pyridostigmine in human plasma. *J Chromatogr* 120: 349–358.

Nisikawa M *et al.* (1991). The analysis of quaternary ammonium compounds in human urine by direct inlet electron impact ionization mass spectrometry. *Forensic Sci Int* 51: 131–138.

Varin F *et al.* (1999). Determination of neostigmine in human plasma and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 723: 319–323.

Williams NE *et al.* (1978). Clearance of neostigmine from the circulation during the antagonism of neuromuscular block. *Br J Anaesth* 50: 1065–1067.

Nevirapine

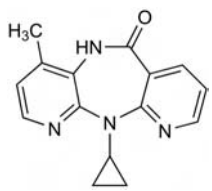
Non-Nucleoside Reverse Transcriptase Inhibitor, Antiretroviral

$C_{15}H_{14}N_4O$ = 266.3

CAS—129618-40-2

Synonyms BI-RG-587; BIRG-0587; 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*]-[1,4]diazepin-6-one.

Proprietary Name *Viramune*

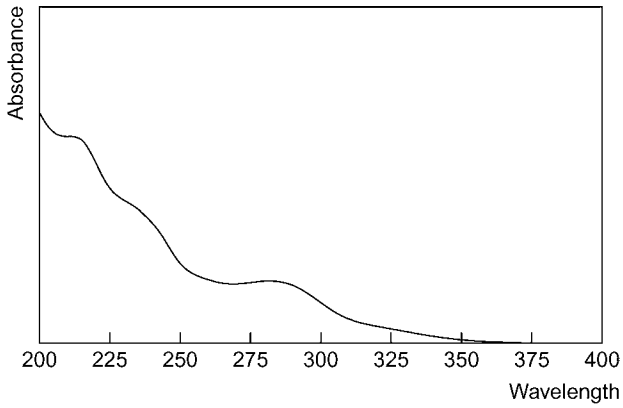


Chemical Properties Crystals from pyridine/water. Mp 247° to 249°. Highly soluble in water at pH <3 but solubility decreases to ~0.1 g/L at neutral pH. pK_a 2.8. Log *P* (octanol/water), 83.

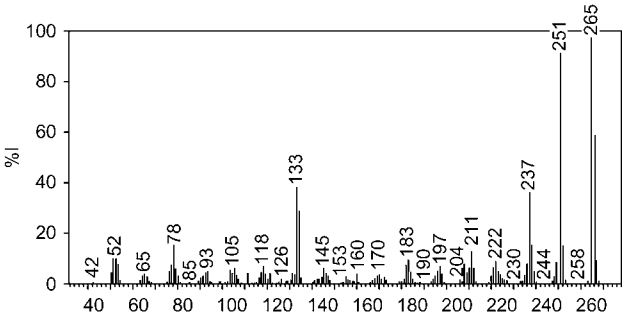
High Performance Liquid Chromatography System HAD—*k* 11.1.

Column: C_{18} Spherisorb (300 × 4.6 mm i.d., 3 µm). Mobile phase: 10 mmol/L phosphate buffer (pH 5) : acetonitrile (82 : 18) with 10 mmol/L triethylamine, flow rate 1.0 mL/min. UV detection (λ=240 nm). Retention time: 2.0 min [Lopez *et al.* 2001].

Ultraviolet Spectrum Aqueous acid—205, 216, 280 nm.



Mass Spectrum Principal ions at *m/z* 265, 251, 267, 133, 237, 135, 78, 211.



Quantification

Plasma **HPLC** UV detection (λ=260 nm). Limit of quantification, 0.1 mg/L [Aymard *et al.* 2000]. UV detection (λ=280 nm). Limit of quantification, 0.1 mg/L, limit of detection, 0.05 mg/L [Hollanders *et al.* 2000]. Column: RP LC-8 Supelco (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L phosphate buffer (pH 6) : methanol : acetonitrile (63 : 21.5 : 15.5) with 25 mmol/L 1-butane-sulfonic acid, flow rate 1.0 mL/min. Internal standard: BIRH-414. UV detection (λ=280 nm). Retention time: nevirapine, 5.5 min; IS, 7.5 min. Limit of quantification, 0.025 mg/L [Pav *et al.* 1999]. Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L phosphate buffer (pH 5.5) : methanol : acetonitrile (7 : 2 : 1) with 25 mmol/L hexane-1-sulfonic acid, flow rate 1.0 mL/min. UV detection (λ=282 nm). Retention time: 9.5 min. Limit of quantification, 0.052 mg/L [van Heeswijk *et al.* 1998]. UV detection (λ=245, 280 nm). Limit of quantification, 0.01 mg/L [Jayaraj *et al.* 1992].

Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 0.084 mg/L [Simon *et al.* 2001]. See Plasma [Pav *et al.* 1999].

Cerebrospinal Fluid **HPLC** See Plasma [Pav *et al.* 1999].

Milk **HPLC** See Plasma [Pav *et al.* 1999].

Disposition in the Body Nevirapine is readily absorbed after oral administration with a peak plasma concentration at 4 h. Absorption is not affected by food. The concentration of the drug in the CNS is 45% of that in plasma. It crosses the placenta and has been detected in breast milk. Nevirapine undergoes extensive metabolism in the liver mainly be the cytochrome P450 isoenzymes of the CYP3A family and the major metabolite is hydroxymethyl-nevirapine. It is excreted via urine as the glucuronide conjugates of the hydroxylated metabolites. The drug is widely distributed in body tissues and the CNS.

Therapeutic Concentration The therapeutic concentration is >1 mg/L.

Eighteen male and 3 HIV-1-infected female patients, aged between 19 and 46 years, with an absolute CD4 count <400/µL were administered with doses of 12.5, 25, 50, 100, 200 and 400 mg nevirapine after an overnight fast (3 patients for each dose). The mean peak plasma concentrations were 0.145, 0.416, 0.560, 1.215, 2.070 and 3.068 mg/L, respectively, to the doses, achieved after 1.0, 1.7, 3.7, 3.8, 2.0 and 2.25 h, respectively [Cheeseman *et al.* 1993].

Bioavailability Oral, about 93%.

Half-life 45 h which decreases on multiple dosing to 20 to 30 h over a 2- to 4-week period.

Volume of Distribution Steady state, 1.2 to 1.5 L/kg. Reported as 1.54 L/kg in females and 1.38 L/kg in males.

Clearance Oral, 0.27 to 0.52 mL/kg/min. Plasma, 3.96 L/h (after a 200 mg dose).

Protein Binding 60%.

Note For a safety profile of nevirapine, see Pollard *et al.* [1998].

Dose Adult: 200 mg once daily which can be increased to 400 mg daily after 2 weeks. Children under 50 kg (2 months to 8 years): 4 mg/kg increased to 7 mg/kg twice daily (maximum 400 mg daily). (8 to 16 years): 4 mg/kg once daily increased to twice daily (maximum 400 mg).

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 744: 227–240.

Cheeseman SH *et al.* (1993). Pharmacokinetics of nevirapine: initial single-rising-dose study in humans. *Antimicrob Agents Chemother* 37: 178–182.

Hollanders RM *et al.* (2000). Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. *J Chromatogr Biomed Sci Appl* 744: 65–71.

Jayaraj A *et al.* (1992). *Pharm Res* 9: S334.

Lopez RM *et al.* (2001). Simple and rapid determination of nevirapine in human serum by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 751: 371–376.

Pav JW *et al.* (1999). HPLC-UV method for the quantitation of nevirapine in biological matrices following solid phase extraction. *J Pharm Biomed Anal* 20: 91–98.

Pollard RB *et al.* (1998). Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection. *Clin Ther* 20: 1071–1092.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1–2): 447–453.

van Heeswijk RP *et al.* (1998). Rapid determination of nevirapine in human plasma by ion-pair reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr Biomed Sci Appl* 713(2): 395–399.

Nialamide

Antidepressant

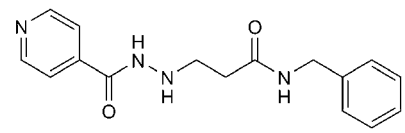
$C_{16}H_{18}N_4O_2$=98.3

CAS—51-12-7

IUPAC Name *N*-Benzyl-3-[2-(pyridine-4-carbonyl)hydrazinyl]propanamide

Synonym 4-Pyridinecarboxylic acid 2-[3-oxo-3-[(phenylmethyl)amino]propyl] hydrazide

Proprietary Names *Espril*; *Niamid(al)*; *Niaquitil*; *Nuredal*; *Nyazin*.



Chemical Properties A white crystalline powder. Mp 151° to 153°. Soluble 1 in 400 of water, 1 in 40 of ethanol, 1 in 150 of chloroform, and 1 in 10 of methanol; freely soluble in acidic solvents. Log *P* (octanol/pH 7.4), 0.9, (octanol/water), 0.87 [Hansch *et al.* 1995].

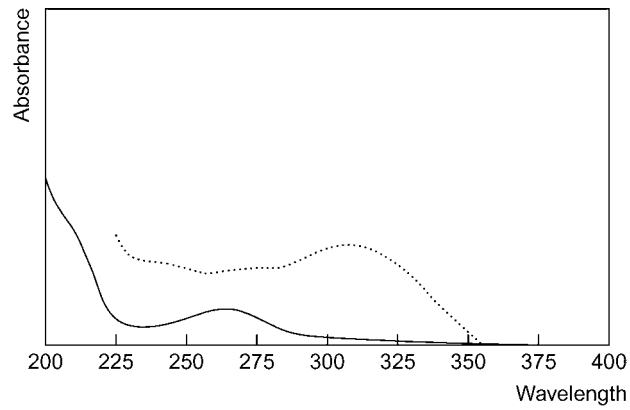
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—red; Nessler's reagent—black.

Thin-layer Chromatography System TA—R_f 70; system TB—R_f 02; system TC—R_f 25; system TAE—R_f 68; system TAF—R_f 64; system TAG—R_f 0.04 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—brown).

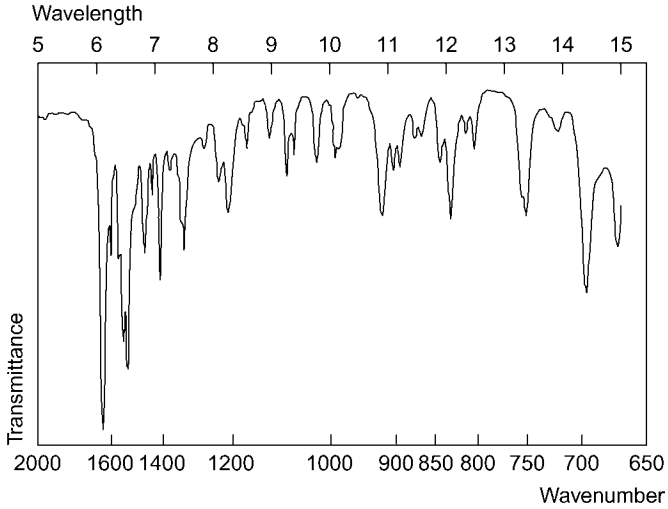
Gas Chromatography System GA—RI 1500.

High Performance Liquid Chromatography System HA—*k* 1.2 (tailing peak); system HX—RI 334.

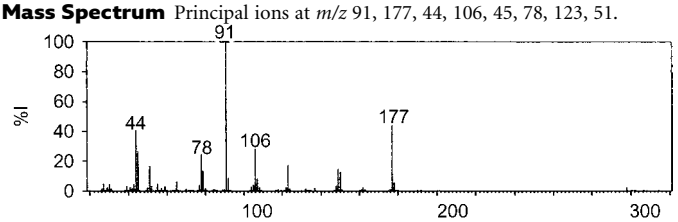
Ultraviolet Spectrum Aqueous acid—266 nm (A_1^1 = 193a); aqueous alkali—307 nm.



Infrared Spectrum Principal peaks at wavenumbers 1625, 1520, 1547, 698, 670, 1600 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 177, 44, 106, 45, 78, 123, 51.



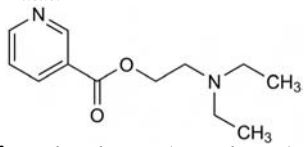
Disposition in the Body
Toxicity A dose of 5 g nialamide can be fatal in humans [Matell, Thorstrand 1967].
Dose 75 to 150 mg daily.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society.
Matell G, Thorstrand C (1967). A case of fatal nialamid poisoning. *Acta Med Scand* 181: 79–82.

Nicametate

Vasodilator
$C_{12}H_{18}N_2O_2 = 222.3$
CAS—3099-52-3

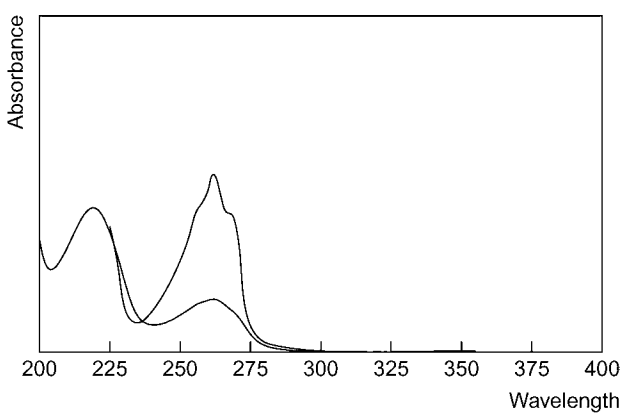
IUPAC Name 2-Diethylaminoethyl pyridine-3-carboxylate
Synonym 3-Pyridinecarboxylic acid 2-(diethylamino)ethyl ester
Proprietary Name *Eucast*



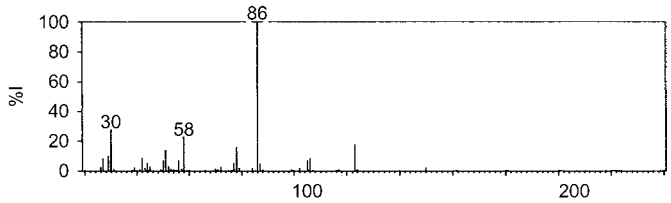
Chemical Properties A liquid. Log *P* (octanol/water), 1.3.

Nicametate Citrate
$C_{12}H_{18}N_2O_2,C_6H_8O_7,H_2O = 432.4$
CAS—1641-74-3
Proprietary Names *Euclidan*; *Nurtin*; *Soclidan*.
Chemical Properties A white crystalline powder.

Colour Test Cyanogen bromide—orange.
Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.41; system TC—R_f 0.35; system TE—R_f 0.68; system TL—R_f 0.20; system TAE—R_f 0.35 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—nicametate RI 1608, nicotinic acid RI 1335.
Ultraviolet Spectrum Aqueous acid—261 nm (A^1_1=120b).



Infrared Spectrum Principal peaks at wavenumbers 1729, 1283, 1594, 1130, 1116, 743 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 30, 58, 123, 78, 51, 29, 42; nicotinic acid 123, 105, 78, 51, 106, 77, 124, 50.

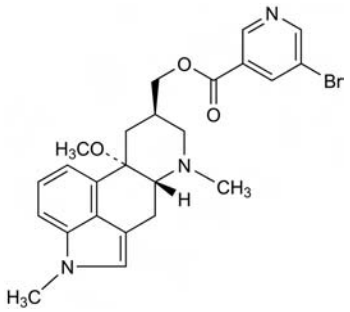


Disposition in the Body Slowly hydrolysed to nicotinic acid and diethylaminoethanol.
Dose 300 mg of nicametate citrate daily.

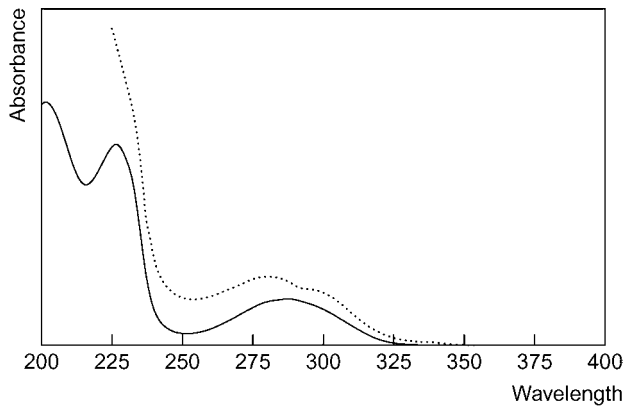
Nicergoline

Vasodilator
$C_{24}H_{26}BrN_3O_3 = 484.4$
CAS—27848-84-6

Synonyms FI-6714; (8β)-10-methoxy-1,6-dimethylergoline-8-methanol 5-bromo-3-pyridinecarboxylate; nicotergoline; nimergoline.
Proprietary Names *Cebran*; *Circo-Maren*; *Duracebrol*; *Ergobel*; *Ergolin*; *Ergotop*; *Fisifax*; *Memoq*; *Neugen*; *Nicer*; *Nicergobeta*; *Nicerium*; *Sermion*; *Varson*.



Chemical Properties A yellowish-white crystalline powder. Mp 136° to 138°. Practically insoluble in water; soluble in ethanol and chloroform; slightly soluble in ether.
Colour Tests Liebermann's reagent—black; Mandelin's test—violet-brown→brown; Marquis test—blue→grey; sulfuric acid—violet.
Thin-layer Chromatography System TA—R_f 0.64; system TAE—R_f 0.43; system TE—R_f 0.73 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HAA—retention time 15.5 min.
Ultraviolet Spectrum Aqueous acid—286 nm; aqueous alkali—280 nm; ethanol—287 nm (A^1_1=200b).



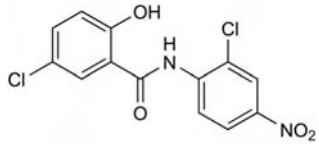
Infrared Spectrum Principal peaks at wavenumbers 1281, 751, 1718, 1306, 1075, 1103 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC–MS** Limit of detection, 2 µg/L for nicergoline and its metabolites [Banno *et al.* 1991].
Radioimmunoassay Limit of detection, <60 ng/L [Bizollon *et al.* 1982].
Urine **HPLC** UV detection. For method of quantification for 10α-methoxy-9,10 dihydrolysergol, a metabolite of nicergoline, see Sioufi *et al.* [1992].
HPLC–MS Limit of detection, 10 µg/L for nicergoline and its metabolites [Banno *et al.* 1991].
Radioimmunoassay See Plasma [Bizollon *et al.* 1982].

Disposition in the Body Nicergoline is extensively metabolised, mainly by hydrolysis and N^1-demethylation, and is excreted in the urine as free and conjugated (glucuronide) metabolites.
Dose Usually 15 mg daily.

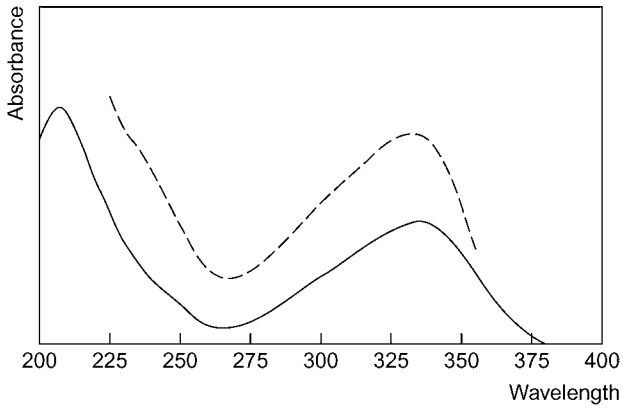
Banno K *et al.* (1991). Assay of nicergoline and three metabolites in human plasma and urine by high-performance liquid chromatography-atmospheric pressure ionization mass spectrometry. *J Chromatogr* 568: 375–384.
Bizollon CA *et al.* (1982). Radioimmunoassay of nicergoline in biological material. *Eur J Nucl Med* 7: 318–321.
Sioufi A *et al.* (1992). Determination of 10 alpha-methoxy-9,10-dihydrolysergol, a nicergoline metabolite, in human urine by high performance liquid chromatography. *Biomed Chromatogr* 6: 9–11.

Niclosamide

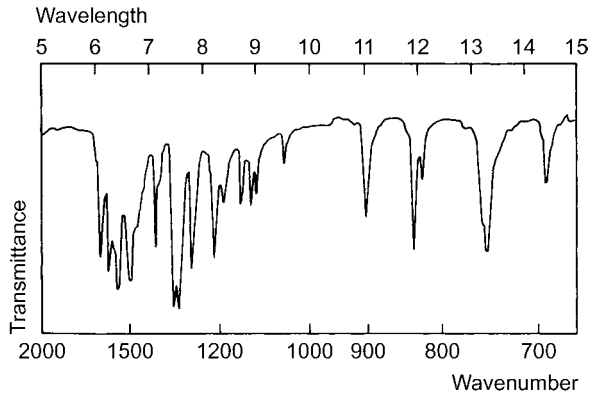
Anthelmintic
$C_{13}H_8Cl_2N_2O_4 = 327.1$
CAS—50-65-7
IUPAC Name 5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide
Synonym Phenasale
Proprietary Names *Atenase*; *Kontal*; *Mansonil* (vet.); *Niclocide*; *Trédémine*; *Yomesan*.



Chemical Properties A cream-coloured powder. Mp 225° to 230°. Practically insoluble in water; soluble 1 in 150 of ethanol, 1 in 400 of chloroform and 1 in 350 of ether; soluble in acetone. Log *P* (octanol/water), 4.6.
Colour Tests Mandelin's test—green; methanolic potassium hydroxide—yellow.
Thin-layer Chromatography System TA—R_f 0.91; system TB—R_f 0.00; system TE—R_f 0.23; system TAE—R_f 0.93 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HX—RI 723; system HAA—retention time 23.9 min.
Ultraviolet Spectrum Aqueous alkali—334, 377 nm; methanol—333 nm (A^1_1=527b).



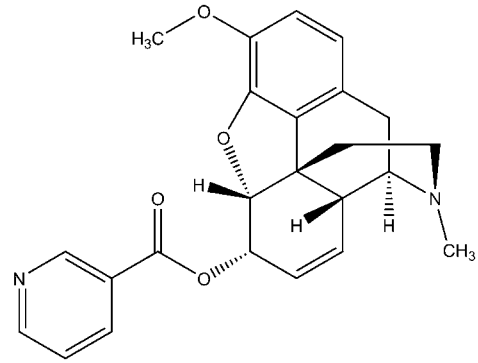
Infrared Spectrum Principal peaks at wavenumbers 1572, 1515, 1613, 1285, 1650, 1218 cm^{-1} (KBr disk).



Dose Usually 2 g as a single dose.

Nicocodine

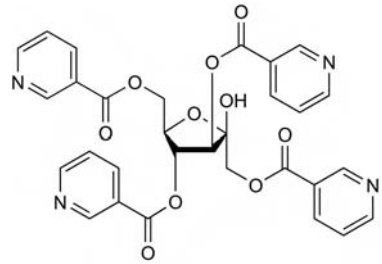
Narcotic, Antitussive
$C_{24}H_{24}N_2O_4 = 404.5$
CAS—3688-66-2
Synonyms 7,8-Dehydro-4,5-epoxy-3-methoxy-*N*-methyl-6-nicotinoyloxymorphinan; 3-O-methyl-6-O-nicotinoylmorphine; 6-nicotinoylcodeine.
Proprietary Names *Lyopect*; *Tusscodin*.



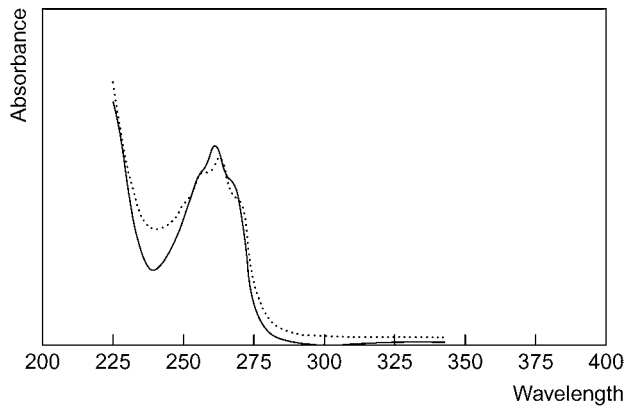
Chemical Properties Nicocodine is extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—green→blue→green (limit of detection, 1 in 1000); sulfuric acid-formaldehyde test—purple→blue-purple (limit of detection, 1 in 1000); Vitali's test—yellow/yellow/orange (limit of detection, 1 in 1000).
Thin-layer Chromatography System T10—R_f 0.36 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—261 and 267 nm with an inflextion at 256 nm.
Dose As the hydrochloride it is given orally in doses of 5 to 7.5 mg up to 3 times daily.

Nicofuranose

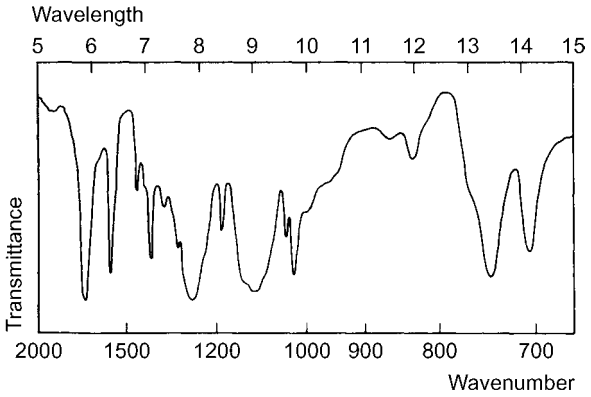
Vasodilator
$C_{30}H_{24}N_4O_{10} = 600.5$
CAS—15351-13-0
IUPAC Name [(2*R*,3*R*,4*S*,5*R*)-5-Hydroxy-3,4-bis(pyridine-3-carbonyloxy)-5-(pyridine-3-carbonyloxymethyl)oxolan-2-yl]methyl pyridine-3-carboxylate
Synonyms β-D-Fructofuranose-1,3,4,6-tetra-3-pyridinecarboxylate; tetranicotinoylfructofuranose; tetranicotinoylfructose.
Proprietary Name *Bradilan*



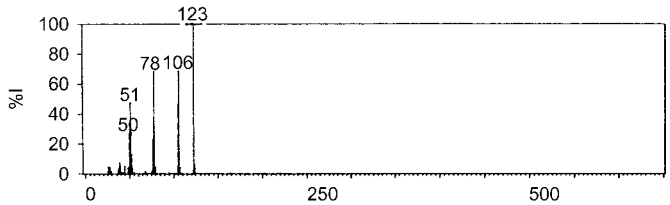
Chemical Properties A creamy-white crystalline powder. Mp 132° to 143°. Practically insoluble in water; soluble 1 in 4 of chloroform and 1 in 70 of methanol; sparingly soluble in dilute solutions of hydrochloric acid and sodium hydroxide. Log *P* (octanol/water), 0.1.
Colour Tests Add 50 mg to 5 mL of potassium cupritartrate solution and heat to boiling for 1 min—red precipitate; Fuse 50 mg with 10 mg of 2,4-dinitrochlorobenzene, cool, add about 3 mL of ethanol and 1 mL of 1 mol/L sodium hydroxide—intense red-violet colour
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.42; system TC—R_f 0.70; system TL—R_f 0.61 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—262 nm (A^1_1=380b); aqueous alkali—263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1721, 1271, 1107, 737, 1587, 1022 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 123, 106, 78, 51, 105, 50, 77, 52; nicotinic acid 123, 105, 78, 51, 106, 77, 124, 50.



Disposition in the Body Nicofuranose is slowly hydrolysed in the small intestine to nicotinic acid, which is readily absorbed.

Therapeutic Concentration

Following a single oral dose of 500 mg to 27 subjects, a mean peak plasma concentration of 0.72 mg/L was attained in 3 h [Salmi, Frey 1974].

Dose 1.5 to 3 g daily.

Salmi HA, Frey H (1974). Nicotinic acid plasma concentrations after ingestion of 1,3,4,6-tetranicotinoyl-fructo-furanose (tetranicotinoylfructose, nicofuranose). *Curr Ther Res* 16: 669–674.

Nicomorphine

Narcotic Analgesic

$C_{29}H_{25}N_3O_5$ = 495.5

CAS—639-48-5

Synonyms (5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol di-3-pyridinecarboxylate (ester); morphine dinicotinate; morphine ester with nicotinic acid; morphine *bis*(nicotinate); morphine *bis*(pyridine-3-carboxylate); nicotinic acid morphine ester.

Proprietary Name *Gewalan*.

Chemical Properties Crystals. Mp 178° to 178.5°. Practically insoluble in water, soluble in ethanol.

Nicomorphine Hydrochloride

$C_{29}H_{25}N_3O_5$, HCl = 532.0

CAS—35055-78-8

Synonyms 3,6-Di-*O*-nicotinoylmorphine hydrochloride; (−)-(5*R*,6*S*)-4,5-epoxy-9*a*-methylmorphin-7-en-3,6-diyl dinicotinate hydrochloride.

Proprietary Name *MorZet*; *Vilan*.

Chemical Properties Soluble in water. Log *P* (octanol/water), 2.87 [Advanced Chemistry Development 2007].

Quantification

Blood **HPLC** Column: Hypersil 5 ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: water-tetramethylammonium hydroxide-acetonitrile : 7 mL 10% tetramethylammonium hydroxide (pH 4.0), total volume 300 mL plus 150 mL acetonitrile, flow rate 1.6 mL/min. UV detection (λ= 265 nm). Retention time 2.7 min. Limit of quantification, 9 µg/L [Koopman-Kimenai *et al.* 1995].

Serum **HPLC** Column: Hypersil 5 ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: water-tetramethylammonium hydroxide-acetonitrile : 7 mL 10% tetramethylammonium hydroxide (pH 4.0), total volume 300 mL plus 150 mL acetonitrile, flow rate 1.6 mL/min. UV detection (λ= 265 nm). Retention time 2.7 min. Limit of detection, 40 µg/L [Koopman-Kimenai *et al.* 1987].

Urine **HPLC** See Blood [Koopman-Kimenai *et al.* 1995].

Disposition in the Body After rectal administration, nicomorphine is rapidly absorbed and quickly metabolised to the active compounds 6-mononicotinoylmorphine, morphine and morphine-6-glucuronide.

Therapeutic Concentration

Eight female patients due to undergo elective gynaecological abdominal surgery were administered 20 mg nicomorphine IM into the deltoid muscle. 6-Mononicotinylmorphine was almost instantaneously formed, with a mean maximum plasma concentration of 55 µg/L at 0.16 h and an elimination half-life of 0.3 h. Morphine reached a mean maximum plasma concentration of 36 µg/L at 0.74 h and was eliminated with a half-life of 1.4 h. Morphine 3-glucuronide and morphine 6-glucuronide appeared almost simultaneously and reached a maximum concentration at 1.37 h. They were eliminated with half-lives of 3.66 and 2.54 h, respectively [Koopman-Kimenai *et al.* 1995].

Eight patients were administered 30 mg nicomorphine rectally. Levels of morphine, morphine 3-glucuronide and morphine 6-glucuronide reached 45 ± 12, 513 ± 125 and 84 ± 31 µg/L, respectively, at 0.53 ± 0.13, 1.19 ± 0.32 and 1.17 ± 0.36 min, respectively [Koopman-Kimenai *et al.* 1994].

IV administration of 30 mg nicomorphine to 6 patients, 20 mg nicomorphine to five patients and 10 mg to five patients resulted in mean peak plasma levels of 1.12, 0.567 and 0.74 mg/L, respectively, attained at at 1.6, 1 and 1 min, respectively. Levels of 6-mononicotinoylmorphine reached 777, 365 and 241 µg/L, respectively, attained at 1.67, 1 and 1 min, respectively; levels of morphine reached 159, 104 and 96.1 µg/L, respectively, attained at 7.83, 1 and 7.2 min, respectively [Koopman-Kimenai *et al.* 1993].

Bioavailability Rectal administration 88%; IM injection, 85%; IV injection 95%.

Half-life 0.32 ± 0.2 h.

Clearance Morphine, 162 mL/min; glucuronide derivatives, 81 mL/min.

Dose Daily dose: 5 to 10 mg orally, or 10 to 20 mg by IM, slow IV or SC injection. It may also be given rectally.

Advanced Chemistry Development (2007). *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development. (www.acdlabs.com).

Koopman-Kimenai PM *et al.* (1987). High-performance liquid chromatography and preliminary pharmacokinetics of nicomorphine and its metabolites 3-nicotinoyl- and 6-nicotinoylmorphine and morphine. *J Chromatogr* 416: 382–387.

Koopman-Kimenai PM *et al.* (1993). Pharmacokinetics of intravenously administered nicomorphine and its metabolites in man. *Eur J Anaesthesiol* 10: 125–132.

Koopman-Kimenai PM *et al.* (1994). Rectal administration of nicomorphine in patients improves biological availability of morphine and its glucuronide conjugates. *Pharm World Sci* 16: 248–253.

Koopman-Kimenai PM *et al.* (1995). The bioavailability of intramuscularly administered nicomorphine (Vilan) with its metabolites and their glucuronide conjugates in surgical patients. *Int J Clin Pharmacol Ther* 33: 442–448.

Nicorandil

Antianginal, Nitrovasodilator, Potassium Channel Activator

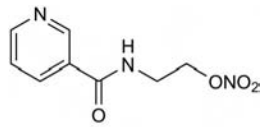
$C_8H_9N_3O_4$ = 211.2

CAS—65141-46-0

IUPAC Name 2-(Pyridine-3-carbonylamino)ethyl nitrate

Synonyms *N*-[2-(Nitrooxy)ethyl]-3-pyridine carboxamide; SG-75.

Proprietary Names *Adancor*; *Dancor*; *Ikorel*; *Perisalol*; *Sigmart*.



Chemical Properties A colourless to off-white crystalline powder. Mp 92° to 93°. Log *P* (octanol/water), 0.43.

High Performance Liquid Chromatography System HZ—retention time 2.2 min.

Column: C_8 Finepak SIL (250 × 4.6 mm i.d., 5 µm). Temperature: 30°. Mobile phase: methanol : water (15 : 85), flow rate 1.2 mL/min. IS: (*N*-[2-(nitrooxy)propyl]-3-pyridine carboxamide) chlorohydrate. UV detection (λ=230 nm). Retention time: nicorandil, 35.3 min; (*N*-(2-hydroxyethyl)nicotinamide), 11.1 min; IS, 59.7 min [Ojha, Pargal 1999].

Quantification

Blood **HPLC** Column: C_{18} µBondapak (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : water : 0.1 mol/L borate buffer (pH 8) (15 : 70 : 15), flow rate 1 mL/min. IS: SG-89. UV detection (λ=220 nm). Retention time: 8 min. Limit of detection, 3 µg/L [Tanikawa *et al.* 1993].

Plasma **GC–MS** Limit of detection, 0.1 to 0.25 µg/L [Frydman 1992].

HPLC Column: Phenyl Nucleosil (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 6.2) : acetonitrile (10 : 3), flow rate 1.0 mL/min. MS detection (APCI, full scan). Retention time: 6 min. Limit of quantification, 1.0 µg/L [Andrensek *et al.* 1999].

Disposition in the Body Nicorandil is rapidly and well absorbed from the gastrointestinal tract, but food can decrease the rate of absorption. There is no significant first-pass metabolism. It is extensively metabolised, mainly via denitration and there are two predominant biotransformation pathways: the formation of nicotamide through degradation of the side chain and the production of *N*-(2-hydroxyethyl)nicotinamide from denitration, which eventually degrades to nicotinic acid. Further metabolism of these products leads to non-toxic water-soluble vitamin B complex substances. The kidney is the major route of elimination, with 20% of an administrated dose being excreted in urine mainly as metabolites. <2% of the dose is excreted via the biliary route. Ten percent of the administered dose can be found in plasma but it is rapidly eliminated.

Therapeutic Concentration After a 5 mg dose, a plasma concentration of 64 µg/L is achieved; 10 mg dose, 107 µg/L; 20 mg dose, 261 µg/L; and a 40 mg dose, 490 µg/L. The maximum plasma concentration occurs 30 to 60 min after oral administration. 8 to 24 h after dosing, the mean plasma concentration remains in the range 0.8

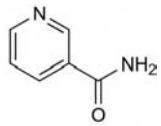
to 1.5 µg/L. There is a linear relationship between dose and increase in maximum plasma concentration. Steady state is achieved 96 to 120 h after continuous dosing.
Bioavailability 75 to 80%.
Half-life Approximately 1 h.
Volume of Distribution 1.0 to 1.4 L/kg; also reported as 100 L.
Clearance The total body clearance is 0.87 to 1.15 L/min (52 to 69 L/h).
Protein Binding Weakly bound to albumin and other plasma proteins at ~25% (19% to albumin).
Dose The initial dose is 10 mg twice daily; the usual dose is 10 to 20 mg twice daily with a maximum of 30 mg twice daily.

Andrensek S *et al.* (1999). Routine and sensitive method for determination of nicorandil in human plasma developed for liquid chromatography with ultraviolet and mass spectrometric detection. *J Chromatogr Biomed Sci Appl* 735(1): 103–109.
Frydman A (1992). Pharmacokinetic profile of nicorandil in humans: an overview. *J Cardiovasc Pharmacol* 20: 3S34–S44.
Ojha A, Pargal A (1999). Determination of nicorandil concentrations in human plasma using liquid chromatography. *J Pharm Biomed Anal* 21: 175–178.
Tanikawa M *et al.* (1993). Sensitive method for determination of nicorandil in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 617: 163–167.

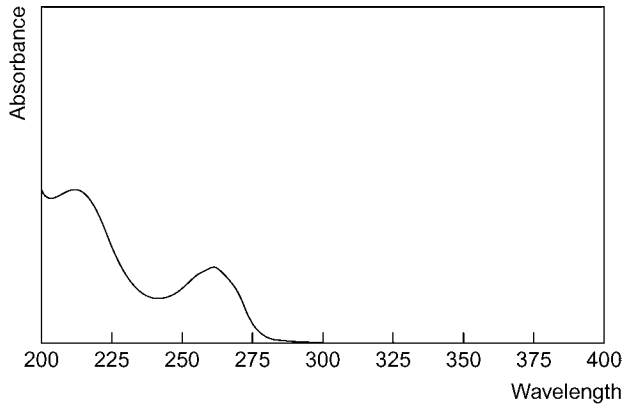
Nicotinamide

Vitamin
$C_6H_6N_2O$ = 122.1
CAS—98-92-0

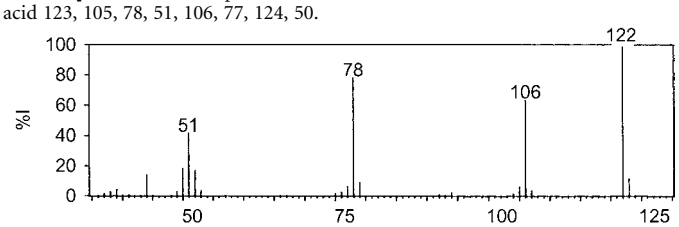
IUPAC Name 3-Pyridinecarboxamide
Synonyms Niacinamide; nicotinic acid amide; nicotylamide; vitamin PP.
Proprietary Names *Nacro*; *Nicobion*; *Nicotinoid*; *Nicovital*; *Papulex*; *Ucemine PP.*



Chemical Properties A white crystalline powder or colourless crystals. Mp 128° to 131°. Soluble 1 in 1 of water, 1 in 1.5 of ethanol and 1 in 10 of glycerol; slightly soluble in chloroform and ether. pK_a 3.3 (20°). Log *P* (octanol/water), −0.4.
Colour Tests Cyanogen bromide—orange; Nessler's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.54, streaking; system TB—R_f 0.00; system TC—R_f 0.21; system TE—R_f 0.40; system TL—R_f 0.27; system TAE—R_f 0.68; system TAF—R_f 0.66.
Gas Chromatography System GA—nicotinamide RI 1436, nicotinic acid RI 1335.
High Performance Liquid Chromatography System HX—RI 168.
Ultraviolet Spectrum Aqueous acid—261 nm (A^1_1=451a).



Infrared Spectrum Principal peaks at wavenumbers 1680, 1698, 703, 1618, 1594, 1026 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 122, 78, 106, 51, 50, 52, 44, 123; nicotinic acid 123, 105, 78, 51, 106, 77, 124, 50.



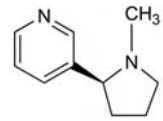
Quantification See also Nicotinic Acid.
Plasma **HPLC** For method of quantification for nicotinamide and *N*-methylnicotinamide, see Gillmor *et al.* [1999]. For method of quantification for nicotinamide and its metabolites, see Stratford, Dennis [1992]. Fluorescence detection. Limit of detection, <2 µg/L for *N*-methylnicotinamide [Somogyi *et al.* 1990].
Urine **HPLC** See Plasma [Stratford, Dennis 1992]. See Plasma [Somogyi *et al.* 1990].
Disposition in the Body It is excreted in the urine as unchanged drug, nicotinic acid, *N*-methylnicotinamide and nicotinuric acid. Nicotinamide is a metabolite of niketamide.
Therapeutic Concentration
In 15 subjects given 6 g of nicotinamide daily orally, a mean maximum plasma concentration of 1.166 mmol/L (range, 0.787 to 2.312) was achieved in 1 to 6 h (1 h in 54% of the subjects) [Bernier *et al.* 1998].
Twenty-two subjects given nicotinamide orally at a dose of 60 mg/kg (maximum 6 g) daily, had a mean peak plasma concentration of 0.793 mmol/L (range, 0.516 to 1.513) at 0.77 h (0.22 to 3.05); co-administration with domperidone did not significantly change these values [Bussink *et al.* 2002].
Dose Up to 250 mg daily.

Bernier J *et al.* (1998). Pharmacokinetics of nicotinamide in cancer patients treated with accelerated radiotherapy: the experience of the Co-operative Group of Radiotherapy of the European Organization for Research and Treatment of Cancer. *Radiother Oncol* 48: 123–133.
Bussink J *et al.* (2002). Pharmacology and toxicity of nicotinamide combined with domperidone during fractionated radiotherapy. *Radiother Oncol* 63: 285–291.
Gillmor HA *et al.* (1999). Measurement of nicotinamide and N-methyl-2-pyridone-5-carboxamide in plasma by high performance liquid chromatography. *Biomed Chromatogr* 13: 360–362.
Somogyi A *et al.* (1990). Determination of endogenous concentrations of N1-methylnicotinamide in human plasma and urine by high-performance liquid chromatography. *Anal Biochem* 187: 160–165.
Stratford MR, Dennis MF (1992). High-performance liquid chromatographic determination of nicotinamide and its metabolites in human and murine plasma and urine. *J Chromatogr* 582: 145–151.

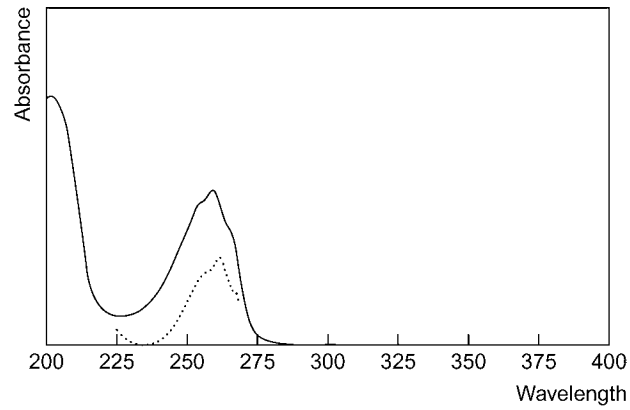
Nicotine

Insecticide, Aid to Smoking Cessation
$C_{10}H_{14}N_2$ = 162.2
CAS—54-11-5

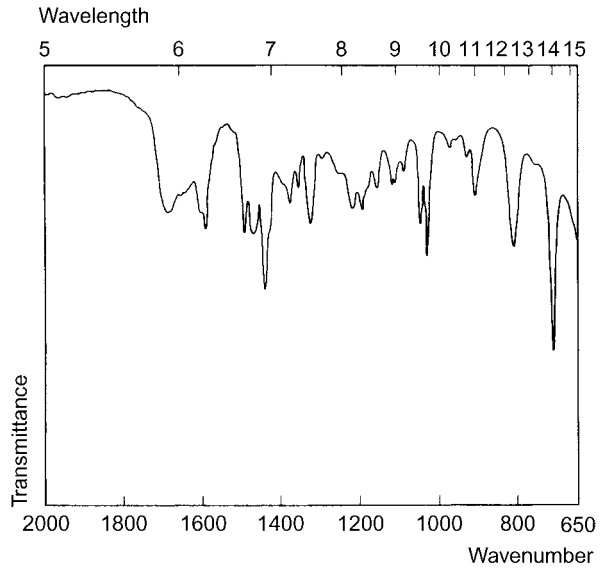
IUPAC Name 3-[(2*S*)-1-Methylpyrrolidin-2-yl]pyridine
Synonym 3-[(2*S*)-1-Methyl-2-pyrrolidinyl]pyridine
Proprietary Names *Commit*; *Habitrol*; *Nicabate*; *Nicoderm*; *Nicogum*; *Nicolan*; *Nicomax*; *Niconil*; *Nicopatch*; *Nicorette*; *Nicotinell*; *Nicotrans*; *Nicotrol*; *Nikofrenon*; *Nikotugg*; *NiQuitin*; *Prostep*; *Quit*; *Stoppers*; *Stubit*; *Tabazur* (anti-smoking preparations).



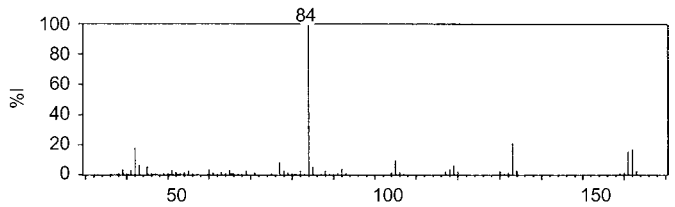
Chemical Properties An alkaloid obtained from the dried leaves of the tobacco plant, *Nicotiana tabacum* (Solanaceae). A colourless to pale yellow, very hygroscopic, oily liquid with an unpleasant pungent odour. It gradually becomes brown on exposure to air or light. Weight per millilitre ~1.01 g. Bp 247°, with decomposition. Refractive index 1.5280. Miscible with water below 60°; very soluble in ethanol, chloroform, petroleum ether, kerosene, oils and ether. pK_{a1} 3.2, pK_{a2}7.9 (25°). Log *P* (octanol/water), 1.2. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005]. Serum samples stable at -20° for 3 months [Baumann *et al.* 2010].
Colour Test Cyanogen bromide—orange.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.39; system TC—R_f 0.35; system TE—R_f 0.61; system TL—R_f 0.13; system TX—R_f 0.04; system TY—R_f 0.01; system TAE—R_f 0.39; system TAF—R_f 0.22; system TAJ—R_f 0.09; system TAK—R_f 0.00; system TAL—R_f 0.11 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).
Gas Chromatography System GA—RI 1350, RI 1715 M (cotinine); system GB—RI 1380, RI 1645 M (cotinine); system GC—RI 1573, RI 2111 M (cotinine); system GF—RI 1525, RI 2195 M (cotinine).
High Performance Liquid Chromatography System HA—*k* 1.1, *k* 0.2 M (cotinine); system HX—RI 69, RI 215 M (cotinine); system HZ—RT 1.73 min, M (cotinine) RT 1.71 min.
Ultraviolet Spectrum Aqueous acid—259 nm (A^1_1 = 338a); aqueous alkali—261 nm.



Infrared Spectrum Principal peaks at wavenumbers 712, 1022, 810, 1575, 1310, 1040 cm^{-1} (thin film).



Mass Spectrum Principal ions at *m/z* 84, 133, 42, 162, 161, 105, 77, 119 (nicotine); 98, 176, 42, 118, 41, 119, 51, 175 (cotinine); 84, 178, 161, 133, 118, –, –, – (nicotine-1′-*N*-oxide).



Quantification

Blood **LC-MS** Column: Atlantis dC_{18} (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 5 mmol/L ammonium acetate (pH 5.0) : acetonitrile (95 : 5 to 80 : 20 in 3 min to 10 : 90 in 2 min for 1 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.35 µg/L for cotinine [Hegstad *et al.* 2009].

Plasma **GC** [Nystrom *et al.* 1997]. Column: Carbowax 1000 100/120 mesh (28 m × 0.3 mm i.d., 0.1 µm). Carrier gas: H_2, 50 cm/s. Temperature programme: 40° for 1 min to 80° at 30°/min for 2 min to 230° at 10°/min for 10 min. FID. Limit of detection, 5 µg/L for nicotine and cotinine and 15 µg/L for nicotine *N*-oxides [Stehlik *et al.* 1982]. Column: WCOT fused silica (12 m 0.2 mm i.d.). Carrier gas: He, 0.7 mL/min. Temperature: 180°. AFID. Retention time: 1.16 min. Limit of detection, 1 µg/L for nicotine and 5 µg/L for cotinine [Verebey *et al.* 1982].

GC-MS Ion-trap detection. Limit of quantification, 10 µg/L for nicotine and 5 µg/L for cotinine (liquid–liquid extraction) and 1 µg/L for nicotine and cotinine (solid-phase extraction) [Cognard, Staub 2003]. Limit of detection, 1.0 µg/L for nicotine and cotinine [Shin *et al.* 2002]. Column: 5% phenyl methyl silicone HP-5 (25 m × 0.31 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 1 min to 123° at 60°/min to 155° at 5.8°/min to 200° at 8°/min. EI ionisation at 70 eV. Limit of quantification, 1 µg/L for nicotine and 2 µg/L for cotinine and mecamylamine [Jacob, *et al.* 2000]. Column: Easy Wax bonded polyethylene glycol (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 100° for 1 min to 220° at 20°/min for 2 min. SIM acquisition mode. Limit of quantification, 0.5 µg/L [Davoli *et al.* 1998].

HPLC Column: Partisil-10 SCX cation exchange. Mobile phase: sodium phosphate buffer : methanol (pH 4.8, 92 : 8), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 µg/L for caffeine, 3′-hydroxycotinine and cotinine, and 20 µg/L for total 3′-hydroxycotinine [Ghosheh *et al.* 2000]. Column: Hichrome $5C_{18}$ (150 × 4.6 mm i.d., 5 µm). Mobile phase: 7% methanol : 2 mmol/L sodium dihydrogen phosphate : 0.1% phosphoric acid : 1 mmol/L heptanosulfonate sodium. UV detection (λ = 260 nm). Limit of quantification, 0.2 µg/L for nicotine and 1.0 µg/L for cotinine [Nakajima *et al.* 2000].

LC-MS Column: Discovery HS F5 (100 × 4.6 mm i.d., 3 µm). Mobile phase: 10 mmol/L ammonium acetate with 0.001% formic acid (pH 4.97) : methanol (85 : 15 to 24 : 76 in 11 min to 85 : 15 after 11.6 min for 3.4 min). ESI, MRM acquisition mode. Retention time: 7.23 min. Limit of quantification, 1.0 µg/L for nicotine and metabolites, 50 µg/L for cotinine-*N*-β-glucuronide, limit of detection, 0.25 µg/L for nicotine and metabolites, 25 µg/L for cotinine-*N*-β-glucuronide [Miller *et al.* 2010]. Mobile phase: 100 mmol/L ammonium formate (pH 3.0) : acetonitrile (15 : 85), flow rate 0.54 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.64 min. Limit of quantification, 1 to 2 µg/L, limit of detection, 1 µg/L [Yue *et al.* 2010]. Column: Synergi Hydro RP (75 × 2.0 mm i.d., 4 µm). Mobile phase: 1 mmol/L ammonium acetate- 0.01% formic acid : 0.01% formic acid in acetonitrile (35 : 65), flow rate 400 µL/min. TIS. Limit of quantification, 1 µg/L, limit of detection, 0.5 µg/L [Shakleya, Huestis 2009]. Column: C_8 base select separation column. Mobile phase: acetonitrile : ammonium formate (pH 3.5). Limit of quantification, 50 µg/L (using APCI) and 1 µg/L (using ESI) for nicotine and cotinine [Beyer *et al.* 2007]. Column: Atlantis HILIC (100 × 0.3 mm i.d., 5 µm). Mobile phase: 7.5 mmol/L ammonium acetate : acetonitrile (23 : 77) containing 0.4% acetic acid, flow rate 15 µL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.15 µg/L for deuterium-labelled D_2-nicotine and 0.25 µg/L for D_2-cotinine [Murphy *et al.* 2007]. See also Kim, Huestis [2006] and Taylor *et al.* [2004]

Serum **GC-MS** See Plasma [Cognard, Staub 2003]. Column: DB-5M5 (15 m × 0.32 mm i.d., 1.0 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 85° to 300° at 30°/min. EI ionisation, SIM acquisition mode. Limit of detection, 2 µg/L [Baskin *et al.* 1998]. Limit of quantification, 1.25 µg/L, limit of detection, 0.16 µg/L for nicotine and cotinine [James *et al.* 1998].

HPLC DAD. Nicotine and cotinine [Wiergowski *et al.* 2006]. Column: Supelcosil LC_8DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: water-acetonitrile (96.4 : 3.6) containing 2 mL/L TEA and 0.012 mol/L sodium heptanosulfonate, dipotassium hydrogen phosphate and citric acid (pH 4.7) : water-acetonitrile (80.3 : 19.7) containing 2 mL/L TEA, 0.012 mol/L sodium heptanosulfonate, dipotassium hydrogen phosphate and citric acid (pH 5.2, 100 : 0 for 15 min to 50 : 50 at 20 min), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 µg/L for nicotine, 3 µg/L for cotinine *N*-oxide and 5 µg/L for cotinine, *trans*-3′-hydroxycotinine and norcotinine [Zuccaro *et al.* 1993].

LC-MS Column: X-Bridge C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water (20 : 80), flow rate 0.2 mL/min. CID, positive ion mode. Limit of quantification, 2 µg/L for nicotine and 5 µg/L for cotinine [Baumann *et al.* 2010]. See Plasma [Yue *et al.* 2010]. Column: Betasil Diol-100 (50 × 2.1 mm i.d., 5 µm). Mobile phase: 2 mmol/L ammonium formate in methanol : acetonitrile (35 : 65), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.8 µg/L [Byrd *et al.* 2005]. Column: Polyhydroxyethyl A (100 cm × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile : ammonium formate : formic acid (200 : 1800 : 1.3 : 15), flow rate 1.0 mL/min. API, positive ion mode. Limit of quantification, 0.5–5 µg/L for nicotine and its metabolites [Moyer *et al.* 2002]. Column: Symmetry C_{18} (75 × 4.6 mm i.d., 3.5 µm). Mobile phase: methanol : 9.2 mmol/L ammonium acetate (pH 5.0, 30 : 70). APCI, MRM acquisition mode. Limit of detection, ~0.1 µg/L for cotinine [Bernert, Jr *et al.* 2000].

Note For a radioimmunoassay and a TLC method see Wielkoszynski *et al.* [2009].

Urine **GC** Column: HP 5% phenyl methyl silicone (25 m × 0.32 mm i.d., 0.52 µm). Temperature programme: 90° for 0.5 min to 180° at 25°/min to 220° at 10°/min to 275° at 25°/min. NPD. Limit of quantification, 1 µg/L for *trans*-3′-hydroxycotinine [Jacob *et al.* 1992]. FID. Limit of detection, 0.03 mg/L for nicotine and cotinine and 0.1 mg/L for nicotine *N*-oxides [Stehlik *et al.* 1982].

GC-MS Column: DB-5MS. EI ionisation. Limit of quantification, 0.2 µg/L for cotinine and 1.25 mg/L for phenylglyoxylic acid and mandelic acid [Wu *et al.* 2009]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 14.5 psi. Temperature programme: 70° to 230° at 25°/min for 1 min. SIM acquisition mode. Limit of detection, 0.2 µg/L for nicotine, 0.5 µg/L for cotinine [Man *et al.* 2006]. Column: DB-1MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 32 cm/min. Temperature programme: 40° for 1 min to 130° at 70°/min for 1 min to 270° at 25°/min for 1 min. Limit of quantification, 0.10 mg/L for nicotine and cotinine and 1 mg/L for thiocyanate, limit of detection, 0.6 µg/L for nicotine and cotinine and 0.06 µg/L for thiocyanate [Toraño, van Kan 2003]. Limit of detection, 0.2 µg/L for nicotine and cotinine [Shin *et al.* 2002]. Column: DB-5 MS (15 m × m i.d., 1 µm). Carrier gas: He, 35 kPa. Temperature programme: 85° for 0.5 min to 300° at 30°/min. SIM acquisition mode. Limit of quantification, 20 µg/L for cotinine and 50 µg/L for *trans*-3′-hydroxycotinine [Ji *et al.* 1999]. See Serum [Baskin *et al.* 1998; James *et al.* 1998]. See also Jacob *et al.* [1992].

HPLC UV detection. Nicotine and its metabolites [Piekoszewski *et al.* 2009]. Column: LiChrospher 100 C_{18} (250 × 4 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 4.0) : acetonitrile (85 : 15 to 50 : 50 at 60 min), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: 3.1 min. Limit of quantification, 50 µg/L [Rabbaa-Khabbaz *et al.* 2006]. See Serum [Wiergowski *et al.* 2006]. Column: reversed phase. Mobile phase: acetate buffer (0.03 mol/L sodium acetate and 0.1 mol/L acetic acid, pH 3.1) : acetonitrile containing 0.02 mol/L sodium octanosulfonate (78 : 22). Limit of detection, 5 µg/L for nicotine and 2 µg/L for cotinine [Doctor *et al.* 2004]. Column: C_8 Symmetry (150 × 3.9 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile in potassium dihydrohen phosphate buffer (pH 3.4, 81 : 10 : 9), flow rate 1 mL/min. DAD (λ = 254 nm). Limit of detection, 5 µg/L [Ceppa *et al.* 2000]. UV detection. Limit of quantification, 5 µg/L for nicotine and 0.5 µg/L for cotinine [Oddoze *et al.* 1998]. Column LC_8DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (80 : 9) containing 5 mL TEA, 670 mg/L sodium heptanosulfonate, 0.034 mol/L potassium dihydrogen phosphate and 0.034 mol/L citric acid (pH 4.4), flow rate 1.6 mL/min. Nicotine and its metabolites [Zuccaro *et al.* 1995]. See also Maskarinec *et al.* [1978].

LC-MS See Plasma. Limit of quantification, 2.5 µg/L for nicotine and metabolites, 50 µg/L for cotinine-*N*-β-glucuronide, limit of detection, 1.0 µg/L for nicotine

and metabolites, 25 μg/L for cotinine-*N*-β-glucuronide [Miller *et al.* 2010]. See Plasma [Yue *et al.* 2010]. Column: Synergi 4 μm POLAR-RP 80A. Mobile phase: 5 mmol/L ammonium formate : methanol (55 : 45), flow rate 0.8 mL/min. ESI, positive mode. Limit of detection, 15–40 ng/L for nicotine, cotinine and related alkaloids [Kataoka *et al.* 2009]. Column: Phenomenex SCX (4.0 × 3.0 mm i.d., 4 μm). Mobile phase: 2 mmol/L ammonium acetate with 0.1% formic acid : methanol containing 2 mmol/L ammonium acetate with 0.1% formic acid : 100 mmol/L ammonium acetate with 0.1% formic acid (70 : 20 : 10 to 0 : 10 : 90 at 0.1 min to 70 : 20 : 10 at 0.7 min for 0.1 min to 10 : 80 : 10 at 1.9 min to 70 : 20 : 10 at 2 min), flow rate 0.6 mL/min except 0.8 mL/min at 0.1 min. ESI, MRM acquisition mode. Limit of quantification, 2.5 μg/L, limit of detection, 0.156 μg/L for cotinine [Chadwick, Keevil 2007]. Column: Restek pentafluorophenyl-propyl (100 × 3.2 mm i.d.). Mobile phase: 2 mmol/L ammonium acetate-10 mmol/L acetic acid in water : 2 mmol/L ammonium acetate-10 mmol/L acetic acid in 99.5% methanol (95 : 5 to 30 : 70 in 2 min), flow rate 1 mL/min to 600 μL/min after 2 min. API. Limit of quantification, 0.53 μg/L [Hoofnagle *et al.* 2006]. Limit of detection, 8 ng/mL for N^7-methylguanine. Cotinine also quantified [Chao *et al.* 2005]. See Serum [Moyer *et al.* 2002]; See also Heavner *et al.* [2005], Meger *et al.* [2002], Xu *et al.* [2004]. See also Hecht *et al.* [1999] and Tuomi *et al.* [1999].

Molecularly imprinted SPE-spectrophotometry Limit of quantification, 1.1 μmol/L [**Figueiredo *et al.* 2009**].

Meconium **HPLC** Column: Lichrosorb C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol : acetate buffer (pH 4.66) : acetonitrile : acetic acid (pH 4.3, 50 : 29 : 20 : 2 : 1), flow rate 1.0 mL/min. DAD. Limit of detection, 10 μg/L [Baranowski *et al.* 1998]

Oral Fluid **GC-MS** See Urine [Toraño, van Kan 2003]. See Plasma [Shin *et al.* 2002].

HPLC See Serum [Wiergowski *et al.* 2006].

LC-MS See Urine [Kataoka *et al.* 2009]. See Serum [Bernert, Jr*et al.* 2000; Byrd *et al.* 2005].

Pericardial Fluid **LC-MS** See Blood [Hegstad *et al.* 2009].

Adipose Tissue **GC-MS** See Kidney [Urakawa *et al.* 1994].

Hair **GC** Column: HP-101 (12.5 m × 0.2 mm i.d., 0.2 μm). Temperature programme: 50° for 2.5 min to 210° at 9°/min. NPD. Retention time: 10.9 min. Limit of quantification, 1.5 μg/L [Mizuno *et al.* 1993].

GC-MS See Urine [Toraño, van Kan 2003]. Column: HP-1 methyl silicone (12 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 30 cm/s. Temperature programme: 60° for 1 min to 150° at 30°/min for 3 min. SIM acquisition mode. Limit of detection, 0.02 μg/g [Zahlsen, Nilsen 1994].

HPLC See Serum [Wiergowski *et al.* 2006]. Column: Prodigy ODS(3) (150 × 4.6 mm i.d., 5 μm). Mobile phase: 50 mmol/L potassium dihydrogen phosphate : methanol : acetonitrile (93.5 : 2.5 : 4), flow rate 1.0 mL/min. Electrochemical detection. Limit of quantification, 0.1 ng/mg, limit of detection, <0.05 ng/mg [Mahoney, Al Delaimy 2001]. Column: LC_8DB (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile (94.6 : 5.3) containing 3 mL/L TEA and 0.002 mol/L sodium heptanosulfonate, 0.018 mol/L dipotassium hydrogen phosphate and 0.013 mol/L citric acid (pH 4.7). UV detection (λ = 254 nm). Limit of detection, 0.2 ng/mg [Pichini *et al.* 1997].

Kidney **GC-MS** Column: Shimadzu CBP1 bonded methyl silicone (12 m × 0.53 mm i.d., 1.0 μm). Carrier gas: He, 40 mL/min. Temperature programme: 90° to 220° at 40°/min. EI ionisation, SIM acquisition mode. Limit of detection, 5 μg/g for nicotine and 10 μg/g for cotinine [Urakawa *et al.* 1994].

Liver **GC-MS** See Kidney [Urakawa *et al.* 1994].

Lung **GC-MS** See Kidney [Urakawa *et al.* 1994].

Muscle **GC-MS** See Kidney [Urakawa *et al.* 1994].

Nail Clippings **LC-MS** Column: Zorbax SB C_{18} (150 × 0.5 mm i.d., 3.5 μm). Mobile phase: methanol : water (35 : 65), flow rate 10 μL/min. ESI, positive ion mode. Limit of detection, 0.02 pg/mg for N′-nitrosonorcornitine [Stepanov, Hecht 2008]. Column: Zorbax SB C_{18} (150 × 0.5 mm i.d., 3.5 μm). Mobile phase: methanol : water (35 : 65), flow rate 10 μL/min. ESI, SRM acquisition mode. Limit of detection, 0.01 μg/g for nicotine, 0.012 μg/g for cotinine and 0.02 ng/g for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) [Stepanov *et al.* 2006].

Spleen **GC-MS** See Kidney [Urakawa *et al.* 1994].

Note For procedures to assess tobacco smoke exposure, see Dhar [2004].

Disposition in the Body Nicotine is readily absorbed from the gastrointestinal tract, the buccal mucosa, the respiratory tract and from intact skin; it is widely distributed throughout the tissues. Nicotine undergoes first-pass metabolism when administered orally, thus reducing its bioavailability. It is widely distributed, crossing the placental barrier and it is also found in breast milk. It is metabolised by oxidation via cytochrome P450 isozyme CYP2A6 to cotinine and nicotine-1′-*N*-oxide, followed by further degradation to produce hydroxycotinine, norcotinine, and a ring cleavage product. Approximately 5% is excreted unchanged in the urine in 24 h and ~10% as cotinine; the excretion of unchanged drug is decreased if the urine is alkaline.

Blood Concentration

In a group of 150 habitual smokers, nicotine concentrations in samples of plasma withdrawn 2 min after smoking were in the range 0.003–0.063 mg/L (mean, 0.019); corresponding cotinine concentrations ranged from 0.02–0.26 mg/L (mean, 0.22) [Kogan *et al.* 1981].

Six smokers received a single dose of nicotine (1 mg nasal spray; average dose absorbed 0.8 mg; range, 0.7–1.0) and 6 others smoked cigarettes (1 puff per min for 10 min; average dose absorbed 4.4 mg; range, 1.5–3.2); all subjects also received a 30 min IV infusion of nicotine (average dose 5.1 mg; range, 4.1–6.3) beginning 70 min after administration of the spray or commencement of smoking. In the subjects who received cigarettes, a mean peak plasma concentration of 0.0398 mg/L was attained at 8.2 min in arterial blood and 0.0186 mg/L at 11.9 min in venous blood. In the subjects who received nicotine intranasally, these values were 0.0104 mg/L in 4.7 min in arterial blood and 0.0054 mg/L in 24.8 min in venous blood. Mean peak plasma concentrations in arterial and venous blood after the IV infusion were 0.049 mg/L in 29 min and 0.0295 mg/L in 30 min, respectively [Gourlay, Benowitz 1997].

Ten non-smoking patients with ulcerative colitis received nicotine tartrate liquid enema at a dose of 3 mg nicotine base nightly for 1 week, then 6 mg for 3 weeks. Only 1 patient had a detectable peak nicotine concentration (0.0023 mg/L) and none had a detectable trough concentration; the mean trough plasma cotinine concentration was 0.012 mg/L [Sandborn *et al.* 1997].

A heavy smoker who abused lozenges containing nicotine (~240 lozenges of 0.4 mg nicotine each daily) had saliva and blood cotinine levels of 1.154 and 0.947 mg/L, respectively. At 20 min after his 70th 0.4 mg lozenge of the day, his plasma nicotine concentration was 0.0321 mg/L [Foulds *et al.* 1998].

In 8 non-smoking subjects who consumed 8 lozenges of 1.1 mg nicotine, mean nicotine levels increased from 0.0003–0.0064 mg/L [Foulds *et al.* 1998].

After 25 subjects were administered nicotine via transdermal patches (1 patch of 15 mg for 16 h coverage, or 2 different 21 mg 24 h patches; each patch applied for 3 consecutive days), mean minimum and maximum plasma nicotine concentrations of 0.00153 and 0.0123 mg/L were reported for the 15 mg patch, 0.013 and 0.0195 mg/L for the first 21 mg patch and 0.0119 and 0.0278 mg/L for the second 21 mg patch. The times taken to reach peak plasma concentration was 6.0, 8.0, and 2.8 h, respectively, for the 3 patches [Fant *et al.* 2000].

Plasma nicotine levels in 5 healthy subjects given nicotine as infusions over 0.5–5 min were dose dependent: 11.5–14.1 μg/L and 19.02–26.3 μg/L with a 0.75 and 1.5 mg dose, respectively. They were generally highest with the shortest infusion time at each dose. Oral fluid levels were highest at the longest infusion time for each dose (19.1–49.1 μg/L and 26–104 μg/L with the 0.75 and 1.5 mg doses, respectively) [Jenkins *et al.* 2005].

Average plasma and urine levels of nicotine in smokers were found to be 1263.1 μg/L and 1618 μg/L, respectively. Cotinine levels in plasma and urine were 379.4 and 865 μg/L, respectively [Massadeh *et al.* 2009].

Toxicity Nicotine is highly toxic and, in acute poisoning, death may occur within a few minutes because respiratory failure arises from paralysis of the respiratory muscles. The lethal dose for an adult is between 40 and 60 mg. Blood concentrations >5 mg/L may be fatal. The maximum permissible atmospheric concentration is 0.5 mg/m^3.

In 5 adults who ingested 20–25 g nicotine and died within 1 h, postmortem blood concentrations were between 11 and 63 mg/L (mean, 29) [Baselt, Cravey 1977].

Nine cases of self-poisoning associated with dermal exposure to 2–20 transdermal nicotine patches has been reported; all subjects recovered. Plasma nicotine/cotinine levels did not correlate with severity of toxicity [Woolf *et al.* 1996].

A report describes 36 exposures to transdermal nicotine in children aged under 16 years; 18 children had dermal exposure, and the other 18 children had bitten, chewed or swallowed part of a patch. Symptoms after dermal exposure were associated with an estimated dose of at least 10 mg nicotine [Woolf *et al.* 1997].

In 8 smokers who committed suicide, levels of nicotine and cotinine in blood were 115 and 405 μg/L and in urine 1940 and 1170 μg/L, respectively. Lower blood levels of nicotine and cotinine (33.2 and 140 μg/L, respectively) and lower urine levels (246 and 179 μg/L, respectively) were found in 5 smokers who did not commit suicide [Moriya, Hashimoto 2004].

Another study also found higher levels of nicotine and cotinine in smokers who committed suicide than in non-suicide smokers [Moriya *et al.* 2007]. High postmortem femoral muscle nicotine and cotinine levels of 213 and 488 μg/g, respectively, suggesting possible suicide, were found in an adipocere body drowned ~5 months earlier [Nishimura *et al.* 2009].

Nicotine and cotinine femoral blood levels of 0.6 and 2 mg/L were found in a subject who had cardiorespiratory failure induced by the additive effects of tramadol and nicotine following self-poisoning [Solarino *et al.* 2010a].

In a fatal poisoning caused by nicotine (as ingested tobacco leaves) and methomyl, nicotine levels in blood ranged from 222–733 μg/L. The stomach contained 170 g of greenish liquid containing a small amount of shredded tobacco leaves [Moriya, Hashimoto 2005].

Life-threatening nicotine intoxication is reported to occur after ingestion of more than 6 cigarettes. However, a case of non-life-threatening toxicity occurred in a 35-year-old man after ingestion of between 7 and 20 cigarettes [Metzler *et al.* 2005].

A 42-year-old man who was found dead had high postmortem femoral venous blood concentrations of nicotine and cotinine (2.2 mg/L) [Solarino *et al.* 2010b].

A 27-year-old nurse survived after self-administering an IV injection of 5 mL of cigarette soakage solution containing approximately 5.7 mg nicotine [Hagiya *et al.* 2010].

For other reports of suicide attempts with transdermal nicotine, see Engel, Parmentier [1993], Montalto *et al.* [1994] and Labelle, Boulay [1999].

Bioavailability ≈30% (oral).

Half-life After inhalation or parenteral administration, plasma half-life, nicotine 0.5–2 h, cotinine 6–16 h (mean, 11).

Volume of Distribution ≈1–3 L/kg.

Clearance Plasma clearance, 10–30 mL/min/kg.

Protein Binding ≈5%.

Note For a review of the clinical pharmacokinetics of the nicotine inhaler, see Schneider *et al.* [2001]; for a review of the pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders, see Balfour, Fagerstrom [1996]; for a review of the clinical pharmacology of nicotine, see Zevin *et al.* [1998]; for a review of the metabolism of nicotine, see Kyerematen, Vesell [1991]; for a report of unintentional child poisonings from ingestion of conventional and novel tobacco products, see Connolly *et al.* [2010].

Dose As an aid to smoking withdrawal, dose depends on the level of smoking and formulation of preparation used.

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Nicotinic Acid

Vitamin, Vasodilator

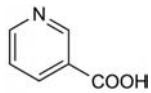
$C_6H_5NO_2$ = 123.1

CAS—59-67-6

IUPAC Name 3-Pyridinecarboxylic acid

Synonym Niacin

Proprietary Names *Hipocol*; *Natinate*; *Niacor*; *Niaspan*; *Nicangin*; *Nico-400*; *Nicobid*; *Nico-Span*; *Nicotinex*; *Pepevit*; *Slo-Niacin*.



Chemical Properties White or creamy-white crystals or crystalline powder. Mp 234° to 237°. Soluble 1 in 60 of water and 1 in 100 of ethanol; very slightly soluble in chloroform; insoluble in ether; freely soluble in boiling water and alcohol and in solutions of alkali hydroxides and carbonates. pK_a 2.0 (-N=), 4.8 (-COOH), (25°). Log *P* (octanol/water), 0.4.

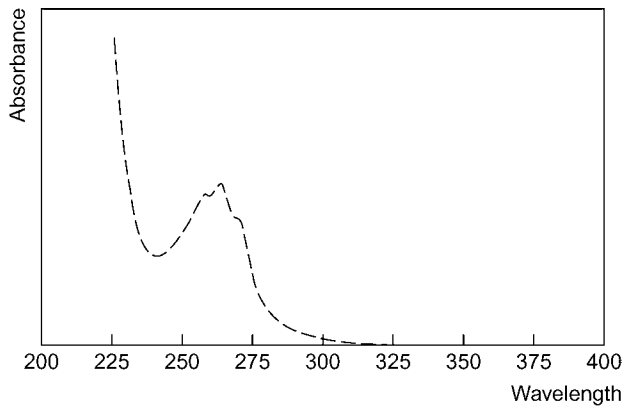
Colour Test Cyanogen bromide—orange.

Thin-layer Chromatography System TA—R_f 0.58; system TC—R_f 0.17; system TD—R_f 0.01; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.04; system TAE—R_f 0.72; system TAJ—R_f 0.04; system TAK—R_f 0.10; system TAL—R_f 0.56.

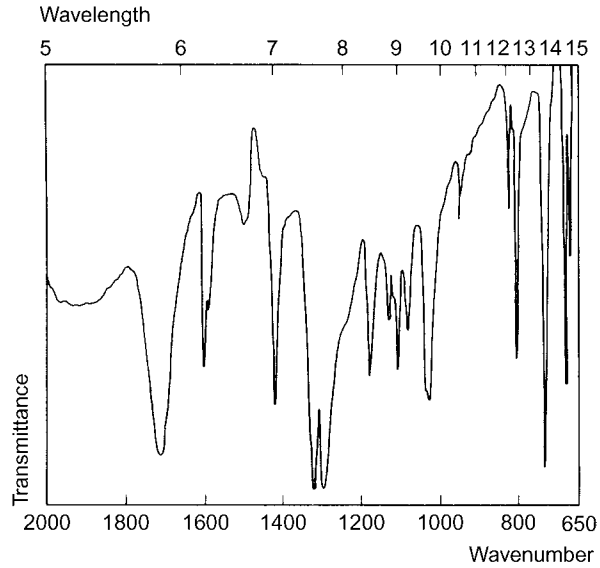
Gas Chromatography System GA—nicotinic acid RI 1335, nicotinic acid-(Me) RI 1390.

High Performance Liquid Chromatography System HAA—retention time 3.2 min.

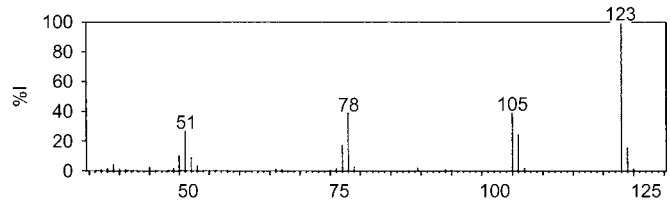
Ultraviolet Spectrum Methanol—263 nm (A^1_1=229a).



Infrared Spectrum Principal peaks at wavenumbers 1300, 744, 1710, 1042, 694, 1180 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 123, 105, 78, 51, 106, 77, 124, 50; *N*-methylnicotinamide 78, 106, 135, 136, 51, 50, 79, 52.



Quantification See also Nicotinamide.

Plasma **HPLC** For method of quantification for nicotinic acid, nicotinamide, and nicotinuric acid, see Miyauchi *et al.* [1993]. Limit of detection, 100 µg/L for nicotinic acid, 500 µg/L for nicotinuric acid, and 1 mg/L for nicotinamide [Takikawa *et al.* 1982]. UV detection. Limit of detection, 500 µg/L for nicotinic acid and nicotinuric acid [Hengen *et al.* 1978].

Urine **HPLC** See Plasma [Hengen *et al.* 1978].

Tissues **GC–MS** Limit of detection, <1 fmol for nicotinic, quinolinic, and picolinic acids [Smythe *et al.* 2002].

Disposition in the Body Readily absorbed after oral administration. Metabolised to *N*-methylnicotinamide, *N*-methyl-6-oxo-pyridine-3-carboxamide, *N*-methyl-4-oxopyridine-3-carboxamide, and by glycine conjugation to nicotinuric acid. It is rapidly excreted in the urine, and after administration of therapeutic doses about 34% is excreted unchanged in 6 h.

Nicotinic acid is a metabolite of nicergoline, nicotinamide, and nicotinyl tartrate.

Therapeutic Concentration

After a single oral dose of 300 mg to 5 subjects, peak plasma concentrations of 3.5 to 18.4 mg/L (mean, 9.3) were attained in about 40 min [Lesne *et al.* 1976].

Half-life Plasma half-life, about 0.3 to 0.8 h.

Distribution in Blood Plasma : whole blood ratio, 0.01.

Note For a review of the clinical pharmacology and pharmacokinetics of nicotinic acid, see Weiner [1979]; for a survey of nicotinic acid and its derivatives, see Hotz [1983]; for a review of the pharmacokinetics of nicotinic acid, see Gugler [1978].

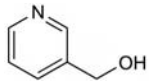
Dose Up to 500 mg daily.

Gugler R (1978). Clinical pharmacokinetics of hypolipidaemic drugs. *Clin Pharmacokinet* 3: 425–439.
Hengen N *et al.* (1978). High-performance liquid-chromatographic determination of free nicotinic acid and its metabolite, nicotinuric acid, in plasma and urine. *Clin Chem* 24: 1740–1743.
Hotz W (1983). Nicotinic acid and its derivatives: a short survey. *Adv Lipid Res* 20: 195–217.
Lesne M *et al.* (1976). [Comparative pharmacokinetic study of 2 galenic forms of nicotinic acid]. *Pharm Acta Helv* 51: 367–370.
Miyauchi Y *et al.* (1993). Simultaneous determination of nicotinic acid and its two metabolites in human plasma using solid-phase extraction in combination with high performance liquid chromatography. *Int J Vitam Nutr Res* 63: 145–149.
Smythe GA *et al.* (2002). Concurrent quantification of quinolinic, picolinic, and nicotinic acids using electron-capture negative-ion gas chromatography-mass spectrometry. *Anal Biochem* 301: 21–26.
Takikawa K *et al.* (1982). High-performance liquid chromatographic determination of nicotinic acid and its metabolites, nicotinuric acid and nicotinamide, in plasma. *J.Chromatogr* 233: 343–348.
Weiner M (1979). Clinical pharmacology and pharmacokinetics of nicotinic acid. *Drug Metab Rev* 9: 99–106.

Nicotinyl Alcohol

Vasodilator
C_6H_7NO = 109.1
CAS—100-55-0

IUPAC Name 3-Pyridinemethanol
Synonyms 3-Hydroxymethylpyridine; nicotinic alcohol; 3-pyridinemethanol; β-pyridylcarbinol.

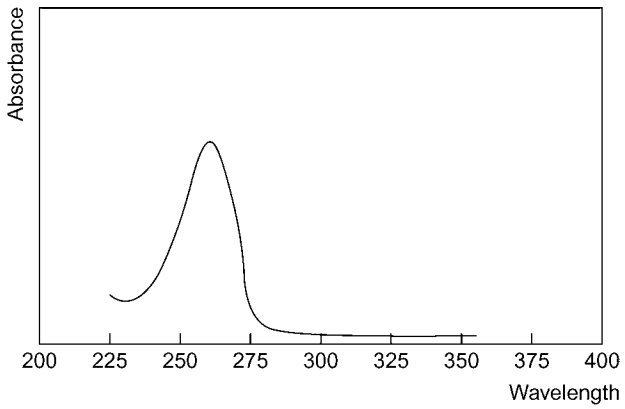


Chemical Properties A very hygroscopic liquid. Refractive index 1.5425. Freely soluble in water and ether; sparingly soluble in petroleum ether. Log *P* (octanol/water), 0.0.

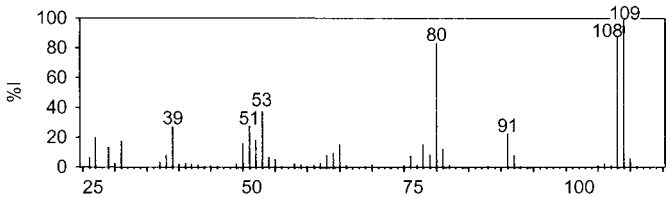
Nicotinyl Tartrate

$C_6H_7NO,C_4H_6O_6$ = 259.2
CAS—6164-87-0
Proprietary Names *Niltuvin*; *Radecol*; *Roniacol*; *Ronicol*.
Chemical Properties A white crystalline powder. Mp 147° to 148°. Soluble in water, ethanol and ether.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.04; system TC—R_f 0.17; system TE—R_f 0.45; system TL—R_f 0.22; system TAE—R_f 0.74; system TAF—R_f 0.69 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—nicotinyl alcohol RI 1215, nicotinic acid RI 1335.
High Performance Liquid Chromatography System HX—RI 55.
Ultraviolet Spectrum Aqueous acid—260 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 675, 1262, 1305, 1556, 1215, 788 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 109, 108, 80, 53, 51, 39, 91, 27; nicotinic acid 123, 105, 78, 51, 106, 77, 124, 50.

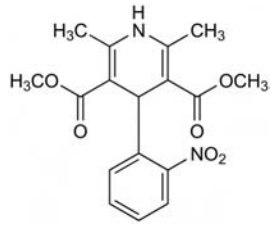


Disposition in the Body Nicotinyl tartrate is absorbed after oral administration and partly metabolised to nicotinic acid. It is excreted in the urine.
Dose The equivalent of 100 to 200 mg of nicotinyl alcohol daily; up to 600 mg daily given as a sustained-release preparation.

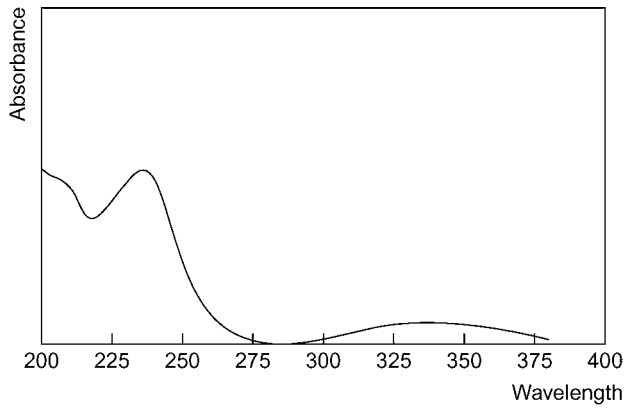
Nifedipine

Antianginal Vasodilator
$C_{17}H_{18}N_2O_6$ = 346.3
CAS—21829-25-4

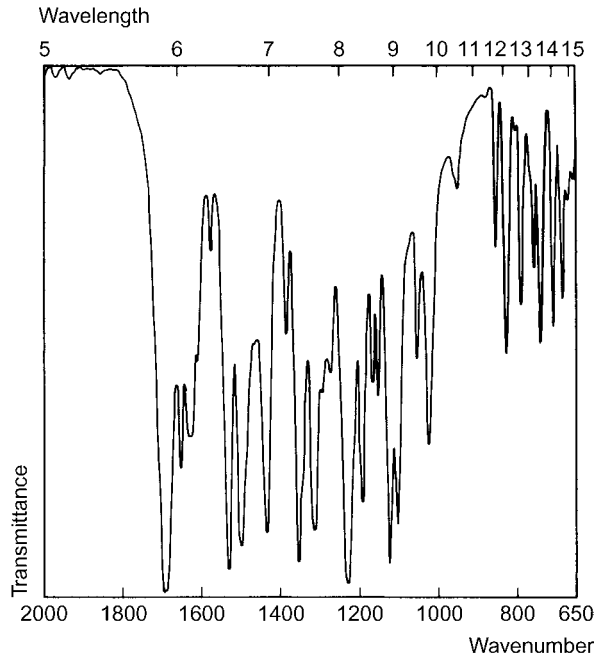
IUPAC Name Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate
Synonyms Bay-a-1040; nifedipina; nifedipinum.
Proprietary Names *Adalat(e)*; *Adipine*; *Angiopine*; *Calanif*; *Calcilat*; *Cardilate MR*; *Coracten*; *Coroday*; *Fortipine*; *Hypolar Retard*; *Nifed*; *Nifedical*; *Nifedipress*; *Nifedotard*; *Nifelease*; *Nifopress*; *Nimodrel*; *Nivaten*; *Pinifed*; *Procardia*; *Slofedipine*; *Systepin*; *Tensipine*; *Unipine XL*; *Vasofed*.



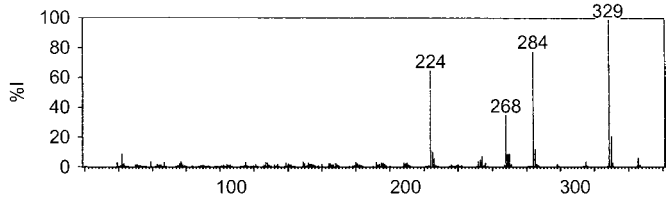
Chemical Properties A yellow crystalline powder. Mp 172° to 174°. Practically insoluble in water; slightly soluble in ethanol; soluble in acetone and chloroform. Log *P* (octanol/water), 2.2. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Methanolic potassium hydroxide—orange; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.01; system TC—R_f 0.65; system TE—R_f 0.71; system TL—R_f 0.68; system TAE—R_f 0.79.
Gas Chromatography System GA—nifedipine RI 2545, M (dehydro-) RI 2250, M (dehydro-COOH-) RI 2290; system GB—nifedipine RI 2708, M (dehydro-) RI 2370; system GP—M (dehydro-) RI 2255, M (dehydro-2-COOH-)-Me RI 2695.
High Performance Liquid Chromatography System HA—*k* 0.2; system HX—RI 527; system HY—RI 464; system HZ—retention time 7.2 min; system HAA—retention time 19.5 min; system HAX—retention time 7.4 min; system HAY—retention time 7.9 min.
Ultraviolet Spectrum Aqueous acid—238 (A^1_1=595b), 338 nm (A^1_1=165b). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1690, 1225, 1527, 1120, 1496, 1310 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 329, 284, 224, 268, 330, 285, 225, 270.



Quantification

Plasma **GC** Limit of detection, 0.5 µg/L [Le Guellec *et al.* 1992]. ECD. Limit of detection, 1 µg/L for nifedipine and dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate [Dokladalova *et al.* 1982].

HPLC Limit of detection, 3 µg/L [Abou-Auda *et al.* 2000]. UV detection. Limit of detection, 1 µg/L [Grundy *et al.* 1994]. Limit of quantification, 7 µg/L [Thongnopnua, Viwatwongsa 1994]. UV detection. For method, see Sadanaga *et al.* [1982].

HPLC–MS Limit of quantification, 0.5 µg/L [Dankers *et al.* 1998].

Serum **GC–MS** Limit of detection, 2 µg/L [Martens *et al.* 1994].

HPLC UV detection. Limit of detection, 3 µg/L [Zaater *et al.* 2000]. For method, see Goldnik *et al.* [1996]. Comparison with GC. Limit of detection, 5 µg/L [Jankowski, Lamparczyk 1994].

Gingival Crevicular Fluid **GC** ECD. For method, see Ellis *et al.* [1993].

Disposition in the Body Rapidly and almost completely absorbed after oral or sublingual administration. It is completely metabolised, undergoing first-pass metabolism. It is found in breast milk. Between 70 and 90% of a dose is excreted in the urine in 24 h as inactive metabolites, including 5-methoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3-carboxylic acid, dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate, and 2-hydroxymethyl-5-methoxycarbonyl-6-methyl-4-(2-nitrophenyl)pyridine-3-carboxylic acid, and its lactone derivative. Up to 15% of a dose is eliminated in the faeces as metabolites in 4 days.

Therapeutic Concentration

Following a single oral dose of 10 mg to 9 subjects, a mean peak plasma concentration of 0.07 mg/L was attained in about 0.6 h; in a further 3 subjects, absorption was much slower with peak plasma concentrations reported at 2 to 6 h [Foster *et al.* 1983].

Following single oral doses of 20, 40 and 60 mg to 8 hypertensive subjects, mean peak plasma concentrations of 0.06, 0.11, and 0.17 mg/L were reported at 1.6, 2.1, and 1.8 h, respectively [Banzet 1983].

Toxicity

A 57-year-old man who took 30 × 20 mg nifedipine retarded-release tablets developed hypotension, tachycardia and flushing; 10 h after the overdose, the nifedipine plasma concentration was 0.604 mg/L and that of the M-I metabolite was 0.110 mg/L [Ferner *et al.* 1990].

Bioavailability Between 45 and 75% for liquid-filled capsules but less for longer-acting formulations.

Half-life Plasma half-life, about 2 to 6 h.

Volume of Distribution About 0.8 L/kg.

Clearance Plasma clearance, about 7 mL/min/kg.

Protein Binding 92 to 98%.

Dose As the long-acting preparation, 10 to 90 mg daily; as liquid-filled capsules, 15 to 60 mg daily.

Abou-Auda HS *et al.* (2000). Liquid chromatographic assay of nifedipine in human plasma and its application to pharmacokinetic studies. *J Pharm Biomed Anal* 22: 241–249.
Banzet O (1983). Acute antihypertensive effect and pharmacokinetics of a tablet preparation of nifedipine. *Eur J Clin Pharmacol* 24: 145–150.
Dankers J *et al.* (1998). Determination of nifedipine in human plasma by flow-injection tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 710: 115–120.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Dokladalova J *et al.* (1982). Occurrence and measurement of nifedipine and its nitropyridine derivatives in human blood plasma. *J Chromatogr* 231: 451–458.
Ellis JS *et al.* (1993). Determination of nifedipine in gingival crevicular fluid: a capillary gas chromatographic method for nifedipine in microlitre volumes of biological fluid. *J Chromatogr* 621: 95–101.
Ferner RE *et al.* (1990). Pharmacokinetics and toxic effects of nifedipine in massive overdose. *Hum Exp Toxicol* 9: 309–311.
Foster TS *et al.* (1983). Nifedipine kinetics and bioavailability after single intravenous and oral doses in normal subjects. *J Clin Pharmacol* 23: 161–170.
Goldnik A *et al.* (1996). Determination of nifedipine in serum by HPLC. *Acta Pol Pharm* 53: 7–8.
Grundy JS *et al.* (1994). Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. *J Chromatogr B Biomed Appl* 654: 146–151.
Jankowski A, Lamparczyk H (1994). Evaluation of chromatographic methods for the determination of nifedipine in human serum. *J Chromatogr A* 668: 469–473.
Le Guellec C *et al.* (1992). Determination of nifedipine in plasma by a rapid capillary gas chromatographic method. *Biomed Chromatogr* 6: 20–23.
Martens J *et al.* (1994). Determination of nifedipine in human serum by gas chromatography-mass spectrometry: validation of the method and its use in bioavailability studies. *J Chromatogr B Biomed Appl* 660: 297–302.
Sadanaga T *et al.* (1982). Determination of nifedipine in plasma by high-performance liquid chromatography. *Chem Pharm Bull* 30: 3807–3809.
Thongnopnua P, Viwatwongsa K (1994). Quantitative analysis of nifedipine in plasma by high-performance liquid chromatography. *J Pharm Biomed Anal* 12: 119–125.
Yriti M *et al.* (2000). Quantitation of nifedipine in human plasma by on-line solid-phase extraction and high-performance liquid chromatography. *J Chromatogr A* 870: 115–119.
Zaater M *et al.* (2000). Trace-level determination of nifedipine in human serum by reversed phase high performance liquid chromatography. *Pol J Pharmacol* 52: 307–312.

Nifekalant

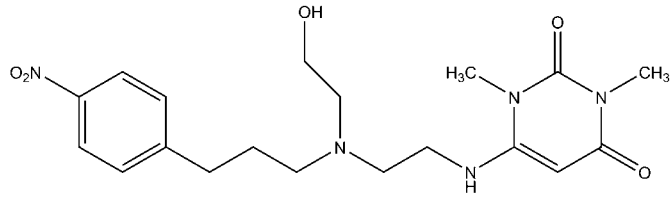
Antiarrhythmic, Potassium Channel Antagonist, Uracil

$C_{19}H_{27}N_5O_5$ = 405.5

CAS—130636-43-0

IUPAC Name 6-[2-[2-Hydroxyethyl-[3-(4-nitrophenyl)propyl]amino]ethylamino]-1,3-dimethylpyrimidine-2,4-dione

Synonym 6-[[2-[(2-Hydroxyethyl)[3-(4-nitrophenyl)propyl]amino]ethyl]amino]-1,3-dimethyl-2,-4(1*H*,3*H*)-pyrimidinedione



Chemical Properties Crystals. Mp 117.5° to 118.5°.

Nifekalant Hydrochloride

$C_{19}H_{27}N_5O_5$, HCl = 441.9

CAS—130656-51-8

IUPAC Name 6-[2-[2-Hydroxyethyl-[3-(4-nitrophenyl)propyl]amino]ethylamino]-1,3-dimethylpyrimidine-2,4-dione hydrochloride

Synonyms 6-[(2-{(2-Hydroxyethyl)[3-(*p*-nitrophenyl)propyl]amino}ethyl)amino]-1,3-dimethyluracil; MS-551.

Proprietary Name *Shinbit*

Chemical Properties Light yellow to yellow crystals. Mp 172° to 174°. Very soluble in water, slightly soluble in methanol, very slightly soluble in ethanol. Practically insoluble in ether. pK_a 7.05 [O'Neil *et al.* 2006].

Disposition in the Body Only the unchanged form is active and it undergoes glucuronate conjugation in the liver. Approximately 30% of the dose is excreted unchanged in the urine.

Therapeutic Concentration Approximately 0.5 mg/L. For a study investigating the IV administration of nifekalant in the prevention of ischaemia-induced ventricular tachyarrhythmia in patients with renal failure undergoing haemodialysis, see Myoishi *et al.* [2003].

Half-life Approximately 1.5 h.

Volume of Distribution Approximately 0.14 L/kg.

Protein Binding Between 86 to 96%.

Dose 300 µg/kg bodyweight by IV injection over 5 min with a maintenance dose of 400 µg/kg/h.

Myoishi M *et al.* (2003). Intravenous administration of nifekalant hydrochloride for the prevention of ischemia-induced ventricular tachyarrhythmia in patients with renal failure undergoing hemodialysis. *Circ J* 67: 898–900.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Nifenazone

Analgesic

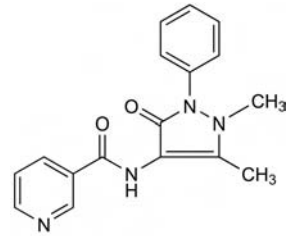
$C_{17}H_{16}N_4O_2$ = 308.3

CAS—2139-47-1

IUPAC Name *N*-(2,3-Dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)nicotinamide

Synonym Nicotinylamidoantipyrine

Proprietary Names *Neopiran*; *Nicopyron*; *Nicoreumal*; *Reumatosil*.



Chemical Properties A pale yellow crystalline powder. Slightly soluble in water and ether. Log *P* (octanol/water), 0.6.

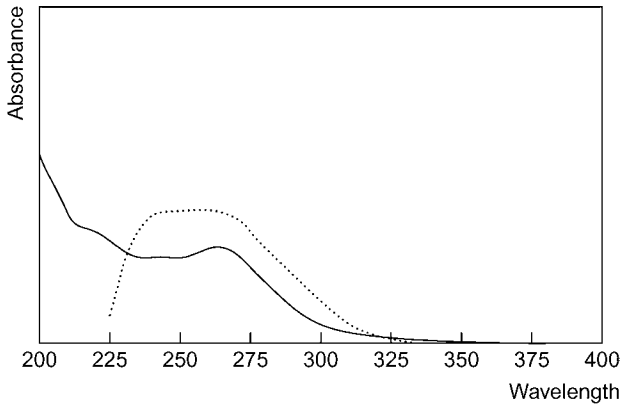
Colour Tests Cyanogen bromide—orange; ferric chloride—brown-red; Liebermann's reagent (100°)—yellow.

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.00; system TE—R_f 0.36; system TAE—R_f 0.58 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—nifenazone RI 3080; M (desacetyl) RI 1955.

High Performance Liquid Chromatography System HD—*k* 0.1; system HW—*k* 0.45; system HX—RI 310.

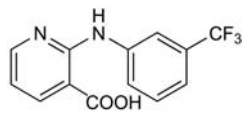
Ultraviolet Spectrum Aqueous acid—262 nm (A_1^1=502a).



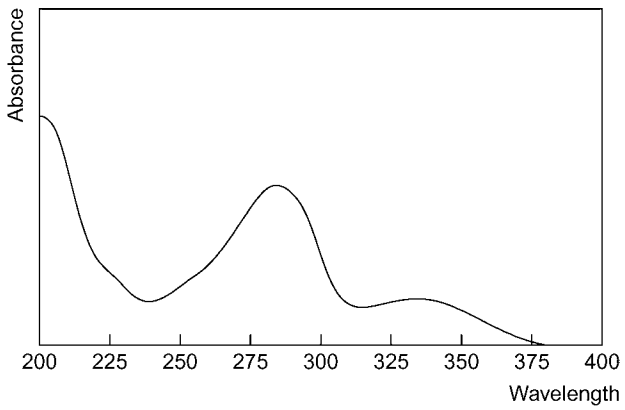
Infrared Spectrum Principal peaks at wavenumbers 1648, 1670, 1588, 1511, 1302, 1495 cm^{-1} (KBr disk).
Dose 0.5 to 1.5 g daily.

Niflumic Acid

Analgesic
$C_{13}H_9F_3N_2O_2$ = 282.2
CAS—4394-00-7
IUPAC Name 2-[[3-(Trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid
Synonym UP-83
Proprietary Names *Actol*; *Flaminon*; *Flunir*; *Niflactol*; *Niflam*; *Niflugel*; *Nifluril*.



Chemical Properties Crystals. Mp 204°. Log *P* (octanol/water), 4.4.
Thin-layer Chromatography System TD—R_f 0.03; system TE—R_f 0.11; system TF—R_f 0.03; system TG—R_f 0.28; system TAD—R_f 0.15; system TAE—R_f 0.88; system TAJ—R_f 0.27; system TAK—R_f 0.56; system TAL—R_f 0.90 (Ludy Tenger reagent, orange-brown).
Gas Chromatography System GA—niflumic acid RI 2085; niflumic acid-Me RI 1955; System GD—methyl derivative retention time 1.38 relative to n-C_6H_{34}; system GL—niflumic acid-Me RI 1960; M (OH-)Me_2 RI 2140.
High Performance Liquid Chromatography System HV—retention time 0.93 relative to meclofenamic acid; system HX—RI 595; system HY—RI 530; system HAA—retention time 22.0 min.
Ultraviolet Spectrum Aqueous acid—255 (A_1^1=471b), 329 nm (A_1^1=202b); aqueous alkali—288 nm (A_1^1=788b).

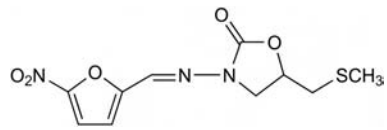


Mass Spectrum Principal ions at *m/z* 282, 236, 237, 281, 263, 145, 44, 93.
Quantification
Plasma **HPLC** For method, see Avgerinos and Malamataris [1990].
Urine **HPLC** For method, see Avgerinos and Malamataris [1990].
Dose 0.5 to 1 g daily.

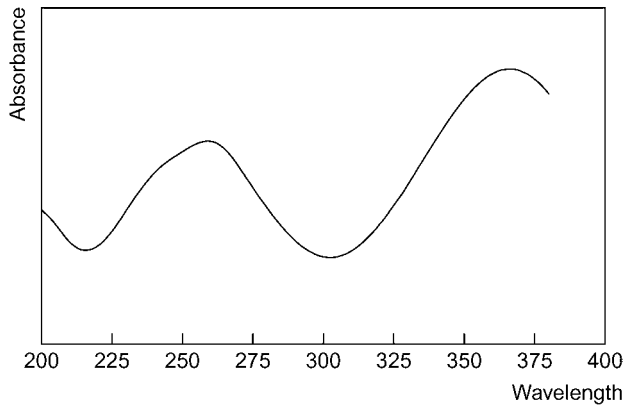
Avgerinos A, Malamataris S (1990). High-performance liquid chromatographic determination of niflumic acid in human plasma and urine. *J Chromatogr* 533: 271–274.

Nifuratel

Antimicrobial, Antiprotozoal
$C_{10}H_{11}N_3O_5S$ = 285.3
CAS—4936-47-4
IUPAC Name 5-[(Methylthio)methyl]-3-[[(5-nitro-2-furanyl)methylene]amino]-2-oxazolidinone
Synonyms Methylmercadone; thiodinone.
Proprietary Names *Inimur*; *Macmiror*; *Magmilor*; *Omnes*; *Polmiror*; *Tydantil*.



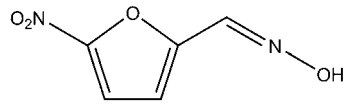
Chemical Properties A yellow crystalline powder. Mp 186° to 188°. Practically insoluble in water; soluble 1 in 400 of chloroform; sparingly soluble in acetone; soluble in dimethylformamide. Log *P* (octanol/water), 1.5.
Colour Tests Methanolic potassium hydroxide—orange; palladium chloride—black.
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.00; system TE—R_f 0.67; system TF—R_f 0.43; system TAE—R_f 0.77 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2590.
High Performance Liquid Chromatography System HX—RI 432.
Ultraviolet Spectrum Aqueous acid—259 (A_1^1=452a), 367 nm.



Infrared Spectrum Principal peaks at wavenumbers 1748, 1253, 1232, 1520, 1027, 1126 cm^{-1} (KBr disk).
Dose 600 mg daily.

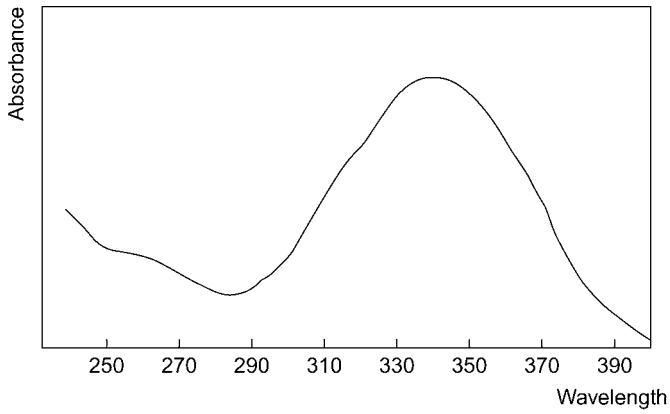
Nifuroxime

Antifungal, Antitrichomonal
$C_5H_4N_2O_4$ = 156.1
CAS—6236-05-1
Synonyms Anti-5-nitro-2-furaldoxime; anti-5-nitrofurfuraldehyde oxime; NF6; 5-nitro-2-furancarboxaldehyde oxime.
Proprietary Name *Micofur*. It is an ingredient of *Tricofuron*.



Chemical Properties A white-to-pale-yellow crystalline powder which darkens on standing; it also discolours on exposure to direct sunlight and on contact with alkaline material. Soluble 1 in 1000 of water, 1 in 25 of ethanol, and 1 in 11 of methanol; very soluble in dimethylformamide.
Colour Tests Ammonium vandate test—green, forming slowly (limit of detection, 0.5 µg); Vitali's test—pale yellow/pale yellow/brown (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.79 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 0.99 (relative to codeine).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—231 and 340 nm; neutral solution (1% dimethylformamide in water)—340 nm [Elsayed *et al.* 1980].



Infrared Spectrum Principal peaks at wavenumbers 1346, 811, 820, 1305, 1531 cm^{-1} (KBr disk).

Disposition in the Body

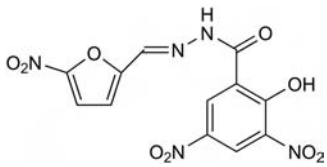
Toxicity When used in conjunction with furazolidine, there has been a report of cholestatic hepatitis [Engel *et al.* 1975] as well as generalised urticaria [Aaronson 1969].

Aaronson CM (1969). Generalized urticaria from sensitivity to nifuroxime. *JAMA* 210: 557–558.
Elsayed L *et al.* (1980). Simultaneous spectrophotometric determination of nifuroxime and furazolidone in pharmaceutical preparations. *J Assoc Off Anal Chem* 63: 992–995.
Engel JJ *et al.* (1975). Cholestatic hepatitis after administration of furan derivatives. *Arch Intern Med* 135: 733–735.

Nifursol

Antiprotozoal (Veterinary)
$C_{12}H_7N_5O_9$ = 365.2
CAS—16915-70-1

IUPAC Name 2-Hydroxy-3,5-dinitro-*N*-[(*E*)-(5-nitrofuran-2-yl)methylideneamino]benzamide
Synonym 3,5-Dinitro-2′-(5-nitrofurfurylidene)salicylohydrazide
Proprietary Name *Salfuride*

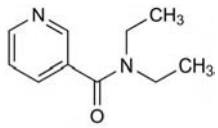


Chemical Properties A bright yellow crystalline powder. Mp 214° to 225°, with decomposition. Very slightly soluble in water, ethanol and chloroform; soluble 1 in 350 of acetone; practically insoluble in ether; slightly soluble in methanol.
Colour Test Methanolic potassium hydroxide—orange.
Thin-layer Chromatography System TA—R_f 0.86 (visible yellow spot; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—not eluted.
Infrared Spectrum Principal peaks at wavenumbers 1251, 1281, 1606, 1176, 1674, 1566 cm^{-1} (KBr disk).

Nikethamide

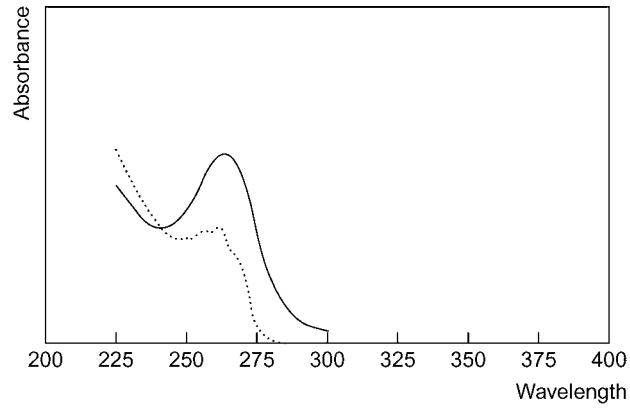
Respiratory Stimulant
$C_{10}H_{14}N_2O$ = 178.2
CAS—59-26-7

IUPAC Name *N,N*-Diethyl-3-pyridinecarboxamide
Synonyms Cardiamide; diethylamide nicotinic acid; nicethamidum; nicorine; nicotinoyldiaethylamidum; nikethylamide.
Proprietary Names *Coramin(e)*; *Cormed*; *Juvacor*; *Kardonyl*.

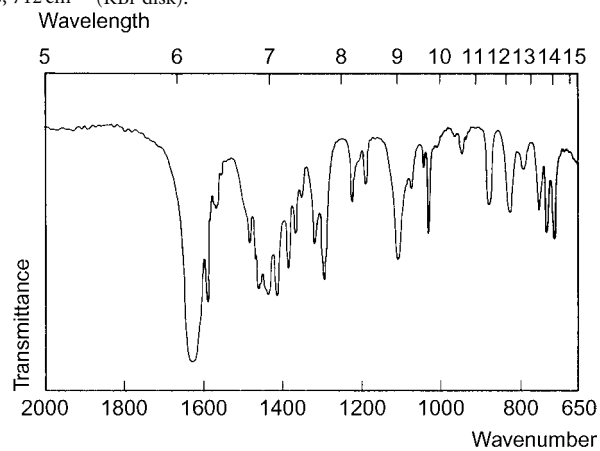


Chemical Properties A colourless or slightly yellow, oily liquid or crystalline solid. Relative density 1.060 to 1.066. Mp 23° to 25°. Bp 280°. Refractive index 1.524 to 1.526. Miscible with water, acetone, ethanol, chloroform and ether. pK_a 3.5 (20°). Log *P* (octanol/water), 0.3.

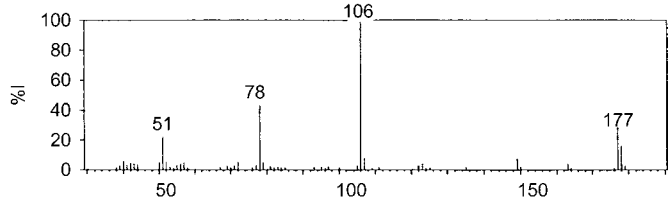
Colour Test Cyanogen bromide—orange.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.15; system TC—R_f 0.56; system TE—R_f 0.59; system TL—R_f 0.29; system TAE—R_f 0.71; system TAF—R_f 0.67; system TAJ—R_f 0.59; system TAK—R_f 0.19; system TAL—R_f 0.89 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—nikethamide RI 1525; M (nicotinamide) RI 1341, M (*N*-ethylnicotinamide) RI 1605; system GB—nikethamide RI 1569; M (nicotinamide) RI 1418; system GC—RI 1852; system GF—RI 1895.
High Performance Liquid Chromatography System HX—RI 304; system HY—RI 239.
Ultraviolet Spectrum Aqueous acid—264 nm (A^1_1=280a); aqueous alkali—256, 261 nm.



Infrared Spectrum Principal peaks at wavenumbers 1635, 1590, 1291, 1103, 1316, 712 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 106, 78, 177, 51, 178, 107, 149, 40; nicotinamide 122, 78, 106, 51, 50, 52, 44, 123.



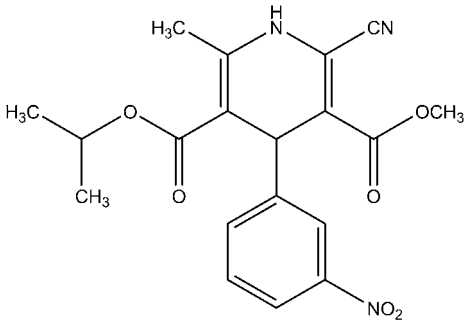
Quantification
Canine Blood GC FID. For method, see Lewis [1979].
Canine Urine GC FID. For method, see Lewis [1979].
Disposition in the Body Rapidly absorbed after oral or parenteral administration and metabolised by *N*-dealkylation to *N*-ethylnicotinamide and nicotinamide; rapidly excreted in the urine; the major urinary metabolite is *N*-ethylnicotinamide.
Dose 0.5 to 2 g IV, repeated if necessary.

Lewis JH (1979). Determination of nikethamide and N-ethylnicotinamide in the blood and urine of greyhounds. *J Chromatogr* 172: 295–302.

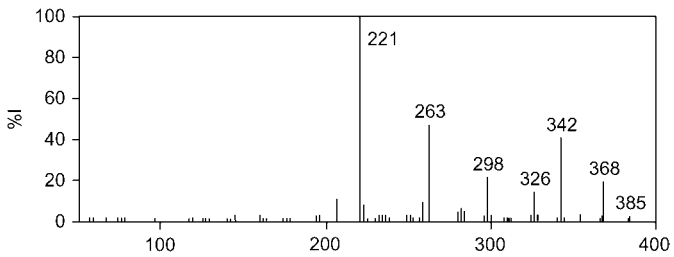
Nilvadipine

Antihypertensive, Calcium Channel Blocker, Dihydropyridine
$C_{19}H_{19}N_3O_6$ = 385.4
CAS—75530-68-6

IUPAC Name 3-*O*-Methyl 5-*O*-propan-2-yl 2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Synonyms CL-287389; 2-cyano-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 3-methyl 5-(1-methylethyl) ester; FK-235; FR-34235; isopropyl 6-cyano-5-methoxycarbonyl-2-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate; 5-isopropyl-3-methyl-2-cyano-1,4-dihydro-6-methyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate; nivadipine; nivaldipine; SKF-102362.
Proprietary Names *Escor*; *Nivadil*; *Peroma*; *Tensan*.



Chemical Properties Yellow prisms. Mp 148° to 150° [O'Neil *et al.* 2006]. Log *P* (octanol/water), 1.73 [ACD 2007], >3 [von Nieciecki *et al.* 1992]. Photolabile. Stable in human plasma at 37° for up to 1 h [Tokuma *et al.* 1987a] and after freezing at –20° for 227 days [Tokuma *et al.* 1985]. Spiked plasma samples were stable for ~2 h under laboratory light. Stable in human plasma for at least 2 months at –20° and for 24 h at room temperature in an autosampler. After 3 freeze–thaw cycles, ~80% of the sample remained [Baranda *et al.* 2006].
Note For the identification of the photodegradation products of nilvadipine, see Mielcarek *et al.* [2000] and Augustyniak *et al.* [2001].
Mass Spectrum Principal ions at *m/z* 221, 263, 342, 298, 368, 326. [Mielcarek *et al* 2000].



Quantification

Plasma **GC-MS** Column: 5% phenylmethylsilicone fused silica capillary (12.5 m × 0.31 mm i.d., 0.52 µm). ECD, negative ion mode, SIM acquisition mode. Limit of detection not reported [Tokuma *et al.* 1987b]. Column: Ultra No. 2 5% phenylmethylsilicone fused silica capillary (25 m × 0.31 mm i.d., 0.52 µm). Carrier gas: He, 1.8 mL/min. ECD. Retention time: 5.8 min. Limit of detection, 0.5 µg/L [Tokuma *et al.* 1987a] Column: 5% phenylmethylsilicone fused silica capillary (12.5 m × 0.31 mm i.d., 0.52 µm). Carrier gas: He, 0.28 kg/cm^2. Temperature programme: 180° for 1 min to 250° at 20°/min to 285° at 10°/min. Positive ion–negative ion CI at 70 eV, SIM acquisition mode. Limit of detection, 0.01 µg/L [Tokuma *et al.* 1985].

HPLC Column: Daicel chiralpak OT(+) (+)-poly(triphenylmethyl methacrylate) polymer (25 cm × 4.6 mm i.d.). Mobile phase: methanol : water (95 : 5), flow rate 0.8 mL/min [Tokuma *et al.* 1987b].
Serum **LC-MS** Column: Luna RP-C_{18}(2) (150 × 2 mm i.d., 3.0 µm). Mobile phase: 0.1% methanoic acid-1 mmol/L ammonium formate (pH 2.7) : acetonitrile-0.1% formic acid-1 mmol/L ammonium formate (95 : 5; 80 : 20 for 3 min to 60 : 40 at 11 min to 30 : 70 at 12 min to 5 : 95 at 12.5 min for 1 min to 80 : 20 at 15.5 min). ESI, MRM acquisition mode. Limit of detection not reported [Baranda *et al.* 2006].
Urine **GC-MS** Following extractive methylation. Column: HP capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 300° at 30°/min. EI ionisation at 70 eV, full scan mode. Limit of detection, 10 µg/L [Maurer, Arlt 1999]. Column: Ultra No. 2 5% phenylmethylsilicone fused silica capillary (25 m × 0.31 mm, 0.52 µm). Carrier gas: He, 1.8 mL/min. ECD. Retention time: 5.8 min. Limit of detection, 0.5 µg/L [Tokuma *et al.* 1987a].
Disposition in the Body Nilvadipine is absorbed quickly and completely. It undergoes marked first-pass metabolism. Biotransformation occurs by almost complete oxidation of the dihydropyridine ring to the corresponding pyridine derivative (M1) and by subsequent splitting of the methyl ester (M3) and the isopropyl ester (M7) in the side chain. The metabolites are pharmacologically inactive. None of the other metabolites (such as the hydroxyl derivative of the isopropyl remnant [M5] or the dihydropyridine precursors of M7) play any significant role or can even be found in humans. Excretion is mainly via the kidneys; ~25% is excreted in the faeces. Approximately 60% is found in the urine within 24 h. Approximately 70% is found in urine and 15 to 20% in faeces within 72 h. Redistribution from tissues determines the rate of elimination, as opposed to rate of metabolism.

Therapeutic Concentration
Forty healthy male volunteers were randomly assigned to receive either placebo or 4, 8, 12, 16 or 20 mg nilvadipine orally. Mean maximum plasma levels were 2.00 ± 0.78, 3.03 ± 1.55, 5.97 ± 3.88, 15.7 ± 7.7 and 14.7 ± 8.6 µg/L, attained at 12.6 ± 4.3, 8.08 ± 2.29, 10.5 ± 1.8, 15.6 ± 6.4 and 12.4 2.3 h, respectively [Cheung *et al.* 1988].

Six healthy male volunteers (aged 20 to 24 years; weight 57 to 69 kg) were given a single oral dose of 4 mg followed by 4 mg every 12 h for 6 days after a washout period of at least 3 days. There was no significant difference in peak plasma concentrations and time to maximum (t_{max}) of single dosing or day 4 or day 6 of multiple dosing, although there was a 2-fold accumulation of trough concentration [Terakawa *et al.* 1988].

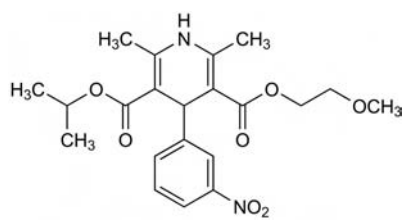
Six healthy male volunteers were given single 2, 4 or 6 mg oral doses of nilvadipine after an overnight fast. Mean maximum plasma levels of 1.48 ± 0.47, 3.48 ± 0.53 and 6.69 ± 1.54 µg/L, respectively, were attained at 1.08 to 1.50 h for each dose. The elimination half-life was independent of dose and averaged 11.0 ± 2.3 h [Terakawa *et al.* 1987a].
The bioavailability of nilvadipine was not affected by the presence of food, apart from a small increase in t_{max} with a larger meal, although this was clinically insignificant [Terakawa *et al.* 1987b].
Note For studies comparing the pharmacokinetics of the (*R*)- and (*S*)-stereoisomers, see Tokuma and Noguchi [1995] and Tokuma *et al.* [1987c].
Bioavailability 14 to 19%.
Half-life Approximately 11 h.
Protein Binding 97.5 to 98.7%.
Dose Up to 16 mg daily.

ACD (2007) *ACD/LogP DB*. Toronto ON: Advanced Chemistry Development www.acdlabs.com (accessed November 2007).
Augustyniak W *et al.* (2001). Spectroscopic and HPLC studies of photodegradation of nilvadipine. *Drug Dev Ind Pharm* 27: 1031–1038.
Baranda AB *et al.* (2006). Instability of calcium channel antagonists during sample preparation for LC-MS-MS analysis of serum samples. *Forensic Sci Int* 156: 23–34.
Cheung WK *et al.* (1988). Pharmacokinetics of nilvadipine after single oral doses in healthy volunteers. *Int J Clin Pharmacol Res* 8: 299–305.
Maurer HH, Arlt JW (1999). Screening procedure for detection of dihydropyridine calcium channel blocker metabolites in urine as part of a systematic toxicological analysis procedure for acidic compounds by gas chromatography–mass spectrometry after extractive methylation. *J Anal Toxicol* 23: 73–80.
Mielcarek J *et al.* (2000). Identification of photodegradation products of nilvadipine using GC-MS. *J Pharm Biomed Anal* 24: 71–79.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.
Terakawa MA *et al.* (1987). Pharmacokinetics of nilvadipine in healthy volunteers. *J Clin Pharmacol* 27: 111–117.
Terakawa M *et al.* (1987). Effect of two different meals on bioavailability of nilvadipine in healthy volunteers. *J Clin Pharmacol* 27: 293–296.
Terakawa M *et al.* (1988). Multiple-dose pharmacokinetics of nilvadipine in healthy volunteers. *J Clin Pharmacol* 28: 350–355.
Tokuma Y, Noguchi H (1995). Stereoselective pharmacokinetics of dihydropyridine calcium antagonists. *J Chromatogr A* 694: 181–193.
Tokuma Y *et al.* (1985). Determination of nilvadipine in human plasma by capillary column gas chromatography–negative–ion chemical-ionization mass spectrometry. *J Chromatogr* 345: 51–58.
Tokuma Y *et al.* (1987a). Determination of nilvadipine in plasma and urine by capillary column gas chromatography with electron-capture detection. *J Chromatogr* 415: 156–162.
Tokuma Y *et al.* (1987b). Determination of (+)- and (−)-nilvadipine in human plasma using chiral stationary-phase liquid chromatography and gas chromatography–mass spectrometry, and a preliminary pharmacokinetic study in humans. *J Pharm Sci* 76: 310–313.
Tokuma Y *et al.* (1987). Plasma levels of (+)- and (−)-nilvadipine after oral dosing with racemic (+)-nilvadipine in man. *Res Commun Chem Pathol Pharmacol* 57: 229–237.
von Nieciecki A *et al.* (1992). Pharmacokinetics of nilvadipine. *J Cardiovasc Pharmacol* 20: 6S22–S29.

Nimodipine

Calcium Antagonist, Calcium Channel Blocker
$C_{21}H_{26}N_2O_7$ = 418.4
CAS—66085-59-4

IUPAC Name 3-*O*-(2-Methoxyethyl) 5-*O*-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Synonyms Bay-e-9736; 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl-1-methylethyl ester.
Proprietary Names *Admon*; *Brainal*; *Calnit*; *Kenesil*; *Modus*; *Nimotop*; *Remontal*; *Periplum*.



Chemical Properties A yellow crystalline powder; crystals from petroleum ether/acetic ester. Mp 125°. Soluble in ethyl acetate, sparingly soluble in absolute alcohol and insoluble in water. Log *P* (octanol/water), 3.05. Exposure to light leads to formation of nitrophenylpyridine derivatives. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Thin-layer Chromatography System TB—R_f 0.01; system TE—R_f 0.78; system TAE—R_f 0.87.

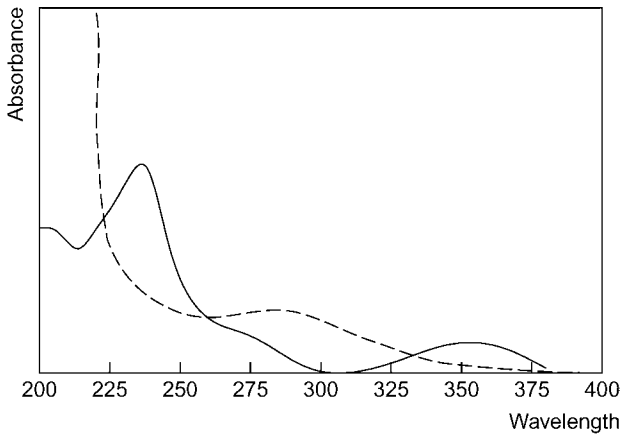
Gas Chromatography System GA—RI 2929; system GB—RI 3096; system GP—RI 2300 M (dehydro-desisopropyl-desmethoxyethyl)-Me_2,RI 2390 M (dehydro-desmethoxyethyl)-Me, RI 2645M (dehydro-desisopropyl-O-desmethyl-HOOC)-Me, RI 2655 M (dehydro-), RI 2665 M (dehydro-desisopropyl-O-desmethyl)-Me, RI 2740M (dehydro-O-desmethyl-HOOC-)-Me.

Column: HP1 methyl silicone (0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, flow rate 0.9 mL/min. Detection: mass spectrometer. Retention index: 2961 [Mills, Roberson 1993].

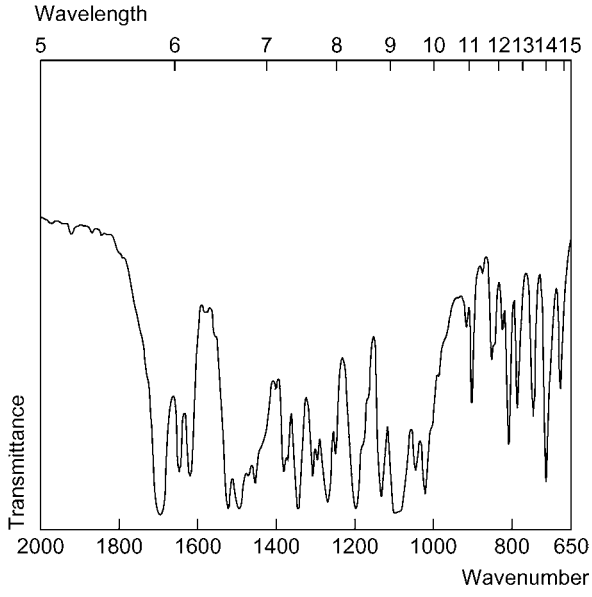
High Performance Liquid Chromatography System HX—RI 668; system HY—RI 584; system HZ—RI 17.7 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L potassium dihydrogen phosphate (pH 3.5, 70 : 30), flow rate 1 mL/min. UV detection (diode array). Retention time: 3.8 min [Mills, Roberson 1993].

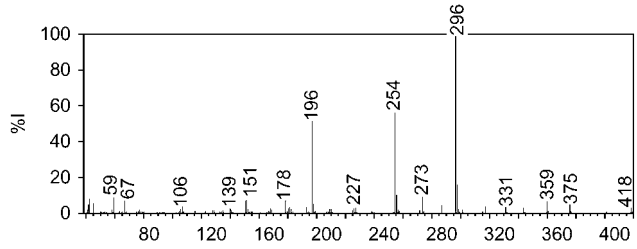
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1689, 1337, 1098 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 296, 254, 196, 273, 59, 67, 151, 178.



Quantification

Plasma **GC** ECD. Limit of detection, 1 µg/L [Krol *et al.* 1984].

HPLC Column: ODS Spherisorb (150 × 4.6 mm i.d., 3 µm). Mobile phase: (A) acetonitrile:water (57 : 43); (B) methanol : water (66 : 34), flow rate 0.5 mL/min. UV detection (λ=238 nm). Retention time: solvent (A) nimodipine, 12.19 min; pyridine analogue, 13.02 min; solvent (B) nimodipine, 17.33 min; pyridine analogue, 19.89 min. Limit of detection, 1 µg/L [Krol *et al.* 1984]. Limit of detection, 3 µg/L [Bach 1983].

Serum **GC** ECD. Limit of detection, 0.2 µg/L [Gengo *et al.* 1987].

GC–MS Limit of quantification, 0.1 µg/L [Fischer *et al.* 1993].

HPLC UV detection (λ=238 nm). Limit of quantification, 0.038 µg/L [Fischer *et al.* 1993]. See Plasma [Bach 1983].

Cerebrospinal Fluid **GC** See Plasma [Krol *et al.* 1984].

HPLC See Plasma [Krol *et al.* 1984].

Disposition in the Body Nimodipine is rapidly and readily absorbed after oral administration and undergoes hepatic metabolism. It is rapidly distributed throughout the body to tissues and organs. Metabolism occurs entirely in the liver and includes dehydrogenation to produce a pyridine analogue, followed by subsequent demethylation, ester hydrolysis and hydroxylation. Three metabolites have been isolated, the *O*-desmethyl metabolite which has slight activity and the pyridine and *O*-desmethyl pyridine metabolites which are both inactive. It is excreted mainly as metabolites in urine (50% of a dose excreted over 4 days) and faeces (32%). Virtually no unchanged drug is present in urine.

Therapeutic Concentration The serum therapeutic concentration is 10 to 50 µg/L. Six patients with biopsy-proven cirrhosis, 38 to 72 years old (mean, 55.3 years) and 5 healthy volunteers, aged between 42 and 68 years (mean, 52.4 years) were administered with a 60 mg oral dose after an overnight fast. The mean peak plasma concentration was 115.8 µg/L (range, 25.3 to 250.9 µg/L) for the patients with liver disease and 80.2 (between 58.5 and 126.5) µg/L for the healthy individuals. These concentrations were reached by 1.75 h (0.5 to 5.0 h) and 0.7 (0.25 to 1.0) h, respectively. Additionally, they were asked to take a 30 mg oral dose by sublingual administration. This meant that the actual dose received ranged between 8 and 18 mg. Peak concentrations of 12.4 (7.7 to 17.9) µg/L and 19 (4.6 to 53.6) µg/L were observed at 2.5 h (range, 1 to 10 h) and 1.3 (0.75 to 3.06) h for the patients and volunteers, respectively [Gengo *et al.* 1987].

Toxicity No specific or general toxic effects with therapeutic treatment, but cardiovascular problems may arise with higher doses.

Bioavailability 13%.

Half-life 9 h (with initial rapid phase with half-life around 1 to 2 h).

Volume of Distribution Steady state, 0.9 to 2.3 L/kg.

Clearance Plasma, 0.84 L/kg/h; 0.23 L/kg/h (patients with renal failure). Also, reported as 1.21 L/kg/h.

Distribution in Blood Erythrocytes : plasma ratio is unity.

Protein Binding 95 to 98%.

Note For a review of nimodipine, see Langley and Sorkin [1989]. For a review of the pharmacokinetics of nimodipine, see Parnetti [1995].

Dose Oral, 360 mg daily; IV, 1 mg/h initially, increased to 2 mg/h after 2 h.

Bach PR (1983). Determination of nifedipine in serum or plasma by reversed-phase liquid chromatography. *Clin Chem* 29(7): 1344–1348.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fischer C *et al.* (1993). Simultaneous assessment of the intravenous and oral disposition of the enantiomers of racemic nimodipine by chiral stationary-phase high-performance liquid chromatography and gas chromatography/mass spectroscopy combined with a stable isotope technique. *J Pharm Sci* 82(3): 244–250.

Gengo FM *et al.* (1987). Nimodipine disposition and haemodynamic effects in patients with cirrhosis and age-matched controls. *Br J Clin Pharmacol* 23: 47–53.

Krol GJ *et al.* (1984). Gas and liquid chromatographic analyses of nimodipine calcium antagonist in blood plasma and cerebrospinal fluid. *J Chromatogr* 305: 105–118.

Langley MS, Sorkin EM (1989). Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease. *Drugs* 37: 669–699.

Mills T, Roberson JC (1993) *Instrumental Data for Drug Analysis*, 2nd edn. Vol. 5. Boca Raton: CRC Press, 308–309.

Parnetti L (1995). Clinical pharmacokinetics of drugs for Alzheimer's disease. *Clin Pharmacokinet* 29: 110–129.

Nimorazole

Antiprotozoal

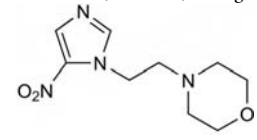
$C_9H_{14}N_4O_3$ = 226.2

CAS—6506-37-2

IUPAC Name 4-[2-(5-Nitro-1*H*-imidazol-1-yl)ethyl]morpholine

Synonym Nitrimidazine

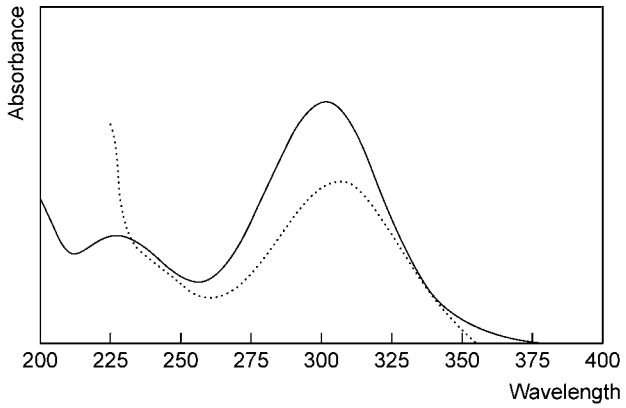
Proprietary Names *Acterol Forte*; *Esclama*; *Naxogin*.



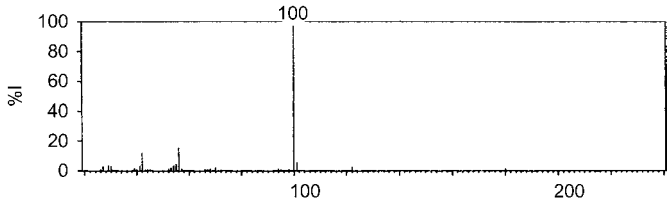
Chemical Properties A whitish crystalline powder. Mp about 110°. Soluble 1 in 33 of water; soluble in acetone, alcohols, chloroform and dilute acetic acid. Log *P* (octanol/water), 0.1.

Colour Test Dissolve 10 mg in 1 mL of methanol, add 10 mL of water, 10 mL of 6 mol/L sodium hydroxide containing 20 mg of alpha-naphthol and 10 mL of

12 mol/L sodium hydroxide, and heat for 30 min on a boiling water-bath—an intense brown colour develops.
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.03; system TC—R_f 0.44; system TE—R_f 0.58; system TL—R_f 0.33; system TAE—R_f 0.60 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1803.
Ultraviolet Spectrum Aqueous acid—297 nm; aqueous alkali—305 nm.



Mass Spectrum Principal ions at *m/z* 100, 56, 42, 101, 55, 54, 41, 30.



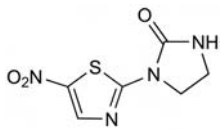
Note For studies of the pharmacokinetic properties of nimorazole, see Overgaard *et al.* [1983].
Dose Usually 2 g as a single dose.

Overgaard J *et al.* (1983). Studies of the pharmacokinetic properties of nimorazole. *Br J Cancer* 48: 27–34.

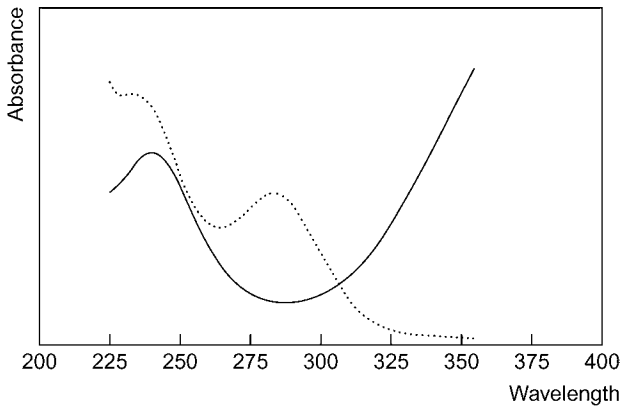
Niridazole

Anthelmintic
$C_6H_6N_4O_3S$ = 214.2
CAS—61-57-4

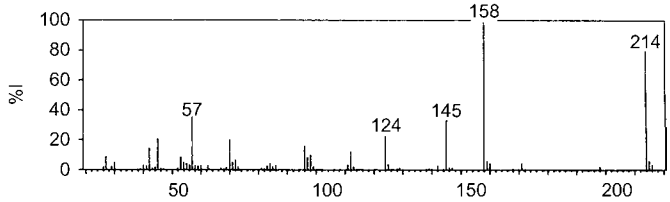
IUPAC Name 1-(5-Nitro-2-thiazolyl)-2-imidazolidinone
Synonym Ba-32644
Proprietary Name *Ambilhar*



Chemical Properties A yellow crystalline powder. Mp 260° to 264°. Practically insoluble in water, ethanol, acetone, chloroform and ether; soluble in dimethyl sulfoxide, dimethylformamide and pyridine. Log *P* (octanol/water), 1.0.
Colour Test Methanolic potassium hydroxide—yellow.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.01; system TC—R_f 0.44; system TL—R_f 0.53 (visible yellow spot.)
Ultraviolet Spectrum Aqueous acid—239 nm; aqueous alkali—233, 282 nm.



Mass Spectrum Principal ions at *m/z* 158, 214, 57, 145, 124, 45, 70, 96.



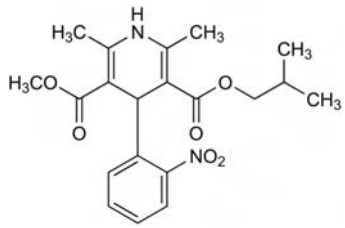
Quantification
Plasma **HPLC** UV detection. Limit of detection, 50 µg/L [Miller *et al.* 1978].
Serum **GC** FID. Limit of detection, 250 µg/L [Miller, Oake 1977].
Urine **GC** See Serum [Miller, Oake 1977].
HPLC See Plasma [Miller *et al.* 1978].
Disposition in the Body Niridazole is slowly absorbed after oral administration. It undergoes extensive first-pass metabolism in the liver to inactive metabolites. About 50% of a dose is excreted in the urine as metabolites in 48 h; niridazole is also excreted as metabolites in bile.
Therapeutic Concentration
After a single oral dose of 25 mg/kg, peak blood concentrations of unchanged drug of about 0.2 mg/L were attained in about 3 h, and peak blood concentrations of metabolites of about 5 to 10 mg/L were reported after 6 to 9 h [Faigle 1971].
Half-life Plasma half-life, 12 to 15 h.
Protein Binding Niridazole metabolites extensively bound.
Dose 25 mg/kg daily, orally, up to a maximum of 1.5 g daily.

Faigle JW (1971). Blood levels of a schistosomicide in relation to liver function and side effects. *Acta Pharmacol Toxicol (Copenh)* 29: 3233–239.
Miller JJ, Oake RJ (1977). Gas-liquid chromatographic determination of niridazole in biological fluids. *J Chromatogr* 131: 442–443.
Miller JJ *et al.* (1978). Improved procedure for the determination of niridazole in biological fluids by high-performance liquid chromatography. *J Chromatogr* 147: 507–508.

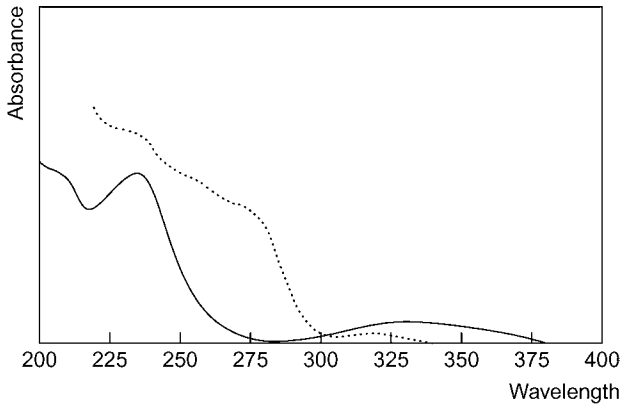
Nisoldipine

Calcium Antagonist, Calcium Channel Blocker
$C_{20}H_{24}N_2O_6$ = 388.4
CAS—63675-72-9

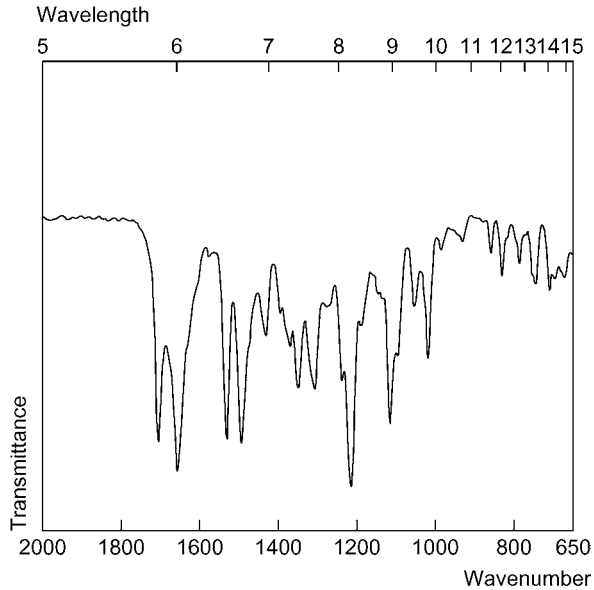
IUPAC Name 3-*O*-Methyl 5-*O*-(2-methylpropyl) 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Synonyms Bay-k-5552; 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl 2-methylpropyl ester.
Proprietary Names *Baymycard*; *Cornel*; *Sular*; *Syscor*; *Zadipina*.



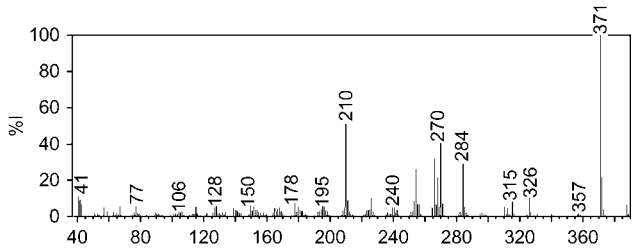
Chemical Properties Yellow crystals. Mp 151° to 152°. Practically insoluble in water; soluble in ethanol. Log *P* (octanol/water), 3.26. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Thin-layer Chromatography System TAE—R_f 0.86; system TB—R_f 0.45; system TE—R_f 0.85; system TF—R_f 0.65.
Gas Chromatography System GP—RI 2255 M (dehydro-desisobutyl-)-Me, RI 2450 M (dehydro-), RI 2615 M (dehydro-OH^-), RI 2695 M (dehydro-desisobutyl-2-$HOOC^-$)-Me_2.
Column: Ultra 1 (12.5 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 100° for 1 min, to 280° at 35°/min, hold for 3 min. Injector and detector temperatures: 280° and 300°, respectively. Carrier gas: He; flow rate, 1.9 mL/min. IS: nitrendipine. MS detection (EI, SIM at *m/z* 270.2 and 371.35 for nisoldipine and *m/z* 360 for I. S.). Retention time: nisoldipine, 6.86 min; IS, 6.77 min [Marques *et al.* 2001].
Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, flow rate 0.9 mL/min. MS detection. Retention index: 2139 [Mills, Roberson 1993].
High Performance Liquid Chromatography System HX—RI 690.
Column: C_{18} Supelcosil (250 × 4.6 mm i.d., 5 µm). Temperature: 30°. Mobile phase: methanol : water (containing 2 mmol/L sodium acetate buffer), (pH 5.0), flow rate 1 mL/min. Electrochemical detection. *k* value: 2.27 [Lopez *et al.* 2000].
Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L potassium dihydrogen phosphate, (pH 7.0), flow rate 1 mL/min. UV diode array detection. Retention time: 4.3 min [Mills, Roberson 1993].
Ultraviolet Spectrum Aqueous acid—235, 331 nm; basic—324 nm.



Infrared Spectrum Principal peaks at wavenumber 1647, 1485, 1210 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 371, 210, 270, 266, 284, 254, 268, 41.



Quantification

Plasma **GC** ECD. Limit of detection, 0.1 µg/L [Soons *et al.* 1990].

HPLC UV detection (λ=230 nm). Limit of quantification, 0.1 µg/L [Heinig *et al.* 1994].

Disposition in the Body Nisoldipine is well absorbed after oral administration from the gastrointestinal tract and the concentration of the active (+)-nisoldipine enantiomer is about 6 times higher than the inactive (−)-nisoldipine enantiomer. It is extensively metabolised in the gut wall and liver to five major urinary metabolites, which include BAYs-1869 and BAYs-4755. A hydroxylated derivative of the side chain appears to be the only active metabolite, having about 10% of the activity of the parent compound. Cytochrome P450 3A4 enzymes are believed to be involved. Between 60 and 80% of an administered dose is excreted in urine and the remainder in faeces, mostly as metabolites. <1% of a single dose is excreted unchanged.

Therapeutic Concentration

Four healthy male volunteers, 22 to 38 years old, were administered with a 40 mg dose of the controlled release formulation. The mean peak plasma concentration was 3.40 µg/L for the (+)-formulation drug and 0.37 µg/L for the (−)-drug 3 h after administration [Heinig *et al.* 1994].

Toxicity Studies have revealed that, when administered with grapefruit juice, nisoldipine mean peak plasma concentrations increase three-fold.

A 72-year-old woman with a history of depression was admitted because of severe shock, hypothermia and renal insufficiency. Routine screening revealed therapeutic drug concentrations of clomipramine (177 µg/L) and desmethylclomipramine (173 µg/L), but a nisoldipine serum concentration of 1544 µg/L was found. The patient was discharged 4 days later without sequelae [Louagie *et al.* 1998].

Bioavailability 5.5% (oral, modified release formulation). Ranges between 4 and 8%.

Half-life 2 h (beta phase) and 10 to 12 h (gamma phase). Terminal elimination half-life is 7 to 12 h.

Volume of Distribution 2.7 L/kg.

Protein Binding >99%.

Dose 10 to 40 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings at the 12th TIAFT*, Seoul: 481–486.

Heinig R *et al.* (1994). Determination of the enantiomers of nisoldipine in human plasma using high-performance liquid chromatography on a chiral stationary phase and gas chromatography with mass-selective detection. *J Chromatogr B Biomed Appl* 655: 286–292.

Louagie HK *et al.* (1998). The measurement of nisoldipine serum concentration in a case of severe nisoldipine overdose. *J Toxicol Clin Toxicol* 36: 125–127.

Lopez JA *et al.* (2000). High-performance liquid chromatography with amperometric detection applied to the screening of 1,4-dihydropyridines in human plasma. *J Chromatogr A* 870: 105–114.

Marques MP *et al.* (2001). Enantioselective assay of nisoldipine in human plasma by chiral high-performance liquid chromatography combined with gas chromatographic-mass spectrometry: applications to pharmacokinetics. *J Chromatogr B Biomed Sci Appl* 762: 87–95.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Vol. 5. Boca Raton: CRC Press, 310–311.

Soons PA *et al.* (1990). Enantioselective determination of felodipine and other chiral dihydropyridine calcium entry blockers in human plasma. *J Chromatogr* 528: 343–356.

Nitrazepam

Benzodiazepine, Hypnotic

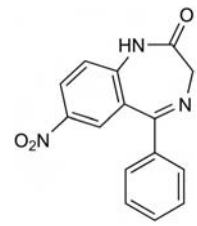
$C_{15}H_{11}N_3O_3 = 281.3$

CAS—146-22-5

IUPAC Name 7-Nitro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one

Synonyms 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one; nitrazepamum; NSC-58775; Ro-4-5360; Ro-5-3059.

Proprietary Names *Alodorm*; *Apodorm*; *Arem*; *Dima*; *Dormalon*; *Dumolid*; *Eatan N*; *Imeson*; *Insoma*; *Insomin*; *Mogadan*; *Mogadon*; *Nelbon*; *Nitrados*; *Nitrazadon*; *Nitrazepol*; *Numbon*; *Ormodon*; *Paxadorm*; *Radedorm*; *Remnos*; *Serenade*; *Somnite*; *Sonebon*; *Sonotrat*; *Surem*; *Unisomnia*.



Chemical Properties A yellow crystalline powder. Mp 224° to 226° (crystals from ethanol). Practically insoluble in water, benzene and hexane; soluble 1 in 120 of ethanol, 1 in 45 of chloroform and 1 in 900 of ether; soluble in acetone and ethyl acetate. pK_{a1} 3.2, pK_{a2} 10.8 (20°). Log *P* (octanol/water), 2.25 [Capella-Peiró *et al.* 2002], (octanol/water pH 7.4), 2.1. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Plasma samples stable for 3 weeks at 4° but there was deterioration at room temperature [Kelly *et al.* 1982].

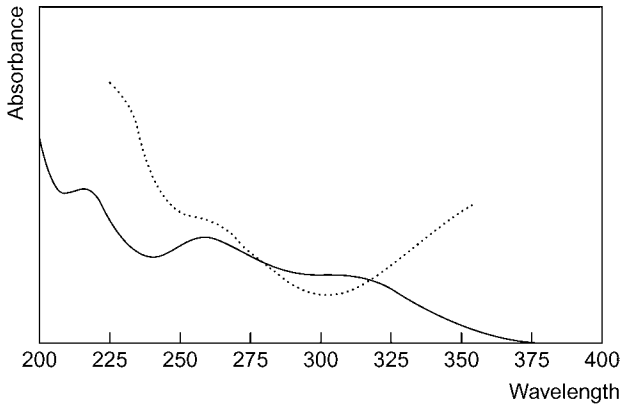
Colour Test Formaldehyde-sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.00; system TC—R_f 0.36; system TD—R_f 0.35; system TE—R_f 0.64; system TF—R_f 0.46; system TL—R_f 0.55; system TAD—R_f 0.53; system TAE—R_f 0.90; system TAF—R_f 0.86; system TAJ—R_f 0.53; system TAK—R_f 0.52; system TAL—R_f 0.92 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).

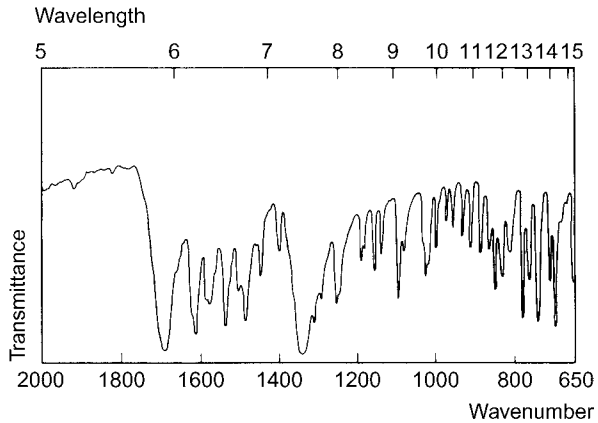
Gas Chromatography System GA—nitrazepam RI 2740, M (7-acetamido-) RI 3205, M (7-amino-) RI 2785, 2-amino-5-nitrobenzophenone RI 2388; system GB—nitrazepam RI 2915, nitrazepam-TMS RI 2642, M (7-amino-) RI 2878, M (7-amino-)-TMS_3 RI 2634; system GG—RI 3450.

High Performance Liquid Chromatography System HA—*k* 0.1; system HI—nitrazepam *k* 2.96, M (7-acetamido-) *k* 0.68, M (7-amino-) *k* 0.46; system HK—nitrazepam *k* 1.49, M (7-acetamido-) *k* 1.93, M (7-amino-) *k* 0.00; system HX—nitrazepam RI 448; system HY—RI 370; system HZ—RT 4.2 min; system HAA—RT 16.9 min; system HAF—RT 9.10 min; system HAL—RT 4.6 min; system HAM—RT 4.5 min; system HAX—RT 6.3 min; system HAY—RT 6.0 min; system HAZ—*k* 0.88; system HBH—*k* 3.22; system HBI—*k* 0.97.

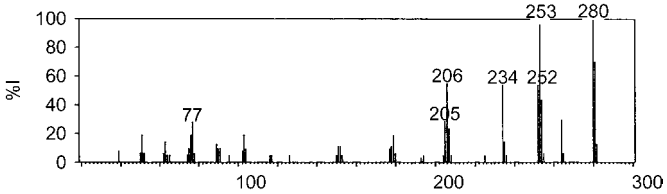
Ultraviolet Spectrum Methanolic acid—280 nm ($A^1_1 = 910$a).



Infrared Spectrum Principal peaks at wavenumbers 1690, 1610, 698, 1536, 745, 784 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 280, 253, 281, 206, 234, 252, 254, 264 (nitrazepam); 293, 265, 264, 292, 43, 222, 223, 294 (7-acetamidonitrazepam); 251, 222, 223, 250, 252, 195, 110, 97 (7-aminonitrazepam); 241, 77, 242, 105, 44, 43, 195, 57 (2-amino-5-nitrobenzophenone).



Quantification

Blood **GC** Columns: DB-1 and DB-1701 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 28° at 30°/min to 230° at 2°/min to 300° at 30°/min for 1 min or 120° for 1 min to 230° at 40°/min to 280° at 8°/min for 9 min. ECD. Limit of detection, 0.088 or 0.082 µmol/L [Gjerde *et al.* 1992]. Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2 to 3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 7.87 min. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 6.49 µg/L, limit of detection, 2.14 µg/L [Papoutsis *et al.* 2010]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 100 µg/L [Pirnay *et al.* 2002].

HPLC Column: Chromspher C_8 (100 × 3.0 mm i.d., 5 µm). Mobile phase: methanol : water (20 : 80 for 2 min to 30 : 70 at 2.20 min until 4 min to 40 : 60 at 4.4 min until 4.5 min to 43 : 57 at 5 min to 45 : 55 at 6 min to 52 : 48 at 7 min to 58 : 42 at 9.5 min to 75 : 25 at 10.5 min for 5 min to 20 : 80 at 15.3 min until 19 min), flow rate 0.7 mL/min. DAD (λ = 450 nm). Limit of quantification, 75 µg/L, limit of detection, 25 µg/L [Lambert *et al.* 1995]. Column: Nova-Pak phenyl-bonded (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 40 mmol/L phosphate buffer (28 : 72), flow rate 0.8 mL/min. UV detection (λ = 240 nm). Limit of quantification, 0.01 mg/L, limit of detection, 8 µg/L [Robertson, Drummer 1995]. Column: Spherisorb S 5W (250 × 4.9 mm i.d.). Mobile phase: methanol : diethyl ether : 1.85 nmol/L perchloric acid (80 : 20 : 0.02), flow rate 2 mL/min. UV detection (λ = 280 nm). Retention time: 2.93 min. Limit of detection, 5 µg/L for nitrazepam and 7-acetamidonitrazepam and 50 µg/L for 7-aminonitrazepam [Kelly *et al.* 1982].

LC-MS Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5 to 50 : 25 : 25 at 7 min to 10 : 45 : 45 at 27 min for 3 min to 95 : 5 : 5 at 31 min), flow rate 0.45 mL/min. APCI. Limit of quantification, 3 µg/L [Dussy *et al.* 2006]. Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 0.006 mol/L formic acid (pH 3.0) : methanol (70 : 30 to 60 : 40 at 5 min to 50 : 50 in 25 min to 40 : 60 at 30 min for 5 min to 70 : 30 at 36 min for 9 min), flow rate 0.2 mL/min. Limit of quantification, 0.7 µg/L, limit of detection, 0.2 µg/L [Smink *et al.* 2004].

Plasma **Spectrofluorimetry** Limit of detection, ~10 µg/L for nitrazepam and 25 µg/L for the sum of 7-amino- and 7-acetoamido-metabolites [Rieder 1973].

GC See Blood [Lillsunde, Seppälä 1990]. Column: 3% OV-17 on 100/120 mesh Chromosorb W or 3% SP-2250 on 100/120 mesh Supelcoport (1.5 or 1.8 m [5 or 6 ft] × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 245° or 275°. ECD. Limit of detection, 1 µg/L for nitrazepam and 200 ng/L for its hydrolysis product [Kangas 1977].

HPLC Column: C_{18} (250 × 4.6 mm). Mobile phase: methanol : water (65 : 35), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Nitrazepam and other benzodiazepines [Liang *et al.* 2009]. Column: Hisep (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.18 mol/L ammonium acetate buffer (pH 2.5, 15 : 85), flow rate 2 mL/min. UV detection (λ = 254 nm). Limit of quantification, 0.5 mg/L, limit of detection, 0.16 mg/L [Pistos, Stewart 2003]. Column: Nova-Pak C_{18} (4 µm). Mobile phase: acetonitrile : methanol : 10 nmol/L dipotassium hydrogen phosphate (pH 3.7, 30 : 2 : 100), flow rate 1.5 mL/min. DAD (λ = 240 nm). Limit of detection, 5 nmol/L [Akerman *et al.* 1996]. Column: Nova-Pak C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 6 mmol/L phosphate buffer : acetonitrile : methanol (64 : 23 : 13), flow rate 1.3 mL/min. UV detection (λ = 242 nm). Limit of detection, 6.0 µg/L [Boukhabza *et al.* 1991]. Column: C_8 reversed phase (250 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : 0.03 mol/L potassium dihydrogen phosphate (pH 4.5, 55 : 45), flow rate 1.1 mL/min. UV detection. Limit of detection, 5 µg/L [Ho *et al.* 1983]. See Blood [Kelly *et al.* 1982].

LC-MS Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.002 mg/L, limit of detection, 0.002 mg/L [Kratzsch *et al.* 2004].

Note For a radioimmunoassay for the measurement of nitrazepam see Dixon *et al.* [1979].

Serum **HPLC** Column: Hypersil Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L acetate buffer (pH 4.1, 60 : 40), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 5 ng [Honeychurch *et al.* 2006]. Column: Symmetry Shield RP8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen phosphate (40 : 60), flow rate 0.9 mL/min. UV detection (λ = 230 nm). Limit of detection, 1.0 µg/L [He *et al.* 2005]. Column: Kromasil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: butanol : 0.06 mol/L SDS (pH 7, 5 : 95), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 4 µg/L [Capella-Peiró *et al.* 2002]. Column: TSK gel Super-ODS (100 × 4.6 mm i.d., 2 µm) or Hypersil ODS-C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L sodium dihydrogen phosphate (pH 6, 45 : 55), flow rate 0.65 mL/min. UV detection (λ = 254 nm). Limit of sensitivity and quantification for nitrazepam and other benzodiazepines, 4–10 times better using column 1 [Tanaka *et al.* 1996]. Column: Supelcosil C_8 DB 5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 0.5 mol/L monobasic potassium phosphate (370 : 600 : 30), flow rate 1.65 mL/min. UV detection (λ = 219 nm). Limit of quantification, 1 µg/L [Welk 1996]. See Plasma [Akerman *et al.* 1996]. See Blood [Kelly *et al.* 1982].

LC-MS Unison UK-C_{18} RP ODS (150 × 2 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate containing 0.1% formic acid : methanol containing 0.1% formic acid (70 : 30 over 20 min to 20 : 80 over 5 min), flow rate 0.25 mL/min. MRM acquisition mode. Limit of quantification, 3.3 µg/L, limit of detection, 1.0 µg/L [Nakamura *et al.* 2009]. See Blood [Dussy *et al.* 2006]. Column: LiChrospher 60 RP-Select B (100 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : water : acetonitrile (1 : 1 : 1), flow rate 100 µL/min. APCI, SRM acquisition mode. Limit of quantification, 2 µg/L [Kleinschnitz *et al.* 1996].

Urine **Spectrofluorimetry** See Plasma [Rieder 1973].

GC Column: DB-17 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 80 kPa. Temperature programme: 150° for 1 min to 230° at 10°/min for 5 min to 300° at 10°/min for 9 min. ECD. Limit of detection, 160 µg/L [Guan *et al.* 1999]. Column: U-shaped borosilicate glass (1.5 m [5 ft] × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 275°. ECD or AFID. Limit of detection, 0.2 µg/L for nitrazepam, 50 µg/L for metabolites [Kangas 1979].

HPLC See Serum [He *et al.* 2005].

LC-MS Column: Hypurity C_8 (150 × 3 mm i.d.). Mobile phase: 4 mmol/L ammonium acetate (pH 6.8) in methanol-water (5 : 95) : 1% propan-2-ol, 0.05% formic acid in methanol (100 : 0 for 1 min to 0 : 100 at 3 min for 1.5 min to 100 : 0 over 0.1 min for 1.4 min). TIS, MRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 1.25 µg/L [Glover, Allen 2010]. Column: Polaris-C_{18}-A (150 × 2.1 mm i.d., 5 µm). Mobile phase: water : acetonitrile (20 : 80 to 90 : 10 at 4 min for 3 min to 20 : 80 at 7.1 min), flow rate 0.25 mL/min. ESI, SRM acquisition mode, positive ion mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.07 µg/L for 7-aminonitrazepam [Kuang *et al.* 2009]. Column: Waters Symmetry C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 5, 10 : 90 to 30 : 70 at 5 min to 80 : 20 at 9 min to 10 : 90 at 9.1 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.01 µmol/L for 7-aminonitrazepam [Hegstad *et al.* 2006]. See Serum [Kleinschnitz *et al.* 1996].

CE Capillary: fused silica (72 cm × 50 µm i.d.). Buffer: 60 mmol/L SDS–6 mmol/L phosphate borate (pH 8.5) : methanol (85 : 15). UV detection (λ = 220 nm). Limit of detection, 0.1–0.2 mg/L for nitrazepam and its metabolites [Tomita *et al.* 1993].

CE-MS Running buffer: methanol : water (50 : 50) containing 0.1% formic acid, flow rate 0.5 mL/min. ESI, TOF. Limit of quantification, 12.5 μg/L, limit of detection, 1.2 μg/L [Blas, McCord 2008].

Oral Fluid **LC-MS** Zorbax Eclipse XDB C_{18} (50 × 4.6 mm i.d., 1.8 μm). Mobile phase: 20 mmol/L ammonium formate : acetonitrile (50 : 50), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Moore *et al.* 2007].

Hair **LC-MS** Mobile phase: 3 mmol/L ammonium formate and 0.001% formic acid in water : acetonitrile (65 : 35 to 20 : 80 after 13 min to 10 : 90 at 13.5 min until 16.5 min to 65 : 35 until 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.05 ng/30 mg, limit of detection, 0.03 ng/30 mg [Miller *et al.* 2006].

Disposition in the Body Nitrazepam is readily absorbed after oral administration. It crosses the blood–brain barrier and the placenta and is found in breast milk. The major metabolites, 7-aminonitrazepam and the 7-acetamido derivative, accumulate to some extent in plasma. Other metabolites include 2-amino-5-nitrobenzophenone and 2-amino-3-hydroxy-5-nitrobenzophenone; all the metabolites are inactive. After oral administration, ~50–70% of a dose is excreted in the urine in 120 h and up to 20% is eliminated in the faeces. Up to ~37% of a dose may be excreted in the first 24 h, with ~5–10% of the dose as free 7-acetamidonitrazepam, 5% as 7-aminonitrazepam, 5% as conjugated 2-amino-3-hydroxy-5-nitrobenzophenone, and 20% as unknown acidic compounds. Approximately 20% of an oral dose is found in the faeces. After IV administration, ~90% is excreted in the urine in 120 h and up to ~10% is eliminated in the faeces. Only a small amount (<4%) is excreted as unchanged nitrazepam after oral or IV administration.

Therapeutic Concentration In plasma, usually in the range 0.03–0.07 mg/L.

Following daily oral doses of 5 mg to 4 subjects, steady-state plasma nitrazepam concentrations of 0.035–0.044 mg/L (mean, 0.04) were reported; combined steady-state concentrations of the 7-amino and 7-acetamido metabolites were in the range 0.018–0.053 mg/L (mean, 0.03) [Rieder, Wendt 1973].

Following a single oral dose of 5 mg to 9 subjects, peak plasma concentrations of 0.028–0.045 mg/L (mean, 0.04) were attained in 0.5–4 h [Breimer *et al.* 1977].

Following single oral doses of 5 mg to 12 healthy subjects, peak concentrations in serum and oral fluid were 40.7 and 1.9 μg/L, respectively, at ~2.5 h [Kangas *et al.* 1979].

Toxicity Blood concentrations greater than 0.2 mg/L may produce toxic effects.

In 2 deaths caused by overdose of nitrazepam, postmortem urine concentrations of 5.9 and 6.0 mg/L were reported; in the first case alcohol was also present at a blood concentration of 2100 mg/L [Oliver, Smith 1974].

In a death caused by ingestion of up to 250 mg nitrazepam, postmortem blood and liver concentrations of 9 mg/L and 4 μg/g, respectively, were reported [Loveland 1974].

The following postmortem tissue concentrations of nitrazepam and 7-aminonitrazepam, respectively, were reported in a 52-year-old woman who died from drowning and cold exposure following nitrazepam overdose: aorta blood 0.741 and 0.498 mg/L, femoral vein blood 0.450 and 1.56 mg/L, CSF 0.074 and 0.098 mg/L, vitreous humour 0.099 and 0.032 mg/L, pericardial fluid 0.188 and 0.780 mg/L, bile 4.08 and 1.67 mg/L, urine 0.580 and 1.09 mg/L, cerebral cortex 3.49 and 2.55 μg/g, diencephalons 6.22 and 2.49 μg/g, cerebellum 2.17 and 5.11 μg/g, myocardium 2.77 and 0.816 μg/g, left lung 0.656 and 0.717 μg/g, right lung 1.27 and 1.01 μg/g, spleen 0.458 and 0.329 μg/g, liver 0.059 and 0.113 μg/g, kidney 0.077 and 1.21 μg/g, femoral muscle 2.12 and 1.04 μg/g, stomach contents (total amount) 201 and 20.9 mg [Moriya, Hashimoto 2003].

For other reports of nitrazepam overdose fatalities, see Brodsgaard *et al.* [1995] and Giusti, Chiarotti [1979]. For a report of post-mortem distribution and redistribution of nitrazepam and other benzodiazepines, see Robertson, Drummer [1998].

Half-life Plasma half-life, 18–38 h (mean, 28).

Volume of Distribution Approximately 2–3 L/kg, increased in elderly subjects.

Clearance Plasma clearance, ~1 mL/min/kg.

Protein Binding ~85–88%.

Note For a review of the pharmacokinetics of nitrazepam see Kangas, Breimer [1981].

Dose Usually 5 to 10 mg.

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Nitrendipine

Calcium Antagonist, Calcium Channel Blocker

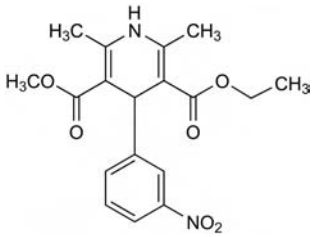
$C_{18}H_{20}N_2O_6 = 360.4$

CAS—39562-70-4

IUPAC Name 5-*O*-Ethyl 3-*O*-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

Synonyms Bay-e-5009; 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic ethyl methyl ester; nitrendipinum.

Proprietary Names *Bayotensin*; *Baypresol*; *Baypress*; *Bylotensin*; *Deiten*; *Gericin*; *Monopress*; *Nidrel*; *Niprina*; *Nitrendepat*; *Nitrepress*; *Subtension*; *Tensogradal*; *Trendinol*; *Vastensium*.



Chemical Properties A yellow crystalline powder. Mp 158°. Practically insoluble in water; sparingly soluble in absolute alcohol and methanol; freely soluble in ethyl acetate. Log *P* (octanol/water), 2.88. Exposure to light leads to formation of nitrophenylpyridine derivatives.

Thin-layer Chromatography System TB—R_f 0.02; system TE—R_f 0.80; system TAE—R_f 0.87.

Plate: silica gel 60 F_{254} (10 × 10 cm, 5 µm). Mobile phase: ethyl acetate : chloroform (1:9). UV detection (λ= 254 nm). R_f 0.68 [Tipre, Vavia 2001].

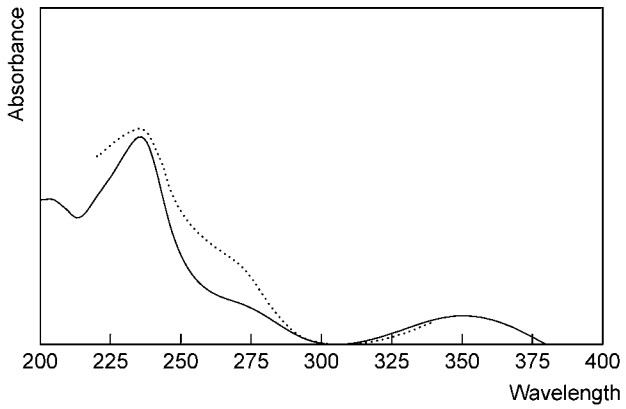
Gas Chromatography System GA—nitrendipine RI 2635, M (dehydro-desethyl-)–CO_2 RI 2275, M (dehydro-desmethyl-)–CO_2 RI 2330, M (dehydro-desethyl-OH-)–H_2O RI 2650, M (dehydro-desethyl-OH-)–H_2O RI 2690; system GP—M (dehydro-desethyl-)-Me RI 2300, M (dehydro-) RI 2370.

Column: DB1 fused-silica (40 m × 0.25 mm i.d., 0.25 mm). Temperature: 250°. Carrier gas: H_2, 32 cm/s. IS: amlodipine. Detection: ^{63}Ni ECD. Retention time: nitrendipine, 6.7 min; IS, 9.9 min [Monkman *et al.* 1996].

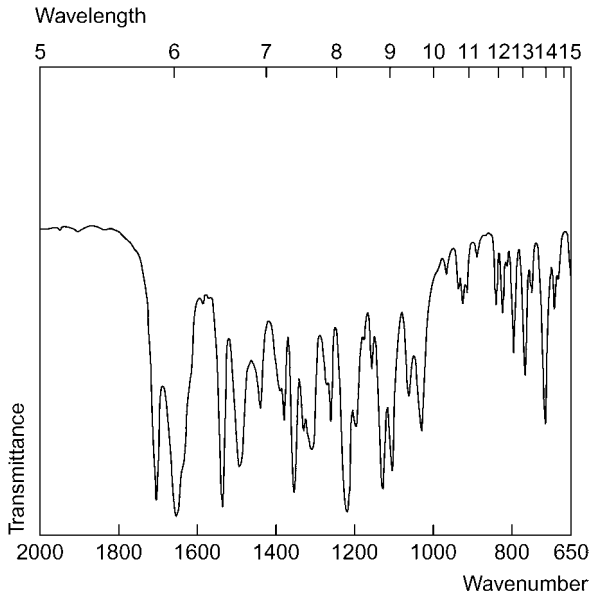
High Performance Liquid Chromatography System HX—RI 625; system HY—RI 554; system HAA—retention time 22.1 min.

Column: Lichrocart (125 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : water : acetonitrile (45 : 45 : 10), flow rate 1.2 mL/min. UV detection (λ= 235 nm). Retention time: 5.94 min [Tipre, Vavia 2001].

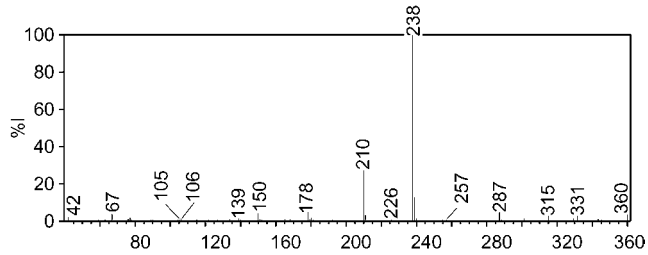
Ultraviolet Spectrum Aqueous acid—236, 203, 350 nm.



Infrared Spectrum Principal peaks at wavenumber 1640, 1203 and 896 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 238, 210, 239, 287, 150, 360, 178, 67.



Quantification

Blood **HPLC** UV detection. Limit of detection, 1 µg/L [Janis *et al.* 1983].

Plasma **GC-MS** Limit of quantification, <0.5 µg/L [Beck, Ryman 1985].

HPLC Column: ODS Jasco (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium dihydrogen phosphate (pH 4.5), flow rate 1.5 mL/min. IS: felodipine. UV detection (λ= 250 nm). Retention time: nitrendipine, 6.93 min; IS, 12.20 min. Limit of detection, 15 µg/L [Patel *et al.* 1998].

Serum **GC-MS** Column: SE-52 fused silica capillary (25 m × 0.32 mm i.d., 0.26 µm). Temperature programme: 110° for 1 min to 320° at 30°/min. Carrier gas: CH_4. NICI, SIM at *m/z*: 360. Retention time: 6.7 min. Limit of quantification, 0.1 µg/L [Fischer *et al.* 1993].

Disposition in the Body Nitrendipine is well absorbed following oral administration and undergoes extensive first pass metabolism in the liver to inactive metabolites. Major routes of metabolism are dehydrogenation to the pyridine analogue, cleavage of ester groups by hydrolysis to carboxylic acids and hydroxylation of methyl groups with subsequent glucuronide conjugation in the bile. All 5 known metabolites are 1000 times less potent than the parent drug; hence the dihydropyridine ring is thought to be essential for activity. It is excreted as inactive polar metabolites, mainly in urine (80%) and a small amount in faeces (8%). <0.1% of an oral dose appears as unchanged drug in the urine.

Therapeutic Concentration

Twenty male and female patients with chronic renal failure and elevated diastolic blood pressure were involved in this study. Ten of the patients required maintenance haemodialysis, mean age of 63.7 years, and the other 10 were at the pre-dialysis stage, mean age of 55.6 years. All were administered with an oral dose of 5 mg nitrendipine as a solution. The peak plasma concentration for the patients undergoing dialysis was 17.3 (9.0–33.0) µg/L observed at 0.5 (0.25–3.0) h and for the non-dialysis patients, 12.4 (6.9–22.2) µg/L at 0.5 (0.25–2.5) h [Kierdorf *et al.* 1993].

Seven elderly hypertensive patients with chronic renal failure, mean age of 72 years (range, 56–87 years) and 6 control subjects with hypertension but normal renal function, mean age of 67 years (range, 48–85 years) were involved in the study. They were all administered with 20 mg nitrendipine, before breakfast, once daily for 7 days. For the patients, peak concentrations for the racemate drug were 39.1 (18.4–64.1) µg/L; for (*S*)-nitrendipine, 26.3 (12.5–40.4) µg/L; and for (*R*)-nitrendipine, 12.8 (5.9–23.7) µg/L. All were observed at 1.9 (0.5–3.0) h. In the control subjects, concentrations were 25.6 (10.9–36.4) µg/L, 16.7 (7.3–22.9) µg/L and 9.0 (3.6–13.5) µg/L for the racemate, (*S*)-nitrendipine and (*R*)-nitrendipine, respectively. Again all concentrations were observed at 1.7 (1.0–2.0) h [Soons *et al.* 1992].

Toxicity There is a potential for seizures precipitously following acute ingestion of large doses.

Bioavailability 10–23%. Depends partly on dosage form. Bioavailability of tablet relative to solution is ~75%.

Half-life 10–22 h.

Volume of Distribution 4–8 L/kg.

Clearance The total oral clearance from plasma is 81–87 L/h.

Protein Binding 98%.

Note For a general review of nitrendipine, see Goa and Sorkin [1987].

Dose 20 mg daily with a maximum of 40 mg.

Beck O, Ryman T (1985). Quantification of nitrendipine in plasma by a capillary column gas chromatographic-mass spectrometric method. *J Chromatogr* 337: 402–407.

Fischer C *et al.* (1993). Simultaneous assessment of the intravenous and oral disposition of the enantiomers of racemic nimodipine by chiral stationary-phase high-performance liquid chromatography and gas chromatography/mass spectroscopy combined with a stable isotope technique. *J Pharm Sci* 82(3): 244–250.

Goa KL, Sorkin EM (1987). Nitrendipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of hypertension. *Drugs* 33: 123–155.

Janis R *et al.* (1983). Radioreceptor and high-performance liquid chromatographic assays for the calcium channel antagonist nitrendipine in serum. *J Clin Pharmacol* 23: 266–273.

Kierdorf H *et al.* (1993). Pharmacodynamics and pharmacokinetics of oral nitrendipine solution in hypertensive patients with advanced renal failure. *Eur J Clin Pharmacol* 45(2): 129–134.

Monkman SC *et al.* (1996). Automated gas chromatographic assay for amlodipine in plasma and gingival crevicular fluid. *J Chromatogr B Biomed Appl* 678: 360–364.

Patel YP *et al.* (1998). Isocratic, simultaneous reversed-phase high-performance liquid chromatographic estimation of six drugs for combined hypertension therapy. *J Chromatogr A* 828: 283–286.

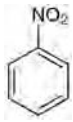
Soons PA *et al.* (1992). Stereoselective pharmacokinetics of oral nitrendipine in elderly hypertensive patients with normal and impaired renal function. *Eur J Clin Pharmacol* 42(4): 423–427.

Tipre DN, Vavia PR (2001). Oxidative degradation study of nitrendipine using stability indicating, HPLC, HPTLC and spectrophotometric method. *J Pharm Biomed Anal* 24: 705–714.

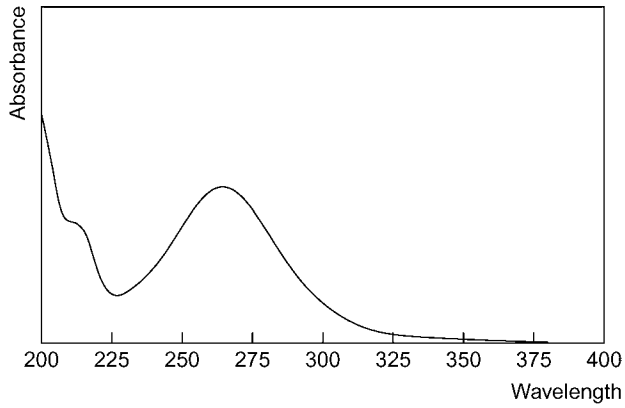
Nitrobenzene

Solvent
$C_6H_5NO_2$ = 123.1
CAS—98-95-3

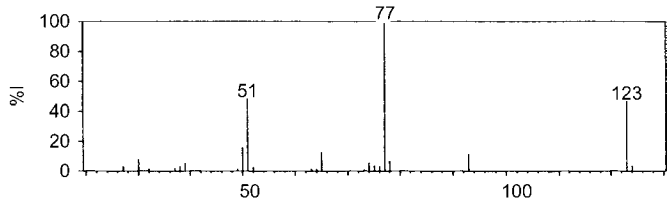
Synonyms Nitrobenzol; oil of Mirbane.



Chemical Properties A pale yellow, oily liquid. Mass per mL about 1.203 g. Bp 210° to 212°. Practically insoluble in water; freely soluble in alcohol, benzene, ether and oils. Log *P* (octanol/water), 1.8.
Ultraviolet Spectrum Ethanol—258 nm (A_1^1=697b).



Mass Spectrum Principal ions at *m/z* 77, 51, 123, 50, 65, 93, 30, 78.



Quantification

Urine **GC-MS** For method of quantification for nitrobenzene and other nitroaromatic compounds, see Bader *et al.* [1998].

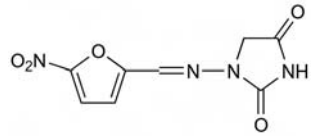
HPLC For method of quantification for metabolites of nitrobenzene and other aromatic compounds, see Astier [1992].

Astier A (1992). Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene. *J Chromatogr* 573: 318–322.
Bader M *et al.* (1998). Analysis of nitroaromatic compounds in urine by gas chromatography-mass spectrometry for the biological monitoring of explosives. *J Chromatogr B Biomed Sci Appl* 710: 91–99.

Nitrofurantoin

Antibacterial (Urinary)
$C_8H_6N_4O_5$ = 238.2
CAS—67-20-9 (anhydrous); 17140-81-7 (monohydrate)

IUPAC Name 1-[[(5-Nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione
Synonym Furadonium
Proprietary Names *Berkfurin*; *Cyantin*; *Furadantin(e)*; *Furadöine*; *Furan*; *Furatine*; *Ituran*; *Macrodantin*; *Nephronex*; *Nifuran*; *Nitrex*; *Novofuran*; *Trantoin*; *Urantoin*; *Urolong*. It is an ingredient of *Ceduran*.



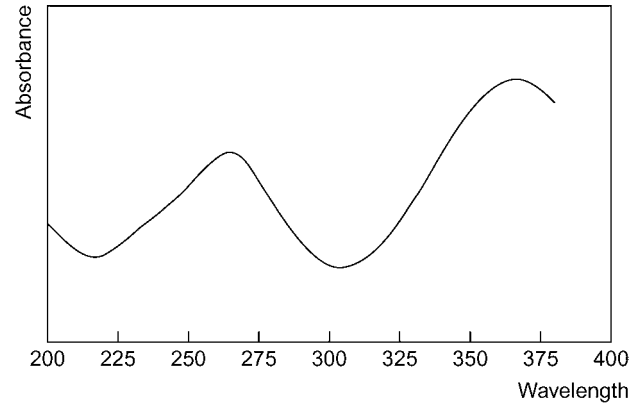
Chemical Properties Yellow crystals or fine powder. Discoloured by alkalis and by exposure to light. Mp about 271°. Soluble 1 in 5000 of water, 1 in 2000 of ethanol, 1 in 200 of acetone and 1 in 16 of dimethylformamide. pK_a 7.2 (25°). Log *P* (octanol/water), −0.5.

Nitrofurantoin Sodium

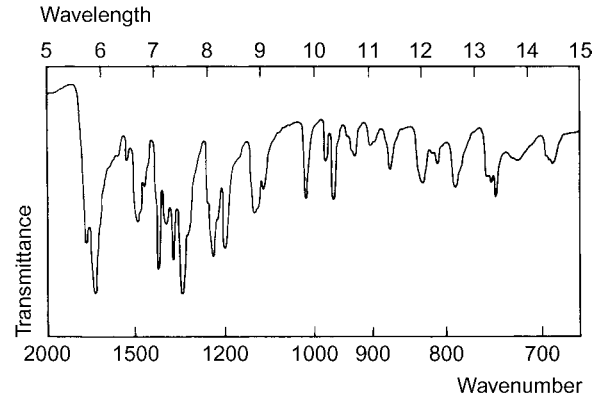
$C_8H_5N_4NaO_5$ = 260.1
CAS-54-87-5

Proprietary Name *Ivadantin*
Chemical Properties A yellow-orange coloured powder.

Colour Test Methanolic potassium hydroxide—yellow-orange.
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.02; system TE—R_f 0.06; system TF—R_f 0.30; system TAD—R_f 0.33; system TAE—R_f 0.84; system TAJ—R_f 0.46; system TAK—R_f 0.09; system TAK—R_f 0.72.
High Performance Liquid Chromatography System HX—RI 319; system HY—RI 288; system HZ—retention time 2.4 min.
Ultraviolet Spectrum After solution in dimethylformamide and dilution with water—266, 367 nm (A_1^1=765a).



Infrared Spectrum Principal peaks at wavenumbers 1718, 1237, 1205, 1770, 1513, 1126 cm^{-1} (KBr disk).



Quantification

Plasma **HPLC** UV detection. Limit of detection, 0.01 mg/L [Muth *et al.* 1996]. UV detection. Limit of detection, 4 mg/L for aminofurantoin and cyanofurantoin [Hoener, Wolff 1980]. UV detection. Limit of detection, 20 µg/L [Vree *et al.* 1979].

Spectrofluorimetry For method, see Watari *et al.* [1980].

Urine **HPLC** UV detection. Limit of detection 0.38 mg/L. [Muth *et al.* 1996]. UV detection. For method of quantification for aminofurantoin and cyanofurantoin, see Hoener and Wolff [1980]. See Plasma [Vree *et al.* 1979].

Spectrofluorimetry See Plasma [Watari *et al.* 1980].

Disposition in the Body Nitrofurantoin is readily and almost completely absorbed after oral administration. About 40% of a dose is excreted in the urine unchanged in 24 h together with small amounts of the reduced metabolite, aminofurantoin.

Therapeutic Concentration

Following a single oral dose of 50 mg to 6 fasting subjects, peak plasma concentrations of 0.29 to 0.66 (mean, 0.43) mg/L were attained in 1 to 4 h [Hoener, Patterson 1981].

Half-life Plasma half-life, about 0.5 to 1 h.
Volume of Distribution About 0.6 L/kg.
Clearance Plasma clearance, about 10 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.3.
Protein Binding About 60%.
Dose Usually 400 mg daily.

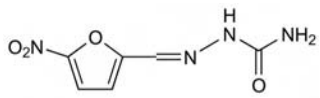
Hoener BA, Wolff JL (1980). High-performance liquid chromatographic assay for the metabolites of nitrofurantoin in plasma and urine. *J Chromatogr* 182: 246–251.
Hoener BA, Patterson SE (1981). Nitrofurantoin disposition. *Clin Pharmacol Ther* 29: 808–816.
Muth P *et al.* (1996). Sensitive determination of nitrofurantoin in human plasma and urine by high-performance liquid chromatography. *J Chromatogr A* 729: 251–258.

Vree TB *et al.* (1979). Determination of nitrofurantoin (Furadantine) and hydroxymethylnitrofurantoin (Urfadyn) in plasma and urine of man by means of high-performance liquid chromatography. *J Chromatogr* 162: 110–116.
Watari N *et al.* (1980). Fluorescence assay of nitrofurantoin with o-aminothiophenol in plasma and urine. *J Pharm Sci* 69: 106–107.

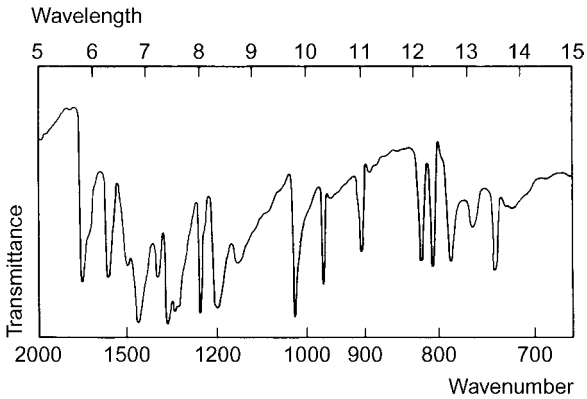
Nitrofurazone

Antibacterial
$C_6H_6N_4O_4$ = 198.1
CAS—59-87-0

IUPAC Name 2-[(5-Nitro-2-furanyl)methylene]hydrazinecarboxamide
Synonyms Furacilinum; nitrofural.
Proprietary Name *Furacin(e)*



Chemical Properties A lemon to brownish-yellow crystalline powder which slowly darkens on exposure to light and discolours in contact with alkalis. Mp 236° to 240°, with decomposition. Soluble 1 in 4200 of water, 1 in 590 of ethanol, 1 in 350 of propylene glycol; soluble in dimethylformamide; soluble in alkaline solutions; practically insoluble in chloroform and ether. pK_a 10.0. Log *P* (octanol/water), 0.2.
Colour Test Methanolic potassium hydroxide—red.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.41; system TF—R_f 0.11; system TAE—R_f 0.78; system TAJ—R_f 0.34; system TAK—R_f 0.36; system TAL—R_f 0.83.
High Performance Liquid Chromatography System HX—RI 316.
Ultraviolet Spectrum Aqueous acid—261 nm (A^1_1=610b); aqueous alkali—284 nm (A^1_1=475b).
Infrared Spectrum Principal peaks at wavenumbers 1023, 1250, 1200, 970, 1718, 1585 cm^{-1} (Nujol mull).

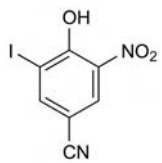


Uses Topically in a concentration of 0.2%; doses of 0.4 to 2 g daily have been given orally.

Nitroxinil

Anthelmintic (Veterinary)
$C_7H_3IN_2O_3$ = 290.0
CAS—1689-89-0

IUPAC Name 4-Hydroxy-3-iodo-5-nitrobenzonitrile
Synonym Nitroxynil

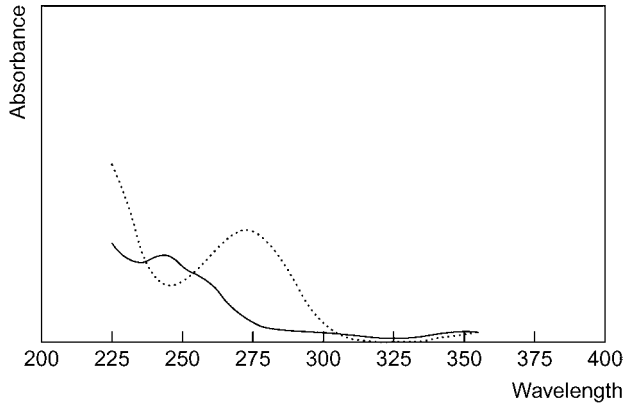


Chemical Properties A yellow powder. Mp 136° to 139°. Sparingly soluble in water; soluble 1 in 120 of ethanol and 1 in 60 of ether; soluble in solutions of alkali hydroxides. Log *P* (octanol/water), 3.2.

Nitroxinil Eglumine Salt

$C_7H_3IN_2O_3$, $C_8H_{19}NO_5$ = 499.3
CAS—27917-82-4
Proprietary Name *Trodax*
Chemical Properties Readily soluble in water.

Colour Tests Iodine test—positive; methanolic potassium hydroxide—yellow.
Thin-layer Chromatography System TA—R_f 0.83 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1754.
Ultraviolet Spectrum Aqueous acid—243, 350 nm; aqueous alkali—271 nm (A^1_1=660a).

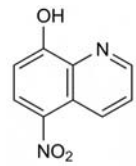


Infrared Spectrum Principal peaks at wavenumbers 1529, 1245, 1123, 730, 1309, 1600 cm^{-1} (KBr disk).

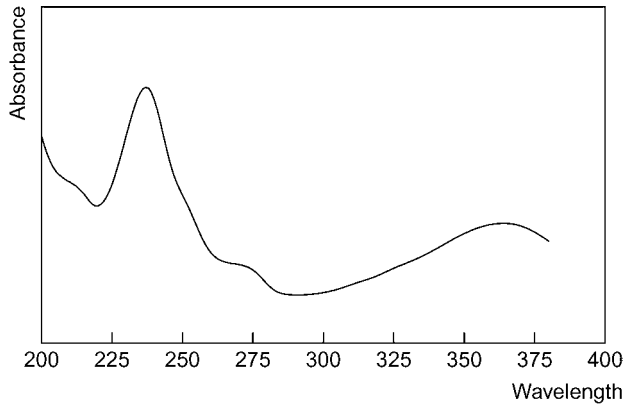
Nitroxoline

Antibacterial (Urinary)
$C_9H_6N_2O_3$ = 190.2
CAS—4008-48-4

IUPAC Name 5-Nitro-8-quinolinol
Proprietary Names *Nibiol*; *Uro-Coli.*



Chemical Properties A yellow crystalline powder. Mp 179° to 182°. Sparingly soluble in ethanol and ether; freely soluble in alkalis and hot hydrochloric acid. Log *P* (octanol/water), 2.0.
Colour Tests Mandelin's test—green; methanolic potassium hydroxide—yellow.
Thin-layer Chromatography System TA—R_f 0.13, streaking (visible yellow spot; acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—249 (A^1_1=1914a), 293 nm.



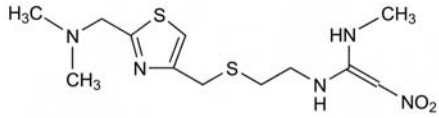
Infrared Spectrum Principal peaks at wavenumbers 1504, 1277, 1307, 1189, 1149, 1565 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** Absorbance detection (λ=436 nm). For method, see Sorel *et al.* [1981].
Urine **HPLC** See Plasma [Sorel *et al.* 1981].
Disposition in the Body Nitroxoline is readily and almost completely absorbed after oral administration. About 60% of a dose is excreted in the urine in 24 h, mainly as conjugated nitroxoline.
Dose 300 to 500 mg daily.

Sorel RH *et al.* (1981). High-performance liquid chromatographic analysis of nitroxoline in plasma and urine. *J Chromatogr* 222: 241–248.

Nizatidine

Histamine H_2-Receptor Antagonist, Ulcer-Healing Drug
$C_{12}H_{21}N_5O_2S_2 = 331.5$
CAS—76963-41-2

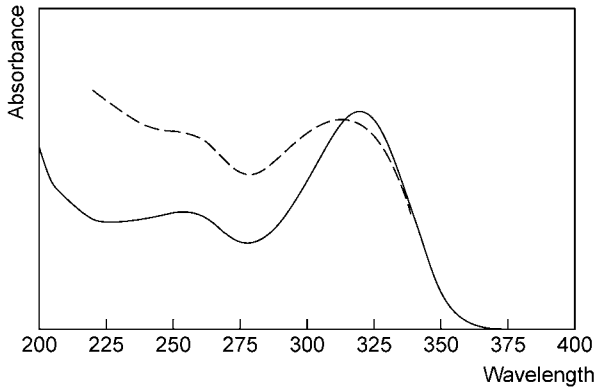
IUPAC Name *N*-[2-[[[2-[(Dimethylamino)methyl]-4-thiazolyl]methyl]thio]ethyl]-*N′*-methyl-2-nitro-1,1-ethenediamine
Synonyms LY-139037; ZE-101; ZL-101.
Proprietary Names *Antizid*; *Axid*; *Calmaxid*; *Cronizat*; *Distaxid*; *Gastrax*; *Naxidine*; *Nizax*; *Nizaxid*; *Panaxid*; *Tazac*; *Ulcosal*; *Ulxit*; *Zanizal*; *Zinga*.



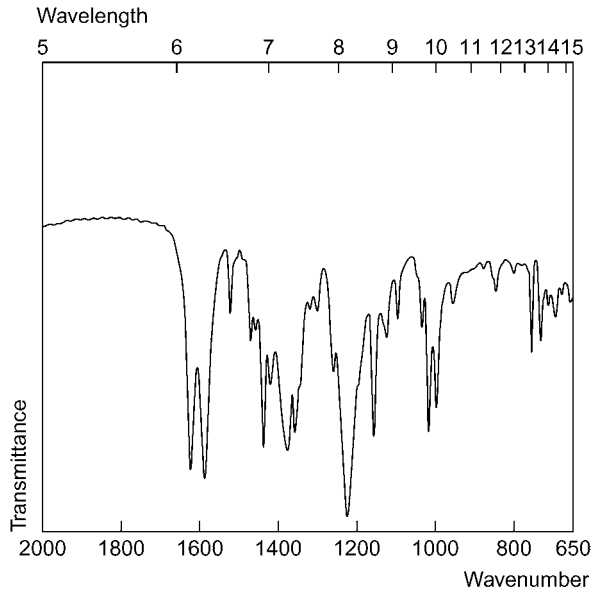
Chemical Properties A white to off-white crystalline solid. Mp 130° to 132°. Freely soluble in chloroform; soluble in methanol; sparingly soluble in water and buffered solutions; slightly soluble in ethyl acetate and isopropane; essentially insoluble in benzene, diethyl ether and octanol. pK_a 2.1, 6.8 (in aqueous media); 6.3, 8.4 (dimethylformamide). Log *P* (octanol/water), −0.43.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.28; system TAE—R_f 0.45.
High Performance Liquid Chromatography System HY—RI 178; system HAA—retention time 3.3 min; system HAX—RT 5.6 min; system HAY—RT 3.1 min.

Column: ODS-2 Inertsil (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.04 mol/L sodium dihydrogen phosphate : acetonitrile : methanol : triethylamine (345 : 20 : 35 : 0.7), flow rate 1 mL/min. IS: procaine hydrochloride. UV detection (λ=230 nm). Retention time: nizatidine, 18.6 min; IS, 10.7 min [Ho *et al.* 1999].

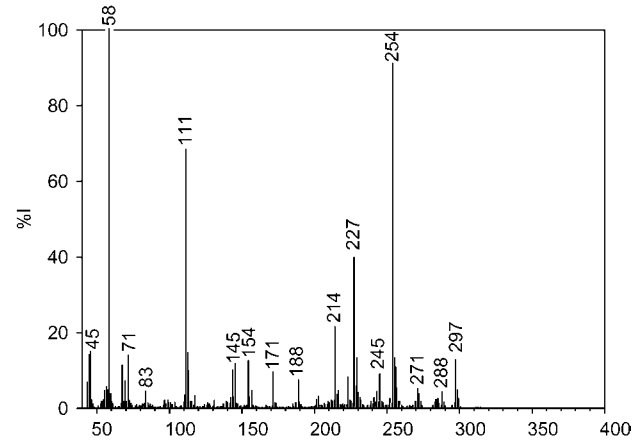
Column: Si-60 Lichrosorb (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water : 25% ammonia solution (1000 : 200 : 20 : 5), flow rate 2.0 mL/min. IS: ranitidine. UV detection (λ=320 nm). Retention time: nizatidine, 2.7 min; *N*-desmethylnizatidine, 4.2 min; nizatidine sulfoxide, 6.2 min; IS, 5.1 min [Tracqui *et al.* 1990].
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—319, 254 nm; methanol—240, 325 nm; basic—313 nm; water—260, 314 nm.



Infrared Spectrum Principal peaks at wavenumber 1227, 1436, 1586 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 58, 254, 111, 227, 214, 45, 44, 71.



Quantification
***Plasma* HPLC** UV detection (λ=313 nm). Limit of detection, 0.01 mg/L [Knadler *et al.* 1986].
***Urine* HPLC** See Plasma [Knadler *et al.* 1986].
***Saliva* HPLC** See Plasma [Knadler *et al.* 1986].
Disposition in the Body Nizatidine is readily and almost completely absorbed after oral administration, and peak plasma concentrations are reached within 1 to 3 h. Absorption is increased by the presence of food and decreased by 10% in the presence of antacids such as aluminium hydroxide gel and magnesium silicate. It is partially metabolised by the liver, but does not inhibit the hepatic mixed function oxidase system. Three metabolites have been identified, nizatidine *N*-2-oxide, nizatidine *S*-oxide and *N*-2-monodesmethylnizatidine (60% activity of nizatidine). Nizatidine is widely distributed throughout the body and has been detected in breast milk (0.1% of the administered dose). 90% of an administered dose is excreted in urine, partly by active tubular secretion, with 60% as the unchanged drug. <6% of a dose is excreted in faeces.
Therapeutic Concentration The serum therapeutic concentration is 0.15 to 1.0 mg/L.

Five lactating and 5 non-lactating women, aged between 19 and 34 years, were administered with a single dose of 150 mg nizatidine and 150 mg every 12 h for a total of 5 doses (multiple dosing study). The mean peak plasma concentrations were 1.455 mg/L for the lactating women and 1.46 mg/L for the non-lactating women after the single dose. Both concentrations were reached at about 1.5 h. The multiple doses produced peak levels of 1.59 and 1.16 mg/L for the lactating and non-lactating women, respectively in 1.8 and 1.3 h [Obermeyer *et al.* 1990].

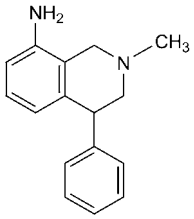
Toxicity Little experience with human overdosing.
Bioavailability >70%.
Half-life 1.3 to 1.6 h (prolonged in renal impairment).
Volume of Distribution Approximately 1.2 to 1.6 L/kg (single 150 mg dose); 1.1 to 1.9 L/kg (multiple doses).
Clearance Serum, 37.5 to 41.4 L/h (single 150 mg dose); 39.1 to 44.6 L/h (multiple doses). Plasma, 40 to 51 L/h.
Protein Binding 35%.
Distribution in Blood Plasma : blood cell ratio is 3 : 1.
Dose Orally: the usual daily dose is 150 to 600 mg. The total IV daily dose should not exceed 480 mg. Patients with renal impairment: 150 mg once or twice daily, or alternate days (creatinine clearance 20 to 50 mL/min) and 150 mg daily, alternate days or every third day (creatinine clearance <20 mL/min).

Ho C *et al.* (1999). Simultaneous high-performance liquid chromatographic analysis for famotidine, ranitidine HCl, cimetidine, and nizatidine in commercial products. *Drug Dev Ind Pharm* 25: 379–385.
Knadler MP *et al.* (1986). Nizatidine, an H2-blocker. Its metabolism and disposition in man. *Drug Metab Dispos* 14: 175–182.
Obermeyer BD *et al.* (1990). Secretion of nizatidine into human breast milk after single and multiple doses. *Clin Pharmacol Ther* 47: 724–730.
Tracqui A *et al.* (1990). Determination of nizatidine and two of its main metabolites in human serum using high-performance liquid chromatography. *J Chromatogr* 529: 369–376.

Nomifensine

Antidepressant
$C_{16}H_{18}N_2 = 238.3$
CAS—24526-64-5

IUPAC Name 2-Methyl-4-phenyl-3,4-dihydro-1*H*-isoquinolin-8-amine
Synonym 1,2,3,4-Tetrahydro-2-methyl-4-phenyl-8-isoquinolinamine



Chemical Properties Mp 179° to 181°. Log *P* (octanol/water), 2.4. Derivatised samples are stable for 60 days at 4° [Vereczkey *et al.* 1976].

Nomifensine Maleate

$C_{16}H_{18}N_2$, $C_4H_4O_4$ = 354.4
CAS—32795-47-4

Proprietary Names *Alival*; *Merital*; *Psicronizer*.

Chemical Properties A white or slightly yellowish powder. Mp ≈195°. Soluble in dimethylformamide and methanol.

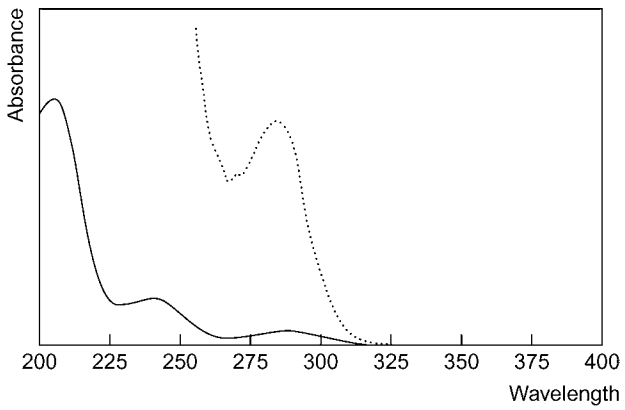
Colour Tests Coniferyl alcohol—yellow; diazotisation—red; Liebermann's reagent—orange; Marquis test—orange (slow).

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.09; system TC—R_f 0.29; system TE—R_f 0.64; system TAE—R_f 0.53; system TAF—R_f 0.52; system TAG—R_f 0.31.

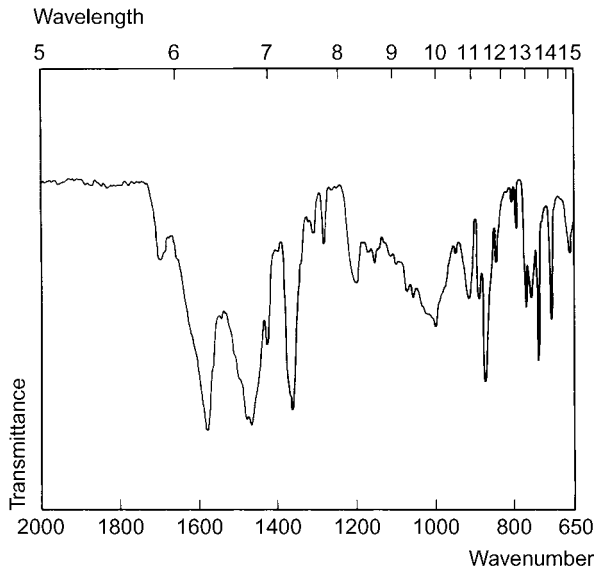
Gas Chromatography System GA—nomifensine RI 2130, M (OH-) RI 2450, M-isomer 1 RI 2505, M-isomer 2 RI 2590; system GB—RI 2239; system GF—RI 2670; system GM—RRT 0.850.

High Performance Liquid Chromatography System HA—*k* 0.9; system HF—*k* 0.42; system HX—RI 349; system HY—RI 296.

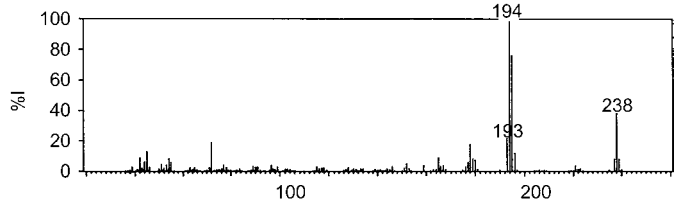
Ultraviolet Spectrum Aqueous alkali—284 nm (A^1_1 = 75b); methanol—241 (A^1_1 = 461b), 292 nm (A^1_1 = 124a).



Infrared Spectrum Principal peaks at wavenumbers: 1580, 877, 741, 1000, 708, 775 cm^{-1} (nomifensine maleate).



Mass Spectrum Principal ions at *m/z* 194, 195, 238, 193, 72, 178, 45, 196.



Quantification

Plasma **GC** Column: 3% OV-17 on 80/100 mesh HP Chrom WAW-DMCS (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 180° to 265° at 3°/min. FID. Retention time: 12.25 min. Limit of quantification, 1 µg/L [Quaglio, Bellini 1984]. Column: 3% OV-101 on Chromosorb WHP 100/120 mesh (1.2 m × 2 mm i.d.). Carrier gas: He or N_2, 30 mL/min. Temperature: 215°. NPD. Limit of detection, 5 µg/L [McIntyre *et al.* 1981]. Column: OV-101 (50 m × 0.25 mm i.d.). Carrier gas: He, 2 mL/min. Temperature: 200°. Limit of detection, 2 µg/L [Bailey *et al.* 1977]. Column: 3% OV-25 on Chromosorb W-HP. Carrier gas: He, 30 mL/min. Temperature: 250°. NSD. Retention time: ~3 min. Limit of detection, 10 µg/L [Chamberlain, Hill 1977]. Column: 3% OV-17 100/120 mesh Chromosorb Q (2 m × 0.46 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 245°. ECD. Limit of detection, 1 µg/L [Vereczkey *et al.* 1976].

HPLC Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.01 mol/L potassium phosphate buffer (pH 2.25) : methanol : dioxane (86.5 : 6.5 : 7), flow rate 2.2 mL/min. UV detection (λ = 210 nm). Limit of detection, 0.007 µmol/L for metabolites of nomifensine [Lindberg 1985].

Note For a radioimmunoassay method, see McIntyre *et al.* [1981].

Serum **GC** Column: 3% OV-17 on 80/100 mesh Chromosorb W HP (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 250°. ECD. Retention time: 2.10 min. Limit of detection, 8 nmol/L [Lindberg *et al.* 1983].

HPLC Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.05 mol/L potassium phosphate buffer (pH 3.5) : acetonitrile (74 : 26), flow rate 1.6 mL/min. UV detection (λ = 205 nm). Retention time: 4.15 min. *k'*: 2.0. Limit of detection, 8 nmol/L. [Lindberg *et al.* 1983].

Disposition in the Body Readily absorbed after oral administration. More than 99% of the dose is excreted within 48 h, ~96% is in urine and ~3% in faeces. Of the material excreted in the urine, ~60% is the glucuronide conjugate of nomifensine; the metabolites found in urine include 4′-hydroxynomifensine, which is active, 4′-hydroxy-3′-methoxynomifensine, and 3′-hydroxy-4′-methoxynomifensine, each of which accounts for ~7% of the dose. A further 4 metabolites, accounting for <1% of the dose, have also been identified and the remaining material is present as unidentified stable conjugates. After a single dose, <5% is usually excreted as unchanged drug. The proportion of free nomifensine found in the urine is dependent on the urinary pH, the time and temperature of storage of the sample, and the extraction procedure, because the glucuronide is readily hydrolysed if the pH is below 7.1 and the sample is not frozen.

Therapeutic Concentration A relationship between plasma concentration and clinical effect has not been established. Nomifensine is present in plasma mainly as the glucuronide conjugate; when both free and conjugated nomifensine concentrations are required to be measured, plasma samples must be deep-frozen immediately after collection to prevent hydrolysis. Steady-state concentrations are usually achieved within 5 days.

Five men (aged 23 to 41 years) and 1 woman (28 years old) were administered 100 mg nomifensine as a single dose or an IV infuson. Following the oral dose, a peak plasma concentration of 0.18 µmol/L was reached after 1.13 h. The peak concentration following the IV infusion was 1.21 µmol/L [Lindberg, Syvalahti 1986; Lindberg *et al.* 1986]

Following a single oral dose of 100 mg to 6 male subjects, peak plasma concentrations of 0.09–0.18 mg/L (mean 0.13) free nomifensine and 2.65–3.68 mg/L (mean 3.1) total nomifensine were attained in 1–2 h; after a single oral dose of 100 mg to 6 female subjects, the corresponding peak plasma concentrations were 0.03–0.05 mg/L (mean 0.04), and 1.95–3.32 mg/L (mean 2.6), for free and total nomifensine, respectively [McIntyre *et al.* 1982].

Following oral administration of 25 mg three times a day to 9 subjects for 6 days, plasma concentrations of 0.005 to 0.017 mg/L (mean 0.011) were reported 6 h after a dose [Bailey *et al.* 1977].

Toxicity Ingestion of 3.5 g has been reported without serious toxic effects.

A 37-year-old man took ~50 tablets, the majority of which were nomifensine. His plasma concentration was 833 µg/L [Horn *et al.* 1986].

In 26 cases of suspected overdose where the estimated mean dose was 1.15 g, there were few serious toxic reactions; of the subjects who had ingested only nomifensine, only one became comatose and all eventually recovered [Dawling *et al.* 1979].

In a fatality involving the ingestion of alcohol and nomifensine, the following postmortem concentrations of nomifensine were reported: blood 17 mg/L, kidney 141 µg/g, liver 32 µg/g, urine 400 mg/L; a blood alcohol concentration of 5000 mg/L was also reported [Reyfer *et al.* 1979].

A 28-year-old woman took 1.5 g nomifensine. Her plasma nomifensine concentration was 2780 µg/L on admission and 1915 µg/L the following morning. She made a complete recovery [Montgomery *et al.* 1978].

Note For cases of dyskinesia of the tongue following nomifensine, see Gibson [1981] and Sandyk [1987]; for acute renal failure following nomifensine overdose,

see Skinner and Ferner [1986] and Prescott *et al.* [1980]. Nomifensine has been associated with hepatitis [Vaz *et al.* 1984].

Half-life Plasma half-life 1.5–4 h; prolonged in severe renal impairment. Half-life approximately 4 h after oral or IV dose; half-life shortens to 2.11 h after dosing for 2 weeks [Lindberg *et al.* 1986]

Volume of Distribution 8.69 L/kg [Lindberg *et al.* 1986].

Protein Binding 60–75%.

Note For a review of nomifensine, see Brogden *et al.* [1979], Kinney [1985] or Pepper [1986].

Dose 75 to 200 mg of nomifensine maleate daily.

Bailey E *et al.* (1977). Automated high-resolution gas chromatographic analysis of psychotropic drugs in biological fluids using open-tubular glass capillary columns. I. Determination of nomifensine in human plasma. *J Chromatogr* 131: 347–355.

Brogden RN *et al.* (1979). Nomifensine: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs* 18: 1–24.

Chamberlain J, Hill HM (1977). A simple gas chromatographic method for the determination of nomifensine in plasma and a comparison of the method with other available techniques. *Br J Clin Pharmacol* 4(Suppl 2): 117S–121S.

Dawling S *et al.* (1979). Nomifensine overdose and plasma drug concentration. *Lancet* 1: 56.

Gibson AC (1981). Nomifensine and dyskinesia. *Br J Psychiatry* 138: 439.

Horn ER *et al.* (1986). Nomifensine overdose with TCAs and MAOIs. *J Clin Psychopharmacol* 6: 315–316.

Kinney JL (1985). Nomifensine maleate: a new second-generation antidepressant. *Clin Pharm* 4: 625–636.

Lindberg RL (1985). Selective and sensitive high-performance liquid chromatographic assay for the metabolites of nomifensine in human plasma. *J Chromatogr* 341: 333–339.

Lindberg RL, Syvalahti EK (1986). Metabolism of nomifensine after oral and intravenous administration. *Clin Pharmacol Ther* 39: 378–383.

Lindberg RL *et al.* (1983). Determination of nomifensine in human serum. A comparison of high-performance liquid and gas–liquid chromatography. *J Chromatogr* 276: 85–92.

Lindberg RL *et al.* (1986). Disposition of nomifensine after acute and prolonged dosing. *Clin Pharmacol Ther* 39: 384–388.

McIntyre IM *et al.* (1981). Determination of nomifensine plasma concentrations: a comparison of radioimmunoassay and gas chromatography. *Br J Clin Pharmacol* 12: 691–694.

McIntyre IM *et al.* (1982). Pharmacokinetics of nomifensine after a single oral dose. *Br JClin Pharmacol* 13: 740–743.

Montgomery S *et al.* (1978). Nomifensine overdose. *Lancet* 1: 828–829.

Pepper GA (1986). Nomifensine (Merital): a second generation antidepressant. *Nurse Pract* 11: 72–74.

Prescott LF *et al.* (1980). Acute haemolysis and renal failure after nomifensine overdosage. *Br Med J* 281: 1392–1393.

Quaglio MP, Bellini AM (1984). Determination of nomifensine in pharmaceutical formulations and simultaneous GLC determination of nomifensine and benzodiazepines in human plasma. *Farmaco Prat* 39: 222–232.

Reyfer AF *et al.* (1979). [Suicide by poisoning with a drug whose active ingredient is nomifensine]. *Beitr Gerichtl Med* 37: 313–318.

Sandyk R (1987). Nomifensine-induced orofacial dyskinesia and Sydenham's chorea. *Int J Neurosci* 35: 91–92.

Skinner R, Ferner RE (1986). Acute renal failure without acute intravascular haemolysis after nomifensine overdosage. *Hum Toxicol* 5: 279–280.

Vaz FG *et al.* (1984). Hepatitis induced by nomifensine. *Br Med J* 289: 1268.

Vereczkey L *et al.* (1976). Gas chromatographic method for the determination of nomifensine in human plasma. *J Chromatogr* 116: 451–456.

Noracymethadol

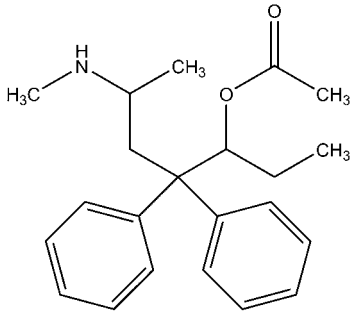
Narcotic Analgesic

$C_{22}H_{29}NO_2$ = 339.5

CAS—1477-39-0

IUPAC Name [6-Methylamino-4,4-di(phenyl)heptan-3-yl] acetate

Synonyms α-*dl*-3-Acetoxy-4,4-diphenyl-6-methylaminoheptane; α-*dl*-3-acetoxy-6-methylamino-4,4-diphenylheptane; ARC I-C-25; α-*dl*-4,4-diphenyl-6-methylamino-3-heptanol acetate; α-ethyl-β-[2-(methylamino)propyl]-β-phenylbenzeneethanol acetate; α-*dl*-6-(methylamino)-4,4-diphenyl-3-heptanol acetate.



Chemical Properties Soluble in dilute acetic acid. Noracymethadol is extracted by organic solvents from aqueous alkaline solutions.

Noracymethadol Hydrochloride

$C_{23}H_{27}NO_5$,HBr = 375.9

CAS—5633-25-0

Synonym NIH-7667

Chemical Properties Crystals from acetone and ether. Mp 216° to 217° [O'Neil *et al.* 2006].

Colour Tests Ammonium molybdate test—dull purple (limit of detection, 0.25 µg); ammonium vandate test—dull purple→grey-green (limit of detection, 0.25 µg); sulfuric acid-formaldehyde test—brown-purple (limit of detection, 0.25 µg); Vitali's test—brown (limit of detection, 0.25 µg).

Thin-layer Chromatography System T10—R_f 0.51 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—253, 258, 269 nm.

Disposition in the Body

Therapeutic Concentration Single oral doses of 2.5 to 30 mg to postpartum patients were 3.25-times as potent as morphine with fewer undesirable effects [Gruber, Bapisti 1963].

Gruber CM,Jr Bapisti AJr (1963). Estimating the acceptability of morphine and noracymethadol in postpartum patients. *Clin Pharmacol Ther* 4: 172–181.

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Noradrenaline

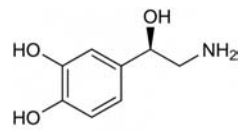
Catecholeamine, Sympathomimetic

$C_8H_{11}NO_3$ = 169.2

CAS—51-41-2

IUPAC Name 4-[(1*R*)-2-Amino-1-hydroxyethyl]-1,2-benzenediol

Synonyms *l*-Arterenol; levarterenol.



Chemical Properties Microcrystals. Mp 216.5° to 218°, with decomposition. Slightly soluble in water, ethanol and ether. pK_{a1} 8.6, pK_{a2} 9.8, pK_{a3} 12.0 (20°). Log *P* (octanol/water), −1.2.

Noradrenaline Acid Tartrate

$C_8H_{11}NO_3$,$C_4H_6O_6$,H_2O = 337.3

CAS—51-40-1 (anhydrous); 69815-49-2 (monohydrate)

Synonyms Norepinephrine tartrate.

Proprietary Names *Levophed*; *Noradrec*.

Chemical Properties A white or faintly grey, crystalline powder which darkens on exposure to air and light. Mp 102° to 104°, with decomposition. Soluble 1 in 2.5 of water and 1 in 300 of ethanol; practically insoluble in chloroform and ether.

Noradrenaline Hydrochloride

$C_8H_{11}NO_3$,HCl = 205.6

CAS—329-56-6

Proprietary Name *Arterenol*

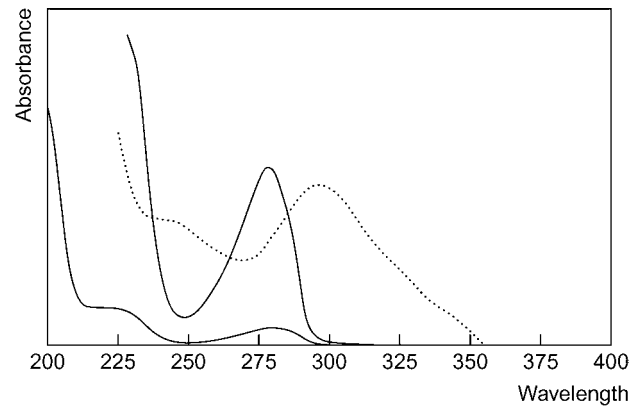
Chemical Properties Crystals. Mp about 145.2° to 146.4°. Freely soluble in water.

Colour Tests Ammoniacal silver nitrate (room temperature)—black; ferric chloride—green; Folin-Ciocalteu reagent—blue; Mandelin's test—orange; Marquis test—brown; methanolic potassium hydroxide—blue→orange; Nessler's reagent—black; potassium dichromate (method 1)—green→brown (30 s).

Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.03; system TL—R_f 0.00; system TC—R_f 0.00; system TAE—R_f 0.43 (acidified potassium permanganate solution, positive).

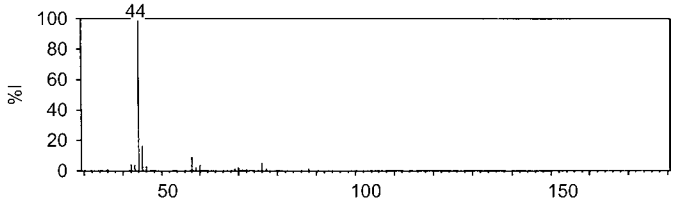
High Performance Liquid Chromatography System HB—*k* 0.10; system HY—RI 21; system HAA—retention time 2.8 min.

Ultraviolet Spectrum Aqueous acid—279 nm (A^{1}_{1}=160a); aqueous alkali—296 nm.



Infrared Spectrum Principal peaks at wavenumbers 1265, 1293, 1216, 1200, 1066, 1137 cm^{-1} (noradrenaline acid tartrate, KBr disk).

Mass Spectrum Principal ions at *m/z* 44, 45, 58, 76, 60, 43, 42, 46 (no peaks above 90); normetanephrine 153, 93, 30, 65, 152, 154, 125, 110.



Quantification

Plasma **GC** FID. For method, see Lovelady and Foster [1975].

HPLC Electrochemical detection. Limit of detection, 0.1 fmol [Forster, Macdonald 1999]. Electrochemical detection. Limit of detection, <10 pg for noradrenaline, adrenaline, dopamine and 3,4-dihydroxyphenylacetic acid [Wang *et al.* 1999].

RIA Limit of detection, 30 ng/L [Hörtnagl *et al.* 1977].

Serum **GC** See Plasma [Lovelady, Foster 1975].

Urine **GC** See Plasma [Lovelady, Foster 1975].

HPLC Electrochemical detection; comparison with GC–MS. For method for quantification of methodnoradrenaline and adrenaline, see Willemsen *et al.* [2001].

LC-MS Noradrenaline and other catecholamines. Limit of detection, 10 µg/L for noradrenaline [Kushnir *et al.* 2002]. Noradrenaline, adrenaline, dopamine and metanephrines. Limit of detection, 5 µg/L for catecholamines and 2.5 µg/L for metanephrines [Chan, Ho 2000].

Erythrocytes **GC** See Plasma [Lovelady, Foster 1975].

Disposition in the Body Noradrenaline is rapidly metabolised before reaching the systemic circulation and is therefore ineffective after oral administration. It is poorly absorbed after SC injection, and is widely distributed throughout the body. The principal metabolic reaction is *O*-methylation catalysed by catechol-*O*-methyltransferase to form normetanephrine; this is followed by oxidative deamination catalysed by monoamine oxidase, to form 4-hydroxy-3-methoxymandelic aldehyde which is converted to 4-hydroxy-3-methoxymandelic acid (vanillylmandelic acid) and to 4-hydroxy-3-methoxyphenyl glycol; the reaction sequence also occurs in reverse, producing 3,4-dihydroxymandelic acid which is methylated to 4-hydroxy-3-methoxymandelic acid; the metabolites are conjugated with glucuronic acid or sulfate or further metabolised. Up to about 16% of an IV dose is excreted unchanged in the urine together with methylated and deaminated metabolites in free and conjugated forms; negligible amounts of endogenous noradrenaline are excreted in the urine in normal subjects.

Noradrenaline is a metabolite of dopamine and levodopa.

Endogenous plasma concentrations are approximately in the range 0.1–0.3 µg/L.

Dose Initially, the equivalent of 8 to 12 µg/min of noradrenaline by IV infusion.

Chan EC, Ho PC (2000). High-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric method for the analysis of catecholamines and metanephrines in human urine. *Rapid Commun Mass Spectrom* 14: 1959–1964.

Forster CD, Macdonald IA (1999). The assay of the catecholamine content of small volumes of human plasma. *Biomed Chromatogr* 13: 209–215.

Hörtnagl H *et al.* (1977). A sensitive radioenzymatic assay for adrenaline and noradrenaline in plasma. *Br J Clin Pharmacol* 4: 553–558.

Kushnir MM *et al.* (2002). Analysis of catecholamines in urine by positive-ion electrospray tandem mass spectrometry. *Clin Chem* 48: 323–331.

Lovelady HG, Foster LL (1975). Quantitative determination of epinephrine and norepinephrine in the picogram range by flame ionization gas-liquid chromatography. *J Chromatogr* 108: 43 –52.

Wang Y *et al.* (1999). A simple high-performance liquid chromatography assay for simultaneous determination of plasma norepinephrine, epinephrine, dopamine and 3,4-dihydroxyphenyl acetic acid. *J Pharm Biomed Anal* 21: 519–525.

Willemsen JJ *et al.* (2001). Evaluation of specific high-performance liquid-chromatographic determinations of urinary adrenaline and noradrenaline by comparison with isotope dilution mass spectrometry. *Ann Clin Biochem* 38: 356–364.

Norandrostenediol

Anabolic Steroid

Synonym 19-Norandrostenediol

Quantification

Plasma **GC-MS** Column: UP Ultra-1 (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 180° to 230° at at 3°/min to 310° at 40°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 8.9 min. Limit of quantification, 0.9 µg/L [Torrado *et al.* 2008a]. Column: HP-5 cross-linked methyl silicone (17 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 190° at 40°/min to 240° at 5°/min to 320° at 40°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 2.3 µg/L [Schrader *et al.* 2006].

Urine **GC-MS** Column: HP Ultra-1 (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 180° to 230° at 3°/min to 310° at 40°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.13 for the free fraction and 0.23 µg/L for the glucuronide fraction [Torrado *et al.* 2008b]. EI ionisation, SIM acquisition mode. Norandrostenediol metabolites (noranderosterone and noretiocholanolone) [Kintz *et al.* 1999].

Disposition in the Body Norandrostenediol is a hormone precursor and is metabolised in the liver to nortestosterone (nandrolone) after oral administration.

Therapeutic Concentration

Following the administration of 100 mg norandrostenediol as a capsule formulation to 8 healthy subjects, maximum total plasma levels (i.e. conjugated and unconjugated compounds) of norandrostenediol were 1.1 µg/L. Levels of metabolites were 4.0 µg/L for nandrolone, 154.8 µg/L for norandrosterone and 37.7 µg/L for noretiocholanolone. Administration of 25 mg norandrostenediol as sublingual tablets resulted in maximum plasma levels of 3.3 µg/L. Peak levels of nandrolone, norandrosterone and noretiocholanolone were 11.0, 106.3 and 28.5 µg/L, respectively [Schrader *et al.* 2006].

Kintz P *et al.* (1999). [Norandrostenolone and noretiocholanolone: metabolite markers]. *Acta Clin Belg Suppl* 1: 68–73.

Schrader Y *et al.* (2006). Quantitative determination of metabolic products of 19-norandrostenediol in human plasma using gas chromatography/mass spectrometry. *Drug Metab Dispos* 34: 1328–1335.

Torrado S *et al.* (2008a). Gas chromatography–mass spectrometry method for the analysis of 19-nor-4-androstenediol and metabolites in human plasma: application to pharmacokinetic studies after oral administration of a prohormone supplement. *Steroids* 73: 751–759.

Torrado S *et al.* (2008b). Urinary metabolic profile of 19-norsteroids in humans: glucuronide and sulphate conjugates after oral administration of 19-nor-4-androstenediol. *Rapid Commun Mass Spectrom* 22: 3035–3042.

Norandrostenedione

Anabolic Steroid

$C_{18}H_{24}O_2$ = 272.4

CAS—734-32-7

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*)-13-Methyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthrene-3,17-dione

Synonyms 4-Estrene-3,17-dione; estr-4-ene-3,17-dione; 19-norandrostenedione.

Proprietary Names *Androbolic*; *Androdyne*; *Androstat Pro Six*; *CycloROID*; *MHP Nor-Stak*; *Nortesten*.

Chemical Properties An almost white powder with Mp 165° (fine product) or an almost yellow powder with Mp 156° (crude product).

Quantification

Urine **GC-MS** EI ionisation, SIM acquisition mode. Norandrostenedione metabolites (norandrosterone and noretiocholanolone) [Kintz *et al.* 1999].

CE Buffer: 15 mmol/L Britton–Robinson buffer, 50 mmol/L sodium cholate and 0.1% Triton-X100 (pH 9.0). UV detection (λ= 241 nm). Limit of detection, 0.20–0.51 mg/L for norandrostenedione and other synthetic anabolic steroids [Zhang *et al.* 2009].

Disposition in the Body Norandrostenedione is a hormone precursor and after administration and absorption is converted to nortestosterone (nandrolone), especially in the liver. Conversion can also occur in the placenta.

Dose A dose of 100 to 250 mg 1 to 3 times daily is recommended for optimum effect.

Kintz P *et al.* (1999). [Norandrostenolone and noretiocholanolone: metabolite markers]. *Acta Clin Belg Suppl* 1: 68–73.

Zhang L *et al.* (2009). A new mixed micellar electrokinetic chromatography method for analysis of natural and synthetic anabolic steroids. *Talanta* 77: 1002–1008.

Norbolethone

Anabolic Steroid

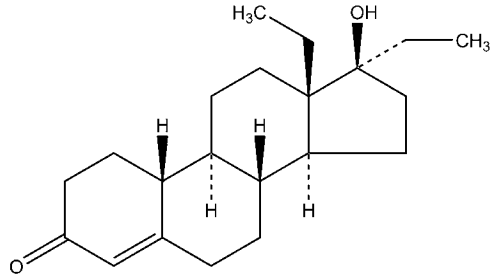
$C_{21}H_{32}O_2$ = 316.5

CAS—1235-15-0

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-13,17-Diethyl-17-hydroxy-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one

Synonyms dl-13β,17α-Diethyl-17β-hydroxygon-4-en-3-one; (17α)-(±)-13-ethyl-17-hydroxy-18,19-dinor-pregn-4-en-3-one; Wy-3475.

Proprietary Name *Genabol*



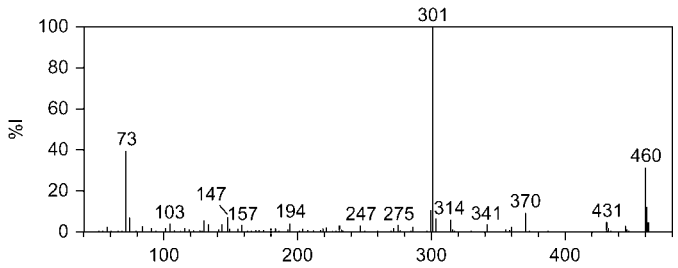
Chemical Properties Crystals. Mp 144° to 145°. Log *P* (octanol/water), 4.6 [Meylan, Howard 1995].

Note Norbolethone is synthesised by the same method used to produce tetrahydrogestrinone, the only difference being that norbolethone needs a longer reaction time to be formed.

***D*-Norbolethone**
Chemical Properties Crystals. Mp 175° to 176°.

***L*-Norbolethone**
Chemical Properties Crystals. Mp 172° to 175.5°.

Ultraviolet Spectrum Peak at 241 nm.
Mass Spectrum Principal ions at *m/z* 301, 73, 460, 302, 461, 300, 370, 147 (di-TMS derivative) [Catlin *et al.* 2002].



Quantification
Urine **GC-MS** Column: Agilent Technologies 6890/5973. Full scan mode. Limit of detection not reported [Catlin *et al.* 2002].

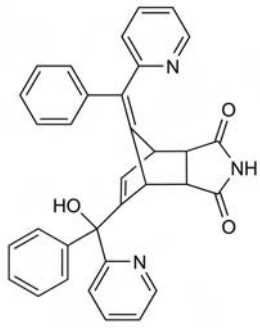
LC-MS Column:Macherey-Nagel Nucleodur C_{18} Pyramid (70 × 40 mm i.d., 5.0 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate containing 0.1% acetic acid (pH 3.5; 10 : 90 to 100 : 0 in 10 min), flow rate 800 µL/min. ESI, positive ion mode. Limit of detection, 50 µg/L [Thevis *et al.* 2005].

Other **LC-MS** Equine Urine. Column: Supelcosil LC-8-DB reversed phase (10 cm × 2.1 mm i.d., 3.0 µm). Mobile phase: 0.1% acetic acid : methanol (60 : 40 to 0 : 100 at 5 min for 5 min), flow rate 0.2 mL/min. API, ESI, positive ion mode, MRM acquisition mode. Limit of detection, 50 µg/L [Yu *et al.* 2005].

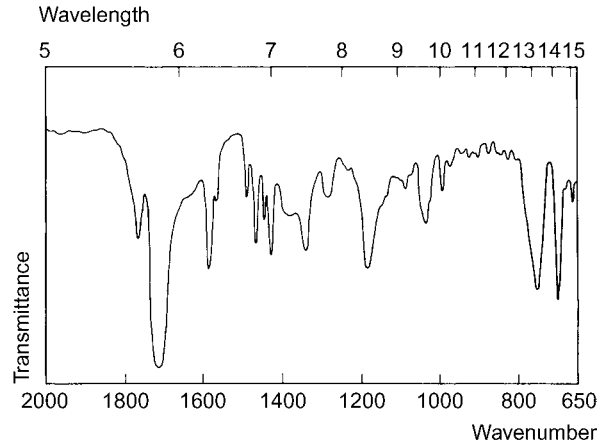
Catlin DH *et al.* (2002). Detection of norbolethone, an anabolic steroid never marketed, in athletes' urine. *Rapid Commun Mass Spectrom* 16: 1273–1275.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Thevis M *et al.* (2005). Screening for unknown synthetic steroids in human urine by liquid chromatography-tandem mass spectrometry. *J Mass Spectrom* 40: 955–962.
Yu NH *et al.* (2005). Screening of anabolic steroids in horse urine by liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 37: 1031–1038.

Norbormide

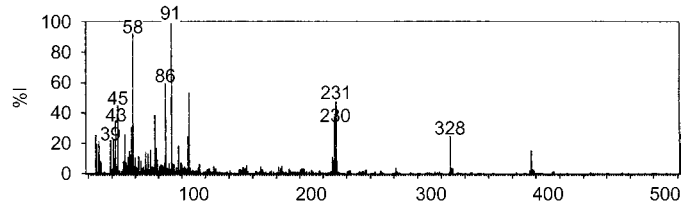
Rodenticide
$C_{33}H_{25}N_3O_3$ = 511.6
CAS—991-42-4
IUPAC Name 3a,4,7,7a-Tetrahydro-5-(hydroxyphenyl-2-pyridinylmethyl)-8-(phenyl-2-pyridinylmethylene)-4,7-methano-1*H*-isoindole-1,3(2*H*)-dione



Chemical Properties A white crystalline powder consisting of a mixture of stereoisomers. Mp 190° to 198°. Practically insoluble in water, ethanol and ether; very slightly soluble in chloroform. Log *P* (octanol/water), 3.2.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—green-brown.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.00; system TC—R_f 0.62; system TL—R_f 0.64 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2050.
Ultraviolet Spectrum Methanolic acid—238, 300 nm (A^{1}_{1}=136b).
Infrared Spectrum Principal peaks at wavenumbers 1708, 701, 751, 1590, 1190, 1754 cm^{-1} (KBr disk).

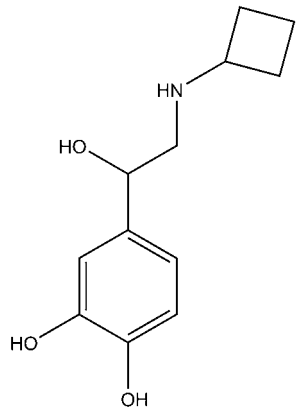


Mass Spectrum Principal ions at *m/z* 91, 58, 86, 106, 231, 45, 77, 230.



Norbudrine

Bronchodilator
$C_{12}H_{17}NO_3$ = 223.3
IUPAC Name 4-[2-(Cyclobutylamino)-1-hydroxyethyl]benzene-1,2-diol
Synonyms 2-Cyclobutylamino-1-(3,4-dihydoxyphenyl)-ethanol; *N*-cyclobutylnoradrenaline; norbudrina; norbutrine.



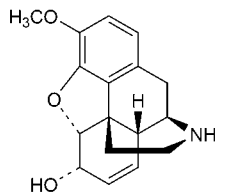
Norbudrine Hydrochloride
$C_{12}H_{17}NO_3$, HCl = 259.8
Synonym RD 9338
Chemical Properties Mp 170°, with decomposition. Very soluble in water; soluble 1 in 10 of ethanol.

Colour Tests Ammonium molybdate test—(yellow) grey-blue (limit of detection, 0.1 µg); ammonium vanadate test—(blue-green) light brown (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—orange (limit of detection, 0.5 µg); Vitali's test—orange/orange/brown (limit of detection, 0.5 µg).
Thin-layer Chromatography System T1—R_f 0.57 (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid—254 (E1%, 1 cm 128) and 285 nm (E1%, 1 cm 6).
Infrared Spectrum Principal peaks at wavenumbers 1247 or 1437, 1614, 1192 cm^{-1} (KBr disk) (Norbutrine base).

Norcodeine

Narcotic Analgesic
$C_{17}H_{19}NO_3$=85.3
CAS—467-15-2

IUPAC Name 3-Methoxy-6α-hydroxy-4,5α-epoxy-7,8-didehydromorphinan
Synonyms *N*-Demethylcodeine; (5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxymorphinan-6-ol; normorphine 3-methyl ether.

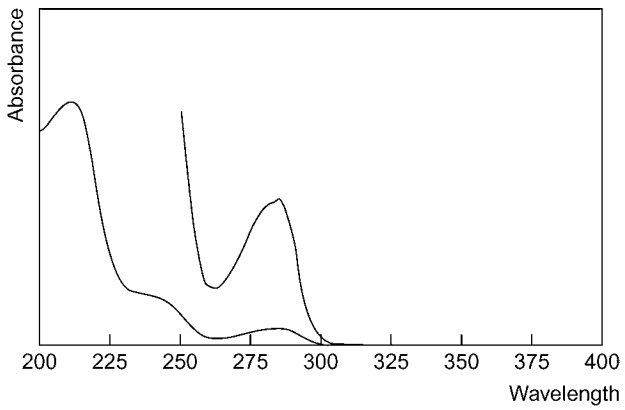


Chemical Properties Crystals. Mp 185°. Sparingly soluble in water and ether; freely soluble in ethanol; moderately soluble in acetone. pK_a 9.23 (25°). Log *P* (octanol/water), 0.69 [Avdeef *et al.* 1996].

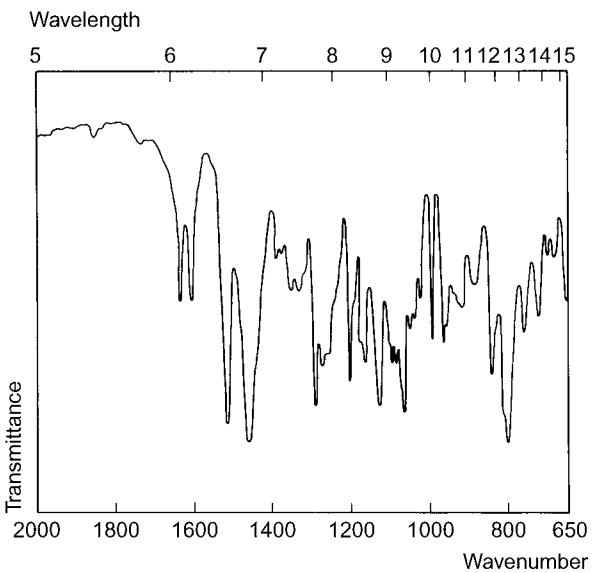
Norcodeine Hydrochloride

$C_{17}H_{19}NO_3$, HCl_3, H_2O = 375.8
CAS—14648-14-7 (anhydrous)
Chemical Properties Crystals. The anhydrous form decomposes at 309°. Sparingly soluble in cold water, more soluble in hot water; freely soluble in ethanol; almost insoluble in acetone.

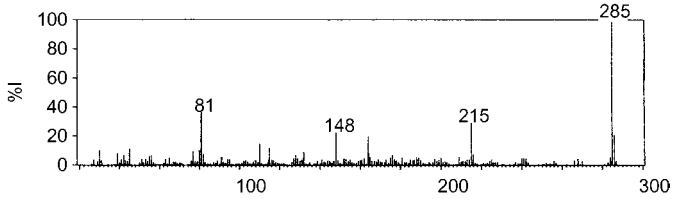
Colour Test Marquis test—yellow→violet.
Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 00; system TC—R_f 0.05 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—blue-violet; ninhydrin spray—positive).
Gas Chromatography System GA—RI 2388; system GB—RI 2535.
High Performance Liquid Chromatography System HA—*k* 3.1 (tailing peak); system HC—*k* 3.51; system HY—RI 235.
Ultraviolet Spectrum Aqueous acid—284 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 800, 1515, 1065, 1130, 1290, 1205 cm^{-1} (KBr disk). Polymorphism may occur.



Mass Spectrum Principal ions at *m/z* 285, 81, 215, 148, 286, 164, 110, 115.



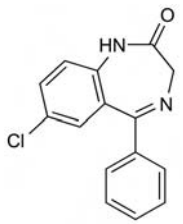
Quantification See under Codeine.
Disposition in the Body Norcodeine is a metabolite of codeine.

Avdeef A *et al.* (1996). Octanol-, chloroform-, and propylene glycol dipelargonat–water partitioning of morphine-6-glucuronide and other related opiates. *J Med Chem* 39: 4377–4381.

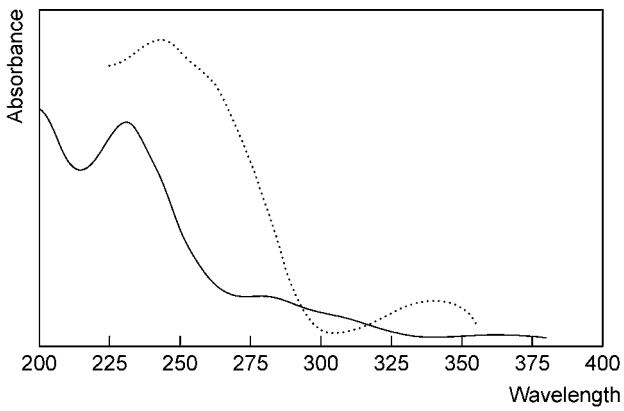
Nordazepam

Benzodiazepine, Tranquilliser
$C_{15}H_{11}ClN_2O$ = 270.7
CAS—1088-11-5

IUPAC Name 7-Chloro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one
Synonyms A-101; 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one; demethyldiazepam; desmethyldiazepam; *N*-desmethyldiazepam; DMDZ; nordiazepam; Ro-5-2180.
Proprietary Names *Calmday*; *Lomax*; *Madar*; *Nordaz*; *Praxadium*; *Sopax*; *Stilny*; *Tranxilium N*; *Vegesan*.

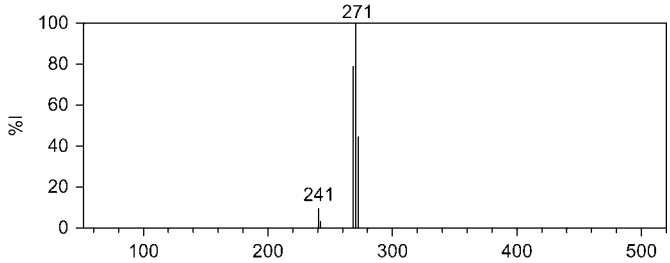


Chemical Properties A white or pale yellow crystalline powder. Mp 216° to 217° (crystals from acetone). Practically insoluble in water; slightly soluble in ethanol and chloroform. pK_{a1} 3.5, pK_{a2} 12.0. Log *P* (octanol/water), 2.93 [Mullett, Pawliszyn 2001]. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].
Colour Test Formaldehyde-sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.03; system TC—R_f 0.55; system TD—R_f 0.34; system TE—R_f 0.67; system TF—R_f 0.45; system TL—R_f 0.60; system TAD—R_f 0.57; system TAE—R_f 0.82; system TAF—R_f 0.83; system TAJ—R_f 0.53; system TAK—R_f 0.60; system TAL—R_f 0.92 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).
Gas Chromatography System GA—nordazepam RI 2490, M (oxazepam) RI 2325; system GB—nordazepam RI 2625, M (oxazepam) RI 2438; system GF—nordazepam RI 3041, M (oxazepam) RI 2803.
High Performance Liquid Chromatography System HA—*k* 0.2; system HI—nordazepam *k* 8.00, M (oxazepam) *k* 4.62; system HK—nordazepam *k* 1.99, M (oxazepam) *k* 0.73; system HX—RI 470; system HY—RI 372; system HAK—RT 7.6 min; system HAL—nordazepam RT 7.9 min, M (oxazepam) RT 4.4 min; system HAX—nordazepam RT 6.7 min, M (oxazepam) RT 6.0 min; system HAY—nordazepam RT 6.8 min, M (oxazepam) RT 4.5 min; system HAZ—nordazepam *k* 1.88, M (oxazepam) *k* 1.23; system HBH—nordazepam *k* 8.97, M (oxazepam) *k* 5.42; system HBI—nordazepam *k* 1.89, M (oxazepam) *k* 1.25.
Ultraviolet Spectrum Aqueous acid—238 (A^{1}_{1} = 1140b), 283, 361 nm; aqueous alkali—240, 340 nm.



Infrared Spectrum Principal peaks at wavenumbers 1680, 700, 1602, 820, 738, 790 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 242, 269, 270, 241, 243, 271, 244, 272 (nordazepam); 257, 77, 268, 239, 205, 267, 233, 259 (oxazepam) .



Quantification See also under Diazepam.

Blood **GC-MS** Column: 100% methylsiloxane or 5% phenylsiloxane, 95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 25°/min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 25 µg/L [Tiscione *et al.* 2008]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 10 µg/L [Pirnay *et al.* 2002].

HPLC Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: 35 mmol/L phosphate buffer (pH 2.1) : acetonitrile (70 : 30), flow rate 2 mL/min. DAD (λ = 220 nm). Limit of quantification, 30 µg/L [Bugey, Staub 2004].

LC-MS Column: Acquity BEH Phenyl (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (80 : 20 for 0.25 min to 65 : 35 over 2.25 min for 2.5 min to 20 : 80 over 1 min to 80 : 20 over 0.01 min for 1.4 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of detection not reported [Gunn *et al.* 2010]. Column: Retek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5), flow rate 0.45 mL/min. DAD. Limit of quantification, 2 µg/L [Dussy *et al.* 2006].

Plasma **GC** Column: CPSIL 8 CB (25 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 150° to 230° at 40°/min for 2 min to 250° at 5°/min for 1 min to 300° at 15°/min for 3 min. NPD. Limit of detection, 0.02 µmol/L [Reubsaet *et al.* 1998]. ECD. Limit of detection, 1 µg/L [Viala *et al.* 1978].

GC-MS Column: HP-1 capillary (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° for 2 min to 310° at 40°/min for 2.5 min. EI ionisation, SIM acquisition mode. Retention time: 6.80 min. Limit of quantification, 0.05 mg/L [Peters *et al.* 2005].

HPLC Column: C_{18}. UV detection. Limit of detection, 31.3 µg/L for nordazepam and 45.0 µg/L for diazepam [Mullett, Pawliszyn 2001]. Column: LiChrospher RP-Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer (pH 2.1, 30 : 70 for 5 min to 35 : 65 at 0.3 mL/min), flow rate 0.5 mL/min for 5 min to 0.3 mL/min at 30 min. DAD (λ = 254 nm). Retention time: 12.4 min. Limit of detection not reported [El Mahjoub, Staub 2000]. Nordazepam and diazepam [Atta-Politou *et al.* 1999].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Retention time: 6.02 min. Limit of quantification, 20 µg/L, limit of detection, 10 µg/L [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.025 mg/L (SIM mode), limit of detection, <0.01 mg/L (scan mode)[Kratzsch *et al.* 2004].

Serum **GC-MS** SIM acquisition mode. Nordazepam, diazepam and oxazepam [Duthel *et al.* 1992].

HPLC Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (52 : 48), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 100 µg/L [Mullett, Pawliszyn 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (54 : 46), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 104.3 µg/L, limit of detection, 31.3 µg/L [Mullett, Pawliszyn 2001]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: flow rate 1.5 mL/min. DAD (λ = 254 nm). Limit of quantification, 107.5 µg/L, limit of detection, 29.8 µg/L [Ahrens *et al.* 2000].

LC-MS See Blood [Dussy *et al.* 2006; Gunn *et al.* 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Note For a radioimmunoassay for the measurement of nordazepam, see Dixon *et al.* [1979].

Urine **GC-MS** See Blood [Goldberger *et al.* 2010]. Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 160° for 1 min to 280° at 20°/min for 3 min. NCI. Limit of detection not reported [Fitzgerald *et al.* 1993]. Column; methyl silicone (12.5 m). Temperature programme; 120° to 310°. Limit of quantification, 5 µg/L, limit of detection, 1 µg/L [West, Ritz 1993].

HPLC UV detection. Limit of detection, 100 µg/L [Mullett, Pawliszyn 2002]. Column: C_{18}. UV detection. Limits of detection, 33.1 µg/L for nordazepam and 52.9 µg/L for diazepam [Mullett, Pawliszyn 2001]. Column: Hypersil C18 (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.04 mol/L phosphate (pH 4)–0.4% octylamine : acetonitrile (73 : 27, 70 : 30, 65; 35 or 60 : 40), flow rate 1 mL/min. DAD (240 nm). Retention time: 14.3 min. Limit of detection, not reported [Segura *et al.* 2001]. Column: 60 RP select B (250 × 4 mm i.d., 5 µm) or LiChrospher 100 RP 8 (200 × 4 mm i.d., 8.5 µm). Mobile phase: 0.01 mol/L sodium phosphate buffer (pH 7.0) : acetonitrile (87.5 : 12.5 to 83.5 : 16.5 at 15 min to 80 : 20 over 8.5 min for 3 min to 75 : 25 in 1 min for 15 min), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 10 µg/L [Franzelius, Besserer 1993].

LC-MS Column: Hypurity C_8 (150 × 3 mm i.d.). Mobile phase: 4 mmol/L ammonium acetate (pH 6.8) in methanol-water (5 : 95) : 1% propan-2-ol, 0.05% formic acid in methanol (100 : 0 for 1 min to 0 : 100 at 3 min for 1.5 min to 100 : 0 over 0.1 min for 1.4 min). TIS, MRM acquisition mode. Limit of quantification, 2.5 µg/L, limit of detection, 1.25 µg/L [Glover, Allen 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 from 3.01 to 6 min to 100 : 0 from 6.01 to 10 min for 5 min), flow rate 0.9 mL/min for 3 min to 0.3 mL/min until 10 min to 0.9 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.1 µg/L [Umezawa *et al.* 2008].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Hair **GC-MS** Column: HP-Ultra 2 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 70° for 2 min to 220° at 25°/min to 255° at 5°/min to 300° for 7 min. EI ionisation at 70 eV. Retention time: 14.2 min. Limit of detection, 0.1 ng/mg [Yegles *et al.* 1997]. Column: HP-5 MS 5% phenylsiloxane, 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 290° at 30°/min for 2 min. NICI. Retention time: 9.04 min. Limit of detection, 0.001 ng/mg [Kintz *et al.* 1996].

LC-MS Mobile phase: 3 mmol/L ammonium formate and 0.001% formic acid in water : acetonitrile (65 : 35 to 20 : 80 after 13 min to 10 : 90 at 13.5 min until 16.5 min to 65 : 35 until 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.41 ng/30 mg, limit of detection, 0.24 ng/30 mg [Miller *et al.* 2006]. Column: Uptisphere ODB C_{18}. Mobile phase: 2 mmol/L formate buffer : acetonitrile. ESI. Limit of detection, <2 ng/g for nordazepam and other benzodiazepines [Chèze *et al.* 2005]. NICI. Nordazepam and oxazepam [Kintz *et al.* 1996; Vayssette *et al.* 1996].

Disposition in the Body Nordazepam is absorbed after oral administration, maximum blood concentrations being attained in 2–4 h; bioavailability is ~50%. It is metabolised to oxazepam and then to oxazepam glucuronide. Nordazepam is a metabolite of several benzodiazepines including chlordiazepoxide, clorazepate, diazepam, medazepam, and prazepam.

Therapeutic Concentration

Following daily oral doses of 20–30 mg to nine subjects for 10 days, plasma concentrations of 0.63–1.84 mg/L (mean, 1.1) were reported 10 h after a dose [Tognoni *et al.* 1975].

Following a single oral dose of 10 mg to 2 subjects, a mean peak plasma concentration of 0.17 mg/L was reported [Pacifici *et al.* 1982].

Toxicity

High concentrations of diazepam and nordazepam were found in 94 cases of driving under the influence of drugs. Mean, median and maximum levels of nordazepam were 1.5, 1.0 and 7.6 µg/g, respectively; corresponding diazepam levels were 2.0, 1.7 and 7.8 µg/g, respectively. In 86 cases, other drugs were also found [Jones *et al.* 2004].

In 20 deaths involving nordazepam, postmortem peripheral and heart blood levels were ~587 µg/L [Pos Pok *et al.* 2008].

Half-life The plasma half-life is very variable and values ranging from 25 to over 200 h have been reported. Mean values reported are usually in the range 40–100 h in normal subjects. The plasma half-life is prolonged in elderly subjects and in subjects with liver disease.

Volume of Distribution 0.5–2.5 L/kg, increased in elderly subjects.

Clearance Plasma clearance, ~0.1–0.3 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.7.

Protein Binding ~97%.

Dose Nordazepam has been given in doses of up to 15 mg daily.

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Kintz P *et al.* (1996). Hair analysis for nordiazepam and oxazepam by gas chromatography–-negative-ion chemical ionization mass spectrometry. *J Chromatogr B Biomed Appl* 677: 241–244.
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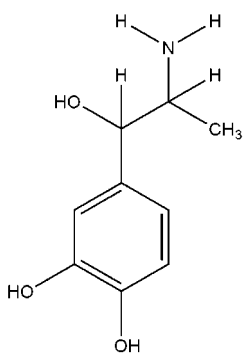
Nordefrin

Vasoconstrictor

$C_9H_{13}NO_3$ = 183.2

IUPAC Name 4-(2-Amino-1-hydroxypropyl)benzene-1,2-diol

Synonyms (±)-2-Amino-1-(3,4-dihydroxyphenyl)propan-1-ol; cordadride; *dl*-3,4-dihydroxynorephedrine; dihydroxyphenylaminopropanol; α-methylnoradrenaline; norhomoepinephrine.



Chemical Properties Mp 212° to 215° with decomposition.

Nordefrin Hydrochloride

$C_9H_{13}NO_3$, HCl = 219.7

Proprietary Names *Corbefrin*; *Corbasil*; *Lirotil*.

Chemical Properties A white crystalline powder which slowly darkens on exposure to air and light. Mp 175° to 180°. Soluble 1 in 1.5 of water and 1 in 12 of ethanol; almost insoluble in chloroform and ether.

Levonordefrin ((−)-isomer)

$C_9H_{13}NO_3$ = 183.2

Proprietary Name *Neo-Cobefrin*

Chemical Properties A white crystalline powder. Mp ~210°. Almost insoluble in water; slightly soluble in ethanol, ether, chloroform and acetone; soluble in aqueous solutions of mineral acids.

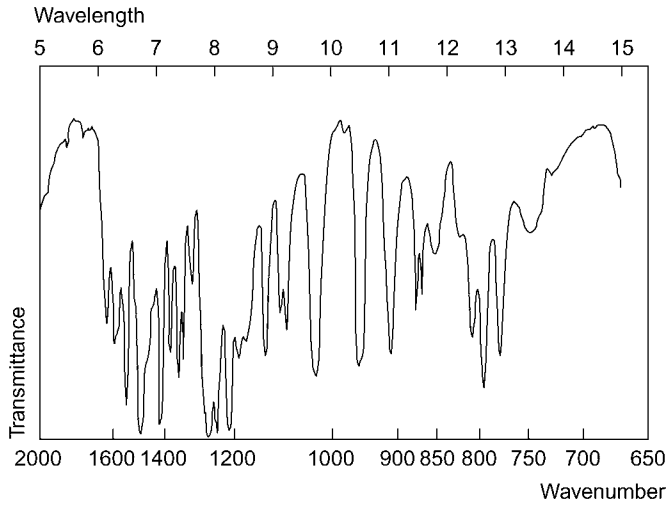
Note Nearly all the vasoconstrictor activity of nordefrin is possessed by levonordefrin.

Colour Test Ammonium molybdate—(brown) grey→green (limit of detection, 1.0 μg); ammonium vandate—(grey) orange (limit of detection, 1.0 μg); formaldehyde–sulfuric acid—faint orange (limit of detection, 1.0 μg); sulfuric acid—yellow (limit of detection, 1.0 μg); Vitali's test—yellow/orange/brown (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.33, streaking (potassium permanganate—positive).

Ultraviolet Spectrum Aqueous acid (0.05 mol/L sulfuric acid)—220 nm (A^1_1 = 330) and 280 (A^1_1 = 156) (maximum), 249 nm (minimum).

Infrared Spectrum Principal peaks at 1258, 1497, 1527, 775 or 1053 cm^{-1} (KBr disk).



Dose In a concentration of 1 in 10 000 in solutions of local anaesthetics.

Norelgestromin

Contraceptive, Progestational Steroid, Progestogen

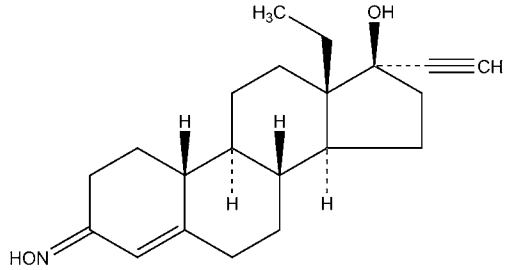
$C_{21}H_{29}NO_2$ = 327.5

CAS—53016-31-2

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-13-Ethyl-17-ethynyl-3-hydroxyimino-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-17-ol

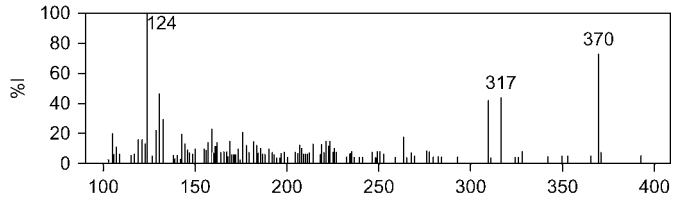
Synonyms 17-Deacetylnorgestimate; (17α)-13-ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one oxime; levonorgestrel 3-oxime; 18-methylnorethindrone oxime; RWJ-10553.

Proprietary Names *Evra*; *Ortho-Evra* (combination drug).



Chemical Properties Crystals. Mp 112°.

Mass Spectrum



Quantification

Serum **LC-MS** Column: Inertsil phenyl (150 × 4.0 mm i.d., 5 µm). Mobile phase: water : acetonitrile (70 : 30 to 30 : 70 over 4 min), flow rate 1.0 mL/min. APCI, positive ion mode, SRM acquisition mode. Retention time: 8.5 min. Limit of quantification, 0.1 µg/L [Wong *et al.* 1999].

Disposition in the Body Norelgestromin is the primary active metabolite of norgestimate and is generally administered as a patch. Hepatic metabolism of norelgestromin occurs and metabolites include norgestrel, which is highly bound to sex-hormone-binding globulin (SHBG), as well as various hydroxylated and conjugated metabolites. Norelgestromin itself is not bound to SHBG. Elimination occurs via the renal and faecal pathways.

Therapeutic Concentration In the range 0.6 to 1.2 µg/L.

A total of 31 women were randomised to 1 of 4 treatment sequences where a combination patch of norelgestromin/ethinylestradiol was applied to different parts of the body for 7 days. The patch delivered 150 µg norelgestromin and 20 µg ethinylestradiol daily to the systemic circulation. Mean peak serum concentrations for norelgestromin were as follows:

Parameter	*Abdomen*	*Arm*	*Buttock*	*Torso*
C_{max} (µg/L)	0.88	1.18	1.17	1.07
Time (h)	72	72	72	72

The half-life was in the range 26 to 30 h [Abrams *et al.* 2002a].

A total of 31 women were administered a daily oral tablet containing 250 µg norgestimate and 35 µg ethinyl estradiol for 7 days. Mean peak serum concentration of norelgestromin on day 1 was 1.52 µg/L after 1.5 h and on day 7 was 2.00 µg/L after 1.2 h [Devineni *et al.* 2007].

The consecutive wearing of 2 patches did not affect serum concentrations of norelgestromin, which remained within the reference ranges of 0.6 to 1.2 µg/L [Abrams *et al.* 2001].

Half-life Elimination, 28 h.

Protein Binding More than 97%, to albumin.

Note For a protein-binding study of norelgestromin, see Hammond *et al.* [2003]; for a pharmacokinetic overview of norelgestromin, see Abrams *et al.* [2002b].

Dose A weekly patch containing 6 mg norelgestromin and 0.75 mg ethinyl estradiol is used for 3 weeks followed by a wash-out period of 1 week before starting a new cycle.

Abrams LS *et al.* (2001). Pharmacokinetics of norelgestromin and ethinyl estradiol delivered by a contraceptive patch (Ortho Evra/Evra) under conditions of heat, humidity, and exercise. *J Clin Pharmacol* 41: 1301–1309.

Abrams LS *et al.* (2002a). Pharmacokinetics of a contraceptive patch (Evra/Ortho Evra) containing norelgestromin and ethinyloestradiol at four application sites. *Br J Clin Pharmacol* 53: 141–146.

Abrams LS *et al.* (2002b). Pharmacokinetic overview of OrthoEvra/Evra. *Fertil Steril* 77: S3–12.

Devineni D *et al.* (2007). Pharmacokinetics and pharmacodynamics of a transdermal contraceptive patch and an oral contraceptive. *J Clin Pharmacol* 47: 497–509.

Hammond GL *et al.* (2003). Serum distribution of the major metabolites of norgestimate in relation to its pharmacological properties. *Contraception* 67: 93–99.

Wong FA *et al.* (1999). Determination of norgestimate and its metabolites in human serum using high-performance liquid chromatography with tandem mass spectrometric detection. *J Chromatogr B Biomed Sci Appl* 734: 247–255.

Norethandrolone

Anabolic Steroid

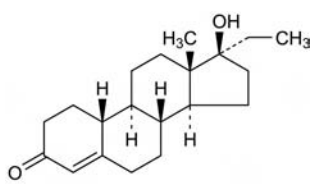
$C_{20}H_{30}O_2$ = 302.5

CAS—52-78-8

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Ethyl-17-hydroxy-13-methyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthren-3-one

Synonyms 17α-Ethyl-19-nortestosterone; (17α)-17-hydroxy-19-norpregn-4-en-3-one.

Proprietary Name *Nilevar*



Chemical Properties A white crystalline powder. Mp about 135°. Insoluble in water; soluble 1 in 8 of ethanol, 1 in 5 of chloroform, and 1 in 3 of methanol; very soluble in acetone; soluble in benzene, ether and ethyl acetate. Log *P* (octanol/water), 3.8.

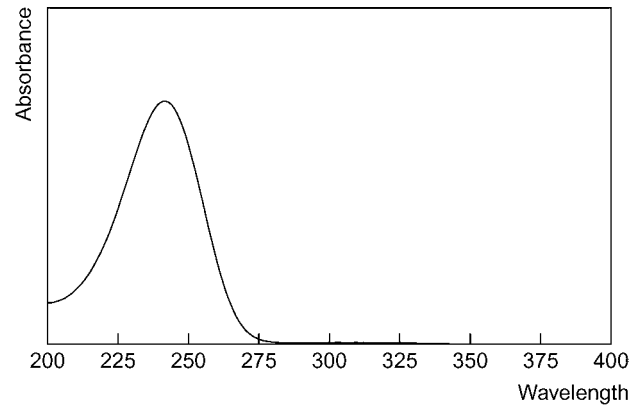
Colour Tests Antimony pentachloride—brown; naphthol–sulfuric acid—orange, green dichroism/red-orange; sulfuric acid—orange (green-yellow fluorescence under UV light).

Thin-layer Chromatography System TP—R_f 0.71; system TQ—R_f 0.20; system TR—R_f 0.95; system TS—R_f 0.78 (*p*-toluenesulfonic acid solution, positive).

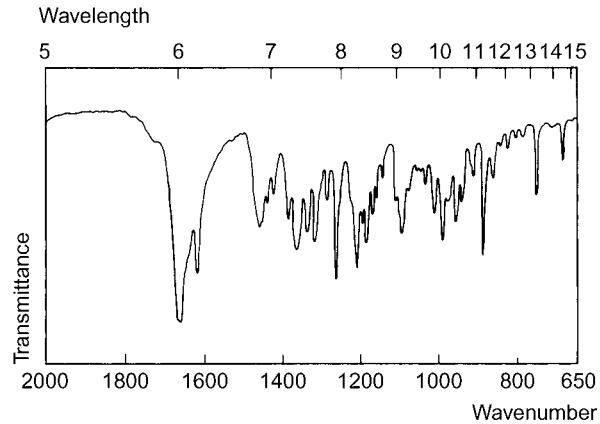
Gas Chromatography System GAI—Urinary metabolite: 17α-(2-hydroxyethyl)-5α-estran-3α,17β-diol RRT 1.078 (relative to 17α-methyl-5α-androstan-3β,17β-diol).

High Performance Liquid Chromatography System HATb—RRT 1.55 (relative to testosterone).

Ultraviolet Spectrum Methanol—240 nm (A_1^1=565a).



Infrared Spectrum Principal peaks at wavenumbers 1650, 1263, 1612, 1210, 894, 1185 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 302, 57, 85, 231, 91, 110, 79, 215.

Dose 10 to 30 mg daily.

Norethisterone

Progestational Steroid

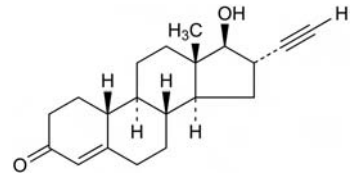
$C_{20}H_{26}O_2$ = 298.4

CAS—68-22-4

IUPAC Name (17α)-17-Hydroxy-19-norpregn-4-en-20-yn-3-one

Synonyms Ethinylnortestosterone; norethindrone; norpregneninolone.

Proprietary Names *Micronor*; *Micronovum*; *Norfor*; *Noriday*; *Norlutin*; *Nor-QD*; *Primolut N*; *Utovlan*.



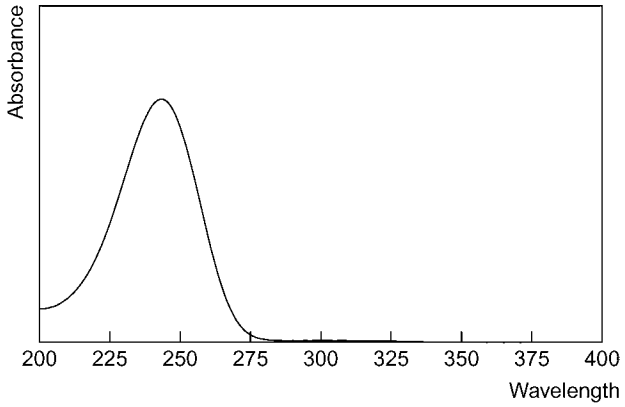
Chemical Properties Norethisterone is an ingredient of many proprietary preparations—see Sweetman [2009]. A white or creamy-white crystalline powder. Mp 201° to 208°. Practically insoluble in water; soluble 1 in 150 of ethanol, 1 in 30 of chloroform and 1 in 5 of pyridine; slightly soluble in ether. Log *P* (octanol/water), 3.0.

Norethisterone Acetate

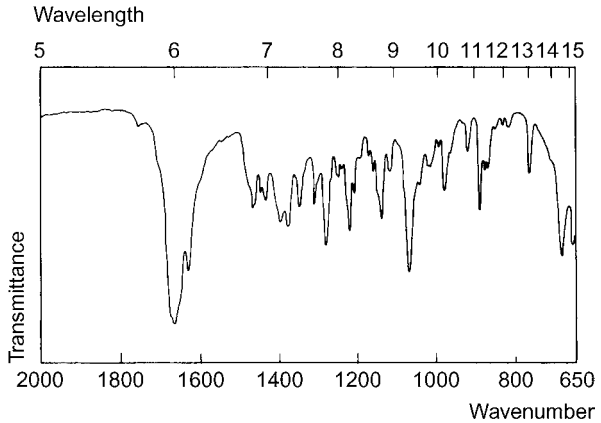
$C_{22}H_{28}O_3$ = 340.5
CAS—51-98-9

Synonym Norethindrone acetate
Proprietary Names *Milligynon*; *Norlutate*; *Primolut Nor*; *SH 420*.
Chemical Properties Norethisterone acetate is an ingredient of many proprietary preparations—see Sweetman [2009]. A white or creamy-white crystalline powder. Mp about 163°. Practically insoluble in water; soluble 1 in about 12 of ethanol, 1 in 4 of acetone, 1 in <1 of chloroform, 1 in 2 of dioxan and 1 in 18 of ether.

Colour Tests Antimony pentachloride—brown; naphthol–sulfuric acid—orange/orange-brown; sulfuric acid—orange (orange fluorescence under ultraviolet light).
Thin-layer Chromatography Norethisterone: System TB—R_f 0.20; system TE—R_f 0.76; system TF—R_f 0.57; system TP—R_f 0.71; system TQ—R_f 0.22; system TR—R_f 0.87; system TS—R_f 0.63, streaking may occur; system TAE—R_f 0.86. Norethisterone acetate: System TP—R_f 0.87; system TQ—R_f 0.39; system TR—R_f 0.98; system TS—R_f 0.90.
Gas Chromatography System GA—RI 2625.
High Performance Liquid Chromatography System HX—RI 536; system HY—RI 676; system HAA—retention time 24.0 min.
Ultraviolet Spectrum Ethanol—240 nm (A^1_1=570a).



Infrared Spectrum Principal peaks at wavenumbers 1649, 1068, 1617, 689, 1272, 660 cm^{-1} (KBr disk).



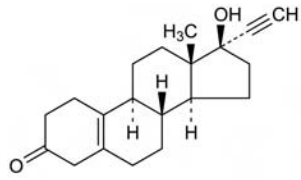
Mass Spectrum Principal ions at *m/z* 43, 340, 298, 325, 91, 41, 231, 280 (norethisterone acetate).
Quantification
Plasma **GC–MS** Limit of detection, <0.1 µg/L [Pommier *et al.* 1996]. Limit of detection, <0.5 to 8 µg/L for norethisterone and its metabolites [Pommier *et al.* 1995]. For method of quantification for norethisterone metabolites, see Rizk *et al.* [1991].
Dose 5 to 25 mg of norethisterone or 2.5 to 15 mg of norethisterone acetate daily; doses of up to 60 mg daily have been given.

Pommier F *et al.* (1995). Simultaneous determination of norethisterone and six metabolites in human plasma by capillary gas chromatography with mass-selective detection. *J Chromatogr B Biomed Appl* 674: 155–165.
Pommier F *et al.* (1996). Quantitative determination of norethisterone acetate in human plasma by capillary gas chromatography with mass-selective detection. *J Chromatogr A* 750: 75–81.
Rizk MS *et al.* (1991). Gas chromatography and mass spectrometry of dimethylethylsilyl ether derivatives of norethisterone metabolites in plasma. *Acta Pharm Nord* 3: 205–210.
Sweetman SC (2009). *Martindale, The Complete Drug Reference*, 36 edn. London: Pharmaceutical Press.

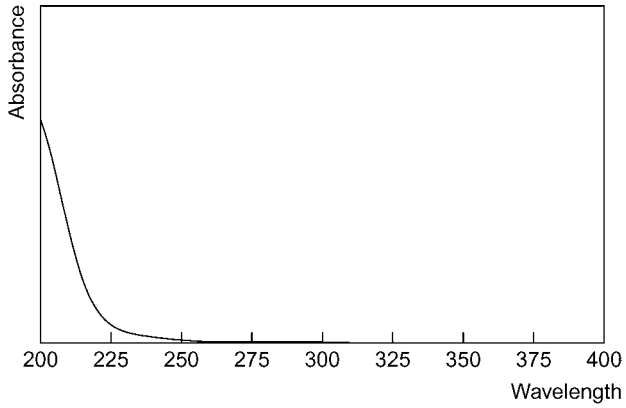
Noretynodrel

Progestational Steroid
$C_{20}H_{26}O_2$ = 298.4
CAS—68-23-5

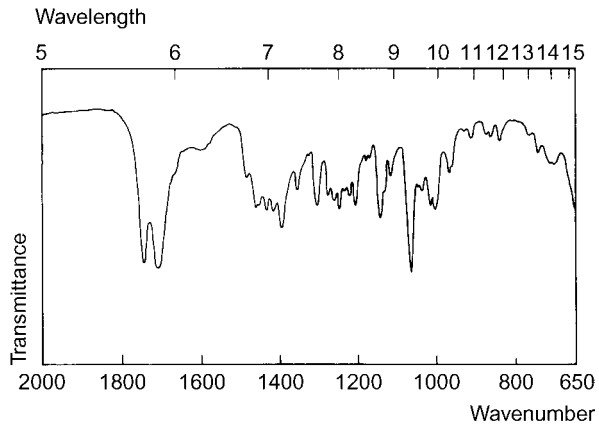
IUPAC Name (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-17-hydroxy-13-methyl-1,2,4,6,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonym (17α)-17-Hydroxy-19-norpregn-5(10)-en-20-yn-3-one



Chemical Properties A white crystalline powder. Mp about 174° to 184°. Practically insoluble in water; soluble 1 in 30 of ethanol, 1 in 7 of chloroform and 1 in 60 of ether; soluble in acetone. Log *P* (octanol/water), 3.5.
Colour Tests Antimony pentachloride—green-brown; naphthol–sulfuric acid—orange-red/brown-red; sulfuric acid—orange (orange fluorescence under ultraviolet light); Dissolve 2 mg in 0.3 mL of a 0.5% solution of dinitrobenzene in ethanol and add 2 drops of benzalkonium chloride solution. Mix, and add 2 mL of dilute ammonia solution—intense violet immediately, changing to red-brown in about 5 min.
Thin-layer Chromatography System TP—R_f 0.79; system TQ—R_f 0.32; system TR—R_f 0.91; system TS—R_f 0.71.
Gas Chromatography System GA—RI 2551.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1066, 1690, 1729, 1140, 1008, 1242 cm^{-1} (KBr disk).

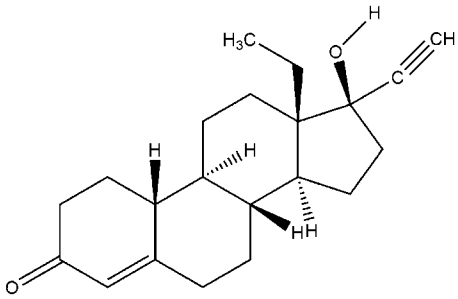


Mass Spectrum Principal ions at *m/z* 91, 215, 79, 105, 77, 55, 41, 298.
Dose 5 to 40 mg daily.

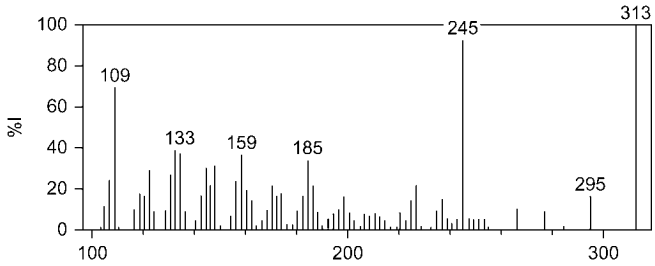
Norgestrel

Progestational Steroid
$C_{21}H_{28}O_2$ = 312.5

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-13-Ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonyms 13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one; levonorgestrel; Wy 3707.
Proprietary Names It is an ingredient of *Anfertil*, *Duoluton*, *Follinyl*, *Gentrol*, *Microlut*, *Nordiol-21*, *Ovral*, *Ovran*, *Stediril*, *Uugynon*.



Chemical Properties A white crystalline powder. Mp 206° to 208.5°. Insoluble in water; slightly soluble in ethanol, chloroform, acetone and dioxin.
Thin-layer Chromatography System T26a—R_f 0.68; system 26b—R_f 0.24; system T26c—R_f 1.00 (*p*-toluenesulfonic acid—positive).
Ultraviolet Spectrum Methanol—maximum at 241 nm (A^1_1= 549).
Infrared Spectrum Principal peaks at 1070, 1620, and 1650 (KBr disk).
Mass Spectrum



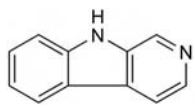
Quantification

Plasma **LC-MS** Column: Hypersil BDS-C_{18} (50 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : water (69 : 31) containing 0.1% formic acid, flow rate 200 µL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.25 µg/L [Zhao *et al.* 2008]. Column: Acquity UPLC BEH C_{18} (50 × 2 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid in acetonitrile : acetonitrile-water (50 : 50, 0 : 100 for 0.65 min to 60 : 40 at 1.85 min to 90 : 10 at 2.3 min to 0 : 100 at 2.35 min until 2.7 min), flow rate 0.75 mL/min to 1.0 mL/min at 2.3 min to 0.75 mL/min at 2.7 min. TIS, positive ion mode. Limit of detection, 0.1 µg/L [Licea-Perez *et al.* 2007]. Column: Phenomenex Luna phenyl-hexyl (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.1% formic acid (45 : 35 : 20), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Retention time: 2.38 min. Limit of quantification, 0.265 µg/L [Theron *et al.* 2004].
Serum **LC-MS** Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (80 : 20) with 0.5% formic acid, flow rate 1.0 mL/min. APCI. Limit of quantification, 0.2 µg/L [Wu *et al.* 2000].
Note For a gel filtration or radioimmunoassay for the measurement of D-norgestrel in human milk, see Thomas *et al.* [1977].
Disposition in the Body Orally administred norgestrel is absorbed and slowly excreted; 21% of a dose is excreted in the urine in 24 h and a further 21% in the following 5 days
Toxicity Doses greater than 5 g/kg did not produce toxic symptoms in mice and rats.

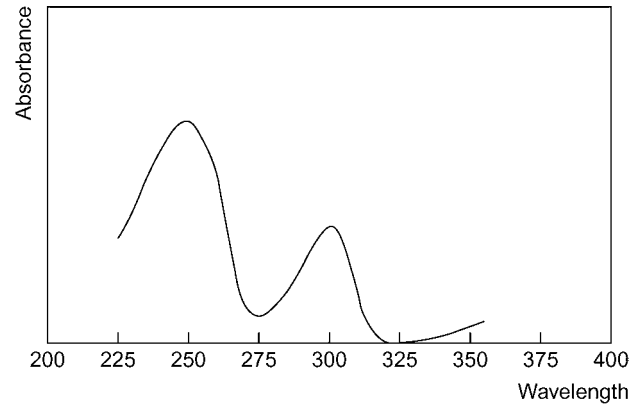
Licea-Perez H *et al.* (2007). A semi-automated 96-well plate method for the simultaneous determination of oral contraceptives concentrations in human plasma using ultra performance liquid chromatography coupled with tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 69–76.
Theron HB *et al.* (2004). Selective and sensitive liquid chromatography–tandem mass spectrometry method for the determination of levonorgestrel in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 813: 331–336.
Thomas MJ *et al.* (1977). The detection and measurement of D-norgestrel in human milk using Sephadex LH 20 chromatography and radioimmunoassay. *Steroids* 30: 349–361.
Wu Z *et al.* (2000). Simultaneous quantitative determination of norgestrel and progesterone in human serum by high-performance liquid chromatography–tandem mass spectrometry with atmospheric pressure chemical ionization. *Analyst* 125: 2201–2205.
Zhao LZ *et al.* (2008). Determination of levonorgestrel in human plasma by liquid chromatography–tandem mass spectrometry method: application to a bioequivalence study of two formulations in healthy volunteers. *Biomed Chromatogr* 22: 519–526.

Norharman

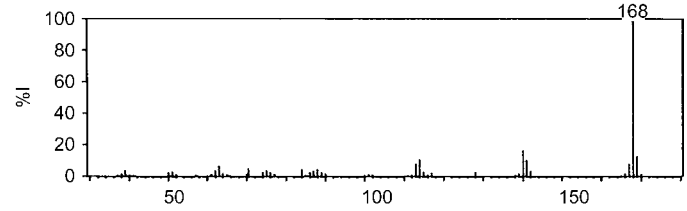
Putrefactive Base
$C_{11}H_8N_2$ = 168.2
CAS—244-63-3
IUPAC Name 9*H*-Pyrido[3,4-*b*]indole
Synonym β-Carboline



Chemical Properties Colourless crystals. Mp 199°. Soluble in hot water, ethanol and ether. Log *P* (octanol/water), 3.2.
Colour Tests Mandelin's test—green→yellow; Marquis test—green.
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.00; system TC—R_f 0.30; system TL—R_f 0.33 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—247 (A^1_1=1723a), 300 nm.

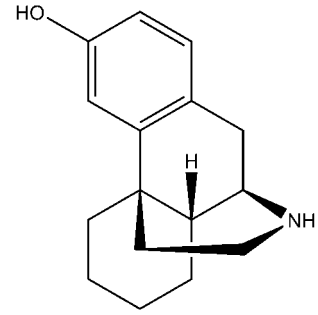


Infrared Spectrum Principal peaks at wavenumbers 1247, 731, 747, 1630, 813, 1280 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 168, 140, 169, 114, 141, 167, 113, 63.



Norlevorphanol

Narcotic Analgesic
$C_{16}H_{21}NO$ = 243.4
CAS—1531-12-0
Synonyms (–)-1,3,4,9,10,10a-Hexahydro-6-hydroxy-2*H*-10,4a-iminoethanophenanthrene; (–)-3-hydroxymorphinan; morphinan-3-ol.



Chemical Properties Crystals from acetone and methanol. Mp 270° to 272°. Norlevorphanol is extracted by organic solvents from aqueous ammoniacal solutions. Stock standard solutions were stable for 4 months at 5° and for 8 days at room temperature. Plasma samples were stable for 3 months at –20° and 3 days at room temperature [Kristensen 1998]. Plasma and urine samples stored at –20° for 16 weeks showed a loss of <5% [Chen *et al.* 1990].

Norlevorphanol Hydrobromide

$C_{23}H_{27}NO_5$, HBr = 324.3
CAS—63732-85-4
Synonym NIH-7539
Chemical Properties Crystals from water. Mp 222° to 224° [O'Neil *et al.* 2006].

(+)-Norlevorphanol

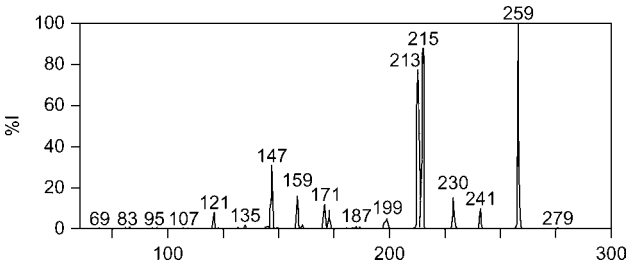
CAS—15676-23-0
Synonyms (9α,13α,14 a)-Morphinan-3-ol; nordextrorphan.

Chemical Properties Crystals from acetone. Mp 258° to 259° [O'Neil *et al.* 2006].

Colour Tests Ammonium molybdate test—blue→yellow-green (limit of detection, 0.25 μg); sulfuric acid-formaldehyde test—olive-green (limit of detection, 1.0 μg); Vitali's test—faint yellow/faint yellow/orange (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.12 (location reagent acidified iodoplatinate spray, positive reaction).

Mass Spectrum Principal ions at *m/z* 259, 215, 213, 147, 159, 230 Vengurlekar *et al* [2002].



Quantification

Plasma **HPLC** Column: Thermo Hypersil-Keystone phenyl (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : potassium hydrogen phosphate (20 : 30 : 50), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 330 nm). Retention time: 6.1 min. Limit of quantification, 1 nmol/L [Lin *et al.* 2007]. Column: Spherisorb (15 cm × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : triethylamine : distilled water (17 : 0.06 : 82.94), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 330 nm). Retention time: 4.2 min. Limit of detection, 0.5 μg/L [Chen *et al.* 1990]. Column: Macherey-Nagel Nucleosil (150 × 4.6 mm i.d., 5 μm). Mobile phase: 2 mmol/L laurylsulfate and 10 mmol/L sodium phosphate buffer (pH 2.1) : acetonitrile (59 : 41). Limit of detection, 10 μg/L [Mortimer *et al.* 1989]. Column: Polygosil C_8 (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.05 mol/L perchloric acid-0.01 mol/L triethylamine (20 : 80), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 275 nm, λ_{em} = 305 nm). Retention time: 3.4 min. Limit of detection, 10 μg/L [Mascher 1987].

CE Capillary: fused silica (40.0 cm to the inlet, 75 μm i.d.). Running buffer: 50 mmol/L sodium borate (pH 9.4). UV detection (λ = 195 nm). Limit of quantification, 1.2 μg/L; limit of detection, 0.5 μg/L [Kristensen 1998].

Serum **HPLC** Column: Waters NovaPak C_{18} (150 × 3.9 mm i.d.). Mobile phase: 50 mmol/L potassium phosphate buffer (pH 3.0) : acetonitrile : triethylamine (66 : 34 : 0.05 to 78 : 22 : 0.05 at 10 min for 12 min), flow rate 0.5 mL/min. Diode array detection (λ = 205 nm). Retention time: 4.3 min. Limit of detection not reported [Carlsson *et al.* 2004].

Urine **HPLC** Column: Phenyl Spherisorb (15 cm × 4.6 mm i.d., 5 μm). Mobile phase: 1.5% glacial acetic acid and 0.1% triethylamine : acetonitrile (75 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 200 nm, no cut-off for emission). Limit of quantification, 47.8 μg/L; limit of detection, 2.43 μg/L [Bendriss *et al.* 2001]. Column: Phenyl (25 cm × 4.6 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : 10 mmol/L potassium phosphate buffer (pH 3.5; 20 : 25 : 55), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 228 nm, λ_{em} no cut off). Retention time: ≈5.2 min. Limit of detection, 60 μg/L [Ducharme *et al.* 1996]. Column: Spherisorb (15 cm × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : triethylamine : distilled water (17 : 0.06 : 82.94), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 330 nm). Retention time: 4.2 min. Limit of detection, 0.5 μg/L [Chen *et al.* 1990].

LC-MS Column: Zorbax RX-C8 (15 cm × 2.1 mm i.d., 5 μm). Mobile phase: 15 mmol/L ammonium acetate (pH 4.75) : acetonitrile-methanol (50 : 50; 99 : 1 to 1 : 99 at 8 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode, positive ion mode. Limit of quantification, 100 μg/L [Vengurlekar *et al.* 2002].

Other **HPLC** Human Liver Microsomes. Column: Hi-chrom phenyl (250 × 4.6 mm i.d.). Mobile phase: acetonitrile-methanol (250 : 200) : 10 mmol/L potassium phosphate buffer (pH 3.5; 50 : 50), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 310 nm). Limit of detection not reported [Yu, Haining 2001].

LC-MS Rat Everted Gut Sacs. Column: Waters Symmetry C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: water : 1% formic acid : methanol (80 : 10 : 10 to 50 : 10 : 40 at 4 min to 25 : 10 : 65 at 8 min to 15 : 10 : 75 at 10 min to 80 : 10 : 10 at 11 min for 9 min), flow rate 0.2 mL/min. SIR acquisition mode, positive ion mode. Retention time: 7.6 min. Limit of detection, 2.5 nmol/L [Arellano *et al.* 2005].

Disposition in the Body Norlevorphanol is a product of (-)-3-phenoxy-*N*-methylmorphinan [Leinweber *et al.* 1981] and dextromethorphan metabolism via the action of CYP3A4, CYP2B6 or CYP2D6 [Yu, Haining 2001]. For phenotypic differences in dextromethorphan metabolism see Vetticaden *et al.* [1989] and Nagai *et al.* [1996].

Dose It is an intermediate in the synthesis of morphinan derivatives.

Arellano C *et al.* (2005). Validation of a liquid chromatography-mass spectrometry method to assess the metabolism of dextromethorphan in rat everted gut sacs. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 105–113.

Bendriss EK *et al.* (2001). High-performance liquid chromatography assay for simultaneous determination of dextromethorphan and its main metabolites in urine and in microsomal preparations. *J Chromatogr B Biomed Sci Appl* 754: 209–215.

Carlsson KC *et al.* (2004). Analgesic effect of dextromethorphan in neuropathic pain. *Acta Anaesthesiol Scand* 48: 328–336.

Chen ZR *et al.* (1990). Simultaneous determination of dextromethorphan and three metabolites in plasma and urine using high-performance liquid chromatography with application to their disposition in man. *Ther Drug Monit* 12: 97–104.

Ducharme J *et al.* (1996). Dextromethorphan as an in vivo probe for the simultaneous determination of CYP2D6 and CYP3A activity. *J Chromatogr B Biomed Appl* 678: 113–128.

Kristensen HT (1998). Simultaneous determination of dextromethorphan and its metabolites in human plasma by capillary electrophoresis. *J Pharm Biomed Anal* 18: 827–838.

Leinweber FJ *et al.* (1981). The metabolism of (-)-3-phenoxy-N-methylmorphinan in dogs. *Drug Metab Dispos* 9: 284–291.

Lin SY *et al.* (2007). Simultaneous analysis of dextromethorphan and its three metabolites in human plasma using an improved HPLC method with fluorometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 859: 141–146.

Mascher H (1987). High-performance liquid chromatographic determination of dextrorphan and 3-hydroxymorphinan in human plasma based on a selective pre-column sample clean-up. *J Chromatogr* 420: 217–222.

Mortimer O *et al.* (1989). Dextromethorphan: polymorphic serum pattern of the O-demethylated and didemethylated metabolites in man. *Br J Clin Pharmacol* 27: 223–227.

Nagai N *et al.* (1996). Pharmacokinetics and polymorphic oxidation of dextromethorphan in a Japanese population. *Biopharm Drug Dispos* 17: 421–433.

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck & Co., Inc.

Vengurlekar SS *et al.* (2002). A sensitive LC-MS/MS assay for the determination of dextromethorphan and metabolites in human urine–application for drug interaction studies assessing potential CYP3A and CYP2D6 inhibition. *J Pharm Biomed Anal* 30: 113–124.

Vetticaden SJ *et al.* (1989). Phenotypic differences in dextromethorphan metabolism. *Pharm Res* 6: 13–19.

Yu A, Haining RL (2001). Comparative contribution to dextromethorphan metabolism by cytochrome P450 isoforms in vitro: can dextromethorphan be used as a dual probe for both CTP2D6 and CYP3A activities? *Drug Metab Dispos* 29: 1514–1520.

Normetadrenaline

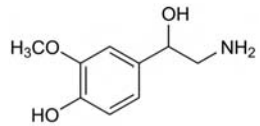
Catecholamine Metabolite

$C_9H_{13}NO_3$ = 183.2

CAS—97-31-4

IUPAC Name α-(Aminomethyl)-4-hydroxy-3-methoxybenzenemethanol

Synonym Normetanephrine



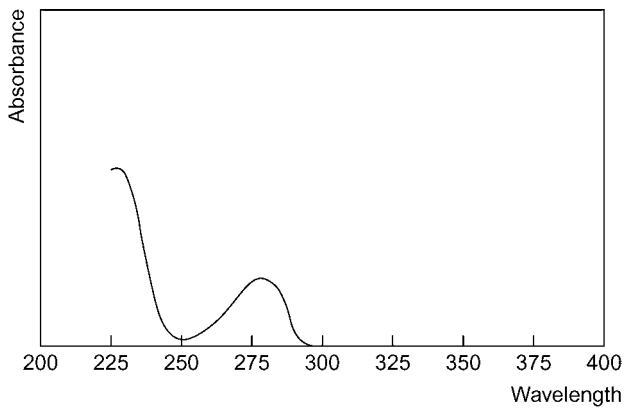
Chemical Properties pK_a 8.8. Log *P* (octanol/water), −1.0.

Colour Tests Liebermann's reagent—black; Mandelin's test—green; Marquis test—orange→violet-brown.

Thin-layer Chromatography System TA—R_f 0.33 (acidified potassium permanganate solution, positive).

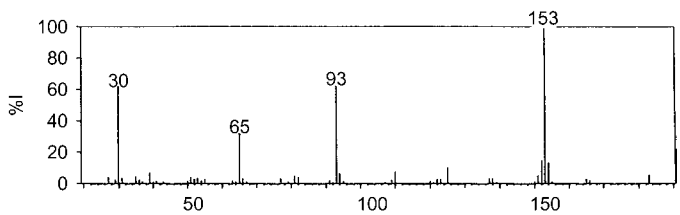
High Performance Liquid Chromatography System HC—*k* 1.08.

Ultraviolet Spectrum Aqueous acid—278 nm (A^1_1=85b).



Infrared Spectrum Principal peaks at wavenumbers 1529, 1162, 1250, 1037, 1290, 1123 cm^{-1} (normetanephrine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 153, 93, 30, 65, 152, 154, 125, 110.



N

Quantification

Plasma **HPLC** Electrochemical detection. For method of quantification for normetadrenaline and metadrenaline, see Lenders *et al.* [1993]. Fluorescence detection. Limit of detection, 0.1–17.5 µg/L for catecholamines, their precursors and metabolites including normetadrenaline [Jeon *et al.* 1992]. Electrochemical detection. For method of quantification for normetadrenaline and metanephrine, see Pagliari *et al.* [1991].

Urine **HPLC** Electrochemical detection (comparison with GC-MS method). For method of quantification for normetadrenaline and metadrenaline, see Willemsen *et al.* [2001]. Fluorimetric detection. For method of quantification for catecholamines and their metabolites, normetadrenaline and metadrenaline, see Chan *et al.* [2000]. Coulometric array detection. Limit of detection, 2.6 µg/L for normetadrenaline and metadrenaline [Gamache *et al.* 1993]. See Plasma [Jeon *et al.* 1992]. Electrochemical detection. For method of quantification for normetadrenaline, metadrenaline and 3-methoxytyramine, see Volin [1992]. Coulometric detection. For method of quantification for normetadrenaline, metadrenaline and catecholamines, see Santagostino *et al.* [1991].

GC–MS Limit of detection, <25 µg/L for normetadrenaline and metadrenaline [Crockett *et al.* 2002]. Limit of detection, 10 µg/L for normetadrenaline and metadrenaline [Taylor, Singh 2002].

Disposition in the Body Normetadrenaline is a metabolite of noradrenaline (norepinephrine).

Chan EC *et al.* (2000). High-performance liquid chromatographic assay for catecholamines and metanephrines using fluorimetric detection with pre-column 9-fluorenylmethyloxycarbonyl chloride derivatization. *J Chromatogr B Biomed Sci Appl* 749: 179–189.

Crockett DK *et al.* (2002). Rapid analysis of metanephrine and normetanephrine in urine by gas chromatography-mass spectrometry. *Clin Chem* 48: 332–337.

Gamache PH *et al.* (1993). Urinary metanephrine and normetanephrine determined without extraction by using liquid chromatography and coulometric array detection. *Clin Chem* 39: 1825–1830.

Jeon HK *et al.* (1992). High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction. *Anal Biochem* 200: 332–338.

Lenders JW *et al.* (1993). Determination of metanephrines in plasma by liquid chromatography with electrochemical detection. *Clin Chem* 39: 97–103.

Pagliari R *et al.* (1991). Determination of free and conjugated normetanephrine and metanephrine in human plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 563: 23–36.

Santagostino G *et al.* (1991). Simultaneous measurement of total catecholamines and metanephrines in human urine by liquid chromatography with coulometric detection. *Farmaco* 46: 1217–1223.

Taylor RL, Singh RJ (2002). Validation of liquid chromatography-tandem mass spectrometry method for analysis of urinary conjugated metanephrine and normetanephrine for screening of pheochromocytoma. *Clin Chem* 48: 533–539.

Volin P (1992). Determination of urinary normetanephrine, metanephrine and 3-methoxytyramine by high-performance liquid chromatography with electrochemical detection: comparison between automated column-switching and manual dual-column sample purification methods. *J Chromatogr* 578: 165–174.

Willemsen JJ *et al.* (2001). Evaluation of specific high-performance liquid-chromatographic determinations of urinary adrenaline and noradrenaline by comparison with isotope dilution mass spectrometry. *Ann Clin Biochem* 38: 356–364 and 722–730.

Normethadone

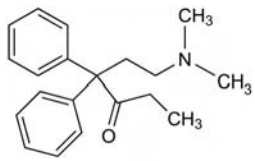
Narcotic Analgesic

$C_{20}H_{25}NO$ = 295.4

CAS—467-85-6

IUPAC Name 6-(Dimethylamino)-4,4-diphenyl-3-hexanone

Synonyms Desmethylmethadone; phenyldimazone.



Chemical Properties An oily liquid. pK_a 9.2. Log *P* (octanol/water), 3.8.

Normethadone Hydrochloride

$C_{20}H_{25}NO$, HCl = 331.9

CAS—847-84-7

Proprietary Name *Ticarda*

Chemical Properties Crystals. Mp 174° to 175°. Soluble in water and ethanol.

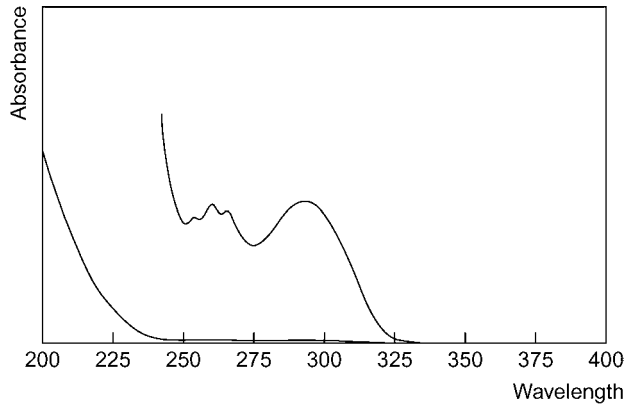
Colour Test Mandelin's test—yellow-green.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.40; system TC—R_f 0.34; system TAJ—R_f 0.19; system TAK—R_f 0.20; system TAL—R_f 0.78 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2095.

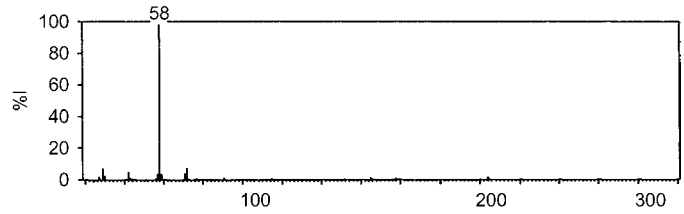
High Performance Liquid Chromatography System HC—*k* 0.53; system HY—RI 366.

Ultraviolet Spectrum Aqueous acid—253, 259, 265, 292 nm (A_1^1=19a).



Infrared Spectrum Principal peaks at wavenumbers 1711, 701, 1495, 1122, 1032, 760 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 72, 29, 42, 71, 57, 59, 224.



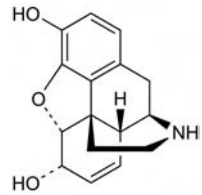
Normorphine

Narcotic Analgesic

$C_{16}H_{17}NO_3$ = 271.3

CAS—466-97-7

Synonyms Desmethylmorphine; (5α,6α)-7,8-didehydro-4,5-epoxymorphinan-3,6-diol.



Chemical Properties Crystals. Mp 273° to 277°. Slightly soluble in water and ethanol; practically insoluble in chloroform and ether. pK_a 9.8 (25°). Log *P* (ether/pH 7.0), −2.8.

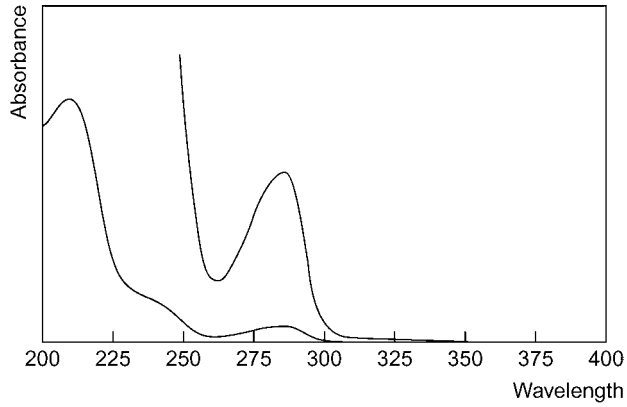
Colour Tests Mandelin's test—grey-green; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.17; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.28 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2459.

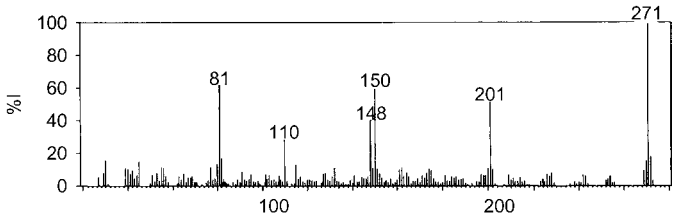
High Performance Liquid Chromatography System HA—*k* 2.9 (tailing peak); system HC—*k* 3.92; system HY—RI 133.

Ultraviolet Spectrum Aqueous acid—285 nm (A_1^1=57b).



Infrared Spectrum Principal peaks at wavenumbers 1312, 790, 1258, 1066, 1275, 1030 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 271, 81, 150, 201, 148, 110, 272, 82.



Quantification

Plasma **HPLC** Diode-array detection. Morphine and its metabolites.Limit of detection, 10 µg/L for normorphine [Wielbo *et al.* 1993]. Fluorometric detection. For method of quantification for morphine and its metabolites, including normorphine, see Glare *et al.* [1991]. Fluorescence detection. Limit of detection, <0.5 µg/L for morphine and its metabolites including normorphine [Venn, Michalkiewicz 1990].

HPLC–MS Morphine and its metabolites. Limit of detection, 0.3 µg/L for normorphine [Schanzle *et al.* 1999].

Serum **HPLC** See Plasma [Venn, Michalkiewicz 1990].

HPLC–MS See Plasma [Schanzle *et al.* 1999].

Cerebrospinal Fluid **HPLC** See Plasma [Venn, Michalkiewicz 1990].

HPLC–MS See Plasma [Schanzle *et al.* 1999].

Disposition in the Body Normorphine is a metabolite of codeine, diamorphine and morphine.

Venn RF, Michalkiewicz A (1990). Fast reliable assay for morphine and its metabolites using high-performance liquid chromatography and native fluorescence detection. *J Chromatogr* 525: 379–388.

Glare PA *et al.* (1991). A simple, rapid method for the simultaneous determination of morphine and its principal metabolites in plasma using high-performance liquid chromatography and fluorometric detection. *Ther Drug Monit* 13: 226–232.

Wielbo D *et al.* (1993). High-performance liquid chromatographic determination of morphine and its metabolites in plasma using diode-array detection. *J Chromatogr* 615: 164–168.

Schanzle G *et al.* (1999). Rapid, highly sensitive method for the determination of morphine and its metabolites in body fluids by liquid chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 721: 55–65.

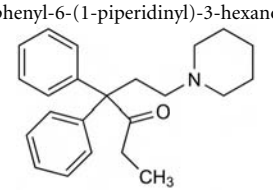
Norpipanone

Narcotic Analgesic

$C_{23}H_{29}NO$ = 335.5

CAS—561-48-8

IUPAC Name 4,4-Diphenyl-6-(1-piperidinyl)-3-hexanone



Chemical Properties Log *P* (octanol/water), 5.1.

Norpipanone Hydrochloride

$C_{23}H_{29}NO,HCl$ = 371.9

CAS—6033-41-6

Chemical Properties Crystals. Mp 181° to 182°. Soluble in water and ethanol.

Norpipanone Hydrobromide

$C_{23}H_{29}NO,HBr$ = 415.4

CAS—6033-42-7

Chemical Properties Crystals. Mp 192° to 193°. Soluble in water and ethanol.

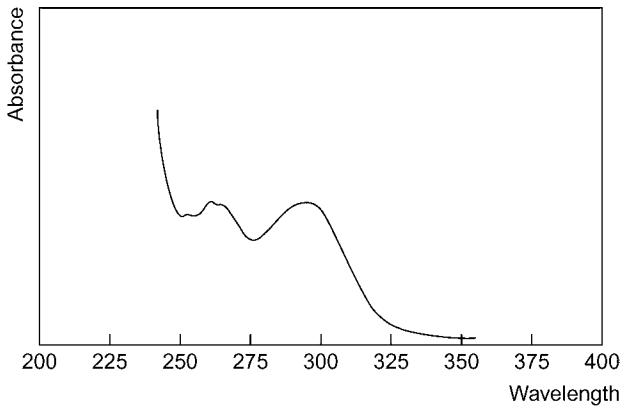
Colour Test Mandelin's test—brown→blue.

Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.58; system TC—R_f 0.50; system TE—R_f 0.80; system TL—R_f 0.38; system TAE—R_f 0.43 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, dull orange).

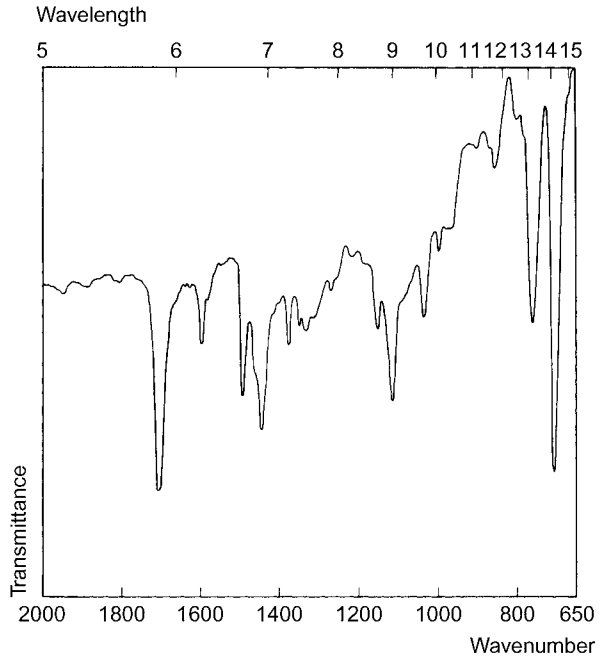
Gas Chromatography System GA—RI 2488.

High Performance Liquid Chromatography System HC—*k* 0.35; system HX—RI 466.

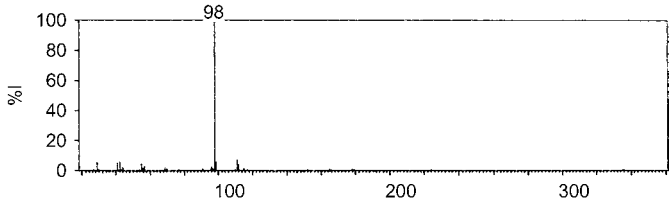
Ultraviolet Spectrum Aqueous acid—260 (A^{1}_{1}=17b), 294 nm.



Infrared Spectrum Principal peaks at wavenumbers 1705, 700, 1115, 1492, 1600, 1157 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 98, 111, 99, 42, 55, 41, 29, 112.



Dose Norpipanone hydrochloride has been given in doses of 18 mg daily.

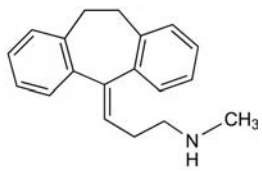
Nortriptyline

Antidepressant

$C_{19}H_{21}N$ = 263.4

CAS—72-69-5

Synonym 3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine



Chemical Properties pK_a 10.1. Log *P* (octanol/water), 4.51. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Nortriptyline Hydrochloride

$C_{19}H_{21}N$, HCl = 299.8

CAS—894-71-3

Synonyms 38489; nortriptylini hydrochloridum.

Proprietary Names *Acetexa*; *Allegron*; *Aventyl*; *Martimil*; *Norfenazin*; *Noritren*; *Norpress*; *Nortab*; *Norterol*; *Nortrilen*; *Ortrip*; *Pamelor*; *Sensaval*; *Vividyl*. It is an ingredient of *Motipress*; *Motival*.

Chemical Properties A white powder. Mp 213° to 215° (crystals from ether and ethanol). Soluble 1 in 50 of water, 1 in 10 of ethanol, and 1 in 5 of chloroform; practically insoluble in ether, acetone and benzene. pK_a 9.7. Log *P* (octanol/water pH 7.4), 1.63 [Meylan, Howard 1995].

Colour Tests Mandelin's test—brown→green; Marquis test—orange-brown; sulfuric acid—orange.

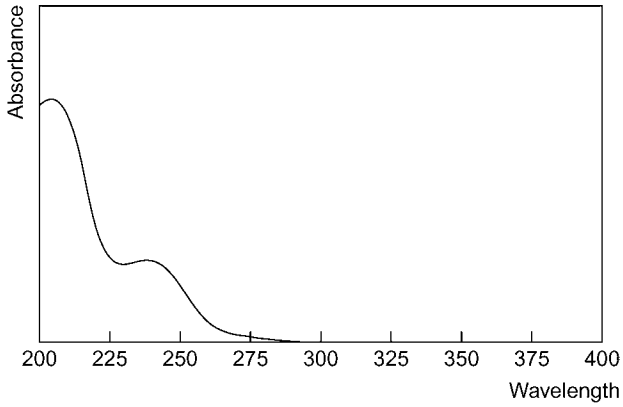
Thin-layer Chromatography System TA—R_f 0.34; system TB—R_f 0.27; system TC—R_f 0.16; system TAJ—R_f 0.01; system TAK—R_f 0.09; system TAL—R_f 0.68 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—brown).

Gas Chromatography System GA—nortriptyline RI 2215, M (*cis*-10-OH-) RI 2375, M (*trans*-10-OH-) RI 2375, M (acetyl-) RI 2660; system GB—nortriptyline RI

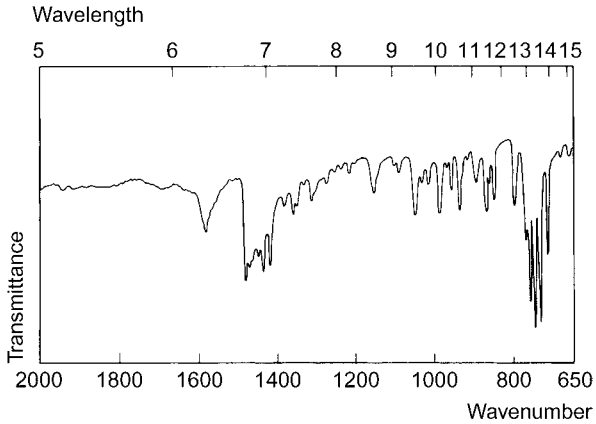
2304, M (*cis*-10-OH-) RI 2480, M (*trans*-10-OH-) RI 2494, M (norcyclobenzaprine) RI 2343, M (acetyl-) RI 2774, M (norcyclobenzaprine)-AC RI 2949; system GM—nortriptyline RRT 0.816, M (*cis*-10-OH-) RRT 1.261, M (*trans*-10-OH-) RRT 1.323, M (norcyclobenzaprine) RRT 0.880 (all relative to iprindole); system GS—RT 17.5 min.

High Performance Liquid Chromatography System HA—nortriptyline *k* 2.0, M (10-hydroxy-) *k* 1.8; system HF—*k* 4.58; system HY—RI 338; system HZ—RT 6.6 min; system HAA—RT 15.6 min; system HAM—not detected; system HAX—RT 13.7 min; system HAY—RT 6.8 min; system HAZ—*k* 1.71.

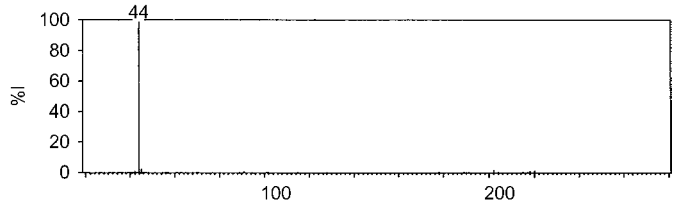
Ultraviolet Spectrum Aqueous acid—239 nm (A_1^1 = 517a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 756, 742, 768, 720, 775, 1590 cm^{-1} (nortriptyline hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 202, 45, 220, 218, 215, 91, – (nortriptyline); 44, 45, 26, 218, 215, 203, 202, 42 (10-hydroxynortriptyline).



Quantification See also under Amitriptyline.

Blood **GC-MS** Limits of detection, 0.02 mg/L for nortriptyline and 0.05 mg/L for amitriptyline [Melent'ev *et al.* 2007].

Plasma **GC** Column: Silica. Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (94 : 6). UV detection. Limit of detection, 10 µg/g for nortriptyline and 5 µg/g for amitriptyline [Kudo *et al.* 1997]. Column: HP 5890, DB-17 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 140° to 220° at 20°/min to 270° at 2°/min. NPD. Limit of quantification, 125 µg/L [Ulrich, Martens 1997]. Column: Supelco SP2250 (30 m × 0.25 mm i.d., 0.2 µm) or HP-17 (25 m × 0.2 mm i.d., 0.17 mm). Carrier gas: Ar : CH_4 (95 : 5), 60 mL/min. Temperature programme: 200° to 275° over 22 min. NPD. Limits of quantification, 1 µg/L for nortriptyline and 3 µg/L for 10-hydroxynortriptyline [Anliker *et al.* 1992].

LC-MS Column: Sunfire C_{18} IS (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate (pH 3), flow rate 0.4 mL/min. ESI, positive ion mode. Retention time: 3.72 min. Limit of quantification, 2 µg/L [De Castro *et al.* 2008]. Column: RP-18 (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 5.5) : acetonitrile (50 : 50). ESI. Limit of detection, 0.1 µg/L for nortriptyline and other tricyclic antidepressants [Alves *et al.* 2007]. Column: Zorbax SB C_{18} (75 × 4.6 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 25 mmol/L ammonium acetate buffer (pH 4.2, 40 : 60), flow rate 1.0 mL/min. APCI. Limit of quantification, 0.8 µg/L for nortriptyline and its metabolite 10-hydroxynortriptyline, limit of detection, 0.2 µg/L [Tybring *et al.* 1998]. Column: Hypersil (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (94 : 6), flow rate 0.4 mL/min. PICI. Limit of detection, 5 µg/g for nortriptyline and 2 µg/g for amitriptyline [Kudo *et al.* 1997].

Serum **GC** Column: HP-5 (25 m × 0.2 mm i.d., 0.33 µm). NPD. Limit of detection, 1.5 µg/L for nortriptyline; amitriptyline and hydroxyamitriptyline and 3 µg/L for 10-hydroxynortriptyline [Ulrich, Martens 1997].

HPLC Column: Spheri-5 C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphoric buffer (pH 2.36, 1 : 1), flow rate 1.0 mL/min. DAD (λ = 254 nm). Limit of quantification, 0.05 mg/L, limit of detection, 0.01 mg/L [Wozniakiewicz *et al.* 2008]. Column: C_{18}. Mobile phase: 0.15 mol/L SDS : 6% pentanol buffered (pH 7), flow rate 1.5 mL/min. Electrochemical detection. Limit of detection, 0.31 µg/L for nortriptyline and 0.25 µg/L for amitriptyline [Bose *et al.* 2005]. Column: Luna C_{18} (150 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.01 mol/L triethylamine buffer (pH 3.0, 34 : 66), flow rate 0.85 mL/min. UV detection (λ = 242 nm). Limit of quantification, 2 µg/L [Olesen *et al.* 2000].

LC-MS Column: Hypersil Gold C_{18} (50 × 2.1 mm i.d.). Mobile phase: water : acetonitrile each with 0.1% formic acid. ESI. Limit of quantification, <22 µg/L, limit of detection, <15 µg/L for nortriptyline and other tricyclic antidepressants [Breaud *et al.* 2009].

Urine **GC-MS** Column: CP-SIL 5CB (10 m × 0.15 mm i.d., 0.12 µm). Carrier gas: H_2, 1 mL/min. Temperature programme: 110° for 0.5 min to 270° at 40°/min to 288° at 54°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 50 µg/L, limit of detection, 25 µg/L [Rana *et al.* 2008].

Oral Fluid **LC-MS** See Plasma [De Castro *et al.* 2008].

Disposition in the Body Nortriptyline is readily absorbed after oral administration and undergoes first-pass metabolism. The main metabolic reactions are *N*-demethylation and 10-hydroxylation; glucuronide conjugation of nortriptyline and its metabolites also occurs. Up to 60% of the daily dose is excreted in the urine within 24 h, with <5% as unchanged drug, 30–50% as free or conjugated 10-hydroxynortriptyline, 6–15% as free and conjugated 10-hydroxydinortriptyline, and ~1% as dinortriptyline. Nortriptyline is a metabolite of amitriptyline.

Therapeutic Concentration In plasma, usually in the range 0.05–0.15 mg/L. Plasma concentrations vary considerably between individuals and are influenced by exposure to other drugs.

Following a single oral dose of 100 mg to 10 subjects, peak plasma concentrations of nortriptyline of 0.015–0.050 mg/L (mean, 0.03) and of 10-hydroxynortriptyline of 0.039–0.172 mg/L (mean, 0.10) were attained in 3–24 h [Nakano, Hollister 1978].

Following average daily oral doses of 82 mg to 62 subjects, the following steady-state plasma concentrations were reported: nortriptyline 0.03–0.34 mg/L (mean, 0.12), 10-hydroxynortriptyline 0.04–0.42 mg/L (mean, 0.16) and conjugated 10-hydroxynortriptyline 0.075–1.07 mg/L (mean, 0.37) [Dawling *et al.* 1982].

Toxicity Relatively few cases of serious intoxication have been attributed to nortriptyline in comparison to the tertiary amine tricyclic antidepressants. Toxic effects are usually associated with blood concentrations greater than 0.25 mg/L; concentrations above 1 mg/L may produce coma. Recovery has occurred after the ingestion of 2.5 g.

The following distribution was reported in nine fatal cases: blood 0 to 26 mg/L (mean, 11; 3 cases), kidney 7–94 µg/g (mean; 43, 5 cases), liver 8 to 220 µg/g (mean, 90; nine cases), urine 25–120 mg/L (mean, 76; 4 cases) [Bonnichsen *et al.* 1970]. Another fatal case has been reported [Rudorfer, Robins 1981].

Postmortem heart blood levels of 84.6 mg/L were found in a 31-year-old man who died after an overdose of nortriptyline [Rohrig, Prouty 1989].

A 42-year-old woman treated with nortriptyline 100 mg daily developed severe side effects and was found to have toxic serum levels (~2100 nmol/L). The patient was a poor metaboliser and treatment with a reduced dose of 25 mg daily was then given without adverse effect [Petersen, Brosen 1991].

A 62-year-old woman survived after an intentional overdose of nortriptyline despite having a peak plasma concentration of 2.29 mg/L; high levels (up to 2 mg/L) of (*E*)-10-hydroxynortriptyline were still detected for 4 to 5 days after drug intake [Franssen *et al.* 2003].

Bioavailability ~50–60%.

Half-life Plasma half-life, 15–90 h (mean, 30).

Volume of Distribution 14–40 L/kg (mean, 23).

Clearance Plasma, 7.2 mL/min/kg.

Protein Binding 90–95%.

Note For a review of tricyclic antidepressants, see Molnar, Gupta [1980]; for a review of the formation of active metabolites of psychotropic drugs, see Caccia, Garattini [1990].

Dose Usually, the equivalent of 30 to 100 mg nortriptyline daily; maximum 150 mg daily.

Alves C *et al.* (2007). Analysis of tricyclic antidepressant drugs in plasma by means of solid-phase microextraction-liquid chromatography–mass spectrometry. *J Mass Spectrom* 42: 1342–1347.

Anliker SL *et al.* (1992). Sensitive method for the quantitation of nortriptyline and 10-hydroxynortriptyline in human plasma by capillary gas chromatography with electron-capture detection. *J Chromatogr* 573: 141–145.

Bonnichsen R *et al.* (1970). A report on autopsy cases involving amitriptyline and nortriptyline. *Z Rechtsmed* 67: 190–200.

Bose D *et al.* (2005). Amitriptyline and nortriptyline serum determination by micellar liquid chromatography. *J Pharmacol Toxicol Meth* 52: 323–329.

Breaud AR *et al.* (2009). A rapid and reliable method for the quantitation of tricyclic antidepressants in serum using HPLC-MS/MS. *Clin Biochem* 42: 1300–1307.

Caccia S, Garattini S (1990). Formation of active metabolites of psychotropic drugs: an updated review of their significance. *Clin Pharmacokinet* 18: 434–459.

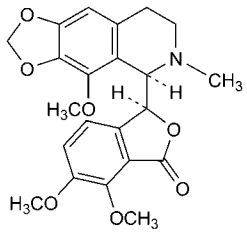
Dawling S *et al.* (1982). Nortriptyline metabolism in chronic renal failure: metabolite elimination. *Clin Pharmacol Ther* 32: 322–329.

De Castro A *et al.* (2008). LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma: study of correlation between venlafaxine concentrations in both matrices. *J Pharm Biomed Anal* 48: 183–193.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481-486.
Franssen EJ *et al.* (2003). Toxicokinetics of nortriptyline and amitriptyline: two case reports. *Ther Drug Monit* 25: 248–251.
Kudo K *et al.* (1997). Selective determination of amitriptyline and nortriptyline in human plasma by HPLC with ultraviolet and particle beam mass spectrometry. *J Anal Toxicol* 21: 185–189.
Melent'ev AB *et al.* (2007). [Gas chromatography with mass-selective detector in testing blood for amitriptyline and nortriptyline]. *Sud Med Ekspert* 50: 31–34.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Molnar G, Gupta RN (1980). Plasma levels and tricyclic antidepressant therapy: Part 2. Pharmacokinetic, clinical and toxicologic aspects. *Biopharm Drug Dispos* 1: 283–305.
Nakano S, Hollister LE (1978). No circadian effect on nortriptyline kinetics in man. *Clin Pharmacol Ther* 23: 199–203.
Olesen OV *et al.* (2000). Determination of nortriptyline in human serum by fully automated solid-phase extraction and on-line high-performance liquid chromatography in the presence of antipsychotic drugs. *J Chromatogr B Biomed Sci Appl* 746: 233–239.
Petersen P, Brosen K (1991). [Severe nortriptyline poisoning in poor metabolizers of the sparteine type]. *Ugeskr Laeger* 153: 443–444.
Rana S *et al.* (2008). A new method for simultaneous determination of cyclic antidepressants and their metabolites in urine using enzymatic hydrolysis and fast GC-MS. *J Anal Toxicol* 32: 355–363.
Rohrig TP, Prouty RW (1989). A nortriptyline death with unusually high tissue concentrations. *J Anal Toxicol* 13: 303–304.
Rudorfer MV, Robins E (1981). Fatal nortriptyline overdose, plasma levels, and in vivo methylation of tricyclic antidepressants. *Am J Psychiatry* 138: 982–983.
Tybring G *et al.* (1998). High-performance liquid chromatography–mass spectrometry for the quantification of nortriptyline and 10-hydroxynortriptyline in plasma. *J Chromatogr B Biomed Sci Appl* 716: 382–386.
Ulrich S, Martens J (1997). Solid-phase microextraction with capillary gas–liquid chromatography and nitrogen–phosphorus selective detection for the assay of antidepressant drugs in human plasma. *J Chromatogr B Biomed Sci Appl* 696: 217–234.
Wozniakiewicz M *et al.* (2008). Microwave-assisted extraction of tricyclic antidepressants from human serum followed by high performance liquid chromatography determination. *J Chromatogr A* 1190: 52–56.

Noscapine

Cough Suppressant
$C_{22}H_{23}NO_7$=13.4
CAS—128-62-1

IUPAC Name (3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-7,8-dihydro-5*H*-[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]-3*H*-2-benzofuran-1-one
Synonyms (3*S*)-6,7-Dimethoxy-3-[(5*R*)-5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]-1(3*H*)-isobenzofuranone; narcotine; lα-narcotine; noscapinum; NSC-5366.
Proprietary Names *Capval*; *Finipect*; *Nipaxon*; *Nitepax* (resin complex).

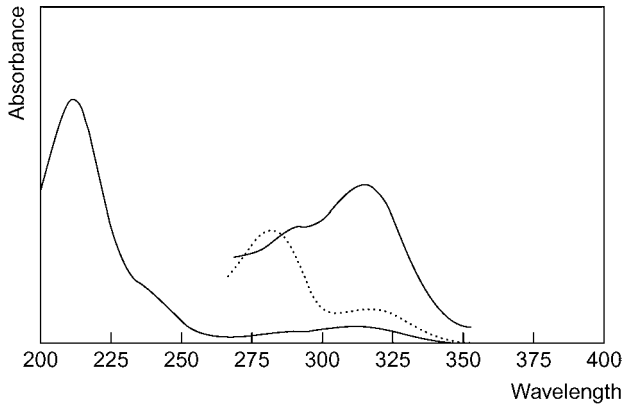


Chemical Properties An alkaloid obtained from opium. Colourless crystals or a fine white crystalline powder. Mp 176°, with decomposition. Practically insoluble in water; slightly soluble in ethanol and ether; soluble in acetone and chloroform. pK_{a1} 6.2 (20°). Log P (octanol/water), 2.0. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

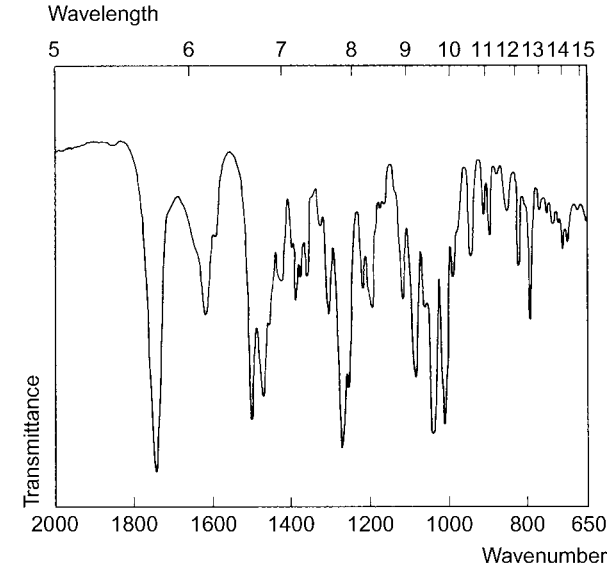
Noscapine Hydrochloride

$C_{22}H_{23}NO_7$, HCl, H_2O = 467.9
CAS—912-60-7 (anhydrous)
IUPAC Name 6,7-Dimethoxy-3-(4-methoxy-6-methyl-7,8-dihydro-5*H*-[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl)-3*H*-2-benzofuran-1-one hydrate hydrochloride
Proprietary Names *Capval*; *Libronchin Prikkelhoest*; *Noscaflex*; *Roter Noscapect*; *Tuscalman*; *Tussanil N*.
Chemical Properties Hygroscopic, colourless crystals or a fine white crystalline powder. Mp ≈200°, with decomposition. Soluble 1 in 4 of water and 1 in 8 of ethanol; freely soluble in chloroform; practically insoluble in ether. Aqueous solutions may deposit the base on standing.

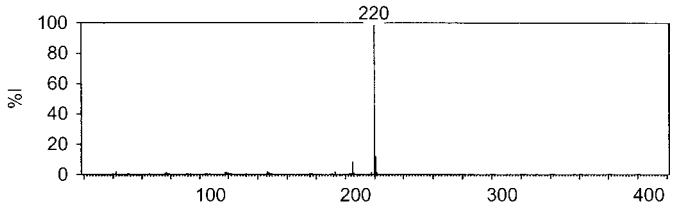
Colour Tests Liebermann's reagent—black; Marquis test—blue-violet (fades).
Thin-layer Chromatography System TA—R_f 0.64; system TB—R_f 0.21; system TC—R_f 0.74; system TE—R_f 0.78; system TL—R_f 0.64; system TAE—R_f 0.72; system TAF—R_f 0.75; system TAJ—R_f 0.65; system TAK—R_f 0.18; system TAL—R_f 0.93 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—RI 3145; system GB—RI 3358.
High Performance Liquid Chromatography System HA—*k* 0.3; system HC—*k* 0.15; system HS—*k* 0.01; system HX—RI 368; system HY—RI 289; system HAA—RT 12.8 min.
Ultraviolet Spectrum Aqueous acid—290, 312 (A_1^1 = 84a); aqueous alkali—281, 315 nm.



Infrared Spectrum Principal peaks at wavenumbers 1745, 1276, 1038, 1010, 1498, 1080 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 220, 221, 205, 147, 42, 193, 77, 118.



Quantification

Plasma **HPLC** Limit of quantification, ~7 µg/L [Chollet *et al.* 1997]. Column: Nucleosil C_{18} (100 × 4.6 mm i.d., 3 um). Mobile phase: 3 mmol/L dodecyl sulfate : acetonitrile in phosphate buffer (pH 2; 60 : 40), flow rate 1.0 mL/min. UV detection (λ = 310 nm). Limit of quantification, 9 µg/L [Johansson *et al.* 1988]. Column: LiChrosorb Si 60 (250 × 4 mm i.d., 5 µm). Mobile phase: hexane : methanol : chloroform : diethylamine (86.5 : 10.1 : 3.4 : 0.034), flow rate 1.0 mL/min. UV detection (λ = 310 nm). *k'*: 2.8. Limit of detection, 5 µg/L [Johansson *et al.* 1983].

LC-MS Column: Diamonsil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (70 : 30 : 0.5), flow rate 0.5 mL/min. APCI, SRM acquisition mode. Limit of quantification, 0.1 µg/L [Zhu *et al.* 2005].

Note For a spectrofluorimetry method in plasma and urine, see Vedsø [1961].

Serum **HPLC** Column: LiChrosorb CN (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : 0.02 mol/L disodium hydrogen phosphate with 0.1 mol/L sodium perchlorate (pH 3.8; 25 : 75), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of detection, 1 µg/L [Haikala 1985]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.6% potassium dihydrogen phosphate (pH 3.0) : acetonitrile (55 : 45), flow rate 0.9 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 µg/L [Jensen 1983].

Disposition in the Body Rapidly absorbed after oral administration. Approximately 1% of a dose is excreted in the urine in 6 h as free and conjugated noscapine. See Tsunoda, Yoshimura [1981].

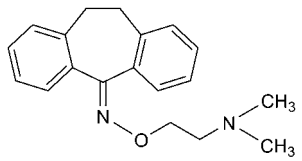
Therapeutic Concentration
Following a single oral dose of 150 mg to 5 subjects, peak plasma concentrations of 0.20–0.35 mg/L (mean 0.27) were attained in 0.5–2.5 h [Dahlström *et al.* 1982].
Protein Binding 93% to albumin and α_1-acid glycoprotein [Karlsson, Dahlström 1990].
Bioavailability Approximately 30%.
Half-life Plasma half-life, 1.5–4 h (mean 2.5).
Volume of Distribution 3–7 L/kg (mean 5) [Dahlström *et al.* 1982].
Clearance Plasma clearance, ~20 mL/min/kg.
Dose Up to 150 mg daily in divided doses.

Chollet DF *et al.* (1997). Determination of noscapine in human plasma using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 701: 81–85.
Dahlström B *et al.* (1982). Pharmacokinetic properties of noscapine. *Eur J Clin Pharmacol* 22: 535–539.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Haikala V (1985). New sensitive method to determine noscapine in serum by reversed-phase liquid chromatography. *J Chromatogr* 337: 429–433.
Jensen KM (1983). Determination of noscapine in serum by high-performance liquid chromatography. *J Chromatogr* 274: 381–387.
Johansson M *et al.* (1983). Determination of noscapine in plasma by liquid chromatography. *J Chromatogr* 275: 355–366.
Johansson M *et al.* (1988). Determination of noscapine and its metabolites in plasma by coupled-column liquid chromatography. *J Chromatogr* 459: 301–311.
Karlsson MO, Dahlström B (1990). Serum protein binding of noscapine: influence of a reversible hydrolysis. *J Pharm Pharmacol* 42: 140–143.
Tsunoda N, Yoshimura H (1981). Metabolic fate of noscapine. III. Further studies on identification and determination of the metabolites. *Xenobiotica* 11: 23–32.
Vedsø S (1961). The determination of noscapine (narcotine) in plasma and urine. *Acta Pharmacol Toxicol (Copenh)* 18: 119–128.
Zhu L *et al.* (2005). Simultaneous determination of methylephedrine and noscapine in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 820: 175–182.

Noxiptiline

Antidepressant
$C_{19}H_{22}N_2O$ = 294.4
CAS—3362-45-6

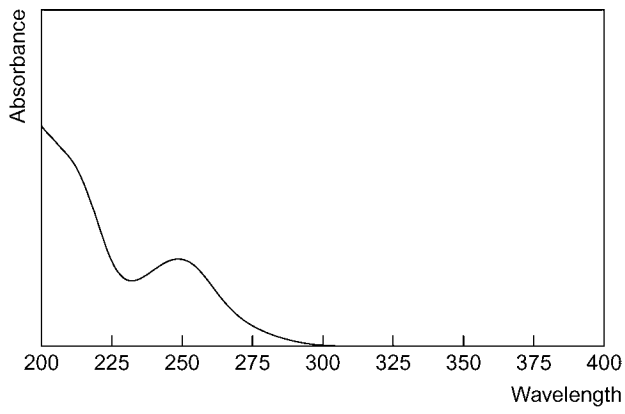
Synonyms Dibenzoxine; 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one-*O*-[2-(dimethylamino)ethyl]oxime; noxiptyline.



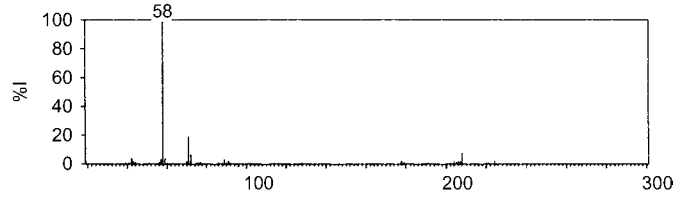
Chemical Properties Soluble in chloroform. Log *P* (octanol/water), 4.3.

Noxiptiline Hydrochloride
$C_{19}H_{22}N_2O$, HCl = 330.9
CAS—4985-15-3
Proprietary Names *Agedal*; *Nogédal*.
Chemical Properties White crystalline powder. Mp 189° to 191°. Soluble in water.

Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.53; system TAE—R_f 0.29; system TAG—R_f 0.18; system TB—R_f 0.43; system TC—R_f 0.35; system TE—R_f 0.66 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—noxiptyline RI 2270, M (dibenzocycloheptanone) RI 1850; system GB—M (OH-dibenzocycloheptanone)-H_2O RI 2200.
High Performance Liquid Chromatography System HF—*k* 1.63; system HY—RI 330.
Ultraviolet Spectrum Aqueous acid—250 nm (A_1^1 = 477a). No alkaline shift.



Mass Spectrum Principal ions at *m/z* 58, 71, 208, 72, 59, 42, 89, 57.



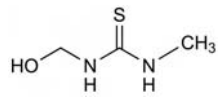
Quantification
Plasma **GC** Column: 1.5% V-17 on Gas Chrom Q (1.8 m × 0.3 cm i.d.). Carrier gas: N_2, 46 cm^3/min. Temperature: 230°. AFID. Limit of detection, 100 µg/L [Szyszko, Wejman 1981].
Urine **GC** See Plasma [Szyszko, Wejman 1981].
Dose The equivalent of 10 to 200 mg of noxiptiline daily.

Szyszko E, Wejman W (1981). Gas chromatographic determination of noxyptyline in substance, tablets and in biological material. *J Chromatogr* 219: 291–296.

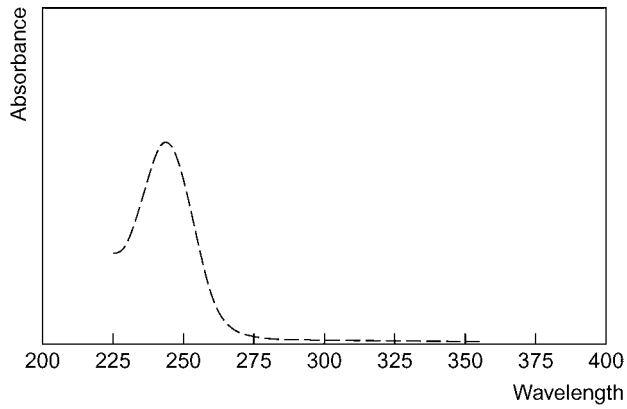
Noxytiolin

Antibacterial, Antifungal
$C_3H_8N_2OS$ = 120.2
CAS—15599-39-0

IUPAC Name [(Methylamino-λ^4-sulfanylidyne)methylamino]methanol
Synonyms *N*-(Hydroxymethyl)-*N'*-methylthiourea; noxythiolin.
Proprietary Name *Noxyflex-S*



Chemical Properties A white crystalline powder. Mp 88° to 90°. Soluble 1 in 10 of water; soluble in chloroform. Log *P* (octanol/water), −1.8.
Colour Test Palladium chloride—brown.
Thin-layer Chromatography System TA—R_f 0.74 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2370.
Ultraviolet Spectrum Methanol—244 nm (A_1^1=1180a).



Infrared Spectrum Principal peaks at wavenumbers 1558, 1042, 1230, 987, 1026, 1517 cm^{-1} (KBr disk).
Quantification
Serum **HPLC** For method, see Debruyne *et al.* [1985].
Use In concentrations of 1 to 2.5%.

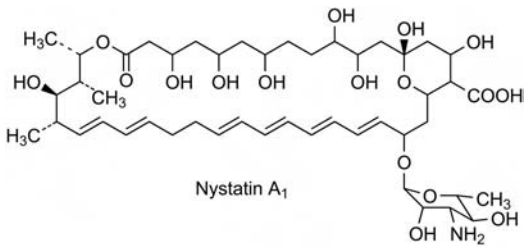
Debruyne D *et al.* (1985). Liquid chromatographic determination of noxytiolin and 1-methyl-2-thiourea in serum: application to pharmacokinetic studies in rabbits and humans. *J Pharm Sci* 74: 224–226.

Nystatin

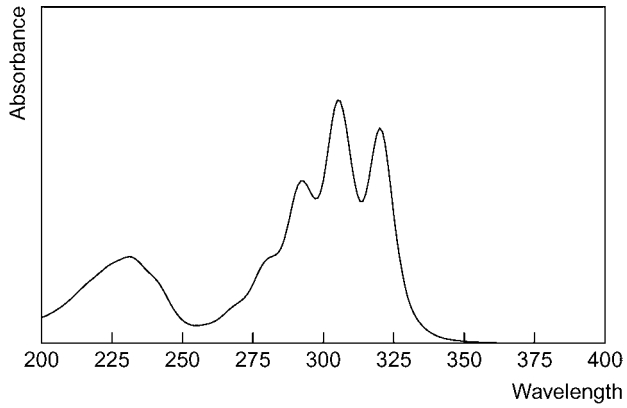
Antifungal
CAS—1400-61-9

IUPAC Name (4*E*,6*E*,8*E*,10*E*,14*E*,16*E*,18*S*,19*R*,20*R*,21*S*,35*S*)-3-[(2*S*,3*S*,4*S*,5*S*, 6*R*)-4Amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-19,25,27,29,32,33,35,37-octahydroxy-18,20,21-trimethyl-23-oxo-22,39-dioxabicyclo[33.3.1]nonatriaconta-4,6, 8, 10,14,16-hexaene-38-carboxylic acid

Synonyms Fungicidin; nistatina.
Proprietary Names *Nyspes*; *Nystan*; *Nystavescent*.



Chemical Properties Nystatin is an ingredient of many proprietary preparations—see Sweetman [2009]. A mixture of antifungal polyenes produced by the growth of certain strains of *Streptomyces noursei*, or by any other means. It consists largely of nystatin A. Approximate molecular formula: $C_{47}H_{75}NO_{17} = 926.1$. A yellow to light brown, hygroscopic powder. Very slightly soluble in water; sparingly soluble in ethanol; practically insoluble in chloroform and ether; freely soluble in dimethylformamide and formamide. Log *P* (octanol/water), 7.1.
Gas Chromatography System GA—RI 1945.
Ultraviolet Spectrum Aqueous alkali—291 (A_1^1=405b), 305 (A_1^1= 600b), 319 nm (A_1^1=530b).



Infrared Spectrum Principal peaks at wavenumbers 1067, 1000, 1570, 1175, 1316, 847 cm^{-1} (Nujol mull).
Uses Topically in preparations containing 100 000 units per g; orally in doses of 1500 000 to 4000 000 units daily.

Sweetman SC, ed. (2009). *Martindale: the Complete Drug Reference*, 36th edn. London: Pharmaceutical Press.

Obidoxime Chloride

Antidote (Organophosphate Insecticide Poisoning)

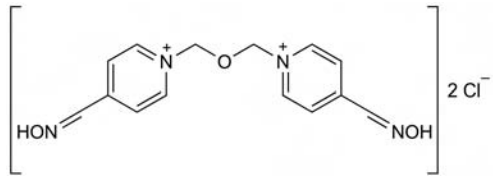
$C_{14}H_{16}Cl_2N_4O_3$ = 359.2

CAS—7683-36-5 (obidoxime); 114-90-9 (chloride)

IUPAC Name Oxo-[[1-[[4-(oxoazaniumylmethylidene)pyridin-1-yl]methoxymethyl]pyridin-4-ylidene]methyl]azanium dichloride

Synonyms BH-6; 1,1′-[oxybis(methylene)]bis[4-(hydroxyimino)methyl]pyridinium dichloride.

Proprietary Name *Toxogonin*

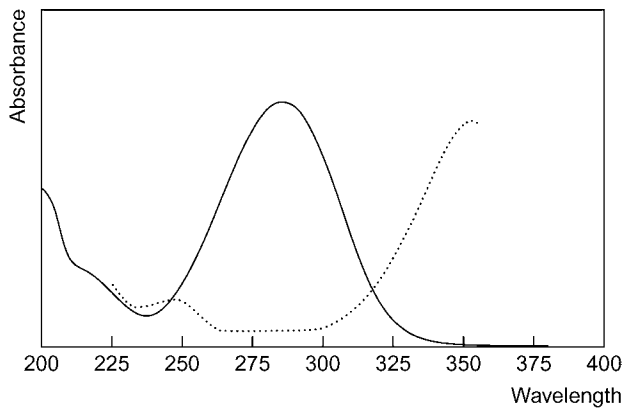


Chemical Properties White crystals, occurring in two interchangeable isomeric forms. Mp 225°, with decomposition. Freely soluble in water; slightly soluble in ethanol and methanol; practically insoluble in chloroform and ether. pK_a 7.6, 8.3 (25°). Log *P* (octanol/water), −2.0.

Colour Tests Mandelin's test—green→blue; methanolic potassium hydroxide—orange.

Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.53; system TAF—R_f 0.08 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—285 nm (A^1_1=947a); aqueous alkali—353 nm (A^1_1=1440b).



Infrared Spectrum Principal peaks at wavenumbers 996, 1090, 1637, 1600, 1282, 847 cm^{-1} (KBr disk).

Quantification

***Serum* Polarography** Limit of detection, 4 mg/L [Korićanac *et al.* 1982].

***Urine* HPLC** UV detection (λ=288 nm). Limit of detection, 1.8 mg/L [Grasshoff *et al.* 2001].

Polarography See Serum [Korićanac *et al.* 1982].

Disposition in the Body Obidoxime chloride is poorly absorbed after oral administration. After IM administration, >80% of a dose is excreted unchanged in the urine in 24 h.

Therapeutic Concentration

Following IM doses of 2.5 to 10 mg/kg to 10 subjects, peak plasma concentrations of 6.3 to 26.5 mg/L were attained in about 20 min [Sidell, Groff 1970].

Obidoxime plasma levels of 35.9 to 71.8 mg/L were achieved when it was administered to 5 subjects as an IV bolus of 250 mg followed by continuous infusion of 750 mg/24 h [Thiermann *et al.* 1997].

Half-life Plasma half-life, about 1.4 h.

Distribution in Blood Plasma : whole blood ratio, 1.9.

Dose Obidoxime chloride has been given parenterally in doses of 250 mg.

Grasshoff C *et al.* (2001). Internal standard high-performance liquid chromatography method for the determination of obidoxime in urine of organophosphate-poisoned patients. *J Chromatogr B Biomed Sci Appl* 753: 203–208.

Korićanac Z *et al.* (1982). *Acta Pharm Jugoslav* 32: 297–302.

Sidell FR, Groff WA (1970). Toxogonin: blood levels and side effects after intramuscular administration in man. *J Pharm Sci* 59: 793–797.

Thiermann H *et al.* (1997). Cholinesterase status, pharmacokinetics and laboratory findings during obidoxime therapy in organophosphate poisoned patients. *Hum Exp Toxicol* 16: 473–480.

Ochratoxins

Isocoumarin Derivative, Mycotoxin

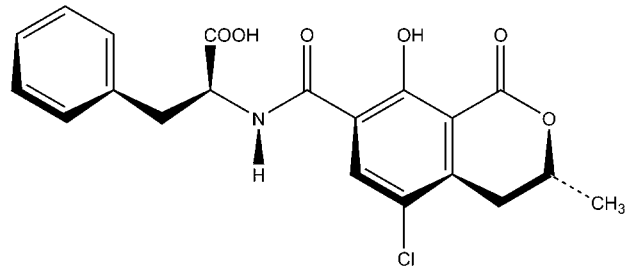
Ochratoxin A

$C_{20}H_{18}ClNO_6$ = 403.8

CAS—303-47-9

IUPAC Name (*R*)-*N*-[(5-Chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine

Synonym OTA



Chemical Properties Crystals from xylene, Mp 169°; also reported as Mp 90° from benzene [O'Neil *et al.* 2006]. Secondary metabolite mainly produced by the fungi *Penicillium verrucosum* and *Aspergillus alutaceus* (formerly *ochraceus*), which are encountered in food crops grown in semitropical and temperate climates [Soleas *et al.* 2001]. It has also been observed that members of the *Aspergillus niger* section *nigri* have colonised grapes, wine and vine fruits [Magan, Aldred 2005]. When treated at 140° with 24% moisture content, OTA content in extruded barley meal is reduced by 53.7% after 30 s and 83.5% after 70 s residence time [Castells *et al.* 2006].

Ochratoxin B

$C_{20}H_{19}NO_6$ = 369.4

CAS—4825-86-9

IUPAC Name (*R*)-*N*-[(3,4-Dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine

Synonym OTB

Chemical Properties Crystals from methanol, Mp 221°; also reported as 208° to 209° [O'Neil *et al.* 2006].

Ochratoxin C

$C_{22}H_{22}ClNO_6$ = 431.9

CAS—4865-85-4

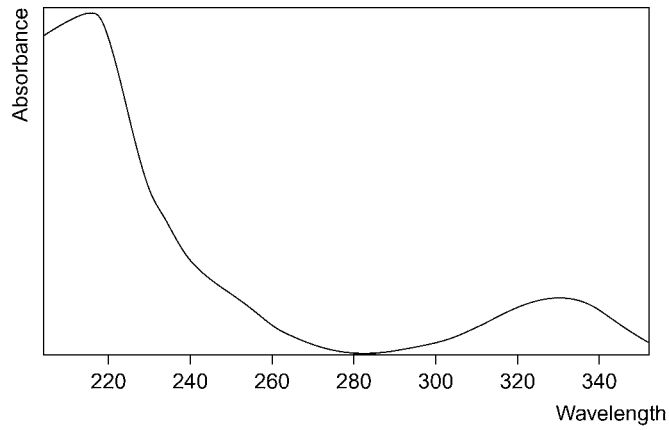
IUPAC Name (*R*)-*N*-[(5-Chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine ethyl ester

Synonym OTC

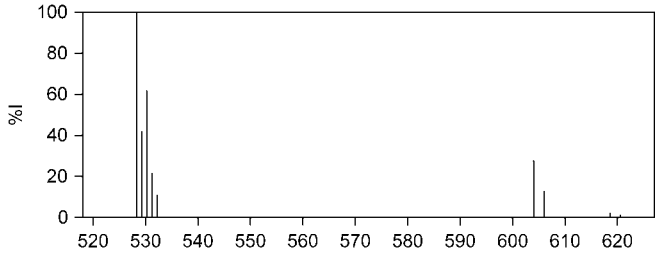
Chemical Properties Amorphous ethyl ester of OTA.

Thin-layer Chromatography Plates: fluorescent silica gel F_{254}. Solvent system: toluene : ethyl acetate : formic acid (6 : 3 : 1). UV detection (λ = 366 nm). Limit of detection, 0.1 μg/spot [Odhav, Naicker 2002].

Ultraviolet Spectrum



Mass Spectrum [Soleas *et al* 2001].



Quantification

Blood **HPLC** Column: Spherisorb C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 9% glacial acetic acid (pH 2.3; 18 : 7), flow rate 1 mL/min. Post-column derivatisation with 25% ammonium hydroxide solution. Fluorescence detection (λ_{ex} = 390 nm, λ_{em} = 440 nm). Retention times: OTA 5 min; relative retention times (relative to OTA) ochratoxin-α 0.71, OTB 0.89, ochratoxin-α methyl ester 0.89, OTA methyl ester 1.32, OTB methyl ester 1.13, OTC 1.50, OTA propyl ester 1.76, OTA butyl ester 2.13. Limit of quantification, 5 pg/g [Zimmerli, Dick 1995].

Plasma **HPLC** Column: Lichrospher C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (pH 3.16; 500 : 500 : 5), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 336 nm, λ_{em} = 464 nm). Retention time: ~9 min. Limit of detection, 0.2 μg/L [Peraica *et al.* 1999]. Column: C_{18} (250 × 3.2 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : 0.15 mol/L orthophosphoric acid (1 : 1 : 1), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 470 nm). Retention time: 4.8 to 6.2 min. Limit of detection, 0.15 μg/L [Scott *et al.* 1998].

LC-MS Column: Inertsil ODS-2 (250 × 2.1 mm i.d., 5 μm). Mobile phase: 0.17% formic acid in acetonitrile : methanol : water (1 : 1 : 1), flow rate 0.2 mL/min. ESI, positive ion mode, SIM and MRM acquisition modes. Limit of detection, 0.5 ppb [Lau *et al.* 2000].

Serum **TLC** Plates: silica gel 60 (20 × 10 cm, 0.2 mm). Solvent system: benzene : methanol : acetic acid (8 : 1 : 1). Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 500 nm). Limit of quantification, 0.1 to 0.5 μg/L [Ruprich, Ostry 1993].

HPLC Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: water : acetonitrile : glacial acetic acid (49.5 : 49.5 : 1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 460 nm). Retention time: 10.9 min. Limit of quantification, 0.05 μg/L [Dinis *et al.* 2007]. Column: Spherisorb C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 9% glacial acetic acid (pH 2.3; 18 : 7), flow rate 1 mL/min. Post-column derivatisation with 25% ammonium hydroxide solution. Fluorescence detection (λ_{ex} = 390 nm, λ_{em} = 440 nm). Retention times: OTA 5 min; relative retention times (relative to OTA) ochratoxin-α 0.71, OTB 0.89, ochratoxin-α methyl ester 0.89, OTA methyl ester 1.32, OTB methyl ester 1.13, OTC 1.50, OTA propyl ester 1.76, OTA butyl ester 2.13. Limit of quantification, 5 ng/kg [Zimmerli, Dick 1995]. Column: Spherisorb C_{18} (150 × 4.6 mm i.d., 3 μm). Mobile phase: methanol : 0.1 mol/L potassium phosphate buffer (pH 7.5; 56 : 44), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 380 nm, λ_{em} = 450 nm). Retention time: OTA 10 min, OTA methyl ester 20 min. Limit of quantification, OTA 0.1 μg/L; limit of detection, OTA 0.05 μg/L [Breitholtz-Emanuelsson *et al.* 1994].

Urine **HPLC** Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: water : acetonitrile : acetic acid (111 : 87 : 2), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 332 nm, λ_{em} = 460 nm). Limit of quantification, 0.05 μg/L; limit of detection, 0.01 μg/L [Vatinno *et al.* 2007].

Milk **HPLC** Column: Lichrosorb C_{18} (250 × 4.0 mm i.d., 10 μm). Mobile phase: 2% acetic acid : acetonitrile : methanol (40 : 35 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 443 nm). Retention times: OTA 7.3 min, aflatoxin M_1 3.1 min. Limit of quantification, OTA, aflatoxin M_1 0.01 μg/L [Navas *et al.* 2005].

Other **GC-MS** White Wine Samples. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 3.7 mL/min. Temperature programme: 150° to 220° at 8°/min for 5 min to 290° at 25°/min. EI ionisation, SIM acquisition mode. Retention time: 14.5 min (silyl derivative). Limit of quantification, 1 μg/L; limit of detection, 0.1 μg/L [Soleas *et al.* 2001]. Food Samples. Column: Supelco PTE-5 fused silica capillary (10 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H, 70 cm/s. Temperature programme: 60° for 2 min to 310° at 25°/min. NICI at 70 eV, MID. Limit of detection, 0.1 ppb (diazomethane derivative) [Jiao *et al.* 1992].

HPLC Rice and Dried Fruit Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (49.5 : 49.5 : 1), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 464 nm). Retention time: approx. 9 min. Limit of quantification, 0.02 μg/kg for rice, 0.03 μg/kg for dried fruits and nuts [Zinedine *et al.* 2007]. Coffee Samples. Column: Nucleosil C_{18} (250 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (pH 3.0; 99 : 99 : 2), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 335 nm, λ_{em} = 475 nm). Limit of detection, 0.8 μg/kg [Fujii *et al.* 2006]. Spice Samples. Column: Lichrospher C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (426 : 564 : 10). Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 460 nm). Retention time: 8.2 min. Limit of detection, 0.2 μg/kg [Fazekas *et al.* 2005]. Rice Samples. Column: NovaPak C_{18} (150 × 3.9 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (57 : 43 : 2), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 440 nm). Limit of detection, 0.5 ppb [Park *et al.* 2005]. Maize Samples. Column: Lichrospher C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: methanol : water : acetic acid (70 : 30 : 2), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 336 nm, λ_{em} = 464 nm). Limit of detection, 0.25 μg/kg [Domijan *et al.* 2005]. White Wine and Grape Juice and Red Wine and Red Grape Juice Samples. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.083 mol/L phosphoric acid : methanol : acetonitrile (1 : 1 : 1 for white wine and white grape juice; 433 : 283 : 283 for red wine and red grape juice), flow rates 0.8 mL/min for white wine and white grape juice; 1.0 mL/min for red wine and red grape juice. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 470 nm). Limit of quantification, 20 ng/L for white wine, 40 ng/L for red wine and red and white grape juice; limit of detection, 4 ng/L for white wine, 8 ng/L for red wine and red and white grape juice [Ng *et al.* 2004]. Cereal-based Products. Column: Inertsil ODS-3 (250 × 4.0 mm i.d., 5 μm). Mobile phase: water : acetonitrile : acetic acid (50 : 49 : 1), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 443 nm). Retention time: ~10 min. Limit of quantification, 0.13 μg/kg; limit of detection, 0.06 μg/kg [Biffi *et al.* 2004]. Dried Vine Fruit Samples. Column: SupelcoSil C_{18} (150 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : water : acetic acid (99 : 99 : 2), flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 440 nm). Retention time: 8.5 min. Limit of quantification, 0.1 μg/kg; limit of detection, 0.03 μg/kg [Lombaert *et al.* 2004]. Cocoa Bean Samples. Column: Tracer Extrasil ODS-2 (250 × 4.0 mm i.d., 5 μm). Mobile phase: methanol : acetonitrile : 5 mmol/L sodium acetate (pH 2.2; 29 : 29 : 42), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 225 nm, λ_{em} = 461 nm). Retention time: 5.7 min. Limit of quantification, 0.1 μg/kg, limit of detection, 0.04 μg/kg [Amezqueta *et al.* 2004]. Wine and Grape Juice Samples. Column: Microsorb C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 9% acetic acid in water (72 : 28), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 335 nm, λ_{em} = 470 nm). Limit of detection, 21 ng/L [Rosa *et al.* 2004]. Ham Samples. Column: Extrasil ODS-2 (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water : acetic acid (99 : 99 : 2), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 460 nm). Limit of detection, 0.04 μg/kg (methyl ester derivative) [Chiavaro *et al.* 2002]. Beer Samples. Column: Hypersil BDS C_{18} (150 × 4.0 mm i.d., 3 μm). Mobile phase: acetonitrile : water : acetic acid (45 : 54 : 1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 332 nm, λ_{em} = 462 nm). Retention time: 8.3 min. Limit of quantification, 10 μg/L, limit of detection, 3 μg/L [Tangni *et al.* 2002]. White Wine, Red Wine and Beer Samples. Column: Nucleosil C_{18} (250 × 4.0 mm i.d., 5 μm). Mobile phase: 3 mmol/L orthophosphoric acid in water (0.2 mL in 1 L, pH 3.1): 3 mmol/L orthophosphoric acid in acetonitrile (0.2 mL in 1 L, pH 3.4; 60 : 40 to 40 : 60 over 5 min for 10 min), flow rate 0.5 mL/min. UV detection (λ = 333 nm). Retention time: 19.2 min (methyl ester derivative). Limit of quantification, 0.1 μg/L; limit of detection, 0.05 μg/L [Soleas *et al.* 2001]. Baby Food samples. Column: C_{18} (250 × 4.7 mm i.d., 5 μm). Mobile phase: 0.011% glacial acetic acid : methanol (40 : 60 to 0 : 100 over 10 min to 40 : 60 over 1 min for 19 min), flow rate 1 mL/min. Post-column derivatisation with 25% ammonium hydroxide, flow rate 0.2 mL/min. Fluorescence detection (λ_{ex} = 390 nm, λ_{em} = 440 nm). Limit of quantification not reported [Burdaspal *et al.* 2001]. Pig-derived Pâté Samples. Column: Spherisorb ODS2 (250 × 3.0 mm i.d., 10 μm). Mobile phase: 5 mmol/L sodium acetate (pH 2.2) : methanol : acetonitrile (62 : 19 : 19), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 225 nm, λ_{em} = 461 nm). Retention time: ~24 min. Limit of quantification, 0.84 μg/kg, limit of detection, 0.56 μg/kg [Jimenez *et al.* 2001]. Grain and Cereal Samples. Column: Lichrospher C_{18} (125 × 4.0 mm i.d., 5 μm). Mobile phase: methanol : water (pH 2.3; 60 : 40). Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 500 nm). Retention time: 12.9 min. Limit of detection, 0.4 μg/L [Vrabcheva *et al.* 2000]. Column: Spherisorb ODS-2 (250 × 3.2 mm i.d.). Mobile phase: 0.1% orthophosphoric acid : acetonitrile (50 : 50), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 333 nm, λ_{em} = 470 nm). Limit of detection, 0.1 μg/kg [Scudamore *et al.* 1999]. Milk and Infant Formulas. Column: Spherisorb ODS-2 (150 × 4.6 mm i.d., 3 μm). Mobile phase: 10 mmol/L tetrabutylammonium bromide in methanol : potassium phosphate buffer (pH 7.5; 51 : 49), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 380 nm, λ_{em} = 450 nm). Limit of detection, 10 ng/L [Skaug 1999].

LC-MS Fungal Cultures. Column: Purospher C_{18} LichroCart 250-3. Mobile phase: methanol : 10 mmol/L ammonium acetate containing 20 μmol/L sodium acetate (20 : 80 for 4 min to 70 : 30 over 4 min for 18 min to 90 : 10 over 1 min for 4 min), flow rate 0.4 mL/min. ESI, positive ion mode. Retention times: OTA 15.4 min; aflatoxins G_2, G_1, B_2, B_1 13.5 min, 13.8 min, 14.2 min, 14.5 min, respectively; citrinine 14.5 min, sterigmatocystin 25.8 min. Limit of quantification, 200 ng [Tuomi *et al.* 2001]. Pig Kidney and Rye Flour. Column: Spherisorb ODS-1 (250 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water : methanol : acetic acid (50 : 30 : 20 : 0.5), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.6 min (methyl ester derivative). Limit of detection, 0.02 μg/kg [Jorgensen, Vahl 1999].

Note For a review of LC-MS methods for the detection of OTA and related substances, see Zollner and Mayer-Helm [2006]; for chromatographic methods for the detection of OTA and related substances, see Valenta [1998]. For an enzyme immunoassay method for the detection of OTA and OTB, see Vrabcheva *et al.* [2000]; for OTA, see Fujii *et al.* [2006] and Kawamura *et al.* [1989]. For the HPLC analysis of OTA and its hydroxyl metabolite in urine, see Castegnaro *et al.* [1990]; for the determination of OTA and other mycotoxins using two-dimensional TLC, see Tapia [1985].

Disposition in the Body Readily absorbed after oral administration from the gastrointestinal tract, especially from the small intestine. Ochratoxins undergo some degree of first-pass metabolism and are extensively bound to serum albumin and other macromolecules, with only 0.02% remaining unbound. OTA crosses the

placental barrier in animal models and is found to be transferred to breast milk in humans. OTA is excreted into bile and urine and is hydrolysed to the non-toxic congener ochratoxin-α (i.e. the isocoumarin moiety lacking the phenylalanine group), which can, in turn, be absorbed; consequently, OTA undergoes enterohepatic circulation. *In vitro* studies in human liver microsomes have shown that OTA is oxidised to 4-OH-OTA epimers by cytochrome P450 enzymes. It has been suggested that OTA is a substrate for the family of organic anion transporter proteins that is found in numerous organ systems including the kidney.

Toxicity OTA was first detected in human serum samples collected in 1977 in Germany and in 1980 in former Yugoslavia. Epidemiological studies have shown that the presence of OTA in blood is associated with Balkan endemic nephropathy and/or urinary system tumours. Several further studies have found significantly higher blood serum or plasma levels of OTA in patients with kidney or urinary disorders compared with healthy people. However, intra-person variations of serum concentrations of OTA over time (as much as 10-fold) have been noted in several countries. In addition to differences seen in epidemiological studies, regional variations have been observed in healthy humans within several countries, which may be explained by differences in diet or climate. OTA is ubiquitous in human blood serum/plasma and indicates continuous exposure to the toxin, mainly from food intake [Scott 2005].

Estimated OTA intake can be calculated by multiplying the plasma concentration (in ng/mL) by a factor of 1.34 to obtain the intake in ng/kg bodyweight. However, the variations described when measuring concentrations in serum/plasma of healthy volunteers contribute to the variability when attempting to relate serum/plasma concentrations to dietary intake [Breitholtz *et al.* 1991]. The EU has set maximum permissible limits for OTA of 5 μg/kg in whole grain and 3 μg/kg for processed products [Scudamore 2005]. The provisional tolerable daily intake (TDI) established by the Joint FAO/WHO Expert Committee on Food Additives is 14 ng/kg bodyweight [JEFCA 1995].

In a study investigating ochratoxin levels in humans and a possible link to nephropathy, serum was collected from healthy individuals and individuals suffering from different kidney disorders and analysed. The mean serum concentrations of OTA were reported as follows:

Group	*Mean OTA (μg/L)*	*Lowest (μg/L)*	*Highest (μg/L)*
Healthy	0.53	0.12	2.0
Transplant recipients	0.47	0.20	1.0
Chronic glomerulonephritis	0.54	0.05–0.1	1.4
Renal calculus or cyst	0.49	0.17	1.0
Chronic renal failure	0.60	0.20	1.4
Patients on dialysis	1.4	0.18	14

[Breitholtz-Emanuelsson *et al.* 1994].

A group of 50 breast-lactating mothers and their infants who were breast-fed for at least 4 months were analysed for OTA concentrations in serum and breast milk. A total of 36 of these volunteers (72%) tested positive for OTA, with serum and breast milk mean concentrations of 4.28 and 1.89 μg/L, respectively. The corresponding OTA serum mean concentration in infants was 1.26 μg/L [Hassan *et al.* 2006].

In another study investigating the presence of OTA in human milk in relation to dietary intake, women with a high dietary intake of liver paste and cakes were more likely to have OTA-contaminated breast milk [Skaug *et al.* 2001].

OTA was detected in 38 out of 92 colostrum samples collected from women in the first 24 h postpartum, with a range of 0.2 to 7.3 μg/L [Kovacs *et al.* 1995].

Half-life Reported as 20 h during the first 6 days and 35.6 days from day 6 onwards.

Clearance Reported as 1.5 mL/kg·h.

Distribution in Blood Serum : blood ratio, 2.0.

Protein Binding >99%, predominantly to serum albumin, with also high affinity binding to macromolecules of lower molecular weight.

Note For proceedings of a workshop on OTA in food, see Supplement 1 to *Food Additives and Contamination*, volume 22 [2005]. For a study on the toxicokinetics of OTA in several species including humans, see Hagelberg *et al.* [1989].

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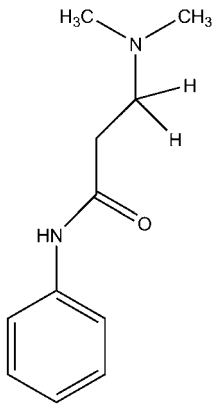
Octacaine

Anaesthetic (Local)

$C_{14}H_{22}N_2O = 234.3$

IUPAC Name 3-(Diethylamino)-*N*-phenylbutanamide

Synonym Diethylaminobutyranilide



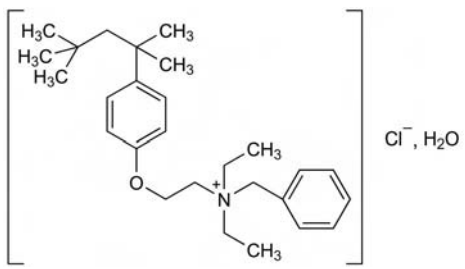
Chemical Properties White crystalline powder. Mp 46° to 47°. Soluble in chloroform, ethanol, ether and benzene. Extracted by chloroform from aqueous alkaline solutions.

Octacaine Hydrochloride
Proprietary Name It is an ingredient of *Risunal*.
Chemical Properties White crystalline powder. Mp 132° to 134°. Soluble in water and ethanol.

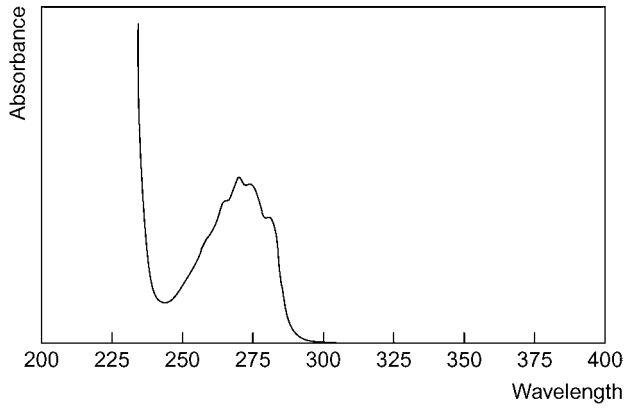
Colour Tests Ammonium molybdate test—faint blue (limit of detection, 1.0 µg); ammonium vanadate test—red (limit of detection, 0.25 µg); Vitali's test—–/–/bright yellow (limit of detection, 0.25 µg).
Thin-layer Chromatography System T1—R_f 0.56 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.13 relative to diphenhydramine, retention time 0.25 relative to codeine.
Ultraviolet Spectrum Octacaine hydrochloride in 0.1 N sulfuric acid, maximum at 240 nm (E1%, 1 cm 430).
Infrared Spectrum Principal peaks at wavenumbers at 1538 or 1587, 1433 or 1656, 755, 1488 cm^{-1} (KBr disk).
Use Octacaine hydrochloride is used as a 5% ointment.

Octafonium Chloride

Cationic Disinfectant
$C_{27}H_{42}$, $ClNOH_2O$ = 450.1
CAS—15687-40-8 (anhydrous)
IUPAC Name (*Z*)-But-2-enedioate; dibutyltin(2+)
Synonyms Benzyldiethyl-{2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethyl} ammonium chloride monohydrate; octaphonium chloride; phenoctide.



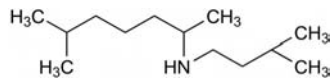
Chemical Properties A white crystalline powder. Soluble 1 in 5 of water; soluble in ethanol and chloroform.
Colour Tests Liebermann's reagent—black; Mandelin's test—violet-brown; Marquis test—grey-brown; sulfuric acid—grey-brown.
Thin-layer Chromatography System TA—R_f 0.15, streaking (Dragendorff spray, positive).
Gas Chromatography System GA—RI 2013.
Ultraviolet Spectrum Aqueous acid—269 (A_1^1=32a), 274, 281 nm; ethanol—263 (A_1^1=25b), 269 (A_1^1=30b), 274 (A_1^1=29b), 282 nm (A_1^1=25b).



Infrared Spectrum Principal peaks at wavenumbers 1240, 840, 760, 1515, 708, 1185 cm^{-1} (Nujol mull).

Octamylamine

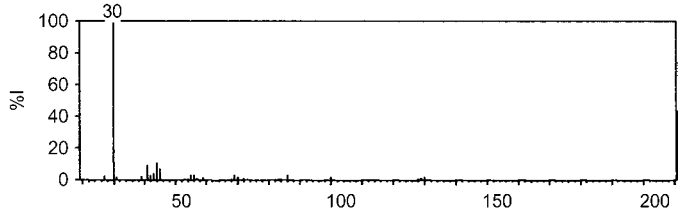
Antispasmodic
$C_{13}H_{29}N$ = 199.4
CAS—502-59-0
IUPAC Name 6-Methyl-*N*-(3-methylbutyl)heptan-2-amine
Synonym *N*-Isopentyl-1,5-dimethylhexylamine; octisamyl.



Chemical Properties An oily liquid. Log *P* (octanol/water), 5.0.

Octamylamine Hydrochloride
$C_{13}H_{29}N$,HCl = 235.8
CAS—5964-56-7
Proprietary Name *Octometine*
Chemical Properties White crystals. Mp 121°. Soluble in water, ethanol and ether.

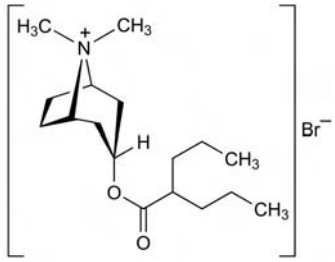
Thin-layer Chromatography System TA—R_f 0.22; system TB—R_f 0.28; system TC—R_f 0.11; system TL—R_f 0.25 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1303.
Mass Spectrum Principal ions at *m/z* 30, 44, 41, 45, 86, 55, 43, 69.



Dose Octamylamine hydrochloride is given in doses of 100 to 200 mg.

Octatropine Methylbromide

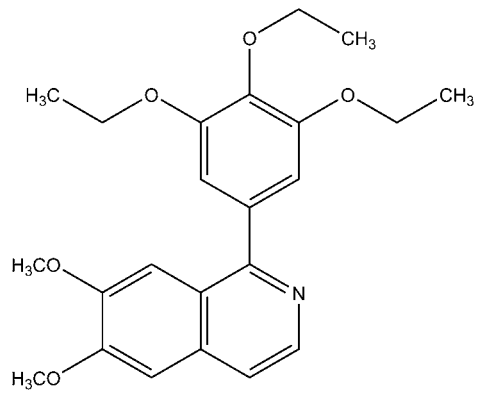
Anticholinergic
$C_{17}H_{32}BrNO_2$ = 362.3
CAS—80-50-2
IUPAC Name [(1*R*,5*S*)-8,8-Dimethyl-8-azoniabicyclo[3.2.1]octan-3-yl] 2-propylpentanoate bromide
Synonyms Anisotropine methobromide; anisotropine methylbromide; *endo*-8,8-dimethyl-3-[(1-oxo-2-propylpentyl)oxy]-8-azoniabicyclo-[3.2.1]octane bromide.
Proprietary Name *Valpin*



Chemical Properties A white, glistening, hygroscopic powder. Mp 329°. Soluble in water; freely soluble in ethanol and chloroform; practically insoluble in ether. Log *P* (octanol/water), 0.6.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 1735, 1155, 1178, 1040, 1237, 1203 cm^{-1} (KBr disk).
Dose 30 to 150 mg daily.

Octaverine

Antispasmodic
$C_{23}H_{27}NO_5$ = 397.5
CAS—549-68-8
IUPAC Name 6,7-Dimethoxy-1-(3,4,5-triethoxyphenyl)isoquinoline
Synonyms Oktaverine; 1-(3,4,5-triethoxyphenyl)-6,7-dimethoxyisoquinoline.



Chemical Properties Free base. Insoluble in water. Octaverine is extracted by organic solvents from aqueous alkaline solutions.

Octaverine Hydrochloride

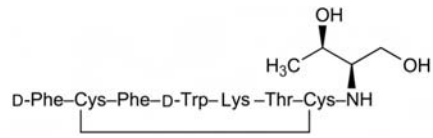
$C_{23}H_{27}NO_5$,HCl = 433.9
CAS—6775-26-4
IUPAC Name 6,7-Dimethoxy-1-(3,4,5-triethoxyphenyl)isoquinoline hydrochloride
Proprietary Name *Gastrolena-Sorbitol*
Chemical Properties Crystals. Mp 199° to 200°. Sparingly soluble in water (1 : 500). Aqueous solutions are acid to litmus [O'Neil *et al.* 2006].

Colour Tests Ammonium molybdate test—green (limit of detection, 0.1 µg); ammonium vanadate test—red (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—green→brown (limit of detection, 0.1 µg); Vitali's test—red-brown/brown/red-orange (limit of detection, 0.1 µg).
Thin-layer Chromatography System T10—R_f 0.75 (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum Ethanol—247, 321, 358 nm.
Disposition in the Body
Therapeutic Concentration Octaverine (94 mg/L) is 4-times as active as papaverine in suppressing spontaneous contractions of the rat uterus [Goldberg, Shapero 1954a]. Octaverine (0.5 to 2.0 mg/kg) is twice as effective as papaverine at causing hypotension and counteracting adrenaline-induced hypertension in rabbits [Goldberg, Shapero 1954a].
Toxicity In mice, the LD_{50} is approximately 200 mg/kg [Goldberg, Shapero 1954b].

Goldberg AA, Shapero M (1954a). A comparative study of the action of octaverine perparine and papaverine on the circulatory and respiratory systems. *J Pharm Pharmacol* 6: 236–245.
Goldberg AA, Shapero M (1954b). A comparative study of the spasmolytic activities of octaverine, perparine and papaverine. *J Pharm Pharmacol* 6: 171–177.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals: Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Octreotide

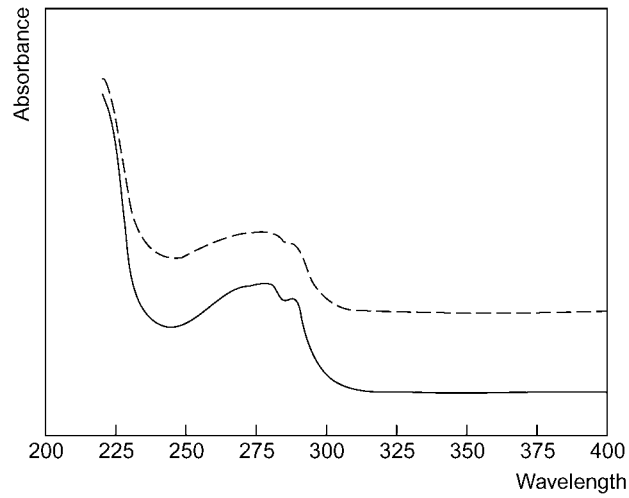
Somatostatin Analogue (Octopeptide)
$C_{49}H_{66}N_{10}O_{10}S_2$ = 1019.3
CAS—83150-76-9
IUPAC Name 10-(4-Aminobutyl)-19-[(2-amino-3-phenylpropanoyl)amino]-16-benzyl-*N*-(1,3-dihydroxybutan-2-yl)-7-(1-hydroxyethyl)-13-(1*H*-indol-3-ylmethyl)-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide
Synonyms D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-*N*-[2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide; SMS-201-995.
Proprietary Name *Longastatin*



Octreotide Acetate

$C_{49}H_{66}N_{10}O_{10}S_2.xC_2H_4O_2$
CAS—79517-01-4
Synonym SMS-201-995
Proprietary Name *Sandostatin*

High Performance Liquid Chromatography Column: Lichrospher-60 RP select B (125 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer (pH 7.4, 35 : 65). UV detection (λ=210 nm). Retention time: 5.5 min [Kyaterekera *et al.* 1999].
Ultraviolet Spectrum Aqueous acid—280, 288 nm (no alkaline shift).



Disposition in the Body Octreotide is rapidly and completely absorbed after SC administration and peak plasma concentrations are reached within 30 min. It undergoes considerable hepatic metabolism, with 30 to 40% of a dose eliminated in this way. Between 11 and 33% of the dose is excreted unchanged in urine and <2% in faeces. It is distributed to body tissues.
Therapeutic Concentration The therapeutic range is 1 to 3 µg/L.
Fourteen acromegalic patients, male and female, aged between 35 and 59 years, were administered with 100 µg of octreotide SC. Peak plasma concentrations of 3.5 µg/L were reached after 27.4 min [Nicholls *et al.* 1990].
Eight healthy male volunteers were administered with IV doses of 25, 50, 100 and 200 µg octreotide. Peak plasma concentrations of 5.7, 9.6, 24.2 and 27.8 µg/L, respectively, were reached within 3.7, 4.1, 3.4 and 7.3 min. Subcutaneous doses were also administered at doses of 50, 100, 200 and 400 µg to the same individuals. Peak levels were observed to be 2.4, 4.4, 10.6 and 23.5 µg/L at 26, 26, 35 and 28 min, respectively [Kutz *et al.* 1986].
Toxicity It has been reported that one patient was accidentally overdosed with Sandostatin at a dose of 250 µg/h for 48 h instead of 25 µg/h, and no side-effects were experienced [Datapharm Communications Ltd 2002]
Half-life 1.5 h (prolonged in the elderly and those with renal failure).
Volume of Distribution 0.27 L/kg; also reported as 13.6 L.
Clearance Total body clearance, 9.6 L/h which may be increased to 18 L/h in patients with acromegaly and reduced to 4.5 L/h in those with chronic renal failure.
Protein Binding 65% (mainly to liprotein and to a lesser extent, albumin).
Distribution in Blood The drug is distributed mainly to the plasma component of blood. The amount of drug bound to blood cells is negligible.
Dose The initial dose is 50 µg once or twice daily which is gradually increased to 200 µg three times daily, if necessary.

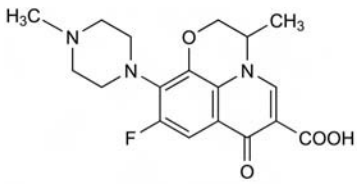
Datapharm Communications Ltd (2002). Sandostatin Ampoules and Multidose Vial in: *Medicines Compendium*. Epsom, Surrey: Virtual Health Network and Pharmaceutical Press: 1762–1763.
Kutz K *et al.* (1986). Pharmacokinetics of SMS 201-995 in healthy subjects. *Scand J Gastroenterol Suppl* 119: 65–72.
Kyaterekera N *et al.* (1999). LC determination of octreotide acetate in compound formulations of Sandostatin and diamorphine hydrochloride. *J Pharm Biomed Anal* 21: 327–330.
Nicholls J *et al.* (1990). Pharmacokinetics of the long-acting somatostatin analogue octreotide (SMS 201-995) in acromegaly. *Clin Endocrinol (Oxf)* 32: 545–550.

Ofloxacin

Quinolone Antibiotic, Antibacterial
$C_{18}H_{20}FN_3O_4 = 361.4$
CAS—82419-36-1; 83380-47-6

Synonyms DL-8280; 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid; HOE-280; ofloxacine.

Proprietary Names *Apo-Oflox*; *Bactoflox*; *Bioquil*; *Exocin*; *Floxal*; *Floxstat*; *Inoflox*; *Konovid*; *Megasin*; *Monoflocet*; *Occidal*; *Oflovir*; *O-Flox*; *Ofloxcin*; *Qinolon*; *Seracin*; *Tarivid*; *Trafloxal*.



Chemical Properties Colourless needles from ethanol. Mp 250° to 257°.

Levofloxacin (Ofloxacin *S*-(-)-form)

$C_{18}H_{20}FN_3O_4 = 361.4$
CAS—100986-85-4

Synonyms DR-3355; HR-355; RWJ-25213; *S*-(−)-ofloxacin.

Proprietary Names *Cravit*; *Quixin*; *Tavanic*.

Chemical Properties Hemihydrate needles from ethanol/ethyl ether. Mp 225° to 227°.

Ofloxacin Hydrochloride

Proprietary Name *Tarivid intravenous infusion*

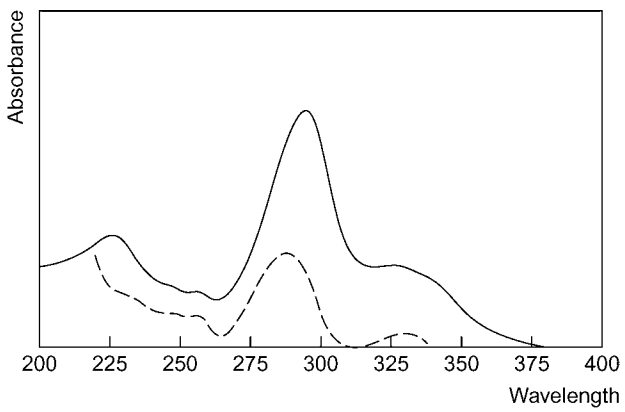
Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00.

Plate: silica F_{254} (10 × 10 cm). Mobile phase: chloroform : methanol : triethylamine (3 : 1 : 1). Diastereometric L-leucinamide derivative of (−)-ofloxacin, R_f 0.57; (+)-ofloxacin derivative, 0.49 [Lehr, Damm 1988].

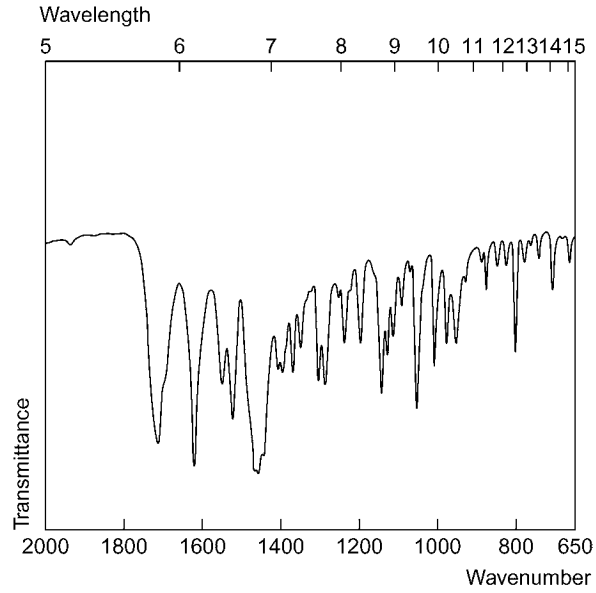
High Performance Liquid Chromatography System HX—RI 314; system HY—RI 260; system HZ—RRT 2.0 min; system HAA—retention time 8.6 min.

Column: ODS.A YMC (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L phosphate buffer (pH 2.6) : acetonitrile (82 : 18), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}=290 nm, λ_{em}=460 nm). Retention time: 7 min [Immanuel *et al.* 2001].

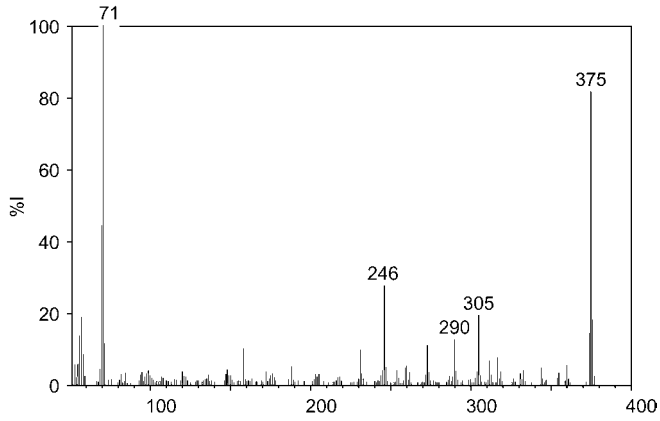
Ultraviolet Spectrum Aqueous acid —225, 226, 256, 326 nm; basic—288, 332 nm.



Infrared Spectrum Principal peaks at wavenumber 1459, 1621, 1713, 1086 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 71, 375, 70, 246, 305, 290 (ofloxacin-Me).



Quantification

***Plasma* HPLC** Limit of detection, 0.01 mg/L [Knoller *et al.* 1998]. Column: ET Resolvosil-BSA-7 (150 × 4 mm i. d.). Mobile phase: 0.2 mol/L phosphate buffer (pH 8) : propan-2-ol (97 : 3), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=298 nm, λ_{em}=458 nm). Retention time: (−)-ofloxacin, 5.2 min; (+)-ofloxacin, 7.5 min. Limit of detection, 0.003 mg/L [Lehr, Damm 1988].

***Serum* HPLC** Fluorescence detection (λ_{ex}=295 nm, λ_{em}=490 nm). Limit of detection, 10 µg/mL [Bottcher *et al.* 2001]. See Plasma. [Knoller *et al.* 1998].

***Urine* HPLC** See Plasma. [Knoller *et al.* 1998]. See Plasma. Limit of detection, 0.08 mg/L [Lehr, Damm 1988].

***Tissue* HPLC** See Serum [Bottcher *et al.* 2001].

Disposition in the Body Ofloxacin is absorbed rapidly after oral administration. The rate of absorption may be affected by food but not the extent. It is widely distributed in body fluids, including CSF, and tissue penetration is good. It also crosses the placenta and is distributed in breast milk. A small amount of the dose is metabolised to desmethyl ofloxacin (moderate activity) and *N*-oxide ofloxacin. Excretion is mainly via the kidneys, with about 80% of the dose being excreted as the unchanged drug in urine over 24 to 48 h and between 4 to 8% being excreted in faeces.

Bioavailability Approximately 100% after oral administration.

Therapeutic Concentration The serum therapeutic concentration range is 1.0 to 4.0 mg/L; trough, 0.05 to 5.0 mg/L and peak, 1.0 to 7.0 mg/L.

Half-life 5 to 8 h; renal impairment 15 to 60 h.

Protein Binding 25%.

Dose The dose varies between 200 and 400 mg daily, and may also be increased to 400 mg twice daily if necessary.

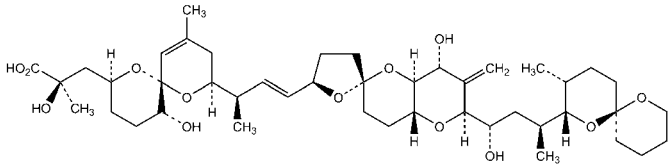
Bottcher S *et al.* (2001). An HPLC assay and a microbiological assay to determine levofloxacin in soft tissue, bone, bile and serum. *J Pharm Biomed Anal* 25: 197–203.

Immanuel C *et al.* (2001). Simple and rapid high-performance liquid chromatography method for the determination of ofloxacin concentrations in plasma and urine. *J Chromatogr B Biomed Sci Appl* 760: 91–95.
Knoller J *et al.* (1998). *J Chromatogr Biomed Sci Appl* 427: 257–267.
Lehr KH, Damm P (1988). Quantification of the enantiomers of ofloxacin in biological fluids by high-performance liquid chromatography. *J Chromatogr* 425: 153–161.

Okadaic Acid

Halochondrine, Protein Phosphatase Inhibitor, Phycotoxin
$C_{44}H_{68}O_{13}$ = 805.0
CAS—78111-17-8

IUPAC Name 9,10-Deepithio-9,10-didehydroacanthifolicin
Synonym Halochondrine A



Chemical Properties Crystals from dichloromethane/hexane. Mp 171° to 175°. Also reported as crystals from benzene–trichloromethane. Mp 164° to 166° [O'Neil *et al.* 2006]. Soluble in dimethyl sulfoxide, ethanol, methanol. First isolated from the black sponge *Holichondria okadaii* but essentially originates from 10 planktonic species of dinoflagellate belonging to the genera *Dinophysis* and *Phalacroma* and 6 benthic species of the genus *Prorocentrum;* it accumulates in bivalve shellfish and other marine life to cause diarrhetic shellfish poisoning (DSP) in consumers of contaminated seafood. More than 20 analogues of okadaic acid have been isolated, including a range of 7-*O*-acetylated derivatives (DTX-3 family), which may be produced in the shellfish by metabolism of the toxins. Other major analogues include 35-methylokadaic acid (known as dinophysistoxin, DTX-1 family) or an isomer of okadaic acid (DTX-2 family) [Holmes, Teo 2002]. DTX-3 originally described a group of DSP toxin derivatives in which saturated or unsaturated fatty acyl groups are attached to the 7-OH group of DTX-1. However, it has been shown that any of the parent toxins, okadaic acid, DTX-1 and DTX-2, can be acylated with a range of saturated and unsaturated fatty acids from C_{14} to C_{18} [Hallegraeff 1995; Wright 1995]. Ultraviolet exposure to an okadaic acid standard derivatised with anthryldiazomethane for 30 min prior to HPLC analysis resulted in a complete disappearance of the anthrylmethylokadaic acid peak. Peak area was inversely proportional to UV exposure time [Rawn *et al.* 2005].

Quantification

Other **HPLC** Shellfish Samples. Column: Nucleosil C_{18} (150 × 4.0 mm i.d., 5 µm) or Hypersil C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20 for Nucleosil, 85 : 15 for Hypersil), flow rate 1.1 mL/min. Fluorescence detection (λ_{ex} = 356 nm, λ_{em} = 440 nm). Limit of detection, 0.1 mg/kg hepatopancreas (Nucleosil column), 0.05 mg/kg hepatopancreas (Hypersil column; 1-bromoacetylpyrene derivative) [Gonzalez *et al.* 1998; 2000]. Phytoplankton Samples (*Dinophysis acuta*). Column: C_{18} (250 × 3.2 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (80 : 5 : 15), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 412 nm). Retention time: okadaic acid 22.2 min, DTX-2 25.2 min, DTX-2B and DTX-2C co-elute at 27.4 min, DTX-1 35.7 min. Limit of detection, okadaic acid 5 µg/L [Draisci *et al.* 1998a]. Shellfish Samples. Column: C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (82 : 18), flow rate 1.1 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 412 nm). Limit of quantification, okadaic acid 1 mg/kg hepatopancreas, DTX-1 0.5 mg/kg hepatopancreas (9-anthrylmethyl derivative) [Aase, Rogstad 1997]. Mussel Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (75 : 25), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 412 nm). Retention times: okadaic acid approx. 19.0 min, DTX-1 approx. 38 min (tetramethylammonium hydroxide derivatives). Limit of quantification, okadaic acid and DTX-1 70 µg/kg hepatopancreas, okadaic acid and DTX-1 15 µg/kg whole tissue [Lawrence *et al.* 1996]. Shellfish Samples. Column: C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20), 1.0 mL/min. Fluorescence detection (λ_{ex} = 254 nm, λ_{em} = 412 nm). Limit of quantification, okadaic acid, DTX-1, DTX-2, DTX-3, 7-*O*-acetylokadaic acid 0.1 mg/kg (9-anthrylmethyl derivatives) [Quilliam 1995]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (75 : 10 : 15), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 412 nm). Limit of detection, 0.05 mg/kg (9-anthrylmethyl derivative) [Lee *et al.* 1987].

LC-MS Cell Extracts. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 2 mmol/L ammonium acetate (pH 5.8) : methanol (30 : 70 for 8 min to 20 : 80 over 22 min for 15 min), flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Retention time: okadaic acid 5.4 min, methyl okadaate 15.7 min, norokadanone 14.9 min, 2-hydroxymethylallyl okadaate 12.4 min, 5-hydroxy-2-methylene-pent-3-enyl okadaate 17.2 min, 7-hydroxy-2,4-dimethyl-hepta-2,4-dienyl okadaate 24.2 min, 7-hydroxy-4-methyl-2-methylene-hept-4-enyl okadaate 25.8 min, 5,7-dihydroxy-2,4-dimethylene-heptyl okadaate 15.0 min, 5-hydroperoxy-7-hydroxy-2,4-dimethylene-heptyl okadaate 15.1 min, 4-formyl-2-methylene-pent-4-enyl okadaate (DTX-6) 13.1 min, 7-hydroxy-2-methyl-hepta-2,4-dienyl okadaate 20.5 min, 7-hydroxymethyl-2-methylene-octa-4,7-dienyl okadaate 28.1 min. Limit of quantification not reported [Paz *et al.* 2007]. Phytoplankton Samples. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 1.0 mmol/L aqueous ammonium acetate : 1.0 mmol/L ammonium acetate in acetonitrile (60 : 40 to 55 : 45 over 0.5 min, to 25 : 75 over 0.1 min, held 5.49 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention times: okadaic acid 2.8 min, DTX-2 3.5 min, DTX-1 6.6 min. Limit of detection, 0.48 µg/kg [Fernandez Puente *et al.* 2004]. Shellfish Samples. Column: C_{18} (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile-water (1 : 9) : acetonitrile-water (9 : 1) : 33 mmol/L ammonium hydroxide–500 mmol/L formic acid in water (85 : 5 : 10 for 2 min to 5 : 85 : 10 over 11 min for 12 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention times: okadaic acid 15.2 min, DTX-1 17.9 min, DTX-2 15.8 min. Limit of detection, 0.01 mg/kg [McNabb *et al.* 2005]. Column: C_8 (50 × 2.1 mm i.d., 3 µm). Mobile phase: 2 mmol/L ammonium formate : 50 mmol/L formic acid in acetonitrile (50 : 50), flow rate 0.25 mL/min. ESI, negative ion mode, SIM acquisition mode. Limit of detection, 41 µg/kg in mussels, 13 µg/kg in cockles, 26 µg/kg in oysters and scallops [Stobo *et al.* 2005]. Mussel and Clam Samples. Column: C_{18} (250 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : water (85 : 15) with 0.1% formic acid, flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: okadaic acid 5.1 min, DTX-1 5.5 min. Limit of detection, 5 µg/kg [Jorgensen *et al.* 2005]. Phytoplankton. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L aqueous trifluoroacetic acid-0.01% heptafluorobutyric acid (5 : 95 to 40 : 60 over 12 min to 70 : 30 over 5 min for 10 min), flow rate 0.7 mL/min. ESI, positive ion mode, MID. Retention times: okadaic acid ~24 min, DTX-1 ~29 min. Limit of detection, okadaic acid, DTX-1 1 ng on-column [Dahlmann *et al.* 2003]. Column: C_{18} (125 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05% acetic acid (65 : 35), flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Limit of detection, 0.01 µg/g digestive gland, 0.25 µg/100 g edible parts [Vale, Sampayo 2002]. Scallop Hepatopancreas. Column: Inertsil ODS-2 (150 × 2.1 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium acetate : methanol (60 : 40 to to 0 : 100 over 20 min for 10 min), flow rate 0.2 mL/min. Sonic spray ionisation, negative ion mode. Retention times: okadaic acid 17.0 min, DTX-1 18.7 min. Limit of detection, okadaic acid, DTX-1 3 µg/L [Ito, Tsukada 2002]. Scallop Samples. Columns: (A) Symmetry C_{18} (150 × 2.1 mm i.d., 3.5 µm) for okadaic acid, DTX-1, pectenotoxins 6 and 2SA; (B) Capcellpak C_{18} (250 × 1.5 mm i.d., 5 µm) for palmitoyl derivatives of okadaic acid and DTX-1. Mobile phases: acetonitrile : 0.05% acetic acid (7 : 3) for column A; methanol : 2.5% acetic acid (98 : 2) for column B; flow rate 0.1 mL/min. ESI, negative ion mode, SIM acquisition mode. Limit of detection, , 5 and 10 g/kg for okadaic acid, 10 and 20 µg/kg for DTX-1, 20 and 40 µg/kg for okadaic acid palmitoyl derivative, 20 and 40 µg/kg for DTX-1 palmitoyl derivative, in muscle and digestive glands, respectively [Goto *et al.* 2001]. Bivalve Molluscs. Column: C_{18} (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (7 : 3) with 0.1% acetic acid, flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Retention times: okadaic acid ~5.0 min, DTX-1 ~7.8 min (post-hydrolysis). Limit of detection, 5 ng/kg in the midgut gland [Suzuki, Yasumoto 2000]. Mussel Hepatopancreas Samples and Phytoplankton Samples. Column: C_{18} (300 × 1.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20) with 0.1% trifluroacetic acid, flow rate 0.04 mL/min. API, positive ion mode, SIM acquisition mode. Retention times: okadaic acid 9.3 min, DTX-1 10.2 min, DTX-2 9.7 min, DTX-2B 9.9 min, DTX-2C 10.3 min, pectenotoxin-2 9.8 min, pectenotoxin-2SA 8.8 min, 7-*epi*-pectenotoxin-2SA 9.4 min, AC1 8.2 min. Limit of quantification not reported [Draisci *et al.* 1999]. Mussel Samples. Column: C_{18} (50 × 1.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (1 : 1) with 1 mmol/L ammonium acetate, flow rate 0.05 mL/min. ESI, negative ion mode, SRM acquisition mode. Retention time: 1.5 min. Limit of detection, 1 ng/g digestive tissue, 0.2 ng/g edible tissue [Holmes *et al.* 1999]. Phytoplankton Samples (*D. acuta*). Column: Vydac 218TP51 (250 × 1.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (60 : 40) with 0.1% trifluoroacetic acid, flow rate 0.04 mL/min. API, positive ion mode, SIM acquisition mode. Retention times: okadaic acid 6.1 min, DTX-2 6.6 min, DTX-2B 7.5 min, DTX-2C 8.3 min, DTX-1 8.5 min. Limit of quantification not reported [Draisci *et al.* 1998b]. Mussel Samples. Column: C_{18} (300 × 1.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20) with 0.1% trifluoroacetic acid, flow rate 0.01 mL/min. API, positive ion mode, SRM acquisition mode. Limit of quantification, 0.2 µg/g for okadaic acid, DTX-1, DTX-2, DTX-2B [Draisci *et al.* 1995]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (90 : 10) with 0.1% trifluoroacetic acid, flow rate 0.8 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times: okadaic acid ~5.0 min, DTX-1 ~6.0 min. Limit of detection, 0.1 mg/L, equivalent to 0.2 µg/g hepatopancreas [Draisci *et al.* 1995].

CE Mussel Samples. Column: uncoated fused-silica capillary (total/effective length: 64.5/56 cm, 50 µm i.d.) Buffers: 12.5 mmol/L borate buffer (pH 9.2, CZE), with 20 mmol/L SDS (MEKC). UV detection (λ = 200 nm). Migration time: CZE 4.2 min, MEKC 4.2 min. Limit of quantification, 10 ng/g whole mussel tissue [Bouaicha *et al.* 1997]. Shellfish Samples. Column: uncoated fused-silica capillary (total/effective length: 37.5/28.5 cm, 50 µm i.d.). Buffer: 12.5 mmol/L borate buffer (pH 9.2) : 40 mmol/L SDS, 10% methanol. UV detection (λ = 200 nm). Migration times: okadaic acid 4.7 min, DTX-1 5.7 min. Limit of quantification, 6.25 mg/L [Consoli, Damerval 2001].

Note Okadaic acid is often measured by mouse bioassay. The mouse bioassay defines the mouse unit (MU) as the minimum amount of toxin needed to kill a 20 g mouse within 24 h: 1 MU is equivalent to 4 to 5 µg okadaic acid [Vieites *et al.* 1996]. For studies comparing HPLC-fluorimetric detection methods using different derivatisation agents, see James *et al.* [1999] and Mak *et al.* [2005]. For a study of

detailed profiles of 7-*O*-acyl esters of okadaic acid and DTX-2 in plankton and shellfish, see Vale [2006]. For the detection of okadaic acid by the protein phosphatase 2A inhibition assay, see Tubaro *et al.* [1996]. For an ultra-performance liquid chromatography–tandem mass spectrometry method for screening lipophilic marine toxins, including okadaic acid derivatives, pectenotoxin derivatives and yessotoxin derivatives, see Fux *et al.* [2007].

Disposition in the Body

Toxicity The regulatory limit set by the European Commission for okadaic acid in shellfish is 16 µg/100 g shellfish tissue. This also applies to analogues of okadaic acid.

Okadaic acid is a tumour promoter, an inducer of lipid peroxidation, and an inducer of cytotoxicity and apoptosis in cultured mammalian cells. At low concentrations, it significantly increases DNA methylation; thus it may interfere with gene regulation and expression and cellular proliferation and participate in epigenetically induced tumours by a gap junction intracellular communication inhibition mechanism [Matias, Creppy 1998].

Note For a study investigating okadaic acid placental transfer in pregnant mice, see Matias and Creppy [1996].

Dose Used as a biochemical tool as tumour promoter and probe of cellular regulation.

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Olanzapine

Antipsychotic, Benzodiazepine

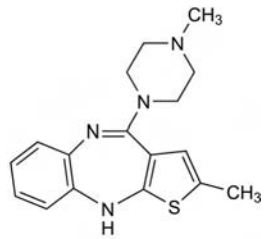
$C_{17}H_2ON_4S$ = 312.4

CAS—132539-06-1

IUPAC Name 2-Methyl-4-(4-methylpiperazin-1-yl)-5*H*-thieno[3,2-*c*][1,5]benzodiazepine

Synonyms LY-170053; 2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine.

Proprietary Name *Zyprexa*.



Chemical Properties A yellow crystalline solid. Mp 195°. It is practically insoluble in water. Slightly soluble in dehydrated alcohol and in methanol; soluble in propyl alcohol; sparingly soluble in acetonitrile. pK_{a1}, 5.0; pK_{a2}, 7.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stock standard solutions were stable for 35 days in propan-2-ol and stored at -20°, also stable at -70° in human plasma and serum for at least 59 and 16 days, respectively [Berna *et al.* 1998].

Olanzapine Embonate

$C_{23}H_{16}O_6$,$C_{17}H_{20}N_4S$,H_2O = 718.8

CAS—221373-18-8

IUPAC Name 4-[(3-Carboxy-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxynaphthalene-2-carboxylic acid; 2-methyl-4-(4-methylpiperazin-1-yl)-5*H*-thieno[3,2*c*][1,5]benzodiazepine; hydrate

Synonym Olanzapine pamoate

Proprietary Name *Zypadhera*

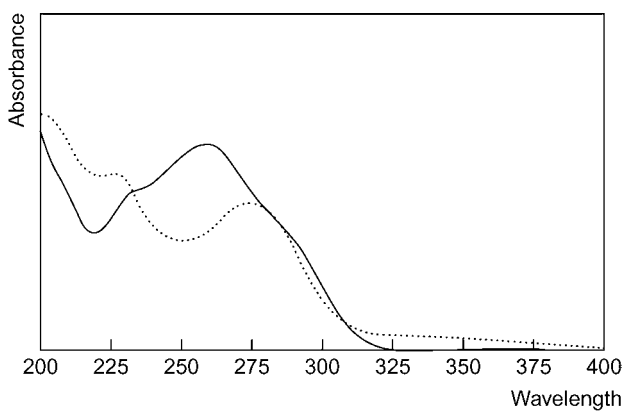
Gas Chromatography System GB—RI 2861, M (nor-) RI 2911; system GT—RI 2780.

Column: 5% phenyl silicone, 95% methyl silicone (HP 5MS; 15 m × 0.25 mm i.d., 0.25 mm). Temperature programme: 60° for 1 min to 300° at 20°/min for 3 min. Carrier gas: He, 1.0 mL/min. IS ethylmorphine. NPD. Retention time: 12.1 min for olanzapine, 10.8 min for IS [Elian 1998].

High Performance Liquid Chromatography System HZ—RT 1.8 min.

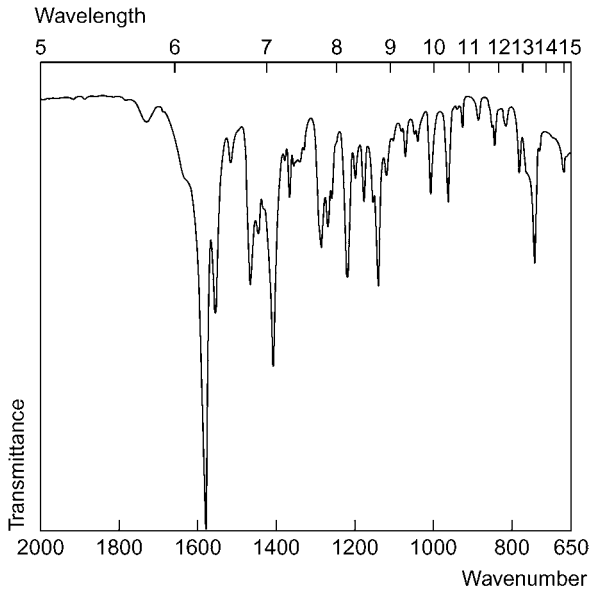
Column: YMC basic (150 × 4.6 mm i.d., 5 µm). Mobile phase: 75 mmol/L sodium phosphate (pH 7) : methanol : acetonitrile (48 : 26 : 26), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 7.0 min [Catlow *et al.* 1995].

Ultraviolet Spectrum

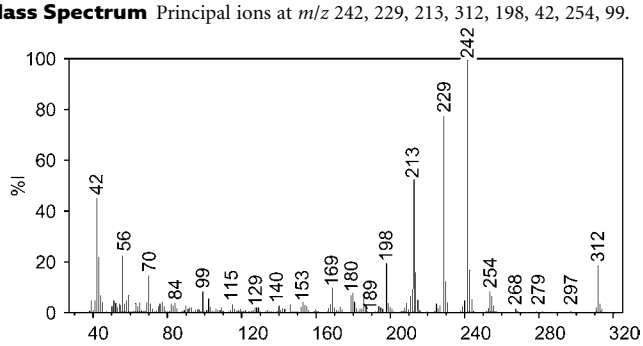


O

Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 242, 229, 213, 312, 198, 42, 254, 99.



Quantification

Blood **GC** Column: Cross-linked fused silica (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Retention time: 9.9 min. Limit of quantification, 218 µg/L, limit of detection, 66 µg/L [Sánchez de la Torre *et al.* 2005]. NPD. Limit of detection, 0.05 mg/L [Merrick *et al.* 2001].

GC-MS Columns (dual): Rtx-50, 50% phenylpolysiloxane, 50% methylpolysiloxane capillary column (30 m × 0.32 mm i.d., 0.25 µm); Rtx-200, cross-bond trifluropropylmethylpolysiloxane capillary column (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 130° for 3 min, to 270° at 10°/min for 10 min to 295° at 10°/min for 5 min. Carrier gas: He, flow rate 3 mL/min. Internal standard: promazine. NPD. Retention time: 20.3 min for olanzapine and 15.1 min for IS (Rtx-200), 20.0 min for olanzapine and 15.8 min for IS (Rtx-50). Limit of quantification, 100 µg/L, limit of detection, 50 µg/L [Jenkins *et al.* 1998].

HPLC Column: Microsorb CN (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : 50 mmol/L sodium phosphate buffer (pH 6.5, 5 : 28 : 67), flow rate 1.5 mL/min. IS clozapine. Retention time: 9.6 min for olanzapine and 12.5 min for IS. Limit of detection, 1 µg/L [Prieto, Hoffman 1997].

LC-MS Column: reversed phase Zorbax Extend-C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium hydroxide (pH 9.0)-acetonitrile (95 : 5) : acetonitrile (97 : 3 to 20 : 80 at 10 min for 0.5 min to 97 : 3), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.005 mg/kg, limit of detection, 0.001 mg/kg [Nielsen, Johansen 2009]. Column: MetaChem Monochrom (150 × 4.6 mm i.d., 5 µm). APCI, MRM acquisition mode, positive ion mode [Berna *et al.* 2002].

Plasma **GC** Column: HP-5 fused silica (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 290° to 296° at 1°/min for 0.5 min. NPD. Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Ulrich 2005].

HPLC Electrochemical detection [Kobylinska *et al.* 2008]. Column: reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L phosphate buffer (pH 3.5, 27 : 73). Electrochemical detection. Olanzapine and its metabolite, *N*-desmethylolanzapine [Saracino *et al.* 2007]. Column: Spherisorb S5 C_6 (250 × 4.6 mm i.d.). Mobile phase: water : acetonitrile (55 : 45) containing 0.009 mol/L heptanosulfonic acid and 0.06 mol/L monobasic potassium phosphate (pH 2.7). UV detection (λ = 254 nm). Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [D'Arrigo *et al.* 2006]. Column: C(8). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 3.8). UV detection (λ = 260, 280 and 240 nm). Limit of quantification, 5 µg/L for olanzapine and other atypical antipsychotics [Titier *et al.* 2003]. Column: RP Select B (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.25% hydrogen phosphate-0.05% TEA (14 : 86), flow rate 1.0 mL/min. UV detection (λ = 270 nm). Retention time: 5.4 min. Limit of quantification, 1.5 µg/L, limit of detection, 1 µg/L [Dusci *et al.* 2002]. Column: reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15.4 mmol/L phosphate buffer (pH 3.8) containing 19.7 mmol/L TEA (20 : 80), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 6.1 min. Limit of quantification, 3 µg/L, limit of detection, 1 µg/L for olanzapine and desmethylolanzapine [Raggi *et al.* 2001]. See Urine [Boulton *et al.* 2001]. See also Aravagiri *et al.* [1997], Catlow *et al.* [1995] and Xiao *et al.* [2001].

LC-MS Column: Luna C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L ammonium acetate buffer (70 : 30) with 0.1% formic acid, flow rate 0.5 mL/min. TIS. Limit of quantification, 0.167 µg/L [Elshafeey *et al.* 2009]. Column: Inertsil reversed phase C_{18} ODS (100 × 3.0 mm i.d., 3.0 µm). Mobile phase: 10 mmol/L ammonium acetate buffer : acetonitrile (10 : 90), flow rate 0.8 mL/min. TIS, positive ion mode, MRM acquisition mode. Limit of quantification, 100 ng/L [Nirogi *et al.* 2006]. Column: Macherey-Nagel C_{18} (125 × 2 mm i.d., 3 µm). Mobile phase: 2.7 mmol/L formic acid-10 mmol/L ammonium acetate : acetonitrile (53 : 47), flow rate 0.16 mL/min. ESI, SIR acquisition mode. Limit of quantification, 1 µg/L [Zhou *et al.* 2004]. Column: Merck LiChroCART with Superspher 60 RP Select B stationary phase. Mobile phase: aqueous ammonium formate and acetonitrile. APCI [Kratzsch *et al.* 2003]. Column: Symmetry C_{18} (150 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (20 : 80), flow rate 0.5 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [Kollroser, Schober 2002]. Column: MetaChem (150 × 4.6 mm i.d., 5 µm). Mobile phase: 100 mmol/L ammonium acetate : 20% propan-1-ol (80 : 20 for 0.1 min to 55 : 45 at 0.2 min to 30 : 70 at 5 min to 15 : 85 at 6 min to 80 : 20 at 6.1 min until 9 min), flow rate 1.0 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of quantification, 250 ng/L [Berna *et al.* 1998].

Serum **HPLC** Column: ODS Hypersil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : tetramethylethylenediamine (pH 6.5, 8 : 92 : 0 to 37.5 : 62.1 : 0.4 at 5 min to 8 : 92 : 0 at 15 min for 10 min), flow rate 1.3 mL/min. UV detection (λ = 254 nm). Retention time: 9.16 min. Limit of quantification, 10 µg/L for olanzapine, its metabolites and other antipsychotic drugs [Sachse *et al.* 2006]. Column: Spherisorb S5W (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L ammonium acetate buffer (pH 9.9, 85 : 15), flow rate 1.1 mL/min. UV detection (λ = 257 nm). Relative retention time: 0.86 min. Limit of detection, 5 nmol/L [Olesen *et al.* 2001]. Column: Hypersil C_{18} ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : tetramethylethylenediamine (pH 6.5, 37 : 62.6 : 0.4), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 4.46 min. Limit of quantification, 10–20 µg/L for olanzapine and its demethylated metabolites [Weigmann *et al.* 2001]. Column: Spherisorb S5W (150 × 4.6 mm i.d.). Mobile phase: 50 mmol/L ammonium acetate buffer (pH 9.9) : methanol (15 : 85), flow rate 1.1 mL/min. UV detection (λ = 270 nm). Retention time: 3.58 min. Limit of quantification, 1.56 µg/L [Olesen, Linnet 1998].

LC-MS Column: monolithic C_{18} (50 × 4.6 mm). Mobile phase: methanol : 5 mmol/L acetate buffer (pH 3.9). Olanzapine and other antipsychotics and antidepressants [Kirchherr, Kühn-Velten 2006]. Column: Superspher RP-18 (125 × 3 mm i.d., 4 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium formate buffer (pH 3.0, 25 : 75), flow rate 0.3 mL/min. APCI, positive ion mode, SIM acquisition mode. Limit of quantification, 1 µg/L [Bogusz *et al.* 1999]. Column: Phenomenex Luna phenyl hexyl (150 × 2 mm i.d., 5 µm). Mobile phases: acetonitrile-20 mol/L ammonium acetate (52 : 48) : formic acid-acetonitrile (0.1 : 100, 98 : 2), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection not reported [Chin *et al.* 2004]. See Plasma [Berna *et al.* 1998].

Urine **GC** See Blood [Merrick *et al.* 2001].

GC-MS Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

HPLC Column: Supelcosil LC-CN (250 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : 50 mmol/L phosphate buffer (pH 6.0, 10 : 25 : 65), flow rate 1.0 mL/min. UV detection (λ = 214 nm). Limit of quantification, 1 µg/L, limit of detection, 0.75 µg/L [Boulton *et al.* 2001].

Bile **GC-MS** See Blood [Jenkins *et al.* 1998]

CSF **GC** See Blood [Merrick *et al.* 2001].

Stomach Contents **GC** See Blood [Merrick *et al.* 2001].

GC-MS See Blood [Jenkins *et al.* 1998].

Brain **GC** See Blood [Merrick *et al.* 2001].

Liver **GC** See Blood [Merrick *et al.* 2001].

Disposition in the Body

Olanzapine is well absorbed from the gastrointestinal tract and is eliminated extensively by first-pass metabolism, mainly by conjugative and oxidative pathways. Approximately 40% of the dose is metabolised before reaching systemic circulation. Food does not affect the rate or extent of absorption. It is extensively distributed and metabolised throughout the body: 7% of the dose is recovered in urine as the unchanged drug, indicating that it is highly metabolised. Approximately 57% of the dose is recovered in urine and 30% in faeces as metabolites. The main metabolites include the 10-*N*-glucuronide, *N*-desmethyl, 2-hydroxymethyl, 2-carboxy, *N*-desmethyl-2-carboxy and 4′-*N*-oxide derivatives of olanzapine; these lack pharmacological activity. Renal impairment has little effect on the pharmacokinetics.

Therapeutic Concentration The serum therapeutic concentration is 0.01–0.05 mg/L.

Fifteen elderly healthy individuals and 5 young were administered with 10 mg olanzapine. Peak concentrations were 9.23 µg/L at 8 h and 12.6 µg/L at 6.1 h, respectively [Calleghen et al 1995].

Six men were fasted overnight before receiving 15 mg olanzapine. Concentrations reached 13.3 μg/L, observed at 5.8 h. In comparison, 6 men administered the drug in a fed state produced levels of 13.8 μg/L approximately 4.7 h after dosing [Henry *et al.* 1995].

Thirty male non-smokers and 19 male smokers were administered with 9.5 mg and 10.5 mg olanzapine, respectively. Peak concentrations of 12.9 μg/L were observed at 6.1 h for the non-smokers and of 13.2 μg/L at 5.5 h for the smokers [Kisicki *et al.* 1995].

Olanzapine 5 mg was given to three groups of patients: 6 patients with hepatic impairment (creatinine clearance, >5.4 L/h/1.73 m^2), 6 patients with creatinine clearance <0.6 L/h/1.73 m^2 (drug given after haemodialysis) and 6 patients with creatinine clearance <0.6 L/h/1.73 m^2 (drug given before haemodialysis). Peak plasma concentrations were 5.9, 8.2 and 9.5 μg/L for the 3 groups, respectively, attained at 6.2, 5.2 and 4.2 h, respectively [Bergstorm *et al.*1996].

Four healthy individuals and eight patients with cirrhosis were administered 7.5 mg olanzapine. Peak plasma concentrations were 9.2 μg/L at 8 h for the healthy individuals and 7.7 μg/L at 6.6 h for the patients with cirrhosis [Thomasson *et al.* 1996].

In a study in patients stabilised on 20 mg olanzapine daily, steady-state maximum plasma levels after further treatment with olanzapine 20 mg daily for 20 days, 30 mg daily for 10 days followed by 40 mg daily for 10 days, or 40 mg daily for 20 days, were 57.8, 75.6, and 94.1 μg/L, respectively. The AUCs were 997, 1220, and 1630 ng.h/mL, respectively [Mitchell *et al.* 2006].

Median serum levels of olanazapine were 32.7 μg/L in 122 children and adolescent patients. Patients with anorexia receiving median doses of 7.5 mg had levels of 18.7 μg/L while those treated with median doses of 12.5 mg daily for schizophrenia had levels of 37.7 μg/L [Theisen *et al.* 2006].

A breast-feeding mother taking 15 mg (270 μg/kg) olanzapine daily had plasma levels of 24 μg/L Levels of olanzapine 12.2 and 11.5 ng/g (with and without additional hydrochloric acid extraction, respectively) were found in breast milk. Levels in the infant's plasma were below the limit of detection (<5 μg/L) [Lutz *et al.* 2008].

For a report of olanzapine excretion in breast milk, see [Croke *et al.* 2002]; for a study showing that the pharmacokinetics of olanzapine in adolescents and adults are similar, see Lobo *et al.* [2010]. For a pharmacokinetic and pharmacodynamic profile of olanzapine, see Callaghan *et al.* [1999].

Toxicity The minimum toxic concentration in blood is 0.1 mg/L.

A patient has recovered after an overdose of 800 mg olanzapine [Cohen *et al.* 1999].

A 29-year-old woman survived an acute overdose with 1110 mg of olanzapine that was associated with respiratory and cardiovascular adverse effects. Blood levels were not reported [Gardner *et al.* 1999].

A 22-year-old man who took 800 mg of olanzapine in a suicide attempt had maximum serum levels of 200 μg/L (approx. 20 times therapeutic levels at a dose of 10 mg). The effects of this overdose were sedation and mild anticholinergic symptoms [Bosch *et al.* 2000].

Severe non-fatal olanzapine toxicity has been reported in a 58-year-old woman who was found unconscious with two empty 28-tablet containers of olanzapine 10 mg. Whole blood levels of olanzapine were 0.41, 0.34, and 0.38 mg/L at ~4, 8 and 12 h after ingestion, respectively [Ballesteros *et al.* 2007].

A blood level of 800 pg/L olanzapine was found in a patient about 1 day after a non-fatal overdose with 550 mg olanzapine. The initial elimination half-life was 24 h for the first 3 days, followed by a second phase with a half-life of ~2.5 days [Lennestål *et al.* 2007].

A 59-year-old woman was found unresponsive in a hotel room and later died. At the scene were 2 empty bottles of Zyprexa (each bottle containing 30 tablets at 10 mg olanzapine each, filled only days earlier) and suicide notes. The blood concentration of olanzapine was 0.049 mg/L with 0.410 mg/L in the gastric contents [Elian 1998].

A 43-year-old male psychiatric outpatient died after ingesting 600 mg olanzapine. Analysis of postmortem blood and urine yielded olanzapine concentrations of 1238 and 6987 μg/L, respectively, which are far in excess of the therapeutic concentration [Stephens *et al.* 1998].

In a report of 2 deaths associated with olanzapine, blood levels were 0.237 and 0.675 μg/L and gastric content concentrations were 0.197 μg/L and 17.4 mg/L [Gerber, Cawthon 2000].

In 58 postmortem blood samples, mean olanzapine levels were 358 μg/L (10–5200 μg/L). Potential toxicity was considered at levels above 100 μg/L and postmortem blood levels as low as 160 μg/L were found in cases where death was caused by olanzapine toxicity [Robertson, McMullin 2000].

A 25-year-old man died from acute poisoning with olanzapine. Postmortem levels of olanzapine were found to be 0.40 mg/L in heart blood, 0.27 mg/L in carotid blood, 0.35 mg/L in urine, 0.61 mg/kg in liver and 0.33 mg/50 mL in gastric contents. Levels in the CSF were below the limit of detection (0.05 mg/L). Levels in the brain were 0.22 mg/kg in hippocampus, 0.86 mg/kg in midbrain, 0.16 mg/kg in amygdala, 0.39 mg/kg in caudate/putamen, 0.17 mg/kg in left frontal cortex and 0.37 mg/kg in right frontal cortex. Olanzapine was not detected in the cerebellum [Merrick *et al.* 2001].

A 40-year-old man who died after an overdose of olanzapine and citalopram had the following postmortem levels of olanzapine and citalopram, respectively: 1.38 and 3.35 mg/L in heart blood, 1.11 and 1.65 mg/L in femoral blood, 60.24 and 32.43 mg/L in urine, 6.47 and 10.71 mg/kg in liver, and 38.36 and 49.16 mg/kg in lung [Horak, Jenkins 2005].

In an analysis of 28 deaths involving olanzapine between 2004 and 2007, there were 6 where olanzapine was the only drug involved. Average olanzapine levels in these 6 were 3.2 mg/L in peripheral blood, 4.5 mg/L in central blood, 40 mg/kg in liver and 1.6 mg/L in vitreous humour. In 10 cases where death was not attributed to olanzapine, levels were 0.26, 0.29 and 0.78 mg/L for peripheral blood, central blood, and vitreous humour, respectively, and 5.6 mg/kg in liver. In 10 cases where death was attributed to olanzapine plus other drugs, levels were 0.59, 0.64 and 0.78 mg/L, respectively, for peripheral blood, central blood and vitreous humour, and 5.9 mg/kg in liver. Concentrations of olanzapine associated with toxicity were in the ranges 1.4–6.2, 1.1–7.4, and 1.1–2.1 mg/L, respectively, for peripheral blood, central blood and vitreous humour and 14–88 mg/kg for liver [Vance, McIntyre 2009].

For reports of olanzapine overdoses in children, see Bond, Thompson [1999], Catalano *et al.* [1999], Kochhar *et al.* [2002] and Yip *et al.* [1998].

Note For a review of olanzapine-associated toxicity and fatality in overdose, see Chue, Singer [2003].

Half-life A mean of 33.8 h (range, 30–60) for young, healthy individuals and 51.8 h for elderly individuals. The half-life tends to be longer in woman than in men.

Bioavailability ≈80%.

Volume of Distribution ≈1150 L (660–1790 L); 22 L/kg; also reported as 10–20 L/kg.

Clearance Plasma clearance ranges from 12–47 L/h with a mean of 25 L/h. Hepatic impairment may reduce clearance. It is generally 30% lower in women than men and 40% higher in smokers than non-smokers.

Protein Binding 93%, primarily to albumin and α_1-acid glycoprotein.

Dose Adults: usual oral dose is 10 mg daily, with a range between 5 and 20 mg. In the elderly, the starting dose may possibly be reduced to 5 mg daily. Patients with renal and/or hepatic impairment need a starting dose of 5 mg daily, only increased with caution.

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Oleandomycin

Antibiotic

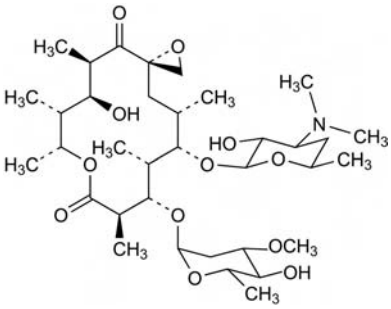
$C_{35}H_{61}NO_{12}$ = 687.9

CAS—3922-90-5

IUPAC Name (3*R*,5*R*,6*S*,7*S*,8*S*,9*S*,12*S*,13*R*,14*R*,15*R*)-6-[(2*S*,3*R*,4*S*,6*S*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-hydroxy-8-[(2*R*,4*S*,5*S*,6*S*)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-2,11-dioxaspiro[2.13]hexadecane-10,16-dione

Synonym PA-105

Proprietary Names *Triolmicina Romicil*; *Landomycin*.



Chemical Properties An antimicrobial substance produced by the growth of *Streptomyces antibioticus*. A white amorphous powder. Moderately soluble in water; freely soluble in butanol, ethanol, acetone and methanol; soluble in dilute acids; practically insoluble in hexane, carbon tetrachloride and dibutyl ether. pK_a 8.8 (25°). Log *P* (octanol/water), 1.7.

Oleandomycin Phosphate

$C_{35}H_{61}NO_{12}$, H_3PO_4 = 785.9

CAS—7060-74-4

Chemical Properties A white crystalline powder. Soluble 1 in 2.5 of water and 1 in 3 of ethanol; slightly soluble in ether.

Colour Tests Mandelin's test—green; Marquis test—green.

Thin-layer Chromatography System TA—R_f 0.45 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Aqueous acid—242 nm (A^{1}_{1}=7b).

Infrared Spectrum Principal peaks at wavenumbers 1111, 1052, 1075, 1010, 1162, 1190 cm^{-1} (oleandomycin phosphate).

Quantification

Serum **HPLC** UV detection. Limit of detection, <0.25 mg/L [Stubbs *et al.* 1986].

Urine **HPLC** UV detection. Limit of detection, <1 mg/L [Stubbs *et al.* 1986].

Dose Oleandomycin phosphate has been given in doses of 1 to 2 g daily.

Stubbs C *et al.* (1986). High-performance liquid chromatographic analysis of oleandomycin in serum and urine. *J Chromatogr* 353: 33–38.

Oleoresin of Capsicum

Capsaicinoid, Food Additive, Incapacitant

Synonyms OC; pepper spray.

Proprietary Names *Cap-Stun*; *Cap-Tor*; *MK4 First Defense*; *Pepperfoam 10%*; *Sabre Defence* (defence spray formulations). It is also an ingredient of *Sabre*.

Chemical Properties OC is a reddish-brown liquid extracted from dried ripe fruits of the genus *Capsicum*. The extracts are highly complex mixtures of over 100 natural compounds, all in varying concentrations. The total concentration of capsaicinoids in a pepper ranges from 0.1 to 2.0% (dry weight) and depends upon the variety of the pepper, the growing conditions, and the time of harvest. The typical capsaicinoid content of pepper spray formulations ranges from 1.2 to 12.6%. Capsaicin is the major ingredient of OC, closely followed by dihydrocapsaicin. Together, they make up more than 80% of the capsaicinoids in OC. Another major component of OC is a dihydroxycarotenone, thought to be involved in the irritant properties of OC. Further capsaicinoids in OC include nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, nonanoic acid vanillylamide and decanoic acid vanillylamide. Owing to the potential for interproduct composition variations, more recent pepper spray formulations use pure capsaicin or its synthetic equivalent, nonivamide [Olajos, Salem 2001; Olajos, Stopford 2004].

Mass Spectrum [Haas *et al* 1997].

Quantification

Other **GC-MS** Pepper Spray. Column: methyl silicone capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 70° for 10 min to 320° at 8°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention times: capsaicin 35.5 min, dihydrocapsaicin 35.8 min, nordihydrocapsaicin 34.6 min, homodihydrocapsaicin 36.7 min (underivatised samples). Limit of quantification not reported [Haas *et al.* 1997].

LC-MS Pepper Spray. Column: YMC Basic S-3 (50 × 2.0 mm i.d.). Mobile phase: 10 mmol/L ammonium formate (pH 4.2) : methanol (35 : 65 for 0.5 min to 10 : 90 over 1 min for 4 min), flow rate 0.35 mL/min. APCI, positive ion mode. Retention time: capsaicin 1.9 min (nitrobenzene diazonium tetrafluoroborate derivative). Limit of quantification not reported [Cavett *et al.* 2004]. Fresh Peppers, OC, and Pepper Spray Products. Column: MetaSil Basic RP (100 × 3.0 mm i.d., 3 µm). Mobile phase: stepwise gradient methanol : water containing 0.1% formic acid. Positive ion mode. Retention times: capsaicin 12.6 min, dihydrocapsaicin 15.8 min, octanoylvanillamide 8.4 min, nordihydrocapsaicin 11.8 min, nonivamide 12.6 min, homocapsaicin 16.1 min, homodihydrocapsaicin 17.5 min. Limit of quantification not reported [Reilly *et al.* 2001].

Note For a study comparing the chemical and elemental composition of 2 formulations of OC spray, see Haas *et al.* [1997]

Disposition in the Body Studies in rats have shown that capsaicin and capsaicinoids are rapidly absorbed from the gastrointestinal tract following intragastric administration. However, at the time of writing (2009), the uptake of OC via inhalation has not been studied in animals or humans. Animal studies have shown that capsaicin and capsaicinoids undergo metabolic conversion via both hepatic oxidative and non-oxidative pathways. Hydroxylation on the vanillyl ring moiety occurs, forming catechol metabolites. The alkyl chain of capsaicin is also susceptible to oxidative deamination. Other pathways include the hepatic mixed-function oxidase system forming highly reactive metabolites (epoxide, phenoxy and methyl radicals, and quinone-type compounds). The majority of capsaicin and capsaicinoid metabolites are excreted in urine, mostly as glucuronide conjugates.

Toxicity OC has remarkable irritant potency and very low toxicity when compared with other agents in the same class (CS or CN). When applied to ocular membranes, OC causes stinging, lacrimation and blepharospasm. The piquancy (or potency) of peppers is measured by the Scoville Organoleptic Test. This is a taste test based upon the greatest dilution of a pepper extract that can be detected by the human tongue and is measured in Scoville heat units (SHU). Pure capsaicin has a value of $\sim 15 \times 10^6$ SHU and standard US-grade pepper sprays have a range of 2×10^6 to 5.3×10^6 SHU. Every variety of pepper is thus graded, with the bell-pepper variety at the bottom of the scale with an SHU of zero. The inhalation toxicity of chemical warfare agents, military chemicals, and riot control agents is, by convention, expressed by the notation Ct. It is defined as the product of the concentration in mg/m^3 multiplied by the exposure time (t) in minutes ($mg_{\bullet}min/m^3$). The terms LCt_{50} and ICt_{50} describe the airborne dosages that are lethal (L) or incapacitating (I) to 50% of the exposed population. At the time of writing LCt_{50} and ICt_{50} values have yet to be determined. The minimum tolerated exposure is reported as $0.002\,mg/m^3$ while the minimum lethal exposure is estimated at $>100\,000\,mg_{\bullet}min/m^3$. Owing to poor water solubility, OC-containing formulations are usually made up of solvents as carriers together with some kind of propellant gas. It is worth noting that some solvents may contribute to unwanted effects of CS use, such as corneal erosion, skin blistering, or even neurotoxicity [Olajos, Salem 2001; Olajos, Stopford 2004]. Reviews of in-custody deaths from pepper spray exposure have concluded that pepper spray was unrelated to the causes of death where sufficient information was available [Steffee *et al.* 1995].

Cavett V *et al.* (2004). Visualization and LC/MS analysis of colorless pepper sprays. *J Forensic Sci* 49: 469–476.

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Olmesartan

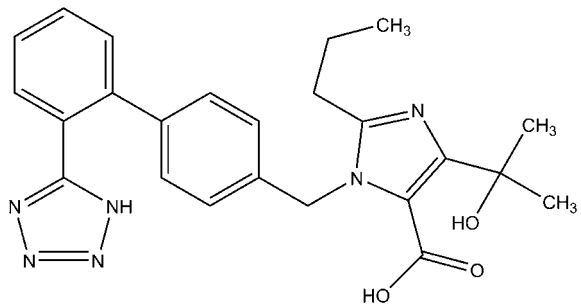
Angiotensin II Receptor Antagonist, Antihypertensive

$C_{24}H_{26}N_6O_3 = 446.5$

CAS—144689-24-7

IUPAC Name 5-(2-Hydroxypropan-2-yl)-2-propyl3-[[4-[2-(2*H*-tetrazol-5-yl) phenyl]phenyl]methyl]imidazole-4-carboxylic acid

Synonyms 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-carboxylic acid; RNH-6270.



Olmesartan Medoxomil

$C_{29}H_{30}N_6O_6 = 558.6$

CAS—144689-63-4

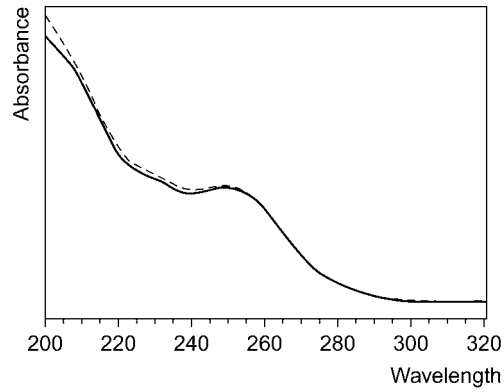
IUPAC Name (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate

Synonyms CS-866; 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-carboxylic acid (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester; (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[4-[2-(tetrazol-5-yl)phenyl]-phenyl] methylimidazole-5-carboxylate.

Proprietary Names *Benicar*; *Olmetec.*

Chemical Properties Crystals. Mp 180° to 182°. Practically insoluble in water; sparingly soluble in methanol.

Ultraviolet Spectrum NaOH/water (0.02 mol/L)—258 nm. Celebier and Altinoz [2007]



Quantification

Plasma **HPLC** Column: YMC-pack A-312 (150 × 6.0 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L ammonium acetate buffer (pH 3.75,; 28 : 72), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 370$ nm). Limit of quantification, 0.2 µg/L [Yoshihara *et al.* 2005].

LC-MS Column: Hypersil C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile Phase: formic acid : methanol : water (0.5 : 70 : 30), flow rate 0.6 mL/min. API, ESI, MRM acquisition mode. Limit of quantification, 0.2 µg/L [Liu *et al.* 2007].

Urine **LC-MS** See Plasma. Limit of quantification, 5 µg/L [Liu *et al.* 2007].

Note For a UV-vis spectrophotometry method for the measurement of olmesartan in tablets, see Celebier and Altinoz [2007].

Disposition in the Body Olmesartan medoxomil is rapidly de-esterified *in vivo* and completely metabolised to the active acid olmesartan, the major metabolite in a reaction catalysed by both aryl esterase and albumin. At the time of writing (2008), no metabolites of olmesartan have been identified in humans. Approximately 5 to 12% of an administered dose is excreted in urine as olmesartan.

Therapeutic Concentration

Fifteen healthy subjects (aged 30.5 ± 7.0 years; weight 70.8 ± 8.8 kg) were administered a daily 20 mg oral dose of olmesartan medoxomil for 7 days. The mean maximum steady-state plasma concentration reached was 479.3 µg/L after 1.5 h. The average amount of administered dose of olmesartan excreted in urine was 9.5%. A separate study showed that the concomitant administration of olmesartan medoxomil with glibenclamide had no significant effects on the steady-state pharmacokinetics of either agent and was well tolerated and safe [Huber *et al.* 2006].

Single oral doses of 10 to 160 mg olmesartan medoxomil were administered to 25 healthy volunteers (aged 24 ± 4 years; weight 76 ± 8 kg). Peak plasma concentrations were as follows:

Dose (mg)	*C_{max} (µg/L)*	*Time (h)*
10	224	2.4
20	419	2.5
40	752	1.4
80	1095	2.0
160	2100	2.8

The elimination half-life in this study was found to be between 12 and 15 h [Schwocho, Masonson 2001].

Note For information on the influence of age and renal and hepatic function on the pharmacokinetics of olmesartan medoxomil, see von Bergmann *et al.* [2001]. For a general review of olmesartan medoxomil, see Brousil and Burke [2003].

Toxicity

Note For a study of olmesartan medoxomil and potential drug interactions, see Laeis *et al.* [2001].

Bioavailability Approximately 29%.

Half-life Elimination, approximately 15 h.

Volume of Distribution Approximately 35 L.

Clearance Approximately 1.39 L/h

Distribution in Blood Blood : plasma ratio 0.4 to 0.7.

Dose Initially, 10 mg daily, increased to 20 mg if necessary; maximum dose is 40 mg.

Brousil JA, Burke JM (2003). Olmesartan medoxomil: an angiotensin II-receptor blocker. *Clin Ther* 25: 1041–1055.

Celebier M, Altinoz S (2007). Determination of olmesartan medoxomil in tablets by UV-vis spectrophotometry. *Pharmazie* 62: 419–422.

Huber M *et al.* (2006). Pharmacokinetics and safety of olmesartan medoxomil in combination with glibenclamide in healthy volunteers. *Clin Exp Hypertens* 28: 631–643.

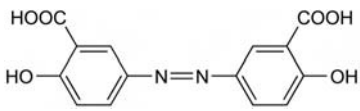
Laeis P *et al.* (2001). The pharmacokinetic and metabolic profile of olmesartan medoxomil limits the risk of clinically relevant drug interaction. *J Hypertens Suppl* 19: S21–S32.

Liu D *et al.* (2007). Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 856: 190–197.
Schwocho LR, Masonson HN (2001). Pharmacokinetics of CS-866, a new angiotensin II receptor blocker, in healthy subjects. *J Clin Pharmacol* 41: 515–527.
von Bergmann K *et al.* (2001). Olmesartan medoxomil: influence of age, renal and hepatic function on the pharmacokinetics of olmesartan medoxomil. *J Hypertens Suppl* 19: S33–S40.
Yoshihara K *et al.* (2005). Population pharmacokinetics of olmesartan following oral administration of its prodrug, olmesartan medoxomil: in healthy volunteers and hypertensive patients. *Clin Pharmacokinet* 44: 1329–1342.

Olsalazine

Antiinflammatory, Aminosalicylate
$C_{14}H_{10}N_2O_6$ = 302.2
CAS—15722-48-2

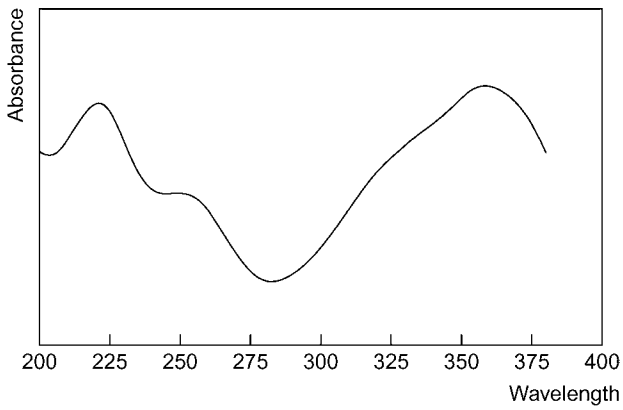
IUPAC Name 5-[(2*Z*)-2-(3-Carboxy-4-oxocyclohexa-2,5-dien-1-ylidene)hydrazinyl]-2-hydroxybenzoic acid
Synonyms 3,3′-Azobis(6-hydroxybenzoic acid); azodisal; CI mordant yellow 5; 5,5′-azobis(salicylic acid).



Olsalazine Sodium

$C_{14}H_8N_2Na_2O_6$ = 364.2
CAS—6054-98-4
IUPAC Name Disodium 5,5′-asodisalicylate
Synonyms Azodisal sodium; CI mordant yellow 5 disodium salt; CJ-91B; CL-14130; sodium azodisalicylate.
Proprietary Names *Dipentum*
Chemical Properties A yellow, fine, crystalline powder. Mp 240°. Sparingly soluble in water; soluble in dimethylsulfoxide; very slightly soluble in methanol; practically insoluble in ethanol, chloroform and ether.

Ultraviolet Spectrum Aqueous acid—358, 221, 249 nm.



Quantification

Serum **HPLC** Column: C_{18} Nucleosil (250 4 mm i.d., 10 µm). Mobile phase: methanol : phosphate buffer (pH 7.4, 52 : 48) with 0.02 mol/L TBA (tetrabutylammonium hydrogen sulfate). UV detection (λ=365 nm); fluorescence detection (λ_{ex}=312 nm, λ_{em}=469 nm). *k* values: olsalazine, 5.2; olsalazine-sulfate, 1.2. Limit of quantification, 0.5 µg/L [Ryde, Ahnfelt 1988].
Urine **HPLC** See Serum. Limit of quantification, 1 µg/L [Ryde, Ahnfelt 1988].
Disposition in the Body Olsalazine is not well absorbed after oral administration and almost the entire dose reaches its site of action in the colon intact. It is broken down by colonic bacteria flora into 5-aminosalicylic acid (mesalazine), some of which is absorbed and acetylated. Both mesalazine and its acetylated metabolite are absorbed from the colon. The presence of food has no significant effect on the absorption. 1 to 2% of the intact olsalazine absorbed is excreted, mainly in urine. A dose of olsalazine is almost completely metabolised by sulfate conjugate in the liver. Approximately 17 to 20% of the total dose is recovered in urine within 24 h of IV administration in patients with active inflammatory bowel disease, but this decreases for an oral dose. <5% of the ingested dose is recovered in faeces as the unchanged drug. Steady state concentrations of conjugated olsalazine are achieved within days of the start of administration. Approximately 0.1% of an oral dose of olsalazine is metabolised in the liver to olsalazine-*O*-sulfate (olsalazine-*S*) and 22 to 33% appears in the urine almost all as acetyl 5-aminosalicylate. Olsalazine-*S* has a half-life of 7 days and accumulates to steady state within 2 to 3 weeks.
Therapeutic Concentration
Seven healthy males, aged 27 to 44 years, administered with a 10 mg IV dose of olsalazine showed peak serum concentrations of 7.5 µg/L (range, 3.8 to 9.2 µg/L) by the end of the infusion.
Eight healthy male volunteers were administered with 1 g olsalazine with or without food, and also 2 g and 4 g in the fasting state. Mean peak serum concentrations of the parent drug were 2.3 (range, 1.1 to 4.6), 3.6, 5.9 (range, 3.9 to 9.8) and 32 (range, 14 to 47) µg/L, for the 1 g (with food), 1, 2 and 4 g (fasting) doses, respectively. Mean peak concentrations for olsalazine sulfate were 0.5, 1.2, 1.9 and 8.3 µg/L, respectively; 5-aminosalicyclic acid (5-ASA) 1.6, 1.2, 4.5 and 3.8 µg/L, respectively; *N*-acetyl-aminosalicyclic acid (ac-5-ASA) 3.2, 4.1, 10.0 and 9.1 µg/L, respectively. Peak olsalazine concentrations were observed approx. 1 h after administration of the parent drug, peak olsalazine sulfate concentrations after 4 h, and peak 5-ASA and ac-5-ASA at approx. 8 h [Ryde, Ahnfelt 1988].
Bioavailability Varies from 2.4, 1.9 to 4.8% after 1, 2 and 4 g doses, respectively.
Half-life Olsalazine, 0.9 to 1 h and the 5-aminosalicylic acid metabolite, 7 days.
Volume of Distribution Mean, 6.2 L.
Distribution in Blood Olsalazine does not enter the erythrocytes.
Protein Binding Olsalazine is bound >99% and the 5-aminosalicylic acid metabolite, 74%.
Dose The usual dose is 1 g daily with a maximum of 3 g daily.

Ryde EM, Ahnfelt NO (1988). The pharmacokinetics of olsalazine sodium in healthy volunteers after a single i.v. dose and after oral doses with and without food. *Eur J Clin Pharmacol* 34(5): 481–488.

Omalizumab

Antiallergic
CAS—242138-07-4

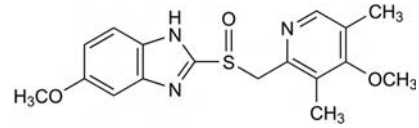
Synonym Rhu-Mab-325
Proprietary Name *Xolair*
Chemical Properties Recombinant humanised monoclonal antibody.
Disposition in the Body Readily absorbed after SC injection, with peak serum concentrations reached after 7 to 8 days. It is removed by IgG and IgE clearance processes in the liver. During treatment with omalizumab, the serum concentration of free IgE decreases but that of total IgE increases because the omalizumab–IgE complex has a slower elimination rate than free IgE.
Therapeutic Concentration In adults with allergic asthma, an initial dose of IV omalizumab (2.0 mg/kg) followed by 6 doses of 1.0 mg/kg administered over 77 days resulted in a mean peak serum concentration at steady state of 30.9 mg/L [Boulet *et al.* 1997]
Bioavailability Approximately 62%.
Half-life Serum elimination half-life, 1 to 4 weeks after SC or IV administration.
Volume of Distribution Approximately 3.6 L for free omalizumab and 2.25 L for the omalizumab–IgE complex.
Clearance Apparent, 7.32 mL/h.
Dose The dose depends on the patient's weight and pre-treatment serum IgE concentrations; regimens range from 75 to 300 mg every 4 weeks to 225 to 375 mg every 2 weeks. Omalizumab is given by SC injection, and not more than 150 mg should be given at one injection site.

Boulet LP *et al.* (1997). Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am J Respir.Crit Care Med* 155: 1835–1840.

Omeprazole

Antiulcerative, Proton Pump Inhibitor
$C_{17}H_{19}N_3O_3S$ = 345.4
CAS—73590-58-6

IUPAC Name 6-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1*H*-benzimidazole
Synonyms H-168/68; 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1*H*-benzimidazole.
Proprietary Names *Antra*; *Gastracid*; *Gastrogard*; *Gastroloc*; *Losec*; *Mepral*; *Mopral*; *Omebeta*; *Omepral*; *Omeprazen*; *Parizac*; *Pepticum*; *Prilosec*; *Zegerid*; *Zoltum.*



Chemical Properties A white or almost white powder. Mp 156°. It is very slightly soluble in water; soluble in alcohol, methyl alcohol and dichloromethane; very soluble in alkaline solutions. pK_a, 4.0. Log *P* (octanol/water), 2.23.

Omeprazole Magnesium

$C_{34}H_{36}N_6O_6S_2$, Mg = 713.1
CAS—95382-33-5
Chemical Properties A white or almost white, hygroscopic powder. Very slightly soluble in water; sparingly soluble in methyl alcohol; practically insoluble in heptane.

Omeprazole Sodium

$C_{17}H_{18}N_3O_3S$, Na = 367.4
CAS—95510-70-6

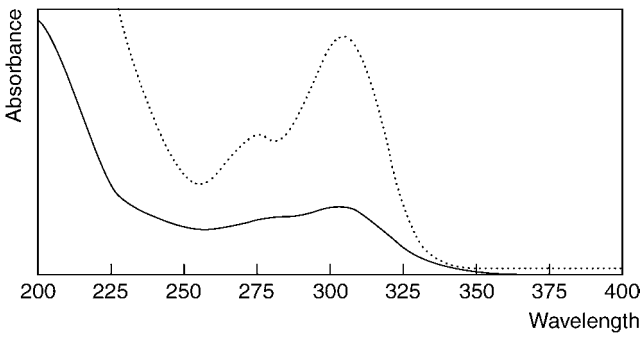
Chemical Properties A white or almost white hygroscopic powder. It is freely soluble in water and alcohol; soluble in propylene glycol; very slightly soluble in dichloromethane.

High Performance Liquid Chromatography System HAA—RT 14.1 min; system HZ—RT 2.8 min.

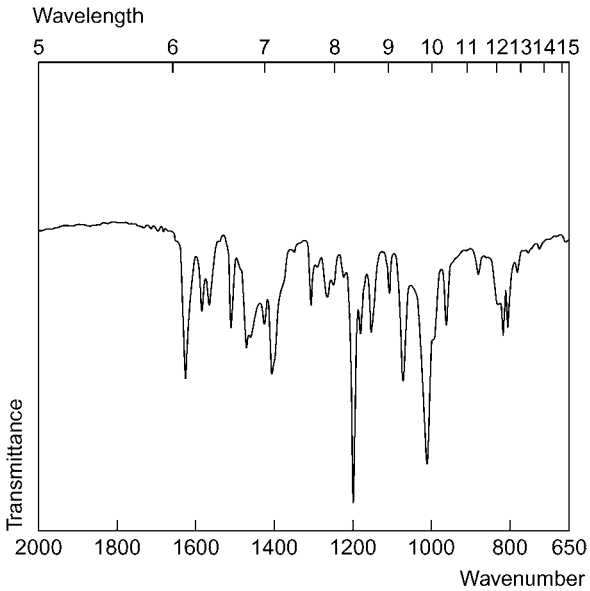
Column: Crestpak C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L disodium hydrogen orthophosphate : acetonitrile (65 : 35, pH 6.5), flow rate 1 mL/min. IS: chloramphenicol. UV detection (λ = 302 nm). Retention time: omeprazole 6.3 min and I.S. 4.5 min [Yuen *et al.* 2001].

Column: C_{18} SG120 Capcell Pak (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L phosphate buffer (pH 8.5, 25 : 75), flow rate 0.8 mL/min. IS: phenacetin. UV detection (λ = 302 nm). Retention time: omeprazole 16.8 min, hydroxyomeprazole 6.6 min, omeprazole sulfone 11.5 min, IS 13.6 min [Kobayashi *et al.* 1992].

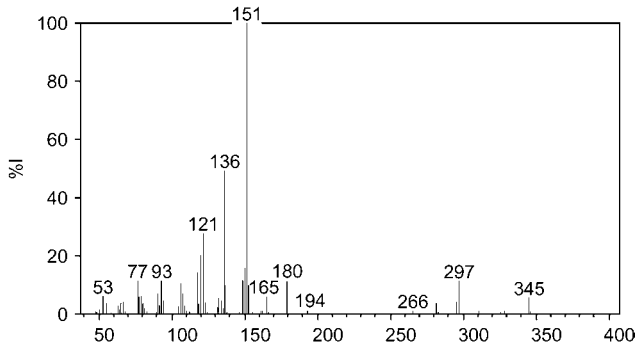
Ultraviolet Spectrum Aqueous acid (0.2 mol/L NH_2SO_4)—277, 303 nm; basic—279 nm.



Infrared Spectrum Principal peaks at wavenumber 1628, 1205, 1015 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 151, 136, 121, 180, 297, 77, 93, 194.



Quantification

Plasma **HPLC** Column: Zorbax C_8. Mobile phase: 0.1% triethylamine (pH 6.0) : acetonitrile (72 : 28), flow rate 1 mL/min. UV detection. Limit of quantification, 20.61 µg/L for omeprazole and other proton pump inhibitors [Bharathi *et al.* 2009]. Column: Zorbax C_{18} (150 × 3.0 mm i.d., 3.5 µm). Mobile phase: 22.0 mmol/L phosphate mono basic (pH 6.0)-methanol (90 : 10) : 22.0 mmol/L phosphate mono basic (pH 6.0)-acetonitrile-methanol (10 : 80 : 10, 74 : 26 to 34 : 66 at 13 min to 4 : 96 at 14 min for 1 min to 74 : 26 at 17 min), flow rate 0.55 mL/min to 0.56 mL/min at 13 min to 0.7 mL/min at 14 min for 1 min to 0.55 mL/min at 17 min. UV detection (λ = 302 nm). Retention time: 7.9 min. Limit of detection, 2 µg/L for omeprazole and its metabolites [Rezk *et al.* 2006]. Column: SK-W precolumn (35 × 4.6 mm i.d., 10 µm) or Inertsil DS-80A (150 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer : acetonitrile (pH 7.0, 92 : 8) or phosphate buffer : acetonitrile : methanol (pH 6.5, 65 : 30 : 5). UV detection (λ = 302 nm). Limit of quantification, 3 µg/L for omeprazole and its metabolites [Shimizu *et al.* 2006]. Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: 0.01 mol/L disodium hydrogen phosphate buffer : acetonitrile (pH 7.1, 73 : 27). UV detection (λ = 302 nm). Limit of quantification, 10 µg/L [Zarghi *et al.* 2006]. Column: amylose tris(3,5-dimethylphenylcarbamate) on APS- Nucleosil (150 × 4.6 mm i.d., 7 µm). Mobile phase: acetonitrile : water (60 : 40), flow rate 0.5 mL/min. UV detection (λ = 302 nm). Limit of detection, 0.0063 mg/L for each enantiomer [Cass *et al.* 2003]. See also Garcia-Encina *et al.* [1999], Orlando, Bonato [2003] and Yuen *et al.* [2001].

LC-MS Column: ODS 3. Mobile phase: 0.01 mol/L ammonium acetate : acetonitrile (40 : 60), flow rate 0.25 mL/min. Limit of quantification, 0.05 µg/L [Vittal *et al.* 2009]. Column: ODS. Mobile phase: methanol : 10 mmol/L ammonium acetate (60 : 40). Positive ion mode, SRM acquisition mode. Limit of quantification, 1.2 µg/L [Macek *et al.* 2007]. Column: ProntoSIL AQ C_{18}. Mobile phase: 10 mmol/L ammonium acetate (pH 7.25) : acetonitrile. SIM acquisition mode. Limit of quantification, 10 µg/L for omeprazole and omeprazole sulfone, and 5 µg/L for 5-hydroxyomeprazole [Hofmann *et al.* 2006]. Column: Betasil silica (50 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : water : formic acid (73.4 : 26.5 : 0.1), flow rate 1.5 mL/min. ESI. Limit of quantification, 2.5 µg/L for omeprazole and 5-hydroxyomeprazole [Song, Naidong 2006]. Column: Symmetry RP_8 (150 × 3.0 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium hydroxide–formic acid (pH 8.2) : methanol (35 : 65), flow rate 400 µL/min. TIS. Limit of quantification, 400 ng/L, limit of detection, 2 to 15 ng/L for omeprazole and hydroxyomeprazole [Frerichs *et al.* 2005]. See also Wang *et al.* [2005] and Woolf, Matuszewski [1998].

CE Capillary: uncoated fused silica (57/50 cm total/effective length, 75 µm i.d.). Buffer: 20 mmol/L borate buffer: 30 mmol/L SDS (pH 9.5). DAD (λ = 300 nm). Limit of detection, 0.04–0.07 mg/L for omeprazole and its metabolites [Pérez-Ruiz *et al.* 2006].

Serum **HPLC** Column: C_{18}. Mobile phase: SDS : propan-2-ol (pH 7.0). UV detection (λ = 305 nm). Limit of quantification, <25 µg/L, limit of detection, <6 µg/L for omeprazole and its metabolites [Rambla-Alegre *et al.* 2009].

LC-MS Column: ReproSil Chiral-CA (250 × 2 mm i.d., 5 µm). Mobile phase: 2-propanol-acetic acid-diethylamine (100 : 4 : 1) : hexane (10 : 90 to 15 : 85 in 10 min for 1 min at 2 : 75 for 1 min to 10 : 90), flow rate 0.35 mL/min. Limit of detection, 0.2 µg/L for (*R*)-omeprazole, (*S*)-omeprazole and omeprazole sulfone, and 1 µg/L for (*R*)-5-hydroxyomeprazole and (*S*)-5-hydroxyomeprazole [Martens-Lobenhoffer *et al.* 2007].

Urine **HPLC** See Serum [Rambla-Alegre *et al.* 2009].

Liver **HPLC** Microsomes. Column: Superspher SI-60 (125 × 4.0 mm i.d., 4 µm). Mobile phase: dichloromethane : 5% ammonium hudroxide in methanol : 2-propanol (191 : 8 : 1), flow rate 1.5 mL/min. UV detection (λ = 302 nm). Limit of detection, 2 ng/L for omeprazole sulfone and 25 ng/L for hydroxyomeprazole [Andersson *et al.* 1993].

Disposition in the Body Omeprazole is rapidly absorbed after oral administration and absorption is not affected by the presence of food. It is almost completely metabolised in the liver, mainly by the cytochrome P450 isoform CYP2C19, and it is excreted in urine. Metabolites include omeprazole sulfone, 5-hydroxymethylomeprazole, 5-carboxyomeprazole and at least three other unidentified compounds. Distribution and elimination of the drug are both rapid. Approximately 80% of metabolites are excreted in urine, the rest in faeces.

Therapeutic Concentration

Ten healthy males, aged 23 to 40 years, were administered with 40 mg and 80 mg omeprazole orally on two separate occasions. The mean maximum concentration was 3.6 µmol/L (range, 2.34–5.54) for the 40-mg dose and 7.99 µmol/L (range, 5.02–12.71) for the 80-mg dose. These concentrations were observed at 0.2 h (range, 0.12–0.32). For the sulfone metabolite, mean concentrations were 0.7 µmol/L (range, 0.54–0.9) and 1.6 µmol/L (range, 1.09–2.37) for the 40- and 80-mg dose, respectively, attained at 0.38 h (range, 0.23–0.61) and 0.52 h (range, 0.33–0.84), respectively. For the hydroxy metabolite, mean concentrations were 2.13 µmol/L (range, 1.81–2.51) and 2.95 µmol/L (range, 2.37 to 3.68) for 40- and 80-mg doses, respectively, attained at 0.27 h (range, 0.18–0.41) and 0.27 h (range, 0.20–0.36), respectively [Andersson, Regårdh 1990].

Thirteen patients aged between 0.3 and 20 years old and undergoing treatment for acute gastrointestinal disease involving gastric acidity, mostly with normal hepatic and renal function, were given an IV median dose of 22 mg omeprazole (range, 6–40) (median 47.4 mg/1.73 m^2 [range, 36.9–60.2]). The mean maximum concentration observed was 0.58 µg/L (range, 0.23–1.66) at 2.0 h (range, 1.0–4.4) [Jacqz-Aigrain *et al.* 1994].

Toxicity

A 26-year-old woman ingested 16 omeprazole capsules (20-mg enteric-coated granules), which she had been prescribed for abdominal pain. She became drowsy, sweaty and complained of a headache. She was admitted to hospital and it was noted that she was flushed and tachycardic (110 beats/min). At 6 h after administration, no more abnormal signs were observed and after 24 h, only abdominal pain was still present. The initial plasma concentration was 1.01 mg/L [Ferner, Allison 1993].

In another case, a 40-year-old lorry driver was admitted to hospital 20 h after overdosing on 20 capsules (20-mg enteric-coated granules). He complained of drowsiness, blurred vision, dry mouth and a throbbing headache. Clinical examination showed that total blood count, creatinine, urea and electrolytes were normal. All symptoms had gone 32 h after ingestion [Ferner, Allison 1993].

Bioavailability Oral bioavailability increases from ~35%, after the first dose to ~50% after several doses.

Half-life 0.5–3 h.

Volume of Distribution 0.27–0.45 L/kg.

Clearance Systemic clearance, 0.23 L/kg/h.

Protein Binding ~95%.

Note For a review of the pharmacokinetics of omeprazole, see Howden [1991]; for a review of the pharmacology and the uses of omeprazole, see Wilde, McTavish [1994].

Dose The usual oral dose is 20 to 40 mg daily for peptic ulcer, gastro-oesophageal reflux and acid aspiration; up to 120 mg daily in Zollinger–Ellison syndrome. The dose is 0.7 to 1.4 mg/kg daily for children over 2 years, with a maximum of 40 mg.

Andersson T, Regårdh C-G (1990). Pharmacokinetics of omeprazole and metabolites following single intravenous and oral doses of 40 and 80 mg. *Drug Invest* 2: 255–263.

Andersson T *et al.* (1993). High-performance liquid chromatographic assay for human liver microsomal omeprazole metabolism. *J Chromatogr* 619: 291–297.

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Cass QB *et al.* (2003). Enantiomeric determination of the plasma levels of omeprazole by direct plasma injection using high-performance liquid chromatography with achiral–chiral column-switching. *J Chromatogr B Analyt Technol Biomed Life Sci* 798: 275–281.

Ferner RE, Allison TR (1993). Omeprazole overdose. *Hum Exp Toxicol* 12: 541–542.

Frerichs VA *et al.* (2005). Analysis of omeprazole, midazolam and hydroxy-metabolites in plasma using liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 824: 71–80.

Garcia-Encina G *et al.* (1999). Validation of an automated liquid chromatographic method for omeprazole in human plasma using on-line solid-phase extraction. *J Pharm Biomed Anal* 21: 371–382.

Hofmann U *et al.* (2006). Sensitive quantification of omeprazole and its metabolites in human plasma by liquid chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 831: 85–90.

Howden CW (1991). Clinical pharmacology of omeprazole. *Clin Pharmacokinet* 20: 38–49.

Jacqz-Aigrain E *et al.* (1994). Pharmacokinetics of intravenous omeprazole in children. *Eur J Clin Pharmacol* 47: 181–185.

Kobayashi K *et al.* (1992). Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkaline-resistant polymer-coated C18 column. *J Chromatogr* 579: 299–305.

Macek J *et al.* (2007). Rapid determination of omeprazole in human plasma by protein precipitation and liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 282–287.

Martens-Lobenhoffer J *et al.* (2007). Enantioselective quantification of omeprazole and its main metabolites in human serum by chiral HPLC–atmospheric pressure photoionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 301–307.

Orlando RM, Bonato PS (2003). Simple and efficient method for enantioselective determination of omeprazole in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 795: 227–235.

Pérez-Ruiz T *et al.* (2006). Determination of omeprazole, hydroxyomeprazole and omeprazole sulfone using automated solid phase extraction and micellar electrokinetic capillary chromatography. *J Pharm Biomed Anal* 42: 100–106.

Rambla-Alegre M *et al.* (2009). Analysis of omeprazole and its main metabolites by liquid chromatography using hybrid micellar mobile phases. *Anal Chim Acta* 633: 250–256.

Rezk NL *et al.* (2006). A simple and sensitive bioanalytical assay for simultaneous determination of omeprazole and its three major metabolites in human blood plasma using RP-HPLC after a simple liquid–liquid extraction procedure. *J Chromatogr B Analyt Technol Biomed Life Sci* 844: 314–321.

Shimizu M *et al.* (2006). Sensitive determination of omeprazole and its two main metabolites in human plasma by column-switching high-performance liquid chromatography: application to pharmacokinetic study in relation to CYP2C19 genotypes. *J Chromatogr B Analyt Technol Biomed Life Sci* 832: 241–248.

Song Q, Naidong W (2006). Analysis of omeprazole and 5-OH omeprazole in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry (HILIC-MS/MS)–eliminating evaporation and reconstitution steps in 96-well liquid/Liquid extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 830: 135–142.

Vittal S *et al.* (2009). Highly sensitive method for the determination of omeprazole in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry: application to a clinical pharmacokinetic study. *Biomed Chromatogr* 23: 390–396.

Wang J *et al.* (2005). Determination of omeprazole in human plasma by liquid chromatography–electrospray quadrupole linear ion trap mass spectrometry. *J Pharm Biomed Anal* 39: 631–635.

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Woolf EJ, Matuszewski BK (1998). Simultaneous determination of omeprazole and 5′-hydroxyomeprazole in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr A* 828: 229–238.

Yuen KH *et al.* (2001). Improved high performance liquid chromatographic analysis of omeprazole in human plasma. *J Pharm Biomed Anal* 24: 715–719.

Zarghi A *et al.* (2006). HPLC determination of omeprazole in human plasma using a monolithic column. *Arzneimittelforschung* 56: 382–386.

Omethoate

Acaricide, Insecticide

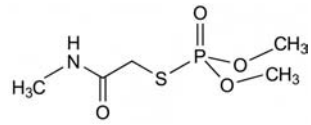
$C_5H_{12}NO_4PS$ = 213.2

CAS—1113-02-6

IUPAC Name 2-Dimethoxyphosphorylsulfanyl-*N*-methylacetamide

Synonyms Dimethoate-met; *O,O*-dimethyl-*S*-methylcarbamoylmethylphosphorothioate.

Proprietary Names *Bay-45432*; *Dimethoxon*; *Folimat*; *S-6876.*

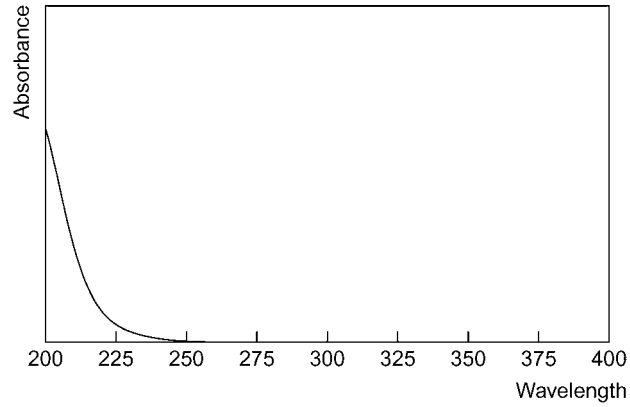


Chemical Properties A colourless to yellow oil. Mp −28° decomposes at 135°. Soluble in water, alcohol, acetone and many hydrocarbons; slightly soluble in diethyl ether; insoluble in petroleum ether. Log *P* (octanol/water), −0.74.

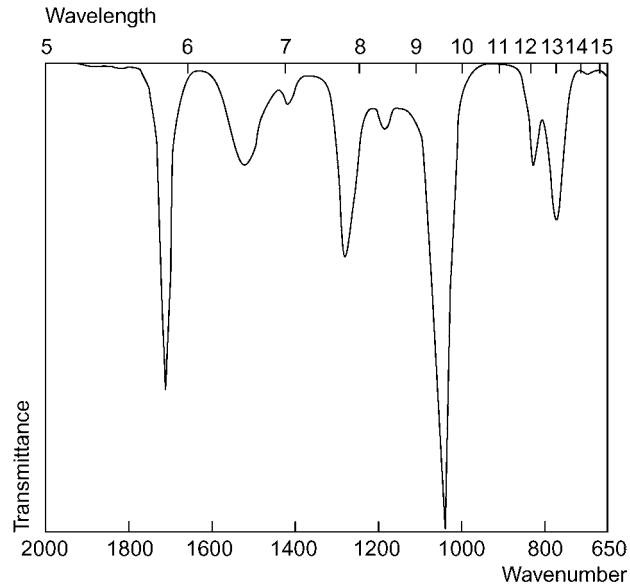
Thin-layer Chromatography System TX—R_f 0.00; system TY—R_f 0.00.

Gas Chromatography System GA—RI 1595; system GK—RRT 0.73 (relative to caffeine).

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1046, 1714, 1278, 775, 2964, 599 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 110, 156, 109, 79, 58, 15, 47, 126.

Disposition in the Body Omethoate is eliminated in urine, mostly within 48 h after ingestion.

Toxicity The allowed daily intake is 0.3 µg/kg.

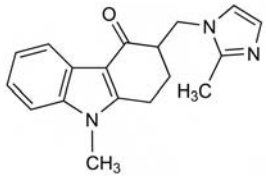
An adult female was admitted to hospital a few hours after ingestion of a pesticide. The omethoate blood level was determined on admission at 1.6 mg/L, fenthion was also detected at 2.9 mg/L. The patient remained clinically well and was discharged three days later [Tsatsakis *et al.* 1998.]

Tsatsakis AM *et al.* (1998). Clinical and toxicological data in fenthion and omethoate acute poisoning. *J Environ Sci Health B* 33(6): 657–670.

Ondansetron

5-HT_3 Receptor Antagonist, Antiemetic
$C_{18}H_{19}N_3O$ = 293.4
CAS—99614-02-5

IUPAC Name 9-Methyl-3-[(2-methylimidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4-one
Synonyms GR-38032; 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one.

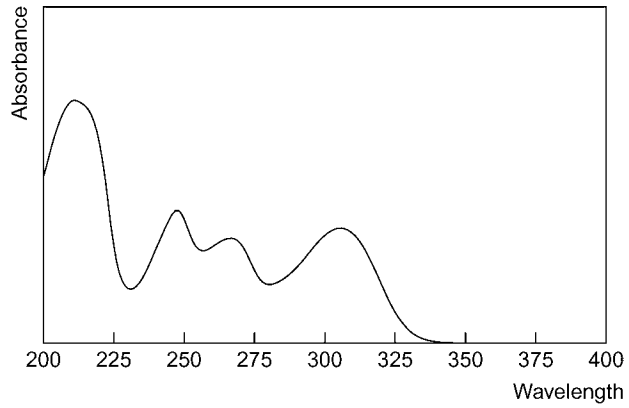


Chemical Properties Crystals from methanol. Mp 231° to 232°. Hydrochloride dihydrate; pK_a 7.4.

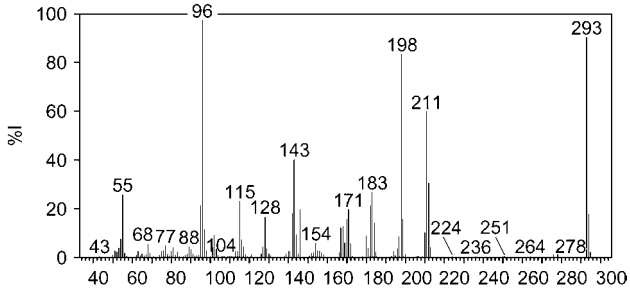
Ondansetron Hydrochloride Dihydrate

$C_{18}H_{19}N_3O$,HCl, $2H_2O$ = 365.9
CAS—99614-01-4
IUPAC Name 1,2,3,9-Tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one hydrochloride dihydrate
Synonyms GR-38032F; GR-C507/75; SN-307.
Proprietary Names *Zofran*; *Zophren*.
Chemical Properties A white crystalline solid from water/isopropanol. Mp 178.5° to 179.5°. Soluble in aqueous solutions, but solubility decreases with pH >5.7.

High Performance Liquid Chromatography System HZ—retention time 2.9 min.
Ultraviolet Spectrum Aqueous acid (pH 2.8)—210, 248, 266, 310 nm.



Mass Spectrum Principal ions at *m/z* 96, 293, 198, 211, 143, 183, 55, 115.



Quantification
Plasma **HPLC** Column: RP C_{18} Spherisorb (100 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : 20 mmol/L sodium phosphate monobasic buffer (pH 3 with phosphoric acid) (60:40), flow rate 1.5 mL/min. IS: loxapine. UV detection (λ=305 nm). Retention time: ondansetron, 3.9 min; IS, 5.5 min. Limit of quantification, 0.5 µg/L [Depot *et al.* 1997]. Column: Spherisorb silica S3W (100 × 4.6 mm i.d., 3 µm). Temperature: 35°. Mobile phase: 25 mmol/L sodium acetate buffer (pH 4.2) : acetonitrile (6:4), flow rate 1 mL/min. UV detection (λ=305 nm). Retention time: 4–5 min. Limit of detection, 1 µg/L [Colthup *et al.* 1991].
Serum **HPLC** MS detection. Limit of detection 0.25 µg/L [Xu *et al.* 2000]. UV detection (λ=210 nm). Limit of quantification, 15 µg/L for each enantiomer, limit of detection, 7 µg/L [Liu, Stewart 1997]. Column: Chiralcel OD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : 95% ethanol : 2-propanol : acetonitrile (65 : 25 : 10 : 1), flow rate 1 mL/min. IS: prazosin. UV detection (λ=216 nm). Retention time: R(−)-ondansetron, 10.0 min; S(+)-ondansetron, 11.6 min; IS, 8.0 min. Limit of quantification, 10 µg/L for each enantiomer, limit of detection, 2.5 µg/L [Kelly *et al.* 1993].
Disposition in the Body Ondansetron is readily and completely absorbed after oral administration with peak plasma concentrations of 0.03 to 0.04 mg/L (after an 8 mg dose) being reached within 2 h. The drug is first detected in plasma after 30 min. It undergoes extensive hepatic metabolism with <5% being excreted via urine unchanged. At least 40% of a single dose is oxidised to 8-hydroxy-ondansetron in the liver, 20% to 7-hydroxy-ondansetron, <5% to 6-hydroxy-ondasetron and a little appears as *N*-demethyl-ondansetron. The metabolites have also been detected in faeces. These intermediates are glucuronide or sulfate conjugated before leaving the liver and being excreted in urine.
Therapeutic Concentration The serum therapeutic concentration range is 30 to 300 µg/L.

Twelve healthy male and female volunteers, aged between 26 and 69 years (mean age, 44.8 years) and 12 patients with mild, moderate and severe liver disease (4 for each), aged between 31 and 65 years (mean, 47.3), were administered with an 8 mg oral dose or an 8 mg IV dose. Peak plasma concentrations for the oral dose were 38.6, 40.1 and 43.0 µg/L for the mild, moderate and severe hepatically impaired patients, respectively, and 27.9 µg/L for the healthy individuals. These concentrations were observed at 1.25, 1.07, 1.5 and 1.9 h, respectively. Peak concentrations for the IV dose were 97.8 µg/L for the patients with mild liver disease, 115.0 µg/L moderate liver disease and 64.5 µg/L for those with severe liver disease, observed at 0.38, 0.25 and 0.31 h, respectively. The healthy individuals reached a peak at 129 µg/L in 0.22 h [**Figg *et al.* 1996**].

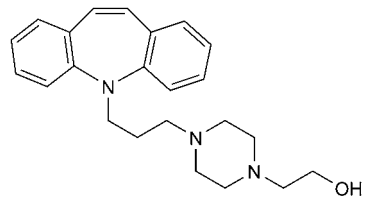
Bioavailability 60% (young healthy subjects), 65% (elderly); 85% (patients with cancer) and 100% (severe hepatic impairment).
Half-life 3 h (young healthy subjects), 5 h (elderly) and 15 to 32 h (severe hepatic impairment).
Volume of Distribution Approximately 140 to 160 L; also reported as 1.3 to 2.9 L/kg. 3.05 L/kg (mild liver disease); 3.36 L/kg (moderate); 3.86 L/kg (severe); 2.5 L/kg (healthy individuals).
Clearance 16.6 L/h (patients with mild liver disease); 15.9 L/h (moderate liver disease); 11.6 L/h (severe); 28.3 L/h (healthy volunteers).
Distribution in Blood Blood : plasma ratio is 0.83. It distributes into erythrocytes and circulates bound within.
Protein Binding 70 to 75%.
Dose Adult: 8 mg (orally) before treatment followed by 8 mg every 12 h. 16 mg daily (by rectum administration) or 32 mg (IV). Children: 5 mg/m^2 (IV) immediately before treatment and then 4 mg orally every 12 h. Alternatively, 100 µg/kg (maximum 4 mg) (over 2 years old).

Colthup PV *et al.* (1991). Determination of ondansetron in plasma and its pharmacokinetics in the young and elderly. *J Pharm Sci* 80(9): 868–871.
Depot M *et al.* (1997). High-resolution liquid chromatographic method using ultraviolet detection for determination of ondansetron in human plasma. *J Chromatogr Biomed Sci Appl* 693: 399–406.
Figg WD *et al.* (1996). Pharmacokinetics of ondansetron in patients with hepatic insufficiency. *J Clin Pharmacol* 36: 206–215.
Kelly JW *et al.* (1993). High-performance liquid chromatographic separation of ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction. *J Chromatogr* 622: 291–295.
Liu J, Stewart JT (1997). High-performance liquid chromatographic analysis of ondansetron enantiomers in human serum using a reversed-phase cellulose-based chiral stationary phase and solid-phase extraction. *J Chromatogr Biomed Sci Appl* 694(1): 179–184.
Xu X *et al.* (2000). Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. *J Mass Spectrom* 35(11): 1329–1334.

Opipramol

Antidepressant
$C_{23}H_{29}N_3O$ = 363.5
CAS—315-72-0

IUPAC Name 2-[4-(3-benzo[*b*][1]benzazepin-11-ylpropyl)piperazin-1-yl] ethanol
Synonym 4-[3-(5*H*-Dibenz[*b*,*f*]azepin-5-yl)propyl]-1-piperazine-ethanol



Chemical Properties Crystals. Mp 100° to 101°.

Opipramol Hydrochloride

$C_{23}H_{29}N_3O$, 2HCl = 436.4
CAS—909-39-7
Proprietary Name *Insidon*
Chemical Properties Light-yellow crystalline powder that develops a reddish tinge on prolonged exposure to light. Mp ≈210°, with decomposition. Soluble in

water and ethanol; sparingly soluble in acetone. pK_a 3.8. Log *P* (octanol/water), 3.41 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Colour Tests Forrest reagent—blue; Mandelin's test—yellow-green.

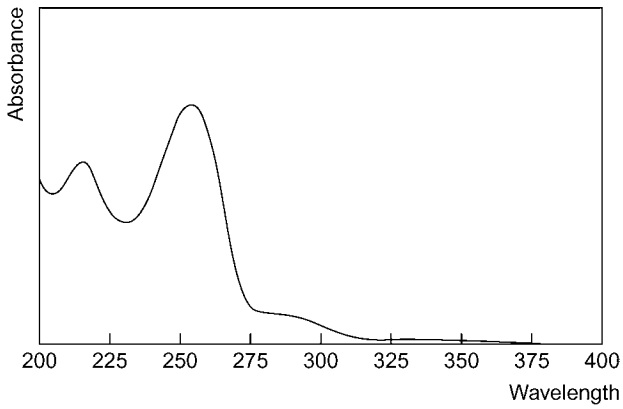
Thin-layer Chromatography System TA—R_f 0.54; system TAE—R_f 0.35; system TAF—R_f 0.39; system TAG—R_f 0.07; system TB—R_f 0.06; system TC—R_f 0.22; system TE—R_f 0.38 (acidified iodoplatinate solution, positive).

For a TLC double-radioisotope technique for the detection of opipramol in blood and other biological samples, see Riess [1977].

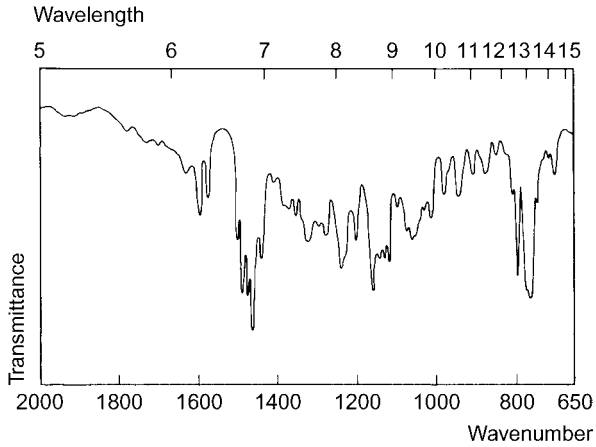
Gas Chromatography System GA—opipramol RI 3050, M (ring) RI 1985; system GB—opipramol RI 3219.

High Performance Liquid Chromatography System HAA—RT 14.2 min; system HA—*k* 2.2; system HF—*k* 1.63; system HX—RI 377; system HY—RI 340; system HZ—RT 3.9 min.

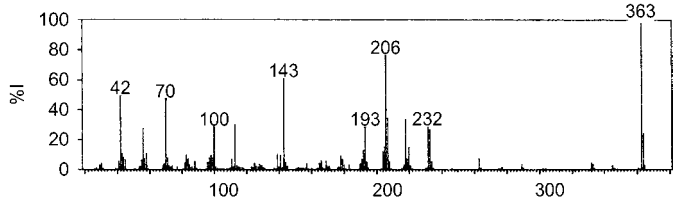
Ultraviolet Spectrum Aqueous acid—253 nm ($A_1^1 = 870$a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 755, 1155, 790, 1237, 1115, 1126 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 363, 206, 143, 42, 70, 207, 218, 113.



Quantification

Plasma **HPLC** Column: Phenomenex Luna CN (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate : 20 mmol/L acetic acid : acetonitrile (240 : 60 : 700), flow rate 1.0 mL/min. Photometric detection. Retention time: 8.7 to 8.9 min. Limit of quantification, 250 ng/L [Kees *et al.* 2001].

LC-MS Column: Symmetry C_{18} (150 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (28 : 72 for 4 min to 70 : 30 in 1 min for 3 min to 28 : 72 in 0.7 min), flow rate 0.6 mL/min. APCI, positive ion mode. Limit of quantification, 50 µg/L, limit of detection, 5 µg/L [Kollroser, Schober 2002].

Serum **LC-MS** Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.83 µg/L [Kirchherr, Kühn-Velten 2006]. Column: Uptisphere (12.5 cm × 2 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0)-acetonitrile : acetonitrile (100 : 0), flow rate 200 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 19 nmol/L [Gutteck, Rentsch 2003].

Disposition in the Body Readily absorbed after oral administration.

Therapeutic Concentration

Following the administration of a single oral dose of 50 mg opipramol hydrochloride to 20 healthy volunteers a mean peak plasma concentration of 15 µg/L was attained after ≈2 h [Kees *et al.* 2001].

Toxicity

Somnolence was reported in a 27-year-old subject following an overdose of opipramol; a maximum serum concentration of 0.115 mg/L was determined; ethanol was also detected [Pedersen *et al.* 1982].

In a fatality involving the intentional ingestion of an unknown quantity (up to 3 g) of opipramol dihydrochloride, postmortem tissue concentrations of opipramol dihydrochloride and deshdroxyethyl opipramol dihydrochloride, respectively, were: blood (heart) 29.6 and 5.2 mg/L; urine 19.1 and 4.7 mg/L; liver 260.7 and 50.6 µg/g; lung 68.9 and 24.0 µg/g; brain 113.5 and 5.1 µg/g; kidney 69.6 and 11.8 µg/g; muscle 9.1 and 1.5 µg/g [Skopp *et al.* 1996].

Dose 150 to 300 mg of opipramol hydrochloride daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Gutteck U, Rentsch KM (2003). Therapeutic drug monitoring of 13 antidepressant and five neuroleptic drugs in serum with liquid chromatography-electrospray ionization mass spectrometry. *Clin Chem Lab Med* 41: 1571–1579.

Kees F *et al.* (2001). Determination of opipramol in human plasma by high-performance liquid chromatography with photometric detection using a cyanopropyl column. *J Chromatogr B Biomed Sci Appl* 753: 337–342.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Kollroser M, Schober C (2002). Simultaneous determination of seven tricyclic antidepressant drugs in human plasma by direct-injection HPLC-APCI-MS-MS with an ion trap detector. *Ther Drug Monit* 24: 537–544.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Pedersen OL *et al.* (1982). Overdosage of antidepressants: clinical and pharmacokinetic aspects. *Eur J Clin Pharmacol* 23: 513–521.

Riess W (1977). The double radio-isotope derivative technique for the assay of drugs in biological material Part II. The simultaneous determination of opipramol and its deshydroxyethyl metabolite. *Anal Chim Acta* 88: 109–115.

Skopp G *et al.* (1996). Fatal poisoning with the antidepressive agent opipramol. *Forensic Sci Int* 77: 45–51.

Opium

Narcotic Analgesic

Synonyms Adormidera; blue bread seed poppy; chandu; gelinjik; gum opium; hashas; kheshkhash abu al noum; ikkanshu; madat; mawseed; O fang; O fu jung; opium crudum; O p'ien; papaver sominferum; pavot; poppy; raw opium; tengkoh; white poppy; ya p'ien; yu mi.

Proprietary Names It is an ingredient of *Bromocod N*; *BandO Supprettes No. 15A*; *Colchimax*; *Digestovital*; *Doveru*; *Lamaline*; *Pectyl*; *Stilpane*; *Tanagel*.

Street Names Ah-pen-yen; aunti; aunti emma; big O; black; black pill; black shit; black stuff; black tar opium; block; boulette; chandoo; chandu; China; Chinese molasses; Chinese tobacco; chocolate; cruz; dopium; Dover's deck; Dover's powder; dream gum; dream gun; dream stick; dreams; Dutch courage; easing powder; fi-donie; gee; God's medicine; goma; gondola; gong; goric; great tobacco; gum; guma; hard stuff; hocus; hop; hops; incense; Indonesian bud; joy plant; midnight oil; mira; mud; O; OP; ope; pen yan; pin gon; pin yen; pox; skee; tar; toxy; toys; when-shee; ze; zero.

Chemical Properties Opium is an air-dried latex obtained by incision from the unripe seed pods/capsules of *Papaver somniferum* (Papaveraceae). It varies in colour from yellow to dark brown and has a characteristic odour and a bitter taste. It is a non-homogeneous material containing poppy capsule fragments. It is sticky, tar-like and dark brown when fresh, and becomes brittle and hard as it ages. Prepared opium is a sticky dark product obtained as a result of various treatments of raw opium, e.g. water extraction, in order to make it suitable for smoking. It contains ~40 alkaloids, which constitute about 25% of the opium, up to 25% water, meconic acid, some lactic and sulfuric acids, albumin, mucilage, sugar, and some resinous and waxy-like substances. The principal active alkaloids are morphine (4 to 21%), codeine (0.7 to 3%), thebaine (0.2 to 1%), papaverine (0.5 to 1.3%), and noscapine (2 to 8%).

Colour Tests Froehde reagent→brown-black; Mecke's reagent→green-black.

Thin-layer Chromatography Plates: HPTLC silica gel F_{254} (10 × 20 cm, 0.1 mm). Solvent system: methanol : dichloromethane : hexane (100 : 0 : 0 to 50 : 50 : 0 to 0 : 100 : 0 to 0 : 0 : 100). Limit of detection, ethylmorphine, thebaine, papaverine, codeine1 µg; morphine, noscapine 2 µg [Pothier, Galand 2005].

Gas Chromatography Column: OV-1 or SE-54 cross-linked glass capillary (25 m × 0.27 mm i.d., 0.15 µm). Carrier gas: H_2, 110 cm/s (OV-1) or 65 cm/s (SE-54). Temperature programme: 150° to 280° at 9°/min. FID. Limit of detection not reported [Neumann 1984].

Note For a GC analysis of the headspace constituents of opium see [Buchbauer *et al.* 1994].

High Performance Liquid Chromatography Column: TSKgel ODS-120 A (250 × 4.6 mm i.d.) Mobile phase: acetonitrile : 10 mmol/L sodium 1-heptanesulfonate (pH 3.5; 20 : 80 to 30 : 70 at 15 min to 40 : 60 at 20 min for 5 min to 50 : 50 at 30 min to 20 : 80 at 35 min), flow rate 1 mL/min. DAD (λ = 284 nm). Limit of detection not reported [Yoshimatsu *et al.* 2005]. Column: Whatman 5 ODS-3 (125 × 3.2 mm) Mobile phase: methanol : phosphate-hexylamine buffer (pH 2.1; 2 : 98 for 3 min to 35 : 65 in 9.5 min for 4.5 min to 100 : 0 in 1 min for 4 min to 2 : 98 in 3 min), flow rate 0.76 mL/min for 14 min to 1.5 mL/min at 16 min for 3 min to 0.76 mL/min in 4 min for 5 min. DAD. Limit of detection, morphine 1.16 mg/L, papaverine 1.11 mg/L, codeine 1.18 mg/L, noscapine 0.94 mg/L, thebaine 1.10 mg/L [Lurie *et al.* 2003]. Column: Spherisorb CN (250 × 4.6 mm, 10 µm). Mobile phase: 1% aqueous ammonium acetate (pH 5.8) : acetonitrile : dioxane (100 : 10 : 5), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Trenerry *et al.* 1995]. Column: MN-Nucleosil 7 C_6H_5 (250 × 4.0 mm i.d., 7 µm). Mobile phase: methanol-water (pH 3.2; 5 : 95) : methanol-water (pH 3.95; 70 : 30; 100 : 0 to 0 : 100 in 20 min for 12 min to 100 : 0 in 8 min), flow rate 1.5 mL/min. UV detection (λ = 280 nm). Limit of detection not reported [Ayyangar, Bhide 1988]. Column: silica (30 cm × 3.9 mm i.d.) Mobile phase: isooctane : diethyl ether : methanol : diethylamine : water (400 : 325 : 175 : 1.5 : 0.5), flow rate 2 mL/min. UV detection. Limit of detection not reported [Wijesekera *et al.* 1988]. Column: µBondapak phenyl (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: 1% aqueous sodium acetate–7.0 mmol/L TEA : methanol (pH 11; 42 : 58), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Ayyangar, Bhide 1986].

Supercritical Fluid Chromatography Column: LiChrosorb Si 60 (120 × 4.6 mm i.d., 5 µm) or aminopropyl bonded silica (230 × 4.6 mm, 10 µm). Mobile phase: carbon dioxide : methanol : methylamine : water (83.37 : 16.25 : 0.15 : 0.23). DAD. Limit of detection not reported [Janicot *et al.* 1988].

Capillary Electrophoresis Capillary: uncoated fused silica (700 × 50 µm i.d., effective length 55 cm). Buffer: 100 mmol/L sodium acetate (pH 3.1) : methanol (3 : 7). UV detection (λ = 224 nm). Limit of detection not reported [Mohana *et al.* 2005]. Capillary: fused silica (320 × 50 µm i.d., effective length 23.5 cm). Buffer: 25 mmol/L HP-β-CD and 75 mmol/L DM-β-cyclodextrin in CElixir Reagent B (pH 2.5). Limit of detection, morphine 1.16 mg/L, papaverine 1.11 mg/L, codeine 1.18 mg/L, noscapine 0.94 mg/L, thebaine 1.10 mg/L [Lurie *et al.* 2003]. Capillary: uncoated fused silica (700 × 50 µm i.d., 55 cm from detector). Buffer: 100 mmol/L sodium acetate (pH 3.1) : methanol (3 : 7). UV detection (λ = 224 nm). Limit of detection, morphine 850 µg/L, thebaine 450 µg/L, codeine and narcotine 500 µg/L, papaverine 550 µg/L [Reddy *et al.* 2003]. Capillary: fused silica (640 × 50 µm i.d., 55 cm to detector). DAD (λ = 214 nm). Limit of detection not reported [Bjornsdottir, Hansen 1995a]. Capillary: fused silica (550 × 50 µm i.d.). Buffer: 0.05 mol/L 6-ACA (pH 4.0) containing Tween 20 and SDS. Limit of detection, morphine 0.3 mg/L, noscapine 0.08 mg/L, normorphine 0.2 mg/L, thebaine 0.2 mg/L, codeine 0.2 mg/L, papaverine 0.2 mg/L [Bjornsdottir, Hansen 1995b]. Capillary: uncoated fused silica (700 × 50 µm i.d., effective length 450 mm). Buffer: DMF : 0.05 mol/L cetyltrimethylammonium bromide-0.01 mol/L potassium dihydrogen orthophosphate-0.01 mol/L sodium tetraborate (pH 8.6; 10 : 90). UV detection (λ = 254 nm). Limit of detection, not reported [Trenerry *et al.* 1995].

Note For a capillary electrophoresis method with electrochemiluminescence for the determination of opiate alkaloids in Chinese herbal medicines, see Gao *et al.* [2006]. For the detection and characterisation of reticuline as a marker of opium use, see Al Amri *et al.* [2004]. For a comparison of raw, prepared and dross opium, see [Lim and Kwok 1981] and for a summary of opium characterisation methods, see Remberg *et al.* [1994].

Quantification

***Blood* HPLC** Column: Hypersil BDS Phenyl Rocket (57 × 7.0 mm i.d., 3 µm). Mobile phase: water-methanol (90 : 10) : methanol-25 mmol/L TEA-30.3 mmol/L formic acid (100 : 0 to 50 : 50 in 10 min to 0 : 100 in 2 min for 1 min to 100 : 0 in 2 min), flow rate 2.0 mL/min. DAD (λ= 240 nm) or fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 335 nm). Limit of detection, morphine 7.6 (DAD) and 3.3 µg/L, codeine 6.3 (DAD) and 2.3 µg/L, papaverine 1.6 µg/L (DAD), noscapine 4.1 µg/L (DAD) [Dams *et al.* 2002].

***Plasma* HPLC** Column: µBondaPak C_{18} reversed phase (300 × 4.0 mm i.d., 10 µm). Mobile phase: 5% methanol : 3% acetonitrile : 0.5 mmol/L sodium acetate : 0.012 mol/L potassium dihydrogen orthophosphate : 0.148 mmol/L phosphoric acid, flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 235 nm, λ_{em} = 349 nm). Limit of detection, 20 µg/L [Ghazi-Khansari *et al.* 2006].

***Urine* GC-MS** Column: DB-5 cross-linked 5% phenylmethyl silicone (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 2 min to 310° at 16°/min for 5 min. SIM or scan acquisition mode. Limit of detection, morphine 20 µg/L, 6-monoacetyl morphine (6-MAM) 25 µg/L in SIM mode, morphine 50 µg/L, 6-MAM 200 µg/L in scan mode [Paterson, Cordero 2006]. Column: HP-1MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 120° for 1 min to 280° at 20°/min for 6 min. SIM acquisition mode. Limit of quantification, morphine 30 µg/L, codeine 40 µg/L; limit of detection, morphine 10 µg/L, codeine 15 µg/L [Liu *et al.* 2006]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 80° for 2 min to 290° at 16°/min for 5.37 min. SIM acquisition mode. Limit of detection, morphine 0.02 mg/L, codeine 0.02 mg/L, 6-MAM 0.02 mg/L [Paterson *et al.* 2000, 2005].

HPLC Column: Hypersil BDS Phenyl Rocket (57 × 7.0 mm i.d., 3 µm). Mobile phase: water-methanol (90 : 10) : methanol-25 mmol/L TEA-30.3 mmol/L formic acid (100 : 0 to 50 : 50 in 10 min to 0 : 100 in 2 min for 1 min to 100 : 0 in 2 min), flow rate 2.0 mL/min. UV (λ = 240 nm) or fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 335 nm). Limit of detection not reported [Dams *et al.* 2002].

***Hair* GC-MS** Column: DB-1 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 150° for 2 min to 280° at 10°/min for 15 min. SIM acquisition mode. Limit of quantification, 0.03 µg/g; limit of detection, 0.016 µg/g [Sabzevari *et al.* 2004].

***Other* LC-MS** Water. Column: Synergi Polar-RP 80 Å (150 × 3 mm, 4 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 4; 10 : 90 for 5 min to 80 : 20 within 13 min to 10 : 90 within 2 min for 10 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, morphine 0.01, 0.005 and 0.001 µg/L in waste water (influent and effluent) and surface water, respectively; codeine 0.02, 0.01 and 0.002 µg/L in waste water (influent and effluent) and surface water, respectively [Hummel *et al.* 2006].

Toxicity

The following 7 cases describe deaths where morphine was detected postmortem.

Case 1: A 26-year-old man was found dead in his bed beside a bag of dried poppy capsules.

Case 2: A 19-year-old man was found dead in his home. For the previous 3 weeks he had injected amfetamine and consumed a large quantity of diazepam. In the previous 2 days he had drunk large quantities of tea made from poppy capsules.

Case 3: A 28-year-old man consumed a large quantity of tea made from poppy capsules and was found dead the next day.

Case 4: An 18-year-old man died shortly after arriving at a friend's home. He had a pot of poppy capsules and a syringe amongst his possessions.

Case 5: A 34-year-old man was found dead and a pot of poppy capsules was found in his flat. He had been prescribed 25 × 5 mg methadone tablets and 200 mL codeine syrup 3 days before death.

Case 6: A 33-year-old woman died after drinking tea made from boiled poppy capsules. She had been prescribed clobazam, clomipramine and levomepromazine 4 days before her death.

Case 7: A 25-year-old man was found dead in his flat. There were boiled poppy capsules and used syringes in his flat.

The following table shows the concentrations of morphine found upon postmortem examination. In all cases morphine was detected in the range normally seen in deaths among addicts following the intake of morphine or diamorphine.

Case	*Morphine (µmol/kg)*	
	Blood/muscle	*Liver*
1	B: 4.8	2.6
2	B: 0.3	0.7
3	B: 0.2	–
4	B: 6.7	2.5
5	B: 0.6	0.3
6	B: 3.3	6.6
7	M: 0.7	4.1

B, blood; M, muscle. [Steentoft *et al.* 1988].

Note For a study of arsenic levels in Indian opium eaters, see Narang *et al.* [1987]; for an overview of opium overdose in Iran, see Karbakhsh and Salehian [2007].

Dose Opium is intended only as the starting material for the manufacture of galenical preparations and is not dispensed as such. It is used as Prepared Opium (Ph. Eur 5.5), as Powdered Opium (USP 29), as Opium Tincture (BP 2005 or USP 29), or as Camphorated Opium Tincture (BP 2005) or Paregoric (USP 29) in various oral preparations. These have included Opiate Squill Linctus (BP 2005; Gee's linctus) for cough.

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Ghazi-Khansari M *et al.* (2006). Determination of morphine in the plasma of addicts in using Zeolite Y extraction following high-performance liquid chromatography. *Clin Chim Acta* 364: 235–238.
Hummel D *et al.* (2006). Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. *Environ Sci Technol* 40: 7321–7328.
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Mohana M *et al.* (2005). Principal opium alkaloids as possible biochemical markers for the source identification of Indian opium. *J Sep Sci* 28: 1558–1565.
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Remberg B *et al.* (1994). Fifty years of development of opium characterization methods. *Bull Narc* 46: 79–108.
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Steentoft A *et al.* (1988). Fatal intoxications in Denmark following intake of morphine from opium poppies. *Z Rechtsmed* 101: 197–204.
Trenerry VC *et al.* (1995). Determination of morphine and related alkaloids in crude morphine, poppy straw and opium preparations by micellar electrokinetic capillary chromatography. *J Chromatogr A* 718: 217–225.
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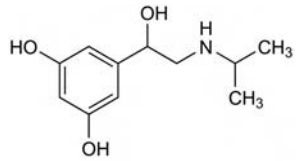
Yoshimatsu K *et al.* (2005). A rapid and reliable solid-phase extraction method for high-performance liquid chromatographic analysis of opium alkaloids from papaver plants. *Chem Pharm Bull (Tokyo)* 53: 1446–1450.

O

Orciprenaline

Sympathomimetic
$C_{11}H_{17}NO_3 = 211.3$
CAS—586-06-1

IUPAC Name 5-[1-Hydroxy-2-[(1-methylethyl)amino]ethyl]-1,3-benzenediol
Synonym Metaproterenol



Chemical Properties Crystals. Mp 100°. pK_a 9.0 (—OH), 10.1 (—NH—), 11.4 (—OH), (25°). Log *P* (octanol/water), 0.2. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

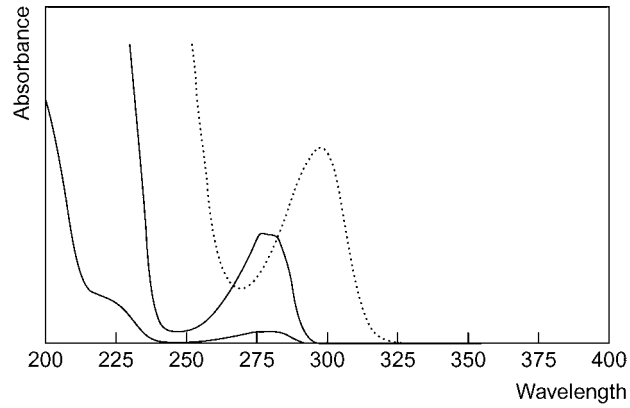
Orciprenaline Sulfate

$(C_{11}H_{17}NO_3)_2, H_2SO_4 = 520.6$
CAS—5874-97-5

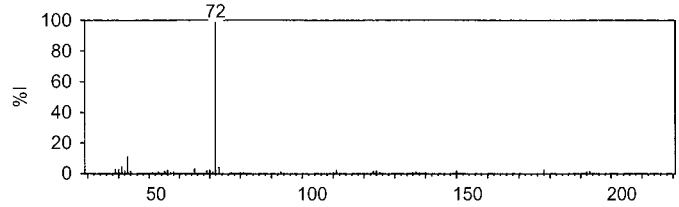
Proprietary Names *Alupent*; *Dosalupent*; *Metaprel.*
Chemical Properties A white crystalline powder. Mp about 205°. Soluble 1 in 2 of water and 1 in 1 of ethanol; practically insoluble in chloroform and ether.

Colour Tests *p*-Dimethylaminobenzaldehyde—orange/violet; Folin–Ciocalteu reagent—blue; Marquis test—yellow; potassium dichromate (method 1)—brown (slow).
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.01; system TC—R_f 0.03; system TE—R_f 0.18; system TL—R_f 0.06; system TAE—R_f 0.21; system TAF—R_f 0.77 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 151; system HY—RI 131.

Ultraviolet Spectrum Aqueous acid—276 nm (A^1_1=89a); aqueous alkali—297 nm (A^1_1=155a).



Infrared Spectrum Principal peaks at wavenumbers 1086, 1173, 1605, 1010, 1305, 995 cm^{-1} (orciprenaline sulfate, KBr disk).
Mass Spectrum Principal ions at *m/z* 72, 43, 73, 41, 70, 65, 40, 39.



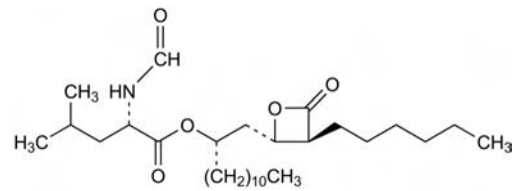
Quantification
Plasma **GC–MS** Limit of detection, 100 ng/L [Leis *et al.* 1990].
Dose 80 mg of orciprenaline sulfate daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings at the 12th TIAFT*, Seoul: 481–486.
Leis HJ *et al.* (1990). Quantitative determination of terbutaline and orciprenaline in human plasma by gas chromatography/negative ion chemical ionization/mass spectrometry. *Biomed Environ Mass Spectrom* 19: 382–386.

Orlistat

Antiobesity Agent, Gastric and Pancreatic Lipase Inhibitor
$C_{29}H_{53}NO_5 = 495.7$
CAS—96829-58-2

IUPAC Name [(2*S*)-1-[(2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl]tridecan-2-yl] (2*S*)-2-formamido-4-methylpentanoate
Synonyms *N*-Formyl-L-leucine (1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]-methyl] dodecyl ester; orlipastat; Ro-18-0647; Ro-18-0647/002; (−)-tetrahydrolipstatin.
Proprietary Name *Xenical*



Chemical Properties A white to off-white crystalline powder. Mp 40° to 43°. Practically insoluble in water; freely soluble in chloroform; very soluble in methanol and ethanol. pK_a is out of physiological range.
Quantification
Plasma **HPLC–MS** Column: Spherisorb C_6 (100 × 2 mm, 2 µm). Mobile phase: (A) acetonitrile : 0.1% formic acid (95 : 5), flow rate 150 µL/min; (B) methanol : water (85 : 15), flow rate 150 µL/min. Rapid change to 100% methanol at 300 µL/min for 3 min then re-equilibration with 85% methanol. MS–MS detection. Retention time: (A) 1.1 min; (B) 2.4 min. Limit of quantification, 0.3 µg/L [Wieboldt *et al.* 1998]. MS–MS detection. Limit of detection, 0.2 µg/L [Bennett *et al.* 1997].
Serum **HPLC–MS** Limit of detection, 0.25 µg/L [Xu *et al.* 2000].

Disposition in the Body Orlistat is not absorbed following oral administration and plasma concentrations of intact orlistat are non-measurable. There is no evidence of accumulation and no defined systemic pharmacokinetics. Metabolism may occur within the gastrointestinal wall. There are two metabolites M1 (4-member lactone ring hydrolysed) and M3 (M1 with *N*-formyl leucine moiety cleaved) which account for ~42% of the total plasma concentration. Elimination is mainly by faecal excretion of the unabsorbed drug, with ~97% of the administered dose excreted in this way, 83% of which is the unchanged drug. Cumulative renal excretion of total orlistat related material was <2% of the dose. Complete excretion occurs within 3 to 5 days. Orlistat, M1 and M3 are all subject to biliary excretion.

Therapeutic Concentration With therapeutic doses, detection of intact orlistat in plasma is difficult and concentrations are extremely low, <10 µg/L. Low plasma levels of M1 (26 µg/L) and M3 (108 µg/L) are observed 2 to 4 h after a therapeutic dose. <5 µg/L 8 h following administration with 360 mg orlistat.

Half-life Ranges between 1 and 2 h for the parent drug; for the metabolites ~2 h for M1 and 13.5 h for M3.

Volume of Distribution Cannot be determined because it is minimally absorbed and no definite systemic pharmacokinetics are observed.

Distribution in Blood Minimally partitions into erythrocytes.

Protein Binding >99%. Mainly lipoprotein and albumin.

Dose 120 mg three times a day.

Bennett PK *et al.* (1997). Quantitative determination of Orlistat (tetrahydrolipostatin, Ro 18-0647) in human plasma by high-performance liquid chromatography coupled with ion spray tandem mass spectrometry. *J Mass Spectrom* 32: 739–749.

Wieboldt R *et al.* (1998). Quantitative liquid chromatographic-tandem mass spectrometric determination of orlistat in plasma with a quadrupole ion trap. *J Chromatogr Biomed Sci Appl* 708: 121–129.

Xu X *et al.* (2000). Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. *J Mass Spectrom* 35(11): 1329–1334.

Orphenadrine

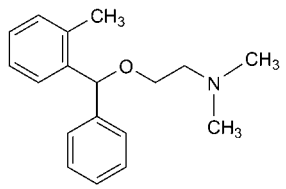
Anticholinergic

$C_{18}H_{23}NO = 269.4$

CAS—83-98-7

IUPAC Name *N*,*N*-Dimethyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine

Synonyms Mephenamine; orphenadin.



Chemical Properties Liquid. pK_a 8.4. Log *P* (octanol/water), 3.8.

Orphenadrine Citrate

$C_{18}H_{23}NO, C_6H_8O_7 = 461.5$

CAS—4682-36-4

Proprietary Names *Banflex*; *Flexoject*; *Flexon*; *Myolin*; *Norflex*; *Orfenace*. It is an ingredient of *Norgesic* and *Orphengesic*.

Chemical Properties White crystalline powder. Mp 134° to 138°. Soluble 1 in 70 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

Orphenadrine Hydrochloride

$C_{18}H_{23}NO, HCl = 305.8$

CAS—341-69-5

Synonym BS-5930

Proprietary Names *Biorphen*; *Disipal*; *Lysantin*; *Orfenal*.

Chemical Properties White crystalline powder. Mp 156° to 157°. Soluble 1 in 1 of water, 1 in 1 of ethanol, and 1 in 2 of chloroform; sparingly soluble in acetone and benzene; practically insoluble in ether.

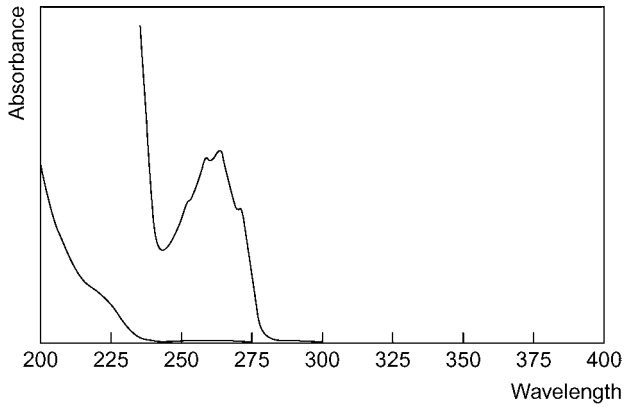
Colour Tests Mandelin's test—orange; Marquis test—yellow-orange; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.48; system TC—R_f 0.33; system TE—R_f 0.68; system TL—R_f 0.16; system TAE—R_f 0.25; system TAF—R_f 0.49; system TAJ—R_f 0.14; system TAK—R_f 0.02; system TAL—R_f 0.47 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, yellow).

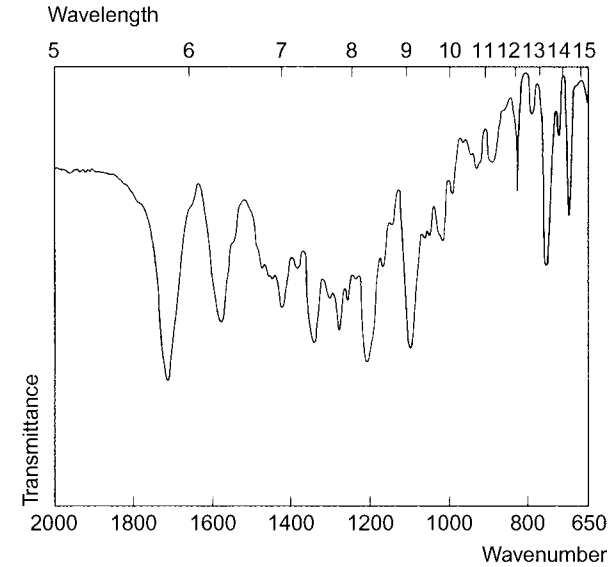
Gas Chromatography System GA—orphenadrine RI 1935, M (nor-) RI 1900, M (methylbenzophenone) RI 1700; system GB—orphenadrine RI 2014, M (nor-) RI 2007, M (methylbenzophenone) RI 1700; system GF—RI 2185.

High Performance Liquid Chromatography System HA—orphenadrine *k* 3.0, *N*-monodesmethylorphenadrine *k* 1.7, orphenadrine *N*-oxide *k* 1.1 (tailing peak); system HX—RI 418; system HY—RI 323; system HZ—RT 6.0 min.

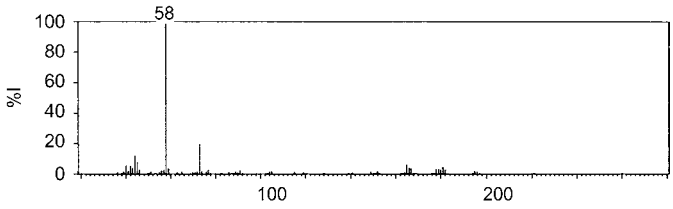
Ultraviolet Spectrum Aqueous acid—258, 264 nm ($A_1^1 = 24a$). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1718, 1216, 1108, 1285, 1585, 1265 cm^{-1} (orphenadrine citrate, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 73, 44, 45, 165, 42, 40, 181 (no peaks above 200); *N*-monodesmethylorphenadrine 44, 59, 165, 166, 181, 179, 178, 43; orphenadrine *N*-oxide 58, 181, 43, 45, 60, 44, 165, 73.



Quantification

Blood **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3 mL/min. Temperature programme: 170° for 1 min to 280° at 10°/min. FID. Limit of detection, 136 µg/L [Nishikawa *et al.* 1997]. Column: DB-1 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. NPD. Retention time: 14.7 min. Limit of detection, 100–250 ng/L [Hattori *et al.* 1992]. Column: 2.5% OV-101 on Supelco Chromosorb AW DMCS 80/100 mesh (1 m × 2 mm i.d.). Temperature: 210°. FID. Limit of detection not reported [Wilkinson *et al.* 1983]. Column: 3.8% W-98 on 80/100 mesh AW-DMCS Chromosorb W (1.8 m × 3.5 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 220°. FID. Retention time: 2.1 min. Limit of detection not reported [Robinson *et al.* 1977].

Plasma **GC** Column: DB-17 (30 m × 0.53 mm i.d.). Carrier gas: N_2, 15 mL/min. Temperature: 220°. NPD. Limit of detection, ≈5 µg/L [Contin *et al.* 1987]. Column: 3% KOH plus 3% Carbowax 20M on 100/120 mesh Gas Chrom Q (1.3 and 1.5 m × 2.3 mm i.d.). Carrier gas: 30 mL/min. AFID. Retention time: 3.65 min. Limit of detection, 1 µg/L [Labout *et al.* 1977].

Urine **GC** See Blood [Nishikawa *et al.* 1997]. See Blood [Wilkinson *et al.* 1983]. See Plasma [Labout *et al.* 1977]. See Blood [Robinson *et al.* 1977].

GC See Blood [Hattori *et al.* 1992].

Bile **GC** See Blood [Robinson *et al.* 1977].
Stomach Contents **GC** See Blood [Wilkinson *et al.* 1983]. See Blood [Robinson *et al.* 1977].
Kidney **GC** See Blood [Robinson *et al.* 1977].
Liver **GC** See Blood [Wilkinson *et al.* 1983]. See Blood [Robinson *et al.* 1977].
Lung **GC** See Blood [Robinson *et al.* 1977].
Spleen **GC** See Blood [Robinson *et al.* 1977].
Disposition in the Body Readily absorbed after oral administration and rapidly distributed. Up to ~60% of an oral dose is excreted in the urine in 3 days. During 24 h after dosage, under uncontrolled conditions, ~4% of a dose is excreted as unchanged drug, ~5% as *N*-monodesmethylorphenadrine (tofenacin), ~3% as *N,N*-didesmethylorphenadrine, ~3% as orphenadrine *N*-oxide, ~8% as a conjugate of 2-methylbenzhydryloxyacetic acid, and ~6% as a conjugate of 2-methylbenzhydrol. The urinary excretion appears to be dependent on urinary pH.
Therapeutic Concentration In plasma, usually 0.1–0.2 mg/L.
Toxicity The lethal dose is estimated to be >2 g. Blood concentrations >0.5 mg/L may cause toxic reactions and concentrations >5 mg/L may be lethal.

In a survey of 9 fatalities attributed to orphenadrine overdose, blood concentrations ranged from 1.1–37 mg/L (mean 15); in 5 cases the bile concentration was 85–234 mg/L (mean 150), and in 7 cases the urine concentration was 3–122 mg/L (mean 53). In 1 case in which a more complete analysis was reported, the concentrations were: blood 33 mg/L, bile 202 mg/L, brain 3.3 µg/g, kidney 15 µg/g, liver 23 µg/g, lung 19.5 µg/g, spleen 26.5 µg/g [Robinson *et al.* 1977].

In a fatality due to an orphenadrine overdose in which death occurred within 2.5 h of ingestion, the following postmortem concentrations were reported: blood 18.1 mg/L, liver 242 µg/g, urine 7 mg/L [Wilkinson *et al.* 1983].

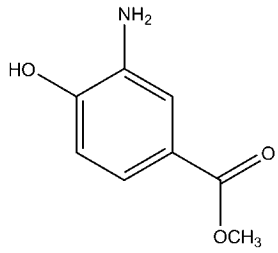
A 38-year-old male whose death was attributed to massive ingestion of orphenadrine and clozapine with suicidal intent, had the following postmortem tissue concentrations: heart blood 183 and 44 mg/L (orphenadrine and clozapine, respectively), urine 331 and 245 mg/L, gastric contents – and 859 mg/L; the heart blood concentration of orphenadrine (183 mg/L) was the highest reported thus far in an acute intoxication [Fucci *et al.* 2001].

Half-life Plasma half-life, ~14 h.
Protein Binding ≈20%.
Dose 150 to 400 mg of orphenadrine hydrochloride daily.

Contin M *et al.* (1987). Simple and rapid GLC method for the determination of orphenadrine in human plasma. *Biomed Chromatogr* 2: 193–194.
Fucci N *et al.* (2001). Acute intoxication with orphenadrine and clozapine. *Forensic Sci Int* 123: 13–16.
Hattori H *et al.* (1992). Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 581: 213–218.
Labout JJ *et al.* (1977). Sensitive and specific gas chromatographic and extraction method for the determination of orphenadrine in human body fluids. *J Chromatogr* 144: 201–208.
Nishikawa M *et al.* (1997). Simple analysis of diphenylmethane antihistaminics and their analogues in bodily fluids by headspace solid-phase microextraction-capillary gas chromatography. *J Chromatogr Sci* 35: 275–279.
Robinson AE *et al.* (1977). Forensic toxicology of some orphenadrine-related deaths. *Forensic Sci* 9: 53–62.
Wilkinson LF *et al.* (1983). A report on the analysis of orphenadrine in post mortem specimens. *J Anal Toxicol* 7: 72–75.

Orthocaine

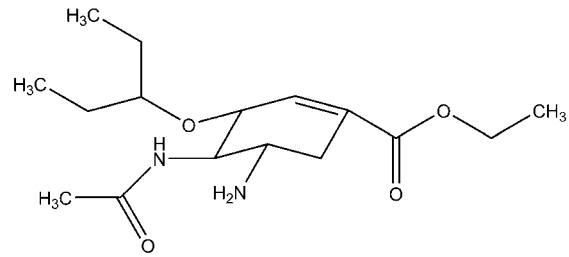
Anaesthetic (Local)
$C_8H_9NO_3$ = 167.2
CAS—536-25-4
IUPAC Name Methyl 3-amino-4-hydroxybenzoate
Synonyms Aminobenz; methyl aminoxybenzoate.
Proprietary Name *Orthoform*



Chemical Properties White crystalline powder. Mp 143°. Almost insoluble in water; soluble 1 in 7 ethanol and 1 in 50 of ether. Orthocaine is extracted by organic solvents from aqueous alkaline solutions.
Colour Tests Ammonium molybdate test—(dull green) brown, fading (limit of detection, 1.0 µg); ammonium vanadate test—(grey-brown) brown (limit of detection, 1.0 µg); *p*-dimethylaminobenzaldehyde test—yellow (limit of detection, 1.0 µg); Vitali's test—yellow→blue-grey/brown/dark brown (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.71 (location reagent: *p*-dimethylaminobenzaldehyde spray, yellow; potassium permanganate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid—253 nm (E1%, 1 cm 836).
Disposition in the Body
Metabolism Owing to its insolubility, orthocaine penetrates the tissues very slowly. It is excreted largely in the conjugated form; a certain amount may be acetylated at the amino-group.
Toxicity The maximum amount stated to be safe for surface anaesthesia is 5 g.
Dose Doses of up to 3 g daily have been given but considerable risk attends its internal administration. Externally, it is employed as a dusting-powder or as a 5 or 10% ointment.

Oseltamivir

Antiviral, Ester, Neuraminidase Inhibitor
$C_{16}H_{28}N_2O_4$ = 312.4
CAS—196618-13-0
IUPAC Name Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-pentan-3-yloxycyclohexene-1-carboxylate
Synonyms Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate; GS-4071.



Chemical Properties Pale solid. pK_a 7.7 (25°), 6.6 (70°) [Oliyai *et al.* 1998]. Log *P* (octanol/water), 0.36 oseltamivir, −2.1 metabolite [Oo *et al.* 2003a]. Low concentrations of oseltamivir in plasma at room temperature have been observed to decrease after 16 h by about 15 to 20%. It has been shown to be stable in plasma stored at –20° for up to 6 months and at room temperature for 60 min [Wiltshire *et al.* 2000].

Oseltamivir Phosphate

$C_{16}H_{28}N_2O_4$, H_3PO_4 = 410.4
CAS—204255-11-8
IUPAC Name Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (1 : 1)
Synonyms GS-4104/002; oseltamivir, fosfato de; Ro-64-0796/002.
Proprietary Name *Tamiflu*
Chemical Properties White crystalline solid. Very soluble in water. Oral solutions of oseltamivir phosphate with sodium benzoate have been shown to be stable for up to 46 days [Albert, Bockshorn 2007].

High Performance Liquid Chromatography Column: C_{18} ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium acetate in acetonitrile-water (7.5 : 92.5) : 50 mmol/L sodium acetate in acetonitrile-water (60 : 40; 100 : 0 to 0 : 100 over 15 min), flow rate 1.5 mL/min. UV detection (λ = 220 nm). Retention time: oseltamivir 9.8 min, oseltamivir isomer 10.7 min, carboxylic acid 4.2 min, carboxylic acid isomer 3.6 min [Oliyai *et al.* 1998].
Quantification
Plasma **HPLC** Column: ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium acetate in acetonitrile : water (27 : 73), flow rate 2 mL/min. Fluorescence detection (λ_{ex} = 420 nm, λ_{em} = 472 nm). Derivatisation agent, naphthalene-2,3-dialdehyde. Retention times: oseltamivir 5.3 min, carboxylate 4.3 min. Limit of quantification, 50 µg/L; limit of detection, 20 µg/L [Eisenberg, Cundy 1998].

LC-MS Column: Nova-Pak CN HP (100 × 5 mm i.d., 4 µm). Mobile phase: methanol : 80 mmol/L aqueous formic acid (pH 3; 50 : 50), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: oseltamivir 5 min, metabolite 3.5 min. Limit of quantification, oseltamivir 1 µg/L, metabolite 10 µg/L [Wiltshire *et al.* 2000].
Urine **LC-MS** Column: Nova-Pak CN HP (100 × 5 mm i.d., 4 µm). Mobile phase: methanol : 80 mmol/L aqueous formic acid (pH 3; 50 : 50), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: oseltamivir 5 min, metabolite 3.5 min. Limit of quantification, oseltamivir 5 µg/L, metabolite 30 µg/L [Wiltshire *et al.* 2000].
Other **HPLC** Oral Solutions. Column: Nucleodur 100-5 C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate : acetonitrile-50 mmol/L ammonium acetate (60 : 40; 90 : 10 to 0 : 100 over 20 min), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: oseltamivir 14.9 min, oseltamivir isomer 16.0 min, oseltamivir carboxylic acid 7.2 min, oseltamivir carboxylic acid isomer 6.1 min. Limit of quantification, oseltamivir 16 mg/L, oseltamivir carboxylic acid 35 mg/L; limit of detection, oseltamivir 7 mg/L, oseltamivir carboxylic acid

10 mg/L [Albert, Bockshorn 2007]. Capsules. Column: Hypersil Gold (150 × 4.6 mm i.d.). Mobile phase: methanol : 0.1 mol/L phosphate buffer (pH 2.5; 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Retention time: ~6.2 min. Limit of quantification not reported [Lindegardh *et al.* 2006]. Capsules. Column: Zorbax CN (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.04 mol/L formic acid (pH 3.0; 50 : 50), flow rate 1.2 mL/min. UV detection (λ = 226 nm). Retention time: oseltamivir phosphate 3.4 min. Limit of quantification, 30 mg/L [Joseph-Charles *et al.* 2007].

Disposition in the Body Pro-drug readily absorbed from the gastrointestinal tract. Approximately 80% of an orally administered dose of oseltamivir reaches the systemic circulation as the single active metabolite, oseltamivir carboxylic acid (Ro 64-0802 or GS4057). This conversion is mediated by hepatic and/or intestinal carboxylesterases. No other metabolites have been identified in humans. *In vitro* studies have shown that neither oseltamivir nor its metabolite interacts with CYP450 mixed-function oxidases or glucuronyltransferases. Measurable plasma concentrations of the metabolite are found within 30 min, reach peak concentrations in 2 to 3 h post-dose, and are roughly 20 times those of oseltamivir. Oseltamivir and its metabolite distribute well into the sites of influenza infection (lung, trachea, nasal mucosa and middle ear) and the concentration at these sites is high enough to inhibit viral replication. Elimination is exclusively by renal excretion, with 60 to 70% of an oral dose appearing in urine as the active metabolite, and <5% as oseltamivir. Renal clearance of oseltamivir and its metabolite exceeds the glomerular filtration rate, indicating that renal tubular secretion contributes to the elimination of the compound. Tubular secretion occurs via the anionic transport process. Less than 20% of an oral dose is recovered in faeces (equal proportions between oseltamivir and the active metabolite).

Therapeutic Concentration

A group of 36 healthy adult male volunteers (18 to 55 years) was administered single doses (20 to 1000 mg) of oral oseltamivir for 7 days. Mean peak plasma concentrations for oseltamivir and its active metabolite were reported as follows:

Dose (mg)	Oseltamivir		Metabolite	
	C_{max} (µg/L)	Time (h)	C_{max} (µg/L)	Time (h)
20	11.5	1.1	67.9	4.9
100	46.0	1.2	250	3.7
500	564	0.8	1682	3.7
1000	809	1.1	3955	3.8

[Massarella *et al.* 2000].

In a separate study, 18 healthy elderly volunteers (>65 years) were administered oral doses of 100, 150 and 200 mg oseltamivir twice daily for 7 days. Mean peak plasma concentrations were reported as follows:

Dose (mg)	Study day	Oseltamivir		Metabolite	
		C_{max} (µg/L)	Time (h)	C_{max} (µg/L)	Time (h)
100	1	95.4	0.93	432	3.8
	7	105	0.58	575	3.3
150	1	179	0.58	579	4.2
	7	192	0.58	897	3.5
200	1	302	0.5	796	3.7
	7	256	0.60	1293	2.8

[Massarella *et al.* 2000].

Two groups of 12 healthy children (group A: 3 to 5 years; group B: 1 to 2 years) were administered a single oral dose of oseltamivir (group A: 45 mg; group B: 30 mg) as a suspension after breakfast. Mean peak plasma concentrations were reported as follows:

	Oseltamivir		Metabolite	
	Group A	Group B	Group A	Group B
C_{max} (µg/L)	57	35	179	121
Time (h)	1.5	1.8	5.0	5.6

Oral clearance of the metabolite was found to be higher in group B than in group A [Oo *et al.* 2003b].

Eight healthy male volunteers were administered oseltamivir (150 mg) to 4 separate sites along the gastrointestinal tract: stomach, proximal small bowel (jejunum), distal small bowel (ileum), and the ascending colon. Pharmacokinetic parameters were reported as follows:

	Stomach	Jejunum	Ileum	Ascending colon
Oseltamivir				
C_{max} (µg/L)	99.5	104	132	48.6
Time (h)	0.5	0.61	0.56	0.84
Metabolite				
C_{max} (µg/L)	487	463	442	197
Time (h)	2.9	3.9	3.2	5.9

[Oo *et al.* 2003a].

Bioavailability Approximately 80%.

Half-life Oseltamivir, 1 to 3 h; oseltamivir carboxylic acid, 6 to 10 h (oral dose), 1.8 h (IV dose).

Volume of Distribution Steady state, 23 to 26 L.

Clearance Approximately 13 to 21.7 L/h; reduced to 1.5 to 7.5 L/h in patients with renal impairment.

Protein Binding Oseltamivir, 42%; oseltamivir carboxylic acid, <3%.

Dose Given as the phosphate, but doses are expressed in terms of the base. Oseltamivir phosphate 98.5 mg is equivalent to ~75 mg oseltamivir. For treatment, doses are given twice daily for 5 days, beginning as soon as possible (within 48 h) after the onset of symptoms. For post-exposure prophylaxis, doses are given once daily for 10 days (or longer in adults) and for up to 6 weeks during an epidemic; therapy should begin within 48 h of exposure. Dosage should be reduced in children and in patients with moderate renal impairment.

Albert K, Bockshorn J (2007). Chemical stability of oseltamivir in oral solutions. *Pharmazie* 62: 678–682.

Eisenberg EJ, Cundy KC (1998). High-performance liquid chromatographic determination of GS4071, a potent inhibitor of influenza neuraminidase, in plasma by precolumn fluorescence derivatization with naphthalenedialdehyde. *J Chromatogr B Biomed Sci Appl* 716: 267–273.

Joseph-Charles J *et al.* (2007). Development and validation of a rapid HPLC method for the determination of oseltamivir phosphate in Tamiflu and generic versions. *J Pharm Biomed Anal* 44: 1008–1013.

Lindegardh N *et al.* (2006). A simple and rapid liquid chromatographic assay for evaluation of potentially counterfeit Tamiflu. *J Pharm Biomed Anal* 42: 430–433.

Massarella JW *et al.* (2000). The pharmacokinetics and tolerability of the oral neuraminidase inhibitor oseltamivir (Ro 64-0796/GS4104) in healthy adult and elderly volunteers. *J Clin Pharmacol* 40: 836–843.

Oliyai R *et al.* (1998). Biexponential decomposition of a neuraminidase inhibitor prodrug (GS-4104) in aqueous solution. *Pharm Res* 15: 1300–1304.

Oo C *et al.* (2003a). Pharmacokinetics and delivery of the anti-influenza prodrug oseltamivir to the small intestine and colon using site-specific delivery capsules. *Int J Pharm* 257: 297–299.

Oo C *et al.* (2003b). Pharmacokinetics of anti-influenza prodrug oseltamivir in children aged 1–5 years. *Eur J Clin Pharmacol* 59: 411–415.

Wiltshire H *et al.* (2000). Development of a high-performance liquid chromatographic–mass spectrometric assay for the specific and sensitive quantification of Ro 64-0802, an anti-influenza drug, and its pro-drug, oseltamivir, in human and animal plasma and urine. *J Chromatogr B Biomed Sci Appl* 745: 373–388.

Ouabain

Cardiac Glycoside, Cardiotonic

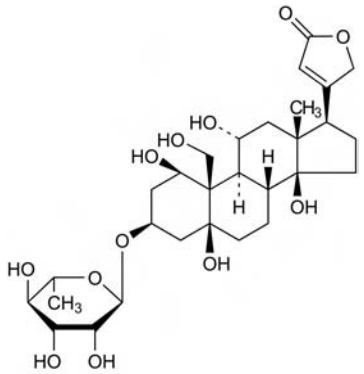
$C_{29}H_{44}O_{12}$, $8H_2O$ = 728.8

CAS—630-60-4 (anhydrous); 11018-89-6 (octahydrate)

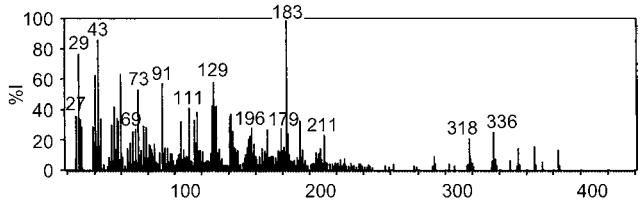
IUPAC Name 3-[(1*R*,3*S*,5*S*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*,17*R*)-1,5,11,14-Tetrahydroxy-10-(hydroxymethyl)-13-methyl-3-[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy-2,3,4,6,7,8,9,11,12,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]-2*H*-furan-5-one

Synonyms (1β,3β,5β,11α)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-1,5,11,14,19-pentahydroxycard-20(22)-enolide; G-strophanthin; strophanthin-G; strophanthinum; strophanthoside-G; uabaina; ubaína.

Proprietary Names *Ouabaine Arnaud*; *Purostrophan*; *Strodival*; *Strophoperm*.



Chemical Properties A glycoside obtained from the seeds of *Strophanthus gratus* or from the wood of *Acokanthera schimperi* or *A. ouabaio* (Apocynaceae). Colourless crystals or white crystalline powder. Mp about 190°, with decomposition. Slowly soluble 1 in 75 of water, 1 in 5 of boiling water; soluble 1 in 100 of ethanol and 1 in 30 of methanol; soluble in amyl alcohol and dioxane; slightly soluble in chloroform, ether and ethyl acetate. Log *P* (octanol/water), −2.0.
Colour Test Sulfuric acidorange-brown (slow); perchloric acid solution, followed by examination under ultraviolet light—yellow-green fluorescence; *p*-anisaldehyde reagent—yellow.
Mass Spectrum Principal ions at *m/z* 183, 43, 29, 60, 41, 129, 91, 73.

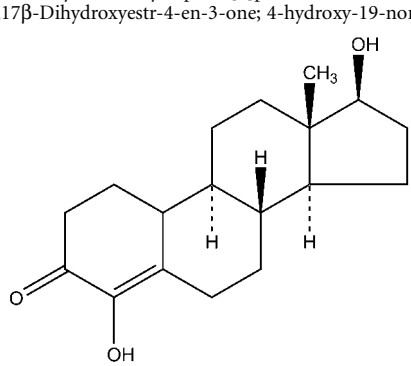


Quantification
Plasma **Radioimmunoassay** Limit of detection, 5 ng/L [Vakkuri *et al.* 2000]. Limit of detection, 50 ng/L [Selden, Smith 1972].
Urine **Radioimmunoassay** See Plasma [Selden, Smith 1972].
Disposition in the Body Irregularly absorbed after oral administration. About 30 to 60% of a dose is excreted in the urine in 24 h and about 25% is eliminated in the faeces in 3 days. Under steady-state conditions about 40% of the daily dose is excreted in the 24-hour urine.
Therapeutic Concentration
After a single IV dose of 0.5 mg to 3 subjects, a plasma concentration of about 0.012 mg/L was reported in 5 min; following daily IV administration of 0.25 mg to 3 subjects, steady-state plasma concentrations of about 0.0005 mg/L were attained on the 4th or 5th day [Selden, Smith 1972].
Half-life Plasma half-life, about 10 to 20 h.
Protein Binding About 5 to 10%.
Dose 250 to 500 µg IV; further injections of 100 µg may be given up to a total dose of 1 mg in 24 h.

Selden R, Smith TW (1972). Ouabain pharmacokinetics in dog and man. Determination by radioimmunoassay. *Circulation* 45: 1176–1182.
Vakkuri O *et al.* (2000). Radioimmunoassay of plasma ouabain in healthy and pregnant individuals. *J Endocrinol* 165: 669–677.

Oxabolone

Anabolic Steroid
$C_{18}H_{26}O_3$ = 290.4
CAS—4721-69-1
IUPAC Name (8*R*,9*S*,13*S*,14*S*,17*S*)-4,17-Dihydroxy-13-methyl-2,6,7,8,9,10,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-one
Synonyms 4,17β-Dihydroxyestr-4-en-3-one; 4-hydroxy-19-nortestosterone.



Chemical Properties Mp 189°. Log *P* (octanol/water), 3.25 [Meylan, Howard 1995].

Oxabolone Cipionate
$C_{26}H_{38}O_4$ = 414.6
CAS—1254-35-9
IUPAC Name 4,17β-Dihydroxyestr-4-en-3-one 17-(β-cyclopentylpropionate)
Synonym FI-5852
Proprietary Names *Steranabol-Depot*; *Steranabol Ritardo.*

Ultraviolet Spectrum Peak at 275 nm
Disposition in the Body Oxabolone cipionate is absorbed slowly from SC or IM depots as the lipid-soluble ester. It is highly protein bound and carried in plasma by the sex-hormone-binding protein globulin. Once de-esterified, oxabolone is metabolised rapidly by hepatic mixed-function oxidases; the duration of the biological effects is determined almost entirely by the rate of absorption from SC or IM depots and on the de-esterification which precedes it.
Protein Binding Highly protein bound.
Dose 400–800 mg/week.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci*,83–92.

Oxalates

Anion

Oxalic Acid
$C_2H_2O_4$ = 90.03
CAS—144-62-7
Synonym Ethanedioic acid
Chemical Properties pK_a 1.25 Log *P* (octanol/water), −2.22. Used in calico printing, bleaching leather, in metal polishes, as a reducing agent, in ceramics and pigments, in the paper industry, in photography, in process engraving, in rubber manufacture industry.

Sodium Oxalate
$C_2Na_2O_4$ = 134.0
CAS—62-76-0
Synonym Ethanedioic acid disodium salt
Chemical Properties White, odourless, crystalline powder. Soluble in water, insoluble in alcohol. Used for finishing textiles, tanning and finishing leather, in standardising potassium permanganate solution.

Calcium Oxalate
C_2CaO_4 = 128.1
CAS—563-72-4
Synonym Ethanedioic acid calcium salt
Chemical Properties The monohydrate appears as crystals. Loses all its water at 200°. Practically insoluble in water or acetic acid; soluble in dilute hydrochloric acid or nitric acid. Used in ceramic glazes, as carrier for separation of rare earth metals.

Potassium Oxalate
$C_2K_2O_4$ = 166.2
CAS—583-52-8
Chemical Properties Occurs as the monohydrate as colourless, odourless crystals. Loses its water at 160°. Soluble in water. Used on cleaning and bleaching straw, removing stains in photography, as an *in vitro* blood anticoagulant, in analytical chemistry.

Quantification
Plasma **HPLC** Column: AS4-A anion exchange. Mobile phase: 2 g/L EDTA (pH 5.0) and 3.0 mol/L sodium hydroxide, flow rate 0.6 mL/min. Enzyme reactor detection. Limit of detection, 0.68 µmol/L [Honow *et al.* 2002]. Column: Capcell Pak C_8 (150 × 6 mm i.d., 5 µm). Mobile phase: 80 mmol/L potassium dihydrogen phosphate : 5 mmol/L terta-*n*-butylammonium phosphate (pH 4.0), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 10 µmol/L [Yamato *et al.* 1994]. Column: AS10 anion exchange. Mobile phase: 40 mmol/L sodium tetraborate (pH 9.4), flow rate 1.0 mL/min. Limit of detection, 0.5 µmol/L [Hagen *et al.* 1993]. Column: AS-4A anion exchanger (25 × 0.4 cm). Mobile phase: 1.4 mmol/L sodium carbonate : 1.8 mmol/L sodium bicarbonate, flow rate 2.0 mL/min. Electrochemical detection. Retention time: ~13.5 min. Limit of detection, 0.5 µmol/L [Petrarulo *et al.* 1993].
Serum **CE** Capillary: Fused silica (total/effective length 100/87.7 cm × 75 µm i.d.). Mobile phase: 250 mmol/L sodium chloride : 7.5 mmol/L cetyltrimethylammonium chloride (pH 2.2). UV detection (λ= 226 nm). Limit of detection, 0.04 mg/L [Hirokawa *et al.* 2004].
Urine **HPLC** Column: LiChrosorb RP18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.25% potassium dihydrogen phosphate : 2.5 mmol/L tetrabutylammonium hydrogen sulfate (pH 2.0). UV detection (λ= 210 nm). Limit of detection, 8.0 µmol/L [Khaskhali *et al.* 1996]. See Plasma [Yamato *et al.* 1994]. Column: AS10 anion exchange. Mobile phase: 40 mmol/L sodium tetraborate (pH 9.4), flow rate 1.0 mL/min. Limit of detection, 0.5 µmol/L [Hagen *et al.* 1993]. Column: Polypropylene minicolumn (50 × 7 mm i.d.). Mobile phase: 0.5% hydrogen phosphate, flow rate 0.7 mL/min. UV detection (λ= 214 nm). Retention time: 8 min. Limit of detection, 10 mg/L [Kataoka *et al.* 1990]. Column: C_{18} reversed phase (51 cm × 4.6 mm i.d.). Mobile phase: 3% methanol and 1% tetrahydrofuran in 0.35 mol/L ammonium acetate, flow rate 1.5 mL/min. UV detection (λ= 314 nm).

Limit of detection not reported [Koolstra *et al.* 1987]. Column; LiChrosorb RP-8 (100 × 4.6 mm i.d., 10 µm). Mobile phase: aqueous phosphate buffer (pH 7.0) : 10% methanol, flow rate 1 mL/min. Electrochemical detection. Limit of detection, 5 ng [Kok *et al.* 1984].

GC Column: Glass (2 m × 2 mm i.d.). Carrier gas: N_2. Temperature: 145°. Limit of detection, ~3 mg/L [Di Corcia *et al.* 1982].

CE See Serum [Hirokawa *et al.* 2004].

AAS Perkin-Elmer 575. Cu hollow cathode lamp (λ = 324.8 nm). Limit of detection, 7.9 µmol/L [Munoz Leyva *et al.* 1990].

Note For a method of determining oxalate in urine using alkylamine glass-bound barley oxalate oxidase, see Chandran *et al.* [2001]; for a photochemical method for the determination of oxalate in urine, see Leon *et al.* [1990]. A method employing oxalate oxidase and sensors based on the injection of a recognition element is described by Hong *et al.* [2003]. For a comparison of 6 methods for measuring urinary oxalate, see Zerwekh *et al.* [1983]; for a method of detecting calcium oxalate using alizarin red S stain, see Proia, Brinn [1985]; for a chemiluminescence method for detecting oxalate in blood, see Gaulier *et al.* [1998]; for the detection of fluphenazine in urine and blood using peroxyoxalate chemiluminescence, see Mann, Grayeski [1991].

Disposition in the Body Oxalic acid is the main product of ethylene glycol metabolism. It is present in rhubarb leaves. The normal concentration of oxalate in the blood is 60 to 230 µg/L [Konta *et al.* 1998].

Toxicity Oxalic acid is corrosive to tissue. When ingested, oxalic acid removes calcium from the blood so kidney damage can be expected as the calcium is removed in the form of calcium oxalate. The calcium oxalate obstructs the kidney tubules.

Inhalation exposure Oxalic acid is harmful if inhaled and can cause severe irritation and burns of the nose, throat and respiratory tract.

Oral exposure Highly toxic: oxalic acid may cause burns, nausea, severe gastroenteritis, vomiting, shock and convulsions. It may cause renal damage, as evidenced by bloody urine. The estimated fatal dose is 5 to 15 g.

Dermal exposure Can cause severe irritation and possible skin burns. It is absorbed through the skin.

Ocular exposure It is an eye irritant and may produce corrosive effects.

Chronic exposure May cause inflammation of the upper respiratory tract. Prolonged skin contact can cause dermatitis, cyanosis of the fingers and possible ulceration.

A 47-year-old man presented with acute renal failure caused by ingestion of an unknown amount of oxalate. His blood oxalate concentration was 302 µg/L [Konta *et al.* 1998].

A 49-year-old man was found unconscious in a hotel room. His serum ethylene glycol concentration was 0.24 mmol/L [Huhn, Rosenberg 1995].

A 58-year-old woman was administered a single 45 g dose of ascorbic acid, a metabolic precursor of oxalate. Her serum oxalate concentration following haemodialysis was 15.4 mg/L. Her plasma and urine oxalate concentrations were 2.3 and 11.6 mg/L, respectively. Postmortem examination of her kidneys revealed crystals that were bifringent under polarised light, many with a 'dumbell' or 'shock-of-wheat' configuration, characteristic of calcium oxalate deposition [Lawton *et al.* 1985].

Note For 4 cases of ethylene glycol poisoning, see Leth and Gregersen [2005]; for an account of massive crystalluria in a further 4 cases of ethylene glycol poisoning, see Jacobsen *et al.* [1982]; for a case of the oral manifestations of oxalosis following ileojejunal intestinal bypass, see Lapointe and Listrom [1988]; for historical accounts of oxalic acid poisoning, see Campbell [1982] and Clarke [1990]. For a fatal case of oxalic acid poisoning following the ingestion of sorrel (*Rumex crispus*), see Farre *et al.* [1989] and for a review of poisoning following ingestion of plants containing oxalate, see Sanz and Reig [1992].

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Oxaliplatin

Antineoplastic

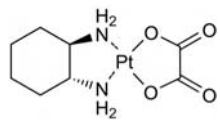
$C_8H_{14}N_2O_4Pt = 397.3$

CAS—61825-94-3

IUPAC Name Cyclohexane-1,2-diamine; oxalic acid; platinum

Synonyms (*SP*-4-2)-[(1*R*,2*R*)-1,2-Cyclohexanediamine-κ*N*,κ*N*′][ethanedioato-(2–)-κO^1,κO^2]platinum; NSC-266046; l-OHP; oxalatoplatin; oxalatoplatinum; RP-54780.

Proprietary Name *Eloxatin*



Chemical Properties Colourless, thin triangular plates with truncated vertices. Soluble in water at 7.9 g/L.

Quantification

Plasma **HPLC** Column: ODS-2 Inertsil (250 × 4.6 mm i.d.). Temperature: 40°. Mobile phase: acetonitrile : 10 mmol/L acetate buffer (pH 5.5, 5 : 95), flow rate 1 mL/min. UV detection (λ=290 nm). Retention time: 11.6 min. Limit of detection, 0.1 µg/L [Kizu *et al.* 1995].

Urine **HPLC** See Plasma [Kizu *et al.* 1995].

Disposition in the Body Biotransformation is extensive and no intact drug is detectable in plasma ultrafiltrate at the end of a 2-h infusion. Renal elimination is the major pathway. 50% or more of the platinum administered was recovered in urine within 1 to 5 days following administration. <5% is eliminated in faeces.

Therapeutic Concentration

The maximum plasma concentration of platinum following a constant rate infusion of oxaliplatin at 20 mg/m^2/day for 5 days was 330 to 720 µg/L [Lévi *et al.* 2000].

Half-life About 24 h.

Volume of Distribution Ranges between 582 and 812 L.

Clearance About 17.6 L/h.

Distribution in Blood Maximum platinum uptake in erythrocytes was 37% of the total platinum. Plasma ultrafiltrate : total plasma : erythrocytes ratio is ~1 : 4 : 3 at the end of a 2-h infusion.

Dose 130 mg/m^2 body surface.

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Oxandrolone

Anabolic Steroid, Androgen

$C_{19}H_{30}O_3 = 306.4$

CAS—53-39-4

IUPAC Name (1*S*,3*aS*,3*bR*,5*aS*,9*aS*,9*bS*,11*aS*)-1-hydroxy-1,9*a*,11*a*-trimethyl-2,3,3*a*,3*b*,4,5,5*a*,6,9,9*b*,10,11-dodecahydroindeno[4,5-h]isochromen-7-one

Synonyms (5α,17β)-17-Hydroxy-17-methyl-2-oxa-androstan-3-one; NSC-67068; SC-11585.

Proprietary Names *Anavar*; *Lonavar*; *Oxandrin*; *Provitar*; *Vasorome*.

Chemical Properties White odourless crystalline powder with Mp 235° to 238°. It is soluble in water (1 in 5200), alcohol (1 in 57), acetone (1 in 69), chloroform (1 in <5) and ether (1 in 860). Log *P* (octanol/water), 2.58 [Meylan, Howard 1995].

Thin-layer Chromatography Plate: silica gel HF_{254} (5 × 20 cm). Mobile phase: chloroform : methanol : glacial acetic acid (94 : 5 : 1). Visualisation by iodine vapour. Detection by zonal scraping method. R_f 0.5. [Karim *et al.* 1973].

Gas Chromatography System GAG—RRT 1.17 (relative to testosterone); system GAI—RRT 1.111 (relative to 17α-methyl-5α-androstan-3β,17β-diol)α-methyl-5α-androstan-3β; system GAR—RT 13.7 min.

Gas Chromatography-Mass Spectrometry Column: 5% phenyl methyl silicone (HP Ultra-2, 25 m × 0.2 mm i.d., 0.33 µm). Temperature: 100° for 1 min to 220° at 16°/min to 300° at 3.8°/min for 10 min. Carrier gas: He, 0.6 mL/min. EI ionisation, SIM acquisition mode at *m/z*: 143.1, 363.3, 378.3). RT: (relative oxandrolone TMS derivative, RT 27.97 min) δ-hydroxy acid metabolite, 0.90; 16-α-hydroxyoxandrolone, 1.13; 16-β-hydroxyoxandrolone, 1.16; 17-epioxandrolone, 0.93. (Relative to 5α-androstan-17-one TMS-enol derivative, RT 17.95 min) oxandrolone, 1.56; δ-hydroxy acid metabolite, 1.40; 16-α-hydroxyoxandrolone, 1.77; 16-β-hydroxyoxandrolone, 1.81; 17-epioxandrolone, 1.44 [Massé *et al.* 1989].

High Performance Liquid Chromatography System HATb—RRT 0.75 (relative to testosterone).

Column: Zorbax ODS (5 cm × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (75 : 25). UV detection (λ = 210 nm). Retention time: 0.75. Limit of detection, 5 mg/L [Walters *et al.* 1990].

Infrared Spectrum Principal peaks at wavenumbers 1717, 1202, 1231, 1258 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 291, 248, 43, 79, 91, 93, 107, 176.

Quantification

Plasma **GC-MS** Column: 1.5% OV-17 on 80-100 mesh Chromosorb W-HP 6′ × 4 mm i.d.). Carrier gas: He, 50.4 mL/min. Temperature: 240°. FID. Retention time: 5.3 min. Limit of detection not reported [Karim *et al.* 1973].

Urine **GC** Column: DB-1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 20 cm/s. Temperature programme: 200° for 4 min to 300° at 4°/min. FID. Limit of detection not reported [Harrison *et al.* 1989].

GC-MS Column: HP Ultra-2 5% phenyl methyl silicone (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° for 1 min to 220° at 16°/min to 300° at 3.8°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection 0.005 mg/L [Massé *et al.* 1989].

LC-MS Column: LiChroCART Purospher RP C_{18} (125 × 3 mm, 5 µm). Mobile phase: 5 mmol/L ammonium acetate- 0.01% acetic acid : 90% methanol (50 : 50 to 0 : 100 at 15 min for 3 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.5 µg/L for oxandrolone metabolite 17-epioxandrolone [Leinonen *et al.* 2004].

Disposition in the Body Oxandrolone is rapidly absorbed from the gastrointestinal tract and excreted mainly in urine as metabolites; 16-β-hydroxyoxandrolone and 16-α-hydroxyoxandrolone (total, 3%), 17-epioxandrolone (8.4%) and a small amount as the δ-hydroxy acid metabolite, and the parent drug (35.8%). The drug is first detected in urine 2 h after dosing and can be detected for up to 72 h. A small amount is excreted in the faeces (≈2.8%) [Karim *et al.* 1973].

Therapeutic Concentration

The maximum plasma concentration of 0.417 mg/L was observed between 30 and 90 min following oral ingestion of a 10 mg dose. [Karim *et al.* 1973].

Toxicity Moderately toxic by ingestion and IP injection. When heated to decomposition temperature will emit acrid smoke and fumes.

Bioavailability 97%.

Half-life Biphasic plasma elimination: from 90 min to 4 h, and 33 min, and from 4 to 48 h, and 9 h.

Volume of Distribution 578 mL/kg.

Clearance Plasma clearance, 43 mL/h/kg.

Protein Binding 94–97%.

Dose The usual dose is 2.5 to 20 mg by mouth. Usual dose 5 to 10 mg daily in divided doses for 2 to 4 weeks.

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Oxazepam

Benzodiazepine, Tranquilliser

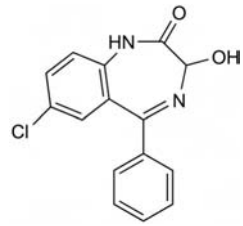
$C_{15}H_{11}ClN_2O_2$ = 286.7

CAS—604-75-1

IUPAC Name 7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one

Synonyms 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one; oxazepamum; Wy-3498.

Proprietary Names *Adumbran*; *Alepam*; *Alopam*; *Anxiolit*; *Azutranquil*; *Benzotran*; *Durazepam*; *Hilong*; *Limbial*; *Mirfudorm*; *Murelax*; *Noctazepam*; *Opamox*; *Oxabenz*; *Oxahexal*; *Oxamin*; *Oxanid*; *Oxapax*; *Oxepam*; *Praxiten*; *Serax*; *Serepax*; *Seresta*; *Serpax*; *Sigacalm*; *Sobril*; *Tranquo*; *Uskan*; *Zaxopam*.



Chemical Properties A white to pale yellow crystalline powder. Mp 205° to 206° to (crystals from alcohol). Practically insoluble in water; soluble 1 in 220 of ethanol, 1 in 270 of chloroform and 1 in 2200 of ether; soluble in dioxan. pK_{a1} 1.7, pK_{a2} 11.6 (20°). Log *P* (octanol/water), 2.24 [Capella-Peiró *et al.* 2002], 2.17 [Mullett, Pawliszyn 2001], (octanol/water pH 7.4), 2.2. Extraction yield (chlorobutane), 0.85 [Demme *et al.* 2005].

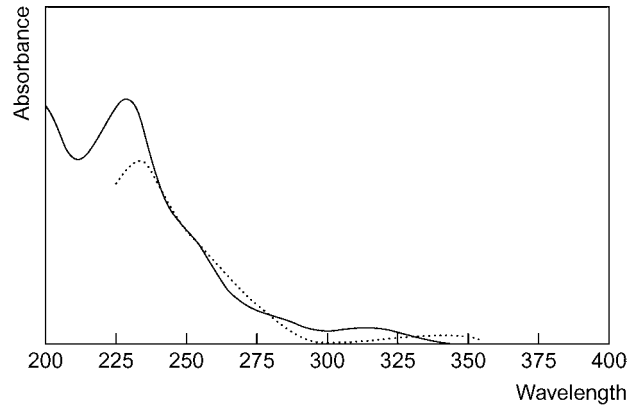
Colour Test Formaldehyde–sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.00; system TC—R_f 0.40; system TD—R_f 0.22; system TE—R_f 0.45; system TF—R_f 0.35; system TL—R_f 0.51; system TAD—R_f 0.42; system TAE—R_f 0.82; system TAF—R_f 0.91; system TAJ—R_f 0.47; system TAK—R_f 0.47; system TAL—R_f 0.89 (acidified iodoplatinate solution—positive; acidified potassium permanganate solution—positive).

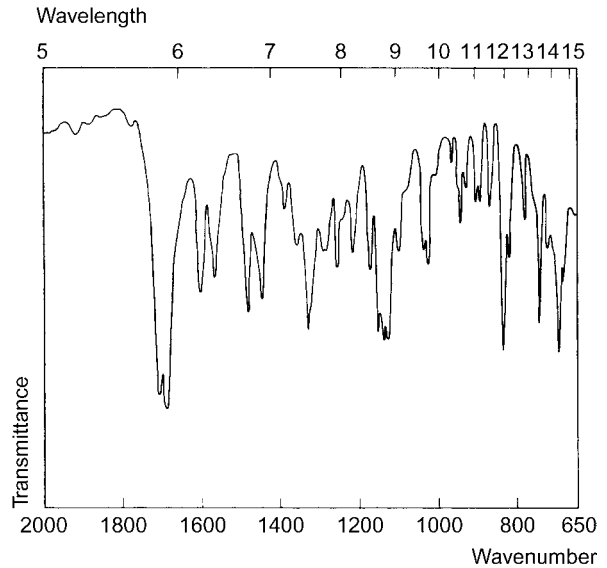
Gas Chromatography System GA—RI 2325; system GB—oxazepam RI 2438, oxazepam-TMS_2 RI 2468; system GG—RI 2803.

High Performance Liquid Chromatography System HI—*k* 4.62; system HK—*k* 0.73; system HX—RI 441; system HY—RI 390; system HZ—RT 3.8 min; system HAL—RT 4.4 min; system HAM—RT 3.4 and RT 4.4 min; system HAX—RT 6.0 min; system HAY—RT 4.5 min; system HAZ—*k* 1.23; system HBH—*k* 5.42; system HBI—*k* 1.25.

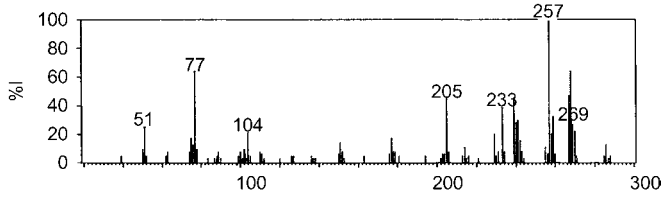
Ultraviolet Spectrum Aqueous acid—234, 280 nm; aqueous alkali—233, 344 nm; ethanol—230 (A_1^1 = 1235a), 315 nm (A_1^1 = 85a; see below).



Infrared Spectrum Principal peaks at wavenumbers 1687, 1706, 693, 830, 1136, 1123 cm^{-1} (KBr disk; see below).



Mass Spectrum Principal ions at *m/z* 257, 77, 268, 239, 205, 267, 233, 259.



Quantification

Blood **GC** Columns: DB-1 and DB-1701 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 28° at 30°/min to 230° at 2°/min to 300° at 30°/min for 1 min or 120° for 1 min to 230° at 40°/min to 280° at 8°/min for 9 min. ECD. Limit of detection, 0.058 µmol/L (DB-1701) [Gjerde *et al.* 1992]. Column: SE-54 5% phenyl methyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2–3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 6.28 min. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: 100% methylsiloxane or 5% phenylsiloxane, 95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 25°/min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation. [Papoutsis *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Tiscione *et al.* 2008]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 10 µg/L [Pirnay *et al.* 2002].

HPLC Column: Chromolith Performance (RP-18e) (100 × 4.6 mm i.d.). Mobile phase: 35 mmol/L phosphate buffer (pH 2.1) : acetonitrile (70 : 30), flow rate 2 mL/min. DAD (λ= 220 nm). Limit of quantification, 30 µg/L [Bugey, Staub 2004]. Column: Lichrospher Select B (125 × 3 mm i.d.). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate (pH 2.1, 30 : 70 to 35 : 65 at 30 min), flow rate 0.5 mL/min to 0.3 mL/min at 30 min. DAD (λ= 254 nm). Limit of quantification, 30 µg/L, limit of detection, 10 µg/L [El Mahjoub, Staub 2001a]. Column: Lichrospher Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 2.1) : acetonitrile (65 : 35), flow rate 0.3 mL/min. UV detection (λ= 220 nm). Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [El Mahjoub, Staub 2000a].

LC-MS MRM acquisition mode [Gunn *et al.* 2010]. Column: Restek Allure C_{18} (150 × 3.2 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (90 : 5 : 5), flow rate 0.45 mL/min. DAD. Limit of quantification, 2 µg/L [Dussy *et al.* 2006].

Plasma **GC** Column: CPSIL 8 CB (25 × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 150° to 230° at 40°/min for 2 min to 250° at 5°/min for 1 min to 300° at 15°/min for 3 min. NPD. Limit of detection, 0.45 µmol/L [Reubsaet *et al.* 1998]. See Blood [Lillsunde, Seppälä 1990]. Column: 3% OV-7 on Chromosorb W 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 40 mL/min. Temperature: 250°. ECD. Limit of detection, 10 µg/L [Giles *et al.* 1978].

HPLC Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: methanol : water (65 : 35), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, 5 µg/L for oxazepam and other benzodiazepines [Liang *et al.* 2009]. Column: Hisep (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.18 mol/L ammonium acetate buffer (pH 2.5, 15 : 85), flow rate 2 mL/min. UV detection (λ= 254 nm). Limit of quantification, 0.5 mg/L, limit of detection, 0.16 mg/L [Pistos, Stewart 2003]. Column: Cyclobond 1-2000 RSP (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1% triethylamine acetate buffer (pH 4.5) : water (18 : 8 : 73), flow rate 0.4 mL/min. DAD (λ= 230 nm). Limit of detection, 10 µg/L [Pham-Huy *et al.* 2002]. Column: C_{18} (300 × 3.9 mm i.d.). Mobile phase: methanol : phosphate buffer (pH 6, 50 : 50), flow rate 1.5 mL/min. UV detection (λ= 230 nm). Limit of detection, 30 µg/L [Zarghi, Jenabi 2001]. Column: LiChrospher Select B C_8 (125 × 3 mm i.d., 5 µm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer (pH 2.1, 30 : 70 for 5 min to 35 : 65 at 0.3 mL/min), flow rate 0.5 mL/min for 5 min to 0.3 mL/min at 30 min. DAD (λ= 254 nm). Retention time: 13.5 min. Limit of quantification, 60 µg/L, limit of detection, 18 µg/L [El Mahjoub, Staub 2000b]. See Urine [Azzam *et al.* 1998; Franzelius, Besserer 1993].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Retention time: 4.93 min. Limit of quantification, 20 µg/L, limit of detection, 10 µg/L [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.025 mg/L, limit of detection, 0.001 mg/L [Kratzsch *et al.* 2004].

Serum **GC-MS** See Blood [Pirnay *et al.* 2002]. Column: SPB-1 (30 m × 0.75 mm i.d., 1.0 µm). Carrier gas: He, 0.2 bar. Temperature programme: 230° for 2 min to 280° at 7°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1.5 ng/L, limit of detection, 1 ng/L [Duthel *et al.* 1992].

HPLC Column: Perfectsil Target ODS-3 reversed phase (125 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium dihydrogen phosphate buffer (pH 5.8) : methanol (50 : 50), flow rate 1.5 mL/min. UV detection (λ= 254 nm) [Kazemifard *et al.* 2007]. Column: Symmetry Shield RP8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L potassium dihydrogen phosphate (40 : 60), flow rate 0.9 mL/min. UV detection (λ= 230 nm). Limit of detection, 1.0 µg/L [He *et al.* 2005]. Column: Kromasil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: butanol : 0.06 mol/L SDS (pH 7, 5 : 95), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, 7 µg/L [Capella-Peiró *et al.* 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (52 : 48), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, 750 µg/L [Mullett, Pawliszyn 2002]. Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: flow rate 1.5 mL/min. DAD (λ= 254 nm). Limit of quantification, 150.7 µg/L, limit of detection, 42 µg/L [Ahrens *et al.* 2000]. See Blood [El Mahjoub, Staub 2000a]. See Plasma [El Mahjoub, Staub 2000b]. See also Goldnik *et al.* [1993].

LC-MS See Blood [Gunn *et al.* 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Urine **GC** See Plasma [Reubsaet *et al.* 1998]. Column: DB-17 (15 m). Carrier gas: N_2. Temperature: 225°. ECD. Retention time: 1.42 min. Limit of detection, 0.13 ng [Beischlag, Inaba 1992].

GC-MS See Blood [Goldberger *et al.* 2010; Pirnay *et al.* 2002]. Column: polydimethylsiloxane. SIM or scan acquisition mode. Limit of detection, 1.0–1.7 µg/L (SIM) and 13–30 µg/L (scan mode) for oxazepam and other benzodiazepines [Borrey *et al.* 2001a; Borrey *et al.* 2001b]. Column: 5% phenylmethylsiloxane (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.5 mL/min. Temperature programme: 240° to 260° at 25°/min to 300° at 30°/min. SIM acquisition mode. Limit of detection, 0.1 mg/L [Black *et al.* 1994]. Column: DB-1 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 160° for 1 min to 280° at 20°/min for 3 min. NCI. Limit of detection not reported [Fitzgerald *et al.* 1993]. Column: methylsiloxane (12.5 m). Temperature programme; 120° to 310°. Limit of quantification, 3 µg/L, limit of detection, 1 µg/L [West, Ritz 1993]. Column: DB-5 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 66 cm/s. Temperature programme: 210° to 300° at 20°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, <10 µg/L, limit of detection, <10 µg/L [Dickson *et al.* 1992].

HPLC UV detection. Limit of detection, 750 µg/L [Mullett, Pawliszyn 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol (54 : 46), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of quantification, 80.7 µg/L, limit of detection, 24.2 µg/L [Mullett, Pawliszyn 2001]. Column: Hypersil C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.04 mol/L phosphate (pH 4)-0.4% octylamine : acetonitrile (73 : 27, 70 : 30, 65; 35 or 60 : 40), flow rate 1 mL/min. DAD (λ= 240 nm). Retention time: 10.45 min. Limit of quantification, 26.0 µg/L, limit of detection, 8.0 µg/L [Segura *et al.* 2001]. Hypersil BDS RP-C_{18} (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: methanol : acetonitrile : 0.05 mol/L potassium dihydrogen phosphate (pH 3.5, 50 : 10 : 40), flow rate 1.2 mL/min. UV detection (λ= 232 nm). Retention time: 7.16 min. Limit of quantification, 10 µg/L [Azzam *et al.* 1998]. Column: LiChrospher 100 RP-C18(e) (250 × 4 mm i.d., 5 µm). Mobile phase: water : methanol : triethylamine (pH 5.5, 70 : 30 : 0.1), flow rate 0.7 mL/min. UV detection (λ= 240 nm). Limit of detection, 2 µg/L for oxazepam and other diazepam metabolites [Chiba *et al.* 1995]. Column: 60 RP-Select B (250 × 4 mm i.d., 5 µm) or LiChrospher 100 RP-C8 (200 × 4 mm i.d., 8.5 µm). Mobile phase: 0.01 mol/L sodium phosphate buffer (pH 7.0): acetonitrile (87.5 : 12.5 to 83.5 : 16.5 at 15 min to 80 : 20 over 8.5 min for 3 min to 75 : 25 in 1 min for 15 min), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, 10 µg/L [Franzelius, Besserer 1993]. Column: Ultrabase C_{18} reversed phase (35 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (60 : 40), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 364 nm, λ_{em}= 469 nm). Limit of detection, 4 µg/L [Berrueta *et al.* 1993]. See also Goldnik *et al.* [1993].

LC-MS Column: Hypurity C_8 (150 × 3 mm i.d.). Mobile phase: 4 mmol/L ammonium acetate (pH 6.8) in methanol-water (5 : 95) : 1% propan-2-ol, 0.05% formic acid in methanol (100 : 0 for 1 min to 0 : 100 at 3 min for 1.5 min to 100 : 0 over 0.1 min for 1.4 min. TIS, MRM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, 1.25 µg/L [Glover, Allen 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 from 3.01 to 6 min to 100 : 0 from 6.01 to 10 min for 5 min), flow rate 0.9 mL/min for 3 min to 0.3 mL/min until 10 min to 0.9 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.1 µg/L [Umezawa *et al.* 2008]. Column: Symmetry C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate (pH 5, 10 : 90 to 30 : 70 at 5 min to 80 : 20 at 9 min to 10 : 90 at 9.1 min), flow rate 0.3 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.01 µmol/L [Hegstad *et al.* 2006].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Oral Fluid **LC-MS** Limit of detection, 0.5–5 µg/L for oxazepam and other benzodiazepines [Moore *et al.* 2007].

Hair **GC-MS** Column: HP-Ultra 2 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 2 mL/min. Temperature programme: 70° for 2 min to 220° at 25°/min to 255° at 5°/min to 300° for 7 min. EI ionisation at 70 eV. Retention time: 9.6 min. Limit of detection, 0.2 ng/mg [Yegles *et al.* 1997]. Column: HP-5 MS 5% phenylsiloxane, 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 290° at 30°/min for 2 min. NICI. Retention time: 9.28 min. Limit of detection, 0.005 ng/mg [Kintz *et al.* 1996].

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HPLC Column: Lichrospher Select-B (125 × 3 mm i.d., 5 μm). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate (pH 2.1, 30 : 70 to 35 : 65 in 30 min), flow rate 0.5 mL/min to 0.3 mL/min at 30 min. UV detection (λ = 254 nm). Limit of quantification, 0.3–0.45 ng/mg, limit of detection, 0.2 ng/mg [El Mahjoub, Staub 2001b].

LC-MS Mobile phase: 3 mmol/L ammonium formate and 0.001% formic acid in water : acetonitrile (65 : 35 to 20 : 80 after 13 min to 10 : 90 at 13.5 min until 16.5 min to 65 : 35 until 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.19 ng/30 mg, limit of detection, 0.11 ng/30 mg [Miller *et al.* 2006].

Disposition in the Body Oxazepam is readily absorbed after oral administration. It crosses the placenta and is found in breast milk. Approximately 70–80% of a single dose is excreted in the urine in 72 h, almost entirely as oxazepam glucuronide with only traces of unchanged oxazepam and other minor metabolites. Up to 10% of a dose is eliminated in the faeces, mostly as unchanged drug. Oxazepam is a metabolite of several benzodiazepines including chlordiazepoxide, clorazepic acid, demoxepam, desmethyldiazepam (nordazepam), diazepam, ketazolam, medazepam, prazepam, and temazepam.

Therapeutic Concentration In plasma, usually in the range 0.5–2 mg/L.
A single oral dose of 45 mg administered to 8 subjects produced serum oxazepam concentrations of 0.88–1.44 mg/L (mean, 1.1) in ~2 h, and oxazepam glucuronide concentrations of 0.7–1.4 mg/L (mean, 0.9) in 2–4 h. Daily oral doses of 10 mg every 6 h administered to 6 subjects produced serum oxazepam concentrations of 0.14–0.56 mg/L (mean, 0.3) 2 h after a dose, and oxazepam glucuronide concentrations of 0.22–0.40 mg/L (mean, 0.3) 4 h after the dose [Knowles, Ruelius 1972].

Toxicity Blood concentrations >2 mg/L may produce toxic effects.

Note For a report of oxazepam overdose associated with alcohol ingestion, see Weinberg *et al.* [1994]; for an assessment of the relative toxicity of benzodiazepines in overdose, see Buckley *et al.* [1995].

Half-life Plasma half-life, 4–15 h (mean, 8).

Volume of Distribution 0.5–2 L/kg.

Clearance Plasma clearance, ~1–2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~0.9.

Protein Binding ~95%.

Note For a review of the clinical pharmacokinetics of oxazepam, see Greenblatt [1981].

Dose 30 to 120 mg daily.

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Oxcarbazepine

Anticonvulsant

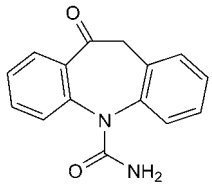
$C_{15}H_{12}N_2O_2 = 252.3$

CAS—28721-07-5

IUPAC Name 5-Oxo-6*H*-benzo[*b*][1]benzazepine-11-carboxamide

Synonyms 10,11-Dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide; GP-47680; oxacarbazepine.

Proprietary Name *Trileptal*



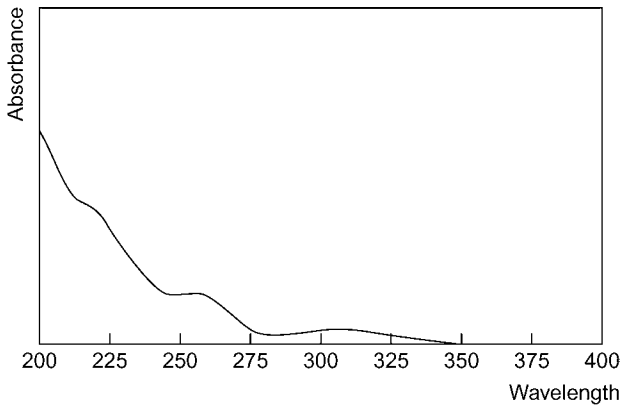
Chemical Properties White to faintly orange crystalline powder. Mp 215° to 216°. Practically insoluble in water, ether and ethanol; slightly soluble in chloroform, dichloromethane, acetone and methanol. Log *P* (octanol/water), 1.11 [Meylan, Howard 1995]. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005]. Samples were stable in extraction solvent in the autosampler for at least 24 h [Mazzucchelli *et al.* 2007]. Stock solutions were stable for at least 6 months when stored at 4°. Plasma samples were stable following 3 freeze-thaw cycles and after 24 h at room temperature. Quality-control samples were stable for at least 30 days stored at –50°. Stable in the autosampler for 24 h [Nirogi *et al* 2006]. Stable for ≈1 year in plasma when stored at –70°. Not stable at –20° in human plasma. Stock solutions were stable for at least 3 months at 4°. Extracts were stable on the autosampler at 4° for at least 33 h [Souppart *et al* 2001].

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.54; system TF—R_f 0.20; system TAE—R_f 0.78.

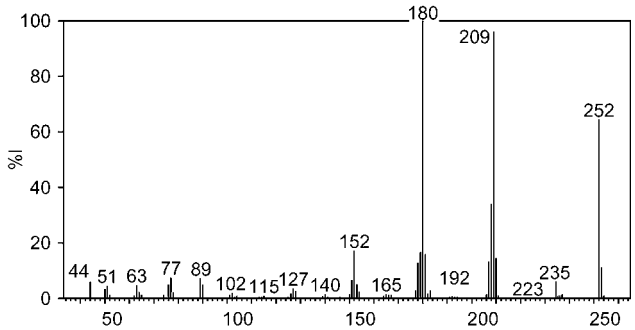
Gas Chromatography System GA—M (formylacridine) RI 2025, M (carbamazepine) RI 2285; system GB—oxcarbamazepine RI 2266, M (formylacridine) RI 2158, M (methylacridine) RI 2054, M (10-OH-) RI 2580, M (carbamazepine) RI 2435; system GAJ—M (carbamazepine) RRT 1.716 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HX—RI 396; system HZ—oxcarbazepine RT 2.9 min, M (10-OH-) RT 2.2 min.

Ultraviolet Spectrum Aqueous acid—256, 306 nm.



Mass Spectrum Principal ions at *m/z* 180, 209, 252, 208, 152, 179, 181, 210.



Quantification

Blood **LC-MS** Column: LiChroCART (125 × 3 mm i.d., 5 µm). Mobile phase: 0.1% formic acid : acetonitrile-0.1% formic acid (95 : 5, 95 : 5 for 2 min to 30 : 70 for 2 min to 95 : 5 for 8 min), flow rate 0.4 mL/min. APCI, positive ion mode. Limit of detection, 0.05 mg/L [Klys *et al.* 2005].

Plasma **GC** Column: HP fused silica (30 m × 0.31 mm i.d., 1 µm). Carrier gas: H_2, 1.4 kg/cm^2. Temperature programme: 140 to 200° at 2 min to 240° at 30°/min. FID. Retention time: 8 min 45 s. Limit of detection, 10 µg/L and 25 µg/L for 10-hydroxycarbazepine and 10,11-dihydro-10-hydroxy-carbamazepine, respectively [von Unruh, Paar 1985].

GC-MS Column: HP 5% phenylmethyl silicone (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He. Temperature: 250°. EI ionisation at 20 eV. Limit of detection, 0.1 µg/L and 1 µg/L for 10-hydroxycarbazepine and 10,11-dihydro-10-hydroxycarbamazepine, respectively [von Unruh, Paar 1986].

HPLC Column: XTERRA C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L potassium dihydrogen phosphate : acetonitrile : *n*-octylamine (76 : 24 : 0.05), flow rate 0.7 mL/min. UV detection (λ = 237 nm). Limit of quantification, 25 µg/L [Kimiskidis *et al.* 2007]. Column: Chiralcel ODR (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : 37.5 mmol/L potassium hexafluorophosphate (18 : 82 to 25 : 75), flow rate 0.5 mL/min. UV detection (λ = 210 nm). Limit of quantification, 0.1 mg/L, limit of detection, 0.05 mg/L [Mazzucchelli *et al.* 2007]. Column: Synergi Hydro-RP (250 × 4.6 mm i.d., 4 µm). Mobile phase: 0.01 mol/L potassium phosphate (pH 7.0) : methanol : acetonitrile 955 : 20 : 30), flow rate 1.1 mL/min. UV detection (λ = 240 nm). Retention time: 4.5 min. Limit of detection not reported [Juenke *et al.* 2006]. Column: Symmetry C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile (52 : 28 : 20), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 50 µg/L, 100 µg/L for 10-hydroxycarbazepine [Nirogi *et al.* 2006]. Column: Synergi Hydro-RP (150 × 4 mm i.d., 4 µm). Mobile phase: 50 mmol/L potassium dihydrogen phosphate (pH 4.5) : acetonitrile-methanol (3 : 1, 65 : 35), flow rate 1.0 mL/min. Retention time: 7.06 min. Limit of quantification, 1.0 mg/L, limit of detection, 0.5 mg/L both for monohydroxycarbamazepine [Contin *et al.* 2005].

See also Franceschi, Furlanut [2005], Mandrioli *et al.* [2003], Souppart *et al.* [2001], Matar *et al.* [1995], Rouan *et al.* [1994], Flesch *et al.* [1992], Hartley *et al.* [1991], van Heiningen *et al.* [1991], Elyas *et al.* [1990], Dickinson *et al* [1989], Menge *et al.* [1987], Menge, Dubois [1983], Noirfalise, Collinge [1983].

LC-MS Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (50 : 50) with 20 mmol/L acetic acid, flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 20 ug/L [de Sousa Maia *et al.* 2007]. Column: Zorbax XD8 C_8 (150 × 4.6 mm i.d., 4 µm). Mobile phase: acetonitrile : 2 mmol/L formate buffer (pH 3.0; 15 : 85 to 60 : 40 over 30 min to 70 : 30 over 10 min for 5 min to 15 : 85 over 1 min for 4 min), flow rate 0.8 mL/min. ESI, positive ion mode, full scan mode. Limit of quantification, 0.4 mg/L [Breton *et al.* 2005]. Column: LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 4 min to 10 : 90 at 7 min to 60 : 40 at 10 min), flow rate 0.4 mL/min for the first 4 min, 0.6 mL/min for the next 3 min followed by 0.4 mL/min. APCI, SIM acquisition mode. Limit of quantification, 0.1 mg/L; limit of detection, 0.01 mg/L [Maurer *et al.* 2002].

CE Capillary: uncoated fused silica (48.5/40.0 total/effective length, 50 µm i.d.). Running buffer: 30 mmol/L phosphate buffer with 60 mmol/L SDS : methanol (80 : 20). UV detection (λ = 205 nm). Limit of quantification, 0.15 mg/L, limit of detection, 0.05 mg/L [Pucci *et al.* 2003].

Serum **HPLC** Column: Betasil C_6 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer : acetonitrile (pH 3.0; 70 : 30), flow rate 1.3 mL/min. DAD (λ = 215 nm). Retention time: 16.05 min. Limit of quantification, 90 µg/L, limit of detection, 30.0 µg/L [Greiner, Haen 2007]. Column: Supelcosil LC-18 (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: 30 mmol/L potassium phosphate buffer (pH 3.7) : acetonitrile (65 : 35), flow rate 1.2 mL/min. UV detection (λ = 230 nm). Limit of quantification, 0.75 mg/L, limit of detection, 0.25 mg/L [Greiner-Sosanko *et al.* 2007]. Column: Alltima 3C_{18} (15 × 0.46 cm). Mobile phase: methanol : acetonitrile : 25 mmol/L phosphate buffer containing 12.5 mmol/L sodium chloride (pH 6.2), flow rate 0.9 mL/min. UV detection (λ = 215 and 275 nm). Retention time: 6.3 min for hydroxycarbazepine. Limit of quantification, 0.017 mg/L, limit of detection, 0.01 mg/L [Vermeij, Edelbroek 2007]. Column: C_{18} Ultrabiosep (150 × 4.6 mm i.d., 5 µm). Mobile phase: 7.3 mmol/L sodium acetate buffer (pH 5.4) : acetonitrile (68 : 32), flow rate 2.0 mL/min. UV detection (λ = 240 nm). Retention time: 2.35 min. Limit of quantification, 0.58 mg/L [Levert *et al.* 2002a; Levert *et al.* 2002b]. Column: Superspher 60 RP-select B (125 × 4 mm i.d., 4 µm). Mobile phase: acetonitrile : 20 mmol/L potassium dihydrogen phosphate (20 : 80) containing 0.05% triethylamine (pH 6.3), flow rate 1.0 mL/min. UV detection (λ = 212 nm). Limit of quantification, 55 µg/L, limit of detection, 12 µg/L [Pienimäki *et al.* 1995]. Column: LiChrosorb RP-8 (25 × 0.4 cm i.d., 10 µm). Mobile phase: acetonitrile : water (18 : 82 to 23 : 77 for 15 min to 80 : 20 at 16 min for 2.5 min to 18 : 82 at 19.5 min for 2 min), flow rate 2.0 mL/min. UV detection (λ = 190 to 600 nm). Retention time: 9.2 min. Limit of detection not reported [Wad 1984].

LC-MS Column: Symmetry C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 0.02% formic acid (40 : 60), flow rate 350 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 3.9 µg/L [Paglia *et al.* 2007].

Urine **HPLC** Column: Chiralcel OC (250 × 4.6 mm i.d.). Mobile phase: *n*-hexane : ethanol : 2-propanol (18 : 2 : 1) with 0.1% glacial acetic acid, flow rate 1.0 min. UV detection (λ = 215 nm). Retention time: 10.6 min for *R*-(–)-MHD, 13.3 min for *S*-(+)-MHD. Limit of quantification 200 µg/L for 10-hydroxycarbazepine and 400 µg/L for the 10,11-dihydro-10-hydroxycarbamazepine metabolite [Volosov *et al.* 2000]. See Plasma. Limit of quantification, 0.625 mg/L [van Heiningen *et al.* 1991].

Hair **LC-MS** See Blood. Limit of detection, 0.5 ng/mg [Klys *et al.* 2005].

Disposition in the Body Oxcarbazepine is rapidly and well absorbed after oral administration and is widely distributed in the body. It is rapidly and extensively metabolised in the liver to 10,11-dihydro-10-hydroxycarbamazepine which possesses antiepileptic activity, and a monohydroxy derivative (MHD). The drug is mainly excreted in urine as the metabolites, with <1% as the unchanged drug. Faecal excretion accounts for <4% of a dose. Approximately 80% of a dose is excreted in urine as glucuronides of MHD (49%) or as unchanged MHD (27%) whereas conjugates of oxcarbazepine account for 13% of the dose [Schütz *et al.* 1986]. The drug and the monohydroxy metabolite cross the placenta and are distributed into breast milk.

Therapeutic Concentration The therapeutic serum concentration range of hydroxycarbazepine is 12–30 mg/L.

Twelve young, healthy (age range, 30–31 years) and 12 elderly (61–82 years) males were administered with a single dose of 300 mg oxcarbazepine on day 1 and 300 mg twice daily on days 5 to 12. Twelve young (18–32 years) and 12 elderly (60–79 years) females were administered with a single 600-mg dose on day 1, 150 mg twice daily on day 5, and 300 mg twice daily on days 6 to 11. The maximum plasma concentration after the single doses was 2.33 and 2.88 mg/L for the young and elderly males, respectively. These were observed at 4 (4–6) and 5 (4–10) h, respectively. For the young females, concentrations were 5.17 mg/L at 4 (2–12) h and for the elderly, 7.20 mg/L at 5 (3–20) h. For multiple dosing, the maximum concentrations were 8.49 and 12.03 mg/L at 2 (2–4) and 4 (2–8) h for the young and elderly males, respectively and 9.34 and 11.72 mg/L at 3 (2–4) h for the young and elderly females. [van Heiningen *et al.* 1991].

A healthy volunteer was administered a single oral dose of 300 mg oxcarbazepine. The plasma concentration of unchanged drug reached a maximum of 0.4 µg/g after 1 h of intake of the drug [Menge, Dubois 1983].

Note For the pharmacokinetic influence of oxcarbazepine on feldopine, see Zaccara *et al.* [1993]. For the pharmacokinetics of oxcarbazepine in the placenta, see Pienimäki *et al.* [1997]. For changes in disposition during pregnancy, see Mazzucchelli *et al.* [2006]. For the pharmacokinetics of enantiomers of 10-hydroxycarbazepine following oxcarbazepine, see Volosov *et al.* [1999].

Toxicity The toxic serum concentration is 45 mg/L.

Note For the postmortem distribution of hydroycarbazepine in 3 cases, see [Levine *et al.* 2004].

Half-life Oxcarbazepine, 1–2.5 h [Dickinson *et al.* 1989]; monohydroxy derivative, 8–14 h.

Volume of Distribution 3.94 L/kg; monohydroxy derivative, 49 L.

Clearance Oral, 2.5 L/kg/h [Dickinson *et al.* 1989].

Protein Binding ≈40% (primarily to albumin).

Dose The initial daily dose is 300 mg with a maintenance dose of 600 to 1200 mg. The dose may need to be reduced in those with moderate-to-severe renal impairment (dose halved).

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Oxedrine

Sympathomimetic

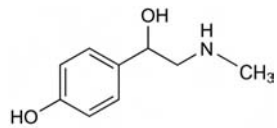
$C_9H_{13}NO_2$ = 167.2

CAS—94-07-5

IUPAC Name 4-Hydroxy-α-[(methylamino)methyl]benzenemethanol

Synonyms Oxyphenylmethylaminoethanol; sympaethaminum; synephrine.

Note *m*-Synephrine has been used as a synonym for phenylephrine.



Chemical Properties Crystals. Mp 184° to 185°. pK_a 9.3 (—OH), 10.2 (—NH—), (25°). Log *P* (octanol/water), −0.4.

Oxedrine Tartrate

$(C_9H_{13}NO_2)_2,C_4H_6O_6$ = 484.5

CAS—16589-24-5; 67-04-9 (±)

Synonym Aethaphenum tartaricum

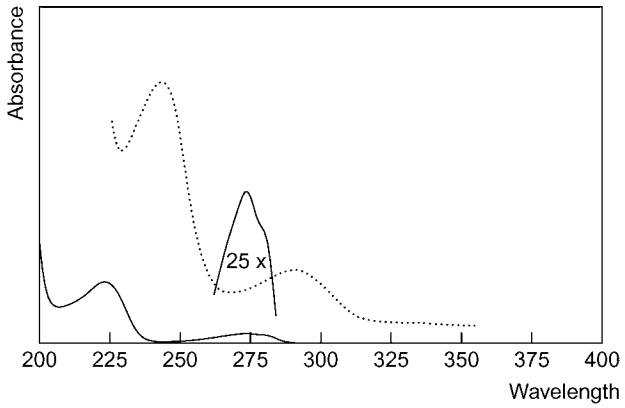
Proprietary Names *Dulcidrine*; *Sympatol*.

Chemical Properties Colourless crystals or white crystalline powder. Mp 188° to 190°, with some decomposition. Soluble 1 in 2 of water and 1 in 400 of ethanol; practically insoluble in chloroform and ether.

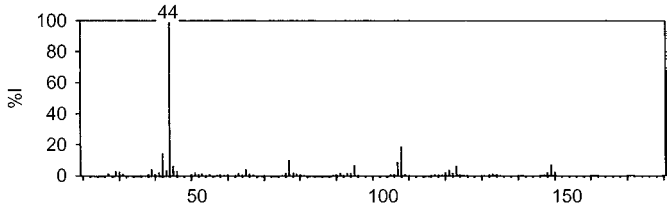
Thin-layer Chromatography System TA—R_f 0.25; system TB—R_f 0.04; system TC—R_f 0.01 (acidified potassium permanganate solution, positive).

High Performance Liquid Chromatography System HB—*k* 0.27.

Ultraviolet Spectrum Aqueous acid—273 nm (A^1_1=81a); aqueous alkali—241 (A^1_1=810b), 291 nm.



Infrared Spectrum Principal peaks at wavenumbers 835, 1510, 1054, 1271, 1098, 1611 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 44, 108, 42, 77, 107, 149, 95, 123.

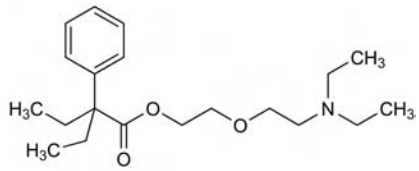


Dose Oxedrine tartrate has been given in doses of about 300 mg daily.

Oxeladin

Cough Suppressant
$C_{20}H_{33}NO_3$ = 335.5
CAS—468-61-1

IUPAC Name 2-[2-(Diethylamino)ethoxy]ethyl-α,α-diethylbenzeneacetate

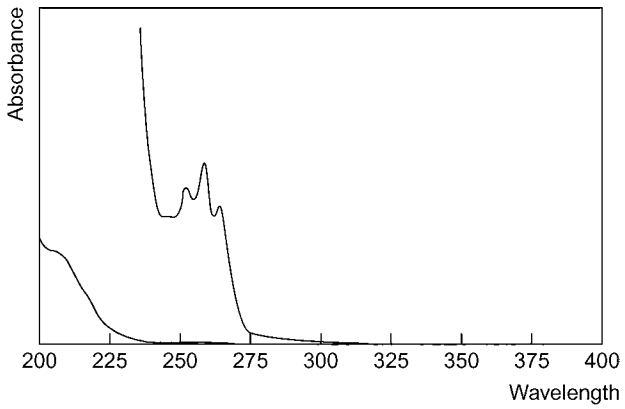


Chemical Properties A yellow oil. Practically insoluble in water; soluble in ethanol, acetone, toluene and ether. Log *P* (octanol/water), 4.3.

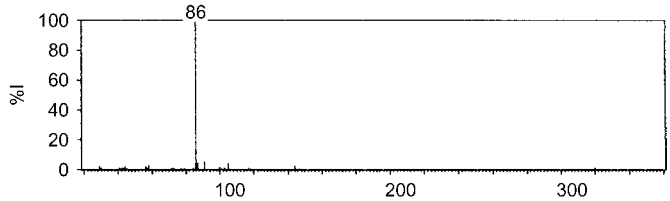
Oxeladin Citrate

$C_{20}H_{33}NO_3$, $C_6H_8O_7$ = 527.6
CAS—52432-72-1
Proprietary Names *Dorex-retard*; *Paxeladine*.
Chemical Properties A white crystalline powder. Mp 90° to 91°. Soluble in water.

Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.51; system TC—R_f 0.22; system TE—R_f 0.67; system TL—R_f 0.19; system TAE—R_f 0.19 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2190.
High Performance Liquid Chromatography System HA—*k* 3.0.
Ultraviolet Spectrum Aqueous acid—252, 258, 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1731, 1128, 1221, 1100, 700, 1080 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 91, 105, 87, 144, 58, 100, 56.

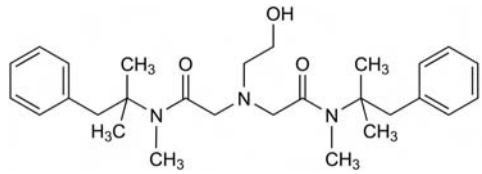


Dose 80 to 120 mg of oxeladin citrate daily.

Oxetacaine

Anaesthetic (Local)
$C_{28}H_{41}N_3O_3$ = 467.6
CAS—126-27-2

IUPAC Name 2-[2-Hydroxyethyl-[2-[methyl-(2-methyl-1-phenylpropan-2-yl)amino]-2-oxoethyl]amino]-*N*-methyl-*N*-(2-methyl-1-phenylpropan-2-yl)acetamide
Synonyms 2,2′-[(2-Hydroxyethyl)imino]bis[*N*-(1,1-dimethyl-2-phenylethyl)-*N*-methylacetamide]; oxethazaine.
Proprietary Names It is an ingredient of *Mucaine*, *Mucoxin*, *Mutesa*, *Muthesa*, *Oxaine* and *Tepilta*.

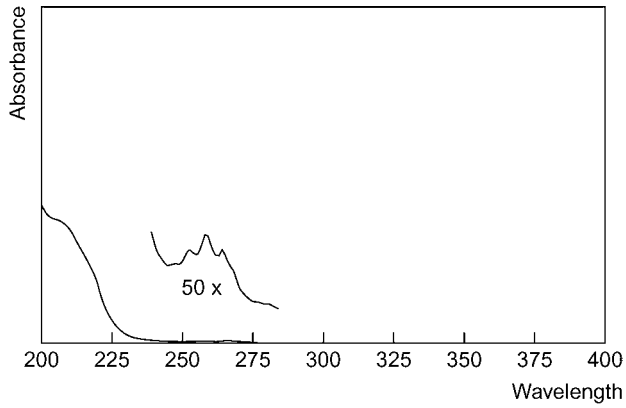


Chemical Properties A white crystalline powder. Mp about 104°. Insoluble in water; soluble in ethanol and in dilute acids. Log *P* (octanol/water), 3.4.

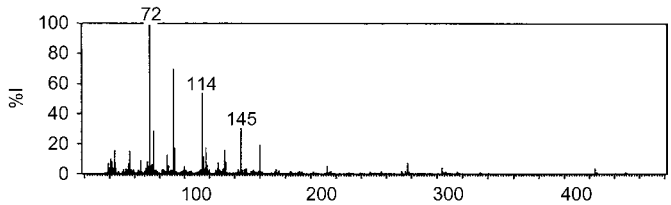
Oxetacaine Hydrochloride

CAS—13930-31-9 (xHCl)
Proprietary Name *Emoren*
Chemical Properties Mp 146° to 147°. Soluble in water.

Colour Tests Mandelin's test—violet-brown; Marquis test—red-brown.
Thin-layer Chromatography System TA—R_f 0.52; system TAE—R_f 0.61; system TB—R_f 0.10; system TC—R_f 0.07; system TE—R_f 0.38; system TL—R_f 0.15 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2525.
High Performance Liquid Chromatography System HR—*k* 4.14.
Ultraviolet Spectrum Aqueous acid—248, 252, 258, 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1645, 756, 707, 1219, 1101, 1068 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 72, 91, 114, 145, 75, 160, 117, 92.



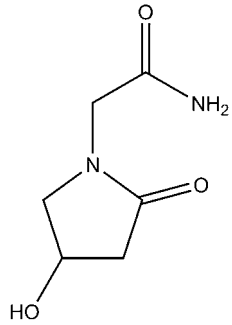
Dose 30 to 80 mg daily, orally.

O

Oxiracetam

Acetamide, Nootropic
$C_6H_{10}N_2O_3$ = 158.2
CAS—62613-82-5

IUPAC Name 2-(4-Hydroxy-2-oxopyrrolidin-1-yl)acetamide
Synonyms CGP-21690E; CT-848; ISF-2522; 4-hydroxy-2-oxo-1-pyrrolidineacetamide; hydroxypiracetam; 2-(4-hydroxypyrrolidin-2-on-1-yl)acetamido; oksirasetami; oxiracetamum; oxracetam.
Proprietary Names *Neupan*; *Neuractiv*; *Neuromet*.

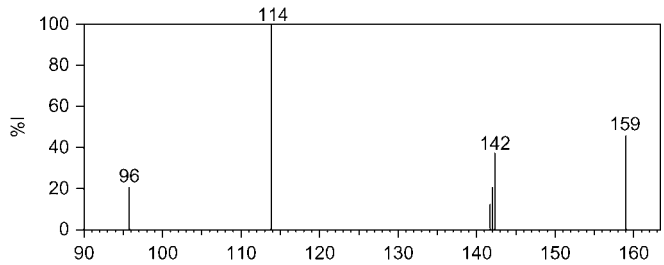


Chemical Properties White microcrystalline powder. Mp 165° to 168°. Log *P* (octanol/buffer pH 7.4) −2.25 [Gschwind *et al.* 1992].

(*R*)-Oxiracetam
Chemical Properties Crystals. Mp 135° to 136°.

(*S*)-Oxiracetam
Chemical Properties Crystals. Mp 135° to 136°.

Mass Spectrum Principal ions at *m/z* 114, 159, 142, 96, 141.



Quantification
Plasma **HPLC** Column switching system. UV detection (λ = 200 nm). Limit of quantification, 240 µg/L [Lecaillon *et al.* 1989].

LC-MS Column: Atlantis dC_{18} (50 × 3.0 mm i.d., 3 µm). Mobile phase: 0.1% acetic acid : methanol (99 : 1 to 20 : 80 over 1 min for 1 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.2 mg/L [Son *et al.* 2004].

Serum **HPLC** Column: Bondapak NH_2 (300 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water (80 : 20), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Retention time: 6.3 min. Limit of detection, 1 µg/L [Jiao *et al.* 1994]. Column: Silica (250 × 4.5 mm i.d., 5 µm). Mobile phase: hexane : propan-2-ol : water (77 : 22.5 : 0.5), flow rate 2 mL/min. UV detection (λ = 230 nm). Retention time: 6.3 min (triphenylsilyl ether derivative). Limit of quantification, 9.7 µmol/L; limit of detection, 3.4 µmol/L [Visconti *et al.* 1987].

Urine **HPLC** Column: Bondapak NH_2 (300 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water (80 : 20), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Retention time: 6.3 min. Limit of detection, 20 µg/L [Jiao *et al.* 1994]. Column switching system. UV detection (λ = 200 nm). Limit of quantification, 12 mg/L [Lecaillon *et al.* 1989]. Column: Silica (250 × 4.5 mm i.d., 5 µm). Mobile phase: hexane : propan-2-ol : water (77 : 22.5 : 0.5), flow rate 2 mL/min. UV detection (λ = 230 nm). Retention time: 6.3 min (triphenylsilyl ether derivative). Limit of quantification, 190 µmol/L [Visconti *et al.* 1987].

Disposition in the Body Well absorbed from the gastrointestinal tract after oral administration, with peak plasma concentrations reached after 1 to 3 h. It undergoes relatively extensive distribution in extravascular spaces. It is mainly excreted by renal clearance, with ~84% recovered unchanged in the urine. Oxiracetam does penetrate the blood–brain barrier to some extent.

Therapeutic Concentration

Four healthy male volunteers (aged 20 to 26 years) were administered a single oral dose of 2 g oxiracetam. The mean peak serum concentration was 40 mg/L after 1.25 h. The same group was administered a 200 mg/min IV infusion of oxiracetam over 5 min. Urinary recoveries of the dose were 88 and 93% after 24 and 48 h, respectively. The corresponding recoveries after oral administration were 61 and 68%, respectively [Perucca *et al.* 1984].

A group of 18 elderly patients (aged 73 to 98 years) was administered 800 mg oxiracetam orally on day 1 and then every 12 h from day 2 evening until the morning of day 10. Mean peak plasma concentrations on days 1 and 10 were 22 mg/L and 31 mg/L, respectively, reached after 2 h. Peak plasma concentrations in healthy non-geriatric volunteers given the same regimen were 5 mg/L on day 1 and 9 mg/L on day 10. The difference was mainly attributed to low values of creatinine clearance in the geriatric patients [Lecaillon *et al.* 1990].

Six patients (aged 53 to 66 years) diagnosed as having probable Alzheimer's disease were administered 2 g oxiracetam as a daily IV infusion over 60 min for 7 consecutive days. At the end of infusion, mean oxiracetam concentrations were 88.1 mg/L in serum and 3.5 mg/L in CSF. One hour after the infusion, oxiracetam concentrations fell to 52.9 mg/L in serum and 2.8 mg/L in CSF. The relative CSF : serum partition was found to be 4.0% at the end of infusion, rising to 5.3% 1 h later. Furthermore, there was an indication that elimination kinetics in CNS compartment are slower than in serum [Parnetti *et al.* 1990].

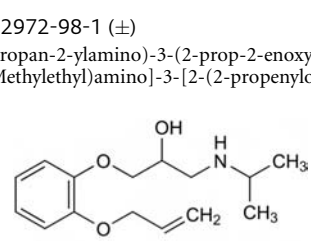
Toxicity No adverse events were observed when oxiracetam was administered concomitantly with valproic acid, clobazam, or carbamazepine. The half-life of oxiracetam decreased to 3.35 h (range 2.72 to 4.60 h) [van Wieringen *et al.* 1990].
Bioavailability Approximately 75% (range 68 to 82%), also reported as 56%.
Half-life Healthy volunteers 8 h; patients with renal impairment 10 to 68 h.
Volume of Distribution Approximately 0.9 to 1.2 L/kg; steady state, 0.55 L/kg.
Clearance Approximately 78.7 to 95.5 mL/min.
Distribution in Blood Blood : plasma ratio, 0.65.
Dose Used as a nootropic in organic brain syndromes and senile dementia in doses of up to 2 g daily.

Gschwind HP *et al.* (1992). Absorption and disposition of ^{14}C-labelled oxiracetam in rat, dog and man. *Eur J Drug Metab Pharmacokinet* 17: 67–82.
Jiao XL *et al.* (1994). Methodological study on the determination of oxiracetam concentration in serum and urine by HPLC. *Yao Xue Xue Bao* 29: 570–575.
Lecaillon JB *et al.* (1989). Determination of oxiracetam in plasma and urine by column-switching high-performance liquid chromatography. *J Chromatogr* 497: 223–230.
Lecaillon JB *et al.* (1990). Pharmacokinetics of oxiracetam in elderly patients after 800 mg oral doses, comparison with non-geriatric healthy subjects. *Eur J Drug Metab Pharmacokinet* 15: 223–230.
Parnetti L *et al.* (1990). Comparative kinetics of oxiracetam in serum and CSF of patients with dementia of Alzheimer type. *Eur J Drug Metab Pharmacokinet* 15: 75–78.
Perucca E *et al.* (1984). Pharmacokinetics of oxiracetam following intravenous and oral administration in healthy volunteers. *Eur J Drug Metab Pharmacokinet* 9: 267–274.
Son J *et al.* (2004). Rapid quantitative analysis of oxiracetam in human plasma by liquid chromatography/electrospray tandem mass spectrometry. *J Pharm Biomed Anal* 36: 657–661.
vanWieringen A *et al.* (1990). Pilot study to determine the interaction of oxiracetam with antiepileptic drugs. *Clin Pharmacokinet* 18: 332–338.
Visconti M *et al.* (1987). Determination of oxiracetam in human serum and urine by high-performance liquid chromatography. *J Chromatogr* 416: 433–438.

Oxprenolol

β-*Blocker*
$C_{15}H_{23}NO_3$ = 265.4
CAS—6452-71-7; 22972-98-1 (±)

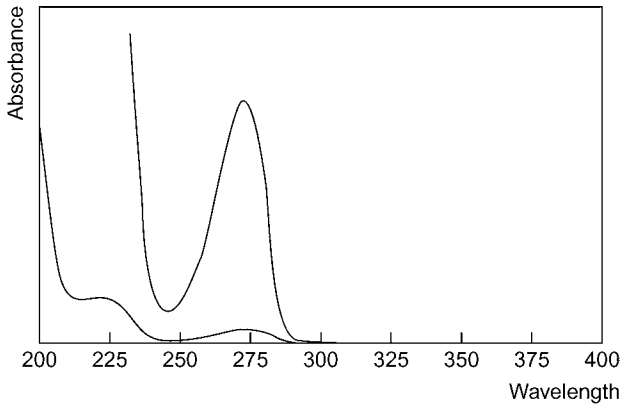
IUPAC Name 1-(Propan-2-ylamino)-3-(2-prop-2-enoxyphenoxy)propan-2-ol
Synonyms 1-[(1-Methylethyl)amino]-3-[2-(2-propenyloxy)phenoxy]-2-propanol; oxyprenolol.



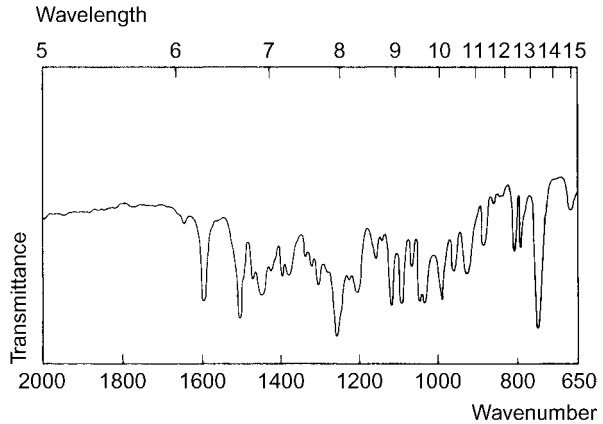
Chemical Properties Crystals. Mp 75° to 80°. pK_a 9.5. Log *P* (octanol/water), 2.1. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Oxprenolol Hydrochloride
$C_{15}H_{23}NO_3$, HCl = 301.8
CAS—6452-73-9; 22972-97-0 (±)
Proprietary Names *Apsolox*; *Laracor*; *Slow-Pren*; *Slow-Trasicor*; *Trasicor*. It is an ingredient of *Trasidrex*.
Chemical Properties A white to slightly cream-coloured crystalline powder. Mp 107° to 109°. Soluble 1 in <1 of water and 1 in 1.5 of ethanol; very slightly soluble in ether.

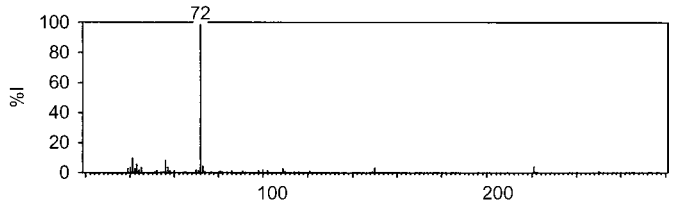
Colour Tests Liebermann's reagent—black; Mandelin's test—grey-violet; Marquis test—violet; sulfuric acid—orange-red (fluoresces under UV light at 350 nm).
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.11; system TC—R_f 0.11; system TE—R_f 0.45; system TL—R_f 0.13; system TAE—R_f 0.20; system TAF—R_f 0.78 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—Oxprenolol RI 1870, Art RI 1985; M (desamino-OH-desalkyl-) RI 1700; system GB—oxprenolol RI 1972, Art RI 2062; system GF—RI 2270.
High Performance Liquid Chromatography System HA—*k* 1.3; system HX—RI 354; system HY—RI 284; system HZ—retention time 3.0 min; system HAA—RT 12.0 min.
Ultraviolet Spectrum Aqueous acid—273 nm (A^{1}_{1}=85a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1258, 750, 1500, 1120, 1092, 1037 cm^{-1} (oxprenolol hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 41, 56, 43, 221, 73, 57, 45.



Quantification

Blood **GC-MS** For method of quantification for oxprenolol and other β-blockers, see Black *et al.* [1996].

HPLC UV detection. Limit of detection, 10 µg/L [Tsuei *et al.* 1980].

Plasma **TLC** Limit of detection, 10 µg/L [Schäfer, Mutschler 1979].

HPLC UV detection. Oxprenolol and celiprolol. Limit of detection, <25 µg/L for oxprenolol [Braza *et al.* 1998]. UV detection. Limit of detection, 20 µg/L [Godbillon *et al.* 1985]. See Blood [Tsuei *et al.* 1980].

Serum **GC** ECD. Limit of detection, 2 pg [DeBruyne *et al.* 1979].

LC-MS Limit of detection, 18–44 µg/L for oxprenolol and other β-blockers [Hyotylainen *et al.* 1997].

Urine **GC-MS** See Blood Black *et al.* [1996].

HPLC See Blood [Tsuei *et al.* 1980].

LC-MS See Serum [Hyotylainen *et al.* 1997].

Disposition in the Body Readily absorbed after oral administration but subject to extensive first-pass metabolism. The major metabolite is oxprenolol glucuronide. About 70–90% of a dose is excreted in the urine in 24 h, with <5% as unchanged drug.

Therapeutic Concentration

Following a single oral dose of 40 mg to 7 subjects, peak blood concentrations of 0.09–0.24 mg/L (mean, 0.15) of oxprenolol and 0.11–0.35 mg/L (mean, 0.24) of oxprenolol glucuronide were attained in 0.5–1.5 h and 1–2.5 h, respectively [Dayer *et al.* 1983].

Following oral administration of 160 mg twice daily to 27 subjects, steady-state plasma concentrations of 0.1–1.8 mg/L (mean, 0.9) were reported, determined 1 h after a dose [Silke *et al.* 1983].

Toxicity

In a fatality attributed to oxprenolol overdose, a postmortem liver concentration of 58 µg/g was reported [Oliver, Watson 1977].

In a fatality in which death occurred 2 h after the ingestion of 4.5 g, the following postmortem concentrations were reported: blood 10 mg/L, brain 71 µg/g, liver 230 µg/g [Khan, Muscat-Baron 1977].

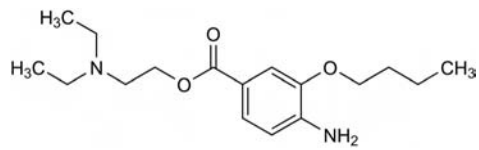
Half-life Plasma half-life, 1–3 h.
Volume of Distribution About 1 L/kg.
Protein Binding About 80%.
Dose 80 to 480 mg of oxprenolol hydrochloride daily.

Black SB *et al.* (1996). Solid-phase extraction and derivatisation methods for beta-blockers in human post mortem whole blood, urine and equine urine. *J Chromatogr Biomed Appl* 685: 67–80.
Braza AJ *et al.* (1998). Determination of celiprolol and oxprenolol in human plasma by high-performance liquid chromatography and the analytical error function. *J Chromatogr B Biomed Sci Appl* 718: 267–272.
Dayer P *et al.* (1983). *Eur J Drug Metab Pharmacokinet* 8: 181–188.
DeBruyne D *et al.* (1979). Improved electron-capture GLC determination of alprenolol and oxprenolol in serum using a wall-coated open tubular column. *J Pharm Sci* 68: 511–512.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Godbillon J *et al.* (1985). Determination of oxprenolol in human plasma by high-performance liquid chromatography, in comparison with gas chromatography and gas chromatography-mass spectrometry. *J Chromatogr* 345: 365–371.
Hyotylainen T *et al.* (1997). Liquid chromatographic sample cleanup coupled on-line with gas chromatography in the analysis of beta-blockers in human serum and urine. *J Chromatogr Sci* 35: 280–286.
Khan A, Muscat-Baron JM (1977). Fatal oxprenolol poisoning. *Brit Med J* 1: 552.
Oliver JS, Watson AA (1977). Oxprenolol (Trasicor) poisoning. *Med Sci Law* 17: 279–281.
Schäfer M, Mutschler E (1979). Fluorimetric determination of oxprenolol in plasma by direct evaluation of thin-layer chromatograms. *J Chromatogr* 164: 247–252.
Silke B *et al.* (1983). Initiation and maintenance of beta-blockade with intravenous oxprenolol. *Eur J Clin Pharmacol* 24: 7–14.
Tsuei S *et al.* (1980). Quantification of oxprenolol in biological fluids using high-performance liquid chromatography. *J Chromatogr* 181: 135–140.

Oxybuprocaine

Anaesthetic (Local)
$C_{17}H_{28}N_2O_3$ = 308.4
CAS—99-43-4

IUPAC Name 2-(Diethylamino)ethyl-4-amino-3-butoxybenzoate
Synonym Benoxinate

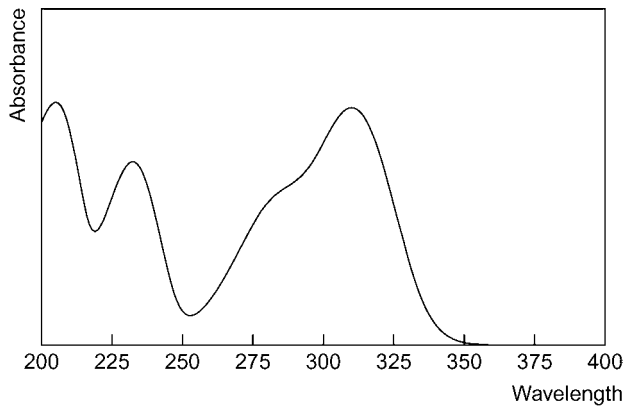


Chemical Properties Log *P* (octanol/water), 3.5.

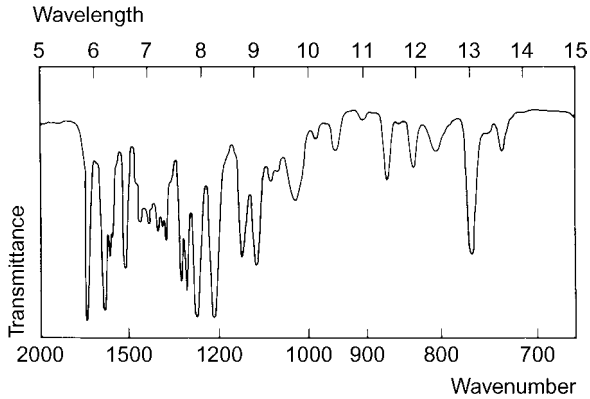
Oxybuprocaine Hydrochloride

$C_{17}H_{28}N_2O_3$, HCl = 344.9
CAS—5987-82-6
Proprietary Names *Cebesine*; *Conjuncain*; *Novesine*.
Chemical Properties White crystals or crystalline powder. Mp about 155° to 160°. Soluble 1 in 0.8 of water, 1 in 2.6 of ethanol and 1 in 2.5 of chloroform; practically insoluble in ether.

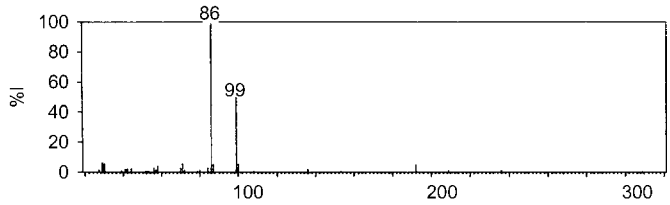
Colour Tests Coniferyl alcohol—orange; diazotisation—red.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.23; system TC—R_f 0.41; system TE—R_f 0.83; system TL—R_f 0.36; system TAE—R_f 0.54 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2471.
High Performance Liquid Chromatography System HQ—*k* 16.25; system HR—*k* 0.86; system HX—RI 405.
Ultraviolet Spectrum Aqueous acid—235 (A_1^1=437b), 298 nm; aqueous alkali—231, 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1709, 1214, 1261, 1613, 1294, 1311 cm^{-1} (oxybuprocaine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 29, 30, 100, 71, 87, 192.



Quantification

Blood **GC** Surface ionisation detection. Limit of detection, 5 to 10 pg for oxybuprocaine and other local anaesthetics [Hattori *et al.* 1991].

Aqueous Humour **HPLC** Electrochemical detection. Oxybuprocaine and diclofenac. Limit of detection, 50 µg/L for oxybuprocaine [Kuhlmann *et al.* 1998].

Cerebrospinal Fluid **GC** See Blood [Hattori *et al.* 1991].

Use Oxybuprocaine hydrochloride is used as a 0.4% solution.

Hattori H *et al.* (1991). Determination of local anaesthetics in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 564: 278–282.

Kuhlmann O *et al.* (1998). Simultaneous determination of diclofenac and oxybuprocaine in human aqueous humor with HPLC and electrochemical detection. *J Pharm Biomed Anal* 17: 1351–1356.

Oxybutynin

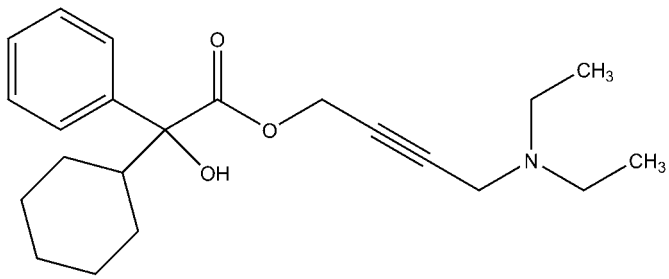
Antimuscarinic, Tertiary Amine, Treatment of Urinary Incontinence

$C_{22}H_{31}NO_3 = 357.5$

CAS—5633-20-5

IUPAC Name 4-Diethylaminobut-2-ynyl 2-cyclohexyl-2-hydroxy-2-phenylacetate

Synonyms α-Cyclohexyl-α-hydroxybenzeneacetic acid 4-(diethylamino)-2-butynyl ester; 4-(diethylamino)-2-butynyl phenylcyclohexaneglycolate; 4-(diethylamino)-2-butynyl α-phenylcyclohexaneglycolic acid ester; α-phenylcyclohexaneglycolic acid 4-(diethylamino)-2-butynyl ester.



Chemical Properties Soluble in water (77 mg/L, pH 1; 0.8 mg/L, pH 6; 0.012, pH >9.6). pK_a 8.04. Log *P* (octanol/water), 2.9 [O'Neil *et al.* 2006].

Oxybutynin Hydrochloride

$C_{22}H_{31}NO_3$, HCl = 393.9

CAS—1508-65-2

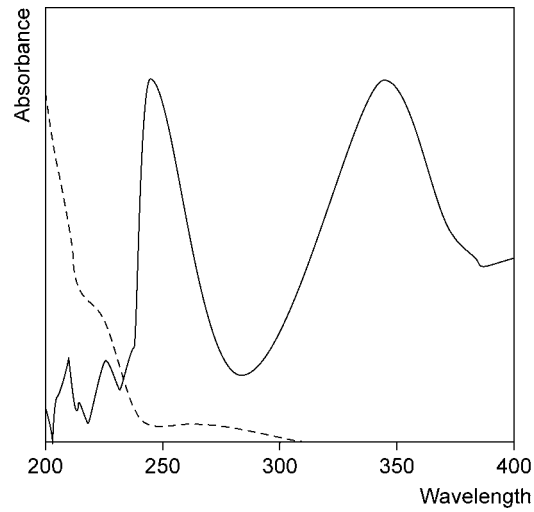
Synonyms 5058; MJ-4309-1; oxybutynin chloride.

Proprietary Names *Cystrin*; *Delak*; *Detrusan*; *Ditropan*; *Dresplan*; *Dridase*; *Driptane*; *Frenurin*; *Incontinol*; *Kentera*; *Lenditro*; *Lyrinel*; *Novitropan*; *Nu-Oxybutyn*; *Odranal*; *Oxi-Q*; *Oxitina*; *Oxybugamma*; *Oxyspas*; *Oxytrol*; *Oxyurin*; *Pollakisu*; *Retebem*; *Retemic*; *Retemicon*; *Reteven*; *Soxsup*; *Tropax*; *Urazol*; *Urequin*; *Uricont*; *Urotron*; *Uroxal*; *Zatur*.

Chemical Properties A white, practically odourless, crystalline powder. Mp 129° to 130°. Freely soluble in water and in alcohol; soluble in acetone; very soluble in chloroform and methyl alcohol; slightly soluble in ether; very slightly soluble in hexane.

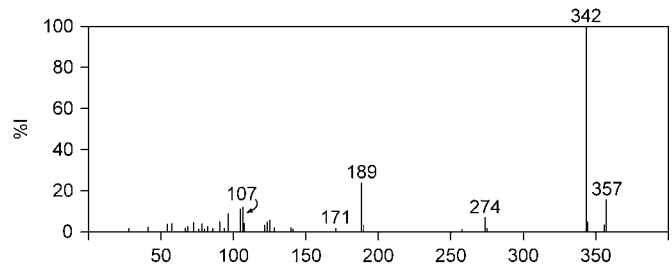
High Performance Liquid Chromatography Column: C_{18} (150 × 4.1 mm i.d., 5 µm). Mobile phase: methanol : water containing 30 mmol/L sodium octane sulfate and 7.5 mmol/L *N*,*N*-dimethyloctamine (pH 5; 65 : 35), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Retention time: 7.9 min. Limit of quantification not reported [De Schutter, De Moerloose 1988].

Ultraviolet Spectrum See Varma *et al.* [2004].



Infrared Spectrum Principal peaks at wavenumbers 1743, 2069, 1210, 2563, 2480, 2250 to 3500 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 342, 189, 357, , 107, 274, 343 [Lindeke *et al.* [1981].



Quantification

Blood **LC-MS** Column: C_{18} (150 × 2.1 mm i.d.). Mobile phase: methanol : 10 mmol/L ammonium acetate (75 : 25), flow rate 0.2 mL/min. Tandem MS. Limit of quantification, 10 µg/L for oxybutynin and *N*-desethyloxybutynin [Mizushima *et al.* 2007a].

Plasma **GC** Column: cross-linked methylsilicone (12 m × 0.2 mm i.d.). Carrier gas: He, 0.3 mL/min. Temperature programme: 140° for 2 min to 280° at 8°/min. NPD. Limit of quantification, 3.5 nmol/L [Aaltonen *et al.* 1984].

GC-MS Column: DB-1 methylsilicone fused-silica (30 m × 0.32 mm i.d.). Carrier gas: He, 50 cm/s. Temperature: 250°. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 3.0 min. Limit of quantification, 0.5 µg/L; limit of detection, 0.25 µg/L [Patrick *et al.* 1989].

HPLC Column: Cyano (100 mm, 5 µm). Mobile phase: methanol : 0.02 mol/L orthophosphoric acid buffer (pH 6.2; 40 : 60), flow rate 0.6 mL/min. Electrochemical detection. Retention times: oxybutynin 17 min, *N*-desethyloxybutynin 12 min. Limit of detection, 0.5 µg/L for oxybutynin, 5 µg/L for desethyloxybutynin [Hughes *et al.* 1992].

LC-MS See Blood. Limit of quantification, 0.2 µg/L for oxybutynin and *N*-desethyloxybutynin. Column: chiral AGP (100 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 4.0; 11 : 89), flow rate 1.3 mL/min. Tandem MS. Limit of quantification, 0.075 µg/L for oxybutynin and *N*-desethyloxybutynin enantiomers [Mizushima *et al.* 2007a]. Column: C_{18} (150 × 2.0 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 3.0; 50 : 50), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 3.2 min. Limit of quantification not reported [Zhang *et al.* 2005]. Column: C_{18} (50 × 2.0 mm i.d., 3 µm). Mobile phase: methanol : 0.01% formic acid (70 : 30), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification not reported [Cho *et al.* 2005]. Column: C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.2% formic acid (44 : 56), flow rate 0.25 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 1.9 min. Limit of quantification not reported [de Jager *et al.* 2002]. Column: chiral AGP (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (90 : 10), flow rate 0.15 mL/min. ESI. Limit of quantification, 0.5 µg/L [Zobrist *et al.* 2001].

Serum **GC-MS** Column: 3% OV-1 on GasChrom Q (80/100) (0.9 m × 2.0 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 220° isothermal. EI ionisation at 70 eV, SIM acquisition mode. Retention times: oxybutynin 2.2 min, *N*-desethyloxybutynin 1.9 min. Limit of detection, 0.5 µg/L [Lindeke *et al.* 1981].
Urine **GC-MS** See Serum [Lindeke *et al.* 1981].
Other **GC** Rat Liver. Column: 3% OV-17 on GasChrom Q (1.8 m × 2.0 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 230°. FID. Retention time: 4.7 min. Limit of quantification not reported [Lindeke *et al.* 1981].

HPLC Human Skin. Column: C_{18} (150 × 6.0 mm i.d.). Mobile phase: 0.02 mol/L potassium dihydrogen phosphate-0.005 mol/L hexane sulfonic acid-0.3% triethylamine (pH 4.5) : acetonitrile (50 : 50), flow rate 1.5 mL/min. UV detection (λ= 214 nm). Limit of quantification, 0.51 mg/L [Mizushima *et al.* 2007b]. Capsules, Tablets, and Syrup. Column: VP-ODS Shim-pack (250 × 4.6 mm i.d., 4.6 µm). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogen phosphate : diethylamine (60 : 40 : 0.2), flow rate 1.5 mL/min. UV detection (λ= 220 nm). Retention time: 2.5 min. Limit of quantification, 5.64 µg/L, limit of detection, 1.69 µg/L [El Gindy 2005]. Tablet Dissolution Studies. Column: Cyano (250 × 4.6 mm i.d., 5 µm). Mobile phase: water-methanol-triethylamine (pH 3.5; 3200 : 800 : 0.9) : acetonitrile (60 : 40), flow rate 1.5 mL/min. UV detection (λ= 203 nm). Retention time: 6.8 min. Limit of quantification, 1.65 mg/L, limit of detection, 0.5 mg/L [Varma *et al.* 2004]. Column: Inertsil phenyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : triethylamine (pH 3.8; 35 : 65 : 0.15). UV detection (λ= 220 nm). Limit of quantification, 0.28 mg/L [Sathyan *et al.* 2004]. Intravaginal Rings. Column: ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.02 mol/L potassium phosphate buffer (pH 5.0; 45 : 55). UV detection (λ= 220 nm). Retention time: 2 min. Limit of quantification not reported [Malcolm *et al.* 2003]. Column: ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% orthophoshoric acid (pH 2.5; 65 : 35), flow rate 1.6 mL/min. UV detection (λ= 220 nm). Retention time: 1.7 min. Limit of quantification not reported [Woolfson *et al.* 2003]. Transdermal Patch Residues. Column: Cosmosil 5CN-MS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L potassium dihydrogen phosphate-0.005 mol/L sodium hexane sulfonate-0.3% triethylamine (pH 4.5) : acetonitrile (65 : 35), flow rate 1.2 mL/min. UV detection (λ= 220 nm). Limit of quantification, 60 µg/L [Zobrist *et al.* 2001]. Human Bladder Tissue. Column: C_{18} (250 × 4.5 mm i.d., 4.6 µm). Mobile phase: acetonitrile : water : 1 mol/L ammonium acetate (pH 7.0; 85 : 13 : 2), flow rate 0.5 mL/min. Electrochemical detection, UV detection (λ= 235 nm). Retention time: 18 min. Limit of quantification, 5 µg/L [Massoud *et al.* 1999]. Capsules, Tablets, and Syrup. Column: C_{18} (150 × 4.1 mm i.d., 5 µm). Mobile phase: methanol : 30 mmol/L sodium octane sulfate and 7.5 mmol/L *N*,*N*-dimethyloctamine (pH 5; 65 : 35), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Retention time: 7.9 min. Limit of quantification not reported [De Schutter, De Moerloose 1988].
Note For UV spectrophotometric determination of oxybutynin, see Varma *et al.* [2004].

Disposition in the Body After oral doses of oxybutynin, peak plasma concentrations are reached within 1 h. Oxybutynin is also absorbed after application to the skin. Transdermal administration of oxybutynin avoids the extensive presystemic gastrointestinal and hepatic metabolism associated with other routes of administration. However, minimal presystemic metabolism occurs via a small amount of CYP3A4 present in the skin, resulting in the limited formation of the major active metabolite, *N*-desethyloxybutynin. It is a chiral compound with the *R*-enantiomer being mainly responsible for its antimuscarinic acitivity. Oxybutynin undergoes extensive first-pass metabolism, particularly by CYP3A4 (also CYP3A5) to *N*-desethyloxybutynin. Oxybutynin and its metabolites are excreted in the urine and faeces. Oxybutynin has been detected in breast milk. Evidence suggests that it may cross the blood–brain barrier.

Therapeutic Concentration

A group of 18 healthy young volunteers was administered oxybutynin either as a transdermal patch (applied to the lower abdomen, dose 36 mg over 96 h) or as an immediate-release tablet (5 mg). Peak oxybutynin plasma concentrations were as follows:

	Patch		Tablet	
	C_{max} (µg/L)	Time (h)	C_{max} (µg/L)	Time (h)
R-oxybutynin	1.2	48	2.2	1.0
R-desethyloxybutynin	1.2	48	15.5	1.0
S-oxybutynin	1.6	48	4.1	0.8
S-desethyloxybutynin	1.4	48	10.9	1.0

[Zobrist *et al.* 2001].

Three groups of volunteers (group A: healthy, mean age 29 years; group B: healthy elderly, mean 76 years; group C: elderly with urinary incontinence, mean age 79 years), were administered a single oral dose of 5 mg oxybutynin.

Peak plasma concentrations for oxybutynin and its *N*-desethyl metabolite in the three groups were as follows:

	Oxybutynin			N-Desethyl metabolite		
	Group A	Group B	Group C	Group A	Group B	Group C
C_{max} (µg/L)	13.4	16.7	32.0	68.9	57.9	63.7
Time (h)	0.76	0.69	0.60	1.0	1.4	1.0

Variations in values appeared to be a result of variation in the rate of absorption and the degree of first-pass metabolism [Hughes *et al.* 1992].

In a further study where group B was administered 5 mg oral oxybutynin 3 times daily for 14 days and group C was administered 5 mg twice daily for 14 days, peak plasma concentrations (C_{max}) for oxybutynin and its *N*-desethyl metabolite were:

	Oxybutynin		N-Desethyl metabolite	
	Group B	Group C	Group B	Group C
C_{max} (µg/L)	18.1	37.7	63.3	82.5
Time (h)	0.65	0.56	1.1	1.1

[Hughes *et al.* 1992].

A group of 24 healthy male subjects (aged 18 to 45 years old) were administered oxybutynin as either a 15 mg controlled-release tablet daily for 5 days (CR group) or as 5 mg immediate-release tablets three times daily for 5 days (IR group). Values for C_{max} for oxybutynin and its *N*-desethyl metabolite were reported as:

	Oxybutynin		N-Desethyl metabolite	
	CR group	IR group	CR group	IR group
C_{max} (µg/L)	5.7	7.5	31.2	57.9
Time (h)	9.2	6.5	8.3	1.8

[Reiz *et al.* 2007].

Toxicity Oxybutynin is a potent inhibitor of CYP3A4 and CYP2D6.
Bioavailability Approximately 6%.
Half-life 2–3 h.
Volume of Distribution 89.2 L after a 1 mg bolus IV administration and 192.9 L after a 5 mg infusion.
Clearance 26–34 L/h.
Protein Binding Both enantiomers bind strongly (>99%), principally to α_1-acid glycoprotein. *S*-Oxybutynin is bound to α_1-acid glycoprotein more strongly than the *R*-enantiomer and this is reflected in the enantioselective protein binding of this drug in plasma [Shibukawa *et al.* 2002a, b].
Note For a review of the clinical pharmacokinetics of different formulations of oxybutynin, see Guay [2003].

Dose Usual dosage of oxybutynin hydrochloride is 5 mg two or three times daily by mouth, increased to 5 mg four times daily if required. In elderly patients, lower doses of 2.5 or 3 mg twice daily initially, increased to 5 mg twice daily if necessary, may be adequate. Modified-release preparations of oxybutynin hydrochloride are also available. The initial dose is 5 mg once daily, increased by 5 mg at weekly intervals if necessary, up to a maximum of 20 or 30 mg daily, depending on the preparation. Oxybutynin is also given via a transdermal patch, which supplies 3.9 mg of oxybutynin daily.

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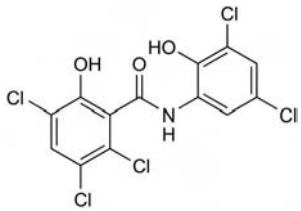
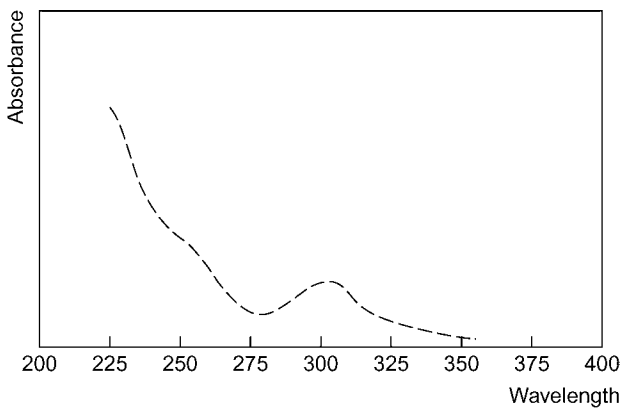
Oxyclozanide

Anthelmintic (Veterinary)
$C_{13}H_6Cl_5NO_3$ = 401.5
CAS—2277-92-1

IUPAC Name 2,3,5-Trichloro-*N*-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide
Proprietary Name *Zanil*



Chemical Properties A cream-coloured powder. Mp 209° to 211°. Very slightly soluble in water; soluble 1 in 20 of ethanol, 1 in 5 of acetone and 1 in 600 of chloroform; soluble in solutions of alkali hydroxides and carbonates. Log *P* (octanol/water), 6.0.
Colour Test Mandelin's test—violet→orange.
Thin-layer Chromatography System TA—R_f 0.87 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous alkali—324 nm (A^1_1=338b); methanol—303 nm (A^1_1=188b).

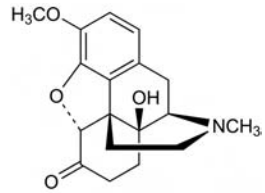


Infrared Spectrum Principal peaks at wavenumbers 1203, 1527, 1166, 1587, 1155, 1219 cm^{-1} (KBr disk).

Oxycodone

Narcotic Analgesic
$C_{18}H_{21}NO_4$ = 315.4
CAS—76-42-6

Synonyms 7,8-Dihydro-14-hydroxycodeinone; dihydrone; (5*R*,9*R*,13*S*,14*S*)-4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one; (5α)-4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one; oxycone; NSC-19043.



Chemical Properties Mp 218° to 220°. It is practically insoluble in water and ether; soluble in ethanol and chloroform. pK_a 8.9 (20°). Log *P* (octanol/water), 0.7. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Oxycodone Hydrochloride

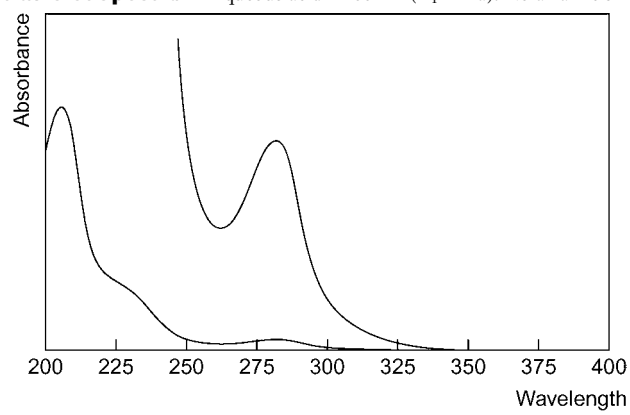
$C_{18}H_{21}NO_4$, HCl,$3H_2O$ = 405.9
CAS—124-90-3 (anhydrous)
Synonym Thecodine
Proprietary Names *Endocodone*; *Endone*; *ETH-Oxydose*; *Eubine*; *Eukodal*; *Oxycod*; *OxyContin*; *Oxyfast*; *Oxygesic*; *OxyIR*; *OxyNorm*; *Percolone*; *Roxicodone*; *Supeudol*; *Targinact*. It is an ingredient of *Combunox*; *Endocet*; *Magnacet*; *Narvox*; *Percocet*; *Percodan*; *Perloxx*; *Pimalev*; *Roxicet*; *Roxilox*; *Roxiprin*; *Tylox*; *Xolox*.
Chemical Properties A white crystalline powder. Mp 270° to 272° (rods from water), with decomposition. It is soluble 1 in 10 of water, 1 in 60 of ethanol and 1 in 600 of chloroform; practically insoluble in ether.

Colour Test Marquis test—yellow→brown→violet.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.25; system TC—R_f 0.51; system TE—R_f 0.62; system TL—R_f 0.39; system TAE—R_f 0.30; system TAF—R_f 0.33; system TAJ—R_f 0.27; system TAK—R_f 0.01; system TAL—R_f 0.36 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—violet.)

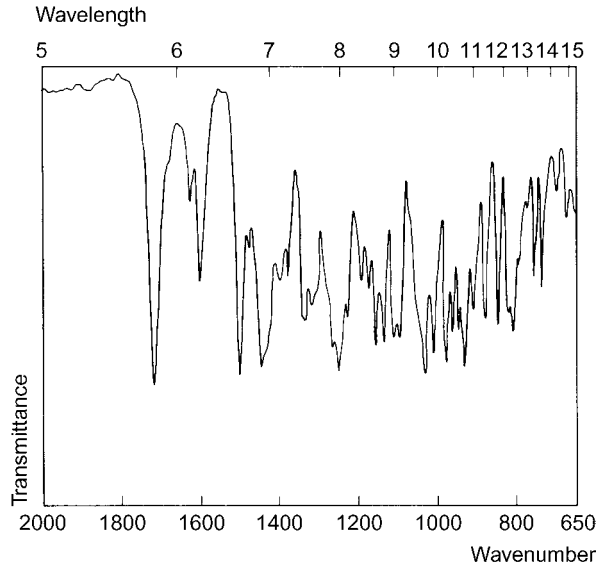
Plate: silica (10 × 20 cm). Mobile phase: propan-2-ol : acetic acid : water: 25% ammonia : methanol (20 : 1 : 1 : 3 : 10). UV detection. R_f 0.6 [Salomies, Salo 2000].
Gas Chromatography System GA—oxycodone RI 2524, oxycodone-AC RI 2555, oxycodone-enol-AC_2 RI 2560, oxycodone-trifluroacetic acid RI 2290, M (nor-)-enol-AC_3 RI 2680, M (dihydro-)-AC_2 RI 2570, M (nor-dihydro-)-AC_3 RI 2935, M (nor-dihydro-)-AC_2 RI 2900, M (oxymorphone) RI 2538; system GB—oxycodone RI 2671, oxycodone-TMS RI 2703, oxycodone-enol-TMS_2 RI 2602, oxycodone-oxime-TMS_2 RI 2740, M (nor-) RI 2703, M (nor-)-TMS_2 RI 2763, M (nor-)-enol-TMS_2 RI 2621, M (nor-)-enol-TMS_3 RI 2746, M (dihydro-) RI 2666, M (oxymorphone) RI 2723; system GC19—RT 5.53 min.

Column: HP-1 fused silica capillary (25 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 150°, to 245° at 35°/min. Carrier gas: He, 1.5 mL/min at 40° and 0.5 mL/min at 150°. NPD. Retention time: 12.8 min. Limit of detection, 1.8 μg/L [Kapil *et al.* 1992].
Gas Chromatography–Mass Spectrometry Column: HP-Ultra-2 capillary (25 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 90°, for 2 min to 310° at 15°/min, for 15 min. Carrier gas: He, 0.8 mL/min. SIM acquisition mode (*m/z* 315, 230, 140). Retention time: 18.1 min. [Drummer *et al.* 1994].
High Performance Liquid Chromatography System HA—oxycodone *k* 6.9 (tailing peak), M (oxymorphone) *k* 6.7 (tailing peak); system HC—*k* 0.85; system HX—RI 277; system HY—RI 246; system HAX—RT 6.5 min; system HAY—RT 5.8 min.

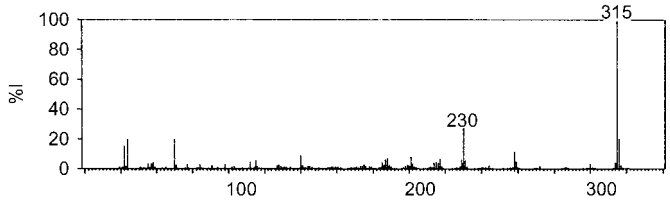
Column: Hypersil BDS-C_{18} (100 × 46 mm i.d., 3 μm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 3) : acetonitrile (93 : 7), 1.0 mL/min flow rate. UV detection, (λ=212 nm). Retention time: 9.1 min [Pascual, Sanagustin 1999].
Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1= 44a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1719, 1501, 1033, 1255, 934, 980 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 315, 230, 316, 70, 44, 42, 258, 140 (oxycodone); 301, 216, 44, 42, 70, 302, 203, 57 (oxymorphone).



Quantification

Blood **GC-MS** Column: 100% methylsiloxane or 5% phenylsiloxane, 95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 20°/min for 2 min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: DB-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° to 170° at 40°/min to 290° at 10°/min for 3.75 min. ESI. Limit of quantification, 1 mg/L, limit of detection, 1 mg/L [Antonides *et al.* 2007]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 305° at 10°/min for 0.5 min. EI ionisation. Retention time: 385 s. Limit of quantification, 10 µg/L, limit of detection, 2 µg/L [Meatherall 2005]. Column: HP-1MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 62 cm/s. Temperature programme: 160° to 195° at 35°/min to 240° at 5°/min to 300° at 30°/min for 2 min. SIM acquisition mode. Limit of quantification, 20 µg/L [Ropero-Miller *et al.* 2002].

LC-MS Column: Waters HSS T3 (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 1% formic acid in water : 1% formic acid in acetonitrile (97 : 3 for 0.1 min to 80 : 20 at 2.5 min to 1 : 99 at 2.55 min for 0.45 min to 97 : 3 at 3.01), flow rate 0.6 mL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L [Dahn *et al.* 2010]. Column: Genesis C_{18} reversed phase. Mobile phase: acetonitrile and ammonium acetate (pH 3.2). MRM acquisition mode. Oxycodone and other opioids [Gergov *et al.* 2009]. Column: Phenomenex Synergi reversed phase. Mobile phase: 10 mmol/L ammonium formate (pH 3) : acetonitrile. Limit of quantification, 0.5–4.09 µg/L, limit of detection, 0.16–1.2 µg/L for oxycodone and other opioids and metabolites [Al Asmari, Anderson 2007]. Oxycodone and other opioids and metabolites [Coles *et al.* 2007].

Plasma **GC** Column: Methyl silicone (HP-1) fused silica (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.5 mL/min. Temperature programme: 150° to 245° at 35°/min. NPD. Retention time: 12.8 min. Limit of quantification, 1.8 µg/L [Kapil *et al.* 1992].

HPLC Column Pyramid CC 250/4 C_{18} (5 µm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer (8 : 92), flow rate 1 mL/min. UV detection (λ = 205 nm). Limit of quantification, 2 µg/L [Cheremina *et al.* 2005]. Column: C_{18} (100 × 8 mm i.d.). Mobile phase: methanol : acetonitrile : 0.0133 mol/L phosphate buffer (pH 7.5, 23 : 2 : 75). Retention time: 9.6 min. Electrochemical detection. Limit of quantification, 14.2 nmol/L [Wright *et al.* 1998]. Electrochemical detection. Limit of detection, 10 µg/L [Smith *et al.* 1991]. Mobile phase: 0.01 mol/L potassium dihydrogen orthophosphate : methanol : acetonitrile (20 : 30 : 50), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 2 µg/L [Schneider *et al.* 1984].

LC-MS See Blood [Dahn *et al.* 2010]. ESI. Limit of detection, 0.1 µg/L for oxycodone and 0.25 µg/L for noroxycodone [Neuvonen, Neuvonen 2008]. See Blood [Coles *et al.* 2007]. Column: Phenomex C_{12} MAX-RP (150 × 2 mm i.d., 4 µm). Mobile phase: ammonium formate buffer (pH 3.5) : acetonitrile. Oxycodone and other opioids [Musshoff *et al.* 2006]. ESI. Limit of quantification, 1 µg/L, estimated limit of detection, 33 ng/L [Dawson *et al.* 2002].

Serum **HPLC** Column: XTerra RP18 (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 5 mmol/L phosphate buffer (pH 8, 2 : 1 : 7). Electrochemical detection. Limit of quantification, 0.5 µg/L [Kokubun *et al.* 2005]

LC-MS See Blood [Coles *et al.* 2007; Dahn *et al.* 2010].

Urine **GC-MS** See Blood [Goldberger *et al.* 2010]. Column: DB-5MS (15 m × 0.249 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 240° to 280° at 40°/min for 1.2 min. SIM acquisition mode. Limit of quantification, 40 µg/L, limit of detection, 40 µg/L [McKinley *et al.* 2007]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 290° at 10°/min for 0.5 min. EI ionisation, full scan mode. Retention time: 385 s. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Meatherall 1999]. Column: DB-5 (15 m × 0.26 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 225° to 280° at 30°/min. SIM acquisition mode. Limit of detection, 1.2 µg/L [Smith *et al.* 1995].

LC-MS See Blood [Dahn *et al.* 2010]. Column: Acquity UPLC BEH C_{18} (50 × 2.1 mm i.d., 1.7 µm). Mobile phase: 5 mmol/L ammonium bicarbonate (pH 10.2) : methanol (95 : 5 for 0.15 min to 70 : 30 at 0.3 min to 50 : 50 in 2.7 min to 10 : 90 at 3.8 min until 4.2 min to 95 : 5 at 4.5 min for 0.5 min), flow rate 0.4 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.005 mg/L, limit of detection, 0.0016 mg/L [Berg *et al.* 2009]. See Blood [Coles *et al.* 2007; Gergov *et al.* 2009]. Column: Agilent Zorbax SB Phenyl (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.05% formic acid with 10 mmol/L ammonium acetate-0.05% formic acid in acetonitrile (95 : 5) : 0.05% formic acid in acetonitrile-0.05% formic acid with 10 mmol/L ammonium formate (95 : 5, 100 : 0 for 0.5 min to 82 : 18 at 3 min for 2.5 min to 100 : 0). ESI. Limit of quantification, 50 µg/L [Edinboro *et al.* 2005].

Meconium See Blood [Coles *et al.* 2007].

Oral Fluid **GC-MS** Column: DB-5 5% phenylsiloxane, 95% methylsiloxane (25 m × 0.2 mm i.d., 0.33 mm). Carrier gas: 1.5 mL/min. Temperature programme: 150° for 1.0 min to 245° at 20°/min for 8 min to 290° at 50°/min. EI ionisation, MSD. Limit of quantification, 2 µg/L, limit of detection, 3 µg/L [Jones *et al.* 2002].

Vitreous Humour **GC-MS** Limit of quantification, 50 µg/L, limit of detection, 10 µg/L [Knittel *et al.* 2009]. See Blood [Antonides *et al.* 2007].

Hair **GC-MS** See Oral Fluid [Jones *et al.* 2002].

Disposition in the Body Oxycodone is absorbed after oral administration and metabolised to some extent by *O*-demethylation to oxymorphone, which is active, and by *N*-demethylation to noroxycodone. Approximately 30–60% of a dose is excreted in the urine in 24 h as free and conjugated oxycodone, conjugated oxymorphone and noroxycodone. Oxymorphone is excreted mainly in the conjugated form and noroxycodone as the unconjugated. After multiple dosing, accumulation of the drug does not occur. Oxycodone is secreted in breast milk.

Therapeutic Concentration

Nine healthy, young, male and female volunteers were administered 0.14 mg/kg oxycodone hydrochloride once IM (equivalent to 0.11 mg/kg free base) in the first study and 0.28 mg/kg oxycodone chloride orally (equivalent to 0.22 mg/kg free base) in 2 additional studies at intervals of 2 weeks. The mean maximum plasma oxycodone concentration was 34 µg/L for the IM dose and 38 µg/L for the oral doses, observed within 1 h of administration. The peak noroxycodone concentration after the IM dose was 4.0 µg/L and after the oral doses,14.78 µg/L. These were observed at 1.75 h (range, 0.75–5.0) and 0.75 h (range, 0.25–2.0), respectively [Pöyhiä *et al.* 1992].

Twelve patients with moderate to severe cancer pain and a mean age of 58 years (range, 23–71) were administered 5 mL oxycodone hydrochloride (concentration 5 mg/mL, equivalent to 4.6 mg/mL base) as an IV infusion and 10 mg oxycodone hydrochloride every 4 h (equivalent to 9.1 mg base). A mean maximum plasma concentration of 34.6 µg/L (range, 17.0–62.2) was observed at 1.6 h for the oral dose. A steady-state concentration of 47.7 µg/L (range, 26.8–68.9) was observed at 1.04 h (range, 0.25–2.0). The minimum steady-state concentration was 23.5 µg/L (range, 10.4–43.1) [Leow *et al.* 1992].

Twenty-four healthy volunteers, aged 21–50 years (mean, 34.5) were administered either a 10-mg controlled-release oxycodone hydrochloride tablet every 12 h for 4 days or 5 mg of immediate-release oxycodone hydrochloride solution every 6 h for 4 days. Volunteers were fasted 2 h before and after drug administration. Steady-state concentrations were observed after 1 day of dosing and maintained until day 4. The mean maximum plasma oxycodone concentration after the controlled-release formulation was 15.1 µg/L, and after the immediate-release formulation, it was 15.6 µg/L. These concentrations were observed at 3.2 and 1.4 h, respectively [Reder *et al.* 1996].

In 28 subjects administered a single 20-mg oral controlled-release oxycodone tablet, mean maximum plasma concentrations of 23.2 µg/L for oxycodone, 15.2 µg/L for noroxycodone and 0.82 µg/L for oxymorphone occurred at 3.2, 4.3, and 4.5 h, respectively, after dosing. When compared for gender and age, young and elderly women (7 subjects in each group) had the highest oxycodone AUC values, the lowest oxymorphone AUC values and the greatest drug effect. Young and elderly men (7 subjects in each group) had the lowest oxycodone AUC values, the highest oxymorphone AUC values, and the lowest drug effect [Kaiko *et al.* 1996].

In 30 children aged 6–91 months, maximum plasma levels after 0.2 mg/kg oxycodone buccally or sublingually were 16 and 22 µg/L, respectively. In 12 of 15 children in each group, oxycodone reached the therapeutic concentration (12 µg/L). Elimination half-lives were 140 and 150 min, respectively [Kokki *et al.* 2006].

Toxicity The estimated minimum lethal dose is 500 mg. Prolonged use of oxycodone may lead to dependence of the morphine type. A toxic serum concentration is 0.2 mg/L and a probable lethal concentration, 0.6 mg/L.

A 28-year-old male heroin addict with acquired immunodeficiency syndrome (AIDS) died while in police custody. Toxic concentrations of oxycodone were found in femoral blood at a concentration of 1.2 mg/L and in his liver at 1.9 mg/kg. Traces of flunitrazepam were also detected in his blood. His death was determined to be caused by a combination of the toxic effects of both oxycodone and flunitrazepam [Drummer *et al.* 1994].

In a total of 9 deaths investigated by the Victorian Institute of Forensic Pathology, Australia, the mean femoral blood oxycodone concentration was 0.9 mg/L (range, 0.6–1.4). Oxycodone was not detected very often in the gastric contents. It was shown that oxycodone femoral blood concentrations of 0.6 mg/L or higher may result in death [Drummer *et al.* 1994].

A 45-year-old woman who was admitted to hospital after suffering generalised convulsion had been discovered next to a nearly full bottle of controlled-release oxycodone tablets; unfortunately the bottle was left near her bedside and approximately 20 min after her arrival, she ingested the remaining contents (when full, the bottle contained 100 tablets of 40 mg each). The patient developed CNS and respiratory depression, and IV naloxone and oral activated charcoal was administered. Naloxone proved inadequate and mechanical ventilation was required for 3 days before the patient recovered completely. Three serum oxycodone levels were noted: the initial and highest was 2400 µg/L 10 h after admission; the level declined to ~1000 µg/L at 15 h and 100 µg/L at 70 h [Schneir *et al.* 2002].

A 2-year-old child was admitted to hospital after exhibiting signs of rubbing of the mouth and staggering. She was discharged after a urine sample was found negative for drugs of abuse and tricyclic antidepressants, but she was found unresponsive and in cardiopulmonary arrest the next morning and she died despite efforts to resuscitate. Oxycodone was found at postmortem with levels of 1.36 mg/L in heart blood, 222.34 mg/L in gastric contents, 0.2 mg/kg in liver and 47.23 mg/L in urine [Armstrong *et al.* 2004].

A 10-month-old infant who went into cardiac arrest and died was found to have postmortem blood and liver oxycodone levels of 0.6 mg/L and 1.6 mg/kg, respectively [Levine *et al.* 2004].

In a review of a 6-year period at a toxicology laboratory, there were 67 cases where oxycodone had been detected and 36 of these were determined to involve controlled-release formulations and could be identified by 'ghost pills' (intact, but drug-free tablets). In these cases, postmortem levels were: 0.12–46 mg/L in heart blood, <0.1–13 mg/L in femoral blood, 0.11–6.1 mg/kg in liver, 2.5–122 mg/L in urine, 0.19–49 mg/L in bile, 0.24–0.82 mg/L in vitreous humour and 0.06–119 mg in gastric contents [Anderson *et al.* 2002].

A review of postmortem and coroner's reports from 10 countries for 2000 and 2001 revealed 88 cases of death caused by oxycodone or hydrocodone exposure, 24 of which were attributed to oxycodone alone. Mean and median postmortem oxycodone blood concentrations of 1.23 and 0.43 mg/L, respectively, were reported (range, 0.12–8.0); in 13 cases, the concentration was ≥0.5 mg/L. In seven cases in which death was attributed to a combination of oxycodone and hydrocodone, the mean concentrations were reported to be 0.34 and 0.14 mg/L, respectively [Spiller 2003].

In a review of 1014 fatalities involving oxycodone, postmortem oxycodone blood levels in those involving oxycodone plus other drugs were 0.93 mg/L (167 cases with controlled release oxycodone [OxyContin]) and 0.73 mg/L (in 579 cases not involving OxyContin). In cases where oxycodone was the only drug identified, levels were 1.55 and 1.70 mg/L for OxyContin (12 cases) and non-OxyContin formulations (15 cases), respectively [Cone *et al.* 2004].

In a review of 172 deaths involving the use of oxycodone, 18 were attributed to oxycodone, 117 to oxycodone combined with other drugs, and the rest from trauma (23), natural causes (9) and other drug(s; 5). The mean postmortem oxycodone blood levels were 0.69, 0.72 and 0.6 mg/L in the cases of oxycodone toxicity, combined drug toxicity and trauma, respectively. Mean oxycodone blood levels of 0.87 mg/L were found in cases attributed to natural causes or other drugs [Wolf *et al.* 2005].

In a review of 67 cases where oxycodone was detected at postmortem between 2000 and 2005, 30 cases were determined to be drug overdoses; of these oxycodone alone was responsible for 7 deaths and mean heart blood free oxycodone levels were 1.060 mg/L (range, 0.27–3.39). Mean oxycodone concentrations in the 23 cases of mixed drug overdoses was 0.82 mg/L (range, 0.014–3.8) [Thompson *et al.* 2008].

In a review of 10 oxycodone-positive postmortem cases, nine were drug-related fatalities. Although there was some overlap between levels of oxycodone in the 5 deaths attributed to oxycodone alone and the 4 deaths attibuted to oxycodone plus other drugs, blood oxycodone levels in those attributed to oxycodone alone were >1 mg/L; in those involving other drugs, blood oxycodone was <1 mg/L [Al Asmari *et al.* 2009].

For a review of controlled-release oxycodone (OxyContin) abuse and overdose, see Aquina *et al.* [2009].

Half-life Elimination, 2–3 h.

Note For reviews of the clinical pharmacokinetics and pharmacodynamics of oxycodone, see Pöyhiä *et al.* [1993] and Lugo, Kern [2004]; for a report on the role of metabolites in the pharmacokinetics and pharmacodynamics of oxycodone, see Lalovic *et al.* [2006]; for a review of the pharmacology and clinical use of oxycodone, see Ordóñez Gallego *et al.* [2007].

Dose The usual adult dose of conventional oral oxycodone hydrochloride preparations is 5 to 15 mg every 4 to 6 h.

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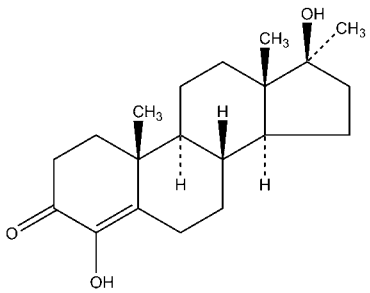
Oxymesterone

Anabolic Steroid, Androgen
$C_{20}H_{30}O_3$ = 318.5
CAS—145-12-0

IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-4,17-Dihydroxy-10,13,17-trimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-3-one
Synonyms (17β)-4,17-Dihydroxy-17-methylandrost-4-en-3-one; 4,17β-dihydroxy-17α-methyl-3-oxo-androst-4-ene; 4-hydroxy-17α-methyltestosterone; 17α-methyl-4-androstene-4,17β-diol-3-one; oxymestrone.
Proprietary Names *Anamidol*; *Oranabol*; *Theranabol*.



Chemical Properties Crystals. Mp 169° to 171°. Practically insoluble in water. Soluble in chloroform, acetone and alcohol. Log *P* (octanol/water), 4.16 [Meylan, Howard 1995].
Mass Spectrum Principal ions at *m/z* 73, 534, 147, 143, 389, 444, 519 17-epi-Oxymesterone (Tris-TMS derivative).
Quantification
Urine **GC-MS** Column 1: HP5 SE54 fused silica capillary cross-linked 5% phenyl methylsilicone (17 m × 0.2 mm i.d., 0.3 μm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. Column 2: OV 1 fused silica capillary cross-linked methylsilicone (20 m × 0.25 mm i.d., 0.33 μm). Carrier gas: He, 1 mL/min. Temperature programme: 200° to 300° at 5°/min. EI ionisation at 70 eV. Limit of detection not reported [Schanzer *et al.* 1992].
Disposition in the Body A 39-year-old male was administered 20 mg oxymesterone orally. The urinary excretion profile was analysed by GC-MS over 72 h. Oxymesterone and its epimer were detected with a total excretion of 16.2 and 0.27% of the administered steroid, respectively. The metabolite 17α-methyl-5α-androstane-3α,17β-diol and its 17β-methyl epimer were only detected after administration of 40 mg oxymesterone with a total excretion of 0.83 and 0.01% of the administered dose, respectively [Schanzer *et al.* 1992].
Toxicity

A previously well 18-year-old football player collapsed while training. He had just completed a 2 km run and was performing warm-down exercises when he had a cardiac arrest. Cardiopulmonary resuscitation was unsuccessful. Postmortem examination revealed that all organs were normal apart from the heart. It weighed 410 g but had a normal external appearance with some petechial haemorrhages. The coronary arteries and valves were all normal. The left ventricular free wall measured 13 mm, the interventricular septum measured 17 mm and the right ventricle measured 4 mm. Histological findings were consistent with hypertrophic cardiomyopathy. Oxymesterone glucuronide was detected in urine by GC-MS [Kennedy, Lawrence 1993].

A previously well 24-year-old man collapsed while playing football. Cardiopulmonary resuscitation was again unsuccessful. Six months previously the man had suffered a flu-like illness that had lasted 2 weeks. Soon after recovery he had commenced gymnasium work and had a sudden increase in muscle bulk. In the 2 months prior to his death he was irritable and prone to sudden rages. Postmortem examination revealed that all organs were normal apart from his heart, which weighed 440 g. There was a slight prominence on the left ventricle with petechial haemorrhages on the posterior surface. There were 2 haemorrhages on the anterior surface of the left ventricle consistent with cardiac puncture. The left ventricle free wall measured 17 mm, the interventricular septum was 12 mm, and the right ventricle was 6 mm. There was an extensive area of scarring on the interventricular septum (25 × 20 mm). Histological findings were consistent with a myocarditis. Oxymesterone glucuronide was detected in urine by GC-MS [Kennedy, Lawrence 1993].

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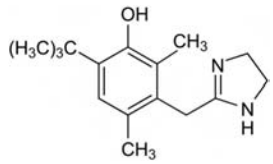
Oxymetazoline

Sympathomimetic
$C_{16}H_{24}N_2O$ = 260.4
CAS—1491-59-4

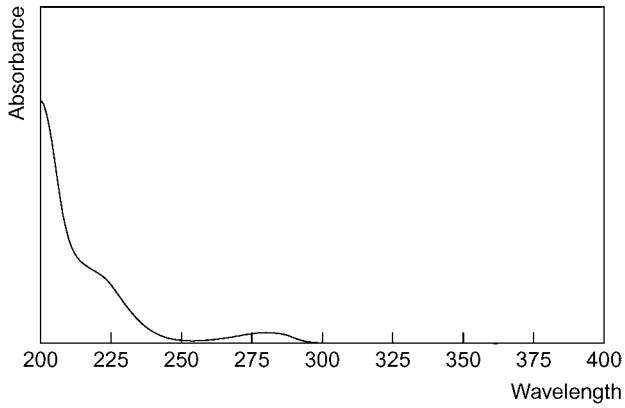
IUPAC Name 6-Tert-butyl-3-(4,5-dihydro-1*H*-imidazol-2-ylmethyl)-2,4-dimethylphenol
Synonym 3-[(4,5-Dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol



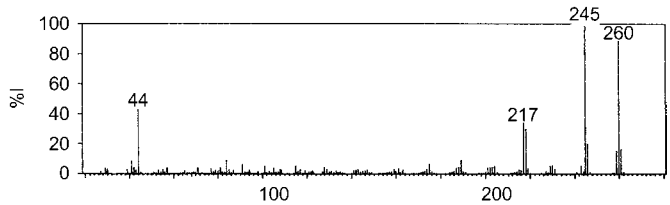
Chemical Properties Crystals. Mp 181° to 183°. Log *P* (octanol/water), 4.9.

Oxymetazoline Hydrochloride
$C_{16}H_{24}N_2O$, HCl = 296.8
CAS—2315-02-8
Proprietary Names *Afrazine*; *Afrin*; *Drixine*; *Durazol*; *Hazol*; *Iliadin-Mini*; *Nafrine*; *Nasivin*; *Rhinolitan*.
Chemical Properties A white, hygroscopic, crystalline powder. Mp 300° to 303°, with decomposition. Soluble 1 in 6.7 of water and 1 in 3.6 of ethanol; practically insoluble in benzene, chloroform and ether.

Colour Test Mandelin's test—green.
Thin-layer Chromatography System TA—R_f 0.09; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.34; system TL—R_f 0.01; system TAE—R_f 0.80; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.25 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2170.
High Performance Liquid Chromatography System HA—*k* 1.7.
Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1=68a); aqueous alkali—303 nm (A^1_1=164b).



Infrared Spectrum Principal peaks at wavenumbers 1595, 1199, 1285, 1250, 1070, 765 cm^{-1} (oxymetazoline hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 245, 260, 44, 217, 218, 246, 261, 259.

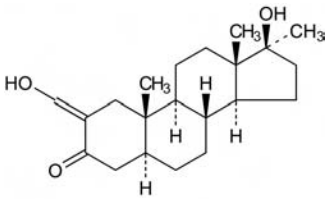


Use Oxymetazoline hydrochloride is used as a 0.05% solution.

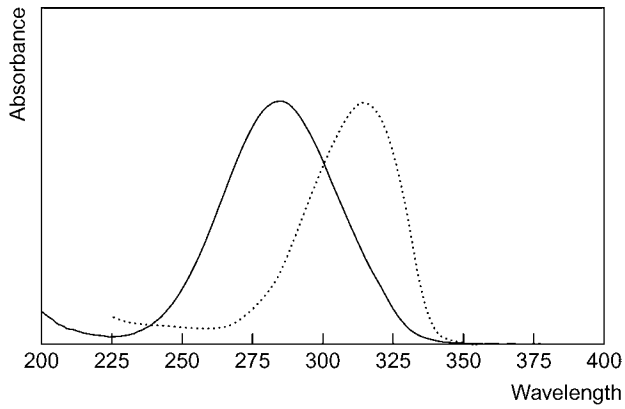
Oxymetholone

Anabolic Steroid
$C_{21}H_{32}O_3$ = 332.5
CAS—434-07-1

IUPAC Name (2*Z*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-2-(hydroxymethylidene)-10,13,17-trimethyl-1,4,5,6,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonym (5α,17β)-17-Hydroxy-2-(hydroxymethylene)-17-methylandrostan-3-one
Proprietary Names *Adroyd*; *Anadrol-50*; *Anapolon*; *Anasteron*; *Nastenon*; *Pardroyd*; *Plenastril*.



Chemical Properties A white to creamy-white crystalline powder. Mp 172° to 180°. Practically insoluble in water; soluble 1 in 50 of ethanol; freely soluble in chloroform; soluble in dioxan; slightly soluble in ether. Log *P* (octanol/water), 3.6.
Colour Tests Antimony pentachloride—brown; naphthol-sulfuric acid—brown/pink-orange; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.95; system TE—R_f 0.09; system TP—R_f 0.69; system TQ—R_f 0.23; system TR—R_f 0.85; system TS—R_f 0.82; system TAJ—R_f 0.70; system TAK—R_f 0.74; system TAL—R_f 0.94; system TAM—R_f 0.86 (*p*-toluenesulfonic acid solution, positive).
Gas Chromatography System GA—oxymetholone RI 3005,oxymetholone enol-TMS_3 RI 2870; system GAI—urinary metabolites: 17α-methyl-5α-androstan-3α,17β-diol RRT 0.925,2-hydroxymethyl-17α-methyl-5-androstan-3,17-diol RRT 1.106, 2-hydroxymethyl-17α-methyl-5-androstan-3, 6, 17-triol RRT 1.180 (relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAG—RRT 1.28 (relative to testosterone); system GAR—oxymetholone, retention time 13.7 min.
High Performance Liquid Chromatography System HATb—RRT 2.64 (relative to testosterone).
Ultraviolet Spectrum Methanolic acid—277 nm (A^{1}_{1}=300b); methanolic alkali—315 (A^{1}_{1}=547a).

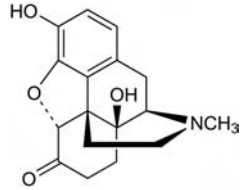


Infrared Spectrum Principal peaks at wavenumbers 1612, 1204, 1219, 1306, 1157, 935 cm^{-1} (KBr disk).
Quantification
Plasma **GC–MS** For method, see Cardoso *et al.* [2002].
Dose Usually 5 to 10 mg daily; doses of 100 to 350 mg daily may be given.

Cardoso CR *et al.* (2002). Validation of the determination of oxymetholone in human plasma analysis using gas chromatography-mass spectrometry. Application to pharmacokinetic studies. *J Chromatogr B Anal Technol Biomed Life Sci* 775: 1–8.

Oxymorphone

Narcotic Analgesic
$C_{17}H_{19}NO_4$ = 301.3
CAS—76-41-5
Synonyms 7,8-Dihydro-14-hydroxymorphinone; 4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-oneoximorphone; oxydimorphone.

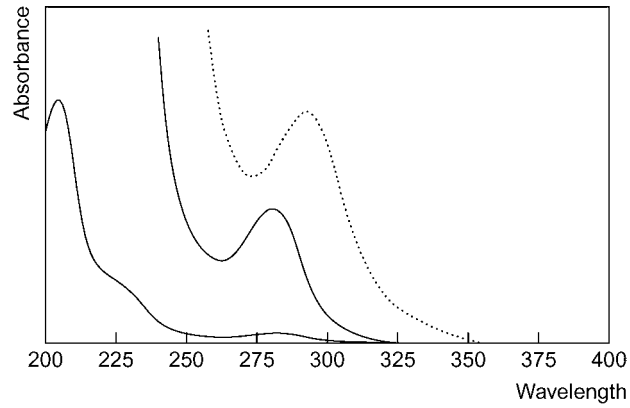


Chemical Properties Crystals. Mp 248° to 249°, with decomposition. Soluble in boiling acetone and chloroform; readily soluble in aqueous alkali; moderately soluble in boiling ethanol; sparingly soluble in benzene. pK_{a1} 8.17 [Sangster 1997], 8.5, pK_{a2} 9.3. Log *P* (octanol/water), 0.83 [Hansch *et al.* 1995], (octanol/pH 7.4), 0.

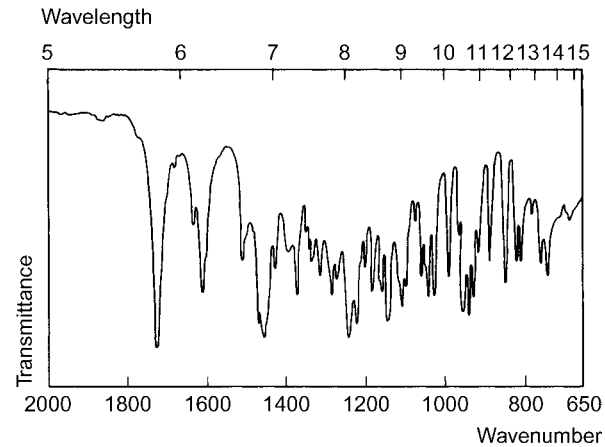
Oxymorphone Hydrochloride

$C_{17}H_{19}NO_4$, HCl = 337.8
CAS—357-07-3
Proprietary Names *Numorphan*; *Opana*.
Chemical Properties A white powder that darkens on exposure to light. Soluble 1 in 4 of water, 1 in 100 of ethanol and 1 in 25 of methanol; very slightly soluble in chloroform and ether.

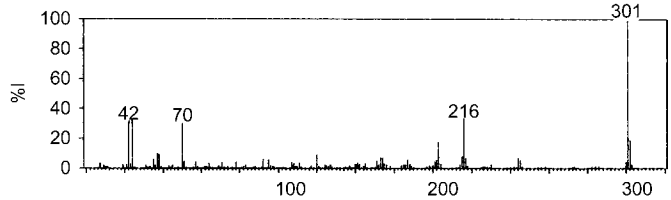
Colour Test Marquis test—grey-violet.
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.10; system TC—R_f 0.37; system TE—R_f 0.33; system TL—R_f 0.30; system TAE—R_f 0.27; system TAF—R_f 0.36; system TAJ—R_f 0.13; system TAK—R_f 0.00; system TAL—R_f 0.13 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—grey).
Gas Chromatography System GA—oxymorphone RI 2723, oxymorphone-TMS RI 2715, oxymorphone-TMS_2 RI 2728, oxymorphone-TMS_3 RI 2641, oxymorphone-oxime-TMS_3 RI 2748, M (nor-) not eluted, M (nor-enol, -TMS_2) RI 2788, M (nor-enol, -TMS_3) RI 2662, M (nor-enol-TMS_4) RI 2773, M (dihydro-) RI 2690.
Gas Chromatography-Mass Spectrometry Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 130° for 1 min to 270° at 10°/min. Carrier gas: He. EI ionisation, full scan mode. RT: 20.5 min. [Nowatzke *et al.* 1999].
High Performance Liquid Chromatography System HA—*k* 6.7 (tailing peak); system HX—RI 217; system HY—RI 184.
Ultraviolet Spectrum Aqueous acid—281 nm (A^{1}_{1} = 34a); aqueous alkali—292 nm.



Infrared Spectrum Principal ions at wavenumbers 1730, 1240, 1225, 1145, 941, 953 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 301, 216, 44, 42, 70, 302, 203, 57.



Quantification

Blood **GC-MS** Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 305° at 10°/min for 0.5 min. EI ionisation. Retention time: 385 s. Limit of quantification, 10 µg/L; limit of detection, 2 µg/L [Meatherall 2005]. Column: HP-1MS capillary (30 m × 0.25 mm, 0.25 µm). Carrier gas: He, 62 cm/s. Limit of quantification, 5 to 20 µg/L [Ropero-Miller *et al.* 2002].

LC-MS Column: Waters HSS T3 (50 × 2.1 mm i.d., 3.0 µm). Mobile phase: 1% formic acid in water : 1% formic acid in acetonitrile (97 : 3 for 0.1 min to 80 : 20 at 2.5 min to 1 : 99 at 2.55 min for 0.45 min to 97 : 3 at 3.01), flow rate 0.6 mL/min. ESI, positive ion mode. Limit of quantification, 10 µg/L [Dahn *et al.* 2010]. Column: Phenomenex Synergi reversed phase. Mobile phase: 10 mmol/L ammonium formate (pH 3) : acetonitrile. Limit of quantification, 0.5 to 4.09 µg/L; limit of detection, 0.16 to 1.2 µg/L [Al Asmari, Anderson 2007].

Plasma **LC-MS** See Blood [Dahn *et al.* 2010]. Column: XBridge C18 (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 5 mmol/L ammonium formate (pH 9.4) : methanol (94 : 6 for 10 min to 45 : 55 for 3.5 min to 15 : 85 for 1.5 min to 94 : 6 for 10 min), flow rate 180 µL/min. ESI. Limit of detection, 0.1 µg/L for oxymorphone and 0.25 µg/L for noroxymorphone [Neuvonen, Neuvonen 2008]. Column: Phenomex C_{12} MAX-RP (150 × 2 mm, 4 µm). Mobile phase: water–acetonitrile (90 : 10)–5 mmol/L ammonium formate (pH 3.5) : water–acetonitrile (10 : 90)–5 mmol/L ammonium formate (pH 3.5; 100 : 0 for 5 min to 0 : 100 at 19 min for 7 min to 100 : 0 at 29 min for 6 min), flow rate 200 µL/min. TIS, positive ion mode, MRM acquisition mode. Limit of quantification, 2.9 µg/L, limit of detection; 0.8 µg/L [Musshoff *et al.* 2006].

Serum **LC-MS** See Blood [Dahn *et al.* 2010].

Urine **GC-MS** Column: DB-5MS bonded phase capillary (15 m × 0.249 mm i.d., 0.25 µm). Carrier gas: He. Temperature programme: 240° to 280° at 40°/min for 1.2 min. SIM acquisition mode. Limit of quantification, 40 µg/L, limit of detection; 20 µg/L [McKinley *et al.* 2007]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 60 cm/s. Temperature programme: 140° for 1 min to 220° at 50°/min to 290° at 10°/min for 0.5 min. EI ionisation, full scan mode. Retention time: 385 s. Limit of quantification, 25 µg/L, limit of detection; 10 µg/L [Meatherall 1999]. Column: DB-5 capillary (15 m × 0.26 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature: 260°. SIM acquisition mode. Limit of detection, 3.7 µg/L [Smith *et al.* 1995].

LC-MS See Blood [Dahn *et al.* 2010]. Column: Agilent Zorbax SB Phenyl (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.05% formic acid with 10 mmol/L ammonium acetate–0.05% formic acid in acetonitrile (95 : 5) : 0.05% formic acid in acetonitrile–0.05% formic acid with 10 mmol/L ammonium formate (95 : 5, 100 : 0 for 0.5 min to 82 : 18 at 3 min for 2.5 min to 100 : 0). ESI. Limit of quantification, 80 µg/L [Edinboro *et al.* 2005].

Disposition in the Body Oxymorphone is absorbed after oral administration. Approximately 50% of an oral dose is excreted in the urine in 5 days (mostly in the first 24 h), mainly as conjugated oxymorphone together with small quantities of unchanged drug and the conjugated 6α- and 6β-hydroxy metabolites. Oxymorphone is a metabolite of oxycodone.

Therapeutic Concentration

Two healthy male volunteers with a history of heroin abuse participated in the study. Prior to the study, each subject was required to test negative for morphine in urine for 3 consecutive days. Each volunteer was administered 1.5 mg and 3 mg IM oxymorphone. Peak plasma concentrations for subject 1 after 24 and 36 h were 164 µg/L and 91 µg/L, respectively, for the 1.5 mg dose and 435 µg/L and 120 µg/L, respectively, for the 3 mg dose. For subject 2, the levels at 24 and 36 h were 434 µg/L and 381 µg/L, respectively, for the 1.5 mg dose and 654 µg/L and 129 µg/L, respectively, for the 3 mg dose [Smith *et al.* 1995].

Toxicity The estimated minimum lethal dose is 50 mg. Prolonged use of oxymorphone may lead to dependence of the morphine type.

In a review of 33 deaths involving oxymorphone, mean postmortem central and peripheral blood levels were 0.15 mg/L. Liver concentrations ranged from 'none detected' to greater than 2 mg/L and most urine samples had levels greater than 0.5 mg/L [Garside *et al.* 2009].

Blood levels of 0.05 mg/L and 0.12 mg/L were found in two oxymorphone-related fatalities [McIntyre *et al.* 2009].

Note For a study of oxymorphone metabolism in human, rat, guinea pig, rabbit and dog, see Cone *et al.* [1983]. For reviews of the pharmacology, pharmacokinetics and use of oxymorphone and an extended-release oxymorphone preparation, see Prommer [2006] and Sloan, Barkin [2008].

Dose 0.5 to 1.5 mg of oxymorphone hydrochloride given parenterally; doses of 5 to 20 mg have been given orally.

Al Asmari AI, Anderson RA (2007). Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 31: 394–408.

Cone EJ *et al.* (1983). Oxymorphone metabolism and urinary excretion in human, rat, guinea pig, rabbit, and dog. *Drug Metab Dispos* 11: 446–450.

Dahn T *et al.* (2010). Quantitation of morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and 6-monoacetylmorphine (6-MAM) in urine, blood, serum, or plasma using liquid chromatography with tandem mass spectrometry detection. *Meth Mol Biol* 603: 411–422.

Edinboro LE *et al.* (2005). Direct analysis of opiates in urine by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 29: 704–710.

Garside D *et al.* (2009). Concentration of oxymorphone in postmortem fluids and tissue. *J Anal Toxicol* 33: 121–128.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants* Washington DC: American Chemical Society.

McIntyre IM *et al.* (2009). Case report: oxymorphone-involved fatalities – a report of two cases. *J Anal Toxicol* 33: 615–619.

McKinley S *et al.* (2007). Rapid quantification of urinary oxycodone and oxymorphone using fast gas chromatography–mass spectrometry. *J Anal Toxicol* 31: 434–441.

Meatherall R (1999). GC-MS confirmation of codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone in urine. *J Anal Toxicol* 23: 177–186.

Meatherall R (2005). GC-MS quantitation of codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone in blood. *J Anal Toxicol* 29: 301–308.

Musshoff F *et al.* (2006). An automated and fully validated LC-MS/MS procedure for the simultaneous determination of 11 opioids used in palliative care, with 5 of their metabolites. *J Mass Spectrom* 41: 633–640.

Neuvonen M, Neuvonen PJ (2008). Determination of oxycodone, noroxycodone, oxymorphone, and noroxymorphone in human plasma by liquid chromatography–electrospray-tandem mass spectrometry. *Ther Drug Monit* 30: 333–340.

Nowatzke W *et al.* (1999). Distinction among eight opiate drugs in urine by gas chromatography–mass spectrometry. *J Pharm Biomed Anal* 20: 815–828.

Prommer E (2006). Oxymorphone: a review. *Support Care Cancer* 14: 109–115.

Ropero-Miller JD *et al.* (2002). Simultaneous quantitation of opioids in blood by GC-EI-MS analysis following deproteination, detautomerization of keto analytes, solid-phase extraction, and trimethylsilyl derivatization. *J Anal Toxicol* 26: 524–528.

Sangster J (1997).*Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry.* Chichester UK: John Wiley.

Sloan PA, Barkin RL (2008). Oxymorphone and oxymorphone extended release: a pharmacotherapeutic review. *J Opioid Manag* 4: 131–144.

Smith ML *et al.* (1995). Forensic drug testing for opiates. VI. Urine testing for hydromorphone, hydrocodone, oxymorphone, and oxycodone with commercial opiate immunoassays and gas chromatography–mass spectrometry. *J Anal Toxicol* 19: 18–26.

Oxypertine

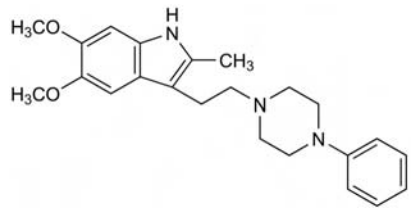
Tranquilliser

$C_{23}H_{29}N_3O_2 = 379.5$

CAS—153-87-7

IUPAC Name 5,6-Dimethoxy-2-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl]-1*H*-indole

Proprietary Names *Equipertine*; *Forit*; *Integrin*; *Opertil*.



Chemical Properties White crystalline powder. Slightly soluble in water and ethanol. Log *P* (octanol/water), 3.8.

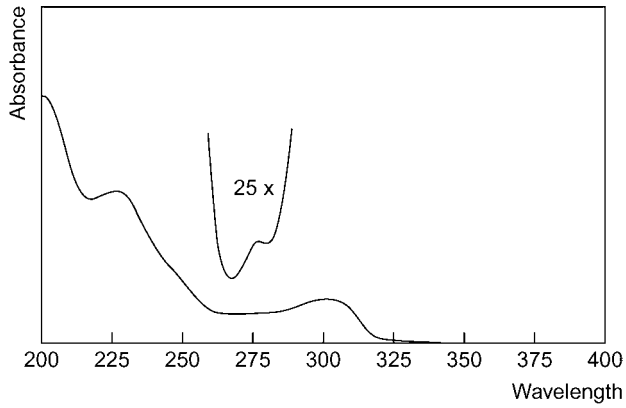
Colour Tests Liebermann's reagent—blue; Mandelin's test—grey; Marquis test—grey-green.

Thin-layer Chromatography System TA—R_f 0.68; system TAE—R_f 0.74; system TAG—R_f 0.58; system TB—R_f 0.04; system TC—R_f 0.65; system TE—R_f 0.78 (acidified iodoplatinate solution, positive).

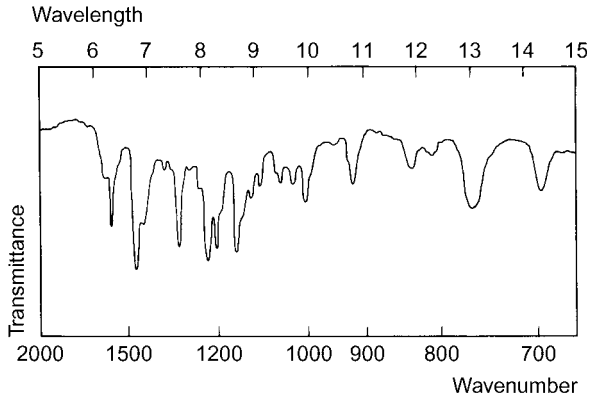
Gas Chromatography System GA—RI 2355.

High Performance Liquid Chromatography System HA—*k* 0.7; system HF—*k* 1.33; system HX—RI 402.

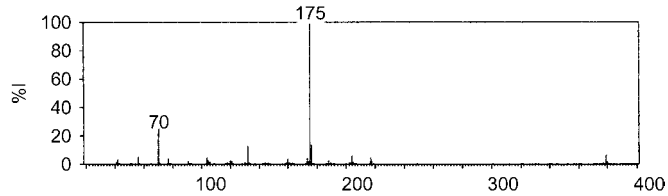
Ultraviolet Spectrum Aqueous acid—301 nm ($A^1_1 = 212b$). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1233, 1153, 1205, 1592, 762, 1003 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 175, 70, 176, 132, 379, 204, 56, 217.



Quantification

Plasma **GC** Column: CBP1-bonded methylsilicone (12 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. NPD. Limit of detection, 0.1 ng [Tokunaga *et al.* 1996].

Serum **HPLC** Column: Zorbax Sil (30 cm × 4 mm i.d., 7–8 µm). Mobile phase: methanol : acetic acid : sodium acetate (200 : 0.3 : 0.1), flow rate 0.8 mL/min. UV detection (λ = 252 nm). Retention time: 6.4 min. Limit of quantification, 20 µg/L [Minatogawa *et al.* 1983].

Dose Usually up to 300 mg daily.

Minatogawa Y *et al.* (1983). Determination of oxypertine in human serum by high-performance liquid chromatography. *J Chromatogr* 274: 413–416.

Tokunaga H *et al.* (1996). Screening of antipsychotic drugs by wide-bore capillary gas chromatography with nitrogen phosphorus detection–detection levels in plasma. *Nihon Hoigaku Zasshi* 50: 196–202.

O

Oxyphenbutazone

Analgesic

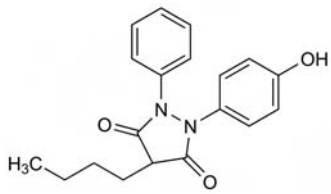
$C_{19}H_{20}N_2O_3$, H_2O = 342.4

CAS—129-20-4 (anhydrous); 7081-38-1 (monohydrate)

IUPAC Name 4-Butyl-1-(4-hydroxyphenyl)-2-phenyl-3,5-pyrazolidinedione

Synonyms G-27202; hydroxyphenylbutazone.

Proprietary Names *Imbun*; *Isobutil*; *Oxalid*; *Oxybutazone*; *Phlogase*; *Rheumapax*; *Tandearil*; *Tanderil*.



Chemical Properties A white to yellowish-white crystalline powder. Mp 96°. Monohydrate. Soluble in ethanol, methanol, chloroform, benzene and ether. Forms a water-soluble sodium salt. pK_a 4.7 (22°). Log *P* (octanol/water), 2.7.

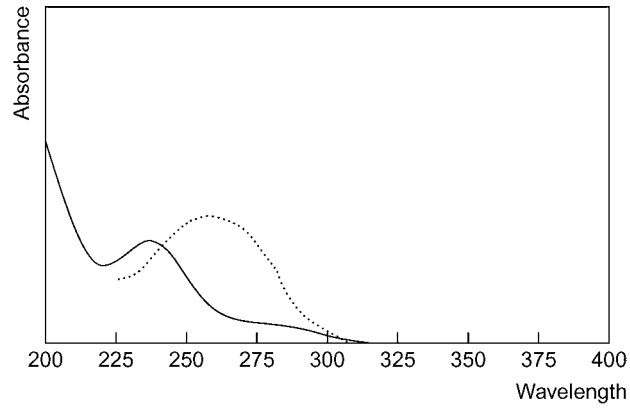
Colour Test Marquis test—yellow.

Thin-layer Chromatography System TA—R_f 0.77; system TB—R_f 0.00; system TD—R_f 0.52; system TE—R_f 0.09; system TF—R_f 0.62; system TG—R_f 0.25; system TAD—R_f 0.57; system TAE—R_f 0.90; system TAJ—R_f 0.56; system TAK—R_f 0.41; system TAL—R_f 0.92 (chromic acid solution, yellow; Ludy Tenger reagent, orange; mercurous nitrate spray, positive; acidified potassium permanganate solution, positive).

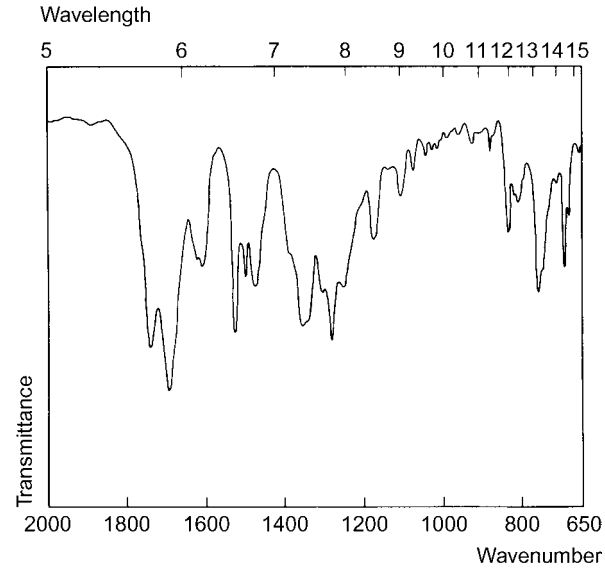
Gas Chromatography System GA—oxyphenbutazone— not eluted; oxyphenbutazone-Me_2 (isomer 1) RI 2545; oxyphenbutazone-Me_2 (isomer 2) RI 2720; system GD—methyl derivative RRT 2.11 (relative to *n*-$C_{16}H_{34}$).

High Performance Liquid Chromatography System HD—*k* 1.95; system HV—RRT 0.69 (relative to meclofenamic acid); system HX—RI 501; system HY—RI 459; system HZ—retention time 6.7 min.

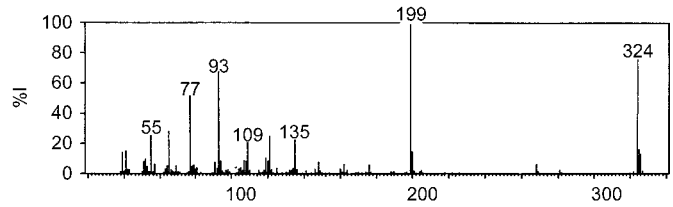
Ultraviolet Spectrum Aqueous acid—237 nm; aqueous alkali—254 nm (A^1_1=750a).



Infrared Spectrum Principal peaks at wavenumbers 1683, 1736, 1275, 1512, 765, 1600 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 199, 324, 93, 77, 65, 55, 121, 135.



Quantification See also Phenylbutazone.

Plasma **GC** AFID. Limit of detection, 2.5 mg/L [Bertrand *et al.* 1979].

HPLC UV detection. Limit of detection, 250 µg/L [Pound, Sears 1975].

Disposition in the Body Almost completely absorbed after oral administration. It is slowly metabolised by glucuronide conjugation and by hydroxylation; it is slowly excreted in the urine, <2% of a dose being excreted as unchanged drug and about 1 to 5% as the *O*-glucuronide conjugate in 24 h.

Oxyphenbutazone is a major metabolite of phenylbutazone.

Therapeutic Concentration

Following a single oral dose of 200 mg to 10 subjects, peak plasma concentrations of 11.4 to 43.7 mg/L were attained in 2 to 12 h [Bertrand *et al.* 1979].

Following daily oral doses of 300 to 400 mg to 6 subjects, steady-state plasma concentrations determined immediately before a dose ranged from 27 to 95 mg/L (mean, 62) [Weiner *et al.* 1967].

Toxicity The estimated minimum lethal dose is 5 g.

Half-Life Plasma half-life, about 2 to 3 days.

Volume of Distribution About 0.1 L/kg.

Protein Binding About 99%.

Dose Oxyphenbutazone has been given in doses of 100 to 800 mg daily.

Bertrand M *et al.* (1979). Quantitative determination of plasma oxyphenbutazone by gas-liquid chromatography with selective nitrogen detection. *J Chromatogr* 171: 377–383.

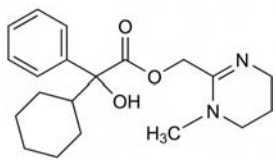
Pound NJ, Sears RW (1975). Simultaneous determination of phenylbutazone and oxyphenbutazone in plasma by high-speed liquid chromatography. *J Pharm Sci* 64: 284–287.

Weiner M *et al.* (1967). Effect of steroids on disposition of oxyphenbutazone in man. *Proc Soc Exp Biol Med* 124: 1170–1173.

Oxyphencyclimine

Anticholinergic
$C_{20}H_{28}N_2O_3$ = 344.5
CAS—125-53-1

IUPAC Name (1-Methyl-5,6-dihydro-4*H*-pyrimidin-2-yl)methyl 2-cyclohexyl-2-hydroxy-2-phenylacetate
Synonym α-Cyclohexyl-α-hydroxybenzeneacetic acid (1,4,5,6-tetrahydro-1-methyl-2-pyrimidinyl)methyl ester
Proprietary Names *Antalcus*; *Zamanil*.

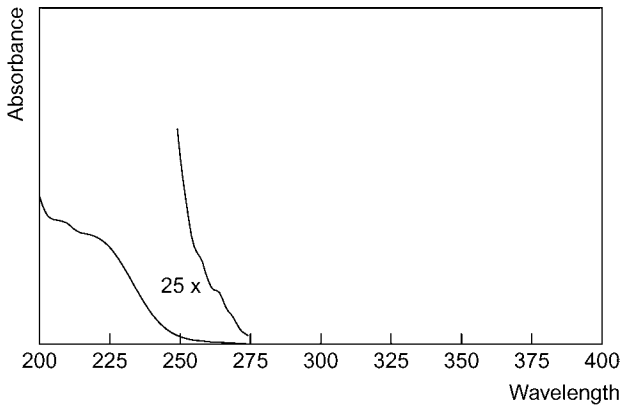


Chemical Properties Log *P* (octanol/water), 3.5.

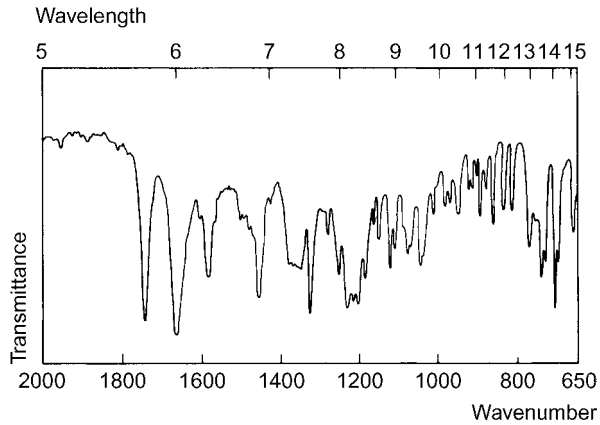
Oxyphencyclimine Hydrochloride

$C_{20}H_{28}N_2O_3$, HCl = 380.9
CAS—125-52-0
Proprietary Names *Daricon*; *Manir*; *Vagogastrin*. It is an ingredient of *Enarax* and *Vistrax*.
Chemical Properties A white crystalline powder. Mp 231° to 232° with decomposition. Soluble 1 in 100 of water, 1 in 75 of ethanol, 1 in 500 of chloroform, 1 in 3000 of ether and 1 in 20 of methanol.

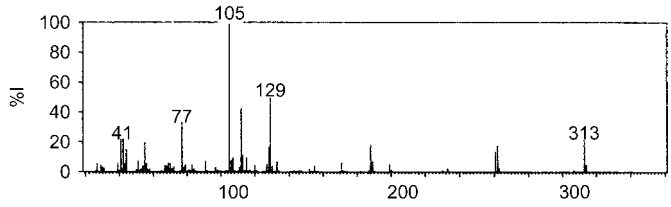
Colour Test Mandelin's test—grey-green.
Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.01; system TC—R_f 0.03; system TE—R_f 0.06; system TL—R_f 0.00; system TAE—R_f 0.02; system TAF—R_f 0.18; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1661 and RI 2250; system GF—RI 2900.
High Performance Liquid Chromatography System HA—*k* 2.8; system HX—RI 424.
Ultraviolet Spectrum Principal peak at 221 nm.



Infrared Spectrum Principal peaks at wavenumbers 1656, 1737, 1227, 705, 1201, 1211 cm^{-1} (oxyphencyclimine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 105, 129, 112, 77, 42, 313, 41, 55.

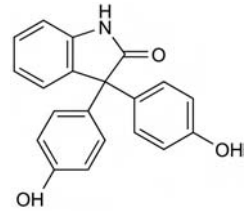


Dose 10 to 20 mg of oxyphencyclimine hydrochloride daily; up to 50 mg daily has been given.

Oxyphenisatine

Purgative
$C_{20}H_{15}NO_3$ = 317.3
CAS—125-13-3

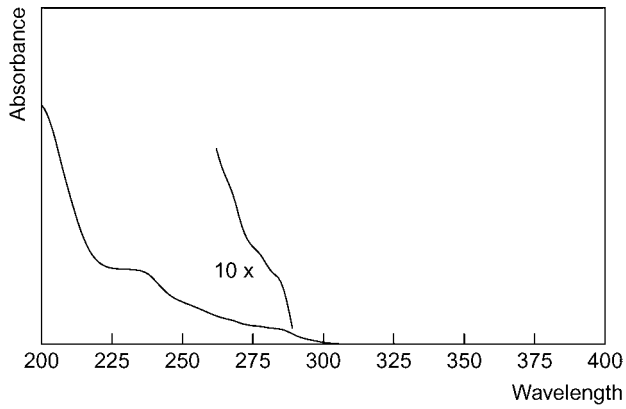
IUPAC Name 3,3-Bis[4-hydroxyphenyl]-1,3-dihydro-2*H*-indol-2-one
Synonym Dihydroxyphenylisatin
Proprietary Name *Veripaque*



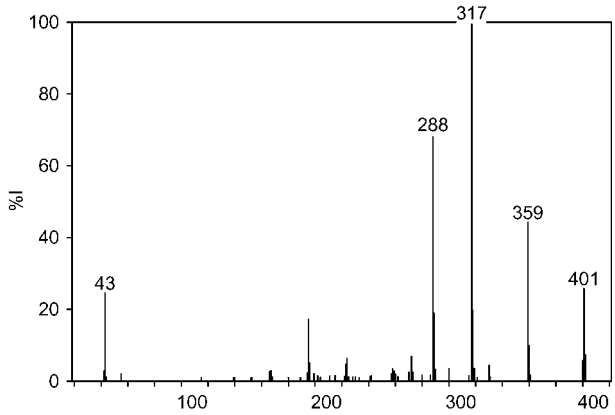
Oxyphenisatine Acetate

$C_{24}H_{19}NO_5$ = 401.4
CAS—115-33-3
Synonyms Acetphenolisatin; bisatin; diacetoxydiphenylisatin; diacetyldiphenolisatin; diasatin; diphesatin; isaphenin; oxyphenisatin acetate.
Chemical Properties A white crystalline powder. Mp about 242°. Practically insoluble in water, ether and dilute hydrochloric acid; soluble 1 in 70 of chloroform; slightly soluble in ethanol. Log *P* (octanol/water), 3.5.

Colour Tests Folin–Ciocalteu reagent—blue; Koppanyi–Zwikker test—violet; Liebermann's reagent—black; Mandelin's test—violet; Marquis test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.82 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Principal peak at 240 nm.



Infrared Spectrum Principal peaks at wavenumbers 1188, 1727, 1495, 1232, 1162, 1011 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 317, 288, 359, 401, 43, 318, 289, 196 (oxyphenisatine acetate).

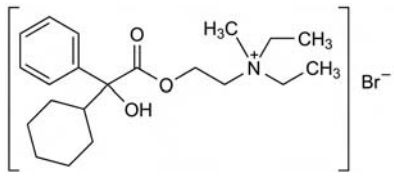


Dose Oxyphenisatine acetate has been given in a dose of 5 to 20 mg.

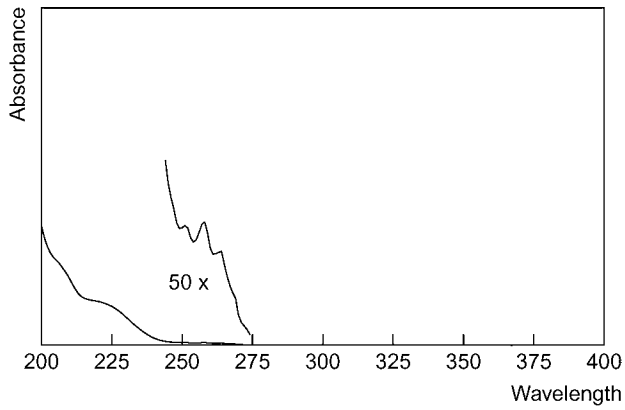
Oxyphenonium Bromide

Anticholinergic
$C_{21}H_{34}BrNO_3$ = 428.4
CAS—14214-84-7 (oxyphenonium); 50-10-2 (bromide)

IUPAC Name 2-[(Cyclohexylhydroxyphenylacetyl)oxy]-*N*,*N*-diethyl-*N*-methylethanaminium bromide
Synonyms Ba-5473; C-5473; spasmophen.
Proprietary Names *Antrenil*; *Antrenyl*.



Chemical Properties A white crystalline powder. Mp about 190°. Freely soluble in water; sparingly soluble in alcohol. Log *P* (octanol/water), 0.2.
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.01; system TL—R_f 0.00; system TAE—R_f 0.02; system TAF—R_f 0.36 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—Art RI 2173.
High Performance Liquid Chromatography System HA—*k* 2.6 (tailing peak); system HX—RI 424.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=5.3a), 264 nm.



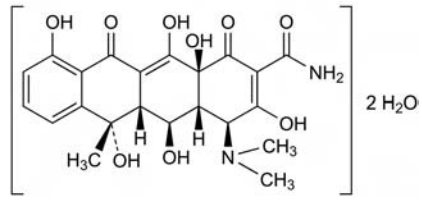
Infrared Spectrum Principal peaks at wavenumbers 1225, 1719, 729, 704, 1030, 1111 cm^{-1} (KBr disk).
Quantification
Plasma **GC** ECD. Limit of detection, 2 µg/L [Greving *et al.* 1977].
Urine **GC** ECD. Limit of detection, 200 µg/L [Greving *et al.* 1977].
Dose 20 to 40 mg daily.

Greving JE *et al.* (1977). Determination of oxyphenonium bromide in plasma and urine by means of ion-pair extraction, derivatization and gas chromatography-electron-capture detection. *J Chromatogr* 142: 611–619.

Oxytetracycline Dihydrate

Antibacterial
$C_{22}H_{24}N_2O_9$, $2H_2O$ = 496.5
CAS—79-57-2 (anhydrous); 6153-64-6 (dihydrate)

IUPAC Name (2*Z*,4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-2-[Amino(hydroxy)methylidene]-4-(dimethylamino)-5,6,10,11,12*a*-pentahydroxy-6-methyl-4,4*a*,5,5*a*-tetrahydrotetracene-1,3,12-trione dihydrate
Synonyms [4*S*-(4α,4aα,5α,5aα,6β,12aα,)]-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a,-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide dihydrate; 5-hydroxytetracycline; oxytetracycline; terrafungine.
Proprietary Names *Abbocin*; *Chemocycline* (tablets); *Galenomycin*; *Imperacin*; *Oxymed*; *Oxymycin*; *Terramycin* (tablets).



Chemical Properties A yellow to tan-coloured crystalline powder. Stable in air but darkens on exposure to strong sunlight. Soluble 1 in 4150 of water, 1 in 66 of dehydrated alcohol and 1 in 6250 of ether; sparingly soluble in ethanol; practically insoluble in chloroform; soluble in dilute acids and alkalis. pK_a 3.3, 7.3, 9.1 (25°). Log *P* (octanol/pH 7.5), −1.6.

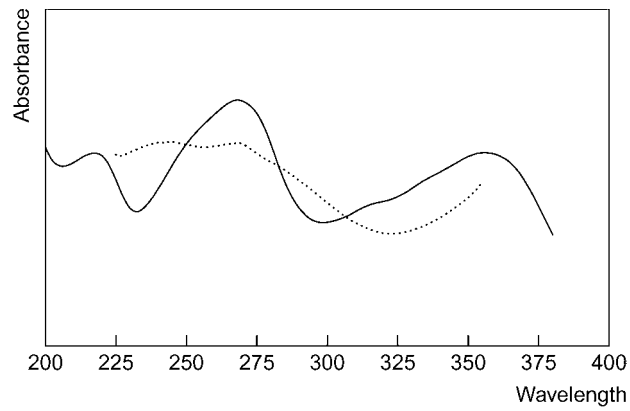
Oxytetracycline Calcium

$C_{44}H_{46}CaN_4O_{18}$ = 958.9
CAS—15251-48-6 (xCa)
Proprietary Names *Chemocycline* (syrup); *Terramycin* (syrup).
Chemical Properties A yellow to light brown crystalline powder. Practically insoluble in water; soluble in dilute solutions of sodium hydroxide.

Oxytetracycline Hydrochloride

$C_{22}H_{24}N_2O_9$,HCl = 496.9
CAS—2058-46-0
Proprietary Names *Macocyn*; *Terramycin* (capsules); *Unimycin*. It is an ingredient of *Bisolvomycin* and *Terra-Cortril*.
Chemical Properties A yellow, hygroscopic, crystalline powder, which darkens on exposure to sunlight or to moist air above 90°. It decomposes above 180°. Soluble 1 in 2 of water, 1 in 45 of ethanol and 1 in 45 of methanol; less soluble in dehydrated alcohol; practically insoluble in chloroform and ether. Solutions in water become turbid on standing owing to precipitation of oxytetracycline base.

Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—brown-red; Liebermann's reagent—red; Mandelin's test—violet→red→orange; Marquis test—orange; sulfuric acid—violet-red.
Thin-layer Chromatography System TA—R_f 0.05, streaking; system TB—R_f 0.00; system TE—R_f 0.00; system TAD—R_f 0.00; system TAE—R_f 0.08 (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HX—RI 299; system HY—RI 260.
Ultraviolet Spectrum Aqueous acid—268 (A_1^1=400a), 352 nm; aqueous alkali—246, 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1616, 1584, 1665, 1235, 1180, 1138 cm^{-1} (oxytetracycline hydrochloride, KBr disk).

Quantification

Blood **Spectrofluorimetry** Limit of detection <100 µg/L [Scales, Assinder 1973].

Plasma **Spectrofluorimetry** See Blood [Scales, Assinder 1973].

Disposition in the Body Incompletely and irregularly absorbed after oral administration and widely distributed throughout the body. About 70% of a dose is excreted in the urine; biliary excretion also occurs.

Therapeutic Concentration

Following oral doses of 500 mg to 4 subjects, peak plasma concentrations of 1.2 to 3.4 mg/L (mean, 2.1) were attained in 2 to 3 h [Scales, Assinder 1973].

Half-life Plasma half-life, about 9 h, increased in renal impairment.

Protein Binding About 20 to 35%.

Dose Up to 3 g of oxytetracycline hydrochloride daily.

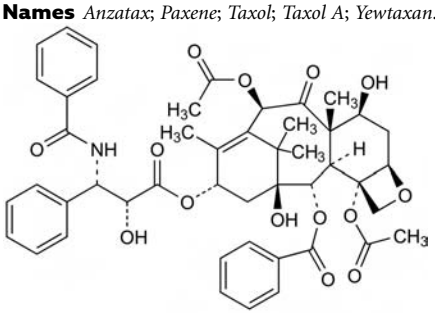
Scales B, Assinder DA (1973). Fluorometric estimation of oxytetracycline in blood and plasma. *J Pharm Sci* 62: 913–917.

Paclitaxel

Antineoplastic, Taxane
$C_{47}H_{51}NO_{14}$ = 853.9
CAS—33069-62-4

Synonyms [2a*R*-[2aα,4β,4aβ,6β,9α(α*R**,β*S**),11α,12α,12aα,12bα]]-β-(Benzoyl-amino)-α-hydroxybenzenepropanoic acid 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca-[3,4]benz[1,2-*b*]oxet-9-yl ester; BMS-181339-01; NSC-125973.

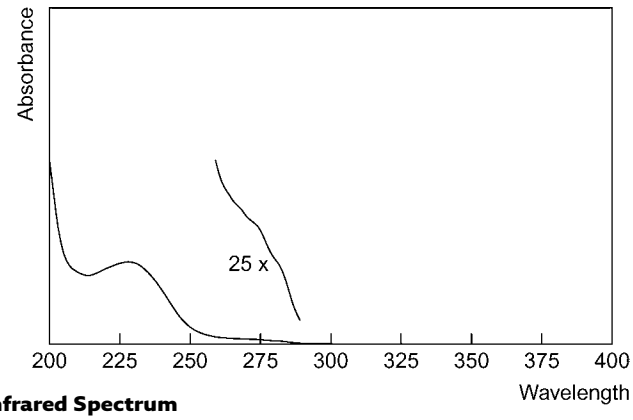
Proprietary Names *Anzatax*; *Paxene*; *Taxol*; *Taxol A*; *Yewtaxan*.



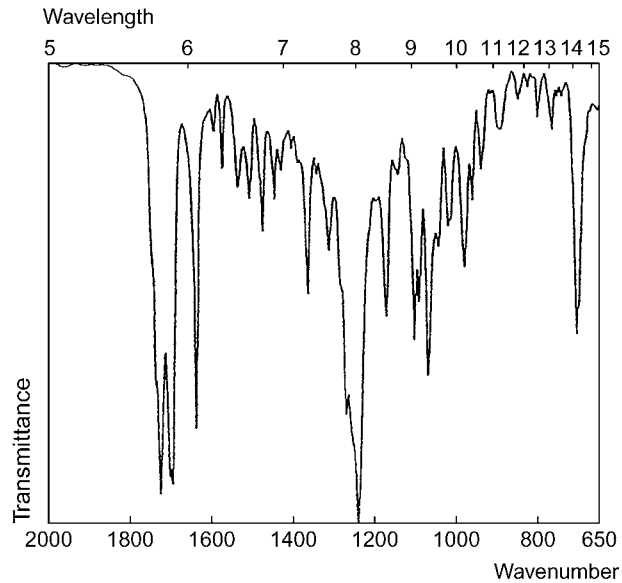
Chemical Properties White to off-white crystalline powder. Mp 216° to 217°, with decomposition. Virtually insoluble in water and shows poor solubility in most pharmaceutically approved solvents.

High Performance Liquid Chromatography Column: C_{18} Radial-Pak cartridge (100 × 8 mm i.d., 10 µm). Mobile phase: water : acetonitrile (65 : 35 to 0 : 100 exponentially over 20 min. IS: *N*-cyclohexylbenzamide. UV detection (λ = 227 nm). Retention time: paclitaxel, 19.5 min; IS, 12.5 min [Longnecker *et al.* 1987].

Ultraviolet Spectrum Aqueous acid—227, 273 nm.



Infrared Spectrum



Quantification

Plasma **HPLC** UV detection (λ = 227 nm). Limit of quantification, 5 µg/L [Supko *et al.* 1999]. UV detection (λ = 227 nm). Limit of quantification 25 µg/L [Martin *et al.* 1998]. Column: C_8 MOS Hypersil (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.02 mol/L acetate buffer (pH 4.5, 65 : 35), flow rate 2 mL/min. UV detection (λ = 227 nm). *k*: 5.5. Limit of detection, 0.10 µg/L [Rizzo *et al.* 1990].

Serum **LC-MS** Limit of detection, 0.25 µg/L [Xu *et al.* 2000].

Urine **HPLC** See Plasma. Limit of quantification, 40 µg/L [Martin *et al.* 1998].

Note For an ELISA for the quantification of paclitaxel, see Leu *et al.* [1992]. For a competitive inhibition enzyme immunoassay for the quantification of paclitaxel, see Grothaus *et al.* [1993].

Disposition in the Body Paclitaxel is not significantly absorbed after oral administration and is metabolised in the liver with the help of the cytochrome P450 isoforms CYP2C8 and CYP3A4. It undergoes extensive extravascular distribution and/or tissue binding, but does not cross the blood–brain barrier. Five metabolites, including 2 mono-hydroxylated and 1 di-hydroxylated derivatives, have been identified in humans. The major metabolite is 6α-hydroxytaxol; minor metabolites include 3′-*p*-hydroxypaclitaxel and 6α,3′-*p*-dihydroxypaclitaxel. <5–10% of a dose is excreted in urine as the unchanged drug and it has also been detected in bile (75% of a dose; mainly as its metabolites and ~20% unchanged).

Therapeutic Concentration

Thirty patients with advanced cancer were administered with either a single IV dose of 210, 250 or 300 mg/m^2 over 3 h. Peak plasma concentrations were reached by the end of infusion at 6, 9.2 and 14.2 µmol/L, respectively [Sonnichsen *et al.* 1994].

Thirty patients aged between 2.3 and 22.8 years were administered with doses of 200, 250, 350 and 420 mg/m^2 paclitaxel over 24 h. Peak concentrations ranged between 1.06 and 1.26 µmol/L, 0.31–1.56, 0.54–2.61 and 1.18–6.77 µmol/L, respectively, and were reached by the end of infusion [Sonnichsen *et al.* 1994].

Toxicity

The major clinical toxicity is myelosuppression which is dose-dependent and reversible. Dose-limiting myelosuppression without granulocyte colony-stimulating factor (G-CSF) was observed at 250 mg/m^2 and the dose-limiting toxicity for paclitaxel with G-CSF was neuropathy at 300 mg/m^2. Therefore, the maximum doses for paclitaxel when administered over a 3-h infusion with or without G-CSF support, are 250 and 210 mg/m^2, respectively [Schiller *et al.* 1994].

Half-life 3–50 h (non-linear pharmacokinetics, independent of dose and wide inter-patient variability).

Volume of Distribution 200–≈700 L/m^2.

Clearance Plasma, 11.6–24.0 L/h/m^2; mean, 0.42 L/m^2/day (from peritoneal cavity).

Protein Binding 88–98%.

Dose The dose varies between 100 and 175 mg/m^2 body surface by IV infusion over 3 h every 2 to 3 weeks depending on condition and response. Dose may be reduced by 20% in some cases.

Grothaus PG *et al.* (1993). An enzyme immunoassay for the determination of taxol and taxanes in Taxus sp. tissues and human plasma. *J Immunol Methods* 158(1): 5–15.

Leu JG *et al.* (1993). Characterization of polyclonal and monoclonal anti-taxol antibodies and measurement of taxol in serum. *Cancer Res* 53(6): 1388–1391.

Longnecker SM *et al.* (1987). High-performance liquid chromatographic assay for taxol in human plasma and urine and pharmacokinetics in a phase I trial. *Cancer Treat Rep* 71(1): 53–59.

Martin N *et al.* (1998). Assay of paclitaxel (Taxol) in plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 709: 281–288.

Rizzo J *et al.* (1990). Analysis of anticancer drugs in biological fluids: determination of taxol with application to clinical pharmacokinetics. *J Pharm Biomed Anal* 8(2): 159–164.

Schiller JH *et al.* (1994). Phase I trial of 3-hour infusion of paclitaxel with or without granulocyte colony-stimulating factor in patients with advanced cancer. *J Clin Oncol* 12(2): 241–248.

Sonnichsen DS *et al.* (1994). Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. *J Clin Oncol* 12: 532–538.

Sparreboom A *et al.* (1998). Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B, Biomed Sci Appl* 705: 159–164.

Supko JG *et al.* (1999). Adaptation of solid phase extraction to an automated column switching method for online sample cleanup as the basis of a facile and sensitive high-performance liquid chromatographic assay for paclitaxel in human plasma. *J Pharm Biomed Anal* 21: 1025–1036.

Xu X *et al.* (2000). Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. *J Mass Spectrom* 35(11): 1329–1334.

Padimate

Sunscreen Agent
$C_{14}H_{21}NO_2$ = 235.3
CAS—14779-78-3; 21245-01-2.

IUPAC Name Pentyl 4-(dimethylamino)benzoate

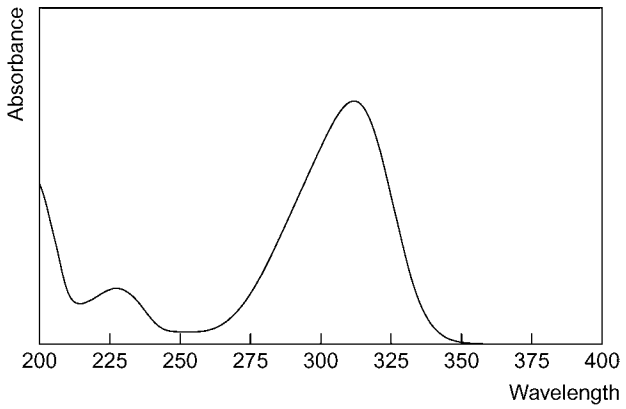
Synonyms Amyl dimethylaminobenzoate; isoamyl dimethylaminobenzoate; padimate A.

Proprietary Names *Chapstick*. It is an ingredient of *Chapstick Sun Block*, *Filter OTC* and *Vaseline Intensive Care Blockout*.
Note A mixture of pentyl, isopentyl and 2-methylbutyl 4-dimethylaminobenzoates.
Chemical Properties A yellow liquid. Practically insoluble in water; soluble in ethanol, chloroform and isopropyl alcohol.

Padimate O

$C_{17}H_{27}NO_2 = 277.4$
CAS—21245-02-3
Synonyms 2-Ethylhexyl 4-(dimethylamino)benzoate; octyl dimethyl PABA.
Proprietary Names *Banana Boat Dark Tanning*; *Chapstick*; *Escalol 507*; *Ice Lipbalm*; *Lipbalm with Sunscreen*; *Lip-Sed*; *Spectraban*. It is an ingredient of many proprietary preparations—see Sweetman [2009].
Chemical Properties A light yellow, mobile liquid. Practically insoluble in water; soluble in ethanol and isopropyl alcohol.

Colour Tests Aromaticity(method2)—yellow/orange;Liebermann'stest(100°)—blue.
Thin-layer Chromatography System TA—R_f 0.75 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1964.
Ultraviolet Spectrum Principal peaks at 231, 314 nm.



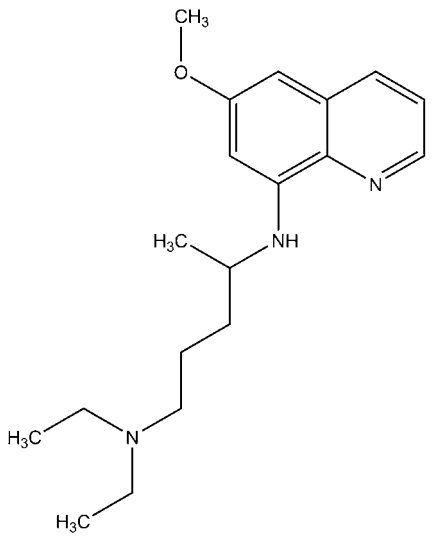
Quantification
Plasma **HPLC** UV detection. Padimate O and other sunscreen agents. Limit of detection, 50 µg/L for padimate O [Jiang *et al.* 1996].
Uses Topically in a concentration of 2.5%.

Jiang R *et al.* (1996). High-performance liquid chromatographic assay for common sunscreening agents in cosmetic products, bovine serum albumin solution and human plasma. *J Chromatogr B Biomed Appl* 682: 137–145.
Sweetman SC, ed. (2009). *Martindale, The complete drug reference*, 36 edn. London: Pharmaceutical Press.

Pamaquin

Antimalarial
$C_{19}H_{29}N_3O,C_{23}H_{16}O_6 = 703.8$
IUPAC Name 1-*N*,1-*N*-diethyl-4-*N*-(6-methoxyquinolin-8-yl)pentane-1,4-diamine
Synonyms Aminoquin; 8-(4-diethylamino-1-methylbutylamino)-6-methoxyquinoline 2,2′-dihydroxy-1,1′-dinaphthylmethane-3,3′-dicarboxylate; gametocidum; pamachin; pamaquine; naphtoate; plasmoquinum; RP 4516; SN 971.
Proprietary Names *Plasmochin Naphthoate*; *Praequine*.

The following is the formula of the free base, 8-(4-diethylamino-1-methybutylamino)-6methoxyquinoline:

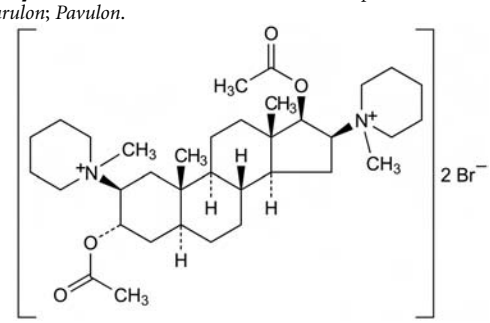


Chemical Properties A yellow-to-yellow-orange powder. Insoluble in water; soluble 1 in 20 of ethanol and 1 in 20 of acetone containing 5% of water. Pamaquin is extracted by organic solvents from aqueous solutions.
Colour Tests Ammonium molybdate test—pale blue (limit of detection, 1.0 µg); ammonium vanadate test—purple-brown (limit of detection, 0.5 µg); sulfuric acid-formaldehyde test—orange (limit of detection, 0.25 µg); Vitali's test—brown/yellow/orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.22 (location reagent diazotized *p*-niroaniline-sodium hydroxyide spray, positive reaction.
Ultraviolet Spectrum Pamaquin in 0.1 N sodium hydroxide, maxima at 238 nm (E1%, 1 cm 4775), 288 nm (E1%, 1 cm 566), about 298 nm (E1%, 1 cm 400) and 362 nm (E1%, 1 cm 400).
Infrared Spectrum Principal peaks at wavenumbers A 1458, B 1393, C 1355 (KBr disk).
Disposition in the Body In humans, pamaquin has been found to be metabolised fairly rapidly into 2 compounds which are excreted in the urine. One metabolite is unstable and can lead to methaemoglobinaemia, a toxic effect of pamaquin; it is suggested that this compound is 5-hydroxypamaquin, which may undergo reversible conversion to the iminoquinone. About 3% of a daily dose is excreted as this metabolite. The second metabolite is a stable, highly flurorescent compound.

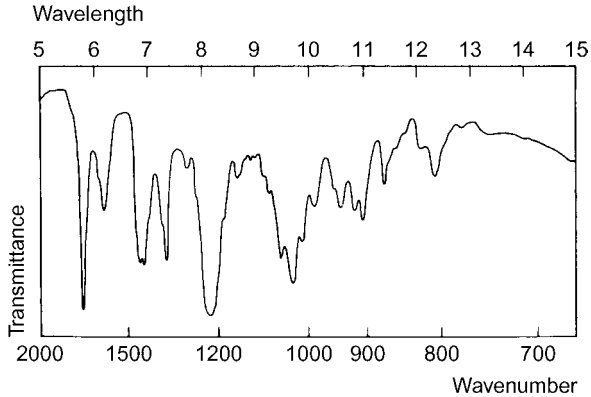
A 5,6-quinone metabolite of pamaquin has been found to be excreted by chickens; it is 16-times as active as pamaquin *in vitro*. The formation of this metabolite involves demethylation and oxidation.
Toxicity The margin between therapeutic and toxic doses is small, 30 mg daily having caused serious toxic effects. Toxic reactions may include methaemoglobinema, jaundice, and IV haemolysis with passage of blood pigments in the urine.
Dose Usually up to 20 mg daily.

Pancuronium Bromide

Muscle Relaxant
$C_{35}H_{60}Br_2N_2O_4 = 732.7$
CAS—15500-66-0
IUPAC Name [(2*S*,3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,16*S*,17*R*)-3-Acetyloxy-10,13-dimethyl-2,16-bis(1-methylpiperidin-1-ium-1-yl)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl] acetate dibromide
Synonyms 1,1′,2β,3α,5α,16β,17β)-3,17-Bis(acetyloxy)androstane-2,16-diyl]bis[1-methylpiperidinium] dibromide; poncuronium bromide.
Proprietary Names *Bromurex*; *Curon-B*; *Minopres*; *Pancuron*; *Pancurox*; *Panlem*; *Parulon*; *Pavulon*.



Chemical Properties White, hygroscopic crystals or crystalline powder. Mp 215°. A solution in water is dextrorotatory. Soluble 1 in 1 of water, 1 in 5 of ethanol, 1 in 5 of chloroform, and 1 in 1 of methanol; practically insoluble in ether. Log *P* (octanol/water), −1.2.
Colour Test Antimony pentachloride—green.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TN—R_f 0.80; system TAE—R_f 0.00; system TAF—R_f 0.15 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HAA—RT 3.0 min.
Ultraviolet Spectrum No significant absorption 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1225, 1745, 1031, 1056, 1013, 907 cm^{-1} (KBr disk; see below).



P

Quantification
Plasma **GC** Column: DB-5 (15 m × 0.32 mm i.d., 0.1 or 0.25 μm) or Ultra 2 cross-linked 5% phenylmethyl silicone (12.5 m × 0.31 mm i.d., 0.17 μm). NSD. Limit of detection, 2 μg/L [Furuta *et al.* 1988].

LC-MS CI, positive ion mode. Limit of detection, 5 μg/L [Baker *et al.* 1990].

Note For a spectrofluorimetry method in serum, see Wingard *et al.* [1979].

Urine **GC** See Plasma [Furuta *et al.* 1988].

MS EI ionisation at 70 eV, Retention time: 4.5 min. Limit of detection, 50 μg/L [Nisikawa *et al.* 1991].

Bile **GC** See Plasma [Furuta *et al.* 1988].

Disposition in the Body Pancuronium is metabolised by hydroxylation to 3-hydroxypancuronium, which is approximately half as active as pancuronium, and to a small extent to the 17-hydroxy and 3,17-dihydroxy derivatives, which are virtually inactive. In ~30 h ~37–44% of a dose is excreted in the urine unchanged, with 15% as the 3-hydroxy metabolite; ~11% of a dose is excreted in the bile [Agoston *et al.* 1973].

Therapeutic Concentration

After an IV injection of 4 mg to 6 subjects, plasma concentrations of ~0.6 mg/L were reported at 5 min, decreasing to 0.07 mg/L at 4 h [McLeod *et al.* 1976].

Toxicity

In the suicide of a 30-year-old man using IV administration of pancuronium bromide, postmortem concentrations were 1.6 and 1.5 mg/L in blood and urine, respectively; thiopental and thioridazine were also detected [Poklis, Melanson 1980].

The following postmortem concentrations were reported in a 25-year-old male anaesthesiologist who was found dead with an IV drip line connected to his leg and several empty ampoules of pancuronium, midazolam and buprenorphine nearby: concentrations of pancuronium and midazolam in blood were 0.3 and 0.1 μg/g and in urine 0.9 and 0.05 μg/g, respectively; death was attributed to pancuronium [Yashiki *et al.* 1992].

A 79-year-old man died 6 days after receiving a total of 105 mg pancuronium bromide by continuous IV infusion over 4 days. The plasma concentration post-infusion was 400 μg/L pancuronium and 400 μg/L 3-desacetylpancuronium; the next day these levels declined to 150 μg/L and 350 μg/L, respectively, and the level of 3-desacetylpancuronium was 240 and 110 μg/L on the third and fourth day post-infusion. The following postmortem tissue concentrations of pancuronium combined with its metabolite were reported: liver 6.38 μg/g, spleen 1.68 μg/g, kidneys 0.68 μg/g, lungs 0.40 μg/g, heart 0.60 μg/g, muscles 0.80 μg/g, gastrointestinal contents 16.67 μg/g [Vandenbrom, Wierda 1988].

Half-life Plasma half-life, ~2 h.

Volume of Distribution 0.1–0.4 L/kg.

Clearance Plasma clearance, ~1–2 mL/min/kg.

Protein Binding Approximately 87%.

Note For a review of the pharmacokinetics of muscle relaxants, see Ramzan *et al.* [1981].

Dose Initially 40 to 100 μg/kg IV

Agoston S *et al.* (1973). The fate of pancuronium bromide in man. *Acta Anaesthesiol Scand* 17: 267–275.

Baker TR *et al.* (1990). Mass spectrometric assay for determination of pancuronium and vecuronium in biological fluids utilizing the moving belt introduction system. *Biomed Environ Mass Spectrom* 19: 69–74.

Furuta T *et al.* (1988). Quantitation of pancuronium, 3-desacetylpancuronium, vecuronium, 3-desacetylvecuronium, pipecuronium and 3-desacetylpipecuronium in biological fluids by capillary gas chromatography using nitrogen-sensitive detection. *J Chromatogr* 427: 41–53.

McLeod K *et al.* (1976). Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Br J Anaesth* 48: 341–345.

Nisikawa M *et al.* (1991). The analysis of quaternary ammonium compounds in human urine by direct inlet electron impact ionization mass spectrometry. *Forensic Sci Int* 51: 131–138.

Poklis A, Melanson EG (1980). A suicide by pancuronium bromide injection: evaluation of the fluorometric determination of pancuronium in postmortem blood, serum and urine. *J Anal Toxicol* 4: 275–280.

Ramzan MI *et al.* (1981). Clinical pharmacokinetics of the non-depolarising muscle relaxants. *Clin Pharmacokinet* 6: 25–60.

Vandenbrom RH, Wierda JM (1988). Pancuronium bromide in the intensive care unit: a case of overdose. *Anesthesiology* 69: 996–997.

Wingard LBJr *et al.* (1979). Modified fluorometric quantitation of pancuronium bromide and metabolites in human maternal and umbilical serums. *J Pharm Sci* 68: 914–916.

Yashiki M *et al.* (1992). [A case of suicide by an intravenous injection of pancuronium.]. *Nihon Hoigaku Zasshi* 46: 282–285.

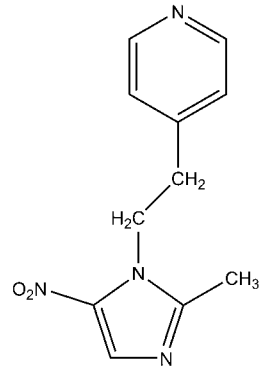
Panidazole

Antiamoebic

$C_{11}H_{12}N_4O_2 = 232.2$

IUPAC Name 4-[2-(2-Methyl-5-nitroimidazol-1-yl)ethyl]pyridine

Synonyms 2-Methyl-5-nitro-1-(2-pyrid-4-ylethyl)imidazole; 4-[2-(2-methyl-5-nitroimidazol-1-yl)ethyl]pyridine; WB-5040/2.



Chemical Properties A white crystalline powder. Mp 120° to 123°. Insoluble in water; slightly soluble in ethanol and other organic solvents; soluble in dilute acetic acid. Panidazole is extracted by chloroform from aqueous alkaline solutions.

Colour Test Vitali's test—red→dull violet (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.56 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—retention time 1.82 (relative to diphenhydramine), retention time 0.41 (relative to codeine).

Ultraviolet Spectrum 0.1 N sulfuric acid—256 (E1%, 1 cm 420), 278 nm (E1%, 1 cm 296); inflexion at 261 nm (E1%, 1 cm 408), minima at 236 and 270 nm.

Infrared Spectrum Principal peaks at wavenumbers 1186, 1359, 1458, 1256, 1422, 1520cm^{-1} (KBr disk).

Pantoprazole

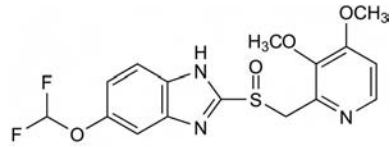
Proton Pump Inhibitor

$C_{16}H_{15}F_2N_3O_4S = 383.4$

CAS—102625-70-7

IUPAC Name 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole

Synonyms B8510-029; SK&F-96022.



Chemical Properties An off-white solid. Mp 139° to 140°. Freely soluble in water; practically insoluble in hexane; very slightly soluble in phosphate buffer (pH 7.4). pK_a 3.92; 8.19 (21° to 22°). Log *P* (octanol/buffer), 2.05 (pH 7.4); −0.26 (pH 11.4) (21–22°).

Pantoprazole Sodium Sesquihydrate

$C_{16}H_{14}F_2N_3NaO_4S,{}^3/_2H_2O = 432.5$

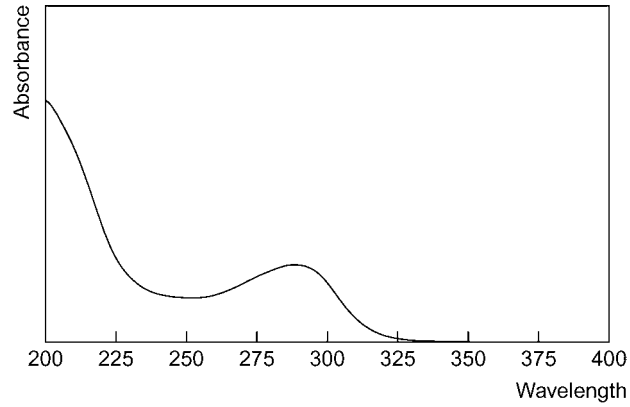
CAS—164579-32-2

Synonyms BY 1023; B8610-023; SK&F-96022-Z.

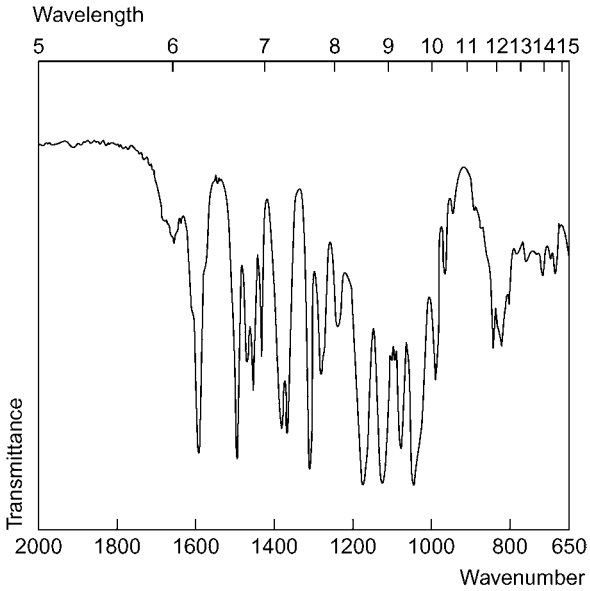
Proprietary Names *Pantozol*; *Protium*; *Protonix*.

Chemical Properties A white to off-white solid.

Ultraviolet Spectrum (Sodium Sesquihydrate) Aqueous acid—290 nm.



Infrared Spectrum Principal peaks at wavenumber 1590, 1493, 1277, 1170, 1122, 1041 cm^{-1}.



Quantification

Serum **HPLC** Column: RP-18 Hypersil (125 × 4.6 mm i.d., 5 µm). Mobile phase: (A : B) methanol : 10 mmol/L diammonium hydrogen phosphate buffer (pH 6.5), flow rate 1 mL/min. Elution programme: (43 : 57) for 2 min, to (83 : 17) in 17 min, to (100 : 0) in 2 min; to (43 : 57) in 7 min. UV detection (λ=290 nm). Retention time: pantoprazole, 16.5 min; sulfone metabolite, 13 min. Limit of detection, 0.004 mg/L [Huber *et al.* 1990].

Disposition in the Body Pantoprazole is rapidly absorbed after administration and almost exclusively metabolised in the liver. Co-administration with food delays absorption. It is extensively metabolised mainly via hepatic cytochrome P450 CYP2C19 isoenzyme. CYP34A, CYP2D6 and CYP2C9 metabolise the drug to a much lesser extent. The main metabolite is desmethylpantoprazole which is conjugated with sulfur and has been detected in plasma and urine. The metabolites are excreted primarily via renal elimination (~80%) and the remainder in faeces.

Therapeutic Concentration

Twelve healthy males, aged between 19 and 29 years, were administered with a 40 mg IV dose over 15 min or a 40 mg oral dose, both after an overnight fast. The mean peak plasma concentrations were 4.62 mg/L (range, 3.21 to 7.05) for the IV dose, observed at the end of the infusion and 2.09 (1.11 to 3.12) mg/L for the oral dose, observed 2 to 4 h after ingestion [Pue *et al.* 1993].

Bioavailability For the enteric coated tablet form, 77%.

Half-life 1 to 2 h in good metabolisers (pantoprazole); 6 h in poor metabolisers (drug); 1.5 h (metabolites).

Volume of Distribution 0.15 L/kg; apparent volume of distribution also reported as 11.0 to 23.6 L.

Clearance Plasma, 0.1 L/h/kg. Total, 7.6 to 14.0 L/h.

Distribution in Blood Pantoprazole and metabolites are essentially located in blood plasma with only negligible penetration in erythrocytes. Blood : plasma ratio is 0.55.

Protein Binding Approximately 98% (primarily to albumin).

Note For a general review of proton pump inhibitors, see Richardson *et al.* [1998].

Dose 20 or 40 mg daily.

Huber R *et al.* (1990). High-performance liquid chromatographic determination of the H+/K+ ATPase inhibitor (BY 1023/SK&F 96,022) and its sulphone metabolite in serum or plasma by direct injection and fully automated pre-column sample clean-up. *J Chromatogr* 529: 389–401.

Pue MA *et al.* (1993). Pharmacokinetics of pantoprazole following single intravenous and oral administration to healthy male subjects. *Eur J Clin Pharmacol* 44: 575–578.

Richardson P *et al.* (1998). Proton pump inhibitors. Pharmacology and rationale for use in gastrointestinal disorders. *Drugs* 56: 307–335.

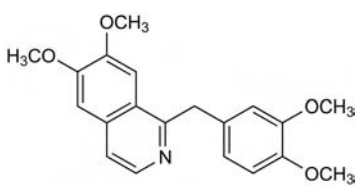
Papaverine

Antispasmodic

$C_{20}H_{21}NO_4$ = 339.4

CAS—58-74-2

IUPAC Name 1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline



Chemical Properties An alkaloid obtained from opium or prepared synthetically. Crystals or white crystalline powder. Mp 147°. Almost insoluble in water; soluble in hot benzene, glacial acetic acid and acetone; slightly soluble in chloroform, carbon tetrachloride and petroleum ether. pK_a 6.4 (25°). Log *P* (ether), 0.5.

Papaverine Hydrochloride

$C_{20}H_{21}NO_4$,HCl = 375.9

CAS—61-25-6

Proprietary Names *Albatran*; *Cerebid*; *Cerespan*; *Genabid*; *Oxadilene*; *Panergon*; *Pavabid*; *Pavacap*; *Pavacen*; *Pavakey*; *Pavarine*; *Pavased*; *Pavatine*; *Paverolan*; *Therapav*; *Vasal*; *Vasospan*.

Chemical Properties White crystals or crystalline powder. Mp 220° to 225° (from water). Soluble 1 in about 40 of water, 1 in 120 of ethanol and 1 in 10 of chloroform; practically insoluble in ether.

Papaverine Sulfate

$(C_{20}H_{21}NO_4)_2$,H_2SO_4,$5H_2O$ = 866.9

CAS—32808-09-6 (anhydrous)

Chemical Properties White crystals or crystalline powder. Soluble 1 in 2 of water, 1 in 20 of ethanol and 1 in 5000 of ether; slightly soluble in chloroform.

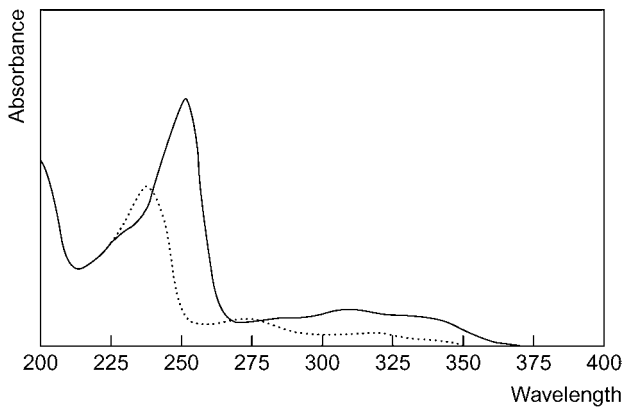
Colour Tests Liebermann's reagent—black; Mandelin's test—grey-green.

Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.08; system TC—R_f 0.65; system TE—R_f 0.69; system TL—R_f 0.47; system TAE—R_f 0.74; system TAF—R_f 0.74; system TAJ—R_f 0.66; system TAK—R_f 0.08; system TAL—R_f 0.93 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).

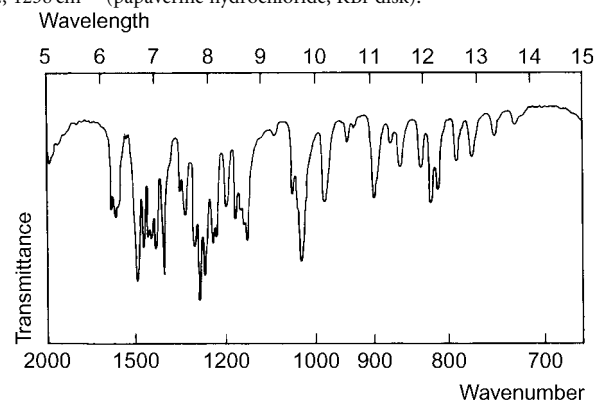
Gas Chromatography System GA—RI 2825; system GB—RI 2973.

High Performance Liquid Chromatography System HA—*k* 0.3; system HC—*k* 0.16; system HS—*k* 0.04; system HX—RI 363; system HY—RI 295; system HAA—retention time 12.1 min.

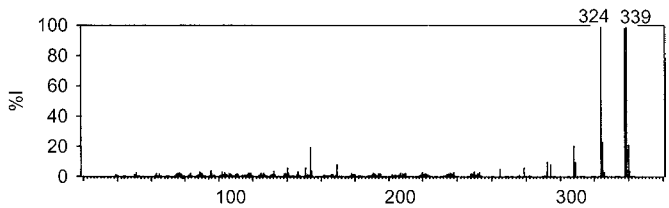
Ultraviolet Spectrum Aqueous acid—250 nm (A_1^1=1830a); aqueous alkali—236 (A_1^1=1994b), 277, 326 nm.



Infrared Spectrum Principal peaks at wavenumbers 1279, 1508, 1263, 1026, 1292, 1238 cm^{-1} (papaverine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 339, 324, 338, 325, 340, 308, 154, 292.



Quantification

Blood **GC** AFID. Limit of detection, 5 µg/L [Bellia *et al.* 1978].

GC–MS Limit of detection, 5 µg/L [De Graeve *et al.* 1977].

HPLC UV detection. Limit of detection, 5 µg/L [Hoogewijs *et al.* 1981].

P

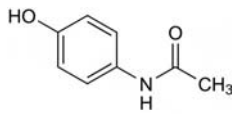
Plasma **HPLC** UV detection. Limit of detection, 2 µg/L [Pierson *et al.* 1979].
Disposition in the Body Readily and completely absorbed after oral administration but undergoes extensive first-pass metabolism. Metabolised by demethylation and glucuronic acid and sulfate conjugation of the resulting phenolic groups. About 50 to 80% of a dose is excreted in the urine in 48 h as conjugated phenolic metabolites, mostly 6-hydroxypapaverine (37% of the dose) and 4′-hydroxypapaverine (about 8.5% of the dose). <1% of a dose is excreted in the urine unchanged.
Therapeutic Concentration
After oral administration of single doses of 150 mg given as an elixir and tablets, respectively, to 6 subjects, mean peak plasma concentrations of 0.58 and 0.53 mg/L were attained in about 1 h [Meyer *et al.* 1980].
Half-life Plasma half-life, about 7 h, but there is wide variation and a biological half-life of 1 to 2 h is also reported.
Bioavailability About 30%.
Protein Binding About 90%.
Dose Up to 600 mg of papaverine hydrochloride daily.

Bellia V *et al.* (1978). Determination of papaverine in blood samples by gas chromatography using a flame-ionization and a nitrogen-phosphorus detector. *J Chromatogr* 161: 231–235.
De Graeve J *et al.* (1977). Determination of papaverine in blood samples by gas-liquid chromatography and mass fragmentography. *J Chromatogr* 133: 153–160.
Hoogewijs G *et al.* (1981). High-performance liquid chromatographic determination of papaverine in whole blood. *J Chromatogr* 226: 423–430.
Meyer MC *et al.* (1980). *J Clin Pharmacol* 19: 435–444.
Pierson SL *et al.* (1979). Simple and rapid high-pressure liquid chromatographic determination of papaverine in plasma. *J Pharm Sci* 68: 1550–1551.

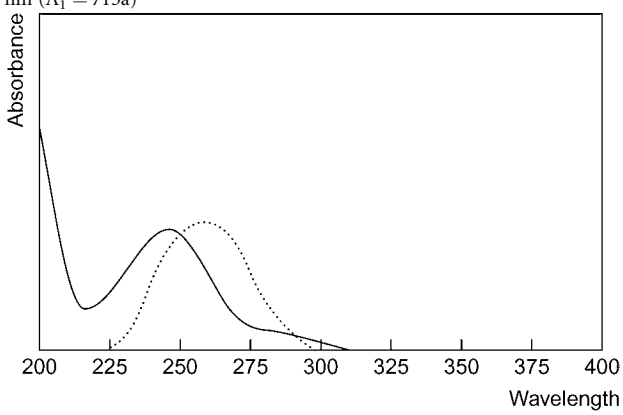
Paracetamol

Analgesic
$C_8H_9NO_2 = 151.2$
CAS—103-90-2

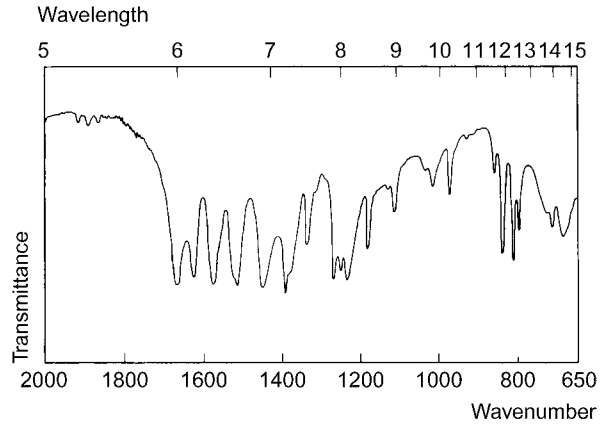
IUPAC Name *N*-(4-Hydroxyphenyl)acetamide
Synonyms Acetaminophen; *N*-acetyl-*p*-aminophenol.
Proprietary Names Paracetamol is an ingredient of *Anadin paracetamol*; *co-bucafAPAP*; *co-codamol*; *co-codAPAP*; *co-dydramol*; *co-hycodAPAP*; *co-methiamol*; *co-oxycodAPAP*; *co-proxamol*; *co-proxAPAP*; *Disprol*; *Galpamol*; *Mandanol*; *Medinol*; *Miradol*; *Obimol*; *Paldesic*; *Panadol*; *Salzone*.



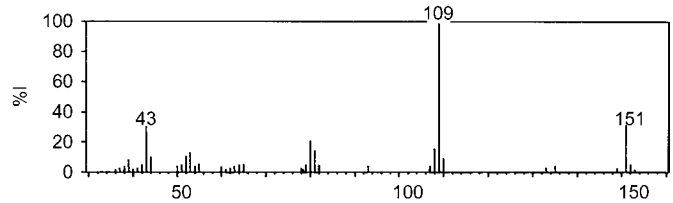
Chemical Properties White crystals or crystalline powder. Mp 169.0° to 170.5°. Very slightly soluble in cold water, considerably more soluble in hot water; soluble in ethanol, methanol, dimethylformamide, ethylene dichloride, acetone and ethyl acetate; very slightly soluble in chloroform; slightly soluble in ether; practically insoluble in petroleum ether, pentane and benzene. pK_a 9.5 (25°). Log *P* (octanol/water), 0.5. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Tests Ferric chloride—blue; Folin–Ciocalteu reagent—blue; Liebermann's reagent—violet; Nessler's reagent—brown (slow).
Potassium dichromate—boil 0.1 g with 1 mL hydrochloric acid for 3 min; add 10 mL of water cool, and add 0.05 mL 0.02 mol/L potassium dichromate—violet, developing slowly (which in contrast to phenacetin does not become red).
Thin-layer Chromatography System TA—R_f 0.95; system TB—Rf 0.00; system TD—R_f 0.15; system TE—R_f 0.45; system TF—R_f 0.32; system TAD—R_f 0.26; system TAE R_f 0.77; system TAJ—R_f 0.30; system TAK—R_f 0.05; system TAL—R_f 0.73 (acidified potassium permanganate solution—positive; ferric chloride—faint blue).
Gas Chromatography System GA—RI 1665, art (*p*-aminophenol) RI 1253, paracetamol-Me RI 1512, art (*p*-aminophenol)-Me_2 RI 1220; system GB—RI 1722, art (*p*-aminophenol) RI 1280; system GL—paracetamol-Me RI 1630.
High Performance Liquid Chromatography System HD—*k* 0.1; system HW—*k* 0.32; system HX—RI 264; system HY—RI 241; system HZ—RT 1.9 min; system HAA—RT 5.6 min; system HAM—RT 2.0 min; system HAX—RT 4.8 min; system HAY—RT 3.7 min.
Ultraviolet Spectrum Aqueous acid—245 nm ($A^1_1 = 668a$); aqueous alkali—257 nm ($A^1_1 = 715a$)



Infrared Spectrum Principal peaks at wave numbers 1506, 1657, 1565, 1263, 1227, 1612 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 109, 151, 43, 80, 108, 81, 53, 52 (paracetamol); 141, 43, 183, 44, 140, 80, 108, 52 (cysteine conjugate); 43, 141, 183, 42, 87, 41, 140, 165 (mercapturic acid conjugate).



Quantification

Blood **GC** Column: HP-5 (25 m × 0.25 mm i.d., 0.33 µm). Temperature programme: 100° for 1 min to 300° at 10°/min for 14 min. FID. Limit of detection not reported [Lo *et al.* 1997].
GC-MS Column: SGE BPX5 (12 m × 0.15 mm i.d., 0.44 mm). Temperature programme: 80° for 1 min to 300° at 20°/min. Limit of detection not reported [Speed *et al.* 2001]. Limit of detection not reported [Takayasu *et al.* 1993].
HPLC Column: LC_8 Nova-Pak (150 mm). Mobile phase: acetonitrile : 0.005 mol/L phosphate buffer (pH 6.0, 1 : 9). DAD. Limit of detection not reported [Singer *et al.* 2007]. Column: Hypersil C_{18} (75 × 4.6 mm i.d., 3 µm). Mobile phase: 20 mmol/L ammonium formate buffer (pH 3.5) : methanol (96 : 4 for 5 min to 46 : 54 at 15 min to 10 : 90 at 16 min for 2 min to 96 : 4 at 19 min for 5 min), flow rate 0.8 mL/min until 15 min when 1.0 mL/min until 19 min to 0.8 mL/min. UV detection (λ= 254 nm). Limit of detection, 600 pg [Oliveira *et al.* 2002]. Column: LC-8DB (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.1 mol/L potassium dihydrogen phosphate : water (20 : 10 : 5 : 65), flow rate 1.1 mL/min. UV detection (λ= 245 nm). Limit of detection not reported [Pufal *et al.* 2000]. Column: ODS-Hypersil (200 × 2.1 mm i.d., 5 µm). Mobile phase: 2 mmol/L phosphate buffer : acetonitrile (95 : 5 to 50 : 50 over 20 min for 10 min, to 95 : 5 over 1 min), flow rate 0.4 mL/min. DAD (λ= 210 nm). Limit of detection not reported [Lo *et al.* 1997]. Apex ODS II (150 × 0.005 mm, 0.5 µm). Mobile phase: water : acetonitrile : glacial acetic acid (425 : 50 : 25). UV/VIS detection. Limit of detection not reported [Williams, Pounder 1997]. Column: Ultrasphere ODS (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.01 mol/L phosphoric acid (7 : 93), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 0.1 mg/L [Whelpton *et al.* 1993]. See also Koves [1995] and West [1981].
Plasma **GC** Column: Chromosorb W HP 100/120 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 235°. FID. Limit of detection, 10 mg/L [Huggett *et al.* 1981]. Column: 3% SP2100 on Supelsoport 100/120 mesh (1.5 m × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 300°. ECD. Limit of quantification, 5 mg/L [Chan, McCann 1979]. Column: 3% SP 2250 on 80/100 mesh chromosorb W, AW/DMCS (2 m × 2 mm i.d.). FID. Limit of detection, >0.1 µg [Evans, Harbison 1977].
GC-MS Column: 3% OBV-17 on 100/120 mesh Gas Chrom Q (1.21 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 180°. SIM acquisition mode. Limit of detection, 0.1 mg/L [Garland *et al.* 1977].
HPLC Column: RP-C_{18}. Mobile phase: 0.1% potassium dihydrogen orthophosphate : propan-2-ol : tetrahydrofuran (100 : 1.5 : 0.1). Spectrophotometric detection (λ= 254 nm) [Jensen *et al.* 2004]. Column: CLC C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (55 : 45), flow rate 0.8 mL/min. UV detection (λ= 230 nm). Limit of quantification, 0.550 mg/L, limit of detection, 0.208 mg/L [Nagaralli *et al.* 2003]. Column: µBondipak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: methanol : acetonitrile : 20 mmol/L orthophosphoric acid (pH 3.2, 2 : 4 : 94). UV detection. Retention time: 9.3 min. Limit of detection, 0.2 mg/L [al Obaidy *et al.* 1995]. Column: Nova-Pak C_{18} (100 × 8 mm i.d., 5 µm). Mobile phase: 0.1 mol/L potassium dihydrogen orthophosphate : acetic acid : propan-2-ol (100 : 0.1 : 0.75), flow rate 1.5 mL/min. UV detection (λ= 254 nm). Retention time: 11.8 min. Limit of detection, 1–2 ng [Lau, Critchley 1994]. See Blood [Whelpton *et al.* 1993].

Column: Spherisorb SI (250 × 4.6 mm i.d., 5 µm). Mobile phase: water-saturated chloroform : heptane : ethanol : acetic acid (225 : 700 : 75 : 1), flow rate 1.6 mL/min. UV detection (λ = 248 nm). Limit of detection, 50 µg/L [Buskin *et al.* 1982]. See also Douidar, Ahmed [1982], Kozer, Koren [2001], Munson *et al.* [1978], Palmer [1986], Rustum [1989] and Surmann [1980].

LC-MS Column: YMC-ODS-AQ C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 0.3% acetic acid : methanol (100 : 0 for 3 min, to 2 : 98 for 1 min, to 100 : 0 for 1 min), flow rate 0.30 mL/min. MS-MS detection. Limit of quantification, 10 µg/L [Lou *et al.* 2010]. Column: Venusil Mp-C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: formic acid : 10 mmol/L ammonium acetate : methanol (1 : 40 : 60), flow rate 1.0 mL/min. ESI ionisation, MRM acquisition mode, MS-MS detection. Limit of quantification, 5.0 µg/L [Li *et al.* 2010]. Column: (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water with 0.5% formic acid (20 : 80). ESI, SRM acquisition mode, MS-MS detection. Limit of quantification, 0.2 mg/L [Feng *et al.* 2009]. Column: Zorbax SB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (80 : 20 : 0.5), flow rate 0.6 mL/min. APCI, SRM acquisition mode, MS-MS detection. Limit of quantification, 50 µg/L [Chen *et al.* 2005]. Column: Thermo Hypersil APS-2 Amino (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.4% glacial acetic acid in water (20 : 80), flow rate 1.0 mL/min. ESI, MRM acquisition mode, MS-MS detection. Limit of quantification, 0.1 mg/L [Yin *et al.* 2005]. See also Celma *et al.* [2000] and Matsumoto *et al.* [2003].

Serum **GC** See Plasma [Huggett *et al.* 1981]. Column: 2.8% OV-210 and 3.2% OV-1 on Chromosorb W HP 80/100 mesh (900 × 2 mm i.d.). Column: Carri (150 × 4.6 mm i.d.). Mobile phase: water: 1.1 mol/L ammonium acetate : methanol : 1 mol/L acetic acid (390 : 34 : 70 : 9), flow rate 1.17 mL/min. gas: N_2, 40 mL/min. Temperature: 120°. FID. Retention time: 1.9 min. Limit of detection, 7.5 mg/L [Kaa 1980]. Column: (150 × 4.6 mm i.d.). Mobile phase: water : 1 mol/L ammonium acetate : methanol : 1 mol/L acetic acid (390 : 34 : 70 : 9), flow rate 1.17 mL/min. ECD. Limit of quantification, 20 µg/L, limit of detection, 2 pg [Miner, Kissinger 1979].

HPLC Column: C_{18}. Mobile phase: 0.02 mol/L SDS. Electrochemical detection. Limit of detection, 0.83 µg/L [Bose *et al.* 2005]. Column: Chromolith RP-C_{18} (100 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L potassium phosphate monobasic : acetonitrile (95 : 5), flow rate 9 mL/min. UV detection (λ = 220 nm). Limit of quantification, 3.75 mg/L, limit of detection, 1.25 mg/L [Pistos, Stewart 2004]. Column: TSK-GEL-ODS-80Tm (250 × 4.6 mm i.d.). Mobile phase: 50 mmol/L sodium acetate : methanol (93 : 7), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 3 pmol/mg [Muldrew *et al.* 2002]. Column: Separon Six C_{18} (150 × 3.7 mm i.d., 5 µm). Mobile phase: methanol : 0.4% phosphoric acid. UV detection (λ = 254 nm). Limit of detection, 0.2 mg/L [Kotal *et al.* 1989]. Mobile phase: 4% acetonitrile : 12% acetic acid in water. Limit of detection, 2 mg/L [Osterloh, Yu 1988].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (2 : 98 for 15 min to 25 : 75 over 14 min, for 20 to 50 min, to 2 : 98 over 20 min), flow rate 0.15 mL/min. Positive ion mode, SIM acquisition mode. Limit of detection, 1 mg/L [Hori *et al.* 2006].

Urine **GC** See Plasma [Chan, McCann 1979].

GC-MS See Blood [Takayasu *et al.* 1993].

HPLC Column: LiChroCART (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.05% trifluoroacetic acid in water (3 : 1 : 96 to 29 : 1 : 70 to 49 : 1 : 50), flow rate 1.2 mL/min. DAD. Limit of quantification, 0.44 mg/L, limit of detection, 0.15 mg/L [Baranowska *et al.* 2009]. See Serum. Limit of detection, 0.74 µg/L [Bose *et al.* 2005]. See Plasma [Jensen *et al.* 2004]. See Blood [Pufal *et al.* 2000]. UV detection [Di Girolamo *et al.* 1998]. Column: RP-18 (250 × 4 mm i.d., 5 µm). Mobile phase: water : acetonitrile : TEA (pH 2.5, 99.99 : 0.001 : 0.5), flow rate 1.7 mL/min. UV detection (λ = 254 nm). Limit of detection, 1 mg/L [Retaco *et al.* 1996]. See also al Obaidy *et al.* [1995], Douidar, Ahmed [1982], Lau, Critchley [1994], Wilson *et al.* [1982] and Wong *et al.* [1986].

LC-MS Column: ChromSpeed monolithic (50 × 4.6 mm i.d.). Mobile phase: 0.1% formic acid : acetonitrile with 0.1% formic acid (100 : 0 to 60 : 40), flow rate 2 mL/min. Limit of detection not reported [Johnson, Plumb 2005]. Column: Acquity (50 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid : acetonitrile with 0.1% formic acid (100 : 0 to 60 : 40), flow rate 500 µL/min. Limit of detection not reported [Johnson, Plumb 2005]. Column: Symmetry C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium acetate solution : acetonitrile (90 : 10 for 5 min, to 20 : 80 over 10 min for 10 min), flow rate 150 µL/min. ESI, SIM acquisition mode. Limit of quantification, 10 µg/L [Matsumoto *et al.* 2003].

CSF **HPLC** See Plasma [Kozer, Koren 2001]. See Blood [Pufal *et al.* 2000]. Partisil 5 ODS-3 (250 × 6.35 mm i.d., 5 µm). Mobile phase: methanol : water (25 : 75), flow rate 1.5 mL/min. UV detection (λ = 245 nm). Limit of detection, 2 µg/L [Bannwarth *et al.* 1992]. Mobile phase: 0.1 mol/L citrate/phosphate buffer : methanol (95 : 5), flow rate 2.7 mL/min. Electrochemical detection [Walsh *et al.* 1982].

Milk **HPLC** See Oral Fluid [Berlin *et al.* 1980].

Oral Fluid **HPLC** Column: TSkgel ODS-80Tm (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 1.0 mL/min. Fluorescence detection (λ_{em} = 560 nm, λ_{ex} = 540 nm). Limit of detection, 0.1 mg/L [Fujino *et al.* 2005]. See Urine. Mobile phase: water : acetonitrile : TEA (94 : 6 : 0.5), flow rate 1 mL/min. Limit of detection, 0.3 mg/L [Retaco *et al.* 1996]. See Plasma [al Obaidy *et al.* 1995]. Column: Bondapak C_{18}. Mobile phase: 0.05 mol/L sodium acetate (pH 4.0) : acetonitrile (93 : 10), flow rate 2.0 mL/min. Retention time: 3–5 min. Limit of detection, 0.5 mg/L [Berlin *et al.* 1980].

Stomach Contents **GC-MS** See Blood [Singer *et al.* 2007; Takayasu *et al.* 1993].

Vitreous Humour **HPLC** See Blood [Pufal *et al.* 2000; Singer *et al.* 2007].

Brain **HPLC** See Blood [Pufal *et al.* 2000].

Hair **HPLC** Column: Symmetry C_8 (250 × 4.6 mm i.d., 5 µm). Column: CP SIL8 CB (25 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 50° for 2 min to 310° at 15°/min for 4.67 min. Mobile phase: acetonitrile : phosphate buffer (pH 3.8). UV detection. Limit of detection not reported [Gaillard, Pepin 1997].

Kidney **HPLC** See Blood [Pufal *et al.* 2000]. Column: Apex (3 µm). Mobile phase: distilled water : acetonitrile (86 : 14). UV detection (λ = 254 nm). Limit of detection, 50 µg/L [Colin *et al.* 1987]. See Blood [West 1981].

Liver **GC-MS** See Blood [Speed *et al.* 2001; akayasu *et al.* 1993].

HPLC See Blood [Pufal *et al.* 2000; Singer *et al.* 2007; West 1981]. See Kidney [Colin *et al.* 1987].

Muscle **HPLC** See Blood [West 1981; Williams, Pounder 1997].

Disposition in the Body Small doses of paracetamol are readily absorbed but the absorption of larger doses varies considerably and is influenced by gastric emptying rate, the presence of food and the time of day. Paracetamol is widely distributed throughout most body fluids and is present in the saliva at concentrations paralleling those in plasma. It crosses the placenta and is found in breast milk. It undergoes first-pass metabolism and is metabolised mainly by conjugation to form glucuronides and ethereal sulfates; 3-hydroxylation also occurs followed by conjugation or *O*-methylation of the hydroxy group. Oxidation to a reactive metabolite thought to be acetylimino-*p*-benzoquinone occurs to a small extent with therapeutic doses but becomes more significant after larger doses, and this metabolite appears to be responsible for hepatic necrosis in paracetamol overdosage; it is normally detoxified by glutathione conjugation to form mercapturic acid and cysteine conjugates but after sources of glutathione are depleted, the free metabolite is available to bind covalently with liver cell protein; this binding occurs ~10–12 h after dosing. Approximately 90% of a therapeutic dose is excreted in the urine in 24 h; of the excreted material, 1–4% is unchanged, 20–30% is conjugated with sulfate, 40–60% is conjugated with glucuronic acid, 5–10% consists of the 3-hydroxy-3-sulfate, the 3-methoxyglucuronide and the 3-methoxy-3-sulfate metabolites, and ~5–10% consists of the mercapturic acid and cysteine conjugates; 3-methylthio-4-hydroxyacetanilide has also been identified at concentrations of <1%. Larger amounts of the mercapturic acid and cysteine conjugates are excreted in overdose.

Paracetamol is a metabolite of benorilate and phenacetin.

Therapeutic Concentration In plasma, usually in the range 10–20 mg/L. Plasma concentrations vary considerably between subjects. The glucuronide and sulfate conjugates accumulate in subjects with impaired renal function.

After a single oral dose of 1.5 g to 14 subjects, peak plasma paracetamol concentrations of 7.4–37 mg/L (mean, 24) were attained in 0.5–3 h (mean, 1.4) [Heading *et al.* 1973].

Following administration of 4 rectal doses of paracetamol, 20 mg/kg every 6 h, to 10 full-term neonates undergoing painful procedures or having painful conditions, mean peak serum concentrations were 10.79, 15.34 and 6.24 mg/L in the whole group, boys, and girls, respectively; the median time to peak serum concentration was 1.5 h after the first dose and 15 h for multiple doses. A starting dose of 30 mg/kg followed by 20 mg/kg rectally at intervals increasing from 6–8 h was proposed for neonates [van Lingen *et al.* 1999].

Of 24 children (over 25 kg) undergoing elective surgery and given paracetamol rectally at a dose of 1 or 40 mg/kg, most children in the 1 g group failed to attain therapeutic plasma levels whereas those in the 40 mg/kg group did (mean peak plasma concentration 7.8 vs. 15.9 mg/L attained at 3.8 and 2.6 h, respectively) [Howell, Patel 2003].

Toxicity The minimum lethal dose is ~10 g. Symptoms of hepatic damage do not occur for at least 12 h after overdosage but may not appear until 4–6 days later. Plasma concentrations have been used to indicate possible hepatic necrosis; at 4 h, hepatic necrosis is possible at concentrations of paracetamol of 120–300 mg/L, probable at concentrations above 300 mg/L, and unlikely at concentrations <120 mg/L. Similarly, at 12 h, concentrations above 120 mg/L indicate the probability of necrosis, concentrations of 50–120 mg/L indicate that it is possible, and concentrations below 50 mg/L indicate that it is unlikely.

The following postmortem tissue concentrations were reported in 3 deaths: 160, 200 and 387 mg/L in blood; 180, – and 900 mg/L in bile; –, – and 385 µg/g in liver; 200, – and 475 mg/L in liver blood; and 180, 620 and – mg/L in urine [Robinson *et al.* 1977].

A 6-year-old child died after receiving paracetamol overdosage over a period of 3 days. The prescribed dose for fever (associated with measles) had been 325 mg every 6 h, but the child's mother, believing paracetamol to be non-toxic, had progressively increased the dose, first in response to the fever and subsequently for abdominal pain (500 mg every 4 h for 48 h followed by 500 mg every 2–3 h for 12 h had been given). On admission to hospital 11 h after the last dose, the child's serum paracetamol concentration was 163 mg/L. Despite appropriate treatment, the child died on the 11th day [Blake *et al.* 1988].

Of 93 patients hospitalised for paracetamol toxicity, 80 were classified as suicidal and 13 had accidentally poisoned themselves in an attempt to relieve pain. Peak plasma levels were higher in the suicidal overdose group (mean, 121.7 mg/L) than in the accidental overdose group (64.5 mg/L) [Gyamlani, Parikh 2002].

Bioavailability 70–90%.

Half-life Plasma half-life after therapeutic doses, ~1–3 h in adults, ~5 h in neonates; plasma half-lives greater than ~4 h in adults are indicative of possible liver damage.

Volume of Distribution ~0.8–1.0 L/kg.
Clearance Plasma clearance, ~5 mL/min/kg.
Protein Binding In plasma, not bound at concentrations <60 μg/mL. In poisoned subjects, protein binding has been reported to vary between ~8 and 40%.
Dose Up to maximum of 4 g daily.
Note For a review of the pharmacokinetics of paracetamol, see Forrest *et al.* [1982]; for a review of its use in infants, see Arana *et al.* [2001]; for a review of paracetamol overdosage, see Kozer, Koren [2001].

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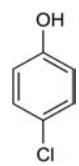
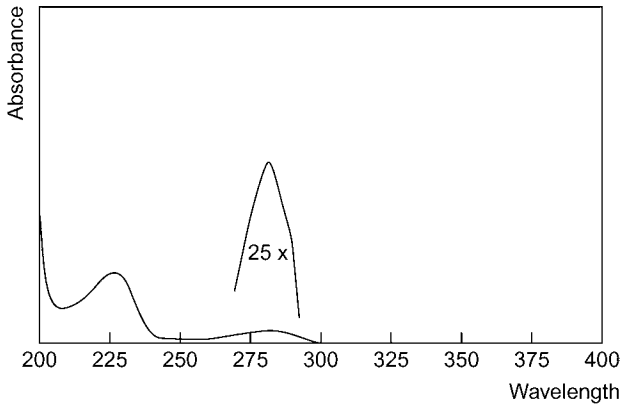
Parachlorophenol

Disinfectant
C_6H_5ClO = 128.6
CAS—106-48-9
IUPAC Name 4-Chlorophenol
Synonym *p*-Chlorophenol
Proprietary Names It is an ingredient of the proprietary preparations *Esofenol Ferri* and *Cresophene*.



Chemical Properties White or pink crystals. Mp about 43°. Soluble 1 in 60 of water; very soluble in ethanol, chloroform and ether. pK_a 9.2 (20°). Log *P* (octanol/water), 2.4.
Colour Test Ferric chloride—blue.
Gas Chromatography System GA—RI 1390.
Ultraviolet Spectrum Aqueous alkali—298 nm (A^1_1=194b); ethanol—284 (A^1_1=149b), inflexion at 291 nm.

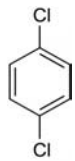


Mass Spectrum Principal ions at *m/z* 128, 130, 65, 39, 64, 63, 129, 99.

Paradichlorobenzene

Insecticide
$C_6H_4Cl_2$ = 147.0
CAS—106-46-7

IUPAC Name 1,4-Dichlorobenzene
Synonym Dichlorbenzol
Proprietary Names *Paracide*; *Para-zene*; *Paramoth*. It is an ingredient of *Cerumol*, *Cerumenol* and *Otoceril*.

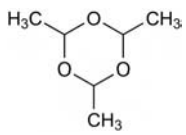


Chemical Properties Colourless shining crystals; slowly volatile in air. Mp 53° to 54°. Practically insoluble in water; soluble in most organic solvents. Log *P* (octanol/water), 3.4.
Ultraviolet Spectrum Ethanol—272 (A_1^1=27b), 265 (A_1^1=20b), 282 nm (A_1^1=17b).
Infrared Spectrum Principal peaks at wavenumbers 1012, 1091, 817, 1121, 951, 1000 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 146, 148, 111, 75, 50, 150, 113, 147.
Disposition in the Body
Toxicity The estimated minimum lethal dose is 25 g and the maximum permissible atmospheric concentration is 75 ppm or 450 mg/m^3. Paradichlorobenzene is very irritant to the eyes, mucous membranes and skin, and it may cause narcosis.

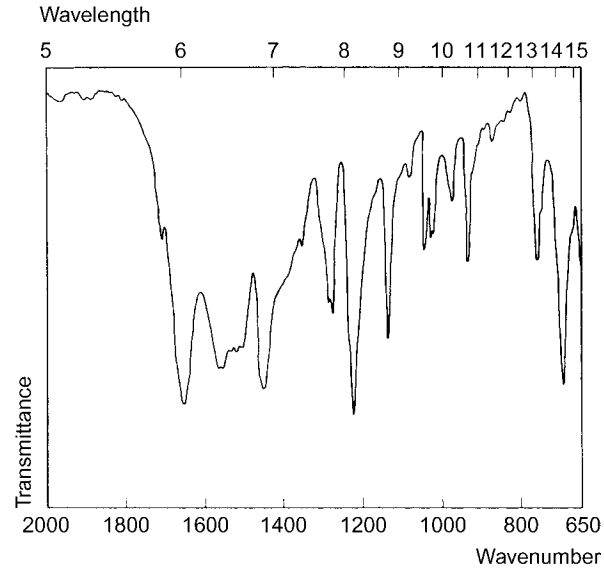
Paraldehyde

Anticonvulsant, Hypnotic, Sedative
$(C_2H_4O)_3$ = 132.2
CAS—123-63-7

IUPAC Name 2,4,6-Trimethyl-1,3,5-trioxane
Synonym Paracetaldehyde
Proprietary Name *Paral*
Note The trimer of acetaldehyde; 2,4,6-trimethyl-1,3,5-trioxane.



Chemical Properties A clear colourless or pale yellow liquid which decomposes and increases in toxicity on storage. Relative density 0.991–0.996. Mp 10° to 13°. Bp 123° to 126°. At low temperatures it solidifies to form a crystalline mass. Soluble 1 in 10 of water, but only 1 in 17 of boiling water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 0.7.
Gas Chromatography System GA—paraldehyde RI 786, M acetaldehyde RI 372; system GI—paraldehyde RT 23.2 min, M acetaldehyde RT 0.7 min.
Infrared Spectrum Principal peaks at wavenumbers 1095, 1170, 946, 860, 846, 763 cm^{-1} (thin film).



Mass Spectrum Principal ions at *m/z* 43, 44, 87, 31, 45, 42, 131, 71; M acetaldehyde 29, 44, 43, 42, 26, 41, 28, 27.
Quantification
Blood GC Headspace analysis. Limit of detection, 2 mg/L [Anthony *et al.* 1978]. Headspace analysis after conversion to acetaldehyde. See Hancock *et al.* [1977].
Plasma GC See Blood [Anthony *et al.* 1978].
Serum GC See Hessel [1988].
Urine GC See Blood [Anthony *et al.* 1978].
Gastric Contents GC See Blood [Hancock *et al.* 1977].
Disposition in the Body Readily absorbed after oral, rectal or IM administration and distributed throughout the tissues. Probably metabolised by depolymerisation to acetaldehyde which is oxidised to acetic acid and ultimately to carbon dioxide. About 7% of an oral dose is exhaled unchanged in 4 h.
Therapeutic Concentration In plasma, usually in the range 30–100 mg/L. Concentrations in the CSF are about 70% of those in plasma.

After oral doses of 100 mg/kg, peak blood concentrations of 100–150 mg/L were attained in 0.5 h; following rectal administration of 100 mg/kg, peak blood concentrations of 50–100 mg/L were reported at 1.5–2 h [Anthony *et al.* 1978.]

Following an IM injection of 10 mL to 1 subject, a peak plasma concentration of 77 mg/L was attained in 1.2 h [Maes *et al.* 1969.]

Toxicity The minimum lethal dose has been estimated to be 25 mL orally and 12 mL rectally, although recovery has occurred after the ingestion of 125 mL. Toxic effects have been associated with blood concentrations of 200–400 mg/L. Blood concentrations of ≈500 mg/L, or less if alcohol has also been ingested, may be lethal. On storage, paraldehyde may depolymerise to acetaldehyde and acetic acid; severe acidosis and fatalities may follow the use of partly depolymerised material.

In 15 fatalities attributed to paraldehyde overdose, blood concentrations of 490–1600 mg/L were reported [Hayward, Boshell 1957.]

The following postmortem tissue concentrations were reported in 3 fatalities, 2 of which involved alcohol: blood 115, 480 and 140 mg/L, brain 150, 300 and 370 µg/g, kidney 190, 50 and 260 µg/g, liver 210, 200 and 600 µg/g, urine –, –, 130 mg/L [Rehling 1967].

Half-life Plasma half-life, 4–10 h.
Volume of Distribution About 1 L/kg.
Clearance Plasma clearance, about 2 mL/min/kg.
Dose 5 to 10 mL orally or by IM injection.

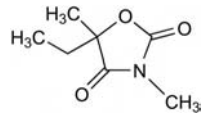
Anthony RM *et al.* (1978). *J Anal Toxicol* 2: 262–264.
Hancock JP *et al.* (1977). *J Anal Toxicol* 1: 161–163.
Hayward JN, Boshell BR (1957). Paraldehyde intoxication with metabolic acidosis; report of two cases, experimental data and a critical review of the literature. *Am J Med* 23: 965–976.
Hessel DW (1988). The analysis of blood serum for paraldehyde by ultrafiltration and gas chromatography with a wide-bore capillary column. *J Anal Toxicol* 12: 350–353.
Maes R *et al.* (1969). The gas chromatographic determination of selected sedatives (ethchlorvynol, paraldehyde, meprobamate, and Carisoprodol) in biological material. *J Forensic Sci* 14: 235–254.
Rehling CJ (1967). Poison Residues in Human Tissues. In: Stolman A, ed. *Progress in Chemical Toxicology*, Vol. 3. New York: Academic Press Inc., 363–386.

Paramethadione

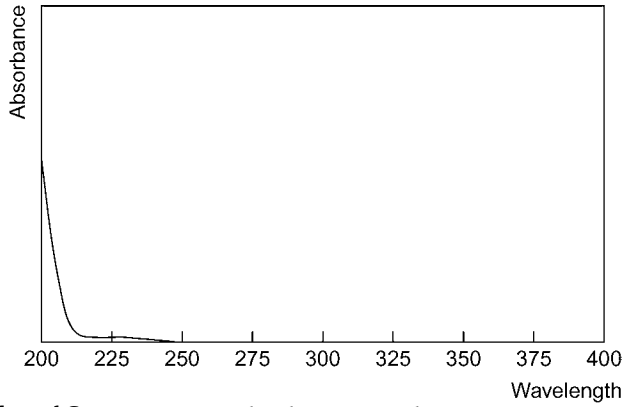
Anticonvulsant
$C_7H_{11}NO_3$ = 157.2
CAS—115-67-3

IUPAC Name 5-Ethyl-3,5-dimethyl-2,4-oxazolidinedione

Proprietary Name *Paradione*



Chemical Properties A clear colourless liquid. Refractive index, at 25°, 1.4485 to 1.4505. Sparingly soluble in water; freely soluble in ethanol, benzene, chloroform and ether. Log *P* (octanol/water), 1.1.
Colour Test Koppanyi-Zwikker test—violet.
Thin-layer Chromatography System TA—R_f 0.86; system TD—R_f 0.00; system TE—R_f 0.07; system TF—R_f 0.60; system TAD—R_f 0.56; system TAJ—R_f 0.87; system TAK—R_f 0.70; system TAL—R_f 0.94.
Gas Chromatography System GA—RI 1115; system GE—paramethadione RRT 0.06, *N*-desmethylparamethadione RRT 0.22 (both relative to phenytoin).
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



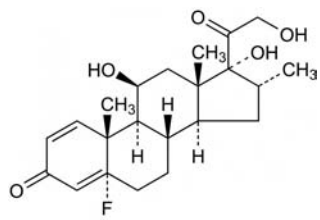
Infrared Spectrum Principal peaks at wavenumbers 1730, 1805, 1100, 1040, 1280, 1070 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 43, 129, 57, 56, 41, 72, 39, 58.
Quantification
Serum **GC** FID. Limit of detection, 200 µg/L for paramethadione and 5-ethyl-5-methyloxazolidine-2,4-dione [Hoffman, Chun 1975].
Disposition in the Body Readily absorbed after oral administration. Metabolised by *N*-demethylation to 5-ethyl-5-methyloxazolidine-2,4-dione which is active and accumulates during chronic administration; 30 to 80% of a dose is excreted slowly in the urine as the *N*-desmethyl metabolite with <1% as unchanged drug.
Therapeutic Concentration
After a single oral dose of 300 mg to 3 subjects, peak serum concentrations of paramethadione of 5.4 to 7.6 mg/L (mean, 6.3) were attained in 0.5 to 1 h; concentrations of the *N*-desmethyl metabolite gradually increased to a plateau of 8.1 to 8.6 mg/L (mean, 8.4) at 32 h [Hoffman, Chun 1975].
Following oral administration of 900 mg daily to 2 subjects for 14 days, peak plasma concentrations of the *N*-desmethyl metabolite of about 240 mg/L were reported [Butler 1955].
Toxicity The estimated minimum lethal dose is 5 g.
Half-life Plasma half-life, paramethadione about 16 h after a single dose, *N*-desmethylparamethadione about 7 to 14 days during chronic administration.
Protein Binding Not significantly bound.
Dose 0.9 to 1.8 g daily.

Butler TC (1955). Metabolic demethylation of 3,5-dimethyl-5-ethyl 2,4-oxazolidinedione (paramethadione, paradione). *J Pharmacol Exp Ther* 113: 178–185.
Hoffman DJ, Chun AHC (1975). Paramethadione and metabolite serum levels in humans after a single oral paramethadione dose. *J Pharm Sci* 64: 1702–1703.

Paramethasone

Corticosteroid
$C_{22}H_{29}FO_5$ = 392.5
CAS—53-33-8

IUPAC Name (6*S*,8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,16*R*,17*R*)-6-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-one
Synonym (6α,11β,16α)-6-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

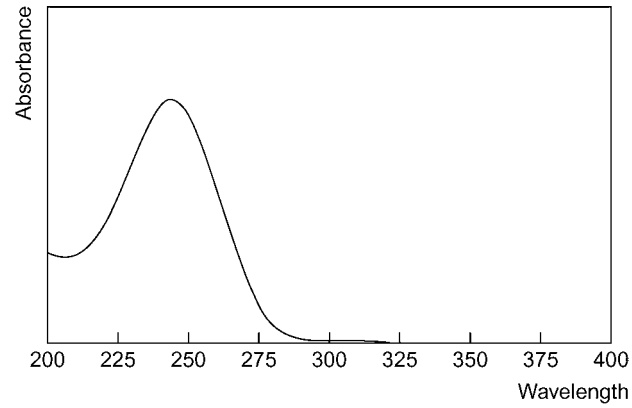


Chemical Properties Log *P* (octanol/water), 1.7.

Paramethasone Acetate

$C_{24}H_{31}FO_6$ = 434.5
CAS—1597-82-6
IUPAC Name 6α-Fluoro-16α-methylprednisolone 21-acetate
Proprietary names *Cordidene*; *Depodillar*; *Dilar*; *Haldrone*; *Metilar*; *Monocortin*; *Paramezone*.
Chemical Properties A white to creamy-white, fluffy, crystalline powder. Mp 228° to 241°, with decomposition. Practically insoluble in water; soluble in ethanol, acetone and ether; soluble 1 in 50 of chloroform.

Thin-layer Chromatography System TA—R_f 0.91; system TE—R_f 0.88; system TAJ—R_f 0.54; system TAK—R_f 0.39; system TAL—R_f 0.91; system TAM—R_f 0.91.
Ultraviolet Spectrum Paramethasone acetate: methanol—241 nm (A_1^1=500b).

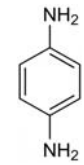


Infrared Spectrum Principal peaks at wavenumbers 1658, 1227, 1600, 905, 1745, 1034 cm^{-1} (Nujol mull).
Quantification
Plasma **HPLC** UV detection. For method of quantification for paramethasone and other corticosteroids, see Saito *et al.* [1979].
Urine **HPLC** See Plasma [Saito *et al.* 1979].
Dose Up to 12 mg of paramethasone acetate daily.

Saito Z *et al.* (1979). The high pressure liquid chromatography of corticoids. II. Analysis of synthetic corticoids in blood and urine (author's transl). *Nippon Naibunpi Gakkai Zasshi* 55: 1296–1306.

Paraphenylenediamine

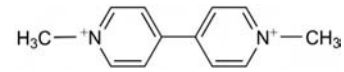
Dye
$C_6H_8N_2$ = 108.1
CAS—106-50-3
IUPAC Name Benzene-1,4-diamine
Synonyms 'Para'; *p*-phenylenediamine.



Chemical Properties White or reddish crystals. Mp 145° to 147°. Soluble 1 in 100 of water; soluble in ethanol, chloroform and ether. pK_a 6.2 (25°). Log *P* (octanol/water), −0.3.
Colour Tests Ferric chloride—green; Mandelin's test—yellow.
Thin-layer Chromatography System TA—R_f 0.61 (acidified iodoplatinate solution, positive).

Paraquat

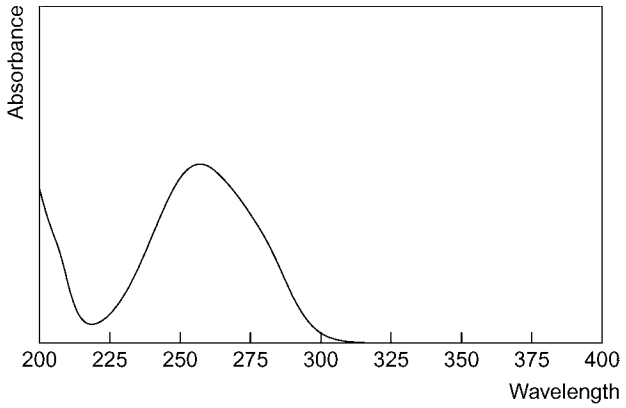
Herbicide
$C_{12}H_{14}N_2$ = 186.3
CAS—4685-14-7
IUPAC Name 1-Methyl-4-(1-methylpyridin-1-ium-4-yl)pyridin-1-ium
Synonyms 1,1′-Dimethyl-4,4′-bipyridinium; parakuat.



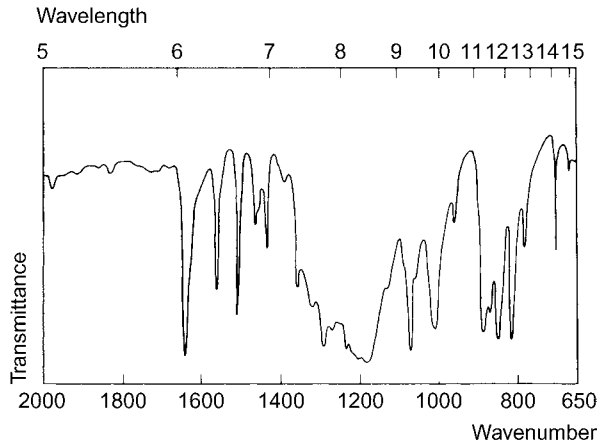
Chemical Properties Log *P* (octanol/water), −4.2.

Paraquat Dichloride
$C_{12}H_{14}Cl_2N_2 = 257.2$
CAS—1910-42-5
Proprietary Names *Dextrone X*; *Gramoxone*. It is an ingredient of *Dexuron*; *Pathclear*; *Weedol*.
Chemical Properties A white crystalline solid. Decomposes at ~300°. Very soluble in water. Hydrolysed by alkalis.

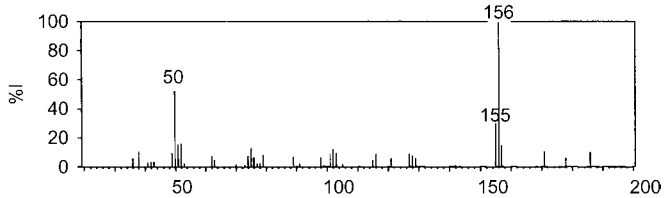
Note Unless otherwise stated, the analytical information given below refers to paraquat dichloride. For information on the monoene and diene reduction products for GC-MS analysis, see under Quaternary Ammonium Herbicides in Chapter 16.
Colour Test Sodium dithionite—blue.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TN—R_f 0.22; system TO—R_f 0.10; system TAB—R_f 0.00; system TAC—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.00 (acidified iodoplatinate solution—positive).
Gas Chromatography System GK—monoene reduction product RRT 0.36, diene reduction product RRT 0.58 (relative to caffeine).
High Performance Liquid Chromatography System HX—RI 00.
Ultraviolet Spectrum Aqueous acid—257 nm (A^1_1 = 693b).



Infrared Spectrum Principal peaks at wavenumbers 1174, 1638, 1064, 1229, 1284, 850 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 96, 42, 70, 72, 150, 122, 194, 43 (monoene reduction product); 96, 42, 148, 192, 94, 122, 134, 44 (diene reduction product).



Quantification

Blood **GC** Paraquat derivatives [Chen *et al.* 2008]. Thermionic specific detector. Limit of detection, 0.002 µg/L [Huang *et al.* 2008].

HPLC Column: Puresil C_{18}. Mobile phase: 10 mmol/L sodium octane sulfonate : 10 mmol/L triethylamine : 0.5 mol/L potassium bromide (pH 3.0), flow rate 1.0 ML/min. UV detection (λ = 290 nm). Limit of detection, 1 ng for paraquat and diquat [Fuke *et al.* 2002]. Column: Alluspher 100 RP-Select B (125 × 4.0 mm i.d., 5 µm). Mobile phase: 0.0125 mol/.L sodium hydroxide in water : 0.0125 mol/L sodium hydroxide in acetonitrile (90 : 10 to 10 : 90 in 15 min), flow rate 1.0 mL/min. DAD (λ = 220 to 300 nm). Limit of detection, 63 µg/L [Arys *et al.* 2000]. Column: µBondapak C_{18} (250 × 4.9 mm i.d., 10 µm). Mobile phase: 25% aqueous methanol : 10 mmol/L octane sulfonic acid : 13.4 mL/L *o*-orthophosphoric acid (pH 3.0), flow rate 1.3 mL/min. UV detection (λ = 254 nm). Limit of detection, 50 µg/L [Querée *et al.* 1985].

LC-MS Column: Atlantis dC_{18}. Mobile phase: 15 mmol/L heptafluorobutyric acid-20 mmol/L ammonium formate buffer (pH 3.3) : methanol (95 : 5 to 10 : 90 in 18 min), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 36.9 µg/L, limit of detection, 11.1 µg/L [Ariffin, Anderson 2006]. Column: Suplex pkb-100 (250 × 2.1 mm i.d., 5 µm). Mobile phase: heptafluorobutyric acid (pH 3.0) : acetonitrile 100 : 0 to 70 : 30 in 20 min). TIS, SRM acquisition mode. Limit of detection, 10 µg/L for paraquat and 5 µg/L for diquat [Lee *et al.* 2004].

Plasma **Colorimetry** Limit of detection, 50 µg/L [Jarvie, Stewart 1979].

TLC Stationary phase: Chromarod SII. Mobile phase: methanol : 2 mol/L hydrochloric acid (2 : 3). FID. Limit of detection, 50 ng [Ikebuchi *et al.* 1988].

GC Column: 3% Poly-A 135 on 80/100 mesh 01-1895 Supelcoport (1.8 m × 3.1 mm [6 ft × 0.125 in] i.d.). Carrier gas: O_2. Temperature: 190°. AFID. Retention time: 4 min 30 s. Limit of detection, 30 µg/L [van Dijk *et al.* 1977].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 80° for 1 min to 200° at 10°/min to 270° at 20°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.05 mg/L for paraquat and diquat [de Almeida, Yonamine 2007].

HPLC Column: LiChrospher RP-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L sodium octane sulfonate in 0.05 mol/L orthophosphoric acid (40 : 60, pH 2.8). UV detection (λ = 258 nm). Limit of detection, 5 µg/L [Brunetto *et al.* 2003]. Column: ODS C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : diethylamine-orthophosphoric acid buffer (pH 3, 10 : 90), flow rate 0.8 mL/min. UV detection (λ = 258 nm) Limit of quantification, 0.4 mg/L, limit of detection, 0.1 mg/L [Paixão *et al.* 2002]. Column: Zorbax RX-Silica (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 g/L sodium chloride : acetonitrile (96 : 4, pH 2.2), flow rate 1 mL/min. UV detection (λ = 260 nm). Limit of quantification, 0.1 mg/L [Taylor *et al.* 2001].

RIA Limit of detection, 5 µg/L [Levitt 1979].

Serum **HPLC** Column: Capcell Pak C_{18} UG120 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 200 mmol/L phosphoric acid-0.1 mol/L diethylamine-12 mmol/L sodium 1-heptane sulfonate (1 : 4), flow rate 0.5 mL/min. UV detection (λ = 391 nm). Limit of quantification, 50 µg/L for paraquat and 100 µg/L for diquat [Hara *et al.* 2007]. Column: Inertsil ODS-3 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% potassium bromide : 5% methanol containing 1 mL/L triethylamine (pH 3.0, 92 : 8), flow rate 1.0 mL/min. UV detection (λ = 256 nm). Limit of detection, 0.05 mg/L for paraquat and diquat [Ito *et al.* 2005]. See Plasma [Paixão *et al.* 2002]. Column: Inertsil ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L phosphate buffer (pH 2.0) containing 10 mmol/L sodium octane sulfonate (17 : 83), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Limit of quantification, 0.1 mg/L [Lee *et al.* 1998]. Column: Microspher C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: 13.5 mL orthophosphoric acid : 10.3 mL diethylamine : 2.02 g sodium heptane sulfonate in 1000 mL water : methanol (80 : 20), flow rate 0.5 mL/min. UV detection (λ = 258 nm). Limit of quantification, 25 µg/L [Croes *et al.* 1993]. Mobile phase: 0.2 mol/L sodium dihydrogen phosphate (pH 3.0)-acetonitrile : 0.2 mol/L sodium dihydrogen phosphate (pH 3.0, 20 : 80), flow rate 1.0 mL/min. UV detection (λ = 290 nm). Limit of detection, 5 mg/L [Nakagiri *et al.* 1989].

LC-MS Column: Luna Polar RP (150 × 2.1 mm i.d.). Mobile phase: heptafluorobutyric acid : acetonitrile. TIS, positive ion mode, MRM acquisition mode. Retention time: 5.78 min. Limit of quantification, 5 µg/L for paraquat and 1 µg/L for diquat [Wang *et al.* 2008].

Note For a spectroscopy method, see Fuke *et al.* [1992]; for a radioimmunoassay see Fatori, Hunter [1980].

Urine **TLC** See Plasma [Ikebuchi *et al.* 1988].

GC See Blood. Limit of detection, 0.004 µg/L [Huang *et al.* 2008].

GC-MS See Plasma [de Almeida, Yonamine 2007].

HPLC See Plasma [Taylor *et al.* 2001]. See Blood. Limit of detection, 32 µg/L [Arys *et al.* 2000]. See Serum [Nakagiri *et al.* 1989]. UV detection. Limit of detection, <1 mg/L [Gill *et al.* 1983].

LC-MS Column: HILIC silica (150 × 2.1 mm i.d., 5.0 µm). Mobile phase: 250 mmol/L ammonium formate (pH 3.7) : acetonitrile (60 : 40), flow rate 400 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.63 µg/L for paraquat, 0.13 µg/L for diquat [Whitehead *et al.* 2009]. See Blood [Lee *et al.* 2004].

ELISA Comparison with LC-MS [Koivunen *et al.* 2005].

Brain **HPLC** See Blood [Fuke *et al.* 2002].

Liver **HPLC** See Blood [Fuke *et al.* 2002]. UV detection. Limit of detection, 100 µg/L [Querée *et al.* 1985].

Tissues **HPLC** Column: ODS. Mobile phase: 0.5% potassium bromide : 5% methanol containing 1 mL/L triethylamine. UV detection (λ = 256 nm for paraquat and 310 nm for diquat). Limit of detection, 0.05 µg/g [Ito *et al.* 1993].

Disposition in the Body Less than 10% of paraquat is absorbed after ingestion. It is slowly excreted unchanged in the urine and faeces; detectable concentrations have been found in the urine for up to 26 days after acute ingestion.

Toxicity Several hundred fatalities caused by the accidental or suicidal ingestion of paraquat have occurred. The estimated minimum lethal dose is ~1 g. The maximum permissible atmospheric concentration is 0.1 mg/m^3 and the maximum acceptable daily intake is 2 μg/kg as the dichloride. Large amounts of paraquat produce death in a few hours or days from pulmonary oedema, haemorrhage and the toxic effects on the kidney, liver and heart. Smaller doses may result in death after a delay through progressive respiratory failure, and death may still ensue even after concentrations in blood or urine are almost undetectable. Tissue concentrations in fatal cases, therefore, vary considerably according to the length of survival after ingestion. Blood concentrations >~2 mg/L at 4 h and 0.1 mg/L at 24 h are likely to be lethal in most subjects but individual response is variable and some subjects with much higher concentrations have recovered.

In a review of 26 fatalities attributed to paraquat poisoning, the following postmortem tissue concentrations (mean, N) were reported:

	Death within 24 h	*Death within 1 to 7 days*	*Death after 8 days or more*
Blood (mg/L)	1.4–52 (14, 5)	0.2–4.4 (1.3, 8)	Not detected
Kidney (μg/g)	22.5–279 (79, 6)	1.4–74 (16, 10)	0.6–2.8 (1.5, 5)
Liver (μg/g)	8.8–141 (41, 6)	0.3–57.6 (8, 10)	0.2, 1.4 (2)
Lung (μg/g)	12.8–186 (78, 3)	1.6–21.4 (7, 6)	–
Urine (mg/L)	1210 (1)	4–15.8 (9, 3)	trace, 2.2 (2)

[Carson, Carson 1976].

In a review of 106 paraquat poisoning cases, of the 55 with known survival times, paraquat plasma levels in those who died within 1 day of ingestion were 2.3–636.6 mg/L (average, 127.6) while those who died 1–4 days after ingestion had plasma paraquat levels of 0.9–25.1 mg/L (average, 7). Those who died within 24 h of paraquat ingestion were likely to have plasma levels of more than 30 mg/L [Lee *et al.* 1999].

In a review of 375 patients who had ingested paraquat, 110 survived. The upper limit of plasma paraquat in survivors was 2.64 mg/L at 3 h and all patients with levels above 3.44 mg/L died. Minimum paraquat levels at deaths were low (0.12 mg/L at 5 h, 0.02 mg/L at 12 h and 0.01 mg/L at 24 h) [Gil *et al.* 2008].

A 71-year-old man who was admitted to hospital 2 days after ingesting Korean weed killer (and had ingested some more the evening before admission), died 6 days later after experiencing increasing respiratory distress. Serum paraquat measured on day 1 after admission was 0.7 mg/L; the quantity of paraquat ingested was ~90 mL of a 25% solution [Erickson *et al.* 1997].

A mother administered a paraquat solution to her 3 children (8 years, 6 years and 15 months) before taking an unknown amount of it herself. At 30 h after the ingestions, the serum paraquat concentrations of the children were 0.06, <0.006 and 0.025 mg/L, respectively. The children recovered following haemoperfusion and, in 2, plasmaphoresis and erythroporesis, but the mother died [Kalabalikis *et al.* 2001].

A crop-dusting accident resulted in the death of a worker who absorbed paraquat through the skin after sustaining 37% total body surface area burns. After 9.5 h of cutaneous exposure, a paraquat level of 169 mg/L was obtained at 20 h in blood [Gear *et al.* 2001].

In a fatality caused by massive ingestion of paraquat, the following postmortem concentrations were reported: blood 5.05 mg/L, urine 6.00 mg/L, stomach contents, 17.2 g/L, liver 4.86 μg/g, kidney 80.6 μg/g [Arys *et al.* 2000].

A 34-year-old woman had a blood paraquat level of 1.98 mg/L ~6 h after ingesting ~20 mL of 24% paraquat (Gramoxone). She developed multiple organ failure within 24 h and died on day 4 [Sittipunt 2005].

A 15-year-old girl survived after ingesting a potentially lethal dose of paraquat (~50 mL of a 20% preparation of paraquat hydrochloride) [Dinis-Oliveira *et al.* 2006].

An 81-year-old man died after minimal skin burn from paraquat exposure [Soloukides *et al.* 2007].

A 17-year-old woman at 36 weeks of gestation survived after attempting suicide by ingesting half a glass of 27.6% paraquat (Gramoxone). A male infant was delivered by emergency caesarean section and survived, but at age 10 months still had evidence of chronic lung disease [Chomchai, Tiawilai 2007].

For a case of fatal IV paraquat poisoning and a discussion of findings from other cases of IV poisoning, see Chen *et al.* [2009]; for a report of survival after a massive dose of paraquat, see Lalloo, Ambaram [2008]. For a review of postmortem analyses in cases of paraquat fatalities and a discussion of the limitations of therapeutic protocols for the treatment of paraquat poisonings, see Dinis-Oliveira *et al.* [2009]. For a comparison of different methods for predicting the outcome after paraquat poisoning, see Senarathna *et al.* [2009]. For a discussion of the clinical outcome of paraquat poisoning, see Yoon [2009]. For reviews of the toxicology of paraquat, see Bismuth *et al.* [1990], Haley [1979] and Vale *et al.* [1987]. For a report of the application and outcome of blood level analysis and a nomogram for the evaluation of paraquat toxicity, see Fukumoto *et al.* [2006].

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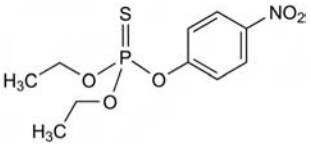
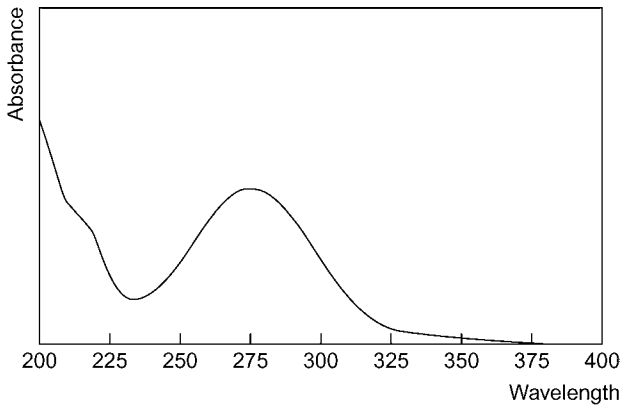
Parathion

Insecticide
$C_{10}H_{14}NO_5PS = 291.3$
CAS—56-38-2

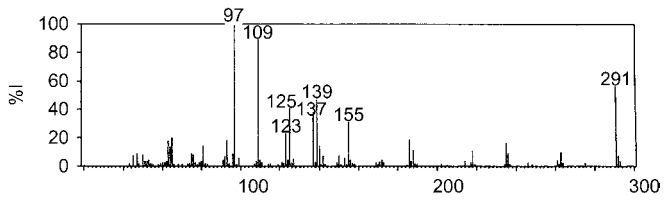
IUPAC Name Phosphorothioic acid *O*,*O*-Diethyl *O*-(4-nitrophenyl) ester
Proprietary Names *Alkron*; *Aphamite*; *Folidol*; *Fostex E*; *Fosferno 20*; *Niran*; *Nitrostigmine*; *Paraphos*; *Rhodiatox*; *Thiophos*.



Chemical Properties A pale yellow liquid which is decomposed by heat and darkens on exposure to light. Mp 6°. Bp 375°. Refractive index, at 25°, 1.5367. Practically insoluble in water; miscible with most organic solvents. It is rapidly hydrolysed in alkaline solution. Log *P* (octanol/water), 3.8. Extraction yield (chlorobutane), 1 (for both parathion ethyl and parathion methyl) [Demme *et al.* 2005].
Colour Tests Palladium chloride—brown; phosphorus test—yellow.
Thin-layer Chromatography System TW—parathion R_f 0.81, parathion methyl R_f 0.77; system TX—parathion ethyl R_f 0.41, parathion methyl R_f 0.30; system TY—parathion ethyl R_f 0.84, parathion methyl R_f 0.73.
Gas Chromatography System GA—parathion ethyl RI 1950; parathion methyl RI 1855; M (amino-) RI 1892; M (*p*-nitrophenol) RI 1525; paraoxon RI 1895; system GK—parathion ethyl RRT 1.04; parathion methylRRT 0.96 (both relative to caffeine).
High Performance Liquid Chromatography System HY—paraoxon RI 428; system HZ—parathion RT 35.5 min; paraoxonRT 6.5 min, paraoxon decomposition RT 3.7 min.
Ultraviolet Spectrum Ethanol—274 nm (A^1_1=343b).



Infrared Spectrum Principal peaks at wavenumbers 1020, 925, 1515, 1219, 1587, 970 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 97, 109, 291, 139, 125, 137, 155, 123.



Quantification
Blood **GC-MS** Limit of detection, 0.01–0.03 µg/g for parathion-ethyl, parathion-methyl and other pesticides [Musshoff *et al.* 2002]. GC with ECD confirmed by GC-MS. For method for quantification of parathion and other pesticides, see García-Repetto *et al.* [2001]. Limit of detection, 0.02–0.05 µg/g [Musshoff *et al.* 1999].
Plasma **Radioimmunoassay** Limit of detection, 100 µg/L [Ercegovich *et al.* 1981].
Urine **GC** ECD. Limit of detection, 20 µg/L for 4-nitrophenol [Bradway, Shafik 1973].

HPLC For method for quantification of paranitrophenyl sulfate, see Oneto *et al.* [1995].
Disposition in the Body Parathion is activated in the liver by metabolism to paraoxon. Parathion and paraoxon are further metabolised to diethylthiophosphoric acid (DETP), diethylphosphoric acid (DEP), and 4-nitrophenol which are the major urinary excretion products although DETP and DEP are unstable in stored urine. Urinary 4-nitrophenol concentrations may be indicative of the extent of exposure to parathion. 4-Nitrophenol is rapidly excreted in the urine and is not detectable 48 h after exposure by inhalation or ingestion, but excretion is more prolonged after exposure of intact skin due to the much slower absorption of parathion by this route. Aminoparathion has been detected in postmortem blood and tissues.

Urinary 4-nitrophenol concentrations ranged from 0.4–13.2 mg/L in 23 occupationally-exposed asymptomatic workers and correlated well with serum-parathion concentrations which were in the range 0.003–0.20 mg/L [Roan *et al.* 1969].

Toxicity The estimated minimum lethal dose by inhalation or ingestion is 20 mg and the maximum permissible atmospheric concentration is 0.1 mg/m^3. Urinary concentrations of about 2 mg/L or more of 4-nitrophenol may be associated with severe toxicity. Numerous fatalities have occurred due to contamination of food by parathion and also from suicidal ingestion.

The following postmortem tissue concentrations were reported in 19 fatalities due to parathion poisoning (determined by a bioassay based on cholinesterase inhibition): blood 0.5–34 mg/L (mean, 9.0, 11 cases); brain 0.9–12.5 µg/g (mean, 4.9, 4 cases); kidney 0.2–11.9 µg/g (mean, 3.3, 9 cases); liver 0.08–120 µg/g (mean, 11, 13 cases); urine 0.4–78 mg/L (mean, 10, 10 cases) [Heyndrickx, De Clerc 1977].

In 2 fatalities due to parathion, the following postmortem tissue concentrations, mg/L or µg/g, were reported:

	Parathion	*Aminoparathion*
Blood	0.01, 0.15	0.27, 6.7
Bile	—, 0.63,	—, 0.31
Liver	ND, ND	0.47, 32
Urine	ND, —	0.02, —

(ND=not detected)
[Chan *et al.* 1983].

Half-life Derived from urinary excretion data, about 8 h.

Bradway DE, Shafik TM (1973). Parathion exposure studies. A gas chromatographic method for the determination of low levels of p-nitrophenol in human and animal urine. *Bull Environ Contam Toxicol* 9: 134–139.
Chan LT *et al.* (1983). Detection and analysis of aminoparathion in human postmortem specimens. *J Forensic Sci* 28: 122–127.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Ercegovich CD *et al.* (1981). Development of a radioimmunoassay for parathion. *J Agric Food Chem* 29: 559–563.
García-Repetto R *et al.* (2001). New method for determination of ten pesticides in human blood. *J AOAC Int* 84: 342–349.
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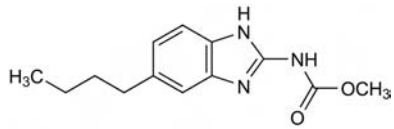
Roan CC *et al.* (1969). *Bull Environ Contam Toxicol* 4: 362–369.

Parbendazole

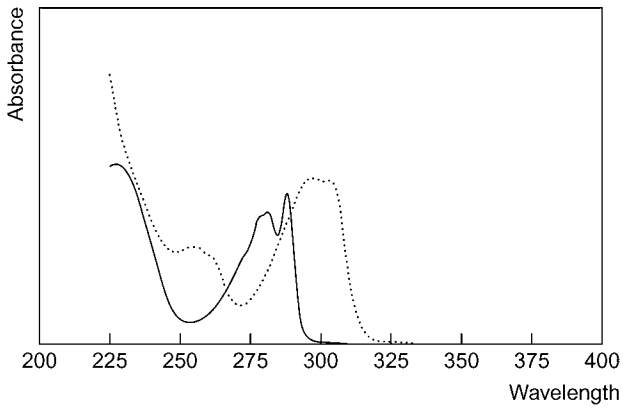
Anthelmintic (Veterinary)
$C_{13}H_{17}N_3O_2 = 247.3$
CAS—14255-87-9

IUPAC Name Methyl *N*-(6-butyl-1*H*-benzimidazol-2-yl)carbamate
Synonym (5-Butyl-1*H*-benzimidazol-2-yl)carbamic acid methyl ester
Proprietary Names *Helmatac*; *Verminum*; *WormGuard*.



Chemical Properties A white crystalline powder. Mp 225° to 227°, with decomposition. Practically insoluble in water; soluble 1 in 900 of ethanol and 1

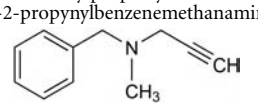
in 300 of chloroform; very slightly soluble in ether; soluble in dilute mineral acids. Log *P* (octanol/water), 3.6.
Colour Test Dissolve 5 mg in 5 mL of 0.1 mol/L hydrochloric acid and add 3 mg of *p*-phenylenediamine dihydrochloride; shake to dissolve and add 0.1 g of zinc powder. After mixing, allow to stand for 2 min and add 10 mL of ferric ammonium sulfate solution—a blue or violet-blue colour develops.
Thin-layer Chromatography System TA—R_f 0.70 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HAA—retention time 18.2 min.
Ultraviolet Spectrum Aqueous acid—281, 288 nm (A_1^1=760a); aqueous alkali—254, 297 (A_1^1=816b), 303 nm.



Infrared Spectrum Principal peaks at wavenumbers 1626, 1600, 1274, 1093, 1100, 1193 cm^{-1} (KBr disk).

Pargyline

Antihypertensive
$C_{11}H_{13}N$ = 159.2
CAS—555-57-7
IUPAC Name *N*-benzyl-*N*-methylprop-2-yn-1-amine
Synonym *N*-Methyl-*N*-2-propynylbenzenemethanamine

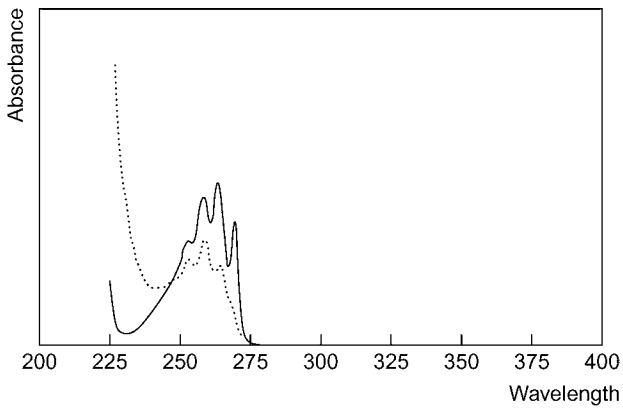


Chemical Properties pK_a 6.9. Log *P* (octanol/water), 2.0.

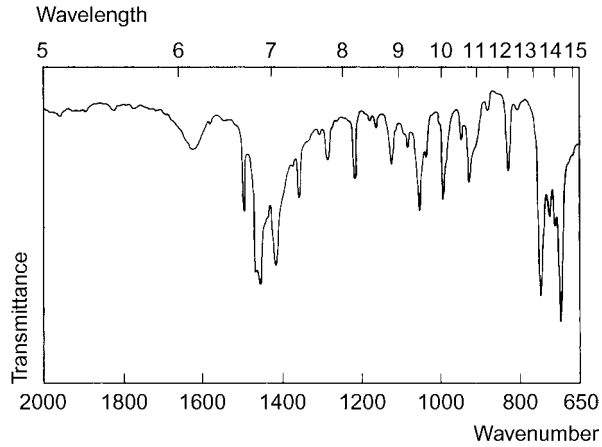
Pargyline Hydrochloride

$C_{11}H_{13}N,HCl$ = 195.7
CAS—306-07-0
Proprietary Name *Eutonyl*
Chemical Properties A white crystalline powder. Mp 154° to 155°; sublimation occurs when kept at raised temperatures. Soluble 1 in 0.6 of water, 1 in 5 of ethanol and 1 in about 7 of chloroform. Aqueous solutions are unstable.

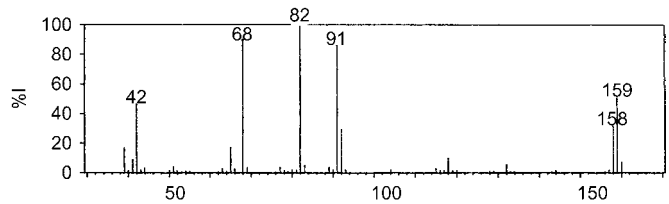
Thin-layer Chromatography System TA—R_f 0.70; system TE—R_f 0.60; system TAE—R_f 0.77; system TAJ—R_f 0.71; system TAK—R_f 0.20; system TAL—R_f 0.93 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1214; system GB—RI 1257; system GC—RI 1440.
High Performance Liquid Chromatography System HA—*k* 0.2; system HY—RI 203.
Ultraviolet Spectrum Aqueous acid—251, 256, 261 (A_1^1=16a), 268 nm; aqueous alkali—252, 257, 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 697, 746, 714, 724, 1052, 1515 cm^{-1} (KBr disk).



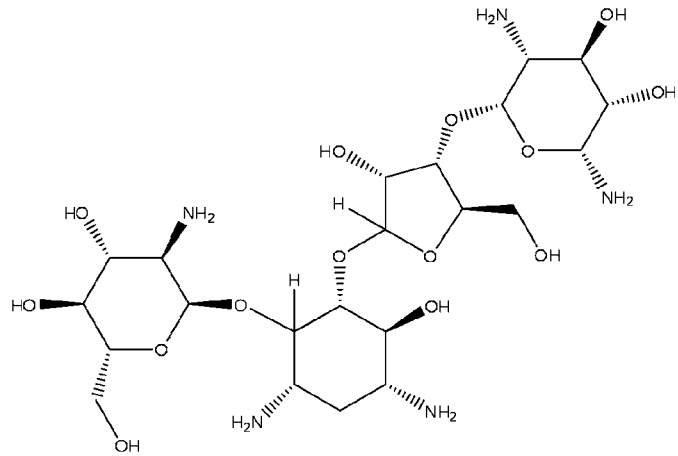
Mass Spectrum Principal ions at *m/z* 82, 68, 91, 159, 42, 158, 92, 65.



Dose Usually 25 to 50 mg of pargyline hydrochloride daily; maximum of 200 mg daily.

Paromomycin

Antibiotic
$C_{23}H_{45}N_5O_{14}$ = 615.6
CAS—7542-37-2
IUPAC Name (2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-6-[(2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(3*R*,4*S*,5*R*)-4-[(2*R*,3*R*,4*R*,5*S*,6*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxy-2-(hydroxymethyl)oxane-3,4-diol
Synonyms Antibiotic 1600; C 1448; FI 5853; D-glucosamine-deoxystreptamine-D-ribosediaminohexose.



Chemical Properties An antibiotic substance derived from cultures of certain *Streptomyces* species, one of which is *Streptomyces rimosus* forma *paromomycinus*. Amorphous white powder. Soluble in water; sparingly soluble in ethanol; moderately soluble in methanol.

Paromomycin Sulfate

Proprietary Names *Crestomicina*; *Gabbromycin(a)*; *Gabbroral*; *Humagel*; *Humatin*; *Humycin*; *Pargonyl*.
Chemical Properties Creamy-white to light-yellow hygroscopic powder. Soluble 1 in 1 of water; insoluble in ethanol, ether, and chloroform; soluble in dilute acids and solutions of alkali hydroxides.

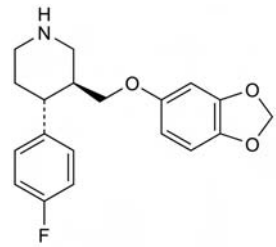
Colour Test Dissolve 10 mg in 5 mL of water, add 0.1 mL of pyridine and 2 mL of a 0.1% solution of ninhydrin, and heat at 70°. After 10 min the solution is a pale straw colour. Heat for a further 5 min at 100°— a deep purple colour develops.
Thin-layer Chromatography System T1—R_f 0.00 (location reagent potassium permanganate spray, positive reaction).

Schmitt and Mathis [1970] describe a system for the separation of antibiotics, including paromomycin sulfate.
Disposition in the Body Orally administered paromomycin is poorly absorbed. After the oral administration of 160 mg/kg of paromomycin to monkeys, about 3% of the dose was excreted in the urine in 24 h [Coffey *et al.* 1959].
Toxicity LD_{50} (oral): in mice >2275 mg/kg and in rats >1625 mg/kg.
Dose Up to 100,000 units/kg daily.

Coffey GL *et al.* (1959). Biological studies of paromomycin. *Antibiot Chemother* 9: 730–738.
Schmitt JP, Mathis C (1970). [Separation of antibiotics by thin-layer chromatography]. *Ann Pharm Fr* 28: 205–210.

Paroxetine

Antidepressant, Selective Serotonin Reuptake Inhibitor (SSRI)
$C_{19}H_{20}FNO_3$ = 329.4
CAS—61869-08-7
IUPAC Name (3S,4*R*)-3-(1,3-Benzodioxol-5-yloxymethyl)-4-(4-fluorophenyl) piperidine
Synonyms (3*S-trans*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine; BRL-29060; FG-7051.

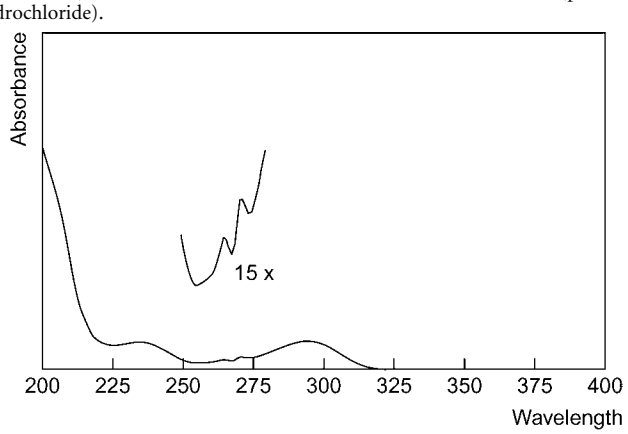


Chemical Properties pK_a 9.9. Log *P* (octanol/water), 3.95. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

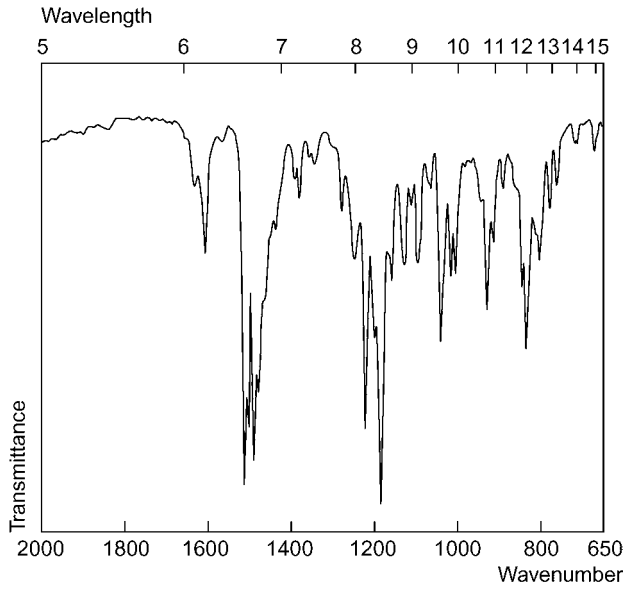
Paroxetine Hydrochloride Hemihydrate

$C_{19}H_{20}FNO_3$,HCl,$^1/_2H_2O$ = 369.85
CAS—110429-35-1
IUPAC Name (3*S*,4*R*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)-piperidine hydrochloride hydrate
Synonyms BRL-29060A; (−)-*trans*-4-(4-fluorophenyl)-3-(3,4-methylenedioxyphenoxymethyl)piperidinehydrochloride hemihydrates; piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)-, hydrochloride, hydrate (2 : 1).
Proprietary Names *Aropax*; *Casbol*; *Deroxat*; *Frosinor*; *Motivan*; *Paxil*; *PaxPar*; *Sereupin*; *Seroxat*; *Tagonis*.
Chemical Properties Crystals. Mp 129° to 131°.

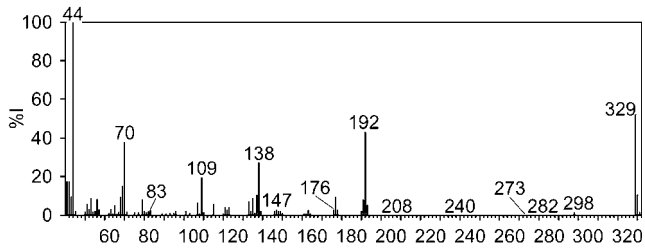
Thin-layer Chromatography System TB—R_f 0.04; system TE—R_f 0.40; system TAE—R_f 0.08.
Gas Chromatography System GB—RI 2691, M (desmethylenyl-3-methyl-) RI 2734, M (paroxetine) RI 2687; system GM—RRT 2.047 (relative to iprindole).
High Performance Liquid Chromatography System HX—RI 426; system HY—RI 337; system HZ—RT 5.6 min; system HAA—RT 15.3 min; system HAX—RT 11.1 min; system HAY—RT 5.8 min.
Ultraviolet Spectrum Aqueous acid (0.1 mol/L ammonium formate, pH 3)—295 nm; (0.025 mol/L sulfuric acid)—292.5, 234.5 nm; Aqueous acid (0.2 mol/L sulfuric acid)—233, 264, 270, 293 nm; basic—233, 264, 270, 292 nm (paroxetine hydrochloride).



Infrared Spectrum Principal peaks at wavenumbers 1185, 1512, 1490 cm^{-1} (paroxetine hydrochloride; KBr pellet).



Mass Spectrum Principal ions at *m/z* 44, 329, 192, 70, 138, 109, 176, 147.



Quantification

Blood **GC** Column: HP (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min, to 300° at 10°/min for 3 min. NPD detection. Limit of quantification, 376 µg/L, limit of detection, 113 µg/L [Martinez *et al.* 2004].

GC-MS Column: DB-5 MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 105° for 1 min, to 300° at 70°/min for 1.22 min. NICI, SRM acquisition mode. Limit of quantification, 20 µg/L, limit of detection, 20 ng/L [Deglon *et al.* 2010]. Column: HP-5 capillary (10 m × 0.25 mm i.d.). Temperature programme: 140° for 1 min to 300° at 20°/min for 3 min. EI ionisation, SIM acquisition mode [Singer, Jones 1997].

HPLC Column: LiChroCART (125 × 4 mm i.d., 5 µm). Mobile phase: water : 250 mmol/L phosphate buffer (pH 2.3) : methanol. DAD (λ= 220–350 nm) [Wille *et al.* 2009]. Fluorescence detection. Limit of quantification, 0.025 µmol/L [Kristoffersen *et al.* 1999].

LC-MS Column: C_{18}. Mobile phase: acetonitrile : 0.1% trifluoroacetic acid (50 : 50), flow rate 0.4 mL/min. ESI, SIM acquisition mode [Pufal, Sykutera 2008]. Column: XTerra RP-18. Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2). ESI, MRM acquisition mode. Limit of quantification, 5 µg/L [Castaing *et al.* 2007].

Plasma **GC** ECD [Lai *et al.* 2000].

GC-MS Column: DB-5 MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 160° for 1 min to 310° at 40°/min for 1 min. NICI. Limit of quantification, 0.469 µg/L [Leis *et al.* 2002]. Limit of quantification, 2 µg/L [Eap *et al.* 1998].

HPLC Column: LichroCART RP-C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: 0.05 mol/L phosphate buffer : methanol (pH 4.5, 55 : 45), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of detection, 20 µg/L [Chaves *et al.* 2009]. Column: Hypersil 120A (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium acetate buffer (pH 3.5) : tetrahydrofuran : acetonitrile (55 : 35 : 10), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex}= 470 nm, λ_{em}= 530 nm). Limit of detection not reported [Khalil 2010]. Column: Nucleosil CN Mobile phase: acetonitrile : 10 mmol/L sodium acetate buffer (pH 3.5) : methanol (47 : 47 : 6), flow rate 1 mL/min. Fluorescence detection (λ_{ex}= 470 nm, λ_{em}= 530 nm). Limit of quantification, 4.14 µg/L, limit of detection, 1.37 µg/L [Darwish *et al.* 2009]. Column: LiChrospher 60 RP-select B (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.25

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mol/L sodium acetate buffer (pH 4.5), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of quantification, 10 μg/L [Malfará *et al.* 2007]. Column: Phenomenex Luna C_{18} (250 × 3.0 mm i.d., 5 μm). Mobile phase: 25 mmol/L aqueous phosphate buffer (pH 2.5) : acetonitrile (66.7 : 33.3), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 294 nm, λ_{em}= 330 nm). Limit of detection, 1.2 μg/L [Mandrioli *et al.* 2007]. See also Brett *et al.* [1987], Chaves *et al.* [2007], Duverneuil *et al.* [2003], Erk, Biryol [2003], Foglia *et al.* [1997], Knoeller *et al.* [1995], Lopez-Calull, Dominguez [1999], Lucca *et al.* [2000], Naidong, Eerkes [2004], Onal, Oztunc [2006], Oztunc *et al.* [2002], Schatz, Saria [2000], Shin *et al.* [1998], Titier *et al.* [2003], Wille *et al.* [2005] and Zainaghi *et al.* [2003].

LC-MS Column: Aquity UPLC Hypersil Gold C_{18} (100 × 2.1 mm i.d., 1.9 μm). Mobile phase: acetonitrile : 0.1% glacial acetic acid (50 : 50), flow rate 0.350 mL/min. Limit of quantification, 26.8 μg/L [Bhatt, Shah 2010]. Column: BDS Hypersil C_{18}. Mobile phase: 0.2% formic acid in methanol : acetonitrile (65 : 35). ESI. Limit of detection not reported [Shah *et al.* 2010]. Column: Sunfire C_{18} (20 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 2 mmol/L ammonium formate (pH 3), flow rate 0.4 mL/min. ESI, MRM acquisition mode. Limit of quantification, 2 μg/L [De Castro *et al.* 2008]. Column: C_{18}. Mobile phase: acetonitrile : 5 mmol/L ammonium formate (4 : 3). ESI, MRM acquisition mode. Limit of quantification, 0.05 μg/L [Jhee *et al.* 2007]. Column: Intersil-C_8 (150 × 2 mm i.d., 5 μm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.10 mL/min. Limit of quantification, 0.10 mg/L, limit of detection, 0.05 mg/L [Shinozuka *et al.* 2006]. See also Juan *et al.* [2005], Massaroti *et al.* [2005], Segura *et al.* [2003] and Zhu, Neirinck [2002].

Serum **GC-MS** Limit of detection, 0.1 μg/L [Misri *et al.* 2000].

HPLC Column: Nucleosil 100-Protect 1 (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 25 mmol/L potassium dihydrogen phosphate buffer (pH 7.0, 40 : 60), flow rate 1 mL/min. UV detection (λ= 230 nm). Limit of detection not reported [Frahnert *et al.* 2003]. Column: Beckman RP-C_{18}. UV detection (λ= 200.4 nm). Limit of quantification, 20 μg/L [Tournel *et al.* 2001]. UV detection (λ= 225 nm). Limit of detection, 1 μg/L [Hendrick *et al.* 2001]. Column: (100 × 2 mm i.d., 3 μm). Mobile phase: 0.02 mol/L potassium phosphate monobasic : *N,N*-demethyloctylamine : acetonitrile, flow rate 0.6 mL/min. UV detection. Limit of detection, 2 μg/L [Stowe *et al.* 2000]. Column: C_8 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 10 mmol/L phosphate buffer : acetonitrile (2 : 1). Fluorescence detection (λ_{ex}= 295 nm, λ_{em}= 365 nm). Limit of detection, 5 μg/L [Gupta 1994].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: 5 mmol/L acetate buffer (pH 3.9), flow rate 1.0 mL/min. ESI, MRM acquisition mode. Limit of quantification, 1.07 μg/L [Kirchherr, Kühn-Velten 2006].

Urine **GC-MS** HP (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation. Limit of detection, at least 100 μg/L [Maurer, Bickeboeller-Friedrich 2000].

LC-MS Column: Synergi 4u MAX-RP 80A (150 × 2 mm i.d., 4 μm). Mobile phase: acetonitrile : 0.02% formic acid (66 : 34), flow rate 0.25 mL/min. Limit of quantification, 0.7 μg/L, limit of detection, 2.20 μg/L [Segura *et al.* 2003].

Milk **GC-MS** See Serum [Misri *et al.* 2000].

HPLC UV detection [Hostetter *et al.* 2004]. See Serum [Stowe *et al.* 2000].

Oral Fluid **GC-MS** Column: Ultra 1 (16.5 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 2 min, to 160° at 30°/min to 170° at 5°/min to 200° at 20°/min 220° at 10°/min to 300° at 30°/min. EI ionisation, SIM acquisition mode [Pujadas *et al.* 2007].

HPLC Column: C_{18}. Mobile phase: 0.05 mol/L sodium phosphate buffer (pH 5.0) : acetonitrile. UV detection (λ= 205 nm). Limit of quantification, 4 μg/L, limit of detection, 1 μg/L [Tsuruta *et al.* 2007].

LC-MS See Plasma [De Castro *et al.* 2008]. Column: Luna C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water: 0.1% formic acid, flow rate 0.5 mL/min. ESI. Limit of detection not reported [Doherty *et al.* 2007].

Stomach Contents **GC-MS** See Blood [Singer, Jones 1997].

Brain **HPLC** See Blood [Wille *et al.* 2009].

Hair **HPLC** See Blood [Wille *et al.* 2009].

LC-MS See Oral Fluid [Doherty *et al.* 2007]. Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: methanol : water : formic acid : trifluoroacetic acid, flow rate 0.5 mL/min. ESI [Smyth *et al.* 2006].

Liver **GC-MS** [Maurer *et al.* 2000]. See Blood [Singer, Jones 1997].

Disposition in the Body Paroxetine is readily absorbed after oral administration and undergoes extensive first-pass hepatic metabolism: *O*-demethylenation in a reaction regulated by CYP2D6 gives rise to a catechol-type metabolite that is further *O*-methylated and conjugated with glucuronic acid and sulfate. Peak plasma concentrations can be observed ~5 h after administration. Paroxetine is distributed throughout the body, including the CNS, with only 1% remaining in the plasma. It is excreted mainly as metabolites in urine (~64%) and faeces (~36%). Less than 2% of a dose is found in urine as the parent drug. It is distributed in breast milk and widely distributed throughout the body.

Therapeutic Concentration The serum therapeutic concentration range is 10–75 μg/L; peak, 15–150 μg/L.

Twelve patients with biopsy-proven alcoholic cirrhosis, aged 28–69 years, and 6 healthy individuals with no signs of liver disease, aged 26–60 years, were administered 30 mg paroxetine hydrochloride daily for 14 days. The maximum steady-state concentration observed for the patients was 4.49 μg/L, measured on days 13 and 14, and the minimum was 3.43 μg/L. For the healthy subjects, the maximum was 2.48 μg/L and the minimum 1.53 μg/L [Dalhoff *et al.* 1991].

Toxicity The toxic serum concentration ranges from 350–400 μg/L.

A young man was found at home shivering and incoherent, followed later by numerous seizures. Four hours after he was found, he suffered from full cardiac arrest and it was impossible for the paramedics to resuscitate him because his jaw was unusually stiff and the paramedics could not open his mouth to intubate him. Toxicological analysis showed a paroxetine subclavian blood concentration of 1.58 mg/L, 15.3 mg/kg in liver and 0.0101 mg/g in the gastric contents. His death was actually attributed to a combined toxicity of moclobemide and paroxetine. Moclobemide was found in his blood at a concentration of 18.5 mg/L, in liver at 28.5 mg/kg and in the gastric contents at 1.116 mg/g [Singer, Jones 1997].

A young nurse was found in her flat with a femoral blood paroxetine concentration of 0.176 mg/L. Doxepine and desmethyldoxepine were also detected [Musshoff *et al.* 1999].

Half-life 12–40 h.

Volume of Distribution 3–28 L/kg.

Protein Binding 95%.

Dose The usual dose is 10 or 20 mg daily, which may be increased to 50 or 60 mg in increments if necessary, with a maintenance dose of 40 mg daily. In the elderly and debilitated patients: maximum 40 mg daily. Those with severe renal or hepatic impairment: 20 mg.

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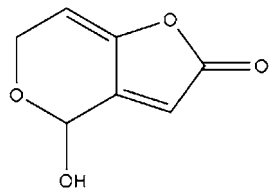
Patulin

Mycotoxin
$C_7H_6O_4 = 154.1$
CAS—149-29-1

IUPAC Name 4-Hydroxy-4,6-dihydrofuro[3,2-*c*]pyran-2-one
Synonyms Clavacin; clavatin; claviformin; expansine; 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one; mycoin C3; penicidin.



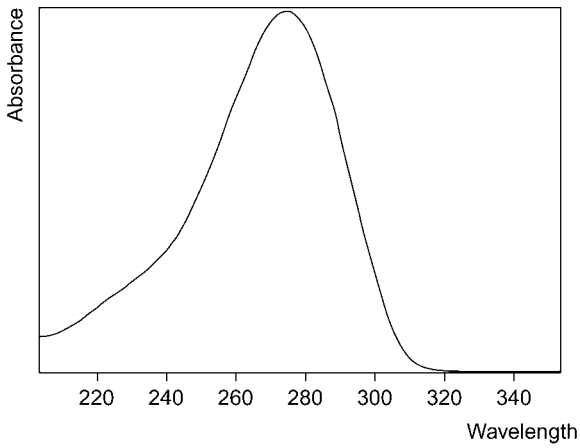
Chemical Properties Toxic metabolite produced by several species of *Penicillium, Aspergillus* and *Byssochlamys*. The most commonly occurring fungus is *Penicillium expansum*, the apple blue mould rot, from which apple juices are the most important source of patulin in the human diet [Sforza *et al.* 2006]. Mp 110°, also reported as 105°–108°. Soluble in water and the common organic solvents apart from petroleum ether; very soluble in ethyl or amyl acetate. Log *P* (octanol/water), −2.4 [Meylan, Howard 1995]; 0.7 [Rychlik *et al.* 2004]. Unstable in alkali with loss of biological activity [O'Neil *et al* 2006]. Stable to heat in acidic media such as fruit juices [Li *et al.* 2007]. Juice extracts are quickly and easily derivatised with bis(trimethylsilyl) trifluoracetamide under mild conditions, and the trimethylsilyl ethers of the analytes are stable for at least several hours [Rupp, Turnipseed 2000]. Patulin is unstable in a basic environment and as a dry film [Trucksess, Tang 1999]. The silylation products of patulin are stable for at least 15 days if stored refrigerated at 4° [Suzuki *et al.* 1975].
Note For a study of the stability of patulin at a range of pH values at 25° see Brackett, Marth. [1979].
Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: A) methanol: water; B) acetonitrile: water; C) tetrahydrofuran: water. Location reagent: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV detection (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as shown in the table below.

	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
DON	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyl-DON	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
NIV	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Patulin	**60 : 40**	**0.70**	**0.94**	**60 : 40**	**0.66**	**0.85**	**60 : 40**	**0.49**	**0.60**
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989]

P

Ultraviolet Spectrum



Quantification

Serum **GC-MS** Column: DB-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 60° for 1 min to 250° at 10°/min. MID. Limit of detection, 200 ng/L [Rychlik 2003].

Other

Note For a TLC method for the quantification of patulin in apple juice see Betina [1985] or Gimeno, Martins [1983] and in cheese see Siriwardana, Lafont [1979].

GC Culture Filtrates of *Penicillium Urticae* NRRL 2159A. Column: Gas-Chrom Q coated with QF-1 or NPGS 80/100 mesh (1.88 m × 2.16 mm i.d.). Carrier gas: N_2, 30 mL/min (QF-1) or 27 mL/min. Temperature programme: 120° for 2 min to 200° at 4°/min for 4 min (QF-1) or 120° for 4 min to 200° at 4°/min for 16 min. FID. Retention time: ≈22.8 min on QF-1, 32.9 min on NPGS. Limit of detection, 12 mg/L for QF-1 [Ehman, Gaucher 1977]. Column: 10% DC-200 plus 15% QF-1 (1 : 1) on Gas-Chrom-Q (2.0 m × 3 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 175°. ECD. Retention time: 7.8 min [Suzuki *et al.* 1975].

GC-MS Apple Juice. Column: HP MS5 cross-linked 5% phenylmethylsilicone (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 2 min to 200° at 10°/min to 300° for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: ≈10.1 min. Limit of quantification, 10 µg/L, limit of detection, 3 µg/L [Marks 2007]. SIM acquisition mode. Limit of detection, 1 µg/L [Llovera *et al.* 2005]. Column: DB-5 capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 60° for 1 min to 250° at 10°/min. EI ionisation at 70 eV. Limit of detection, 0.2 µg/L [Rychlik *et al.* 2004]. Column: HP Ultra 2 crosslinked 5% phenylmethylsilicone (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 2 min to 200° at 10°/min to 300° at 20°/min for 3 min. EI ionisation at 70 eV, MSD. Retention time ≈9.8 min. Limit of detection, 30 µg/L [Rupp, Turnipseed 2000]. Column: HP-5 MS cross-linked methylsilicone capillary (30 m × 0.25 mm i.d.). Carrier gas: He. Temperature programme: 80° for 1 min to 250° at 15°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 4 µg/L [Llovera *et al.* 1999]. Column: DB-5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 60° for 1 min to 250° at 10°/min. EI ionisation at 70 eV, MID. Retention time: 14.25 min. Limit of detection, 12 ng/L [Rychlik, Schieberle 1999]. Column: HP-5MS (30 m ×0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 50° for 2 min to 280° at 10°/min for 3 min. EI ionisation, SIM acquisition mode. Limit of quantification, 10 µg/L [Sheu, Shyu 1999].

HPLC Lactic Acid Bacteria. Column: SC-02-100 Prontosil C_{18} (100 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : water (1 : 99), flow rate 0.3 mL/min. UV detection (λ = 276 nm). Limit of detection, 25 µg/L [Fuchs *et al.* 2008]. Apple-Based Products. Column: Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: water : acetonitrile (95 : 5), flow rate 0.5 mL/min. UV detection (λ = 276 nm). Retention time: ≈15 min. Limit of quantification, 0.3 µg/L, limit of detection, 0.1 µg/L [Gonzalez-Osnaya *et al.* 2007]. Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: tetrahydrofuran : water (0.8 : 99.2), flow rate 0.2 mL/min. UV detection (λ = 276 nm). Retention time: 5.6 min. Limit of quantification, 23 µg/L, limit of detection, 8 µg/L [Li *et al.* 2007]. Column: Hypersil ODS C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : water-acetic acid (99 : 1, 1 : 99 for 5 min to 10 : 90 for 10 min to 100 : 0 for 5 min to 1 : 99 for 10 min), flow rate 0.7 mL/min. DAD (276 nm). Retention time: 6.5 min. Limit of detection, 5 ppb [Mhadhbi *et al.* 2007]. Apple Juice. Column: Shim-Pack CLC-ODS (250 × 4 mm i. d., 5 µm). Mobile phase: water : ethanol-water (3 : 7, 84 : 16 for 19 min to 50 : 50 in 5 min for 14 min to 84 : 16 in 5 min for 7 min), flow rate 0.5 mL/min. DAD (λ = 230–320 nm). Retention time: 17.4 min. Limit of quantification, 7 µg/L, limit of detection, 3 µg/L [Iha & Sabino 2006]. Dried Figs. Column: Phenomenex C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: tetrahydrofuran : 3% acetonitrile (4 : 96), flow rate 0.5 mL/min. DAD (λ = 272 nm). Limit of detection, 0.1 ppb [Karaca, Nas 2006]. Apple-Based Products. Column: Synergi Hydro RP80 (250 × 4.6 mm i.d., 4 µm). Mobile phase: water : acetonitrile : 60% perchloric acid (990 : 10 : 1), flow rate 1.0 mL/min. UV detection (λ = 276 nm). Limit of detection, 12 µg/L [Arranz *et al.* 2005]. Conventional and Organic Fruit. Column: Synergi Hydro-RP (250 × 4.6 mm i.d., 4 µm). Mobile phase: water : acetonitrile : perchloric acid (96 : 4 : 0.1 for 18 min to 35 : 65 : 0 for 5 min), flow rate 1.0 mL/min. DAD (λ = 276 nm). Retention time: 13.7 min. Limit of quantification, 0.5 µg/kg [Piemontese *et al.* 2005]. Apple Juice. Column: Puresil C_{18} (250 × 4.6 mm i.d.). Mobile phase: 0.095% acetonitrile : acetonitrile (97 : 3), flow rate 1.0 mL/min. UV detection (λ = 276 nm). Retention time: ≈14.6 min. Limit of quantification, 10 µg/L, limit of detection, 4 µg/L [Watanabe, Shimizu 2005]. Column: Licrosphere 100 RP-18 (250 nm × 4 mm i.d.). Mobile phase: water : acetonitrile (99 : 1), flow rate 1.0 mL/min. UV detection (λ = 276 nm). Limit of detection, 20 µg/L [Bissessur *et al.* 2001]. Column: Phenomenex C_{18}(150 × 4 mm i.d., 5 µm). Mobile phase: water : acetonitrile (99 : 1), flow rate 1.0 mL/min. UV detection (λ = 250–300 nm). Retention time: 8 min. Limit of detection, <5 µg/L [Gökmen, Acar 1999]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-0.05% TFA (2 : 98) : acetonitrile-water (1 : 1, 100 : 0 for 22 min to 0 : 100 until 30 min to 100 : 0 until 45 min), flow rate 1.0 mL/min. UV detection (λ = 276 nm). Limit of detection, 5 µg/L [Trucksess, Tang 1999].

See also Gökmen, Acar [1996], Bartolomé *et al.* [1994], Prieta *et al.* [1993], Priest, Light [1990], Frisvad, Thrane [1987], Hurst *et al.* [1987], Torres *et al.* [1986].

LC-MS Apple Juice. Column: Mightysil RP-18 (250 × 2 mm i.d., 5 µm). Mobile phase: water : acetonitrile (100 : 0 to 0 : 100 in 30 min), flow rate 0.2 mL/min. ESI, SIM acquisition mode. Limit of quantification, 5 pg, limit of detection, 2.5 pg [Ito *et al.* 2004]. Column: Zorbax Eclipse XDB C_{18} (12.5 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate : methanol : 10 mmol/L ammonium acetate (0 : 2 : 98), flow rate 0.5 mL/min. APCI, ESI, SIM acquisition mode. Limit of detection, 1.03–1.5 µg/L [Takino *et al.* 2003]. Column: Luna ODS C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (10 : 90), flow rate 0.5 mL/min. APCI, positive and negative ion mode, full scan mode. Limit of detection, 4 µg/L [Sewram *et al.* 2000]. Column: Nucleosil RP18 (250 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (5 : 95 for 5 min to 95 : 5 within 20 min), flow rate 0.4 mL/min. ESI, negative ion mode. Retention time: 16.5 min. Limit of detection, 120 ng/L [Rychlik, Schieberle 1999].

Note For a review of GC-MS and LC-MS methods for the determination of patulin in food see Sforza *et al.* [2006].

CE Apple Juice. Column: Agilent (56 cm effective length, 75 µm i.d.). Running buffer: 33.3 mmol/L sodium tetraborate : 66.6 mmol/L SDS : 5% acetonitrile (pH 9.0). DAD (λ = 276 nm). Retention time: ≈9 min. Limit of quantification, 2.5 µg/L, limit of detection, 0.7 µg/L [Murillo *et al.* 2008]. Apple Juice Microemulsion. Column: Agilent (64.5/56 cm total/effective length, 75 µm i.d.). Running buffer: 33.3 mmol/L sodium tetraborate : 66.6 mmol/L SDS : 5% acetonitrile (pH 9.0). DAD (λ = 276 nm). Limit of quantification, 8.0 µg/L, limit of detection, 3.2 µg/L [Murillo-Arbizu *et al.* 2008]. Apple Cider. Column: fused silica capillary (50 cm effective length, 7.5 µm i.d.). Running buffer: 0.1 mol/L sodium tetraborate : 0.2 mol/L SDS : water (25 : 25 : 50, pH 9.0). DAD (λ = 273 nm). Limit of detection, 3.8 µg/L [Tsao, Zhou 2000].

Note For a review of chromatographic methods for the determination of patulin see Shephard, Leggott [2000].

Therapeutic Concentration

A healthy male volunteer (age, 36 years) consumed apple juice that contained ≈50 µg/L patulin. No patulin was detectable in the volunteer's serum before or after consumption [Rychlik 2003].

Toxicity Patulin can cause gastrointestinal hyperemia, bleeding, and ulcers in animals [Watanabe, Shimizu 2005]. The acute toxic effects in humans include nausea, vomiting, and other gastrointestinal trauma with accompanying kidney damage. Chronic exposure has been shown to induce the formation of cancerous tumours, cause genetic mutations, and cause embryonic developmental defects [Ito *et al.* 2004]. The WHO recommends to limit its content in food to 50 µg/kg [Sewram *et al.* 2000].

LD_{50} (SC) in mice, 10–15 mg/kg. LD_{50} (oral, SC and IP) in rats, 55, 11, and 10 mg/kg, respectively [McKinley *et al.* 1982].

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Pecazine

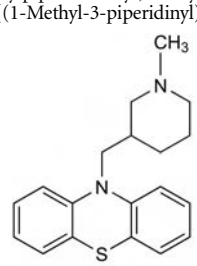
Tranquilliser

$C_{19}H_{22}N_2S = 310.5$

CAS—60-89-9

IUPAC Name 10-[(1-methylpiperidin-3-yl)methyl]phenothiazine

Synonyms Mepazine; 10-[(1-Methyl-3-piperidinyl)methyl]-10*H*-phenothiazine.



Chemical Properties pK_a 9.7 (24°). Log *P* (octanol/water), 5.4.

Pecazine Hydrochloride

$C_{19}H_{22}N_2S,HCl,H_2O = 364.9$

CAS—2975-36-2

Chemical Properties A white crystalline powder. Mp 180° to 181°. Very slightly soluble in water; freely soluble in dehydrated alcohol; soluble in chloroform; practically insoluble in ether and benzene.

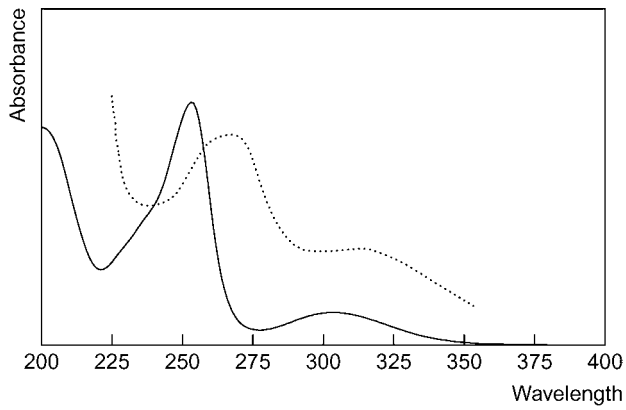
Colour Tests Mandelin's test—green→violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.47; system TC—R_f 0.44; system TE—R_f 0.65; system TF—R_f 0.01; system TL—R_f 0.16; system TAE—R_f 0.27 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2540; system GB—RI 2669.

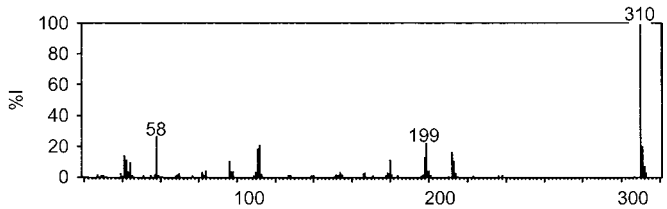
High Performance Liquid Chromatography System HA—*k* 3.9; system HX—RI 443; system HY—RI 382; system HAX—retention time 15.3 min; system HAY—retention time 7.0 min.

Ultraviolet Spectrum Aqueous acid—253 (A_1^1=892a), 300 nm; aqueous alkali—265 nm.



Infrared Spectrum Principal peaks at wavenumbers 750, 768, 1219, 1250, 1162, 1282 cm^{-1} (pecazine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 310, 58, 199, 112, 311, 111, 212, 41.

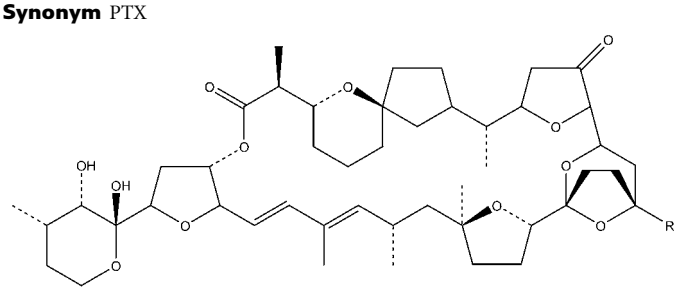


Dose Pecazine hydrochloride has been given in doses equivalent to 37.5 to 75 mg of the base daily.

Pectenotoxins

Polyether Macrolactone, Toxin

Synonym PTX



Chemical Properties Pectenotoxins (PTXs) were first isolated from scallops (*Patinopecten yessoensis*) cultivated in Japan. PTX-2 (methyl group at C-43 position), considered the main toxin in this group, is found in many species of the toxic dinoflagellate genus *Dinophysis*. The majority of the known PTX analogues are a result of bioconversion in shellfish or as artefacts during isolation [Suzuki *et al.* 2001; Yasumoto 2001]. These arise mainly from successive oxidation of the methyl group at the C-43 position, forming PTX-1 (hydroxymethyl at C-43), PTX-3

(aldehyde at C-43) and PTX-6 (carboxylic acid at C-43). PTX-2 is also rapidly metabolised to PTX-2 seco acid (PTX-2SA) and its epimer in most bivalve species. Further analogues include PTX-11 (hydroxyl at C-34), a natural metabolite in the dinoflagellate *Dinophysis acuta*, and PTX-12 (not characterised). Log *P* (octanol/water), 3.7 for PTX-2 [Takahashi *et al.* 2007].

Quantification

Other **HPLC** Dinoflagellate (*Dinophysis acuta*) and Mussel Samples. Column: C_{18} (250 × 3.2 mm i.d., 5 μm). Mobile phase: acetonitrile : methanol : water (80 : 5 : 15), flow rate 0.5 mL/min. Fluorescence detection ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 412$ nm). Retention times: PTX-2SA 13.0 min, 7-*epi*-PTX-2SA 16.2 min, okadaic acid 15.5 min, dinophysistoxin-2 16.9 min (9-anthryldiazomethane [ADAM] derivatives). Limit of quantification not reported [James *et al.* 1999; Pavela-Vrancic *et al.* 2001]. Dinoflagellate Samples (*Dinophysis fortii*). Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : water (6 : 4), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 370$ nm, $\lambda_{em} = 440$ nm). Limit of detection, PTX-2 1 ng (4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione [DMEQ-TAD] derivative) [Sasaki *et al.* 1999]. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (60 : 40) containing 0.1% trifluroacetic acid, flow rate 1.0 mL/min. UV detection ($\lambda = 235$ nm). Retention time: 6.6 min for PTX-2. Limit of quantification not reported [Draisci *et al.* 1996].

LC-MS Shellfish Samples. Column: C_{18} (100 × 2.1 mm i.d., 1.7 μm). Mobile phase: water : acetonitrile-water (95 : 5) both containing 2 mmol/L ammonium formate and 50 mmol/L formic acid (70 : 30 to 10 : 90 over 3 min for 1.5 min), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: PTX-1 2.38 min, PTX-6 2.46 min, PTX-2SA 2.77 min, 7-*epi*-PTX-2SA 2.97 min, PTX-2 3.08 min. Limit of quantification, 0.16 μg/L, limit of detection, 47.7 ng/L for PTX-2 [Fux *et al.* 2007]. Column: C_8 (50 × 2.0 mm i.d., 3 μm). Mobile phase: water : acetonitrile–water (95 : 5) both containing 2 mmol/L ammonium formate and 50 mmol/L formic acid (70 : 30 to 10 : 90 over 8 min for 3 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 10 μg/kg for PTX-2SA [Villar-Gonzalez *et al.* 2007]. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile-water (1 : 9) : acetonitrile-water (9 : 1) : 33 mmol/L ammonium hydroxide-500 mmol/L formic acid in water. (85 : 5 : 10 for 2 min to 5 : 85 : 10 over 11 min for 12 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention times: 16.2 min for PTX-2, 14.7 min for PTX-2SA, 15.4 min for 7-*epi*-PTX-2SA, 13.7 min for PTX-1, 14.0 min for PTX-6, 14.5 min for PTX-11SA. Limit of detection, PTX-2 5 μg/kg [McNabb *et al.* 2005]. Column: C_8 (50 × 2.1 mm i.d., 3 μm). Mobile phase: water : acetonitrile-water (95 : 5) both containing 5 mmol/L ammonium acetate (80 : 20 to 0 : 100 over 12.5 min, for 5 min), flow rate 0.25 mL/min. API, positive and negative ion modes, SIM acquisition mode. Relative retention times: 1.47 for PTX-2, 1.26 for PTX-1 (relative to okadaic acid). Limit of detection, PTX-2 9–11 μg/kg [Stobo *et al.* 2005]. Dinoflagellate Samples (*D. acuta*). Column: C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: water with 1.0 mmol/L ammonium acetate: acetonitrile with 1.0 mmol/L ammonium acetate : (60 : 40 for 0.5 min to 25 : 75 over 5 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention times: 9.6 min for PTX-2, 4.1 min for 7-*epi*-PTX-2SA, 3.3 min for dinophysistoxin-2, 2.8 min for okadaic acid. Limit of quantification not reported [Puente *et al.* 2004]. Dinoflagllate Samples. Column: Hypersil C_8 (50 × 2.0 mm i.d., 3 μm). Mobile phase: water : acetonitrile-water (95 : 5), both containing 2 mmol/L ammonium formate and 50 mmol/L formic acid (50 : 50), flow rate 0.2 mL/min. ESI, positive ion mode (ammonium adduct ions), SIM acquisition mode. Retention times: 2.2 min for PTX-1, 3.5 min for PTX-4, 5.3 min for PTX-8, 2.5 min for PTX-6 , 3.6 min for PTX-7, 5.3 min for PTX-9, 4.8 min for PTX-2, 8.6 min for PTX-2b, 11.7 min for PTX-2c, 4.0 min for PTX-11, 6.5 min for PTX-11b, 8.1 min for PTX-11c. Limit of quantification not reported [Suzuki *et al.* 2003]. Shellfish Samples. Column: Lichrospher C_{18} (125 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.05% acetic acid (65 : 35), 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Retention times: 4.3 min for PTX-2SA, 5.1 min for 7-*epi*-PTX-2SA, 6.1 min for PTX-2, 4.7 min for okadaic acid. Limit of quantification not reported [Vale and de Sampayo 2002]. Column: Inertsil C_{18} (150 × 2.1 mm i.d., 5 μm). Mobile phase: 1.0 mmol/L ammonium acetate in water : methanol (60 : 40 to 0 : 100 over 20 min for 10 min), flow rate 0.2 mL/min. SSI, negative ion mode. Limit of detection, 6 μg/L for PTX-6 and yessotoxin, 1.3 μg/L for okadaic acid and dinophysistoxin [Ito, Tsukada 2002]. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.05% acetic acid (7 : 3), flow rate 0.1 mL/min. ESI, positive ion mode (ammonium ion adduct), SIM acquisition mode. Limits of detection in muscle and digestive glands, 40 and 80 μg/kg, respectively, for PTX-1, PTX-2, PTX-6 and PTX-2SA [Goto *et al.* 2001]. Dinoflagellate (*D. acuta*) and Greenshell and Blue Mussel Samples. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (67 : 33) containing 50 mmol/L formic acid and 2 mmol/L ammonium formate, flow rate 0.1 mL/min. ESI, negative ion mode. Retention times: 15.8 min for PTX-2, 7.9 min for PTX-6; PTX-2SA and 7-*epi*-PTX2SA also detected. Limit of quantification not reported [Suzuki *et al.* 2001]. Shellfish Samples. Column: C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (7 : 3) containing 0.1% acetic acid, flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Limit of detection, 5 μg/kg [Suzuki, Yasumoto 2000].

Disposition in the Body

Toxicity PTXs were formerly included in the causative toxins associated with diarrhetic shellfish poisoning (DSP) because they were found in shellfish relating to DSP together with okadaic acid and dinophysistoxins. However, PTXs do not actually induce diarrhoea and are no longer included in the DSP group of toxins [Miles *et al.* 2006]. The LD_{50} of PTX-2 in mice by IP injection is reported as 219 μg/kg. Oral doses of up to 5000 μg/kg in mice did not produce overt toxic effects, possibly reflecting poor absorption from the gastrointestinal tract, or conversion in the gut to a less toxic material, such as PTX-2SA [Miles *et al.* 2004]. Studies have shown that PTX-2 exhibits nanomolar cytotoxicity against breast, colon and lung cancer lines [Spector *et al.* 1999].

Draisci R *et al.* (1996). First report of pectenotoxin-2 (PTX-2) in algae (*Dinophysis fortii*) related to seafood poisoning in Europe. *Toxicon* 34: 923–935.

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Goto H *et al.* (2001). Quantitative determination of marine toxins associated with diarrhetic shellfish poisoning by liquid chromatography coupled with mass spectrometry. *J Chromatogr A* 907: 181–189.

Ito S, Tsukada K (2002). Matrix effect and correction by standard addition in quantitative liquid chromatographic–mass spectrometric analysis of diarrhetic shellfish poisoning toxins. *J Chromatogr A* 943: 39–46.

James KJ *et al.* (1999). Liquid chromatographic methods for the isolation and identification of new pectenotoxin-2 analogues from marine phytoplankton and shellfish. *J Chromatogr A* 844: 53–65.

McNabb P *et al.* (2005). Multiresidue method for determination of algal toxins in shellfish: single-laboratory validation and interlaboratory study. *J AOAC Int* 88: 761–772.

Miles CO *et al.* (2004). Isolation of pectenotoxin-2 from *Dinophysis acuta* and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. *Toxicon* 43: 1–9.

Miles CO *et al.* (2006). Isolation of yessotoxin 32-*O*-[beta-L-arabinofuranosyl-(5′→1′)-beta-L-arabinofuranoside] from *Protoceratium reticulatum*. *Toxicon* 47: 510–516.

Pavela-Vrancic M *et al.* (2001). The occurrence of 7-*epi*-pectenotoxin-2 seco acid in the coastal waters of the central Adriatic (Kastela Bay). *Toxicon* 39: 771–779.

Puente PF *et al.* (2004). Studies of polyether toxins in the marine phytoplankton, *Dinophysis acuta*, in Ireland using multiple tandem mass spectrometry. *Toxicon* 44: 919–926.

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Spector I *et al.* (1999). New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. *Microsc Res Tech* 47: 18–37.

Stobo LA *et al.* (2005). Liquid chromatography with mass spectrometry: detection of lipophilic shellfish toxins. *J AOAC Int* 88: 1371–1382.

Suzuki T, Yasumoto T (2000). Liquid chromatography–electrospray ionization mass spectrometry of the diarrhetic shellfish-poisoning toxins okadaic acid, dinophysistoxin-1 and pectenotoxin-6 in bivalves. *J Chromatogr A* 874: 199–206.

Suzuki T *et al.* (2001). Pectenotoxin-2 seco acid: a toxin converted from pectenotoxin-2 by the New Zealand Greenshell mussel, *Perna canaliculus*. *Toxicon* 39: 507–514.

Suzuki T *et al.* (2003). Liquid chromatography–mass spectrometry of spiroketal stereoisomers of pectenotoxins and the analysis of novel pectenotoxin isomers in the toxic dinoflagellate *Dinophysis acuta* from New Zealand. *J Chromatogr A* 992: 141–150.

Takahashi E *et al.* (2007). Occurrence and seasonal variations of algal toxins in water, phytoplankton and shellfish from North Stradbroke Island, Queensland, Australia. *Mar Environ Res* 64: 429–442.

Vale P, deSampayo MA (2002). Pectenotoxin-2 seco acid, 7-*epi*-pectenotoxin-2 seco acid and pectenotoxin-2 in shellfish and plankton from Portugal. *Toxicon* 40: 979–987.

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P

Pemoline

CNS Stimulant

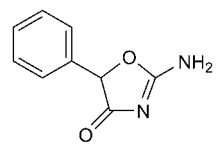
$C_9H_8N_2O_2 = 176.2$

CAS—2152-34-3

IUPAC Name 2-Amino-5-phenyl-1,3-oxazol-4-one

Synonyms 2-Amino-5-phenyl-4(5*H*)-oxazolone; phenilone; phenoxazole; phenylisohydantoin; phenylpseudohydantoin.

Proprietary Names *Cylert*; *Deltamine*; *Dynalert*; *Hyperilex*; *Nitan*; *PemADD*; *Sigmadyn*; *Senior*; *Stimul*; *Tradon*; *Volital*.



Chemical Properties White crystalline powder. Mp 256° to 257°, with decomposition. Practically insoluble in water, acetone, and ether; soluble 1 in 100 of propylene glycol. pK_a 10.5. Log *P* (octanol/water), 0.5. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

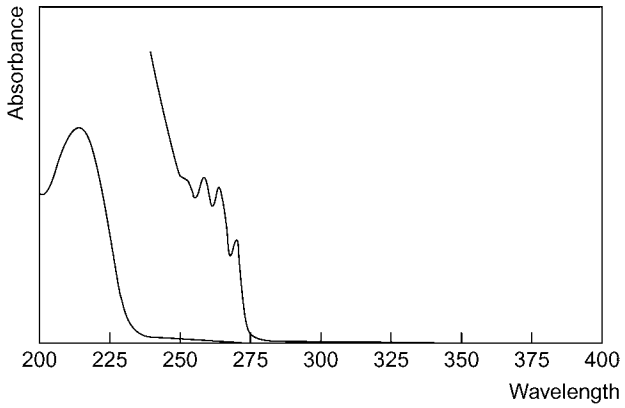
Colour Test Koppanyi–Zwikker test—violet.

Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.00; system TC—R_f 0.23; system TE—R_f 0.36; system TF—R_f 0.11; system TL—R_f 0.40; system TAE—R_f 0.81; system TAF—R_f 0.81 (Marquis reagent, yellow-brown; ninhydrin spray, positive; acidified potassium permanganate solution, positive).

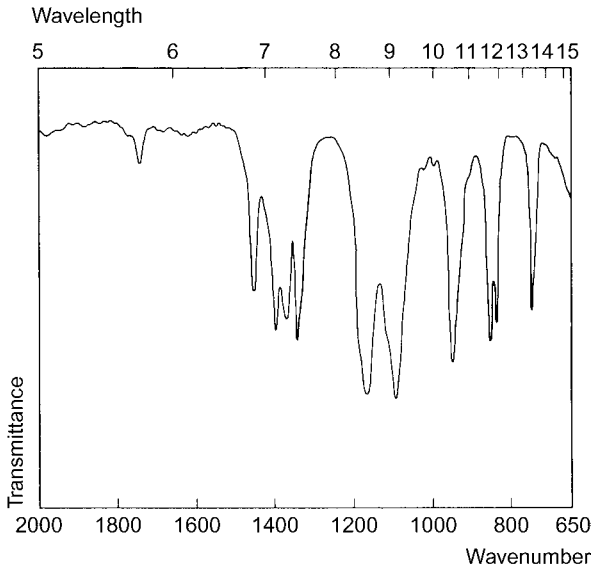
Gas Chromatography System GA—pemoline RI 1969, pemoline-Me_2 RI 1590, mandelic acid RI 1485; system GB—pemoline RI 2081.

High Performance Liquid Chromatography System HA—*k* 0.2; system HC—*k* 0.1; system HX—RI 307; system HY—RI 271.

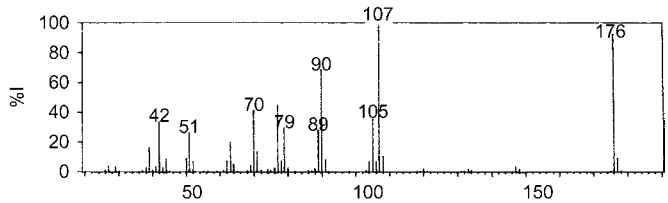
Ultraviolet Spectrum Aqueous acid—256 ($A^1_1 = 20a$), 262, 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1218, 1653, 695, 1560, 1137, 1275 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 107, 176, 90, 77, 70, 105, 42, 79; mandelic acid 107, 79, 77, 51, 152, 105, 50, 78; 5-phenyloxazolidine-2,4-dione 90, 177, 105, 77, 106, 51, 89, 50.



Quantification

Blood **GC** Column: 2%OV-17 on 100/120 mesh Gas-Chrom Q or 2% OV-225 on Chromosorb W-HP (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 180° or 190°. FID. Limit of detection, 20 µg/L [van Boven, Daenens 1977].

Plasma **GC** Column: 3% phenylmethyl dimethyl silicone on 100/120 mesh (2 m × 2.5 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 180°. FID. Limit of detection, 20 µg/L [Libeer, Schepens 1978]. Column: SCOT (8 m × 0.5 mm i.d.). Carrier gas: He, 5 mL/min. Temperature: 190°. NPD. Limit of detection, 1 ng [Vermeulen *et al.* 1978].

GC-MS Column: 3% OV-101 Chromosorb W-HP 100/120 mesh (5′ × 2 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 240°. ECD. Retention time: 38.0 min. Limit of detection, 50 µg/L [Igwe, Blake 1981].

HPLC Column: Nucleosil C_{18} (12.5 cm, 5 µm). Mobile phase: 0.01 mol/L dibasic potassium phosphate buffer (pH 5.0) : acetonitrile (82 : 18), flow rate 1.0 mL/min. UV detection (λ = 215 nm). Retention time: 7.6 min. Limit of detection, 10 µg/L [Sallee *et al.* 1985]. Column: Nucleosil 7C_{18} (25 cm × 4 mm i.d., 7.5 µm). Mobile phase: acetonitrile : 0.01 mol/L acetate buffer (pH 4.0; 9 : 91), flow rate 1.0 mL/min. UV detection (λ = 218 nm). Retention time: 14.0 min. Limit of detection, 20 µg/L [Nishihara *et al.* 1984].

Serum **GC** AFID. Limit of detection, 50 µg/L [Hoffman 1979]. See Plasma [Libeer, Schepens 1978].

HPLC Column: µBondapak C_{18} (30 × 4 cm i.d.). Mobile phase: 0.01 mol/L phosphate buffer (pH 5.0) : acetonitrile (83 : 17), flow rate 2.0 mL/min. UV detection (λ = 215 nm). Limit of detection, 0.1 mg/L [Tomkins *et al.* 1980].

Urine **GC** Column: 5% OV-17 on 80/100 mesh Chromosorb 750 (0.8 × 1 mm i.d.). Carrier gas: He, 40 mL/min. Temperature programme: 100° to 25° at 30°/min for 4 min. NPD. Limit of detection, 0.1 mg/L [Krylov, Khlebnikova 1982]. See Serum [Hoffman 1979]. See Plasma [Libeer, Schepens 1978]. See Plasma. Limit of detection, 100 µg/L [Vermeulen *et al.* 1978]. See Blood [van Boven, Daenens 1977]. Column: 2% OV-17 and 1% OV 225 on Gas-Chrom Q (120 × 0.12 cm i.d.). Carrier gas: He, 5 mL/min. Temperature: 190°. NSD. Limit of detection, 500 µg/L [Vermeulen *et al.* 1977].

GC-MS See Plasma. Limit of detection, 100 µg/L [Igwe, Blake 1981].

HPLC See Plasma [Nishihara *et al.* 1984]. Column: LiChrosorb RP-8 (25 cm × 4.6 mm i.d.). Mobile phase: water : methanol (85 : 15), flow rate 2.0 mL/min. UV detection (λ = 220 nm). Retention time: 8.2 min. Limit of detection, 0.1–0.05 mg/L [Cartoni *et al.* 1980]. Column: Zorbax (25 cm × 2.1 mm i.d.). Mobile phase: *n*-hexane : isopropanol : concentrated ammonia (37 : 12 : 1), flow rate 0.5 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Cartoni, Natalizia 1976].

Saliva **HPLC** See Plasma [Nishihara *et al.* 1984]. See Plasma [Vermeulen *et al.* 1978].

Disposition in the Body Slowly absorbed after oral administration. Approximately 50% of a dose is excreted in the urine as unchanged drug in 48 h and ~4% as 5-phenyloxazolidine-2,4-dione; the remainder is excreted in the urine as conjugated pemoline and unidentified polar metabolites; mandelic acid is also a metabolite of pemoline. Less than 1% of a dose is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 50 mg given to 3 subjects, peak plasma concentrations of 0.77 –1.22 mg/L (mean 1.0) were attained in about 2–3 h; peak concentrations in saliva of 0.47–0.75 mg/L (mean 0.59) were reported after 2–4 h; following administration of 37.5 mg to 1 subject, peak plasma and saliva concentrations of 0.89 and 0.58 mg/L, respectively, were attained in about 4 h [Vermeulen *et al.* 1979].

After daily oral doses of 37.5 and 75 mg to 28 hyperactive children for 7 days, mean plasma concentrations of about 2 and 4 mg/L respectively, were reported 3 h after the last dose [Tomkins *et al.* 1980].

Peak plasma concentrations of 4.3 (± 1.0) mg/L were attained in 2.8 (±1.8) h after a single 2 mg/kg oral dose of pemoline to 7 prepubescent boys [Sallee *et al.* 1985].

Toxicity Estimated minimum lethal dose is 2 g for an adult, 0.2 g for children up to 2 years (even though recovery has occurred following the ingestion of up to 1.8 g by young children).

Half-life Plasma half-life, ≈10–18 h.

Protein Binding ≈30% [Nishihara *et al.* 1984].

Dose Usually 40 mg daily; maximum of 120 mg daily.

Cartoni GP, Natalizia F (1976). Determination of pemoline high-pressure liquid chromatography. *J Chromatogr* 123: 474–478.

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Igwe OJ, Blake JW (1981). Gas/liquid chromatographic analysis of pemoline in biological fluids using electron capture detection. *J Chromatogr Sci* 19: 617–624.

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Libeer JC, Schepens P (1978). GLC determination of pemoline in biological fluids. *J Pharm Sci* 67: 419–421.

Nishihara K *et al.* (1984). Determination of pemoline in plasma, plasma water, mixed saliva, and urine by high-performance liquid chromatography. *Ther Drug Monit* 6: 232–237.

Sallee F *et al.* (1985). Oral pemoline kinetics in hyperactive children. *Clin Pharmacol Ther* 37: 606–609.

Tomkins CP *et al.* (1980). Analysis of pemoline in serum by high performance liquid chromatography: clinical application to optimize treatment of hyperactive children. *Ther Drug Monit* 2: 255–260.

Van Boven M, Daenens P (1977). Combined gas-liquid chromatographic-mass spectrometric analysis of pemoline in biological samples. *J Chromatogr* 134: 415–421.

Vermeulen NP *et al.* (1977). Gas chromatographic determination of pemoline as 5-phenyl-2 4-oxazolidinedione in human urine. *J Chromatogr* 137: 333–342.

Vermeulen NP *et al.* (1978). Assay of pemoline in human plasma, saliva and urine by capillary gas chromatography with nitrogen-selective detection. *J Chromatogr* 157: 133–140.

Vermeulen NP *et al.* (1979). Pharmacokinetics of pemoline in plasma, saliva and urine following oral administration. *Br J Clin Pharmacol* 8: 459–463.

Pempidine

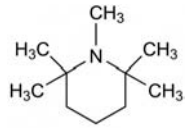
Antihypertensive

$C_{10}H_{21}N$ = 155.3

CAS—79-55-0

IUPAC Name 1,2,2,6,6-pentamethylpiperidin-1-ium

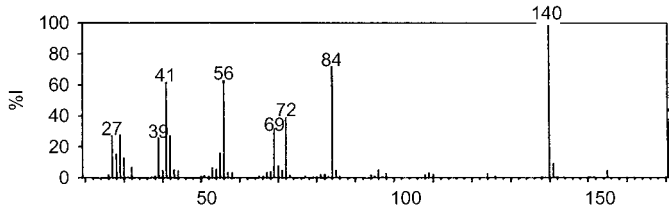
Synonym 1,2,2,6,6-Pentamethylpiperidine



Chemical Properties A liquid.

Pempidine Tartrate
$C_{10}H_{21}N,C_4H_6O_6 = 305.4$
CAS—546-48-5
Proprietary Names *Pempidil*; *Pempiten*; *Perolysen*; *Tenormal*; *Tensinol*; *Tensoral*.
Chemical Properties A white crystalline powder. Mp 160°. Soluble 1 in 2 of water and 1 in 14 of ethanol; very slightly soluble in acetone; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.24; system TB—R_f 0.68; system TC—R_f 0.03; system TL—R_f 0.10 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 1308, 1261, 1210, 1136, 1063, 675 cm^{-1} (pempidine tartrate, KBr disk).
Mass Spectrum Principal ions at *m/z* 140, 84, 56, 41, 72, 69, 29, 39.

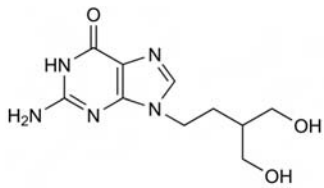


Dose 7.5 to 80 mg of pempidine tartrate daily.

Penciclovir

Antiviral
$C_{10}H_{15}N_5O_3 = 253.3$
CAS—39809-25-1

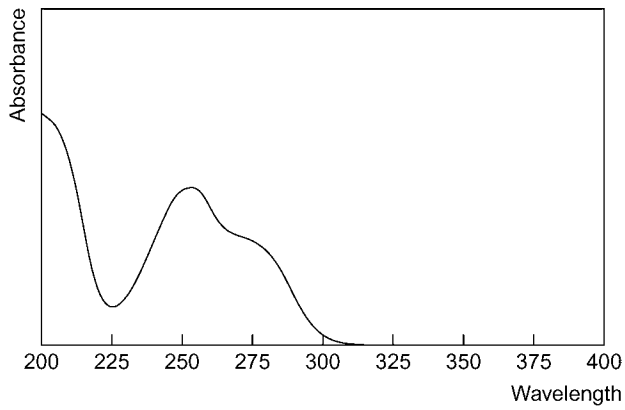
IUPAC Name 2-amino-9-[4-hydroxy-3-(hydroxymethyl)butyl]-3*H*-purin-6-one
Synonyms BRL-39123; PCV; 2-Amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6*H*-purin-6-one.
Proprietary Names *Denavir*; *Famvir*; *Vectavir*.



Chemical Properties White crystalline solid. Mp 275° to 277°. Also reported as colourless matted needles. Mp 272° to 275°. Soluble in water (1.7 mg/mL, 20°, pH 7). Log *P* (octanol/water), −1.62.

Penciclovir Sodium
$C_{10}H_{15}N_5O_3Na,H_2O = 294.3$
CAS—97845-62-0
Synonym BRL-39123A
Chemical Properties Crystalline solid. Soluble in water.

Ultraviolet Spectrum Principal peaks at 253, 278 nm.



Quantification
Plasma **HPLC** UV detection. Limit of quantification, 0.1 mg/L [Fowles, Pierce 1989].
Urine **HPLC** Limit of quantification, 50 mg/L, see Plasma [Fowles, Pierce 1989].
Disposition in the Body The drug is poorly absorbed from the gastrointestinal tract. For systemic use, it is usually administered orally as the prodrug, famciclovir, which is rapidly converted to penciclovir, producing peak plasma concentrations to the dose after 45 min to 1 h. Metabolites include a triphosphate derivative which is active. Minor metabolites include 6-deoxypenciclovir, monoacetylated penciclovir and monoacetylated 6-deoxypenciclovir. It is mainly excreted unchanged in the urine.
Therapeutic Concentration
Following the administration of a single 500 mg dose of famciclovir to 9 patients with normal renal function, mean peak plasma penciclovir concentrations were 2.83 mg/L (range, 1.30 to 3.82 mg/L) reached in 0.89 h (mean range, 0.5 to 1.5 h). Another 18 patients, split into three groups according to their renal impairment: mild, moderate and severe, were also administered the drug regimen. Mean peak plasma concentrations were 3.26 mg/L, 4.45 mg/L and 5.31 mg/L for the three groups, respectively, and reached in 0.79, 1.38 and 1.13 h (all mean times) [Boike *et al.* 1994].

Twenty healthy males (mean age, 36 years; range, 23 to 59 years), fasted from both fluids and solids for 10 hours pre-dosing, were administered 4 single oral doses of famciclovir (125, 250, 500 and 750 mg) on 4 separate days and at least one week apart. Penciclovir concentrations were detected 15 to 30 min after oral administration at all four dosages. Mean peak plasma penciclovir concentrations were 0.84 mg/L, 1.59 mg/L, 3.34 mg/L and 5.09 mg/L for the 125, 250, 500 and 750 mg dose, respectively, reached at 0.51, 0.75, 0.75 and 0.75 h, respectively [Pue *et al.* 1994].

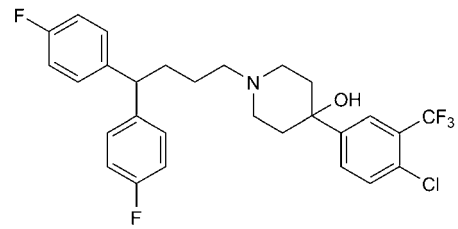
Following the IV administration of 10, 15 or 20 mg/kg penciclovir over 60 min to 15 healthy male subjects, mean peak plasma concentrations were 12.1, 19.6 and 22.7 mg/L, respectively. Peak plasma concentrations coincided with the end of the infusion [Fowles *et al.* 1992].
Bioavailability About 77%.
Half-life In plasma, ≈2 h.
Volume of Distribution Reported as 111.9 L or 1.5 L/kg (mean values).
Clearance Reported as 25.5 to 32.0 L/h in young healthy subjects.
Protein Binding <20%.
Dose Topically as 1% cream every 2 h for 4 days in the treatment of herpes labialis. For systemic use, penciclovir is administered orally as the prodrug famciclovir.

Boike SC *et al.* (1994). Pharmacokinetics of famciclovir in subjects with varying degrees of renal impairment. *Clin Pharmacol Ther* 55: 418–426.
Fowles SE, Pierce DM (1989). High-performance liquid chromatographic method for the determination of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL-39123) in human plasma and urine. *Analyst* 114: 1373–1375.
Fowles SE *et al.* (1992). The tolerance to and pharmacokinetics of penciclovir (BRL 39,123A), a novel antiherpes agent, administered by intravenous infusion to healthy subjects. *Eur J Clin Pharmacol* 43: 513–516.
Pue MA *et al.* (1994). Linear pharmacokinetics of penciclovir following administration of single oral doses of famciclovir 125, 250, 500 and 750 mg to healthy volunteers. *J Antimicrob Chemother* 33: 119–127.

Penfluridol

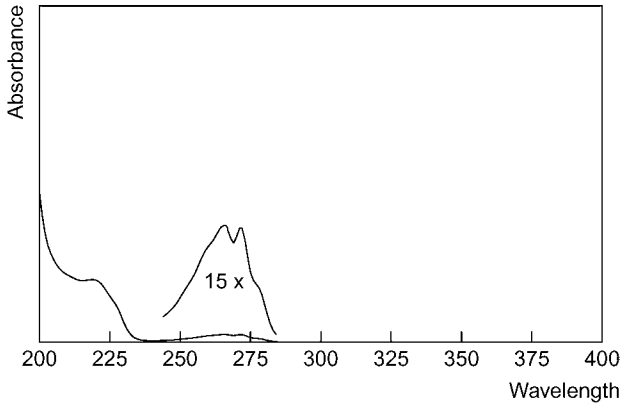
Tranquilliser
$C_{28}H_{27}ClF_5NO = 524.0$
CAS—26864-56-2

IUPAC Name 1-[4,4-Bis(4-fluorophenyl)butyl]-4-[4-chloro-3-(trifluoromethyl)phenyl]piperidin-4-ol
Synonym 1-[4,4-Bis(4-fluorophenyl)butyl]-4-[4-chloro-3-(trifluoromethyl) phenyl]-4-piperidinol
Proprietary Name *Semap*

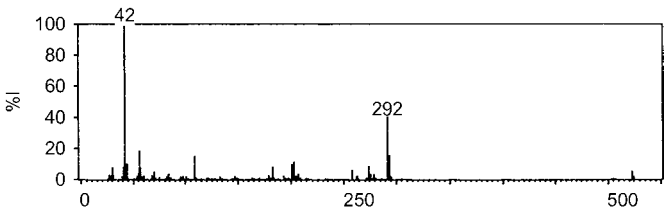


Chemical Properties White crystalline or microcrystalline powder. Mp 105° to 107°. Practically insoluble in water; very soluble in ethanol, acetone, and chloroform; freely soluble in ether. Log *P* (octanol/water), 2.7.
Colour Test Liebermann's reagent—brown.
Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.17; system TC—R_f 0.60; system TE—R_f 0.84; system TL—R_f 0.60; system TAE—R_f 0.72; system TAF—R_f 0.89 (Dragendorff spray).
Gas Chromatography System GA—penfluridol RI 3360, M (*N*-desalkyl-) RI 2210, M (*N*-desalkyl-oxo-)-2H_2O RI 1920, M (desamino-OH-) RI 2120, M (desamino-carboxy-) RI 2230.

High Performance Liquid Chromatography System HAA—RT 20.2 min; system HX—RI 659; system HY—RI 656; system HZ—RT 43.4 min.
Ultraviolet Spectrum Aqueous acid—267 ($A_1^1 = 47a$), 273 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1500, 1138, 1225, 1315, 1157, 825 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 42, 292, 56, 294, 109, 203, 201, 44.



Quantification
***Plasma* GC** Column: 3% OV-17 on 80/100 mesh (6′ × 0.25" i.d.). Carrier gas: Ar : CH_4 (95 : 5), 100 mL/min. Temperature: 280°. ECD. Retention time: 9 min. Limit of detection not reported [Jacobsson *et al.* 1976]. Column: 3% OV-3 (phenylmethyldimethylsilicone) on Gas Chrom Q 60/80 mesh (6′ × 3.5 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 48 mL/min. Temperature: 275°. ECD. Retention time: 17 min. Limit of detection 1–2 µg/L [Cooper *et al.* 1975a].
***Hair* LC-MS** MRM acquisition mode. Limit of detection, <0.05 ng/mg [Weinmann *et al.* 2002].
Disposition in the Body Incompletely absorbed after oral administration. It has a very long duration of action. Approximately 5% of a dose is excreted in the urine in 7 days, mainly as 4,4′-bis(4-fluorophenyl)butyric acid; ~80% of a dose is eliminated in the faeces, mostly as unchanged drug.
Therapeutic Concentration
Following weekly oral administration of 30, 60 and 120 mg for 13 weeks to 7, 7 and 8 subjects, mean steady-state plasma concentrations of about 0.004 to 0.01, 0.006 to 0.012 and 0.008 to 0.025 mg/L were reported. In all subjects maximum concentrations were attained within 12 h of a dose; concentrations declined rapidly between 12 and 24 h followed by a much slower decline during the next 120 h and were still significant (about 0.001 mg/L) 168 h after the dose [Cooper *et al.* 1975b].

After 4 weeks of treatment of 20, 40, 60 or 80 mg penfluridol daily there was a tenfold variation in plasma levels in the 47 patients [Jacobsson *et al.* 1976].
Dose Usually 20 to 60 mg weekly, by mouth; doses of up to 120 mg weekly have been given.

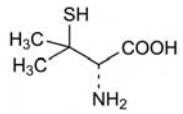
Cooper SF *et al.* (1975a). Gas-liquid chromatographic determination of penfluridol in plasma. A new specific technique. *Int Pharmacopsychiatry* 10: 78–88.
Cooper SF *et al.* (1975b). Penfluridol steady-state kinetics in psychiatric patients. *Clin Pharmacol Ther* 18: 325–329.
Jacobsson L *et al.* (1976). Penfluridol and thiothixene. Dosage, plasma levels and changes in psychopathology. *Int Pharmacopsychiatry* 11: 206–214.
Weinmann W *et al.* (2002). LC-MS-MS analysis of the neuroleptics clozapine, flupentixol, haloperidol, penfluridol, thioridazine, and zuclopenthixol in hair obtained from psychiatric patients. *J Anal Toxicol* 26: 303–307.

Penicillamine

Chelating Agent
$C_5H_{11}NO_2S = 149.2$
CAS—52-67-5

IUPAC Name (2*S*)-2-amino-3-methyl-3-sulfanylbutanoic acid
Synonyms (−)-β,β-Dimethylcysteine; 3,3-dimethyl-(*s*)-cysteine; D-3-mercaptovaline; D-penicillamine.

Proprietary Names *Adalken*; *Artamin*; *Atamir*; *Cuprimine*; *Cupripen*; *Depen*; *Distamine*; *Kelatin*; *Mercaptyl*; *Metalcaptase*; D-*Penamine*; *Pendramine*; *Rheumantin*; *Sufortan*; *Sufortanon*; *Trisorcin*; *Trolovol*.



Chemical Properties A white, finely crystalline powder. Mp 202° to 206°. Soluble 1 in 9 of water and 1 in 530 of ethanol; practically insoluble in chloroform and ether. pK_a 1.8, 7.9, 10.5. Log *P* (octanol/water), −1.8.

Penicillamine Hydrochloride
$C_5H_{11}NO_2S,HCl = 185.7$
CAS—2219-30-9
Proprietary Name *Pemine*
Chemical Properties A white, hygroscopic, finely crystalline powder. Mp about 175°, with decomposition. Soluble 1 in 1 of water, 1 in 1.5 of ethanol and 1 in 230 of chloroform; practically insoluble in ether.

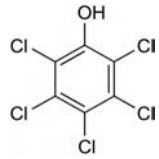
Colour Tests Mandelin's test—yellow; palladium chloride—yellow.
Thin-layer Chromatography System TA—R_f 0.36; system TB—R_f 0.01; system TC—R_f 0.03; system TE—R_f 0.03; system TL—R_f 0.03; system TAE—R_f 0.44 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 1595, 1525, 1615, 1550, 1090, 1055 cm^{-1} (Nujol mull). Two polymorphic forms occur.
Mass Spectrum Principal ions at *m/z* 75, 41, 57, 70, 43, 59, 56, 47.
Quantification
***Blood* HPLC** Electrochemical detection. Penicillamine, penicillamine disulfide and penicillamine glutathione mixed disulfide. Limit of detection, 30 µg/L for penicillamine disulfide [Yamashita, Rabenstein 1989]. Electrochemical detection. Limit of detection, 10 µg/L [Drummer *et al.* 1986]. Electrochemical detection. Limit of detection, 450 µg/L [Bergström *et al.* 1981].
***Plasma* HPLC** Electrochemical detection. Penicillamine, homocysteine, homocystine and disulfides [Rabenstein, Yamashita 1989]. See Blood [Yamashita, Rabenstein 1989]. Electrochemical detection. D-penicillamine-albumin sulfide. Limit of detection, 1.2 µmol/L [Joyce, Wade 1988]. Limit of detection, 75 µg/L, see Blood [Bergström *et al.* 1981].
***Serum* HPLC** Fluorescence detection. Limit of detection, 130 µg/L [Lankmayr *et al.* 1981].
***Urine* HPLC** See Plasma [Rabenstein, Yamashita 1989]. See Blood [Yamashita, Rabenstein 1989]. Limit of detection, 75 µg/L, see Blood [Bergström *et al.* 1981].
Reviews For reviews of assay methods, see Lecavalier and Crawhall [1981] and Kucharczyk and Shahinian [1981].
Disposition in the Body Readily but incompletely absorbed after oral administration. About 50% of an oral dose is excreted in the urine in 48 h with about 10% as unchanged drug, up to 25% as cysteine-penicillamine disulfide, about 15% as penicillamine disulfide and <10% as *S*-methyl-D-penicillamine; about 35% of an oral dose is eliminated in the faeces in 3 days. After IV administration, about 80% of the dose is excreted in the urine in 24 h; traces of penicillamine remain in the plasma after 48 h owing to protein binding.
Therapeutic Concentration
Following daily oral doses of 500 to 750 mg to 10 subjects, peak plasma concentrations of 1.7 to 5.6 mg/L (mean, 3.7) were attained 1.5 to 3 h after a dose [Butler *et al.* 1982].
Half-life Plasma half-life, about 2 to 6 h.
Protein Binding Up to 90%.
Note For further details of penicillamine metabolism and kinetics see Bergström *et al.* [1981] and Crawhall *et al.* [1979].
Dose 0.125 to 4 g daily.

Bergström RF *et al.* (1981). High-performance liquid chromatographic determination of penicillamine in whole blood, plasma, and urine. *J Chromatogr* 222: 445–452.
Bergström RF *et al.* (1981). Penicillamine kinetics in normal subjects. *Clin Pharmacol Ther* 30: 404–413.
Butler M *et al.* (1982). Pharmacokinetics of reduced D-penicillamine in patients with rheumatoid arthritis. *Arthritis Rheum* 25: 111–116.
Crawhall JC *et al.* (1979). Penicillamine, its metabolism and therapeutic applications: a review. *Biopharm Drug Dispos* 1: 73–95.
Drummer OH *et al.* (1986). Measurement of penicillamine and N-acetylcysteine in human blood by high-performance liquid chromatography and electrochemical detection. *J Chromatogr* 374: 251–257.
Joyce DA, Wade DN (1988). Assay for D-penicillamine-protein conjugate in human plasma utilising chemical reduction followed by high-performance liquid chromatography with gold/mercury electrochemical detection. *J Chromatogr* 430: 319–327.
Kucharczyk N, Shahinian S (1981). An overview of assay methods for D-penicillamine. *J Rheumatol Suppl* 7: 28–34.
Lankmayr EP *et al.* (1981). Determination of D-penicillamine in serum by fluorescence derivatization and liquid column chromatography. *J Chromatogr* 222: 249–255.
Lecavalier DR, Crawhall JC (1981). A survey of analytical approaches to the measurement of D-penicillamine and penicillamine disulfides. *J Rheumatol Suppl* 7: 20–27.
Rabenstein DL, Yamashita GT (1989). Determination of homocysteine, penicillamine, and their symmetrical and mixed disulfides by liquid chromatography with electrochemical detection. *Anal Biochem* 180: 259–263.
Yamashita GT, Rabenstein DL (1989). Determination of penicillamine, penicillamine disulfide and penicillamine-glutathione mixed disulfide by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 491: 341–354.

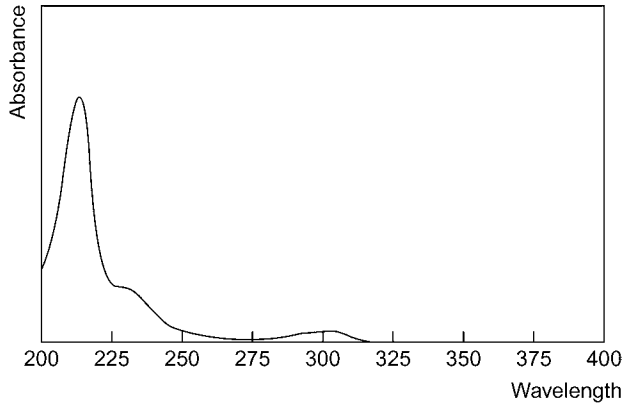
Pentachlorophenol

Preservative
C_6HCl_5O = 266.3
CAS—87-86-5

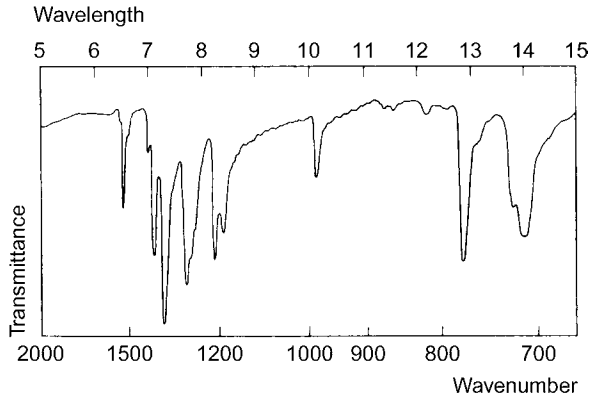
IUPAC Name 2,3,4,5,6–Pentachlorophenol
Synonyms PCP; penta.
Note The name PCP has also been used as a synonym for phencyclidine hydrochloride.



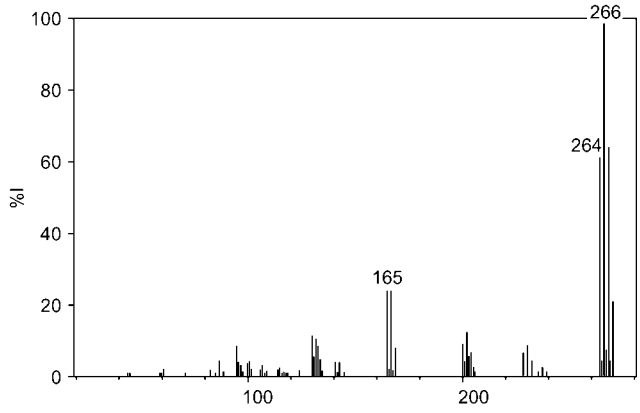
Chemical Properties White crystals. Mp 190° to 191°. Practically insoluble in water; freely soluble in ethanol and ether; soluble in benzene. pK_a 4.7 (25°). Log *P* (octanol/water), 5.1.
Colour Test Nitric acid, fuming—red/red/brown-violet.
Gas Chromatography System GA—pentachlorophenol RI 1760, pentachlorophenol-Me RI 1815.
Ultraviolet Spectrum Aqueous alkali—319 nm (A^1_1=194b); ethanol—295 (A^1_1=89b), 303 nm (A^1_1=115b).



Infrared Spectrum Principal peaks at wavenumbers 1307, 774, 1222, 711, 1196, 1553 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 266, 268, 264, 167, 165, 270, 202, 132.



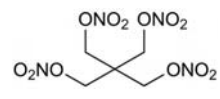
Quantification
Blood **GC** ECD. Limit of detection, 1 µg/L [Needham *et al.* 1981].
Plasma **GC** ECD. Limit of detection, 10 µg/L [Rick *et al.* 1982].
Serum **GC** ECD [Noren, Sjovall 1987]. See Blood [Needham *et al.* 1981].
Urine **GC** See Plasma [Rick *et al.* 1982]. See Blood [Needham *et al.* 1981].
GC-MS SIM acquisition mode. Pentachlorophenol and other chlorophenols. Limit of detection for pentachlorophenol, 3.6 µg/L [Kontsas *et al.* 1995].
HPLC UV detection. Limit of detection, 30 µg/L [Pekari, Aitio 1982].
Postmortem Tissues **GC-MS** Pentachlorophenol and other chlorinated compounds [Wagner *et al.* 1991].
Disposition in the Body Readily absorbed after ingestion, inhalation or through intact skin. Pentachlorophenol and its oxidised metabolite, tetrachlorohydroquinone, are excreted in the urine in free and conjugated forms, with about 74% of a dose being excreted unchanged in 7 days. About 4% of a dose is eliminated in the faeces. Urinary concentrations in 130 pest-control operators exposed to pentachlorophenol ranged from 0.003–35.7 mg/L (mean, 1.8) [Bevenue *et al.* 1967].
Blood Concentration
Plasma concentrations of 0.99–9.1 mg/L were reported in 6 occupationally exposed subjects and blood concentrations of 0.34–6.0 mg/L were reported in 7 exposed workers [Bevenue *et al.* 1968].
A sample of 15 women with long-term (5–17 years) low exposure to pentachlorophenol contained in wood-preservative had a mean pentachlorophenol serum level of 43.6 µg/L [Peper *et al.* 1999].
Toxicity The estimated minimum lethal dose is 1 g and the maximum permissible atmospheric concentration is 0.5 mg/m^3. Blood concentrations >30 mg/L are usually toxic. Numerous fatalities after absorption, inhalation or ingestion have been reported.
In 2 fatalities, the following postmortem tissue concentrations (mg/L or µg/g) were reported: blood 113, 156; brain 14, 35; kidney –, 123; liver 94, 134; urine –, 520 [Mason *et al.* 1965].
In 1 fatality, the following postmortem tissue concentrations were reported: blood 173 mg/L, kidney 116 µg/g, liver 225 µg/g, urine 75 mg/L [Cretney 1976].
Half-life About 30 h.
Distribution in Blood Plasma : whole blood ratio, 1.8.
Protein Binding About 99%.

Bevenue A *et al.* (1967). *Bull Environ Contam Toxicol* 2: 319–332.
Bevenue A *et al.* (1968). A sensitive gas chromatographic method for the determination of pentachlorophenol in human blood. *J Chromatogr* 38: 467–472.
Cretney MJ (1976). *Bull Int Assoc Forensic Toxicol* 12(3): 10.
Kontsas H *et al.* (1995). Gas chromatographic-mass spectrometric determination of chlorophenols in the urine of sawmill workers with past use of chlorophenol-containing anti-stain agents. *Analyst* 120: 1745–1749.
Mason MF *et al.* (1965). Pentachlorophenol poisoning: report of two cases. *J Forensic Sci* 10: 136–147.
Needham LL *et al.* (1981). Determining pentachlorophenol in body fluids by gas chromatography after acetylation. *J Anal Toxicol* 5: 283–286.
Noren K, Sjovall J (1987). Analysis of pentachlorophenol in water and urine by enrichment with Lipidex 5000. *J Chromatogr* 414: 55–63.
Pekari K, Aitio A (1982). A simple liquid chromatographic method for the analysis of penta- and tetrachlorophenols in urine of exposed workers. *J Chromatogr* 232: 129–136.
Peper M *et al.* (1999). Long-term exposure to wood-preserving chemicals containing pentachlorophenol and lindane is related to neurobehavioral performance in women. *Am J Ind Med* 35: 632–641.
Rick DL *et al.* (1982). Determination of phenol and pentachlorophenol in plasma and urine samples by gas liquid chromatography. *J Anal Toxicol* 6: 297–300.
Wagner SL *et al.* (1991). Residues of pentachlorophenol and other chlorinated contaminants in human tissues: analysis by electron capture gas chromatography and electron capture negative ion mass spectrometry. *Arch Environ Contam Toxicol* 21: 596–606.

Pentaerithrityl Tetranitrate

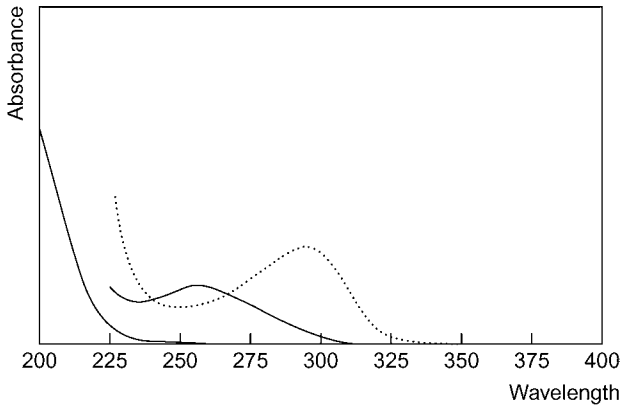
Antianginal Vasodilator
$C_5H_8N_4O_{12}$ = 316.1
CAS—115-77-5 (pentaerithrityl); 78-11-5 (tetranitrate)

IUPAC Name [3–Nitrooxy-2,2–bis(nitrooxymethyl)propyl]nitrate
Synonyms 2,2-Bis[(nitrooxy)methyl]-1,3-propanediol dinitrate (ester); erynite; nitropentaerythrol; nitropenthrite; pentaerythritol tetranitrate; pentanitrol.
Proprietary Names *Cardiacap*; *Dilcoran*; *Hasethrol*; *Lentrat*; *Mycardol*; *Neo-Corovas*; *Nirason S*; *Nitrodex*; *Nitropenton*; *Pentafin*; *Pentalong*; *Pentanitrine*; *Pentral 60*; *Pentrite*; *Pentritol*; *Pentryate*; *Pentylan*; *Peritrate*; *Pergitral*; *Perityl*; *Prevangor*; *Quintrate*; *Subicard*; *Terpate*; *Vasodiatol*.



Chemical Properties A white crystalline powder. Mp 140°. Practically insoluble in water; slightly soluble in ethanol and ether; soluble in acetone. Log *P* (octanol/water), 2.4.
Note For medicinal purposes pentaerithrityl tetranitrate is supplied diluted with an inert substance such as lactose because the undiluted compound may explode upon percussion or on exposure to heat.
Thin-layer Chromatography System TE—R_f 0.72; system TAE—R_f 0.92.

High Performance Liquid Chromatography System HX—RI 663 (pentaerithrityl tetranitrate); system HAA—retention time 23.1 (pentaerithrityl) min.
Ultraviolet Spectrum Aqueous acid—255 nm; aqueous alkali—294 nm.

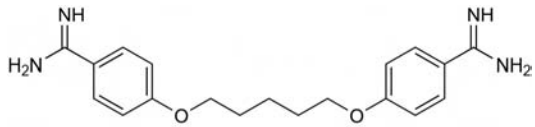


Mass Spectrum Principal ions at *m/z* 46, 76, 57, 55, 56, 60, 47, 97.
Quantification
Blood **GC** Pentaerithrityl tetranitrate and de-esterified metabolites. ECD. Limits of detection, 100 ng/L to 2 µg/L in blood [Neurath, Düünger 1977].
Plasma **GC-MS** Pentaerithrityl tetranitrate and its metabolites. Limit of detection, <50 ng/L [Stalleicken *et al.* 1997].
HPLC Limit of detection, 100 pg [Yu, Goff 1983].
Urine **GC** See Blood Neurath and Düünger [1977].
Disposition in the Body Incompletely absorbed from the gastro-intestinal tract; it may be absorbed through intact skin. The main metabolic reaction is stepwise de-esterification ultimately to pentaerithrityl. The nitrate ester metabolites may be conjugated with glucuronic acid. About 50 to 60% of a dose is excreted in the urine in 48 h, mainly as pentaerithrityl and pentaerithrityl mononitrate, with small amounts of the dinitrate ester. About 30 to 40% of a dose is eliminated in the faeces in 72 h, partly as unchanged drug, with the main metabolite present being pentaerithrityl, together with small quantities of the dinitrate and mononitrate esters.
Half-life About 7 h.
Protein Binding About 85%.
Dose 30 to 240 mg daily.

Neurath GB, Düünger M (1977). Blood levels of the metabolites of glyceryl trinitrate and pentaerythritol tetranitrate after administration of a two-step preparation. *Arzneimittelforschung* 27: 416–419.
Stalleicken D *et al.* (1997). Quantitative determination of pentaerythrityl tetranitrate and its metabolites in human plasma by gas chromatography/mass spectrometry. *Arzneimittelforschung* 47: 347–352.
Yu WC, Goff EU (1983). Determination of vasodilators and their metabolites in plasma by liquid chromatography with a nitrosyl-specific detector. *Anal Chem* 55: 29–32.

Pentamidine

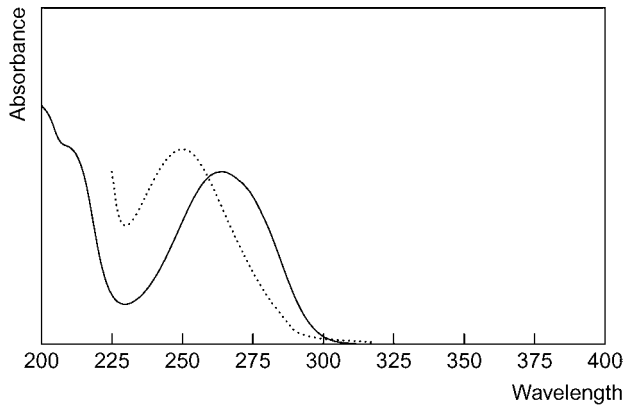
Antiprotozoal
$C_{19}H_{24}N_4O_2$ = 340.4
CAS—100-33-4
IUPAC Name 4-[5-(4-Carbamimidoylphenoxy)pentoxy]benzenecarboximidamide
Synonym 4,4′-[1,5-Pentanediylbis(oxy)]bisbenzenecarboximidamide



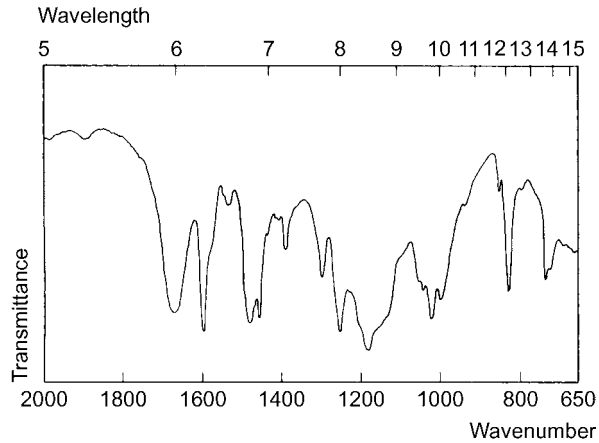
Chemical Properties Log *P* (octanol/water), 2.8.

Pentamidine Isetionate
$C_{19}H_{24}N_4O_2,2C_2H_6O_4S$ = 592.7
CAS—140-64-7
Synonyms Pentamidine di-isetionate; pentamidine isethionate
Proprietary Names *NebuPent*; *Pentacarinet*; *Pentam*; *Pentamina*; *Pneumopent*.
Chemical Properties White, hygroscopic crystals or powder. Mp about 180°. Soluble 1 in 10 of water; slightly soluble in ethanol; insoluble in acetone, chloroform and ether.

Pentamidine Mesilate
$C_{19}H_{24}N_4O_2,2CH_4O_3S$ = 532.6
CAS—6823-79-6
Synonyms Pentamidine dimethylsulfonate; pentamidine mesylate; pentamidine methanesulfonate.
Proprietary Name *Lomidine*
Chemical Properties A white or very faintly pink granular powder. Slightly soluble in water and ethanol; practically insoluble in chloroform and ether.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.01; system TC—R_f 0.00; system TL—R_f 0.00; system TAD—R_f 0.00; system TAE—R_f 0.00 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3010.
Ultraviolet Spectrum Aqueous acid—262 nm (A^1_1=840a); aqueous alkali—249 nm.



Infrared Spectrum Principal peaks at wavenumbers (pentamidine isetionate) 1188, 1604, 1260, 1028, 1676, 1003 cm^{-1} (KBr disk).



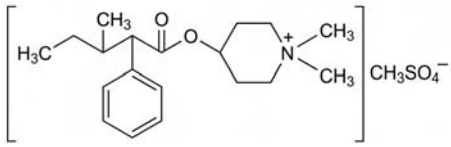
Quantification
Blood **HPLC** Fluorescence detection. Limit of detection, 16 nmol/L [Ericsson, Rais 1990].
Plasma **HPLC** See Blood [Ericsson, Rais 1990]. UV detection. Limit of detection, 15 nmol/L [Dusci *et al.* 1987].
Urine **HPLC** Limit of detection, 27.7 nmol/L, see Blood [Ericsson, Rais 1990].
Disposition in the Body
Therapeutic Concentration
In 6 subjects treated for *Pneumocystis carinii* pneumonia with IV pentamidine isetionate 3 mg/kg given once daily as a 2 h IV infusion, the plasma concentration at the end of the first infusion was 249 (±80) µg/L; this value was 179 (±185) µg/L in a further 4 patients. In 9 patients undergoing haemodialysis, the mean peak plasma concentration was 275 (±184) µg/L after a 3 mg/kg dose and 227 (±110) µg/L after a 4 mg/kg dose [Conte 1991].
Dose Usually the equivalent of 2 to 4 mg/kg of pentamidine daily, by IM injection.

Conte JE (1991). Pharmacokinetics of intravenous pentamidine in patients with normal renal function or receiving hemodialysis. *J Infect Dis* 163: 169–175.
Dusci LJ *et al.* (1987). High-performance liquid chromatographic method for measurement of pentamidine in plasma and its application in an immunosuppressed patient with renal dysfunction. *Ther Drug Monit* 9: 422–425.
Ericsson O, Rais M (1990). Determination of pentamidine in whole blood, plasma, and urine by high-performance liquid chromatography. *Ther Drug Monit* 12: 362–365.

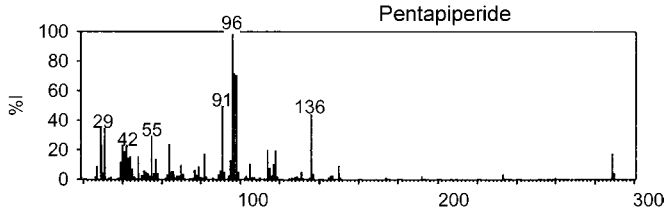
Pentapiperide Metilsulfate

Anticholinergic
$C_{19}H_{30}NO_2,CH_3SO_4$ = 415.5
CAS—7009-54-3 (pentapiperide); 26372-86-1 (pentapiperium); 7681-80-3 (pentapiperide metilsulfate or pentapiperium methylsulfate)

IUPAC Name (1,1-Dimethylpiperidin-1-ium-4-yl)3-methyl-2-phenylpentanoate; methyl sulfate
Synonyms 1,1-Dimethyl-4-(3-methyl-2-phenylvaleryloxy)piperidinium methylsulfate; pentapiperide methylsulfate; pentapiperium metilsulfate; valpipamate methylsulfate.
Note Pentapiperide metilsulfate and pentapiperium methylsulfate are identical but pentapiperide differs from pentapiperium in having one less methyl group.
Proprietary Names *Crilin*; *Crylène*.



Chemical Properties A white crystalline powder. Mp 140° to 148°. Freely soluble in water, chloroform and methanol; soluble in ethanol.
Colour Tests Liebermann's reagent—red-orange; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.00 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid —253, 258, 265 nm.
Infrared Spectrum Principal peaks at wavenumbers 1210, 1730, 1252, 755, 1008, 1162 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 96, 97, 98, 91, 136, 31, 29, 55.

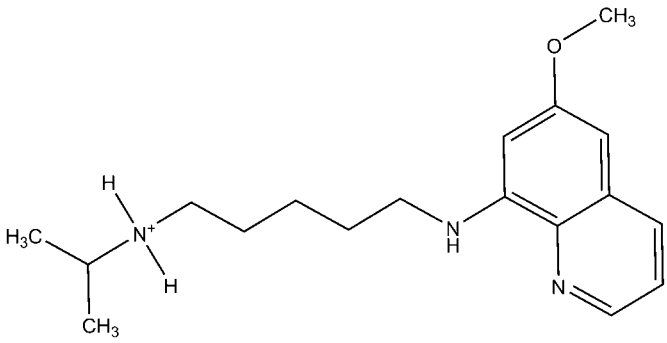


Dose 20 to 30 mg daily.

Pentaquin

Antimalarial
$C_{18}H_{27}N_3O$ = 301.4
CAS—86-78-2

IUPAC Name 5-[(6-Methoxyquinolin-8-yl)amino]pentyl-propan-2-ylazanium
Synonyms 8-(5-Isopropylaminopentylamino)-6-methoxyquinolone; SN 13276.



Chemical Properties pK_a 3.2 [Whichard *et al.* 1968]. Pentaquin is extracted by organic solvents from aqueous alkaline solutions.

Pentaquin Phosphate

$C_{18}H_{27}N_3O,H_2PO_4$ = 387.9
CAS—5428-64-8
Synonyms CB-11; hepagin; heptalin; Hoechst 10600.
Proprietary Name *Heptalgin*
Chemical Properties Yellow crystalline powder. Soluble 1 in 25 of water, almost insoluble in ethanol; insoluble in chloroform and ether. Log *P* (octanol/water) 4.52 [Meylan, Howard 1995].

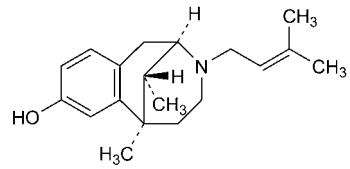
Colour Tests Ammonium molybdate test—pale blue (limit of detection, 1.0 µg); ammonium vandate test—purple (limit of detection, 0.5 µg); sulfuric acid-formaldehyde test—orange (limit of detection, 0.25 µg); Vitali's test—grey-brown/yellow/yellow (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.20 (location reagent diazotised *p*-nitroaniline-sodium hydroxide spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.70 (relative to codeine).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—222, 265, 277, 331, 400 nm.
Disposition in the Body
Therapeutic Concentration
A dose of 60 mg pentaquin base per day concomitantly with 2 g of quinine sulfate per day prevented relapses of St Elizabeth strain *vivax* malaria [White *et al.* 1948] but did not prevent Chesson *vivax* malaria [Coatney *et al.* 1950].
Toxicity The toxic effects of pentaquin are similar to those of pamaquin, but are less likely to occur with therapeutic dosage [Coggeshall, Rice 1949; Craige *et al.* 1948]. Plasma concentrations are sustained longer with pentaquin [Eldin, Morcos 1952]. For a study of the toxicity of pentaquin in rhesus monkeys see Schmidt and Schmidt [1951].
Dose Up to 60 mg (base) daily [Alving *et al.* 1948].

Alving AS *et al.* (1948). Pentaquine (SN-13,276), a therapeutic agent effective in reducing the relapse rate in viv ax malaria. *J Clin Invest* 27: 25–33.
Coatney GR *et al.* (1950). Studies in human malaria. XXVII. Observations on the use of pentaquine in the prevention and treatment of Chesson strain vivax malaria. *J Natl Malar Soc* 9: 222–233.
Coggeshall LT, Rice FA (1949). Cure of chronic vivax malaria with pentaquine. *J Am Med Assoc* 139: 437–439.
Craige B *et al.* (1948). The toxicity of large doses of pentaquine (SN-13,276), a new antimalarial drug. *J Clin Invest* 27: 17–24.
Eldin GN, Morcos F (1952). Pentaquine in the treatment of malaria. *J Egypt Med Assoc* 35: 330–334.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Schmidt IG, Schmidt LH (1951). Neurotoxicity of the 8-aminoquinolines. III. The effects of pentaquine, isopentaquine, primaquine, and pamaquine on the central nervous system of the rhesus monkey. *J Neuropathol Exp Neurol* 10: 231–256.
Whichard LP *et al.* (1968). The binding of primaquine, pentaquine, pamaquine, and plasmocid to deoxyribonucleic acid. *Mol Pharmacol* 4: 630–639.
White WC *et al.* (1948). Studies in human malaria; the cure of St. Elizabeth strain vivax malaria by pentaquine-quinine, administered during acute attacks or during latency. *J Natl Malar Soc* 7: 316–321.

Pentazocine

Narcotic Analgesic
$C_{19}H_{27}NO$ = 285.4
CAS—359-83-1

Synonyms IH-7958; (2*R**,6*R**,11*R**)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol; NSC-107430; pentazocinum; Win 20228.
Proprietary Names *Fortal*; *Fortalgesic*; *Fortral*; *Fortralin*; *Ospronim*; *Pentalgina*; *Rafazocine*; *Sosegon*; *Sosenol*; *Talwin*; *Talwin NX*. It is an ingredient of *Emergent-Ez*, *Fortagesic* and *Talacen*. (Includes proprietary names of pentazocine salts.)



Chemical Properties White or pale tan-coloured powder. Mp 145.4° to 147.2°. Practically insoluble in water; soluble 1 in about 15 of ethanol, 1 in 2 of chloroform, and 1 in 33 of ether. pK_a 8.5, 10.0 (20°). Log *P* (octanol/pH 7.4), 2.0. Extraction yield (chlorobutane), 0.8 [Demme *et al.* 2005].

Pentazocine Hydrochloride

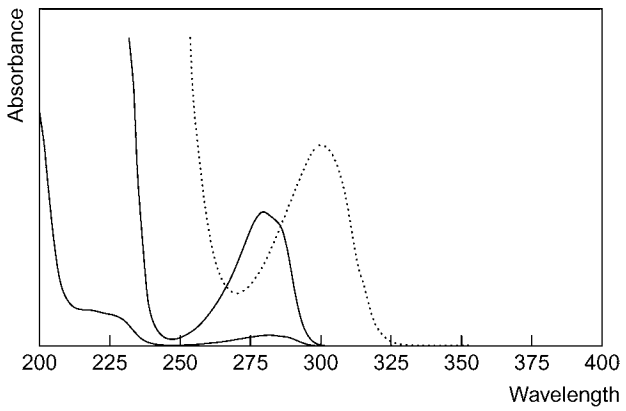
$C_{19}H_{27}NO,HCl$ = 321.9
CAS—2276-52-0; 64024-15-3
Chemical Properties White to pale cream-coloured crystalline powder. There are 2 forms, melting at ~218° and ~254°. Soluble 1 in 30 of water, 1 in 16 of ethanol, and 1 in 4 of chloroform; practically insoluble in ether.

Pentazocine Lactate

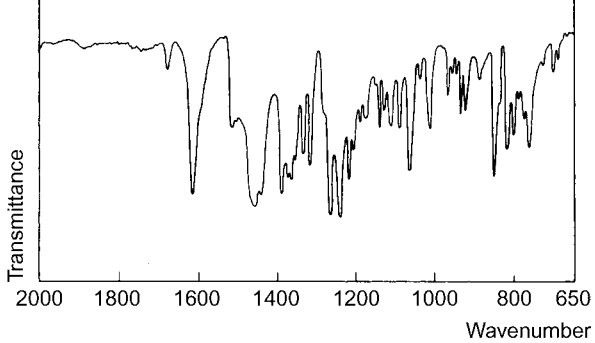
$C_{19}H_{27}NO,C_3H_6O_3$ = 375.5
CAS—17146-95-1
Chemical Properties White to cream-coloured powder. Soluble 1 in 25 of water, 1 in 12 of ethanol, 1 in 25 of chloroform, and 1 in 1500 of ether.

Colour Tests Folin–Ciocalteu reagent—blue; Mandelin's test—green; Marquis test—red→green.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.16; system TC—R_f 0.12; system TE—R_f 0.70; system TL—R_f 0.28; system TAE—R_f 0.34; system TAF—R_f 0.72; system TAJ—R_f 0.02; system TAK—R_f 0.03; system TAL—R_f 0.57 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—pentazocine RI 2280, pentazocine-AC RI 2330, pentazocine-PFP RI 2120, pentazocine-TFA RI 2075, pentazocine-TMS RI 2320, M (desalkyl-)-AC_2 RI 2380, M (OH-) RI 2545; system GB—pentazocine RI 2356, M (desalkyl-) RI 2019, M (OH-) RI 2649; system GC—RI 2225; system GF—RI 3030; system GM—RRT 0.870 (relative to iprindole).

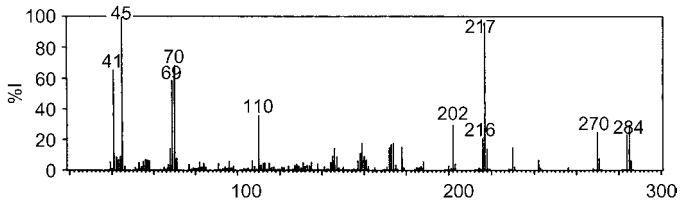
High Performance Liquid Chromatography System HA—k 1.8; system HC—k 0.67; system HX—RI 372; system HY—RI 288; system HZ—RT 3.8 min; system HAA—RT 12.5 min; system HAX—RT 9.9 min; system HAY—RT 5.5 min.
Ultraviolet Spectrum Aqueous acid—278 ($A_1^1 = 69a$); aqueous alkali—240 ($A_1^1 = 330a$), 300 nm ($A_1^1 = 106a$).



Infrared Spectrum Principal peaks at wavenumbers 1238, 1264, 1609, 1214, 854, 1054 cm^{-1} (KBr disk). Polymorphism may occur.



Mass Spectrum Principal ions at m/z 45, 217, 70, 41, 69, 110, 285, 202.



Quantification

Blood **GC** Column: 3% OV-17 on Chromosorb W HP 80/100 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. AFID. Retention time: 6.9 min. Limit of detection, 0.5 mg/L [Mackell, Poklis 1982]. Column: OV-17 Chromosorb WHP 80/100 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. FID or NPD. Retention time: 6.9 min. Limit of detection not reported [Poklis, Mackell 1982]. Column: 3% Dexil 300 on Gas-Chrom Q 100/120 mesh (1.8 m × 3.2 mm i.d.). Carrier gas: Ar: CH_4 (95 : 5), 50 mL/min. Temperature: 265°. ECD. Retention time: 4.8 min. Limit of detection, 0.5 µg/L [Swezey *et al.* 1978]. Column: OV-17 (180 × 0.3 cm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 245°. Limit of detection not reported [Gelbke *et al.* 1978]. Column: 2.5% SE-30 80/100 mesh Chromosorb G (2 m × 2 m long). Carrier gas: N_2, 60 mL/min. Temperature: 200°. FID. Retention time: 5.3 min. Limit of detection not reported [Beckett *et al.* 1970].

See also Berkowitz *et al.* [1969].

GC-MS SIM acquisition mode. Limit of detection 0.5 µg/L [Seno *et al.* 2000].

HPLC Column: Ultrasphere-ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L citrate buffer (pH 4.0) : acetonitrile : pyridine : 70 : 30 : 0.2), flow rate 1.4 mL/min. Electrochemical detection. Limit of detection, 1 ng [Shibanoki *et al.* 1987].

LC-MS Mobile phase: acetonitrile : 10 mmol/L ammonium acetate solution (20 : 80, pH 4.0) containing 0.5% glycerol, flow rate 25 µL/min. FAB, SIM and scan acquisition mode. Limit of detection, 1 and 0.1 ng/g in scan and SIM mode, respectively [Imamura *et al.* 1999].

Plasma **GC** Column: BP 10 (SGE, 25 m × 0.22 mm i.d.). Carrier gas: N_2, 6.2 mL/min. Temperature programme: 200° to 270° at 10°/min for 23 min. NPD. Retention time: 8.88 min. Limit of detection, 4.5 µg/L [Kintz *et al.* 1990]. Column: Nucleosil (25 cm × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L hydrogen phosphate (33 : 67), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 278$ nm, $\lambda_{em} = 324$ nm). Retention time: 6.2 min. Limit of quantification, 4 µg/L; limit of detection, ≈1 µg/L [Moeller *et al.* 1990]. Column: 3% OV-17 Gas Chrom Q 120/140 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 190° to 260° at 10°/min. Retention time: 4.5 min. Limit of detection not reported [Yeh *et al.* 1986]. See Blood [Swezey *et al.* 1978]. Column: 5% OV-17 on Gas Chrom Q 80/100 mesh. Carrier gas: N_2, 22 mL/min for FID, 30 mL/min for ECD. Temperature: 220° for FIB, 235° for ECD. Limit of detection, 8.0×10^{-17} mole/sec for PFB-pentazocine [Brötell *et al.* 1973]. Column: 10% OV-1 on 100/120 mesh Gas Chrom Q (1.5 m × 2 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 215°. ECD. Limit of quantification, 0.1–0.2 mg/L for HFB derivatives [Pittman & Davison 1973].

GC-MS Column: 3% SE-30 Gas-Chrom Q 100/120 mesh (1 m × 3 mm i.d.). Carrier gas: He, 25 mL/min. Temperature: 210°. EI ionisation at 50 eV. MID. Limit of detection, 2 µg/L [Agurell *et al.* 1974].

HPLC Column: LiChrosorb RP-18 (250 × 4 mm i.d., 10 µm). Mobile phase: 0.001 mol/L methanolic acetonitrile : 0.003 mol/L octane-1-sulfonic acid : sodium acetate (2 : 6 : 2), flow rate 1 mL/min. UV detection ($\lambda = 230$ nm). Retention time: 2.75 min. Limit of detection not reported [Misztal, Przyborowski 1991].

Serum **HPLC** Column: Prodigy ODS(3) 100A (150 × 3.2 mm i.d., 5 µm). Mobile phase: 10 mmol/L potassium dihydrogen phosphate (pH 5.8) : absolute ethanol (80 : 20), flow rate 0.7 mL/min. UV detection ($\lambda = 220$ nm). Limit of quantification, 20 µg/L, limit of detection, 15 µg/L [Ameyibor & Stewart 1997]. Column: Ultron ES-OVM (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 10 mmol/L potassium dihydrogen phosphate (pH 5.8; 20 : 5, 3 : 74.7), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 275$ nm, $\lambda_{em} = 335$ nm). Limit of detection, 5 µg/L [Kelly *et al.* 1994]. Column: µBondapak phenyl (30 cm × 4 mm i.d.). Mobile phase: acetonitrile : 0.01% phosphoric acid-0.01% NaCl (35 : 65, pH 2.8), flow rate 1.5 mL/min. UV detection ($\lambda = 210$ nm). Limit of detection, 10 µg/L [Hackett *et al.* 1987].

Urine **GC** Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: CH_4, 30 mL/min. Temperature programme: 150° to 250° at 10°/min. FID. Limit of detection not reported [Reid & Gerbeck 1981]. See Blood [Swezey *et al.* 1978]. Column: 2.5% SE 30 80/100 mesh chromosorb G (2 m × 0.25" i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 220°. FID. Retention time: 9.5 min. Limit of detection not reported [Vaughan, Beckett 1974]. See Plasma [Beckett *et al.* 1970].

Cerebrospinal Fluid **GC-MS** See Plasma [Agurell *et al.* 1974].

Faeces **GC** See Plasma [Beckett *et al.* 1970].

Disposition in the Body Well absorbed after oral, IM, or rectal administration, but undergoes extensive first-pass metabolism after oral administration. Metabolism occurs mainly by extensive oxidation of the methyl groups of the dimethylallyl side-chain to produce the *cis*-hydroxy and the *trans*-carboxylic acid metabolites; the *trans*-hydroxy metabolite appears to be rapidly oxidised to the acid and is not detectable. Glucuronic acid conjugation of unchanged drug and metabolites also occurs; the 3-(3-hydroxy-3-methylbutyl) derivative has also been detected in the urine after overdoses. There is considerable inter-subject variation in the rate of metabolism, and smokers appear to metabolise 40% more pentazocine than non-smokers. After IM administration, 65–76% of a dose is excreted in the urine in 48 h. After an oral dose, up to 13% is excreted as unchanged drug and 12–30% as the conjugate in 24 h, the major proportion being excreted in the first 12 h [Beckett *et al.* 1970; Berkowitz 1971; Pittman 1970]. Less than 2% of a dose is eliminated in the faeces [Beckett *et al.* 1970; Burt, Beckett 1971]. Pentazocine crosses the placenta.

Therapeutic Concentration In plasma, usually in the range 0.05–0.20 mg/L. Blood concentrations may be erratic and more than one peak may occur after IM or oral administration.

Two volunteers were administered an IM injection of pentazocine. Over 1–6 h the pentazocine concentration in whole blood was 13.5–59.3 µg/L, and in urine it was 0.39–4.0 mg/L [Seno *et al.* 2000].

Four patients were administered 30 mg pentazocine IV for analgesia during surgery. At 1 min the blood concentration was 3.2 mg/L, falling to 137 µg/L at 30 min [Shibanoki *et al.* 1987].

Six healthy former drug abusers, aged between 21 and 55 years, were administered 40 or 80 mg IM pentazocine. Mean peak plasma concentrations at 15 min were 102 and 227 µg/L, respectively [Yeh *et al.* 1986].

After an IM dose of 45 mg given to 8 subjects, peak plasma concentrations of 0.11–0. 24 mg/L (mean 0.14) were attained in 1 h. After a single oral dose of 75 mg given to 5 subjects, peak plasma concentrations of 0.11–0.30 mg/L (mean 0.16) were attained in 1–3 h [Berkowitz *et al.* 1969].

Toxicity The estimated minimum lethal dose is 0.3 g, although recovery after ingestion of 1.2 g has been reported. Blood concentrations of 1 mg/L or more may be lethal. Some cases of abuse have been reported, resulting in a mild form of addiction. Pentazocine abuse has been reported to be widespread among 'street addicts' in the USA, in combination with tripelennamine ("T's and Blues').

A 26-year-old black female was found dead at home. Postmortem examination revealed the following pentazocine concentrations: stomach contents, 2.15 mg/g; blood, 41 mg/L; liver, 12.1 µg/g. Zopiclone was also found at the following concentrations: stomach contents, 0.15 mg/g; blood, 1.18 mg/L; liver 5.10 µg/g [van Bocxlaer *et al.* 1996].

In 2 fatalities due to pentazocine ingestion, the following postmortem tissue concentrations were reported: blood 9.2 and 3.3 mg/L, liver 43 and 34 µg/g, urine, –, 4.5 mg/L; ethanol was also detected in blood at concentrations of 200 and 1760 mg/L. Blood- and liver-pentazocine concentrations in other cases taken from the literature were reported to range from 0.8 to 38 mg/L and from 3 to 197 µg/g respectively [Poklis, Mackell 1982].

In 17 fatalities involving IV abuse of pentazocine and tripelennamine, pentazocine blood concentrations of 0 to 11 mg/L (mean, 3) were reported; tripelennamine concentrations ranged from 0–3.0 mg/L (mean, 0.5) [Monforte *et al.* 1983].

Note For a case in which there were problems with putrefaction in the detection of pentazocine, see De Zeeuw *et al.* [1977].

Bioavailability ≈20%.

Half-life Plasma half-life, ≈4 h.

Volume of Distribution ≈5 L/kg.

Clearance Plasma clearance, ≈18 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ≈0.93.

Protein Binding ≈60% in control subjects and 48 to 75% in intracranial tumour or aneurysm patients [Ehrnebo *et al.* 1974].

Note For a review of pentazocine, see Goldstein [1985]. For a review of the pharmacokinetics of narcotic agonists-antagonists, see Bullingham *et al.* [1983].

Dose The equivalent of up to 600 mg of pentazocine daily, by mouth; up to 360 mg daily, given parenterally.

Agurell S *et al.* (1974). Plasma and cerebrospinal fluid concentrations of pentazocine in patients: assay by mass fragmentography. *J Pharm Pharmacol* 26: 1–8.

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Brötell H *et al.* (1973). Gas chromatographic determination of pentazocine in human plasma by means of electron-capture detection. *J Chromatogr* 78: 293–301.

Bullingham RE *et al.* (1983). Clinical pharmacokinetics of narcotic agonist-antagonist drugs. *Clin Pharmacokinet* 8: 332–343.

Burt RA, Beckett AH (1971). The absorption and excretion of pentazocine after administration by different routes. *Br J Anaesth* 43: 427–435.

De Zeeuw RA *et al.* (1977). Analytical problems with putrefaction in a fatal case involving ergotamine and pentazocine. *J Forensic Sci* 22: 550–557.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Ehrnebo M *et al.* (1974). Pentazocine binding to blood cells and plasma proteins. *Clin Pharmacol Ther* 16: 424–429.

Gelbke HP *et al.* (1978). Isolation of drugs from blood by column chromatography on Amberlite XAD-2. *Arch Toxicol* 39: 211–217.

Goldstein G (1985). Pentazocine. *Drug Alcohol Depend* 14: 313–323.

Hackett LP *et al.* (1987). The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography. *J AnalToxicol* 11: 269–271.

Imamura T *et al.* (1999). Liquid chromatography-fast atom bombardment mass spectrometry for detection and determination of pentazocine in human tissues. *J Chromatogr B Biomed Sci Appl* 731: 149–154.

Kelly JW *et al.* (1994). HPLC separation of pentazocine enantiomers in serum using an ovomucoid chiral stationary phase. *Biomed Chromatogr* 8: 255–257.

Kintz P *et al.* (1990). Simultaneous screening and quantification of several nonopiate narcotic analgesics and phencyclidine in human plasma using capillary gas chromatography. *Methods Find Exp Clin Pharmacol* 12: 193–196.

Mackell MA, Poklis A (1982). Determination of pentazocine and tripelennamine in blood of T's and Blue addicts by gas-liquid chromatography with a nitrogen detector. *J Chromatogr* 235: 445–452.

Misztal G, Przyborowski L (1991). Determination of pentazocine in human plasma by high performance liquid chromatography. *Pharmazie* 46: 464–465.

Moeller N *et al.* (1990). High-performance liquid chromatographic determination of pentazocine in plasma. *J Chromatogr* 530: 200–205.

Monforte JR *et al.* (1983). Toxicological and pathological findings in fatalities involving pentazocine and tripelennamine. *J Forensic Sci* 28: 90–101.

Pittman K (1970). Human metabolism of orally administered pentazocine. *Biochem Pharmacol* 19: 1833–1836.

Pittman KA, Davison C (1973). Quantitative determination of pentazocine in plasma and of pentazocine and metabolites in urine. *J Pharm Sci* 62: 765–769.

Poklis A, Mackell MA (1982). Toxicological findings in deaths due to ingestion of pentazocine: a report of two cases. *Forensic Sci Int* 20: 89–95.

Reid RW, Gerbeck CM (1981). Detection of pentazocine and tripelennamine in urine. *Clin Chem* 27: 10–13.

Seno H *et al.* (2000). Determination of pentazocine in human whole blood and urine by gas chromatography/surface ionization organic mass spectrometry. *J Mass Spectrom* 35: 33–38.

Shibanoki S *et al.* (1987). Application of high-performance liquid chromatography with electrochemical detection for monitoring the concentration of pentazocine in human blood. *J Chromatogr* 421: 425–429.

Swezey SE *et al.* (1978). Gas chromatographic analysis of pentazocine. *J Chromatogr* 154: 256–260.

Van Bocxlaer J *et al.* (1996). Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection. *J Anal Toxicol* 20: 52–54.

Vaughan DP, Beckett AH (1974). An analysis of the inter-subject variation in the metabolism of pentazocine. *J Pharm Pharmacol* 26: 789–798.

Yeh SY *et al.* (1986). The pharmacokinetics of pentazocine and tripelennamine. *Clin Pharmacol Ther* 39: 669–676.

Pentetrazol

Respiratory Stimulant

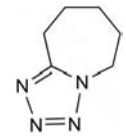
$C_6H_{10}N_4$ = 138.2

CAS—54-95-5

IUPAC Name 6,7,8,9-Tetrahydro-5*H*-tetrazolo[1,5-*a*]azepine

Synonyms Corazol; leptazol; pentamethazol; 1,5-pentamethylenetetrazole; pentazol; pentylenetetrazol.

Proprietary Names *Cardiazol*; *Cenalene-M*; *Coranormol*; *Corazole*; *Corvasol*; *Deumacard*; *Gewazol*; *Korazol*; *Metrazol*; *Phrenazol*; *Ventrazol*.



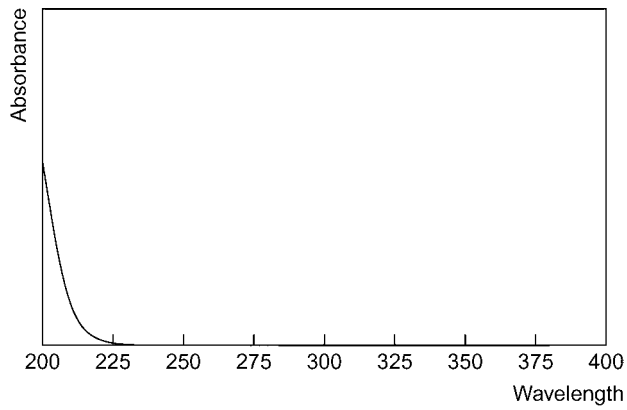
Chemical Properties Colourless crystals or white crystalline powder. Mp 57° to 60°. Soluble 1 in <1 of water, of ethanol, and of chloroform, and 1 in <4 of ether. Log *P* (octanol/water), 0.1.

Thin-layer Chromatography System TA—R_f 0.72; system TB—R_f 0.07; system TC—R_f 0.64; system TE—R_f 0.60; system TL—R_f 0.63; system TAE—R_f 0.72; system TAF—R_f 0.74; system TAJ—R_f 0.70; system TAK—R_f 0.41; system TAL—R_f 0.95 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1550; system GB—RI 1579; system GC—RI 2021.

High Performance Liquid Chromatography System HX—RI 290.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1535, 1115, 1250, 1000, 900, 800 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 55, 82, 41, 39, 54, 42, 56, 109.

Quantification

Plasma **GC** AFID. Limit of detection, 100 µg/L [Bo *et al.* 1979]. FID. Limit of detection, 500 µg/L [Jun *et al.* 1975].

Urine **GC** See Plasma [Jun *et al.* 1975].

Disposition in the Body Readily absorbed after oral or parenteral administration and rapidly metabolised. About 60% of an oral dose is excreted in the urine in 24 h with about 10% as unchanged drug and the remainder as metabolites.

Therapeutic Concentration

Following an oral dose of 100 mg to 3 subjects, who were taking the drug regularly, peak plasma concentrations of 1.5 to 3.1 mg/L (mean, 2.2) were attained in 1.3 to 5 h [Jun *et al.* 1975].

Dose Usually 100 mg parenterally.

Bo LD *et al.* (1979). *J Pharmacol Methods* 2: 29–33.

Jun HW *et al.* (1975). Quantitative GLC determination of pentylenetetrazol in biological fluids. *J Pharm Sci* 64: 1843–1846.

Penthienate Methobromide

Anticholinergic

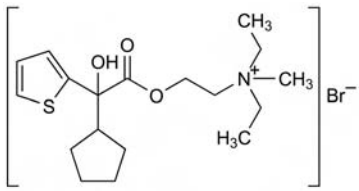
$C_{18}H_{30}BrNO_3S$ = 420.4

CAS—22064-27-3 (penthienate) ($C_{18}H_{30}NO_3S$); 60-44-6 (methobromide)

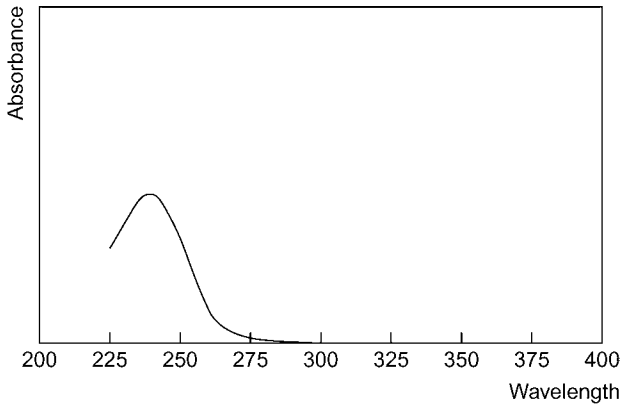
IUPAC Name 2-(2-Cyclopentyl-2-hydroxy-2-thiophen-2-ylacetyl)oxyethyl-diethyl-methylazanium bromide

Synonyms 2-[(Cyclopentylhydroxy-2-thienylacetyl)oxy]-*N*,*N*-diethyl-*N*-methylethanaminium bromide; penthienate bromide

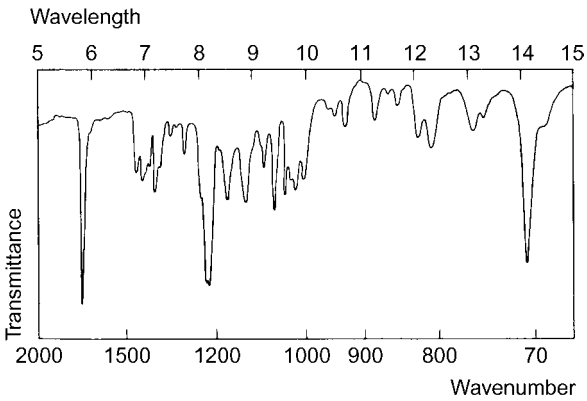
Proprietary Name *Monodral*



Chemical Properties A white crystalline powder. Mp about 125°. Soluble 1 in 5 of water; freely soluble in ethanol and chloroform; insoluble in ether. Log *P* (octanol/water), −0.5.
Colour Tests The following tests are performed on the nitrate: Liebermann's reagent—violet; Mandelin's test—violet; Marquis test—violet; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.02; system TE—R_f 0.03; system TAE—R_f 0.09 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HA—*k* 3.2.
Ultraviolet Spectrum Aqueous acid—238 nm (A^1_1=188a).



Infrared Spectrum Principal peaks at wavenumbers 1739, 1227, 1234, 704, 1064, 1130 cm^{-1} (KBr disk).

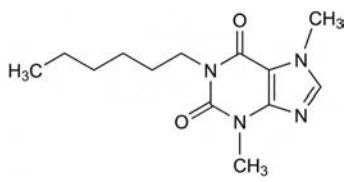


Dose 7.5 to 40 mg daily.

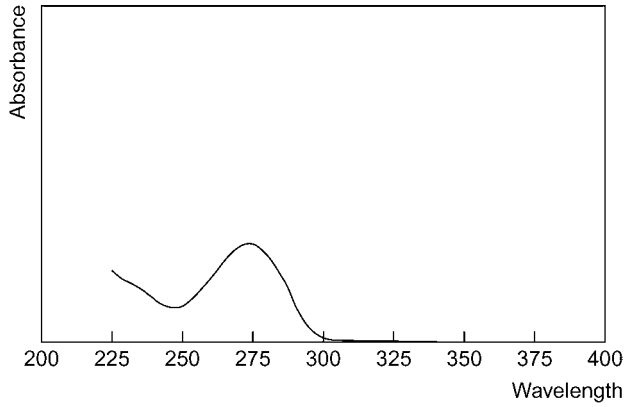
Pentifylline

Vasodilator
$C_{13}H_{20}N_4O_2$ = 264.3
CAS—1028-33-7

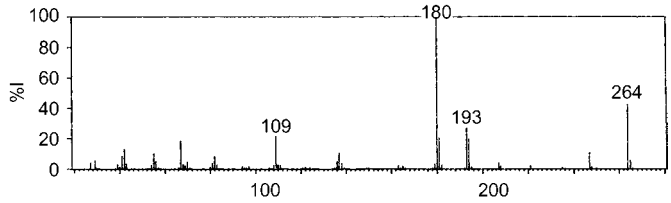
IUPAC Name 1-Hexyl-3,7-dimethylpurine-2,6-dione
Synonyms 1-Hexyl-3,7-dihydro-3,7-dimethyl-1*H*-purine-2,6-dione; 1-hexyltheobromine.
Proprietary Name *Cosaldon*



Chemical Properties A crystalline solid. Mp 82° to 83°. Practically insoluble in water; soluble in ethanol, chloroform and hydrochloric acid. Log *P* (octanol/water), 2.6.
Colour Test Amalic acid test—pink-orange/violet.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.06; system TC—R_f 0.66; system TE—R_f 0.66; system TL—R_f 0.46; system TAE—R_f 0.72 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—pentifylline RI 2240, M (OH-)-AC RI 2560, M (OH-) isomer-1 RI 2295, M (OH-) isomer-2 RI 2505, M (di-OH-)-H_2O isomer-1 RI 2250, M (di-OH-)-H_2O isomer-2 RI 2285, M (di-OH-) RI 2700.
Ultraviolet Spectrum Aqueous acid—275 nm (A^1_1=335b).



Infrared Spectrum Principal peaks at wavenumbers 1652, 1700, 1548, 1220, 760, 750 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 180, 264, 193, 109, 194, 181, 67, 42.

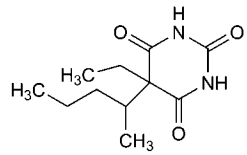


Dose Pentifylline has been given in doses of up to 800 mg daily.

Pentobarbital

Hypnotic, Barbiturate
$C_{11}H_{18}N_2O_3$ = 226.3
CAS—76-74-4

IUPAC Name 5-Ethyl-5-pentan-2-yl-1,3-diazinone-2,4,6-trione
Synonyms Aethaminalum; 5-ethyl-5-(1-methylbutyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; mebubarbital; mebumal; pentobarbitalum; pentobarbitone.
Proprietary Name *Hypnol*



Chemical Properties Colourless crystals or a white crystalline powder. Mp 129° to 130° (from alcohol). A polymorphic form may occur, with an mp of about 115°; it gradually reverts to the more stable form on heating at about 110°. Very slightly soluble in water; soluble 1 in 4.5 of ethanol, 1 in 4 of chloroform and 1 in 10 of ether; very soluble in acetone and methanol. pK_a 8.0 (20°). Log *P* (octanol/buffer pH 7.4), 1.9. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].

Pentobarbital Calcium

$(C_{11}H_{17}N_2O_3)_2Ca$ = 490.6
CAS—7563-42-0 ($C_{11}H_{18}N_2O_3$,xCa)
Synonym Pentobarbitone calcium
Proprietary Name *Repocal*
Chemical Properties A fine white crystalline powder. Sparingly soluble in water; slightly soluble in ethanol; practically insoluble in ether.

Pentobarbital Sodium

$C_{11}H_{17}N_2NaO_3$ = 248.3
CAS—57-33-0
Synonyms Ethaminal sodium; mebumalnatrium; pentobarbitone sodium; soluble pentobarbitone.
Proprietary Names *Nembutal*; *Nova Rectal*; *Pentone*; *Petab*. It is an ingredient of *Cafatine PB* and *Cafergot-PB*.

P

Chemical Properties A white, hygroscopic, crystalline powder or granules. Decomposes at 127°. Freely soluble in water and ethanol; practically insoluble in benzene and ether. A solution in water slowly decomposes.

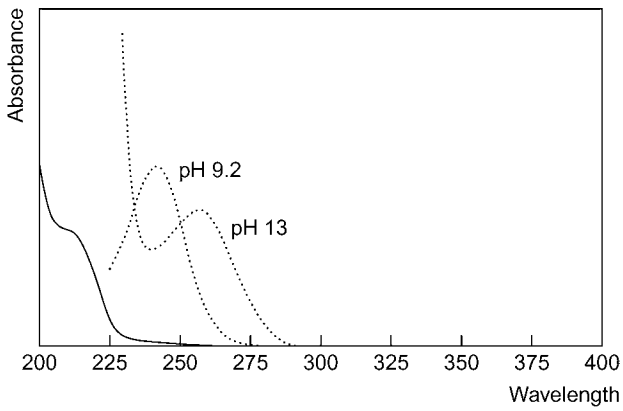
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown-red/violet.

Thin-layer Chromatography System TD—R_f 0.55; system TE—R_f 0.45; system TF—R_f 0.66; system TH—R_f 0.76; system TAD—R_f 0.59; system TAE—R_f 0.90 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; Zwikker's reagent, pink).

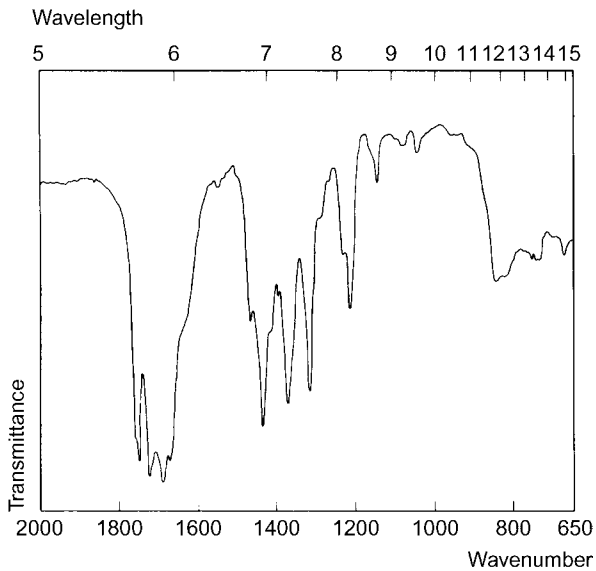
Gas Chromatography System GA—pentobarbital RI 1735, pentobarbital-Me_2 RI 1630, M (3′-OH-) RI 1955, M (3′-OH-)-Me_2 RI 1820; system GB—pentobarbital RI 1776, M (3′-OH-) RI 2039; system GF—RI 2465; system GAJ—RRT 0.803 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HG—*k* 10.96; system HH—*k* 8.07; system HX—RI 424; system HY—RI 383; system HZ—retention time 4.1 min; system HAX—retention time 5.9 min; system HAY—retention time 5.6 min.

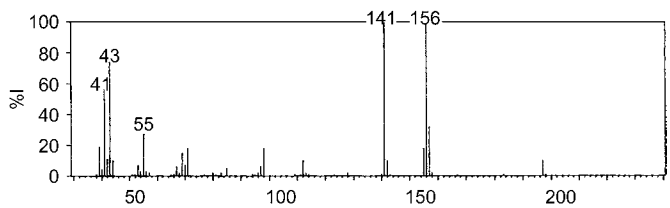
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=438a); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=327b).



Infrared Spectrum Principal peaks at wavenumbers 1685, 1719, 1744, 1315, 1218, 845 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 141, 156, 43, 41, 157, 55, 39, 98; 3′-hydroxypentobarbital 45, 156, 141, 69, 41, 43, 157, 55.



Quantification

Plasma **GC** FID. Limit of detection, 500 µg/L [Hulshoff *et al.* 1979].

GC-MS Pentobarbital and thiopental. Limits of detection, 5 µg/L for pentobarbitaland and 10 µg/L for thiopental [Martens-Lobenhoffer 1999].

Serum **GC** AFID. Limit of detection, 80 µg/L [Sun, Hoffman 1979].

See also under Amobarbital.

Disposition in the Body More than 90% of the sodium salt is absorbed after oral administration. About 80% of a dose is excreted in the urine in 5 days, with about 7% as (+)-3′-hydroxypentobarbital, 30% as the (−)-3′-hydroxy isomer, up to 13% as the *N*-hydroxy metabolite, 7 to 14% as the 3′-oxo metabolite, and about 10 to 15% as the 3′-carboxy derivative. About 1% of a dose is excreted in the urine unchanged.

Pentobarbital is a metabolite of thiopental.

Therapeutic Concentration In plasma, usually in the range 1 to 10 mg/L.

Following a single oral dose of 50 mg to 5 fasting subjects, peak plasma concentrations of 0.62 to 0.88 mg/L (mean 0.73) were attained in 1 h [Smith *et al.* 1973].

After an IV injection of 100 mg to 7 subjects, mean plasma concentrations of 3 mg/L were reported in 6 min [Ehrnebo 1974].

Toxicity The estimated minimum lethal dose is 1 g. Toxic effects are usually associated with blood concentrations greater than about 8 mg/L and concentrations of 12 mg/L or more may produce coma. Fatalities have been associated with plasma concentrations of 8 to 24 to 73 mg/L.

In 55 fatalities, the following postmortem concentrations were reported: blood 5 to 169 mg/L (mean, 30), liver 23 to 550 µg/g (mean, 130) [Baselt, Cravey 1977].

A 51-year-old veterinary official, who committed suicide by self-injecting pentobarbital, had the following postmortem tissue concentrations, 24 h after discovery of the corpse (mg/L or µg/g): peripheral blood 13.5, plasma from peripheral blood 21.7, urine 7.2, gastric contents 18.1 (0.7 mg total), vitreous humour 12.6, brain cortex 33.2, CSF 13.9, bile 67.4, liver 27.5; approximately 1.6 g of pentobarbital was thought to have been administered [Romain *et al.* 2003].

In 3 fatalities caused by pentobarbital, the following postmortem tissue distribution, mg/L or µg/g, was reported:

	Pentobarbital	*3′-Hydroxy metabolite*
Blood	51, 10, 25	ND, ND, 4
Bile	152, 148, 59	-, -, 13
Kidney (left)	46, 16, 38	4, 7, 7
Liver	165, 20, 46	5, 8, 9
Urine	7, 5, 62	-, 82, 65

(ND = not detected)
[Robinson, McDowall 1979].

Half-life Plasma half-life, about 15 to 50 h (mean, 27).

Volume of Distribution About 0.7 to 1 L/kg.

Clearance Plasma clearance, about 0.3 to 0.5 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.93.

Saliva Plasma : saliva ratio, about 3.

Protein Binding About 60 to 70%.

Note For a review of the pharmacokinetics of hypnotic drugs, see Breimer [1977].

Dose 100 mg by mouth or up to 200 mg rectally, as a hypnotic.

Baselt RC, Cravey RH (1977). *J Anal Toxicol* 1: 81–103.
Breimer DD (1977). Clinical pharmacokinetics of hypnotics. *Clin Pharmacokinet* 2: 93–109.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Ehrnebo M (1974). Pharmacokinetics and distribution properties of pentobarbital in humans following oral and intravenous administration. *J Pharm Sci* 63: 1114–1118.
Hulshoff A *et al.* (1979). *Anal Chim Acta* 105: 139–146.
Martens-Lobenhoffer J (1999). Stability of thiopental and pentobarbital in human plasma determined with a new easy and specific gas chromatography-mass spectrometry assay. *Pharmazie* 54: 597–599.
Robinson AE, McDowall RD (1979). The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man. *J Pharm Pharmacol* 31: 357–365.
Romain N *et al.* (2003). Suicide by injection of a veterinarian barbiturate euthanasia agent: report of a case and toxicological analysis. *Forensic Sci Int* 131: 103–107.
Smith RB *et al.* (1973). *J Pharmacokinet Biopharm* 1: 5–16.
Sun SR, Hoffman DJ (1979). Rapid, sensitive GLC determination of pentobarbital and other barbiturates in serum using nitrogen-specific detector. *J Pharm Sci* 68: 386–388.

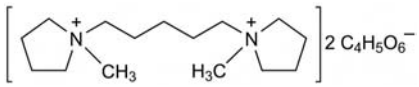
Pentolonium Tartrate

Antihypertensive

$C_{23}H_{42}N_2O_{12}$ = 538.6

CAS—144-44-5 (pentolonium); 52-62-0 (tartrate)

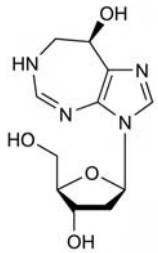
IUPAC Name 1-Methyl-1-[5-(1-methylpyrrolidin-1-ium-1-yl)pentyl]pyrrolidin-1-ium; 2,3,4-trihydroxy-4-oxobutanoate
Synonyms 1,1′-(1,5-Pentanediyl)bis[1-methylpyrrolidinium] salt with [*R*-(*R**, *R**)]-2,3-dihydroxybutanedioic acid (1:2); pentapyrrolidinium bitartrate; pentolinium tartrate.
Proprietary Names *Ansolysen*; *Pentilium*.



Chemical Properties A white powder. Mp 203°, with decomposition. Soluble 1 in 0.4 of water and 1 in 810 of ethanol; insoluble in chloroform and ether.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.00; system TAF—R_f 0.01 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HX—RI 48.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1299, 1256, 1064, 1208, 1129, 676 cm^{-1} (KBr disk).
Disposition in the Body Incompletely and irregularly absorbed after oral administration. Excreted in the urine as unchanged drug.
Dose 2.5 to 25 mg IV. Doses of 20 to 900 mg daily have been given by mouth.

Pentostatin

Antineoplastic
$C_{11}H_{16}N_4O_4 = 268.3$
CAS—53910-25-1
IUPAC Name (8*R*)-3-[(2*R*,4*S*,5*R*)-4-Hydroxy-5-hydroxymethyl)oxolan-2-yl]-7,8-dihydro-4H-imidiazo[4,5-*d*][1,3]diazepin-8-ol
Synonyms CI-825; CL-67310405; covidarabine; DCF; 2′-dCF; 2′-deoxycoformycin; (8*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,7,8-tetrahydroimidazole [4,5-*d*][1,3]diazepin-8-ol; NSC-218321; PD-81565.
Proprietary Name *Nipent*

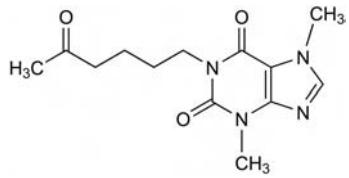


Chemical Properties White crystals from methanol/water. Mp 220° to 225°; also reported as 204° to 209.5° with darkening at >150°. pK_a 5.2. Log *P* (octanol/water), −2.54.
Quantification
Plasma **Enzyme Inhibition Assay** Limit of quantification, 1 µg/L [Staubus *et al.* 1984].
Disposition in the Body Pentostatin penetrates the blood–brain barrier and the concentration in the cerebrospinal fluid reaches 10 to 12% of that observed in plasma within 2–4 h after an IV administration of a 5–30 mg/m^2 (0.25 mg/kg) dose. The drug is metabolised by phosphorylation to mono-, di- and triphosphate derivatives, and is recovered in urine unchanged. 50–96% of a single dose is excreted through the kidney in 24 h.
Therapeutic Concentration
An IV dose of 0.25–1 mg/kg (7.5–30 mg/m^2) generates peak plasma concentrations of 2–6 µmol/L observed at 1 h. No accumulation of the drug occurs with greater than 3 consecutive doses of 1 mg/kg pentostatin [Venner *et al.* 1981].
Toxicity
In early clinical trials, considerable toxicity was observed with the administration of pentostatin, affecting the kidneys (acute renal failure), central nervous system (depression, seizures and coma) and the immune system with severe and often fatal opportunistic infections. These are frequently associated with the higher doses; 10–20 mg/m^2 daily for 3–5 consecutive days) [Brogden, Sorkin 1993].
Half-life Plasma 3–15 h (measured with sampling for 24 h); may be increased in patients with impaired renal function.
Volume of Distribution 42.4 L (terminal phase); 36.9 L (steady state).
Clearance Total body plasma, 52.5–64 mL/min/m^2 (3.14–3.84 L/h/m^2); 27 mL/min/m^2 (1.6 L/h/m^2) in patients with renal impairment.
Protein Binding 1.9–4.9% (at therapeutic plasma concentrations).
Note For a review of the pharmacokinetics of pentostatin, see Brogden and Sorkin [1993].
Dose 4 mg/m^2 every 1 to 2 weeks.

Brogden RN, Sorkin EM (1993). Pentostatin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in lymphoproliferative disorders. *Drugs* 46: 652–677.
Staubus AE *et al.* (1984). An enzymatic kinetic method for the determination of 2′-deoxycoformycin in biological fluids. *Biochem Pharmacol* 33(10): 1633–1637.
Venner PM *et al.* (1981). Levels of 2′-deoxycoformycin, adenosine, and deoxyadenosine in patients with acute lymphoblastic leukemia. *Cancer Res* 41: 4508–4511.

Pentoxifylline

Vasodilator
$C_{13}H_{18}N_4O_3 = 278.3$
CAS—6493-05-6
IUPAC Name 3,7-Dimethyl-1-(5-oxohexyl)purine-2,6-dione
Synonyms 3,7-Dihydro-3,7-dimethyl-1-(5-oxohexyl)-1*H*-purine-2,6-dione; oxpentifylline.
Proprietary Names *Torental*; *Trental*.



Chemical Properties White crystalline powder. Mp 102° to 105°. Soluble in water; sparingly soluble in ethanol; freely soluble in chloroform and methanol; slightly soluble in ether. pK_a 0.3. Log *P* (octanol/water), 0.3. Stable in breast milk when stored at –15° for 3 weeks [Bauza *et al.* 1984].
Colour Test Amalic acid test—pink/violet.
Thin-layer Chromatography System TAE—R_f 0.64; system TE—R_f 0.55.
Gas Chromatography System GA—RI 2406.
High Performance Liquid Chromatography System HAA—RT 11.5 min; system HX—RI 355; system HY—RI 274; system HZ—RT 2.1 min; system HAX—RT 5.0 min; system HAY—RT 4.2 min.
Ultraviolet Spectrum Aqueous acid—274 nm (A^{1}_{1} = 380a). No alkaline shift.
Infrared Spectrum Principal peaks at wavenumbers 1660, 1700, 1720, 1550, 762, 752 cm^{-1} (KBr disk).
Quantification
Blood **GC** Column: Restek RTX-50 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min for 12 min to 3 mL/min over 5 min for 3.5 min. Temperature programme: 220° for 3 min to 280° at 10°/min for 2 min to 300° at 40°/min for 5.5 min. NPD. Limit of detection, 0.16 mg/L [Engelhart *et al.* 1997].
HPLC Column: LC-8-DB (25 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (24 : 76), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention time: 6.4 min. Limit of detection, 5 µg/L [Grasela, Rocci, Jr. 1987].
Plasma **GC** Column: DB-5 phenylmethyl (30 × 0.32 mm i.d.). NPD. Limit of detection, 5 µg/L [Cleary *et al.* 1999]. Column; HP-ultra 2 cross-linked methylphenylsilicone (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 130° for 1 min to 250° at 15°/min for 5 min. FID Retention time: 14.8 min. Limit of detection not reported [Suarez-Penaranda *et al.* 1998]. Column: 3% V-73 on Chromosorb W HP 100/120 mesh (25 m × 0.31 mm i.d., 0.52 µm). Carrier gas: He, 35 cm/s. Temperature: 250°. NSD. Limit of detection, 5 µg/L [Burrows 1987]. Column: 3% OV-1 on Chromosorb W HP 100/120 mesh (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 43 cm/s. Temperature: 230°. NSD. Limit of detection, 2 µg/L [Burrows, Jolley 1985]. Column: 3% OV-25 on Chromosorb W HP 100/120 mesh (2 m × 1.75 mm i.d.). Carrier gas: He, 25 mL/min. Temperature: 235°. Retention time: 8.8 min. AFID. Limit of detection 3–10 µg/L for pentoxifylline and 5-hydroxyhexyl metabolite [Bryce, Burrows 1980].
HPLC Column: KR100-5-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.02 mol/L phosphoric acid (pH 4.0) : methanol : tetrahydrofuran (55 : 45 : 1), flow rate 1.0 mL/min. UV detection (λ = 273 nm). Limit of quantification, 12.5 µg/L, limit of detection, 3 µg/L [Wong *et al.* 1998]. Column: LiChrospher 100 RP-18 (5 µm). Mobile phase: water : dioxane : acetonitrile (87 : 6.5 : 6.5) with 0.5% acetic acid. UV detection (λ = 275 nm). Limit of detection, 25 µg/L [Mancinelli *et al.* 1992]. Column: Zorbax ODS (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water: acetic acid (22 : 77.9 : 0.1), flow rate 0.75 mL/min. UV detection (λ = 274 nm). Limit of detection, 53 nmol/L [Lambert *et al.* 1989]. Column: LiChrosorb cyanopropyl (12.5 × 0.4 cm i.d., 5 µm). Mobile phase: acetonitrile : water (1 : 99), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Limit of detection, 10 µg/L [Musch *et al.* 1989]. Column: µBondapak C_{18} (30 cm × 3.9 mm i.d.). Mobile phase; water : methanol (55 : 45), flow rate 1.5 mL/min. UV detection (λ = 273 nm). Retention time: 5.3 min. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Garnier-Moiroux *et al.* 1987]. Column: LiChrosorb RP 18 (25 cm × 4.9 mm i.d., 10 µm). Mobile phase: methanol : water (48 : 52), flow rate 2.0 mL/min. UV detection (λ = 268 nm). Limit of detection, 10 µg/L for pentoxifylline and 5-hydroxyhexyl metabolite [Rieck, Platt 1984].
See also Chivers *et al.* [1981].
LC-MS Column: Phenomenex Luna $C_8(2)$ (150 × 1.0 mm i.d., 5 µm). Mobile phase: 0.5 mmol/L ammonium acetate (pH 3.5) : methanol (50 : 50). TIS, positive ion mode, MRM acquisition mode. Limit of detection, 1 µg/L [Kyle *et al.* 2005].
Serum **GC-MS** Column: HP1 (30 m × 0.53 mm i.d., 2.65 µm). Carrier gas: N_2, 25 mL/min. Temperature programme: 200° for 0.01 min to 250° at 10°/min for 7 min. NPD. Limit of detection, 2 and 10 µg/L for pentoxifylline and its major metabolite, respectively [Marko, Bauerová 1991].

P

HPLC Column: Octadecyl silane reversed phase (15 cm × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid (18 : 81.9 : 0.1), flow rate 1.0 mL/min. UV detection (λ= 274 nm). Retention time: 8.0 min. Limit of detection not reported [Poondru *et al.* 2000].

Urine **GC** See Blood [Engelhart *et al.* 1997]. See Plasma. Limit of detection, 0.1–0.2 mg/L [Burrows, Jolley 1985].

HPLC Column: ODS Spherisorb (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.2 mol/L formic acid : tetrahydrofuran (93 : 7), flow rate 1 mL/min. UV detection (λ= 274 nm). Limit of detection not reported [Bryce *et al.* 1989]. See Plasma [Lambert *et al.* 1989]. Column: Spherisorb-ODS 1 (15 cm × 3 mm i.d., 5 µm). Mobile phase: methanol : 0.02 mol/L orthophosphoric acid (pH 4, 1 : 2.5), flow rate 1.0 mL/min. UV detection (λ= 274 nm). Retention time: 18 min. Limit of detection, 1 mg/L for CB-DMX [Bryce *et al.* 1985].

Bile **GC** See Blood [Engelhart *et al.* 1997].

Breast Milk **GC** Column: 3% OV-17 on Chromosorb W-HP 100/120 mesh. Carrier gas: He, 25 mL/min. Temperature: 245°. NPD. Retention time: ≈8 min. Limit of detection, 10 µg/L [Bauza *et al.* 1984].

Gastric Contents **GC-MS** Column: Column; HP-ultra 2 cross-linked methylphenylsilicone (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 130° for 1 min to 250° at 15°/min for 5 min. EI ionisation at 70 eV, MSD. Retention time: 14.8 min. Limit of detection not reported [Suarez-Penaranda *et al.* 1998]. See Blood [Engelhart *et al.* 1997].

Disposition in the Body Readily absorbed after oral administration. It is extensively metabolised and several metabolites have been identified in the urine. About 60% of a dose is excreted in the urine as metabolites in 24 h, mostly in the first 4 h; the major urinary metabolite, 1-(3-carboxypropyl)-3,7-dimethylxanthine (inactive), accounts for about 80% of the material excreted in the urine; <1% is excreted as unchanged drug. Other metabolites include 1-(5-hydroxyhexyl)-3,7-dimethylxanthine, which is active and is the major metabolite detected in blood, 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine, 1-(4,5-dihydroxyhexyl)-3,7-dimethylxanthine, 1-(4-carboxybutyl)-3,7-dimethylxanthine, 3-methyl-1-(5-oxohexyl)xanthine and 1-(5-hydroxyhexyl)-3-methylxanthine, all of which are inactive. Pentoxyfylline and its major metabolites are present in human milk 2 h after administration to the mother [Witter, Smith 1985].

Therapeutic Concentration

After a single oral dose of 400 mg to 16 fasting subjects, mean peak plasma concentrations of 1.3 mg/L of pentoxifylline and 1.8 mg/L of the 5-hydroxy metabolite were attained in 0.8 and 1.3 h, respectively [Wills *et al.* 1980].

After the administration of a 400 mg oral dose to the mother, 4 h later a 2.5 kg infant taking 4 oz of milk would have 1.69 ± 1.25 µg/kg pentoxyfylline [Witter, Smith 1985].

After the administration of 100, 200 and 400 mg pentoxyfylline to healthy volunteers mean maximum plasma concentrations of 272, 683 and 1607 µg/L were reached at 0.39, 0.41 and 0.29 h, respectively [Smith *et al.* 1986].

Note For the pharmacokinetics of pentoxifylline in patients with renal insufficiency see Paap *et al.* [1996].

Toxicity A 54-year-old male died after intentional ingestion of a massive overdose of pentoxifylline (estimated to be 20–24 g); plasma concentrations were as high as 32.5 g/L [Suarez-Penaranda *et al.* 1998].

Half-life Plasma half-life, pentoxifylline, about 1 h, 5-hydroxy metabolite about 1 h; 1-(3-carboxypropyl)-3,7-dimethylxanthine, derived from urinary excretion data, about 1.4 h.

Volume of Distribution ≈5 L/kg.

Clearance Plasma clearance, ≈70 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ≈1.2.

Dose Usually 300 to 600 mg daily; sustained-release tablets are given in doses of 0.8 to 1.2 g daily.

Bauza MT *et al.* (1984). Gas chromatographic determination of pentoxifylline and its major metabolites in human breast milk. *J Chromatogr* 310: 61–69.

Bryce TA, Burrows JL (1980). Determination of oxpentifylline and a metabolite, 1-(5′-hydroxyhexyl)-3,7-dimethylxanthine, by gas–liquid chromatography using a nitrogen-selective detector. *J Chromatogr* 181: 355–361.

Bryce TA *et al.* (1985). Determination of 1-(3′-carboxypropyl)-3,7-dimethylxanthine and 1-(4′-carboxybutyl)-3,7-dimethylxanthine, two major metabolites of oxpentifylline, in urine by high-performance liquid chromatography. *J Chromatogr* 344: 397–402.

Bryce TA *et al.* (1989). Metabolism and pharmacokinetics of 14C-pentoxifylline in healthy volunteers. *Arzneimittelforschung* 39: 512–517.

Burrows JL (1987). Determination of oxpentifylline and three metabolites in plasma by automated capillary gas chromatography using nitrogen-selective detection. *J Chromatogr* 423: 139–146.

Burrows JL, Jolley KW (1985). Determination of oxpentifylline and four metabolites in plasma and urine by automated capillary gas chromatography using nitrogen-selective detection. *J Chromatogr* 344: 187–198.

Chivers DA *et al.* (1981). Simultaneous determination of pentoxifylline and its hydroxy metabolite in plasma by high-performance liquid chromatography. *J Chromatogr* 225: 261–265.

Cleary JD *et al.* (1999). Administration of crushed extended-release pentoxifylline tablets: bioavailability and adverse effects. *Am J Health Syst Pharm* 56: 1529–1534.

Engelhart DA *et al.* (1997). Diltiazem and pentoxifylline determination in postmortem specimens. *J Anal Toxicol* 21: 576–579.

Garnier-Moiroux A *et al.* (1987). High-performance liquid chromatographic determination of pentoxifylline and its hydroxy metabolite in human plasma. *J Chromatogr* 416: 183–188.

Grasela DM, Rocci MLJr (1987). High-performance liquid chromatographic analysis of pentoxifylline and 1-(5′-hydroxyhexyl)-3,7-dimethylxanthine in whole blood. *J Chromatogr* 419: 368–374.

Kyle PB *et al.* (2005). Use of liquid chromatography-tandem mass spectrometry for the analysis of pentoxifylline and lisofylline in plasma. *Biomed Chromatogr* 19: 231–236.

Lambert WE *et al.* (1989). Simultaneous determination of pentoxifylline and three metabolites in biological fluids by liquid chromatography. *Clin Chem* 35: 298–301.

Mancinelli A *et al.* (1992). Determination of pentoxifylline and its metabolites in human plasma by high-performance liquid chromatography with solid-phase extraction. *J Chromatogr* 575: 101–107.

Marko V, Bauerová K (1991). Study of the solid phase extraction of pentoxifylline and its major metabolite as a basis of their rapid low concentration gas chromatographic determination in serum. *Biomed Chromatogr* 5: 256–261.

Musch G *et al.* (1989). Determination of pentoxifylline and its 5-hydroxy metabolite in human plasma by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 495: 215–226.

Paap CM *et al.* (1996). Multiple-dose pharmacokinetics of pentoxifylline and its metabolites during renal insufficiency. *Ann Pharmacother* 30: 724–729.

Poondru S *et al.* (2000). Correlation of serum and salivary levels of pentoxifylline. *Ther Drug Monit* 22: 432–436.

Rieck W, Platt D (1984). Determination of 3,7-dimethyl-1-(5-oxohexyl)-xanthine (pentoxifylline) and its 3,7-dimethyl-1-(5-hydroxyhexyl)-xanthine metabolite in the plasma of patients with multiple diseases using high-performance liquid chromatography. *J Chromatogr* 305: 419–427.

Smith RV *et al.* (1986). Pharmacokinetics of orally administered pentoxifylline in humans. *J Pharm Sci* 75: 47–52.

Suarez-Penaranda *et al.* (1998). A fatal case of suicidal pentoxifylline intoxication. *Int J Legal Med* 111: 151–153.

Wills RJ *et al.* (1980). Influence of food on the bioavailability of Trental® (pentoxifylline) in man. *Drug Dev Ind Pharm* 7: 385–396.

Witter FR, Smith RV (1985). The excretion of pentoxifylline and its metabolites into human breast milk. *Am J Obstet Gynecol* 151: 1094–1097.

Wong JW *et al.* (1998). Simple high-performance liquid chromatographic method for determination of pentoxifylline in human plasma. *J Chromatogr B Biomed Sci Appl* 716: 387–391.

Perazine

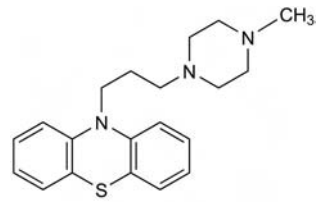
Tranquilliser

$C_{20}H_{25}N_3S$ = 339.5

CAS—84-97-9

IUPAC Name 10-[3-(4-Methylpiperazin-1-yl)propyl]phenothiazine

Synonyms 10-[3-(4-Methyl-1-piperazinyl)propyl]-10*H*-phenothiazine; pemazine; P-725.



Chemical Properties Crystals. Mp 51° to 53°. pK_a 8.0. Log *P* (octanol/pH 7.4), 2.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005] Serum samples were stable for up to 8 h at room temperature and up to 2 weeks when stored at 4° and -20° [Tanaka *et al.* 2007]. Standard solutions are stable for several months at -20° in the dark [Sachse *et al.* 2006].

Perazine Dimalonate

$C_{20}H_{25}N_3S,2C_3H_4O_4$ = 547.6

CAS—14777-25-4

Proprietary Name *Taxilan*

Chemical Properties A white crystalline powder. Mp 114° to 116°. Soluble 1 in 1 of water, 1 in 33 of ethanol, and 1 in 250 of chloroform.

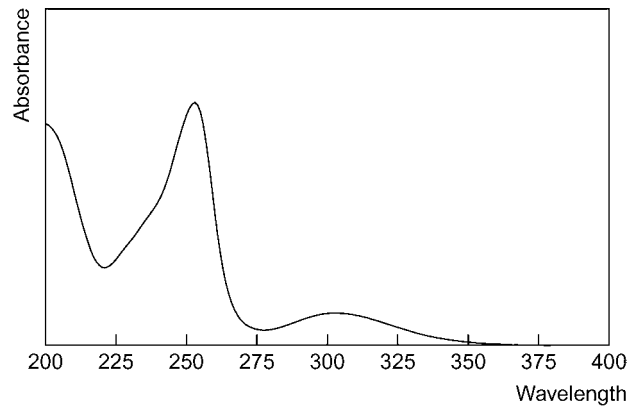
Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—brown-orange; FPN reagent—brown-orange; Mandelin's test—green→violet; Marquis test—violet; Sulfuric acid—violet.

Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.25; system TC—R_f 0.37; system TE—R_f 0.47; system TL—R_f 0.03; system TAE—R_f 0.21; system TAF—R_f 0.23 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—perazine RI 2798; M (OH-) RI 3175; M (phenothiazine) RI 2120.

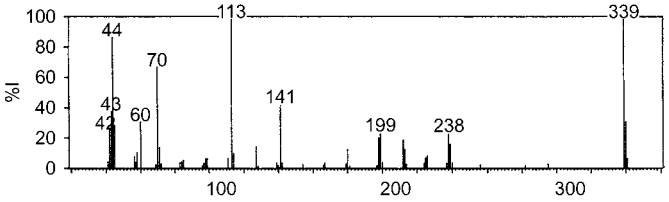
High Performance Liquid Chromatography System HX—RI 403; system HY—RI 371; system HZ—RT 6.3 min.

Ultraviolet Spectrum Aqueous acid—251 (A^1_1 = 875a), 300 nm[Breyer 1969].



Infrared Spectrum Principal peaks at wavenumbers 748, 1279, 1244, 1163, 1143, 1570 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 113, 339, 44, 70, 141, 43, 60, 340.



Quantification

Blood **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 80° for 1 min to 300° at 10°/min for 5 min. Limit of detection not reported [Musshoff *et al.* 1998].

HPLC Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : tetramethylethylenediamine (8 : 92 : 0 to 37.5 : 62.1 : 0.4 at 5 min for 10 min to 8 : 92 : 0 at 15 min for 10 min), flow rate 1.3 mL/min. UV detection (λ = 254 nm). Retention time: 19.6 min. Limit of detection, 50 µg/L [Sachse *et al.* 2006]. Column: Lichrosorb RP8 (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : potassium phosphate buffer (pH 2.3, 30 : 70), flow rate 1.0 mL/min. DAD. Limit of detection not reported [Musshoff *et al.* 1998].

Plasma **GC** Column: 3% XE-60 on 80/100 mesh GasChrom Q (1.83 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 240°. FID. Retention time: 0.41 min. Limit of detection, 100 µg/L [Vanderheeren, Theunis 1976].

HPLC Column: SymmetryShield RP8 (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate and 0.1% formic acid : acetonitrile (70 : 30 to 0 : 100 at 15 min), flow rate 0.2 mL/min. UV detection (λ = 250 nm). Limit of detection, 0.104 mg/L [Marumo *et al.* 2005]. Column: Nucleosil $5C_{18}$ (25 × 0.46 cm i.d.). Mobile phase: perchloric acid (pH 2.5) : acetonitrile (60 : 40), flow rate 1.2 mL/min. Electrochemical detection. Retention time: 6.4 min. Limit of detection, 1 µg/L [Breyer-Pfaff *et al.* 1988]

Serum **GC** Column: 3% OV-17 Gaschrom Q 100/120 mesh (1.9 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 270°. FID. Limit of detection, 20 µg/L [Schley *et al.* 1978].

HPLC Column: Inersil ODS-SP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 300 : 200 : 500), flow rate 0.9 mL/min. UV detection (λ = 250 nm). Retention time: 21.1 min. Limit of quantification, 4.6 µg/L [Tanaka *et al.* 2007].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 7.3 µg/L [Kirchherr, Kühn-Velten 2006].

Note For a TLC method for the detection of perazine in serum, see Kresse *et al.* [1980].

Urine **HPLC** See Plasma. Limit of detection, 0.049 mg/L [Marumo *et al.* 2005].

Brain **GC-MS** See Blood [Musshoff *et al.* 1998].

HPLC See Blood [Musshoff *et al.* 1998].

Kidney **GC-MS** See Blood [Musshoff *et al.* 1998].

HPLC See Blood [Musshoff *et al.* 1998].

Liver **GC-MS** See Blood [Musshoff *et al.* 1998].

HPLC See Blood [Musshoff *et al.* 1998].

Disposition in the Body Perazine is absorbed after oral administration but undergoes extensive metabolism by demethylation, sulfoxidation, *N*-oxidation and aromatic hydroxylation, followed by conjugation with glucuronic acid. Approximately 15 to 30% of an oral dose is excreted in the urine, mainly as conjugated phenolic metabolites. CYP1A2 and CYP3A4 are the main P450 isoforms catalysing 5-sulfoxidation (32 and 30%, respectively), while CYP2C19 is the main isoenzyme involved in *N*-demethylation (68%) [Wójcikowski *et al.* 2004].

Therapeutic Concentration Following daily oral doses of 300 to 400 mg given to 14 subjects for at least 4 days, plasma concentrations of 0.02–0.34 mg/L (mean 0.11) perazine and 0.04–0.54 mg/L (mean 0.17) desmethyl metabolite were reported [Breyer, Villumsen 1976].

Toxicity Perazine has been associated with acute toxic hepatitis [Pantel, Schroder 1996].

A 22-year-old woman who died after ingesting perazine had the following postmortem tissue concentrations: heart blood 9.6 µg/g, muscle 4.1 µg/g, brain 32 µg/g, liver 176 µg/g, kidney 64 µg/g and lungs 83 µg/g. Analytical results led to the assumption that 14 to 30 Taxilan 100-mg tablets had been ingested, probably intentionally [Kaferstein *et al.* 2000].

A 51-year-old woman who committed suicide by ingesting perazine and moclobemide had blood concentrations of 1.27 and 49.9 mg/L, respectively; some metabolites were also detected in the blood [Musshoff *et al.* 1998].

Note For a case of acute psychotic disorder following perazine, see Hesslinger *et al.* [1996].

Protein Binding ≈94–97%.

Dose Usually the equivalent of 25 to 600 mg perazine daily; up to 1000 mg daily in resistant cases.

Breyer U (1969). Urinary metabolites of 10-[3′-(4″-methylpiperazinyl)-propyl]-phenothiazine (perazine) in psychiatric patients. I. Isolation, identification and determination of metabolites. *Biochem Pharmacol* 18: 777–788.

Breyer U, Villumsen K (1976). Measurement of plasma levels of tricylic psychoactive drugs and their metabolites by UV reflectance photometry of thin layer chromatograms. *Eur J Clin Pharmacol* 09: 457–465.

Breyer-Pfaff U *et al.* (1988). Single-dose kinetics of the neuroleptic drug perazine in psychotic patients. *Psychopharmacology (Berl)* 95: 374–377.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Hesslinger B *et al.* (1996). An acute psychotic disorder caused by pefloxacin: a case report. *Prog Neuropsychopharmacol Biol Psychiatry* 20: 343–347.

Kaferstein H *et al.* (2000). [Poisoning by perazine: organ distribution and interpretation.]. *Arch Kriminol* 206: 82–87.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Kresse M *et al.* (1980). Reliable routine method for determination of perazine in serum by thin-layer chromatography with an internal standard. *J Chromatogr* 183: 475–482.

Marumo A *et al.* (2005). Analysis of phenothiazines in human body fluids using disk solid-phase extraction and liquid chromatography. *J AOAC Int* 88: 1655–1660.

Musshoff F *et al.* (1998). Suicide with moclobemide and perazine. *Int J Legal Med* 111: 196–198.

Pantel J, Schroder J (1996). Acute hepatitis, rhabdomyolisis and pancytopenia associated with perazine therapy. *Pharmacopsychiatry* 29: 43.

Sachse J *et al.* (2006). Automated analysis of quetiapine and other antipsychotic drugs in human blood by high performance-liquid chromatography with column-switching and spectrophotometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 830: 342–348.

Schley J *et al.* (1978). Determination of perazine serum levels by gas liquid chromatography under clinical routine conditions. *J Clin Chem Clin Biochem* 16: 307–311.

Tanaka E *et al.* (2007). Simple and simultaneous determination for 12 phenothiazines in human serum by reversed-phase high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 116–120.

Vanderheeren FA, Theunis DJ (1976). Gas–liquid chromotographic determination of perazine, thioridazine and thioridazine metabolites in human plasma. *J Chromatogr* 120: 123–128.

Wójcikowski J *et al.* (2004). The metabolism of the piperazine-type phenothiazine neuroleptic perazine by the human cytochrome P-450 isoenzymes. *Eur Neuropsychopharmacol* 14: 199–208.

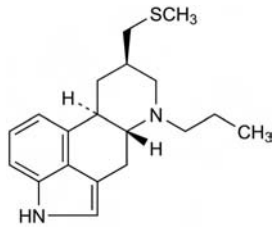
Pergolide

Dopaminergic Agent, Antiparkinsonian

$C_{19}H_{26}N_2S$ = 314.5

CAS—66104-22-1

Synonyms LY-141B; (8β)-8-[(Methylthio)methyl]-6-propylergoline.



Chemical Properties Mp 206° to 209°. pK_a 7.8. Log *P* (octanol/water), 4.02; log *P* (chloroform/water, pH 2.19), 6.14.

Pergolide Mesilate

$C_{19}H_{26}N_2S,CH_3SO_3H$ = 410.6

CAS—66104-23-2

Synonym LY-127809

Proprietary Names *Celance*; *Nopar*; *Permax*.

Chemical Properties A white to off-white crystalline powder. Mp 225°; also reported as 258° to 260° (decomposition). Sparingly soluble in dimethylformamide and methanol; slightly soluble in water, 0.01 mol/L hydrochloric acid, chloroform, acetonitrile, dichloromethane and dehydrated ethanol; very slightly soluble in acetone; practically insoluble in 0.1 mol/L sodium hydroxide and 0.1 mol/L hydrochloric acid and ether. Log *P* (chloroform/water, pH 4.02), 119.6.

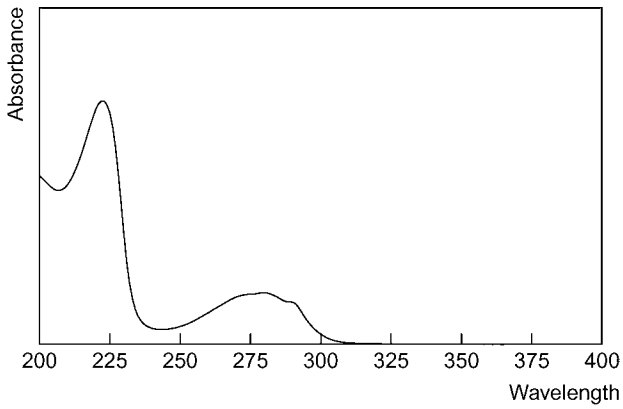
Colour Test Neubauer rhode—deep purplish blue.

Thin-layer Chromatography Plate: silica gel 60 F_{254}. Mobile phase: methanol : $CHCl_3$: acetone : NH_4OH (7 : 63 : 27 : 3). UV detection (λ = 254 and 280 nm). R_f 0.70 [Rubin *et al.* 1981].

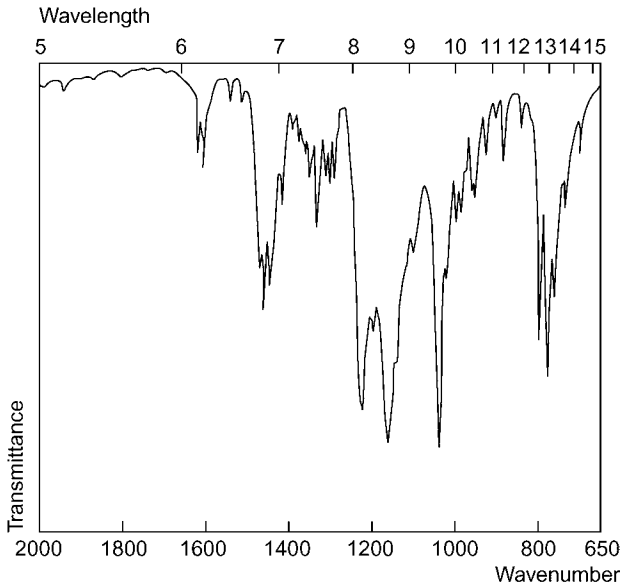
High Performance Liquid Chromatography System HZ—retention time 5.4 min.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. UV diode array detection. Retention time: 3.6 min [Mills, Roberson 1993].

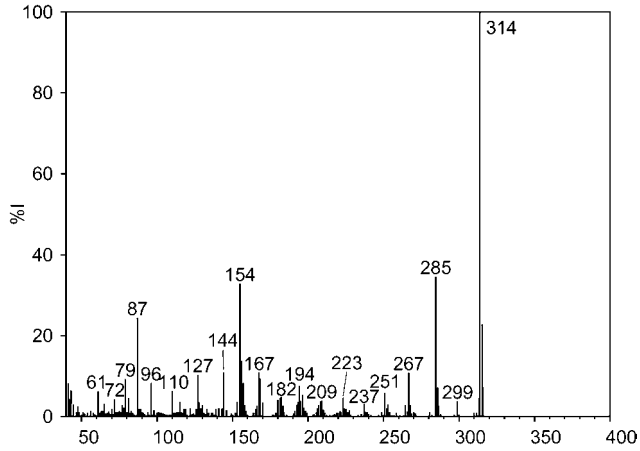
Ultraviolet Spectrum (Pergolide mesylate) Aqueous acid (0.2 mol/L H_2SO_4)—222, 279 nm; (methanol)—280 nm; (dehydrated ethanol)—281 nm; basic—279 nm.



Infrared Spectrum Principal peaks at wavenumbers (pergolide mesilate) 1456, 1157, 1038 and 775 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 314, 285, 154, 87, 315, 155, 144, 167.



Disposition in the Body Pergolide is rapidly absorbed after oral administration from the gastrointestinal tract and appears in plasma 15–30 min after dosing. Ten metabolites have been identified including *N*-despropylpergolide, pergolide sulfoxide and pergolide sulfone. It is known that these are not produced by β-glucuronidation or sulfate conjugation. It is excreted mainly as the metabolites in urine (55% of an administered dose), 40% in faeces, 5% in expired carbon dioxide and the remainder is absorbed.

Therapeutic Concentration

Three healthy male volunteers, aged 31, 42 and 47 years, were administered a dose equivalent to 0.138 mg pergolide base. Peak plasma concentrations of 1.8 mg/L were reached within 1–2 h. The dose was completely cleared within 4–5 days [Rubin *et al.* 1981].

Toxicity

Overdoses of 60 mg on one day, 19 mg daily for 3 days, and 14 mg daily for 23 days have occurred. Vomiting, hypotension, agitation, severe hallucinations, severe involuntary movements and tingling sensations were observed. Another patient administered with 7 mg pergolide, instead of 0.7 mg suffered from palpitations and hypotension [Eli Lilly and Company Ltd].

Protein Binding 90%.

Dose The initial dose is 50 μg daily for 2 days. This may be increased over the next 12 days as 100–150 μg steps every third day. Further increases of 250 μg every third day until an optimal therapeutic dosage is reached. The usual maintenance dose is 2 to 2.5 mg daily.

Eli Lilly and Company Ltd. Supplied data.
Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn. Vol. 6. Boca Raton: CRC Press, 196–197.
Rubin A *et al.* (1981). Physiologic disposition of pergolide. *Clin Pharmacol Ther* 30: 258–265.

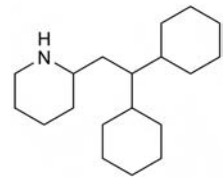
Perhexiline

Antianginal

$C_{19}H_{35}N = 277.5$

CAS—6621-47-2

IUPAC Name 2-(2,2-Dicyclohexylethyl)piperidine



Chemical Properties Log *P* (octanol/water), 7.5.

Perhexiline Maleate

$C_{19}H_{35}N,C_4H_4O_4 = 393.6$

CAS—6724-53-4

Proprietary Names *Pexid*; *Pexsig*.

Chemical Properties A white crystalline powder. Mp 188° to 191°. Slightly soluble in water; soluble in chloroform and methanol.

Thin-layer Chromatography System TA—R_f 0.41; system TB—R_f 0.57; system TC—R_f 0.08; system TE—R_f 0.59; system TL—R_f 0.06; system TAE—R_f 0.08 (acidified iodoplatinate solution, strong reaction).

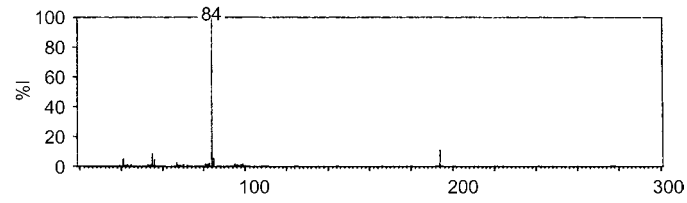
Gas Chromatography System GA—Perhexiline RI 2245; M (OH-) RI 2485; M (diOH-) RI 2660; M (diOH-)-H_2O RI 2510.

High Performance Liquid Chromatography System HA—*k* 0.2.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers (perhexiline maleate) 1582, 873, 990, 730, 1029, 1081 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 84, 194, 55, 85, 56, 41, 30, 99.



Quantification

Plasma **GC** AFID. Limit of detection, 20 μg/L [Grgurinovich 1983].

HPLC UV detection. Perhexiline and its monohydroxy metabolite. Limits of detection, 0.03 mg/L for perhexiline and 0.02 mg/L for its monohydroxy metabolite [Grgurinovich 1997]. ECD. Perhexiline and its monohydroxy metabolites. Limits of detection, 0.1 mg/L (perhexiline) and 0.025 mg/L (metabolites) [Cooper *et al.* 1986]. Fluorescence detection. Limit of detection, 5 μg/L [Horowitz *et al.* 1981].

Urine **HPLC** See Plasma [Cooper *et al.* 1986].

Bile **HPLC** See Plasma [Cooper *et al.* 1986].

Disposition in the Body Incompletely absorbed after oral administration. It is metabolised by hydroxylation to mono- and dihydroxylated derivatives which are slowly excreted in the urine and faeces; very little unchanged drug is excreted in the urine.

Therapeutic Concentration

After a single oral dose of 150 mg given to 5 subjects, peak plasma concentrations of 0.02 to 0.12 mg/L (mean, 0.07) were attained in about 6 h; following a single oral dose of 300 mg to the same subjects, peak plasma concentrations of 0.14 to 0.44 mg/L (mean, 0.27) were reported at 7 h; absorption was considerably delayed in one subject with both doses [Horowitz *et al.* 1981].

Following chronic oral doses of 100 to 400 mg daily to 14 subjects, minimum steady-state plasma-perhexiline concentrations of 0.35 to 2.8 mg/

L (mean, 1.07) were reported; steady-state plasma concentrations of the major monohydroxylated metabolite ranged from 1.25 to 7.4 mg/L (mean, 3.8). Steady-state plasma concentrations in 13 subjects who had been receiving similar daily doses but had developed toxic effects were: perhexiline 2.2 to 6.5 mg/L (mean, 3.8), monohydroxy metabolite 0.6 to 3.8 mg/L (mean, 1.6) [Singlas *et al.* 1978].

Forty subjects receiving perhexiline maleate 400 mg daily for 3 days followed by 200 mg daily thereafter had widely varying plasma perhexiline levels at 72 to 96 h (mean, 0.46 mg/L; range, 0.11 to 1.77) [Stewart *et al.* 1996].

Half-life Plasma half-life, about 2 to 6 days; the rate of elimination appears to be decreased in subjects with toxic effects, and may be dose-dependent.

Dose 100 to 400 mg of perhexiline maleate daily.

Cooper RG *et al.* (1986). Simultaneous determination of perhexiline and its monohydroxy metabolites in biological fluids by gas chromatography-electron-capture detection. *J Chromatogr* 381: 305–314.

Grgurinovich N (1983). A simple and sensitive method for the determination of perhexiline in plasma using gas-liquid chromatography with nitrogen-phosphorus detection. *J Chromatogr* 274: 361–365.

Grgurinovich N (1997). Method for the analysis of perhexiline and its hydroxy metabolite in plasma using high-performance liquid chromatography with precolumn derivatization. *J Chromatogr B Biomed Sci Appl* 696: 75–80.

Horowitz JD *et al.* (1981). High-performance liquid chromatographic assay of perhexiline maleate in plasma. *J Pharm Sci* 70: 320–322.

Singlas E *et al.* (1978). Pharmacokinetics of perhexiline maleate in anginal patients with and without peripheral neuropathy. *Eur J Clin Pharmacol* 14: 195–201.

Stewart S *et al.* (1996). Relationship between plasma perhexiline concentration and symptomatic status during short-term perhexiline therapy. *Ther Drug Monit* 18: 635–639.

Pericyazine

Tranquilliser

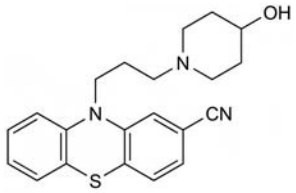
$C_{21}H_{23}N_3OS = 365.5$

CAS—2622-26-6

IUPAC Name 10-[3-(4-Hydroxypiperidin-1-yl)propyl]phenothiazine-2-carbonitrile

Synonyms 10-[3-(4-Hydroxy-1-piperidinyl)propyl]-10*H*-phenothiazine-2-carbonitrile; periciazine; propericiazine; RP-8909; SKF-20716.

Proprietary Names *Aolept*; *Neulactil*; *Neuleptil*.



Chemical Properties A yellow crystalline powder. Mp 116° to 117°. Practically insoluble in water; soluble in ethanol and acetone; freely soluble in chloroform; slightly soluble in ether. Log *P* (octanol/water), 3.5.

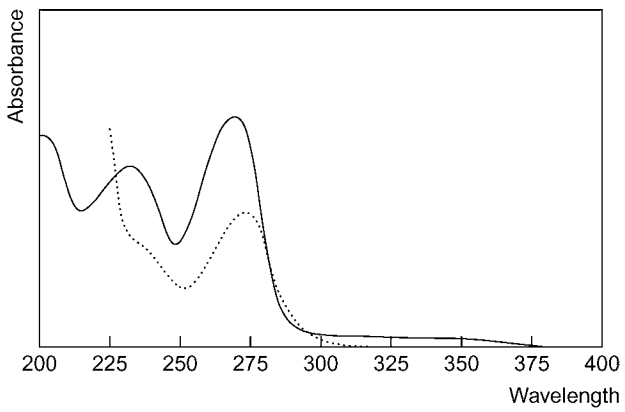
Colour Tests Formaldehyde-sulfuric acid—red; Forrest reagent—brown-orange; FPN reagent—orange; Mandelin's test—red; Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.04; system TC—R_f 0.16; system TE—R_f 0.51; system TL—R_f 0.18; system TAE—R_f 0.46; system TAF—R_f 0.61 (acidified iodoplatinate solution, positive).

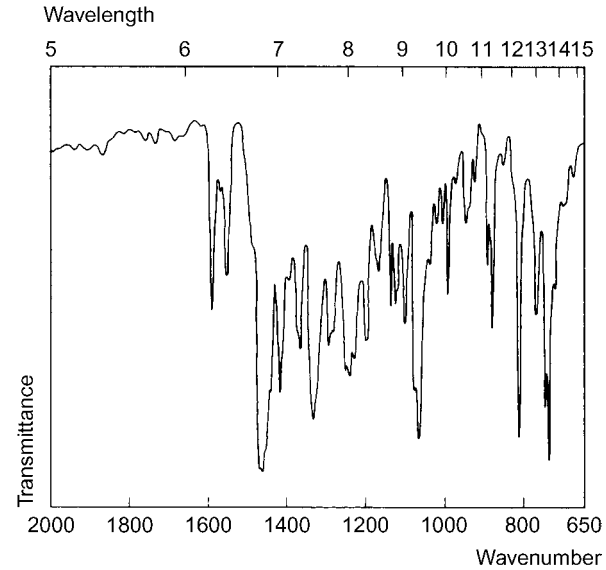
Gas Chromatography System GA—pericyazine RI 3260, M (ring) RI 2555; system GB—RI 3486.

High Performance Liquid Chromatography System HA—*k* 1.3; system HX—RI 410; system HY—RI 356; system HZ—retention time 4.4 min; system HAX—retention time 10.2 min; system HAY—retention time 5.1 min.

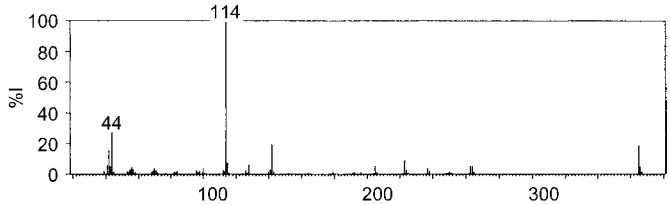
Ultraviolet Spectrum Aqueous acid—232, 268 nm (A^1_1=761a); aqueous alkali—272 nm.



Infrared Spectrum Principal peaks at wavenumbers 736, 1066, 811, 746, 1245, 1260 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 114, 44, 142, 365, 42, 223, 115, 205.



Dose 15 to 30 mg daily for severe anxiety or behaviour disorders; initially 75 mg daily in psychoses increasing up to 300 mg daily in severe cases.

Perindopril

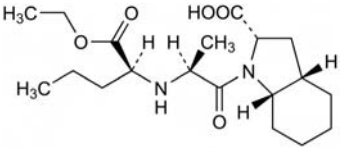
Antihypertensive

$C_{19}H_{32}N_2O_5 = 368.5$

CAS—82834-16-0

IUPAC Name (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxopentan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid

Synonyms (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)butyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid; McN-A-2833; S-9490.



Perindoprilat

$C_{17}H_{28}N_2O_5 = 340.4$

CAS—95153-31-4

Synonym S-9780

Perindopril Erbumine (Perindopril Tert-Butylamine)

$C_{19}H_{32}N_2O_5,C_4H_{11}N = 441.6$

CAS—107133-36-8

Synonyms MCN-A-2833-109; S-9490-3.

Proprietary Names *Aceon*; *Coversum*; *Coversyl*; *Procaptan*.

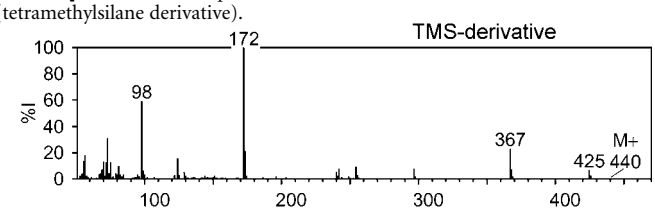
Chemical Properties White crystalline powder. Freely soluble in water, alcohol and chloroform.

Thin-layer Chromatography System TE—R_f 0.06 (perindopril), R_f 0.03 (perindoprilat); system TF—R_f 0.00 (perindopril), R_f 0.00 (perindoprilat).

Gas Chromatography System GP—RI 2450perindopril-ME; RI 2470 M (perindoprilat)-ME3; RI 2560 M (perindoprilat-H_2O)-ME3.

High Performance Liquid Chromatography System HX—perindoprilat RI 314; system HZ—perindoprilat retention time 1.6 min; system HAA—retention time 13.7 min.

Mass Spectrum Principal ions at *m/z* 172, 98, 73, 367, 173, 56, 124, 55 TMS, (tetramethylsilane derivative).



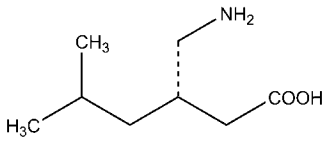
Quantification
Plasma **GC-MS** Limit of detection, 2 μg/L [Diez Ibañez *et al.* 1989].
Disposition in the Body Perindopril is rapidly and extensively absorbed after oral administration and approx. 30 to 50 % is hydrolysed to the active drug perindoprilat. Other metabolites include perindopril and perindoprilat glucuronides, dehydrated perindopril and the diastereoisomers of dehydrated perindoprilat. Peak plasma concentrations of perindopril are reached within 1 h and of perindoprilat in 3 to 4 h. The metabolism of perindopril to perindoprilat is affected by the presence of food in the gastro-intestinal tract. Around 75% of the dose is excreted in urine as perindopril, perindoprilat and other metabolites, and the remainder in faeces. Only 4 to 12% of the dose is recovered as unchanged drug in urine. Excretion is reduced in those with renal impairment.
Therapeutic Concentration
Twelve healthy males, 19 to 33 years old, were administered a single 4 mg oral dose of perindopril erbumine after 12 h fasting or during breakfast. Peak plasma perindopril concentrations were 64.2 μg/L for those in the fasted state and 52.4 μg/L for those in the fed state. These levels were reached within 0.9 and 1.1 h, respectively. The peak concentrations of the metabolite, pendoprilat were 4.7 and 3.6 μg/L in the fasted and fed individuals, observed 3.6 to 3.9 h after administration [Lecocq *et al.* 1990].
Bioavailability Perindopril, 65 to 70%; perindoprilat, 25%.
Half-life Perindopril: 2.9 h (fasted individuals) and 3.4 h (fed). Perindoprilat: 10.9 h (fasted) and 12.0 h (fed). For perindoprilat, the distribution half-life is also reported to be 5 h with an elimination half-life of 25 to 30 h.
Volume of Distribution Perindoprilat, 0.2 L/kg; also reported as 4.3 L/kg.
Clearance Perindoprilat, 4.9 L/h.
Protein Binding Perindopril, 60%; perindoprilat, <30% (concentration dependent).
Note For a review of the pharmacokinetics of ACE (angiotensin-converting enzyme) inhibitors, see Song and White [2002].
Dose 2 to 4 mg daily with a maximum of 8 mg daily.

Diez Ibañez MA *et al.* (1989). Gas chromatographic-mass spectrometric study of deacetylation and oxidation of 2-acetylaminofluorene by rat liver epithelial cell lines upon cocarcinogen induction. *J Chromatogr* 488: 237–248.
Lecocq B *et al.* (1990). Influence of food on the pharmacokinetics of perindopril and the time course of angiotensin-converting enzyme inhibition in serum. *Clin Pharmacol Ther* 47: 397–402.
Song JC, White CM (2002). Clinical pharmacokinetics and selective pharmacodynamics of new angiotensin converting enzyme inhibitors: an update. *Clin Pharmacokinet* 41: 207–224.

Perospirone

Antipsychotic
$C_{23}H_{30}N_4O_2S$ = 426.6
CAS—150915-41-6

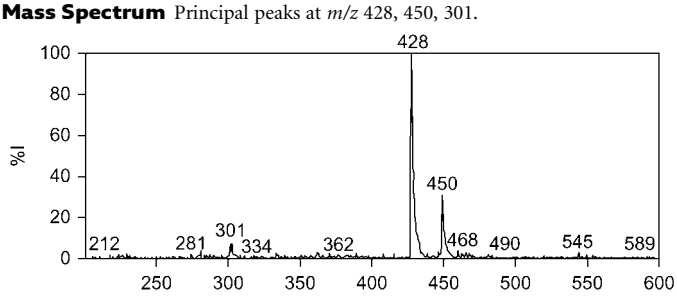
IUPAC Name (3a*S*,7a*R*)-2-[4-[4-(1,2-Benzothiazol-3-yl)piperazin-1-yl]butyl]-3a,4,5,6,7,7a-hexahydroisoindole-1,3-dione
Synonyms (3a*R*,7a*S*)-rel-2-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl] butyl]hexahydro-1*H*-isoindole-1,3(2*H*)-dione; *cis*-*N*-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]-1,2-cyclohexanedicarboximide.



Chemical Properties Stock solutions were stable at –40° for at least a month. Stable in plasma for 24 h at 4°, 1 month at –40°, and for 1 month after 3 freeze–thaw cycles [Ma *et al.* 2007].

Perospirone Hydrochloride
$C_{23}H_{30}N_4O_2S.HCl$ = 463.1
CAS—129273-38-7
Synonym SM-9018
Chemical Properties Mp 192° to 193°.

Mass Spectrum Principal peaks at *m/z* 428, 450, 301.



Quantification
Plasma **HPLC** Column: C_{18} STR ODS-II (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L phosphate buffer (pH 4.6) : 6 mol/L perchloric acid : acetonitrile (58 : 0.5 : 41.5), flow rate 0.6 mL/min. Fluorescence detection (λ_{ex} = 315 nm, λ_{em} = 405 nm). Limit of quantification, 0.1 μg/L, limit of detection, 0.06 μg/L [Yasui-Furukori *et al.* 2003].

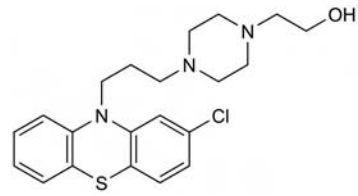
LC-MS Column: Acquity BEH C_{18} (100 × 2.1 mm i.d., 1.7 μm). Mobile phase: acetonitrile : 30 mmol/L ammonium acetate (62 : 38), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 50 ng/L; limit of detection, 5 ng/L [Li *et al.* 2007]. Column: Xterra C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: methanol : 10 mmol/L ammonium acetate (84 : 16), flow rate 0.25 mL/min. ESI, SIM acquisition mode. Limit of quantification, 0.1 μg/L [Ma *et al.* 2007]
Disposition in the Body Metabolism of perospirone occurs mainly in the liver, catalysed by CYP3A4 and, to a lesser extent, CYP2C8 and CYP2D6. The active metabolite hydroxyperospirone may play a role in the antipsychotic action.
Therapeutic Concentration
Twelve healthy Chinese volunteers (6 male, 6 female) received an oral dose of 8 mg perospirone hydrochloride. The mean peak plasma concentration was 2.79 ± 0.78 μg/L at 1.79 ± 0.45 h. The elimination half-life was 6.78 ± 1.38 [Ma *et al.* 2007].
A study of 10 schizophrenic patients receiving 16 mg perosprirone twice daily monitored plasma concentrations before and up to 12 h after administration. The mean peak plasma concentration was 8.8 μg/L at 0.8 h. The elimination half-life was 1.9 h. The active metabolite hydroxyperospirone reached a mean peak plasma concentration of 29.4 μg/L at 1.1 h [Yasui-Furukori *et al.* 2004]
Note For pharmacokinetic parameters of perospirone in combination with carbamazepine, itraconazole and tandospirone, see Masui *et al.* [2006a] and Masui *et al.* [2006b], respectively.
Clearance 423 ± 156 L/h [Ma *et al.* 2007].
Dose Oral dose, 8 to 48 mg once daily.

Li KY *et al.* (2007). Ultra-performance liquid chromatography-tandem mass spectrometry for the determination of atypical antipsychotics and some metabolites in in vitro samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 581–585.
Ma N *et al.* (2007). Determination of perospirone by liquid chromatography/electrospray mass spectrometry: application to a pharmacokinetic study in healthy Chinese volunteers. *J Chromatogr B Analyt Technol Biomed Life Sci* 847: 210–216.
Masui T *et al.* (2006). Effect of carbamazepine on the single oral dose pharmacokinetics of perospirone and its active metabolite. *Prog Neuropsychopharmacol Biol Psychiatry* 30: 1330–1333.
Masui T *et al.* (2006). Effects of itraconazole and tandospirone on the pharmacokinetics of perospirone. *Ther Drug Monit* 28: 73–75.
Yasui-Furukori N *et al.* (2003). Determination of a new atypical antipsychotic agent perospirone and its metabolite in human plasma by automated column-switching high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 789: 239–245.
Yasui-Furukori N *et al.* (2004). Steady-state pharmacokinetics of a new antipsychotic agent perospirone and its active metabolite, and its relationship with prolactin response. *Ther Drug Monit* 26: 361–365.

Perphenazine

Tranquilliser
$C_{21}H_{26}ClN_3OS$ = 404.0
CAS—58-39-9

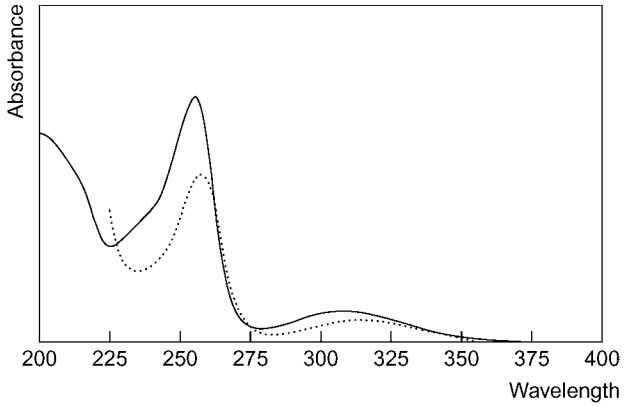
IUPAC Name 2-[4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl] ethanol
Synonyms 4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]-1-piperazineethanol; chlorpiprazine; chlorpiprozine; perphenazinum.
Proprietary Names *Decentan*; *Fentazin*; *Peratsin*; *Perphenan*; *Phenazine*; *Trilafon*; *Trilifan*. It is an ingredient of *Etrafon*, *Triavil*, *Triptafen*.



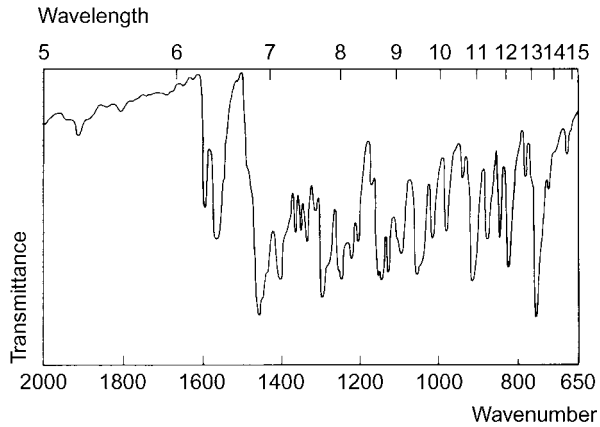
Chemical Properties A white or creamy-white powder, sensitive to light. Mp 94° to 110°. Practically insoluble in water; soluble 1 in 20 of ethanol, 1 in 1 of chloroform, and 1 in 80 of ether. pK_a 7.8 (24°). Log *P* (octanol/pH 7.0), 3.1. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Formaldehyde–sulfuric acid—violet; Forrest reagent—violet-red; FPN reagent—violet-red; Liebermann's reagent—red-brown; Mandelin's test—violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.07; system TC—R_f 0.29; system TE—R_f 0.42; system TL—R_f 0.09; system TAE—R_f 0.40; system TAF—R_f 0.40; system TAJ—R_f 0.00; system TAK—R_f 0.03; system TAL—R_f 0.56 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; FPN reagent—pink; Marquis test–pink; ninhydrin spray—positive).
Gas Chromatography System GA—perphenazine RI 3380, M (ring) RI 2100; system GB—RI 3594.
High Performance Liquid Chromatography System HA—*k* 1.9; system HX—RI 428; system HY—RI 395; system HZ—RT 7.2 min; system HAA—RT

16.0 min; system HAX—RT 13.1 min; system HAY—RT 6.3 min; system HAZ—*k* 3.28.

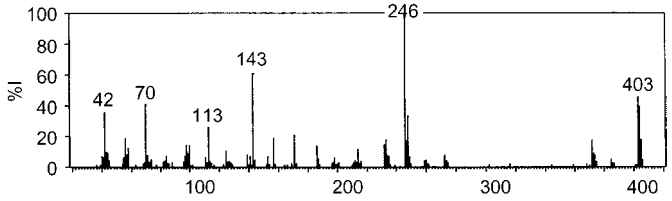
Ultraviolet Spectrum Aqueous acid—254 ($A_1^1 = 805a$), 307; aqueous alkali—257 ($A_1^1 = 791a$), 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 757, 1294, 917, 1145, 1247, 1155 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 246, 143, 403, 70, 404, 42, 248, 113.



Quantification

Blood **GC** Column: HP-5 (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 200° at 30°/min to 260° at 10°/min to 300° at 20°/min. NPD, EI ionisation. Limit of quantification, 0.2 mg/L [Levine *et al.* 1999]. Column: JJ CQ 100/120 mesh BS (0.9 m × 4 mm i.d.). Carrier gas: Ar : CH_4 20 mL/min. Temperature: 250°. Limit of quantification, 0.2 µg/L [Hansen, Larsen 1974]. Column: JJ CQ 100/120 mesh BS (0.9 m × 4 mm i.d.). Carrier gas: Ar : CH_4 20 mL/min. Temperature: 250°. Limit of detection, 10 µg/L [Larsen, Naestoft 1973].

LC-MS Column: Zorbax Stable Bond Cyano (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 2 : 8 : 90) : methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 24 : 36 : 40, 80 : 20 to 20 : 80 in 4.5 min for 2.5 min to 80 : 20 for 2.5 min). TIS, MRM acquisition mode. Retention time: 5.9 min. Limit of detection, 0.8 µg/L [Roman *et al.* 2008].

Plasma **GC** Column: CBP1-bonded methyl silicone (12 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. Limit of detection, 50 µg/L [Tokunaga *et al.* 1996]. Column: 1% OV-17 Chromosorb W on 100/120 mesh (2 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 50 mL/min. Temperature: 280°. Limit of detection, 5 µg/L [Cooper *et al.* 1979]. Column; OV-17 on Celite JJ CQ 100/120 mesh (1.5 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (90 : 10) 60 mL/min. Temperature: 305°. ECD. Limit of quantification, 0.2 µg/L [Larsen, Naestoft 1975].

HPLC Column; Nucleosil C_{18} (120 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate buffer with 5 mmoll/L tetramethylammonium chloride (pH 2.4) : acetonitrile : methanol (70 : 26 : 4), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 15.01 min. Limit of quantification, 0.5 µg/L [Foglia *et al.* 1995]. Column: C_{18} (20 × 4.6 mm i.d.). Mobile phase: methanol : water : methylene dichloride : ammonia (200 : 40 : 10 : 3), flow rate 1.0 mL/min. UV detection (λ = 257 nm). Retention time: 2.1 min. Limit of quantification, <0.5 nmol/L [Larsen *et al.* 1985].

Note For a radioimmunoassay in plasma, see Midha *et al.* [1981].

Serum **HPLC** Column: S5 CN Spherisorb (150 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate buffer : methanol (1 : 9), flow rate 0.6–1.7 mL/min. UV detection (λ = 256 and 245 nm). Limit of quantification, 1 nmol/L [Angelo, Petersen 2001]. Column: µBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: 0.01 mol/L dodecylhydrogensulfate in acetonitrile : water : acetic acid (65 : 35 : 3). UV detection (λ = 257 nm). Limit of detection, 0.3 µg/L [Larsson, Forsman 1983].

Bile **GC** See Blood [Levine *et al.* 1999]

Gastric Contents **GC** See Blood [Levine *et al.* 1999].

Kidney **GC** See Blood [Levine *et al.* 1999].

Liver **GC** See Blood [Levine *et al.* 1999].

Disposition in the Body Perphenazine is absorbed after oral administration but subject to first-pass metabolism. Small amounts are found in breast milk. The main metabolic reactions are oxidation to the sulfoxide, which may have some pharmacological activity, *N*-dealkylation to form the *N*-deshydroxyethyl metabolite, *N*-oxidation, cleavage of the piperazine ring and phenolic hydroxylation, followed by glucuronide conjugation. After oral administration, ~1–2% of the daily dose is excreted in the urine as unchanged drug, with approx. 13% as the sulfoxide and 30% as perphenazine glucuronide; after chronic administration of perphenazine enantate IM, excretion of unchanged drug and the sulfoxide is increased to ~6% and 22% of the dose, respectively.

Therapeutic Concentration

A 22-year-old mother was admitted because of postpartum psychosis. At a 24 h intake of 24 mg perphenazine (480 µg/kg), the drug concentration in the milk was 7.8 nmol/L (3.2 µg/L). The 24 h milk production was 510 mL, thus 1.59 µg perphenazine (0.45 µg/kg) would have been passed on to the child [Olesen *et al.* 1990].

After daily oral doses of 12–48 mg to 16 subjects, steady-state plasma concentrations of 0.0003–0.025 mg/L (mean 0.004 mg/L) of perphenazine and 0.0004–0.018 mg/L (mean 0.004 mg/L) of the sulfoxide were reported [Hansen, Larsen 1977].

Toxicity

The following postmortem tissue concentrations were reported in a fatality caused by ingestion of ~1 g perphenazine: blood 3 mg/L, brain 11 µg/g, liver 149 µg/g; a blood-alcohol concentration of 1700 mg/L was also reported [Cravey 1980].

A 34-year-old woman whose death was attributed to suicide by means of multiple drug intoxication was found to have a heart blood concentration of 4.4 mg/L perphenazine and 950 mg/L valproic acid at postmortem. The distribution of perphenazine was as follows:

Specimen	*Concentration (mg/L or mg/kg)*
Bile	40
Blood (peripheral)	3.5
Kidney	23
Liver	57
Stomach (mg)	230

[Levine *et al.* 1999]

Bioavailability Approximately 60–80%.

Half-life Approximately 8–12 h in plasma.

Volume of Distribution 10–35 L/kg.

Clearance Approximately 12–38 mL/min/kg from plasma.

Note For a study of the pharmacological profile of perphenazine metabolites, see Sweet *et al.* [2000].

Dose Usually 12 to 24 mg daily; up to 64 mg daily has been given.

Angelo HR, Petersen A (2001). Therapeutic drug monitoring of haloperidol, perphenazine, and zuclopenthixol in serum by a fully automated sequential solid phase extraction followed by high-performance liquid chromatography. *Ther Drug Monit* 23: 157–162.

Cooper S *et al.* (1979). Gas chromatographic determination of amitriptyline, nortriptyline and perphenazine in plasma of schizophrenic patients after administration of the combination of amitriptyline with perphenazine. *Arzneimittelforschung* 29: 158–161.

Cravey RH (1980). Perphenazine. Personal Communication. In Basalt RC ed. *Disposition of Toxic Chemicals and Drugs in Man*, 5th edn. Davis, CA: Biomedical Publications, pp. 672–674.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Foglia JP *et al.* (1995). Quantitative determination of perphenazine and its metabolites in plasma by high-performance liquid chromatography and coulometric detection. *J Chromatogr B Biomed Appl* 668: 291–297.

Hansen CE, Larsen NE (1974). Perphenazine concentrations in human whole blood. A pilot study during anti-psychotic therapy using different administration forms. *Psychopharmacologia* 37: 31–36.

Hansen LB, Larsen NE (1977). Plasma concentrations of perphenazine and its sulphoxide metabolite during continuous oral treatment. *Psychopharmacology (Berl)* 53: 127–130.

Larsen NE, Naestoft J (1973). Determination of perphenazine and fluphenazine in whole blood by gas chromatography. *Med Lab Technol* 30: 129–132.

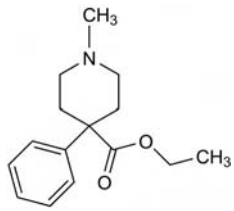
Larsen NE, Naestoft J (1975). Determination of perphenazine and its sulphoxide metabolite in human plasma after therapeutic doses by gas chromatography. *J Chromatogr* 109: 259–264.

Larsen NE *et al.* (1985). Quantitative determination of perphenazine and its dealkylated metabolite using high-performance liquid chromatography. *J Chromatogr* 341: 244–250.
Larsson M, Forsman A (1983). A high-performance liquid chromatographic method for the assay of perphenazine and its dealkylated metabolite in serum after therapeutic doses. *Ther Drug Monit* 5: 225–228.
Levine B *et al.* (1999). Perphenazine distribution in a postmortem case. *J Anal Toxicol* 23: 127–129.
Midha KK *et al.* (1981). Radioimmunoassay for perphenazine in human plasma. *Br J Clin Pharmacol* 11: 85–88.
Olesen OV *et al.* (1990). Perphenazine in breast milk and serum. *Am J Psychiatry* 147: 1378–1379.
Roman M *et al.* (2008). Quantitation of seven low-dosage antipsychotic drugs in human postmortem blood using LC-MS-MS. *J Anal Toxicol* 32: 147–155.
Sweet RA *et al.* (2000). Pharmacologic profile of perphenazine's metabolites. *J Clin Psychopharmacol* 20: 181–187.
Tokunaga H *et al.* (1996). Screening of antipsychotic drugs by wide-bore capillary gas chromatography with nitrogen phosphorus detection—detection levels in plasma. *Nihon Hoigaku Zasshi* 50: 196–202.

Pethidine

Narcotic Analgesic
$C_{15}H_{21}NO_2 = 247.3$
CAS—57-42-1

IUPAC Name Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate
Synonyms Isonipecaine; meperidine; 1-methyl-4-phenyl-4-piperidinecarboxylic acid ethyl ester.

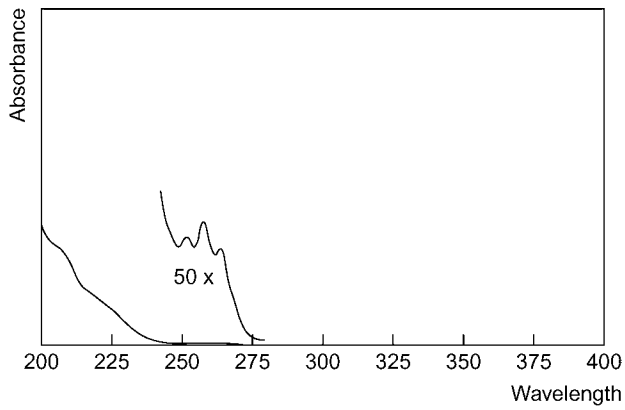


Chemical Properties An oily liquid which slowly crystallises. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

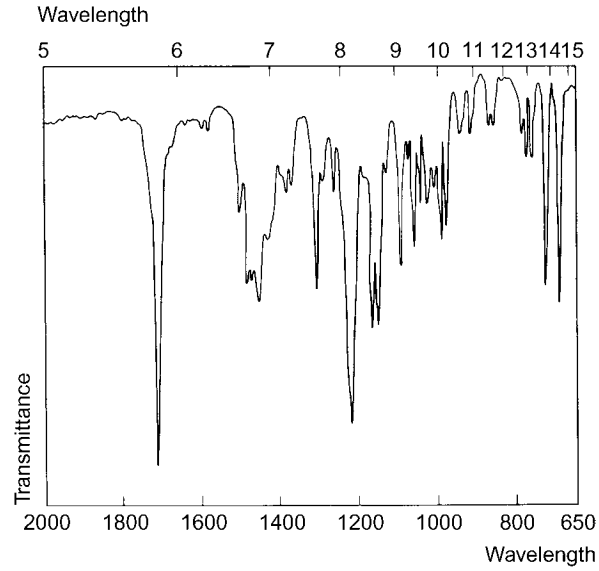
Pethidine Hydrochloride

$C_{15}H_{21}NO_2$,HCl = 283.8
CAS—50-13-5
Proprietary Names *Alodan*; *Centralgine*; *Demerol*; *Dispadol*; *Dolantine*; *Dolantin (a)*; *Dolestine*; *Dolosal*; *Mefedina*; *Pethoid*. It is an ingredient of *Mepergan*; *Meprozin*; *Pamergan P100*.
Chemical Properties A white crystalline powder. Mp 186° to 189°. Soluble in water, acetone and ethyl acetate; slightly soluble in alcohol, isopropanol; practically insoluble in ether and benzene. pK_a 8.7 (20°). Log *P* (octanol/water), 2.7.

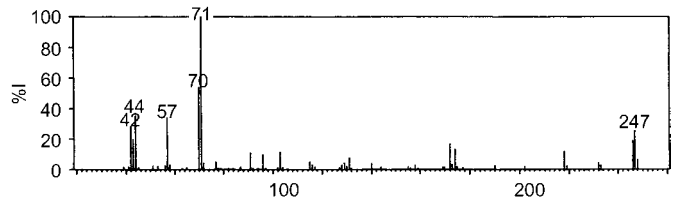
Colour Tests Liebermann's reagent—red-orange; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.37; system TC—R_f 0.34; system TE—R_f 0.60; system TL—R_f 0.11; system TAE—R_f 0.34; system TAF—R_f 0.40; system TAJ—R_f 0.14; system TAK—R_f 0.06; system TAL—R_f 0.72 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—brown).
Gas Chromatography System GA—pethidine RI 1754, M (nor-) RI 1885, M (nor-)-AC RI 2240, M (OH-) RI 2045, M (OH-)-AC RI 2205, M (nor-OH-)-AC_2 RI 2600; system GB—pethidine RI 1809, M (nor-) RI 1842, M (nor-)-AC RI 2256, M (OH-) RI 2145; system GC—RI 2025; system GF—RI 1995; system GM—pethidine RRT 0.319, M (nor-) RRT 0.357 (both relative to iprindole).
High Performance Liquid Chromatography System HA—pethidine *k* 2.8 (tailing peak), M (nor-) *k* 1.7 (tailing peak), pethidinic acid *k* 2.8 (tailing peak); system HC—pethidine *k* 0.55, M (nor-) *k* 2.04; system HX—RI 345; system HY—RI 281; system HZ—RT 3.2 min; system HAA—RT 11.8 min; system HAX—RT 9.2 min; system HAY—RT 4.8 min.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1 = 8.5a), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1708, 1218, 1166, 1148, 688, 730cm^{-1} (pethidine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 71, 70, 44, 57, 42, 247, 43, 246 (pethidine); 57, 233, 42, 56, 158, 43, 160, 103 (norpethidine); 71, 70, 57, 43, 219, 42, 218, 44 (pethidinic acid).



Quantification

Blood **GC** Column: 2% polyethylene glycol and 2% potassium hydroxide on 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. NPD. Limit of detection, 5 µg/L for pethidine and 2.5 µg/L for norpethidine [Jacob *et al.* 1982].

GC-MS Column: CP-SIL 8CB-MS. Carrier gas: He, 1.6 mL/min. Temperature programme: 100° for 1 min to 290° at 20°/min. EI ionisation at 70 eV. Limit of detection, 0.5 µg/L [Ishii *et al.* 2003]. SIM acquisition mode. Limit of detection, 0.2 µg/L [Ishii *et al.* 2001].

HPLC Column: C_{18} (10 µm). Mobile phase: methanol : 25 mmol/L potassium dihydrogen phosphate (90 : 10), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.05 mg/L [Wang *et al.* 1999].

LC-MS Mobile phase: Phenomenex Gemini C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile with 0.1% formic acid : ammonium acetate (pH 3.2, 15 : 85 for 9 min to 30 : 70 in 13 min to 80 : 20 in 10 min to 95 : 5 in 1 min), flow rate 150 µL/min. Positive ion mode, MRM acquisition mode. Limit of detection, 0.0002 mg/L [Gergov *et al.* 2009].

Plasma **GC** Column: 3% OV-17 Gas Chrom Q 100/120 mesh (1.8 m × 2 mm i.d.). Temperature programme: 210° for 2 min to 270° at 15°/min for 10 min. NPD. Retention time: 1.69 min. Limit of quantification, 5 µg/L for pethidine and norpethidine [Kintz *et al.* 1989]. Column: 3% OV-17 on Gas-Chrom Q 100/120 mesh (1.5 m × 2.0 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 260°. Limit of detection, 20 µg/L for pethidine and norpethidine [Hartvig, Fagerlund 1983]. See Blood [Jacob *et al.* 1982].

GC-MS Limit of detection, 170 ng/L for pethidine and 500 ng/L for norpethidine [Todd *et al.* 1979].

Serum **GC-MS** See Plasma [Todd *et al.* 1979].

HPLC Column: Supelcosil LC-PCN (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L phosphate buffer (pH 7.0) : methanol (55 : 25 : 20), flow rate 2.5 mL/min. UV detection (λ = 205 nm). Limit of detection, 2 µg/L [Meatherall *et al.* 1985]

Urine **GC** NPD. Limits of detection, 0.007–0.015 mg/L for pethidine, and some stimulants, narcotics and anti-oestrogens [Qiu *et al.* 2009]. Carrier gas: N_2, 1.0 mL/min. Temperature programme: 100° for 0.5 min to 300° at 20°/min for 3 min. NPD. Limit of detection, <1 µg/L [Myung *et al.* 1999]. FID. Limit of detection, 20 µg/L for pethidine and its metabolites [Hu *et al.* 1994]. Column: SE-54 (24 m × 0.24 mm i.d., 0.25 µm). Temperature programme: 100° to 250° at 10°/min for 8 min. FID. Limit of detection, 20 µg/L [Liu *et al.* 1994]. Column: SP2250 on Chromosorb W (80-100 mesh). NPD. Pethidine and its metabolites [Chan *et al.* 1991]. See Blood [Jacob *et al.* 1982].

GC-MS Limit of detection, 20 ng [Song *et al.* 1999]. Limit of detection, 20 µg/L for pethidine and its metabolites [Hu *et al.* 1994]. Pethidine and its metabolites [Liu *et al.* 1994]. Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (9.0 m × 2.0 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 205° for 2 min to 235° at 10°/min. MID. Limit of detection, 500 µg/L for pethidinic acid and norpethidinic acid [Lindberg *et al.* 1978]. See Blood [Ishii *et al.* 2001; Ishii *et al.* 2003].

HPLC See Serum [Meatherall *et al.* 1985].

LC-MS See Blood [Gergov *et al.* 2009].

Milk **GC-MS** Column: HP-1 Ultra (12 m × 0.2 mm i.d.). Temperature programme: 120° for 30 s to 250° at 30°/min for 4 min. Limit of detection, 20 µg/L [Borgatta *et al.* 1997].

Disposition in the Body Pethidine is readily absorbed after oral administration and rapidly and extensively distributed throughout the tissues. It undergoes considerable first-pass metabolism; bioavailability is ~55%. The major metabolites are the *N*-demethylated derivative norpethidine and the hydrolysis product pethidinic acid, and its conjugates. Norpethidine is about half as active an analgesic and about twice as toxic as pethidine; it may accumulate in the plasma on chronic administration, particularly in subjects with renal failure. The excretion of pethidine and its metabolites is dependent on the urinary pH. In normal subjects, ~70% of a dose is excreted in the urine in 24 h, up to 10% as unchanged drug, 10% as norpethidine, 20% as pethidinic acid, 16% as conjugated pethidinic acid, 8% as norpethidinic acid and 10% as conjugated norpethidinic acid; other metabolites identified in the urine in minor amounts include the *N*-hydroxy and *N*-hydroxyphenyl derivatives and pethidine *N*-oxide. Urinary excretion of pethidine and norpethidine may both be enhanced to ~30% of a dose if the urine is acid; in alkaline urine, <5% is excreted as pethidine and norpethidine. In pregnant women and in women taking oral contraceptives, the excretion of unchanged pethidine appears to be increased; less unchanged drug is eliminated in older subjects; excretion of pethidine is delayed in subjects with cirrhosis. Pethidine and norpethidine are distributed in the CSF. Pethidine crosses the placenta and is excreted in breast milk. Pethidine is a metabolite of phenoperidine.

Therapeutic Concentration In plasma, usually in the range 0.2–0.8 mg/L. Higher plasma concentrations are found in elderly subjects.

Following a single oral dose of 50 mg to 6 subjects, a mean peak serum concentration of 0.14 mg/L was attained in 2 h; after an IM injection of 50 mg to the same subjects, a mean peak serum concentration of 0.20 mg/L in 1 h was reported, and a mean serum concentration of 0.52 mg/L was obtained 1 min after an IV injection of 50 mg [Stambaugh *et al.* 1976].

Following multiple oral doses of 75 to 100 mg to four subjects, plasma pethidine concentrations of 0.16 to 0.54 mg/L (mean, 0.36) and norpethidine concentrations of 0.13 to 0.48 mg/L (mean, 0.27) were reported 1 to 4 h after a dose [Szeto, Inturrisi 1976].

Toxicity The estimated minimum lethal dose is 1 g. Toxic effects are usually associated with blood concentrations greater than 2 mg/L, but fatalities involving pethidine are uncommon.

Half-life Plasma half-life, pethidine 3 to 6 h, increased in neonates and in renal impairment; norpethidine ~20 h.

Bioavailability ≈55%

Volume of Distribution ≈4.4 L/kg.

Clearance Plasma clearance, ~17 mL/min/kg; decreased in subjects with liver disease and in the elderly.

Distribution in Blood Plasma : whole blood ratio, ~0.9.

Protein Binding ≈50–60%.

Note For reviews of the pharmacokinetics of pethidine, see Edwards *et al.* [1982] and Mather, Meffin [1978]; for a general review of pethidine, see Latta *et al.* [2002].

Dose Usually 50 to 150 mg of pethidine hydrochloride, by mouth or 25 to 100 mg by IM injection every 4 h.

Borgatta L *et al.* (1997). Clinical significance of methohexital, meperidine, and diazepam in breast milk. *J Clin Pharmacol* 37: 186–192.

Chan K *et al.* (1991). Determination of pethidine and its major metabolites in human urine by gas chromatography. *J Chromatogr* 565: 247–254.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Edwards DJ *et al.* (1982). Clinical pharmacokinetics of pethidine: 1982. *Clin Pharmacokinet* 7: 421–433.

Gergov M *et al.* (2009). Simultaneous screening and quantification of 25 opioid drugs in postmortem blood and urine by liquid chromatography–tandem mass spectrometry. *Forensic Sci Int* 186: 36–43.

Hartvig P, Fagerlund C (1983). A simplified method for the gas chromatographic determination of pethidine and norpethidine after derivatization with trichloroethyl chloroformate. *J Chromatogr* 274: 355–360.

Hu XY *et al.* (1994). [Analysis of meperidine and its metabolites in urine of an addict by GC/FID and GC/MS]. *Yao Xue Xue Bao* 29: 116–121.

Ishii A *et al.* (2001). Sensitive determination of pethidine in body fluids by surface ionization organic mass spectrometry. *J Chromatogr B Biomed Sci Appl* 758: 117–121.

Ishii A *et al.* (2003). Sensitive determination of pethidine in body fluids by gas chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 792: 117–121.

Jacob PIII *et al.* (1982). Gas chromatographic analysis of meperidine and normeperidine: determination in blood after a single dose of meperidine. *J Pharm Sci* 71: 166–168.

Kintz P *et al.* (1989). Simultaneous determination of pethidine (meperidine), phenoperidine, and norpethidine (normeperidine), their common metabolite, by gas chromatography with selective nitrogen detection. *Forensic Sci Int* 43: 267–273.

Latta KS *et al.* (2002). Meperidine: a critical review. *Am J Ther* 9: 53–68.

Lindberg C *et al.* (1978). Determination of pethidinic acid and norpethidinic acid in urine by mass fragmentography after extractive alkylation. *Acta Pharm Suec* 15: 327–336.

Liu F *et al.* (1994). Investigation of meperidine and its metabolites in urine of an addict by gas chromatography–flame ionization detection and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 658: 375–379.

Mather LE, Meffin PJ (1978). Clinical pharmacokinetics of pethidine. *Clin Pharmacokinet* 3: 352–368.

Meatherall RC *et al.* (1985). Analysis of meperidine and normeperidine in serum and urine by high-performance liquid chromatography. *J Chromatogr* 338: 141–149.

Myung SW *et al.* (1999). Solid-phase microextraction for the determination of pethidine and methadone in human urine using gas chromatography with nitrogen–phosphorus detection. *Analyst* 124: 1283–1286.

Qiu L *et al.* (2009). [Simultaneous determination of stimulant, narcotics and antiestrogen in urine by gas chromatography–nitrogen phosphorous detection]. *Se Pu* 27: 364–367.

Song F *et al.* (1999). Quantitative analysis of pethidine using liquid secondary ion and tandem mass spectrometry. *Rapid Commun Mass Spectrom* 13: 478–480.

Stambaugh JE *et al.* (1976). The clinical pharmacology of meperidine: comparison of routes of administration. *J Clin Pharmacol* 16: 245–256.

Szeto HH, Inturrisi CE (1976). Simultaneous determination of meperidine and normeperidine in biofluids. *J Chromatogr* 125: 503–510.

Todd EL *et al.* (1979). Determination of meperidine and normeperidine in serum by gas chromatography/mass spectrometry. *J Anal Toxicol* 3: 256–259.

Wang Y *et al.* (1999). [Separation and determination of morphine, pethidine and diazepam by high performance liquid chromatography]. *Se Pu* 17: 399–400.

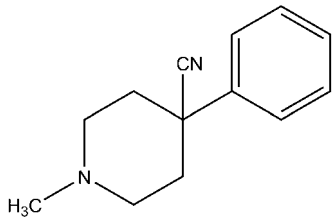
Pethidine Intermediate A

Narcotic Intermediate

$C_{13}H_{16}N_2 = 200.3$

IUPAC Name 1-Methyl-4-phenylpiperidine-4-carbonitrile

Synonym 4-Cyano-1-methyl-4-phenylpiperidine



Chemical Properties White powder. Soluble in dilute acetic acid and organic solvents. Pethidine intermediate A is extracted by organic solvents from aqueous alkaline solutions.

Thin-layer Chromatography System T1—R_f 0.52 (location reagent acidified iodoplatinate spray, positive reaction).

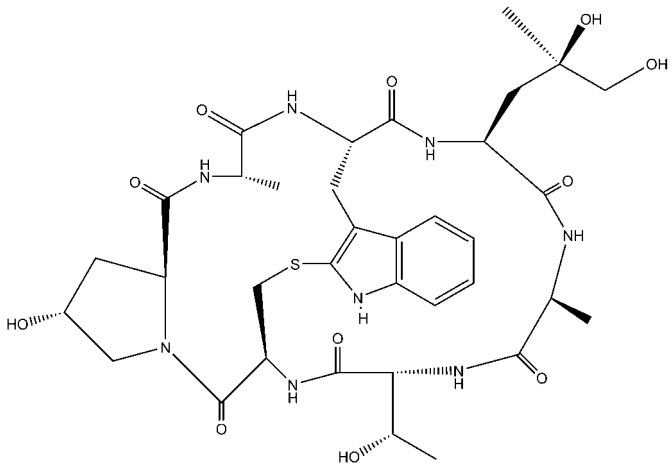
Phallotoxin

Toxin

$C_{35}H_{48}N_8O_{11}S = 788.9$

CAS—17466-45-4

Synonyms Phalloidin; phalloidine.



Chemical Properties Hexahydrate, needles from water. Mp 280° to 282°. More soluble in hot water as opposed to cold; freely soluble in methanol, ethanol, butanol and pyridine. Log *P* (octanol/water), −5.58 [Meylan, Howard 1995]. Phalloidin is the best known of the toxins isolated from the poisonous green fungus *Amanita phalloides,* also known as the green death cap or deadly agaric.

Capillary Electrophoresis Capillary: fused silica (84 cm length), Running buffer: aqueous solution of 20 mmol/L ammonium formate (pH 10.8). ESI, negative ion mode, SIM acquisition mode. Limit of detection, phalloidin 17 nmol/L, phallacidin 41 nmol/L [Rittgen *et al.* 2008].

Quantification

Serum **HPLC** Column: Develosil RP AQUEOUS. Mobile phase: acetonitrile containing 0.01 mol/L ammonium acetate (pH 5.0). UV detection (λ = 295, 302,and 230 nm). Limit of detection, 0.2 mg/L [Nishizawa, Yamaura 2003]. Column: Lichrosorb RP-18 (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L acetic acid-ammonium acetate buffer (pH 5.0; 7 : 93 for 7 min to 25 : 75 at 30 min), flow rate 1 mL/min. UV detection (λ = 302 nm). Retention time: 27.8 min. Limit of detection, 5 ng [Caccialanza *et al.* 1985].

Urine **HPLC** Column: Inertsil ODS-3 (150 × 4.6 mm i.d.). Mobile phase: 0.01 mol/L ammonium acetate buffer (pH 5.0) : chloroform (84 : 16), flow rate 1.0 mL/min. DAD. Limit of detection not reported [Gonmori, Yoshioka 2003]. Column: Lichrosorb RP-18 (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L acetic acid-ammonium acetate buffer (pH 5.0; 7 : 93 for 7 min to 25 : 75 at 30 min),

flow rate 1 mL/min. UV detection (λ = 302 nm). Retention time: 27.8 min. Limit of detection, 5 ng Caccialanza *et al.* 1985.

Other **HPTLC** *A. phalloides*. Plates: Silicagel 60 for nano-TLC (10 × 10 cm). Solvent system: chloroform : methanol : acetic acid : water (75 : 33 : 5 : 7.5) or 2-butanol : ethylacetate : water (56 : 48 : 20). Visualisation with 1 mL cinnamaldehyde in 100 mL methanol in a tank of fuming hydrochloric acid. Limit of detection, 500 mg/kg [Stijve, Seeger 1979].

HPLC *A. phalloides*. Column: Reversed phase Ultrasphere ODS (250 × 4.6 mm i. d., 5 µm). Mobile phase: 0.02 mol/L aqueous ammonium acetat-acetonitrile (pH 5.0, 90 : 10) : 0.02 mol/L aqueous ammonium acetate-acetonitrile (pH 5.0, 76 : 24; 100 : 0 for 4 min to 43 : 57 for 16 min to 0 : 100 for 10 min to 100 : 0), flow rate 1 mL/min. UV detection (λ = 214 and 295 nm). Limit of detection, 10 µg/L [Enjalbert *et al.* 1992].

Note For a study of the distribution of amatoxins and phallotoxins in *A. phalloides*, see Enjalbert *et al.* [1999].

Disposition in the Body

Toxicity

A 56-year-old man was admitted to hospital 42 h after mushroom ingestion. His main complaints of nausea and vomiting had begun 12 h after the ingestion. His laboratory findings were normal apart from the renal and liver function and cardiac markers, which were all elevated. The patient was treated with activated charcoal, high-dose IV penicillin G, and IV silibinin (5 mg/kg bolus and 20 mg/kg/24 h continuous infusion for 3 days). Because of the elevated cardiac enzymes, he was also given acetylsalicylic acid and metoprolol. After 480 h, the majority of the patient's biochemical parameters had returned to normal. The patient was discharged but with a haemodialysis schedule as a result of chronic renal failure [Unverir *et al.* 2007].

Note For a report on acetylcysteine as a life-saving antidote in *A. phalloides* poisoning, see Montanini *et al.* [1999]. For a report of amanita toxicosis in a dog, see Puschner *et al.* [2007]. For a review of the clinical characteristics of amanita and non-amanita mushroom poisoning in Turkish children, see Erguven *et al.* [2007];in Turkish adults see Yilmaz *et al.* [2006]. For a review investigating the geographical distribution of mushroom poisoning in Japan, see Gonmori and Yoshioka [2003].

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Enjalbert F *et al.* (1992). Simultaneous assay for amatoxins and phallotoxins in *Amanita phalloides* Fr. by high-performance liquid chromatography. *J Chromatogr* 598: 227–236.

Enjalbert F *et al.* (1999). Distribution of the amatoxins and phallotoxins in *Amanita phalloides*. Influence of the tissues and the collection site. *C R Acad Sci III* 322: 855–862.

Erguven M *et al.* (2007). Mushroom poisoning. *Indian J Pediatr* 74: 847–852.

Gonmori, K, Yoshioka, N. (2003) The examination of mushroom poisonings at Akita University. *Leg Med (Tokyo)* 5(Suppl.1): S83–S86.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Montanini S *et al.* (1999). Use of acetylcysteine as the life-saving antidote in *Amanita phalloides* (death cap) poisoning. Case report on 11 patients. *Arzneimittelforschung* 49: 1044–1047.

Nishizawa C, Yamaura Y (2003). Determination of amanitatoxins by HPLC. *Chudoku Kenkyu* 16: 441–445.

Puschner B *et al.* (2007). Diagnosis of *Amanita* toxicosis in a dog with acute hepatic necrosis. *J Vet Diagn Invest* 19: 312–317.

Rittgen J (2008). Identification of toxic oligopeptides in Amanita fungi employing capillary electrophoresis-electrospray ionization-mass spectrometry with positive and negative ion detection. *Electrophoresis* 29: 2094–2100.

Stijve T, Seeger T (1979). Determination of alpha-, beta-, and gamma-amanitin by high performance thin-layer chromatography in *Amanita phalloides* (Vaill. ex Fr.) secr. from various origin. *Z Naturforsch (C)* 34: 1133–1138.

Unverir P *et al.* (2007). Renal and hepatic injury with elevated cardiac enzymes in *Amanita phalloides* poisoning, a case report. *Hum Exp Toxicol* 26: 757–761.

Yilmaz A *et al.* (2006). Emergency room cases of mushroom poisoning. *Saudi Med J* 27: 858–861.

Phanquinone

Antiamoebic, Antibacterial

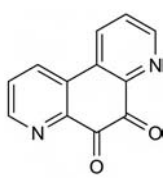
$C_{12}H_6N_2O_2$ = 210.2

CAS—84-12-8

IUPAC Name 4,7-Phenanthroline-5,6-dione

Synonyms Ciba 11925; phanquone.

Proprietary Name *Entobex*

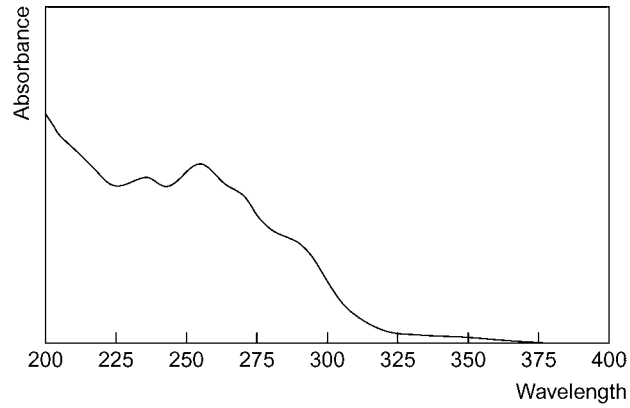


Chemical Properties An orange crystalline powder. Mp 295°, with decomposition. Slightly soluble in water; practically insoluble in ethanol and chloroform; soluble in dilute mineral acids. Log *P* (octanol/water), 1.2.

Colour Tests Marquis test—yellow; methanolic potassium hydroxide—yellow→brown-violet.

Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.03; system TC—R_f 0.45; system TE—R_f 0.54; system TL—R_f 0.17; system TAE—R_f 0.86; system TAJ—R_f 0.11; system TAK—R_f 0.00; system TAL—R_f 0.00 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—265 (A_1^1=500a), 272 (A_1^1=500a), 292 nm (A_1^1=480b).



Infrared Spectrum Principal peaks at wavenumbers 1700, 1283, 1584, 1275, 1683, 950 cm^{-1} (KBr disk).

Quantification

Biological Fluids **GC** Limit of detection, 15 µg/L [Degen *et al.* 1976].

Dose 150 to 300 mg daily.

Degen PH *et al.* (1976). The determination of phanquone in biological material by gas-liquid chromatography. *J Chromatogr* 118: 363–370.

Phenacaine

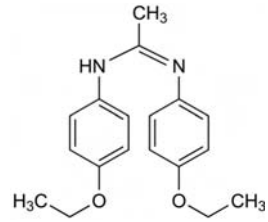
Anaesthetic (Local)

$C_{18}H_{22}N_2O_2$ = 298.4

CAS—101-93-9

IUPAC Name *N,N′*-Bis(4-ethoxyphenyl)ethanimidamide

Synonym Phenetidylphenacetin



Chemical Properties Log *P* (octanol/water), 5.6.

Phenacaine Hydrochloride Monohydrate

$C_{18}H_{22}N_2O_2$,HCl,H_2O = 352.9

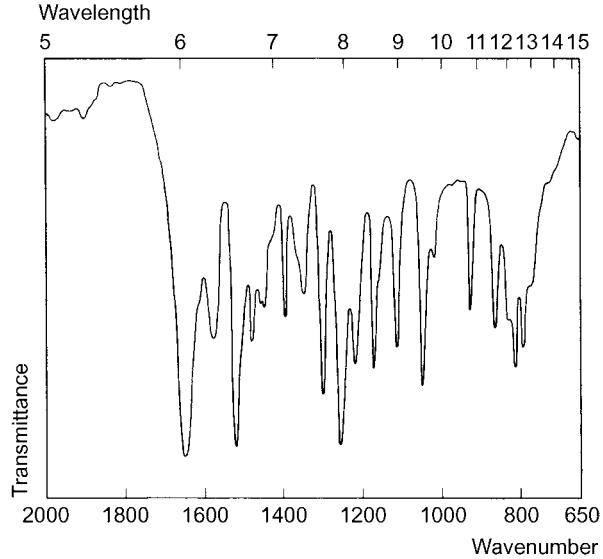
CAS—620-99-5 (anhydrous); 6153-19-1 (monohydrate)

Proprietary Name *Holocaine*

Chemical Properties White crystals. Mp 190° to 192°. Soluble 1 in 50 of water; freely soluble in ethanol and chloroform; insoluble in ether.

Gas Chromatography System GA—RI 2617.

Infrared Spectrum Principal peaks at wavenumbers 1646, 1520, 1256, 1300, 1046, 815 cm^{-1} (KBr disk).



Uses Phenacaine hydrochloride has been used as a 1% aqueous solution or as a 1 or 2% ointment.

Phenacemide

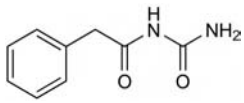
Anticonvulsant

CAS—63-98-9

IUPAC Name *N*-carbamoyl-2-phenylacetamide

Synonyms *N*-(aminocarbonyl)benzeneacetamide; carbamidum phenylaceticum; phenacetylurea.

Proprietary Name *Phenurone*



Chemical Properties $C_9H_{10}N_2O_2$=178.2. A white crystalline powder. Mp about 212° to 216°. Very slightly soluble in water; slightly soluble in acetone, ethanol, chloroform, ether and methanol. Log *P* (octanol/water), 0.9.

Colour Tests Liebermann's reagent—red-orange; Nessler's reagent—brown-orange (slow).

Thin-layer Chromatography System TD—R_f 0.22; system TE—R_f 0.65; system TF—R_f 0.40; system TAD—R_f 0.50.

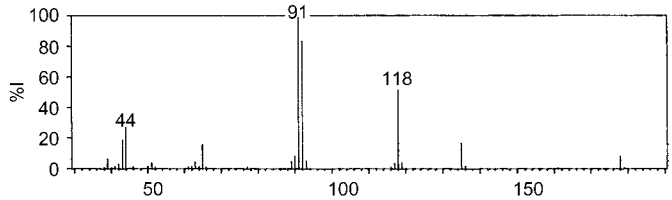
Gas Chromatography System GA—RI 1473.

High Performance Liquid Chromatography System HX—RI 339; system HY—RI 266.

Ultraviolet Spectrum Methanol—258 (A_1^1=18a), 265 nm.

Infrared Spectrum Principal peaks at wavenumbers 1660, 1090, 716, 1618, 1170, 970 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 91, 92, 118, 44, 43, 135, 65, 178.



Disposition in the Body Well absorbed after oral administration. It is extensively metabolised to inactive metabolites, principally by *p*-hydroxylation of the phenyl ring; the metabolites are excreted in the urine.

Toxicity The estimated minimum lethal dose is 5 g.

Dose 1.5 to 3 g daily.

Phenacetin

Analgesic

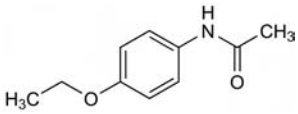
$C_{10}H_{13}NO_2$ = 179.2

CAS—62-44-2

IUPAC Name *N*-(4-Ethoxyphenyl)acetamide

Synonyms *p*-Acetiphenetidide; aceto-*p*-phenetidide; acetophenetidin; acetylphenetidin; fenacetina; paracetophenetidin.

Proprietary Names It is an ingredient of *APC tablets* and was included in *Gripponyl*, *Hemagene Tailleur*, *Polypirine* and *Cratodin Rectal*.



Chemical Properties White glistening crystalline scales or fine white crystalline powder. Mp 134° to 135°. Soluble 1 in 1300 of water, 1 in 15 of ethanol, 1 in 14 of chloroform and 1 in 90 of ether. pK_a 2.2. Log *P* (octanol/water), 1.6.

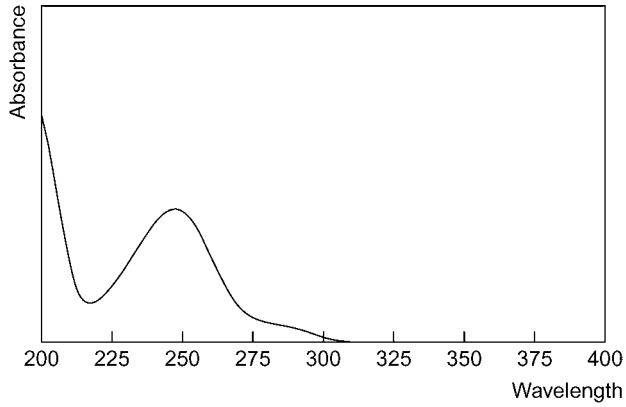
Colour Tests Liebermann's reagent—violet; boil 0.1 g with 1 mL of hydrochloric acid for 3 min, dilute with 10 mL of water, cool, filter, and add one drop of 0.02 mol/L potassium dichromate to the filtrate—violet→red.

Thin-layer Chromatography System TD—R_f 0.38; system TE—R_f 0.68; system TF—R_f 0.37; system TAD—R_f 0.52; system TAE—R_f 0.83; system TAJ—R_f 0.58; system TAK—R_f 0.41; system TAL—R_f 0.89 (acidified potassium permanganate solution, positive). See also under Paracetamol.

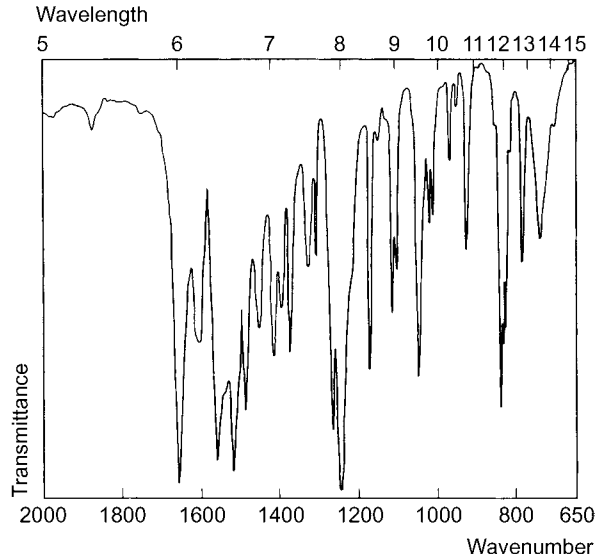
Gas Chromatography System GA—phenacetin RI 1675, M (hydroquinone) RI 1240, M (p-phenetidine) RI 1275, paracetamol RI 1687; system GB—RI 1730; system GF—RI 2325.

High Performance Liquid Chromatography System HD—phenacetin *k* 0.6, paracetamol *k* 0.1; system HW—phenacetin *k* 4.40, paracetamol *k* 0.30; system HX—phenacetin RI 377, paracetamol RI 264; system HY—phenacetin RI 335, paracetamol RI 241; system HZ—phenacetin retention time 3.0 min, paracetamol retention time 1.9 min.

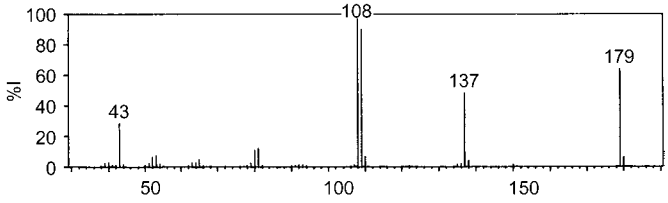
Ultraviolet Spectrum Aqueous acid—244 nm (A_1^1=649a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1244, 1655, 1513, 1555, 1265, 836 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 108, 109, 179, 137, 43, 81, 80, 110; paracetamol 109, 151, 43, 80, 108, 81, 53, 52.



Quantification

Plasma **GC** FID. Phenacetin and paracetamol. Limit of detection, <100 µg/L [Evans, Harbison 1977].

GC-MS Limit of detection, 1 pg [Murray, Boobis 1991]. Phenacetin and paracetamol. Limit of detection, 1 µg/L for phenacetin and 100 µg/L for paracetamol [Garland *et al.* 1977].

HPLC UV detection. Phenacetin and paracetamol. Limit of detection, 500 µg/L [Gotelli *et al.* 1977].

See also under Paracetamol.

Disposition in the Body Readily absorbed after oral administration, but subject to extensive first-pass metabolism. It is metabolised mainly in the liver by *O*-dealkylation to paracetamol and acetaldehyde, followed by conjugation of the paracetamol with sulfate or glucuronic acid (see under Paracetamol); other reactions are deacetylation to phenetidine (*p*-ethoxyaniline), *N*-, 2-, and α-hydroxylation forming mainly 2-hydroxyphenetidine and also 2-hydroxyphenacetin, sulfate conjugation of deacetylated metabolites, and glutathione conjugation to form *S*-(1-acetamido-4-hydroxyphenyl)cysteine and its corresponding mercapturic acid.

2-Hydroxyphenetidine appears to be the nephrotoxic metabolite and is possibly involved in the formation of methaemoglobinaemia. As the dose is increased so the percentage of the dose which is deacetylated is increased, producing proportionately

P

more phenetidine and 2-hydroxyphenetidine; production of these metabolites is also increased by concomitant administration of aspirin, caffeine, and codeine. Phenacetin is not hepatotoxic despite being converted to paracetamol; this is because the enzyme systems which convert paracetamol to its toxic metabolite are also involved in the conversion of phenacetin to paracetamol and the two conversions therefore compete, resulting in reduced paracetamol oxidation. Phenacetin metabolism is stimulated by cigarette smoking. The extent of *O*-dealkylation appears to be genetically determined. About 80 to 90% of a dose is excreted in the urine in 24 h with 50 to 80% of the dose as the sulfate and glucuronide conjugates of paracetamol, 6 to 8% as 2-hydroxyphenetidine sulfate, about 2% as *S*-(1-acetamido-4-hydroxyphenyl)cysteine, about 0.3% as phenetidine and 2-hydroxyphenacetin, 0.2% as unchanged drug, and 2 to 3% as unconjugated paracetamol.

Therapeutic Concentration

After a single oral dose of 0.9 g to 9 subjects, peak plasma-phenacetin concentrations of 0.2 to 7.4 mg/L (mean, 2.3) were attained in 1 to 2 h, and peak plasma-paracetamol concentrations of 3.1 to 11.9 mg/L (mean, 7.9) were attained in 2 to 3.5 h; plasma-phenacetin concentrations were reported to be lower in 9 smokers [Pantuck *et al.* 1974].

Toxicity The estimated minimum lethal dose is 5 g. Prolonged use of phenacetin is associated with analgesic nephropathy; it may also produce haemolytic anaemia and methaemoglobinaemia.

In a fatality involving the intentional overdosage of a preparation containing codeine, aspirin, phenacetin and caffeine, a blood–phenacetin concentration of 136 mg/L was reported; the codeine and salicylate blood concentrations were 5.3 and 265 mg/L respectively [Wright *et al.* 1975].

Half-life Plasma half-life, phenacetin 0.7 to 1.5 h, paracetamol 1.5 to 3 h.

Volume of Distribution About 1 to 2 L/kg.

Clearance Plasma clearance, about 20 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.93.

Protein Binding About 30%.

Note For a review of the general properties of phenacetin, see Clissold [1986].

Dose Phenacetin has been given as a single dose of 300 to 600 mg; maximum daily dose of 3 g.

Clissold SP (1986). Paracetamol and phenacetin. *Drugs* 32: 446–59.

Evans MA, Harbison RD (1977). GLC microanalyses of phenacetin and acetaminophen plasma levels. *J Pharm Sci* 66: 1628–1629.

Garland WA *et al.* (1977). Quantitative determination of phenacetin and its metabolite acetaminophen by GLC-chemical ionization mass spectrometry. *J Pharm Sci* 66: 340–344.

Gotelli GR *et al.* (1977). Determination of acetaminophen and phenacetin in plasma by high-pressure liquid chromatography. *Clin Chem* 23: 957–959.

Murray S, Boobis AR (1991). Combined assay for phenacetin and paracetamol in plasma using capillary column gas chromatography-negative-ion mass spectrometry. *J Chromatogr* 568: 341–350.

Pantuck EJ *et al.* (1974). Effect of cigarette smoking on phenacetin metabolism. *Clin Pharmacol Ther* 15: 9–17.

Wright JA *et al.* (1975). Blood codeine concentrations in fatalities associated with codeine. *Clin Toxicol* 8: 457–463.

P

Phenadoxone

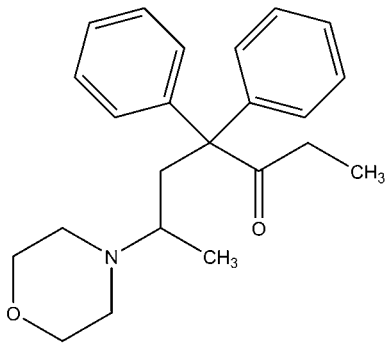
Narcotic Analgesic

$C_{23}H_{29}NO_2$ = 351.5

CAS—467-84-5

IUPAC Name 6-Morpholin-4-yl-4,4-di(phenyl)heptan-3-one

Synonyms Heptaxone; morphodone; 6-morpholino-4,4-diphenylheptan-3-one; 6-(4-morpholinyl)-4,4-diphenyl-3-heptanone; 6-tetrahydrooxazine-4,4-diphenyl-3-heptanone.



Chemical Properties Mp 75° to 76°. Log *P* (octanol/water) 4.2 [National Institutes of Health 2008], 1.56 [Meylan, Howard, 1995]. Phenadoxone is extracted by organic solvents from aqueous alkaline solutions; hydrolysis of biological material is required to improve yield.

Phenadoxone Hydrochloride

$C_{23}H_{29}NO_2$,HCl = 387.9

CAS—545-91-5

Synonyms CB-11; hepagin; heptalin; heptazone; Hoechst 10600.

Proprietary Name *Heptalgin*

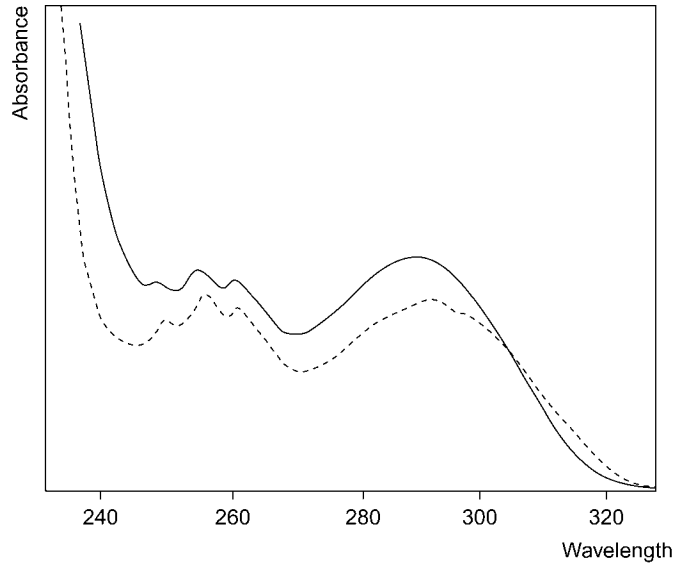
Chemical Properties A colourless crystalline powder. Mp 224° to 225° (dec). Soluble 1 in 25 of water and 1 in 10 of ethanol; very soluble in chloroform; almost insoluble in benzene and ethyl acetate. pKa (25°) 6.7 in 40% alcohol [O'Neil *et al.* 2006].

Colour Test Ammonium vandate test—deep green→blue (limit of detection, 0.5 μg).

Thin-layer Chromatography System T10—R_f 0.77 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—retention time 1.60 (relative to codeine); system G4—retention time 0.96 (relative to codeine).

Ultraviolet Spectrum Ethanol—259, 296 nm; aqueous acid (0.1 N sulfuric acid)—253, 259, 266, 292 nm.



Note For a review of the pharmacology of phenadoxone, see Basil *et al.* [1950].

Dose Up to 50 mg orally, up to 15 mg SC or IM.

References

Basil B *et al.* (1950). The pharmacology of phenadoxone or dl-6-morpholino-4 : 4-diphenyl-heptan-3-one hydrochloride. *Br J Pharmacol Chemother* 5: 125–141.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

National Institutes of Health (2008). Phenadoxone. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=10089&loc=ec_rcs. (accessed 1 July 2008).

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Phenaglycodol

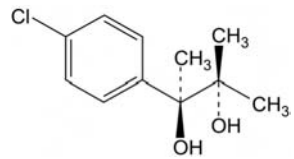
Tranquilliser

$C_{11}H_{15}ClO_2$ = 214.7

CAS—79-93-6

IUPAC Name 2-(4-Chlorophenyl)-3-methylbutane-2,3-diol

Proprietary Name *Felixyn*



Chemical Properties A crystalline solid. Mp 77° to 78°. Practically insoluble in water; soluble in ethanol.

Colour Tests Liebermann's reagent—brown; Mandelin's test—blue.

Thin-layer Chromatography System TE—R_f 0.71; system TAE—R_f 0.84.

Ultraviolet Spectrum Ethanol—259, 266 (A^1_1=13.5a), 275 nm.

Infrared Spectrum Principal peaks at wavenumbers 1095, 1085, 1015, 1180, 807, 830 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 43, 59, 155, 121, 156, 31, 157, 158.

Quantification

Blood **UV spectrophotometry** [Wallace 1968].

Urine **UV spectrophotometry** See Blood [Wallace 1968].

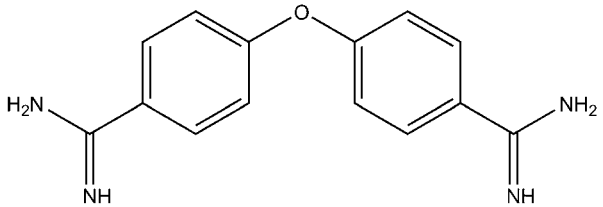
Dose 0.9 to 1.2 g daily.

Wallace JE (1968). Determination of phenaglycodol in biologic specimens by ultraviolet spectrophotometry. *J Pharm Sci* 57: 426–429.

Phenamidine

Antiprotozoal (Veterinary)
$C_{14}H_{14}N_4O = 254.3$
CAS—101-62-2

IUPAC Name 4-(4-Carbamimidoylphenoxy)benzenecarboximidamide
Synonyms 4,4′-Diamidinodiphenyl ether; 4,4′-oxybisbenzenecarboximidamide; 4,4′-oxydibenzamidine.



Chemical Properties Irregular plates from water. Mp 215° to 216°. Log *P* (octanol/water) 2.7 [National Institutes of Health 2008], 1.56 [Meylan, Howard 1995]. Phenamidine is extracted by organic solvents from aqueous alkaline solutions.

Phenamidine Isethionate

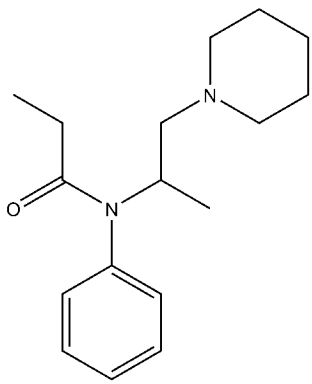
$C_{14}H_{14}N_4O,2C_2H_6O_4S = 506.6$
CAS—620-90-6
Synonym M 736; B 736.
Proprietary Name *Lomadine*
Chemical Properties White crystals or powder. Mp 225° (dec). Soluble 1 in 1.4 of water and 1 in 300 of ethanol; insoluble in ether and chloroform

Thin-layer Chromatography System T10—R_f 0.04, streaking (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—265 nm.
Quantification
Serum **CE** Capillary: fused silica (570 mm × 75 μm i.d., length to detector 500 mm). Buffer: 25 mmol/L citrate. UV detection (λ = 200 nm). Retention time: 8.6 min. Limit of detection, 0.25 mg/L [Rabanal *et al.* 2000].
Urine **CE** See Serum. Retention time: 8.0 min. [Rabanal *et al.* 2000].
Disposition in the Body
Toxicity Phenamidine is usually well tolerated when used in single doses in the treatment of babesiasis in animals [Groves, Vanniasingham 1970]. The repeated use of large doses in the treatment of demodectic mange in dogs has caused toxic symptoms, probably associated with hepatic damage due to a cumulative effect. For case studies, see Nye *et al.* [1950], Koutz [1952] and van Heerden [1981].
Dose Horses and cattle: 0.03 mL of a 40% solution/kg bodyweight; dogs: 0.3 mL of a 5% solution/kg bodyweight.

Groves MG, Vanniasingham JA (1970). Treatment of *Babesia gibsoni* infections with phenamidine isethionate. *Vet Rec* 86: 8–10.
Koutz FR (1952). Demodex folliculorum studies. I. The use of phenamidine as internal medication for the treatment of demodectic mange in the dog. *J Am Vet Med Assoc* 121: 470–473.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health. Phenamidine. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=64948&loc=ec_rcs (accessed 1 July 2008).
Nye SS *et al.* (1950). Toxic reactions following the use of phenamidine (May and Baker) in the dog. *Vet Rec* 62: 533–534.
Rabanal B *et al.* (2000). Determination by capillary zone electrophoresis of berenil, phenamidine, diampron and dibromopropamidine in serum and urine. *J Chromatogr B Biomed Sci Appl* 738: 293–303.
van Heerden J (1981). Diamidine poisoning in a dog. *J S Afr Vet Assoc* 52: 338–339.

Phenampromide

Narcotic Analgesic
$C_{17}H_{26}N_2O = 274.4$
IUPAC Name *N*-Phenyl-*N*-(1-piperidin-1-ylpropan-2-yl)propanamide
Synonyms DEA No. 9638; EINECS 204-967-1; fenampromide; *N*-(1-methyl-2-piperidinoethyl)propionanilide; phenampromid.

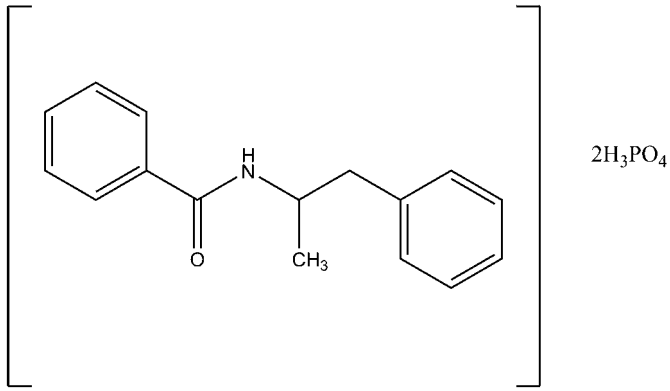


Chemical Properties Bp 124° to 128° at 0.2 mmHg. Refractive index 1.518. Log *P* (octanol/water) 2.8 [National Institutes of Health 2008]. Phenampromide is extracted by organic solvents from aqueous alkaline solutions. (–)-Phenampromide has greater analgesic efficacy than the (+)-enantiomer [Portoghese 1965].
Thin-layer Chromatography System T10—R_f 0.73 (location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—250, 257 nm with inflexions at 260, 262 and 266 nm.

National Institutes of Health. Phenampromid. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=8523&loc=ec_rcs (accessed 1 July 2008).
Portoghese PS (1965). Stereochemical studies on medicinal agents II. Absolute configuration of (–)-phenampromide. *J Med Chem* 8: 147–150.

Phenatine

Central Stimulant
$C_{15}H_{16}N_2O,2H_3PO_4 = 436.3$
IUPAC Name *N*-(1-Phenylpropan-2-yl)pyridine-3-carboxamide; phosphoric acid
Synonyms *N*-(α-Methylphenethyl)pyridine-3-carboxamide diphosphate; nicotinic acid β-phenylisopropylamide diphosphate.

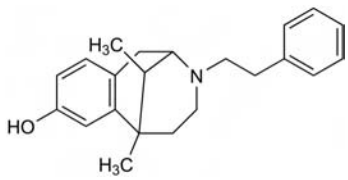


Chemical Properties Colourless crystals or a white crystalline powder. Mp 162° to 166°. Soluble in water and ethanol; almost insoluble in ether. The base, $C_{15}H_{16}N_2O$, occurs as crystals with a Mp of 99° to 100°; insoluble in water and soluble in chloroform. Phenatine is extracted by organic solvents from aqueous alkaline solutions.
Colour Test Sulfuric acid–formaldehyde test—orange (limit of detection, 0.5 μg).
Thin-layer Chromatography System T1—R_f 0.69 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 2.00 (relative to diphenhydramine), retention time 0.43 (relative to codeine); system G4/225—retention time 0.89 (relative to codeine).
Ultraviolet Spectrum 0.1 N sulfuric acid—262 nm (E1%, 1 cm 275), minimum at 242 nm.
Infrared Spectrum Principal peaks at wavenumbers 1650, 1527, 704, 749 cm^{-1} (KBr disk).
Dose Up to 600 mg daily.

Phenazocine

Narcotic Analgesic
$C_{22}H_{27}NO = 321.5$
CAS—127-35-5

Synonyms 1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(2-phenethyl)-2,6-methano-3-benzazocin-8-ol; phenethylazocine; phenobenzorphan.



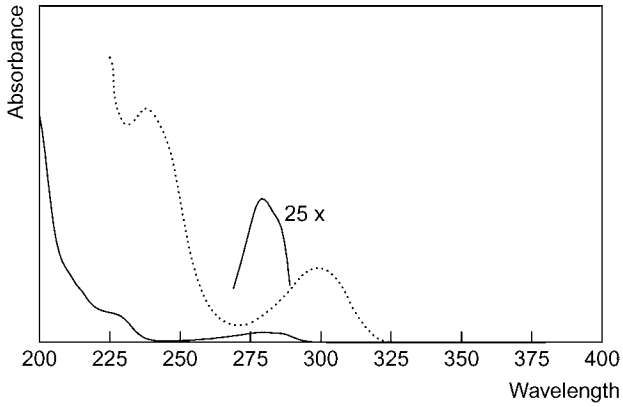
Chemical Properties Crystals. Mp 181° and Mp about 159°. pK_a 8.5. Log *P* (octanol/water), 5.2.

Phenazocine Hydrobromide

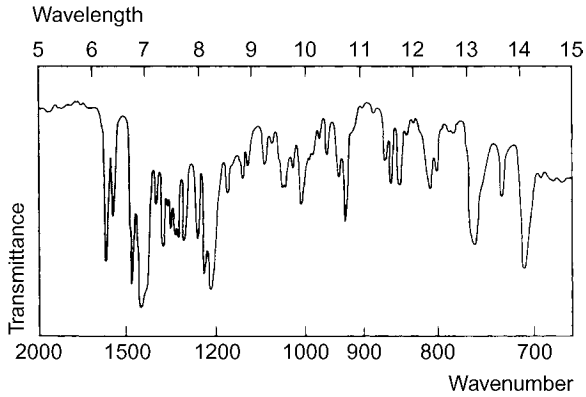
$C_{22}H_{27}NO,HBr,{}^1/_2H_2O = 411.4$
CAS—1239-04-9 (anhydrous)

Proprietary Name *Narphen*
Chemical Properties A white microcrystalline powder, which can exist in 3 polymorphic forms. Mp about 166° to 170°. Soluble 1 in 350 of water, 1 in 45 of ethanol and 1 in 140 of chloroform; practically insoluble in ether.

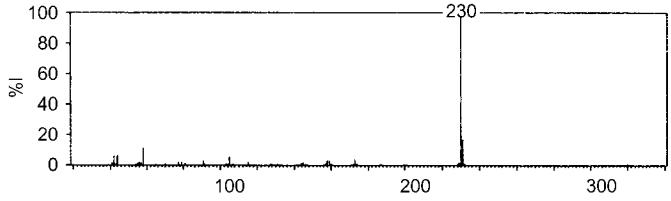
Colour Test Marquis test—brown.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.16; system TC—R_f 0.39; system TE—R_f 0.74; system TL—R_f 0.49; system TAE—R_f 0.50; system TAF—R_f 0.81; system TAJ—R_f 0.26; system TAK—R_f 0.20; system TAL—R_f 0.90 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, brown).
Gas Chromatography System GA—RI 2686; system GB—RI 2833.
High Performance Liquid Chromatography System HA—*k* 1.3; system HC—*k* 0.3; system HX—RI 409; system HY—RI 299.
Ultraviolet Spectrum Aqueous acid—278 nm (A^1_1=64a); aqueous alkali—238, 298 nm.



Infrared Spectrum Principal peaks at wavenumbers (phenazocine hydrobromide, Nujol mull) 1224, 1497, 1241, 705, 1613, 758 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 230, 231, 58, 44, 42, 105, 173, 159.



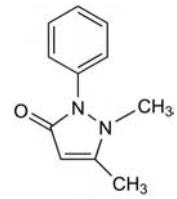
Disposition in the Body Readily absorbed after oral, sublingual or IM administration; metabolised in the liver.
Toxicity The estimated minimum lethal dose is 0.2 g. Phenazocine is addictive although tolerance develops more slowly and to a lesser extent than with morphine.
Dose Usually 5 mg of phenazocine hydrobromide every 4 to 6 h; single doses of 20 mg may be given.

Phenazone

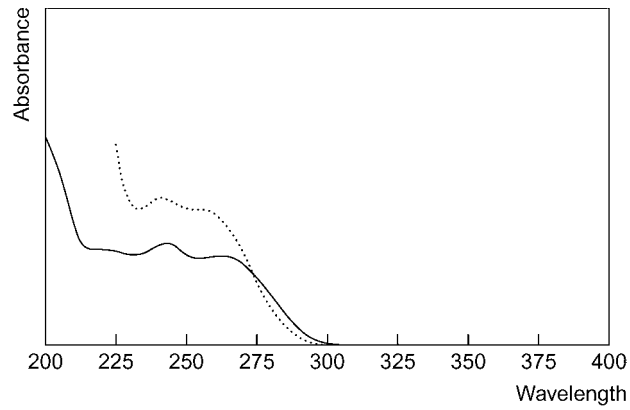
Analgesic
$C_{11}H_{12}N_2O$ = 188.2
CAS—60-80-0

IUPAC Name 1,5-Dimethyl-2-phenylpyrazol-3-one
Synonyms Analgésine; antipyrin(e); azophenum; 1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one; fenazona; phenazonum; phenyldimethylpyrazolone.

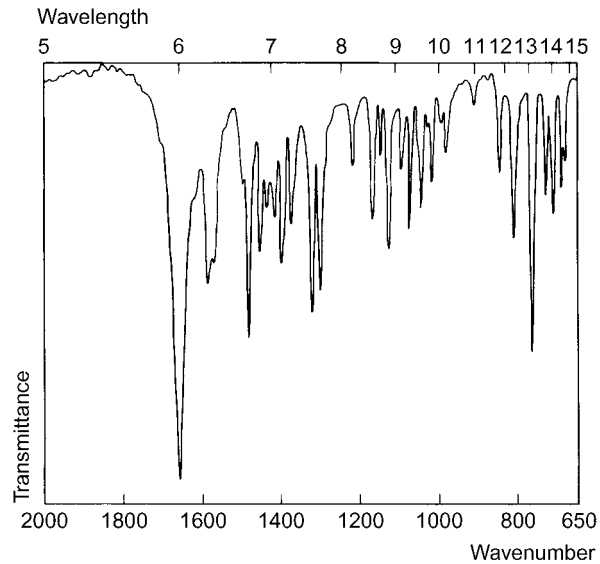
Proprietary Names *Aequiton-P*; *Aurone*; *Erasol*; *Migrane-Kranit-mono*; *Oto-Phen*; *Tropex*. It is an ingredient of *Allergen*; *Auralgan*, *Auralgicin*; *Auroto*, *Otocalm* and *Tympagesic*.



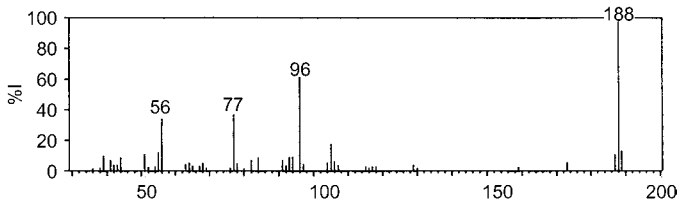
Chemical Properties Small, colourless crystals or white crystalline powder. Mp 111° to 113°. Soluble 1 in <1 of water, 1 in 1.3 of ethanol, 1 in 1 of chloroform and 1 in 43 of ether. pK_a 1.5 (25°). Log *P* (octanol/pH 7.4), 0.4. Extraction yield (chlorobutane), 0.35 [Demme *et al.* 2005].
Colour Tests *p*-Dimethylaminobenzaldehyde (100°, 5 min)—red/violet; ferric chloride—red; Liebermann's reagent (100°)—orange; Mandelin's test—green; nitrous acid—green.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.04; system TD—R_f 0.18; system TE—R_f 0.45; system TF—R_f 0.14; system TAD—R_f 0.50; system TAE—R_f 0.66; system TAF—R_f 0.66; system TAJ—R_f 0.51; system TAK—R_f 0.18; system TAL—R_f 0.83 (ferric chloride solution, red-brown; acidified iodoplatinate solution, positive; mercuric chloride–diphenylcarbazone reagent, positive; acidified potassium permanganate solution, positive; Van Urk reagent, pink).
Gas Chromatography System GA—phenazone RI 1835, M (4-OH-) RI 1855; system GB—RI 1951; system GF—RI 2445.
High Performance Liquid Chromatography System HA—*k* 0.2; system HD—*k* 0.1; system HW—*k* 0.95; system HX—RI 333; system HY—RI 299; system HZ—RT 2.1 min; system HAX—RT 5.4 min; system HAY—RT 4.5 min.
Ultraviolet Spectrum Aqueous acid—230 nm (A^1_1=590a); aqueous alkali—242 (A^1_1=494a), 256 nm.



Infrared Spectrum Principal peaks at wavenumbers 1660, 770, 1318, 1305, 1590, 1580 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 188, 96, 77, 56, 105, 189, 55, 51; norphenazone 174, 77, 91, 105, 132.



Quantification

Plasma **GC** AFID. Limit of detection, 1 mg/L [Abernethy *et al.* 1981].

HPLC UV detection. Phenazone and its major metabolites [Lanchote *et al.* 1997]. UV detection. Limit of detection, 500 µg/L [Campbell *et al.* 1979].

LC-MS Limit of detection, 0.3 µg/L [Coolen *et al.* 1999].

RIA Limit of detection, 10 µg/L [Chang *et al.* 1976].

Urine **HPLC** Phenazone and its metabolites [Sarkar *et al.* 1992]. Phenazone metabolites [Palette *et al.* 1991]. UV detection. Phenazone, 3-hydroxymethylphenazone, 4-hydroxyphenazone and norphenazone. Limit of detection, 1–2 mg/L [Eichelbaum *et al.* 1981].

Biological Fluids **HPLC** Phenazone sulfo- and glucuro-conjugated metabolites [Palette *et al.* 1994].

Saliva **HPLC** [Gartzke, Jager 1991].

RIA See Plasma [Chang *et al.* 1976].

Disposition in the Body Rapidly and completely absorbed after oral administration. It is widely distributed in body fluids, with saliva and breast milk levels being about the same as those found in plasma. It is metabolised to 3-hydroxymethylphenazone, 4-hydroxyphenazone, and norphenazone, followed by glucuronic acid conjugation; demethylation also occurs. The rate of biotransformation is variable and appears to be genetically determined. Phenazone and its metabolites are excreted in the urine mainly as glucuronides, although sulfate conjugation has also been reported. About 30–40% of a dose is excreted in the urine in 48 h as conjugated 4-hydroxyphenazone, 10–40% as the 3-hydroxymethyl derivative, up to about 20% as norphenazone, 3–6% as 4,4′-dihydroxyphenazone, 8–12% as 4,5-dioxypyrazoline, and <5% as unchanged drug; 3-carboxyphenazone has also been identified in the urine. A total of about 95% of a dose is excreted in the urine in 4 days.

Therapeutic Concentration

After a single oral dose of 10 mg/kg to 5 subjects, peak plasma concentrations of 10–15.5 mg/L (mean, 13.4) were attained in 1 h. Concentrations in saliva were similar to those in plasma [van Boxtel *et al.* 1976].

Toxicity The estimated minimum lethal dose is 5 g, but fatalities from acute poisoning are rare. Prolonged therapeutic administration may give rise to agranulocytosis.

In a suicide due to ingestion of phenazone, a postmortem blood concentration of 110 mg/L was reported; alcohol was also detected in the blood at a concentration of 260 mg/L [Péclet, Rousseau 1981].

During the course of a clinical trial of the efficacy of oxerutins in venous ulceration, an 87-year-old woman died within 2 h of the IV injection of phenazone 500 mg (administered over 10 min). Postmortem analysis revealed a blood phenazone concentration of 22 mg/L and the cause of death was certified as 'cardiac arrest following injection of antipyrine' [Travers 1991].

Half-life Plasma half-life, about 7–15 h (mean, 10); increased in subjects with renal impairment or hypothyroidism and in the elderly, decreased in subjects with hyperthyroidism; the half-life is also readily influenced by concomitant administration of other drugs.

Volume of Distribution About 0.6 L/kg.

Clearance Plasma clearance, about 0.7 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, about 0.93.

Protein Binding <10%.

Note For a review of the pharmacokinetics of phenazone, see Vital-Durand *et al.* [1988].

Dose Phenazone has been given in doses of 300 to 600 mg; maximum daily dose of 4 g.

Abernethy DR *et al.* (1981). Antipyrine determination in human plasma by gas–liquid chromatography using nitrogen–phosphorus detection. *J Chromatogr* 223: 432–437.

Campbell TM *et al.* (1979). Determination of antipyrine in plasma by reversed-phase high-performance liquid chromatography. *J Chromatogr* 163: 236–238.

Chang RL *et al.* (1976). Antipyrine: radioimmunoassay in plasma and saliva following administration of a high dose and a low dose. *Clin Pharmacol Ther* 20: 219–226.

Coolen SA *et al.* (1999). Determination of phenolic derivatives of antipyrine in plasma with solid-phase extraction and high-performance liquid chromatography-atmospheric-pressure chemical ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl* 732: 103–113.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Eichelbaum M *et al.* (1981). HPLC determination of antipyrine metabolites. *Pharmacology* 23: 192–202.

Gartzke J, Jager H (1991). The determination of antipyrine elimination in saliva by liquid chromatography. *J Pharm Biomed Anal* 9: 977–979.

Lanchote VL *et al.* (1997). Determination of antipyrine and metabolites in plasma of a patient with mild renal failure. *Ther Drug Monit* 19: 705–710.

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Palette C *et al.* (1994). Isolation and purification of three glucuronides of antipyrine. Proposal for an original analytical method for quantitation of sulpho- and glucuroconjugated metabolites. *Biomed Chromatogr* 8: 77–84.

Péclet C, Rousseau M (1981). *Bull Int Assoc Forensic Toxicol* 16(1): 32.

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Travers AF (1991). A fatality after antipyrine administration. *Clin Pharmacol Ther* 49: 695–696.

van Boxtel CJ *et al.* (1976). Comparison of the half-life of antipyrine in plasma, whole blood and saliva of man. *Eur J Clin Pharmacol* 9: 327–332.

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Phenazopyridine

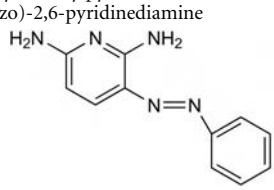
Analgesic

$C_{11}H_{11}N_5$ = 213.2

CAS—94-78-0

IUPAC Name 3-Phenyldiazenylpyridine-2,6-diamine

Synonym 3-(Phenylazo)-2,6-pyridinediamine



Chemical Properties Mp 139°. Log *P* (octanol/water), 2.8.

Phenazopyridine Hydrochloride

$C_{11}H_{11}N_5$,HCl = 249.7

CAS—136-40-3

Proprietary Names *Phenazo*; *Pyridacil*; *Pyridium*. It is an ingredient of *Azo Gantanol*, *Azo Gantrisin*, *Azo-Mandelamine*, *Azotrex*, and *Uromide*.

Chemical Properties A light or dark red to dark violet crystalline powder. Mp about 235°, with decomposition. Soluble 1 in 300 of cold water, 1 in 20 of boiling water, 1 in about 60 of ethanol, and 1 in about 330 of chloroform; very slightly soluble in ether; soluble in glacial acetic acid. Phenazopyridine hydrochloride readily forms supersaturated aqueous solutions which deposit slowly on storage.

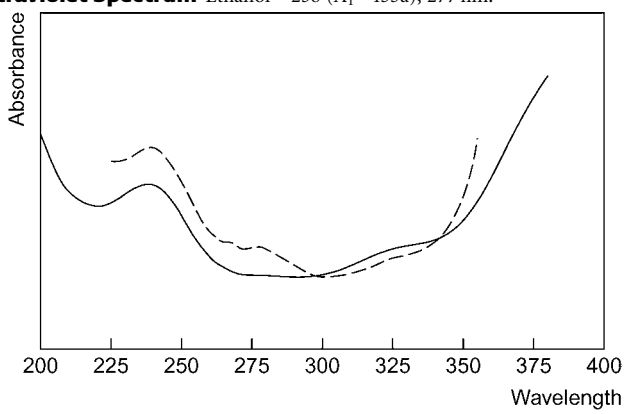
Colour Tests Mandelin's test—green; Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.01; system TC—R_f 0.50; system TE—R_f 0.70; system TL—R_f 0.53; system TAE—R_f 0.80; system TAJ—R_f 0.46; system TAK—R_f 0.56; system TAL—R_f 0.91 (acidified potassium permanganate solution, positive).

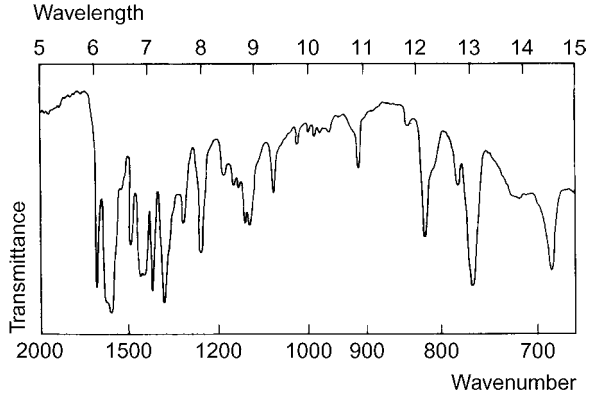
Gas Chromatography System GA—phenazopyridine RI 2245, 4-aminophenol RI 1265, aniline RI 1158; system GB—RI 2370.

High Performance Liquid Chromatography System HY—RI 314.

Ultraviolet Spectrum Ethanol—238 (A_1^1=455a), 277 nm.

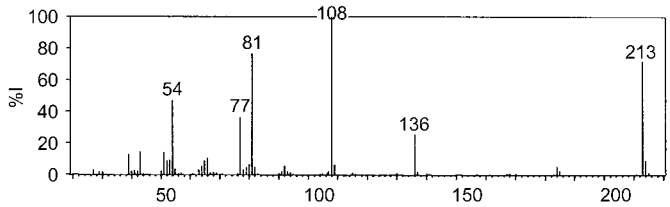


Infrared Spectrum Principal peaks at wavenumbers 1585, 1656, 762, 685, 1248, 1493 cm^{-1} (Nujol mull).



P

Mass Spectrum Principal ions at *m/z* 108, 81, 213, 54, 77, 136, 51, 43; 4-aminophenol 109, 80, 53, 81, 108, 52, 54, 110.



Disposition in the Body Absorbed after oral administration. Rapidly eliminated by the kidneys, giving an orange-red colour to the urine; about 90% of a dose is excreted in the urine in 24 h, of which 7% is aniline, 20% is *N*-acetyl-4-aminophenol, 25% is 4-aminophenol, and 45% is unchanged drug.

Toxicity Methaemoglobinaemia and acute renal failure have been reported after overdoses.

Dose Usually 600 mg of phenazopyridine hydrochloride daily.

Phencyclidine

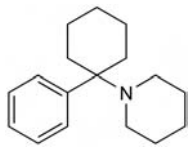
Hallucinogen

$C_{17}H_{25}N$ = 243.4

CAS—77-10-1

IUPAC Name 1-(1-Phenylcyclohexyl)piperidine

Street Names Angel dust, PCP. For other names applied to illicitly used phencyclidine and phencyclidine hydrochloride, see Sweetman [2007].



Chemical Properties Crystals. Mp 46° to 46.5°. pK_a 8.5. Log *P* (octanol/water), 4.7. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Phencyclidine Hydrochloride

$C_{17}H_{25}N,HCl$ = 279.9

CAS—956-90-1

Synonyms CI-395; CN-25253-2; GP-121; NSC-40902; PCP.

Chemical Properties A white crystalline powder. Mp 233° to 235°. Soluble 1 in 6 of water, 1 in 7 of ethanol and 1 in 2 of chloroform; very slightly soluble in ether.

Phencyclidine Hydrobromide

Chemical Properties Crystals. Mp 214° to 218°.

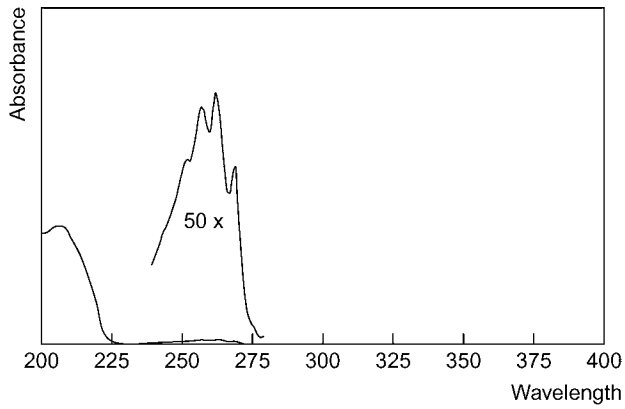
Colour Test *p*-Dimethylaminobenzaldehyde (100°, 3 min)—red.

Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.73; system TC—R_f 0.35; system TE—R_f 0.84; system TL—R_f 0.66; system TAE—R_f 0.23; system TAF—R_f 0.69; system TAJ—R_f 0.24; system TAK—R_f 0.04; system TAL—R_f 0.48 (acidified iodoplatinate solution—positive).

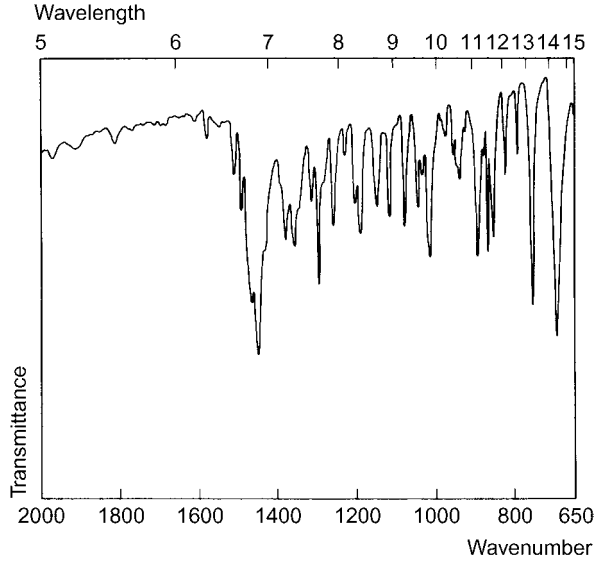
Gas Chromatography System GA—RI 1900; system GB—RI 1981; system GF—RI 2150.

High Performance Liquid Chromatography System HA—*k* 2.4 (tailing peak); system HX—RI 375; system HY—RI 285.

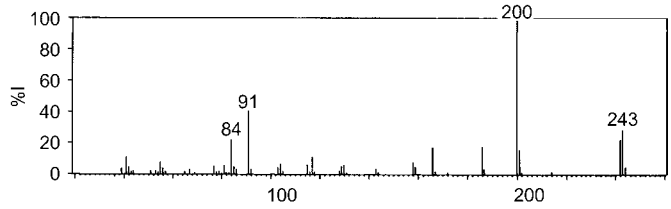
Ultraviolet Spectrum Aqueous acid—252, 258, 263 (A_1^1 = 13.5a), 270 nm.



Infrared Spectrum Principal peaks at wavenumbers 700, 760, 1298, 1020, 892, 869 cm^{-1} (phencyclidine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 200, 91, 243, 242, 84, 186, 166, 201 (phencyclidine); 91, 216, 77, 259, –, –, –, – (1-(1-phenylcyclohexyl)-4-hydroxypiperidine); 200, 91, 84, 86, 186, 259, –, – (4-phenyl-4-piperidinocyclohexanol).



Quantification

Blood **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3 mL/min. Temperature programme: 150° for 1 min to 280° at 10°/min. NPD. Retention time: 8.3 min. Limit of detection, 0.75 µg/L [Ishii *et al.* 1996].

GC-MS SIM acquisition mode [Ferguson, Garg 2010]. EI ionisation. Limit of detection, 0.05 µg/L. SIM acquisition mode. Limit of detection, 0.01 µg/L (whole blood) [Ishii *et al.* 2000].

Immunoassay For an immunoassay for phencyclidine and other drugs including amfetamines and methadone, see Klinger *et al.* [1990].

Plasma **GC** Column: BP 10 SGE (25 m × 0.22 mm i.d.). Carrier gas: N_2, 6.2 mL/min. Temperature programme: 200° to 270° at 10°/min for 23 min. NPD. Retention time: 6.42 min. Limit of detection, 1.2 µg/L [Kintz *et al.* 1990]. Column: 3% OV-17 on 80/100 mesh Chromosorb W HP (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 220° for 8 min to 270° at 50°/min for 1 min. NPD. Limit of detection, 5 ng/L [Pitts *et al.* 1980].

HPLC FID (λ_{ex}= 380 nm, λ_{em}= 445 nm). Phencyclidine, methadone and their metabolites [Derendorf, Garrett 1983].

LC-MS Column: Alltima C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L formic acid (10 : 90 for 2 min to 25 : 75 in 16 min to 35 : 65 in 0.1 min to 100 : 0 in 12 min), flow rate 1.0 mL/min. MRM acquisition mode, positive-ion mode. Limit of quantification, 1.3 µg/L, limit of detection, 0.22 µg/L [Sergi *et al.* 2009].

Immunoassay See Blood [Klinger *et al.* 1990].

Serum **GC** Column: SP-2250 on 80/100 Supelco mesh (1.84 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 220°. NPD. Limit of detection, 0.5 µg/L [Werner *et al.* 1986]. Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (1.8 m [6′] × 2 mm i.d.). Carrier gas: He, 26 mL/min. Temperature: 192°. NPD. Retention time: 2.8 min. Limit of detection, 5 µg/L [Miceli *et al.* 1981]. See Plasma [Pitts *et al.* 1980].

Radioimmunoassay Limit of detection, <0.5 µg/L [Owens *et al.* 1982]. See Blood [Klinger *et al.* 1990]

Urine **GC** Column: HP5 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 140° to 300° at 20°/min. PDHID. Limit of detection, 0.07 mg/L [Casari, Andrews 2001]. See Blood [Ishii *et al.* 1996]. Column: Chromosorb W AW-DMCS 80/100 mesh (914 × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 230°. NPD. Retention time: 1.29 min. Limit of detection, 15 µg/L [Kandiko *et al.* 1990]. SE-30 3% on Gas-Chrom Q 100/120 mesh (1.83 m × 2 mm i.d.). Carrier gas: CH_4, 16 mL/min. Temperature: 190°. CI at 70 eV, SIM acquisition mode. Relative retention time: 0.79. Limit of detection, 10 µg/L for phencyclidine and 50 µg/L for metabolites [Cone *et al.* 1981]. See Serum [Miceli *et al.* 1981]. See Plasma [Pitts *et al.* 1980].

GC-MS See Blood [Ferguson, Garg 2010; Ishii *et al.* 2000]. Column: J &W DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 115° for 1 min to

300° at 30°/min for 2 min. Comparisons of SIM, SIS, or full scan mode. Limit of detection not reported [Vorce *et al.* 2000]. Column: cross-linked 5% phenyl methyl silicone (12 × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 50 mL/min. Temperature programme: 120° for 1 min to 190° at 40°/min for 6 min. Limit of quantification, 1.38 µg/L, limit of detection, 0.47 µg/L [Stevenson *et al.* 1992].

LC-MS Column: XTerra MS C_{18} (50 × 3.0 mm i.d., 2.5 µm). Mobile phase: 5 mmol/L ammonium acetate with 0.05% acetic acid : acetonitrile (96 : 4 to 88 : 12 at 8 min to 74 : 26 at 8.5 min to 63 : 37 at 14 min to 20 : 80 at 14.1 min for 2.2 min to 96; 4 at 16.4 min for 3.7 min), flow rate 0.8 mL/min. TIS, MRM acquisition mode. Retention time: 11.1 min. Limit of detection, 0.0766 µg/L [Feng *et al.* 2007].

CSF **GC** See Serum [Miceli *et al.* 1981].

Oral Fluid **GC** See Plasma [Pitts, Jr. *et al.* 1980].

LC-MS Column: Allure PFP Propyl LC (50 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% formic acid-2 mmol/L ammonium acetate-2% acetonitrile : 0.1% formic acid-2 mmol/L ammonium acetate-10% HPLC grade water in acetonitrile (90 : 10 for 0.5 min to 65 : 35 over 1.5 min for 2 min to 5 : 5 in 1 min to 2 : 98 in 1 min for 2 min to 90 : 10 for 3 min), flow rate 800 µL/min. ESI, positive ion mode. Limit of quantification, 0.4 µg/L, limit of detection, 0.2 µg/L [Fritch *et al.* 2009]. See Plasma [Sergi *et al.* 2009].

Vitreous Humour **GC** FID [Jenkins, Oblock 2008].

Hair **GC-MS** Column: cross-linked methylsilicone TC-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 4 psi. Temperature programme: 80° for 0.5 min to 280° at 15°/min. Limit of quantification, 0.2 ng/mg [Nakahara *et al.* 1997].

Tissues **GC** See Plasma [Pitts *et al.* 1980].

Note For a review of the analysis of phencyclidine and its metabolites, see Veselovskaia *et al.* [1999].

Disposition in the Body Phencyclidine is a drug of abuse that is self-administered orally, IV, by nasal insufflation, or by smoking impregnated plant material. The main metabolic reaction is hydroxylation and 2 metabolites have been identified in the urine as glucuronide conjugates: 4-phenyl-4-piperidinocyclohexanol and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP). The product of *N*-dealkylation (1-phenylcyclohexylamine) has also been identified in urine, together with an oxidised derivative, 5-(1-phenylcyclohexylamino)valeric acid. Approximately 73% of a dose is excreted in the urine and 5% is eliminated in the faeces in 10 days, of which ~16% of the dose is unchanged and 30% is conjugated hydroxylated metabolites; the rate of excretion of unchanged drug is increased in acidic urine.

Concentration in Blood

Following an oral dose of 1 mg, given to 5 subjects, a mean peak plasma concentration of 0.0027 mg/L was reported at 2.5 h [Cook *et al.* 1982].

Toxicity Blood concentrations in the region of 0.1 mg/L produce abnormal behaviour and concentrations of ~0.3 mg/L or more cause severe toxic symptoms, which may be followed by death although there is considerable intersubject variation. Many deaths have resulted, not from the toxic effects of phencyclidine itself, but from the effects of irrational behaviour caused by the drug. In 53 cases of death reported in the literature as not directly caused by phencyclidine, blood concentrations ranged from 0.01–2.1 mg/L (mean, 0.36); in 22 of these cases urine concentrations ranged from 0.1–10.6 mg/L (mean, 2.1); alcohol was also present in a number of these cases. In one study of postmortem phencyclidine blood concentrations, most were in the range 0.01–9.0 mg/L [Budd, Liu 1982].

In 9 deaths directly attributable to phencyclidine overdose, the following postmortem concentrations were reported: blood 0.3–12 mg/L (mean, 2.4; 8 cases), brain 0.1–32 µg/g (mean, 7.3; 6 cases), liver 0.9–80 µg/g (mean, 20; 9 cases) and urine 0.4–48.6 mg/L (mean, 21.7; 5 cases) [Cravey *et al.* 1979].

A 28-year-old man survived a massive overdose of phencyclidine, despite plasma concentrations reaching a maximum of 1.879 mg/L 2 days after admission to hospital (the highest recorded so far in a survivor). On hospital day 11 (13 days after the ingestion), he passed 2 plastic bags (1 of which was ruptured) via his rectum and his CSF phencyclidine concentration was 0.245 mg/L; the patient made a rapid neurological recovery starting on hospital day 12 [Jackson 1989].

In a 29-year-old man who swallowed a plastic bag containing phencyclidine, serum levels were persistently elevated for several weeks, with the patient remaining in a coma for 24 days. On hospital day 20, following nasogastric administration of oral colonic lavage solution on days 11–14, a plastic bag (presumed to have contained phencyclidine) was passed via the rectum; the serum phencyclidine levels declined and the patient recovered neurological function. The following course of serum phencyclidine concentrations was reported: 0.85 mg/L on day 4, 1.01 mg/L on day 7, 1.69 mg/L on day 10, 1.65 mg/L on day 14, 1.16 mg/L on day 16, 0.57 mg/L on day 18, 0.62 mg/L on day 21 and 0.06 mg/L on day 23 [Young, Crapo 1992].

Half-life Plasma half-life, 7–46 h (mean, 17), increased up to 4 days in severe poisoning cases.

Volume of Distribution ≈6 L/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.0.

Protein Binding ≈65–80%.

Budd RD, Liu Y (1982). Phencyclidine concentrations in postmortem body fluids and tissues. *J Toxicol Clin Toxicol* 19: 843–850.

Casari C, Andrews AR (2001). Application of solvent microextraction to the analysis of amphetamines and phencyclidine in urine. *Forensic Sci Int* 120: 165–171.

Cone EJ *et al.* (1981). Simultaneous determination of phencyclidine and monohydroxylated metabolites in urine of man by gas chromatography–mass fragmentography with methane chemical ionization. *J Chromatogr* 223: 331–339.

Cook CE *et al.* (1982). Phencyclidine disposition after intravenous and oral doses. *Clin Pharmacol Ther* 31: 625–634.

Cravey RH *et al.* (1979). Phencyclidine-related deaths: a report of nine fatalities. *J Anal Toxicol* 3: 199–201.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Derendorf H, Garrett ER (1983). High-performance liquid chromatographic assay of methadone, phencyclidine, and metabolites by postcolumn ion-pair extraction and on-line fluorescent detection of the counterion with applications. *J Pharm Sci* 72: 630–635.

Feng J *et al.* (2007). Simultaneous determination of multiple drugs of abuse and relevant metabolites in urine by LC-MS-MS. *J Anal Toxicol* 31: 359–368.

Ferguson AM, Garg U (2010). Quantitation of phencyclidine (PCP) in urine and blood using gas chromatography–mass spectrometry (GC-MS). *Meth Mol Biol* 603: 461–467.

Fritch D *et al.* (2009). Identification and quantitation of amphetamines, cocaine, opiates, and phencyclidine in oral fluid by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 33: 569–577.

Ishii A *et al.* (1996). Simple and sensitive detection of phencyclidine in body fluids by gas chromatography with surface ionization detection. *Int J Legal Med* 108: 244–247.

Ishii A *et al.* (2000). Ultrasensitive determination of phencyclidine in body fluids by surface ionization organic mass spectrometry. *Anal Chem* 72: 404–405.

Jackson JE (1989). Phencyclidine pharmacokinetics after a massive overdose. *Ann Intern Med* 111: 613–615.

Jenkins AJ, Oblock J (2008). Phencyclidine and cannabinoids in vitreous humor. *Leg Med (Tokyo)* 10: 201–203.

Kandiko CT *et al.* (1990). Detection of low nanogram quantities of phencyclidine extracted from human urine: preparation of an acetylated column packing material for use in gas chromatography with nitrogen–phosphorus detection. *J Chromatogr* 528: 208–213.

Kintz P *et al.* (1990). Simultaneous screening and quantification of several nonopiate narcotic analgesics and phencyclidine in human plasma using capillary gas chromatography. *Meth Find Exp Clin Pharmacol* 12: 193–196.

Klinger RA *et al.* (1990). Direct automated EMIT d.a.u. analysis of *N,N*-dimethylformamide-modified serum, plasma, and postmortem blood for amphetamines, barbiturates, methadone, methaqualone, phencyclidine, and propoxyphene. *J Anal Toxicol* 14: 288–291.

Miceli JN *et al.* (1981). An improved method for the quantitation of phencyclidine (PCP) in biological samples utilizing nitrogen-detection gas chromatography. *J Anal Toxicol* 5: 29–32.

Nakahara Y *et al.* (1997). Hair analysis for drugs of abuse. XVII. Simultaneous detection of PCP, PCHP, and PCPdiol in human hair for confirmation of PCP use. *J Anal Toxicol* 21: 356–362.

Owens SM *et al.* (1982). Radioimmunoassay for phencyclidine (PCP) in serum. *Clin Chem* 28: 1509–1513.

Pitts FNJr *et al.* (1980). Capillary gas chromatography with a nitrogen detector for measurement of phencyclidine, ketamine and other arylcycloalkylamines in the picogram range. *J Chromatogr* 193: 157–159.

Sergi M *et al.* (2009). Multiclass analysis of illicit drugs in plasma and oral fluids by LC-MS/MS. *Anal Bioanal Chem* 393: 709–718.

Stevenson CC *et al.* (1992). Solid phase extraction of phencyclidine from urine followed by capillary gas chromatography/mass spectrometry. *J Anal Toxicol* 16: 337–339.

Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35 edn. London: Pharmaceutical Press.

Veselovskaia NV *et al.* (1999). [The chromatographic analysis of phencyclidine, its metabolites and analogs in biological fluids]. *Sud Med Ekspert* 42: 20–25.

Vorce SP *et al.* (2000). Assessment of the ion-trap mass spectrometer for routine qualitative and quantitative analysis of drugs of abuse extracted from urine. *J Anal Toxicol* 24: 595–601.

Werner M *et al.* (1986). Gas–liquid chromatography of phencyclidine in serum, with nitrogen–phosphorus detection. *Clin Chem* 32: 1921–1924.

Young JD, Crapo LM (1992). Protracted phencyclidine coma from an intestinal deposit. *Arch Intern Med* 152: 859–860.

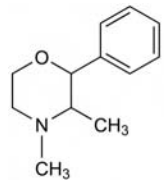
Phendimetrazine

Anorectic

$C_{12}H_{17}NO$ = 191.3

CAS—634-03-7

IUPAC Name 3,4-Dimethyl-2-phenylmorpholine



Chemical Properties pK_a 7.6. Log *P* (octanol/water), 1.7.

Phendimetrazine Tartrate

$C_{12}H_{17}NO,C_4H_6O_6$ = 341.4

CAS—50-58-8

Synonyms Phendimetrazine acid tartrate; phendimetrazine bitartrate.

Proprietary Names *Adipost*; *Adphen*; *Anoran*; *Anorex*; *Bacarate*; *Bontril*; *Dyrexan-OD*; *Melfiat*; *Obalan*; *Obesan-X*; *Obex-LA*; *Plegine*; *Prelu-2*; *Rexigen Forte*; *Statobex*; *Trimstat*; *Trimtabs*; *Wehless*; *Weightrol*; *X-trozine*.

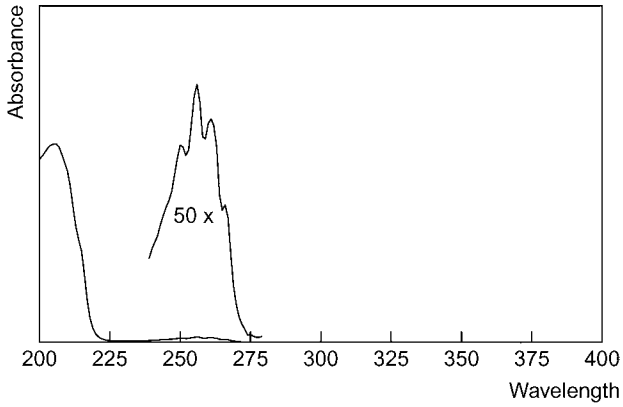
Chemical Properties A white crystalline powder. Freely soluble in water; very slightly soluble in ethanol; practically insoluble in acetone, benzene, chloroform and ether.

Colour Test Liebermann's reagent—red-orange.

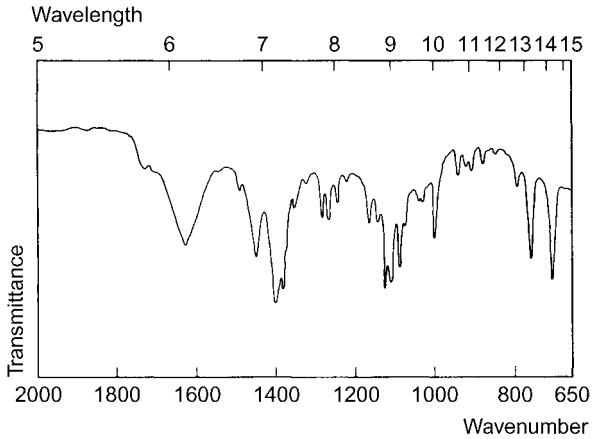
Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.36; system TC—R_f 0.51; system TE—R_f 0.62; system TL—R_f 0.24; system TAE—R_f 0.49; system TAF—R_f 0.41 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive). See also Phenmetrazine.

Gas Chromatography System GA—phendimetrazine RI 1334, phenmetrazine RI 1432; system GB—phendimetrazine RI 1504, phenmetrazine RI 1483; system GC—phendimetrazine RI 1735, phenmetrazine RI 1873.

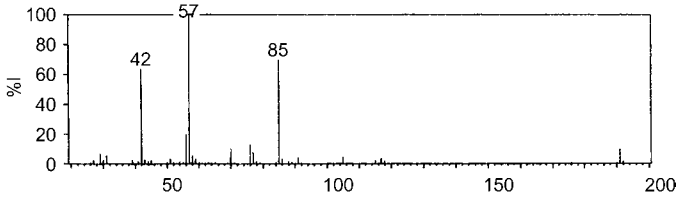
High Performance Liquid Chromatography System HA—*k* 0.9 phendimetrazine; *k* 1.7 phenmetrazine; system HC—*k* 0.3; system HX—phendimetrazine RI 263; phenmetrazine RI 258; system HY—phendimetrazine RI 218; phenmetrazine RI 241.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=12.7a), 261, 267 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1120, 1105, 697, 1083, 752, 1630 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 57, 85, 42, 56, 76, 191, 70, 77; phenmetrazine 71, 42, 56, 43, 177, 77, 178, 105.

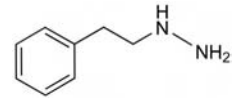


Quantification
Plasma **GC** FID. Phendimetrazine and other CNS stimulants [Kintz *et al.* 1989]. AFID. Limit of detection, 2 µg/L [Rudolph *et al.* 1983].
Serum **GC** See Plasma [Rudolph *et al.* 1983]. AFID. Phendimetrazine and phenmetrazine. Limit of detection, 10 µg/L [Long *et al.* 1982].
Urine **GC** See Plasma [Kintz *et al.* 1989]; [Rudolph *et al.* 1983]. See Serum [Long *et al.* 1982].
Disposition in the Body Readily absorbed after oral administration. Metabolised by *N*-demethylation to phenmetrazine, which is active, and by *N*-oxidation. About 5 to 30% of a dose is excreted in the urine unchanged in 24 h together with up to 30% as phenmetrazine and 20% as the *N*-oxide.
Therapeutic Concentration
Following a single oral dose of 35 mg to 20 subjects, a mean peak plasma concentration of about 0.07 mg/L was attained in about 1 h [Rudolph *et al.* 1983].
Toxicity
A blood concentration of 300 µg/L was reported in a fatality attributed to phendimetrazine [Hood *et al.* 1988].
Half-life Plasma half-life, about 2 to 3 h.
Dose Usually 70 to 105 mg of phendimetrazine tartrate daily; maximum of 210 mg daily.

Hood I *et al.* (1988). Fatality from illicit phendimetrazine use. *J Toxicol Clin Toxicol* 26: 249–255.
Kintz P *et al.* (1989). A simple gas chromatographic identification and determination of 11 CNS stimulants in biological samples. Application on a fatality involving phendimetrazine. *Forensic Sci Int* 40: 153–159.
Long G *et al.* (1982). *Drug Dev Ind Pharm* 8: 203–213.
Rudolph GR *et al.* (1983). GLC determination of phendimetrazine in human plasma, serum, or urine. *J Pharm Sci* 72: 519–521.

Phenelzine

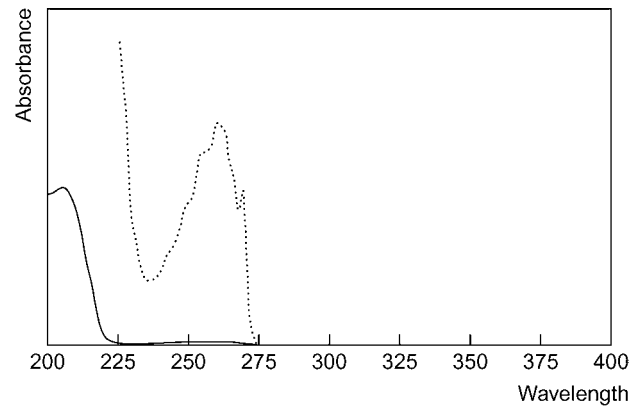
Antidepressant, Monoamine Oxidase Inhibitor
$C_8H_{12}N_2$ = 136.2
CAS—51-71-8
IUPAC Name Phenethylhydrazine
Synonym (2-Phenethyl)hydrazine



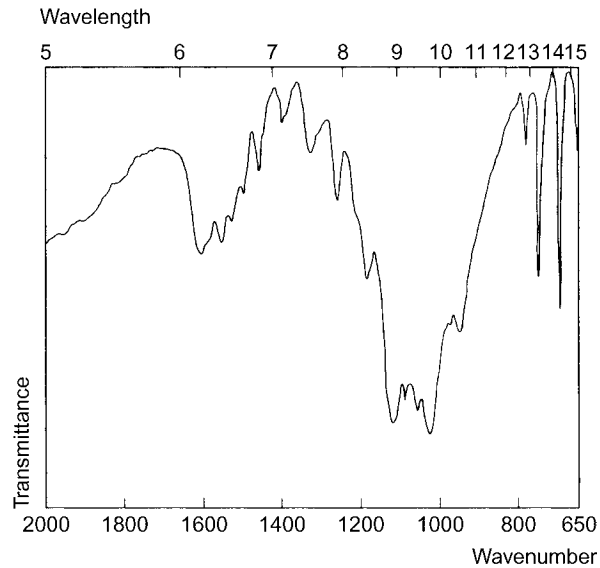
Chemical Properties Practically insoluble in water; soluble in chloroform and ether. Log *P* (octanol/water) 1.0.

Phenelzine Sulfate
$C_8H_{12}N_2,H_2SO_4$ = 234.3
CAS—156-51-4
Proprietary Names *Nardelzine*; *Nardil*.
Chemical Properties A white or yellowish-white powder or pearly platelets. Mp 164° to 168°. Soluble 1 in 7 of water; practically insoluble in ethanol, chloroform and ether.

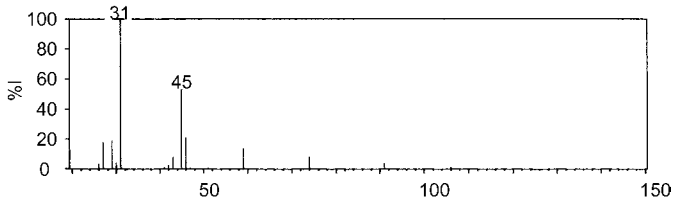
Colour Tests Benedict's reagent—orange; Liebermann's reagent—orange; Mandelin's test—brown; Nessler's reagent—black; palladium chloride—black.
Thin-layer Chromatography System TA—R_f 0.77; system TB—R_f 0.37; system TC—R_f 0.12; system TE—R_f 0.83; system TAE—R_f 0.29; system TAF—R_f 0.82; system TAG—R_f 0.63 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—brown; Ninhydrin (spray)—positive).
Gas Chromatography System GA—RI 1335; system GB—RI 1278; system GC—RI 1460.
High Performance Liquid Chromatography System HA—*k* 1.0; system HB—*k* 5.91; system HC—*k* 0.37; system HX—RI 184.
Ultraviolet Spectrum Aqueous acid—247, 252, 257 (A_1^1= 12.9a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1016, 1108, 1050, 1080, 940, 699 cm^{-1} (phenelzine sulfate, KBr disk).



Mass Spectrum Principal ions at *m/z* 31, 45, 46, 29, 27, 59, 74, 43.



Quantification

Plasma **GC** Column: OV-17 (25 m × 0.32 mm i.d., 0.15 µm). Carrier gas: He, 4.0 mL/min. Temperature programme: 170° to 300° at 32°/min. NSD. Limit of detection, <1 µg/L [Lichtenwalner *et al.* 1988]. Column: 10% OV-17 on gas chrom Q (2 m × 1.88 mm i.d.). Temperature: 240°. AFID. Limit of detection, 2 µg/L [McGilveray *et al.* 1982].

GC-MS Column: 1% OV-17 (1.8 m). Carrier gas: N_2, 30 mL/min. Temperature: 190°. Limit of quantification, 2 µg/L [Jindal *et al.* 1980].ECD [Cooper *et al.* 1978].

Urine **GC** Column: 10% m/m FFAP on Gas-Chrom Q 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 150°. FID. Limit of detection, 50 µg/L [Caddy, Stead 1977].

GC-MS Column: SE-54 fused silica capillary (15 m × 0.255 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 105° for 0.5 min to 300° at 25°/min for 10 min. ECD. Limit of detection, <10 pg [Rao *et al.* 1987].

Brain **GC** See Urine [Rao *et al.* 1987].

Disposition in the Body Phenelzine is readily absorbed after oral administration and widely distributed throughout the body. It is believed to be metabolised by acetylation, followed by glutamine conjugation, producing phenylacetylglutamine. Aromatic hydroxylation and conjugation with glucuronic acid may also occur. <5% of a dose is excreted in the urine unchanged.

Therapeutic Concentration

Following a single oral dose of 50 mg phenelzine solution in 1 subject, a peak plasma concentration of 0.05 mg/L was reported at 0.25 h [McGilveray *et al.* 1982].

Following a single oral dose of 30 mg in 1 subject, a peak plasma concentration of 0.002 mg/L was attained in 2 h. In a subject who had been receiving daily oral doses of 30 mg for 6 weeks, a trough steady-state plasma concentration of 0.0002 mg/L was reported [Cooper *et al.* 1978].

Toxicity

In a fatality attributed to phenelzine and alcohol, postmortem urine concentrations were phenelzine 58 mg/L, alcohol 1700 mg/L; in a second subject who took a non-fatal overdose, blood and plasma concentrations of 1.5 and 1.26 mg/L phenelzine, respectively, were reported 12 h after ingestion [Caddy, Stead 1978].

Phenelzine was held to be the cause of death in 2 subjects, even though other drugs were present in elevated concentrations. The postmortem blood concentration in the first was 3.1 mg/L and a total of 62 g was found in the stomach contents. Postmortem tissue concentrations in the second were blood 0.68 mg/L, urine 4.60 mg/L, and the antemortem serum concentration was 0.72 mg/L [Lichtenwalner *et al.* 1995].

Half-life Plasma half-life, ~7 h.

Dose The equivalent of 45 to 60 mg phenelzine daily.

Caddy B, Stead AH (1977). Indirect determination of phenelzine in urine. *Analyst* 102: 42–49.

Caddy B, Stead AH (1978). Three cases of poisoning involving the drug phenelzine. *J Forensic Sci Soc* 18: 207–208.

Cooper TB *et al.* (1978). Phenelzine measurement in human plasma: a sensitive GLC-ECD procedure. *Commun Psychopharmacol* 2: 505–512.

Jindal SP *et al.* (1980). Determination of phenelzine in human plasma with gas chromatography–mass spectrometry using an isotope labeled internal standard. *J Chromatogr* 221: 301–308.

Lichtenwalner M *et al.* (1988). Quantitative determination of phenelzine in human fluids by gas chromatography with nitrogen specific detection. *J Anal Toxicol* 12: 98–101.

Lichtenwalner MR *et al.* (1995). Two fatalities involving phenelzine. *J Anal Toxicol* 19: 265–266.

McGilveray IJ *et al.* (1982). Determination of phenelzine plasma concentrations in piglet and man. *J Pharm Pharmacol* 3498P.

Rao TS *et al.* (1987). Analysis of the antidepressant phenelzine in brain tissue and urine using electron-capture gas chromatography. *J Pharmacol Meth* 17: 297–304.

Phenethylamine

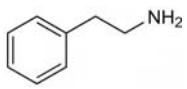
Putrefactive Base

$C_8H_{11}N = 121.2$

CAS—64-04-0

IUPAC Name 2-Phenylethanamine

Synonym β-Aminoethylbenzene; benzeneethanamine



Chemical Properties A strongly basic liquid which absorbs carbon dioxide from the air. Bp 194° to 195°. Soluble in water; freely soluble in ethanol and ether. pK_a 10.0 (19°). Log *P* (octanol/water), 1.4.

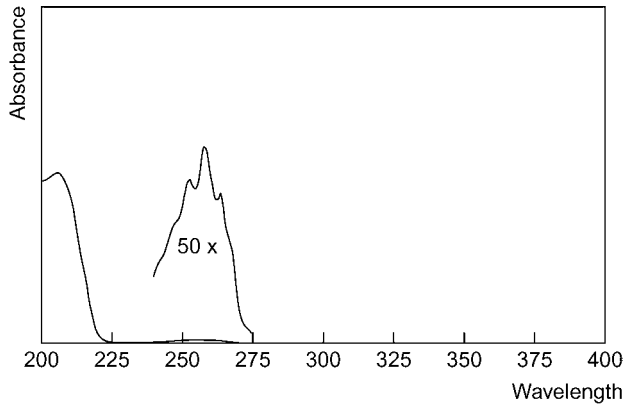
Colour Test Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.28; system TC—R_f 0.28; system TE—R_f 0.54; system TL—R_f 0.39; system TAE—R_f 0.44; system TAJ—R_f 0.03; system TAK—R_f 0.16; system TAL—R_f 0.50 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 1111.

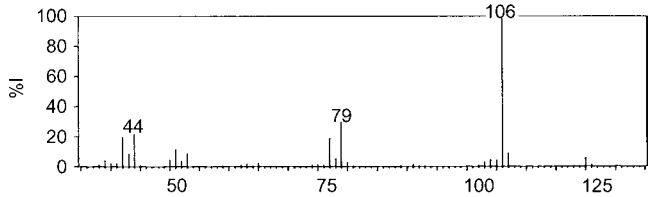
High Performance Liquid Chromatography System HA—*k* 1.2; system HB—*k* 3.60; system HC—*k* 1.3.

Ultraviolet Spectrum Aqueous acid—242 (A^1_1=15b), 247 (A^1_1=17.2b), 252 (A^1_1=19.8b), 257 (A^1_1=21.3b), 263 nm (A^1_1=16b).



Infrared Spectrum Principal peaks at wavenumbers 695, 745, 755, 942, 1146, 1608 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 30, 91, 65, 92, 51, 39, 121, 103.



Disposition in the Body Phenethylamine is the putrefactive base obtained from phenylalanine by decarboxylation.

P

Pheneticillin

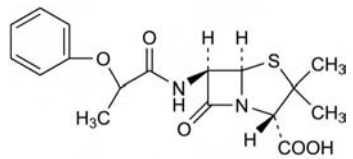
Antibiotic

$C_{17}H_{20}N_2O_5S = 364.4$

CAS—147-55-7

IUPAC Name (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenoxypropanoylamino)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Synonyms (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(1-oxo-2-phenoxypropyl) amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; penicillin B; phenethicillin; α-phenoxyethylpenicillin.



Chemical Properties pK_a 2.7 (25°). Log *P* (octanol/water), 2.2.

Pheneticillin Potassium

$C_{17}H_{19}KN_2O_5S = 402.5$

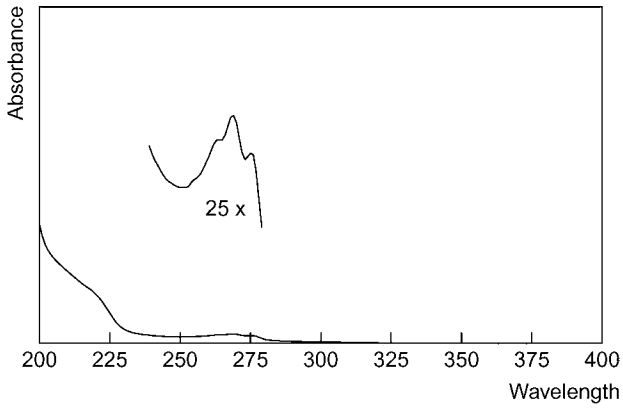
CAS—132-93-4

Synonym Phenethicillin potassium

Proprietary Names *Alfacillin*; *Brocosil*; *Broxil*; *Chemipen*; *Darcil*; *Dramcillin-S*; *Maxipen*; *Optipen*; *Peniplus*; *Penorale*; *Penova*; *Pensig*; *Syncillin*; *Synthecilline*; *Synthepen*.

Chemical Properties A white, fine, crystalline powder. Mp 230° to 232°, with decomposition. Soluble 1 in 1.5 of water, 1 in 85 of ethanol, and 1 in 800 of dehydrated alcohol; slightly soluble in chloroform; practically insoluble in ether.

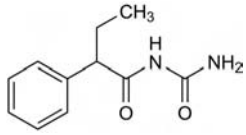
Ultraviolet Spectrum Pheneticillin potassium: aqueous acid—271 (A^1_1=44b), 275 nm. No alkaline shift.



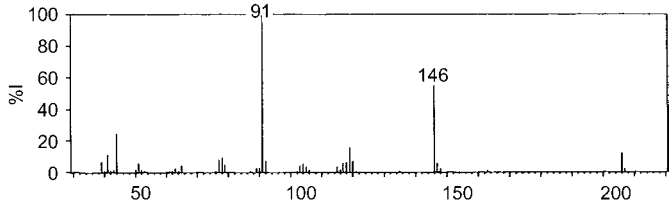
Infrared Spectrum Principal peaks at wavenumbers 1610, 1778, 1510, 1238, 1690, 752 cm^{-1} (pheneticillin potassium, KBr disk).
Dose The equivalent of 1 to 2 g of pheneticillin daily.

Pheneturide

Anticonvulsant
$C_{11}H_{14}N_2O_2 = 206.2$
CAS—90-49-3
IUPAC Name *N*-Carbamoyl-2-phenylbutanamide
Synonyms *N*-(Aminocarbonyl)-α-ethylbenzeneacetamide; EPA; ethylphenacemide; PBU.
Proprietary Names *Benuride*; *Laburide*.



Chemical Properties A white crystalline powder. Practically insoluble in water and ether; slightly soluble in ethanol; very soluble in glacial acetic acid. Log *P* (octanol/water), 1.6.
Colour Test Nessler's reagent—orange-brown (slow).
Thin-layer Chromatography System TA—R_f 0.76; system TD—R_f 0.38; system TE—R_f 0.71; system TF—R_f 0.53; system TAD—R_f 0.59.
Gas Chromatography System GA—RI 1465.
High Performance Liquid Chromatography System HE—*k* 6.8.
Ultraviolet Spectrum Chloroform—259 (A^{1}_{1} = 18b), 265 nm (A^{1}_{1s} = 11b).
Infrared Spectrum Principal peaks at wavenumbers 1672, 1700, 1093, 694, 1176, 1615 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 91, 146, 44, 119, 206, 41, 78, 77.



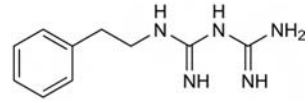
Quantification

Plasma **TLC-UV spectrophotometry** [Galeazzi *et al.* 1979].
HPLC UV detection. Pheneturide and other anticonvulsants. Limit of detection, 1.4 mg/L [Christofides, Fry 1980].
Serum **TLC-UV spectrophotometry** see Plasma. Limit of detection, 500 µg/L [Galeazzi *et al.* 1979].
HPLC See Plasma [Christofides, Fry 1980].
Urine **TLC-UV spectrophotometry** See Plasma [Galeazzi *et al.* 1979].
Disposition in the Body Slowly absorbed after oral administration. Pheneturide is thought to be completely metabolised as only traces of unchanged drug have been found in the urine. It may induce its own metabolism.
Toxicity Pheneturide has been reported to be less likely to cause toxic effects than phenacemide, to which it is chemically related.
Half-life Plasma half-life, 30–90 h (mean, 54) after single doses and about 40 h after chronic administration.
Volume of Distribution About 3 L/kg.
Clearance Plasma clearance, ≈0.7 mL/min/kg.
Dose Pheneturide has been given in doses of 0.6 to 1 g daily.

Christofides JA, Fry DE (1980). Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin Chem* 26: 499–501.
Galeazzi RL *et al.* (1979). Pharmacokinetics of phenylethylacetylurea (pheneturide), an old antiepileptic drug. *J Pharmacokinet Biopharm* 7: 453–462.

Phenformin

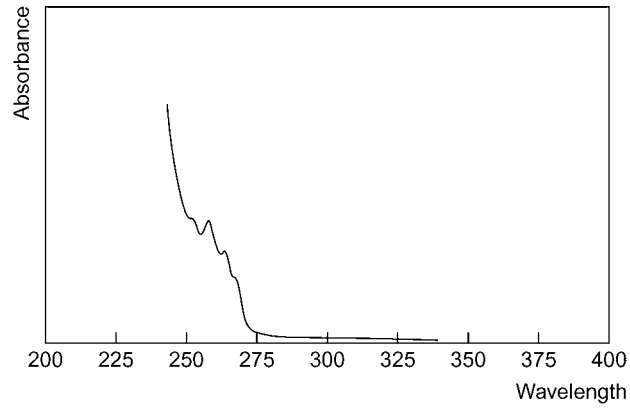
Antidiabetic
$C_{10}H_{15}N_5 = 205.3$
CAS—114-86-3
IUPAC Name 1-(Diaminomethylidene)-2-phenethylguanidine
Synonyms Fenformina; phenethyldiguanide β-PEBG; *N*-(2-phenylethyl)imidodicarbonimidic diamide; PEDG.



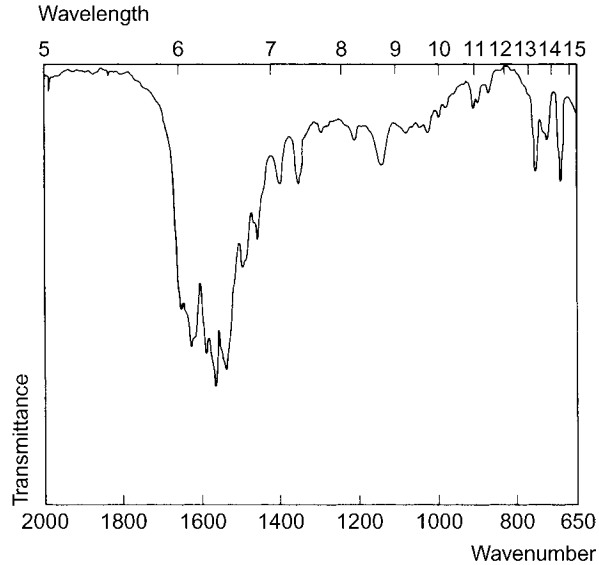
Chemical Properties pK_a 2.7, 11.8 (32°). Log *P* (octanol/water), −0.8.

Phenformin Hydrochloride

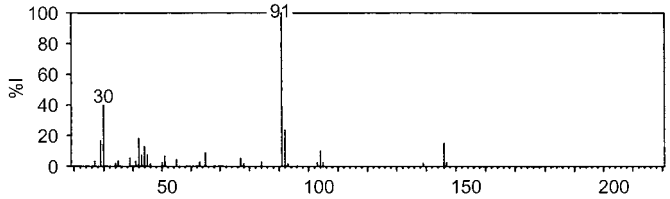
$C_{10}H_{15}N_5$,HCl = 241.7
CAS—834-28-6
Proprietary Names *Azucaps*; *Debei*; *Debein*; *Debeuna*; *Debeone*; *Debinyl*; *Dibotin*; *Dipar*; *Feguanide*; *Glucopostin*; *Insoral*; *Lentobetic*; *Meltrol*; *Normoglucina*.
Chemical Properties A white crystalline powder. Mp 175° to 178°. Soluble 1 in 8 of water and 1 in 15 of ethanol; practically insoluble in chloroform and ether.
Colour Tests Mandelin's test—green; Marquis test—orange→brown.
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00; system TAJ—R_f 0.00; system TAK—R_f 0.03; system TAL—R_f 0.29 (Marquis reagent, positive).
High Performance Liquid Chromatography System HX—RI 250.
Ultraviolet Spectrum Aqueous acid—251 (A^{1}_{1} = 11a), 258 (A^{1}_{1} = 11a), 264, 267 nm.



Infrared Spectrum Principal peaks at wavenumbers 1568, 1537, 1590, 1625, 1650, 1500 cm^{-1} (phenformin hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 30, 92, 42, 29, 146, 44, 104 (no peaks above 150).



Quantification

Plasma **GC** ECD. Phenformin and other biguanides. Limit of detection, <1 µg/L in plasma [Matin *et al.* 1975].

HPLC UV detection. Limit of detection, 10 µg/L in plasma [Hill, Chamberlain 1978].

Serum **HPLC** Fluorimetric detection. Phenformin and buformin [Kobayashi *et al.* 1988].

Urine **GC** See Plasma [Matin *et al.* 1975].

HPLC UV detection. Phenformin and 4-hydroxyphenformin. Limit of detection, 1 mg/L for phenformin and 500 µg/L for 4-hydroxyphenformin [Oates *et al.* 1980]. See Plasma [Hill, Chamberlain 1978].

Disposition in the Body Well absorbed after oral administration. The major metabolic reaction is aromatic hydroxylation to form 4-hydroxyphenformin which is then conjugated with glucuronic acid. Up to about 50% of a dose is excreted in the urine in 24 h, about two-thirds in the form of unchanged drug and one-third as the hydroxy metabolite.

Therapeutic Concentration

Following a single oral dose of 50 mg to 8 subjects, peak plasma concentrations of 0.08–0.18 mg/L (mean, 0.13) were attained in about 3 h; plasma concentrations were higher in 4 subjects who were poor metabolisers of debrisoquine in comparison with the four extensive metabolisers [Oates *et al.* 1983].

Following daily oral doses of 50 mg three times a day to 8 subjects, plasma concentrations of 0.10–0.24 mg/L (mean, 0.18) were reported 2 h after a dose [Karam *et al.* 1974].

Toxicity

A 44-year-old woman ingested 1.5 g in slow-release capsules and died 30 h later. A postmortem blood concentration of 3 mg/L and liver concentration of 60 µg/g were reported [Bingle *et al.* 1970].

Half-life Plasma half-life, 10–15 h.

Protein Binding About 12–20%.

Note For a review of phenformin-induced lactic acidosis, see Kwong and Brubacher [1998].

Dose 50 to 100 mg of phenformin hydrochloride daily.

Bingle JP *et al.* (1970). Fatal self-poisoning with phenformin. *Brit Med J* 3: 752.

Hill HM, Chamberlain J (1978). Determination of oral anti-diabetic agents in human body fluids using high-performance liquid chromatography. *J Chromatogr* 149: 349–358.

Karam J *et al.* (1974). *Diabetes* 23: 1375.

Kobayashi Y *et al.* (1988). Fluorometric determination of biguanides in serum by high-performance liquid chromatography with reagent-containing mobile phase. *J Chromatogr* 430: 65–71.

Kwong SC, Brubacher J (1998). Phenformin and lactic acidosis: a case report and review. *J Emerg Med* 16: 881–886.

Matin SB *et al.* (1975). Simple elcetron capture gas chromatographic method for the determination of oral hypoglycemic biguanidis in biological fluids. *Anal Chem* 47(3): 545–548.

Oates NS *et al.* (1980). On the urinary disposition of phenformin and 4-hydroxy-phenformin and their rapid simultaneous measurement. *J Pharm Pharmacol* 32: 731–732.

Oates NS *et al.* (1983). Influence of oxidation polymorphism on phenformin kinetics and dynamics. *Clin Pharmacol Ther* 34: 827–834.

Phenglutarimide

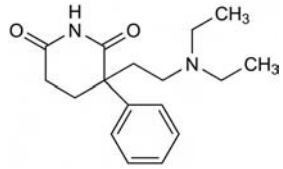
Anticholinergic, Antiparkinsonian

$C_{17}H_{24}N_2O_2$ = 288.4

CAS—1156-05-4

IUPAC Name 3-(2-Diethylaminoethyl)-3-phenylpiperidine-2,6-dione

Synonym 3-[2-(Diethylamino)ethyl]-3-phenyl-2,6-piperidinedione



Chemical Properties Crystals. Mp 125° to 127°. Log *P* (octanol/water), 1.9.

Phenglutarimide Hydrochloride

$C_{17}H_{24}N_2O_2$,HCl = 324.8

CAS—1674-96-0

Proprietary Names *Aturbal*; *Aturbane*.

Chemical Properties A white crystalline powder. Mp 168° to 172° and also 176° to 177°, with decomposition. Soluble 1 in 1 of water and 1 in 1.5 of ethanol; very slightly soluble in ether.

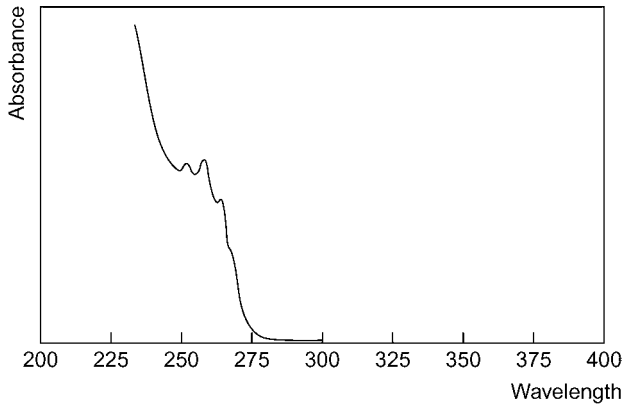
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.40; system TB—R_f 0.17; system TC—R_f 0.08; system TL—R_f 0.08 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—Phenglutarimide RI 2235, M (desethyl-) RI 2370.

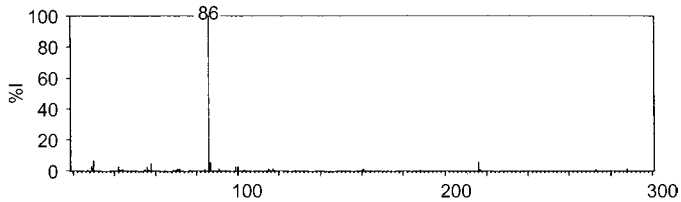
High Performance Liquid Chromatography System HA—*k* 2.9.

Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=13.3a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1698, 1194, 1181, 1250, 700, 1150 cm^{-1} (phenglutarimide hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 86, 30, 216, 87, 58, 100, 99, 56.



Dose Phenglutarimide hydrochloride has been given in doses of 10 to 50 mg daily.

Phenindamine

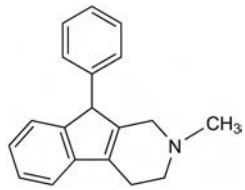
Antihistamine

$C_{19}H_{19}N$ = 261.4

CAS—82-88-2

IUPAC Name 2-Methyl-9-phenyl-1,3,4,9-tetrahydroindeno[2,1-*c*]pyridine

Synonyms Nu-1504; 2,3,4,9-tetrahydro-2-methyl-9-phenyl-1*H*-indeno[2,1-*c*]pyridine.



Chemical Properties Crystals. Mp 90° to 91°. pK_a 8.3 (25°). Log *P* (octanol/water), 4.2.

Phenindamine Tartrate

$C_{19}H_{19}N,C_4H_6O_6$ = 411.5

CAS—569-59-5

Proprietary Names *Nolahist*; *Thephorin*.

Chemical Properties A white voluminous powder. Mp 160°, with decomposition. Soluble 1 in 70 of water and 1 in 300 of ethanol; practically insoluble in chloroform and ether.

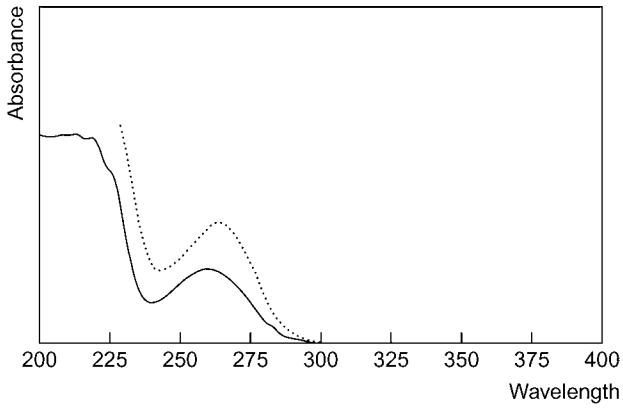
Colour Tests Mandelin's test—green; Marquis test—grey-green.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.45; system TC—R_f 0.57; system TE—R_f 0.68; system TL—R_f 0.21; system TAD—R_f 0.41; system TAF—R_f 0.49; system TAJ—R_f 0.37; system TAK—R_f 0.02; system TAL—R_f 0.82 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, grey-brown).

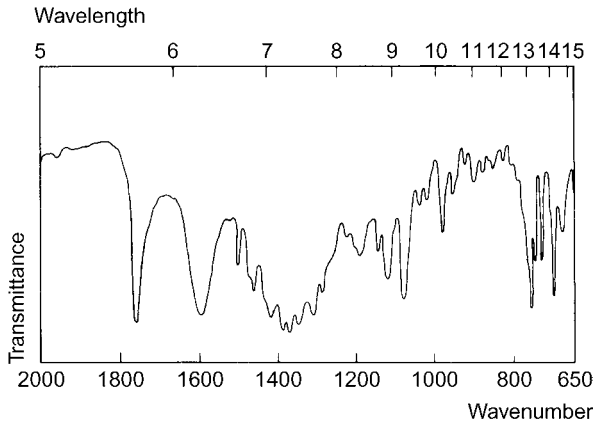
Gas Chromatography System GA—Phenindamine RI 2165, M (OH-) RI 2300, M (*N*-oxide) RI 2230, M (nor-) RI 2210, M (nor-OH-) RI 2590; system GB—RI 2245; system GC—RI 2926; system GF—RI 2515.

High Performance Liquid Chromatography System HA—*k* 2.5; system HX—RI 397.

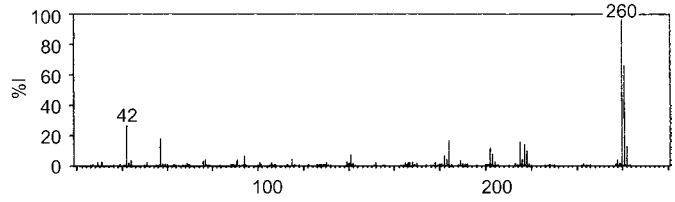
Ultraviolet Spectrum Aqueous acid—259 nm (A_1^1=347a); aqueous alkali—263 nm (A_1^1=385a).



Infrared Spectrum Principal peaks at wavenumbers 1751, 1302, 1585, 752, 1072, 693 cm^{-1} (phenindamine tartrate, KBr disk).



Mass Spectrum Principal peaks at *m/z* 260, 261, 42, 57, 184, 215, 217, 262.

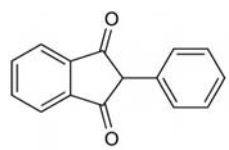


Dose Up to 150 mg of phenindamine tartrate daily.

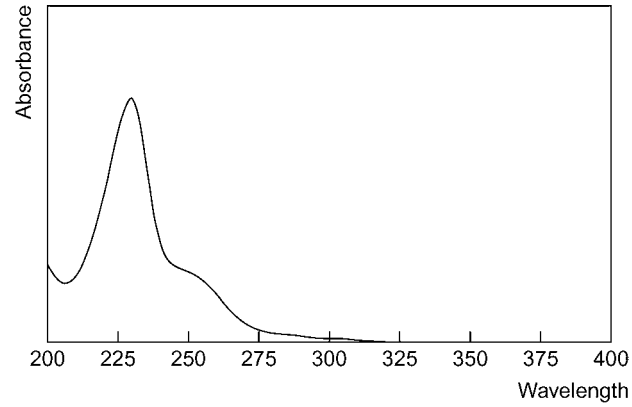
Phenindione

Anticoagulant
$C_{15}H_{10}O_2$ = 222.2
CAS—83-12-5

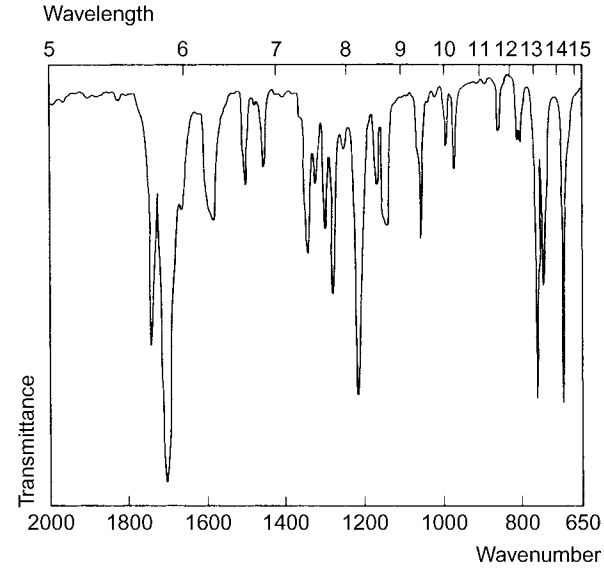
IUPAC Name 2-Phenylindene-1,3-dione
Synonyms Phenylindanedione; 2-phenyl-1*H*-indene-1,3(2*H*)-dione; phenylinium PID.
Proprietary Names *Danilone*; *Dindevan*; *Fenilin*; *Hedulin*; *Pindione*; *Rectadione*.



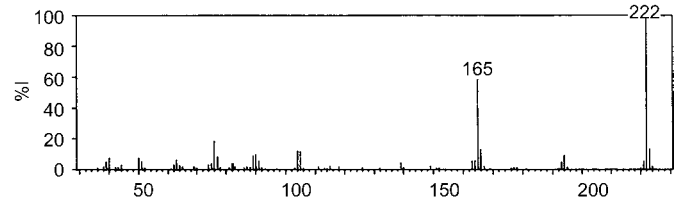
Chemical Properties Soft, white or creamy-white or pale yellow crystals or crystalline powder. Mp 149° to 151°. Very slightly soluble in water; soluble 1 in about 125 of ethanol, 1 in 6.5 of chloroform, and 1 in 110 of ether; soluble in methanol, benzene, and acetone; forms yellow to red solutions. pK_a 4.1. Log *P* (octanol/water), 2.9.
Colour Tests Methanolic potassium hydroxide—red; sulfuric acid—violet.
Thin-layer Chromatography System TD—R_f 0.65; system TE—R_f 0.21; system TF—R_f 0.56; system TAD—R_f 0.70.
Gas Chromatography System GA—RI 2055.
High Performance Liquid Chromatography System HAA—retention time 18.1 min.
Ultraviolet Spectrum Aqueous alkali—278 (A^1_1=1310a), 330 nm (A^1_1=400b).



Infrared Spectrum Principal peaks at wavenumbers 1700, 700, 766, 1219, 1740, 1280 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 222, 165, 76, 223, 166, 105, 104, 90.



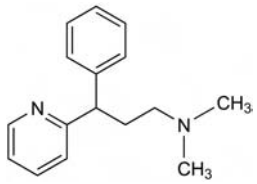
Quantification
Serum **Polarography** Limit of detection, 4 mg/L [Jacobsen, Klevan 1972].
Disposition in the Body Well absorbed after oral administration. The urine may be coloured red-orange due to the excretion of a metabolite.
Toxicity Fatalities have occurred after therapeutic doses.
Half-life Plasma half-life, about 6 h.
Dose Maintenance, 25 to 150 mg daily.

Jacobsen E, Klevan KH (1972). Electroreduction and polarographic determination of 2-phenylindan-1,3-dione in serum. *Anal Chim Acta* 62: 405–413.

Pheniramine

Antihistamine
$C_{16}H_{20}N_2$ = 240.3
CAS—86-21-5

IUPAC Name *N*,*N*-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine
Synonyms *N*,*N*-Dimethyl-γ-phenyl-2-pyridinepropanamine; prophenpyridamine



Chemical Properties A slightly yellow oily liquid. Practically insoluble in water; soluble in ethanol, benzene, chloroform and ether. pK_a 4.2, 9.3 (25°). Log *P* (octanol/water), 3.2.

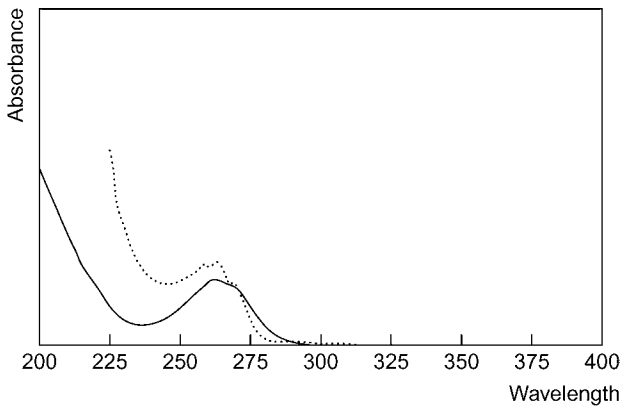
Pheniramine Aminosalicylate

$C_{16}H_{20}N_2,C_7H_7NO_3 = 393.5$
CAS—3269-83-8
Chemical Properties Crystals. Mp 142°, with decomposition. Soluble 1 in 10 of water; freely soluble in ethanol; sparingly soluble in ethyl acetate, ether and acetone.

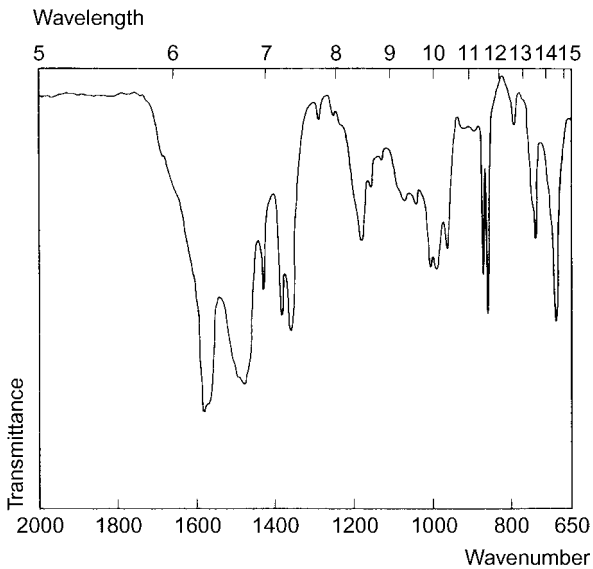
Pheniramine Maleate

$C_{16}H_{20}N_2,C_4H_4O_4 = 356.4$
CAS—132-20-7
Proprietary Names *Avil*; *Daneral*; *Fenamine*; *Inhiston*.
Chemical Properties A white crystalline powder. Mp 107°. Soluble 1 in 0.3 of water, 1 in 2.5 of ethanol and 1 in 1.5 of chloroform; slightly soluble in ether and benzene.

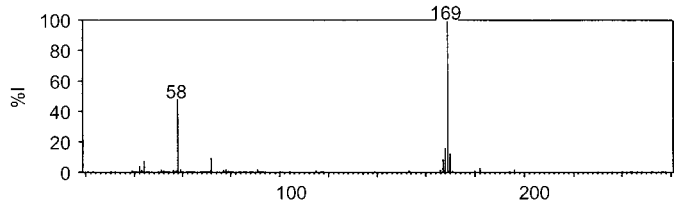
Colour Tests Cyanogen bromide—orange; Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.35; system TC—R_f 0.13; system TE—R_f 0.46; system TL—R_f 0.03; system TAE—R_f 0.14; system TAF—R_f 0.26 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—pheniramine RI 1805, M (nor-) RI 2080; system GB—pheniramine RI 1874, M (nor-) RI 1890; system GF—RI 2100.
High Performance Liquid Chromatography System HA—*k* 4.1; system HX—RI 283; system HY—RI 206; system HAX—retention time 9.5 min; system HAY—retention time 4.5 min.
Ultraviolet Spectrum Aqueous acid—261 (A^1_1=305a), 266 nm (A^1_1=310a); aqueous alkali—258, 263 (A^1_1=206a), 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 1584, 690, 860, 870, 990, 1010 cm^{-1} (pheniramine maleate, KBr disk).



Mass Spectrum Principal ions at *m/z* 169, 58, 168, 170, 72, 167, 44, 42 (no peaks above 200).

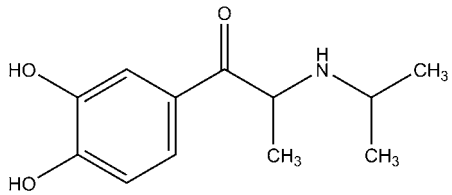


Quantification
Blood **GC** AFID. Limit of detection, 50 µg/L [Querée *et al.* 1979].
Serum **GC-MS** Pheniramine and loratadine. Limit of detection for pheniramine, 0.5 µg/L [Martens 1995].
Urine **GC** See Blood [Querée *et al.* 1979].
Tissues **GC** See Blood [Querée *et al.* 1979].
Disposition in the Body Absorbed after oral administration. About 17% of a dose is excreted in the urine as unchanged drug in 24 h, increasing to 23% over a period of 6 days; after chronic oral administration about 24% is excreted daily as monodesmethylpheniramine, and 0.5% as didesmethylpheniramine.
Therapeutic Concentration
After a single oral dose of 75 mg to 6 subjects, blood concentrations of 0.01 to 0.19 mg/L (mean, 0.11) were reported at 2 h [Querée *et al.* 1979].
Toxicity The estimated minimum lethal dose is 25 mg/kg.
In a fatality due to pheniramine overdose, the following postmortem tissue concentrations were reported: blood 1.9 mg/L, brain 5.3 µg/g, kidney 4.0 µg/g, liver 6.6 µg/g, urine 149 mg/L; in a second fatality in which methadone, pentobarbital, phenobarbital and alcohol had also been ingested, postmortem blood and liver concentrations of 30 mg/L and 115 µg/g, respectively, were reported [Querée *et al.* 1979]
In a fatality due to the ingestion of 3.75 g of pheniramine, the following postmortem tissue concentrations were reported: blood 10.7 mg/L, bile 109 mg/L, liver 33 µg/g, urine 362 mg/L [Chan, Allender 1983].
Note For a review of the clinical pharmacokinetics of antihistamines, see Paton and Webster [1985].
Dose Up to 90 mg of pheniramine maleate daily.

Chan LFT, Allender WJ (1983). *Bull Int Assoc Forensic Toxicol* 17(2): 25–26.
Martens J (1995). Determination of loratadine and pheniramine from human serum by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Appl* 673: 183–188.
Paton DM, Webster DR (1985). Clinical pharmacokinetics of H1-receptor antagonists (the antihistamines). *Clin Pharmacokinet* 10: 477–497.
Querée EA *et al.* (1979). *J Anal Toxicol* 3: 253–255.

Phenisonone

Bronchodilator
$C_{12}H_{17}NO_3 = 223.3$
IUPAC Name 1-(3,4-Dihydroxyphenyl)-2-(propan-2-ylamino)propan-1-one
Synonym 3,4-Dihydroxy-α-(isopropylamino)propiophenone



Chemical Properties Insoluble in water and organic solvents. Phenisonone is extracted by organic solvents from aqueous alkaline solutions.

Phenisonone Hydrobromide

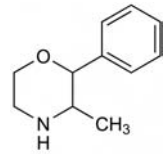
$C_{12}H_{17}NO_3,HBr = 304.2$
Proprietary Name *Dapanone*
Chemical Properties Crystals. Mp 141° to 143°. Soluble 1 in 3.3 of water and 1 in 4 of ethanol; insoluble in ether and chloroform. Aqueous solutions are unstable.

Colour Tests Ammonium molybdate test—(yellow) blue-green (limit of detection, 0.5 µg); ammonium vanadate test—(green) yellow (limit of detection, 1.0 µg); sulfuric acid-formaldehyde test—yellow (limit of detection, 0.5 µg); Vitali's test—yellow/brown/brown (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.30, streaking (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum 0.1 N sulfuric acid—233 (E1%, 1 cm 473), 284 (E1%, 1 cm 409) and 313 nm (E1%, 1 cm 350); water—233 (E1%, 1 cm 38.2), 284 nm (E1%, 1 cm 32.5) (hydrobromide).

Phenmetrazine

Anorectic
$C_{11}H_{15}NO = 177.2$
CAS—134-49-6

IUPAC Name 3-Methyl-2-phenylmorpholine



Chemical Properties A liquid. pK_a 8.4 (25°). Log *P* (octanol/water), 1.5.

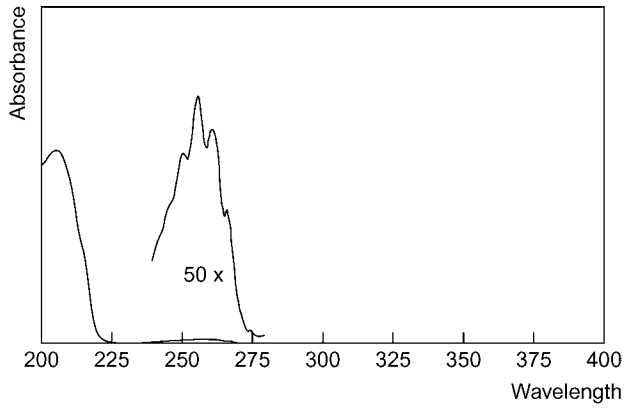
Phenmetrazine Hydrochloride

$C_{11}H_{15}NO,HCl = 213.7$
CAS—1707-14-8
Synonym Oxazimédrine
Proprietary Name *Preludin*
Chemical Properties A white crystalline powder. Mp 182°. Soluble 1 in 0.4 of water, 1 in 2 of ethanol and 1 in 2 of chloroform; sparingly soluble in ether.

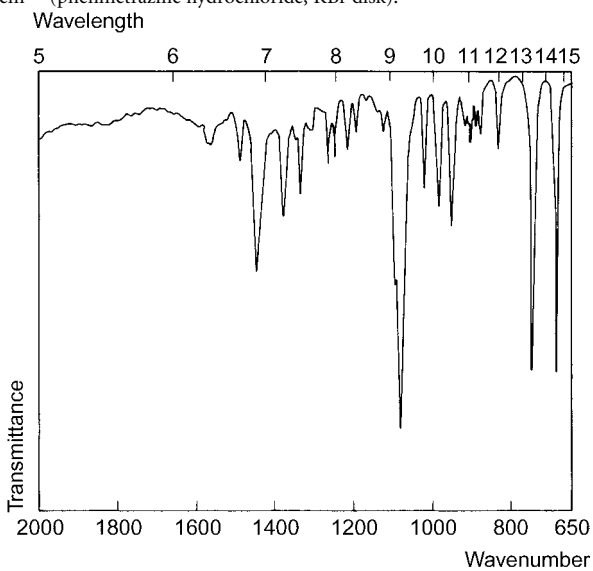
Phenmetrazine Teoclate

$C_{11}H_{15}NO,C_7H_7ClN_4O_2 = 391.9$
CAS—13931-75-4
Synonyms Phenmetrazine 8-chlorotheophyllinate; phenmetrazine theoclate.
Chemical Properties A white powder. Soluble in water and ethanol; slightly soluble in acetone and ether.

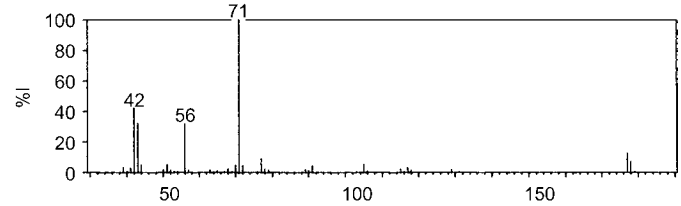
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.14; system TC—R_f 0.27; system TE—R_f 0.46; system TL—R_f 0.14; system TAE—R_f 0.34; system TAF—R_f 0.45; system TAJ—R_f 0.20; system TAK—R_f 0.08; system TAL—R_f 0.60 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—phenmetrazine RI 1432, phenmetrazine-TFA RI 1530, phenmetrazine-TMS RI 1620, phenmetrazine-AC RI 1810, M (OH-) isomer 1 RI 1830, M (OH-) isomer 2 RI 1865, M (OH-methoxy-) RI 1900, M (OH-) isomer 1-AC_2 RI 2150, M (OH-) isomer 2-AC_2 RI 2200, M (OH-methoxy-)-AC_2RI 2320; system GB—phenmetrazine RI 1483; system GC—RI 1873.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 258; system HY—RI 241.
Ultraviolet Spectrum Aqueous acid—250, 256 (A_1^1= 13.3a), 261, 263, 267 nm.



Infrared Spectrum Principal peaks at wavenumbers 1083, 757, 695, 965, 990, 1030 cm^{-1} (phenmetrazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 71, 42, 56, 43, 177, 77, 178, 105.



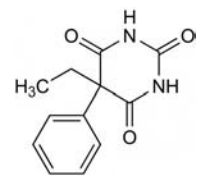
Quantification
Serum **GC** AFID. Phendimetrazine and phenmetrazine. Limit of detection, 10 µg/L [Long *et al.* 1982].
Urine **GC** NPD. Phenmetrazine, phentermine and amfetamines [Jonsson *et al.* 1996]. See Serum [Long *et al.* 1982].
GC-MS Limit of detection, 0.2–0.5 mg/L [Dasgupta, Mahle 1997; Dasgupta *et al.* 1998]. Limit of quantification, 50 µg/L [Kronstrand *et al.* 1996].
Disposition in the Body Readily absorbed after oral administration. About 70% of a dose is excreted in the urine in 24 h with about 19% as unchanged drug, 19% as the lactam (5-methyl-3-oxo-6-phenylmorpholine), 12% as free 4-hydroxyphenmetrazine, 10% as conjugated 4-hydroxyphenmetrazine and about 5% as the *N*-oxide.
Phenmetrazine is a metabolite of phendimetrazine.
Therapeutic Concentration
After a single oral dose of 75 mg given to 5 subjects, peak plasma concentrations of 0.10–0.24 mg/L (mean 0.16) were attained in about 2 h. Following a single oral dose of 75 mg of a slow-release preparation, average peak plasma concentrations of about 0.07 mg/L were reported at 5 h; concentrations remained almost constant over the period 5–24 h [Quinn *et al.* 1967].
Toxicity Estimated minimum lethal dose for children up to 2 years of age, 200 mg. Large doses and prolonged treatment may lead to severe mental depression and dependence of the amfetamine type.
In a fatality following IV overdosage, the following postmortem distribution was reported: blood 4 mg/L, bile 7 mg/L, liver 5 µg/g, urine 24 mg/L [Norheim 1973].
The following postmortem tissue concentrations were reported in 12 fatalities: blood 0.1–4.9 mg/L (mean, 1.1), bile 0.2–23 mg/L (mean, 5.5), brain 0.1–15 µg/g (mean, 2.9), kidney 0.1–8 µg/g (mean, 1.5), liver 0.1–20 µg/g (mean, 3.1), urine 0.1–90 mg/L (mean, 21) [Gottschalk, Baselt 2000].
Half-life Plasma half-life, ≈8 h.
Dose 25 to 75 mg of phenmetrazine hydrochloride daily.

Dasgupta A, Mahle CE (1997). Determination of phenmetrazine in urine by gas chromatography-mass spectrometry after liquid-liquid extraction and derivatization with perfluorooctanoyl chloride. *J Forensic Sci* 42: 937–941.
Dasgupta A *et al.* (1998). A convenient derivatization method for gas chromatography/mass spectrometric determination of phenmetrazine in urine using 2,2,2-trichloroethyl chloroformate. *J Forensic Sci* 43: 630–635.
Dasgupta A *et al.* (1998). Gas chromatography-electron ionization and chemical ionization mass spectrometric analysis of urinary phenmetrazine after derivatization with 4-carbethoxyhexafluorobutyryl chloride–a new derivative. *J Forensic Sci* 43: 636–640.
Gottschalk L, per Baselt RC (2000). *Disposition of toxic chemicals and drugs in man*, 5th edn. Davis, California: Biomedical Publications, 687–688.
Jonsson J *et al.* (1996). A convenient derivatization method for the determination of amphetamine and related drugs in urine. *J Forensic Sci* 41: 148–151.
Kronstrand R *et al.* (1996). Determination of Phenmetrazine in urine by gas chromatography-mass spectrometry. *J Anal Toxicol* 20: 277–280.
Long G *et al.* (1982). *Drug Dev Ind Pharm* 8: 203–213.
Norheim G (1973). A fatal case of phenmetrazine poisoning. *J Forensic Sci Soc* 13: 287–289.
Quinn GP *et al.* (1967). The effect of formulation on phenmetrazine plasma levels in man studied by a sensitive analytic method. *Clin Pharmacol Ther* 8: 369–373.

Phenobarbital

Hypnotic, Sedative, Barbiturate
$C_{12}H_{12}N_2O_3 = 232.2$
CAS—50-06-6

IUPAC Name 5-Ethyl-5-phenyl-1,3-diazinane-2,4,6-trione
Synonyms 5-Ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; fenobarbital; phenemalum; phenobarbitalum; phenobarbitone; phenylethylbarbituric acid; phenylethylmalonylurea.
Proprietary Names *Aparoxal*; *Comizial*; *Edhanol*; *Fenemal*; *Fenocriz*; *Gardenal (e)*; *Kaneuron*; *Luminal(e)*; *Luminaletas*; *Luminalette*; *Luminaletten*; *Neurobiol*; *Phenaemal*; *Phenaemaletten*; *Solfoton*. It is an ingredient of many proprietary preparations [Sweetman 2002].



Chemical Properties Colourless crystals or a white crystalline powder which may exhibit polymorphism. Mp 174° to 178°. Soluble 1 in 1000 of water, 1 in 8 of ethanol, 1 in 40 of chloroform, 1 in 13 of ether and 1 in about 700 of benzene. pK_a 7.4 (25°). Log *P* (octanol/water), 1.5.

Phenobarbital Sodium

$C_{12}H_{11}N_2NaO_3$ = 254.2
CAS—57-30-7

Synonyms Phenemalnatrium; phenobarbitone sodium; sodium phenylethylbarbiturate; soluble phenobarbitone.

Proprietary Names *Garnenal(e)*; *Luminal(e)*. It is an ingredient of *Garoin*.

Chemical Properties A white hygroscopic powder, granules, or flakes. Mp about 175°. Soluble 1 in about 1 of water and 1 in about 10 of ethanol; practically insoluble in chloroform and ether.

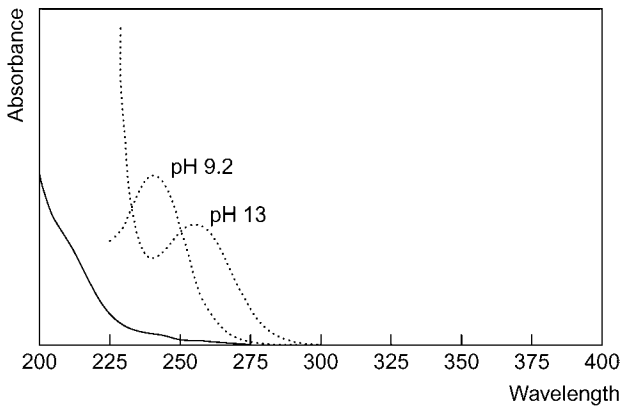
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.47; system TE—R_f 0.28; system TF—R_f 0.65; system TH—R_f 0.38; system TAD—R_f 0.53; system TAE—R_f 0.85 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; Zwikker's reagent, pink).

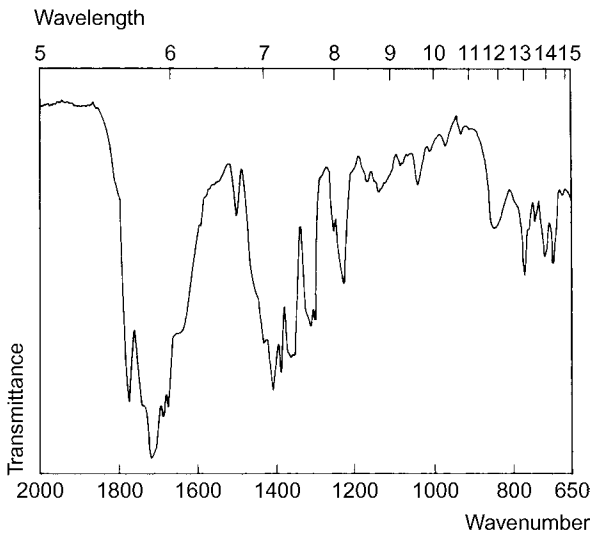
Gas Chromatography System GA—phenobarbital RI 1953, M (4-OH-) RI 2295, phenobarbital-Me_2 RI 1855, M (4-OH-)-Me_3 RI 2200; system GB—phenobarbital RI 2031, M (4-OH-) RI 2378; system GE—RRT 0.74 (relative to phenytoin); system GF—RI 2960; system GAJ—RRT 1.150 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HE—*k* 2.76; system HG—*k* 3.09; system HH—*k* 1.23; system HX—RI 379; system HY—RI 335; system HZ—retention time 3.0 min; system HAA—retention time 14.0 min; system HAK—not detected; system HAM—retention time 2.8 min; system HAX—retention time 5.6 min; system HAY—retention time 4.7 min; system HAZ—phenobarbital *k* 0.39, M (4-OH-) *k* 0.14.

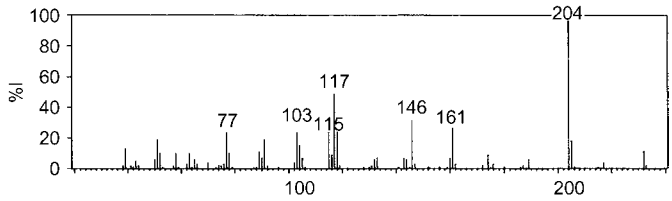
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A_1^1=452a); 1 mol/L sodium hydroxide (pH 13)—254 nm (A_1^1=342b).



Infrared Spectrum Principal peaks at wavenumbers 1712, 1684, 1670, 1770, 1310, 1300 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 204, 117, 146, 161, 77, 103, 115, 118; 4-hydroxyphenobarbital 219, 248, 148, 220, 120, 218, 133, 64.



Quantification See also under Amobarbital.

Blood **GC-MS** Phenobarbital and other anticonvulsants [Speed *et al.* 2000].

Plasma **GC-MS** Phenobarbital and 4-hydroxyphenobarbital. Limit of detection, 100 µg/L [Patel *et al.* 1980].

HPLC Phenobarbital and other anticonvulsants [Matar *et al.* 1999]. UV detection. Phenobarbital and other anticonvulsants [Johansen *et al.* 1995]. UV detection. Phenobarbital and other anticonvulsants [Romanyshyn *et al.* 1994]. UV detection. Phenobarbital and other anticonvulsants. Limit of detection, about 600 µg/L for phenobarbital [Christofides, Fry 1980].

HPLC-MS Phenobarbital and other sedatives [Kanazawa *et al.* 1998].

Polarisation Fluoroimmunoassay Limit of detection, 800 µg/L [Sidki *et al.* 1982].

Serum **GC** Thermionic specific detection. Phenobarbital and other anticonvulsants, Limits of quantification, 50 to 200 µg/L [Queiroz *et al.* 2002]. FID. Limit of detection, 500 µg/L [Di Corcia *et al.* 1982].

HPLC UV detection. Phenobarbital and other anticonvulsants. Limit of quantification for phenobarbital, about 1 µg/L [Levert *et al.* 2002].UV detection. Phenobarbital and other anticonvulsants [Kouno *et al.* 1997]. UV detection. Phenobarbital and other anticonvulsants [Kouno *et al.* 1993]. Photodiode-array detection. Phenobarbital, other anticonvulsants, and their metabolites [Liu *et al.* 1993]. Phenobarbital, phenytoin, and theophylline, comparison with immunoassay [Hannak *et al.* 1992]. See Plasma [Christofides, Fry 1980].

Polarisation Fluoroimmunoassay Phenobarbital and other anticonvulsants [Steijns *et al.* 2002]. See Plasma [Sidki *et al.* 1982].

Urine **GC-MS** See Plasma [Patel *et al.* 1980].

HPLC UV detection. Phenobarbital and its metabolites [Paibir, Soine 1997]. See Serum [Liu *et al.* 1993].

Saliva **HPLC** See Serum [Liu *et al.* 1993].

Hair **GC-MS** Phenobarbital, phenytoin and their metabolites [Saisho *et al.* 2001; Goulle *et al.* 1995].

Liver **GC-MS** See Blood [Speed *et al.* 2000].

Biological Samples **HPLC** Phenobarbital and other barbiturates [Tanaka *et al.* 1997].

Disposition in the Body Readily absorbed after oral administration. The major metabolites are *N*-glucopyranosylphenobarbital and 4-hydroxyphenobarbital, together with its glucuronide conjugate. Other metabolites include two dihydrodiol compounds and hydroxymethylphenylbarbituric acid. During chronic dosing, up to about 25% of a dose is excreted in the urine in 24 h as unchanged drug and up to about 17% as total 4-hydroxyphenobarbital, about half of which is the glucuronide conjugate. Urinary excretion of unchanged drug is increased when the urine is alkaline or when the urinary volume is increased; the excretion of conjugated 4-hydroxyphenobarbital is reduced in patients with liver disease. After a single dose, about 80 to 90% is excreted in the urine in 16 days; the *N*-glucoside accounts for about 30% of a single dose. Phenobarbital crosses the placenta and is found in breast milk.

Phenobarbital is a metabolite of methylphenobarbital and primidone.

Therapeutic Concentration In plasma, usually in the range 2 to 30 mg/L. However, there is considerable intersubject variation and there does not appear to be any correlation between plasma concentration and clinical effect. Concentrations in CSF reach about 50% of those in plasma.

Following a single oral dose of 100 mg to 6 subjects, peak serum concentrations of 2.1 to 3.8 mg/L (mean, 2.9) were attained in 0.5 to 4 h (mean, 1.5) [Wilensky *et al.* 1982].

Following daily oral doses of 100 mg to 10 subjects for 15 days, serum concentrations of 10.6 to 22.1 mg/L (mean, 17) were reported 12 h after a dose [Luoma *et al.* 1982].

Toxicity The estimated minimum lethal dose is 1.5 g although recovery has occurred after ingestion of as much as 16 g. Toxic effects have been associated with blood concentrations of 4 to 17 to 90 mg/L and fatalities with concentrations of 4 to 45 to 120 mg/L. However, a degree of tolerance may develop in chronic dosing.

Blood concentrations ranged from 10 to 300 mg/L (mean 86) in 113 fatalities attributed to phenobarbital overdose [Parker *et al.* 1970].

In 20 fatalities caused by phenobarbital overdose, the following postmortem concentrations were reported: blood 15 to 540 mg/L (mean, 113), brain 0 to 833 µg/g (mean, 135), kidney 17 to 867 µg/g (mean, 152), liver 24 to 1450 µg/g (mean, 236), spleen 0 to 850 µg/g (mean, 137) [Sunshine, Hackett 1957].

Bioavailability Almost 100%.

Half-life Plasma half-life, about 90 to 100 h in adults, prolonged in neonates and reduced to about 65 to 70 h in children; increased in subjects with liver disease.

Volume of Distribution About 0.5 L/kg.

Clearance Plasma clearance, about 0.06 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.93.

P

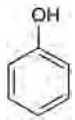
Saliva Plasma : saliva ratio, about 3.
Protein Binding About 50%.
Dose 60 to 180 mg daily.

Christofides JA, Fry DE (1980). Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin Chem* 26: 499–501.
Di Corcia A *et al.* (1982). Chromatographic micro-procedure for trace determination of phenobarbital in blood serum. *J Chromatogr* 229: 365–372.
Goulle JP *et al.* (1995). Phenobarbital in hair and drug monitoring. *Forensic Sci Int* 70: 191–202.
Hannak D *et al.* (1992). *Wien Klin Wochenschr Suppl* 191: 27–31.
Johansen K *et al.* (1995). Automated analysis of free and total concentrations of three antiepileptic drugs in plasma with on-line dialysis and high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 669: 281–288.
Kanazawa H *et al.* (1998). Determination of sedatives and anesthetics in plasma by liquid chromatography-mass spectrometry with a desalting system. *J Chromatogr A* 797: 227–236.
Kouno Y *et al.* (1993). Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring. *J Chromatogr* 622: 47–52.
Kouno Y *et al.* (1997). Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera. *J Chromatogr B Biomed Sci Appll* 695: 349–353.
Levert H *et al.* (2002). Simultaneous determination of four antiepileptic drugs in serum by high-performance liquid chromatography. *Biomed Chromatogr* 16: 19–24.
Liu H *et al.* (1993). Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. *J Chromatogr* 616: 105–115.
Luoma PV *et al.* (1982). Phenobarbital pharmacokinetics and salivary and serum concentrations in pregnancy. *Ther Drug Monit* 4: 65–68.
Matar KM *et al.* (1999). Liquid chromatographic determination of six antiepileptic drugs and two metabolites in microsamples of human plasma. *Ther Drug Monit* 21: 559–566.
Paibir SG, Soine WH (1997). High-performance liquid chromatographic analysis of phenobarbital and phenobarbital metabolites in human urine. *J Chromatogr B Biomed Sci Appl* 691: 111–117.
Parker KD *et al.* (1970). Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide, or diphenylhydantoin. *Clin Toxicol* 3: 131–145.
Patel IH *et al.* (1980). Simultaneous analysis of phenobarbital and p-hydroxyphenobarbital in biological fluids by GLC-chemical-ionization mass spectrometry. *J Pharm Sci* 69: 1218–1219.
Queiroz ME *et al.* (2002). Determination of lamotrigine simultaneously with carbamazepine, carbamazepine epoxide, phenytoin, phenobarbital, and primidone in human plasma by SPME-GC-TSD. *J Chromatogr Sci* 40: 219–223.
Romanyshyn LA *et al.* (1994). Simultaneous determination of felbamate, primidone, phenobarbital, carbamazepine, two carbamazepine metabolites, phenytoin, and one phenytoin metabolite in human plasma by high-performance liquid chromatography. *Ther Drug Monit* 16: 90–99.
Saisho K *et al.* (2001). Hair analysis for pharmaceutical drugs. I. Effective extraction and determination of phenobarbital, phenytoin and their major metabolites in rat and human hair. *Biol Pharm Bull* 24: 59–64.
Sidki AM *et al.* (1982). Direct determination of phenobarbital in serum or plasma by polarization fluoroimmunoassay. *Ther Drug Monit* 4: 397–403.
Speed DJ *et al.* (2000). Analysis of six anticonvulsant drugs using solid-phase extraction, deuterated internal standards, and gas chromatography-mass spectrometry. *J Anal Toxicol* 24: 685–690.
Steijns LS *et al.* (2002). Evaluation of fluorescence polarization assays for measuring valproic acid, phenytoin, carbamazepine and phenobarbital in serum. *Ther Drug Monit* 24: 432–435.
Sunshine I, Hackett E (1957). *J Forensic Sci* 2: 149–158.
Sweetman SC (2002). *Martindale, The complete drug reference*, 33 edn. London: Pharmaceutical Press.
Tanaka E *et al.* (1997). Forensic analysis of 10 barbiturates in human biological samples using a new reversed-phase chromatographic column packed with 2-micrometre porous microspherical silica-gel. *Forensic Sci Int* 85: 73–82.
Wilensky AJ *et al.* (1982). Kinetics of phenobarbital in normal subjects and epileptic patients. *Eur J Clin Pharmacol* 23: 87–92.

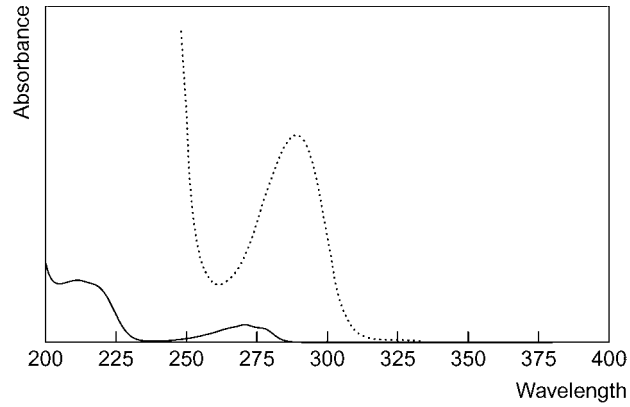
Phenol

Disinfectant, Anaesthetic (Topical)
C_6H_5O = 94.11
CAS—108-95-2

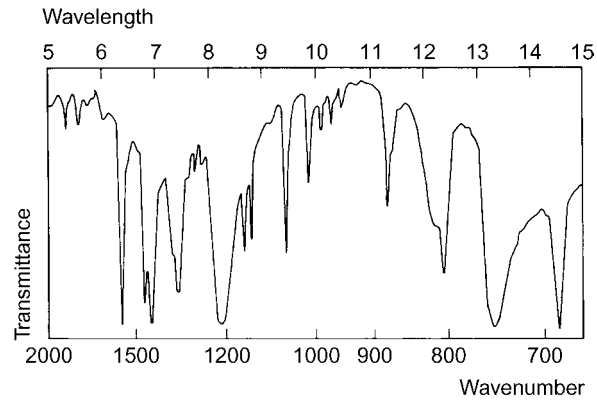
IUPAC Name Phenol
Synonyms Carbolic acid; fenol; hydroxybenzene; oxybenzene; phenyl hydrate; phenylic acid.
Proprietary Names *Chloraseptic*; *Phenaseptic*; *Septosol*.



Chemical Properties Colourless or faintly pink, deliquescent crystals or crystalline masses, becoming pink on storage. Mp 40° to 41°. Commercial products may contain impurities which raise the bp to about 182°. Soluble 1 in about 15 of water, 6 in 1 of ethanol, 2 in 1 of chloroform and 5 in 1 of ether; soluble in glycerol. pK_a 10.0 (25°). Log *P* (octanol/water), 1.5.
Caution Phenol is caustic to the skin.
Colour Tests *p*-Dimethylaminobenzaldehyde—orange/violet; ferric chloride—blue; Folin–Ciocalteu reagent—blue; Liebermann's reagent—green; potassium dichromate (method 1)—yellow→brown (2 min).
Gas Chromatography System GA—RI 981.
High Performance Liquid Chromatography System HAA—retention time 13.4 min.
Ultraviolet Spectrum Aqueous acid—270 nm (A_1^1=172b); aqueous alkali—287 nm (A_1^1=281a).



Infrared Spectrum Principal peaks at wavenumbers 690, 755, 1235, 1595, 1497, 811 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 94, 66, 39, 65, 40, 55, 38, 95.
Quantification
Plasma **GC-MS** [Harrison *et al.* 1991].
Serum **GC-MS** Limit of detection, 1 mg/L [Hart, Dasgupta 1997].
Urine **GC** Phenol and other phenolic compounds. Limit of detection, 0.1 to 0.2 mg/L [Bieniek 1996]. Limit of detection, 1 mg/L [Ahmed, Hale 1994]. FID [Rick *et al.* 1982]. FID [Baldwin *et al.* 1981].

HPLC Phenol and *p*-cresol. Fluorescence detection. Limits of detection, 0.17 pmol and 0.25 pmol per injection respectively [Tsuruta *et al.* 1996]. UV detection. Phenol and *p*-cresol [Birkett *et al.* 1995]. Fluorescence detection. Limit of detection, 10 ppb [Jen, Tsai 1994]. Fluorimetric detection. Phenol, hydroquinone, and catechol [Lee 1993]. UV detection. Limit of detection, <500 µg/L [Eadsforth, Coveney 1984].
Faeces **HPLC** See Urine [Birkett *et al.* 1995].
Disposition in the Body Rapidly absorbed from the gastrointestinal tract and readily penetrates the skin. It is metabolised by conjugation to yield phenyl glucuronide and phenyl sulfate; small amounts are oxidised to catechol and quinol which are also conjugated. Oxidation of these metabolites to quinones may tint the urine green. Acid-labile phenol conjugates are present endogenously in serum at concentrations of about 0.1 mg/L. Phenol is a metabolite of benzene. Endogenous phenol concentrations in urine average about 5 to 10 mg/L.
Toxicity Phenol denatures and precipitates cellular proteins and thus may cause rapid poisoning. The minimum lethal dose by mouth is about 1 g. Severe and even fatal poisoning may also arise from absorption of phenol from open wounds or through the intact skin. The maximum permissible atmospheric concentration is 5 ppm.

In a fatality due to the deliberate ingestion of a mixture containing phenol, the following postmortem tissue concentrations were reported: blood, phenol 56 mg/L, alcohol 300 mg/L; liver, phenol 74 µg/g. In a second fatality due to accidental percutaneous absorption, postmortem blood concentrations of 27 mg/L of phenol and 300 mg/L of alcohol were reported [Soares, Tift 1982].

In a fatality resulting from the ingestion of phenol, the following postmortem concentrations were reported: blood 130 mg/L, urine 47 mg/L, bile 187 mg/L, brain 486 µg/g, kidney 331 µg/g, muscle 204 µg/g, liver 228 µg/g, stomach content 668 mg [Lo Dico *et al.* 1989].

In a fatality due to accidental dermal exposure to phenol, the following postmortem concentrations were reported: blood 4.7 mg/L, unhydrolysed liver 3.3 µg/g, hydrolysed liver 7.1 µg/g [Lewin, Cleary 1982].

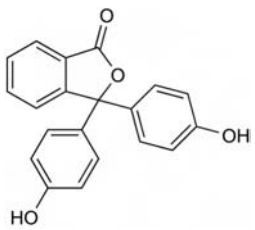
Ahmed N, Hale K (1994). A microassay for urinary phenol using capillary gas chromatography and optimised enzymic hydrolysis. *Clin Chim Acta* 230: 201–208.
Baldwin MK *et al.* (1981). Measurement of phenol in urine by the method of Van Haaften and Sie: a critical appraisal. *Analyst* 106: 763–767.
Bieniek G (1996). Simultaneous determination of phenol, cresol, xylenol isomers and naphthols in urine by capillary gas chromatography. *J Chromatogr B Biomed Appl* 682: 167–172.

Birkett AM *et al.* (1995). Simple high-performance liquid chromatographic analysis of phenol and p-cresol in urine and feces. *J Chromatogr B Biomed Appl* 674: 187–191.
Eadsforth CV, Coveney PC (1984). Measurement of phenol in urine using a high-performance liquid chromatographic method. *Analyst* 109: 175–176.
Harrison LM *et al.* (1991). Microtechnique for quantifying phenol in plasma by gas chromatography-mass spectrometry. *Clin Chem* 37: 1739–1742.
Hart AP, Dasgupta A (1997). A novel derivatization of phenol after extraction from human serum using perfluorooctanoyl chloride for gas chromatography-mass spectrometric confirmation and quantification. *J Forensic Sci* 42: 693–696.
Jen JF, Tsai MY (1994). Determination of phenol in urine by high-performance liquid chromatography with on-line precolumn enzymatic hydrolysis of the conjugates. *J Chromatogr B Biomed Appl* 658: 87–92.
Lee BL *et al.* (1993). Simultaneous determination of hydroquinone, catechol and phenol in urine using high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 619: 259–266.
Lewin JF, Cleary WT (1982). An accidental death caused by the absorption of phenol through skin. A case report. *Forensic Sci Int* 19: 177–179.
Lo Dico C *et al.* (1989). Phenol: tissue distribution in a fatality. *J Forensic Sci* 34: 1013–1015.
Rick DL *et al.* (1982). Determination of phenol and pentachlorophenol in plasma and urine samples by gas liquid chromatography. *J Anal Toxicol* 6: 297–300.
Soares ER, Tift JP (1982). Phenol poisoning: three fatal cases. *J Forensic Sci* 27: 729–731.
Tsuruta Y *et al.* (1996). Fluorometric determination of phenol and p-cresol in urine by precolumn high-performance liquid chromatography using 4-(N-phthalimidinyl)benzenesulfonyl chloride. *Anal Biochem* 243: 86–91.

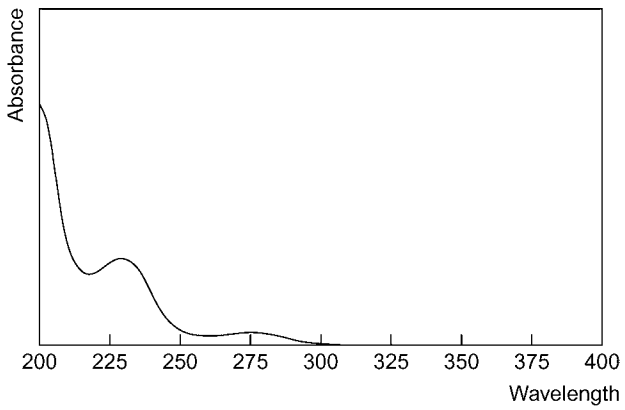
Phenolphthalein

Purgative
$C_{20}H_{14}O_4 = 318.3$
CAS—77-09-8

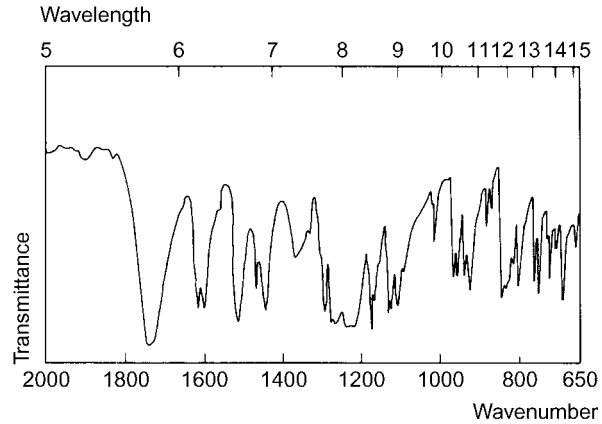
IUPAC Name 3,3-Bis(4-hydroxyphenyl)-2-benzofuran-1-one
Synonyms 3,3-Bis(4-hydroxyphenyl)-1-(3*H*)-isobenzofuranone; dihydroxyphthalophenone; fenolftaleina.
Proprietary Names *Alophen Pills*; *Bonomint*; *Brooklax*; *Easylax*; *Euchessina*; *Laxen Busto*; *Laxettes*; *Laxicaps P*; *Nylax*; *Purmolax*; *Regulim*; *Sure Lax*. It is an ingredient of *Agarol* and *Alophen*. For a list of other preparations which contain phenolphthalein, see Sweetman [2002].



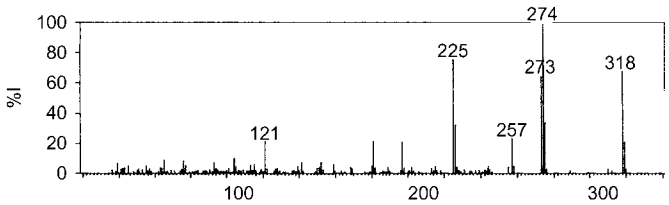
Chemical Properties A white or yellowish-white crystalline or amorphous powder. Mp 258° to 262°. Practically insoluble in water; soluble 1 in 12 of ethanol and 1 in about 100 of ether; soluble in dilute solutions of alkali hydroxides. pK_a 9.7 (25°). Log *P* (octanol/water), 2.4. Extraction yield (chlorobutane), 0.7 [Demme *et al.* 2005].
Colour Test Folin–Ciocalteu reagent—blue.
Thin-layer Chromatography System TD—R_f 0.38; system TE—R_f 0.53; system TF—R_f 0.59; system TAD—R_f 0.39; system TAE—R_f 0.86 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HX—RI 436; system HZ—retention time 4.2 min.
Ultraviolet Spectrum Methanol—277 nm (A^1_1=150a).



Infrared Spectrum Principal peaks at wavenumbers 1740, 1178, 1230, 1265, 1278, 1510 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 274, 225, 318, 273, 275, 226, 257, 121.



Quantification
Serum **Colorimetry** [Morris, Powell 1979].
Urine **Colorimetry** See Serum [Morris, Powell 1979].
Disposition in the Body Poorly absorbed after oral administration (up to about 15%), and partly excreted in the bile as the active glucuronide, and in the urine; if the urine is alkaline the excreted compound will impart a red colour. The greater part of the ingested material is eliminated in the faeces in both free and conjugated forms.
Toxicity Phenolphthalein is usually non-toxic even in relatively large doses, although fatalities have occurred in young children after ingestion of 0.6 to 1.8 g.

> A 32-year-old woman (suspected of being a chronic abuser of laxatives) eventually died due to massive liver necrosis after ingesting an unknown quantity of Nylax tablets containing phenolphthalein. The serum-phenolphthalein concentration on the day after hospital admission was 0.4 µg/L [Sidhu *et al.* 1989].

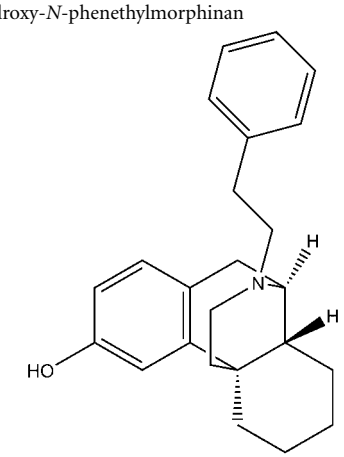
Dose 50 to 300 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Morris SM, Powell DW (1979). Spectrophotometric assay of phenolpthalein in biological fluids. *Anal Biochem* 95: 465–471.
Sidhu PS *et al.* (1989). Fatal phenolphthalein poisoning with fulminant hepatic failure and disseminated intravascular coagulation. *Hum Toxicol* 8: 381–384.
Sweetman SC (2002). *Martindale: The Complete Drug Reference*, 33 edn. London: Pharmaceutical Press.

Phenomorphan

Narcotic
$C_{24}H_{29}NO = 347.5$
CAS—468-07-5

Synonym 3-Hydroxy-*N*-phenethylmorphinan



Chemical Properties Phenomorphan is extracted by organic solvents from aqueous ammoniacal solutions; hydrolysis of biological material is required to improve yield.

Phenomorphan Hydrobromide

$C_{24}H_{29}NO,HBr = 428.4$

Chemical Properties Mp 289° to 292°.

Colour Test Ammonium molybdate test—blue (limit of detection, 0.25 µg); Vitali's test—yellow/yellow/orange (limit of detection, 0.25 µg).

Thin-layer Chromatography System T1—R_f 0.72 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Ethanol—280 nm and inflexions at about 230 and 288 nm; 0.1 N sodium hydroxide—242, 300 nm; 0.1 N sulphuric acid—279 nm (E1%, 1 cm 43.7) and an inflexion at about 284 nm (E1%, 1 cm 41).

Phenoperidine

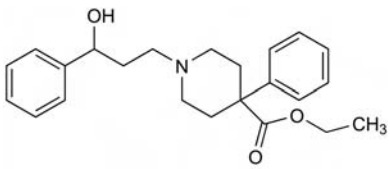
Narcotic Analgesic

$C_{23}H_{29}NO_3 = 367.5$

CAS—562-26-5

IUPAC Name Ethyl 1-(3-hydroxy-3-phenylpropyl)-4-phenylpiperidine-4-carboxylate

Synonym 1-(3-Hydroxy-3-phenylpropyl)-4-phenyl-4-piperidinecarboxylic acid ethyl ester



Chemical Properties Log *P* (octanol/water), 4.0. Plasma samples were stable for 7 days at –20° [Chan *et al.* 1981].

Phenoperidine Hydrochloride

$C_{23}H_{29}NO_3,HCl = 403.9$

CAS—3627-49-4

IUPAC Name Ethyl 1-(3-hydroxy-3-phenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride

Proprietary Names *Lealgin*; *operidine*; *R 1406*.

Note Operidine has been used as a synonym for pethidine hydrochloride in Japan Pharmacopeia.

Chemical Properties A white crystalline powder. Mp 200° to 202°. Soluble 1 in 50 of water, 1 in 10 of ethanol (90%) and 1 in 3 of chloroform; practically insoluble in ether.

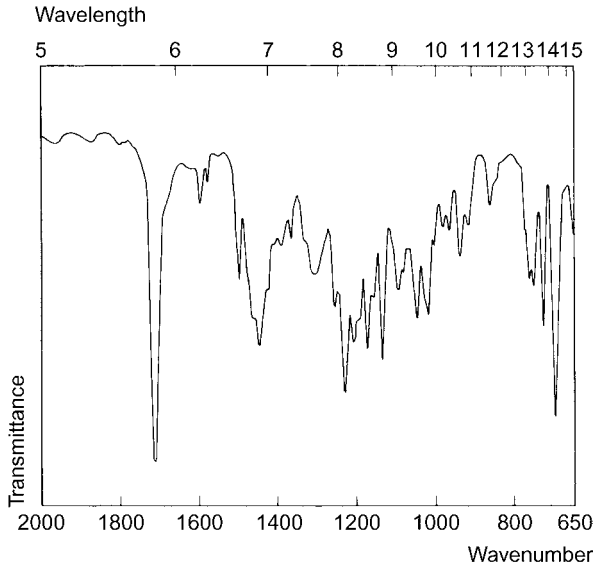
Colour Test Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.26; system TC—R_f 0.64; system TE—R_f 0.76; system TAE—R_f 0.70; system TAF—R_f 0.82; system TAG—R_f 0.58 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—faint red). See also Pethidine monograph.

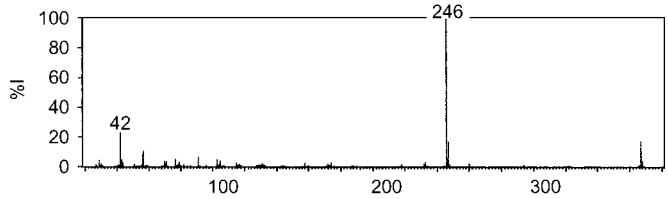
Gas Chromatography System GA—phenoperidine RI 2872, norpethidine RI 1885, pethidine RI 1754; system GB—phenoperidine RI 2983, norpethidine RI 1842, pethidine RI 1809.

High Performance Liquid Chromatography System HA—phenoperidine *k* 0.8, norpethidine *k* 1.7 (tailing peak), pethidine *k* 2.8 (tailing peak); system HC—phenoperidine *k* 0.10, norpethidine *k* 2.04, pethidine *k* 0.55; system HX—RI 434. See also Pethidine monograph.

Infrared Spectrum Principal peaks at wavenumbers 1710, 698, 1230, 1136, 1175, 1205 cm^{-1} (phenoperidine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 246, 42, 367, 247, 57, 56, 91, 77 (phenoperidine); 57, 233, 42, 56, 158, 43, 160, 103 (norpethidine); 71, 70, 44, 57, 42, 247, 43, 246 (pethidine).



Quantification

***Plasma* GC** Column: SGE BP 10 (25 m × 0.22 mm i.d.). Carrier gas: N_2, 6.2 mL/min. Temperature programme: 200° to 270° at 10°/min for 23 min. NSD. Limit of detection, 0.9 µg/L [Kintz *et al.* 1990]. Column: Alltech 3% OV-17 Gas Chrom Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 210° for 2 min to 270° at 15°/min for 10 min. NSD. Retention time: 14.35 min. Limit of detection, 1.2 µg/L [Kintz *et al.* 1989]. Column: 3% OV-1 or 10% SP-2100 on Supelcoport 80/100 mesh (1 m × 4 mm o.d.). Carrier gas: N_2, 30 mL/min. Temperature: 350°. NPD. Retention time: 3 or 6 min, respectively, for the two columns. Limit of detection, 2 µg/L [Chan *et al.* 1981].

Disposition in the Body Phenoperidine is absorbed from the gastrointestinal tract but there is extensive first-pass metabolism; bioavailability is ~30%. It is metabolised to pethidine and norpethidine. Up to ~5% of a dose is excreted unchanged in the urine, with ~18% as norpethidine and 2% as pethidine, in 2–3 days. The urinary excretion of unchanged phenoperidine is increased to ~7% when the urine is acidified.

Therapeutic Concentration

After a single IV dose of 2 mg administered to five patients during general anaesthesia, a mean plasma concentration of 0.02 mg/L was reported at 2 min [Milne *et al.* 1980].

Half-life Plasma half-life, ~1 h.

Volume of Distribution ~2 L/kg.

Clearance Plasma clearance, ~20 mL/min/kg.

Protein Binding ~80%.

Dose Phenoperidine hydrochloride 0.5 to 1 mg IV; with assisted ventilation, an initial IV dose of 2 to 5 mg may be given.

Chan K *et al.* (1981). Quantitative gas–liquid chromatographic method for the determination of phenoperidine in human plasma. *J Chromatogr* 223: 213–218.

Kintz P *et al.* (1989). Simultaneous determination of pethidine (meperidine), phenoperidine, and norpethidine (normeperidine), their common metabolite, by gas chromatography with selective nitrogen detection. *Forensic Sci Int* 43: 267–273.

Kintz P *et al.* (1990). Simultaneous screening and quantification of several nonopiate narcotic analgesics and phencyclidine in human plasma using capillary gas chromatography. *Meth Find Exp Clin Pharmacol* 12: 193–196.

Milne L *et al.* (1980). Plasma concentration and metabolism of phenoperidine in man. *Br J Anaesth* 52: 537–540.

Phenothiazine

Anthelmintic (Veterinary)

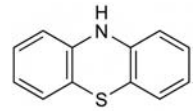
$C_{12}H_9NS = 199.3$

CAS—92-84-2

IUPAC Name 10H-Phenothiazine

Synonyms Bibenzothiazine; thiodiphenylamine.

Proprietary Names *AFI-Tiazin*; *Antiverm*; *Fentiazin*; *Helmetina*; *Lethelmin*; *Nemazine*; *Orimon*; *Phenegic*; *Phenoverm*; *Phenovis*; *Phenoxur*; *Reconox*; *Souframine*; *Vermitin*.



Chemical Properties A yellow crystalline powder. Slowly oxidised in air and darkens on exposure to light acquiring a greenish-brown tint. Mp about 185°. Practically insoluble in water and chloroform; freely soluble in benzene; soluble 1 in 60 of ethanol and 1 in 5 of acetone. pK_a 2.5. Log *P* (octanol/water), 4.2.

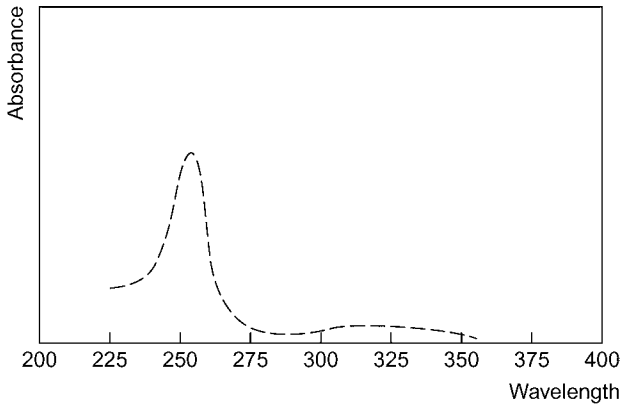
Colour Tests Ferric chloride—green; formaldehyde-sulfuric acid—red-violet; Forrest reagent—orange; FPN reagent—orange; Liebermann's reagent—green; sulfuric acid—green.

Thin-layer Chromatography System TA—R_f 0.79; system TB—R_f 0.11; system TC—R_f 0.79; system TE—R_f 0.82; system TL—R_f 0.71; system TAE—R_f 0.82; system TAF—Tf 89; system TAJ—R_f 0.90; system TAK—R_f 0.77; system TAL—R_f 0.96.

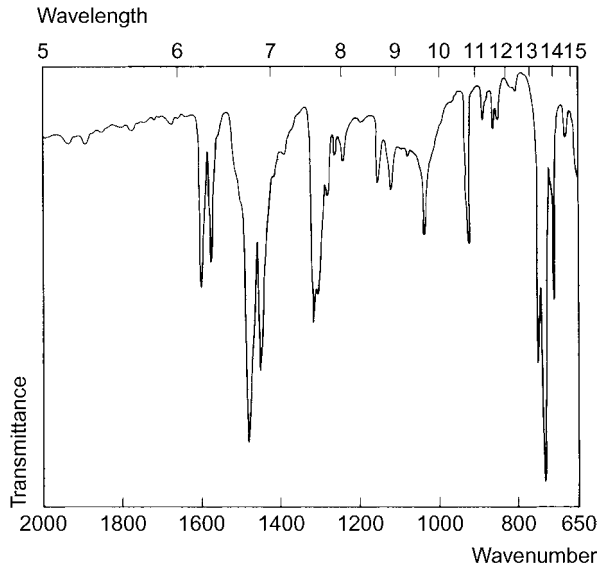
Gas Chromatography System GA—RI 2120; system GB—RI 2130; system GF—RI 2845.

High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 665; system HY—RI 637; system HAA—retention time 14.1 min (phenothiazine ring), retention time 14.3 min (phenothiazine ring).

Ultraviolet Spectrum Ethanol—254 (A^{1}_{1}=2160b), 317 nm (A^{1}_{1}=266b).



Infrared Spectrum Principal peaks at wavenumbers 735, 750, 1310, 710, 1300, 1590 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 199, 167, 198, 166, 99, 154, 69, 77.

Phenoxybenzamine

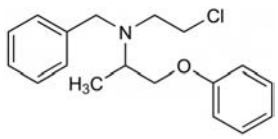
Antihypertensive

$C_{18}H_{22}ClNO$ = 303.8

CAS—59-96-1

IUPAC Name *N*-Benzyl-*N*-(2-chloroethyl)-1-phenoxypropan-2-amine

Synonyms 688-A; bensylyt; *N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl) benzenemethanamine.



Chemical Properties Crystals. Mp 38° to 40°. Soluble in benzene. Log *P* (octanol/water), 4.6.

Phenoxybenzamine Hydrochloride

$C_{18}H_{22}ClNO,HCl$ = 340.3

CAS—63-92-3

Proprietary Names *Dibenyline*; *Dibenzyline*; *Dibenzyran*.

Chemical Properties A white crystalline powder. Mp 137° to 141°. Soluble 1 in 25 of water, 1 in 9 of ethanol and 1 in 9 of chloroform; practically insoluble in ether. Neutral and alkaline solutions are unstable.

Caution Phenoxybenzamine hydrochloride in powder form should not be allowed to come into contact with eyes or skin, as it may cause irritation.

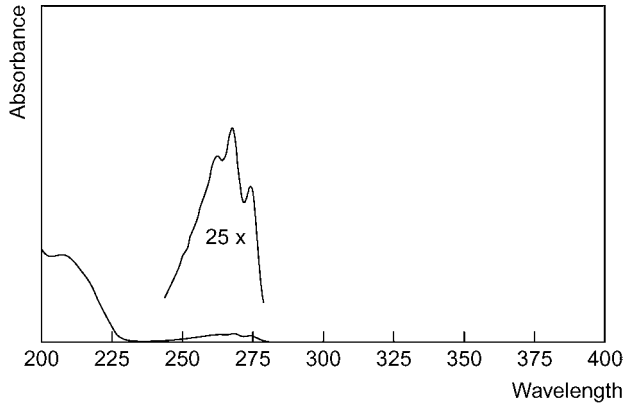
Colour Tests Mandelin's test—green→violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.63; system TC—R_f 0.76; system TE—R_f 0.87; system TL—R_f 0.68; system TAE—R_f 0.84; system TAF—R_f 0.97; system TAJ—R_f 0.65; system TAK—R_f 0.04; system TAL—R_f 0.51 (acidified iodoplatinate solution, positive).

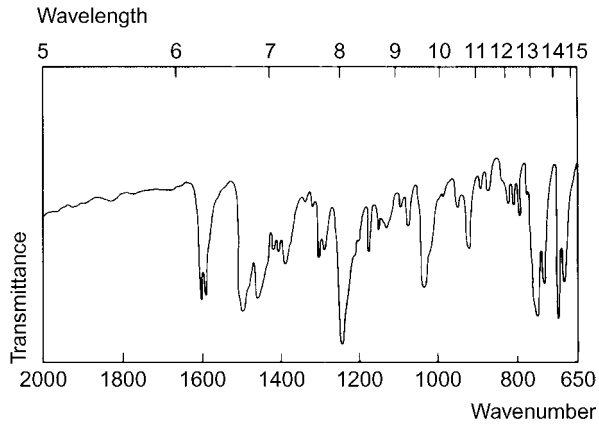
Gas Chromatography System GA—RI 2235; system GB—RI 2332; system GF—RI 2600.

High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 396.

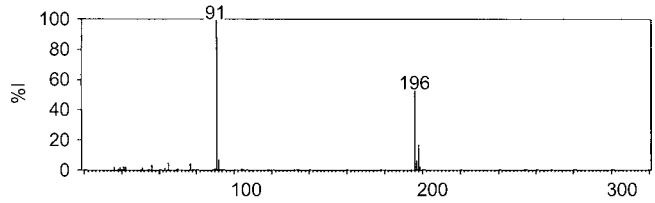
Ultraviolet Spectrum Aqueous acid—262, 268 (A_1^1=48a), 274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1240, 750, 698, 1494, 1598, 1586 cm^{-1} (phenoxybenzamine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 196, 198, 92, 197, 65, 77, 56.



Dose 10 to 60 mg of phenoxybenzamine hydrochloride daily; up to 200 mg daily has been given.

Phenoxymethylpenicillin

Antibiotic

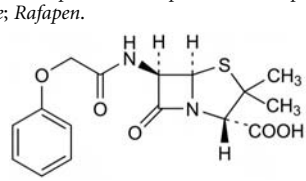
$C_{16}H_{18}N_2O_5S$ = 350.4

CAS—87-08-1

IUPAC Name (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Synonyms Fenoximetilpenicilina; penicillin V; phenomycilline.

Proprietary Names *Acipen V*; *Compocillin*; *Fenospen*; *Kopen*; *Meracilina*; *Ospen*; *Pen-V*; *Pen-Vee*; *Rafapen*.



Chemical Properties An antimicrobial acid produced by the growth of certain strains of *Penicillium notatum* or related organisms. A white crystalline powder. Mp

P

120° to 128°, with decomposition. Soluble 1 in 1700 of water, 1 in 7 of ethanol and 1 in 6 of acetone; soluble in chloroform. pK_a 2.7 (25°). Log *P* (octanol/water), 2.1.

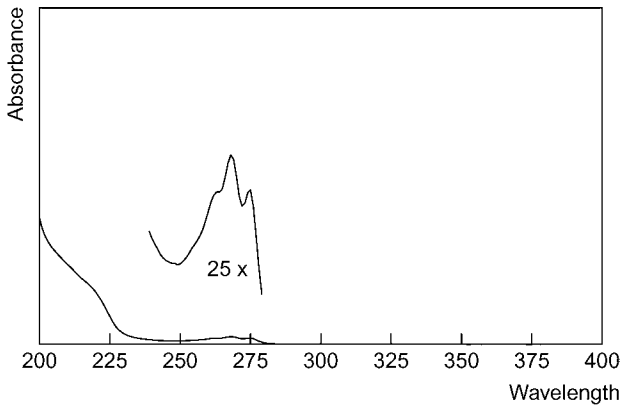
Phenoxymethylpenicillin Calcium

$(C_{16}H_{17}N_2O_5S)_2Ca,2H_2O = 774.9$
CAS—147-48-8 (anhydrous); 73368-74-8 (dihydrate)
Synonym Penicillin V calcium
Proprietary Names *Calcipen*; *Calvepen*; *Milcopen*.
Chemical Properties A white, finely crystalline powder. Slowly soluble 1 in 20 of water.

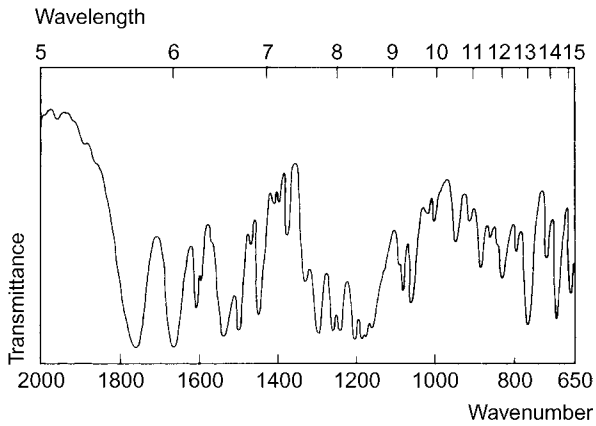
Phenoxymethylpenicillin Potassium

$C_{16}H_{17}KN_2O_5S = 388.5$
CAS—132-98-9
Synonyms Fenoximetilcilina potassica; penicillin V potassium.
Proprietary Names *Acipen*; *Apocillin*; *Apo-Pen-VK*; *Apsin VK*; *Arcasin*; *Beepen-VK*; *Betapen-VK*; *Brunocillin*; *Calciopen*; *Cilicaine VK*; *Cliacil*; *Colipen VK*; *Copen*; *Darocillin*; *Deltacillin*; *Distaquaine V-K*; *Fenoxicillin*; *InfectoCillin*; *Isocillin*; *Ledercillin VK*; *Len V.K*; *LPV*; *Mack Pen*; *Megacillin(e)*; *Milcopen*; *Nadopen-V*; *NovoPen-VK*; *Oracilin*; *Oracilline*; *Ospen*; *Pen*; *Penbene*; *Penhexal VK*; *Peni-Oral*; *Penstad*; *Pen-V*; *Pen-Vee-K*; *Pen-Ve-Oral*; *Pen-Vi-K*; *PVFK*; *PVK*; *Robicillin VK*; *Rocilin*; *Tikacillin*; *V-Cil-K*; *V-Cillin K*; *Veekay*; *Veetids*; *Vepenicillin*.
Note *Megacillin* is also used as a proprietary name for benzylpenicillin potassium or benzathine benzylpenicillin and *Megacilline* for clemizole penicillin. *Oracilline*, *Pen-V* and *Pen-Vee* are also used as proprietary names for benzathine phenoxymethylpenicillin and *Arcasin* is used for cisapride.
Chemical Properties A white crystalline powder. Soluble 1 in 1.5 of water and 1 in 150 of ethanol; practically insoluble in ether; insoluble in acetone.

Ultraviolet Spectrum Phenoxymethylpenicillin potassium : water—268 (A^1_1 = 31b), 275 nm (A^1_1 = 25b).



Infrared Spectrum Principal peaks at wavenumbers 1754, 1660, 1181, 1202, 1174, 1532 cm^{-1} (KBr disk).



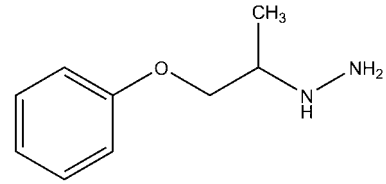
Quantification

Plasma **HPLC** UV detection. Limit of detection, 0.1 mg/L [Krauwinkel, Volkers-Kamermans 1996].
Serum **HPLC** UV detection. Phenoxymethylpenicillin and other penicillins. Limit of detection, <0.5 mg/L [Mendez-Alvarez *et al.* 1991]. Limit of detection, 30 µg/L [Lindberg *et al.* 1984].
Disposition in the Body Rapidly but incompletely absorbed after oral administration; peak plasma concentrations are attained ≈2 h after a dose. About 20–35% of an oral dose is excreted in the urine unchanged in 24 h and ≈34% is excreted as penicilloic acid in the same period.
Half-life Plasma half-life, ≈0.5 h.
Protein Binding About 80%.
Dose 0.5 to 3 g daily.

Krauwinkel WJ, Volkers-Kamermans NJ (1996). Determination of penicillin-V in human plasma by high-performance liquid chromatography and solid-phase extraction. *J Chromatogr B Biomed Appl* 679: 129–135.
Lindberg RL *et al.* (1984). Rapid high-pressure liquid chromatographic method for analysis of phenoxymethylpenicillin in human serum. *Antimicrob Agents Chemother* 26: 300–302.
Mendez-Alvarez E *et al.* (1991). A reversed phase liquid chromatographic method for the simultaneous determination of several common penicillins in human serum. *Biomed Chromatogr* 5(2): 78–82.

Phenoxypropazine

Monoamine Oxidase Inhibitor, Antidepressant
$C_9H_{14}N_2O = 166.2$
CAS—3818-37-9
IUPAC Name 1-(Phenoxy)propan-2-ylhydrazine
Synonyms Fenoxipropazinum; 1-methyl-2-phenoxyethyl)hydrazine.



Chemical Properties Log *P* (octanol/water) 1.4 [National Institutes of Health 2008]. Phenoxypropazine is extracted by organic solvents from aqueous alkaline solutions.

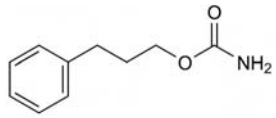
Phenoxypropazine Maleate

$C_9H_{14}N_2O,C_4H_4O_4 = 282.3$
CAS—3818-37-9
Synonyms HP 1275; phenoxypropazine hydrogen maleate.
Proprietary Name *Drazine*
Chemical Properties Prismatic needles from isopropanol. A white crystalline powder. Mp 107° to 110°. Very soluble in water; soluble in ethanol or isopropanol.
Colour Tests Ammonium molybdate test—(grey) blue (limit of detection, 0.5 µg); ammonium vandate test—(brown) brown (limit of detection, 0.5 µg); sulfuric acid-formaldehyde test—pale red (limit of detection, 0.5 µg); Vitali's test—brown→yellow/yellow/yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.52 (location reagent acidified iodoplatinate spray, white).
Gas Chromatography System G2/120—retention time 1.5 (relative to ephedrine).
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—263, 268, 319 nm.
Infrared Spectrum Maleate: Principal peaks at wavenumbers 1465, 1500, 1582, 760, and 1353 cm^{-1} (KBr disk).
Disposition in the Body Phenoxypropazine is structurally similar to amphetamine [Davis, Horlington 1964].
Therapeutic Concentration The efficacy of phenoxypropazine has been demonstrated in the treatment of depression that is sufficiently severe to warrant hospitalisation [Leahy *et al.* 1963], an effect that was comparable with amitryptiline [Rose *et al.* 1963] and improved by the concomitant administration of chlordiazepoxide [Rose 1964]. It has also been shown to be effective against milder forms of depression [Imlah 1963].
Toxicity See under phenelzine. LD_{50} (oral) in mice: 460 mg/kg.
Dose Up to 20 mg daily.

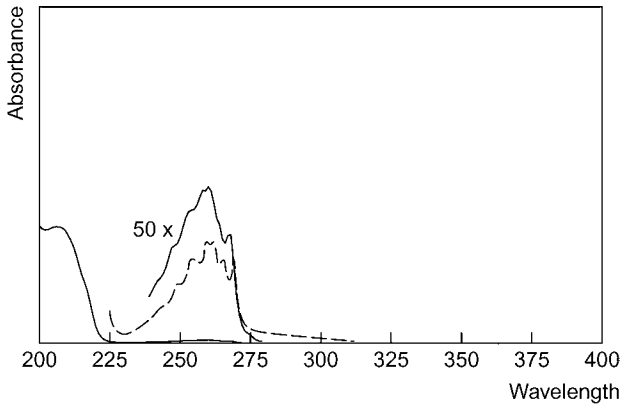
Davis RA, Horlington M (1964). Effects of reserpine pretreatment on the protective action of amphetamine and phenoxypropazine in the phenylbenzoquinone-induced writhing syndrome in mice. *Nature* 201: 306–307.
Imlah NW (1963). Preliminary report on phenoxypropazine. *Am J Psychiatry* 119: 1091–1092.
Leahy MR *et al.* (1963). A preliminary study of phenoxypropazine in the treatment of depression. *Am J Psychiatry* 119: 986–987.
National Institutes of Health. Fenoxipropazinum. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=71467&loc=ec_rcs (accessed 27 June 2008).
Rose JT (1964). Phenoxypropazine and chlordiazepoxide in depression. *Am J Psychiatry* 120: 899–900.
Rose JT *et al.* (1963). A comparison of phenoxypropazine and amytryptiline in depression. *Am J Psychiatry* 120: 393–395.

Phenprobamate

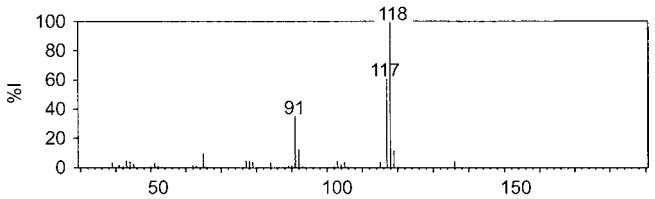
Skeletal Muscle Relaxant, Tranquilliser
$C_{10}H_{13}NO_2 = 179.2$
CAS—673-31-4
IUPAC Name 3-Phenylpropyl carbamate
Synonyms Benzenepropanol carbamate; MH-532; proformiphen.
Proprietary Names *Extacol*; *Gamaquil*; *Spantol*.



Chemical Properties A fine, white, crystalline powder. Mp 101° to 104°. Practically insoluble in water; soluble in ethanol and chloroform; slightly soluble in ether. Log *P* (octanol/water), 2.0.
Colour Test Marquis test—red-brown.
Thin-layer Chromatography System TA—R_f 0.75; system TD—R_f 0.47; system TE—R_f 0.73; system TF—R_f 0.55; system TAD—R_f 0.60; system TAE—R_f 0.82 (furfuraldehyde reagent, positive).
Gas Chromatography System GA—RI 1520.
Ultraviolet Spectrum Ethanol—254, 259 (A_1^1= 11.6b), 261 (A_1^1= 11.6b), 265 nm, 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1690, 1088, 1064, 700, 1605, 1623 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 118, 117, 91, 92, 119, 65, 77, 103.



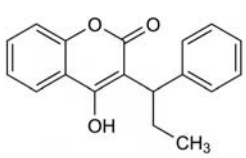
Quantification
Blood **TLC** Phenprobamate and metabolites [Schatz, Jahn 1966].
Plasma **HPLC** UV detection [Sun *et al.* 1987].
Urine **TLC** See Blood [Schatz, Jahn 1966].
Disposition in the Body Rapidly absorbed after oral administration and metabolised by oxidative degradation of the side chain and *p*-hydroxylation of the phenyl ring. About 7% of a dose is excreted unchanged in the urine in 48 h and ≈76% as metabolites, mostly as hippuric acid and free and conjugated 3-hydroxyphenprobamate. The 3-hydroxy metabolite, which is active, is present in blood at concentrations greater than those of unchanged drug.
Half-life Plasma half-life, 5–8 h.
Dose 1.2 to 2.4 g daily.

Schatz F, Jahn U (1966). Studies on the resorption and metabolism of gamma-phenylpropyl carbamate (Phenprobamate). *Arzneimittelforschung* 16: 866–870.
Sun JX *et al.* (1987). High-performance liquid chromatographic analysis, plasma protein binding and red blood cell partitioning of phenprobamate. *Biopharm Drug Dispos* 8: 341–351.

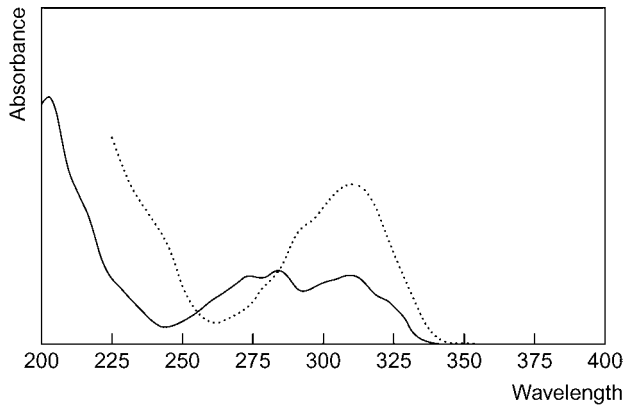
Phenprocoumon

Anticoagulant
$C_{18}H_{16}O_3$ = 280.3
CAS—435-97-2

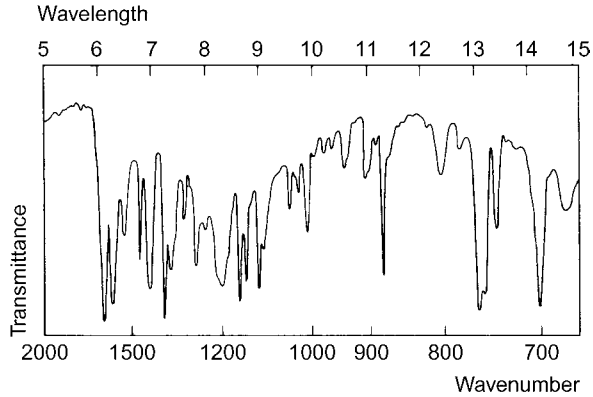
IUPAC Name 2-Hydroxy-3-(1-phenylpropyl)chromen-4-one
Synonym 4-Hydroxy-3-(1-phenylpropyl)-2*H*-1-benzopyran-2-one; phenylpropylhydroxycoumarin
Proprietary Names *Falithrom*; *Liquamar*; *Marcoumar*; *Marcumar*; *Marcuphen*; *Phenpro*.



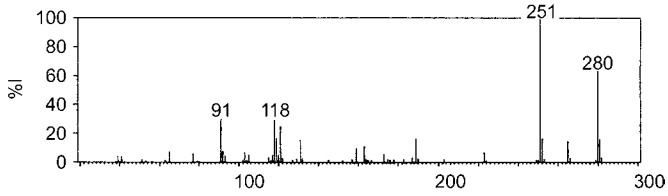
Chemical Properties A fine white crystalline powder. Mp 179° to 180°. Practically insoluble in water; soluble in chloroform, methanol and solutions of alkali hydroxides. Log *P* (octanol/water), 3.6. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].
Thin-layer Chromatography System TD—R_f 0.62; system TE—R_f 0.19; system TF—R_f 0.58; system TAD—R_f 0.61; system TAE—R_f 0.93.
Gas Chromatography System GA—phenprocoumon isomer 1-Me RI 2375, isomer 2-Me RI 2395; M (OH-)isomer-1 Me_2 RI 2655; isomer-2 Me_2 RI 2675, isomer 3 Me_2 RI 2705; M (OH-methoxy)Me_2 RI 2770.
High Performance Liquid Chromatography System HX—RI 616; system HZ—retention time 12.6 min.
Ultraviolet Spectrum Aqueous alkali—310 nm (A_1^1= 540b); methanol—285 (A_1^1= 394a), 310 nm (A_1^1= 440a).



Infrared Spectrum Principal peaks at wavenumbers 1656, 759, 696, 1613, 1163, 1113 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 251, 280, 118, 91, 121, 119, 252, 189.



Quantification
Plasma **TLC–fluorescence densitometry** Limit of detection, 5 µg/L [Haefelfinger 1979].
GC FID. Limit of detection, 125 µg/L [Midha *et al.* 1976].
HPLC UV and fluorescence detection. Phenprocoumon and its metabolites [de Vries, Schmitz-Kummer 1994]. UV detection. Limit of detection, 100 µg/L [De Vries *et al.* 1982].
LC-MS Phenprocoumon, warfarin, and acenocoumarol. Limit of detection, 1 µg/L (phenprocoumon and warfarin) and 10 µg/L (acenocoumarol) [Kollroser, Schober 2002].
Serum **HPLC** [Petersen *et al.* 1993].
Urine **HPLC** See Plasma [de Vries, Schmitz-Kummer 1994]. Phenprocoumon and its metabolites [Edelbroek *et al.* 1990]. See Plasma [De Vries *et al.* 1982].
Breast Milk **HPLC** See Plasma [de Vries, Schmitz-Kummer 1994].
Disposition in the Body Well absorbed after oral administration. The *S*-enantiomer is stated to be considerably more potent as an anticoagulant than the *R*-form but there appears to be little difference in pharmacokinetic properties. Phenprocoumon is thought to be excreted almost entirely as a glucuronide conjugate with <10% of the dose as unchanged drug.

Therapeutic Concentration

Following an IV injection of 3 mg to 1 subject, a plasma concentration of 0.8 mg/L was reported at 5 min; in a subject receiving daily doses of 1.6 mg, a steady-state plasma concentration of 1.8 mg/L was reported [Haefelfinger 1979].

Half-life Plasma half-life, 3–9 days (mean, 5).

Volume of Distribution 0.1–0.2 L/kg.

Clearance Plasma clearance, ≈0.01 mL/min/kg.

Protein Binding >99%.

Note For a review of the clinical pharmacokinetics of oral anticoagulants, see Kelly and O'Malley [1979].

Dose Maintenance, 0.75 to 4.5 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

de Vries JX, Schmitz-Kummer E (1994). Determination of the coumarin anticoagulant phenprocoumon and metabolites in human plasma, urine and breast milk by high-performance liquid chromatography after solid-phase extraction. *J Chromatogr B Biomed Appl* 655: 63–71.

De Vries JX *et al.* (1982). Determination of the anticoagulant phenprocoumon in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 231: 83–92.

Edelbroek PM *et al.* (1990). Analysis of phenprocoumon and its hydroxylated and conjugated metabolites in human urine by high-performance liquid chromatography after solid-phase extraction. *J Chromatogr* 530: 347–358.

Haefelfinger P (1979). A specific and sensitive method for the determination of the anticoagulant phenprocoumon in plasma. *J Chromatogr* 162: 215–222.

Kelly JG, O'Malley K (1979). Clinical pharmacokinetics of oral anticoagulants. *Clin Pharmacokinet* 4: 1–15.

Kollroser M, Schober C (2002). Determination of coumarin-type anticoagulants in human plasma by HPLC-electrospray ionization tandem mass spectrometry with an ion trap detector. *Clin Chem* 48: 84–91 and 1372.

Midha KK *et al.* (1976). GLC determination of plasma concentrations of phenprocoumon. *J Pharm Sci* 65: 387–391.

Petersen D *et al.* (1993). Concentrations of phenprocoumon in serum and serum water determined by high-performance liquid chromatography in patients on oral anticoagulant therapy. *Haemostasis* 23: 83–90.

Phensuximide

Anticonvulsant

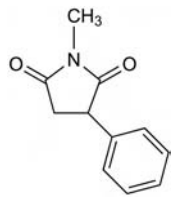
$C_{11}H_{11}NO_2$ = 189.2

CAS—86-34-0

IUPAC Name 1-Methyl-3-phenylpyrrolidine-2,5-dione

Synonym Fensuximid

Proprietary Names *Lifene*; *Milontin*; *Mirontin*; *Succitimal*.



Chemical Properties A white crystalline powder. Mp 71° to 73°. Soluble 1 in 250 of water, 1 in 20 of ethanol, 1 in 1.5 of chloroform and 1 in 35 of ether; readily soluble in methanol. Log *P* (octanol/water), 1.0.

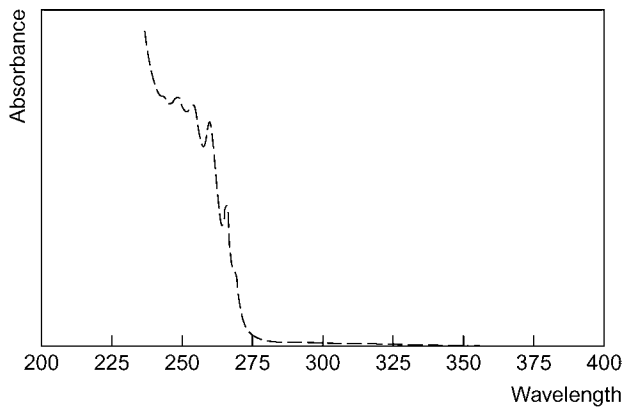
Colour Test Liebermann's reagent—red-orange.

Thin-layer Chromatography System TA—R_f 0.75; system TD—R_f 0.71; system TE—R_f 0.77; system TF—R_f 0.59; system TAD—R_f 0.72; system TAJ—R_f 0.81; system TAK—R_f 0.71; system TAL—R_f 0.96 (acidified potassium permanganate solution, positive).

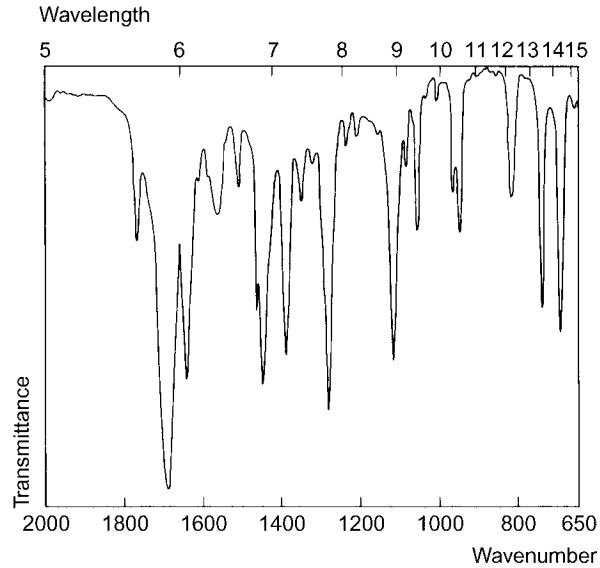
Gas Chromatography System GA—RI 1634; system GE—retention time 0.4 relative to phenytoin.

High Performance Liquid Chromatography System HY—RI 347.

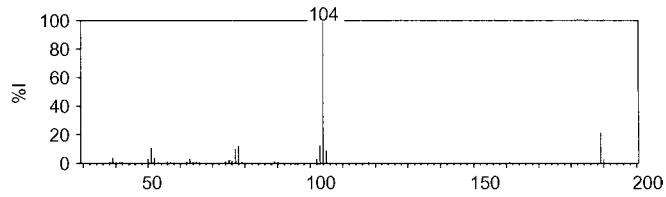
Ultraviolet Spectrum Ethanol—247 (A^1_1=17.2b), 252 (A^1_1=16.2b), 258 (A^1_1=14.5b), 264 nm (A^1_1=9.6b).



Infrared Spectrum Principal peaks at wavenumbers 1686, 1275, 1637, 1111, 695, 751 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 104, 189, 103, 78, 51, 77, 105, 52.



Quantification

Plasma **GC** FID. Limit of detection, 2.5 mg/L [van der Kleijn *et al.* 1973].

Serum **GC** FID. Phensuximide and other succinimide anticonvulsants. Limit of detection, 1 to 5 mg/L for phensuximide [Bonitati 1976].

Urine **GC** See Plasma [van der Kleijn *et al.* 1973].

Disposition in the Body Readily absorbed after oral administration and rapidly metabolised to the *N*-desmethyl derivative, which is active. About 27% of a dose is excreted in the urine in 48 h, mostly as 4′-hydroxyphensuximide and its conjugate.

Therapeutic Concentration

After a single oral dose of 1 g to 12 subjects, peak plasma concentrations of 10.6 to 15.6 mg/L were attained in 0.5 to 1.5 h [Glazko, Dill 1972].

Following daily oral doses of 3 g to 5 subjects with intractable seizures, steady-state plasma concentrations of 3.9 to 7.9 mg/L (mean, 5.7) of phensuximide and 1.5 to 2.1 mg/L (mean, 1.7) of *N*-desmethylphensuximide, were reported [Porter *et al.* 1977].

Toxicity The estimated minimum lethal dose is 5 g.

Half-life Plasma half-life, 4 to 12 h (mean, 8).

Protein Binding Not significantly bound.

Dose 0.5 to 3 g daily.

Bonitati J (1976). Gas-chromatographic analysis for succinimide anticonvulsants in serum: macro- and micro-scale methods. *Clin Chem* 22: 341–345.

Glazko AJ, Dill WA. Woodbury DM *et al.* eds.(1972). *Other Succinimides: Methuximide and Phensuximide, in Antiepileptic Drugs*. New York: Raven Press, 455–464.

Porter RJ *et al.* (1977). Diagnostic and therapeutic reevaluation of patients with intractable epilepsy. *Neurology* 27: 1006–1011.

van der Kleijn E *et al.* (1973). Gas chromatographic determination of ethosuximide and phensuximide in plasma and urine of man. *J Pharm Pharmacol* 25: 324–327.

Phentermine

Anorectic

$C_{10}H_{15}N$ = 149.2

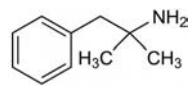
CAS—122-09-8

IUPAC Name 2-Methyl-1-phenylpropan-2-amine

Synonym α,α-Dimethylbenzeneethanamine

Note Phentermine is an isomer of metamfetamine.

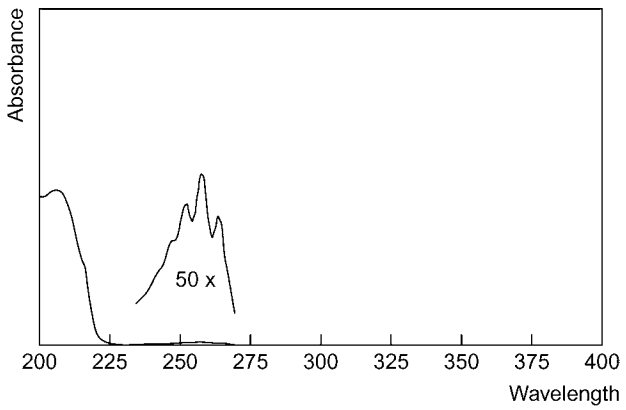
Proprietary Names *Duromine*; *Ionamin(e)*; *Mirapront* (all as resin complexes).



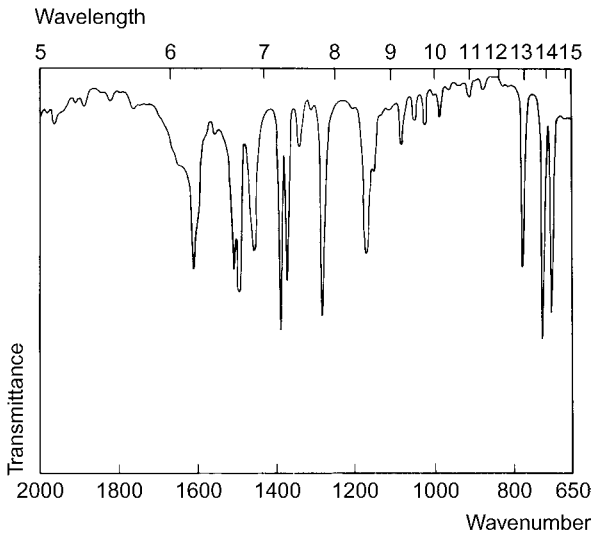
Chemical Properties A colourless oily liquid. Slightly soluble in water; soluble in ethanol, chloroform and ether. pK_a 10.1. Log *P* (octanol/water), 1.9.

Phentermine Hydrochloride
$C_{10}H_{15}N,HCl = 185.7$
CAS—1197-21-3
Proprietary Names *Adipex-P*; *Fastin*; *Panbesy*; *Pronidin*; *Umine*.
Chemical Properties A white crystalline powder. Mp 198°. Very soluble in water, ethanol and chloroform; practically insoluble in ether.

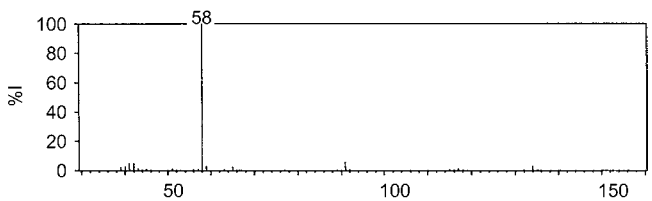
Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.26; system TC—R_f 0.24; system TE—R_f 0.48; system TL—R_f 0.12; system TAE—R_f 0.11; system TAF—R_f 0.78; system TAJ—R_f 0.02; system TAK—R_f 0.05; system TAL—R_f 0.36 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—phentermine RI 1155, phentermine-TFA RI 1100, phentermine-PFP RI 1305, phentermine-TMS RI 1195, phentermine-AC RI 1510; system GB—RI 1191; system GC—RI 1450.
High Performance Liquid Chromatography System HA—*k* 0.6; system HB—*k* 19.46; system HC—*k* 0.86; system HY—RI 245; system HZ—retention time 2.4 min.
Ultraviolet Spectrum Aqueous acid—247, 251, 257 (A_1^1=13.9b), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 730, 1285, 705, 1495, 1610, 1510 cm^{-1} (phentermine hydrochloride, KCl disk).



Mass Spectrum Principal ions at *m/z* 58, 91, 42, 41, 134, 65, 59, 40.



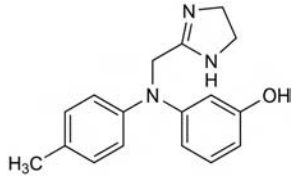
Quantification
Blood **GC** FID. Amfetamine and phentermine. Limit of detection, 10 µg/L in blood [O'Brien *et al.* 1972].

Plasma **HPLC** Fluorescence detection. Phentermine and fenfluramine [Kaddoumi *et al.* 2001]. UV detection. Phentermine and other sympathomimetic amines. Limits of detection, about 0.3 to 23 pmol [Kaddoumi *et al.* 2001].
Serum **HPLC** Phentermine and other amfetamine-related drugs. Limits of detection, 0.01 to 0.03 mg/L (UV detection, single wavelength) or 0.05 to 0.1 mg/L (diode-array detection); also MS detection [Bogusz *et al.* 1997].
HPLC-MS Phentermine and other amfetamine-related drugs. Limits of detection,1 to 5 µg/L [Bogusz *et al.* 2000].
Urine **GC** NPD. Phentermine and amfetamines. Limit of quantification for phentermine, 0.07 mg/L [Jonsson *et al.* 1996]. FID. Phentermine, chlorphentermine, and mephentermine [Beckett, Brookes 1971]. See Blood [O'Brien *et al.* 1972].
GC-MS Phentermine and other amfetamine-related drugs [Namera *et al.* 2002]. Phentermine and fenfluramine [Palmer *et al.* 2000].
HPLC See Serum [Bogusz *et al.* 1997].
Disposition in the Body Readily absorbed after oral administration. About 70 to 80% of a dose is excreted in the urine as unchanged drug in 24 h together with small amounts of the *N*-hydroxy and nitroso metabolites and traces of free and conjugated 4-hydroxyphentermine.
Phentermine is a metabolite of mephentermine.
Therapeutic Concentration
The following postmortem tissue concentrations were reported in a subject who had been receiving daily oral doses of 40 mg of a slow-release preparation and who died of natural causes: blood 0.9 mg/L, bile 6.5 mg/L, liver 4 µg/g, urine 50 mg/L [Price 1974].
Following single oral doses of 0.375 mg/kg, peak blood concentrations of about 0.1 mg/L were attained in about 4 h [Hinsvark *et al.* 1973].
Toxicity
In a fatality involving the ingestion of phentermine, the following postmortem tissue concentrations were reported: blood 7.6 mg/L, kidney 16 µg/g, liver 14 µg/g, urine 88 mg/L; amobarbital and ethchlorvynol were also detected in blood at concentrations of 10 mg/L and 12 mg/L, respectively [Levine *et al.* 1984].
Half-life Plasma half-life, about 19 to 24 h.
Volume of Distribution About 3 to 4 L/kg.
Dose 15 to 30 mg daily as the resin complex; 15 to 37.5 mg daily as the hydrochloride.

Beckett AH, Brookes LG (1971). The metabolism and urinary excretion in man of phentermine, and the influence of N-methyl and p-chloro-substitution. *J Pharm Pharmacol* 23: 288–294.
Bogusz MJ *et al.* (1997). Determination of phenylisothiocyanate derivatives of amphetamine and its analogues in biological fluids by HPLC-APCI-MS or DAD. *J Anal Toxicol* 21: 59–69.
Bogusz MJ *et al.* (2000). Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Anal Toxicol* 24: 77–84.
Hinsvark ON *et al.* (1973). *J Pharmacokinet Biopharm* 1: 319–328.
Jonsson J *et al.* (1996). A convenient derivatization method for the determination of amphetamine and related drugs in urine. *J Forensic Sci* 41: 148–151.
Kaddoumi A *et al.* (2001). High performance liquid chromatography with UV detection for the simultaneous determination of sympathomimetic amines using 4-(4,5-diphenyl-1H-imidazole-2-yl)benzoyl chloride as a label. *Biomed Chromatogr* 15: 379–388.
Kaddoumi A *et al.* (2001). Fluorometric determination of DL-fenfluramine, DL-norfenfluramine and phentermine in plasma by achiral and chiral high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 763: 79–90.
Levine B *et al.* (1984). A fatality involving phentermine. *J Forensic Sci* 29: 1242–1245.
Namera A *et al.* (2002). Automated headspace solid-phase microextraction and in-matrix derivatization for the determination of amphetamine-related drugs in human urine by gas chromatography-mass spectrometry. *J Chromatogr Sci* 40: 19–25.
O'Brien JE *et al.* (1972). Determination of amphetamine and phentermine in biological fluids. *J Chromatogr Sci* 10: 336–341.
Palmer RB *et al.* (2000). Simultaneous determination of fenfluramine and phentermine in urine using gas chromatography mass spectrometry with pentafluoropropionic anhydride derivatization. *Ther Drug Monit* 22: 418–422.
Price K (1974). *Bull Int Assoc Forensic Toxicol* 10(1): 12–13.

Phentolamine

Antihypertensive
$C_{17}H_{19}N_3O = 281.4$
CAS—50-60-2
IUPAC Name 3-[*N*-(4,5-dihydro-1*H*-imidazol-2-ylmethyl)-4-methylanilino] phenol
Synonyms C-7337; 3-[[(4,5-dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)amino]-phenol



Chemical Properties A white crystalline powder. Mp 174° to 175°. pK_a 7.7. Log *P* (octanol/water), 3.4.

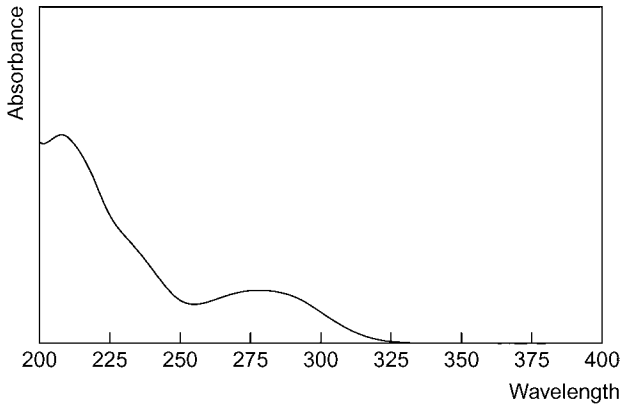
Phentolamine Hydrochloride

$C_{17}H_{19}N_3O,HCl = 317.8$
CAS—73-05-2
Chemical Properties A white or faintly cream-coloured crystalline powder. Mp 239° to 240°. Soluble 1 in 50 of water and 1 in about 70 of ethanol; very slightly soluble in chloroform and ether; practically insoluble in acetone. Solutions in water foam on shaking.

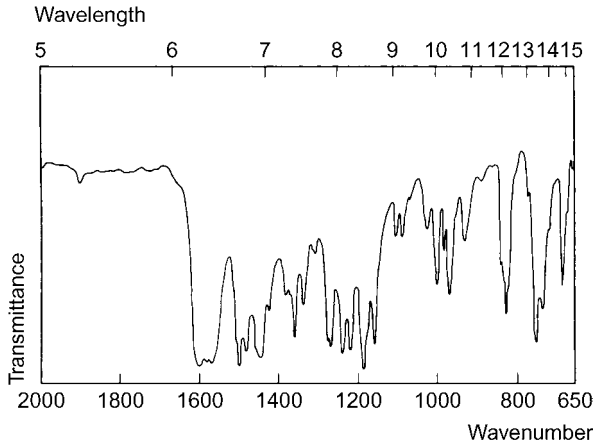
Phentolamine Mesilate

$C_{17}H_{19}N_3O,CH_3SO_3H = 377.5$
CAS—65-28-1
Synonyms Phentolamine mesylate; phentolamine methanesulfonate.
Proprietary Names *Q-Tech*; *Regitine*; *Rogitine*; *Z-Max*.
Chemical Properties A white, slightly hygroscopic, crystalline powder. Mp 177° to 181°. Soluble 1 in 1 of water, 1 in 4 of ethanol and 1 in 700 of chloroform.

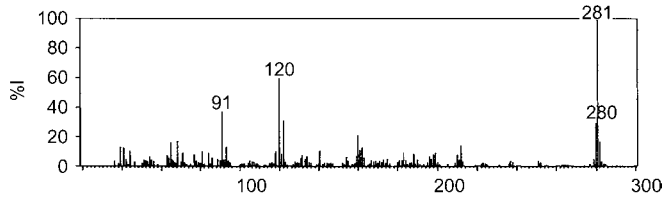
Colour Tests Mandelin's test—blue-green; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.32; system TB—R_f 0.01; system TC—R_f 0.03; system TE—R_f 0.33; system TL—R_f 0.02; system TAE—R_f 0.06; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.34 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 368; system HZ—retention time 3.0 min.
Ultraviolet Spectrum Aqueous acid—278 nm; aqueous alkali—291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1195, 1508, 1610, 1580, 1592, 1247 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 281, 120, 91, 122, 280, 160, 68, 282.



Quantification

Plasma **GC** ECD. Phentolamine and conjugates. Limit of detection, 5 µg/L (phentolamine in plasma) [Sioufi *et al.* 1981].

HPLC UV detection. Phentolamine metabolite. Limit of detection, 200 µg/L [Godbillon, Carnis 1981].

Urine **GC** Limit of detection, 25 µg/L (phentolamine and conjugates in urine), see Plasma [Sioufi *et al.* 1981].

HPLC See Plasma [Godbillon, Carnis 1981].

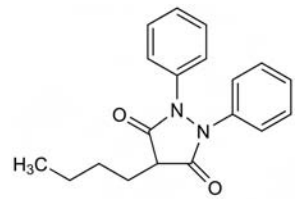
Dose 5 to 10 mg of phentolamine mesilate IV. Phentolamine hydrochloride has been given orally in doses of 200 to 300 mg daily.

Godbillon J, Carnis G (1981). Determination of the major metabolite of phentolamine in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 222: 461–466.
Sioufi A *et al.* (1981). Gas chromatographic determination of phentolamine (Regitine) in human plasma and urine. *J Chromatogr* 222: 429–435.

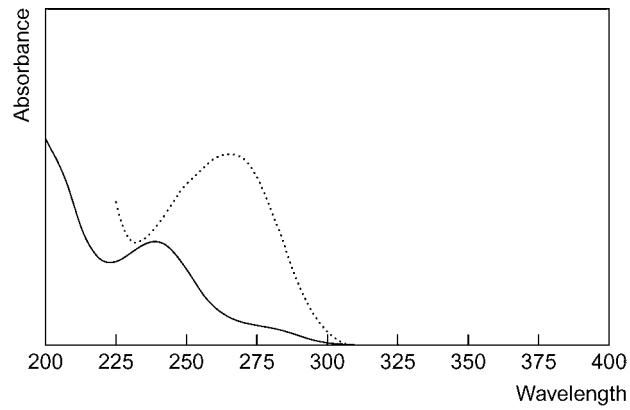
Phenylbutazone

Analgesic
$C_{19}H_{20}N_2O_2 = 308.4$
CAS—50-33-9

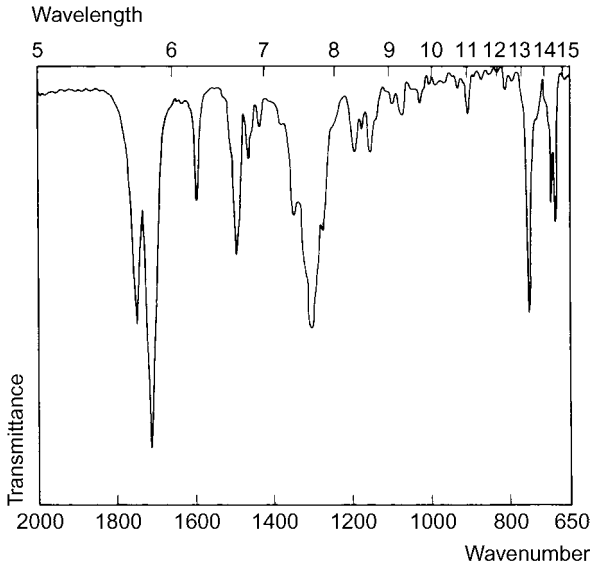
IUPAC Name 4-Butyl-1,2-diphenylpyrazolidine-3,5-dione
Synonyms Butadione; fenilbutazona.
Proprietary Names *Aflamina*; *Ambene*; *Basireuma*; *Bresal*; *Buta*; *Butacote*; *Butadion*; *Butazolidina*; *Butazolidin(e)*; *Butazolon*; *Butazona*; *Butazone*; *Butazonil*; *Carudol*; *Delbulasa*; *Demoplas*; *Exrheudon OPT*; *Feniben*; *Fezona*; *Inflazone*; *Kadol*; *Peralgin*; *Rudesol*; *Tisatin*.



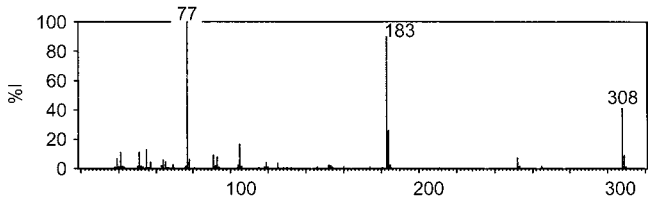
Chemical Properties A fine, white, crystalline powder. Mp 105°. Practically insoluble in water; soluble 1 in 28 of ethanol, 1 in 1.25 of chloroform and 1 in 15 of ether; freely soluble in acetone; soluble in alkaline solutions. pK_a 4.4 (20°). Log *P* (octanol/pH 7.4), 0.7; (octanol/water), 3.2.
Colour Tests Liebermann's reagent (100°)—brown; Mandelin's test—violet.
Thin-layer Chromatography System TA—R_f 0.79; system TD—R_f 0.78; system TE—R_f 0.65; system TF—R_f 0.68; system TG—R_f 0.23; system TAD—R_f 0.76; system TAE—R_f 0.87; system TAJ—R_f 0.90; system TAK—R_f 0.76; system TAL—R_f 0.97 (chromic acid solution, brown; Ludy Tenger reagent, orange; mercurous nitrate spray, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—phenylbutazone RI 2367, phenylbutazone-Me RI 2290, oxyphenbutazone not eluted; system GB—RI 2472; system GD—phenylbutazone RRT 2.05, oxyphenbutazone RRT 2.11, both as methyl derivatives relative to *n*-$C_{16}H_{34}$; system GF—RI 2860; system GL—phenylbutazone-Me RI 2290, M (OH-alkyl)-Me RI 2500.
High Performance Liquid Chromatography System HD—phenylbutazone *k* 6.5, oxyphenbutazone *k* 1.95; system HV—phenylbutazone RRT 0.95, oxyphenbutazone RRT 0.7, both relative to meclofenamic acid; system HX—phenylbutazone RI 672, oxyphenbutazone RI 501; system HY—phenylbutazone RI 643, oxyphenbutazone RI 459; system HZ—phenylbutazone RT 19.5 min, oxyphenbutazone RT 6.7 min; system HAA—RT 24.1 min.
Ultraviolet Spectrum Aqueous acid—237 nm (A^1_1 = 456a); aqueous alkali—264 nm (A^1_1 = 660a).



Infrared Spectrum Principal peaks at wavenumbers 1714, 1300, 1755, 755, 1492, 1275 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 77, 183, 308, 184, 105, 55, 51, 41; oxyphenbutazone 199, 324, 93, 77, 65, 55, 121, 135.



Quantification

Blood **GC** Limit of detection, 1 mg/L [Budd 1982].

Plasma **GC** See Blood [Budd 1982]. ECD. Sensitivity, 10 µg/L [Sioufi *et al.* 1978].

HPLC UV detection. Phenylbutazone, oxyphenbutazone and other NSAIDs. Limit of detection, 0.05 mg/L [Caturla, Cusido 1992]. UV detection. Phenylbutazone and other NSAIDs [Owen *et al.* 1987]. UV detection. Phenylbutazone, oxyphenbutazone and 3′-hydroxyphenylbutazone. Limit of detection, 5.5 mg/L for phenylbutazone [Aarons, Higham 1980]. UV detection. Phenylbutazone, oxyphenbutazone and 3′-hydroxyphenylbutazone. Limit of detection, 50 µg/L [Marunaka *et al.* 1980].

Serum **HPLC** UV detection. Limit of detection, 0.1 mg/L and 1 mg/L for phenylbutazone and oxyphenbutazone, respectively [Simmons *et al.* 1995].

Urine **GC** See Blood [Budd 1982].

HPLC See Plasma [Marunaka *et al.* 1980].

Bile **GC** See Blood [Budd 1982].

Disposition in the Body Readily absorbed after oral or rectal administration and slowly absorbed after IM injection. The major metabolic reactions are *C*-glucuronidation at the 4-position of the pyrazolidine ring and 4-hydroxylation of one of the phenyl rings to form the active metabolite, oxyphenbutazone; 3-hydroxylation of the butyl side-chain to form 3′-hydroxyphenylbutazone, and formation of 4,3′-dihydroxyphenylbutazone also occur. About 61% of a dose is slowly excreted in the urine over a period of about 21 days, together with up to 27% in the faeces. Of the material excreted in the urine, the 4-*C*-glucuronide of phenylbutazone accounts for ≈40%, free phenylbutazone and free oxyphenbutazone ≈1%, 4,3′-dihydroxyphenylbutazone ≈6%, 3′-hydroxyphenylbutazone ≈3%, and the remainder consists of the *C*-glucuronide of 3′-hydroxyphenylbutazone (≈12%), oxyphenbutazone *O*-glucuronide and 2 other metabolites.

Therapeutic Concentration In plasma, usually in the range 50–100 mg/L.

Following single oral doses of 100, 300 and 600 mg to 6 subjects, peak plasma concentrations of 12.5–17.1 mg/L (mean, 14), 30.8–46.3 mg/L (mean, 38), and 51.2–88.1 mg/L (mean, 75), respectively, were obtained in 1–7 h (mean, 3) [Sioufi *et al.* 1980].

After daily oral doses of 100, 200, and 300 mg to 7 subjects, mean steady-state plasma concentrations of 52, 83, and 95 mg/L, respectively, were reported; in 3 subjects given 400 mg daily, the mean steady-state concentration was 99 mg/L [Orme *et al.* 1976].

Following daily oral doses of 200 mg to 1 subject for 7 days, the following plasma concentrations were found: phenylbutazone about 50 mg/L, oxyphenbutazone about 20 mg/L, and 3′-hydroxyphenylbutazone about 12 mg/L [Midha *et al.* 1974].

Toxicity The estimated minimum lethal dose is 5 g. Toxic effects during treatment are frequent and may occur even when the dose does not exceed 400 mg daily; they are usually associated with plasma concentrations above 100 mg/L. A considerable number of deaths have occurred, especially from blood disorders.

In a fatality due to an accidental overdose by a 5-year-old child, the following postmortem concentrations were reported: blood 400 mg/L, bile 475 mg/L, kidney 250 µg/g, liver 250 µg/g [Lam, Chien 1976].

A plasma concentration of 670 mg/L was reported in 1 subject, one day after ingestion of 8 g. The concentration of phenylbutazone declined rapidly with a half-life of 23 h but concentrations of 3′-hydroxyphenylbutazone were unusually high and exceeded those of unchanged drug after 48 h; the subject recovered after about 3 days [Prescott *et al.* 1980].

A racetrack worker ingested at least 17 g of phenylbutazone (intended for equine use) over a 24-h period in an attempt to treat toothache. He had a serum-phenylbutazone concentration of 900 mg/L and a serum-oxyphenbutazone concentration of 60 mg/L approximately 8 h after presentation [Newton, Rose 1991].

Half-life Plasma half-life, phenylbutazone 2–5 days (mean, 3), but dose-dependent and increased in subjects with renal failure and in the elderly; oxyphenbutazone about 2–3 days, 3′-hydroxyphenylbutazone ≈32 h.

Distribution in Blood Plasma : whole blood ratio, 1.8.

Protein Binding About 99% at therapeutic concentrations; decreased at higher concentrations when the binding sites become saturated.

Note For a review of the pharmacokinetics of phenylbutazone, see Aarbakke [1978].

Dose Initially 300 to 600 mg daily; maintenance, 100 to 400 mg daily.

Aarbakke J (1978). Clinical pharmacokinetics of phenylbutazone. *Clin Pharmacokinet* 3: 369–380.
Aarons L, Higham C (1980). An improved HPLC assay for monitoring phenylbutazone and its two major oxidised metabolites in plasma. *Clin Chim Acta* 105: 377–382.
Budd RD (1982). Gas chromatographic determination of butazolidin (phenylbutazone) in biological fluids. *J Chromatogr* 243: 368–371.
Caturla MC, Cusido E (1992). Solid-phase extraction for the high-performance liquid chromatographic determination of indomethacin, suxibuzone, phenylbutazone and oxyphenbutazone in plasma, avoiding degradation of compounds. *J Chromatogr* 581: 101–107.
Lam KL, Chien K (1976). *Bull Int Assoc Forensic Toxicol* 12(2): 20.
Marunaka T *et al.* (1980). Simultaneous determination of phenylbutazone and its metabolites in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 183: 331–338.
Midha KK *et al.* (1974). GLC determination of gamma-hydroxyphenylbutazone in plasma. *J Pharm Sci* 63: 1751–1754.
Newton TA, Rose SR (1991). Poisoning with equine phenylbutazone in a racetrack worker. *Ann Emerg Med* 20: 204–207.
Orme M *et al.* (1976). Plasma concentration of phenylbutazone and its therapeutic effect-studies in patients with rheumatoid arthritis. *Br J Clin Pharmacol* 3: 185–191.
Owen SG *et al.* (1987). Rapid high-performance liquid chromatographic assay for the simultaneous analysis of non-steroidal anti-inflammatory drugs in plasma. *J Chromatogr* 416: 293–302.
Prescott LF *et al.* (1980). Phenylbutazone overdosage: abnormal metabolism associated with hepatic and renal damage. *BMJ* 281: 1106–1107.
Simmons BR *et al.* (1995). A supercritical fluid chromatographic method using packed columns for phenylbutazone and oxyphenbutazone in serum, and for phenylbutazone in a dosage form. *J Pharm Biomed Anal* 13: 59–64.
Sioufi A *et al.* (1978). GLC determination of phenylbutazone in human plasma. *J Pharm Sci* 67: 243–245.
Sioufi A *et al.* (1980). Pharmacokinetics of phenylbutazone in healthy subjects after oral administration of single and multiple doses. *J Pharm Sci* 69: 1413–1416.

Phenylephrine

Sympathomimetic

$C_9H_{13}NO_2 = 167.2$

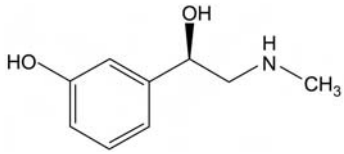
CAS—59-42-7

IUPAC Name 3-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]phenol

Synonyms (α*R*)-3-Hydroxy-α-[(methylamino)methyl]benzenemethanol; neosynephrine; *m*-synephrine.

Note Synephrine has been used as a synonym for oxedrine.

Proprietary Name It is an ingredient of *Vibrocil*.



Chemical Properties A white or almost white crystalline powder. Mp about 174°. Slightly soluble in water and ethanol; sparingly soluble in methanol; soluble in dilute mineral acids and solutions of alkali hydroxides. pK_a 8.9 (–OH), 10.1 (–NH–), (20°). Log *P* (octanol/water), −0.3.

Phenylephrine Hydrochloride

$C_9H_{13}NO_2.HCl = 203.7$

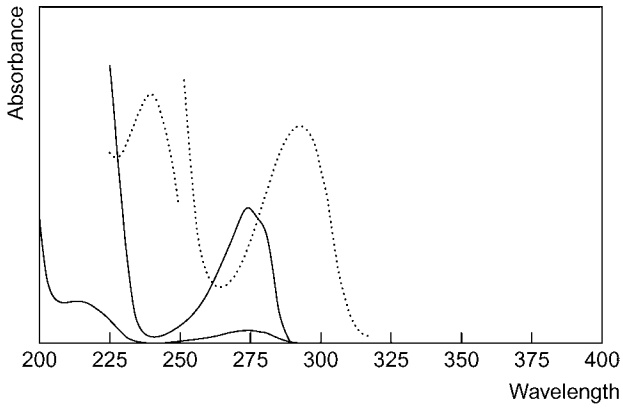
CAS—61-76-7

Synonyms Mesatonum; metaoxedrini chloridum.

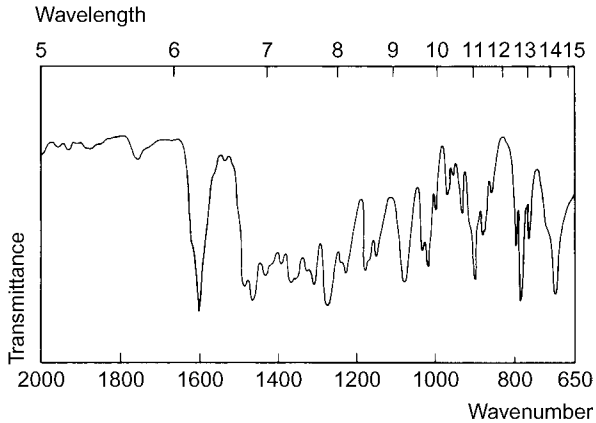
Proprietary Names *Ak-Dilate*; *Ak-Nefrin*; *Albalon*; *Analux*; *Dionephrine*; *Fenox*; *Isonefrine*; *Isopto Frin*; *Mydfrin*; *Neofrin*; *Neo-Mydrial*; *Neo-Synephrine*; *Ocu-Phrin*; *Phenoptic*; *Prefrin*; *Rectacaine*; *Releif*; *Rhinall*; *Ricobid D*; *Sinex*; *Visadron*; *Vistafrin*. It is an ingredient of many preparations for the relief of nasal congestion – see Sweetman [2002] or the latest edition of the British National Formulary.

Chemical Properties A white crystalline powder. Mp 140° to 145°. A solution in water is laevorotatory. Soluble 1 in 2 of water and 1 in 4 of ethanol.

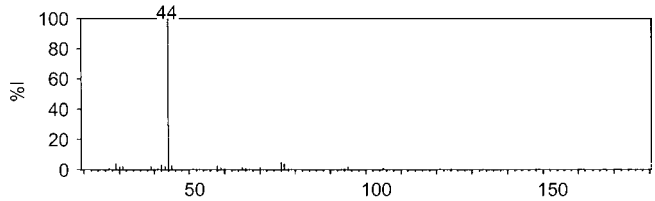
Colour Tests Mandelin's test—brown; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.12; system TL—R_f 0.00; system TAE—R_f 0.08; system TAF—R_f 0.67; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.22 (acidified potassium permanganate solution, positive).
Gas Chromatography System GC—RI 1934.
High Performance Liquid Chromatography System HA—*k* 1.3; system HC—*k* 1.6; system HX—RI 80; system HY—RI 79.
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1=110a); aqueous alkali—238 (A_1^1=534a), 291 nm (A_1^1=182a).



Infrared Spectrum Principal peaks at wavenumbers 1594, 1273, 784, 696, 1304, 900 cm^{-1} (phenylephrine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 76, 77, 29, 45, 42, 95, 65.



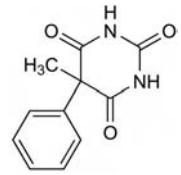
Quantification
Plasma **GC** ECD. Limit of detection, 12.5 µg/L [Dombrowski *et al.* 1973].
HPLC Electrochemical detection. Phenylephrine and its conjugates. Limits of detection, 2 µg/L for phenylephrine and 25 µg/L for its conjugates [Gumbhir, Mason 1996]. Fluorescence detection. Limit of detection, 0.5 µg/L [Chien, Schoenwald 1985].
Serum **HPLC** Coulometric detection. Limit of detection, about 0.3 µg/L [Vuma, Kanfer 1996]. Fluorescence detection. Limit of detection, 5 µg/L [Yamaguchi *et al.* 1994].
Urine **GC-MS** Phenylephrine and four metabolites [Ibrahim *et al.* 1983].
Aqueous humour **HPLC** UV detection. Phenylephrine and tropicamide [Galmier *et al.* 2000].
Disposition in the Body Readily absorbed after oral administration, but undergoes extensive first-pass metabolism; bioavailability about 38%. It is metabolised by conjugation with sulfate and glucuronic acid and by oxidative deamination to *m*-hydroxymandelic acid (MHMA) and *m*-hydroxyphenylglycol (MHPG). About 80% of an oral dose is excreted in the urine in 24 h, mainly as sulfate conjugates of phenylephrine and MHPG; about 30% of a dose is excreted as unconjugated MHMA.
Therapeutic Concentration
After a single oral dose of 9 mg of ^{3}H-labelled phenylephrine hydrochloride to 6 subjects, peak plasma concentrations of 0.23 to 0.47 mg/L (mean, 0.31) of total phenylephrine and metabolites were attained in 1 to 2 h [Cavallito *et al.* 1963].

Following a single oral dose of 1 mg to 3 subjects, a mean peak plasma concentration of unconjugated phenylephrine of 0.0009 mg/L was attained in about 1.3 h [Hengstmann, Goronzy 1982].
Toxicity The estimated minimum lethal dose, for children up to 2 years is 100 mg intranasally and for adults is 1 g.
Half-life Plasma half-life, about 2 to 3 h.
Volume of Distribution About 5 L/kg.
Clearance Plasma clearance, about 30 mL/min/kg.
Dose 30 to 40 mg of phenylephrine hydrochloride daily, orally.

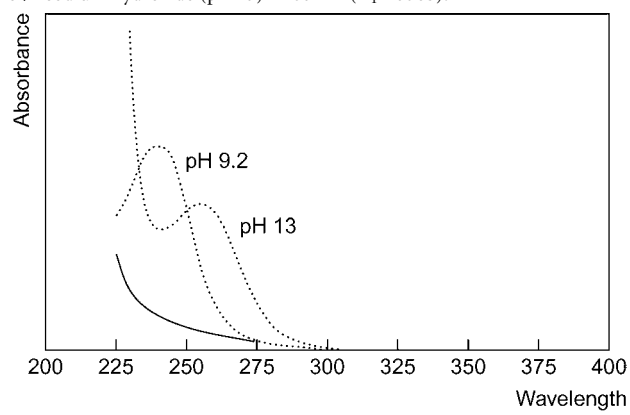
London: BMA and RPSGB. *British National Formulary.*
Cavallito CJ *et al.* (1963). Some studies of a sustained release principle. *J Pharm Sci* 52: 259–263.
Chien DS, Schoenwald RD (1985). Fluorometric determination of phenylephrine hydrochloride by liquid chromatography in human plasma. *J Pharm Sci* 74: 562–564.
Dombrowski LJ *et al.* (1973). GLC determination of phenylephrine hydrochloride in human plasma. *J Pharm Sci* 62: 1761–1763.
Galmier MJ *et al.* (2000). High-performance liquid chromatographic determination of phenylephrine and tropicamide in human aqueous humor. *Biomed Chromatogr* 14: 202–204.
Gumbhir K, Mason WD (1996). High-performance liquid chromatographic determination of phenylephrine and its conjugates in human plasma using solid-phase extraction and electrochemical detection. *J Pharm Biomed Anal* 14: 623–630.
Hengstmann JH, Goronzy J (1982). Pharmacokinetics of 3H-phenylephrine in man. *Eur J Clin Pharmacol* 21: 335–341.
Ibrahim KE *et al.* (1983). The mammalian metabolism of R-(-)-m-synephrine. *J Pharm Pharmacol* 35: 144–147.
Sweetman SC (2002). *Martindale: The Complete Drug Reference*, 33 edn. London: Pharmaceutical Press.
Vuma V, Kanfer I (1996). High-performance liquid chromatographic determination of phenylephrine in human serum with coulometric detection. *J Chromatogr B Biomed Appl* 678: 245–252.
Yamaguchi M *et al.* (1994). High-performance liquid chromatographic determination of phenylephrine in human serum using column switching with fluorescence detection. *J Chromatogr B Biomed Appl* 661: 93–99.

Phenylmethylbarbituric Acid

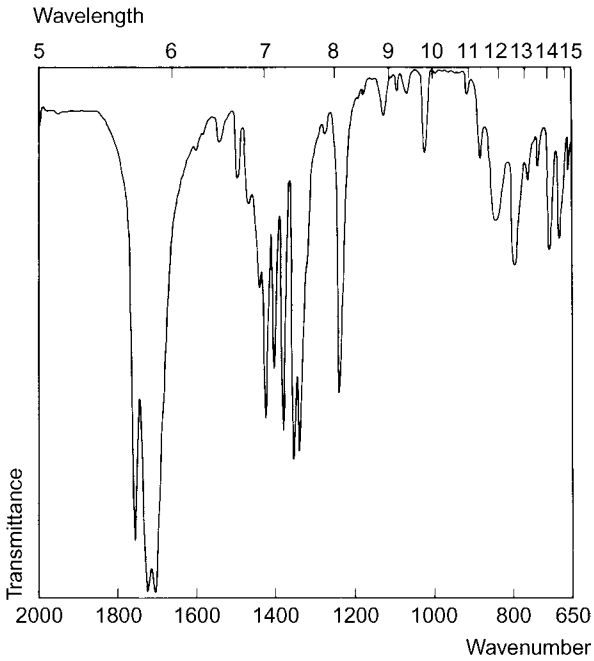
Anticonvulsant, Barbiturate
$C_{11}H_{10}N_2O_3$ = 218.2
CAS—76-94-8
IUPAC Name 5-Methyl-5-phenyl-1,3-diazinane-2,4,6-trione
Synonyms Heptobarbitalum (distinguish from heptabarbital); 5-methyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione.
Proprietary Name *Rutonal*



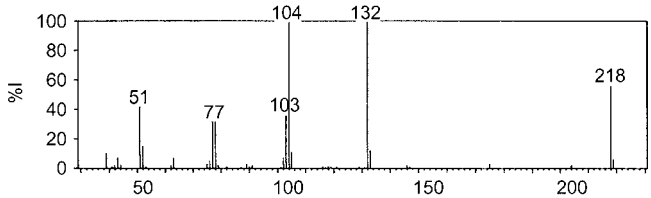
Chemical Properties A white crystalline powder. Mp about 226°. Practically insoluble in water; soluble 1 in 60 of ethanol; soluble in ether and aqueous solutions of alkalis. pK_a 7.7 (25°). Log *P* (octanol/water), 0.9.
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TD—R_f 0.29; system TE—R_f 0.24; system TF—R_f 0.61; system TH—R_f 0.27 (mercurous nitrate spray, black; Zwikker's reagent, pink).
Gas Chromatography System GA—phenylmethylbarbituric acid RI 1880, phenylmethylbarbituric acid-Me_2 RI 1790; system GAJ—RRT 1.087 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HG—*k* 1.50; system HH—*k* 0.90.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—238 nm (A_1^1= 494b); 1 mol/L sodium hydroxide (pH 13)—253 nm (A_1^1=350b).



Infrared Spectrum Principal peaks at wavenumbers 1702, 1720, 1751, 1240, 802, 716 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 104, 132, 218, 51, 103, 77, 78, 52.

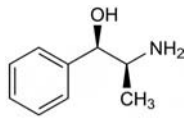


Dose Phenylmethylbarbituric acid has been given in doses of 400 mg daily.

Phenylpropanolamine

Sympathomimetic
$C_9H_{13}NO$ = 151.2
CAS—14838-15-4

IUPAC Name (1*S*,2*R*)-2-Amino-1-phenylpropan-1-ol
Synonyms (α*S*)-*rel*-α-[(1*R*)-1-Aminoethyl]benzenemethanol; DL-norephedrine



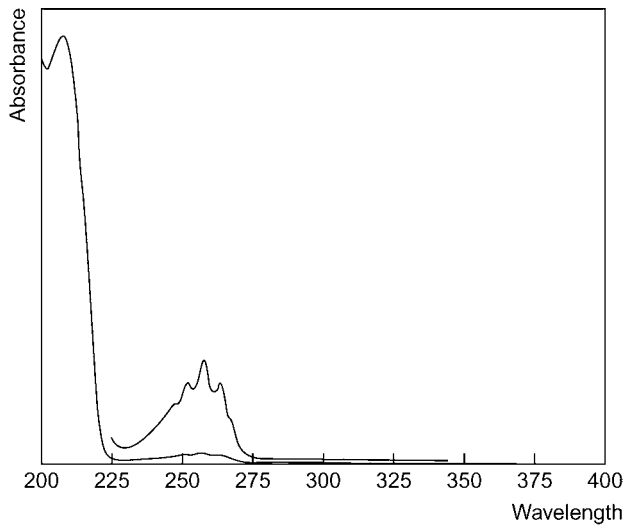
(+)-Form

Chemical Properties Mp about 101°. pK_a 9.4 (20°). Log *P* (octanol/water), 0.7.

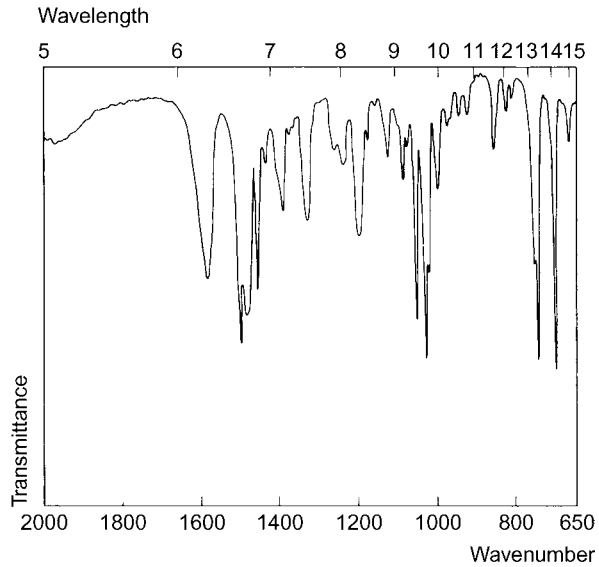
Phenylpropanolamine Hydrochloride

$C_9H_{13}NO,HCl$ = 187.7
CAS—154-41-6
Synonym Mydriatin
Proprietary Names *Acutrim*; *Appedrine*; *Capton Diet*; *Centapp*; *Control*; *Dexatrim*; *Fansia*; *Fasupond*; *Just One Per Day*; *Kontexin*; *Merex*; *Monydrin*; *Phenoxine*; *Phenyldrine*; *Procol*; *Propagest*; *Recatol mono*; *Restaslim*; *Rinexin*; *Slimomin*; *Spray-U-Thin*; *Stay Trim*; *Unitrol*. It is an ingredient of many preparations for the relief of cold and cough symptoms – see Sweetman [2002] or the latest edition of the British National Formulary.
Chemical Properties A white to creamy-white crystalline powder. Mp 190° to 194°. Soluble 1 in 1 of water and 1 in about 7 of ethanol; practically insoluble in chloroform and ether.

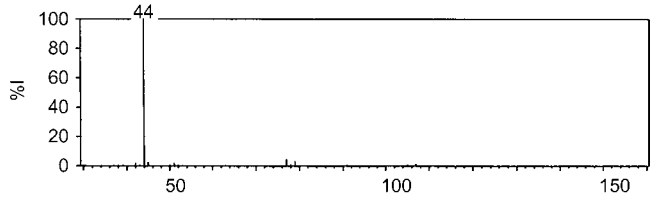
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.04; system TC—R_f 0.04; system TAJ—R_f 0.01; system TAK—R_f 0.02; system TAL—R_f 0.29 (Dragendorff spray, positive; FPN reagent, violet; acidified iodoplatinate solution, positive; Marquis reagent, yellow; ninhydrin spray, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1313; system GB—RI 1353; system GC—RI 1383.
High Performance Liquid Chromatography System HA—*k* 0.9; system HB—*k* 3.90; system HC—*k* 0.7; system HY—RI 201.
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=11.7a), 262 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 700, 746, 1030, 1500, 1055, 1590 cm^{-1} (phenylpropanolamine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 77, 79, 51, 45, 42, 107, 105.



Quantification
Plasma **GC** ECD. Limit of detection, 1 µg/L [Crisologo *et al.* 1984].
HPLC UV detection. Phenylpropanolamine enantiomers. Limit of detection, <10 µg/L [Stockley *et al.* 1991]. UV detection. Limit of detection, 0.4 µg/L [Yamashita *et al.* 1990]. Fluorescence detection. Limit of detection, 5 µg/L [Mason, Amick 1981].
Urine **HPLC** Limit of detection, 8 µg/L, see Plasma [Yamashita *et al.* 1990].
Disposition in the Body Absorbed after oral administration. More than 90% of a dose is excreted in the urine as unchanged drug in 24 h, together with small

amounts of hippuric acid. Phenylpropanolamine is a metabolite of amfetamine, diethylpropion, and ephedrine.

Therapeutic Concentration

Following a single oral dose of 25 mg to 12 subjects, a mean peak plasma concentration of about 0.08 mg/L was attained in about 2 h [Mason, Amick 1981].

Toxicity

In a fatality due to a phenylpropanolamine overdose, the following postmortem tissue concentrations were reported: blood 48 mg/L, brain 86 µg/g, liver 460 µg/g [Cravey, Baselt 2002].

A 9-month-old infant died due to non-intentional overdosage with over-the-counter cough and cold preparations. Postmortem analysis revealed the following phenylpropanolamine levels: heart blood 1.4 mg/L, liver 0.5 mg/kg; elevated blood levels of pseudoephedrine and dextromethorphan were also found [Gunn *et al.* 2001].

Half-life Plasma half-life, about 4 h.

Dose 75 to 200 mg of phenylpropanolamine hydrochloride daily.

BMA and RPSGB, London. *British National Formulary.*
Cravey RH, per Baselt RC (2002). *Disposition of Toxic Chemicals and Drugs in Man*, 5th edn. Davis, California: Biomedical Publications, 700–702.
Crisologo N *et al.* (1984). Electron-capture capillary gas chromatographic determination of phenylpropanolamine in human plasma following derivatization with trifluoroacetic anhydride. *J Pharm Sci* 73: 1313–1315.
Gunn VL *et al.* (2001). Toxicity of over-the-counter cough and cold medications. *Pediatrics* 108: e52.
Mason WD, Amick EN (1981). High-pressure liquid chromatographic analysis of phenylpropanolamine in human plasma following derivatization with O-phthalaldehyde. *J Pharm Sci* 70: 707–709.
Stockley CS *et al.* (1991). Stereospecific high-performance liquid chromatographic assay for the enantiomers of phenylpropanolamine in human plasma. *Ther Drug Monit* 13: 332–338.
Sweetman SC (2002). *Martindale: The complete drug reference*, 33 edn. London: Pharmaceutical Press.
Yamashita K *et al.* (1990). High-performance liquid chromatographic determination of phenylpropanolamine in human plasma and urine, using column switching combined with ion-pair chromatography. *J Chromatogr* 527: 103–114.

Phenylpropylmethylamine

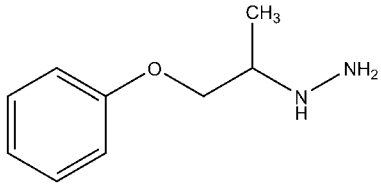
α-Adrenoceptor Agonist, Decongestant

$C_{10}H_{15}N$ = 149.2

CAS—93-88-9

IUPAC Name 2-Methyl-2-phenylpropan-1-amine

Synonyms *N*,β-Dimethylbenzeneethanamine; *dl-N*,β-dimethylphenyethylamine; 1-methylamino-2-methyl-2-phenylethane; 1-methylamino-2-phenylpropane; *dl-N*-methyl-2-phenylpropylamine; phenpromethamine; vonedrine.



Chemical Properties Volatile liquid. Bp 205° to 210°. Refractive index 1.51. Slightly soluble in water (1.2 g/100 mL). Freely soluble in alcohol, ether and benzene. Log *P* (octanol/water) 1.8 [National Institutes of Health 2008]. Phenylpropylmethylamine is extracted by organic solvents from aqueous alkaline solutions.

Phenylpropylmethylamine Hydrochloride

$C_{10}H_{15}N,HCl$ = 185.7

Proprietary Name *Vonedrine hydrochloride*

Chemical Properties Mp 144° to 148°.

Colour Test Sulfuric acid-formaldehyde test—orange→brown (limit of detection, 0.25 µg).

Thin-layer Chromatography System T1—R_f 0.30 (location reagent Marquis reagent, positive reaction; ninhydrin spray, positive reaction; potassium permanganate spray, positive reaction).

Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—252, 257, 263 nm

National Institutes of Health. Phenylpropylmethylamine http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=210602&loc=ec_rcsv. (accessed 27 June 2008).

Phenyltoloxamine

Antihistamine

$C_{17}H_{21}NO$ = 255.4

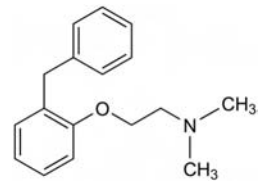
CAS—92-12-6

IUPAC Name 2-(2-Benzylphenoxy)-*N*,*N*-dimethylethanamine

Synonyms C-5581H; *N*,*N*-dimethyl-2-[2-(phenylmethyl)phenoxy]ethanamine; phenyltolyloxamine; PRN.

Note Phenyltoloxamine is a structural isomer of diphenhydramine.

Proprietary Name It is an ingredient of *Pholtex.*



Chemical Properties An oily liquid. pK_a 9.1. Log *P* (octanol/water), 3.8.

Phenyltoloxamine Citrate

$C_{17}H_{21}NO,C_6H_8O_7$ = 447.5

CAS—1176-08-5

Proprietary Names It is an ingredient of *Aceta-Gesic*, *Anabar*, *Codipront*, *Comhist LA*, *Mobigesic*, *Momentum*, *Naldecon*, *Nalex-A*, *Percogesic*, *Phenylgesic*, *Poly-Histine*, *Sinutab Nightime* and *Tetra-Mag*.

Chemical Properties Crystals. Mp 138° to 140°. Soluble in water.

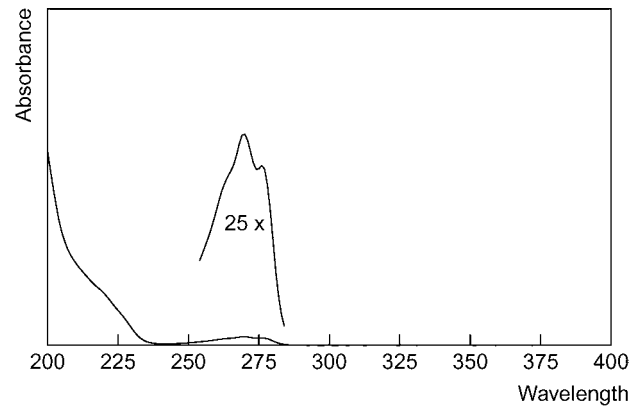
Colour Tests Mandelin's test—green; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.38; system TC—R_f 0.48; system TE—R_f 0.67; system TL—R_f 0.15; system TAE—R_f 0.32; system TAJ—R_f 0.27; system TAK—R_f 0.15; system TAL—R_f 0.69 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—phenyltoloxamine RI 1940, M ((*N*-oxide)-ME_2 NOH) RI 1500, M (nor-) RI 2140, M (*O*-desalkyl-) RI 1680, M (*O*-desalkyl-OH-) RI 2220, M (OH-) isomer 1 RI 2280, M (OH-) isomer 2 RI 2300, M (nor-OH-) isomer 1 RI 2320, M (nor-OH-) isomer 2 RI 2340, M (OH-methoxy-) RI 2320, M (desamino-OH-) RI 1830; system GB—phenyltoloxamine RI 2030, M ((*N*-oxide)-ME_2 NOH) RI 1580, M (nor-) RI 2002, M (*O*-desalkyl-) RI 1724, M (nor-OH-) isomer 1 RI 2398, M (nor-OH-) isomer 2 RI 2402, M (desamino-OH-) RI 1928.

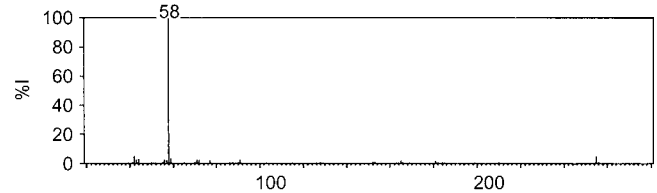
High Performance Liquid Chromatography System HA—*k* 3.1; system HX—RI 415.

Ultraviolet Spectrum Aqueous acid—270 (A^1_1=73b), 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1245, 1491, 752, 1030, 697, 1111 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 58, 255, 42, 71, 59, 44, 181, 165.



Dose 75 to 200 mg of phenyltoloxamine citrate daily.

Phenytoin

Anticonvulsant

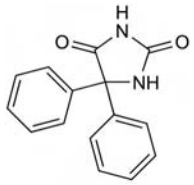
$C_{15}H_{12}N_2O_2$ = 252.3

CAS—57-41-0

IUPAC Name 5,5-Diphenylimidazolidine-2,4-dione

Synonyms Diphenylhydantoin; fenitoína; phenantoinum; phenytoinum.

Proprietary Names *Aurantin*; *Dantoin*; *Di-Hydan*; *Dilantin*; *Diphantoine*; *Diphenylan*; *Epanutin*; *Epelin*; *Epilan-D*; *Epilantine*; *Epinat*; *Hidantal*; *Hydantin*; *Neosidantoina*; *Phenhydan*; *Pyorédol*; *Sinergina*; *Zentropil*.



Chemical Properties A white crystalline powder. Mp 295° to 298°. Practically insoluble in water; soluble 1 in ~60 of ethanol, 1 in 500 of chloroform, 1 in ~30 of acetone and 1 in 600 of ether; soluble in solutions of alkali hydroxides. pK_a 8.3 (25°). Log *P* (octanol/water), 2.5. Extraction yield (chlorobutane), 0.5 [Demme *et al.* 2005].

Phenytoin Sodium

$C_{15}H_{11}N_2NaO_2 = 274.3$
CAS—630-93-3

Synonyms Diphenin; phenytoinum natricum; sodium diphenylhydantoin; soluble phenytoin.

Proprietary Name It is an ingredient of *Garoin*.

Chemical Properties A white, slightly hygroscopic, crystalline powder that on exposure to air absorbs carbon dioxide with the liberation of phenytoin. Soluble 1 in ~66 of water and 1 in 10.5 of alcohol; practically insoluble in chloroform and ether. In aqueous solution it is partly hydrolysed to the base and turbidity develops.

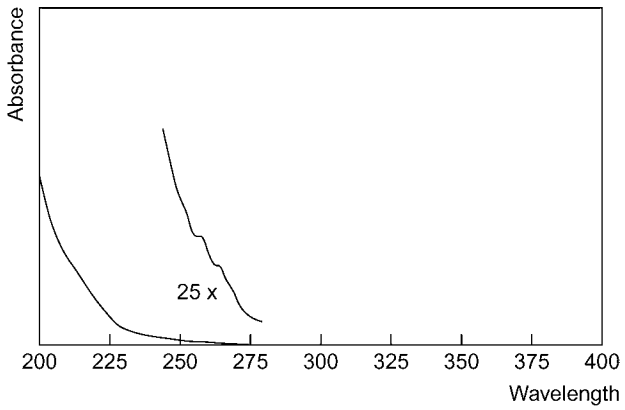
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-orange; mercurous nitrate—black.

Thin-layer Chromatography System TD—R_f 0.33; system TE—R_f 0.41; system TF—R_f 0.55; system TAD—R_f 0.53; system TAE—R_f 0.86; system TAJ—R_f 0.48; system TAK—R_f 0.84; system TAL—R_f 0.96 (mercuric chloride-diphenylcarbazone reagent—positive; mercurous nitrate spray—black).

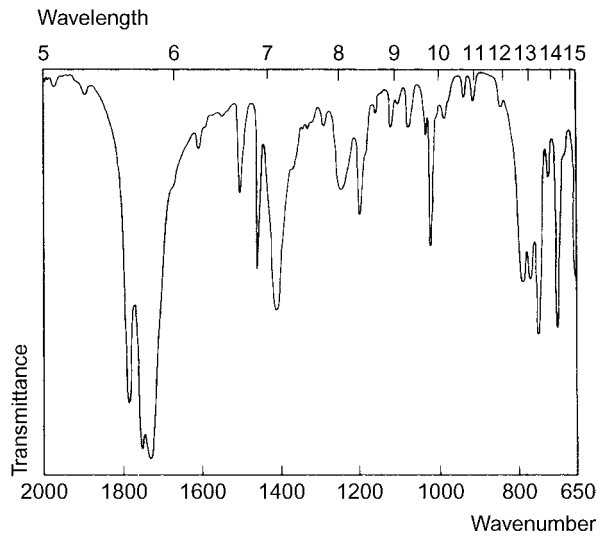
Gas Chromatography System GA—phenytoin RI 2320, M (OH-)RI 2795, phenytoin-Me RI 2245, M (4-OH-)-Me_2 RI 2720, M (4-OH-methoxy-) RI 2770, M (4-OH-methoxy-)-Me_2 RI 2740. Phenytoin is used as the reference substance in System GE.

High Performance Liquid Chromatography System HE—*k* 9.71; system HX—RI 431; system HY—RI 381; system HZ—RT 3.7 min; system HAA—RT 16.3 min; system HAK—not detected; system HAM—RT 3.5 min; system HAX—RT 6.1 min; system HAY—RT 5.3 min; system HAZ—*k* 0.60.

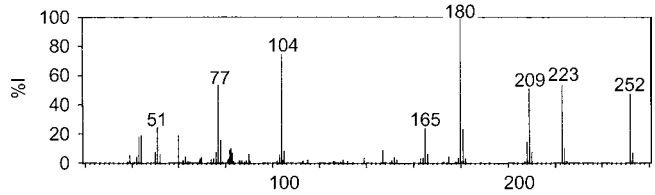
Ultraviolet Spectrum Methanol—258 (A_1^1 = 27a), 264 nm (A_1^1 = 16a).



Infrared Spectrum Principal peaks at wavenumbers 1720, 1740, 1774, 748, 698, 785 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 180, 104, 223, 77, 209, 252, 51, 165 (phenytoin); 239, 196, 268, 120, 197, 225, 77, 104 (5-(4-hydroxyphenyl)-5-phenylhydantoin [HPPH]).



Quantification

Blood **GC-MS** Column: 5% phenylmethylpolysiloxane (12 m × 0.15 mm i.d., 0.4 µm). Temperature programme: 80° for 1 min to 300° at 20°/min. Limit of detection, 0.5 mg/L [Speed *et al.* 2000].

Plasma **GC** Column: DB 1 (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: N_2, 12 psi. Temperature programme: 90° for 4 min to 170° at 15°/min to 240° at 6°/min to 300° at 15°/min for 1 min. TSD. Limit of quantification, 0.05 mg/L [Queiroz *et al.* 2002]. Column: 1.5% OV17, 1.95 OV210 (2 m × 2 mm i.d.). FID. Limit of detection, 200 µg/L [Zarghi *et al.* 1999]. Column: 3% OV-225 on Chromosorb W HP (100/120 mesh). Carrier gas: N_2, 35 mL/min. Temperature: 234°. FID, Limit of detection, 1 mg/L [Gordos *et al.* 1977].

GC-MS Column: DB-5MS (30 m × 0.25 mm i.d., 0.5 µm). Carrier gas: He, 1.5 kg/cm^2. Temperature programme: 270° for 1 min to 300° at 10°/min for 1 min. Limit of quantification, 2.5 mg/L [Nelson *et al.* 1998].

HPLC Column: RP-18E (100 × 3 mm i.d., 3 mm). Mobile phase: 0.1 mol/L phosphate buffer (pH 6.5) : methanol : acetonitrile (77 : 20 : 3), flow rate 2.1 mL/min. UV detection (λ = 210 nm). Retention time: 12.0 min. Limit of quantification, 2.2 mg/L [Heideloff *et al.* 2010]. Column: Shimdazu Shimpack XR-ODS (50 × 4.6 mm i.d., 2.2 µm). Mobile phase: 20 mmol/L acetate buffer (pH 5.6) : methanol-acetonitrile-tetrahydrofuran (61.3 : 32.2 : 6.5; 86 : 14 for 2 min to 77 : 23 at 5 min for 4 min to 64 : 36 at 12 min to 59 : 41 at 16.5 min to 86 : 14 at 17.5 min for 3.5 min), flow rate 0.8 mL/min. APCI, SIM acquisition mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.63 µg/L [Subramanian *et al.* 2008]. Column: LiChrospher 100 RP-18 C_{18} (125 × 4 mm i.d., 5 µm). Mobile phase: water : acetonitrile (78 : 22), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of quantification, 125 µg/L [Queiroz *et al.* 2008]. Column: Supelcosil LC-18. Mobile phase: 0.01 mol/L phosphate buffer : methanol : acetonitrile (pH 7.5, 65 : 18 : 17), flow rate 1 mL/min. UV detection (λ = 220 nm) [Matar *et al.* 1999]. Column: Spherisorb ODS2. Mobile phase: acetonitrile : methanol : potassium phosphate buffer (18 : 18 : 70). Retention time 9.9 min. UV detection (λ = 210 nm). Limit of quantification, 50 µg/L [Bhatti *et al.* 1998]. See also Andresen *et al.* [1993], Christofides, Fry [1980], Maya *et al.* [1992], Romanyshyn *et al.* [1994], Sawchuk, Cartier [1980] and Shimoyama *et al.* [1998].

LC-MS Column: C_{18} (150 × 3 mm i.d., 3 µm). Mobile phase: water-methanol (95 : 5) : water-methanol (10 : 90) both with 0.1% formic acid (100 : 0 to 20 : 80 in 3 min for 2 min to 100 : 0 at 6 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 12.2 µg/L [Zhang *et al.* 2008].

Serum **HPLC** See Plasma [Heideloff *et al.* 2010]. Column: Alltima 3C_{18} (150 × 4.6 mm i.d.) Mobile phase: acetonitrile : methanol : 25 mmol/L phosphate buffer (14.5 : 19.5 : 66), flow rate 0.9 mL/min. DAD (λ = 215 or 275 nm). Retention time: 15.65 min. Limit of quantification, 0.065 mg/L, limit of detection, 0.039 mg/L [Vermeij, Edelbroek 2007]. Column: Hypersil ODS Nova-Pak C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 µmol/L phosphate buffer : methanol : acetonitrile : acetone (pH 7.0, 55 : 22 : 12 : 11). UV detection (λ = 210 nm). Retention time: 9.97 min. Limit of quantification, 200 µg/L [Patil, Bodhankar 2005]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : glacial acetic acid (67 : 33 : 1), flow rate 1 mL/min. UV detection (λ = 230 nm). Limit of quantification, 0.05 mg/L [Kishore *et al.* 2003]. Column: Hypersil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 7.3 mmol/L sodium acetate buffer (pH 5.4) : acetonitrile (68 : 32), flow rate 2.0 mL/min. UV detection (λ = 240 nm). Limit of quantification, ~3 mg/L [Levert *et al.* 2002].

See also Christofides, Fry [1980], Hannak *et al.* [1992], Kouno *et al.* [1993], Lensmeyer *et al.* [1997], Liu *et al.* [1993], May *et al.* [1998] and Rambeck *et al.* [1994].

Urine **HPLC** Column: reversed phase C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 6.0, 60 : 40), flow rate 1 mL/min. DAD (λ = 210 nm). Limit of quantification, 300 µg/L, limit of detection, 80 µg/L [Santagati *et al.* 2005]. See Plasma [Maya *et al.* 1992; Sawchuk, Cartier 1980]. See Serum [Liu *et al.* 1993].

Breast Milk **HPLC** Column: Develosil C_8-5 (150 × 4.6 mm i.d.). Mobile phase: 0.5% potassium dihydrogen phosphate (pH 4.5) : acetonitrile (70 : 30), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 0.025 mg/L [Shimoyama *et al.* 1998].

Oral Fluid **HPLC** Column: ODS-Hypersil (250 × 2 mm i.d., 3 µm). Mobile phase: 0.01 mol/L potassium phosphate buffer (pH 7.0) : acetonitrile : methanol (110 : 50 : 30), flow rate 0.2 mL/min. DAD (215, 254 and 285 nm). Limit of detection, 0.78 mg/L [Liu *et al.* 1993].

Hair **HPLC** Column: S5 ODS2 (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : water (20 : 80), flow rate 2.0 mL/min. UV detection (λ = 220 nm). Limit of detection, 0.63 µg/g [Tsatsakis *et al.* 2000]. Column: reversed phase. Mobile phase: acetonitrile : methanol : water (9 : 37 : 54). UV detection (λ = 214 nm). Limit of detection, 2 ng/mg [Mei, Williams 1997].

Liver **GC-MS** See Blood [Speed *et al.* 2000].

Disposition in the Body Phenytoin is slowly but almost completely absorbed after oral administration; the rate of absorption is variable, being prolonged after large doses, and bioavailability may vary considerably between different formulations. It is metabolised mainly by P450 isozyme CYP2C9 and to a lesser extent by CYP2C19. Metabolism is affected by genetic polymorphism. Phenytoin undergoes enterohepatic recirculation. Approximately 50–70% of a dose may be excreted as free or conjugated HPPH in 24 h; the excretion of this metabolite is dose dependent and decreases as the dose is increased. Phenytoin hydroxylation is capacity limited and is, therefore, readily inhibited by agents that compete for its metabolic pathways. <5% of a dose is excreted as unchanged drug. Minor metabolites include 5-(3-hydroxyphenyl)-5-phenylhydantoin, 3,4-dihydro-3,4-dihydroxyphenytoin, catechol, and 3-*O*-methylcatechol. Up to ~15% of a dose may be eliminated in the faeces. Phenytoin crosses the placenta; low concentrations are found in breast milk

Therapeutic Concentration In plasma, usually in the range 10–20 mg/L.

In 332 adults treated for epilepsy with phenytoin for an average of 7.7 months, the average optimised dose was 305.8 mg (range, 100–500) daily, which produced an average steady-state serum concentration of 62.7 µmol/L (range, 15–133) [Valodia *et al.* 1999].

Following daily oral doses of 300 mg, mean steady-state plasma concentrations of 1.3–6.6 mg/L (mean, 3.6) were reported in 5 subjects with short phenytoin half-lives, in comparison with mean steady-state plasma concentrations of 5.0–27.2 mg/L (mean, 13.4) in 4 subjects with long phenytoin half-lives; the corresponding concentrations of HPPH were 1.3–2.7 mg/L (mean, 2.0) and 0.7–1.3 mg/L (mean, 1.0), respectively [Glazko *et al.* 1982].

A single oral dose of 15 mg/kg phenytoin produced a peak serum concentration of 11.95 mg/L at 7.26 h in 19 healthy subjects. To achieve a target serum concentration of 15 mg/L, an oral loading dose of 18.7 mg/kg was needed in males and 24.8 mg/kg in females; a peak serum concentration of 23.89 mg/L was attained in 9.71 h in males and 21.46 mg/L was attained in 7.71 h in females [Ratanakorn *et al.* 1997].

Toxicity The estimated minimum lethal dose is 5 g but few deaths from overdosage have been reported. Side effects have been associated with plasma concentrations greater than 20 mg/L and severe toxic effects with concentrations greater than 40 mg/L. Fatalities have been associated with blood concentrations >70 mg/L.

A $4^1/_2$-year-old girl ingested 2 g phenytoin; 24 h later, her blood concentration was found to be 94 mg/L. She died on the third day and postmortem concentrations were blood 45 mg/L, brain 78 µg/g, kidney 112 µg/g and liver 272 µg/g [Laubscher 1966].

In 9 patients displaying signs of phenytoin toxicity during the course of therapy, initial serum concentrations ranged from 34–57.5 mg/L. In 3 patients, concentrations remained relatively constant for 2–5 days before declining steadily; in the remaining 6 patients levels declined almost linearly. In 7 patients, levels declined between 4.6 and 5.9 mg/L per day [Chua *et al.* 2000].

A 15-year-old boy survived despite having a peak serum level of 100.8 mg/L after ingesting 19.6 g (15 g verifiable; 392 mg/kg) phenytoin sodium in a suicide attempt [Mellick *et al.* 1989].

Following an apparent suicide attempt by an adult non-epileptic man who ingested an unkown amount of phenytoin, an unusual absorption profile was observed. Twelve hours after the ingestion, the serum phenytoin concentration was 45 mg/L. This steadily increased, reaching a maximum of 114 mg/L 4 days later, fluctuated at ~100 mg/L for a week and then slowly declined to undetectable levels over the following week. The protracted absorption appeared to result from the presence of a large concretion of phenytoin in the gastrointestinal tract, slowly disintegrating and dissolving and also from the patient's diminished intestinal motility [Chaikin, Adir 1987].

Half-life Plasma half-life varies considerably within the approximate range of 7 to 42 h; it is dose dependent, increasing as the dose increases; at steady state it is ~22 h.

Volume of Distribution ≈0.5–1.2 L/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.6.

Saliva Plasma : saliva ratio, ~10.

Protein Binding ≈90%.

Note For a review of phenytoin disposition and toxicity, see Edeki, Brase [1995]; for a review of phenytoin dosing methods, see Pryka *et al.* [1991]; for a review of the clinical features and management of poisoning with phenytoin, see Larsen, Larsen [1989]; for a review of the pharmacokinetics of phenytoin, see Richens [1979].

Dose 300 to 600 mg of phenytoin or phenytoin sodium daily.

Andresen AT *et al.* (1993). Automated determination of free phenytoin in human plasma with on-line equilibrium dialysis and column-switching high-performance liquid chromatography. *J Chromatogr* 621: 189–198.

Bhatti MM *et al.* (1998). Simultaneous determination of phenytoin, carbamazepine, and 10,11-carbamazepine epoxide in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* 16: 1233–1240.

Chaikin P, Adir J (1987). Unusual absorption profile of phenytoin in a massive overdose case. *J Clin Pharmacol* 27: 70–73.

Christofides JA, Fry DE (1980). Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin Chem* 26: 499–501.

Chua HC *et al.* (2000). Elimination of phenytoin in toxic overdose. *Clin Neurol Neurosurg* 102: 6–8.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul:. 481–486.

Edeki TI, Brase DA (1995). Phenytoin disposition and toxicity: role of pharmacogenetic and interethnic factors. *Drug Metab Rev* 27: 449–469.

Glazko AJ *et al.* (1982). Phenytoin metabolism in subjects with long and short plasma half-lives. *Ther Drug Monit* 4: 281–292.

Gordos J *et al.* (1977). Micro-determination of plasma diphenylhydantoin by gas–liquid chromatography. *J Chromatogr* 143: 171–181.

Hannak D *et al.* (1992). Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline. *Wien Klin Wochenschr Suppl* 191: 27–31.

Heideloff C *et al.* (2010). A novel HPLC method for quantification of 10 antiepileptic drugs or metabolites in serum/plasma using a monolithic column. *Ther Drug Monit* 32: 102–106.

Kishore P *et al.* (2003). Validated high performance liquid chromatographic method for simultaneous determination of phenytoin, phenobarbital and carbamazepine in human serum. *Arzneimittelforschung* 53: 763–768.

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Santagati NA *et al.* (2005). Simultaneous determination of phenytoin and dextromethorphan in urine by solid-phase extraction and HPLC-DAD. *J Sep Sci* 28: 1157–1162.

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Pholcodine

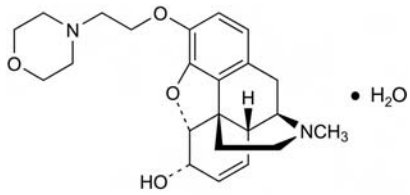
Cough Suppressant

$C_{23}H_{30}N_2O_4,H_2O = 416.5$

CAS—509-67-1 (anhydrous)

Synonyms (5α,6α)-7,8-Didehydro-4,5-epoxy-17-methyl-3-[2-(4-morpholinyl)-ethoxy]morphinan-6-ol monohydrate; folcodina; MEM; morpholinylethylmorphine; pholcodinum.

Proprietary Names *Benylin Childrens Dry Coughs*; *Evaphol*; *Expulin Dry Cough*; *Famel Linctus*; *Galenphol*; *Hill's Balsam Dry Cough*; *Pavacol-D*; *Pholcomed*; *Tixylix Daytime*. It is an ingredient of many proprietary preparations, see Sweetman [2002].



Chemical Properties Colourless crystals or a white crystalline powder. Mp 91°. Soluble 1 in 50 of water and 1 in 3 of dehydrated alcohol; soluble in benzene and chloroform. pK_a 8.0, 9.3 (37°). Log *P* (octanol/water), 0.6.

Pholcodine Tartrate

$C_{23}H_{30}N_2O_4,2C_4H_6O_6,3H_2O = 752.7$

Chemical Properties A white crystalline powder. Soluble 1 in 8 of water; very slightly soluble in ethanol; slightly soluble in chloroform and ether.

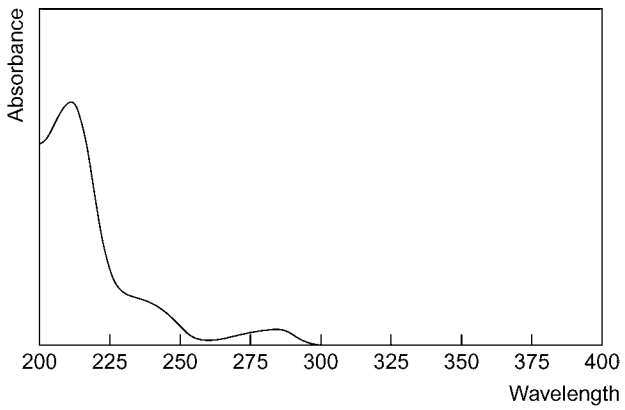
Colour Tests Liebermann's reagent—black; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.36; system TB—R_f 0.03; system TC—R_f 0.18; system TE—R_f 0.25; system TL—R_f 0.02; system TAE—R_f 0.15 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis Reagent, violet).

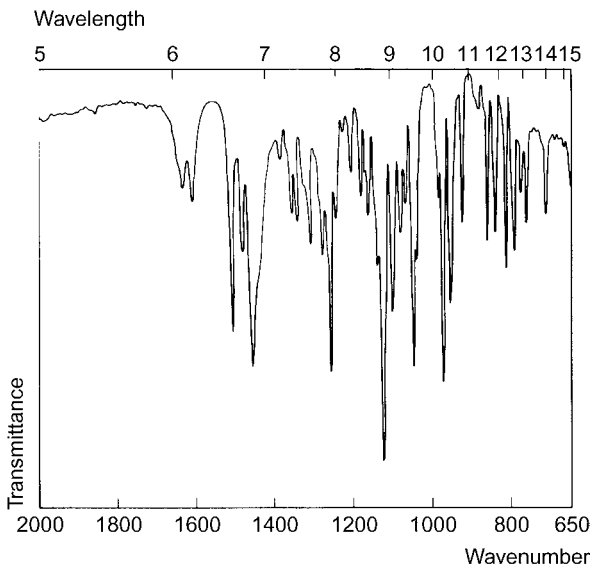
Gas Chromatography System GA—phocoldine RI 3070, phocoldine-AC RI 3260, phocoldine-PFP RI 2980, phocoldine-TFA RI 2800, phocoldine-TMS RI 3140; system GB—pholcodine RI 3348, pholcodine-TMS RI 3410.

High Performance Liquid Chromatography System HA—*k* 6.0 (tailing peak); system HC—*k* 1.63; system HX—RI 65; system HY—RI 92; system HAA—retention time 2.7 min.

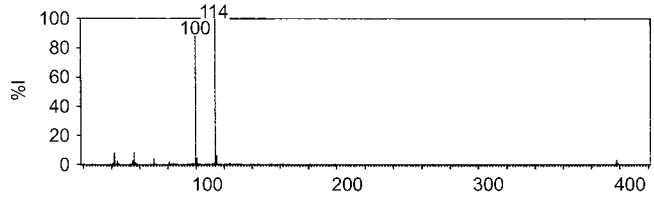
Ultraviolet Spectrum Aqueous acid—283 nm (A_1^1=40a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1118, 969, 1250, 1043, 1500, 1098 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 114, 100, 56, 42, 115, 101, 70, 398.



Quantification

Blood **GC-MS** Pholcodine and other opioids [Krogh *et al.* 1993].

HPLC Fluorescence detection [Andresen *et al.* 1992].

Plasma **GC-MS** See Blood [Krogh *et al.* 1993].

HPLC See Blood [Andresen *et al.* 1992].

Urine **GC** Nitrogen detection. Pholcodine and its metabolites [Johansen *et al.* 1990].

GC-MS Pholcodine and other opioids [Sautou *et al.* 1994]. Comparison with immunoassay. Pholcodine and its metabolites [Maurer, Fritz 1990].

HPLC Comparison with EMIT. Pholcodine and other opioids., Limits of detection, 4 to 20 µg/L [Low, Taylor 1995]. Fluorescence and electrochemical detection. Pholcodine and its metabolites [Johansen *et al.* 1992].

Hair **GC-MS** See Urine [Maurer, Fritz 1990].

Disposition in the Body Pholcodine is partly metabolised to morphine and other metabolites; it also forms a glucuronic acid conjugate. About 29% of an oral dose is excreted in the urine as unchanged pholcodine and a further 15% as the glucuronide; about 0.5 to 1% is excreted in the urine as morphine glucuronide. Other metabolites have been identified as desmorpholinohydroxy-, nordesmorpholinohydroxy-, hydroxy-, oxo-, nor- and noroxo-pholcodine.

Therapeutic Concentration

Six healthy subjects received single oral doses of 20 and 60 mg pholcodine (with 3 weeks between the doses) and subsequently received 20 mg pholcodine orally every 8 h for 10 days. After single doses pholcodine was absorbed rapidly (t_{max}=1.6 h), with the mean peak plasma concentration being 12.8 µg/L after the 20-mg dose and 26.3 µg/L after the 60-mg dose. Multiple dosing did not alter the pharmacokinetics [Chen *et al.* 1988].

Note For interpretation of GC–MS opiate results in the presence of pholcodine, see Meadway *et al.* [2002].

Dose Usually up to 40 mg daily.

Andresen AT *et al.* (1992). On-line dialysis and weak cation-exchange enrichment of dialysate. Automated high-performance liquid chromatography of pholcodine in human plasma and whole blood. *J Chromatogr* 582: 123–130.

Chen ZR *et al.* (1988). Pharmacokinetics of pholcodine in healthy volunteers: single and chronic dosing studies. *Br J Clin Pharmacol* 26: 445–453.

Johansen M *et al.* (1990). Determination of pholcodine and its metabolites in urine by capillary gas chromatography. *J Chromatogr* 532: 277–284.

Johansen M *et al.* (1992). Column-switching high-performance liquid chromatographic detection of pholcodine and its metabolites in urine with fluorescence and electrochemical detection. *J Chromatogr* 573: 283–288.

Krogh M *et al.* (1993). Automated sample preparation by on-line dialysis and trace enrichment. Analysis of morphine, 6-monoacetylmorphine, codeine, ethylmorphine and pholcodine in plasma and whole blood by capillary gas chromatography and capillary gas chromatography-mass spectrometry. *J Chromatogr* 621: 41–48.

Low AS, Taylor RB (1995). Analysis of common opiates and heroin metabolites in urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 663: 225–233.

Maurer HH, Fritz CF (1990). Toxicological detection of pholcodine and its metabolites in urine and hair using radio immunoassay, fluorescence polarisation immunoassay, enzyme immunoassay, and gas chromatography-mass spectrometry. *Int J Legal Med* 104: 43–46.

Meadway C *et al.* (2002). Interpretation of GC-MS opiate results in the presence of pholcodine. *Forensic Sci Int* 127: 131–135.

Sautou V *et al.* (1994). Analysis of opiates in the urine of children with gas chromatography coupled to mass spectrometry. *J Pharm Belg* 49: 383–389.

Sweetman SC. (2002). *Martindale: The Complete Drug Reference*, 33 edn. London: Pharmaceutical Press.

Pholedrine

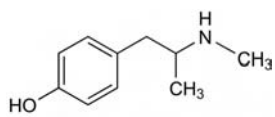
Sympathomimetic

$C_{10}H_{15}NO = 165.2$

CAS—370-14-9

IUPAC Name 4-[2-(Methylamino)propyl]phenol

Synonyms Isodrine; sympropaminum.

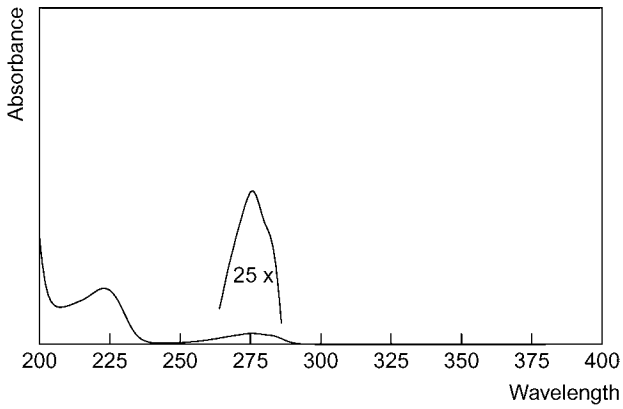


Chemical Properties Crystals. Mp 162° to 163°. Slightly soluble in water; soluble in ethanol and ether. pK_a 9.4 (25°). Log *P* (octanol/water), 1.7. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Pholedrine Sulfate

$(C_{10}H_{15}NO)_2,H_2SO_4 = 428.5$
CAS—6114-26-7
Synonym Pholedrine sulfate
Proprietary Name *Paredrinol*; *Pulsotyl*; *Veritol*
Chemical Properties A white crystalline powder. Mp about 320° to 323°, with decomposition. Soluble 1 in 20 of water; practically insoluble in ethanol, chloroform and ether.

Colour Test Marquis test—grey→green.
Thin-layer Chromatography System TA—R_f 0.29; system TB—R_f 0.03; system TC—R_f 0.03; system TE—R_f 0.27; system TL—R_f 0.03; system TAE—R_f 0.09 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1490.
High Performance Liquid Chromatography System HX—RI 205.
Ultraviolet Spectrum Aqueous acid—275 nm (A^1_1=82a); aqueous alkali—238 (A^1_1=640b), 295 nm (A^1_1=150b).



Infrared Spectrum Principal peaks at wavenumbers 1258, 1513, 1274, 1590, 1610, 810 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 30, 107, 59, 77, 56, 42, 43.
Quantification
Plasma **Radioimmunoassay** [Peinhardt 1997].
Serum **HPLC** Amperometric detection [Peinhardt 1999].
Disposition in the Body
Toxicity
In a fatality involving the ingestion of pholedrine, the following postmortem concentrations were reported: blood 15 µg/g, kidney 122 µg/g, liver 73 µg/g [Hammer *et al.* 1980].
Dose Pholedrine sulfate has been given parenterally in doses of up to 20 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Hammer HJ *et al.* (1980). *Dtsch GesundhWes* 35: 1352–1354.
Peinhardt GP (1999). High-performance liquid chromatographic determination of pholedrine in human serum using ion-pair extraction and amperometric detection. *J Chromatogr B Biomed Sci Appl* 726: 309–312.
Peinhardt G (1997). Radioimmunoassay for the determination of pholedrine. *Pharmazie* 52: 937–939.

Phosgene

Halocarbon, Vesicant, War Gas
$COCl_2 = 98.9$
CAS—75-44-5
IUPAC Name Carbonyl Chloride
Synonyms Carbonic dichloride; carbonyl chloride; chloroformyl chloride.
Chemical Properties Colourless, highly toxic gas; suffocating odour; when much diluted with air, there is an odour reminiscent of mouldy hay. Condenses at ~0° to a clear, colourless fuming liquid. Mp −118°. Bp (760 mmHg) 8.2°. Slightly soluble in water and slowly hydrolysed by it; freely soluble in benzene, toluene, glacial acetic acid and most liquid hydrocarbons [O'Neil *et al.* 2006]. Thermal decomposition studies of phosgene in air have shown that phosgene decomposes by 60% at 500°, 90% at 600°, and 100% at 800° with chlorine as a decomposition by-product formed only at 700° or above [Noweir *et al.* 1973]. It is widely used as an intermediate in industrial processes, in the preparation of isocyanates, polycarbonates, polyurethanes, aniline dyes and pesticides. It may also be formed by the thermal decomposition of chlorinated hydrocarbons (e.g. polyvinylchloride), and high concentrations may be released in fires [Bjerre 1984]. It is highly reactive toward amino, hydroxyl and thiol groups [Noort *et al.* 2000] It is known to be generated photochemically from organochlorine precursors [Hatch *et al.* 2001]. It has also been widely reported that phosgene was first used as a chemical warfare agent in 1915 during the First World War.
Colour Tests Paper soaked in alcoholic or carbon tetrachloride solution containing 10% of a mixture of equal parts of *p*-dimethylaminobenzaldehyde and colourless diphenylamine, then dried, will turn from yellow to deep orange in the presence of phosgene [O'Neil *et al.* 2006]. Several reagents when dissolved in *o*-dichlorobenzene will react when exposed to phosgene [Linch *et al.* 1965], described as shown in the table below.
Mass Spectrum Principal ions at *m/z* 154, 153, 182, 121, 69, 155, 77, 110 (dimercaptotoluene derivative) ; 151, 96, 123, 69 (aminothiophenol derivative); 83, 141, 55, 41, 42, 100, 125, 86 (bis(2-hydroxymethylpiperidine) derivative) [Muir *et al* 2005].
Quantification
Other **GC** Air Samples. Column: DB-5 fused silica capillary (30 m × 0.32 mm i.d.). Carrier gas: He, 24 mL/min. Temperature programme: 150° for 16 min to 190 at 16°/min for 16 min. FID. Retention time: ~15 min (tetra-*n*-butyl urea derivative). Limit of quantification not reported [Hendershott 1986].
GC-MS Air Samples and Synthetic GasMixtures. Column: DB-1701 capillary (25 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 40° for 1 min to 280° at 20°/min for 1 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: phosgene 3,4-dimercaptotoluene derivative 12.2 min; phosgene 2-aminothiophenol derivative 13.4 min. Limit of detection, phosgene 3,4-dimercaptotoluene derivative 3 ng/m^3, phosgene 2-aminothiophenol derivative 3 ng/m^3[Muir *et al.* 2005].
Note For a method for the monitoring of toxic compounds in air using a handheld ion trap mass spectrometer, see Keil *et al.* [2008]; for purity analysis of phosgene by quantitative ^{13}C NMR spectroscopy, see Henderson and Cullinan [2007]; for the detection of phosgene using fluorescence resonance energy transfer, see Zhang and Rudkevich [2007]; for the LC-MS detection of phosgene in blood *in vitro* as a glutathione adduct, see Fabrizi *et al.* [2003], or as a pentapeptide fragment adduct of albumin or haemoglobin, see Noort *et al.* [2000]; for the detection of phosgene in air using ion mobility spectrometry, see Bocos-Bintintan *et al.* [2002]; for a spectrophotometric method for the determination of phosgene in air, see Dangwal [1994]; for a GC study comparing different column packings for phosgene analysis, see Esposito *et al.* [1977]; for a colorimetric method for the quantification of phosgene in air, see Thompson *et al.* [1976].
Disposition in the Body
Toxicity A concentration of 0.1 ppm phosgene has been recommended by several agencies in the USA and Europe as a safe workplace time-weighted average

Reagent structure		*Solution colour*	
Component A	*Component B*	*Initial*	*After phosgene exposure*
p-Dimethyl aminobenzaldehyde	*N*-Phenyl-β-naphthylamine	Colourless-slight yellow	Orange
p-Dimethyl aminobenzaldehyde	*N*-Methyl-α-naphthylamine	Slight violet	No change
p-Dimethyl aminobenzaldehyde	Diphenyl-*p*-phenylene-diamine	Violet	Red-Brown
p-Dimethyl aminobenzaldehyde	4,4′-Dimethoxydiphenylamine	Colourless	Yellow
p-Dimethyl aminobenzaldehyde	Diphenylamine	Near colourless	Yellow
o-Chlorobenzaldehyde	*N*-Phenyl-β-naphthylamine	Colourless	Yellow
o-Chlorobenzaldehyde	Diphenylamine	Colourless	Faint yellow
o-Chlorobenzaldehyde	Diphenyl-*p*-phenylene-diamine	Brown	No change
m-Diethylaminophenol	Nitroso-*m*-diethylaminophenol	Faint brown	No change
4,4` -Bis(dimethylamino) benzophenone	–	Faint violet	Deep blue
4,4` -Bis(dimethylamino) benzophenone	Dimethylaniline	Faint blue	Deep blue
4,4` -Bis(dimethylamino) benzophenone	2,4-Dinitrophenol	Faint blue	Blue
4,4` -Bis(diethylamino)benzophenone	–	Faint blue	Blue-green

exposure for repeated 8 h per work day [Pauluhn *et al.* 2007]. The lethal exposure for humans is ~800 ppm for 2 min [Cucinell 1974]. Phosgene is capable of damaging a variety of biological materials in an oxidant-like fashion, its activity resulting from at least 2 separate chemical reactions: acylation and hydrolysis. Acylation, the more important mechanism, results from the reaction of phosgene with nucleophilic moieties such as amino, hydroxyl, and sulfhydryl groups of tissue macromolecules. This results in the denaturation of proteins and lipids, irreversible alterations of membrane structures, and disruption of enzyme and other cell functions. In addition, hydrolysis of phosgene results in hydrochloric acid formation on moist membranes, which may provoke irritation and tissue damage [Borak, Diller 2001; Pauluhn *et al.* 2007].

Symptoms of poisoning, which may be delayed for up to 24 h (and rarely 72 h), include burning of the eyes and throat, cough, dyspnoea, cyanosis, pulmonary congestion, and oedema. Death may result from anoxia. It has been observed that among those affected who die, 80% may be expected to die during the first 24–48 h post-exposure [Karalliedde *et al.* 2000]. Exposure to 50 ppm may be rapidly fatal. Massive exposure may cause intravascular haemolysis, thrombus formation and immediate death. Exertional dyspnoea may persist for months after exposure to high concentrations. After inhalation of phosgene or absorption from the skin, treatment consists of complete rest and inhalation of oxygen. The mouth, eyes, nose and skin should be irrigated with copious amounts of water. Oral or parenteral corticosteroids have been used for bronchospasm, but the role of inhaled corticosteroids is considered to be controversial. Antibacterials may reduce respiratory infections [Sweetman 2007]. Premedication with hexamethylenetetramine (HMT) has been reported to be effective in protecting humans against a lethal dose of phosgene; however, this is limited at higher doses of phosgene and the necessary high HMT doses cause side effects [Diller 1980].

Note For case histories of acute lung injury in humans after phosgene inhalation, see Lim *et al.* [1996] and Snyder *et al.* [1992]. For a review of the concentration–effect relationship of phosgene exposure in humans, see Diller [1978], [1985a]. For a case study of contact dermatitis caused by phosgene (chlorophenyl)hydrazones found in bleached sweaters, see Kojima *et al.* [1990] and Kojima and Momma [1989]. For proceedings of a conference on phosgene poisoning, see Diller [1985b]. For a review on vesicants, see McManus and Huebner [2005].

Protein Binding Binds to haemoglobin and albumin.

Bjerre A (1984). Health hazard assessment of phosgene formation in gases of combustion of polyvinyl chloride using a simplified method of mathematical modelling. *Ann Occup Hyg* 28: 49–59.

Bocos-Bintintan V *et al.* (2002). Characterisation of the phosgene response of a membrane inlet ^{63}Ni ion mobility spectrometer. *Analyst* 127: 1211–1217.

Borak J, Diller WF (2001). Phosgene exposure: mechanisms of injury and treatment strategies. *J Occup Environ Med* 43: 110–119.

Cucinell SA (1974). Review of the toxicity of long-term phosgene exposure. *Arch Environ Health* 28: 272–275.

Dangwal SK (1994). A spectrophotometric method for determination of phosgene in air. *Ind Health* 32: 41–47.

Diller WF (1978). Medical phosgene problems and their possible solution. *J Occup Med* 20: 189–193.

Diller WF (1980). The methenamine misunderstanding in the therapy of phosgene poisoning. *Arch Toxicol* 46: 199–206.

Diller WF (1985). Pathogenesis of phosgene poisoning. *Toxicol Ind Health* 1: 7–15.

Diller WF (1985). Phosgene induced edema: diagnosis and therapeutic countermeasures. An international symposium. September 23–24, 1982. *Toxicol Ind Health* 1: 1–160.

Esposito GG *et al.* (1977). Determination of phosgene in air by gas chromatography and infra-red spectrophotometry. *Anal Chem* 49: 1774–1778.

Fabrizi L *et al.* (2003). Adduction of the chloroform metabolite phosgene to lysine residues of human histone H2B. *Chem Res Toxicol* 16: 266–275.

Hatch G *et al.* (2001). An 'injury–time integral' model for extrapolating from acute to chronic effects of phosgene. *Toxicol Ind Health* 17: 285–293.

Hendershott JP (1986). The simultaneous determination of chloroformates and phosgene at low concentrations in air using a solid sorbent sampling–gas chromatographic procedure. *Am Ind Hyg Assoc J* 47: 742–746.

Henderson TJ, Cullinan DB (2007). Purity analysis of hydrogen cyanide, cyanogen chloride and phosgene by quantitative (13)C NMR spectroscopy. *Magn Reson Chem* 45: 954–961.

Karalliedde L *et al.* (2000). Possible immediate and long-term health effects following exposure to chemical warfare agents. *Public Health* 114: 238–248.

Keil A *et al.* (2008). Monitoring of toxic compounds in air using a handheld rectilinear ion trap mass spectrometer. *Anal Chem* 80: 734–741.

Kojima S, Momma J (1989). Phosgene (2,5-dichlorophenyl)hydrazone, a new strong sensitizer. *Contact Dermatitis* 20: 235–236.

Kojima S *et al.* (1990). Phosgene (chlorophenyl)hydrazones, strong sensitizers found in yellow sweaters bleached with sodium hypochlorite, defined as causative allergens for contact dermatitis by an experimental screening method in animals. *Contact Dermatitis* 23: 129–141.

Lim SC *et al.* (1996). Acute lung injury after phosgene inhalation. *Korean J Intern Med* 11: 87–92.

Linch AL *et al.* (1965). Phosgene in air: development of improved detection procedures. *Am Ind Hyg Assoc J* 26: 465–474.

McManus J, Huebner K (2005). Vesicants. *Crit Care Clin* 21: 707–718.

Muir B *et al.* (2005). Analysis of chemical warfare agents III. Use of bis-nucleophiles in the trace level determination of phosgene and perfluoroisobutylene. *J Chromatogr A* 1098: 156–165.

Noort D *et al.* (2000). *In vitro* adduct formation of phosgene with albumin and hemoglobin in human blood. *Chem Res Toxicol* 13: 719–726.

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Phthalylsulfacetamide

Antibacterial, Sulfonamide

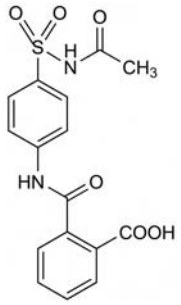
$C_{16}H_{14}N_2O_6S = 362.4$

CAS—131-69-1

IUPAC Name 2-[[4-(Acetylsulfamoyl)phenyl]carbamoyl]benzoic acid

Synonyms 2-[[[4-[(Acetylamino)sulfonyl]phenyl]amino]carbonyl]benzoic acid; ftalicetimida; phthaloylsulfacetamide; phthalylsulfacetamide; sulfanilacetamidum phthalylatum.

Proprietary Names *Enterocid*; *Enterosulfamid*; *Enterosulfon*; *Talecid*; *Thalacet*; *Rabalan*; *Sterathal*.



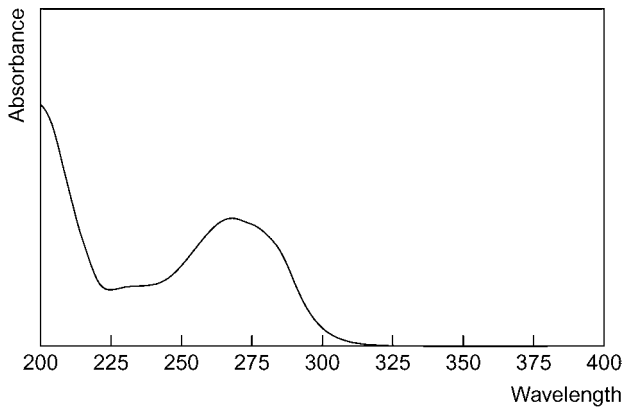
Chemical Properties White or creamy-white crystals or crystalline powder. Mp 196°. Very slightly soluble in water; slightly soluble in ethanol; soluble in acetone; freely soluble in solutions of alkali hydroxides. Log *P* (octanol/water), 1.3.

Colour Test Copper sulfate (method 1)—blue.

Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.00.

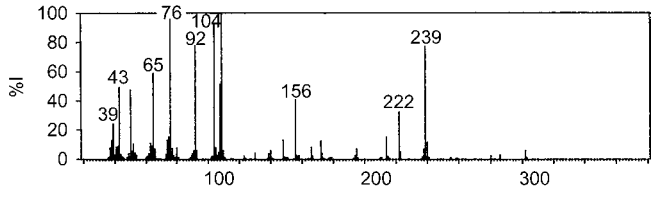
Gas Chromatography See Sulfacetamide.

Ultraviolet Spectrum Aqueous acid—268 nm (broad); aqueous alkali—265 nm (broad).



Infrared Spectrum Principal peaks at wavenumbers 1170, 1681, 1527, 837, 1302, 1592 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 109, 76, 104, 92, 239, 65, 108, 43.



Dose 6 to 12 g daily.

P

Phthalylsulfathiazole

Antibacterial, Sulfonamide

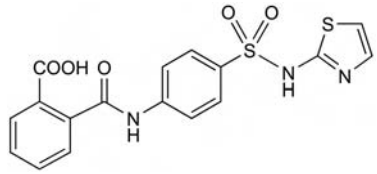
$C_{17}H_{13}N_3O_5S_2$ = 403.4

CAS—85-73-4

IUPAC Name 2-[[4-(1,3-Thiazol-2-ylsulfamoyl)phenyl]carbamoyl]benzoic acid

Synonyms Ftalilsulfatiazol; phthalazolum; phthalylsulfathiazole; sulfaphtalylthiazol; 2-[[[4-[(2-thiazolylamino)sulfonyl]phenyl]amino]carbonyl]benzoic acid.

Proprietary Names *AFI-Ftalyl*; *Entexidina*; *Ftalazol*; *Intestiazol*; *Kaotalil*; *Sulfathalidine*; *Sulfalyl*; *Taleudron*; *Talidine*; *Thalazole*; *Ultratiazol*.



Chemical Properties White or yellowish-white crystals, or powder, which darken on prolonged exposure to light. Effervesces at 244° to 250°. Mp 272° to 277°, with decomposition. Practically insoluble in water, chloroform and ether; soluble 1 in 600 of ethanol; freely soluble in dimethylformamide and solutions of alkali hydroxides and carbonates, and in hydrochloric acid. Log *P* (octanol/water), 0.9.

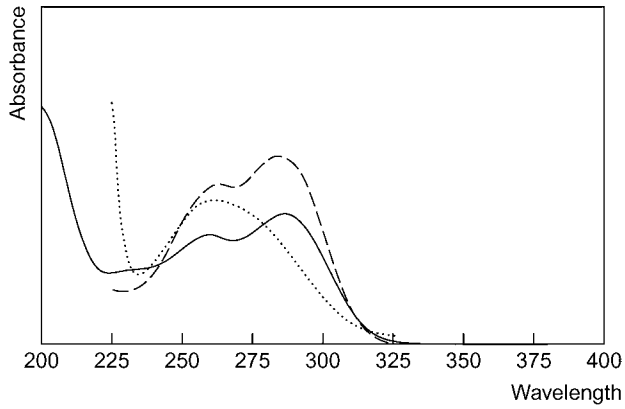
Colour Test Copper sulfate (method 1)—green.

Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TT—R_f 0.02; system TU—R_f 0.04; system TV—R_f 0.04; system TAD—R_f 0.00; system TAJ—R_f 0.00; system TAK—R_f 0.04; system TAL—R_f 0.42.

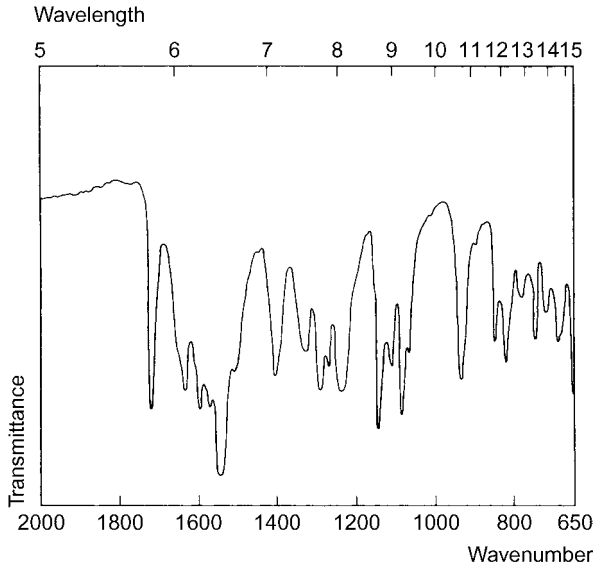
Gas Chromatography See Sulfathiazole.

High Performance Liquid Chromatography System HU—*k* 14.0.

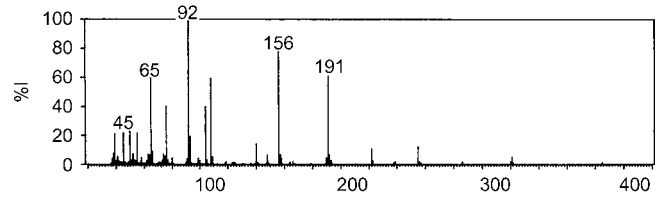
Ultraviolet Spectrum Aqueous acid—282; aqueous alkali—260 nm ($A^{1\%}_1$=600a); methanol—260 nm ($A^{1\%}_1$=497a), 288 nm ($A^{1\%}_1$=648a).



Infrared Spectrum Principal peaks at wavenumbers 1532, 1142, 1084, 1710, 1585, 1560 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 92, 156, 191, 65, 108, 76, 104, 50.



Disposition in the Body Only about 5% of a dose is absorbed after oral administration and blood concentrations are therefore very low compared to other sulfonamides, usually <15 mg/L. It is slowly hydrolysed to sulfathiazole in the gastro-intestinal tract.

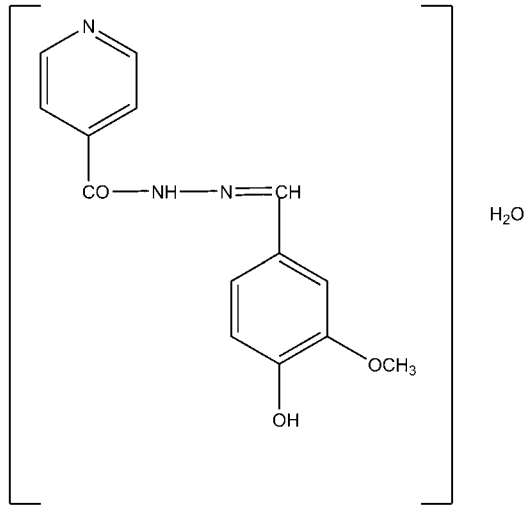
Dose 2 to 12 g daily.

Phthivazid

Tuberculostatic

$C_{14}H_{13}N_3O_3$ = 289.3

Synonyms Ftivazide; *N′*-[4-hydroxy-3-methoxybenzylidene)methyl]pyridine-4-carbohydrazide monohydrate; isonicotinic acid vanillylidenehydrazide.



Chemical Properties A light-yellow crystalline powder. Almost insoluble in water; slightly soluble in ethanol; freely soluble in inorganic acids and alkalis; soluble in glacial acetic acid. Extracted by chloroform from aqueous alkaline solutions.

Colour Tests Dissolve 50 mg, heating slightly, in 10 mL of ethanol; cool and add a few drops of 2 N sodium hydroxide—the light yellow colour of the solution darkens to canary yellow. Acidify with dilute hydrochloric acid—the solution becomes almost colourless and then acquires a yellow colour.

To 50 mg add 50 mg of dinitrochlorobenzene and 3 mL of ethanol; boil for 2 to 3 minutes, cool and add 2 drops of 2N sodium hydroxide—a brownish red colour develops, which becomes more intense on standing.

Thin-layer Chromatography System T1—R_f 0.60 (location reagent potassium permanganate spray, positive reaction).

Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.

Ultraviolet Spectrum Phthivazid in 0.1 N sulfuric acid, maxima at 228 (E1%, 1 cm 670), 273 (E1%, 1 cm 537) and 307 nm (E1%, 1 cm 359), minima at 246 and 297 nm; in ethanol maxima at 275 (E1%, 1 cm 360) and 333 nm (E1%, 1 cm 830), minimum at 250 nm.

Infrared Spectrum Principal peaks at wavenumbers 1282 or 1590, 1668, 1506 cm^{-1} (KBr disk).

Dose Up to 2 g daily.

Physostigmine

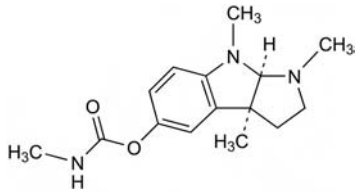
Anticholinesterase

$C_{15}H_{21}N_3O_2$ = 275.3

CAS—57-47-6

IUPAC Name [(3a*R*,8b*S*)-3,4,8b-Trimethyl-2,3a-dihydro-1*H*-pyrrolo[2,3-b]indol-7-yl] *N*-methylcarbamate

Synonyms Eserine; (3a*S*-*cis*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]-indol-5-ol methylcarbamate ester; physostigmina.



Chemical Properties An alkaloid obtained from the calabar bean (ordeal bean; chopnut), the seed of *Physostigma venenosum* (Leguminosae). Colourless crystals, or a white microcrystalline powder. Mp 105° to 106°; also unstable, low-melting form, mp 86° to 87°. Soluble 1 in 75 of water, 1 in 10 of ethanol, 1 in 1 of chloroform and 1 in 30 of ether. Physostigmine, its salts and their aqueous solutions become red on exposure to heat, light, air and on contact with traces of metals, owing to the formation of rubreserine; the change is less rapid in acid solution. pK_a 1.8, 7.9 (25°). Log *P* (octanol/water), 1.6.

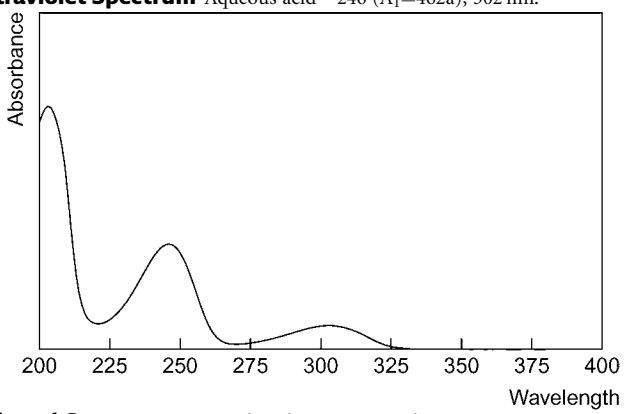
Physostigmine Salicylate

$C_{15}H_{21}N_3O_2,C_7H_6O_3 = 413.5$
CAS—57-64-7
Synonym Eserine salicylate
Proprietary Names *Anticholium*; *Antilirium*.
Chemical Properties Colourless or white crystals or white powder. Mp 185° to 187°. Soluble 1 in 75 to 1 in 90 of water, 1 in 16 of ethanol, 1 in 6 of chloroform, and 1 in 250 of ether.

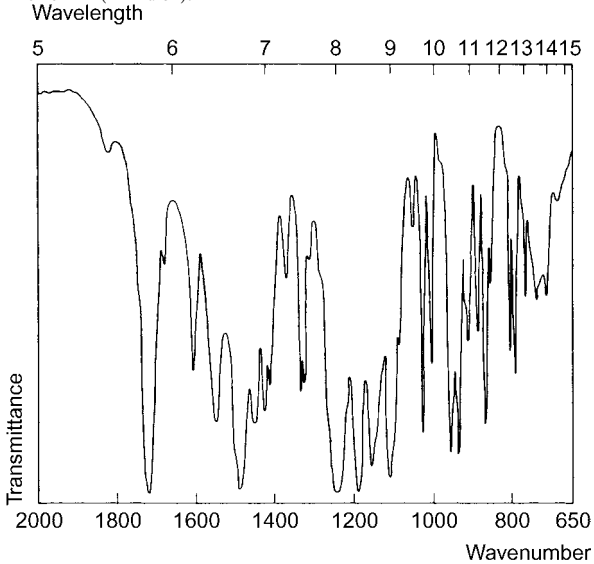
Physostigmine Sulfate

$(C_{15}H_{21}N_3O_2)_2,H_2SO_4 = 648.8$
CAS—64-47-1
Synonym Eserine sulfate
Chemical Properties A white deliquescent, microcrystalline powder. Mp 140°. Soluble 1 in 4 of water and 1 in 0.4 of ethanol; soluble in chloroform; very slightly soluble in ether.

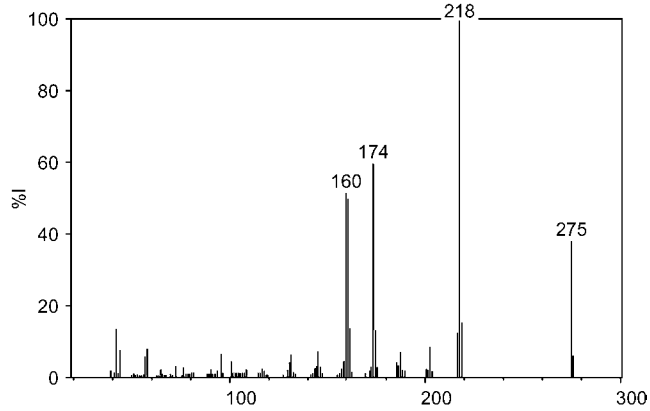
Colour Test Mandelin's test—yellow-brown.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.12; system TC—R_f 0.36; system TE—R_f 0.55; system TL—R_f 0.18; system TAE—R_f 0.40; system TAF—R_f 0.38; system TAJ—R_f 0.18; system TAK—R_f 0.03; system TAL—R_f 0.50 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1804, RI 2035 and RI 2190.
High Performance Liquid Chromatography System HA—*k* 2.6; system HX—RI 296; system HY—RI 240; system HZ—retention time 2.2 min.
Ultraviolet Spectrum Aqueous acid—246 (A^{1}_{1}=462a), 302 nm.



Infrared Spectrum Principal peaks at wavenumbers 1724, 1243, 1200, 1495, 1120, 1162 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 218, 174, 160, 161, 275, 219, 175, 162.

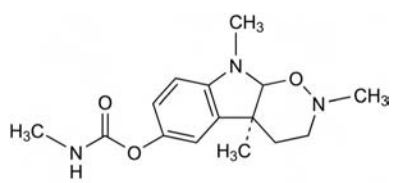


Quantification
Blood **HPLC** Electrochemical detection. Physostigmine and eseroline [Lawrence, Yatim 1990].
Plasma **HPLC** See Blood [Lawrence, Yatim 1990]. Fluorescence detection. Limit of detection, 100 ng/L [Elsayed *et al.* 1989]. Electrochemical detection. Limit of detection, 500 ng/L [Unni *et al.* 1989]. Electrochemical detection. Limits of detection, 25 to 50 pg/mol [Whelpton, Moore 1985]. Electrochemical detection. Limit of detection, 500 ng/L [Whelpton 1983].
Serum **HPLC** Fluorescence detection. Physostigmine and eseroline. Limit of detection, 2 µg/L for physostigmine and 5 µg/L for eseroline [Quinn, Stewart 1991].
Cerebrospinal Fluid **HPLC** See Blood [Lawrence, Yatim 1990]. See Plasma [Unni *et al.* 1989].
Disposition in the Body Readily absorbed after oral or SC administration and from mucous membranes. Physostigmine readily penetrates the CNS. It is rapidly hydrolysed by cholinesterases and most of a dose is destroyed in 2 h. Very little is excreted in the urine.
Therapeutic Concentration
After a single SC injection of 1 mg of physostigmine salicylate (equivalent to 0.67 mg base) to one subject, a peak plasma concentration of 0.0036 mg/L was achieved after 15 min [Whelpton 1983].
After a single oral dose of 4 mg of physostigmine salicylate to one subject, a peak plasma concentration of about 0.001 mg/L was attained in 0.75 h [Gibson *et al.* 1985].
Toxicity Fatalities have occurred following ingestion of 6 mg of physostigmine orally and administration of 1.2 mg IV. The ingestion of 6 calabar beans by a child has proved fatal.

Elsayed NM *et al.* (1989). Determination of physostigmine in plasma by high-performance liquid chromatography and fluorescence detection. *Anal Biochem* 177: 207–211.
Gibson M *et al.* (1985). Physostigmine concentrations after oral doses. *Lancet* 1: 695–696.
Lawrence GD, Yatim N (1990). Extraction of physostigmine from biologic fluids and analysis by liquid chromatography with electrochemical detection. *J Pharmacol Methods* 24: 137–143.
Quinn KD, Stewart JT (1991). A high performance liquid chromatographic post-column fluorescent ion pair extraction system: application to physostigmine and its metabolite eseroline in human serum. *Biomed Chromatogr* 5: 8–13.
Unni LK *et al.* (1989). Determination of physostigmine in plasma and cerebrospinal fluid by liquid chromatography with electrochemical detection. *Clin Chem* 35: 292–295.
Whelpton R (1983). Analysis of plasma physostigmine concentrations by liquid chromatography. *J Chromatogr* 272: 216–220.
Whelpton R, Moore T (1985). Sensitive liquid chromatographic method for physostigmine in biological fluids using dual-electrode electrochemical detection. *J Chromatogr* 341: 361–371.

Physostigmine Aminoxide

Anticholinesterase
$C_{15}H_{21}N_3O_3 = 291.3$
CAS—25573-43-7
IUPAC Name [(4a*S*,9a*S*)-2,4a,9-Trimethyl-4,9a-dihydro-3*H*-oxazino[6,5-b]indol-6-yl] *N*-methylcarbamate
Synonyms Eseridine; eserine aminoxide; (4a*S*-*cis*)-2,3,4,4a,9,9a-hexahydro-2,4a,9-trimethyl-1,2-oxazino[6,5-*b*]indol-6-ol methylcarbamate ester; physostigmine *N*-oxide.



Chemical Properties Crystals. Mp 129°. Practically insoluble in water; soluble in ethanol, chloroform, benzene, acetone and ether. Log *P* (octanol/water), 2.1.

Physostigmine Aminoxide Salicylate

$C_{15}H_{21}N_3O_3,C_7H_6O_3 = 429.5$
CAS—5995-96-0

P

Synonyms Eseridine salicylate; eserine aminoxide salicylate; physostigmine *N*-oxide salicylate.
Proprietary Name *Geneserine 3*
Chemical Properties Crystals which become red on exposure to heat, light and air. Mp 90°.

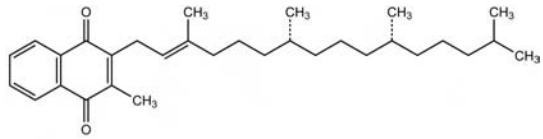
Thin-layer Chromatography System TA—R_f 0.31 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—241 (A^1_1=411b), 300 nm.
Infrared Spectrum Principal peaks at wavenumbers 1720, 1250, 1204, 1268, 1495, 1289 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC-MS** Limit of detection, 25 ng/L [Pruvost *et al.* 2000].
Dose Physostigmine aminoxide salicylate has been given in doses of 9 mg daily.

Pruvost A *et al.* (2000). Fully automated determination of eserine N-oxide in human plasma using on-line solid-phase extraction with liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J Mass Spectrom* 35: 625–633.

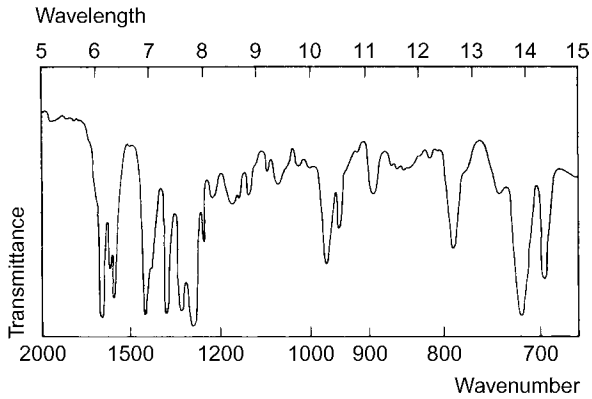
Phytomenadione

Vitamin
$C_{31}H_{46}O_2$ = 450.7
CAS—84-80-0

IUPAC Name 2-Methyl-3-[(*E*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione
Synonyms 2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl]-1,4-naphthalenedione; methylphytylnaphthochinonum; phylloquinone; phytonadione; vitamin K_1.
Proprietary Names *Aquamephyton*; *Kanakion*; *Kanavit*; *Konakion*; *Mephyton*; *Vitamin K*.



Chemical Properties A clear, deep yellow, very viscous oil which is stable in air but decomposes on exposure to light. Sp. gr. about 0.967. Refractive index 1.5255 to 1.5285. Practically insoluble in water; soluble 1 in 70 of ethanol; more soluble in dehydrated alcohol; sparingly soluble in methanol; freely soluble in chloroform and ether. Log *P* (octanol/water), 11.7.
Colour Test Methanolic potassium hydroxide—green-yellow→violet→brown.
Gas Chromatography System GA—RI 3287.
Ultraviolet Spectrum Methanol—244, 249 (A^1_1=394a), 263 (A^1_1=350b), 270 (A^1_1=349a), 331 nm (A^1_1=68a).
Infrared Spectrum Principal peaks at wavenumbers 1285, 1650, 714, 1587, 690, 1610 cm^{-1}.



Quantification
Plasma **HPLC** Fluorescence detection. Phytomenadione and vitamins A and E, and beta-carotene. Limit of detection, 0.04 µg/L for phytomenadione [Jakob, Elmadfa 1995]. Fluorescence detection. Phytomenadione, its epoxides, and menaquinone-4. Limit of detection, 30 to 50 ng/L [Hirauchi *et al.* 1988]. Fluorescence detection. Limit of detection, 0.05 µg/L [Haroon *et al.* 1986]. UV detection. Limit of detection, 500 pg [Shearer *et al.* 1982].
Serum **HPLC** Fluorescence detection. Limit of detection,about, 20 ng/L MacCrehan, Schonberger 1995]. Fluorescence detection. Phytomenadione. Limit of detection, 30 pg/inj. [Cham *et al.* 1989].
Liver **HPLC** Fluorescence detection. Phytomenadione and menaquinone [Usui *et al.* 1989].
Disposition in the Body Absorbed from the gastro-intestinal tract in the presence of bile. Rapidly metabolised and excreted as conjugates in the urine, bile and faeces. Endogenous plasma concentrations are usually in the range 0.0001 to 0.0007 mg/L.

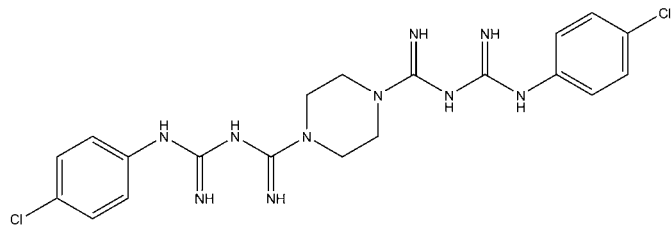
Therapeutic Concentration
Phytomenadione given in a dose of 10 mg as a mixed micellar preparation to 30 subjects, produced a mean maximum plasma concentration of 67 µg/L (range, 16 to 113) after 9.2 h (range, 2 to 24) after IM injection [Soedirman *et al.* 1996].

Phytomenadione given by i.v. injection to 18 neonates at a dose of 0.3 mg/kg, resulted in a mean blood concentration of 191.3 µg/L after 22.9 h; a further blood sample taken after 111.8 h from 10 of the neonates revealed a concentration of 98.7 µg/L [Raith *et al.* 2000].
Half-life Plasma half-life, about 2 h.
Dose Initially 2.5 to 25 mg by SC or IM injection, or 5 to 20 mg by mouth.

Cham BE *et al.* (1989). Simultaneous liquid-chromatographic determination of vitamin K1 and vitamin E in serum. *Clin Chem* 35: 2285–2289.
Haroon Y *et al.* (1986). Liquid-chromatographic determination of vitamin K1 in plasma, with fluorometric detection. *Clin Chem* 32: 1925–1929.
Hirauchi K *et al.* (1988). Simultaneous determination of vitamin K1, vitamin K1 2,3-epoxide and menaquinone-4 in human plasma by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 430: 21–29.
Jakob E, Elmadfa I (1995). Rapid HPLC assay for the assessment of vitamin K1, A, E and beta-carotene status in children (7-19 years). *Int J Vitam Nutr Res* 65: 31–35.
MacCrehan WA, Schonberger E (1995). Determination of vitamin K1 in serum using catalytic-reduction liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Appl* 670: 209–217.
Raith W *et al.* (2000). Plasma concentrations after intravenous administration of phylloquinone (vitamin K(1)) in preterm and sick neonates. *Thromb Res* 99: 467–472.
Shearer MJ *et al.* (1982). Plasma vitamin K1 in mothers and their newborn babies. *Lancet* 2: 460–463.
Soedirman JR *et al.* (1996). Pharmacokinetics and tolerance of intravenous and intramuscular phylloquinone (vitamin K1) mixed micelles formulation. *Br J Clin Pharmacol* 41: 517–523.
Usui Y *et al.* (1989). Measurement of vitamin K in human liver by gradient elution high-performance liquid chromatography using platinum-black catalyst reduction and fluorimetric detection. *J Chromatogr* 489: 291–301.

Picloxydine

Disinfectant
$C_{20}H_{24}C_{l2}N_{10}$ = 475.4
IUPAC Name 1-*N*′,4-*N*′-Bis[*N*′-(4-chlorophenyl)carbamimidoyl]piperazine-1,4-dicarboximidamide
Synonyms Bisguanizine; 1,4-di(*p*-chlorophenylguanidinoformimidoyl)piperazine; pikloxidin.



Chemical Properties White amorphous powder. Mp 247° to 251° with decomposition. Very sparingly soluble water and organic solvents. Picloxydine is extracted by chloroform : isopropanol (3 : 1) from aqueous alkaline solutions.

Picloxydine Dihydrochloride
Chemical Properties White crystalline solid. Mp 274° to 278° with decomposition. Soluble 1 in 500 of water; very slightly soluble in ethanol, methanol and acetone; insoluble in ether and chloroform.

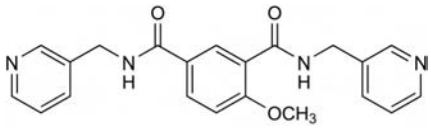
Picloxydine Digluconate
Proprietary Name It is an ingredient of *Resiguard*.
Chemical Properties A colourless or pale-yellow gel which melts to a clear yellow liquid at about 33°. Soluble in water and warm methanol; insoluble in ether and chloroform.

Thin-layer Chromatography System T1—R_f 0.10; R_f 0.33, streaking (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.
Ultraviolet Spectrum pH 4.6 buffer—233 (E1%, 1 cm 571), 253 nm (E1%, 1 cm 610).
Infrared Spectrum Principal peaks at wavenumbers 1000, 1560, 1570, 1650 cm^{-1} (KBr disk) (picloxydine dihydrochloride).
Use In a disinfectant fluid concentrate containing 1% as the digluconate.
Disposition in the Body
Toxicity LD_{50} in rats, >5 g/kg (oral).

Picotamide

Anticoagulant, Antithrombotic
$C_{21}H_{20}N_4O_3$ = 376.4
CAS—32828-81-2

IUPAC Name 4-Methoxy-1-*N*,3-*N*-bis(pyridin-3-ylmethyl)benzene-1,3-dicarboxamide
Synonyms G-137; 4-methoxy-*N*,*N*′-bis(3-pyridinylmethyl)-1,3-benzenedicarboxamide.



Chemical Properties A white or almost white crystalline powder. Mp 124°. Slightly soluble in water; soluble in ethanol, methanol and dichloromethane. It dissolves in dilute mineral acids. Log *P* (octanol/water), 1.53.

Picotamide Monohydrate

$C_{21}H_{20}N_4O_3,H_2O = 394.4$
Synonym Picotamidum monohydricum
Proprietary Name *Plactidil*

Thin-layer Chromatography Plate: Merck F-254 silica gel plates (0.25 mm). Mobile phase: (A) chloroform : methanol (9 : 1); (B) methanol : concentrated ammonia (100 : 105); (C) benzene : ethylacetate : acetic acid (2 : 1 : 0.1); (D) ethylacetate : ethanol (2 : 1). UV detection (λ=254 nm). Picotamide monohydrate (A) R_f 0.41; Picotamide (B) R_f 0.60; (C) R_f 0.0; (D) R_f 0.21 [Orzalesi *et al.* 1989].
High Performance Liquid Chromatography Column: LiChrosorb RP-Select B C_8 (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L sodium dihydrogen phosphate (pH 5.5; 28 : 72), flow rate 1 mL/min. IS: bamifylline. UV detection (λ=230 nm). Retention time: picotamide, 10 min; IS, 7 min [Fossati *et al.* 1992].

Column: Spherisorb S5 ODS1 (250 × 40 mm, 6 mm i.d.). Mobile phase: methanol : phosphate buffer (pH 7.9; 60 : 40), flow rate 0.8 mL/min. Retention time: 9.35 min. Limit of detection, 0.1 µg[Orzalesi *et al.* 1989].

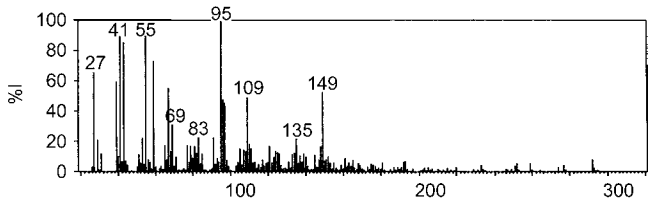
Quantification
Blood **HPLC** UV detection (λ=230 nm). Limit of quantification, 5 µg/L [Fossati *et al.* 1992].
Urine **HPLC** Limit of quantification, 1 µg/L, see Blood [Fossati *et al.* 1992].
Dose Initial daily dose, 900 to 1200 mg; maintenance dose of 300 to 600 mg daily.

Fossati T *et al.* (1992). Determination of picotamide in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 577: 382–386.
Orzalesi G *et al.* (1989). *Int J Pharm* 52: 225–229.

Picrotoxin

Respiratory Stimulant
$C_{30}H_{34}O_{13} = 602.6$
CAS—124-87-8

Synonym Cocculin
Chemical Properties An active principle from the seeds of *Anamirta cocculus* (=*A. paniculata*) (Menispermaceae). The fruits of *A. cocculus* are sometimes known as 'fish berries' or 'Levant berries'. Colourless, flexible, shining prismatic crystals or white microcrystalline powder. Mp 203°. Soluble 1 in 350 of water and 1 in 16 of ethanol; soluble in glacial acetic acid and solutions of acids and alkali hydroxides; slightly soluble in chloroform and ether. Log *P* (octanol/water), −2.8.
Colour Tests Dissolve in sulfuric acid—yellow→red-brown (slow); add 1 drop of nitric acid, fuming, to the material—blue-green, discharged by excess acid.
Infrared Spectrum Principal peaks at wavenumbers 1780, 1755, 1795, 1123, 990, 1162 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 95, 55, 41, 43, 59, 27, 39, 67.

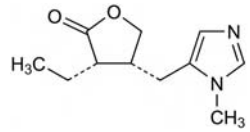


Dose Picrotoxin was formerly given in doses of 3 to 6 mg IV.

Pilocarpine

Parasympathomimetic
$C_{11}H_{16}N_2O_2 = 208.3$
CAS—92-13-7

IUPAC Name (3*S*,4*R*)-3-Ethyl-4-[(3-methylimidazol-4-yl)methyl]oxolan-2-one
Synonym (3*S*-*cis*)-3-Ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2(3*H*)-furanone
Proprietary Names *Ocusert Pilo*; *Pilocarpol*; *Piloftal*; *Pilomann-Ol*; *Piloplex*.



Chemical Properties An alkaloid obtained from the leaves of jaborandi, *Pilocarpus microphyllus* (Rutaceae) and other species of *Pilocarpus*. A viscous, hygroscopic, colourless, oily liquid or crystals. Mp about 34°. Soluble in water, ethanol and chloroform; sparingly soluble in ether and benzene. pK_a 1.6, 7.1 (15°). Log *P* (octanol/water), 0.1.

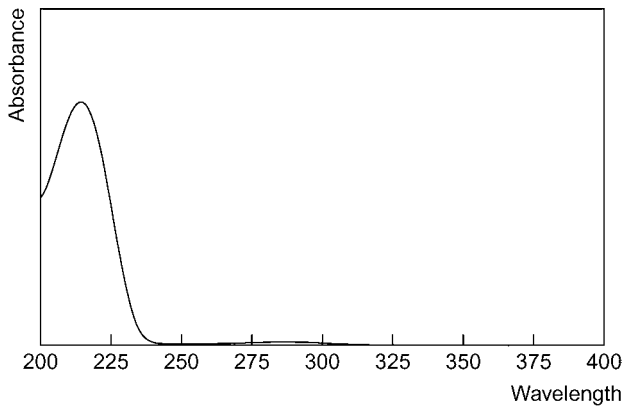
Pilocarpine Hydrochloride

$C_{11}H_{16}N_2O_2,HCl = 244.7$
CAS—54-71-7
Proprietary Names *Adsorbocarpine*; *Akarpine*; *Asthenopin*; *Borocarpin-S*; *Isopto Carpine*; *Diocarpine*; *Dropilton*; *Glaucocarpine Liocarpina*; *Miocarpine*; *Mi-Pilo*; *Ocu-Carpine*; *Pilax*; *Pilo*; *Pilocar*; *Pilocarcil*; *Pilogel*; *Pilomann*; *Pilopt*; *Piloptic*; *Pilostat*; *Pilo-Stulln*; *Pilotonina*; *PV Carpine*; *Salagen*; *Sno Pilo*; *Spersacarpin(e)*; *Storzine*; *Vistacarpin*. It is an ingredient of *E-Pilo* and *PE*.
Chemical Properties Hygroscopic, colourless crystals or white crystalline powder. Mp 204° to 205°. Soluble 1 in <1 of water, 1 in 3 of ethanol and 1 in 360 of chloroform; practically insoluble in ether.

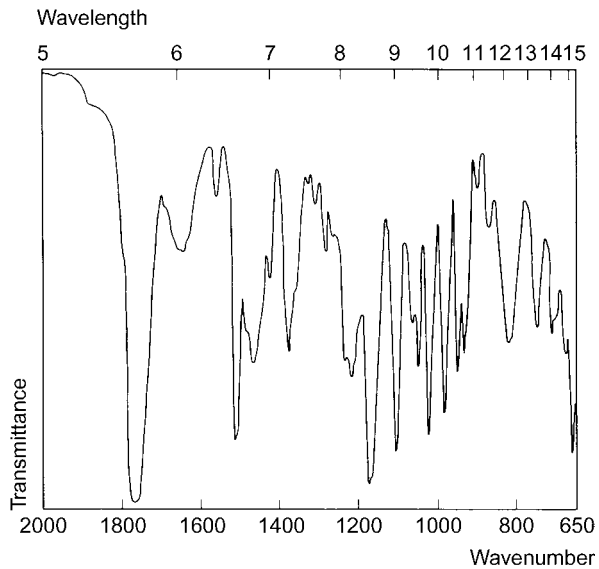
Pilocarpine Nitrate

$C_{11}H_{16}N_2O_2,HNO_3 = 271.3$
CAS—148-72-1
Proprietary Names *Chibro-Pilocarpine*; *Licarpin*; *Pilagan*; *Pilo*; *Pilopos*.
Chemical Properties Colourless crystals or white crystalline powder. Mp 173° to 174°, with decomposition. Soluble 1 in 4 of water and 1 in 75 of ethanol; insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.00; system TC—R_f 0.32; system TE—R_f 0.44; system TL—R_f 0.12; system TAE—R_f 0.52; system TAF—R_f 0.45; system TAJ—R_f 0.22; system TAK—R_f 0.00; system TAL—R_f 0.44 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2014.
High Performance Liquid Chromatography System HAA—retention time 4.6 min; system HY—RI 158.

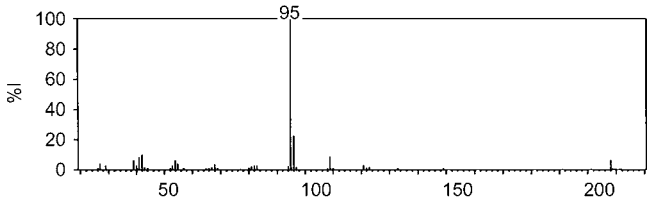


Infrared Spectrum Principal peaks at wavenumbers 1752, 1168, 1104, 660, 1505, 1020 cm^{-1} (thin film).



P

Mass Spectrum Principal ions at *m/z* 95, 96, 42, 109, 41, 208, 54, 39.



Quantification

Plasma **GC-MS** Pilocarpic acid. Limit of detection, <1 µg/L [Birk *et al.* 1998].

HPLC Limit of detection, 10 µg/L [Weaver *et al.* 1992].

HPLC-MS Pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid [van de Merbel *et al.* 1998].

Urine **HPLC-MS** See Plasma [van de Merbel *et al.* 1998].

Biological Fluids **HPLC** Limit of detection, 1 µg/L [Aromdee *et al.* 1996].

Therapeutic Concentration

Eighteen subjects receiving a single oral dose (2.5 to 20 mg) of pilocarpine had a mean peak plasma concentration of 20.5 µg/L (range, 12.5 to 33.2) at 1.21 h (0.50 to 1.87); the mean peak plasma concentration in 8 patients with renal failure receiving a similar dose was 30.9 µg/L (range, 12.7 to 47.0) at 0.61 h (range, 0.57 to 0.65) [St Peter *et al.* 2000].

Eight subjects given 15 mg of a controlled-release pilocarpine formulation every 12 h for 3 doses had a mean peak plasma concentration of 8.2 µg/L approx. 1 h after the first dose and 11.5 µg/L after the third dose; 24 h after the final dose the level had declined to 0.06 µg/L [Lockhart *et al.* 1996].

Uses Pilocarpine hydrochloride is used as a 0.5 to 5% ophthalmic solution and in the treatment of xerostomia/xerophthalmia.

Aromdee C *et al.* (1996). Sensitive high-performance liquid chromatographic assay for pilocarpine in biological fluids using fluorescence derivatisation. *J Chromatogr B Biomed Appl* 677: 313–318.

Birk KL *et al.* (1998). Determination of pilocarpic acid in human plasma by capillary gas chromatography with mass-selective detection. *J Chromatogr B Biomed Sci Appl* 719: 93–102.

Lockhart PB *et al.* (1996). Pilot study of controlled-release pilocarpine in normal subjects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 82: 517–524.

St Peter JV *et al.* (2000). Pharmacokinetics of pilocarpine in subjects with varying degrees of renal function. *J Clin Pharmacol* 40: 1470–1475.

van de Merbel NC *et al.* (1998). Determination of pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid in human plasma and urine by high-performance liquid chromatography with tandem mass spectrometric detection. *J Chromatogr B Biomed Sci Appl* 708: 103–112.

Weaver ML *et al.* (1992). High-performance liquid chromatographic determination of pilocarpine in plasma. *J Chromatogr* 581: 293–296.

Piminodine

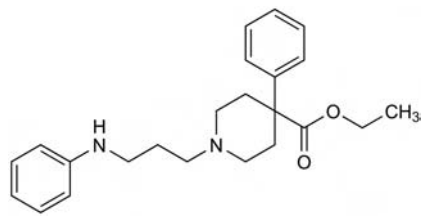
Narcotic Analgesic

$C_{23}H_{30}N_2O_2$ = 366.5

CAS—13495-09-5

IUPAC Name Ethyl 1-(3-anilinopropyl)-4-phenylpiperidine-4-carboxylate

Synonym 4-Phenyl-1-[3-(phenylamino)propyl]-4-piperidinecarboxylic acid ethyl ester



Chemical Properties Log *P* (octanol/water), 4.8.

Piminodine Esilate

$C_{23}H_{30}N_2O_2.C_2H_6O_3S$ = 476.6

CAS—7081-52-9

Synonyms Piminodine esylate; piminodine ethanesulfonate.

Chemical Properties A colourless crystalline powder. Mp 128° to 135°. Slightly soluble in water and ether; soluble 1 in 6 of ethanol and 1 in 2 of chloroform.

Colour Test Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.36; system TC—R_f 0.64; system TE—R_f 0.88; system TL—R_f 0.59; system TAE—R_f 0.63; system TAF—R_f 0.77; system TAJ—R_f 0.51; system TAK—R_f 0.24; system TAL—R_f 0.90 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 2884.

High Performance Liquid Chromatography System HA—*k* 1.0.

Ultraviolet Spectrum Aqueous acid—247, 253, 257, 263 nm.

Mass Spectrum Principal ions at *m/z* 246, 45, 42, 366, 58, 57, 43, 106.

Dose Piminodine esilate has been given in doses of 25 to 50 mg orally and 10 to 20 mg parenterally.

Pimozide

Tranquilliser

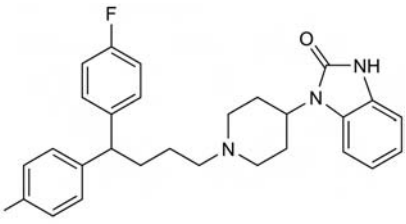
$C_{28}H_{29}F_2N_3O$ = 461.6

CAS—2062-78-4

IUPAC Name 3-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]- 1*H*-benzimidazol-2-one

Synonyms 1-[1-[4,4-Bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one; R-6238.

Proprietary Names *Antolan*; *Opiran*; *Orap*; *Pizide*.



Chemical Properties A colourless microcrystalline powder. Mp 214° to 218°. Practically insoluble in water; soluble 1 in 1000 of ethanol, ether and methanol, 1 in 10 of chloroform and 1 in 100 of acetone. pK_a 7.3. Log *P* (octanol/water), 6.3. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

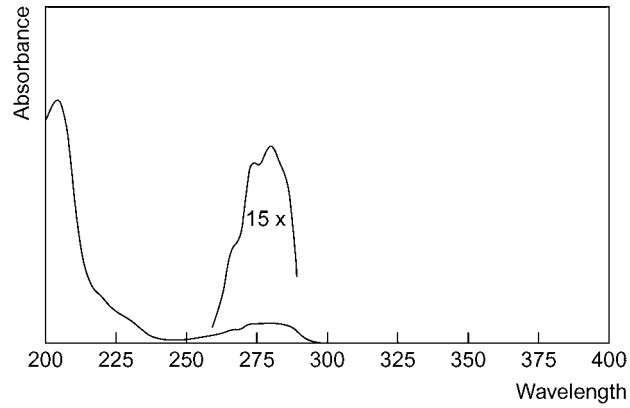
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—brown; Mandelin's test—brown; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.03; system TC—R_f 0.60; system TE—R_f 0.71; system TL—R_f 0.40; system TAE—R_f 0.73; system TAF—R_f 0.82 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—pimozide RI 3870, M (*N*-desalkyl-) RI 2415, M (benzimidazolone) RI 1950, M (desamino-OH-) RI 2120, M (Desamino-Carboxy-) RI 2130; system GB—not eluted.

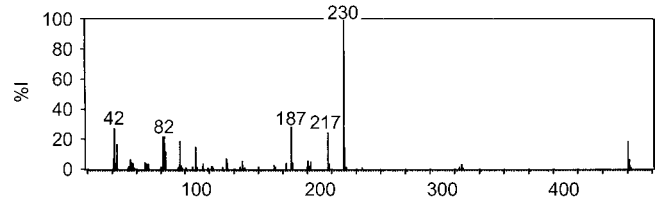
High Performance Liquid Chromatography System HA—*k* 0.7; system HX—RI 504; system HZ—retention time 11.9 min; system HAA—retention time 17.2 min.

Ultraviolet Spectrum Acid isopropyl alcohol—273, 280 nm (A^1_1=145a).



Infrared Spectrum Principal peaks at wavenumbers 1695, 1505, 753, 1220, 1230, 825 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 230, 187, 42, 217, 83, 82, 461, 96.



Quantification

Plasma **GC** FID. Limit of detection, 30 µg/L [Quaglio *et al.* 1982].

HPLC Fluorescence detection. Limit of detection, 1 µg/L [Kerbusch *et al.* 1997]. Fluorescence detection. Limit of detection, <5 µg/L [Miyao *et al.* 1983].

Radioimmunoassay Limit of detection, 50 pg [Michiels *et al.* 1975].

Disposition in the Body Readily but slowly absorbed after oral administration. About 40% of a dose is excreted in the urine in 4 days. Two metabolites which have been identified in the urine are 4-bis(4-fluorophenyl)butyric acid and 1-(4-piperidyl)benzimidazolin-2-one. Very little unchanged drug is excreted in the urine. Both unchanged drug and the butyric acid metabolite are eliminated in the faeces.

Therapeutic Concentration

Following a single oral dose of 6 mg to 9 subjects, a mean peak plasma concentration of about 0.004 mg/L was attained in 4 to 12 h (mean, 8) [McCreadie *et al.* 1979].

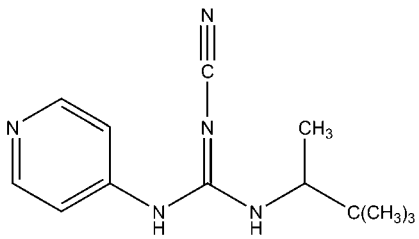
Half-life Plasma half-life, about 2 days.
Note For a review of pimozide, see Pinder *et al.* [1976].
Dose 2 to 20 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Kerbusch T *et al.* (1997). Sensitive assay for pimozide in human plasma using high-performance liquid chromatography with fluorescence detection: application to pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 694: 163–168.
McCreadie RG *et al.* (1979). Plasma pimozide profiles in chronic schizophrenics. *Br J Clin Pharmacol* 7: 533–534.
Michiels LJ *et al.* (1975). Radioimmunoassay of the neuroleptic drug pimozide. *Life Sci* 16: 937–944.
Miyao Y *et al.* (1983). A sensitive assay method for pimozide in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 275: 443–449.
Pinder RM *et al.* (1976). Pimozide: a review of its pharmacological properties and therapeutic uses in psychiatry. *Drugs* 12: 1–39.
Quaglio MP *et al.* (1982). Determination of benperidol, droperidol and pimozide in human plasma by GLC. *Boll Chim Farm* 121: 276–284.

Pinacidil

Antihypertensive, Guanidine, Potassium Channel Activator
$C_{13}H_{19}N_5 = 245.3$
CAS—60560-33-0

IUPAC Name 3-Cyano-2-(3,3-dimethylbutan-2-yl)-1-pyridin-4-ylguanidine
Synonyms *N*-Cyano-*N*"-4-pyridinyl-*N*"-(1,2,2-trimethylpropyl)guanidine; (±)-2-cyano-1-(4-pyridyl)-3-(1,2,2-trimethylpropyl)guanidine; P-1134; pinacidilum; pinasidiili.



Chemical Properties Very soluble in water (31.9 g/L). Log *P* (octanol/water), 1.06 [Meylan, Howard 1995].

Pinacidil Monohydrate

$C_{13}H_{19}N_5,H_2O = 263.3$
CAS—85371-64-8
Proprietary Name *Pindac*
Chemical Properties Crystals. Mp 164° to 165°. Soluble in water (166 mg/L). Log *P* (octanol/water), 2.55 [Meylan, Howard 1995].

Quantification

Plasma **HPLC** Column: LiChrosolv RP select B (250 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L dipotassium phosphate (pH 7.5; 51 : 49), flow rate 1.1 mL/min. UV detection (λ = 254 nm). Retention time: 9.0 min. Limit of quantification, 1 µg/L [Bareggi *et al.* 1999]. Column: C_8 (250 × 4.6 mm i.d., 6 µm). Mobile phase: methanol : 0.05 mol/L ammonium bicarbonate (pH 8; 50 : 50), flow rate 1 mL/min. Scintillation counting. Retention time: pinacidil 13 to 15 min, pinacidil-*N*-oxide 8 to 10 min, polar metabolites 2 to 5 min. Limit of detection, 5 µg/L [DeLong *et al.* 1988].
Serum **HPLC** Column: Hypersil ODS (100 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L diammonium hydrogen phosphate (33 : 67), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of quantification, not reported [Ward *et al.* 1984].
Urine **HPLC** Column: Hypersil ODS (100 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L diammonium hydrogen phosphate (20 : 80), flow rate 1.0 mL/min. UV detection (λ = 294 nm). Limit of quantification not reported [Ward *et al.* 1984].
Disposition in the Body Well absorbed from the gastrointestinal tract. Bioavailability is increased when administered with food, resulting in an increase in peak serum concentration of ~45%. Eliminated mainly by renal excretion of hepatic metabolites, the principal one being the pyridine-*N*-oxide form. Urinary excretion over 24 h accounts for 86% of a dose, with <4% recovered in faeces. Urinary recovery as unchanged pinacidil and its oxide metabolite accounts for ~10% and 60% of a dose, respectively; the remainder is excreted as free and conjugated analogues of pinacidil and metabolites.
Therapeutic Concentration Reported as ~80 to 300 µg/L in serum [Ward *et al.* 1984].

A group of 18 healthy volunteers (age: 22 to 48 years) was administered either half a 25 mg pinacidil slow-release tablet (group A) or a 12.5 mg standard slow-release capsule (group B) every 12 h for 6 days and on the morning of day 7. Mean peak plasma concentrations for groups A and B were 69.7 and 64.4 µg/L attained after 2.4 and 2.9 h, respectively. No significant difference was observed between the 2 formulations [Bareggi *et al.* 1999].

In a multicentre trial, 406 hypertensive patients were administered pinacidil orally twice daily in doses of 12.5 to 75 mg for varying periods of time. Mean plasma concentrations of pinacidil and its pyridine-*N*-oxide metabolite were reported as follows:

Dose (mg)	*Compound*	*Plasma Concentration (µg/L)*
12.5	Pinacidil	69.1
	Pinacidil-*N*-oxide	63.6
37.5	Pinacidil	190.0
	Pinacidil-*N*-oxide	109.2
75	Pinacidil	318.2
	Pinacidil-*N*-oxide	177.9

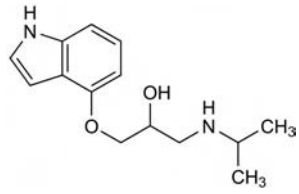
Age, race, and estimated creatinine clearance significantly influenced the plasma clearance of pinacidil [Goldberg *et al.* 1989].
Toxicity Facial flushing, uncomfortable chest sensation and distressing postural hypotension have been observed with serum concentrations above 300 µg/L [Ward *et al.* 1984].
Bioavailability Approximately 57%.
Half-life Approximately 3.4 h for pinacidil and 3.9 h for pinacidil-*N*-oxide.
Volume of Distribution Apparent, 90.3 L.
Clearance Plasma, 31 L/h in patients <45 years of age and 27 L/h in patients >60 years.
Distribution in Blood Blood : plasma ratio, 0.8 to 0.9.
Protein Binding Approximately 40%.
Dose Administered as oral doses of 12.5 mg to 75 mg twice daily.

Bareggi SR *et al.* (1999). Pharmacodynamics and pharmacokinetics of pinacidil in normotensive volunteers after repeated doses of a new slow-release tablet formulation. *Arzneimittelforschung* 49: 21–25.
DeLong AF *et al.* (1988). Disposition of [^{14}C]pinacidil in humans. *J Pharm Sci* 77: 153–156.
Goldberg MR *et al.* (1989). Clinical pharmacokinetics of pinacidil, a potassium channel opener, in hypertension. *J Clin Pharmacol* 29: 33–40.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Ward JW *et al.* (1984). Pharmacokinetics and hypotensive effect in healthy volunteers of pinacidil, a new potent vasodilator. *Eur J Clin Pharmacol* 26: 603–608.

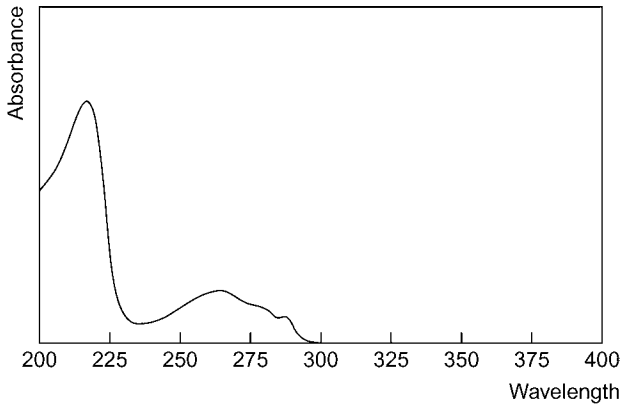
Pindolol

β-*Blocker*
$C_{14}H_{20}N_2O_2 = 248.3$
CAS—13523-86-9

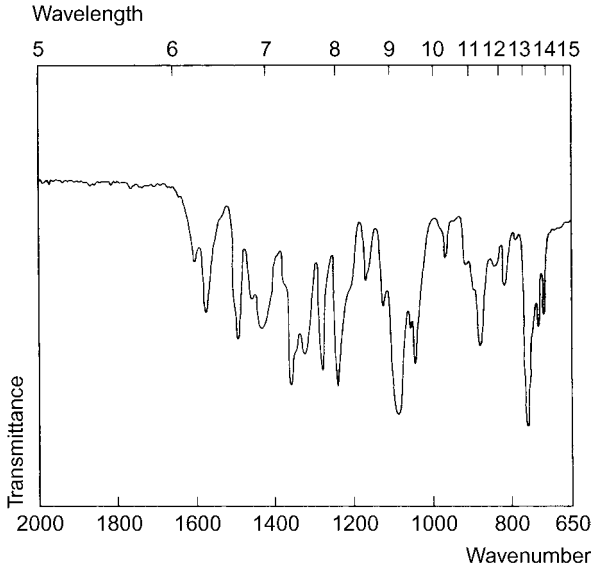
IUPAC Name 1-(1*H*-Indol-4-yloxy)-3-(propan-2-ylamino)propan-2-ol
Synonyms 1-(1*H*-Indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol; prindolol; prinodolol; LB-46.
Proprietary Names *Apo-Pindol*; *Barbloc*; *Betapindol*; *durapindol*; *Hexapindol*; *Novo-Pindol*; *Nu-Pindol*; *Pinden*; *Pindocor*; *Pindol*; *Pindoptan*; *Pinloc*; *Pinsken*; *Viskeen*; *Visken*; *Viskene*; *Vypen*. It is an ingredient of *Viskaldix*, *Viskazide* and *Viskenit*.



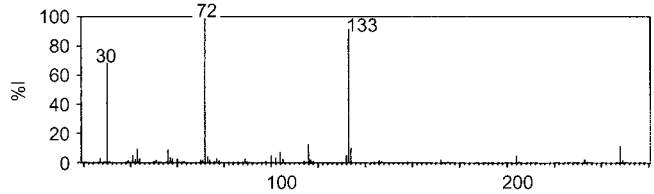
Chemical Properties A white or almost white crystalline powder. Mp 171° to 173°. Practically insoluble in water; slightly soluble in dehydrated alcohol, methanol and chloroform; soluble in mineral acids. pK_a 9.7 (24°). Log *P* (octanol/pH 7.0), −0.9. Extraction yield (chlorobutane), 0.4, 0.2 [Demme *et al.* 2005].
Colour Tests *p*-Dimethylaminobenzaldehyde—red/violet; Liebermann's reagent—blue-green; Mandelin's test—green; Marquis test—yellow→brown.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.02; system TC—R_f 0.05; system TE—R_f 0.43; system TL—R_f 0.08; system TAE—R_f 0.18; system TAF—R_f 0.78; system TAJ—R_f 0.00; system TAK—R_f 0.06; system TAL—R_f 0.56.
Gas Chromatography System GA—RI 2245; system GB—RI 2335.
High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 300; system HY—RI 253; system HZ—RT 2.4 min; system HAA—RT 8.6 min.
Ultraviolet Spectrum Aqueous acid—264 (A^1_1 = 292b), 287 nm; methanol—264 (A^1_1 = 330a), 287 nm (A^1_1 = 182a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 768, 1096, 1250, 1288, 1053, 890 cm^{-1}.



Mass Spectrum Principal peaks at *m/z* 72, 133, 30, 116, 248, 134, 56, 41.



Quantification

Plasma **GC** ECD. Limit of detection, 500 ng/L [Guerret 1980].

HPLC Fluorescence detection [Beal, Tett 1998]. Electrochemical detection [Telting-Diaz *et al* 1991]. UV or fluorescence detection. Pindolol and other β-blockers [Musch *et al.* 1989; Shields *et al.* 1986]. Fluorescence detection. Limit of detection, 2 µg/L [Bangah *et al.* 1980; Smith 1987].

Serum **HPLC** Fluorescence detection. Pindolol enantiomers. Limit of detection, 1.2 µg/L (R+) and 4.3 µg/L (R–) [Zhang *et al.* 1995]. Limit of detection, 2 µg/L [Chmielowiec *et al.* 1991].

Urine **HPLC** See Plasma [Beal, Tett 1998]. Limit of detection, 21 µg/L (R+) and 76 µg/L (R–) [Zhang *et al.* 1995]. See Plasma [Shields *et al.* 1986].

Disposition in the Body Well absorbed after oral administration. Metabolised by conjugation with glucuronic acid and sulfate. About 20–30% of an oral dose is excreted in the urine in 24 h as unchanged drug.

Therapeutic Concentration

After a single oral dose of 5 mg given to 12 subjects, peak plasma concentrations of 0.02–0.08 mg/L (mean, 0.04) were attained in 0.5–3 h [Gugler *et al.* 1974].

After daily oral dosing of 6 subjects with 5 mg three times a day, a mean steady-state plasma concentration of 0.015 mg/L was reported [Gugler, Bodem 1978].

Half-life Plasma half-life, 2–4 h.

Volume of Distribution About 1–2 L/kg.

Clearance Plasma clearance, about 7 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.5.

Protein Binding 40–70%.

Note For a review of the pharmacokinetics of the β-blocking agents, see Johnsson and Regärdh [1976].

Dose Up to 45 mg daily.

Bangah M *et al.* (1980). Determination of pindolol in human plasma by high-performance liquid chromatography. *J Chromatogr* 183: 255–259.

Beal JL, Tett SE (1998). Determination of pindolol enantiomers in human plasma and urine by simple liquid-liquid extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 715: 409–415.

Chmielowiec D *et al.* (1991). Determination of pindolol in human serum by HPLC. *J Chromatogr Sci* 29: 37–39.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Guerret M (1980). Determination of pindolol in biological fluids by an electron-capture gas-liquid chromatographic method on a wall-coated open tubular column. *J Chromatogr* 221: 387–392.

Gugler R *et al.* (1974). Pharmacokinetics of pindolol in man. *Eur J Clin Pharmacol* 7: 17–24.

Gugler R, Bodem G (1978). Single and multiple dose pharmacokinetics of pindolol. *Eur J Clin Pharmacol* 13: 13–16.

Johnsson G, Regärdh CG (1976). Clinical pharmacokinetics of beta-adrenoreceptor blocking drugs. *Clin Pharmacokinet* 1: 233–263.

Musch G *et al.* (1989). A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography. *J Pharm Biomed Anal* 7: 483–497.

Shields BJ *et al.* (1986). Determination of pindolol in human plasma and urine by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 378: 163–171.

Smith HT (1987). High-performance liquid chromatographic method for the determination of pindolol in human plasma. *J Chromatogr* 415: 95–103.

Telting-Diaz M *et al.* (1991). High-performance liquid chromatographic determination of nifedipine, nicardipine and pindolol using a carbon fibre flow-through amperometric detector. *J Pharm Biomed Anal* 9: 889–893.

Zhang H *et al.* (1995). High-performance liquid chromatographic analysis of pindolol enantiomers in human serum and urine using a reversed-phase cellulose-based chiral column. *J Chromatogr B Biomed Appl* 668: 309–313.

Pioglitazone

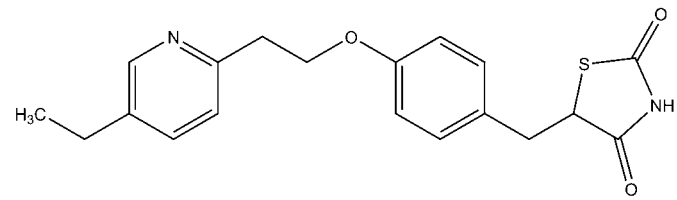
Antidiabetic, Thiazolidinedione

$C_{19}H_{20}N_2O_3S = 356.4$

CAS—111025-46-8

IUPAC Name 5-[[4-[2-(5-Ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione

Synonyms AD-4833; 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazoilidinedione; (±)-5-{*p*-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl}-2,4-thiazolidinedione; U-7210E.



Chemical Properties Colourless needles from DMF and water. Mp 183° to 184°. pK_a 6.35 in acidic media, 5.56 in basic media [Giaginis *et al.* 2007]. Log *P* (octanol/water), 3.31 [Giaginis *et al.* 2007]. Parent compound and metabolites stable in serum and urine for at least 2 months at –20° [Yamashita *et al.* 1996].

Pioglitazone Hydrochloride

$C_{19}H_{20}N_2O_3S,HCl = 392.9$

CAS—112529-15-4

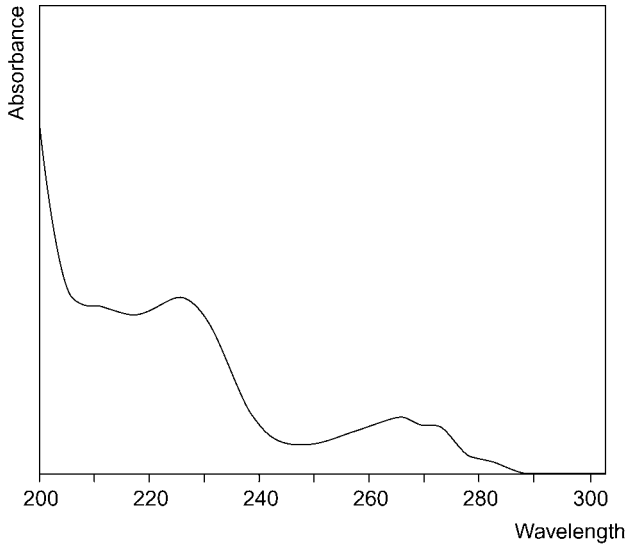
Synonym U-72107A

Proprietary Names *Actos*; *Cereluc*; *Diabestat*; *Diaglit*; *Glita*; *Glizone*; *Glustin*; *G-Tase*; *Higlucem*; *Opam*; *Pepar*; *P-Glitz*; *Pioglit*; *Piomed*; *Piosafe*; *Piotamax*; *Piozulin*; *Prialta*; *Tiazac*; *Zactos*; *Zypi*.

Chemical Properties Colourless prisms from ethanol. Mp 193° to 194°. Soluble in DMF; slightly soluble in ethanol; very slightly soluble in acetone, acetonitrile; practically insoluble in water; insoluble in ether.

High Performance Liquid Chromatography Column: Hichrom-RPB (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogen phosphate (pH 6.0; 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 225 nm). Retention times: 8.7 min pioglitazone; 3.4 min, 5.6 min and 18.0 min for impurities I, II, III, respectively. Limit of detection not reported [Kumar *et al.* 2004]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L ammonium formate buffer (pH 4.1; 55 : 45), flow rate 1.0 mL/min. UV detection (λ = 266 nm). Retention time: 6.1 min. Limit of quantification, 125 µg/L, limit of detection, 41.7 µg/L [Jedlicka *et al.* 2004]. Column: Zorbax XDB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L disodium hydrogen phosphate (pH 7.1; 34 : 66) containing 5 mmol/L SDS, flow rate 1.0 mL/min. UV detection (λ = 226 nm). Retention time: 7.8 min. Limit of quantification, 10 µg/L; limit of detection, 3 µg/L [Kolte *et al.* 2004].

Ultraviolet Spectrum Methanol : acetonitrile (30 : 70)—226, 266 nm.



Infrared Spectrum Principal peaks at wavenumbers 3082, 2964, 1736, 1690, 1472, 1331, 1254, 1040, 841, 728 cm^{-1} (KBr disc).

Mass Spectrum Principal ions at *m/z* 150, 134, 121, 356.

Quantification

Plasma **HPLC** Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 10 mmol/L potassium phosphate buffer (pH 2.6, 40 : 12 : 48), flow rate 1.2 mL/min. UV detection (λ = 269 nm). Retention time: 8.2 min. Limit of quantification, 50 µg/L [Sripalakit *et al.* 2006].

LC-MS Column: C_{18} (50 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile : water (60 : 40) with 10 mmol/L ammonium acetate and 0.02% trifluoroacetic acid, flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: pioglitazone 1.72 min, keto-metabolite 1.09 min, hydroxy-metabolite 0.93 min. Limit of quantification, 0.5 µg/L [Lin *et al.* 2003]. Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate in acetonitrile-water (10 : 90) : water-acetonitrile (10 : 90; 50 : 50), flow rate 1.35 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 1.9 min. Limit of quantification, 9 µg/L [Xue *et al.* 2003]. Column: Zorbax SB-C_{18} (75 × 4.6 mm i.d., 3.5 µm). Mobile phase: methanol : ammonium acetate (1 : 1), flow rate 1.0 mL/min. API, positive ion mode, MRM acquisition mode. Limit of quantification, pioglitazone 10 µg/L, hydroxy-metabolite 0.99 µg/L [Wong *et al.* 2004].

Serum **HPLC** Column: Inertsil ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol-0.05 mol/L phosphate buffer (pH 6.0, 1 : 9) : methanol-acetonitrile-0.05 mol/L phosphate buffer (pH 6.0, 2 : 4 : 4; 80 : 20 to 55 : 45 over 18 min, to 0 : 100 over 24 min for 7 min), flow rate 1.0 mL/min. UV detection (λ = 269 nm). Retention time: ~9 min. Limit of detection, pioglitazone, its keto- and hydroxyl-metabolites 0.01 mg/L, minor metabolites 0.02 to 0.05 mg/L [Yamashita *et al.* 1996]. Column: Zorbax RX-C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water-3 mL/L acetic acid (pH 5.5; 40 : 60), flow rate 1.2 mL/min (0 to 10 min) and 1.6 mL/min (10 to 20 min). UV detection (λ = 269 nm). Retention time: ~14.2 min. Limit of quantification, pioglitazone, its keto- and hydroxy-metabolites 0.2 mg/L, minor metabolites 0.2 to 0.5 mg/L [Zhong, Williams 1996].

Urine **HPLC** Column: Inertsil ODS-2 (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol–0.05 mol/L phosphate buffer (pH 6.0; 1 : 9) : methanol-acetonitrile-0.05 mol/L phosphate buffer (pH 6.0, 2 : 4 : 4; 95 : 5 to 0 : 100 over 42 min for 7 min), flow rate 1.0 mL/min. UV detection (λ = 269 nm). Retention time: ~42 min. Limit of detection, pioglitazone, its keto- and hydroxy-metabolites 0.1 mg/L, minor metabolites 0.2 to 0.5 mg/L [Yamashita *et al.* 1996].

Other **HPLC** Tablets. Column: Hypersil ODS-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : acetic acid (pH 5.5; 75 : 25 : 0.3), flow rate 0.5 mL/min. UV detection (λ = 230 nm). Retention time: 8.5 min. Limit of detection, 9.9 µg/L [Shankar *et al.* 2005]. Column: Zorbax XDB C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L disodium hydrogen phosphate (pH 7.1; 34 : 66) containing 5 mmol/L SDS, flow rate 1.0 mL/min. UV detection (λ = 226 nm). Retention time: 7.8 min. Limit of quantification, 10 µg/L; limit of detection, 3 µg/L [Kolte *et al.* 2004].

CE Tablets. Capillary: fused silica (34.5 cm × 50 µm i.d.). Buffer: 20 mmol/L sodium borate (pH 9.3)-50 mmol/L SDS : acetonitrile (80 : 20). UV detection (λ = 210 nm). Limit of quantification, 0.74 mg/L; limit of detection, 0.29 mg/L [Radhakrishna *et al.* 2002].

Disposition in the Body Rapidly absorbed after oral doses, with peak plasma concentrations occurring within 2 h. It is extensively metabolised in the liver by oxidation and hydroxylation of aliphatic methylene groups, primarily by CYP2C8 and CYP3A4 to form 5 primary metabolites and 2 secondary metabolites. These include both active (keto- and hydroxy-derivatives) and inactive metabolites. Three of the metabolites have pharmacological activity and have an anti-hyperglycaemic potency ~40 to 60% of that of pioglitazone. Secondary metabolic pathways involve CYP2C9 and CYP1A1/2. Approximately 15 to 30% of a pioglitazone dose is recovered in urine as metabolites. Renal elimination of unchanged pioglitazone is negligible. It is presumed that the remainder is excreted in bile either unchanged or as metabolites and eliminated in the faeces.

Therapeutic Concentration

Twelve healthy young adults (mean age 23 years) were administered pioglitazone 15 or 30 mg/day for 8 days. Peak plasma concentrations for the 2 doses at steady state were 0.7 and 1.7 mg/L reached at 2.5 and 3 h, respectively. Following the same regimen, plasma concentrations obtained in healthy elderly volunteers (mean age 70 years) were 0.7 and 1.2 mg/L, respectively, for the 2 doses, reached at 4.8 and 3.7 h, respectively [Ogiwara *et al.* 1997].

A group of 24 healthy male volunteers was administered a single oral dose of 30 mg pioglitazone. A mean peak plasma concentration of 1.6 mg/L was reached at 1.5 h [Sripalakit *et al.* 2006].

Toxicity When pioglitazone is given with gemfibrozil, an inhibitor of CYP2C8, there is a 3-fold increase in the AUC of pioglitazone; a decrease in pioglitazone dose may be needed if it is given with gemfibrozil or similar CYP2C8 inhibitors. Equally, rifampicin, a potent inducer of CYP540, halves the AUC of pioglitazone when given concomitantly, and the pioglitazone dose may need to be increased. Studies have shown that AUC values for pioglitazone and its 2 major metabolites (keto- and hydroxy-forms) decrease with increasing impairment of renal function [Edwards, Eckland 1999].

Bioavailability >80%.

Half-life Pioglitazone, up to 7 h; active metabolites, up to 24 h.

Protein Binding >99%, predominantly to albumin.

Dose Given as pioglitazone hydrochloride but doses are expressed in terms of the base; pioglitazone hydrochloride 1.1 mg is equivalent to ~1 mg pioglitazone. The usual dose is 15 or 30 mg once daily. This may be increased to a maximum of 45 mg once daily if necessary. It may be taken with or without food.

Edwards G, Eckland D (1999). Pharmacokinetics of pioglitazone in patients with renal impairment. *Diabetologia* 48(Suppl.1)A230.

Giaginis *et al.* (2007). Investigation of the lipophilic behaviour of some thiazolidinediones. Relationships with PPAR-gamma activity. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 181–187.

Jedlicka A *et al.* (2004). Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets. *Pharmazie* 59: 178–182.

Kolte BL *et al.* (2004). Simultaneous high-performance liquid chromatographic determination of pioglitazone and metformin in pharmaceutical-dosage form. *J Chromatogr Sci* 42: 27–31.

Kumar YR *et al.* (2004). Structural characterization of impurities in pioglitazone. *Pharmazie* 59: 836–839.

Lin ZJ *et al.* (2003). Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS. *J Pharm Biomed Anal* 33: 101–108.

Ogiwara T *et al.* (1997). The pharmacokinetics of AD-4833 in elderly patients. *Rinsho to Kenkyu* 74: 1307–1318.

Radhakrishna T *et al.* (2002). Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods. *J Pharm Biomed Anal* 29: 593–607.

Shankar MB *et al.* (2005). Estimation of pioglitazone hydrochloride and metformin hydrochloride in tablets by derivative spectrophotometry and liquid chromatographic methods. *J AOAC Int* 88: 1167–1172.

Sripalakit P *et al.* (2006). High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 164–169.

Wong H *et al.* (2004). In vivo bioequivalence of oral antidiabetic agents: pioglitazone tablets. *Arzneimittelforschung* 54: 618–624.

Xue YJ *et al.* (2003). Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci* 795: 215–226.

Yamashita K *et al.* (1996). High-performance liquid chromatographic determination of pioglitazone and its metabolites in human serum and urine. *J Chromatogr B Biomed Appl* 677: 141–146.

Zhong WZ, Williams MG (1996). Simultaneous quantitation of pioglitazone and its metabolites in human serum by liquid chromatography and solid phase extraction. *J Pharm Biomed Anal* 14: 465–473.

Pipamazine

Antiemetic

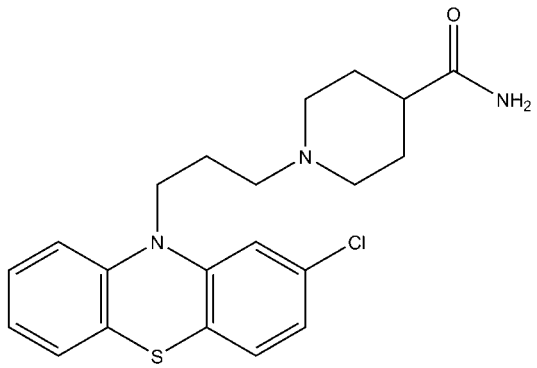
$C_{21}H_{24}ClN_3OS$ = 402

CAS—84-04-8

IUPAC Name 1-[3-(2-Chlorophenothiazin-10-yl)propyl]piperidine-4-carboxamide

Synonyms 10-[3-(4-Carbamoylpiperidino)propyl]-2-chlorophenothiazine; 10-[3-(4-carbamoylpiperidin-1-yl)propyl]-2-chlorophenothiazine; 2-chloro-10-[3-(4-carbamoylpiperidinyl)propyl]phenothiazine; 1-[3-(2-chlorophenothiazin-10-yl)propyl]isonipecotamide; 1-[3-(2-chloro-10*H*-phenothiazin-10-yl)propyl]-4-piperidinecarboxamide; EINECS 201-512-9; mometine; nometine; SC-9387.

Proprietary Names *Mornidine*; *Nausidol*.



Chemical Properties Crystals from 2-propanol and petroleum ether. Mp ≈140°. Soluble 1 in 500 of water, 1 in 60 of ethanol, 1 in 200 of ether and 1 in 5 of chloroform. Log *P* (octanol/water) 4.1 [National Institutes of Health 2008]. Pipamazine is extracted by organic solvents from aqueous organic solvents.

Pipamazine Hydrochloride

$C_{21}H_{24}ClN_3OS,HCl = 438.4$

Chemical Properties Crystals. Mp ≈196° to 197° with formation of bubbles [O'Neil *et al.* 2006].

Colour Tests Ammonium molybdate test—purple (limit of detection, 0.1 µg); ammonium vandate test—green→purple (limit of detection, 0.1 µg); Vitali's test—purple→yellow/yellow/yellow (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—purple (limit of detection, 0.1 µg).

Thin-layer Chromatography System T1—R_f 0.62 (location reagent acidified iodoplatinate spray, positive reaction); system T6—R_f 0.83 (the spot may be converted on the plate to the sulfoxide, R_f 0.53; location of both spots under ultraviolet light).

Ultraviolet Spectrum Aqueous acid (0.01 N hydrochloric acid)—255 nm.

Infrared Spectrum Principal peaks at wavenumbers 1644, 1455, 747 cm^{-1} (KBr disk).

Dose Up to 30 mg daily.

National Institutes of Health (2008). *Pipamazine*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=67618loc=ec_rcs. (accessed 27 June 2008).

O'Neil MJ *et al.* (2006). *The Merck Index: an Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Pipamperone

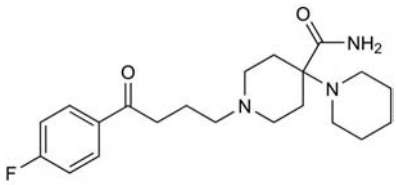
Tranquilliser

$C_{21}H_{30}FN_3O_2 = 375.5$

CAS—1893-33-0

IUPAC Name 1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-piperidin-1-ylpiperidine-4-carboxamide

Synonyms 1′-[4-(4-Fluorophenyl)-4-oxobutyl]-[1,4′-bipiperidine]-4′-carboxamide; floropipamide; R-3345.



Chemical Properties Log *P* (octanol), 2.4; (octanol/water), 2.0. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Pipamperone Hydrochloride

$C_{21}H_{30}FN_3O_2,2HCl = 448.4$

CAS—2448-68-2

Proprietary Names *Dipiperon*; *Piperonil*.

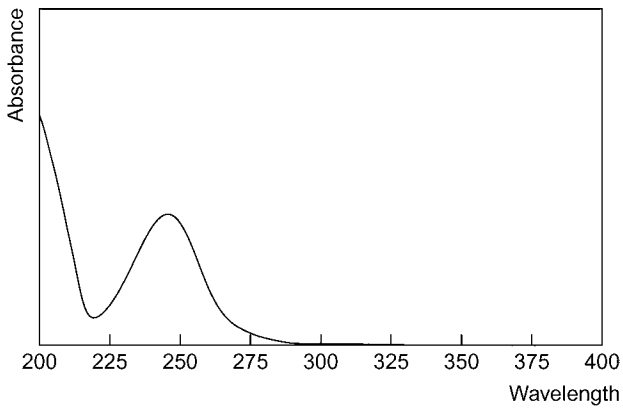
Chemical Properties Crystals. Mp 124.5° to 126.0°.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.01; system TC—R_f 0.12; system TE—R_f 0.43; system TL—R_f 0.08; system TAE—R_f 0.33; system TAF—R_f 0.61 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—pipamperone RI 3040, M (OH-) RI 3250.

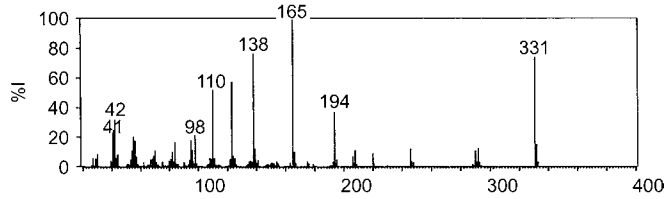
High Performance Liquid Chromatography System HX—RI 299; system HY—RI 241; system HZ—retention time 2.7 min; system HAA—retention time 10.9 min.

Ultraviolet Spectrum Aqueous acid—248 nm (A_1^1=315a).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1685, 1600, 1239, 1157, 1212 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 165, 138, 331, 123, 110, 194, 42, 41.



Quantification

Blood **GC** SID. Pipamperone and other butyrophenones. Limit of detection, about 0.1 nmol/L [Seno *et al.* 1993].

Plasma **HPLC** UV detection. Limit of detection, 2 µg/L [Luhmann *et al.* 1992].

Urine **GC** See Blood [Seno *et al.* 1993].

Disposition in the Body

Therapeutic Concentration

Pipamperone dihydrochloride (≡120 mg pipamperone), given as either a reference tablet or one of two test tablets, to 24 subjects as a single oral dose, produced mean maximum plasma concentrations of 0.266 mg/L (range, 0.161 to 0.455) for the reference product, 0.266 mg/L (range, 0.087 to 0.509) for one of the test products and 0.263 mg/L (range, 0.123 to 0.502) for the other. The time taken to reach maximum plasma concentrations was 1 h for all preparations [Potgieter *et al.* 2002].

Mean plasma concentrations of 0.04403 (±0.01744) mg/L were attained 1.5 h after a single oral dose of 40 mg to 12 subjects [Luhmann *et al.* 1992].

Dose The equivalent of 120 to 360 mg of pipamperone daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul 481–486.

Luhmann I *et al.* (1992). Determination of pipamperone in human plasma by high performance liquid chromatography. *Arzneimittelforschung* 42: 1069–1072.

Potgieter GE *et al.* (2002). Pharmacokinetics of pipamperone from three different tablet formulations. *Arzneimittelforschung* 52: 430–434.

Seno H *et al.* (1993). Determination of some butyrophenones in body fluids by gas chromatography with surface ionization detection. *Nippon Hoigaku Zasshi* 47: 367–371.

Pipazetate

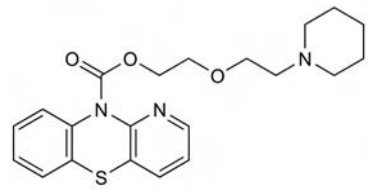
Cough Suppressant

$C_{21}H_{25}N_3O_3S = 399.5$

CAS—2167-85-3

IUPAC Name 2-(2-Piperidin-1-ylethoxy)ethyl pyrido[3,2-b][1,4]benzothiazine-10-carboxylate

Synonyms D-254; pipazethate; piperestazine; 10*H*-pyrido[3,2-*b*][1,4]benzothiadiazine-10-carboxylic acid 2-(2-piperidinoethoxy)ethyl ester.



Chemical Properties Log *P* (octanol/water), 4.9.

Pipazetate Hydrochloride

$C_{21}H_{25}N_3O_3S,HCl = 436.0$

CAS—6056-11-7

Proprietary Names *Selvigon*; *Selvjgon*; *Transpulmin*; *Lenopect*; *Theratuss*.

Chemical Properties A white crystalline powder. Mp 160° to 161°. Very soluble in water; soluble in ethanol and methanol. Practically insoluble in acetone.

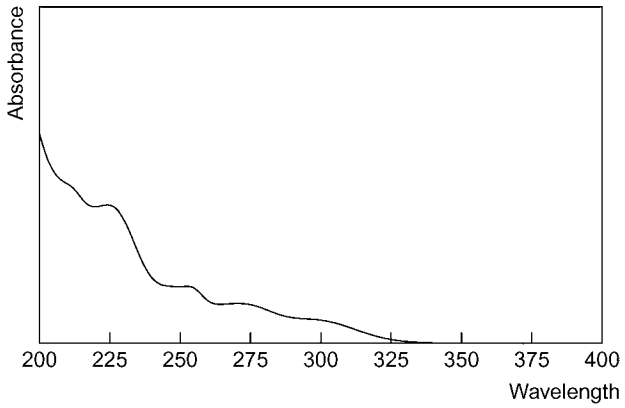
Colour Tests Aromaticity (method 2)—colourless/yellow; Liebermann's test—brown (→red at 100°).

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.17; system TC—R_f 0.13; system TE—R_f 0.48; system TL—R_f 0.06; system TAE—R_f 0.12 (acidified iodoplatinate solution, positive).

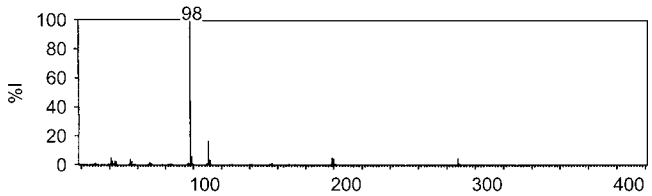
Gas Chromatography System GA—RI 2037.

High Performance Liquid Chromatography System HA—*k* 5.4; system HX—RI 385.

Ultraviolet Spectrum Aqueous acid—251 nm (A_1^1=210a).



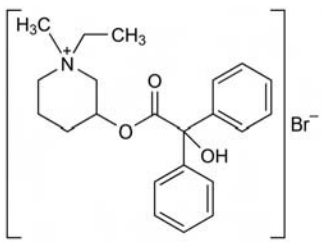
Infrared Spectrum Principal peaks at wavenumbers 1717, 1225, 1315, 1300, 1095, 1050 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 111, 99, 199, 41, 288, 200, 55.



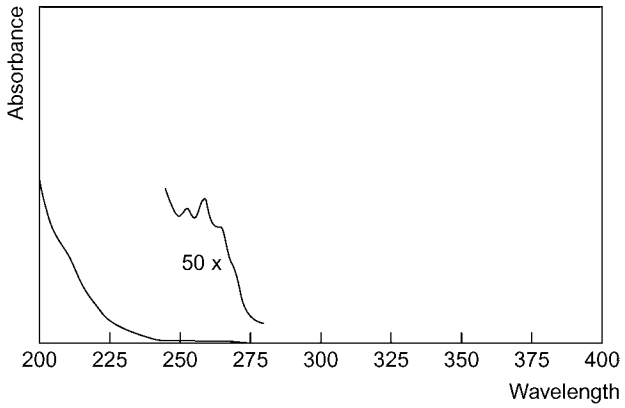
Dose Pipazetate hydrochloride has been given in doses of 60 to 120 mg daily.

Pipenzolate Bromide

Anticholinergic
$C_{22}H_{28}BrNO_3 = 434.4$
CAS—13473-38-6 (pipenzolate); 125-51-9 (bromide)
IUPAC Name (1-Ethyl-1-methylpiperidin-1-ium-3-yl) 2-hydroxy-2,2-diphenylacetate bromide
Synonyms 1-Ethyl-3-[(hydroxydiphenylacetyl)-oxy]-1-methylpiperidinium bromide; JB-323; pipenzolate methobromide; pipenzolone bromide.
Proprietary Names *Expal*; *Ila-med m*; *Piptal*; *Poliptal*; *Propedil*. It is an ingredient of *Finprod*, *Pedriachol* and *Piptalin*.



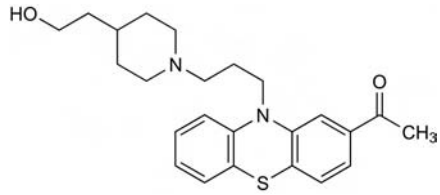
Chemical Properties A white crystalline powder. Mp 179° to 180°. Freely soluble in water. Log *P* (octanol/water), −0.3.
Colour Tests The following tests are performed on pipenzolate nitrate. Mandelin's test—orange→green; Marquis test—orange→green→blue.
Thin-layer Chromatography System TA—R_f 0.04 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=11a), 262 nm.



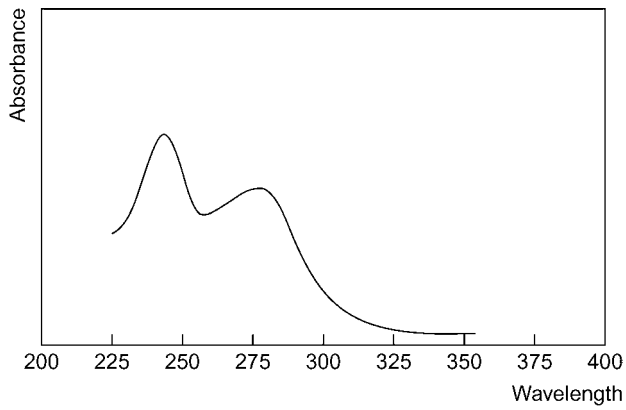
Infrared Spectrum Principal peaks at wavenumbers 1726, 698, 1220, 1160, 763, 1055 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 105, 111, 77, 96, 97, 183, 42, 51.
Dose Usually 20 to 25 mg daily.

Piperacetazine

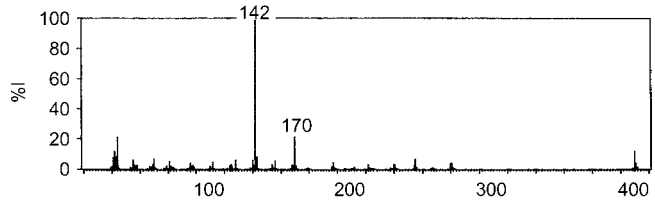
Tranquilliser
$C_{24}H_{30}N_2O_2S = 410.6$
CAS—3819-00-9
IUPAC Name 1-[10-[3-[4-(2-Hydroxyethyl)piperidin-1-yl]propyl]phenothiazin-2-yl]ethanone
Synonyms 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperidinyl]propyl]-10*H*-phenothiazin-2-yl]ethanone; PC-1421.
Proprietary Name *Quide*



Chemical Properties A yellow granular powder. Mp 100° to 110°. Practically insoluble in water; soluble 1 in 11 of ethanol, 1 in 1.3 of chloroform and 1 in 1200 of ether. Log *P* (octanol/water), 5.1.
Colour Tests Formaldehyde-sulfuric acid—blue-violet; Forrest reagent—red; FPN reagent—brown-orange; Mandelin's test—green→red→violet; Marquis test—red; sulfuric acid—yellow→red.
Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.06; system TC—R_f 0.19; system TL—R_f 0.17; system TAJ—R_f 0.04; system TAK—R_f 0.00; system TAL—R_f 0.30 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 1.9.
Ultraviolet Spectrum Aqueous acid—243, 278 nm.



Infrared Spectrum Principal peaks at wavenumbers 1675, 1222, 1269, 1592, 748, 1560 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 142, 170, 44, 410, 42, 143, 43, 41.

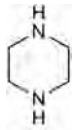


Dose 20 to 160 mg daily.

Piperazine

Anthelmintic
$C_4H_{10}N_2 = 86.1$
CAS—110-85-0
IUPAC Name Piperazine
Proprietary Names *Lombrimade*; *Lu-Peracina*; *Ortovermin*; *Oxiurazina*; *Overpon*; *Pipemed*; *Piperazil*; *Pipercream*; *Pipermel*; *Pipertox*; *Pipervermin*; *Pirzinol*; *Trivermon*; *Verfid*; *Vermilen*; *Vermin*; *Versol*.

P



Chemical Properties Crystals. Mp 106°. Soluble in water and ethanol; practically insoluble in ether. pK_a 5.7, 9.8 (20°). Log *P* (octanol/water), −1.5.

Piperazine Adipate

$C_4H_{10}N_2,C_6H_{10}O_4 = 232.3$
CAS—142-88-1
Proprietary Names *Adelmintex*; *Antepar*; *Entacyl*; *Vermi*.
Chemical Properties A white crystalline powder. Mp 256° to 257°. Soluble 1 in 18 of water; practically insoluble in ethanol, chloroform and ether.

Piperazine Calcium Edetate

$C_4H_{10}N_2,C_{10}H_{14}CaN_2O_8 = 416.4$
CAS—12002-30-1
Synonym Piperazine calcium edathamil
Chemical Properties Freely soluble in water; very slightly soluble in ethanol and chloroform; practically insoluble in ether.

Piperazine Citrate

$(C_4H_{10}N_2)_3,2C_6H_8O_7,xH_2O = 642.7$ (anhydrous)
CAS—144-29-6 (anhydrous); 41372-10-5 (hydrate)
Synonym Hydrous tripiperazine dicitrate
Proprietary Names *Antepar*; *Citrazine*; *Citropiperazina*; *Ectodyne*; *Helmifar*; *Pipermed*; *Piprine*; *Pripsen Elixir*; *Tasnon*; *Veriga*; *Verimex*; *Vermex*; *Worm*; *Wormex*; *Wormilex*.
Chemical Properties A fine, white, crystalline or granular powder. Mp about 190°. Soluble 1 in 1.5 of water; practically insoluble in ethanol, chloroform and ether.

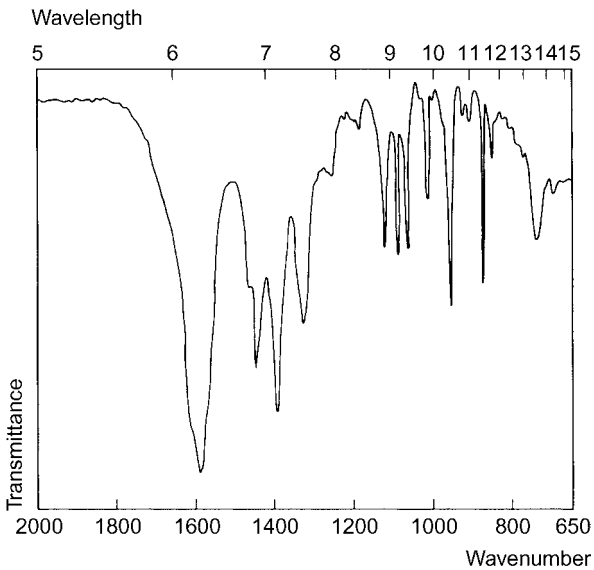
Piperazine Hydrate

$C_4H_{10}N_2,6H_2O = 194.2$
CAS—142-63-2
Synonyms Diethylenediamine; hexahydropyrazine; piperazidine.
Proprietary Names *Antelmina*; *Antepar*; *Ascarin*; *Desparasil*; *Pipralen*; *Solucamphre*; *Vermifran*; *Vermifuge*.
Chemical Properties Colourless, glassy, deliquescent crystals. Mp about 43°. Soluble 1 in 3 of water and 1 in 1 of ethanol; very slightly soluble in ether.

Piperazine Phosphate

$C_4H_{10}N_2,H_3PO_4,H_2O = 202.1$
CAS—14538-56-8 (anhydrous); 18534-18-4 (monohydrate)
Proprietary Name It is an ingredient of *Pripsen*.
Chemical Properties A white crystalline powder. Soluble 1 in about 60 of water; practically insoluble in ethanol, chloroform and ether; soluble in dilute hydrochloric acid.

Colour Test Simon's test—blue.
Thin-layer Chromatography System TA—R_f 0.05; system TB—R_f 0.01; system TC—R_f 0.01; system TL—R_f 0.00; system TAE—R_f 0.01; system TAF—R_f 0.04 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 813.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1592, 955, 870, 1080, 1055, 1115 cm^{-1} (piperazine citrate, KBr disk).

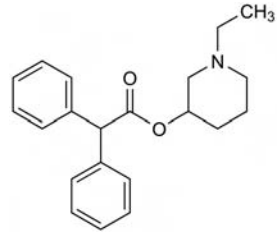


Mass Spectrum Principal ions at *m/z* 44, 100, 41, 60, 29, 27, 30, 86 (piperazine adipate).
Quantification
Urine **Colorimetry** [Hanna, Tang 1973].
GC Limit of detection, 20 and 1 µg/L with NSD or MSD, respectively [Skarping *et al.* 1986].
Disposition in the Body Readily absorbed after oral administration and excreted in the urine as unchanged drug and metabolites. There is wide variation in the rate of excretion between individuals, 15–75% of a dose being excreted in 24 h.
Toxicity Toxic effects are rare and are usually due to accumulation after large doses. Three children had symptoms of toxicity after doses of 6, 9 and 12 g but they subsequently recovered.
Dose The equivalent of 4 g of piperazine hydrate as a single dose, or 2 g daily for 7 days.

Hanna S, Tang A (1973). Human urinary excretion of piperazine citrate from syrup formulations. *J Pharm Sci* 62: 2024–2025.
Skarping G *et al.* (1986). Determination of piperazine in working atmosphere and in human urine using derivatization and capillary gas chromatography with nitrogen- and mass-selective detection. *J Chromatogr* 370: 245–258.

Piperidolate

Anticholinergic
$C_{21}H_{25}NO_2 = 323.4$
CAS—82-98-4
IUPAC Name (1-Ethylpiperidin-3-yl) 2,2-diphenylacetate
Synonyms JB-305; α-phenylbenzeneacetic acid 1-ethyl-3-piperidinyl ester.

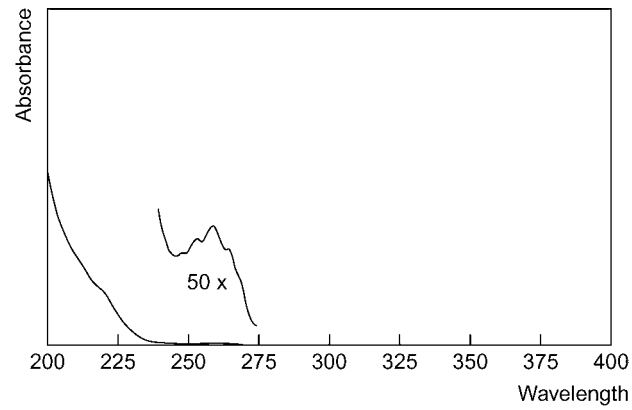


Chemical Properties A liquid. Bp 191° to 192°. Log *P* (octanol/water), 4.7.

Piperidolate Hydrochloride

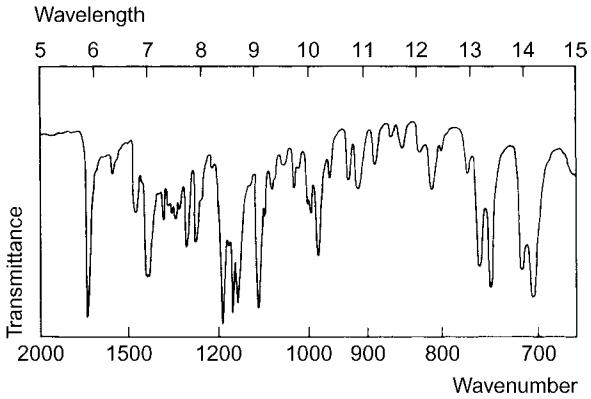
$C_{21}H_{25}NO_2,HCl = 359.9$
CAS—129-77-1
Proprietary Name *Dactil*
Chemical Properties A white or cream-coloured powder. Mp 195° to 196°. Soluble 1 in about 18 of water, 1 in about 52 of ethanol, 1 in about 4 of chloroform and 1 in about 5 of methanol.

Colour Tests Liebermann's reagent—orange-brown; Mandelin's test—brown; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.55; system TC—R_f 0.81; system TE—R_f 0.82; system TL—R_f 0.55; system TAE—R_f 0.54; system TAF—R_f 0.52; system TAJ—R_f 0.49; system TAK—R_f 0.11; system TAL—R_f 0.64 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, yellow).
Gas Chromatography System GA—RI 2318; system GF—RI 2660.
High Performance Liquid Chromatography System HA—*k* 1.7; system HX—RI 429.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=13.4a), 264 nm. No alkaline shift.

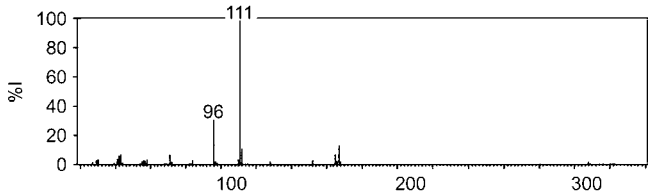


Infrared Spectrum Principal peaks at wavenumbers 1192, 1715, 1163, 1101, 700, 1149 cm^{-1} (piperidolate hydrochloride, KBr disk).

P



Mass Spectrum Principal ions at *m/z* 111, 96, 167, 112, 165, 71, 43, 42.

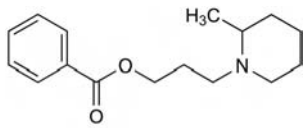


Dose 200 mg of piperidolate hydrochloride daily.

Piperocaine

Anaesthetic (Local)
$C_{16}H_{23}NO_2$ = 261.4
CAS—136-82-3

IUPAC Name 3-(2-Methylpiperidin-1-yl)propyl benzoate
Synonym 2-Methyl-1-piperidinepropanol benzoate

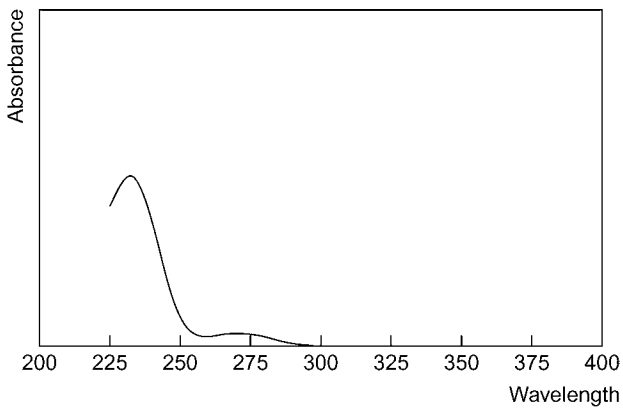


Chemical Properties Log *P* (octanol/water), 3.8.

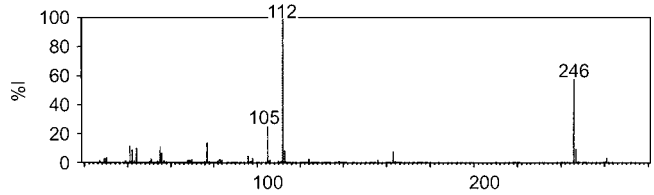
Piperocaine Hydrochloride

$C_{16}H_{23}NO_2$,HCl = 297.8
CAS—533-28-8
Proprietary Name *Metycaine Hydrochloride*
Chemical Properties Small white crystals or white crystalline powder. Mp 167° to 169°. Soluble 1 in 1.5 of water and 1 in 4.5 of ethanol; soluble in chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.53; system TC—R_f 0.37; system TE—R_f 0.76; system TL—R_f 0.27; system TAE—R_f 0.24; system TAF—R_f 0.56 (Dragendorff spray, positive; Acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1980.
High Performance Liquid Chromatography System HQ—*k* 4.59; system HX—RI 357; system HY—RI 312.
Ultraviolet Spectrum Aqueous acid—231, 274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1278, 1703, 711, 1115, 1310, 1040 cm^{-1} (piperocaine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 112, 246, 105, 77, 55, 41, 44, 247.

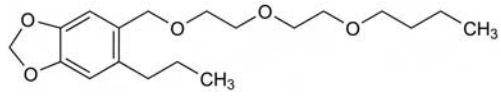


Uses Piperocaine hydrochloride is used in concentrations of 0.5 to 10%.

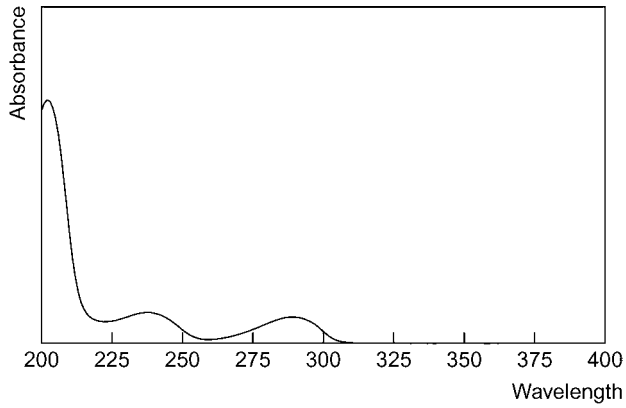
Piperonyl Butoxide

Insecticide Synergist
$C_{19}H_{30}O_5$ = 338.4
CAS—51-03-6

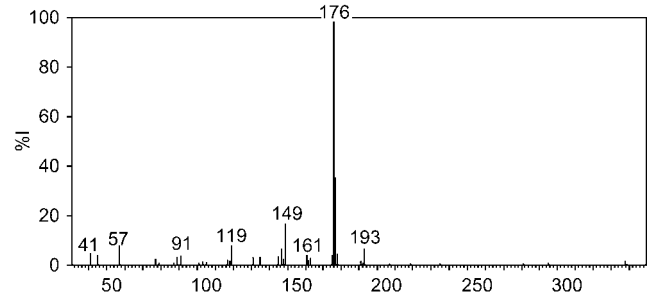
IUPAC Name 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole
Synonym ENT 14250
Proprietary Names *Butacide*; *Nusyn-noxfish*; *Prentox*; *Pybuthrin*; *Synpren-fish*.



Chemical Properties A yellow-pale brown oily liquid. Mp <25° and Bp 180°. Miscible with methanol, ethanol, benzene, freons, geons, other organic solvents and oils. Very slightly soluble in water (14.3 mg/L at 25°). Log *P* (octanol/water), 4.75.
Gas Chromatography System GA—RI 2376.
High Performance Liquid Chromatography System HAA—retention time 27.6 min.



Mass Spectrum Principal ions at *m/z* 176, 177, 149, 57, 119, 193, 41, 147.



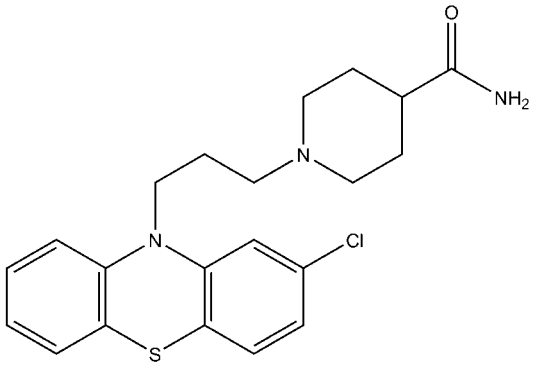
Disposition in the Body Piperonyl is eliminated as the glucuronide or amino acid derivative.
Toxicity Gastrointestinal tract effects and mild CNS depression: coma, convulsions, hepatic and renal damage. Poison via skin contact; moderately toxic by ingestion and i.p. routes.

Piperoxan

Benzodioxane, α_2-Adrenoceptor Antagonist, Antihypertensive, Diagnostic Aid (Pheochromocytoma)
$C_{14}H_{19}NO_2$ = 233.3
CAS—59-39-2

P

IUPAC Name 1-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)piperidine
Synonyms Benzodioxane; fourneau; 2-piperidinomethyl-1,4-benzodioxan; 2-piperidinomethyl-1,4-benzodioxan; piperoxane; US 2056046.



Chemical Properties Bp 193°. Log *P* (octanol/water) 2.4 [National Institutes of Health 2008]. Piperoxan is extracted by organic solvents from aqueous alkaline solutions.

Piperoxan Hydrochloride (*dl*-Form)

$C_{14}H_{19}NO_2$,HCl = 269.8
CAS—4546-39-8
Synonyms Compound 933 F; fourneau 933.
Proprietary Name *Benodaine*
Chemical Properties White crystalline powder. Mp 232° to 237°. Soluble 1 in 3.5 water and 1 in 12 of ethanol. Almost insoluble in ether, soluble in chloroform.

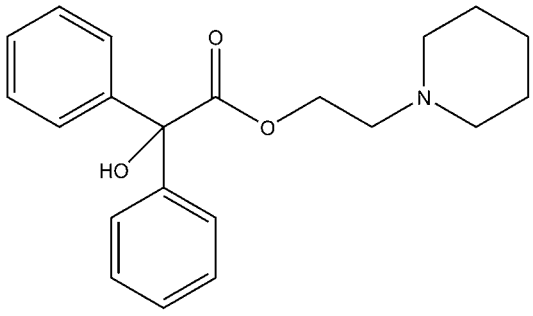
Colour Tests Ammonium molybdate test—blue-green (limit of detection, 0.1 µg); ammonium vandate test—green (limit of detection, 0.1 µg); Vitali's test—–/–/faint red (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—deep purple (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.65 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 0.98 relative to diphenylhydramine; system G4—retention time 0.96 relative to diphenhydramine.
UV Spectrum Aqueous acid (0.1 N sulfuric acid)—274 nm.
Dose Up to 20 mg IV.
Note For a study of piperoxan in paradoxical sleep, see Gaillard [1983].

Gaillard JM (1983). Biochemical pharmacology of paradoxical sleep. *Br J Clin Pharmacol* 16 (suppl2): 205S–230S.
National Institutes of Health (2008). *Piperoxan*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6040&loc=ec_rcs. (accessed 27 June 2008).

P

Pipethanate

Antimuscarinic, Antispasmodic, Tranquilliser
$C_{21}H_{25}NO_3$ = 339.4
CAS—4546-39-8
IUPAC Name 2-Piperidin-1-ylethyl 2-hydroxy-2,2-di(phenyl)acetate
Synonyms Benzilic acid 1-piperidineethanol ester; α-hydroxy-α-phenylbenzeneacetic acid 2-(1-piperidinyl)ethyl ester; pentamethate; 1-piperidineethanol benzilate; 2-(1-piperidino)ethyl benzilate; β-piperidylethyl benzilate; piperilate.



Chemical Properties Log *P* (octanol/water) 3.5 [National Institutes of Health 2008]. Pipethanate is extracted by organic solvents from aqueous alkaline solutions.

Pipethanate Hydrochloride

$C_{21}H_{25}NO_3$,HCl = 375.9
CAS—4544-15-4
Proprietary Names *Daipisate*; *Norticon*; *Pensanate*; *Pipenale*; *Sycotrol*.
Chemical Properties Crystals from acetone or ethanol. Mp 170° to 171°. Log *P* (octanol/water) 1.07 [Meylan, Howard 1995].

Pipethanate Ethobromide

$C_{23}H_{30}BrNO_3$ = 448.4
CAS—23182-46-9
Synonyms Ethylpipethanate bromide; piperilate ethobromide.
Proprietary Names *Nospasmin*; *Panpurol*; *Spalgin*; *Spasmodene*; *Spasmodil*.
Chemical Properties Pipethanate ethobromide is an antimuscarinic with actions similar to those of atropine. It has been used in the symptomatic treatment of visceral spasms in doses by mouth of up to 160 mg daily in divided doses. Pipethanate ethobromide has also been given IM or IV in doses of 10 to 20 mg daily and rectally in doses of 60 or 120 mg daily.

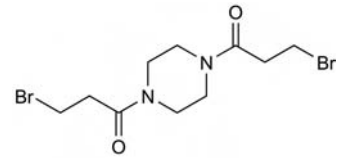
Colour Tests Ammonium molybdate test—orange (limit of detection, 1.0 µg); sulfuric acid–formaldehyde test—yellow→green→blue (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.68 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.30 relative to codeine.

Column: DB-1 non-polar fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. SID and NPD. Retention time, 21.0 min. Limit of detection, 20–50 pg [Hattori *et al.* 1992].
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—252, 258, 262 nm.
Quantification
Blood **GC** Column: DB-1 non-polar fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. SID and NPD. Retention time, 21.0 min. Limit of detection, 1 to 2.5 µg/L [Hattori *et al.* 1992].
Urine **GC** Column: DB-1 non-polar fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. SID and NPD. Retention time, 21.0 min. Limit of detection, 1 to 2.5 µg/L [Hattori *et al.* 1992].
Dose Up to 18 mg daily.

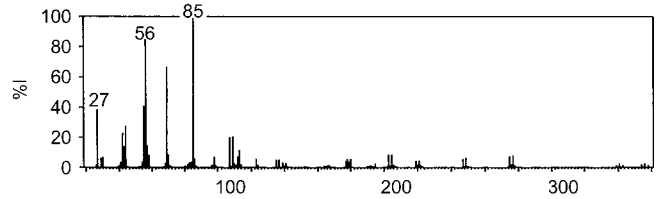
Hattori H *et al.* (1992). Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 581: 213–218.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health (2008). *Piperilate*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=20674&loc=ec_rcs. (accessed 27 June 2008).

Pipobroman

Antineoplastic
$C_{10}H_{16}Br_2N_2O_2$ = 356.1
CAS—54-91-1
IUPAC Name 3-Bromo-1-[4-(3-bromopropanoyl)piperazin-1-yl]propan-1-one
Synonym 1,4-Bis-(3-bromo-1-oxopropyl)piperazine
Proprietary Names *Amedel*; *Vercite 25*; *Vercyte*.



Chemical Properties A white crystalline powder. Mp 106° to 107°. Soluble 1 in 230 of water, 1 in 35 of ethanol, 1 in 4.8 of chloroform and 1 in 530 of ether; soluble in acetone. Log *P* (octanol/water), 0.4.
Colour Test Add 2 mg to 2 mL of water and heat to dissolve; add 2 mL of hydroxylamine hydrochloride solution and 2 mL of 3 mol/L sodium hydroxide, mix, and allow to stand for 30 min; acidify with dilute hydrochloric acid and add 2 mL of a 9% ferric chloride solution—red–orange.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.02; system TC—R_f 0.58; system TL—R_f 0.41 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1811.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1626, 1219, 1003, 1266, 923, 892 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 85, 56, 69, 55, 27, 44, 42, 109.

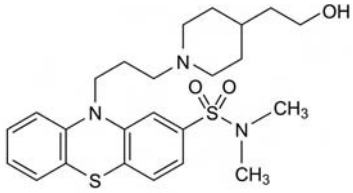


Dose Initially 1 to 1.5 mg/kg daily, orally; maintenance, 7 to 175 mg daily.

Pipotiazine

Tranquilliser
$C_{24}H_{33}N_3O_3S_2 = 475.7$
CAS—39860-99-6

IUPAC Name 10-[3-[4-(2-Hydroxyethyl)piperidin-1-yl]propyl]-*N*,*N*-dimethylphenothiazine-2-sulfonamide
Synonyms 10-[3-[4-(2-Hydroxyethyl)-1-piperidinyl]propyl]-*N*,*N*-dimethyl-10*H*-phenothiazine-2-sulfonamide; pipothiazine; RP-19366.
Proprietary Name *Piportil*



Chemical Properties Log *P* (octanol/water), 4.4.

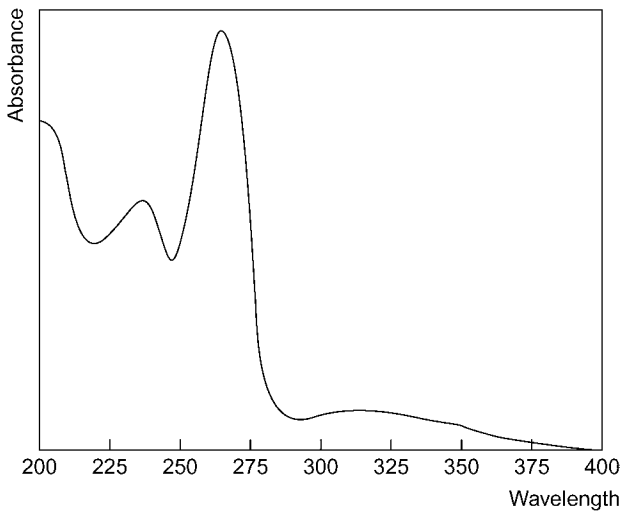
Pipotiazine Palmitate

$C_{40}H_{63}N_3O_4S_2 = 714.1$
CAS—37517-26-3
Synonym Pipothiazine palmitate; RP-19552.
Proprietary Names *Lonseren*; *Piportil L4*.
Chemical Properties A pale yellow crystalline powder. Practically insoluble in water; sparingly soluble in ethanol; freely soluble in chloroform and ether.

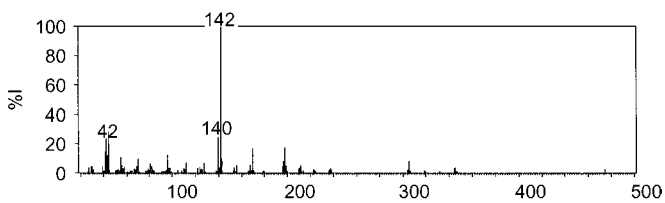
Pipotiazine Undecylenate

$C_{35}H_{51}N_3O_4S_2 = 641.9$
Synonym RP-19551
Proprietary Name *Piportil M2*.

Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.03; system TC—R_f 0.32; system TE—R_f 0.53; system TL—R_f 0.21; system TAE—R_f 0.40; system TAF—R_f 0.59.
Gas Chromatography System GA—RI 2932; system GB—not eluted.
High Performance Liquid Chromatography System HX—RI 431; system HAA—retention time 14.7 min.
Ultraviolet Spectrum Pipotiazine palmitate: ethanol—265 (A^{1}_{1}=500b), 315 nm.



Infrared Spectrum Principal peaks at wavenumbers 1165, 1735, 710, 745, 1250, 955 cm^{-1} (pipotiazine palmitate, solution in carbon tetrachloride–carbon disulfide).
Mass Spectrum Principal ions at *m/z* 142, 44, 140, 42, 198, 170, 41, 96.



Quantification
Plasma **GC** ECD. Limit of detection, 10 µg/L [Cooper, Lapierre 1981].
HPLC [Ogden *et al.* 1989]. Fluorescence detection. Limit of detection, 250 ng/L for plasma [Le Roux *et al.* 1982].
Urine **HPLC** Limit of detection, 2 µg/L for urine, see Plasma [Le Roux *et al.* 1982].

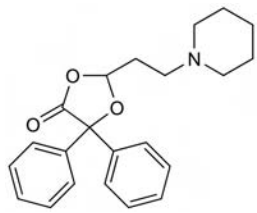
Disposition in the Body Pipotiazine palmitate is very slowly absorbed from the site of IM injections and gradually releases pipotiazine into the body. After oral administration of pipotiazine, about 1% of a dose is excreted in the urine unchanged in 24 h.
Therapeutic Concentration
Following monthly IM injections of 25 to 112.5 mg to 5 subjects, plasma concentrations of 0.018 to 0.058 mg/L (mean, 0.038) were reported immediately before a dose [Cooper, Lapierre 1981].
Dose Usually 10 to 20 mg of pipotiazine daily, orally. Pipotiazine palmitate is given IM in doses of 25 to 200 mg, at intervals of about 4 weeks.

Cooper SF, Lapierre YD (1981). Gas–liquid chromatographic determination of pipotiazine in plasma of psychiatric patients. *J Chromatogr* 222: 291–296.
Le Roux Y *et al.* (1982). High-performance liquid chromatographic determination of pipotiazine in human plasma and urine. *J Chromatogr* 230: 401–408.
Ogden DA *et al.* (1989). Determination of pipothiazine in human plasma by reversed-phase high-performance liquid chromatography. *J Pharm Biomed Anal* 7: 1273–1280.

Pipoxolan

Antispasmodic
$C_{22}H_{25}NO_3 = 351.4$
CAS—23744-24-3

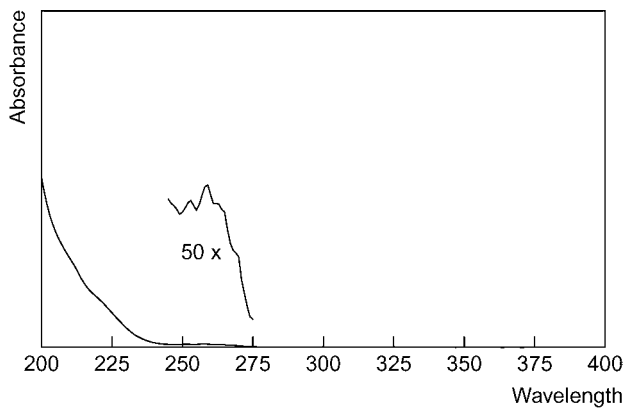
IUPAC Name 5,5-Diphenyl-2-(2-piperidin-1-ylethyl)-1,3-dioxolan-4-one
Synonym BR-18



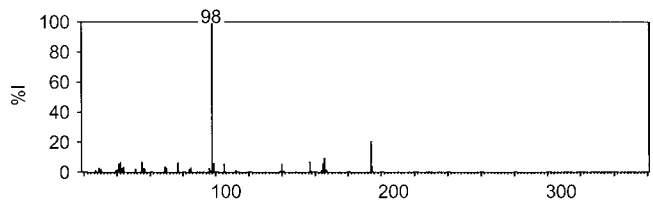
Pipoxolan Hydrochloride

$C_{22}H_{25}NO_3$,HCl = 387.9
CAS—18174-58-8
Proprietary Name *Rowapraxin*
Chemical Properties A white crystalline powder. Mp 207° to 209°. Soluble in water.

Colour Tests Liebermann's reagent—brown; Mandelin's test—green→brown; Marquis test—violet→grey; sulfuric acid—red.
Thin-layer Chromatography System TA—R_f 0.77; system TB—R_f 0.53; system TC—R_f 0.68; system TL—R_f 0.56 (acidified iodoplatinate solution, positive).



Infrared Spectrum Principal peaks at wavenumbers 1786, 1176, 1205, 1111, 1149, 698 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 194, 166, 157, 55, 42, 165, 99.



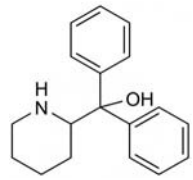
Dose Pipoxolan hydrochloride has been given in doses of 20 to 90 mg daily.

P

Pipradrol

CNS Stimulant
$C_{18}H_{21}NO$ = 267.4
CAS—467-60-7

IUPAC Name Diphenyl(piperidin-2-yl)methanol
Synonyms Alpha-pipradrol; α,α-diphenyl-2-piperidinemethanol; MRD-108; pipradol.

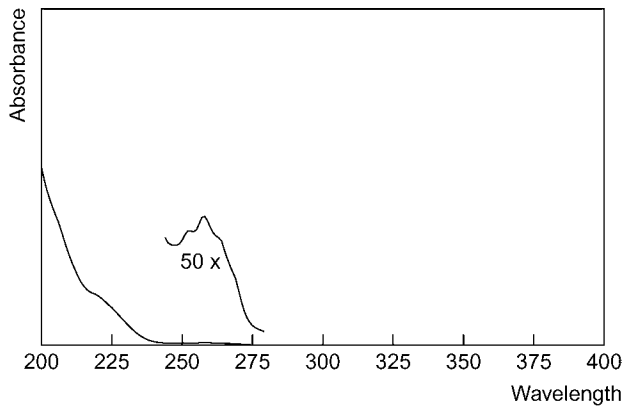


Chemical Properties Log *P* (octanol/water), 3.4.

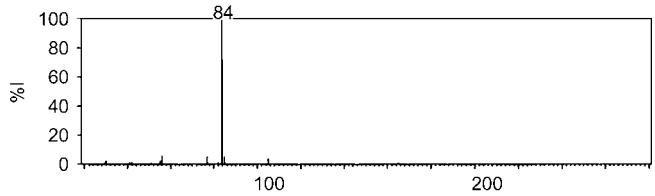
Pipradrol Hydrochloride

$C_{18}H_{21}NO,HCl$ = 303.8
CAS—71-78-3
Proprietary Names *Meratran*; *Stimolag*. It is an ingredient of *Alertonic*.
Chemical Properties Small white crystals or white crystalline powder. Mp 308° to 309° (crystals from butanone). Soluble 1 in 60 of hot water, 1 in 35 of ethanol, 1 in 1000 of chloroform and 1 in 8 of methanol; practically insoluble in ether.

Colour Test Marquis test—yellow-orange.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.59; system TC—R_f 0.38; system TE—R_f 0.81; system TL—R_f 0.39; system TAE—R_f 0.19; system TAF—R_f 0.79. (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, red-brown; ninhydrin spray, positive.)
Gas Chromatography System GA—RI 2145; system GB—RI 2242; system GC—RI 2478.
High Performance Liquid Chromatography System HA—*k* 1.2; system HC—*k* 0.69; system HX—RI 355.
Ultraviolet Spectrum Aqueous acid—252, 258 nm (A_1^1=17a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 701, 740, 690, 1585, 1300, 1065 cm^{-1} (pipradrol hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 84, 56, 85, 77, 105, 55, 30, 42.

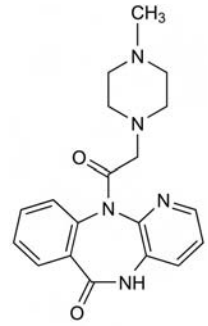


Dose Pipradrol hydrochloride has been given in doses of 2 to 6 mg daily.

Pirenzepine

Treatment of Gastric Ulcers, Antiulcerative
$C_{19}H_{21}N_5O_2$ = 351.4
CAS—28797-61-7

IUPAC Name 11-[2-(4-Methylpiperazin-1-yl)acetyl]-5*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one
Synonyms 5,11-Dihydro-11-[(4-methyl-1-piperazinyl)acetyl]-6*H*-pyrido[2,3-*b*]-[1,4]benzodiazepin-6-one; LS-519

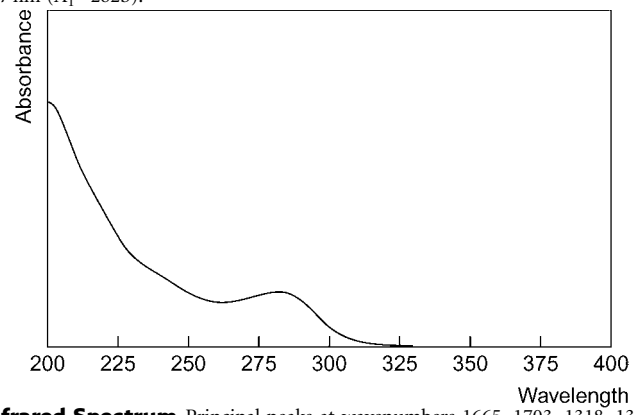


Chemical Properties pK_a 2.1, 8.1. Log *P* (octanol/water), 1.7.

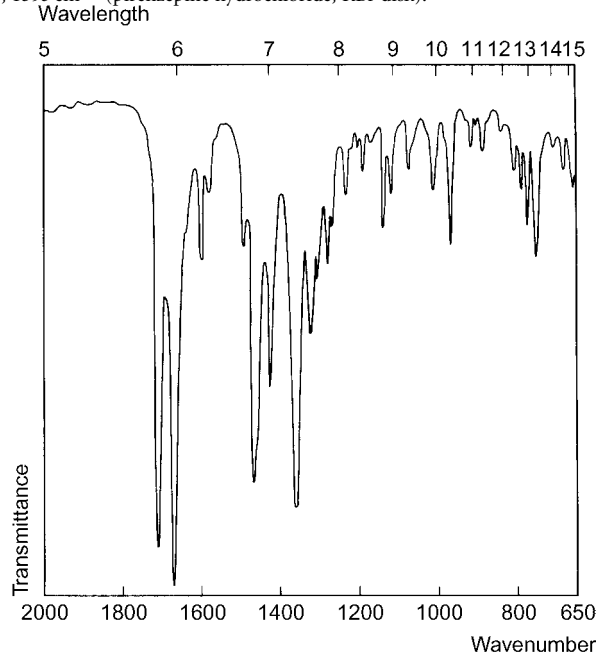
Pirenzepine Hydrochloride

$C_{19}H_{21}N_5O_2,2HCl$ = 424.3
CAS—29868-97-1
Synonym LS-519-cl2
Proprietary Names *Cevanil*; *Duogastral*; *Frazim*; *Gastricur*; *Gastrol*; *Gastropin*; *Gastropiren*; *Gastrosed*; *Gastrozepin(a)*; *Ulcepin*; *Ulcin*; *Ulcoprotect*; *Ulcosafe*; *Leblon*; *Maghen*; *Tabe*.
Chemical Properties A white crystalline powder. Mp 248° to 253°, with decomposition. Freely soluble in water; practically insoluble in ether; very slightly soluble in methanol.

Thin-layer Chromatography System TB—pirenzepine R_f 0.00; M (desamide) R_f 0.04; M (*N*-desmethyl) R_f 0.00; system TE—pirenzepine R_f 0.16; M (desamide) R_f 0.70; M (*N*-desmethyl) R_f 0.03; system TAE—pirenzepine R_f 0.18; M (desamide) R_f 0.81; M (*N*-desmethyl) R_f 0.04.
High Performance Liquid Chromatography System HA—*k* 2.7; system HX—pirenzepine RI 293; M (desamide) RI 373;M (*N*-desmethyl) RI 291.
Ultraviolet Spectrum Aqueous acid—280 (A_1^1=237a); aqueous alkali—297 nm (A_1^1=282b).

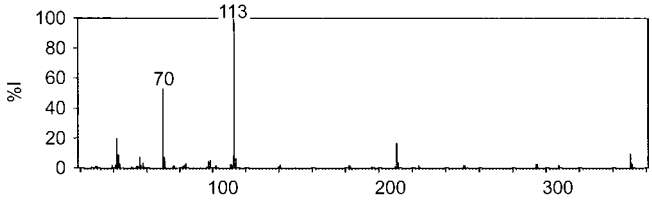


Infrared Spectrum Principal peaks at wavenumbers 1665, 1703, 1318, 1300, 1278, 1593 cm^{-1} (pirenzepine hydrochloride, KBr disk).



P

Mass Spectrum Principal ions at *m/z* 113, 70, 42, 211, 351, 43, 71, 56.



Quantification

Plasma **HPLC** Limit of detection, 2.5 µg/L [Meineke *et al.* 1986].

Radioimmunoassay Limit of detection, 0.25 µg/L in plasma [Tanswell *et al.* 1986].

Urine **Radioimmunoassay** Limit of detection, 4 µg/L in urine, see Plasma [Tanswell *et al.* 1986].

Disposition in the Body Incompletely absorbed after oral administration. Excreted in the urine and faeces largely as unchanged drug with <10% of a dose as the desmethyl metabolite.

Therapeutic Concentration

Following a single oral dose of 50 mg to 87 subjects, a mean peak plasma concentration of 0.05 mg/L was attained in 2 h [Bozler, Hammer 1980].

Bioavailability About 20 to 30%.

Half-life Plasma half-life, about 11 h.

Clearance Plasma clearance, about 3.5 mL/min/kg.

Protein Binding About 10%.

Dose 100 to 150 mg of pirenzepine hydrochloride daily.

Bozler G, Hammer R (1980). An international pharmacokinetic study on pirenzepine following a single oral dose. *Scand J Gastroenterol* 15: 27–33.

Meineke I *et al.* (1986). Sensitive high-performance liquid chromatographic determination of pirenzepine in plasma. *J Chromatogr* 375: 369–375.

Tanswell P *et al.* (1986). Automated monoclonal radioimmunoassays for pirenzepine, a selective muscarinic receptor antagonist, in plasma and urine. *J Immunol Methods* 93: 247–258.

Piritramide

Narcotic Analgesic

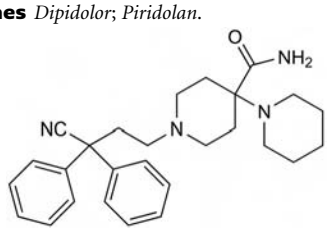
$C_{27}H_{34}N_4O = 430.6$

CAS—302-41-0

IUPAC Name 1-(3-Cyano-3,3-diphenylpropyl)-4-piperidin-1-ylpiperidine-4-carboxamide

Synonyms 1′-(3-Cyano-3,3-diphenylpropyl)-[1,4′-bipiperidine]-4′-carboxamide; pirinitramide.

Proprietary Names *Dipidolor*; *Piridolan*.



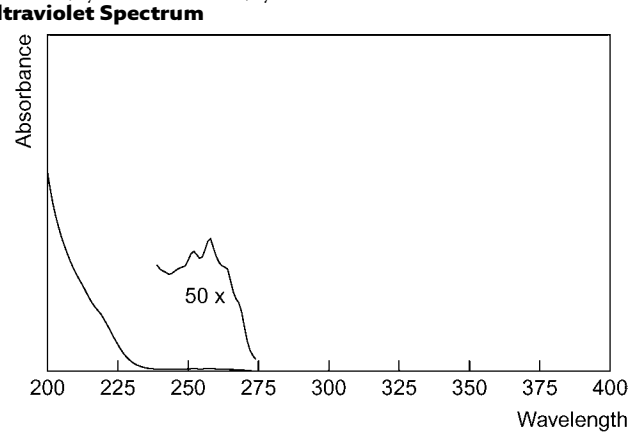
Chemical Properties Crystalline powder. Mp 149° to 150°. Soluble in dilute acetic acid. Log *P* (octanol/water), 3.9. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005]. Stability was verified after 3 freeze-thaw cycles, although it was advisable to store plasma samples at -80° for no longer than 6 months due to slight degradation [Kahlich *et al.* 2006].

Thin-layer Chromatography System TA—R_f 0.70; system TAE—R_f 0.73; system TAF—R_f 0.74; system TAG—R_f 0.42; system TB—R_f 0.01; system TC—R_f 0.45; system TE—R_f 0.61 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, orange; ninhydrin spray, positive).

Gas Chromatography System GA—RI 3560.

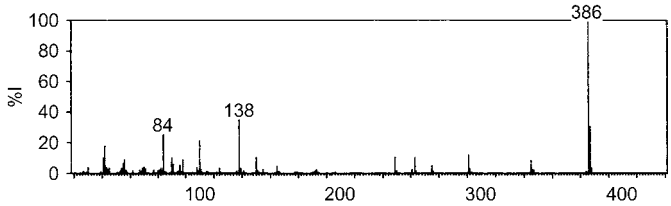
High Performance Liquid Chromatography System HA—*k* 0.6; system HC—*k* 0.1; system HX—RI 377; system HY—RI 343.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1669, 701, 757, 1087, 1190, 1592 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 386, 138, 387, 84, 110, 42, 301, 263.



Quantification

Plasma **GC** Column: OV-1 (4 m × 0.32 mm i.d., 0.5 µm). Carrier gas: He, 4.2 mL/min. Temperature: 260°. NPD. Limit of detection, 1.5 µg/L [Kietzmann *et al.* 1996]. Column: J & W DB-1 (30 m × 0.53 mm i.d., 1.5 µm). Carrier gas: He, 19 mL/min. Temperature programme: 290° for 9 min to 310° at 50°/min for 10 min. NPD. Limit of detection, 1–2 µg/L [Michaelis *et al.* 1991].

LC-MS Column: C_{18} Grom Sil 120 ODS-3 CP (150 × 2 mm i.d., 5 µm). Mobile phase: water : acetonitrile : formic acid (70 : 30 : 0.1), flow rate 200 µL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.05 µg/L [Kahlich *et al.* 2006]. Column: Phenomenex C_{12} MAX-RP (150 × 2 mm i.d., 4 µm). Mobile phase: water-acetonitrile (90 : 10) with 5 mmol/L ammonium formate (pH 3.5) : water-acetonitrile (10 : 90) with 5 mmol/L ammonium formate (pH 3.5, 100 : 0 for 5 min to 0 : 100 at 19 min for 7 min to 100 : 0 in 3 min for 6 min), flow rate 200 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2.6 µg/L, limit of detection, 1.1 µg/L [Musshoff *et al.* 2006].

Serum **LC-MS** Merck Supersphere 100 RP-18 (125 × 4 mm i.d.). Mobile phase: 0.02% trifluoroacetic acid : methanol (55 : 45 to 30 : 70 in 0.5 min for 10 min), flow rate 0.8 mL/min. APCI, positive ion mode. Retention time: 5.6 min. Limit of detection, 0.3 µg/L [Martens-Lobenhoffer, Römhild 2003]

Urine **GC** See Plasma [Kietzmann *et al.* 1996].

LC-MS See Plasma [Kahlich *et al.* 2006].

Hair **LC-MS** Phenomenex C_{12} Synergi Max-RP (150 × 2 mm i.d., 4 µm). Mobile phase: water-acetonitrile (90 : 10) with 5 mmol/L ammonium formate (pH 3.5) : water-acetonitrile (10 : 90) with 5 mmol/L ammonium formate (pH 3.5, 100 : 0 for 5 min to 0 : 100 at 19 min for 7 min to 100 : 0 in 3 min for 6 min), flow rate 200 µL/min. TIS, ESI, positive ion mode. Limit of quantification, 9.1 pg/mg, limit of detection, 2.2 pg/mg [Musshoff *et al.* 2007].

Protein Binding ≈94% [Kietzmann *et al.* 1996].

Volume of Distribution 4.7 L/kg.

Clearance 7.8 mL/min/kg.

Note For the pharmacokinetics of piritramide in patients see Kietzmann *et al.* [1997], Bouillon *et al.* [1999], or Bouillon *et al.* [2004].

Dose 20 mg by IM injection.

Bouillon T *et al.* (1999). Population pharmacokinetics of piritramide in surgical patients. *Anesthesiology* 90: 7–15.

Bouillon T *et al.* (2004). The pharmacokinetics of piritramide after prolonged administration to intensive care patients. *Eur J Anaesthesiol* 21: 673–678.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Kahlich R *et al.* (2006). Quantitative determination of piritramide in human plasma and urine by off- and on-line solid-phase extraction liquid chromatography coupled to tandem mass spectrometry. *Rapid Commun Mass Spectrom* 20: 275–283.

Kietzmann D *et al.* (1996). Pharmacokinetics of piritramide after an intravenous bolus in surgical patients. *Acta Anaesthesiol Scand* 40: 898–903.

Kietzmann D *et al.* (1997). Pharmacodynamic modelling of the analgesic effects of piritramide in postoperative patients. *Acta Anaesthesiol Scand* 41: 888–894.

Martens-Lobenhoffer J, Römhild W (2003). Quantitative determination of piritramide in human serum applying liquid chromatography-two-stage mass spectrometry. *J Chromatogr B* 783: 53–59.

Michaelis HC *et al.* (1991). Sensitive determination of piritramide in human plasma by gas chromatography. *J Chromatogr* 571: 257–262.

Musshoff F *et al.* (2006). An automated and fully validated LC-MS/MS procedure for the simultaneous determination of 11 opioids used in palliative care, with 5 of their metabolites. *J Mass Spectrom* 41: 633–640.

Musshoff F *et al.* (2007). Determination of opioid analgesics in hair samples using liquid chromatography/tandem mass spectrometry and application to patients under palliative care. *Ther Drug Monit* 29: 655–661.

Piroxicam

Analgesic

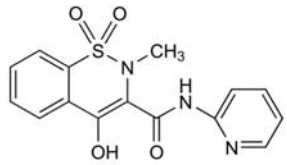
$C_{15}H_{13}N_3O_4S = 331.3$

CAS—36322-90-4

IUPAC Name (3E)-3-[Hydroxy-(pyridin-2-ylamino)methylidene]-2-methyl-1,1-dioxo-1λ⁶,2-benzothiazin-4-one

Synonyms CP-16171; 4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide

Proprietary Names *Antiflog*; *Brexidol*; *Brexin(e)*; *Candyl*; *Cycladol*; *Fasax*; *Felden(e)*; *Fexicam*; *Flamatrol*; *Flexase*; *Flexirox*; *Geroxicam*; *Inflaced*; *Jenapirox*; *Larapam*; *Olcam*; *Pericam*; *Piro*; *Pirocam*; *Piroflam*; *Piroxil*; *Piroxistad*; *Proxalyoc*; *Zofora*; *Sasulen*; *Zunden*.



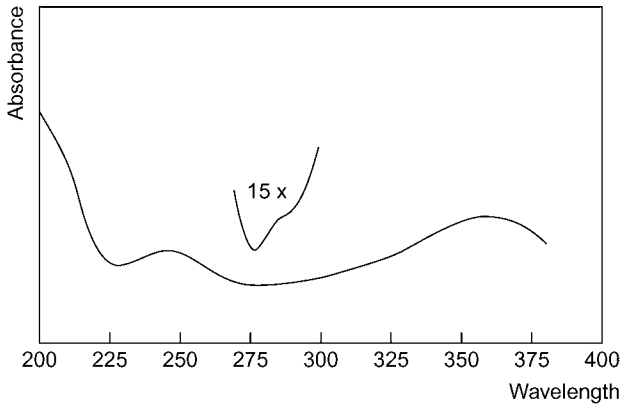
Chemical Properties Crystals. Mp 198° to 200°. Practically insoluble in water; slightly soluble in dehydrated ethanol and aqueous alkaline solutions. pK_a 6.3. Log *P* (octanol/water), 3.1. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Colour Tests Koppanyi–Zwikker test (omit pyrrolidine)—orange; Liebermann's reagent—yellow.

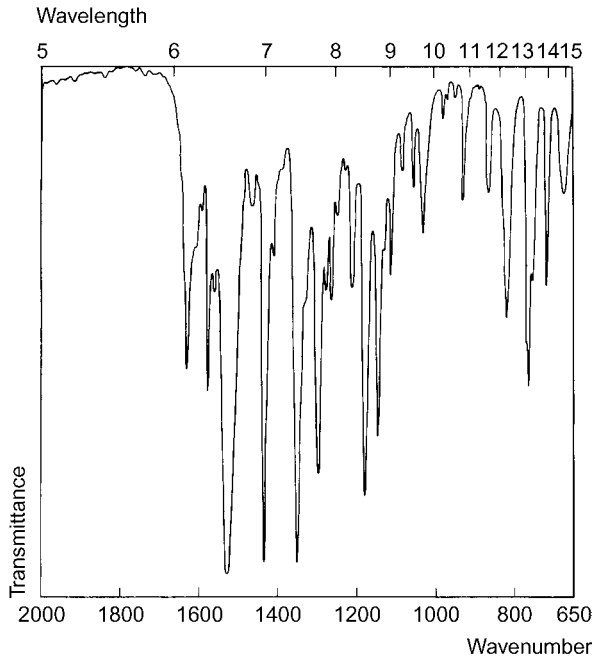
Thin-layer Chromatography System TB—M (5-hydroxy) R_f 0.00; system TD—piroxicam R_f 0.51, M (5-hydroxy) R_f 0.08; system TE—piroxicam R_f 0.17, M (5-hydroxy-) R_f 0.03; system TF—piroxicam R_f 0.38, M (5-hydroxy-) R_f 0.18; system TAD—R_f 0.71; system TAE—piroxicam R_f 0.88, M (5-hydroxy-) R_f 0.88; system TAJ—R_f 0.69; system TAK—R_f 0.45; system TAL—R_f 0.94.

Gas Chromatography System GA—RI 1413.

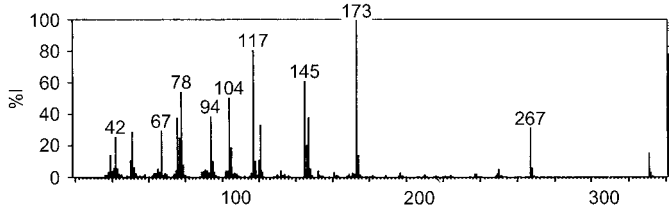
High Performance Liquid Chromatography System HD—*k* 0.6; system HW—*k* 7.70; system HX—piroxicam RI 431, M (5-hydroxy-) RI 446; system HY—RI 382; system HZ—retention time 4.9 min; system HAA—retention time 16.6 min.



Infrared Spectrum Principal peaks at wavenumbers 1524, 1180, 1298, 1147, 1573, 770 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 173, 117, 145, 78, 104, 94, 76, 147.



Quantification

Plasma **HPLC** Electrochemical detection. Limit of detection, 0.72 µg/L in plasma [de Jager *et al.* 1999]. UV detection. Limit of detection, 50 to 100 µg/L [Yritia *et al.* 1999]. UV detection. Piroxicam and 5′-hydroxypiroxicam. Limit of detection, 50 µg/L [Avgerinos *et al.* 1995]. UV detection. Piroxicam and its metabolites. Limit of detection, 50 µg/L [Milligan 1992]. UV detection. Limit of detection, 0.2 mg/L [Saeed, Becher 1991]. UV detection. Piroxicam and other NSAIDs [Streete 1989].

Serum **HPLC** See Plasma [Streete 1989]. UV detection. Limit of detection, 500 µg/L [Fraser, Woodbury 1983].

Urine **HPLC** UV detection. Piroxicam and other NSAIDs. Limit of detection, 50 µg/L [Hirai *et al.* 1997]. See Plasma [Avgerinos *et al.* 1995; Milligan 1992].

Bile **HPLC** See Plasma [Milligan 1992].

Synovial Fluid **HPLC** See Plasma [de Jager *et al.* 1999].

Subcutaneous Tissue **HPLC** See Plasma [de Jager *et al.* 1999].

Synovial Capsule **HPLC** See Plasma [de Jager *et al.* 1999].

Disposition in the Body Readily absorbed after oral or rectal administration. It is extensively metabolised to inactive metabolites; the major metabolite is produced by hydroxylation of the pyridyl ring and exists free and conjugated with glucuronic acid. About 10% of an oral dose is excreted in the urine as unchanged drug in 8 days.

Therapeutic Concentration Piroxicam accumulates on repeated administration; steady-state plasma concentrations are attained after approximately 7 days. The normal therapeutic level is between 5 and 10 mg/L.

Following oral administration of 40 mg to 20 subjects, peak plasma concentrations of 1.7 to 6.8 mg/L (mean, 4.3) were reported; multiple peak concentrations were observed in 16 subjects, suggesting possible enterohepatic circulation. After daily oral administration of 10, 20, and 30 mg to 5 subjects for 14 days, maximum steady-state plasma concentrations of 2.1 to 4.3 (mean, 3.2), 3.6 to 6.5 (mean, 4.5), and 9.0 to 16.5 (mean, 11.7) mg/L were reported [Hobbs, Twomey 1979].

Administration of 20 mg piroxicam daily for at least 4 weeks to 85 osteoarthritis patients produced mean steady-state free plasma concentrations of 0.057 mg/L when all patients were considered and 0.004 to 0.117 mg/L in newly diagnosed patients (fraction of unbound piroxicam was 0.87%) [Hundal, Rugstad 1993].

Toxicity

After ingesting approximately 1800 mg of piroxicam, a 54-year-old woman had a maximum serum concentration of 241.6 mg/L (measured at about 12 h after the overdosage), which is about 30 times the normal therapeutic level (5 to 10 mg/L) [Mosvold *et al.* 1984].

Half-life Plasma half-life, about 30 to 60 h, increased in elderly subjects.

Volume of Distribution About 0.1 L/kg.

Clearance Plasma clearance, about 0.03 mL/min/kg.

Protein Binding About 99%.

Note For a review of the pharmacokinetics of piroxicam, see Brogden *et al.* [1984].

Dose Usually 20 mg daily; doses of 40 mg daily may be given for 7 days.

Avgerinos A *et al.* (1995). Extractionless high-performance liquid chromatographic method for the simultaneous determination of piroxicam and 5′-hydroxypiroxicam in human plasma and urine. *J Chromatogr B Biomed Appl* 673: 142–146.

Brogden RN *et al.* (1984). Piroxicam. A reappraisal of its pharmacology and therapeutic efficacy. *Drugs* 28: 292–323.

de Jager AD *et al.* (1999). High-performance liquid chromatographic determination with amperometric detection of piroxicam in human plasma and tissues. *J Chromatogr B Biomed Sci Appl* 729: 183–189.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fraser AD, Woodbury JFL (1983). Liquid chromatographic determination of piroxicam in serum. *Ther Drug Monit* 5: 239–242.

Hirai T *et al.* (1997). Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 692: 375–388.

Hobbs DC, Twomey TM (1979). Piroxicam pharmacokinetics in man: aspirin and antacid interaction studies. *J Clin Pharmacol* 19: 270–281.

Hundal O, Rugstad HE (1993). Free plasma concentrations of piroxicam in patients with osteoarthritis: relation to age, sex and efficacy. *Clin Rheumatol* 12: 226–230.

Milligan PA (1992). Determination of piroxicam and its major metabolites in the plasma, urine and bile of humans by high-performance liquid chromatography. *J Chromatogr* 576: 121–128.

Mosvold J *et al.* (1984). Overdosage of piroxicam. *Acta Med Scand* 216: 335–336.

Saeed K, Becher M (1991). On-line solid-phase extraction of piroxicam prior to its determination by high-performance liquid chromatography. *J Chromatogr* 567: 185–193.

Streete PJ (1989). Rapid high-performance liquid chromatographic methods for the determination of overdose concentrations of some non-steroidal anti-inflammatory drugs in plasma or serum. *J Chromatogr* 495: 179–193.

Yritia M *et al.* (1999). Piroxicam quantitation in human plasma by high-performance liquid chromatography with on- and off-line solid-phase extraction. *J Chromatogr A* 846: 199–205.

Pivampicillin

Antibiotic

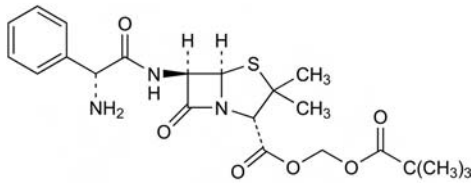
$C_{22}H_{29}N_3O_6S$ = 463.5

CAS—33817-20-8

IUPAC Name 2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

Synonyms (2*S*,5*R*,6*R*)-6-[[(2*R*)-Aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (2,2-dimethyl-1-oxopropoxy)methyl ester; MK-191

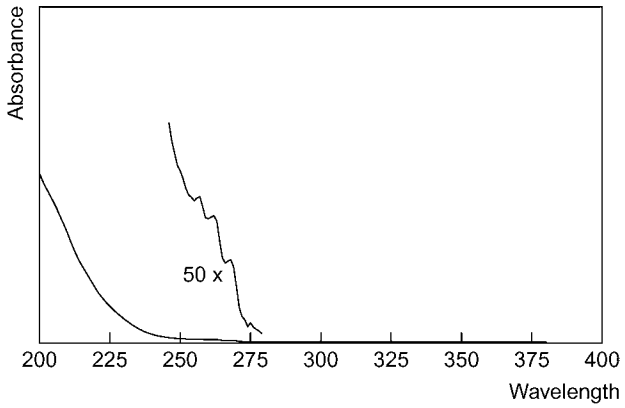
Proprietary Names *Lervipan*; *Pondocil*; *Pondocillin*; *Proampi*.



Chemical Properties pK_a 7.0.

Pivampicillin Hydrochloride
$C_{22}H_{29}N_3O_6S,HCl = 500.0$
CAS—26309-95-5
Proprietary Name *Pivamiser*; *Pondocil*; *Sanguicillin*.
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 2 of water and 1 in 1.5 of chloroform; very slightly soluble in ether.

High Performance Liquid Chromatography System HAA—retention time 15.8 min.
Ultraviolet Spectrum Aqueous acid—256, 261, 268 nm; aqueous alkali—257, 311 nm.



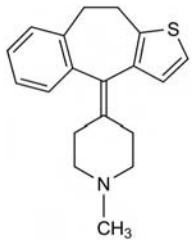
Infrared Spectrum Principal peaks at wavenumbers 1760, 1742, 1104, 1683, 972, 1150 cm^{-1} (KBr disk).
Disposition in the Body
Therapeutic Concentration
Ten cystic fibrosis patients given pivampicillin 50 mg/kg daily alone or combined with pivmecillinam 40 mg/kg daily for 14 days displayed mean peak pivampicillin serum concentrations of 6.0 mg/L and 6.9 mg/L, respectively, 2 h after oral dosing [Johansen *et al.* 1999].
Dose 1 to 2 g daily.

Johansen HK *et al.* (1999). Randomised trial of pivampicillin plus pivmecillinam vs. pivampicillin in children and young adults with chronic obstructive pulmonary disease and infection with Haemophilus influenzae. *Curr Med Res Opin* 15: 300–309.

Pizotifen

Antimigraine, Antihistamine (Sedating)
$C_{19}H_{21}NS = 295.4$
CAS—15574-96-6

Synonyms BC-105; 4-(9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thien-4-ylidene)-1-methylpiperidine; pizotyline.

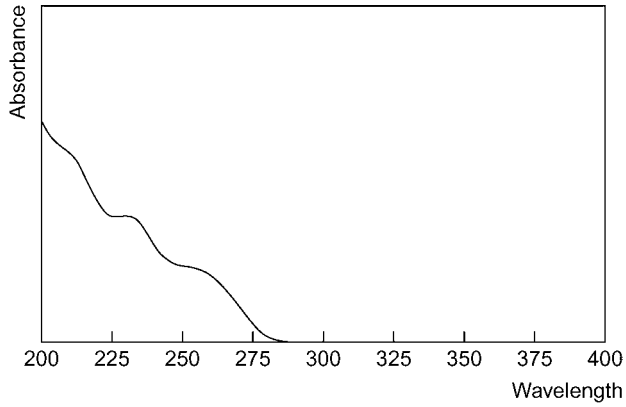


Chemical Properties A white to yellow crystalline powder. Mp 261° to 263°, with decomposition. Soluble in dilute acetic acid and in chloroform : methanol (1 : 1). pK_a 7.0. Log *P* (octanol/water), 5.2.

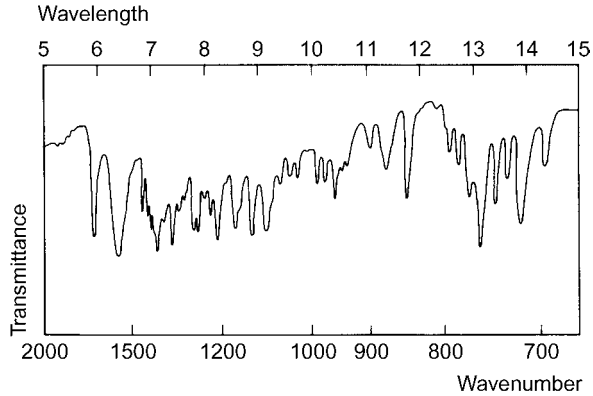
Pizotifen Malate
$C_{19}H_{21}NS,C_4H_6O_5 = 429.5$
CAS—5189-11-7

Proprietary Names *Anorsia*; *Litec*; *Mosegor*; *Pizomed*; *Sandomigran*; *Sanmigran*; *Sanomigran*; *Zofen*.
Chemical Properties Crystals. Mp 185° to 186°, with decomposition.

Colour Tests Liebermann's reagent—black; Mandelin's test—violet→green; Marquis test—orange→red; sulfuric acid—orange-yellow→violet.
Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.45; system TE—R_f 0.64; system TAE—R_f 0.28. (Acidified iodoplatinate solution, positive.)
Gas Chromatography System GA—RI 2375.
High Performance Liquid Chromatography System HA—*k* 3.4; system HX—RI 435; system HZ—retention time 6.6 min; system HAA—retention time 15.2 min.



Infrared Spectrum Principal peaks at wavenumbers 1577, 756, 1212, 1703, 1124, 1274 cm^{-1} (KBr disk).

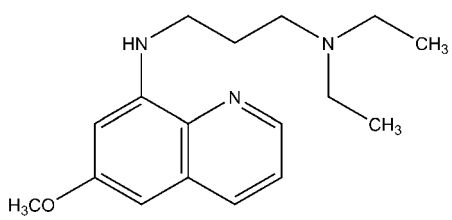


Disposition in the Body Absorbed after oral administration. Peak blood concentrations of total radioactivity were attained in 5 to 7 h after a single oral dose of 1 mg of ^{3}H-pizotifen. About 62% of a dose is excreted in the urine and 24% is eliminated in the faeces in 120 h, with about 36% of the dose being excreted in the first 24 h.
Half-life Derived from urinary excretion data (total radioactivity), about 26 h.
Dose The equivalent of 0.5 to 6 mg of pizotifen daily.

Plasmocide

Antimalarial
$C_{17}H_{25}N_3O = 287.4$
CAS—551-01-9

IUPAC Name *N,N*-Diethyl-*N*′-(6-methoxyquinolin-8-yl)propane-1,3-diamine
Synonyms Antimalarine; *N,N*-diethyl-N′-(6-methoxy-8-quinolinyl)-1,3-propanediamine; 8-(3-diethylaminopropylamino)-6-methoxyquinoline; 710-F; fourneau 710; 6-methoxy-8-(3-diethylaminopropylamino)quinoline; rhodoquine; SN-3115.



Chemical Properties Oily liquid. Bp_1 182°. Log *P* (octanol/water) 3.6 [National Institutes of Health 2008]. Plasmocide is extracted by organic solvents from aqueous alkaline solutions.

Plasmocide Dihydrochloride

$C_{17}H_{25}N_3O,2HCl$ =360.32

Chemical Properties Yellow crystals. Mp 218° to 220°. Slightly soluble in water, alcohol.

Plasmocide Diphosphate

Chemical Properties Yellow crystals. Mp 169° to 171°. Slightly soluble in water, alcohol.

Colour Tests Ammonium molybdate test—pale blue (limit of detection, 1.0 µg); ammonium vandate test—purple→orange (limit of detection, 0.5 µg); sulfuric acid–formaldehyde test—orange (limit of detection, 0.25 µg); Vitali's test—yellow/brown/yellow-brown (limit of detection, 0.1 µg).

Thin-layer Chromatography System T1—R_f 0.35 (location reagent acidified iodoplatinate spray, positive reaction).

Disposition in the Body

Toxicity For a review of antimalarial drugs and glucose-6-phosphate dehydrogenase deficiency, see Beutler and Duparc [2007].

Dose Up to 60 mg daily.

Beutler E, Duparc S (2007). Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am J Trop Med Hyg* 77: 779–789.

National Institutes of Health (2008). *Plasmocid*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=120202&loc=ec_rcs. (accessed 27 June 2008) 2008.

Poldine Metilsulfate

Anticholinergic

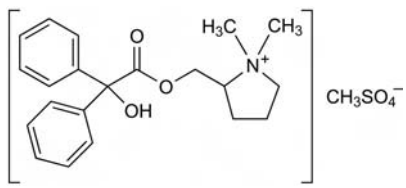
$C_{21}H_{26}NO_3,CH_3SO_4$ = 451.5

CAS—596-50-9 (poldine); 545-80-2 (metilsulfate)

IUPAC Name (1,1-Dimethylpyrrolidin-1-ium-2-yl)methyl-2-hydroxy-2,2-diphenylacetate; methyl sulfate

Synonyms 2-[[(Hydroxydiphenylacetyl)oxy]methyl]-1,1-dimethylpyrrolidinium methyl sulfate; IS-499; MeN-R-726-47; poldine methosulfate; poldine methylsulfate.

Proprietary Names *Nactate*; *Nacton*.



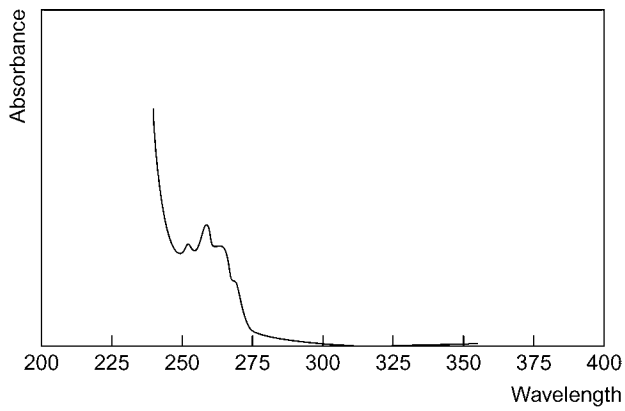
Chemical Properties A white to creamy-white crystalline powder. Mp 154° to 155°. Soluble 1 in 1 of water, 1 in 20 of ethanol and 1 in 1000 of chloroform; practically insoluble in ether. Log *P* (octanol/water), 0.9.

Colour Tests Mandelin's test—orange→green→violet; Marquis test—orange→green→blue.

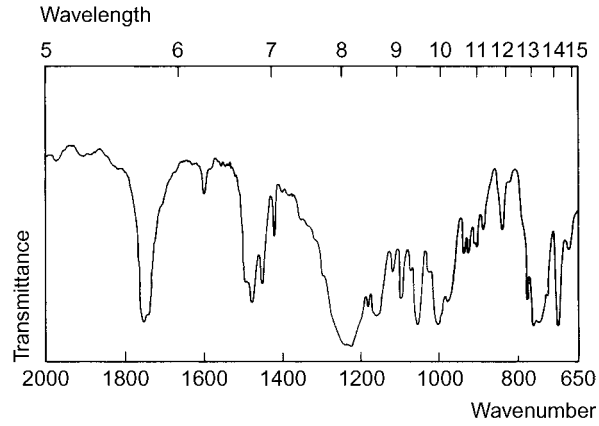
Thin-layer Chromatography System TA—R_f 0.02. (Acidified iodoplatinate solution, positive.)

High Performance Liquid Chromatography System HA—*k* 3.3 (tailing peak).

Ultraviolet Spectrum Aqueous acid—252, 258 nm (A^1_1=10.5a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1222, 701, 1061, 1008, 765, 1751 cm^{-1} (KBr disk).



Dose 8 to 16 mg daily.

Polythiazide

Diuretic

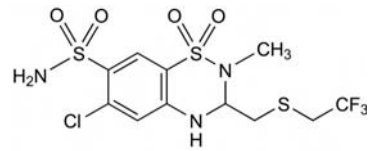
$C_{11}H_{13}ClF_3N_3O_4S_3$ = 439.9

CAS—346-18-9

IUPAC Name 6-Chloro-2-methyl-1,1-dioxo-3-(2,2,2-trifluoroethylsulfanylmethyl)-3,4-dihydro-1λ^6,2,4-benzothiadiazine-7-sulfonamide

Synonym 6-Chloro-3,4-dihydro-2-methyl-3-[[(2,2,2-trifluoroethyl)thio]methyl]-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Proprietary Names *Drenusil*; *Nephril*; *Renese*.



Chemical Properties A white crystalline powder. Mp about 214°, with decomposition. Practically insoluble in water and chloroform; soluble 1 in 40 of ethanol; soluble in acetone and methanol. Log *P* (octanol/water), 1.9.

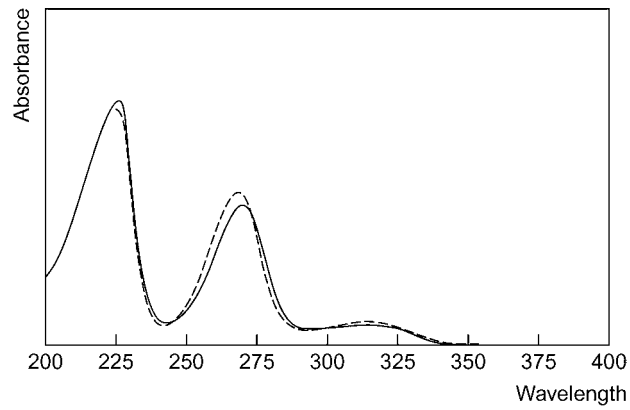
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—red-brown; palladium chloride—orange; sulfuric acid—orange.

Thin-layer Chromatography System TD—R_f 0.22; system TE—R_f 0.63; system TF—R_f 0.60; system TAD—R_f 0.32; system TAJ—R_f 0.35; system TAK—R_f 0.08; system TAL—R_f 0.70.

Gas Chromatography System GA—polythiazide RI 2380, polythiazide-ME3 RI 2985; system GX—polythiazide-ME3 retention time 11.0 min.

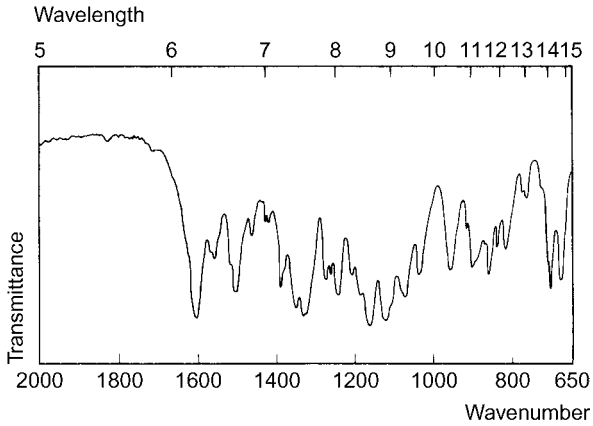
High Performance Liquid Chromatography System HN—*k* 15.09.

Ultraviolet Spectrum Methanol—268 (A^1_1=500a), 317 nm.

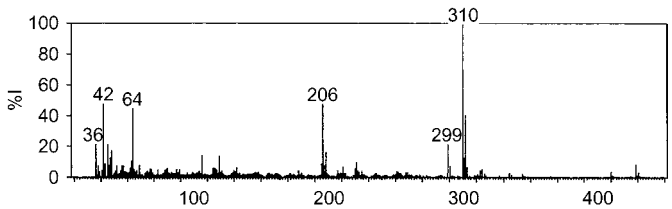


Infrared Spectrum Principal peaks at wavenumbers 1162, 1120, 1597, 1071, 1240, 1500 cm^{-1} (KBr disk).

P



Mass Spectrum Principal ions at *m/z* 310, 206, 42, 64, 312, 299, 45, 48.

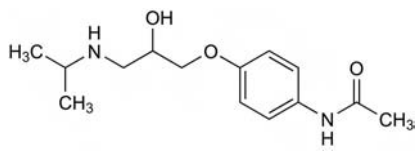


Quantification
Plasma **GC** ECD. Limit of detection, 200 ng/L [Hobbs, Twomey 1978].
HPLC Spectrophotometric detection. Limit of detection, 0.5 µg/L [Dokladalova *et al.* 1981].
Disposition in the Body Well absorbed after oral administration. About 20% of a single oral dose is excreted in the urine as unchanged drug in 48 h.
Therapeutic Concentration
After a single oral dose of 1 mg to 18 subjects, peak plasma concentrations of 0.002 to 0.007 mg/L (mean, 0.004) were attained in 5 to 12 h [Hobbs, Twomey 1978].
Half-life Plasma half-life, about 26 h.
Protein Binding About 80 to 85%.
Dose 1 to 4 mg daily.

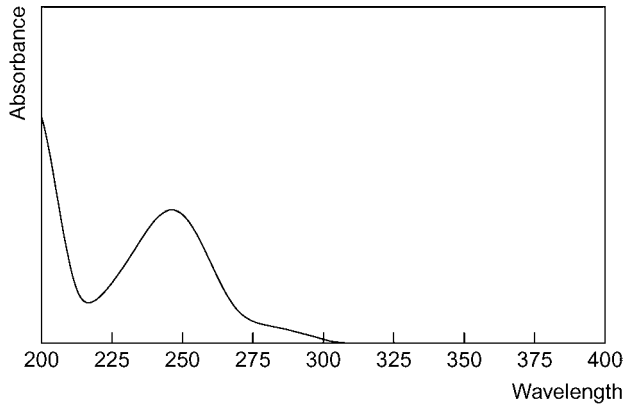
Dokladalova J *et al.* (1981). Determination of polythiazide and prazosin in human plasma by high-performance liquid chromatography. *J Chromatogr* 224: 33–41.
Hobbs DC, Twomey TM (1978). Kinetics of polythiazide. *Clin Pharmacol Ther* 23: 241–246.

Practolol

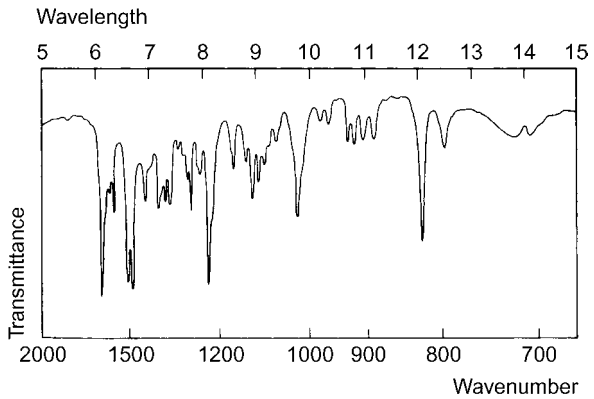
β-*Blocker*
$C_{14}H_{22}N_2O_3$ = 266.3
CAS—6673-35-4; 23313-50-0 (±)
IUPAC Name *N*-[4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide
Synonyms AY-21011; *N*-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetamide; ICI-50172.
Proprietary Name *Dalzic*; *Eraldin*.



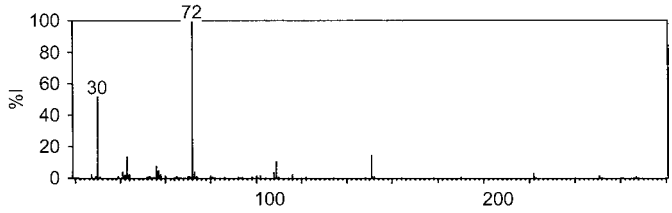
Chemical Properties A fine, white powder. Mp 134° to 136°. Soluble 1 in 400 of water, 1 in 40 of ethanol and 1 in 200 of chloroform; very soluble in dilute solutions of acetic acid. pK_a 9.5 (20°). Log *P* (octanol/pH 8.0), −1.3.
Colour Test Liebermann's reagent—black.
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.04. (Acidified potassium permanganate solution, positive.)
Gas Chromatography System GA—not eluted; system GB—RI 2440.
High Performance Liquid Chromatography System HA—*k* 0.5.
Ultraviolet Spectrum Aqueous acid—243 nm (A^1_1=467a); methanol—248 nm (A^1_1=620a).



Infrared Spectrum Principal peaks at wavenumbers 1642, 1497, 1234, 1515, 822, 1019 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 30, 151, 43, 109, 56, 57, 108.



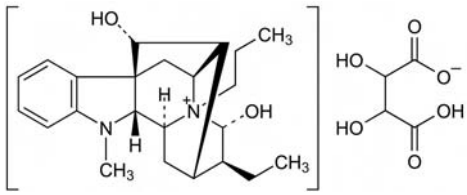
Quantification
Blood **Spectrofluorimetry** Limit of detection, 200 µg/L [Bodem, Chidsey 1972].
Plasma **GC** ECD. Limit of detection, 10 µg/L [Besager, Harvengt 1975].
HPLC Practolol and other beta-blockers [Musch *et al.* 1989]. UV detection. Limit of detection, 30 ng/mL [Mehta, Calvert 1983].
Urine **Spectrofluorimetry** See Blood [Bodem, Chidsey 1972].
GC See Plasma [Besager, Harvengt 1975].
Disposition in the Body Completely absorbed after oral administration and almost entirely excreted in the urine as unchanged drug. Up to about 70% of a dose is excreted in the urine in 24 h.
Therapeutic Concentration
After a single oral dose of 200 mg to 13 subjects, a mean peak plasma concentration of 1.3 mg/L was attained in 2 h [Castleden *et al.* 1975].
Daily oral doses of 200 mg, 400 mg, and 800 mg to 8 subjects produced mean steady-state serum concentrations of 1.6, 2.9, and 4.5 mg/L, respectively [Alderman *et al.* 1973].
Toxicity The use of practolol has been restricted by serious adverse effects, notably the oculomucocutaneous syndrome. This is characterised by adverse effects on the skin, eyes and mucous membranes, deafness, systemic lupus erythematosus and sclerosing peritonitis.
Half-life Plasma half-life, 10 to 13 h.
Volume of Distribution About 1.6 L/kg.
Clearance Plasma clearance, about 2 mL/min/kg.
Protein Binding <10%.
Dose Practolol has been given in doses of 0.2 to 1.2 g daily.

Alderman EL *et al.* (1973). Practolol in patients with angina pectoris. *Clin Pharmacol Ther* 14: 175–181.
Besager JP, Harvengt C (1975). An improved gas-liquid chromatographic method for determining practolol in plasma and urine. *J Pharm Pharmacol* 27: 52–54.
Bodem G, Chidsey CA (1972). Simple fluorometric method for estimating practolol (1-(4-aminophenoxy)-3-isopropylaminopropan-2-0l) in blood and urine. *Clin Chem* 18: 363–365.

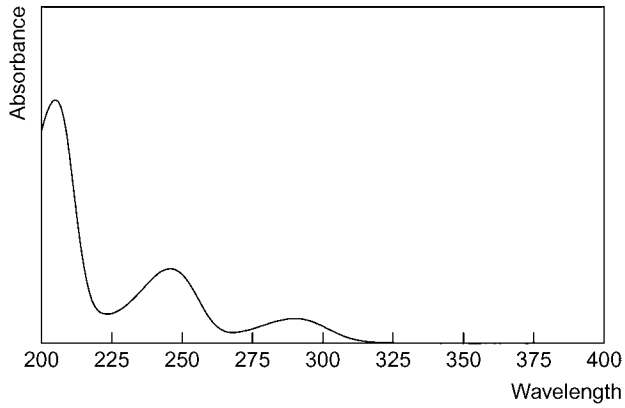
Castleden CM *et al.* (1975). The effect of age on plasma levels of propranolol and practolol in man. *Br J Clin Pharmacol* 2: 303–306.
Mehta AC, Calvert RT (1983). High-performance liquid chromatographic determination of practolol in plasma. *J Chromatogr* 276: 208–212.
Musch G *et al.* (1989). A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography. *J Pharm Biomed Anal* 7: 483–497.

Prajmalium Bitartrate

Antiarrhythmic
$C_{23}H_{33}N_2O_2,C_4H_5O_6 = 518.6$
CAS—35080-11-6 (prajmalium); 2589-47-1 (bitartrate)
Synonyms (17*R*,21α)-17,21-Dihydroxy-4-propylajmalinium hydrogen tartrate; GT-1012; NPAB.
Proprietary Names *Neo Aritmina*; *Neo-Gilurytmal*; *Neorythmin*.



Chemical Properties A white to pale yellow powder. Mp 149° to 152°. Soluble in water; readily soluble in ethanol, glacial acetic acid and dilute mineral acids; moderately soluble in chloroform; practically insoluble in ether.
Colour Tests Liebermann's reagent—red; Mandelin's test—red.
Thin-layer Chromatography System TA—R_f 0.59; system TAE—R_f 0.08. (Acidified potassium permanganate solution, positive.)
Gas Chromatography System GA—prajmalium RI 2925; M (OH-) Art RI 3130, M (methoxy-) Art RI 2895, M (OH-methoxy-) Art RI 3200.
High Performance Liquid Chromatography System HA—*k* 2.2 (tailing peak); system HY—RI 340.
Ultraviolet Spectrum Aqueous acid—246, 290 nm.



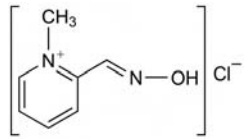
Quantification
Plasma **GC** AFID. Limit of detection, 5 µg/L [Thoma *et al.* 1981].
HPLC Fluorescence detection. Limit of detection, about 5 µg/L [Grundevik, Persson 1982].

Disposition in the Body
Therapeutic Concentration
Following oral administration of 20 mg four times a day to 8 subjects, steady-state plasma concentrations of 0.08 to 0.55 mg/L (mean, 0.2) were reported [Trompler *et al.* 1983].
Half-life Plasma half-life, about 7 h.
Dose 20 to 40 mg daily.

Grundevik I, Persson BA (1982). *J Liq Chromatogr* 5: 141–150.
Thoma M *et al.* (1981). [Gas chromatographic method for determining N-propylajmalin bitartrate in human plasma (author's transl)]. *Arzneimittelforschung* 31: 1020–1021.
Trompler AT *et al.* (1983). Pharmacokinetics and Antiarrhythmic effects of Prajmalium Bitartrate. *Arzneimittelforschung* 33: 436–439.

Pralidoxime Chloride

Cholinesterase Reactivator, Antidote (Nerve Gas and Organophosphate Insecticide Poisoning)
$C_7H_9ClN_2O = 172.6$
CAS—6735-59-7 (pralidoxime); 51-15-0 (chloride)
IUPAC Name [(*E*)-(1-Methylpyridin-2-ylidene)methyl]-oxoazanium chloride
Synonyms Chloride oxime; 2-[(hydroxyimino)methyl]-1-methylpyridinium chloride; 2-PAM chloride; 2-PAMCl; pyraloxime chloride; 2-pyridine aldoxime methochloride.
Proprietary Name *Protopam Chloride*



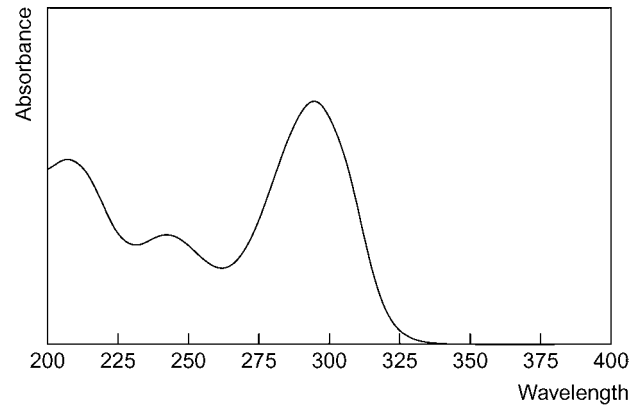
Chemical Properties A white or pale yellow crystalline powder. Mp 235° to 238°, with decomposition. Soluble 1 in <2 of water and 1 in 100 of ethanol. pK_a 8.0 (20°).

Pralidoxime Iodide
$C_7H_9IN_2O = 264.1$
CAS—94-63-3
Synonyms PAM; P-2-AM; 2-PAM iodide; 2-PAMI; pamium; pyraloxime iodide; 2-pyridine aldoxime methiodide.
Chemical Properties A yellow, hygroscopic, crystalline powder. Mp 225° to 226°, with decomposition. Soluble 1 in 20 of water; practically insoluble in ethanol, chloroform and ether. Aqueous solutions are unstable.

Pralidoxime Mesilate
$C_8H_{12}N_2O_4S = 232.3$
CAS—154-97-2
Synonyms 2-PAMM; pralidoxime mesylate; pralidoxime methanesulfonate; P2S; 2-pyridine aldoxime methyl mesilate.
Chemical Properties A colourless or white, hygroscopic, crystalline or granular powder. Mp 155°. Soluble 1 in 2 of water and 1 in 12 of ethanol; practically insoluble in chloroform and ether.

Pralidoxime Metilsulfate
$C_8H_{12}N_2O_5S = 248.3$
CAS—1200-55-1
Synonym Pralidoxime methylsulfate
Proprietary Name *Contrathion*
Chemical Properties A white crystalline powder. Mp about 111°. Very soluble in water; slightly soluble in chloroform.

Thin-layer Chromatography System TA—R_f 0.05; system TB—pralidoxime mesilate R_f 0.00; system TE—pralidoxime mesilate R_f 0.00; system TF—pralidoxime mesilate R_f 0.00; system TAE—pralidoxime mesilate R_f 0.00 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HAA—retention time 2.9 min.
Ultraviolet Spectrum Aqueous acid—242, 292 nm; aqueous alkali—332, inflexion at 280 nm.

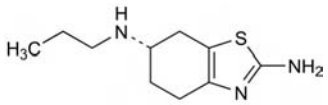


Disposition in the Body Pralidoxime chloride is slowly absorbed from the gastro-intestinal tract; the mesilate is more readily absorbed. After IV administration as the chloride, about 86% of a dose is excreted in the urine in 24 h, mostly in the first 3 h. After oral administration as the mesilate, about 30% of a dose is excreted in the 24-h urine. 1-Methyl-2-cyanopyridinium ion has been detected in urine as a metabolite.
Half-life Plasma half-life, about 1 h.
Volume of Distribution About 0.6 L/kg.
Clearance Plasma clearance, about 7 mL/min/kg.
Protein Binding Not significantly bound.
Dose 1 to 2 g of pralidoxime chloride parenterally, repeated if necessary; up to 15 g daily, orally.

Pramipexole

Antiparkinsonian, Dopamine D_2 Agonist
$C_{10}H_{17}N_3S = 211.3$
CAS—104632-26-0

IUPAC Name (6*S*)-6-*N*-Propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine
Synonyms (*S*)-2-Amino-4,5,6,7-tetrahydro-6-(propylamino)benzothiazole; SUD919Y; U-98528E.

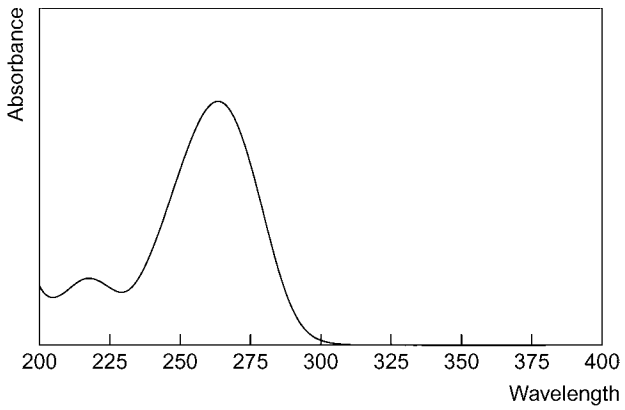


Chemical Properties White to off-white crystals. pKa_1 5.0; pKa_2 9.6. Log *P* (octanol/water), 0.87. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

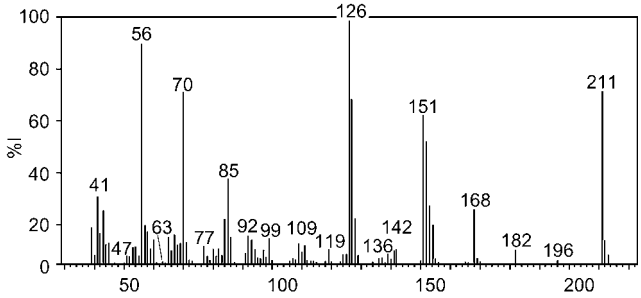
Pramipexole Dihydrochloride Monohydrate

$C_{10}H_{17}N_3S,2HCl,H_2O = 302.3$
CAS—191217-81-9
Synonyms PNU-98528-E; SND-919-CL-2Y.
Proprietary Names *Mirapex*; *Mirapexin*; *Sifrol*.
Chemical Properties White to off-white crystals. Mp 296° to 301°. Soluble in water (>20%); 8% soluble in methanol; 0.5% soluble in ethanol; practically insoluble in dichloromethane.

High Performance Liquid Chromatography Column: Zorbax Rx C_8 (250 × 4.6 mm, 5 µm). Mobile phase: 10.2 g potassium dihydrogen phosphate, 10.2 g sodium acetate and 4.5 g heptanesulfonic acid sodium salt in 3 L water, pH 3.5, flow rate 1.2 mL/min. Electrochemical and UV detection (λ = 286 nm). Retention time: 14.4 min [Lau *et al.* 1996a].
Ultraviolet Spectrum Aqueous acid (pH 2.38)—216, 264 nm.



Mass Spectrum Principal ions at *m/z* 126, 56, 70, 211, 127, 151, 152, 85.

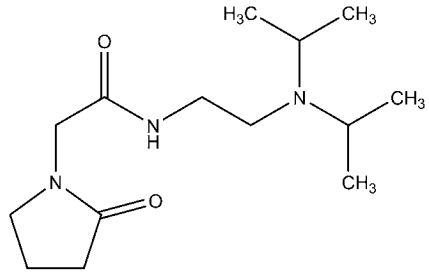


Quantification
Plasma **HPLC** Electrochemical (plasma) detection. Limit of detection, 0.05 µg/L [Lau *et al.* 1996b]. Limit of quantification, 0.05 µg/L [Wright *et al.* 1997].
LC-MS MS-MS detection. Limit of quantification, 0.05 µg/L [Lau *et al.* 1996a].
Urine **HPLC** UV detection. Limit of detection, 10 µg/L [Lau *et al.* 1996a]. See Plasma. Limit of quantification, 10 µg/L, [Wright *et al.* 1997].
Disposition in the Body Pramipexole is readily, rapidly and completely absorbed from the gastrointestinal tract. It is widely and extensively distributed throughout the body. Metabolism is minimal. More than 90% of the dose is excreted unchanged in urine via renal tubular secretion. Steady state is achieved by 2 days. No metabolites have been identified in plasma or urine.
Therapeutic Concentration
Sixteen healthy males (aged 19.5–52.1 years, mean, 28 years) and females (42.6–53.7 years, mean age, 48.3 years) were administered 0.125, 0.5, 1.0 and 1.5 mg doses of pramipexole dihydrochloride monohydrate every 8 h. The maximum steady state concentrations, when measured, reached 0.39, 1.65, 3.55 and 5.44 µg/L for the males at doses 0.125, 0.5, 1.0 and 1.5 mg. For the females, these concentrations were 0.50, 2.12, 4.63 and 7.17 µg/L, respectively. These concentrations were observed by day 3 [Wright *et al.* 1997].
Toxicity
No clinical experience with massive overdose. One patient with a 10 year history of schizophrenia took 11 mg daily for 2 days (2–3 times the recommended amount) and showed no adverse reaction [Datapharm Communications 2002].
Bioavailability 90%.
Half-life Men, 11.6 h; women, 14.1 h; also reported as 8–12 h.
Volume of Distribution Men, 7.34 L/kg; women, 7.01 L/kg; also reported as 400 L.
Clearance Total 500 mL/min. Renal clearance is ≈400 mL/min (3 times higher than the glomerular filtration rate) or 5.5 mL/min/kg. Clearance is ≈30% lower in women compared with men, elderly compared with young people and those with Parkinson's disease compared with healthy people. It is ≈75% lower for people with renal impairment.
Distribution in Blood The erythrocyte : plasma ratio is 2.
Protein Binding 15%.
Dose 125 to 500 µg three times daily. Maximum 4.5 mg daily.

Datapharm Communications (2002). *ABPI Medicines Compendium*, Epsom, Surrey.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Lau Y *et al.* (1996a). Determination of pramipexole (U-98,528) in human plasma and urine by high-performance liquid chromatography with electrochemical and ultraviolet detection. *J Chromatogr B Biomed Appl* 683: 217–223.
Lau Y *et al.* (1996b). Determination of pramipexole (U-98,528) in human plasma by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *J Chromatogr B Biomed Appl* 683: 209–216.
Wright CE *et al.* (1997). Steady-state pharmacokinetic properties of pramipexole in healthy volunteers. *J Clin Pharmacol* 37: 520–525.

Pramiracetam

Acetamide, Nootropic
$C_{14}H_{27}N_3O_2 = 269.4$
CAS—68497-62-1
IUPAC Name *N*-[2-(Di(propan-2-yl)amino)ethyl]-2-(2-oxopyrrolidin-1-yl) acetamide
Synonyms Amacetam; *N*-[2-[bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrolidineacetamide; *N*-[2-(diisopropylamino)ethyl]-2-oxo-1-pyrrolidineacetamide.



Chemical Properties Mp 47° to 48° (monohydrate). Bp 162° to 164°.

Pramiracetam Sulfate

$C_{14}H_{27}N_3O_2,H_2SO_4 = 367.5$
CAS—72869-16-0
IUPAC Name *N*-[2-(Diisopropylamino)ethyl]-2-oxo-1-pyrrolidineacetamide sulfate
Synonyms Amacetam sulphate; CI-879; pramiracetam sulphate; pramiracetam, sulfato de.
Proprietary Names *Neupramir*; *Pramistar*; *Remen*.

Quantification
Plasma **GC** Column: 3% OV-225 on 100-120 mesh Gas-Chrom Q (1.22 m × 2 mm i.d.). Carrier gas: N_2, 8 mL/min. Temperature: 225°. NPD. Retention time: 6.8 min. Limit of detection, 20 µg/L [Chang, Young 1983].
HPLC Column: Spherisorb ODS (100 mm, 3 µm). Mobile phase: acetonitrile : 0.07 mol/L potassium phosphate buffer (pH 5.5; 30 : 70). UV detection (λ = 215 nm). Limit of detection, 0.1 mg/L [Auteri *et al.* 1992].
Disposition in the Body Oral absorption of pramiracetam is moderately rapid, with peak plasma concentrations reached 2 to 3 h post-dose. The drug is widely distributed into tissues and >90% of a dose is excreted in the urine.
Therapeutic Concentration
A group of 12 healthy volunteers was administered single oral doses (400, 800, 1200 or 1600 mg) of pramiracetam in gelatine capsules. Mean peak plasma concentrations were reported as follows:

	400 mg	*800 mg*	*1200 mg*	*1600 mg*
C_{max} (mg/L)	2.71	5.40	6.13	8.98
Time (h)	2.30	2.80	3.20	3.20

[Chang *et al.* 1985].

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A group of 11 healthy volunteers was administered single oral doses (600 mg) of pramiracetam in either an aqueous solution or tablet formulation. Mean peak plasma concentrations were 6.8 and 5.8 mg/L for the solution and tablet form reached after 4.7 and 4.3 h, respectively [Auteri *et al.* 1992].

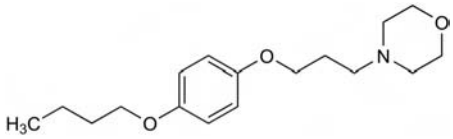
Half-life Approximately 4.5 to 6.5 h.
Volume of Distribution Apparent, 1.8 to 2.9 L/kg.
Clearance 1.8 to 3.0 mL/kg/min.
Distribution in Blood CSF : plasma ratio, 0.12 to 0.38.
Dose Used in age-related memory impairment and senile dementia, in doses equivalent to 600 mg of pramiracetam twice daily by mouth. It has also been tried, without much success, as an adjunct to electroconvulsive therapy in severe depression.

Auteri A *et al.* (1992). Pharmacokinetics of pramiracetam in healthy volunteers after oral administration. *Int J Clin Pharmacol Res* 12: 129–132.
Chang T, Young RM (1983). Gas chromatographic assay of pramiracetam in human plasma using nitrogen specific detection. *J Chromatogr* 274: 346–349.
Chang T *et al.* (1985). Pharmacokinetics of oral pramiracetam in normal volunteers. *J Clin Pharmacol* 25: 291–295.

Pramocaine

Anaesthetic (Local)
$C_{17}H_{27}NO_3$ = 293.4
CAS—140-65-8

IUPAC Name 4-[3-(4-Butoxyphenoxy)propyl]morpholine
Synonyms Pramoxine; proxazocain.

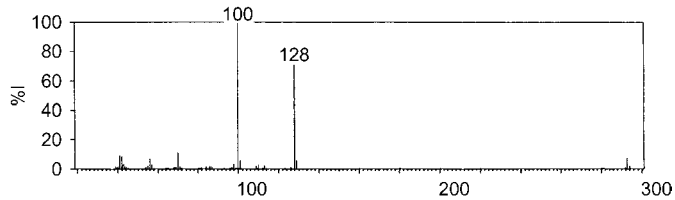


Chemical Properties Log *P* (octanol/water), 3.4.

Pramocaine Hydrochloride

$C_{17}H_{27}NO_3$,HCl = 329.9
CAS—637-58-1
Proprietary Names *Anti Itch*; *Balsabit*; *PramOtic*; *Prax*; *Proctofoam-NS*; *Tronotene*; *Tronothane*.
Chemical Properties A white crystalline powder. Mp 181° to 183°. Freely soluble in water and ethanol; soluble 1 in 35 of chloroform; very slightly soluble in ether.

Colour Test Marquis test—yellow→green.
Thin-layer Chromatography System TA—R_f 0.70; system TB—R_f 0.43; system TC—R_f 0.55; system TE—R_f 0.73; system TL—R_f 0.41; system TAE—R_f 0.62; system TAF—R_f 0.60; system TAJ—R_f 0.59; system TAK—R_f 0.20; system TAL—R_f 0.90. (Dragendorff spray, positive; Acidified iodoplatinate solution, positive; Marquis reagent, green.)
Gas Chromatography System GA—RI 2281; system GF—RI 2600.
High Performance Liquid Chromatography System HA—*k* 0.6; system HR—*k* 2.48; system HX—RI 415; system HZ—retention time 6.5 min.
Ultraviolet Spectrum Aqueous acid—286 nm (A^1_1=80c). No alkaline shift.
Infrared Spectrum Principal peaks at wavenumbers 1510, 1230, 1118, 1210, 824, 1635 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 100, 128, 70, 41, 42, 293, 56, 101.

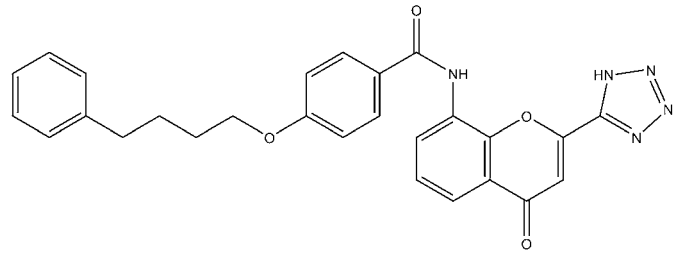


Uses Pramocaine hydrochloride is used as a 1% cream or jelly.

Pranlukast

Amide, Antiasthmatic, Leukotriene D_4 Antagonist
$C_{27}H_{23}N_5O_4$ = 481.5
CAS—103177-37-3

IUPAC Name *N*-[4-oxo-2-(2*H*-tetrazol-5-yl)chromen-7-yl]-4-(4-phenylbutoxy)benzamide
Synonyms ONO-1078; ONO-RS-411; *N*-[4-oxo-2-(1*H*-tetrazol-5-yl)-4*H*-1-benzopyran-8-yl]-*p*-(4-phenylbutoxy)benzamide; 8-[4-(4-phenylbutoxy)benzamido]-2-(tetrazol-5-yl)-4*H*-1-benzopyran-4-one; 8-[*p*-(4-phenylbutoxy)benzoyl]amino-2-(5-tetrazolyl)-4-oxo-4*H*-1-benzopyran.
Proprietary Names *Azlaire*; *Onon*.



Chemical Properties Crystals (hemihydrate). Mp 244° to 245°.
Quantification
Plasma **HPLC** Column: C_{18} (300 × 4.6 mm i.d.). Mobile phase: 0.07 mol/L ammonium formate buffer : acetonitrile (55 : 45), flow rate 1.0 mL/min. UV detection (λ = 262 nm). Limit of quantification, 10 µg/L [Brocks *et al.* 1996a].

LC-MS Column: Hypersil BDS C_{18} (30 × 2.0 mm i.d., 3 µm). Mobile phase: 20 mmol/L ammonium acetate : methanol (75 : 25 for 0.3 min to 30 : 70 over 0.1 min for 2 min to 10 : 90 over 0.1 min for 0.8 min), flow rate 300 µL/min. ESI, negative ion mode, SRM acquisition mode. Limit of quantification, pranlukast 10 µg/L, hydroxylated metabolites 1 µg/L [Marchese *et al.* 1998].

Disposition in the Body A minimum of 12.5% of an oral dose of pranlukast administered after food is absorbed from the gastrointestinal tract, with peak plasma concentrations reached after 2 to 6 h. The absorption of oral pranlukast is characterised by an apparent lag-time of 1 to 2 h. Bioavailability is increased when the drug is administered just before or after a meal. Furthermore, the bioavailability is greater after evening than after morning administration. An *in vitro* study has demonstrated that pranlukast is metabolised mainly to mono- and dihydroxylated molecules and their glucuronide conjugates by CYP enzymes. However, in a clinical setting, pranlukast appears to have low potential for drug–drug interactions via this system although caution is recommended when pranlukast is co-administered with drugs that inhibit or are predominantly metabolised by CYP3A4. The majority of an oral dose is eliminated by faecal excretion (87% as unchanged drug, 12% as metabolites) within 72 h. Less than 0.25% of an oral dose is excreted renally, predominantly as metabolites (>95%).

Therapeutic Concentration

A group of 37 healthy volunteers (19 young, 18 elderly) was administered a 300 mg oral dose of pranlukast 30 min after breakfast. Mean peak plasma concentrations in the young and elderly were 673 and 632 µg/L, respectively, after 4.5 h for both groups. There appeared to be a delay in the absorption of pranlukast in both groups, as quantifiable levels of the drug 1 h post-dose could only be measured in a few subjects [Brocks *et al.* 1996a].

In a dose-ranging study, six groups of 8 healthy male volunteers were administered oral doses of pranlukast (112.5 to 675 mg) twice daily (morning and evening) for 8 days. Mean peak plasma concentrations were reported as follows:

Dose twice daily	*Day*	*C_{max} (µg/L)*	*Time (h)*
112.5 mg	1	291	4.0
	8	445	5.3
337.5 mg	1	438	5.0
	8	631	4.5
675.0 mg	1	898	3.5
	8	1010	2.5

A great disparity between the morning trough concentration on the last day of dosing and the concentration measured 12 h after the last dose was observed, suggesting that the pharmacokinetics of pranlukast are influenced by the time of dose administration. It has been observed that the absorption of lipophilic compounds is often more rapid in the early morning than when the drug is administered during the evening [Brocks *et al.* 1996b].

Following administration of 300 mg pranlukast as an oral tablet to a healthy volunteer, peak plasma concentrations of pranlukast and 3 of its hydroxylated metabolites were 1320, 293, 337 and 12.9 µg/L, respectively [Marchese *et al.* 1998].

Half-life Approximately 4.5 h (range: 2 to 9 h).
Protein Binding Approximately 99%.
Dose Used in the management of asthma in adults at a usual dose of 225 mg pranlukast hydrate twice daily. In children with asthma, and weighing at least 12 kg, a dose of 3.5 mg/kg twice daily may be used, up to no more than the adult dose. In the management of allergic rhinitis, a dose of 225 mg twice daily is usually used in adults.

Brocks DR *et al.* (1996a). The pharmacokinetics of pranlukast in healthy young and elderly subjects. *Int J Clin Pharmacol Ther* 34: 375–379.
Brocks DR *et al.* (1996b). The single and multiple dose pharmacokinetics of pranlukast in healthy volunteers. *Eur J Clin Pharmacol* 51: 303–308.

Marchese A *et al.* (1998). Determination of pranlukast and its metabolites in human plasma by LC/MS/MS with PROSPEKT on-line solid-phase extraction. *J Mass Spectrom* 33: 1071–1079.

Pravastatin

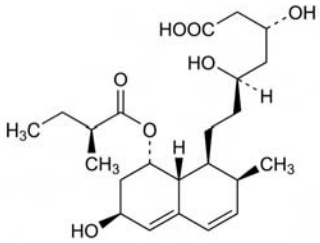
HMG-CoA Reductase Inhibitor, Antihyperlipoproteinaemic

$C_{23}H_{36}O_7$ = 425.5

CAS—81093-37-0

IUPAC Name (3*R*,5*R*)-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-Hydroxy-2-methyl-8-[(2*S*)-2-methylbutanoyl]oxy-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid

Synonym (β*R*,δ*R*,1*S*,2*S*,6*S*,8*S*,8a*R*)-1,2,6,7,8,8a-Hexahydro-β,δ,6-trihydroxy-2-methyl-8-[(2*S*)-2-methyl-1-oxobutoxy]-1-naphthaleneheptanoic acid



Chemical Properties Log *P* (octanol/water), −0.23 (pravastatin sodium).

Pravastatin Sodium

$C_{23}H_{35}O_7Na$ = 446.5

CAS—81131-70-6

Synonyms CS-514; eptastatin sodium; 3β-hydroxycompactin sodium; SQ-31000.

Proprietary Names *Elisor*; *Lipostat*; *Liprevil*; *Mevalotin*; *Oliprevin*; *Pravachol*; *Pravaselect*; *Selectin*; *Selipran*; *Vasten*.

Chemical Properties A hygroscopic white to off-white crystalline powder. Freely soluble in water and methanol; relatively insoluble in chloroform, ether, acetone and acetonitrile.

Pravastatin Lactone

$C_{23}H_{34}O_6$ = 406.5

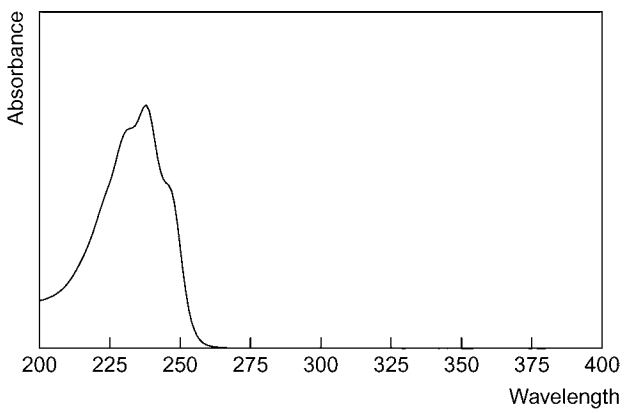
Chemical Properties Colourless plate-like crystals. Mp 138° to 142°.

High Performance Liquid Chromatography Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. UV diode array detection. Retention time: pravastatin sodium, 2.9 min [Mills, Roberson 1993].

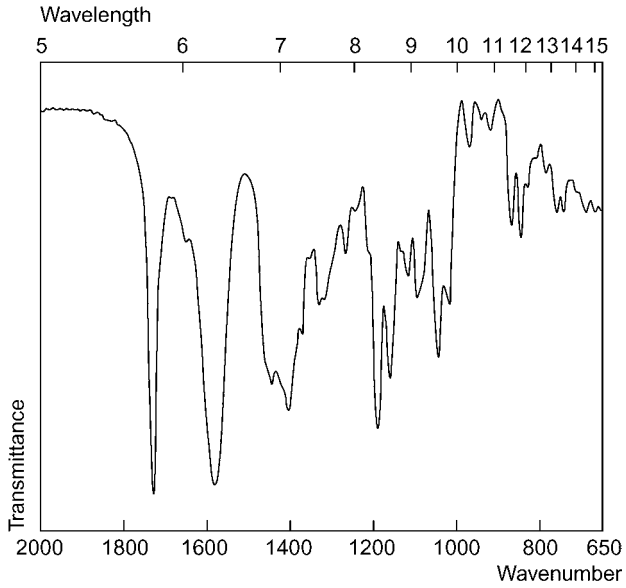
Column: ODS-2 Inertsil (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : ammonium acetate (0.01 mol/L) (60 : 40) containing 0.02% glacial acetic acid and 0.06% triethylamine, flow rate, 1 mL/min. MS detection (NIAPCI, *m/z* 423). Retention time: 2.1 min; metabolite, 2.5 min [Kawabata *et al.* 1998].

Column: RP C_{18} endcapped Purospher (250 × 4.0 mm i.d., 5 µm) with an RP C_{18} LiChrospher 100 (4 × 4 mm i.d., 5 µm). Mobile phase: sodium dihydrogen phosphate (20 mmol/L containing 1 mmol/L dodecyl sulfate):acetonitrile (65 : 35), adjusted to pH 2 with phosphoric acid, flow rate 1 mL/min. IS: triamcinolone acetonide. UV detection (λ=239 nm). Retention time: pravastatin, 6.8 min; IS, 11.0 min [Otter *et al.* 1998].

Ultraviolet Spectrum (Sodium salt) methanol—230, 237, 245 nm; aqueous acid—238 nm; basic—238 nm.(Lactone) methanol—230, 237, 245 nm.



Infrared Spectrum (Sodium salt) Principal peaks at wavenumbers 1727, 1579, 1187 cm^{-1} (KBr disk).



Quantification

Plasma **GC-MS** Limit of detection, 0.3 µg/L [Funke *et al.* 1989].

HPLC MS detection. Limit of quantification, 0.625 µg/L [Kawabata *et al.* 1998]. UV detection (λ=239 nm). Limit of quantification, 2 µg/L [Otter, Mignat 1998].

Serum **GC-MS** Limit of quantification ,0.5 µg/L for pravastatin and its metabolites [Pan *et al.* 1990]. See Plasma [Funke *et al.* 1989].

HPLC MS–MS detection. Limit of quantification, 0.5 µg/L for the drug and analytes [Mulvana *et al.* 2000].

Disposition in the Body Pravastatin is rapidly absorbed after oral administration and peak plasma concentrations are observed 1 to 1.5 h after dosing. Approx. 34% of a dose is absorbed after ingestion. It undergoes extensive hepatic metabolism to a 3-α-hydroxy isomeric compound (SQ-31906; R-416), which has one-tenth to one-fortieth the activity of the parent compound, and a 3-α,5-β,6-β-trihydroxy isomeric metabolite (SQ-31945). Peak plasma concentrations of the metabolite, SQ-31906, are observed between 0.9 and 1.3 h after ingestion of pravastatin. It is excreted mainly in faeces (approx. 70%) via bile and also, urine (20%) with 80% of urine excretion being completed within the first 12 h. The drug and/or its metabolites may accumulate in patients with renal or hepatic insufficiency. A negligible amount of the drug is distributed into breast milk.

Therapeutic Concentration

Twenty healthy male volunteers, 19 to 36 years old (mean, 25), fasted overnight and were administered an oral daily dose of 40 mg pravastatin, followed by an additional 1 h fast. The mean peak plasma concentration was 64.1 µg/L observed 1.05 h after ingestion [Pan *et al.* 1990].

Sixteen healthy males (18 to 40 years old) were administered a 20 mg single dose after an 8 h fast and fasted for another 4 h after ingestion or alternatively, co-administered with 40 mg twice daily propranolol. The peak plasma drug concentrations were 38.4 and 29.1 µg/L, respectively. The peak concentration for the metabolite SQ-31906 was 16.4 µg/L for pravastatin alone and 7.8 µg/L for co-administration with propranolol. Peak concentrations for the metabolite, SQ-31945, were 5.3 and 4.4 µg/L, respectively. All peak concentrations were reached within 1 to 1.4 h [Pan *et al.* 1991].

Bioavailability 17%.

Half-life 1.3 to 2.6 h (pravastatin); 0.8 to 1.3 h (metabolites).

Volume of Distribution 0.46 L/kg at steady state.

Protein Binding 50%.

Dose The usual dose is 10 to 40 mg daily.

Funke PT *et al.* (1989). Determination of pravastatin sodium and its major metabolites in human serum/plasma by capillary gas chromatography/negative ion chemical ionization mass spectrometry. *Biomed Env Mass Spectrom* 18(10): 904–909.

Kawabata K *et al.* (1998). An automated method for the simultaneous determination of pravastatin and its main metabolite in human plasma by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Biomed Chromatogr* 12: 271–275.

Mills T, Roberson JC (1993). *Instrumental Data for Drug Analysis*, 2nd edn., Vol. 6. Boca Raton: CRC Press, 206–207.

Mulvana D *et al.* (2000). Quantitative determination of pravastatin and its biotransformation products in human serum by turbo ion spray LC/MS/MS. *J Pharm Biomed Anal* 23(5): 851–866.

Otter K, Mignat C (1998). Determination of pravastatin in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 708: 235–241.

Otter K *et al.* (1998). Determination of pravastatin in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B, Biomed Sci Appl* 708: 235–241.

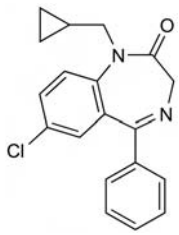
Pan HY *et al.* (1990). Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. *J Clin Pharmacol* 30: 1128–1135.

Pan HY *et al.* (1991). Pharmacokinetic interaction between propranolol and the HMG-CoA reductase inhibitors pravastatin and lovastatin. *Br J Clin Pharmacol* 31: 665–670.

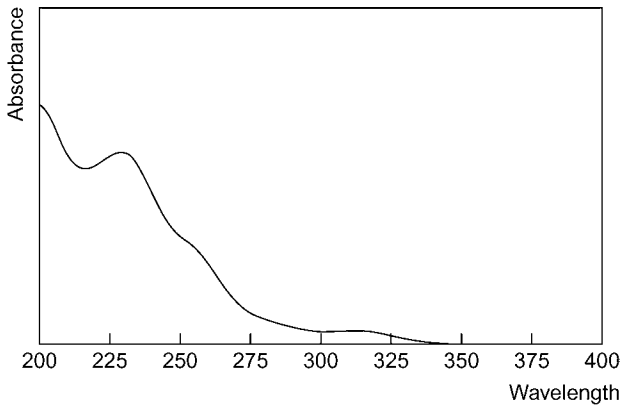
Prazepam

Benzodiazepine, Tranquilliser
$C_{19}H_{17}ClN_2O$ = 324.8
CAS—2955-38-6

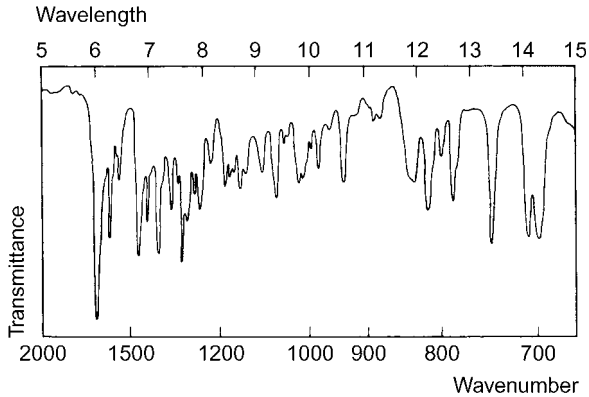
IUPAC Name 7-Chloro-1-(cyclopropylmethyl)-5-phenyl-3*H*-1,4-benzodiazepin-2-one
Synonyms 7-Chloro-1-(cyclopropylmethyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one; prazepamum; W-4020.
Proprietary Names *Centrax*; *Demetrin*; *Lysanxia*; *Pozapam*; *Prasepine*; *Prazene*; *Reapam*; *Trepidan*.



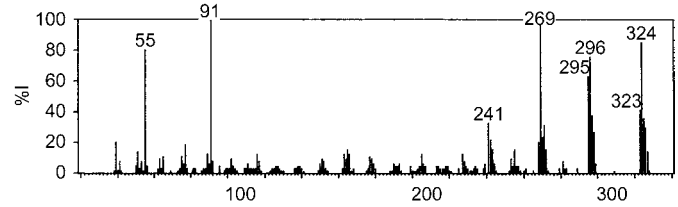
Chemical Properties A white crystalline powder. Mp 145° to 146° (crystals from methanol). Soluble in ethanol, chloroform, and dilute mineral acids. pK_a 2.7. Log *P* (octanol/pH 7.4), 3.7. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Test Formaldehyde–sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.36; system TC—R_f 0.74; system TD—R_f 0.64; system TE—R_f 0.81; system TF—R_f 0.55; system TL—R_f 0.63; system TAD—R_f 0.72; system TAE—Rf 0.84; system TAF—R_f 0.89; system TAJ—R_f 0.75; system TAK—R_f 0.69; system TAL—R_f 0.94 (acidified iodoplatinate solution—positive; acidified potassium permanganate—positive).
Gas Chromatography System GA—prazepam RI 2648, M (3-OH-) RI 2860, nordazepam RI 2490, oxazepam RI 2325; system GB—prazepam RI 2783, nordazepam RI 2625, oxazepam RI 2438; system GC—prazepam RI 3145, oxazepam RI 2803.
High Performance Liquid Chromatography System HB—*k* 29.99; system HJ—*k* 4.60; system HK—prazepam *k* 2.19, nordazepam *k* 1.99, oxazepam *k* 0.73; system HX—RI 648; system HY—RI 570; system HZ—RT 17.6 min; system HAA—RT 23.4 min; system HAX—RT 10.5 min; system HAY—RT 12.8 min; system HBI—*k* 4.28.
Ultraviolet Spectrum Aqueous acid—240 (A_1^1= 1760a), 285, 360 nm.



Infrared Spectrum Principal peaks at wavenumbers 1667, 1316, 740, 694, 1602, 704 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 269, 324, 55, 296, 295, 323, 297 (prazepam); 242, 269, 270, 241, 243, 271, 244, 272 (nordazepam); 257, 55, 311, 77, 259, 313, 44, 312 (3-hydroxyprazepam); 257, 77, 268, 239, 205, 267, 233, 259 (oxazepam).



Quantification

Serum **GC** Column: 3% SP-2250 on Supelcoport 100/120 mesh (2 m). Carrier gas: N_2, 20 mL/min. Temperature: 260°. ECD, SIM acquisition mode. Limit of detection, 1 µg/L [Nau *et al.* 1978].

LC-MS Column: Unison UK-C_{18} ODS (150 × 2 mm i.d., 3 µm) or Cadenza CD-C_{18} ODS (150 × 2.0 mm i.d., 3 µm). Mobile phase: 10 mmol/L aqueous ammonium formate-0.1% formic acid : methanol-0.1% formic acid (70 : 30 to 20 : 80 at 20 min for 5 min), flow rate 0.25 mL/min. ESI. Limit of detection, 0.3–11.4 µg/L [Nakamura *et al.* 2009].

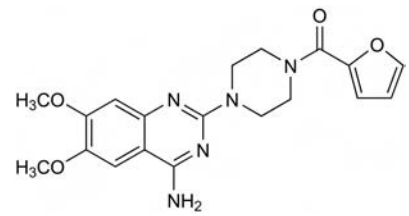
Disposition in the Body Prazepam is slowly and variably absorbed after oral administration. Desmethyldiazepam (nordazepam) is the major metabolite found in plasma and accounts for most of the activity. Prazepam is excreted in the urine mainly as glucuronide conjugates of oxazepam and 3-hydroxyprazepam, with only trace amounts of desmethyldiazepam or unchanged drug. Up to ~20% of a dose is excreted in the urine in 24 h and ~60% over a period of 7 days; ~7% of a dose is eliminated in the faeces in 48 h. Desmethyldiazepam is found in breast milk after administration of prazepam.
Therapeutic Concentration After a single oral dose of 30 mg to 10 subjects, peak plasma concentrations of 0.19–0.40 mg/L (mean, 0.3) desmethyldiazepam were reported in 2–8 h; a second peak plasma concentration was reported for each subject. Following oral administration of 20 mg three times a day for 3 days to 5 subjects, plasma concentrations averaged 0.8 mg/L 12 h after the final dose [Brodie *et al.* 1981].
Half-life Plasma half-life, ~40 to 100 h for desmethyldiazepam, but there is considerable intersubject variation (see Nordazepam monograph).
Volume of Distribution Desmethyldiazepam, 0.5–2.5 L/kg, increased in elderly subjects.
Clearance Plasma clearance, ~0.1–0.3 mL/min/kg for desmethyldiazepam.
Distribution in Blood Plasma : whole blood ratio, desmethyldiazepam ~1.7.
Milk Plasma : breast milk ratio, desmethyldiazepam 9.6.
Protein Binding Desmethyldiazepam, ~97%.
Dose 10 to 60 mg daily.

Brodie RR *et al.* (1981). Concentrations of *N*-descyclopropylmethylprazepam in whole-blood, plasma, and milk after administration of prazepam to humans. *Biopharm Drug Dispos* 2: 59–68.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography–tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.
Nau H *et al.* (1978). Quantitative analysis of prazepam and its metabolites by electron capture gas chromatography and selected ion monitoring. Application to diaplacetal passage and fetal hepatic metabolism in early human pregnancy. *J Chromatogr* 146: 227–239.

Prazosin

Antihypertensive
$C_{19}H_{21}N_5O_4$ = 383.4
CAS—19216-56-9

IUPAC Name [4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(furan-2-yl)methanone
Synonym 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)-piperazine; furazosin



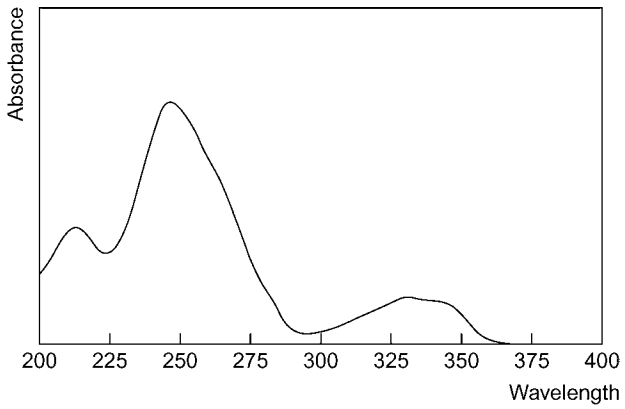
Chemical Properties Crystals. Mp 278° to 280°. pK_a 6.5.

Prazosin Hydrochloride

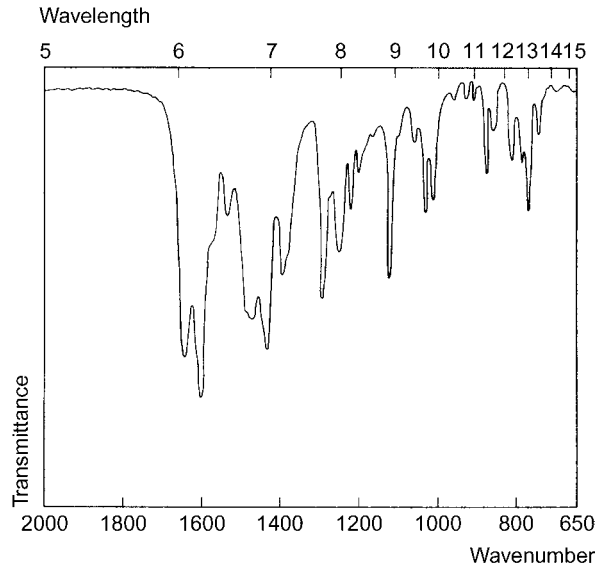
$C_{19}H_{21}N_5O_4$,HCl = 419.9
CAS—19237-84-4
Synonym CP-12299-1

Proprietary Names *Adversuten*; *Alphavase*; *Alpress*; *Apo-Prazo*; *Duramipress*; *Hexapress*; *Hypovase*; *Hyprosin*; *Hypotens*; *Minipress*; *Mipraz*; *Novo-Prazin*; *Nu-Prazo*; *Parabowl*; *Patsolin*; *Peripress*; *Prasig*; *Pratsiol*; *Prazac*; *Prazocor*; *Prazohexal*; *Pressin*.
Chemical Properties A white to tan-coloured powder. Mp about 264°, with decomposition. Very slightly soluble in water; slightly soluble in ethanol and methanol; practically insoluble in acetone and chloroform.

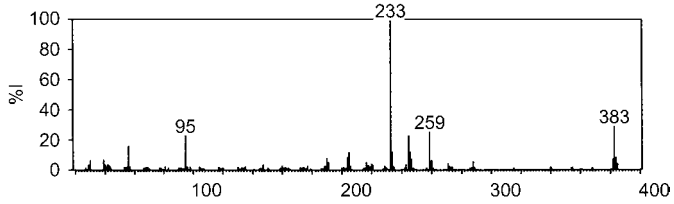
Colour Test Liebermann's reagent—brown-pink (→red-orange at 100°).
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.01; system TC—R_f 0.47; system TE—R_f 0.59; system TL—R_f 0.49; system TAE—R_f 0.68; system TAF—R_f 0.74; system TAJ—R_f 0.39; system TAK—R_f 0.00; system TAL—R_f 0.54.
Gas Chromatography System GA—Not eluted.
High Performance Liquid Chromatography System HA—*k* 0.8; system HX—RI 352; system HZ—retention time 2.5 min; system HAA—retention time 10.6 min.
Ultraviolet Spectrum Aqueous acid—247 (A_1^1=1470b), 331 nm (A_1^1=281b); aqueous alkali—252 (A_1^1=1642b), 345 nm (A_1^1=150b).



Infrared Spectrum Principal peaks at wavenumbers 1603, 1643, 1293, 1125, 1252, 1540 cm^{-1} (prazosin hydrochloride).



Mass Spectrum Principal ions at *m/z* 233, 383, 259, 245, 95, 56, 246, 234.



Quantification
Blood **HPLC** Fluorescence detection. Limit of detection, 1 µg/L [Lin *et al.* 1980].
Plasma **HPLC** See Blood [Lin *et al.* 1980]. Fluorescence detection. Limit of detection, 500 mg/L [Reece 1980].
Urine **HPLC** See Blood [Lin *et al.* 1980].
Disposition in the Body Readily absorbed after oral administration; bioavailability about 60%. Extensively metabolised by 6-*O*- and 7-*O*-demethylation and glucuronic acid conjugation; <5% of a dose is excreted in the urine as unchanged drug in 24 h.

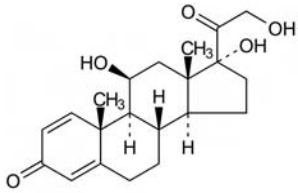
Therapeutic Concentration
After single oral doses of 0.5 mg and 1.5 mg given to 5 subjects, peak plasma concentrations of 0.001 to 0.005 mg/L (mean, 0.003) and 0.004 to 0.009 mg/L (mean, 0.006), respectively, were attained in 1 to 3 h [Dynon *et al.* 1980].

Following oral administration of 0.5 mg three times a day to 8 subjects, mean steady-state plasma concentrations of 0.001 to 0.007 mg/L (mean, 0.003) were reported; after dosing with 2 mg three times a day in 7 subjects, mean steady-state plasma concentrations were 0.008 to 0.019 mg/L (mean 0.013) [Grahnén *et al.* 1981].
Half-life Plasma half-life, about 3 h.
Volume of Distribution About 0.6 L/kg.
Clearance Plasma clearance, about 3 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, 1.4.
Protein Binding About 95%.
Note For a review of the pharmacokinetics of prazosin, see Jaillon [1980].
Dose 0.5 to 20 mg of prazosin hydrochloride daily.

Dynon MK *et al.* (1980). Pharmacokinetics of prazosin in normotensive subjects after low oral doses. *Clin Pharmacokinet* 5: 583–590.
Grahnén A *et al.* (1981). Prazosin kinetics in hypertension. *Clin Pharmacol Ther* 30: 439–446.
Jaillon P (1980). Clinical pharmacokinetics of prazosin. *Clin Pharmacokinet* 5: 365–376.
Lin ET *et al.* (1980). High-performance liquid chromatographic determination of prazosin in human plasma, whole blood and urine. *J Chromatogr* 183: 367–371.
Reece PA (1980). Quantification of prazosin in plasma by high-performance liquid chromatography. *J Chromatogr* 221: 188–192.

Prednisolone

Corticosteroid
$C_{21}H_{28}O_5$ = 360.4
CAS—50-24-8 (anhydrous); 52438-85-4 (sesquihydrate)
IUPAC Name (8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-3-one
Synonyms 1,2-Dehydrohydrocortisone; deltahydrocortisone; Δ^1-hydrocortisone; hydroretrocortine; metacortandralone; (11β)-11,17,21-trihydroxypregna-1,4-diene-3,20-dione.
Proprietary Names *Codelcortone*; *Cortalone*; *Decaprednil*; *Decortin H*; *Delta-Cortef*; *Deltacortril*; *Deltasolone*; *Flamasone*; *Hydeltra*; *Hydrodeltalone*; *Klismacort*; *Meticortolone*; *Paracortol*; *Precortilon*; *Precortisyl*; *Prednelan*; *Solone*; *Sterolone*.



Chemical Properties A white hygroscopic crystalline powder. Mp 240° to 241°, with decomposition. Soluble 1 in 1300 of water, 1 in 30 of ethanol, 1 in 27 of dehydrated alcohol, 1 in 180 of chloroform and 1 in 50 of acetone; soluble in dioxan and methanol. Log *P* (octanol/water), 1.6.

Prednisolone Acetate
$C_{23}H_{30}O_6$ = 402.5
CAS—52-21-1
Synonym Prednisolone 21-acetate
Proprietary Names *Deltastab*; *Econopred*; *Pred Forte*; *Pred Mild*; *Ultracortenol.*
Chemical Properties A white crystalline powder. Mp 237° to 239°, with decomposition. Practically insoluble in water; soluble 1 in 120 of ethanol, 1 in 170 of dehydrated alcohol and 1 in 150 of chloroform; slightly soluble in acetone.

Prednisolone Pivalate
$C_{26}H_{36}O_6$ = 444.6
CAS—1107-99-9
Synonyms Prednisolone 21-pivalate; prednisolone trimethylacetate.
Proprietary Name *Ultracortenol*
Chemical Properties A white crystalline powder. Mp 233° to 236°. Practically insoluble in water; soluble 1 in 150 of ethanol and 1 in 16 of chloroform.

Prednisolone Sodium Phosphate
$C_{21}H_{27}Na_2O_8P$ = 484.4
CAS—125-02-0
Synonym Prednisolone 21-(disodium orthophosphate)
Proprietary Names *Codelsol*; *Hefasolon*; *Hydeltrasol*; *Inflamase*; *Metreton*; *Prednesol*; *Predsol*; *Solucort*; *Solu-Predalone*.
Chemical Properties A white or slightly yellow hygroscopic powder or granules. Soluble 1 in 3 or 1 in 4 of water, 1 in 1000 of dehydrated alcohol and 1 in 13 of methanol; practically insoluble in chloroform.

Prednisolone Steaglate
$C_{41}H_{64}O_8$ = 684.9
CAS—5060-55-9
Synonym Prednisolone 21-stearoylglycolate
Proprietary Name *Sintisone*
Chemical Properties A white powder. Mp 105° to 107°. Soluble in ethanol, acetone and methanol.

P

Prednisolone Succinate

$C_{25}H_{32}O_8$ = 460.5
CAS—2920-86-7; 1715-33-9 (sodium salt)
Synonym Prednisolone 21-hydrogen succinate
Proprietary Name *Solu-Dacortin (sodium salt)*
Chemical Properties A fine creamy-white powder with friable lumps. Mp about 205°, with decomposition. Very slightly soluble in water; soluble 1 in about 6 of ethanol and 1 in about 250 of ether; soluble in acetone; very slightly soluble in chloroform.

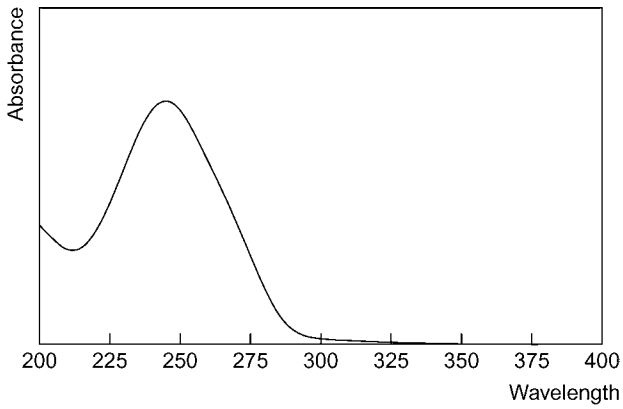
Prednisolone Tebutate

$C_{27}H_{38}O_6,H_2O$ = 476.6
CAS—7681-14-3 (anhydrous)
Synonyms Prednisolone butylacetate; prednisolone 21-(3,3-dimethylbutyrate); prednisolone tertiary-butylacetate.
Proprietary Names *Codelcortone TBA*; *Hydeltra-TBA*.
Chemical Properties A white to slightly yellow hygroscopic powder. Very slightly soluble in water; sparingly soluble in ethanol and methanol; soluble in acetone; freely soluble in chloroform and dioxan.

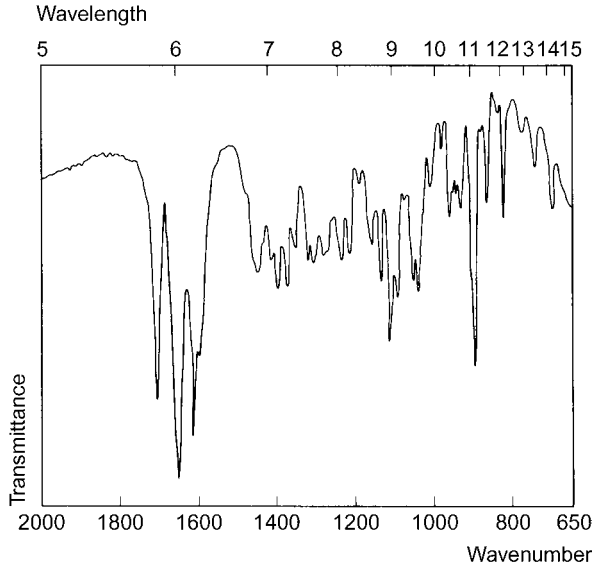
Colour Tests Naphthol-sulfuric acid—brown/brown; sulfuric acid—orange-pink.
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.41; system TF—R_f 0.24; system TP—R_f 0.20; system TQ—R_f 0.00; system TR—R_f 0.02, streaking may occur; system TS—R_f 0.00; system TAE—R_f 0.86; system TAJ—R_f 0.19; system TAK—R_f 0.03; system TAL—R_f 0.65; system TAM—R_f 0.54. (DPST solution.)

System TP—pivalate R_f 0.69; system TQ—pivalate R_f 0.04; system TR—pivalate R_f 0.44; system TS—pivalate R_f 0.00 (DPST solution).

System TP—sodium phosphate R_f 0.00; system TQ—sodium phosphate R_f 0.00; system TR—sodium phosphate R_f 0.00; system TS—sodium phosphate R_f 0.00.
High Performance Liquid Chromatography System HT—*k* 8.4; system HX—RI 401; system HY—RI 361; system HZ—RT 2.5 min; system HAA—RT 14.1 min.
Ultraviolet Spectrum Ethanol—240 nm (A_1^1=415a).



Infrared Spectrum Principal peaks at wavenumbers 1654, 1612, 1708, 887, 1112, 1085 cm^{-1} (KBr disk).



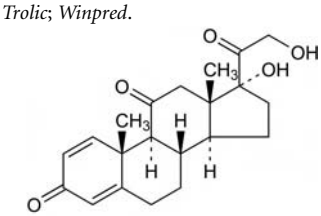
Mass Spectrum Principal peaks at *m/z* 121, 122, 91, 147, 225, 43, 135, 120.
Quantification
Plasma **GC-MS** Prednisolone and prednisone [Matin, Amos 1978].

HPLC Prednisolone and other corticosteroids. Limit of detection, 10 µg/L [al-Habet, Rogers 1989]. UV detection. Prednisolone, prednisone and hydrocortisone. Limit of detection, 5 µg/L [Rose, Jusko 1979]. UV detection. Limit of detection, 10 µg/L [Hartley, Brocklebank 1982].
Serum **RIA** Limit of detection, 10 µg/L [Olivesi *et al.* 1983].
Urine **HPLC** See Plasma. Limit of detection, 25 µg/L [al-Habet, Rogers 1989]. See Plasma [Rose, Jusko 1979].
Saliva **HPLC** See Plasma. Limit of detection, 10 µg/L [al-Habet, Rogers 1989]. See Plasma [Rose, Jusko 1979].
Dose Usually 5 to 60 mg of prednisolone daily.

al-Habet SM, Rogers HJ (1989). Two chromatographic methods for the determination of corticosteroids in human biological fluids: pharmacokinetic applications. *J Pharm Sci* 78: 660–666.
Hartley R, Brocklebank JT (1982). Determination of prednisolone in plasma by high-performance liquid chromatography. *J Chromatogr* 232: 406–412.
Matin SB, Amos B (1978). Quantitative determination of prednisone and prednisolone in human plasma using GLC and chemical-ionization mass spectrometry. *J Pharm Sci* 67: 923–926.
Olivesi A *et al.* (1983). Specific micro-radioimmunoassay for prednisolone in serum. *Clin Chem* 29: 1358–1362.
Rose JQ, Jusko WJ (1979). Corticosteroid analysis in biological fluids by high-performance liquid chromatography. *J Chromatogr* 162: 273–280.

Prednisone

Corticosteroid
$C_{21}H_{26}O_5$ = 358.4
CAS—53-03-2
IUPAC Name (8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Hydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,9,12,14,15,16-octahydrocyclopenta[a]phenanthrene-3,11-dione
Synonyms 1,2-Dehydrocortisone; deltacortisone; deltadehydrocortisone; 17,21-dihydroxypregna-1,4-diene-3,11,20-trione; metacortandracin; NSC-10023.
Proprietary Names *Colisone*; *Contancyl*; *Dacortin*; *Deltacortone*; *Deltason(e)*; *Meprosona-F*; *Meticorten*; *Ofisolona*; *Orasone*; *Panafcort*; *Paracort*; *Predeltin*; *Predicor*; *Prednicort*; *Prednicorten*; *Prednidib*; *Prednitone*; *Promifen*; *Pulmison*; *Rectodelt*; *Sterapred*; *Trolic*; *Winpred*.

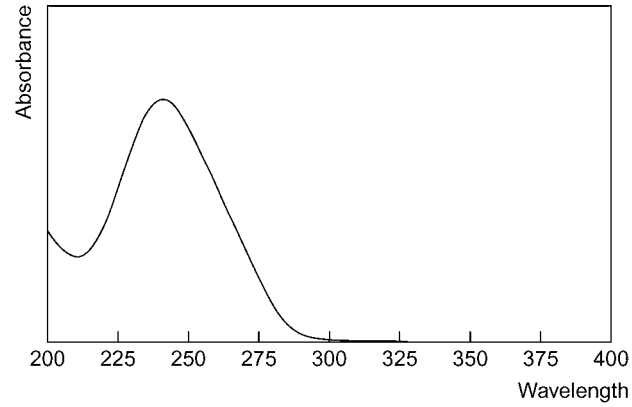


Chemical Properties A white crystalline powder. Mp 233° to 235°, with decomposition. Practically insoluble in water; soluble 1 in about 150 of ethanol, 1 in 300 of dehydrated alcohol and 1 in 200 of chloroform; slightly soluble in methanol. Log *P* (octanol/water), 1.5.

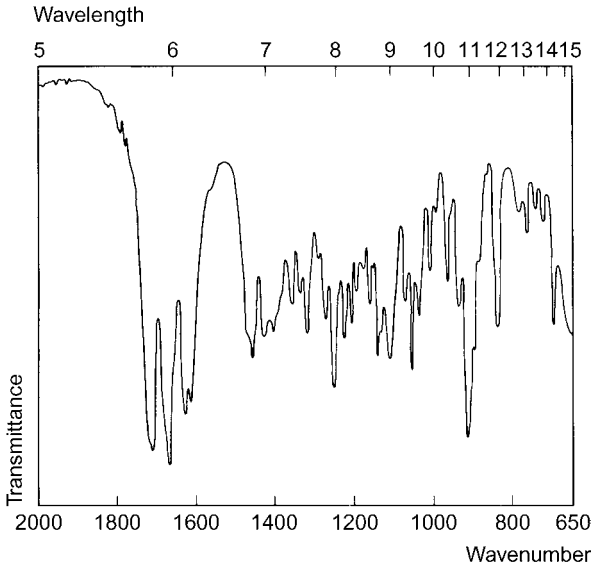
Prednisone Acetate

$C_{23}H_{28}O_6$ = 400.5
CAS—125-10-0
Synonym Prednisone 21-acetate
Chemical Properties A white crystalline powder. Mp 226° to 232°, with decomposition. Practically insoluble in water; soluble 1 in about 120 of ethanol, 1 in 160 of dehydrated alcohol and 1 in 6 of chloroform.

Colour Tests Naphthol-sulfuric acid—brown/orange; sulfuric acid—yellow (green fluorescence under UV light).
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.45; system TF—R_f 0.28; system TP—R_f 0.41; system TQ—R_f 0.00; system TR—R_f 0.10; system TS—R_f 0.00; system TAE—R_f 0.84; system TAJ—R_f 0.33; system TAK—R_f 0.04; system TAL—R_f 0.74; system TAM—R_f 0.60 (DPST solution).
High Performance Liquid Chromatography System HT—*k* 3.4; system HX—RI 250; system HY—RI 340; system HZ—retention time 2.6 min; system HAA—retention time 14.2 min.
Ultraviolet Spectrum Ethanol—240 nm (A_1^1=420a).



Infrared Spectrum Principal peaks at wavenumbers 1668, 1707, 904, 1622, 1610, 1246 cm^{-1} (KBr disk).

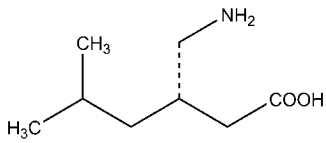


Quantification See under Prednisolone.
Dose Usually 5 to 60 mg daily.

Pregabalin

Antiepileptic
$C_8H_{17}NO_2 = 159.2$
CAS—148553-50-8

IUPAC Name (3*S*)-3-(Aminomethyl)-5-methylhexanoic acid
Synonyms (*S*)-(+)-4-Amino-3-(2-methylpropyl)butanoic acid; CI-1008; (*S*)-(+)-3-Isobutyl-γ-aminobutyric acid; PD-144723.
Proprietary Name *Lyrica*



Chemical Properties A white crystalline solid. Mp 186° to 188°. pK_{a1} 4.2, pK_{a2} 10.6 [Wishart 2006]. Log *P* (octanol/water), 0.844 [Wishart 2006]. Pregabalin was stable for 3 to 4 weeks under all conditions examined (4° and –20°), except for room temperature [Berry, Millington 2005].

Quantification

Plasma **HPLC** Column: Hypersil 5 MOS (25 cm × 4 mm i.d.). Mobile phase: acetate buffer (pH 4.6) : acetonitrile (50 : 50), flow rate 1.9 mL/min. UV detection (λ = 340 nm). Limit of quantification, 0.2 mg/L for the picrylsulfonic acid derivative [Berry, Millington 2005].

Serum **HPLC** Column: reversed phase packed with Alltina $3C_{18}$ (15 × 0.46 cm i.d.). Mobile phase: methanol : acetonitrile : 20 mmol/L phosphate buffer (pH 7.0; 8 : 17.5 : 74.5), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 450 nm). Limit of quantification, 0.13 mg/L; limit of detection, 44 µg/L for the *o*-phtaldialdehyde derivative [Vermeij, Edelbroek 2004].

Disposition in the Body Rapidly absorbed after administration and mainly (>90%) eliminated as the parent drug in urine.

Therapeutic Concentration

A group of patients with doses escalated to 600 mg/day had serum concentrations of 2.8 to 8.2 mg/L at steady state [Berry, Millington 2005].

Male and female patients (n = 38) suffering from degrees of renal insufficiency were administered a single dose of 50 mg pregabalin after an 8 h fast. Mean peak plasma concentrations were 1.86, 1.53, 1.90, 1.69 and 1.24 mg/L after 1.0, 1.29, 1.93, 1.0 and 3.18 h for patients with creatinine clearance of >60, 30–60, 15–29 and <15 mL/min, and on haemodialysis, respectively [Randinitis *et al.* 2003].

Healthy male and female volunteers were administered single doses of pregabalin ranging from 1 to 300 mg. Peak plasma concentrations, ranging from 0.038 to 9.46 mg/L, were observed within 1.3 h [Busch *et al.* 1998].

Toxicity

A 29-year-old male epileptic was found in a coma. He had left a suicide note, had slashed his wrists, and had taken approx. 11.5 g pregabalin and 32 g lamotrigine. He was admitted to hospital and was found to have plasma serum levels of approximately 62 and 45 mg/L pregabalin and lamatrogine, respectively, after 1 day. He was administered a loading dose of phenytoin followed by 100 mg three times a day. He was discharged after 28 days in hospital, 19 of which were spent in critical care [Braga, Chidley 2007].

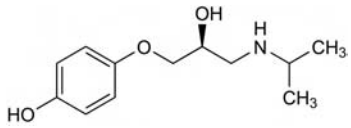
Bioavailability Approximately 90%.
Half-life 4.6 to 6.8 h.
Protein Binding Does not bind to plasma proteins.
Dose 150 to 600 mg/day orally.

Berry D, Millington C (2005). Analysis of pregabalin at therapeutic concentrations in human plasma/serum by reversed-phase HPLC. *Ther Drug Monit* 27: 451–456.
Braga AJ, Chidley K (2007). Self-poisoning with lamotrigine and pregabalin. *Anaesthesia* 62: 524–527.
Busch JA *et al.* (1998). Pregabalin (C1-1008) single-dose pharmacokinetics and safety/tolerance in healthy subjects after oral administration of pregabalin solution or capsule doses. *Epilepsia* 3958.
Randinitis EJ *et al.* (2003). Pharmacokinetics of pregabalin in subjects with various degrees of renal function. *J Clin Pharmacol* 43: 277–283.
Vermeij TA, Edelbroek PM (2004). Simultaneous high-performance liquid chromatographic analysis of pregabalin, gabapentin and vigabatrin in human serum by precolumn derivatization with *o*-phtaldialdehyde and fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 810: 297–303.
Wishart DS *et al.* (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Prenalterol

Sympathomimetic
$C_{12}H_{19}NO_3 = 225.3$
CAS—57526-81-5

IUPAC Name 4-[(2*S*)-2-Hydroxy-3-(propan-2-ylamino)propoxy]phenol
Synonym 4-[(2*S*)-2-Hydroxy-3-[(1-methylethyl)amino]propoxy]phenol



Chemical Properties pK_a 9.5 (ammonium ion), 10.0 (phenol). Log *P* (octanol/water), 0.9.

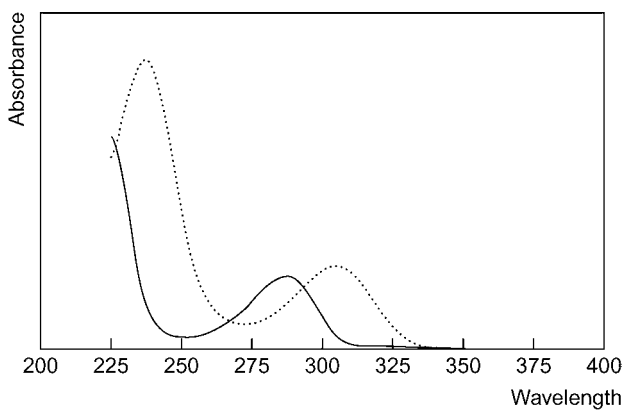
Prenalterol Hydrochloride

$C_{12}H_{19}NO_3$,HCl = 261.7
CAS—61260-05-7
Synonym CGP-7760B; H-133/22; (–)H-80/62.
Proprietary Names *Hyprenan*; *Varbian*.
Chemical Properties A white crystalline powder. Mp about 128°. Freely soluble in water and ethanol; slightly soluble in acetone; very slightly soluble in ether.

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.01; system TC—R_f 0.09; system TD—R_f 0.02; system TE—R_f 0.25; system TF—R_f 0.02. (Dragendorff spray, positive; Ferric chloride solution, violet; Acidified iodoplatinate solution, positive; Marquis reagent, green→black; acidified potassium permanganate solution, positive.)

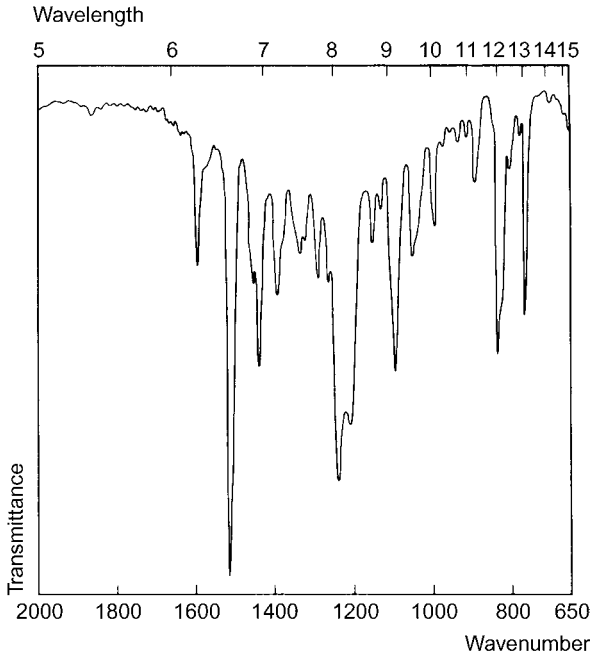
Gas Chromatography System GA—RI 1933.

Ultraviolet Spectrum Aqueous acid—287 nm (A_1^1=105a); aqueous alkali—235 (A_1^1=444b), 305 nm.

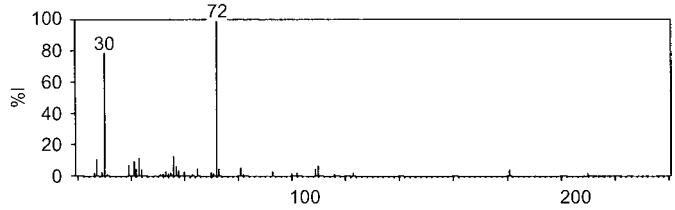


Infrared Spectrum Principal peaks at wavenumbers 1518, 1242, 1213, 1099, 840, 772 cm^{-1} (prenalterol hydrochloride, KBr disk).

P



Mass Spectrum Principal ions at *m/z* 72, 30, 56, 43, 27, 41, 39, 110.



Quantification
Plasma **GC** ECD. Limit of detection, 2 µg/L [Degen, Ervik 1981].
GC-MS Limit of detection, 1 µg/L [Ervik *et al.* 1982].
HPLC UV and fluorescence detection. Prenalterol and other beta-blockers [Musch *et al.* 1989]. Fluorescence detection. Limit of detection, 1 µg/L [Oddie *et al.* 1982].
Urine **GC** See Plasma [Degen, Ervik 1981].
GC-MS See Plasma [Ervik *et al.* 1982].

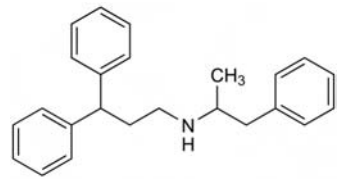
Disposition in the Body Rapidly and completely absorbed after oral administration, but subject to extensive first-pass metabolism. The main metabolic reaction is conjugation with sulfate at the phenol position; minor metabolites formed are β-(4-hydroxyphenoxy)lactic acid and prenalterol glucuronide. About 90% of a dose is excreted in the urine in 24 h. After an IV dose, about 60% is excreted unchanged with about 35% as the sulfate; after oral administration, about 15% is excreted unchanged, with about 80% as the sulfate.
Bioavailability About 25%.
Half-life Plasma half-life, about 2 h.
Volume of Distribution About 3.5 L/kg.
Clearance Plasma clearance, about 20 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, about 0.9.
Protein Binding <5%.
Dose 0.5 mg of prenalterol hydrochloride per min by IV infusion, to a total of not more than 20 mg.

Degen PH, Ervik M (1981). Determination of prenalterol in plasma and in urine by gas-liquid chromatography. *J Chromatogr* 222: 437–444.
Ervik M *et al.* (1982). Assay of prenalterol in plasma and urine by gas chromatography-mass spectrometry. *J Chromatogr* 229: 87–94.
Musch G *et al.* (1989). A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography. *J Pharm Biomed Anal* 7: 483–497.
Oddie CJ *et al.* (1982). Determination of prenalterol in plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 231: 473–477.

Prenylamine

Antianginal
$C_{24}H_{27}N$ = 329.5
CAS—390-64-7

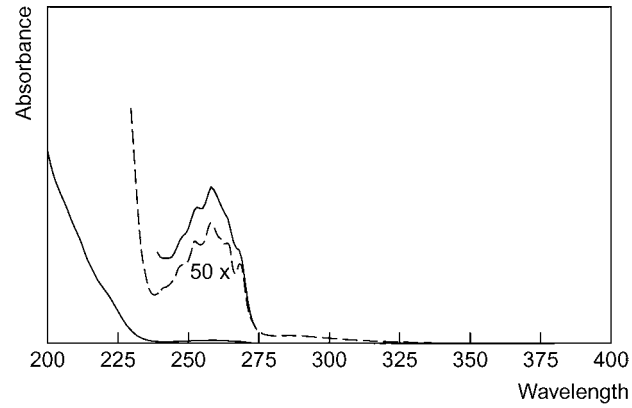
IUPAC Name 3,3-Diphenyl-*N*-(1-phenylpropan-2-yl)propan-1-amine
Synonyms B-436; *N*-(1-methyl-2-phenylethyl)-γ-phenylbenzenepropanamine
Proprietary Name *Elecor*



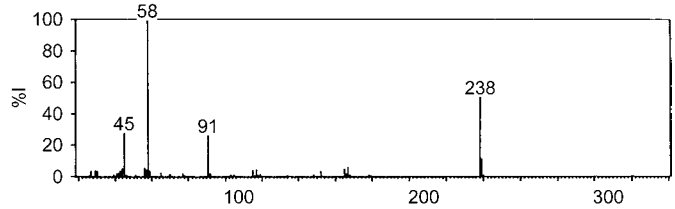
Chemical Properties Mp 36.5° to 37.5°. Log *P* (octanol/water), 6.0.

Prenylamine Lactate
$C_{24}H_{27}N,C_3H_6O_3$ = 419.6
CAS—69-43-2
Proprietary Names *Angormin*; *Bismetin*; *Carditin-Same*; *Coredamin*; *Corontin*; *Crepasin*; *Daxauten*; *Hostaginan*; *Incoran*; *Irrorin*; *Lactamin*; *Plactamin*; *Reocorin*; *Roinin*; *Seccidin*; *Sedolatan*; *Segontin(e)*; *Synadrin*.
Chemical Properties A white crystalline powder. Mp 140° to 142°. Soluble 1 in about 200 of water, 1 in 5 of ethanol and 1 in 2 of chloroform; very soluble in glacial acetic acid.

Colour Tests Mandelin's test—green; Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.55; system TC—R_f 0.68; system TE—R_f 0.84; system TL—R_f 0.56; system TAE—R_f 0.43; system TAF—R_f 0.85; system TAJ—R_f 0.47; system TAK—R_f 0.63; system TAL—R_f 0.90. (Acidified iodoplatinate solution, positive.)
Gas Chromatography System GA—prenylamine RI 2555, M (desamino-OH-)-H_2O RI 1940.
High Performance Liquid Chromatography System HA—*k* 1.0.
Ultraviolet Spectrum Methanol—253, 258 (A^1_1=18.8a), 268 nm.



Infrared Spectrum Principal peaks at wavenumbers 700, 747, 1136, 1027, 1592, 1075 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 238, 91, 45, 239, 167, 165, 56.



Quantification
Plasma **HPLC** Fluorimetric detection. *R*- and *S*-prenylamine. Limit of detection, 1 µg/L in plasma [Gietl *et al.* 1988].
Serum **GC** FID. Prenylamine and metabolites. Limits of detection, 10 to 50 µg/L [Eichelbaum *et al.* 1973].
Urine **GC** See Serum [Eichelbaum *et al.* 1973].
HPLC Limit of detection, 2 µg/L in urine, see Plasma [Gietl *et al.* 1988].

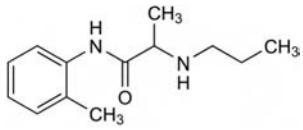
Disposition in the Body Readily absorbed after oral administration but extensively metabolised. Numerous metabolites, including traces of amfetamine, have been detected in the urine; some unchanged drug is eliminated in the faeces.
Half-life Plasma half-life, about 7 h.
Protein Binding About 97%.
Dose The equivalent of 180 to 300 mg of prenylamine daily.

Eichelbaum M *et al.* (1973). A specific and sensitive method for the analysis of prenylamine and its derivatives in biological materials. *Arzneimittelforschung* 23: 74–77.
Gietl Y *et al.* (1988). Simultaneous determination of R- and S-prenylamine in plasma and urine by reversed-phase high-performance liquid chromatography. *J Chromatogr* 426: 304–314.

Prilocaine

Anaesthetic (Local)
$C_{13}H_{20}N_2O$ = 220.3
CAS—721-50-6

IUPAC Name *N*-(2-Methylphenyl)-2-(propylamino)propanamide
Synonym Propitocaine

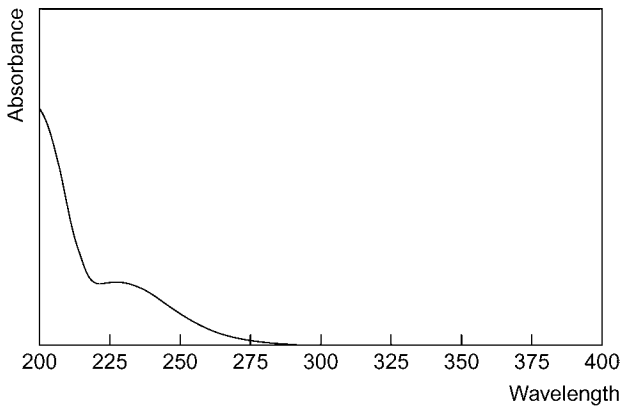


Chemical Properties A white crystalline powder. Mp 37° to 38°. Freely soluble in ethanol, chloroform and ether. pK_a 7.9 (25°). Log *P* (octanol/water), 2.1. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

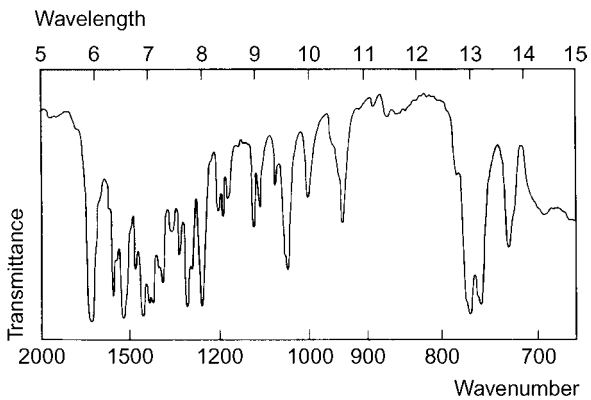
Prilocaine Hydrochloride

$C_{13}H_{20}N_2O,HCl = 256.8$
CAS—1786-81-8
Synonyms Astra-1512; L-67.
Proprietary Names *Citanest*; *Citocaina*; *Xylonest*. It is an ingredient of *EmLa*.
Chemical Properties A white crystalline powder. Mp 167° to 168°(crystals from ethanol and isopropyl ether). Soluble 1 in 5 of water, 1 in 6 of ethanol and 1 in 175 of chloroform; practically insoluble in ether.

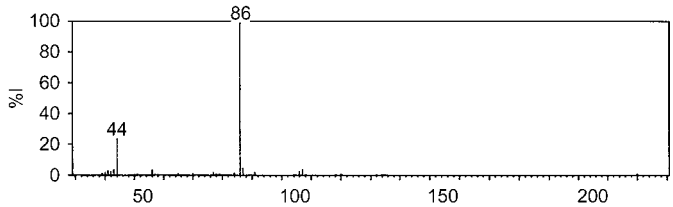
Colour Test Mandelin's test—violet.
Thin-layer Chromatography System TA—R_f 0.77; system TB—R_f 0.29; system TC—R_f 0.64; system TE—R_f 0.75; system TL—R_f 0.60; system TAE—R_f 0.62; system TAF—R_f 0.79; system TAJ—R_f 0.50; system TAK—R_f 0.22; system TAL—R_f 0.69.
Gas Chromatography System GA—prilocaine RI 1823, M (OH-) RI 2155, M (OH-desacyl-) RI 1160, prilocaine-AC RI 2060, M (desacyl-)-AC RI 1350; system GQ—retention time 5.7 min.
High Performance Liquid Chromatography System HA—*k* 1.0; system HQ—*k* 1.38; system HZ—retention time 2.7 min.
Ultraviolet Spectrum Methanol—230 nm (A_1^1=295a).



Infrared Spectrum Principal peaks at wavenumbers 1695, 1543, 766, 754, 1299, 1258 cm^{-1} (prilocaine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 44, 87, 107, 43, 106, 56, 41.



Quantification

Blood **GC-MS** Prilocaine and other local anaesthetics [Watanabe *et al.* 1998].
Plasma **GC-MS** (SIM, *m/z* 86). Limit of quantification, 100 µg/L, limit of detection is 80 µg/L [Ohshima, Takayasu 1999]. Limit of detection, 10 mg/L [Bachmann-Mennenga *et al.* 1991]. Prilocaine enantiomers [Tucker *et al.* 1990].
Serum **HPLC** UV detection. Prilocaine enantiomers. Limit of quantification, 10 µg/L [Siluveru, Stewart 1996].
Urine **GC-MS** See Plasma [Ohshima, Takayasu 1999].
Biological Samples **GC** Nitrogen sensitive detection. Prilocaine and other local anaesthetics [Lau *et al.* 1991].
Disposition in the Body Metabolised by hydrolysis and hydroxylation resulting in deacetylated and hydroxylated metabolites which are excreted mainly as conjugates. About 35% of a dose is excreted in the urine as 4-hydroxy-2-methylaniline (*p*-hydroxytoluidine), 3% as 6-hydroxy-2-methylaniline, 1% as 2-methylaniline (*o*-toluidine), and less than 5% as unchanged drug. Prilocaine crosses the placenta and is excreted in breast milk.
Therapeutic Concentration In plasma, usually in the range 1 to 5 mg/L. 4-Hydroxy-2-methylaniline has been detected in plasma at concentrations similar to those of prilocaine.

In 30 patients receiving 40 mL of a 0.5% solution of prilocaine IV, the mean peak plasma concentration was 4.4 mg/L reached immediately after release of the tourniquet [Simon *et al.* 1997].

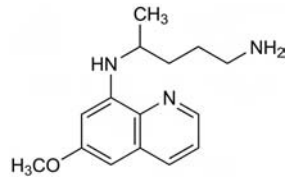
After the epidural administration of 200, 400, and 600 mg to 10 subjects, mean peak plasma concentrations determined within 10 to 20 min were 1.69, 2.67, and 4.47 mg/L, respectively; after the intercostal administration of 20 mL of a 2% solution, the mean peak concentration was 4.5 mg/L [Scott *et al.* 1972].

Toxicity Toxic effects are usually associated with plasma concentrations greater than 5 mg/L. 4-Hydroxy-2-methylaniline is thought to be responsible for the methaemoglobinaemia which may occur after large doses.
Half-life Plasma half-life, about 1 to 2 h.
Distribution in Blood Plasma : whole blood ratio, 0.80.
Protein Binding About 50%.
Dose Maximum dose of prilocaine hydrochloride, 400 mg, or 300 mg in solutions with felypressin.

Bachmann-Mennenga B *et al.* (1991). Ultrafiltration as a fast and simple method for determination of free and protein bound prilocaine concentration. Clinical study following high-dose plexus anesthesia. *Arzneimittelforschung* 41: 520–524.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Lau OW *et al.* (1991). Gas-liquid chromatographic determination and pharmacological studies of six clinically-used local anesthetics. *Methods Find Exp Clin Pharmacol* 13: 475–481.
Ohshima T, Takayasu T (1999). Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography-mass spectrometry with solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 726: 185–194.
Scott DB *et al.* (1972). Factors affecting plasma levels of lignocaine and prilocaine. *Br J Anaesth* 44: 1040–1049.
Siluveru M, Stewart JT (1996). Stereoselective determination of R(-)- and S(+)-prilocaine in human serum using a brush-type chiral stationary phase, solid-phase extraction and UV detection. *J Pharm Biomed Anal* 15: 389–392.
Simon MA *et al.* (1997). Intravenous regional anesthesia with 0.5% articaine, 0.5% lidocaine, or 0.5% prilocaine. A double-blind randomized clinical study. *Reg Anesth* 22: 29–34.
Tucker GT *et al.* (1990). Plasma concentrations of the stereoisomers of prilocaine after administration of the racemate: implications for toxicity? *Br J Anaesth* 65: 333–336.
Watanabe T *et al.* (1998). Simple analysis of local anaesthetics in human blood using headspace solid-phase microextraction and gas chromatography-mass spectrometry-electron impact ionization selected ion monitoring. *J Chromatogr B Biomed Sci Appl* 709: 225–232.

Primaquine

Antimalarial
$C_{15}H_{21}N_3O = 259.4$
CAS—90-34-6
IUPAC Name 4-*N*-(6-Methoxyquinolin-8-yl)pentane-1,4-diamine
Synonyms N^4-(6-Methoxy-8-quinolinyl)-1,4-pentadiamine; primachin.

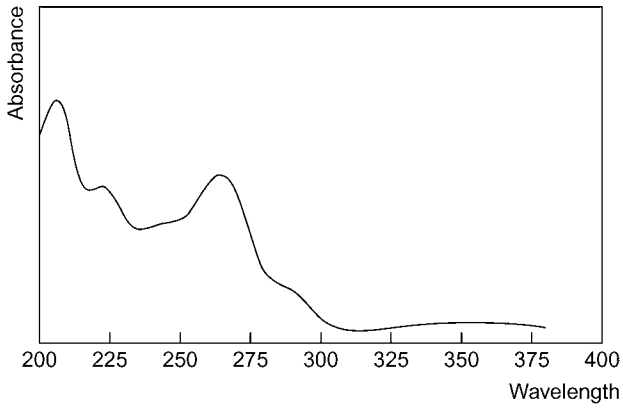


Chemical Properties A viscous liquid. Moderately soluble in water; soluble in ether. Log *P* (octanol/water), 3.2.

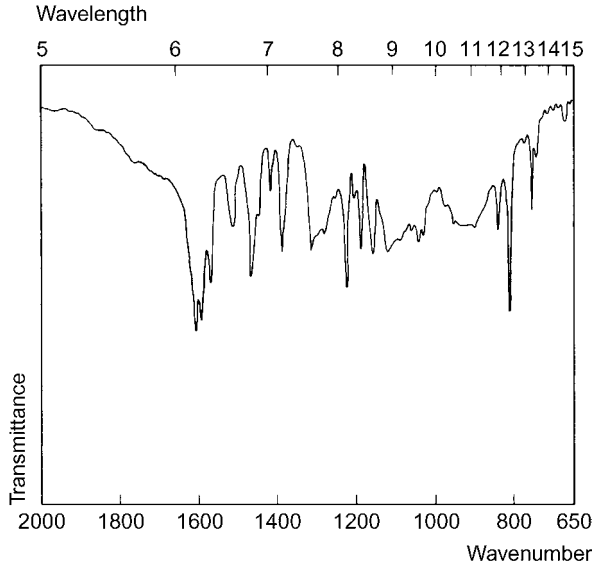
Primaquine Phosphate

$C_{15}H_{21}N_3O,2H_3PO_4 = 455.3$
CAS—63-45-6
Proprietary Name *Primacin*
Chemical Properties An orange-red crystalline powder. Mp 197° to 198°. Soluble 1 in about 15 of water; practically insoluble in ethanol, chloroform and ether.

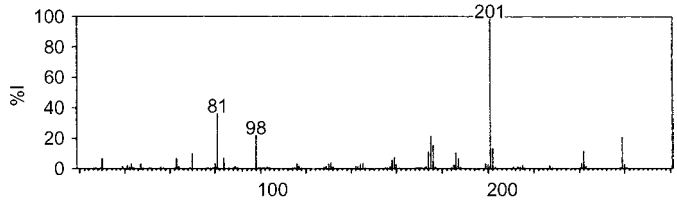
Colour Tests Mandelin's test—violet→orange; Marquis test—orange; Dissolve 10 mg in 5 mL of water and add 1 mL of a 5% solution of ceric ammonium sulfate in dilute nitric acid—violet (distinction from chloroquine).
Thin-layer Chromatography System TA—R_f 0.19; system TB—R_f 0.13; system TC—R_f 0.05; system TL—R_f 0.15 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2314.
High Performance Liquid Chromatography System HA—*k* 1.4; system HY—RI 276.
Ultraviolet Spectrum Aqueous acid—265 (A^1_1= 579a), 282 (A^1_1= 574a), 334 nm.



Infrared Spectrum Principal peaks at wavenumbers 1611, 1595, 815, 1230, 1572, 1170 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 201, 81, 98, 175, 259, 176, 202, 242.



Quantification
Blood **GC** ECD. Limit of detection, 10 µg/L [Rajagopalan *et al.* 1981].
HPLC UV detection. Primaquine and carboxyprimaquine [Dua *et al.* 1996].
Plasma **GC-MS** [Greaves *et al.* 1979].
HPLC See Blood [Dua *et al.* 1996]. Electrochemical detection. Primaquine and carboxyprimaquine. Limit of detection, 5 µg/L for primaquine and 20 µg/L for carboxyprimaquine [Dean *et al.* 1994]. Carboxyprimaquine and *N*-acetylprimaquine. Limit of detection, 75 µg/L for carboxyprimaquine and 10 µg/L for *N*-acetylprimaquine [Mihaly *et al.* 1984]. UV detection. Limit of detection, 1 µg/L [Ward *et al.* 1983].
Urine **GC-MS** See Plasma [Greaves *et al.* 1979].
HPLC See Plasma [Mihaly *et al.* 1984].
Disposition in the Body Readily absorbed after oral administration. It is extensively metabolised by oxidation to carboxyprimaquine which is the major plasma metabolite. Other reactions which may occur are demethylation and oxidation to the 5,6-diol, which is then converted to an active quinone, *N*-dealkylation to 8-amino-6-methoxyquinoline, and *N*-acetylation. Less than 5% of a dose is excreted in the urine unchanged in 24 h.

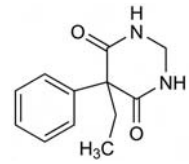
Therapeutic Concentration
Following a single oral dose of 45 mg to 5 subjects, peak plasma concentrations of 0.13–0.18 mg/L (mean, 0.15) were attained in 2–3 h; peak plasma concentrations of carboxyprimaquine of 1.1–1.8 mg/L (mean, 1.4) were attained in 2–12 h (mean, 7) [Mihaly *et al.* 1984].
Half-life Plasma half-life, about 4–10 h (mean, 7).
Volume of Distribution About 3–4 L/kg.
Distribution in Blood Plasma : whole blood ratio, 0.80
Protein Binding Extensively bound.
Dose The equivalent of 15 to 45 mg of primaquine daily.

Dean RA *et al.* (1994). Simultaneous determination of primaquine and carboxyprimaquine in plasma using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* 655: 89–96.
Dua VK *et al.* (1996). High-performance liquid chromatographic determination of primaquine and carboxyprimaquine concentrations in plasma and blood cells in Plasmodium vivax malaria cases following chronic dosage with primaquine. *J Chromatogr B Biomed Appl* 675: 93–98.
Greaves J *et al.* (1979). A selected ion monitoring assay for primaquine in plasma and urine. *Biomed Mass Spectrom* 6: 109–112.
Mihaly GW *et al.* (1984). Pharmacokinetics of primaquine in man: identification of the carboxylic acid derivative as a major plasma metabolite. *Br J Clin Pharmacol* 17: 441–446.
Rajagopalan TG *et al.* (1981). *J Chromatogr* 224: 265–273.
Ward SA *et al.* (1983). *J Chromatogr* 305: 239–243.

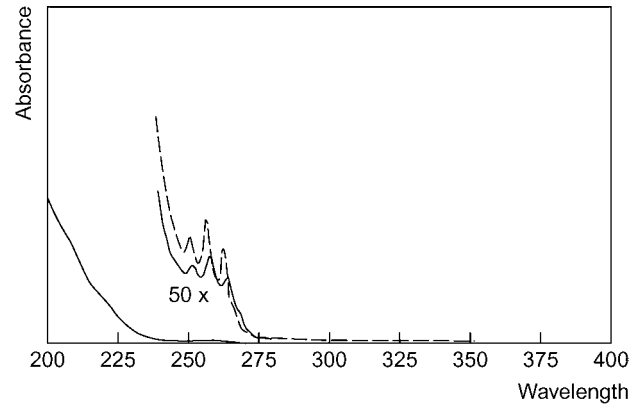
Primidone

Anticonvulsant
$C_{12}H_{14}N_2O_2$ = 218.3
CAS—125-33-7

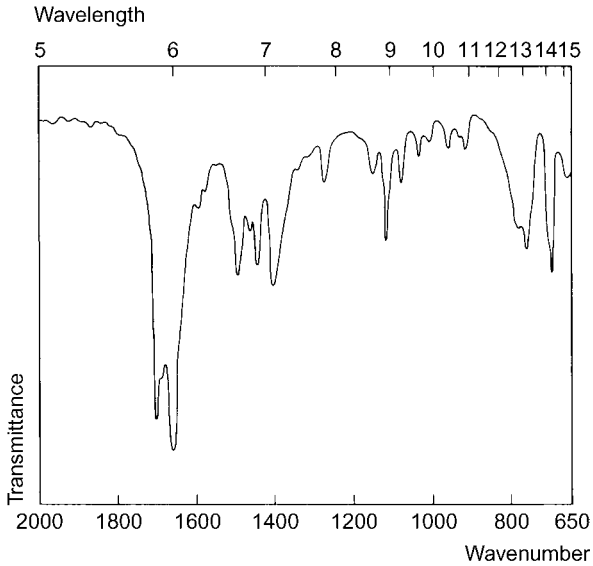
IUPAC Name 5-Ethyl-5-phenyl-1,3-diazinane-4,6-dione
Synonyms 5-Ethyldihydro-5-phenyl-4,6(1*H*,5*H*)-pyrimidinedione; hexamidinum; primaclone; primidonum.
Proprietary Names *Cyral*; *Liskantin*; *Midone*; *Mylepsin*; *Mylepsinum*; *Mysoline*; *Prysoline*; *Resimatil*; *Sertan*.
Note The name Hexamidine is applied to a compound with antibacterial and fungistatic properties.



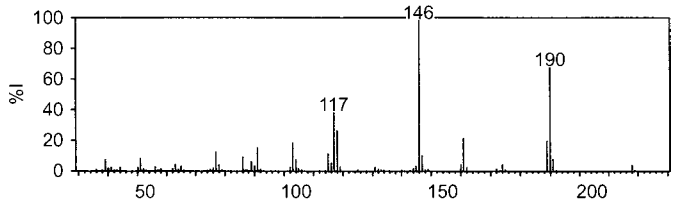
Chemical Properties A white crystalline powder. Mp 281° to 282°. Soluble 1 in 2000 of water and 1 in 170 of ethanol; practically insoluble in most other organic solvents. Log *P* (octanol/water), 0.9. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Thin-layer Chromatography System TA—R_f 0.88; system TD—R_f 0.08; system TE—R_f 0.41; system TF—R_f 0.23; system TAD—R_f 0.28; system TAE—R_f 0.76; system TAJ—R_f 0.29; system TAK—R_f 0.60; system TAL—R_f 0.86 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, positive).
Gas Chromatography System GA—primidone RI 2250, M (phenobarbital) RI 1953, M (phenylethylmalondiamide (PEMA)) RI 1884, M (diamide) RI 1935, M (acetyl-) RI 2115; system GB—primidone RI 2384, primidone-Me_2 RI 2161, M (PEMA) RI 1996, M (acetyl-) RI 2189; system GE—primidone RRT 0.89, M (phenobarbital) RRT 0.74, and M (PEMA) RRT 0.65 (all relative to phenytoin); system GAJ—primidone RRT 1.674, M (PEMA) RRT 1.074 (both relative to methylphenobarbital).
High Performance Liquid Chromatography System HE—primidone *k* 1.35, M (phenobarbital) *k* 2.76; system HX—RI 322; system HY—RI 288; system HZ—retention time 2.1 min; system HAA—retention time 11.1 min; system HAM—retention time 2.2 min.
Ultraviolet Spectrum Methanol—252, 258 (A^1_1=9.7c), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 1662, 1705, 1496, 700, 765, 1120 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 146, 190, 117, 118, 161, 189, 103, 91; phenobarbital 204, 117, 146, 161, 77, 103, 115, 118; phenylethylmalondiamide 163, 148, 91, 103, 117, 120, 44, 77.



Quantification

Blood **GC-MS** Primidone and other anticonvulsants [Speed *et al.* 2000].

Plasma **GC** Thermionic specific detection. Primidone and other anticonvulsants. Limits of quantification, 0.05 to 0.2 mg/L [Queiroz *et al.* 2002].

HPLC UV detection. Primidone and other anticonvulsants [Romanyshyn *et al.* 1994]. UV detection. Primidone and phenobarbital. Limit of detection, about 550 µg/L for primidone [Christofides, Fry 1980].

Serum **GC-MS** Primidone, phenobarbital, 4-hydroxyphenobarbital and phenylethylmalondiamide. Limits of detection, between 1.4 and 3.7 µg/L [Nau *et al.* 1980].

HPLC Photodiode-array detection. Primidone and other anticonvulsants [Liu *et al.* 1993]. See Plasma [Christofides, Fry 1980].

Urine **GC-MS** See Serum [Nau *et al.* 1980].

HPLC See Serum [Liu *et al.* 1993].

Breast milk **GC-MS** See Serum [Nau *et al.* 1980].

Saliva **GC-MS** See Serum [Nau *et al.* 1980].

HPLC See Serum [Liu *et al.* 1993].

Liver **GC-MS** See Blood [Speed *et al.* 2000].

Tissues **GC-MS** See Serum [Nau *et al.* 1980].

Disposition in the Body Readily absorbed after oral administration. Rapidly metabolised to phenylethylmalondiamide (PEMA) and more slowly metabolised to phenobarbital; both metabolites are active anticonvulsants. Aromatic hydroxylation to form 4-hydroxyphenobarbital also occurs followed by glucuronic acid conjugation. The formation of phenobarbital is stimulated by phenytoin. During chronic treatment, about 92% of the daily dose is excreted in the urine in 24 h, of which 15 to 65% (mean, 42%) is unchanged drug, 16 to 65% (mean, 45%) is PEMA, and 1 to 8% (mean, 5%) is phenobarbital or its hydroxylated metabolites and conjugates. Primidone is widely distributed in all organs and tissues. It crosses the blood–brain barrier and placental barrier and is excreted in breast milk.

Therapeutic Concentration In plasma, usually in the range 5 to 12 mg/L but there is considerable intersubject variation. At the beginning of treatment there is a delay of several days before phenobarbital appears in the plasma. Concentrations of phenobarbital and PEMA accumulate during chronic treatment, and steady-state plasma-phenobarbital concentrations are often used to monitor therapy.

After daily oral dosing of a number of subjects at various dose levels, steady-state plasma concentrations were as follows: 26 subjects at 250 mg per day, 0 to 13 mg/L (mean, 3.2); 26 subjects at 500 mg per day, 1 to 14 mg/L (mean, 6.7); 78 subjects at 750 mg per day, 2 to 23 mg/L (mean, 8.7); 52 subjects at 1000 mg per day, 3 to 20 mg/L (mean, 10.5); 9 subjects at 1250 mg per day, 10 to 19 mg/L (mean, 14.7). In 30 subjects given primidone but no barbiturate, the mean primidone concentration was 9.2 mg/L and the mean phenobarbital concentration was 31 mg/L [Booker *et al.* 1970].

Following oral doses of 75 to 250 mg three times a day to 7 subjects, the following steady-state serum concentrations were reported: primidone 5.0 to 13.4 mg/L (mean, 8.3), phenobarbital 3.3 to 34.6 mg/L (mean, 12.6), PEMA 1.4 to 9.9 mg/L (mean, 4.6) [Cloyd *et al.* 1981].

Toxicity The estimated minimum lethal dose is 5 g but instances of survival after the ingestion of 25 to 30 g have been reported. Toxic effects are usually associated with plasma concentrations greater than 10 mg/L and concentrations greater than 50 mg/L may be lethal.

In a fatality due to primidone ingestion, postmortem blood concentrations of 65 mg/L of primidone and 3 mg/L of phenobarbital were reported [Wright, Baselt 2000].

Serum concentrations of 95 mg/L of primidone and 175 mg/L of phenobarbital were reported about 12 h after a non-fatal suicide attempt by a 10-year-old epileptic subject; the corresponding urine concentrations were 1570 and 50 mg/L, respectively [Cate, Tenser 1975].

Half-life Plasma half-life, primidone about 10 to 15 h, phenobarbital 50 to 150 h, PEMA 24 to 48 h.

Volume of Distribution In children after oral dosing, about 0.6 L/kg.

Clearance Plasma clearance, after oral administration, about 0.7 mL/min/kg.

Saliva Plasma : saliva ratio, about 1.0.

Protein Binding <20%.

Dose Initially 125 mg daily, increasing to 0.5 to 1.5 g daily.

Booker HE *et al.* (1970). A clinical study of serum primidone levels. *Epilepsia* 11: 395–402.

Cate JC, Tenser R (1975). Acute primidone overdosage with massive crystalluria. *Clin Toxicol* 8: 385–389.

Christofides JA, Fry DE (1980). Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin Chem* 26: 499–501.

Cloyd JC *et al.* (1981). Primidone kinetics: effects of concurrent drugs and duration of therapy. *Clin Pharmacol Ther* 29: 402–407.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Liu H *et al.* (1993). Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. *J Chromatogr* 616: 105–115.

Nau H *et al.* (1980). Microassay for primidone and its metabolites phenylethylmalondiamide, phenobarbital and p-hydroxyphenobarbital in human serum, saliva, breast milk and tissues by gas chromatography–mass spectrometry using selected ion monitoring. *J Chromatogr* 182: 71–79.

Queiroz ME *et al.* (2002). Determination of lamotrigine simultaneously with carbamazepine, carbamazepine epoxide, phenytoin, phenobarbital, and primidone in human plasma by SPME-GC-TSD. *J Chromatogr Sci* 40: 219–223.

Romanyshyn LA *et al.* (1994). Simultaneous determination of felbamate, primidone, phenobarbital, carbamazepine, two carbamazepine metabolites, phenytoin, and one phenytoin metabolite in human plasma by high-performance liquid chromatography. *Ther Drug Monit* 16: 90–99.

Speed DJ *et al.* (2000). Analysis of six anticonvulsant drugs using solid-phase extraction, deuterated internal standards, and gas chromatography-mass spectrometry. *J Anal Toxicol* 24: 685–690.

Wright JA, per Baselt RC (2000). *Disposition of Toxic Chemicals and Drugs in Man*, 5th edn. Davis, California: Biomedical Publications, 724–726.

P

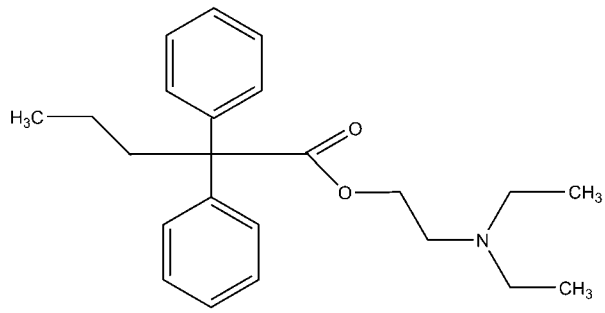
Proadifen

Non-specific Synergist

$C_{23}H_{31}NO_2 = 353.5$

IUPAC Name 2-Diethylaminoethyl 2,2-diphenylpentanoate

Synonyms 2-Diethylaminoethyl α,α-diphenylpentanoate; propyladiphenine.



Proadifen Hydrochloride

$C_{23}H_{31}NO_2,HCl = 389.5$

Synonyms RP 5171; SKF 525-A; U 5446.

Chemical Properties White crystalline powder. Mp 122° to 123°. Soluble in water. Extracted by organic solvents from aqueous alkaline solutions.

Thin-layer Chromatography System T1—R_f 0.70 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Proadifen hydrochloride in 0.1 N sulfuric acid, maxima at 252 (E1%, 1 cm 16.3), 259 (E1%, 1 cm 14.8), 264 (E1%, 1 cm 12.1), 270 (E1%, 1 cm 7.3), 327 (E1%, 1 cm 4.9) and 337 nm (E1%, 1 cm 4.4).

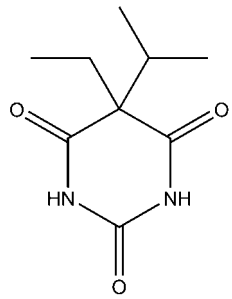
Probarbital

Barbiturate

$C_9H_{14}N_2O_3$ = 198.2

IUPAC Name (1,3-Dimethyl-4-phenylazepan-4-yl) propanoate

Synonyms DEA Number 9643; 1,3-dimethyl-4-phenyl-4-propionyloxyazacycloheptane; EINECS 201-006-8.



Chemical Properties Log *P* (octanol/water) 0.9 [National Institutes of Health 2008].

Probarbital Sodium

Synonym Ethypropymalnatrium

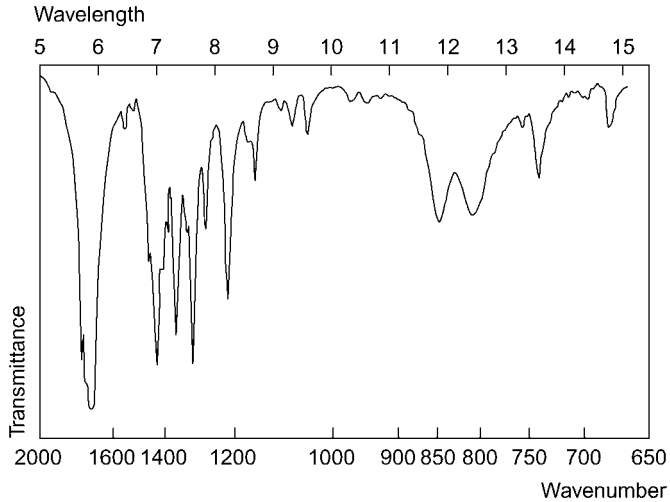
Proprietary Name *Ipral sodium*

Chemical Properties White hygroscopic powder. Very soluble in water, slightly soluble in ethanol, insoluble in ether and chloroform. Aqueous solutions slowly decompose.

Thin-layer Chromatography System T10—R_f 0.24 (location reagents: mercurous nitrate spray, black; Zwikker's reagent spray, pink).

Gas Chromatography System G1/180—retention time 0.44 relative to diphenhydramine; system G7—retention time 1.1 relative to barbitone.

Infrared Spectrum



Disposition in the Body

Toxicity The estimated minimum lethal dose is 2 g. It is an intermediate-acting barbiturate.

Dose 250 mg.

National Institutes of Health (2008). *Probarbitone.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6455&loc=ec_rcs. (accessed 27 June 2008).

Probenecid

Uricosuric

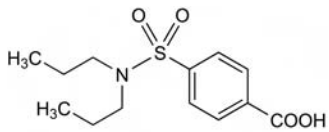
$C_{13}H_{19}NO_4S$ = 285.4

CAS—57-66-9

IUPAC Name 4-(Dipropylsulfamoyl)benzoic acid

Synonym 4-[(Dipropylamino)sulfonyl]benzoic acid

Proprietary Names *Bencid*; *Benemid(e)*; *Benuryl*; *Probalan*; *Probecid*; *Proben*; *Procid*.



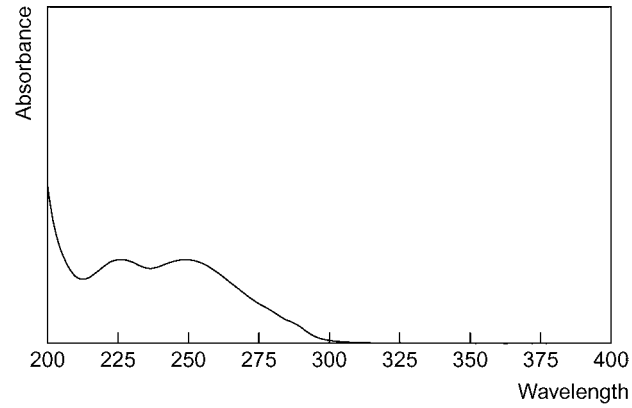
Chemical Properties A white crystalline powder. Mp 194° to 196°. Practically insoluble in water and dilute acids; soluble 1 in 25 of ethanol and 1 in 12 of acetone; soluble in chloroform and dilute alkalis. pK_a 3.4 (20°). Log *P* (octanol/water), 3.2.

Thin-layer Chromatography System TD—R_f 0.13; system TE—R_f 0.05; system TF—R_f 0.23; system TAD—R_f 0.24; system TAE—R_f 0.87; system TAJ—R_f 0.28; system TAK—R_f 0.71; system TAL—R_f 0.90.

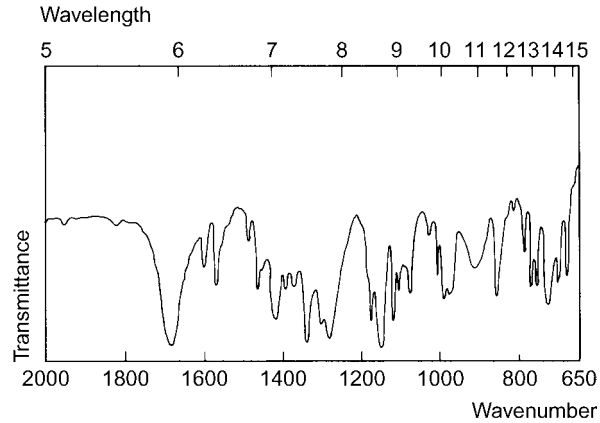
Gas Chromatography System GA—RI 2336.

High Performance Liquid Chromatography System HX—RI 526; system HY—RI 507; system HZ—retention time 7.8 min.

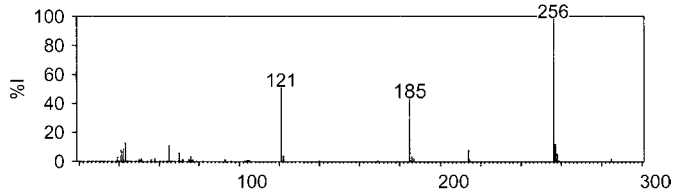
Ultraviolet Spectrum Ethanolic acid—248 nm (A_1^1=332a).



Infrared Spectrum Principal peaks at wavenumbers 1683, 1156, 1285, 1307, 1125, 1180 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 256, 121, 185, 43, 257, 65, 42, 214.



Quantification

Plasma **Spectrofluorimetry** Plasma [Cunningham *et al.* 1978].

GC ECD. Limit of detection, 20 µg/L [Roos *et al.* 1980].

HPLC Probenecid and its metabolites [Vree *et al.* 1992]. UV detection. Limit of detection, 500 µg/L [Hekman *et al.* 1980].

Urine **HPLC** See Plasma [Vree *et al.* 1992]. Probenecid and its glucuronide [Hansen-Moller, Schmit 1991]. See Plasma [Hekman *et al.* 1980].

Cerebrospinal Fluid **GC** See Plasma [Roos *et al.* 1980].

Disposition in the Body Readily absorbed after oral administration. Metabolised by side-chain oxidation, glucuronic acid conjugation and *N*-dealkylation. Up to about 90% of a dose is excreted in the urine, the major urinary metabolite, probenecid acyl glucuronide, accounting for up to 50% of a dose; up to 25% of a dose is excreted as hydroxylated and carboxylic acid metabolites, and 5 to 15% as *N*-dealkylated metabolites. About 5 to 10% of a dose is excreted as unchanged drug but this appears to be variable, increasing with increasing urinary pH values and urinary flow.

Therapeutic Concentration

Following single oral doses of 0.5, 1.0 and 2.0 g to 5 subjects, mean peak plasma concentrations of 35.3, 69.6, and 149 mg/L were reported at 3 to 4 h [Selen *et al.* 1982].

After daily oral administration of 0.5 g four times a day to 19 subjects for 4 weeks, a mean plasma concentration of 22.5 mg/L was reported [Cunningham *et al.* 1981].

Toxicity Probenecid is relatively non-toxic and recovery has occurred after the ingestion of 47 g.

A 36-year-old man died after deliberately ingesting approximately 75 g of probenecid. Postmortem tissue concentrations were highest in serum (710 mg/L) and the liver (550 µg/g); ethanol was also detected in blood (1.3 g/L) [McIntyre *et al.* 1992].

Half-life Plasma half-life, 4 to 17 h (dose-dependent).

Volume of Distribution About 0.1 to 0.2 L/kg.

Protein Binding About 90%.

Note For a review of the clinical pharmacokinetics of probenecid, see Cunningham *et al.* [1981].

Dose 0.5 to 2 g daily.

Cunningham RF *et al.* (1978). New spectrophotofluorometric assay for probenecid. *J Pharm Sci* 67: 434–436.

Cunningham RF *et al.* (1981). Clinical pharmacokinetics of probenecid. *Clin Pharmacokinet* 6: 135–151.

Hansen-Moller J, Schmit U (1991). Rapid high-performance liquid chromatographic assay for the simultaneous determination of probenecid and its glucuronide in urine. Irreversible binding of probenecid to serum albumin. *J Pharm Biomed Anal* 9: 65–73.

Hekman P *et al.* (1980). Rapid high-performance liquid chromatographic method for the determination of probenecid in biological fluids. *J Chromatogr* 182: 252–256.

McIntyre IM *et al.* (1992). A death involving probenecid. *J Forensic Sci* 37: 1190–1193.

Roos BE *et al.* (1980). Quantitation of CSF concentrations and biological activity of probenecid metabolites. *Eur J Clin Pharmacol* 17: 223–226.

Selen A *et al.* (1982). Pharmacokinetics of probenecid following oral doses to human volunteers. *J Pharm Sci* 71: 1238–1242.

Vree TB *et al.* (1992). Direct measurement of probenecid and its glucuronide conjugate by means of high pressure liquid chromatography in plasma and urine of humans. *Pharm Weekbl (Sci.)* 14: 83–87.

Procainamide

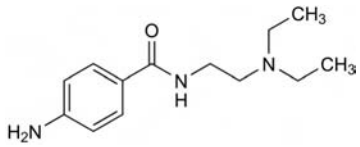
Antiarrhythmic

$C_{13}H_{21}N_3O = 235.3$

CAS—51-06-9

IUPAC Name 4-Amino-*N*-(2-diethylaminoethyl)benzamide

Synonym Procaine amide



Chemical Properties pK_a 9.2 (20°). Log *P* (ethyl acetate/pH 7.4), −1.5; (octanol/water), 0.9. Extraction yield (chlorobutane), 0.1 [Demme *et al.* 2005].

Procainamide Hydrochloride

$C_{13}H_{21}N_3O,HCl = 271.8$

CAS—614-39-1

Synonym Novocainamidum

Proprietary Names *Biocoryl*; *Novocamid*; *Procamide*; *Procan SR*; *Procanbid*; *Procapan*; *Pronestyl*.

Chemical Properties A white to tan-coloured, hygroscopic crystalline powder. Mp 165° to 169°. Soluble 1 in 0.25 of water, 1 in 2 of ethanol and 1 in 140 of chloroform; slightly soluble in acetone; practically insoluble in ether.

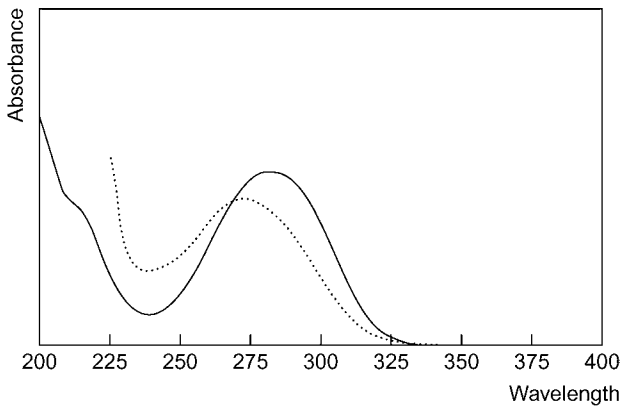
Colour Tests Coniferyl alcohol—orange; diazotisation—red; ninhydrin—yellow.

Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.01; system TC—R_f 0.05; system TE—R_f 0.39; system TAE—R_f 0.17; system TAF—R_f 0.33; system TAG—R_f 0.09 (acidified iodoplatinate solution—positive).

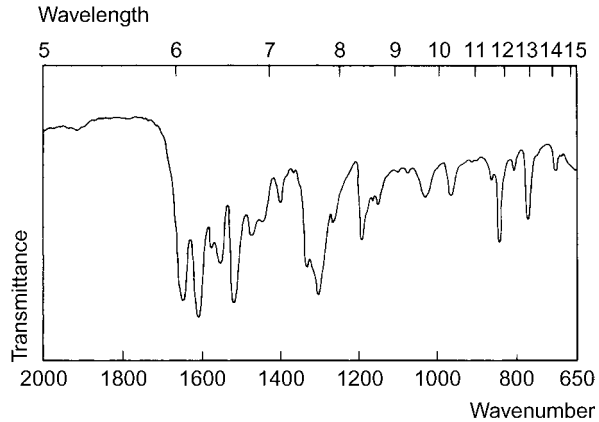
Gas Chromatography System GA—procainamide RI 2255, M (*N*-acetyl-) RI 2550; system GB—procainamide RI 2332, M (*N*-acetyl-) RI 2724; system GF—RI 2965.

High Performance Liquid Chromatography System HA—procainamide *k* 1.3, M (*N*-acetyl-) *k* 3.0; system HX—RI 208; system HY—procainamide RI 160; system HZ—RT 1.9 min, M (*N*-acetyl-) RT 1.8 min.

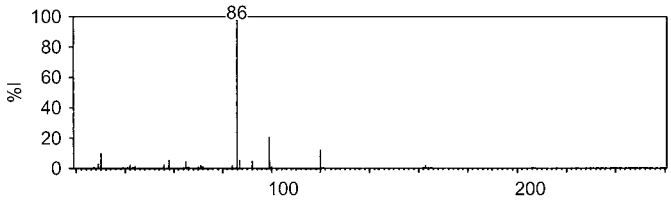
Ultraviolet Spectrum Aqueous alkali—275 nm ($A^1_1 = 693a$).



Infrared Spectrum Principal peaks at wavenumbers 1600, 1512, 1639, 1297, 1545, 1570 cm^{-1} (procainamide hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 120, 30, 92, 87, 58, 65 (procainamide); 86, 58, 99, 56, 162, 132, 149, 205 (*N*-acetylprocainamide).



Quantification

Plasma **HPLC** Column: Ultraspher octyl C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: water : methanol : acetic acid : TEA (pH 5.5, 78 : 22 : 1 : 0.01), flow rate 1.3 mL/min. UV detection (λ = 280 nm). Retention time, 4 min. Limit of quantification, 4 µg/L, limit of detection, 2 µg/L [Lessard *et al.* 1998]. Column: Spherisorb hexyl (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 15 mmol/L potassium dihydrogen phosphate plus 0.01% TEA (pH 3.0, 10 : 10 : 80), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 0.4 mg/L [Verbesselt *et al.* 1991]. Column: Nucleosil C_{18} (150 × 4.8 mm i.d., 5 µm). Mobile phase: methanol : acetic acid : triethylamine : water (200: 10 : 5 : 785), flow rate 0.2 mL/min. Fluorescence detection (λ_{ex} = 290 nm, λ_{em} = 335 nm). Limit of detection, 100 µg/L [Raphanaud *et al.* 1986]. Column: silica (250 mm, 10 µm). Mobile phase: 3.0 pmol/L ammonium hydroxide : 0.05 mol/L ammonium nitrate : methanol (1 : 12 : 87), flow rate 2.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 500 µg/L [Bridges, Jennison 1983]. Column: alkylphenyl (300 × 4 mm i.d.). Mobile phase: acetonitrile : phosphate buffer (60 : 40), flow rate 2.0 mL/min. UV detection (λ = 280 nm). Retention time: 4.4 min. Limit of detection, 50 µg/L [Lai *et al.* 1980]. See also Carr *et al.* [1976].

Serum **GC** Column: 5% OV-17 on 100/120 mesh Gas Chrom Q (1.0 m × 4 mm i.d.). Carrier gas: Ar : CH_4 (95 : 5), 40 mL/min. Temperature: 235°. ECD. Limit of detection, 100 ng [Ludden *et al.* 1978].

HPLC Column: IBM phenyl (150 × 4.5 mm i.d., 5 µm). Mobile phase: 0.075 mol/L acetate buffer (pH 4.3) : acetonitrile (20 : 3), flow rate 1 mL/min. UV detection (λ = 270 nm). Limit of detection, 50 µg/L [Coyle *et al.* 1987].

Urine **HPLC** See Serum [Coyle *et al.* 1987]. See Plasma. Limit of detection, 500 µg/L [Lai *et al.* 1980].

Disposition in the Body Procainamide is readily absorbed after oral administration and widely distributed throughout the body; bioavailability is 75–95%. The major metabolite, *N*-acetylprocainamide (acecainide), has similar pharmacological activity to procainamide; the acetylation of procainamide is subject to genetic polymorphism. Up to ~80% of a dose is excreted in the urine of normal subjects in 24 h, ~50–60% as unchanged drug and up to ~30% as *N*-acetylprocainamide (less in slow acetylators). Other metabolites include monodesethylprocainamide and monodesethyl-*N*-acetylprocainamide.

Therapeutic Concentration There is considerable intersubject variation in plasma concentrations. The therapeutic effect has been correlated with plasma concentrations of ~4–10 mg/L procainamide and 5–30 mg/L combined procainamide and *N*-acetylprocainamide. *N*-Acetylprocainamide may accumulate during chronic administration in fast acetylators and in subjects with renal impairment.

After a single oral dose of 1 g to 5 normal subjects, peak plasma procainamide concentrations of 3.5–5.3 mg/L (mean, 4.2) were attained in 1–2 h; concentrations of *N*-acetylprocainamide reached a peak of 0.6–2.1 mg/L (mean, 1.6) in 3–8 h [Giardina *et al.* 1976].

The following steady-state plasma concentrations were reported in 10 subjects receiving maintenance treatment: procainamide 2.6 to 20.7 mg/L (mean, 10.6), *N*-acetylprocainamide 4.6 to 43.6 mg/L (mean, 15.9) and monodesethyl-*N*-acetylprocainamide 0.4 to 11.9 mg/L (mean, 2.7) [Ruo *et al.* 1981].

Following IV infusion of 84–374 mg/h procainamide to 34 subjects, steady-state plasma procainamide concentrations of 1.7–17 µg/mL (mean, 6.5) and

N-acetylprocainamide concentrations of 1.1–20 µg/mL (mean, 5.7) were reported [Lima *et al.* 1979].

Subjects received 1, 2 or 4 g procainamide daily by mouth, given either in 4 divided doses as a conventional-release capsule or in 2 divided doses as an extended-release tablet. The peak plasma concentrations of procainamide after twice daily and 4 times daily administration, respectively, were 2.74 and 2.77 mg/L for all dose levels (at 4.0 and 2.0 h). Corresponding values for *N*-acetylprocainamide were 2.61 and 2.92 mg/L (at 4.4 and 2.6 h) [Yang *et al.* 1996].

Toxicity Toxic effects are usually associated with plasma-procainamide concentrations of ~12 mg/L or more and fatalities with concentrations >20 mg/L. As little as 200 mg IV can be fatal.

In 4 fatalities attributed to procainamide intoxication in subjects receiving therapeutic doses, plasma procainamide concentrations shortly before death ranged from 17.6–25.2 mg/L [Koch-Weser, Klein 1971].

The following postmortem concentrations were reported in a fatality attributed to procainamide: blood 114 mg/L, liver 283 µg/g and urine 556 mg/L [Kopjak, Jennison 1976].

A 14-year-old boy had a serum procainamide level of 63 mg/L and an *N*-acetylprocainamide level of 80.4 mg/L ~2.5 h after intentionally ingesting ~21 g procainamide; after 43 h, the levels were 0.3 and 0.6 mg/L for procainamide and *N*-acetylprocainamide, respectively [White *et al.* 2002]

Half-life Plasma half-life: procainamide ~3 h in normal subjects, increased in subjects with renal failure or with heart disease; *N*-acetylprocainamide ~6–9 h.

Volume of Distribution ≈2 L/kg.

Clearance Plasma clearance, ~5–15 mL/min/kg.

Protein Binding ≈15%.

Note For a review of the clinical pharmacokinetics of procainamide, see Karlsson [1978].

Dose Maintenance, 0.5 to 1 g of procainamide hydrochloride, by mouth, every 4 to 6 h.

Bridges RR, Jennison TA (1983). An HPLC method for the simultaneous quantitation of quinidine, procainamide, *N*-acetylprocainamide, and disopyramide. *J Anal Toxicol* 7: 65–68.

Carr K *et al.* (1976). Simultaneous quantification of procainamide and *N*-acetylprocainamide with high-performance liquid chromatography. *J Chromatogr* 129: 363–368.

Coyle JD *et al.* (1987). Reversed-phase liquid chromatography method for measurement of procainamide and three metabolites in serum and urine: percent of dose excreted as deethyl metabolites. *J Pharm Sci* 76: 402–405.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Giardina EG *et al.* (1976). Metabolism of procainamide in normal and cardiac subjects. *Clin Pharmacol Ther* 19: 339–351.

Karlsson E (1978). Clinical pharmacokinetics of procainamide. *Clin Pharmacokinet* 3: 97–107.

Koch-Weser J, Klein SW (1971). Procainamide dosage schedules, plasma concentrations, and clinical effects. *JAMA* 215: 1454–1460.

Kopjak L, Jennison TA (1976). *TIAFT Bull* 12: 12–13.

Lai CM *et al.* (1980). Determination of procainamide and *N*-acetylprocainamide in biological fluids by high-pressure liquid chromatography. *J Pharm Sci* 69: 982–984.

Lessard E *et al.* (1998). Improved high-performance liquid chromatographic assay for the determination of procainamide and its *N*-acetylated metabolite in plasma: application to a single-dose pharmacokinetic study. *J Chromatogr Sci* 36: 49–54.

Lima JJ *et al.* (1979). Safety and efficacy of procainamide infusions. *Am J Cardiol* 43: 98–105.

Ludden TM *et al.* (1978). Microdetermination of procainamide in human serum. *J Pharm Sci* 67: 371–373.

Raphanaud D *et al.* (1986). High performance liquid chromatography of procainamide and *N*-acetylprocainamide in human blood plasma. *Ther Drug Monit* 8: 365–367.

Ruo TI *et al.* (1981). Identification of desethyl procainamide in patients: a new metabolite of procainamide. *J Pharmacol Exp Ther* 216: 357–362.

Verbesselt R *et al.* (1991). High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Ther Drug Monit* 13: 157–165.

White SR *et al.* (2002). The case of the slandered Halloween cupcake: survival after massive pediatric procainamide overdose. *Pediatr Emerg Care* 18: 185–188.

Yang BB *et al.* (1996). Pharmacokinetic and pharmacodynamic comparisons of twice daily and four times daily formulations of procainamide in patients with frequent ventricular premature depolarization. *J Clin Pharmacol* 36: 623–633.

Procaine

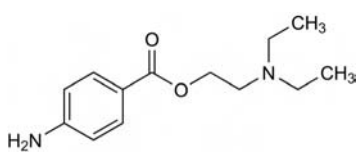
Anaesthetic (Local)

$C_{13}H_{20}N_2O_2 = 236.3$

CAS—59-46-1

IUPAC Name 2-Diethylaminoethyl 4-aminobenzoate

Synonym Ethocaine



Chemical Properties Mp 61°. Soluble 1 in 200 of water (when freshly precipitated); soluble in ethanol, benzene, chloroform and ether. pK_a 9.0 (20°). Log *P* (octanol/water), 2.1. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Procaine Hydrochloride

$C_{13}H_{20}N_2O_2,HCl = 272.8$

CAS—51-05-8

Synonyms Allocaine; novocainum; procaini hydrochloridum; procainii chloridum; procainum chloride; syncaine.

Proprietary Names *Geroaslan H3*; *Gerovital H3*; *Lenident*; *Novanaest*; *Novocain*; *Procaneural*; *Syntocaine*; *Venocaina*.

Chemical Properties Colourless crystals or a white crystalline powder. Mp 153° to 156°. Soluble 1 in 1 of water, 1 in 30 of ethanol and 1 in 30 of dehydrated alcohol; slightly soluble in chloroform; practically insoluble in ether.

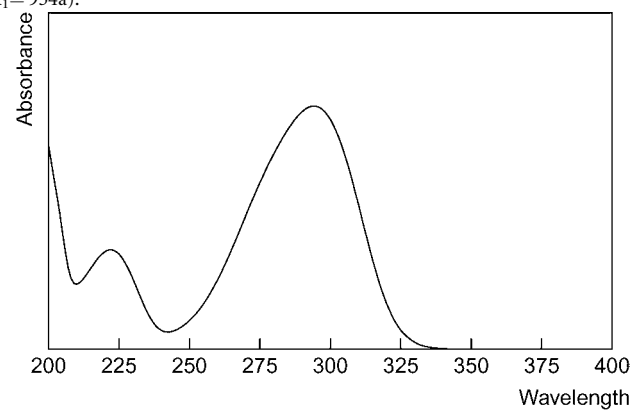
Colour Test Diazotisation—red-orange.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.05; system TC—R_f 0.31; system TE—R_f 0.71; system TL—R_f 0.30; system TAE—R_f 0.36; system TAF—R_f 0.42; system TAJ—R_f 0.06; system TAK—R_f 0.00; system TAL—R_f 0.22 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; ninhydrin spray, positive).

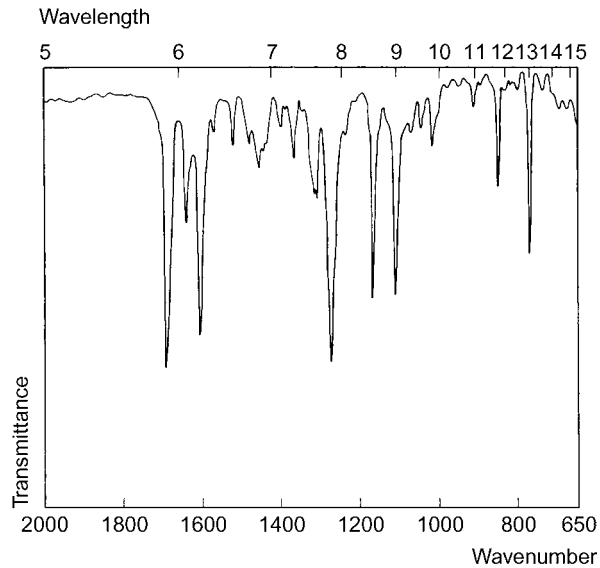
Gas Chromatography System GA—procaine RI 2010, procaine-AC RI 2350, 4-aminobenzoic acid RI 1547; system GF—RI 2580; system GQ—retention time 6.8 min.

High Performance Liquid Chromatography System HA—*k* 1.9; system HQ—*k* 0.00; system HX—RI 264; system HY—RI 225; system HAA—RT 5.2 min; system HAX—RT 7.3 min; system HAY—RT 4.0 min.

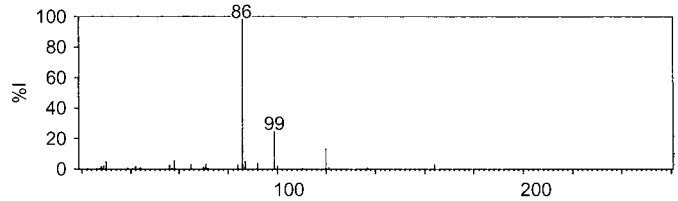
Ultraviolet Spectrum Aqueous acid—294, 222 nm; methanol—296 nm ($A^{1\%}_{1cm}$ = 954a).



Infrared Spectrum Principal peaks at wavenumbers 1690, 1274, 1605, 1174, 1116, 772 cm^{-1} (procaine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 120, 58, 87, 30, 92, 71; 4-aminobenzoic acid 137, 120, 92, 65, 39, 138, 121, 63.



Quantification
Blood **GC** SID. Limit of detection, 5–10 pg per injection for procaine and other local anaesthetics [Hattori *et al.* 1991].
Plasma **GC** FID. Limit of detection, 0.1 mg/L for procaine and chloroprocaine [Smith *et al.* 1978]. FID. For method, see Green *et al.* [1974].
GC-MS SIM, *m/z* 86. Limit of quantification, 100 µg/L, limit of detection, 80 µg/L [Ohshima, Takayasu 1999].
Serum **GC** NPD. Limit of detection, ≈60–70 pg per injection for procaine and other local anaesthetics [Terada *et al.* 1996].
Urine **GC–MS** SIM, *m/z* 86. Limit of quantification, 100 µg/L, limit of detection, 80 µg/L [Ohshima, Takayasu 1999].
Cerebrospinal Fluid **GC** SID. Limit of detection, 5–10 pg per injection for procaine and other local anaesthetics [Hattori *et al.* 1991].

Disposition in the Body Poorly absorbed from mucous membranes; readily absorbed after parenteral administration and rapidly hydrolysed in the plasma to 4-aminobenzoic acid and diethylaminoethanol. About 80% of the 4-aminobenzoic acid is excreted unchanged or conjugated in the urine, together with about 30% of the diethylaminoethanol, the remainder being metabolised in the liver; about 2% of a dose is excreted in the urine as unchanged drug.

Therapeutic Concentration
A mean steady-state plasma concentration of 12.7 mg/L was reported during the continuous IV infusion of 1.0 mg/kg/min to 6 subjects; following continuous IV infusion of 1.5 mg/kg/min to a further 6 subjects, a mean steady-state plasma concentration of 42.7 mg/L was reported [Seifen *et al.* 1979].

Toxicity
Following IV injection to 10 subjects until convulsions were induced (total dose 18–55 mg/kg), plasma concentrations of 21–86 mg/L (mean 49) of procaine and 0.6–80 mg/L (mean 23) of 4-aminobenzoic acid were reported [Usubiaga *et al.* 1966].
Following the accidental IV injection of 4 g to 1 subject, a peak blood concentration of 96 mg/L was reported; the subject recovered [Wikinski *et al.* 1970].

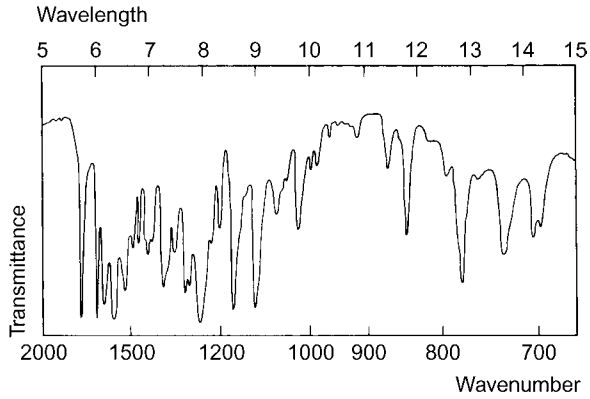
Half-life Plasma half-life, ≈0.1 h.
Protein Binding ≈6%.
Dose Up to 1 g of procaine hydrochloride may be administered by injection with adrenaline.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Green RL *et al.* (1974). Elevated plasma procaine concentrations after administration of procaine penicillin G. *New Engl J Med* 291: 223–226.
Hattori H *et al.* (1991). Determination of local anaesthetics in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 564: 278–282.
Ohshima T, Takayasu T (1999). Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography-mass spectrometry with solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 726(1–2): 185–194.
Seifen AB *et al.* (1979). Pharmacokinetics of intravenous procaine infusion in humans. *Anesth Analg Curr Res* 58: 382–386.
Smith RH *et al.* (1978). Measurement of chloroprocaine and procaine in plasma by flame ionization gas-liquid chromatography. *Clin Chem* 24: 1599–1601.
Terada M *et al.* (1996). Determination of ester-type local anesthetic drugs (procaine, tetracaine, and T-caine) in human serum by wide-bore capillary gas chromatography with nitrogen-phosphorus detection. *J Anal Toxicol* 20(5): 318–322.
Usubiaga JE *et al.* (1966). Local anesthetic-induced convulsions in man–an electroencephalographic study. *Anesth Analg Curr Res* 45: 611–620.
Wikinski JA *et al.* (1970). Cardiovascular and neurological effects of 4,000 mg of procaine. *JAMA* 213: 621–623.

Procaine Benzylpenicillin

Antibacterial
$C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$ = 588.7
CAS—54-35-3 (anhydrous); 6130-64-9 (monohydrate)

IUPAC Name 2-Diethylaminoethyl 4-aminobenzoate; (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; hydrate
Synonyms Benzylpenicillin novocaine; (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate; penicillin G procaine; procaine penicillin; procaine penicillin G.
Proprietary Names *Aqucilina*; *Atracilina*; *Ayercillin*; *Cilicaine*; *Crysticillin AS*; *Farmaproina*; *Fradicilina*; *Jenacillin O*; *Novocillin*; *Pfizerpen AS*; *Probecilin*; *Procillin*; *Wycillin*. It is an ingredient of *Bicillin*.
Note *Bicillin* is also used as a proprietary name for benzathine benzylpenicillin.
Chemical Properties A white crystalline powder. Mp 106° to 110°, with decomposition. Soluble 1 in 200 of water, 1 in 30 of ethanol and 1 in 60 of chloroform; very slightly soluble in ether.
Ultraviolet Spectrum Aqueous acid—278 (A_1^1=53b); aqueous alkali—280 (A_1^1=256b); methanol—295 nm (A_1^1=367b).
Infrared Spectrum Principal peaks at wavenumbers 1266, 1600, 1779, 1692, 1176, 1120 cm^{-1} (KBr disk).

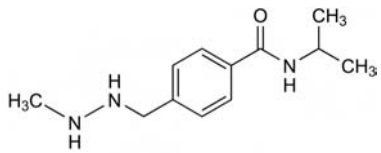


Dose 300 to 600 mg daily, by deep IM injection.

Procarbazine

Antineoplastic
$C_{12}H_{19}N_3O$ = 221.3
CAS—671-16-9

IUPAC Name 4-[(2-Methylhydrazinyl)methyl]-*N*-propan-2-ylbenzamide
Synonyms Ibenzmethyzin; *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl] benzamide; MIH; Ro-4-6467.

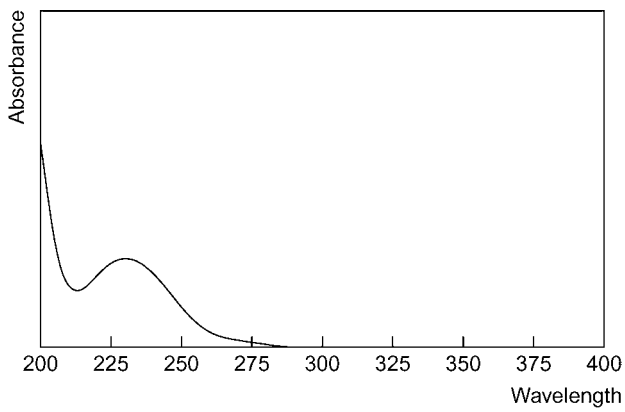


Chemical Properties pK_a 6.8. Log *P* (octanol/water), 0.1.

Procarbazine Hydrochloride

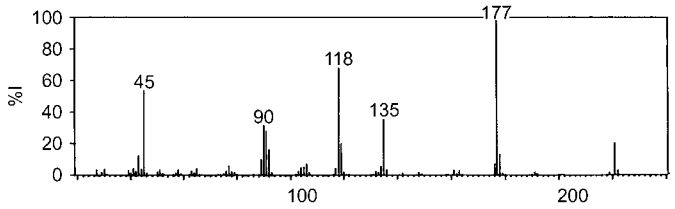
$C_{12}H_{19}N_3O,HCl$ = 257.8
CAS—366-70-1
Proprietary Names *Matulane*; *Natulan*.
Chemical Properties A white to pale yellow crystalline powder. Mp 223° to 226°, with decomposition. Freely soluble in water; sparingly soluble in ethanol; slightly soluble in chloroform; practically insoluble in ether; soluble in methanol. Solutions in water are unstable.

Colour Tests Liebermann's reagent (100°)—blue (15 s); Nessler's reagent (room temperature)—black; palladium chloride—black.
Thin-layer Chromatography System TA—R_f 0.49; system TB—R_f 0.02; system TC—R_f 0.10; system TE—R_f 0.80; system TL—R_f 0.04; system TAD—R_f 0.68; system TAE—R_f 0.88; system TAJ—R_f 0.34; system TAK—R_f 0.03; system TAL—R_f 0.90. (Acidified potassium permanganate solution, positive.)
Gas Chromatography System GA—RI 1990.
Ultraviolet Spectrum Aqueous acid—232 nm (A_1^1=570a).



Infrared Spectrum Principal peaks at wavenumbers 1632, 1555, 1661, 1301, 861, 1176 cm^{-1} (procarbazine hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 177, 118, 45, 135, 90, 91, 221, 119.

P



Quantification

Plasma **GC–MS** Limit of detection, 10 µg/L for procarbazine and metabolites [Gorsen *et al.* 1980].

HPLC UV detection. For method for quantification of procarbazine metabolites, see Shiba and Weinkam [1982]. Electrochemical detection. Limit of detection, 10 ng [Rucki, Moros 1980].

Urine **HPLC** Electrochemical detection. Limit of detection, 10 ng [Rucki, Moros 1980].

Disposition in the Body Readily absorbed after oral administration; peak plasma concentrations are attained in 0.5 to 1 h. Rapidly metabolised to active azo derivatives and by oxidation to *N*-isopropylterephthalamic acid. Up to about 75% of a dose is excreted in the urine with about 5% of a dose consisting of unchanged drug.

Dose Initially the equivalent of 50 mg of procarbazine daily, increasing to 250 or 300 mg daily.

Gorsen RM *et al.* (1980). Analysis of procarbazine and metabolites by gas chromatography-mass spectrometry. *J Chromatogr* 221: 309–318.

Rucki RJ, Moros SA (1980). Application of an electrochemical detector to the determination of procarbazine hydrochloride by high-performance liquid chromatography. *J Chromatogr* 190: 359–365.

Shiba DA, Weinkam RJ (1982). Quantitative analysis of procarbazine, procarbazine metabolites and chemical degradation products with application to pharmacokinetic studies. *J Chromatogr* 229: 397–407.

Prochlorperazine

Tranquilliser

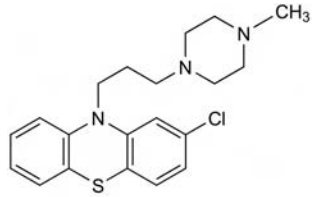
$C_{20}H_{24}ClN_3S = 373.9$

CAS—58-38-8

IUPAC Name 2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine

Synonyms Chlormeprazine; 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10*H*-phenothiazine; prochlorpemazine; proclorperazine.

Proprietary Names *Compazine*; *Compro*; *Stemetil* (suppositories).



Chemical Properties Clear, pale-yellow, viscous liquid that is sensitive to light. Very slightly soluble in water; freely soluble in ethanol, chloroform, and ether. pK_a 8.1 (24°). Log *P* (octanol/water), 4.9. Plasma samples were stable for 24 h at 4° and room temperature, and when stored for 20 days at –20°. Stock solutions were stable for at least 1 month and there was no significant deterioration following 3 freeze-thaw cycles [Yan *et al.* 2009].

Prochlorperazine Edisilate

$C_{20}H_{24}ClN_3S,C_2H_6O_6S_2 = 564.1$

CAS—1257-78-9

Synonyms Prochlorperazine edisylate; prochlorperazine ethanedisulfonate.

Proprietary Name *Compazine*

Chemical Properties White to very light-yellow, crystalline powder. Soluble 1 in 2 of water and 1 in 1500 of ethanol; practically insoluble in chloroform and ether.

Prochlorperazine Maleate

$C_{20}H_{24}ClN_3S,2C_4H_4O_4 = 606.1$

CAS—84-02-6

Synonyms Prochlorperazine dihydrogen maleate; prochlorperazine dimaleate.

Proprietary Names *Anti-Naus*; *Buccastem*; *Compazine*; *Dhaperazine*; *Meterazine*; *Mitil*; *Proclozine*; *Proziere*; *Scripto-Metic*; *Stemetil* (tablets); *Stemzine*.

Chemical Properties White or pale-yellow, crystalline powder. Mp 228°. Practically insoluble in water, ethanol, and ether; slightly soluble in warm chloroform.

Prochlorperazine Mesilate

$C_{20}H_{24}ClN_3S,2CH_3SO_3H = 566.2$

CAS—51888-09-6

Synonyms Prochlorperazine dimethanesulfonate; prochlorperazine mesylate; prochlorperazine methanesulfonate.

Proprietary Name *Stemetil* (injection and syrup).

Chemical Properties White powder. Mp about 242°. Soluble 1 in less than 0.5 of water and 1 in 40 of ethanol; slightly soluble in chloroform; practically insoluble in ether.

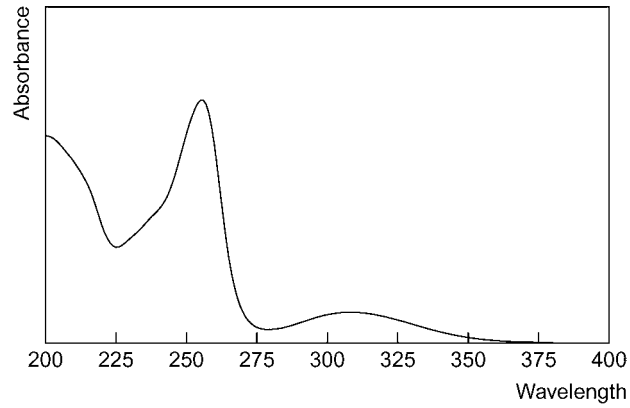
Colour Tests Formaldehyde–sulfuric acid—red-violet; Forrest reagent—red; FPN reagent—red; Mandelin's test—brown→violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.49; system TAE—R_f 0.26; system TAF—R_f 0.26; system TAG—R_f 0.07; system TB—R_f 0.34; system TC—R_f 0.37; system TE—R_f 0.55 (acidified iodoplatinate solution, positive).

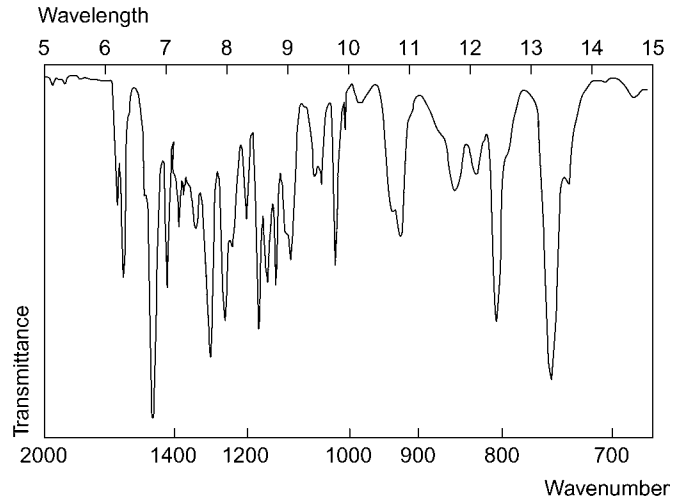
Gas Chromatography System GA—prochlorperazine RI 2954; M (*N*-oxide) RI 2100; system GB—prochlorperazine RI 3129; M (*N*-oxide) RI 2356; M (norsulfoxide) RI 3571; M (sulfoxide) RI 3758.

High Performance Liquid Chromatography System HA—*k* 3.9; system HX—RI 450; system HY—RI 323; system HZ—retention time 10.4 min.

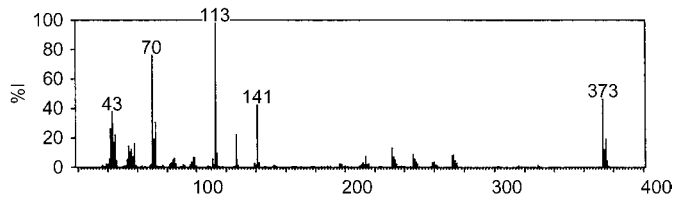
Ultraviolet Spectrum Aqueous acid—254 ($A_1^1 = 875$a), 305 nm.



Infrared Spectrum Principal peaks at wavenumbers 752, 1280, 1569, 1165, 1242, 1145 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 113, 70, 373, 141, 43, 72, 42, 127.



Quantification

Plasma **HPLC** Column: SymmetryShield RP8 (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate and 0.1% formic acid : acetonitrile (70 : 30 to 0 : 100 at 15 min), flow rate 0.2 mL/min. UV detection (λ = 250 nm). Limit of detection, 0.052 mg/L [Marumo *et al.* 2005]. Column: Spherisorb C_8 reversed phase. Mobile phase: acetonitrile : methanol : buffer (60 : 15 : 25). Limit of quantification, 10 µg/L, limit of detection, 2 mg/L [Mou *et al.* 1997]. Column: Octyl (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : phosphoric acid : diethylamine (70 : 0.08 : 0.08, pH 2.3). Electrochemical detection. Limit of detection, 10 µg/L [Sridhar *et al.* 1994]. Electrochemical detection. [McKay *et al.* 1982]. Column:

Spherisorb (15 cm × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L aqueous dipotassium hydrogen phosphate (pH 6.5) : acetonitrile : methanol (7 : 6 : 4). Electrochemical detection. Limit of detection, ≈200 ng/L [Sankey *et al.* 1982].

LC-MS Column: Thermo Hypersil-Hypurity C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 3.6) : methanol : acetonitrile (27 : 68 : 5), flow rate 0.22 mL/min. ESI, MSD, SIM acquisition mode. Limit of quantification, 0.2 µg/L [Yan *et al.* 2009].

Note For a radioimmunoassay in plasma see Midha *et al.* [1983] and for a spectrofluorometric method see Tompsett [1968].

***Serum* HPLC** Column: Inersil ODS-SP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 300 : 200 : 500), flow rate 0.9 mL/min. UV detection (λ = 250 nm). Retention time: 43.2 min. Limit of quantification, 4.9 µg/L [Tanaka *et al.* 2007].

***Urine* HPLC** Column: LiChrosorb C_8 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : triethylamine (40 : 40 : 20 : 2), flow rate 0.5 mL/min. UV detection (λ = 250 nm). Limit of quantification, 190.2 µg/L, limit of detection, 57.1 µg/L [Cruz-Vera *et al.* 2009]. See Plasma. Limit of detection, 0.025 mg/L [Marumo *et al.* 2005].

Disposition in the Body Absorbed after oral administration but extensively metabolised.

Therapeutic Concentration

After a single oral dose of 12.5 mg to 1 subject, a peak plasma concentration of about 0.0008 mg/L was attained in 6 h [Sankey *et al.* 1982].

High plasma levels of prochlorperazine (>600 µg/L) were sustained for up to 24 h in patients treated with 135 mg/m^2 by IV infusion given over 2 h, as a doxorubicin-efflux blocker [Sridhar *et al.* 1994].

Toxicity Serum concentrations of 1.2–1.6 mg/L were found in 4 cases of non-fatal overdose [Tompsett 1968].

Dose Usually 15 to 30 mg of prochlorperazine maleate daily, as an antiemetic; 50 to 100 mg daily for the treatment of psychoses.

Cruz-Vera M *et al.* (2009). Determination of phenothiazine derivatives in human urine by using ionic liquid-based dynamic liquid-phase microextraction coupled with liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 37–42.

Marumo A *et al.* (2005). Analysis of phenothiazines in human body fluids using disk solid-phase extraction and liquid chromatography. *J AOAC Int* 88: 1655–1660.

McKay G *et al.* (1982). Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses. *J Chromatogr* 233: 417–422.

Midha KK *et al.* (1983). Radioimmunoassay for prochlorperazine in human plasma. *Ther Drug Monit* 5: 117–121.

Mou C *et al.* (1997). Simultaneous quantitation of plasma doxorubicin and prochlorperazine content by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 703: 217–224.

Sankey MG *et al.* (1982). A simple and sensitive HPLC method for the assay of prochlorperazine in plasma. *Br J Clin Pharmacol* 13: 578–580.

Sridhar KS *et al.* (1994). Phase I and pharmacokinetics studies of prochlorperazine 2-h i.v. infusion as a doxorubicin-efflux blocker. *Cancer Chemother Pharmacol* 34: 377–384.

Tanaka E *et al.* (2007). Simple and simultaneous determination for 12 phenothiazines in human serum by reversed-phase high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 116–120.

Tompsett SL (1968). The spectrofluorimetric determination of phenothiazine drugs in blood serum. *Acta Pharmacol Toxicol (Copen)* 26: 298–302.

Yan M *et al.* (2009). Quantification of prochlorperazine maleate in human plasma by liquid chromatography-mass spectrometry: application to a bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 3243–3247.

Procyclidine

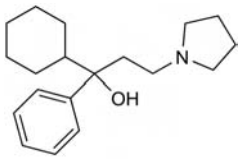
Anticholinergic

$C_{19}H_{29}NO$ = 287.4

CAS—77-37-2

IUPAC Name 1-Cyclohexyl-1-phenyl-3-pyrrolidin-1-ylpropan-1-ol

Synonym α-Cyclohexyl-α-phenyl-1-pyrrolidinepropanol



Chemical Properties Crystals. Mp 85.5° to 86.5° (crystals from petroleum ether). Log *P* (octanol/water), 4.8. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Procyclidine Hydrochloride

$C_{19}H_{29}NO,HCl$ = 323.9

CAS—1508-76-5

Proprietary Names *Arpicolin*; *Kemadren*; *Kemadrin*; *Muscinil*; *Osnervan*; *Procyclid*.

Chemical Properties A white crystalline powder. Mp 226° to 227° (crystals from ethanol and ethyl acetate), with decomposition. Soluble 1 in about 40 of water, 1 in 15 of ethanol and 1 in 6 of chloroform; very slightly soluble in ether.

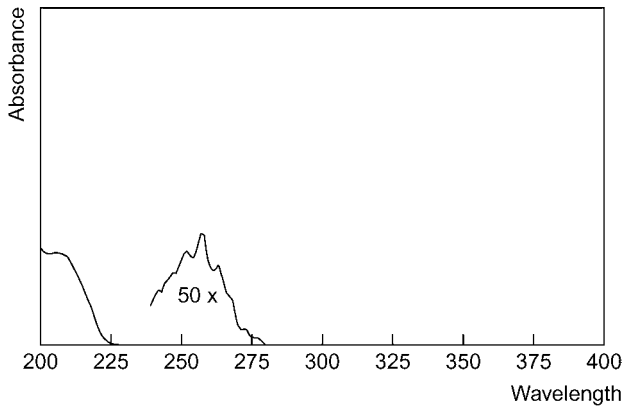
Colour Tests Mandelin's test—black; Marquis test—violet; sulfuric acid—yellow.

Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.62; system TC—R_f 0.31; system TE—R_f 0.74; system TL—R_f 0.23; system TAE—R_f 0.20; system TAF—R_f 0.68; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.36 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, violet-brown).

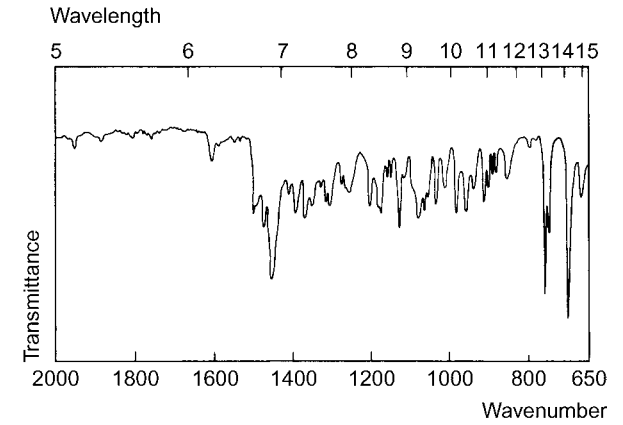
Gas Chromatography System GA—procyclidine RI 2156, M (oxo-)-H_2O RI 2490; system GB—procyclidine RI 2261, M (OH-isomer-1) RI 2487, M (OH-isomer-2) RI 2517, M (oxo-)-H_2O RI 2669; system GF—RI 2485.

High Performance Liquid Chromatography System HA—*k* 2.0; system HX—RI 406; system HZ—RT 6.2 min; system HAX—not eluted after 20 min; system HAY—RT 4.7 min.

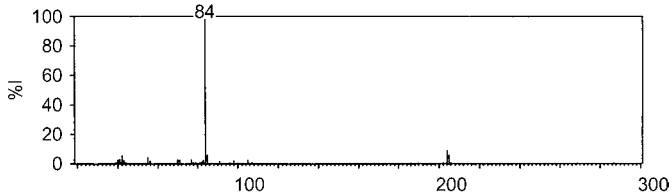
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1 = 6.9a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 700, 756, 746, 1128, 1075, 1175 cm^{-1} (procyclidine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 84, 204, 205, 85, 42, 55, 105, 77.



Quantification

***Blood* GC** AFID. Limit of detection, 100 µg/L [Missen *et al.* 1978].

***Plasma* GC** AFID. Limit of detection 20 µg/L [Dean *et al.* 1980].

***Urine* GC** See Plasma [Dean *et al.* 1980].

***Biological Fluids* GC** Flame thermionic detection. Limit of detection, 50–100 ng/L for procyclidine, cycrimine and trihexyphenidyl [Owen *et al.* 1989].

Disposition in the Body Absorbed after oral administration. It rapidly disappears from the tissues. Metabolised by hydroxylation of the benzyl or cyclohexyl rings and then conjugated with glucuronic acid.

Therapeutic Concentration

Following a single oral dose of 10 mg to 6 subjects, a mean peak plasma concentration of 0.12 mg/L was reported; in 5 of the subjects the peak concentrations were attained in about 1 h, whereas in the sixth subject the time to peak concentration was 8 h [Whiteman *et al.* 1985].

In 6 patients undergoing therapy with daily oral doses of 10–30 mg, mean steady-state blood concentrations of 0.15–0.63 mg/L were reported [Missen *et al.* 1978].

Toxicity

In 2 fatalities involving procyclidine, postmortem tissue concentrations were: blood 4 and 4.4 mg/L, liver 15 and 11 μg/g, urine 7 and 1.8 mg/L; in the second case benzatropine and chlorpromazine were also detected [Ashton 1980].

Bioavailability About 75%.

Half-life Plasma half-life, 8–16 h (mean 12).

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, ≈1 mL/min/kg.

Dose 7.5 to 30 mg of procyclidine hydrochloride daily; up to 60 mg daily has been given.

Ashton PG (1980). *Bull Int Assoc Forensic Toxicol* 15(2): 9–11.

Dean K *et al.* (1980). Analysis of procyclidine in human plasma and urine by gas-liquid chromatography. *J Chromatogr* 221: 408–413.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Missen AW *et al.* (1978). *J Anal Toxicol* 2: 238–240.

Owen JA *et al.* (1989). Capillary gas chromatography of trihexyphenidyl, procyclidine and cycrimine in biological fluids. *J Chromatogr* 494: 135–142.

Whiteman PD *et al.* (1985). Pharmacokinetics and Pharmacodynamics of procyclidine in man. *Eur J Clin Pharmacol* 28: 73–78.

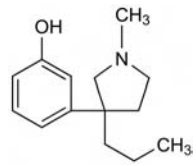
Profadol

Narcotic Analgesic

$C_{14}H_{21}NO$ = 219.3

CAS—428-37-5

IUPAC Name 3-(1-Methyl-3-propylpyrrolidin-3-yl)phenol



Profadol Hydrochloride

$C_{14}H_{21}NO,HCl$ = 255.8

CAS—2324-94-9

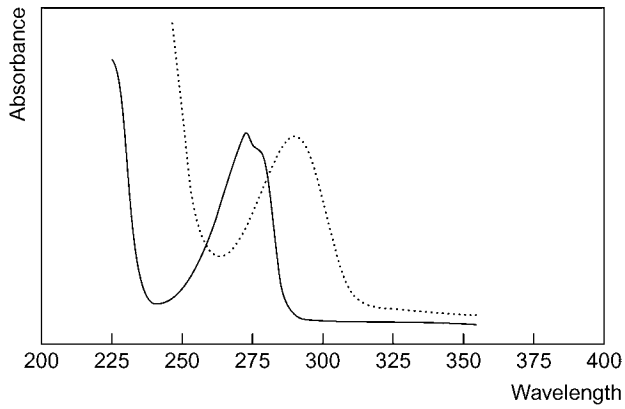
Chemical Properties A white solid. Mp 145° to 146°. Soluble in water and chloroform.

Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—blue-green→green; Marquis test—orange→red-brown.

Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.08; system TC—R_f 0.06; system TL—R_f 0.08. (Acidified iodoplatinate solution, positive.)

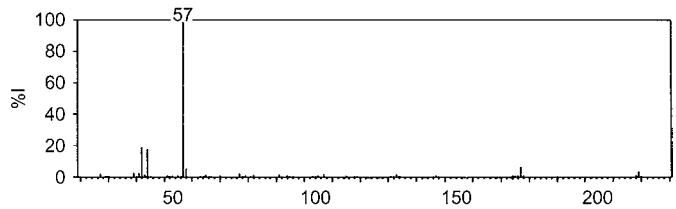
Gas Chromatography System GA—RI 1748.

Ultraviolet Spectrum Aqueous acid—272 nm (A_1^1=87b); aqueous alkali—237, 290 nm.



Infrared Spectrum Principal peaks at wavenumbers 1575, 1590, 1234, 752, 702, 1267 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 57, 42, 44, 177, 58, 219, 133, 107.



Profenamine

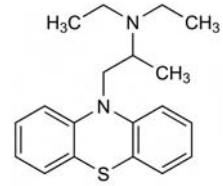
Anticholinergic

$C_{19}H_{24}N_2S$ = 312.5

CAS—522-00-9

IUPAC Name *N*,*N*-Diethyl-1-phenothiazin-10-ylpropan-2-amine

Synonyms *N*,*N*-Diethyl-α-methyl-10*H*-phenothiazine-10-ethanamine; ethopropazine; isothazine; phenopropazine.



Chemical Properties Crystals. Mp 53° to 55°. pK_a 9.6 (20°). Log *P* (octanol/water), 4.8.

Profenamine Hibenzate

$C_{19}H_{24}N_2S,C_{14}H_{10}O_4$ = 554.7

Synonym Profenamine hybenzate

Chemical Properties A white crystalline powder. Mp about 188°, with decomposition. Practically insoluble in water; slightly soluble in methanol.

Profenamine Hydrochloride

$C_{19}H_{24}N_2S,HCl$ = 348.9

CAS—1094-08-2

Synonyms Profenamini hydrochloridum; prophenamini chloridum.

Proprietary Name *Parsitan*

Chemical Properties A white crystalline powder, which darkens in colour on exposure to light. Mp 223° to 225°, with some decomposition. Soluble 1 in 400 of water at 20° and 1 in 20 at 40°, 1 in 35 of ethanol and 1 in 7 of chloroform; sparingly soluble in acetone; practically insoluble in ether and benzene.

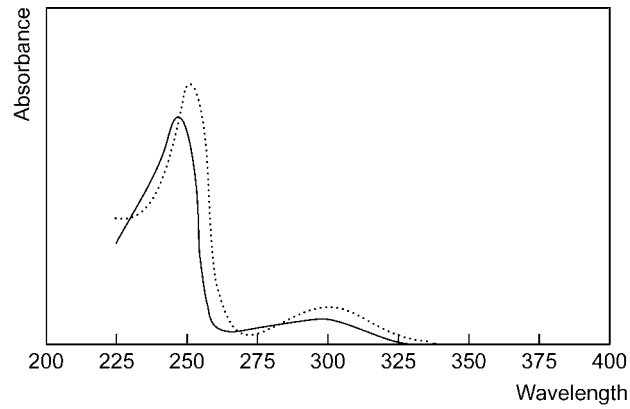
Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—pink; FPN reagent—orange→yellow; Liebermann's reagent—red-brown; Mandelin's test—green→violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.64; system TC—R_f 0.47; system TE—R_f 0.83; system TL—R_f 0.66; system TAE—R_f 0.31; system TAF—R_f 0.55; system TAJ—R_f 0.22; system TAK—R_f 0.10; system TAL—R_f 0.57. (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, orange; ninhydrin spray, positive.)

Gas Chromatography System GA—profenamine RI 2340, M (bis-desethyl-)-AC RI 2450, M (bis-desethyl-OH-)-AC_2 RI 2900, M (desethyl-)-AC RI 2515, M (desethyl-OH-)-AC_2 RI 2880; system GF—RI 2775.

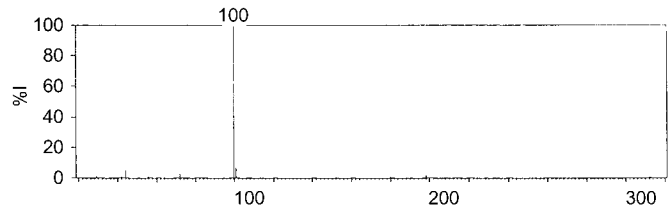
High Performance Liquid Chromatography System HA—*k* 2.4; system HX—RI 444; system HY—RI 338; system HAX—retention time 16.6 min; system HAY—retention time 8.3 min.

Ultraviolet Spectrum Aqueous acid—250 (A_1^1=881a), 299 nm; aqueous alkali—253, 302 nm.



Infrared Spectrum Principal peaks at wavenumbers 748, 1248, 1590, 1282, 1568, 1125 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 100, 101, 44, 72, 198, 180, 42, 29.

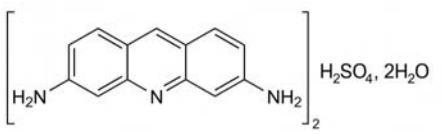


Dose 50 to 500 mg of profenamine hydrochloride daily.

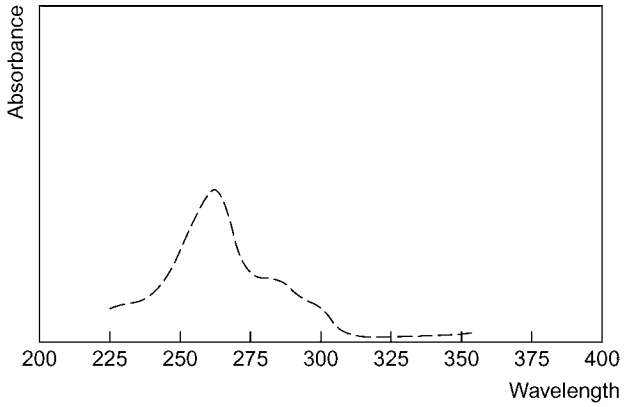
Proflavine Hemisulfate

Disinfectant, Antiseptic (Topical)
$(C_{13}H_{11}N_3)_2,H_2SO_4,2H_2O = 552.6$
CAS—92-62-6 (proflavine); 553-30-0 (sulfate, anhydrous)

IUPAC Name (6-Aminoacridin-3-yl)azanium sulfate
Synonyms Neutral proflavine sulfate; proflavine hemisulfate.



Chemical Properties An orange to red, hygroscopic, crystalline powder. Soluble 1 in 300 of water, 1 in 1 of boiling water, and 1 in 35 of glycerol; very slightly soluble in ethanol; practically insoluble in chloroform and ether. A saturated solution in water is deep orange in colour and gives a green fluorescence when freely diluted. Proflavine: pK_a 8.1 (20°). Proflavine: Log *P* (octanol/water), 1.8.
Colour Tests Mandelin's test—green; Marquis test—yellow→orange.
Thin-layer Chromatography System TA—R_f 0.16.
Ultraviolet Spectrum Ethanol—261 nm (A^1_1 = 1091b).

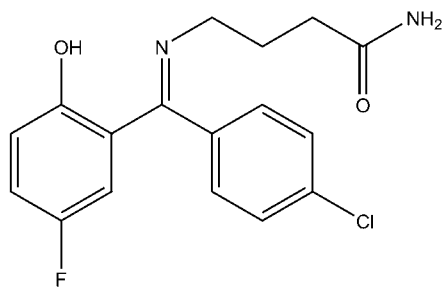


Infrared Spectrum Principal peaks at wavenumbers 1605, 1625, 1125, 1160, 1218, 1263 cm^{-1} (KBr disk).

Progabide

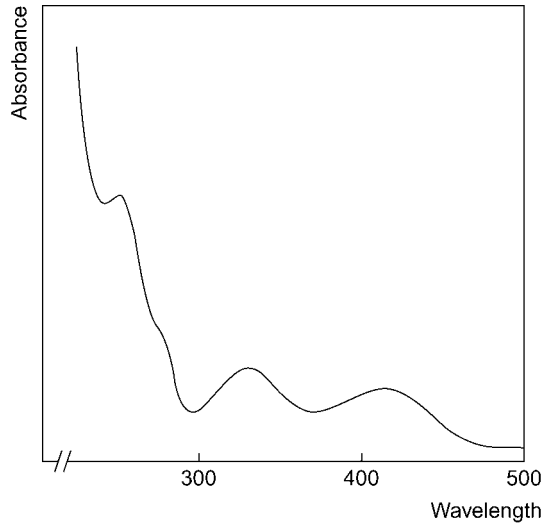
Anticonvulsant, GABA Analogue, GABA Receptor Agonist
$C_{17}H_{16}ClFN_2O_2 = 334.8$
CAS—62666-20-0

IUPAC Name 4-[[(*Z*)-(4-chlorophenyl)-(3-fluoro-6-oxo-1-cyclohexa-2,4-dienylidene)methyl]amino]butanamide
Synonyms 4-(4′-Chloro-5-fluoro-2-hydroxybenzhydrylideneamino)butyramide; 4-[[α-(*p*-chlorophenyl)-5-fluoro-2-hydroxybenzylidene]amino]butyramide; 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanamide; 4-[[α-(*p*-chlorophenyl)-5-fluorosalicylidene]amino]butyramide; halogabide; SL-76.002.
Proprietary Name *Gabrene*

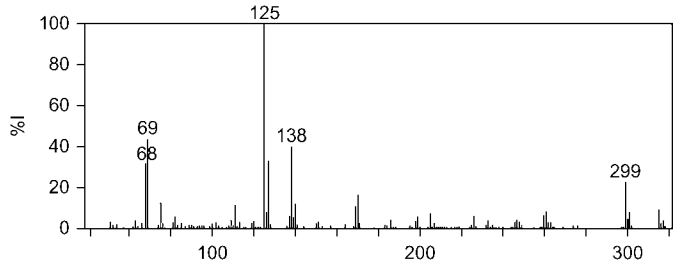


Chemical Properties Crystals. Mp 133° to 135°. Soluble in water (70.9 mg/L), increased solubility with decreasing pH. Log *P* (octanol/water), 3.09. Stable in solutions at pH 6 to 7 [Farraj *et al.* 1988].
High Performance Liquid Chromatography Column: Spherisorb ODS1 (247 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : McIlvaine buffer (pH 5.25; 35 : 30 : 35), flow rate 1.7 mL/min. UV detection (λ = 255 nm). Retention time: progabide, 3.7 min; progabide acid, 2.6 min [Farraj *et al.* 1987].

Ultraviolet Spectrum Methanol—332, 250, 210 nm [Farraj *et al* 1987].



Mass Spectrum Principal ions at *m/z* 125, 69, 138, 511, 68, 458 [Gillet *et al* 1982].



Quantification

Plasma **HPLC** Column: Nucleosil C_{18} (300 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (9.0 g potassium dihydrogen phosphate and 0.14 g disodium hydrogenphosphate heptahydrate in 1 L water, pH 5.05) : methanol : acetonitrile : 0.15 mol/L sodium chloride (36 : 27 : 27 : 10), flow rate 2.5 mL/min. UV detection (λ = 340 nm). Retention time: progabide, 7.9 min; progabide acid (PGA), 6.9 min. Limit of detection, progabide 20 µg/L, PGA 8 µg/L [Decourt *et al.* 1990]. Column: Spherisorb ODS1 (247 × 4.5 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : McIlvaine buffer (pH 5.25; 35 : 30 : 35), flow rate 1.4 mL/min. UV detection (λ = 255 nm). Retention time: progabide 5.4 min, PGA 4.2 min. Limit of quantification, progabide 30 µg/L, PGA 100 µg/L [Farraj *et al.* 1987].

GC-MS Column: Gas-Chrom Q 3% V-17 (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 230°. ECD. Limit of detection, 1 µg/L [Gillet *et al.* 1982].

Urine **GC-MS** Column: Gas-Chrom Q 3% V-17 (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: at 230°. ECD. Limit of detection not reported [Gillet *et al.* 1982].

Disposition in the Body Progabide is a GABA analogue pro-drug. Progabide is absorbed after oral administration with peak plasma levels reached after 2 to 3 h. The plasma half-life is 10 to 12 h and the compound is mostly metabolised, with only trace amounts being found in the urine. It is transformed by deamidation to its acid form PGA ([α-(chloro-4′-phenyl)-fluoro-5-hydroxy-2-benzylidene-amino]-4-butonate sodium; SL 75102) or can be cleaved at the imine link to yield the carrier moiety and GABAmide, which is further metabolised to GABA. PGA also undergoes the same metabolic transformation to GABA. The metabolites and GABA appear in the CNS and peripherally a few minutes after administration. Steady-state plasma levels are achieved in 2 to 4 days [Bergmann 1985].

Therapeutic Concentration A single 300 mg oral dose will result in a mean maximum plasma concentration of 1350 µg/L at ~2 h. The maximum allowable doses in the USA were 45 mg/kg/day in FDA-approved clinical trials [Bergmann 1985].

Twenty therapy-resistant epileptic patients were enrolled in a double-blind trial. Daily oral doses of progabide ranging from 19.3 to 36 mg/kg resulted in plasma concentrations ranging from <50 to 1476 µg/L [Martinez-Lage *et al.* 1984].

Fifty-two adult epileptic outpatients were administered daily doses of 30 to 45 mg/kg progabide in addition to other therapy and were monitored over a period of 6 to 12 months. Differences were observed in therapeutic effect between patients. Subgroup A (14; the responders) showed a persistent therapeutic response with a reduction in seizure frequency consistently >50%, which was associated with blood concentrations of progabide and

PGA of 689 and 1012 µg/L, respectively. Subgroup B (21; the non-responders) had a reduction in seizure frequency of <50%, which was associated with blood concentrations of progabide and PGA of 256 and 633 µg/L, respectively. Subgroup C (17; the fluctuant responders) showed varying degrees of reduction in seizure frequency over the period. For this subgroup, a satisfactory response was associated with progabide and PGA blood concentrations of 718 and 1266 µg/L, while lack of therapeutic efficacy was associated with concentrations of 748 µg/L for PGA and 432 µg/L for progabide [Benassi *et al.* 1988].

Four groups of 6 healthy volunteers were administered oral doses of valproic acid (500 mg, group 1), phenytoin (100 mg, group 2), carbamazepine (400 mg, group 3), or phenobarbital (100 mg, group 4) on day 1 of the study. From day 5 to 19, progabide was administered at a dosage of 600 mg three times daily. Blood concentrations of progabide and PGA were measured at days 13, 15 and 17, and were found to be steady within the groups but differed considerably among the 4 groups (290 to 736 µg/L for progabide, 748 to 1641 µg/L for PGA) [Bianchetti *et al.* 1987].

Toxicity Liver disorders, indicated by elevation of liver enzyme values, have been reported to occur in ~9% of patients receiving progabide, usually in the first few months of treatment, and have progressed to jaundice, hepatitis, encephalopathy and death in some patients.

Half-life Approximately 10 to 12 h.

Protein Binding Progabide 98.5%, PGA 99.8%; mainly to serum albumin [Hamberger *et al.* 1987].

Dose In the range 15 to 60 mg/kg/day.

Benassi E *et al.* (1988). Blood levels of progabide and its active metabolite in epileptic patients: relationships to the therapeutic outcome. *Int J Clin Pharmacol Res* 8: 409–413.

Bergmann KJ (1985). Progabide: a new GABA-mimetic agent in clinical use. *Clin Neuropharmacol* 8: 13–26.

Bianchetti G *et al.* (1987). Pharmacokinetic interactions of progabide with other antiepileptic drugs. *Epilepsia* 28: 68–73.

Decourt JP *et al.* (1990). Simultaneous determination of progabide and its acid metabolite by reversed-phase high-performance liquid chromatography. *J Chromatogr* 527: 214–219.

Farraj NF *et al.* (1987). A reversed-phase high-performance liquid chromatography assay procedure for progabide and its related metabolic derivatives. *Pharm Res* 4: 28–32.

Farraj NF *et al.* (1988). The stability and solubility of progabide and its related metabolic derivatives. *Pharm Res* 5: 226–231.

Gillet G *et al.* (1982). Gas chromatographic method for the determination of progabide (SL 76.002) in biological fluids. *J Chromatogr* 230: 154–161.

Hamberger C *et al.* (1987). Progabide and SL 75102 binding to plasma proteins and red blood cells in humans. *Int J Clin Pharmacol Ther Toxicol* 25: 178–184.

Martinez-Lage JM *et al.* (1984). Progabide treatment in severe epilepsy: a double-blind cross-over trial versus placebo. *Epilepsia* 25: 586–593.

Progesterone

Progestational Steroid

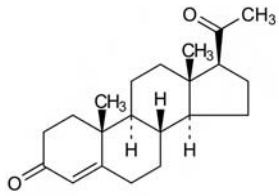
$C_{21}H_{30}O_2$ = 314.5

CAS—57-83-0

IUPAC Name (8*S*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Acetyl-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one

Synonyms Corpus luteum hormone; luteal hormone; luteine; pregn-4-ene-3,20-dione; pregnenedione; progestin.

Proprietary Names *Crinone*; *Cutifitol*; *Cyclogest*; *Esolut*; *Estima*; *Evapause*; *Gesterol*; *Gestone*; *Lugesteron*; *Progeffik*; *Progenar*; *Progestan*; *Progestasert*; *Progestilin*; *Progestogel*; *Progestol*; *Progestosol*; *Prolidon*; *Proluton*; *Prometrium*; *Prontogest*; *Utrogest*; *Utrogestan*.



Chemical Properties Colourless crystals or a white or slightly yellowish-white crystalline powder. There are two forms, one known as the α-form melts at 127° to 131° and the other, known as β-progesterone, at about 121°. Practically insoluble in water; soluble 1 in 8 of ethanol, 1 in less than 1 of chloroform and 1 in 16 of ether. Log *P* (octanol/water), 3.9.

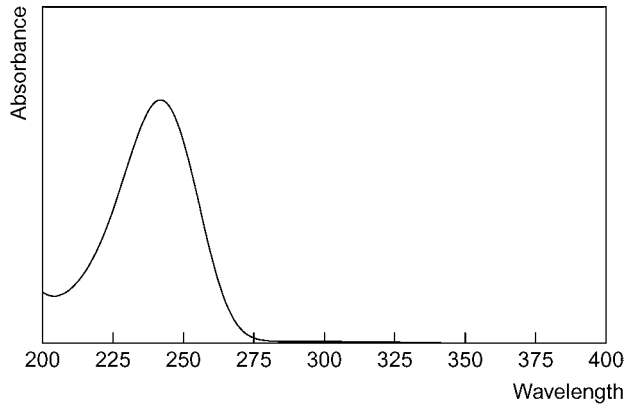
Colour Tests Naphthol-sulfuric acid—brown/yellow; sulfuric acid—yellow (green fluorescence under UV light).

Thin-layer Chromatography System TB—R_f 0.36; system TE—R_f 0.79; system TF—R_f 0.56; system TP—R_f 0.81; system TQ—R_f 0.20; system TR—R_f 0.99; system TS—R_f 0.95; system TAE—R_f 0.83; system TAF—R_f 0.89; system TAJ—R_f 0.76; system TAK—R_f 0.68; system TAL—R_f 0.95; system TAM—R_f 0.97.

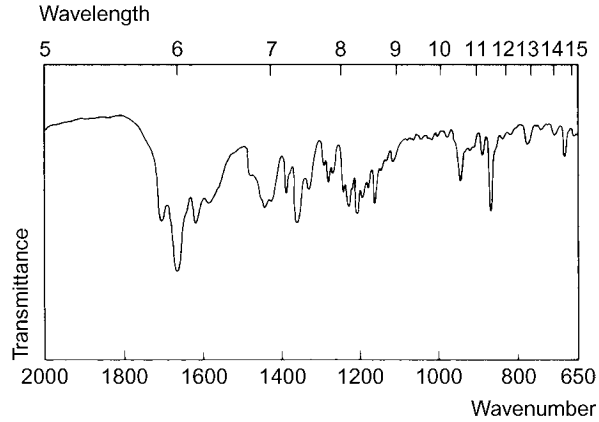
Gas Chromatography System GA—RI 2793.

High Performance Liquid Chromatography System HX—RI 672; system HY—RI 698; system HAA—retention time 23.8 min.

Ultraviolet Spectrum Dehydrated alcohol—240 nm (A^1_1=540a).



Infrared Spectrum Principal peaks at wavenumbers 1662, 1614, 1700, 872, 1209, 1232 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 124, 43, 314, 79, 91, 229, 272, 105.

Dose Up to 50 mg daily by IM injection.

Proguanil

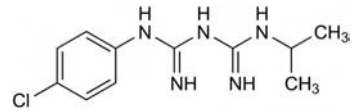
Antimalarial

$C_{11}H_{16}ClN_5$ = 253.7

CAS—500-92-5

IUPAC Name (1*E*)-1-[Amino-(4-chloroanilino)methylidene]-2-propan-2-yl-guanidine

Synonyms Chloriguane; chlorguanide; chloroguanide; *N*-(4-chlorophenyl)-*N*′-(1-methylethyl)imidodicarbonimidic diamide; proguanide.



Chemical Properties Colourless crystals. Mp 129°. pK_a 2.3, 10.4 (22.5°). Log *P* (octanol/water), 2.5.

Proguanil Hydrochloride

$C_{11}H_{16}ClN_5$,HCl = 290.2

CAS—637-32-1

Synonyms Bigumalum; M-4888; RP-3359; SN-12837.

Proprietary Names *Paludrine*. It is an ingredient of *Malarone*.

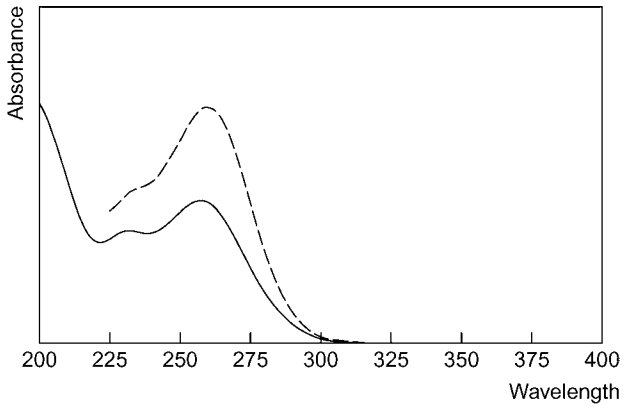
Chemical Properties A white crystalline powder. Mp 243° to 244°. Soluble 1 in 110 of water and 1 in 40 of ethanol; practically insoluble in chloroform and ether.

Colour Test To 10 mL of a saturated solution of proguanil hydrochloride add 1 drop of 10% copper sulfate solution and 2.5 mL of dilute ammonia solution and shake well; add 5 mL of toluene and shake again—toluene layer is violet-red.

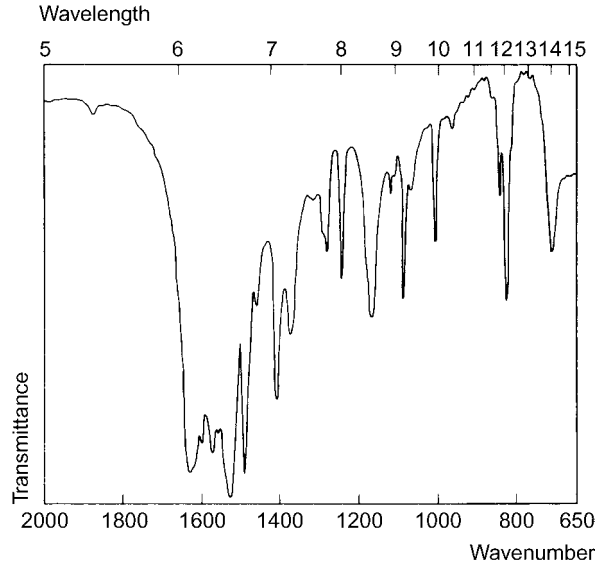
Thin-layer Chromatography System TA—R_f 0.03; system TB—R_f 0.00; system TC—R_f 0.01; system TE—R_f 0.18; system TL—R_f 0.01; system TAE—R_f 0.07; system TAF—R_f 0.79 (acidified iodoplatinate solution, positive).

High Performance Liquid Chromatography System HX—RI 379; system HZ—RT 3.8 min; system HAA—RT 13.6 min.

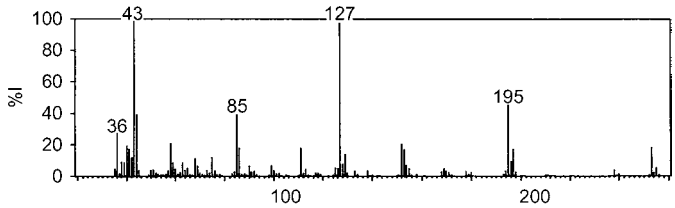
Ultraviolet Spectrum Methanol—259 nm (A^1_1= 886b).



Infrared Spectrum Principal peaks at wavenumbers 1534, 1635, 1492, 1575, 1600, 1170 cm^{-1} (proguanil hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 127, 195, 85, 44, 36, 152, 58.



Quantification

Blood **HPLC** Limit of detection, 50 nmol/L [Bergqvist *et al.* 1998a]. Proguanil, cycloguanil and 4-chlorophenylbiguanide (in blood dried on sampling paper). Limit of detection, 125 nmol/L for proguanil [Bergqvist *et al.* 1998b]. Proguanil, cycloguanil and 4-chlorophenylbiguanide (in blood dried on filter paper). Limit of detection, ≈12 µg/L for proguanil [Kolawole *et al.* 1995].

Plasma **HPLC** Limit of detection, 20 nmol/L [Bergqvist *et al.* 1998a]. Limit of detection, 1 µg/L for proguanil, cycloguanil and 4-chlorophenylbiguanide [Kusaka *et al.* 1996]. Proguanil, cycloguanil and 4-chlorophenylbiguanide. Limit of detection, ≈12 µg/L for proguanil [Kolawole *et al.* 1995]. UV detection. Proguanil, chloroquine and their metabolites. Limit of detection, 9 µg/L for proguanil [Chaulet *et al.* 1994].

Serum **HPLC** UV detection. Limit of detection, 60 µg/L for proguanil, cycloguanil and 4-chlorophenylbiguanide [Moody *et al.* 1980].

Urine **HPLC** For method, see Bergqvist *et al.* [1998a]. Limit of detection, 5 µg/L for proguanil, cycloguanil and 4-chlorophenylbiguanide [Kusaka *et al.* 1996]. UV detection. Proguanil, chloroquine and their metabolites. Limit of detection, 9 µg/L for proguanil [Chaulet *et al.* 1994].

Erythrocytes **HPLC** UV detection. Proguanil, chloroquine and their metabolites. Limit of detection, 9 µg/L for proguanil [Chaulet *et al.* 1994].

Disposition in the Body Readily absorbed after oral administration; peak plasma concentrations are attained in ≈3 h. About 60% of a dose is excreted in the urine unchanged, together with ≈30% as the active metabolite cycloguanil; 4-chlorophenylbiguanide is also a metabolite.

Therapeutic Concentration

Six healthy subjects given proguanil 200 mg daily, orally, for 7 days, co-administered with dapsone (10 mg), had peak plasma concentrations of 151 µg/L 3.5 h after the final dose; the peak plasma concentration of cycloguanil was 55.5 µg/L after 6 h [Edstein *et al.* 1990].

Children given proguanil 100–300 mg daily, orally (mean, 7 mg/kg daily) for 3 days after having received atovaquone (250–750 mg; mean, 17 mg/kg daily) for 3 days, had peak plasma concentrations of 306 µg/L 6 h after the final dose; the peak plasma concentration of cycloguanil was 44.3 µg/L after 6 h [Sabchareon *et al.* 1998].

Protein Binding ≈75%.

Dose 100 to 300 mg of proguanil hydrochloride daily.

Bergqvist Y *et al.* (1998a). Improved validated assay for the determination of proguanil and its metabolites in plasma, whole blood, and urine using solid-phase extraction and high-performance liquid chromatography. *Ther Drug Monit* 20: 325–330.

Bergqvist Y *et al.* (1998b). Improved method for the simultaneous determination of proguanil and its metabolites by high-performance liquid chromatography and solid-phase extraction of 100-microl capillary blood samples dried on sampling paper. *J Chromatogr B Biomed Sci Appl* 719: 141–149.

Chaulet JF *et al.* (1994). Simultaneous determination of chloroquine, proguanil and their metabolites in human biological fluids by high-performance liquid chromatography. *J Pharm Biomed Anal* 12: 111–117.

Edstein MD *et al.* (1990). Multiple-dose kinetics in healthy volunteers and in vitro antimalarial activity of proguanil plus dapsone. *Chemotherapy* 36: 169–176.

Kolawole JA *et al.* (1995). Determination of proguanil and metabolites in small sample volumes of whole blood stored on filter paper by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 674: 149–154.

Kusaka M *et al.* (1996). Simultaneous measurement of proguanil and its metabolites in human plasma and urine by reversed-phase high-performance liquid chromatography, and its preliminary application in relation to genetically determined S-mephenytoin 4′-hydroxylation status. *Am J Trop Med Hyg* 54(2): 189–196.

Moody RR *et al.* (1980). High-performance liquid chromatography of proguanil, cycloguanil and 4-chlorophenylbiguanide using hydrophobic pairing ion and its application to serum assay. *J Chromatogr* 182: 359–367.

Sabchareon A *et al.* (1998). Efficacy and pharmacokinetics of atovaquone and proguanil in children with multidrug-resistant Plasmodium falciparum malaria. *Trans R Soc Trop Med Hyg* 92: 201–208.

Proheptazine

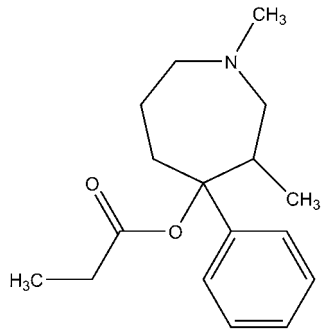
Opiate, Narcotic

$C_{17}H_{25}NO_2$ = 275.4

CAS—77-14-5

IUPAC Name (1,3-Dimethyl-4-phenylazepan-4-yl) propanoate

Synonyms DEA Number 9643; dimepheprimine; DL-α-1,3-dimethyl-4-phenyl-4-propionoxyazacycloheptane; 1,3-dimethyl-4-phenyl-4-propionoxyhexamethylenimine; EINECS 201-006-8; hexahydro-1,3-dimethyl-4-phenyl-1*H*-azepin-4-ol propanoate (ester); 4-phenyl-4-propionoxy-1,3-dimethylazacycloheptane; 4-propionoxy-1,3-dimethyl-4-phenylhexamethylenimine; WY-757.



Chemical Properties Liquid [O'Neil *et al.* 2006]. White crystals. Soluble in dilute acetic acid. Log *P* (octanol/water) 3 [National Institutes of Health 2008].

Proheptazine Hydrobromide

$C_{17}H_{25}NO_2$,HBr = 356.3

Chemical Properties Crystals from acetone and methanol. Soluble 1 to 2% in water at 25° [O'Neil *et al.* 2006].

Proheptazine Hydrochloride

$C_{17}H_{25}NO_2$,HCl = 311.9

Chemical Properties Crystals.

Colour Tests Ammonium molybdate test—blue-grey (limit of detection, 0.25 µg); ammonium vandate test—greyish-purple, fading (limit of detection, 0.25 µg) ; sulfuric acid–formaldehyde—dull orange (limit of detection, 0.25 µg).

Thin-layer Chromatography System T1—R_f 0.40 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—retention time 0.48 relative to diphenhydramine; system G4—retention time 0.46 relative to diphenhydramine.

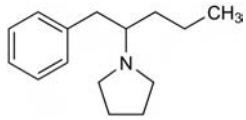
National Institutes of Health (2008). *Proheptazine*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=60969&loc=ec_rcs. (accessed 30 June 2008).

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Prolintane

CNS Stimulant
$C_{15}H_{23}N$ = 217.4
CAS—493-92-5

IUPAC Name 1-(1-Phenylpentan-2-yl)pyrrolidine
Synonym 1-[1-(Phenylmethyl)butyl]pyrrolidine

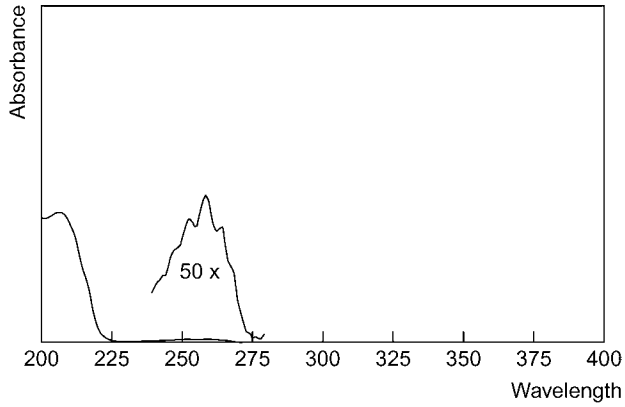


Chemical Properties Log *P* (octanol/water), 4.3.

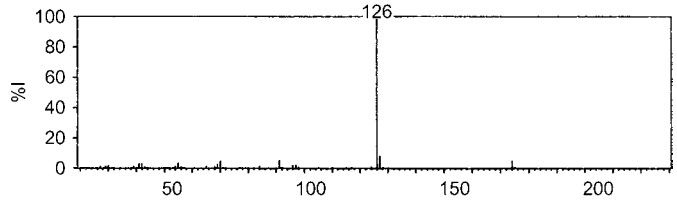
Prolintane Hydrochloride

$C_{15}H_{23}N,HCl$ = 253.8
CAS—1211-28-5
Proprietary Names *Catorid*; *Catovit N*; *Promotil*. It is an ingredient of *Katovit* and *Villescon*.
Chemical Properties A white powder. Mp about 133° to 134°. Soluble in water, ethanol and chloroform; practically insoluble in ether.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—red; Marquis test—orange→brown.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.67; system TC—R_f 0.32; system TE—R_f 0.79; system TL—R_f 0.25; system TAE—R_f 0.22 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, orange-brown; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—prolintane RI 1634; M (oxo-) RI 1895;M (OH-phenyl-) RI 2135, M (OH-phenyl-)-AC RI 2110, M (OH-methoxy-phenyl-)-AC RI 2115; M (oxo-OH-alkyl-) RI 2200, M (oxo-OH-alkyl-)-AC RI 2255; M (oxo-OH-methoxy-phenyl-) RI 2240, M (oxo-OH-methoxy-phenyl-)-AC RI 2360; M (oxo-di-OH-)-AC_2 RI 2485, M (oxo-di-OH-phenyl-) RI 2475, M (oxo-di-OH-phenyl-)-AC_2 RI 2450, M (oxo-OH-phenyl-)-AC RI 2275, M (oxo-di-OH-methoxy-)-AC_2 RI 2560, M (di-OH-phenyl-)-AC_2 RI 2295, M (tri-OH-)-AC_3 RI 2630, system GB—prolintane RI 1660; system GC—prolintane RI 1849.
High Performance Liquid Chromatography System HA—*k* 2.0; system HC—*k* 1.3; system HX—RI 370.
Ultraviolet Spectrum Aqueous acid—252, 258 (A_1^1=8.6a), 264 nm.



Infrared Spectrum Principal peaks at wavenumbers 776, 714, 752, 1501, 1141, 1020 cm^{-1} (prolintane hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 126, 127, 174, 91, 70, 69, 55, 42.

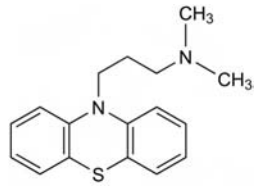


Dose 20 mg of prolintane hydrochloride daily.

Promazine

Tranquilliser
$C_{17}H_{20}N_2S$ = 284.4
CAS—58-40-2

IUPAC Name *N*,*N*-Dimethyl-3-phenothiazin-10-ylpropan-1-amine
Synonyms A-145; *N*,*N*-dimethyl-10*H*-phenothiazine-10-propanamine; RP-3276; Wy-1094.



Chemical Properties An oily liquid. pK_a 9.4 (25°). Log *P* (octanol/pH 7.4), 2.5. Extraction yield (promazine), 1 [Demme *et al.* 2005].

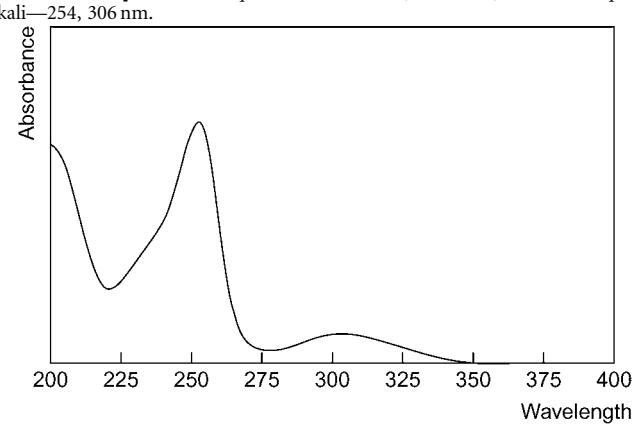
Promazine Embonate

$(C_{17}H_{20}N_2S)_2,C_{23}H_{16}O_6$ = 957.2
Synonym Promazine pamoate
Proprietary Names *Protactyl (suspension)*; *Sparine (suspension)*.

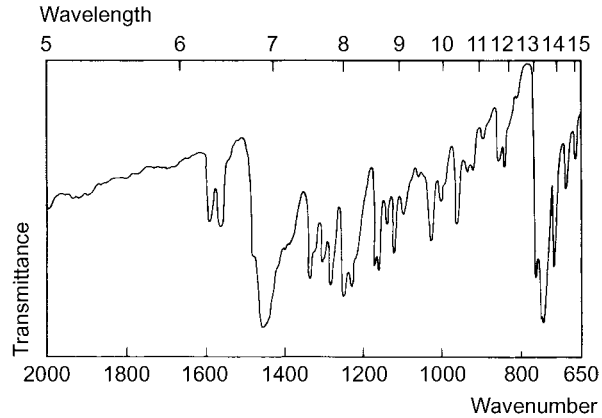
Promazine Hydrochloride

$C_{17}H_{20}N_2S,HCl$ = 320.9
CAS—53-60-1
Synonym Propazinum
Proprietary Names *Liranol*; *Prazine*; *Promabec*; *Promanyl*; *Promwill*; *Protactyl (injection and tablets)*; *Sparine (injection and tablets)*; *Talofen*.
Chemical Properties A white or slightly yellow, slightly hygroscopic, crystalline powder. It is affected by air and light and traces of heavy metals. Decomposed solutions may be coloured pink, red or blue. Mp 181°, with decomposition. Soluble 1 in 3 of water, 1 in 2 of ethanol and 1 in 2 of chloroform; soluble in methanol; practically insoluble in ether and benzene.

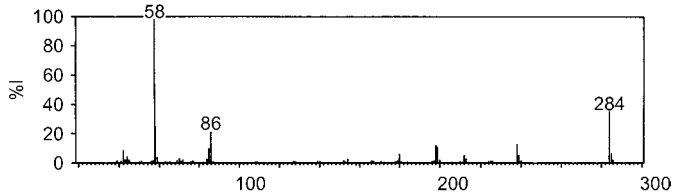
Colour Tests Formaldehyde-sulfuric acid—red; Forrest reagent—red-brown; FPN reagent—red-brown; Liebermann's reagent—green-brown; Mandelin's test—green→violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.44; system TB—R_f 0.38; system TC—R_f 0.30; system TE—R_f 0.62; system TL—R_f 0.11; system TAE—R_f 0.18; system TAF—R_f 0.35; system TAJ—R_f 0.06; system TAK—R_f 0.02; system TAL—R_f 0.41. (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, red; ninhydrin spray, positive.)
Gas Chromatography System GA—Promazine RI 2315, M (nor-) RI 2405, M (sulfoxide) RI 2705, M (phenothiazine) RI 2120, M (OH-) RI 2685; system GB—promazine RI 2425, M (nor-) RI 2452, M (sulfoxide) RI 2840, M (norsulfoxide) RI 2875, M (phenothiazine) RI 2130, M (OH-) RI 2781, M (nor-OH-) RI 2797; system GF—RI 2745.
High Performance Liquid Chromatography System HA—*k* 5.9; system HX—RI 407; system HY—RI 326; system HZ—retention time 5.9 min.
Ultraviolet Spectrum Aqueous acid—251 (A_1^1=1055a), 302 nm; aqueous alkali—254, 306 nm.



Infrared Spectrum Principal peaks at wavenumbers 751, 1250, 1234, 1287, 770, 1162 cm^{-1} (promazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 284, 86, 238, 198, 199, 85, 42.



Quantification

Blood **GC** SID. Limit of detection, 250 to 500 ng/L for promazine and other phenothiazines [Hattori *et al.* 1992].

Plasma **HPLC** Electrochemical detection. For method, see Larsimont *et al.* [1998].

Urine **GC** SID. Limit of detection, 250 to 500 ng/L for promazine and other phenothiazines [Hattori *et al.* 1992].

Dose Usually up to 400 mg of promazine hydrochloride daily; maximum of 1 g daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Hattori H *et al.* (1992). Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 579(2): 247–252.

Larsimont V *et al.* (1998). Validated high-performance liquid chromatographic assay for the determination of promazine in human plasma. Application to pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 719: 222–226.

Promethazine

Antihistamine

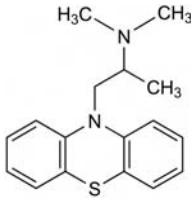
$C_{17}H_{20}N_2S$ = 284.4

CAS—60-87-7

IUPAC Name *N*,*N*-Dimethyl-1-phenothiazin-10-ylpropan-2-amine

Synonym *N*,*N*,α-Trimethyl-10*H*-phenothiazine-10-ethanamine

Proprietary Names *Fenergan Creme*; *Phenergan*.



Chemical Properties A crystalline solid. Mp 60°. pK_a 9.1 (25°). Log *P* (octanol/pH 7.4), 2.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Promethazine Hydrochloride

$C_{17}H_{20}N_2S,HCl$ = 320.9

CAS—58-33-3

Synonyms Diprazinum; proazamine chloride; promethazini hydrochloridum; promethazinium chloride.

Proprietary Names *Anergan*; *Atosil*; *Fenergan*; *Histantil*; *Insomn-Eze*; *Pamergan*; *Phenergan*; *Phenhalal*; *Prothiazine*; *Q-Mazine*; *Sominex*; *Ziz*. It is an ingredient of *Medised*, *Mepergan*, *Night Nurse*, *Pamergan P100*, *Phensedyl Plus*and *Tixylix Night-Time*.

Chemical Properties A white or faintly yellow, crystalline powder, which is slowly oxidised on prolonged exposure to air, becoming blue in colour. Mp 230° to 232° (crystals from ethylene dichloride), with some decomposition. Soluble 1 in 0.6 of water, 1 in 9 of ethanol and 1 in 2 of chloroform; practically insoluble in acetone, ether and ethyl acetate.

Promethazine Teoclate

$C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$ = 499.0

CAS—17693-51-5

Synonyms Promethazine chlorotheophyllinate; promethazine theoclate.

Proprietary Names *Avomine*.

Chemical Properties A white powder. Very slightly soluble in water; soluble 1 in 70 of ethanol and 1 in 2.5 of chloroform; practically insoluble in ether.

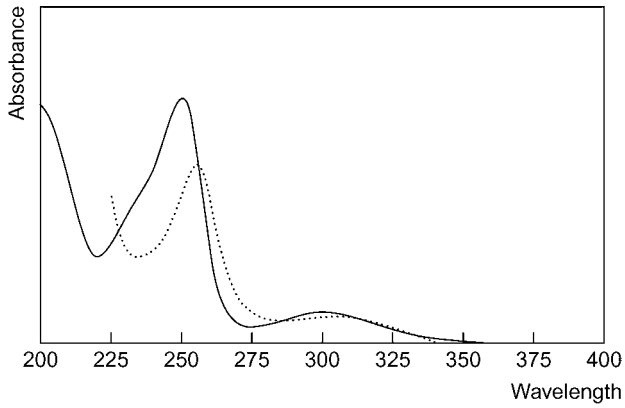
Colour Tests Formaldehyde-sulfuric acid—blue–violet; Forrest reagent—red; FPN reagent—orange; Mandelin's test—green→violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.36; system TC—R_f 0.35; system TE—R_f 0.65; system TL—R_f 0.17; system TAE—R_f 0.30; system TAF—R_f 0.44. (Dragendorff spray, positive; FPN reagent, positive; acidified iodoplatinate solution, positive; Marquis reagent, violet; ninhydrin spray, positive.)

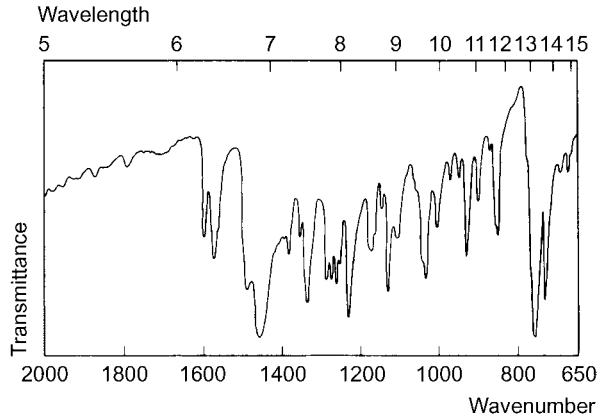
Gas Chromatography System GA—promethazine RI 2259, M (nor-) RI 2250, M (phenothiazine) RI 2020, M (sulfoxide) RI 2710, M (nor-OH-) RI 2580, M (OH-) RI 2590; system GB—promethazine RI 2383, M (nor-) RI 2333, M (phenothiazine) RI 2120, M (sulfoxide) RI 2797, M (norsulfoxide) RI 2732, M (nor-OH-) RI 2717; system GC—RI 2546; system GF—RI 2675.

High Performance Liquid Chromatography System HA—*k* 5.0; system HX—RI 409; system HY—RI 324; system HZ—retention time 5.7 min; system HAA—retention time 14.5 min; system HAX—retention time 13.2 min; system HAY—retention time 6.4 min.

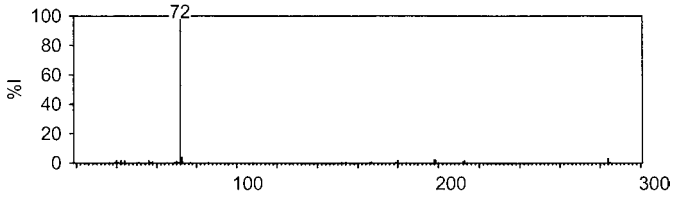
Ultraviolet Spectrum Aqueous acid—249 (A_1^1=1032a), 298 nm; aqueous alkali—254, 305 nm.



Infrared Spectrum Principal peaks at wavenumbers 758, 1229, 733, 1129, 1259, 1287 cm^{-1} (promethazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 73, 284, 198, 213, 199, 180, 56; *N*-monodesmethylpromethazine (M (nor-))58, 213, 198, 180, 214, 57, 212, 270.



Quantification

Blood **HPLC** UV detection. Promethazine, *N*-monodesmethylpromethazine and promethazine sulfoxide. Limit of detection, 200 ng/L for promethazine [Taylor, Houston 1982].

Plasma **HPLC** UV detection. Promethazine and its metabolites. Limit of detection, 1 µg/L for promethazine [Vanapalli *et al.* 2001]. Electrochemical detection. Limit of detection, 200 ng/L [Wallace *et al.* 1981].

Serum **HPLC** UV detection. Limit of detection, 2 µg/L for promethazine enantiomers [Liu, Stewart 1997]. Coulometric electrochemical detection. Promethazine and other neuroleptics. Limit of detection, 0.1 µg/L for promethazine [Bagli *et al.* 1994]. Electrochemical detection. Limit of quantification, 0.2 µg/L [Fox, McLoughlin 1993]. Electrochemical detection. Limit of detection, 200 ng/L [Wallace *et al.* 1981].

Postmortem Samples **HPLC** UV detection. For method for quantification of promethazine and metabolites, see Allender and Archer [1984].

Disposition in the Body Readily absorbed after oral administration but bioavailability is low because of extensive first-pass metabolism. About 2% of a dose is excreted in the urine unchanged. It is excreted slowly via urine and bile. The major urinary metabolite is the sulfoxide; glucuronic acid conjugation also occurs. *N*-Monodesmethylpromethazine has been detected in plasma and urine. Promethazine crosses the blood–brain and placental barriers and it is excreted in breast milk.

Therapeutic Concentration

Following an oral dose of 25 mg to 10 subjects, a mean peak blood concentration of 0.005 mg/L was attained in 2 h; saliva concentrations averaged 0.008 mg/L consistently from 1 to 6 h after administration. After an IM dose of 25 mg to the same subjects, mean peak concentrations of 0.022 mg/L at 4 h and 0.003 mg/L at 2 h were reported for blood and saliva, respectively [DiGregorio, Ruch 1980].

Following single oral doses of 25 mg to 7 subjects, peak blood concentrations of 0.002 to 0.018 mg/L were attained in 1.5 to 3 h [Taylor *et al.* 1983].

Toxicity The estimated minimum lethal dose is 200 mg/kg.

In 2 fatalities involving the ingestion of promethazine, the following postmortem tissue concentrations were reported (mg/L or μg/g):

	Promethazine	*Promethazine sulfoxide*	*N-Monodesmethyl-promethazine*
Blood	5.2, 2.4	3.1, 0.8	0.8, 1.2
Bile	82.6, 11.6	23.5, 27.0	14.5, 3.0
Liver	30.6, 23.2	8.4, 5.4	6.3, 16.2
Urine	6.0, 14.0	26.0, 42.0	1.0, 3.0

Other drugs were detected in both cases [Allender, Archer 1984].

Half-life Plasma half-life, 10 to 15 h.
Volume of Distribution About 13 L/kg.
Clearance Plasma clearance, about 16 mL/min/kg.
Distribution in Blood Plasma:whole blood ratio, 1.5.
Protein Binding About 75 to 93%.
Dose 20 to 100 mg of promethazine hydrochloride daily.

Allender WJ, Archer AW (1984). Liquid chromatographic analysis of promethazine and its major metabolites in human postmortem material. *J Forensic Sci* 29: 515–526.

Bagli M *et al.* (1994). Quantification of chlorprothixene, levomepromazine and promethazine in human serum using high-performance liquid chromatography with coulometric electrochemical detection. *J Chromatogr B Biomed Appl* 657: 141–148.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

DiGregorio GJ, Ruch E (1980). Human whole blood and parotid saliva concentrations of oral and intramuscular promethazine. *J Pharm Sci* 69: 1457–1459.

Fox AR, McLoughlin DA (1993). Rapid, sensitive high-performance liquid chromatographic method for the quantification of promethazine in human serum with electrochemical detection. *J Chromatogr* 631: 255–259.

Liu J, Stewart JT (1997). Quantitation of promethazine enantiomers in human serum using a chiralcel OJ-R column and mixed-mode disc solid-phase extraction. *J Pharm Biomed Anal* 16: 303–309.

Taylor G, Houston JB (1982). Simultaneous determination of promethazine and two of its circulating metabolites by high-performance liquid chromatography. *J Chromatogr* 230: 194–198.

Taylor G *et al.* (1983). Pharmacokinetics of promethazine and its sulphoxide metabolite after intravenous and oral administration to man. *Br J Clin Pharmacol* 15: 287–293.

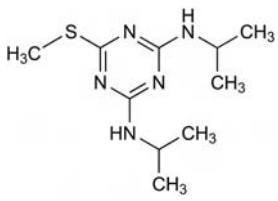
Vanapalli SR *et al.* (2001). A liquid chromatographic method for the simultaneous determination of promethazine and three of its metabolites in plasma using electrochemical and UV detectors. *J Chromatogr Sci* 39: 70–72.

Wallace JE *et al.* (1981). Determination of promethazine and other phenothiazine compounds by liquid chromatography with electrochemical detection. *Anal Chem* 53: 960–962.

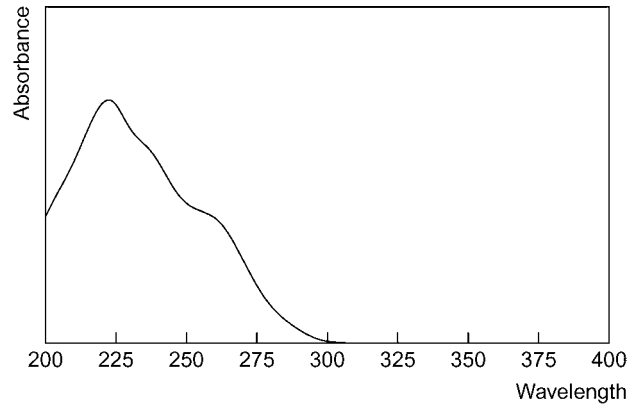
Prometryne

Herbicide
$C_{10}H_{19}N_5S$ = 241.4
CAS—7287-19-6

IUPAC Name 6-Methylsulfanyl-2-*N*,4-*N*-di(propan-2-yl)-1,3,5-triazine-2,4-diamine
Synonyms *N*,*N*′-Bis(1-methylethyl)-6-methylthio-1,3,5-triazine-2,4-diamine; prometryn.
Proprietary Names *Caparol*; *Gesagard*.



Chemical Properties A white crystalline solid. Mp 118° to 120°. Practically insoluble in water; readily soluble in organic solvents. pK_a 4.1. Log *P* (octanol/water), 3.5.
Thin-layer Chromatography System TA—R_f 0.76; system TX—R_f 0.32; system TY—R_f 0.31 (Dragendorff spray, positive).
Gas Chromatography System GA—RI 1930.
High Performance Liquid Chromatography System HAA—retention time 21.9 min.

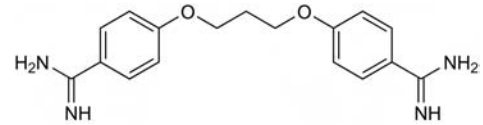


Infrared Spectrum Principal peaks at wavenumbers 1510, 1592, 1171, 1299, 809, 1217 cm^{-1} (KBr disk).

Propamidine

Antibacterial, Antifungal
$C_{17}H_{20}N_4O_2$ = 312.4
CAS—104-32-5

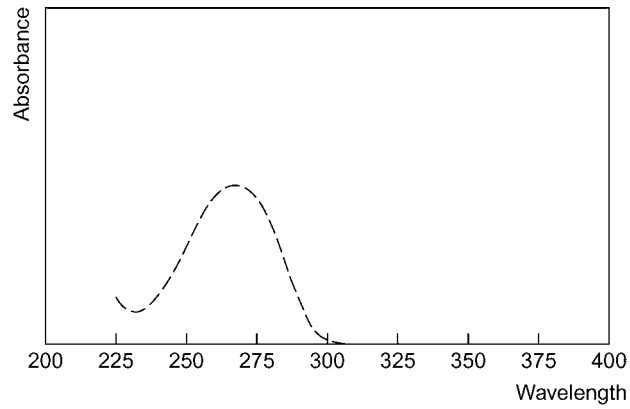
IUPAC Name 4-[3-(4-Carbamimidoylphenoxy)propoxy]benzenecarboximidamide
Synonym 4,4′-[1,3-Propanediylbis(oxy)]bisbenzenecarboximidamide



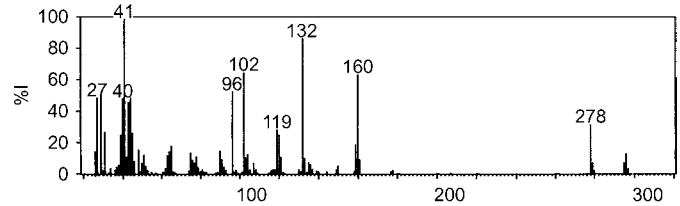
Chemical Properties Log *P* (octanol/water), 1.8.

Propamidine Isetionate
$C_{17}H_{20}N_4O_2,2C_2H_6O_4S$ = 564.6
CAS—140-63-6
Synonym Propamidine isethionate
Proprietary Name *Brolene*
Chemical Properties A white, granular, hygroscopic powder. Mp 235°. Soluble 1 in 5 of water and 1 in 33 of ethanol; practically insoluble in organic solvents.

Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.01; system TC—R_f 0.01; system TL—R_f 0.01 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Ethanol—267 nm (A^1_1=1224b).



Infrared Spectrum Principal peaks at wavenumbers 1200, 1219, 1613, 1048, 1280, 1495 cm^{-1} (propamidine isetionate, KBr disk).
Mass Spectrum Principal ions at *m/z* 41, 132, 102, 160, 96, 29, 27, 44.

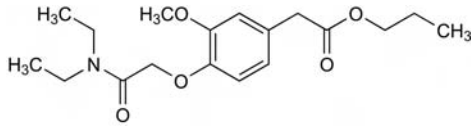


Uses Propamidine isetionate is used in concentrations of 0.1 to 0.15%.

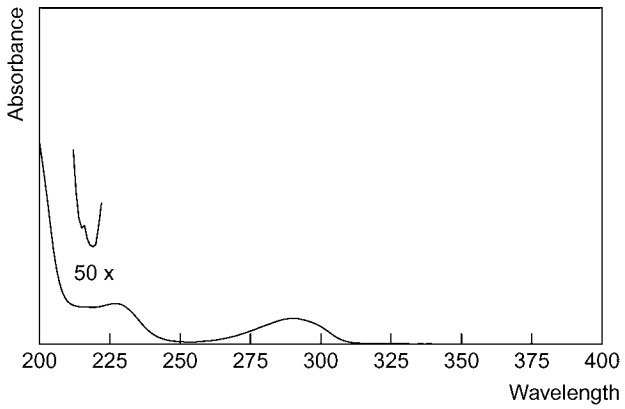
Propanidid

Anaesthetic (General)
$C_{18}H_{27}NO_5$ = 337.4
CAS—1421-14-3

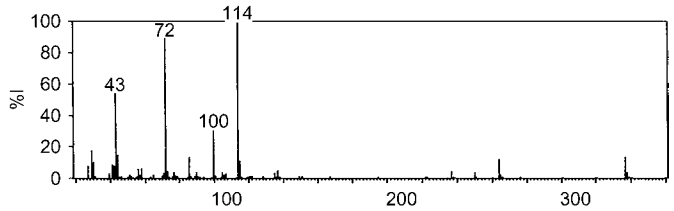
IUPAC Name Propyl 2-[4-[2-(diethylamino)-2-oxoethoxy]-3-methoxyphenyl] acetate
Synonym 4-[2-(Diethylamino)-2-oxoethoxyl]-3-methoxybenzeneacetic acid propyl ester
Proprietary Names *Epontol*; *Inductol*; *Panitol*; *Sombrevin*.



Chemical Properties A pale greenish-yellow, hygroscopic, viscous liquid. Bp 210° to 212°. Very slightly soluble in water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 2.4.
Colour Tests Aromaticity (method 2)—yellow/orange; Liebermann's test—black.
Thin-layer Chromatography System TA—R_f 0.66; system TB—R_f 0.20; system TC—R_f 0.70; system TE—R_f 0.72; system TL—R_f 0.55; system TAE—R_f 0.80 (Dragendorff spray, positive).
Gas Chromatography System GA—RI 2433.
Ultraviolet Spectrum Ethanol—280 nm (A^1_1=82a).



Infrared Spectrum Principal peaks at wavenumbers 1258, 1508, 1637, 1142, 1724, 1220 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 114, 72, 43, 100, 29, 44, 337, 86.



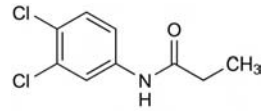
Disposition in the Body Rapidly metabolised by hydrolysis and demethylation to inactive metabolites which are excreted in the urine. About 90% of a dose is excreted in the urine in 2 h and up to 6% is eliminated in the faeces.
Therapeutic Concentration
Following IV administration of 7 mg/kg to 10 subjects, a mean serum concentration of about 14 mg/L was reported at 1 min [Doenicke *et al.* 1968].
Half-life Plasma half-life, about 0.2 h.
Protein Binding About 75%.
Dose Usually 5 to 10 mg/kg IV.

Doenicke A *et al.* (1968). Experimental studies of the breakdown of Epontol: determination of propanidid in human serum. *Br J Anaesth* 40: 415–428.

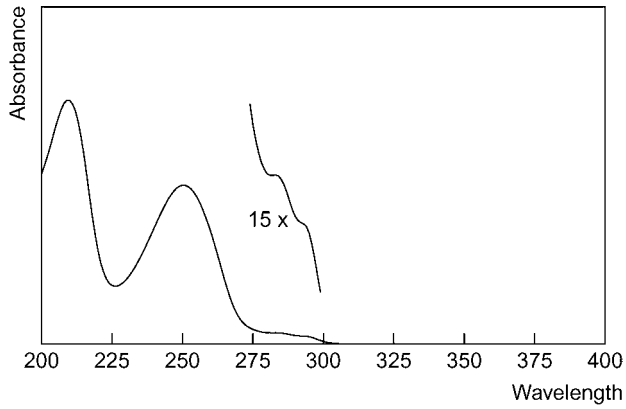
Propanil

Herbicide, Nematocide
$C_9H_9Cl_2NO$ = 218.1
CAS—709-98-8

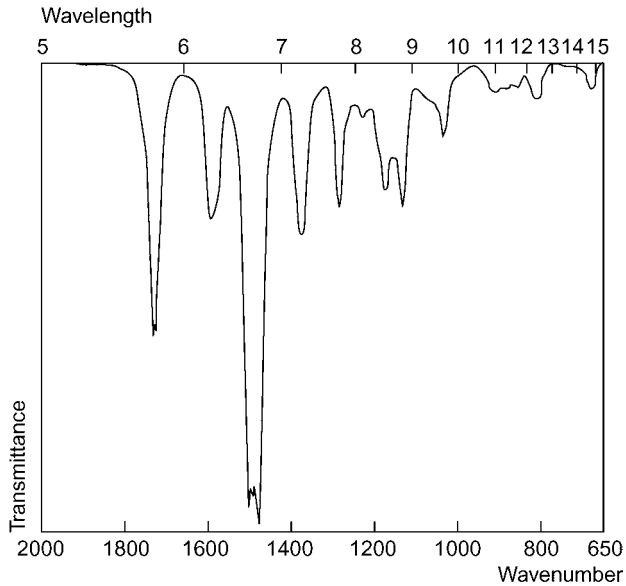
IUPAC Name *N*-(3,4-Dichlorophenyl)propanamide
Synonyms Bay 30130; Cekupropanil; DCPA; DPA; FW-734; S10145.
Proprietary Names *Arrosol*; *ChemRice*; *Dropaven*; *Erban*; *Herbax*; *Propanex*; *Riselect*; *Rogue*; *Stam*; *Stampede*; *Strel*; *Surcopur*; *Surpur*; *Vertac*.



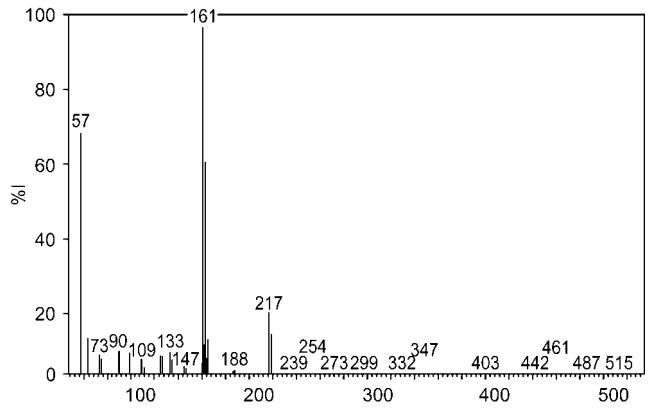
Chemical Properties A white crystalline solid. Mp 91° to 93° (the technical state is brownish in colour). Soluble in water (19.73 mg/L at 25°), toluene, isopropanol and xylene, and very soluble in benzene (70 g/L at 25°), ethanol (1100 g/L at 25°), acetone (1700 g/L at 25°), cyclohexane (350 g/L at 20°) and methyl ethyl ketone (250 g/L at 20°). Log *P* (octanol/water), 3.07.
Gas Chromatography System GK—RRT 0.94 (relative to caffeine).



Infrared Spectrum Principal peaks at wavenumbers 1729, 1282, 1590, 1136 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 161, 57, 163, 217, 219, 63, 165, 90.



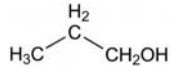
P

Disposition in the Body Propanil is absorbed into the bloodstream after ingestion and metabolised in the liver to aniline derivatives: 3,4-dichloroaniline and *N*-hydroxy-3,4-dichloroaniline. Metabolism is responsible for the formation of methaemoglobin. Propanil is lipid/fat soluble. It is recovered in faeces and urine.

Propanol

Solvent
C_3H_7OH = 60.1.
CAS—71-23-8

IUPAC Name Propan-1-ol
Synonyms Normal propyl alcohol; primary propyl alcohol; propyl alcohol.
Proprietary Name *Satinazid*

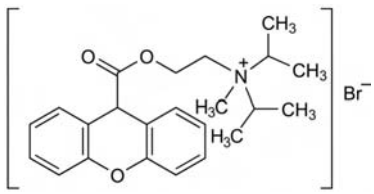


Chemical Properties A clear, colourless, flammable liquid. Weight per mL about 0.804 g. Bp about 96°. Miscible with water, ethanol, chloroform and ether. pK_a 16.1. Log *P* (octanol/water), 0.3.
Colour Test Potassium dichromate (method 2)—green.
Gas Chromatography System GA—RI 571; system GI—retention time 5.5 min.
Mass Spectrum Principal ions at *m/z* 31, 59, 42, 60, 27, 29, 45, 41.

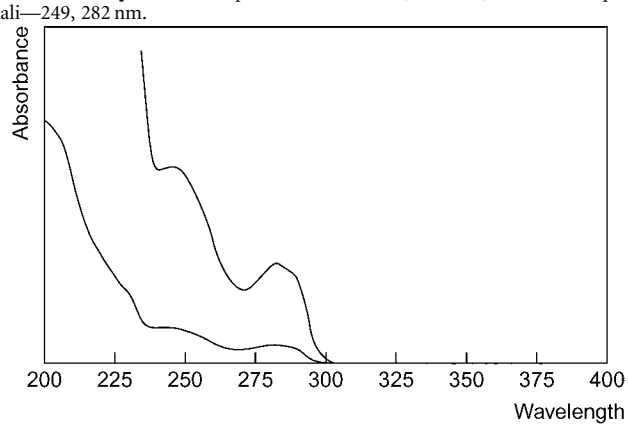
Propantheline Bromide

Anticholinergic
$C_{23}H_{30}BrNO_3$ = 448.4
CAS—298-50-0 (propantheline); 50-34-0 (bromide)

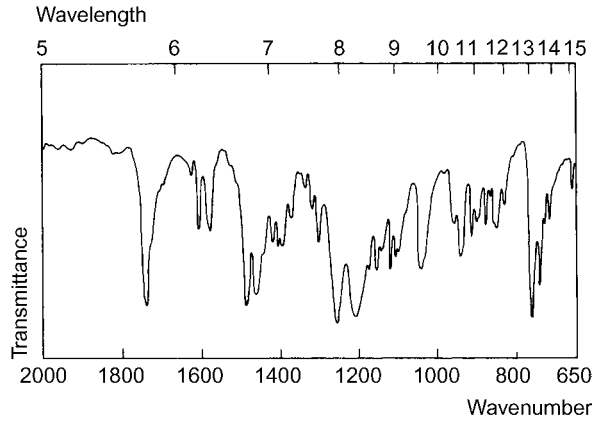
IUPAC Name Methyl-di(propan-2-yl)-[2-(9H-xanthene-9-carbonyloxy)ethyl] azanium bromide
Synonym *N*-Methyl-*N*-(1-methylethyl)-*N*-[2-[(9*H*-xanthen-9-ylcarbonyl)oxy] ethyl]-2-propanaminium bromide
Proprietary Names *Bropantil*; *Ercoril*; *Ercotina*; *Pantheline*; *Pro-Banthine*; *Propantel*; *Propanthel*.



Chemical Properties A white or yellowish-white powder. Mp 159° to 160°. Very soluble in water, ethanol and chloroform; practically insoluble in ether and benzene. Log *P* (octanol/water), 2.5.
Note The dust is irritant to mucous membranes.
Colour Tests The following tests are performed on the nitrate: Mandelin's test—orange; Marquis test—orange.
Thin-layer Chromatography System TA—propantheline bromide R_f 0.04; system TB—propantheline bromide R_f 0.00; system TC—propantheline bromide R_f 0.04; system TE—propantheline bromide R_f 0.04, xanthanoic acid R_f 0.05; system TF—xanthanoic acid R_f 0.08; system TL—R_f 0.00; system TAE—propantheline bromide R_f 0.03, xanthanoic acid R_f 0.87; system TAF—propantheline bromide R_f 0.31; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.20 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 4.4; system HX—RI 454 (propantheline bromide), RI 499 (xanthanoic acid).
Ultraviolet Spectrum Aqueous acid—243 (A^1_1=103a), 281 nm; aqueous alkali—249, 282 nm.



Infrared Spectrum Principal peaks at wavenumbers 1255, 761, 1208, 1733, 745, 1158 cm^{-1} (KBr disk).



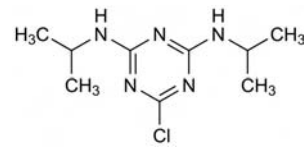
Mass Spectrum Principal ions at *m/z* 86, 181, 43, 44, 41, 114, 42, 152 (propantheline).
Quantification
Plasma **GC–MS** Limit of detection, 5 µg/L [Ford *et al.* 1977].
Serum **HPLC** UV detection. Limit of detection, 2 µg/L [Moses *et al.* 1983].
Urine **GC–MS** Limit of detection, 10 µg/L [Ford *et al.* 1977].
MS Limit of detection, 20 to 150 µg/L for propantheline bromide and other quaternary ammonium compounds [Nisikawa *et al.* 1991].
Disposition in the Body Poorly absorbed after oral administration due to decomposition in the small intestine. It undergoes extensive metabolism mainly by hydrolysis and glucuronide conjugation; aromatic hydroxylation also occurs. About 50 to 65% of an oral dose is excreted in the urine in 24 h with <10% as unchanged drug. The major urinary metabolite is xanthanoic acid glucuronide conjugate; other metabolites include free xanthanoic acid, hydroxyxanthanoic acid, propantheline and hydroxypropantheline.
Therapeutic Concentration Following single oral doses of 30 mg and 60 mg to 6 subjects, peak plasma concentrations of 0.0 to 0.038 mg/L (mean 0.024) and 0.033 to 0.162 mg/L (mean 0.06), respectively, were attained in about 0.5 to 1 h [Vose 1980].
Half-life Plasma half-life, 1 to 3 h.
Clearance Plasma clearance, about 20 mL/min/kg.
Dose Usually 75 mg daily; maximum of 120 mg daily.

Ford GC *et al.* (1977). The measurements of propantheline ion in biological fluids after administering propantheline bromide to man. *Biomed Mass Spectrom* 4: 94–97.
Moses DK *et al.* (1983). Food reduces the oral bioavailability of propantheline bromide in healthy subjects. *Br J Clin Pharmacol* 16: 758–759.
Nisikawa M *et al.* (1991). The analysis of quaternary ammonium compounds in human urine by direct inlet electron impact ionization mass spectrometry. *Forensic Sci Int* 51: 131–138.
Vose CW *et al.* (1980). Plasma levels and urinary excretion of orally administered propantheline bromide in man. *Eur J Drug Metab Pharmacokinet* 5: 29–34.

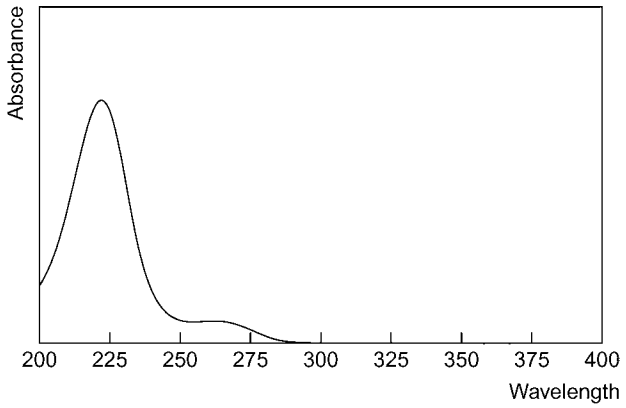
Propazine

Herbicide
$C_9H_{16}ClN_5$ = 229.7
CAS—139-40-2

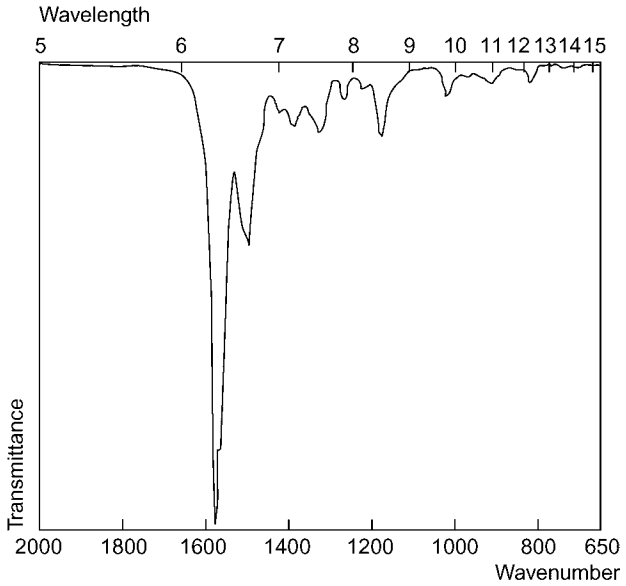
IUPAC Name 6-Chloro-2-*N*,4-*N*-di(propan-2-yl)-1,3,5-triazine-2,4-diamine
Synonyms 6-Chloro-*N*,*N*′-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine; G-30028; Geigy 30028.
Proprietary Names *Gesamil*; *Milocep*; *Milogard*; *Milo-Pro*; *Plantulin*; *Primatol P*; *Propazin*; *Propinex*; *Prozinex*.



Chemical Properties A colourless crystalline solid. Mp 213°. Practically insoluble in water (5 mg/L at 20°, 8.6 mg/L at 22°). Slightly soluble in benzene (6.2 g/kg at 20°), diethyl ether (5.0 g/kg at 20°), carbon tetrachloride (2.5 g/kg at 20°) and toluene (6.2 g/kg at 20°). pK_a 1.7 (21°). Log *P* (octanol/water), 2.93.
Gas Chromatography System GA—RI 1738.



Infrared Spectrum Principal peaks at wavenumbers 1571, 1496, 1173, 1323 cm^{-1}.

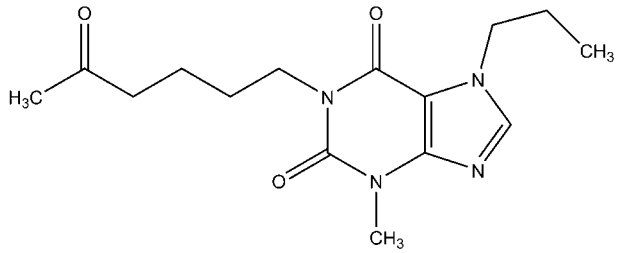


Mass Spectrum Principal ions at *m/z* 58, 214, 229, 43, 172, 187, 216, 69.
Disposition in the Body Propazine is readily absorbed into the bloodstream after ingestion and metabolised in the body. It is excreted in both urine (≈65%) and faeces (25%).
Toxicity Slightly toxic by ingestion.

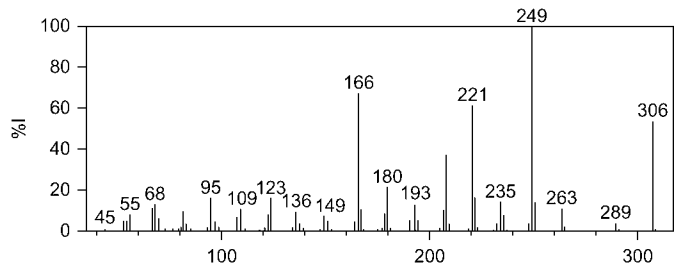
Propentofylline

Adenosine A_1 Antagonist, Nootropic, Xanthine Derivative
$C_{15}H_{22}N_4O_3$ = 306.4
CAS—55242-55-2

IUPAC Name 3-Methyl-1-(5-oxohexyl)-7-propylpurine-2,6-dione
Synonyms Albert-285; 3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl-1*H*-purine-2,6-dione; HOE-285; HWA-285; 3-methyl-1-(5-oxohexyl)-7-propylxanthine; 1-(5′-oxohexyl)-3-methyl-7-propyl-xanthine; propentofilina; propentofyllin; propentofylliini; propentofyllinum.
Proprietary Names *Hextol*; *Karsivan*.



Chemical Properties Mp 69° to 70°. Soluble in water, ethanol and DMSO. Log *P* (octanol/water), 1.52 [Meylan, Howard 1995].
Mass Spectrum Principal ions at *m/z* 249, 166, 221, 306 (3-Methyl-1-(5-hydroxyhexyl)-7-propylxanthine); 166, 209, 221, 180, 308, 123, 95, 45.



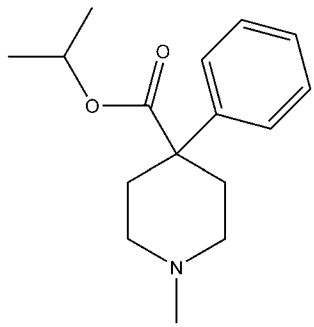
Quantification
Plasma **GC** Column: Cross-linked 5% phenylmethylsilicone (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 1.08 mL/min. Temperature programme: 180° to 220° at 10°/min to 280° at 3°/min. NPD. Retention time: 20.6 min. Limit of detection, 5 µg/L [Kwon *et al.* 1998].
GC-MS Column: Cross-linked 5% phenylmethylsilicone (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 1.08 mL/min. Temperature programme: 180° to 220° at 10°/min to 280° at 3°/min. EI ionisation at 70 eV. Retention time: 20.6 min. Limit of detection, 5 µg/L [Kwon *et al.* 1998].
Serum **HPLC** Column switching system. UV detection (λ = 270 nm). Retention time: ~21 min. Limit of detection, 0.13 µmol/L [Kuroda *et al.* 1999].
Disposition in the Body Undergoes extensive first-pass metabolism, forming 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine, the main active metabolite, and at least 2 further minor metabolites.
Therapeutic Concentration
Ten healthy young volunteers (mean age 22 years) were administered 200 mg oral propentofylline in the fasted state. Mean peak plasma concentrations for propentofylline and its hydroxyl metabolite were 828 and 269 µg/L attained at 2.2 and 2.5 h, respectively [Kwon *et al.* 1998].
Half-life Approximately 45 min.
Dose Has been investigated in cerebrovascular disorders including dementia. It is also used in veterinary medicine as a vasodilator (peripheral and cerebral). For the treatment of cognitive dysfunction in dogs, a daily dose of ~10 mg/kg bodyweight (range 8–12) is administered, either in a single dosage unit or preferably in multiple dosage units, which accumulate to 1–20 mg/kg bodyweight per day.

Kuroda N *et al.* (1999). Simple and rapid high-performance liquid chromatography analysis of propentofylline and its main metabolites in serum using a direct injection technique. *Biomed Chromatogr* 13: 340–343.
Kwon OS *et al.* (1998). Pharmacokinetics of propentofylline and the quantitation of its metabolite hydroxypropentofylline in human volunteers. *Arch Pharm Res* 21: 698–702.
Meylan W, Howard P (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Properidine

Narcotic Analgesic
$C_{16}H_{23}NO_2$ = 261.4
CAS—561-76-2

IUPAC Name Propan-2-yl methyl-4-phenylpiperidine-4-carboxylate
Synonyms DEA Number 9644; EINECS 209-222-4; gevelina; ipropethidine; isopedine; isopropyl 1-methyl-4-phenylisonipecotate; isopropyl 1-methyl-4-phenylpiperidine-4-carboxylate.



Chemical Properties Log *P* (octanol/water) 3.97 [Meylan, Howard 1995]; 3.1 [National Institutes of Health 2008]. Properidine is extracted by organic solvents from aqueous alkaline solutions.

Properidine Hydrochloride
$C_{16}H_{23}NO_2$,HCl = 297.9
Synonym NU 896
Proprietary Name *Dolisina B*

Colour Tests Sulfuric acid–formaldehyde test—dull orange (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.53 (location reagent acidified iodoplatinate spray, positive reaction).

P

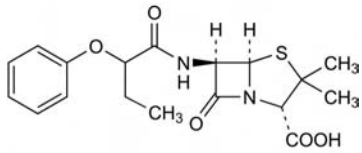
Gas Chromatography System G2/225—retention time 0.75 relative to diphenhydramine; system G4—retention time 0.65 relative to diphenhydramine.
Ultraviolet Spectrum Aqueous acid (0.1 N sulfuric acid)—251, 257, 263 nm.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health, (2008). *Ipropethidine*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=62373&loc=ec_rcs. (accessed 30 June 2008).

Propicillin

Antibacterial
$C_{18}H_{22}N_2O_5S$ = 378.4
CAS—551-27-9

IUPAC Name (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxybutanoylamino)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Synonyms (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(1-oxo-2-phenoxybutyl) amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; α-phenoxypropylpenicillin.

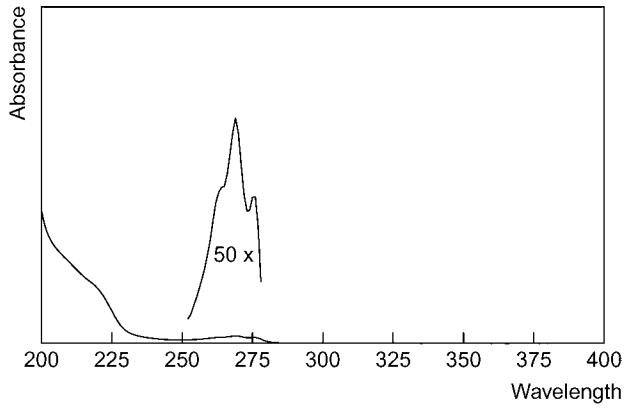


Chemical Properties pK_a 2.7 (25°). Log *P* (octanol/water), 2.6.

Propicillin Potassium

$C_{18}H_{21}KN_2O_5S$ = 416.5
CAS—1245-44-9
Synonyms Potassium α-phenoxypropylpenicillin; BRL-284; PA-248.
Proprietary Names *Baycillin*; *Oricillin*; *Propibay*; *Ultrapen*.
Chemical Properties A white, hygroscopic, finely crystalline powder. Mp 195° to 197°, with decomposition. Soluble 1 in about 1 of water, 1 in about 25 of ethanol and 1 in 65 of dehydrated alcohol; practically insoluble in chloroform and ether.

Ultraviolet Spectrum Propicillin potassium: water—269 (A^1_1=27b), 276 nm (A^1_1=21.5b).

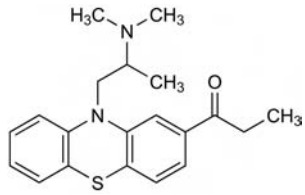


Infrared Spectrum Principal peaks at wavenumbers 1600, 1757, 1669, 1224, 1314, 750 cm^{-1} (propicillin potassium, KBr disk).
Dose The equivalent of 0.5 to 1.5 g of propicillin daily.

Propiomazine

Antihistamine, Sedative
$C_{20}H_{24}N_2OS$ = 340.5
CAS—362-29-8

IUPAC Name 1-[10-[2-(Dimethylamino)propyl]phenothiazin-2-yl]propan-1-one
Synonyms 1-[10-[2-(Dimethylamino)propyl]-10*H*-phenothiazin-2-yl]-1-propanone; Wy-1359.



Chemical Properties pK_a 6.6. Log *P* (octanol/water), 4.8.

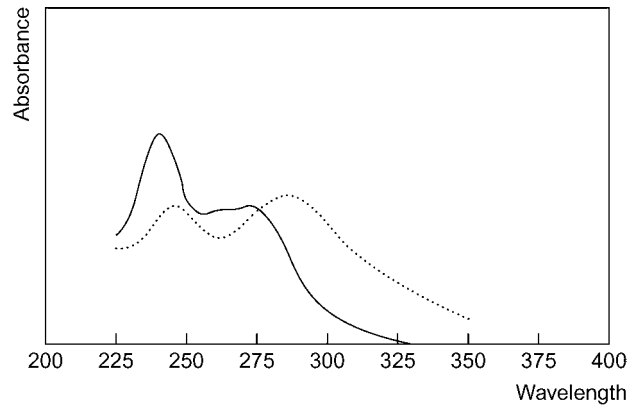
Propiomazine Hydrochloride

$C_{20}H_{24}N_2OS,HCl$ = 376.9
CAS—1240-15-9
Proprietary Name *Largon*
Chemical Properties A yellow powder. Soluble 1 in <1 of water, 1 in 6 of ethanol and 1 in 2 of chloroform; practically insoluble in ether and benzene.

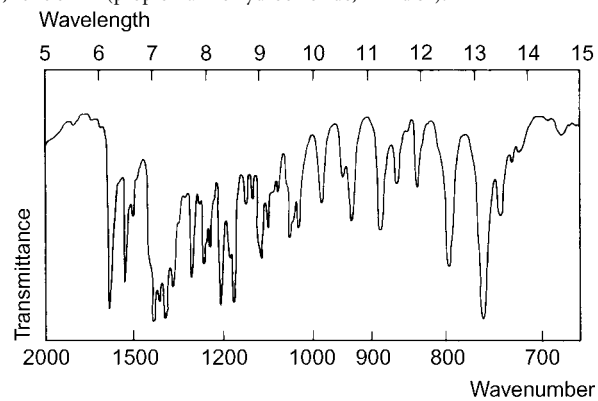
Propiomazine Maleate

$C_{20}H_{24}N_2OS,C_4H_4O_4$ = 456.6
CAS—3568-23-8
Synonym Propiomazine hydrogen maleate
Proprietary Name *Propavan*
Chemical Properties A yellow microcrystalline powder. Mp 160° to 161° (from propan-2-ol). Soluble 1 in 500 of water, 1 in 60 of ethanol and 1 in 140 of dehydrated alcohol.

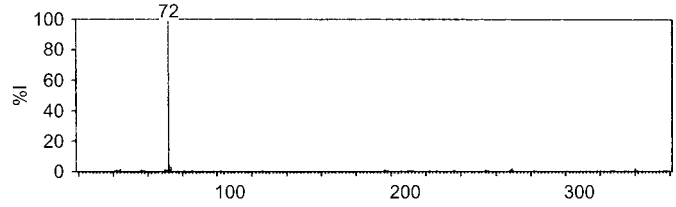
Colour Tests Formaldehyde-sulfuric acid—blue–violet; Forrest reagent—red; FPN reagent—pink–orange→red→fades; Liebermann's reagent—violet; Mandelin's test—violet; Marquis test—red–violet.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.34; system TC—R_f 0.42; system TE—R_f 0.68; system TL—R_f 0.26; system TAE—R_f 0.30; system TAF—R_f 0.52; system TAJ—R_f 0.34; system TAK—R_f 0.16; system TAL—R_f 0.81 (location under UV light, pink fluorescence; Dragendorff spray, positive; FPN reagent, yellow; acidified iodoplatinate solution, positive; Marquis reagent, red).
Gas Chromatography System GA—RI 2738; system GF—RI 3225.
High Performance Liquid Chromatography System HA—*k* 2.1; system HX—RI 440; system HY—RI 359; system HAX—retention time 14.1 min; system HAY—retention time 7.1 min.
Ultraviolet Spectrum Aqueous acid—241 (A^1_1=749a), 273, 360 nm; aqueous alkali—243, 283 nm.



Infrared Spectrum Principal peaks at wavenumbers 754, 1653, 1221, 1186, 1575, 1316 cm^{-1} (propiomazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 73, 340, 269, 197, 71, 70, 56.



Quantification

Plasma **GC** AFID. Limit of detection, 10 to 20 µg/L for propiomazine and *N*-monodesmethylpropiomazine [Hartvig *et al.* 1980].

Disposition in the Body Slowly absorbed after oral administration. Metabolised by *N*-demethylation.

Therapeutic Concentration

After a single oral dose of 50 mg to one subject, a peak serum concentration of about 0.05 mg/L was attained in 2 to 3 h. In several subjects, low concentrations (<0.01 mg/L) of *N*-monodesmethylpropiomazine were also observed [Hartvig *et al.* 1981].

Bioavailability About 33%.

Half-life Plasma half-life, 2 to 15 h (mean 9).

Volume of Distribution About 2 to 3 L/kg.

Clearance Plasma clearance, 3 to 10 mL/min/kg (mean 5).

Dose The equivalent of 25 to 50 mg of propiomazine daily, orally.

Hartvig P *et al.* (1980). Determination of propiomazine and its N-demethyl metabolite in plasma by gas chromatography with alkali flame ionization detection. *J Chromatogr* 183: 229–233.
Hartvig P *et al.* (1981). *Curr Ther Res* 29: 351–362.

Propofol

Anaesthetic

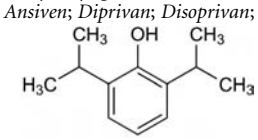
$C_{12}H_{18}O$ = 178.3

CAS—2078-54-8

IUPAC Name 2,6-Di(propan-2-yl)phenol

Synonyms 2,6-Bis(1-methylethyl)phenol; ICI-35868; disoprofol.

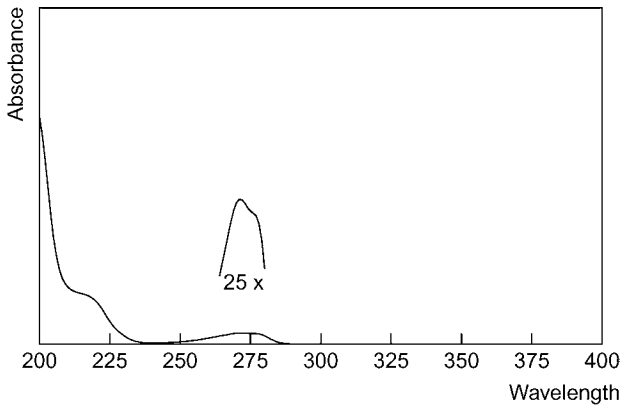
Proprietary Names *Ansiven*; *Diprivan*; *Disoprivan*; *Rapinovet*.



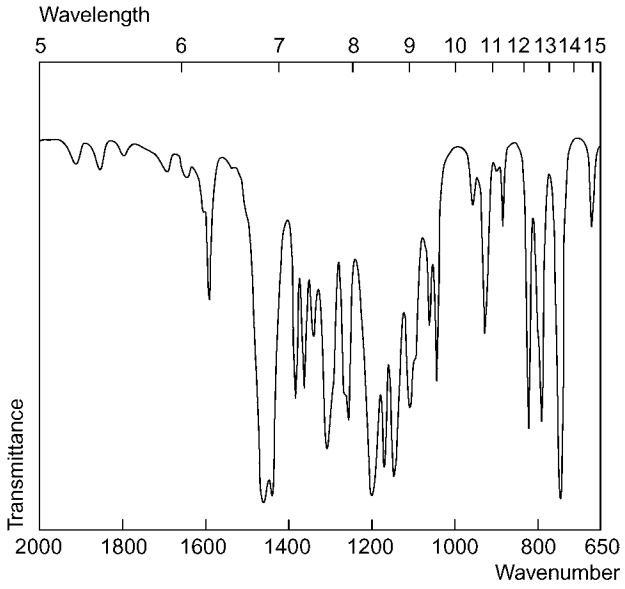
Chemical Properties Mp 19°. Very slightly soluble in water. pK_a 11.1. Log *P* (octanol/water), 3.79. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

High Performance Liquid Chromatography System HZ—retention time 35.0 min; system HAX—retention time 10.1 min; system HAY—retention time 15.2 min.

Ultraviolet Spectrum Aqueous acid—271 nm; basic—240, 291 nm.



Infrared Spectrum Principal peaks at wavenumbers 1461, 1202, 748 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 163, 178, 91, 117, 41, 164, 77, 121 (propofol); *m/z* 235, 73, 250, 207, 161, 115, 128, 177 (TMS (tetramethylsilane) derivative).

Quantification

Blood **HPLC** Electrochemical detection. Limit of detection, 0.02 mg/L [Mazzi, Schinella 1990].

Plasma **GC–MS** Limit of detection, 0.02 µg/L [Stetson *et al.* 1993].

Serum **HPLC** UV detection (λ=270 nm). Limit of detection, 0.1 mg/L [Pavan *et al.* 1992]. Coulometric detection. Limit of detection, 0.08 mg/L [Uebel *et al.* 1990].

Disposition in the Body Propofol is rapidly absorbed and distributed from circulation into the tissues. Extensive tissue distribution occurs. It has a two-phase distribution, with the second phase being the slower phase when significant hepatic metabolism (conjugation) takes place before excretion via urine. Inactive conjugates of propofol are formed and its corresponding quinol. Also detected in urine is the unchanged drug, propofol glucuronide, 1-glucuronide, 4-glucuronide and the 4-sulfate conjugates of 2,6-diisopropyl 1,4-quinol. Approximately 2% of a dose is excreted in faeces. Propofol crosses the placental barrier and is also excreted in breast milk. It is extensively distributed and rapidly cleared from the body.

Therapeutic Concentration Sedation can be maintained at serum concentrations of 0.001 to 0.004 mg/L.

Seven male patients, 38 to 67 years old, admitted for oral or neck surgery, were administered a dose between 403 and 1800 mg to reach a state of anaesthesia. Doses were administered at a maintenance infusion rate of 3.5 and 6.1 mg/kg lean body mass/h and after 15 min of infusion, blood concentrations were relatively stable ranging between 1.5 and 2.5 mg/L [Morgan *et al.* 1990].

Toxicity The toxic blood concentration is 0.22 mg/L, as noted from abuse.

A 29-year-old female committed suicide by IV injection of 400 mg propofol. Postmortem concentrations were 0.22 mg/L in femoral blood and 1.4 mg/kg in liver. [Drummer 1992].

Half-life 2 to 4 min (first phase); 30 to 60 min (second phase); 3 to 12 h (terminal half-life).

Volume of Distribution Steady state, 171 to 349 L; elimination, 209 to 1008 L. Also reported as 2 to 12 L/kg and 60 L/kg.

Clearance Total, 94 to 139 L/h.

Protein Binding >95% (haemoglobin, erythrocytes and other serum proteins); hypoalbuminaemia may increase free fraction.

Dose Adults: Induction of anaesthesia: 1.5 to 2.5 mg/kg. Maintenance of anaesthesia: 25 to 50 mg repeated according to response, or 4 to 12 mg/kg/h. Children >3 years: 9 to 15 mg/kg/h. Sedation: 0.3 to 4.5 mg/kg/h.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Drummer OH (1992). A fatality due to propofol poisoning. *J Forensic Sci* 37: 1186–1189.
Mazzi G, Schinella M (1990). Simple and practical high-performance liquid chromatographic assay of propofol in human blood by phenyl column chromatography with electrochemical detection. *J Chromatogr* 528: 537–541.
Morgan DJ *et al.* (1990). Pharmacokinetics of propofol when given by intravenous infusion. *Br J Clin Pharmacol* 30: 144–148.
Pavan I *et al.* (1992). Monitoring propofol serum levels by rapid and sensitive reversed-phase high-performance liquid chromatography during prolonged sedation in ICU patients. *J Chromatogr Sci* 30(5): 164–166.
Stetson PL *et al.* (1993). Determination of plasma propofol levels using gas chromatography-mass spectrometry with selected-ion monitoring. *J Chromatogr* 620: 260–267.
Uebel RA *et al.* (1990). Electrochemical determination of 2,6-diisopropylphenol after high-performance liquid chromatography of extracts from serum. *J Chromatogr* 526: 293–295.

Propoxycaine

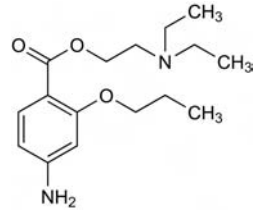
Anaesthetic (Local)

$C_{16}H_{26}N_2O_3$ = 294.4

CAS—86-43-1

IUPAC Name 2-Diethylaminoethyl 4-amino-2-propoxybenzoate

Synonym 4-Amino-2-propoxybenzoic acid 2-diethylaminoethyl ester



Chemical Properties pK_a 8.6. Log *P* (octanol/water), 1.5.

Propoxycaine Hydrochloride

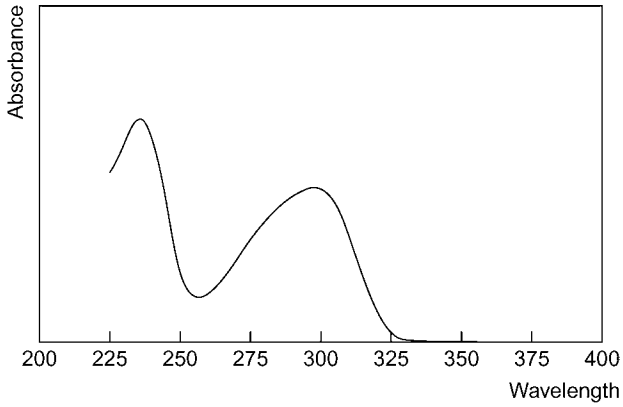
$C_{16}H_{26}N_2O_3$,HCl = 330.9

CAS—550-83-4

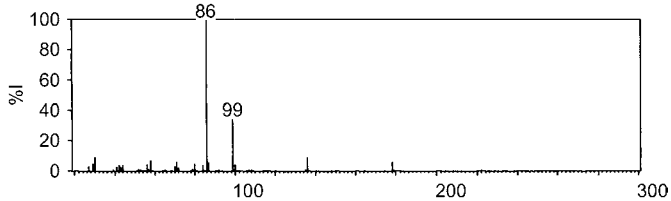
Proprietary Names It is an ingredient of *Ravocaine* and *Novocain*.

Chemical Properties A white crystalline powder which discolours on prolonged exposure to light and air. Mp 148° to 150°. Soluble 1 in 2 of water, 1 in 10 of ethanol and 1 in 80 of ether; practically insoluble in acetone and chloroform.

Colour Tests Coniferyl alcohol—orange; diazotisation—red; Mandelin's test—brown.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.03; system TC—R_f 0.33; system TL—R_f 0.28; system TAJ—R_f 0.11; system TAK—R_f 0.00; system TAL—R_f 0.45 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2335.
High Performance Liquid Chromatography System HQ—*k* 1.10.
Ultraviolet Spectrum Aqueous acid—235 (A^1_1=270b), 298 nm.



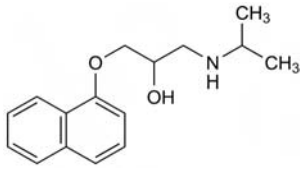
Infrared Spectrum Principal peaks at wavenumbers 1605, 1278, 1246, 1670, 1210, 1700 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 99, 136, 30, 58, 178, 87, 71.



Uses Propoxycaine hydrochloride has been used as a 0.5% solution.

Propranolol

β-*Adrenoceptor Antagonist*
$C_{16}H_{21}NO_2$ = 259.3
CAS—525-66-6; 13013-17-7 (±)
IUPAC Name 1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol

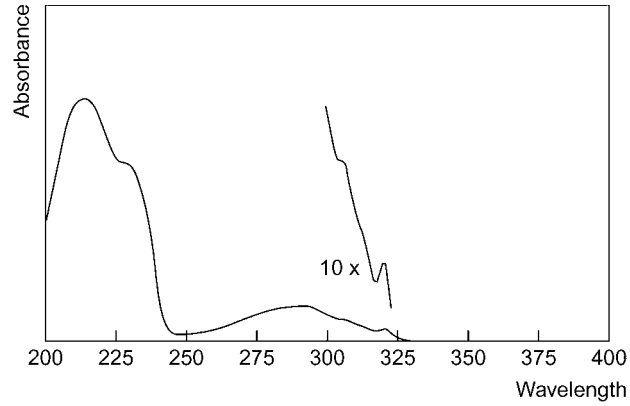


Chemical Properties Crystals. Mp 96° (crystals from cyclohexane). pK_a 9.5 (24°). Log *P* (octanol/water pH 7.4), 1.2. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

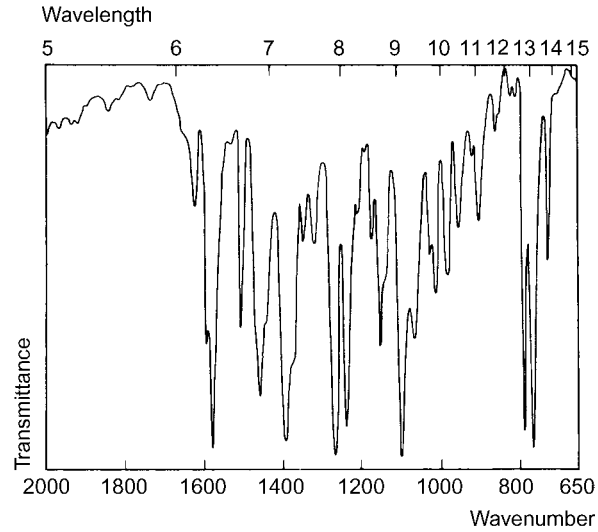
Propranolol Hydrochloride

$C_{16}H_{21}NO_2$,HCl = 295.8
CAS—318-98-93506-09-0 (±)
Synonyms AY-64043; ICI-45520; NSC-91523; propranololi hydrochloridum.
Proprietary Names *Adrexan*; *Angilol*; *Apsolol*; *Avlocardyl*; *Bedranol*; *Berkolol*; *Betachron*; *Beta-Prograne*; *Betadur CR*; *Cardinol*; *Dociton*; *Efektolol*; *Elbrol*; *Half Beta-Prograne*; *Half Betadur*; *Half Inderal*; *Indobloc*; *Hemipralon*; *Inderal*; *Lopranol LA*; *Obsidan*; *Probeta LA*; *Propabloc*; *Propanix*; *Prophylux*; *Proprnur*; *Syprol*; *Tiperal*. It is an ingredient of *Indereti*c; *Inderex*; *Inderide*.
Chemical Properties A white powder. Mp 163° to 164° (crystals from propan-1-ol). Soluble 1 in 20 of water and ethanol; slightly soluble in chloroform; practically insoluble in ether, benzene and ethyl acetate.

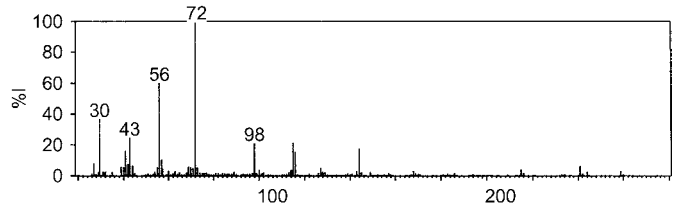
Colour Tests Mandelin's test—green; Marquis test—green.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.06; system TC—R_f 0.10; system TE—R_f 0.49; system TL—R_f 0.07; system TAE—R_f 0.21; system TAF—R_f 0.79; system TAJ—R_f 0.00; system TAK—R_f 0.05; system TAL—R_f 0.49 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—propranolol RI 2147, M (1-naphthol-) RI 1505, M (desamino-OH-) RI 2065; system GB—propranolol RI 2234, M (4-OH-) RI 2546, M (1-naphthol-) RI 1534.
High Performance Liquid Chromatography System HA—propranolol *k* 1.3, M (4-hydroxy-) *k* 1.1; system HX—RI 377; system HY—RI 299; system HZ—RT 3.7 min; system HAA—RT 13.1 min; system HAM—not detected; system HAV—*k* 2.3; system HAX—RT 9.2 min; system HAY—RT 4.9 min.
Ultraviolet Spectrum Aqueous acid—288 (A^1_1= 222a), 305, 319 nm; methanol—290 (A^1_1= 240a), 306 (A^1_1= 143a), 319 nm (A^1_1= 86a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1103, 1270, 772, 1580, 795, 1240 cm^{-1} (propranolol hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 72, 56, 30, 43, 98, 115, 144, 41 (propranolol); 72, 30, 116, 56, 43, 160, 41, 158 (4-hydroxypropranolol).



Quantification

Blood **GC-MS** Column: DB-1 (25 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 0.6 mL/min. Temperature programme: 140° for 2.5 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.01 mg/L [Black *et al.* 1996].

HPLC UV and ESI-ion trap MS detection [Buszewski *et al.* 2009]. Column: LiChrospher RP-8 ADS (25 × 4.6 mm i.d., 25 µm). Mobile phase: 10 mmol/L phosphate buffer (pH 3.0) : acetonitrile : methanol (70 : 15 : 15), flow rate 1.0 mL/min. DAD (λ= 280 nm). Limit of detection, 50 µg/L [Mislanova, Hutta 2001].

Plasma **GC** Column: OV7 phenyl methyl silicone with 20% phenyl (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature programme: 190° for 3 min to 270° at 3°/min. FID. Limit of detection, 50 µg/L [Quaglio *et al.* 1992]. ECD. Limit of detection, <1 µg/L [Kates, Jones 1977].

GC-MS Column: HP methyl silicone (12 m × 0.22 mm i.d., 0.33 µm) or phenyl methyl silicone (12 m × 0.22 mm i.d., 0.22 µm). Temperature programme: 200° to 300° at 3°/min. Limit of detection, 10 µg/L, limit of detection, with FID, 50 µg/L [Quaglio *et al.* 1993].

HPLC Column: Hypurity C_{18} (250 × 4.6 mm, i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8), flow rate 1.0 mL/min. DAD (λ= 220 nm). Limit of detection, 5–10 µg/L [Delamoye *et al.* 2004]. Column: Altex C_{18}. Mobile phase: acetonitrile : water : phosphoric acid : triethylamine (58 : 42 : 0.1 : 0.06). Fluorescence detection (λ_{ex}= 280 nm, λ_{em}= 325 nm) [Wu *et al.* 1997]. Column: Hypersil CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : aqueous acetic acid (1%) containing 0.2% triethylamine (35 : 65, pH 3.6), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}= 230 nm, λ_{em}= 340 nm). Retention time 9.5 min. Limit of quantification, 5 µg/L, limit of detection, 2 µg/L [Rekhi *et al.* 1995]. Column: β-cyclodextrin (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : absolute ethanol : glacial acetic acid : triethylamine (96 : 4 : 0.4 : 0.3), flow rate 1.0 mL/min. Retention time: 16–18 min. Fluorescence detection (λ_{ex}= 222 nm, λ_{em}= 340 nm). Limit of detection, ~1.5 µg/L [Pham-Huy *et al.* 1995]. See also Nation *et al.* [1978] and Spahn-Langguth *et al.* [1991].

LC-MS Column: MSpak GF-310 (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 at 4 min until 9.5 min), flow rate 0.55 mL/min. ESI, SRM acquisition mode. Limit of quantification, 10 µg/L. Limit of detection, 3 µg/L [Umezawa *et al.* 2008]. Column: Chirobiotic V (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetic acid : triethylamine (100 : 0.05 : 0.04 to 100 : 0.05 : 0.1 within 25 min to 100 : 0.05 : 0.04 for 5 min), flow rate 1 mL/min. APCI, SIM acquisition mode. Limit of detection, 0.03 µg/L [Siluk *et al.* 2007]. Column: LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (80 : 20 for 2.2 min to 60 : 40 at 2.21 min until 5.5 min to 10 : 90 at 5.51 min until 8 min to 80 : 20 at 8.01 min until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min at 5.51 mL/min to 6.5 mL/min at 8.01 min to 0.4 mL/min at 9.51 min. APCI, positive ion mode, SIM acquisition mode. Limit of detection, 0.01 mg/L [Maurer *et al.* 2004].

Note For a radioimmunoassay for the quantification of propranolol see Mould *et al.* [1981].

Serum **HPLC** Limit of detection, 4 µg/L [Rumiantsev, Ivanova 1995]. See Plasma [Rekhi *et al.* 1995].

LC-MS Column: Hypersil BDS-C_{18}. Mobile phase: acetonitrile : methanol : water : acetic acid (15 : 15 : 70 : 1). Limit of detection, 0.1 to 1.2 µg/L [Kataoka *et al.* 1999].

Urine **GC-MS** Column: HP-5 (14 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 30° for 2 min to 220° at 15°/min for 1 min to 260° at 5°/min to 320° at 15°/min for 3 min. Limit of detection, 40 µg/L [Hartonen, Riekkola 1996]. See Blood [Black *et al.* 1996]. Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° to 260° at 10°/min. EI ionisation, SIM acquisition. Limit of detection, 1 µmol/L for the acetyl conjugate of *N*-desisopropylpropranolol (1-acetamino-3-(1-naphthyloxy)-2-propanol) [Noda *et al.* 1995].

HPLC Column: reversed phase. Mobile phase: acetonitrile : methanol : 0.05% trifluoroacetic acid. DAD [Baranowska *et al.* 2009]. See Blood [Buszewski *et al.* 2009]. See Plasma [Pham-Huy *et al.* 1995]. Column: Chiracel OD (250 × 4.6 mm i.d., 10 µm). Mobile phase: hexane : ethanol : diethylamine (91 : 9 : 0.1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 240 nm, λ_{em}= 320 nm). Limit of detection, 0.1 mg/L [Herring, Johnson 1993]. See Plasma [Spahn-Langguth *et al.* 1991]. See also Pritchard *et al.* [1979].

LC-MS See Serum [Kataoka *et al.* 1999]

Note For a review of HPLC methods for propranolol and other β-blockers, see Egginger *et al.* [1993].

Disposition in the Body Propranolol is rapidly and almost completely absorbed after oral administration but undergoes extensive first-pass metabolism with considerable intersubject variation. It is very soluble in lipids. It is rapidly distributed throughout the body with highest levels being found in the lungs, liver, kidney, brain and heart. It crosses the placenta; it is excreted in breast milk. Metabolic reactions include ring hydroxylation, *N*-demethylation, oxidative deamination and conjugation. In 48 h, ~10% of a dose is excreted in the urine as propranolol glucuronide and <4% as unchanged drug. Metabolites found in the urine, in free and conjugated forms, include naphthoxylactic acid (14–22% of the dose in 48 h) and the active metabolite, 4-hydroxypropranolol and its glucuronide (5–8% of the dose); propranolol glycol, *N*-desisopropylpropranolol, and naphthoxyacetic acid each account for <2% of the dose and 4-hydroxypropranolol sulfate accounts for ~18% of the dose. After IV doses, a greater proportion of naphthoxylactic acid (up to 40% of the dose) is excreted in the urine and 4-hydroxypropranolol is not found in the urine. <5% of a dose is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 0.05–1 mg/L. However, there is considerable intersubject variation and a correlation between plasma propranolol concentration and therapeutic effect has not been established.

After a single oral dose of 80 mg propranolol administered to 6 subjects, the following mean peak concentrations of drug and metabolites were found in the plasma after 2 h: propranolol 0.05 mg/L, naphthoxylactic acid 0.53 mg/L, 4-hydroxypropranolol 0.009 µg/mL, naphthoxyacetic acid 0.024 µg/mL, propranolol glycol 0.005 mg/L [Schneck *et al.* 1980].

Following oral administration of 160 mg twice a day to 12 subjects, mean steady-state plasma concentrations of 0.38 mg/L propranolol and 0.03 mg/L 4-hydroxypropranolol were reported [Charles *et al.* 1982].

Administration of a single 40-mg dose of propranolol hydrochloride either sublingually or orally to 14 severely hypertensive patients produced mean peak plasma concentrations of 0.147 mg/L at 34 min and 0.041 mg/L at 52 min [Mansur *et al.* 1998].

Toxicity Toxic effects have been associated with plasma concentrations >2 mg/L and fatalities with concentrations >4 mg/L.

In a suicide attributed to propranolol overdose, the following concentrations were found: blood 14 mg/L, brain 67 µg/g, liver 171 µg/g and urine 0.9 mg/L; alcohol was also present [Kristinsson, Johannesson 1977].

In a fatality resulting from the ingestion of 9.6 g of propranolol, in which death occurred 8 h after ingestion, the following postmortem tissue concentrations were reported: blood 20 mg/L, brain 6 µg/g, kidney 26 µg/g, liver 10 µg/g and lung 69 µg/g [Jones *et al.* 1982].

In a postmortem performed 5 days after a 60-year-old man was found dead near an empty box of Inderal, the following propranolol concentrations were reported: blood 7 mg/L, urine 0.28 mg/L and gastric contents 2000 mg/L; death was attributed to cardiocirculatory failure resulting from the suicidal ingestion of a massive dose of Inderal [Fucci, Offidani 2000].

Bioavailability 10–50% (mean, 30).

Half-life Plasma half-life, 3–6 h (dose-dependent elimination has been reported).

Volume of Distribution ≈4 L/kg.

Clearance Plasma clearance, ~10–20 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.3.

Protein Binding ≈90%.

Note For a review of the pharmacokinetics of long-acting propranolol, see Nace, Wood [1987]; see also Routledge, Shand [1979] and Silber *et al.* [1983]

Dose Propranolol hydrochloride 30 to 400 mg daily.

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Kristinsson J, Johannesson T (1977). A case of fatal propranolol intoxication. *Acta Pharmacol Toxicol (Copenh)* 41: 190–192.

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Mould GP *et al.* (1981). A propranolol radioimmunoassay and its use in the study of its pharmacokinetics following low doses. *Biopharm Drug Dispos* 2: 49–57.

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Nation RL *et al.* (1978). High-pressure liquid chromatographic method for the simultaneous quantitative analysis of propranolol and 4-hydroxypropranolol in plasma. *J Chromatogr* 145: 429–436.

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Siluk D *et al.* (2007). HPLC-atmospheric pressure chemical ionization mass spectrometric method for enantioselective determination of *R,S*-propranolol and *R,S*-hyoscyamine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 859: 213–221.
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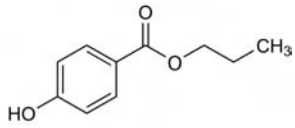
Propyl Hydroxybenzoate

Preservative
$C_{10}H_{12}O_3$ = 180.2
CAS—94-13-3

IUPAC Name Propyl 4-hydroxybenzoate
Synonyms 4-Hydroxybenzoic acid propyl ester; propagin; propyl parahydroxybenzoate; propylis oxybenzoas; propylparaben.
Proprietary Names *Nipasol M*; *Propyl Parasept*; *Solbrol P.* It is an ingredient of *Nipasept* and *Nipastat.*

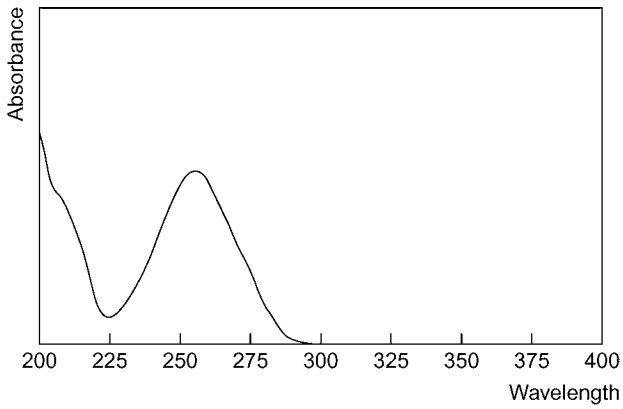


Chemical Properties Colourless crystals or a white crystalline powder. Mp 96° to 97°. Soluble 1 in 2500 of cold water, 1 in 400 of boiling water, 1 in 1.5 of ethanol, 1 in 4 of chloroform and 1 in 3 of ether. pK_a 8.4 (22°). Log *P* (octanol/water), 3.0.

Sodium Propyl Hydroxybenzoate

$C_{10}H_{11}NaO_3$ = 202.2
CAS—35285-69-9
Synonyms Sodium propylparaben; soluble propyl hydroxybenzoate.
Proprietary Name *Nipasol M Sodium*
Chemical Properties A white, hygroscopic, crystalline powder. Soluble 1 in 1 of water, 1 in 50 of ethanol and 1 in 2 of ethanol (50%).

Thin-layer Chromatography System TE—R_f 0.53; system TF—R_f 0.67; system TAD—R_f 0.56; system TAE—R_f 0.90.
Gas Chromatography System GA—RI 1567.
High Performance Liquid Chromatography System HX—RI 473; system HY—RI 458; system HAA—RT 18.3 min.
Ultraviolet Spectrum Aqueous acid—255 nm (A_1^1= 877b); aqueous alkali—296 nm (A_1^1= 1324b).

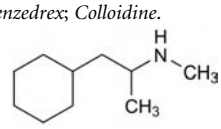


Infrared Spectrum Principal peaks at wavenumbers 1276, 1165, 1220, 1317, 1665, 1600 cm^{-1} (KBr disk).

Propylhexedrine

Sympathomimetic, Anorectic
$C_{10}H_{21}N$ = 155.3
CAS—101-40-6; 3595-11-7 (±)

IUPAC Name 1-Cyclohexyl-*N*-methylpropan-2-amine
Synonyms *N*,α-Dimethylcyclohexaneethanamine; hexahydrodesoxyephedrine; propylhexed.
Proprietary Names *Benzedrex*; *Colloidine.*

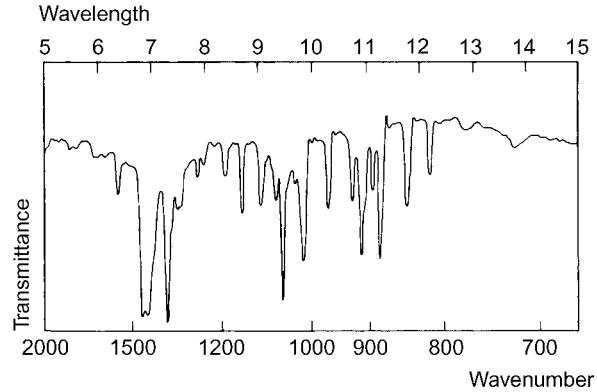


Chemical Properties A clear colourless liquid which slowly volatilises at room temperature and absorbs carbon dioxide from the air. Mass per mL 0.853–0.861 g. Bp about 204°. Very slightly soluble in water; miscible with ethanol, chloroform and ether. pK_a 10.7 (25°). Log *P* (octanol/water), 3.5.

Propylhexedrine Hydrochloride

$C_{10}H_{21}N$,HCl = 191.7
CAS—1007-33-6; 6192-95-6 (±)
Proprietary Name *Eventin(e)*
Chemical Properties A crystalline solid. Mp 127° to 128°, with decomposition. Soluble in water, ethanol and chloroform; slightly soluble in ether.

Thin-layer Chromatography System TA—R_f 0.26; system TB—R_f 0.34; system TE—R_f 0.34; system TAE—R_f 0.14; system TAJ—R_f 0.00; system TAK—R_f 0.24; system TAL—R_f 0.65 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—propylhexedrine RI 1175, propylhexedrine-TFA RI 1385, propylhexedrine-PFP RI 1385, propylhexedrine-AC RI 1570, M (OH-) RI 1475, M (OH-)-AC_2 RI 1915; system GB—RI 1192; system GC—RI 1500.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1058, 1015, 885, 913, 1153, 966 cm^{-1} (propylhexedrine hydrochloride, Nujol mull).



Mass Spectrum Principal ions at *m/z* 58, 140, 67, 72, 83, 81, 155, 156.
Quantification
Serum **HPLC** Propylhexedrine and other sympathomimetics and amfetamines. Limit of detection, 0.01–0.03 mg/L (UV detection single wavelength) and 0.05–0.1 mg/L (DAD) [Bogusz *et al.* 1997].
LC–MS Limit of detection, 1–5 µg/L for propylhexedrine and other sympathomimetics and amfetamines [Bogusz *et al.* 2000].
Urine **HPLC** See Serum [Bogusz *et al.* 1997].
Disposition in the Body Norpropylhexedrine, cyclohexylacetoxime and *cis/trans*-4-hydroxypropylhexedrine have been identified as urinary metabolites.
Toxicity The estimated minimum lethal dose for children up to 2 years of age is 200 mg.

In a fatality attributed to propylhexedrine ingestion, the following postmortem tissue concentrations were reported: blood 35 mg/L, bile 20 mg/L, brain 24 µg/g, kidney 30 µg/g, liver 36 µg/g, lung 50 µg/g, urine 60 mg/L [Riddick, Reisch 1981].

The following postmortem tissue concentrations were reported in 2 fatalities involving IV abuse: blood 1.8 and 2.7 mg/L, kidney 1.5 and 9.5 µg/g, liver 2.8 and 11.8 µg/g, urine 12.6 mg/L (first case) [Sturner *et al.* 1974].

In a fatality attributed to acute IV propylhexedrine abuse, postmortem tissue concentrations were: blood 2 mg/L, bile 9.4 mg/L, kidney 4.0 µg/g, liver 7.4 µg/g, urine 69.5 mg/L [DiMaio, Garriott 1977].

Note For a review of propylhexedrine, see Wesson [1986].
Dose Usually 75 to 100 mg of propylhexedrine hydrochloride daily.

Bogusz MJ *et al.* (1997). Determination of phenylisothiocyanate derivatives of amphetamine and its analogues in biological fluids by HPLC-APCI-MS or DAD. *J Anal Toxicol* 21: 59–69.
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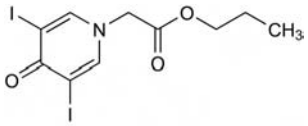
Riddick L, Reisch R (1981). Oral overdose of propylhexedrine. *J Forensic Sci* 26: 834–839.
Sturner WQ *et al.* (1974). Two propylhexedrine-associated fatalities: Benzedrine revisited. *J Forensic Sci* 19: 572–574.
Wesson DR (1986). Propylhexdrine. *Drug Alcohol Depend* 17: 273–278.

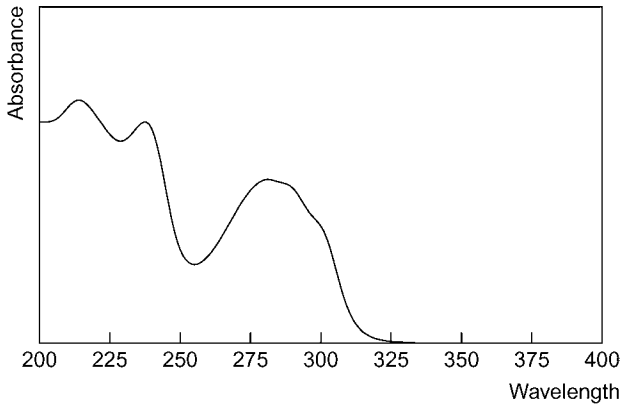
Propyliodone

Diagnostic Aid (Radiopaque Medium)
$C_{10}H_{11}I_2NO_3$ = 447.0
CAS—587-61-1

IUPAC Name Propyl 2-(3,5-diiodo-4-oxopyridin-1-yl)acetate
Synonyms 3,5-Diiodo-4-oxo-1(4*H*)-pyridineacetic acid propyl ester; propiodone.
Proprietary Name *Dionosil*



Chemical Properties A white crystalline powder. Mp 186° to 187°. Practically insoluble in water; soluble 1 in 500 of ethanol and 1 in 150 of chloroform; soluble in acetone and ether. Log *P* (octanol/water), 2.1.
Colour Test Iodine test—positive.
Thin-layer Chromatography System TA—R_f 0.81 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Dehydrated alcohol—239 (A^1_1=320a), 281 nm (A^1_1=260a).

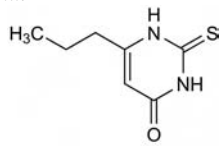


Infrared Spectrum Principal peaks at wavenumbers 1730, 1200, 1567, 1608, 751, 1227 cm^{-1} (KBr disk).
Uses Administered as a 50% aqueous suspension or as a 60% oily suspension.

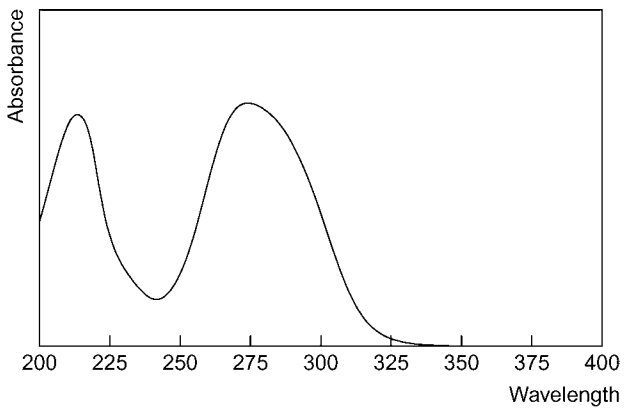
Propylthiouracil

Antihyperthyroid
$C_7H_{10}N_2OS$ = 170.2
CAS—51-52-5

IUPAC Name 6-Propyl-2-sulfanylidene-1*H*-pyrimidin-4-one
Synonym 2,3-Dihydro-6-propyl-2-thioxo-4(1*H*)pyrimidinone
Proprietary Names *Propacil*; *Propycil*; *Propylthiocil*; *Propyl-Thyracil*; *Prothiucil*; *Thyreostat II*; *Tiotil*.



Chemical Properties White or pale cream-coloured crystals or crystalline powder. Mp 219° to 221°. Soluble 1 in about 900 of water, 1 in about 60 of ethanol and 1 in 60 of acetone; slightly soluble in chloroform and ether; soluble in solutions of alkali hydroxides. pK_a 8.3 (20°). Log *P* (octanol/water), 1.0.
Ultraviolet Spectrum Aqueous acid—275 nm (A^1_1= 987b); aqueous alkali—260 nm (A^1_1= 703a).



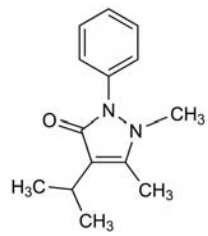
Infrared Spectrum Principal peaks at wavenumbers 1658, 1563, 1193, 1625, 1166, 1241 cm^{-1} (KBr disk).
Quantification
Blood **HPLC** UV detection. Limit of detection, 300 µg/L [Kim 1983].
Plasma **HPLC** Limit of detection, 40 µg/L [Cannell *et al.* 1991].
Serum **RIA** Limit of detection, 2.5 ng [Cooper *et al.* 1981].
Disposition in the Body Rapidly and almost completely absorbed after oral administration; concentrated in the thyroid. Metabolised by conjugation with glucuronic acid and sulfate. Less than 10% of a dose is excreted in the urine as unchanged drug.
Therapeutic Concentration
Following a single oral dose of 400 mg to 17 subjects, serum concentrations of 1.6–7.5 mg/L (mean 4.2) were reported at 1 h [Kampmann, Hansen 1981a].
A single oral administration of 300 mg to 7 subjects produced peak plasma concentrations of 3.50 mg/L about 2 h after a conventional-release tablet and 0.50–1.25 mg/L about 3 h after sustained-release tablets [Kabanda *et al.* 1996].
Half-life Plasma half-life, ≈1–2 h, increased in renal failure.
Volume of Distribution About 0.4 L/kg.
Clearance Plasma clearance, ≈4 mL/min/kg.
Protein Binding ≈80%.
Note For a review of the pharmacokinetics of antithyroid drugs, see Kampmann and Hansen [1981b].
Dose Initially 200 to 600 mg daily; maintenance, 50 to 200 mg daily.

Cannell GR *et al.* (1991). Selective liquid chromatographic assay for propylthiouracil in plasma. *J Chromatogr* 564: 310–314.
Cooper DS *et al.* (1981). Studies of propylthiouracil using a newly developed radioimmunoassay. *J Clin Endocr Metab* 52: 204–213.
Kabanda L *et al.* (1996). In-vivo evaluation in man of a hydrophilic matrix containing propylthiouracil. *J Pharm Pharmacol* 48: 1023–1026.
Kampmann JP, Hansen JEM (1981a). Correlation between antithyroid effect and serum concentrations of propylthiouracil in patients with hyperthyroidism. *Br J Clin Pharmacol* 12: 681–686.
Kampmann JP, Hansen JEM (1981b). Clinical pharmacokinetics of antithyroid drugs. *Clin Pharmacokinet* 6: 401–428.
Kim C (1983). Simple and sensitive method for the determination of propylthiouracil in blood by high-performance liquid chromatography. *J Chromatogr* 272: 376–379.

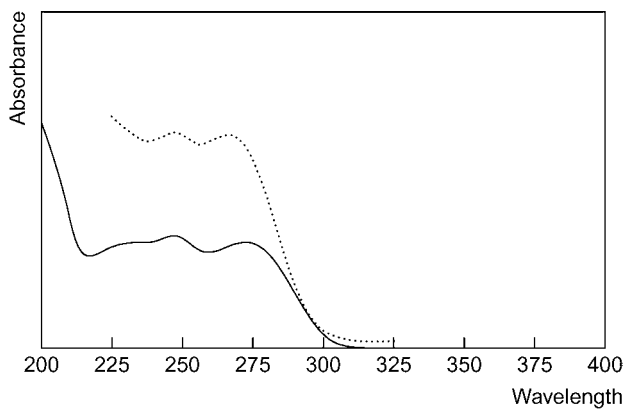
Propyphenazone

Analgesic
$C_{14}H_{18}N_2O$ = 230.3
CAS—479-92-5

IUPAC Name 1,5-Dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one
Synonyms 1,2-Dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl-3*H*-pyrazol-3-one; isopropylantipyrine; isopropylphenazone; propifenazona.
Proprietary Names *Cibalgina*; *Demex*; *Dim-Antos*; *Eufibron*; *Isoprochin P*; *Pireuma*.

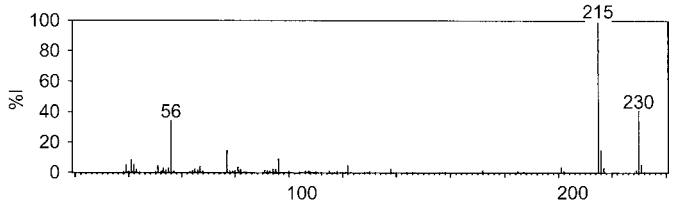


Chemical Properties White crystals or white crystalline powder. Mp 103°. Soluble 1 in 400 of water; freely soluble in ethanol and chloroform; soluble in ether. Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].
Colour Tests Ferric chloride—orange; Liebermann's reagent (100°)—blue (red with water).
Thin-layer Chromatography System TA—R_f 0.71; system TB—R_f 0.32; system TD—R_f 0.61; system TE—R_f 0.74; system TF—R_f 0.49; system TAD—R_f 0.65; system TAE—R_f 0.81 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1920; system GB—RI 2030; system GF—RI 2310.
High Performance Liquid Chromatography System HD—*k* 1.3; system HW—*k* 11.0; system HX—RI 441; system HY—RI 370; system HZ—retention time 4.7 min.
Ultraviolet Spectrum Aqueous acid—240 nm (A^1_1=400a); aqueous alkali—245 (A^1_1=385b), 265 nm.



P

Infrared Spectrum Principal peaks at wavenumbers 1650, 1618, 1131, 1590, 1500, 750 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 215, 230, 56, 77, 216, 96, 41, 39.

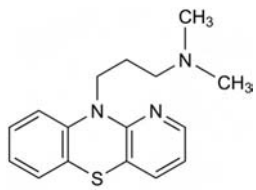


Quantification
***Plasma* HPLC** UV detection. Limit of detection, 100 µg/L [Rouan *et al.* 1992].
Dose Propyphenazone has been given in doses of 0.3 to 1.5 g daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Rouan MC *et al.* (1992). Rapid determination of propyphenazone in plasma by high-performance liquid chromatography. *J Chromatogr* 577: 387–390.

Prothipendyl

Tranquilliser
$C_{16}H_{19}N_3S$ = 285.4
CAS—303-69-5
IUPAC Name *N,N*-Dimethyl-3-pyrido[3,2-*b*][1,4]benzothiazin-10-ylpropan-1-amine
Synonyms *N,N*-Dimethyl-10*H*-pyrido[3,2-*b*][1,4]benzothiazine-10-propanamine; phrenotropin.
Proprietary Name *Dominal*.

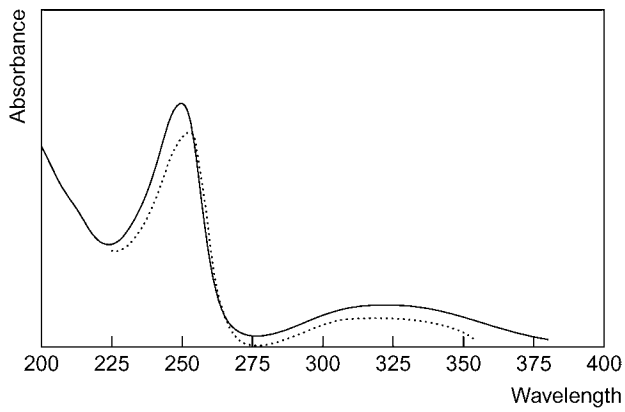


Chemical Properties pK_a 2.3 (25°). Log *P* (octanol/water), 4.0. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

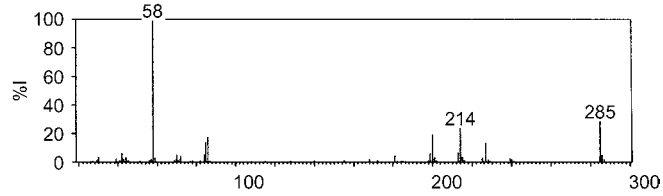
Prothipendyl Hydrochloride

$C_{16}H_{19}N_3S,HCl,H_2O$ = 339.9
CAS—1225-65-6 (anhydrous)
Chemical Properties A crystalline powder. Mp 108° to 112°. Freely soluble in water and methanol; practically insoluble in ether.

Colour Test Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.47; system TAE—R_f 0.15; system TAF—R_f 0.29; system TAG—R_f 0.09; system TB—R_f 0.43; system TC—R_f 0.23; system TE—R_f 0.59 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; FPN reagent—yellow; Marquis test—orange).
Gas Chromatography System GA—prothipendyl RI 2345, M (OH-) RI 2720, M (OH-)(ring) RI 2800, M (ring) RI 2045, M (sulfoxide) RI 2750; system GF—RI 2800.
High Performance Liquid Chromatography System HA—*k* 4.4; system HX—RI 388.
Ultraviolet Spectrum Aqueous acid—242 nm (A^1_1 = 928a); aqueous alkali—250 nm (A^1_1 = 909a), 320 nm (A^1_1 = 163b).



Infrared Spectrum Principal peaks at wavenumbers 757, 1295, 780, 1592, 1162, 1110 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 285, 214, 200, 86, 227, 85, 213.

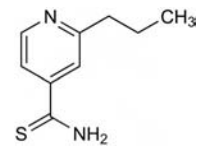


Quantification
***Plasma* HPLC** Chrompack CP sphere C_8 (100 × 3.0 mm i.d.). Mobile phase: 0.05 mol/L sodium dihydrogen phosphate : acetonitrile (30 : 70, pH 2.2), flow rate 0.8 mL/min. UV detection (λ = 210 nm). Limit of detection not reported [Debailleul *et al.* 1991].
Disposition in the Body
Toxicity
In 2 cases of fatal poisoning with prothipendyl, the highest concentrations were found in the liver (1.2–1.8 mg/g) and kidney (0.6 mg/g), which far exceeded those found in blood (which in one death, was 200 times over the therapeutic range). It was suggested that more than 4 g prothipendyl is fatal [Wu *et al.* 1994].
Dose The equivalent of up to 960 mg of prothipendyl daily.

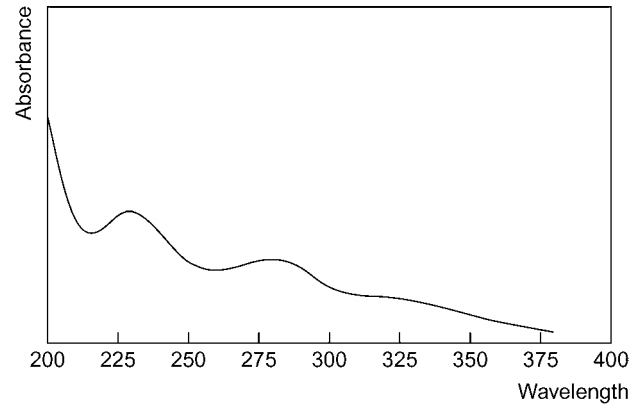
Debailleul G *et al.* (1991). HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication. *J Anal Toxicol* 15: 35–37.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Wu M *et al.* (1994). *[Suicide with prothipendyl]. Arch Kriminol* 193: 158–162.

Protionamide

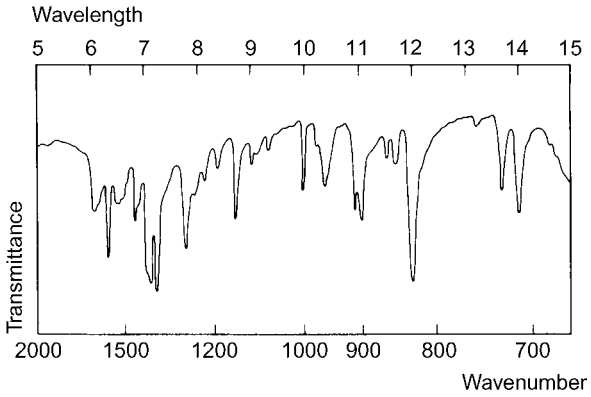
Tuberculostatic
$C_9H_{12}N_2S$ = 180.3
CAS—14222-60-7
IUPAC Name 2-Propylpyridine-4-carbothioamide
Synonyms 2-Propyl-4-pyridinecarbothioamide; prothionamide.
Proprietary Names *Ektebin*; *Peteha*; *Trevintix*.



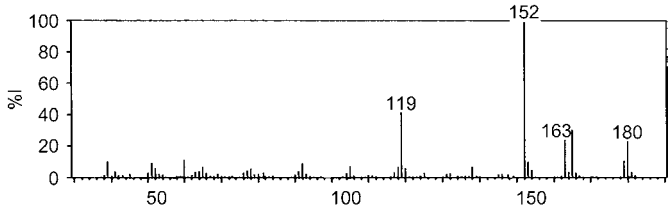
Chemical Properties Yellow crystals or crystalline powder. Mp 142°. Practically insoluble in water; soluble 1 in 30 of ethanol, 1 in 200 of chloroform, 1 in 300 of ether and 1 in 16 of methanol; soluble in acetone. Log *P* (octanol/water), 2.0.
Colour Tests Nessler's reagent—brown-orange; palladium chloride—brown.
Thin-layer Chromatography System TA—R_f 0.66; system TAE—R_f 0.77; system TL—R_f 0.57; system TB—R_f 0.01; system TC—R_f 0.38 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1816.
Ultraviolet Spectrum Ethanol—291 nm (A^1_1=390b).



Infrared Spectrum Principal peaks at wavenumbers 828, 1590, 1284, 902, 1148, 710 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 152, 119, 165, 163, 180, 60, 179, 153.

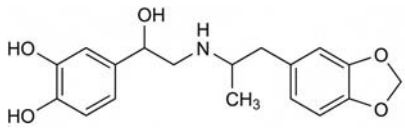


Quantification
Plasma **HPLC** UV detection. Limit of detection, 10 µg/L [Jenner, Ellard 1981].
Serum **HPLC** UV detection. Limit of detection, 27 µg/L [Bartels, Bartels 1998]. UV detection. Limit of detection, 10 µg/L [Jenner, Ellard 1981].
Urine **HPLC** UV detection. Limit of detection, 10 µg/L [Jenner, Ellard 1981].
Disposition in the Body Readily absorbed after oral administration. Metabolised to the sulfoxide and excreted in the urine mainly as metabolites.
Therapeutic Concentration
Following a single oral dose of 500 mg to 10 subjects, mean peak plasma concentrations of 3 mg/L of unchanged drug and 2.7 mg/L of the sulfoxide were attained in 1 h [Pütter 1972].
Dose Usually 0.5 to 1 g daily.

Bartels H, Bartels R (1998). Simple, rapid and sensitive determination of protionamide in human serum by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 707: 338–341.
Jenner PJ, Ellard GA (1981). High-performance liquid chromatographic determination of ethionamide and prothionamide in body fluids. *J Chromatogr* 225: 245–251.
Pütter J (1972). [Determination of prothionamide and ethionamide and their sulfoxides in blood plasma]. *Arzneimittelforschung* 22: 1027–1031.

Protokylol

Bronchodilator
$C_{18}H_{21}NO_5$ = 331.4
CAS—136-70-9
IUPAC Name 4-[2-[1-(1,3-Benzodioxol-5-yl)propan-2-ylamino]-1-hydroxyethyl]benzene-1,2-diol
Synonyms 4-[2-[[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]amino]-1-hydroxyethyl]-1,2-benzenediol; protochylol.

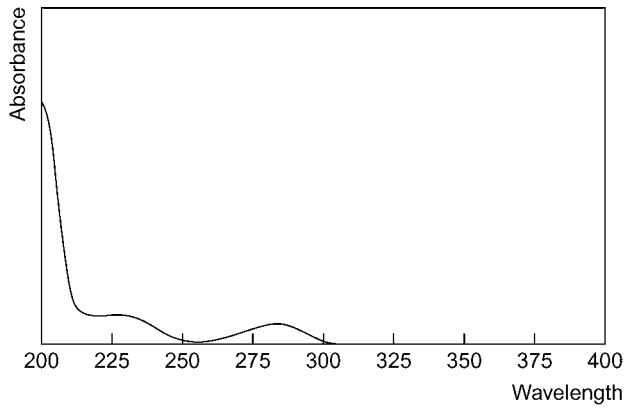


Chemical Properties Log *P* (octanol/water), 1.8.

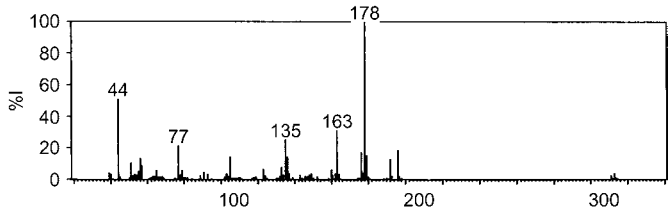
Protokylol Hydrochloride
$C_{18}H_{21}NO_5$,HCl = 367.8
CAS—136-69-6
Synonym JB-251
Proprietary Names *Caytime*; *Ventaire*.
Chemical Properties A white crystalline powder. Mp 126° to 127°. Soluble in water.

Colour Tests Ammoniacal silver nitrate—red-grey/brown; ferric chloride—green; Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—yellow→brown; Marquis test—grey-green; methanolic potassium hydroxide—orange→yellow; Nessler's reagent—black; potassium dichromate—brown→red-brown (on warming).

Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.01; system TC—R_f 0.03; system TL—R_f 0.06; system TAJ—R_f 0.00; system TAK—R_f 0.02; system TAL—R_f 0.48 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 1487.
High Performance Liquid Chromatography System HA—*k* 3.1 (tailing peak).
Ultraviolet Spectrum Aqueous acid—281 nm (A_1^1=168b).



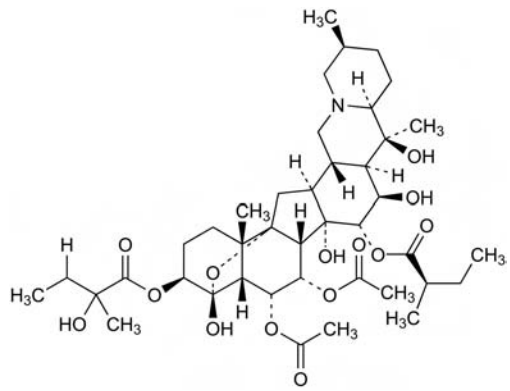
Infrared Spectrum Principal peaks at wavenumbers 1492, 1250, 1271, 1227, 1036, 1128 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 178, 44, 163, 135, 77, 196, 176, 179.



Dose Protokylol hydrochloride has been given in doses of 8 to 16 mg daily.

Protoveratrine A and B

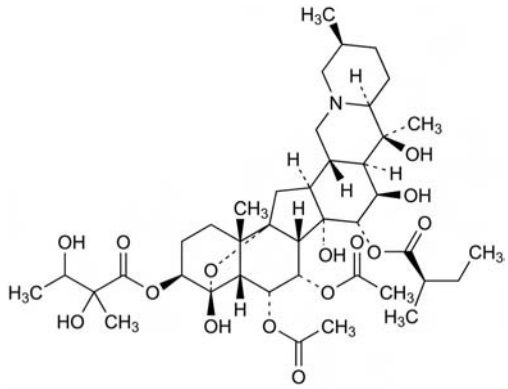
Antihypertensive
$C_{41}H_{63}NO_{14}$ = 793.9
CAS—143-57-7
IUPAC Name [3β(*S*),4α,6α,7α,15α(*R*),16β]-4,9-Epoxycevane-3,4,6,7,14,15,16,20-octol 6,7-diacetate
Proprietary Name *Protalba*



Chemical Properties An alkaloid obtained from the roots and rhizomes of white veratrum, *Veratrum album* (Liliaceae). White crystals. Mp 267° to 269°, with decomposition. Practically insoluble in water; soluble in hot ethanol and in chloroform.

Protaveratrine B
$C_{41}H_{63}NO_{15}$ = 809.9
CAS—124-97-0
IUPAC Name [3β(2*R*,3*R*),4α,6α,7α,15α(*R*),16β]-4,9-Epoxycevane-3,4,6,7,14,15,16,20-octol 6,7-diacetate
Synonyms Neoprotoveratrine; veratetrine.

P

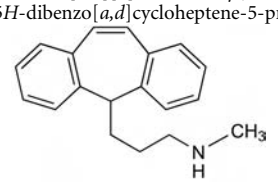


Protoveratrines A and B
CAS—8053-18-7
Proprietary Names *Provell*; *Tensatrin*.
Chemical Properties A white crystalline powder with a strong sternutatory action. Mp 256° to 262°, with decomposition. Practically insoluble in water; soluble in chloroform; very slightly soluble in ether. It is rapidly decomposed in alkaline and alcoholic solutions.

Thin-layer Chromatography Protoveratrine A: System TA—R_f 0.72. Protoveratrine B: System TA—R_f 0.70 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—protoveratrines A and B, RI 2465.
Infrared Spectrum Principal peaks at wavenumbers 1742, 1200, 1182, 1240, 1150, 1055 cm^{-1} (protoveratrines A and B, KCl disk).
Dose Protoveratrines A and B have been given in a dose of 1.6 to 6 mg daily.

Protriptyline

Tricyclic Antidepressant
$C_{19}H_{21}N$ = 263.4
CAS—438-60-8
IUPAC Name 3-(5*H*-Dibenzo[*a*,*d*][7]annulen-5-yl)-*N*-methylpropan-1-amine
Synonym *N*-Methyl-5*H*-dibenzo[*a*,*d*]cycloheptene-5-propanamine

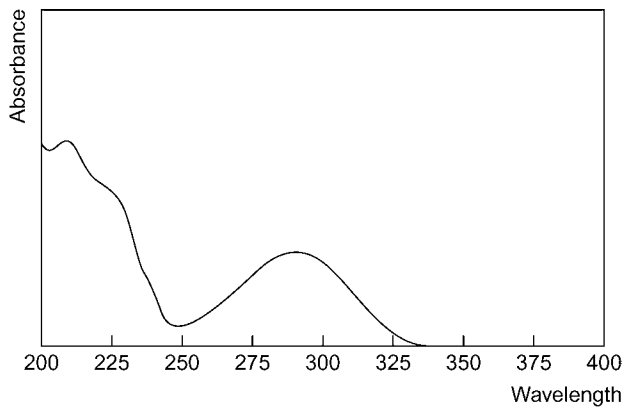


Chemical Properties Log *P* (octanol/water), 4.9.

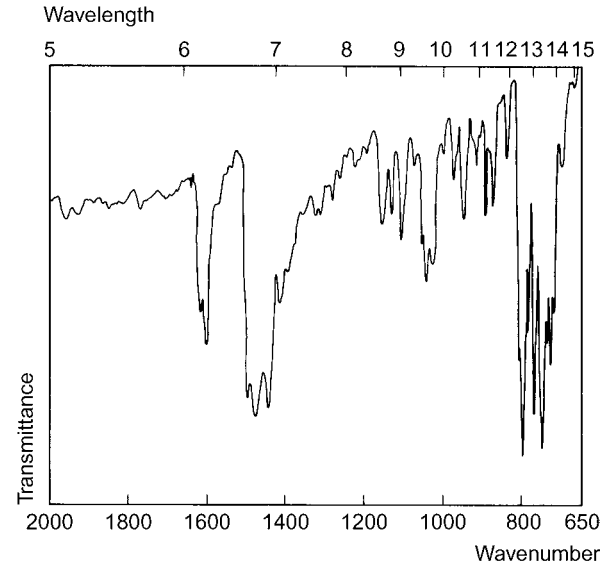
Protriptyline Hydrochloride

$C_{19}H_{21}N,HCl$ = 299.8
CAS—1225-55-4
Proprietary Names *Concordin(e)*; *Triptil*; *Vivactil*.
Note The name *Vivactil* has also been applied to a vitamin/amino acid preparation.
Chemical Properties A white to yellowish powder. Mp ≈169° to 171°. Soluble 1 in 2 of water, 1 in 3.5 of ethanol and 1 in 2.5 of chloroform; practically insoluble in ether. pK_a 8.2.

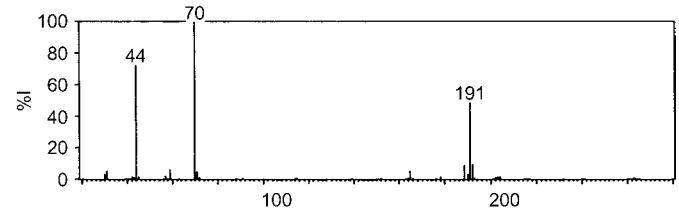
Colour Tests Mandelin's test—violet-brown; Marquis test—green; sulfuric acid—green.
Thin-layer Chromatography System TA—R_f 0.19; system TB—R_f 0.18; system TC—R_f 0.07; system TE—R_f 0.38; system TAE—R_f 0.6; system TAF—R_f 0.69; system TAG—R_f 0.02 (acidified iodoplatinate solution—positive).
Gas Chromatography System GA—RI 2253; system GB—RI 2329, M (nor-) RI 2343; system GF—RI 2590; system GM—RRT 0.878.
High Performance Liquid Chromatography System HA—*k* 2.10; system HF—*k* 3.60; system HX—RI 418; system HY—RI 362.
Ultraviolet Spectrum Methanolic acid—292 nm (A_1^1 = 530a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 792, 750, 768, 1490, 730, 1595 cm^{-1} (protriptyline hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 70, 44, 191, 192, 188, 59, 165, 71 (protriptyline) ; 44, 70, 179, 178, 207, 280, 250, 236 (10,11-dihydro-10,11-dihydroxyprotriptyline) ; 70, 44, 207, 178, 279, 249, –, – (10-hydroxyprotriptyline).



Quantification
Plasma **GC-MS** Column: 3% OV-17 or 1% SE-30 on 100/120 mesh Gas Chrom Q (500 × 6 mm o.d.). Carrier gas: He, 30 mL/min. Temperature programme: 180° to 220°. EI ionisation at 70 eV. Limit of detection, 10 µg/L [Biggs *et al.* 1976].

HPLC Column: µBondapak C_{18} (300 × 4 mm i.d.). Mobile phase: acetonitrile : 45 mmol/L potassium dihydrogen phosphate (pH 3.0; 35 : 65), flow rate 1.5 mL/min. UV detection (λ = 235 nm). Retention time: 8.2 min. Limit of detection, 60 µg/L [Hackett, Dusci 1979].

Serum **GC** Column: 3% OV-225 on 100/120 mesh Supelcoport (1 m × 2 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature programme: room temperature to 250° at 2°/min. NPD. Retention time: 0.93 min. Limit of detection, 10 to 20 µg/L [Kristinsson 1981]. Column: SPB-1 100% polymethylsiloxane (60 m × 0.75 mm i.d., 1.0 µm). Carrier gas: He, 8 mL/min. Temperature: 260°. NSD. Retention time: ~7 min. Limit of detection, 25 µg/L [Rifai *et al.* 1988].

Disposition in the Body Protriptyline is slowly but well absorbed after oral administration; bioavailability is 75–90% (10–25% first-pass metabolism). It is metabolised by oxidation to form the 10-hydroxy and 10,11-dihydro-10,11-dihydroxy derivatives and 5,10-dihydro-10-formylanthracen-5-ylpropylamine; glucuronic acid conjugation also occurs. Approximately 50% of a dose is excreted in the urine in 16 days, with only small amounts of unchanged drug; 2% of a dose is eliminated in the faeces.

Therapeutic Concentration In plasma, usually in the range 0.07–0.25 mg/L.
After a single oral dose of 30 mg in 8 subjects, peak plasma concentrations of 0.010–0.022 mg/L (mean, 0.014) were attained in 6–12 h [Biggs *et al.* 1976; Ziegler *et al.* 1978].
Following daily oral doses of 40 mg to 30 subjects, steady-state plasma concentrations of 0.11–0.38 mg/L (mean, 0.22) were reported [Moody *et al.* 1977].

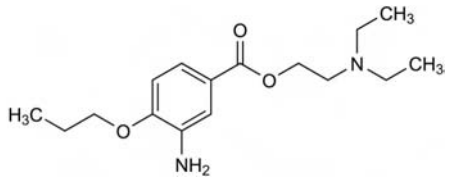
Toxicity Toxic effects are associated with plasma concentrations greater than 0.5 mg/L; concentrations above 1 mg/L may be lethal.
Half-life Plasma half-life, during chronic dosing, ~3–8 days (mean, 4).
Volume of Distribution ~22 L/kg.
Clearance Plasma clearance, 2–5 mL/min/kg.
Protein Binding ~92%.
Note For a review of tricyclic antidepressants see Molnar, Gupta [1980].
Dose 15 to 60 mg of protriptyline hydrochloride daily.

Biggs JT *et al.* (1976). Electron beam ionization mass fragmentographic analysis of tricyclic antidepressants in human plasma. *J Pharm Sci* 65: 261–268.
Hackett LP, Dusci LJ (1979). The use of high-performance liquid chromatography in clinical toxicology. II. Tricyclic antidepressants. *Clin Toxicol* 15: 55–61.
Kristinsson J (1981). A gas chromatographic method for the determination of antidepressant drugs in human serum. *Acta Pharmacol Toxicol (Copenh)* 49: 390–398.
Molnar G *et al.* (1980). Plasma levels and tricyclic antidepressant therapy: Part 2 Pharmacokinetic, clinical and toxicologic aspects. *Biopharm Drug Dispos* 1: 283–305.
Moody JP *et al.* (1977). Pharmacokinetic aspects of protriptyline plasma levels. *Eur J Clin Pharmacol* 11: 51–56.
Rifai N *et al.* (1988). Measurement of antidepressants using solid-phase extraction and wide-bore capillary gas chromatography with nitrogen-selective detection. *Ther Drug Monit* 10: 194–196.
Ziegler VE *et al.* (1978). Protriptyline kinetics. *Clin Pharmacol Ther* 23: 580–584.

Proxymetacaine

Anaesthetic (Local)
$C_{16}H_{26}N_2O_3$ = 294.4
CAS—499-67-2

IUPAC Name 2-Diethylaminoethyl 3-amino-4-propoxybenzoate
Synonyms 3-Amino-4-propoxybenzoic acid 2-(diethylamino)ethyl ester; proparacaine.

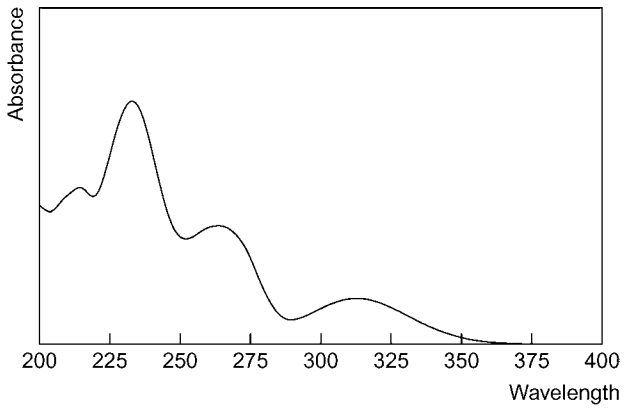


Chemical Properties Log *P* (octanol/water), 3.2.

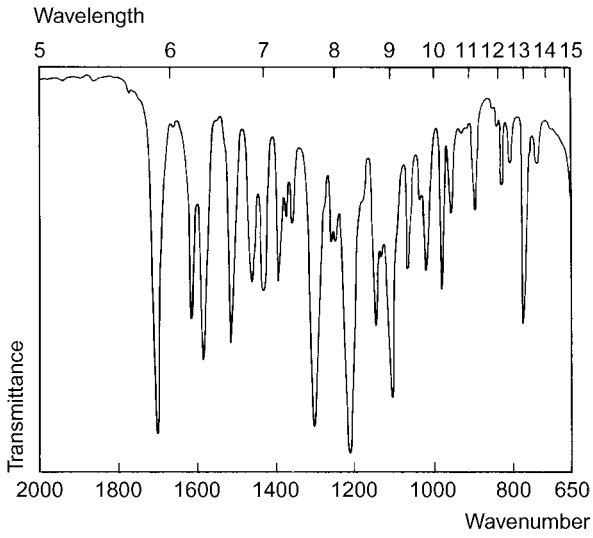
Proxymetacaine Hydrochloride

$C_{16}H_{26}N_2O_3$,HCl = 330.9
CAS—5875-06-9
Proprietary Names *Ak-Taine*; *Alcaine*; *Anestalcon*; *Diocaine*; *Kéracaine*; *Ocu-Caine*; *Ophthaine*; *Ophthetic*; *Parcaine*; *Visonest*.
Chemical Properties A white or faintly buff-coloured crystalline powder. It discolours on heating or exposure to air and solutions exposed to air become yellow and then dark brown. Mp 182° to 183°. Soluble 1 in 30 of water and 1 in 50 of ethanol; practically insoluble in ether; soluble in chloroform and methanol.

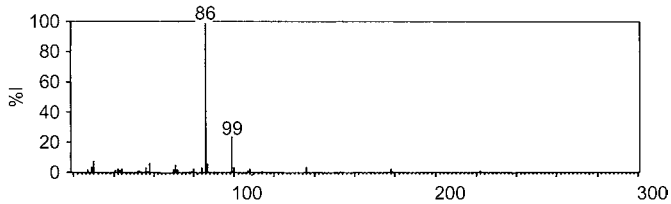
Colour Tests Coniferyl alcohol—orange; diazotisation—red; ninhydrin—yellow.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.26; system TC—R_f 0.41; system TL—R_f 0.35 (acidified iodoplatinate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—RI 2323.
High Performance Liquid Chromatography System HA—*k* 2.1; system HQ—*k* 1.38; system HY—RI 269.
Ultraviolet Spectrum Water—231 nm, 268 nm (A_1^1=326b), 310 nm (A_1^1=182a).



Infrared Spectrum Principal peaks at wavenumbers 1213, 1708, 1305, 1104, 1590, 1520 cm^{-1} (proxymetacaine hydrochloride, KCl disk).



Mass Spectrum Principal ions at *m/z* 86, 99, 30, 87, 58, 71, 136, 100.

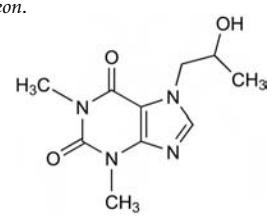


Use Proxymetacaine hydrochloride is used as a 0.5% ophthalmic solution.

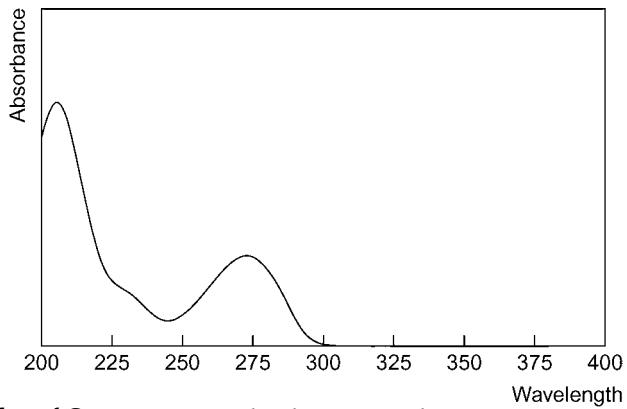
Proxyphylline

Xanthine Bronchodilator
$C_{10}H_{14}N_4O_3$ = 238.2
CAS-603-00-9

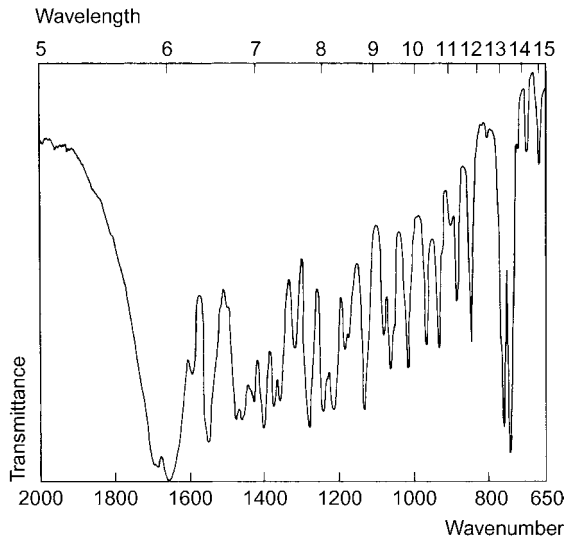
IUPAC Name 7-(2-Hydroxypropyl)-1,3-dimethylpurine-2,6-dione
Synonym 3,7-Dihydro-7-(2-hydroxypropyl)-1,3-dimethyl-1*H*-purine-2,6-dione
Proprietary Names *Brontyl*; *Neofyllin*; *Pantafillina*; *Purofilina*; *Spantin*; *Spasmolysin*; *Thean*; *Theon*.



Chemical Properties A white crystalline powder. Mp 135° to 136°. Soluble 1 in 1 of water, 1 in 12 of ethanol, 1 in 6 of chloroform and 1 in 500 of ether. Log *P* (octanol/water), −0.8.
Colour Test Amalic acid test—yellow/violet.
Thin-layer Chromatography System TA—R_f 0.58; system TB—R_f 0.02; system TC—R_f 0.33; system TE—R_f 0.49; system TL—R_f 0.29; system TAE—R_f 0.71 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2103.
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 293.
Ultraviolet Spectrum Aqueous acid—273 nm (A_1^1=372a). No alkaline shift.

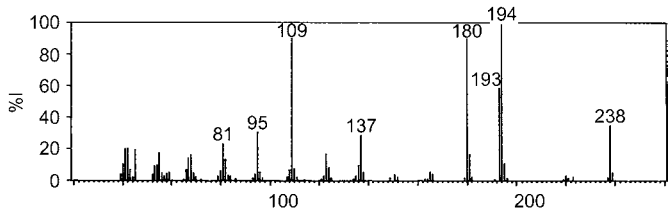


Infrared Spectrum Principal peaks at wavenumbers 1656, 1690, 750, 1538, 763, 1282 cm^{-1} (KBr disk).



P

Mass Spectrum Principal ions at *m/z* 194, 180, 109, 193, 238, 95, 137, 81.



Quantification

Plasma **GC** ECD. Limit of detection, 100 µg/L [Arbin *et al.* 1976].

HPLC Limit of detection, 0.2 mg/L for (*R*)- and (*S*)-proxyphylline [Ruud-Christensen *et al.* 1989].

Serum **HPLC** UV detection. Limit of detection, 12 µg/L [Selvig, Bjerve 1977].

Disposition in the Body Proxyphylline is rapidly and completely absorbed after oral administration. More than 95% of a dose is excreted in the urine in 96 h with about 30% as unchanged drug, 58% as 1-methyl-7-(2-hydroxypropyl)xanthine, 10% as proxyphylline glucuronide and 2% as 1-methyl-7-(2-hydroxypropyl) uric acid.

Therapeutic Concentration

After a single oral dose of 400 mg given to 5 subjects, plasma concentrations of 7 to 13 (mean 10) mg/L were attained in 1 h. Oral dosing of 5 subjects with 400 mg three times a day for 5 days, produced peak plasma concentrations of 12 to 25 (mean 18) mg/L on the 5th day [Graffner *et al.* 1973].

Half-life Plasma half-life, 8 to 12 (mean 9) h.

Volume of Distribution About 0.6 L/kg.

Clearance Plasma clearance, about 0.8 mL/min/kg.

Dose Usually 900 mg daily.

Arbin A *et al.* (1976). Determination of proxyphylline and dyphylline in capillary blood samples by means of electron capture gas chromatography. *Acta Pharm Suec* 13: 235–240.

Graffner C *et al.* (1973). Pharmacokinetic studies on proxyphylline administered intravenously and orally to man. *Acta Pharm Suec* 10: 425–434.

Ruud-Christensen M *et al.* (1989). High-performance liquid chromatographic determination of (R)-and (S)-proxyphylline in human plasma. *J Chromatogr* 491: 355–366.

Selvig K, Bjerve KS (1977). Determination of serum proxiphylline by high-pressure liquid chromatography. *Scand J Clin Lab Invest* 37: 373–378.

Pseudoephedrine

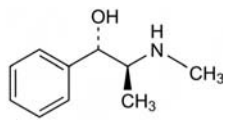
Sympathomimetic

$C_{10}H_{15}NO = 165.2$

CAS—90-82-4

IUPAC Name (1*S*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol

Synonyms *d*-ψ-Ephedrine; *d*-isoephedrine; (α*S*)-α-[(1*S*)-1-(methylamino) ethyl]benzenemethanol.



Chemical Properties An alkaloid obtained from Ephedra spp. It is a stereoisomer of ephedrine. Mp 118.0° to 118.7° (crystals from ether). Sparingly soluble in water; freely soluble in ethanol and ether. pK_a 9.8. Log *P* (octanol/water), 0.9. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005].

Pseudoephedrine Hydrochloride

$C_{10}H_{15}NO,HCl = 201.7$

CAS—345-78-8

Proprietary Names *Afrin*; *Allermed*; *Bronalin Decongestant*; *Cenafed*; *Contac*; *Decofed*; *DeFed*; *Dimetapp Decongestant*; *Dorcol Children's Decongestant*; *Drixoral Non-Drowsy*; *Efidac*; *Galpseud*; *Genaphed*; *Halofed*; *Seudotabs*; *Silfedrine*; *Sinustop Pro*; *Sudafed*; *Triaminic Decongestant*. It is an ingredient of many proprietary preparations [Sweetman 2002].

Chemical Properties White crystals or powder. Mp 182.5° to 183.5°. A solution in water is dextrorotatory. Soluble 1 in 1.6 of water, 1 in 4 of ethanol and 1 in 60 of chloroform; very slightly soluble in ether.

Pseudoephedrine Sulfate

$(C_{10}H_{15}NO)_2,H_2SO_4 = 428.5$

CAS—7460-12-0

Synonym Sch-4855

Proprietary Names *Drixora*; *Afrinol*.

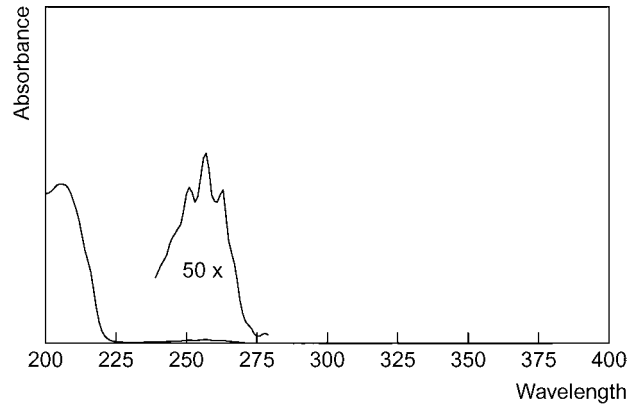
Chemical Properties A white crystalline powder. Mp 174° to 179°. A solution in water is dextrorotatory. Soluble in water.

Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.05; system TC—R_f 0.04; system TE—R_f 0.17; system TL—R_f 0.63; system TAE—R_f 0.09; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.30 (acidified potassium permanganate solution, positive).

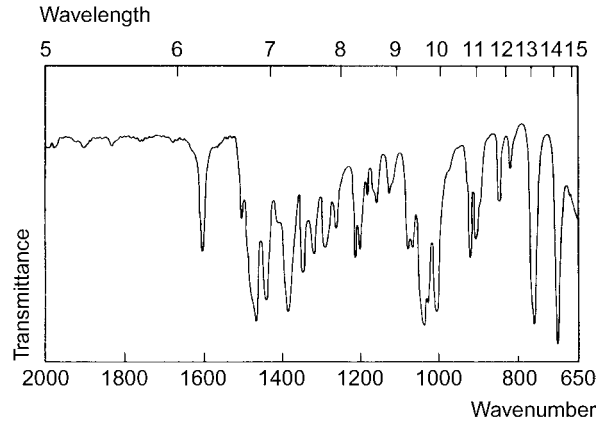
Gas Chromatography System GA—RI 1365; system GB—RI 1410; system GC—RI 1543.

High Performance Liquid Chromatography System HA—pseudoephedrine *k* 1.2, norpseudoephedrine *k* 1.0; system HB—*k* 5.90; system HC—*k* 1.77; system HX—RI 237; system HY—RI 230.

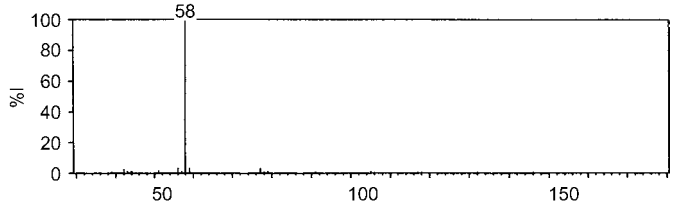
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1= 11.9a), 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 704, 1036, 764, 1005, 1210, 1595 cm^{-1} (pseudoephedrine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 77, 59, 56, 51, 42, 105, 91.



Quantification

Plasma **GC** ECD. Pseudoephedrine and norpseudoephedrine. Limit of detection, 20 µg/L for pseudoephedrine [Lo *et al.* 1981].

HPLC Spectrophotometric detection. Limit of quantification, 5.8 µg/L [Macek *et al.* 2002]. Limit of detection, 12 µg/L [Guo *et al.* 1999]. Fluorescence detection. Limit of quantification, 1 µg/L for pseudoephedrine and ephedrine [Shao *et al.* 1995].

RIA Limit of detection, 0.2–2.5 µg/L [Findlay *et al.* 1981].

Serum **GC** ECD. Limit of detection, 20 µg/L [Sun, Leveque 1979].

Urine **GC** NPD. For method for quantification of pseudoephedrine and other ephedrines, see Van Eenoo *et al.* [2001]. ECD. For method for quantification of pseudoephedrine and norpseudoephedrine, see Lo *et al.* [1981].

HPLC UV detection. Imaz *et al* 2005]. Limit of quantification, 0.5 mg/L for pseudoephedrine and other ephedrines [van der Merwe *et al.* 1994]. ECD. For method for quantification of pseudoephedrine and norpseudoephedrine, see Lo *et al.* [1981].

Disposition in the Body Rapidly and completely absorbed after oral administration. Up to about 90% of a dose is excreted unchanged in the urine in 24 h with <1% as norpseudoephedrine (cathine) (pH-dependent).

Therapeutic Concentration

After a single oral dose of 180 mg to 3 subjects, peak plasma concentrations of 0.72–0.81 mg/L were attained in 2–3 h [Kuntzman *et al.* 1971].

Following oral administration of 60 mg 4 times a day to 17 subjects, maximum steady-state plasma concentrations of 0.41–0.79 mg/L (mean 0.55) were reported about 1.5 h after a dose [Perkins *et al.* 1980].

Toxicity Toxic effects have occurred after single doses of 60 mg.

The following postmortem tissue concentrations were reported in a fatality involving pseudoephedrine: blood 19 mg/L, brain 22 µg/g, liver 33 µg/g, urine 105 mg/L [*Registry of Human Toxicology*, 1978].

Half-life Plasma half-life, 5–8 h but may be increased when the urine is alkaline and decreased when it is acid.

Volume of Distribution About 3 L/kg.

Dose Usually 180 to 240 mg of pseudoephedrine hydrochloride daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Findlay JW *et al.* (1981). Stereospecific radioimmunoassays for d-pseudoephedrine in human plasma and their application to bioequivalency studies. *J Pharm Sci* 70: 624–631.

Guo P *et al.* (1999). Direct injection of plasma to determine pseudoephedrine by high performance liquid chromatography with column switching. *Biomed Chromatogr* 13: 61–64.

Imaz C *et al.* (1993). Determination of ephedrines in urine by high-performance liquid chromatography. *J Chromatogr* 631: 201–205.

Kuntzman RG *et al.* (1971). The influence of urinary pH on the plasma half-life of pseudoephedrine in man and dog and a sensitive assay for its determination in human plasma. *Clin Pharmacol Ther* 12: 62–67.

Lo LY *et al.* (1981). Sensitive assay for pseudoephedrine and its metabolite, norpseudoephedrine in plasma and urine using gas–liquid chromatography with electron-capture detection. *J Chromatogr* 222: 297–302.

Macek J *et al.* (2002). Rapid determination of pseudoephedrine in human plasma by high-performance liquid chromatography. *J Chromatogr B Anal Technol Biomed Life Sci* 766: 289–294.

Perkins JG *et al.* (1980). *Curr Ther Res* 28: 650–668.

(1978) *Registry of Human Toxicology*. American Academy of Forensic Sciences.

Shao G *et al.* (1995). Quantitative analysis of (l)-ephedrine and (d)-pseudoephedrine in plasma by high-performance liquid chromatography with fluorescence detection. *Yao Xue Xue Bao* 30: 384–389.

Sun SR, Leveque MJ (1979). Electron-capture GLC determination of pseudoephedrine in serum. *J Pharm Sci* 68: 1567–1568.

Sweetman SC, ed. (2002). *Martindale, The complete drug reference*, 33 edn. Pharmaceutical Press: London

van der Merwe PJ *et al.* (1994). Simultaneous quantification of ephedrines in urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 661: 357–361.

Van Eenoo P *et al.* (2001). Simultaneous quantitation of ephedrines in urine by gas chromatography-nitrogen-phosphorus detection for doping control purposes. *J Chromatogr B Biomed Sci Appl* 760: 255–261.

Pseudomorphine

Morphine Impurity

$C_{34}H_{36}N_2O_6,3H_2O = 622.7$

CAS—125-24-6 (anhydrous); 6472-73-7 (trihydrate)

IUPAC Name (5′α,6′α)-7,7′,8,8′-Tetradehydro-4,5:4′,5′-diepoxy-17,17′-dimethyl-[2,2′-bimorphinan]-3,3′,6,6′-tetrol

Synonyms 2,2′-Bimorphine; oxydimorphine.

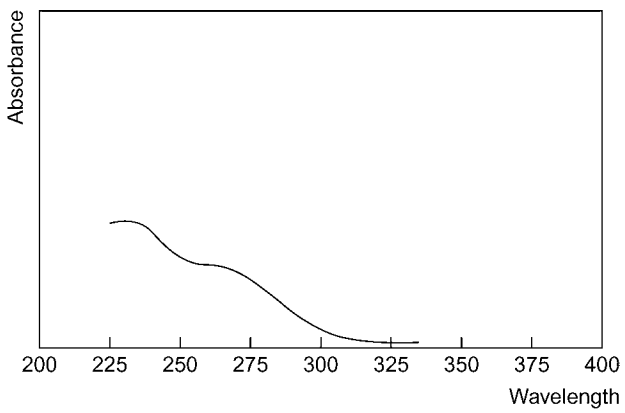
Chemical Properties White crystals (trihydrate). Becomes anhydrous at 150°. Mp 327°, with decomposition. Practically insoluble in water, ethanol and ether; soluble in dilute hydrochloric acid, dilute acetic acid and pyridine (pseudomorphine trihydrate).

Colour Test Marquis test—green.

Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.00; system TC—R_f 0.00; system TL—R_f 0.00; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.02 (acidified iodoplatinate solution, positive).

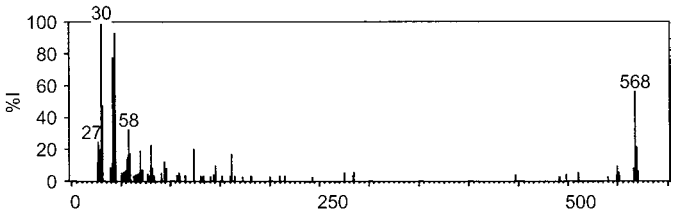
Gas Chromatography System GA—RI 2770.

Ultraviolet Spectrum Aqueous acid—231 nm (A^1_1 = 308b).



Infrared Spectrum Principal peaks at wavenumbers 1118, 1098, 1069, 1044, 1195, 1210 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 30, 44, 42, 568, 31, 58, 27, 81.



Note Pseudomorphine may be found as an oxidation product of morphine in decomposed viscera.

Psilocin

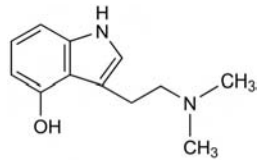
Hallucinogen

$C_{12}H_{16}N_2O = 204.3$

CAS—520-53-6

IUPAC Name 3-[2-(Dimethylamino)ethyl]-1*H*-indol-4-ol

Synonyms 4-Hydroxy-*N*,*N*-dimethyltryptamine; psilocin.



Chemical Properties An indole alkaloid obtained from the Mexican mushroom (teonanácatl) *Psilocybe mexicana* (Agaricaceae). White crystals. Mp 173° to 176°. Soluble in ethanol and dilute acetic acid. Unstable in solution, especially alkaline solution. Log *P* (octanol/water), 1.5.

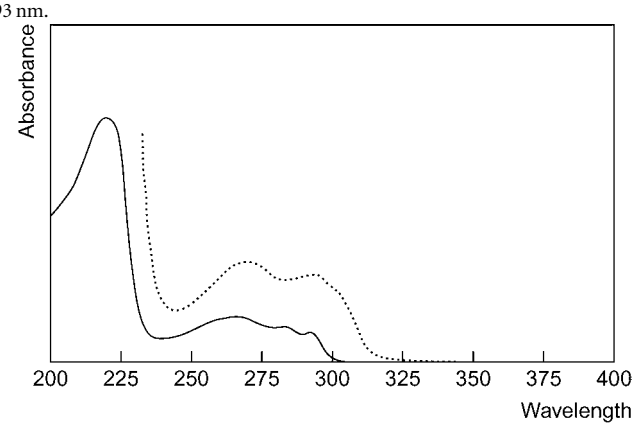
Colour Test Marquis test—green-brown.

Thin-layer Chromatography System TA—R_f 0.39; system TB—R_f 0.05, psilocin-ethyl R_f 0.09; system TC—psilocin R_f 0.09, psilocin-ethyl R_f 0.15; system TE—psilocin R_f 0.47, psilocin-ethyl *k* 0.65; system TL—psilocin R_f 0.09, psilocin-ethyl *k* 0.25; system TAE—psilocin R_f 0.14, psilocin-ethyl *k* 0.15; system TAF—psilocin R_f 0.48, psilocin-ethyl *k* 0.67 (van Urk reagent—faint blue).

Gas Chromatography System GA—psilocin RI 1985, psilocin-AC RI 2270, psilocin-AC_2 RI 2340; system GB—RI 2080.

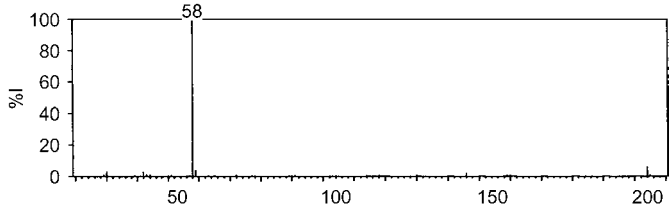
High Performance Liquid Chromatography System HA—*k* 3.1 (tailing peak); system HX—RI 240; system HY—RI 226.

Ultraviolet Spectrum Aqueous acid—266, 283, 292 nm; aqueous alkali—270, 293 nm.



Infrared Spectrum Principal peaks at wavenumbers 836, 1261, 1236, 1042, 1061, 733 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 58, 204, 59, 42, 30, 146, 77, 44.



Quantification

Plasma **GC-MS** Column: HP Ultra-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 5.0 mL/min. Temperature programme: 200° to 250° at 5°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 µg/L [Sticht, Kaferstein 2000].

HPLC Column: Supersphere 60RP select B (250 × 4 mm i.d., 5 µm). Mobile phase: 0.1 mol/L sodium acetate-0.1 mol/L citric acid-0.03 mmol/L sodium EDTA (pH 4.1) : acetonitrile (83 : 17), flow rate 700 µL/min. Electrochemical detection.

P

Limit of quantification, 10 µg/L [Lindenblatt *et al.* 1998]. Electrochemical detection [Hasler *et al.* 1997].

Urine **GC-MS** Column: DB-5 (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 5 psi (34.9 kPa). Temperature programme: 70° to 240° at 20°/min for 2 min. ECD, SIM acquisition mode. Limit of quantification, 10 µg/L [Grieshaber *et al.* 2001]. See Plasma [Sticht, Kaferstein 2000].

LC-MS Column: Hypersil GOLD (100 × 2.1 mm i.d., 5 µm). Mobile phase: 1% acetonitrile in 10 mmol/L formic acid : 60% acetonitrile in 10 mmol/L formic acid (100 : 0 to 0 : 100 to 100 : 0 at 10 min for 4 min), flow rate 200 µL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: ~5 min. Limit of detection, 4 µg/L [Björnstad *et al.* 2009].

Disposition in the Body Psilocin is absorbed from the gastrointestinal tract. It is a metabolite of psilocybine. An oral dose of several milligrams may produce hallucinations and related effects.

Björnstad K *et al.* (2009). A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1162–1168.
Grieshaber AF *et al.* (2001). The detection of psilocin in human urine. *J Forensic Sci* 46: 627–630.
Hasler F *et al.* (1997). Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* 72: 175–184.
Lindenblatt H *et al.* (1998). Quantitation of psilocin in human plasma by high-performance liquid chromatography and electrochemical detection: comparison of liquid–liquid extraction with automated on-line solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 709: 255–263.
Sticht G, Kaferstein H (2000). Detection of psilocin in body fluids. *Forensic Sci Int* 113: 403–407.

Psilocybine

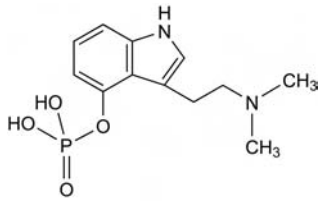
Hallucinogen

$C_{12}H_{17}N_2O_4P$ = 284.3

CAS—520-52-5

IUPAC Name 3-[2-(Dimethylamino)ethyl]-1H-indol-4-ol dihydrogen phosphate ester

Synonyms CY-39; 4-phosphoryloxy-*N*,*N*-dimethyltryptamine; psilocybin.



Chemical Properties The main indole alkaloid present in the sacred Mexican mushroom (teonanácatl), *Psilocybe mexicana* (Agaricaceae). In the UK, psilocybine is present in the indigenous mushroom *Psilocybe semilanceata* (magic mushroom; liberty cap). Psilocybine is also present in the mushrooms *Stropharia cubensis* and *Conocybe* spp. White crystals. Mp 185° to 195° (crystals from boiling methanol), with decomposition. Soluble in 20 parts boiling water and in 120 parts boiling methanol; practically insoluble in chloroform and benzene. Log *P* (octanol/water), 1.00.

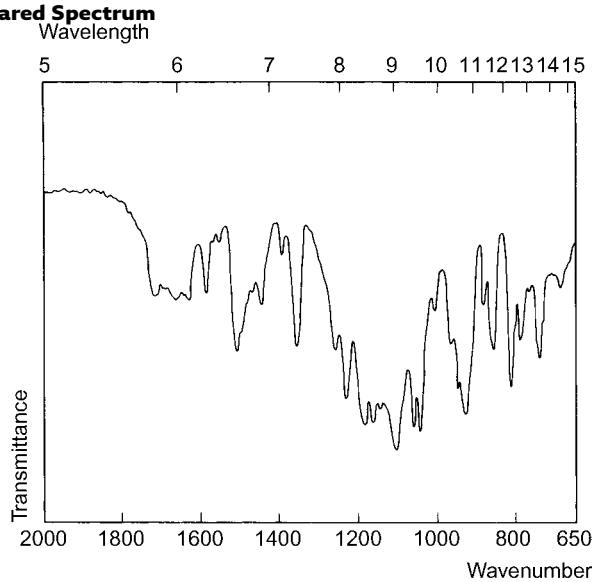
Colour Test Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.05; system TB—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TAE—R_f 0.80; system TAF—R_f 0.01 (van Urk reagent—grey-violet).

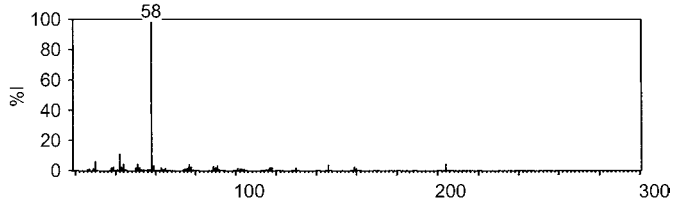
Gas Chromatography System GA—RI 2046; system GAM—RT 6.9 min.

High Performance Liquid Chromatography System HY—RI 185; system HBF—*k* 3.20.

Infrared Spectrum



Mass Spectrum



Quantification

Blood **GC-MS** Column: HP Ultra-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 5.0 mL/min. Temperature programme: 200° to 250° at 5°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 µg/L [Sticht, Kaferstein 2000].

Plasma **HPLC** Column: Superspher 60RP select B (250 × 4 mm i.d., 5 µm). Mobile phase: 0.1 mol/L sodium acetate-0.1 mol/L citric acid-0.03 mmol/L sodium EDTA (pH 4.1) : acetonitrile (83 : 17), flow rate 700 µL/min. Electrochemical detection. Limit of quantification, 10 µg/L [Lindenblatt *et al.* 1998]. Electrochemical detection [Hasler *et al.* 1997].

Urine **GC-MS** Column: DB-5 (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 5 psi (34.9 kPa). Temperature programme: 70° to 240° at 20°/min for 2 min. ECD, SIM acquisition mode. Limit of quantification, 10 µg/L [Grieshaber *et al.* 2001]. See Blood [Sticht, Kaferstein 2000].

Disposition in the Body Psilocybine is absorbed from the gastrointestinal tract; hallucinogenic effects usually occur within 30 min of ingestion with a duration of effect of ~6 h. It is rapidly dephosphorylated to psilocin, which appears to be the psychoactive compound.

Therapeutic Concentration

Six healthy volunteers were administered a single 10–20 mg (mean, 0.22 mg/kg bodyweight) oral dose or a 1 mg IV psilocybin. After the oral dose, the mean peak plasma psilocin concentration was 8.2 µg/L at 105 min and the peak 4-hydroxyindole-3-acetic acid concentration was 150 µg/L at 113 min. After the IV dose, the mean psilocin concentration was 12.9 µg/L 1.9 min after administration [Hasler *et al.* 1997].

Toxicity

In 2 young men who ingested 4–5 g of 'magic mushroom', the majority of psilocybin was metabolised to psilocin and excreted as the glucuronide. Psilocin was detected in serum at a concentration of 0.018 mg/L (free psilocin) and 0.052 mg/L (total). The concentration of free psilocin in urine was 0.23 mg/L, with a total amount of 1.76 mg/L. It was noted that they showed unstable and sluggish walking, inarticulate speech, enlarged pupils, dazed consciousness, depression and retarded behaviour 5–6 h after ingestion [Sticht, Kaferstein 2000].

Note For a study of the renal excretion profiles of psilocin in man following oral administration of psilocybin, see Hasler *et al.* [1997].

Grieshaber AF *et al.* (2001). The detection of psilocin in human urine. *J Forensic Sci* 46: 627–630.
Hasler F *et al.* (1997). Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* 72: 175–184.
Lindenblatt H *et al.* (1998). Quantitation of psilocin in human plasma by high-performance liquid chromatography and electrochemical detection: comparison of liquid–liquid extraction with automated on-line solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 709: 255–263.
Sticht G, Kaferstein H (2000). Detection of psilocin in body fluids. *Forensic Sci Int* 113: 403–407.

Pulegium Oil

Essential Oil

Synonym Pennyroyal oil

Chemical Properties An oil obtained by distillation from fresh pennyroyal herb, *Mentha pulegium* (Labiatae); it contains not less than 85% of pulegone ($C_{10}H_{18}O$=152.2). A yellow to greenish-yellow oil with aromatic mint-like odour. Refractive index 1.482 to 1.487. Slightly soluble in water; soluble 1 in 3 of ethanol (70%); soluble in ether and chloroform. Pulegium oil is volatile and may be isolated by distillation.

Colour Tests Mix a drop with a drop of sulphuric acid—orange; mix a drop with a drop of fuming nitric acid—deep yellow.

Disposition in the Body Pulegone has been shown in rabbits to be reduced first to pulegol and then to menthol; both of these compounds may be excreted as glucuronides.

Toxicity The effect of the oil on a particular individual is unpredictable; a teaspoonful has caused convulsions and 15 mL of pennyroyal essence (about 1 in 10 of the oil) has resulted in coma.

A healthy female, aged 24, in the third month of pregnancy, took pulegium oil (dose unknown). The drug produced abortion, vaginal bleeding, haemolytic anaemia, and acute tunular necrosis of the kidney, with death from uraemia on the 14th day.

Pumactant

Pulmonary Surfactant

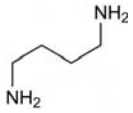
Synonym ALEC

Chemical Properties Dipalmitoylphosphatidylcholine and egg phosphatidylglycerol in a 7:3 (w/w) ratio. Fine crystalline powder.
Note For a study of pumactant for treatment of respiratory distress syndrome in neonates, see Ainsworth *et al.* [2000]; for a review of natural versus synthetic surfactants in neonatal respiratory distress syndrome, see Halliday [1996].

Ainsworth SB *et al.* (2000). Pumactant and poractant alfa for treatment of respiratory distress syndrome in neonates born at 25-29 weeks' gestation: a randomised trial. *Lancet* 355: 1387–1392.
Halliday HL (1996). Natural vs synthetic surfactants in neonatal respiratory distress syndrome. *Drugs* 51: 226–237.

Putrescine

Putrefactive Base
$NH_2[CH_2]_4NH_2 = 88.2$
CAS—110-60-1
IUPAC Name Butane-1,4-diamine
Synonyms 1,4-Butanediamine; tetramethylenediamine.

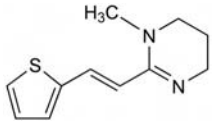


Chemical Properties Crystals with a strong odour. Mp 23° to 24°. Bp 158° to 160°. Very soluble in water. pK_a 10.8 (20°). Log *P* (octanol/water), −0.7.
Colour Test Ninhydrin—violet.
Thin-layer Chromatography System TA—R_f 0.09 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 930.
Infrared Spectrum Principal peaks at wavenumbers 1118, 874, 1288, 923, 1026, 1036 cm^{-1} (KBr disk).
Quantification
Serum **GC** FID. Limit of detection, 0.5 to 0.6 ng/injection for putrescine and cadaverine [Khuhawar *et al.* 1999].
Urine **GC** NPD. For method for quantification of putrescine and other polyamines and their metabolites, see Muskiet *et al.* [1984].
HPLC Limit of detection, 2 to 10 µg/L for putrescine and other polyamines [Molins-Legua *et al.* 1999].
Cancer Cell Homogenates **HPLC** Fluorescence detection. Putrescine and other natural polyamines and monoacetyl conjugates. Limit of detection, 30 to 130 fmol for putrescine [Weiss *et al.* 1997].

Khuhawar MY *et al.* (1999). Capillary gas chromatographic determination of putrescine and cadaverine in serum of cancer patients using trifluoroacetylacetone as derivatizing reagent. *J Chromatogr B, Biomed Sci Appl* 723: 17–24.
Molins-Legua C *et al.* (1999). Urine polyamines determination using dansyl chloride derivatization in solid-phase extraction cartridges and HPLC. *Analyst* 124: 477–482.
Muskiet FA *et al.* (1984). Total polyamines and their non-alpha-amino acid metabolites simultaneously determined in urine by capillary gas chromatography, with nitrogen-phosphorus detector; and some clinical applications. *Clin Chem* 30: 687–695.
Weiss T *et al.* (1997). High-resolution reversed-phase high-performance liquid chromatography analysis of polyamines and their monoacetyl conjugates by fluorescence detection after derivatization with N-hydroxysuccinimidyl 6-quinolinyl carbamate. *Anal Biochem* 247: 294–304.

Pyrantel

Anthelmintic
$C_{11}H_{14}N_2S = 206.3$
CAS—15686-83-6
IUPAC Name 1-Methyl-2-[(*E*)-2-thiophen-2-ylethenyl]-5,6-dihydro-4*H*-pyrimidine
Synonyms Pirantel; (*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl] pyrimidine.



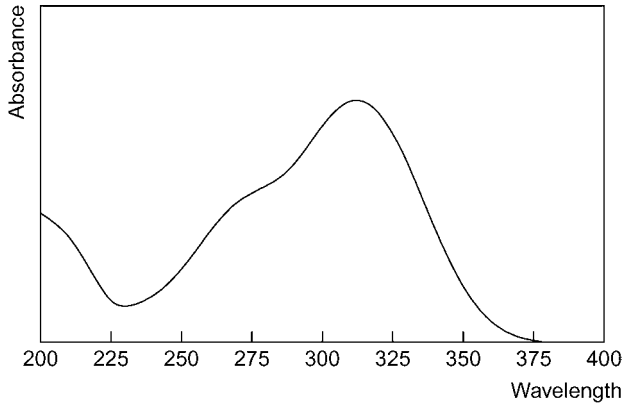
Chemical Properties Crystals. Mp 178° to 179°. pK_a 11.0 (20°). Log *P* (octanol/water), 3.1.

Pyrantel Embonate

$C_{11}H_{14}N_2S,C_{23}H_{16}O_6 = 594.7$
CAS—22204-24-6
Synonyms Pyrantel pamoate; CP-10423-16.
Proprietary Names *Anthel*; *Antiminth*; *Ascarical*; *Banminth* (vet.); *Bantel*; *Canex* (vet.); *Combantrin*; *Early Bird*; *Helmex*; *Helmintox*; *Lombriareu*; *Nemex* (vet.); *Pin-Rid*; *Pin-X*; *Pirantrim*; *Pyral*; *Pyrantrin*; *Pyrapam*; *Reese's Pinworm*; *Strongid* (vet.); *Trilombrin*.
Chemical Properties A yellow to tan-coloured crystalline powder. Practically insoluble in water, ethanol and methanol; soluble in dimethyl sulfoxide.

Pyrantel Tartrate

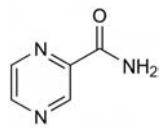
$C_{11}H_{14}N_2S,C_4H_6O_6 = 356.4$
CAS—33401-94-4
Synonym CP-10423-18
Chemical Properties A white to pale greenish-yellow, crystalline powder. Mp 148° to 150°. Soluble 1 in 5 of water and 1 in 9 of methanol; slightly soluble in chloroform; practically insoluble in ether.
Colour Tests Liebermann's reagent—black; Mandelin's test—violet; Marquis test—blue-violet; sulfuric acid—orange→violet.
Thin-layer Chromatography System TA—R_f 0.06 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HX—RI 299.
Ultraviolet Spectrum Methanolic acid—315 nm (A^1_1=920a).



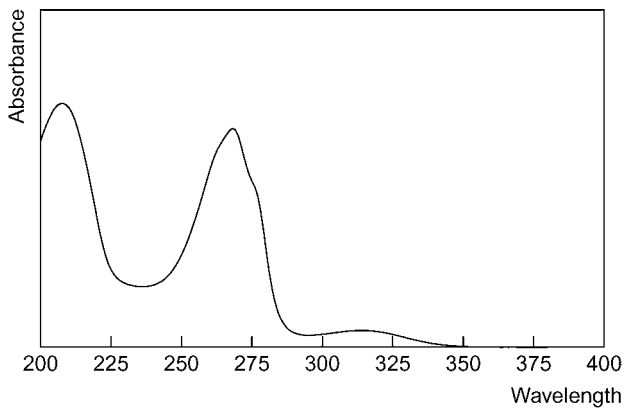
Infrared Spectrum Principal peaks at wavenumbers 1640, 1605, 1305, 1208, 1132, 1065 cm^{-1} (pyrantel tartrate, KBr disk).
Mass Spectrum Principal ions at *m/z* 205, 42, 135, 173, 206, 123, 145, 45.
Dose The equivalent of 10 to 20 mg/kg of pyrantel, as a single dose.

Pyrazinamide

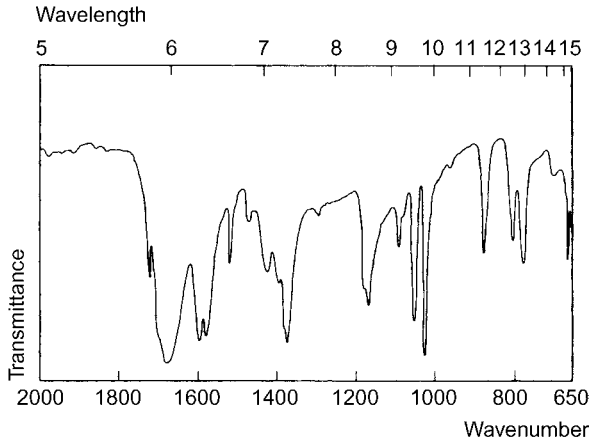
Tuberculostatic
$C_5H_5N_3O = 123.1$
CAS—98-96-4
IUPAC Name Pyrazine-2-carboxamide
Synonyms Pyrazinecarboxamide; pyrazinoic acid amide; D-50.
Proprietary Names *Braccopiral*; *Pirafoid*; *Piraldina*; *Piraside*; *Pirazer*; *Pirilene*; *Pramide*; *Premox*; *Pyrafat*; *Pyratab*; *PZA*; *Rozide*; *Tebezide*; *Tebrazid*; *Tisamid*; *Zinamide*.



Chemical Properties A white crystalline powder. Mp 189° to 191°; begins to sublime at 60°. Soluble 1 in about 60 of water and 1 in 175 of dehydrated ethanol, 1 in 135 of chloroform, 1 in 1000 of ether and 1 in 72 of methanol. pK_a 0.5. Log *P* (octanol/water), −0.6.
Colour Test Nessler's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.03; system TC—R_f 0.42; system TE—R_f 0.54; system TL—R_f 0.46; system TAD—R_f 0.44; system TAE—R_f 0.71; system TAF—R_f 0.70.
Gas Chromatography System GA—RI 1250.
High Performance Liquid Chromatography System HAA—retention time 3.8 min.
Ultraviolet Spectrum Aqueous acid—269 (A^1_1=659a), 312 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1685, 1020, 1600, 1584, 1050, 1165 cm^{-1} (KBr disk).



Quantification

Blood **HPLC** UV detection. Limit of detection, 0.11 ng/5 µL for pyrazinamide, 0.2 ng/5 µL for isoniazid and 13 ng/5 µL for rifampicin [Khuhawar, Rind 2002].

Plasma **HPLC** UV detection. For method, see Conte *et al.* [2000]. For method for quantification of pyrazinamide, rifampicin and isoniazid, see Smith *et al.* [1999]. For method for quantification of pyrazinamide and its metabolites, see Kraemer *et al.* [1998]. Limit of detection, 0.2 mg/0.2 mL [Revankar *et al.* 1994]. UV detection. For method for quantification of pyrazinamide, rifampicin and isoniazid, see Walubo *et al.* [1994].

Serum **GC–MS** Pyrazinamide, pyrazine-2-carboxylic acid and 5-hydroxypyrazine-2-carboxylic acid. Limit of detection, 10 µg/L for pyrazinamide [Roboz *et al.* 1978].

HPLC Limit of detection, about 90 µg/L for pyrazinamide and other antituberculosis drugs [Gennaro *et al.* 2001].

Urine **GC–MS** Limit of detection, about 90 µg/L for pyrazinamide and other antituberculosis drugs [Gennaro *et al.* 2001].

Bronchoalveolar Lavage **HPLC** UV detection. For method, see Conte *et al.* [2000].

Alveolar Cells **HPLC** UV detection. For method, see Conte *et al.* [2000].

Disposition in the Body Pyrazinamide is well absorbed after oral administration. It is metabolised by hydrolysis to pyrazine-2-carboxylic acid and subsequent hydroxylation to 5-hydroxypyrazine-2-carboxylic acid. In 24 h, about 30 to 40% of a dose is excreted in the urine as pyrazine-2-carboxylic acid and <4% as unchanged drug; the 5-hydroxy metabolite is also excreted in the urine.

Therapeutic Concentration

After an oral dose of 3 g to one subject, a peak serum concentration of 134 mg/L was attained in 1 h; the serum concentration of pyrazine-2-carboxylic acid was 20 to 30 mg/L after the first hour and the concentration of 5-hydroxypyrazine-2-carboxylic acid ranged between 3 and 8 mg/L over a period of 12 h [Roboz *et al.* 1978].

Half-life Plasma half-life, 4 to 10 h.

Protein Binding About 50%.

Dose 20 to 35 mg/kg to a maximum of 3 g daily.

Conte JE *et al.* (2000). High-performance liquid chromatographic determination of pyrazinamide in human plasma, bronchoalveolar lavage fluid, and alveolar cells. *J Chromatogr Sci* 38: 33–37.

Gennaro MC *et al.* (2001). Ion interaction reagent reversed-phase high-performance liquid chromatography determination of anti-tuberculosis drugs and metabolites in biological fluids. *J Chromatogr B Biomed Sci Appl* 754: 477–486.

Khuhawar MY, Rind FM (2002). Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 357–363.

Kraemer HJ *et al.* (1998). Quantification of pyrazinamide and its metabolites in plasma by ionic-pair high-performance liquid chromatography. Implications for the separation mechanism. *J Chromatogr B Biomed Sci Appl* 706: 319–328.

Revankar SN *et al.* (1994). Determination of pyrazinamide in human by high performance liquid chromatography. *J Postgrad Med* 40: 7–9.

Roboz J *et al.* (1978). Mass fragmentographic determination of pyrazinamide and its metabolites in serum and urine. *J Chromatogr* 147: 337–347.

Smith PJ *et al.* (1999). Determination of rifampicin, isoniazid and pyrazinamide by high performance liquid chromatography after their simultaneous extraction from plasma. *Int J Tuberc Lung Dis* 3: 3S325–S328 and S351–S352.

Walubo A *et al.* (1994). Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography. *J Chromatogr B Biomed Appl* 658: 391–396.

Pyrazophos

Fungicide

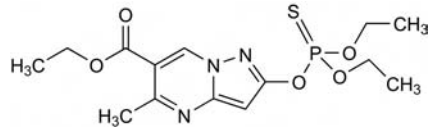
$C_{14}H_{20}N_3O_5PS = 373.4$

CAS—13457-18-6

IUPAC Name Ethyl 2-diethoxyphosphinothioyloxy-5-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate

Synonyms 2-[(Diethoxyphosphinothioyl)oxy]-5-methylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic acid ethyl ester; HOE 2873; W11099.

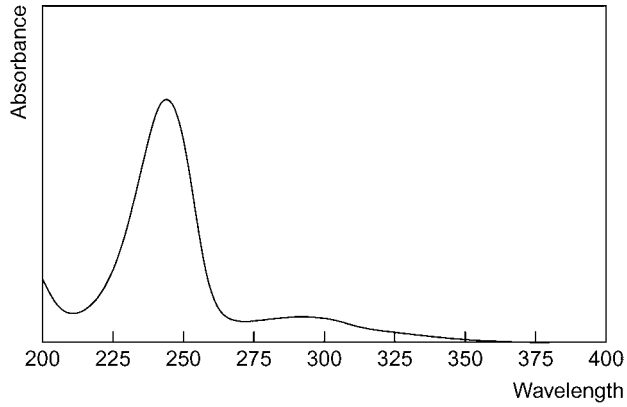
Proprietary Names *Afugan*; *Curamil*; *Missile*; *Pokon Mildew Spray*; *Siafos*.



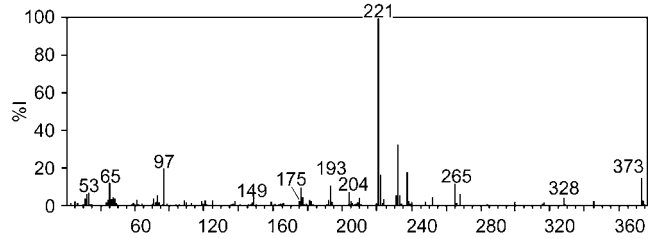
Chemical Properties A yellow oil. Mp 38° to 40°. Soluble in water (4.2 mg/L at 20°), acetone (1212 g/L at 25°), toluene (>980 g/L at 25°), ethyl acetate (898 g/L at 25°), ethanol (95 g/L at 25°) and hexane (11 g/L at 25°); readily soluble in most organic solvents. Log *P* (octanol/water), 3.80.

Thin-layer Chromatography System TX—R_f 0.32; system TY—R_f 0.47.

Gas Chromatography System GA—RI 2590.



Mass Spectrum Principal ions at *m/z* 221, 232, 97, 237, 373, 65, 265, 193.



Disposition in the Body

Toxicity The allowed daily intake is 0.004 mg/kg bodyweight.

Pyrethrum

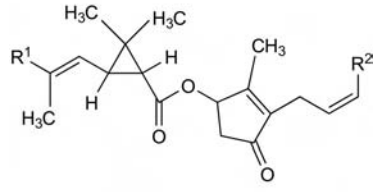
Insecticide, Scabicide

$C_{(20-22)}H_{(28,30)}O_{(3,5)} = 316–374$

CAS—8000-33-47

Synonyms Pyrethrum flower; chrysantheme insecticide; insektenbluten; pyrethriflos.

Proprietary Names *Allethrin*; *Anerin*; *Buhach*; *Chrysanthenum Cinerariaefolium*; *Cypermethrin*; *Dalmatian Insect Powder*; *Decamethrin*; *Firmotox*; *Flucythrinate*; *Moskill*; *Ofirmotox*; *Parexan*; *Persian Insect Powder*; *Piretro*; *Prothrin*; *Resmethrin*.



	R^1	R^2
Pyrethrin-I	CH_3	$CH{=}CH_2$
Pyrethrin-II	$COOCH_3$	$CH{=}CH_2$
Cinerin-I	CH_3	CH_3
Cinerin-II	$COOCH_3$	CH_3

Chemical Properties Pyrethrum extract is a mixture of three naturally occurring esters of chrysanthemic acid (Pyrethrins I: pyrethrin 1, cinerin 1 and jasmolin

1) and three esters of pyrethric acid (Pyrethrins II: pyrethrin 2, cinerin 2 and jasmolin 2) with the molecular weight varying between 316 and 374. It is a pale yellow to brown viscous liquid or oil. Practically insoluble in water but soluble in liquid paraffin and most organic solvents including alcohol, kerosene, nitromethane, petroleum ether, carbon tetrachloride and ethylene dichloride.

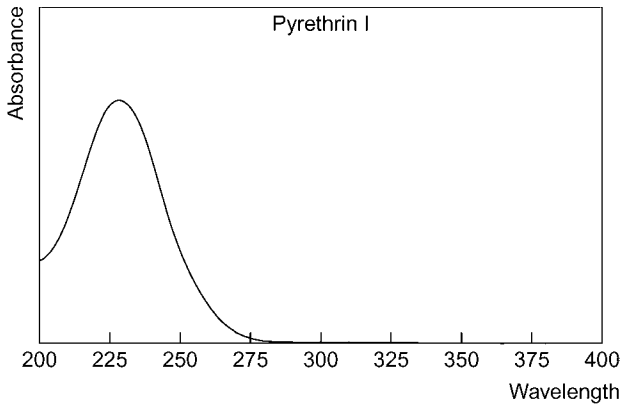
Pyrethrin I

$C_{21}H_{28}O_3 = 328.5$
CAS—121-21-1
Synonyms Chrysanthemummonocarboxylic acid pyrethrolone ester; pyrethronyl (+)-*trans*-chrysanthemate.
Chemical Properties A viscous liquid with solubility as for the pyrethrum extract.

Pyrethrin II

$C_{22}H_{28}O_5 = 372.5$
CAS—121-29-9
Synonyms Pyrethrine; pyrethronyl (+)-pyrethrate.
Chemical Properties A viscous liquid with solubility as for the pyrethrum extract.

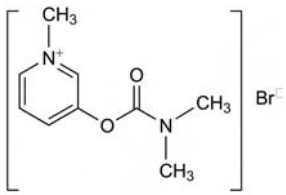
Gas Chromatography System GK—Pyrethrin I RRT 1.27, pyrethrin II RRT 1.59 (both relative to caffeine).
Ultraviolet Spectrum Ethanol—225 nm (Pyrethrin I) and 229 nm (Pyrethrin II).



Disposition in the Body Pyrethrum is absorbed through the stomach, intestines and the skin fairly slowly and more quickly through the lungs during respiration. It is metabolised to chrysanthemumic acid and alcohol which can be further metabolised to aldehyde and acid or conjugated with glucuronide. It is excreted in urine and faeces. The metabolites are less toxic than the parent compound. Pyrethrins I and II are excreted unchanged in faeces and the other pyrethrum components undergo rapid destruction and detoxification in the liver and gastrointestinal tract.
Toxicity The lowest lethal oral dose for children is 750 mg/kg bodyweight and for adults, 1000 mg/kg. The allowed daily intake is 0.04 mg/kg bodyweight.

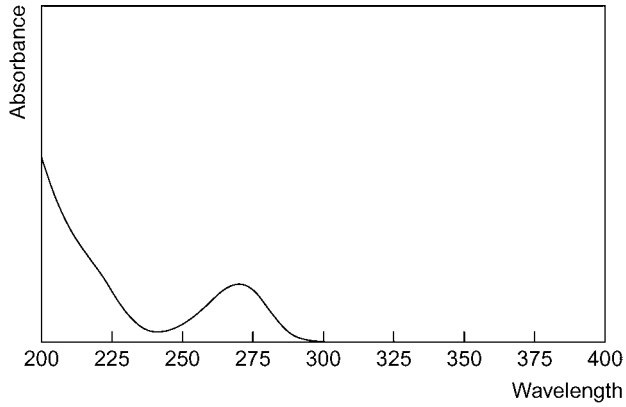
Pyridostigmine Bromide

Anticholinesterase
$C_9H_{13}BrN_2O_2 = 261.1$
CAS—155-97-5 (pyridostigmine); 101-26-8 (bromide)
IUPAC Name (1-Methylpyridin-1-ium-3-yl) *N*,*N*-dimethylcarbamate
Synonym 3-[[(Dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide
Proprietary Names *Mestinon*; *Regonol*.

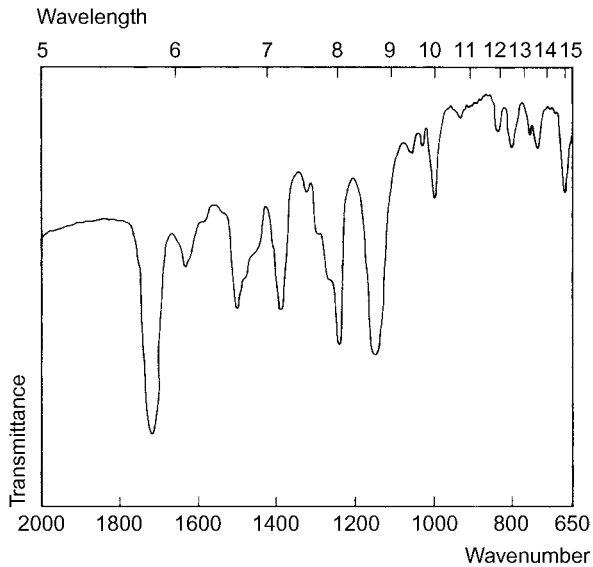


Chemical Properties A white, deliquescent, crystalline powder. Mp 152° to 154°. Soluble 1 in >1 of water, 1 in >1 of ethanol and 1 in 1 of chloroform; practically insoluble in ether, acetone and benzene. Log *P* (octanol/water), −3.7.
Thin-layer Chromatography System TA—R_f 0.04; system TB—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.00 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1515.
High Performance Liquid Chromatography System HA—*k* 6.3 (tailing peak); system HX—RI 65; system HZ—retention time 1.8 min; system HAA—retention time 3.2 min.

Ultraviolet Spectrum Aqueous acid—270 nm (A_1^1=186a).



Infrared Spectrum Principal peaks at wavenumbers 1728, 1156, 1249, 1500, 1630, 1010 cm^{-1} (KBr disk).



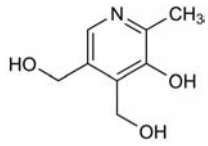
Mass Spectrum Principal ions at *m/z* 72, 39, 42, 38, 94, 56, 51, 81.
Quantification
Blood **HPLC** Limit of detection, 40 µg/L [Ellin *et al.* 1982].
Plasma **GC** AFID. Limit of detection, 5 µg/L [Chan *et al.* 1976].
GC–MS Limit of detection, 50 µg/L [Aquilonius *et al.* 1980].
HPLC–MS Limit of detection, 1 µg/L [Malcolm *et al.* 1990].
Urine **HPLC** Limit of detection, 40 µg/L [Ellin *et al.* 1982].
Disposition in the Body Pyridostigmine bromide is poorly and irregularly absorbed after oral administration and may undergo significant first-pass metabolism. Up to about 16% of an oral dose is excreted in the urine unchanged, together with small amounts of 3-hydroxy-*N*-methylpyridinium. After IV administration, up to about 90% is excreted in the urine unchanged.
Therapeutic Concentration In plasma, usually in the range 0.05 to 0.1 mg/L. After a single oral dose of 120 mg to 5 subjects, peak plasma concentrations of about 0.04 to 0.07 mg/L were attained in 1 to 2 h [Aquilonius *et al.* 1980].
Following daily oral doses of 60 to 660 mg to 6 subjects, maximum steady-state plasma concentrations of 0.04 to 0.08 mg/L were reported [Calvey, Chan 1977].
Half-life Plasma half-life, 0.3 to 2 h, increased in subjects with renal disease.
Volume of Distribution About 1 L/kg.
Clearance Plasma clearance, about 8 to 16 mL/min/kg.
Dose Usually 0.3 to 1.2 g daily.

Aquilonius SM *et al.* (1980). Pharmacokinetics and oral bioavailability of pyridostigmine in man. *Eur J Clin Pharmacol* 18: 423–428.
Calvey TN, Chan K (1977). Plasma pyridostigmine levels in patients with myasthenia gravis. *Clin Pharmacol Ther* 21: 187–193.
Chan K *et al.* (1976). A quantitative gas-liquid chromatographic method for the determination of neostigmine and pyridostigmine in human plasma. *J Chromatogr* 120: 349–358.
Ellin RI *et al.* (1982). Method for isolation and determination of pyridostigmine and metabolites in urine and blood. *J Chromatogr* 228: 235–244.
Malcolm SL *et al.* (1990). Thermospray mass spectrometer as a quantitative specific, sensitive, detector for liquid chromatography. Its application to the analysis of pyridostigmine in human plasma. *J Pharm Biomed Anal* 8: 771–776.

Pyridoxine

Vitamin
$C_8H_{11}NO_3$ = 169.2
CAS—65-23-6

IUPAC Name 4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol
Synonyms Adermine; 5-hydroxy-6-methyl-3,4-pyridinedimethanol; pyridoxol; vitamin B_6.

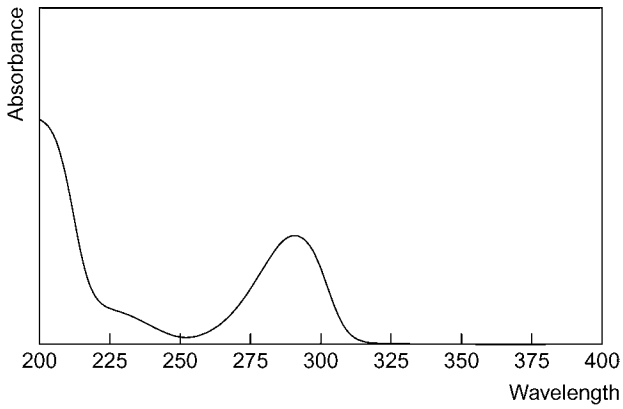


Chemical Properties pK_a 5.0 (–N), 9.0 (–OH), (25°). Log *P* (octanol/water), −0.8 (pyridoxine).

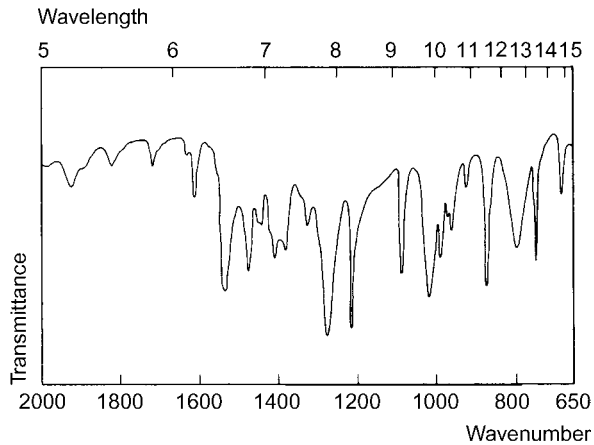
Pyridoxine Hydrochloride

$C_8H_{11}NO_3$,HCl = 205.6
CAS—58-56-0
Proprietary Names *Aminoxin*; *Anacrodyne*; *Bécilan*; *Bedoxine*; *Beesix*; *Benadon*; *Bonasanit*; *Carthamex*; *Complument*; *Conductasa*; *Dermo 6*; *Heksavit*; *Hexobion*; *Lactosec*; *Memosprint*; *Metadoxil*; *Pyroxin*; *Surfoxide*; *Vicotrat*; *Vita-B6*; *Xanturenasi*.
Chemical Properties A white crystalline powder or crystals. Mp 205° to 212°, with decomposition. Soluble 1 in 5 of water and 1 in about 90 of ethanol; practically insoluble in chloroform and ether; sparingly soluble in acetone.

Colour Test Mandelin's Test—blue→grey-green.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.00; system TC—R_f 0.08; system TE—R_f 0.15; system TL—R_f 0.05; system TAE—R_f 0.75; system TAF—R_f 0.67 (acidified potassium permanganate solution, positive).
High Performance Liquid Chromatography System HAA—retention time 2.9 min; system HX—RI 65.
Ultraviolet Spectrum Aqueous acid—290 nm (A^{1}_{1}=523a); phosphate buffer (pH 6.88)—254 nm (A^{1}_{1}=219a), 324 (A^{1}_{1}=426a).



Infrared Spectrum Principal peaks at wavenumbers 1277, 1212, 1015, 1540, 870, 1086 cm^{-1} (pyridoxine hydrochloride, KBr disk).



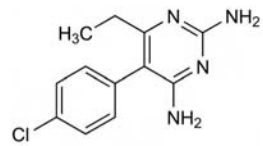
Mass Spectrum Principal ions at *m/z* 151, 94, 122, 106, 51, 53, 149, 150.
Quantification
Plasma **HPLC** Fluorescence detection. For method for quantification of pyridoxine, pyridoxal 5′-phosphate, pyidoxal, pyridoxamine and pyridoxamine 5′-phosphate, see Bisp *et al.* [2002]. Fluorescence detection. For method for quantification of pyridoxal and pyridoxal 5′-phosphate, see Kimura *et al.* [1996]. Fluorescence detection. Limit of detection, 50 fmol for pyridoxal 5′-phosphate [Kurioka *et al.* 1993]. Fluorescence detection. Limit of detection, 2 µg/L for pyridoxal [Mascher 1993]. Fluorescence detection. For method for quantification of pyridoxal 5′-phosphate, see Hirose *et al.* [1990]. UV detection. Limit of detection, 300 µg/L for pyridoxine, pyridoxal and 4-pyridoxic acid [O'Reilly *et al.* 1980].
Urine **HPLC** UV detection. Limit of detection, 500 µg/L for pyridoxine, pyridoxal and 4-pyridoxic acid [O'Reilly *et al.* 1980].
Disposition in the Body Pyridoxine is absorbed from the gastro-intestinal tract and converted to the active form, pyridoxal phosphate. It is excreted in the urine, mainly as 4-pyridoxic acid.
Protein Binding In plasma, pyridoxine not significantly bound, pyridoxal phosphate almost completely bound.
Dose 20 to 200 mg of pyridoxine hydrochloride daily.

Bisp MR *et al.* (2002). Determination of vitamin B6 vitamers and pyridoxic acid in plasma: development and evaluation of a high-performance liquid chromatographic assay. *Anal Biochem* 305: 82–89.
Hirose N *et al.* (1990). Highly sensitive determination of PLP in human plasma with HPLC method. *J Nutr Sci Vitaminol (Tokyo)* 36: 521–529.
Kimura M *et al.* (1996). Highly sensitive and simple liquid chromatographic determination in plasma of B6 vitamers, especially pyridoxal 5′-phosphate. *J Chromatogr A* 722: 296–301.
Kurioka S *et al.* (1993). Assay of vitamin B6 in human plasma with graphitic carbon column. *Biomed Chromatogr* 7: 162–165.
Mascher H (1993). Determination of total pyridoxal in human plasma following oral administration of vitamin B6 by high-performance liquid chromatography with post-column derivatization. *J Pharm Sci* 82: 972–974.
O'Reilly WJ *et al.* (1980). High-performance liquid chromatographic determination of pyridoxine and congeners in biological fluids of man after high-dose therapy. *J Chromatogr* 183: 492–498.

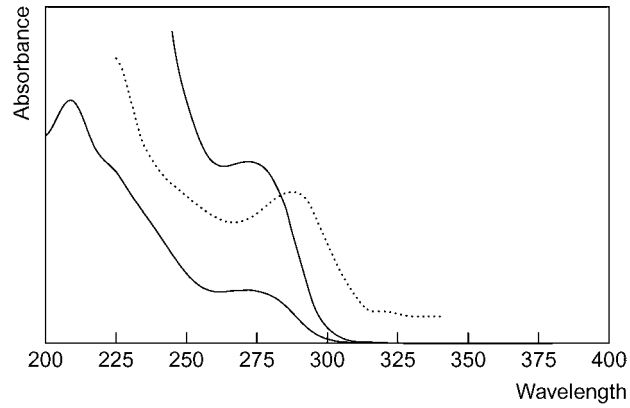
Pyrimethamine

Antimalarial
$C_{12}H_{13}ClN_4$ = 248.7
CAS—58-14-0

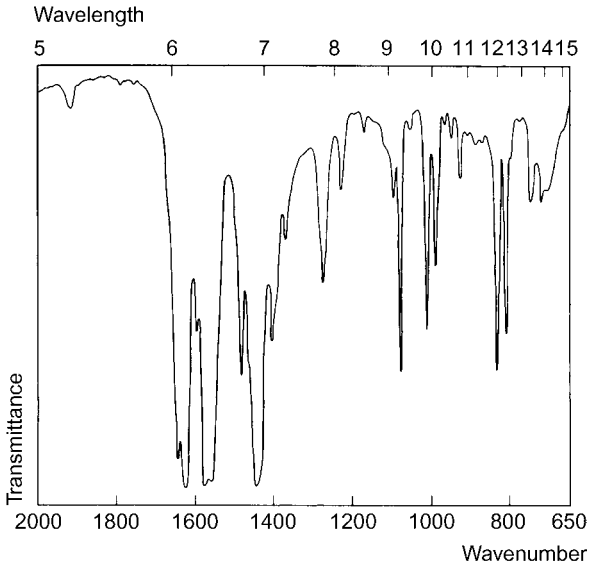
IUPAC Name 5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine
Synonym 5-(4-Chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
Proprietary Names *Daraprim*; *Erbaprelina*; *Malocide*. It is an ingredient of *Fansidar*, *Maloprim*, *Madomine*; *Supacox* (vet.), *Vivaxine* and *Whitsyn S* (vet.).



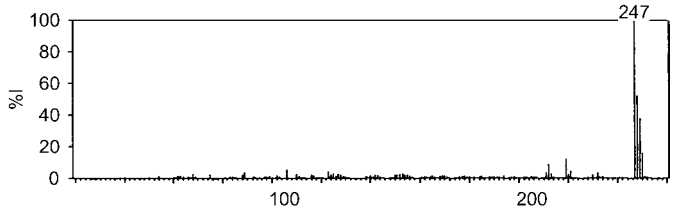
Chemical Properties A white crystalline powder. Mp 233°; Mp 240° to 242° (copper block). Practically insoluble in water; soluble 1 in 200 of ethanol and 1 in 125 of chloroform; slightly soluble in acetone; soluble in warm dilute mineral acids. pK_a 7.3 (20°). Log *P* (octanol/water), 2.7.
Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.02; system TC—R_f 0.31; system TE—R_f 0.58; system TL—R_f 0.21; system TAE—R_f 0.66 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2138.
High Performance Liquid Chromatography System HA—*k* 1.0; system HY—RI 289; system HAA—RT 12.5 min.
Ultraviolet Spectrum Aqueous acid—272 nm (A^{1}_{1}= 320a); aqueous alkali—286 nm (A^{1}_{1}= 381b).



Infrared Spectrum Principal peaks at wavenumbers 1628, 1575, 1640, 1075, 835, 805 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 247, 248, 249, 250, 219, 212, 106, 221.



Quantification

Blood **HPLC** Limit of detection, 1–10 mg/L when sample dried on filter paper [Green *et al.* 2002].

Plasma **GC** ECD. Limit of detection, 5 µg/L [Midskov 1984].

HPLC UV detection. Pyrimethamine and sulfadoxine. Limit of detection, 10 µg/L for pyrimethamine [Astier *et al.* 1997]. UV detection. Limit of detection, 3 µg/L [Na-Bangchang *et al.* 1997]. UV detection. Pyrimethamine and other antimalarials. Limit of detection, 15 µg/L for pyrimethamine [Eljaschewitsch *et al.* 1996]. Enzyme inhibition analysis. For method, see Roberts *et al.* [1995]. UV detection. For method for quantification of pyrimethamine, sulfadoxine and mefloquine, see Bergqvist *et al.* [1991]. Limit of detection, 1 µg/L [Le Liboux *et al.* 1991]. UV detection. Limit of detection, 10 µg/L for pyrimethamine and mefloquine [Guenzi *et al.* 1989]. Fluorescence detection. Limit of detection, 10 µg/L [Timm, Weidekamm 1982].

Urine **GC** ECD. Limit of detection, 5 µg/L [Midskov 1984].

Disposition in the Body Pyrimethamine is well absorbed after oral administration, and very slowly excreted in the urine, ≈20% of a dose being excreted in 7 days.

Therapeutic Concentration

A single oral dose of 50 mg given to 5 subjects produced a peak plasma concentration of 0.21–0.43 (mean 0.34) mg/L in 2–4 h, followed by a slow decline over several days [Donno *et al.* 1980].

Six subjects given 8 weekly doses of 12.5 mg pyrimethamine plus 100 mg dapsone, had a pyrimethamine peak plasma concentrations of 116.2 µg/L 3.7 h after the last dose [Edstein *et al.* 1990].

In 3 severely ill patients with advanced AIDS (acquired immunodeficiency syndrome), maximum plasma pyrimethamine concentrations were 254, 34 and 610 µg/L after receiving maintenance doses of 0.5, 1.4 and 1.6 mg/kg, respectively, for cerebral toxoplasmosis (lower end of therapeutic range: 750 mg/L) [Winstanley *et al.* 1995].

Toxicity Children have died following ingestion of 0.4–1 g.

Accidental prolonged overdosage with pyrimethamine (30 mg daily instead of the usual dose of 3 mg daily, for 10 days) in a 7-week-old infant treated for toxoplasmosis produced initial plasma concentrations of 6.22 mg/L. Plasma levels were still within the normal therapeutic range 7 days after stopping therapy [Tracqui *et al.* 1993].

Half-life Plasma half-life, about 4 days.

Dose Up to 75 mg daily.

Astier H *et al.* (1997). Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction. *J Chromatogr B, Biomed Sci Appl* 698: 217–223.

Bergqvist Y *et al.* (1991). Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma. *J Chromatogr* 571: 169–177.

Donno L *et al.* (1980). *Curr Ther Res* 27: 346–355.

Edstein MD *et al.* (1990). Multiple-dose pharmacokinetics and in vitro antimalarial activity of dapsone plus pyrimethamine (Maloprim) in man. *Br J Clin Pharmacol* 30: 259–265.

Eljaschewitsch J *et al.* (1996). High-performance liquid chromatography determination of pyrimethamine, dapsone, monoacetyldapsone, sulfadoxine, and N-acetyl-sulfadoxine after rapid solid-phase extraction. *Ther Drug Monit* 18: 592–597.

Green MD *et al.* (2002). High-performance liquid chromatographic assay for the simultaneous determination of sulfadoxine and pyrimethamine from whole blood dried onto filter paper. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 159–162.

Guenzi A *et al.* (1989). Simultaneous determination of pyrimethamine and mefloquine in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 494: 219–230.

Le Liboux A *et al.* (1991). Pharmacokinetics of pyrimethamine in healthy young volunteers using a new solid phase extraction/HPLC method. *Eur J Drug Metab Pharmacokinet* Spec No 3: 284–290.

Midskov C (1984). Rapid gas chromatographic determination of pyrimethamine in human plasma and urine. *J Chromatogr* 306: 388–393.

Na-Bangchang K *et al.* (1997). Alternative method for determination of pyrimethamine in plasma by high-performance liquid chromatography. *J Chromatogr B, Biomed Sci Appl* 689: 433–437.

Roberts WL *et al.* (1995). Pyrimethamine analysis by enzyme inhibition and HPLC assays. *Am J Clin Pathol* 104: 82–88.

Timm U, Weidekamm E (1982). Determination of pyrimethamine in human plasma after administration of fansidar of fansidar-mefloquine by means of high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 230: 107–114.

Tracqui A *et al.* (1993). Nonfatal prolonged overdosage of pyrimethamine in an infant: measurement of plasma and urine levels using HPLC with diode-array detection. *J Anal Toxicol* 17: 248–250.

Winstanley P *et al.* (1995). Marked variation in pyrimethamine disposition in AIDS patients treated for cerebral toxoplasmosis. *J Antimicrob Chemother* 36: 435–439.

Pyriproxyfen

Insecticide

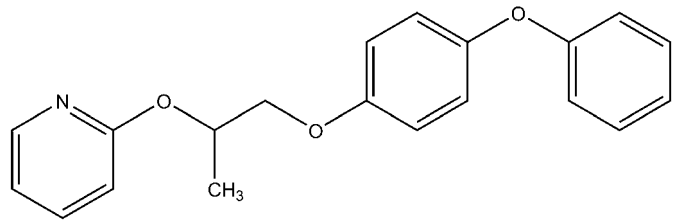
$C_{20}H_{19}NO_3$ = 321.4

CAS—95737-68-1

IUPAC Name 2-[1-[4-(Phenoxy)phenoxy]propan-2-yloxy]pyridine

Synonyms 2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine; 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether; S-9318; S-31183.

Proprietary Name *Sumilarv*



Chemical Properties A pale yellow liquid. Mp 46°. Log *P* (octanol/water), 5.55 [Meylan, Howard 1995].

Quantification

Other **LC-MS** Fruit. Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium formate in methanol : 10 mmol/L ammonium formate in water (70 : 30 to 90 : 10 in 10 min for 10 min to 70 : 30 at 30 min), flow rate 0.6 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 µg/kg [Soler *et al.* 2007]. Column: Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (70 : 30 to 90 : 10 at 35 min), flow rate 0.6 mL/min. ESI, positive ion mode, full scan mode. Limit of quantification, 0.2 mg/kg; limit of detection, 0.05 mg/L [Soler *et al.* 2005]. Column: Phenomenex Luna C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (70 : 30 for 5 min to 90 : 10 from 20 to 40 min), flow rate 0.6 mL/min. ESI, positive ion mode, full scan and MRM acquisition mode. Limit of quantification, 0.05 mg/kg [Soler *et al.* 2004]. Column: Luna C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (40 : 60 for 5 min to 80 : 20 at 8 min for 10 min to 90 : 10 at 20 min for 5 min), flow rate 0.8 mL/min. APCI, positive ion mode, full scan and SIM acquisition mode. Limit of quantification, 8 µg/kg [Blasco *et al.* 2002].

CE Fruit and Vegetables. Capillary: fused silica (total/effective length 60/50 cm, 75 µm i.d.). Buffer: 20 mmol/L phosphate buffer (pH 2.3) containing 25 mmol/L SDS and 10% methanol. DAD (λ = 230 nm). Limit of quantification, 8, 6, and 7 µg/kg for sweeping, normal stacking with reversed migration and water plug, and stacking with reversed migration and removal of sample matrix using polarity switching, respectively. Limit of detection, 0.3, 0.04, 0.01 and 0.01 mg/L for conventional injection, sweeping, normal stacking with reversed migration and water plug, and stacking with reversed migration and removal of sample matrix using polarity switching, respectively [Juan-Garcia *et al.* 2007]. Capillary: fused silica (total/effective length 57/50 cm, 75 µm). Buffer: 6 mmol/L sodium tetraborate decahydrate containing 75 mmol/L cholic acid sodium solution (pH 9.2). DAD (λ = 214 nm). Limit of quantification, 0.5 mg/kg; limit of detection, 0.1 mg/L for solid-phase extraction [Juan-Garcia *et al.* 2005].

Blasco C *et al.* (2002). Comparison of microextraction procedures to determine pesticides in oranges by liquid chromatography–mass spectrometry. *J Chromatogr A* 970: 201–212.

Juan-Garcia A *et al.* (2005). Capillary electrophoresis for analyzing pesticides in fruits and vegetables using solid-phase extraction and stir-bar sorptive extraction. *J Chromatogr A* 1073: 229–236.

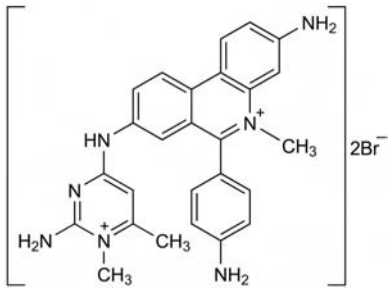
Juan-Garcia A *et al.* (2007). On-line preconcentration strategies for analyzing pesticides in fruits and vegetables by micellar electrokinetic chromatography. *J Chromatogr A* 1153: 104–113.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.
Soler C *et al.* (2004). Liquid chromatography–electrospray quadrupole ion-trap mass spectrometry of nine pesticides in fruits. *J Chromatogr A* 1048: 41–49.
Soler C *et al.* (2005). Routine application using single quadrupole liquid chromatography–mass spectrometry to pesticides analysis in citrus fruits. *J Chromatogr A* 1088: 224–233.
Soler C *et al.* (2007). Capabilities of different liquid chromatography tandem mass spectrometry systems in determining pesticide residues in food. Application to estimate their daily intake. *J Chromatogr A* 1157: 73–84.

Pyritidium Bromide

Trypanocide (Veterinary)
$C_{26}H_{27}Br_2N_7$ = 597.4
CAS—3616-05-5 (pyritidium); 14222-46-9 (bromide)

IUPAC Name 8-[(2-Amino-1,6-dimethylpyrimidin-4-ylidene)amino]-6-(4-aminophenyl)-5-methylphenanthridin-5-ium-3-amine bromide hydrobromide
Synonyms 3-Amino-8-(2-amino-1,6-dimethylpyrimidinium-4-ylamino)-6-(4-amino-phenyl)-5-methylphenanthridinium dibromide; pyrithidium bromide.

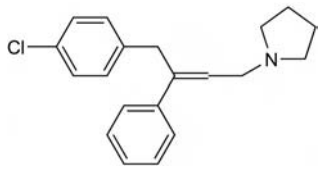


Chemical Properties A brick-red to reddish-purple, hygroscopic powder. Soluble 1 in 40 of water and 1 in 1900 of ethanol.
Thin-layer Chromatography System TA—R_f 0.01 (location under ultraviolet light, yellow fluorescence; Acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—256 (A^1_1=425a), 313 nm (A^1_1=813a).
Infrared Spectrum Principal peaks at wavenumbers 1615, 1642, 1515, 1250, 1562, 1298 cm^{-1} (KBr disk).

Pyrrobutamine

Antihistamine
$C_{20}H_{22}ClN$ = 311.9
CAS—91-82-7

IUPAC Name 1-[(*E*)-4-(4-chlorophenyl)-3-phenylbut-2-enyl]pyrrolidine
Synonym 1-[4-(4-Chlorophenyl)-3-phenyl-2-butenyl]pyrrolidine
Proprietary Name *Pyronil*

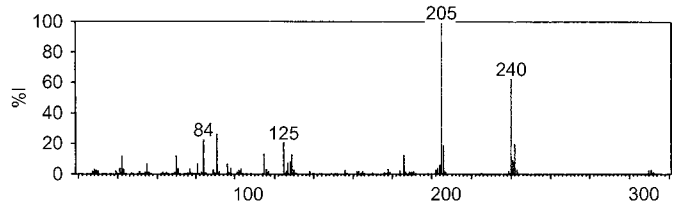


Chemical Properties An oily liquid. On standing gives crystals. Mp 48° to 49°. pK_a 8.8 (25°). Log *P* (octanol/water), 6.3.

Pyrrobutamine Phosphate

$C_{20}H_{22}ClN,2H_3PO_4$ = 507.8
CAS—135-31-9
Proprietary Name It is an ingredient of *Co-Pyronil*
Chemical Properties A white crystalline powder. Mp 129° to 130°. Soluble in water; soluble 1 in 20 of ethanol; practically insoluble in chloroform and ether. pK_a 8.8, 5.2.

Colour Tests Liebermann's reagent—brown; Mandelin's test—violet; Marquis test—grey-violet.
Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.54; system TC—R_f 0.37; system TE—R_f 0.71; system TL—R_f 0.18; system TAE—R_f 0.25; system TAF—R_f 0.66; system TAJ—R_f 0.24; system TAK—R_f 0.25; system TAL—R_f 0.86 (Dragendorff spray, positive; Acidified iodoplatinate solution, positive; Marquis reagent, grey).
Gas Chromatography System GA—pyrrobutamine RI 2419; M (oxo-) RI 2920; system GF—RI 2815.
High Performance Liquid Chromatography System HA—*k* 2.8; system HX—RI 477.
Infrared Spectrum Principal peaks at wavenumbers 995, 1018, 1087, 772, 1492, 820 cm^{-1} (pyrrobutamine phosphate, KBr disk).
Mass Spectrum Principal ions at *m/z* 205, 240, 91, 84, 125, 242, 206, 186.

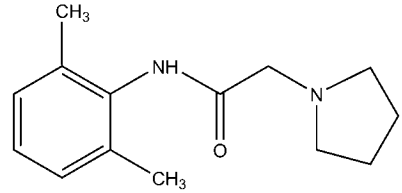


Dose 30 to 90 mg of pyrrobutamine phosphate daily.

Pyrrocaine

Anaesthetic (Local)
$C_{14}H_{20}N_2O$ = 232.3

IUPAC Name *N*-(2,6-Dimethylphenyl)-2-pyrrolidin-1-ylacetamide
Synonyms 2,6-Dimethyl-α-pyrrolidin-1-ylacetanilide; *N*-(pyrrolidin-1-ylacetyl)-2,6-xylidine.



Chemical Properties Pyrrocaine melts at 84°. Soluble 1 in 350 of water, 1 in 20 of ether, and 1 in 7 of methanol. Pyrrocaine is extracted by ether from aqueous alkaline solutions.

Pyrrocaine Hydrochloride

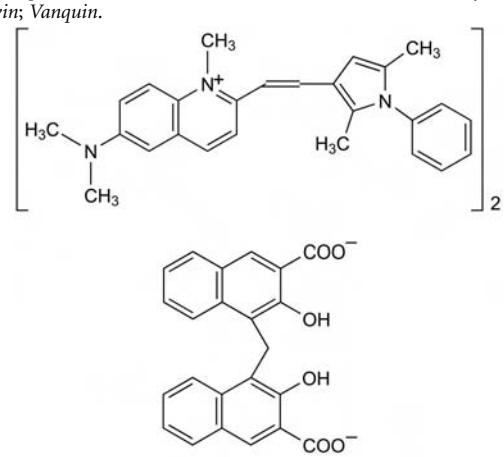
Synonym EN-1010
Proprietary Name *Dynacaine*
Chemical Properties White crystalline powder. Mp 200° to 205°. Soluble 1 in 1.5 of water and 1 in 12 of ethanol; soluble in chloroform; almost insoluble in ether.

Thin-layer Chromatography System T1—R_f 0.71 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.32 relative to diphenhydramine, retention time 0.31 relative to codeine.
Ultraviolet Spectrum 0.1 N hydrochloric acid—263 nm (E1%, 1 cm 17) (pyrrocaine hydrochloride).
Infrared Spectrum Principal peaks at wavenumbers 775, 1219, 1450, 1492, 1515, 1667 cm^{-1} (pyrrocaine hydrochloride) (KBr disk) .
Disposition in the Body
Toxicity LD_{50} in mice 480 mg/kg (oral).
Dose Usually 1 to 2 mL of a 2% solution.

Pyrvinium Embonate

Anthelmintic
$C_{52}H_{56}N_6,C_{23}H_{14}O_6$ = 1151.4
CAS—3546-41-6

IUPAC Name 4-[(3-Carboxylato-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxynaphthalene-2-carboxylate; 2-[2-(2,5-dimethyl-1-phenylpyrrol-3-yl)ethenyl]-*N*,*N*,1-trimethylquinolin-1-ium-6-amine
Synonyms 6-(Dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl-1*H*-pyrrol-3-yl) ethenyl]-1-methylquinolinium salt with 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylic acid] (2:1); pyrvinium pamoate; viprynium embonate; viprynium pamoate.
Proprietary Names *Molevac*; *Pamovin*; *Pamoxan*; *Povan*; *Povanyl*; *Pyrcon*; *Pyr-Pam*; *Pyrvin*; *Vanquin*.



Chemical Properties A bright orange or orange-red to almost black, crystalline powder. Mp 210° to 215° (softens at 190°). Practically insoluble in water and ether; very slightly soluble in ethanol; slightly soluble in chloroform and methoxyethanol; freely soluble in glacial acetic acid.

Pyrvinium Chloride

$C_{26}H_{27}N_3$,HCl = 418.0
CAS—548-84-5
Synonyms Viprynium chloride; SN-4395.
Chemical Properties Deep-red powder. Mp 249° to 251°, with decomposition. Sparingly soluble in water.
Colour Tests Mandelin's test—yellow; Marquis test—yellow
Thin-layer Chromatography System TA—R_f 0.67 (acidified potassium permanganate solution, weak reaction).
Ultraviolet Spectrum 2-Methoxyethanol—239, 358 nm (A_1^1=380b).
Infrared Spectrum Principal peaks at wavenumbers 1592, 1618, 1294, 1188, 1500, 1513 cm^{-1} (KBr disk).
Dose The equivalent of 5 mg/kg of pyrvinium as a single dose.

Quazepam

Benzodiazepine, Sedative, Hypnotic

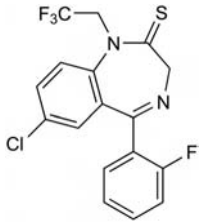
$C_{17}H_{11}ClF_4N_2S = 386.8$

CAS—36735-22-5

IUPAC Name 7-Chloro-5-(2-fluorophenyl)-1-(2,2,2-trifluoroethyl)-3*H*-1,4-benzodiazepine-2-thione

Synonym Sch-16134

Proprietary Names *Doral*; *Dormalin*; *Dorme*; *Oniria*; *Prosedar*; *Quazium*; *Quiedorm*; *Selepam*.



Chemical Properties Off-white to yellowish powder with Mp 137.5° to 139°. It is insoluble in water; soluble in alcohol. Log *P* (octanol/water), 4.03.

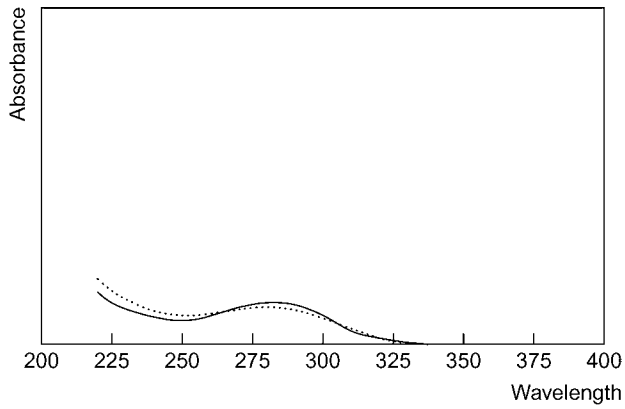
Thin-layer Chromatography System TA—(quazepam) R_f 0.74, (quazepam-CFTB) R_f 0.79, (M2-oxo-) R_f 0.78, (M3-hydroxy-2-oxo-) R_f 0.71, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.67, (M-*N*-dealkyl-2-oxo-) R_f 0.75; system TAD—(quazepam) R_f 0.78, (quazepam-CFTB) R_f 0.80, (M2-oxo-) R_f 0.70, (M3-hydroxy-2-oxo-) R_f 0.58, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.35, (M-*N*-dealkyl-2-oxo-) R_f 0.60; system TAE—(quazepam) R_f 0.87, (quazepam-CFTB) R_f 0.77, (M2-oxo-) R_f 0.87, (M3-hydroxy-2-oxo-) R_f 0.88, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.88, (M-*N*-dealkyl-2-oxo-) R_f 0.88; system TAF—(quazepam) R_f 0.96, (quazepam-CFTB) R_f 0.93, (M-2-oxo-) R_f 0.90, (M3-hydroxy-2-oxo-) R_f 0.90, (M-3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.89, (M-*N*-dealkyl-2-oxo-) R_f 0.88; system TAG—(quazepam) R_f 0.76, (quazepam-CFTB) R_f 0.73, (M2-oxo-) R_f 0.71, (M3-hydroxy-2-oxo-) R_f 0.59, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.38, (M-*N*-dealkyl-2-oxo-) R_f 0.58; system TB—(quazepam) R_f 0.44, (quazepam-CFTB) R_f 0.44, (M2-oxo-) R_f 0.16, (M3-hydroxy-2-oxo-) R_f 0.02, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0, (M-*N*-dealkyl-2-oxo-) R_f 0.2; system TC—(quazepam) R_f 0.77, (quazepam-CFTB) R_f 0.77, (M-2-oxo-) R_f 0.89, (M-3-hydroxy-2-oxo-) R_f 0.55, (M-3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.30, (M-*N*-dealkyl-2-oxo-) R_f 0.56; system TD—(quazepam)R_f 0.78, (quazepam-CFTB) R_f 0.74, (M2-oxo-) R_f 0.59, (M3-hydroxy-2-oxo-) R_f 0.42, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.15, (M-*N*-dealkyl-2-oxo-) R_f 0.34; system TE—(quazepam) R_f 0.83, (quazepam-CFTB) R_f 0.85, (M2-oxo-) R_f 0.80, (M3-hydroxy-2-oxo-) R_f 0.69, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.49, (M-*N*-dealkyl-2-oxo-) R_f 0.72; system TF—(quazepam) R_f 0.71, (quazepam-CFTB) R_f 0.73, (M-2-oxo-) R_f 0.83, (M3-hydroxy-2-oxo-) R_f 0.57, (M3-hydroxy-*N*-dealkyl-2-oxo-) R_f 0.28, (M-*N*-dealkyl-2-oxo-) R_f 0.45.

Gas Chromatography System GA—RI 2440; M (2-oxo--) RI 2255; system GB—RI 2576; system GZ—retention time 6.8 min.

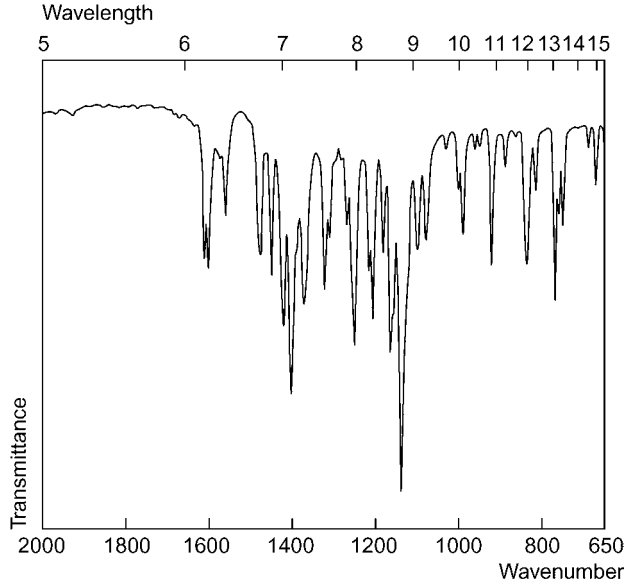
High Performance Liquid Chromatography System HAX—retention time 11.9 min; system HAY—retention time 17.7 min; system HY—RI 766; system HZ—retention time 37.5 min.

Column: Si-10 porous silica (300 × 4 mm, 10 µm). Mobile phase: ethanol, flow rate 0.5 mL/min. UV detection. Retention time: 2.7 min [Mills, Roberson 1993].

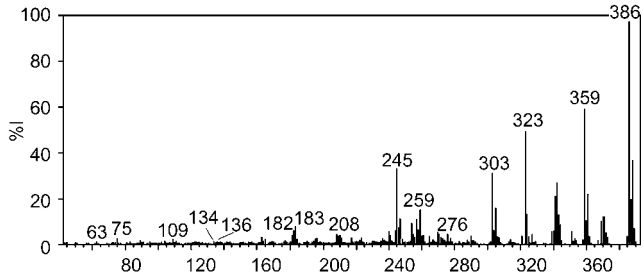
Ultraviolet Spectrum Aqueous acid (0.2 M H_2SO_4)—283 nm; basic—281 nm.



Infrared Spectrum Principal peaks at wavenumbers 1141, 1405, and 1253 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 386, 359, 323, 245, 303, 388, 342.



Quantification

Blood **HPLC** Column: Nova-Pak, C_{18} (75 × 3.9 mm i.d.). Mobile phase: methanol : 2 mmol/L phosphate buffer (pH 7.2, 60 : 40), flow rate 1 mL/min. IS: diazepam. UV detection (λ= 265 nm). Retention time: quazepam, 17.6 min; IS, 8.1 min. Limit of quantification, 2 µg/L [Gupta, Ellinwood, Jr. 1988].

Plasma **GC** Column: WSCOT CP-Sil 5 (25 m × 0.5 mm i.d., 1 µm). Carrier gas: He, 0.4 bar. Temperature: 220°. ECD. Retention time: 20.5 min. Limit of detection, 0.2 µg/L [Bun *et al.* 1986]. Column: 3% OV-25 on 80/100 mesh Supelcoport (1.83 m × 2 mm i.d.). Carrier gas: N_2, 28 mL/min. Temperature: 220°. ECD. Retention time: 6.8 min. Limit of quantification, 0.75 µg/L [Hilbert *et al.* 1984b].

Serum **LC-MS** ESI. Limit of detection, 0.3–11.4 µg/L [Nakamura *et al.* 2009]

For an enzyme immunoassay in serum see Huang, Moody [1995].

Urine **GC-MS** Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

Disposition in the Body Quazepam is readily and rapidly absorbed from the gastrointestinal tract after oral administration. It is distributed widely throughout the body into most body tissues and fluids, and metabolised extensively in the liver. The principal active metabolites are 2-oxo-quazepam (half-life 40 h), *O,N*-desalkyl-2-oxo-quazepam (half-life, 69 h) and 3-hydroxy-2-oxoquazepam glucuronide. It is excreted in urine (31%) and faeces (23%), mainly as the conjugated metabolites. Only trace amounts of the unchanged drug are present in urine. Elimination is prolonged in the elderly. Steady state is achieved by day 7 and maintained up to 14 days due to slow elimination. Quazepam and its metabolites readily cross the blood–brain barrier.

Therapeutic Concentration The serum therapeutic concentration range is 10–150 µg/L.

Two healthy male volunteers were administered 15 mg quazepam sublingually. Maximum plasma concentrations of 44.1 and 38.9 µg/L were attained at 1.27 and 1.73 h, respectively [Gupta, Ellinwood, Jr. 1988].

Six healthy males were administered 25 mg quazepam in solution. Peak plasma concentration of the unchanged drug was 148 µg/L and observed at 1.5 h post-dosing [Zampaglione *et al.* 1985].

Ten geriatric patients were administered a 15 mg tablet of quazepam. Mean peak plasma concentration was 29.3 µg/L reached at 2.7 h [Hilbert *et al.* 1984a].

Toxicity Quazepam has the same toxic potential as the benzodiazepines.

Half-life 39 h.

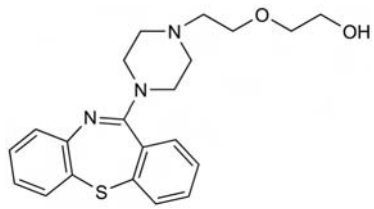
Bioavailability ≈80%.
Volume of Distribution At bedtime, 5 L/kg and in the morning, 8.6 L/kg.
Clearance The total body clearance is 890 mL/min.
Protein Binding >95%.
Note Active metabolites accumulate with chronic use.
Dose An oral dose of 15 mg is administered at bedtime which may be reduced to 7.5 mg in the elderly and debilitated.

Bun H *et al.* (1986). Plasma quantification of quazepam and its 2-oxo and *N*-desmethyl metabolites by capillary gas chromatography. *J Chromatogr* 378: 137–145.
Gupta SK, Ellinwood EHJr (1988). Liquid chromatographic assay and pharmacokinetics of quazepam and its metabolites following sublingual administration of quazepam. *Pharm Res* 5: 365–368.
Hilbert JM *et al.* (1984a). Quazepam kinetics in the elderly. *Clin Pharmacol Ther* 36: 566–569.
Hilbert JM *et al.* (1984b). Gas chromatographic determination of quazepam and two major metabolites in human plasma. *J Pharm Sci* 73: 516–519.
Huang W, Moody DE (1995). Immunoassay detection of benzodiazepines and benzodiazepine metabolites in blood. *J Anal Toxicol* 19: 333–342.
Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography-mass spectrometry. *J Chromatogr* 422: 85–101.
Mills TI, Roberson JC (1993). *Instrumental Data for Drug Analysis, Vol. 4-5*, 2nd edn. Boca Raton, FL: CRC Press.
Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography-tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.
Zampaglione N *et al.* (1985). Disposition and metabolic fate of 14C-quazepam in man. *Drug Metab Dispos* 13: 25–29.

Quetiapine

Antipsychotic
$C_{21}H_{25}N_3O_2S = 383.5$
CAS—111974-69-7

IUPAC Name 2-[2-(4-Benzol[*b*][*p*1,5]benzothiazepin-6-ylpiperazin-1-yl)ethoxy] ethanol
Synonym 2-[2-(4-Dibenzo[*b,f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol

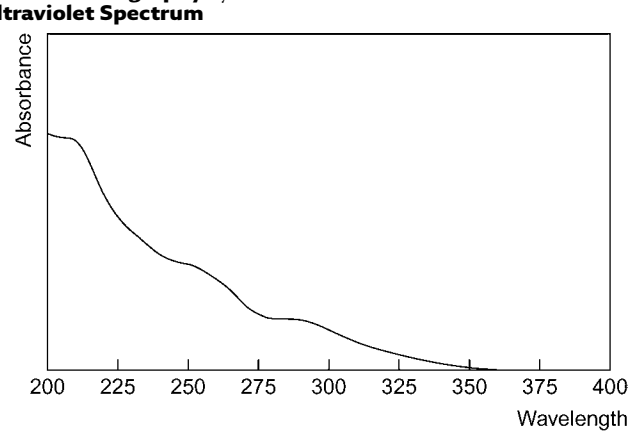


Chemical Properties White to off-white crystalline powder. It is moderately soluble in water. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Standard solutions were stable after 4 freeze-thaw cycles and at room temperature for 2 h. Plasma samples were stable for 2 h following alkinisation and for 24 h at 10° [Barrett *et al.* 2007]. Standard solutions are stable for several months at –20° in the dark [Sachse *et al.* 2006]. Standard solutions in methanol were stable for 3 months when stored at –20°. Samples were stable for at least 30 days when stored at –20°, following freeze-thaw cycles and for 24 h at 20° [Zhou *et al.* 2004]. Stable in plasma for 15 months at –20° and after 8 freeze-thaw cycles [Davis *et al.* 1999].

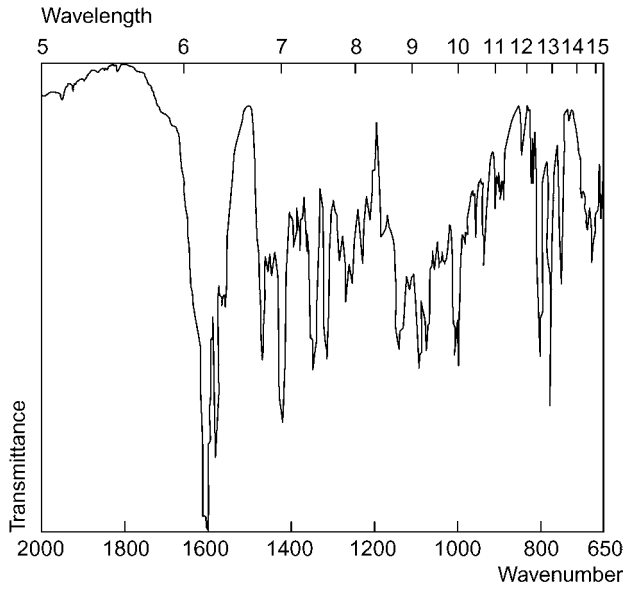
Quetiapine Fumarate

$(C_{21}H_{25}N_3O_2S)_2C_4H_4O_4 = 883.1$
CAS-111974-72-2
Synonym ICI-204636
Proprietary Name *Seroquel*
Chemical Properties Crystals from ethanol. Mp 172° to 173°. pKa_1 3.3; pKa_2 6.8. Stable in plasma for 12 months at –20° or –70° and after 3 freeze-thaw cycles [Pullen *et al.* 1992].

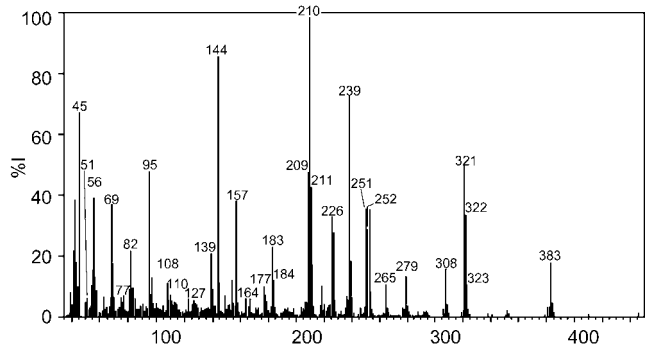
Gas Chromatography System GB—RI 3400.
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1600, 1575, 1410, 765 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 210, 144, 239, 45, 321, 209, 95, 211.



Quantification

Blood **GC** Column: HP-5 (15 m × 0.25 mm i.d., 0.25 µm) and HP-35 (15 m × 0.32 mm i.d., 0.32 µm). Temperature programme: 140° for 0.5 min to 300° at 10°/min for 10.5 min. NPD. Limit of quantification, 0.1 mg/L [Anderson, Fritz 2000].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 140° to 300° at 30°/min for 7.17 min. MSD, SIM acquisition mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.05 mg/L [Flammia *et al.* 2006].

HPLC Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water: tetramethylethylenediamine (8 : 92 : 0 : 0: to 37.5 : 62.1 : 0.4 at 5 min for 10 min to 8 : 92 : 0 at 15 min for 10 min), flow rate, 1.3 mL/min. UV detection (λ= 254 nm). Retention time: 13.86 min. Limit of detection, 20 µg/L [Sachse *et al.* 2006]. Column: XTerra MS C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L ammonium hydroxide (20 : 80 for 4 min to 60 : 40 over 3 min for 6 min), flow rate, 0.4 mL/min. DAD (λ= 258 nm). Retention time: 9.1 min. Limit of detection, 0.1 mg/L [Hopenwasser *et al.* 2004].

Plasma **GC-MS** Column: cross-linked methylsilicone (30 m × 0.25 mm i.d., 0.1 µm). Carrier gas: He, 0.75 mL/min. Temperature programme: 190° for 1.6 min to 295° at 30°/min for 8.9 min. EI ionisation at 70 eV, SIM acquisition mode at *m/z*: 322 and 340. Retention time: TMS (tetramethylsilane) derivatives: quetiapine, 10.1 min; 7-hydroxyquetiapine, 13.0 min. Limit of quantification, 15 µg/L [Pullen *et al.* 1992].

HPLC Column: Chromsep C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 30 mmol/L phosphate buffer containing 0.5% triethylamine (pH 3.0, 30 : 70), flow rate, 1.0 mL/min. UV detection (λ= 238 nm). Limit of quantification, 1.5 µg/L, limit of detection, 0.5 µg/L [Mercolini *et al.* 2007]. Column: Varian ResElut C_8 (!50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10.5 mmol/L phosphate buffer (pH 3.5) containing 0.12% triethylamine (30 : 70), flow rate 1.2 mL/min. UV detection (λ= 254 nm). Retention time: 7.3 min. Limit of quantification, 2.5 µg/L, limit of detection, 0.8 µg/L [Saracino *et al.* 2006]. Column: Zorbax Stablebond phenyl (150 × 2.1 mm i.d., 5 µm). Mobile phase: 20 mmol/L

phosphate buffer (30 mmol/L potassium chloride, pH 7.4) : methanol : acetonitrile (40 : 10 : 50), flow rate 0.25 mL/min. Electrochemical detection. Retention time: 7.8 min. Limit of quantification, 2.5 µg/L [Davis *et al.* 1999]. Column: ODS Hypersil (250 × 4.6 mm i.d., 5 µm); Mobile phase: acetonitrile : methanol : 5 mmol/L potassium phosphate (pH 7.0; 2 : 1 : 2), flow rate 1 mL/min. UV detection (λ = 250 nm). Retention time: quetiapine, 9.7 min; 7-hydroxyquetiapine, 4.0 min. Limit of quantification, 2 µg/L [Pullen *et al.* 1992].

LC-MS Column: Atlantis dC_{18} (100 × 3.0 mm i.d., 3 µm). Mobile phase: acetonitrile : methanol : 0.01 mol/L ammonium acetate (pH 3.5, 31 : 19 : 50), flow rate 0.4 mL/min. ESI, positive ion mode, SRM acquisition mode, CID. Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Barrett *et al.* 2007]. Column: Kromasil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 1.7 mmol/L formic acid and 5.8 mmol/L ammonium acetate : acetonitrile (65 : 35), flow rate 0.95 mL/min. ESI, SIR acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 0.3 µg/L [Li *et al.* 2004]. Column: Macherey-Nagel C_{18} (125 × 2.0 mm i.d., 3 µm). Mobile phase: 2.7 mmol/L formic acid and 10 mmol/L ammonium acetate : acetonitrile (53 : 47), flow rate 0.16 mL/min. ESI, positive ion mode, SIR acquisition mode. Limit of detection, 1.0 µg/L [Zhou *et al.* 2004].

Serum **HPLC** Column: RP-18e (100 × 4.6 mm i.d.). Mobile phase: methanol : 0.1% trifluoroacetic acid (15 : 85 to 35 : 65 over 7 min to 100 : 0 for 3 min), flow rate 3.0 mL/min. MSD. Retention time: 5.8 min. Limit of detection, 0.2 nmol/L [Bellomarino *et al.* 2009]. Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogenphosphate (pH 7.0) : acetonitrile (60 : 40), flow rate, 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 11.7 min. Limit of detection not reported [Frahnert *et al.* 2003]. Column: Spherisorb S5W (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 20 mmol/L ammonium acetate buffer (pH 5.0, 99 : 1), flow rate 1.0 mL/min. UV detection (λ = 257 nm). Limit of detection, 10.3 nmol/L [Hasselstrøm, Linnet 2003].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.17 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **GC** See Blood [Anderson, Fritz 2000].

GC-MS See Blood [Flammia *et al.* 2006].

HPLC See Serum. Limit of detection, 70 mmol/L [Bellomarino *et al.* 2009]. See Blood [Hopenwasser *et al.* 2004].

Bile **GC** See Blood [Anderson, Fritz 2000].

GC-MS See Blood [Flammia *et al.* 2006].

HPLC See Blood [Hopenwasser *et al.* 2004].

Gastric Contents **GC** See Blood [Anderson, Fritz 2000].

GC-MS See Blood [Flammia *et al.* 2006].

HPLC See Blood [Hopenwasser *et al.* 2004].

Vitreous Humour **GC-MS** See Blood [Flammia *et al.* 2006].

HPLC See Blood [Hopenwasser *et al.* 2004].

Brain **HPLC** See Blood [Hopenwasser *et al.* 2004].

Liver **GC** See Blood [Anderson, Fritz 2000].

GC-MS See Blood [Flammia *et al.* 2006].

HPLC See Blood [Hopenwasser *et al.* 2004].

Muscle **HPLC** See Blood [Hopenwasser *et al.* 2004].

Spleen **GC** See Blood [Anderson, Fritz 2000].

HPLC See Blood [Hopenwasser *et al.* 2004].

Disposition in the Body Quetiapine is well and rapidly absorbed following oral administration. It is widely distributed throughout the body and extensively metabolised in the liver by sulfoxidation, carboxylic acid formation on the ethoxyethanol side chain and 7-hydroxylation. At least 20 metabolites have been detected but only 7-hydroxyquetiapine has significant pharmacological activity. CYP3A4 is the main isoenzyme responsible for cytochrome P450-mediated metabolism. Elimination is mainly through hepatic metabolism. The drug is excreted as its inactive metabolite: 73% in urine and 20% in faeces. One percent of an administrated dose is excreted unchanged. Only small quantities are excreted in the breast milk [Lee *et al.* 2004]. Peak plasma concentrations are observed within 1.5–2 h after administration. Steady state is achieved within 2 days of the dose. Linear pharmacokinetics are observed.

Therapeutic Concentration The effective dose is 200–750 mg daily. The therapeutic concentration is 0.4 mg/L.

Twenty-four healthy male Thai volunteers were administered 200 mg quetiapine as 2 separate preparations. The mean maximum plasma concentration was 811.3 µg/L reached at 1.1 h following Seroquel and 886.6 µg/L at 1.08 h following Quantia200 [Mahatthanatrakul *et al.* 2008].

A patient receiving a dose of 150 mg quetiapine fumarate had a maximum plasma concentration of 437 ± 173 µg/L at 2 h [Davis *et al.* 1999].

Twelve hospitalised patients with schizophrenia were administered increasing doses of quetiapine over 21 days. A 75-mg oral dose produced a peak serum concentration of 0.278 mg/L on the last day of treatment. [Fabre, Jr. *et al.* 1995].

Toxicity The lethal concentration is 7 mg/L.

A 34-year-old female ingested ≈24 g quetiapine. Approximately 2 h after admission her plasma concentration was 22 mg/L which decreased with a $t_{1/2}$ of 4.1 h. Interestingly there was a small increase in quetiapine concentration between 24 and 37 h, suggesting redistribution from tissues. The patient recovered after 2 days [Bodmer *et al.* 2008].

The distribution of quetiapine was investigated in 20 postmortem cases. The quetiapine mean and range of concentrations in each tissue were as follows: peripheral blood, 7.7 mg/L (0.14–37 mg/L, n = 17); heart blood, 23.6 mg/L (0.53–76 mg/L, n = 4); liver, 71 mg/kg (1.1–510 mg/kg, n = 19); bile, 44 mg/L (6.0–96 mg/L, n = 4); urine, 15 mg/L (1.9–37 mg/L, n = 8); gastric, 897 mg total (3.5–3960 mg, n = 7); and vitreous humour, 1.4 mg/L (0.2–3.2 mg/L, n = 5). The mean of all blood concentrations in 18 cases in which quetiapine contributed to the cause of death was 7.95 mg/L (0.4–76 mg/L) [Flammia *et al.* 2006].

The postmortem concentrations from 8 cases are shown in the table below (mg/L or mg/kg). Quetiapine was the cause of death in case 2.

A physically healthy 40-year-old female with an extensive psychiatric history was being treated with 100 mg quetiapine fumarate twice daily as well as clonazepam, chloral hydrate and valproic acid. After being discharged from hospital (after treatment for another overdose) she overdosed on 3000 mg quetiapine fumerate. Upon admission at hospital, she was drowsy, confused, suffering from hypotension, tachycardia and was mildly obtunded; other symptoms were also present. She was treated with activated charcoal and after 90 min was more obtunded and could not continue with this treatment. The serum quetiapine concentration was 1.824 mg/L. She became progressively worse but improved after systolic blood pressure support and saline solution. Eight hours after overdosing, serum concentration was 0.963 mg/L; 22 h, 0.180 mg/L; 42 h, 0.015 mg/L and 48 h, 0.005 mg/L. The drug was still detectable after 7 days. Tachycardia persisted until 42 h after overdosing [Pollak, Zbuk 2000].

A 40-year-old male was found decomposed in a hotel room. Postmortem examination revealed the following quetiapine concentrations: 49 mg/L in heart blood, 112 mg/kg in liver, 7.6 mg total gastric content, 4.0 mg/kg in spleen. Other substances detected in the heart blood were: alprazolam (2.6 mg/L), fluoxetine (2.1 mg/L), norfluoxetine (2.1 mg/L), prochlorperazine (0.83 mg/L) and ethanol (0.41 g%) [Anderson, Fritz 2000].

A young female who overdosed with 10 000 mg quetiapine was initially treated with gastric lavage and activated charcoal within 75 min of overdosing. Serum drug concentrations reached 12.7 mg/L. Her level of consciousness decreased 180 min after ingestion requiring intubation for 240 min. Alertness was regained at 16 h but her persistent tachycardia was not resolved until 42 h after ingestion. A full recovery was made [Harmon *et al.* 1998].

Note For a review of postmortem cases see Parker, McIntyre [2005].

Half-life 6–7 h; also reported as 2.7–9.3 h.

Bioavailability 100% bioavailability of tablets relative to solution. Absolute bioavailability 9%.

Volume of Distribution 10 L/kg.

Protein Binding 83%.

Milk : Plasma Ratio 0.29 [Rampono *et al.* 2007].

Tissue	*Case*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Blood	present	19.8	2.7	–	0.15	1.3	2.7	0.37
Liver	1.42	12.6	2.8	4.83	1.3	3.3	9.5	2.2
Bile	–	161.5	46.2	–	10.1	16.4	16.5	–
Vitreous	<0.05	–	0.11	–	0.06	0.08	–	0.15
Spleen	–	81.9	–	0.43	–	–	–	–
Brain	–	40.9	–	0.49	–	–	–	–
Muscle	–	25.3	–	0.17	–	–	–	–
Urine	–	–	–	0.12	4.8	3.0	3.1	0.89
Stomach	0.85	–	63	2.3	1.3	25	208	0.06

[Hopenwasser *et al.* 2004]

Dose 25 mg twice daily on day 1; 50 mg twice daily on day 2; 100 mg twice daily on day 3; 150 mg twice daily on day 4; then increased as necessary. The usual dose is 300 to 450 mg daily in two divided doses with a maximum of 750 mg. In the elderly the initial dose is 25 mg.

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Quinagolide

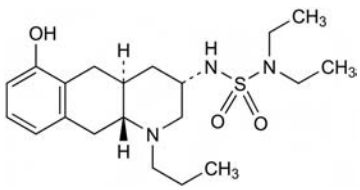
Prolactin Inhibitor, Dopaminergic Agent

$C_{20}H_{33}N_3O_3S = 395.6$

CAS—87056-78-8

IUPAC Name (3*S*,4a*S*,10a*R*)-3-(Diethylsulfamoylamino)-6-hydroxy-1-propyl-3,4,4a,5,10,10a-hexahydro-2*H*-benzo[*g*]quinolin

Synonym (3*R*,4a*R*,10a*S*)-*rel*-*N*,*N*-Diethyl-*N*′-(1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-propylbenzo[*g*]quinolin-3-yl)sulfamide



Chemical Properties A beige powder. Mp 122.5° to 124°.

Quinagolide Hydrochloride

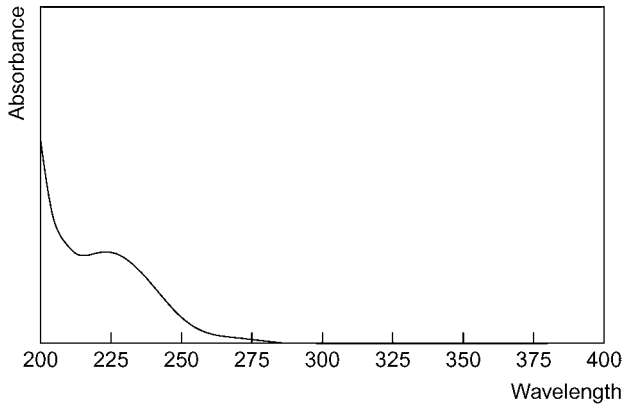
$C_{20}H_{33}N_3O_3S$, HCl = 432.0

CAS—94424-50-7

Proprietary Name *Norprolac*

Chemical Properties Crystals. Mp 234° to 236°.

UV Spectrum



Disposition in the Body Quinagolide is rapidly and well absorbed from the gastro-intestinal tract. There is extensive first-pass metabolism to the *N*-desethyl analogue which is biologically active and the *N*,*N*-didesethyl analogue. 95% of the drug is excreted as its metabolites with approximately equal amounts of a dose appearing in urine and in faeces. Excretion occurs in urine as sulfate or glucuronide conjugates of quinagolide and its metabolites and in faeces as the unconjugated forms.

Bioavailability 100% after oral administration.

Half-life 11.5 h after a single dose and 17 h at steady state.

Volume of Distribution Approximately 100 L after single a oral dose.

Protein Binding About 90%.

Dose 25 to 150 µg daily. Maximum 300 µg.

Quinapril

Antihypertensive

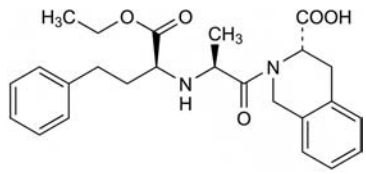
$C_{25}H_{30}N_2O_5 = 438.5$

CAS—85441-61-8

IUPAC Name (3*S*)-2-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-propanoyl]-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid

Synonym (3*S*)-2-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid

Proprietary Names *Accuretic* (Quinapril with Hydrochlorothiazide); *Acequide*; *Koretic*.



Chemical Properties Log *P* (octanol/water), 3.72.

Quinaprilat (Diacid of Quinapril)

$C_{23}H_{26}N_2O_5 = 410.5$

CAS—82768-85-2

Synonym CI-928

Chemical Properties Hydrate crystals from methanol–ethyl ether. Mp 166° to 168°.

Quinapril Hydrochloride

$C_{25}H_{30}N_2O_5$, HCl = 475.0

CAS—82586-55-8

Synonyms CI-906; PD-109452-2.

Proprietary Names *Accupril*; *Accuprin*; *Accupro*; *Acequin*; *Acuitel*; *Korec*; *Quinazil*.

Chemical Properties Crystals from ethyl acetate–toluene. Mp 120° to 130°. Also reported as white crystalline solid (from acetonitrile). Mp 119° to 121.5°.

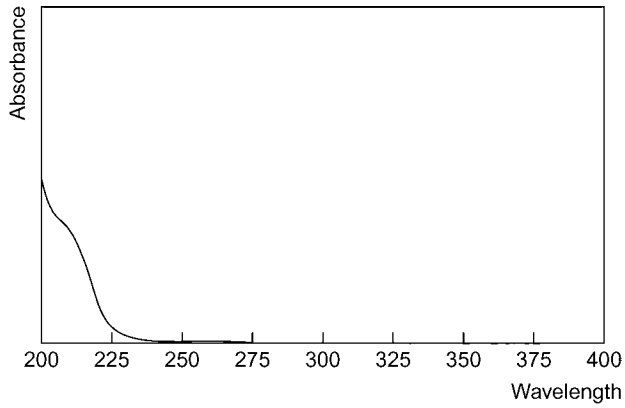
Gas Chromatography System GP—quinapril-ME RI 3110; M (quinaprilate)-ME_3 RI 3080; M (quinaprilate-H_2O)-ME_3 RI 3310.

Gas Chromatography-Mass Spectrometry Column: fused-silica capillary DB-1 (10 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 150° for 0.1 min, to 300° at 25°/min for 1.5 min. Injector temperature: 70° to 300° at 10°/min, held for 5 min. Carrier gas: He. NICI, SIM at *m/z* 302 for quinapril and 288 for quinaprilat. Retention times: quinapril, 6.20 min; quinaprilat, 6.15 min [Hammes *et al.* 1995].

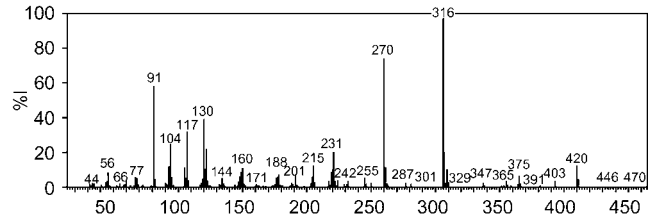
High Performance Liquid Chromatography System HZ—retention time 5.4 min; system HAA—retention time 16.8 min.

Column: C_{18} μBondapak (300 × 3.9 mm i.d., 10 μm). Column temperature: 30°. Mobile phase: 1-propanol: acetonitrile: phosphoric acid (0.01 mol/L) (20:15:78), flow rate 1.0 mL/min. UV detection (λ=206 nm). Retention times: quinapril, 7.6 min; quinaprilat, 5.7 min [Prieto *et al.* 2001].

UV Spectrum



Mass Spectrum Principal ions at *m/z* 316, 270, 91, 130, 117, 104, 132, 231.



Quantification

Plasma **GC** ECD. Limit of detection 10 μg/L [Ferry *et al.* 1987].

GC-MS Limit of quantification, 0.05 μg/L for quinapril and quinaprilat [Goto *et al.* 1992a]. Limit of quantification, 0.2 μg/L for dioxopiperazine metabolites of quinapril, limit of detection, 0.1 μg/L [Goto *et al.* 1992b].

Urine **GC** ECD. Limit of detection 50 μg/L [Ferry *et al.* 1987].

GC-MS Limit of quantification, 0.5 μg/L for quinapril and quinaprilat [Goto *et al.* 1992a]. Limit of quantification, 1.0 μg/L for dioxopiperazine metabolites of quinapril, limit of detection, 0.5 μg/L [Goto *et al.* 1992b].

HPLC UV detection (λ= 206 nm). Limit of quantification, 190 μg/L for quinapril and 160 μg/L for quinaprilat, limit of detection, 60 μg/L for quinapril and 50 μg/L for quinaprilat [Prieto *et al.* 2001].

Disposition in the Body Approximately 60% of a dose is absorbed after oral administration and is metabolised mainly in the liver to quinaprilat and inactive metabolites. Peak plasma concentrations of the active metabolite are reached within 2 h of administration. Up to 60% of the dose is excreted in urine as the unchanged drug, quinaprilat and inactive metabolites. The remainder is excreted in faeces. Elimination of quinaprilat is reduced in elderly patients (>65 years).

Therapeutic Concentration

Twelve patients with terminal renal failure were involved in the study, 6 of whom were on maintenance haemodialysis (at 4 h, three times a week), mean age 58.5 years (range, 25–71 years) and 6 on continuous peritoneal dialysis (CAPD) treatment (4 exchanges daily), mean 50.2 years old (range, 32–68 years). Patients were administered a single oral dose of 2.5 mg quinapril. Peak plasma concentrations for those undergoing haemodialysis were 83.5 (range, 45.8–103.2) μg/L observed at 4 h and for those being treated by CAPD, 64.3 (47.1–83.8) μg/L at 4.7 (4–8) h [Wolter, Fritschka 1993].

Twenty-two normotensive subjects, aged 18–75 years, were divided into 3 groups according to their creatinine clearance rates and treated with 20 mg quinapril hydrochloride on day 1 and on days 4 to 10. The dose was administered after an 8 h fast which was continued for another 4 h after dosing. Group 1: creatinine clearance, >60 mL/min (6 subjects); group 2: 30–59 mL/min (6 subjects); group 3: <29 mL/min (10 subjects). For the single dose, peak plasma quinapril concentrations reached 153, 136 and 161 μg/L for groups 1, 2 and 3, respectively. These were observed at 0.75, 0.8 and 1.29 h, respectively. The peak quinaprilat concentrations were 607, 663 and 885 μg/L at 1.88, 2.25 and 3.57 h, respectively, for groups 1, 2 and 3. For the multiple dosing, peak drug concentrations for groups 1, 2 and 3, were 149, 115 and 211 μg/L observed at 0.75, 1.0 and 1.07 h, respectively. The metabolite concentrations were 578, 686 and 1330 μg/L at 1.75, 2.33 and 2.43 μg/L for the 3 groups, respectively. These concentrations were measured on day 10 [Halstenson *et al.* 1992].

Bioavailability Quinapril, 60%.

Half-life Quinapril, ≈1 h; quinaprilat, 3 h (increased in those with creatinine clearance ≤40 mL/min).

Volume of Distribution 0.31 L/kg (range 0.18–0.52 L/kg) for patients undergoing haemodialysis; 0.3 (0.26–0.4) L/kg for those undergoing continuous peritoneal dialysis treatment.

Clearance Quinapril, 111 L/h; quinaprilat 13 L/h.

Protein Binding 97% (quinapril and its metabolites).

Dose Hypertension: An initial dose of 10 mg once daily is administered. In the elderly, those with renal impairment and those taking diuretics: 2.5 mg. The usual maintenance dose is 20 to 40 mg daily. Heart failure: initially 2.5 mg daily followed by a maintenance dose of 10 to 20 mg.

Ferry JJ *et al.* (1987). Determination of quinapril and its active metabolite in human plasma and urine by gas chromatography with electron-capture detection. *J Chromatogr* 421: 187–191.

Goto N *et al.* (1992a). Trace analysis of quinapril and its active metabolite, quinaprilat, in human plasma and urine by gas chromatography-negative-ion chemical ionization mass spectrometry. *J Chromatogr* 578(2): 195–201.

Goto N *et al.* (1992b). Determination of dioxopiperazine metabolites of quinapril in biological fluids by gas chromatography-mass spectrometry. *J Chromatogr* 578(2): 203–206.

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Prieto JA *et al.* (2001). Solid-phase extraction and high-performance liquid chromatography applied to the determination of quinapril and its metabolite quinaprilat in urine. *J Chromatogr Sci* 39(4): 153–159.

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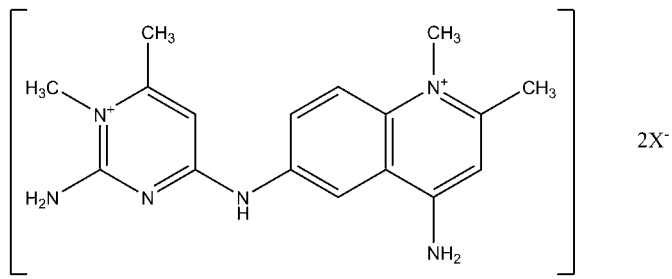
Quinapyramine

Trypanocide (Veterinary)

Synonyms 4-Amino-6-[(2-amino-1,6-dimethyl-4(1*H*)-pyrimidinylidene) amino]-1,2-dimethylquinolinium conjugate monoacid; 4-amino-6-[(2-amino-1,6-dimethyl-4-pyrimidinyl)amino]-1,2-dimethylquinoline salts; 4-amino-6-[(2-amino-6-methyl-4-pyrimidinyl)amino]-1-methylquinaldinium methosalts; 4-amino-6-(2-amino-6-methyl-4-pyrimidylamino)quinaldine-1,1′-dimethosalts; M-7555.

Proprietary Name *Antrycide*



Quinapyramine Dimethosulfate

$C_{19}H_{28}N_6O_8S_2$

Chemical Properties The 1,1′-dimetho(methylsulphate) salt of quinapyramine. White crystalline powder. Mp 266°, with decomposition. Soluble 1 in 2 of water; almost insoluble in ethanol, ether, and chloroform. The quinapyramine salts are quaternary ammonium compounds.

Quinapyramine Dichloride

$C_{17}H_{22}Cl_2$, $N_{62}H_2O$ = 417.3

Chemical Properties White crystalline powder. Mp 310°, with decomposition. Soluble 1 in 850 of water and 1 in 50 of boiling water; almost insoluble in ethanol, ether, and chloroform.

Quinapyramine Diiodide

Chemical Properties Pale-cream needles, Mp 312° to 313° with decomposition. Sparingly soluble in water.

Colour Test Vitali's test—blue-grey/yellow/orange (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.02 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum Quinapyramine sulfate in 0.1 mol/L sulfuric acid, maxima at 248 (A^1_1 415), 297 (A^1_1 771), 325 nm (A^1_1 218).

Disposition in the Body The 1,1′-dimethochloride dihyrate salt is absorbed very much more slowly than the 1,1′-dimetho(methylsulphate), so the 2 salts are commonly used prophylactically as 'pro-salt R.F', a mixture of 4 parts of the chloride to 3 of the sulfate. Rapid absorption of the sulfate gives an initial high blood-level which is maintained by slow release from the chloride depot at the injection site.

Toxicity Overdose causes tremours, inco-ordination, increase in respiration and heart rate, and dementia. Acute inflammation of the gastrointestinal tract and nephrosis have been found postmortem.

Dose 4.4 mg/kg SC.

Quinethazone

Diuretic

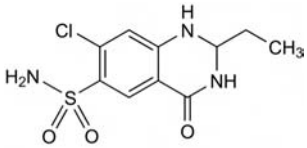
$C_{10}H_{12}ClN_3O_3S$ = 289.7

CAS—73-49-4

IUPAC Name 7-Chloro-2-ethyl-4-oxo-2,3-dihydro-1*H*-quinazoline-6-sulfonamide

Synonyms Chinethazonum; 7-chloro-2-ethyl-1,2,3,4-tetrahydro-4-oxo-6-quinazolinesulfonamide; CL-36010.

Proprietary Names *Aquamox*; *Hydromox*.



Chemical Properties A white to yellowish-white, crystalline powder. Mp 250° to 252°. Very slightly soluble in water; slightly soluble in ethanol; freely soluble in solutions of alkali hydroxides and carbonates. pK_a 9.3, 10.7. Log *P* (octanol/water), 0.2.

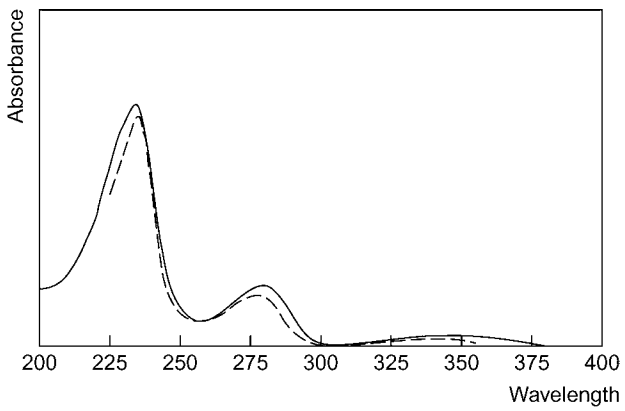
Colour Test Koppanyi–Zwikker test—violet.

Thin-layer Chromatography System TA—R_f 0.75; system TAD—R_f 0.15; system TD—R_f 0.04; system TE—R_f 0.40; system TF—R_f 0.21; system TAJ—R_f 0.11; system TAK—R_f 0.06; system TAL—R_f 0.56 (location under ultraviolet light, blue fluorescence; acidified iodoplatinate solution, positive).

Gas Chromatography System GA—not eluted.

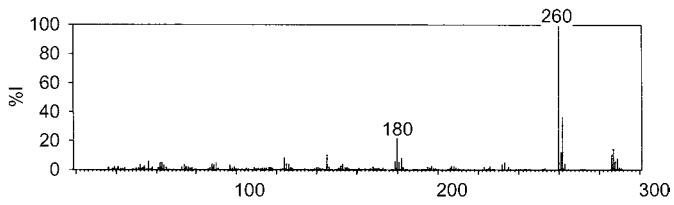
High Performance Liquid Chromatography System HN—*k* 0.67.

Ultraviolet Spectrum Ethanol—234 (A_1^1=1532a), 278, 345 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1650, 1160, 960, 1510, 1040 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 260, 262, 180, 287, 261, 145, 286, 124.



Quantification

Urine **HPLC** UV detection. Average limit of detection, 1 mg/L for quinethazone and other diuretics [Cooper *et al.* 1989].

Dose 50 to 200 mg daily.

Cooper SF *et al.* (1989). Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography. *J Chromatogr* 489: 65–88.

Quinidine

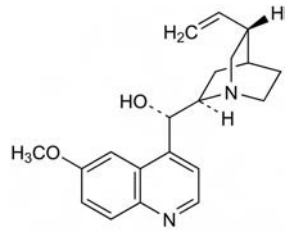
Antiarrhythmic

$C_{20}H_{24}N_2O_2$, $2H_2O$ = 360.5

CAS—56-54-2 (anhydrous); 63717-04-4 (dihydrate)

IUPAC Name (*S*)-[(2*R*,5*H*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl)methanol dihydrate

Synonyms Chinidinum; (9*S*)-6′-Methoxycinchonan-9-ol; quinidina.



Chemical Properties A dextrorotatory stereoisomer of quinine, obtained from the bark of species of *Cinchona* (Rubiaceae). Commercial samples may contain 20 to 30% of hydroquinidine. A white amorphous powder or acicular crystals. Mp 174° to 175° (anhydrous). The anhydrous alkaloid is soluble 1 in about 2000 of cold water, 1 in 800 of boiling water, 1 in 36 of alcohol, 1 in 1.6 of chloroform and 1 in 56 of ether; very soluble in methanol; practically insoluble in petroleum ether. pK_a 4.2, 8.8 (20°). Log *P* (octanol/water), 3.4. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

Quinidine Bisulfate

$C_{20}H_{24}N_2O_2$, H_2SO_4, $4H_2O$ = 494.6

CAS—747-45-5 (anhydrous); 6151-39-9 (tetrahydrate)

Proprietary Names *Biquin*; *Biquinate*; *Kiditard*; *Kinidin(e)*; *Kiniduron*; *Quini*; *Quiniduran*; *Quinidurule*.

Chemical Properties Colourless crystals. Soluble 1 in 8 of water and 1 in 3 of ethanol; practically insoluble in ether.

Quinidine Gluconate

$C_{20}H_{24}N_2O_2$, $C_6H_{12}O_7$ = 520.6

CAS—7054-25-3

Proprietary Names *Quinaglute*; *Quinate*.

Chemical Properties A white powder. Mp 175.0° to 176.5°. Soluble 1 in 9 of water and 1 in 60 of alcohol.

Quinidine Polygalacturonate

$C_{20}H_{24}N_2O_2$, $(C_6H_{10}O_7)_x$, xH_2O

CAS—27555-34-6 (anhydrous); 65484-56-2 (hydrate)

Proprietary Names *Cardioquin(e)*; *Galactoquin*.

Chemical Properties An amorphous powder. Mp 180°, with decomposition. The anhydrous product is sparingly soluble in water; insoluble in ethanol, chloroform, ether, acetone, dioxane and methanol.

Quinidine Sulfate

$(C_{20}H_{24}N_2O_2)_2$, H_2SO_4, $2H_2O$ = 783.0

CAS—50-54-4 (anhydrous); 6591-63-5 (dihydrate)

Synonyms Chinidini sulfas; chinidin sulfate; chinidinum sulfuricum; quinidini sulfas.

Proprietary Names *Kinidin*; *Quinicardina*; *Quinicardine*; *Quinidex*; *Quinora*; *Systodin*.

Chemical Properties White acicular crystals or fine white powder, darkening on exposure to light. Mp about 207°, with decomposition. Soluble 1 in about 90 of water (1 in 15 boiling water), 1 in 10 of ethanol, 1 in 3 of methanol and 1 in 12 of chloroform; insoluble in ether and benzene.

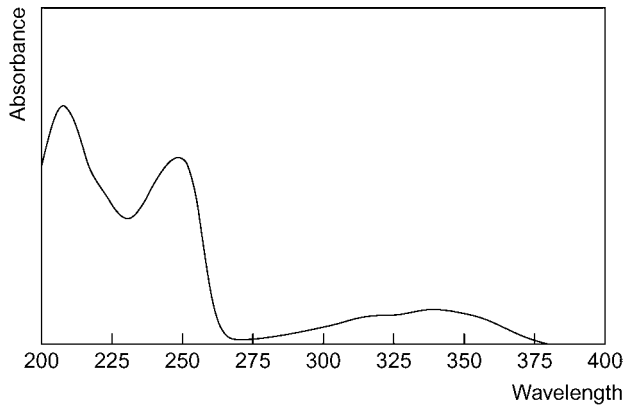
Colour Tests Sulfuric acid—yellow (fluoresces under UV light); Thalleioquin test—green.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.04; system TC—R_f 0.12; system TE—R_f 0.49; system TL—R_f 0.06; system TAE—R_f 0.30; system TAF—R_f 0.63; system TAJ—R_f 0.00; system TAK—R_f 0.02; system TAL—R_f 0.68 (location under UV light, blue fluorescence; Dragendorff spray, positive; acidified iodoplatinate solution, positive).

Gas Chromatography System GA—quinidine RI 2790, M (*N*-oxide) RI 2950; system GB—quinidine RI 2979, M (*N*-oxide) RI 3086.

High Performance Liquid Chromatography System HA—*k* 2.1; system HX—RI 322; system HY—RI 245; system HZ—RT 2.6 min; system HAA—RT 11.0 min; system HAX—RT 8.7 min; system HAY—RT 4.6 min.

Ultraviolet Spectrum Aqueous acid—250 (A_1^1= 959a), 317, 345 nm; aqueous alkali—280, 330 nm.



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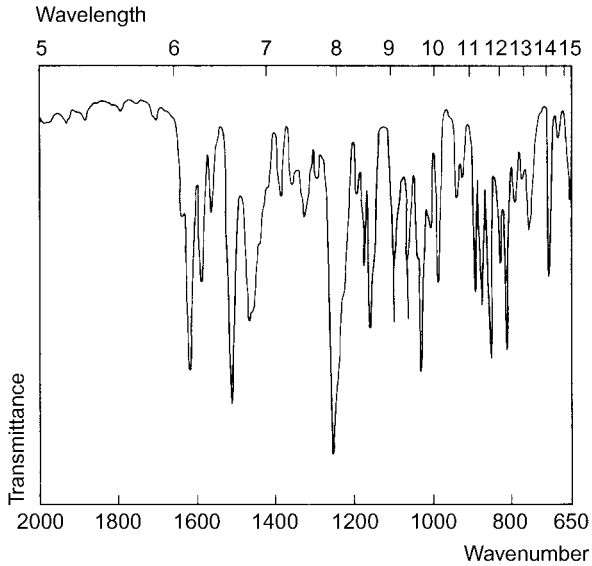
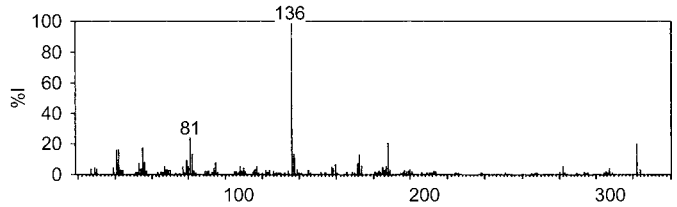
Infrared Spectrum Principal peaks at wavenumbers 1258, 1514, 1619, 1040, 860, 820 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 136, 81, 322, 188, 55, 42, 41, 172.



Quantification

Plasma **HPLC** UV and fluorescence detection. Quinidine and diltiazem. Limit of quantification, 4 µg/L for quinidine [Carignan *et al.* 1995]. For method, see Brandsteterova *et al.* [1994]. Fluorescence detection. Limit of quantification, 3.6 µg/L for quinidine and its metabolites [Nielsen *et al.* 1994]. Fluorescence detection. For method for quantification of quinidine and quinine, see Edstein *et al.* [1990]. UV detection. Limit of detection, 500 µg/L for quinidine and other antiarrhythmic agents [Bridges, Jennison 1983]. Fluorescence detection. Quinidine, hydroquinidine and 2 quinidine metabolites. Limit of detection, 50 µg/L for quinidine [Pershing *et al.* 1982]. Fluorescence and UV detection. Quinidine, hydroquinidine and quinidine metabolites. Limit of detection, 10 µg/L for quinidine [Guentert *et al.* 1980].

Serum **GC** AFID. For method for quantification of quinidine and other antiarrhythmic agents, see Kessler *et al.* [1982].

HPLC UV detection. Limit of detection, 0.1 mg/L for quinidine and metabolites [Hoyer *et al.* 1991].

Urine **HPLC** Fluorescence detection. Limit of quantification, 3.6 µg/L for quinidine and its metabolites [Nielsen *et al.* 1994]. Fluorescence and UV detection. Quinidine, hydroquinidine and quinidine metabolites. Limit of detection, 10 µg/L for quinidine [Guentert *et al.* 1980].

Disposition in the Body Rapidly absorbed after oral administration and undergoes first-pass metabolism in the liver. Extensively metabolised, mainly by the cytochrome P450 isoenzyme CYP3A4. The major metabolites, 3-hydroxyquinidine and quinidin-2′-one, appear to be pharmacologically active. Hydroquinidine, which may occur in quinidine as an impurity at concentrations of up to ≈30%, has similar pharmacological activity to quinidine. About 10–30% of a dose is excreted in the urine as unchanged drug in 48 h and the remainder is mostly excreted in the urine as metabolites; *O*-desmethylquinidine accounts for ≈2% of a dose; quinidine-10,11-dihydrodiol and an *N*-oxide metabolite have been detected in plasma and urine. The proportion of unchanged drug is dependent on the urinary pH, being decreased if the urine is alkaline.

Quinidine crosses the placenta and is excreted in breast milk.

Therapeutic Concentration In plasma, usually in the range 2–6 mg/L; there is, however, considerable intersubject variation. Concentrations appear to be higher in subjects with congestive heart failure.

After single oral doses of 4.5 mg/kg of quinidine sulfate to 10 subjects, peak plasma concentrations of 0.9–1.8 mg/L (mean 1.2) were attained in about 50 min [Guentert *et al.* 1979].

Following daily oral doses of 8–19 mg/kg (mean 12) to 5 subjects, steady-state serum concentrations of 0.5–2.5 mg/L (mean 1.8) of quinidine, 0.38–0.94 mg/L (mean 0.55) of 3-hydroxyquinidine and 0.02–0.12 mg/L (mean 0.07) of quinidin-2′-one, were reported; following daily oral doses of 8.6–13.3 mg/kg (mean 10.7) to 8 subjects with mild renal dysfunction, steady-state serum-quinidine concentrations ranged from 1.4–3.6 mg/L (mean 2.3) [Drayer *et al.* 1978].

Toxicity Plasma concentrations greater than 6 mg/L are progressively associated with toxicity, and concentrations of about 15 mg/L may cause toxic reactions in about 50% of patients; concentrations in excess of about 30 mg/L may be lethal.

The following postmortem tissue concentrations were reported in one fatality: blood 75 mg/L, brain 11 µg/g, kidney 89 µg/g, liver 145 µg/g, spleen 136 µg/g, urine 52 mg/L; 3-hydroxyquinidine, and lactone conjugates of quinidine and 3-hydroxyquinidine were also detected [Leferink *et al.* 1977].

In a fatality involving the ingestion of about 5 g of a sustained-release preparation by a 2-year-old child, the following postmortem tissue concentrations were reported: blood 45 mg/L, kidney 180 µg/g, liver 220 µg/g; death occurred 28 h after ingestion [McBay, Turk 1972].

Bioavailability About 70–80% but there is considerable intersubject variation.

Half-life Plasma half-life, about 4–12 h (mean 7).

Volume of Distribution About 2–3 L/kg, decreased in subjects with congestive heart failure.

Clearance Plasma clearance, ≈5 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ≈1.0.

Protein Binding Quinidine about 75–95%, 3-hydroxyquinidine ≈60% and quinidin-2′-one ≈55%.

Note For a review of the pharmacokinetic interactions of antimalarial agents, see Giao and de Vries [2001]. For a general review of quinidine, see Grace and Camm [1998]. For a review of the pharmacokinetics of quinine and quinidine in malaria, see White [1987]. For a review of the pharmacokinetics of quinidine, see Ochs *et al.* [1980].

Dose Usually 0.6 to 1.6 g of quinidine sulfate daily.

Brandsteterova E *et al.* (1994). Automatic solid-phase extraction and high-performance liquid chromatographic determination of quinidine in plasma. *J Chromatogr A* 665: 101–104.
Bridges RR, Jennison TA (1983). An HPLC method for the simultaneous quantitation of quinidine, procainamide, N-acetylprocainamide, and disopyramide. *J Anal Toxicol* 7: 65–68.
Carignan G *et al.* (1995). Simultaneous determination of diltiazem and quinidine in human plasma by liquid chromatography. *J Chromatogr B Biomed Appl* 672: 261–269.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Drayer DE *et al.* (1978). Steady-state serum levels of quinidine and active metabolites in cardiac patients with varying degrees of renal function. *Clin Pharmacol Ther* 24: 31–39.
Edstein MD *et al.* (1990). Simultaneous measurement of quinine and quinidine in human plasma, whole blood, and erythrocytes by high-performance liquid chromatography with fluorescence detection. *Ther Drug Monit* 12: 493–500.
Giao PT, de Vries PJ (2001). Pharmacokinetic interactions of antimalarial agents. *Clin Pharmacokinet* 40: 343–373.
Grace AA, Camm AJ (1998). Quinidine. *N Engl J Med* 338: 35–45.
Guentert TW *et al.* (1979). Quinidine pharmacokinetics in man: choice of a disposition model and absolute bioavailability studies. *J Pharmacokinet Biopharm* 7: 315–330.
Guentert TW *et al.* (1980). An integrated approach to measurements of quinidine and metabolites in biological fluids. *J Chromatogr* 183: 514–518.
Hoyer GL *et al.* (1991). High-performance liquid chromatographic method for the quantitation of quinidine and selected quinidine metabolites. *J Chromatogr* 572: 159–169.
Kessler KM *et al.* (1982). Simultaneous quantitation of quinidine, procainamide, and *N*-acetylprocainamide in serum by gas-liquid chromatography with a nitrogen-phosphorus selective detector. *Clin Chem* 28: 1187–1190.
Leferink JG *et al.* (1977). *J Anal Toxicol* 1: 62–65.
McBay AJ, Turk RF (1972). *Bull Int Assoc Forensic Toxicol* 8(4): 2–3.
Nielsen F *et al.* (1994). Determination of quinidine, dihydroquinidine, (3S)-3-hydroxyquinidine and quinidine N-oxide in plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 660: 103–110.
Ochs HR *et al.* (1980). Clinical pharmacokinetics of quinidine. *Clin Pharmacokinet* 5: 150–168.
Pershing LK *et al.* (1982). An HPLC method for the quantitation of quinidine and its metabolites in plasma: an application to a quinidine-phenytoin drug interaction study. *J Anal Toxicol* 6: 153–156.
White NJ (1987). The pharmacokinetics of quinine and quinidine in malaria. *Acta Leiden* 55: 65–76.

Quinine

Antimalarial

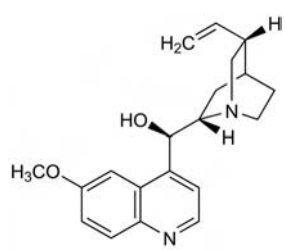
$C_{20}H_{24}N_2O_2$, $3H_2O$ = 378.5

CAS—130-95-0 (anhydrous)

IUPAC Name (8α,9*R*)-6′-Methoxycinchonan-9-ol trihydrate

Synonyms Chinina; chininum; quinina.

Proprietary Name It is an ingredient of *Nicobrevin*.



Chemical Properties The chief alkaloid of various species of Cinchona (Rubiaceae). It is a laevorotatory stereoisomer of quinidine. A white, slightly

efflorescent, flaky, granular or microcrystalline powder. Mp 57°. The anhydrous form is soluble 1 in 1900 of water, 1 in 760 of boiling water, 1 in 0.8 of alcohol, 1 in 80 of benzene, 1 in 1.2 of chloroform, 1 in 250 of dry ether and 1 in 20 of glycerol; almost insoluble in petroleum ether. pK_a 4.1, 8.5 (20°). Log *P* (octanol/water), 3.4. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Quinine Bisulfate

$C_{20}H_{24}N_2O_2$, H_2SO_4, $7H_2O$ = 548.6
CAS—549-56-4 (anhydrous); 6183-68-2 (heptahydrate)
Synonyms Chininum bisulfuricum; neutral quinine sulfate; quinine acid sulfate; quinine bisulphate; quinini bisulfas.
Proprietary Names *Biquin*; *Biquinate*; *Myoquin*; *Quinbisul*.
Chemical Properties Colourless crystals or white crystalline powder. It effloresces in dry air and becomes yellow when exposed to light. Soluble 1 in 8 of water, 1 in 50 of ethanol and 1 in 625 of chloroform.

Quinine Dihydrobromide

$C_{20}H_{24}N_2O_2$, 2HBr, $3H_2O$ = 540.3
Synonyms Neutral quinine hydrobromide; quinine acid hydrobromide.
Chemical Properties Yellowish or white crystals or powder. Soluble 1 in 7 of water; soluble in ethanol; practically insoluble in ether.

Quinine Dihydrochloride

$C_{20}H_{24}N_2O_2$, 2HCl = 397.3
CAS—60-93-5
Synonyms Chinini dihydrochloridum; neutral quinine hydrochloride; quinine acid hydrochloride; quinini dihydrochloridum.
Chemical Properties A white powder. Soluble 1 in about 0.6 of water, 1 in about 12 of ethanol and 1 in 7 of chloroform; very slightly soluble in ether.

Quinine Ethyl Carbonate

$C_{23}H_{28}N_2O_4$ = 396.5
CAS—83-75-0
Synonyms Euquinina; euquinine; quinine etabonate.
Chemical Properties White masses of silky crystals which darken on exposure to light. Mp 91° to 95°. Very slightly soluble in water; soluble 1 in 2 of ethanol, 1 in 1 of chloroform and 1 in 10 of ether.

Quinine Hydrobromide

$C_{20}H_{24}N_2O_2$, HBr, $2H_2O$ = 441.4
CAS—549-49-5 (anhydrous)
Synonyms Basic quinine hydrobromide; chinini bromidum; quinine monohydrobromide.
Chemical Properties White, silky, efflorescent crystals which darken on exposure to light. Soluble 1 in about 55 of water, 1 in 0.7 of ethanol and 1 in 1 of chloroform, the solution in chloroform being turbid due to separation of water.

Quinine Hydrochloride

$C_{20}H_{24}N_2O_2$, HCl, $2H_2O$ = 396.9
CAS—130-89-2 (anhydrous); 6119-47-7 (dihydrate)
Synonyms Basic quinine hydrochloride; chinini hydrochloridum; chinini chloridum; chininium chloratum; chininum hydrochloricum; quinine monohydrochloride.
Proprietary Names *Kinin*; *Surquina*.
Chemical Properties Colourless, fine, silky, acicular crystals which effloresce in dry air and gradually become yellowish on exposure to light. Soluble 1 in 16 of water (1 in 0.5 of boiling water), 1 in 1 of ethanol, 1 in 7 of glycerol, 1 in about 350 of ether and 1 in about 1 of chloroform to give a turbid solution.

Quinine Salicylate

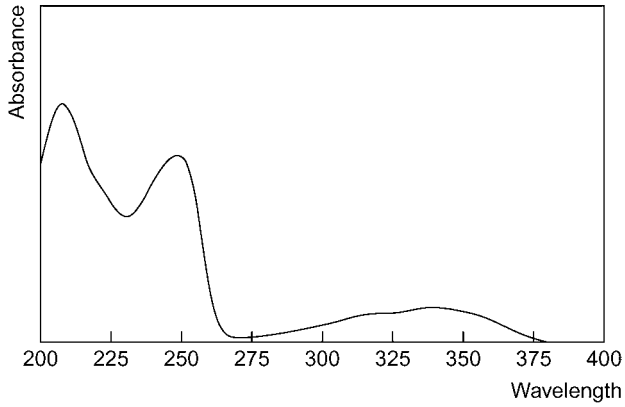
$C_{20}H_{24}N_2O_2$, $C_7H_6O_3$, H_2O = 480.6
CAS—750-90-3 (anhydrous)
Chemical Properties White silky crystals or a crystalline powder; it becomes pink on storage. Very slightly soluble in water; soluble 1 in 24 of ethanol and 1 in 25 of chloroform.

Quinine Sulfate

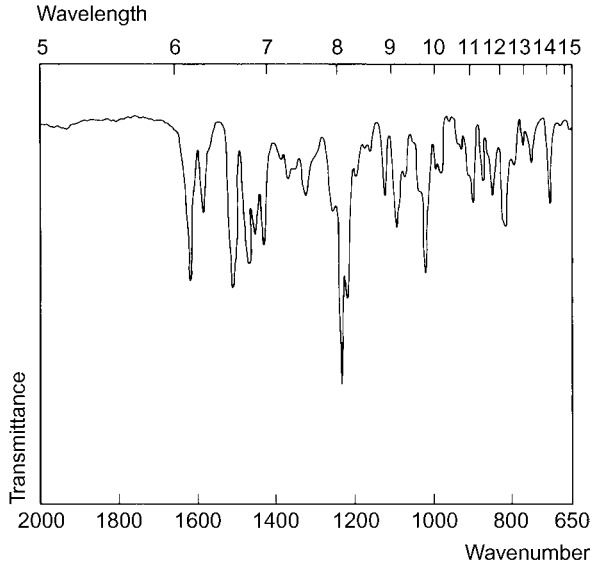
$(C_{20}H_{24}N_2O_2)_2$, H_2SO_4, $2H_2O$ = 783.0
CAS—804-63-7 (anhydrous); 6119-70-6 (dihydrate)
Synonyms Basic quinine sulphate; chinini sulfas; chininum sulfuricum; quinine sulphate.
Proprietary Names *Q200*; *Q300*; *Quinate*; *Quinoc*; *Quinoctal*; *Quinora*; *Quinsul*.
Chemical Properties Colourless acicular crystals or a white crystalline powder, becoming brown on exposure to light. Soluble 1 in about 810 of water (1 in 32 of boiling water) and 1 in 120 of ethanol; slightly soluble in chloroform and ether; readily soluble in a 2:1 mixture of chloroform and dehydrated alcohol.

Colour Tests Sulfuric acid—yellow (fluoresces under UV light); Thalleioquin test—green.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.02; system TC—R_f 0.11; system TE—R_f 0.45; system TL—R_f 0.04; system TAE—R_f 0.26; system TAF—R_f 0.65 (location under UV light, blue fluorescence; Dragendorff spray, positive; acidified iodoplatinate solution, positive).
Gas Chromatography System GA—quinine RI 2800, quinine-AC RI 2760, M (OH-)-AC_2 RI 3195, M (*N*-oxide)-AC RI 2945, M (di-OH-dihydro-)-AC_3 RI 3360.
High Performance Liquid Chromatography System HA—*k* 2.4; system HS—*k* 2.02; system HX—RI 327; system HY—RI 246; system HZ—RT 2.6 min; system HAA—RT 11.3 min; system HAX—RT 8.3 min; system HAY—RT 4.5 min.

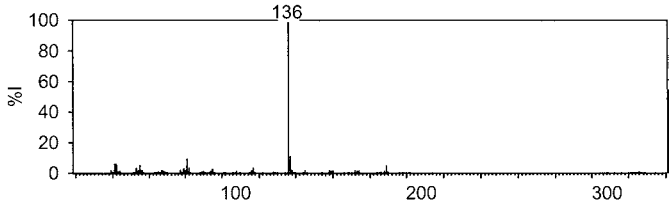
Ultraviolet Spectrum Aqueous acid—250 (A^1_1 = 959a), 317, 346 nm; aqueous alkali—280, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1235, 1215, 1510, 1619, 1030, 1105 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 136, 137, 81, 42, 41, 189, 55, 117.



Quantification

Blood **HPLC** UV and fluorescence detection. Limit of quantification, 0.1 mg/L (on filter paper) [Kolawole, Mustapha 2000]. Fluorescence detection. Quinine and chloroquine and related compounds in whole blood (or dried on filter paper). Limit of quantification, 50 µg/L for quinine [Croes *et al.* 1994]. Fluorescence detection. Limit of detection, 50 nmol/L in whole blood and dried samples [Ericsson *et al.* 1993].

Plasma **HPLC** Fluorescence detection. Limit of quantification, 0.024–0.081 µmol/L for quinine and its metabolites [Mirghani *et al.* 2001]. UV and fluorescence detection. Limit of quantification, 0.1 mg/L [Kolawole, Mustapha 2000]. Fluorescence detection. Limit of quantification, 4.5 nmol/L for 3-hydroxyquinine [Mirghani *et al.* 1998]. UV detection. Limit of detection, 10 µg/L [Babalola *et al.* 1993]. Fluorescence detection. Quinine and chloroquine. Limit of detection, ≈23 µg/L for quinine [Chaulet *et al.* 1993]. Fluorescence detection. For method, see Dua *et al.* [1993]. Fluorescence detection. Limit of detection, 2 nmol/L [Ericsson *et al.* 1993]. Fluorescence detection. For method for quantification of quinine and quinidine, see Edstein *et al.* [1990].

Spectrofluorimetry See Hall *et al.* [1973].

Serum **HPLC** Fluorescence detection. Quinine and chloroquine and related compounds. Limit of quantification, 50 µg/L for quinine [Croes *et al.* 1994].

Q

Fluorescence detection. See Dua *et al.* [1993]. UV detection. For comparison with spectrofluorometric method, see Galloway *et al.* [1990].

Urine **GC–MS** See Hall *et al.* [1973].

HPLC Fluorescence detection. Limit of quantification, 0.024–0.081 µmol/L for quinine and its metabolites [Mirghani *et al.* 2001]. UV detection. Limit of detection, 10 µg/L [Babalola *et al.* 1993]. Fluorescence detection. Quinine and chloroquine. Limit of detection, ≈23 µg/L for quinine [Chaulet *et al.* 1993]. Fluorescence detection. Limit of detection, 50 nmol/L [Ericsson *et al.* 1993].

Spectrofluorimetry See Hall *et al.* [1973].

Saliva **HPLC** UV detection. Limit of detection, 10 µg/L [Babalola *et al.* 1993].

Erythrocytes **HPLC** Fluorescence detection. Quinine and chloroquine. Limit of detection, about 23 µg/L for quinine [Chaulet *et al.* 1993].

Red Blood Cells **HPLC** Fluorescence detection. See Dua *et al.* [1993].

Disposition in the Body Readily absorbed after oral administration but more slowly absorbed after IM or SC doses. Hydroquinine may occur in quinine as an impurity at concentrations up to ≈10%. Metabolised by oxidation to hydroxylated metabolites, mainly the 2-hydroxyquinoline and 6-hydroxyquinoline derivatives, 3-hydroxyquinine, and the corresponding dihydro compounds; quinine-10,11-epoxide and quinine-10,11-dihydrodiol have also been detected in urine. Up to ≈20% of a single dose is excreted in the urine in 24 h with <5% as unchanged drug. <5% of a dose is eliminated in the faeces. The urinary excretion of quinine is increased when the urine is acid; quinine is excreted to some extent in the bile and saliva. Quinine crosses the placenta and is excreted in breast milk.

Therapeutic Concentration In plasma, usually in the range 3–7 mg/L. Malarial infection inhibits hepatic metabolism and thus plasma concentrations resulting from a given dose will vary according to the severity of the infection.

Single oral doses of 300 mg quinine bisulfate administered to 8 healthy subjects and to 8 patients with chronic renal failure on haemodialysis, resulted in mean maximum plasma concentrations of 2.60 mg/L in healthy subjects and 4.56 mg/L in dialysis patients; the time to reach peak concentrations was similar in both groups (1.6 vs 1.9 h) [Roy *et al.* 2002].

Following a single oral dose of quinine sulfate 600 mg to 4 healthy subjects, a mean maximum concentration of 2.88 mg/L was attained in the plasma at 2.75 h and 0.90 mg/L in the saliva at 4.25 h [Babalola *et al.* 1996].

Oral doses of 540 mg, given 8-hourly for 3 days to 11 subjects, resulted in a mean peak plasma concentration of 4 mg/L after 72 h [Hall *et al.* 1973].

Toxicity The estimated minimum lethal dose is 8 g. Blood concentrations greater than about 10 mg/L may cause toxic effects and can be lethal.

Fatal quinine poisoning occurred in three young females, and the following postmortem concentrations (mg/L or µg/g) were reported: blood –, 11, –; kidney 300, 96, 410; liver 1500, 39, 150; spleen 600, 87, –; urine –, –, 860 [Walker GW, personal communication, 1964].

In a fatality involving the ingestion of 32.5 g of quinine sulfate, postmortem brain and liver concentrations of 292 and 3000 µg/g, respectively, were reported [Andrtauskas *et al.* 1974].

Half-life Plasma half-life, 4–15 h (mean 9); it appears to be increased during malarial infection.

Volume of Distribution About 2 L/kg; reduced in malaria infection.

Distribution in Blood Plasma : whole blood ratio, ≈0.93 in normal subjects. In malarial subjects, the plasma concentration rises but this does not seem to be accompanied by a rise in the amount taken up by erythrocytes; under these conditions there may be twice as much in the plasma as in the erythrocytes.

Protein Binding In plasma, 70% in healthy subjects increasing to 90% or higher in malaria infection.

Note For a review of the pharmacokinetics of quinine, chloroquine and amodiaquine, see Krishna and White [1996]. For a review of the pharmacokinetics of quinine and quinidine in malaria, see White [1987]; for a review of the pharmacokinetic interactions of antimalarial agents, see Giao and de Vries [2001]. For a toxicity study of quinine when given with doxycycline and mefloquine, see Karbwang *et al.* [1994].

Dose 1.8 g of quinine sulfate or other salt, by mouth daily, for 7 days for treatment of falciparum malaria. For nocturnal leg cramps 200 to 300 mg of sulfate or bisulfate at night.

Andrtauskas S *et al.* (1974). *Bull Int Assoc Forensic Toxicol* 10(3): 7–8.

Babalola CP *et al.* (1993). Column liquid chromatographic analysis of quinine in human plasma, saliva and urine. *J Chromatogr* 616: 151–154.

Babalola CP *et al.* (1996). Relationship between plasma and saliva quinine levels in humans. *Ther Drug Monit* 18: 30–33.

Chaulet JF *et al.* (1993). Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography. *J Chromatogr* 613: 303–310.

Croes K *et al.* (1994). Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood. *J Anal Toxicol* 18: 255–260..

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dua VK *et al.* (1993). Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and Plasmodium falciparum malaria cases by high-performance liquid chromatography. *J Chromatogr* 614: 87–93.

Edstein MD *et al.* (1990). Simultaneous measurement of quinine and quinidine in human plasma, whole blood, and erythrocytes by high-performance liquid chromatography with fluorescence detection. *Ther Drug Monit* 12: 493–500.

Ericsson O *et al.* (1993). Reversed-phase high-performance liquid chromatography determination of quinine in plasma, whole blood, urine, and samples dried on filter paper. *Ther Drug Monit* 15: 334–337.

Galloway JH *et al.* (1990). A simple and rapid method for the estimation of quinine using reversed-phase high-performance liquid chromatography with UV detection. *J Anal Toxicol* 14: 345–347.

Giao PT, de Vries PJ (2001). Pharmacokinetic interactions of antimalarial agents. *Clin Pharmacokinet* 40: 343–373.

Hall AP *et al.* (1973). Human plasma and urine quinine levels following tablets, capsules, and intravenous infusion. *Clin Pharmacol Ther* 14: 580–585.

Karbwang J *et al.* (1994). Quinine toxicity when given with doxycycline and mefloquine. *Southeast Asian J Trop Med Public Health* 25: 397–400.

Kolawole JA, Mustapha A (2000). Improved RP-HPLC determination of quinine in plasma and whole blood stored on filter paper. *Biopharm Drug Dispos* 21: 345–352.

Krishna S, White NJ (1996). Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. *Clin Pharmacokinet* 30: 263–299.

Mirghani RA *et al.* (1998). High-performance liquid chromatographic method for the determination of the major quinine metabolite, 3-hydroxyquinine, in plasma and urine. *J Chromatogr B Biomed Sci Appl* 708: 209–216.

Mirghani RA *et al.* (2001). Simultaneous determination of quinine and four metabolites in plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 754: 57–64.

Roy L *et al.* (2002). Quinine pharmacokinetics in chronic haemodialysis patients. *Br J Clin Pharmacol* 54: 604–609.

White NJ (1987). The pharmacokinetics of quinine and quinidine in malaria. *Acta Leiden* 55: 65–76.

Quinisocaine

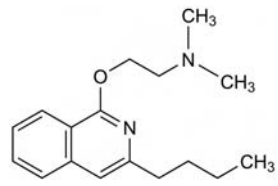
Anaesthetic (Local)

$C_{17}H_{24}N_2O$ = 272.4

CAS—86-80-6

IUPAC Name 2-(3-Butylisoquinolin-1-yl)oxy-*N*,*N*-dimethylethanamine

Synonyms 2-[(3-Butyl-1-isoquinolinyl)oxy]-*N*,*N*-dimethylethanamine; chinisocaine; dimethisoquin.



Chemical Properties A liquid. Bp 155° to 157°. Log *P* (octanol/water), 4.4.

Quinisocaine Hydrochloride

$C_{17}H_{24}N_2O$, HCl = 308.9

CAS—2773-92-4

Proprietary Names *Haenal*; *Isochinol*; *Pruralgan*; *Pruralgin*; *Quotane*.

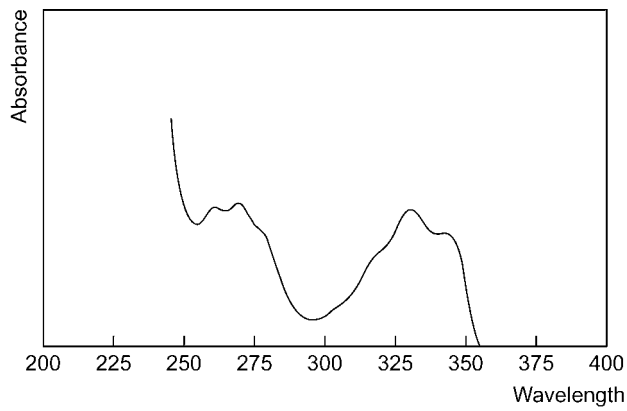
Chemical Properties A white crystalline powder. Mp 144° to 148°. Soluble 1 in 8 of water, 1 in 3 of ethanol and 1 in 2 of chloroform; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 0.55; system TC—R_f 0.46; system TL—R_f 0.28; system TAJ—R_f 0.25; system TAK—R_f 0.13; system TAL—R_f 0.66 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2030.

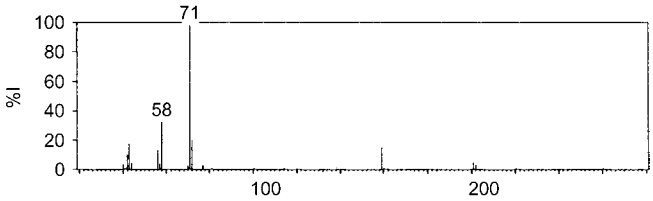
High Performance Liquid Chromatography System HA—*k* 2.2; system HR—*k* 11.24.

Ultraviolet Spectrum Aqueous acid—261, 269 (A_1^1=159a), 331 nm.



Infrared Spectrum Principal peaks at wavenumbers 1576, 1631, 1499, 1155, 1115, 751 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 71, 58, 72, 43, 159, 56, 42, 201.



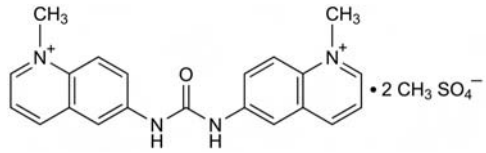
Use Quinisocaine hydrochloride is used in a concentration of 0.5%.

Quinuronium Sulfate

Antiprotozoal (Veterinary)
$C_{23}H_{26}N_4O_9S_2$ = 566.6
CAS—135-14-8

IUPAC Name 1,3-Bis(1-methylquinolin-1-ium-6-yl)urea; methyl sulfate
Synonyms SN-5870; 6,6′-Ureylenebis[1,1′-dimethylquinolinium]sulfate.

Proprietary Names *Acaprin*; *Atral*; *Babesan*; *Baburan*; *Pirevan*; *Pyroplasmin*; *Zothelone*.

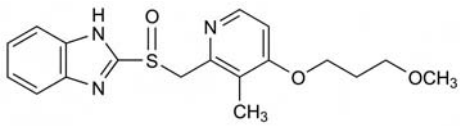


Chemical Properties A creamy-white to yellow, crystalline powder. Mp 237°, with decomposition. Very soluble in water; very slightly soluble in organic solvents. On heating, aqueous solutions darken and some decomposition may occur.
Colour Test Aromaticity (method 2)—yellow/orange.
Thin-layer Chromatography System TA—R_f 0.00 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—274 ($A^{1\%}_{1cm}$=1527a), 323, 359 nm.
Infrared Spectrum Principal peaks at wavenumbers 1210, 1248, 1276, 1572, 1025, 1525 cm^{-1} (KBr disk).

Rabeprazole

Proton Pump Inhibitor
$C_{18}H_{21}N_3O_3S = 359.4$
CAS—117976-89-3

IUPAC Name 2-[[4-(3-Methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl]-1*H*-benzimidazole
Synonyms LY-307640; 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole; pariprazole.

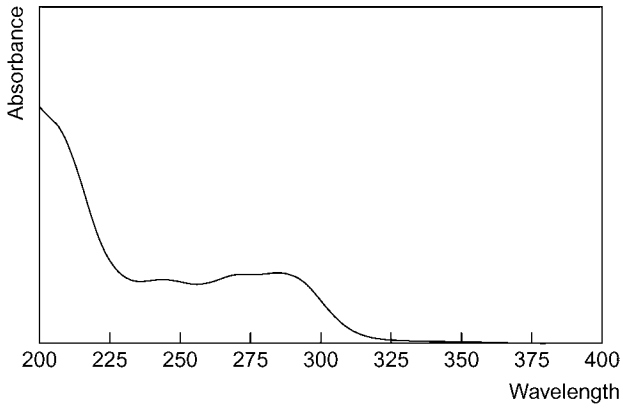


Chemical Properties White crystals. Mp 99° to 100°.

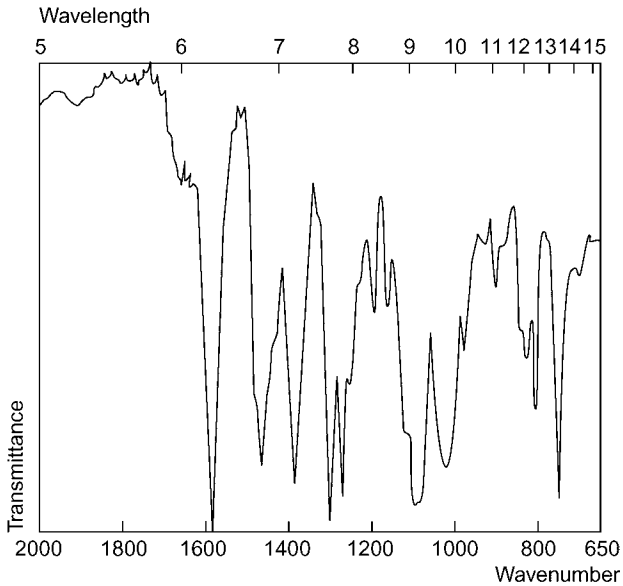
Rabeprazole Sodium

$C_{18}H_{20}N_3NaO_3S = 381.4$
CAS—117976-90-6
Synonyms E-3810; SHKA.
Proprietary Names *AcipHex*; *Pariet*.
Chemical Properties A white to slightly yellowish white, crystalline solid. Mp 140° to 141°. Very soluble in water and methanol; freely soluble in ethanol, chloroform and ethyl acetate; insoluble in ether and *n*-hexane.

High Performance Liquid Chromatography Column: C_8 Inertsil (150 × 4.6 mm i.d., 5 µm). Column temperature: 40°. Mobile phase: acetonitrile: phosphate buffer (0.1 mol/L, pH 7.0) (28:72), flow rate 1.4 mL/min. UV detection (λ=288 nm). Retention times: rabeprazole sodium, 6.5 min; demethylated metabolite, 2.8 min; demethylated thioether metabolite, 6.0 min; sulfone metabolite, 8.0 min; thioether metabolite, 19.7 min [Nakai *et al.* 1994].
Ultraviolet Spectrum Aqueous base (0.01 mol/L NaOH)—292 nm.



Infrared Spectrum Principal peaks at wavenumbers 1584, 1298, 1462, 1081 cm^{-1}.



Quantification
Plasma **HPLC** UV detection (λ=290 nm). Limit of quantification, 0.03 mg/L [Mano *et al.* 1996]. UV detection (λ=288 nm). Limit of quantification, 5 µg/L for the drug and 20 µg/L for the metabolites [Nakai *et al.* 1994].
Disposition in the Body Rabeprazole is well absorbed (at least 30%) and can be detected in the plasma within 1 h. Food delays absorption. It is extensively and rapidly metabolised; this is regulated by the CYP2C19 isoenzyme in the liver and to some extent by CYP3A4. The primary metabolite is the rabeprazole thioether. Other metabolites are sulfone E3810, desmethyl E3810, desmethylated thioether E3810 and thioether carboxylic acid E3810. 90% of the administered drug is excreted in urine with 30% as the thioether carboxylic acid metabolite and its glucuronide. No unchanged rabeprazole has been recovered in urine or faeces. Renal excretion accounts for 30 to 35%. There is no accumulation following multiple dosing.
Therapeutic Concentration A maximum plasma concentration of 0.406 mg/L can be achieved with a single 20 mg dose of rabeprazole. A maximum concentration of 0.418 mg/L is reached after 40 mg for 7 days. Time to reach maximum plasma concentration is about 3.1 h [Prakash, Faulds 1998].

Six healthy volunteers were administered with doses between 10 and 80 mg rabeprazole sodium. The maximum plasma concentration reached for the 20 mg dose was 0.406 mg/L, observed after 3.5 h. The 80 mg dose produced concentrations of ≈1.4 mg/L at 4 h; 40 mg dose ≈0.9 mg/L at 3.5 h; and the 10 mg dose, ≈0.15 mg/L at 4.5 h [Nakai *et al.* 1994].

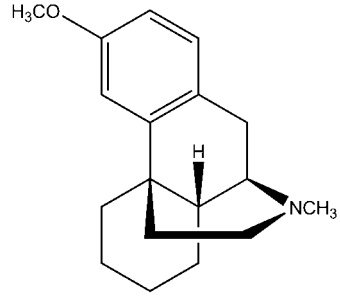
Bioavailability 52%.
Half-life 1 to 2 h.
Clearance 35.3 L/h; 4.37 to 8.40 mL/min/kg.
Protein Binding 96.3%.
Dose 20 mg daily.

Mano N *et al.* (1996). Plasma direct injection high-performance liquid chromatographic method for simultaneously determining E3810 enantiomers and their metabolites by using flavoprotein-conjugated column. *J Pharm Sci* 85: 903–907.
Nakai H *et al.* (1994). Determination of a new H(+)-K+ ATPase inhibitor (E3810) and its four metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 660: 211–220.
Prakash A, Faulds D (1998). Rabeprazole. *Drugs* 55(2): 261–267.

Racemethorphan

Narcotic, Antitussive
$C_{18}H_{25}NO = 271.4$
CAS—510-53-2

Synonyms Deoxydihydrothebacodine; *dl-cis*-1,2,3,9,10,10a-hexahydro-6-methoxy-11-methyl-4*H*-10-4a-iminoethanophenanthrene; *dl-cis*-1,3,4,9,10,10a-hexahydro-6-methoxy-11-methyl-2*H*-10-4a-iminoethanophenanthrene; methorphan; (±)-3-methoxy-17-methylmorphinan.



Chemical Properties Log *P* (octanol/water) 3.97 [Meylan, Howard 1995]; 3.6 [National Institutes of Health 2008]. Racemethorphan is extracted by organic solvents from aqueous alkaline solutions.

Racemethorphan Hydrobromide

$C_{18}H_{25}NO,HBr = 352.3$
CAS—6031-86-3
Synonym Ro-1-5470
Chemical Properties Crystals.

Racemethorphan Hydrobromide (*d*-Form)

CAS—125-69-9
Synonym Demorphan hydrobromide; dextromethorphan hydrobromide; Ro-1-5470/5.
Proprietary Names *Benylin*; *Canfodion*; *Cosylan*; *Hihustan*.
Chemical Properties Crystals. Mp 122° to 124°. Solubility in water 1.5% at 25°, 5% at 50° and 25% at 85°. Solubility 25% in 95% ethanol at room temperature, 10% in glycerol. Soluble in propylene glycol and chloroform, practically insoluble in ether. Long range stability of aqueous solutions obtained by adjusting the pH to 4–5.6. Reacts with alkalis forming the free base which is practically insoluble in water [O'Neil *et al.* 2006].

Racemethorphan Hydrobromide (*l*-Form)
CAS—125-68-8
Synonyms Levomethorphan hydrobromide; Ro-1-5470/6; Ro 1-7788.
Chemical Properties Crystals. Mp 124° to 126°.

Colour Test Ammonium molybdate test—blue→green (limit of detection, 0.25 μg).
Thin-layer Chromatography System T1—R_f 0.25 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—Retention time 0.50 relative to codeine
Ultraviolet Spectrum Ethanol—220–221, 281, 289 nm; aqueous acid (0.1 N sulfuric acid)—277 nm with an inflexion at 283 nm.

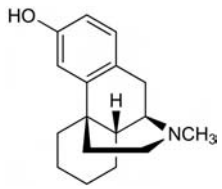
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health, (2008). *Racemethorphan*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5359423&loc=ec_rcs. (accessed 30 June 2008).
O'Neil MJ *et al.* (2006). The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Racemoramide

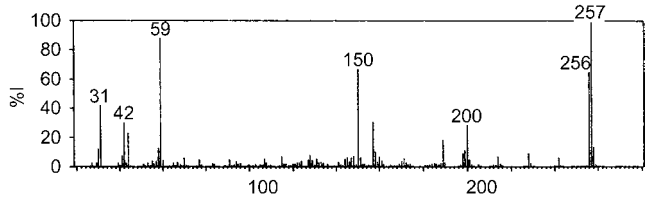
Narcotic Analgesic
$C_{25}H_{32}N_2O_2$ = 392.5
IUPAC Name 3-Methyl-4-morpholino-2,2-diphenylbutyrl)pyrrolidine
Synonyms NIH 7421; R 610; SKF 5137.
Proprietary Name *Eupharma*.
Chemical Properties A white crystalline powder. A racemic mixture of dextromoramide (shown) and levomoramide.
Thin-layer Chromatography System T1—R_f 0.70 (acidified iodoplatinate spray—positive reaction).
Gas Chromatography System G2/225—RT 4.70 min (relative to codeine).
Ultraviolet Spectrum Aqueous acid (0.05 mol/L sulfuric acid)—253.5 and 264 nm (maxima) 267 and 270 nm (minor inflexions).
Dose Up to 20 mg.

Racemorphan

Narcotic Analgesic
$C_{17}H_{23}NO$ = 257.4
CAS—297-90-5
Synonyms 17-Methylmorphinan-3-ol; methorphinan.

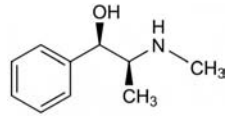


Chemical Properties White powder. Mp 251° to 253°. Soluble in dilute acetic acid.
Thin-layer Chromatography System TA—R_f 0.34; system TAG—R_f 0.02; system TB—R_f 0.14; system TC—R_f 0.09 (Dragendorff spray, positive, acidified iodoplatinate solution, positive).
Gas Chromatography System GA—racemetorphan RI 2138; M (nor--) RI 2193; system GB—racemetorphan RI 2237; M (nor--) RI 2244; M (OH-) RI 2420.
Ultraviolet Spectrum Aqueous acid—279 (A^1_1 = 79a); aqueous alkali—240 (A^1_1 = 339a), 299 nm (A^1_1 = 119a)
Infrared Spectrum Principal peaks at wavenumbers 1242, 1280, 1580, 1495, 1505, 760 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 257, 59, 150, 256, 31, 157, 42, 200.



Racephedrine

Sympathomimetic
$C_{10}H_{15}NO$ = 165.2
CAS—90-81-3
IUPAC Name 2-(Methylamino)-1-phenylpropan-1-ol
Synonyms *dl*-Ephedrine; [α-(methylamino)ethyl]benzenemethanol; racemic ephedrine.
Proprietary Name It is an ingredient of *Riddofan*.



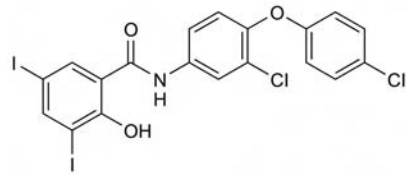
Chemical Properties Crystals. Mp 75°. Soluble in water, ethanol, chloroform and ether. Log *P* (octanol/water), 0.7.
Note For analytical data, see under Ephedrine.

Racephedrine Hydrochloride
$C_{10}H_{15}NO,HCl$ = 201.7
CAS—134-71-4
Proprietary Names *Efetonina*; *Ephetonin*.
Chemical Properties Fine white crystals or powder. Mp 189°. Soluble 1 in about 4 of water and 1 in about 4 of ethanol; practically insoluble in ether.

Dose 50 to 200 mg of racephedrine hydrochloride daily.

Rafoxanide

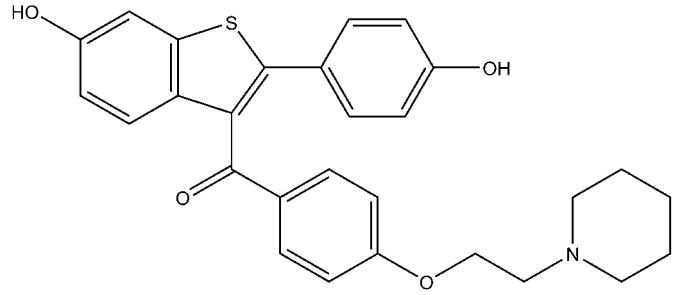
Anthelmintic (Veterinary)
$C_{19}H_{11}Cl_2I_2NO_3$ = 626.0
CAS—22662-39-1
IUPAC Name *N*-[3-Chloro-4-(4-chlorophenoxy)phenyl]-2-hydroxy-3,5-diiodobenzamide
Synonym MK-990
Proprietary Names *Flukanide*; *Flukex*; *Ranide*; *Ridafluke*.



Chemical Properties A greyish-white to brown powder. Mp 168° to 170°. Practically insoluble in water; soluble 1 in 25 of acetone, 1 in 40 of chloroform, 1 in 35 of ethyl acetate and 1 in 200 of methanol. Log *P* (octanol/water), 8.1.
Colour Test Iodine test—positive.
Thin-layer Chromatography System TA—R_f 0.89 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Methanolic acid—280 (A^1_1=243b), 335 nm (A^1_1=149a).
Infrared Spectrum Principal peaks at wavenumbers 1215, 1562, 1592, 1257, 1087, 1275 cm^{-1} (KBr disk).

Raloxifene

Antiosteoporotic, Benzothiophene, Bone Modulator, Selective Oestrogen Receptor Modulator
$C_{28}H_{27}NO_4S$ = 473.6
CAS—84449-90-1
IUPAC Name [6-Hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4-(2-piperidin-1-ylethoxy)phenyl]methanone
Synonyms 6-Hydroxy-2-(*p*-hydroxyphenyl)benzo[b]thien-3-yl-*p*-(2-piperidinoethoxy)phenyl ketone; keoxifene; LY-139481.



Chemical Properties Crystals from acetone. Off-white to pale yellow non-volatile solid. Mp 143° to 147°. Very slightly soluble in water. pK_{a1} 8.95, pK_{a2} 9.83, pK_{a3} 10.91. Log *P* (octanol/water), 3.12 [Trontelj *et al.* 2005], 5.75 [Wishart 2006].

Raloxifene Hydrochloride
$C_{28}H_{27}NO_4S,HCl$ = 510.0
CAS—82640-04-8
Synonyms Keoxifene hydrochloride; LY-156758.

Proprietary Names *Bonmax*; *Celvista*; *Estroact*; *Evista*; *Ketidin*; *Optruma*; *Oseofem*; *Ralista*; *Raxeto*.
Chemical Properties Crystals from methanol/water. Mp 258°.

High Performance Liquid Chromatography Column: Nucleosil C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 3.0; 36 : 64), flow rate 0.7 mL/min. UV detection (λ = 287 nm) and colorimetric detection. Retention time: 9 min. Limit of quantification, 0.61 and 0.34 mg/L for UV and colorimetric detection, respectively, limit of detection, 0.2 and 0.11 mg/L, for UV and colorimetric detection, respectively [Trontelj *et al.* 2005].
Ultraviolet Spectrum Neutral—287 nm.
Quantification
***Plasma* HPLC** Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L sodium dodecyl sulfate (pH 7; 11 : 9). UV detection (λ = 286 nm). Limit of quantification not reported [Perez-Ruiz *et al.* 2004].

LC-MS Column: C_{18} (50 × 2.0 mm i.d.). Mobile phase: 0.1% formic acid in acetonitrile : 0.1% formic acid in water (10 : 90 for 0.25 min to 31 : 69 over 4.75 min to 63 : 37 over 1 min to 100 : 0 over 1 min for 3 min), flow rate 0.5 mL/min for 7 min to 0.8 mL/min over 1 min for 2 min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.088 µg/L for raloxifene, 0.2 µg/L for raloxifene 6-glucuronide, 1.6 µg/L for raloxifene 4′-glucuronide [Trontelj *et al.* 2007]. Column: C_{18} (30 × 1.0 mm i.d., 3 µm). Mobile phase: methanol : acetonitrile : water (28 : 32 : 30) containing 5 mmol/L ammonium acetate, flow rate 0.5 mL/min. MRM acquisition mode. Retention time: 8 min. Limit of quantification, 38 µg/L [Zweigenbaum, Henion 2000].

CE Column: fused silica capillary (total/effective length: 80/50 cm, 75 µm). UV detection (λ = 286 nm). Limit of quantification, 20.5 µg/L; limit of detection, 6.1 µg/L [Perez-Ruiz *et al.* 2004].
***Other* HPLC** Human Tissues. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (80 : 20 to 50 : 50 over 25 min). UV detection (λ = 290 nm). Retention time: raloxifene monosulfate 6 min; raloxifene disulfate 8 min. Limit of quantification not reported [Falany, Falany 2007]. Rat Lung. Column: Cosmosil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: 0.05 mol/L ammonium acetate (pH 4.0; 33 : 67), flow rate 1.0 mL/min. UV detection (λ = 289 nm). Retention time: 5.2 min. Limit of quantification, 0.008 mg/L [Yang *et al.* 2007]. Rat Liver. Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate : methanol (70 : 30 to 20 : 80 over 40 min for 20 min). UV detection (λ = 254 nm). Retention time: raloxifene 43.1 min; dihydroxyraloxifene 33.0 min; piperidine-OH/c-ring-OH 34.7 min; piperidine-OH/piperidine-OH 36.9 min; OH in a or b-ring 38.9 min; OH in a or b-ring 40.0 min; c-ring-OH 42.0 min. Limit of quantification not reported [Van Liempd *et al.* 2006]. Tablets. Column: Nucleosil C_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 3.0; 36 : 64), flow rate 0.7 mL/min. UV detection (λ = 287 nm) and colorimetric detection. Retention time: 9 min. Limit of quantification, 0.61 and 0.34 mg/L for UV and colorimetric detection, respectively; limit of detection, 0.2 and 0.11 mg/L for UV and colorimetric detection, respectively [Trontelj *et al.* 2005]. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L sodium dodecyl sulfate (pH 7; 11 : 9). UV detection (λ = 286 nm). Limit of quantification not reported [Perez-Ruiz *et al.* 2004]. Cultured Cells. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.25% perchloric acid-0.25% ethanoic acid (20 : 80 to 75 : 25 over 40 min), flow rate 0.7 mL/min. UV detection (λ = 280 nm). Retention time: ~28 min. Limit of quantification not reported [Toader *et al.* 2003]. Rat Plasma. Column: LiChrosorb C_8 or Zorbax C_8 (10 µm). Mobile phase: 0.1 mol/L sodium acetate buffer (pH 4.0) : acetonitrile (50 : 50), flow rate 2 mL/min. Electrochemical detection. Retention time: 5.8 min. Limit of quantification not reported [Lindstrom *et al.* 1984].

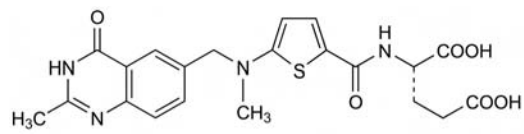
CE Tablets. Column: fused silica capillary (total/effective length: 80/50 cm, 75 µm). UV detection (λ = 286 nm). Limit of quantification, 20.5 µg/L, limit of detection, 6.1 µg/L [Perez-Ruiz *et al.* 2004].
Disposition in the Body Raloxifene is rapidly absorbed from the gastrointestinal tract, with peak plasma concentrations reached within 0.5 h. It is metabolically activated and distributed as the active form in various tissues, including the liver, lungs, spleen, bone, uterus and kidneys. It undergoes extensive first-pass metabolism, forming the raloxifene conjugates 4′-glucuronide (M2), 6-glucuronide (M1) and 4′,6-diglucuronide. Very small amounts of the parent compound are found in the circulation. No other metabolites of raloxifene are formed, indicating that it is not metabolised by the CYP enzyme system. Raloxifene is primarily eliminated in the bile and excreted via the faeces, with small amounts excreted in urine. Raloxifene undergoes enterohepatic circulation.
Therapeutic Concentration
Ten healthy male volunteers and 10 renally impaired patients were administered a single oral dose of two 60 mg tablets of raloxifene hydrochloride (a total of 111.42 mg of the free raloxifene base) after an overnight fast. Mean peak plasma raloxifene concentrations for the healthy group and renally impaired group were 0.7 and 1.0 µg/L, reached at 6 and 8 h, respectively [Czock *et al.* 2005].

A group of 47 postmenopausal women with osteoporosis taking a daily dose of 60 mg raloxifene were assessed for plasma concentrations of raloxifene and its metabolites M1 and M2. These were reported together with coefficients of variation (CV%) as 1.97 µg/L (63%), 204 µg/L (79%), and 43.6 µg/L (81%) for raloxifene, M2 and M1, respectively. The large interindividual variations are thought to reflect genetic polymorphisms in gene for UDP-glucuronosyltransferase (UGT), as the glucuronidation efficiency of various UGT polymorphic variants can affect the clearance of raloxifene and thereby its plasma concentrations [Trontelj *et al.* 2007].
Toxicity Colestyramine reduces the absorption and enterohepatic recycling of raloxifene, and they should not be given together. Raloxifene may decrease the efficacy of warfarin.
Bioavailability Approximately 60% is absorbed, but owing to extensive first-pass metabolism the absolute bioavailability is 2% [Clemett, Spencer 2000].
Half-life Approximately 27.7 h [Clemett, Spencer 2000].
Volume of Distribution 2348 L [Clemett, Spencer 2000].
Clearance Approximately 44.1 L/h/kg (range, 40 to 60) [Clemett, Spencer 2000].
Protein Binding More than 95%, primarily to albumin and α_1-acid glycoprotein. It does not bind to sex-hormone-binding globulin.
Dose Raloxifene is used in doses of 60 mg daily by mouth for the prevention and treatment of postmenopausal osteoporosis.

Clemett D, Spencer CM (2000). Raloxifene: a review of its use in postmenopausal osteoporosis. *Drugs* 60: 379–411.
Czock D *et al.* (2005). Raloxifene pharmacokinetics in males with normal and impaired renal function. *Br J Clin Pharmacol* 59: 479–482.
Falany JL, Falany CN (2007). Interactions of the human cytosolic sulfotransferases and steroid sulfatase in the metabolism of tibolone and raloxifene. *J Steroid Biochem Mol Bio* 107: 202–210.
Lindstrom TD *et al.* (1984). Disposition and metabolism of a new benzothiophene antiestrogen in rats, dogs and monkeys. *Xenobiotica* 14: 841–847.
Perez-Ruiz T *et al.* (2004). Development and validation of a quantitative assay for raloxifene by capillary electrophoresis. *J Pharm Biomed Anal* 34: 891–897.
Toader V *et al.* (2003). Nitrosation, nitration, and autoxidation of the selective estrogen receptor modulator raloxifene by nitric oxide, peroxynitrite, and reactive nitrogen/oxygen species. *Chem Res Toxicol* 16: 1264–1276.
Trontelj J *et al.* (2005). HPLC analysis of raloxifene hydrochloride and its application to drug quality control studies. *Pharmacol Res* 52: 334–339.
Trontelj J *et al.* (2007). Development and validation of a liquid chromatography–tandem mass spectrometry assay for determination of raloxifene and its metabolites in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 855: 220–227.
VanLiempd SM *et al.* (2006). On-line formation, separation, and estrogen receptor affinity screening of cytochrome P450-derived metabolites of selective estrogen receptor modulators. *Drug Metab Dispos* 34: 1640–1649.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.
Yang Z *et al.* (2007). The determination of raloxifene in rat tissue using HPLC. *Biomed Chromatogr* 21: 229–233.
Zweigenbaum J, Henion J (2000). Bioanalytical high-throughput selected reaction monitoring-LC/MS determination of selected estrogen receptor modulators in human plasma: 2000 samples/day. *Anal Chem* 72: 2446–2454.

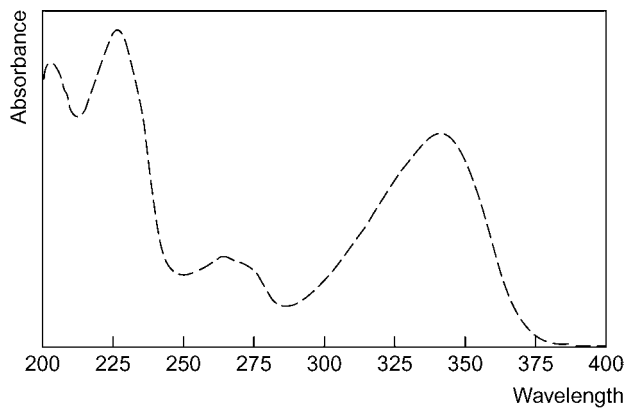
Raltitrexed

Antineoplastic
$C_{21}H_{22}N_4O_6S$ = 458.5
CAS—112887-68-0
IUPAC Name (2*S*)-2-[[5-[Methyl-[(2-methyl-4-oxo-1*H*-quinazolin-6-yl)methyl]amino]thiophene-2-carbonyl]amino]pentanedioic acid
Synonyms *N*-[[5-[[(1,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-methylamino]-2-thienyl]carbonyl]-L-glutamic acid; ICI-D-1694; ZD-1694.
Proprietary Name *Tomudex*



Chemical Properties Pale yellow powder. Soluble in water.

Raltitrexed Monohydrate
Chemical Properties Crystals. Mp 180° to 184°.
Ultraviolet Spectrum



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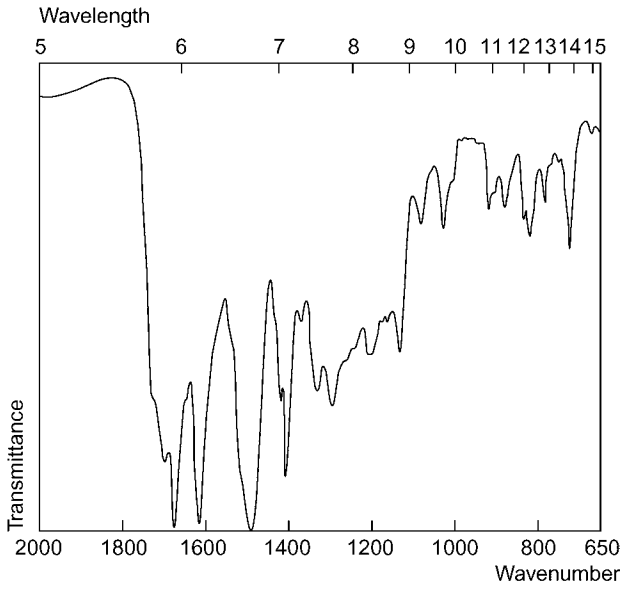
Infrared Spectrum



Disposition in the Body Following IV administration of raltitrexed, triphasic pharmacokinetics are displayed. There is a rapid decline from peak plasma concentration followed by a slow terminal elimination phase. The maximum plasma concentration is reached within 15 min. The drug is actively transported into cells and metabolised to active polyglutamate forms. The reminder of the dose is excreted unchanged with approx. 50% of the dose appearing in urine and 15% in faeces. No accumulation occurs of the drug after multiple dosing.

Therapeutic Concentration

The peak plasma concentration after a 3 mg/m^2 dose was 833 µg/L. The mean observed peak plasma concentration in cancer patients was 700.6 µg/L [Beale *et al.* 1998].

Toxicity

Increasing the dosage above 3 mg/m^2, increases the incidence of life threatening or fatal toxicity [ABPI Data Sheets].

Bioavailability Following IP administration, it is fully bioavailable. Following oral administration, 10 to 20%.

Half-life 8 days (257 h).

Volume of Distribution Steady state, 548 L.

Clearance Following a 3 mg/m^2 dose clearance is 51.6 mL/min and the renal clearance is 25.1 mL/min. It is reduced in those with renal impairment by 25%.

Protein Binding 93%.

Dose 3 mg/m^2 IV every 3 weeks.

Beale P *et al.* (1998). Metabolism, excretion and pharmacokinetics of a single dose of [14C]-raltitrexed in cancer patients. *Cancer Chemother Pharmacol* 42: 71–76.

Ramelteon

Propanamide, Melatonin Antagonist, Sedative

$C_{16}H_{21}NO_2 = 259.3$

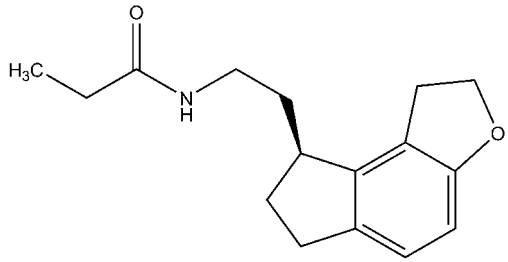
CAS—196597-26-9

IUPAC Name (−)-*N*-{2-[(8*S*)-1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl] ethyl}propanamide

Synonym TAK-375

Proprietary Name *Rozerem*

Chemical Properties Crystals. Mp 113° to 115°. Log *P* (octanol/water), 2.4 [Wishart 2006].



Quantification

Serum **LC-MS** Column: C_{18} (150 × 2.0 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile : methanol (60 : 30 : 10), flow rate 0.2 mL/min. TIS, positive ion mode, MRM acquisition mode. Limit of quantification, ramelteon 0.05 µg/L, metabolites 0.5 µg/L [Karim *et al.* 2006].

Disposition in the Body Rapidly and extensively absorbed after oral administration. Absorption is reduced when administered with food. It undergoes extensive first-pass metabolism. The primary metabolic pathway of ramelteon is oxidation to hydroxyl and carbonyl groups via CYP1A2 and, to a lesser degree, the CYP2C subfamily and CYP3A4, followed by glucuronidation. Four main metabolites are formed, only one of which (MII, oxidation of indene ring) is active (melatonin MT_1 and MT_2 receptor affinity is about one fifth to one tenth of the parent drug and it is 17- to 25-fold less potent than ramelteon in *in vitro* functional assays). Approximately 84% of the drug is eliminated in the urine, with 4% excreted in the faeces. Negligible urinary excretion (<2%) of unchanged drug occurs. Renal clearance of ramelteon is reduced in elderly patients.

Therapeutic Concentration

Five groups of 12 healthy volunteers were administered a single oral dose of either 4, 8, 16, 32 or 64 mg ramelteon following an overnight fast. Mean peak serum concentrations and half-life values of ramelteon and its metabolite MII were reported as follows:

Parameter	*Analyte*	*Ramelteon single dose (mg)*				
		4	**8**	**16**	**32**	**64**
C_{max} (µg/L)	Ramelteon	1.15	5.73	6.92	14.4	25.9
	MII	34.4	73.0	129	284	463
Time (h)	Ramelteon	0.78	0.75	0.78	0.88	0.94
	MII	0.88	0.94	1.06	1.06	1.28
Half-life h)	Ramelteon	0.83	1.36	1.28	1.59	1.90
	MII	2.27	2.62	2.56	3.17	3.39

Half-life values were observed to be dose dependent [Karim *et al.* 2006].

A total of 48 volunteers, 24 men and 24 women divided into 2 groups (young, aged 18 to 35 years; elderly, aged 60 to 79 years) were administered a single 16 mg oral dose of ramelteon 90 min after a light breakfast. Mean peak serum concentrations of ramelteon and MII together with other pharmacokinetic variables were reported as:

	Young male	*Young female*	*Elderly male*	*Elderly female*
Ramelteon				
C_{max} (µg/L)	5.9	7.9	12.0	11.2
Time (h)	1.6	1.6	1.5	1.3
Oral clearance (L/min)	59	56	33	25
MII				
C_{max} (µg/L)	103	118	125	125
Half-life (h)	2.2	2.3	3.1	3.0

Results were also presented as follows:

	All young	*All elderly*	*All male*	*All female*
Ramelteon				
C_{max} (µg/L)	6.9	11.6	8.9	9.6
Time (h)	1.6	1.4	1.5	1.4
Oral Clearance (L/min)	58	29	46	40
MII				
C_{max} (µg/L)	110	125	114	121
Half-life (h)	2.3	3.0	2.6	2.6

Differences in values between young and elderly groups were attributed to known reduced clearance of CYP1A4 substrate drugs in elderly subjects [Greenblatt *et al.* 2007].

Toxicity Drug interactions may occur with inhibitors or inducers of CYP1A4, CYP3A4 and the CYP2C subfamily. Co-administration of ramelteon with fluvoxamine (CYP1A2 inhibitor) resulted in a 190-fold increase in the AUC for ramelteon, whereas co-administration of ramelteon with rifampicin (CYP1A4 inducer) resulted in an 80% reduction in the AUC for ramelteon and MII. However, co-administration of ramelteon with theophylline (CYP1A2 substrate) or dextromethorphan (CYP2D6 substrate) had no clinically significant effect on exposure to ramelteon [Takeda Pharmaceuticals 2008].

Bioavailability Less than 2%.

Half-life Approximately 1 to 2 h.

Volume of Distribution Approximately 73.6 L.

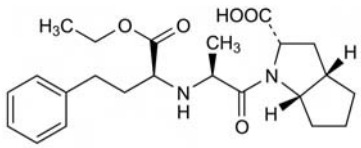
Clearance Reported as ~58 L/min in young adults; reduced to 29 L/min in the elderly.
Protein Binding Approximately 82%, primarily to albumin.
Note For reviews of ramelteon, see Borja, Daniel [2006] and McGechan *et al* [2005].
Dose Usual dose is 8 mg by mouth taken within 30 min of bedtime; it should not be taken with or immediately after a high-fat meal.

Borja N, Daniel K (2006). Ramelteon for the treatment of insomnia. *Clin Ther* 28: 1540–1555.
Greenblatt DJ *et al.* (2007). Age and gender effects on the pharmacokinetics and pharmacodynamics of ramelteon, a hypnotic agent acting via melatonin receptors MT_1 and MT_2. *J Clin Pharmacol* 47: 485–496.
Karim A *et al.* (2006). Disposition kinetics and tolerance of escalating single doses of ramelteon, a high-affinity MT_1 and MT_2 melatonin receptor agonist indicated for treatment of insomnia. *J Clin Pharmacol* 46: 140–148.
McGechan A *et al.* (). Ramelteon. *CNS Drugs* 19: 1057–1065.
Takeda Pharmaceuticals (2008). *Rozerem (Ramelteon) Tablets: Prescribing Information.* Deerfield IR: Takeda Pharmaceuticals
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Ramipril

Antihypertensive
$C_{23}H_{32}N_2O_5$ = 416.5
CAS—87333-19-5

IUPAC Name (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2*H*-cyclopenta[b]pyrrole-2-carboxylic acid
Synonyms (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid; HOE-498; ramiprilum.
Proprietary Names *Acovil*; *Altace*; *Cardace*; *Delix*; *Pramace*; *Quark*; *Ramace*; *Triatec*; *Tritace*; *Unipril*; *Vespril*. It is also an ingredient of *Arelix*; *Composto*; *Delix Plus* and *Triapin*.



Chemical Properties Needle-shaped crystals (from ether). Mp 109°. Soluble in methyl alcohol; sparingly soluble in water. Log *P* (octanol/water), 3.32 for ramipril; 0.22 for ramiprilat.

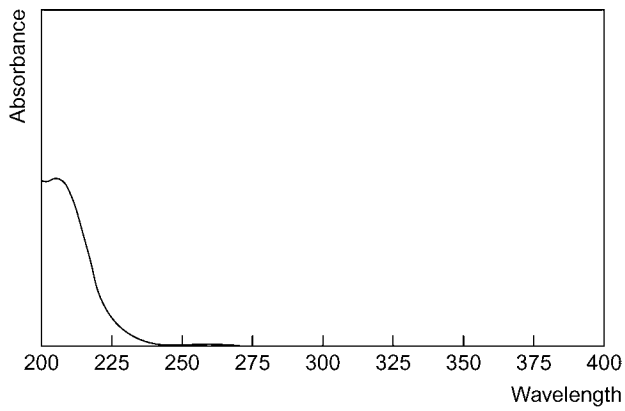
Ramiprilat

$C_{21}H_{28}N_2O_5$ = 388.5
CAS—87269-97-4

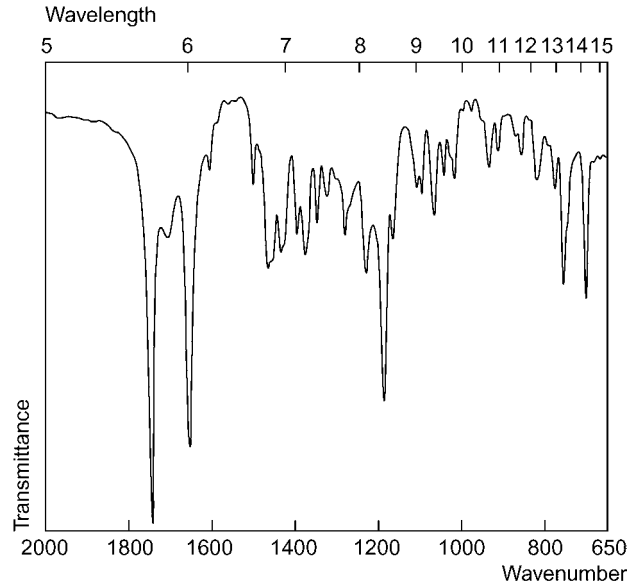
Gas Chromatography System GP—RI 2880 ramipril-ME; RI 2865 M (ramiprilat)-ME3; RI 2925 M (ramiprilate-H_2O)-ME3.
High Performance Liquid Chromatography System HAA—RT 15.7 min; system HZ—RT 4.2 min.

Column: LC-8 Supelcosil (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L0.1 mol/Lsodium perchlorate solution, pH 2.5, flow rate 1.5 mL/min. IS: clobazam. UV detection (λ= 210 nm). Retention times: ramipril, 5.1 min; IS, 3.4 min [Belal *et al.* 2001].

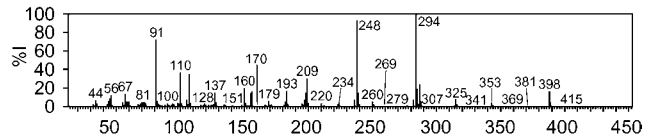
Ultraviolet Spectrum Aqueous acid (0.2 mol/L H_2SO_4)—252, 258 nm; basic—252, 258, 267 nm.



Infrared Spectrum Principal peaks at wavenumbers 1743, 1652, 1187, 756 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 294, 248, 91, 110, 117, 209, 160, 297.



Disposition in the Body Ramipril is rapidly absorbed after oral administration, with 54–65% of the dose absorbed. Absorption is not affected by the presence of food. It is metabolised in the liver to the active drug ramiprilat, a dicarboxylic acid, by hydrolysis, as well as ramipril glucuronide, ramiprilat glucuronide and diketopiperazine derivatives. Peak plasma concentrations of the parent drug are reached within 0.25 and 1 h and for the metabolite, ramiprilat, 2–4 h. The drug is excreted mainly in urine (60%) and faeces (40%) with little unchanged drug detected. The drug is rapidly cleared from the blood and distributed to all body tissues especially to the liver, kidneys and lungs where high concentrations are found. The drug can still be detected in serum up to 24 h after dosing.

Therapeutic Concentration

Twelve healthy male volunteers were administered 2.5 mg ramipril orally, IV and ramiprilat 2.5 mg IV on three separate occasions, after an overnight fast. The peak plasma ramipril concentration after the oral ramipril dose was 6.2 µg/L and 2.7 µg/L for the metabolite, ramiprilat. These were observed at 48 and 151 min, respectively. The peak drug concentration was 261 µg/L after the IV dose and 14 µg/L for the metabolite at 5.3 and 158 min, respectively. For the ramiprilat dose, the peak concentration was 301 µg/L at 6.9 min [van Griensven *et al.* 1995].

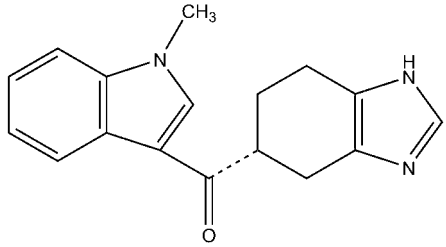
Bioavailability Ramipril, 28%; ramiprilat, 44%.
Half-life Ramipril, 1–2 h; ramiprilat, 9–18 h.
Volume of Distribution Ramipril, 90 L.
Clearance Ramiprilat clearance reduced in renal impairment.
Protein Binding Ramipril, 73%; ramiprilat, 56%.
Dose 1.25 to 5 mg daily with a maximum of 10 mg daily.

Belal F *et al.* (2001). A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. *J Pharm Biomed Anal* 24: 335–342.
van Griensven JM *et al.* (1995). Pharmacokinetics, pharmacodynamics and bioavailability of the ACE inhibitor ramipril. *Eur J Clin Pharmacol* 47: 513–518.

Ramosetron

5-HT_3 Receptor Antagonist, Antiemetic
$C_{17}H_{17}N_3O$ = 279.3
CAS—132036-88-5

IUPAC Name (1-Methylindol-3-yl)-[(5*R*)-4,5,6,7-tetrahydro-3*H*-benzimidazol-5-yl]methanone
Synonyms (*R*)-5-[(1-Methylindol-3-yl)carbonyl]-4,5,6,7-tetrahydrobenzimidazole; (1-methyl-1*H*-indol-3-yl)[(5*R*)-4,5,6,7-tetrahydro-1*H*-benzimidazol-5-yl]methanone; Nor-YM-060.



Chemical Property Foam

Ramosetron Hydrochloride
$C_{17}H_{17}N_3O,HCl = 315.8$
CAS—132907-88-5
Synonym YM-060
Proprietary Name *Nasea*
Chemical Properties A slightly yellowish-white, odourless crystalline powder. Mp 215° to 230°. Soluble in water.

High Performance Liquid Chromatography Column: Crestpak C_{18} S-10 (150 × 4.0 mm i.d.). Mobile phase: acetonitrile : 3% triethylamine hydrogen phosphate (pH 2.0; 15 : 85), flow rate 2 mL/min. UV detection. Retention time: ramosetron, 7.3 min; nor-ramosetron, 3.7 min. Limit of quantification not reported [Ishiwata *et al.* 1995].

Quantification
***Plasma* HPLC** Column: TSK-gel ODS-80-Tm (250 × 4.6 mm i.d.). Mobile phase: 0.1 mol/L potassium biphosphate : 0.1 mol/L phosphoric acid : acetonitrile (3 : 3 : 2), flow rate 1.0 mL/min. UV detection (λ = 311 nm). Limit of detection, 0.2 µg/L [Miura *et al.* 1994].
***Urine* HPLC** See Plasma. Column: Cosmosil $_5C_{18\ AR}$ (250 × 4.6 mm i.d.). Limit of detection, 1 µg/L [Miura *et al.* 1994].

Disposition in the Body Ramosetron has no effect on CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 [Niwa *et al.* 2006].

Therapeutic Concentration A dose of 6 µg/kg ramosetron has proved effective at preventing postoperative vomiting in the paediatric population after strabismus surgery [Fujii *et al.* 2005] and tonsillectomy [Fujii, Tanaka 2003]. A plasma concentration of 1.68 µg/L was obtained 4 h after IV administration of 0.4 mg of ramosetron [Miura *et al.* 1994].

Dose Ramosetron is given for its antiemetic properties in the management of nausea and vomiting induced by cancer chemotherapy. The usual dose is 300 µg once daily IV, or 100 µg once daily by mouth. A dose of 6 µg/kg is used in children to reduce postoperative vomiting.

Fujii Y, Tanaka H (2003). Results of a prospective, randomized, double-blind, placebo-controlled, dose-ranging trial to determine the effective dose of ramosetron for the prevention of vomiting after tonsillectomy in children. *Clin Ther* 25: 3135–3142.

Fujii Y *et al.* (2005). A randomized clinical trial of a single dose of ramosetron for the prevention of vomiting after strabismus surgery in children: a dose-ranging study. *Arch Ophthalmol* 123: 25–28.

Ishiwata K *et al.* (1995). Synthesis of 5-HT_3 receptor antagonists. [^{11}C]Y-25130 and [^{11}C]YM060. *Appl Radiat Isot* 46: 907–910.

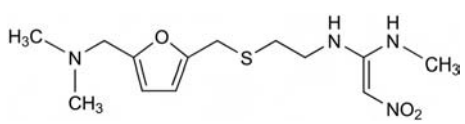
Miura H *et al.* (1994). A simple method for the determination of YM060 in plasma and urine by high performance liquid chromatography. *Biomed Chromatogr* 8: 103–104.

Niwa T *et al.* (2006). Effects of serotonin-3 receptor antagonists on cytochrome P450 activities in human liver microsomes. *Biol Pharm Bull* 29: 1931–1935.

Ranitidine

Histamine H_2-Receptor Antagonist
$C_{13}H_{22}N_4O_3S = 314.4$
CAS—66357-35-5

IUPAC Name (*E*)-1-*N*′-[2-[[5-(Dimethylaminomethyl)furan-2-yl]methylsulfanyl]ethyl]-1-*N*-methyl-2-nitroethene-1,1-diamine
Synonym *N*-[2-[[[-5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine



Chemical Properties A solid. Mp 69° to 70°. pK_a 2.3, 8.2. Log *P* (octanol/water), 0.3. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

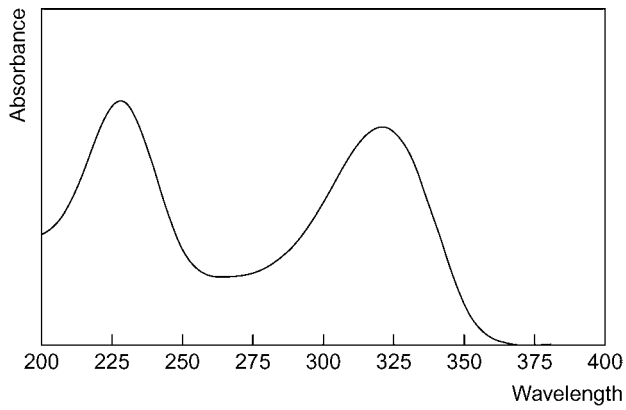
Ranitidine Hydrochloride
$C_{13}H_{22}N_4O_3S,HCl = 350.9$
CAS—66357-59-3
Synonyms AH-19065; ranitidini hydrochloridum.
Proprietary Names *Ausran*; *Azantac*; *Azuranit*; *Digestosan*; *Gertac*; *Ranaps*; *Rani*; *Ranic*; *Ranihexal*; *Raniplex*; *Raniprotect*; *Ranitic*; *Ranopine*; *Ranoxyl*; *Rantec*; *Sostril*; *Ulcidine*; *Ulcirex*; *Ulsal*; *Xanomel*; *Zaedoc*; *Zandin(e)*; *Zantac*; *Zantic*; *Zidac*.

Chemical Properties A yellowish-grey powder. Mp 133° to 134°. Freely soluble in water and acetic acid; soluble in methanol; sparingly soluble in ethanol; practically insoluble in chloroform.

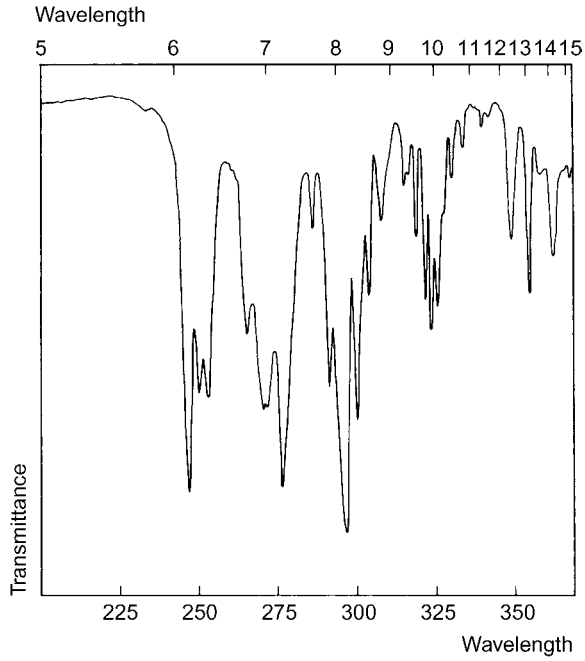
Ranitidine Bismuth Citrate
$C_{13}H_{22}N_4O_3S.C_6H_5BiO_7 = 712.5$
CAS—128345-62-0
IUPAC Name Compound with bismuth (3+)citrate (1:1)
Synonyms GR-122311X; ranitidine bismutrex.
Proprietary Names *Pylorid*; *Tritec*.

Thin-layer Chromatography System TA—R_f 0.50; system TE—R_f 0.30; system TAJ—R_f 0.02; system TAK—R_f 0.00; system TAL—R_f 0.10.
Gas Chromatography System GA—RI 2087.
High Performance Liquid Chromatography System HA—*k* 2.3; system HAA—RT 3.7 min; system HAX—RT 5.9 min; system HAY—RT 3.3 min; system HY—RI 175; system HZ—RT 1.8 min.

Column: (analytical) C_{18} µBondapak (250 × 4.6 mm i.d., 10 µm); (guard) C_{18} µBondapak (6 × 5.0 mm i.d., 10 µm). Mobile phase: 21 mmol/L disodium hydrogen phosphate : TEA: acetonitrile (pH 3.5, 1000:60:150), flow rate 1.3 mL/min. Retention time: 6.7 min [Ahmadiani, Amini 2001].
Ultraviolet Spectrum Water—313 nm (A_1^1 = 499a).



Infrared Spectrum Principal peaks at wavenumbers 1220, 1620, 1192, 1570, 1590, 1260 cm^{-1} (ranitidine hydrochloride, KBr disk).



Quantification
***Plasma* HPLC** UV detection. Limit of detection, 5 µg/L [Ahmadiani, Amini 2001]. UV detection. Limit of detection, 2 µg/L [Hare *et al.* 2001]. Limit of detection, 1 µg/L [Wong *et al.* 1998]. UV detection. Limit of detection, 10 µg/L [Farthing *et al.* 1997]. Fluorescence detection. Ranitidine and its *N*-oxide and *S*-oxide metabolites. Limit of detection, 32 µg/L for ranitidine [Vinas *et al.* 1997]. Limit of quantification, 10 µg/L [Lloyd *et al.* 1992]. UV detection. Limit of detection, 5 µg/L for ranitidine, 15 µg/L for desmethylranitidine [Mihaly *et al.* 1980].

Serum **HPLC** UV detection. Limit of detection, 2 μg/L [Lopez-Calull *et al.* 1997]. Limit of quantification, 10 μg/L [Lloyd *et al.* 1992].
Urine **HPLC** Fluorescence detection. Ranitidine and its *N*-oxide and *S*-oxide metabolites. Limit of detection, 32 μg/L for ranitidine [Vinas *et al.* 1997]. UV detection. For method for quantification of ranitidine and metabolites, see Carey *et al.* [1981]. UV detection. Limit of detection, 5 μg/L for ranitidine, 15 μg/L for desmethylranitidine [Mihaly *et al.* 1980].
Biological Fluids **RIA** Limit of detection, 2 μg/L [Jenner *et al.* 1981].
Disposition in the Body Readily absorbed after oral administration. It is metabolised by *N*-oxidation, *S*-oxidation and demethylation, its major metabolite being ranitidine *N*-oxide, but is excreted mainly as unchanged drug. After oral administration, about 30% is excreted unchanged in the urine in 24 h (dose-dependent), together with small amounts of the metabolites; after an IV dose, about 70–80% is excreted unchanged in the urine in 24 h, mainly by active tubular secretion. Ranitidine crosses the placenta and is excreted in breast milk.

The pharmacokinetics of the ranitidine component of ranitidine bismuth citrate is dose-proportional and similar to that observed after administration of ranitidine alone. Ranitidine bismuth citrate is rapidly absorbed and dissociates into ranitidine and bismuth compounds after oral administration. About 0.5% of the bismuth dose is absorbed and accumulates in plasma after repeated dosing (peak plasma concentration around 19 μg/L) and is eliminated via urine. Bismuth absorption is increased more than proportionally with the dose at 1600 mg. Low levels of bismuth can be detected in plasma and urine up to 5 months after the last dose. Because of the risk of bismuth accumulation, ranitidine bismuth citrate is not suitable for those with renal impairment or for long-term maintenance therapy.

Therapeutic Concentration

Following single oral administration of an over-the-counter 75-mg preparation of ranitidine to 19 children with heartburn, a median peak plasma concentration of 0.477 mg/L was attained in 2.5 h [Orenstein *et al.* 2002].

A study of IV ranitidine administration to 23 critically ill children revealed that a mean bolus dose of 1.37 mg/kg rapidly reduced gastric acidity, producing a peak plasma concentration of 0.373 mg/L at 2.3 h. Subsequent administration of ranitidine as a continuous IV infusion to 18 of the same subjects showed that a dose of 0.17 mg/kg/h and steady-state concentration of 0.287 mg/L was associated with gastric pH control. It was determined that a loading dose of 0.45 mg/kg followed by a continuous infusion of 0.15 mg/kg/h would be necessary to achieve a steady-state concentration of 0.287 mg/L [Lugo *et al.* 2001].

A total of 60 healthy males, aged between 19 and 40 years, were administered with either a 200, 400, 800 or 1600 mg dose of ranitidine bismuth citrate (equal number of volunteers for each dose), after an overnight fast and followed by a further 4 h after ingestion. The peak plasma ranitidine concentrations were 182, 480, 956 and 1983 μg/L for the doses, respectively, and these were observed between 1 and 5 h. The peak bismuth concentrations were 2.89, 2.85, 3.31 and 11.6 μg/L for the 200, 400, 800 and 1600 mg dose, respectively, and were reached 0.25–1.0 h after ingestion [Koch 1996].

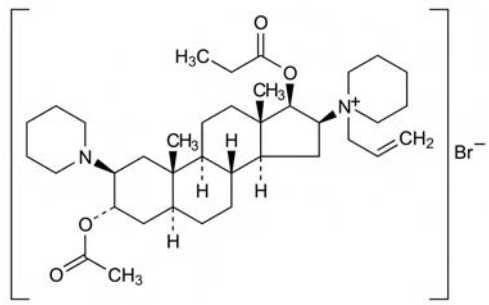
Bioavailability About 50% but there is considerable intersubject variability.
Half-life Plasma half-life, about 2–3 h, increased in elderly subjects, and in renal impairment.
Volume of Distribution 1–2 L/kg.
Clearance Plasma clearance, about 10 mL/min/kg.
Protein Binding About 15%.
Note For a review of ranitidine bismuth citrate in the treatment of *Helicobacter pylori* infection and duodenal ulcer, see Vondracek [1998]; for a general review of ranitidine, see Grant *et al.* [1989]; for a review of the drug interaction potential of ranitidine, see Klotz and Kroemer [1991].
Dose The equivalent of 150 to 600 mg of ranitidine daily. Ranitidine bismuth citrate is used in a 7 day triple therapy regimen: 400 mg twice daily or 14 day dual therapy with clarithromycin, amoxicillin and/or metronidazole. It is not used in those with moderate to severe renal impairment.

Ahmadiani A, Amini H (2001). Rapid determination of ranitidine in human plasma by high-performance liquid chromatography without solvent extraction. *J Chromatogr B Biomed Sci Appl* 751: 291–296.
Carey PF *et al.* (1981). Determination of ranitidine and its metabolites in human urine by reversed-phase ion-pair high-performance liquid chromatography. *J Chromatogr* 225: 161–168.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Farthing D *et al.* (1997). Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method utilizing midbore chromatography. *J Chromatogr B Biomed Sci Appl* 688: 350–353.
Grant SM *et al.* (1989). Ranitidine. An updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in peptic ulcer disease and other allied diseases. *Drugs* 37: 801–870.
Hare LG *et al.* (2001). The use of polymeric solid phase extraction and HPLC analysis for the determination of ranitidine in routine plasma samples obtained from paediatric patients. *J Pharm Pharmacol* 53: 1265–1272.
Jenner WN *et al.* (1981). The development of a radioimmunoassay for ranitidine in biological fluids. *Life Sci* 28: 1323–1329.
Klotz U, Kroemer HK (1991). The drug interaction potential of ranitidine: an update. *Pharmacol. Ther* 50: 233–244.
Koch KM (1996). Pharmacokinetics of bismuth and ranitidine following single doses of ranitidine bismuth citrate. *Br J Clin Pharmacol* 42: 201–205.
Lloyd TL *et al.* (1992). Robotic solid phase extraction and high performance liquid chromatographic analysis of ranitidine in serum or plasma. *Biomed Chromatogr* 6: 311–316.
Lopez-Calull C *et al.* (1997). Simple and robust high-performance liquid chromatographic method for the determination of ranitidine in microvolumes of human serum. *J Chromatogr B Biomed Sci Appl* 693: 228–232.
Lugo RA *et al.* (2001). Pharmacokinetics and pharmacodynamics of ranitidine in critically ill children. *Crit Care Med* 29: 759–764.
Mihaly GW *et al.* (1980). High-pressure liquid chromatographic determination of ranitidine, a new H2-receptor antagonist, in plasma and urine. *J Pharm Sci* 69: 1155–1157.
Orenstein SR *et al.* (2002). Ranitidine, 75 mg, over-the-counter dose: pharmacokinetic and pharmacodynamic effects in children with symptoms of gastro-oesophageal reflux. *Aliment Pharmacol Ther* 16: 899–907.
Vinas P *et al.* (1997). Use of post-column fluorescence derivatization to develop a liquid chromatographic assay for ranitidine and its metabolites in biological fluids. *J Chromatogr B Biomed Sci Appl* 693: 443–449.
Vondracek TG (1998). Ranitidine bismuth citrate in the treatment of Helicobacter pylori infection and duodenal ulcer. *Ann Pharmacother* 32: 672–679.
Wong CF *et al.* (1998). Simple high-performance liquid chromatographic method for the determination of ranitidine in human plasma. *J Chromatogr B Biomed Sci Appl* 718: 205–210.

Rapacuronium Bromide

Anaesthetic
$C_{37}H_{61}N_2O_4,Br = 677.8$
CAS—156137-99-4

IUPAC Name [(2*S*,3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,16*S*,17*R*)-3-acetyloxy-10,13-dimethyl-2-piperidin-1-yl-16-(1-prop-2-enylpiperidin-1-ium-1-yl)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl] propanoate bromide
Synonyms 1-[(2β,3α,5α,16β,17β)-3-(Acetyloxy)-17-(1-oxopropoxy)-2-(1-piperidinyl)androstan-16-yl]-1-(2-propenyl)piperidinium bromide; Org-9487.
Proprietary Name *Raplon*



Disposition in the Body Rapacuronium rapidly enters the neuromuscular junction (its effect compartment) and is subsequently rapidly cleared from the plasma. After IM injection, peak plasma concentrations are observed at 4 and 5 min in infants and children, respectively. After 30 min, <25% of a dose has been absorbed. It is metabolised by spontaneous hydrolysis and/or hepatic metabolism. The major metabolite is 3-desacetylrapacuronium and represents 10% of the drug plasma concentration. Rapacuronium is excreted by the hepatic and renal pathways; 22% of the drug and metabolite is excreted in urine over a 24 h period.

Therapeutic Concentration

Ten patients undergoing elective surgery, aged 18 to 57 years (mean 28 years) were treated with fentanyl and propofol to induce anaesthesia. They were then treated with rapacuronium as an IV bolus dose of 1.5 mg/kg, followed by an infusion of 3.4 mg/kg/h for 45 min, to maintain a neuromuscular block of 83%, and in the final 15 min, 2.5 mg/kg/h. 10 min after the beginning of the infusion, peak plasma concentrations were 5979 μg/L for the drug and 340 μg/L for its metabolite; 60 min afterwards the concentrations were 5029 and 650 μg/L for the drug and metabolite, respectively. However, 240 min after the infusion period had finished, the plasma concentrations were 89 and 205 μg/L, respectively [Van den Broek *et al.* 1994].

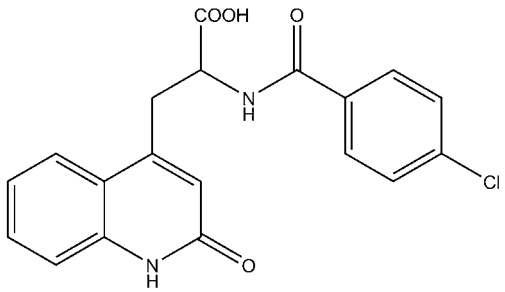
Toxicity Rapacuronium is associated with bronchospasm which may lead to death.
Bioavailability IM bioavailability, 56%.
Half-life Approximately 90 min (range, 72 to 184 min).
Volume of Distribution Total apparent, 221 (124 to 285) mL/kg (healthy individuals); 331 (284 to 488) mL/kg (patients with cirrhosis); steady state, 0.193 to 0.457 L/kg; lower in those with chronic renal failure compared to those with normal function and in men compared with women.
Clearance Plasma, 4.77 mL/min/kg (infants and children); 5.3 mL/min/kg (healthy adults); 6.9 mL/min/kg (patients with cirrhosis).
Dose The recommended dose is 1.5 to 2.5 mg/kg as a rapid IV bolus after anaesthetic induction.

Van den Broek L *et al.* (1994). Pharmacodynamics and pharmacokinetics of an infusion of Org 9487, a new short-acting steroidal neuromuscular blocking agent. *Br J Anaesth* 73: 331–335.

Rebamipide

Propionic Acid, Antiulcerative
$C_{19}H_{15}ClN_2O_4 = 370.8$
CAS—90098-04-7; 111911-87-6.

IUPAC Name 2-[(4-Chlorobenzoyl)amino]-3-(2-oxo-1*H*-quinolin-4-yl)propanoic acid
Synonyms α-[(4-Chlorobenzoyl)-amino]-1,2-dihydro-2-oxo-4-quinolinepropanoic acid; 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propionic acid; OPC-12759; proamipide.
Proprietary Name *Mucosta*



Chemical Properties White powder. Mp 288° to 290°. Soluble in DMF; very slightly soluble in methanol and ethanol; practically insoluble in ether and water [Shin *et al.* 2004].

(–)-Rebamipide

Chemical Properties Colourless needles from DMF. Mp 305° to 306°.

(+)-Rebamipide

Chemical Properties Colourless needles from DMF. Mp 305° to 306°.

High Performance Liquid Chromatography Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L phosphate buffer (pH 2.5; 50 : 50), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 380$ nm). Retention time: 5.4 min. Limit of quantification, 2 µg/L [Shin *et al.* 2004].

Quantification

Plasma **HPLC** Column: YMC Pack A-303 ODS (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : tetrahydrofuran : acetic acid : water (32 : 3 : 1 : 64), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 375$ nm). Retention time: 6.2 min. Limit of quantification, 0.01 mg/L; limit of detection, 10 µg/L [Shioya, Shimizu 1988].

LC-MS Column: Inertsil ODS-3 (150 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (75 : 25 : 0.1), flow rate 0.2 mL/min.ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1 µg/L [Hasegawa *et al.* 2003].

Serum **HPLC** Column: TSK-gel ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L sodium sulfate : tetrahydrofuran : acetic acid (750 : 1250 : 20 : 6), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 375$ nm). Limit of quantification, 0.01 mg/L [Naito *et al.* 1996]. Column: ODS-80 TM (150 × 6.0 mm i.d.). Mobile phase: acetonitrile : 5 mmol/L sodium sulfate (3 : 5) with 1% acetic acid and 0.3% tetrahydrofuran, flow rate 1.5 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 370$ nm). Retention time: 9.5 min. Limit of quantification, 20 µg/L [Miyake *et al.* 2006].

Urine **HPLC** Column: YMC Pack A-303 ODS (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : tetrahydrofuran : acetic acid : water (32 : 3 : 1 : 64), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 375$ nm). Retention time: 6.2 min. Limit of quantification, 0.5 mg/L; limit of detection, 10 µg/L [Shioya, Shimizu 1988].

Gastric Mucosa **HPLC** Column: TSK-gel ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 5 mmol/L sodium sulfate : tetrahydrofuran : acetic acid (750 : 1250 : 20 : 6), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 375$ nm). Limit of quantification, 0.3 µg/g [Naito *et al.* 1996].

Disposition in the Body Poorly absorbed in the systemic circulation following oral administration owing to its low solubility and low membrane permeability.

Therapeutic Concentration

Twenty-nine patients with chronic gastritis (aged 20 to 89 years) were administered a single oral dose of 100 mg rebamipide. The mean peak rebamipide concentration in gastric mucosa between 30 and 120 min after ingestion was 60.0 µg/g. The corresponding rebamipide serum concentration was 0.25 µg/mL. The gastric/serum ratio was reported as 1221 [Naito *et al.* 1996].

Two groups of 14 volunteers were administered a single dose of 100 mg rebamipide in either granules or tablets. Following administration of the granule formulation, the mean peak plasma concentration was 241.8 µg/L after 2.5 h, with a plasma elimination half-life of 1.97 h. Corresponding values for the tablet formulation were 216.2 µg/L after 2.4 h, with a half-life of 1.94 h [Hasegawa *et al.* 2003].

Twenty healthy volunteers were administered single doses of either 100, 200 or 300 mg rebamipide. Mean peak gastric mucosa, gastric mucus and serum rebamipide concentrations were reported as follows:

Specimen	*Dose (mg)*	C_{max}	*Time (h)*
Mucosa (µg/g tissue)	100	244.7	1.9
	200	143.4	1.0
	300	186.7	1.1
Mucus (µg/mL)	100	75.8	1.1
	200	119.2	1.0
	300	372.0	1.1
Serum (µg/mL)	100	0.28	2.5
	200	0.36	2.4
	300	0.49	2.8

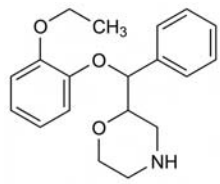
[Akamatsu *et al.* 2002].

Half-life Approximately 2 h.
Dose Usual dose of 100 mg by mouth three times daily. It has also been used rectally for the treatment of intestinal inflammation. Rebamipide eye drops are under investigation in the treatment of dry eye.

Akamatsu T *et al.* (2002). Local gastric and serum concentrations of rebamipide following oral ingestion in healthy volunteers. *Dig Dis Sci* 47: 1399–1404.
Hasegawa S *et al.* (2003). Bioequivalence of rebamipide granules and tablets in healthy adult male volunteers. *Clin Drug Investig* 23: 771–779.
Miyake M *et al.* (2006). Optimization of suppository preparation containing sodium laurate and taurine that can safely improve rectal absorption of rebamipide. *Biol Pharm Bull* 29: 330–335.
Naito Y *et al.* (1996). Local gastric and serum concentrations of rebamipide following oral administration to patients with chronic gastritis. *Arzneimittelforschung* 46: 698–700.
Shin BS *et al.* (2004). Oral absorption and pharmacokinetics of rebamipide and rebamipide lysinate in rats. *Drug Dev Ind Pharm* 30: 869–876.
Shioya Y, Shimizu T (1988). High-performance liquid chromatographic procedure for the determination of a new anti-gastric ulcer agent, 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propioni c acid, in human plasma and urine. *J Chromatogr* 434: 283–287.

Reboxetine

Antidepressant
$C_{19}H_{23}NO_3 = 313.4$
CAS—71620-89-8; 98769-81-4
IUPAC Name (2*R*)-2-[(*R*)-(2-Ethoxyphenoxy)phenylmethyl]morpholine



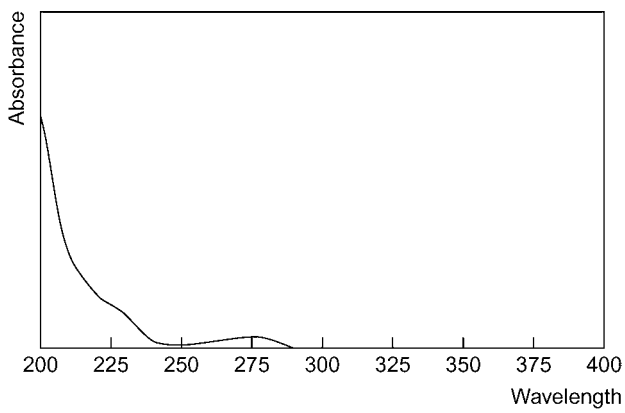
Chemical Properties Mp 170° to 171°. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Reboxetine Methanesulfonate (Mesylate)

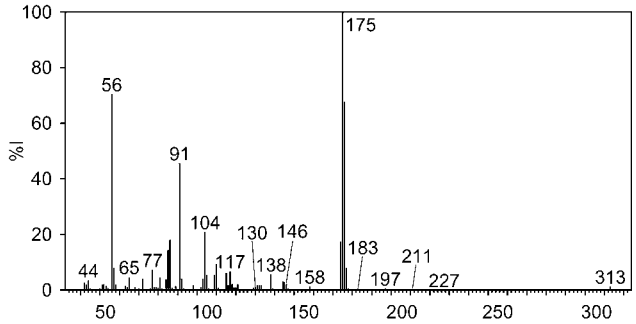
$C_{19}H_{23}NO_3,CH_3SO_3H = 409.5$
CAS—98769-82-5
Synonyms FCE-20124; PNU-155950E.
Proprietary Names *Edronax*; *Prolift*; *Vestra*.
Chemical Properties Mp 145° to 146°.

High Performance Liquid Chromatography Column: Zorbax phenyl (150 × 3.0 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L phosphate buffer (pH 4.9, 27 : 73), flow rate 0.5 mL/min. UV detection (λ= 210 nm). Retention time: 13.0 min [Ohman *et al.* 2001].

Ultraviolet Spectrum Aqueous acid (0.025 mol/L sulfuric acid)—276.5 nm.



Mass Spectrum Principal peaks at *m/z* 175, 56, 176, 91, 104, 86, 174, 85.



Quantification

Blood **GC-MS** Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He 1 mL/min. Temperature: 300° for 1 min. Limit of detection, 20 ng/L [Déglon *et al.* 2010].

HPLC Column: Partisphere C_8 (110 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L phosphate buffer (pH 2.3, 64 : 36), flow rate 0.45 mL/min. UV detection (λ = 210 nm). Limit of quantification, 10 µg/L. [Edwards *et al.* 1995].

Plasma **HPLC** Column: Hypersil Phenyl 120A (250 × 4.6 mm i.d., 5 µm). Mobile phase: sodium acetate buffer (pH 3.5) : tetrahydrofuran : acetonitrile (55 : 35 : 10), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 470 nm, λ_{em} = 530 nm). Limit of quantification, 1.7 µg/L. Limit of detection, 0.5 µg/L [Khalil 2010]. Fluorimetric detection. Limit of quantification, 11 µg/L, limit of detection, 4 µg/L [Ragg *et al.* 2002]. UV detection (λ = 210 nm). Limit of quantification, 10 µg/L [Coulomb *et al.* 2000]. Mobile phase: 0.1 mol/L dibasic ammonium phosphate buffer (pH 7.5) : tetrahydrofuran (53.5 : 46.5). Fluorescence detection (λ_{ex} = 260 nm, λ_{em} = 315 nm). Limit of quantification, 1.1 µg/L [Denolle *et al.* 1999]. Fluorescence detection (λ_{ex} = 260 nm, λ_{em} = 315 nm). Column: LiChroCART Supersphere 60 RP-8 (250 × 4.6 mm i.d., 4 µm). Mobile phase: 0.1 mol/L dibasic ammonium phosphate buffer (pH 7.5) : tetrahydrofuran (53.5 : 46.5), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex} = 260 nm, λ_{em} = 315 nm). Limit of quantification, 1 µg/L [Frigerio *et al.* 1994].

Serum **GC-MS** Column: Uptisphere C_{18} (125 × 2 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium acetate buffer (pH 4)-acetonitrile (60 : 40, 100 : 0), flow rate 200 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quanification, 19 nmol/L [Gutteck, Rentsch 2003].

HPLC Column: Nucleosil 100-Protect 1. Mobile phase: acetonitrile : potassium dihydrogen phosphate buffer [Frahnert *et al.* 2003]. Column: Lichrospher CN (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.008 mol/L aqueous potassium phosphate buffer (pH 6.4, 50 : 50). UV detection (λ = 273 or 226 nm). Limit of quantification, 15 µg/L and 4 µg/L [Härtter *et al.* 2000].

Urine **HPLC** See Blood [Edwards *et al.* 1995].

Disposition in the Body Reboxetine is rapidly and well absorbed from the gastrointestinal tract and peak plasma levels occur in ~2 h. The presence of food decreases the peak concentration reached and lengthens the time to reach this. The drug is metabolised by dealkylation, hydroxylation and oxidation followed by glucuronide or sulfate conjugation. Traces of hydroxyl, desethyl and 3-morpholine metabolites have been identified in plasma as well as glucuronide and sulfate conjugates of these. Elimination is mainly renal via urine (78%) with 10% excreted as the unchanged drug. Pharmacokinetics is linear. Multiple dosing and gender do not affect the pharmacokinetics. Steady-state concentrations are reached within 4 days.

Therapeutic Concentration

Nine healthy males, 19–29 years (mean, 23), were administered a single oral dose of 4-mg reboxetine and 2 mg (*R,R*)-enantiomer after an overnight fast. The peak plasma concentration after parent drug administration was 109 µg/L observed at 1.9 h for reboxetine, 77 µg/L at 1.8 h for the (*R,R*)-enantiomer and 35 µg/L for the (*S,S*)-enantiomer 2.3 h. The peak concentration of the (*R,R*)-enantiomer observed after its administration was 75 µg/L at 1.4 h [Denolle *et al.* 1999].

Six patients with mild renal dysfunction (mean age 54 years; creatinine clearance 60–80 mL/min), 6 with moderate dysfunction (mean age 45 years; creatinine clearance 30–50 mL/min), 6 with severe dysfunction (mean age 55 years; creatinine clearance 10–20 mL/min) and healthy volunteers were administered a 4-mg dose reboxetine after an overnight fast and continued fasting for 4 h after dosing. Peak plasma concentrations were 151, 176, 203 and 111 µg/L for the 4 groups, attained at 1.83, 1.08, 1.58 and 2.4 h, respectively [Coulomb *et al.* 2000].

Bioavailability 60%.

Half-life 13–15 h.

Volume of Distribution Reboxetine 32 L; at steady state: 0.39 L/kg (*R,R*)-(−)-reboxetine and 0.92 L/kg (*S,S*)-(+)-reboxetine.

Clearance Plasma, 2.21 L/h.

Protein Binding Approximately 97%, primarily to α_1-acid glycoprotein; 92% in the elderly.

Dose A usual dose of 8 mg daily. Maximum 12 mg.

Coulomb F *et al.* (2000). Pharmacokinetics of single-dose reboxetine in volunteers with renal insufficiency. *J Clin Pharmacol* 40: 482–487.

Déglon J *et al.* (2010). Use of the dried blood spot sampling process coupled with fast gas chromatography and negative-ion chemical ionization tandem mass spectrometry: application to fluoxetine, norfluoxetine, reboxetine, and paroxetine analysis. *Anal Bioanal Chem* 396: 2523–2532.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Denolle T *et al.* (1999). Hemodynamic effects of reboxetine in healthy male volunteers. *Clin Pharmacol Ther* 66: 282–287.

Edwards DM *et al.* (1995). Pharmacokinetics of reboxetine in healthy volunteers: single oral doses, linearity and plasma protein binding. *Biopharm Drug Dispos* 16: 443–460.

Frahnert C *et al.* (2003). Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. *J Chromatogr B Analyt Technol Biomed Life Sci* 794: 35–47.

Frigerio E *et al.* (1994). Sensitive procedure for the determination of reboxetine enantiomers in human plasma by reversed-phase high-performance liquid chromatography with fluorimetric detection after chiral derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate. *J Chromatogr A* 660: 351–358.

Gutteck U, Rentsch KM (2003). Therapeutic drug monitoring of 13 antidepressant and five neuroleptic drugs in serum with liquid chromatography–electrospray ionization mass spectrometry. *Clin Chem Lab Med* 41: 1571–1579.

Härtter S *et al.* (2000). Automated determination of reboxetine by high-performance liquid chromatography with column-switching and ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 740: 135–140.

Khalil NY (2010). A highly sensitive HPLC method with automated on-line sample pre-treatment and fluorescence detection for determination of reboxetine in human plasma. *Talanta* 80: 1251–1256.

Ohman D *et al.* (2001). Bioanalysis of racemic reboxetine and its desethylated metabolite in a therapeutic drug monitoring setting using solid phase extraction and HPLC. *Ther Drug Monit* 23: 27–34.

Ragg MA *et al.* (2002). Determination of reboxetine, a recent antidepressant drug, in human plasma by means of two high-performance liquid chromatography methods. *J Chromatogr A* 949: 23–33.

Remifentanil

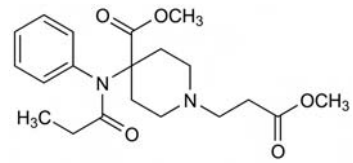
Analgesic

$C_{20}H_{28}N_2O_5$ = 376.5

CAS—132875-61-7

IUPAC Name Methyl 1-(3-methoxy-3-oxopropyl)-4-(*N*-propanoylanilino) piperidine-4-carboxylate

Synonyms GI-87084; 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid methyl ester; remifentanyl.



Chemical Properties White to off-white lyophilised powder. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stability of remifentanil in acetonitrile stock solutions stored at 4° was at least 18 months [Grosse *et al.* 1994]. Unstable at room temperature. Blood samples should be extracted by organic solvent to avoid degradation [Zhang *et al.* 2008]. Plasma samples were stable in the autosampler (5°) for 12 h, at room temperature for 24 h and for 1 month at –70° [Bossù *et al.* 2006]. Stable in blood for 1 month when stored at –20° [Bender *et al.* 1999].

Remifentanil Hydrochloride

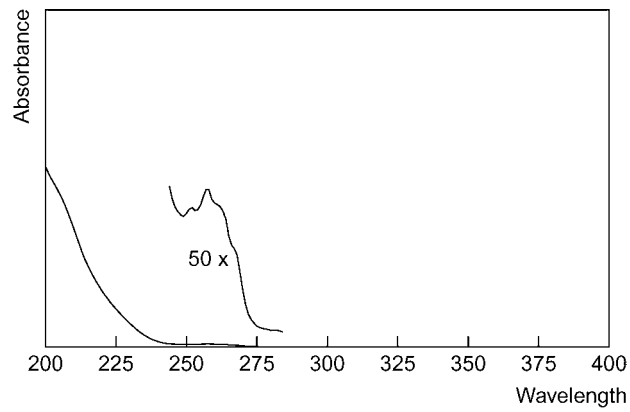
$C_{20}H_{28}N_2O_5$,HCl = 412.9

IUPAC Name 4-(Methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid methyl ester hydrochloride

Synonym GI-87084B

Chemical Properties pK_a 7.1.

Ultraviolet Spectrum



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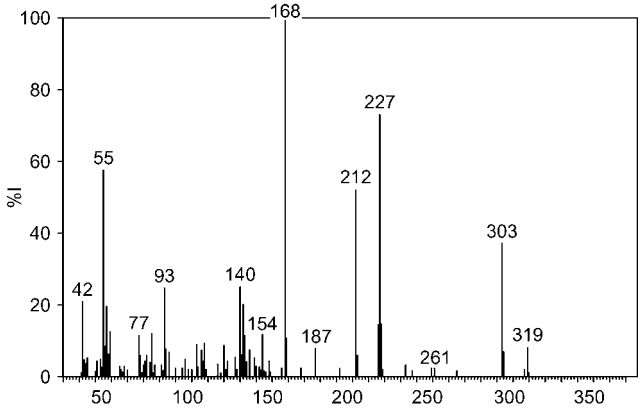
Mass Spectrum Principal ions at *m/z* 168, 227, 55, 212, 303, 140, 93, 42.



Quantification

Blood **GC-MS** Column: RTx-1 dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 130° to 210° at 50°/min to 280° at 6°/mL to 320° at 50°/min for 2 min. EI ionisation, SIM acquisition mode. Limit of quantification, 0.1 µg/L [Grosse *et al.* 1994].

HPLC Column: Zorbax SB-CN (250 × 4.6 mm i.d., 5 µm). Temperature: 30°. Mobile phase: acetonitrite : methanol : water : 0.03 mol/L phosphate buffer (pH 3.0; 14 : 28 : 52 : 6), flow rate 1.5 mL/min. UV detection (λ= 210 nm). RT: remifentanil, 8.5 min; carboxylic acid metabolite, 4.5 min. Limit of quantification, 1 µg/L [Selinger *et al.* 1994].

LC-MS Column: Phenomenex Gemini C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile containing 0.1% formic acid : ammonium acetate buffer (85 : 15 for 9 min to 30 : 7- in 13 min to 80 : 20 in 10 min to 95 : 5 in 1 min), flow rate 150 µL/min. TIS, MRM acquisition mode. Retention time: 8.5 min. Limit of detection, 0.5 ng/L [Gergov *et al.* 2009]. Mobile phase: acetonitrile : chloroform (1 : 1) containing 2 mmol/L ammonium acetate, flow rate 0.3 mL/min. TIS, positive ion mode. Limit of quantification 0.1 µg/L [Bender *et al.* 1999].

Plasma **LC-MS** Column: XTerra MS C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (65 : 35), flow rate 0.3 mL/min. TIS, positive ion mode, MRM acquisition mode. Limit of detection, 0.5 µg/L [Zhang *et al.* 2009]. Column: Intersil ODS-3 (50 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : chloroform (50 : 50) containing 2 mmol/L ammonium acetate, flow rate 0.3 mL/min. Positive ion mode, full scan mode. Limit of quantification, 0.5 µg/L [Zhang *et al.* 2008]. Column: XTerra MS C_8 (150 × 2.1 mm i.d., 5 µm). Mobile phase: water : methanol : acetonitrile (86 : 10 : 4) containing 0.1% formic acid, flow rate 0.2 mL/min. ESI, positive ion mode, SIR acquisition mode. Limit of quantification, 0.5 µg/L, limit of detection, 0.18 µg/L [Bossù *et al.* 2006].

Other **HPLC** Rat Blood. Column: RP C_8 column (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L sodium hydrogen phosphate buffer (pH 3.5; 27 : 73) with 300 µl/L TEA, flow rate 1.5 mL/min. UV detection (λ= 210 nm). Retention time: 6 min. Limit of detection, 2.5 µg/L [Haidar *et al.* 1996].

Disposition in the Body Parenteral administration results in rapid onset and there is short duration of action. Remifentanil is metabolised by non-specific plasma and tissue esterases that hydrolyse the drug prior to its secretion by the kidneys to an inactive carboxylic acid metabolite (with a half-life of 90 min). There is rapid distribution throughout the body and elimination is independent of duration of the drug. Ninety-five percent of a dose is excreted in urine as the metabolite. Linear and dose-independent pharmacokinetics are seen while the pharmacokinetics are gender independent. Age and lean body mass alter pharmacokinetics. Pharmacokinetics are not changed in renal or hepatic impairment.

Therapeutic Concentration 1–40 µg/L after an infusion rate of 0.04–2 µg/kg bodyweight per min. Steady state is achieved within 5–10 min with a volume of 350 mL/kg.

Half-life 6–16 min (independent of dose).

Volume of Distribution 0.39 L/kg; steady state volume of distribution, 24–40 L; children (2–12 years old), steady state, 0.21 L/kg.

Clearance 40 mL/min/kg (reduced by 25% in the elderly); total clearance, 2.5–3.8 L/min; systemic clearance, 2.8 L/min.

Protein Binding 70% bound.

Note For a review of remifentanil see Egan [1995].

Dose 0.5 to 1 µg/kg/min. Initial dose reduced in the elderly by 50%.

Bender J *et al.* (1999). Determination of remifentanil in human heparinised whole blood by tandem mass spectrometry with short-column separation. *J Pharm Biomed Anal* 21: 559–567.

Bossù E *et al.* (2006). LC-MS Determination of remifentanil in maternal and neonatal plasma. *J Pharm Biomed Anal* 42: 367–371.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Egan TD (1995). Remifentanil pharmacokinetics and pharmacodynamics: a preliminary appraisal. *Clin Pharmacokinet* 29: 80–94.

Gergov M *et al.* (2009). Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry. *Forensic Sci Int* 186: 36–43.

Grosse CM *et al.* (1994). Determination of remifentanil in human blood by liquid-liquid extraction and capillary GC-HRMS-SIM using a deuterated internal standard. *J Pharm Biomed Anal* 12: 195–203.

Haidar SH *et al.* (1996). Determination of remifentanil, an ultra-short-acting opioid anesthetic, in rat blood by high performance liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* 14: 1727–1732.

Selinger K *et al.* (1994). Determination of remifentanil in human and dog blood by HPLC with UV detection. *J Pharm Biomed Anal* 12: 243–248.

Zhang C *et al.* (2008). Population pharmacokinetics study of remifentanil in Chinese adult patients determined by an LC-MS/MS method. *Int J Clin Pharmacol Ther* 46: 477–488.

Zhang LP *et al.* (2009). Population pharmacokinetics of remifentanil in patients undergoing orthotopic liver transplantation. *Chin Med J (Engl)* 122: 1032–1038.

Remoxipride

Dopamine D_2 Receptor Antagonist, Benzamide, Antipsychotic

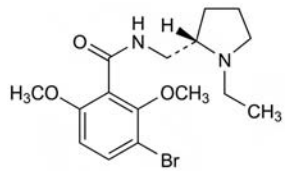
$C_{16}H_{23}BrN_2O_3$ = 371.3

CAS—80125-14-0

IUPAC Name 3-Bromo-*N*-[[(2*S*)-1-ethyl-2-pyrrolidinyl]methyl]-2,6-dimethoxybenzamide

Synonyms A-33547; BRN4323708; FLA-731.

Proprietary Names *Psyloc*; *Roxiam*.



Chemical Properties Log *P* (octanol/water), 2.10.

Remoxipride Hydrochloride

$C_{16}H_{23}BrN_2O_3$,HCl,H_2O = 425.8

CAS—73220-03-8 (anhydrous); 117591-79-4 (monohydrate)

IUPAC Name 3-Bromo-*N*-[[(2*S*)-1-ethyl-2-pyrrolidinyl]methyl]-2,6-dimethoxybenzamide hydrochloride monohydrate

Chemical Properties White to off-white crystalline solid with Mp 173°. It is soluble in water, ethanol, dichloromethane and acetone. pK_a 8.9 [Nilsson 1990]. Stable in plasma and urine for 48 h at 20°, 2 weeks at 4° and 13.5–15 months at –20° [Nilsson 1990].

Thin-layer Chromatography System TAE—R_f 0.26 (remoxipride); R_f 0.08 (M-FLA-838); R_f 0.82 (M-NCM-001); R_f 0.82 (M-NCM-009); system TB—R_f 0.14 (remoxipride); R_f 0.00 (M-FLA-838); R_f 0.00 (M-NCM-001); R_f 0.00 (M-NCM-009); system TE—R_f 0.54 (remoxipride); R_f 0.23 (M-FLA-838); R_f 0.41 (M-NCM-001); R_f 0.27 (M-NCM-009).

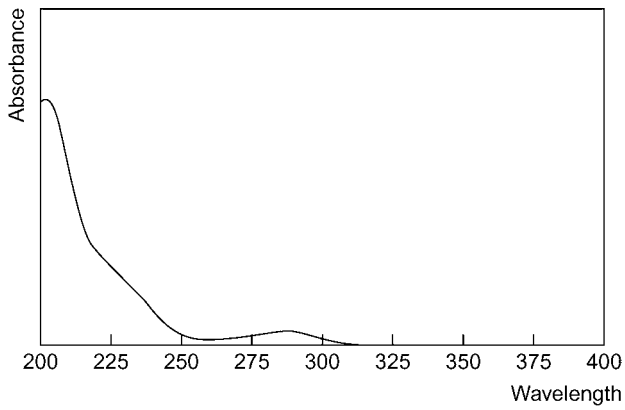
Plate: Merck 254. Mobile phase: acetone : toluene : ethanol : concentrated ammonia (150 : 150 : 20 : 2). Detection: Dragendorff's reagent. R_f 0.4. [Segerberg-Konttinen *et al.* 1989].

Gas Chromatography System GB—RI 2588.

High Performance Liquid Chromatography

System HAX—RT 8.8 min; system HAY—RT 4.6 min; system HX—RI 334 (remoxipride); RI 316 (M-FLA-838); RI 364 (M-NCM-001); RI 341 (M-NCM-009); system HZ—RT 3.0 min.

Ultraviolet Spectrum Aqueous solution (neutral)—286 nm; aqueous acid (ethanol)—287 nm; 0.1 mol/L hydrochloric acid—286 nm.



Mass Spectrum Principal peaks at *m/z* 98, 99, 70, 228, 230, 243, 245, 185.

Quantification

Plasma **HPLC** Column: Analytichem C_{18} (50 × 4.6 mm i.d., 3 µm). Mobile phase: aqueous buffer (0.2 mol/L sodium perchlorate, 0.1 mol/L phosphoric acid, pH 1.7) : acetonitrile (25 : 75), flow rate 1.3 mL/min. UV detection (λ = 214 nm). Retention time: 2.8 min. Limit of quantification, 12.5 µg/L [Chiou, Lo 1992]. Column: Nucleosil 120-3 C_{18} (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : phosphate buffer (pH 2; 30 : 70), containing 0.4 mmol/L *N*,*N*-dimethyloctylamine

R

and 0.5 mmol/L decylsulfate, flow rate 1.3 mL/min. IS: 3-bromo-*N*-[(1-propyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide. UV detection (λ= 206 nm). Retention time: remoxipride, 3 min; IS 5.3 min. Limit of quantification, 0.002 µg/L [Nilsson 1990].

Urine **HPLC** See Plasma. Mobile phase: aqueous buffer (0.2 mol/L sodium perchlorate, 0.1 mol/L phosphoric acid, pH 1.7) : acetonitrile (69 : 31). Retention time: 1.8 min. Limit of quantification, 50 µg/L [Chiou, Lo 1992]. See Plasma. Mobile phase: acetonitrile : phosphate buffer (pH 2; 25 : 75) containing 0.2 mmol/L *N*,*N*-dimethyloctylamine acetonitrile : phosphate buffer (pH 2; 25 : 75) containing 0.2 mmol/L *N*,*N*-dimethyloctylamine. Retention time: remoxipride, 2.2 min; IS, 3.6 min [Nilsson 1990].

Disposition in the Body Remoxipride is almost completely absorbed following oral administration in healthy subjects, mainly in the small intestine (although some absorption takes place in the large intestine). Distribution is rapid. Peak plasma levels are reached within 1–2 h of oral administration. The drug is metabolised to lactam, hydroxy-substituted lactam, *N*-de-ethylated lactam and *N*-de-ethylated hydroxy-substituted lactam metabolites. Most of the oral dose is excreted in urine, 10–40% unchanged and the remainder as metabolites. Elimination may be impaired in the elderly and individuals with severe renal dysfunction. Steady state was achieved within 2 days of initial treatment. Pharmacokinetics are not altered by alcohol, warfarin or diazepam.

Therapeutic Concentration The recommended therapeutic level should not exceed 7–8 mg/L. Doses of 70–140 mg are safe but above 140 mg, side effects become apparent. A single dose of 75 mg results in a therapeutic plasma level of 1 mg/L after 1 h [Malhotra, Cooper 1997].

Six healthy volunteers were administered 100 mg remoxipride hydrochloride monohydrate, and plasma concentrations reached 3.8 µmol/L. The concentrations for the lactam, hydroxy-substituted lactam, *N*-de-ethylated lactam and *N*-de-ethylated hydroxy-substituted lactam metabolites were 1.5, 0.5, 0.3 and 0.2 µmol/L, respectively [Widman *et al.* 1990].

Toxicity

A 23-year-old female was found dead at her home and was known to be receiving psychiatric treatment. At the scene an empty bottle of triazolam and 3 empty bottles of remoxipride were found. Remoxipride was detected at concentrations of 230 mg/L, 220 mg/L and 490 mg/kg in blood, urine and liver, respectively. No triazolam was detected and ethanol was found at a concentration of 0.048 g/100 mL in urine. Death was attributed to remoxipride poisoning [Segerberg-Konttinen *et al.* 1989].

Half-life 4–7 h. Longer in elderly patient's and those with renal dysfunction.

Bioavailability >90%.

Volume of Distribution 0.5–0.7 L/kg.

Clearance Plasma, 120 mL/min.

Protein Binding 80%

Dose By mouth, IM or IV, starting dose of 300 mg in 2 divided doses and adjusted to control symptoms. Initial dose may be halved for elderly patients. Most patients are administered 300 to 450 mg daily.

Aravagiri M *et al.* (1993). Determination of risperidone in plasma by high-performance liquid chromatography with electrochemical detection: application to therapeutic drug monitoring in schizophrenic patients. *J Pharm Sci* 82: 447–449.

Chiou RH, Lo MW (1992). Determination of remoxipride in human plasma and urine by reversed-phase ion-pair high-performance liquid chromatography. *J Chromatogr* 581: 300–305.

Malhotra R, Cooper C (1997). Concentrations of remoxipride in biological fluids following a post-mortem study. *TIAFT Bull Case Notes* 27(3).

Nilsson LB (1990). Determination of remoxipride in plasma and urine by reversed-phase column liquid chromatography. *J Chromatogr* 526: 139–150.

Segerberg-Konttinen M *et al.* (1989). Fatal intoxication by remoxipride. *J Forensic Sci* 34: 500–503.

Widman M *et al.* (1990). Pharmacokinetics of remoxipride and metabolites following a single oral dose of ^{14}C-remoxipride. *Eur J Pharmacol* 1831871.

R

Repaglinide

Antidiabetic

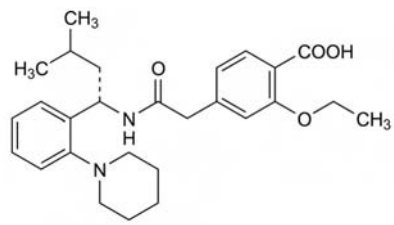
$C_{27}H_{36}N_2O_4$ = 452.6

CAS—135062-02-1

IUPAC Name 2-Ethoxy-4-[2-[[(1*S*)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid

Synonyms AG-EE-338 ZW; AG-EE-623 ZW; AG-EE-6232W; 2-ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

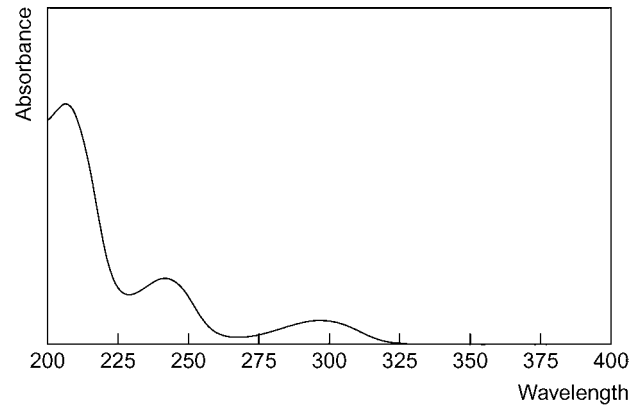
Proprietary Names *Novonorm*; *Prandin*.



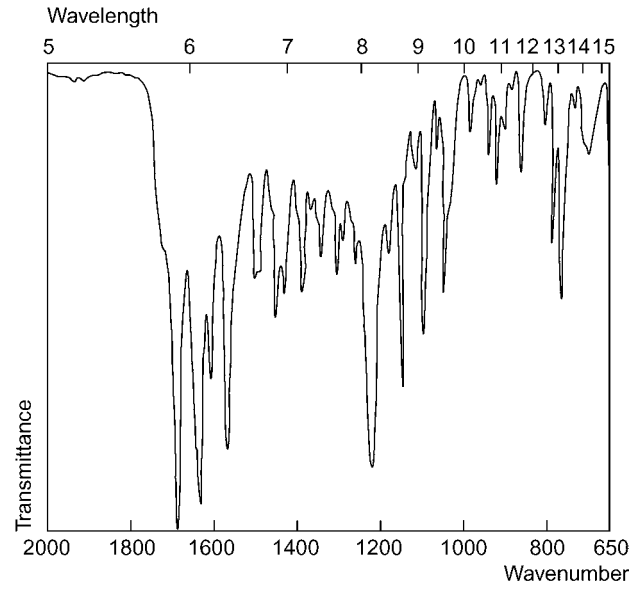
Chemical Properties A white, odourless, crystalline powder. Mp 126° to 128°.

High Performance Liquid Chromatography Columns: (analytical) 100 RP_{18} LiChrospher (125 × 4 mm i.d., 5 µm); (guard) ODS Hypersil (17 × 4.6 mm i.d., 5 µm); (precolumn) Perisorb RP_2 (17 × 2.9 mm i.d., 30 to 40 µm). Mobile phase: methanol-acetonitrile-dioxane (68:24:8) : 21 mmol/L potassium dihydrogenphosphate with lithium perchlorate (0.5 g/L), pH 2.7, 76 : 24), flow rate 1 mL/min. Electrochemical detection. Retention time(s): 8.8–9.1 min [Greischel *et al.* 1991].

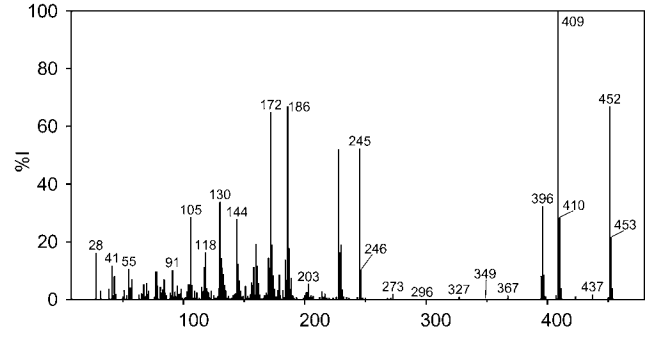
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1688, 1636, 1215, 1588, 1150, 1607 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 409, 172, 452, 186, 245, 228, 130, 396.



Quantification

Plasma **HPLC** Electrochemical detection. Limit of detection, 5 µg/L [Greischel *et al.* 1991].

Serum **ELISA** Limit of quantification, 1.88 µg/L, limit of detection, 0.35 µg/L [Hatorp *et al.* 2000].

Disposition in the Body Repaglinide is rapidly and almost completely absorbed after oral administration and peak concentrations are observed in ≈1 h.

The presence of food does not affect the absorption or pharmacokinetics of repaglinide. Plasma levels decrease rapidly once the peak concentration has been reached. It undergoes almost complete hepatic metabolism involving cytochrome P450 CYP3A4. Major metabolites include the oxidised dicarboxylic acid, aromatic amine and the acyl glucuronide, none of which are active with clinically relevant hypoglycaemic activity. The drug is eliminated rapidly, usually within 4–6 h, and excreted primarily via bile as both the parent compound and its metabolites. Less than 8% of an administered dose is excreted in urine, mainly as the metabolites, and <1% of the dose is detected in faeces as the unchanged drug.

Therapeutic concentration

Twelve male volunteers aged between 37 and 62 years (mean age 52.9 years) with moderate to severe chronic liver damage, and 12 healthy males, 42–62 years old (mean age 53.2 years) participated in this study. All subjects were fasted overnight and administered 4 mg repaglinide. An additional fast of 1 h after dosing was required. The peak plasma concentration for the patients was 105.4 µg/L at 0.8 h and for the healthy individuals, 46.7 µg/L at 0.8 h [Hatorp *et al.* 2000].

Twelve healthy volunteers; young adults aged 18–40 years (mean 33 years) and elderly individuals (mean 67 years) participated in this study. On day 1, after a 10 h fast, subjects were administered 2 mg repaglinide and continued fasting until noon that day. On days 2 to 8: 2 mg repaglinide 15 min before each of the 3 meals was administered and on day 9: a single 2 mg dose was administered. The mean peak plasma concentrations for the young adults were 47.9 µg/L (range, 18.0–127.0 µg/L) on day 1 and 58.5 (range, 26.0–212.0) µg/L on day 9. These concentrations were observed at 0.8 (0.5–2.5) h and 0.6 (0.5–0.8) h, respectively. For the elderly adults, the peak concentrations on day 1 were 47.5 (range, 20.0–115.0) µg/L at 0.7 (0.5–1.3) h and on day 9, 52.8 (range, 21.0–109.0) µg/L at 0.8 (0.3–1.5) h [Hatorp *et al.* 1999].

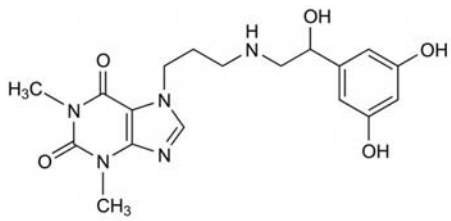
Bioavailability Approximately 63%; also reported as 56%.
Half-life 1 h.
Clearance Plasma, 143 mL/min.
Volume of Distribution 20–30 L.
Protein Binding >98%.
Dose The initial dose is 0.5 to 2.0 mg administered 30 min before meals. 1.0 mg or above is administered to patients who have had previous hypoglycaemic treatment. The dose is adjusted every 1 to 2 weeks up to 4.0 mg. A total daily dose of 16.0 mg should not be exceeded.

Greischel A *et al.* (1991). Quantitation of the new hypoglycaemic agent AG-EE 388 ZW in human plasma by automated high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 568: 246–252.
Hatorp V *et al.* (1999). Repaglinide pharmacokinetics in healthy young adult and elderly subjects. *Clin Ther* 21(4): 702–710.
Hatorp V *et al.* (2000). Single-dose pharmacokinetics of repaglinide in subjects with chronic liver disease. *J Clin Pharmacol* 40(2): 142–152.

Reproterol

Bronchodilator
$C_{18}H_{23}N_5O_5 = 389.4$
CAS—54063-54-6

IUPAC Name 7-[3-[[2-(3,5-Dihydroxyphenyl)-2-hydroxyethyl]amino]propyl]-1,3-dimethylpurine-2,6-dione
Synonyms 7-[3-[[2-(3,5-Dihydroxyphenyl)-2-hydroxyethyl]amino]propyl]-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione; D-1959.
Proprietary Name *Allergospasmin*

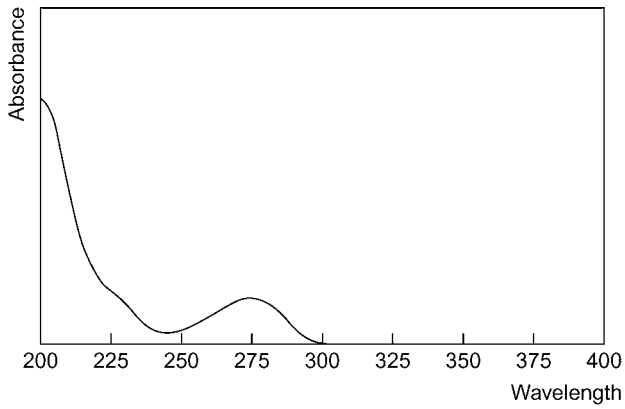


Chemical Properties Log *P* (octanol/water), −0.39.

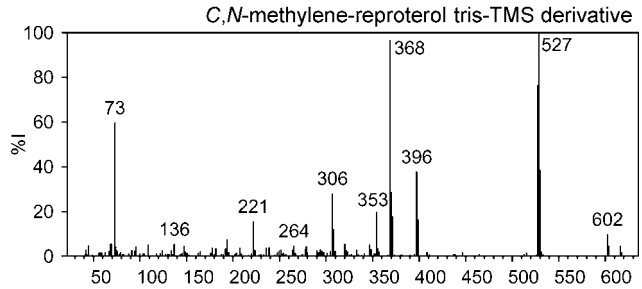
Reproterol Hydrochloride
$C_{18}H_{23}N_5O_5,HCl = 425.9$
CAS- 13055-82-8
Synonym W-2946M
Proprietary Names *Aarane*; *Asmaterolo*; *Bronchodil*; *Bronchospasmin*.
Chemical Properties Crystals. Mp 249° to 250°.

Gas Chromatography-Mass Spectrometry Column: HP Ultra-1 OV1 (16.8 m × 0.22 mm i.d., 0.11 µm). Temperature programme: 140° to 320° at 20°/min, for 3 min. Carrier gas: He, split flow 11 mL/min. EI, SIM *m/z* at 527, 369, 368, 356, 262, 250. Retention time: 9.5 min TMS derivative [Henze *et al.* 2001].

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 527, 368, 526, 73, 396, 528, 306, 369 (C *N*-methylene-reproterol tris-TMS)



Quantification
Plasma **HPLC** Limit of quantification, 0.4 µg/L [Knebel, Winkler 1997].
Urine **GC-MS** Limit of detection, 10 µg/L [Henze *et al.* 2001].
Disposition in the Body Reproterol is rapidly and well absorbed after oral, IV and aerosol administration. Intravenously: the drug is rapidly distributed and very low plasma levels are observed. Elimination is predominantly renal. By aerosol: it is quickly absorbed and is present at very low concentrations in plasma; peak concentrations are reached in 10 min. Orally: reproterol is rapidly and well absorbed with peak plasma concentrations at 1–3 h. Elimination is mainly renal but some biotransformation does take place. The drug is metabolised mainly in the liver and also in the small intestine. Up to 80% is excreted in urine unchanged. There is substantial extracellular distribution of the administered dose. Pharmacokinetics are linear.

Therapeutic Concentration

Three healthy volunteers, aged 20 years, were orally administered 20 or 40 mg reproterol hydrochloride or 270 or 540 µg reproterol IV over 10 to 15 min or were administered with 1 mg as 2 separate aerosol puffs, after an overnight fast. Peak plasma concentrations were observed shortly after administration of an oral dose. Plasma concentrations were 3.1 and 6.0 µg/L at 90 to 120 min for the 20 mg dose and 9.9 and 18.5 µg/L for the 2 individuals at 90 to 180 min for 40 mg. For the IV dose, the maximum concentrations were 8.4 µg/L for the 270 µg dose and 17.9 and 21.2 µg/L for the 2 individuals receiving the 540 µg dose. Peak plasma concentrations of 0.6 µg/L were observed with the inhaled dose (after the second puff) after 6 to 30 min [Hageman *et al.* 1988].

Bioavailability 50%, after oral administration.
Protein Binding Negligible.
Dose Adults: 0.5 to 1 mg aerosol inhalation 3 times a day. 10 to 20 mg orally 3 times a day. Single or repeated dose of 90 µg ampoule IV. Child: 500 µg.

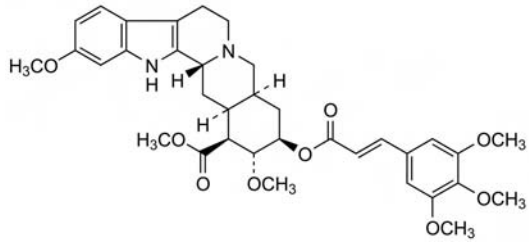
Hageman RJ *et al.* (1988). Plasma levels, heart rate, and blood pressure after intravenous, oral, and aerosol administration of reproterol in man. *Biopharm Drug Dispos* 9(3): 301–314.
Henze MK *et al.* (2001). Screening of beta-2 agonists and confirmation of fenoterol, orciprenaline, reproterol and terbutaline with gas chromatography-mass spectrometry as tetrahydroisoquinoline derivatives. *J Chromatogr B Biomed Sci Appl* 751(1): 93–105.
Knebel N, Winkler M (1997). Rapid and automated determination of the beta2-agonist reproterol in human plasma by atmospheric pressure chemical ionisation high-performance liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 702: 119–129.

Rescinnamine

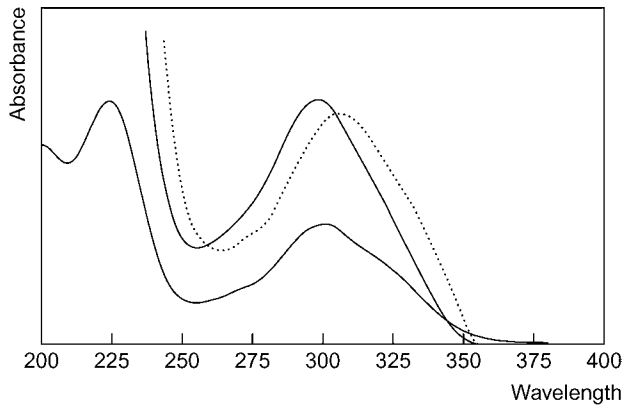
Antihypertensive
$C_{35}H_{42}N_2O_9 = 634.7$
CAS—24815-24-5

Synonyms (3β,16β,17α,18β,20α)-11,17-Dimethoxy-18-[[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]oxy]-3,20-yohimban-16-carboxylic acid methyl ester; reserpinine.

Proprietary Names *Anaprel*; *Apoterin S*; *Cartric*; *Cinnaloid*; *Moderil*.



Chemical Properties An alkaloid obtained from the roots of certain species of *Rauwolfia* (Apocynaceae), mainly *R. serpentina* and *R. vomitoria* or by synthesis. A white or pale buff to cream-coloured, crystalline powder. It slowly darkens on exposure to light but more rapidly when in solution. Mp 238° to 239°. Practically insoluble in water; soluble in methanol, ethanol, benzene, acetic acid and chloroform. Log *P* (octanol/water), 3.8.
Colour Tests Mandelin's test—brown; Marquis test—grey-green.
Thin-layer Chromatography System TA—R_f 0.73; system TB—R_f 0.01; system TC—R_f 0.75; system TE—R_f 0.81; system TL—R_f 0.64; system TAE—R_f 0.77; system TAF—R_f 0.79 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2180.
High Performance Liquid Chromatography System HA—*k* 0.6; system HX—RI 496; system HY—RI 407.
Ultraviolet Spectrum Aqueous acid—299 nm; aqueous alkali—306 nm.

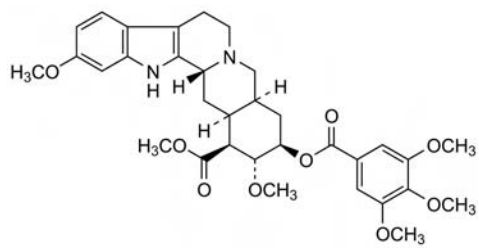


Infrared Spectrum Principal peaks at wavenumbers 1266, 1147, 1124, 1165, 1233, 1715 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 221, 199, 200, 186, 395, 251, 77, 214.
Dose 0.25 to 1 mg daily.

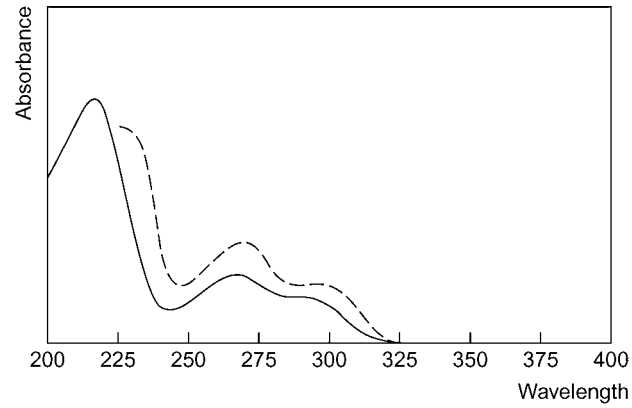
Reserpine

Antihypertensive
$C_{33}H_{40}N_2O_9 = 608.7$
CAS—50-55-5

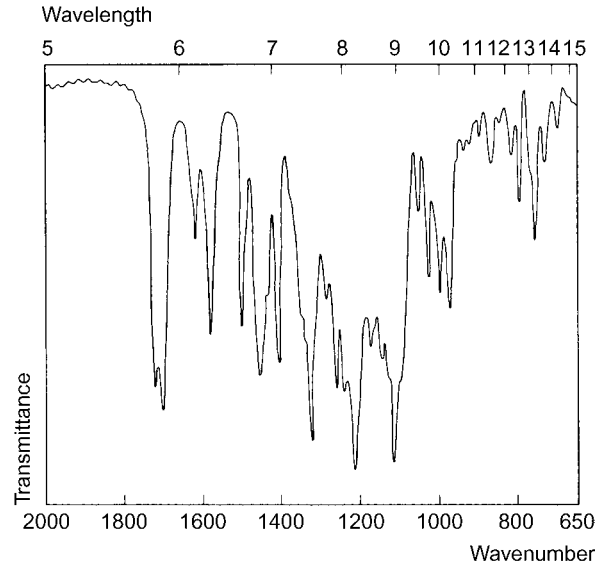
IUPAC Name (3β,16β,17α,18β,20α-11,17-Dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]yohimban-16-carboxylic acid methyl ester.
Proprietary Names *Crystoserpine*; *Eskaserp*; *Ortoserpina*; *Rau-Sed*; *Rauserpin*; *Reserpoid*; *Rivasin*; *Sandril*; *Sedaraupin*; *Serfin*; *Serfinato*; *Serpalan*; *Serpasil*. It is an ingredient of *Diupres*, *Hydropres*, *Metatensin*, *Naquival*, *Regroton*, *Renese-R*, *Salutensin*, *Tri-Hydroserpine*.



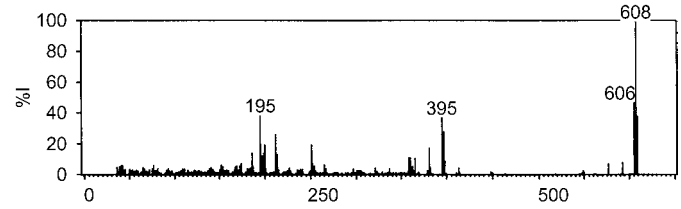
Chemical Properties An alkaloid obtained from the roots of certain species of *Rauwolfia* (Apocynaceae), mainly *R. serpentina* and *R. vomitoria,* or by synthesis. Fine, white or pale buff to slightly yellow-coloured crystals or crystalline powder. It darkens slowly on exposure to light, but more rapidly when in solution. Mp 264° to 265°, with decomposition. Practically insoluble in water and ether; soluble 1 in 2000 of ethanol and 1 in 6 of chloroform; freely soluble in glacial acetic acid; slightly soluble in acetone and methanol. pK_a 6.6 (25°). Log *P* (octanol/water), 3.3.
Colour Test Mandelin's test—green; Marquis test—grey-green→brown.
Thin-layer Chromatography System TA—R_f 0.69; system TAE—R_f 0.76; system TAF—R_f 0.80; system TAG—R_f 0.63; system TB—R_f 0.02; system TC—R_f 0.74; system TE—R_f 0.77 (acidified potassium permanganate—positive).
Gas Chromatography System GA—reserpine not eluted, M (trimethoxybenzoic acid) RI 1780, M (trimethoxyhippuric acid) RI 2085, M (trimethoxybenzoic acid-ME) RI 1740, M (trimethoxyhippuric acid-ME) RI 2350.
High Performance Liquid Chromatography System HX—RI 467; system HY—RI 351; system HAA—RT 16.4 min.
Ultraviolet Spectrum Ethanol—267 (A^1_1= 272a), 295 nm.



Infrared Spectrum Principal peaks at wavenumbers 1220, 1120, 1700, 1240, 1720, 1265 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 608, 606, 195, 609, 395, 397, 212, 396.



Quantification
Plasma **TLC** Limit of detection, 50 ng/L [Tripp *et al.* 1975].
HPLC Column: LC-1 TMS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L acetate buffer (pH 4.2) : acetonitrile (50 : 50) with 5 mmol/L heptane sulfonate and 0.01 mol/L TEA, flow rate, 1.6 mL/min. Fluorescence detection (λ_{ex}= 460 nm, λ_{em}= 570 nm). Limit of detection, 300 ng/L [Suckow *et al.* 1983].
Disposition in the Body Reserpine is readily but somewhat erratically absorbed after oral administration. It is rapidly and extensively metabolised by hydrolysis and *O*-demethylation; the major metabolites are trimethoxybenzoic acid and methyl reserpate, together with reserpic acid, syringomethyl reserpate and syringic acid; metabolites may be conjugated with glucuronic acid or sulfate. Approximately 6% of an oral dose is excreted in the urine in 24 h and 8% in the first 4 days, mainly as trimethoxybenzoic acid; <3% of a dose is excreted in the urine

unchanged. Approximately 60% of the dose is eliminated in the faeces in 4 days, mainly as unchanged drug.

Therapeutic Concentration

After a single oral dose of 1 mg to 2 subjects, peak plasma concentrations of 0.0004–0.0006 mg/L were attained in 2–3 h [Tripp *et al.* 1975].

Toxicity High dosage may occasionally cause a Parkinsonian-like syndrome and severe depression; several cases of accidental poisoning have been reported in children but fatalities are unlikely.

Half-life Plasma half-life, extremely variable and may be up to 11 days.

Distribution in Blood Plasma : whole blood ratio, 1.1.

Protein Binding ~40%.

Dose Usually 250 to 500 µg daily, for hypertension. Doses of 0.1 to 1 mg or more daily have been given as a sedative.

Suckow RF *et al.* (1983). An improved method for the determination of reserpine in plasma using liquid chromatography with fluorescence detection. *J Liq Chromatogr* 6: 1111–1122.

Tripp SL *et al.* (1975). A specific assay for subannogram concentrations of reserpine in human plasma. *Life Sci* 16: 1167–1177.

Resorantel

Anthelmintic (Veterinary)

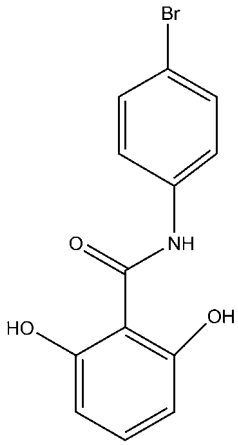
$C_{13}H_{10}BrNO_3$ = 308.1

CAS—20788-07-2

IUPAC Name *N*-(4-Bromophenyl)-2,6-dihydroxybenzamide

Synonym *p′*-Bromo-2,6-dihydroxybenzanilide

Proprietary Name *Terenol*



Chemical Properties White powder. Mp 229° to 230°. Insoluble in water; soluble in methanol. Resorantel is extracted by chloroform from aqueous alkaline solutions.

Colour Tests Ammonium vanadate test—brown (limit of detection, 0.5 µg); Vitali's test—dull yellow/orange-brown/orange-brown (limit of detection, 0.1 µg).

Thin-layer Chromatography System T1—R_f 0.85 (location reagent acidified iodoplatinate spray, positive reaction).

Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.

Dose In cattle and sheep, ≈70 mg/kg.

Resorcinol

Dermatological Agent

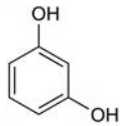
$C_6H_6O_2$ = 110.1

CAS—108-46-3

IUPAC Name Benzene-1,3-diol

Synonyms 1,3-Benzenediol; *m*-dihydroxybenzene; dioxybenzolum; resorcin.

Proprietary Names *Astriderm*; *Castel*. It is an ingredient of *Acnomel* and *Eskamel*.



Chemical Properties Colourless or slightly pinkish-grey, acicular crystals or crystalline powder. It becomes red on exposure to air and light. Mp 109° to 111°; it sublimes on further heating. Soluble 1 in <1 of water and 1 in 1 of ethanol; slightly soluble in chloroform; freely soluble in ether and glycerol. pK_a 9.5, 10.1 (20°). Log *P* (octanol/water), 0.8.

Resorcinol Monoacetate

$C_8H_8O_3$ = 152.1

CAS—102-29-4

Synonyms 3-Acetoxyphenol; resorcin acetate.

Proprietary Name *Euresol*

Chemical Properties A pale yellow or amber viscous liquid. Relative density 1.203–1.207. Bp about 283°, with decomposition. Sparingly soluble in water; soluble in ethanol and most organic solvents.

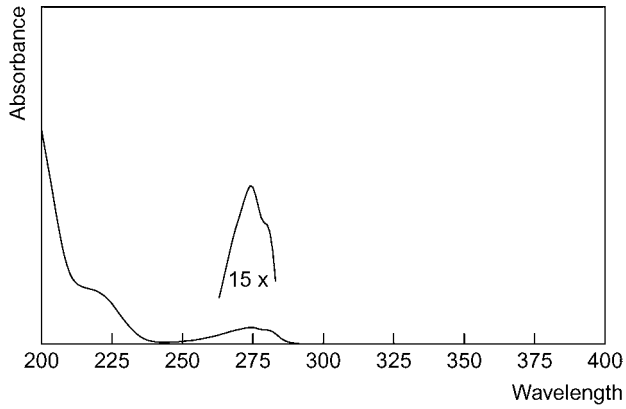
Colour Test Liebermann's reagent—violet.

Thin-layer Chromatography System TAE—R_f 0.86; system TAF—R_f 0.91; system TB—R_f 0.00; system TE—R_f 0.50; system TAJ—R_f 0.34; system TAK—R_f 0.20; system TAL—R_f 0.68.

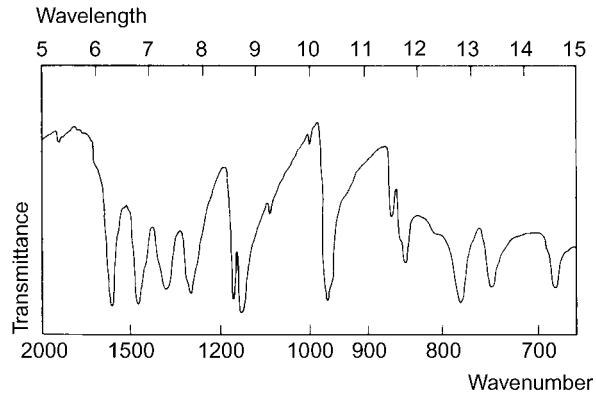
Gas Chromatography System GA—RI 1258.

High Performance Liquid Chromatography System HX—RI 243; system HY—RI 211; system HAA—RT 8.0 min.

Ultraviolet Spectrum Aqueous acid—273 nm (A^1_1 = 172a); aqueous alkali—290 nm (A^1_1 = 315a).



Infrared Spectrum Principal peaks at wavenumbers 1149, 1603, 774, 962, 1164, 1289 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 110, 82, 39, 81, 53, 69, 55, 111.

Quantification

Plasma **HPLC** UV detection. Limit of detection, 500 µg/L [Yeung *et al.* 1981].

Urine **HPLC** See Plasma [Yeung *et al.* 1981].

Disposition in the Body Resorcinol may be absorbed through the skin or from ulcerated surfaces.

Toxicity The estimated minimum lethal dose is 2 g and the maximum permissible atmospheric concentration is 10 ppm. The systemic effects of resorcinol are similar to those of phenol but convulsions may be more frequent.

Use Topically in concentrations of 2 to 5%.

Yeung D *et al.* (1981). *J Chromatogr* 224: 513–518.

Reteplase

Thrombolytic, Treatment of Acute Myocardial Infarction

$C_{1736}H_{2653}N_{499}O_{522}S_{22}$ = 39571.1

CAS—133652-38-7

IUPAC Name 173-L-Serine-174-L-tyrosine-175-L-glutamine-173-527-plasminogen activator

Synonyms BM-06.022; r-PA.

Proprietary Names *Rapilysin*; *Retavase*.

Disposition in the Body Elimination is both hepatic and renal. Hepatic clearance appears to be less than that of renal clearance. The drug is also cleared

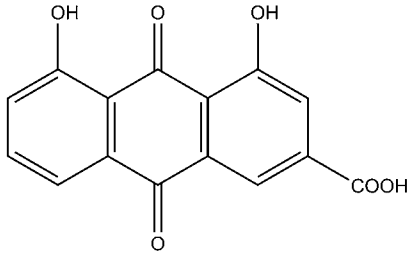
from circulation by α_2-antiplasmin, C_1 inactivator and α_1-antitrypsin, however the extent by these factors is unknown.
Half-life 170–173 min.
Volume of Distribution 6 L in healthy individuals.
Clearance The total plasma clearance is 17.4–22.3 L/h. In individuals with acute myocardial infarction, 7.3 L/h.
Note For a review of reteplase, see Noble and McTavish [1996].
Dose Ten units over 2 min by IV administration followed by another 10 units after 30 min. 10 units=1.16 g.

Noble S, McTavish D (1996). Reteplase. A review of its pharmacological properties and clinical efficacy in the management of acute myocardial infarction. *Drugs* 52: 589–605.

Rhein

Hydroxyanthraquinone, Laxative, Stimulant
$C_{15}H_8O_6$ = 284.2
CAS—478-43-3

IUPAC Name 4,5-Dihydroxyanthraquinone-2-carboxylic acid
Synonyms Cassic acid; chrysazin-3-carboxylic acid; 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthracenecarboxylic acid; 1,8-dihydroxyanthraquinone-3-carboxylic acid; monorhein; parietic acid; rheic acid; rhubarb yellow.
Proprietary Names *Bukosan*; *Laxitab*; *Phytoestrol N*; *Radirex*; *Rzewex*.

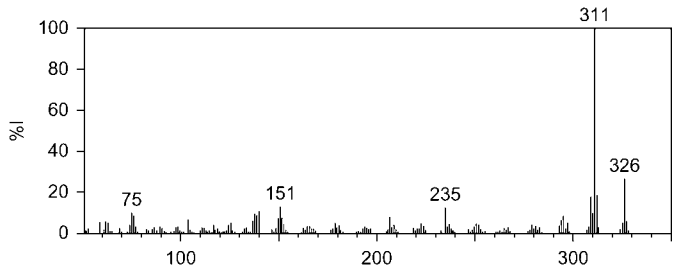


Chemical Properties Found in the free state and as a glucoside in *Rheum* (Polygonaceae) (rhubarb) and in Senna leaves. It is also found in several species of *Cassia* (Leguminosae). Yellow needles by sublimation. Mp 321° to 322°. Soluble in alkalis and pyridine; slightly soluble in alcohol, benxene, chloroform, ether and petroleum ether. pK_a 8.28 [Huitao *et al.* 2004]. Log *P* (octanol/water), 2.03 [Kagedal *et al.* 1999]. Stable in rat plasma at room temperature and 4° for 12 h and at –70° for 7 days [Yan, Ma 2007]. When 10 mL of urine spiked with 100 µg/mL rhein was incubated with extraction buffer at room temperature, degradation of rhein was pronounced and followed a mathematical model of monoexponential decay [Beyer *et al.* 2005].

Rhein Diacetate

See Diacerein.

Ultraviolet Spectrum Methanol—229, 258, 435 nm.
Mass Spectrum Principal peaks at *m/z* 311, 326, 235, 151, 75 (3ME derivative) [Beyer *et al.* 2005].



Quantification

Urine **TLC** Plates: C_{18} SepPak. Solvent system: chloroform : propan-2-ol (95 : 5). R_f 0.45. Limit of detection, 10–20 mg/L [Perkins, Livesey 1993]. Plates: Schleicher, Schuell F1500. Solvent system: chloroform : acetone (4 : 1). R_f 0.50 [Morton 1987].

HPLC Column: Hypersil ODS (100 × 2 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 5.0; 20 : 80 to 45 : 55 at 3 min), flow rate 0.5 mL/min. DAD (λ= 225 nm). Limit of detection, 0.29 mg/L [Fullinfaw *et al.* 1988].

GC-MS Column: HP-1 capillary cross-linked methylsilicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1 mL/min. Temperature programme: 100° to 310° at 30°/min. EI ionisation at 70 eV, full scan mode. Limit of detection, 25 µg/L [Beyer *et al.* 2005].

Other **HPLC** Rat Plasma. Column: Inertsil ODS-A C_{18} reversed phase (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.2% acetic acid (pH 3.83; 89 : 11), flow rate 1 mL/min. UV detection (λ= 254 nm). Limit of quantification, 15 µg/L, limit of detection, 7.5 µg/L [Tang *et al.* 2007]. Column: Thermo Hypersil-Keystone C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: water-phosphoric acid (100 : 0.1) : methanol (24 : 76 for 18 min to 10 : 90 at 19 min for 5 min to 0 : 100 at 25 min for 9 min to 24 : 76 at 35 min), flow rate 1 mL/min. Fluorescence detection (λ_{ex}= 435 nm, λ_{em}= 515 nm). Limit of quantification, 20 µg/L [Yan, Ma 2007]. Rhubarb, Rat Plasma, Urine and CSF. Column: Zorbax SB-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.5% acetic acid (85 : 15), flow rate 0.6 mL/min. DAD (λ= 254 nm). Limit of detection, 1.12 ng [Ding *et al.* 2003]. Mouse Urine and Bile. Column: Waters µBondapak C_{18} (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: 0.025 mol/L sodium acetate : acetonitrile : acetic acid (85 : 15 : 4 to 40 : 60 : 4 over 15 min), flow rate 1.5 mL/min. Amperometric detection. Limit of detection not reported [Moreau *et al.* 1985]. See also Qu *et al.* [2007] for Chinese Medicine, Koyama *et al.* [2007] for Rhubarb, and Yi *et al.* [2006] for Rat Plasma.

CE Huangdan Yinchen Keli. Capillary: Polyimide-coated fused silica (70 cm × 25 µm i.d.). Buffer: 30 mmol/L borate (pH 9.5). Amperometric detection. Limit of detection, 150 nmol/L [Wang *et al.* 2004]. Chinese Medicinal Preparations. Capillary: fused silica (50 cm [42.4 cm to the detector] × 75 µm i.d.). Buffer: 0.1 mol/L borate (pH 9.2). UV detection (λ= 254 nm). Limit of detection, 10 mg/L [Huitao *et al.* 2004]. Capillary: fused silica (50 cm [42.6 cm to the detector] × 75 µm i.d.). Buffer: 10 mmol/L borate : 20 mmol/L SDS : 10% acetonitrile (pH 9.55). UV detection (λ= 254 nm). Limit of detection, 0.4 mg/L [Liu *et al.* 2000].

Disposition in the Body It is generally accepted that the lipophilicity of the aglycones facilitates their absorption through the upper part of the intestine. They undergo a first-pass effect, after which they are excreted in bile as glucuronides [Moreau *et al.* 1985].

Therapeutic Concentration Rhein has been shown to effectively inhibit the uptake of glucose into tumour cells, change membrane-associated functions, and lead to cell death at concentrations of up to 113.7 mg/L (400 µmol/L) *in vitro* [Huang *et al.* 2007].

Fifteen postpartum women were administered 15 mg/day of a senna laxative (Agiolax, active ingredients senna and ispaghula). Breast milk concentrations reached a maximum of 27 µg/L, equivalent to 0.3% of the rhein intake of the mother, below the dose necessary for any laxative effect [Faber, Strenge-Hesse 1989].

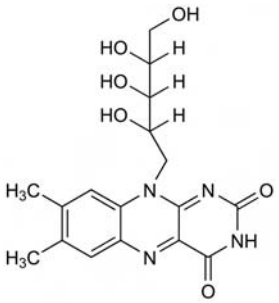
Toxicity Studies have shown that rhein is not mutagenic and that it does not induce chromosomal aberrations [Heidemann *et al.* 1993]. It is, however, a substrate for the multidrug resistance-associated protein 1 efflux pump and could, therefore, be cytotoxic by inducing apoptosis [van Gorkom *et al.* 2002]. It also generates the production of free oxygen radicals [Kagedal *et al.* 1999].
Bioavailability Approximately 35%.
Half-life In healthy subjects, 4.3 h.
Volume of Distribution Approximately 13.3 L.
Clearance Approximately 0.13 L/h.
Protein Binding Approximately 99%.
Dose As a laxative: adults, 2 to 3 5 mg tablets in a single dose; children over 12 years of age, one 5 mg tablet in a single dose, preferably at bedtime. The onset of laxative action is expected within 6 to 8 h. The optimal individual dose is the smallest dose resulting in soft stool.

Beyer J *et al.* (2005). Screening procedure for detection of stimulant laxatives and/or their metabolites in human urine using gas chromatography–mass spectrometry after enzymatic cleavage of conjugates and extractive methylation. *Ther Drug Monit* 27: 151–157.
Ding M *et al.* (2003). Simultaneous determination of hydroxyanthraquinones in rhubarb and experimental animal bodies by high-performance liquid chromatography. *Anal Sci* 19: 1163–1165.
Faber P, Strenge-Hesse A (1989). Senna-containing laxatives: excretion in the breast milk? Geburtshilfe Frauenheilkd *Geburtshilfe Frauenheilkd* 49: 958–962.
Fullinfaw RO *et al.* (1988). Screening procedure for stimulant laxatives in urine using high-performance liquid chromatography with diode array detection. *J Chromatogr* 433: 131–140.
Heidemann A *et al.* (1993). The genotoxicity status of senna. *Pharmacology* 47: 1178–186.
Huang Q *et al.* (2007). Anti-cancer properties of anthraquinones from rhubarb. *Med Res Rev* 27: 609–630.
Huitao L *et al.* (2004). Determination of rhein, baicalin and berberine in traditional Chinese medicinal preparations by capillary electrophoresis with two-marker technique. *Biomed Chromatogr* 18: 288–292.
Kagedal K *et al.* (1999). Anthraquinone cytotoxicity and apoptosis in primary cultures of rat hepatocytes. *Free Radic Res* 31: 419–428.
Koyama J *et al.* (2007). Simultaneous determination of anthraquinones in rhubarb by high-performance liquid chromatography and capillary electrophoresis. *J Chromatogr A* 1145: 183–189.
Liu HT *et al.* (2000). Electrophoretic behavior study and determination of some active components in Chinese medicinal preparations by capillary electrophoresis. *Analyst* 125: 1083–1086.
Moreau JP *et al.* (1985). Comparative physiological disposition of some anthraquinone glycosides and aglycones. *Biopharm Drug Dispos* 6: 325–334.
Morton J (1987). The detection of laxative abuse. *Ann Clin Biochem* 24: 107–108.
Perkins S *et al.* (1993). A rapid high-performance thin-layer chromatographic urine screen for laxative abuse. *Clin Biochem* 26: 179–181.
Qu H *et al.* (2007). Simultaneous determination of eight active components in Chinese medicine 'YIQING' capsule using high-performance liquid chromatography. *J Pharm Biomed Anal* 43: 66–72.
Tang WF *et al.* (2007). Determination and pharmacokinetic comparison of rhein in rats after oral dosed with Da-Cheng-Qi decoction and Xiao-Cheng-Qi decoction. *Biomed Chromatogr* 21: 1186–1190.
vanGorkom BA *et al.* (2002). Cytotoxicity of rhein, the active metabolite of sennoside laxatives, is reduced by multidrug resistance-associated protein 1. *Br J Cancer* 86: 1494–1500.
Wang A *et al.* (2004). Determination of active ingredients in Huangdan Yinchen Keli by CZE with amperometric detection. *J Pharm Biomed Anal* 35: 959–964.
Yan D, Ma Y (2007). Simultaneous quantification of five anthraquinones in rat plasma by high-performance liquid chromatography with fluorescence detection. *Biomed Chromatogr* 21: 502–507.
Yi L *et al.* (2006). Simultaneous determination of baicalin, rhein and berberine in rat plasma by column-switching high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 838: 50–55.

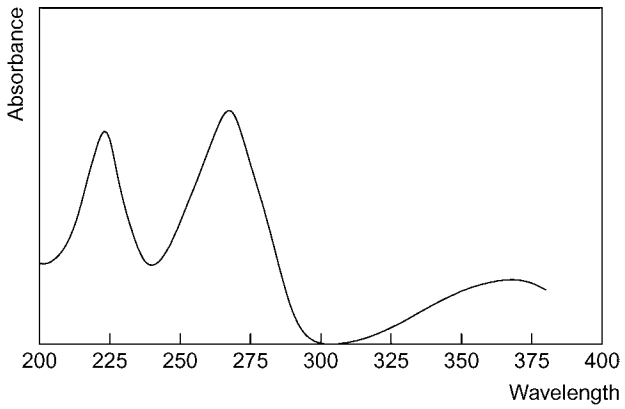
Riboflavin

Vitamin
$C_{17}H_{20}N_4O_6 = 376.4$
CAS—83-88-5

IUPAC Name 7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione
Synonyms 7,8-Dimethyl-10-(D-*ribo*-2,3,4,5-tetrahydroxypentyl)isoalloxazine; lactoflavin; riboflavine; vitamin B_2; vitamin G.
Proprietary Names *Beflavin(e)*; *Berivine*; *Boflavin*; *Flavaxin*; *Hibon*; *Ribon*.



Chemical Properties A yellow or orange-yellow, crystalline powder. Mp 278° to 282°, with decomposition (darkens at about 240°). Solutions deteriorate on exposure to light. Soluble 1 in 3000 to 1 in 20 000 of water, the variation in solubility being due to the variation in the internal crystalline structure; practically insoluble in ethanol, acetone, chloroform and ether; very soluble in dilute solutions of alkali hydroxides. pK_a 1.9, 10.2 (20°). Log *P* (octanol/water), −1.5.
Colour Test Dissolve 1 mg in 100 mL of water—the solution is green-yellow when viewed by transmitted light and shows an intense yellow-green fluorescence which disappears on adding mineral acids, alkalis and reducing agents such as sodium dithionite.
Ultraviolet Spectrum Aqueous acid—267 nm (A_1^1=820a); aqueous alkali—270, 356 nm.



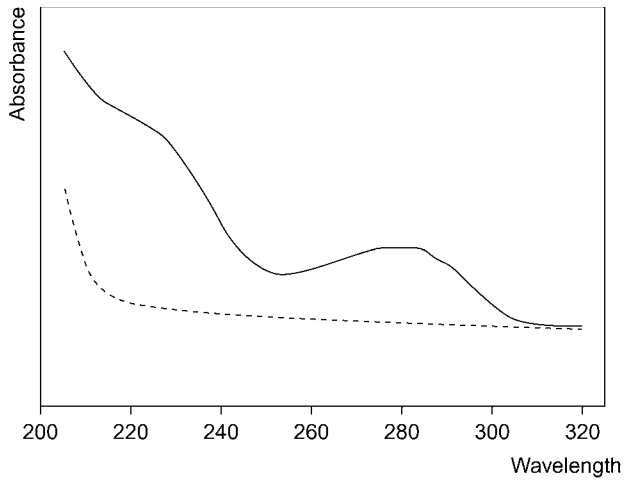
Infrared Spectrum Principal peaks at wavenumbers 1544, 1575, 1641, 1715, 1235, 1070 cm^{-1} (KBr disk).
Quantification
Blood **HPLC** UV and fluorescence detection. For method for quantification of riboflavin, thiamine and pyridoxine, see Botticher and Botticher [1987].
Plasma **HPLC** For method for quantification of riboflavin and riboflavin cofactors, see Capo-chichi *et al.* [2000]. Fluorescence detection. Limit of detection, <3 nmol/L for riboflavin and 9 nmol/L for flavocoenzymes [Zempleni 1995]. Fluorimetric detection. For method for quantification of riboflavin, riboflavin 5′-phosphate and flavin adenine dinucleotide, see Lopez-Anaya and Mayersohn [1987].
Serum **HPLC** UV and fluorescence detection. For method for quantification of riboflavin, thiamine and pyridoxine, see Botticher and Botticher [1987].
Urine **HPLC** Fluorimetric detection. For method for quantification of riboflavin, riboflavin 5′-phosphate and flavin adenine dinucleotide, see Lopez-Anaya and Mayersohn [1987]. Fluorescence detection. Limit of detection, 50 µg/L [Smith 1980].
Disposition in the Body Riboflavin is readily absorbed after oral administration. It is widely distributed throughout the body but little is stored, and amounts in excess of the body's requirements are excreted in the urine. It is metabolised by phosphorylation to flavine mononucleotide (FMN) and then to flavine adenine dinucleotide (FAD). The recommended daily intake is up to 500 µg/kg. Endogenous plasma concentrations are usually <0.1 µg/mL.
Half-life Plasma half-life, about 1 h.
Protein Binding About 60%.
Note For a review of the chemical and biological actions of riboflavin, see Massey [2000].
Dose 2 to 10 mg daily.

Botticher B, Botticher D (1987). A new HPLC-method for the simultaneous determination of B1-, B2- and B6-vitamers in serum and whole blood. *Int J Vitam Nutr Res* 57: 273–278.
Capo-chichi CD *et al.* (2000). Analysis of riboflavin and riboflavin cofactor levels in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 739: 219–224.
Lopez-Anaya A, Mayersohn M (1987). Quantification of riboflavin, riboflavin 5′-phosphate and flavin adenine dinucleotide in plasma and urine by high-performance liquid chromatography. *J Chromatogr* 423: 105–113.
Massey V (2000). The chemical and biological versatility of riboflavin. *Biochem Soc Trans* 28: 283–296.
Smith MD (1980). Rapid method for determination of riboflavin in urine by high-performance liquid chromatography. *J Chromatogr* 182: 285–291.
Zempleni J (1995). Determination of riboflavin and flavocoenzymes in human blood plasma by high-performance liquid chromatography. *Ann Nutr Metab* 39: 224–226.

Ricin

Protein Toxin, Type 2 Ribosome-inactivating Protein
66 kDa approximately
CAS—9009-86-3

IUPAC Name Not available
Chemical Properties White powder. Stable over a wide pH range, inactivated by heat, 80° in aqueous solution for 1 h [Audi *et al.* 2005]. A toxic lectin and haemagglutinin isolated from castor bean *Ricinus communis* L., Euphorbiaceae. Ricin is among the most toxic compounds known. Seeds of *R. communis*, if thoroughly masticated, can result in serious poisoning and death. For a paper on the photophysical properties of ricin, see Gaigalas *et al.* [2007].
Liquid Chromatography-Mass Spectrometry Column: Zorbax C_{18}. Mobile phase: 0.1% formic acid. APEI, positive ion mode, MRM acquisition mode. Limit of detection, 2.4 µg/L for adenine, equivalent to ~100 µg/L ricin. Coupled with immunoaffinty, limit of detection, 0.1 µg/L ricin [Becher *et al.* 2007]. Column: PepMap C_{18} (15 cm × 1.0 mm i.d., 5 µm). Mobile phase: 0.1% formic acid, flow rate 120 µL/min. ESI, SIM acquisition mode. Limit of quantification, 0.27 µmol/L for adenine; limit of detection, 2.4 µg/L, for adenine [Hines *et al.* 2004].
Ultraviolet Spectrum 280 nm.



Quantification
Other **LC-MS** Crude CastorBean Extracts. Column: PepMap C_{18} (150 × 0.3 mm i.d., 5 µm). Mobile phase: acetonitrile (5 to 10% over 4 min to 80% over 42 min) with 0.2% formic acid and 0.02% trifluoroacetic acid, flow rate 7 µL/min. TOF. Limit of detection not reported [Ostin *et al.* 2007]. Column: PepMap C_{18} (15 cm × 300 µm i.d., 3 µm). Mobile phase: 0.2% formic acid : 0.2% formic acid in acetonitrile (100 : 0 to 20 : 80 at 60 min), flow rate 0.1 mL/min for 5 min to 0.5 mL/min. MALDI. Limit of detection not reported [Fredriksson *et al.* 2005]. *R. communis* Beans. Mobile phase: acetonitrile : water (50 : 50) containing 0.5% formic acid, flow rate 5 µL/min. ESI. Limit of detection not reported [Despeyroux *et al.* 2000].

CE Crude Castor Bean Extracts. Capillary: fused silica (34 cm [26 cm to detector] × 50 µm i.d.). Buffer: 50 mmol/L phosphate buffer (pH 2.5). DAD. Limit of detection not reported [Ostin *et al.* 2007]. *R. communis* Beans. Capillary: LPA coated Biocap (78 cm [60 cm to detector] × 50 µm i.d.). Buffer: 50 mmol/L β-alanine (pH 3.5). UV detection (λ = 219 nm). Limit of detection not reported [Despeyroux *et al.* 2000].
Note Feltis *et al.* [2008] have developed a hand-held biosensor that can detect ricin at levels of 200 µg/L in 10 min. For a cytotoxicity assay for the measurement of ricin in beverages and food matrices, see Brzezinski and Craft [2007]. For a review article on the detection methods for ricin, see Ler *et al.* [2006]. For a Raman spectroscopy method for the detection of ricin in A549 lung cells, see Notingher *et al.* [2004]. For a comparison of factors affecting the capillary electrophoresis of ricin, see Hines and Brueggemann [1994].
Disposition in the Body Since ricin is a large protein, it is unlikely to be extensively absorbed in the gastrointestinal tract. Animal studies have shown that orally administered ricin is predominantly found in the large intestine after 24 h, with limited systemic uptake. Dermal absorption is unlikely to occur unless there are open wounds. For a review of the pharmacokinetics of ricin in rats following IV or intrathecal infusion, see Candiani *et al.* [2001].

Toxicity The lethal oral dose in humans is estimated to be 1 to 20 mg of ricin/kg (~8 beans). Toxicity results from the inhibition of protein synthesis, but other mechanisms may also be involved, including apoptosis pathways, direct cell membrane damage, alteration of membrane structure and function, and release of cytokine inflammatory mediators. The number of beans ingested in reports documenting clinical symptoms (mild to lethal) range from one half to 30. The minimum number of beans associated with death was 2 [Audi *et al.* 2005].

A 36-year-old chemist self-administered 2 IM injections of ricin prepared from a single castor bean. It was calculated that he had injected 150 mg ricin, although this would be impossible from a single bean as the maximum amount of ricin in a single castor bean is ≈10 mg, equivalent to no more than 140 µg/kg bodyweight. He complained of headache and rigors 10 h after the injections and he developed anorexia and nausea, a sinus tachycardia, and erythematous areas and lymphadenopathy at the sites of injection. He had mildly increased hepatic transaminase activity. He was discharged after 10 days with no sequelae.

A 20-year-old man was admitted to hospital 36 h after the SC injection of castor bean extract. He complained of nausea, weakness, dizziness, chest and abdominal pain, and myalgia with parasthaesia of the extremities. Upon examination, the patient presented with hypotension, anuria, and metabolic acidosis, and there was fresh blood present in the rectum, possibly from a bleeding diathesis. He developed hepatorenal and cardiorespiratory failure and died 18 h after admission, following an asystolic arrest.

Ricin A chain has been proposed as a possible agent for anti-tumour immunotherapy.

In a study of 56 patients who were treated with ricin A chain immunotoxin, 12 required interruption or termination of treatment owing to severe adverse reactions, and 2 patients died as a result of vascular leak syndrome [Bradberry *et al.* 2003].

Audi J *et al.* (2005). Ricin poisoning: a comprehensive review. *JAMA* 294: 2342–2351.

Becher F *et al.* (2007). Detection of functional ricin by immunoaffinity and liquid chromatography–tandem mass spectrometry. *Anal Chem* 79: 659–665.

Bradberry SM *et al.* (2003). Ricin poisoning. *Toxicol Rev* 22: 65–70.

Brzezinski JL, Craft DL (2007). Evaluation of an in vitro bioassay for the detection of purified ricin and castor bean in beverages and liquid food matrices. *J Food Prot* 70: 2377–2382.

Candiani C *et al.* (2001). Pharmacokinetics of intrathecal transferrin-ricin a chain immunotoxin. *Life Sci* 69: 335–346.

Despeyroux D *et al.* (2000). Characterization of ricin heterogeneity by electrospray mass spectrometry, capillary electrophoresis, and resonant mirror. *Anal Biochem* 279: 23–36.

Feltis BN *et al.* (2008). A hand-held surface plasmon resonance biosensor for the detection of ricin and other biological agents. *Biosens Bioelectron* 23: 1131–1136.

Fredriksson SA *et al.* (2005). Forensic identification of neat ricin and of ricin from crude castor bean extracts by mass spectrometry. *Anal Chem* 77: 1545–1555.

Gaigalas AK *et al.* (2007). Photophysical properties of ricin. *Photochem PhotoBiol* 83: 1149–1156.

Hines HB, Brueggemann EE (1994). Factors affecting the capillary electrophoresis of ricin, a toxic glycoprotein. *J Chromatogr A* 670: 199–208.

Hines HB *et al.* (2004). High-performance liquid chromatography–mass selective detection assay for adenine released from a synthetic RNA substrate by ricin A chain. *Anal Biochem* 330: 119–122.

Ler SG *et al.* (2006). Trends in detection of warfare agents. Detection methods for ricin, staphylococcal enterotoxin B and T-2 toxin. *J Chromatogr A* 1133: 1–12.

Notingher I *et al.* (2004). Discrimination between ricin and sulphur mustard toxicity in vitro using Raman spectroscopy. *J R Soc Interface* 1: 79–90.

Ostin A *et al.* (2007). Solvent-assisted trypsin digestion of ricin for forensic identification by LC-ESI MS/MS. *Anal Chem* 79: 6271–6278.

Rifabutin

R

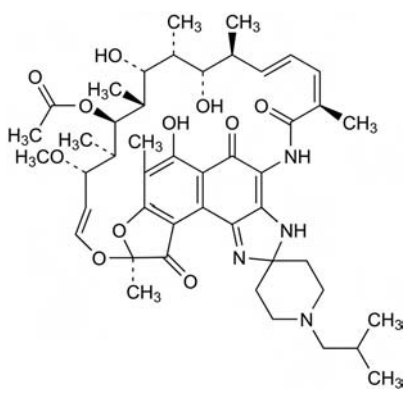
Antimycobacterial, Rifamycin Antibiotic

$C_{46}H_{62}N_4O_{11}$ = 847.0

CAS—72559-06-9

Synonyms Ansamicin; ansamycin; LM-427; rifabutine; (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,16,18,20-tetrahydroxy-1′-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca-[1,11,13]trienimino)-2*H*-furo-[2′,3′:7,8]naphth[1,2-d]imidazole-2,4′-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate.

Proprietary Names *Alfacid*; *Ansatipine*; *Mycobutin*.



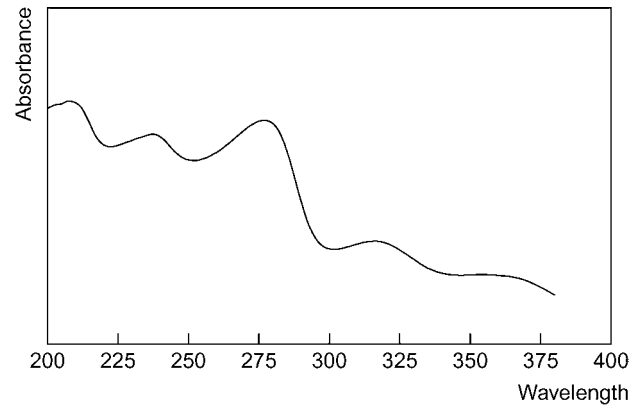
Chemical Properties A violet/red crystalline powder. Highly soluble in chloroform; soluble in methanol; sparingly soluble in ethanol; minimal solubility in water. Log *P* (*n*-octanol/water), 3.2.

High Performance Liquid Chromatography System HAA—retention time 17.6 min.

Column: RP C_8 Symmetry Shield (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: potassium dihydrogen phosphate buffer (0.05 mol/L, pH 4.10) (48.7:51.3), flow rate 1 mL/min. Internal standard (IS): medazepam. UV detection (λ=275 nm). Retention times: rifabutin, 4.5 min; IS, 6.4 min [Gatti *et al.* 1999].

Column: (analytical) ODS Ultrasphere (250 × 4.6 mm i.d., 5 µm); (guard) RP-18 Newguard (15 × 3.2 mm i.d.). Mobile phase: acetonitrile: monobasic potassium phosphate (0.05 mol/L, pH 4.2):TEA (38:61.5:0.5), flow rate 1 mL/min. IS: medazepam. UV detection (λ=275 nm). Retention times: rifabutin, 22 min; 25-deacetylrifabutin, 9 min; IS, 19 min [Lewis *et al.* 1991].

Ultraviolet Spectrum Aqueous acid—208, 237, 277, 326 nm.



Quantification

Plasma **HPLC** UV detection (λ=275 nm). Limit of quantification, 5 µg/L [Gatti *et al.* 1999]. UV detection (λ=275 nm). Limit of quantification, 5 µg/L for rifabutin and 2.5 µg/L for the metabolite, 25-deacetylrifabutin [Lewis *et al.* 1991]. UV detection (λ=275 nm). Limit of quantification, 30 µg/L [Skinner *et al.* 1989].

Urine **HPLC** UV detection (λ=275 nm). Limit of quantification, 100 µg/L for rifabutin and the metabolite, 25-deacetylrifabutin [Lewis *et al.* 1991].

Disposition in the Body The absorption of rifabutin from the gastro-intestinal tract is poor but rapid with peak concentrations (0.4 to 0.7 mg/L) observed at 2 to 4 h. Absorption is delayed by co-administration with food but the extent is not affected. Rifabutin undergoes extensive tissue distribution, especially with lung tissues (concentrations can be 5 to 10 times higher than those detected in plasma over 24 h). This drug induces hepatic enzymes and is thought to induce its own metabolism. It appears to have more than 20 metabolites, out of which 25-deacetylrifabutin (25-*o*-deacetyl-rifabutin-*N*-oxide) has antibacterial activity similar to the parent drug. Following administration, rifabutin and its main metabolite, 25-deacetylrifabutin, are excreted in urine and faeces (30%). 31-Hydroxy-rifabutin (another predominant metabolite), 32-hydroxy-rifabutin and 32-hydroxydeactyl-rifabutin have also been identified in urine.

Therapeutic Concentration The trough serum therapeutic concentration is 50 µg/L and peak, 150 µg/L.

Fifteen healthy male volunteers with a mean age of 24.7 years (19 to 39 years old) were either administered with 150 mg rifabutin solution in a fasted state, 150 mg capsule in a fasted state or 150 mg capsule after breakfast. The peak plasma concentrations for the fasted and fed volunteers were 156.2 and 187.9 µg/L, respectively for rifabutin, and 23.3 and 27.2 µg/L for the metabolite, 25-deacetylrifabutin. These concentrations were observed 5.4 and 3 h after administration, respectively. The peak concentration detected for the solution was higher than that observed for the capsule formulation; 237.5 µg/L compared with 187.9 µg/L for the drug and 34.5 versus 27.2 µg/L for the metabolite. These were observed at 2.5 and 3 h [Narang *et al.* 1992].

Toxicity

Dose-limiting toxicity was observed in 9 out of 10 patients (with AIDS-related complex) administered with doses greater than 1050 mg over 4 to 66 weeks. A reversible syndrome of arthritis was observed [Siegal *et al.* 1990].

Bioavailability 20%.

Half-life Mean, 45 h (range, 16 to 69 h).

Volume of Distribution 8 to 9 L/kg (in HIV-infected patients).

Clearance Systemic, healthy volunteers: mean, 0.69 L/h/kg (range, 0.46 to 1.34 L/h/kg).

Protein Binding 70 to 90%.

Dose Dose varies between 150 and 600 mg daily depending on symptoms and use, but the recommended dose is 300 mg once daily.

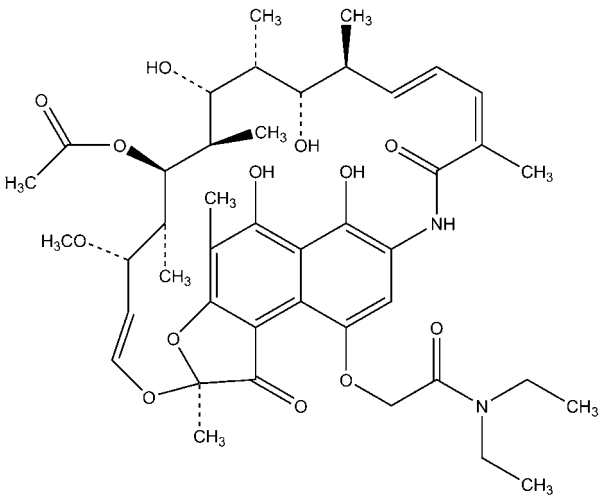
Gatti G *et al.* (1999). Specific high-performance liquid chromatography assay for determination of rifabutin plasma concentration following Extrelut column extraction. *J Chromatogr B Biomed Sci Appl* 728: 233–239.

Lewis RC *et al.* (1991). A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by high-performance liquid chromatography (HPLC). *Pharm Res* 8 (11): 1434–1440.

Narang PK *et al.* (1992). Rifabutin absorption in humans: relative bioavailability and food effect. *Clin Pharmacol Ther* 52: 335–341.
Siegal FP *et al.* (1990). Dose-limiting toxicity of rifabutin in AIDS-related complex: syndrome of arthralgia/arthritis. *AIDS* 4: 433–441.
Skinner MH *et al.* (1989). Pharmacokinetics of rifabutin. *Antimicrob Agents Chemother* 33: 1237–1241.

Rifamide

Antibiotic
$C_{43}H_{58}N_2O_{13}$ = 810.9
Synonyms Rifamycin B; *N,N*-diethylamide.
Proprietary Name *Rifamycin M-14*



Chemical Properties Orange-yellow crystalline powder that softens at 140° and melts completely at 170°, with decomposition. Soluble in methanol and aqueous alkaline solutions. Rifamide is extracted by chloroform from aqueous solutions.

Rifamide Sodium

Proprietary Name *Rifocin M*
Chemical Properties Orange-yellow powder. Soluble in 1 in 7 of water.

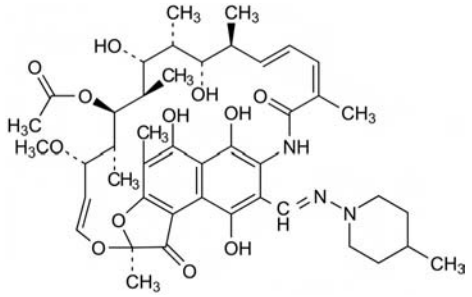
Colour Tests Ammonium molybdate test—(orange) orange-brown (limit of detection, 0.1 µg); ammonium vanadate test—(orange) dark brown (limit of detection, 1.0 µg); Vitali's test—(yellow) light brown/yellow-brown/orange-brown (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.86 (location reagent potassium permanganate spray, positive reaction).
Ultraviolet Spectrum Phosphate buffer (pH 7.38)—220 (E1%, 1 cm 528) and 302 nm (E1%, 1 cm 256).
Quantification A differential spectrophotometric method for the determination of rifamycin antibiotics in dosage forms and fermentation broths, based on the oxidation by sodium nitrite of the hydroquinone moiety of the compounds, is described by Pasqualucci *et al.* [1970]. Fürész *et al.* [1965] describe a microbiological assay procedure for the estimation of rifamide in blood.
Disposition in the Body Absorption of orally administered rifamide is irregular. Peak plasma concentrations of 0.1–0.2 mg% are obtained 30 to 60 min after an IM injection of 150 mg of rifamide; the half-life of the drug is 1–2 h. A 150 mg dose produces peak concentrations of 1–2 mg% in the urine. Urinary excretion accounts for ≈4 and 7% of 100 and 500 mg doses, respectively. The principal route of excretion, accounting for ≈80% of the dose, is in the bile, in which concentrations of up to 160 mg% may occur after the administration of 150 mg of rifamide [Fürész *et al.* 1965].
Toxicity LD_{50} in mice 2.45 and in rats >4.0 g/kg (oral).
Dose Up to 600 mg daily by IM injection.

Fürész S *et al.* (1965). Rifamycin B diethylamide (rifamide): activity in experimental infections, absorption and elimination in man. *Arzneimittelforschung* 15: 802–804.
Pasqualucci CR *et al.* (1970). Improved differential spectrophotometric determination of rifamycins. *J Pharm Sci* 59: 685–687.

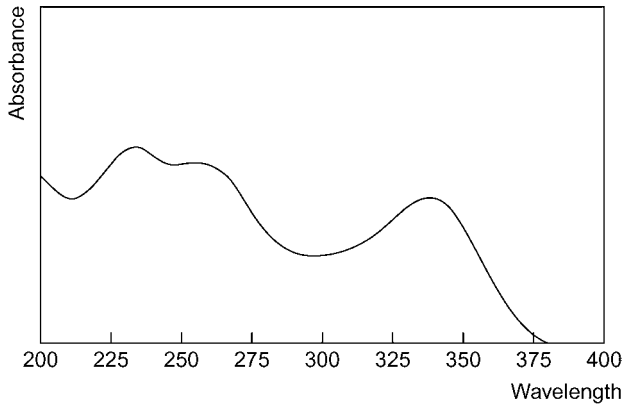
Rifampicin

Tuberculostatic
$C_{43}H_{58}N_4O_{12}$ = 822.9
CAS—13292-46-1

Synonyms 3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin; rifaldazine; rifampin; rifamycin AMP.
Proprietary Names *Eremfat*; *Fampin*; *Ramfin*; *Rifa*; *Rifadin(e)*; *Rifagen*; *Rifaldin*; *Rifapiam*; *Rifasynt*; *Rifcin*; *Rifex*; *Rifocina*; *Rifoldin(e)*; *Rimactan(e)*; *Rimapen*; *Rofact*. It is an ingredient of *Rifater*, *Rifinah* and *Rimactazid*.



Chemical Properties A brick-red to reddish-brown, crystalline powder. Mp 183° to 188°, with decomposition. Slightly soluble in water, ethanol, acetone and ether; freely soluble in chloroform; soluble in ethyl acetate and methanol. pK_a 1.7, 7.9. Log *P* (octanol/water), 4.2.
Colour Test Mandelin's test—orange-brown.
Thin-layer Chromatography System TA—R_f 0.79; system TAE—R_f 0.83. (Visible red-brown spot; acidified potassium permanganate solution, positive.)
High Performance Liquid Chromatography System HAA—retention time 16.2 min; system HY—RI 417.
Ultraviolet Spectrum Aqueous acid—231 (A^1_1=320a), 263, 336 nm (A^1_1=250a).



Infrared Spectrum Principal peaks at wavenumbers 1250, 1567, 976, 1098, 1064, 1650 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 45, 60, 58, 95, 99, 398, 206.
Quantification
Plasma **HPLC** Limit of detection, 0.5 mg/L [Conte *et al.* 2000]. UV detection. Limit of detection, 100 µg/L for rifampicin and metabolites [Lecaillon *et al.* 1978].
Serum **HPLC** Limit of detection, 17 and 10 µg/L for rifampicin and desacetylrifampicin, respectively [Guillaumont *et al.* 1982].
Urine **HPLC** UV detection. Limit of detection, 100 µg/L for rifampicin and metabolites [Lecaillon *et al.* 1978].
Bronchoalveolar Lavage **HPLC** Limit of detection, 0.015 mg/L [Conte *et al.* 2000].
Saliva **HPLC** UV detection. Limit of detection, 100 µg/L for rifampicin and metabolites [Lecaillon *et al.* 1978].
Alveolar Cells **HPLC** Limit of detection, 0.03 mg/L [Conte *et al.* 2000].
Disposition in the Body Rifampicin is readily absorbed after oral administration; peak plasma concentrations are attained in about 2 h after a single dose. It is widely distributed in the tissues and fluids, and undergoes enterohepatic circulation. It is metabolised to desacetylrifampicin, which is active and is excreted in the urine and bile.
Therapeutic Concentration
Fourteen subjects ingested single doses of 600 mg rifampicin, under fasting conditions and after a high-fat meal and with an aluminium–magnesium antacid. The fasting mean peak plasma concentration was 10.54 mg/L at 2.42 h; this was unaltered by the antacid (10.89 mg/L at 2.36 h) but was reduced and delayed by the high-fat meal (7.27 mg/L at 4.43 h) [Peloquin *et al.* 1999].
Half-life Plasma half-life, about 2 to 6 h (dose-dependent), decreased during chronic dosing; prolonged in subjects with liver disease.
Volume of Distribution About 1 L/kg.
Protein Binding About 80%.
Note For a review of the pharmacokinetics of rifampicin, see Acocella [1978].
Dose Usually 600 mg daily.

R

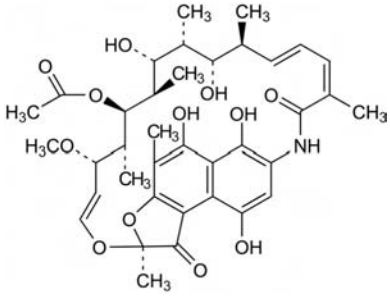
Acocella G (1978). Clinical pharmacokinetics of rifampicin. *Clin Pharmacokinet* 3: 108–127.
Conte JE *et al.* (2000). Liquid chromatographic determination of rifampin in human plasma, bronchoalveolar lavage fluid, and alveolar cells. *J Chromatogr Sci* 38: 72–76.
Guillaumont M *et al.* (1982). Determination of rifampicin, desacetylrifampicin, isoniazid and acetylisoniazid by high-performance liquid chromatography: application to human serum extracts, polymorphonucleocytes and alveolar macrophages. *J Chromatogr* 232: 369–376.
Lecaillon JB *et al.* (1978). Quantitative assay of rifampicin and three of its metabolites in human plasma, urine and saliva by high-performance liquid chromatography. *J Chromatogr* 145: 319–324.
Peloquin CA *et al.* (1999). Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. *Chest* 115: 12–18.

Rifamycin SV

Antibacterial
$C_{37}H_{47}NO_{12}$ = 697.8
CAS—6998-60-3

Synonyms 5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-2,7-(epoxypentadeca[1,11,13]trienimino)-naphtho[2,1-*b*]furan-1,11(2*H*)dione 21-acetate; rifamicine SV; rifomycin SV.
Chemical Properties A semi-synthetic antibiotic derived from rifamycin B, a substance produced during growth of certain strains of *Streptomyces mediterranei*. Dark yellow crystals. Mp 300°, decomposes at 140°. Practically insoluble in water; very soluble in ethanol, methanol, acetone and ethyl acetate; soluble in chloroform and ether. Log *P* (octanol/water), 5.0.

Rifamycin Sodium
$C_{37}H_{46}NNaO_{12}$ = 719.8
CAS—15105-92-7; 14897-39-3
Synonyms Rifamycin SV sodium; rifamastene.
Proprietary Names *Otofa*; *Rifocin(e)*; *Rifocina*; *Rifocyna*.



Chemical Properties A brick-red, fine or slightly granular powder. Soluble in water and chloroform; freely soluble in dehydrated alcohol and methanol; practically insoluble in ether.

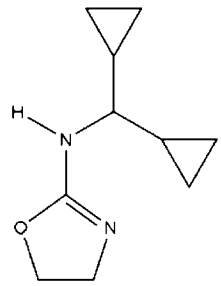
Colour Test Mandelin's test—yellow-brown.
Thin-layer Chromatography System TA—R_f 0.84. (Visible orange spot.)
High Performance Liquid Chromatography System HAA—retention time 20.9 min.
Ultraviolet Spectrum Ethanol—315 nm (A^1_1=420b).
Dose 0.5 to 1 g of rifamycin sodium daily, by IM injection.

R

Rilmenidene

Antihypertensive
$C_{10}H_{16}N_2O$ = 180.2
CAS—54187-04-1

IUPAC Name *N*-(Dicyclopropylmethyl)-4,5-dihydro-1,3-oxazol-2-amine
Synonyms 2-[(Dicyclopropylmethyl)amino]-2-oxazoline;oxaminozoline



Chemical Properties Crystals. Mp 106° to 107°.

Rilmenidene Phosphate
$C_{10}H_{16}N_2O,H_3PO_4$ = 278.2
CAS—85409-38-7
Synonyms Rilmenidine dihydrogen phosphate; rilmenidine hydrogen phosphate; S-3341-3.
Proprietary Names *Albarel*; *Hyperdix*; *Hyperium*; *Iterium*; *Tenaxum*.
Chemical Properties A white or almost white powder. Freely soluble in water; slightly soluble in alcohol; practically insoluble in dichloromethane. pK_a 9.3.

Rilmenidene Fumarate
$C_{10}H_{16}N_2O,½C_4H_4O_4$ = 238.3
CAS—207572-68-7
Synonym Rilmenidine hemifumarate

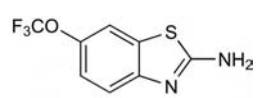
Quantification
Plasma **GC-MS** NICI, SIM acquisition mode. Limit of detection, 0.2 µg/L [Ung *et al.* 1987]. Negative ion detection (*m/z* 424). Limit of detection, ~1 pg [Ehrhardt 1985]. Negative ion detection (*m/z* 420). Limit of quantification, 2 µg/L, limit of detection, 0.1 µg/L [Murray *et al.* 1985].
Urine **GC-MS** See Plasma [Ehrhardt 1985; Ung *et al.* 1987].
Disposition in the Body Rilmenidene is rapidly and extensively absorbed, with peak plasma levels at ~2 h. It is mainly eliminated by renal excretion (two-thirds of total clearance), with 65% of rilmenidine excreted unchanged in urine. Some metabolites are excreted in urine; no metabolites detected in plasma [Genissel *et al.* 1988; Genissel, Bromet 1989].
Therapeutic Concentration
In patients, oral doses of rilmenidine 1 mg and 2 mg produced peak plasma concentrations of 3.3 µg/L at 1.8 h and 7.6 µg/L at 1.4 h, respectively [Ehrhardt 1985].
In healthy subjects, single doses of 2 oral formulations of rilmenidine phosphate 1.544 mg produced peak plasma levels of 3.73 and 3.97 µg/L, respectively, at 1.33 h [Groenewoud *et al.* 2009].
Half-life Elimination half-life, 6.7–8 h [Ehrhardt 1985]; prolonged by 50% in the elderly [Singlas *et al.* 1988].
Bioavailability Absolute bioavailability factor close to 1 [Genissel *et al.* 1988; Genissel, Bromet 1989].
Volume of Distribution 5 L/kg (315 L) [Genissel *et al.* 1988; Genissel, Bromet 1989]. Decreased by 12% in the elderly [Singlas *et al.* 1988].
Clearance Renal clearance 330 mL/min [Aparicio *et al.* 1994].
Protein Binding <10% [Genissel, Bromet 1989].
Dose The equivalent of rilmenidine 1 mg daily, increased, if necessary, after 1 month to 2 mg daily in divided doses. (Note. Rilmenidine phosphate 1.5 mg is equivalent to approx. 1 mg rilmenidine.)
Note For a clinical overview of rilmenidine, see Reid [2000].

Aparicio M *et al.* (1994). Pharmacokinetics of rilmenidine in patients with chronic renal insufficiency and in hemodialysis patients. *Am J Cardiol* 74: 43A–50A.
Ehrhardt JD (1985). Gas chromatographic negative ion mass spectrometric assay of 2-dicyclopropylmethylamino-2-oxazoline (*S*-3341), a new antihypertensive drug. *Biomed Mass Spectrom* 12: 593–595.
Genissel P, Bromet N (1989). Pharmacokinetics of rilmenidine. *Am J Med* 87: 18S–23S.
Genissel P *et al.* (1988). Pharmacokinetics of rilmenidine in healthy subjects. *Am J Cardiol* 61: 47D–53D.
Groenewoud G *et al.* (2009). Bioequivalence evaluation of rilmenidine in healthy volunteers. *Arzneimittelforschung* 59: 233–237.
Murray S *et al.* (1985). Bistrifluoromethylaryl derivatives for drug analysis by gas chromatography electron capture negative ion chemical ionization mass spectrometry. Application to the measurement of (*N*-dicyclopropylmethyl)amino-2-oxazoline in plasma. *Biomed Mass Spectrom* 12: 230–237.
Reid JL (2000). Rilmenidine: a clinical overview. *Am J Hypertens* 13: 106S–111S.
Singlas E *et al.* (1988). Pharmacokinetics of rilmenidine. *Am J Cardiol* 61: 54D–59D.
Ung HL *et al.* (1987). Quantitative analysis of S3341 in human plasma and urine by combined gas chromatography–negative ion chemical ionization mass spectrometry: 15 month inter-day precision and accuracy validation. *Biomed Environ Mass Spectrom* 14: 289–293.

Riluzole

Glutamatergic Antagonist, Neuroprotective
$C_8H_5F_3N_2OS$ = 234.2
CAS—1744-22-5

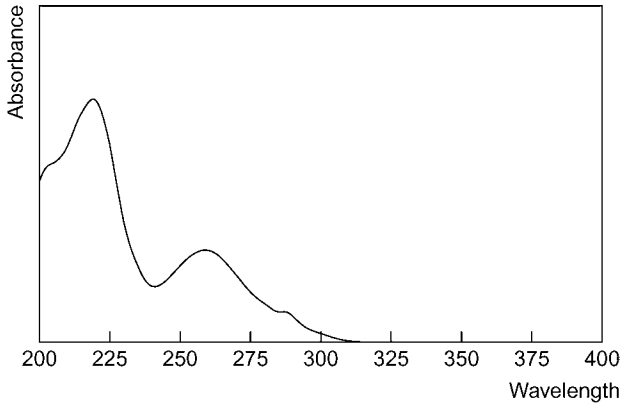
IUPAC Name 6-(Trifluoromethoxy)-1,3-benzothiazol-2-amine
Synonyms PK-26124; RP-54274; 6-(trifluoromethoxy)-2-benzothiazolamine.
Proprietary Name *Rilutek*



Chemical Properties A white to slightly yellow crystalline powder. Mp 119°. Very slightly soluble in water and 0.1 mol/L sodium hydroxide; very soluble in dimethylformamide and methanol; sparingly soluble in 0.1 mol/L hydrochloric acid.
High Performance Liquid Chromatography Column: (analytical) C_{18} µBondapak (300 × 4.6 mm i.d.); (guard) C_{18} µBondapak (39 × 4.6 mm i.d.). Mobile phase: acetonitrile: methanol: acetic acid: dipotassium hydrogen phosphate (10:55:1:35), flow rate 1 mL/min. Internal standard (IS): riluzole *N*-methyl

derivative. UV detection (λ=265 nm). Retention times: riluzole, 9.2 min; IS, 12.4 min [Le Liboux *et al.* 1997].

Ultraviolet Spectrum



Quantification

Blood **HPLC** UV detection (λ=265 nm). Limit of detection, 5 μg/L [Le Liboux *et al.* 1997].

Plasma **HPLC** MS–MS detection. Limit of quantification, 0.5 μg/L [Le Liboux *et al.* 1999].

Urine **HPLC** UV detection (λ=265 nm). Limit of detection, 10 μg/L [Le Liboux *et al.* 1997].

Disposition in the Body At least 90% of an administered dose is absorbed 1 to 1.5 h after an oral dose. Absorption is rapid from the gastro-intestinal tract following oral administration and metabolism occurs via hepatic hydroxylation and glucuronidation with several inactive metabolites. The rate and extent of absorption may be reduced when a high-fat meal is taken with the dose. The drug is widely and extensively distributed throughout the body. The main metabolites are three phenolic derivatives, one ureido derivative and unchanged riluzole. Excretion is mainly renal via urine and predominately as glucuronides. 2% of a dose is excreted unchanged in urine and a small amount in faeces. Decreased renal function will result in higher plasma concentrations. The drug displays linear pharmacokinetics, which are dose independent. Steady state is achieved in less than 5 days. The unchanged drug is the main component in plasma. There is no change in pharmacokinetics in the elderly or those with renal impairment.

Therapeutic Concentration

Sixteen healthy, young, white males (18 to 40 years) were administered with a single dose of 100 mg riluzole with or without a high-fat meal. The peak plasma concentrations were decreased when the drug was administered with food; 216 μg/L compared with 387 μg/L for those in the fasting state. These concentrations were observed at 2 h and 0.8 h, respectively.

Sixteen males were administered with a single IV dose of 50 mg and two single oral doses of 100 mg riluzole. The peak concentration for the oral dose was 318 μg/L at 1.1 h and 584 μg/L for the IV dose at 0.5 h.

Twenty-four males were administered with four incremental doses of 25, 75, 150 and 250 mg (group 1); 50, 100, 200 and 300 mg (group 2). The peak plasma concentrations observed were 91, 247, 557 and 889 μg/L at 1.0, 0.9, 1.1, 1.3 h (group 1) and 214, 428, 1117 and 1611 μg/L at 1.4, 1.4, 1.5 and 1.3 h, respectively for group 2.

In the final part of this study, 12 males were administered with single oral doses of 25, 50 and 100 mg riluzole and then multiple dosing twice daily for 10 days. For the single doses, the peak concentrations were 52, 180 and 282 μg/L at 1.1, 0.9 and 1.6 h for the 25, 50 and 100 mg doses. The multiple doses produced peak concentrations of 77, 173 and 357 μg/L at 0.8, 0.8 and 1.3 h, respectively [Le Liboux *et al.* 1997].

Bioavailability 60%.

Half-life 9 to 15 h.

Volume of Distribution 245 L (3.4 L/kg).

Clearance This can vary between individuals because CYP 1A2 has considerable inter-individual variability. Cigarette smoking and reduced hepatic function may reduce or delay clearance. Japanese patients clear riluzole 50% less efficiently than Caucasians. Possible genetic and environmental factors might be involved.

Protein Binding 97% (mainly to albumin and lipoproteins).

Dose 100 mg daily.

Le Liboux A *et al.* (1997). Single- and multiple-dose pharmacokinetics of riluzole in white subjects. *J Clin Pharmacol* 37(9): 820–827.

Le Liboux A *et al.* (1999). A comparison of the pharmacokinetics and tolerability of riluzole after repeat dose administration in healthy elderly and young volunteers. *J Clin Pharmacol* 39: 480–486.

Rimantadine

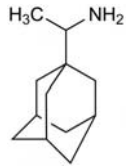
Antiviral

$C_{12}H_{21}N = 179.3$

CAS—13392-28-4

IUPAC Name 1-(1-Adamantyl)ethanamine

Synonyms α-Methyl-1-adamantanemethylamine; α-methyltricyclo[3.3.1.1^{3,7}]decane-1-methenamine.



Rimantadine Hydrochloride

$C_{12}H_{21}N,HCl = 215.8$

CAS—1501-84-4

Synonym EXP-126

Proprietary Names *Flumadine*; *Meradan(e)*; *Roflual.*

Chemical Properties A white crystalline powder. Mp 373° to 375° (sealed tube). Soluble 1 in 17 of water, 1 in 20 of ethanol and 1 in 6 of chloroform.

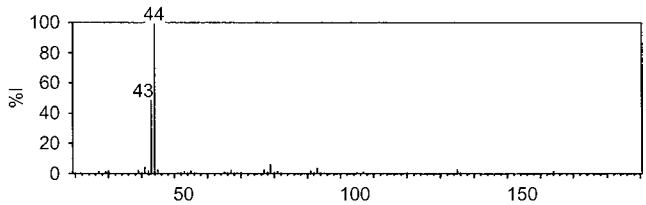
Colour Test Ninhydrin—pink-violet.

Thin-layer Chromatography System TA—R_f 0.37; system TL—R_f 0.35; system TB—R_f 0.50; system TC—R_f 0.11 (acidified iodoplatinate solution, strong reaction).

Gas Chromatography System GA—RI 1388.

Infrared Spectrum Principal peaks at wavenumbers 1538, 1600, 1266, 1277, 1160, 1117 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 44, 43, 79, 93, 41, 135, 91, 81.



Note For a review of the clinical use of rimantadine, see Wintermeyer and Nahata [1995].

Dose 300 to 400 mg of rimantadine hydrochloride daily.

Wintermeyer SM, Nahata MC (1995). Rimantadine: a clinical perspective. *Ann Pharmacother* 29: 299–310.

Rimiterol

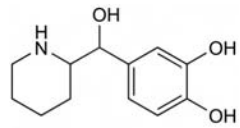
Bronchodilator

$C_{12}H_{17}NO_3 = 223.3$

CAS—32953-89-2

IUPAC Name 4-[(*S*)-Hydroxy-[(2*R*)-piperidin-2-yl]methyl]benzene-1,2-diol

Synonym 4-(Hydroxy-2-piperidinylmethyl)-1,2-benzenediol



Chemical Properties Crystals. Mp 203° to 204°. pK_a 8.7, 10.3 (25°). Log *P* (octanol/water), 0.6.

Rimiterol Hydrobromide

$C_{12}H_{17}NO_3,HBr = 304.2$

CAS—31842-61-2

Synonyms R-798; WG-253.

Proprietary Name *Pulmadil*

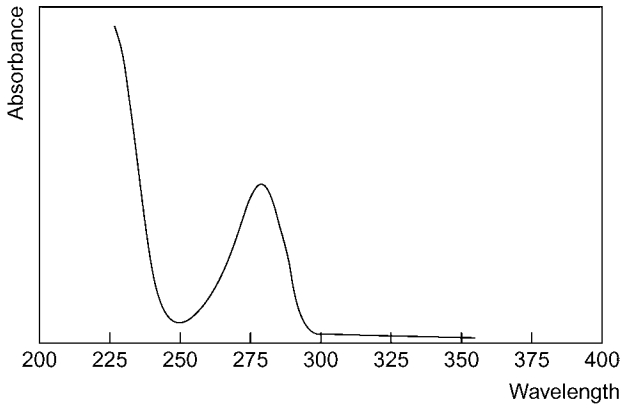
Chemical Properties A white or pale grey, crystalline powder. Mp about 220°, with decomposition. Soluble 1 in 10 of water and 1 in 20 of methanol.

Colour Tests Ammoniacal silver nitrate—red/brown; ferric chloride—green; Liebermann's reagent—black; Marquis test—brown→black; methanolic potassium hydroxide—orange-pink; Nessler's reagent—black; palladium chloride—orange; potassium dichromate—green→brown; sulfuric acid—yellow.

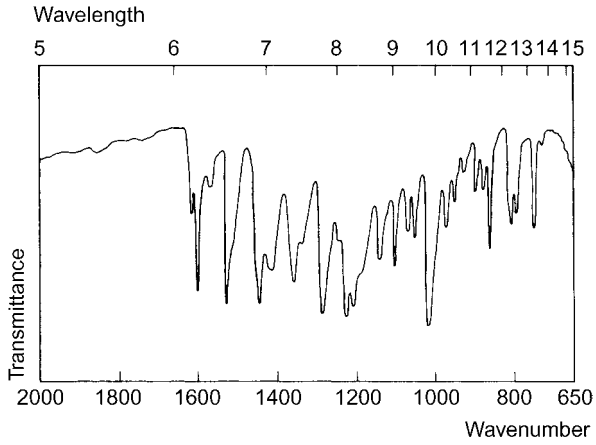
Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.06; system TAE—R_f 0.07.

High Performance Liquid Chromatography System HX—RI 150.

Ultraviolet Spectrum Aqueous acid—280 nm (A^1_1=131a).



Infrared Spectrum Principal peaks at wavenumbers 1020, 1230, 1290, 1210, 1530, 1605 cm^{-1} (rimiterol hydrobromide, KCl disk).



Disposition in the Body Rimiterol is rapidly absorbed from the lungs, but most of an inhaled dose is swallowed; it is readily absorbed from the gastrointestinal tract. It is subject not only to extensive first-pass metabolism by sulfate and glucuronide conjugation, but also to metabolism by catechol-*O*-methyltransferase (COMT), forming 3-*O*-methylrimiterol. It also appears to be metabolised by COMT in the lungs. After intrabronchial administration, about 70% of a dose is excreted in the urine in 24 h, mainly as free and conjugated 3-*O*-methylrimiterol, together with some free and conjugated rimiterol. A proportion of a dose is excreted in the bile. After oral or aerosol administration, <50% of a dose is excreted in the urine, mainly as conjugates of rimiterol.

Half-life Plasma half-life, about 3 min after IV administration.

Dose 200 to 600 µg of rimiterol hydrobromide, by aerosol inhalation; maximum of 8 doses daily.

R

Risedronic Acid

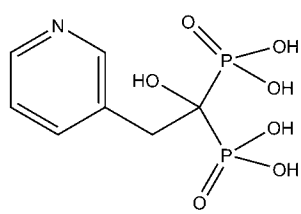
Aminobiphosphonate, Bone Resorption Inhibitor

$C_7H_{11}NO_7P_2$ = 283.1

CAS—105462-24-6

IUPAC Name (1-Hydroxy-1-phosphono-2-pyridin-3-ylethyl)phosphonic acid

Synonym [1-Hydroxy-2-(3-pyridinyl)ethylidene]diphosphonic acid



Risedronate Sodium

$C_7H_{10}NNaO_7P_2$ = 305.1

CAS—115436-72-1

IUPAC Name Sodium hydroxy-(1-hydroxy-1-phosphono-2-pyridin-3-ylethyl) phosphinate

Synonyms Monosodium risedronate; NE-58095.

Proprietary Names *Actonel*; *Benet*; *Ductonar*; *Optinate*; *Rentop*; *Ribastamin*; *Ridron*; *Risedon*; *Risofos*; *Seralis*. It is also an ingredient in *Actonel Combi*; *Ribastamin Duo*; *Ridron Pack*.

Chemical Properties Fine, white to off-white, ordourless crystalline powder. Occurs as the hemipentahydrate. Soluble in water. Essentially insoluble in common organic solvents [O'Neil *et al.* 2006]. Log *P* (octanol/water), −3.6 [Wishart 2006].

Quantification

Serum **LC-MS** Column: Zorbax 300-SCX (50 × 3.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 2.5; 75 : 25), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.2 µg/L for the diazomethane derivative [Zhu *et al.* 2006].

Urine **HPLC** Column: Synergi Polar RP (150 × 4.6 mm i.d., 4 µm). Mobile phase: 5 mmol/L 1-octyltriethylammonium phosphate in 11 mmol/L sodium phosphate : 1.1 mmol/L etidronate in acetonitrile (pH 6.25, 87 : 13), flow rate 1.0 mL/min. UV detection (λ= 262 nm). Retention time: 18.5 min. Limit of quantification, 7.5 µg/L [Vallano *et al.* 2003].

LC-MS See Serum [Zhu *et al.* 2006].

Other **HPLC** Tablets. Column: Hypersil BDS C_{18} (250 × 4.6 mm i.d.). Mobile phase: 5 mmol/L tetrabutylammonium hydroxide–5 mmol/L sodium pyrophosphate (pH 7.0) : acetonitrile (78 : 22), flow rate 1.0 mL/min. UV detection (λ= 262 nm). Retention time: 6.0 min. Limit of quantification, 1.61 mg/L, limit of detection, 0.48 mg/L [Kyriakides, Panderi 2007]. Rat Plasma. Column: C_{18} (150 × 3.0 mm i.d., 3 µm). Mobile phase: 33 mmol/L ammonium dihydrogen phosphate-17 mmol/L diammonium hydrogen phosphate-0.32 mmol/L EDTA-7.3 mmol/L trimethyl(tetradecyl)ammonium bromide : acetonitrile (72 : 28), flow rate 0.5 mL/min. UV detection (λ= 263 nm). Limit of quantification not reported [Sakuma *et al.* 2007].

Disposition in the Body Like other bisphosphonates, risedronate is poorly absorbed after oral administration. Absorption is reduced by food, especially by products containing calcium or other polyvalent cations. Risedronate is not metabolised. Approximately half of the absorbed portion is excreted in the urine within 24 h; the remainder is sequestered to bone for a prolonged period. Unabsorbed drug is excreted unchanged in the faeces.

Therapeutic Concentration

A group of 30 healthy volunteers was administered 30 mg risedronate, as a film-coated tablet or aqueous solution, or 0.3 mg risedronate IV. Mean peak serum concentrations (C_{max}) were as follows:

	Tablet	*Solution*	*Intravenous*
Dose-adjusted C_{max} (µg/L)	0.16	0.14	50
Time (h)	1.03	1.23	0.99

[Mitchell *et al.* 2001].

Four groups of healthy volunteers were administered a single dose of 30 mg risedronate 4 h before lunch (group 1), 1 h before breakfast (group 2), 0.5 h before breakfast (group 3), or 2 h after dinner (group 4). Mean peak serum concentrations were as follows:

	Group 1	*Group 2*	*Group 3*	*Group 4*
C_{max} (µg/L)	3.93	3.38	2.68	0.97
Time (h)	0.58	0.38	0.31	1.64

[Mitchell *et al.* 1999].

A group of 8 healthy male volunteers was administered risedronate solution (40 mg in 30 mL of water) directly into the stomach, the second part of the duodenum, or the terminal ileum via a nasoenteral tube. Mean serum concentrations were as follows:

	Stomach	*Duodenum*	*Terminal ileum*
C_{max} (µg/L)	13.9	13.3	21.3
Time (h)	1.01	0.69	0.63

[Mitchell *et al.* 1998].

In a separate study, 8 healthy volunteers were administered 40 mg risedronate solution as a rapid (over 1 min) or slow (over 1 h) infusion into the duodenum. Peak serum concentrations for the rapid and slow infusions were 14.2 and 10.7 µg/L, reached after 0.78 and 1.84 h, respectively [Mitchell *et al.* 1998].

A total of 67 healthy volunteers were administered a single oral dose of risedronate as a 2.5, 5, or 30 mg film-coated tablet. Subjects fasted for

10 h pre-dose and for 4 h post-dose. Mean peak serum concentrations for the 3 doses were as follows:

Dose	*2.5 mg*	*5.0 mg*	*30 mg*
C_{max} (μg/L)	0.41	0.94	5.1
Time (h)	0.87	0.87	0.81

[Mitchell *et al.* 2000].

Toxicity Risedronate is not known to induce or inhibit hepatic CYP450 enzymes.
Bioavailability Approximately 0.63% in the fasting state and reduced by 30% when given 1 h before breakfast, and by 55% when given 0.5 h before breakfast.
Half-life Approximately 200 h, also reported as 480 h. The long half-life values reflect the gradual release of the drug from bone surfaces.
Volume of Distribution Aproximately 6.3 L/kg at steady state.
Clearance 0.092 L/h/kg.
Protein Binding Approximately 24%.
Note For a review of risedronate use in resorptive bone disease, see Dunn and Goa [2001].
Dose The recommended dosage for Paget's disease of bone is 30 mg risedronate sodium once daily for 2 months. The recommended dosage for the treatment or prevention of postmenopausal or corticosteroid-induced osteoporosis is 5 mg daily. Alternatively, for postmenopausal osteoporosis, 35 mg once weekly may be given, or 75 mg may be taken on 2 consecutive days of each month. For men with osteoporosis, the recommended dose is 35 mg once weekly.

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Sakuma S *et al.* (2007). Effect of administration site in the gastrointestinal tract on bioavailability of poorly absorbed drugs taken after a meal. *J Control Release* 118: 59–64.
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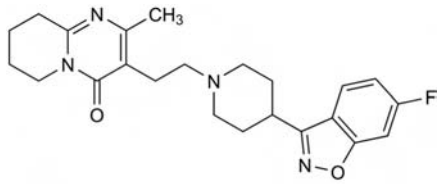
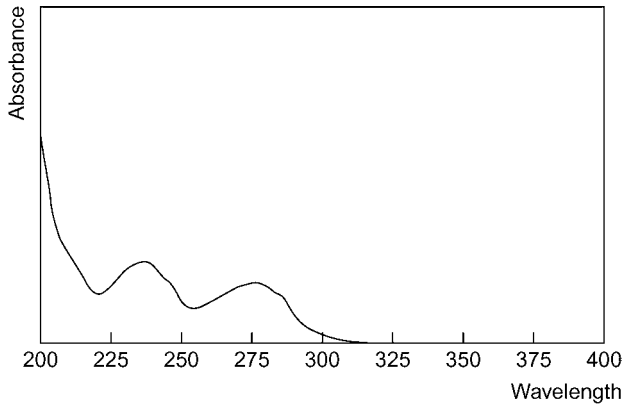
Risperidone

Atypical Antipsychotic
$C_{23}H_{27}FN_4O_2 = 410.5$
CAS—106266-06-2

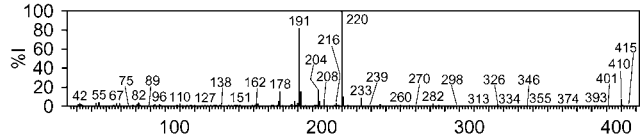
IUPAC Name 3-[2-[4-(6-Fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydropyrido[1,2-*a*]pyrimdidn-4-one.
Synonym 3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, R-64766.
Proprietary Names *Belivon*; *Risperdal*; *Rispolin*.



Chemical Properties White or almost white powder. Mp 170°. Practically insoluble in water; sparingly soluble in alcohol; freely soluble in dichloromethane and dilute acid solutions. pK_{a1} 8.24, pK_{a2} 3.11 [Woestenborghs *et al.* 1992]. Log *P* (octanol/water), 3.04 [Woestenborghs *et al.* 1992], 3.49. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Plasma samples stable for up to 11 months at –20° and at room temperature for up to 72 h, and methanolic stock solutions can be stored for 6 months at –20° for 1 month at 4°. No racemisation was observed for (–)-9-OH-risperidone and (+)-9-OH-risperidone in plasma samples kept at –20° for 6 months. Plasma samples were stable for 24 h in the autosampler at 5° [Locatelli *et al.* 2009]. Processed serum samples were stable at room temperature for at least 24 h and over 3 freeze-thaw cycles [Huang *et al.* 2008]. Stable in plasma for at least 6 h at room temperature and 24 h in the autosampler at 10°. Repeated freeze-thaw had no effect. Stable in plasma at –20° for at least 57 days [Bhatt *et al.* 2006]. Stable in plasma and saliva at ambient temperature, –20° and –80° for 3 weeks and over 6 freeze-thaw cycles. Standard solutions were stable for 1 week at ambient temperature [Flarakos *et al.* 2004]. Standard solutions left on the benchtop were stable for 24 h. Plasma samples were stable through 2 freeze-thaw cycles and for 490 days at –20° [Moody *et al.* 2004].
High Performance Liquid Chromatography System HAX—RT 9.1 min; system HAY—RT 4.6 min; system HZ—RT 3.1 min; RT 2.8 min (9-hydroxyrisperidone)
Ultraviolet Spectrum Aqueous acid (0.025 mol/L sulfuric acid)—238.5, 274.5 nm; aqueous acid (0.1 mol/L $HCOONH_4$, pH 3)—277 nm; aqueous acid (pH 2.38)—194, 236, 270 nm; ethanol—237, 279, 285 nm.



Mass Spectrum Principal ions at *m/z* 220, 191, 204, 178, 192, 221, 233, 410.



Quantification

Blood **HPLC** Column: Silica. Mobile phase: acetonitrile : methanol: iso-PrOH: ammonium hydroxide (84 : 12 : 3.6 : 0.4), flow rate 2 mL/min. UV detection (λ= 280 nm). Limit of detection not reported [Springfield, Bodiford 1996].

LC-MS Column: Zorbax Stable Bond Cyano (50 × 2.1 mm i.d., 3.5 μm). Mobile phase: methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 2 : 8 : 90) : methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 24 : 36 : 40, 80 : 20 to 20 : 80 in 4.5 min for 2.5 min to 80 : 20 for 2.5 min). TIS, MRM acquisition mode. Retention time: 4.0 min. Limit of detection, 0.8 μg/L [Roman *et al.* 2008].

Plasma **HPLC** Column: Prevail C_{18} (150 × 3 mm i.d. 5 μm). Mobile phase: 0.05 mol/L dipotassium hydrogen orthophosphate and 0.3% triethylamine (pH 3.7), flow rate 0.6 mL/min. UV detection (λ= 280 nm). Retention time: 3.7 min. Limit of quantification, 5 μg/L, limit of detection, 1.5 μg/L [Jones *et al.* 2009]. Column: Chiral-AGP (100 × 4.0 mm i.d., 5 μm). Mobile phase: 0.1 mol/L phosphate buffer (pH 6.2) : methanol (85 : 15), flow rate 0.8 mL/min for 4 min to 0.9 mL/min between 4.0 and 11.0 min. Electrochemical detection. Limit of detection, 0.5 μg/L [Locatelli *et al.* 2009]. ResElut C_8 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 34 mmol/L phosphate buffer containing 11.5 mmol/L triethylamine (pH 3.0) : acetonitrile (73 : 27, pH 3.5), flow rate 1.0 mL/min. DAD (λ= 240 nm). Retention time: 3.8 min. Limit of quantification, 4 μg/L, limit of detection, 2 μg/L [Raggi *et al.* 2005]. Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 3 μm). Mobile phase: acetonitrile : water (28 : 72) containing 5.44 g/L potassium dihydrogen phosphate and 400 μL DMOA, flow rate 0.8 mL/min. UV detection (λ= 278 nm). Retention time: 5.0 min. Limit of detection, 10 nmol/L [LLerena *et al.* 2003]. Column: Ultrasphere cyano (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.04 mol/L ammonium acetate buffer (pH 6.8) : methanol : acetonitrile (10 : 8 : 82), flow rate 1.5 mL/min. Internal standard (IS): remoxipride. Electrochemical detection. RT: risperidone, 14 min; IS, 17 min. Limit of quantification, 0.1 μg/L. [Aravagiri *et al.* 1993].

See also Le Moing *et al.* [1993] and Woestenborghs *et al.* [1992].

LC-MS Column: Chiralcel OJ (50 × 4.6 mm, 10 μm). Mobile phase: hexane : 0.01 mol/L ammonium acetate in isopropanol : 0.01 mol/L ammonium acetate in ethanol (80 : 10 : 10 for 7 min to 10 : 45 : 45 until 10.6 min to 80 : 10 : 10 until 13.5 min), flow rate 1.5 mL/min. TIS, MRM acquisition mode. Limit of quantification, 0.2 μg/L [De Meulder *et al.* 2008]. Column: Alltima-C_{18} (100 × 2.1 mm i.d., 3.0 μm). Mobile phase: 0.1% formic acid : acetonitrile (40 : 60), flow rate 0.2 mL/min. ESI, SRM acquisition mode, positive ion mode. Limit of quantification, 0.25 μg/L [Huang *et al.* 2008]. Column: Chiralcel OJ (50 × 4.6 mm i.d., 10 μm). Mobile phase: hexane : 0.01 mol/L ammonium acetate in isopropanol (pH 9.0) : 0.01 mol/L ammonium acetate in ethanol (pH 9.0, 80 : 10 : 10 for 7 min

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to 10 : 45 : 45), flow rate 1.0 mL/min. MRM acquisition mode. Limit of quantification, 0.2 μg/L [Aman *et al.* 2007]. Column: Chiralcel OJ (50 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate in ethanol-propanol (50 : 50) : hexane (20 : 80 to 90 : 10 over 4.5 min for 2 min to 20 : 80 at 6.6 min for 1.5 min), flow rate 1.0 min. MRM acquisition mode. Limit of quantification, 0.2 μg/L [Cabovska *et al.* 2007]. Column: Betasil C_{18} (100 × 3 mm i.d., 3 μm). Mobile phase: 10 mmol/L ammonium acetate (pH 4.6) : acetonitrile (40 : 60), flow rate 0.3 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.1 μg/L [Bhatt *et al.* 2006].

See also Flarakos *et al.* [2004] Kratzsch *et al.* [2004], Moody *et al.* [2004], Zhou *et al.* [2004], Remmerie *et al.* [2003].

Serum **HPLC** Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 25 mmol/L potassium dihydrogenphosphate (pH 7.0) : acetonitrile (60 : 40), flow rate, 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 11.1 min. Limit of detection not reported [Frahnert *et al.* 2003]. Column: LiChrochart (250 × 4.6 mm i.d.). Mobile phase: 40 mmol/L ammonium acetate buffer (pH 7.0) : methanol (100 : 900), flow rate 1.0 mL/min. UV detection (λ = 280 nm). Retention time: ≈4.5 min. Limit of quantification, 1.2 μg/L [Olesen, Linnet 1997]. Column: Microsorb-MV (10 cm × 4.6 mm i.d., 3 μm). Mobile phase: 50 mmol/L sodium phosphate (40 mmol/L monobasic and 10 mmol/L dibasic) : methanol : acetonitrile (pH 6.5, 67 : 28 : 5), flow rate 0.8 mL/min. Electrochemical detection. Retention time: 13.3 min. Limit of detection, 0.5 μg/L [Price, Hoffman 1997].

LC-MS Column: Phenomenex Luna cyano (50 × 2 mm i.d., 5 μm). Mobile phase: 0.1% formic acid : acetonitrile-2-propanol (4 : 1, 100 : 0 for 1 min to 5 : 95 for 2 min to 100 : 0 for 6 min), flow rate 300 μL/min. TIS. Limit of quantification, 0.2 μg/L, limit of detection, 0.05 μg/L [Lostia *et al.* 2009]. Column: C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.67 μg/L [Kirchherr, Kühn-Velten 2006].

Urine **HPLC** See Plasma [Woestenborghs *et al.* 1992].

LC-MS See Plasma. Limit of quantification, 1 μg/L [De Meulder *et al.* 2008].

Saliva **LC-MS** See Plasma [Aman *et al.* 2007]. [Doherty *et al.* 2007]. See Plasma [Flarakos *et al.* 2004].

Hair **LC-MS** Column: XTerra MS C_{18} (150 × 3.9 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium formate buffer (pH 3.8) : acetonitrile (70 : 30 for 0.5 min to 5 : 95 at 4 min for 4 min to 70 : 30 at 8.25 min for 0.25 min), flow rate 0.4 mL/min. ESI, positive ion mode. Limit of quantification, 1.8 pg/mg, limit of detection, 0.9 pg/mg [Schneider *et al.* 2009]. See Saliva [Doherty *et al.* 2007]. Column: Luna C_{18} (15 cm × 4.6 mm i.d.). Mobile phase: 0.02 mol/L ammonium acetate and 0.1% acetic acid in water : acetonitrile (35 : 65), flow rate 0.5 mL/min. ESI. Limit of detection, 20 nmol/L [McClean *et al.* 2000].

Note For a bioassay, sensitivity 0.1–0.2 μg/L, see [Reid, Wilson, 1990].

Disposition in the Body Risperidone is well absorbed after oral administration with peak plasma concentrations reached within 1–2 h. It undergoes metabolism by hydroxylation and oxidative *N*-dealkylation to 9-hydroxyrisperidone, which is active. Other metabolites include 7-hydroxy-risperidone (an acid derivative of the drug and metabolite), as well as glucuronides. The hydroxylation process is mediated by the cytochrome P450 isoenzyme CYP2D6 [Berecz *et al.* 2004] which is subject to genetic polymorphism, but the effect of this on the pharmacokinetics of risperidone and 9-hydroxyrisperidone is not thought to be clinically significant because the absolute bioavailability of the active (antipsychotic) components remains unaffected [Mihara *et al.* 2003]. The drug and metabolites are extensively distributed within the body. Excretion is via urine (70%), mainly as the metabolite, and faeces (15%) over 7 days following a dose.

Therapeutic Concentration The serum therapeutic concentration range is between 10 and 90 μg/L and the total sum of parent drug and its metabolite, hydroxyrisperidone, 10–100 μg/L.

Eighteen healthy male volunteers administered a single oral dose of 2 mg risperidone reached a mean peak plasma concentration of ≈17 μg/L at ≈1.3 h [Huang *et al.* 2008].

Twelve healthy males (mean age, 24 years; range, 19–41 years) were treated, on 3 separate occasions, with 1 mg risperidone as an IV infusion (over a 30 min period), as an IM injection or orally. At least 2 weeks were allowed between treatments. The individuals were fasted overnight for at least 10 h and for an additional 2 h after dosing. After the oral dose, the peak plasma drug concentrations were 7.90 μg/L for the extensive metabolisers (9 subjects); 4.97 μg/L for the intermediate metaboliser, and 20.8 and 12.1 μg/L for the poor metabolisers (2 subjects). These concentrations were observed at 0.8, 3.0, 1.0 and 1.5 h, respectively. After the IM injection, peak concentrations were 13.9, 7.54, and 28.1 and 18.4 μg/L for the extensive, intermediate and poor metabolisers, respectively observed at 0.35, 0.13, and 0.5 and 0.13 h. Peak concentrations for the metabolite, 9-hydroxy-risperidone, were 6.5, 1.61, and 0.85 and 1.05 μg/L for the extensive, intermediate and poor metabolisers observed at 3.2, 8.0, and 48 and 24 h, respectively, after the oral dose. For the IM dose, the peak metabolite concentrations were 5.0, 1.3, and 1.8 and 0.86 μg/L at 6.9, 5.0, and 8.0 and 16.0 h, respectively for the different metabolisers [Huang *et al.* 1993].

Toxicity

A 45-year-old male was found dead with several empty bottles of risperidone by his side. Postmortem examination revealed the following risperidone concentrations: blood, 1.8 mg/L; urine, 14.4 mg/L and gastric contents, 34.6 mg/L. The 9-hydroxy-risperidone metabolite was not detected in the blood or gastric contents, however, the urine concentration was found to be 17.8 mg/L [Springfield, Bodiford 1996].

Note For a study of postmortem redistribution of risperidone see Rodda, Drummer [2006].

Half-life Approximately 19 h (active fraction). Also reported as extensive metabolisers: risperidone $t_{1/2\beta}$ 2.8 h, 9-hydroxyrisperidone $t_{1/2\beta}$ 20.5 h. Poor metabolisers: risperidone 16 h. Both groups, total active moiety 24 h. Increases in the elderly and those with moderate to severe renal impairment.

Bioavailability 66% (extensive metabolisers), 82% (slow metabolisers).

Volume of Distribution 1.1 L/kg (extensive metabolisers); 2.1 L/kg (intermediate); 0.7 L/kg (poor).

Clearance Total plasma, 394 mL/min (extensive metabolisers); 192 (intermediate); 54 mL/min (poor metabolisers).

Protein Binding Risperidone, 88–90%; 9-hydroxyrisperidone, 77%.

Dose Day 1: 2 mg; day 2: 4 mg. The usual dose ranges between 4 and 6 mg daily with a maximum of 16 g. In the elderly and those with hepatic or renal impairment: 500 μg twice daily increased in 500 μg steps to 1–2 mg daily.

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Remmerie BM *et al.* (2003). Validated method for the determination of risperidone and 9-hydroxyrisperidone in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 783: 461–472.

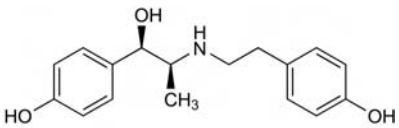
Rodda KE, Drummer OH (2006). The redistribution of selected psychiatric drugs in post-mortem cases. *Forensic Sci Int* 164: 235–239.

Roman M *et al.* (2008). Quantitation of seven low-dosage antipsychotic drugs in human postmortem blood using LC-MS-MS. *J Anal Toxicol* 32: 147–155.
Schneider S *et al.* (2009). Time resolved analysis of risperidone and 9-hydroxy-risperidone in hair using LC/MS-MS. *J Chromatogr B* 877: 2589–2592.
Springfield AC, Bodiford E (1996). An overdose of risperidone. *J Anal Toxicol* 20: 202–203.
Woestenborghs R *et al.* (1992). Determination of risperidone and 9-hydroxyrisperidone in plasma, urine and animal tissues by high-performance liquid chromatography. *J Chromatogr* 583: 223–230.
Zhou Z *et al.* (2004). Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 802: 257–262.

Ritodrine

Sympathomimetic
$C_{17}H_{21}NO_3$ = 287.4
CAS—26652-09-5

IUPAC Name 4-[2-[[(1*R*,2*S*)-1-hydroxy-1-(4-hydroxyphenyl)propan-2-yl]amino]ethyl]phenol
Synonym (α*S*)-*rel*-4-Hydroxy-α-[(1*R*)-1-[[2-(4-hydroxyphenyl)ethyl]amino]-ethyl]benzenemethanol

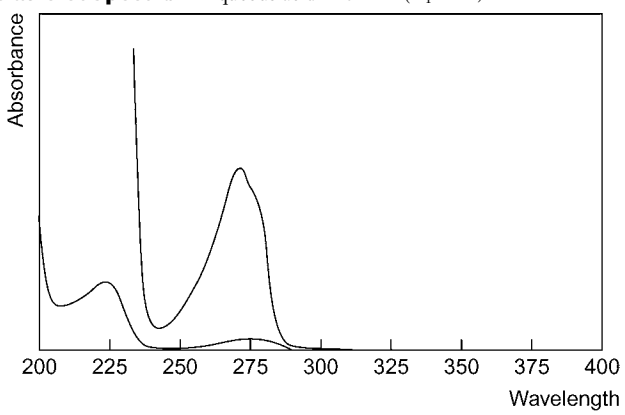


Chemical Properties Mp 88° to 90°.

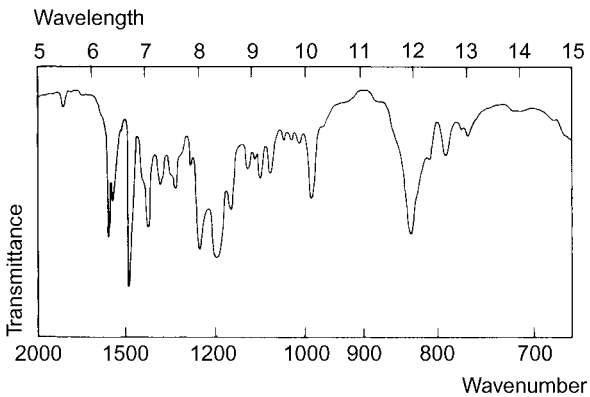
Ritodrine Hydrochloride

$C_{17}H_{21}NO_3$,HCl = 323.8
CAS—23239-51-2
Synonym DU-21220
Proprietary Names *Miodrina*; *Miolene*; *Prempar*; *Pre-Par*; *Utemerin*; *Utopar*; *Yutopar*.
Chemical Properties A white crystalline powder. Mp 193° to 195°, with decomposition. Soluble in chloroform and dilute acetic acid.

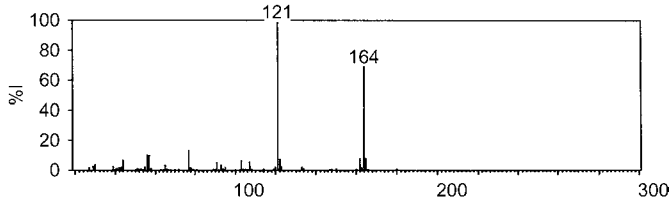
Colour Tests Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—green; Marquis test—yellow-brown.
Thin-layer Chromatography System TA—R_f 0.73 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
Ultraviolet Spectrum Aqueous acid—274 nm (A^1_1=98a).



Infrared Spectrum Principal peaks at wavenumbers 1510, 1209, 1256, 1610, 833, 1170 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 121, 164, 77, 57, 56, 165, 162, 122.



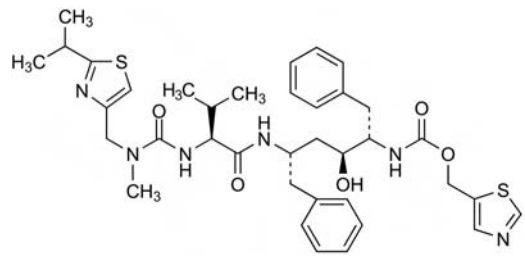
Quantification
Plasma **RIA** Limit of detection, 300 ng/L [Gandar *et al.* 1980].
Serum **HPLC** Electrochemical detection. Limit of detection, 2 µg/L [Lin *et al.* 1984].
RIA Limit of detection, 300 ng/L [Gandar *et al.* 1980].
Disposition in the Body Ritodrine is readily absorbed after oral administration but subject to considerable first-pass metabolism. About 70–90% of an oral dose is excreted in the urine in 24 h, mainly as sulfate and glucuronide conjugates with about 5% of the dose as unchanged drug.
Therapeutic Concentration
Following oral and IM administration of 10 mg to 4 subjects, mean peak serum concentrations of 0.01 and 0.02 mg/L were attained in 20–40 min and 10 min, respectively [Gandar *et al.* 1980].
Intravenous ritodrine hydrochloride, administered at a dose of 50 µg/min and increased by 50 µg/min every 30 min, if necessary, to a maximum of 350 µg/min, produced average plasma ritodrine levels of 27.8–113.3 µg/L in patients undergoing tocolytic therapy, whereas levels fell to 9.8–13.8 µg/L upon switching to oral therapy at a dose of 60–120 mg per 24 h [Schiff *et al.* 1993].
Bioavailability About 30%.
Half-life Plasma half-life, about 1 to 2 h; a longer elimination half-life of about 15–20 h has also been reported.
Protein Binding About 32%.
Note For a review of ritodrine, see Finklestein [1981].
Dose 50 to 350 µg/min of ritodrine hydrochloride by IV infusion; up to 120 mg daily, orally.

Finklestein BW (1981). *Drug Intell Clin Pharm* 15: 425–433.
Gandar R *et al.* (1980). Serum level of ritodrine in man. *Eur J Clin Pharmacol* 17: 117–122.
Lin LS *et al.* (1984). Analysis of ritodrine in serum by high-performance liquid chromatography with electrochemical detection. *J Pharm Sci* 73: 131–133.
Schiff E *et al.* (1993). Currently recommended oral regimens for ritodrine tocolysis result in extremely low plasma levels. *Am J Obstet Gynecol* 169: 1059–1064.

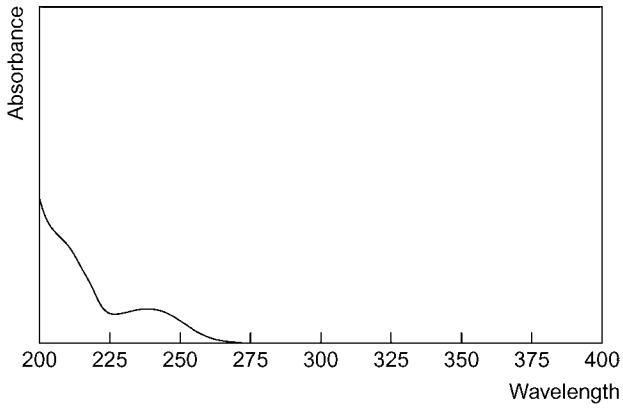
Ritonavir

Protease Inhibitor, Antiviral
$C_{37}H_{48}N_6O_5S_2$ = 721.0
CAS—155213-67-5

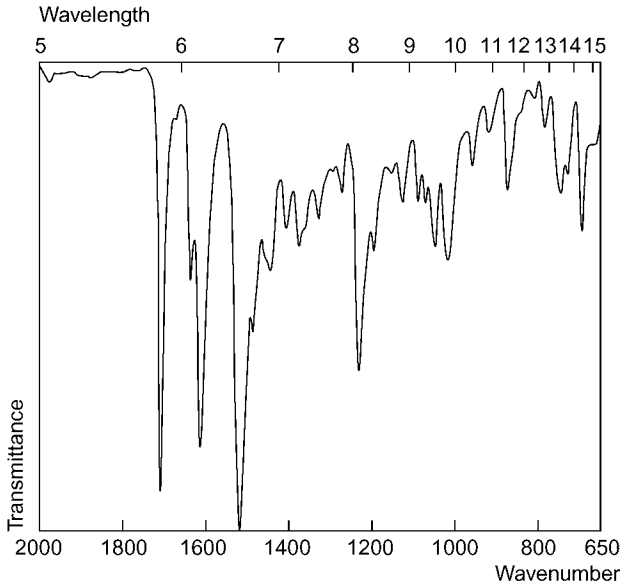
IUPAC Name 1,3-Thiazol-5-ylmethyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[[(2*S*)-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]butanoyl]amino]-1,6-diphenylhexan-2-yl]carbamate
Synonyms A-84538, Abbott-84538, ABJ-538; [5*S*,8*S*,10*S*,11*S*)]-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester.
Proprietary Name *Norvir*



Chemical Properties White to light-tan powder. Practically insoluble in water, freely soluble in methanol and ethanol; soluble in isopropanol.
High Performance Liquid Chromatography System HAC—*k* 7.1; system HAE—retention time 22.2 min.
Column: ODS-AQ (50 × 4.0 mm i.d., 3 µm). Mobile phase: acetonitrile: methanol: 0.1% trifluoroacetic acid (with 0.01 mol/L tetramethylammonium perchlorate) (40:5:55), flow rate 1 mL/min. UV detection (λ=205 nm). Retention time: 8 min [Marsh *et al.* 1997].
Ultraviolet Spectrum Aqueous acid—238 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumber 3355, 3328, 2964, 1714, 1618, 1522, 1235 cm^{-1}.



Quantification

Plasma **HPLC** Limit of quantification, 0.05 mg/L [Aymard *et al.* 2000]. UV detection (λ=260 nm). Limit of quantification, 0.1 mg/L [Proust *et al.* 2000]. UV detection (λ=205 nm). Lower limit of quantification, 0.012 mg/L [Marsh *et al.* 1997].

Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 0.51 mg/L [Simon *et al.* 2001].

Disposition in Body Approximately 60 to 80% of a dose is absorbed. Ritonavir is extensively metabolised in the liver by oxidation principally by cytochrome P450, CYP3A and CYP2D6, to three major metabolites: desthiazolyl carbamate, an isopropylthiazolyl oxidative product and a des-isopropylthiazolyl product. It induces its own metabolism. Ritonavir is excreted mainly in faeces (86%) with a minor amount in urine (11% over a 148 h period). The plasma concentration is generally higher during the first few days of administration. Linear pharmacokinetics have been observed after multiple dosing and steady state is achieved after administration for 2 weeks.

Therapeutic Concentration

Twelve HIV-positive male patients, aged 40 to 49 years, were fasted overnight and co-administered ritonavir as 3 dosing regimens. Group 1: 800 mg indinavir twice daily and 100 mg ritonavir twice daily. Group 2: 1200 mg indinavir twice daily and 100 mg ritonavir twice daily. The peak plasma ritonavir concentration for group 1 was 1.4 mg/L (range, 0.7 to 2.1 mg/L) and group 2, 1.9 mg/L. These levels were observed at 2.8 (1.5 to 6.9) and 1.5 to 5.0 h, respectively [van Heeswijk *et al.* 1999].

Eighteen males confirmed as asymptomatic HIV positive but otherwise healthy, mean age 37.4 years (range, 28 to 49 years), were administered 300 mg ritonavir every 6 h for 4 days. The peak plasma concentration was 12.31 mg/L at 3.8 h, and the minimum concentration was 4.93 mg/L after multiple dosing [Cato *et al.* 1998].

Sixty-four HIV-positive males, mean 29 years old (range, 21 to 45 years) with no AIDS-defining illness other than non-visceral Kaposi's sarcoma, were involved in the study. They were divided into equal groups and received ritonavir in the following doses. Group 1: 200 mg every 12 h; group 2: 300 mg/12 h; group 3: 400 mg/12 h; group 4: 500 mg/12 h. Peak plasma concentrations on day 1 were 2.0, 4.4, 9.0 and 9.6 g/L at 4.3, 3.9, 3.6 and 3.9 h, respectively, for the 4 groups. On day 16, the peak concentrations were 4.5, 6.5, 11.7 and 14.2 g/L for groups 1, 2, 3 and 4, respectively, at 3.8, 4.6, 4.3 and 4.3 h. The minimum concentrations on day 16 were 0.6, 0.7, 1.1 and 2.3 g/L, respectively [Hsu *et al.* 1997].

Half-life 3 to 5 h.

Volume of Distribution 20 to 40 L (after single 600 mg dose). Also reported as 0.41 L/kg.

Clearance Apparent oral, 40 L/h (100 mg dose); 5 L/h (600 mg); clearance increases in children.

Protein Binding 98 to 99%.

Dose An oral dose of 1.2 g daily in two divided doses.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

Cato A III *et al.* (1998). Multidose pharmacokinetics of ritonavir and zidovudine in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 42(7): 1788–1793.

Hsu A *et al.* (1997). Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. *Antimicrob Agents Chemother* 41(5): 898–905.

Marsh KC *et al.* (1997). Determination of ritonavir, a new HIV protease inhibitor, in biological samples using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 704: 307–313.

Proust V *et al.* (2000). Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma. *J Chromatogr B Biomed Sci Appl* 742: 453–458.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1–2): 447–453.

van Heeswijk RP *et al.* (1999). The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. *AIDS* 13(14): F95–F99.

Rituximab

Immunosuppressant

CAS—174722-31-7

IUPAC Name IgG_1 kappa immunoglobulin (145 kDa)

Synonyms IDEC-102; IDEC-C2B8.

Proprietary Names *MabThera*; *Rituxan*.

Quantification

Serum **ELISA** Limit of quantification, 6.6 mg/L, limit of detection, 2.0 mg/L [Iacona *et al.* 2000].

Disposition in the Body Serum concentrations of rituximab are proportional to the dose administered. The drug can be detected in serum for a long period of time after the completion of treatment, on average 3 to 6 months, and accumulation of the drug occurs.

Therapeutic Concentration

Patients were administered with either a 125, 250 or 375 mg/m^2 body surface dose of rituximab once weekly for 4 weeks. The serum antibody concentration increased with increasing dose. For the 375 mg/m^2 dose, peak serum concentrations were 238.7 mg/L after the first infusion and 480.7 mg/L after the fourth infusion [Roche Products Ltd, 1999].

Seven patients with follicular non-Hodgkin's lymphoma were administered 375 mg/m^2 rituximab infusion once weekly, after chemotherapy, for 4 weeks. Maximum serum concentrations were 349.1 mg/L after the first infusion; 332.0 mg/L after the second; 447.5 mg/L after the third infusion and 660.0 mg/L after the fourth. These concentrations were observed by the end of the infusion [Iacona *et al.* 2000].

Fourteen patients with relapsed indolent lymphoma were administered 375 mg/m^2 rituximab once weekly for 4 weeks. After the first infusion, the maximum serum concentration was 206 mg/L at the end and following the fourth infusion, the maximum was 465 mg/L [Berinstein *et al.* 1998].

Toxicity Rituximab is associated with cytokine-release syndrome including fevers and rigors, usually within 2 h of beginning the treatment. Tumour lysis syndrome, respiratory failure and cardiac arrhythmias (both serious and life-threatening) and even death may occur.

Half-life The serum half-life is proportional to the dose administered. A mean serum half-life is 60 (range, 11–105) h after a 10 mg/m^2 IV infusion and 174 (26–442) h after 250 mg/m^2.

Clearance Plasma, 0.046 L/h (after an 375 mg/m^2 body surface dose, first infusion); 0.015 L/h (after fourth infusion).

Dose The recommended dose is 375 mg/m^2 body surface once weekly as an IV dose for 4 doses. The first infusion is given at a rate of 50 mg/h which is increased in steps of 50 mg/h every 30 min to a maximum of 400 mg/h.

Berinstein NL *et al.* (1998). Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. *Ann Oncol* 9: 995–1001.

Iacona I *et al.* (2000). Rituximab (IDEC-C2B8): validation of a sensitive enzyme-linked immunoassay applied to a clinical pharmacokinetic study. *Ther Drug Monit* 22(3): 295–301.

Roche Products Limited (1999). Product information.

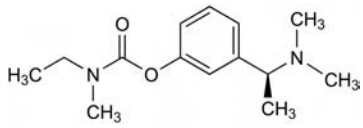
Rivastigmine

Anticholinesterase, Nootropic

$C_{14}H_{22}N_2O_2$ = 250.3

CAS—123441-03-2

IUPAC Name [3-[(1*S*)-1-(dimethylamino)ethyl]phenyl] *N*-ethyl-*N*-methylcarbamate
Synonym Ethylmethylcarbamic acid 3-[(1*S*)-1-(dimethylamino)ethyl]phenyl ester

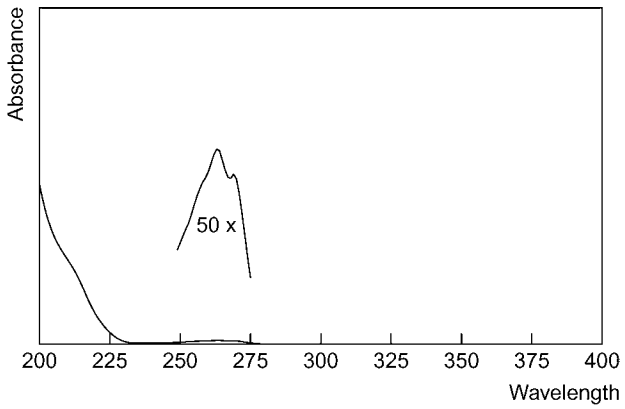


Chemical Properties Log *P* (octanol/buffer pH 7.0), 3.0.

Rivastigmine Hydrogen Tartrate

$C_{14}H_{22}N_2O_2$, $C_4H_6O_6$ = 400.4
CAS—129101-54-8
Synonyms ENA-713; SDZ-ENA-713; SDZ-212-713.
Proprietary Names *Exelon*; *Prometax*.
Chemical Properties White to off-white fine crystalline powder. Mp 123° to 125°. Very soluble in water, soluble in ethanol and acetonitrile, slightly soluble in *n*-octanol and very slightly soluble in ethyl acetate.

Gas Chromatography Column: wide-bore fused silica coated with cross bonded 50% phenyl/5% methyl silicone. Carrier gas: helium, flow rate 5 mL/min. MS detection: Retention time: 3.2 min [Habucky *et al.* 1998].
High Performance Liquid Chromatography System HZ—retention time 2.3 min.
Ultraviolet Spectrum



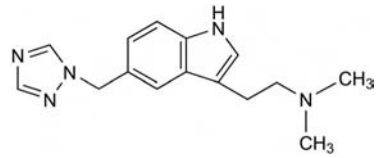
Quantification
Blood **GC–MS** Limit of quantification, 0.25 µg/L [Tse, Laplanche 1998].
Disposition in the Body Rivastigmine is rapidly and completely absorbed from the gastro-intestinal tract after administration. Peak plasma concentrations are reached in about 1 h. Administration with food delays absorption by 1.5 h and reduces the maximum plasma concentration. The drug is rapidly and extensively metabolised by cholinesterase-mediated hydrolysis to a weakly active decarbamylated metabolite. Rivastigmine undergoes a significant first-pass effect and it is widely distributed throughout the body. 90% of the dose is excreted in urine within 24 h and <1% appears in faeces. Metabolism and excretion of rivastigmine and its metabolite may be altered by renal impairment. The drug has a prolonged duration of action. There is no accumulation of the parent drug or its metabolite.
Toxicity Most accidental overdoses resulted in no clinical signs or symptoms. After ingestion of a 46 mg dose full recovery was made within 24 h following conservative management. Nausea, vomiting and diarrhoea may occur.
Bioavailability Approximately 36% following a 3 mg dose which may increase when taken with food.
Half-life Approximately 1 h.
Volume of Distribution Apparent, 1.8 to 2.7 L/kg.
Clearance Plasma, 130 L/h (0.2 mg IV dose); 70 mL/h (2.7 mg).
Distribution in Blood Blood : plasma ratio is 0.8 to 0.9 with 40 to 50% of the drug associated with red blood cells; independent of concentration.
Protein Binding 40%.
Dose The initial dose is 1.5 mg twice daily which is increased to 3 mg twice daily over a 2-week interval; 4.5 mg twice daily to a maximum 6 mg twice daily. The effective dose is 3 to 6 mg daily.

Habucky K *et al.* (1998). Disposition of SDZ ENA 713, an acetylcholinesterase inhibitor, in the rabbit. *Biopharm Drug Dispos* 19: 285–290.
Tse F, Laplanche R (1998). Absorption, metabolism, and disposition of [14C]SDZ ENA 713, an acetylcholinesterase inhibitor, in minipigs following oral, intravenous, and dermal administration. *Pharm Res* 15: 1614–1620.

Rizatriptan

5-HT$_1$ Receptor Agonist, Antimigraine
$C_{15}H_{19}N_5$ = 269.4
CAS—144034-80-0

IUPAC Name *N*,*N*-Dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl] ethanamine
Synonym *N*,*N*-Dimethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indole-3-ethanamine

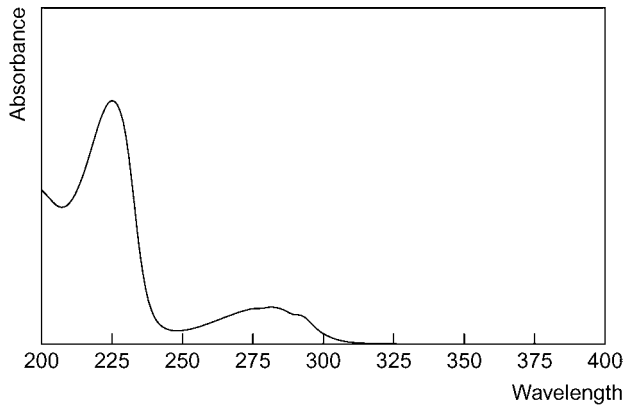


Chemical Properties A white to off-white crystalline solid. Mp 120° to 121°. Soluble in water (42 g/L at 25°) as the free base.

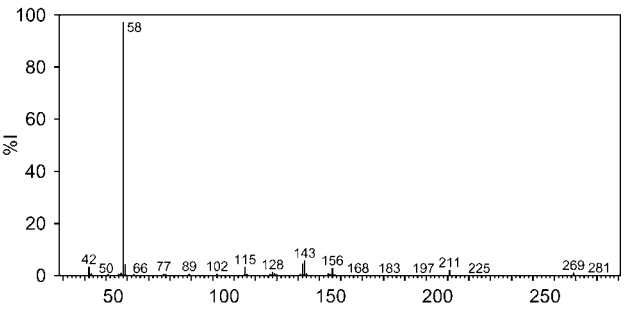
Rizatriptan Benzoate

$C_{15}H_{19}N_5$,C_6H_5COOH = 391.5
CAS—145202-66-0
Synonyms MK-0462; MK-462.
Proprietary Name *Maxalt*
Chemical Properties A white to off-white crystalline solid. Mp 178° to 180°.

High Performance Liquid Chromatography Column: (analytical) silica Alltech (150 × 2.1 mm i.d., 5 µm); (guard) C_8 Optiguard (1 mm).
Mobile phase: ammonium acetate (20 mmol/L, pH 4 with glacial acetic acid, then to pH 2.7 with formic acid): methanol: acetonitrile (80:10:10), flow rate 300 µL/min. MS–MS detection (ESI, SIM *m/z* at 270→201). Retention time: 3.48 min [Vishwanathan *et al.* 2000].
Column: SB-phenyl Zorbax (250 × 4.6 mm i.d., 5 µm). Mobile phase: (A) acetonitrile:0.1% trifluroacetic acid in water (16:84); (B) acetonitrile:0.1% phosphoric acid in water (12:88), flow rate 1.5 mL/min. UV detection (λ=280 nm). Retention times: rizatriptan, (A) 6.3 min; (B) 6.7 min [Antonucci *et al.* 1998].
Ultraviolet Spectrum Aqueous acid—225, 282 nm.



Mass Spectrum Principal ions at *m/z* 58, 143, 115, 42, 156, 211, 128, 269.



Quantification
Plasma **HPLC–MS** MS–MS detection. Limit of quantification, 0.5 µg/L [Lee *et al.* 1999]. MS detection. Limit of quantification, 0.5 µg/L [McLoughlin *et al.* 1996].
Serum **HPLC–MS** MS–MS detection. Limit of detection, 0.1 µg/L [Vishwanathan *et al.* 2000].
Urine **HPLC–MS** MS–MS detection. Limit of quantification, 1 µg/L [Lee *et al.* 1999].
Disposition in the Body Rizatriptan is rapidly and completely absorbed after an oral dose. Peak plasma concentrations are reached after 1 to 1.5 h or 1.6 to 2.5 h (disintegrating tablets) depending on formulation. Food may delay time to peak plasma concentration by up to 1 h. It is metabolised primarily by monoamine oxidase type A to the inactive indole acetic acid derivative. The active *N*-monodesmethyl-rizatriptan metabolite is formed to a small degree. Other minor non-active metabolites include *N*-oxide, 6-hydroxy compound and the sulfate conjugate of the 6-hydroxy metabolite. Eighty percent of a dose is excreted in urine with approximately 14% as the unchanged drug, 51% as the indole acetic acid metabolite

and 1% as *N*-monodesmethyl-rizatriptan. This indicates substantial first-pass metabolism. Ten percent is excreted in the faeces. No accumulation occurs. The drug is rapidly cleared from the plasma. Renal impairment did not effect pharmacokinetics. Hepatic impairment increased plasma concentrations by 30%.

Therapeutic Concentration

Six healthy males were administered with 60 mg rizatriptan. The mean peak plasma concentration was 19.8 µg/L which was observed at 1.4 h [Vyas *et al.* 2000].

Twenty-four healthy male and female individuals, aged between 19 and 49 years (mean, 32.9 years) were administered with a single dose of 10 mg rizatriptan followed, 48 h later, by a second 10 mg dose every 2 h for three doses on 4 consecutive days. The mean peak plasma rizatriptan concentration reached on day 1 was 19.6 µg/L at 0.9 h; day 3, 35.7 µg/L at 5.0 h and on day 6, 37.0 µg/L at 4.8 h. For the metabolite, *N*-monodesmethyl-rizatriptan, peak concentrations were 2.2, 3.4 and 3.8 g/L at 1.5, 5.3 and 5.0 h, respectively, on days 1, 3 and 6 [Goldberg *et al.* 2000].

Bioavailability 40 to 45%.

Half-life 2 to 3 h.

Volume of Distribution 140 L in males and 110 L in females.

Clearance 1000 to 1500 mL/min (males); 900 to 1100 mL/min (females). About 20 to 30% of this is renal clearance.

Protein Binding 14%.

Dose 10 mg.

Antonucci V *et al.* (1998). *J Liq Chromatogr Rel Technol* 21: 1649–1670.

Goldberg MR *et al.* (2000). Rizatriptan, a novel 5-HT1B/1D agonist for migraine: single- and multiple-dose tolerability and pharmacokinetics in healthy subjects. *J Clin Pharmacol* 40(1): 74–83.

Lee Y *et al.* (1999). Pharmacokinetics and tolerability of oral rizatriptan in healthy male and female volunteers. *Br J Clin Pharmacol* 47: 373–378.

McLoughlin DA *et al.* (1996). Quantitation of the 5HT1D agonists MK-462 and sumatriptan in plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr A* 726: 115–124.

Vishwanathan K *et al.* (2000). Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14(3): 168–172.

Vyas KP *et al.* (2000). Disposition and pharmacokinetics of the antimigraine drug, rizatriptan, in humans. *Drug Metab Dispos* 28(1): 89–95.

Rocuronium

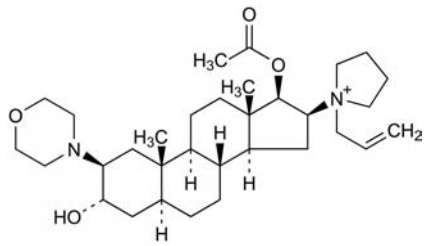
Neuromuscular Blocker, Muscle Relaxant

$[C_{32}H_{53}N_2O_4]^+ = 529.8$

CAS—143558-00-3

IUPAC Name [(2*S*,3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,16*S*,17*R*)-3-Hydroxy-10,13-dimethyl-2-morpholin-4-yl-16-(1-prop-2-enylpyrrolidin-1-ium-1-yl)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl] acetate

Synonym 1-[(2β,3α,5α,16β,17β)-17-(Acetyloxy)-3-hydroxy-2-(4-morpholinyl)-androstan-16-yl]-1-(2-propenyl)pyrrolidinium



Chemical Properties Log *P* (octanol/water), 0.5.

Rocuronium Bromide

$C_{32}H_{53}BrN_2O_4 = 609.7$

CAS—119302-91-9

Synonym Org-9426

Proprietary Names *Esmeron*; *Zemuron*.

Chemical Properties Crystals. Mp 161° to 169°.

Thin-layer Chromatography Silica gel plates (20 × 20 cm). Mobile phase: solution of 2% sodium iodide in 2-propanol. Detected with iodo-platinate reagent. R_f 31.8 [Kleef *et al.* 1993].

Quantification

Plasma **HPLC** Fluorescence detection (λ_{ex}=385 nm; λ_{em}=452 nm). Limit of quantification, 10 µg/L, limit of detection, 3 µg/L [Kleef *et al.* 1993].

Urine **HPLC** Fluorescence detection (λ_{ex}=385 nm; λ_{em}=452 nm). Limit of quantification, 25 µg/L, limit of detection, 4 µg/L [Kleef *et al.* 1993].

Bile **HPLC** Fluorescence detection (λ_{ex}=385 nm; λ_{em}=452 nm). Limit of quantification, 100 µg/L, limit of detection, 4 µg/L [Kleef *et al.* 1993].

Stoma Fluid **HPLC** Fluorescence detection (λ_{ex}=385 nm; λ_{em}=452 nm). Limit of quantification, 20 µg/L [Kleef *et al.* 1993].

Tissue Homogenates **HPLC** Fluorescence detection (λ_{ex}=385 nm; λ_{em}=452 nm). Limit of quantification, 100 µg/L, limit of detection, 5 µg/L [Kleef *et al.* 1993].

Disposition in the Body Rocuronium has two distribution phases following IV administration. The initial phase has a half-life of 1 to 2 min and the slower phase a half-life of 14 to 18 min. It undergoes metabolism and the metabolite 17-desacetylrocuronium has weak neuromuscular blocking activity. Up to 30% of the dose is excreted in urine with the majority being excreted in bile.

Therapeutic Concentration

Nine patients with renal failure with a mean age of 51 years (range, 22 to 61 years) requiring regular haemodialysis and 9 subjects with normal renal function, mean 46 years (range, 27 to 64 years), undergoing elective dental or ophthalmic surgery were involved in this study. After anaesthesia was induced with nitrous oxide, fentanyl and isoflurane, a 0.6 mg/kg single bolus dose of rocuronium was administered. The plasma concentration during clinical relaxation was 1.229 (range, 0.843 to 1.657) mg/L for those with renal failure and 0.891 (0.430 to 1.560) mg/L for those with normal renal function. The duration of clinical relaxation was 55 min for the patients with renal failure and 42 min for those without [Cooper *et al.* 1993].

Half-life 1.4 to 1.6 h, may be increased by hepatic impairment.

Volume of Distribution Steady state, 0.28 L/kg (0.6 mg/kg dose); 0.27 L (1.0 mg/kg dose).

Clearance 0.17 L/kg/h (0.6 mg/kg dose); 0.24 L/kg/h (1.0 mg/kg dose); 0.32 L/kg/h (individuals with hepatic dysfunction after a 0.6 mg/kg dose).

Protein Binding 30%.

Dose IV injection: 600 µg/kg with a maintenance dose of 150 µg/kg. IV infusion: 300 to 600 µg/kg/h.

Cooper RA *et al.* (1993). Time course of neuromuscular effects and pharmacokinetics of rocuronium bromide (Org 9426) during isoflurane anaesthesia in patients with and without renal failure. *Br J Anaesth* 71: 222–226.

Kleef UW *et al.* (1993). Determination of rocuronium and its putative metabolites in body fluids and tissue homogenates. *J Chromatogr* 621: 65–76.

Rofecoxib

Antiinflammatory, COX Inhibitor

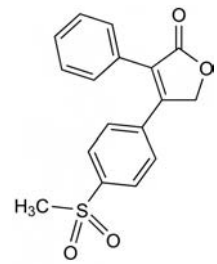
$C_{17}H_{14}O_4S = 314.4$

CAS—162011-90-7

IUPAC Name 3-(4-Methylsulfonylphenyl)-4-phenyl-2*H*-furan-5-one

Synonyms 4-[4-(Methylsulfonyl)phenyl]-3-phenyl-2(5*H*)-furanone; MK-0966.

Proprietary Name *Vioxx*

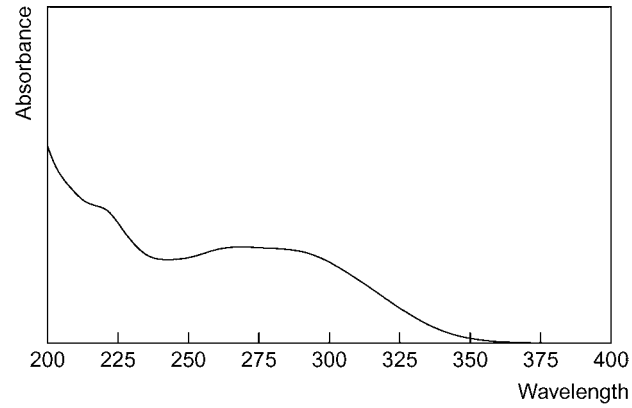


Chemical Properties White to off-white yellow powder. Insoluble in water, sparingly soluble in acetone, slightly soluble in methanol and isopropyl acetate, very slightly soluble in ethanol and practically insoluble in octanol.

Gas Chromatography System GB—RI 3119.

High Performance Liquid Chromatography Column: C_{18}(100 x 4.6 mm i.d., 5 µm). Mobile phase: water: acetonitrile: acetic acid: triethylamine (770:230:1:0.3), flow rate 1 mL/min. Internal standard: ketoprofen. UV detection (λ=272 nm). Retention times: rofecoxib 13 min, internal standard 24 min [Jamali, Sattari 2000].

Ultraviolet Spectrum



Quantification

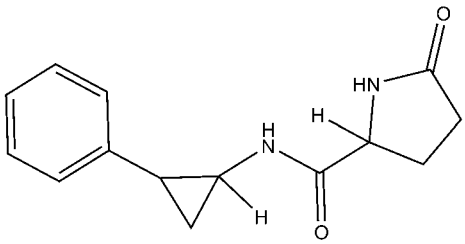
Plasma **HPLC** UV detection (λ=272 nm). Limit of quantification, 10 µg/L [Jamali and Sattari 2000]. Fluorescence detection. Limit of quantification, 0.5 µg/L [Woolf *et al.* 1999].

HPLC–MS MS–MS detection. Limit of quantification, 0.1 µg/L [Chavez-Eng *et al.* 2002].
Serum **HPLC** Limit of quantification, 20 µg/L [Aravind *et al.* 2002].
Disposition in the Body Rofecoxib is well absorbed from the gastro-intestinal tract after oral administration. Peak plasma concentrations are reached in approximately 2 h. It is extensively metabolised in the liver, mainly by reduction to *cis*- and *trans*-dihydrorofecoxib, accounting for 56% of a dose recovered in urine and an additional 8.8% as the 5-hydroxy glucuronide metabolite. None of the metabolites demonstrates measurable activity as cyclo-oxygenase inhibitors. Excretion is mainly via the urine (72%) with only 14% of a dose appearing in the faeces.
Bioavailability ≈93%.
Half-life Steady state, 17 h.
Volume of Distribution Approximately 91 to 100 L (1.55 L/kg).
Clearance About 120 mL/min.
Protein Binding About 85%.
Toxicity Administration of single doses of up to 1000 mg rofecoxib have not resulted in significant toxicity.
Dose In osteoarthritis, 12.5 mg once daily to a maximum of 25 mg. Patients with mild hepatic impairment should not exceed 12.5 mg daily. An initial dose of 50 mg once daily is permitted for the short-term treatment of acute symptomatic pain.

Aravind MK *et al.* (2002). A rapid and sensitive high-performance liquid chromatography assay for rofecoxib in human serum. *J Chromatogr Sci* 40: 26–28.
Chavez-Eng CM *et al.* (2002). High-performance liquid chromatographic-tandem mass spectrometric evaluation and determination of stable isotope labeled analogs of rofecoxib in human plasma samples from oral bioavailability studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 117–129.
Jamali F, Sattari S (2000). High performance liquid chromatographic determination of cyclooxygenase II inhibitor rofecoxib in rat and human plasma. *J Pharm Pharm Sci* 3: 312–317.
Woolf E *et al.* (1999). Determination of rofecoxib, a cyclooxygenase-2 specific inhibitor, in human plasma using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 730: 221–227.

Rolicyprine

Antidepressant
$C_{14}H_{16}N_2O_2 = 244.3$
IUPAC Name (+)-5-Oxo-*N*-(*trans*-2-phenylcyclopropyl-L-pyrrolidine-2-carboxamide
Synonyms EX 4883; rolicypram.
Proprietary Name *Cypromin*

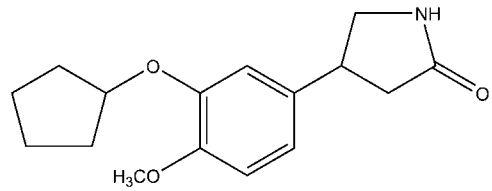


Chemical Properties White crystalline powder. Mp 144° to 149°. Soluble 1 in 50 of water; soluble in chloroform. Rolicypram is extracted by chloroform from aqueous acid solutions at pH 2.0.
Colour Tests Ammonium molybdate test—grey-blue (limit of detection, 1.0 µg); sulfuric acid test—pale yellow (limit of detection, 1.0 µg); sulphuric acid–formaldehyde test—red→brown (limit of detection, 0.1 µg); Vitali's test—pale yellow/–/pale yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.67 (location reagent acidified iodoplatinate spray, strong reaction).
Gas Chromatography System G2/225—no peaks within 30 min.
Ultraviolet Spectrum Methanol—219, 260, 266, 273 nm.
Disposition in the Body
Metabolism Rolicypram undergoes biotransformation to tranylcypromine and pyrrolidone carboxylic acid. This biotransformation is necessary before the drug exerts any monoamine oxidase inhibitor activity [Lowe, Horita 1973; McMonigle, Horita 1969].
Toxicity LD_{50} in rats 97 mg/kg (oral).

Lowe MC, Horita A (1973). The role of biotransformation on the pharmacology of the monoamineoxidase inhibitor 5-oxo-*N*-(d-*trans*-2-phenylcyclopropyl)-1-2-pyrrolidone-carboxamide (EX-4883). *Eur J Pharmacol* 21: 46–52.
McMonigle JJ, Horita A (1969). Bioactivation of 5-oxo-(D-*trans*-2-phenylcyclopropyl)-L-2-pyrrolidone carboxamide (EX 4883) into a monoamine oxidase inhibitor by a soluble fraction enzyme system. *Arch Int Pharmacodyn Ther* 178: 53–61.

Rolipram

Antidepressant, Phosphodiesterase 4 Inhibitor
$C_{16}H_{21}NO_3 = 275.3$
CAS—61413-54-5
IUPAC Name 4-[3-(Cyclopentyloxy)-4-methoxyphenyl] pyrrolidin-2-one
Synonym ZK-62711

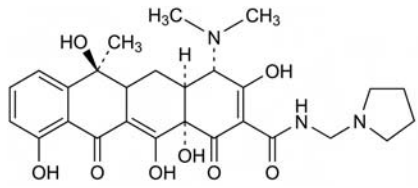


Chemical Properties White solid. Mp 132°. Soluble in DMSO and ethanol. Log *P* (octanol/water), 1.66 [Wishart 2006]. Rolipram is stable at room temperature for at least 12 weeks [Pfeffer *et al.* 1990]
Quantification
Plasma **HPLC** Column: Reversed phase Lichrosorb RP-18 (250 × 4.6 mm i.d., 10.0 µm). Mobile phase: methanol : water (65 : 35). UV detection (λ = 240 nm). Limit of quantification, 50 µg/L [Pfeffer *et al.* 1990].
Disposition in the Body Rolipram is rapidly absorbed after oral administration. It is metabolised by ether cleavage at the methoxy and cyclopentyloxy groups and by hydroxylation in positions 2 or 3 of the cyclopentyloxy ring, followed by sulfation. The 5-position of the pyrrolidone ring also undergoes hydroxylation.
Therapeutic Concentration
After oral administration of 1.0 mg rolipram to 6 healthy male volunteers after an overnight fast, the mean peak plasma concentration reached 16 µg/L after 0.5 h [Krause *et al.* 1990].
Bioavailability Approximately 73%.
Half-Life Plasma levels decline in three phases of 0.2, 0.6 to 0.9, and 6 to 8 h, respectively.
Volume of Distribution Approximately 0.40 L/kg.
Clearance Approximately 6 mL/min/kg.

Krause W *et al.* (1990). Pharmacokinetics of (+)-rolipram and (−)-rolipram in healthy volunteers. *Eur J Clin Pharmacol* 38: 71–75.
Pfeffer M *et al.* (1990). In-vitro and in-vivo characterisation of two sustained release formulations for the antidepressant rolipram. *Arzneimittelforschung* 40: 1191–1194.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Rolitetracycline

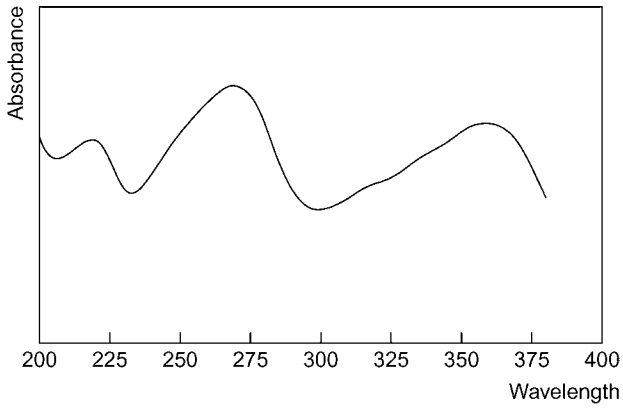
Antibacterial
$C_{27}H_{33}N_3O_8 = 527.6$
CAS—751-97-3
IUPAC Name (2*Z*,4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-6,10,11,12a-tetrahydroxy-2-[hydroxy-(pyrrolidin-1-ylmethylamino)methylidene]-6-methyl-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione
Synonyms [4*S*-(4α,4aα,5aα,6β,12aα)]-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-*N*-(1-pyrrolidinylmethyl)-2-naphthacenecarboxamide; PMT; pyrrolidinomethyltetracycline.
Proprietary Names *Reverin*; *Syntetrin*; *Tetraverin*; *Transcycline*.



Chemical Properties A light yellow crystalline powder. Mp 162° to 165°, with decomposition. Soluble 1 in 1.1 of water and 1 in 200 of ethanol; soluble in acetone; very slightly soluble in ether; soluble in dilute acids and alkalis. pK_a 7.4. Log *P* (octanol/water), −0.7.

Rolitetracycline Nitrate
$C_{27}H_{33}N_3O_8,HNO_3,\ 1\frac{1}{2}H_2O = 617.6$
CAS—20685-78-3 (anhydrous); 26657-13-6 (sesquihydrate)
Chemical Properties A yellow crystalline powder. Soluble 1 in 40 of water.

Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—green-yellow (→yellow-brown); Mandelin's test—violet→red→orange; Marquis test—orange; sulfuric acid—violet.
Thin-layer Chromatography System TA—R_f 0.05, streaking; system TAE—R_f 0.00 (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—272 (A^{1}_{1}=325a), 357 nm; aqueous alkali—248 (A^{1}_{1}=261b), 268 nm (A^{1}_{1}=266b).

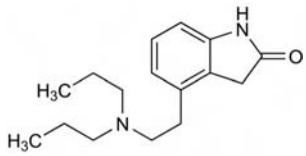


Dose Up to 700 mg daily, given parenterally.

Ropinirole

Dopamine D_2 Agonist, Antiparkinsonian
$C_{16}H_{24}N_2O = 260.4$
CAS—91374-21-9

IUPAC Name 4-[2-(Dipropylamino)ethyl]-1,3-dihydroindol-2-one
Synonyms 4-[2-(Dipropylamino)ethyl]-1,3-dihydro-2*H*-indol-2-one; SKF-101468.



Chemical Properties Log *P* (octanol/water), 2.70.

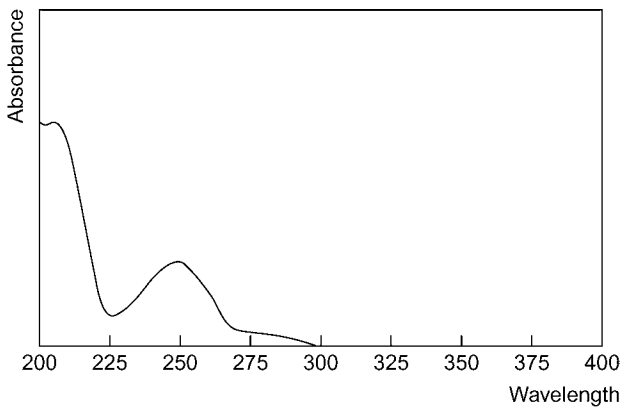
Ropinirole Hydrochloride

$C_{16}H_{25}ClN_2O = 296.8$
CAS—91374-20-8
Synonym SKF-101468A
Proprietary Name *Requip*
Chemical Properties A white to greenish-yellow crystalline powder. Mp 243° to 250°. Soluble in water (133 g/L).

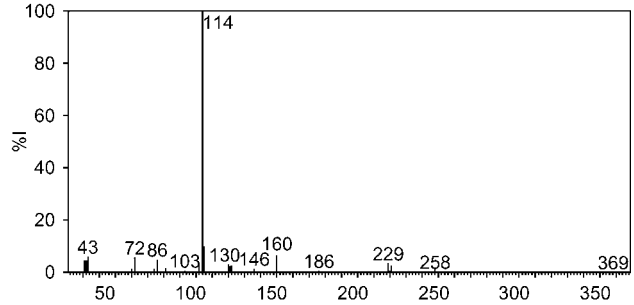
High Performance Liquid Chromatography Column: LC-ABZ Supelcosil (150 × 4.6 mm i.d., 5 µm). Temperature: 40°. Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (pH 4, 0:100 to 15:85 in 10 min to 100:0 in 12 min, for 15 min, flow rate 1 mL/min. UV detection (λ= 250 nm). Retention time: 15 min [Bloomer *et al.* 1997].

Column: RP C_{18} Ultrasphere (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.07 mol/L ammonium formate buffer (pH 3.8, containing 0.3% EDTA and 0.005% sodium octyl sulfate; 25 : 75), flow rate 1 mL/min. UV detection (λ=250 nm). Retention time: 9.4 min [Swagzdis, Mico 1986].

Ultraviolet Spectrum Aqueous acid—205, 248 nm.



Mass Spectrum Principal ions at *m/z* 114, 115, 160, 72, 43, 86, 229, 42.



Quantification
Plasma **HPLC** UV detection (λ= 250 nm). Limit of quantification, 10 µg/L, limit of detection, 5 µg/L, [Swagzdis, Mico 1986].

Disposition in the Body Ropinirole is rapidly and completely absorbed from the gastrointestinal tract after oral administration and is widely distributed throughout the body. The rate, but not the extent, of absorption may be reduced if taken with food. Extensive metabolism in the liver occurs primarily by oxidative metabolism via cytochrome P450 isoenzyme CYP1A2 and excreted in urine as metabolites. The major metabolic pathways are *N*-despropylation and hydroxylation to form the inactive *N*-despropyl and hydroxy metabolites. *N*-despropyl ropinirole is the predominant metabolite in urine (40%) followed by the carboxylic acid metabolite (10%) and the glucuronide of the hydroxy metabolite (10%). Of the dose, 88% is recovered in urine with <10% as the unchanged drug. There is wide inter-individual variability with its pharmacokinetics. Steady state is achieved within 2 days. The drug displays linear pharmacokinetics over dosing range 1–8 mg twice daily. Accumulation from multiple dosing is predictive from single dosing.

Therapeutic Concentration
Mean maximum plasma concentrations of 1.16 µg/L after a 1 mg dose and 5.11 µg/L after 5 mg have been observed. These concentrations are reached within 1.5 h after oral administration [Boothman, Spokes 1990].

Toxicity
Ten patients ingested more than 24 mg daily and there were no significant effects. The largest overdose in pre-marketing clinical trials was 435 mg taken over a 7-day period (62.1 mg daily) [Winthrop Pharmaceuticals 2008].

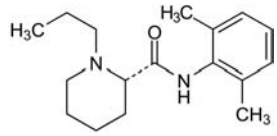
Bioavailability Absolute approximately, 50–55%. Relative bioavailability from tablet to an oral solution is 85%.
Half-life 6 h.
Volume of Distribution 7.5–8 L/kg.
Clearance 47 L/h.
Distribution in Blood Blood : plasma ratio is 1:1.
Protein Binding 10–40%.
Dose 3 to 9 mg daily.

Bloomer JC *et al.* (1997). In vitro identification of the P450 enzymes responsible for the metabolism of ropinirole. *Drug Metab Dispos* 25: 840–844.
Boothman B, Spokes E (1990). Pharmacokinetic data for ropinirole. *Lancet* 336: 814.
Swagzdis J, Mico B (1986). Liquid chromatographic determination of 4-(2-di-N,N-propylaminoethyl)-2-(3H)-indolone in rat, dog, and human plasma with ultraviolet detection. *J Pharm Sci* 75(1): 90–91.
Winthrop Pharmaceuticals (2008). Ropinirole 0.25 mg film-coated tablet. UK Summary of Product Characteristics.

Ropivacaine

Anaesthetic (Local)
$C_{17}H_{26}NO_2 = 274.4$
CAS—84057-95-4

IUPAC Name (2*S*)-*N*-(2,6-Dimethylphenyl)-1-propyl-2-piperidinecarboxamide
Synonyms AL-281; LEA-103.



Chemical Properties White crystalline powder with Mp 144° to 146°. pK_a 8.16. Log *P* (octanol/water), 2.9 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

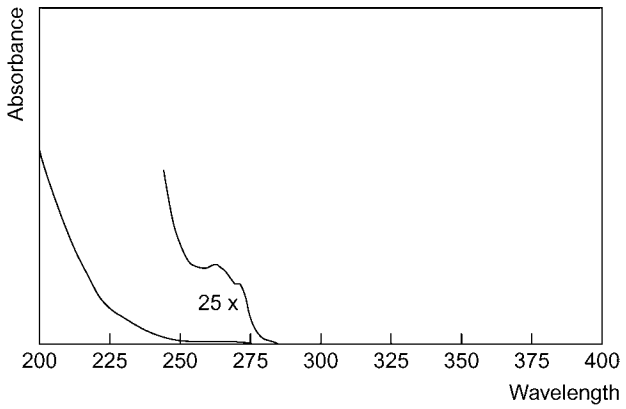
Ropivacaine Hydrochloride

$C_{17}H_{26}N_2O,HCl = 310.9$
CAS—98717-15-8
Chemical Properties Crystals with Mp 260° to 262°.

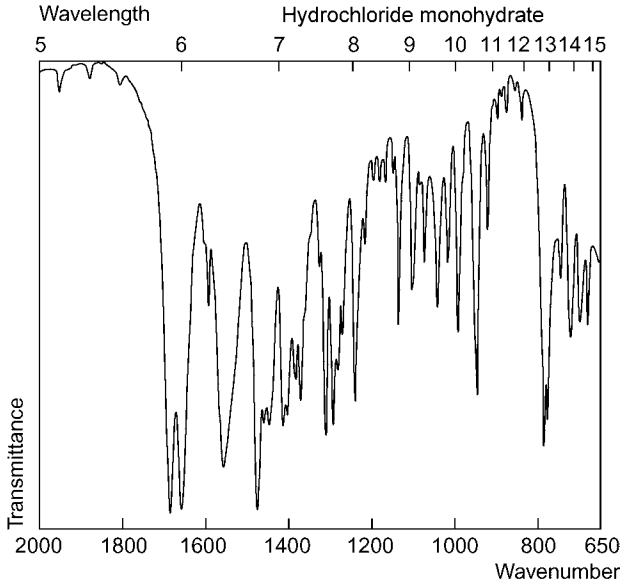
Ropivacaine Hydrocloride Monohydrate

$C_{17}H_{26}N_2O,HCl,H_2O = 328.9$
CAS—132112-35-7
Proprietary Name *Naropin.*
Chemical Properties White crystalline powder with Mp 269.5° to 270.6°. It is soluble in water (53.8 g/L).

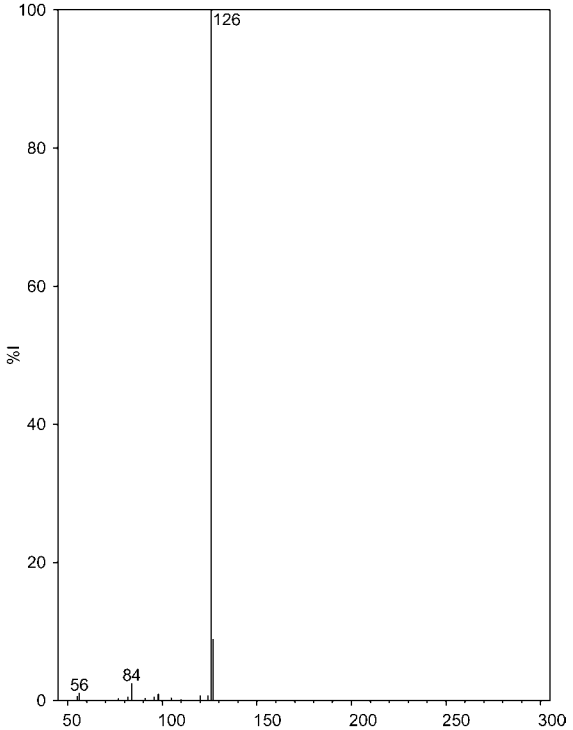
Ultraviolet Spectrum



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 126, 84, 56.



Quantification

Plasma **GC-MS** Column: fused silica (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He. Temperature programme: 150° to 230° at 40°/min. PICI, SIM acquisition mode at *m/z* 275. Retention time: 4.02 min. Limit of quantification, 3 µg/L [Emanuelsson *et al.* 1997]. NSD. Limit of detection, 10 µg/L [Erichsen *et al.* 1996].

HPLC Column: TSK-GEL ODS (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol: 0.05 mol/L phosphate buffer (pH 4.0, 10 : 30 : 60), flow rate 0.8 mL/min. UV detection (λ = 215 nm). Retention time: 8.4 min. Limit of detection, 25 µg/L [Kawata *et al.* 2005]. Column: Lichrospher RP-SelectB-C_8 (125 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L potassium dihydrogen phosphate (pH 2.1; 20 : 80), flow rate 1 mL/min. Internal standard (IS): mepivacaine. UV detection (λ = 205 nm). *k* value: ropivacaine, 11.3; IS, 15.9. Limit of detection, 0.9 µg/L [Reif *et al.* 1998].

LC-MS Column: Zorbax eclipse XD_8 C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05% trimethylamine in acetonitrile : 2 mmol/L ammonium formate (pH 3.0, 15 : 85 for 5 min to 35 : 65 at 10 min to 50 : 50 in the next 0.1 min until 15 min to 80 : 20 for 1 min to 15 : 85 for 4 min), flow rate 1 mL/min to 0.5 mL/min from 1 to 5 min for 5 min to 1 mL/min in 0.1 min. ESI, positive ion mode, SIM acquisition mode. Retention time: 8.9 min. Limit of quantification, 1 µg/L [Mathieu *et al* 2006]. Column: *Ace*III C_{18} (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 0.1% formic acid. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2.74 µg/L [Simon *et al.* 2006]. Column: Luna C_{18} (3 cm × 4.6 mm i.d.). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (86.5 : 13.5). MSD, SIM acquisition mode. Limit of quantification, 2.5 nmol/L [Cobb, Andersson 2005]. Column: Optiguard C_8 (10 × 1 mm i.d.). Mobile phase: 0.1% formic acid in methanol : water (1 : 1), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2 nmol/L [Abdel-Rehim *et al.* 2004].

Serum **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 100 : 100 : 300), flow rate 1 mL/min. UV detection (λ = 210 nm). Retention time: 9.7 min. Limit of quantification, 5 µg/L [Tanaka *et al.* 2006].

LC-MS Column: Synergy Polar-RP 80A (150 × 2 mm i.d., 4 µm). Mobile phase: acetonitrile-2 mmol/L ammonium acetate-formic acid (5 : 95 : 0.2) : acetonitrile-2 mmol/L ammonium acetate-formic acid (95 : 5 : 0.2, 100 : 0 for 0.1 min to 20 : 80 at 2.5 min for 0.5 min to 100 : 0 at 3.2 min for 3.8 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 1 µg/L [Koehler *et al.* 2005].

Urine **GC-MS** See Plasma [Emanuelsson *et al.* 1997]. See Plasma [Erichsen *et al.* 1996].

LC-MS Column: Optiguard C_8 (20 mm × 1 mm i.d.). Mobile phase: 0.1% formic acid in methanol : 0.1% formic acid in water (1 : 1), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 5.0 nmol/L.

Placenta Perfusate **HPLC** Diamonsil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogen phosphate : acetonitrile (79 : 21), flow rate 1.0 mL/min. UV detection (λ = 210 nm). Limit of detection, 10 µg/L [Zuo *et al.* 2004].

Disposition in the Body Ropivacaine is readily absorbed and extensively metabolised in the liver, predominately by aromatic hydroxylation, which is mediated by CYP1A. The metabolites are mainly excreted in urine, with 86% of the dose excreted after IV administration. 1% of the dose is excreted as the unchanged drug. The major metabolites are 3-hydroxy-ropivacaine, 37% of which is excreted in urine mainly in the conjugated form, and 4-hydroxy-ropivacaine, the *N*-dealkylated metabolite and 4-hydroxy-dealkylated metabolite. The drug displays linear pharmacokinetics and has a long duration of action, and has been shown to cross the placenta.

Therapeutic Concentration Plasma concentrations are dependent upon the dose, route of administration and the vascularity of injection site. The mean maximum tolerated and free arterial plasma concentration is 4.3 and 0.6 mg/L.

An 80 mg IV dose resulted in a mean peak plasma concentration of 1.9 ± 0.3 mg/L, and an infusion of 50 mg over a 15 min period produced mean plasma concentrations of 1.5 mg/L. [Markham, Faulds 1996].

Eight healthy male volunteers (age: 26–38 years) were administered 80 mg ropivacaine as an IV infusion over 25 min, 40 mg of which was the [$^2H_3$] form. At the end of the infusion the amounts of ropivacaine and [$^2H_3$]-ropivacaine were similar being 0.7–1.2 mg/L and 0.6–1.3 mg/L, respectively [Emanuelsson *et al.* 1997].

Toxicity Ropivacaine is associated with CNS toxicity, in particular symptoms resembling those caused by seizure activity in the temporal lobe (psychomotor epilepsy), including spasms of facial muscles, behavioural manifestations and motor movements. Accidental IV injection results in immediate toxic effects. The toxic plasma concentration is >4 mg/L.

A 45-year-old female with rheumatoid arthritis was treated with ropivacaine for regional anaesthesia prior to surgery on her right wrist. A total of 40 mL ropivacaine (7.5 g/L) was slowly injected in doses of 5 mL 1–2 min apart (the total dose was 6 mg/kg). Three minutes after the injection had been completed, the patient experienced oral numbness and twitching in her throat. Her speech became irrational, her arterial blood pressure increased, she experienced sinus tachycardia and her heart beat increased. She finally lost consciousness but regained after subsequent treatment. The total plasma concentration of ropivacaine was 6.0 mg/L 40 min after injection; 5.4 mg/L after 60 min; 4.6 mg/L after 80 min and 4.0 mg/L after 98 min. Six weeks later, a second attempt at the surgery was planned. A total dose of 30 mL ropivacaine (7.5 g/L) was administered, total dose 4.5 mg/kg followed by 10 mL lidocaine-adrenaline (20 g/L). Her heart rate increased over the first 10 min and after

R

25 min, she experienced a strange feeling in her tongue and speech disorders due to loss of muscle control relating to the CNS. She did not respond when spoken to and experienced involuntary spasms around the head and neck. The total plasma concentrations of ropivacaine were 4.0 mg/L 25 min after injection, 2.5 mg/L after 37 min and 2.0 mg/L after 56 min [Ala-Kokko *et al.* 2000].

A 13-year-old male patient with chronic pain due to bladder spasm was given an epidural IV infusion of ropivacaine hydrochloride (2 mL at a concentration of 10 mg/mL) over 15 s. Facial numbness was experienced immediately and within 1 min, classical grand mal seizure. Convulsions lasted for 2 min and he returned to a normal state of consciousness within the next 15 min. The blood ropivacaine concentration, 30–40 min after the convulsion, was 1.4 mg/L and at the time of the seizure this would have been considerably higher, possibly a concentration of 6–30 mg/L. [Plowman *et al.* 1998].

Half-life 1.8 h.

Volume of Distribution Steady state, 41–59 L.

Clearance Total plasma clearance, 387 ± 107 mL/min; unbound plasma clearance, 7.2 ± 1.6 L/min.

Protein Binding 94% (α_1-acid glycoprotein).

Note For a review of the pharmacology of ropivacaine see Markham, Faulds [1996].

Dose Maximum single dose is 200 mg.

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Rosiglitazone

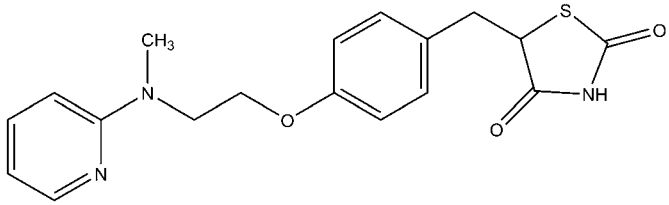
Antidiabetic, Thiazolidinedione

$C_{18}H_{19}N_3O_3S = 357.4$

CAS—122320-73-4

IUPAC Name 5-[[4-[2-(Methyl-pyridin-2-ylamino)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione

Synonym (±)-5-{*p*-[2-(Methyl-2-pyridylamino)ethoxy]benzyl}-2,4-thiazolidinedione



Chemical Properties Colourless crystals from methanol. Mp 153° to 155° [O'Neil *et al.* 2006].

Rosiglitazone Maleate

$C_{18}H_{19}N_3O_3S,C_4H_4O_4 = 473.5$

CAS—155141-29-0

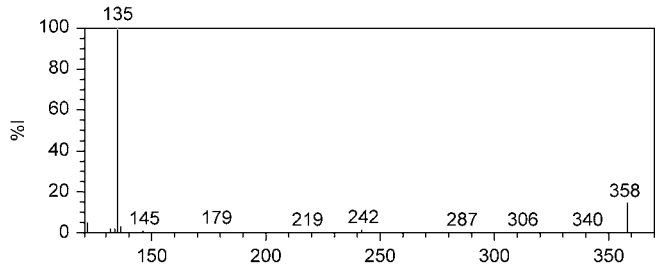
Synonym BRL-49653-C

Proprietary Names *Avandia*; *Diaben*; *Gaudil*; *Glimide*; *Gliximina*; *Gludex*; *Roglin*; *Rosicon*; *Rosiglit*. It is also an ingredient in *Avandamet*; *Avaglim*; *Glyroz*; *Roglin-M*.

Chemical Properties White to off-white solid. Mp 122° to 123°. Readily soluble in ethanol and in buffered aqueous solution with pH of 2.3; solubility decreases with increasing pH in the physiological range. pK_{a1} 6.1, pK_{a2} 6.8 [O'Neil *et al.* 2006].

High Performance Liquid Chromatography Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.025 mol/L sodium dihydrogen phosphate (pH 6.2) : acetonitrile (50 : 50), flow rate 1.0 mL/min. UV detection (λ = 245 nm). Retention time: 7.4 min. Limit of quantification, 180 mg/L [Radhakrishna *et al.* 2002].

Mass Spectrum Principal peaks at *m/z* 135, 358.



Quantification

Plasma **HPLC** Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 10 mmol/L phosphate buffer (pH 2.6, 40 : 12 : 48), flow rate 1.2 mL/min. UV detection (λ = 269 nm). Retention time: 4.1 min. Limit of quantification not reported [Sripalakit *et al.* 2006]. Column: C_{18} Inertsil ODS-III (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water-acetonitrile (5 : 95) : water-methanol (10 : 90; 100 : 0 : 0 for 1 min, to 90 : 0 : 10 over 1 min to 20 : 50 : 30 over 4 min to 30 : 55 : 15 over 6 min to 10 : 85 : 5 over 10 min to 20 : 65 : 15 over 2 min to 40 : 50 : 10 over 1 min), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention times: rosiglitazone 11.4 min; repaglinide 25.4 min; pioglitazone 13.3 min; glipizide 14.8 min; glyburide 20.7 min. Limit of quantification, 0.1 mg/L [Venkatesh *et al.* 2006]. Column: phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium acetate (pH 5) : acetonitrile (60 : 40), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 247 nm, λ_{em} = 367 nm). Retention time: 7.9 min. Limit of quantification, 5 µg/L [Hruska, Frye 2004]. Column: Capcell Pak MG120 (250 × 1.5 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate (52 : 48; pH 7.5 with triethylamine), flow rate 0.2 mL/min. Fluorescence detection (λ_{ex} = 247 nm, λ_{em} = 367 nm). Retention time: 4.5 min. Limit of quantification, 10 µg/L, limit of detection, 5 µg/L [Kim, Park 2004]. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L phosphate buffer (pH 2.6) : methanol (70 : 30), flow rate 1.2 mL/min. UV detection (λ = 245 nm). Retention time: 8.3 min. Limit of quantification, 5 µg/L [Kolte *et al.* 2003].

See also Mamidi *et al.* [2002], Muxlow *et al.* [2001]and Cox *et al.* [2000].

LC-MS Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.02 mol/L ammonium acetate buffer (pH 6.5) : acetonitrile (47 : 53), flow rate 0.20 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 5.7 min. Limit of quantification, 1 µg/L [He *et al.* 2007]. Column: Cyano (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 30 mmol/L ammonium acetate (pH 5.0; 80 : 20), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 3.2 min. Limit of quantification, 1.5 µg/L [Zhang *et al.* 2007]. Column: Thermo Fluophase PFP-RP (50 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% formic acid in acetonitrile : 10 mmol/L ammonium formate (40 : 60 for 1 min, to 95 : 5 over 2.5 min, to 40 : 60 over 2 min), flow rate 0.6 mL/min. Retention time: 1.7 min. Limit of quantification, 1 µg/L [Wang, Miksa 2007]. Column: phenyl (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate-0.02% trifluoroacetic acid (50 : 50), flow rate 0.3 mL/min. ESI, positive ion mode, MRM. Retention time: 3.5 min. Limit of quantification, 1 µg/L [Lin *et al.* 2004].

Urine **HPLC** Column: Hypersil BDS C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile-methanol (1 : 1) : 50 mmol/L ammonium acetate (pH 8.0; 0 : 100 for 5 min to 50 : 50 over 50 min to 95 : 5 over 5 min for 5 min), flow rate 1.0 mL/min. UV detection (λ = 247 nm). Retention time: rosiglitazone 59.3 min; phenoxyacetic acid derivative 17.4 min; *N*-desmethyl glucuronide-1 27.8 min; *N*-despyridinyl 30.8 min; *N*-desmethyl glucuronide-2 35.1 min; *o*-*O*-glucuronide 35.9 min; *N*-desmethyl-*p*-*O*-sulfate 36.9 min; *p*-*O*-glucuronide 38.4 min; *N*-desmethyl-*o*-*O*-sulfate 38.9 min; *N*-desmethyl-*o*-hydroxy 41.3 min; *N*-desmethyl-*p*-hydroxy 41.7 min; *o*-*O*-sulfate 41.9 min; unknown 43.6 min; *p*-*O*-sulfate 44.3 min; *o*-hydroxy 49.9 min; *N*-desmethyl 51.7 min; *p*-hydroxy 53.7 min. Limit of quantification not reported [Cox *et al.* 2000].

LC-MS Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate buffer (pH 5.0; 72 : 28), flow rate 0.5 mL/min. APCI,

positive ion mode, SRM acquisition mode. Retention time: 7.8 min. Limit of quantification, 0.3 μg/L, limit of detection, 0.1 μg/L [Chou *et al.* 2005].

Faeces **HPLC** See Urine [Cox *et al.* 2000].

Other **HPLC** Tablets. Column: Inertsil ODS-III (250 × 4.6 mm i.d., 5 μm). Mobile phase: buffer (3.45 g ammonium dihydrogen phosphate, 4.08 g potassium dihydrogen orthophosphate, 3 mL triethylamine in 3 L water, pH 5.0) : acetonitrile (50 : 50), flow rate 1.0 mL/min. UV detection (λ = 242 nm). Limit of quantification, 1 mg/L [Shaikh *et al.* 2007]. Column: C_{18} Inertsil ODS-III (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water-acetonitrile (5 : 95) : water-methanol (10 : 90; 100 : 0 : 0 for 1 min to 90 : 0 : 10 over 1 min to 20 : 50 : 30 over 4 min to 30 : 55 : 15 over 6 min to 10 : 85 : 5 over 10 min to 20 : 65 : 15 over 2 min to 40 : 50 : 10 over 1 min), flow rate 1.0 mL/min. UV detection (λ = 260 nm). Retention time: rosiglitazone 11.4 min; repaglinide 25.4 min; pioglitazone 13.3 min; glipizide 14.8 min; glyburide 20.7 min. Limit of quantification, 0.1 mg/L [Venkatesh *et al.* 2006]. Rat Bile. Column: C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate : acetonitrile : methanol (pH 6.5; 40 : 50 : 10), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 247 nm, λ_{em} = 367 nm). Retention time: rosiglitazone 8.2 min; desmethyl metabolite 4.8 min. Limit of quantification, 5 μg/L [Muzeeb *et al.* 2006]. Tablets. Column: C_{18} (150 × 4.6 mm i.d., 5 μm). Mobile phase: 10 mmol/L disodium hydrogen phosphate-5 mmol/L sodium dodecyl sulfate : acetonitrile (pH 7.1; 66 : 34), flow rate 1.0 mL/min. UV detection (λ = 226 nm). Retention time: 7.6 min. Limit of quantification, 0.2 mg/L [Kolte *et al.* 2004]. Column: C_{18} (125 × 4.0 mm i.d.). Mobile phase: 25 mmol/L potassium dihydrogen phosphate : acetonitrile (pH 6.2; 55 : 45), flow rate 0.8 mL/min. UV detection (λ = 247 nm). Retention time: 6.1 min. Limit of quantification, 0.71 mg/L, limit of detection, 0.23 mg/L [Gomes *et al.* 2004].

LC-MS Equine Urine or Plasma. Column: C_8 (100 × 2.1 mm i.d., 3 μm). Mobile phase: 10 mmol/L ammonium formate (pH 3.0) : methanol (70 : 30 to 0 : 100 over 8 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 6.5 min. Limit of detection, <1 μg/L [Ho *et al.* 2004].

CE Tablets. Column: fused silica capillary (total/effective length: 48/39.5 cm, 75 μm i.d.). Buffer: 10 mmol/L sodium tetraborate (pH 9.0) with 30 mmol/L sodium dodecyl sulfate. UV detection (λ = 247 nm). Migration time: 5.3 min. Limit of quantification, 4.26 mg/L; limit of detection, 1.41 mg/L [Gomes *et al.* 2004].

Note For a chiral separation of rosiglitazone enantiomers using CE, see Jamali *et al.* [2004]; for a spectrophotometric determination of rosiglitazone in tablets, see Gomes and Steppe [2006].

Disposition in the Body

Rosiglitazone is well absorbed from the gastro-intestinal tract after oral dosing, with peak plasma concentrations occurring within 1 h. There are no clinically relevant effects of food on rosiglitazone absorption or exposure. It is extensively metabolised via *N*-demethylation and hydroxylation, with subsequent sulfate and glucuronic acid conjugation. This is primarily mediated by CYP2C8, with CYP2C9 contributing to a lesser extent. Practically no unchanged parent drug is excreted. Elimination is predominantly via the urine (64%) and faeces (23%). Elimination of metabolites is slow, which may be explained by the high plasma protein binding of the *p*-hydroxysulfate metabolite.

Note The hepatic uptake transporter organic anion transporting polypeptide (OATP) $1B_1$ plays no significant role in the disposition of rosiglitazone [Kalliokoski *et al.* 2008].

Therapeutic Concentration

Four healthy volunteers were administered [^{14}C]rosiglitazone either as an IV infusion (2 mg in 20 mL over 60 min) or as an oral solution (8 mg in 80 mL). After a wash-out period of 50 days, they were given non-radiolabelled rosiglitazone tablets (8 mg as two 4 mg tablets). Mean peak plasma concentrations (C_{max}) were as follows:

Dose	*C_{max} (μg/L)*	*Time (h)*
Oral [^{14}C]rosiglitazone (8 mg)	564	0.5
IV [^{14}C]rosiglitazone (2 mg)	146	1.0
Rosiglitazone (8 mg)	603	0.75

The percentage of plasma radioactivity for rosiglitazone and its metabolites after the 8 mg oral dose of [^{14}C]rosiglitazone was as follows:

Compound	*Percentage of plasma radioactivity*				
	1 h	*4 h*	*8 h*	*24 h*	*Day 4*
N-Desmethyl-*p*-*O*-sulfate	0	2	3	10	11
o-*O*-Sulfate	0	2	2	1	2
p-*O*-Sulfate	18	46	60	65	80
N-Desmethyl	11	15	6	17	4
Rosiglitazone	71	34	19	2	0
Total identified	100	99	90	95	97

The mean percentages of dose (8 mg oral dose [^{14}C]rosiglitazone) associated with each identified metabolite in 0–8 day pooled urine and 0–7 day pooled faecal extracts were as follows:

Compound	*Urine (% dose)*	*Faeces (% dose)*
Phenoxyacetic acid derivative	3.7	ND
N-Desmethyl glucuronide	1.6	ND
N-Despyridinyl	1.2	ND
N-Desmethyl glucuronide	1.8	ND
o-*O*-Glucuronide	0.3	ND
N-Desmethyl-*p*-*O*-sulfate	17.7	ND
p-*O*-Glucuronide	3.8	ND
N-Desmethyl-*o*-*O*-sulfate	0.7	ND
N-Desmethyl-*o*-hydroxy	1.1	0.7
N-Desmethyl-*p*-hydroxy	ND	6.1
ortho-*O*-Sulfate	3.5	ND
Unknown	0.3	ND
p-*O*-Sulfate	15.9	ND
o-Hydroxy	0.1	0
N-Desmethyl	0.5	ND
p-Hydroxy	0.1	9.0
Rosiglitazone	0.1	ND
Total excretion assigned	52.3	15.8
Total excretion	55.3	17.7

ND, not determined.
[Cox *et al.* 2000].

Thirty-one women receiving a legal termination of pregnancy between 8 and 13 weeks of gestation were administered 8 mg rosiglitazone before surgery (one 4 mg tablet the night before surgery and another 4 mg on the day of surgery). The mean duration between the first and second doses and termination of pregnancy was 15.7 h and 3.8 h, respectively. The mean maternal serum rosiglitazone concentration was 110.3 μg/L. Rosiglitazone was detectable in 19 fetal samples at a mean of 52.7 ng/g and was more likely to be detected if the gestation at termination was 10 weeks or more compared with earlier gestation. The mean concentration of rosiglitazone in the coelomic fluid was 22.8 μg/L (only 22 samples collected). Amniotic fluid was collected from all participants. Rosiglitazone was only detectable in 2 of the samples: 10.3 and 12.6 μg/L [Chan *et al.* 2005].

A group of 57 patients with varying degrees of renal impairment was administered a single oral dose of 8 mg rosiglitazone. Mean peak plasma concentrations were as follows:

	Normal	*Renal impairment*		
		Mild	*Moderate*	*Severe*
C_{max} (μg/L)	461	454	475	359
Time (h)	2.0	2.0	2.0	2.0

[Chapelsky *et al.* 2003].

Toxicity Consistent with the extensive metabolism of rosiglitazone by CYP2C8 and CYP2C9, co-administration with CYP2C8 inhibitors (gemfibrozil, ketoconazole and trimethoprim) or inducers (rifampicin) can increase or reduce plasma concentrations of rosiglitazone, respectively [Hruska *et al.* 2005; Niemi *et al.* 2003, 2004]. However, quercetin, a potent inhibitor of CYP2C8, had no effect on rosiglitazone pharmacokinetics [Kim *et al.* 2005]. Rosiglitazone does not markedly alter CYP3A4-mediated drug metabolism, although there is evidence it may act as an extremely weak inducer of CYP3A4 [Harris *et al.* 1999]. Co-administration of rosiglitazone with nevirapine results in a significant reduction in nevirapine peak plasma concentration (44%); however, this is not seen with other antiviral drugs such as efavirenz or lopinavir [Oette *et al.* 2005]. Sitagliptin, sucralfate or ranitidine when co-administered individually with rosiglitazone do not alter the pharmacokinetics of rosiglitazone in healthy subjects [Miller *et al.* 2002; Mistry *et al.* 2007; Rao *et al.* 2002]. Acarbose has a small, but clinically insignificant, effect on rosiglitazone pharmacokinetics [Miller *et al.* 2001].

Bioavailability 99%.

Half-life Between 3 to 4 h.

Volume of Distribution Approximately 17.6 L.

Clearance Approximately 3.0 L/h.

Protein Binding Approximately 99.8% for both rosiglitazone and its *p*-hydroxysulfate form (major metabolite).

Dose Rosiglitazone is given as rosiglitazone maleate, but doses are expressed in terms of the base: 1.32 mg rosiglitazone maleate is equivalent to approx. 1 mg rosiglitazone. The usual initial dose is 4 mg daily, given in a single dose or 2 divided doses. The dose may be increased to a maximum of 8 mg daily if necessary after 8 to 12 weeks in patients receiving monotherapy or combination oral therapy.

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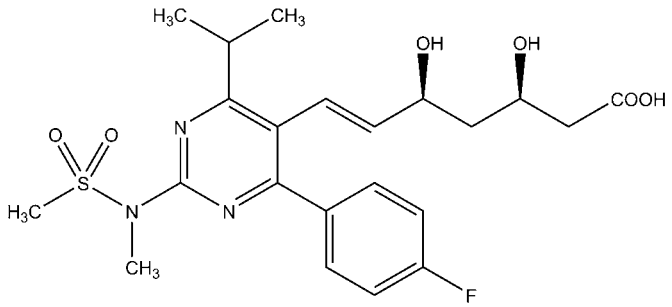
Rosuvastatin

Antihyperlipidaemic, HMG-CoA Reductase Inhibitor, Statin
$C_{22}H_{27}FN_3O_6S$ = 481.5
CAS—287714-41-4

IUPAC Name (*E*)-(3*R*,5*S*)-7-{4-(4-Fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino]pyrimidin-5-yl}-3,5-dihydroxyhept-6-enoic acid
Synonym ZD-4522

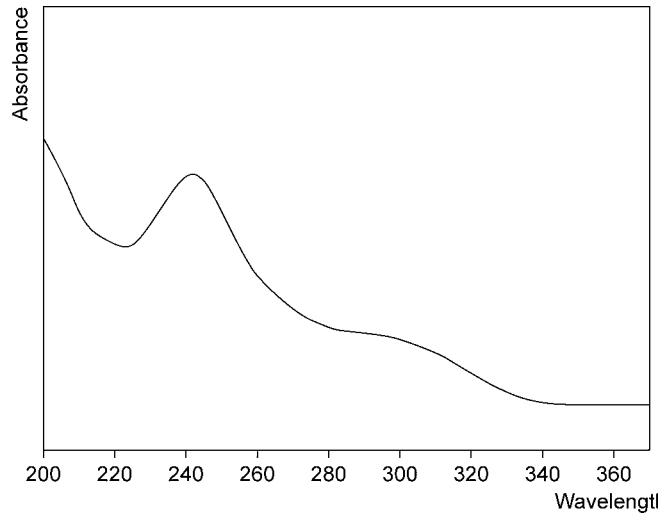


Chemical Properties Log *P* (octanol/water), 0.13 [O'Neil *et al.* 2006].

Rosuvastatin Calcium

$(C_{22}H_{27}FN_3O_6S)_2Ca$ = 1001.1
CAS—147098-20-2
Synonym S-4522
Proprietary Names *Cresadex*; *Crestor*; *Provisacor*; *Rosuvast*; *Rosuvas*; *Rovartal*; *Simestat*; *Sinlip*.
Chemical Properties White powder from water as the monohydrate. Begins to melt at 155° with no definitive Mp. Sparingly soluble in water, methanol; slightly soluble in ethanol [O'Neil *et al.* 2006].

High Performance Liquid Chromatography Column: C_{18} Inertsil ODS-3 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium dihydrogen phosphate dehydrate (pH 2.0) : acetonitrile : methanol (40 : 20 : 40), flow rate 1.0 mL/min. UV detection (λ = 242 nm). Retention time: 12.5 min. Limit of quantification, 30 µg/L; limit of detection, 10 µg/L [Mehta *et al.* 2005].
Ultraviolet Spectrum 250 nm



Quantification

Plasma **LC-MS** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 2% formic acid (80 : 20), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.38 min. Limit of quantification, 0.1 µg/L [Lan *et al.* 2007]. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (pH 6; 75 : 25), flow rate 0.5 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention time: 2.35 min. Limit of quantification, 0.2 µg/L [Gao *et al.* 2007]. Column: Inertsil ODS-3 (100 × 4.6 mm i.d., 3 µm). Mobile phase: 0.05 mol/L formic acid : acetonitrile (20 : 80), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.5 min. Limit of quantification, 0.05 µg/L [Kallem *et al.* 2007]. Column: C_{18} microbore (50 × 1.0 mm i.d., 3 µm). Mobile phase: water with 0.2% formic acid : methanol-water (95 : 5) with 0.2% formic acid (58 : 42 for 0.1 min to 5 : 95 over 1.4 min for 2 min), flow rate 0.06 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 1.7 min. Limit of quantification, 0.3 µg/L. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (7 : 3) with 0.2% formic acid, flow rate 1 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3.7 min. Limit of quantification, 0.3 µg/L [Oudhoff *et al.* 2006]. Column: Atlantis C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.2% formic acid : methanol (30 : 70), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 4.1 min. Limit of quantification, 0.2 µg/L [Xu *et al.* 2006]. Column: C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: 2.0 mmol/L ammonium formate (pH 3.4) : acetonitrile (70 : 30 for 2 min to 10 : 90 over 3 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1 µg/L; limit of detection, 0.3 µg/L [Bergman *et al.* 2006]. See also Trivedi *et al.* [2005]; Hull *et al.* [2002], [2004]; Martin *et al.* [2003].
Urine **LC-MS** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.2% formic acid in water (70 : 30), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 10 µg/L [Martin *et al.* 2003].
Bile **LC-MS** Column: C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: 2.0 mmol/L ammonium formate (pH 3.4) : acetonitrile (70 : 30 for 2 min to 10 : 90 over 3 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 20 µg/L, limit of detection, 2 µg/L [Bergman *et al.* 2006].
Other **HPLC** Rat Plasma. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L formic acid : acetonitrile (55 : 45), flow rate 1.0 mL/min. UV detection (λ = 240 nm). Retention time: 8.6 min. Limit of quantification, 20 µg/L [Kumar *et al.* 2006]. Tablets. Column: C_{18} Inertsil ODS-3 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium dihydrogen phosphate dehydrate (pH 2.0) : acetonitrile: methanol (40 : 20 : 40), flow rate 1.0 mL/min. UV detection (λ = 242 nm). Retention time: 12.5 min. Limit of quantification, 30 µg/L; limit of detection, 10 µg/L [Mehta *et al.* 2005].

Disposition in the Body Incompletely absorbed from the gastrointestinal tract, with peak plasma concentrations achieved ~5 h after an oral dose. It is taken up extensively by the liver, its primary site of action, and undergoes limited metabolism, mainly by CYP2C9. Approximately 90% of an oral dose of rosuvastatin is excreted in the faeces, including absorbed and non-absorbed drug, and the remainder is excreted in the urine; ~5% of a dose is excreted unchanged in urine. The pharmacodynamic effects and pharmacokinetics of rosuvastatin are not dependent on time of dosing. Systemic exposure to rosuvastatin has been observed to be ~2-fold higher in Japanese subjects living in Japan compared with Caucasian subjects in Western Europe or the USA.

Therapeutic Concentration

A group of healthy volunteers (male and female; young aged 18 to 35 years, elderly aged >65 years) was administered a single oral dose of 40 mg rosuvastatin after an overnight fast. Mean peak plasma rosuvastatin concentrations were reported as follows:

	Age		*Gender*	
	Young	*Elderly*	*Male*	*Female*
C_{max} (μg/L)	24.7	19.9	19.3	25.3
Time (h)	3.0	4.0	5.0	3.0

[Martin *et al.* 2002].

A study was conducted to determine whether the rosuvastatin pharmacokinetic differences observed between Japanese and white subjects extended to other Asian ethnic groups and to determine whether polymorphisms in the gene *SLCO1B1* contribute to this variation. The organic anion-transporting polypeptide 1B1 contributes to the hepatic uptake of rosuvastatin. Polymorphisms in *SLCO1B1* (521T→C) can lead to reduced transport function *in vitro*. Groups of Caucasian, Chinese, Malay and Asian-Indian subjects, living in the same environment, were genotyped for *SLCO1B1* polymorphisms (388A→G and 521T→C) and then administered a single oral 40 mg dose of rosuvastatin. Mean peak rosuvastatin plasma concentrations in the four groups were reported as follows:

	Chinese	*Asian-Indian*	*Malay*	*Cauacasian*
C_{max} (μg/L)	59.1	42.0	50.0	25.0
Time (h)	3.1	3.9	3.1	4.1

Similar increases in exposure to *N*-desmethylrosuvastatin and rosuvastatin-lactone were observed between the Chinese, Asian-Indian, and Malay subjects, compared with Caucasian subjects. However, *SLCO1B1* genotypes in this study did not account for the observed pharmacokinetic differences between the groups [Lee *et al.* 2005]. A similar study has shown that polymorphism affecting the breast cancer resistance protein (BCRP) (421C→A) may play an important role in the pharmacokinetics of rosuvastatin in healthy Chinese male subjects [Zhang *et al.* 2006].

A group of 32 healthy Caucasian volunteers with different *SLCO1B1* genotypes (c.521TT, c.521TC, c.521CC) was administered a single 10 mg oral dose of rosuvastatin. Mean peak plasma concentrations were reported as follows:

SLCO1B1 genotype	C_{max} *(μg/L)*	*Time (h)*
c.521TT	4.2	5.0
c.521TC	6.4	4.0
c.521CC	7.5	5.0

[Pasanen *et al.* 2007].

Toxicity Rosuvastatin undergoes limited metabolism, principally by CYP2C9, and may not have the same interactions with enzyme inhibitors as simvastatin. However, increased rosuvastatin plasma concentrations (up to 10-fold) have been reported with ciclosporin and, to a lesser extent, with gemfibrozil, and such combinations should be avoided.

The co-administration of itraconazole (an inhibitor of CYP3A4) with rosuvastatin produced modest increases in rosuvastatin plasma concentrations, supporting earlier findings that CYP3A4 plays a minor role in the limited metabolism of rosuvastatin [Cooper *et al.* 2003a]. Similar results were observed when erythromycin (a potent inhibitor of CYP3A4) was co-administered with rosuvastatin [Cooper *et al.* 2003b]. The co-administration of fluconazole (a potent inhibitor of CYP2C9 and CYP2C19) with rosuvastatin produced only small increases in rosuvastatin plasma concentrations [Cooper *et al.* 2002]. The co-administration of gemfibrozil with rosuvastatin increased rosuvastatin plasma concentrations ~2-fold, which is similar to the effect of gemfibrozil on other statins. Gemfibrozil inhibition of the organic anion transporter 2-mediated (OATP2) rosuvastatin hepatic uptake may contribute to the mechanism of the drug–drug interaction [Schneck *et al.* 2004].

Bioavailability Approximately 20%.

Half-life Approximately 19 h.

Volume of Distribution 134 L at steady state.

Protein Binding Approximately 90%.

Note For a review of drug interactions with lipid-lowering drugs, see Neuvonen *et al.* [2006].

Dose Given orally as the calcium salt, although doses are expressed in terms of the base; 10.4 mg of rosuvastatin calcium is equivalent to ≈10 mg base. The initial dose is 5 to 10 mg once daily, increased at intervals of 4 weeks, if necessary, to a usual maximum of 20 mg once daily. A higher dose of 40 mg once daily may be given under specialist supervision in severe hypercholesterolaemia but should not be given to patients at high risk of myopathy, including those receiving fibrates, and Asian patients; use with ciclosporin is contraindicated.

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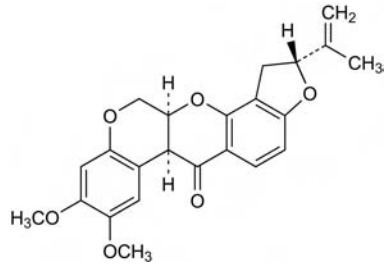
Rotenone

Ectoparasiticide

$C_{23}H_{22}O_6$ = 394.4

CAS—83-79-4

Synonym [2*R*-(2α,6*a*α,12*a*α)]-1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano[3,4-*b*]furo[2,3-*h*][1]benzopyran-6(6a*H*)-one



Chemical Properties Rotenone is the principal active insecticidal constituent of derris, the dried rhizome and roots of *Derris elliptica*, *D. malaccensis* (Leguminosae) and other species of *Derris* (also known as tuba root or akertuba), and of lonchocarpus, the dried root of *Lonchocarpus utilis*, *L. urucu* and other species of *Lonchocarpus* (Leguminosae). Colourless to brownish crystals, or a white

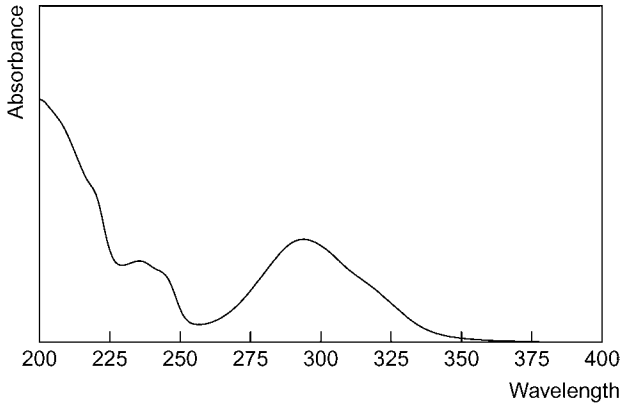
to brownish-white crystalline powder; it decomposes on exposure to light and air. Mp 165° to 166°. A diamorphic form melts at 185° to 186°. Practically insoluble in water; soluble 1 in 300 of ethanol, 1 in 12 of acetone, 1 in 3 of chloroform and 1 in 200 of ether. Log *P* (octanol/water), 4.1.

Caution Inhalation may cause severe pulmonary irritation. Symptoms of overexposure are irritation of eyes, skin and respiratory system, numbness of mucous membranes, nausea, vomiting, abdominal pain, muscle tremors, incontinence, clonic convulsions and stupor.

Colour Tests Antimony pentachloride—brown; Liebermann's reagent—black; sulfuric acid—orange.

Gas Chromatography System GA—RI 3195.

Ultraviolet Spectrum Aqueous acid—235 (A^1_1=480b), 292 nm (A^1_1=550b).



Roxithromycin

Antibacterial

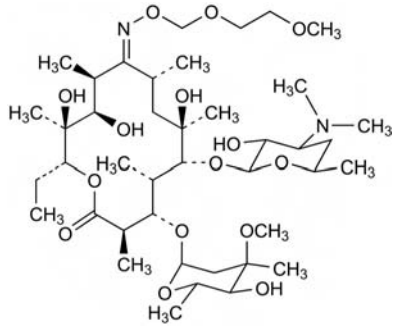
$C_{41}H_{76}N_2O_{15}$ = 837.0

CAS—80214-83-1

IUPAC Name (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,10*E*,11*S*,12*R*,13*S*,14*R*)-6-[4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-(5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl)oxy-10-(2-methoxyethoxymethoxyimino)-3,5,7,9,11,13-hexamethyl-oxacyclotetradecan-2-one

Synonyms Erythromycin 9-[*O*-[(2-methoxyethoxy)methyl]oxime]; roxithromycinum; RU-965; RU-28965.

Proprietary Names *Assoral*; *Biaxin*; *Biaxsig*; *Cirumyan*; *Claramid*; *Forilin*; *Macrosil*; *Overal*; *Rossitrol*; *Rotesan*; *Rotramin*; *Rulid*; *Surlid*.



Chemical Properties A white crystalline powder which exhibits polymorphism. Very slightly soluble in water; freely soluble in alcohol, acetone and dichloromethane; slightly soluble in dilute hydrochloric acid. Log *P* (octanol/water), 2.75.

High Performance Liquid Chromatography System HAA—retention time 15.8 min; system HBA—retention time 17.1 min; system HBB—retention time 16.3 min.

Column: (analytical) C_{18} Nucleosil 100-3 (150 × 4.6 mm i.d., 3 µm); (guard) C_{18} Nucleosil 120-5 (10 × 4.0 mm i.d., 5 µm). Column temperature: 60°. Mobile phase: methanol: potassium dihydrogen phosphate buffer (15 mmol/L, pH 6.0 with potassium hydroxide) (70:30), flow rate 1.2 mL/min. Internal standard (IS): clarithromycin. UV detection (λ=220 nm). Retention times: roxithromycin, 8.5 min; IS, 10.2 min [Macek *et al.* 1999].

Column: ODS Kromasil (200 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: methanol: aqueous buffer (3 mmol/L EDTA and 60 mmol/L sodium dihydrogen phosphate, pH 7.0 with sodium hydroxide) (39:11:49), flow rate 1.0 mL/min. Electrochemical detection. Retention times: roxithromycin, 31.5 min; descladinosine derivative, 13.4 min; erythromycin-oxime, 14.5 min; *N*-demethylated derivative, 19.8 min; *O*-demethylated derivative, 14.1 min; *N,O*-di-demethylated derivative, 10.2 min; *N*-mono-demethylated derivative of the erythromycin-oxime metabolite, 10.7 min; *N*-di-demethylated derivative of the erythromycin-oxime metabolite, 9.96 min [Zhong *et al.* 2000].

Quantification

Plasma **HPLC** Limit of quantification, 0.1 mg/L [Taninaka *et al.* 2000]. Electrochemical detection. Limit of detection, 0.01 mg/L [Zhong *et al.* 2000]. UV detection (λ=220 nm). Limit of quantification, 0.5 mg/L [Macek *et al.* 1999].

Urine **HPLC** Electrochemical detection. Limit of detection, 0.01 mg/L [Zhong *et al.* 2000].

Bile **HPLC** Electrochemical detection. Limit of detection, 0.01 mg/L [Zhong *et al.* 2000].

Disposition in the Body Roxithromycin is well absorbed but this is reduced when taken with food. It is widely distributed in tissue and body fluids. A small amount is metabolised in the liver by isomerisation, *O*-demethylation, *N*-demethylation, hydrolysis and dealkylation. Secondary metabolism may also occur involving these pathways. Metabolites include a descladinosine derivative of the drug, an erythromycin-oxime, *N*-, *O*-, and *N,O*-di-demethylated derivatives of the drug and *N*-mono- and *N*-di-demethylated derivatives of the erythromycin-oxime metabolite. The majority of the dose is excreted in faeces as the unchanged drug and as its metabolites. Approximately 7 to 12% is excreted in urine and 15% via the lungs. It crosses the placenta and is distributed in breast milk. Steady state is achieved by day 4.

Therapeutic Concentration

Eighteen paediatric patients suffering from a respiratory tract infection were administered with a 2.5 mg/kg oral dose of roxithromycin. The patients were divided into 3 groups; group 1: patients aged <18 months; 2: aged <5 years; 3: aged <13 years. The maximum plasma concentration on day 6 was observed at 1 to 2 h post-administration and was measured at 10.1, 8.7 and 8.8 mg/L, respectively, for the three groups [Demotes-Mainard *et al.* 1989].

Twelve healthy men with a mean age of 20.8 years (range, 18 to 28 years) were administered with 150 mg roxithromycin after an overnight fast and continued fasting for 4 h post-dosing. In a multiple dosing study for 5 days, 150 mg 12-hourly was also administered. The peak plasma concentration after the single dose was 5.69 (1.59 to 9.57) mg/L observed at 2.19 (0.5 to 8.0) h. The peak concentration for the multiple dosing was 6.76 (2.32 to 9.35) mg/L at 2.4 (0.5 to 6.17) h [Birkett *et al.* 1990].

Bioavailability 50%.

Half-life 8 to 13 h (prolonged in patients with hepatic and renal impairment); 20 to 21 h in children aged <18 months to 13 years.

Protein Binding 96% (mainly α_1-acid glycoprotein). Binding is saturable and only 86% is bound at peak concentrations.

Note For a review of roxithromycin antibacterial activity, pharmacokinetic properties and clinical efficacy, see Young *et al.* [1989].

Dose 150 mg twice daily.

Birkett DJ *et al.* (1990). Single oral dose pharmacokinetics of erythromycin and roxithromycin and the effects of chronic dosing. *Ther Drug Monit* 12(1): 65–71.

Demotes-Mainard FM *et al.* (1989). Pharmacokinetics of a new macrolide, roxithromycin, in infants and children. *J Clin Pharmacol* 29(8): 752–756.

Macek J *et al.* (1999). Determination of roxithromycin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B Biomed Sci Appl* 723: 233–238.

Taninaka C *et al.* (2000). Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *J Chromatogr B Biomed Sci Appl* 738: 405–411.

Young RA *et al.* (1989). Roxithromycin. A review of its antibacterial activity, pharmacokinetic properties and clinical efficacy. *Drugs* 37: 8–41.

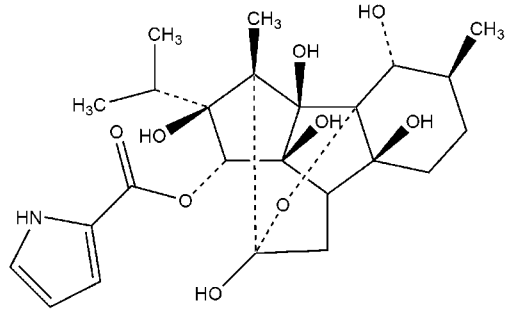
Zhong D *et al.* (2000). Identification of the metabolites of roxithromycin in humans. *Drug Metab Dispos* 28(5): 552–559.

Ryanodine

Insecticide, Alkaloid

$C_{25}H_{35}O_9$ = 493.6

CAS—15662-33-6



Chemical Properties Toxic alkaloid found in *Ryania speciosa* (Flacourtiaceae). Crystals. Soluble in water, alcohol, acetone, ether, chloroform. Practically insoluble in benzene and petroleum ether. Log *P* (octanol/water), 1.75 [Meylan, Howard 1995].

Ultraviolet Spectrum Alcohol—268.5 nm [O'Neil *et al.* 2006].

Toxicity Ryanodine has a high affinity for a type of calcium channel found in skeletal and cardiac muscle. Mutations of ryanodine receptor 1 (RyR 1) in skeletal muscle have been associated with malignant hyperthermia [Matsusue *et al.* 2009; Miyatake *et al.* 1996]. Nanomolar concentrations of ryanodine lock the receptors in the half-open state while micromolar concentrations close them. It is the nanomolar binding that leads to the release of calcium from intracellular stores in the sarcoplasmic reticulum, causing massive muscular contraction.

Matsusue A *et al.* (2009). Genetic analysis of ryanodine receptor 1 gene and carnitine palmitoyltransferase II gene: an autopsy case of neuroleptic malignant syndrome related to vegetamin. *Leg Med (Tokyo)* 11: 1S570–S572.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

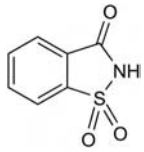
Miyatake R *et al.* (1996). No association between the neuroleptic malignant syndrome and mutations in the RYR1 gene associated malignant hyperthermia. *J Neurolog Sci* 143: 161–165.

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn, Whitehouse Station, NJ: Merck Research Laboratories.

Saccharin

Sweetener
$C_7H_5NO_3S$ = 183.2
CAS—81-07-2

IUPAC Name 1,1-Dioxo-1,2-benzothiazol-3-one
Synonyms 1,2-Benzisothiazol-3(2*H*)-one 1,1-dioxide; benzoic acid sulphimide; benzoic sulfimide; benzosulphimide; garantose; gluside; sacarina; saccarina; zaharina.



Chemical Properties White crystals or crystalline powder. Mp 228° to 230°. Soluble 1 in 290 of water, 1 in 31 of ethanol and 1 in 12 of acetone; slightly soluble in chloroform and ether; readily soluble in dilute ammonia solution and in solutions of alkali hydroxides and, with the evolution of carbon dioxide, in solutions of alkali bicarbonates and carbonates. pK_a 1.6 (20°); 1.3 (25°). Log *P* (octanol/water), 0.9.

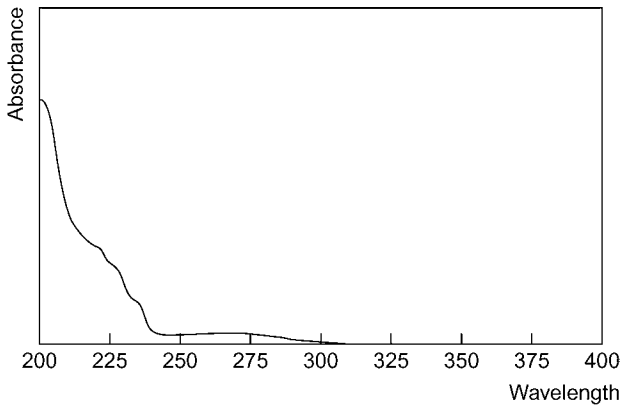
Saccharin Calcium

$C_{14}H_8CaN_2O_6S_2,3\frac{1}{2}H_2O$ = 467.5
CAS—6485-34-3 (anhydrous); 6381-91-5 (hydrate)
Synonym Calcium benzosulphimide
Chemical Properties White crystals or crystalline powder. Soluble 1 in about 2.5 of water and 1 in about 5 of ethanol.

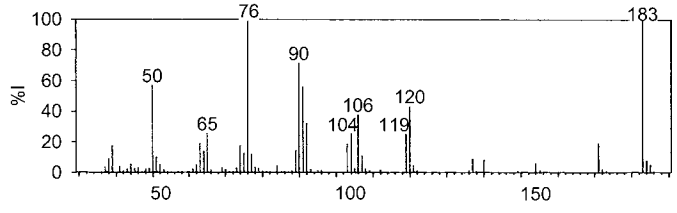
Saccharin Sodium

$C_7H_4NNaO_3S,2H_2O$ = 241.2
CAS—128-44-9 (anhydrous); 6155-57-3 (dihydrate)
Synonyms Saccharoidum natricum; sodium benzosulphimide; soluble gluside; soluble saccharin.
Chemical Properties White efflorescent crystals or a white crystalline powder. Soluble 1 in 1.5 of water and 1 in 50 of ethanol; practically insoluble in chloroform and ether.

Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.01; system TE—R_f 0.07; system TF—R_f 0.01; system TAD—R_f 0.04; system TAE—R_f 0.87; system TAJ—R_f 0.00; system TAK—R_f 0.19; system TAL—R_f 0.25.
Gas Chromatography System GA—RI 1819.
High Performance Liquid Chromatography System HX—RI 291; system HY—RI 234; system HAA—retention time 5.9 min.
Ultraviolet Spectrum Aqueous alkali—235 (A^1_1=351b), 268 nm (A^1_1=82a).



Infrared Spectrum Principal peaks at wavenumbers 1260, 1154, 1646, 750, 678, 1120 cm^{-1} (saccharin sodium, KBr disk).
Mass Spectrum Principal ions at *m/z* 183, 76, 90, 50, 91, 120, 106, 92.



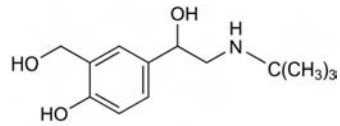
Quantification
Plasma **GC–MS** Limit of detection, 50 µg/L [Pantarotto *et al.* 1981].
Urine **GC** ECD. Limit of detection, 10 µg/L [Hartvig *et al.* 1978].
GC–MS Limit of detection, 50 µg/L [Pantarotto *et al.* 1981].
HPLC UV detection. Limit of detection, 10 ppm for saccharin sodium [Holder, Bowman 1980].
Disposition in the Body Saccharin is rapidly absorbed after oral administration; peak plasma concentrations are attained in 0.5 to 1 h. About 60% of a dose is excreted in the urine unchanged in 6 h and 80% in 24 h.
Half-life Plasma half-life, about 5 to 9 h in regular saccharin users.

Hartvig P *et al.* (1978). Determination of saccharin in urine by electron-capture gas chromatography after extractive methylation. *J Chromatogr* 151: 232–236.
Holder CL, Bowman MC (1980). Determination of sodium saccharin in animal feed, wastewater and human urine by high-pressure liquid chromatography. *Toxicol Lett* 5: 27–38.
Pantarotto C *et al.* (1981). GLC-mass fragmentographic determination of saccharin in biological fluids. *J Pharm Sci* 70: 871–874.

Salbutamol

β$_2$-Adrenoceptor Antagonist, Sympathomimetic
$C_{13}H_{21}NO_3$ = 239.3
CAS—18559-94-9

IUPAC Name α′-[[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol
Synonyms Albuterol; AH-3365; salbutamolum; Sch-13949W.
Proprietary Names *Accuneb*; *Aerolin*; *Airet*; *Airomir*; *Asmasal*; *Asmaven*; *Buventol*; *Kentamol*; *Libetist*; *Maxivent*; *Proventil*; *Rimasal*; *Salamol*; *Salapin*; *Salbulin*; *Ventmax*; *Ventodisks*; *Ventolin(e)*; *Volmax*. It is an ingredient of *Aerocrom*, *Combivent*, *DuoNeb*, *Ventide* (includes proprietary names of *Salbutamol*, *Salbutamol Sulfate*).

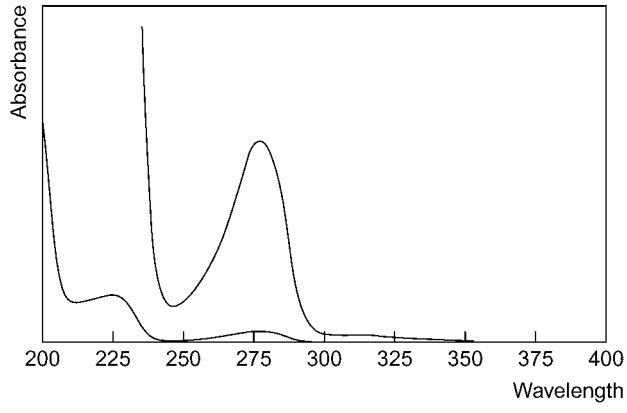


Chemical Properties A white crystalline powder. Mp reported as 151° or 157° to 158°. Soluble 1 in 70 of water and 1 in 25 of ethanol; slightly soluble in ether.

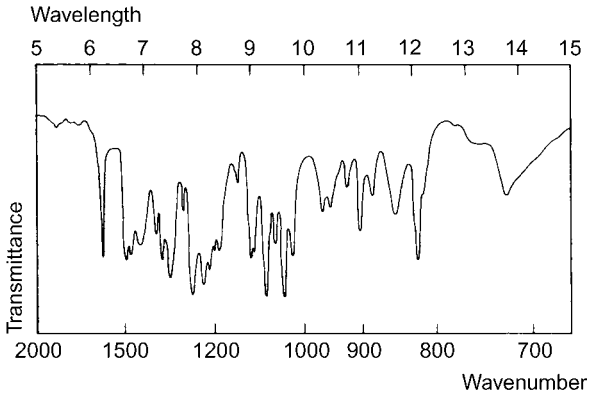
Salbutamol Sulfate

$C_{13}H_{21}NO_3,\frac{1}{2}H_2SO_4$ = 288.4
CAS—51022-70-9
Synonyms Albuterol sulfate; salbutamol hemisulfate; salbutamol sulphate.
Chemical Properties A white powder. Soluble 1 in 4 of water; slightly soluble in ethanol, chloroform, and ether. pK_a 9.3, 10.3. Log *P* (octanol/water), 0.6.

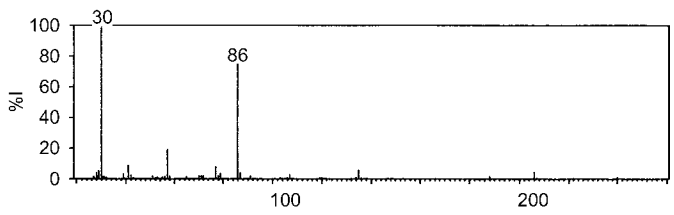
Colour Test Liebermann's reagent—black; Mandelin's test—blue rim→brown rim; Marquis test—yellow; sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.20; system T_f—R_f 0.00; system TL—R_f 0.04; system TAE—R_f 0.16; system TAF—R_f 0.74 (acidified potassium permanganate—positive).
Gas Chromatography System GA—salbutamol-AC_2 RI 2230, salbutamol-AC_3 RI 2250, salbutamol-H_2O RI 1850.
High Performance Liquid Chromatography System HA—*k* 1.0; system HX—RI 220; system HY—RI 238.
Ultraviolet Spectrum Aqueous acid—276 (A^1_1= 71a); aqueous alkali—245 (A^1_1= 510a), 295 nm (A^1_1= 133a).



Infrared Spectrum Principal peaks at wavenumbers 1075, 1038, 1263, 1228, 1213, 822 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 30, 86, 57, 41, 77, 135, 29, 206.



Quantification

Blood **GC-MS** Column: Ultra-2, cross-linked 5% phenyl methyl silicone column (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: H_2. Temperature: 250° and 210°. Limit of quantification, 1 µg/L [Couper, Drummer 1996].

Plasma **GC-MS** Column: DB-5 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2. Temperature: 250°. Limit of quantification, 50 ng/L [Logsdon *et al.* 1997].

HPLC Column: CT-CSP (250 × 4.5 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : glacial acetic acid : diethylamine (40 : 60 : 0.3 : 0.2), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 310 nm). Limit of detection, 125 ng/L [Fried *et al.* 1998]. Column: Chirex (250 × 4.0 mm). Mobile phase: hexane : 1,2-dichloromethane : methanol : trifluoroacetic acid (243 : 140 : 17 : 1), flow rate 1.0 mL/min. Florescence detection (λ_{ex} = 220 nm, λ_{em} = 309 nm). Limit of detection, 250 ng/L [Boulton, Fawcett 1995]. Column: Spherisorb S5 SCX (250 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : water (40 : 40 : 20), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 200 nm). Limit of detection, 1 µg/L [McCarthy *et al.* 1993]. Column: Spherisorb ODS (250 × 4.6 mm i.d., 10 µm). Mobile phase: 0.06 mol/L phosphate buffer (pH 5.0) : methanol : 40 g/L SDS : diethanolamine (45 : 55 : 0.5 : 0.02), flow rate 1.0 mL/min. Electrochemical detection. Limit of detection, 1 µg/L [Sagar *et al.* 1993]. Column: Kromasil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: propan-2-ol : methanol : acetonitrile : 0.1 mol/L phosphate buffer (pH 6.8; 2 : 2 : 3 : 93) with 0.4 mol/L sodium perchlorate, flow rate 1.7 mL/min. Electrochemical detection. Retention time: 7.5 min. Limit of detection, 0.25 µg/L [Tamisier-Karolak *et al.* 1992]. Column: Partisil SCX (50 × 4.6 mm i.d., 10 µm) or LiChrosorb RP-2 (250 × 4.6 mm i.d., 10 µm). Mobile phase: phosphate buffer (pH 7.5) containing 2.58 g sodium hydrogen phosphate and 0.3 g/L potassium hydrogen phosphate : 40 g/L sodium perchlorate-45 mL/L propan-2-ol in phosphate buffer (pH 7.0) containing 14 g/L sodium hydrogen phosphate and 7.5 g/L potassium hydrogen phosphate, flow rate 2.9 mL/min. Electrochemical detection. Limit of detection, 500 ng/L [Oosterhuis, van Boxtel 1982].

LC-MS Column: Bischoff (125 × 2.1 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 5.0; 75 : 25), flow rate 300 µL/min. MRM acquisition mode. Limit of quantification, 0.2 µg/L [Schmeer *et al.* 1997].

Serum **GC-MS** Column: HP-5 5% phenylmethylsiloxane (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 10.4 psi (72.6 kPa). EI ionisation at 70 eV. SIM acquisition and full scan mode. Limit of detection, 2 µg/L [Saleh *et al.* 2000]. Column: CP-Sil 8 (20 m × 0.5 mm i.d.). Carrier gas: He, 56 kPa. Temperature: 210°. Limit of detection, 1 µg/L [Leferink *et al.* 1982].

HPLC Column: Sumichiral OA 4700. Mobile phase: hexane : methylene chloride : methanol : trifluoroacetic acid (350 : 410 : 30 : 2), flow rate 1.0 mL/min. Fluorescence detection (λ = 228 nm). Limit of detection, 2 µg/L [He, Stewart 1992].

Urine **HPLC** Column: Chirex 3022 (250 × 4.0 mm i.d.). Mobile phase: hexane : dichlormethane : methanol : trifluoroacetic acid (250 : 218 : 31 : 1), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 309 nm). Limit of quantification, 36.1 and 34.6 µg/L for (*S*)(+)- and R(−)-salbutamol, respectively, limit of detection, 10.8 and 10.4 µg/L for (*S*)(+)- and R(−)-salbutamol, respectively [Berges *et al.* 1999]. Salbutamol enantiomers. Electrochemical detection. Limit of detection, 20 µg/L [Kim, Kim 1998]. See Plasma [Boulton, Fawcett 1995].

Disposition in the Body Salbutamol is rapidly absorbed after oral administration and after inhalation. It is not metabolised in the lung. Approximately 10–20% of an inhaled dose reaches the lower airways; the remainder is retained in the delivery system or deposited on the oropharynx and swallowed. Orally administered salbutamol, or the swallowed portion of an inhaled dose, undergoes first-pass metabolism in the liver and possibly in the gut wall to the phenolic sulfate. Approximately 60–90% of a dose is excreted in the urine in 24 h, of which ~50% is unchanged salbutamol and 50% is the 4′-*O*-sulfate of salbutamol. Up to ~12% is eliminated in the faeces.

Therapeutic Concentration

Following oral or rectal administration of racemic salbutamol solution (dose 0.1 mg/kg) to 5 healthy subjects, a peak serum concentration of 0.0179 mg/L was attained in 0.67 h after rectal administration compared with a concentration of 0.017 mg/L in 1.5 h after oral administration [Kurosawa *et al.* 1993]. In 11 asthmatic patients attending hospital with acute asthma who had been receiving salbutamol as part of their maintenance therapy, the plasma salbutamol concentration before treatment was <0.003–0.0346 mg/L (median, <0.003); 1 h after a dose of 5 mg nebulised salbutamol, the concentration was <0.003–0.056 mg/L (median, 0.0074) [Lewis *et al.* 1990]. After a single oral dose of 8 mg was given to 3 subjects, peak plasma concentrations of 0.096–0.117 mg/L were attained in 1–3 h. An inhaled dose of 0.04–0.1 mg given to 6 subjects produced peak plasma concentrations of 0.0006–0.0014 mg/L in 3–5 h. The ratio of salbutamol to an unidentified metabolite in the plasma was found to be 1 : 4 [Walker *et al.* 1972].

Toxicity Hypokalaemia has been reported after overdosage; recovery has occurred after the ingestion of up to 240 mg.

In a study of 17 patients admitted to emergency departments who had recently ingested an overdose of salbutamol, the mean dose ingested was 89 mg, which resulted in plasma concentrations of 0.018– 0.449 mg/L (median, 0.166) [Lewis *et al.* 1993].

Half-life Plasma half-life, 4–6 h.

Bioavailability After oral administration, ~50%.

Protein Binding ~10%.

Note For a review of salbutamol clinical efficacy in the 1980s, see Price, Clissold [1989]. For a review of the pharmacokinetics of levosalbutamol, see Boulton, Fawcett [2001].

Dose Usually the equivalent of 6 to 16 mg salbutamol daily orally.

Berges R *et al.* (1999). Analytical methodology for enantiomers of salbutamol in human urine for application in doping control. *J Chromatogr B Biomed Sci Appl* 723: 173–184.

Boulton DW, Fawcett JP (1995). Determination of salbutamol enantiomers in human plasma and urine by chiral high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 672: 103–109.

Boulton DW, Fawcett JP (2001). The pharmacokinetics of levosalbutamol: what are the clinical implications. *Clin Pharmacokinet* 40: 23–40.

Couper FJ, Drummer OH (1996). Gas chromatographic-mass spectrometric determination of beta 2-agonists in postmortem blood: application in forensic medicine. *J Chromatogr B Biomed Appl* 685: 265–272.

Fried KM *et al.* (1998). Determination of the enantiomers of albuterol in human and canine plasma by enantioselective high-performance liquid chromatography on a teicoplanin-based chiral stationary phase. *Chirality* 10: 484–491.

He L, Stewart JT (1992). A high performance liquid chromatographic method for the determination of albuterol enantiomers in human serum using solid phase extraction and chemical derivatization. *Biomed Chromatogr* 6: 291–294.

Kim KH, Kim TK (1998). Resolution of salbutamol enantiomers in human urine by reversed-phase high performance liquid chromatography after derivatization with 2,3,4,6-tetra-*O*-acetyl-beta-D-glucopyranosyl isothiocyanate. *Arch Pharm Res* 21: 217–222.

Kurosawa N *et al.* (1993). Serum concentration and cardiovascular effects of salbutamol after oral and rectal administration in healthy volunteers. *J Clin Pharm Ther* 18: 103–108.

Leferink JG *et al.* (1982). A time-saving method for the determination of the beta 2 sympathomimetics terbutaline, salbutamol and fenoterol. Preliminary results. *J Chromatogr* 229: 217–221.

Lewis LD *et al.* (1990). Plasma concentrations of salbutamol in acute severe asthmatics. *Aust NZ J Med* 20: 204–207.

Lewis LD *et al.* (1993). A study of self poisoning with oral salbutamol: laboratory and clinical features. *Hum Exp Toxicol* 12: 397–401.

Logsdon TW *et al.* (1997). Determination of albuterol in plasma after aerosol inhalation by gas chromatography–mass spectrometry with selected-ion monitoring. *J Chromatogr B Biomed Sci Appl* 692: 472–477.

McCarthy PT *et al.* (1993). Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection. *Biomed Chromatogr* 7: 25–28.

Oosterhuis B, van Boxtel CJ (1982). Determination of salbutamol in human plasma with bimodal high-performance liquid chromatography and a rotated disc amperometric detector. *J Chromatogr* 232: 327–334.

Price AH, Clissold SP (1989). Salbutamol in the 1980s. A reappraisal of its clinical efficacy. *Drugs* 38: 77–122.

Sagar KA *et al.* (1993). Simultaneous determination of salbutamol and terbutaline at overdose levels in human plasma by high performance liquid chromatography with electrochemical detection. *Biomed Chromatogr* 7: 29–33.

Saleh MI *et al.* (2000). Clean-up, detection and determination of salbutamol in human urine and serum. *Analyst* 125: 1569–1572.

Schmeer K *et al.* (1997). Rapid pharmacokinetic screening of salbutamol in plasma samples by column-switching high-performance liquid chromatography–electrospray mass spectrometry. *J Chromatogr A* 777: 67–72.

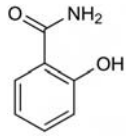
Tamisier-Karolak L *et al.* (1992). Plasma assay of salbutamol by means of high-performance liquid chromatography with amperometric determination using a loop column for injection of plasma extracts. Application to the evaluation of subcutaneous administration of salbutamol. *Ther Drug Monit* 14: 243–248.

Walker SR *et al.* (1972). The clinical pharmacology of oral and inhaled salbutamol. *Clin Pharmacol Ther* 13: 861–867.

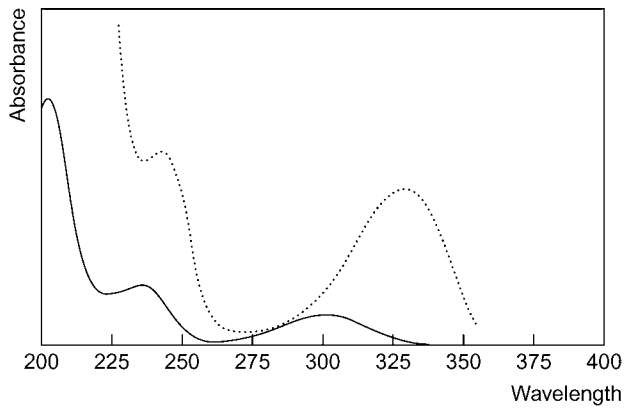
Salicylamide

Analgesic
$C_7H_7NO_2$ = 137.1
CAS—65-45-2

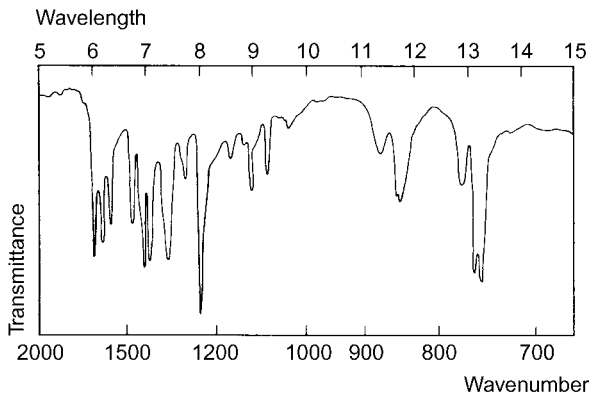
IUPAC Name 2-Hydroxybenzamide
Proprietary Names *Isosal*. It is an ingredient of *Anabar*, *Lobac*, *Intralgin*, *Saleto* and *Trim-Elim*.



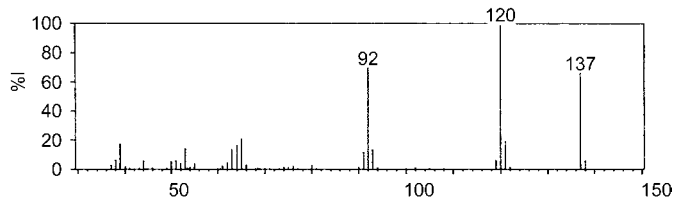
Chemical Properties A white crystalline powder. Mp 140°. Soluble 1 in 500 of water, 1 in 15 of ethanol, 1 in 100 of chloroform and 1 in 35 of ether; soluble in solutions of alkalis. pK_a 8.2 (37°). Log *P* (octanol/water), 1.3.
Colour Tests Ferric chloride—blue-violet (after hydrolysis); McNally's test—orange; Nessler's reagent—brown-orange (weak).
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.38; system TE—R_f 0.50; system TF—R_f 0.55; system TAD—R_f 0.43; system TAE—R_f 0.83; system TAF—R_f 0.84 (location under UV light, violet fluorescence; ferric chloride solution, violet; acidified potassium permanganate solution, positive).
Gas Chromatography System GA—salicylamide RI 1414, salicylamide-AC RI 1660, M (OH-)-AC_2 RI 1860; system GB—RI 1489.
High Performance Liquid Chromatography System HD—*k* 0.4; system HW—*k* 2.50; system HX—RI 327; system HY—RI 289.
Ultraviolet Spectrum Aqueous acid—235 (A_1^1=572a), 298 nm; aqueous alkali—241 (A_1^1=547a), 328 nm.



Infrared Spectrum Principal peaks at wavenumbers 1250, 752, 758, 1670, 1626, 1587 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 120, 92, 137, 65, 121, 39, 64, 53.



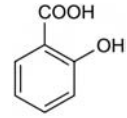
Quantification
Blood **Spectrofluorimetry** Limit of detection, 100 µg/L [Veresh *et al.* 1971].
Plasma **GC** AFID. Limit of detection, 1 mg/L [de Boer *et al.* 1979].
HPLC Fluorescence detection. Limit of detection, 500 pg [Gautam *et al.* 1981].
Serum **HPLC** UV detection. Limit of detection, 1 to 2 mg/L for salicylamide and metabolites [Morris, Levy 1983].
Spectrofluorimetry Limit of detection, 100 µg/L [Veresh *et al.* 1971].
Urine **GC** AFID. Limit of detection, 5 mg/L [de Boer *et al.* 1979].
HPLC UV detection. Limit of detection, 1 to 2 mg/L for salicylamide and metabolites [Morris, Levy 1983].
Spectrofluorimetry Limit of detection, 100 µg/L [Veresh *et al.* 1971].
Saliva **GC** AFID. Limit of detection, 1 mg/L [de Boer *et al.* 1979].
HPLC UV detection. Limit of detection, 1 to 2 mg/L for salicylamide and metabolites [Morris, Levy 1983].
Disposition in the Body Readily absorbed after oral administration but undergoes significant first-pass metabolism; widely distributed throughout the body. It is rapidly excreted in the urine mainly as the sulfate and glucuronide conjugates together with small amounts of gentisamide glucuronide and traces of gentisamide and gentisamide sulfate; only traces are excreted unchanged.
Therapeutic Concentration
After a single oral dose of 1.3 g given to 5 subjects, mean peak plasma concentrations of about 2 mg/L of free salicylamide and about 18 mg/L of total (free + conjugated) salicylamide were attained in 0.3 and 2 h respectively; following similar doses of a solution of sodium salicylamide, peak plasma concentrations were higher and were attained more rapidly [Fleckenstein *et al.* 1976].
Following a single oral dose of 1.5 g of sodium salicylamide given as an aqueous solution to 5 subjects, peak plasma concentrations of 21.8 to 44.6 mg/L (mean 31) of salicylamide were attained in 5 to 10 min [de Boer *et al.* 1983].
Half-life Derived from urinary excretion data, about 1 h.
Dose Salicylamide is given in doses of about 1 to 2.5 g daily, usually with other analgesics.

de Boer AG *et al.* (1979). Assay of underivatized salicylamide in plasma, saliva and urine. *J Chromatogr* 162: 457–460.
de Boer AG *et al.* (1983). First-pass elimination of salicylamide in man following oral and rectal administration. *Biopharm Drug Dispos* 4: 321–330.
Fleckenstein L *et al.* (1976). Sodium salicylamide: relative bioavailability and subjective effects. *Clin Pharmacol Ther* 19: 451–458.
Gautam SR *et al.* (1981). *Anal Lett (Part B)* 14: 577–582.
Morris ME, Levy G (1983). Determination of salicylamide and five metabolites in biological fluids by high-performance liquid chromatography. *J Pharm Sci* 72: 612–617.
Veresh SA *et al.* (1971). Spectrophotofluorometric determination of salicylamide in blood serum and urine. *J Pharm Sci* 60: 1092–1095.

Salicylic Acid

Dermatological Agent, Analgesic
$C_7H_6O_3$ = 138.1
CAS—69-72-7

IUPAC Name 2-Hydroxybenzoic acid
Synonyms Acido ortóxibenzoico; acidum salicylicum; salizylsäure.
Proprietary Names It is an ingredient of many proprietary preparations (Sweetman 2007).



Chemical Properties Colourless, feathery crystals or a white crystalline powder. Mp 159°. Soluble 1 in ~550 of water, 1 in ~4 of ethanol, 1 in 45 of chloroform and 1 in 3 of ether. Extraction yield (chlorobutane), 0 (salicylate) [Demme *et al.* 2005].

Choline Salicylate
$C_{12}H_{19}NO_4$ = 241.3
CAS—2016-36-6
Synonym (2-Hydroxyethyl)trimethylammonium salicylate
Proprietary Names *B Arthropan*; *Audax*; *Teejel*. It is an ingredient of *Bonjela*.
Chemical Properties A white, crystalline, very hygroscopic powder. Mp 49.5° to 50.0°. Very freely soluble in water; soluble in ethanol and acetone; practically insoluble in ether, petroleum ether, benzene, and oils.

Sodium Salicylate
$C_7H_5NaO_3$ = 160.1
CAS—54-21-7
Synonym Sodium 2-hydroxybenzoate
Proprietary Names *Dodds*; *Jackson's Febrifuge*. It is an ingredient of *Cystex*, *Doans Backache Pills*, *TCP Cream*.
Chemical Properties Colourless crystals, crystalline flakes, or white or faintly pink powder. Mp 440°. Soluble 1 in 1 of water and 1 in 11 of ethanol; practically insoluble in chloroform and ether. Concentrated aqueous solutions are liable to deposit crystals of the hexahydrate on standing. pK_a 3.0, 13.4 (25°). Log *P* (octanol/water), 2.3.

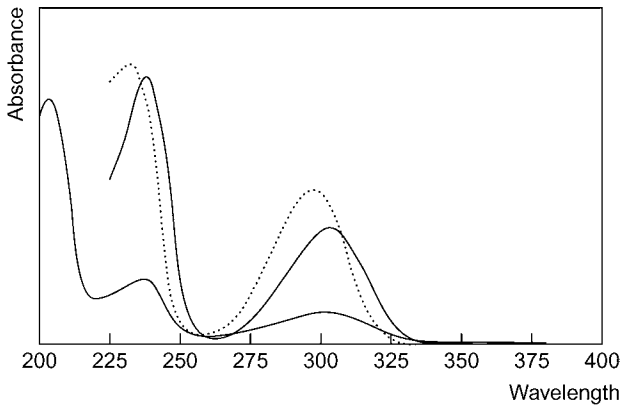
Colour Test Ferric chloride—blue-violet; Folin-Ciocalteu reagent—blue; McNally's test—red.

Thin-layer Chromatography System TD—salicylic acid R_f 0.07, salicyluric acid R_f 0.00; system TE—salicylic acid R_f 0.10, salicyluric acid R_f 0.00; system TF—salicylic acid R_f 0.01, salicyluric acid R_f 0.00; system TAD—R_f 0.24; system TAE—R_f 086; system TAJ—R_f 0.12; system TAK—R_f 0.71; system TAL—R_f 0.70 (acidified potassium permanganate—positive; ferric chloride—violet; location under UV light—violet fluorescence).

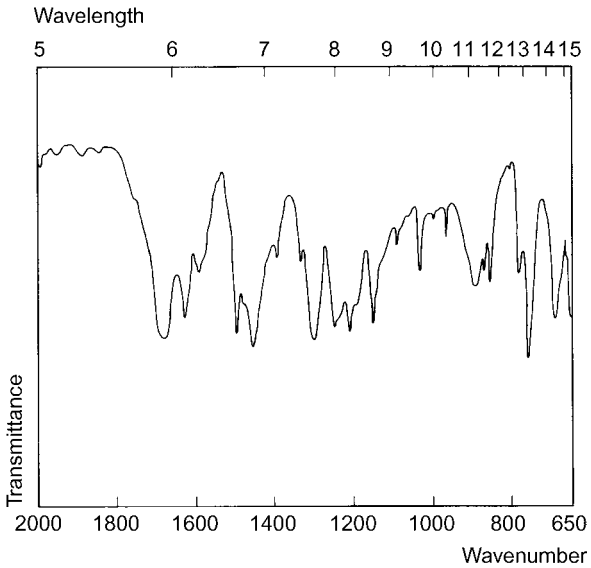
Gas Chromatography System GA—salicylic acid RI 1307, M (-Me) RI 1200, M ($-Me_2$) RI 1195, M (glycine conjugate) RI 1825, M (glycine conjugate-Me) RI 1810, M (glycine conjugate-Me_2) RI 1845; system GB—salicylic acid RI 1340, M (-Me) RI 1228; system GL— M ($-Me_2$)RI 1210, M (5-OH-Me_3) RI 1530.

High Performance Liquid Chromatography System HD—salicylic acid *k* 0.7, choline salicylate *k* 0.7; system HW—salicylic acid *k* 4.60, choline salicylate *k* 4.80; system HX—RI 350; system HY—RI 355; system HAA—RT 12.1 min; system HAX—RT 5.2 min; system HAY—RT 4.4 min.

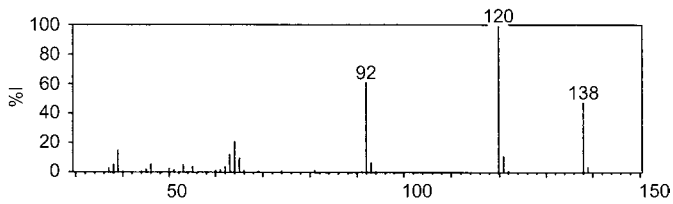
Ultraviolet Spectrum Aqueous acid—236 (A_1^1= 647a), 303 nm; aqueous alkali—(salicylic acid) 298 nm (A_1^1= 259a); methanol—(choline salicylate) 298 nm (A_1^1= 170b).



Infrared Spectrum Principal peaks at wavenumbers 758, 1657, 1288, 1210, 1250, 1150 (salicylic acid, KBr disk) ; 1587, 1724, 1176, 1515, 699, 1041 cm^{-1} (choline salicylate).



Mass Spectrum Principal ions at *m*/*z* 120, 92, 138, 64, 39, 63, 121, 65 (salicylic acid); 121, 120, 69, 92, 195, 39, 93, 45 (salicyluric acid).



Quantification See Aspirin monograph.

Disposition in the Body Salicylic acid is rapidly absorbed and distributed throughout the body. It is metabolised by conjugation with glucuronic acid and glycine to give salicyluric acid, salicyl *O*-glucuronide and salicyl ester glucuronide; hydroxylation to gentisic acid, gentisuric acid, and dihydroxy and trihydroxy derivatives also occurs. It is excreted in the urine as unchanged drug and metabolites. Salicylic acid is the major metabolite of aspirin, methyl salicylate and salsalate.

Therapeutic Concentration

Following daily oral doses of 9 mg/kg sodium salicylate to 20 female and 20 male subjects, mean peak plasma concentrations were 57 and 58 mg/L, respectively, attained in 0.9 and 0.5 h, respectively [Miaskiewicz *et al.* 1982].

After daily oral doses of choline salicylate, equivalent to 3.8 g aspirin, a mean steady-state serum concentration of 166 mg/L was reported [Hansten, Hayton 1980].

Toxicity The estimated minimum lethal dose is 15 g. Plasma concentrations greater than 300 mg/L are likely to produce toxic effects and concentrations greater than 500 mg/L are associated with moderate to severe intoxication.

In 80 subjects who had ingested an overdose of salicylate either in the form of aspirin tablets (n= 42) or as topical medicaments containing methyl salicylate or wintergreen oil (n= 38), the plasma salicylate concentrations on admission to hospital were generally higher in the subjects who had ingested aspirin tablets, although the 2 highest readings (4.3 and 3.5 nmol/L) belonged to subjects who had taken topical medicaments [Chan 1996].

A blood salicylic acid concentration of 6.04 mmol/L was reported in a patient with psoriasis 19 h after he had applied 40% salicylic acid ointment to ~41% of his body surface; following haemodialysis, the patient was discharged after 14 days [Pec *et al.* 1992].

Half-life Plasma half-life, dose dependent (2–4 h after salicylate doses of <3 g, increasing to ~19 h after large doses).

Volume of Distribution Approximately 0.1–0.2 L/kg (dose dependent).

Clearance Plasma clearance, ~1–2 mL/min/kg.

Protein Binding Approximately 50–90% (dose dependent, ~90% bound at concentrations below 100 µg/mL, decreasing to 50% at concentrations above 400 µg/mL).

Note For a review of the pharmacokinetics of salicylates, see Needs, Brooks [1985]; for a review of drug interactions involving aspirin and salicylic acid, see Miners [1989]; for a brief review of ototoxicity associated with salicylates, see Brien [1993].

Dose Salicylic acid is applied topically in concentrations usually ranging from 1 to ~6% as a keratolytic; up to 60% is used as a caustic in preparations for the removal of warts. Sodium salicylate is given orally in doses of up to 5.4 g daily.

Brien JA (1993). Ototoxicity associated with salicylates: a brief review. *Drug Saf* 9: 143–148.
Chan TY (1996). The risk of severe salicylate poisoning following the ingestion of topical medicaments or aspirin. *Postgrad Med J* 72: 109–112.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Hansten PD, Hayton WL (1980). Effect of antacid and ascorbic acid on serum salicylate concentration. *J Clin Pharmacol* 20: 326–331.
Miaskiewicz SL *et al.* (1982). Sex differences in absorption kinetics of sodium salicylate. *Clin Pharmacol Ther* 31: 30–37.
Miners JO (1989). Drug interactions involving aspirin (acetylsalicylic acid) and salicylic acid. *Clin Pharmacokinet* 17: 327–344.
Needs CJ, Brooks PM (1985). Clinical pharmacokinetics of the salicylates. *Clin Pharmacokinet* 10: 164–177.
Pec J *et al.* (1992). Salicylate intoxication after use of topical salicylic acid ointment by a patient with psoriasis. *Cutis* 50: 307–309.
Sweetman S, ed. (2007). *Martindale: The Complete Drug Reference*, 35th edn. London: Pharmaceutical Press.

Salinazid

Tuberculostatic

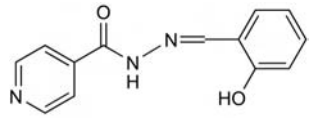
$C_{13}H_{11}N_3O_2$ = 241.2

CAS—495-84-1

IUPAC Name N′-[(6-Oxocyclohexa-2,4-dien-1-ylidene)methyl]pyridine-4-carbohydrazide

Synonyms *o*-Hydroxybenzal isonicotinylhydrazone; [(2-hydroxyphenyl)methylene]hydrazide 4-pyridinecarboxylic acid; INSH; SAH; salizid.

Proprietary Names *Acozid*; *Nupa-Sal*.

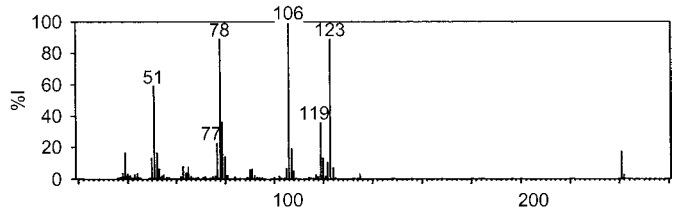


Chemical Properties A pale cream-coloured powder. Mp 232° to 233°; also reported as mp 251°. Very slightly soluble in water; soluble in dilute acids and alkalis. Log *P* (octanol/water), 1.8.

Colour Tests Aromaticity (method 2)—yellow/orange; ferric chloride—yellow-brown; Folin–Ciocalteu reagent—blue; Liebermann's reagent—orange; Mandelin's test—brown; Marquis test—yellow; Nessler's reagent (100°)—black; sulfuric acid—yellow.

S

Thin-layer Chromatography System TA—R_f 0.84; system TL—R_f 0.25; system TB—R_f 0.01; system TC—R_f 0.20. (Acidified iodoplatinate solution, positive.)
Ultraviolet Spectrum Aqueous acid—257 (A^1_1=697a), 327 nm (broad); aqueous alkali—264 nm.
Infrared Spectrum Principal peaks at wavenumbers 1290, 1680, 1565, 1270, 687, 772 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 106, 123, 78, 51, 119, 79, 77, 107.

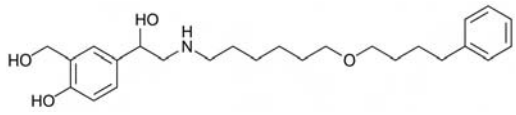


Dose Salinazid was formerly given in doses of 0.2 to 3 g daily.

Salmeterol

β_2-Adrenoceptor Agonist, Bronchodilator
$C_{25}H_{37}NO_4$ = 415.6
CAS—89365-50-4

IUPAC Name 2-(Hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl]phenol
Synonyms GR-33343X; 4-hydroxy-α^1-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-1,3-benzenedimethanol.

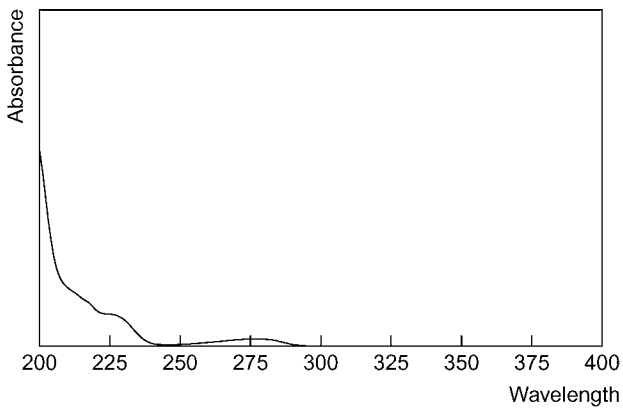


Chemical Properties Mp 75.5° to 76.5°.

Salmeterol Xinafoate

$C_{25}H_{37}NO_4.C_{11}H_8O_3$ = 603.7
CAS—94749-08-3
Synonym GR-33343G
Proprietary Names *Arial*; *Aeromax*; *Beglan*; *Betamican*; *Dilamax*; *Inaspir*; *Salmetedur*; *Seretide*; *Serevent*.
Chemical Properties White to off-white powder. Mp 137° to 138°. Freely soluble in methanol; sparingly soluble in water; slightly soluble in ethanol, chloroform and isopropanol.

High Performance Liquid Chromatography Column: (analytical) Spherisorb ODS II cartridge (100 × 4.6 mm i.d., 5 µm); (guard) ODS II (10 × 4.6 mm i.d.). Mobile phase: 0.05 mol/L phosphate buffer (pH 5) : methanol (60:40), flow rate 1 mL/min. Fluorescence detection (λ_{ex}=310 nm; λ_{em}=410 nm). Retention time: salmeterol xinafoate, 4.4 min [Chilton *et al.* 1995].
Ultraviolet Spectrum



Quantification

Plasma **HPLC** Fluorescence detection (λ_{ex}=310 nm; λ_{em}=410 nm). Limit of detection, 10 µg/L [Chilton *et al.* 1995].
Disposition in the Body Salmeterol is extensively metabolised by P450 cytochrome CYP3A4 by aliphatic oxidation to α-hydroxy-salmeterol, which is primarily excreted in faeces (57%) and also urine (23%). Less than 5% of the unchanged drug is detected in urine. After taking salmeterol xinafoate, xinafoic acid is detected in the systemic circulation and steady-state concentrations are approximately, 100 µg/L. This is 1000-fold less than the steady-state concentration observed in toxicity studies. Very low plasma concentrations have been observed at therapeutic doses (<200 ng/L) after an inhaled dose.

Therapeutic Concentration

Healthy individuals administered with single oral doses of 50 µg and 400 µg salmeterol produced peak plasma concentrations of 0.1–0.2 µg/L and 1–2 µg/L, respectively within 5 to 15 min. After a 1 mg dose, peak concentrations were approximately 0.6 µg/L at 45 min.

Repeated dosing of 50 µg twice daily to 6 patients with asthma, resulted in levels of 0.15 µg/L and dosing was continued for 10 months, a second peak could be observed between 0.07 and 0.2 µg/L 45 to 90 min after administration [Glaxo Wellcome 1996].

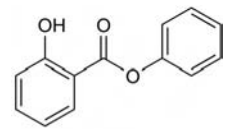
Toxicity Associated with cardiovascular and other non-pulmonary effects including headaches, tremors and palpitations.
Half-life 5.5 h for salmeterol; 15 days (healthy individuals); 30 days (patients with renal impairment) for xinafoic acid.
Volume of Distribution Steady state, 7.26 L.
Clearance Plasma, 18.6 mL/h (healthy); 10.8 mL/h (renal impairment).
Protein Binding 94–98%.
Note For a review of salmeterol, see Adkins, McTavish [1997].
Dose 50 to 100 µg twice daily (by inhalation).

Adkins JC, McTavish D (1997). Salmeterol. A review of its pharmacological properties and clinical efficacy in the management of children with asthma. *Drugs* 54: 331–354.
Chilton AS *et al.* (1995). The determination in human plasma of 1-hydroxy-2-naphthoic acid following administration of salmeterol xinafoate. *J Pharm Biomed Anal* 13(2): 165–169.
Glaxo Wellcome (1996). Data on file.

Salol

Analgesic, Antipyretic, Antiinflammatory
$C_{13}H_{10}O_3$ = 214.2
CAS—118-55-8

IUPAC Name Phenyl-2–hydroxybenzoate
Synonyms 2-Hydroxybenzoic acid phenyl ester; Phenyl salicylate.
Proprietary Name *Aussie Tan Sunstick*

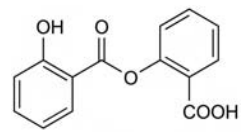


Chemical Properties Colourless acicular crystals, or white crystalline powder. Mp 41° to 43°. Soluble 1 in about 7000 of water, 1 in 6 of ethanol and 1 in 0.3 of chloroform and of ether. Log *P* (octanol/water), 3.8.
Gas Chromatography System GA—RI 1685.
High Performance Liquid Chromatography System HD—*k* 15.6.
Ultraviolet Spectrum Ethanol—242 (A^1_1=664a), 310 nm (A^1_1=248a).
Use Topically in concentrations of 5 to 10%.

Salsalate

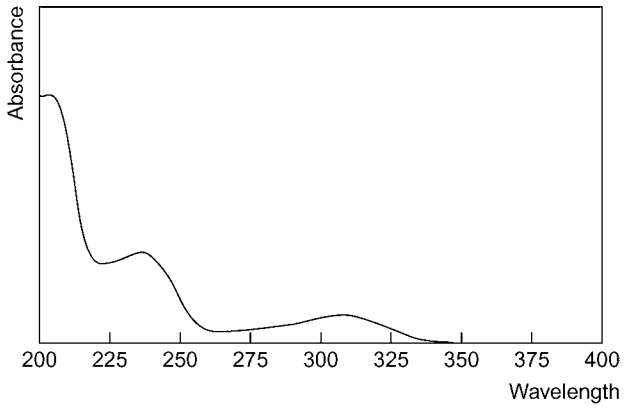
Analgesic
$C_{14}H_{10}O_5$ = 258.2
CAS—552-94-3

IUPAC Name 2-(2-Hydroxybenzoyl)oxybenzoic acid
Synonyms 2-Hydroxybenzoic acid 2-carboxyphenyl ester; salicyl salicylate; salicylosalicylic acid; salicylsalicylic acid; salysal; sasapyrine; NSC-49171.
Proprietary Names *Amigesic*; *Argesic-SA*; *Artha-G*; *Disalcid*; *Marthritic*; *Mono-Gesic*; *Salflex*; *Salsitab*; *Umbradol*.



Chemical Properties Colourless crystals or white crystalline powder. Mp about 147° to 149°. Practically insoluble in water but gradually hydrolysed to 2 molecules of salicylic acid; soluble in ethanol and ether; sparingly soluble in benzene. pK_a 3.5, 9.8 (25°). Log *P* (octanol/water), 3.4.
Thin-layer Chromatography System TG—R_f 0.23 (chromic acid solution, brown).
Gas Chromatography System GD—methyl derivative RRT 0.4 and RRT 0.6 (relative to *n*-$C_{16}H_{34}$).
High Performance Liquid Chromatography System HD—*k* 3.6; system HV—RRT 0.69 relative to meclofenamic acid.

Ultraviolet Spectrum Aqueous acid—204, 235, 310 nm; aqueous alkali—341 nm.



Infrared Spectrum Principal peaks at wavenumbers 1690, 1205, 1170, 1310, 1620, 1270 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** UV detection. Limit of detection, 1 mg/L for salsalate and salicylic acid [Harrison *et al.* 1980].

Urine **HPLC** See Plasma [Harrison *et al.* 1980].

Disposition in the Body Salsalate is rapidly absorbed after oral administration and extensively hydrolysed to salicylic acid. Less than 1% of a dose is excreted in the urine as unchanged salsalate in 24 h, and the remainder is excreted in the urine by the normal metabolic route for salicylic acid.

Therapeutic Concentration

Following a single oral dose of 1 g of salsalate to 12 subjects, a mean peak plasma concentration of 21 mg/L of salsalate was attained in 1.5 h and peak plasma-salicylic acid concentrations of 38–77 mg/L (mean 54) were reported at 2–4 h [Harrison *et al.* 1981].

Half-life Plasma half-life, ≈1 h.

Dose Up to 4 g daily.

Harrison LI *et al.* (1980). High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine. *J Pharm Sci* 69: 1268–1271.

Harrison LI *et al.* (1981). Absorption, biotransformation, and pharmacokinetics of salicylsalicylic acid in humans. *J Clin Pharmacol* 21: 401–404.

Santonin

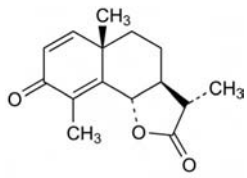
Anthelmintic

$C_{15}H_{18}O_3$ = 246.3

CAS—481-06-1

IUPAC Name (3*S*,3a*S*,5a*S*,9b*S*)-3,5a,9-trimethyl-3a,4,5,9*b*-tetrahydro-3*H*-benzo[*g*][1]benzofuran-2,8-dione

Synonyms Santolactone; α-santonin; [3*S*-(3α,3aα,5aβ,9bβ)]-3a5,5a,9b-tetrahydro-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,8(3*H*,4*H*)-dione.

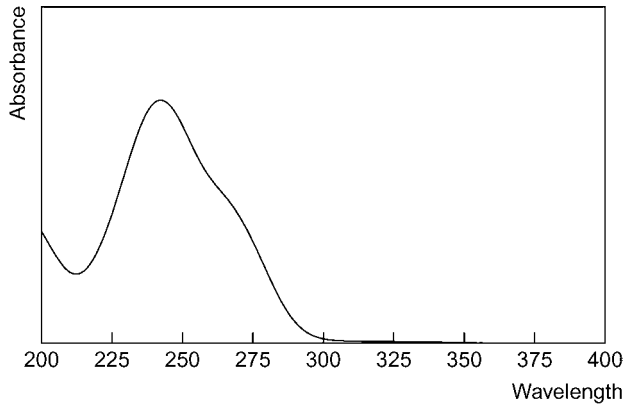


Chemical Properties A crystalline lactone obtained from the dried unexpanded flowerheads of *Artemesia cina* (santonica, wormwood) and other species of *Artemesia* (Compositae). Colourless crystals, or white crystalline powder, becoming yellow on exposure to light. Mp about 173°. Practically insoluble in water; soluble 1 in 50 of ethanol, 1 in about 4 of chloroform and 1 in 125 of ether. Log *P* (octanol/water), 1.8.

Thin-layer Chromatography System TD—R_f 0.64; system TE—R_f 0.75; system TF—R_f 0.50; system TAD—R_f 0.69; system TAF—R_f 0.79 (acidified iodoplatinate solution, positive; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 2174.

Ultraviolet Spectrum Methanol—239 nm (A^1_1=493a).



Mass Spectrum Principal ions at *m/z* 41, 173, 91, 135, 77, 55, 44, 69.

Dose Santonin was formerly given in doses of 60 to 200 mg daily for 3 days.

Saquinavir

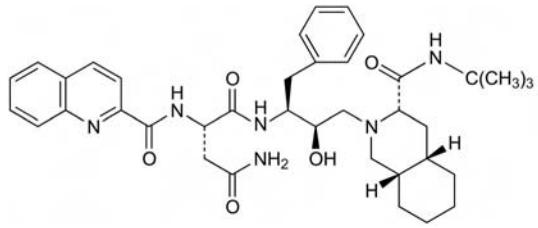
Antiviral

$C_{38}H_{50}N_6O_5$ = 670.8

CAS—127779-20-8

IUPAC Name (2*S*)-*N*-[(2*S*,3*R*)-4-[(3*S*,4a*S*,8a*S*)-3-(Tert-butylcarbamoyl)-3,4,4a,5,6,7,8,8a-octahydro-1H-isoquinolin-2-yl]-3-hydroxy-1-phenylbutan-2-yl]-2-(quinoline-2-carbonylamino)butanediamide

Synonym (2*S*)-N^1-[(1*S*,2*R*)-3-[(3*S*,4a*S*,8a*S*)-3-[[(1,1-Dimethylethyl)amino]carbonyl]octahydro-2(1*H*)-isoquinolinyl]-2-hydroxy-1-(phenylmethyl)propyl]-2-[(2-quinolinylcarbonyl)amino]butanediamide



Chemical Properties A white crystalline solid. Solubility: 0.22 g/100 mL water.

Saquinavir Mesilate

$C_{38}H_{50}N_6O_5.CH_3SO_3H$ = 767.0

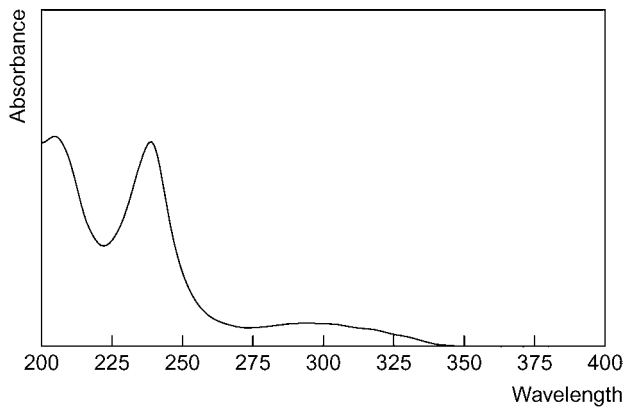
CAS—149845-06-7

Synonyms Ro-31-8959/003; Ro-31-8959.

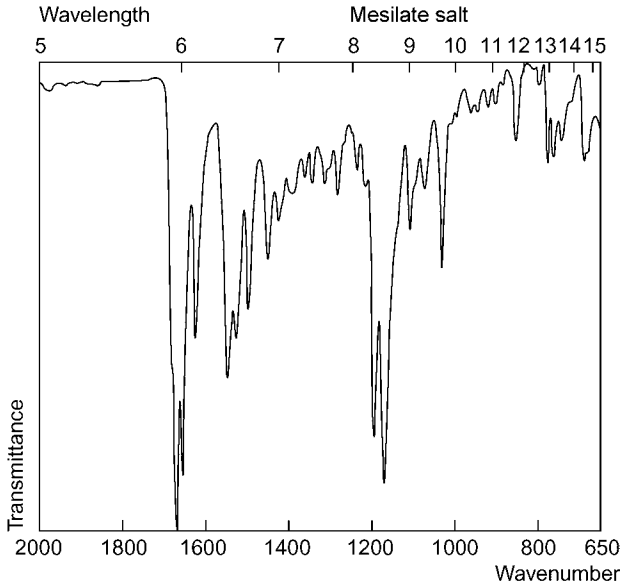
Proprietary Names *Invirase*; *Fortovase*.

High Performance Liquid Chromatography System HAC—*k* 9.5; system HAE—retention time 22.2 min.

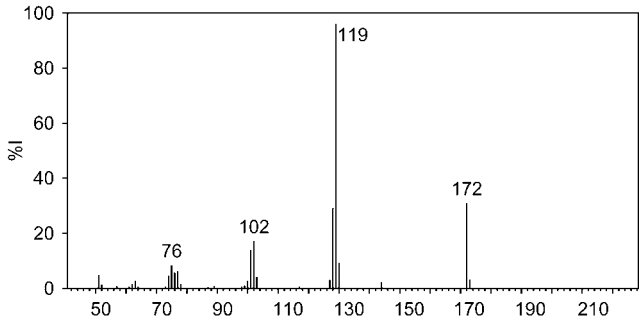
Ultraviolet Spectrum Aqueous acid—204, 238, 292 nm (saquinavir mesilate).



Infrared Spectrum Principal peaks at wavenumber 1670, 1658, 1198, 1173 cm^{-1} (saquinavir mesilate).



Mass Spectrum Principal ions at *m/z* 129, 172, 128, 102, 101, 130, 76, 77 (saquinavir mesilate).



Quantification

Plasma **HPLC** UV detection (λ = 241 nm). Limit of quantification, 5 µg/L [Aymard *et al.* 2000]. Limit of quantification, 100 µg/L [Proust *et al.* 2000]. UV detection (λ = 240 nm). Limit of detection, 1 µg/L [Ha *et al.* 1997].

Serum **HPLC** UV detection (λ = 250 nm). Limit of detection, 100 µg/L [Simon *et al.* 2001].

Disposition in the Body Saquinavir is absorbed but to a limited extent (30%) following oral administration and undergoes extensive first-pass metabolism in the liver. Peak plasma concentrations are observed approximately 0.77 h after administration of a suspension formulation and 3 to 4 h after capsules. It is extensively distributed into tissues but CNS concentrations are minimal. Metabolism is rapid by the cytochrome P450 system (CYP3A4) and a number of inactive monohydroxylated and dihydroxylated metabolites are formed. The drug is excreted mainly in faeces (88%) with only 1% in urine.

Therapeutic Concentration

Eight starved volunteers were administered with 600 mg saquinavir mesilate. Mean peak plasma concentrations of 20 µg/L were reached in less than an hour with a second peak of approximately, 0.4 µg/L after 5 h (after lunch) [Ha *et al.* 1997].

One hundred HIV-infected patients with $CD4^+$ cell counts between 50 and 301 cells/mm^3, mean age 41.5 years, were administered with 600 mg saquinavir three times a day plus 200 mg zidvudine three times a day for 24 weeks. The mean weekly peak saquinavir concentration was 70.8 µg/L after 3.11 h [Vanhove *et al.* 1997].

Bioavailability The oral bioavailability of saquinavir is low and is affected by the presence of food. In healthy volunteers receiving a single 600 mg dose after a heavy breakfast the bioavailability was found to be around 4%.

Half-life 13.2 h.

Volume of Distribution 700 L following an IV dose of 12 mg.

Clearance 1.14 L/h/kg. Plasma, 98.8 L/h.

Protein Binding Approximately 98% (independent of concentration over range 15 to 700 ng/mL).

Dose 1800 mg daily as 600 mg 3 times a day.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

Ha HR *et al.* (1997). Determination of saquinavir in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 694: 427–433.

Proust V *et al.* (2000). Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma. *J Chromatogr B Biomed Sci Appl* 742: 453–458.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1–2): 447–453.

Vanhove GF *et al.* (1997). Pharmacokinetics of saquinavir, zidovudine, and zalcitabine in combination therapy. *Antimicrob Agents Chemother* 41(11): 2428–2432.

Sarin

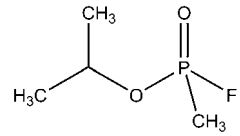
Organophosphate Nerve Agent, Anticholinesterase

$C_4H_{10}FO_2P$ = 140.1

CAS—107-44-8

IUPAC Name Isopropoxymethylphosphoryl fluoride

Synonyms GB; isopropyl methylphosphonofluoridate; methylphosphonofluoridic acid 1-methylethyl ester.



Chemical Properties Colourless, odourless liquid. Mp −57°. Bp 147°. Miscible in water. Log *P* (octanol/water), 0.299 [Munro *et al.* 1999]. Analytes were stable for at least 2 weeks in urine stored at 4° and for at least 2 years in urine samples stored at –70° [Barr *et al.* 2004].

Thin-layer Chromatography Plate: Silica gel 60–Kieselguhr F_{254}. Solvent system: *n*-Hexane : pyridine : dioxane (7 : 2;1). Reagent: ChE solution : β-naphthol acetate-*o*-dianisidine. Retention time: 0.57 min [Witkiewicz *et al.* 1990].

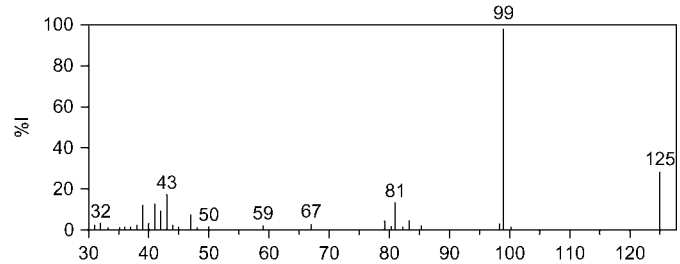
Gas Chromatography-Mass Spectrometry Column: SGE BPX5 5% phenyl 95% methylpolysiloxane (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 50° to 280° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Dubey *et al.* 2005; Gupta *et al.* 2005]. Column: DB5-MS (25 m × 0.22 mm i.d., 0.33 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 40° for 2 min to 160° at 20°/min to 310° at 30°/min for 5 min. Automatic thermal desorption, full scan mode. Limit of detection, 50 ng [Carrick *et al.* 2001]. Column: DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°/min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 ng/mL for isopropylmethylphosphonic acid metabolite (IMPA) [Shih *et al.* 1991].

High Performance Liquid Chromatography Column: C_9 Spherisorb (250 × 4.6 mm i.d., 10 µm) or LiChrosorb Hibar RP-18 (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: methanol : water (50 : 50) or methanol : phosphate buffer (50 : 50). UV detection (λ = 254 nm). Limit of detection, 3.3 ng [Witkiewicz *et al.* 1990]. Column: LiChrosorb RP_{18} (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: methanol : water (15 : 85 to 65 : 35). UV detection (λ = 254 nm). Limit of detection, 10 pg [Witkiewicz *et al.* 1990]. Column: LiChrosorb Hibar RP_{18} (250 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : water (15 : 85 to 65 : 35 over 35 min for 3 min). UV detection (λ = 254 nm). Limit of detection, 10 pg [Sipponen 1987].

Liquid Chromatography-Mass Spectrometry Column: Waters Atlantis HILIC (50 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate (86 : 14). ESI, negative ion mode, SRM acquisition mode. Limit of detection, 240 ng/L for IMPA [Mawhinney *et al.* 2007a]. The effect of post-column addition of organic solvents on this method is reported in Mawhinney *et al.* [2007b].

Capillary Electrophoresis Capillary: fused silica (56 cm (effective length) × 75 µm i.d.). Buffer: 200 mmol/L boric acid : 10 mmol/L phenylphosphonic acid : 0.03% Triton X-100 : 0.35 mmol/L didodecyldimethylammonium hydroxide. UV detection (λ = 210 nm). Limit of quantification, 0.5 mg/L; limit of detection, 0.1 mg/L for IMPA [Nassar *et al.* 1998].

Mass Spectrum Principal ions at *m/z* 99, 125, 43, 81, 41, 39, 42, 47; IMPA-PFB derivative 181, 256, 80, 303, 97, 276, 161, 179.



Quantification

Blood **GC-MS** Column: 5890B capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 2 min to 150° at 8°/min to 280° at 15°/min for 10 min). EI ionisation at 70 eV. Limit of detection not reported

[Nagao *et al.* 1997, 2003]. Column: DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°/min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Shih *et al.* 1991]. Column: HP-SE-54 bonded methylsilicon (15 m). Temperature: 60°. EI ionisation at 70 eV. Limit of detection not reported [Singh *et al.* 1985]

Plasma **GC-MS** DB-5MS bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 2 min to 100° at 10°/min for 3 min. EI ionisation at 70 eV. SIM acquisition mode. Limit of detection, 0.5 μg/L [Adams *et al.* 2004].

Serum **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 μg/L for IMPA [Miki *et al.* 1999].

HPLC Column: Shim-pack IC-A3 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.5 mmol/L phthalic acid : 0.1 mmol/L Tris : 5% acetonitrile, flow rate 1 mL/min. UV detection (λ = 266 nm). Limit of detection, IMPA 40 μg/L [Katagi *et al.* 1997].

LC-MS Column: μs-CrestPak C_{18}S (150 × 2.2 mm i.d.). Mobile phase: 0.22% formic acid : formic acid-methanol (0.2 : 99.8; 95 : 5 to 50 : 50 at 50 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 4 ng butyrylcholinesterase (BuChE) per injection [Tsuge, Seto 2006]. Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45) containing 0.1% glycerol, flow rate 100 μL/min. FAB ionisation. Limit of detection, IMPA 1 μg/L [Katagi *et al.* 1999].

Urine **GC** Column: HR-1 fused silica (50 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2 mL/min. Temperature programme: 50° to 80° at 4°/min for 7.5 min to 200° at 25°/min for 4.8 min. FID. Limit of detection, 0.01 μg/L [Hui, Minami 2000]. Column: HP-5 (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 1 min to 250° at 10°/min for 25 min. FPD. Limit of detection, 10 μg/L for methyl phosphonic acid metabolite (MPA) [Nakajima *et al.* 1998]. Column: Shimadzu CBPI-M50-0.25 fused silica (50 m × 0.25 mm i.d.). Carrier gas: He, 2.0 mL/min. Temperature programme: 100° to 200° in 5 min for 5 min to 290° in 5 min for 5 min. Flame photometric detection (λ = 526 nm). Limits of detection, IMPA 0.025 μg/L, MPA 0.625 μg/L [Minami *et al.* 1997].

GC-MS Column: Restek Rtx-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 40 cm/s. Temperature programme: 60° for 1 min to 180° at 30°/min to 280° at 10°/min for 2 min. CI, negative ion mode, SRM acquisition mode. Limit of detection,0.1 μg/L for IMPA [Riches *et al.* 2005]. Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 70° for 1.5 min to 250° in 5 min at 40°/min for 3 min. MRM acquisition mode. Limit of detection, 0.6 μg/L for IMPA [Barr *et al.* 2004]. Column: DB-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 1.5 min to 250° at 40°/min in 5 min for 5 min. SRM acquisition mode. Limit of detection, 3 μg/L [Driskell *et al.* 2002]. Column: DB-1 megabore (30 m × 0.53 mm i. d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 2.5 μg/L for IMPA [Miki *et al.* 1999]. Column: DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°/min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, IMPA 10 μg/L [Shih *et al.* 1991].

LC-MS Column: Xterra MS (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.5% formic acid : methanol (80 : 20 for 1.5 min to 10 : 90 at 2 min to 0 : 100 at 2.5 min until 8.5 min to 80 : 20 at 8.6 min for 2.4 min), flow rate 200 μL/min. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 0.5 ng/L [Ciner *et al.* 2007].

Albumin **GC-MS** DB-5MS bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 2 min to 100° at 10°/min for 3 min. EI ionisation at 70 eV. SIM acquisition mode. Limit of detection, 0.5 μg/L [Adams *et al.* 2004].

Oral Fluid **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 μg/L for IMPA [Miki *et al.* 1999].

Brain **GC-MS** Cerebella. HP-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° for 2 min to 150° at 8°/min to 280° at 5°/min for 10 min. EI ionisation at 70 eV. Limit of detection not reported [Matsuda *et al.* 1998].

Other **GC** Military-related Waste. Column: DB-5 (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 20 mL/min. Temperature programme: 60°t o 90° at 10°/min for 1 min to 220° at 45°/min for 1.1 min. FPD. Limit of detection, non-contaminated metal 8.2 μg/kg, non-contaminated decontamination fluid 2.2 μg/L, suspect-contaminated decontamination fluid 3.7 μg/L, non-contaminated soil 4.8 μg/kg, suspect-contaminated soil 4.4 μg/kg [O'Neill *et al.* 2002]. Water. Column: CP-Sil 19 (50 m × 0.32 mm i.d., 0.4 μm). Temperature programme: 60° for 3 min to 70° at 5°/min for 7 min to 180° at 10°/min to 240° at 15°/min for 12 min. Limit of detection, ng/L range [Degenhardt-Langelaan, Kientz 1996].

GC-MS Airborne Particles. Column: DB1-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 45 cm/s. Temperature programme: 35° for 1 min to 150° at 20°/min. EI ionisation at 70 eV. Limit of quantification, 2.5 mg/m^3; limit of detection, 0.1 mg/m^3 [Hook *et al.* 2004]. Clothing, Grave Debris, Soil, and Munition Fragments. Column: BP5 SGE (25 m × 0.22 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 40° for 0.5 min to 300° at 15°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 to 50 ng/sample [Black *et al.* 1994].

LC-MS Minipig Plasma. Column: Hichrome RPB C_8/C_{18} (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.1% formic acid in methanol : 0.1% formic acid (5 : 95 for 5 min to 80 : 20 at 15 min for 1 min to 5 : 95 over 2 min), flow rate 0.8 mL/min. ESI, negative ion mode, TOF. Limit of quantification, 5 μg/L for IMPA, limit of detecton, 2 μg/L for IMPA [Evans *et al.* 2008]. Spiked Food, Bottled Water, Canola Oil, Cornmeal, and Honey. Column: Agilent Zorbax SB-C_{18} (50 × 0.3 mm i.d., 1.8 μm). Mobile phase: 0.1% trifluoroacetic acid in water : acetonitrile (95 : 5 to 50 : 50 over 15 min), flow rate 10 μL/min. ESI, APCI, orFAIMS. Limit of quantification, 2 and 129 μg/L using APCI with and without FAIMS, respectively; limit of detection, 0.5 and 39 μg/L using APCI with and without FAIMS, respectively. Limit of quantification, 800 and 2000 μg/L for IMPA using ESI with and without FAIMS, respectively; 3900 μg/L for IMPA using APCI with FAIMS. Limit of detection, 200 and 750 μg/L for IMPA using ESI with and without FAIMS, respectively; 1200 μg/L for IMPA using APCI with FAIMS [Kolakowski *et al.* 2007]. Office Media. Column: Zorbax C_{18} SB fused silica capillary (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid : acetonitrile (95 : 5 to 25 : 75 over 30 min), flow rate 10 μL/min. ESI. Limit of detection, ≥ mg/kg [D'Agostino *et al.* 2006]. Soil. Column: Zorbax C_{18} SB fused silica capillary (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 99 : 1 to 25 : 75 over 30 min), flow rate 200 μL/min. ESI. Limit of detection not reported [D'Agostino *et al.* 2001]. River Water. Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45), containing 0.1% glycerol, flow rate 100 μL/min. FAB ionisation. Limit of detection, 1 μg/L for IMPA [Katagi *et al.* 1999]. Aqueous Samples. Column: Zorbax C_{18} SB (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 99 : 1 to 25 : 75 over 30 min). Limit of detection not reported [D'Agostino *et al.* 1999].

Note Hand-held detectors for the quantitative detection of sarin are in development. For information on photoionisation instruments, see Smith *et al.* [2007]; for studies of biomarkers as indicators of exposure to sarin, see Williams *et al.* [2007] or Abu-Qare and Abou-Donia [2001]. For a review article on the chromatographic analysis of chemical warfare agents, see Witkiewicz *et al.* [1990].

Disposition in the Body Sarin is rapidly absorbed and distributed in tissue following absorption from vapour or liquid. It is generally metabolised very rapidly after administration in animals and humans, although the rate depends on the route of administration and species studied. It is widely accepted that the lethality of sarin increases with increasing temperature. Esterase enzymes, such as paraoxonase, spontaneously hydrolyse sarin to inactive metabolites, first to IMPA and then to MPA. The major route of excretion of sarin and its metabolites is via the kidneys [Abu-Qare, Abou-Donia 2002].

Toxicity

A 19-year-old who opened the window from his third floor apartment located ~50 m away from the site where sarin was released was found unconscious 2 h later. Urine was collected on the first, third, and seventh day of hospitalisation and analysed for IMPA and MPA by GC-FID. The following urine concentrations were reported:

Metabolite	*Urine (mg/L) on day*		
	1	3	7
IMPA	0.76	0.08	0.01
MPA	0.14	0.02	–

The total excretion of IMPA and MPA in the urine was 2.1 and 0.45 mg, respectively. The subject was considered to have been exposed to 2.79 mg sarin at the incident [Nakajima *et al.* 1998].

Serum samples of the Japanese victims of the Matsumoto incident and the Tokyo subway incident were analysed by LC-MS for exposure to sarin. Activity of BuChE was also determined within 1.5 to 2.5 h after the incidents. The results were reported as per the table overleaf.

The results appear to show that the degree of initial inhibition of BuChE correlates with the amount of IMPA in the serum samples [Noort *et al.* 1998].

The cerebella from 4 of those who died in the Tokyo subway attacks were obtained at postmortem and stored in fixative (formalin) for ~2 years. They were then analysed by GC-MS for MPA as indicator of sarin exposure. The reported concentrations were 31, 77, 109 and 46 ng/g. [Matsuda *et al.* 1998].

Note For a study on the role of diazepam in the treatment of nerve agent poisoning, see Marrs [2004]; for a review of the toxicity of tabun, sarin and VX, see Munro [1994].

Half-life A time-course analysis of IMPA in urine from an intoxicated patient showed that the maximum IMPA concentration was obtained within 12 h, suggesting that the half-life of sarin is short.

Abu-Qare AW, Abou-Donia MB (2001). Combined exposure to sarin and pyridostigmine bromide increased levels of rat urinary 3-nitrotyrosine and 8-hydroxy-2′-deoxyguanosine, biomarkers of oxidative stress. *Toxicol Lett* 123: 51–58.

Abu-Qare AW, Abou-Donia MB (2002). Sarin: health effects, metabolism, and methods of analysis. *Food Chem Toxicol* 40: 1327–1333.

Adams TK *et al.* (2004). The application of the fluoride reactivation process to the detection of sarin and soman nerve agent exposures in biological samples. *Drug Chem Toxicol* 27: 77–91.

Barr JR *et al.* (2004). Quantitation of metabolites of the nerve agents sarin, soman, cyclohexylsarin, VX, and Russian VX in human urine using isotope-dilution gas chromatography–tandem mass spectrometry. *J Anal Toxicol* 28: 372–378.

Black RM *et al.* (1994). Application of gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry to the analysis of chemical warfare samples, found to

Incident and victim number [a]	*BuChE activity (arbitrary units)*	*IMPA (μg/L)*
Tokyo		
1	21	100
1[b]	28	86
2	126	24
2[b]	134	9
3	126	26
3[b]	116	15
4	126	16
5	583	4
6	818	2
7[c]	1100	ND
8	1131	6
9[c]	804	24
10	66	43
11[c]	172	66
Matsumoto		
12	166	78
13	52	136
14[c]	224	2
15[c]	1460	ND
16[c]	761	11
17[c]	1172	ND
18[c]	1186	3

[a]Victims were male, unless noted otherwise.
[b]Second sample taken from a victim at 2 to 2.5 h after arrival in hospital, whereas all other samples were taken within 1.5 h after hospitalisation.
[c]Female victim.
ND, not determined.

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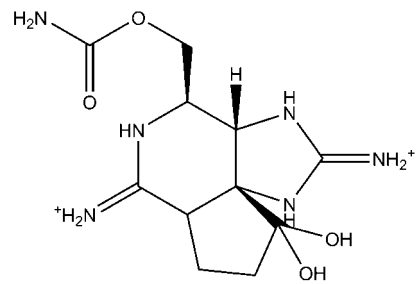
Saxitoxin

Neurotoxin, Sodium Transport Inhibitor, Tetrahydropurine
$[C_{10}H_{17}N_7O_4]^{2+} = 299.3$
CAS—35554-08-6

Synonyms Clam poison; gonyaulax toxin; mussel poison; paralytic shellfish poison; STX.



Chemical Properties Paralytic shellfish poisoning (PSP) toxins are present in some genera of dinoflagellates and one species of blue-green algae. Several species of the genus *Alexandrium* (formerly named *Gonyaulax* or *Protogonyaulax*) are identified as contaminators in shellfish. These are *Alexandrium tamarensis, A. minutum* (syn. *A. excavata*), *A. catenella, A. fraterculus, A. fundyense,* and *A. cohorticula*. Other clearly distinct dinoflagellates have also been recognised as sources of the saxitoxin (STX) family of toxins. These are *Pyrodinium bahamense* and *Gymnodinium catenatum*. The toxicity of the dinoflagellates results from a mixture of saxitoxin derivatives the composition of which differs with the producing species and/or the region of occurrence. There is also an immobile form of some dinoflagellates, the resting cyst or the hypnozygote. The cysts sink to the bottom of the sea and accumulate at the borderline of water and sediment, where they over-winter. When favourable growth conditions return, the cysts may germinate and reinoculate the water with swimming cells that can then bloom. The PSP toxins form a group of closely related tetrahydropurine compounds that make up four subgroups: (1) carbamate components (STX, neoSTX and gonyautoxins [GTX-1 to GTX-4]; (2) *N*-sulfo-carbamoyl components (GTX-5. GTX-6, C1 to C4); (3) decarbamoyl (dc-) components (dcSTX, dcneoSTX, dcGTX-1 to dcGTX-4); and (4) deoxydecarbamoyl (do-) components (doSTX, doneoSTX and doGTX-1) components. At least 21 PSP toxins, mainly from marine dinoflagellates and shellfish that feed on toxic algae, have been identified [Mons *et al.* 1998; Quilliam 2001]. A further 3 hydroxybenzoate saxitoxin analogues have been isolated from the dinoflagellate *Gymnodinium catenatum* [Negri *et al.* 2003]. The boiling or steaming of lobster has been shown to reduce saxitoxin, GTX-2, and GTX-3 (combined) concentrations

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by about 60% and 100%, respectively, when compared with raw lobsters [Lawrence *et al.* 1994].

Saxitoxin Dihydrochloride

Chemical Properties White, hygroscopic solid. pK_a 8.24, 11.60. Very soluble in water, methanol; sparingly soluble in ethanol, glacial acetic acid; practically insoluble in lipid solvents. Stable in acid solutions; decomposes rapidly in alkaline media. Boiling 3–4 h at pH 3 causes loss of activity. [O'Neil *et al.* 2006].

Thin-layer Chromatography Plates: precoated silica gel $60F_{254}$. Solvent system: *n*-butanol : acetic acid : water (2 : 1 : 1). Spray with hydrogen peroxide : 1 mol/L sodium hydroxide : methanol (1 : 50 : 49) and heat at 100° for 5 min. Fluorescence detection ($\lambda = 365$ nm). R_f values: STX 0.30, neoSTX 0.36. Limit of quantification not reported [Naseem, Creasia 1994].

Quantification

Urine **HPLC** Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L 1-heptenesulfonic acid in 30 mmol/L ammonium phosphate buffer (pH 7.1) : acetonitrile (100 : 3), flow rate 0.7 mL/min. Post-column derivatisation. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 395$ nm). Limit of quantification not reported [Garcia *et al.* 2004].

Bile **HPLC** See Urine [Garcia *et al.* 2004].

CSF **HPLC** See Urine [Garcia *et al.* 2004].

Stomach Contents **HPLC** See Urine [Garcia *et al.* 2004].

Vitreous Humour **HPLC** See Urine [Garcia *et al.* 2004].

Brain **HPLC** See Urine [Garcia *et al.* 2004].

Heart **HPLC** See Urine [Garcia *et al.* 2004].

Kidney **HPLC** See Urine [Garcia *et al.* 2004].

Liver **HPLC** See Urine [Garcia *et al.* 2004].

Lung **HPLC** See Urine [Garcia *et al.* 2004].

Muscle **HPLC** See Urine [Garcia *et al.* 2004].

Other **HPLC** Shellfish Samples and Algae Cell Samples (*A. catenella* and *G. catenatum*). Column: zwitterionic HILIC (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium formate and 10 mmol/L formic acid in water : acetonitrile-water (80 : 20) with 8 mmol/L ammonium formate (18 : 82 for 24 min, to 30 : 70 over 0.1 min for 10.9 min to 35 : 65 over 0.1 min for 14.9 min to 30 : 70 over 5 min), flow rate 0.7 mL/min. Post-column derivatisation. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 395$ nm). Retention times: STX 53 min, neoSTX 51.4 min, dcSTX 55.9 min; GTXs also eluted. Limit of detection, 0.10 ng/injection for STX analytes [Diener *et al.* 2007]. Rat Brain. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium formate : 0.1 mol/L ammonium formate in 5% acetonitrile (pH 6; 100 : 0 to 95 : 5 over 5 min, to 30 : 70 over 4 min, to 0 : 100 over 9 min), flow rate 1.5 mL/min. Pre-column derivatisation. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 395$ nm). Retention time: 14.2 min. Limit of quantification, 16 µg/L; limit of detection, 6 µg/L [Cervantes Cianca *et al.* 2007]. Reservoir Water Samples and Cyanobacterial Samples. Columns: Source 15Q PE 4.6/100 anion exchange column and 2 Source 15S PE 4.6/100 cation exchange columns, connected in series. Mobile phase: 20 mmol/L sodium acetate : 450 mmol/L sodium acetate (both pH 6.9; 100 : 0 for 6 min to 0 : 100 over 25 min for 20 min), flow rate 0.8 mL/min. Post-column derivatisation. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 390$ nm). Retention times: STX 51.0 min, neoSTX 47.4 min; GTXs also eluted. Limit of quantification, STX 47 ppb, neoSTX 48 ppb [Papageorgiou *et al.* 2005]. Cultured Microalgae Samples. Column: µBondapak NH_2 (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.05 mol/L sodium acetate buffer (pH 6.5) : water (0 : 100 for 5 min, to 100 : 50 over 20 min), flow rate 1 mL/min. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 390$ nm). Retention times: STX 5.1 min, neoSTX 4.5 min. Limit of detection, STX 0.32 µg/L, neoSTX 1.1 µg/L [He *et al.* 2005]. Shellfish Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium formate : 0.1 mol/L ammonium formate in 5% acetonitrile (pH 6; 100 : 0 to 95 : 5 over 5 min, to 30 : 70 over 4 min, to 100 : 0 over 2 min), flow rate 2.0 mL/min. Pre-column derivatisation. Fluorescence detection ($\lambda_{ex} = 340$, $\lambda_{em} = 395$ nm). Retention times: STX ~10 min, neoSTX ~6.5 min; GTX also eluted. Limit of quantification, 22 µg/kg [Lawrence, Niedzwiadek 2001; Lawrence *et al.* 2005]. Mussel Samples. Column: C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L sodium 1-heptanesulfonate in 30 mmol/L ammonium phosphate buffer (pH 7.1) : acetonitrile (96 : 4). Post-column derivatisation. Fluorescence detection ($\lambda_{ex} = 330$, $\lambda_{em} = 390$ nm). Limit of detection, STX 0.13 µmol/kg (4 µg/100 g) shellfish meat, neoSTX 0.20 µmol/kg (6 µg/100 g) shellfish meat [Asp *et al.* 2004]. Shellfish Samples. Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L sodium heptane sulfonate in 30 mmol/L ammonium phosphate (pH 7.1) with 5.7% aceotnitrile, flow rate 0.8 mL/min. Post-column electrochemical oxidation system. Fluorescence detection ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 390$ nm). Retention times: STX 9.5 min, neoSTX 6.0 min. Limit of detection, 3 µg/100 g shellfish meat [Boyer, Goddard 1999]. Rat Urine. Column: C_{18} (150 × 4.6 mm i.d., 3 µm). Mobile phase: 0.1 mol/L ammonium formate (pH 6.0) : acetonitrile (100 : 0 to 95 : 5 over 15 min for 10 min, to 0 : 100 over 13 min). Pre-column derivatisation. Fluorescence detection ($\lambda_{ex} = 346$ nm, $\lambda_{em} = 408$ nm). Retention time: 15 min. Limit of quantification, 2 µg/L [Stafford, Hines 1994].

LC-MS Shellfish Samples and Algae Cell Samples (*A. catenella* and *G. catenatum*). Column: zwitterionic HILIC (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium formate and 10 mmol/L formic acid in water : acetonitrile-water (80 : 20) with 5 mmol/L ammonium formate and 2 mmol/L formic acid (20 : 80 to 35 : 65 over 5 min, to 40 : 60 over 5 min for 10 min to 20 : 80 over 5.1 min), flow rate 0.7 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention times: STX 29.2 min, neoSTX 28.3 min, dcSTX 31.3 min; GTX also eluted. Limits of detection, STX 0.50 ng/injection, dcSTX 0.30 ng/injection [Diener *et al.* 2007]. Plankton and Mussel Samples. Column: TSK-gel Amide-80 (250 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile-water (95 : 5) : water (65 : 35), both with 2.0 mmol/L ammonium formate and 3.6 mmol/L formic acid (pH 3.5), flow rate 0.2 mL/min. ESI, positive ion mode, SIM and SRM acquisition modes. Retention times: STX 20.3 min, neoSTX 21.0 min, 11(α,β)-OH-saxitoxin 24.9 min, dcSTX 21.1 min, dcneoSTX 20.8 min; GTX also eluted. Limit of detection, STX 800 nmol/L, neoSTX 900 nmol/L (SIM, API-165); STX and neoSTX 7000 nmol/L (SIM, API-III+); STX 20 nmol/L, neoSTX 30 nmol/L (SRM, API-4000) [Dell'Aversano *et al.* 2004, 2005]. Shellfish Samples. Column: C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol-water (20 : 80) with 0.05% heptafluorobutyric acid : methanol-water (15 : 85) with 0.05% acetic acid (100 : 0 for 1.5 min then 0 : 100 for 10 min), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, STX, dcSTX 0.1 mg/kg sample [Fang *et al.* 2002].

CE-MS Mussel Samples. Column: uncoated fused silica capillary (100 cm, 50 µm i.d.). Buffer: 35 mmol/L morpholine (pH 5.0 with formic acid). API, SIM acquisition mode. Migration times: STX 18.7 min, dcSTX 17.7 min. Limit of quantification not reported [Gago-Martinez *et al.* 1996].

Note For a study on the development of STX-conjugated affinity gels, see Watanabe *et al.* [2006]; for an inter-laboratory QA study comparing different HPLC methods for the detection of PSP toxins, see Van Egmond *et al.* [1994]; for a study comparing flow injection analysis, HPLC-fluorescence, LC-MS, CE-UV and CE-MS methods, see Pleasance *et al.* [1992].

Disposition in the Body

Toxicity In most countries the action level for closure of fisheries is 400 MU/100 g shellfish or 80 µg STX equivalents/100 g. One Mouse Unit (MU) is the amount injected toxin which would kill a 20 g mouse in 15 min and is equivalent to 0.18 µg STX. The limit of detection of the mouse bioassay is ~40 µg STX/100 g shellfish tissue with a precision of ±15–20% [Hollingworth, Wekell 1990]. The LD_{50} in mice is 10 µg/kg (IP), 263 µg/kg (oral), 3.4 µg/kg (IV). The oral LD_{50} values in rat, monkey, cat, rabbit, dog, guinea pig and pigeon are reported as 192 to 212 µg/kg, 277 to 800 µg/kg, 254 to 280 µg/kg, 181 to 200 µg/kg, 180 to 200 µg/kg, 128 to 135 µg/kg and 91 to 100 µg/kg, respectively [Mons *et al.* 1998].

The Australia New Zealand Food Authority have reported that 120 to 180 µg PSP toxins can produce moderate symptoms in humans, 400 to 1060 µg can cause death, and 2000 to 10 000 µg is more likely to constitute a fatal dose [ANZFA 2001]. The level at which PSP intoxications occur in humans varies considerably. This variation mainly reflects individual difference in sensitivity and fluctuation in the method of determination. Oral intake causing mild symptoms varies from 144 to 1660 µg STX equivalents/person. Fatal intoxications have been reported after a calculated consumption of 456 to 12 400 µg STX equivalents/person. These values are only reconstructed from what remained of the toxic mussels and vary greatly. An oral consumption of 300 µg PSP toxin per person is in some cases reported as fatal, whereas others note the absence of toxic symptoms after an oral dose of 320 µg toxin per person. In Alaska, PSP was fatal for one fisherman, while two others eventually recovered. The stomach contents of patients contained 370 µg PSP toxin (STX equivalents)/100 g [Mons *et al.* 1998]. Other sources report mild poisoning at doses of PSP toxins between 304 and 4128 µg/person, while severe poisonings are caused by doses between 576 and 8272 µg/person [Aune 2001]. The effect of alcohol consumption on PSP is still unclear. Some consider that alcohol might be a protective factor against the adverse effects of PSP toxins but the mechanism through which alcohol might reduce the risk is unknown. Since the elimination of PSP toxins occurs at least in part through the urine, alcohol may influence illness by a diuretic effect. Alternatively, alcohol may cause hepatic enzyme induction.

In patients from 4 outbreaks of PSP in Alaska during May and June 1994, shellfish toxin levels ranged from 1778 to 19 418 µg/100 g mussel tissue. The median dose estimate was 9176 µg, corresponding to a median toxin dose of 167 µg/kg bodyweight. The lowest dose which caused illness was 21 µg/kg bodyweight and among 4 persons with respiratory arrest, who may be considered to have eaten a lethal dose, toxin dose ranged from 230 to 411 µg/kg bodyweight. PSP toxin levels of 2.8 to 47 nmol/L and 65 to 372 nmol/L in serum and urine, respectively, were detected at acute illness and after acute symptom resolution. Severe hypertension was observed in the patients although only nanomolar serum levels were detected. The PSP toxin profile differed between mussels and human biological specimens, suggesting human metabolism had occurred. Clearance of PSP toxins from serum was evident within 24 h and urine was identified as a major route of excretion [Gessner *et al.* 1997; Gessner, Middaugh 1995].

In 1987, an outbreak of PSP with 187 cases and 26 deaths was reported after consumption of a clam (*Amphichaena kindermani*) soup. The fatalities were the highest among young children: 50% compared with 7% in adults. Some of the children who died ingested an estimated dose of 140 to 160 MU/kg bodyweight [Rodrigue *et al.* 1990]. It was reported that the minimal lethal dose in this incident was estimated to be ≈25 µg STX equivalents/kg bodyweight for a child weighing 25 kg, compared to 86 to 788 µg STX equivalents/kg bodyweight in the four adults who died [Aune 2001].

Two fishermen harvesting sea urchin ≈140 miles from Puerto Natales, Chile, died 3 to 4 h after the ingestion of 7 to 9 ribbed mussels (*Aulacomya ater*). The mussel sample showed a toxicity measured by mouse bioassay of 8575 µg STX equivalent per 100 g of shellfish meat. Saxitoxin concentrations (µg/g tissue, except for fluid samples, mg/L) in postmortem material in one individual were reported as follows:

Samples	NeoSTX	DcSTX	STX
Thyroid glands	ND	ND	2.86
Stomach	ND	ND	14.24
Gastric content	ND	ND	39.69
Spleen	0.22	ND	0.43
Liver	ND	0.04	0.55
Pancreas	1.30	ND	8.18
Kidney	ND	0.01	0.26
Adrenal glands	ND	ND	1.52
Bile	0.69	ND	1.53
CSF	0.77	ND	ND
Urine	22.33	ND	1.80
Vitreous humour	ND	ND	ND
Papillary muscle	ND	ND	0.63
Aorta	ND	ND	ND
Lung	ND	0.06	0.75
Brain			
Grey matter	ND	ND	0.65
White matter	ND	ND	0.08
Heart			
Pericardium	ND	ND	0.37
Miocardium	ND	ND	0.67
Endocardium	ND	ND	ND

ND, Not determined.

GTXs were also present in the samples analysed. [Garcia *et al.* 2004].

A 32-year-old man was found dead 2–3 h after eating ≈10 g of a cooked xanthid crab (*Zosimus aeneus*). Concentrations of PSTs, expressed as μg SXT equivalents/100 mL or 100 g tissue, measured by sodium channel and saxiphilin radioreceptor assays) in crab specimens and postmortem samples were reported as follows:

Sample	Toxin level (sodium channel assay)	Toxin level (saxiphilin assay)
Crab 1	461.5	291.6
Crab 2	289.6	346.7
Deceased		
Urine	15.8	10.1
Liver	0.9	4.0
Bile	ND	ND
Plasma	4.2	8.9
Blood cells	10.3	ND
Gut contents pellet	577.1	257.5
Gut contents supernatant	385.4	376.2

Toxin profiles of crab 2 and the victim's urine and gut contents supernatant were reported as per the table below as well as total toxicity (expressed as μg saxitoxin equivalents/100 mL or 100 g tissue).

The dose consumed by the victim was estimated to be between 1 and 2 μg saxitoxin equivalents/kg bodyweight. However, the victim's meal did not consist solely of the toxic crab eaten [Llewellyn *et al.* 2002].

Note For a first report of saxitoxin in Finnish lakes and possible effects on human health, see Rapala *et al.* [2005]; for a review of PSP cases in Chile, see Montebruno [1993]; for case studies of PSP in South Africa, see Popkiss *et al.* [1979].

Dose Used as a tool in neurochemical research.

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Rodrigue DC *et al.* (1990). Lethal paralytic shellfish poisoning in Guatemala. *Am J Trop Med Hyg* 42: 267–271.

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Van Egmond HP *et al.* (1994). Paralytic shellfish poison reference materials: an intercomparison of methods for the determination of saxitoxin. *Food Addit Contam* 11: 39–56.

Watanabe R *et al.* (2006). Development of saxitoxin-conjugated affinity gels. *Bioconjug Chem* 17: 459–465.

Secbutabarbital

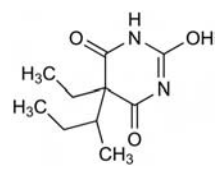
Barbiturate, Sedative

$C_{10}H_{16}N_2O_3 = 212.2$

CAS—125-40-6

IUPAC Name 5-Butan-2-yl-5-ethyl-1,3-diazinane-2,4,6-trione

Synonyms Butabarbital; butabarbitone, (distinguish from butobarbital butobarbitone); 5-ethyl-5-(1-methylpropyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; secbutobarbital; secbutobarbitone.



Chemical Properties A fine, white, microcrystalline powder. Mp 165° to 168°. Soluble 1 in 1400 of water, 1 in 12 of ethanol, 1 in 30 of chloroform and 1 in 30 of

	Toxin profiles (mol%)					Total toxicity
	GTX-2	GTX-3	NeoSXT	DcSXT	SXT	
Crab 2	45.5	17.3	0	0	37.2	162.8
Gut contents supernatant	5.5	10.2	2.6	ND	81.8	29.9
Urine	0.1	0.3	9.6	41.3	48.7	15.3

ether; soluble in aqueous solutions of alkali hydroxides and carbonates. pK_a 8.0 (20°). Log *P* (octanol/water), 1.6.

Secbutabarbital Sodium

$C_{10}H_{15}N_2NaO_3$ = 234.2
CAS—143-81-7

Synonyms Butabarbital sodium; butabarbitone sodium; secumalnatrium; secbutobarbital sodium; secbutobarbitone sodium; sodium butabarbital.

Proprietary Names *Asturidon*; *Busotran*; *Butabon*; *Butak*; *Buta-Kay*; *Butalan*; *Butanotic*; *Butased*; *Butatran*; *Butazem*; *Butex*; *Butisol*; *Butrate*; *Butte*; *Carrbutabarb*; *Loubarb*; *Neravan*; *Prelital*; *Sarisol*. It is an ingredient of *Butibel*.

Chemical Properties A white powder. Soluble 1 in 2 of water and 1 in 7 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

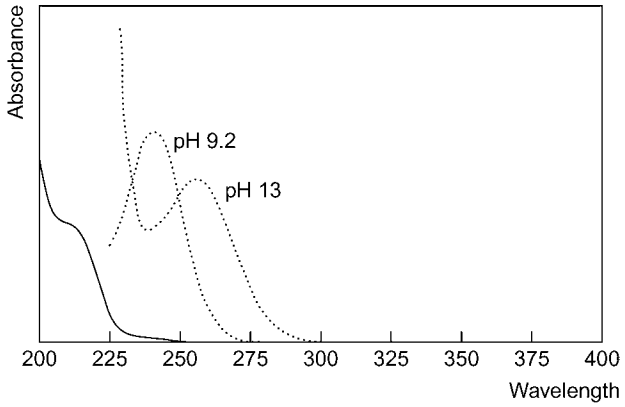
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—orange/violet.

Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.48; system TF—R_f 0.64; system TH—R_f 0.69; system TAD—R_f 0.57; system TAE—R_f 0.88 (mercurous nitrate spray, black).

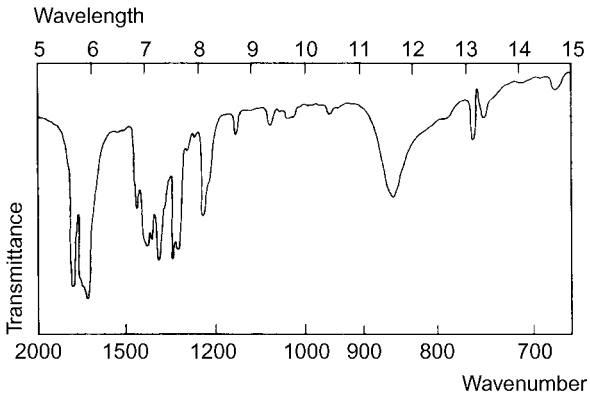
Gas Chromatography System GA—secbutabarbital RI 1655, secbutabarbital-Me_2 RI 1565, 2′-hydroxysecbutabarbital RI 1926.

High Performance Liquid Chromatography System HG—*k* 4.90; system HH—*k* 3.30; system HX—RI 377; system HY—RI 331.

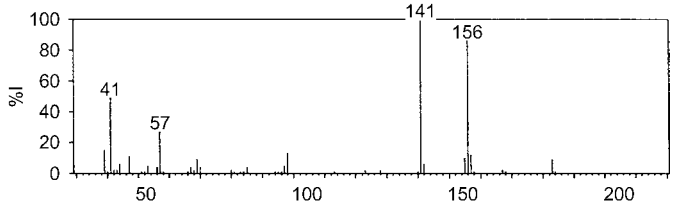
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1= 428a); 1 mol/L sodium hydroxide (pH 13)—254 nm (A^1_1=354b).



Infrared Spectrum Principal peaks at wavenumbers 1675, 1760, 1317, 1303, 1230, 853 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 141, 156, 41, 57, 39, 98, 157, 47.



Quantification See also under Amobarbital.

Urine **GC-MS** For method for quantification of secbutabarbital and other barbiturates, see Pocci *et al.* [1992].

Disposition in the Body Well absorbed after oral administration. Rapidly metabolised by *ω*- and (*ω*-1)-oxidation of the methylpropyl side-chain. About 40–60% of a dose is slowly excreted in the urine, 5–9% as unchanged drug, 30% as 5-(2-carboxy-1-methylethyl)-5-ethylbarbituric acid, 3% as 2′-hydroxysecbutabarbital and 1% as 2′-oxosecbutabarbital; greater amounts of unchanged drug may be excreted after excessive doses.

Toxicity The estimated minimum lethal dose is 2 g. Blood concentrations greater than about 10 mg/L are likely to cause toxic reactions, and concentrations greater than 30 mg/L may be fatal.

Following oral administration of 600 mg, given in 3 doses over a period of 3 h to 5 subjects, peak blood concentrations of 7.6–16.9 mg/L (mean 12.3) were reported, 0.5 h after the third dose. Tests 2 h after the first dose indicated depression of the central nervous system in all subjects [Parker *et al.* 1970].

In 4 fatalities attributed to secbutabarbital overdose, blood concentrations of 30–88 mg/L (mean 58) and liver concentrations of 51–250 µg/g (mean 112) were reported [Baselt, Cravey 1977].

Half-life Plasma half-life, 34–42 h.

Protein Binding About 26%.

Dose Usually 45 to 120 mg daily.

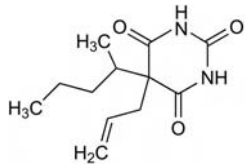
Baselt RC, Cravey RH (1977). *J Anal Toxicol* 1: 81–102.
Parker KD *et al.* (1970). Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide, or diphenylhydantoin. *Clin Toxicol* 3: 131–145.
Pocci R *et al.* (1992). Solid-phase extraction and GC/MS confirmation of barbiturates from human urine. *J Anal Toxicol* 16: 45–47.

Secobarbital

Barbiturate
$C_{12}H_{18}N_2O_3$ = 238.3
CAS—76-73-3

IUPAC Name 5-Pentan-2-yl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

Synonyms Meballymal; 5-(1-methylbutyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; quinalbarbitone; secobarbitalum; secobarbitone.



Chemical Properties A white amorphous or crystalline powder. Mp 100°. Very slightly soluble in water; freely soluble in ethanol and ether; soluble in chloroform. pK_a 7.9 (20°). Log *P* (octanol/water), 2.0.

Secobarbital Sodium

$C_{12}H_{17}N_2NaO_3$ = 260.3
CAS—309-43-3

Synonyms Meballymalnatrium; quinalbarbitone sodium; secobarbitalum natricum; secobarbitone sodium.

Proprietary Names *Seconal*. It is an ingredient of *Somolose* (vet.) and *Tuinal*.

Chemical Properties A white hygroscopic powder. Soluble 1 in 3 of water and 1 in 5 of ethanol; practically insoluble in chloroform and ether.

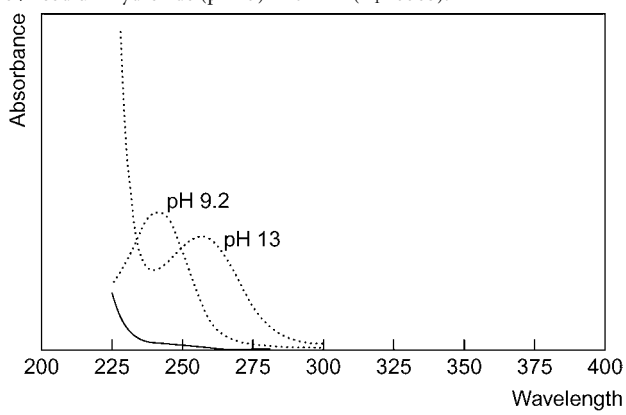
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown-red/violet.

Thin-layer Chromatography System TD—R_f 0.55; system TE—R_f 0.45; system TF—R_f 0.68; system TH—R_f 0.78; system TAD—R_f 0.62; system TAE—R_f 0.88 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).

Gas Chromatography System GA—secobarbital RI 1786, M (3′-OH-) RI 1865, M (desallyl-) RI 1665, secobarbital-Me_2 RI 1690; system GB—secobarbital RI 1827, M (3′-OH-) RI 2029; system GF—RI 2510; system GAJ—secobarbital RRT 0.865, M (3′-OH-) RRT 1.206 (both relative to methylphenobarbital).

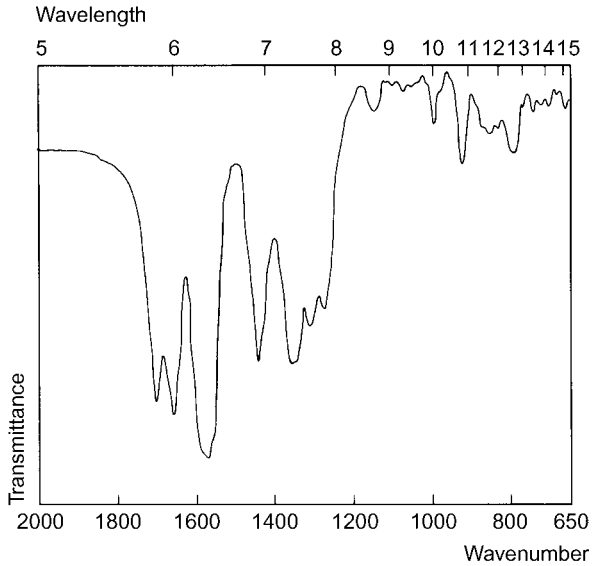
High Performance Liquid Chromatography System HG—*k* 16.28; system HH—*k* 11.47; system HX—RI 437; system HY—RI 407; system HZ—retention time 4.7 min; system HAA—retention time 17.4 min; system HAX—retention time 6.2 min; system HAY—retention time 5.9 min.

Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=393a); 1 mol/L sodium hydroxide (pH 13)—254 nm (A^1_1=330b).

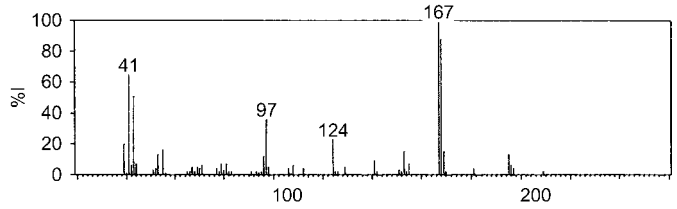


S

Infrared Spectrum Principal peaks at wavenumbers 1559, 1648, 1690, 1298, 1270, 925 cm^{-1} (secobarbital sodium, KBr disk).



Mass Spectrum Principal ions at *m/z* 167, 168, 41, 43, 97, 124, 39, 55; 5-(2 3-dihydroxypropyl)secobarbital 171, 43, 143, 41, 128, 55, 141, 159; 3′-hydroxysecobarbital 41, 45, 43, 168, 39, 70, 69, 167; 3′-ketosecobarbital 43, 69, 168, 41, 85, 167, 86, 169.



Quantification See also under Amobarbital.
Plasma **GC** FID. Limit of detection, 200 µg/L [Valentine *et al.* 1982].
Serum **HPLC** UV detection. Limit of detection, 20 µg/L [Levine *et al.* 1982].
Urine **GC** FID. For method, see Valentine *et al.* [1982].
Disposition in the Body About 90% of a dose is absorbed after oral administration. The major metabolic reactions are hydroxylation of both side-chains at the C_5-position with further oxidation of the ω-position on the butyl side-chain. Less than 5% of an oral dose is excreted unchanged in the urine. Two diastereoisomeric forms of the following metabolites have been found in the urine, each metabolite accounting for about 4% of the dose in 24 h: 5-allyl-5-(3-hydroxy-1-methylbutyl) barbituric acid (3′-hydroxysecobarbital), 5-allyl-5-(3-carboxy-l-methylpropyl)barbituric acid, and 5-(2,3-dihydroxy-propyl)secobarbital; an additional metabolite, 5-allyl-5-(1-methyl-3-oxobutyl)barbituric acid (3′-ketosecobarbital), accounts for about 3% of the dose in 96 h. A total of about 45% of the dose is excreted in the urine over a period of 108 h.
Therapeutic Concentration In plasma, usually in the range 2 to 10 mg/L.
After an oral dose of 3.3 mg/kg to 6 subjects, peak blood concentrations of 2.0 to 2.2 mg/L (mean 2.1) were attained in about 3 h [Clifford *et al.* 1974].
Toxicity The estimated minimum lethal dose is 2 g. Toxic effects are usually associated with blood concentrations greater than about 8 mg/L. In 276 reported fatalities attributed to secobarbital, blood concentrations ranged from 4 to 132 mg/L (mean 30).
The following postmortem tissue distribution was reported in 5 fatalities: blood 35 to 123 mg/L (mean 66), brain 109 µg/g (1 case), kidney 67 to 270 µg/g (mean 152, 3 cases), liver 25 to 605 µg/g (mean 271, 4 cases), spleen 42 µg/g (1 case), urine 12 to 182 mg/L (mean 72, 3 cases) [Rehling, 1967].
The following postmortem tissue concentrations (mg/L or µg/g) were reported in 3 fatalities due to secobarbital:

	Secobarbital 3′	*Hydroxysecobarbital*
Blood	12, 9, 13	0, –, 2
Bile	–, 42, 79	–, –, 0
Left kidney	37, 25, 25	3, –, 17
Right kidney	69, 24, 28	4, –, 16
Liver	77, 51, 44	4, –, 25
Urine	6, –, 3, 32	0, –, 33

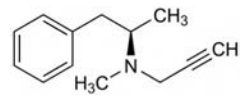
[Robinson, McDowall 1979].

Following a fatality involving the suicidal ingestion of secobarbital, nitrazepam, and codeine, the respective blood concentrations were reported as 11.48, 1.72 and 0.036 mg/L [Tracqui *et al.* 1989].
Half-life Plasma half-life, 19 to 34 h (mean 28).
Volume of Distribution About 1.5 L/kg.
Clearance Plasma clearance, about 0.8 mL/min/kg.
Saliva Plasma : saliva ratio, about 3.3.
Protein Binding About 70%.
Dose 100 mg daily as secobarbital sodium.

Clifford JM *et al.* (1974). Absorption and clearance of secobarbital, heptabarbital, methaqualone, and ethinamate. *Clin Pharmacol Ther* 16: 376–389.
Levine HL *et al.* (1982). An improved high-pressure liquid chromatographic assay for secobarbital in serum. *J Pharm Sci* 71: 1281–1283.
Rehling CJ (1967). Poison Residues in Human Tissues. In: Stolman A, ed. *Progress in Chemical Toxicology*, Vol. 3. New York: Academic Press, 371.
Robinson AE, McDowall RD (1979). The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man. *J Pharm Pharmacol* 31: 357–365.
Tracqui A *et al.* (1989). A fatality involving secobarbital, nitrazepam, and codeine. *Am J Forensic Med Pathol* 10: 130–133.
Valentine JL *et al.* (1982). *Anal Lett (Part B)* 15: 343–361.

Selegiline

Antiparkinsonian
$C_{13}H_{17}N$ = 187.3
CAS—14611-51-9
IUPAC Name (2*R*)-*N*-Methyl-1-phenyl-*N*-prop-2-ynylpropan-2-amine
Synonyms Synonyms used in the literature are confusing and include (−)-deprenil; L-deprenil; deprenyl; (−)-deprenyl; L-deprenyl; L-deprenaline;(αR)-*N*,α-dimethyl-*N*-2-propynylbenzeneethanamine and E-250. It is the (−)-isomer which is active.

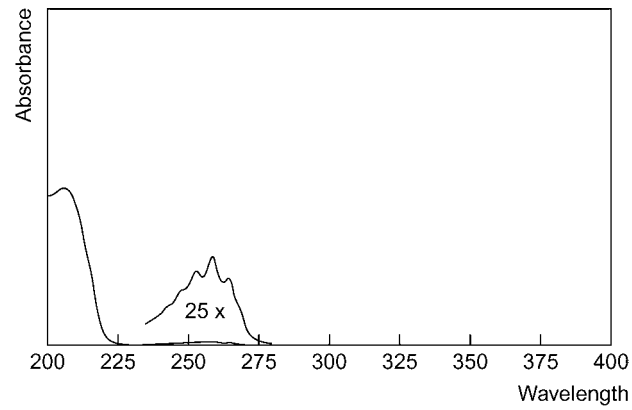


Chemical Properties An oil.

Selegiline Hydrochloride

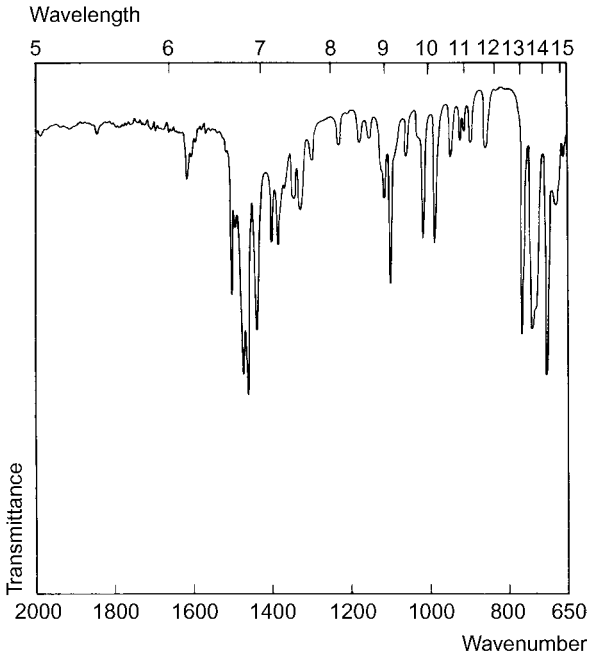
$C_{13}H_{17}N,HCl$ = 223.7
CAS—14611-52-0
Proprietary Names *Amboneural*; *Amindan*; *Antiparkin*; *Atapryl*; *Carbex*; *Centrapryl*; *Cognitiv*; *Deprilan*; *Egibren*; *Eldepryl*; *Elepril*; *Jumex*; *Jumexil*; *MAOtil*; *Movergan*; *Niar*; *Regepar*; *Selegam*; *Selgene*; *Seline*; *Selpar*; *Stilline*; *Tremorex*; *Vivapryl*; *Zelapar*.
Chemical Properties A white crystalline solid. Mp 141° to 142°.

Thin-layer Chromatography System TA—R_f 0.74; system TB—R_f 0.57; system TC—R_f 0.69 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, orange).
Gas Chromatography System GA—selegiline RI 1450, M (OH-) RI 1580, M (OH-)-AC RI 1860; M (nor-) RI 1350, M (nor-)-AC RI 1735, M (nor-OH-) RI 1550, M (nor-OH-)-AC_2 RI 2030, amfetamine RI 1125, metamfetamine RI 1175, system GB—selegiline RI 1453, amfetamine RI 1150, metamfetamine RI 1200.
High Performance Liquid Chromatography System HAA—retention time 10.7 min.
Ultraviolet Spectrum Aqueous acid—252, 258 (A^1_1=9.1b), 264 nm; aqueous alkali—259, 269 nm.

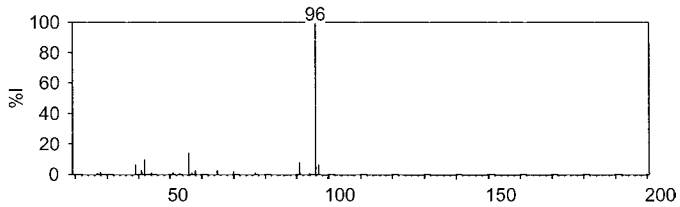


Infrared Spectrum Principal peaks at wavenumbers 704, 765, 740, 1492, 1092, 981 cm^{-1} (selegiline hydrochloride, KBr disk).

S



Mass Spectrum Principal ions at *m/z* 96, 56, 42, 91, 97, 39, 65, 58; amfetamine 44, 91, 40, 42, 65, 45, 39, 43; metamfetamine 58, 91, 59, 134, 65, 56, 42, 57.



Quantification See also under Amfetamine and Metamfetamine.
Plasma **GC** For method for quantification of (−)-metamfetamine, (−)-amfetamine and norselegiline (or (+)-metamfetamine and (+)-amfetamine associated with metamfetamine abuse), see Hasegawa *et al.* [1999].

HPLC Fluorescence detection. Limit of detection, 0.5 µg/L for norselegiline, amfetamine and metamfetamine [La Croix *et al.* 1994].
Urine **GC** For method for quantification of (−)-metamfetamine, (−)-amfetamine and norselegiline (or (+)-metamfetamine and (+) -amfetamine associated with metamfetamine abuse), see Hasegawa *et al.* [1999].

HPLC Fluorescence detection. Limit of detection, 2.8 to 8.8 fmol/5 mL for enantiomers of metamfetamine and amfetamine [al-Dirbashi *et al.* 1998]. Fluorescence detection. Limit of detection, 0.5 µg/L for 2-phenylethylamine [La Croix *et al.* 1996].

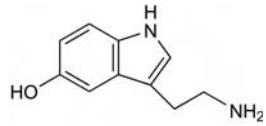
HPLC–MS ESI–MS detection. Limit of detection, 0.1 to 0.5 µg/L for selegiline-*N*-oxide, metamfetamine, amfetamine and desmethylselegiline [Katagi *et al.* 2001].
Hair **GC–MS** SIM. For method for quantification of selegiline, desmethylselegiline, metamfetamine and amfetamine, see Kikura and Nakahara [1995].
Disposition in the Body Rapidly absorbed after oral administration and distributed to the tissues. Selegiline is almost completely metabolised to form metamfetamine and amfetamine. About 50% of a dose is excreted in the urine in 24 h and 75% in 72 h; excreted mainly as metamfetamine with lesser amounts of amfetamine. The excretion of metabolites is increased in acid urine. About 15% of a dose is eliminated in the faeces in 72 h.
Half-life Derived from urinary excretion data (total radioactivity), about 39 h.
Note For a review of the pharmacology of selegeline, see Gerlach *et al.* [1996].
Dose 5 to 10 mg of selegiline hydrochloride daily.

al-Dirbashi O *et al.* (1998). Enantioselective high-performance liquid chromatography with fluorescence detection of methamphetamine and its metabolites in human urine. *Analyst* 123: 2333–2337.
Gerlach M *et al.* (1996). Pharmacology of selegiline. *Neurology* 47 (Suppl. 3): S137–S145.
Hasegawa M *et al.* (1999). Stereoselective analyses of selegiline metabolites: possible urinary markers for selegiline therapy. *Forensic Sci Int* 101: 95–106.
Katagi M *et al.* (2001). Simultaneous determination of selegiline-N-oxide, a new indicator for selegiline administration, and other metabolites in urine by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl* 759: 125–133.
Kikura R, Nakahara Y (1995). Hair analysis for drugs of abuse. IX. Comparison of deprenyl use and methamphetamine use by hair analysis. *Biol Pharm Bull* 18: 267–272.
La Croix R *et al.* (1994). Sensitive high-performance liquid chromatographic method for the determination of the three main metabolites of selegiline (L-deprenyl) in human plasma. *J Chromatogr* 656: 251–258.
La Croix R *et al.* (1996). Sensitive high-performance liquid chromatographic method for the determination of 2-phenylethylamine in human urine. *J Chromatogr Biomed Appl* 681: 185–190.

Serotonin

Neurotransmitter
$C_{10}H_{12}N_2O = 176.2$
CAS—50-67-9

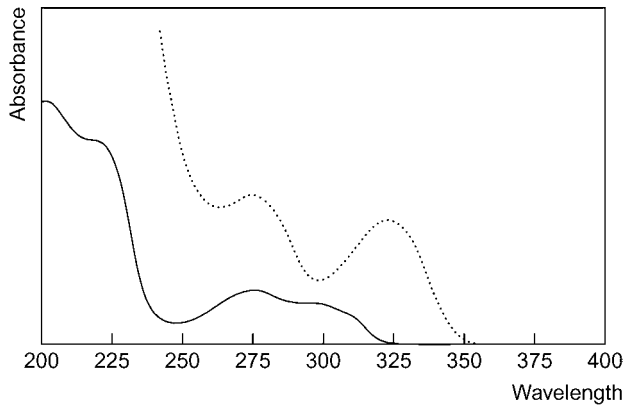
IUPAC Name 3-(2-Aminoethyl)-1*H*-indol-5-ol
Synonyms Enteramine; 5-HT; 5-hydroxytryptamine; thrombocytin; thrombotonin.



Chemical Properties pK_a 9.1, 9.8. Log *P* (octanol/water), 0.2.

Serotonin Hydrochloride
Chemical Properties White crystals. Mp about 167°. Soluble in water.

Colour Test Marquis test—brown (slow).
Thin-layer Chromatography System TA—R_f 0.25 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—276 nm; aqueous alkali—274, 323 nm.



Infrared Spectrum Principal peaks at wavenumbers 1195, 1225, 1582, 1627, 720, 1105 cm^{-1} (serotonin oxalate, KBr disk).
Quantification
Plasma **GC-MS** SIM. For method, see Baba *et al.* [1984].
Urine **HPLC** Fluorescence detection. For method for quantification of serotonin, catecholamines and their metabolites, see Panholzer *et al.* [1999]. Fluorescence detection. Serotonin and 5-hydroxyindoleacetic acid. Limit of quantification, 2.4 nmol/L for serotonin [Kai *et al.* 1998].
Disposition in the Body Serotonin is widely distributed in the body, arising from the hydroxylation and subsequent decarboxylation of tryptophan. It is metabolised chiefly by oxidative deamination to 5-hydroxyindol-3-ylacetic acid; *N*-methylation of the side-chain may also occur.

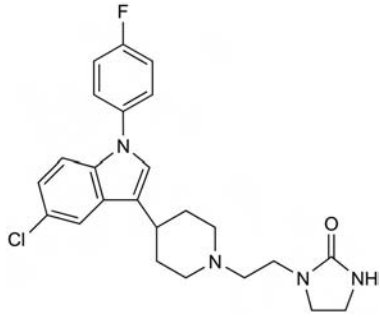
Baba S *et al.* (1984). Revised method for the quantitative determination of 5-hydroxytryptamine in human plasma by gas chromatography–mass spectrometry–selected ion monitoring. *J Chromatogr* 307: 1–9.
Kai M *et al.* (1998). Fluorescence derivatizing procedure for 5-hydroxytryptamine and 5-hydroxyindoleacetic acid using 1,2-diphenylethylenediamine reagent and their sensitive liquid chromatographic determination. *J Chromatogr B Biomed Sci Appl* 720: 25–31.
Panholzer TJ *et al.* (1999). Coupled-column liquid chromatographic analysis of catecholamines, serotonin, and metabolites in human urine. *Clin Chem* 45: 262–268.

Sertindole

Atypical Antipsychotic
$C_{24}H_{26}ClFN_4O = 440.9$
CAS—106516-24-9

IUPAC Name 1-[2-[4-[5-Chloro-1-(4-fluorophenyl)indol-3-yl]piperidin-1-yl]ethyl]imidazolidin-2-one
Synonyms 1-[2-[4-[5-Chloro-1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-piperidinyl]ethyl]-2-imidazolidinone; LU-23-174.

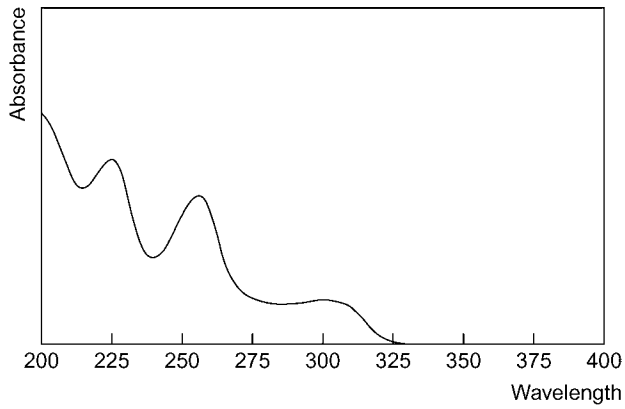
Proprietary Name *Serdolect*



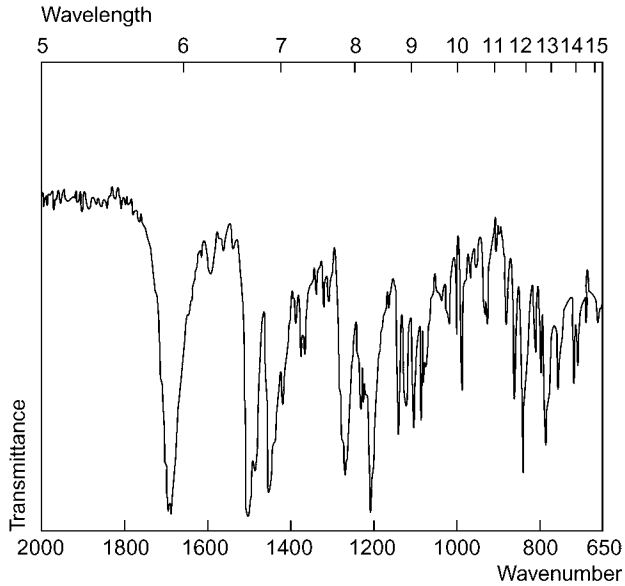
Chemical Properties White to off-white fine powder with Mp 166°. It is soluble in 0.1 mol/L acetic acid; practically insoluble in water; sparingly soluble in ethanol 96%; freely soluble in dichloromethane. pK_{a1} 9.06 [Choong *et al.* 2009], pK_{a2} 8.9. Log *P* (octanol/water), 5.26 [Choong *et al.* 2009], (octanol/phosphate buffer pH 7.4), 3.9. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

High Performance Liquid Chromatography System HZ—RT 9.8 min.

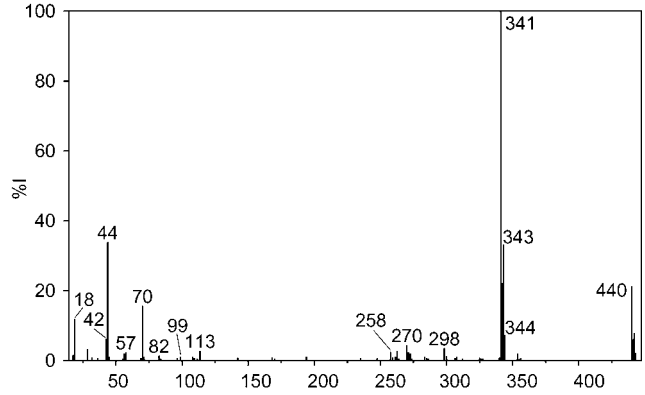
Ultraviolet Spectrum Aqueous acid (methanol)—227, 258, 302 nm; (ethanol)—226, 259, 302 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 341, 44, 343, 440, 70, 18, 42, 344.



Quantification

***Plasma* HPLC** Column: XTerra RP18 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L ammonium acetate buffer (pH 8, 45 : 55), flow rate 1.5 mL/min. UV detection (λ= 256 nm). Retention time: 5.96. Limit of quantification, 5 µg/L [Canal-Raffin *et al.* 2005]. Column: Spherisorb C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: hexane : isopropyl alcohol : methanol : tetrahydrofuran : 28% ammonium hydroxide (73.6 : 15 : 9.6 : 1.3 : 0.5), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 260 nm; λ_{em} 340 nm). Retention time: 12 min. Limit of quantification 0.025 µg/L, limit of detection, 0.01 µg/L [Tzeng *et al.* 1994].

LC-MS Column: Xbridge C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 8.1) : acetonitrile (84 : 16 to 66.5 : 33.5 at 1.31 min to 40 : 60 at 7.51 min to 10.9 min to 15 : 85 at 11 min for 2 min to 84 : 16 for 5 min). ESI, positive ion mode, SIM acquisition mode. Retention time: 10.7 min. Limit of quantification, 2 µg/L [Choong *et al.* 2009].

Disposition in the Body Sertindole is absorbed slowly after oral administration with peak plasma concentrations being reached after about 10 h. It is extensively metabolised in the liver by the cytochrome P450 isoenzymes CYP2D6 and CYP3A. Poor metabolisers (deficient in isoenzyme CYP2D6) may have plasma concentrations of sertindole that are 2–3 times greater. Two metabolites have been identified: dehydrosertindole and norsertindole which appear to be inactive. Excretion is slow and mainly via faeces with a small amount in urine. It readily crosses the placenta.

Therapeutic Concentration

Twelve young, healthy males (mean age, 29 years), 12 elderly males (mean, 71 years), 11 young females (mean, 22 years) and 12 elderly females (mean, 73 years) participated in this study. Each received 4 mg sertindole daily for 3 consecutive days, 8 mg daily for 3 consecutive days and 12 mg daily for 10 consecutive days. After the 4 mg single dose, peak plasma concentrations for sertindole were 1.41 µg/L for the young males and the elderly males, 2.34 µg/L for the young females and 1.83 µg/L for the elderly females. These were observed at 9.7, 10.2, 10.2 and 11.8 h, respectively. Peak dehydro-sertindole concentrations were 0.78 and 0.90 µg/L for the young and elderly males, respectively, and 0.89 and 0.97 µg/L for the young and elderly females. These concentrations were seen at 23.0, 23.3, 22.2 and 21.7 h for the 4 groups, respectively. After 12 mg sertindole daily for 10 days, the peak concentrations were 53.13, 43.91, 63.74 and 57.6 µg/L for the young males, elderly males, young females and elderly females, respectively, at 7.5, 9.3, 8.9 and 10.3 h, respectively. Peak concentrations for the metabolite, dehydrosertindole were 38.34, 34.06, 43.29 and 51.58 µg/L for the 4 groups of volunteers at 8.5, 9.6, 6.0 and 7.3 h, respectively [Wong *et al.* 1997].

Seven healthy males, aged 18 to 37 years, were administered with 4 mg sertindole daily for 2 days and then the dose was increased 4 mg every 3 days until 20 mg daily was reached. The peak serum concentration for the 20 mg dose was 69.8 µg/L on day 11, observed at 8.9 h. Six alternative healthy males were administered with 4 mg daily for 7 days followed by 8 mg daily for an additional 7 days. After a 4 mg dose (day 1), the peak concentration was 2.3 µg/L at 9.3 h and 9.6 µg/L at 10.0 h (day 7). For the 8 mg dose on day 14, peak concentration was 28.7 µg/L at 9.3 h [Wong, Granneman 1998].

Toxicity Cardiac arrhythmia, sudden cardiac death.

Half-life 3 days.

Volume of Distribution 1887–2447 L (single doses of 4 and 8 mg); 809 L (multiple dosing 20 mg).

Clearance Plasma, 22–31 L/h (single doses of 4 and 8 mg); 9.8 L/h (multiple dosing of 20 mg).

Protein Binding 99.5%.

Dose An initial dose of 4 mg daily which may be increased by 4 mg every 4 to 5 days. The usual maintenance dose is 12–20 mg daily with a maximum of 24 mg.

Canal-Raffin M *et al.* (2005). Simplified ultraviolet liquid chromatographic method for determination of sertindole, dehydrosertindole and norsertindole, in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 814: 61–67.

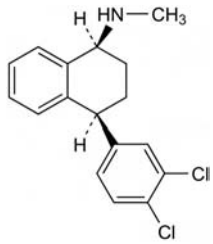
Choong E *et al.* (2009). Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC-MS. *J Pharm Biomed Anal* 50: 1000–1008.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Tzeng TB *et al.* (1994). Sensitive method for the assay of sertindole in plasma by high-performance liquid chromatography and fluorimetric detection. *J Chromatogr B Biomed Appl* 661: 299–306.
Wong SL, Granneman GR (1998). Modeling of sertindole pharmacokinetic disposition in healthy volunteers in short term dose-escalation studies. *J Pharm Sci* 87: 1629–1631.
Wong SL *et al.* (1997). Pharmacokinetics of sertindole in healthy young and elderly male and female subjects. *Clin Pharmacol Ther* 62: 157–164.

Sertraline

Antidepressant
$C_{17}H_{17}Cl_2N$ = 306.2
CAS—79617-96-2

IUPAC Name (1*S*,4*S*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine
Synonym CP-51974



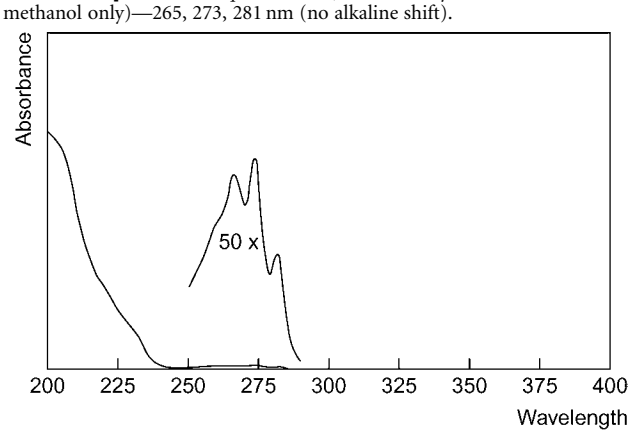
Chemical Properties Crystals. Mp ~207°. Blood, plasma and cardiac tissue samples were stable for at least 6 months at -80° [Läer *et al.* 1996]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Sertraline Hydrochloride

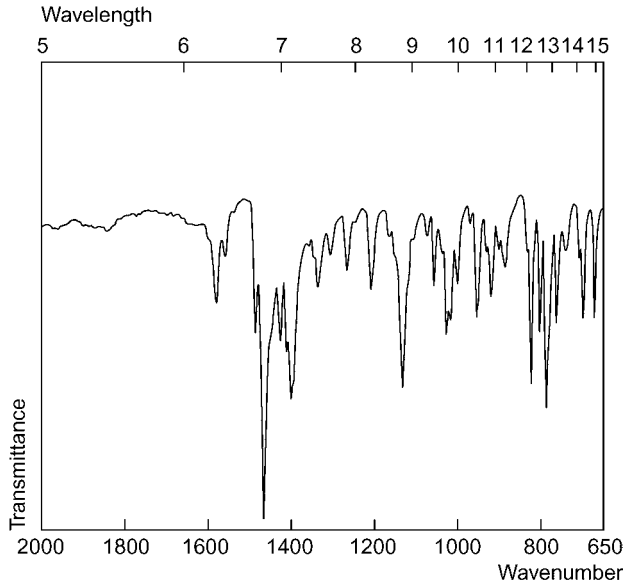
$C_{17}H_{17}Cl_2N$,HCl = 342.7
CAS—79559-97-0
IUPAC Name (1*S*,4*S*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride
Proprietary Names *Altruline*; *Aremis*; *Besitran*; *Gladem*; *Lustral*; *Sealdin*; *Serad*; *Tatig*; *Zoloft*.
Chemical Properties A white to off-white crystalline powder (irritant). Mp onset of change 160° to 180°, partial melt 218°, total melt 243° to 245°. Solubility: water 3.8 mg/mL (pH 5.3), pH dependent. It is soluble in chloroform : methanol (1 : 1), methanolic 0.1 mol/L hydrochloric acid, dimethyl sulfoxide, ethanol and *N,N*-dimethylformamide; practically insoluble in aqueous 0.1 mol/L hydrochloric acid, aqueous 0.1 mol/L sodium hydroxide, propan-2-ol, acetone, ethyl acetate and acetonitrile. pK_a 9.48 ± 0.04 (water), 8.5 (ethanol: water (1 : 1)) and 8.6 (methanol : water (40 : 60)). Log *P* (octanol/water), 5.29.

Thin-layer Chromatography System TB—R_f 0.46; system TE—R_f 0.72; system TAE—R_f 0.25.
Gas Chromatography System GB—sertraline RI 2481, M (nor-) RI 2468, M (ketone) RI 2496; system GM—sertraline RRT 1.166, M (nor-) RRT 1.218. (both relative to iprindole).
Gas Chromatography-Mass Spectrometry Column: HP1 methyl silicone (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, 0.9 mL/min. RI: 2401 [Mills, Roberson 1993].

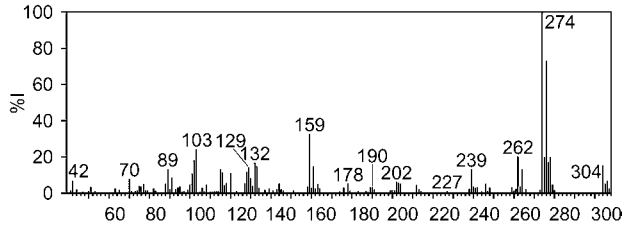
Column: 3% Silar 10C on Gas Chrom Q 80-100 mesh (0.8 m × 0.2 mm i.d.). Temperature: 255°. Injector temperature: 200°. Carrier gas: He, 40 mL/min. EI ionisation, SIM acquisition mode at *m/z*:274. RT: 3.9 min. [Fouda *et al.* 1987].
High Performance Liquid Chromatography System HX—RI 460; system HZ—sertraline RT 8.2 min, M (desmethyl-) RT 7.0 min; system HAX—RT 14.5 min; system HAY—RT 7.7 min.
Ultraviolet Spectrum Aqueous acid (0.01 mol/L hydrochloric acid/methanol or methanol only)—265, 273, 281 nm (no alkaline shift).



Infrared Spectrum Principal peaks at wavenumber 1470, 1135, 825, 800, 790, 760 cm^{-1} (KBr pellet).



Mass Spectrum Principal ions at *m/z* 274, 159, 276, 103, 262, 132, 129, 161.



Quantification

Plasma **HPLC** Column: Ultraspher octyl C_8 (250 × 4.6 mm, 5 µm). Mobile phase: acetonitrile : 0.086% w/v 1-octane sulfonic acid and 0.01% v/v *N,N,N,N*-tetramethylenediamine (pH 2.5, 43 : 57), flow rate 1.6 mL/min. UV detection (λ = 210 nm). Limit of detection, 5 µg/L [Kristensen *et al.* 1998].

GC Column: SE-54 (12 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 165° for 0.5 min to 210° at 50°/min for 12 min. Limit of quantification, 1 µg/L [Tremaine, Joerg 1989].

Serum **HPLC** Column: LC-8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water: diethylamine (1000 : 800 : 1200 : 2), flow rate 1.5 mL/min. UV detection (λ = 600 nm for 2 min followed by 214 nm). Limit of detection, 0.01 mg/L [Rogowsky *et al.* 1994].

GC-MS Column: RTX-5 (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° to 210° at 50°/min. Limit of detection, 10 µg/L [Rogowsky *et al.* 1994].

Milk **HPLC** See Plasma [Kristensen *et al.* 1998].

Disposition in the Body Sertraline is absorbed slowly after oral administration, with peak plasma concentrations being reached within 4.5–8.4 h. Steady-state levels are achieved after 1 week of daily dosing. It is widely distributed throughout the body. Sertraline and its metabolite *N*-desmethylsertraline (inactive in *in vivo* models of depression) are extensively metabolised. Sertraline is metabolised by *N*-demethylation, oxidative deamination and subsequent reduction, hydroxylation and glucuronide conjugation; *N*-desmethylsertraline is metabolised by oxidative deamination and subsequent reduction, hydroxylation and glucuronide conjugation. The metabolites are excreted in urine and faeces; 40–45% of an administered dose is excreted in urine over 9 days with less than 0.2% as the unchanged drug. It is distributed in breast milk.

Therapeutic Concentration The serum therapeutic range is 50–250 µg/L.

Forty-four healthy volunteers (male and female), in 2 groups (young aged between 18 and 45 years and older aged >65 years) were administered with a single 50 mg daily dose of sertraline. The initial dose was titrated to 200 mg over 30 days, which is the maximum dose. On day 30, peak sertraline plasma concentrations were determined as 118 µg/L for the young males and 166 µg/L for the young females. Concentrations were reached in 6.9 and 6.7 h, respectively. Peak plasma concentrations of the metabolite *N*-demethylsertraline were 156 µg/L in 9.1 h and 244 µg/L in 5.9 h for the males and females, respectively. For the older patients, peak plasma concentrations of the drug were 135 µg/L and 147 µg/L for the males and females, respectively, and were reached in 7.8 and 6.4 h, respectively. For the metabolite, these values were 237 µg/L and 274 µg/L, respectively, attained in 13.8 and 8.2 h, respectively [Ronfeld *et al.* 1997].

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Ten patients, aged 35–69 years, with stable chronic hepatic insufficiency caused by cirrhosis (mild to severe) and 10 equally matched healthy individuals were administered with a single 100 mg sertraline base dose after an overnight fast. For patients, mean peak plasma concentration for the drug was 35.2 µg/L (range, 20.0–40.8), observed at 4 h (range, 2–6); mean peak plasma concentration for *N*-demethylsertraline was 7.9 µg/L (range, 5.5–9.7), observed at 168 h (range, 96–216). For the healthy volunteers, mean peak drug concentrations were 20.6 µg/L (range 12.6–27.5) at 6 h (4–6) for the drug and 11.2 µg/L (10.6–13.0) at 6 h (6–10) for the metabolite [Demolis *et al.* 1996].

Toxicity

A 51-year-old white woman was admitted into hospital after ingesting 8000 mg sertraline. She had been treated 3 days before for acute alcohol intoxication. Symptoms included somnolence, nausea, tachycardia, anxiety and dilated pupils. On admission, serum concentrations were 2930 µg/L and 1679 µg/L for sertraline and desmethylsertraline, respectively. Other drugs detected included norpropoxyphene (268 µg/L) and nortriptylene (26 µg/L). No ethanol was found. On day 4, sertraline and desmethylsertraline serum concentrations were 453 µg/L and 1132 µg/L, respectively. The drug concentration reduced further to 305 µg/L on day 5 and the patient was discharged on day 6 after receiving supportive measures [Brendel *et al.* 2000].

Half-life Sertraline, approximately 26 h (mean for young and elderly 22–36 h); *N*-desmethylsertraline, 62–104 h; prolonged in hepatic impairment.

Volume of Distribution 20 L/kg.

Protein Binding ≈98%.

Dose 50 to 200 mg daily.

Brendel DH *et al.* (2000). Massive sertraline overdose. *Ann EmergMed* 36: 524–526.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Demolis JL *et al.* (1996). Influence of liver cirrhosis on sertraline pharmacokinetics. *Br J Clin Pharmacol* 42: 394–397.

Fouda HG *et al.* (1987). Gas chromatographic-mass spectrometric analysis and preliminary human pharmacokinetics of sertraline, a new antidepressant drug. *J Chromatogr* 417: 197–202.

Kristensen JH *et al.* (1998). Distribution and excretion of sertraline and *N*-desmethylsertraline in human milk. *Br J Clin Pharmacol* 45: 453–457.

Läer S *et al.* (1996). Determination of sotalol in human cardiac tissue by high-performance liquid chromatography. *JChromatogr B Biomed Appl* 681: 291–298.

Mills TI, Roberson JC *AT Instrumental Data for Drug Analysis*, 2nd edn, Vol. 4-5. Boca Raton *FL: CRC Press* 222-223

Rogowsky D *et al.* (1994). Determination of sertraline and desmethylsertraline in human serum using copolymeric bonded-phase extraction, liquid chromatography and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Appl* 655: 138–141.

Ronfeld RA *et al.* (1997). Pharmacokinetics of sertraline and its *N*-demethyl metabolite in elderly and young male and female volunteers. *Clin Pharmacokinet* 32(Suppl 1): 22–30.

Tremaine LM, Joerg EA (1989). Automated gas chromatographic-electron-capture assay for the selective serotonin uptake blocker sertraline. *J Chromatogr* 496: 423–429.

Setiptiline

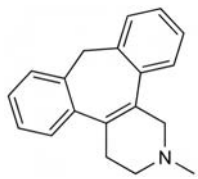
Antidepressant

$C_{19}H_{19}N = 261.4$

CAS—57262-94-9

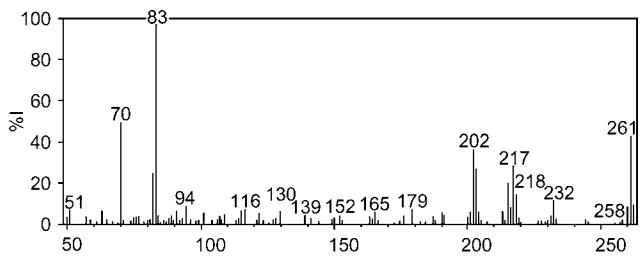
Synonym 2,3,4,9-Tetrahydro-2-methyl-1*H*-dibenzo[3,4,6,7]cyclohepta[1,2-*c*]pyridine

Proprietary Name *Tecipul.*



Gas Chromatography System GU—RT 14.1 min.

Mass Spectrum Principal ions at *m/z* 83, 70, 261, 202, 217, 203, 82, 232.



Quantification

Blood **GC-MS** Column: Supelco SPB-1 fused silica (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 100° for 5 min, to 280° at 20°/min. Carrier gas: He, 0.8 mL/min. Internal standard (IS): imipramine. SIM acquisition mode (*m/z* 202, 217, 261 for setiptiline; 243 for IS). RT: setiptiline, 14.1 min; IS, 13.7 min. Limit of detection, 0.005 µg/g [Namera *et al.* 1998].

Plasma **LC-MS** Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.1 mL/min. SSI, positive ion mode, full scan mode. Retention time: 7.1 min. Limit of quantification, 0.15 mg/L, limit of detection, 0.1 mg/L [Shinozuka *et al.* 2006].

Disposition in the Body Setiptiline is metabolised via glucuronide conjugation, demethylation and hydroxylation in the liver. About 10.7% of a dose is excreted in urine as the glucuronide conjugate and ~9% as the other metabolites, in 48 h, resulting from aromatisation and hydroxylation following demethylation.

Therapeutic Concentration

Forty-five outpatients, aged between 22 and 86 years (mean for males, 48.8 years; mean for females, 59.3 years), being treated for depression were administered with setiptiline at doses and the frequency determined by the attending physicians according to symptoms and illness. The mean dose administered to the female volunteers was 0.08 mg/kg daily (range, 0.04–0.16 mg/kg) and to the male volunteers was 0.07 mg/kg daily (0.04–0.12 mg/kg). The mean plasma concentration observed was 3.54 (0.05–1.12) µg/L for the females and 2.24 (0.08–6.40) µg/L for the males. In terms of age, the mean plasma concentrations for mean ages of 23.8, 33.4, 44.0, 54.8, 65.6, 74.8 and 83.1 years were 1.92, 2.00, 3.06, 2.17, 2.61, 3.32 and 5.63 µg/L, respectively. The daily doses for these ages were 0.051, 0.057, 0.079, 0.062, 0.094, 0.086 and 0.064 mg/kg, respectively [Kamimura *et al.* 1994].

Toxicity Heart blood samples were taken from a subject whose death was suspected to be caused by acute setiptiline poisoning. A concentration of setiptiline of 1.77 µg/g was determined in blood taken from the left hand side of the heart and 0.78 µg/g from the right [Namera *et al.* 1998].

Half-life 11 h.

Kamimura M *et al.* (1994). The effect of age on plasma level of setiptiline maleate in depressed patients. *Prog Neuropsychopharmacol Biol Psychiatry* 18: 1015–1026.

Namera A *et al.* (1998). Simple analysis of tetracyclic antidepressants in blood using headspace-solid-phase microextraction and GC-MS. *J Anal Toxicol* 22: 396–400.

Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.

Sevoflurane

Anaesthetic

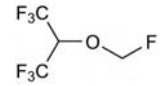
$C_4H_3F_7O = 200.1$

CAS—28523-86-6

IUPAC Name 1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane

Synonyms BAX-3084; MR-654.

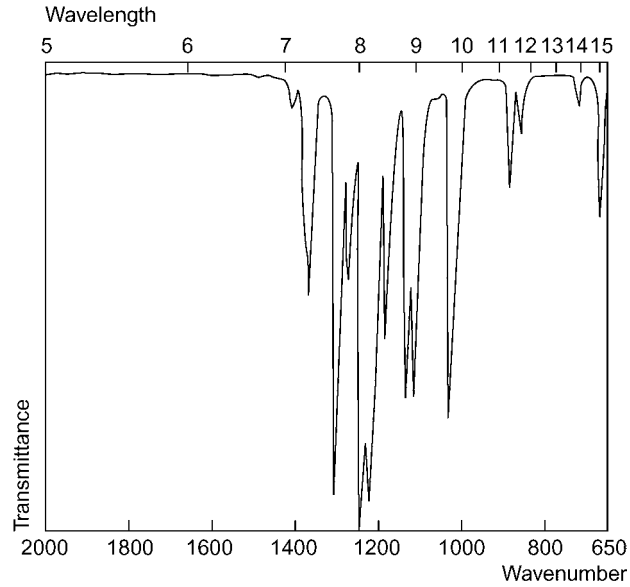
Proprietary Names *Sevofrane*; *Sevorane*; *Ultane.*



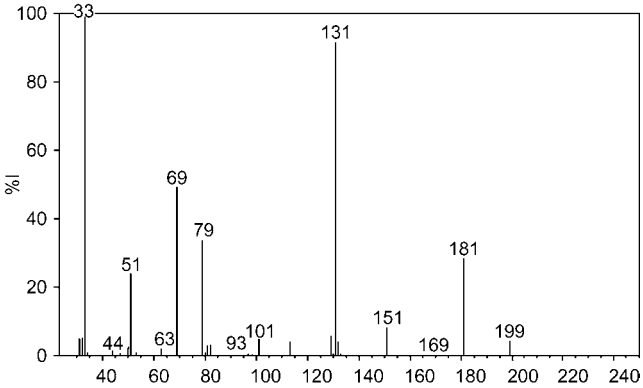
Chemical Properties A clear, colourless, volatile liquid with a non-pungent light odour. Non-flammable. Mp 25°. Bp 58.5°. Slightly soluble in water and miscible with ethanol, ether, chloroform and petroleum benzene. Log *P* (octanol/water), 1.75.

Gas Chromatography System GR—retention time 2.8 min.

Column: PoraPLOT Q (25 m × 0.32 mm i.d., 10 µm). Temperature programme: 30° for 2 min, to 250° at 15°/min for 5 min. Carrier gas: He, flow rate 3.8 mL/min. Detection: flame ionisation/FT-IR. IS: diethyl ketone. Retention time: (relative to IS) 0.758 [Ojanpera *et al.* 1998].



Mass Spectrum Principal ions at *m/z* 33, 131, 69, 79, 181, 51, 151, 101.



Quantification

Blood **GC** FT–IR detection. Limit of detection, 0.01 mg/L [Ojanpera *et al.* 1998].

GC-MS Pulse-heating method. Limit of detection, 0.2 mg/L [Saito *et al.* 1995].

Disposition in the Body Sevoflurane is readily absorbed via the pulmonary capillary system. Up to 5% is metabolised by the hepatic cytochrome P450-2E1 isoenzyme to hexafluroisopropanol (HFIP) with the release of inorganic fluoride and carbon dioxide. HFIP is rapidly conjugated with glucuronic acid and eliminated in the urine. No other metabolic pathway has been identified, suggesting that only 5% of the dose is metabolised. Approximately 3.5% of an administered dose appears in urine as the fluoride. Low solubility facilitates rapid uptake and elimination via the lungs. The blood/gas coefficient is low (0.60). Changes in the clinical effects rapidly follow changes in the inspired concentration. The rapid and extensive pulmonary elimination minimises the amount available for metabolism. Alveolar equilibration is rapid (85% within 30 min). Between 95 and 98% of the amount taken up is eliminated through the lungs. Distribution partition coefficients at 37° water/gas 0.36 and oil/gas 47.2.

Toxicity Toxic effects are related to the metabolism.

Half-life Sevoflurane, 9.5 min; HFIP glucuronide, 55 h.

Clearance Mean pulmonary elimination clearance, 3.58 L/min; total body clearance, 3.6 L/min.

Dose Administered by inhalation using a calibrated vaporiser. In adults, inspired concentrations of up to 5% sevoflurane usually produce surgical anaesthesia in <2 min. In children, inspired concentrations of up to 7% sevoflurane usually produce surgical anaesthesia in <2 min. Surgical levels may be sustained with concentrations of 0.5 to 3% sevoflurane with or without nitrous oxide. Less in the elderly.

Ojanpera I *et al.* (1998). Identification limits for volatile organic compounds in the blood by purge-and-trap GC-FTIR. *J Anal Toxicol* 22: 290–295.

Saito K *et al.* (1995). Determination of the volatile anesthetics halothane, enflurane, isoflurane, and sevoflurane in biological specimens by pulse-heating GC-MS. *J Anal Toxicol* 19: 115–119.

Sildenafil

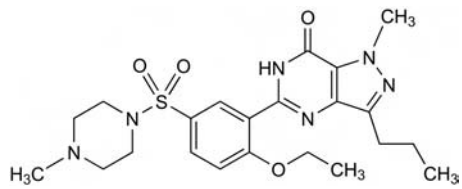
Phosphodiesterase 5 Inhibitor, Vasodilator

$C_{22}H_{30}N_6O_4S = 474.6$

CAS—139755-83-2

IUPAC Name 5-[2-Ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-4H-pyrazolo[4,3-*d*]pyrimidin-7-one

Synonym UK-92480-10



Chemical Properties Crystals. Mp 187° to 189°. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Reconstituted extracts were stable when stored for 24 h at 4° [Kim *et al.* 2003].

Sildenafil Citrate

$C_{22}H_{30}N_6O_4S,C_6H_8O_7 = 666.7$

CAS—171599-83-0

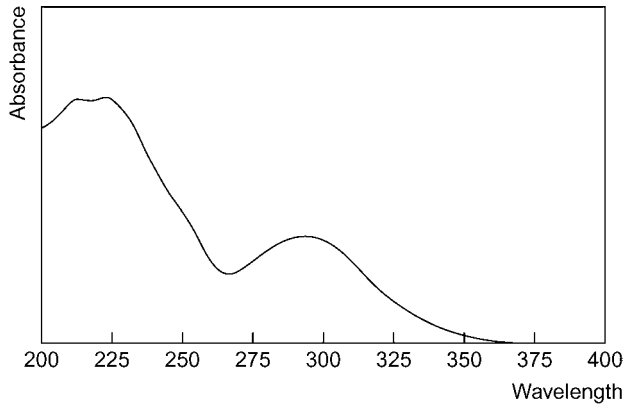
Proprietary Names *Viagra*

Chemical Properties A white to off-white crystalline powder. pK_a 8.7.

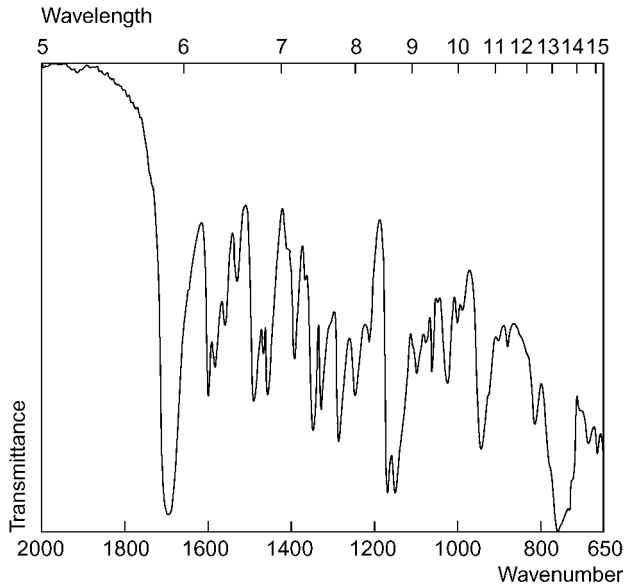
High Performance Liquid Chromatography Column: Kromasil RP C_4 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.5 mol/L potassium dihydrogen phosphate-10 mmol/L diethylamine hydrochloride (32 : 68), flow rate 0.7 mL/min. UV detection (λ = 230 nm). RT: 10.3 min [Lee, Min 2001].

Column: µBondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: 0.2 mol/L ammonium acetate (pH 7.0) : acetonitrile (1 : 1), flow rate 1 mL/min. UV detection (λ = 240 nm). RT: 9.0 min [Daraghmeh *et al.* 2001].

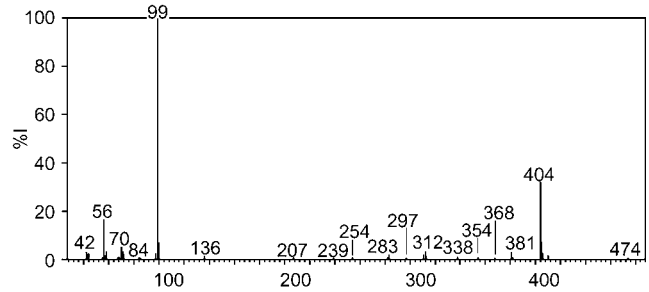
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 760, 1695, 1169, 946 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 99, 404, 56, 70, 312, 381, 42, 474.



Quantification

Plasma **HPLC** Column: Kromasil C_4 (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 500 mmol/L potassium phosphate buffer (pH 4.5) : water (28 : 4 : 68), flow rate 1.5 mL/min. UV detection (λ = 230 nm). Limit of quantification, 1 µg/L. [Cooper *et al.* 1997].

LC-MS Column: Acquity UPLC BEH C_{18} (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in methanol (40 : 60 to 10 : 90 over 1 min to 40 : 60 in 1 min), flow rate 0.3 mL/min. MRM acquisition mode. Limit of quantification, 1 µg/L [Witjes *et al.* 2010]. Column: Luna phenylhexyl (100 × 2 mm i.d., 3 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium formate (pH 6.0; 60 : 40), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.6 min. Limit of quantification, 2 µg/L [Kim *et al.* 2003].

Hair **GC-MS** Column: DB-5MS cross-linked methyl silicone (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 220° for 0.5 min to 280° at 20°/min.

SIM acquisition mode. Limit of quantification, 0.1 ng/mg, limit of detection 0.05 ng/mg. [Saisho *et al.* 2001].

Disposition in the Body Sildenafil is rapidly absorbed after oral administration. Peak plasma concentrations occur within ~30–120 min. Rate of absorption is reduced when taken with food. It is widely distributed into tissues and metabolised in the liver primarily by CYP3A4; some is also metabolised by CYP2C9. The major metabolite formed, *N*-desmethylsildenafil (UK-103,320), has some activity and makes up 30–40% of the total plasma drug concentration after a single dosing regimen. Sidenafil is excreted predominantly as metabolites in faeces (~80% of administered dose) and to a lesser extent in urine (~13% of administered dose with >2% as the parent drug). Clearance may be reduced in the elderly and in patients with severe renal or hepatic impairment.

Therapeutic Concentration

Four groups of healthy males, approximately 14 per group, aged between 18 and 45 years, were administered with a single oral dose of 100 mg sildenafil. The mean peak drug concentrations were 0.296, 0.302, 0.321 and 0.325 mg/L observed at 2.1, 3.1, 2.0 and 2.3 h, respectively. Peak concentrations for the metabolite *N*-desmethylsildenafil were 0.116, 0.118, 0.132 and 0.160 mg/L at 2.5, 3.5, 2.0 and 2.5 h. [Muirhead *et al.* 2000].

Toxicity

A 43-year-old man, treated for cardiovascular disease and erectile dysfunction with sildenafil citrate (Viagra), was found dead in a hotel room. Several tablets of verapamil, trimetazidine, yohimbine and bromazepam were found at the scene as well as a box of Viagra with two 25 mg tablets missing. Postmortem examination revealed sildenafil concentrations of 105, 246, 1206 and 754 µg/L in blood, urine, bile and gastric contents, respectively. Hair concentration was 177 ng/g. The desmethyl metabolite was measured as 143 µg/L in urine. Blood levels of verapamil and trimetazidine were found to be 659 and 2133 µg/L, respectively. Trinitrine and yohimbine were not detected [Dumestre-Toulet *et al.* 2002].

A 42-year-old female ingested 20 tablets of Viagra (100 mg/tablet) in a suicide attempt. Upon admission, she was oriented but nervous and complained of general weakness, palpitations, headache, dizziness and abdominal fullness. She underwent a gastrointestinal decontamination procedure, recovered and was discharged 12 h later [Hung, Yang 2001].

Over the period in which 6 million prescriptions were dispensed for sildenafil, the US Food and Drug Administration have reported 130 deaths in males taking sildenafil. The cause of death was unknown for 48 of these; 41 were caused by known or suspected myocardial function, 27 suffered from cardiac arrest, 6 with other cardiac symptoms and 3 with coronary artery disease. All were with a known risk factor or contraindication to use of the drug, for example co-administration with another drug or substance [US Food and Drug Administration 1998].

Half-life Plasma half-life: sildenafil, 3–5 h; *N*-desmethylsildenafil, 4 h.

Volume of Distribution ≈105 L.

Bioavailability 40%

Clearance Plasma clearance, 41 L/h.

Protein Binding Sildenafil and *N*-desmethylsildenafil, both 96%.

Note For a review of sildenafil see Langtry, Markham [1999].

Dose Usually the equivalent of 50 mg sildenafil.

Cooper JD *et al.* (1997). Development of an assay for the simultaneous determination of sildenafil (Viagra) and its metabolite (UK-103,320) using automated sequential trace enrichment of dialysates and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 701: 87–95.

Daraghmeh N *et al.* (2001). Determination of sildenafil citrate and related substances in the commercial products and tablet dosage form using HPLC. *J Pharm Biomed Anal* 25: 483–492.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Dumestre-Toulet V *et al.* (2002). Last performance with VIAGRA: postmortem identification of sildenafil and its metabolites in biological specimens including hair sample. *Forensic Sci Int* 126: 71–76.

Hung DZ, Yang DY (2001). Sildenafil overdose in a female patient. *J Toxicol Clin Toxicol* 39: 423–424.

Kim J *et al.* (2003). Simultaneous determination of sildenafil and its active metabolite UK-103,320 in human plasma using liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 32: 317–322.

Langtry HD, Markham A (1999). Sildenafil: a review of its use in erectile dysfunction. *Drugs* 57: 967–989.

Lee M, Min DI (2001). Determination of sildenafil citrate in plasma by high-performance liquid chromatography and a case for the potential interaction of grapefruit juice with sildenafil citrate. *Ther Drug Monit* 23: 21–26.

Muirhead GJ *et al.* (2000). Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir. *Br J Clin Pharmacol* 50: 99–107.

Saisho K *et al.* (2001). Hair analysis for pharmaceutical drugs II. Effective extraction and determination of sildenafil (Viagra) and its N-desmethyl metabolite in rat and human hair by GC-MS. *Biol Pharm Bull* 24: 1384–1388.

Witjes BC *et al.* (2010). Simultaneous assay of sildenafil and desmethylsildenafil in neonatal plasma by ultra-performance liquid chromatography–tandem mass spectrometry. *Biomed Chromatogr* 24: 180–185.

US Food and Drug Administration (1998). *Postmarketing Safety of Sildenafil Citrate (Viagra). Summary of Reports of Death in Viagra Users Received from Marketing (late March) through mid-November 1998*. Rockville, MD: US Food and Drug Administration.Food US, Drug Administration (1998). .

Simazine

Herbicide

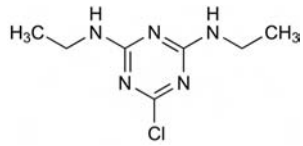
$C_7H_{12}ClN_5$ = 201.7

CAS—122-34-9

IUPAC Name 6-Chloro-2-*N*,4-*N*-diethyl-1,3,5-triazine-2,4-diamine

Synonym 6-Chloro-*N*,*N*′-diethyl-1,3-5-triazine-2,4-diamine

Proprietary Names *Gesatop*; *Princep*; *Simadex*. It is an ingredient of *Pathclear*.



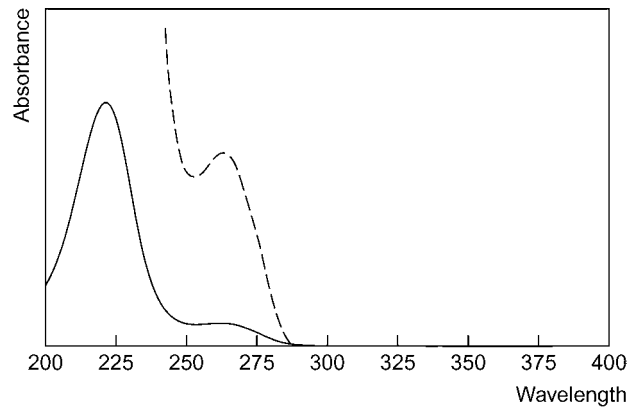
Chemical Properties A white crystalline powder. Mp 226° to 227°. Practically insoluble in water; very slightly soluble in chloroform and ether. pK_a 1.6 (20°). Log *P* (octanol/water), 2.2.

Thin-layer Chromatography System TA—R_f 0.73; system TX—R_f 0.22; system TY—R_f 0.18.

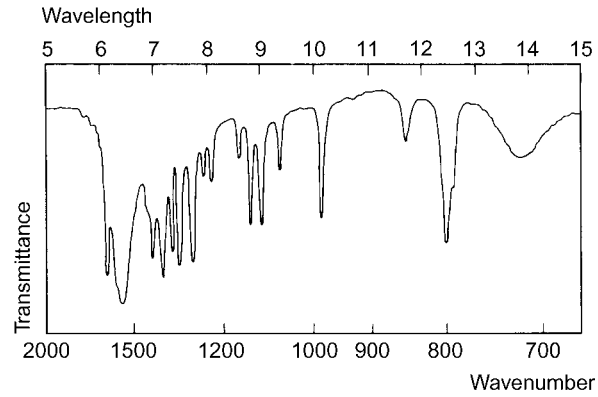
Gas Chromatography System GA—RI 1690; system GK—RRT 0.8 (relative to caffeine).

High Performance Liquid Chromatography System HAA—retention time 15.7 min.

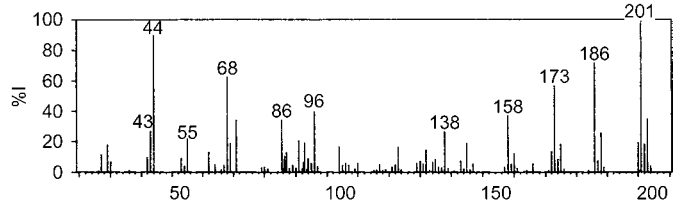
Ultraviolet Spectrum Ethanol—262 nm (A_1^1=162b).



Infrared Spectrum Principal peaks at wavenumbers 1553, 1621, 1289, 797, 1103, 1129 cm^{-1} (KBr disk).



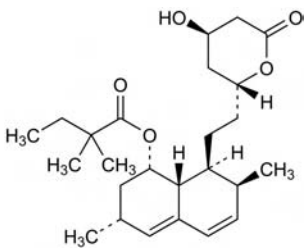
Mass Spectrum Principal ions at *m/z* 201, 44, 186, 68, 173, 96, 158, 203.



Simvastatin

HMG-CoA Reductase Inhibitor, Antihyperlipidaemic
$C_{25}H_{38}O_5$ = 418.6
CAS—79902-63-9

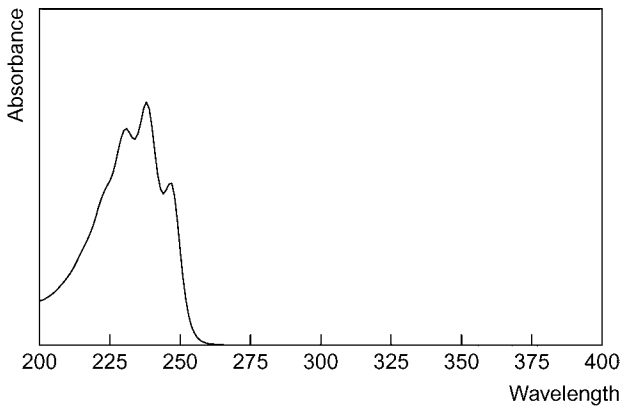
IUPAC Name [(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl] ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate
Synonyms 2,2-Dimethylbutanoic acid (1*S*,3*R*,7*S*,8*S*,8a*R*)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl] ethyl]-1-naphthalenyl ester; L-644128-000U; MK-733; synvinolin; velastatin.
Proprietary Names *Denan*; *Lipex*; *Liponorm*; *Lipovas*; *Lodalès*; *Simovil*; *Sinvacor*; *Sivastin*; *Zocor*; *Zocord*.



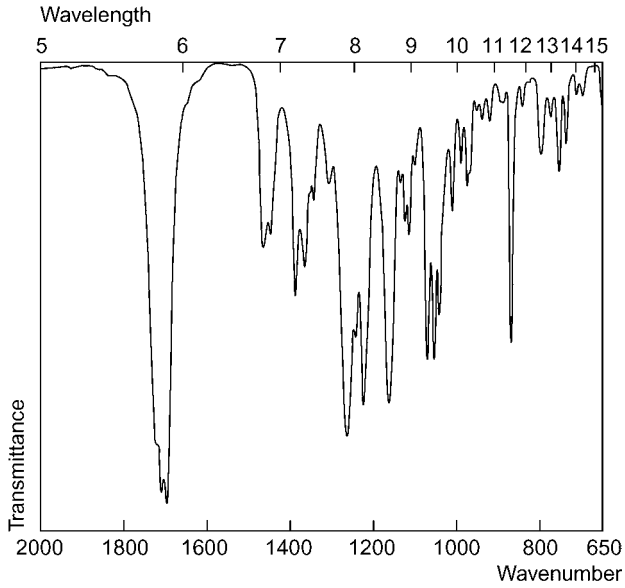
Chemical Properties A white crystalline powder. Mp 135° to 138°. Insoluble in water (0.03 g/L), *n*-hexane (0.15 g/L) and hydrochloric acid (0.1 mol/L) (0.06 g/L); soluble in chloroform (610 g/L), dimethyl sulfoxide (540 g/L), methanol (200 g/L), ethanol (160 g/L), polyethylene glycol (70 g/L), sodium hydroxide (0.1 mol/L) (70 g/L) and propylene glycol (30 g/L). Log *P* (octanol/water), 4.68.
Thin-layer Chromatography System TE—R_f 0.68; system TF—R_f 0.44.
Gas Chromatography Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Column temperature: 280°. Carrier gas: helium, flow rate 0.9 mL/min. MS detection. RI: 3158 [Mills, Roberson 1996].
High Performance Liquid Chromatography System HX—RI 814.

Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol, flow rate 0.5 mL/min. UV diode array detection. Retention time: 3.4 min [Mills, Roberson 1996].

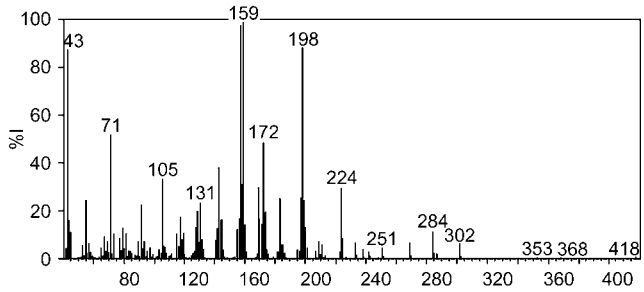
Column: (analytical) ODS (Hypersil, 250 × 4.6 mm i.d., 5 µm); (guard) Pelliguard precolumn (20 mm). Mobile phase: sodium dihydrogen phosphate (25 mmol/L, pH 4.5): acetonitrile (35:65), flow rate 1.5 mL/min. UV detection (λ =238 nm). Retention times: 7.2 min for simvastatin; 3.6 min for simvastatin β,δ-dihydroxyacid [Carlucci *et al.* 1992].
Ultraviolet Spectrum Acetonitrile—231, 238, 247 nm; basic—229, 237, 246 nm.



Infrared Spectrum Principal peaks at wavenumber 1718, 1459, 1389, 1267 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 159, 157, 43, 198, 71, 172, 143, 105.



Quantification

Plasma **GC–MS** Limit of detection, 0.1 µg/L [Scoppola *et al.* 1991]. Limit of quantification, 0.1 µg/L [Takano *et al.* 1990].

HPLC Fluorescence detection (λ_{ex} = 360 nm, λ_{em} = 430 nm). Limit of quantification, 0.1 µg/L [Ochiai *et al.* 1997].

Disposition in the Body Simvastatin is a pharmacologically inactive pro-drug which is rapidly metabolised mainly to simvastatin β-hydroxy acid analogue (with maximum concentrations being reached within 1.3 to 2.4 h after dosing) which is an inhibitor of HMC-CoA reductase. Other metabolites include 3-hydroxy-, 3-hydroxy-3-methyl- and 3-oxomethylene derivatives and analogues of 6-hydroxymethyl- and 6-carboxylic acid, of which the chiral centre at position 6 has been inverted (these are the biliary metabolites). The drug and its metabolites are excreted in urine (13%) and faeces (60%) which includes both the unabsorbed drug and drug excreted in bile.

Therapeutic Concentration

Twelve healthy males, aged between 22 and 29 years, were administered with a single 40 mg dose of simvastatin. The peak plasma concentration of the active inhibitors was 10.3 µg (equivalent)/L and total inhibitors 34.5 µg (equivalent)/L, reached in 2.5 and 2.3 h, respectively [Pentikainen *et al.* 1992].

Bioavailability β-Hydroxyacid, 5%.
Half-life Simvastatin, 2 h; β-hydroxyacid, 1.9 h.
Protein Binding Simvastatin, 98%; β-hydroxyacid, 94%.
Note For a review of simvastatin, see Plosker and McTavish [1995].
Dose The usual daily dose is 10 to 80 mg.

Carlucci G *et al.* (1992). Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high-performance liquid chromatography with UV detection. *J Pharm Biomed Anal* 10: 693–697.
Mills T, Roberson JC (1996) *Instrumental Data for Drug Analysis*, 2nd edn, Vol. 6. Boca Raton, FL: CRC Press, 224–225
Ochiai H *et al.* (1997). Determination of simvastatin and its active metabolite in human plasma by column-switching high-performance liquid chromatography with fluorescence

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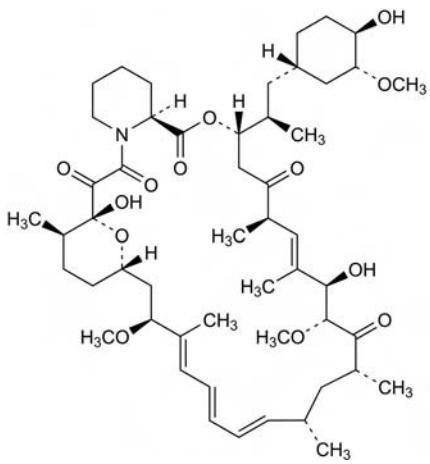
detection after derivatization with 1-bromoacetylpyrene. *J Chromatogr B Biomed Sci Appl* 694: 211–217.
Pentikainen PJ *et al.* (1992). Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J Clin Pharmacol* 32: 136–140.
Plosker GL, McTavish D (1995). Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. *Drugs* 50: 334–363.
Scoppola A *et al.* (1991). Quantitation of plasma mevalonic acid using gas chromatography-electron capture mass spectrometry. *J Lipid Res* 32: 1057–1060.
Takano T *et al.* (1990). A selected ion monitoring method for quantifying simvastatin and its acid form in human plasma, using the ferroceneboronate derivative. *Biomed Environ Mass Spectrom* 19(9): 577–581.

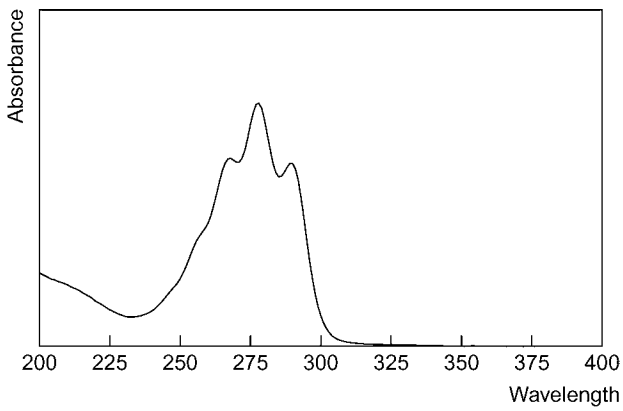
Sirolimus

Immunosuppressant
$C_{51}H_{79}NO_{13}$ = 914.2
CAS—53123-88-9

Synonyms AY-22989; AY-022989; (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-hexadecahydro-9,27-dihydroxy-3-((1*R*)-2-((1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl)-1-methylethyl)-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-c][1,4]oxaazacyclohentriacontine-1,5,11,28,29-(4*H*,6*H*,31*H*)-pentone; NSC 226080; rapamycin; RPM; SILA 9268A; WY-090217.
Proprietary Name *Rapamune*



Chemical Properties Colourless crystalline solid. Mp 183° to 185°. Insoluble in water; soluble in ether, chloroform, acetone, methanol and dimethylformamide; very sparingly soluble in hexane and petroleum ether.
High Performance Liquid Chromatography Column: BDS C_{18} Hypersil (100 × 3.0 mm i.d., 3 µm). Temperature: 70°. Mobile phase: acetonitrile : methanol: water (38 : 34 : 28), flow rate 0.3 mL/min. UV detection (λ = 278 nm). Retention time: 8.4 min [Svensson *et al.* 1997].
Liquid Chromatography-Mass Spectrometry
Column: C_{18} Novapak (150 × 2.1 mm i.d., 4 µm). Temperature: 50°. Mobile phase: methanol : 50 mmol/L ammonium acetate buffer (pH 5.1; 80 : 20), flow rate 0.2 mL/min. IS: 32-*O*-desmethoxysirolimus. ESI, SIM at *m/z* 931.8→864.6 for sirolimus; 901.8→834.4 for IS. Retention time: sirolimus, 7.8 min; IS, 8.4 min [Taylor, Johnson 1998].
Ultraviolet Spectrum Aqueous acid—267, 277, 288 nm.

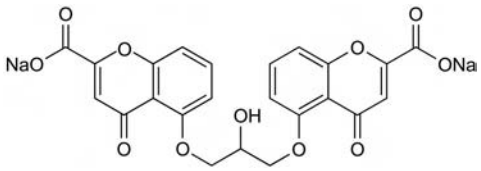


Quantification
Blood **HPLC** UV detection (λ = 278 nm). Limit of quantification, 6.5 µg/L [Holt *et al.* 2000]. UV detection (λ = 278 nm). Limit of detection, 2.5 µg/L [Maleki *et al.* 2000]. UV detection (λ = 278 nm). Limit of detection, 0.4 µg/L [Svensson *et al.* 1997].
LC-MS Limit of quantification, 0.25 µg/L [Taylor *et al.* 2000]. Tandem-MS detection. Limit of quantification, 0.2 µg/L [Taylor, Johnson 1998].
Immunoassay Limit of detection, 1.0 µg/L [Jones *et al.* 2000].
Disposition in the Body Sirolimus is rapidly but incompletely absorbed (within 1 h); it is more slowly absorbed with a high-fat meal compared with those in the fasting state. Peak concentrations are reduced and the times taken to reach these levels are increased. Peak whole blood concentrations increase proportionally with the dose administered. Sirolimus is extensively distributed to a variety of tissues and organs. It is a substrate for both cytochrome P4503A4 and *p*-glycoprotein. It is metabolised in both the intestine and liver by *O*-demethylation and hydroxylation to 7 known metabolites including hydroxyl, demethyl and hydroxydemethyl derivatives. It is excreted mainly in faeces (90%) and a small amount in urine (2%).
Therapeutic Concentration
Thirty patients treated with a stable renal transplant were administered 0.5, 1.0, 1.5, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.5 mg/m^2 every 12 h for 13 days and a single dose on day 14. Each participant was treated with cyclosporin for at least 3 months beforehand and steady-state therapeutic blood concentrations had been achieved. The steady-state concentrations were 10.1, 20.4, 22.0, 40.5, 44.9, 112.0, 93.6, 110.0 and 210.0 µg/L for the doses, respectively. These concentrations were observed at 1.1, 0.8, 0.9, 2.7, 0.8, 1.3, 3.0, 1.0 and 0.7 h, respectively [Zimmerman, Kahan 1997].
Sixteen patients, aged 24 to 63 years, who had received a renal transplant at least 6 months before inclusion in the study and diagnosed as clinically stable for at least 3 months before treatment participated in the study. After an overnight fast, all were administered either 3, 5, 10 or 15 mg/m^2 as an oral solution (5 mg/mL). Mean peak plasma concentrations were 1.00, 0.83, 2.93 and 3.23 µg/L for the 4 doses observed at 1.3, 3.7, 3.0, 2.3 h, respectively. The peak whole blood concentrations were 78.0, 46.3, 119.7 and 190.7 µg/L for the 3, 5, 10 and 15 mg/m^2 doses at 0.83, 2.67, 1.17 and 1.67 h, respectively [Brattstrom *et al.* 1997].
Toxicity
Sirolimus toxicity is associated with thrombocytopenia, leucopenia, anaemia and hyperlipidaemia and whole blood concentrations of >15 µg/L increase this risk. Concentrations of <6 µg/L are associated with acute rejection [Meier-Kriesche, Kaplan 2000].
Bioavailability ≈15%.
Half-life Mean, 57 h (range, 44–87 h).
Volume of Distribution Steady-state, mean, 23 L/kg (healthy); 11.2 L/kg (patients treated by renal transplant).
Clearance Mean, 278 mL/h/kg (healthy); 199.5 mL/h/kg (patients with renal transplant).
Distribution in Blood Whole blood : plasma concentration ratio is 49.1.
Protein Binding 92%, mainly to albumin.
Dose 2 mg daily.

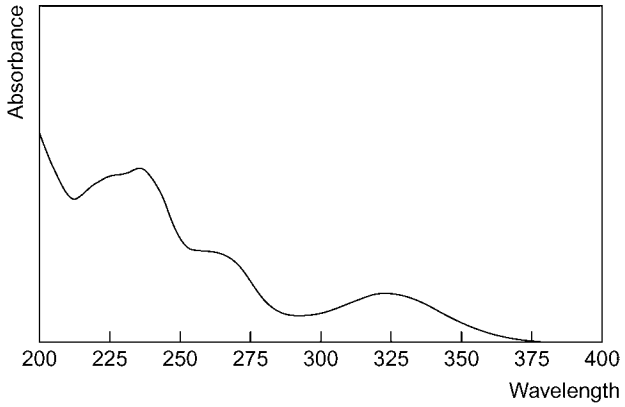
Brattstrom C *et al.* (1997). Kinetics and dynamics of single oral doses of sirolimus in sixteen renal transplant recipients. *Ther Drug Monit* 19(4): 397–406.
Holt DW *et al.* (2000). Measurement of sirolimus in whole blood using high-performance liquid chromatography with ultraviolet detection. *Clin Ther* 22 (Suppl. B): B38–B48.
Jones K *et al.* (2000). An immunoassay for the measurement of sirolimus. *Clin Ther* 22 (Suppl. B): B49–B61.
Maleki S *et al.* (2000). Therapeutic monitoring of sirolimus in human whole-blood samples by high-performance liquid chromatography. *Clin Ther* 22 (Suppl. B): B25–B37.
Meier-Kriesche HU, Kaplan B (2000). Toxicity and efficacy of sirolimus: relationship to whole-blood concentrations. *Clin Ther* 22: BB93–B100.
Svensson JO *et al.* (1997). Determination of rapamycin in whole blood by HPLC. *Ther Drug Monit* 19 (1): 112–116.
Taylor PJ, Johnson AG (1998). Quantitative analysis of sirolimus (Rapamycin) in blood by high-performance liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 718(2): 251–257.
Taylor PJ *et al.* (2000). Simultaneous quantification of tacrolimus and sirolimus, in human blood, by high-performance liquid chromatography-tandem mass spectrometry. *Ther Drug Monit* 22(5): 608–612.
Zimmerman JJ, Kahan BD (1997). Pharmacokinetics of sirolimus in stable renal transplant patients after multiple oral dose administration. *J Clin Pharmacol* 37: 405–415.

Sodium Cromoglicate

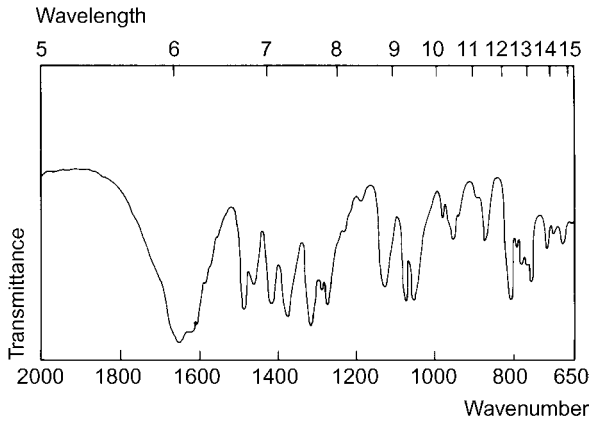
Antiallergic
$C_{23}H_{14}Na_2O_{11}$ = 512.3
CAS—16110-51-3 (cromoglycic acid); 15826-37-6 (disodium salt)
IUPAC Name Disodium 5-[3-(2-carboxylato-4-oxochromen-5-yl)oxy-2-hydroxypropoxy]-4-oxochromene-2-carboxylate
Synonyms Cromolyn sodium; disodium cromoglicate; disodium-5,5′-[(2-hydroxy-1,3-propanediyl)bis(oxy)]bis[4-oxo-4*H*-1-benzopyran-2-carboxylate]; sodium cromoglycate; FPL-670; DSCG.
Proprietary Names *Acromax*; *Allercrom*; *Brol-eze*; *Clariteyes*; *Colimune*; *Cromabak*; *Cromogen*; *Cromo(l)*; *Cromolyn*; *Flenid*; *Frenal*; *Gastrocrom*; *Glinor*; *Hay-Crom*; *Intal*; *Lecrolyn*; *Lomudal*; *Lomusol*; *Nalcrom*; *Nasalcrom*; *Opticrom*; *Rynacrom*; *Vividrin*.



Chemical Properties A white, hygroscopic, crystalline powder. Mp 241° to 242°, with decomposition (cromoglycic acid). Soluble 1 in 20 of water; practically insoluble in ethanol, ether and chloroform. Cromoglycic acid: pK_a 2.5 (20°). Cromoglycic acid: Log *P* (octanol/water), 1.9.
Colour Tests Copper sulfate (method 2)—blue (1 to 2 min); Mandelin's test—red; Marquis test—yellow; methanolic potassium hydroxide—yellow; sulfuric acid—yellow.
Thin-layer Chromatography Cromoglycic acid: System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.00.
Ultraviolet Spectrum Phosphate buffer (pH 7.4)—238 (A^1_1=600b), 326 nm (A^1_1=164a).



Infrared Spectrum Principal peaks at wavenumbers 1635, 1305, 1264, 805, 1069, 1047 cm^{-1} (KBr disk).



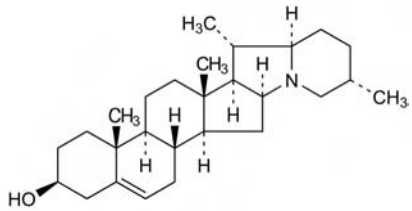
Mass Spectrum Principal ions at *m/z* 44, 206, 162, 108, 51, 207, 201, 178 (cromoglycic acid).
Quantification
Plasma **Spectrofluorimetry** Limit of detection, 100 µg/L [Moss *et al.* 1971].
HPLC-MS Limit of detection, 0.1 µg/L [Ozou *et al.* 2001].
Urine **HPLC** UV detection. Limit of detection, 250 µg/L [Aswania *et al.* 1997]. Fluorescence detection [Mawatari *et al.* 1997]. UV detection. Limit of detection, 50 µg/L [Gardner 1984].
Polarography Limit of detection, 500 µg/L [Fogg, Fayad 1978].
Disposition in the Body Sodium cromoglicate is poorly absorbed after oral or SC administration. After inhalation most of a dose is swallowed with <10% reaching the lungs, from where it is rapidly absorbed; the amount absorbed after inhalation is dependent on particle size, smaller particles being absorbed better than large ones. It does not appear to be metabolised. Up to about 3% of an inhaled dose is excreted unchanged in the urine in 24 h and up to about 87% is eliminated unchanged in the faeces in 3 days. After oral administration <1% is excreted in the urine, and after an IV dose about 50% is excreted in the urine and 50% is eliminated in the faeces.
Therapeutic Concentration
Following inhalation of a ^{14}C-labelled 20-mg dose by 12 asthmatic subjects, peak plasma concentrations of 0.006 to 0.012 mg/L (mean, 0.009) were attained in 15 min [Walker *et al.* 1972].

After inhalation of a single 60 mg dose by 3 subjects, peak plasma concentrations of about 0.2 to 0.3 mg/L were reported in 10 to 15 min [Moss *et al.* 1971].
Half-life Plasma half-life, about 1 to 1.5 h.
Clearance Plasma clearance, about 8 mL/min/kg.
Protein Binding About 60 to 70%.
Dose 20 mg inhaled as a dry powder 4 times daily; 800 mg daily, orally.

Aswania OA *et al.* (1997). Development and validation of an ion-pair liquid chromatographic method for the quantitation of sodium cromoglycate in urine following inhalation. *J Chromatogr B Biomed Sci Appl* 690: 373–378.
Fogg AG, Fayad N (1978). *Anal Chim Acta* 102: 205–210.
Gardner JJ (1984). Determination of sodium cromoglycate in human urine by high-performance liquid chromatography on an anion-exchange column. *J Chromatogr* 305: 228–232.
Mawatari K *et al.* (1997). Determination of disodium cromoglycate in human urine by high-performance liquid chromatography with post-column photoirradiation-fluorescence detection. *Analyst* 122: 715–717.
Moss GF *et al.* (1971). Plasma levels and urinary excretion of disodium cromoglycate afer inhalation by human volunteers. *Toxicol Appl Pharmacol* 20: 147–156.
Ozou ML *et al.* (2001). Determination of sodium cromoglycate in human plasma by liquid chromatography-mass spectrometry in the turbo ion spray mode. *J Chromatogr B Biomed Sci Appl* 765: 179–185.
Walker SR *et al.* (1972). The fate of (14 C)disodium cromoglycate in man. *J Pharm Pharmacol* 24: 525–531.

Solanidine

Alkaloid
$C_{27}H_{43}NO$ = 397.6
CAS—80-78-4
Synonyms (3β)-Solanid-5-en-ol; solatubine.

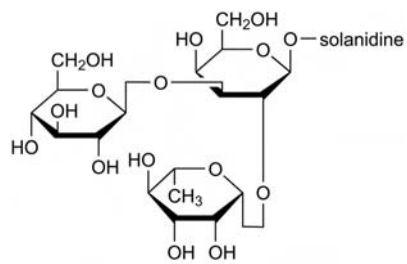


Chemical Properties An alkaloid present in various species of *Solanum* (Solanaceae). It is the aglycone of solanine. A white crystalline powder. Mp 218° to 219°, with decomposition. Practically insoluble in water and ether; slightly soluble in ethanol; freely soluble in chloroform; soluble in dilute acids. Log *P* (octanol/water), 7.3.
Colour Tests Mandelin's test—orange→violet→blue; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.68.
Quantification
Plasma **Radioimmunoassay** Limit of detection, 200 ng/L [Matthew *et al.* 1983].
Serum **HPLC** UV detection. Solanidine and glycoalkaloids. Limit of detection, 0.3 µg/L [Hellenas *et al.* 1992].

Hellenas KE *et al.* (1992). Determination of potato glycoalkaloids and their aglycone in blood serum by high-performance liquid chromatography. Application to pharmacokinetic studies in humans. *J Chromatogr* 573: 69–78.
Matthew JA *et al.* (1983). Determination of solanidine in human plasma by radioimmunoassay. *Food Chem Toxicol* 21: 637–640.

Solanine

Alkaloid
$C_{45}H_{73}NO_{15}$ = 868.1
CAS—20562-02-1
Synonym Solatunine



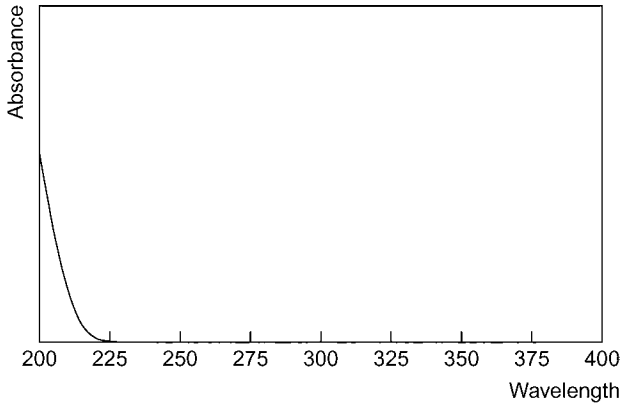
Chemical Properties A glycoalkaloid present in various species of *Solanum*, particularly *S. tuberosum* (Solanaceae). A white crystalline powder which decomposes at 285°. Practically insoluble in water, chloroform and ether; soluble in hot ethanol, dilute acids and amyl alcohol. pK_a 6.65 (15°). Log *P* (octanol/water), 2.0.

S

Colour Tests Mandelin's test—orange→violet→blue; Marquis test—yellow→violet.

Thin-layer Chromatography System TA—R_f 0.52 (Marquis reagent, positive).

Ultraviolet Spectrum



Quantification See under Solanidine.

Soman

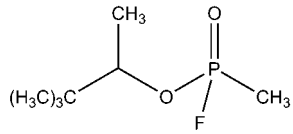
Anticholinesterase, Organophosphate Nerve Agent

$C_7H_{16}FO_2P = 182.2$

CAS—96-64-0

IUPAC Name Pinacoloxymethylphosphoryl fluoride

Synonyms GD; methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester; pinacolyl methylphosphonofluoridate.



Chemical Properties Colourless liquid with a fruity/oil of camphor odour. Mp −42°. Bp 198°. Very soluble in water. Log *P* (octanol/water), 1.78. Analytes were stable for at least 2 weeks in urine stored at 4° and for at least 2 years in urine samples stored at –70° [Barr *et al.* 2004].

Note Soman consists of a mixture of 4 stereoisomers. These isomers are assigned $C_{(-)}P_{(-)}$, $C_{(-)}P_{(+)}$, $C_{(+)}P_{(-)}$ and $C_{(+)}P_{(+)}$, where C stands for the pinacolyl moiety and P for the phosphorus atom. The (−) and (+) refer to laevorotation or dextrorotation, respectively [Benschop *et al.* 1981].

Thin-layer Chromatography Plates: Silica gel (5 × 10 cm). Solvent system: methanol : chloroform (5 : 95) or acetone: methanol : chloroform : concentrated ammonia (25 : 25 : 45 : 5). Liquid scintillation counting. R_f value: 0.64 for soman, with its metabolite pinacoylmethylphosphonic acid (PMPA) remaining at the origin in the methanol : chloroform system; 0.90 and 0.39 for soman and PMPA, respectively, in the acetone : methanol : chloroform : concentrated ammonia system [Reynolds *et al.* 1985].

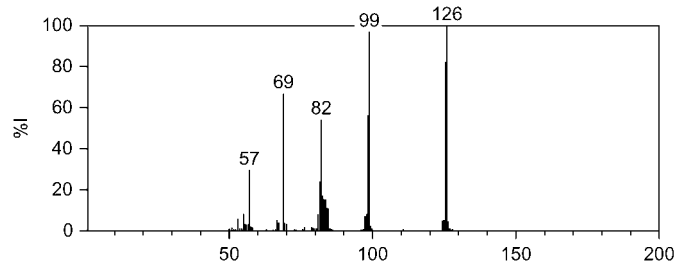
Gas Chromatography-Mass Spectrometry Column: Chiraldex γ-cyclodextrin trifluoroacetyl (20 m × 0.25 mm i.d., 0.125 μm). Carrier gas: He, 45 cm/s. Temperature programme: 80° for 14 min to 90° at 5°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 12.0, 12.8, 13.2 and 13.6 min for $C_{(-)}P_{(-)}$, $C_{(-)}P_{(+)}$, $C_{(+)}P_{(-)}$ and $C_{(+)}P_{(+)}$, respectively. Limit of detection, not reported [Yeung *et al.* 2007]. Column: DB5-MS (25 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 0.9 mL/min. Temperature programme: 40° for 2 min to 160° at 20°/min to 310° at 30°/min for 5 min. ATD, full scan mode. Limit of detection, 50 ng [Carrick *et al.* 2001]. Column: CP-Sil 19 (50 m × 0.32 mm i.d., 0.4 μm). Temperature programme: 60° for 3 min to 70° at 5°/min for 7 min to 180° at 10°/min to 240° at 15°/min for 12 min. Limit of detection, ng/L range [Degenhardt-Langelaan, Kientz 1996]. DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, PMPA 1 ng/mL [Shih *et al.* 1991].

High Performance Liquid Chromatography Column: LiChrosorb Hibar RP-18 (250 × 4.0 mm i.d., 5 μm). Mobile phase: methanol : water (15 : 85 to 65 : 35 over 35 min for 3 min), flow rate 0.7 mL/min. UV detection (λ = 254 nm). Limit of detection, 10 pg [Sipponen 1987].

Liquid Chromatography-Mass Spectrometry Column: Waters Atlantis HILIC (50 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate (86 : 14). ESI, negative ion mode, SRM acquisition mode. Limit of detection, PMPA 30 ng/L [Mawhinney *et al.* 2007a]. The effect of post-column addition of organic solvents on this method is reported in Mawhinney *et al.* [2007b].

Capillary Electrophoresis Capillary: fused silica (56 cm (effective length) × 75 μm i.d.). Buffer: 200 mmol/L boric acid : 10 mmol/L phenylphosphonic acid : 0.03% Triton X-100 : 0.35 mmol/L didodecyldimethylammonium hydroxide. UV detection (λ = 210 nm). Limit of quantification, PMPA 0.5 mg/L; limit of detection, PMPA 0.1 mg/L [Nassar *et al.* 1998].

Mass Spectrum Principal ions at *m/z* 126, 99, 69, 82, 57 [Beck *et al.* 1981]; 181, 256, 123, 277, 97, 303, 41, 80, 57, 161, 345 (PFB derivative) [Shih *et al.* 1991].



Quantification

Blood **GC-MS** Column: DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°/min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Shih *et al.* 1991]. Column: HP-SE-54 bonded methylsilicon (15 m). Temperature: 75°. EI ionisation at 70 eV. Limit of detection not reported [Singh *et al.* 1985]

Plasma **GC** Column CPSil 8CB fused silica (51 m × 0.32 mm i.d., 1.3 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 87° to 140° at 6.2/min or 87° to 130° at 5°/min. FID. Limit of detection, 0.1 mg/L [de Jong *et al.* 1988].

GC-MS Column Chiraldex γ-cyclodextrin trifluoroacetyl (2.0 m × 0.25 mm i.d., 0.125 μm). Carrier gas: He, 45 cm/s. Temperature programme: 80° for 14 min to 90° at 5°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 11.8, 12.6, 13.0 and 13.4 min for $C_{(-)}P_{(-)}$, $C_{(-)}P_{(+)}$, $C_{(+)}P_{(-)}$, and $C_{(+)}P_{(+)}$, respectively. Limit of detection not reported [Yeung *et al.* 2008]. Column: DB-5MS bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 2 min to 100° at 10°/min for 3 min. EI ionisation at 70 eV. SIM acquisition mode. Limit of detection, 0.5 μg/L [Adams *et al.* 2004].

Serum **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, PMPA 5 μg/L [Miki *et al.* 1999].

HPLC Column: Shim-pack IC-A3 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.5 mmol/L phthalic acid : 0.1 mmol/L Tris : 5% acetonitrile, flow rate 1 mL/min. UV detection (λ = 266 nm). Limit of detection, PMPA 80 μg/L [Katagi *et al.* 1997].

LC-MS Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45, containing 0.1% glycerol), flow rate 100 μL/min. FAB ionisation. Limit of detection, PMPA 5 μg/L [Katagi *et al.* 1999].

Urine **GC-MS** Column: Restek Rtx-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 40 cm/s. Temperature programme: 60° for 1 min to 180° at 30°/min to 280° at 10°/min for 2 min. CI, negative ion mode, SRM acquisition mode. Limit of detection, PMPA 0.1 μg/L [Riches *et al.* 2005]. Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 70° for 1.5 min to 250° in 5 min at 40°/min for 3 min. MRM acquisition mode. Limit of detection, PMPA 0.5 μg/L [Barr *et al.* 2004]. Column: DB-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 1.5 min to 250° at 40°/min in 5 min for 5 min. SRM acquisition mode. Limit of detection, 3 μg/L [Driskell *et al.* 2002]. Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, PMPA 5 μg/L [Miki *et al.* 1999]. Column: DB-17 bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 60° for 1 min to 200° at 20°/min for 4 min to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, PMPA 1 μg/L [Shih *et al.* 1991].

Albumin **GC-MS** DB-5MS bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 2 min to 100° at 10°/min for 3 min. EI ionisation at 70 eV. SIM acquisition mode. Limit of detection, 0.5 μg/L [Adams *et al.* 2004].

Oral Fluid **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, PMPA 5 μg/L [Miki *et al.* 1999].

Other **GC** Rabbit and Mouse Plasma and Tissue. Column: Alltech Chirasil-Val (50 m × 0.32 mm i.d., 0.2 μm). Carrier gas: He, 1 mL/min. Temperature programme: 60° for 1.6 min to 90° at 5°/min for 10 min to 130° at 5°/min to 200° at 10°/min for 1 min. NPD. Retention time: 19 to 20 min. Limit of detection, 0.1 μg/L [Li *et al.* 2003]. Military-related Waste. Column: DB-5 (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 20 mL/min. Temperature programme: 60°to 90° at 10°/min for 1 min to 220° at 45°/min for 1.1 min. FPD. Limit of detection, non-contaminated metal 0.39 μg/kg, non-contaminated decontamination fluid 0.96 μg/L, suspect-contaminated decontamination fluid 0.49 μg/L, non-contaminated soil 1.2 μg/kg, suspect-contaminated soil 0.57 μg/kg [O'Neill *et al.* 2002].

Water. Column: CP-Sil 19 (50 m × 0.32 mm i.d., 0.4 μm). Temperature programme: 60° for 3 min to 70° at 5°/min for 7 min to 180° at 10°/min to 240° at 15°/min for 12 min. Limit of detection, ng/L range [Degenhardt-Langelaan, Kientz 1996].

GC-MS Rat Plasma. Column: Restek Rtx-1701 (30 m × 0.32 mm i.d., 1.0 μm). Carrier gas, He, 3.0 mL/min. Temperature programme: 35° for 5 min to 164° at 25°/min to 170° at 2°/min to 270° at 50°/min for 3 min. CI, positive ion mode. Retention time: 11.4 min. Limit of quantification, 18.83 pg; limit of detection, 5.65 pg [Renner *et al.* 2008]. Pesticide Solutions. Column: HP-5 5% phenylmethylpolysiloxane capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 0.9 L/min. Temperature programme: 80° for 1 min to 280° at 20°/min for 4 min. ICP-MS detection. Limit of detection not reported [Richardson, Caruso 2007a, b]. Guinea-pig Plasma and Tissue. Column: DB-5MS bonded phase (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 2 min to 100° at 10°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.5 μg/L [Adams *et al.* 2004]. Porcine CSF. Column: Chirasil-L-Val fused silica capillary (25 m × 0.25 mm i.d., 0.12 μm). Carrier gas: He, 30 cm/s. Temperature programme: 40° to 150° at 10°/min. EI ionisation at 40 eV, SIM acquisition mode Limit of detection not reported [Goransson-Nyberg *et al.* 1998]. Guinea-pig Blood and CSF. Column: Chirasil-L-Val fused silica capillary (25 m × 0.25 mm i.d., 0.12 μm). Carrier gas: He, 30 cm/s. Temperature programme: 40° to 150° at 10°/min. EI ionisation at 40 eV, SIM acquisition mode. Limit of detection not reported [Goransson-Nyberg *et al.* 1998]. Canine Blood. Column: HP-SE-54 bonded methylsilicon (15 m). Temperature: 75°. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Singh *et al.* 1985]. Mouse Blood and Tissue. Column: SP 1000 WCOT capillary (20 m × 0.3 mm i.d.). Carrier gas: He, 2 mL/min. Temperature: 100°. EI ionisation at 70 eV. Limit of detection, 0.5 μg/L or kg of tissue [Beck *et al.* 1981].

LC-MS Spiked Food, Bottled Water, Canola Oil, Cornmeal and Honey. Column: Agilent Zorbax SB-C_{18} (50 × 0.3 mm i.d., 1.8 μm). Mobile phase: 0.1% trifluoroacetic acid : acetonitrile (95 : 5 to 50 : 50 over 15 min), flow rate 10 μL/min. ESI, APCI, or FAIMS. Limit of quantification, 8 and 33 μg/L using APCI with and without FAIMS, respectively; limit of detection, 2 and 10 μg/L using APCI with and without FAIMS, respectively. Limit of quantification, PMPA 2000 and 2500 μg/L using ESI with and without FAIMS, respectively; PMPA 2000 μg/L using APCI with FAIMS. Limit of detection, PMPA 700 and 840 μg/L using ESI with and without FAIMS, respectively; PMPA 2200 μg/L using APCI with FAIMS [Kolakowski *et al.* 2007]. Soil. Column: Zorbax C_{18} SB fused silica capillary (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 99 : 1 to 25 : 75 over 30 min), flow rate 200 μL/min. ESI. Limit of detection not reported [D'Agostino *et al.* 2001]. River Water. Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45, containing 0.1% glycerol), flow rate 100 μL/min. FAB ionisation. Limit of detection, PMPA 1 μg/L [Katagi *et al.* 1999]. Aqueous Samples. Column: Zorbax C_{18} SB (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 99 : 1 to 25 : 75 over 30 min). Limit of detection not reported [D'Agostino *et al.* 1999].

Note For a review article on the chromatographic analysis of chemical warfare agents, see Witkiewicz *et al.* [1990].

Disposition in the Body Soman is quickly absorbed at all sites of administration. It is highly lipophilic and thus has a high affinity for brain acetylcholinesterase (AChE). The rapid monodealkylation of soman-inhibited AChE prevents spontaneous reactivation as well as reactivation induced by oximes, a process known as ageing [Bajgar 1996]. Soman is hydrolysed to PMPA and very slowly hydrolysed to methylphosphonic acid (MPA) [Katagi *et al.* 1999]. The rate of hydrolysis is 5 times slower than that of tabun [Munro *et al.* 1999]. Soman is detoxified in the liver, plasma and lung, excluding these tissues from toxicity. Only 1 to 3% of a soman dose is responsible for the toxic effect, the loss being attributed to binding to non-specific esterases. There is some evidence to suggest that a depot of soman can build up in skin, muscles and lung, which can result in a second bout of intoxication [Bajgar 1996].

Toxicity Both of the $P_{(-)}$ isomers ($C_{(-)}P_{(-)}$ and $C_{(+)}P_{(-)}$) are much more toxic *in vivo* and more readily inhibit AChE *in vitro* than the $P_{(+)}$ isomers [Yeung *et al.* 2007].

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Somatropin

Growth Stimulant

$C_{990}H_{1529}N_{263}O_{299}S_7$ = 22124.1

CAS—12629-01-5

Synonyms Adenohypophyseal growth hormone; CB-311; hGH; human growth hormone; LY-137998; somatotropin.

Proprietary Names *Asellacrin*; *Crescormon*; *Genotropin*; *Grorm*; *Humatrope*; *Nanormon*; *Norditropin*; *Nutropin*; *Saizen*; *Umatrope*; *Zomacton*.

Chemical Properties A white or almost white powder.

High Performance Liquid Chromatography Column: G3000SW (TSK, 300 × 21.5 mm). Mobile phase: 20 mmol/L sodium borate with 1.44 mmol/L EDTA (pH 7.3), flow rate 0.5 mL/min. UV detection (λ = 280 nm). Retention time: dimer, 13.3 min; monomer, 14.5 min [Chang *et al.* 1996].

Column: Vydac 218TP104. Mobile phase: acetonitrile : 0.1% trifluoroacetic acid : water (10.5 : 70 : 19.5)-acetonitrile : 0.1% trifluoroacetic acid : water (21 : 70 : 9; 100 : 0 to 0 : 100 in 30 min), flow rate 1 mL/min. UV detection (λ = 214 nm). Retention time: monomer, 22.5 min; dimer, 25.0 min [Chang *et al.* 1996].

Disposition in the Body Somatropin is well absorbed after SC or IM injection. It is metabolised in the liver and the kidney and excreted in bile and a little in urine. Maximum plasma concentrations are reached within 2–6 h.

Therapeutic Concentration

Adult male volunteers administered with 100 μg/kg somatropin reached peak serum concentrations of approximately 55 μg/L after infusion/injection [Eli Lilly &amp Ltd.].

Toxicity Acromegaly reported. Results in premature death.

Bioavailability 60–80%.

Half-life After IV administration: 20–30 min; after IM or SC administration: 3–5 h due to prolonged release from the injection site.

Volume of Distribution At steady state, 50 mL/kg body weight.

Clearance After IV administration, 116–174 mL/h/kg.

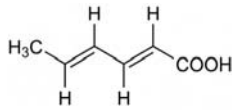
S

Protein Binding 20%.
Dose In paediatric patients: recommended dose is 25 to 35 µg/kg bodyweight daily by SC or IM injection. In adults: 6 µg/kg bodyweight daily as SC injection. This can be increased to 12 µg/kg daily as required. Patients with Turner's Syndrome and paediatric patients with chronic renal insufficiency: 45 to 50 µg/kg daily by SC administration.

Chang JP *et al.* (1996). Improved potency assay for recombinant bovine somatotropin by high-performance size-exclusion chromatography. *J Chromatogr A* 736: 97–104.
Eli Lilly & Company Ltd. Supplied data.

Sorbic Acid

Preservative
$C_6H_8O_2$ = 112.1
CAS—110-44-1; 22500-92-1
IUPAC Name (2*E*,4*E*)-Hexa-2,4-dienoic acid
Proprietary Name *Sorbistat*

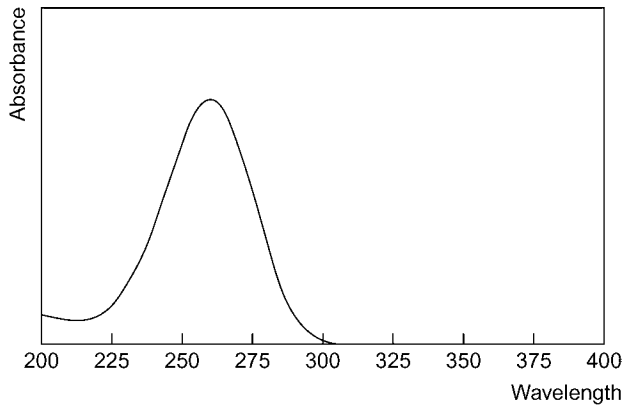


Chemical Properties A white or creamy-white crystalline powder. Mp about 134°. Soluble 1 in 1000 of water, 1 in 10 of ethanol, 1 in 15 of chloroform, 1 in 30 of ether, 1 in 8 of methanol and 1 in 19 of propylene glycol. pK_a 4.8. Log *P* (octanol/water), 1.3.
Caution Sorbic acid is irritant to the eyes and possibly also to the skin.

Potassium Sorbate

$C_6H_7KO_2$ = 150.2
CAS—590-00-1; 24634-61-5
Proprietary Name *Sorbistat-K*
Chemical Properties White or creamy-white crystals or powder. Mp about 270°, with decomposition. Soluble 1 in 4.5 of water and 1 in 35 of ethanol; very slightly soluble in acetone, chloroform and ether; soluble in propylene glycol.

Ultraviolet Spectrum Aqueous acid—264 nm (A^1_1=2343a); aqueous alkali—254 nm (A^1_1=2211b).

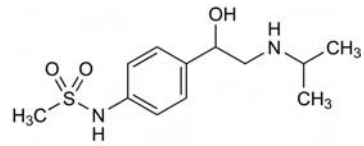


Infrared Spectrum Principal peaks at wavenumbers 1550, 1005, 1616, 1602, 1647, 882 cm^{-1} (potassium sorbate, KBr disk).
Quantification
Urine **GC-MS** Limit of detection, 0.7 µg/L [Renner *et al.* 1999].
Use In concentrations of 0.1 to 0.2%.

Renner T *et al.* (1999). Determination of sorbic acid in urine by gas chromatography-mass spectrometry. *J Chromatogr A* 847: 127–133.

Sotalol

Antihypertensive, Antiarrhythmic, Antianginal
$C_{12}H_{20}N_2O_3S$ = 272.4
CAS—3930-20-9
IUPAC Name *N*-[4-[1-hydroxy-2-(propan-2-ylamino)ethyl]phenyl]methanesulfonamide
Synonym *N*-[4-[1-Hydroxy-2-[(1-methylethyl)amino]ethyl]phenyl] methanesulfonamide.

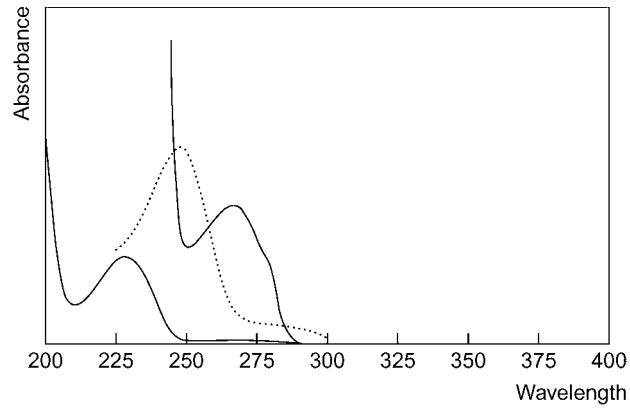


Chemical Properties Crystals. Mp about 207°. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005]. Blood, plasma and cardiac tissue samples were stable for at least 6 months at -80° [Läer *et al.* 1996].

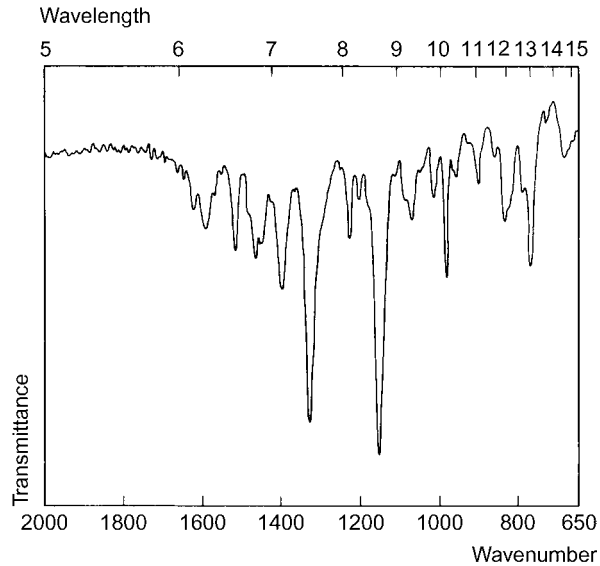
Sotalol Hydrochloride

$C_{12}H_{20}N_2O_3S,HCl$ = 308.8
CAS—959-24-0
Proprietary Names *Beta-Cardone*; *Cardol*; *CorSotalol*; *Darob*; *Dutacor*; *Favorex*; *Gilucor*; *Rentibloc*; *Rylosol*; *Rytmobeta*; *Sotab*; *Sotabet*; *Sotacor*; *Sotahexal*; *Sotalex*; *Sotalin*; *Sotamed*; *Sotamol*; *Sotaper*; *Sotapor*; *Sotaryt*; *Sotastad*; *Tachytalol.*
Chemical Properties Off-white to pale-cream crystalline powder. Mp 206° to 207°, with decomposition. Freely soluble in water; sparingly soluble in chloroform. pK_{a1} 8.2, pK_{a2} 9.8. Log *P* (octanol/water), 0.2.

Colour Test Liebermann's reagent (100°)—brown; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.53; system TAE—R_f 0.19; system TAG—R_f 0.05; system TB—R_f 0.01; system TC—R_f 0.03; system TE—R_f 0.30.
Gas Chromatography System GA—RI 2413; system GB—RI 2520.
High Performance Liquid Chromatography System HA—*k* 1.2; system HAA—RT 3.8 min; system HX—RI 226; system HZ—RT 2.0 min.
Ultraviolet Spectrum Aqueous acid—269 nm (A^1_1 = 16a); aqueous alkali—249 nm (A^1_1 = 552b; see below).

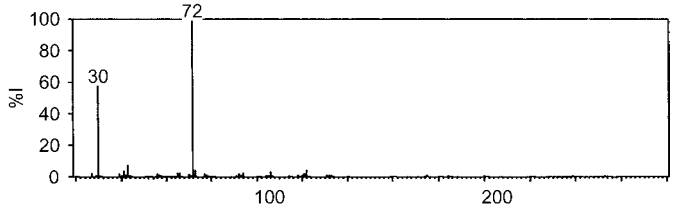


Infrared Spectrum Principal peaks at wavenumbers 1160, 992, 785, 1512, 1230, 1585 cm^{-1} (sotalol hydrochloride, KBr disk; see below).



Mass Spectrum Principal ions at *m/z* 72, 30, 43, 122, 73, 41, 106, 121.

S



Quantification

Blood **HPLC** Column: Spherisorb C_6 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium phosphate buffer (pH 3.0, 17 : 83), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 5.8 min. Limit of detection not reported [Läer *et al.* 1996].

GC-MS Column: 3% OV-17 Gas Chrom Q 100/120 mesh (200 cm × 4 mm i.d.). Carrier gas: N_2, 70 mL/min. Temperature: 275°. ECD. Limit of detection, ≈20 pg [Montagna, Groppi 1980].

LC-MS Column: Atlantis dC_{18} (150 × 2.1 mm i.d., 3.0 µm). Mobile phase: 10 mmol/L ammonium formate (pH 3.1) : acetonitrile (90 : 10 for 10 min to 10 : 90 for 3 min to 90 : 10 for 5 min), flow rate 0.3 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 0.069 µmol/L, limit of detection, 0.021 µmol/L [Kristoffersen *et al.* 2007]. Column: Hypersil Polar-RP (150 × 3.0 mm i.d.). Mobile phase: acetonitrile : 20 mmol/L ammonium formate buffer (pH 3, 10 : 90 for 2 min to 80 : 20 over 7 min for 8 min), flow rate 0.25 mL/min. TIS, positive ion mode, MRM acquisition mode. Retention time: 4.7 min. Limit of detection not reported [Josefsson, Sabanovic 2006].

Plasma **HPLC** Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: 10% acetonitrile with 0.001 mol/L heptane sulfonic acid and 0.02 mol/L sodium dihydrogen phosphate to 100% with water (pH 5.5), flow rate 1.8 mL/min. Fluorescence detection (λ_{ex} = 235 nm, λ_{em} = 300 nm). Retention time: 3.9 min. Limit of quantification, 10 µg/L [Zarghi *et al.* 2006]. Column: HyPurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 3.8) : acetonitrile (90 : 10 to 65 : 35 at 25 min for 1 min), flow rate 1.0 mL/min. DAD (λ = 220 nm). Retention time: 5.6 min. Limit of quantification, 25 µg/L, limit of detection, 8 µg/L [Delamoye *et al.* 2004]. Column: LiChrocart 60-RP-Select B (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L potassium dihydrogen phosphate (pH 7.0) containing 1 mmol/L 1-octanesulfonic acid sodium salt (20 : 80), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 5 ug/L [Rbeida *et al.* 2003]. Column: C_{18}. Fluorescence detection (λ_{ex} = 235 nm, λ_{em} = 310 nm). Retention time: 20 and 22 min for *R*- and *S*-sotalol, respectively. Limit of detection, 12.5 µg/L [da Cunha *et al.* 2001]. Column: Spherisorb C_6 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15 mmol/L potassium phosphate buffer (pH 3.0, 70 : 30), flow rate 1 mL/min. Fluorescence detection (λ_{ex} = 235 nm, λ_{em} = 300 nm). Limit of detection, 90 µg/L [Läer *et al.* 2001].

See also Santos *et al.* [2000], Läer *et al.* [1996], Hooper, Baker [1995], Shimizu *et al.* [1995], Fiset *et al.* [1993], Perrot *et al.* [1988], and Lefebvre *et al.* [1980].

LC-MS Column: Capcell Pak C_{18} (50 × 2.0 mm i.d., 5 µm). Mobile phase: 0.02% formic acid in acetonitrile : 0.02% formic acid in water (5 : 95 to 50 : 50 in 3.5 min to 95 : 5 over 0.5 min to 5 : 95 over 0.5 min for 3 min), flow rate 0.3 mL/min. TIS, positive ion mode. Retention time: 2.0 min. Limit of quantification, 20 µg/L [Li *et al.* 2007]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (80 : 20 for 2.2 min to 60 : 40 at 5.5 min to 10 : 90 at 8.0 min to 80 : 20 at 9.5 min for 0.5 min), flow rate 0.4 mL/min to 0.7 mL/min at 8 min to 0.65 mL/min at 9.5 min to 0.4 mL/min at 10.0 min. APCI, SIM acquisition mode. Limit of quantification, 0.25 mg/L, limit of detection, <0.1 mg/L [Maurer *et al.* 2004]. Column: TE-CSP (250 × 4.5 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : acetic acid : triethylamine (70 : 30 : 0.025 : 0.025), flow rate 1.5 mL/min. ESI, SIR acquisition mode. Limit of quantification, 4 µg/L, limit of detection, 1.0 µg/L [Badaloni *et al.* 2003].

Serum **HPLC** Column: Lichrosorb RP-18. Mobile phase: 0.05 mol/L hydrogen phosphate buffer : methanol (90 : 10) plus 2.5mL/L di-*n*-butylamine and 0.25g/L camphorosulfonic acid (pH 2.1). Fluorescence detection. Limit of detection not reported [Gustavsson *et al.* 1997].

Urine **HPLC** Column: LiChroCART Purospher C_{18}e (125 × 3 mm i.d., 5 µm). Mobile phase: 0.05% trifluoroacetic acid : methanol : acetonitrile (94 : 3 : 3 to 56 : 28 : 16 at 10 min to 25 : 25 : 50 at 20 min to 94 : 3 : 3 at 25 min), flow rate 0.7 mL/min for 10 min to 0.8 mL/min until 20 min to 0.85 mL/min until 25 min to 0.7 mL/min. UV detection (λ = 227 nm) or fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 340 nm). Limit of quantification, 3.23 (UV) and 0.045 mg/L, limit of detection, 1.07 (UV) and 0.015 mg/L [Baranowska, Wilczek 2009]. See Plasma [da Cunha *et al.* 2001]. See Plasma. Limit of detection, 220 ng/mL [Shimizu *et al.* 1995]. See Plasma [Fiset *et al.* 1993].

GC-MS See Blood [Montagna, Groppi 1980].

Bile **HPLC** See Plasma [Perrot *et al.* 1988].

Brain **GC-MS** See Blood [Montagna, Groppi 1980].

HPLC See Plasma [Perrot *et al.* 1988].

Heart **HPLC** See Blood. Limit of detection, 0.27 ng/mg [Läer *et al.* 1996]. See Plasma [Perrot *et al.* 1988].

GC-MS See Blood [Montagna, Groppi 1980].

HPLC See Plasma [Perrot *et al.* 1988].

Liver **GC-MS** See Blood [Montagna, Groppi 1980].

HPLC See Plasma [Perrot *et al.* 1988].

Lung **GC-MS** See Blood [Montagna, Groppi 1980].

HPLC See Plasma [Perrot *et al.* 1988].

Muscle **HPLC** See Plasma [Perrot *et al.* 1988].

Disposition in the Body

Almost completely absorbed after oral administration; bioavailability about 90%. It is excreted almost entirely in the urine as unchanged drug, 50–80% of a dose being excreted in 24 h. Less than 10% is eliminated in the faeces.

Therapeutic Concentration

A single oral dose of 160 mg given to 8 subjects produced peak plasma concentrations of 1.1–1.4 mg/L in about 3 h [Anttila *et al.* 1976].

After daily oral doses of 400 mg for 5 days to 5 subjects, a mean plasma concentration of 6 mg/L was reported 3 h after the last dose [McDewitt, Shanks 1977].

Mean peak plasma concentrations of 0.78 and 0.77 mg/L of the (+) and (-) enantiomers were reached after 4.01 h in 12 subjects after an oral dose of 160 mg [Hooper, Baker 1995].

Mean peak plasma concentrations were ≈0.4 mg/L for both enantiomers at ≈5 h in 6 volunteers given 80 mg [Shimizu *et al.* 1995].

A 6-year-old child was treated with a daily dose of 3.2 mg/kg per day typically used for the treatment of tachycardia. The maximal sotalol concentration was 1.13 mg/L reached after 1–2 h [Läer *et al.* 2001].

After the administration of an single oral dose of 80 mg (*R*,*S*)-sotalol to 5 young volunteers maximum plasma concentration were 0.47–0.75 mg/L reached at 2–6 h [Badaloni *et al.* 2003].

Toxicity

A male ingested about 3 g of sotalol and subsequently died; the following postmortem concentrations were reported: blood 40 mg/L, brain 0.59 µg/g, heart 42.7 µg/g, kidney 116 µg/g, liver 88 µg/g, lung 59.7 µg/g, urine 416 mg/L [Montagna, Groppi 1980].

Postmortem tissue concentrations in a woman who ingested 14.4 g of sotalol (and 50 mg triazolam) were: plasma 40 mg/L, brain 11.5 µg/g, heart 104.4 µg/g, lung 242.2 µg/g, liver 102.6 µg/g, kidney 103.2 µg/g, muscle 352.2 µg/g, bile 37.1 mg/L; the plasma sotalol concentration at admission had been 65 mg/L [Perrot *et al.* 1988].

A 37-year-old male who took 11.2 g of sotalol in a suicide attempt had serum concentrations of 20.6 mg/L after 3 h and 1.8 mg/L 59 h later [Gustavsson *et al.* 1997].

Half-life Plasma half-life, 10–17 h, increased in renal insufficiency and in elderly subjects.

Bioavailability ≈90%, 100% after oral dosing [Montagna, Groppi 1980].

Volume of Distribution ≈1–2 L/kg.

Clearance Plasma clearance, ≈1–2 mL/min/kg.

Protein Binding Not significantly bound [Gustavsson *et al.* 1997].

Dose Usually 120 to 600 mg of sotalol hydrochloride daily.

Anttila M *et al.* (1976). Human pharmacokinetics of sotalol. *Acta Pharmacol Toxicol (Copen)* 39: 118–128.

Badaloni E *et al.* (2003). Enantioselective liquid chromatographic-electrospray mass spectrometric assay of beta-adrenergic blockers: application to a pharmacokinetic study of sotalol in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 796: 45–54.

Baranowska I, Wilczek A (2009). Simultaneous RP-HPLC determination of sotalol, metoprolol, alpha-hydroxymetoprolol, paracetamol and its glucuronide and sulfate metabolites in human urine. *Anal Sci* 25: 769–772.

daCunha LC *et al.* (2001). An improved HPLC-fluorescence stereoselective method for analysis of (+)-*S*- and (-)-*R*-sotalol enantiomers in plasma sample. *Boll Chim Farm* 140: 448–454.

Delamoye M *et al.* (2004). Simultaneous determination of thirteen beta-blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection. *Forensic Sci Int* 141: 23–31.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fiset C *et al.* (1993). Stereoselective high-performance liquid chromatographic assay for the determination of sotalol enantiomers in biological fluids. *J Chromatogr* 612: 231–237.

Gustavsson CG *et al.* (1997). Pharmacokinetic evaluation of a case of massive sotalol intoxication. *Ann Pharmacother* 31: 856–859.

Hooper WD, Baker PV (1995). Enantioselective analysis of sotalol in plasma by reversed-phase high-performance liquid chromatography using diastereomeric derivatives. *J Chromatogr B Biomed Appl* 672: 89–96.

Josefsson M, Sabanovic A (2006). Sample preparation on polymeric solid phase extraction sorbents for liquid chromatographic-tandem mass spectrometric analysis of human whole blood: a study on a number of beta-agonists and beta-antagonists. *J Chromatogr A* 1120: 1–12.

Kristoffersen L *et al.* (2007). Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhythmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry: method development and robustness testing by experimental design. *J Chromatogr B Analyt Technol Biomed Life Sci* 850: 147–160.

Läer S *et al.* (1996). Determination of sotalol in human cardiac tissue by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 681: 291–298.

Läer S *et al.* (2001). Small blood volumes from children for quantitative sotalol determination using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 753: 421–425.

Lefebvre MA *et al.* (1980). Fluorometric high-performance liquid chromatographic determination of sotalol in biological fluids. *J Pharm Sci* 69: 1216–1217.

Li S *et al.* (2007). Simultaneous determination of ten antiarrhythic drugs and a metabolite in human plasma by liquid chromatography: tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 847: 174–181.

Maurer HH *et al.* (2004). Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization. *J Chromatogr A* 1058: 169–181.

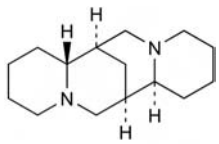
McDewitt DG, Shanks RG (1977). Evaluation of once daily sotalol administration in man. *Br J Clin Pharmacol* 4: 153–156.

Montagna M, Groppi A (1980). Fatal sotalol poisoning. *Arch Toxicol* 43: 221–226.
Perrot D *et al.* (1988). A case of sotalol poisoning with fatal outcome. *J Toxicol Clin Toxicol* 26: 389–396.
Rbeida O *et al.* (2003). Fully automated LC method for the determination of sotalol in human plasma using restricted access material with cation exchange properties for sample clean-up. *J Pharm Biomed Anal* 32: 829–838.
Santos SR *et al.* (2000). A simple HPLC-fluorescence method for the measurement of R,S-sotalol in the plasma of patients with life-threatening cardiac arrhythmias. *Braz J Med Biol Res* 33: 199–204.
Shimizu T *et al.* (1995). Enantioselective determination of sotalol enantiomers in biological fluids using high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 674: 77–83.
Zarghi A *et al.* (2006). Development an ion-pair liquid chromatographic method for determination of sotalol in plasma using a monolithic column. *J Pharm Biomed Anal* 41: 1433–1437.

Sparteine

Treatment of Cardiac Insufficiency
$C_{15}H_{26}N_2 = 234.4$
CAS—90-39-1

Synonyms [7*S*-(7α,7aα,14α,14aβ)]-Dodecahydro-7,14-methano-2*H*,6*H*-dipyrido-[1,2-a:1′,2′-e][1,5]diazocine; lupinidine; (−)-sparteine; *l*-sparteine.

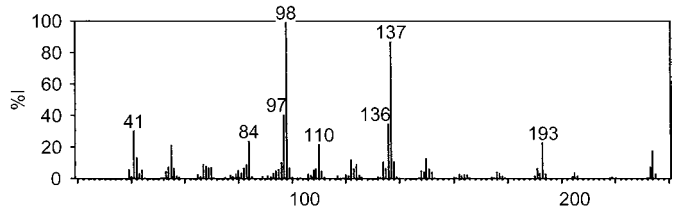


Chemical Properties A dibasic alkaloid obtained from scoparium, the dried tops of broom, *Sarothamnus scoparius* (=*Cytisus scoparius*) (Leguminosae). A viscous oily liquid. Soluble 1 in 325 of water; very soluble in ethanol, chloroform and ether. Log *P* (octanol/water), 2.7.

Sparteine Sulfate

$C_{15}H_{26}N_2,H_2SO_4,\ 5H_2O = 422.5$
CAS—299-39-8 (anhydrous); 6160-12-9 (pentahydrate)
Synonyms Sparteine sulphate; sparteinum sulfuricum; sulfato de esparteina.
Proprietary Names *Depasan*; *Tocosamine*.
Chemical Properties Colourless crystals or white crystalline granules or powder which decompose at 136°. Soluble 1 in about 1 of water and 1 in 3 of ethanol; practically insoluble in chloroform and ether.

Thin-layer Chromatography System TA—R_f 0.05; system TL—R_f 0.05; system TB—R_f 0.67; system TC—R_f 0.03; system TE—R_f 0.40; system TAE—R_f 0.04; system TAF—R_f 0.10; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.27 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1801.
Ultraviolet Spectrum No significant absorption, 230–360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1118, 1070, 1045, 980, 962, 1015 cm^{-1} (sparteine sulfate, KBr disk).
Mass Spectrum Principal ions at *m/z* 98, 137, 97, 136, 41, 193, 84, 110.



Quantification
Urine **GC** Sparteine metabolites [Inaba *et al.* 1986]. FID. Sparteine and metabolites. Limit of detection, 50 µg/L [Eichelbaum *et al.* 1979a]. FID. Sparteine and metabolites. Limit of detection, 30 µg/L [Eichelbaum *et al.* 1979b].

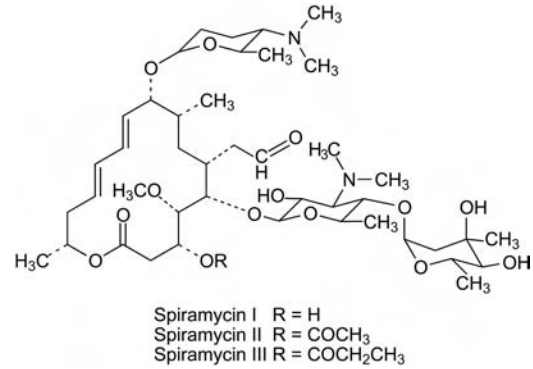
HPLC Electrochemical detection. Sparteine and dehydro metabolites [Moncrieff 1990].
Biological Fluids **GC** NPD [Kintz *et al.* 1989].
Disposition in the Body Sparteine is metabolised by *N*-oxidation followed by rearrangement to 2- and 5-dehydrosparteine. About 5% of the population are non-metabolisers (>90% of a dose excreted in the urine unchanged in 24 h). In metabolisers, about 55% of a dose is excreted in the urine in 24 h, about 30% of the dose as unchanged drug, 7% as 5-dehydrosparteine and 18% as 2-dehydrosparteine.
Half-life Plasma half-life, ≈3 h in metabolisers and 7 h in non-metabolisers.
Dose Sparteine sulfate has been given IM in a dose of 150 mg.

Eichelbaum M *et al.* (1979a). Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur J Clin Pharmacol* 16: 183–187.
Eichelbaum M *et al.* (1979b). Influence of the defective metabolism of sparteine on its pharmacokinetics. *Eur J Clin Pharmacol* 16: 189–194.
Inaba T *et al.* (1986). A simple borohydride/GC method for measuring sparteine metabolites in man. *Br J Clin Pharmacol* 21: 473–480.
Kintz P *et al.* (1989). Subnanogram GC/NPD method for the determination of sparteine in biological fluids. *Methods Find Exp Clin Pharmacol* 11: 115–118.
Moncrieff J (1990). Simultaneous determination of sparteine and its 2-dehydro and 5-dehydro metabolites in urine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 529: 194–200.

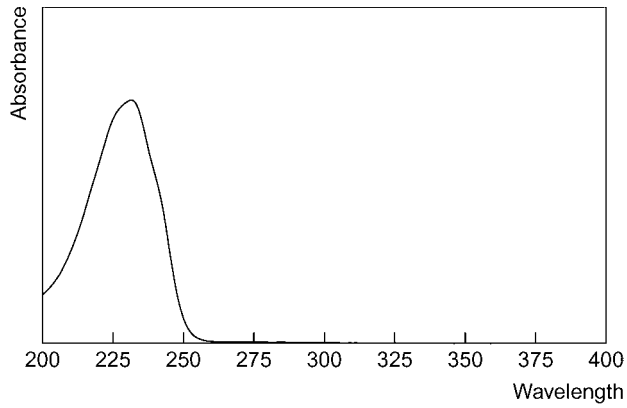
Spiramycin

Antibiotic
CAS—8025-81-8

IUPAC Name 2-[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[(2*S*,3*R*,4*R*,5*S*,6*R*)-5-[(2*S*,4*R*,5*S*,6*S*)-4,5-Dihydroxy-4,6-dimethyloxan-2-yl]oxy-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-10-[(2*R*,5*S*,6*S*)-5-(dimethylamino)-6-methyloxan-2-yl]oxy-4-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-1-oxacyclohexadeca-11,13-dien-7-yl]acetaldehyde
Synonyms 2-[(1*R*,3*R*,4*R*,5*E*,7*E*,10*R*,14*R*,15*S*,16*S*)-16-[5-(4,5-Dihydroxy-4,6-dimethyloxan-2-yl)oxy-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-4-[5-(dimethylamino)-6-methyloxan-2-yl]oxy-14-hydroxy-15-methoxy-3,10-dimethyl-12-oxo-11-oxacyclohexadeca-5,7-dien-1-yl]acetaldehyde; espiramicina; RP-5337; spiramycinum.
Proprietary Names *Dicorvin*; *Provamicina*; *Rovamycin(e)*; *Rovamicina*; *Selectomycin*.
A mixture of basic antimicrobial substances produced by the growth of *Streptomyces ambofaciens* and consisting of spiramycin I, $C_{43}H_{74}N_2O_{14} = 843.1$ (about 63%), spiramycin II, $C_{45}H_{76}N_2O_{15} = 885.1$ (about 24%) and spiramycin III, $C_{46}H_{78}N_2O_{15} = 899.1$ (about 13%).
Note The stated molecular formulae for the spiramycins may vary.



Chemical Properties A white or slightly yellowish amorphous powder. Soluble 1 in 50 of water; very soluble in ethanol, methanol, acetone and chloroform. pK_a 8.0.
Colour Tests Mandelin's test—orange-brown; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.42; system TAE—R_f 0.46 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Aqueous acid—233 nm (A^{1}_{1}=160b). No alkaline shift.



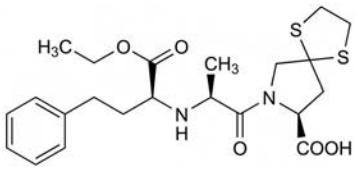
Infrared Spectrum Principal peaks at wavenumbers 1052, 1163, 1122, 994, 1016, 1730 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** UV detection. Limit of detection, 200 µg/L [Carlhant *et al.* 1989; Dow *et al.* 1985].
Vitreous **HPLC** See Plasma [Carlhant *et al.* 1989].
Dose 2 to 4 g daily.

Carlhant D *et al.* (1989). Solid phase extraction and HPLC determination of spiramycin in plasma and vitreous concentrations. *Biomed Chromatogr* 3: 1–4.
Dow J *et al.* (1985). Automated high-performance liquid chromatographic determination of spiramycin by direct injection of plasma, using column-switching for sample clean-up. *J Chromatogr* 344: 275–283.

Spirapril

Antihypertensive
$C_{22}H_{30}N_2O_5S_2$ = 466.6
CAS—83647-97-6

IUPAC Name (2*S*)-3-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-6,9-dithia-3-azaspiro[4.4]nonane-2-carboxylic acid
Synonym (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid; 8-[2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-1,4-dithia-8-azaspiro[4.4]nonane-7-carboxylic acid.



Chemical Properties White foam (hemihydrate). Log *P* (octanol/water), 3.89.

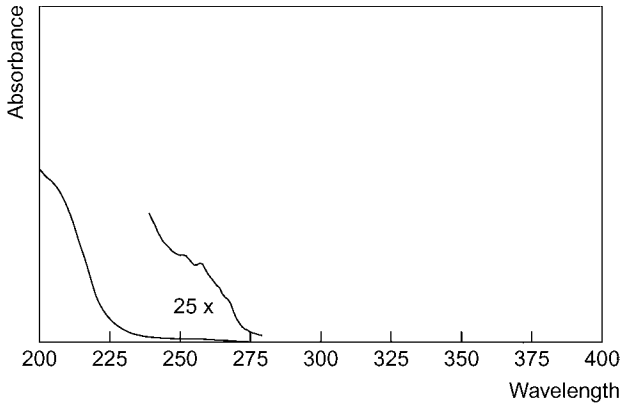
Spiraprilat

$C_{20}H_{26}N_2O_5S_2$ = 438.6
CAS—83602-05-5
Synonyms Sch-33861; spiraprilic acid.
Chemical Properties White foam (hemihydrate). Mp 163° to 165°.

Spirapril Hydrochloride

$C_{22}H_{30}N_2O_5S_2$.HCl = 503.1
CAS—94841-17-5
Synonyms Sch-33844; TI-211-950.
Proprietary Names *Cardiopril*; *Quadropril*; *Renormax*, *Renpress*, *Sandopril*; *Setrilan*.
Chemical Properties A white solid. Mp 192° to 194° with decomposition.

Ultraviolet Spectrum



Disposition in the Body After oral administration, spirapril is rapidly absorbed with peak plasma concentrations observed at 0.65–1.1 h. Elimination is mainly via conversion to the active metabolite spiraprilat (≈42%) and a small amount (3%) is eliminated unchanged in urine. The metabolite is observed at peak concentrations at 1.8–3 h. Sprilaprilat is excreted via both the renal (12.5%) and hepatobiliary routes; it is not detected in plasma or urine.

Therapeutic Concentration

Eighteen patients with liver cirrhosis (mean age of 46 years old) or chronic, non-cirrhotic disease (41 years old) and 16 healthy male and female volunteers (mean age 29 years) were administered with a 25 mg dose after an overnight fast. The peak plasma concentrations for spirapril were 0.430 mg/L for the healthy individuals, 0.415 mg/L for those with liver cirrhosis and 0.289 mg/L for patients with non-cirrhotic disease. These were observed at 0.9, 1.1 and 0.8 h, respectively. Peak concentrations of the metabolite, spiraprilat, were 0.345 mg/L for the healthy volunteers, 0.203 mg/L for patients with liver cirrhosis and 0.257 mg/L for those with non-cirrhotic liver disease. These concentrations were reached at 2.0, 2.2 and 1.9 h, respectively [Krähenbühl *et al.* 1993].

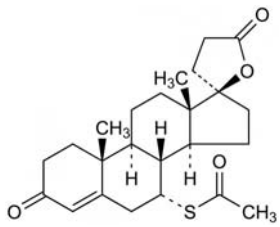
Bioavailability After oral administration in healthy volunteers: spirapril, the mean bioavailability is 50% (range, 28–69%).
Half-life Spirapril (oral administration in healthy volunteers) 0.9–1.6 h; spiraprilat (biphasic disposition) ≈1–2 h and 30–40 h.
Volume of Distribution 57.5 L (healthy individuals); 48.5 L (patients with liver cirrhosis); 83.3 L (non-cirrhotic liver disease).
Clearance Spirapril (total plasma clearance in healthy volunteers) 50–55 L/h; value of 117.8 L/h (renal 3.3 L/h; non-renal 114.5 L/h) has also been reported; spiraprilat 24 L/h.
Dose The recommended dose is 6 mg daily.

Krähenbühl S *et al.* (1993). Pharmacokinetics and haemodynamic effects of a single oral dose of the novel ACE inhibitor spirapril in patients with chronic liver disease. *Eur J Clin Pharmacol* 45: 247–253.

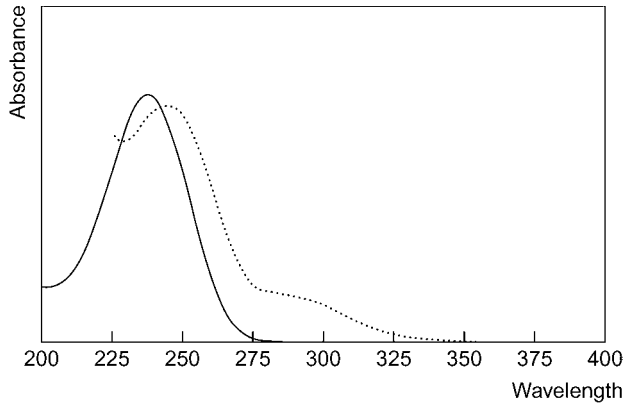
Spironolactone

Diuretic
$C_{24}H_{32}O_4S$ = 416.6
CAS—52-01-7

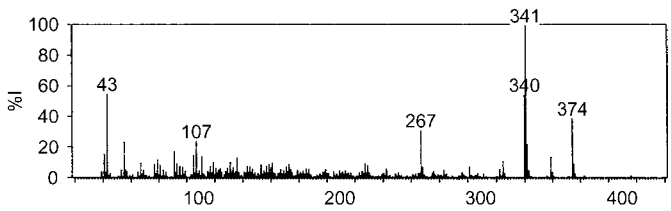
IUPAC Name *S*-[(7*R*,8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-10,13-Dimethyl-3,5′-dioxospiro[2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthrene-17,2′-oxolane]-7-yl] ethanethioate
Synonyms (7α,17α)-7-(Acetylthio)-17-hydroxy-3-oxopregn-4-ene-21-carboxylic acid γ-lactone; *S*-[7*R*,8*R*,9*S*,10*R*,13*S*,14*S*17,*R*)-10,13-dimethyl-3,5′-dioxospiro[2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthrene-17,2′-oxolane]-7-yl] ethanethioate; espironolactona; spirolactone.
Proprietary Names *Aldactone*; *Aldopur*; *Altone*; *Deverol*; *Hexalacton*; *Laractone*; *Osiren*; *Spiractin*; *Spiretic*; *Spirix*; *Spiro(x)*; *Spiroctan*; *Spirobene*; *Spirolone*; *Spironex*; *Spirospare*; *Spirotone*. It is an ingredient of *Aldactide*, *Aldactazide* and *Lasilactone*.
Note The name Aldactone is also used in some countries as a proprietary name for canrenoate potassium.



Chemical Properties A white to light tan powder. Mp 134° to 135°. Practically insoluble in water; soluble 1 in 80 of ethanol, 1 in 3 of chloroform and 1 in 100 of ether; slightly soluble in methanol and fixed oils. Log *P* (octanol/water), 2.8.
Colour Tests Palladium chloride—brown; sulfuric acid—orange→yellow-green (yellow-green fluorescence under ultraviolet light).
Thin-layer Chromatography System TD—R_f 0.66; system TE—R_f 0.78; system TF—R_f 0.51; system TAD—R_f 0.75; system TAE—R_f 0.84; system TAJ—R_f 0.73; system TAK—R_f 0.64; system TAL—R_f 0.96.
Gas Chromatography System GA—spironolactone RI 3280, M (canrenone) RI 3250, M (canrenoic acid) RI 3100, M (canrenoic acid-ME) RI 3130.
High Performance Liquid Chromatography System HX—RI 592; system HY—RI 539; system HZ—retention time 9.5 min; system HAA—retention time 20.7 min.
Ultraviolet Spectrum Aqueous acid—242 nm (A^1_1=464a); aqueous alkali—247 nm (A^1_1=405a).



Infrared Spectrum Principal peaks at wavenumbers 1773, 1676, 1170, 1127, 1115, 1185 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 341, 43, 340, 374, 267, 107, 55, 342.



S

Quantification

Plasma **HPLC** UV detection. Spironolactone and major metabolites [Jankowski *et al.* 1996; Varin *et al.* 1992].

Serum **HPLC** UV detection. Spironolactone and metabolites [Overdiek *et al.* 1985]. UV detection. Canrenone. Limit of detection, 5 µg/L [Besenfelder, Endele 1981]. UV detection. Canrenone. Limit of detection, 5 µg/L [Neurath, Ambrosius 1979].

Urine **GC** FID. Canrenone. Limit of detection, 20 ng [Fehér *et al.* 1976].

HPLC Spironolactone and canrenone. Limit of detection, 20 µg/L [Herraez-Hernandez *et al.* 1994]. See Plasma [Varin *et al.* 1992]. See Serum [Neurath, Ambrosius 1979].

Note Plasma-canrenone concentrations are apparently overestimated when determined by spectrofluorimetry in comparison to HPLC methods [Abshagen *et al.* 1979].

Disposition in the Body Spironolactone is rapidly but incompletely absorbed after oral administration; it is subject to extensive first-pass metabolism and enterohepatic circulation. The metabolism of spironolactone is very complex and there are a large number of metabolites. The initial step appears to be the formation of a thiol intermediate which is subsequently hydrolysed to canrenone, and which may also be methylated to thiomethylspironolactone. The transformation is rapid and spironolactone cannot be measured in the plasma. Canrenone, which is an active metabolite, is in enzymatic equilibrium with canrenoic acid, which may be conjugated with glucuronic acid; canrenone may also be hydroxylated to 15α-hydroxycanrenone. Other active sulfur-containing metabolites are thought to be formed from hydroxylation and sulfoxidation of thiomethylspironolactone. About 25 to 55% of a dose is excreted in the urine in 6 days and up to 40% may be eliminated in the faeces. About 11% of a dose is excreted in the urine as canrenone, canrenoic acid and its glucuronide conjugate, and about 6% as the 6β-hydroxysulfoxide metabolite; the 6β-hydroxysulfone derivative has also been detected in the urine.

Therapeutic Concentration

Following a single oral dose of 100 mg to 20 subjects, a mean peak serum-canrenone concentration of 0.13 mg/L was attained in 2.5 to 3 h; after daily oral doses of 100 mg to 20 subjects, the mean maximum steady-state serum concentration was reported to be 0.20 mg/L [Abshagen *et al.* 1979].

Half-life Plasma half-life, canrenone 13 to 24 (mean, 18) h.

Distribution in Blood Plasma : whole blood ratio, canrenone 1.85.

Protein Binding Canrenone, about 98%.

Note For a review of spironolactone, see Karim [1978].

Dose 100 to 400 mg daily.

Abshagen U *et al.* (1979). *Eur J Clin Pharmacol* 16: 255–262.

Besenfelder E, Endele R (1981). *J High Resolut Chromatogr., Chromatogr Commun* 4: 419–421.

Fehér T *et al.* (1976). Simple gas chromatographic method with flame ionization detection for the determination of aldadiene in human urine. *J Chromatogr* 123: 460–462.

Herraez-Hernandez R *et al.* (1994). High-performance liquid chromatographic determination of spironolactone and its major metabolite canrenone in urine using ultraviolet detection and column-switching. *J Chromatogr B Biomed Appl* 658: 303–310.

Jankowski A *et al.* (1996). Simultaneous determination of spironolactone and its metabolites in human plasma. *J Pharm Biomed Anal* 14: 1359–1365.

Karim A (1978). Spironolactone: disposition, metabolism, pharmacodynamics, and bioavailability. *Drug Metab Rev* 8: 151–188.

Neurath GB, Ambrosius D (1979). High-performance liquid chromatographic determination of canrenone, a major metabolite of spironolactone, in body fluids. *J Chromatogr* 163: 230–235.

Overdiek JW *et al.* (1985). Determination of the serum concentration of spironolactone and its metabolites by high-performance liquid chromatography. *J Chromatogr* 341: 279–285.

Varin F *et al.* (1992). High-performance liquid chromatographic determination of spironolactone and its metabolites in human biological fluids after solid-phase extraction. *J Chromatogr* 574: 57–64.

S

Stanozolol

Anabolic Steroid

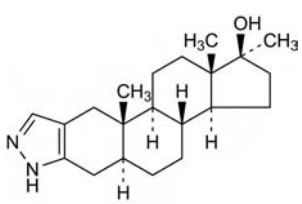
$C_{21}H_{32}N_2O = 328.5$

CAS—10418-03-8

IUPAC Name 7β-Hydroxy-17-methyl-5α-androstano[3,2-*c*]pyrazole

Synonyms Androstanazole; (5α,17β)-17-methyl-2′*H*-androst-2-enol[3,2-*c*]pyrazol-17-ol; methylstanazole.

Proprietary Names *Stromba*; *Strombaject*; *Winstrol*.



Chemical Properties A white crystalline powder. It exists as needles, mp 155°, or prisms, Mp ≈235°. Practically insoluble in water; soluble 1 in ~40 of ethanol, 1 in ~75 of chloroform and 1 in 370 of ether; slightly soluble in acetone and ethyl acetate; soluble in dimethylformamide.

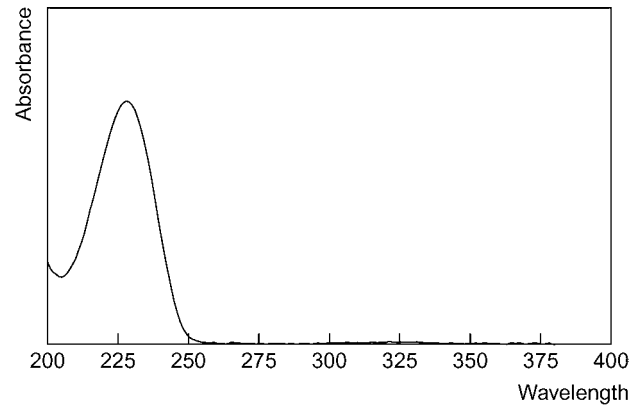
Colour Tests Mandelin's test—brown; Marquis test—orange-yellow.

Thin-layer Chromatography System TA—R_f 0.78 (acidified iodoplatinate solution, positive).

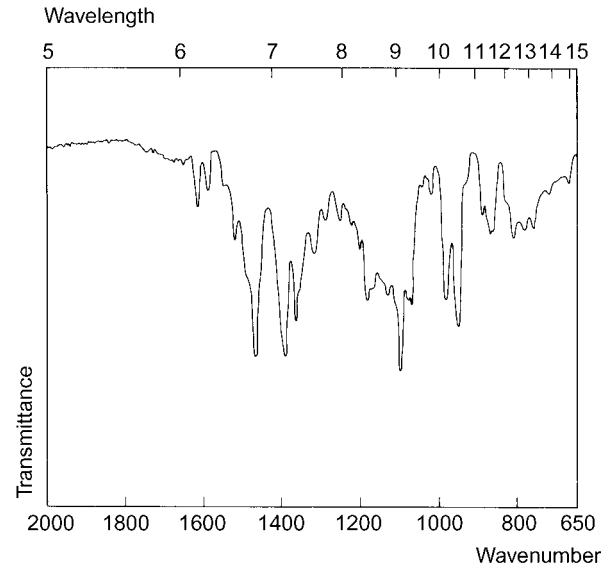
Gas Chromatography System GA—stanozolol RI 3085, M (-TMS_2) RI 3025, M (-AC) RI 2120; system GAI—stanozolol RRT 310, M (3′-OH) RRT 1.380, M (4β-OH) RRT 1.393 (all relative to 17α-methyl-5α-androstan-3β17β-diol); system GAR—stanozolol RT 15.4 min.

High Performance Liquid Chromatography System HX—RI 693.

Ultraviolet Spectrum Methanolic acid—230 nm ($A_1^1 = 200$b)



Infrared Spectrum Principal peaks at wavenumbers 1096, 950, 1068, 982, 1175, 1125 cm^{-1}.



Quantification

Urine **GC-MS** Column: HP-1 100% methylsiloxane (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 140° for 1 min to 180° at 40°/min to 280° at 3°/min to 320° at 40°/min. EI ionisation at 70 eV. Limit of detection, ~2 µg/L [Silva *et al.* 2009]. Column: Omnispher C_{18} (50 × 3 mm i.d., 3 µm). Mobile phase: 0.1% formic acid : methanol (40 : 60), flow rate 0.3 mL/min. ESI. Limit of detection, 2 µg/L for 3′-hydroxystanozolol [Deventer *et al.* 2006]. Column: DB-XLB (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 300° at 25°/min for 4 min. EI ionisation at 70 eV. Retention time: 7.2 min for 3′-hydroxystanozolol. Limit of detection, 0.5 µg/L for 3′-hydroxystanzolol [Mateus-Avois *et al.* 2005]. Column: HP Ultra-1 cross-linked methyl silicone (25 m × 0.2 mm i.d., 0.11 µm). Temperature programme: 120° for 1.6 s to 200° at 50°/min to 245° at 2°/min to 300° at 25°/min for 5 min. CID. Retention time, 31.7 min for 3′-hydroxxystanzolol. Limit of detection, 3 µg/L [Muñoz-Guerra *et al.* 1997]. Column: HP-1 Ultra cross-linked methyl silicone (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 180° to 229° at 3°/min to 310° at 40°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Schänzer *et al.* 1996]. See also Choo *et al.* [1990], Lantto *et al.* [1981], Massé *et al.* [1989a], Massé *et al.* [1989b], Schänzer *et al.* [1990] and Schänzer *et al.* [1992].

HPLC Column: Spherisorb phenyl silica (100 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : water (50 : 50) with 2.5 mmol/L ammonium acetate, flow rate 1.0 mL/min. APCI, SIM acquisition mode. Limit of detection, 5 µg/L [Mück, Henion 1990].

LC-MS Column: Omnispher C_{18} (100 × 2 mm i.d., 3 µm). Mobile phase: 1 mmol/L ammonium acetate-0.01% acetic acid : 1 mmol/L ammonium

acetate-0.01% acetic acid in methanol (70 : 30 for 1.5 min to 45 : 55 at 8 min for 7 min to 5 : 95 at 29.5 min for 1 min to 70 : 30 at 31 min for 3 min), flow rate 250 µL/min. ESI, SRM acquisition mode. Limit of detection, 0.5 µg/L [Pozo *et al.* 2009]. Column: Zorbax C_{18} (50 × 2.1 mm i.d., 1.8 µm). Mobile phase: 0.1% acetic acid : 0.1% acetic acid in acetonitrile (85 : 15 to 40 : 60 in 7 min to 0 : 100 in 14 min or 85 : 15 to 40 : 60 in 5 min to 0 : 100 in 7 min), flow rate 0.25 mL/min or 0.3 mL/min for the fast method. MRM acquisition mode. Limit of detection, 2 µg/L for 3′-hydroxystanozolol [Mazzarino, Botré 2006]. Column: LiChroCART Purospher RP C_{18} (125 × 3 mm, 5 µm). Mobile phase: 5 mmol/L ammonium acetate-0.01% acetic acid : 90% methanol (50 : 50 to 0 : 100 at 15 min for 3 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 2.0 µg/L for 3′-hydroxystanozolol [Leinonen *et al.* 2004].

***Hair* GC-MS** NICI. Limit of quantification, 5 pg/mg, limit of detection, 2 ng/g [Cirimele *et al.* 2000]. Column: HP5-MS 5% phenylsiloxane, 95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 295° at 30°/min for 6 min. EI ionisation. Retention time: 11.71 min. Limit of detection, 10 pg/mg [Kintz *et al.* 1999]. Column: CP-Sil 5 CB (15 m × 0.25 mmh i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.3 min. Limit of quantification, 0.01 ng/mg, limit of detection, 0.05 ng/mg [Deng *et al.* 1999].

Disposition in the Body

Toxicity

Note For a case of toxic hepatitis following the ingestion of testosterone enanthate, stanozolol and methylandrostenediol, see Stimac *et al.* [2002].

Dose 5 to 10 mg daily.

Choo HY *et al.* (1990). Quantitative determination of stanozolol and its metabolite in urine by gas chromatography/mass spectrometry. *J Anal Toxicol* 14: 109–112.

Cirimele V *et al.* (2000). Testing of the anabolic stanozolol in human hair by gas chromatography–negative ion chemical ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl* 740: 265–271.

Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.

Deventer K *et al.* (2006). Screening for amphetamine and amphetamine-type drugs in doping analysis by liquid chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 20: 877–882.

Kintz P *et al.* (1999). Testing for anabolic steroids in hair from two bodybuilders. *Forensic Sci Int* 101: 209–216.

Lantto O *et al.* (1981). Detection and quantitation of stanozolol (Stromba) in urine by isotope dilution–mass fragmentography. *J Steroid Biochem* 14: 721–727.

Leinonen A *et al.* (2004). Screening of free 17-alkyl-substituted anabolic steroids in human urine by liquid chromatography–electrospray ionization tandem mass spectrometry. *Steroids* 69: 101–109.

Massé R *et al.* (1989). Studies on anabolic steroids. III. Detection and characterization of stanozolol urinary metabolites in humans by gas chromatography–mass spectrometry. *J Chromatogr* 497: 17–37.

Massé R *et al.* (1989). Studies on anabolic steroids. I. Integrated methodological approach to the gas chromatographic-mass spectrometric analysis of anabolic steroid metabolites in urine. *J Chromatogr* 489: 23–50.

Mateus-Avois L *et al.* (2005). Use of ion trap gas chromatography–multiple mass spectrometry for the detection and confirmation of 3′-hydroxystanozolol at trace levels in urine for doping control. *J Chromatogr B Analyt Technol Biomed Life Sci* 816: 193–201.

Mazzarino M, Botré F (2006). A fast liquid chromatographic/mass spectrometric screening method for the simultaneous detection of synthetic glucocorticoids, some stimulants, anti-oestrogen drugs and synthetic anabolic steroids. *Rapid Commun Mass Spectrom* 20: 3465–3476.

Mück WM, Henion JD (1990). High-performance liquid chromatography/tandem mass spectrometry: its use for the identification of stanozolol and its major metabolites in human and equine urine. *Biomed EnvironMass Spectrom* 19: 37–51.

Muñoz-Guerra J *et al.* (1997). Use of ion trap gas chromatography–tandem mass spectrometry for detection and confirmation of anabolic substances at trace levels in doping analysis. *J Chromatogr B Biomed Sci Appl* 704: 129–141.

Pozo OJ *et al.* (2009). Detection and structural investigation of metabolites of stanozolol in human urine by liquid chromatography tandem mass spectrometry. *Steroids* 74: 837–852.

Schänzer W *et al.* (1990). Metabolism of stanozolol: identification and synthesis of urinary metabolites. *J Steroid Biochem* 36: 153–174.

Schänzer W *et al.* (1992). 17-Epimerization of 17alpha-methyl anabolic steroids in humans: metabolism and synthesis of 17alpha-hydroxy-17 beta-methyl steroids. *Steroids* 57: 537–550.

Schänzer W *et al.* (1996). Long-term detection and identification of metandienone and stanozolol abuse in athletes by gas chromatography-high-resolution mass spectrometry. *J Chromatogr B Biomed Appl* 687: 93–108.

Silva A *et al.* (2009). Analytical challenges in doping control: Comprehensive two-dimensional gas chromatography with time of flight mass spectrometry, a promising option. *J Chromatogr A* 1216: 2913–2922.

Stimac D *et al.* (2002). Androgenic/anabolic steroid-induced toxic hepatitis. *J Clin Gastroenterol* 35: 350–352.

Stavudine

Nucleoside Reverse Transcriptase Inhibitor, Antiviral

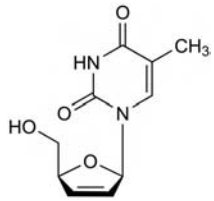
$C_{10}H_{12}N_2O_4$ = 224.2

CAS—3056-17-5

IUPAC Name 1-[(2*R*,5*S*)-5-(Hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4-dione

Synonyms BMY-27857; 2′,3′-didehydro-3′-deoxy-thymidine; D4T; 1-[(2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4-dione.

Proprietary Names *Zerit*; *Zeritavir*.

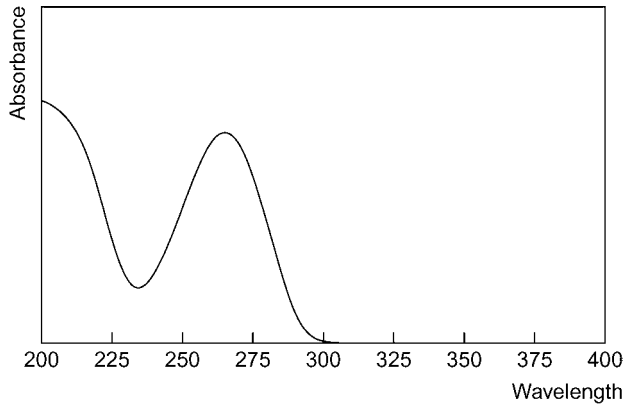


Chemical Properties Crystals from ethanol/ether. Mp 174°. Also reported as a colourless, granular solid from ethanol/benzene. Mp 165° to 166°. Soluble in water and in propylene glycol. Log *P* (octanol/water), −0.72 (also quoted as 0.14).

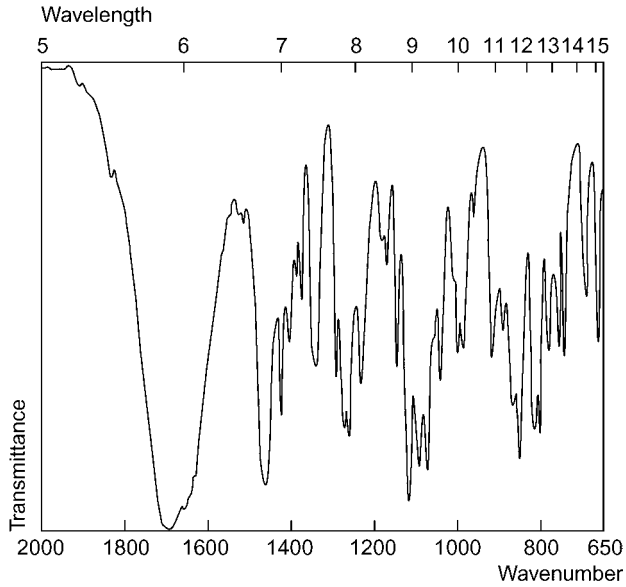
High Performance Liquid Chromatography System HAD—*k* 3.80.

Column: ODS Apex (250 × 4.6 mm i.d., 5 µm). Mobile phase: ammonium phosphate (10 mmol/L) : acetonitrile (9 : 1) (with 7.2 mmol/L TEA, pH 2.5 with 85% phosphoric acid), flow rate 0.8 mL/min. UV detection (λ = 266 nm). Retention time: 7 to 7.5 min [Janiszewski *et al.* 1992].

Ultraviolet Spectrum Aqueous acid—266 nm.



Infrared Spectrum



Quantification

***Plasma* HPLC** UV detection (λ = 260 nm). Limit of quantification, 0.01 mg/L [Aymard *et al.* 2000]. UV detection (λ = 266 nm). Limit of quantification, 0.025 mg/L [Janiszewski *et al.* 1992].

***Urine* HPLC** Limit of quantification, 0.5 mg/L, see Plasma [Janiszewski *et al.* 1992].

***Serum* HPLC** UV detection (λ = 250 nm). Limit of detection, 0.04 mg/L [Simon *et al.* 2001].

Disposition in the Body Stavudine is rapidly absorbed after oral administration and is metabolised intracellularly to an active form, stavudine triphosphate. Absorption is delayed but not reduced in the presence of food. Around 40% of the dose is excreted via urine 6 to 24 h after administration with approximately half as the unchanged drug. Stavudine crosses the blood–brain barrier and the CSF : plasma ratio is 0.4 after about 4 h. It also distributes into total body water and crosses the placenta.

Therapeutic Concentration

Male and female patients with hepatic impairment (creatinine clearance, >50 mL/min), at least 18 years of age, and healthy individuals were administered with a 40 mg oral dose of stavudine after an 8 h fast. The mean peak concentrations were 0.856 and 1.215 mg/L, respectively, and were reached at about 0.6 h [Schaad *et al.* 1997].

Twenty-two patients with a HIV infection (8 people with AIDS and 14 with the AIDS-related complex), aged between 22 and 59 years (mean, 35 years old) were split into 4 groups for different treatment. Group 1: 2 mg/kg daily (0.67 mg/kg three times daily). Group 2: 4 mg/kg daily (1.33 mg/kg three times daily). Group 3: 8 mg/kg (2.67 mg/kg three times daily). Group 4: 12 mg/kg (4 mg/kg three times daily). The mean peak plasma concentrations observed for the groups were 1.19, 1.56, 3.49 and 4.15 mg/L, respectively, all measured within 2 h of administration [Dudley 1992].

Bioavailability 86% (adults); 61 to 78% (children).

Half-life Elimination, 1.0 to 1.5 h (single dose); intracellular stavudine triphosphate, 3.5 h (measured *in vitro*); 0.82 to 1.24 h (children).

Volume of Distribution 0.88 to 1.06 L/kg (also reported as 58±21 L); 16.8 to 19 L/m^2 (children).

Clearance Oral, 0.46 to 0.6 L/h/kg (also reported as 35.5 L/h); children, 212 to 256 mL/min/m^2 (also reported as 333 mL/min/m^2).

Protein Binding Negligible.

Dose Patients >60 kg: recommended dose is 40 mg every 12 h. Patients <60 kg: 30 mg every 12 h. Children >3 years and <30 kg: 1 mg/kg bodyweight every 12 h. Dose may be reduced in those with renal impairment.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

Dudley MN *et al.* (1992). Pharmacokinetics of stavudine in patients with AIDS or AIDS-related complex. *J Infect Dis* 166: 480–485.

Janiszewski JS *et al.* (1992). High-performance liquid chromatographic determination of 2′,3′-didehydro-3′-deoxythymidine, a new anti-human immunodeficiency virus agent, in human plasma and urine. *J Chromatogr* 577(1): 151–156.

Schaad HJ *et al.* (1997). Pharmacokinetics and safety of a single dose of stavudine (d4T) in patients with severe hepatic impairment. *Antimicrob Agents Chemother* 41: 2793–2796.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1–2): 447–453.

Stenbolone

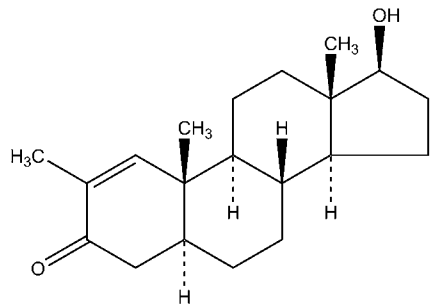
Anabolic Steroid

$C_{20}H_{30}O_2$ = 302.5

CAS—5197-58-0

IUPAC Name (5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-2,10,13-trimethyl-4,5,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one

Synonyms (5α,17β)-17-Hydroxy-2-methylandrost-1-en-3-one; 2-methyl-5α-androst-1-en-17β-ol-3-one; 2-methyl-17β-hydroxy-5α-androst-1-en-3-one; stenobolone.



Chemical Properties Crystals. Mp 155° to 158°. Log *P* (octanol/water), 4.03 [ACD 2007].

Stenbolone Acetate

$C_{22}H_{32}O_3$ = 344.5

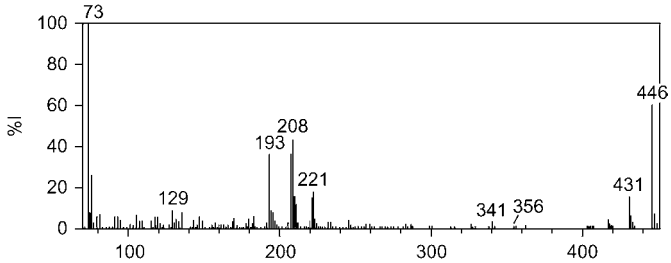
CAS—1242-56-4

Proprietary Name *Anatrofin*

Chemical Properties Crystals. Mp 146° to 149°.

Ultraviolet Spectrum 95% Ethanol—241 nm.

Mass Spectrum Principal ions at *m/z* 446, 208, 193, 221 (TMS-EE derivative) [Goudreault, Massé 1991].



Quantification

Urine **GC-MS** Column: HP-5 cross-linked 5% phenylmethylsilicone fused silica capillary (25 m × 0.2 mm i.d., 0.33 μm). SIM acquisition mode. Limit of quantification, 1 mg/L [Goudreault, Massé 1991].

Other **LC-MS** Equine Urine. Column: Reversed phase Supelcosil LC-8-DB (10 cm × 2.1 mm i.d., 3.0 μm). Mobile phase: 0.1% acetic acid : methanol (60 : 40 to 0 : 100 at 5 min for 5 min), flow rate 0.2 mL/min. API, ESI, positive ion mode, MRM acquisition mode. Limit of detection, 20 μg/L [Yu *et al.* 2005].

Disposition in the Body Stenbolone acetate is metabolised predominantly by 17-dehydrogenation of the 17β-hydroxy group by hepatic 17β-hydroxy steroid dehydrogenases. Hydroxylation at C-16 is also a major metabolic pathway, forming 2 hydroxylated isomers. These are further reduced at their 3-keto group by a minor reaction. Another minor metabolic route is the direct hydroxylation of stenbolone at C-18. Most urinary metabolites are glucuronide conjugates; only isomeric metabolites bearing a 16α- or a 16β-hydroxyl group are detected as sulfates. Less than 20% of an oral dose is excreted in urine.

A healthy non-smoking volunteer was administered a single 50 mg oral dose of stenbolone acetate. Urine samples were collected during the 6 days after administration and analysed by GC-MS The cumulative urinary excretion of unchanged stenbolone was >3 mg after 120 h. A peak level of 7.7 mg/L occurred 4.25 h after administration, only to decrease to 0.10 mg/L after 32 h. Interestingly, the urinary level of stenbolone then rose periodically within the time intervals 37 to 57 h and 57 to 82 h. This probably reflects the enterohepatic circulation of stenbolone, although the retention of the steroid or its acetate in specific tissues and their redistribution may also contribute to this phenomenon [Goudreault, Massé 1991].

ACD (2007). *ACD/LogP DB.* Toronto ON: Advanced Chemistry Development www.acdlabs.com. (accessed November 2007).

Goudreault D, Massé R (1991). Studies on anabolic steroids: 6. Identification of urinary metabolites of stenbolone acetate (17 beta-acetoxy-2-methyl-5 alpha-androst-1-en-3-one) in human by gas chromatography/mass spectrometry. *J Steroid Biochem Mol Biol* 38: 639–655.

Yu NH *et al.* (2005). Screening of anabolic steroids in horse urine by liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 37: 1031–1038.

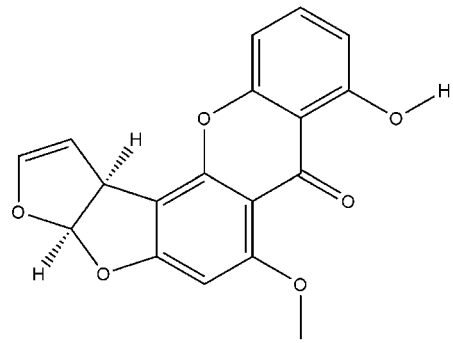
Sterigmatocystin

Carcinogen, Dermatoxin, Mycotoxin

$C_{18}H_{12}O_6$ = 324.28

CAS—10048-13-2

Synonyms Sterigmatocystine, STC.



Chemical Properties Sterigmatocystin (STC) is a mycotoxin produced by fungi of many different *Aspergillus* species. Other species such as *Bipolaris, Chaetomium, Emiricella* are also able to produce STC. STC-producing fungi were frequently isolated from different foodstuffs, while STC was regularly detected in grains, corn, bread, cheese, spices, coffee beans, soya beans, pistachio nuts, animal feed and silage. STC shows different toxicological, mutagenic and carcinogenic effects in animals and has been recognized as a 2B carcinogen (possible human carcinogen) by the International Agency for Research on Cancer. Log *P* (octanol/water), 3.81 [Meylan, Howard 1995].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as per the table overleaf.

Note For a quantitative analysis by TLC of sterigmatocystin in foodstuffs, see Tapia [1985].

Quantification

Other **LC-MS** Food (peanuts, pistachios, wheat, maize, cornflakes, raisins, figs). Column: Alltima C_{18} (150 × 3.2 mm i.d., 5 μm). Mobile phase: 0.1% formic acid in water : 0.1% formic acid in acetonitrile (90 : 10 to 30 : 70 at 12 min for 4 min to 10 : 90 at 17.5 min for 2.5 min to 90 : 10 at 21 min for 4 min), flow rate 0.3 mL/min. ESI. MRM acquisition mode, positive ion mode. Limit of detection, 10 μg/kg [Spanjer *et al.* 2008]. Mould. Column: Polaris C_{18} (150 × 2.0 mm i.d., 5 μm). Mobile phase: 10 mmol/L ammonium acetate : 20 μmol/L sodium acetate in methanol-aqueous buffer, flow rate 0.2 mL/min. ESI. Limit of detection not reported

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	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Deoxynivalenol	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyldeoxynivalenol	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Nivalenol	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	**90 : 10**	**0.37**	**0.62**	**90 : 10**	**0.59**	**0.85**	**70 : 30**	**N/A**	**N/A**
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989].

[Bloom *et al.* 2007]. Fungi. Column: LiChroCART 250-3 Purospher RP-18 (4 × 4 mm i.d.). Mobile phase: methanol : water (20 : 80 for 4 min to 70 : 30 in 4 min for 18 min to 90 : 10 in 1 min for 4 min), flow rate 400 µL/min. ESI, CID. Limit of quantification, 200 ng, limit of detection, 100 ng [Tuomi *et al.* 2001].

Abramson D *et al.* (1989). Chromatography of mycotoxins on precoated reverse-phase thin-layer plates. *Arch Environ Contam Toxicol* 18: 327–330.

Bloom E *et al.* (2007). Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. *Appl Environ Microbiol* 73: 4211–4217.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *J Pharm Sci* 84: 83–92.

Spanjer MC *et al.* (2008). LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Addit Contam* 25: 472–489.

Tapia MO (1985). A quantitative thin layer chromatography method for the analysis of aflatoxins, ochratoxin A, zearalenone, T-2 toxin and sterigmatocystin in foodstuffs. *Rev Argent Microbiol* 17: 183–186.

Tuomi T *et al.* (2001). Detection of aflatoxins (G(1-2), B(1-2)), sterigmatocystin, citrinine and ochratoxin A in samples contaminated by microbes. *Analyst* 126: 1545–1550.

Stilbamidine

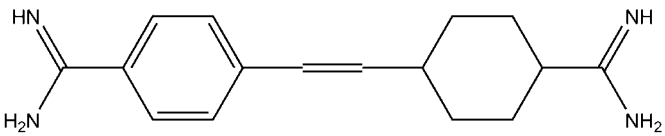
Antiprotozoal

$C_{16}H_{16}N_4$ = 264.3

CAS—122-06-5

IUPAC Name 4-[(*E*)-2-(Carbamimidoylphenyl)ethenyl]benzenecarboximidamide

Synonyms Diamidino stilbene; 4,4′-diamidinostilbene; 4,4′-(1,2-ethenediyl) bisbenzenecarboximidamide; stilbadinum; 4,4′-stilbenedicarboxamidine.



Chemical Properties Log *P* (octanol/water) 2.04 [Meylan, Howard 1995]; 3.9 [National Institutes of Health 2008].

Stilbamidine Dihydrochloride

$C_{16}H_{16}N_4$,2HCl = 337.3

Chemical Properties Needles from water.

Stilbamidine Isethionate

$C_{20}H_{28}N_4O_8S_2$ = 516.6

CAS—140-59-0

Synonym M & B 744

Chemical Properties Crystals discoloured by light. Solubility 1 g in 2.5 to 3 mL water, 1.5 g in 100 mL ethanol.

Colour Test Ammonium molybdate test—pale blue (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.04 (location reagent acidified iodoplatinate spray, positive reaction).

Disposition in the Body

Toxicity Stilbamidine sometimes causes hypoglycaemia and, if administered IV, may produce sudden hypotension. A serious late reaction is a neuropathy that may not develop until 2 to 5 months after a course of treatment.

Dose 3 to 5 mg/kg, on alternate days, by IV injection has been given.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

National Institutes of Health (2008). *Biphenamine.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=21720&loc=ec_rcs.(accessed 19 June 2008).

Stilbazium Iodide

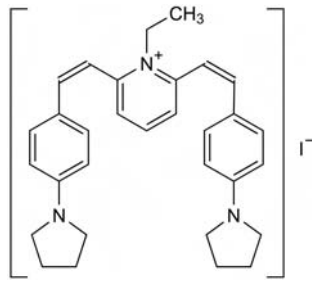
Anthelmintic

$C_{31}H_{36}IN_3$ = 577.6

CAS—3784-99-4

IUPAC Name 1-Ethyl-2,6-bis[(*E*)-2-(4-pyrrolidin-1-ylphenyl)ethenyl]pyridin-1-ium iodide

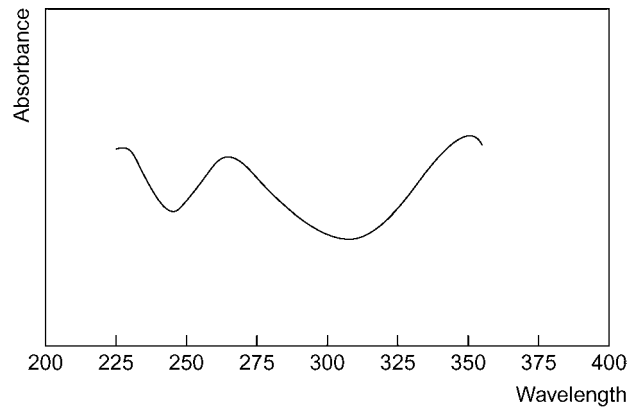
Synonym 1-Ethyl-2,6-bis[4-(pyrrolidin-1-yl)styryl]pyridinium iodide



Chemical Properties Mp about 282°. Soluble in ethanol and dilute acetic acid.

Thin-layer Chromatography System TA—R_f 0.10 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—264 (A^1_1=274b), 355 nm (A^1_1=310b).



Infrared Spectrum Principal peaks at wavenumbers 1175, 1538, 1582, 1510, 1307, 1274 cm^{-1} (KBr disk).

Dose Stilbazium iodide has been given in doses of 10 mg/kg once or twice daily for up to 3 days.

Streptomycin

Antibacterial

$C_{21}H_{39}N_7O_{12}$ = 581.6

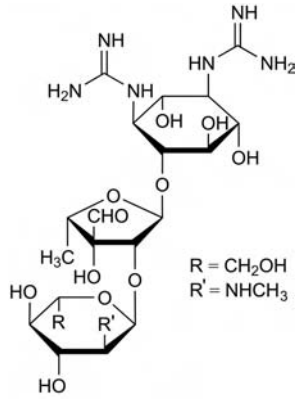
CAS—57-92-1

IUPAC Name 2-[(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-3-(Diaminomethylideneamino)-4-[(2*R*,3*R*,-4*R*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-(methylamino)

S

oxan-2-yl]oxy-4-formyl-4-hydroxy-5-methyloxolan-2-yl]oxy-2,5,6-tri-hydroxy-cyclohexyl]guanidine

Synonyms *O*-2-Deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-*N*,*N*′-bis(aminoiminomethyl)-D-streptamine; estreptomicina.



Chemical Properties An antimicrobial organic base produced by the growth of certain strains of *Streptomyces griseus*, or by any other means. Log *P* (octanol/water), −7.5.

Caution Streptomycin may cause severe dermatitis in sensitised persons.

Streptomycin Sulfate

$(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4 = 1457.4$
CAS—3810-74-0

Synonyms Streptomycin sesquisulfate; streptomycin sulfate.

Proprietary Names *Agristrep* (vet.); *Novostrep*; *Solustrep*; *Strepto*; *Streptobrettin*; *Streptocol*; *Sulfestrep*; *Vetstrep* (vet.).

Chemical Properties A white hygroscopic powder. Very soluble in water; practically insoluble in ethanol, chloroform and ether.

Colour Tests Benedict's reagent—orange-brown; boil a small quantity of streptomycin sulfate with 1 mol/L sodium hydroxide, add a slight excess of hydrochloric acid and a few drops of ferric chloride solution—violet.

Thin-layer Chromatography System TA—R_f 0.00; system TAE—R_f 0.00 (acidified potassium permanganate solution, positive).

Quantification

Serum **HPLC** Fluorescence detection. Limit of detection, 1 mg/L [Kubo *et al.* 1987]. UV detection [Kurosawa *et al.* 1985].

Disposition in the Body Streptomycin is poorly and irregularly absorbed from the gastrointestinal tract. It is rapidly absorbed after IM administration and widely distributed throughout the body. About 30 to 90% of a parenteral dose is excreted unchanged in the urine in 24 h.

Half-life Plasma half-life, about 2 to 4 h, increased in neonates, in elderly subjects and in renal impairment.

Distribution in Blood Plasma : whole blood ratio, about 1.9.

Protein Binding About 20 to 30%.

Dose The equivalent of 0.5 to 1 g of streptomycin daily, by IM injection.

Kubo H *et al.* (1987). Fluorometric determination of streptomycin in serum by high-performance liquid chromatography using mobile phase containing fluorogenic reagent. *Anal Biochem* 162: 219–223.

Kurosawa N *et al.* (1985). Determination of streptomycin in serum by high-performance liquid chromatography. *J Chromatogr* 343: 379–385.

S

Strophanthin-K

Cardiac Glycoside
$C_{37}H_{56}O_{13} = 708.8$
CAS—11005-63-3

IUPAC Name (14*R*)-5,14-Dihydroxy-3-[(2*R*,4*R*,6*R*)-4-methoxy-6-methyl-5-[(1*S*,2*S*,3*R*,4*S*,5*S*)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexyl]oxyoxan-2-yl]oxy-13-methyl-17-(5-oxo-2*H*-furan-3-yl)-2,3,4,6,7,8,9,11,12,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthrene-10-carbaldehyde

Synonyms Estrofantina; kombe strophanthin; K-strophanthin; strophanthoside-K.

Note Do not confuse with K-strophanthin-α which is cymarin.

Proprietary Names *Kombetin*; *Laevostrophan*.

Chemical Properties A mixture of glycosides from strophanthus, the dried ripe seeds of *Strophanthus kombe* (Apocynaceae), adjusted by admixture with a suitable diluent such as lactose so as to possess 40% of the activity of anhydrous ouabain. A white or yellowish-white powder containing microscopic crystals. Soluble in water and ethanol; practically insoluble in chloroform and ether.

Colour Tests Antimony pentachloride—orange→red; dissolve in a cold mixture of sulfuric acid and water (4 : 1)—green, immediately (distinction from ouabain).

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.00; system TAE—R_f 0.81.

High Performance Liquid Chromatography System HX—RI 356.

Infrared Spectrum Principal peaks at wavenumbers 1071, 1035, 1733, 1140, 1165, 1640 cm^{-1} (KBr disk).

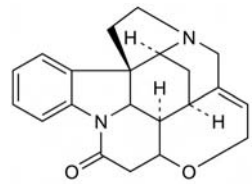
Dose Strophanthin-K has been given IV in doses of 125 to 500 μg daily.

Strychnine

Alkaloid, Glycine Receptor Antagonist
$C_{21}H_{22}N_2O_2 = 334.4$
CAS—57-24-9

IUPAC Name Strychnidin-10-one

Synonym Estricnina



Chemical Properties Strychnine is an alkaloid obtained from nux vomica and the seeds of other species of *Strychnos*. Translucent colourless crystals or white crystalline powder. Mp 275° to 285° (crystals from chloroform-ether). Very slightly soluble in water and ether; soluble 1 in 182 of ethanol, 1 in 250 of methanol, 1 in 150 of benzene, 1 in 83 of pyridine and 1 in 6.5 of chloroform. pK_a 2.3, 8.0 (25°). Log *P* (octanol/water), 1.9. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Strychnine Hydrochloride

$C_{21}H_{22}N_2O_2,HCl,2H_2O = 406.9$
CAS—1421-86-9 (anhydrous); 6101-04-8 (dihydrate)

Chemical Properties Colourless crystals or white crystalline powder. Soluble 1 in ~40 of water and 1 in ~80 of ethanol; practically insoluble in ether.

Strychnine Nitrate

$C_{21}H_{22}N_2O_2,HNO_3 = 397.4$
CAS—66-32-0

Proprietary Names It is an ingredient of *Dysurgal*, *Neuroftal*, *Tessopalmed Forte cum Yohimbine*.

Chemical Properties Colourless glistening crystals. Soluble 1 in 42 of water (1 in 10 of boiling water), 1 in 150 of ethanol, 1 in 50 of glycerol and 1 in 105 of chloroform; practically insoluble in ether.

Strychnine Sulfate

$(C_{21}H_{22}N_2O_2)_2,H_2SO_4,5H_2O = 857.0$
CAS—60-41-3 (anhydrous); 60491-10-3 (pentahydrate)

Synonym Strychnine sulphate

Proprietary Name It is an ingredient of *Hemo-Cyto-Serum*.

Chemical Properties Colourless crystals or white crystalline powder. It is efflorescent in dry air. Mp ~200° with decomposition when anhydrous. Soluble 1 in 35 of water (1 in 7 of boiling water), 1 in 81 of ethanol, 1 in 220 of chloroform and 1 in 6 of glycerol; practically insoluble in ether.

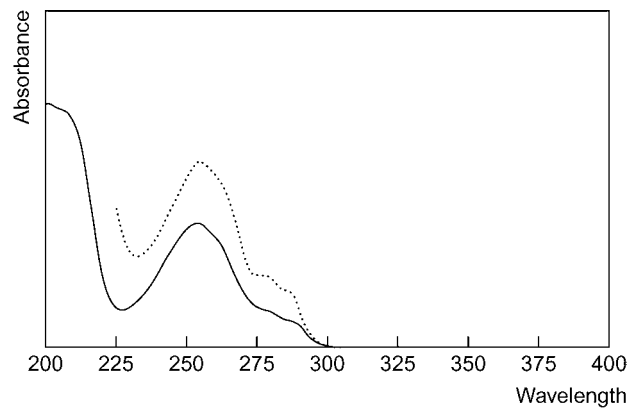
Colour Test Mandelin's test—violet.

Thin-layer Chromatography System TA—R_f 0.26; system TB—R_f 0.08; system TC—R_f 0.19; system TE—R_f 0.32; system TL—R_f 0.02; system TAE—R_f 0.08; system TAF—R_f 0.11; system TAJ—R_f 0.04; system TAK—R_f 0.02; system TAL—R_f 0.44 (acidified iodoplatinate solution—positive; Dragendorff spray—positive).

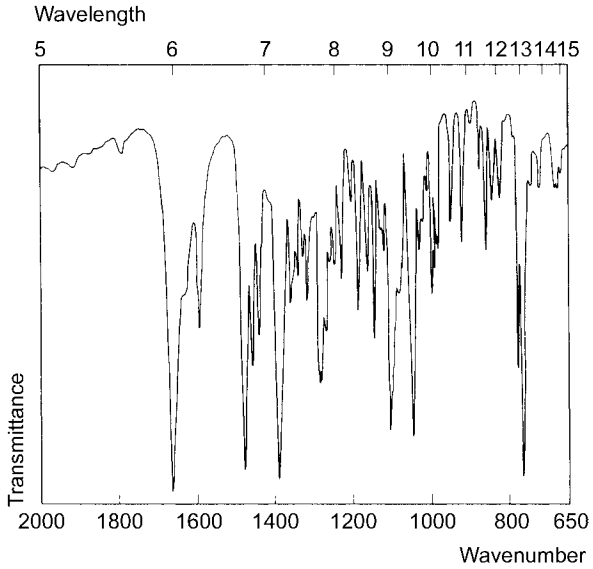
Gas Chromatography System GA—RI 3116.

High Performance Liquid Chromatography System HA—*k* 13.0 (tailing peak); system HS—*k* 2.43; system HX—RI 302; system HY—RI 257; system HZ—RT 2.2 min; system HAA—RT 9.2 min; system HAX—RT 7.5 min; system HAY—RT 3.9 min.

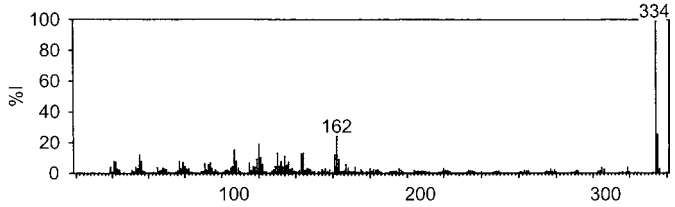
Ultraviolet Spectrum Aqueous acid—254 nm ($A^{1\%}_{1cm}$ = 373a); aqueous alkali—255, 278 nm.



Infrared Spectrum Principal peaks at wavenumbers 1664, 764, 1050, 1110, 1282, 775 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 334, 335, 162, 120, 107, 144, 143, 130.



Quantification

Blood **GC** Column: 3% OV-17 on Chromosorb WHP 100/200 mesh. Carrier gas: He, 30 mL/min. Temperature programme: 240° for 1 min to 280° at 16°/min for 4 min. FID. Relative retention time: 1.49 min [Winek *et al.* 1986].

GC-MS Column: 5% phenylmethylsiloxane (12 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 200° at 35°/min for 1 min to 270° at 40°/min for 7 min. EI ionisation at 70 eV. Limit of quantification, 8.91 µg/L, limit of detection, 6.83 µg/L [Barroso *et al.* 2005a]. Column: 5% phenylmethylsiloxane (12 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 200° at 35°/min for 1 min to 270° at 40°/min for 7 min. EI ionisation at 70 eV. Limit of quantification, 0.10 mg/L, limit of detection, 0.03 mg/L [Barroso *et al.* 2005b]. Column: HP1 methylsiloxane (12.5 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 170° for 1 min to 270° at 20°/min for 7 min. EI ionisation at 70 eV. Limit of quantification, 0.1 mg/L or 0.1 µg/g [Marques *et al.* 2000]. Column: DB-5MS (30 m × 0.25 mm i.d.). Carrier gas: He, 1.8 mL/min. Temperature programme: 200° to 320° at 15°/min. EI ionisation at 70 eV. Limit of quantification, 0.01 mg/L [Cingolani *et al.* 1999].

HPLC Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer (pH 3.8, 10 : 90 to 40 : 60 in 9 min), flow rate 1.0 mL/min. DAD (λ = 254 nm). Limit of quantification, 0.5 mg/L, limit of detection, 0.06 mg/L [Duverneuil *et al.* 2004]. Column: XTerra RP18 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium bicarbonate (pH 10) : acetonitrile (70 : 30), flow rate 1.0 mL/min. DAD (λ = 254 nm). Limit of quantification, 5 µg/L, limit of detection, 0.5 µg/L for strychnine and brucine [Wang *et al.* 2004].

LC-MS Column: XBridge Shield RP18 (250 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium bicarbonate (100 : 0 to 40 : 60 at 3.5 min to 20 : 80 at 7.5 min), flow rate 0.5 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.05 µg/L [Qiu *et al.* 2008].

Plasma **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999].

HPLC Column (250 × 2.6 mm i.d., 10 µm). Mobile phase ammonium hydroxide : methanol (0.75 : 99.25), flow rate 1.1 mL/min. UV detection (λ = 254 nm). Limit of detection, 625 µg/L [Alliot *et al.* 1982].

Serum **HPLC** Column: Nucleodur (250 × 4.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 6.5, 10 : 90 for 15 min to 75 : 25 in 23.5 min for 9 min), flow rate 1.0 mL/min. DAD (λ = 253 nm). Retention time: 14.7 min. Limit of quantification, 9.1 µg/L [Pietsch *et al.* 2008].

Urine **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999; Marques *et al.* 2000].

HPLC See Serum. Limit of quantification, 7.5 µg/L [Pietsch *et al.* 2008]. See Plasma [Alliot *et al.* 1982]. Column: LiChrosorb Si-60. Mobile phase: methanol : water : ammonia (85 : 14.2 : 0.8), flow rate 2.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 20 µg/L [Egloff *et al.* 1982].

LC-MS See Blood [Qiu *et al.* 2008]. Column: Chrompack cyanopropyl (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 1% acetic acid (10 : 90 to 36 : 64 in 6.5 min), flow rate 1.0 mL/min. APCI, positive ion mode. Limit of quantification, 1 µg/L, limit of detection, 0.5 µg/L [Van Eenoo *et al.* 2006].

CE Column: fused silica capillary (570/500 mm total/effective length, 50 µm i.d.). Buffer: 20 mol/L acetate (pH 3.8) with applied voltage of 20 kV. Limit of quantification, 2.0 µg/L for strychnine and 2.5 µg/L for brucine, limit of detection, 8 µg/L for strychnine and 10 µg/L for brucine [Li, Jiang 2010].Capillary: fused silica (500/400 mm total/effective length, 75 µm). UV detection (λ = 203 nm). Limit of detection, 1 µg/L for strychnine and 2 µg/L for brucine [Wang *et al.* 2007].

Bile **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999].

CSF **GC-MS** See Blood [Cingolani *et al.* 1999].

Stomach Contents **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999].

HPLC See Blood [Wang *et al.* 2004].

Brain **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999].

Kidney **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999; Marques *et al.* 2000].

Liver **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999; Marques *et al.* 2000].

Lung **GC-MS** See Blood [Cingolani *et al.* 1999; Marques *et al.* 2000].

Muscle **GC-MS** See Blood [Cingolani *et al.* 1999].

Heart **GC-MS** See Blood [Marques *et al.* 2000].

Intestines **GC** See Blood [Winek *et al.* 1986].

GC-MS See Blood [Cingolani *et al.* 1999].

Tissues **HPLC** See Urine [Egloff *et al.* 1982].

Disposition in the Body Strychnine is readily absorbed after oral or parenteral administration and rapidly oxidised. It is taken up to some extent by the red blood cells. Approximately 20% of a dose is excreted unchanged in the urine.

Toxicity The minimum lethal dose of strychnine is ~15–30 mg for children and 50–100 mg for adults, although with adequate treatment recovery may occur after the ingestion of 250 mg or more.

The following postmortem tissue concentrations were reported in acute strychnine poisoning: bile 2.40 mg/L, stomach contents 14.2 mg, liver 6.68 µg/g and kidney 2.68 µg/g. After preservation of the tissues in formaldehyde solution for 8 weeks, the concentrations were liver 1.59 µg/g, formaldehyde from liver 1.80 mg/L, kidney 0.98 µg/g and formaldehyde from kidney 1.11 mg/L [Cingolani *et al.* 1999].

In a fatal strychnine poisoning, strychnine concentrations were 1.82 mg/L in subclavian blood, 3.32 mg/L in inferior vena cava blood, 3.35 mg/L in urine, 11.4 mg/L in bile, 98.6 µg/g in liver, 12.3 µg/g in lung, 11.8 µg/g in spleen, 2.42 µg/g in brain and 2.32 µg/g in skeletal muscle [Rosano *et al.* 2000].

A 50-year-old woman was admitted to hospital with classic signs of strychnine poisoning, including marked pain in the muscles of her lower limbs, dermal sensitivity and stiffness of the jaw. A strychnine concentration of 0.196 mg/L was detected in her plasma and 6.85 mg/L in her urine. She did not die from this amount [Greene, Meatherall 2001].

A 42-year-old man survived after ingesting an unknown quantity of strychnine powder despite his serum level being 4.73 mg/L at 1.5 h. After respiratory arrest, the patient underwent intensive supportive management and his serum concentration fell to 0.38 mg/L at 74 h post-ingestion (and to zero at 100 h) [Wood *et al.* 2002].

A blood concentration of strychnine 25 mg/L was found in a fatal case of strychnine poisoning [Duverneuil *et al.* 2004].

The following postmortem levels of strychnine were found in a 52-year-old man who had taken strychnine-treated gopher pellets: heart blood 0.96 mg/L, femoral blood 0.31 mg/L, vitreous fluid 0.36 mg/L, bile 1.17 mg/L, urine 2.92 mg/L, liver 4.59 µg/g and brain 0.86 µg/g [Lindsey *et al.* 2004].

For a report of non-fatal strychnine poisoning in a 6-year-old child, see Starretz-Hacham *et al.* [2003]. Two cases of survival after ingestion of 250 mg and 1.5 g of strychnine have been reported [Scheffold *et al.* 2004; Shadnia *et al.* 2004].

Note For the pharmacokinetics of strychnine in overdose, see Sgaragli, Mannaioni [1973]; for a review of the toxicokinetics of acute strychnine poisoning, see Palatnick *et al.* [1997]; for a discussion of strychnine use and overdose, see Radosavljevic *et al.* [2006]; for reviews of strychnine poisoning, see Smith [1990] and Makarovsky *et al.* [2008]. For a review of symptoms of strychnine and other plant alkaloid intoxication and detection methods in biological fluids, see Beyer *et al.* [2009].

Dose Strychnine has been given, as a bitter and analeptic, in doses of 2 to 8 mg, usually as its salts.

Alliot L *et al.* (1982). Measurement of strychnine by high-performance liquid chromatography. *J Chromatogr* 232: 440–442.

Barroso M *et al.* (2005a). Application of solid phase microextraction to the determination of strychnine in blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 816: 29–34.

Barroso M *et al.* (2005b). Determination of strychnine in human blood using solid-phase extraction and GC-EI-MS. *J Anal Toxicol* 29: 383–386.

Beyer J *et al.* (2009). Analysis of toxic alkaloids in body samples. *Forensic Sci Int* 185: 1–9.

Cingolani M *et al.* (1999). Analytical detection and quantitation of strychnine in chemically fixed organ tissues. *J Anal Toxicol* 23: 219–221.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Duverneuil C *et al.* (2004). Liquid chromatography/photodiode array detection for determination of strychnine in blood: a fatal case report. *Forensic Sci Int* 141: 17–21.
Egloff T *et al.* (1982). A new high performance liquid chromatography (HPLC) method for the quantitation of strychnine in urine and tissue extracts. *J Clin Chem Clin Biochem* 20: 203–206.
Greene R, Meatherall R (2001). Dermal exposure to strychnine. *J Anal Toxicol* 25: 344–347.
Li J, Jiang Y (2010). Rapid and sensitive determination of strychnine and brucine in human urine by capillary electrophoresis with field-amplified sample stacking. *Biomed Chromatogr* 24: 186–194.
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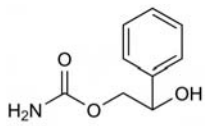
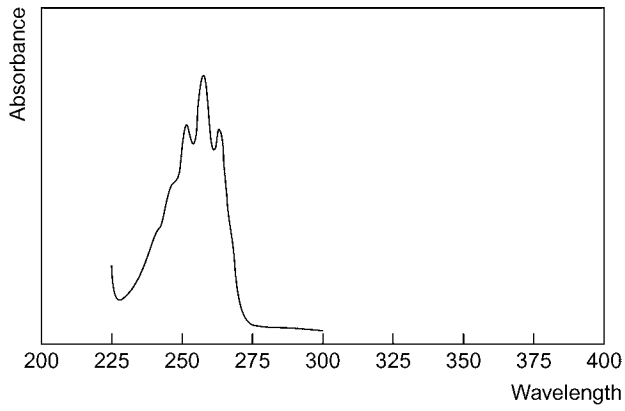
Styramate

Muscle Relaxant
$C_9H_{11}NO_3 = 181.2$
CAS—94-35-9

IUPAC Name (2-Hydroxy-2-phenylethyl) carbamate
Synonym β-Hydroxyphenethyl carbamate
Proprietary Name *Sinaxar*

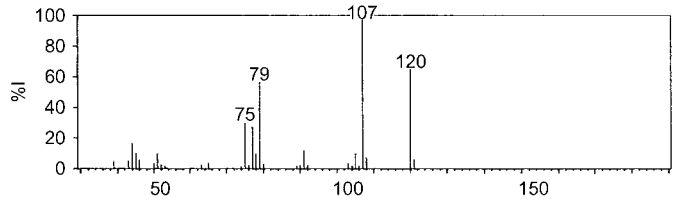


Chemical Properties A crystalline powder. Mp 110° to 111°. Sparingly soluble in water; soluble in ethanol, chloroform and ether. Log *P* (octanol/water), 0.2.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.01; system TC—R_f 0.20; system TD—R_f 0.13; system TE—R_f 0.53; system TF—R_f 0.39; system TL—R_f 0.53; system TAD—R_f 0.30 (furfuraldehyde reagent, positive; Van Urk reagent, positive).
Gas Chromatography System GA—RI 1667.
Ultraviolet Spectrum Aqueous acid—251, 257 (A^1_1=11b), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1059, 1630, 750, 699, 1115 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 107, 120, 79, 75, 77, 44, 91, 45.

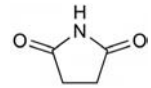


Dose Usually 0.8 to 1.6 g daily.

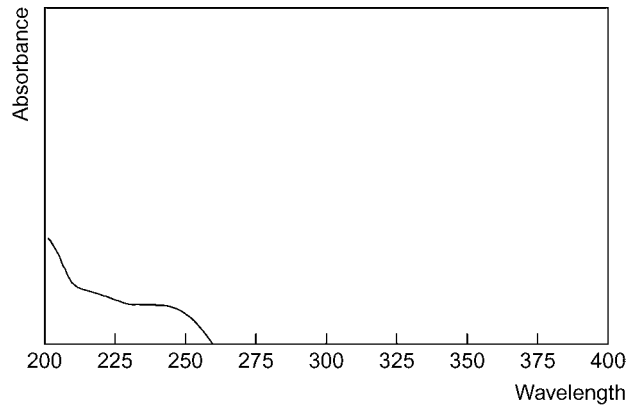
Succinimide

Antiurolithic
$C_4H_5NO_2 = 99.1$
CAS—123-56-8

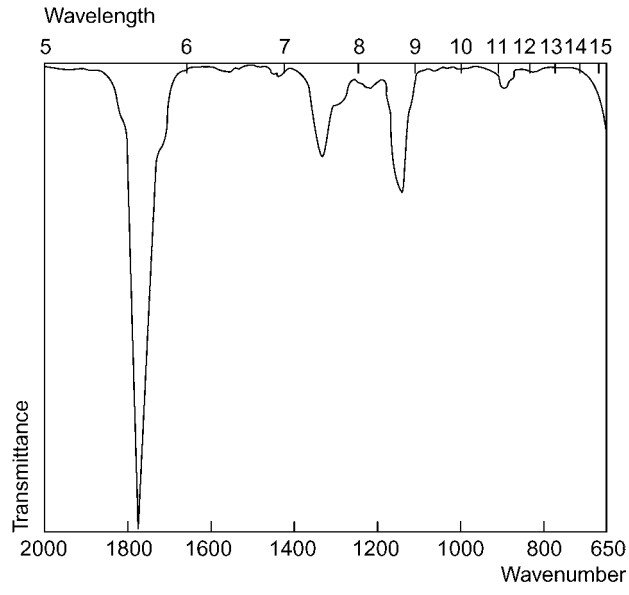
IUPAC Name Pyrrolidine-2,5-dione
Synonyms Butanimide; 3,4-dihydropyrrolidine; 3,4-dihydropyrrole-2,5-dione; dihydro-3-pyrroline-2,5-dione; 2,5-diketopyrrolidine; 2,5-dioxopyrrolidine; lubrizol 6406; 2,5-pyrrolidinedione; succinic acid imide; succinic imide; succinimide-sauba.
Proprietary Name *Orotric*



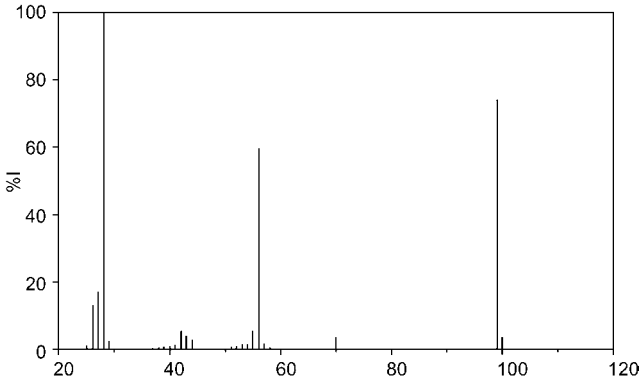
Chemical Properties A white to off-white crystalline nitrogenous powder. Mp 122° to 124°. Bp 287° to 289°. Very soluble in water, slightly soluble in alcohol and insoluble in ether and chloroform. pK_a 9.5. Log *P* (octanol/water), −0.85.
High Performance Liquid Chromatography System HZ—retention time 2.4 min (dimethyl-methyl-succinimide).
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumber 1777, 1145, 648, 896 cm^{-1}.



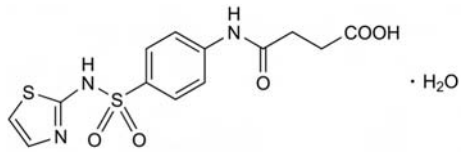
Mass Spectrum Principal ions at *m/z* 28, 99, 56, 27, 26, 55, 42, 100.



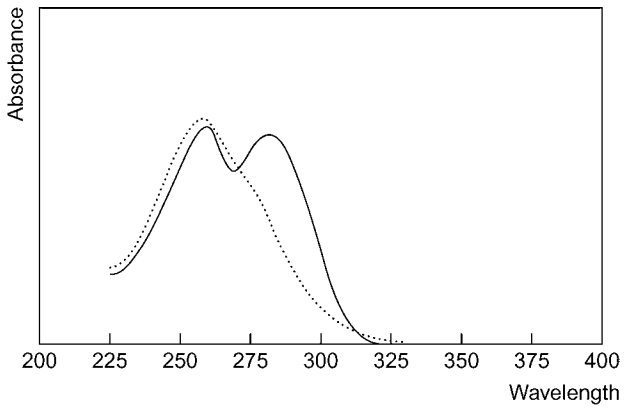
Dose The usual oral dose is 3 g three times a day.

Succinylsulfathiazole

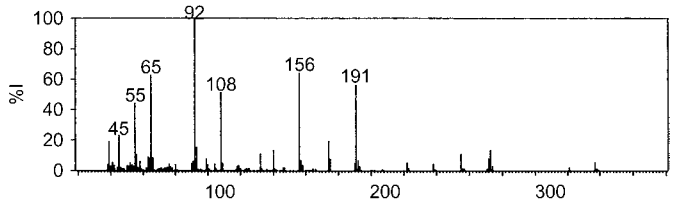
Antibacterial, Sulfonamide
$C_{13}H_{13}N_3O_5S_2,H_2O = 373.4$
CAS—116-43-8 (anhydrous)
IUPAC Name 4-Oxo-4-[[4-[(2-thiazolylamino)sulfonyl]phenyl]amino]butanoic acid monohydrate
Synonyms Succinilsolfatiazolo; succinylsulfathiazole; sulfasuccithiazole.
Proprietary Name *Sulfasuxidine*



Chemical Properties White or yellowish-white crystals or powder which darken on exposure to light. Mp 184° to 186°; also reported as 192° to 195°. Soluble 1 in about 4800 of water, 1 in 200 of ethanol and 1 in 150 of acetone; practically insoluble in chloroform and ether; soluble in aqueous solutions of alkali hydroxides and carbonates. pK_a 4.5. Log *P* (octanol/water), 1.2.
Colour Tests Copper sulfate (method 1)—green (→violet); Koppanyi–Zwikker test—red-violet; mercurous nitrate—black.
Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TT—R_f 0.02; system TU—R_f 0.01; system TV—R_f 0.01; system TAD—R_f 0.02.
Gas Chromatography See Sulfathiazole.
High Performance Liquid Chromatography System HU—*k* 16.8.
Ultraviolet Spectrum Aqueous acid—258 (A^1_1=570a), 281 nm; aqueous alkali—257 nm (A^1_1=651a).



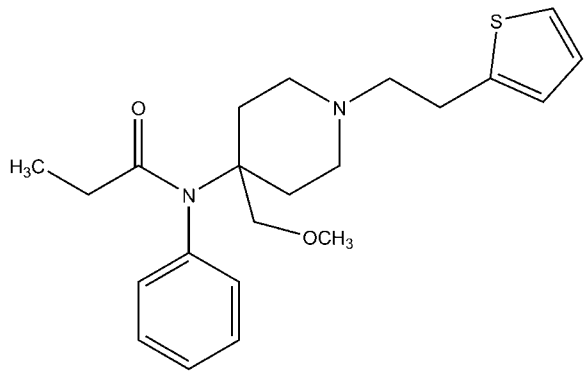
Infrared Spectrum Principal peaks at wavenumbers 1143, 1585, 1091, 840, 1529, 1667 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 92, 156, 65, 191, 108, 55, 45, 174.



Disposition in the Body Only about 5% of a dose is absorbed after oral administration and blood concentrations are therefore very low compared to other sulfonamides, usually <40 mg/L. It is slowly hydrolysed to sulfathiazole in the gastrointestinal tract. Considerable amounts of sulfathiazole are eliminated in the faeces.
Dose Usually 10 to 20 g daily.

Sufentanil

Narcotic Analgesic
$C_{22}H_{30}N_2O_2S = 386.6$
CAS—56030-54-7
IUPAC Name *N*-[4-(Methoxymethyl)-1-(2-thiophen-2-ylethyl)piperidin-4-yl]-*N*-phenylpropanamide
Synonyms *N*-[4-(Methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide; *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidyl] propionanilide; *N*-[4-(methoxymethyl)-1-(2-thiophen-2-ylethyl)-4-piperidyl]-*N*-phenyl-propanamide; R-30730; sufentanyl.

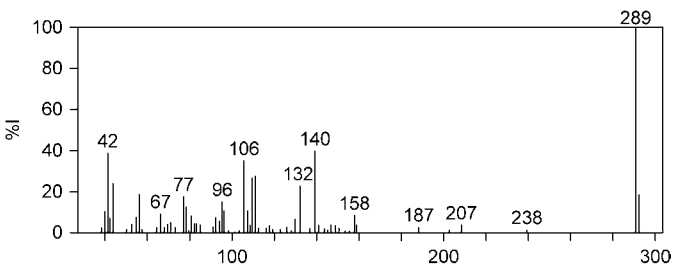


Chemical Properties Mp 96.6°. Soluble in water. pK_a 8.01 [Wishart 2006]. Log *P* (octanol/water), 3.38 [Wishart 2006], 3.95 [Sangster 1997]. Extraction yield (chlorobutane), 1.0 [Demme *et al.* 2005]. Sufentanil was stable in plasma under freeze-thaw conditions and long-term stability was ascertained over 6 months under storage at –40°. Stock solutions were stable at room temperature [Schmidt *et al.* 2006]. Stability in urine was established over a period of 4 weeks regardless of storage temperature (room temperature, 4°, or –20°) [Thevis *et al.* 2005]. No significant loss of analyte occurred upon storage of spiked urine for 1 or 2 months [Van Nimmen *et al.* 2004].

Sufentanil Citrate

$C_{22}H_{30}N_2O_2S,C_6H_8O_7 = 578.7$
CAS—60561-17-3
Synonyms R-33800; R-30730; sufentanil citras.
Proprietary Name *Sufenta*
Chemical Properties A white to almost white powder. Mp 136.5°. Soluble in water; soluble to sparingly soluble in alcohol; sparingly soluble in acetone and chloroform; freely soluble in methyl alcohol. pK_a 3.6 [Wishart 2006]. Log *P* (octanol/water), 3.95 [Janssen-Cilag 2007].

Note For stability studies of sufentanil citrate in various containers, see Roos *et al.* [1992], Dufresne *et al.* [2001].
Mass Spectrum Principal ions at *m/z* 289, 140, 42, 106, 113, 111, 44, 132.



Quantification

Plasma **GC-MS** Column: PTA-5 base-deactivated fused silica capillary (30 m × 0.25 mm i.d, 0.5 μm). Carrier gas: He, 1 mL/min. Temperature programme: 80° for 4 min to 270° at 25°/min for 16.4 min. EI ionisation at 70eV, SIM acquisition mode. Limit of quantification, 6 μg/L [Paradis *et al.* 2002].

LC-MS Column: Alltima HP HILIC (50 × 2.1 mm i.d., 3 μm). Mobile phase: water-formic acid-1 mol/L ammonium acetate (100 : 0.25 : 0.5, pH 2.8) : acetonitrile-formic acid-1 mol/L ammonium acetate (100 : 0.25 : 0.5; 0 : 100 to 30 : 70 at 2.2 min to 50 : 50 at 2.5 min for 0.4 min to 0 : 100 at 3.6 min for 0.3 min), flow rate 0.7 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.25 ng/L [Schmidt *et al.* 2006]. Column: reversed phase μ-HPLC (300 × 1 mm i.d., 5 μm). Mobile phase: acetonitrile : water (80 : 20) containing 0.2% trifluroacetic acid, flow rate 50 μL/min. API, positive ion mode, full scan mode. Limit of quantification, 0.3 μg/L [Palleschi *et al.* 2003].

Serum **LC-MS** Column: Macherey-Nagel Nucleosil CC 100–5 C_{18} (70 × 2 mm i.d.). Mobile phase: 0.02% trifluroacetic acid in water : acetonitrile (85 : 15 to 50 : 50 at 0.4 min to 6.5 min to 85 : 15 at 6.9 min), flow rate 0.4 mL/min to 0.15 mL/min at 2.4 min to 0.4 mL/min at 6.3 min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 10 ng/L; limit of detection, 3 ng/L [Martens-Lobenhoffer 2002].

Urine **GC-MS** Column: DB35-MS (30 m × 0.25 mm i.d., 0.15 μm). Carrier gas: He, 2.5 mL/min. Temperature programme: 70° to 280° at 60°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 2.5 ng/L [Van Nimmen *et al.* 2004].

LC-MS Column: Waters Xterra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.192 g ammonium acetate in 500 mL water : 0.192 g ammonium acetate in 500 mL acetonitrile-methanol (95 : 5; 85 : 15 to 71 : 29 in 4 min to 40 : 60 for a further 9 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 9 ng/L [Wang, Bernert 2006]. Column: Macherey-Nagel Pyramid C_{18} (70 × 4.0 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium acetate containing 1% acetic acid : acetonitrile (100 : 0 for 1 min to 0 : 100 at 7 min to 100 : 0 at 9.7 min), flow rate 800 μL/min. Positive ion mode, MRM acquisition mode. Limit of detection, 0.5 μg/L [Thevis *et al.* 2005].

Disposition in the Body Extensively metabolised by CYP3A4, through oxidative *N*-dealkylation to norsufentanil and the *O*-desmethyl alcohol. Approximately 80% of the administered dose is excreted within 24 h and only 2% of the dose is eliminated as unchanged drug.

Half-Life Approximately 2.7 h.

Volume of Distribution 1.1 to 2.8 L/kg.

Protein Binding Approximately 92.5%, primarily to α_1-acid glycoprotein.

Dose Sufentanil citrate, 30 to 50 μg/kg by epidural administration.

Demme U *et al.* (2005). Systemic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. In *Proceedings of the 12th Annual Meeting of the International Association of Forensic Toxicologists*, Seoul, pp. 481–486.

Dufresne C *et al.* (2001). Stability of sufentanil in human plasma samples. *Ther Drug Monit* 23: 550–552.

Janssen-Cilag (2007). Sufentanil citrate. Beerse,. Belgium: Janssen-Cilag.

Martens-Lobenhoffer J (2002). Very sensitive and specific determination of sufentanil in human serum applying liquid chromatography–two stage mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 769: 227–233.

Palleschi L *et al.* (2003). Quantitative determination of sufentanil in human plasma by liquid chromatography–tandem mass spectrometry. *J Pharm Biomed Anal* 32: 329–336.

Paradis C *et al.* (2002). Solid-phase microextraction of human plasma samples for determination of sufentanil by gas chromatography–mass spectrometry. *Ther Drug Monit* 24: 768–774.

Roos PJ *et al.* (1992). Stability of sufentanil citrate in a portable pump reservoir, a glass container and a polyethylene container. *Pharm Weekbl Sci* 14: 196–200.

Sangster J (1997). *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry*. Chischester, UK: Wiley.

Schmidt R *et al.* (2006). High sensitive determination of sufentanil in human plasma of parturients and neonates following patient-controlled epidural analgesia (PCEA). *J Chromatogr B Analyt Technol Biomed Life Sci* 836: 98–107.

Thevis M *et al.* (2005). Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. *Eur J Mass Spectrom (Chichester, Eng)*. 11: 419–427.

VanNimmen NF *et al.* (2004). Highly sensitive gas chromatographic–mass spectrometric screening method for the determination of picogram levels of fentanyl, sufentanil and alfentanil and their major metabolites in urine of opioid exposed workers. *J Chromatogr B Analyt Technol Biomed Life Sci* 804: 375–387.

Wang L, Bernert JT (2006). Analysis of 13 fentanils, including sufentanil and carfentanil, in human urine by liquid chromatography–atmospheric-pressure ionization–tandem mass spectrometry. *J Anal Toxicol* 30: 335–341.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Sulfacarbamide

Sulfonamide

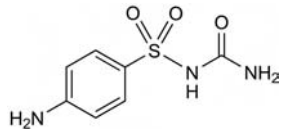
$C_7H_9N_3O_3S,H_2O$ = 233.2

CAS—547-44-4 (anhydrous); 6101-35-5 (monohydrate)

IUPAC Name (4-Aminophenyl)sulfonylurea

Synonyms 4-Amino-*N*-(aminocarbonyl)benzenesulfonamide monohydrate; sulfanilcarbamide; sulfaurea; sulphacarbamide; sulphanilylurea; sulphaurea; urosulphanum.

Proprietary Names *Euvernil*; *Uractyl*; *Uramid*; *Urenil*; *Urosulfan*.



Chemical Properties A white crystalline powder. Mp 125° to 127°. Soluble 1 in 430 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether; soluble in acetone, dilute mineral acids and solutions of alkali hydroxides. Log *P* (octanol/water), −1.0.

Colour Tests Copper sulfate (method 1)—blue; Koppanyi–Zwikker test—violet; palladium chloride—black.

Thin-layer Chromatography System TA—R_f 0.76; system TB—R_f 0.00; system TC—R_f 0.01; system TL—R_f 0.08; system TAD—R_f 0.14; system TAE—R_f 0.81.

Ultraviolet Spectrum Aqueous acid—266 (A^1_1=188b), 272 nm; aqueous alkali—255 nm.

Infrared Spectrum Principal peaks at wavenumbers 1694, 1149, 1587, 1086, 1612, 684 cm^{-1}.

Disposition in the Body Readily absorbed after oral administration. It is rapidly excreted in the urine, most of a single dose being eliminated in 12 h. About 10 to 15% of the material in the urine is the inactive N^4-acetyl metabolite and the remainder is unchanged drug.

Half-life Plasma half-life, about 2.5 h.

Dose 3 g daily.

Sulfacetamide

Antibacterial, Sulfonamide

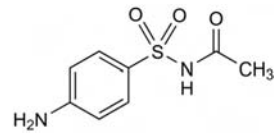
$C_8H_{10}N_2O_3S$ = 214.2

CAS—144-80-9

IUPAC Name *N*-(4-Aminophenyl)sulfonylacetamide

Synonym Acetosulfaminum

Proprietary Names *Sulfanil*; *Vista-Cetamide*. It is an ingredient of *Sultrin*.



Chemical Properties A white or yellowish-white crystalline powder. Mp 182° to 184°. Soluble 1 in 150 of water, 1 in 15 of ethanol and 1 in 7 of acetone; very slightly soluble in chloroform; slightly soluble in ether; soluble in mineral acids and alkali hydroxides. pK_a 1.8, 5.4. Log *P* (octanol/water), −1.0.

Sulfacetamide Sodium

$C_8H_9N_2NaO_3S,H_2O$ = 254.2

CAS—127-56-0 (anhydrous); 6209-17-2 (monohydrate)

Synonyms Soluble sulfacetamide; sulfacylum natrium; sulfacetamide sodium.

Proprietary Names *Acetopt*; *Ak-Sulf*; *Albucid*; *Antebor*; *Beocid Puroptal*; *Bleph-10*; *Cetamide*; *Cetazin*; *Diosulf*; *Optamid(e)*; *Optisol*; *Optosulfex*; *Prontamid*; *Sebizon*; *Sodium Sulamyd*; *Sulf-10*; *Sulfac*; *Sulfex*; *Ultra*; *Vanocin*. It is an ingredient of *Ocusol*.

Chemical Properties White or yellowish-white crystals or crystalline powder. It slowly darkens on exposure to light; on exposure to moist air it absorbs carbon dioxide and becomes less soluble. Soluble 1 in 1.5 of water; slightly soluble in ethanol; sparingly soluble in acetone; practically insoluble in chloroform and ether.

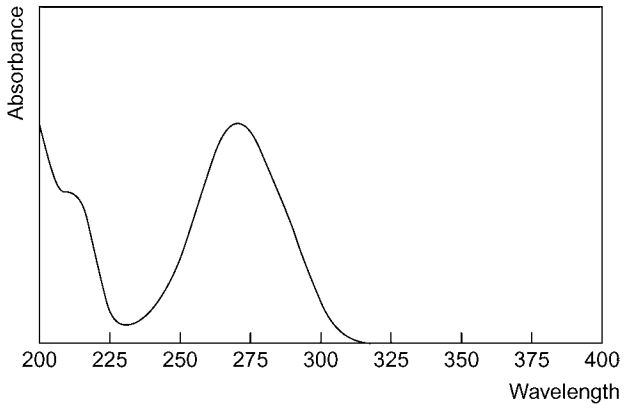
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—blue; Koppanyi–Zwikker test—blue-violet.

Thin-layer Chromatography System TA—R_f 0.70; system TD—R_f 0.17; system TE—R_f 0.04; system TF—R_f 0.42; system TT—R_f 0.53; system TU—R_f 0.37; system TV—R_f 0.04; system TAD—R_f 0.28; system TAE—R_f 0.87 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).

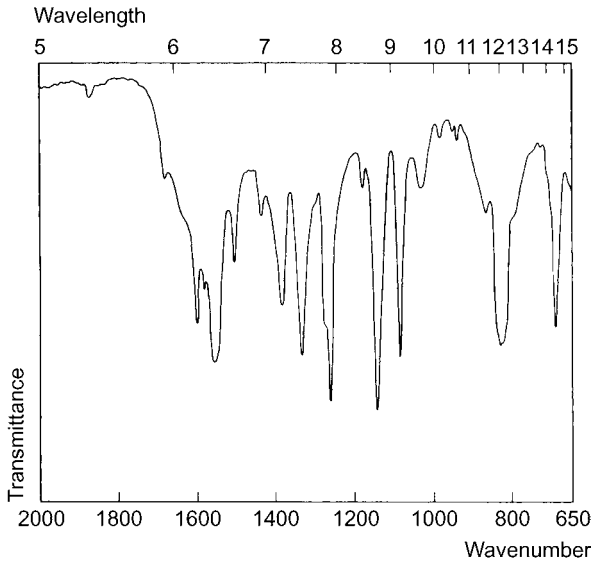
Gas Chromatography System GA—RI 2132; System GJ—methyl derivative RRT 0.16 (relative to griseofulvin).

High Performance Liquid Chromatography System HU—*k* 7.7; system HY—RI 241.

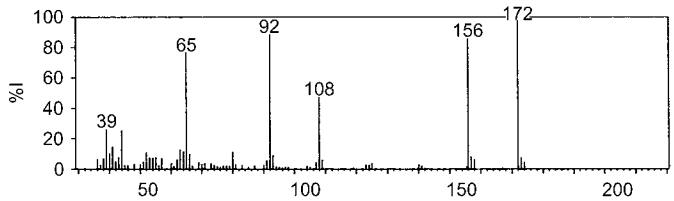
Ultraviolet Spectrum Aqueous acid—271 nm (A^1_1=260a); aqueous alkali—256 nm (A^1_1=750a).



Infrared Spectrum Principal peaks at wavenumbers 1145, 1264, 1552, 1090, 825, 1600 cm^{-1} (sulfacetamide sodium, KBr disk).



Mass Spectrum Principal ions at *m/z* 172, 92, 156, 65, 108, 39, 44, 41.



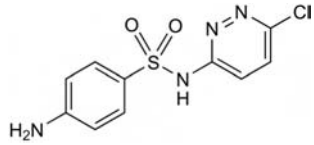
Quantification
HPLC The methods referred to under sulfamethoxazole may be used.
Disposition in the Body After oral administration sulfacetamide is readily absorbed and rapidly excreted in the urine, mainly as unchanged drug. However, it is usually only used topically in the treatment of eye infections; absorption into the blood may occur after application to the eye if the conjunctiva is inflamed.
Half-life Plasma half-life, 7 to 14 h.
Protein Binding 15 to 18%.
Use Sulfacetamide sodium is used as a 10 to 30% ophthalmic solution.

Sulfachlorpyridazine

Antibacterial, Sulfonamide
$C_{10}H_9ClN_4O_2S$ = 284.7
CAS—80-32-0

IUPAC Name 4-Amino-*N*-(6-chloropyridazin-3-yl)benzenesulfonamide
Synonyms Sulphachlorpyridazine; Ba-10370.

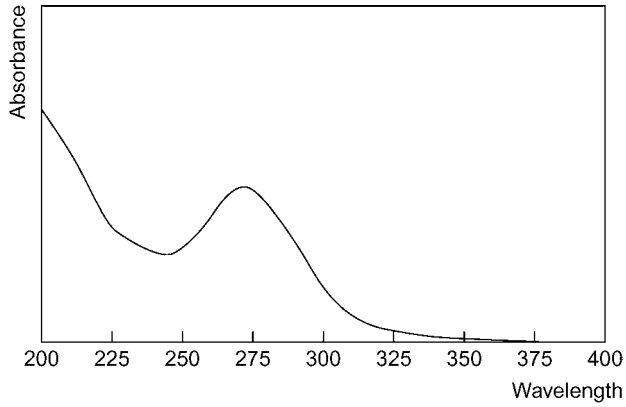
Proprietary Names *Cosulid*; *Cosumix*; *Nefrosul*; *Sonilyn*.



Chemical Properties A yellowish crystalline powder. Soluble in bicarbonate solutions. Log *P* (octanol/water), 0.3.

Sulfachlorpyridazine Sodium
$C_{10}H_8ClN_4O_2S,Na$ = 306.7
CAS—23282-55-5
Proprietary Names *Prinzone*; *Vetisulid*.

Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—pink; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.70; system TAJ—R_f 0.41; system TAK—R_f 0.23; system TAL—R_f 0.80 (Van Urk reagent, positive).
High Performance Liquid Chromatography System HU—*k* 3.3.
Ultraviolet Spectrum Aqueous acid—256 nm; aqueous alkali—257 nm.



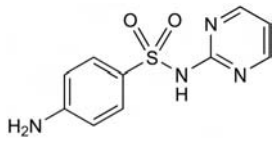
Infrared Spectrum Principal peaks at wavenumbers 1124, 1220, 1087, 1208, 963, 1618 cm^{-1} (KBr disk).
Disposition in the Body Readily absorbed after oral administration and rapidly excreted in the urine.
Therapeutic Concentration
After a single oral dose of 500 mg to 10 subjects, peak plasma concentrations of 39.7 to 85.5 mg/L (mean, 59.6) were attained in about 1 h [Marino *et al.* 1980].
Half-life Plasma half-life, about 3 to 4 h.
Volume of Distribution About 0.1 L/kg.
Clearance Plasma clearance, about 0.5 mL/min/kg.
Protein Binding About 94 to 99%.
Dose Sulfachlorpyridazine has been given in an initial dose of 4 g, followed by 2 to 4 g daily.

Marino EL *et al.* (1980). Influence of hepatic and renal disorders on the pharmacokinetics of sulfachloropyridacine. *Int J Clin Pharmacol Ther Toxicol* 18: 10–14.

Sulfadiazine

Antibacterial, Sulfonamide
$C_{10}H_{10}N_4O_2S$ = 250.3
CAS—68-35-9

IUPAC Name 4-Amino-*N*-pyrimidin-2-ylbenzenesulfonamide
Synonyms Solfadiazina; solfapirimidina; sulfadiazine.
Proprietary Names *Adiazine*; *Balin*; *Diazyl*; *Labdiazina*; *Sulfolex*. It is an ingredient of *Geatrim-Boli*, *Norodine*, *Scorprin*, *Synutrim*, *Tribrissen*, *Triglobe* and *Vesuprim*.



Chemical Properties White, yellowish-white or pinkish-white crystals or powder, slowly darkening on exposure to light. Mp 255°, with decomposition. Practically insoluble in water, chloroform and ether; very slightly soluble in ethanol; soluble 1 in 300 of acetone; soluble in dilute mineral acids and in solutions of alkali hydroxides and carbonates. pK_a 6.5 (25°). Log *P* (octanol/pH 7.5), −1.3.

Sulfadiazine Sodium

$C_{10}H_9N_4NaO_2S = 272.3$
CAS—547-32-0

Synonyms Soluble sulfadiazine; sulfadiazine sodium.

Chemical Properties A white or yellowish-white powder. It slowly darkens on exposure to light; on exposure to moist air it absorbs carbon dioxide with the liberation of sulfadiazine and becomes incompletely soluble in water. Soluble 1 in 2 of water; slightly soluble in ethanol; practically insoluble in chloroform and ether.

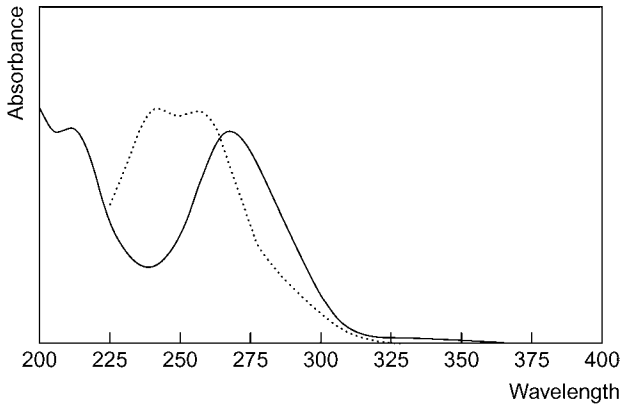
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—violet-brown; Koppanyi–Zwikker test—violet-pink; mercurous nitrate—black.

Thin-layer Chromatography System TA—R_f 0.64; system TD—R_f 0.22; system TE—R_f 0.04; system TF—R_f 0.39; system TT—R_f 0.24; system TU—R_f 0.22; system TV—R_f 0.03; system TAD—R_f 0.38; system TAE—R_f 0.81; system TAJ—R_f 0.40; system TAK—R_f 0.11; system TAL—R_f 0.70.

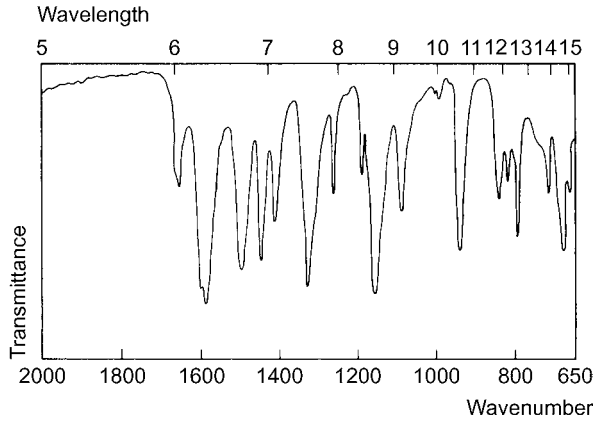
Gas Chromatography System GA—sulfadiazine RI 2502, sulfadiazine-Me RI 2625, M (acetyl-)-Me_2 RI 3710; system GJ—sulfadiazine-Me RRT 0.66, N^4-acetylsulfadiazine-Me RRT 1.69 (both relative to griseofulvin).

High Performance Liquid Chromatography System HU—*k* 8.7; system HY—RI 234; system HAA—retention time 8.4 min.

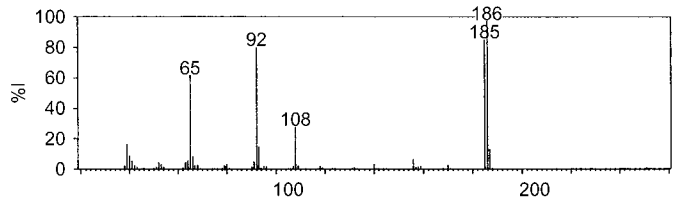
Ultraviolet Spectrum Aqueous acid—242 nm (A^1_1=587a); aqueous alkali—240 (A^1_1=867a), 254 nm (A^1_1=868a).



Infrared Spectrum Principal peaks at wavenumbers 1580, 1159, 1494, 682, 940, 797 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 186, 185, 92, 65, 108, 39, 93, 187.



Quantification

Plasma **GC** ECD. Sulfadiazine and acetyl derivative. Limit of detection, 1 ng [Bye, Land 1977].

HPLC [Metz *et al.* 1996]. UV detection. Limit of detection, 400 µg/L for sulfadiazine in plasma [Westerlund, Wijkström 1982].

Serum **HPLC** UV detection [Springolo, Coppi 1989; Alkaysi *et al.* 1991]

Urine **GC** See Plasma [Bye, Land 1977].

HPLC Sulfadiazine and other sulfonamides. Limit of detection, 0.1 to 0.3 mg/L [Simo-Alfonso *et al.* 1995]. See Plasma [Metz *et al.* 1996]. See Serum [Springolo, Coppi 1989; Alkaysi *et al.* 1991]. Limit of detection, 5 mg/L for sulfadiazine and 7 mg/L for acetyl derivative, see Plasma [Westerlund, Wijkström 1982].

Disposition in the Body Readily absorbed after oral administration. It is acetylated in the body and up to 15% of the sulfadiazine in the blood is in the form of the inactive N^4-acetyl derivative. About 50% of a dose is excreted in the urine in 24 h; up to about 40% of the excreted material is the acetyl derivative and up to about 50% is unchanged drug. Excretion is influenced by the pH of the urine, the rate being increased when the urine is alkaline.

Therapeutic Concentration

After a single oral dose of 820 mg to 5 subjects, peak serum concentrations of sulfadiazine plus metabolites of 19 to 40 mg/L (mean, 33) were attained in 4 h [Bergan *et al.* 1977].

Half-life Plasma half-life, 6 to 17 h.

Volume of Distribution About 0.3 L/kg.

Clearance Plasma clearance, about 0.3 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 0.93.

Protein Binding About 50%.

Dose An initial dose of 2 to 4 g, followed by 1 g every 4 to 6 h.

Alkaysi HN *et al.* (1991). High performance liquid chromatographic analysis of tetroxoprim and sulphadiazine in serum and urine. *Biomed Chromatogr* 5: 265–268.

Bergan T *et al.* (1977). Kinetics of a sulfadiazine-trimethoprim combination. *Clin Pharmacol Ther* 22: 211–224.

Bye A, Land G (1977). Gas-liquid chromatographic determination of sulphadiazine and its major metabolite in human plasma and urine. *J Chromatogr* 139: 181–185.

Metz R *et al.* (1996). Improved determination of sulfadiazine in human plasma and urine by high-performance liquid chromatography. *J Chromatogr A* 729: 243–249.

Simo-Alfonso EF *et al.* (1995). Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography. *J Chromatogr B Biomed Appl* 670: 183–187.

Springolo V, Coppi G (1989). HPLC determination of tetroxoprim and sulphadiazine in pharmaceutical dosage forms and in biological fluids. *J Pharm Biomed Anal* 7: 57–65.

Westerlund D, Wijkström A (1982). Determination of sulfadiazine and N4-acetylsulfadiazine in biological fluids by liquid chromatography on silica gel with an aqueous buffer as mobile phase. *J Pharm Sci* 71: 1142–1145.

Sulfadimethoxine

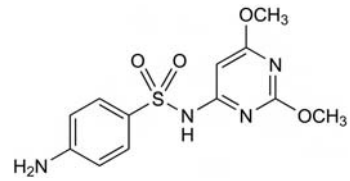
Antibacterial, Sulfonamide

$C_{12}H_{14}N_4O_4S = 310.3$
CAS—122-11-2

IUPAC Name 4-Amino-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide

Synonym Solfadimetossina

Proprietary Names *Agribon*; *Albon*; *Arnosulfan*; *Diasulfa*; *Madribon*; *Maxulvet*; *Neostreptal*; *Sudine*; *Suldixine*; *Sulfabon*; *Sulxin*; *Symbio*; *Ultrasulfon*.



Chemical Properties A white or creamy-white crystalline powder. Mp 201° to 204°. Very slightly soluble in water; soluble 1 in 200 of ethanol, 1 in 800 of chloroform and 1 in 2000 of ether; soluble in dilute mineral acids and in solutions of alkali hydroxides and carbonates. pK_a 5.9 (25°). Log *P* (octanol/water), 1.6.

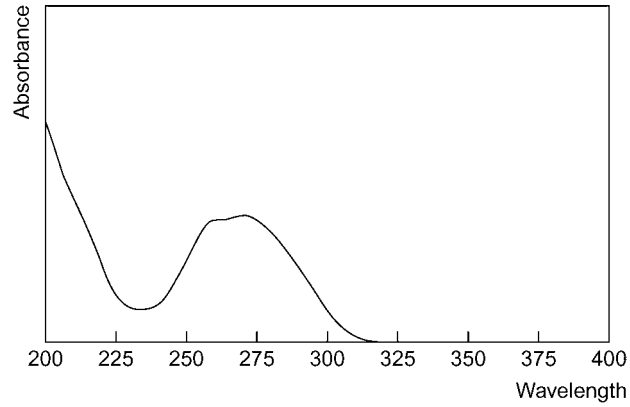
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—violet-pink; mercurous nitrate—black.

Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.31; system TE—R_f 0.10; system TF—R_f 0.51; system TT—R_f 0.85; system TU—R_f 0.52; system TV—R_f 0.34; system TAD—R_f 0.48; system TAE—R_f 0.86; system TAJ—R_f 0.53; system TAK—R_f 0.28; system TAL—R_f 0.98.

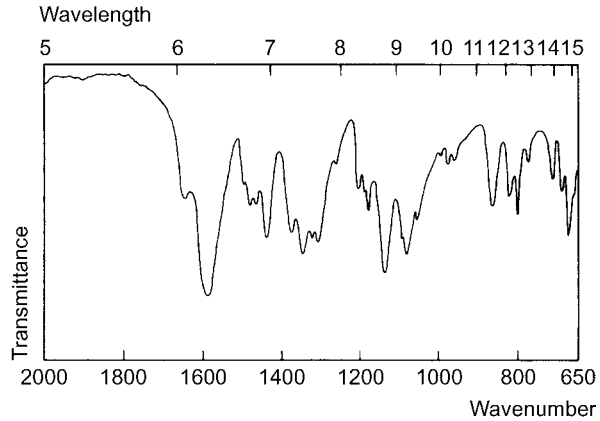
Gas Chromatography System GA—sulfadimethoxine-TMS RI 3060.

High Performance Liquid Chromatography System HY—RI 352; system HAA—retention time 14.7 min.

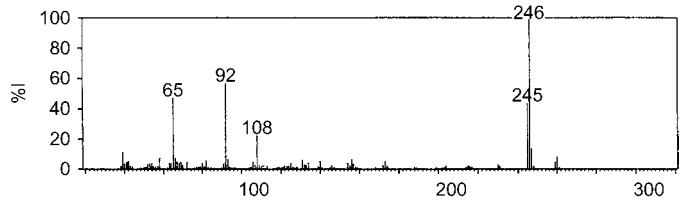
Ultraviolet Spectrum Aqueous acid—275 nm (A^1_1=449a); aqueous alkali—269 nm (A^1_1=845a).



Infrared Spectrum Principal peaks at wavenumbers 1590, 1147, 1090, 1314, 685, 1066 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 246, 92, 65, 245, 108, 247, 39, 260.

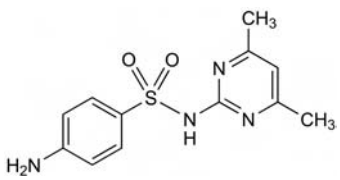


Quantification
Plasma **HPLC** Sulfadimethoxine and its N^1-glucuronide, N^4-acetyl and N^4-acetyl-N^1-glucuronide metabolites [Vree *et al.* 1990].
Urine **HPLC** See Plasma [Vree *et al.* 1990].
Disposition in the Body Sulfadimethoxine is a long-acting sulfonamide which is readily absorbed after oral administration. After a single dose, peak blood concentrations are attained in about 4–6 h. It is acetylated in the body and about 10% of the sulfadimethoxine in the blood is present as the inactive N^4-acetyl derivative, and about 5% as the N^1-glucuronide. About 50% of a dose is excreted in the urine in 48 h, about 20% of the excreted material being the acetyl derivative, about 5% unchanged drug, and 60–80% the N^1-glucuronide. Both unchanged drug and the N^1-glucuronide are excreted in the bile.
Therapeutic Concentration In plasma, usually in the range 50–100 mg/L. Following an oral dose of 2 g, peak blood concentrations of 120–180 mg/L were attained [Seneca 1966].
Half-life Plasma half-life, 20–40 h.
Volume of Distribution About 0.1–0.2 L/kg.
Protein Binding 90–99%.
Dose An initial dose of 1 or 2 g, followed by 0.5 to 1 g daily.

Seneca H (1966). Long-acting sulfonamides in urinary tract infections. *JAMA* 198: 975–980.
Vree TB *et al.* (1990). High-performance liquid chromatography of sulphadimethoxine and its N1-glucuronide, N4-acetyl and N4-acetyl-N1-glucuronide metabolites in human plasma and urine. *J Chromatogr* 526: 119–128.

Sulfadimidine

Antibacterial, Sulfonamide
$C_{12}H_{14}N_4O_2S = 278.3$
CAS—57-68-1
IUPAC Name 4-Amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide
Synonyms Solfametazina; sulfadimerazine; sulfadimezinum; sulfamethazine; sulfadimethylpyrimidine; sulfamethazine.
Note Sulfadimethylpyrimidine has been used as a synonym for sulfisomidine. Care should be taken to avoid confusion between the two compounds, which are isomeric.
Proprietary Names *Diazil*; *Dimidin-R*; *Neazina*; *S-Dimidine Sulfadine*; *Sulmet*. It is an ingredient of *Trisulfaminic* and *Sulfatril*.

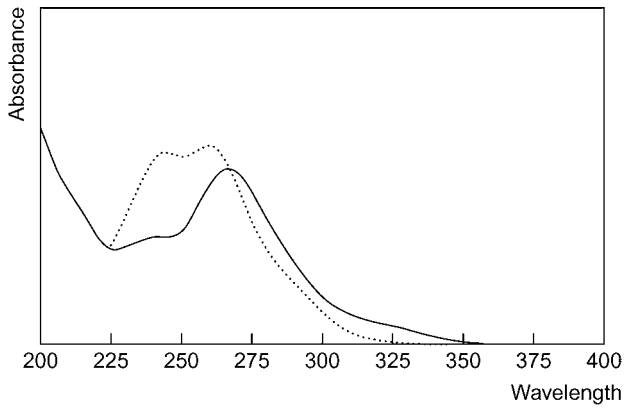


Chemical Properties White or yellowish-white crystals or powder which darken and decompose on exposure to light. Mp 176°; also reported as 178° to 179°, 198° to 199° and 205° to 207°. Very slightly soluble in water; soluble 1 in 120 of ethanol, 1 in 30 of acetone, 1 in 600 of chloroform and 1 in 2500 of ether; soluble in dilute mineral acids and in aqueous solutions of alkali hydroxides and carbonates. pK_a 7.4 (25°). Log *P* (octanol/water), 0.9.

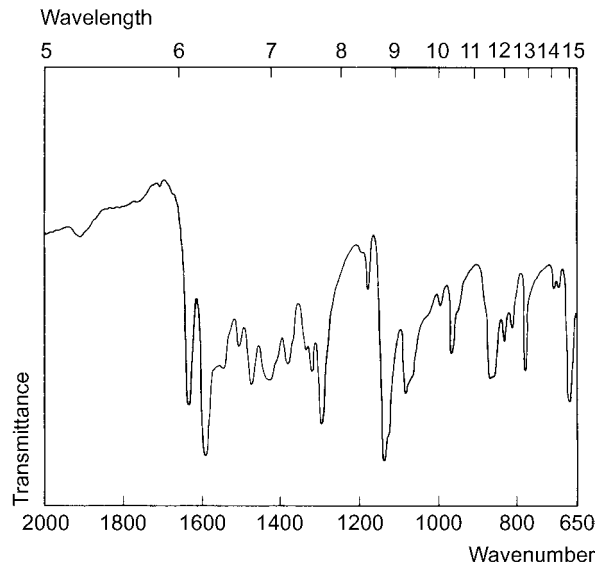
Sulfadimidine Sodium

$C_{12}H_{13}N_4NaO_2S = 300.3$
CAS—1981-58-4
Synonyms Soluble sulfadimethylpyrimidine; soluble sulfadimidine; soluble sulfamethazine; sulfadimidine sodium.
Proprietary Names *Intradine*; *Sulfoxine 33*; *Vesadin*.
Chemical Properties White or creamy-white hygroscopic crystals or powder. It slowly discolours and decomposes on exposure to light; on exposure to air it absorbs carbon dioxide and becomes less soluble in water. Soluble 1 in 2.5 of water and 1 in 60 of ethanol.

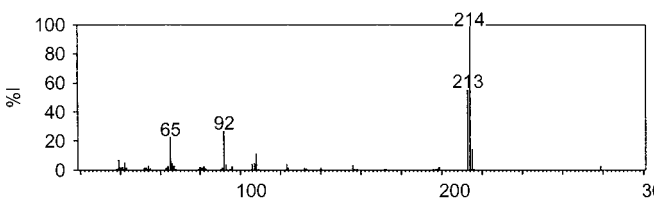
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green→brown; Koppanyi–Zwikker test—violet-pink; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.62; system TD—R_f 0.23; system TE—R_f 0.13; system TF—R_f 0.45; system TT—R_f 0.50; system TU—R_f 0.27; system TV—R_f 0.62; system TAD—R_f 0.44; system TAE—R_f 0.79; system TAJ—R_f 0.43; system TAK—R_f 0.17; system TAL—R_f 0.95 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—RI 2613; System GJ—methyl derivative RRT 0.71 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 7.1; system HX—RI 333; system HY—RI 257.
Ultraviolet Spectrum Aqueous acid—243 (A^1_1=541a), 301 nm; aqueous alkali—242 (A^1_1=760a), 258 nm (A^1_1=783a).



Infrared Spectrum Principal peaks at wavenumbers 1145, 1595, 1304, 1635, 682, 1090 cm^{-1} (KCl disk).



Mass Spectrum Principal ions at *m/z* 214, 213, 92, 65, 215, 108, 39, 42.



S

Quantification
Blood **HPLC** Sulfadimidine and acetyl metabolite. Limit of detection, 6 mg/L [Whelpton *et al.* 1981].
Plasma **HPLC** See Blood [Whelpton *et al.* 1981].
Disposition in the Body Readily absorbed after oral administration. It is acetylated in the body, the degree of acetylation being dependent on the acetylator status of the subject. In rapid acetylators, 60 to 90% of the sulfadimidine in the blood is present as the N^4-acetyl derivative, whereas in slow acetylators, the proportion is only about 16 to 37%. About 50% of a dose is excreted in the urine in 48 h. Of the excreted material, up to about 15% is unchanged drug and up to about 95% may be the acetyl derivative.
Therapeutic Concentration In plasma, free sulfadimidine, usually in the range 50 to 100 mg/L.

Following a single oral dose of 1.5 g to 8 slow acetylators, plasma-sulfadimidine concentrations of 64.0 to 88.4 mg/L (mean, 80.7) were attained in 3 to 4 h; after a similar dose to 8 rapid acetylators, peak plasma concentrations ranged from 38.8 to 64.1 mg/L (mean, 45.7) at 3 h [Woolhouse, Atu-Taylor 1982].

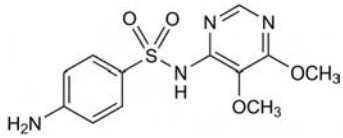
Half-life Derived from urinary excretion data, 1 to 5 h for rapid acetylators and 3 to 11 h (mean, 6) for slow acetylators.
Volume of Distribution About 0.6 L/kg.
Protein Binding 60 to 90%.
Dose An initial dose of up to 3 g, followed by 0.5 to 1.5 g every 6 h.

Whelpton R *et al.* (1981). Bratton-Marshall and liquid-chromatographic methods compared for determination of sulfamethazine acetylator status. *Clin Chem* 27: 1911–1914.
Woolhouse NM, Atu-Taylor LC (1982). Influence of double genetic polymorphism on response to sulfamethazine. *Clin Pharmacol Ther* 31: 377–383.

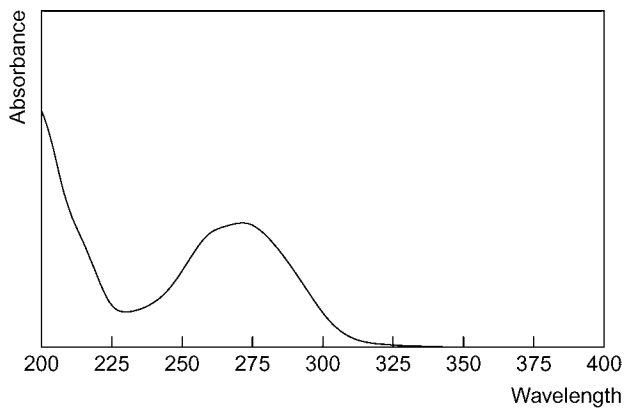
Sulfadoxine

Antibacterial, Sulfonamide
$C_{12}H_{14}N_4O_4S = 310.3$
CAS—2447-57-6

IUPAC Name 4-Amino-*N*-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide
Synonyms Sulformethoxine; sulforthomidine; sulfadoxine; sulformethoxine; sulforthodimethoxine.
Proprietary Names *Fanasil*; *Fanzil*. It is an ingredient of *Bimotrim* (vet.), *Borgal* (vet.), *Fansidar* and *Trivetrin* (vet.).



Chemical Properties A white or creamy-white crystalline powder. Mp 190° to 194°. Very slightly soluble in water; slightly soluble in ethanol and in methanol; practically insoluble in ether; soluble in solutions of alkali hydroxides and mineral acids. Log *P* (octanol/water), 0.7.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.63; system TD—R_f 0.37; system TE—R_f 0.79; system TF—R_f 0.51; system TAD—R_f 0.55; system TAE—R_f 0.78; system TAJ—R_f 0.53; system TAK—R_f 0.28; system TAL—R_f 0.90.
High Performance Liquid Chromatography System HU—*k* 4.4; system HX—RI 364; system HAA—retention time 13.3 min.
Ultraviolet Spectrum Aqueous acid—264 nm; aqueous alkali—272 nm (A^1_1=762a).



Infrared Spectrum Principal peaks at wavenumbers 1583, 1161, 1596, 1315, 1091, 1305 cm^{-1} (KBr disk).

Quantification
Blood **HPLC** Sulfadoxine and pyrimethamine [Green *et al.* 2002]. UV detection. Sulfadoxine and other sulfonamides. Limit of detection, 50 µg/L [Dua *et al.* 1994].
Plasma **HPLC** UV detection. Sulfadoxine and pyrimethamine. Limits of detection, 22 mg/L (sulfadoxine) and 10 µg/L (pyrimethamine) [Astier *et al.* 1997]. UV detection. Sulfadoxine and other antimalarials. Limit of detection, 25 µg/L for sulfadoxine [Eljaschewitsch *et al.* 1996]. See Blood [Dua *et al.* 1994].
Note The methods referred to under Sulfamethoxazole may also be used.
Disposition in the Body Sulfadoxine is a long-acting sulfonamide which is readily absorbed after oral administration. The main metabolic reaction is N^4-acetylation together with glucuronidation. High concentrations of sulfadoxine are attained in the blood in about 4 h with about 5% as the acetyl derivative and 2% as the glucuronide. It is excreted very slowly in the urine, about 8% of a dose being excreted in 24 h and 30% in 7 days. The excreted material consists of 30 to 60% acetyl derivative and 30 to 60% unchanged sulfadoxine, of which up to 40% may be conjugated with glucuronic acid and up to 10% with sulfate.
Therapeutic Concentration

Blood concentrations of 130 to 200 mg/L were attained by 31 patients receiving 1.5 to 2 g weekly [Haegi 1966].

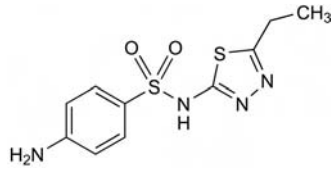
Half-life Plasma half-life, about 4 to 8 days.
Protein Binding About 90%.
Dose Initially 2 g, followed by 1 to 1.5 g weekly.

Astier H *et al.* (1997). Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction. *J Chromatogr B Biomed Sci Appl* 698: 217–223.
Dua VK *et al.* (1994). Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and Plasmodium falciparum malaria cases after treatment with Fansidar using high-performance liquid chromatography. *J Pharm Biomed Anal* 12: 1317–1323.
Eljaschewitsch J *et al.* (1996). High-performance liquid chromatography determination of pyrimethamine, dapsone, monoacetyldapsone, sulfadoxine, and N-acetyl-sulfadoxine after rapid solid-phase extraction. *Ther Drug Monit* 18: 592–597.
Green MD *et al.* (2002). High-performance liquid chromatographic assay for the simultaneous determination of sulfadoxine and pyrimethamine from whole blood dried onto filter paper. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 159–162.
Haegi V (1966). Experimental and clinical experiences with Fanasil, a new ultralong acting sulfonamide. *Schweiz Med Wochenschr* 96: 1308–1314.

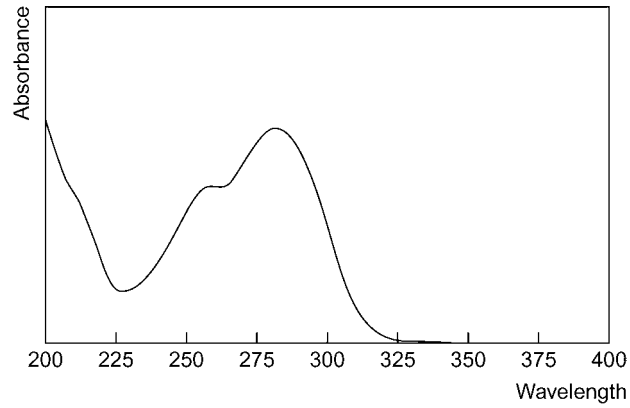
Sulfaethidole

Antibacterial, Sulfonamide
$C_{10}H_{12}N_4O_2S_2 = 284.4$
CAS—94-19-9

IUPAC Name 4-Amino-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide
Synonyms Aethazolum; ethazol; SETD; sulfaethidole; sulfaethylthiadiazole.
Proprietary Name *Sul-Spantab*



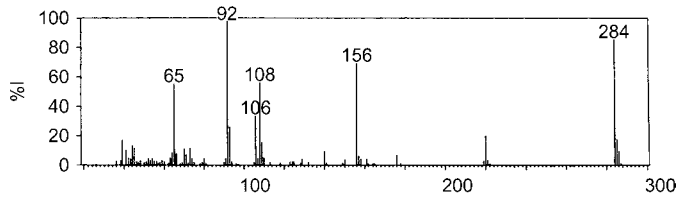
Chemical Properties A white or yellowish-white crystalline powder. Mp about 186°. Very slightly soluble in water; soluble 1 in 30 of ethanol, 1 in 40 of methanol, 1 in 10 of acetone, 1 in 2800 of chloroform and 1 in 1350 of ether; freely soluble in solutions of alkali hydroxides. pK_a 5.6. Log *P* (octanol/water), 1.0.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—violet; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.67; system TD—R_f 0.14; system TE—R_f 0.08; system TF—R_f 0.35; system TAD—R_f 0.34.
Gas Chromatography System GA—sulfaethidole RI 2620, sulfaethidole-Me RI 3060, sulfaethidole-Me_2 RI 2840, M (acetyl-) RI 2490, M (acetyl-)-Me_2 RI 3410.
High Performance Liquid Chromatography System HY—RI 314.
Ultraviolet Spectrum



S

Infrared Spectrum Principal peaks at wavenumbers 1086, 1298, 1538, 1149, 689, 917 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 92, 284, 156, 108, 65, 106, 93, 220.



Quantification

HPLC The methods referred to under sulfamethoxazole may be used.

Disposition in the Body Readily absorbed after oral administration and only slightly acetylated in the body. More than 75% of a dose is excreted in the urine in 48 h as unchanged drug.

Therapeutic Concentration

Following oral administration of 3.9 g to 6 subjects, peak blood concentrations of 138 to 275 mg/L (mean, 227) were attained in about 2 h [Nicholson *et al.* 1960].

Half-life Plasma half-life, about 10 to 12 h.

Volume of Distribution About 0.1 L/kg.

Protein Binding 96 to 99%.

Dose Sulfaethidole has been given in sustained-release form in a dose of up to 3.9 g.

Nicholson AE *et al.* (1960). Sulfaethylthiadiazole. VI. Blood and urine concentrations from sustained and immediate relase tablets. *J Am Pharm Assoc Am Pharm Assoc* 49: 40–44.

Sulfafurazole

Antibacterial, Sulfonamide

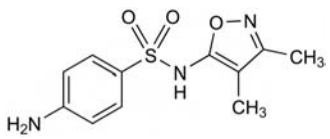
$C_{11}H_{13}N_3O_3S = 267.3$

CAS—127-69-5

IUPAC Name 4-Amino-*N*-(3,4-dimethyl-1,2-oxazol-5-yl)benzenesulfonamide

Synonyms 4-Amino-*N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide; sulfafuraz; sulfafurazolum; sulfisoxazole; sulfafurazole.

Proprietary Names *Gantrisin* (tablets); *Koro-Sulf*; *Novo-Soxazole*; *Sosol*; *Soxisol*; *Soxomide*; *Sulfalar*; *Sulfazin*; *Sulfizax*; *Sulfoxol*.



Chemical Properties A white or yellowish-white crystalline powder. Mp 191° to 194°. Soluble 1 in 7700 of water, 1 in 50 of ethanol, 1 in 1000 of chloroform and 1 in 800 of ether; freely soluble in acetone; soluble in methanol. pK_a 5.0 (20°). Log *P* (octanol/pH 7.5), −0.9.

Acetyl Sulfafurazole

$C_{13}H_{15}N_3O_4S = 309.3$

CAS—80-74-0

Synonym Sulfisoxazole acetyl.

Proprietary Names *Gantrisin* (suspension and syrup). It is an ingredient of *Pediazole*.

Note Acetyl sulfafurazole (*N*-[(4-aminophenyl)sulfonyl]-*N*-(3,4-dimethyl-5-isoxazolyl)acetamide) should be distinguished from the N^4-acetyl derivative formed from sulfafurazole by acetylation in the body.

Chemical Properties A white to slightly yellow crystalline powder. Mp 193° to 194°. Practically insoluble in water; soluble 1 in about 180 of ethanol, 1 in 35 of chloroform, 1 in about 1100 of ether and 1 in about 200 of methanol.

Sulfafurazole Diethanolamine

$C_{11}H_{13}N_3O_3S,C_4H_{11}NO_2 = 372.4$

CAS—4299-60-9

Synonyms Sulfisoxazole diolamine; sulfafurazole diolamine.

Proprietary Name *Gantrisin* (injection)

Chemical Properties A fine, white, crystalline powder. Mp 119° to 124°. Soluble 1 in 2 of water, 1 in 16 of ethanol, 1 in 1000 of chloroform and 1 in 4 of methanol; practically insoluble in ether.

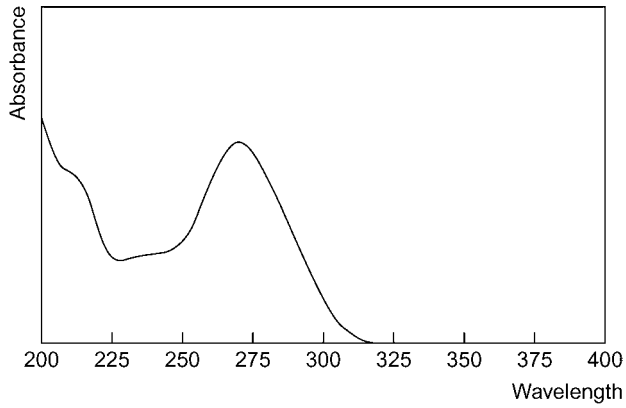
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—brown; Koppanyi–Zwikker test—blue-violet; mercurous nitrate—black; nitrous acid—orange.

Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.25; system TE—R_f 0.06; system TF—R_f 0.52; system TT—R_f 0.74; system TU—R_f 0.48; system TV—R_f 0.04; system TAD—R_f 0.33; system TAE—R_f 0.81; system TAJ—R_f 0.39; system TAK—R_f 0.11; system TAL—R_f 0.70 (mercuric chloride-diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).

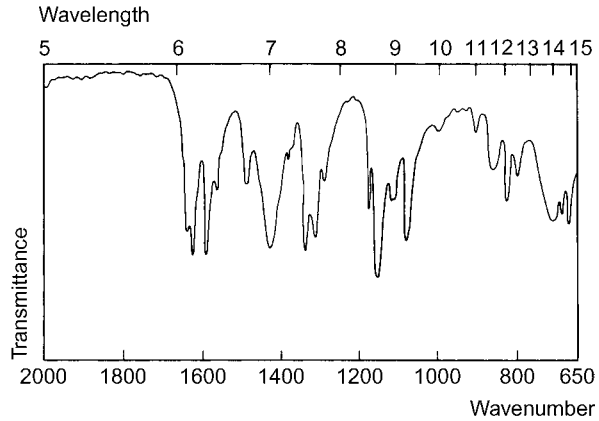
Gas Chromatography System GA—RI 1212; system GJ—methyl derivative RRT 0.42 (relative to griseofulvin).

High Performance Liquid Chromatography System HU—*k* 6.0; system HY—RI 333.

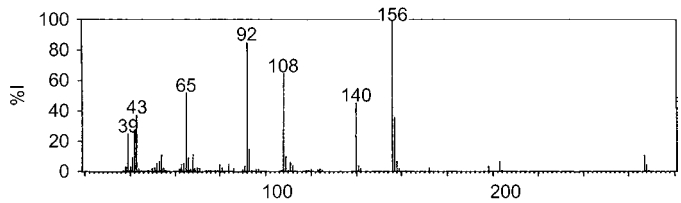
Ultraviolet Spectrum Aqueous acid—265 nm; aqueous alkali—253 nm (A^1_1=783a).



Infrared Spectrum Principal peaks at wavenumbers 1166, 1633, 1598, 1092, 1647, 690 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 156, 92, 108, 65, 140, 43, 157, 42.



Quantification

Plasma **HPLC** Electrochemical detection. Sulfafurazole and other sulfonamides [Klimes, Mokry 1997]. UV detection. Sulfafurazole and acetylsulfafurazole. Limit of detection, 50 µg/L [Jung, Oie 1980].

Urine **HPLC** See Plasma [Jung, Oie 1980].

Disposition in the Body Rapidly absorbed after oral administration. It is acetylated in the body, about 30% of the sulfafurazole in the blood being in the form of the inactive N^4-acetyl derivative. It is rapidly excreted in the urine, almost the entire dose being excreted within 48 h, with up to about 60% as unchanged drug and up to about 30% as the acetyl derivative. Excretion is influenced by the pH of the urine, the rate being increased when the urine is alkaline.

Therapeutic Concentration

After a single oral dose of 2 g to 7 subjects, peak plasma concentrations of sulfafurazole of 127 to 210 mg/L (mean, 169) were attained in 1 to 4 h [Kaplan *et al.* 1972].

Half-life Plasma half-life, 4 to 7 h.

Volume of Distribution About 0.1 to 0.2 L/kg.

Clearance Plasma clearance, about 0.3 mL/min/kg.

Protein Binding 85 to 95%.

Dose An initial dose of 2 to 4 g, followed by 1 to 2 g every 4 to 6 h.

Jung D, Oie S (1980). "High-pressure" liquid chromatography of sulfisoxazole and N4-acetylsulfisoxazole in body fluids. *Clin Chem* 26: 51–54.

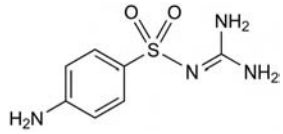
Kaplan SA *et al.* (1972). Pharmacokinetic profile of sulfisoxazole following intravenous, intramuscular, and oral administration to man. *J Pharm Sci* 61: 773–778.

Klimes J, Mokry M (1997). High performance liquid chromatographic analysis of selected sulfonamides in plasma. *Pharmazie* 52: 448–450.

Sulfaguanidine

Antibacterial, Sulfonamide
$C_7H_{10}N_4O_2S = 214.3$
CAS—57-67-0

IUPAC Name 2-(4-Aminophenyl)sulfonylguanidine
Synonyms 4-Amino-*N*-(aminoiminomethyl)benzenesulfonamide; solfaguanidina; sulfamidinum; sulfanilylguanidine; sulginum.
Proprietary Names *Diacta*; *Enteropathyl*; *Ganidan*; *Guanicil*; *Resulfon*; *Shigatox*.

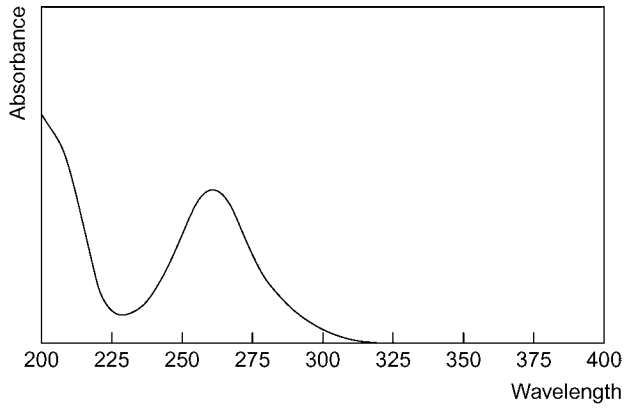


Chemical Properties Log *P* (octanol/water), −1.2.

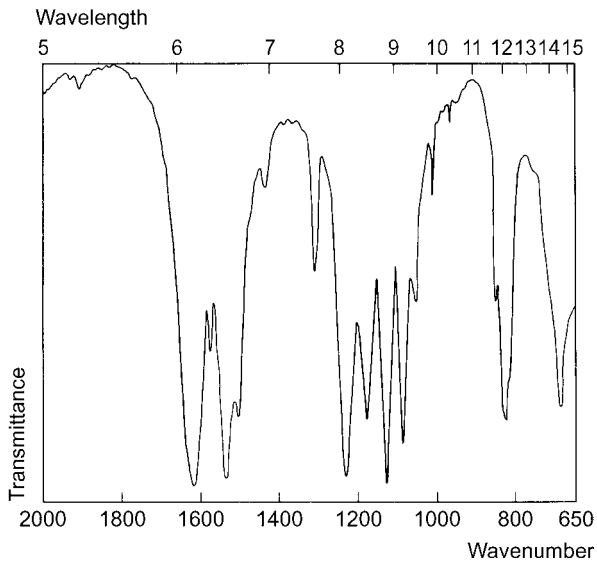
Sulfaguanidine Monohydrate

$C_7H_{10}N_4O_2S,H_2O = 232.3$
CAS—6190-55-2
Chemical Properties White crystals or powder, slowly darkening on exposure to light. Mp 190° to 193°. Soluble 1 in 1000 of water and 1 in 250 of ethanol; slightly soluble in acetone; practically insoluble in ether; readily soluble in dilute mineral acids.

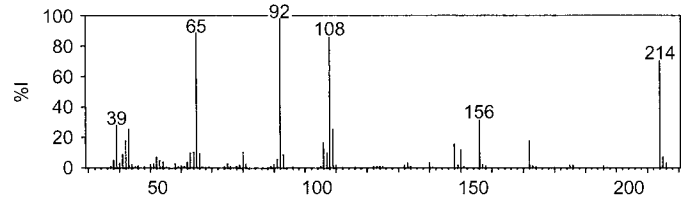
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—blue; Koppanyi–Zwikker test—violet (transient).
Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.01; system TE—R_f 0.25; system TF—R_f 0.06; system TT—R_f 0.21; system TU—R_f 0.90; system TV—R_f 0.48; system TAD—R_f 0.07; system TAE—R_f 0.75.
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HY—RI 92; system HAA—retention time 3.8 min.
Ultraviolet Spectrum Aqueous acid—264 (A^1_1=115a), 271 nm (A^1_1=107c); aqueous alkali—259 nm (A^1_1=758a).



Infrared Spectrum Principal peaks at wavenumbers 1620, 1129, 1230, 1537, 1075, 1176 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 92, 65, 108, 214, 156, 39, 109, 43.



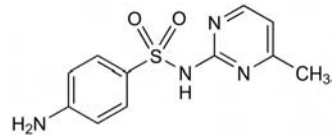
Quantification
Plasma **HPLC** UV detection. Limit of detection, 10 µg/L [Suber, Edds 1980].
Urine **HPLC** Sulfaguanidine and other sulfonamides. Limit of detection, 0.1 to 0.3 mg/L [Simo-Alfonso *et al.* 1995].
Disposition in the Body Absorption is variable after oral administration. It is rapidly excreted in the urine, about 30% of the excreted material being in the form of the inactive N^4-acetyl derivative. Large amounts are also eliminated in the faeces.
Therapeutic Concentration In plasma, usually in the range 15 to 40 mg/L.
Protein Binding About 8%.
Dose Sulfaguanidine has been given in doses of 9 g daily for 3 days.

Simo-Alfonso EF *et al.* (1995). Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography. *J Chromatogr B Biomed Appl* 670: 183–187.
Suber RL, Edds GT (1980). *J Liq Chromatogr* 3: 257–268.

Sulfamerazine

Antibacterial, Sulfonamide
$C_{11}H_{12}N_4O_2S = 264.3$
CAS—127-79-7

IUPAC Name 4-Amino-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide
Synonyms Solfamerazina; sulfamethyldiazine; sulfamethylpyrimidine; sulfamerazine.
Proprietary Names It is an ingredient of *Berlocombin*, *Sulfatril* and *Trisulfaminic*.

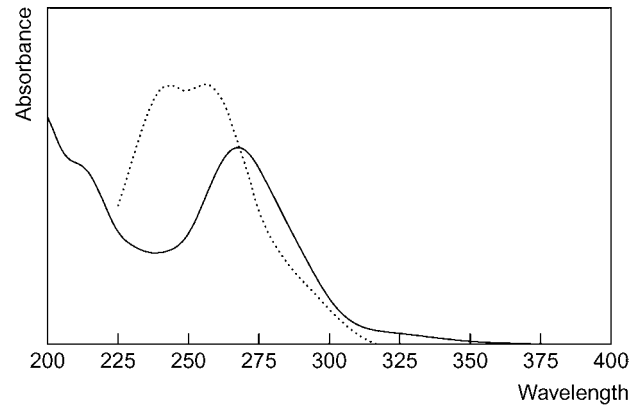


Chemical Properties A white or faintly yellowish-white crystalline powder which slowly darkens on exposure to light. Mp 234° to 238°, with decomposition. Soluble 1 in 6250 of water, 1 in 550 of ethanol and 1 in 60 of acetone; very slightly soluble in ether and chloroform; soluble in dilute mineral acids and in solutions of alkali hydroxides and carbonates. pK_a 7.1 (20°). Log *P* (octanol/pH 7.5), −0.1.

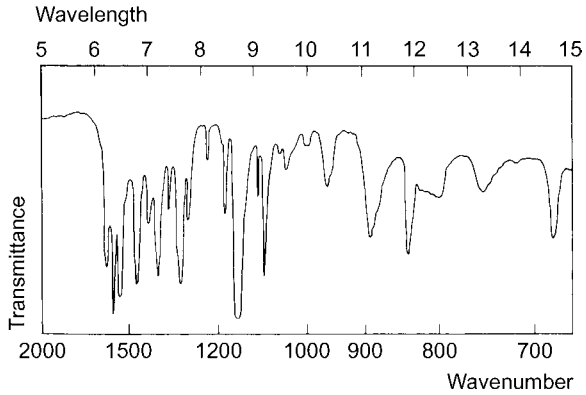
Sulfamerazine Sodium

$C_{11}H_{11}N_4NaO_2S = 286.3$
CAS—127-58-2
Synonyms Soluble sulfamerazine; sulfamerazine sodium.
Chemical Properties A white or yellowish-white powder. It slowly darkens on exposure to light; on exposure to moist air it absorbs carbon dioxide and becomes less soluble in water. Soluble 1 in 3.6 of water; slightly soluble in ethanol; insoluble in chloroform and ether.

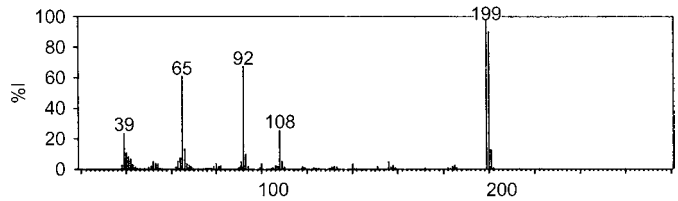
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green→brown; Koppanyi–Zwikker test—pink; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.23; system TE—R_f 0.08; system TF—R_f 0.41; system TT—R_f 0.33; system TU—R_f 0.18; system TV—R_f 0.07; system TAD—R_f 0.42; system TAE—R_f 0.80.
Gas Chromatography System GA—sulfamerazine RI 2566, sulfamerazine-Me RI 2625; System GJ—sulfamerazine-Me RRT 0.69 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 8.1; system HY—RI 247.
Ultraviolet Spectrum Aqueous acid—242 (A^1_1=596a), 304 nm; aqueous alkali—242 (A^1_1=820b), 255 nm (A^1_1=828b).



Infrared Spectrum Principal peaks at wavenumbers 1149, 1590, 1560, 1316, 1088, 1618 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 199, 200, 92, 65, 108, 39, 66, 201.



Quantification

Plasma **HPLC** UV detection. Sulfamerazine and acetylsulfamerazine. Limit of detection, 300 µg/L [Vree *et al.* 1983].

Urine **HPLC** [Vree *et al.* 1983].

Disposition in the Body Rapidly absorbed after oral administration. It penetrates into the CSF to produce concentrations about one-half of those in blood. Metabolised by N^4-acetylation, the extent of which is genetically determined; in slow acetylators about 15% of the sulfamerazine in the blood is in the form of the acetyl derivative compared with 35% in rapid acetylators. About 60% of a dose is excreted in the urine in 48 h; approximately half of the excreted material is in the form of the acetyl derivative.

Toxicity Serious toxic effects, sometimes fatal, have occurred in young children after therapeutic doses.

Half-life Plasma half-life, 9 to 14 h in rapid acetylators and about 24 h in slow acetylators.

Volume of Distribution About 0.4 L/kg.

Protein Binding About 80 to 90%.

Dose 4 g daily.

Vree TB *et al.* (1983). Pharmacokinetics, acetylation-deacetylation, renal clearance, and protein binding of sulphamerazine, N4-acetylsulphamerazine, and N4-trideuteroacetylsulphamerazine in 'fast' and 'slow' acetylators. *Biopharm Drug Dispos* 4: 271–291.

Sulfamethizole

Antibacterial, Sulfonamide

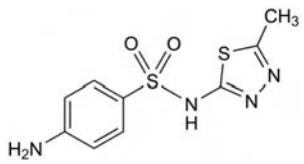
$C_9H_{10}N_4O_2S_2 = 270.3$

CAS—144-82-1

IUPAC Name 4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide

Synonym Sulfamethylthiadiazole

Proprietary Names *Famet*; *Luco-Oph*; *Lucosil*; *Methazol*; *Renisul*; *Rufol*; *Salimol*; *Sulfapyelon*; *Thidicur*; *Thiosulfil*; *Urolex*; *Urolucosil*.



Chemical Properties Colourless crystals or white or creamy-white crystalline powder. Mp 208°. Soluble 1 in 2000 of water, 1 in 30 of ethanol, 1 in 40 methanol, 1 in 10 to 1 in 13 of acetone, 1 in 1370 of ether and 1 in 2800 of chloroform; soluble in solutions of alkali hydroxides and in dilute mineral acids. pK_a 5.5 (25°). Log *P* (octanol/pH 7.5), −1.1; (octanol/water), 0.5.

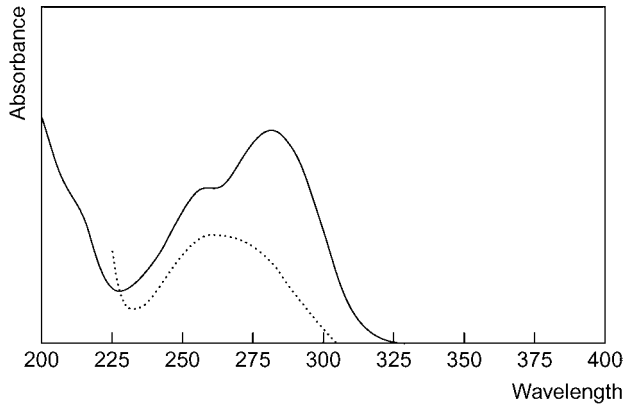
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—red-violet; mercurous nitrate—black; nitrous acid—yellow.

Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.12; system TE—R_f 0.04; system TF—R_f 0.23; system TT—R_f 0.46; system TU—R_f 0.36; system TV—R_f 0.02; system TAD—R_f 0.27; system TAE—R_f 0.83; system TAJ—R_f 0.34; system TAK—R_f 0.14; system TAL—R_f 0.75 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).

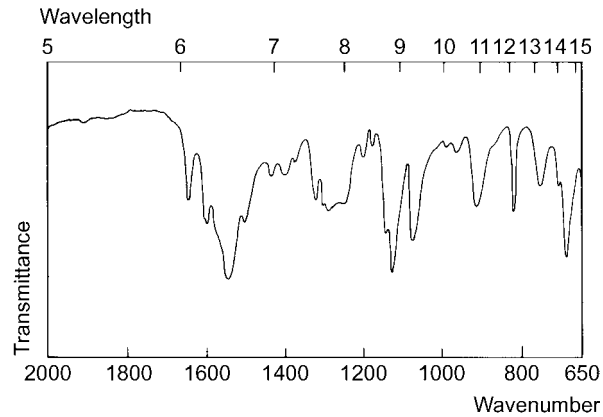
Gas Chromatography System GA—sulfamethizole-Me RI 2660; system GJ—methyl derivative RRT 0.98 (relative to griseofulvin).

High Performance Liquid Chromatography System HZ—retention time 2.3 min.

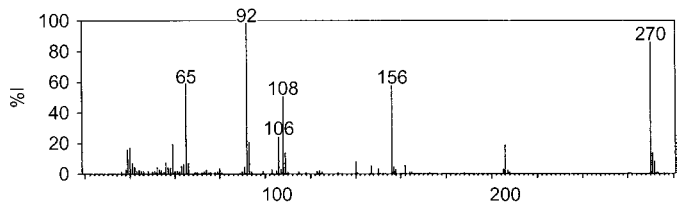
Ultraviolet Spectrum Aqueous acid—268 nm (A_1^1=542a); aqueous alkali—260 nm.



Infrared Spectrum Principal peaks at wavenumbers 1549, 1134, 699, 1084, 1152, 1600 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 92, 270, 65, 156, 108, 106, 93, 59.



Quantification

Urine **HPLC** Sulfamethizole and other sulfonamides. Limit of detection, 0.1 to 0.3 mg/L [Simo-Alfonso *et al.* 1995].

Note The methods referred to under sulfamethoxazole may also be used.

Disposition in the Body Readily absorbed after oral administration and only slightly acetylated in the body. It is rapidly excreted in the urine, up to 90% of a dose being excreted within 24 h, mainly as unchanged drug.

Therapeutic Concentration

Following oral administration of 0.5 g of sulfamethizole to 5 subjects, a mean peak blood concentration of 20 mg/L was attained in about 2 h [Mattok, McGilveray 1972].

Half-life Plasma half-life, 1 to 2 h.

Volume of Distribution About 0.3 L/kg.

Clearance Blood clearance, about 3 mL/min/kg.

Protein Binding About 90%.

Dose 1 to 4 g daily.

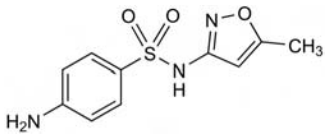
Mattok GL, McGilveray IJ (1972). Comparison of bioavailabilities and dissolution characteristics of commercial tablet formulations of sulfamethizole. *J Pharm Sci* 61: 746–749.

Simo-Alfonso EF *et al.* (1995). Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography. *J Chromatogr B Biomed Appl* 670: 183–187.

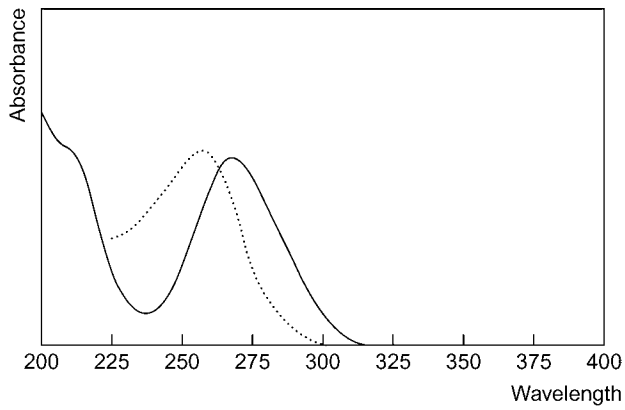
Sulfamethoxazole

Sulfonamide
$C_{10}H_{11}N_3O_3S = 253.3$
CAS—723-46-6

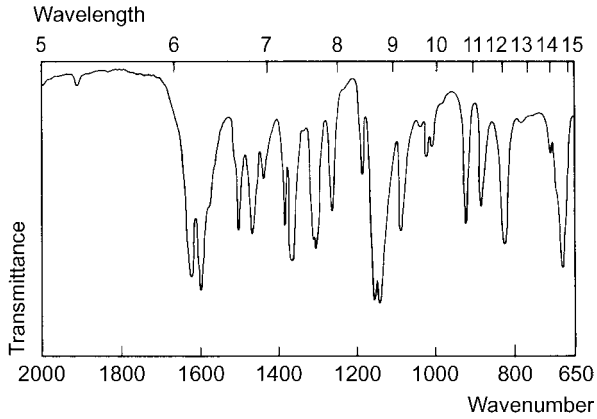
IUPAC Name 4-Amino-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide
Synonyms 4-Amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; Ro-4-2130; sulfamethoxazole; sulfisomezole.
Proprietary Names *Gantanol*; *Urobak*. It is an ingredient of many proprietary preparations, see Sweetman [2002].



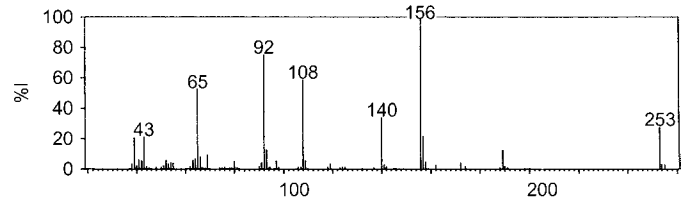
Chemical Properties A white or yellowish-white crystalline powder. Mp 167° (crystals from dilute ethanol). Very slightly soluble in water; soluble 1 in 50 of ethanol and 1 in 3 of acetone; practically insoluble in chloroform and ether; soluble in solutions of alkali hydroxides. pK_a 5.6 (25°). Log *P* (octanol/water), 0.9.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—blue-violet; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.26; system TE—R_f 0.05; system TF—R_f 0.54; system TT—R_f 0.88; system TU—R_f 0.33; system TV—R_f 0.02; system TAD—R_f 0.41; system TAE—R_f 0.79; system TAJ—R_f 0.45; system TAK—R_f 0.26; system TAL—R_f 0.81 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—sulfamethoxazole-Me RI 2500, sulfamethoxazole-Me_2 RI 2460, M (N^4-acetyl-)-Me RI 3255; system GJ—sulfamethoxazole-Me RRT 0.40, M (N^4-acetyl-)-MeRRT 0.91 (both relative to griseofulvin).
High Performance Liquid Chromatography system HU—sulfamethoxazole *k* 4.8, (N^4-acetyl-) *k* 4.9; system HY—RI 320; system HZ—retention time 2.9 min; system HAA—retention time 13.4 min; system HAM—retention time 2.7 min.
Ultraviolet Spectrum Aqueous acid—265 nm (A_1^1=175a); aqueous alkali—256 nm (A_1^1=673a).



Infrared Spectrum Principal peaks at wavenumbers 1145, 1160, 1599, 1621, 685, 1306 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 156, 92, 108, 65, 140, 253, 157, 43.



Quantification
Blood **HPLC** Sulfamethoxazole and trimethoprim. Limit of quantification, 1 mg/L for sulfamethoxazole and 0.1 mg/L for trimethoprim [Ronn *et al.* 1999].
Plasma **HPLC** UV detection. Sulfamethoxazole and its metabolites. Limit of quantification for sulfamethoxazole, 0.1 mg/L [Vree *et al.* 1994].
Serum **HPLC** UV detection. Limit of detection, 0.5 mg/L [Vree *et al.* 1978].
Urine **HPLC** See Plasma [Vree *et al.* 1994]. See Serum [Vree *et al.* 1978].
Disposition in the Body Readily absorbed after oral administration and widely distributed; it is found in saliva, sweat, bile, CSF, peritoneal, ocular and synovial fluids; it is found in pleural and other effusions. It crosses the placenta and is excreted in breast milk. It is metabolised mainly by acetylation with the formation of the N^1-acetyl and N^4-acetyl derivatives; about 15% of the sulfamethoxazole in the blood is present as acetylated metabolites. It is excreted in the urine mostly as the N^4-acetyl derivative and unchanged sulfamethoxazole together with some glucuronide conjugates. Urinary excretion is variable and is dependent on the pH of the urine, the proportion of unchanged drug excreted being increased when the urine is alkaline. Up to about 25% of the dose is excreted unchanged when the urine is acid, rising to 40% or more in alkaline urine. The amount of the N^4-acetyl derivative excreted may be 30 to 70% of the dose. Sulfamethoxazole is oxidised to form a hydroxylamine which may be involved in adverse reactions to sulfonamides.

Sulfamethoxazole is frequently administered together with trimethoprim, but this does not affect its metabolism.

Therapeutic Concentration

Seven healthy males (aged 22 to 32 years; mean, 28.2) were administered with a mean trimethoprim dose of 20.2 (range, 19.3 to 20.9) mg/kg daily and sulfamethoxazole 101.1 (96.3 to 104.7) mg/kg daily for 3 days, as even doses administered every 6 h. Volunteers were fasted for 1 h before dosing and 2 h after administration. The mean peak serum concentrations observed were 13.6 (range, 10.6 to 16.1) mg/L trimethoprim, 372 (277 to 469) mg/L sulfamethoxazole and 50.1 (39.5 to 70.3) mg/L acetylsulfamethoxale. These concentrations were observed at 1.9 (0.3 to 2.7), 2.6 (0.7 to 3.5) and 2.8 (0.3 to 6.0) h, respectively [Stevens *et al.* 1991].

Administration of a single oral dose of sulfamethoxazole and trimethoprim (800 mg and 160 mg, respectively) and metronidazole (2 g) to 24 patients, 1.5 to 10.5 h before colorectal surgery, produced sulfamethoxazole serum concentrations of 15 to 65 mg/L (median 35) at the start of the operation and 13 to 70 mg/L (median 33) at the end. Corresponding concentrations of trimethoprim were 0.7 to 2.6 mg/L (median 1.4) and 1.0 to 2.8 mg/L (median 1.3) at the start and end of surgery, respectively [Raab *et al.* 2001].

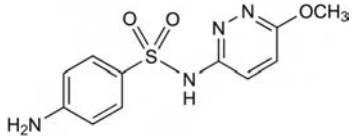
Toxicity Plasma concentrations greater than 400 mg/L may be associated with toxic effects.
Half-life Plasma half-life, 9 to 12 h.
Volume of Distribution About 0.25 L/kg.
Clearance Plasma clearance, about 0.3 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, about 1.35.
Saliva Plasma : saliva ratio, about 14.
Protein Binding 50 to 70%.
Note For a review of the clinical pharmacokinetics of sulfonamides and their N^4-acetyl derivatives, see Hekster and Vree [1982]; for a review of the clinical pharmacokinetics of sulfamethoxazole and trimethoprim, see Patel and Welling [1980].
Dose Usually 2 g initially, followed by 1 g twice daily; maximum of 3 g daily.

Hekster CA, Vree TB (1982). Clinical pharmacokinetics of sulphonamides and their N4-acetyl derivatives. *Antibiot Chemother* 31: 22–118.
Patel RB, Welling PG (1980). Clinical pharmacokinetics of co-trimoxazole (trimethoprim-sulphamethoxazole). *Clin Pharmacokinet* 5: 405–423.
Raab Y *et al.* (2001). Trimethoprim-sulphamethoxazole and metronidazole as prophylaxis in colorectal surgery: a study of bioavailability after an oral single dose. *Eur J Surg* 167: 46–49.
Ronn AM *et al.* (1999). A reversed-phase high-performance liquid chromatography method for the determination of cotrimoxazole (trimethoprim/ sulphamethoxazole) in children treated for malaria. *Ther Drug Monit* 21: 609–614.
Stevens RC *et al.* (1991). Pharmacokinetics and adverse effects of 20-mg/kg/day trimethoprim and 100-mg/kg/day sulfamethoxazole in healthy adult subjects. *Antimicrob Agents Chemother* 35: 1884–1890.
Sweetman SC. *Martindale: The complete drug reference*, 33 edn. London: Pharmaceutical Press, 2002.
Vree TB *et al.* (1978). Determination of trimethoprim and sulfamethoxazole (co-trimoxazole) in body fluids of man by means of high-performance liquid chromatography. *J Chromatogr* 146: 103–112.
Vree TB *et al.* (1994). Isolation, identification and determination of sulfamethoxazole and its known metabolites in human plasma and urine by high-performance liquid chromatography. *J Chromatogr Biomed Appl* 658: 327–340.

Sulfamethoxypyridazine

Antibacterial, Sulfonamide
$C_{11}H_{12}N_4O_3S = 280.3$
CAS—80-35-3

IUPAC Name 4-Amino-*N*-(6-methoxypyridazin-3-yl)benzenesulfonamide
Synonyms CL-13494; RP-7522.
Proprietary Names *Dibasul*; *Exasul*; *Kynex*; *Lederkyn*; *Midicel*; *Midikel*; *Sulfalex*; *Sulfdurazin*; *Sultirene*.



Chemical Properties A white or yellowish-white crystalline powder which slowly darkens on exposure to light. Mp 182° to 183°. Very slightly soluble in water; soluble 1 in 200 of ethanol, 1 in 50 of acetone and 1 in 400 of chloroform; slightly soluble in methanol; practically insoluble in ether; freely soluble in dilute mineral acids and solutions of alkali hydroxides. pK_a 7.2 (20°). Log *P* (octanol/water), 0.3.

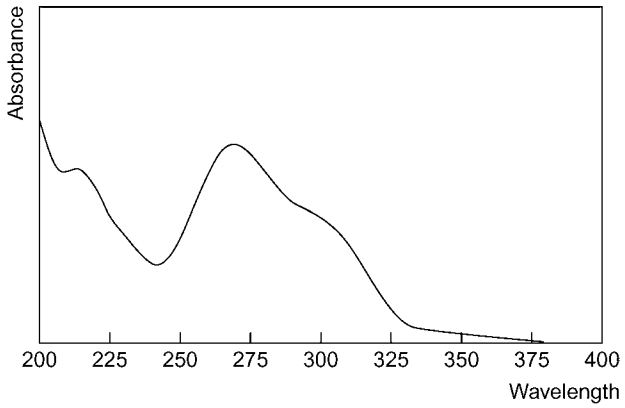
Sulfamethoxypyridazine Sodium

$C_{11}H_{12}N_4NaO_3S = 303.3$
CAS—2577-32-4
Proprietary Names *Davosin*, *Sulfoxine LA*.

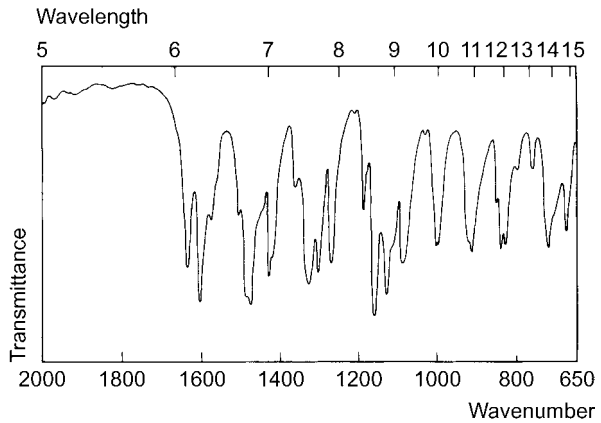
Acetyl Sulfamethoxypyridazine

$C_{13}H_{14}N_4O_4S = 322.3$
CAS—3568-43-2
Note Acetyl sulfamethoxypyridazine [*N*-(6-methoxypyridazin-3-yl)-*N*-sulfanylacetamide] should be distinguished from the N^4-acetyl derivative formed from sulfamethoxypyridazine by acetylation in the body.
Chemical Properties Crystals. Mp 186° to 187°, with decomposition. Very soluble in water.

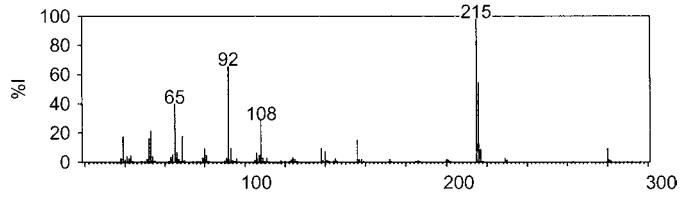
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green-brown; Koppanyi–Zwikker test—red-violet; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.65; system TD—R_f 0.19; system TE—R_f 0.09; system TF—R_f 0.39; system TT—R_f 0.53; system TU—R_f 0.26; system TV—R_f 0.50; system TAD—R_f 0.43; system TAE—R_f 0.83; system TAJ—R_f 0.46; system TAK—R_f 0.20; system TAL—R_f 0.84.
Gas Chromatography System GJ—methyl derivative RRT 0.93 (relative to griseofulvin).
High Performance Liquid Chromatography System HAA—retention time 11.2 min; system HU—*k* 7.5; system HX—RI 335.
Ultraviolet Spectrum Aqueous acid—316 nm (A^1_1=163b); aqueous alkali—250 nm (A^1_1= 712a).



Infrared Spectrum Principal peaks at wavenumbers 1159, 1599, 1130, 1305, 1630, 1088 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 215, 92, 216, 65, 108, 53, 69, 39.



Quantification

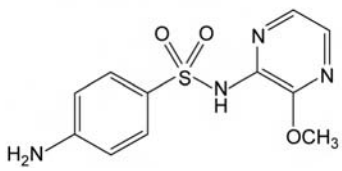
HPLC The methods referred to under sulfamethoxazole may be used.
Disposition in the Body Sulfamethoxypyridazine is a long-acting sulfonamide which is readily absorbed after oral administration and may be detected in blood for up to 7 days after discontinuation of treatment. It is acetylated in the body and 10–15% of the sulfamethoxypyridazine in the blood is present as the inactive N^4-acetyl derivative. About 45% of a dose is excreted in the urine in 48 h, with 40–70% of the excreted material as the acetyl derivative, ≈20% as unchanged drug and ≈13% as glucuronide conjugates. Excretion is influenced by the pH of the urine, the rate being increased when the urine is alkaline.
Therapeutic Concentration
Blood concentrations of 110–180 mg/L were attained in 5 h following an oral dose of 4 g [Seneca 1966].
Half-life Plasma half-life, 1–3 days.
Volume of Distribution About 0.2 L/kg.
Distribution in Blood Small amounts are taken up by the erythrocytes.
Protein Binding 80–90%.
Dose An initial dose of 1 to 2 g, followed by 500 mg daily.

Seneca H (1966). Long-acting sulfonamides in urinary tract infections. *JAMA* 198: 975–980.

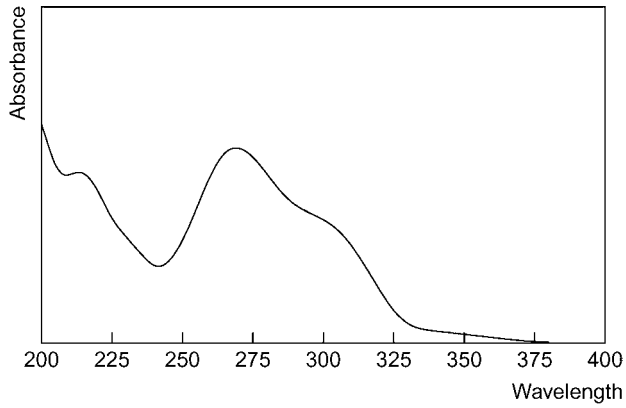
Sulfametopyrazine

Antibacterial, Sulfonamide
$C_{11}H_{12}N_4O_3S = 280.3$
CAS—152-47-6

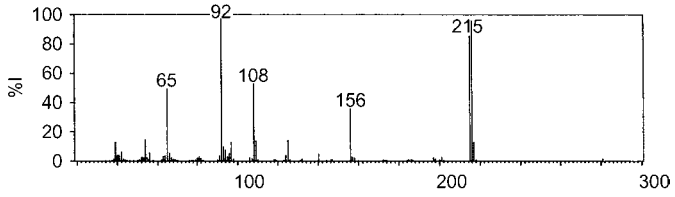
IUPAC Name 4-Amino-*N*-(3-methoxypyrazin-2-yl)benzenesulfonamide
Synonyms Solfametopirazina; solfametossipirazina; sulfalene; sulfamethoxypyrazine; sulfapyrazin methoxyne; sulfalene.
Proprietary Names *Dalysep*; *Kelfizina*; *Kelfizine W*; *Longum*; *Policydal*; *Vetkelfizina*.



Chemical Properties A white or yellowish-white crystalline powder. Mp 176°. Practically insoluble in water; slightly soluble in ethanol and chloroform; freely soluble in acetone and in dilute mineral acids and solutions of alkali hydroxides. pK_a 7.0. Log *P* (octanol/water), 0.7.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.67; system TD—R_f 0.40; system TE—R_f 0.08; system TF—R_f 0.50; system TAD—R_f 0.54 (Van Urk reagent, positive).
Gas Chromatography System GJ—sulfametopyrazine-Me RRT 0.69 (relative to griseofulvin).
High Performance Liquid Chromatography System HX—RI 353.



Infrared Spectrum Principal peaks at wavenumbers 1145, 1315, 1162, 1601, 1642, 1085 cm^{-1} (KBr disk).



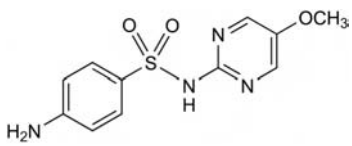
Quantification
Blood **HPLC** UV detection. Limit of detection, 50 µg/L [Dua *et al.* 1991; 1998].
Plasma **HPLC** See Blood [Dua *et al.* 1991; 1998].
Disposition in the Body Sulfametopyrazine is a long-acting sulfonamide and is readily absorbed after oral administration. It is acetylated in the body, 5 to 10% of the sulfametopyrazine in the blood being in the form of the N^4-acetyl derivative which is inactive. It is slowly excreted in the urine, about 70% of the excreted material being the acetyl derivative and about 14 to 20% being unchanged drug.
Therapeutic Concentration
After a single oral dose of 500 mg to 3 subjects, peak plasma concentrations of 25 to 33 mg/L (mean, 29) were attained in 2 to 4 h [Herting *et al.* 1964].
Maximum steady-state plasma concentrations of 51 to 69 mg/L (mean, 58) were reported on the seventh day after 6 subjects had received an initial dose of 600 mg followed by 200 mg daily [Reeves *et al.* 1980].
Half-life Plasma half-life, 2 to 4 days.
Volume of Distribution About 0.2 to 0.3 L/kg.
Protein Binding 65 to 80%.
Dose 2 g once a week.

Dua VK *et al.* (1991). Determination of sulfalene in plasma, red blood cells and whole blood by high-performance liquid chromatography. *J Chromatogr* 563: 333–340.
Dua VK *et al.* (1998). Sulfalene concentrations in plasma and blood cells of Plasmodium falciparum malaria cases after treatment with metakelfin using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 714: 390–394.
Herting RL *et al.* (1964). Clinical pharmacology of 2-sulfanilamido-3-methoxypyrazine. *Antimicrob Agents Chemother* 10: 554–561.
Reeves DS *et al.* (1980). Pharmacokinetic study of a sulfametopyrazine/trimethoprim combination (Kelfiprim) in human volunteers. *J Antimicrob Chemother* 6: 647–656.

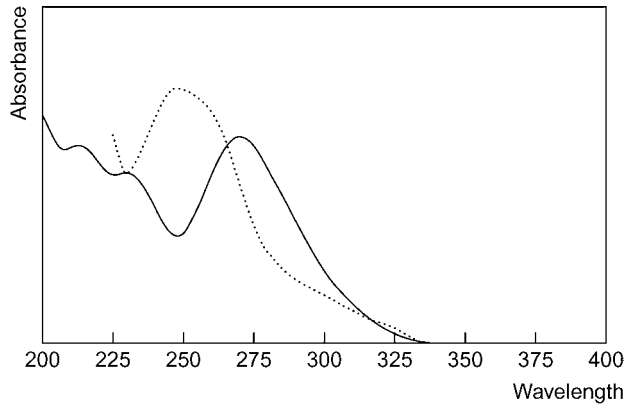
Sulfametoxydiazine

Antibacterial, Sulfonamide
$C_{11}H_{12}N_4O_3S = 280.3$
CAS—651-06-9

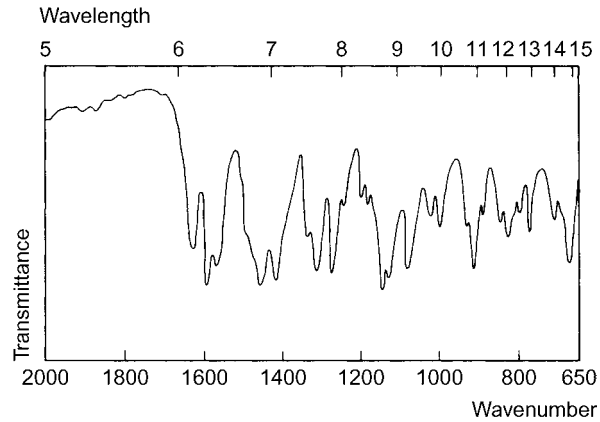
IUPAC Name 4-Amino-*N*-(5-methoxypyrimidin-2-yl)benzenesulfonamide
Synonyms Sulfameter; sulfametin; sulfametorinum; sulfamethoxydiazine; sulfamethoxydin.
Proprietary Names *Bayrena*; *Durenat*; *Kinecid*; *Kirocid*; *Sulla*; *Ultrax*.



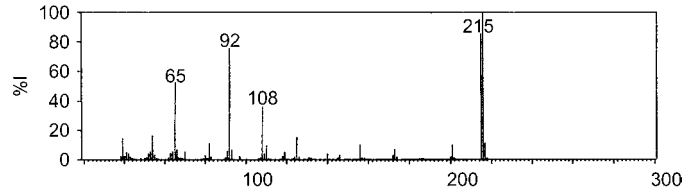
Chemical Properties A white or yellowish-white crystalline powder. Mp 214° to 216°. Very sparingly soluble in water, ethanol and ether; sparingly soluble in acetone; freely soluble in aqueous solutions of alkali hydroxides and carbonates. pK_a 7.0. Log *P* (octanol/water), 0.4.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—violet-brown; Koppanyi–Zwikker test—pink; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.60; system TD—R_f 0.24; system TE—R_f 0.12; system TF—R_f 0.43; system TT—R_f 0.55; system TU—R_f 0.17; system TV—R_f 0.15; system TAD—R_f 0.47; system TAE—R_f 0.82.
Gas Chromatography System GA—sulfamethoxydiazine-Me_3 RI 2925, M (acetyl-)-Me RI 3620; System GJ—methyl derivative RRT 1.38 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 8.2.
Ultraviolet Spectrum Aqueous alkali—245 nm (A_1^1=920a).



Infrared Spectrum Principal peaks at wavenumbers 1153, 1597, 1140, 1284, 1090, 925 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 216, 215, 92, 65, 108, 54, 125, 39.



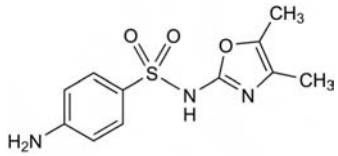
Quantification
Plasma **HPLC** Electrochemical detection. Sulfametoxydiazine and other sulfonamides [Klimes, Mokry 1997].
Disposition in the Body Sulfametoxydiazine is a long-acting sulfonamide which is readily absorbed after oral administration. It is acetylated in the body, about 10% being present in the blood as the inactive N^4-acetyl derivative. It is excreted slowly in the urine, 20 to 40% of a dose being excreted in 24 h and about 70% in 3 days. About 50% of the excreted material is unchanged drug, about 25% is the acetyl derivative, and about 20% is a glucuronide conjugate.
Therapeutic Concentration
After a single oral dose of 1 g to 4 subjects, peak serum concentrations of 62 to 90 mg/L (mean, 80) were attained in about 6 to 8 h; following a single oral dose of 2 g to a further 4 subjects, peak serum concentrations of 113 to 148 mg/L (mean, 134) were reported at 6 to 8 h; mean peak serum concentrations of 86 mg/L and 166 mg/L respectively were reported following daily oral doses of 0.5 and 1 g for 3 days [Chew *et al.* 1965].
Half-life Plasma half-life, 29 to 59 h (mean, 37).
Volume of Distribution About 0.3 L/kg.
Protein Binding About 80%.
Dose An initial dose of 1 or 1.5 g, followed by 500 mg daily.

Chew WH *et al.* (1965). Studies of sulfamethoxydiazine. I. Absorption and excretion. *Clin Pharmacol Ther* 6: 307–315.
Klimes J, Mokry M (1997). High performance liquid chromatographic analysis of selected sulfonamides in plasma. *Pharmazie* 52: 448–450.

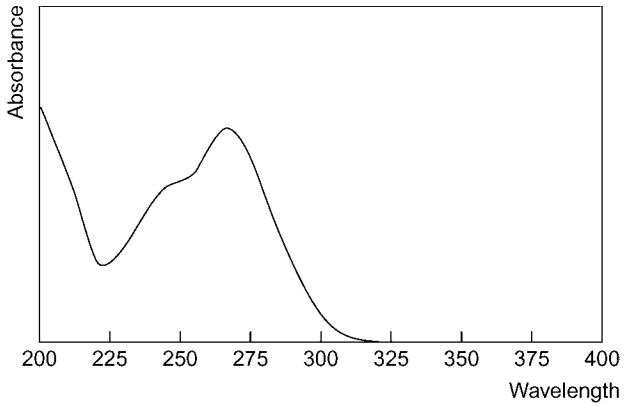
Sulfamoxole

Antibacterial, Sulfonamide
$C_{11}H_{13}N_3O_3S = 267.3$
CAS—729-99-7

IUPAC Name 4-Amino-*N*-(4,5-dimethyl-1,3-oxazol-2-yl)benzenesulfonamide
Synonyms 4-Amino-*N*-(4,5-dimethyl-2-oxazolyl)benzenesulfonamide; SDMO; sulphadimethyloxazole; sulphamoxole.
Proprietary Names *Justamil*; *Sulfmidil*; *Sulfuno*; *Tardamide*. It is an ingredient of *Co-Fram* and *Supristol*.



Chemical Properties A white crystalline powder. Mp about 193°. Slightly soluble in water. pK_a 7.4. Log *P* (chloroform/pH 7.4), −0.3; (octanol/water), 1.0.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green-brown; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.67 (Van Urk reagent, positive).
Gas Chromatography System GA—not eluted; system GJ—methyl derivative RRT 0.40 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 12.6.
Ultraviolet Spectrum Aqueous acid—249 nm (A^1_1=375b); aqueous alkali—250 nm (A^1_1=685b).



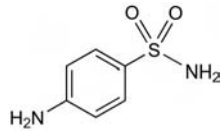
Infrared Spectrum Principal peaks at wavenumbers 1605, 1626, 1127, 1145, 1276, 1094 cm^{-1} (KBr disk).
Quantification
***Plasma* HPLC** Electrochemical detection. Sulfamoxole and other sulfonamides [Klimes, Mokry 1997].
Disposition in the Body Rapidly absorbed after oral administration. About 65 to 71% of a dose is excreted in the urine, mainly as unchanged drug, the N^4-acetyl conjugate, and sulfanilamide.
Therapeutic Concentration
Following single oral doses of 1, 2 and 4 g to 4 subjects, peak serum concentrations of 73.5 to 94.8 (mean, 88), 138 to 182 (mean, 157), and 215 to 290 mg/L (mean, 241), respectively, were attained in about 2 h [vom Bruck *et al.* 1960].
Following oral administration of 400 mg twice a day to 10 subjects (in combination with trimethoprim 80 mg), a mean maximum steady-state plasma concentration of 75 mg/L and a mean trough concentration of 42 mg/L were reported [Watson *et al.* 1982].
Half-life Plasma half-life, 4 to 11 h.
Volume of Distribution About 0.1 L/kg.
Distribution in Blood Plasma : whole blood ratio, about 1.8.
Protein Binding About 25%.
Dose 2 g daily for 1 or 2 days, followed by 1 g daily.

Klimes J, Mokry M (1997). High performance liquid chromatographic analysis of selected sulfonamides in plasma. *Pharmazie* 52: 448–450.
vom Bruck CG *et al.* (1960). *Arzneimittelforschung* 10: 621–626.
Watson ID *et al.* (1982). Comparative pharmacokinetics of co-trifamole and co-trimoxazole to 'steady state' in normal subjects. *Br J Clin Pharmacol* 14: 437–443.

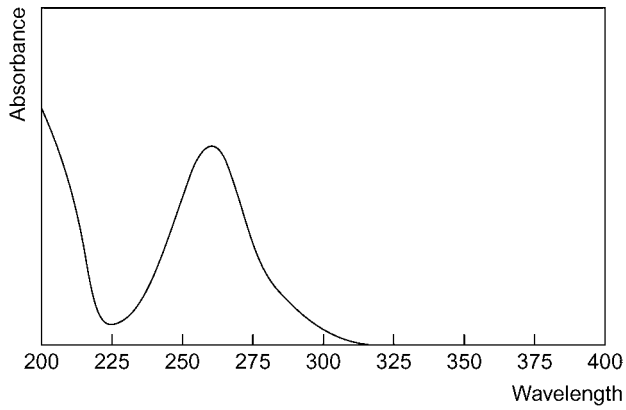
Sulfanilamide

Antibacterial, Sulfonamide
$C_6H_8N_2O_2S$ = 172.2
CAS—63-74-1

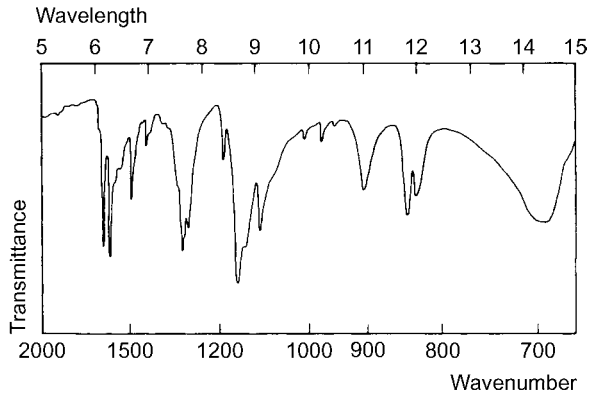
IUPAC Name 4-Aminobenzenesulfonamide
Synonyms 1162-F; solfammide; streptocidum; sulfaminum.
Proprietary Names *Astreptine*; *AVC*; *Azol*; *Prontosil album*; *Prontylin*; *Streptozide*.



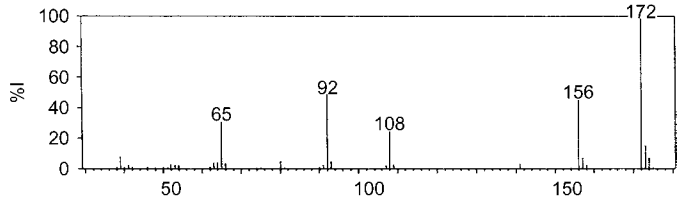
Chemical Properties A white crystalline powder. On heating the dry powder it becomes violet-blue and eventually aniline and ammonia are produced. Mp about 165°. Soluble 1 in 170 of water, 1 in 37 of ethanol and 1 in 5 of acetone; practically insoluble in chloroform and ether; soluble in hydrochloric acid and solutions of alkali hydroxides. pK_a 10.6 (20°). Log *P* (octanol/water), −0.6.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—blue; Koppanyi–Zwikker test—blue-violet; mercurous nitrate—black.
Thin-layer Chromatography System TA—R_f 0.67; system TD—R_f 0.13; system TE—R_f 0.51; system TF—R_f 0.46; system TT—R_f 0.61; system TU—R_f 0.96; system TV—R_f 0.66; system TAD—R_f 0.22; system TAE—R_f 0.83; system TAJ—R_f 0.22; system TAK—R_f 0.05; system TAL—R_f 0.50 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—sulfanilamide RI 2185; sulfanilamide-Me RI 2135; sulfanilamide-Me_4 RI 2095; M (acetyl-) RI 2690; M (acetyl-)-Me RI 2600.
High Performance Liquid Chromatography System HU—sulfanilamide *k* 8.9, N^4-acetylsulfanilamide *k* 9.6; system HY—RI 86.
Ultraviolet Spectrum Aqueous acid—262 (A^1_1=106a), 269 nm (A^1_1=85a); aqueous alkali—250 nm (A^1_1=932b).



Infrared Spectrum Principal peaks at wavenumbers 1149, 1603, 1316, 1637, 1099, 1294 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 172, 92, 156, 65, 108, 173, 39, 174.

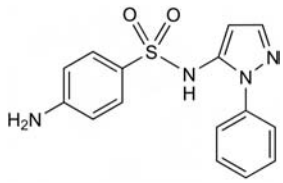


Dose Sulfanilamide has been given in an initial dose of 3 g, followed by 1 to 1.5 g every 4 h.

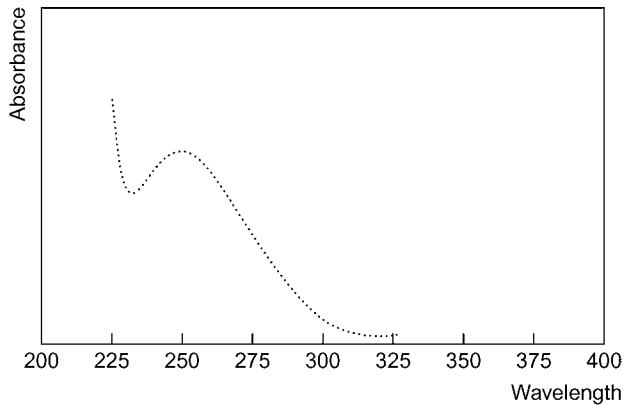
Sulfaphenazole

Antibacterial, Sulfonamide
$C_{15}H_{14}N_4O_2S = 314.4$
CAS—526-08-9

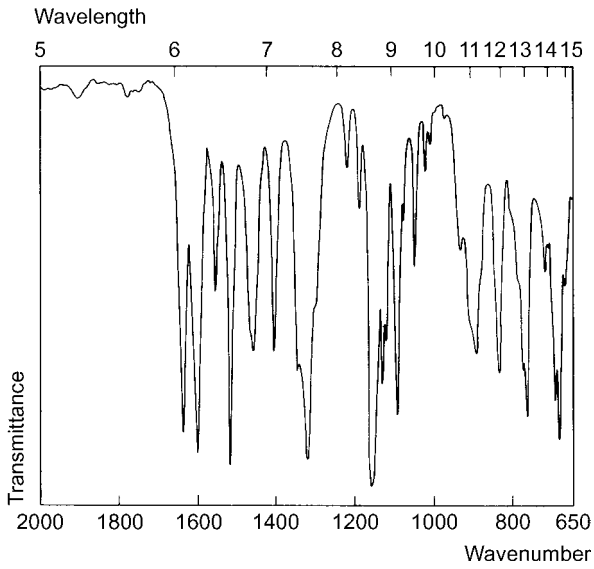
IUPAC Name 4-Amino-*N*-(2-phenylpyrazol-3-yl)benzenesulfonamide
Synonyms 4-Amino-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide; sulphaphenazole; sulphaphenylpyrazol.
Proprietary Names *Isarol V*; *Orisul*; *Orisulf*.



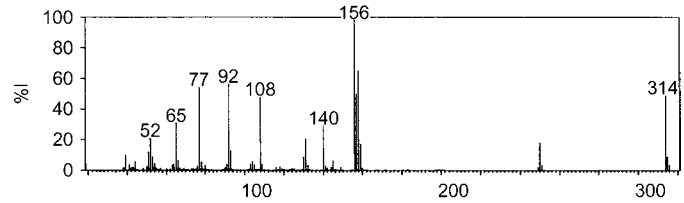
Chemical Properties A white crystalline powder. When heated, the powder becomes brown; when further heated, it produces yellow fumes and an odour of sulfur dioxide. Mp 179° to 183°. Very slightly soluble in water, ethanol, chloroform and ether; soluble in acetone, mineral acids and solutions of alkali hydroxides. pK_a 6.5. Log *P* (octanol/water), 1.5.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—blue; Koppanyi–Zwikker test—blue-violet; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.69; system TD—R_f 0.29; system TE—R_f 0.09; system TF—R_f 0.51; system TT—R_f 0.89; system TU—R_f 0.70; system TV—R_f 0.13; system TAD—R_f 0.43; system TAE—R_f 0.89.
Gas Chromatography System GJ—methyl derivative RRT 1.71 (relative to griseofulvin).
Ultraviolet Spectrum Aqueous alkali—249 nm (A_1^1=700b).



Infrared Spectrum Principal peaks at wavenumbers 1148, 1507, 1313, 1592, 675, 1630 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 156, 158, 92, 77, 157, 314, 108, 65.



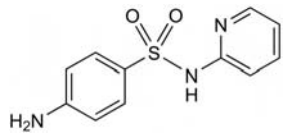
Quantification
Urine **HPLC** Sulfaphenazole–N^2-glucuronide [Vree *et al.* 1990].
Note The methods referred to under sulfamethoxazole may also be used.
Disposition in the Body Readily absorbed after oral administration. It is acetylated in the body and slowly excreted in the urine, about 70 to 80% of a dose being excreted in 72 h, 30% as the acetyl derivative.
Half-life Plasma half-life, 8 to 12 h.
Protein Binding About 90 to 99%.
Dose 2 to 3 g daily for 2 days, followed by 1 g daily.

Vree TB *et al.* (1990). High pressure liquid chromatographic analysis and preliminary pharmacokinetics of sulfaphenazole and its N2-glucuronide and N4-acetyl metabolites in plasma and urine of man. *Pharm Weekbl (Sci)* 12: 243–246.

Sulfapyridine

Antibacterial, Sulfonamide
$C_{11}H_{11}N_3O_2S = 249.3$
CAS—144-83-2

IUPAC Name 4-Amino-*N*-pyridin-2-ylbenzenesulfonamide
Synonym Sulphapyridine
Proprietary Names *Coccoclase*; *Dagenan*; *Eubasin*; *M & B 693*; *Pyriamid*; *Septipulmon*. It is an ingredient of *Trinamide* (vet.).

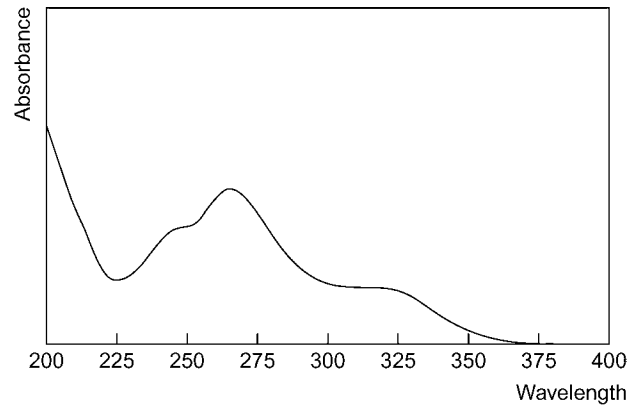


Chemical Properties A white or yellowish-white crystalline powder or granules. It slowly darkens on exposure to light. Mp 190° to 192°. Soluble 1 in 3500 of water, 1 in about 440 of ethanol and 1 in 65 of acetone; practically insoluble in chloroform and ether; soluble in dilute mineral acids and aqueous solutions of alkali hydroxides. pK_a 8.4 (25°). Log *P* (octanol/water), 0.4.

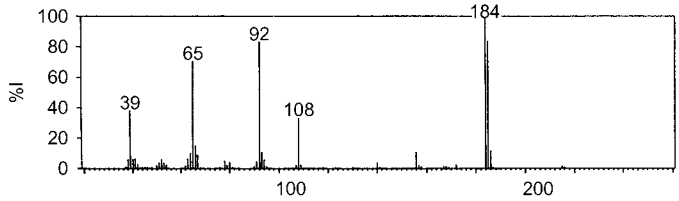
Sulfapyridine Sodium Monohydrate

$C_{11}H_{10}N_3NaO_2S,H_2O = 289.3$
CAS—127-57-1 (anhydrous); 6101-41-3 (monohydrate)
Synonym Soluble sulfapyridine
Proprietary Names *Izopiridina*; *Soludagenan*.
Chemical Properties A white or yellowish-white crystalline powder. On exposure to air, it slowly absorbs carbon dioxide with the liberation of sulfapyridine and becomes incompletely soluble in water. Soluble 1 in 1.5 of water and 1 in 10 of ethanol.

Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green→brown-green; Koppanyi–Zwikker test—pink; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.67; system TD—R_f 0.16; system TE—R_f 0.24; system TF—R_f 0.42; system TT—R_f 0.47; system TU—R_f 0.43; system TV—R_f 0.73; system TAD—R_f 0.34; system TAJ—R_f 0.50; system TAK—R_f 0.19; system TAL—R_f 0.85.
Gas Chromatography System GA—RI 2600; System GJ—methyl derivative RRT 0.47 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 3.8; system HX—RI 312; system HY—RI 242; system HZ—retention time 2.2 min (sulfapyridine); retention time 1.9 min (N^4-acetylsulfapyridine).
Ultraviolet Spectrum Aqueous acid—240 nm (A_1^1= 392a); aqueous alkali—247 nm.



Infrared Spectrum Principal peaks at wavenumbers 1585, 1264, 1127, 1078, 1639, 950 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 184, 92, 185, 65, 39, 108, 66, 186.



Quantification

***Plasma* Colorimetry** Limit of detection, 1.5 mg/L for sulfapyridine or the acetyl metabolite [Bates, Pieniaszek 1978].

HPLC Comparison with GC. Limits of detection, 50 ng for HPLC (fluorescence detection), 10 ng for GC (electrochemical detection) [Sharp *et al.* 1980]. UV detection. Sulfapyridine and metabolites. Limit of detection, 1 mg/L [Fischer, Klotz 1978].

***Serum* Colorimetry** See Plasma [Bates, Pieniaszek 1978].
***Urine* HPLC** See Plasma [Sharp *et al.* 1980].
***Saliva* Colorimetry** See Plasma [Bates, Pieniaszek 1978].

HPLC See Plasma [Sharp *et al.* 1980].

See also under Sulfasalazine.

Disposition in the Body Irregularly absorbed after oral administration. It is acetylated in the body, ≈30–70% of the sulfapyridine in the blood being present as the inactive N^4-acetyl derivative; the extent of acetylation is genetically determined. The rate of excretion appears to be irregular but ≈25% of a dose may be excreted in the urine as the acetyl derivative and ≈20% as unchanged drug in 60 h. Other metabolites include the 5-hydroxy derivative and its glucuronide conjugate. Sulfapyridine is a metabolite of sulfasalazine.

Therapeutic Concentration

After an oral dose of 2.5 g given to 5 subjects, peak serum concentrations of sulfapyridine plus metabolites of about 35–60 mg/L were attained in 3–5 h [Schröder, Campbell 1972].

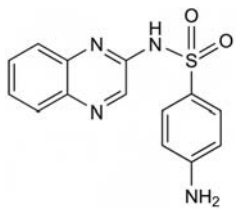
Toxicity Toxic effects are associated with plasma concentrations >50 mg/L.
Half-life Plasma half-life, ≈6 h in rapid acetylators, increased in slow acetylators to ≈13 h.
Volume of Distribution About 0.4 L/kg.
Saliva Plasma : saliva ratio, ≈1.8.
Protein Binding About 40–60%.
Dose Initially 3 or 4 g daily, followed by 0.5 to 1 g daily.

Bates TR, Pieniaszek HJ (1978). *Anal Lett (Part B)* 11: 709–720.
Fischer C, Klotz U (1978). Determination of sulfapyridine and its major metabolites in plasma by high pressure liquid chromatography. *J Chromatogr* 146: 157–162.
Sharp ME *et al.* (1980). *Can J Pharm Sci* 15: 35–38.
Schröder H, Campbell DE (1972). Absorption, metabolism, and excretion of salicylazosulfapyridine in man. *Clin Pharmacol Ther* 13: 539–551.

Sulfaquinoxaline

Coccidiostat (Veterinary)
$C_{14}H_{12}N_4O_2S$ = 300.3
CAS—59-40-5

IUPAC Name 4-Amino-*N*-quinoxalin-2-ylbenzenesulfonamide
Synonyms Solfabenzpyrazine; sulfabenzpyrazine; sulfaquinoxaline.
Proprietary Names *Sulfa-Q*; *Sul-Q-Nox*. It is an ingredient of *Tribrissen (SQX)*.



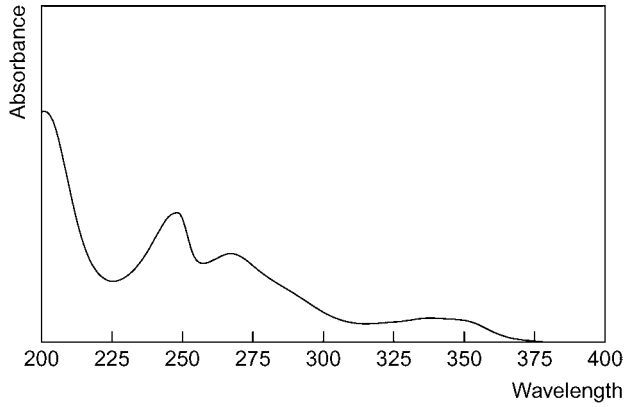
Chemical Properties A yellow powder. Mp 247° to 248°, with decomposition. Practically insoluble in water and ether; very slightly soluble in ethanol and chloroform; soluble in dilute solutions of mineral acids and in solutions of alkalis. pK_a 5.5 (20°). Log *P* (octanol/water), 1.7.

Sulfaquinoxaline Sodium

$C_{14}H_{11}N_4O_2S,Na$ = 322.3
Proprietary Name *Aviochina*.
Chemical Properties Very soluble in water.

Colour Test Copper sulfate (method 1)—green.

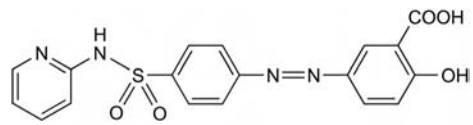
Thin-layer Chromatography System TA—R_f 0.71.
High Performance Liquid Chromatography System HU—*k* 4.8.
Ultraviolet Spectrum Aqueous acid—248 (A^1_1=648b), 263 (A^1_1=540b), 348 nm (A^1_1=263b); aqueous alkali—252 (A^1_1=1085a), 357 nm (A^1_1=265b).



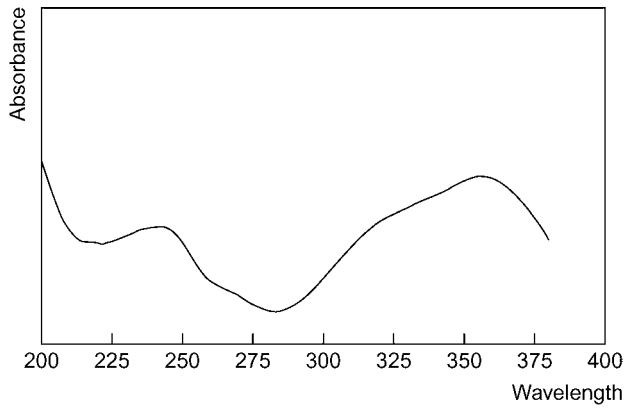
Sulfasalazine

Antiinflammatory, Antibacterial, Sulfonamide
$C_{18}H_{14}N_4O_5S$ = 398.4
CAS—599-79-1

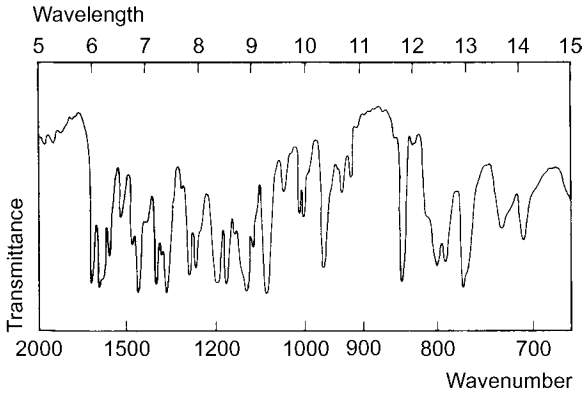
IUPAC Name (3*Z*)-6-Oxo-3-[[4-(pyridin-2-ylsulfamoyl)phenyl]hydrazinylidene]cyclohexa-1,4-diene-1-carboxylic acid
Synonyms 2-Hydroxy-5-[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo]benzoic acid; 6-oxo-3-[[4-(pyridin-2-ylsulfamoyl)phenyl]hydrazinylidene]cyclohexa-1,4-diene-1-carboxylic acid; salazosulfapyridine; salicylazosulphapyridine; sulphasalazine.
Proprietary Names *Aculfin*; *Azulfidine*; *Azulfin*; *Colo-Pleon*; *Pleon RA*; *Pyralin*; *Salazine*; *Salazopirina*; *Salazopyrin(e)*; *Salazopyrina*; *Saridine*; *SAS*; *Sulazine*; *Ucine*.



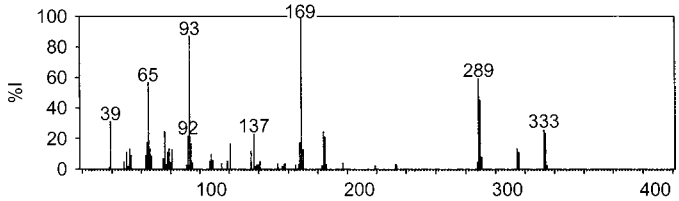
Chemical Properties A fine bright yellow to brownish-yellow powder. Mp about 240° to 245°, with decomposition. Practically insoluble in water, chloroform and ether; soluble 1 in 2900 of ethanol and 1 in 1500 of methanol; soluble in aqueous solutions of alkali hydroxides. pK_a 0.6, 2.4, 9.7, 11.8. Log *P* (octanol/water), 3.8.
Colour Tests Copper sulfate (method 1)—orange-brown; palladium chloride—black.
Thin-layer Chromatography System TA—R_f 0.66; system TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAD—R_f 0.02; system TAE—R_f 0.85; system TAJ—R_f 0.00; system TAK—R_f 0.30; system TAL—R_f 0.39 (visible orange spot).
High Performance Liquid Chromatography System HX—RI 433; system HZ—retention time 1.9 min.
Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1078, 1123, 772, 1634, 1175, 1672 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 169, 92, 289, 65, 290, 39, 333, 184.



Quantification

Plasma **HPLC** UV, fluorescence or electrochemical detection. Sulfasalazine metabolites and conjugates. Limit of detection, 1 mg/L or less [Hansen 1989]. UV and fluorescence detection. Sulfapyridine, acetylsulfapyridine, 5-aminosalicylic acid and 5-acetamidosalicylic acid. Limit of detection, 500 µg/L [Shaw *et al.* 1983]. Fluorescence detection. 5-aminosalicylic acid and 5-acetamidosalicylic acid. Limit of detection, 20 µg/L [Fischer *et al.* 1981].

Serum **HPLC** UV detection. Sulfasalazine, sulfapyridine and *N*-acetylsulfapyridine. Limit of detection, 0.1 to 0.25 mg/L [Bugge *et al.* 1990].

Urine **HPLC** See Plasma [Hansen 1989; Fischer *et al.* 1981].

Bile **HPLC** Fluorescence detection. 5-aminosalicylic acid and 5-acetamidosalicylic acid [Fischer *et al.* 1983].

Faeces **HPLC** See Plasma [Hansen 1989].

Disposition in the Body Partially and irregularly absorbed after oral administration. The absorbed material is not metabolised but is excreted unchanged in the urine and accounts for up to about 10% of the dose. The greater part of the dose passes unchanged into the colon where it is metabolised by bacteria to sulfapyridine and 5-aminosalicylic acid, which is thought to be the active moiety. The sulfapyridine so formed is absorbed, and metabolised by N^4-acetylation, ring hydroxylation and glucuronidation. The degree of acetylation is dependent on the acetylator status of the subject. About 60% of the dose is excreted in the urine as free and acetylated sulfapyridine and their glucuronides, and about 25% of the dose is eliminated as sulfapyridine in the faeces. The 5-aminosalicylic acid is partially absorbed and metabolised by *N*-acetylation; about 30% is excreted in the urine as unchanged drug and 5-acetamidosalicylic acid, and most of the remainder is eliminated unchanged in the faeces.

Therapeutic Concentration

Following a single oral dose of 2 g to 8 subjects, peak plasma concentrations of 7 to 32 mg/L (mean, 17) of sulfasalazine were attained in about 3 h; peak plasma concentrations of sulfapyridine averaged about 20 mg/L [Ryde, Lima 1981].

Following daily oral doses of 3 g to 6 subjects, minimum steady-state plasma concentrations of 0.04 to 0.34 mg/L of 5-aminosalicylic acid and 0.2 to 1.4 mg/L of 5-acetamidosalicylic acid were reported [Fischer *et al* 1981].

After daily oral doses of 4 g to 9 subjects, steady-state serum concentrations of 37 to 92 mg/L (median, 50) for total sulfapyridine and its metabolites, 4.7 to 45 mg/L (median, 12) for sulfasalazine, and <2 mg/L for 5-aminosalicylic acid metabolites were reported on the 5th day [Schröder, Campbell 1972].

Toxicity Toxic effects are associated with plasma concentrations of sulfapyridine >50 mg/L.

The serum salicylate concentration in a 26-year-old man who had taken 50 g of sulfasalazine at some point in the previous 12 h was 128 mg/L [Dunn 1998].

Half-life Plasma half-life, sulfasalazine, 6 to 17 h (mean, 10).

Note For a review of the pharmacokinetics of sulfasalazine, see Das and Dubin [1976].

Dose Initially 4 to 8 g daily; maintenance, 1.5 to 2 g daily.

Bugge CJ *et al.* (1990). Simultaneous determination of sulfasalazine and its metabolites sulfapyridine and N-acetylsulfapyridine in human serum by ion-pair high-performance liquid chromatography using a polymer-based column. *J Pharm Sci* 79: 1095–1098.

Das KM, Dubin R (1976). Clinical pharmacokinetics of sulphasalazine. *Clin Pharmacokinet* 1: 406–425.

Dunn RJ (1998). Massive sulfasalazine and paracetamol ingestion causing acidosis, hyperglycemia, coagulopathy, and methemoglobinemia. *J Toxicol Clin Toxicol* 36: 239–242.

Fischer C *et al.* (1981). Simplified high-performance liquid chromatographic method for 5-aminosalicylic acid in plasma and urine. *J Chromatogr* 225: 498–503.

Fischer C *et al.* (1983). Specific measurement of 5-aminosalicylic acid and its acetylated metabolite in human bile. *Br J Clin Pharmacol* 15: 273–274.

Hansen SH (1989). Simple and rapid method for the simultaneous determination of the eight main metabolites and conjugates of sulphasalazine in human plasma, urine and faeces using dynamically modified silica. *J Chromatogr* 491: 175–185.

Ryde EM, Lima JJ (1981). *Curr Ther Res* 29: 728–737.

Schröder H, Campbell DES (1972). Absorption, metabolism, and excretion of salicylazosulfapyridine in man. *Clin Pharmacol Ther* 13: 539–551.

Shaw PN *et al.* (1983). A rapid method for the simultaneous determination of the major metabolites of sulphasalazine in plasma. *J Chromatogr* 274: 393–397.

Sulfasomizole

Sulfonamide, Antibacterial

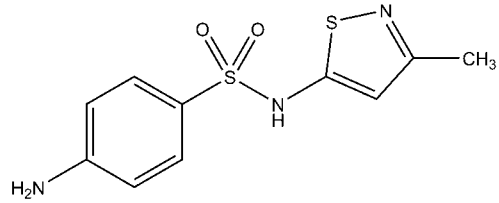
$C_{10}H_{11}N_3O_2S_2$ = 269.3

CAS—632-00-8

IUPAC Name 4-Amino-*N*-(3-methyl-1,2-thiazol-5-yl)benzenesulfonamide

Synonyms 5-*p*-Aminobenzenesulfonamido-3-methylisothiazole; 4-amino-*N*-(3-methyl-5-isothiazolyl)benzenesulfonamide; N^1-(3-methylisothiazol-5-yl)sulfanilamide; 3-methyl-5-sulfanilamidoisothiazole; 5-sulfanilamido-3-methylisothiazole; sulfasomizol; sulphasomizole.

Proprietary Names *Amidozol*; *Amidozole*; *Bidizole.*



Chemical Properties White powder. Mp 189° [Bridges, Williams 1963]. Soluble 1 in 2000 of water; almost insoluble in ether and chloroform. Log *P* (octanol/water) 0.5 [National Institutes of Health 2008]. Sulfasomizole may be extracted by ether from aqueous acid solutions; addition of an equal volume of acetone to the aqueous phase prior to extraction greatly increases the yield.

Sulfasomizole Sodium Monohydrate

$C_{10}H_{10}N_3NaO_2S_2,H_2O$ = 309.3

Chemical Properties Crystals. Freely soluble in water.

Colour Test Copper sulfate test—brown.

Thin-layer Chromatography System T1—R_f 0.93 (location by diazotisation followed by alkaline betanaphthol spray).

Note For paper chromatography of sulfasomizole, see Bridges and Williams [1963] and for the colorimetric determination of sulfanilamides, see Bratton and Marshal [1939].

Ultraviolet Spectrum Aqueous acid (0.1 N hydrochloric acid)—245, 301 nm; aqueous alkali (0.1 N sodium hydroxide)—257, 280 nm.

Disposition in the Body Sulfasomizole is rapidly absorbed. The major urinary metabolite is *N*-acetylsulfasomizole together with the unchanged drug. Approximately 60% of an oral dose of 30 mg/kg is excreted in the urine in 24 h. *N*-4-Glucuronide was identified but thought to be an artefact, not a true metabolite, and there was a trace of *N*-4-sulfosulfasomizole [Bridges, Williams 1963].

Therapeutic Concentration

A patient suffering from meningococcal meningitis was given a 2 g IM injection of sulfasomizole. The CSF concentration was 1.85 mg/100 mL (16.5% of the blood concentration) after 9 h. Two days later after another four IM injections of 1 g each the CSF concentration was 3.25 mg/100 mL (17.2% of the blood concentration) 5 h after the final dose [Adams *et al.* 1960].

Toxicity Sulfasomizole has a fairly long duration of action, so it may produce toxic effects similar to those caused by sulfamethoxypyridazine.

Dose Up to 1 g daily.

Adams A *et al.* (1960). Sulphasomizole (5-*p*-amino-benzenesulphonamido-3-methylisothiazole): a new antibacterial sulphonamide. *Nature* 186: 221–222.

Bratton AC, Marshal EK (1939). A new coupling component for sulfanilamide determination. *J Biol Chem* 128: 537–550.

Bridges JW, Williams RT (1963). The metabolism of 5-*p*-aminobenzenesulphonamido-3-methylisothiazole (sulphasomizole). *J Pharm Pharmacol* 15: 565–573.

National Institutes of Health (2008). *Sulfasomizole.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=69433&loc=ec_rcs.(accessed 26 June 2008).

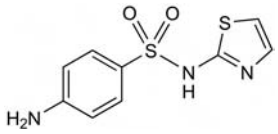
Sulfathiazole

Antibacterial, Sulfonamide

$C_9H_9N_3O_2S_2$ = 255.3

CAS—72-14-0

IUPAC Name 4-Amino-*N*-(1,3-thiazol-2-yl)benzenesulfonamide
Synonyms 4-Amino-*N*-2-thiazolylbenzenesulfonamide; norsulfazole; solfatiazolol; sulfanilamidothiazolum; sulfathiazolum; sulfonazolum; sulphathiazole.
Proprietary Names *Cibazol: Stopex*; *Sulfagine*; *Sulfamul*; *Sulfavitina*; *Sulzol*; *Thiazamide*. It is an ingredient of *Sultrin* and *Triple Sulfa*.

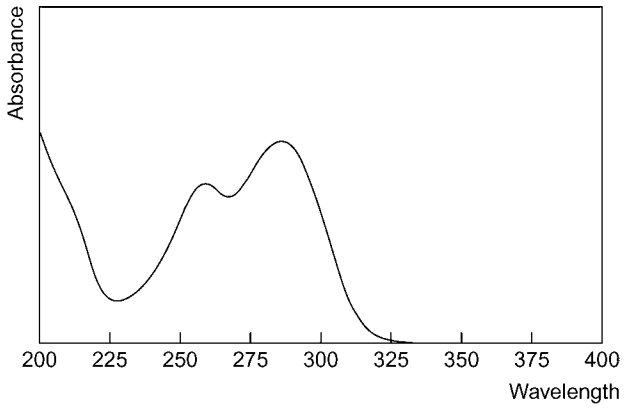


Chemical Properties A white crystalline powder which slowly darkens on exposure to light. Mp about 202°. Soluble 1 in 2500 of water and 1 in 120 of ethanol; practically insoluble in chloroform and ether; soluble in acetone, dilute mineral acids and solutions of alkali hydroxides and carbonates. pK_a 7.1 (25°). Log *P* (octanol/pH 7.5), −0.4.

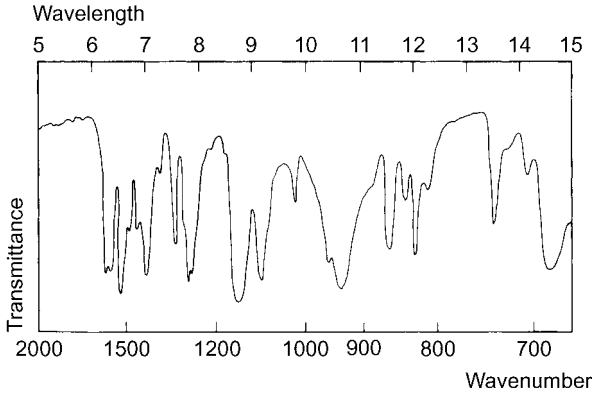
Sulfathiazole Sodium

$C_9H_8N_3NaO_2S_2,5H_2O = 367.4$
CAS—144-74-1 (anhydrous); 6791-71-5 (pentahydrate)
Synonym Soluble sulfathiazole
Chemical Properties A white or yellowish-white microcrystalline powder. It slowly darkens on exposure to light; on exposure to moist air it absorbs carbon dioxide and becomes incompletely soluble in water. Soluble 1 in 3 of water and 1 in 20 of ethanol.

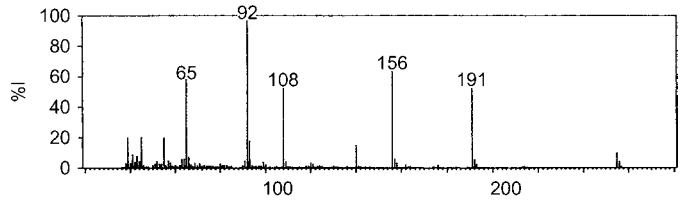
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—violet-brown; Koppanyi–Zwikker test—red-violet; mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.66; system TD—R_f 0.09; system TE—R_f 0.08; system TF—R_f 0.20; system TT—R_f 0.53; system TU—R_f 0.40; system TV—R_f 0.05; system TAD—R_f 0.27; system TAE—R_f 0.79 (mercuric chloride–diphenylcarbazone reagent, blue; acidified potassium permanganate solution, positive; Van Urk reagent, yellow).
Gas Chromatography System GA—not eluted; system GJ—methyl derivative RRT 0.49 (relative to griseofulvin).
High Performance Liquid Chromatography System HU—*k* 13.4; system HX—RI 310; system HAA—retention time 9.0 min.
Ultraviolet Spectrum Aqueous acid—280 nm (A_1^1=498a); aqueous alkali—256 nm (A_1^1=716b).



Infrared Spectrum Principal peaks at wavenumbers 1130, 1527, 929, 1274, 1082, 1592 cm^{-1} (Nujol mull).



Mass Spectrum Principal ions at *m/z* 92, 156, 65, 108, 191, 45, 39, 55.



Quantification

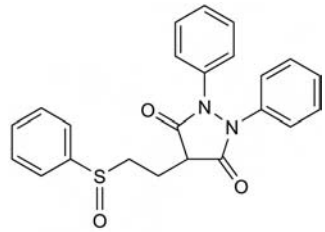
Plasma **HPLC** UV detection. Limit of detection, 250 µg/L in plasma [Sioufi *et al.* 1980].
Urine **HPLC** Sulfathiazole and other sulfonamides. Limit of detection, 0.1 to 0.3 mg/L [Simó-Alfonso *et al.* 1995]. Limit of detection, 2.5 mg/L, see Plasma [Sioufi *et al.* 1980].
Disposition in the Body Readily absorbed after oral administration and acetylated in the body, about 20% of the sulfathiazole in the blood is in the form of the inactive N^4-acetyl derivative. It is rapidly excreted in the urine, about 60 to 90% of a dose being excreted in 24 h; about 20% of the excreted material is the acetyl derivative. Sulfathiazole is a metabolite of phthalylsulfathiazole and succinylsulfathiazole.
Half-life Plasma half-life, about 4 h.
Protein Binding 55 to 80%.
Dose An initial dose of 2 to 3 g, followed by 1 g every 4 to 6 h.

Simó-Alfonso EF *et al.* (1995). Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography. *J Chromatogr B Biomed Appl* 670: 183–187.
Sioufi A *et al.* (1980). High-performance liquid chromatographic determination of sulphathiazole in human plasma and urine. *J Chromatogr* 221: 419–424.

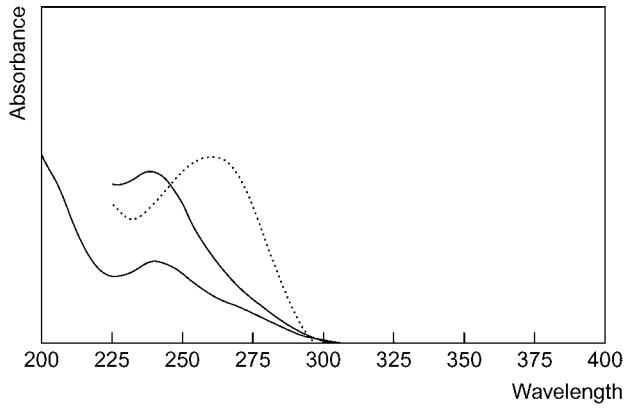
Sulfinpyrazone

Uricosuric, Antithrombotic
$C_{23}H_{20}N_2O_3S = 404.5$
CAS—57-96-5

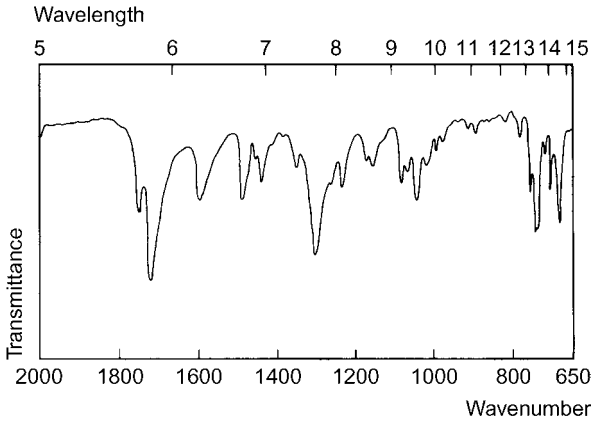
IUPAC Name 4-[2-(Benzenesulfinyl)ethyl]-1,2-diphenylpyrazolidine- 3,5-dione
Synonyms 1,2-Diphenyl-4-[2-(phenylsulfinyl)ethyl)-3,5-pyrazolidinedione; sulphinpyrazone; sulfoxyphenylpyrazolidine.
Proprietary Names *Anturan(e)*; *Enturen*; *Sulfinona*.



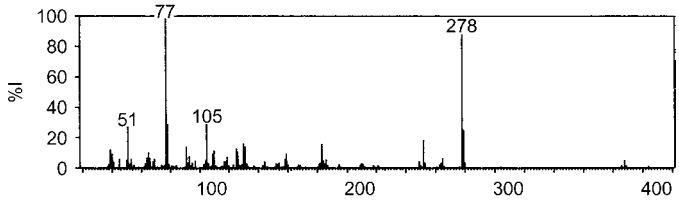
Chemical Properties A white powder. Mp 136° to 137°. Practically insoluble in water; soluble 1 in 40 of ethanol, 1 in about 10 of acetone, 1 in 2 of chloroform and 1 in 750 of ether. pK_a 2.8 (22°). Log *P* (octanol/water), 2.3.
Colour Tests Copper sulfate (method 1)—blue; Koppanyi–Zwikker test—violet (transient); Liebermann's reagent—orange; nitrous acid—yellow.
Thin-layer Chromatography System TAD—R_f 0.30; system TD—R_f 0.04; system TE—R_f 0.16; system TF—R_f 0.04.
Gas Chromatography System GA—RI 2253.
High Performance Liquid Chromatography System HY—RI 474; system HZ—retention time 4.7 min.
Ultraviolet Spectrum Aqueous acid—237 nm (A_1^1=436a); aqueous alkali—260 nm (A_1^1=559a).



Infrared Spectrum Principal peaks at wavenumbers 1716, 1305, 750, 688, 1750, 1595 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 77, 278, 105, 78, 51, 279, 252, 130.



Quantification

Plasma **GC** Sulfinpyrazone and its *p*-hydroxy and sulfone metabolites. Limit of detection for sulfinpyrazone, 100 µg/L using AFID or 10 µg/L using ECD [Jakobsen, Pedersen 1979].

GC-MS Sulfide and *p*-hydroxysulfide metabolites. Limit of detection, 5 µg/L for sulfide and 30 µg/L for *p*-hydroxysulfide [Jakobsen, Pedersen 1981].

HPLC Sulfinpyrazone and its *p*-hydroxy, sulfone, sulfide and *p*-hydroxysulfide metabolites. Limits of detection, 10 µg/L (sulfinpyrazone and sulfide metabolite) and 50 µg/L (other metabolites) [Lentjes *et al.* 1985]. UV detection. Sulfinpyrazone and metabolites. Limit of detection, 30 µg/L [Kuo *et al.* 1984]. UV detection. Sulfinpyrazone and its *p*-hydroxy and sulfone metabolites. Limit of detection, 50 µg/L for sulfinpyrazone [Bjornsson *et al.* 1980].

Urine **GC** See Plasma [Jakobsen, Pedersen 1979].

GC-MS See Plasma Jakobsen, Pedersen 1981].

HPLC See Plasma [Bjornsson *et al.* 1980; Lentjes *et al.* 1985].

Disposition in the Body Sulfinpyrazone is readily absorbed after oral administration. It is metabolised by reduction to the sulfide which is active and is the predominant circulating metabolite, reaching a concentration about 25% of that of sulfinpyrazone. It is also oxidised to the sulfone (active) and there are several hydroxy metabolites. All metabolites are excreted in the urine as the *C*-glucuronides, only a small proportion being excreted in *O*-glucuronidated or unconjugated form. Up to 50% of a dose is excreted in the urine as unchanged drug or its glucuronide. About 80% of a single dose is recovered within the first 24 h and >95% within 4 days (85% from urine and 10% from faeces).

Therapeutic Concentration

Following single oral doses of 200 mg to 6 subjects, peak plasma concentrations of 6.0 to 17.0 (mean, 13) mg/L of sulfinpyrazone were attained in 1 to 4 h; peak plasma concentrations of the metabolites attained in about 4, 4 and 11 h, respectively, were: sulfone 0.2 to 1.1 mg/L, *p*-hydroxysulfinpyrazone 0.1 to 0.48 mg/L, sulfide 0.6 to 8.9 mg/L [Mahony *et al.* 1983].

Following oral administration of 200 mg four times a day to 6 subjects, mean maximum steady-state plasma concentrations were: sulfinpyrazone 20.7 mg/L about 2 h after a dose, sulfide 13.9 mg/L about 7 h after a dose, sulfone 3.4 mg/L 2 h after a dose [Rosenkranz *et al.* 1983].

Daily oral doses of 600 to 800 mg to 8 people with diabetes, treated for 2.5 years or more, produced sulfinpyrazone plasma concentrations of 2.5 to 13.2 (mean, 7) mg/L before the first morning dose of 200 mg and 9.1 to 23.2 (mean, 16) mg/L 2 h later. The corresponding sulfide plasma concentrations were 1.0 to 6.0 (mean, 2.8) µg/mL and 1.5 to 8.6 (mean, 4.3) µg/mL [Pedersen *et al.* 1981].

Half-life Plasma half-life, about 3 to 5 h, sulfide metabolite about 14 to 21 h.

Volume of Distribution About 0.06 L/kg.

Clearance Plasma clearance, about 0.3 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.8.

Protein Binding About 98%.

Note For a review of the pharmacokinetics of sulfinpyrazone, see Pedersen *et al.* [1982].

Dose 100 to 800 mg daily.

Bjornsson TD *et al.* (1980). High-performance liquid chromatographic analysis of sulfinpyrazone and its metabolites in biological fluids. *J Chromatogr* 181: 417–425.

Jakobsen P, Pedersen AK (1979). Determination of sulfinpyrazone and two of its metabolites in human plasma and urine by gas chromatography and selective detection. *J Chromatogr* 163: 259–269.

Jakobsen P, Pedersen AK (1981). Two metabolites of sulphinpyrazone and their identification and determination by mass spectrometry. *J Pharm Pharmacol* 33: 89–92.

Kuo BS *et al.* (1984). High-performance liquid chromatographic determination of sulfinpyrazone and its metabolites in plasma. *Arzneimittelforschung* 34: 548–550.

Lentjes EG *et al.* (1985). Determination of sulfinpyrazone and four metabolites in plasma and urine by high pressure liquid chromatography. *Pharm Weekbl (Sci.)* 7: 252–259.

Mahony C *et al.* (1983). Kinetics and metabolism of sulfinpyrazone. *Clin Pharmacol Ther* 33: 491–497.

Pedersen AK *et al.* (1981). Sulphinpyrazone metabolism during long-term therapy. *Br J Clin Pharmacol* 11: 597–603.

Pedersen AK *et al.* (1982). Clinical pharmacokinetics and potentially important drug interactions of sulphinpyrazone. *Clin Pharmacokinet* 7: 42–56.

Rosenkranz B *et al.* (1983). Plasma levels of sulfinpyrazone and of two of its metabolites after a single dose and during the steady state. *Eur J Clin Pharmacol* 24: 231–235.

Sulfiram

Parasiticide

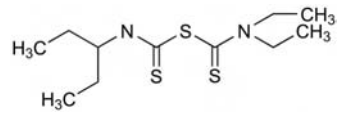
$C_{10}H_{20}N_2S_3 = 264.5$

CAS—95-05-6

IUPAC Name Diethylcarbamothioyl *N*,*N*-diethylcarbamodithioate

Synonyms Monosulfiram; tetraethylthiodicarbonic diamide.

Proprietary Names *Kutkasin*; *Tetmosol*; *Tiosol*; *Thiosan*.

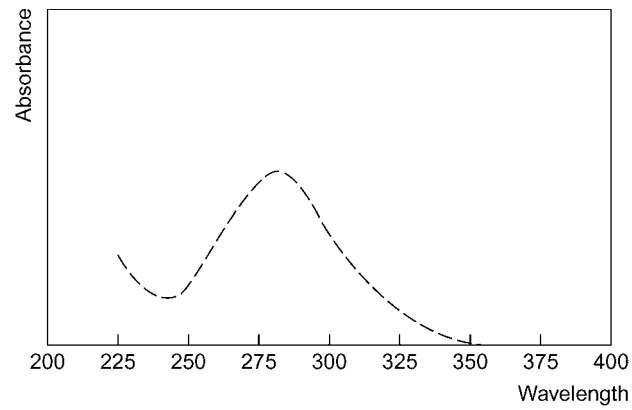


Chemical Properties A yellow or yellowish-brown, soft crystalline powder. Mp 28.5° to 32°. Practically insoluble in water; soluble 1 in 3 of ethanol; freely soluble in chloroform, ether and most other organic solvents. Log *P* (octanol/water), 2.7.

Colour Test Sodium nitroprusside (method 3)—violet.

Thin-layer Chromatography System TA—R_f 0.75 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Methanol—281 nm (A_1^1=650a).



Infrared Spectrum Principal peaks at wavenumbers 1270, 1490, 1198, 1142, 968, 915 cm^{-1} (KBr disk).

Use As a 25% alcoholic solution, diluted with 2 or 3 parts of water immediately before use.

Sulfisomidine

Antibacterial, Sulfonamide

$C_{12}H_{14}N_4O_2S = 278.3$

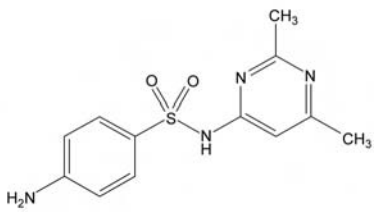
CAS—515-64-0

IUPAC Name 4-Amino-*N*-(2,6-dimethylpyrimidin-4-yl)benzenesulfonamide

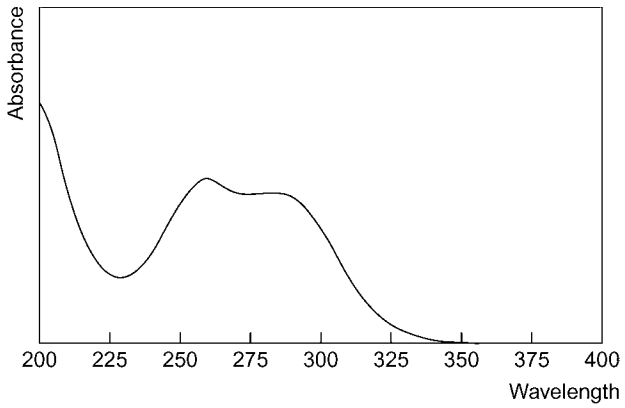
Synonyms Sulfa-isodimérazine; sulfaisodimidine; sulfasomidine; sulphasomidine.

Note Sulfadimethylpyrimidine has been used as a synonym for sulfisomidine and is sometimes used as a synonym for sulfadimidine. Care should be taken to avoid confusion between the two compounds, which are isomeric.

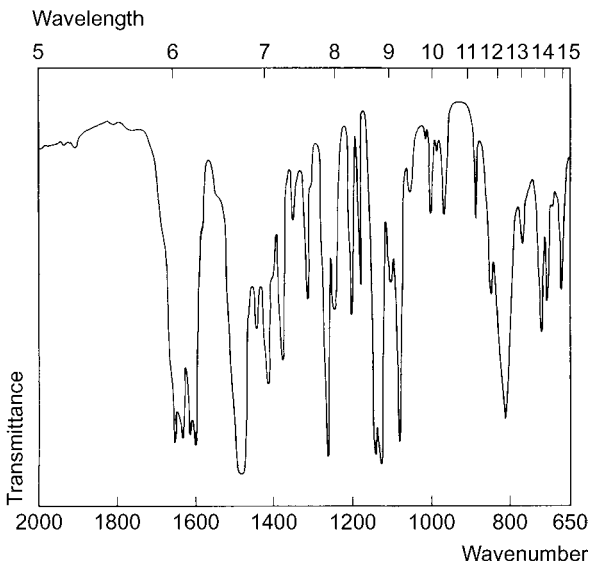
Proprietary Names *Aristamid*; *Domain*; *Elcosine*; *Elkosil*; *Elkosin(e)*.



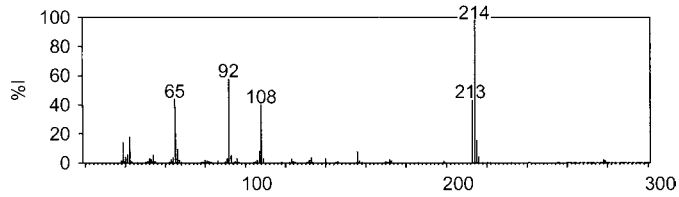
Chemical Properties A white or creamy-white finely crystalline powder, which slowly darkens on exposure to light. Mp 243°. Very slightly soluble in water, chloroform and ether; slightly soluble in ethanol and acetone; readily soluble in dilute mineral acids and solutions of alkali hydroxides. pK_a 7.5 (27°). Log *P* (octanol/water), −0.3.
Colour Tests Coniferyl alcohol—orange; copper sulfate (method 1)—green; Koppanyi–Zwikker test—blue-violet (transient); mercurous nitrate—black; nitrous acid—yellow.
Thin-layer Chromatography System TA—R_f 0.64; system TD—R_f 0.05; system TE—R_f 0.05; system TF—R_f 0.16; system TT—R_f 0.11; system TU—R_f 0.49; system TV—R_f 0.20; system TAD—R_f 0.27; system TAE—R_f 0.80.
Gas Chromatography System GJ—methyl derivative RRT 0.50 (relative to griseofulvin).
High Performance Liquid Chromatography System HX—RI 290.
Ultraviolet Spectrum Aqueous acid—262 nm; aqueous alkali—262 nm (A_1^1=764b); methanol—272 nm (A_1^1=745a).



Infrared Spectrum Principal peaks at wavenumbers 1125, 1260, 1138, 1595, 1650, 1070 cm^{-1} (KBr disk).



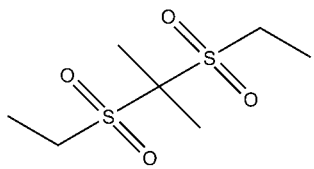
Mass Spectrum Principal ions at *m/z* 214, 92, 65, 213, 108, 42, 215, 39.



Quantification
HPLC The methods referred to under sulfamethoxazole may be used.
Disposition in the Body Absorbed after oral administration. It is acetylated in the body to only a small extent, about 10 to 25% of the material in the blood being in the form of the inactive N^4-acetyl derivative. It is rapidly excreted in the urine, about 87% of the dose being excreted within 48 h as unchanged drug together with about 6% as the acetyl derivative.
Half-life Plasma half-life, 6 to 8 h.
Protein Binding 40 to 90% (dose-dependent).
Dose Sulfisomidine has been given in an initial dose of 2 to 4 g, followed by 6 g daily.

Sulfonal

Hypnotic
$C_7H_{16}O_4S_2$ = 228.3
IUPAC Name 2,2-Bis(ethylsulfonyl)propane
Synonyms Acetone diethylsulfone; acetone diethyl sulfone; 2,2-di(ethylsulfonyl)propane; propanediethyl sulfone; sulfonmethane; sulfonomethane; sulphonal.

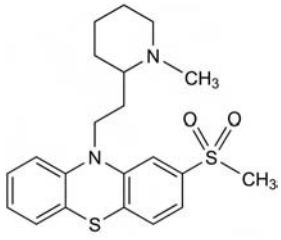


Chemical Properties White powder. Mp 125° to 127°. Soluble 1 in 450 in water, 1 in 15 of boiling water, 1 in 80 of ethanol, 1 in 90 of ether, and 1 in 3 of chloroform. Log *P* (octanol/water) 0.9 [National Institutes of Health 2008].
Colour Test Heat with a few drops of sulfuric acid containing a trace of phenol—green.
Gas Chromatography System G2/225—retention time 0.35 relative to diphenhydramine; system G4—retention time 1.15 relative to diphenhydramine.
Disposition in the Body Sulfonal is largely metabolised by oxidation of the sulfone groups to unknown metabolites, possibly sulfonic acids. It is excreted very slowly and successive doses may have a dangerous cumulative effect.
Toxicity A single dose of 2 to 30 g may cause acute fatal poisoning but recovery has followed very much larger doses. The effects caused by accumulation of sulfonal makes it one of the most dangerous of the hypnotics. It may lead to the destruction of haemoglobin in the blood, with the appearance of haematoporphyrins in the urine, which acquires a cherry-red colour and reduces Fehling's solution [With 1971a; With 1971b].
Dose Up to 1.2 g.

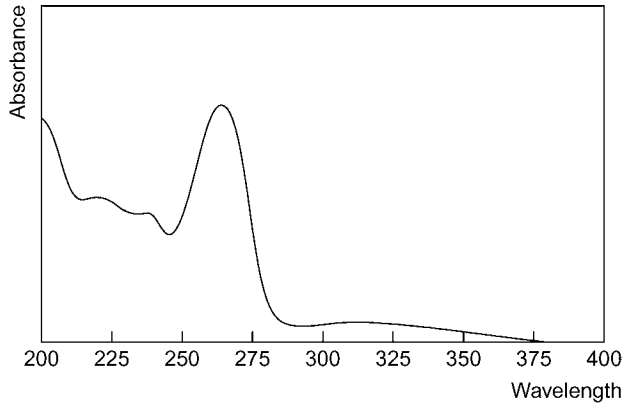
National Institutes of Health (2008). *Sulfonmethane*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=8262&loc=ec_rcs.(accessed 20 June 2008).
With TK (1971a). Acute porphyria, toxic and genuine in the light of history. A re-evaluation of sulphonal-trional porphyria, the first pharmakon-provoked inborn error of metabolism. *Dan Med Bull* 18: 112–121.
With TK (1971b). Toxic porphyria after treatment with sulphonal and trional. *S Afr Med J*,133–137.

Sulforidazine

Tranquilliser
$C_{21}H_{26}N_2O_2S_2$ = 402.6
CAS—14759-06-9
IUPAC Name 10-[2-(1-Methylpiperidin-2-yl)ethyl]-2-methylsulfonylphenothiazine
Synonyms 10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylsulfonyl)-10*H*-phenothiazine; sulphoridazine.
Proprietary Names *Imagotan*; *Inofal*.



Chemical Properties Crystals. Mp 121° to 123°. Log *P* (octanol/water), 4.5.
Thin-layer Chromatography System TE—R_f 0.54; system TF—R_f 0.00.
Gas Chromatography System GA—sulforidazine RI 3415, M (ring) RI 3180; system GB—RI 3690.
High Performance Liquid Chromatography System HX—RI 421; system HZ—retention time 4.8 min.
Ultraviolet Spectrum Aqueous acid—265 (A_1^1=700b), 311 nm (A_1^1=75b); aqueous alkali—266 nm (A_1^1=659b).



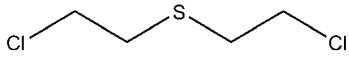
Quantification
Plasma **Radioimmunoassay** Limit of detection, 40 pg [Chakraborty *et al.* 1988]. See also under Thioridazine.
Disposition in the Body Sulforidazine is an active metabolite of mesoridazine and thioridazine.
Dose Sulforidazine has been given in doses of 150 to 300 mg daily.

Chakraborty BS *et al.* (1988). Development of a radioimmunoassay procedure for sulforidazine and its comparison with a high-performance liquid chromatographic method. *Ther Drug Monit* 10: 205–214.

Sulfur Mustard

Vesicant
$C_4H_8Cl_2S$ = 159.1
CAS—505-60-2

IUPAC Name 1-Chloro-2-(2-chloroethylsulfanyl)ethane
Synonyms Bis(2-chloroethyl)sulfide; blister gas; β,β′-dichloroethyl sulfide; HD; kampfstoff 'lost'; mustard gas; S-lost; senfgas; 1,1′-thiobis[2-chloroethane]; yellow cross liquid; yperite.



Chemical Properties Yellow-brown oily liquid with a weak, sweet mustard-like odour. Mp 14.4°. Bp 215° to 217°. Very slightly soluble in water; freely soluble in ethanol, ether and chloroform. Log *P* (octanol/water), 2.41.

1,1′-Sulfonylbis-[2-*S*-(methylthio)ethane] (metabolite)
$C_6H_{14}O_2S_3$ = 214.4
Synonym SBMTE

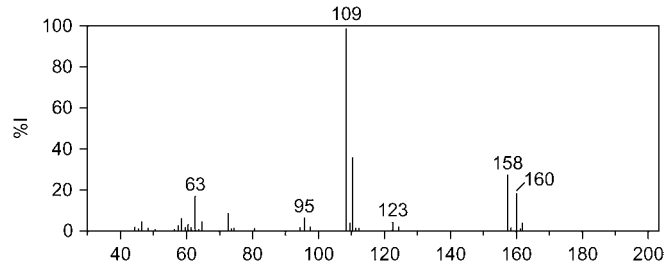
Thiodiglycol (metabolite)
$C_4H_{10}O_2S$ = 122.2
CAS—111-48-8
Synonyms Bis(hydroxyethyl)sulfide; TDG; 2,2′-thiodiethanol; thiodiethylene glycol.
Chemical Properties Liquid. Mp −16°. Slightly soluble in ether.

Thin-Layer Chromatography Plates: (1) Analtech Uniplate silica gel GF (10 × 2.5 cm, 250 μm) or (2) Universal Absorbents silica gel GF (10 × 2.5 cm, 250 μm). Solvent system: (A) chloroform : methanol (10 : 1); (B) chloroform : acetonitrile (5 : 1); (C) ecetonitrile : ethanol (10 : 1); chloroform : acetone (5 : 4); (E) acetonitrile. Visualistion with 1% potassium permanganate in 6% sodium carbonate. R_f values were as follows:

Compound	*Plate system 1, solvent*					*Plate system 2, solvent*	
	A	*B*	*C*	*D*	*E*	*A*	*B*
HD	0.93	0.94	0.92	0.89	0.96	0.92	0.93
HHD	0.75	0.64	0.88	0.77	0.89	0.64	0.63
HDO	0.67	0.33	0.74	0.48	0.64	0.51	0.26
HDO_2	0.86	0.84	0.88	0.84	0.94	0.81	0.80
TDG	0.58	0.24	0.62	0.48	0.59	0.27	0.17
TDGO	0.23	0.00	0.21	0.05	0.06	0.06	0.00
$TDGO_2$	0.44	0.10	0.76	0.42	0.73	0.15	0.08

HHD, 2-chloro-2-hydroxyethyl sulfide; HDO, mustard sulfoxide; HDO_2, mustard sulfone; TDGO, thiodiglycol sulfoxide; $TDGO_2$, thoidyglycol sulfone.

Limit of detection, 56.3 μg/spot for HD [Munavalli, Pannella 1988].
Plates: silica gel G (500 μm). Mobile phase: dichloromethane. Visualisation with 4-(*p*-nitrobenzyl)pyridine followed by an aqueous solution of 5% sodium perchlorate; heat at 105° for 10 min; after cooling, spray with piperidine. Result: blue spot, R_f 0.77 [Sass, Stutz 1981].
Gas Chromatography-Mass Spectrometry Column: DB5-MS (25 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 0.9 mL/min. Temperature programme: 40° for 2 min to 160° at 20°/min to 310° at 30°/min for 5 min. ATD, full scan mode. Limit of detection, 50 ng [Carrick *et al.* 2001].
Note For a GC-AES-MS analysis of an yperite block found in the Baltic Sea, see Mazurek *et al.* [2001].
Mass Spectrum Principal ions at *m/z* 109, 111, 158, 63, 160, 73. [Black *et al.* 1994] and [Creasy *et al.* 1997].



Quantification
Blood **GC-MS** System 1: Column: PepMap C_{18} or Vydac C_{18} (both 150 mm × 300 μm i.d., 3 μm). Mobile phase: 0.2% formic acid in water : 0.2% formic acid in acetonitrile, flow rate 6 μL/min. System 2: Column: 2 × Phenomenex Luna C_{18} (both 150 × 1 mm i.d., 5 μm). Mobile phase 1% formic acid in water : acetonitrile-1% formic acid in water (80 : 20; 100 : 0 to 0 : 100 at 25 min), flow rate 50 μL/min. MRM acquisition mode. Limit of detection, ~0.17 μg/L [Noort *et al.* 2004]. Column: CP-SIL 5 CB fused silica capillary (50 m × 0.32 mm i.d., 0.25 μm). Temperature programme: 120° for 5 min to 275° at 15°/min for 10 min. NICI at 70 eV. Limit of detection not reported [Benschop *et al.* 1997]. Column: CP-SIL 5 CB fused silica capillary (50 m × 0.32 mm i.d., 0.25 um). Temperature programme: 120° for 5 min to 275° at 15°/min for 10 min. NICI at 70 eV, SIM acquisition mode. Limit of detection, 0.1 μmol/L [Fidder *et al.* 1996].
HPLC Column: Polygosil C_{18} (25 cm × 4 mm i.d., 5 μm). Mobile phase: acetonitrile : water (52 : 48), flow rate 1.4 mL/min. UV detection (λ = 200 nm). Limit of detection, 0.25 mg/L [Dangi *et al.* 1994].
LC-MS Column: Phenomenex Luna C_{18} reversed phase (150 × 1.0 mm i.d., 5 μm). ESI, positive ion mode. Limit of detection, 50 nmol/L [Yeo *et al.* 2008]. Column: Spherisorb ODS2 S5 (250 × 0.32 mm i.d., 5 μm). Mobile phase: 0.05% trifluroacetic acid in water; 0.05% trifluroacetic acid in methyl cyanide-water (80 : 20; 100 : 0 for 5 min to 0 : 100 at 35 min for 5 min to 0 : 100 at 45 min), flow rate 1 mL/min. ESI. Limit of detection not reported [Noort *et al.* 1996].
AAS Drying: 120°, 20 s ramp time, 20 s hold time. Charring: 250°, 10 s ramp time, 10 s hold time. Atomising: 2400°, no ramp time, 3 s hold time. Cleaning: 2650°, 1 s ramp time, 4 s hold time. Purge gas: Ar, 300 mL/min. λ = 242.8 nm. Limit of detection, 0.1 mg/L for sulfur mustard–gold complex [Drasch *et al.* 1987].
Note For a method of quantifying sulfur mustard adducts in blood, see Noort *et al.* [1999].
Plasma **TLC** Plates: glass coated with silica gel 60 F_{254} (20 × 20 cm). Solvent system; methylene chloride. R_f 0.77. Limit of detection, 5 ng/spot [Heyndrickx *et al.* 1984].
GC-MS Column: DB-5MS bonded phase capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 80° for 1 min to 225° at 30°/min to 300° at 50°/min for 3 min. NICI, SIM acquisition mode. Limit of quantification, 1.56 nmol/L [Lawrence *et al.* 2008]. Column: DB-5MS bonded phase capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 80° for 1 min to 225° at 30°/min for 11 min. NICI, SIM acquisition mode. Limit of quantification, 20.88 nmol/L; limit of detection, 6.26 nmol/L [Capacio *et al.* 2008]. Column: CP *tm* Sil 5 fused silica capillary (25 m × 0.32 mm i.d.). Carrier gas: argon : methane (90 : 10), 0.5 bar. Temperature programme: 55° for 5 min to 120° at 5°/min to 300° at 10°/min for 10 min. ^{63}Ni ECD. Limit of detection, <100 pg [Heyndrickx *et al.* 1984].

Urine **GC-MS** Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 90° for 2 min to 250° at 50°/min for 3 min. MRM acquisition mode. Limit of detection, 38 ng/L [Barr *et al.* 2008]. Column: Rtx-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 40 cm/s. Temperature programme: 80° for 1 min to 280° at 20°/min for 2 min. CI, positive ion mode, SRM acquisition mode. Limit of detection, <0.2 μg/L for thiodiglycol [Riches *et al.* 2007]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 90° for 2 min to 250° at 50°/min for 3 min. CI, positive ion mode, MRM acquisition mode. Limit of detection, thiodiglycol 0.5, 1,1′-sulfonylbis[2-(methylthio)ethane] 0.25 μg/L [Boyer *et al.* 2004] Column: DB5-MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 90° for 2 min to 320° at 70°/min for 3 min. CI, MRM acquisition mode. Limit of quantification, 0.126 μg/L; limit of detection, SBMTE 38 ng/L [Young *et al.* 2004]. Column: DB5-MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 90° for 1 min to 290° at 20°/min for 5 min. EI ionisation at 70 eV. Limit of detection, thiodiglycol 5.4 μg/L [Ohsawa *et al.* 2004]. See also Black, Read [1995a], Jakubowski *et al.* [1990] and Wils *et al.* [1988].

LC-MS Column: Hypersil Gold-aQ (150 × 2.1 mm i.d., 3.0 μm). Mobile phase: water : acetonitrile-isopropyl alcohol (95 : 5; 95 : 5 for 4 min to 65 : 35 at 12.5 min to 35 : 65 at 15 min to 5 : 95 at 18.5 min), flow rate 300 μL/min for 12.5 min to 400 μL/min at 15 min to 500 μL/min at 18.5 min to 750 μL/min for 10 min. APCI, negative ion mode, MRM acquisition mode. Limit of detection, sesqui- and ethyl ether oxy-mustard metabolites 1 μg/L [Ash *et al.* 2008]. Column: Luna C18(2) (150 × 2.0 mm i.d., 5 μm). Mobile phase: 2 mmol/L ammonium formate : methanol (60 : 40), flow rate 0.5 mL/min. Turbospray ionisation, positive ion mode, MRM acquisition mode. Limit of quantification, 0.08 μg/L; limit of detection, SBMTE 0.02 μg/L [Daly *et al.* 2007]. Column: Hamilton PRP-1 (150 × 2 mm i.d., 3 μm). Mobile phase: 0.05% formic acid in water; 0.05% formic acid in acetonitrile (95 : 5 for 2 min to 10 : 90 at 15 min for 5 min), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection, SBMTE 0.5 to 1 μg/L [Read, Black 2004a]. Column: Hypercarb (150 × 2.1 mm i.d., 2 μm). Mobile phase: 0.02 mol/L ammonium formate in water : 0.02 mol/L ammonium formate in methanol (95 : 5 for 5 min to 20 : 80 at 15 min for 5 min), flow rate 0.2 mL/min. ESI, SRM acquisition mode. Limit of detection, β-lyase metabolites 0.1 to 0.5 μg/L [Read, Black 2004b].

AAS Drying: 120°, 20 s ramp time, 20 s hold time. Charring: 250°, 10 s ramp time, 10 s hold time. Atomising: 2400°, no ramp time, 3 s hold time. Cleaning: 2650°, 1 s ramp time, 4 s hold time. Purge gas: Ar, 300 mL/min. λ = 242.8 nm. Limit of detection, 0.1 mg/L for sulfur mustard–gold complex [Drasch *et al.* 1987].

CSF **AAS** Drying: 120°, 20 s ramp time, 20 s hold time. Charring: 250°, 10 s ramp time, 10 s hold time. Atomising: 2400°, no ramp time, 3 s hold time. Cleaning: 2650°, 1 s ramp time, 4 s hold time. Purge gas: Ar, 300 mL/min. λ = 242.8 nm. Limit of detection, 0.1 mg/L for sulfur mustard–gold complex [Drasch *et al.* 1987].

Brain **AAS** See Urine [Drasch *et al.* 1987].

Fat **GC-MS** Column: SE 54 fused silica (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 45 cm/s. Temperature programme: 55° for 2 min to 120° at 10°/min. EI ionisation at 70 eV. Limit of detection, 0.1 mg/kg [Drasch *et al.* 1987].

Kidney **AAS** See Urine [Drasch *et al.* 1987].

Liver **AAS** See Urine [Drasch *et al.* 1987].

Lung **AAS** See Urine [Drasch *et al.* 1987].

Muscle **AAS** See Urine [Drasch *et al.* 1987].

Skin **AAS** See Urine [Drasch *et al.* 1987]

Spleen **AAS** See Urine [Drasch *et al.* 1987].

Skin Blister Fluid **AAS** See Urine [Drasch *et al.* 1987].

Other **TLC** Soil. Plates: glass coated with silica gel 60 F_{254} (20 × 20 cm). Solvent system: methylene chloride. R_f 0.77. Limit of detection, 5 ng/spot [Heyndrickx *et al.* 1984].

GC Rat Skin Microdialysates. Column: CP-Sil 8 CB (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: H_2, 1.0 mL/min. Temperature programme: 60° for 2 min to 175° at 10°/min to 250° at 40°/min for 1 min. Pulsed flame photometric detection. Limit of quantification, thiodiglycol (2,2^1-sulfobisethanol) 44.4 μg/L; limit of detection, thiodiglycol (2,2^1-sulfobisethanol) 24.4 μg/L [Karvaly *et al.* 2005].

GC-MS Concrete. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 36 cm/s. Temperature programme: 45° for 5 min to 250° at 10°/min. Retention times: 2-chloroethyl vinyl sulfide (CEVS) 7.92 min, 2-hydroxyethyl vinyl sulfide (HOEVS) 8.34 min, sulfur mustard HD 11.32 min, 1,2-bis(vinylthio)ethane (BVTE) 13.02 min, (2-chloroethylthio)ethyl vinyl sulfide (CETEVS) 16.74 min. Limit of detection not reported [Brevett *et al.* 2007]. Marmoset Blood and Tissue. Column: VF-5MS fused silica (30 m × 0.25 mm i.d., 1.0 μm). Carrier gas: He, 30 mL/min. Temperature programme: 30° to 220° at 60°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, blood 10 ng/L, tissue 10 μg/kg [Oostdijk *et al.* 2007]. ChemicalWeapons. Column: DB-5 fused silica capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 100° for 5 min to 280° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Hanaoka *et al.* 2006]. Pig and Monkey Blood. Column: DB-5MS bonded phase capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 80° for 1 min to 225° at 30°/min for 11 min. NICI. SIM acquisition mode. Limit of detection, TDG 2.0 ng/kg protein [Capacio *et al.* 2004]. Suspected Contaminants. Column: SGE BPX5 fused silica capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 50° for 2 min to 280° at 10°/min for 5 min. EI ionisation at 70 eV or NICI. Limit of detection, 100 μg/L (NICI) [Pardasani *et al.* 2004]. See also Kimm *et al.* [2002] and Heyndrickx *et al.* [1984] for soil; Fidder *et al.* [1996] for guinea-pig blood; Black *et al.* [1994] for clothing, grave debris, soil and munition fragments; and Black *et al.* [1991] for rat urine.

LC-MS Rat Blood. Column: PepMap C_{18} (15 cm × 1 mm i.d., 3 μm). Mobile phase: 0.2% formic acid in water : 0.2% formic acid in acetonitrile (100 : 0 for 5 min to 30 : 70 at 50 min), flow rate 0.1 mL/min to 0.6 mL/min at 5 min. Quadrupole TOF, ESI. Limit of detection not reported [Noort *et al.* 2008]. Soil. Column: MicroTech Zorbax C_{18} fused silica capillary (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5). ESI. Limit of detection not reported [D'Agostino *et al.* 2004].

Note For the analysis of the degradation compounds of chemical warfare agents using LC-MS, see Smith, Shih [2001] and for a review of the chromatographic identification of chemical-weapons-related compounds in decontamination solutions and other matrices see Creasy *et al.* [1997].

Disposition in the Body

Sulfur mustard is readily absorbed through the skin, lung or eyes. It may also be absorbed through the gastrointestinal tract following consumption of contaminated food. When absorbed through the skin, ~80% of the liquid form evaporates; of the 20% that penetrates, 2% is retained in the skin and ~18% is absorbed systemically. A study in rabbits where sulfur mustard was administered as vapour showed that ~80% is adsorbed in the upper airways. Postmortem tissue distribution data after inhalation and/or dermal exposure to sulfur mustard indicated higher concentrations in the brain, followed by the kidney, liver, spleen and lung. The metabolism of sulfur mustard has not been studied extensively but it is thought to undergo intramolecular cyclisation to create a hyperactive compound. This, in turn, reacts with alkylate electron-rich molecular structures such as the sulfhydryl and amino groups of proteins and nucleic acids, forming compounds such as N^7-(2-hydroxyethylthioethyl)-2′-deoxyguanosine, the major adduct formed in DNA after exposure to sulfur mustard. Other pathways include reaction with glutathione, forming a bis-glutathione conjugate, which is then metabolised to a bis-cysteinyl conjugate. The cysteinyl C–S bond is cleaved by the enzyme β-lyase and the resulting thiol methylated and oxidised. Non-enzymatic hydrolysis and alcohol

Day after exposure	*SBMTE (μg/L)*	*SBMTE (mg/kg creatinine)*	*TDG (μg/L)*	*TDG (mg/kg creatinine)*	*TDG + TDGO (μg/L)*	*TDG + TDGO (mg/kg creatinine)*	*Bismercapturate (μg/L)*	*Bismercapturate (mg/kg creatinine)*
Patient 1								
2	41	23	24	14	50	28	3.1	1.8
3	7.0	8.6	8.9	11	17	20	ND	NA
4	3.3	4.5	5.4	7.4	11	15	NM	NA
5	1.3	1.2	14	13	28	26	NM	NA
6	0.67	0.58	11	9.9	24	21	NM	NA
7	0.04	0.07	5.7	9.2	14	23	NM	NA
8	0.05	0.06	2.4	3.3	4.5	6.0	NM	NA
9	0.05	0.04	1.8	1.7	9.1	8.5	NM	NA
10	0.06	0.15	1.5	3.5	4.8	11	NM	NA
11	0.02	0.06	1.2	3.1	5.8	15	NM	NA
28	ND	NA	ND	NA	ND	NA	NM	NA
34	ND	NA	ND	NA	ND	NA	NM	NA
41	ND	NA	ND	NA	ND	NA	NM	NA
Patient 2								
2	2.6	1.6	ND	NA	1.8	1.1	NS	NA
4	0.85	0.31	ND	NA	3.0	1.1	NS	NA
7	0.08	0.04	ND	NA	4.4	2.3	NS	NA

NA, not applicable; ND, not detected; NM, not measured after the first ND value; NS, not measured because of insufficient quantity of urine; TDGO, TDG sulfoxide. [Barr *et al.* 2008].

dehydrogenase reactions also occur, forming mercapturic acids. The main urinary metabolites include TDG, TDG sulfoxide, 2 β-lyase metabolites (1-methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane and 1,1′-sulfonylbis[2-(methylsulfinyl)ethane]) and SBMTE. Only the bis-cysteinyl conjugate, the 2 β-lyase metabolites, and SBMTE provide unequivocal confirmation of exposure to sulfur mustard. Urinary excretion is the primary route of elimination for sulfur mustard and its metabolites but there is also evidence of excretion in the faeces.

Toxicity

In July 2004, two subjects developed blistering after the destruction of a WW1-era munition. Urine samples were collected and the concentrations of the metabolites of sulfur mustard were as per table on previous page.

Sulfur mustard biomarkers of adducts to cysteine-34 of serum albumin (HAS) and alkylation sites of glutamic and aspartic acids of plasma proteins were measured in hospitalised patient 1. The concentrations were as follows:

Days after exposure	*HSA adduct concentration (nM-ExB)*[a]	*Plasma protein concentration (nM-ExP)*[b]
2	354	97
3	332	74
4	299	66
5	NS	52
6	NS	53
7	NS	61
8	257	52
9	250	46
10	227	38
29	NS	23
35	NS	22
42	90.8	20

[a]Concentration based on the *in vitro* exposure of whole blood to HD; the measured units nM-ExB are relative to the *in vitro* protocol.
[b]Concentration based on the *in vitro* exposure of whole blood to HD; the measured units nM-ExP are relative to the *in vitro* protocol.
[Smith *et al.* 2008].

Two subjects who were accidentally exposed to sulfur mustard suffered extensive blistering. Urine samples were analysed for TDG and TDG sulfoxide as their bis-pentafluorobenzoyl derivatives and the 2 β-lyase metabolites after reduction with titanium dichloride as the single analyte SBMTE; a further analysis was carried out for TDG and its sulfoxide after a similar reduction. The following concentrations were reported:

Metabolite	*Mean concentration (μg/L)*		
	Subject 1	*Subject 2*	*Control*
TDG	2	2	<1
$TDGO_2$	69	45	5
TDG + $TDGO_2$[a]	77	54	4.5
SBMTE[a]	42	56	<0.1

$TDGO_2$, TDG sulfone.
[(a)] After reduction with titanium trichloride.

The above data support that the detection of the β-lyase metabolites or their reduced form, SBMTE, provides a forensic or diagnostic indicator of mustard poisoning [Black, Read 1995b].

Sulfur mustard exposure in 2 victims was demonstrated by the analysis of blood samples taken 22 and 26 days after the alleged exposure. Immunochemical analysis for the detection of the N^7-guanine adduct of the agent in DNA from lymphocytes and granulocytes was carried out while GC-MS was used to detect the *N*-terminal valine adduct in globin. The following concentrations were reported:

Patient	*N^7-adduct of 2′-deoxyguanosine (μmol/L)*		*Valine adduct (μmol/L)*
	Lymphocytes	*Granulocytes*	
1	0.22	0.16	0.9
2	0.43	0.15	0.9

The adduct levels correspond with those found in human blood after *in vitro* treatment with 0.9 μmol/L sulfur mustard [Benschop *et al.* 1997].

The urine samples of several Iranian patients allegedly attacked with sulfur mustard were measured for their TDG content. With the exception of one high value (330 μg/L), the TDG concentrations were in the range 10 to 100 μg/L. Male control levels did not exceed 20 μg/L [Wils *et al.* 1988].

A 24-year-old Iranian soldier died 7 days after vesicant exposure. Sulfur mustard levels in postmortem samples were as follows:

Tissue	*Concentration (mg/kg or mg/L)*
Brain	10.7
CSF	1.9
Liver	2.4
Kidney	5.6
Spleen	1.5
Lung	0.8
Muscle (thigh)	3.9
Fat (thigh)	15.1
Abdominal skin	8.4
Skin with subcutaneous fat	11.8
Liquid from a skin blister	< LoD
Blood	1.1
Urine	< LoD

[Drasch *et al.* 1987].

Note For an overview of the pharmacology and toxicology of sulfur mustard, see Dacre and Goldman [1996] and Balali-Mood and Hefazi [2005]; for an evaluation of analogues of DRDE-07 as prophylactic agents in sulfur mustard toxicity, see Kulkarni *et al.* [2006]; for methods for detecting exposure to sulfur mustards by analysis of adducts in blood, see Noort *et al.* [2000, 1997, 1999] and Black *et al.* [1997].

Protein Binding Approximately 20% bound to albumin [Noort *et al.* 2000].

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Sulindac

Analgesic

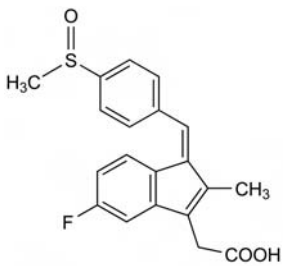
$C_{20}H_{17}FO_3S$ = 356.4

CAS—38194-50-2

IUPAC Name 2-[(3*Z*)-6-Fluoro-2-methyl-3-[(4-methylsulfinylphenyl)methylidene]inden-1-yl]acetic acid

Synonyms (1*Z*)-5-Fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-1*H*-indene-3-acetic acid; MK-231.

Proprietary Names *Aclin*; *Apo-Sulin*; *Artribid*; *Arthrocine*; *Cenlidac*; *Citireuma*; *Clinoril*; *Copal*; *Daclin*; *Kenalin*; *Lyndak*; *Novo-Sundac*; *Saldac*; *Sulartrene*; *Sulene*; *Sulindal*; *Sulreuma*.



Chemical Properties A yellow crystalline powder. Mp about 182° to 185°. Practically insoluble in water; slightly soluble in ethanol, acetone, chloroform and methanol. pK_a 4.7. Log *P* (octanol/water), 3.4.

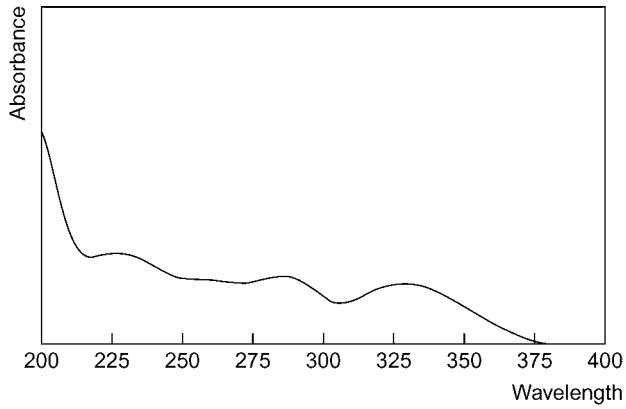
Colour Tests Liebermann's reagent—brown; Mandelin's test—orange; Marquis test—green (slow); sulfuric acid—brown.

Thin-layer Chromatography System TD—R_f 0.14; system TE—R_f 0.04; system TF—R_f 0.10; system TG—R_f 0.13; system TAD—R_f 0.34; system TAE—R_f 0.87; system TAJ—R_f 0.39; system TAK—R_f 0.40; system TAL—R_f 0.92 (chromic acid solution, white; Ludy Tenger reagent, orange-brown).

Gas Chromatography System GA—sulindac RI 2890; M (sulfide) RI 2896; sulindac-ME RI 3220; system GD—methyl derivative RRT 0.49 relative to n-$C_{16}H_{34}$.

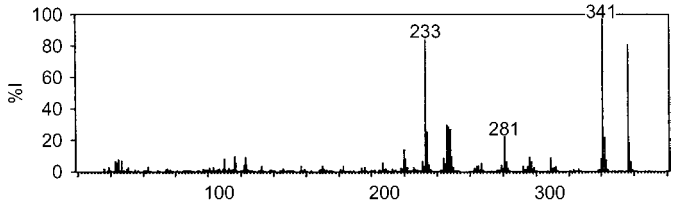
High Performance Liquid Chromatography System HD—*k* 1.25; system HV—RRT 0.78 (relative to meclofenamic acid); system HX—RI 488; system HY—RI 462; system HZ—retention time 3.9 min (sulindac), retention time 7.2 min (sulindac sulfoxide); system HAA—retention time 16.6 min.

Ultraviolet Spectrum Methanolic acid—284 (A^1_1=420a), 327 nm (A^1_1=373a); aqueous alkali—280 (A^1_1=442b), 327 nm (A^1_1=364b).



Infrared Spectrum Principal peaks at wavenumbers 1704, 1006, 1018, 1160, 1270, 1603 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 341, 233, 356, 246, 247, 248, 234, 281.



Quantification

Plasma **HPLC** UV detection. Sulindac and its sulfide and sulfone metabolites. Limit of detection, 0.1 mg/L [Stubbs *et al.* 1987]. UV detection. Sulindac and its sulfide and sulfone metabolites. Limit of detection, 250 µg/L [Musson *et al.* 1984]. UV detection. Sulindac and its sulfide and sulfone metabolites. Limit of detection, 100 µg/L in plasma [Swanson, Boppana 1981].

Serum **HPLC** Fluorescence detection. Sulindac and its sulfide and sulfone metabolites. Limit of detection, 10 µg/L [Siluveru, Stewart 1995].

Urine **HPLC** See Plasma [Swanson, Boppana 1981; Musson *et al.* 1984; Stubbs *et al.* 1987].

Bile **HPLC** See Plasma [Musson *et al.* 1984].

Gastric fluid **HPLC** See Plasma [Musson *et al.* 1984].

Disposition in the Body Sulindac is readily absorbed after oral administration. It is metabolised by oxidation to the sulfone and by reduction to the sulfide, which is thought to be responsible for most of the pharmacological activity. A total of about 36% of a dose is excreted in the urine in 24 h and about 75% in 4 days. In 4 days, about 30% of a dose is excreted as the sulfone and its conjugate, and 20% as unchanged drug and its conjugate; no significant amount of the sulfide or its conjugate is found in the urine. Sulindac and its metabolites are excreted in the bile and undergo extensive enterohepatic circulation. Up to about 25% of a dose is eliminated in the faeces in 4 days, with <2% as unchanged drug.

Therapeutic Concentration

A single dose of 200 mg to 14 subjects produced a mean peak plasma concentration of about 4 mg/L after 1 h; mean peak plasma concentrations for the sulfide and sulfone were about 3 and about 2 mg/L, respectively, after 2 h [Duggan *et al.* 1977].

Following daily oral doses of 200 mg, twice a day to 12 subjects, mean maximum steady-state plasma concentrations were: sulindac 5.0 mg/L, sulfide 6.9 mg/L, sulfone 2.6 mg/L; a diurnal variation in plasma concentrations was reported with lower concentrations being attained after the evening dose. After oral administration of 400 mg once daily to 12 subjects, maximum steady-state plasma concentrations were: sulindac 8.7 mg/L, sulfide 8.8 mg/L, sulfone 3.9 mg/L [Swanson *et al.* 1982].

In 6 patients with end-stage renal failure a single 300-mg oral dose of sulindac produced a mean maximum plasma concentration of 6.26 mg/L and 0.87 mg/L of sulindac and sulindac sulfide, respectively, at 1.6 h and 2.6 h, compared with 11.4 mg/L and 7.68 mg/L at 1.67 h and 7.68 h in normal subjects [Ravis *et al.* 1993].

Toxicity

Postmortem concentrations of sulindac in a 4-year-old child who accidentally overdosed with disopyramide and sulindac were: blood 12.2 mg/L, liver 12.4 µg/g, bile 1.251 g/L and urine 29.8 mg/L [Singer, Mozayani 1995].

Half-life Plasma half-life, sulindac about 7 h, sulfide metabolite about 16 to 18 h.

Protein Binding Sulindac, sulfone and sulfide, about 95%.

Note For a review of the pharmacokinetics of sulindac, see Brogden *et al.* [1978].

Dose 200 to 400 mg daily.

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Sulpiride

Tranquilliser

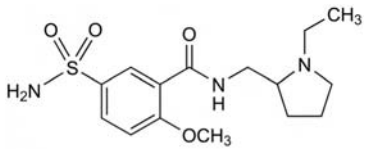
$C_{15}H_{23}N_3O_4S$ = 341.4

CAS—15676-16-1

IUPAC Name *N*-[(1-Ethylpyrrolidin-2-yl)methyl]-2-methoxy-5-sulfamoylbenzamide

Synonym 5-(Aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide

Proprietary Names *Abilit*; *Aiglonyl*; *Arminol*; *Championyl*; *Depex*; *Desisulpid*; *Dobren*; *Dogmatil*; *Dolmatil*; *Eglonyl*; *Equilid*; *Espiride*; *Guastil*; *Intrasil*; *Levobren*; *Levopraid*; *Lisopride*; *Meresa*; *Neogama*; *Omperan*; *Sernevin*; *Sulp*; *Sulpitil*; *Sulpivert*; *Sulpor*; *Sulpril*; *Synedil*.



Chemical Properties A white to almost white crystalline powder. Mp 178° to 180°. Soluble 1 in ~2200 of water, 1 in 100 of ethanol, 1 in ~220 of chloroform, 1 in ~2600 of ether and 1 in 50 of methanol; practically insoluble in benzene. Dissolves in dilute solutions of mineral acids and alkali hydroxides. pK_a 8.9; 10.19. Log *P* (octanol/water), 0.57. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

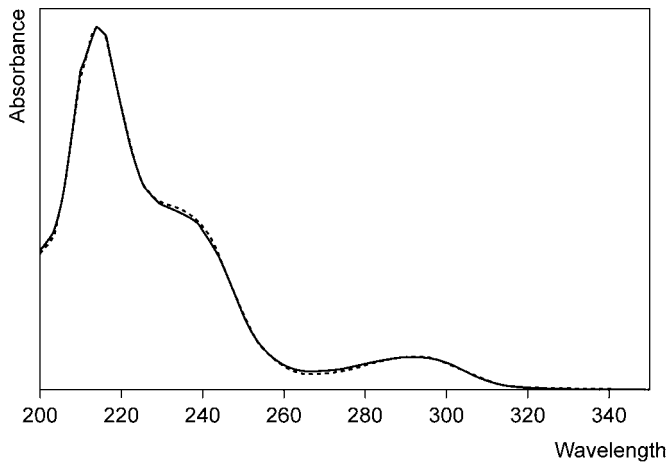
Thin-layer Chromatography System TA—R_f 0.38; system TB—R_f 0.00; system TE—R_f 0.34; system TAE—R_f 0.17.

Plate: Silica gel F_{254} (20 × 10 cm, 0.2 mm layer). Mobile phase: methylene chloride : methanol : 25% ammonia solution (18 : 2.8 : 0.4). Developed with ninhydrin solution and UV. R_f (× 100), 84.8 [Agbaba *et al.* 1999].

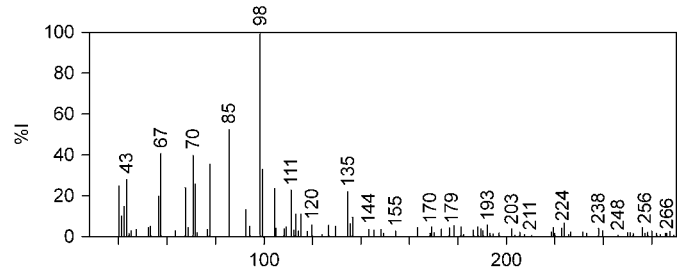
Gas Chromatography System GA—sulpiride RI 3102, M (-Me) RI 3125, M (-Me_2) RI 2995, art (-SO_2NH) RI 2295; system GB—not eluted.

High Performance Liquid Chromatography System HX—RI 259; system HY—RI 235; system HZ—RT 2.0 min; system HAA—RT 3.9 min; system HAZ—*k* 0.02.

Ultraviolet Spectrum Aqueous acid—292 nm (A^1_1 = 70a) No alkaline shift.



Mass Spectrum Principal ions at *m/z* 98, 70, 214, 111, 134, 341, 199, 326.



Quantification

Blood **GC-MS** Column: BPX5 (15 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 70° for 1 min to 220° at 15°/min to 260° at 5°/min to 330° at 25°/min. MSD. Limit of detection, 25 µg/L [Rop *et al.* 1999].

Plasma **HPLC** Column: LiChrospher Si 60 (250 × 4 mm i.d., 5 µm). Mobile phase: 0.5% TEA (pH 4.0): methanol : acetonitrile (10 : 5 : 85), flow rate 1.8 mL/min. Fluorescence detection (λ_{ex} = 300 nm, λ_{em} = 365 nm). Limit of quantification, 20 µg/L [Huang *et al.* 2001]. Column: Spherisorb ODS-II-5 (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L monopotassium phosphate (pH 3.0, 50 : 50), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 300 nm, λ_{em} = 365 nm). Retention time: 7.7 min. Limit of detection, 1 µg/L [Tokunaga *et al.* 1997]. Column: silica. Mobile phase: acetonitrile : 0.1 mol/L ammonium acetate (94 : 6). UV detection (λ = 240 nm). Limit of quantification, 5 µg/L [Jitsufuchi *et al.* 1997]. Column: Spherisorb ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: 2.5 mmol/L sodium heptane sulfonate : methanol : diethylamine (pH 3.5, 700 : 300 : 1), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex}=300 nm, λ_{em}=365 nm). Retention time: 8.0 min. Limit of detection, 10 µg/L [Nicolas *et al.* 1986]. Column: LiChrosorb RP-18 (120 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.05 mol/L phosphate buffer (pH 7.98, 14 : 28 : 58), flow rate 1.0 mL/min. Electrochemical detection. Limit of quantification, 1 µg/L [Sugnaux *et al.* 1983].

LC-MS Column: Inertsil C_8 (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol : 10 mmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 1.0 mL/min. Positive ion mode, full scan mode. Retention time: 4.3 min. Limit of quantification, 0.15 mg/L, limit of detection, 0.087 mg/L [Shinozuka *et al.* 2006].

HPLC Full scan or SIM acquisition mode. Limit of quantification, 10 µg/L [Jitsufuchi *et al.* 1997]. Column: µBondapak C_{18}. Mobile phase: acetonitrile : acetic acid : water (10 : 1 : 89), flow rate 1.5 mL/min. EI ionisation. Limit of detection not reported [Imondi *et al.* 1978].

Serum **HPLC** Column: CBH (150 × 4.0 mm i.d., 5 µm). Mobile phase: propan-2-ol : 1.25 mmol/L EDTA : 0.01 mol/L sodium phosphate buffer (pH 7.0, 40 : 75 : 885), flow rate 0.9 mL/min. UV detection (λ = 214 nm). Limit of detection, 50 µg/L [Müller *et al.* 2001]. Column: Nucleosil C_{18} (150 × 4 mm i.d., 5 µm). Mobile phase: water-acetic acid (99 : 1) : acetonitrile-acetic acid-water (50 : 1 : 49, 88 : 12 to 40 : 60 in 10 min), flow rate 1.2 to 1.6 mL/min (400 psi). Fluorescence detection (λ_{ex} = 299 nm, λ_{em} = 342 nm). Limit of detection, 10 µg/L [Alfredsson *et al.* 1979]

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 8.3 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **HPLC** Column: Shimpack ODS. Mobile phase: 10 mmol/L phosphate buffer (pH 2.5) : acetonitrile (89 : 11), flow rate 1.5 mL/min. UV detection (λ = 240 nm). Limit of detection, 0.1 mg/L [Shinkuma *et al.* 1989]. Column: Lichrosorb PR 8 (250 × 3 mm i.d., 10 µm). Mobile phase: acetonitrile : disodium hydrogen phosphate-0.05 mol/L sodium dihydrogen phosphate (pH 6.0, 12 : 88), flow rate 1.5 mL/min. UV detection (λ = 215 nm). Retention time: ~2.5 min. Limit of detection, 5 mg/L [Verbiese-Genard *et al.* 1980]. See Serum. Limit of detection, 200 µg/L [Alfredsson *et al.* 1979]

Note For a fluorescence method for the detection of sulpride in urine see Nie *et al.* [2007].

CSF **HPLC** See Serum [Alfredsson *et al.* 1979].

Disposition in the Body Sulpiride is slowly and incompletely absorbed after oral administration. It is rapidly distributed to tissues; passage across the blood brain–barrier, however, is not significant. Sulpiride is not metabolised to a significant extent. Approximately 70% of an IV dose is excreted as unchanged drug in the urine in 36 h [Shinkuma *et al.* 1989]; up to ~20% of an oral dose is excreted in the urine as unchanged drug. A high percentage of the drug is recovered in faeces. Sulpiride has been detected in breast milk.

Therapeutic Concentration The therapeutic serum concentration is 0.04–0.6 mg/L.

After a single oral dose of 200 mg given to 10 subjects, peak plasma concentrations of 0.18–0.32 mg/L (mean 0.24) were attained in 3–4 h [Sugnaux *et al.* 1983].

Following oral administration of 50 mg, three times a day, to 14 subjects, steady-state plasma concentrations of 0.03–0.6 mg/L (mean 0.18) were reported, 4–7 h after the final dose [Salminen *et al.* 1980].

Toxicity

A 38-year-old woman with a lengthy history of mental illness was found dead at her home. Empty containers of sulpiride medication were found in her bedroom along with a variety of other pharmaceutical drugs, including cyamemazine, clorazepate and maprotiline. Ingestion of approximately 34 times the therapeutic concentration of sulpiride resulted in death of the

individual. The postmortem blood sulpiride concentration was 38 mg/L; and the other drugs were also detected in minute amounts [Rop *et al.* 1999].

A 55-year-old woman poisoned herself with sulpiride and was admitted to the intensive care unit with cardiovascular shock. The woman died 10 h after admission to hospital despite medical treatment. The postmortem sulpiride concentrations were 38.7 mg/L in blood, 803.2 mg/L in urine and 155.6 mg/L in the gastric contents. The blood drug concentration was ~60 times the therapeutic level; the cause of death was determined as sulpiride intoxication [Kintz *et al.*1994].

Bioavailability ≈30%.

Half-life Plasma half-life, after IV administration, 4–13 h; after oral administration, variously reported as 6–15 h (mean 10) and 13–41 h (mean 25).

Volume of Distribution ≈2–3 L/kg.

Clearance ≈6 mL/min/kg from plasma.

Protein Binding <40%.

Dose Adults: oral dosage 200 to 400 mg twice daily, maximum 1200 mg twice daily; IM dosage 200 to 800 mg daily. Children over 14 years of age: 3 to 5 mg/kg body weight daily.

Agbaba D *et al.* (1999). Quantitative analysis of sulpiride and impurities of 2-aminomethyl-1-ethylpyrrolidine and methyl-5-sulphamoyl-2-methoxybenzoate in pharmaceuticals by high-performance thin-layer chromatography and scanning densitometry. *J AOAC Int* 82: 825–829.

Alfredsson G *et al.* (1979). Quantitative analysis of sulpiride in body fluids by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 164: 187–193.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Huang MC *et al.* (2001). Development of a high-performance liquid chromatographic method for bioanalytical applications with sulpiride. *J Chromatogr B Biomed Sci Appl* 763: 157–163.

Imondi AR *et al.* (1978). Metabolism of sulpiride in man and rhesus monkeys. *Arch Int Pharmacodyn Ther* 232: 79–91.

Jitsufuchi N *et al.* (1997). Selective determination of sultopride in human plasma using high-performance liquid chromatography with ultraviolet detection and particle beam mass spectrometry. *J Chromatogr B Biomed Sci Appl* 690: 153–159.

Kintz P *et al.* (1994). Fatal Sulpiride Intoxication. *TIAFT Bull Case Notes* 24http://www.tiaft.org/tmembers/cnrarchive/24_4_5.php.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Müller MJ *et al.* (2001). Serum levels of sulpiride enantiomers after oral treatment with racemic sulpiride in psychiatric patients: a pilot study. *Pharmacopsychiatry* 34: 27–32.

Nicolas P *et al.* (1986). Improved determination of sulpiride in plasma by ion-pair liquid chromatography with fluorescence detection. *J Chromatogr* 381: 393–400.

Nie JF *et al.* (2007). Determination of sulpiride in human urine using excitation-emission matrix fluorescence coupled with second-order calibration. *Anal Sci* 23: 1377–1382.

Rop PP *et al.* (1999). Toxicological analysis of sulpiride in a lethal poisoning case. *J Anal Toxicol* 23: 294–296.

Salminen JK *et al.* (1980). Sulpride in depression: plasma levels and effects. *Curr Ther Res* 27: 109–115.

Shinkuma D *et al.* (1989). The bioavailability of sulpiride taken as a film-coated tablet with sodium bicarbonate, cimetidine, natural orange juice or hydrochloric acid. *Int J Clin Pharmacol Ther Toxicol* 27: 499–502.

Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.

Sugnaux FR *et al.* (1983). Dose-dependent pharmacokinetics of sulpride and sulpride-induced prolactin secretion in man. *Eur J Drug Metab Pharmacokinet* 8: 189–200.

Tokunaga H *et al.* (1997). Sensitive determination of sulpiride in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 691: 203–207.

Verbiese-Genard N *et al.* (1980). High performance liquid chromatographic determination of sulpiride (Dogmatil) in urine. *J Pharm Belg* 35: 24–30.

Sultiame

Anticonvulsant

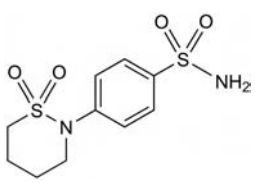
$C_{10}H_{14}N_2O_4S_2$ = 290.4

CAS—61-56-3

IUPAC Name 4-(1,1-Dioxothiazinan-2-yl)benzenesulfonamide

Synonyms Sulthiame; 4-(tetrahydro-2*H*-1,2-thiazin-2-yl)benzenesulfonamide-*S*,*S*-dioxide.

Proprietary Name *Ospolot*



Chemical Properties A white crystalline powder. Mp 180° to 182°. Soluble 1 in 2000 of water, 1 in 350 of ethanol, 1 in 700 of chloroform and 1 in 500 of ether; readily soluble in alkaline solutions. pK_a 10.0. Log *P* (octanol/water), 1.0. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

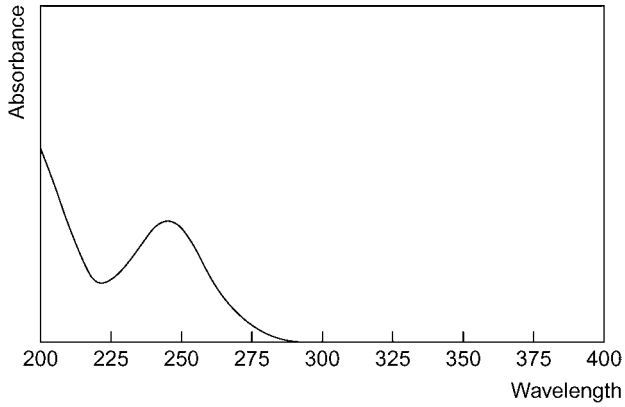
Colour Tests Copper sulfate (method 1)—blue; Koppanyi–Zwikker test—violet-blue.

Thin-layer Chromatography System TD—R_f 0.23; system TE—R_f 0.57; system TF—R_f 0.43; system TAD—R_f 0.42; system TAE—R_f 0.81.

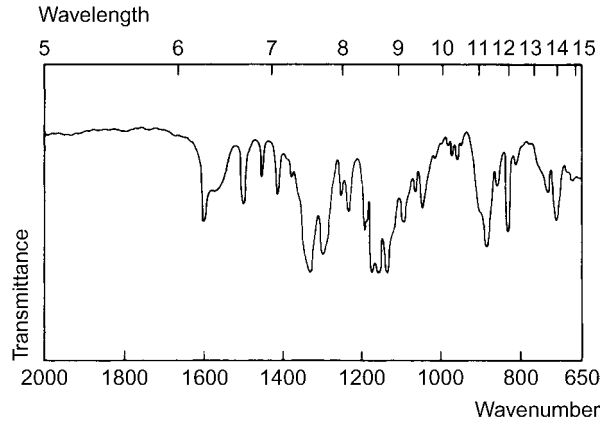
Gas Chromatography System GA—sultiame RI 3000, sultiame-Me RI 2880, sultiame-Me_2 RI 2815.

High Performance Liquid Chromatography System HE—*k* 1.57; system HX—RI 344; system HY—RI 275.

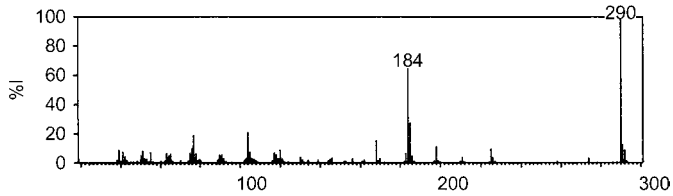
Ultraviolet Spectrum Methanol—246 nm (A^1_1= 390a).



Infrared Spectrum Principal peaks at wavenumbers 1138, 1158, 1172, 1293, 889, 1192 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 290, 184, 185, 104, 77, 168, 291, 198.



Quantification

Plasma **HPLC** UV detection. Limit of detection, 150 µg/L [Sadanaga *et al.* 1981]. UV detection [Berry *et al.* 1979].

Serum **HPLC** [Juergens 1991].

Disposition in the Body Readily but variably absorbed after oral administration. During long-term therapy 17–70% (mean, 32) of the daily dose is excreted in 24 h, of which about 60% is unchanged drug and the remainder is an inactive metabolite. Up to 15% of a dose may be eliminated in the faeces.

Therapeutic Concentration

Following daily oral doses of 3–14.5 mg/kg to 36 subjects, serum concentrations ranged from 0.5–12.5 mg/L; peak concentrations were attained 1–5 h after a dose [Olesen 1968].

Toxicity Recovery has occurred after the ingestion of 4–5 g. Blood concentrations of 12–26 mg/L have been reported in fatalities.

Dose 200 to 600 mg daily.

Berry DJ *et al.* (1979). Determination of sulthiame, tetrahydro-2-p-sulphamoyl-phenyl-2h-1,2-thiazine-1,1-dioxide, in plasma at therapeutic concentrations, using high-performance liquid chromatography. *J Chromatogr* 171: 363–370.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Juergens UH (1991). Analysis of sulthiame in serum by narrow-bore high-performance liquid chromatography. Comparison of direct sample injection with pre-column switching and extrelut extraction. *J Chromatogr* 553: 7–13.

Olesen OV (1968). Determination of sultiam (ospolot) in serum and urine by thin-layer chromatography: serum levels and urinary output in patients under long term treatment. *Acta Pharmacol Toxicol* 26: 22–28.

Sadanaga T *et al.* (1981). Determination of sulthiame in plasma by high-performance liquid chromatography. *Chem Pharm Bull (Tokyo)* 29: 872–874.

Sumatriptan

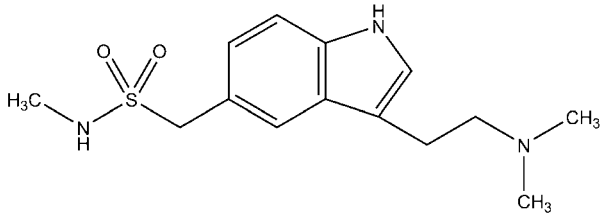
5-HT$_1$ Receptor Agonist, Antimigraine, Sulfonamide

$C_{14}H_{21}N_3O_2S = 295.4$

CAS—103628-46-2

IUPAC Name 1-[3-(2-Dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide

Synonyms 3-(2-Dimethylaminoethyl)indol-5-yl-*N*-methylmethanesulfonamide; GR-43175X.



Chemical Properties White to pale yellow powder. Mp 169° to 171°. Store in airtight containers at below 30°. Do not allow to freeze. Protect from light. Log *P* (octanol/water), 0.8 [O'Neil *et al.* 2006].

Sumatriptan Succinate

$C_{14}H_{21}N_3O_2S,C_4H_6O_4 = 413.5$

CAS—103628-46-2; 103628-47-3 (sumatriptan hemisuccinate)

Synonym GR-43175C

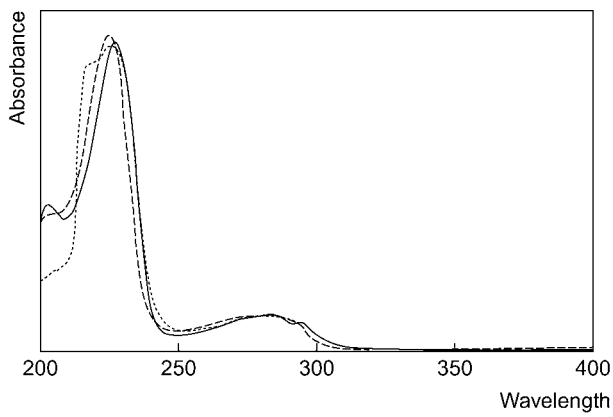
Proprietary Names *Amigrenin*; *Arcoiran*; *Cinie*; *Diletan*; *Glaxotriptan*; *Imigran*; *Imitrex*; *Micranil*; *Migraneitor*; *Migraval*; *Sumamigren*; *Somatran*; *Sumagran*; *Sumax*; *Suvalan*.

Chemical Properties White or almost white powder. Freely soluble in water; practically insoluble in dichloromethane; sparingly soluble in methyl alcohol. A 1% solution in water has a pH of 4.5 to 5.3. Protect from light [O'Neil *et al.* 2006]. Oral liquid preparations of sumatriptan succinate prepared from crushed tablets in 3 different syrups were stable for at least 21 days when stored at 4° and protected from light [Fish *et al.* 1997]. Sumatriptan succinate 12 mg/mL, stored in polypropylene syringes and exposed to fluorescent light or kept in the dark, was stable for 72 h at 2° to 5° and for at least 24 h at 25° [Nii *et al.* 1999].

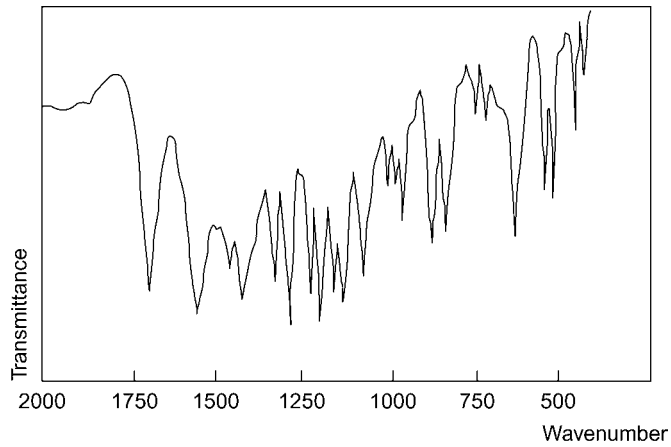
Thin-layer Chromatography Plates: silica gel GF_{254} (20 × 20 cm, 0.25 mm). Solvent system: cyclohexane : dichloromethane : diethylamine (50 : 40 : 10). R_f: sumatriptan 0.17, degradation products 0.00 and 0.32. Limit of quantification, 0.8 mg/L; limit of detection, 0.5 mg/L [Bebawy *et al.* 2003].

High Performance Liquid Chromatography Column: ethylsilane (220 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile-methanol (6 : 1) : 0.1% aqueous formic acid (10 : 90), flow rate 0.6 mL/min. DAD. Retention time: 11.0 min [Xu *et al.* 2001].

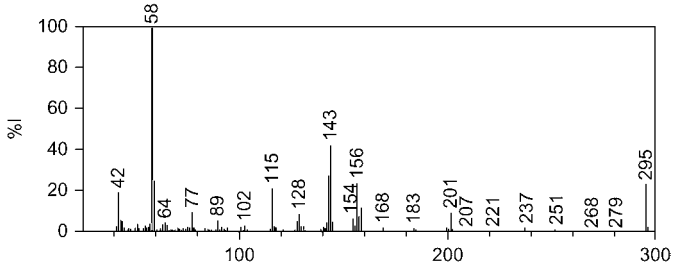
Ultraviolet Spectrum Aqueous acid (0.1 mol/L HCl)—227, 277, 283, 292 nm; aqueous alkali (0.1 mol/L NaOH)—228, 278, 284, 294 nm; methanol—228, 278, 284, 294 nm. [Rochholz et al 1995 and Bebawy et al 2003]



Infrared Spectrum Bebawy et al 2003.



Mass Spectrum Principal ions at *m/z* 58, 143, 142, 295, 156, 59, 115, 42 (MSTFA derivative) Rochholz et al 1995.



Quantification

Plasma **HPLC** Column: C_{18} (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 25 mmol/L sodium phosphate monobasic (pH 7.5; 60 : 40), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 225$ nm, $\lambda_{em} = 350$ nm). Retention time: 2.75 min. Limit of quantification, 1 µg/L [Ge *et al.* 2004]. Column: LiChrospher 60 RP Select B (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.02 mol/L phosphate buffer (pH 7.0; 22 : 78), flow rate 1.0 mL/min. Electrochemical detection. Retention time: sumatriptan 4 min, metabolite 10.2 min. Limit of quantification, 1 µg/L [Wojnar-Horton *et al.* 1996]. Column: CN-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.04 mol/L potassium phosphate (pH 6.6) : methanol (55 : 45), flow rate 1 mL/min. Electrochemical detection. Retention time: 8.8 min. Limit of quantification, 0.5 µg/L [Franklin *et al.* 1996].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (20 : 80) with 5 mmol/L ammonium acetate, flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1 µg/L [Tan *et al.* 2007]. Column: C_8 (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water : formic acid (90 : 10 : 0.1), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3.8 min. Limit of quantification, 0.7 µg/L [Boulton *et al.* 2003]. Column: C_{18} (300 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate (pH 7) : methanol (85 : 15), flow rate 1.0 mL/min. APCI, MRM acquisition mode. Retention time: 2.5 min. Limit of quantification, 0.2 µg/L [Cheng *et al.* 1998]. Column: CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : water (36 : 6 : 58) with 0.1% trifluoroacetic acid, flow rate 1.2 mL/min. APCI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 µg/L [McLoughlin *et al.* 1996]. Column: C_{18} (110 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 0.1 mol/L ammonium acetate (60 : 40), flow rate 1.0 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 2.1 min. Limit of quantification, 2 µg/L [Oxford, Lant 1989].

Serum **HPLC** Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 7.0) : methanol (60 : 40), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 5.1 min. Limit of quantification, 1 µg/L [Poondru *et al.* 2000]. Column: Spherisorb ODS-1 (100 × 4.6 mm i.d., 5 µm). Mobile phase: 75 mmol/L phosphate buffer (pH 7.0) : methanol (35 : 65), flow rate 1.0 mL/min. Electrochemical detection. Retention time: 6 min. Limit of quantification, 1 µg/L [Dunne, Andrew 1996].

LC-MS Column: Alltech Solvent Miser Silica (150 × 2.1 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 4 to final pH 2.7) : methanol : acetonitrile (80 : 10 : 10), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: sumatriptan 2.68 min, naratriptan 2.71 min, zolmitriptan 2.95 min, rizatriptan 3.48 min. Limit of quantification, 0.25 µg/L [Vishwanathan *et al.* 2000].

Urine **TLC** Plates: silica gel $60F_{254}$ (sprayed with Bratton–Marshall reagent, 4-dimethylamino-cinnamaldehyde or fluorescamine solution). Solvent systems: ethylacetate : methanol : ammonia (A, 56 : 36 : 3), dichloromethane : ethanol : ammonia (B, 80 : 16 : 2), ethylacetate : propan-2-ol : water : ammonia (C, 50 : 30 : 16 : 4). R_f: (A) sumatriptan 0.10, indole acetic acid metabolite (M1) 0.57; (B) sumatriptan 0.48, M1 1; (C) sumatriptan 0.73, M1 0.38. Limit of quantification, 40 ng/spot [Rochholz *et al.* 1995].

GC-MS Column: OV-1 (12 m × 0.2 mm i.d., 0.33 μm). Temperature programme: 60° for 1 min to 150° at 20°/min to 280° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention indices: sumatriptan 2800, M1 2900 (MSTFA derivatives). Limit of quantification not reported [Rochholz *et al.* 1995].

LC-MS Column: C_{18} (300 × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L ammonium acetate (pH 7) : methanol (85 : 15), flow rate 1.0 mL/min. APCI, MRM acquisition mode. Retention time: 2.5 min. Limit of quantification, 0.2 μg/L [Cheng *et al.* 1998].

***Other* HPLC** Cleaning Validation Samples. Column: C_{18} (150 × 4.6 mm i.d., 4 μm). Mobile phase: 0.05 mol/L ammonium phosphate monobasic : acetonitrile (pH 3.3; 84 : 16), flow rate 1.0 mL/min. UV detection (λ = 228 nm). Retention time: 4.1 min. Limit of quantification, 9 μg/L; limit of detection, 3 μg/L [Nozal *et al.* 2002]. Polypropylene Syringes. Column: C_{18} (250 × 4.5 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.01 mol/L dibutylamine phosphate in 0.025 mol/L sodium dihydrogen phosphate (pH 7; 25 : 75), flow rate 1.0 mL/min. UV detection (λ = 282 nm). Retention time: 6.5 min. Limit of quantification not reported [Nii *et al.* 1999]. Breast Milk. Column: LiChrospher 60 RP Select B (250 × 4.6 mm i.d.). Mobile phase: acetonitrile: 0.02 mol/L phosphate buffer (pH 7.0; 22 : 78), flow rate 1.0 mL/min. Electrochemical detection. Retention time: sumatriptan 4 min, metabolite 10.2 min. Limit of quantification, 1 μg/L [Wojnar-Horton *et al.* 1996].

LC-MS Rabbit plasma. Column: Nova-Pak C_8 (150 × 2.0 mm i.d., 4 μm). Mobile phase: 20 mmol/L ammonium acetate-acetonitrile (90 : 10) : 20 mmol/L ammonium acetate-acetonitrile (20 : 80; 80 : 20 to 20 : 80 over 20 min), flow rate 0.5 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Duléry *et al.* 1997].

Note For a degradation study of sumatriptan succinate, see Xu *et al.* [2001]; for a spectrophotometric stability-indicating method for the determination of sumatriptan succinate, see Bebawy *et al.* [2003].

Disposition in the Body Rapidly but incompletely absorbed when given orally and undergoes first-pass metabolism, resulting in a low absolute bioavailability. Peak plasma concentrations after oral doses are achieved in ~2 h. Sumatriptan is extensively metabolised in the liver predominantly by MAO type A and is excreted mainly in the urine as the inactive indole acetic acid derivative and its glucuronide (3% of a dose as unchanged sumatriptan and 42% as the major metabolite either as a free acid or glucuronide conjugate). Sumatriptan and its metabolites also appear in the faeces. Distribution of sumatriptan in the CNS is poor and penetration across the blood–brain barrier is slow. Small amounts of sumatriptan are distributed into breast milk (~0.24% of a 6 mg SC dose). It crosses the placenta; although only a very small quantity reaches the fetus.

Therapeutic Concentration

In a study investigating the chronopharmacokinetics of oral sumatriptan, a group of 12 healthy male volunteers was administered a 100 mg dose at 4 different times (7.00 am, 1.00 pm, 7.00 pm and 1.00 am) with a 7-day washout period between each administration. Mean peak serum concentrations were reported as follows:

	Time of administration			
	7 am	1 pm	7 pm	1 am
C_{max} (μg/L)	59.1	52.5	41.9	51.6
Time (h)	1.96	1.96	1.67	2.00

The circadian variations in the activity of MAO responsible for oxidative deamination of sumatriptan are presumed to have affected the first-pass metabolism of sumatriptan, contributing to the observed changes in peak plasma concentrations in the study [Poondru *et al.* 2000].

A group of adolescents (aged 12 to 17 years old) with a history of common classical migraine was administered a single 20 mg sumatriptan as a nasal spray. The mean peak sumatriptan serum concentration reached was 13.9 μg/L after ~2 h [Christensen *et al.* 2003].

In a dose-ranging study of nasal sumatriptan, groups of healthy volunteers were administered single doses of 5, 10 or 20 mg sumatriptan. Peak plasma concentrations for the 3 doses were 4.7, 8.5 and 14.4 μg/L, respectively, reached within 1.5 h. In a separate multiple dosing study, healthy volunteers were administered 20 mg sumatriptan intranasally 3 times daily for 10 doses. Mean peak serum concentrations of sumatriptan and its metabolite were reported as follows:

	Sumatriptan		***Metabolite***	
	Day 1	Day 4	Day 1	Day 4
C_{max} (μg/L)	13.1	16.4	63.3	72.0
Time (h)	1.75	0.88	3.00	2.00

[Moore *et al.* 1997].

Five lactating women were administered 6 mg sumatriptan SC. Peak plasma and breast milk sumatriptan concentrations were reported as 80.2 μg/L after 0.25 h and 87.2 μg/L after 2.5 h, respectively [Wojnar-Horton *et al.* 1996].

A group of 24 healthy subjects received in random order at least 3 days apart and on 4 separate occasions: 6 mg SC sumatriptan, 25 mg sumatriptan oral tablet, 25 mg sumatriptan suppository, and 20 mg sumatriptan intranasal spray. Mean peak serum sumatriptan concentrations for the different routes of administration were reported as follows:

	Route of administration			
	Subcutaneous	Oral	Suppository	Intranasal
C_{max} (μg/L)	69.5	16.5	22.9	12.9
Time (h)	0.17	1.50	1.00	1.50

[Duquesnoy *et al.* 1998].

In a study investigating pharmacokinetics of different sumatriptan formulations, a group of 8 healthy volunteers was administered sumatriptan iontophoretically, orally (50 mg) or SC (6 mg). Four different iontophoretic patches were designed to deliver 1.5 mg over 1.5 h (A), 1.5 mg over 3.0 h (B), 6 mg over 3.0 h (C) or 12 mg over 6 h (D). Mean peak serum concentrations for the different formulations were reported as follows:

	Subcutaneous	***Oral***	***Patch A***	***Patch B***	***Patch C***	***Patch D***
C_{max} (μg/L)	58.4	26.4	8.3	5.2	28.7	34.6
Time (h)	0.28	1.31	1.50	2.30	2.22	4.14

[a][Siegel *et al.* 2007].

Toxicity The pharmacokinetics of oral sumatriptan in adult volunteers are not affected by the concomitant oral administration of contraceptives [Moore *et al.* 2002a], clarithromycin [Moore *et al.* 2002b], naproxen [Srinivasu *et al.* 2000], or by propranolol [Scott *et al.* 1991]. However, the extent of systematic sumatriptan exposure increased significantly in patients receiving pre-treatment with the MAO inhibitor moclobemide [Fuseau *et al.* 2002].

Bioavailability Approximately 14%, although is much greater following SC administration (96%). Bioavailability after intranasal doses is 16% of that achieved SC, with peak concentrations occurring in ~1.5 h.

Half-life Approximately 2 h.

Volume of Distribution Approximately 170 to 203 L.

Clearance Approximately 316 L/hr.

Protein Binding From 14% to 21%.

Dose May be given by mouth or SC as the succinate, and intranasally as the base. Doses are expressed in terms of the base; sumatriptan succinate 70 mg is equivalent to ≈50 mg of sumatriptan. It is given by mouth to adults aged 18 years and over; the recommended dose in the UK is 50 mg, although some patients may require 100 mg. When used intranasally, a clinical response can be expected in 15 min. In the UK, a single dose of 20 mg is given into one nostril, although 10 mg may be effective in some patients. In the USA, a dose of 5, 10 or 20 mg may be used. In patients aged 18 years and over, sumatriptan may be self-administered by subcutaneous injection in a single dose of 6 mg; a clinical response may be expected after 10 to 15 min.

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Moore KH *et al.* (2002b). The pharmacokinetics of sumatriptan when administered with clarithromycin in healthy volunteers. *Clin Ther* 24: 583–594.
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Suprofen

Analgesic

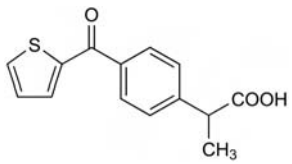
$C_{14}H_{12}O_3S = 260.3$

CAS—40828-46-4

IUPAC Name 2-[4-(Thiophene-2-carbonyl)phenyl]propanoic acid

Synonyms α-Methyl-4-(2-thienylcarbonyl)benzeneacetic acid; sutoprofen.

Proprietary Names *Masterfen*; *Profenol*; *Srendam*; *Sulprotin*; *Supranol*; *Suprocil*; *Suprol*; *Topalgic*.



Chemical Properties A white to slightly yellowish powder. Mp 124°. Sparingly soluble in water; soluble 1 in 5 of ethanol, 1 to 3 of acetone, 1 in about 4 of chloroform and 1 in about 20 of ether. pK_a 3.9. Log *P* (octanol/water), 2.8.

Thin-layer Chromatography System TAJ—R_f 0.37; system TAK—R_f 0.62; system TAL—R_f 0.87.

High Performance Liquid Chromatography System HY—RI 95.

Ultraviolet Spectrum Isopropyl alcohol—265 (A^1_1=550b), 291 nm (A^1_1=550b).

Infrared Spectrum Principal peaks at wavenumbers 1590, 1220, 1722, 1050, 1308, 743 cm^{-1} (KBr disk).

Quantification

Plasma **HPLC** Limit of detection, <0.05 mg/L [Muller *et al.* 1982]. UV detection. Limit of detection, 100 µg/L [Alton, Patrick 1978].

Urine **HPLC** See Plasma [Muller *et al.* 1982].

Disposition in the Body Suprofen is rapidly and well absorbed after oral administration. About 72% of a dose is excreted in the urine in 6 h, mainly as dihydrosuprofen glucuronide.

Therapeutic Concentration

After a single oral dose of 200 mg to 6 subjects, peak plasma concentrations of 4.5 to 28.9 (mean, 17.5) mg/L were attained in 0.25 to 2 h [Zulliger, Fassolt 1983].

Half-life Plasma half-life, about 2 h.

Protein Binding About 99.5%.

Dose 600 to 800 mg daily.

Alton KB, Patrick JE (1978). High-performance liquid chromatographic assay for suprofen, a potent new analgesic, in plasma. *J Pharm Sci* 67: 985–987.
Muller H *et al.* (1982). Quantitative determination of suprofen in human plasma and urine by fully automated high-performance liquid chromatography. *Arzneimittelforschung* 32: 257–260.
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Suxamethonium Chloride

Muscle Relaxant

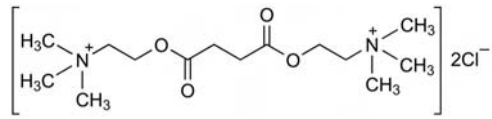
$C_{14}H_{30}Cl_2N_2O_4,2H_2O = 397.3$

CAS—306-40-1 (suxamethonium); 71-27-2 (chloride, anhydrous); 6101-15-1 (chloride, dihydrate)

IUPAC Name Trimethyl-[2-[4-oxo-4-[2-(trimethylazaniumyl)ethoxy]butanoyl]oxyethyl]azanium dichloride dihydrate

Synonyms Choline chloride succinate; 2,2′[(1,4-dioxo-1,4-butanediyl)bis(oxy)]bis[*N,N,N*-trimethylethanaminium] dichloride dihydrate; succicurarium chloride; succinylcholine chloride.

Proprietary Names *Anectine*; *Celocurin*; *Curacit*; *Ectinex*; *Ethicholine*; *Lysthenon*; *Midarine*; *Mioflex*; *Myotenlis*; *Pantolax*; *Quelicin*; *Scoline*; *Succicuran*; *Succinolin*; *Succinyl*; *Sucostrin*; *Sukolin*; *Uxicolin*.



Chemical Properties A white, hygroscopic, crystalline powder. Mp about 156° to 163°; anhydrous form Mp about 190°. Soluble 1 in 1 of water and 1 in 350 of ethanol; practically insoluble in chloroform and ether; soluble in methanol. Log *P* (octanol/water), −7.94.

Suxamethonium Bromide

$C_{14}H_{30}Br_2N_2O_4,2H_2O = 486.2$

CAS—55-94-7

Synonyms Choline bromide succinate; succinylcholine bromide.

Proprietary Name *Brevidil M*

Chemical Properties A white or creamy-white powder. Mp about 225°. Soluble 1 in 0.3 of water and 1 in 5 of ethanol; practically insoluble in chloroform and ether.

Suxamethonium Iodide

$C_{14}H_{30}I_2N_2O_4 = 544.2$

CAS—541-19-5

Synonyms Dithylinum; succinylcholine iodide.

Proprietary Name *Célocurine*

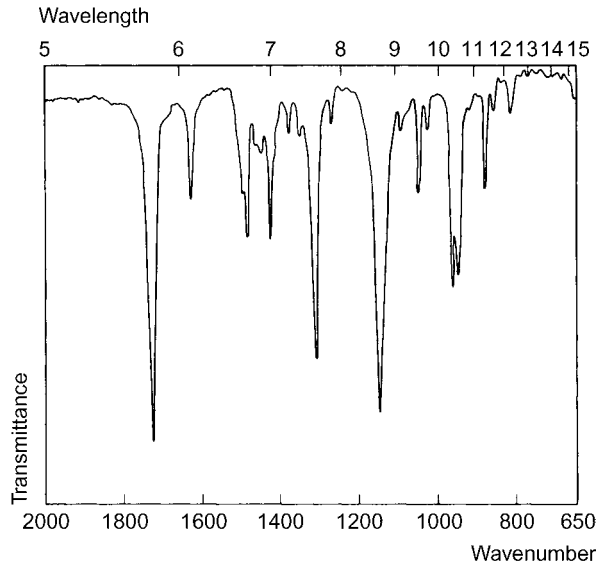
Chemical Properties A white, slightly hygroscopic, crystalline powder. Mp about 243° to 245°. Freely soluble in water; very slightly soluble in ethanol; practically insoluble in ether.

Thin-layer Chromatography System TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TN—R_f 0.35; system TO—R_f 0.10; system TAE—R_f 0.00; system TAF—R_f 0.00 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—not eluted.

Ultraviolet Spectrum No significant absorption, 230–360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1724, 1148, 1308, 961, 952, 1612 cm^{-1} (KBr disk).



Quantification

Plasma **MS** [Roy *et al.* 2001].

HPLC Electrochemical detection. Limit of detection, 156 nmol [Pitts *et al.* 2000]. Electrochemical detection. Limit of detection, 250 µg/L [Gao *et al.* 1998]. Fluorescence detection. Limit of detection, 100 µg/L [Lagerwerf *et al.* 1991].

Disposition in the Body Suxamethonium chloride is slowly and incompletely absorbed after oral administration; it is absorbed after IM administration but generally given by the IV route. It is rapidly distributed into the extracellular fluids throughout the body. Suxamethonium is rapidly hydrolysed in plasma and body tissues to succinylmonocholine (weak activity) and choline. Succinylmonocholine is then slowly hydrolysed to succinic acid and choline. <3% of a dose is excreted unchanged in the urine.

Toxicity

A 33-year-old anaesthesiologist, who committed suicide by injecting suxamethonium and thiobarbital, had a postmortem blood suxamethonium concentration of 14.3 mg/L 3 h after administration [Somogyi *et al.* 1989].

Half-life Plasma half-life, ≈3 min.

Dose Usually 20 to 100 mg of suxamethonium chloride, IV.

Gao H *et al.* (1998). Determination of succinylcholine in human plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl* 718: 129–134.

Lagerwerf AJ *et al.* (1991). Rapid determination of succinylcholine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 570: 390–395.

Pitts NI *et al.* (2000). Determination of succinylcholine in plasma by high-pressure liquid chromatography with electrochemical detection. *Br J Anaesth* 85: 592–598.

Roy JJ *et al.* (2001). Measurement of succinylcholine concentration in human plasma by electrospray tandem mass spectrometry. *Anal Biochem* 290: 238–244.

Somogyi G *et al.* (1989). Drug identification problems in two suicides with neuromuscular blocking agents. *Forensic Sci Int* 43: 257–266.

Suxethonium Bromide

Muscle Relaxant

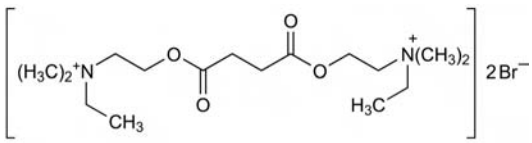
$C_{16}H_{34}Br_2N_2O_4 = 478.3$

CAS—111-00-2

IUPAC Name Ethyl-[2-[4-[2-[ethyl(dimethyl)azaniumyl]ethoxy]-4-oxobutanoyl]oxyethyl]-dimethylazanium dibromide

Synonym 2,2′-Succinyldioxybis(diethyldimethylammonium) dibromide

Proprietary Name *Brevidil E*



Chemical Properties A slightly hygroscopic, white or slightly cream, crystalline powder. Mp 158°. Readily soluble in water, yielding weakly acidic solutions. Log *P* (octanol/water) −7.0.

Thin-layer Chromatography System TA—R_f 0.01; system TN—R_f 0.40; system TO—R_f 0.23 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1740, 1163, 1638, 1270, 962, 1012 cm^{-1} (KBr disk).

Disposition in the Body After IV administration it is rapidly hydrolysed in plasma and body tissues to succinic acid and choline.

Dose 1.5 to 1.9 mg/kg IV.

Syrosingopine

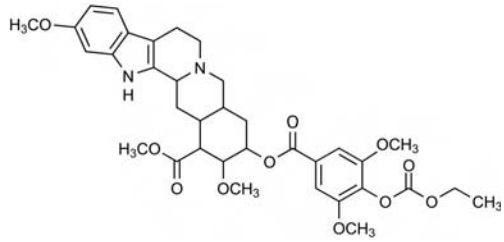
Antihypertensive

$C_{35}H_{42}N_2O_{11} = 666.7$

CAS—84-36-6

Synonyms 18-[[4-[(Ethoxycarbonyl)oxy]-3,5-dimethoxybenzoyl]oxy]-11,17-dimethoxyyohimban-16-carboxylic acid methyl ester; methyl carbethoxysyringoyl reserpate.

Proprietary Names *Isotense*; *Londomin*; *Raunova*; *Serinamin*; *Singoserp*; *Siringina.*

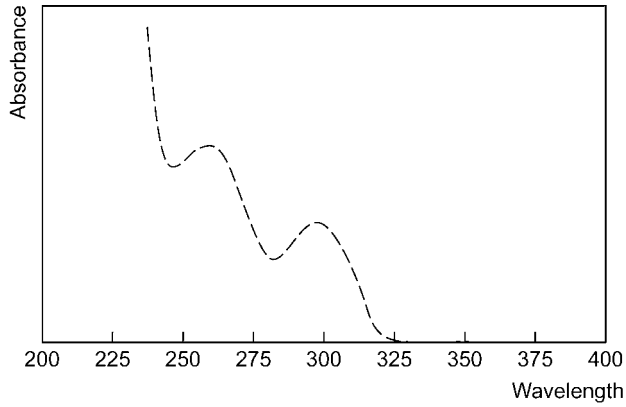


Chemical Properties A white or slightly yellowish crystalline powder. Mp 175° to 179°. Practically insoluble in water; freely soluble in chloroform and acetic acid; slightly soluble in ether.

Colour Tests Mandelin's test—green-brown; Marquis test—brown.

Thin-layer Chromatography System TA—R_f 0.78 (acidified potassium permanganate solution, positive).

Ultraviolet Spectrum Ethanol—258 (A_1^1=242a), 298 nm.



Infrared Spectrum Principal peaks at wavenumbers 1209, 1130, 1247, 1180, 1770, 1727 cm^{-1} (KBr disk).

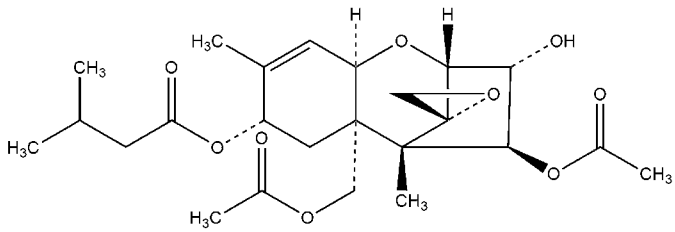
Mass Spectrum Principal ions at *m/z* 181, 395, 198, 251, 397, 396, 199, 666.

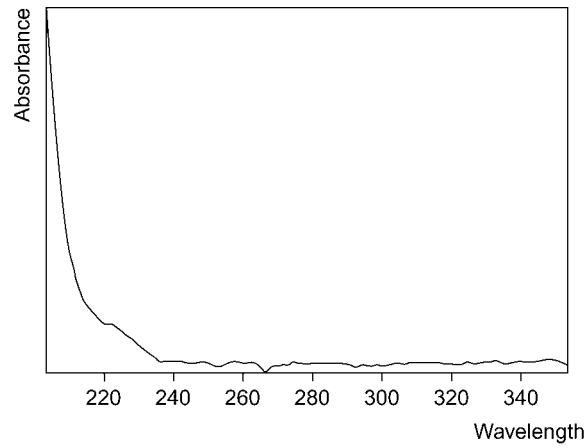
Dose 0.5 to 3 mg daily.

T-2 Toxin

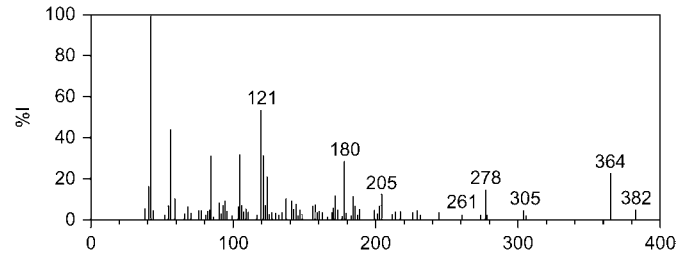
Trichothecene Mycotoxin
$C_{24}H_{34}O_9$ = 466.5
CAS—21259-20-1

IUPAC Name (2α,3α,4β,8α)-4,15-Bis(acetyloxy)-3-hydroxy-12,13-epoxytrichothec-9-en-8-yl 3-methylbutanoate
Synonyms 4β,15-Diacetoxy-3α-hydroxy-8α-[3-methylbutyrloxy]-12,13-epoxytrichothec-9-ene, fusariotoxin T 2; insariotoxin; mycotoxin T 2.





Mass Spectrum Principal ions at *m/z* 121, 180, 364, 278, 205, 382, 305, 261 [Sydenham *et al.* 1996].



Chemical Properties Type-A trichothecene mycotoxin, associated primarily with *Fusarium sporotrichioides*. Surveys have shown that T-2 occurs in cereals at relatively low levels although it is one of the most potent of the naturally occurring tricothecenes affecting animals as a food and feed contaminant [WHO/JECFA 2002]. Crystals, Mp 151° to 152° [O'Neil 2006], 146° to 149° [Sydenham *et al.* 1996]. Freely soluble in ethyl alcohol, ethyl acetate, chloroform, DMSO and other organic solvents; slightly soluble in petroleum ether; very slightly soluble in water [O'Neil 2006]. Log *P* (octanol/water), 2.27 [Meylan, Howard 1995]. For a study of the stability of tricothecenes in calibrant samples see Widestrand, Pettersson [2001].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: A) methanol : water; B) acetonitrile : water; C) tetrahydrofuran : water. Location reagent: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ = 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as shown in the table below.

Quantification
Blood **GC-MS** Column: BP-5 (12 m × 0.22 mm i.d., 0.25 µm). Carrier gas: He, 8 psi. Temperature programme: 90° for 2 min to 180° at 20°/min to 240° at 5°/min for 2 min. CI, negative ion mode, SIM acquisition mode. Limit of detection, 2–7 ppb for DON, NIV, T-2, HT-2, T-2 tetraol, diacetoxyscirpenol, scirpentriol, 15-

	Mobile-phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
DON	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyl-DON	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
NIV	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	**65 : 35**	**0.17**	**0.22**	**65 : 35**	**0.51**	**0.43**	**N/A**	**N/A**	**N/A**
Zearalenone	90 : 10	0.53	0.70	80 : 20	0.59	0.85	65 : 35	0.32	N/A

[Abramson *et al.* 1989]

High Performance Liquid Chromatography Column: C_{18} (25 cm × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : water (70 : 30), flow rate 3 mL/min. UV detection (λ = 278 nm). Retention time 9.6 min. Limit of detection, 30 ng [Yagen *et al.* 1986].
Ultraviolet Spectrum No UV absorption [Sydenham *et al.* 1996], Cyclohexane—187 nm [Rajakylä *et al.* 1987].

MAS (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986]. Column: Varian 3700 J & W DB-5 (15 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 35 cm/s. Temperature programme: 50° for 2 min to 300° at 10°/min for 8 min. CI, SIM acquisition mode. Limit of detection, 0.7 ng/g [D'Agostino *et al.* 1986].
Urine **GC-MS** Column: BP-1 methylsilicone (25 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 15 psi. Temperature programme: 160° for 1 min to 275° at 10°/min,

for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of detection, 2–7 ppb for DON, NIV, T-2, HT-2, T-2 tetraol, diacetoxyscirpenol, scirpentriol, 15-MAS (heptafluorobutyrylimidazole derivatives) [Black *et al.* 1986].

***Other* GC** *In-vitro* Bovine Ruminal Fluid. Column: DB1701 capillary (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 250° to 275° at 5°/min for 5 min for TMS or 225° to 275° at 5°/min for 5 min for TFA. Retention time: 8.26 min [Swanson *et al.* 1987]. Grain Samples. Column: SE-30 fused silica (25 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 35 cm/s. Temperature programme: 180° to 220° at 4°/min to 250° at 15°/min for 5 min. Retention time: 15.1 min. Limit of detection, 25 μg/L [Sydenham, Thiel 1987]. Pig Plasma and Urine. Column: glass packed with 3% OV-17 on 100-120 mesh Supelcoport (1.8 m × 2.0 mm i.d.). Carrier gas: He, 35 mL/min. Temperature: 165°. ECD (Ni^{63}). Retention times: DON 6 min, T-2 tetraol 5 min, scirpenetriol 4.5 min (trifluoroacetic acid derivatives). Limit of detection <25 ppb [Rood, Jr. *et al.* 1986]. Cereal Samples. Column: SE-52 (12 m × 0.25 mm i.d.). Carrier gas: H_2, 40 kPa. Temperature programme: 180° to 260° at 4°/min. FID. Limit of detection, 100 ppb [Bata *et al.* 1983].

GC-MS Wheat Samples. Column: HP-5 capillary (30 m × 0.25 mm i.d., 0.25 mm). Carrier gas: N_2, 1 mL/min. Temperature programme: 80° for 1 min to 160° at 30°/min to 183° at 1°/min to 280° at 12°/min for 5 min. ECD. Limit of quantification, 20 μg/kg, limit of detection, 10 μg/kg [González *et al.* 2008]. Soy Sauce Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 140° for 2 min to 275° at 7°/min for 2 min to 290° at 30°/min for 5 min. Limit of detection, 4 μg/kg for T-2, 7 μg/kg for T-2 tetraol and 5 μg/kg for T-2 triol [Schollenberger *et al.* 2007]. Food Samples. Column: Rtx-200 (60 m × 0.25 mm i.d., 0.1 μm). Carrier gas: He, 1.3 mL/min. Temperature programme: 115° for 5 min to 125° at 50°/min to 300° at 5°/min for 10 min. EI ionisation at 70 eV, positive ion mode. Retention time: 24.6 min for DON, 28.8 min for 3-acetyl-DON, 28.3 min for 15-acetyl-DON, 26.9 min for NIV, 26.4 min for fusarenone-X, 29.4 min for diacetoxyscirpenol, 30.1 min for neosolaniol, 33.7 min for HT-2, 34.5 min for T-2. Limit of quantification, 0.30 μg/kg for DON, 0.26 μg/kg for 3-acetyl-DON, 0.19 μg/kg for 15-acetyl-DON, 0.28 μg/kg for NIV, 0.16 μg/kg for fusarenone-X, 0.36 μg/kg for diacetoxyscirpenol, 0.37 μg/kg for neosolaniol, 0.26 μg/kg for HT-2, 0.18 μg/kg for T-2, limit of detection, 0.16 μg/kg [Schothorst *et al.* 2005]. Grain Samples. Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 80° to 245° at 60°/min for 3 min to 260° at 3°/min to 270° at 10°/min for 7 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Limit of quantification, 30 μg/kg for DON, 3-acetyl-DON, fusarenone-X, and diacetoxyscirpenol, 20 μg/kg for HT-2 and T-2, 30 μg/kg for nivalenol (*N*,*O*-bis(trimethylsilyl)acetamide: trimethylchlorosilane: *N*-trimethylsilylimidazole derivatives) [Jestoi *et al.* 2004]. *In vitro* Bacterial Suspensions. Column: DB-5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 0.6 mL/min. Temperature programme: 80° to 245° at 60°/min for 3 min to 260° at 4°/min to 270° at 10°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [El Nezami *et al.* 2002]. Fungal Extracts. Column: HP-5 (30 m × 0.25 mm i.d., 0.1 μm). Carrier gas: He, 40 cm/s. Temperature programme: 80° for 1 min to 160° at 40°/min to 205° at 4°/min to 240° at 8°/min to 300° at 40°/min for 3 min. EI ionisation, positive ion mode, CID or NICI mode. Limit of detection, 30–70 pg or 50–120 pg (NICI) [Nielsen, Thrane 2001]. Barley Samples. Column: Rtx-200 (30 m × 0.25 μm i.d., 0.25 μm). Carrier gas: He, 0.75 mL/min. Temperature programme: 90° for 0.2 min to 210° at 30°/min to 300° at 6°/min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 12.3 min for DON, 13.8 min for 3-acetyl-DON, 14.8 min for fusarenone-X, 14.0 min for diacetoxyscirpenol, 13.8 min for 15-MAS, 17.9 min for T-2, 12.9 min for scirpentriol, 17.6 min for zearalenone. Limit of detection, 0.1–0.5 mg/kg [Onji *et al.* 1998]. Spiked Barley. Column: HP-5 5% phenylmethylsilicone capillary (25 m × 0.2 mm i.d., 0.3 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 333 K for 1 min to 473 K at 20 K/min to 553 K at 10 K/min for 10 min. EI ionisation at 70 eV. Limit of detection, 30 pg [Kostiainen, Nokelainen 1990]. 'Yellow Rain' Powder Sample. Column: glass packed with 3% OV-17 on 100/120 mesh Chromosorb (1.0 m × 2.0 mm i.d.). Carrier gas: He, 25 mL/min. Temperature programme: 180° to 260° at 12°/min for 5 min. EI ionisation at 70 eV, positive ion mode, SIM acquisition mode. Retention time: 2.3 min for DON, 6.2 min for T-2, 5.5 min for HT-2, 6.5 min for zearalenone, 4.0 min for diacetoxyscirpenol (trimethylsilyl derivatives). Limit of quantification not reported [Rosen, Rosen 1982].

HPLC Grain Samples. Column: C_{18} (250 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (65 : 35) containing 0.75% acetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 292 nm, λ_{em} = 425 nm). Limit of detection, 0.4 μg/kg for DON and HT-2, 0.2 μg/kg for nivalenol and diacetoxyscirpenol, 1.0 μg/kg for 15-acetyl-DON and 3-acetyl-DON, 0.6 μg/kg for fusarenone-X and T-2 (coumarin-3-carbonyl derivatives) [Dall'Asta *et al.* 2004; Mateo *et al.* 2001]. Column: LiChrospher 100 C_{18} (250 × 4.0 mm i.d., 5 μm). Mobile phase: acetonitrile : water (65 : 35) containing 0.75% acetic acid, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 292 nm, λ_{em} = 425 nm). Retention time: ≈8.5 min [Jimenez *et al.* 2000].

LC-MS Fungal Cultures. Column: C_{18} (150 × 3.0 mm i.d., 3.5 μm). Mobile phase: water : methanol both containing 10 mmol/L ammonium acetate and 20 μmol/L sodium acetate (70 : 30 to 50 : 50 over 1 min to 40 : 60 over 9.5 min to 15 : 85 over 1 min for 7 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 5.6 min for DON, 3.9 min for NIV, 7.0 min for de-epoxy-DON, 7.7 min for verrucarol, 6.7 min for neosolaniol, 9.4 min for aflatoxin G_2, 11.4 min for aflatoxin B_2, 10.5 min for aflatoxin G_1, 12.5 min for aflatoxin B_1, 12.7 min for diacetoxyscirpenol, 16.8 min for ochratoxin A, 17.6 min for T-2 toxin, 17.5 min for verrucarin A, 17.7 min for roridin A, 18.9 min for zearalenone, 19.5 min for sterigmatocystin. Limit of detection, 1.5 μg/L [Delmulle *et al.* 2006]. Corn, Wheat, Cornflakes and Biscuits. Column: Zorbax Eclipse XDB C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : methanol (90 : 10 to 0 : 100 in 40 min), flow rate 200 μL/min. APCI. Limit of detection, 0.2 ng/g [Tanaka *et al.* 2006]. Wheat Flour. Column: LC_{18} Supelcosil (250 × 4.6 mm, 5 μm). Mobile phase: water : methanol (80 : 20 to 35 : 65 in 9 min for 8 min to 10 : 90 for 5 min to 80 : 20 for 7 min), flow rate 1.0 mL/min. ESI, positive ion mode. Limit of quantification, 5 ng/g [Biancardi *et al.* 2005]. Column: SB-RP18-Zorbax (150 × 3.0 mm i.d., 3.5 μm). Mobile phase: methanol : water (30 : 70 to 100 : 0 in 8 min), flow rate 250 μL/min. ESI, negative and positive ion mode, MRM acquisition mode. Retention time: 13.6 min. Limit of quantification, 10 ppb, limit of detection, 0.2 μg/kg [Biselli, Hummert 2005]. Spiked Oats. Column: ODS-2 C_{18} (200 × 2.1 mm i.d., 5 μm). Mobile phase: 1 mmol/L ammonium acetate : acetonitrile (80 : 20 to 0 : 100 in 5 min for 3 min to 80 : 20 for 10 min), flow rate 0.3 mL/min. APCI positive ion mode, SIM acquisition mode. Limit of quantification, 50 ng/g [Razzazi-Fazeli *et al.* 2002]. Mouldy Interior Finishes. Column: Lichrocart 250-3 Purospher RP18. Mobile phase: methanol : 10 mmol/L ammonium acetate (20 : 80 for 4 min to 70 : 30 at 8 min for 11.5 min to 90 : 10 in 1 min for 15.5 min), flow rate 400 μL/min. ESI, positive ion mode. Retention time: 18.1 min. Limit of detection, 0.02 ng [Tuomi *et al.* 2000]. Wheat Samples. Column: C_{18} (125 × 2.0 mm i.d., 3 μm). Mobile phase: methanol : water (25 : 75 to 2 : 98 over 12 min), flow rate 0.25 mL/min. APCI, positive ion mode, full-scan. Limit of quantification, 50 ppb for DON and neosolaniol, 100 ppb for NIV and 15-acetyl-DON, 40 ppb for fusarenone-X, 25 ppb for 3-acetyl-DON, 20 ppb for diacetoxyscirpenol, 10 ppb for HT-2, 60 ppb for T-2, limit of detection, 3 ppb [Berger *et al.* 1999]. Rat, Cow and Hen Urine and Faeces. Column: Zorbax ODS (25 cm × 4.6 mm i.d.). Mobile phase: methanol: 0.1 mol/L ammonium acetate (20 : 80 to 70 : 30 in 20 min for 5 min), flow rate 1.5 mL/min. TIS, CI, positive ion mode, full scan or MID mode. Limit of detection, 1 ng (full scan) and 50 pg (MID) [Voyksner *et al.* 1987].

Note For an LC-MS method for the quantification of multiple mycotoxins in a variety of nuts and dried fruits, see Spanjer *et al.* [2008]; in infant foods, see Lattanzio *et al.* [2008]. For the determination of 39 mycotoxins in wheat and maize by LC-MS, see Sulyok *et al.* [2006]; for Type-A and -B trichothecenes in cereals by LC-MS, see Klotzel *et al.* [2005]; for 17 mycotoxins using HPLC-DAD, see Kuronen [1989] and also Jimenez, Mateo [1997]. For a purity assessment study of mycotoxin standards comparing UV, HPLC-UV, GC-ECD, GC-FID, GC-MS, LC-MS, DSC, NMR and FT-IR methods, see Krska *et al.* [2005]. For an ELISA assay for T-2 toxin see Nikulin *et al.* [1996] or Stratton *et al.* [1993].

Disposition in the Body

At the time of writing, there is no T-2 toxin toxicokinetic information available in humans. Animal studies have shown that the oral bioavailability of all trichothecenes is generally low as a result of physiological instability and first-pass metabolism. T-2 is rapidly absorbed after ingestion and it is distributed in the organism with little or no accumulation in any specific organs [Schlatter 2004]. Metabolism can result in glucuronide conjugates and complete or partial de-esterification may occur if ester functionalities are present. Mixed function oxidase activity is important in the metabolism of T-2 toxin to 3′-hydroxy T-2 toxin. The pattern of metabolism and excretion of T-2 toxin is very complex [Voyksner *et al.* 1987]. T-2 is rapidly metabolised to HT-2 *in vivo* [Schlatter 2004].

Toxicity LD_{50} (oral) in chickens (mg/kg): 4.97 [Ueno 1983]; LD_{50} (IP) in mice 9.1 mg/kg [Thompson & Wannemacher, Jr. 1986].

A chemical attack occurred on February 13, 1982 at Tuol Chrey, Kampucchea. One of the victims died on March 16 and the concentrations of T-2 toxin were measured at postmortem, as shown in the table below.

Tissue	*Oesophagus*	*Kidney*	*Lung*	*Large Intestine*
Concentration (ppb)	25.1	6.8	8.5	88

[Mirocha *et al.* 1983].

Note For a review of trichothecene toxicology and potential effects on humans, see Pestka, Smolinski [2005]; Rotter *et al.* [1996]; Sudakin [2003].

Half-life <20 min.

Dose It has been reported that the trichothecenes were used as an agent of biological/chemical warfare ('yellow rain') [Mirocha *et al.* 1983; Rosen, Rosen 1982; Stark 2005].

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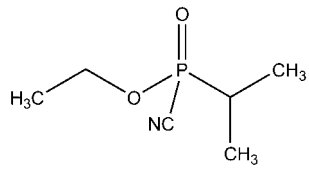
Tabun

Anticholinesterase, Organophosphate Nerve Agent
$C_5H_{11}N_2O_2P = 162.1$
CAS—77-81-6

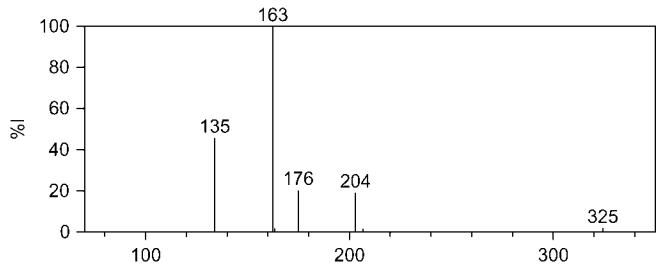
IUPAC Name Ethyl *N*-dimethylphosphoroamidocyanidate
Synonyms Dimethylamidoethoxyphosphoryl cyanide; dimethylphosphoramidocyanidic acid ethyl ester; EA1205; GA; Gelan; Le100; Stoff-83; Trilon-83.



Chemical Properties Clear, colourless liquid with a faint fruity odour reminiscent of bitter almonds. Mp −50°. Bp 240°. Readily soluble in organic solvents. Freely soluble in water. Log *P* (octanol/water), −1.4.

Gas Chromatography-Mass Spectrometry Column: SGE BPX5 capillary 5% phenyl 95% methylpolysiloxane (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.2 mL/min. Temperature programme: 50° for 2 min to 280° at 10°/min. EI ionisation at 70 eV. Limit of detection not reported [Gupta *et al.* 2005]. Column: DB5-MS (25 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 0.9 mL/min. Temperature programme: 40° for 2 min to 160° at 20°/min to 310° at 30°/min for 5 min. ATD, full scan mode. Limit of detection, 50 ng [Carrick *et al.* 2001].

Mass Spectrum



Quantification

Urine **GC-MS** Column: DB-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 1.5 min to 250° at 40°/min in 5 min for 5 min. SRM acquisition mode. Limit of detection, 17 μg/L [Driskell *et al.* 2002].

Other **GC** Water. Column: CP-Sil 19 (50 m × 0.32 mm i.d., 0.4 μm). Temperature programme: 60° for 3 min to 70° at 5°/min for 7 min to 180° at 10°/min to 240° at 15°/min for 12 min. Limit of detection, ng/L range [Degenhardt-Langelaan, Kientz 1996].

GC-MS Soil. Column: J and W DB-35MS capillary (15 m × 0.25 mm i.d.). Temperature programme: 40° for 2 min to 280° at 10°/min for 5 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [D'Agostino *et al.* 2003].

LC-MS Spiked Food, Bottled Water, Canola Oil, Cornmeal and Honey. Column: Agilent Zorbax SB-C_{18} (50 × 0.3 mm i.d., 1.8 μm). Mobile phase: 0.1% trifluoroacetic acid in water : acetonitrile (95 : 5 to 50 : 50 over 15 min), flow rate 10 μL/min. ESI, APCI, or FAIMS. Limit of quantification, 3 and 398 μg/L using APCI with and without FAIMS, respectively; limit of detection, 0.9 and 10 μg/L using APCI with and without FAIMS, respectively [Kolakowski *et al.* 2007]. Soil. Column: Zorbax C_{18} SB (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 95 : 5 to 25 : 75 at 30 min), flow rate 10 μL/min. ESI. Limit of detection not reported [D'Agostino *et al.* 2003]. Aqueous Samples. Column: Zorbax C_{18} SB (150 × 0.32 mm i.d., 5 μm). Mobile phase: 0.1% trifluoroacetic acid in water : 0.1% trifluoroacetic acid in acetonitrile-water (95 : 5; 99 : 1 to 25 : 75 over 30 min). ESI. Limit of detection not reported [D'Agostino *et al.* 1999].

Note For a review article on the chromatographic analysis of chemical warfare agents, see Witkiewicz *et al.* [1990] and Creasy *et al.* [1997].

Disposition in the Body Under neutral conditions, the principal hydrolysis pathway of tabun produces *O*-ethyl *N,N*-dimethylamidophosphoric acid and hydrogen cyanide. The former is then hydrolysed to dimethyl phosphoramidate followed by phosphoric acid. The first stage is relatively rapid while the latter stages of the pathway are relatively slow. Phosphorocyanidate may also be formed from dimethyl phosphoramidate. Under acidic conditions, hydrolysis to ethylphosphoryl cyanidate and dimetylamine occurs. The final product by all pathways is phosphoric acid [Munro *et al.* 1999].

Toxicity For a review of the toxicity of tabun, sarin and VX, see Munro [1994].

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Tacrine

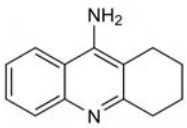
Anticholinesterase, Central Stimulant

$C_{13}H_{14}N_2$ = 198.3

CAS—321-64-2

IUPAC Name 1,2,3,4-Tetrahydroacridin-9-amine

Synonyms 1,2,3,4-Tetrahydro-9-acridinamine; tetrahydroaminoacridine.



Chemical Properties Mp 183° to 184°. pK_a 9.95 (20°). Log *P* (octanol/water), 2.71.

Tacrine Hydrochloride

$C_{13}H_{14}N_2$,HCl = 234.7

CAS—1684-40-8

Synonyms Cl-970; tetrahydroaminoacridine hydrochloride; THA.

Proprietary Names *Cognex*; *Tacrinal*.

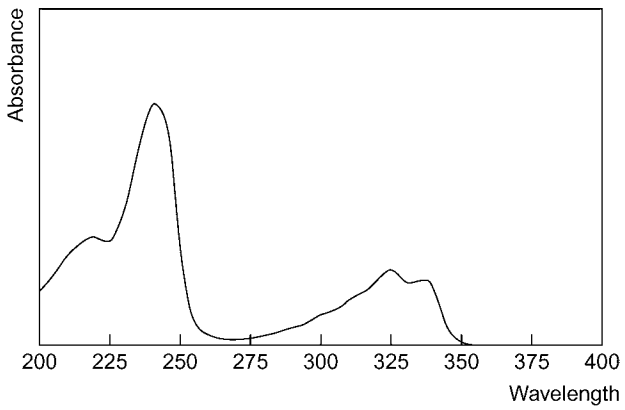
Chemical Properties A pale yellow crystalline powder. Mp 283° to 284°. Soluble in water.

Thin-layer Chromatography System TA—R_f 0.43; system TB—R_f 0.05; system TC—R_f 0.04; system TL—R_f 0.10 (acidified iodoplatinate solution—positive).

Gas Chromatography System GA—RI 2165.

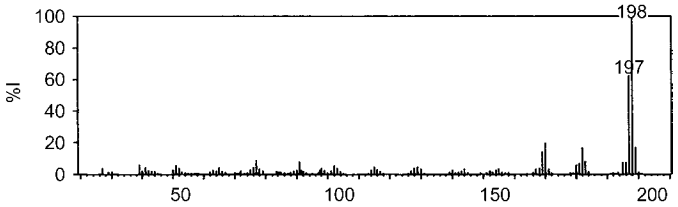
High Performance Liquid Chromatography System HA—*k* 1.6.

Ultraviolet Spectrum Aqueous acid—240 (A^1_1 = 2032a), 323, 336 nm; aqueous alkali—237, 317 nm.



Infrared Spectrum Principal peaks at wavenumbers 1650, 1590, 765, 1496, 774, 1176 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 198, 197, 170, 199, 182, 169, 77, 183.



Quantification

Plasma **HPLC** Column: Nucleosil CN (125 × 4.0 mm i.d., 5 µm). Mobile phase: 0.05 mol/L sodium phosphate buffer (pH 7.0) : acetonitrile (45 : 55), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 365 nm). Retention time: 6.5 min. Limit of quantification, 1.0 µg/L, limit of detection, 0.4 µg/L [Chollet *et al.* 2000]. Column: Hypersil Phenyl (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 4.4 mmol/L potassium dihydrogen phosphate (39 : 61), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 360 nm). Limit of quantification, 0.5 µg/L for tacrine and its metabolites [Aymard *et al.* 1998]. Column: LiChrospher RP-Select B (250 × 4 mm i.d., 5 µm). Mobile phase: 0.2 mol/L acetate buffer (pH 4) : acetonitrile (87 : 13), flow rate 1.25 mL/min (0 to 16 min) and 2.5 mL/min (16 to 40 min). Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 365 nm). Retention time: 29–30 min. Limit of quantification, 2 nmol/L for tacrine, 2.5 nmol/L for 2- and 4-hydroxytacrine and 10 nmol/L for 1-hydroxytacrine, limit of detection, 2 nmol/L for tacrine and 1-hydroxytacrine and 0.5 nmol/L for 2- and 4-hydroxytacrine [Hansen *et al.* 1998]. Column: Brownlee-Spheri-5 Cyano (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L sodium acetate buffer (pH 4.0, 30 : 70), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 240 nm, λ_{em} = 355 nm). Limit of quantification, 0.5 µg/L for tacrine and 4-hydroxytacrine, 1 µg/L for 2-hydroxytacrine and 0.925 µg/L for 1-hydroxytacrine [Haughey *et al.* 1994]. Column: Hypersil (100 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 0.02 mol/L ammonium formate buffer (pH 2.75, 70 : 30), flow rate 1.5 mL/min. UV detection (λ = 240 nm). Limit of detection, 1 µg/L [Hsu *et al.* 1990].

Serum **HPLC** Fluorescence detection [Forsyth *et al.* 1988].

Urine **HPLC** See Plasma. Limit of quantification, 1202 nmol/L for tacrine, 1- and 2-hydroxytacrine and 4-hydroxytacrine; limit of detection, 80 nmol/L for tacrine, 30 nmol/L for 1-hydroxytacrine and 60 nmol/L for 2- and 4-hydroxytacrine [Hansen *et al.* 1998].

Disposition in Body Tacrine is rapidly absorbed from the gastrointestinal tract after oral administration, but there is large inter-individual variation in oral bioavailability and the extent of absorption decreases when administered with food. It is metabolised in the liver by the cytochrome P450 isozymes, principally CYP1A2, to several metabolites, the main one being 1-hydroxytacrine (velnacrine). Little unchanged drug is excreted in urine.

Therapeutic Concentration

Twelve patients with Alzheimer's disease sequentially given 9 doses of 10, 20 or 30 mg every 6 h had maximum steady-state plasma levels of 5.1, 20.7 and 33.9 µg/L, respectively [Cutler *et al.* 1990].

Twenty-four healthy mature volunteers (aged 50–76 years; mean, 64) were administered with a single 40-mg dose of tacrine after an 8 h overnight fast, 1-h before a standard breakfast, 15 min after the start of breakfast (i.e. during breakfast) and 2 h after a standard breakfast was finished. There was a 1 week washout period between doses. The mean maximum plasma concentration was 15.8 µg/L for the fasting individuals (i.e. after the overnight fast and before breakfast), 17.9 µg/L for the dose given 1 h before breakfast, 9.9 µg/L when tacrine was administered during breakfast and 11.6 µg/L when the dose was administered 2 h after breakfast. These concentrations were observed at 1.9, 1.7, 3.6 and 2.8 h, respectively [Welty *et al.* 1994].

Five patients with Alzheimer's disease who had had the disease for 2–12 years (aged 57 to 72 years) were administered 40–160 mg tacrine daily during a 12- to 31-month treatment period. The mean steady-state plasma concentrations were 1.3, 5.5, 11.9 and 30.0 µg/L for the 40-, 80-, 120- and 160-mg doses, respectively. After the morning 80-mg dose, the mean maximum plasma concentration was 8.7 µg/L observed at 1.3 h. The mean plasma concentration was 2.6–5.6 µg/L [Johansson *et al.* 1996].

Seven patients received tacrine 40–140 mg daily for Alzheimer's disease as part of a 6-week clinical trial. At the end of this period, patients had a mean concentration of 8.01 ± 7.07 µg/L in the plasma and 5.21 ± 6.00 µg/L in CSF 30 min after the morning dose (10–40 mg, given in the fasting state) [Grothe *et al.* 1998].

Toxicity A serum concentration >20 µg/L is associated with a high risk of adverse reaction.

A 75-year-old woman with Alzheimer's disease was treated with 40 mg tacrine daily for 14 months. She was admitted to hospital with jaundice and was very confused. Her liver span measured 13 cm. She had excessive levels of bilirubicin in the blood and decreased transaminase levels. Drug-induced hepatotoxicity was suspected. All medications were stopped but transaminase levels decreased further and she had urosepsis and acute renal failure. She died 3 weeks after admission to hospital. The postmortem showed massive hepatic necrosis, which was believed to have been caused by tacrine

and possibly the production of toxic metabolites in the liver [Blackard *et al.* 1998].

Bioavailability 17% with a large inter-individual variation.

Half-life 1.5–2 h.

Protein Binding 55%.

Note For a review of the clinical pharmacokinetics of tacrine, see Madden *et al.* [1995]; for a review of the pharmacokinetics of cholinesterase inhibitors, see Jann *et al.* [2002]; for a review of the use and adverse effects of tacrine in patients with Alzheimer's disease, see Qizilbash *et al.* [2007].

Dose Up to 160 mg daily by mouth.

Aymard G *et al.* (1998). High-performance liquid chromatography with ultraviolet and fluorimetric detection for the simultaneous determination of tacrine, nimodipine, and their respective metabolites in the plasma of patients with Alzheimer disease. *Ther Drug Monit* 20: 422–429.

Blackard WGJr *et al.* (1998). Tacrine: a cause of fatal hepatotoxicity? *J Clin Gastroenterol* 26: 57–59.

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Cutler NR *et al.* (1990). Steady-state pharmacokinetics of tacrine in patients with Alzheimer's disease. *Psychopharmacol Bull* 26: 231–234.

Forsyth DR *et al.* (1988). Determination of tacrine hydrochloride in human serum by chloroform extraction, reversed-phase high-performance liquid chromatography and fluorimetric detection. *J Chromatogr* 433: 352–358.

Grothe DR *et al.* (1998). Penetration of tacrine into cerebrospinal fluid in patients with Alzheimer's disease. *J Clin Psychopharmacol* 18: 78–81.

Hansen LL (1998). Determination of tacrine and its metabolites in human plasma and urine by high-performance liquid chromatography and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 712: 183–191.

Haughey DB *et al.* (1994). Simultaneous determination of tacrine and 1-hydroxy-, 2-hydroxy-, and 4-hydroxytacrine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Pharm Sci* 83: 1582–1585.

Hsu RS *et al.* (1990). High-performance liquid chromatography for the determination of tacrine and its metabolites in plasma. *J Chromatogr* 530: 170–176.

Jann MW *et al.* (2002). Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clin Pharmacokinet* 41: 719–739.

Johansson M *et al.* (1996). Steady-state pharmacokinetics of tacrine in long-term treatment of Alzheimer patients. *Dementia* 7: 111–117.

Madden S *et al.* (1995). Clinical pharmacokinetics of tacrine. *Clin Pharmacokinet* 28: 449–457.

Qizilbash, N *et al.* (2007) WITHDRAWN: Tacrine for Alzheimer's disease. *Cochrane Database Syst Rev* CD000202. [update of *Cochrane Database Syst Rev* 2000; (3):CD000202].

Welty DF *et al.* (1994). The temporal effect of food on tacrine bioavailability. *J Clin Pharmacol* 34: 985–988.

Tacrolimus

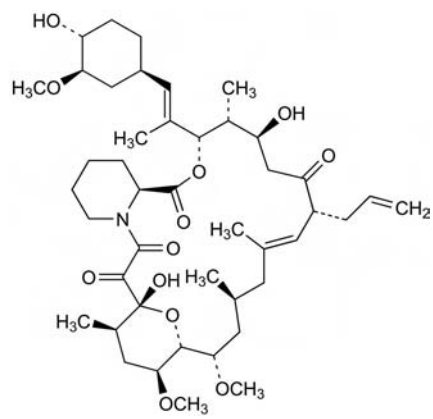
Immunosuppressant

$C_{44}H_{69}NO_{12}$ = 804.0

CAS—104987-11-3

Synonyms FK506; FR-900506; (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26*aS*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5,19-dihydroxy-3-[(1*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

Proprietary Names *Prograf*; *Protopic*.



Tacrolimus Monohydrate

$C_{44}H_{69}NO_{12},H_2O$ = 822.0

CAS—109581-93-3

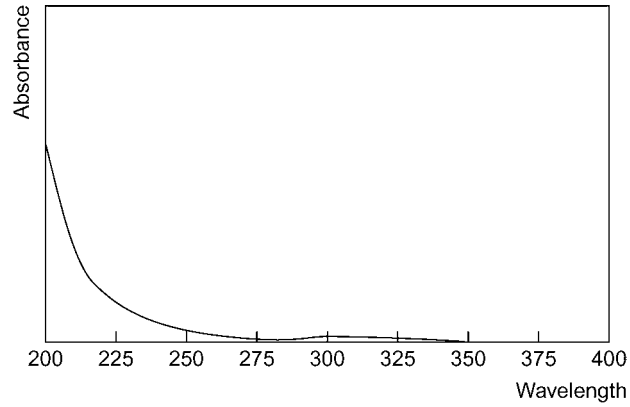
Chemical Properties A white crystalline powder. Mp 127° to 129°. Practically insoluble in water; soluble in methanol, ethanol, acetone, ethyl acetate, trichloromethane and diethylether; sparingly soluble in hexane and petroleum ether.

Liquid Chromatography-Mass Spectrometry Column: ODS Hypersil (100 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L ammonium acetate (pH 9.0; 99 : 1), flow rate 0.3 mL/min. SIM *m/z* 802.5→168.2. Retention time: 0.97 min [Alak *et al.* 1997].

Column: RP C_{18} Spherical (150 × 3.9 mm i.d., 5 µm). Mobile phase: methanol : water (90 : 10), flow rate 0.4 mL/min. NICI, SIM acquisition mode at *m/z* 803.7. Retention time: 5.2 min [Gonschior *et al.* 1995].

Column: C_{18} Novapak (150 × 2.1 mm i.d., 4 µm). Column temperature: 50°. Mobile phase: methanol : 40 mmol ammonium acetate buffer (pH 5.1; 76 : 24), flow rate 0.2 mL/min. ESI, SIM acquisition mode at *m/z* 821.5→768.5). Retention time: 7.3 min (ammoniated adduct) [Taylor *et al.* 2000].

Ultraviolet Spectrum



Quantification

Blood **LC-MS** Limit of quantification, 0.25 µg/L [Taylor *et al.* 2000] Limit of detection, 0.10 µg/L [Alak *et al.* 1997]. Limit of quantification, 0.025 µg/L [Hill *et al.* 1997]. Limit of quantification, 200 µg/L [Gonschior *et al.* 1995].

Serum **Immunoassay** Limit of detection, 0.1 µg/L [Friob *et al.* 1991].

Urine **LC-MS** See Blood [Gonschior *et al.* 1995].

Disposition in the Body Absorption of tacrolimus after oral administration is erratic and generally poor. It undergoes extensive tissue distribution and metabolism is hepatic with >99% of the drug being metabolised. At least 15 metabolites have been detected and are produced mainly by demethylation and hydroxylation, via the hepatic cytochrome P450 enzyme (CYP3A), and some metabolism may occur in the gastrointestinal tract. Several hydroxylated and demethylated metabolites have been identified, including 13-*O*-desmethyl tacrolimus and 15-*O*-desmethyl tacrolimus. It is widely and extensively distributed throughout the tissues of the body after IV administration. Elimination is primarily via bile (>90% of an absorbed dose is excreted in bile as metabolites. <1% of the dose is excreted in urine as the unchanged drug. A peak plasma concentration of 0.4–5.6 µg/L is reached after a single oral dose of 0.15 mg/kg and 10–24 µg/L after IV infusion of 0.5 mg/kg over 2 h. Hepatic impairment causes increased plasma concentration, prolonged half-life and reduced clearance. Renal impairment causes no change in the pharmacokinetics.

Therapeutic Concentration The reference range is 0.5–2 µg/L and the therapeutic peak is 0.2–6 µg/L. In blood, the therapeutic reference concentration at its trough is 3–15 µg/L and its peak, 10–25 µg/L.

Ten African American (mean age, 32.2 years), 12 white (mean age, 44.6 years) and 12 Latin-American (mean age, 35.7 years) healthy patients were administered with a single, oral dose of 5 mg tacrolimus after an overnight fast. The mean maximum drug concentrations after the oral dose were 20.8, 37.8 and 33.0 µg/L for the 3 ethnic groups observed at 1.6, 1.3 and 1.3 h, respectively. For the IV dose, the mean concentrations were 28.3, 26.4 and 27.4 µg/L detected at the end of the infusion. The metabolite, 13-*O*-desmethyl tacrolimus, was observed at concentrations of 2.0, 4.8 and 3.6 µg/L for the ethnic groups at 1.3, 1.5 and 1.6 h, respectively following oral administration [Mancinelli *et al.* 2001].

Toxicity The toxic blood reference concentration is 12–15 µg/L.

A 54-year-old male patient waiting for a liver transplant was co-administered with mibefradil for the treatment of hypertension. He subsequently developed dizziness, fatigue and shoulder muscle ache as well as reversible renal impairment. Tacrolimus levels were observed at 54 µg/L (originally in the therapeutic range, 5–8 µg/L). Treatment with mibefradil was stopped and tacrolimus levels decreased to normal and all symptoms and clinical changes were reversed. It was decided that these toxic effects were due to an interaction between the 2 drugs [Ocran *et al.* 1999].

Bioavailability Oral: 5–67% (mean, 30%).

Half-life 43 h in healthy volunteers and 12–16 h in transplant patients.

Volume of Distribution Plasma, 5–65 L/kg; blood: 0.5–1.4 L/kg (1300 L).

Clearance Plasma, 0.6–5.4 (mean, 1.8) L/h/kg; blood: 0.03–0.09 (mean, 0.06) L/h/kg; also reported as 2.43 L/kg.

Distribution in Blood Tacrolimus is primarily associated with red blood cells and the blood : plasma ratio is between 4 and 39.

Protein Binding 77–99%. Predominantly to the non-lipoprotein fractions containing albumin and α_1-acid glycoprotein.

Dose IV administration: 0.1 mg/kg/day. Oral: 0.3 mg/kg/day.

Alak A *et al.* (1997). An HPLC/MS/MS assay for tacrolimus in patient blood samples. Correlation with results of an ELISA assay. *J Pharm Biomed Anal* 16: 7–13.

Friob MC *et al.* (1991). A combined HPLC-ELISA evaluation of FK 506 in transplant patients. *Transplant Proc* 23(6): 2750–2752.
Gonschior A *et al.* (1995). Simplified high-performance liquid chromatography-mass spectrometry assay for measurement of tacrolimus and its metabolites and cross-validation with microparticle enzyme immunoassay. *Ther Drug Monit* 17: 504–510.
Hill H *et al.* (1997). *Pharm Res* 14S261.
Mancinelli LM *et al.* (2001). The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. *Clin Pharmacol Ther* 69(1): 24–31.
Ocran KW *et al.* (1999). Tacrolimus toxicity due to drug interaction with mibefradil in a patient after liver transplantation. *Z Gastroenterol* 37(10): 1025.
Taylor PJ *et al.* (2000). Simultaneous quantification of tacrolimus and sirolimus, in human blood, by high-performance liquid chromatography-tandem mass spectrometry. *Ther Drug Monit* 22(5): 608–612.

Tadalafil

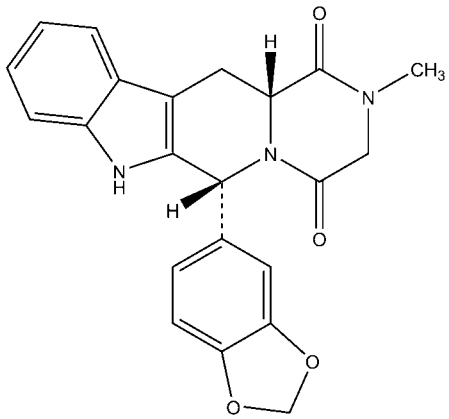
Phosphodiesterase 5 Inhibitor, Treatment of ED

$C_{22}H_{19}N_3O_4 = 389.4$

CAS—171596-29-5

Synonyms GF-196960; (6*R*,12a*R*)-2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl]pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione; IC-351; weekend pill.

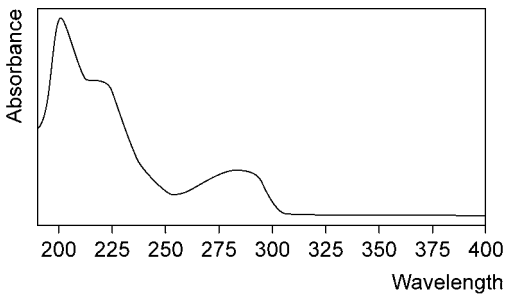
Proprietary Names *Cialis*; *Forzest*; *Tadacip*; *Zydalis*.



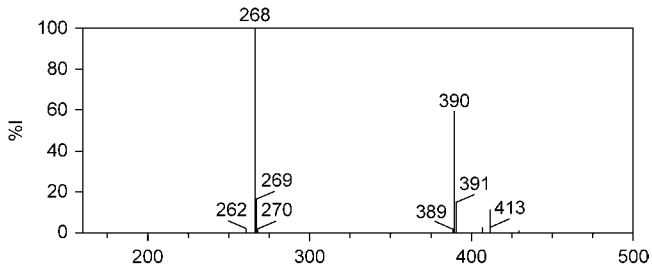
Chemical Properties White crystals from propan-2-ol. Mp 302° to 303°. Very slightly soluble in ethanol. Practically insoluble in water [O'Neil *et al.* 2006]. Log *P* (octanol/water), 1.7 [Wishart 2006].

High Performance Liquid Chromatography Column: C_{18} (250 × 9.4 mm i.d., 5 µm). Mobile phase: acetonitrile : water (1 : 1), flow rate 2 mL/min. UV detection (λ = 225 nm). Retention time: 7.4 min. Limit of quantification not reported [Zou *et al.* 2006a].

Ultraviolet Spectrum Principal peaks at 200, 285 nm [Cheng, Chou 2005]



Mass Spectrum Principal ions at *m/z* 268, 390, 269, 391, 413, 262, 270, 389.



Quantification

***Plasma* HPLC** Hypersil ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L phosphate buffer (pH 7; 35 : 65), flow rate 1.0 mL/min. UV detection (λ = 290 nm). Retention time: 15.5 min. Limit of quantification, 10 µg/L [Cheng, Chou 2005].

LC-MS Column: C_{18} (100 × 3.0 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate : acetonitrile (pH 3.0; 10 : 90), flow rate 0.8 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 0.65 min. Limit of quantification, 10 µg/L [Ramakrishna *et al.* 2004].

***Other* HPLC** Tablet. Column: Chiralpak AD packed with silica gel coated with amylase *tris*-(3,5-dimethylphenylcarbamate), 10 µm. Mobile phase: hexane : isopropyl alcohol (1 : 1), flow rate 0.75 mL/min. UV detection (λ = 220 nm). *k′*: 6*R*,12a*S*-isomer 3.12; 6*R*,12a*R*-isomer 6.61; 6*S*,12a*S*-isomer 9.72; 6*S*,12a*R*-isomer 12.05. Limit of quantification (on-column), 6*R*,12a*S*-isomer 0.60; 6*R*,12a*R*-isomer 0.90; 6*S*,12a*S*-isomer 1.20; 6*S*,12a*R*-isomer 1.80 [Gao *et al.* 2007]. Dietary Supplements. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L ammonium formate (38 : 62), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: tadalafil 12.6 min, vardenafil 15.7 min, sildenafil 16.6 min. Limit of quantification, tadalafil 1.02 mg/L, vardenafil 0.61 mg/L, sildnafil 0.67 mg/L [Zou *et al.* 2006b].

LC-MS Herbal Tablets. Column: Zorbax SB-C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 0.1% formic acid : acetonitrile (95 : 5 to 5 : 95 over 15 min for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification not reported [Gratz *et al.* 2006]. Dietary Supplements. Column: C_{18} (150 × 2.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L ammonium formate (38 : 62), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification (on column), tadalafil 0.70 ng, vardenafil 0.07 ng, sildenafil 0.10 ng [Zou *et al.* 2006b]. Herbal Remedies. Column: C_{18} (125 × 3.0 mm i.d., 4 µm). Mobile phase: 10 mmol/L ammonium formate (pH 3.0) : acetonitrile (95 : 5 for 5 min to 20 : 80 over 25 min for 10 min to 5 : 95 over 5 min for 25 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 23.7 min. Limit of detection (on column), 0.02 ng [Bogusz *et al.* 2006]. Herbal Preparations. Column: C_{18} (5 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate-0.2% formic acid (35 : 65 for 10 min to 80 : 30 over 5 min for 5 min), flow rate 1.0 mL/min. ESI, positive ion mode, SIR and UV detection (λ = 292 nm). Retention time: tadalafil 14.8 min, vardenafil 8.8 min, sildenafil 7.9 min. Limit of quantification, 1.1 µg/L [Zhu *et al.* 2005]. Herbal Tablets. Column: Novapak C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: 0.1% formic acid : acetonitrile : methanol (20 : 10 : 70). ESI, positive ion mode, SIM acquisition mode. Retention times: tadalafil 6.2 min, sildenafil 25 min, vardenafil 27 min. Limit of quantification not reported [Fleshner *et al.* 2005]. Tablets. Column: C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: 0.1% formic acid : acetonitrile (85 : 15 for 5 min to 10 : 90 over 10 min for 10 min), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: tadalafil 13.1 min, vardenafil 10.8 min, sildenafil 11.6 min. Limit of detection on-column, 0.3 ng [Gratz *et al.* 2004].

CE Herbal Tablets. Column: fused silica capillary (total/effective length: 60/50 cm, 75 µm). Running buffer: 10 mmol/L phosphate buffer (pH 12.0) with 25 mmol/L SDS. UV detection (λ = 222 nm). Migration times: tadalafil 1.75 min, vardenafil 1.15 min, sildenafil 1.0 min. Limit of quantification, 2 mg/L; limit of detection, 0.61 mg/L [Rodriguez Flores *et al.* 2004].

Note For structure elucidation of tadalafil analogues in herbal products, see Blok-Tip *et al.* [2004]

Disposition in the Body Well absorbed after oral administration, with peak plasma concentrations attained within 2 h; the rate and extent of absorption are not affected by food. It is widely distributed into tissues. It is metabolised in the liver mainly by CYP3A4 and the major metabolite, the methylcatechol glucuronide, is inactive. Tadalafil is excreted, mainly as metabolites, in the faeces (61% of the dose) and to a lesser extent the urine (36% of the dose). Clearance may be reduced in the elderly and in patients with renal impairment.

Therapeutic Concentration

Fifteen healthy volunteers were administered 20 mg tadalafil daily for 15 days. Mean peak plasma concentrations of tadalafil and its methylcatechol glucuronide metabolite were reported as follows:

	Day 1	*Day 5*	*Day 15*
Tadalafil			
C_{max} (µg/L)	352	514	481
Time (h)	2.0	2.0	2.0
Metabolite			
C_{max} (µg/L)	179	469	442
Time (h)	24	3.0	3.0

In separate studies, no diurnal effect was observed in tadalafil pharmacokinetics, and food had negligible effects on rate and extent of absorption [Forgue *et al.* 2006].

In a multi-centre study investigating the effects of intrinsic factors on tadalafil pharmacokinetics, groups of patients with various conditions were administered a single oral dose of 10 mg tadalafil. Peak plasma concentrations of tadalafil for patients grouped by age (young, mean age 36.3 years; elderly, mean age 70.7 years) or sex were reported as follows:

	Male	Female	Elderly	Young
C_{max} (μg/L)	142	140	196	183
Time (h)	3.5	3.5	2.0	2.5

Grouped by level of renal impairment:

	Normal function	Mild impairment	Moderate impairment
C_{max} (μg/L)	183	217	220
Time (h)	1.0	2.0	2.0

Grouped by level of renal impairment:

	Normal function	Very mild impairment	Mild impairment	Moderate impairment
C_{max} (μg/L)	180	133	146	101
Time (h)	2.5	3.0	2.0	2.5

No changes in pharmacokinetics of tadalafil were observed in patients with diabetes mellitus [Forgue *et al.* 2007].

Toxicity Rifampicin, an inducer of CYP3A4, has been shown to decrease the plasma concentrations of tadalafil. Phosphodiesterase type 5 inhibitors may potentiate the hypotensive effects of organic nitrates and are, therefore, contraindicated in patients receiving such drugs. The licensed product information recommends that if nitrate treatment is needed in a life-threatening situation then it should only be given at least 48 h after the last dose of tadalafil and under close medical supervision. There have been numerous reports of tadalafil (and related compounds) being misused recreationally, often in combination with 'party' drugs such as MDMA, GHB, ketamine and amyl nitrite ('poppers') [Smith, Romanelli 2005]

Bioavailability 25%.

Half-life Approximately 17.5 h.

Volume of Distribution Approximately 63.8 L.

Clearance Approximately 1.6 L/h.

Protein Binding Approximately 94%.

Dose Given orally in a usual dose of 10 mg at least 30 min before sexual intercourse and may be taken with or without food; the dose may be increased to 20 mg, or decreased to 5 mg, if necessary. In patients taking potent inhibitors of CYP3A4, such as ketoconazole or ritonavir, the dose of tadalafil should not exceed 10 mg once every 72 h.

Blok-Tip L *et al.* (2004). Structure elucidation of sildenafil analogues in herbal products. *Food Addit Contam* 21: 737–748.

Bogusz MJ *et al.* (2006). Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. *J Pharm Biomed Anal* 41: 554–564.

Cheng CL, Chou CH (2005). Determination of tadalafil in small volumes of plasma by high-performance liquid chromatography with UV detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 822: 278–284.

Fleshner N *et al.* (2005). Evidence for contamination of herbal erectile dysfunction products with phosphodiesterase type 5 inhibitors. *J Urol* 174: 636–641.

Forgue ST *et al.* (2006). Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 61: 280–288.

Forgue ST *et al.* (2007). Effects of gender, age, diabetes mellitus and renal and hepatic impairment on tadalafil pharmacokinetics. *Br J Clin Pharmacol* 63: 24–35.

Gao W *et al.* (2007). Chiral separation of two pairs of enantiomers of tadalafil by high-performance liquid chromatography. *J Chromatogr Sci* 45: 540–543.

Gratz SR *et al.* (2004). Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal* 36: 525–533.

Gratz SR *et al.* (2006). Accurate mass measurement using Fourier transform ion cyclotron resonance mass spectrometry for structure elucidation of designer drug analogs of tadalafil, vardenafil and sildenafil in herbal and pharmaceutical matrices. *Rapid Commun Mass Spectrom* 20: 2317–2327.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Ramakrishna NV *et al.* (2004). Quantitation of tadalafil in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionization. *J Chromatogr B Analyt Technol Biomed Life Sci* 809: 243–249.

Rodriguez Flores J *et al.* (2004). Development of a Micellar electrokinetic capillary chromatography method for the determination of three drugs employed in the erectile dysfunction therapy. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 231–236.

Smith KM, Romanelli F (2005). Recreational use and misuse of phosphodiesterase 5 inhibitors. *J Am Pharm Assoc* 45: 63–72.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Zhu X *et al.* (2005). Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography–electrospray ionization mass spectrometry. *J Chromatogr A* 1066: 89–95.

Zou P *et al.* (2006a). Structural elucidation of a tadalafil analogue found as an adulterant of a herbal product. *Food Addit Contam* 23: 446–451.

Zou P *et al.* (2006b). Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography–electrospray ionization tandem mass spectrometry. *J Chromatogr A* 1104: 113–122.

Talbutal

Sedative, Barbiturate

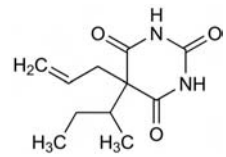
$C_{11}H_{16}N_2O_3 = 224.3$

CAS—115-44-6

IUPAC Name 5-Butan-2-yl-5-prop-2-enyl-1,3-diazinane-2,4,6-trione

Synonym 5-(1-Methylpropyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione

Proprietary Name *Lotusate*



Chemical Properties A white crystalline powder. It occurs in 2 polymorphic forms. Mp about 108°. Soluble 1 in 500 of water, 1 in 1 of ethanol, 1 in 2 of chloroform and 1 in 40 of ether. pK_a 7.9 (20°). Log *P* (octanol/pH 7.4), 1.3; (octanol/water), 1.5.

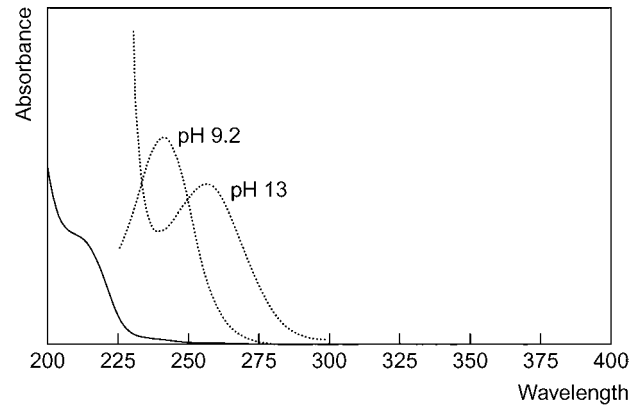
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—brown-orange/violet.

Thin-layer Chromatography System TD—R_f 0.53; system TE—R_f 0.46; system TF—R_f 0.67; system TH—R_f 0.71; system TAD—R_f 0.60; system TAE—R_f 0.92.

Gas Chromatography System GA—talbutal RI 1703, talbutal-Me_2 RI 1600.

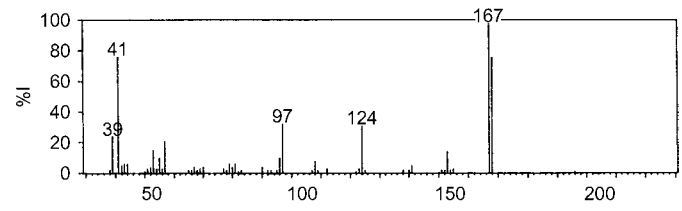
High Performance Liquid Chromatography System HG—*k* 7.20; system HH—*k* 4.70; system HX—RI 403; system HY—RI 370.

Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—239 nm (A^1_1=415a); 1 mol/L sodium hydroxide (pH 13)—255 nm (A^1_1=313b).



Infrared Spectrum Principal peaks at wavenumbers 1703, 1728, 1752, 1315, 1211, 829 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 167, 168, 41, 97, 124, 39, 57, 53.



Disposition in the Body

Toxicity The estimated minimum lethal dose is 2 g.

In one fatality involving talbutal, a blood concentration of 13 mg/L and a liver concentration of 305 μg/g was reported [Baselt, Cravey 1977].

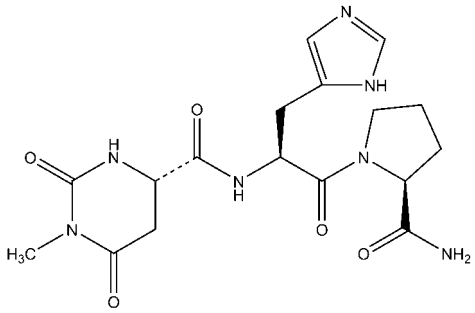
Dose 60 to 150 mg daily.

Baselt RC, Cravey RH (1977). A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. *J Anal Toxicol* 1: 81–102.

Taltirelin

Nootropic, Thyroid Agent, Thyrotropin Releasing Hormone Analogue
$C_{17}H_{23}N_7O_5$ = 405.4
CAS—103300-74-9

IUPAC Name (4*S*)-*N*-[(2*S*)-1-[(2*S*)-2-Carbamoylpyrrolidin-1-yl]-3-(3*H*-imidazol-4-yl)-1-oxopropan-2-yl]-1-methyl-2,6-dioxo-1,3-diazinane-4-carboxamide
Synonyms *N*-[[(4*S*)-Hexahydro-1-methyl-2,6-dioxo-4-pyrimidinyl]carbonyl]-L-histidyl-L-prolinamide; (*S*)-*N*-(1-methyl-4,5-dihydroorotyl)-L-histidyl-L-prolinamide.



Chemical Properties Crystals. Mp 72° to 75°.

Taltirelin Tetrahydrate

$C_{17}H_{23}N_7O_5,4H_2O$ = 477.4
CAS—201677-75-0
Synonym TA-0910
Proproprietary Name *Ceredist*
Chemical Properties White, odourless crystals. Bp 72° to 75°. Soluble in water, acetic acid, and ethanol; slightly soluble in methanol and acetonitrile.

Quantification

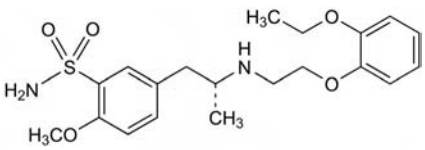
Plasma **LC-MS** Column: Daisopak Sp-120-30DS-BP (150 × 2.0 mm i.d., 5 µm). Mobile phase: methanol-0.1% formic acid (3 : 37) : methanol-0.1% formic acid (9 : 1; 100 : 0 for 3 min to 0 : 100 over 1 min for 3 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 3 min. Limit of quantification, 17 ng/L [Horimoto *et al.* 2002].
Other **LC-MS** Rat Plasma. Column: L-column ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : 1 mol/L ammonium formate (80 : 20 : 0.01), flow rate 1 mL/min. API, MRM acquisition mode. Limit of quantification, 5 µg/L [Urayama *et al.* 2001].
Dose Taltirelin hydrate is used in the treatment of spinocerebellar degeneration at doses from 5 mg twice daily.

Horimoto S *et al.* (2002). Determination of taltirelin, a new stable thyrotropin-releasing hormone analogue, in human plasma by high-performance liquid chromatography turbo-ionspray ionization tandem mass spectrometry. *J Pharm Biomed Anal* 30: 1361–1369.
Urayama A *et al.* (2001). Brain receptor binding characteristics and pharmacokinetic-pharmacodynamic analysis of thyrotropin-releasing hormone analogues. *Life Sci* 70: 647–657.

Tamsulosin

α_1-Adrenoceptor Antagonist, Treatment of BPH
$C_{20}H_{28}N_2O_5S$ = 408.5
CAS—106133-20-4

IUPAC Name 5-[(2*R*)-2-[2-(2-Ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzene-sulfonamide
Synonym 5-[(2*R*)-2-[[2-(2-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide.

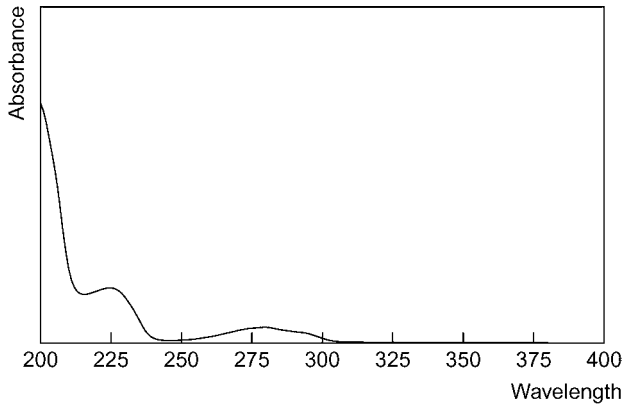


Chemical Properties Mp 254° to 256°. pK_a 8.4 (secondary amine), 10.2 (sulfonamide). Log *P* (octanol/pH 6.9), 0.51. Stock solutions were stable for at least 6 months. Tamsulosin in serum and aqueous humour was stable after 3 freeze-thaw cycles and after 4 h at room temperature [Keski-Rahkonen *et al.* 2007]. Stock solutions were stable after multiple freeze-thaw cycles. Processed plasma samples were stable at room temperature for 6 days. Plasma samples were stable at -18° for 5 months and thawed plasma samples were stable for 24 h at room temperature [Macek *et al.* 2004]. Tamsulosin was stable for at least a year in plasma and urine and for at least 3 months in plasma dialysate stored at -20° [Matsushima *et al.* 1997].

Tamsulosin Hydrochloride

$C_{20}H_{28}N_2O_5S,HCl$ = 445.0
CAS—106463-17-6
Synonyms LY-253351; R-(-)-YM-12617; YM-617; YM-12617-1.
Proprietary Names *Alna*; *Flomax*; *Harnal*; *Omiz*; *Omnic*; *Pradif*.
Chemical Properties White crystals. Mp 228° to 230°. It is sparingly soluble in water and methanol; slightly soluble in glacial acetic acid and ethanol; practically insoluble in ether.

High Performance Liquid Chromatography Column: Nucleosil $5C_{18}$ (150 × 4.0 mm i.d., 5 µm). Mobile phase: 100 mmol/L potassium dihydrogen phosphate (pH 4.5) : acetonitrile-100 mmol/L potassium dihydrogen phosphate (pH 4.9, 20 : 80 for 5 min to 100 : 0 for 5 min, to 0 : 100 over 60 min for 60 min), flow rate 1 mL/min. UV detection (λ= 275 nm). Retention time: 80 min [Soeishi *et al.* 1996a].
Ultraviolet Spectrum Aqueous acid (methanol)—225 nm, 280 nm.



Infrared Spectrum Principal peaks at wavenumber 1500, 1340, 1255, 1165 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 409, 271, 257, 228, 208, 70.

Quantification

Plasma **HPLC** Column: Lichrocart Purospher StarRP 18e (55 × 4 mm i.d., 3 µm). Mobile phase: acetonitrile : 30 mmol/L potassium dihydrogenphosphate (25 : 75), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}=228 nm, λ_{em}= 326 nm). Limit of quantification, 0.397 µg/L [Macek *et al.* 2004]. Column: Nucleosil S1100-5 normal phase (250 × 4 mm i.d.). Mobile phase: benzene : methanol (100 : 1). Fluorescence detection (λ_{ex}= 352 nm, λ_{em}= 500 nm). Limit of quantification, 0.5 µg/L [Matsushima *et al.* 1997].
LC-MS [Ksycinska *et al.* 2006]. Column: Symmetry C_{18}. Mobile phase: 0.03% formic acid : acetonitrile (30 : 70). ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 100 ng/L [Ramakrishna *et al.* 2005]. Column: C_{18} reversed phase. Mobile phase: methanol : water : acetic acid : triethylamine (620 : 380 : 1.5 : 1.5). ESI, positive ion mode, SIM acquisition mode. Limit of detection, 0.2 µg/L [Din *et al.* 2002]. Column: J'sphere ODS-80H (75 × 4.6mm i.d., 5 µm). Mobile phase: 50 mmol/L acetic acid (pH 4), flow rate 0.5 mL/min. API, ESI, SRM acquisition mode. Limit of detection, 0.5 µg/L [Matsushima *et al.* 1997].
Serum **LC-MS** Column: XTerra C_8 (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: water-formic acid (100 : 0.1) : water-acetonitrile-formic acid (50 : 50 : 0.1; 90 : 10 to 0 : 100 at 5 min to 90 : 10 at 6 min for 2 min), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of quantification, 0.1 µg/L [Keski-Rahkonen *et al.* 2007].
Urine **LC-MS** See Plasma. Limit of detection, 1 µg/L [Matsushima *et al.* 1997].
Aqueous Humour **LC-MS** See Serum [Keski-Rahkonen *et al.* 2007].
Disposition in the Body Tamsulosin is absorbed from the gastrointestinal tract; 90% after oral administration. The extent and rate of absorption is reduced in the presence of food. It is distributed into extracellular fluid in the body. The drug is metabolised slowly in the liver by hepatic metabolism whilst first-pass metabolism is negligible. It is mainly metabolised by the cytochrome P_{450} CYP3A enzyme and less than 10% of the dose is excreted in urine unchanged. The metabolites of tamsulosin hydrochloride undergo extensive conjugation to glucuronide or sulfate prior to renal excretion. It is excreted mainly in urine as metabolites and some unchanged drug. There are linear kinetics following single and multiple doses. Steady-state concentrations have been observed by the fifth day. Tamsulosin is highly bound to α_1-acid glycoprotein. There appears to be no or little possibility of binding interactions between tamsulosin and other drugs in clinically concomitant use, despite its strong binding to α_1-acid glycoprotein [Matsushima *et al.* 1999]. In patients with renal impairment, the pharmacokinetics of tamsulosin are affected by the change in protein binding that is associated with alteration of plasma α_1-acid glycoprotein concentration, but are not largely affected by the decrease in the renal excretion. Although total tamsulosin levels increased as plasma protein binding increased, unbound tamsulosin levels (which are directly associated with the pharmacological effects) remained unchanged in these patients [Koiso *et al.* 1996], indicating that no dose modification is required in symptomatic BPH patients with renal impairment [Wolzt *et al.* 1998]. There are no data available on the hepatic-impaired.

Therapeutic Concentration

Young, healthy, male volunteers were administered with 0.4 mg tamsulosin (as modified-release tablets) and the median peak plasma concentration was 16 µg/L. This was reached after 5 h and decreased to 2 µg/L at around 23.5 h [Taguchi *et al.* 1998].

Four healthy males were administered with a single oral dose of 0.2 mg tamsulosin. The mean peak plasma concentration of the unchanged drug was 13.0 µg/L observed within the hour [Soeishi *et al.* 1996b].

Toxicity

Note For a case of contact dermatitis in a 46-year-old male after tamsulosin ingestion see Lijnen, de Graaf [2003] and for a case of unintentional overdose in a 78-year-old female see Anand *et al.* [2005].

Half-life 5–7 h. Prolonged in the elderly. Due to absorption rate-controlled pharmacokinetics, the apparent half-life is ≈9 –13 h in healthy individuals and 14–15 h in the target population.

Bioavailability 100%.

Volume of Distribution Steady state after intravenous administration, 16 L or 0.2 L/kg.

Clearance 2.88 L/h or 48 mL/min.

Distribution in Blood Blood : plasma ratio is 0.53.

Protein Binding 94–99%, primarily to α_1 acid glycoprotein.

Dose 0.4 to 0.8 mg daily.

Anand JS *et al.* (2005). Acute intoxication with tamsulosin hydrochloride. *Clin Toxico (Phila)* 43: 311.

Din L *et al.* (2002). Quantitation of tamsulosin in human plasma by liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 75–81.

Keski-Rahkonen P *et al.* (2007). Determination of tamsulosin in human aqueous humor and serum by liquid chromatography-electrospray ionization tandem mass spectrometry. *J Pharm Biomed Anal* 43: 606–612.

Koiso K *et al.* (1996). Pharmacokinetics of tamsulosin hydrochloride in patients with renal impairment: effects of alpha 1-acid glycoprotein. *J Clin Pharmacol* 36: 1029–1038.

Ksycinska H *et al.* (2006). Validated LC-MS method for determination of tamsulosin in human plasma and its application to pharmacokinetic study. *Acta Pol Pharm* 63: 417–419.

Lijnen RL, de Graaf L (2003). Systemic contact dermatitis from tamsulosin. *Contact Dermatitis* 49: 50–51.

Macek J *et al.* (2004). Rapid determination of tamsulosin in human plasma by high-performance liquid chromatography using extraction with butyl acetate. *J Chromatogr B Analyt Technol Biomed Life Sci* 809: 307–311.

Matsushima H *et al.* (1997). Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 695: 317–327.

Matsushima H *et al.* (1999). Plasma protein binding of tamsulosin hydrochloride in renal disease: role of alpha1-acid glycoprotein and possibility of binding interactions. *Eur J Clin Pharmacol* 55: 437–443.

Ramakrishna NV *et al.* (2005). Rapid, simple and highly sensitive LC-ESI-MS/MS method for the quantification of tamsulosin in human plasma. *Biomed Chromatogr* 19: 709–719.

Soeishi Y *et al.* (1996a). Metabolism of tamsulosin in rat and dog. *Xenobiotica* 26: 355–365.

Soeishi Y *et al.* (1996b). Absorption, metabolism and excretion of tamsulosin hydrochloride in man. *Xenobiotica* 26: 637–645.

Taguchi K *et al.* (1998). Radioreceptor assay analysis of tamsulosin and terazosin pharmacokinetics. *Br J Clin Pharmacol* 45: 49–55.

Wolzt M *et al.* (1998). Pharmacokinetics of tamsulosin in subjects with normal and varying degrees of impaired renal function: an open-label single-dose and multiple-dose study. *Eur J Clin Pharmacol* 54: 367–373.

Tandospirone

Anxiolytic, Antidepressant

$C_{21}H_{29}N_5O_2 = 383.5$

CAS—87760-53-0

Synonym (3aα,4β,7β,7aα)-Hexahydro-2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl]-4,7-methano-1*H*-isoindole-1,3(2*H*)-dione

Chemical Properties Crystals. Mp 112° to 113.5°.

Tandospirone Citrate

$C_{21}H_{29}N_5O_2,C_6H_8O_7 = 575.6$

CAS—112457-95-1

Synonyms Metanopirone citrate; SM-3997.

Proprietary Name *Sediel*

Chemical Properties Crystals. Mp 169.5° to 170°.

Tandospirone Hydrochloride

$C_{21}H_{29}N_5O_2,HCl = 420.0$

Chemical Properties Crystals from isopropanol. Mp 227° to 229°.

Disposition in the Body Tandospirone is metabolised to 1-(2-pyrimidinyl)-piperazine (1-PP). Maximum plasma concentrations after oral administration are reached in 0.5 to 2 h and are 3- to 8-fold higher for the metabolites than the parent drug. Approximately 70% of the administered drug is excreted in urine, with only 0.1% of the dose excreted as the unchanged drug. Dose-dependent pharmacokinetics do not seem to occur.

Therapeutic Concentration The maximum plasma concentration after a 30 mg dose is 3 µg/L.

Half-life Parent drug, 2 to 3 h; metabolite, 3 to 5 h.

Protein Binding Parent drug, 30.4%; metabolite, 1-PP, 87.5%.

Note For a review of tandospirone, see Barradell and Fitton [1996].

Dose 30 or 60 mg daily.

Barradell LB, Fitton A (1996). Tandospirone. *CNS Drugs* 5(2): 147–153.

Taurolin

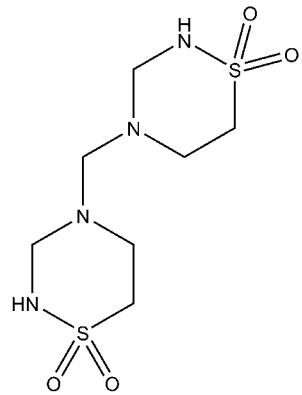
Antibacterial

$C_7H_{16}N_4O_4S_2 = 284.4$

IUPAC Name 4-[(1,1-Dioxo-1,2,4-thiadiazinan-4-yl)methyl]-1,2,4-thiadiazinane 1,1-dioxide

Synonyms 4,4′-Methylenebis(perhydro-1,2,4-thiadiazine-1,1-dioxide)

Proprietary Name *Tauroflex*



Chemical Properties White crystalline powder. Mp 172° to 174°. Soluble 1 in 100 of water. Poorly extracted by ether from aqueous acid or alkaline solutions.

Thin-layer Chromatography System T1—R_f 0.52 (location reagent potassium permanganate spray, positive reaction).

Gas Chromatography System G2/225—no peaks within 30 min; system G4/225—no peaks within 30 min.

Infrared Spectrum Principal peaks at wavenumbers 913 or 1143 or 1312 cm^{-1} (KBr disk).

Teclothiazide

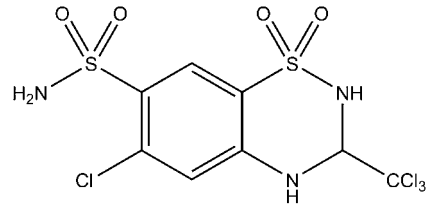
Peripheral Vasodilator, Rubefacient

$C_8H_7Cl_4N_3O_4S_2 = 415.1$

CAS—4267-05-4

IUPAC Name 6-Chloro-1,1-dioxo-3-(trichloromethyl)-3,4-dihydro-2*H*-benzo[e][1,2,4]thiadiazine-7-sulfonamide

Synonyms 6-Chloro-3,4-dihydro-7-sulfamoyl-3-trichloromethyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide; 6-chloro-3,4-dihydro-3-trichloromethyl-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; teclothiazidium; tetrachlormethiazide; 3-trichloromethylhydrochlorothiazide.



Chemical Properties Colourless liquid. Bp 114° to 116°. Soluble in water, oil and chloroform. Log *P* (octanol/water) 1.16 [Meylan, Howard 1995] 1.1 [National Institutes of Health 2008].

Thin-layer Chromatography System T1—R_f 0.62 (location reagent acidified iodoplatinate spray, positive reaction).

Dose It is used as a topical application in creams and ointments.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

National Institutes of Health (2008). *Thurfyl Nicotinate.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6260&loc=ec_rcs.(accessed 20 June 2008).

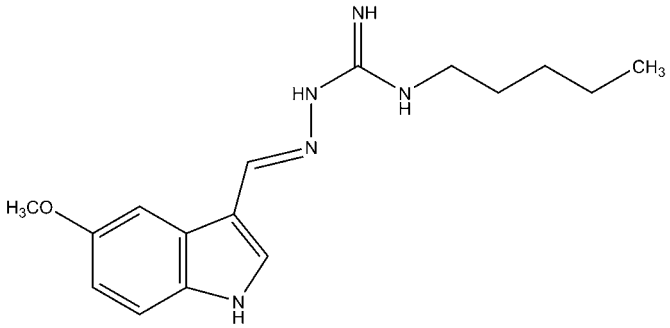
Tegaserod

5-HT$_4$ Partial Agonist, Treatment of Irritable Bowel Syndrome

$C_{16}H_{23}N_5O = 301.4$

CAS—145158-71-0

IUPAC Name 1-[[(*Z*)-[5-(Hydroxymethyl)indol-3-ylidene]methyl]amino]-2-pentylguanidine
Synonym 2-[(5-Methoxy-1*H*-indol-3-yl)methylene]-*N*-pentylhydrazinecarboximidamide

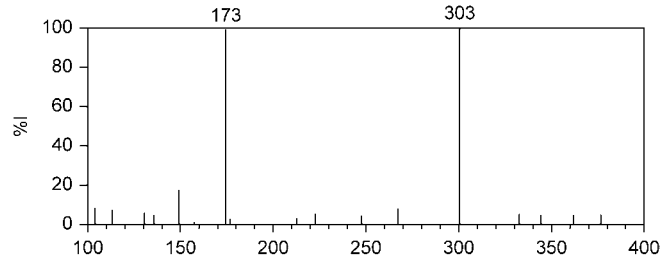


Chemical Properties Mp 155°.

Tegaserod Maleate

$C_{16}H_{23}N_5O,C_4H_4O_4 = 417.5$
CAS—189188-57-6
IUPAC Name 1-{[(5-Methoxyindol-3-yl)methylene]amino}-3-pentylguanidine maleate
Synonym SDZ-HTF-919
Proprietary Names *Altezerod*; *Colonaid*; *Coloserod*; *Distimax*; *Procinet*; *Tegarod*; *Tegasir*; *Tegibs*; *Tegod*; *Ther*; *Zelmac*; *Zelnorm*.
Chemical Properties A white/off-white crystalline powder. Slightly soluble in ethanol and very slightly soluble in water. Plasma samples were stable for 8 h at room temperature (25°), over three freeze–thaw cycles in 30 days, and for 30 days at below –70° [Zou *et al.* 2008].

Mass Spectrum Principal ions at *m/z* 303, 173.



Quantification
***Plasma* GC-MS** Column: CP SIL8 CB fused silica capillary (25 × 0.25 mm). Carrier gas: He. CI, negative ion mode. Limit of quantification, 0.1 µg/L [Appel-Dingemanse *et al.* 1999].

HPLC Column: Agilent Zorbax SB C18 (100 × 2.1 mm, 3.5 µm). Mobile phase: methanol : 5 mmol/L ammonium acetate (pH 3.5; 75 : 25), flow rate 0.2 mL/min. Positive ion mode, MRM acquisition mode. Limit of quantification, 0.05 µg/L [Zou *et al.* 2008].

Disposition in the Body Metabolised in the gastrointestinal tract by hydrolysis, oxidation and conjugation. The main metabolite produced is 5-methoxyindole-3-carboxylic acid glucuronide. Direct glucuronidation may also occur, producing 3 isomeric *N*-glucuronide metabolites. Approximately two-thirds of the drug is excreted unchanged in faeces, with <3% of the remainder excreted in urine as the main metabolite.

Therapeutic Concentration

Thirty-six healthy Chinese volunteers were administered 4, 6 or 12 mg tegaserod maleate orally. Peak plasma concentrations were 1.25 ± 0.53, 2.21 ± 0.52 and 4.34 ± 1.66 µg/L, respectively, attained at 1.00 ± 0.21, 1.05 ± 0.28 and 1.04 ± 0.16 h, respectively [Zou *et al.* 2008].

Ten healthy men and women (mean age 45 years) and 10 patients with severe renal impairment (mean age 44 years) were administered a single 12 mg oral dose of tegaserod maleate. Mean peak plasma concentrations of 5.1 ± 2.2 and 4.7 ± 2.3 µg/L were reached, respectively, ~1 h after dosing [Swan *et al.* 2003].

Eighteen healthy male and female volunteers (aged 21 to 36 years) were administered 2, 6 or 12 mg doses of tegaserod after an overnight fast on day 1. They continued to fast for another 4 h and were then administered the same doses twice daily for 5 days. There was no accumulation in this dose range and the maximum plasma concentrations were 0.7 ± 0.3, 2.7 ± 1.2 and 5.6 ± 2.9 µg/L for the 3 dose regimens, respectively, reached after ~1 h. [Appel-Dingemanse *et al.* 2001a].

Four groups of 10 healthy volunteers (young male, young female, elderly male, and elderly female) were administered a single 12 mg dose of tegaserod maleate after an overnight fast and fasted for 4 h after dosing. Mean peak plasma concentrations of 6.5 ± 2.3 and 5.2 ± 1.3 µg/L were reached for the elderly and young women, respectively, and 5.1 ± 1.9 and 5.5 ± 3.1 µg/L for the elderly and young men, respectively. These concentrations were observed 1 h after dosing. [Appel-Dingemanse *et al.* 2001b].

A single-dose and subsequent twice-daily multiple dose study in which 3 cohorts of 12 men (8 treated, 4 controls) received 25, 50 or 100 mg tegaserod for 14 days showed that peak plasma concentrations were reached between 1.3 and 1.7 h. Mean peak plasma concentrations were 10 ± 4, 16 ± 6 and 36 ± 12 µg/L for 25, 50 and 100 mg after a single dose and 8 ± 3, 12 ± 5 and 38 ± 17 µg/L after multiple doses at each dose, respectively [Appel *et al.* 1997].

Note For a review article on the pharmacokinetics of tegaserod, see Appel-Dingemanse [2002].

Toxicity On March 30 2007, the FDA asked for *Zelnorm* to be withdrawn from the US market. Data collected in over 18 000 patients showed adverse cardiovascular events in 13 of 11 614 patients on *Zelnorm* treatment (0.11%) compared with 1 of 7031 patients on placebo (0.01%).

Bioavailability Approximately 10%.

Half-life Approximately 8.5 h.

Volume of Distribution Approximately 368 L.

Clearance Plasma, approximately 77 L/h.

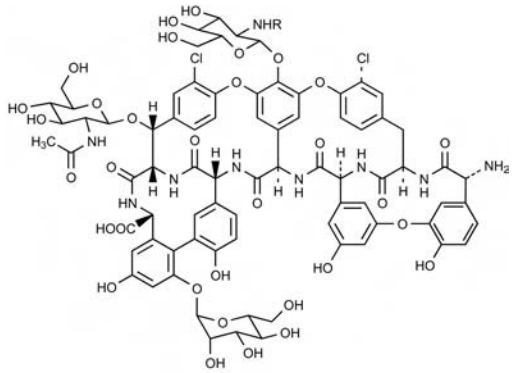
Protein Binding Approximately 98% to α1-acid glycoprotein.

Dose 6 mg of tegaserod maleate twice daily.

Appel S *et al.* (1997). First pharmacokinetic-pharmacodynamic study in humans with a selective 5-hydroxytryptamine4 receptor agonist. *J Clin Pharmacol* 37: 229–237.
Appel-Dingemanse S (2002). Clinical pharmacokinetics of tegaserod, a serotonin 5-HT(4) receptor partial agonist with promotile activity. *Clin Pharmacokinet* 41: 1021–1042.
Appel-Dingemanse S *et al.* (1999). Integrated modelling of the clinical pharmacokinetics of SDZ HTF 919 a novel selective 5-HT4 receptor agonist, following oral and intravenous administration. *Br J Clin Pharmacol* 47: 483–491.
Appel-Dingemanse S *et al.* (2001a). Multiple-dose pharmacokinetics confirm no accumulation and dose proportionality of the novel promotile drug tegaserod (HTF 919). *Eur J Clin Pharmacol* 56: 889–891.
Appel-Dingemanse S *et al.* (2001b). The pharmacokinetics of the novel promotile drug, tegaserod, are similar in healthy subjects-male and female, elderly and young. *Aliment Pharmacol Ther* 15: 937–944.
Swan SK *et al.* (2003). Tegaserod pharmacokinetics are similar in patients with severe renal insufficiency and in healthy subjects. *J Clin Pharmacol* 43: 359–364.
Zou JJ *et al.* (2008). Determination of tegaserod by LC-ESI-MS/MS and its application to a pharmacokinetic study in healthy Chinese volunteers. *J Chromatogr B Analyt Technol Biomed Life Sci* 861: 151–157.

Teicoplanin

Antibacterial
CAS—61036-62-2 (teichoplanin); 61036-64-4 (teichoplanin A_2)
Synonyms A-8327; DL-507-IT; L-12507; MDL-507; teichomycin A_2.
Proprietary Names *Targocid*; *Targosid*; *Teicomid*.



Chemical Properties A glycopeptide antibiotic obtained from cultures of *Actinoplanes teichomyceticus*. An amorphous powder. Mp 260°. Soluble in aqueous solutions at pH 7, in propylene glycol, dimethylformamide and dimethylsulfoxide; partially soluble in methanol and ethanol; insoluble in carbon tetrachloride, acetone, butanol, ethylether and in diluted mineral acids.

High Performance Liquid Chromatography Column: RP C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 15.4 mmol/L sodium dihydrogen phosphate(5 : 95)- acetonitrile : sodium dihydrogen phosphate (75 : 25; 90 : 10 for 5 min; to 70 : 30 in 10 min, for 20 min; to initial conditions over 12 min), flow rate 1 mL/min. UV detection (λ = 214 nm). Retention time: component A_3, 13.7 min; A_2-1, 18.7 min; A_2-2, 20.0 min; A_2-3, 21.0 min; A_2-4, 25.0 min; A_2-5, 26.3 min [Jehl *et al.* 1988].

Column: RP C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: 0.01 mol/L aqueous tetrabutylammonium phosphate solution (pH 7.5) : methanol : *n*-butanol

(10 : 10 : 1), flow rate 1.3 mL/min. Fluorescence detection ($\lambda_{ex} = 390$ nm; $\lambda_{em} = 490$). Retention time: A_2-1, 10.2 min; A_2-2, 13.7 min; A_2-3, 15.7 min; A_2-3a, 17.9 min; A_2-4, 23.2 min; A_2-5, 25.4 min [Joos, Lüthy 1987].

Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—278 nm; aqueous alkali (0.1 mol/L sodium hydroxide)—297 nm.

Quantification

Blood **HPLC** UV detection ($\lambda = 214$ nm). Limit of detection, 0.5 mg/L [Jehl *et al.* 1988].

Plasma **HPLC** Limit of quantification, 0.09 mg/L, limit of detection 0.03 mg/L [Cociglio *et al.* 1998]. UV detection ($\lambda = 210$ nm). Limit of detection, 0.05 mg/L [Taylor *et al.* 1991]. UV detection ($\lambda = 240$ nm). Limit of detection, 0.2 mg/L [Georgopoulos *et al.* 1989]. UV detection ($\lambda = 240$ nm). Limit of detection, 0.1 mg/L [Riva *et al.* 1987].

Serum **HPLC** Fluorescence detection ($\lambda_{ex} = 390$ nm; $\lambda_{em} = 490$ nm). Limit of detection, 0.5 mg/L [Joos, Lüthy 1987]. UV detection ($\lambda = 210$ nm). Limit of detection, 5 mg/L [Levy *et al.* 1987].

Urine **HPLC** See Blood [Jehl *et al.* 1988]. See Plasma [Riva *et al.* 1987].

Review For a comparison of methods for the determination of teicoplanin, see Awni *et al.* [1991].

Disposition in the Body Teicoplanin is a mixture of several components and the pharmacokinetics of individual components vary slightly depending on their lipophilicity. The drug is poorly absorbed after oral administration and therefore administered by the parenteral route. It is well absorbed after IM injection with a bioavailability of 90% and it rapidly penetrates tissues including skin, fat and bone. Highest concentrations are observed in the kidneys, trachea, lungs and adrenals. Penetration into CSF is poor. It is taken up into white blood cells. No metabolites have been identified and it is excreted almost entirely by glomerular filtration in the urine as unchanged drug. Approximately 80% is excreted in urine and a minor amount in faeces. It is not removed by haemodialysis.

Therapeutic Concentration The trough serum therapeutic concentration range is 10–40 mg/L.

Teicoplanin was administered to 6 healthy males, aged 22–23 years old, as a single IM dose of 3 mg/kg, an IV dose of 3 mg/kg or 6 mg/kg after an overnight fast and continued fasting for 2 h after dosing. The mean peak plasma concentrations were 7.12, 53.48 and 111.81 mg/L at 2.02 h and by the end of the infusion, respectively [Verbist *et al.* 1984].

Toxicity The toxic serum concentration is 200 mg/L.

Several overdoses of 100 mg/kg daily administered to a 4-year-old and an 8-year-old neutropenic paediatric patient by mistake, resulted in high plasma concentrations up to 300 mg/L but no symptoms or laboratory abnormalities were observed [Sanofi-aventis 2006].

Bioavailability 90%.

Half-life Plasma half-life, 30 to over 160 h in normal subjects (depending on the sampling time), increased in renal impairment; an effective half-life of about 60 h has been suggested in calculating dosage regimens.

Volume of Distribution 0.9–1.6 L/kg.

Clearance Plasma clearance, 0.18 mL/min/kg.

Protein Binding 90–95%.

Note For reviews of teicoplanin, see Wilson [2000] and Brogden and Peters [1994].

Dose Initially 400 mg IV followed by 200 mg IV or IM on each subsequent day of treatment.

Awni WM *et al.* (1991). Teicoplanin measurement in patients with renal failure: comparison of fluorescence polarization immunoassay, microbiological assay, and high-performance liquid chromatographic assay. *Ther Drug Monit* 13: 511–517.

Brogden RN, Peters DH (1994). Teicoplanin. A reappraisal of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 47: 823–854.

Cociglio M *et al.* (1998). Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma. *J Chromatogr B Biomed Sci Appl* 705: 79–85.

Georgopoulos A *et al.* (1989). High-performance liquid chromatographic determination of teicoplanin in plasma: comparison with a microbiological assay. *J Chromatogr* 494: 340–346.

Jehl F *et al.* (1988). HPLC quantitation of the six main components of teicoplanin in biological fluids. *J Antimicrob Chemother* 21: 53–59.

Joos B, Lüthy R (1987). Determination of teicoplanin concentrations in serum by high-pressure liquid chromatography. *Antimicrob Agents Chemother* 31: 1222–1224.

Levy J *et al.* (1987). High pressure liquid chromatographic quantitation of teicoplanin in human serum. *J Antimicrob Chemother* 19: 533–539.

Riva E *et al.* (1987). Determination of teicoplanin in human plasma and urine by affinity and reversed-phase high-performance liquid chromatography. *J Chromatogr* 421: 99–110.

Sanofi-aventis (2006). Targocid/teicoplanin, 200mg/400mg, powder for injection. UK Summary of Product Characteristics.

Taylor RB *et al.* (1991). Determination of teicoplanin in plasma using microbore high-performance liquid chromatography and injection-generated gradients. *J Chromatogr* 563: 451–457.

Verbist L *et al.* (1984). In vitro activity and human pharmacokinetics of teicoplanin. *Antimicrob Agents Chemother* 26: 881–886.

Wilson AP (2000). Clinical pharmacokinetics of teicoplanin. *Clin Pharmacokinet* 39: 167–183.

Telmisartan

Angiotensin II Receptor Antagonist

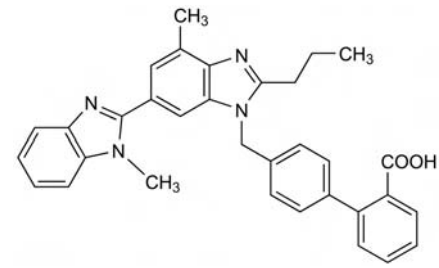
$C_{33}H_{30}N_4O_2 = 514.6$

CAS—144701-48-4

IUPAC Name 2-[4-[[4-Methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid

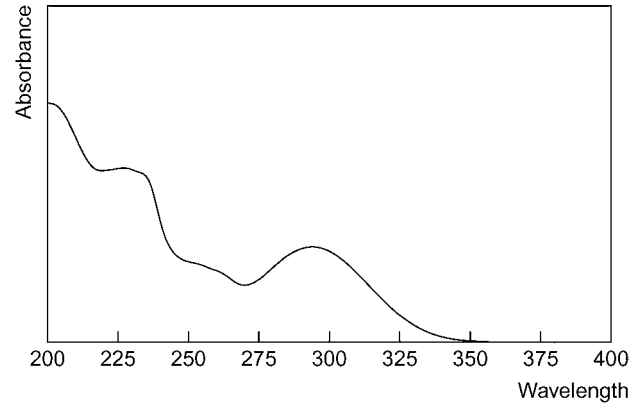
Synonyms BIBR-277-SE; BIBR-277; 4′-[(1,4′-dimethyl-2′-propyl[2,6′-bi-1*H*-benzimidazol]-1′-yl)methyl]-[1,1′-biphenyl]-2-carboxylic acid.

Proprietary Names *Micardis*; *Pritor*.



Chemical Properties A white to off-white, odourless crystalline powder. Mp 261° to 263°. Practically insoluble in water; sparingly soluble in strong acid; soluble in strong base.

Ultraviolet Spectrum



Quantification

Plasma **HPLC** Fluorescence detection ($\lambda_{ex} = 300$ nm; $\lambda_{em} = 385$ nm). Limit of quantification, 1 µg/L [Stangier *et al.* 2000a]. Fluorescence detection ($\lambda_{ex} = 305$ nm; $\lambda_{em} = 365$ nm). Limit of quantification, 0.5 µg/L [Stangier *et al.* 2000b].

Dialysate samples **HPLC** See Plasma [Stangier *et al.* 2000b].

Disposition in the Body After oral administration, telmisartan is rapidly absorbed and the peak concentration is achieved within 0.5 to 1 h. Most of a dose is eliminated unchanged in faeces via biliary excretion (>97%) with minute amounts detected in urine. Telmisartan is metabolised to form its acylglucuronide (pharmacologically inactive) and this is the only metabolite that has been detected in human plasma and urine. The glucuronide makes up approximately 11% of the plasma concentration of the drug.

Therapeutic Concentration

Thirteen healthy males (mean age, 51.2 years; range, 33 to 62 years) and 13 patients with hepatic impairment (mean, 53.5 years; range, 44 to 60 years) were fasted overnight and then administered 20 mg telmisartan. After a 14 day 'wash-out' period, another dose of 120 mg was given to the individuals. After the 20 mg dose, mean peak plasma concentrations of 105 (range, 10.5 to 265) µg/L were observed for the patients and 16.2 (range, 7.8 to 267) µg/L for the healthy individuals. These were detected in a time range between 0.5 and 4.0 h. After the 120 mg, the mean peak concentration for the patients was 1520 (514 to 3280) µg/L and 475 (167 to 760) µg/L for the healthy individuals. These concentrations were observed at a time range between 0.5 and 2.0 h [Stangier *et al.* 2000c].

Six patients with severe renal insufficiency and undergoing regular maintenance dialysis, aged 37 years (range, 21 to 46 years) and 12 healthy males (mean age, 51.2 years; range, 33 to 62 years) were fasted overnight and then administered with a 120 mg dose telmisartan. The healthy individuals reached a mean peak plasma concentration of 438 µg/L after 1 h. The patients receiving the dose between dialysis periods reached concentrations of 100 µg/L at 1 h and for those receiving telmisartan during dialysis, the peak concentration was 169 µg/L at 0.5 h [Stangier *et al.* 2000b].

Bioavailability Administration with food slightly reduces the bioavailability and this is also dose dependent. With a 40 mg dose the absolute bioavailability is 42% and with a 160 mg dose, 58%.

Half-life 24 h.

Volume of Distribution 500 L.

Clearance Plasma clearance, >800 mL/min.

Protein Binding >99.5% bound to, mostly, albumin and α_1-acid glycoprotein.

Dose The usual daily dose is 40 mg.

Stangier J *et al.* (2000a). Absorption, metabolism, and excretion of intravenously and orally administered [14C]telmisartan in healthy volunteers. *J Clin Pharmacol* 40: 1312–1322.

Stangier J *et al.* (2000b). Pharmacokinetics of single-dose telmisartan 120 mg given during and between hemodialysis in subjects with severe renal insufficiency: comparison with healthy volunteers. *J Clin Pharmacol* 40: 1365–1372.
Stangier J *et al.* (2000c). Pharmacokinetics and safety of intravenous and oral telmisartan 20 mg and 120 mg in subjects with hepatic impairment compared with healthy volunteers. *J Clin Pharmacol* 40: 1355–1364.

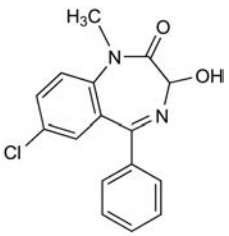
Temazepam

Benzodiazepine, Hypnotic
$C_{16}H_{13}ClN_2O_2 = 300.7$
CAS—846-50-4

IUPAC Name 7-Chloro-3-hydroxy-1-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-one

Synonyms 7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one; ER-115; K-3917; *N*-methyloxazepam; oxydiazepam; Ro-5-5345; temazepamum; Wy-3917.

Proprietary Names *Euhypnos*; *Euipnos*; *Gelthix*; *Levanxene*; *Levanxol*; *Maeva*; *Nocturne*; *Normison*; *Nortem*; *Perdorm*; *Planum*; *Remestan*; *Restoril*; *Somapam*; *Temaze*; *Temtabs*; *Tenox*.



Chemical Properties A white to almost white crystalline powder. Mp 156° to 159° (crystals from cyclohexane). Practically insoluble to very slightly soluble in water; soluble 1 in 10 of ethanol and 1 in 10 of chloroform; freely soluble in dichloromethane. pK_a 1.6. Log *P* (octanol/water), 2.20 [Mullett, Pawliszyn 2001]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

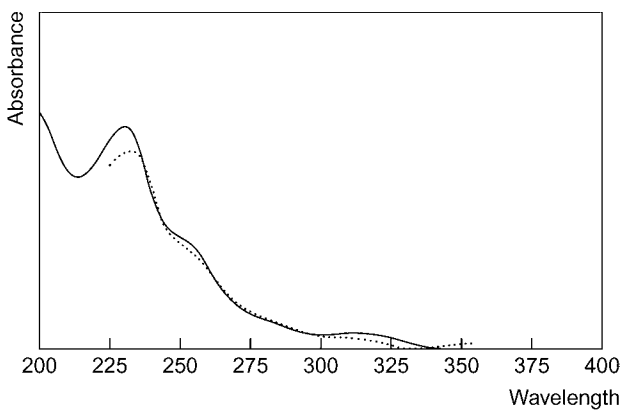
Colour Test Formaldehyde–sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.08; system TC—R_f 0.59; system TD—R_f 0.51; system TE—R_f 0.62; system TF—R_f 0.47; system TL—R_f 0.53; system TAD—R_f 0.65; system TAE—R_f 0.82; system TAF—R_f 0.82; system TAJ—R_f 0.65; system TAK—R_f 0.54; system TAL—R_f 0.92 (acidified iodoplatinate solution—positive; acidified potassium permanganate solution—positive; Dragendorff spray—positive).

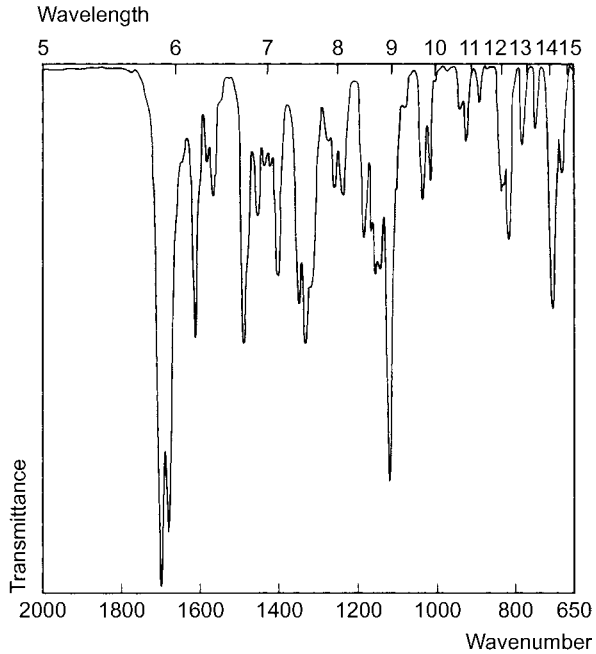
Gas Chromatography System GA—temazepam RI 2595, temazepam-AC RI 2730, temazepam-Me RI 2600, M (OH-) RI 2670, M (OH-)-AC RI 2790, M (oxazepam) RI 2325; system GB—temazepam RI 2727, temazepam-TMS RI 2713, M (oxazepam) RI 2438; system GG—temazepam RI 3125, M (oxazepam) RI 2803.

High Performance Liquid Chromatography System HI—temazepam *k* 5.68, M (oxazepam) *k* 4.62; system HK—temazepam *k* 0.60, M (oxazepam) *k* 0.73; system HX—RI 472; system HY—RI 438; system HZ—RT 5.5 min; system HAA—RT 18.6 min; system HAX—RT 8.9 min; system HAY—RT 6.7 min; system HAZ—*k* 1.43; system HBH—*k* 6.80; system HBI—*k* 1.49.

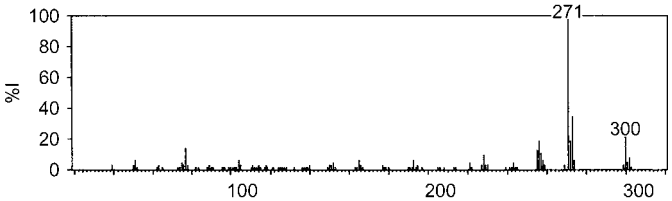
Ultraviolet Spectrum Aqueous acid—237 (A_1^1 = 980b), 284 (A_1^1 = 283b), 358 nm (A_1^1 = 68b); aqueous alkali—231, 313 nm; methanol—230 (A_1^1 = 1090a), 314 nm (A_1^1 = 76b).



Infrared Spectrum Principal peaks at wavenumbers 1687, 1670, 1112, 1603, 705, 1150 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 271, 273, 300, 272, 256, 77, 255, 257 (temazepam); 257, 77, 268, 239, 205, 267, 233, 259 (oxazepam).



Quantification

Blood **GC** Column: SE-54 5% phenylmethylsiloxane (25 m × 0.31 mm i.d., 0.17 µm). Carrier gas: He, 2 to 3 mL/min. Temperature programme: 200° for 1 min to 290° at 10°/min. NPD or ECD. Retention time: 6.68 min. Limit of detection not reported [Lillsunde, Seppälä 1990].

GC-MS Column: 100% methylsiloxane or 5% phenylsiloxane, 95% methylsiloxane. Carrier gas: 1.0 mL/min. Temperature programme: 140° for 0.5 min to 320° at 25°/min. SIM acquisition mode. Limit of detection, 12.5 µg/L [Goldberger *et al.* 2010]. Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° to 295° at 10°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 14 µg/L, limit of detection, 4.61 µg/L [Papoutsis *et al.* 2010]. Column: 5% diphenyldimethylsiloxane DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 2 mL/min. Temperature programme: 150° for 0.6 min to 230° at 40°/min to 310° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, 25 µg/L [Tiscione *et al.* 2008]. Column: DB5-MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° to 230° at 8°/min to 313° at 4°/min. EI ionisation at 70 eV or CI in positive ion mode. Limit of detection, 100 µg/L [Pirnay *et al.* 2002].

HPLC [Scott, Oliver 1997].

LC-MS MRM acquisition mode. Temazepam and other benzodiazepines [Gunn *et al.* 2010].

Plasma **GC** ECD. Limit of detection, 5 µg/L [Divoll, Greenblatt 1981].

HPLC Column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.). Mobile phase: 10 mmol/L phosphate buffer (pH 2.5)-methanol-acetonitrile (63 : 10 : 27), flow rate 2 mL/min. UV detection (λ = 230 nm). Limit of quantification, 2 µg/L for temazepam and diazepam and its other metabolites [Rouini *et al.* 2008]. Column: Cyclobond 1-2000 RSP (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1% triethylamine acetate buffer (pH 4.5) : water (18 : 8 : 73), flow rate 0.4 mL/min. DAD (λ = 230 nm). Limit of detection not reported [Pham-Huy *et al.* 2002]. Hypersil BDS RP_{18} (250 × 4.0 mm i.d., 5.0 µm). Mobile phase: methanol : acetonitrile : 0.05 mol/L potassium dihydrogen phosphate (pH 3.5, 50 : 10 : 40), flow rate 1.2 mL/min. UV detection (λ = 232 nm). Retention time: 9.70 min. Limit of quantification, 10 µg/L [Azzam *et al.* 1998]. Column: 60 RP-Select B (250 × 4 mm i.d., 5 µm) or LiChrospher 100 RP 8 (200 × 4 mm i.d., 8.5 µm). Mobile phase: 0.01 mol/L sodium phosphate buffer (pH 7.0) : acetonitrile (87.5 : 12.5 to 83.5 : 16.5 at 15 min to 80 : 20 over 8.5 min for 3 min to 75 : 25 in 1 min for 15 min), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 5 µg/L [Franzelius, Besserer 1993]. Column: C_8 reversed phase (250 × 4.6 mm i.d., 10 µm). Mobile phase: methanol : 0.03 mol/L

potassium dihydrogen phosphate (pH 4.5, 55 : 45), flow rate 1.1 mL/min. UV detection. Limit of detection, <10 μg/L [Ho *et al.* 1983].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : water : 100 mmol/L ammonium formate (pH 3.0, 55 : 40 : 5), flow rate 0.125 mL/min. ESI, MRM acquisition mode. Retention time: 5.95 min. Limit of quantification, 20 μg/L, limit of detection, 10 μg/L [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Merck LiChroCART (125 × 2 mm i.d.). Mobile phase: 5 mmol/L ammonium formate : acetonitrile (60 : 40 for 5.5 min to 10 : 90 in 8 min to 60 : 40 in 9.5 min for 10 min). APCI, SIM acquisition mode. Limit of quantification, 0.01 mg/L, limit of detection, 0.005 mg/L [Kratzsch *et al.* 2004].

Serum **HLPC** Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol (52 : 48), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 333 μg/L [Mullett, Pawliszyn 2002]. Column: Supelcosil C_{18} (50 × 4.6 mm i.d., 5 μm). Mobile phase: water : methanol (54 : 46), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of quantification, 105.7 μg/L, limit of detection, 31.7 μg/L [Mullett, Pawliszyn 2001]. Column: μBondapak C_{18} (300 × 3.9 mm i.d., 10 μm). Mobile phase: flow rate 1.5 mL/min. DAD (λ = 254 nm). Limit of quantification, 259.5 μg/L, limit of detection, 73.9 μg/L [Ahrens *et al.* 2000]. Column: C_8 reversed phase. Mobile phase: methanol : water : phosphate buffer. UV detection (λ = 230 nm). Limit of quantification, 46.5 μg/L, limit of detection, 15.5 μg/L for temazepam and its glucuronide [Kunsman *et al.* 1991].

LC-MS See Blood [Gunn *et al.* 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Supelco LC-18. Mobile phase: methanol-50 mmol/L ammonium acetate (60 : 40). SIM acquisition mode. Limit of detection, 0.02–2 μg/L for temazepam and other benzodiazepines [Yuan *et al.* 2000].

Urine **GC** Column: DB-17 (15 m). Carrier gas: N_2. Temperature: 225°. ECD. Retention time: 3.56 min. Limit of detection, 0.13 ng [Beischlag, Inaba 1992],

GC-MS See Blood [Goldberger *et al.* 2010]. Column: DB-1 (15 m × 0.32 mm i. d., 0.25 μm). Carrier gas: He. Temperature programme: 160° for 1 min to 280° at 20°/min for 3 min. NCI. Limit of detection, not reported [Fitzgerald *et al.* 1993]. Column; methylsilicone (12.5 m). Temperature programme; 120° to 310°. Limit of quantification, 14 μg/L, limit of detection, 5 μg/L [West, Ritz 1993]. Column: DB-5 (15 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 66 cm/s. Temperature programme: 210° to 300° at 20°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, <10 μg/L, limit of detection, <10 μg/L [Dickson *et al.* 1992].

HPLC UV detection. Limit of detection, 333 μg/L [Mullett, Pawliszyn 2002]. See Plasma [Azzam *et al.* 1998]. Column: LiChrospher 100 RP-18(e) (250 × 4 mm i.d., 5 μm). Mobile phase: water : methanol : triethylamine (pH 5.5, 70 : 30 : 0.1), flow rate 0.7 mL/min. UV detection (λ = 240 nm). Limit of detection, 2 μg/L for temazepam and other diazepam metabolites [Chiba *et al.* 1995]. See Plasma [Franzelius, Besserer 1993]. See Serum [Kunsman *et al.* 1991].

LC-MS Column: Hypurity C_8 (150 × 3 mm i.d.). Mobile phase: 4 mmol/L ammonium acetate (pH 6.8) in methanol-water (5 : 95) : 1% propan-2-ol, 0.05% formic acid in methanol (100 : 0 for 1 min to 0 : 100 at 3 min for 1.5 min to 100 : 0 over 0.1 min for 1.4 min). TIS, MRM acquisition mode. Limit of quantification, 1.25 μg/L, limit of detection, 0.31 μg/L [Glover, Allen 2010]. See Plasma [Marin *et al.* 2008; Marin, McMillin 2010]. Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 μm). Mobile phase: 10 mmol/L ammonium acetate : acetonitrile (100 : 0 for 3 min to 0 : 100 from 3.01 to 6 min to 100 : 0 from 6.01 to 10 min for 5 min), flow rate 0.9 mL/min for 3 min to 0.3 mL/min until 10 min to 0.9 mL/min. ESI, positive ion mode. Limit of quantification, 0.5 μg/L, limit of detection, 0.1 μg/L [Umezawa *et al.* 2008]. See Serum [Yuan *et al.* 2000].

Meconium **LC-MS** See Plasma [Marin *et al.* 2008; Marin, McMillin 2010].

Oral Fluid **LC-MS** Zorbax Eclipse XDB C_{18} (50 × 4.6 mm i.d., 1.8 μm). Mobile phase: 20 mmol/L ammonium formate : acetonitrile (50 : 50), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 0.5 μg/L [Moore *et al.* 2007].

Hair **LC-MS** Mobile phase: 3 mmol/L ammonium formate and 0.001% formic acid in water : acetonitrile (65 : 35 to 20 : 80 after 13 min to 10 : 90 at 13.5 min until 16.5 min to 65 : 35 until 20 min), flow rate 0.3 mL/min. ESI, positive ion mode. Limit of quantification, 0.16 ng/30 mg, limit of detection, 0.09 ng/30 mg [Miller *et al.* 2006].

Disposition in the Body Temazepam is rapidly absorbed after oral administration. The exact rate of absorption is determined by the formulation of the drug administered. It is metabolised principally by glucuronic acid conjugation; demethylation to oxazepam occurs to a small extent and it is also found as a conjugate. Approximately 80% of a dose is excreted in the urine, mostly as the inactive glucuronide conjugates; <2% of a dose is excreted as unchanged drug. Approximately 12% of a dose is eliminated in the faeces. Small amounts of the demethylated derivative, oxazepam, and its conjugated form have also been detected. Temazepam can be found in breast milk and CSF. Temazepam is a metabolite of several benzodiazepines, including diazepam, ketazolam and medazepam.

Therapeutic Concentration The therapeutic serum concentration is 0.3–0.9 mg/L.

Thirteen male patients were administered 20 mg temazepam orally 1–2 h before undergoing spinal anaesthesia for a urological procedure. The total plasma temazepam concentrations just before insertion of the anaesthetic were 385–1776 nmol/L (mean, 1029). The mean concentration in the CSF was 5.2% of that in the plasma [Badcock *et al.* 1990].

Twenty-four healthy, male and female volunteers (aged 18–43 years; mean, 29) were administered a single oral 20-mg dose temazepam as a soft-gelatin capsule and a liquid-filled capsule. Administration was after a 10 h fast, which continued for 4 h after dosing, and a 1-week washout period was allowed between doses. The peak plasma concentration observed for the first type of formulation was 0.617 mg/L, observed at ~40 min (median value). For the liquid-filled capsule, however, the peak plasma concentration was 0.708 mg/L at 30 min [Drake *et al.* 1991].

Ten healthy male volunteers (21–52 years of age) were rectally administered, after an overnight fast, 10 mg temazepam as a polyethylene glycol-based suppository, a liquid-filled capsule and a micro-enema. The same dose was also administered orally as a liquid-filled capsule. A 1-week washout period was allowed between doses. The mean peak serum concentrations of temazepam were 0.202, 0.182 and 0.205 mg/L for the suppository, capsule (administered rectally) and micro-enema, respectively. These were observed at 1.48, 4.08 and 0.49 h, respectively. For the capsule administered orally, the maximum concentration was 0.326 mg/L at 0.8 h [Hanff, Rutten 1996].

Toxicity The toxic serum concentration is 1.0 mg/L.

Half-life Plasma half-life, ~8 to 15 h; there is considerable inter-subject variation, and sex differences have been reported.

Volume of Distribution ≈1 L/kg.

Clearance Plasma clearance, ~1–2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.9.

Protein Binding ≈96%.

Note For a review of the pharmacokinetics of temazepam see Heel *et al.* [1981]; for a review of temazepam and new trends in its clinical application, see Fraschini, Stankov [1993]; for a report of the relative toxicity of benzodiazepines in overdose, see Buckley *et al.* [1995].

Dose For insomnia, 10 to 20 mg at night; exceptional circumstances, 30 to 40 mg; premedication usually 20 to 40 mg.

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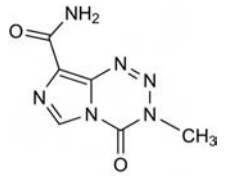
Temozolomide

Antineoplastic
$C_6H_6N_6O_2 = 194.2$
CAS—85622-93-1

IUPAC Name 3-Methyl-4-oxoimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxamide
Synonyms BRN-5547136; CCRG-81045; 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide; MB 39831; methazolastone; NSC-632856.
Proprietary Name *Temodal*



Chemical Properties A white to light tan/pink powder. Mp 212°.
High Performance Liquid Chromatography Column: ODS Ultrasphere (150 × 4.6 mm i.d., 5 µm). Mobile phase: aqueous acetic acid (0.1%) : acetonitrile (90 : 10), flow rate 1.0 mL/min. IS: ethazolastone. UV detection (λ = 316 nm). Retention time: temozolomide, 2.7 min; IS, 5.0 min [Kim *et al.* 2001].

Column: SCD-100 Synchropak, (150 × 4.6 mm i.d., 5 µm). Mobile phase: ammonium acetate (0.02 mol/L) : acetonitrile (92 : 8), flow rate 1.1 mL/min. IS: hydrochlorothiazide. UV detection (λ = 316 nm). Retention time: MTIC, 4.5 min; IS, 6.5 min [Kim *et al.* 1997].

Column: (analytical) RP HP ODS Hypersil (100 × 4.6 mm i.d., 5 µm); (guard) HP ODS Hypersil (20 × 4.0 mm i.d., 5 µm). Mobile phase: methanol : acetic acid (0.5%) (10 : 90), flow rate 1.0 mL/min. UV detection (λ = 330 nm). IS: ethazolastone. Retention time: 3.2 min; IS, 7.4 min [Shen *et al.* 1995].
Ultraviolet Spectrum Ethanol—327 nm.
Quantification
Plasma **HPLC** UV detection (λ = 316 nm). Limit of quantification, 0.1 mg/L [Kim *et al.* 2001]. UV detection (λ = 316 nm). Limit of quantification, 0.01 mg/L [Kim *et al.* 1997]. Limit of quantification, 0.2 mg/L [Shen *et al.* 1995].
Urine **HPLC** Limit of quantification, 2.0 mg/L, see Plasma [Shen *et al.* 1995].
Disposition in the Body Temozolomide is rapidly and completely absorbed after oral administration, with peak plasma concentrations observed within 0.5 to 1.5 h after administration. Food reduces the rate and the extent of absorption. Peak concentrations are reduced by ~32%, and the time to reach these concentrations is extended to over 2 h after a high-fat breakfast. Temozolomide undergoes spontaneous non-enzymatic hydrolysis to its active metabolite 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC), the hydrolysis of which is faster in alkaline pH compared with neutral pH. MTIC is further hydrolysed to 5-amino-imidazole-4-carboxamide (AIC). Temozolomide is also metabolised to the temozolomide acid metabolite. The drug is largely eliminated by the kidneys, about 5 to 10% as the unchanged drug. The majority of the dose is recovered in urine, and a small amount in the faeces. Temozolomide is detected mainly as AIC, the unchanged drug, the temozolomide acid metabolite and other unidentified polar metabolites.
Therapeutic Concentration

Twenty-four patients with solid malignancies untreatable with conventional therapy, aged 32 to 78 years, were fasted 8 h prior to treatment and for an additional 2 h after dosing. All were administered a single oral dose of 100, 150 or 200 mg/m^2 daily for 5 consecutive days. On day 1, the mean peak plasma concentration was 5.4 mg/L for the 100 mg/m^2 dose, 7.7 mg/L for the 150 mg/m^2 dose and 11.0 mg/L for the 200 mg/m^2 dose. These concentrations were observed at 1.0, 0.85 and 0.89 h, respectively. On day 5 the concentrations were 6.4, 9.9 and 16.0 mg/L for the three doses at 1.0, 0.64 and 0.53 h, respectively [Hammond *et al.* 1999].

Fifteen patients with a short life expectancy of ~12 weeks, and with histologically confirmed malignancies untreatable by standard therapy, aged 25 to 71 years (mean, 50 years) participated in the study. All were fasted overnight (and for 2 h after dosing) and administered 100, 150, 200 or 250 mg/m^2 daily for 5 days. On day 1, the peak plasma concentration was 7.0 mg/L for the 100 mg/m^2 dose; 5.84 mg/L for the 150 mg/m^2 dose; 13.9 mg/L for 200 mg/m^2 and 13.7 mg/L for 250 mg/m^2. These concentrations were observed at 0.5, 0.94, 0.94 and 1.0 h, respectively. On day 5, the concentrations were 6.92, 5.71, 13.0 and 12.2 mg/L for the 4 doses at 0.39, 1.17, 1.25 and 1.33 h, respectively.

Fifteen patients were also administered 200 mg/m^2 daily for 5 days, either in the fasting state (overnight fast and an additional 4 h after dosing), or in the fed state (ate a high-fat breakfast 1 h before dosing). This was repeated every 4 weeks. The peak plasma concentration for those who were administered temozolomide while fasting was 9.55 mg/L at 1.07 h, and for those who were allowed food 6.51 mg/L at 2.25 h [Brada *et al.* 1999].

Advanced cancer patients were administered an oral dose of 250 mg/m^2 temozolomide daily for 5 consecutive days. On day 1, the peak plasma concentration was 13.7 mg/L observed at 1 h. On day 5, the peak concentration was 12.2 mg/L at 1.33 h. The drug could be quantified in plasma between 0.25 and 12.0 h after dosing [Kim *et al.* 2001].

Toxicity Haematologic toxicity is dose-limiting with values of 1 g/m^2 and 1.25 g/m^2 being associated. Myelosuppression and thrombocytopenia are also dose-limiting.
Bioavailability 100%.
Half-life 1.8 h (temozolomide); 2.5 min (MTIC).
Volume of Distribution Apparent, 0.4 L/kg; 17 L/m^2.
Clearance Systemic clearance, mean, 115 mL/min/m^2 after a single dose and 102 mL/min/m^2 after 5 days multiple dosing.
Protein Binding 15%.
Dose Orally, 150 to 200 mg/m^2 body surface daily for 5 days, repeated every 28 days.

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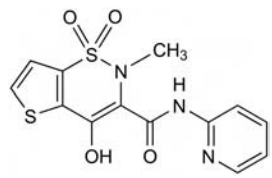
Tenoxicam

Analgesic
$C_{13}H_{11}N_3O_4S_2 = 337.4$
CAS—59804-37-4

IUPAC Name (3*E*)-3-[Hydroxy-(pyridin-2-ylamino)methylidene]-2-methyl-1,1-dioxothieno[2,3-*e*]thiazin-4-one
Synonyms 4-Hydroxy-2-methyl-*N*-(2-pyridyl)-2*H*-thieno[2,3-*e*][1,2]thiazine-3-carboxamide1,1-dioxide; Ro-12-0068.
Proprietary Names *Alganex*; *Artriunic*; *Dolmen*; *Liman*; *Mobiflex*; *Reutenox*; *Rexalgan*; *Tilatil*; *Tilcotil*.



Chemical Properties A yellow, polymorphic, crystalline powder. Mp 205° to 213°. Practically insoluble in water; very slightly soluble in alcohol; sparingly soluble in dichloromethane; it dissolves in solutions of acids and alkalis. pK_a 5.3; 1.1. Log *P* (octanol/water), 2.40; log *P* (octanol/pH 7.4 buffer), 0.3.
Thin-layer Chromatography System TE—R_f 0.14; system TF—R_f 0.06; system TAE—R_f 0.87.
Gas Chromatography System GA—RI 2690(tenoxicam-Me_2)
High Performance Liquid Chromatography System HX—RI 366; system HAA—retention time 12.7 min.

Column: RP C_{18} Nucleosil (250 × 4.6 mm, 5 µm). Mobile phase: sodium dihydrogen orthophosphate (10 mmol/L), sodium lauryl sulfate (1 mmol/L) and acetonitrile : water (pH 2.8) (35 : 65), flow rate 1.5 mL/min. UV detection (λ = 355 nm). IS: ketorolac. Retention time: tenoxicam, 4.6 min; IS, 10.3 min [Mason, Hobbs 1995].

Column: 100 RP-18 Lichrospher (125 × 4 mm i.d., 5 µm). Mobile phase: phosphate buffer (0.1 mol/L, pH 7.4) : methanol (3 : 2), flow rate 1.1 mL/min. UV detection (λ = 335 nm). IS: prioxicam. Retention time: tenoxicam, 3.4 min; IS, 4.5 min [Múnera-Jaramillo, Botero-Garcés 1993].
Ultraviolet Spectrum Aqueous acid—263, 377 nm; basic—263, 289 nm.

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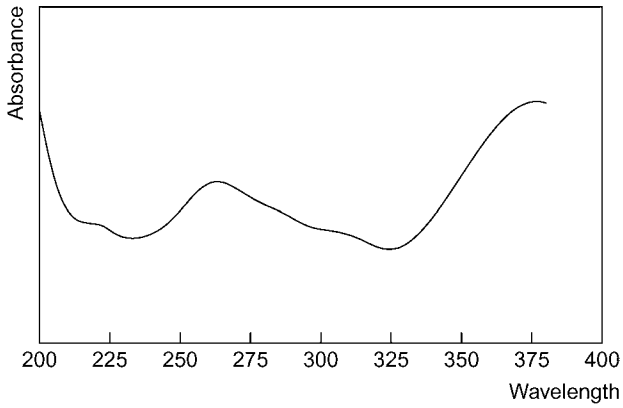
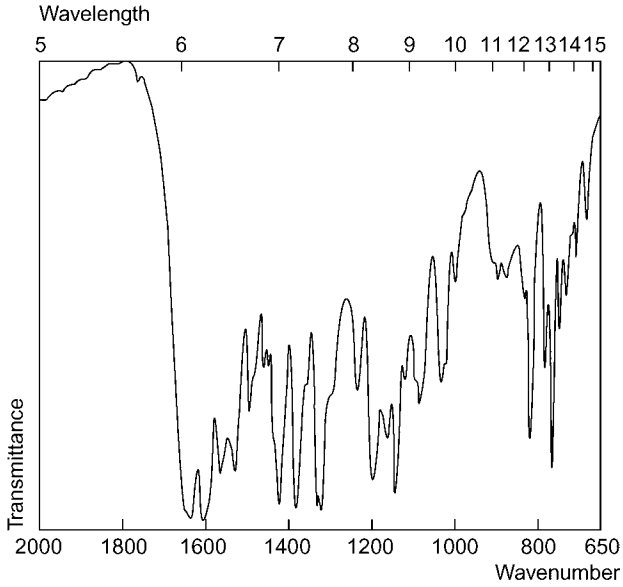
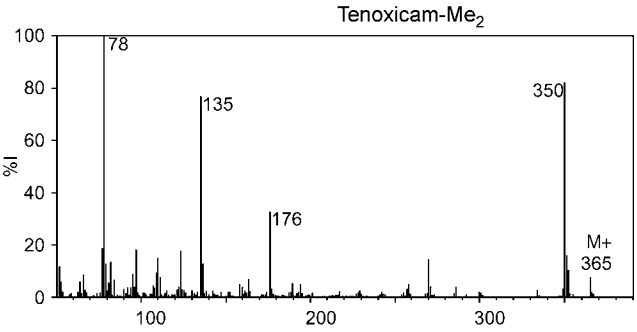


Infrared Spectrum Principal peaks at wavenumber 1607, 1325, 1423 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 78, 350, 135, 176, 77, 97, 123, 110 (tenoxicam-Me_2).



Quantification

Plasma **HPLC** UV detection ($\lambda = 355$ nm). Limit of detection, 0.04 mg/L [Mason, Hobbs 1995]. UV detection ($\lambda = 355$ nm). Limit of detection, 0.005 mg/L [Múnera-Jaramillo, Botero-Garcés 1993]. UV detection ($\lambda = 371$ nm). Limit of detection, 0.02 mg/L [Heizmann *et al.* 1986]. UV detection ($\lambda = 361$ nm). Limit of detection, 0.2 mg/L [Dixon *et al.* 1984]. UV detection ($\lambda = 361$ nm). Limit of detection, 0.05 mg/L [Pickup *et al.* 1981].

Urine **HPLC** UV detection ($\lambda = 371$ nm). Tenoxicam and hydroxy-metabolites. Limit of detection, 0.05 mg/L [Dell *et al.* 1984].

Disposition in the Body Tenoxicam is rapidly and completely absorbed after oral administration. Peak plasma concentrations occur within about 2 h in fasting subjects, but may be delayed up to 6 h after food, although the extent of absorption is not affected. It is over 98.5% protein bound and penetrates synovial fluid. Tenoxicam is completely metabolised to its inactive metabolites, and about two-thirds of the administered dose is excreted in urine, primarily as the active 5-hydroxypyridyl metabolite. The remainder is excreted in bile as glucuronide conjugates (glucuronidated 6-*O*-metabolite and glucuronide metabolites of the hydroxy-metabolite). 0.4% of the drug is excreted unchanged in urine. Steady-state concentrations are achieved within 10 to 15 days. The drug is not known to accumulate.

Therapeutic Concentration

After oral administration of 20 mg to 12 healthy subjects, aged between 21 and 52 years, in the fasting state and then following food, mean peak plasma concentrations of 3.0 mg/L and 2.4 mg/L, respectively, were obtained at 1.8 and 5.8 h [Day *et al.* 1987].

Six healthy male volunteers with a mean age of 25.5 years (between 20 and 31 years) were administered with 20 mg tenoxicam after an overnight fast. The mean maximum concentration was 7.66 µmol/L after 1.3 h [Benveniste *et al.* 1990].

Half-life Plasma half-life, 60 to 70 h.

Volume of Distribution 10 to 12 L.

Clearance Plasma, 0.09 to 0.14 L/h.

Protein Binding Over 98.5%.

Note For reviews of tenoxicam, see Gonzalez andTodd [1987] and Todd and Clissold [1991].

For a review of the pharmacokinetics of tenoxicam, see Nilsen [1994].

Dose Usually 20 mg daily.

Benveniste C *et al.* (1990). Indirect assessment of the enterohepatic recirculation of piroxicam and tenoxicam. *Eur J Clin Pharmacol* 38: 547–549.

Day RO *et al.* (1987). Effect of food and various antacids on the absorption of tenoxicam. *Br J Clin Pharmacol* 24: 323–328.

Dell D *et al.* (1984). Determination of tenoxicam, and the isolation, identification and determination of Ro 17-6661, its major metabolite, in human urine. *J Chromatogr* 317: 483–492.

Dixon JS *et al.* (1984). Rapid method for the determination of either piroxicam or tenoxicam in plasma using high-performance liquid chromatography. *J Chromatogr* 310: 455–459.

Gonzalez JP, Todd PA (1987). Tenoxicam. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* 34: 289–310.

Heizmann P *et al.* (1986). Determination of tenoxicam in human plasma by high-performance liquid chromatography. *J Chromatogr* 374: 95–102.

Mason JL, Hobbs GJ (1995). Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 665: 410–415.

Múnera-Jaramillo MI, Botero-Garcés S (1993). Determination of tenoxicam in plasma by high-performance liquid chromatography. *J Chromatogr* 616: 349–352.

Nilsen OG (1994). Clinical pharmacokinetics of tenoxicam. *Clin Pharmacokinet* 26: 16–43.

Pickup ME *et al.* (1981). Determination of Ro12-0068, a new anti-inflammatory and analgesic compound, in plasma by means of high-performance liquid chromatography. *J Chromatogr* 225: 493–497.

Todd PA, Clissold SP (1991). Tenoxicam. An update of its pharmacology and therapeutic efficacy in rheumatic diseases. *Drugs* 41: 625–646.

Terazosin

α_1-Adrenoceptor Antagonist

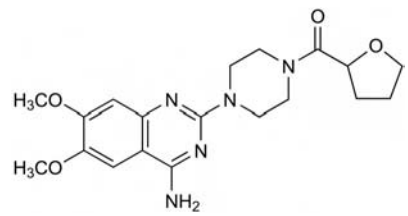
$C_{19}H_{25}N_5O_4 = 387.4$

CAS—63590-64-7

IUPAC Name [4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(oxolan-2-yl)methanone

Synonym 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)-carbonyl]-piperazine

Proprietary Names *Abbott-45975*; *Deflox*; *Flotrin*; *Heitrin*; *Hytrin*; *Hytrinex*; *Itrin*; *Magnurol*; *Sinalfa*; *Teraprost*; *Urodie*; *Uroflow*; *Vasocard*; *Vicard*.



Chemical Properties A white, fine crystalline powder with no discernible odour. Mp 272° to 274°. Soluble in water (29.7 g/L), methanol (33.7 g/L), ethanol (4.1 g/L), 0.1 mol/L aqueous hydrochloric acid (3.8 g/L), chloroform (1.2 g/L) and acetone (0.01 g/L); freely soluble in isotonic saline; practically insoluble in hexane. pK_a 7.1 (0.1 mol/L sodium hydroxide). Log *P* (octanol/water), 1.47.

Terazosin Hydrochloride

$C_{19}H_{25}N_5O_4,HCl = 423.9$

CAS—63074-08-8

Chemical Properties Hygroscopic crystals. Mp 278° to 279°. Soluble in water (761.2 g/L).

Terazosin Hydrochloride Dihydrate

$C_{19}H_{25}N_5O_4,HCl,\ 2H_2O = 459.9$

CAS—70024-40-7

Chemical Properties Mp 271° to 274°. Soluble in water (24.2 g/L).

Thin-layer Chromatography Plate: silica gel 60 F_{254}. Mobile phase: chloroform:toluene:methanol (9:1:6). Fluorescence detection ($\lambda_{ex} = 257$ nm; $\lambda_{em} = 345$ nm). Rf 0.60 [Chang, Bauer 1991].

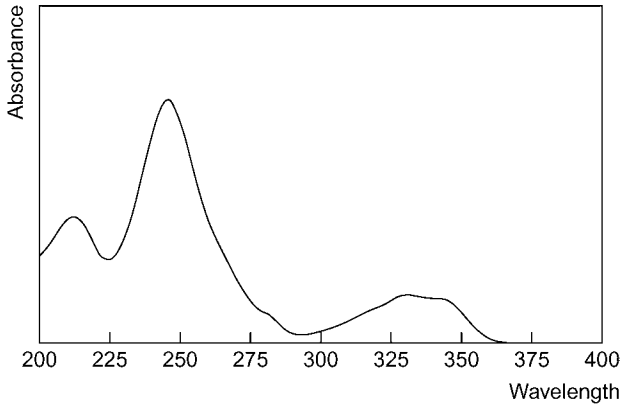
High Performance Liquid Chromatography System HZ—Retention time 2.2 min.

Column: KR100-5-C_{18} metaphase (150 × 4 mm i.d., 5 µm); (guard) RP_{18} Perisorb (30 to 40 µm pellicular). Mobile phase: disodium hydrogen phosphate (0.01 mol/L) : acetonitrile : tetrahydrofuran (76 : 22 : 2) (pH 6.5), flow rate 1 mL/min. IS: prazosin. Fluorescence detection (λ_{ex} = 250 nm; λ_{em} = 370 nm). Retention time: terazosin, 4.1 min; IS, 8.24 min [Cheah *et al.* 2000].

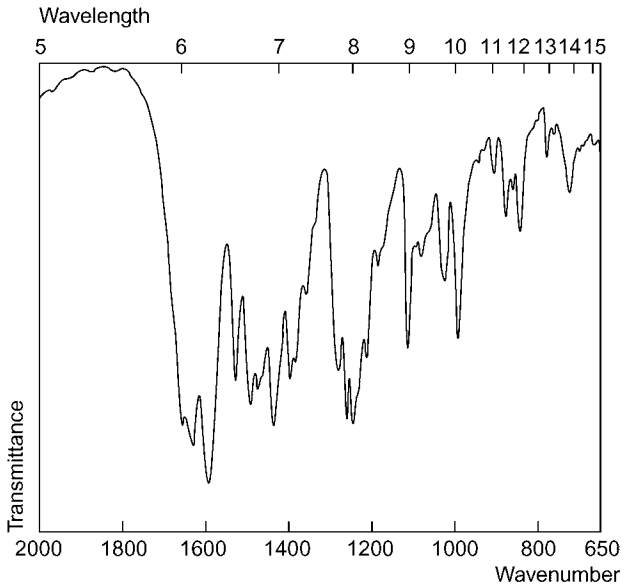
Column: AD Chiralpak (100 × 2.1 mm i.d., 10 µm). Mobile phase: hexane : 2-propanol containing 0.05% diethylamine (65 : 35), flow rate 0.15 mL/min. IS: prazosin. Detection: MS–MS (terazosin, *m/z* →388.2; IS, *m/z* →384.2). Retention time: terazosin, 4.8 min; IS, 4.92 min [Zavitsanos, Alebic-Kolbah 1998].

Column: ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : disodiumhydrogen orthophosphate (0.04 mol/L) (22 : 22 : 56), flow rate 1.2 mL/min. IS: prazosin. Fluorescence detection (λ_{ex} = 250 nm; λ_{em} = 370 nm). Retention time: terazosin 3.8 min; IS, 4.9 min [Sekhar *et al.* 1998].

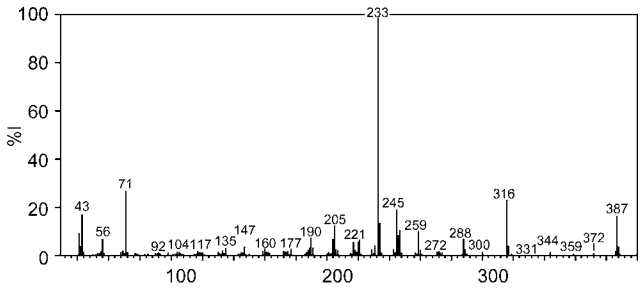
Ultraviolet Spectrum Aqueous solution (water)—212 (A_1^1 = 65.7), 245 (A_1^1 = 127.5), 330 nm (A_1^1 = 24.0); aqueous acid—246, 212, 331 nm; basic—250, 273 nm.



Infrared Spectrum Principal peaks at wavenumber 1594, 1437, 1247 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 233, 71, 316, 245, 43, 387, 234, 205, 259.



Quantification

Blood **HPLC** Limit of detection, 1 µg/L [Sekhar *et al.* 1998]. Limit of quantification, 0.0625 µg/L [Zavitsanos, Alebic-Kolbah 1998].

Plasma **HPLC** Limit of quantification, 0.25 µg/L [Cheah *et al.* 2000].

Disposition in the Body The onset of action occurs after ~15 min and the duration is about 24 h. Terazosin is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration, and is extensively metabolised in the liver to yield piperazine and three other inactive metabolites. Absorption is not affected by the presence of food. The major route of elimination is via the biliary tract, and the drug is excreted in faeces (60%) and urine (40%). 10% is excreted as the parent drug and the remainder as its metabolites. Renal impairment shows no significant effect on pharmacokinetics. The drug displays linear kinetics after oral and IV doses.

Therapeutic Concentration

Young, healthy, male volunteers were administered with 5 mg terazosin and the median peak plasma concentration was 91 µg/L. This was reached after 1 h and decreased to 11 µg/L at around 23.5 h [Taguchi *et al.* 1998].

Bioavailability 90 to 94%.

Half-life 8 to 13 h; increased in the elderly.

Volume of Distribution 15 to 30 L.

Clearance Plasma, 76.3 to 86.6 mL/min (after an 0.5 to 2.0 mg IV dose); decreased in patients with hypertension, 53 to 57 mL/min.

Protein Binding 90 to 95%.

Dose The usual dose is 1 to 3 mg daily but up to 20 mg daily may be administered.

Chang ZL, Bauer JF (1991). Terazosin. *Anal Profiles Drug Subs Excip* 20: 693–727.

Cheah PY *et al.* (2000). Improved high-performance liquid chromatographic analysis of terazosin in human plasma. *J Chromatogr B Biomed Sci Appl* 745(2): 439–443.

Sekhar E *et al.* (1998). Determination of terazosin in human plasma, using high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 710: 137–142.

Taguchi K *et al.* (1998). Radioreceptor assay analysis of tamsulosin and terazosin pharmacokinetics. *Br J Clin Pharmacol* 45(1): 49–55.

Zavitsanos AP, Alebic-Kolbah T (1998). Enantioselective determination of terazosin in human plasma by normal phase high-performance liquid chromatography-electrospray mass spectrometry. *J Chromatogr A* 794: 45–56.

Terbinafine

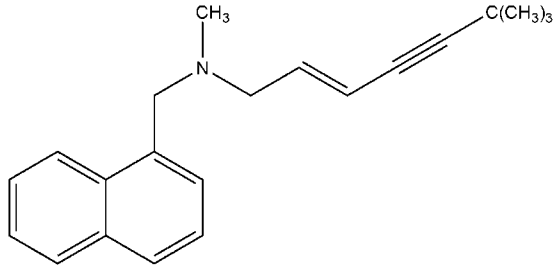
Antifungal

$C_{21}H_{25}N$ = 291.4

CAS—91161-71-6

IUPAC Name (*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine

Synonyms (*E*)-6,6-Dimethylhept-2-en-4-ynl(methyl)-(1-naphthylmethyl) amine; SF-86-327; SF-86327.



Terbinafine Hydrochloride

$C_{21}H_{25}N,HCl$ = 327.9

CAS—78628-80-5

Proprietary Names *DesenexMax*; *Lamisil*; *Terbinex*.

Chemical Properties A white or almost white powder. Mp 195° to 198°. Very slightly or slightly soluble in water; freely soluble in dehydrated alcohol and in methanol; slightly soluble in acetone. Log *P* (octanol/water), 6.00; see also Oremusova, Vitkova [2007].

Quantification

Plasma **HPLC** Mobile phase: acetonitrile and 0.012 mol/L TEA : 0.020 mol/L orthophosphoric acid (50 : 50). UV detection (λ= 224 nm). Limit of quantification, 2 µg/L for terbinafine and desmethylterbinafine [Denouel *et al.* 1995]. Column: phenyl column. UV detection (λ= 224 nm). Terbinafine and 5 metabolites [Zehender *et al.* 1995]. UV detection. Limit of detection, 50 µg/L for terbinafine and desmethylterbinafine (ECD), 100 µg/L for carboxyterbinafine [Schatz, Haberl 1989].

LC-MS Column: reversed phase. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 µg/L [Dotsikas *et al.* 2007]. ESI, positive ion mode. Limit of quantification, 1 µg/L [de Oliveira *et al.* 2001]. Limit of quantification, 0.0679 µg/L [Brignol *et al.* 2000].

Serum **Differential pulse voltammetry** Limit of detection, 2.5×10^{-8} mol/L (25 nmol/L) [Wang *et al.* 2008].

Urine **GC** Column: fused silica capillary. FID. Limit of detection, for naphthoic acid, 50 µg/L [Schatz, Haberl 1989].

HPLC Column: reversed phase. Mobile phase: acetonitrile : methanol : 0.05% trifluoroacetic acid. DAD. Terbinafine and other drugs [Baranowska *et al.* 2009]. See Plasma [Zehender *et al.* 1995]. UV detection. Limit of detection, 300 µg/L for terbinafine, desmethylterbinafine, carboxyterbinafine and desmethylcarboxyterbinafine [Schatz, Haberl 1989].

Milk **HPLC** ECD. Limit of detection, 50 µg/L for terbinafine and 100 µg/L for desmethylterbinafine [Schatz, Haberl 1989].

Sebum **HPLC** See Plasma [Denouel *et al.* 1995].

Hair **LC-MS** Column: reversed phase microbore (50 × 1 mm i.d.). Mobile phase: 12.5% formic acid : propan-2-ol (85 : 15). ESI, positive ion mode. Limit of quantification, 10 ng/g [Majumdar *et al.* 2000].

Nail **HPLC** Terbinafine and its metabolite [Dykes *et al.* 1990]. See Plasma [Denouel *et al.* 1995].

Skin **HPLC** See Plasma [Denouel *et al.* 1995].

Disposition in the Body Terbinafine is well absorbed from the gastrointestinal tract; bioavailability ~40% as it undergoes first-pass hepatic metabolism. Terbinafine rapidly diffuses through the dermis and is concentrated in the lipophilic stratum corneum. It is secreted in sebum and high levels occur in hair follicles, hair and sebum-rich skin. It is distributed in to the nail plate within the first few weeks of treatment. It is metabolised to inactive metabolites, which are excreted mainly in the urine. The main plasma metabolites are unconjugated carboxy compounds. Approximately 70% of an oral dose is excreted in the urine. Less than 5% of topical dose is absorbed.

Therapeutic Concentration Peak plasma levels of ~1 mg/L terbinafine occur ~2 h after a single oral dose of 250 mg.

In 10 healthy subjects, peak plasma levels of 1.7 mg/L occurred 1.2 h after an oral dose of 250 mg terbinafine. Levels exhibited a triphasic decline, with a terminal deposition half-life of 16.5 days [Kovarik *et al.* 1995].

Maximum plasma levels of 1.656 and 1.552 mg/L, respectively, were obtained in healthy subjects after single doses of 2 different oral formulations of terbinafine [Jiang *et al.* 2008].

In 12 patients treated with 250 mg terbinafine daily for up to 48 weeks, terbinafine was detectable in distal nail clippings after 3–18 weeks. Levels of 0.25–0.55 µg/g in nails were quickly achieved and remained stable [Finlay 1992].

Twenty patients given oral doses of 125 mg daily for 4 weeks had detectable levels in the stratum corneum after 1 week and in some cases peak levels of 247.8 ng/g were reached 1 week after completion of treatment. Levels of 50.73 ng/g were detected at 6 weeks after treatment, but terbinafine was not detected at 8 weeks after treatment [Kikuchi *et al.* 2008a; , 2008b].

Topical application of 1% terbinafine hydrochloride produced levels of 170, 228.5 and 249.2 mg/g in the stratum corneum, 2, 4 and 12 weeks after starting treatment, respectively [Tanuma *et al.* 2000].

Toxicity A few cases of overdose (up to 5 g) have been reported. The main symptoms are headache, nausea, epigastric pain and dizziness. Treatment consists in eliminating the drug, usually with activated charcoal, and symptomatic supportive therapy if required.

Half-life Absorption half-life, ~0.8 h. Plasma elimination half-life, ~17–36 h; terminal elimination half-life, up to 400 h on prolonged oral therapy.

Bioavailability Approximately 40% after oral dosing.

Volume of Distribution 11 L/kg [Hosseini-Yeganeh, McLachlan 2002].

Clearance Reduced by ~50% in renal impairment.

Protein Binding >99%.

Note For other reports of the pharmacokinetcs of terbinafine and its metabolites after oral administration, see Humbert *et al.* [1995], Kovarik *et al.* [1992] and Nedelman *et al.* [1996]; for the pharmacokinetics of terbinafine and its metabolites in children, see Abdel-Rahman *et al.* [2005] and Humbert *et al.* [1998].

Dose Terbinafine 250 mg once daily by mouth.

Abdel-Rahman SM *et al.* (2005). Pharmacokinetics of terbinafine in young children treated for tinea capitis. *Pediatr Infect Dis J* 24: 886–891.

Baranowska I *et al.* (2009). Development and validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples. *Anal Sci* 25: 1307–1313.

Brignol N *et al.* (2000). Quantitative analysis of terbinafine (Lamisil) in human and minipig plasma by liquid chromatography tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 141–149.

deOliveira CH *et al.* (2001). Terbinafine quantification in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry: application to a bioequivalence study. *Ther Drug Monit* 23: 709–716.

Denouel J *et al.* (1995). Determination of terbinafine and its desmethyl metabolite in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 663: 353–359.

Dotsikas Y *et al.* (2007). An improved high-throughput liquid chromatographic/tandem mass spectrometric method for terbinafine quantification in human plasma, using automated liquid-liquid extraction based on 96-well format plates. *Biomed Chromatogr* 21: 201–208.

Dykes PJ *et al.* (1990). Determination of terbinafine in nail samples during systemic treatment for onychomycoses. *Br J Dermatol* 123: 481–486.

Finlay AY (1992). Pharmacokinetics of terbinafine in the nail. *Br J Dermatol* 126(Suppl 39): 28–32.

Hosseini-Yeganeh M, McLachlan AJ (2002). Physiologically based pharmacokinetic model for terbinafine in rats and humans. *Antimicrob Agents Chemother* 46: 2219–2228.

Humbert H *et al.* (1995). Pharmacokinetics of terbinafine and of its five main metabolites in plasma and urine, following a single oral dose in healthy subjects. *Biopharm Drug Dispos* 16: 685–694.

Humbert H *et al.* (1998). Pharmacokinetics of terbinafine and five known metabolites in children, after oral administration. *Biopharm Drug Dispos* 19: 417–423.

Jiang X *et al.* (2008). Pharmacokinetics and comparative bioavailability of two terbinafine hydrochloride formulations after single-dose administration in Chinese healthy subjects. *Arzneimittelforschung* 58: 363–366.

Kikuchi I *et al.* (2008). Usefulness and pharmacokinetic study of oral terbinafine for hyperkeratotic type tinea pedis. *Mycoses* 51: 7–13.

Kikuchi I *et al.* (2008). Usefulness and pharmacokinetic study of oral terbinafine for hyperkeratotic-type tinea pedis. *Mycoses* 51: 523–531.

Kovarik JM *et al.* (1992). Dose-proportional pharmacokinetics of terbinafine and its N-demethylated metabolite in healthy volunteers. *Br J Dermatol* 126(Suppl 39): 8–13.

Kovarik JM *et al.* (1995). Multiple-dose pharmacokinetics and distribution in tissue of terbinafine and metabolites. *Antimicrob Agents Chemother* 39: 2738–2741.

Majumdar TK *et al.* (2000). Determination of terbinafine (Lamisil) in human hair by microbore liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14: 1214–1219.

Nedelman JR *et al.* (1996). Pharmacokinetics and pharmacodynamics of multiple-dose terbinafine. *J Clin Pharmacol* 36: 452–461.

Oremusova J, Vitkova Z (2007). Adsorption, partition and release balances of terbinafine hydrochloride, part I. *Pharmazie* 62: 273–277.

Schatz F, Haberl H (1989). Analytical methods for the determination of terbinafine and its metabolites in human plasma, milk and urine. *Arzneimittelforschung* 39: 527–532.

Tanuma H *et al.* (2000). Usefulness of 1% terbinafine HCl (Lamisil) cream for hyperkeratotic-type tinea pedis and its transfer into the horny layer. *Mycoses* 43: 417–432.

Wang C *et al.* (2008). Voltammetric determination of terbinafine in biological fluid at glassy carbon electrode modified by cysteic acid/carbon nanotubes composite film. *Bioelectrochemistry* 72: 107–115.

Zehender H *et al.* (1995). Simultaneous determination of terbinafine (Lamisil) and five metabolites in human plasma and urine by high-performance liquid chromatography using on-line solid-phase extraction. *J Chromatogr B Biomed Appl* 664: 347–355.

Terbutaline

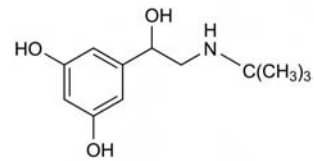
Sympathomimetic

$C_{12}H_{19}NO_3$ = 225.3

CAS—23031-25-6

IUPAC Name 5-[2-(Tert-butylamino)-1-hydroxyethyl]benzene-1,3-diol

Synonym 5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-1,3-benzenediol



Chemical Properties Crystals. Mp 119° to 122°. pK_a 8.7, 10.0, 11.0 (20°).

Terbutaline Sulfate

$(C_{12}H_{19}NO_3)_2,H_2SO_4$ = 548.6

CAS—23031-32-5

Proprietary Names *Aerodur*; *Arubendol*; *Asmaline*; *Asthmoprotect*; *Ataline*; *Brethaire*; *Brethine*; *Bricalin*; *Bricanyl*; *Butaliret*; *Butalitab*; *Butylin*; *Contimit*; *Dhatalin*; *Monovent*; *Taziken*; *Tedipulmo*; *Terbasmin*; *Terbul(in)*; *Terbuturmant*; *Tolbin*; *Vacanyl*.

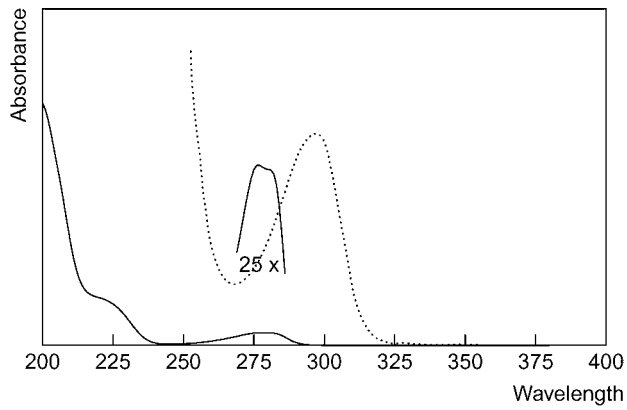
Chemical Properties A white to greyish-white crystalline powder. Mp 246° to 248°. Soluble 1 in 4 of water; slightly soluble in ethanol and methanol; practically insoluble in chloroform and ether.

Colour Tests *p*-Dimethylaminobenzaldehyde—orange/violet; Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—grey-green; Marquis test—yellow; potassium dichromate (method 1)—brown (slow).

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.01; system TC—R_f 0.01; system TE—R_f 0.21; system TL—R_f 0.05; system TAE—R_f 0.18; system TAF—R_f 0.77; system TAJ—R_f 0.00; system TAK—R_f 0.00; system TAL—R_f 0.29 (acidified potassium permanganate solution, strong reaction).

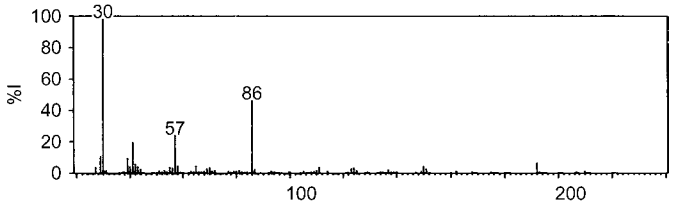
High Performance Liquid Chromatography System HA—*k* 0.9; system HAA—retention time 3.7 min; system HX—RI 225.

Ultraviolet Spectrum Aqueous acid—276 nm (A_1^1=85a); aqueous alkali—297 nm (A_1^1=150b).



Infrared Spectrum Principal peaks at wavenumbers 1210, 1231, 1155, 1069, 1610, 1042 cm^{-1} (terbutaline sulfate, KBr disk).

Mass Spectrum Principal ions at *m/z* 30, 86, 57, 41, 29, 39, 192, 42.



Quantification

Blood **GC-MS** SIM. Terbutaline and other beta-agonists [Black, Hansson 1999].

Plasma **GC-MS** Unconjugated terbutaline. Limit of detection, 100 ng/L [Jacobsson *et al.* 1980]. Unconjugated and total terbutaline. Limit of detection, 300 ng/L [Clare *et al.* 1979].

HPLC Fluorescence detection. Terbutaline enantiomers. Limit of detection, 1 µg/L [Kim *et al.* 2000]. Fluorescence detection. Limit of detection, 2.5 µg/L [McCarthy *et al.* 1993]. Electrochemical detection. Limit of detection, 0.8 to 1 µg/L [Sagar *et al.* 1992; 1993; Herring, Johnson 2000]. Electrochemical detection. Limit of detection about, 1 µg/L [Bergquist, Edholm 1983].

Urine **GC-MS** See Blood [Black, Hansson 1999]. See Plasma [Clare *et al.* 1979].

HPLC Fluorescence detection. Terbutaline enantiomers. Limit of detection, 0.3 µg/L [Kim *et al.* 2001].

Postmortem tissues **GC-MS** Unconjugated terbutaline. Limit of detection, 1.5 ng/g [Leferink *et al.* 1978].

Disposition in the Body Terbutaline is incompletely absorbed after oral administration. Extensive first-pass metabolism occurs in the liver and gut wall to produce the sulfate conjugate (the main metabolite) but some glucuronic acid conjugation also occurs. <15% of an oral dose is present as free drug in the plasma. It is excreted in the urine as unchanged drug and the inactive conjugates, the concentration of each being dependent upon the route of administration. After oral administration, up to about 50% of a dose is excreted in the urine, predominantly as the sulfate conjugate, with up to 10% as unchanged drug. Up to about 60% of a dose is eliminated in the faeces, mainly in unchanged form. After IV or SC administration, more than 80% of a dose is excreted in the urine, with up to about 60% as unchanged drug and only about 2 to 3% is eliminated in the faeces.

Therapeutic Concentration

After single oral doses of 5 mg to 8 subjects, peak serum concentrations of 0.002 to 0.005 (mean, 0.003) mg/L were attained in 2 to 4 h. In 3 subjects there were 2 peaks; the first occurred in 1 to 2 h and the second in 3 to 4 h. After single subcutaneous doses of 0.5 mg to the same subjects, peak serum concentrations of 0.005 to 0.011 (mean, 0.007) mg/L were attained in 0.2 to 0.6 h [Leferink *et al.* 1982].

Toxicity

A female subject took several tablets of terbutaline during a severe night attack of asthma and died the next morning. The following postmortem tissue concentrations were reported: serum 0.014 mg/L, heart 0.036 µg/g, kidney 0.054 µg/g, liver 0.055 µg/g, lung 0.026 µg/g, muscle 0.063 µg/g [Leferink *et al.* 1978].

Approximately 6 h after taking 500 mg of terbutaline, a 22-year-old woman presented with a plasma concentration of 0.2 mg/L, about 50 times the normal therapeutic concentration, which fell to therapeutic levels within 36 h [Heath, Hultén 1987].

Half-life Plasma half-life, about 3 to 4 h.

Volume of Distribution About 1 L/kg.

Clearance Plasma clearance, about 4 mL/min/kg.

Protein Binding About 15 to 25%.

Dose 10 to 15 mg of terbutaline sulfate daily.

Bergquist S, Edholm LE (1983). Quantitative analysis of terbutaline (Bricanyl®) in human plasma with liquid chromatography and electrochemical detection using on-line enrichment. *J Liq Chromatogr* 6(3): 559–574.

Black SB, Hansson RC (1999). Determination of salbutamol and detection of other beta-agonists in human postmortem whole blood and urine by GC-MS-SIM. *J Anal Toxicol* 23: 113–118.

Clare RA *et al.* (1979). The analysis of terbutaline in biological fluids by gas chromatography electron impace mass spectrometry. *Biomed Mass Spectrom* 6: 31–37.

Heath A, Hultén BA (1987). Terbutaline concentrations in self-poisoning: a case report. *Hum Toxicol* 6: 525–526.

Herring VL, Johnson JA (2000). Simple method for determination of terbutaline plasma concentration by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 741: 307–312.

Jacobsson SE *et al.* (1980). Determination of terbutaline in plasma by gas chromatography chemical ionization mass spectrometry. *Biomed Mass Spectrom* 7: 265–268.

Kim KH *et al.* (2000). Determination of terbutaline enantiomers in human plasma by coupled achiral-chiral high performance liquid chromatography. *Arch Pharm Res* 23: 441–445.

Kim KH *et al.* (2001). Determination of terbutaline enantiomers in human urine by coupled achiral-chiral high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 751: 69–77.

Leferink JG *et al.* (1978). Determination of terbutaline in postmortem human tissues by gas chromatography-mass spectrometry. *J Anal Toxicol* 2(3): 86–88.

Leferink JG *et al.* (1982). Pharmacokinetics of terbutaline, a beta 2-sympathomimetic, in healthy volunteers and asthmatic patients. *Arzneimittelforschung* 32: 159–164.

McCarthy PT *et al.* (1993). Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection. *Biomed Chromatogr* 7: 25–28.

Sagar KA *et al.* (1992). Analysis of terbutaline in human plasma by high-performance liquid chromatography with electrochemical detection using a micro-electrochemical flow cell. *J Chromatogr* 577: 109–116.

Sagar KA *et al.* (1993). Simultaneous determination of salbutamol and terbutaline at overdose levels in human plasma by high performance liquid chromatography with electrochemical detection. *Biomed Chromatogr* 7: 29–33.

Terbutryne

Herbicide

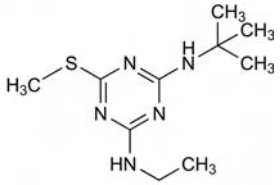
$C_{10}H_{19}N_5S = 241.4$

CAS—886-50-0

IUPAC Name 2-*N*-Tert-butyl-4-*N*-ethyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine

Synonyms Terbutryn; 2-*tert*-Butylamino-4-ethylamino-6-methylthio-1,3,5-triazine.

Proprietary Names *Clarosan*; *Prebane*. It is an ingredient of *Opogard*.



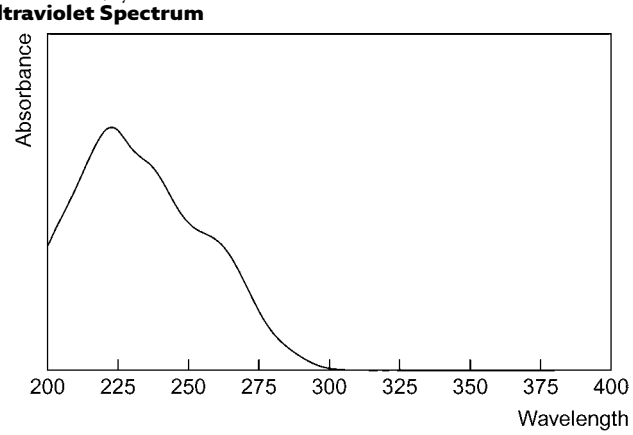
Chemical Properties A white crystalline powder. Mp 104°. Practically insoluble in water; readily soluble in chloroform, ether and methanol. pK_a 4.3. Log *P* (octanol/water), 3.7.

Thin-layer Chromatography System TA—R_f 0.77; system TX—R_f 0.32; system TY—R_f 0.32; system TAB—R_f 0.07; system TAC—R_f 0.12 (Dragendorff spray, positive).

Gas Chromatography System GA—RI 1940; system GK—RRT 0.99 (relative to caffeine).

High Performance Liquid Chromatography System HY—RI 442; system HAO—*k* 12.09; system HAP—*k* 1.68.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1520, 1587, 1215, 806, 1266, 1133 cm^{-1} (KBr disk).

Terbutylazine

Herbicide

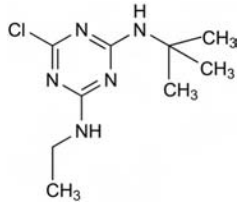
$C_9H_{16}ClN_5 = 229.7$

CAS—5915-41-3

IUPAC Name 2-*N*-Tert-butyl-6-chloro-4-*N*-ethyl-1,3,5-triazine-2,4-diamine

Synonyms 6-Chloro-*N*-(1,1-dimethylethyl)-*N′*-ethyl-1,3,5-triazine-2,4-diamine; diethylterbutylazine; GS-13529; terbuthylazine.

Proprietary Names *Gardoprim*; *Primatol M*; *Sorgoprim*.



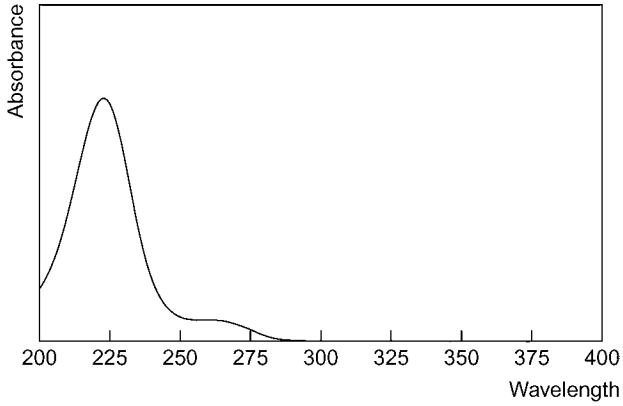
Chemical Properties A colourless powder/white solid. Mp 177° to 179°. Soluble in water (8.5 mg/L at 20°), dimethylformamide (100 g/L at 20°), ethyl

T

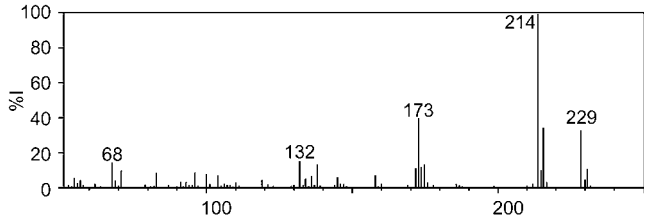
acetate (40 g/L at 20°), isopropanol (10 g/L at 20°), tetralin (10 g/L at 20°), xylene (10 g/L at 20°) and octanol (14.3 g/L at 20°). pK_a 2.0. Log *P* (octanol/water), 3.21.

Gas Chromatography System GA—RI 1805.

Ultraviolet Spectrum



Mass Spectrometry Principal ions at *m/z* 214, 173, 216, 229, 132, 68, 138, 175.



Disposition in the Body Terbutylazine is excreted in urine and faeces, ~85% of a dose is excreted within the first 24 h.

Terodiline

Calcium Antagonist, Antimuscarinic

$C_{20}H_{27}N$ = 281.4

CAS—15793-40-5

IUPAC Name *N*-(1,1-Dimethylethyl)-α-methyl-γ-phenylbenzenepropanamine

Chemical Properties Yellow liquid. Bp 130° to 132°. Slightly soluble in water (7.9 mg/L). Log *P* (octanol/water) 5.42 [Meylan, Howard 1995].

Terodiline Hydrochloride

$C_{20}H_{27}N,HCl$ = 317.9

CAS—7082-21-5

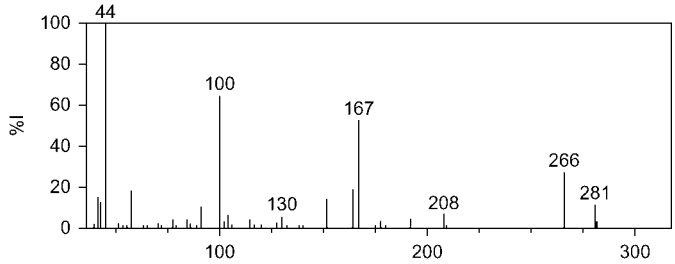
Proprietary Names *Mictrol*; *Micturin*.

Thin-layer Chromatography System TA—R_f 52; system TB—R_f 62; system TC—R_f 19; system TE—R_f 80; system TL—R_f 26; system TAE—R_f 18.

Gas Chromatography System GA—RI 1919.

High Performance Liquid Chromatography Column: Inertsil ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.01 mol/L phosphate buffer (pH 3.5; 38 : 62) containing 0.6% nonylamine. UV detection (λ = 200 nm). Limit of quantification not reported [Ogiso *et al.* 1995].

Mass Spectrum Principal ions at *m/z* 44, 100, 167, 266, 281.



Quantification

Blood **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. NPD. Retention time: 14.9 min. Limit of quantification, 100 µmol/L [Hattori *et al.* 1992].

GC-MS Column: HP-1 methylsilicone (12 m). Carrier gas: He, 1 mL/min. Temperature programme: 45° for 1.5 min to 300° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification not reported [Cattini *et al.* 1989].

Serum **GC-MS** Column: methylsilicon (25 m × 0.32 mm, 0.11 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 85° for 1 min to 300° at 40°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 µg/L [Hallen *et al.* 1994].

Urine **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 22 cm/s. Temperature programme: 100° to 280° at 8°/min. NPD. Retention time: 14.9 min. Limit of quantification, 100 µmol/L [Hattori *et al.* 1992].

GC-MS Column: methylsilicone (25 m × 0.32 mm, 0.11 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 85° for 1 min to 300° at 40°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 µg/L [Hallen *et al.* 1994]. Column: HP-1 methylsilicone (12 m). Carrier gas: He, 1 mL/min. Temperature programme: 45° for 1.5 min to 300° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification not reported [Cattini *et al.* 1989].

Faeces **GC-MS** Column: methylsilicon (25 m × 0.32 mm, 0.11 µm). Carrier gas: He, 0.9 mL/min. Temperature programme: 85° for 1 min to 300° at 40°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1 µg/L [Hallen *et al.* 1994].

Disposition in the Body Rapidly and completely absorbed from the gastrointestinal tract with peak plasma concentrations reached within 4 h. It is extensively metabolised with only 15% of an oral dose recovered as unchanged drug. Hydroxylation of the aromatic rings is the major metabolic pathway, followed by glucuronic acid conjugation. Approximately 75% of an oral dose is recovered in the urine as conjugates, and 25% in faeces. Unchanged terodiline in faeces accounts for about 1% of a dose. Metabolites include *p*-hydroxyterodiline which is the major active metabolite and 3,4-dihydroxyterodiline.

Therapeutic Concentration

Eight healthy volunteers were administered either racemic terodiline hydrochloride (200 mg, 88.5 mg free base for each enantiomer), *R*(+)-terodiline (88.5 mg free base) or *S*(-)-terodiline (65.4 mg free base), as oral doses. Peak plasma concentrations were as shown below.

	R(+)-Terodiline		*S(-)-Terodiline*	
	C_{max} (µg/L)	*Time (h)*	*C_{max} (µg/L)*	*Time (h)*
Racemic dose	324	3.3	260	3.3
Individual enantiomers	260	3.0	179	3.4

[a][Hartigan-Go *et al.* 1996].

Two groups of geriatric patients (mean age, 82 years) were administered either a single 25 mg oral dose of terodiline (Group A) or repeated doses of 12.5 mg twice-daily for 6 weeks (Group B). The mean peak serum concentration for Group A was 110 µg/L after 4 h. A steady-state serum concentration of 642 µg/L was reached for Group B. When compared with healthy young volunteers a difference in various pharmacokinetic parameters was observed: Group A had higher serum terodiline concentrations, increased half-life, lower renal clearance and lower total clearance [Hallen *et al.* 1988].

Toxicity

A 20-year-old male was found dead after taking several terodiline tablets (at least 10, 12.5 mg tablets). Blood and urine terodiline concentrations were found to be 20 mg/L and 108 mg/L, respectively [Cattini *et al.* 1989].

In 1991, the manufacturer withdrew terodiline from all countries because of its association with cardiac arrhythmias.

Bioavailability ≈92%.

Half-life 60 h.

Volume of Distribution Apparent, 479 L (range 260 to 886 L).

Clearance Total (from serum) 4.5 to 4.8 L/h; renal 0.66 L/h.

Protein Binding ≈85%.

Dose Up to 25 mg daily, orally.

Cattini RA *et al.* (1989). An apparent fatal overdose of terodiline. *J Anal Toxicol* 13: 110–112.

Hallen B *et al.* (1988). Single- and multiple-dose pharmacokinetics of terodiline in geriatric patients. *Eur J Clin Pharmacol* 34: 291–297.

Hallen B *et al.* (1994). Bioavailability and disposition of terodiline in man. *J Pharm Sci* 83: 1241–1246.

Hartigan-Go *et al.* (1996). Stereoselective cardiotoxic effects of terodiline. *Clin Pharmacol Ther* 60: 89–98.

Hattori H *et al.* (1992). Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 581: 213–218.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Ogiso T *et al.* (1995). Comparison of the *in vitro* skin penetration of propiverine with that of terodiline. *Biol Pharm Bull* 18: 968–975.

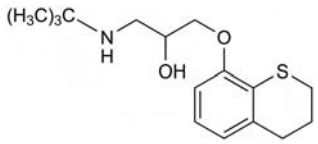
Tertatolol

β-Blocker, Antihypertensive

$C_{16}H_{25}NO_2S$ = 295.4

CAS—34784-64-0

IUPAC Name 1-(Tert-butylamino)-3-(3,4-dihydro-2*H*-thiochromen-8-yloxy) propan-2-ol
Synonym 1-[(3,4-Dihydro-2*H*-1-benzothiopyran-8-yl)oxy]-3-[(1,1-dimethyl-ethyl)-amino]-2-propanol



Chemical Properties Mp 180° to 183° pK_a 9.8. Log *P* (octanol/water), 2.5 (pH 7, 24°). Extraction yield, 1 [Demme *et al.* 2005].

Tertatolol Hydrochloride

$C_{16}H_{25}NO_2S,HCl = 331.9$
CAS—33580-30-2
Proprietary Names *Artex*; *Artexal*; *Prenalex*.
Chemical Properties White crystals. Mp 180° to 183°. Freely soluble in aqueous solution and alcohol, and has high lipid solubility.

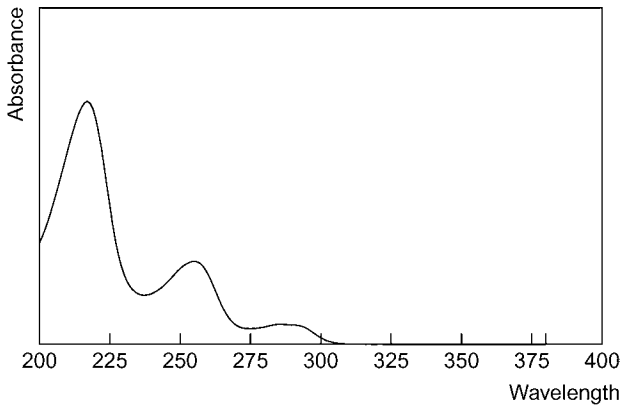
Thin-layer Chromatography System TB—R_f 0.13; system TE—R_f 0.43; system TF—R_f 0.01; system TAE—R_f 0.17.
Gas Chromatography System GA—RI 2310.

Column: 3% SE-30 on 100-120 mesh Chromosorb W AW DMCS (glass, 2.1 m x 2 mm i.d.). Column temperature: oven, 260°. Carrier gas: helium, flow rate 20 mL/min. MS detection (SIM at *m/z* 368 for tertatolol; 456 for IS). IS: 4-hydroxytertalolol. Retention time: tertatolol, 2.13 min; IS, 2.92 min [Efthymiopoulos *et al.* 1987].

High Performance Liquid Chromatography System HX—RI 381.

Column: XLODS Ultrasphere (70 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : water (40 : 60), flow rate 2 mL/min. IS: (−)-alprenolol. Fluorescence detection (λ_{ex}=220 nm; λ_{em}=320 nm). Retention time: (−)-tertatolol, 15 min; (+)-tertatolol, 17 min; IS, 12.5 min [Lave *et al.* 1991].

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 86, 166, 251, 280, 57, 151, 70, 165.
Quantification
Blood **HPLC** Limit of quantification, 6 µg/L [Lave *et al.* 1991].
Urine **GC-MS** Limit of detection, 1 µg/L [Efthymiopoulos *et al.* 1987].
HPLC See Blood [Lave *et al.* 1991].
Disposition in the Body Tertatolol is rapidly and almost completely absorbed, and peak plasma levels are reached within 1 to 2 h after administration. First-pass hepatic metabolism is low and there is limited extravascular distribution. There are linear pharmacokinetics in the dose range of 1 to 10 mg. The three main metabolic routes are sulfoxidation, hydroxylation and conjugation. Inactive metabolites make up 70% of urine excretion. 4-OH-tertatolol is the active metabolite and makes up <10% of urine. Repeated dose results in no change in the pharmacokinetics and there is no change in pharmacokinetics in the elderly or renal impaired. Steady state is achieved from first administration without accumulation.
Therapeutic Concentration

Twenty-two hypertensive patients (12 men and 10 women) with a mean age of 52.6 years and chronic renal failure were used in the study. The patients were orally administered 5 mg tertatolol daily for 4 weeks. The mean peak concentration of the drug was 160 µg/L observed at 1.2 h [Rainfray *et al.* 1989].

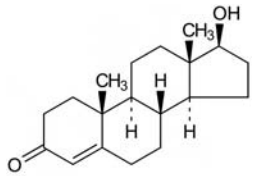
Ten patients, with cirrhosis and oesophageal varices, were administered with 2.5 mg tertatolol orally and 1.25 mg IV. The mean peak plasma concentration was 70 µg/L at 0.75 h [Cales *et al.* 1993].

Toxicity High therapeutic safety index; 3800 to 13 000 higher than human clinical dose.
Bioavailability 60% after oral administration.
Half-life 3 h.
Volume of Distribution 30 to 40 L (0.7 L/kg); increased to 50 L in patients with cirrhosis.
Clearance Plasma clearance, 130 mL/min; decreased to 49 mL/min in patients with cirrhosis. Systemic clearance, 0.1 L/h.
Protein Binding 95% to mainly α_1-glycoprotein; 85% in patients with cirrhosis.
Dose 5 mg daily.

Cales P *et al.* (1993). Hemodynamic and pharmacokinetic study of tertatolol in patients with alcoholic cirrhosis and portal hypertension. *J Hepatol* 19(1): 43–50.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Efthymiopoulos C *et al.* (1987). Simultaneous quantitative determination of tertatolol and its hydroxylated metabolite in human plasma and urine by gas chromatography-mass spectrometry. *J Chromatogr* 421: 360–366.
Lave T *et al.* (1991). Determination of tertatolol enantiomers in biological fluids by high-performance liquid chromatography. *J Chromatogr* 572: 203–210.
Rainfray M *et al.* (1989). Tertatolol in chronic renal failure. A pharmacokinetic study. *Am J Hypertens* 2: 266S–277S.

Testosterone

Androgen
$C_{19}H_{28}O_2 = 288.4$
CAS—58-22-0
IUPAC Name (8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[*a*]phenanthren-3-one
Synonyms 17β-Hydroxyandrost-4-en-3-one; 17-hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[*a*]phenanthren-3-one.
Proprietary Names *Androderm*; *Androgel*; *Andropatch*; *Atmos*; *Histerone*; *Hydrotest*; *Malogen Aqueous*; *Oreton*; *Tesamone*; *Testandro*; *Testoderm*; *Testopel*; *Testotop*; *Testozzard*.



Chemical Properties White or creamy-white crystals or crystalline powder. Mp 152° to 157°. Practically insoluble in water; soluble 1 in 6 of dehydrated alcohol, 1 in 2 of chloroform and 1 in 100 of ether. Log *P* (octanol/water), 3.3.

Testosterone Cipionate

$C_{27}H_{40}O_3 = 412.6$
CAS—58-20-8
Synonyms Testosterone cyclopentylpropionate; testosterone cypionate.
Proprietary Names *Andronate*; *depAndro*; *Depotesteron*; *Duratest*; *Pertestis*; *Testex*; *Testiormina*; *Virilon*.
Chemical Properties A white or creamy-white crystalline powder. Mp 101° to 102°. Insoluble in water; freely soluble in ethanol, chloroform and ether.

Testosterone Enantate

$C_{26}H_{40}O_3 = 400.6$
CAS—315-37-7
Synonyms Testosterone enanthate; testosterone heptanoate; testosterone oenanthate.
Proprietary Names *Andro*; *Andropository*; *Androtardyl*; *Delatestryl*; *Durathate Everone*; *Malogen LA*; *Malogex*; *Primoteston-Depot*; *Testate*; *Testo-Enant*; *Testone LA*; *Testostroval-PA*; *Testoviron-Depot*.
Chemical Properties A white or creamy-white crystalline powder. Mp 36° to 38°. Practically insoluble in water; soluble 1 in 0.3 of ethanol, chloroform and ether.

Testosterone Isocaproate

$C_{25}H_{38}O_3 = 386.6$
CAS—15262-86-9
Synonym Testosterone isohexanoate
Proprietary Names It is an ingredient of *Durateston*, *Estandron P*, *Mixogen*, *Sostanon* and *Sustanon*.
Chemical Properties White to creamy-white crystals or crystalline powder. Mp about 80°. Practically insoluble in water; very soluble in ethanol and chloroform.

Testosterone Phenylpropionate

$C_{28}H_{36}O_3 = 420.6$
CAS—1255-49-8
Proprietary Names It is an ingredient of *Durateston*, *Estandron P*, *Mixogen*, *Sostanon* and *Sustanon*.
Chemical Properties A white crystalline powder. Mp 114° to 117°. Practically insoluble in water; soluble 1 in 40 of ethanol.

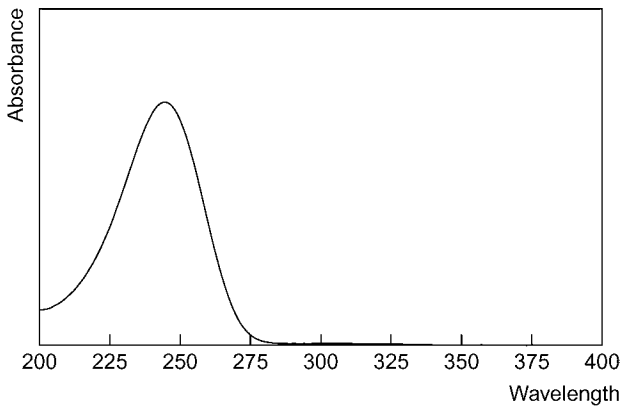
Testosterone Propionate

$C_{22}H_{32}O_3 = 344.5$
CAS—57-85-2
Proprietary Names *Malogen in Oil*; *Testoviron*; *Tesurene*; *Virormone*. It is an ingredient of *Durateston*, *Estandron P*, *Mixogen*, *Sostenon* and *Sustanon*.
Chemical Properties Colourless or yellowish-white crystals or a white or creamy-white crystalline powder. Mp 118° to 122°. Practically insoluble in water; soluble 1 in 6 of ethanol and 1 in 4 of acetone; very soluble in chloroform; freely soluble in ether and in methanol.

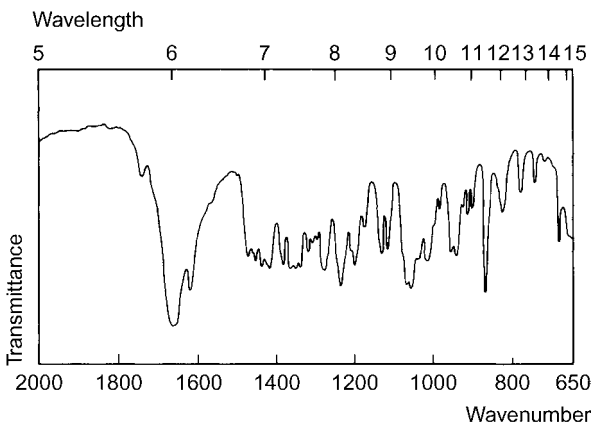
Testosterone Undecylate

$C_{29}H_{48}O_3$ = 444.7
CAS—5949-44-0
Synonym Testosterone undecanoate
Proprietary Names *Andriol*; *Androxon*; *Panteston(e)*; *Restandol*; *Undestor*.

Colour Tests Naphthol-sulfuric acid—yellow/green, brown dichroism; sulfuric acid—no initial colour (green fluorescence under ultraviolet light).
Thin-layer Chromatography Testosterone: system TB—R_f 0.14; system TE—R_f 0.70; system TF—R_f 0.45; system TP—R_f 0.60; system TQ—R_f 0.07; system TR—R_f 0.90; system TS—R_f 0.63; system TAE—R_f 0.85; system TAF—R_f 0.88; system TAJ—R_f 0.59; system TAK—R_f 0.63; system TAL—R_f 0.92; system TAM—R_f 0.92. Testosterone phenylpropionate: system TP—R_f 0.86; system TQ—R_f 0.28; system TR—R_f 0.99; system TS—R_f 0.98. Testosterone propionate: system TP—R_f 0.78; system TQ—R_f 0.12; system TR—R_f 0.99; system TS—R_f 0.98.
Gas Chromatography System GA—testosterone RI 2620, testosterone-AC RI 2750, testosterone enol-TMS_2 RI 2690, testosterone propionate RI 2815, testosterone dipropionate RI 3350; system GAG—methyltestosterone RRT 1.05, testosterone acetate RRT 1.21, testosterone propionate RRT 1.43, testosterone isobutyrate RRT 1.54, testosterone cipionate RRT 2.19, testosterone enantate RRT 1.92, testosterone undecylate RRT 2.56, testosterone isocaproate RRT 1.77, testosterone decanoate RRT 2.36 (all relative to testosterone); system GAI—RRT 0.970 (relative to 17α-methyl-5α-androstan-3β,17β-diol); system GAR—testosterone retention time 12.9 min, methyltestosterone RT 13.1 min, testosterone acetate RT 13.5 min, testosterone propionate RT 14.2 min, testosterone isocaproate RT 15.9 min, testosterone enantate RT 16.7 min, testosterone benzoate RT 18.0 min, testosterone cipionate RT 18.7 min, testosterone decanoate RT 19.8 min, testosterone phenylpropionate RT 20.2 min.
High Performance Liquid Chromatography System HX—RI 534; system HY—testosterone RI 508, testosterone acetate RI 894, testosterone propionate RI 1003; system HAR—methyltestosterone RRT 1.17, testosterone acetate RRT 1.76, testosterone propionate RRT 2.01, testosterone isobutyrate RRT 2.17, testosterone cipionate RRT 2.63, testosterone enantate RRT 2.60, testosterone undecanoate RRT 3.18 (all relative to testosterone); system HATa—testosterone propionate RRT 1.31, testosterone phenylpropionate RRT 1.48, testosterone isocaproate RRT 1.62, testosterone enantate RRT 1.80, testosterone cipionate RRT 2.05, testosterone undecenoate RRT 2.53, testosterone decanoate RRT 2.78, testosterone undecylate RRT 3.27 (all relative to testosterone); system HATb—methyltestosterone RRT 1.27, testosterone acetate RRT 2.59, testosterone propionate RRT 4.06 (all relative to testosterone).
Ultraviolet Spectrum Dehydrated alcohol—240 nm (A^1_1 = 560a).



Infrared Spectrum Principal peaks at wavenumbers 1660, 871, 1615, 1057, 1236, 1066 cm^{-1} (KBr disk).

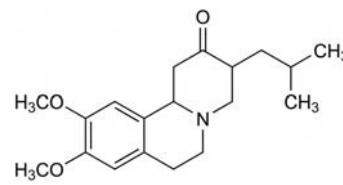


Quantification
Plasma **RIA** Limit of detection, 8 pg [Corker, Davidson 1978].
ELISA Limit of detection, 4 pg [Turkes *et al.* 1979].
Serum **HPLC** Testosterone and other sex hormones. Limit of detection, 5 µg/L [Suzuki *et al.* 1988].
Urine **GC-MS** Testosterone and metabolites [Becchi *et al.* 1994].
HPLC UV detection (λ=240 nm). Limit of quantification, 20 µg/L for testosterone and 30 µg/L for epitestosterone [Navajas *et al.* 1995].
Saliva **ELISA** See Plasma [Turkes *et al.* 1979].
Semen **HPLC** Testosterone and other sex hormones [Hampl *et al.* 2003].
Biological Fluids **RIA** See Plasma [Corker, Davidson 1978].
Hair **RIA** [Wheeler *et al.* 1998].
Note For a review of methods in urinary testosterone analysis, see Venturelli *et al.* [1995].
Disposition in the Body Testosterone is the androgenic hormone formed in the testes. In humans, it is metabolised to 5α-androstane-3α,17β-diol, androsterone, etiocholanolone and 5α-androstene-3,17-dione. In a horse, the major metabolites are 5α-androstane-3β,17α-diol, which is excreted in the urine as the glucuronide conjugate, and the 17β-epimer which is excreted in the urine as the sulfate conjugate.
Therapeutic Concentration
Administration of weekly injections of 25, 50, 125, 300 or 600 mg testosterone enantate to eugonadal males who had received a long-acting gonadotropin-releasing hormone agonist to suppress endogenous testosterone secretion resulted in mean peak concentrations of 25.3, 30.6, 54.2, 134.5 and 237.0 ng/L, respectively [Bhasin *et al.* 2001].
In 45 hypogonadal males receiving long-term treatment with 200 mg testosterone enantate or cipionate IM every 2 weeks, the mean blood testosterone concentration was 31.09 ± 2.00 ng/L (mean bioavailable testosterone was 4.25 ± 0.29 ng/L) [Hajjar *et al.* 1997].
Dose 10 to 30 mg daily, sublingually; 200 to 600 mg by implantation.

Becchi M *et al.* (1994). Gas chromatography/combustion/isotope-ratio mass spectrometry analysis of urinary steroids to detect misuse of testosterone in sport. *Rapid Commun Mass Spectrom* 8: 304–308.
Bhasin S *et al.* (2001). Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 281: E1172–E1181.
Corker CS, Davidson DW (1978). A radioimmunoassay for testosterone in various biological fluids without chromatography. *J Steriod Biochem* 9: 373–374.
Hajjar RR *et al.* (1997). Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. *J Clin Endocrinol Metab* 82: 3793–3796.
Hampl R *et al.* (2003). The content of four immunomodulatory steroids and major androgens in human semen. *J Steroid Biochem Mol Biol* 84: 307–316.
Navajas R *et al.* (1995). Determination of epitestosterone and testosterone in urine by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 673: 159–164.
Suzuki Y *et al.* (1988). Automated direct assay system for the measurement of sex steroid hormones in serum using high-performance liquid chromatography. *J Chromatogr* 426: 33–40.
Turkes A *et al.* (1979). A sensitive solid phase enzymeimmunoassay for testosterone in plasma and saliva. *Steroids* 33: 347–359.
Venturelli E *et al.* (1995). Methods for urinary testosterone analysis. *J Chromatogr B Biomed Appl* 671: 363–380.
Wheeler MJ *et al.* (1998). The measurement of testosterone in hair. *J Endocrinol* 159: R5–R8.

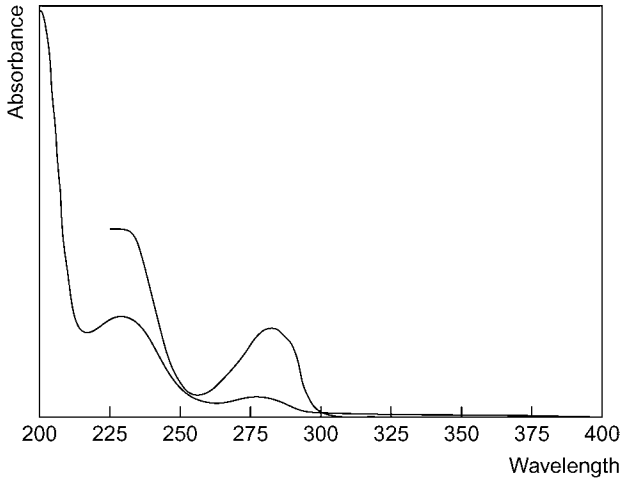
Tetrabenazine

Tranquilliser
$C_{19}H_{27}NO_3$ = 317.4
CAS—58-46-8
IUPAC Name 9,10-Dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydrobenzo[a]quinolizin-2-one
Synonyms 9,10-Dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydropyrido[2,1-a]isoquinolin-2-one; 1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2*H*-benzo[*a*]quinolizin-2-one; TBZ.
Proprietary Names *Nitoman*; *Xenazine*.

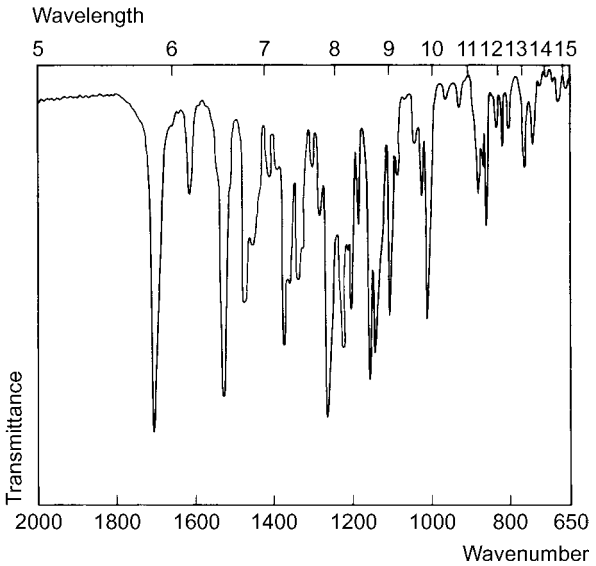


Chemical Properties A white crystalline powder. Mp 125° to 128°. Soluble in hot water, ethanol and chloroform. Log *P* (octanol/water) 2.7.
Colour Tests Liebermann's reagent—black; Mandelin's test—brown; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.41; system TC—R_f 0.78; system TE—R_f 0.79; system TL—R_f 0.67; system TAE—R_f 0.80; system TAJ—R_f 0.83; system TAK—R_f 0.33; system TAL—R_f 0.97 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—tetrabenazine RI 2490; M (*O*-desmethyl-OH-) RI 2500; system GB—tetrabenazine RI 2579; M (*O*-desmethyl-OH-) RI 2638.
High Performance Liquid Chromatography System HZ—retention time 4.4 min.

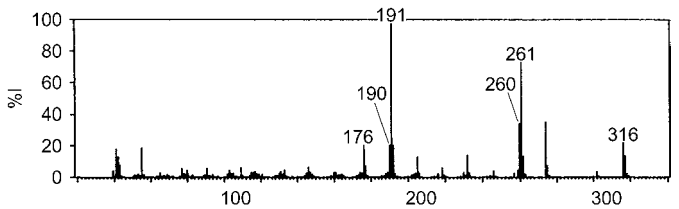
Ultraviolet Spectrum Aqueous acid—282 nm (A_1^1=108a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1694, 1260, 1520, 1155, 1140, 1222 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 191, 261, 274, 260, 316, 192, 190, 176.



Quantification

Plasma **HPLC** Fluorescence detection. Tetrabenazine and 2-hydroxy metabolite. Limits of detection, 100 to 500 ng/L and 1 to 2 µg/L, respectively [Roberts *et al.* 1981; Mehvar *et al.* 1986].

Disposition in the Body Absorbed after oral administration. It is extensively metabolised and excreted in the urine as free and conjugated metabolites; 9 metabolites have been identified in the urine. 2-Hydroxytetrabenazine is an active metabolite.

Therapeutic Concentration

Following a single oral dose of 50 mg to 1 subject, peak plasma concentrations of about 0.015 mg/L of tetrabenazine and 0.085 mg/L of the 2-hydroxy metabolite were attained in 1 and 2 h, respectively [Roberts *et al.* 1981].

Dose 75 to 200 mg daily.

Mehvar R *et al.* (1986). Direct injection high-performance liquid chromatography of tetrabenazine and its metabolite in plasma of humans and rats. *J Pharm Sci* 75: 1006–1009.

Roberts MS *et al.* (1981). Determination of therapeutic plasma concentrations of tetrabenazine and an active metabolite by high-performance liquid chromatography. *J Chromatogr* 226: 175–182.

Tetracaine

Anaesthetic (Local)

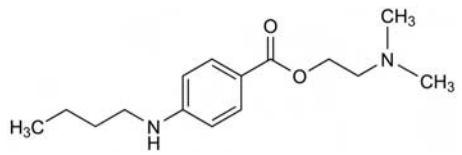
$C_{15}H_{24}N_2O_2$ = 264.4

CAS—94-24-6

IUPAC Name 2-Dimethylaminoethyl 4-(butylamino)benzoate

Synonyms Amethocaine; 4-(butylamino)benzoic acid 2-(dimethylamino)ethyl ester.

Proprietary Names *Ametop*; *Anestesico*; *Pontocaine*. It is an ingredient of *Stypto-Caine*.



Chemical Properties A white or light yellow, waxy solid. Mp 41° to 46°. Very slightly soluble in water; soluble 1 in 5 of ethanol, 1 in 2 of chloroform and 1 in 2 of ether. pK_a 8.2. Log *P* (octanol/water), 3.5.

Tetracaine Hydrochloride

$C_{15}H_{24}N_2O_2$,HCl = 300.8

CAS—136-47-0

Synonyms Butethanol; dicainum.

Proprietary Names *Ametop*; *Anestesico*; *Anethaine*; *Cepacol Viractin*; *Covostet*; *Hemonet*; *Pontocaine*. It is an ingredient of *Anestesiol*; *Anevrase*; *Cetacaine*; *Dynexan*; *Eludril (spray)*; *Herviros*; *Hexomedine*; *Neocones*; *Osmogenol*.

Chemical Properties A white, hygroscopic, crystalline powder. Mp 148°. 134° to 147°. Soluble 1 in 7.5 of water, 1 in 40 of ethanol and 1 in 30 of chloroform; practically insoluble in acetone, benzene and ether.

Colour Tests Aromaticity (method 2)—yellow/red; Liebermann's test (100°)—blue.

Thin-layer Chromatography System TA—R_f 0.57; system TB—R_f 0.15; system TC—R_f 0.32; system TE—R_f 0.64; system TL—R_f 0.16; system TAE—R_f 0.43; system TAF—R_f 0.39; system TAJ—R_f 0.12; system TAK—R_f 0.00; system TAL—R_f 0.25 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).

Gas Chromatography System GA—tetracaine RI 2220, 4-aminobenzoic acid RI 1547; system GF—RI 2715; system GQ—retention time 8.0 min.

Gas Chromatography-Mass Spectrometry

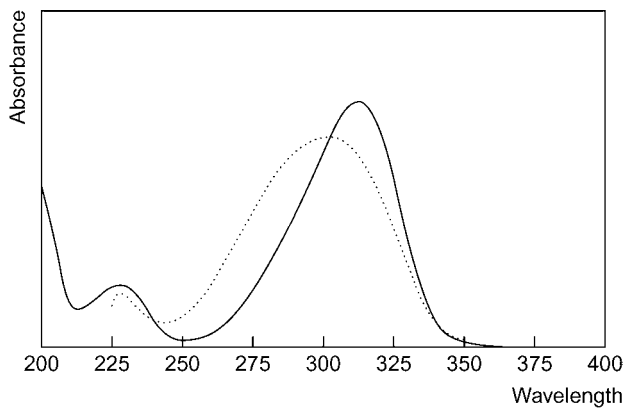
Column: HP-1 100% dimethylpolysiloxane (12 m × 0.2 mm i.d., 0.33 µm). Column temperature: 100° held for 2 min, ramp to 300° at 20°/min, held for 1 min. Injector temperature: 280°. Carrier gas: He, flow rate 1 mL/min. SIM acquisition mode. Retention time 8.7 min [Kudo *et al.* 2001].

Column: methylsilicone fused-silica wide-bore DB-1 (15 m × 0.53 mm i.d., 1.0 µm). Column temperature: 60° to 220° at 8°/min. Injector temperature: 250°. Carrier gas: He, 15 mL/min. NPD [Terada *et al.* 1996].

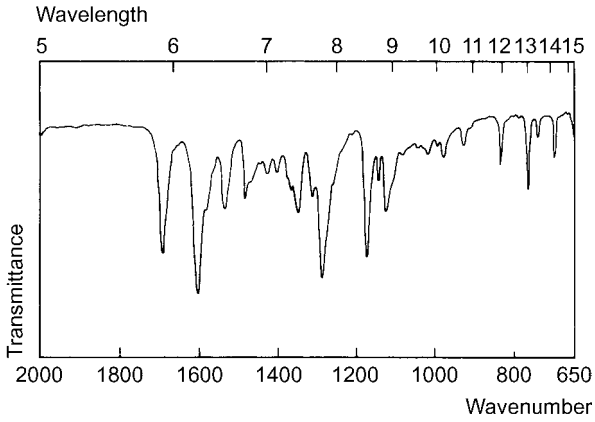
High Performance Liquid Chromatography System HA—*k* 2.0; system HQ—*k* 16.25; system HR—*k* 1.33; system HX—RI 389; system HY—RI 321; system HZ—retention time 4.4 min; system HAX—retention time 10.8 min; system HAY—retention time 5.4 min.

Column: C_{18} µBondapak (300 × 4 mm i.d., 10 µm). Mobile phase: water : acetonitrile : methanol (60 : 20 : 20) containing 0.06% sulfuric acid, 0.5% sodium sulfate, 0.02% sodium heptane sulfonate, pH 2.6. Flow rate, 2 mL/min. UV detection (λ=305 nm). Retention time 6.0 min [Menon and Norris 1981].

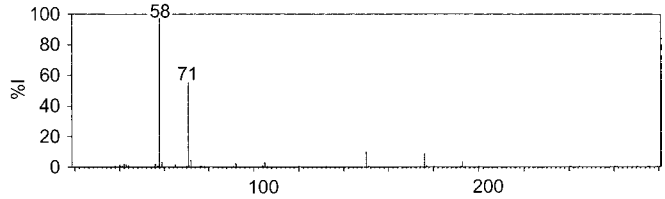
Ultraviolet Spectrum Aqueous acid—229 (A_1^1= 561a), 281, 312 nm; aqueous alkali—227, 303 nm.



Infrared Spectrum Principal peaks at wavenumbers 1600, 1286, 1174, 1688, 1126, 1532 cm^{-1} (tetracaine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 71, 150, 176, 72, 193, 105, 59 (no peaks above 200); 137, 120, 92, 65, 39, 138, 121, 63 (4-aminobenzoic acid).



Quantification

Blood **GC-MS** Tetracaine and its metabolites [Kudo *et al.* 2001]. Tetracaine and 4-butylaminobenzoic acid. Limit of detection, 10 ng/g [Hino *et al.* 2000].

Plasma **GC-MS** (SIM, *m/z* 58). Limit of quantification, 100 µg/L, limit of detection, 80 µg/L [Ohshima, Takayasu 1999].

HPLC Multiwavelength detection. Tetracaine, *p*-butylaminobenzoic acid, and mepivacaine. Limit of detection, 50 µg/L for tetracaine and *p*-butylaminobenzoic acid [Murtaza *et al.* 2001]. Tetracaine and *p-n*-butylaminobenzoic acid [Mazumdar *et al.* 1991].

Serum **GC** NPD. Tetracaine and other local anaesthetics. Limit of quantification, 24 µg/L [Terada *et al.* 1996].

Urine **GC-MS** See Plasma [Ohshima, Takayasu 1999].

Biological Samples **GC** NSD. Tetracaine and other local anaesthetics [Lau *et al.* 1991].

Tissues **GC-MS** see Blood. Limit of detection, 0.6 ng/g [Hino *et al.* 2000].

HPLC Limit of detection, 0.4 g/L for tetracaine, 0.003 g/L for *p-n*-butylaminobenzoic acid [Menon, Norris 1981].

Disposition in the Body Tetracaine is rapidly hydrolysed in the blood to 4-aminobenzoic acid by plasma pseudocholinesterase; it is completely metabolised within 1 h of injection. It can rarely be isolated from blood even within a few minutes of administration. Metabolites are excreted mainly by kidneys. The maximum amount that may be safely administered by injection is probably 50 mg.

Therapeutic Concentration

Following intraspinal administation of 8–14 mg of tetracaine to 10 patients, no trace of tetracaine was revealed in the blood, but mean concentrations of the metabolite, 4-aminobenzoic acid, at 1, 2 and 6 h after injection were 126.5, 97.9 and 43.3 µg/L respectively [Kudo *et al.* 2001].

Dose 5 to 20 mg of tetracaine hydrochloride (0.5% solution) is injected for spinal anaesthesia; 0.5 to 2% solutions have been used for surface anaesthesia.

Hino Y *et al.* (2000). Sensitive and selective determination of tetracaine and its metabolite in human samples by gas chromatography-mass spectrometry. *J Anal Toxicol* 24: 165–169.

Kudo K *et al.* (2001). Blood concentrations of tetracaine and its metabolite following spinal anesthesia. *Forensic Sci Int* 116: 9–14.

Lau OW *et al.* (1991). Gas-liquid chromatographic determination and pharmacological studies of six clinically-used local anesthetics. *Methods Find Exp Clin Pharmacol* 13: 475–481.

Mazumdar B *et al.* (1991). Preliminary study to assay plasma amethocaine concentrations after topical application of a new local anaesthetic cream containing amethocaine. *Br J Anaesth* 67: 432–436.

Menon GN, Norris BJ (1981). Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography. *J Pharm Sci* 70(5): 569–570.

Murtaza R *et al.* (2001). Simultaneous determination of mepivacaine, tetracaine, and p-butylaminobenzoic acid by high-performance liquid chromatography. *J Pharmacol Toxicol Methods* 46: 131–136.

Ohshima T, Takayasu T (1999). Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography-mass spectrometry with solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 726(1–2): 185–194.

Terada M *et al.* (1996). Determination of ester-type local anesthetic drugs (procaine, tetracaine, and T-caine) in human serum by wide-bore capillary gas chromatography with nitrogen-phosphorus detection. *J Anal Toxicol* 20(5): 318–322.

Tetrachloroethane

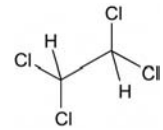
Solvent

$C_2H_2Cl_4$ = 167.8

CAS—79-34-5

IUPAC Name 1,1,2,2-Tetrachloroethane

Synonym Acetylene tetrachloride



Chemical Properties A clear, colourless, heavy, non-flammable, mobile liquid. Wt per mL 1.590 to 1.595 g. Mp 44°. Bp 146°. Refractive index, at 25°, 1.4918. Soluble 1 in 350 of water; miscible with ethanol, chloroform and ether. Log *P* (octanol/water), 2.4.

Gas Chromatography System GA—RI 910; system GI—retention time 24.9 min.

Disposition in the Body Tetrachloroethane is absorbed from the gastrointestinal tract, lungs, and through the skin.

Toxicity Tetrachloroethane is probably the most toxic of the chlorinated hydrocarbons. The estimated lethal dose is 3 mL.

Tetrachloroethylene

Anthelmintic, Solvent

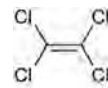
C_2Cl_4 = 165.8

CAS—127-18-4

IUPAC Name 1,1,2,2-Tetrachloroethene

Synonyms Ethylene tetrachloride; perchloroethylene; tetrachloroethene.

Proprietary names *Ankilostin*; *Didakene*; *Nema*; *Perclene*; *Tetracap*; *Tetropil*.

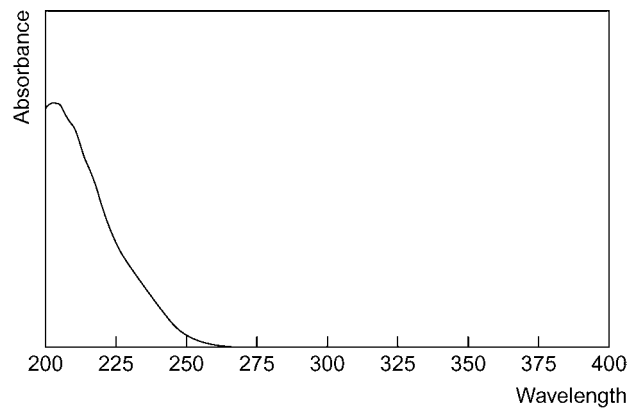


Chemical Properties A colourless, heavy, non-inflammable, mobile liquid. Weight (g/mL) 1.620 to 1.626. Fp -22°. Bp 121°. Tetrachloroethylene (B.P.) contains thymol 0.01% as a preservative. Practically insoluble in water; soluble in ethanol; miscible with chloroform and ether. Log *P* (octanol/water), 3.4.

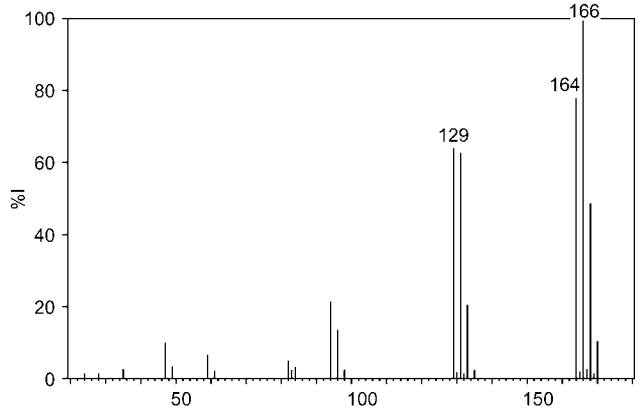
Colour Test Tetrachloroethylene may be distinguished from carbon tetrachloride and chloroform by the following test: to 5 mL in a stoppered cylinder add 5 mL of bromine solution and shake vigorously at intervals of 15 min for 1 h. The bromine colour fades and there is a white turbidity in the lower layer.

Gas Chromatography System GA—RI 789; system GI—RT 24.3 min; system GAA—RI 811.

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 166, 164, 129, 131, 168, 94, 133, 96.



Quantification

Blood **GC** Column: SPB-1 100% polydimethylsiloxane (30 m × 0.25 mm i.d.). Carrier gas: N_2, 3 mL/min. Temperature programme: 60° for 1 min to 130° at 10°/min for 1.5 min. Retention time: 7.72 min. Limit of detection, 3 ng/g [Dehon *et al.* 2000]. Column: 5% Carbowax on 60/80 mesh Carbopack B (6ft × 0.125 in i.d.) Carrier gas: CH_4 : Ar (10 : 90), 30 mL/min. Temperature: 110°. ECD. Retention time: 6.02 min. [Isenschmid *et al.* 1998]. Column: 10% OV-1 on 80/100 mesh GasChrom Q (0.9 m × 2 mm i.d.). Carrier gas: CH_4 : Ar (10 : 90), 30 mL/min. Temperature: 100°. ECD. Limit of detection not reported [Levine *et al.* 1981]

GC-MS Column: SE-30 vitreous silica capillary (25 m × 0.2 mm i.d.). Temperature: 90°. ECD. Limit of detection, ≤0.03 µmol/L [Pekari, Aitio 1985]. Column: 3% OV-1 on 80/100 mesh chromosorb WHP (6 ft × 0.25 in i.d.). Carrier gas: He. EI ionisation, SIM acquisition mode. Limit of detection not reported [Lukaszewski 1979].

Urine **GC** See Blood [Dehon *et al.* 2000]. FID. Tetrachloroethylene and other solvents [Ghittori *et al.* 1987].

GC-MS See Blood [Lukaszewski 1979].

Brain **GC** See Blood [Dehon *et al.* 2000; Levine *et al.* 1981].

GC-MS See Blood [Lukaszewski 1979].

Heart **GC** See Blood [Dehon *et al.* 2000].

Kidney **GC** See Blood [Dehon *et al.* 2000; Levine *et al.* 1981].

Liver **GC** See Blood [Dehon *et al.* 2000; Isenschmid *et al.* 1998; Levine *et al.* 1981].

Lung **GC** See Blood [Isenschmid *et al.* 1998; Levine *et al.* 1981].

GC-MS See Blood [Lukaszewski 1979].

Muscle **GC** See Blood [Dehon *et al.* 2000].

Disposition in the Body Tetrachloroethylene is slightly absorbed from the gastro-intestinal tract and through the skin, and well absorbed from the lungs. It has an affinity for lipid-rich tissues from which it is slowly released. Approximately 25% of an inhaled dose is excreted unchanged in expired air in 40 h and this is thought to be the major route of excretion; small amounts are eliminated through the skin. Less than ~3% of a dose is metabolised and excreted in the urine, partly as trichloroacetic acid, in 3 days.

Toxicity The maximum permissible atmospheric concentration is 100 ppm. Exposure to air concentrations of more than 1000 ppm may cause unconsciousness within a short time.

A 45-year-old woman was found unconscious. Despite treatment she died on the seventh day of hospitalisation. On the second hospital day, the tetrachloroethylene concentration in blood was 1.32 mg/L [Dehon *et al.* 2000].

A 26-year-old individual was found dead at work. Tetrachloroethylene concentrations were 62 mg/L in blood, 341 mg/kg in liver and 47 mg/kg in lung [Isenschmid *et al.* 1998].

Postmortem tissue concentrations in a 2-year-old child accidentally fatally exposed to tetrachloroethylene vapour were as follows: blood 66 mg/L, urine 0.3 mg/L (also expressed as 1.7 mg/L trichloroethanol and 0.9 mg/L trichloroacetic acid, to which trichloroethylene is metabolised), brain 79.9 µg/g, lungs 46 µg/g [Gaillard *et al.* 1995].

A 53-year-old man was found dead after exposure to tetrachloroethylene vapour. The following postmortem concentrations were reported: blood 4.5 mg/L, brain 69 µg/g, kidney 71 µg/g, liver 240 µg/g and lung 30 µg/g [Levine *et al.* 1981].

In a case of fatal exposure to tetrachloroethylene vapour, a 33-year-old man had the following postmortem concentrations: blood 44 mg/L, brain 360 µg/g and lung 3 g/g [Lukaszewski 1979].

Half-life ≈72 h.

Dose Usually 0.1 mL/kg, to a maximum of 5 mL, as a single dose.

Dehon B *et al.* (2000). Tetrachloroethylene and trichloroethylene fatality: case report and simple headspace SPME-capillary gas chromatographic determination in tissues. *J Anal Toxicol* 24: 22–26.

Gaillard Y *et al.* (1995). Tetrachloroethylene fatality: case report and simple gas chromatographic determination in blood and tissues. *Forensic Sci Int* 76: 161–168.

Ghittori S *et al.* (1987). [Use of gas chromatography with flame ionization (GC-FID) in the measurement of solvents in the urine]. *G Ital Med Lav* 9: 21–24.

Isenschmid DS *et al.* (1998). Tetrachloroethylene intoxication in an autoerotic fatality. *J Forensic Sci* 43: 231–234.

Levine B *et al.* (1981). A tetrachloroethylene fatality. *J Forensic Sci* 26: 206–209.

Lukaszewski T (1979). Acute tetrachloroethylene fatality. *Clin Toxicol* 15: 411–415.

Pekari K, Aitio A (1985). *IARC Publication 68: Determination of Tetrachloroethylene in Blood*. Lyon, France: International Agency for Research on Cancer; pp. 451–455.

Tetracosactrin

Corticotrophic Peptide

$C_{136}H_{210}N_{40}O_{31}S = 2933.5$

Synonyms Ciba 30,920; cosyntropin; synthetic β1–24 corticotropin; tetracosactide.

Chemical Properties Synthetic polypeptide identical with the first 24 of the 39 amino-acids of corticotrophin.

Tetracosactrin Acetate

Synonym Tetracosactrin hexa-acetate

Proprietary Names *Cortosyn*; *Synacthen*.

Chemical Properties White crystalline powder. Soluble 1 in 70 of water.

Colour Tests Ammonium molybdate test—blue (limit of detection, 1.0 µg); Vitali's test—pale yellow/pale yellow/orange (limit of detection, 1.0 µg).

Thin-layer Chromatography System T1—R_f 0.00 (location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum 0.1 N hydrochloric acid, maximum at 275 nm.

Disposition in the Body Tetracosactrin is inactivated if administered orally. After IV administration the plasma half-life was about 7 min [Wolf *et al.* 1965].

IV administration produces a rapid rise in plasma hydrocortisone; after IM administration blood hydrocortisone concentrations reach a peak within 1 h and are raised for ≈4 h. Subcutaneous or IM injection of the long-acting depot preparations produce peak blood hydrocortisone concentrations in 8 h with raised values for 24 to 48 h.

Toxicity Allergic reactions, including pruritus and flushing, have been reported, but are much less common than with corticotrophin. Depot preparations produce a prolonged rise in blood corticosteroid concentrations and may lead to Cushing's syndrome and fluid retention.

Dose Up to 2 g daily.

Wolf RL *et al.* (1965). Simultaneous urinary assays for the combined metanephrines and 3-methoxy-4-hydroxyphenylglycol in patients with pheochromocytoma and primary hypertension. *N Engl J Med* 273: 1459–1463.

Tetracycline

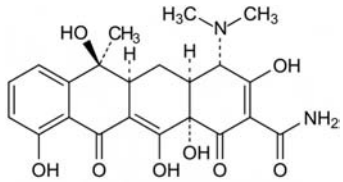
Antibiotic

$C_{22}H_{24}N_2O_8 = 444.4$

CAS—60-54-8 (anhydrous); 6416-04-2 (trihydrate)

IUPAC Name A hydrated form of [4*S*-(4α,4aα,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Proprietary Names Tetracycline and tetracycline hydrochloride are ingredients of many proprietary preparations, see Sweetman [2002].



Chemical Properties A yellow, crystalline, amphoteric powder, which darkens in moist air on exposure to strong sunlight. Soluble 1 in 2500 of water and 1 in 50 of ethanol; practically insoluble in chloroform and ether; freely soluble in dilute acids and, with decomposition, in solutions of alkali hydroxides. pK_a 3.3, 7.7, 9.7 (25°). Log *P* (octanol/pH 7.4) 1.4.

Tetracycline Hydrochloride

$C_{22}H_{24}N_2O_8,HCl = 480.9$

CAS—64-75-5

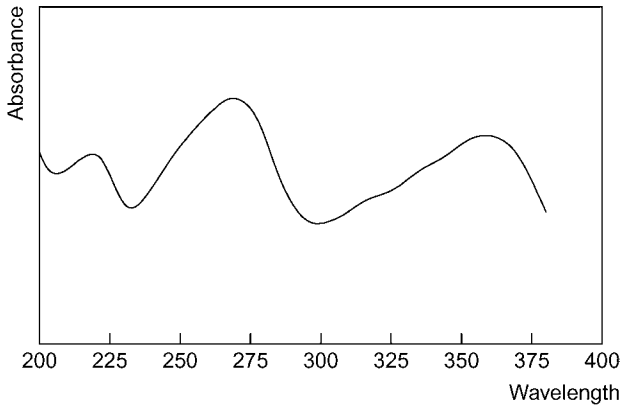
Chemical Properties A yellow, hygroscopic, crystalline, amphoteric powder, which darkens in moist air on exposure to strong sunlight. Mp about 214°, with decomposition. Soluble 1 in 10 of water and 1 in 100 of ethanol; practically insoluble in chloroform and ether; soluble in methanol and in aqueous solutions of alkali hydroxides and carbonates. Solutions in water become turbid on standing owing to hydrolysis and precipitation of tetracycline.

Colour Tests Benedict's reagent—red; formaldehyde-sulfuric acid—green-yellow→yellow-brown; Liebermann's reagent—black; Mandelin's test—violet→red→orange; Marquis test—orange; sulfuric acid—violet.

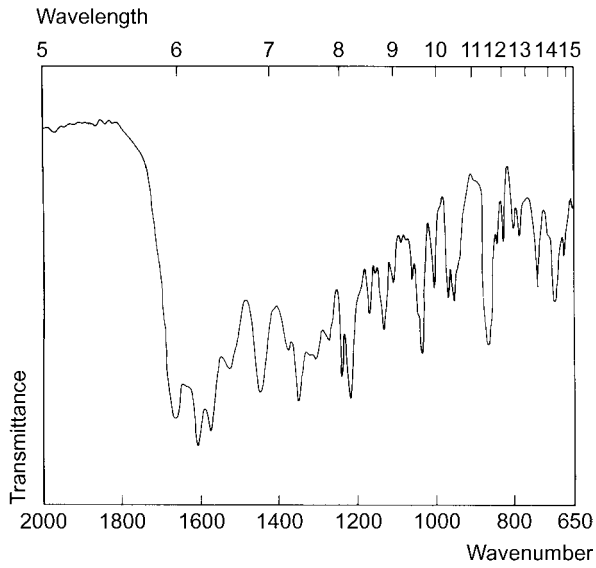
Thin-layer Chromatography System TA—R_f 0.05, streaking; system TB—R_f 0.00; system TAE—R_f 0.88 (location under ultraviolet light, orange fluorescence; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 1950.

High Performance Liquid Chromatography System HX—RI 314; system HY—RI 265; system HAA—retention time 9.9 min.
Ultraviolet Spectrum Aqueous acid—270 (A^1_1=417a), 356 nm.



Infrared Spectrum Principal peaks at wavenumbers 1612, 1580, 1660, 1226, 1248, 1530 cm^{-1} (tetracycline hydrochloride, KCl disk).

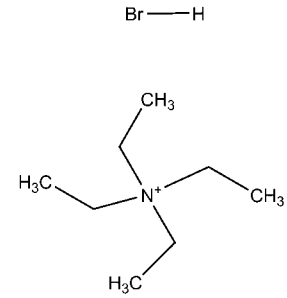


Quantification
Plasma **HPLC** UV detection. Limit of detection, 100 µg/L [Hermansson 1982].
Radioimmunoassay Limit of detection, about 20 µg/L [Faraj, Ali 1981].
Urine **HPLC** See Plasma [Hermansson 1982].
Radioimmunoassay See Plasma [Faraj, Ali 1981].
Disposition in the Body Irregularly and incompletely absorbed after oral administration; widely distributed throughout the body. Up to about 60% of an IV dose and 20 to 50% of an oral dose is excreted in the urine unchanged in 48 h. Excreted in the bile and undergoes some enterohepatic circulation.
Therapeutic Concentration In plasma, usually in the range 1 to 5 mg/L.
Six patients with symptomatic pleural effusions and 1 patient with recurrent pneumothorax were administered 150 mg lidocaine, immediately followed by 20 mg/kg tetracycline instilled in the pleural space through a chest tube. Mean peak plasma concentrations for lidocaine and tetracaine were 1.3 mg/L and 3.6 mg/L, respectively, and these were reached after 86 and 96 min, respectively [Wooten *et al.* 1988].
Half-life Plasma half-life, about 9 h.
Volume of Distribution About 1.3 L/kg.
Clearance Plasma clearance, about 2 mL/min/kg.
Distribution in Blood Plasma : whole blood ratio, about 0.97.
Protein Binding 25 to 65%.
Dose 1 to 2 g daily.

Faraj BA, Ali FM (1981). Development and application of a radioimmunoassay for tetracycline. *J Pharmacol Exp Ther* 217: 10–14.
Hermansson J (1982). Rapid determination of tetracycline and lumecycline in human plasma and urine using high-performance liquid chromatography. *J Chromatogr* 232: 385–393.
Sweetman SC (2002). *Martindale: The complete drug reference*, 33rd edn. London: Pharmaceutical Press.
Wooten SA *et al.* (1988). Systemic absorption of tetracycline and lidocaine following intrapleural instillation. *Chest* 94: 960–963.

Tetraethylammonium Bromide

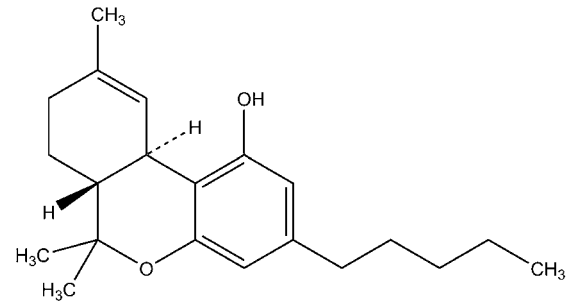
Antihypertensive
$C_8H_{20}BrN$ = 210.2
IUPAC Name Tetraethylazanium hydrobromide
Synonyms TEAB; tetramone bromide; tetrylammonium bromide; TMD 10.
Proprietary Names *Beparon*; *Bromethyl*; *Etambro*; *Etamon(in)*; *Sympatektoman*; *Teamon*; *Tetramon.*



Chemical Properties Tetraethylammonium bromide is a quaternary ammonium compound. White crystalline powder. Soluble 1 in less than 1 of water, 1 in 1 of ethanol and 1 in 4 of chloroform; freely soluble in acetone.
Thin-layer Chromatography System T1—R_f 0.03 (location reagent acidified iodoplatinate spray, positive reaction).
Dose Up to 500 mg IV.

Δ^9-Tetrahydrocannabinol

Antiemetic, Appetite Stimulant, Hallucinogen
$C_{21}H_{30}O_2$ = 314.5
CAS—1972-08-3
IUPAC Name (6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[c]chromen-1-ol
Synonyms Dronabinol; QCD-84924; (–)-Δ1-3,4-*trans*-tetrahydrocannabinol; (6a*R*,10a*R*)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[b,d]pyran-1-ol; Δ^9-THC; Δ^1-THC.
Proprietary Name *Marinol*



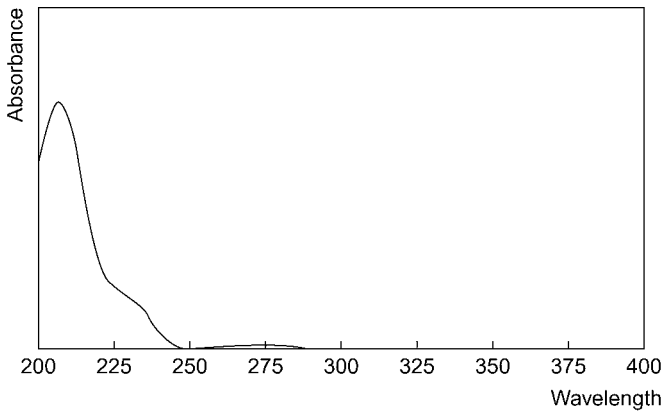
Chemical Properties Light yellow resinous oil that hardens upon refrigeration. Bp 200°. Essentially insoluble in water; soluble 1 in 1 in ethanol and acetone; readily soluble in chloroform and light petroleum. pK_a 10.6 [O'Neil *et al.* 2006]. Log *P* (octanol/water), 7.6. Binds readily to glass and plastic, limiting recoveries during analytical procedures. Absorption can be minimised by storing solutions in amber silylated glassware and by maintenance of the compound in a basic solution or organic solvent. For a study on the adsorptive loss of 11-nor-Δ^9-THC-9-carboxylic acid, see Jamerson *et al.* [2005a]. Stability of Δ^9-THC, 11-hydroxy-Δ^9-THC, and 11-nor-Δ^9-THC-9-carboxylic acid in urine was established after 3 freeze–thaw cycles, 72 h at 4°, and 24 h at room temperature [Abraham *et al.* 2007]. Δ^9-THC concentrations showed a decrease of 26% in urine at –70° in silanised glass. The concentration of Δ^9-THC in EDTA-plasma decreased steadily, with a decrease of 30% after 21 days and 43% after 41 days [Grauwiler *et al.* 2007].
Colour Test *p*-Dimethylaminobenzaldehyde—red/violet; Duquenois reagent—violet-blue/violet.
Thin-layer Chromatography System TA—R_f 11; system TE—R_f 31; system TI—R_f 30; system TJ—R_f 29; system TAH—R_f 50 (Fast blue B solution—red colour); system TAJ—R_f 00; system TAK—R_f 01; system TAL—R_f 31.
Plates: Merck 60F 254 silica gel. Solvent system: hexane : chloroform : dioxane (89 : 8.75 : 2.25). UV detection (λ = 220 nm). R_f 0.27. Limit of detection not reported [Debruyne *et al.* 1994].
Gas Chromatography System GA—RI 2473; system GB—RI 2350.
Gas Chromatography-Mass Spectrometry Column: methyl silicone capillary (25 m × 0.32 mm i.d., 0.25 µm). Carrier gas: N_2. Temperature: 240°. FID. Retention time: 4.6 min. Limit of detection not reported [Debruyne *et al.* 1994].

High Performance Liquid Chromatography System HL—*k* 13.35.

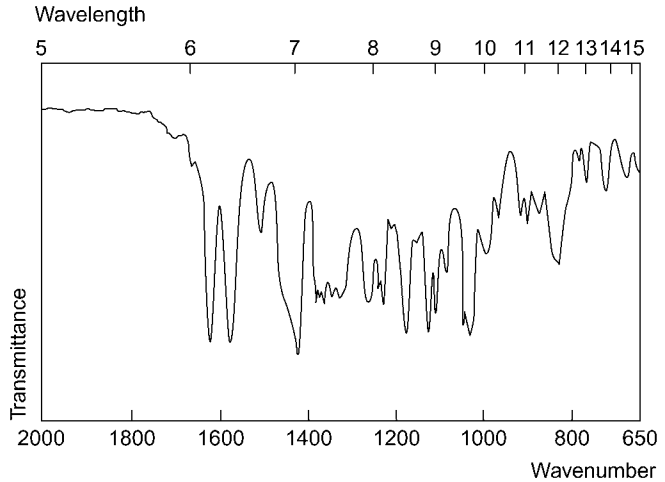
Column: Waters μPorasil (150 × 4.6 mm i.d.). Mobile phase: hexane : chloroform (90 : 10), flow rate 2 mL/min. UV detection (λ = 220 nm). Retention time: 4.9 min. Limit of detection not reported [Debruyne *et al.* 1994].

Supercritical Fluid Chromatography Column: Cyanopropyl silica (250 × 4.6 mm i.d). Mobile phase: methanol in CO_2 (2% to 7% at 15 min), flow rate 2 mL/min. APCI, positive ion mode, full scan and SIM acquisition mode. Retention time: 5.19 min. Limit of detection, 0.69 ng [Backstrom *et al.* 1997].

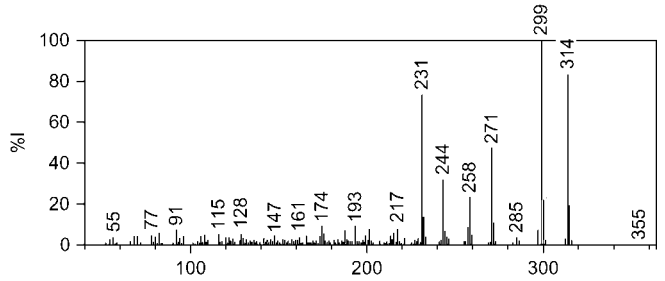
Ultraviolet Spectrum Ethanol—283, 276 nm.



Infrared Spectrum Principal peaks at wavenumbers 1580, 1040, 1620, 1180, 1130, 1050 cm^{-1} (KBr disc).



Mass Spectrum Principal ions at *m/z* 299, 231, 314, 43, 41, 295, 55, 271.



Quantification

Blood **GC-MS** Column: DB-5MS fused silica capillary (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 1 min to 190° at 25°/min to 210° at 25°/min to 290° at 30°/min for 2.5 min. CI, negative ion mode, SRM acquisition mode. Limit of quantification, 0.5 μg/L, limit of detection, 0.1 to 0.2 μg/L [Thomas *et al.* 2007]. Column: Restek Rtx 5-MS capillary (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 70° for 1 min to 260° at 15°/min for 10 min. Limit of quantification, 1 ng/L; limit of detection, 0.5 ng/L [Ondra *et al.* 2006]. Column: rtx-200 (20 m × 0.18 mm i.d., 0.2 μm) and DB-17 (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 120° to 200° at 20°/min to 250° at 10°/min to 300° at 25°/min. SIM acquisition mode. Limit of quantification, 1.0 μg/L for both Δ^9-THC and 11-nor-Δ^9-THC-9-carboxylic acid [Scurlock *et al.* 2006]. Column: 5% phenylmethyl silicone capillary (12 m × 0.32 mm i.d., 0.33 μm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 2 min to 200° at 50°/min to 280° at 15°/min for 5 min. EI ionisation. Limit of detection, ≈1 ng/L [Chiarotti, Costamagna 2000]. Column: HP-5MS 5% phenyl 95% methyl siloxane capillary (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 290° at 30°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.4 μg/L Δ^9-THC, 0.2 μg/L 11-nor-Δ^9-THC-9-carboxylic acid [Kintz, Cirimele 1997]. See also Bergman *et al.* [1981]; McCallum *et al.* [1978] and McCallum, Shaw [1981].

LC-MS Column: Symmetry C_{18} (150 × 1.5 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1% formic acid (70 : 30), flow rate 0.3 mL/min. ESI, positive and negative ion modes. Limit of quantification, 2 μg/L, limit of detection, 0.5 μg/L [Teixeira *et al.* 2007].

Plasma **GC-MS** Column: DB-1MS capillary (15 m × 0.25 mm i.d., 0.25 μm). EI ionisation, SIM acquisition mode. Limit of quantification and detection, 0.125 μg/L [Lowe *et al.* 2007]. Column: HP-5MS 95% dimethylsiloxane 5% diphenylsiloxane capillary (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 130° for 2 min to 300° at 20°/min for 5 min. SIM acquisition mode. Limit of quantification, 0.80 μg/L Δ^9-THC, 0.51 μg/L 11-hydroxy-Δ^9-THC, 0.88 μg/L 11-nor-9-carboxy-Δ^9-THC, limit of detection, 0.24 μg/L Δ^9-THC, 0.15 μg/L 11-hydroxy-Δ^9-THC, 0.26 μg/L 11-nor-Δ^9-THC-9-carboxylic acid [Nadulski *et al.* 2005a].

HPLC Column: Nucleosil C_{18} (125 × 4.6 mm i.d., 3 μm). Mobile phase: methanol : acetonitrile : 5 mmol/L tetrabutylammonium perchlorate solution (pH 3.2; 50 : 25 : 25), flow rate 1.2 mL/min. UV detection (λ = 215 nm). Limit of quantification, 5 μg/L [Abbara *et al.* 2006]. Column: LiChroCart Superspher 60 RP select B (250 × 4 mm i.d., 5 μm). Mobile phase: 5.6 mmol/L tetrabutylammonium hydrogen sulfate (pH 2.3) : acetonitrile : tetrahydrofuran (44 : 46 : 10) with 160 μmol/L Na_2EDTA, flow rate 850 μL/min. Electrochemical detection. Limit of quantification, 5 μg/L for Δ^9-THC and 11-nor-Δ^9-THC-9-carboxylic acid [Kramer, Kovar 1999]. Column: Zorbax C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : 1 mol/L sulfuric acid (35 : 15 : 50), flow rate 1.8 mL/min. Electrochemical detection. Limit of detection, <0.5 μg/L [Nakahara *et al.* 1989].

LC-MS Column: Synergi MAX-RP 80A C_{12} (75 × 2 mm i.d., 4 μm). Mobile phase: 10 mmol/L ammonium formate (pH 3.0) : acetonitrile-10 mmol/L ammonium formate (90 : 10; 50 : 50 for 1 min to 21 : 79 at 12 min to 5 : 95 at 12.5 min for 2.5 min to 50 : 50 at 25 min), flow rate 400 μL/min. APCI, SIM acquisition mode. Limit of quantification, 0.2 μg/L for Δ^9-THC, 11-hydroxy-Δ^9-THC, and 11-nor-Δ^9-THC-9-carboxylic acid, limit of detection, 0.1 μg/L [Grauwiler *et al.* 2007]. Column: Phenomenex Luna phenylhexyl (50 × 2 mm i.d., 3 μm). Mobile phase: 5 mmol/L ammonium acetate (pH 6.5) : acetonitrile (70 : 30 for 0.4 min to 10 : 90 at 7.8 min for 0.4 min to 70 : 30 at 8.5 min for 2.5 min), flow rate 0.25 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.8 μg/L for Δ^9-THC, 11-hydroxy-Δ^9-THC and 4.3 μg/L for 11-nor-9-carboxy-Δ^9-THC, limit of detection, 0.2 μg/L for Δ^9-THC and 11-hydroxy-Δ^9-THC, 1.6 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Maralikova, Weinmann 2004].

Serum **GC-MS** Column: VF-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. EI ionisation at 70 eV. Limit of quantification, 0.5 μg/L Δ^9-THC, 0.3 μg/L 11-hydroxy-Δ^9-THC, 6.2 μg/L 11-nor-Δ^9-THC-9-carboxylic acid, respectively, limit of detection, 0.2 μg/L Δ^9-THC, 0.1 μg/L 11-hydroxy-Δ^9-THC, 1.9 μg/L 11-nor-Δ^9-THC-9-carboxylic acid [Kauert *et al.* 2007].

LC-MS Column: Luna phenylhexyl (50 × 2 mm i.d., 3 μm). Mobile phase: 5 mmol/L ammonium acetate (pH 6.5) : acetonitrile (70 : 30 for 1 min to 10 : 90 at 4 min for 1 min to 70 : 30 at 6 min for 4 min), flow rate 0.25 mL. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 7.5 μg/L, limit of detection, 2.5 μg/L [Jung *et al.* 2007].

Urine **TLC** Plates: TOXI-GRAMS MS (THC) biphasic with alkyl-silica extraction layer (14 × 5.5 cm). Solvent system: *n*-heptane : acetone : glacial acetic acid (50 : 50 : 1). R_f 0.78 for 11-nor-Δ^9-THC-9-carboxylic acid. Limit of detection, 20 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [King *et al.* 1989].

GC-MS Column: DB-35MS capillary (15 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 100° for 1 min to 200° at 35°/min to 250° at 23°/min to 325° at 20°/min for 1 min. SIM acquisition mode. Limit of quantification, 1.0 μg/L Δ^9-THC, 2.5 μg/L 11-hydroxy-Δ^9-THC, 2.5 μg/L 11-nor-Δ^9-THC-9-carboxylic acid, limit of detection, 2.5 μg/L Δ^9-THC, 2.5 μg/L 11-hydroxy-Δ^9-THC, 2.5 μg/L 11-nor-Δ^9-THC-9-carboxylic acid [Abraham *et al.* 2007]. Column: CP Sil fused silica capillary (12.5 m × 0.25 mm i.d., 0.4 μm). Temperature programme: 160° for 2 min to 320° at 20°/min for 2 min. SIM acquisition mode. Limit of quantification, 2.6 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid; limit of detection, 0.8 μg/L, for 11-nor-Δ^9-THC-9-carboxylic acid [Dietz *et al.* 2007]. Column: Zebron ZB-5MS Phenomenex capillary (30 m × 0.25 mm, 0.25 μm). Carrier gas: He, 1.4 mL/min. Temperature programme: 120° to 235 at 20°/min to 250° at 3°/min to 340° at 25°/min. SIM acquisition mode. Limit of detection, 1 ng [Huq *et al.* 2005]. Column: Supelco 5% phenylmethyl silicone capillary (17 m × 0.2 mm i.d., 0.33 μm) or HP1 methyl silicone capillary (30 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 1.1 mL/min. Temperature programme: 160° for 0.5 min to 280° at 15°/min to 320° at 50°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Strano-Rossi *et al.* 2005]. Column: DB-5MS bonded phase capillary (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 230° to 290° at 55°/min for 1.0 min. SIM acquisition mode. Retention time: 1.8 min. Limit of quantification, 3.8 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid, limit of detection, 3.8 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Jamerson *et al.* 2005a]. See also Chiarotti, Costamagna [2000]; Jamerson *et al.* [2005b] and Kintz *et al.* [1995].

HPLC Column: LiChroCart Superspher 60 RP select B (250 × 4 mm i.d., 5 μm). Mobile phase: 5.6 mmol/L tetrabutylammonium hydrogen sulfate (pH 2.3) : acetonitrile: tetrahydrofuran (44 : 46 : 10) with 160 μmol/L Na_2EDTA, flow rate

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850 μL/min. Electrochemical detection. Limit of quantification, 15 μg/L for 11-nor-9-carboxy-Δ^9-THC [Kramer, Kovar 1999]. Column: RP-8 reversed phase (250 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.125 mol/L phosphate buffer (55 : 45), flow rate 1 mL/min. DAD (λ = 212 nm). Retention time: ≈12 min. Limit of detection, 10 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Bianchi, Donzelli 1996]. Column: ODS (100 × 3.2 mm i.d., 3 μm). Mobile phase: 100 mL 0.5 mol/L monochloroacetic acid (pH 3.0) and 1.4 mL TEA to 1 L with water-methanol (30 : 70) : 100 mL 0.5 mol/L monochloroacetic acid (pH 3.0) and 1.4 mL TEA to 1 L with water-tetrahydrofuran-methanol (5 : 15 : 80; 90 : 10 to 70 : 30 at 15 min to 40 : 60 at 30 min for 5 min), flow rate 1.0 mL/min. Electrochemical detection. Limit of quantification, 12 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Fisher *et al.* 1996]. Column: Zorbax C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : 0.1 mol/L sulfuric acid (35 : 15 : 50), flow rate 1.8 mL/min. Electrochemical detection. Limit of detection, <0.5 μg/L [Nakahara *et al.* 1989].

LC-MS Column: Synergi MAX-RP 80A C12 (75 × 2 mm i.d., 4 μm). Mobile phase: 10 mmol/L ammonium formate (pH 3.0) : acetonitrile-10 mmol/L ammonium formate (90 : 10; 50 : 50 for 1 min to 21 : 79 at 12 min to 5 : 95 at 12.5 min for 2.5 min to 50 : 50 at 25 min), flow rate 400 μL/min. APCI, SIM acquisition mode. Limit of quantification, 1 μg/L for Δ^9-THC and 11-nor-Δ^9-THC-9-carboxylic acid, 2 μg/L for 11-hydroxy-Δ^9-THC; limit of detection, 0.5 μg/L for Δ^9-THC and 11-nor-Δ^9-THC-9-carboxylic acid, 1 μg/L for 11-hydroxy-Δ^9-THC [Grauwiler *et al.* 2007]. Column: Luna phenylhexyl (50 × 2 mm i.d., 3 μm). Mobile phase: 5 mmol/L ammonium acetate (pH 6.5) : acetonitrile (70 : 30 for 1 min to 10 : 90 at 4 min for 1 min to 70 : 30 at 6 min for 4 min), flow rate 0.25 mL. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 5 μg/L, limit of detection, 2.5 μg/L [Jung *et al.* 2007]. Column: Symmetry C_{18} (150 × 1.5 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1% formic acid (70 : 30), flow rate 0.3 mL/min. ESI, positive and negative ion modes. Limit of quantification, 2 μg/L; limit of detection, 0.5 μg/L [Teixeira *et al.* 2007]. Column: Waters 2695 HPLC-Micromass Quattro II reversed phase C_{18} (200 × 4.6 mm i.d.). Mobile phase: methanol : water (85 : 15). APCI, positive ion mode, SIM acquisition mode. Limit of detection not reported [Yang, Xie 2006]. Column: RP-C8-select B (125 × 2 mm i.d., 5 μm). Mobile phase: 5 mmol/L ammonium formate-0.1% formic acid (pH 3) : acetonitrile-0.1% formic acid (90 : 10 for 6.6 min to 70 : 30 at 26.6 min to 30 : 70 at 33.3 min to 10 : 90 for 4 min). ESI, positive ion mode, MRM acquisition mode. Limit of detection, <10 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid [Weinmann *et al.* 2000]. Column: Zorbax Eclipse XDB-C_8 reversed phase (150 × 3.0 mm i.d., 5 μm). Mobile phase: 4 mmol/L formic acid in water : 4 mmol/L formic acid in acetonitrile (40 : 60 for 0.5 min to 20 : 80 at 3 min for 4 min to 40 : 60 over 3 min), flow rate 0.5 mL/min. API, ESI, positive ion mode, SIM acquisition mode. Limit of detection, 2 μg/L [Breindahl, Andreasen 1999].

Meconium **GC-MS** Column: Agilent DB-5 MS (15 m × 0.25 mm i.d., 0.25 μm) and Agilent DB-17 MS (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 200° for 0.2 min to 280° at 15°/min for 3.8 min. EI ionisation, FID, SIM acquisition mode. Limit of quantification, 10 μg/kg, limit of detection, 5 μg/kg [Marin *et al.* 2007]. Column: DB-5MS (15 m × 0.2 mm i.d., 0.25 μm). Temperature programme: 200° for 0.2 min to 275° at 60°/min for 3 min to 300° at 20°/min to 325° at 60°/min for 1.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 μg/kg; limit of detection, 5 μg/kg [Coles *et al.* 2005].

Oral Fluid **GC-MS** Column: Ultra 1 methyl silicone capillary (16.5 m × 0.2 mm i.d., 0.11 μm). Carrier gas: He, 0.8 mL/min. Temperature programme: 70° for 2 min to 160° at 30°/min to 170° at 5°/min to 200° at 20°/min to 220° at 10°/min to 300° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 1.9 μg/L, limit of detection, 0.6 μg/L [Pujadas *et al.* 2007]. Column: HP-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 1.8 mL/min. Temperature programme: 70° for 1 min to 300° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 2 μg/L, limit of detection, 1 μg/L [Drummer *et al.* 2007]. Column: VF-1MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 2.4 μg/L, limit of detection, 0.5 μg/L [Kauert *et al.* 2007]. Column: DB-5MS (15 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 125° for 5 min to 250° at 40°/min for 1.3 min to 300° at 70°/min. SIM acquisition mode. Limit of quantification, 0.5 μg/L [Moore *et al.* 2007]. Column: J and W DB-5 (15 m × 0.25 mm i.d., 1.0 μm). Temperature programme: 130° to 300° at 30°/min. Negative ion mode. Limit of quantification, 10 ng/L [Day *et al.* 2006]. See also Gunnar *et al.* [2005].

HPLC Column: LiChrospher Select B RP-8 (125 × 4 mm i.d., 5 μm). Mobile phase: methanol-0.15 mol/L aqueous sodium chloride (20 : 80) : methanol-0.15 mol/L aqueous sodium chloride (82 : 18; 100 : 0 for 5 min to 0 : 100 at 5.1 min until 25 min to 100 : 0 at 25.1 min for 10 min), flow rate 0.8 mL/min. UV detection (λ = 220 nm). Limit of detection, 2 mg/L [Kircher, Parlar 1996].

LC-MS Column: Xterra C_{18} MS reversed phase (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L aqueous ammonium formate (pH 3.5) : methanol (77 : 23 for 0.2 min to 4 : 96 at 6 min for 3 min), flow rate 200 μL/min. TOF-ESI, positive ion mode. Limit of quantification, 0.1 μg/L, limit of detection, 0.05 μg/L [Quintela *et al.* 2007]. Column: Symmetry C_{18} (150 × 1.5 mm i.d., 5 μm). Mobile phase: acetonitrile : 0.1% formic acid (70 : 30), flow rate 0.3 mL/min. ESI, positive and negative ion modes. Limit of quantification, 5 μg/L, limit of detection, 2 μg/L [Teixeira *et al.* 2007]. Column: Atlantis dC_{18} (50 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 5 mmol/L aqueous ammonium acetate (pH 5; 10 : 90 to 40 : 60 at 4 min to 90 : 10 at 4.1 min for 3.9 min to 10 : 90 at 8.1 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, <0.16 μg/L [Oiestad *et al.* 2007]. Column: Xterra MS C_{18} (50 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.05% ammonia (70 : 30), flow rate 0.3 mL/min. ESI, positive ion mode, single ion recording. Limit of quantification, 2 μg/L, limit of detection, 1 μg/L [Teixeira *et al.* 2004, 2005]. Column: Xterra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.1% formic acid : acetonitrile (15 : 85), flow rate 0.25 mL/min. ESI, positive ion mode. Limit of quantification, 2 μg/L [Concheiro *et al.* 2004].

Sweat **GC-MS** CI, negative ion mode. Limit of quantification, 0.4 ng [Huestis *et al.* 2008].

Brain **GC-MS** Column: 6% OV-1 or Hi-EFF 9BP. FPD. Limit of detection, 10 μg/kg [McCallum *et al.* 1978].

Hair **GC-MS** Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 275° at 35°/min for 3 min to 300° at 25°/min for 3.5 min. CI, negative ion mode, MRM acquisition mode. Limit of quantification, 0.05 ng/kg for 11-nor-Δ^9-THC-9-carboxylic acid, limit of detection, 0.02 ng/kg for 11-nor-Δ^9-THC-9-carboxylic acid [Kim, In 2007]. Column: DB-5MS capillary (15 m × 0.25 mm i.d., 1.0 μm). Temperature programme: 180° to 278° at 30°/min to 282° at 5°/min to 300° at 35°/min for 2.5 min. CI, positive ion mode for Δ^9-THC, negative ion mode for 11-nor-Δ^9-THC-9-carboxylic acid, SRM acquisition mode. Limit of quantification, 1 ng/kg for Δ^9-THC, 0.1 ng/kg for 11-nor-Δ^9-THC-9-carboxylic acid [Huestis *et al.* 2007]. Column: HP-5MS capillary. Temperature programme: 130° for 2 min to 300° at 20°/min. SIM acquisition mode. Limit of quantification, 37 ng/kg, limit of detection, 12 ng/kg [Nadulski, Pragst 2007]. Column: DB-35 MS (40 m × 0.18 mm i.d., 0.18 μm) and DB-1 (15 m × m i.d., 0.25 mm). Carrier gas: He, 1.6 mL/min. Temperature programme: 100° for 0.5 min to 200° at 50°/min to 260° at 10°/min for 2.9 min to 220° at 120°/min. CI, negative ion mode, SIM acquisition mode. Limit of quantification, 0.05 ng/kg [Moore *et al.* 2006]. Column: Supelco Equity 1 capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 70° for 1 min to 240° at 40°/min for 1 min to 280° at 20°/min for 5 min. CI, negative ion mode. Limit of quantification, 100 ng/kg, limit of detection, 50 ng/kg [Marsili *et al.* 2005]. See also Baptista *et al.* [2002]; Chiarotti and Costamagna [2000]; Deveaux *et al.* [2000]; Kim *et al.* [2005]; Sachs and Dressler [2000] and Strano-Rossi and Chiarotti [1999].

Nail **GC-MS** Column: HP-1 cross linked dimethyl silicone capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 150° to 300° at 10°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, <0.1 μg/kg for Δ^9-THC and 11-nor-Δ^9-THC-9-carboxylic acid [Lemos *et al.* 1999].

Other **GC-MS** Indoor Air. Column: HP-5MS fused silica capillary (25 m × 0.2 mm i.d., 0.33 μm). Carrier gas: He, 1 mL/min. Temperature programme: 40° for 1 min to 250° at 25°/min for 25 min. EI ionisation at 70 eV, CI, positive ion mode. Retention time, 13.7 min. Limit of quantification, 125 ng/m^3, limit of detection, 100 ng/m^3 [Chou *et al.* 2007].

HPLC Foods Containing Hemp. Column: Nucleosil 120-3 C_{18} (125 × 2 mm i.d.). Mobile phase: acetonitrile : 8.6 g/L 85% phosphoric acid (55 : 45 to 80 : 20 at 25 min to 90 : 10 at 26 min for 4 min to 55 : 45 at 31 min for 9 min). UV detection (λ = 210 nm). Limit of detection, 0.1 ng [Zoller *et al.* 2000]. Rat Cortex and Corpus Striatum. Column: ODS μBondapak C_{18} reversed phase (300 × 4 mm i.d., 10 μm). Mobile phase: methanol : acetonitrile: 0.01 mol/L sulfuric acid (21 : 24 : 55), flow rate 3.0 mL/min. Electrochemical detection. Limit of detection, 1.5 ng [Nyoni *et al.* 1996].

LC-MS Mouse Brain and Blood. Column: Waters 2695 HPLC-Micromass Quattro II reversed phase C_{18} (200 × 4.6 mm i.d.). Mobile phase: methanol : water (85 : 15). APCI, positive ion mode, SIM acquisition mode. Limit of detection not reported [Yang, Xie 2006]. Wastewater. Column: Xterra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: 0.05% TEA : acetonitrile (100 : 0 to 55 : 45 in 12 min to 0 : 100 in 2 min for 2 min to 100 : 0 in 2 min for 8 min), flow rate 200 μL/min. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 1.75 and 0.94 μg/L for 11-nor-Δ^9-THC-9-carboxylic acid in influent and effluent waste water, respectively, limit of detection, 307 pg [Castiglioni *et al.* 2006].

Note For a clinical evaluation of the Toxi Prep system for the screening of THC metabolites in urine, see Steinberg *et al.* [1997].

Disposition in the Body Δ^9-THC is absorbed from the gastrointestinal tract but absorption is slow and irregular. However, Δ^9-THC can be measured in plasma within seconds of inhalation of marijuana smoke. Δ^9-THC is lipophilic and is widely distributed in the body. It is hydroxylated to the active metabolite 11-hydroxy-Δ^9-THC by the hepatic CYP2C9 enzyme, and this is further oxidised to the intermediate 11-oxo-THC, followed by oxidation to 11-nor-Δ^9-THC-9-carboxylic acid. Many other polar mono- and dicarboxylic acids are formed, which are conjugated with glucuronic acid to a variable extent. The inactive substances 8α-hydroxy-Δ^9-THC and 8α,11-dihydroxy-Δ^9-THC are also formed by the action of CYP3A4. 11-nor-Δ^9-THC-9-carboxylic acid undergoes glucuronidation of the carboxy group and this is excreted in the urine and can be detected up to 4 days after smoking one marijuana cigarette and up to 4 weeks after frequent use. Levels of THC increase rapidly after smoking, peak before the end of smoking, and quickly dissipate. Mean peak levels of 11-hydroxy-Δ^9-THC are substantially lower than THC levels and occur directly after the cessation of smoking. 11-nor-Δ^9-THC-9-carboxylic acid levels increase slowly and plateau for a long period of time. 11-nor-Δ^9-THC-9-carboxylic acid glucuronide is present in blood at higher concentrations than the unconjugated acid and is unstable to hydrolysis, a common cause of misinterpretation for the prediction of cannabis exposure [Maurer *et al.* 2006]. Enterohepatic recirculation of metabolites may occur. Up to ≈25% of a dose is excreted in the urine in 3 days, mainly as 11-nor-Δ^9-THC-9-carboxylic acid glucuronide, together with the other carboxylic acids in free and conjugated form. The Δ^9-THC-*O*-glucuronide has been detected in urine. The major excretion route is via

the faeces, with up to ~65% of a dose being excreted in 5 days, mainly as 11-hydroxy-Δ^9-THC and the carboxylic acids in conjugated form. Δ^9-THC metabolites have been detected in urine for up to 12 days following a single oral dose. Δ^9-THC crosses the placenta and is distributed into breast milk.

Blood Concentrations

Six volunteers with histories of marijuana use ingested liquid hemp oil (0.39 and 14.8 mg Δ^9-THC/day), hemp oil in capsules (0.47 mg Δ^9-THC/day), dronabinol capsules (7.5 mg Δ^9-THC/day), or placebo. Plasma Δ^9-THC and 11-hydroxy-Δ^9-THC levels were low and never exceeded 6.1 µg/L. Analytes were detectable 1.5 h after the initial dosing with the 7.5 mg Δ^9-THC/day regimen and 4.5 h after the start of the 14.8 mg Δ^9-THC/day protocol. 11-nor-Δ^9-THC-9-carboxylic acid levels peaked at 3.1 µg/L during dosing with the low dose hemp oils. Fifteen and a half hours following the last THC dose, all plasma samples were negative for Δ^9-THC and 11-hydroxy-THC, although plasma 11-nor-Δ^9-THC-9-carboxylic acid levels persisted for at least 39.5 h after the end of dosing [Goodwin *et al.* 2006].

The distribution of 11-nor-Δ^9-THC-9-carboxylic acid was investigated in postmortem specimens of 50 deaths in traffic accidents in New Jersey, USA. The mean concentration in heart blood, urine and bile was 0.081 (0.016 to 0.33), 0.314 (0.044 to 2.33) and 12.9 (1.03 to 43.7) mg/L, respectively. Vitreous humour samples were negative or below the limit of detection, [Lin, Lin 2005].

Twenty-four volunteers (12 male, 12 female; aged 18 to 45 years) were administered one of 10 mg Δ^9-THC, cannabis extract (10 mg Δ^9-THC + 5.4 mg cannabidiol), or water, and blood samples were obtained. Mean peak concentrations of Δ^9-THC were 3.19 and 4.05 µg/L, respectively, attained at 63.6 and 56 min, respectively, for the dosed groups, although there was some evidence that cannabidiol partially inhibited the CYP2C-catalysed hydroxylation of Δ^9-THC to 11-hydroxy-Δ^9-THC [Nadulski *et al.* 2005b].

Ingestion of 20 mg Δ^9-THC resulted in mean peak plasma concentrations of 6 µg/L, with a range from 4.4 to 121 µg/L. Higher plasma concentrations of 11-hydroxy-Δ^9-THC were observed, possibly as a result of first-pass metabolism [Kochanowski, Kala 2005]. Passive inhalation of marijuana smoke has resulted in plasma Δ^9-THC levels of 1 to 7 µg/L and urine 11-nor-Δ^9-THC-9-carboxylic acid levels as high as 39 µg/L [Baselt 2004].

Seven healthy participants with histories of marijuana use received 0, 0.39, 0.47, 7.5 and 14.8 mg Δ^9-THC/day orally for 5 days in a double-blind randomised study. There were no differences in mean time of maximum excretion rate, mean maximum excretion rate, and mean terminal half-life between the 4 Δ^9-THC doses, with respective ranges of 67.4 to 94.9 h, 0.9 to 16.3 µg/h and 44.2 to 64.0 h [Gustafson *et al.* 2004].

Six healthy men with histories of marijuana use smoked a single marijuana cigarette (placebo, 1.75% or 3.55% Δ^9-THC). The smoking protocol consisted of a 2 s inhalation, a 10 s hold period, and a 72 s exhalation and rest period. A total of eight puffs were inhaled in 11.2 min. Mean plasma levels of 7.0 and 18.1 µg/L Δ^9-THC were observed after the first inhalation of the 1.75% and 3.55% Δ^9-THC cigarettes, respectively. Levels continued to increase rapidly to mean peak plasma concentrations of 79.0 and 152.0 µg/L for the 1.75% and 3.55% Δ^9-THC cigarettes, respectively, attained after 9 min (before inhalation of the last puff sequence at 9.8 min) [Huestis *et al.* 1992].

After 11 subjects had smoked 11.6 to 15.6 mg (mean 13.0) of Δ^9-THC from a cigarette over a period of 5 to 7 min, peak plasma concentrations of 0.03 to 0.12 mg/L (mean 0.08) Δ^9-THC were observed within 3 min of termination of smoking; the concentration fell rapidly to 0.003 to 0.01 mg/L (mean 0.007) at 1 h and to 0.0006 to 0.003 mg/L (mean 0.0016) at 4 h, after smoking. When 20 mg Δ^9-THC was given orally to the same subjects, peak plasma concentrations of 0.004 to 0.01 mg/L Δ^9-THC were produced between 60 and 300 min after ingestion [Ohlsson *et al.* 1980].

Note For information on vaporisation as a smokeless cannabis delivery system, see Abrams *et al.* [2007]. For a trial investigating the efficacy of Δ^9-THC in the treatment of spasticity in spinal cord injury, see Hagenbach *et al.* [2007], and for its efficacy in Tourette's syndrome, see Muller-Vahl *et al.* [2002].

Toxicity Δ^9-THC intoxication may result in loss of consciousness, or even death, but reports of fatalities are rare. The following Δ^9-THC levels were reported following 6 cardiovascular fatalities after cannabis use: 22, 4, 2, 5, 3 and 7 µg/L; no other drugs were detected apart from 400 mg/L ethanol in the fourth case [Bachs, Morland 2001].

The following postmortem levels were reported following a case of fatal poisoning by Δ^9-THC: liver 37.5 µg/g, kidney 42 µg/g, and spleen 12 µg/g [Tewari, Sharma 1980].

Bioavailability After ingestion, ≈6 to 20%. During smoking, ≈18 to 50%.

Half-life In plasma of frequent users ≈2 h.

Volume of Distribution Approximately 10 L/kg.

Distribution in Blood Plasma : whole blood ratio 1.8.

Protein Binding 94 to 99%.

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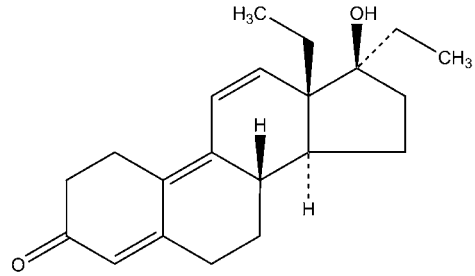
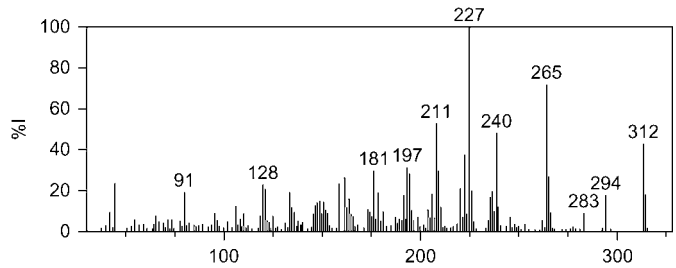
Tetrahydrogestrinone

Anabolic Steroid
$C_{21}H_{28}O_2 = 312.5$
CAS—618903-56-3

IUPAC Name (8*S*,13*S*,14*S*,17*S*)-13,17-Diethyl-17-hydroxy-1,2,6,7,8,14,15,16-octahydrocyclopenta[a]phenanthren-3-one
Synonyms (17α)-13-Ethyl-17-hydroxy-18,19-dinorpregna-4,9,11-trien-3-one; 18a-homopregna-4,9,11-trien-17β-ol-3-one; THG.



Chemical Properties Stock solutions of tetrahydrogestrinone (THG) were stable at –20° for at least a year. THG in human urine was stable for at least 4 h at room temperature, for at least 15 days at –20°, and after freeze–thaw cycles [Marques *et al.* 2007]. THG is stable in equine plasma for 24 h at room temperature, 13 days at 4°, and 34 days at –20° and –70° [Guan *et al.* 2005].
Mass Spectrum Principal ions at *m/z* 227, 265, 211, 240, 312, 225, 197, 181.



Quantification

Urine **GC-MS** Column: HP-1 100% methylsiloxane capillary (17 m × 0.2 mm i.d, 0.11 μm). Carrier gas: He, 1 mL/min. Temperature programme: 140° for 1 min to 180° at 40°/min to 240° at 3°/min to 300° at 40°/min for 3 min. Limit of quantification, 6 μg/L, limit of detection, 3 μg/L [Marques *et al.* 2007]. Column: Hyperclone BDS (50 × 2 mm i.d., 5.0 μm). Carrier gas: 1 mL/min. Temperature programme: 200° for 0.5 min, to 273° at 5°/min, to 310° at 30°/min for 2 min. SIM acquisition mode. Limit of detection, <0.5 μg/L [Catlin *et al.* 2004].

LC-MS Column: Acquity BEH C_{18} (50 × 2.1 mm i.d., 1.7 μm). Mobile phase: 1% formic acid in acetonitrile : 0.1% formic acid in water (5 : 95 to 30 : 70 after 2 min for 1.3 min to 90 : 10 in 2.9 min to 100 : 0 in 0.7 min). Limit of quantification, 0.8 μg/L; limit of detection, 0.2 μg/L [Touber *et al.* 2007]. Column: Waters Symmetry C_{18} (150 × 3.0 mm i.d., 5.0 μm). Mobile phase: water-acetonitrile (90 : 10) : water-acetonitrile (10 : 90; 65 : 35 to 0 : 100 in 20 min). ESI, positive ion mode. Limit of detection, 15 μg/L [Nielen *et al.* 2006]. Column: Zorbax C_{18} (50 × 2.1 mm i.d., 1.8 μm). Mobile phase: 0.1% acetic acid : acetonitrile with 0.1% acetic acid (85 : 15 to 40 : 60 at 5 min to 0 : 100 at 7 min), flow rate 0.3 mL/min. Positive ion mode, MRM acquisition mode. Limit of detection, 1 to 30 μg/L [Mazzarino, Botre 2006]. Column: Macherey-Nagel Nucleodur C_{18} Pyramid (70 × 40 mm i.d., 5.0 μm). Mobile phase: acetonitrile : 5 mmol/L ammonium acetate containing 0.1% acetic acid (pH 3.5; 10 : 90 to 100 : 0 in 10 min), flow rate 800 μL/min. ESI, positive ion mode. Limit of detection, 50 μg/L [Thevis *et al.* 2005]. Column: Hyperclone BDS (50 × 2 mm i.d., 5.0 μm). Mobile phase: 0.1% acetic acid : methanol (15 : 85), flow rate 0.2 mL/min. MRM acquisition mode. Limit of detection, < 0.5 μg/L [Catlin *et al.* 2004].

Other **LC-MS** Bovine Urine. Column: Acquity BEH C_{18} (50 × 2.1 mm i.d., 1.7 μm). Mobile phase: 1% formic acid in acetonitrile : 0.1% formic acid in water (5 : 95 to 30 : 70 after 2 min for 1.3 min to 90 : 10 in 2.9 min to 100 : 0 in 0.7 min). Limit of detection, 2 μg/L [Touber *et al.* 2007]. Equine Plasma. Column: Ace C_8 (50 × 2.1 mm i.d., 5.0 μm). Mobile phase 2 mmol/L ammonium formate : methanol (40 : 60 for 4 min to 10 : 90 at 8 min to 40 : 60 at 8.1 min), flow rate 300 μL/min for 8.1 min to 400 μL/min until 10.4 min to 300 μL/min at 10.5 min. Limit of detection, 25 ng/L [Guan *et al.* 2005].

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Touber ME *et al.* (2007). Multi-detection of corticosteroids in sports doping and veterinary control using high-resolution liquid chromatography/time-of-flight mass spectrometry. *Anal Chim Acta* 586: 137–146.

Tetramisole

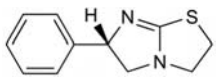
Anthelmintic (Veterinary)

$C_{11}H_{12}N_2S = 204.3$

CAS—5036-02-2

IUPAC Name 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*][1,3]thiazole

Synonym 2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole



Chemical Properties Crystals. Mp 87° to 89°. Practically insoluble in water; soluble in chloroform.

Tetramisole Hydrochloride

$C_{11}H_{12}N_2S,HCl = 240.8$

CAS—5086-74-8

Proprietary Names *Ascarotrat*; *Ascaverm*; *Cofasol*; *Tetramizotil*.

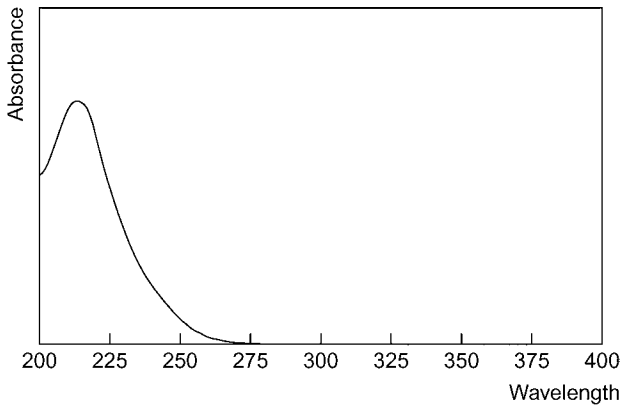
Chemical Properties A white to pale cream-coloured crystalline powder. Mp 264° to 265°. Soluble 1 in 5 of water, 1 in 50 of ethanol, 1 in 3000 of chloroform and 1 in 10 of methanol; very slightly soluble in ether.

For analytical data see also under Levamisole.

Thin-layer Chromatography System TA—R_f 0.69; system TB—R_f 0.18; system TC—R_f 0.54; system TE—R_f 0.66; system TL—R_f 0.46; system TAE—R_f 0.51; system TAF—R_f 0.53; system TAJ—R_f 0.09; system TAK—R_f 0.00; system TAL—R_f 0.06.

High Performance Liquid Chromatography System HX—RI 261; system HY—RI 222; system HAA—retention time 7.0 min.

Ultraviolet Spectrum



Disposition in the Body

Note For a review of the pharmacokinetics of anthelmintic drugs, see Edwards and Breckenridge [1988].

Dose 2.5 to 5 mg/kg of tetramisole hydrochloride as a single dose.

Edwards G, Breckenridge AM (1988). Clinical pharmacokinetics of anthelmintic drugs. *Clin Pharmacokinet* 15: 67–93.

Tetrazepam

Benzodiazepine, Muscle Relaxant, Tranquilliser

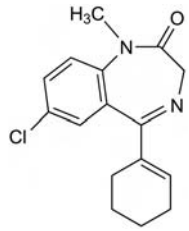
$C_{16}H_{17}ClN_2O = 288.8$

CAS—10379-14-3

IUPAC Name 7-Chloro-5-cyclohexen-1-yl-1-methyl-3*H*-1,4-benzodiazepin-2-one

Synonym 7-Chloro-5-(1-cyclohexen-1-yl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one

Proprietary Names *Megavix*; *Miolastan*; *Mobiforton*; *Musapam*; *Musaril*; *Muskelat*; *Myolastan*; *Myospasmal*; *Panos*; *Rilex*; *Tepam*; *Tethexal*; *Tetra-saar*; *Tetramdura*; *Tetrazep*.



Chemical Properties Yellow-brown crystals. Mp 144°. Log *P* (octanol/water), 3.2 [Capella-Peiro *et al.* 2002]. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].

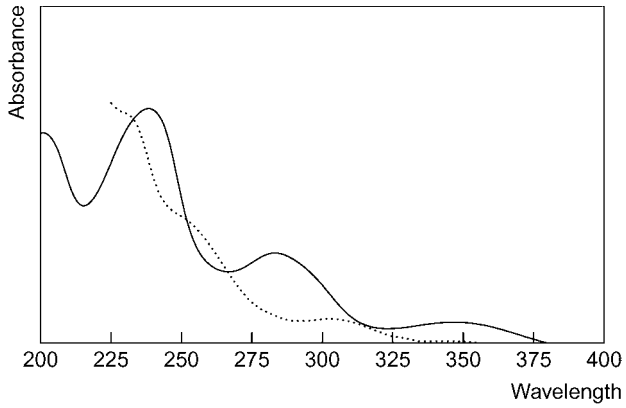
Colour Test Formaldehyde–sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.78 (tetrazepam); R_f 0.77 (M-nor) 0.05; system TAD—R_f 0.67 (tetrazepam); R_f 0.56 (M-nor); system TAE—R_f 0.84 (tetrazepam); R_f 0.85 (M-nor); system TAF—R_f 0.89 (tetrazepam); R_f 0.92 (M-nor); system TAG—R_f 0.66 (tetrazepam); R_f 0.60 (M-nor); system TB—R_f 0.32 (tetrazepam); R_f 0.5 (M-nor); system TC—R_f 0.69 (tetrazepam); R_f 0.58 (M-nor); system TD—R_f 0.57 (tetrazepam); R_f 0.35 (M-nor); system TE—R_f 0.79 (tetrazepam); R_f 0.73 (M-nor); system TF—R_f 0.49 (tetrazepam); R_f 0.45 (M-nor).

Gas Chromatography System GA—RI 2430 tetrazepam; RI 2530 M (nor-); RI 2570 M (OH-) isomer 1; RI 2580 M (OH-) isomer 2; RI 2430 M (oxo-). (Tetrazepam does not form a TMS derivative).

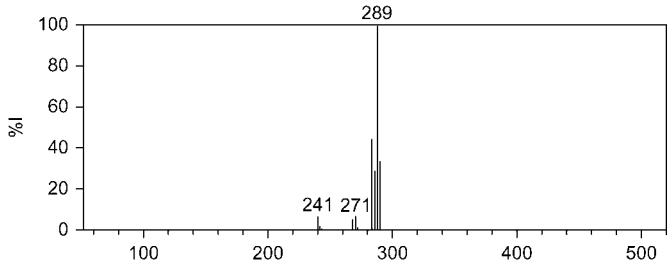
High Performance Liquid Chromatography System HAA—RT 22.4 min; system HX—RI 511; system HY—RI 483.

Ultraviolet Spectrum Aqueous acid—239 ($A_1^1 = 940$b), 284, 345; aqueous alkali—305 nm ($A_1^1 = 84$b) nm.



Infrared Spectrum Principal peaks at wavenumbers 1678, 1602, 825, 1132, 1310, 800 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 253, 288, 287, 289, 225, 259, 254, 41.



Quantification

Plasma **GC** Column: HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 1.5 mL/min. Temperature programme: 100° to 220° at 20°/min for 15 min to 300° at 20°/min for 5 min. ECD. Limit of detection, 20 µg/L [Pavlic *et al.* 2007].

LC-MS Column: LiChroCart (125 × 2 mm i.d.). Mobile phase: 5 mmol/L aqueous ammonium formate (pH 3.0) : acetonitrile (60 : 40 for 5.5 min to 10 : 90 until 8 min to 60 : 40 until 10 min), flow rate 0.4 mL/min for 5.5 min to 0.7 mL/min until 8 min to 0.65 mL/min until 9.5 min to 0.4 mL/min for 0.5 min. APCI, SIM or scan acquisition mode, positive ion mode. Limit of quantification, 025 µg/L (SIM), limit of detection, 5 µg/L (scan) [Kratzsch *et al.* 2004].

Serum **HPLC** Column: Eclipse XDBC-8 (150 × 4.6 mm i.d., 5 µm) or Kromasil C_{18} (120 × 4.6 mm i.d., 5 µm). Mobile phase: 0.06 mol/L SDS : butanol (pH 7.0, 95 : 5), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Limit of detection, 10 µg/L [Capella-Peiro *et al.* 2002]. Column: C_{18} reversed phase (250 × 4 mm i.d., 10 µm). Mobile phase: acetonitrile : 5 mmol/L perchloric acid and 10 mmol/L sodium perchlorate (40 : 60), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 8.2 min. Limit of detection, 500 ng/L for tetrazepam and 100 ng/L for desmethyltetrazepam [Baumgärtner *et al.* 1984].

Urine **GC** See Plasma [Pavlic *et al.* 2007].

T

GC-MS Column: 5% OV-101 Chromosorb G HP 100/120 mesh (60 cm × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 100° to 310° at 30°/min. EI ionisation at 70 eV. Limit of detection not reported [Maurer, Pfleger 1987].

CE Column: fused silica capillary (60/50 cm total/effective length × 75 μm i.d.). Running buffer: 15 mmol/L borate buffer (pH 10.2) containing 40 mmol/L sodium dodecylsulfate. UV detection (λ = 228 nm). Limit of detection, 300 μg/L [Berzas Nevado *et al.* 2006].

Saliva **LC-MS** Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.1% formic acid (5 : 95 to 80 : 20 at 10 min), flow rate 200 μL/min. API, positive ion mode, MRM acquisition mode. Retention time: 11.7 min. Limit of quantification, 0.1 μg/L [Kintz *et al.* 2005].

Hair **LC-MS** Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.1% formic acid (5 : 95 to 80 : 20 at 10 min), flow rate 200 μL/min. API, positive ion mode, MRM acquisition mode. Retention time: 11.7 min. Limit of quantification, 5 ng/g [Villain *et al.* 2005].

Disposition in the Body Rapidly absorbed after oral administration. Extensively metabolised by *N*-demethylation and hydroxylation. Urinary metabolites include 3-hydroxytetrazepam, 3-hydroxydesmethyltetrazepam, 3′-hydroxytetrazepam, and 3′-hydroxydesmethyltetrazepam. Tetrazepam is also metabolised to diazepam and further to nordazepam, the former being subject to CYP 3A4 metabolism [Pavlic *et al.* 2007].

Therapeutic Concentration

After a single oral dose of 50 mg to 12 subjects, peak serum concentrations of 0.49–0.63 mg/L (mean 0.57) of tetrazepam and 0.005–0.008 mg/L of desmethyltetrazepam were attained in about 2 h and 4 h respectively [Baumgärtner *et al.* 1984].

Half-life Plasma half-life, 10–25 h (mean 15).

Dose 25 to 150 mg daily.

Baumgärtner MG *et al.* (1984). Biotransformation and pharmacokinetics of tetrazepam in man. *Arzneimittelforschung* 34: 724–729.

Berzas Nevado JJ *et al.* (2006). Determination of ibuprofen and tetrazepam in human urine by micellar electrokinetic capillary chromatography. *Anal Bioanal Chem* 384: 208–214.

Capella-Peiro (2002). *et al* Direct injection micellar liquid chromatographic determination of benzodiazepines in serum. *J Chromatogr B Analyt Technol Biomed Life Sci* 780: 241–249.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Kintz P *et al.* (2005). Screening and confirmatory method for benzodiazepines and hypnotics in oral fluid by LC-MS/MS. *Forensic Sci Int* 150: 213–220.

Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.

Maurer HH, Pfleger K (1987). Identification and differentiation of benzodiazepines and their metabolites in urine by computerized gas chromatography-mass spectrometry. *J Chromatogr* 422: 85–101.

Pavlic M *et al.* (2007). Medicolegal aspects of tetrazepam metabolism. *Int J Legal Med* 121: 169–174.

Villain M *et al.* (2005). Screening method for benzodiazepines and hypnotics in hair at pg/mg level by liquid chromatography-mass spectrometry/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 825: 72–78.

Tetrodotoxin

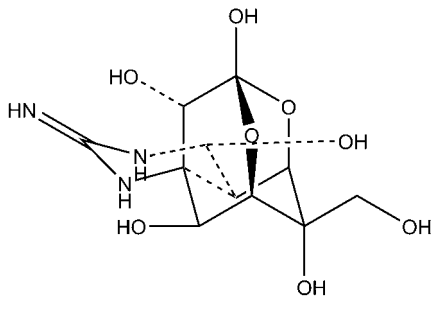
Natural Toxin, Pyrimidinyl Alcohol, Sodium Channel Blocker

$C_{11}H_{17}N_3O_8$ = 319.3

CAS—4368-28-9

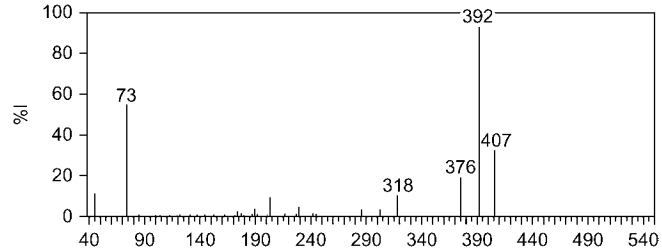
IUPAC Name Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-10a*H*-[1,3]dioxocino[6,5-*d*]pyrimidine-4,7,10,11,12-pentol

Synonyms Arareigai toxin; fugu poison; maculotoxin; spheroidine; tarichatoxin; tetrodonic acid; tetrodontoxin; TTX.



Chemical Properties Found mainly in certain organs (ovaries and liver) of the puffer fish of the genus *Fugu* along with a number of other forms of sea and freshwater animals (e.g. globe fish, *Spheroides rubipres*). The toxin is not made by the fish but appears to be made by relatively common marine bacteria, specifically *Pseudoalteromonas haloplanktis tetraodonis* that associates with these animals. Darkens above 220° without decomposition. Soluble in dilute acetic acid; slightly soluble in water, dry alcohol and ether. Practically insoluble in other organic solvents. Toxin destroyed in strong acids and in alkaline solutions. pK_a 8.76 [O'Neil *et al.* 2006].

Mass Spectrum Principal ions at *m/z* 392, 73, 407, 376, 318 (tri-TMS derivative).



Quantification

Blood **GC-MS** Column: HP-1 (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 165° to 230° at 3°/min. EI ionisation at 70 eV. Retention time: C_9 base-TMS derivative 20.8 min. Limit of quantification not reported [Tsai *et al.* 2006].

LC-MS Column: Zorbax 300SB-C3 (150 × 4.6 mm i.d.). Mobile phase: 1% acetonitrile : 10 mmol/L trimethylamine : 10 mmol/L ammonium formate (pH 4.0), flow rate 0.4 mL/min. ESI, positive ion mode. Limit of detection, 15.6 nmol/L [Tsai *et al.* 2006].

Serum **TLC** Stationary phase: quartz rods coated with silica gel. Mobile phase: *n*-butanol : acetic acid : water (60 : 15 : 30). FID. R_f 0.40. Limit of detection, 0.04 mg/L [Ikebuchi *et al.* 1988].

GC-MS Column: glass with 1.5% OV-17 on 60–80 mesh Chromosorb W (1 m × 30 mm i.d.). Carrier gas: He, 30 mL/min. EI ionisation at 70 eV. Retention time: C_9 base-TMS derivative 1.8 min. Limit of quantification, 0.01 mg/kg [Moriya *et al.* 1992].

HPLC Column: NovaPak C_{18} (100 × 8.0 mm i.d., 4 μm). Mobile phase: 5 mmol/L heptanesulfonic acid-3% acetonitrile in water (pH 4.5), flow rate 0.3 mL/min. Post-column derivatisation with 4 mol/L sodium hydroxide. Fluorescence detection (λ_{ex} = 380 nm, λ_{em} = 505 nm). Retention times: TTX 18 min, anhydroTTX 19 min. Limit of quantification, 5 μg/L [O'Leary *et al.* 2004].

LC-MS Column: Shodex RSpak NN-414 (150 × 4.6 mm i.d.). Mobile phase: 20 mmol/L ammonium acetate (pH 6.8) : acetonitrile (90 : 10), flow rate 0.6 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 18.6 min. Limit of quantification, not reported [Hayashida *et al.* 2003].

Urine **TLC** Stationary phase: quartz rods coated with silica gel. Mobile phase: *n*-butanol : acetic acid : water (60 : 15 : 30). FID. R_f 0.40. Limit of detection, 0.04 μg/mL [Ikebuchi *et al.* 1988].

GC-MS Column: HP-1 (30 m × 0.25 mm i.d.). Carrier gas: He, 1.0 mL/min. Temperature programme: 165° to 230° at 3°/min. EI ionisation at 70 eV. Retention time: C_9 base-TMS derivative 20.8 min. Limit of quantification not reported [Tsai *et al.* 2006]. Column: glass with 1.5% OV-17 on 60–80 mesh Chromosorb W (1 m × 30 mm i.d.). Carrier gas: He, 30 mL/min. EI ionisation at 70 eV. Retention time: C_9 base-TMS derivative 1.8 min. Limit of quantification, 0.01 μg/g [Moriya *et al.* 1992].

HPLC Column: NovaPak C_{18} (100 × 8.0 mm i.d., 4 μm). Mobile phase: 5 mmol/L heptanesulfonic acid 3% acetonitrile in water (pH 4.5), flow rate 0.3 mL/min. Post-column derivatisation with 4 mol/L sodium hydroxide. Fluorescence detection (λ_{ex} = 380 nm, λ_{em} = 505 nm). Retention times: TTX 18 min, anhydroTTX 19 min. Limit of quantification, 20 μg/L [O'Leary *et al.* 2004]. Column: Zorbax 300SB-C3 (150 × 4.6 mm i.d.). Mobile phase: 1% acetonitrile : 10 mmol/L trimethylamine : 10 mmol/L ammonium formate (pH 4.0), flow rate 0.4 mL/min. ESI, positive ion mode. Limit of detection, 15.6 nmol/L [Tsai *et al.* 2006].

Stomach Contents **GC-MS** Column: glass with 1.5% OV-17 on 60–80 mesh Chromosorb W (1 m × 30 mm i.d.). Carrier gas: He, 30 mL/min. EI ionisation at 70 eV. Retention time: C_9 base-TMS derivative 1.8 min. Limit of quantification, 0.01 μg/g [Moriya *et al.* 1992].

Other **TLC** Puffer Fish. Plates: precoated silica gel. Solvent system: methanol : acetic acid (96 : 4). UV detection (λ = 365 nm) after spraying with 10% potassium hydroxide solution. Limit of quantification not reported [Wu *et al.* 2005]. Gastropods (*Zeuxis samiplicutus*). Plates: precoated silica gel $60F_{254}$ (5 × 20 cm, 2 mm thickness). Solvent system: pyridine : ethylacetate : acetic acid : water (15 : 5 : 3 : 4). UV detection (λ = 365 nm) after spraying with 10% potassium hydroxide and heating at 110° for 10 min. R_f 0.62. Limit of quantification not reported [Sui *et al.* 2002].

HPLC Blue-ringed Octopus (*Hapalochlaena maculosa*) and Puffer Fish (*Fugu pardalis*). Column: Develosil C30 UG-5 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 1% acetonitrile : 30 mmol/L ammonium heptafluorobutyrate : 10 mmol/L ammonium formate (pH 5.0), flow rate 0.4 mL/min. Post-column derivatisation with 4 mol/L sodium hydroxide. Fluorescence detection (λ_{ex} = 365 nm, λ_{em} = 510 nm). Limit of quantification not reported [Jang, Yotsu-Yamashita 2006; Yotsu-Yamashita *et al.* 2007]. Freshwater Puffer Fish (*Colomesus asellus*). Column: C_{18} (250 × 4.6 mm i.d.). Mobile phase: 60 mmol/L heptanesulfonic acid : 10 mmol/L ammonium phosphate buffer (pH 5.0). Post-column derivatisation with 4 mol/L sodium hydroxide at 110°. Fluorescence detection (λ_{ex} = 380 nm, λ_{em} = 500 nm). Limit of detection, 0.1 μg/L [Oliveira *et al.* 2006]. Gastropods (*Z. samiplicutus*, *S. japonica*) and Goby (*Yongeichthys nebulosus*). Column: C_{18} (200 × 4.0 mm i.d.). Mobile phase: 2 mmol/L 1-heptane sulfonate in methanol : 0.05 mol/L potassium

phosphate (pH 7.0; 1 : 99). Post-column derivatisation with 3 N sodium hydroxide (1 : 1) and heating at 99° for 0.4 min. Fluorescence detection ($\lambda_{ex} = 381$ nm, $\lambda_{em} = 505$ nm). Retention times: TTX 11 min, anhydroTTX 14.5 min. Limit of quantification not reported [Lin *et al.* 1999; Sui *et al.* 2002]. Gastropods and Goby. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L heptanesulfonic acid in 0.05 mol/L potassium phosphate buffer (pH 7.0), flow rate 0.5 mL/min. Post-column derivatisation with 4 mol/L sodium hydroxide (1 : 1). Fluorescence detection ($\lambda_{ex} = 381$ nm, $\lambda_{em} = 505$ nm). Retention times: TTX 13.0 min, anhydroTTX 16.5 min. Limit of detection, 0.2 µg/g [Chen *et al.* 2002]. Puffer Fish Tissue. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 3% acetonitrile : 0.045 mol/L ammonium heptafluorobutyrate : 0.05 mol/L ammonium acetate buffer (pH 5.0). Post-column derivatisation with 4 mol/L sodium hydroxide at 105°. Fluorescence detection ($\lambda_{ex} = 365$ nm, $\lambda_{em} = 510$ nm). Limit of detection, TTX 0.2 µg/g, 4-*epi*TTX 0.3 µg/g, 4,9-anhydroTTX 0.4 µg/g [Nuñez-Vázquez *et al.* 2000].

LC-MS Puffer Fish (*F. poecilonotus*) Eggs, Newt (*Cynops ensicauda*) Skin and Puffer Fish (*Fugu pardalis*) Tissues. Column: TSKgel Amide-80 (150 × 2.0 mm i.d., 5 µm). Mobile phase: 16 mmol/L ammonium formate buffer (pH 5.5) : acetonitrile (3 : 7), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times: TTX 16.3 min, 5,6,11-trideoxyTTX 5.6 min, 5-deoxyTTX 10.3 min, 11-deoxyTTX 11.0 min, 4,9-anhydroTTX 11.4 min, 11-norTTX-6(*S*)-ol 12.8 min, tetrodonic acid 13.4 min, 4-epiTTX 14.5 min, 11-norTTX-6(*R*)-ol 16.6 min, 6-epiTTX 17.8 min. Limit of detection, 10 pmol/L [Jang, Yotsu-Yamashita 2006; Nakagawa *et al.* 2006]. Mouse Serum. Column: Shodex RSpak NN-414 (150 × 4.6 mm i.d.). Mobile phase: 20 mmol/L ammonium acetate (pH 6.8) : acetonitrile (90 : 10), flow rate 0.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 18.6 min. Limit of quantification, 1 µg/L; limit of detection, 0.5 µg/L [Hayashida *et al.* 2004]. Puffer Fish Liver and Muscles. Column: Shodex RSpak NN-414 (150 × 4.6 mm i.d.). Mobile phase: 20 mmol/L ammonium acetate : methanol (75 : 25), flow rate 0.5 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention time: 13.5 min. Limit of quantification, 0.01 mg/L [Horie *et al.* 2002]. Puffer Fish samples. Column: Develosil C30 UG-5 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 1% acetonitrile : 20 mmol/L ammonium heptafluorobutyrate : 10 mmol/L ammonium formate (pH 4.0), flow rate 0.4 mL/min. ESI, positive ion mode, SIM acquisition mode. Retention times: TTX 17.4 min, 5-deoxyTTX 29.3 min, 11-deoxyTTX 22.5 min, 4,9-anhydroTTX 24.4 min, 11-norTTX-6(*S*)-ol 20.3 min, 4-epiTTX 21.3 min, 11-norTTX-6(*R*)-ol 23.5 min, 6-epiTTX 18.3 min. Limit of quantification, 50 pmol/L [Shoji *et al.* 2001].

Note A mouse bioassay of TTX has been developed and is the current Japanese official method. It defines one mouse unit (MU) as the amount of TTX required to kill a 20 g male mouse of the ddy strain in 30 min. One MU is presumed to be equivalent to 0.22 µg TTX [Horie *et al.* 2002]. For the detection of TTX in postmortem material by gas chromatography, see [Suenaga and Kotoku 1980].

Distribution in the Body

Toxicity TTX binds to the sodium channel of nerves, preventing the passage of sodium ions through the cell membrane and therefore the propagation of an action potential. Exposure typically comes from eating TTX-containing sea- and freshwater animals. Onset of symptoms occurs between 20 min and 3 h and include tingling, numbness, weakness, light-headedness, and limp paralysis, leading to dyspnoea, cyanosis, cardiac arrhythmia, and death. There is no known antidote. LD_{50} in mice 8.7 µg/kg (IV), 10 µg/kg (IP).

Detection of TTX from the urine (100 mL) of a poisoned patient was reported as 9.6 µg 1 day after ingestion of contaminated seafood. Five days later, the amount of TTX detected in 100 mL of urine had fallen to 0.6 µg. In another case, 0.8 µg TTX was detected in 100 mL urine 1 day after ingestion of contaminated seafood, which fell to 0.6 µg 2 days later. In a third case, TTX amounts in 100 mL urine were 7.4, 1.3, 0.6 and 0.7 µg at 1, 5, 6 and 7 days post-ingestion of contaminated seafood [Kawatsu *et al.* 1999].

Serum and urine concentrations for 7 patients with puffer fish poisoning were reported as follows:

Age (years)/sex	Serum TTX (µg/L)	Urine TTX (µg/L)
33/M	–	28
47/F	<5	258
39/M	<5	125
41/M	<5	132
35/F	<5	–
47/M	<5	199
50/M	5	143

Serum samples were collected in hospital ≈24 h after ingestion. Urine samples were single collections and no information on total urine collected over a period of time was available [O'Leary *et al.* 2004].

Six fishermen were victims (including 1 death) of food poisoning from an unknown fish in central Taiwan Strait. Estimated fish ingested and TTX urine and blood levels were reported in 4 of the patients as follows:

Age (years)	Estimated fish (g)	Urine TTX (nmol/L)	Blood TTX (nmol/L)
45	90	325	40.6
44	70	190	21.1
49	100	344	28.6
38	15	47	4.5

Urine and blood specimens were collected ≈10 h after ingestion. Urine levels were higher than blood levels, suggesting that TTX is easily metabolised and excreted in urine [Tsai *et al.* 2006].

A 54-year-old man, found dead 3 to 4 h after ingesting 2 small portions of liver and milt of a puffer fish had a stomach content of 0.053 µg/g TTX. However, no toxin was detected in his urine or serum [Moriya *et al.* 1992].

Chen CY *et al.* (2002). Detection of tetrodotoxin by HPLC in shellfishes and goby from south Taiwan. *J Nat Toxins* 11: 63–68.

Hayashida M *et al.* (2003). A column-switching LC/MS/ESI method for detecting tetrodotoxin and *Aconitum* alkaloids in serum. *Leg Med (Tokyo)* 5: 1S101–S104.

Hayashida M *et al.* (2004). Sensitive determination of tetrodotoxin using column-switching liquid chromatography–mass spectrometry with electrospray ionization in mouse serum. *J Anal Toxicol* 28: 46–49.

Horie M *et al.* (2002). Determination of tetrodotoxin in puffer-fish by liquid chromatography–electrospray ionization mass spectrometry. *Analyst* 127: 755–759.

Ikebuchi J *et al.* (1988). Thin-layer chromatography with flame ionization detection for the determination of tetrodotoxin in biological fluids. *J Chromatogr* 432: 401–406.

Jang J, Yotsu-Yamashita M (2006). Distribution of tetrodotoxin, saxitoxin, and their analogs among tissues of the puffer fish *Fugu pardalis*. *Toxicon* 48: 980–987.

Kawatsu K *et al.* (1999). Application of immunoaffinity chromatography for detection of tetrodotoxin from urine samples of poisoned patients. *Toxicon* 37: 325–333.

Lin SJ *et al.* (1999). Acute goby poisoning in southern Taiwan. *J Nat Toxins* 8: 141–147.

Moriya F *et al.* (1992). The use of mass fragmentography for the detection of tetrodotoxin in human body fluids. *Nihon Hoigaku Zasshi* 46: 117–120.

Nakagawa T *et al.* (2006). Hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry of tetrodotoxin and its analogs. *Anal Biochem* 352: 142–144.

Nuñez-Vázquez EJ *et al.* (2000). Toxicities and distribution of tetrodotoxin in the tissues of puffer fish found in the coast of the Baja California Peninsula, Mexico. *Toxicon* 38: 729–734.

O'Leary MA *et al.* (2004). Use of high performance liquid chromatography to measure tetrodotoxin in serum and urine of poisoned patients. *Toxicon* 44: 549–553.

Oliveira JS *et al.* (2006). Toxicity and toxin identification in *Colomesus asellus*, an Amazonian (Brazil) freshwater puffer fish. *Toxicon* 48: 55–63.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck Research Laboratories.

Shoji Y *et al.* (2001). Electrospray ionization mass spectrometry of tetrodotoxin and its analogs: liquid chromatography/mass spectrometry, tandem mass spectrometry, and liquid chromatography/tandem mass spectrometry. *Anal Biochem* 290: 10–17.

Suenaga K, Kotoku S (1980). Detection of tetrodotoxin in autopsy material by gas chromatography. *Arch Toxicol* 44: 291–297.

Sui LM *et al.* (2002). Identification of tetrodotoxin in marine gastropods implicated in food poisoning. *J Nat Toxins* 11: 213–220.

Tsai YH *et al.* (2006). Determination of tetrodotoxin in human urine and blood using C_{18} cartridge column, ultrafiltration and LC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 832: 75–80.

Wu Z *et al.* (2005). Toxicity and distribution of tetrodotoxin-producing bacteria in puffer fish *Fugu rubripes* collected from the Bohai Sea of China. *Toxicon* 46: 471–476.

Yotsu-Yamashita M *et al.* (2007). Distribution of tetrodotoxin in the body of the blue-ringed octopus (*Hapalochlaena maculosa*). *Toxicon* 49: 410–412.

Tetryzoline

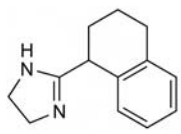
Sympathomimetic

$C_{13}H_{16}N_2 = 200.3$

CAS—84-22-0

IUPAC Name 2-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*- imidazole

Synonyms 4,5-Dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-1*H*-imidazole; tetrahydrozoline.



Chemical Properties A white crystalline powder. Soluble in water and chloroform. Log *P* (octanol/water) 3.7.

Tetryzoline Hydrochloride

$C_{13}H_{16}N_2.HCl = 236.7$

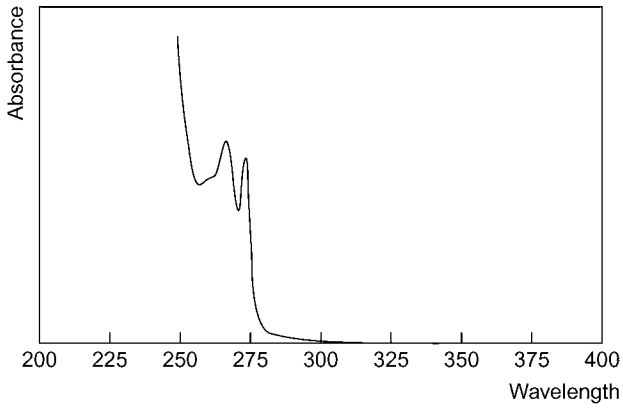
CAS—522-48-5

Proprietary Names *Azoline*; *Caltheon*; *Constrilia*; *Demetil*; *Diabenyl T*; *Edolzine*; *Exrhinin*; *Mallazine*; *Octilia*; *Ophtalmin N*; *Optazine Fresh*; *Optizoline*; *Rhinopront*; *Stilla*; *Tetrasine*; *Tetrilin*; *Tyzine*; *Vasopos N*; *Visine*; *Vispring*; *V-Zoline*; *Yxin*.

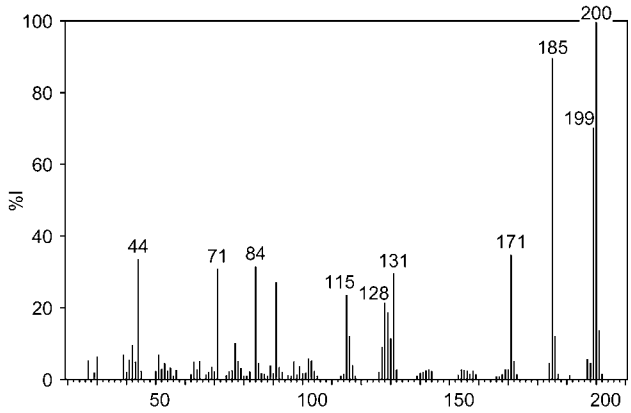
Chemical Properties A white crystalline powder. Mp about 256° to 257°, with decomposition. Soluble 1 in 3.5 of water and 1 in 7.5 of ethanol; very slightly soluble in chloroform; practically insoluble in ether.

Colour Tests Aromaticity (method 2)—colourless/yellow; Liebermann's reagent—brown-orange.

Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 0.07; system TC—R_f 0.02; system TE—R_f 0.26; system TL—R_f 0.02; system TAE—R_f 0.05; system TAF—R_f 0.60 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1833.
Ultraviolet Spectrum Aqueous acid—265 (A_1^1=21a), 272 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1600, 742, 1293, 1250, 763, 1500 cm^{-1} (tetryzoline hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 200, 185, 199, 171, 44, 84, 71, 131.



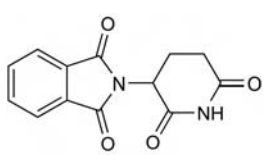
Use Tetryzoline hydrochloride is used as a 0.1% nasal solution.

Thalidomide

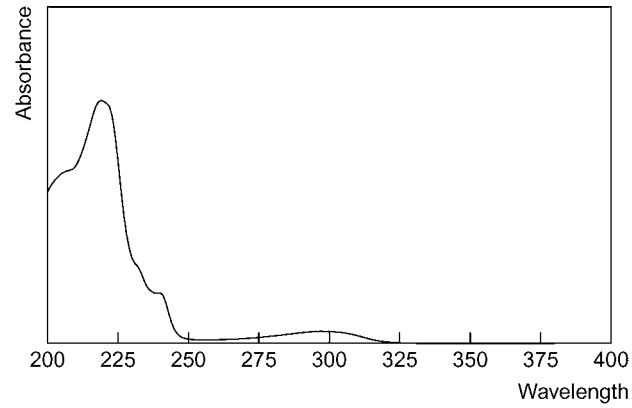
Hypnotic, Immunosuppressant
$C_{13}H_{10}N_2O_4$ = 258.2
CAS—50-35-1

T

IUPAC Name 2-(2,6-Dioxopiperidin-3-yl)isoindole-1,3-dione
Synonyms 2-(2,6-Dioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione; E-217; K-17; NSC-66847; talidomid.
Proprietary Name *Thalomid*



Chemical Properties A white crystalline powder. Mp 269° to 271°. Sparingly soluble in water, methanol, ethanol and acetone; practically insoluble in chloroform, ether and benzene; very soluble in dimethylformamide, dioxan and pyridine. Log *P* (octanol/water), 0.3. Stable in buffered non-extracted plasma and semen samples at room temperature for 24 h, following 3 freeze-thaw cycles and when stored at 4° for 24 h [Teo *et al.* 2002].
Ultraviolet Spectrum Aqueous acid—240 (A_1^1= 385b), 300 nm (A_1^1= 94b).



Infrared Spectrum Principal peaks at wavenumbers 1681, 727, 1205, 1250, 1316, 1110 cm^{-1} (Nujol mull) .
Mass Spectrum Principal ions at *m/z* 76, 173, 104, 111, 148, 50, 169, 130.
Quantification
Blood **HPLC** Column: Chiralpak AD-RH (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : 0.025 mol/L citrate buffer (pH 3.0, 10 : 20 : 70), flow rate 0.5 mL/min. UV detection (λ= 220 nm). Limit of quantification, 0.025 mg/L [Sembongi *et al.* 2008].
Plasma **HPLC** Column: BDS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 5.5) : acetonitrile (75 : 25), flow rate 1.5 mL/min. UV detection (λ= 220 nm). Limit of detection, 0.05 mg/L [Saccomanni *et al.* 2008]. Column: Spherisorb ODS-II (100 × 5 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile (4 : 1) 29.2% in water, flow rate 3.0 mL/min. UV detection (λ= 225 nm). Limit of detection, 0.2 mg/L [Boughton *et al.* 1995].

LC-MS Column: ODS C_{18} (35 × 3.2 mm i.d., 3 µm). Mobile phase: water : acetonitrile : acetic acid (75 : 25 : 0.1), flow rate 0.5 mL/min. APCI, negative ion mode. Limit of quantification, 7 µg/L [Teo *et al.* 2002].
Serum **HPLC** Column: Chirobiotic V (250 × 4 mm i.d.). Mobile phase: acetonitrile : 20 mmol/L ammonium formate (pH 5.4, 14 : 86), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Limit of quantification, 0.05 mg/L for thalidomide enantiomers [Murphy-Poulton *et al.* 2006]. Column: Supelco C_{18} (125 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate (pH 3.0) : methanol (25 : 75), flow rate 1.5 mL/min. DAD (λ= 220 nm). Limit of quantification, 0.222 mg/L [Torano *et al.* 1999]. Column: LiChrosorb RP-8 (125 × 4.6 mm i.d., 5 µm) or Spherisorb S5 C_{18} (126 × 4.6 mm i.d.). Mobile phase: water : methanol : propan-1-ol (180 : 30 : 20), flow rate 0.9 mL/min or water : acetonitrile (80 : 20), flow rate 1.5 mL/min. UV detection, (λ= 254 or 290 nm). Limit of detection, 1 mg/L [Czejka, Koch 1987].
Semen **LC-MS** See Plasma. Limit of quantification, 8 ng/g [Teo *et al.* 2002].
Tissues **HPLC** See Serum [Murphy-Poulton *et al.* 2006].
Disposition in the Body Thalidomide is absorbed from the gastrointestinal tract. It is hydrolysed in the body, forming mainly 2-(*o*-carboxybenzamido)glutarimide, although many other hydrolysis products are also detectable.
Therapeutic Concentration

Forty-five patients with refractory multiple myeloma were treated with 100 mg thalidomide daily for 1 week, then with 200 mg daily. Mean plasma thalidomide trough concentrations were 0.343 and 0.875 mg/L on the third day after treatment with 100-mg and 200-mg doses, respectively [Kodama *et al.* 2009].

Patients with erythema nodosum leprosum treated with 100 mg thalidomide daily had plasma levels of 0.82–1.03 mg/L during treatment [Vieira, Valente 2009].

Peak levels of 1.15 mg/L were achieved in healthy subjects at 4.39 h after a single dose of 200 mg thalidomide [Chen *et al.* 1989].

Toxicity Thalidomide has a low acute toxicity; in 2 cases of reported overdose there was no evidence of respiratory or cardiac depression. Thalidomide has teratogenic effects when administered to women early in pregnancy.
Half-life Absorption half-life 1.7 h. Elimination half-life reported as 7.3 h and 8.7 h [Chung *et al.* 2004].
Volume of Distribution 120.7 L [Chen *et al.* 1989].
Clearance Renal clearance 0.08 L/h (0.6% of dose); therefore, major route of elimination is non-renal [Chen *et al.* 1989]
Note For a review of the clinical pharmacokinetics of thalidomide, see Teo *et al.* [2002].
Dose Thalidomide 50 to 400 mg daily in the treatment of lepra reactions; up to 800 mg daily in the treatment of multiple myeloma.

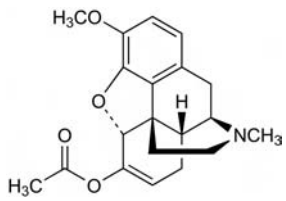
Boughton BJ *et al.* (1995). High-performance liquid chromatographic assay of plasma thalidomide: stabilization of specimens and determination of a tentative therapeutic range for chronic graft-versus-host disease. *Ann Clin Biochem* 32(Pt1): 79–83.
Chen TL *et al.* (1989). Plasma pharmacokinetics and urinary excretion of thalidomide after oral dosing in healthy male volunteers. *Drug Metab Dispos* 17: 402–405.

Chung F *et al.* (2004). Thalidomide pharmacokinetics and metabolite formation in mice, rabbits, and multiple myeloma patients. *Clin Cancer Res* 10: 5949–5956.
Czejka MJ, Koch HP (1987). Determination of thalidomide and its major metabolites by high-performance liquid chromatography. *J Chromatogr* 413: 181–187.
Kodama T *et al.* (2009). A pharmacokinetic study evaluating the relationship between treatment efficacy and incidence of adverse events with thalidomide plasma concentrations in patients with refractory multiple myeloma. *Clin Lymphoma Myeloma* 9: 154–159.
Murphy-Poulton SF *et al.* (2006). Thalidomide enantiomers: determination in biological samples by HPLC and vancomycin-CSP. *J Chromatogr B Analyt Technol Biomed Life Sci* 831: 48–56.
Saccomanni G *et al.* (2008). High performance liquid chromatographic determination of thalidomide in patients affected by hepatocellular carcinoma. *J Pharm Biomed Anal* 48: 447–451.
Sembongi K *et al.* (2008). A new method for determination of both thalidomide enantiomers using HPLC systems. *Biol Pharm Bull* 31: 497–500.
Teo SK *et al.* (2002). Sensitive and rapid method for the determination of thalidomide in human plasma and semen using solid-phase extraction and liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 767: 145–151.
Torano JS *et al.* (1999). Quantitative determination of thalidomide in human serum with high-performance liquid chromatography using protein precipitation with trichloroacetic acid and ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 734: 203–210.
Vieira JL, Valente MS (2009). Thalidomide levels in patients with erythema nodosum leprosum. *Ther Drug Monit* 31: 602–603.

Thebacon

Narcotic Analgesic
$C_{20}H_{23}NO_4 = 341.4$
CAS—466-90-0

Synonyms Acethydrocodone; acetyldihydrocodeinone; (5α)-6,7-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol acetate; dihydrocodeinone enol acetate.

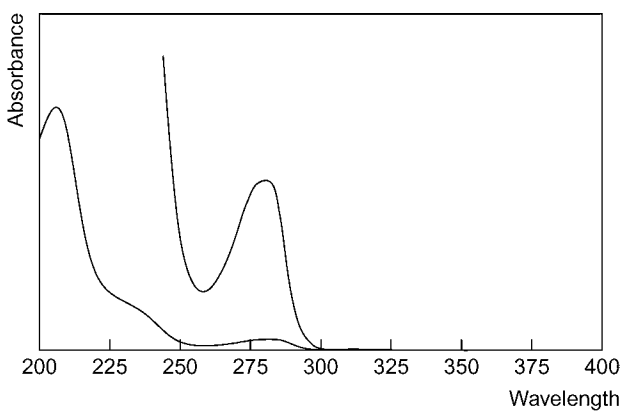


Chemical Properties White powder. Mp 154°. Practically insoluble in water; soluble in ethanol, chloroform and ether. Log *P* (octanol/water), 2.4.

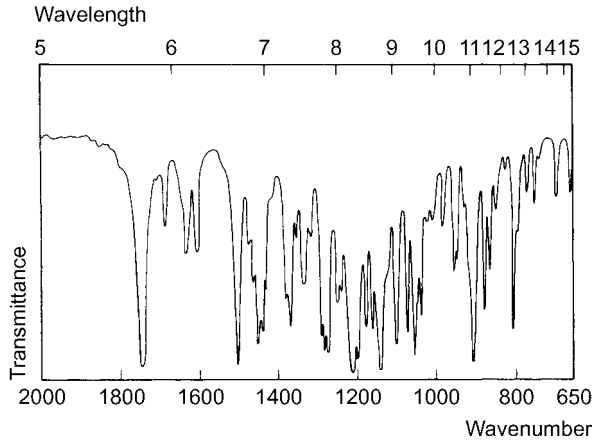
Thebacon Hydrochloride

$C_{20}H_{23}NO_4$,HCl = 377.9
CAS—20236-82-2
Proprietary Name *Acedicone*
Chemical Properties White crystals. Mp 132° to 135°, with decomposition. Soluble in water.

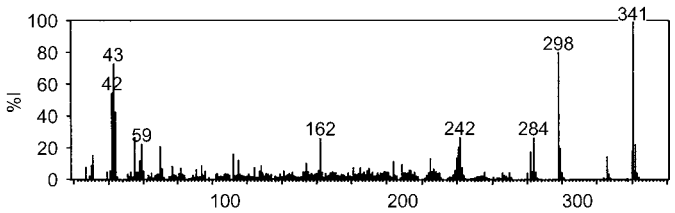
Colour Test Marquis test—yellow→violet.
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.20; system TC—R_f 0.34; system TE—R_f 0.49; system TL—R_f 0.11; system TAE—R_f 0.24; system TAF—R_f 0.25 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2533; system GB—RI 2559. Also metabolised to dihydrocodeine.
High Performance Liquid Chromatography System HA—*k* 3.7 (tailing peak); system HC—*k* 0.85; system HX—RI 333.
Ultraviolet Spectrum Aqueous acid—281 nm (A^1_1 = 41b).



Infrared Spectrum Principal peaks at wavenumbers 1208, 1138, 1744, 1500, 905, 1194 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 341, 298, 43, 44, 55, 284, 242.

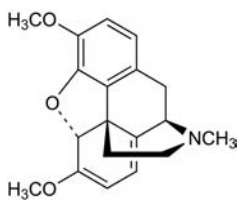


Dose Usually 10 mg of thebacon hydrochloride daily.

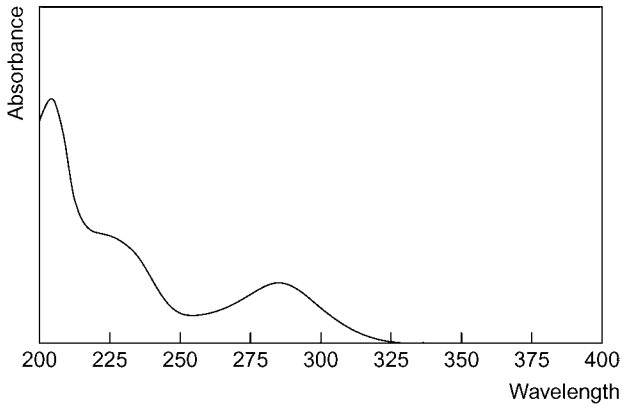
Thebaine

Alkaloid
$C_{19}H_{21}NO_3 = 311.4$
CAS—115-37-7

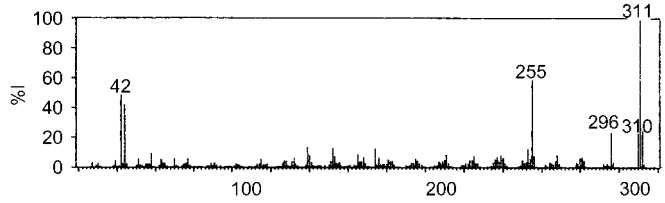
Synonyms Paramorphine; (5α)-6,7,8,14-tetradehydro-4,5-epoxy-3,6 dimethoxy-17-methylmorphinan.



Chemical Properties An alkaloid present in opium. A white powder. Mp 193°. Soluble 1 in 1460 of water, 1 in about 15 of hot ethanol, 1 in 13 of chloroform, 1 in 200 of ether and 1 in 25 of benzene. pK_a 6.1 (15°); 8.2 (20°). Log *P* (octanol/buffer pH 7.5), 0.3; (octanol/water), 2.0.
Colour Tests Mandelin's test—orange→brown; Marquis test—red→orange.
Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.24; system TC—R_f 0.37; system TE—R_f 0.45; system TL—R_f 0.05; system TAE—R_f 0.23; system TAF—R_f 0.32; system TAJ—R_f 0.22; system TAK—R_f 0.11; system TAL—R_f 0.76 (Dragendorff spray, positive; FPN reagent, faint brown; acidified iodoplatinate solution, positive; Marquis reagent, yellow).
Gas Chromatography System GA—RI 2517.
High Performance Liquid Chromatography System HA—*k* 4.6 (tailing peak); system HC—*k* 0.9; system HS—*k* 0.80; system HX—RI 340; system HY—RI 276.
Ultraviolet Spectrum Aqueous acid—284 nm (A^1_1=253a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1234, 1605, 1144, 1270, 1030, 910 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 311, 255, 42, 44, 296, 310, 312, 174.

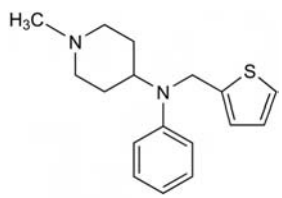


Quantification
Urine **GC-MS** Limit of detection, 2 to 81 µg/L [Cassella *et al.* 1997].
Hair **GC-MS** Thebaine and drugs of abuse [Achilli *et al.* 1996].

Achilli G *et al.* (1996). Determination of illicit drugs and related substances by high-performance liquid chromatography with an electrochemical coulometric-array detector. *J Chromatogr A* 729: 273–277.
Cassella G *et al.* (1997). The analysis of thebaine in urine for the detection of poppy seed consumption. *J Anal Toxicol* 21: 376–383.

Thenalidine

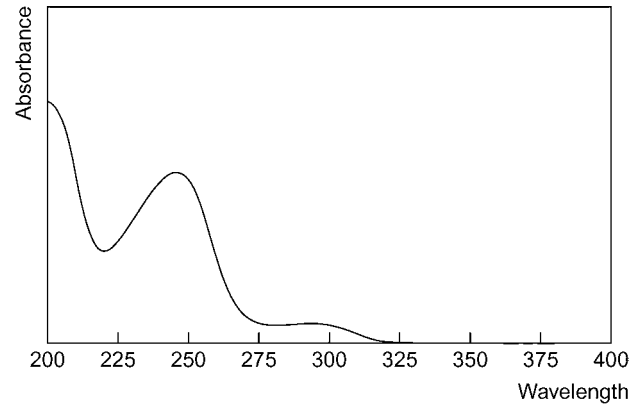
Antihistamine
$C_{17}H_{22}N_2S = 286.4$
CAS—86-12-4
IUPAC Name 1-Methyl-*N*-phenyl-*N*-(thiophen-2-ylmethyl)piperidin- 4-amine
Synonyms Thenaldine; thenophenopiperidine; thenopiperidine.



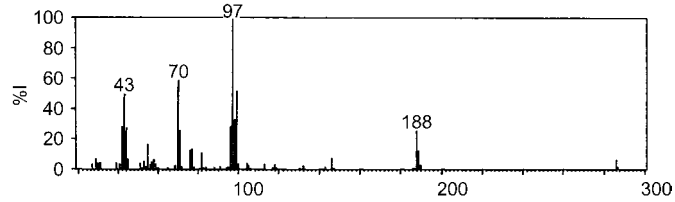
Chemical Properties Mp 95° to 97°. Practically insoluble in water; soluble in chloroform. Log *P* (octanol/water), 3.9.

Thenalidine Tartrate
$C_{17}H_{22}N_2S,C_4H_6O_6 = 436.5$
CAS—16509-35-6
Chemical Properties A white crystalline powder. Mp 170° to 172°. Soluble in water.

Colour Tests Mandelin's test—orange→brown; Marquis test—grey-violet.
Thin-layer Chromatography System TA—R_f 0.50; system TB—R_f 0.38; system TC—R_f 0.44; system TE—R_f 0.52; system TL—R_f 0.12; system TAE—R_f 0.20 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2318.
High Performance Liquid Chromatography System HA—*k* 3.5.
Ultraviolet Spectrum Aqueous acid—234 nm (A_1^1=280b).



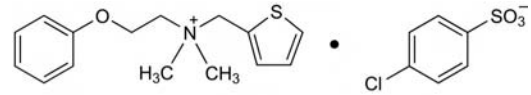
Infrared Spectrum Principal peaks at wavenumbers 1505, 748, 1600, 693, 1279, 755 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 97, 70, 99, 43, 98, 44, 42, 188.



Dose Thenalidine tartrate has been given in doses of 100 to 150 mg daily.

Thenium Closilate

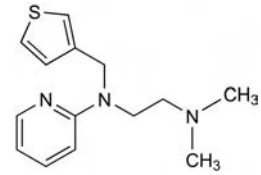
Anthelmintic (Veterinary)
$C_{21}H_{24}ClNO_4S_2 = 454.0$
CAS—16776-64-0 (thenium); 4304-40-9 (closilate)
IUPAC Name 4-Chlorobenzenesulfonate; dimethyl-(2-phenoxyethyl)-(thiophen-2-ylmethyl)azanium
Synonyms 611CSS; *N*,*N*-dimethyl-*N*-(2-phenoxyethyl)-2-thiophenemethanaminium salt with 4-chlorobenzenesulfonic acid (1 : 1); thenium closylate.



Chemical Properties A white crystalline powder. Mp 159° to 160°. Soluble 1 in 200 of water, 1 in 25 of ethanol and 1 in 35 of chloroform.
Colour Tests Mandelin's test—green; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—268 (A_1^1=45b), 275 nm (A_1^1=36b).
Infrared Spectrum Principal peaks at wavenumbers 1210, 1174, 1042, 1135, 760, 1010 cm^{-1} (KBr disk).

Thenyldiamine

Antihistamine
$C_{14}H_{19}N_3S = 261.4$
CAS—91-79-2
IUPAC Name *N*,*N*-Dimethyl-*N*′-pyridin-2-yl-*N*′-(thiophen-3-ylmethyl)ethane-1,2-diamine



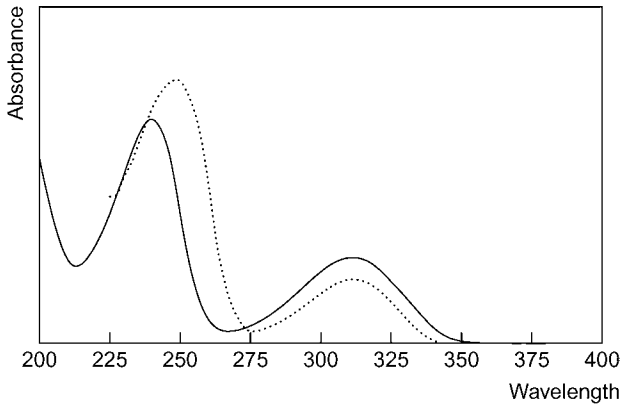
Chemical Properties A liquid. Practically insoluble in water; soluble in chloroform. pK_a 3.9, 8.9 (25°). Log *P* (octanol/water), 2.6.

Thenyldiamine Hydrochloride
$C_{14}H_{19}N_3S,HCl = 297.8$
CAS—958-93-0

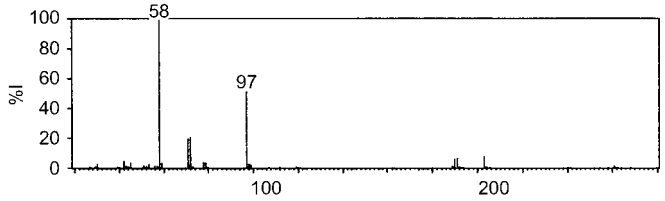
T

Proprietary Names It is an ingredient of *Asafen*, *NTR* and *Sinefricol*.
Chemical Properties A white crystalline powder. Mp about 170°. Soluble 1 in 5 of water, 1 in 5 of ethanol and 1 in 5 of chloroform; practically insoluble in ether.

Colour Tests Mandelin's test—green; Marquis test—black→violet.
Thin-layer Chromatography System TA—R_f 0.53; system TL—R_f 0.12; system TB—R_f 0.42; system TC—R_f 0.25; system TE—R_f 0.65; system TAE—R_f 0.21; system TAF—R_f 0.36 (Dragendorff spray, positive; Acidified iodoplatinate solution, positive; Marquis reagent, violet).
Gas Chromatography System GA—RI 1999; system GB—RI 2034; system GC—RI 2300; system GF—RI 2340.
High Performance Liquid Chromatography System HA—*k* 4.0; system HX—RI 317.
Ultraviolet Spectrum Aqueous acid—239 (A^1_1=703a), 315 nm; aqueous alkali—246, 310 nm.



Infrared Spectrum Principal peaks at wavenumbers 1597, 766, 1242, 976, 1159, 1311 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 58, 97, 72, 71, 203, 191, 190, 42.

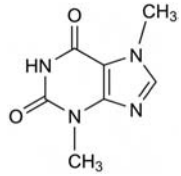


Dose Thenyldiamine hydrochloride has been given in doses of up to 90 mg daily.

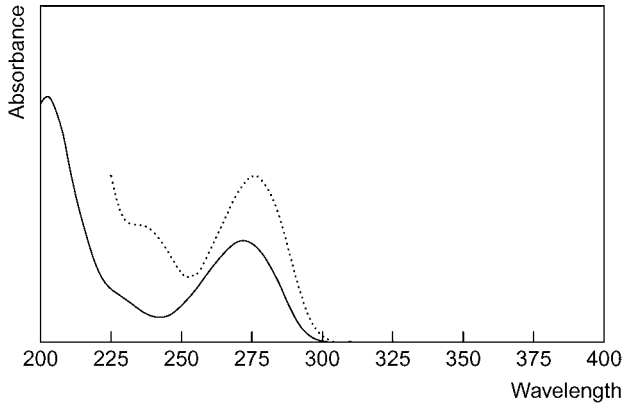
Theobromine

Xanthine Derivative
$C_7H_8N_4O_2$ = 180.2
CAS—83-67-0

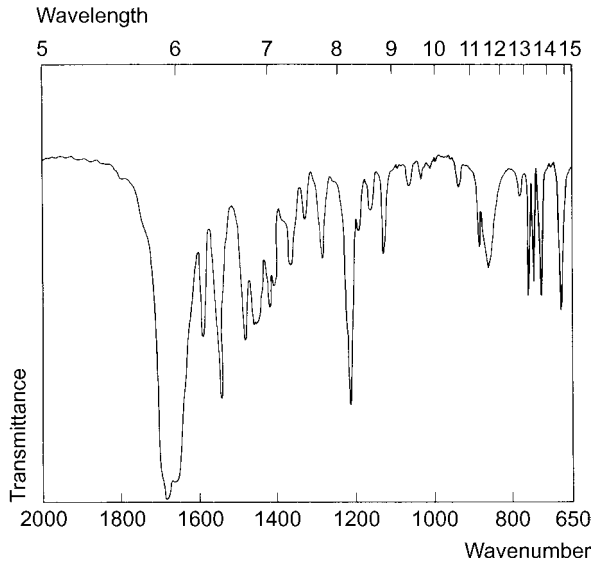
IUPAC Name 3,7-Dimethylpurine-2,6-dione
Synonyms 3,7-Dihydro-3,7-dimethyl-(1*H*)-purine-2,6-dione; 3,7-dimethylxanthine; santheose; theobrominum.
Proprietary Names It is an ingredient of *Asthma-Hilfe*, *Propyre T* and *Urodonal*.



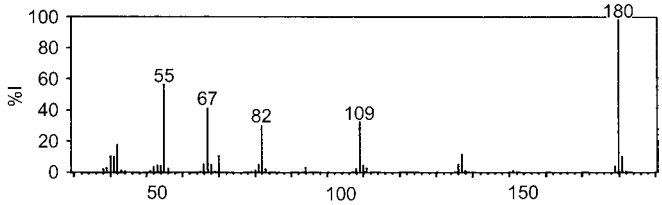
Chemical Properties An alkaloid contained in the seeds of *Theobroma cacao* (Sterculiaceae). A white microcrystalline powder which sublimes at 290° to 295°. Soluble 1 in 2000 of water, 1 in 2220 of 95% ethanol and 1 in 6000 of chloroform; almost insoluble in benzene, carbon tetrachloride and ether; freely soluble in dilute mineral acids and aqueous solutions of alkali hydroxides. pK_a <1, 10.0 (25°). Log *P* (octanol/water), −0.8. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Colour Test Amalic acid test—yellow/violet.
Thin-layer Chromatography System TA—R_f 0.53; system TB—R_f 0.01; system TC—R_f 0.31; system TE—R_f 0.34; system TF—R_f 0.04; system TG—R_f 0.47; system TL—R_f 0.21; system TAE—R_f 0.59; system TAF—R_f 0.54; system TAJ—R_f 0.32; system TAK—R_f 0.08; system TAL—R_f 0.65 (Ludy Tenger reagent, orange).
Gas Chromatography System GA—RI 1807; system GB—RI 1920.
High Performance Liquid Chromatography System HA—*k* 0.11; system HX—RI 262; system HY—RI 201; system HZ—retention time 1.6 min; system HAA–retention time 3.8 min.
Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=563a); aqueous alkali—274 nm.



Infrared Spectrum Principal peaks at wavenumbers 1690, 1665, 1221, 1550, 1595, 680 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 180, 55, 67, 109, 82, 42, 137, 70; 166, 68, 95, 41, 53, 123, –, – (3-methylxanthine); 166, 68, 123, 53, 42, 41, 95, – (7-methylxanthine).



Disposition in the Body Well absorbed after oral administration. It is metabolised by demethylation, mainly to 7-methylxanthine, together with some 3-methylxanthine. The 7-methylxanthine is further oxidised to 7-methyluric acid. About 90% of a dose is excreted in the urine in 48 h, of which ≈40% is 7-methylxanthine, 20% is 3-methylxanthine, 11% is 7-methyluric acid and 15% is unchanged drug. An additional metabolite, 6-amino-5-(*N*-methylformylamino)-1-methyluracil, accounts for ≈7% of a dose.

Theobromine is a metabolite of caffeine.

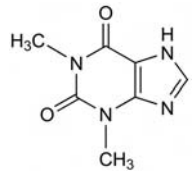
Half-life Plasma half-life, 5–11 h (mean, 8) after a single dose, increased on chronic administration.
Volume of Distribution About 0.5–1 L/kg.
Clearance Plasma clearance, about 0.8 mL/min/kg.
Saliva Plasma : saliva ratio, ≈1.0.
Dose 300 to 600 mg.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Theophylline

Xanthine Bronchodilator
$C_7H_8N_4O_2$ = 180.2
CAS—58-55-9

IUPAC Name 1,3-Dimethyl-7*H*-purine-2,6-dione
Synonyms Anhydrous theophylline; 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione; 1,3-dimethylxanthine; teocina; teofilina; theophyllinum.
Proprietary Names *Accurbron*; *Aerolate*; *Aquaphyllin*; *Asmalix*; *Bronkodyl*; *Bronchoretard*; *Elixomin*; *Elixophyllin*; *Etheophyl*; *Euphylline*; *Euphylong*; *Lasma*; *Nuelin* (tablets); *Quibron-T*; *Respbid*; *Slo-Bid*; *Slo-Phyllin*; *Solosin*; *Sustaire*; *Teosona*; *Theo-24*; *Theobid*; *Theochron*; *Theoclear*; *Theo-Dur*; *Theograd*; *Theolair*; *Theon*; *Theospan*; *Theostat*; *Theovent*; *Theo-X*; *T-Phyl*; *Uni-Dur*; *Uniphyl*; *Uniphyllin Continus* (includes proprietary names of the hydrate). It is an ingredient of *Elixophyllin-GG*, *Elixophyllin-KI*, *Franol*, *Franol Plus*, *Franolyn Expectorant*, *Franyl*, *Glyceryl-T*, *Hydrophed*, *Marax*, *Neoasma*, *Quadrinal*, *Quibron*, *Slo-Phyllin GG*, *Tedrigen*, *Theodrine* and *Theomax DF*.



Chemical Properties A white crystalline powder. Mp 270° to 274°. Soluble 1 in 120 of water, 1 in 80 of ethanol and 1 in 110 of chloroform; sparingly soluble in ether; soluble in dilute acids, ammonia and alkali hydroxide solutions. pK_a <1, 8.6 (25°). Log *P* (octanol/water), −0.02. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Aminophylline

$(C_7H_8N_4O_2)_2,C_2H_4(NH_2)_2,2H_2O$ = 456.5 (composition approximately corresponds to this formula)
CAS—317-34-0 (anhydrous)
IUPAC Name 1,3-Dimethyl-7*H*-purine-2,6-dione; ethane-1,2-diamine
Synonyms Euphyllinum; metaphyllin; theophyllaminum; theophylline and ethylenediamine; theophylline ethylenediamine compound.
Proprietary Names *Aminiodur*; *Aminocont*; *Aminomal*; *Amnivent*; *Cardophylin*; *Clonofilin*; *Elixophyllin*; *Escophylline*; *Euphyllin(a)*; *Filotempo*; *Mundiphyllin*; *Norphyllin SR*; *Pecram*; *Phyllocontin*; *Phyllotemp*; *Planphylline*; *Tefamin*; *Teofylamin*; *Truphylline*. It is an ingredient of *Emergent-EzMudrane*, *Mudrane GG*, *Mudrane GG-2* and *Theodrox*.
Chemical Properties A stable mixture containing 78–84% of anhydrous theophylline and 13–14% of ethylenediamine with a variable quantity of water. White or slightly yellowish granules or powder. Soluble 1 in 5 of water at 25°, but the addition of ethylenediamine or ammonia solution may be necessary to give complete solution. Practically insoluble in ethanol and ether.

Choline Theophyllinate

$C_{12}H_{21}N_5O_3$ = 283.3
CAS—4499-40-5
IUPAC Name 1,3-Dimethyl-2-oxopurin-6-olate; 2-hydroxyethyl(trimethyl) azanium
Synonyms Oxytrimeethylline; oxtriphylline; theophylline cholinate.
Proprietary Names *Brondecon Elixir*; *Choledyl*; *Euspirax*; *Rouphylline*; *Sabidal*; *Teovent*.
Chemical Properties A white crystalline powder. Mp 185° to 192°. Soluble 1 in <1 of water and 1 in 10 of ethanol; very slightly soluble in chloroform and ether.

Theophylline Hydrate

$C_7H_8N_4O_2,H_2O$ = 198.2
CAS—5967-84-0
Synonym Theophylline monohydrate
Chemical Properties A white crystalline powder. Mp ~272°, after drying. Soluble 1 in 120 of water and 1 in 80 of ethanol; very slightly soluble in ether; soluble in solutions of alkali hydroxides.

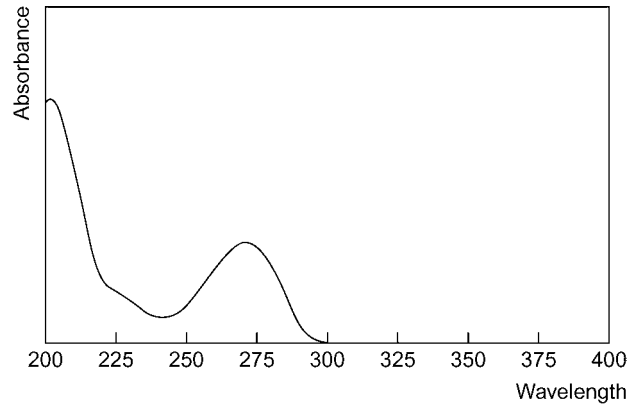
Theophylline Monoethanolamine

$C_7H_8N_4O_2,C_2H_7NO$ = 241.2
CAS—573-41-1
IUPAC Name 2-Aminoethanol; 1,3-dimethyl-7*H*-purine-2,6-dione
Synonyms Monotheamine; theophylline olamine.
Proprietary Names *Inophyline*; *Teosona*.
Chemical Properties An equimolecular compound of anhydrous theophylline and monoethanolamine. A white crystalline powder. Soluble 1 in 20 of water.

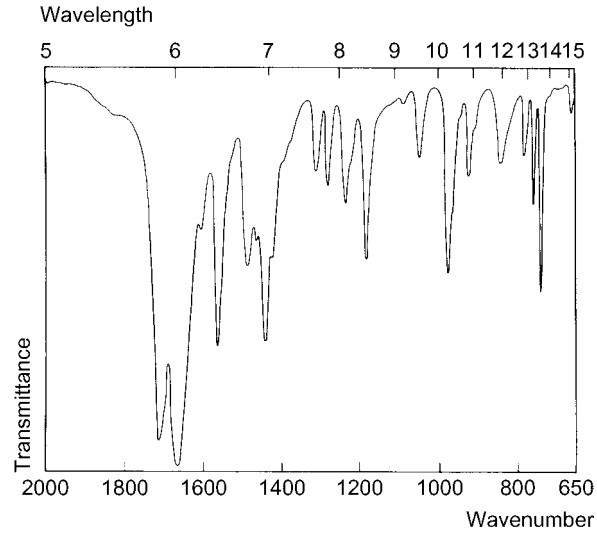
Theophylline Sodium Glycinate

$C_7H_7N_4NaO_2 : C_2H_5NO_2$ = 202.1 : 75.07 (~equimolecular proportions)
CAS—8000-10-0
IUPAC Name Sodium; 2-aminoacetate; 1,3-dimethyl-7*H*-purine-2,6-dione
Synonym Theophylline sodium aminoacetate
Proprietary Name *Nuelin* (liquid)
Chemical Properties An equilibrium mixture of theophylline sodium and glycine in approximately equimolecular proportions, buffered with an additional 1 mol glycine. A white crystalline powder. Soluble 1 in 6 of water; very slightly soluble in ethanol; practically insoluble in chloroform.

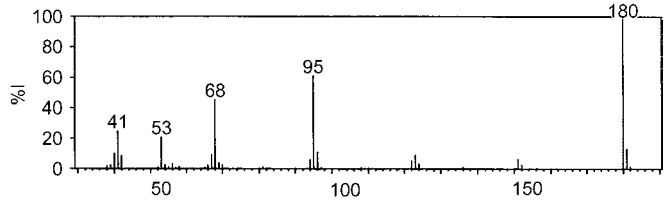
Colour Tests Amalic acid test—yellow/violet; Folin–Ciocalteu reagent—blue.
Thin-layer Chromatography System TA—R_f 0.75; system TB—R_f 0.01; system TC—R_f 0.30; system TE—R_f 0.11; system TF—R_f 0.09; system TG—R_f 0.33; system TL—R_f 0.11; system TAE—R_f 0.74; system TAF—R_f 0.66; system TAJ—R_f 0.40; system TAK—R_f 0.21; system TAL—R_f 0.78 (Ludy Tenger reagent—orange).
Gas Chromatography System GA—RI 2005; system GF—RI 2745.
High Performance Liquid Chromatography System HA—*k* 0.1; system HX—RI 276; system HY—RI 249; system HZ—RT 1.7 min; system HAA—RT 4.9 min; system HAM—RT 1.8 min; system HAX—RT 4.5 min; system HAY—RT 3.5 min.
Ultraviolet Spectrum Aqueous acid—270 nm (A_1^1 = 536a); aqueous alkali—275 nm (A_1^1 = 650a)



Infrared Spectrum Principal peaks at wave numbers 1670, 1717, 1567, 745, 980, 1190 cm^{-1} (KBr disk)



Mass Spectrum Principal ions at *m/z* 180, 95, 68, 41, 53, 181, 96, 40 (theophylline); 166, 68, 95, 41, 53, 123, –, – (3-methylxanthine).



Quantification

Blood **LC-MS** Column: Luna ODS2 (75 × 4.6 mm i.d., 3 µm). Mobile phase: methanol : water (20 : 80), flow rate 0.5 mL/min. SIM acquisition mode. Limit of quantification, 12 µg/L [Watson *et al.* 2001].
Plasma **TLC** Plates: silica. Mobile phase: acetic acid : propan-2-ol : toluene (1 : 12 : 6). Limit of detection, 100 µg/L [Mirfazaelian *et al.* 2002]. Plate: silca gel 60 F254 (0.25 mm). Mobile phase: chloroform : methanol (9 : 1). UV detection (λ= 254 nm). R_f 0.54 Limit of quantification, 20 ng [Devarajan *et al.* 1999].
HPLC Column: Inertsil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate buffer : methanol : acetonitrile (86 : 7 : 7), flow rate 1.0 mL/

min. UV detection (λ= 272 nm). Limit of quantification, 100 µg/L for theophylline and etophylline [Nirogi *et al.* 2007]. Column: TSK gel ODS-80 TM (150 × 4.6 mm i. d., 5 µm). Mobile phase: methanol : 0.01 mol/L phosphate buffer (pH 3.5, 30 : 70). UV detection (λ= 270 nm). Limit of detection, 24.1 µg/L [Emara 2004]. Column: ODS (150 × 6 mm i.d., 5 µm). Mobile phase: 5% acetic acid : acetonitrile-methanol (20 : 80, 80 : 20 for 10 min to 40 : 60 at 15 min to 20 : 80 at 20 min to 10 : 90 at 25 min), flow rate 1 mL/min. UV detection (λ= 280 nm). Retention time: 8 min. Limit of quantification, 0.5 mg/L [Kamberi *et al.* 1999]. Column: ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L acetate buffer (pH 4.0) : methanol (91 : 9), flow rate 1.0 mL/min for 11 min to 1.5 mL/min for 6 min to 2.5 mL/min at 17 min for 13 min. UV detection (λ= 273 nm). Limit of detection, 0.2 µmol/L for theophylline and 0.1 µmol/L for theophylline metabolites (1-methyluric acid, 3-methylxanthine, 1,3 dimethyluric acid) [Rasmussen, Brosen 1996]. Column: RP ODS-2 column packed with Spherisorb C_{18} (150 × 4.6 mm i.d., 5 µm), stainless steel. Mobile phase: acetonitrile : tetrahydrofuran : concentrated acetic acid : water (20 : 20 : 5 : 955), flow rate 1 mL/min. UV detection (λ= 273 nm). Retention time: 5.0 min. Limit of detection, 0.1 mg/L [Schreiber-Deturmeny, Bruguerolle 1996].

LC-MS Column: C_{18}. (150 × 3 mm i.d., 3 µm). Mobile phase: water-methanol (95 : 5) : water-methanol (10 : 90), both containing 0.05% formic acid (100 : 0 to 20 : 80 in 3 min for 2 min to 100 : 0 at 6 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 24.4 µg/L [Zhang *et al.* 2008]. Column: C_{18} (50 × 2.0 mm i.d., 3 µm). Mobile phase: methanol : 0.5 mL/L acetic acid (70 : 3), flow rate 0.25 mL/min. MRM acquisition mode. Limit of quantification, 0.0625 mg/L, limit of detection, 0.028 mg/L [Song *et al.* 2004]. Isotope dilution MS [Kress *et al.* 2002a].

Chemiluminescent assay Limit of quantification, 0.2 mg/L [Malliaros *et al.* 1997].

Serum **GC** Column: 2% SP 2510-DA or 2% SP 2110/1% SP 2510-DA on 100/120 mesh Supelcoport (2 m × 4 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature programme: 25° to 250° at 2°/min. Limit of detection, 2.5 mg/L [Schwertner 1979].

GC-MS Column: DB-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 300° at 20°/min for 2 min. EI ionisation at 70 eV. SIM acquisition mode. Retention time: 11.03 min. Limit of detection, 1 ng [Saka *et al.* 2007].

HPLC Column: Nautilus C_{18} (150 × 4.6 mm i.d.). Mobile phase: 0.02 mol/L acetate : phosphate buffer (pH 3.0) containing 9.6% acetonitrile. UV detection (λ= 273 nm). Theophylline and other substituted xanthines. Comparison with GC-IDMS [Kress *et al.* 2002b]. Column: ODS modified with CHAPS (3-[(3-cholamidopropyl)- dimethylammonio]-1-propanesulfonate). Mobile phase: 0.2 mmol/L sodium hydrogen phosphate (pH 7.4). UV detection (λ= 273 nm). Limit of detection, 0.2 mg/L for theophylline and 0.5 mg/L for caffeine [Umemura *et al.* 1998].

LC-MS Column: XTerra MS C_{18} (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 7.0, 2 : 98 for 15 min to 25 : 75 between 15 and 29 min until 50 min to 2 : 98 between 50 and 70 min), flow rate 0.15 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 0.5 mg/L [Hori *et al.* 2006]. See Plasma [Kress *et al.* 2002a].

Chemiluminescent assay See Plasma [Malliaros *et al.* 1997].

Urine **HPLC** Column: LiChrospher 100 RP-18 (244 × 4.4 mm i.d., 5 µm). Mobile phase water : ethanol : acetic acid (75 : 24 : 1), flow rate 1.0 mL/min. UV detection (λ= 273 nm). Limit of detection, 0.06 µg/L for theophylline, 0.1 µg/L for caffeine and 0.07 µg/L for theobromine [de Aragão *et al.* 2005]. Column: LC-18-DB. Mobile phase: methanol : 5 mmo/L citric acid buffer (pH 5, 20 : 80). UV detection. Theophylline, caffeine, theobromine and paraxanthine. Limit of quantification, 0.8 mg/L for theophylline, 1.2 mg/L for theobromine, limit of detection, 0.15 mg/L for theophylline, 0.3 mg/L for theobromine [Zambonin *et al.* 2004]. Column: Bondesil C_{18}. Mobile phase: methanol : water : acetic acid or ethanol : water : acetic acid (20 : 75 : 5), flow rate 0.7 mL/min. UV detection (λ= 273 nm). Limit of detection, 0.1 ng/L for theophylline, caffeine and theobromine [Bispo *et al.* 2002]. See Serum [Kress *et al.* 2002a]. See Plasma. Mobile phase: (93 : 7). Limit of detection, 2 µmol/L for theophylline and 1 µmol/L for theophylline metabolites (1-methyluric acid, 3-methylxanthine, 1,3 dimethyluric acid) [Rasmussen, Brosen 1996]. Column: RP Ultrasphere-ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L sodium acetate : acetonitrile : tetrahydrofuran (96.9 : 3.0 : 0.1) containing 5 mmol/L tetrabutylammonium hydrogen sulfate (pH 4.7), flow rate 1.5 mL/min. UV detection (λ= 280 nm). *k* value: 4.31 (RT, 8.5 min). Limit of detection, 1 mg/L for theophylline and 0.5 mg/L for theophylline metabolites [Tajerzadeh, Dadashzadeh 1995].

Disposition in the Body Orally administered aminophylline is readily absorbed, but absorption is less rapid for orally administered theophylline; there is irregular absorption after rectal administration. The presence of food or antacids does not affect absorption. Theophylline is rapidly and widely distributed throughout the tissues; unbound theophylline distributes into body water but poorly into body fat. It also passes freely across the placenta, into breast milk and into CSF. It is metabolised by *N*-demethylation to form 3-methylxanthine (which is active but less potent than theophylline), 1,3-dimethyluric acid and 1-methyluric acid (by further hydroxylation of 1-methylxanthine). Approximately 90% of a dose is metabolised in adults and children over 1 year of age; 6% to caffeine by *N*-methylation. In adults, ~13% of a dose is excreted in the urine in 24 h as unchanged drug, with approx. 15% as 3-methylxanthine, 35–50% as 1,3-dimethyluric acid and ~20% as 1-methyluric acid. In premature neonates, most of a dose is excreted as unchanged drug with up to 10% as caffeine. Theophylline is a metabolite of caffeine.

Therapeutic Concentration There is considerable inter-subject variation in serum theophylline concentrations; therapeutic effect has been correlated with concentrations of 10–15 mg/L. 3-Methylxanthine accumulates in the serum to concentrations of ~25% of those of theophylline. Diurnal variations in plasma concentrations have been reported, with higher concentrations in the morning. Steady-state concentrations are reached within 30–65 h (mean, 40) and levels reach 60% of the mean peak therapeutic concentration.

Eighteen healthy men, aged 20–30 years, were administered either a 1000 mg once daily dose in the evening (equivalent to 13 mg/kg) or two 500 mg doses in the morning and evening. Each dose was administered 30 min after a standard meal. The mean peak plasma concentration for the once daily dose was 15.2 mg/L (range, 11.9–19.4) on days 5/6 and 15.0 mg/L (range, 11.6–19.3) on days 8/9. For the twice daily dosing, the peak concentration was 11.8 mg/L (range, 9.0–15.3) on days 5/6 and 12.1 mg/L (range, 9.0–16.2) on days 8/9 [Götz *et al.* 1994].

Twelve healthy, young, non-smoking men, aged 23 to 30 years, were administered a single oral dose of 200 mg theophylline in solution diluted with either water or grapefruit juice, after an overnight fast. The mean peak plasma concentration was 6.2 mg/L (range, 4.7–9.6) for the dose administered with the water and 5.6 mg/L (range, 4.2–7.8) for the dose with grapefruit, observed at 0.25–2.0 h and 0.5–1.5 h, respectively [Fuhr *et al.* 1995].

Twelve debilitated elderly patients were administered theophylline either orally (dose 360 mg daily) or via a nasogastric tube (320 mg daily, by means of emptying capsule contents and mixing with 10 mL of water; dose not significantly different from that given orally). Trough and peak levels in the nasogastric-tube group were 0.5–10.7 mg/L (mean, 3.7) and 1.3–13.3 mg/L (mean, 6.5), respectively, the time to reach peak concentration being 6.5 h. Trough and peak levels in the orally administered group were 0–15.61 mg/L (mean, 8.63) and 4.3–16.2 mg/L (mean, 10.5), respectively, with a time to peak concentration of 5.5 h [Berkovitch *et al.* 2002].

Toxicity The estimated minimum lethal dose after IV administration is 0.1 g; fatalities have occurred after oral doses of 8.4 mg/kg in a child and after 25–100 mg/kg aminophylline given as a suppository. Recovery has been reported after ingestion of choline theophyllinate equivalent to 12.8 g theophylline. Toxic effects are usually associated with plasma concentrations >30 mg/L, and fatalities with concentrations above 50 mg/L; premature neonates appear to be relatively resistant to theophylline poisoning. Premature neonates, patients with hepatic cirrhosis, the elderly and women during the third trimester of pregnancy may show toxic signs at serum concentrations in the therapeutic range, 10–20 mg/L, owing to an increase in unbound pharmacologically active drug.

A 50-year-old patient who attempted suicide with theophylline had peak levels of 148 mg/L [Shechter *et al.* 1996].

In a 44-year-old woman who was found dead next to an empty container of theophylline capsules (maximum possible dose ingested was 100 tablets of 350 mg), toxicological analysis revealed a serum theophylline concentration of 127 mg/L and a concentration of 154 mg/L in the fluid gathered from cutaneous blisters on regions exposed to pressure [Tsokos, Sperhake 2002].

A 17-year-old man was found dead the following morning after complaining of a headache the previous night, which he treated with 1 or 2 Panadol tablets (500 mg paracetamol). Postmortem toxicological analysis showed a serum theophylline concentration of 145 mg/L, liver 91 µg/g, urine 182 mg/L and vitreous humour 48 mg/L. A total of 320 mg and 3 part tablets were detected in the stomach contents. All tissue/fluid theophylline concentrations were at a fatal level [Ryall 1997].

Of 249 patients classed as having theophylline intoxication (defined as a peak serum concentration of 30 mg/L; 167 µmol/L), 119 had acute intoxication without having previously received theophylline therapy. These patients had a mean peak serum theophylline concentration of 375 µmol/L (882 µmol/L in 4 who died). In 38 patients who had acute intoxication while receiving theophylline therapy, the mean peak serum concentration was 381 µmol/L. In 92 patients who had intoxication caused by chronic overmedication, the mean peak serum level was 279 µmol/L [Shannon 1993].

In a further 10-year propective analysis involving 356 patients with serum theophylline levels >30 mg/L (167 µmol/L), the mean peak theophylline concentration was 336 µg/L. In this group, 162 and 144 had acute and chronic poisoning, respectively, and 50 had acute-on-therapeutic poisoning; 15 patients died, 11 of whom had chronic overmedication [Shannon 1999].

Half-life Plasma half-life, 3–13 h in normal subjects (mean, 7) but decreased in smokers and in children, and increased in premature neonates and in certain disease states such as hepatic disease, heart failure and chronic obstructive pulmonary disease. The half-life may also be affected by the amount of dietary methylxanthines ingested.

Volume of Distribution Approximately 0.5 L/kg; increases in premature neonates, patients with hepatic cirrhosis, the elderly and in women during the third trimester of pregnancy.

Clearance Plasma clearance, ~0.5–2 mL/min/kg in normal subjects; decreases by ~30% in the elderly and 50% in patients with hepatic impairment and congestive heart failure.

Distribution in Blood Plasma : whole blood ratio, 1.2.

Saliva Plasma : saliva ratio, ~2, but there is considerable inter-subject variation.

Protein Binding Approximately 40% (temperature and pH dependent); decreased in neonates and in subjects with hepatic cirrhosis.

Note For reviews of the clinical pharmacokinetics of theophylline, see Ogilvie [1978] and Haley [1983]; for a review of theophylline intoxication, see Stegeman, Jordans [1991].

Dose 0.18 to 1 g daily based on serum theophylline concentrations.

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Thialbarbital

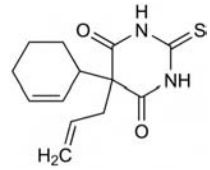
Anaesthetic, Barbiturate

$C_{13}H_{16}N_2O_2S = 264.3$

CAS—467-36-7

IUPAC Name 5-Cyclohex-2-en-1-yl-5-prop-2-enyl-2-sulfanylidene-1,3-diazinane-4,6-dione

Synonyms 5-(2-Cyclohexen-1-yl)dihydro-5-(2-propenyl)-2-thioxo-4,6(1*H*,5*H*)-pyrimidinedione; thialbarbitone.



Chemical Properties Crystals. Mp 148° to 150°. Sparingly soluble in water; readily soluble in most organic solvents. Log *P* (octanol/water), 3.4.

Thialbarbital Sodium

$C_{13}H_{15}N_2NaO_2S = 286.3$

CAS-3546-29-0

Synonyms Natrium cyclohexenylallylthiobarbituricum; thiohexallymalnatrium.

Chemical Properties A pale yellow hygroscopic powder. Mp 130° to 132°. Very soluble in water and ethanol; slightly soluble in chloroform; practically insoluble in ether.

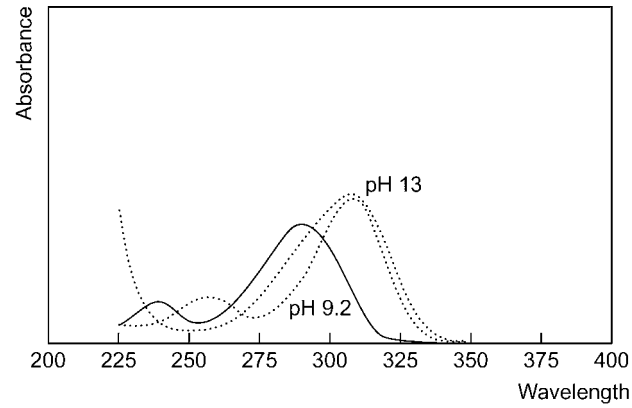
Colour Tests Palladium chloride—orange-yellow; vanillin reagent—brown-orange/violet (transient).

Thin-layer Chromatography System TD—R_f 0.77; system TE—R_f 0.43; system TF—R_f 0.72; system TH—R_f 0.75; system TAD—R_f 0.69 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, green).

Gas Chromatography System GA—RI 2116.

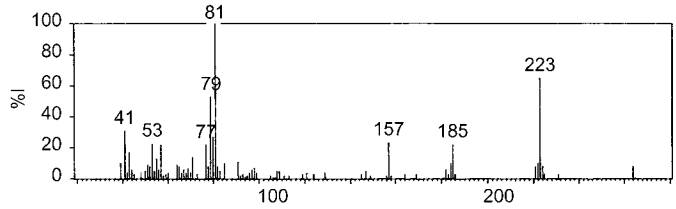
High Performance Liquid Chromatography System HX—RI 480.

Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—256, 307 nm (A^1_1=1236b); M sodium hydroxide (pH 13)—306 nm (A^1_1=1271b).



Infrared Spectrum Principal peaks at wavenumbers 1685, 1610, 1300, 1130, 1270, 1000 cm^{-1} (thialbarbital sodium, KBr disk).

Mass Spectrum Principal ions at *m/z* 81, 223, 79, 41, 80, 157, 185, 77.



Dose Thialbarbital sodium has been given in doses of 0.2 to 1 g IV.

Thiamazole

Antithyroid Agent

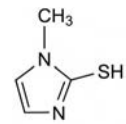
$C_4H_6N_2S = 114.2$

CAS—60-56-0

IUPAC Name 3-Methyl-1*H*-imidazole-2-thione

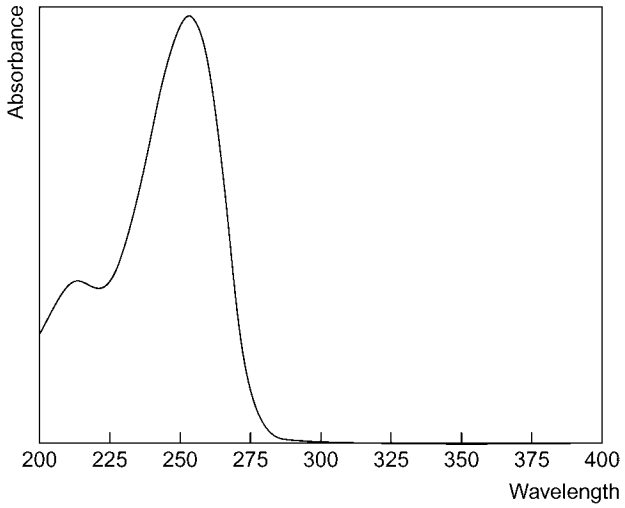
Synonyms 1,3-Dihydro-1-methyl-2*H*-imidazole-2-thione; mercazolylum; methimazole; tiamazol.

Proprietary Names *Favistan*; *Mercaptizol*; *Metibasol*; *Strumazol*; *Tapazol(e)*; *Thacapzol*; *Thycapzol*; *Thyrozol*; *Tirodril*.

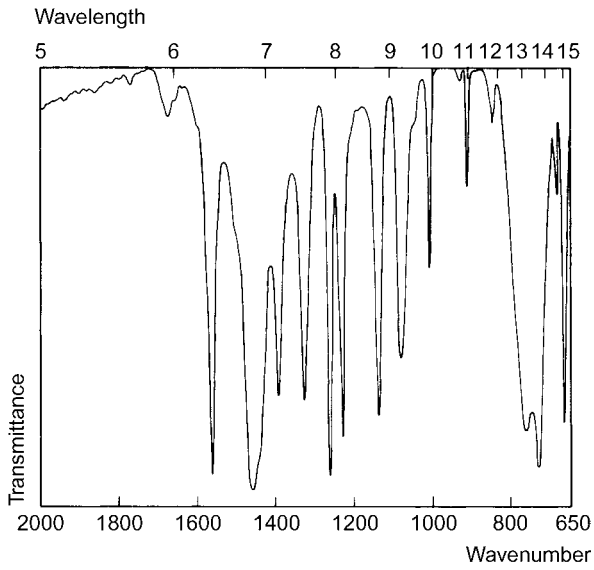


Chemical Properties A white to pale buff, crystalline powder. Mp 144° to 147°. Soluble 1 in 5 of water, 1 in 5 of ethanol and 1 in about 5 of chloroform;

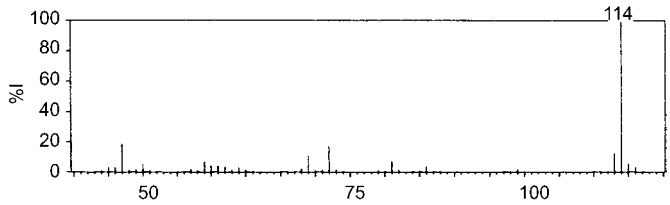
freely soluble in acetone; sparingly soluble in ether, petroleum ether, and benzene. Log *P* (octanol/water), −0.3.
Colour Test Palladium chloride—orange.
Thin-layer Chromatography System TA—R_f 0.62; system TB—R_f 0.01; system TC—R_f 0.52; system TE—R_f 0.41; system TL—R_f 0.59; system TAE—R_f 0.80 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1550.
Ultraviolet Spectrum Aqueous acid—252 nm (A^1_1=1505a).



Infrared Spectrum Principal peaks at wavenumbers 1570, 1271, 740, 1250, 765, 675 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 114, 42, 72, 113, 69, 81, 54, 115.



Quantification See under Carbimazole.
Disposition in the Body Absorbed after oral administration and widely distributed throughout the body. About 10% of a dose is excreted in the urine as unchanged drug. 3-Methyl-2-thiohydantoin has been identified in plasma and urine as a minor metabolite.

Thiamazole is the active metabolite of carbimazole.

Therapeutic Concentration

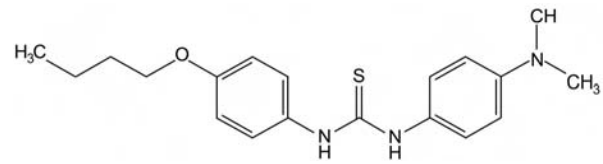
Following a single oral dose of 60 mg given to 11 subjects, peak serum concentrations of 0.5 to 2.5 mg/L (mean, 1.3) were attained in about 1 to 3 h [Melander *et al.* 1980].

Half-life Plasma half-life, about 3 to 5 h.
Volume of Distribution About 0.5 L/kg.
Protein Binding Not significantly bound.
Note For a review of the pharmacokinetics of antithyroid agents, see Kampmann and Hansen [1981].
Dose Initially, 15 to 60 mg daily; maintenance, 5 to 15 mg daily.

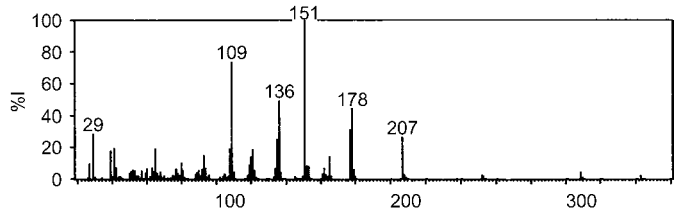
Kampmann JP, Hansen JM (1981). Clinical pharmacokinetics of antithyroid drugs. *Clin Pharmacokinet* 6: 401–428.
Melander A *et al.* (1980). Comparative in vitro effects and in vivo kinetics of antithyroid drugs. *Eur J Clin Pharmacol* 17: 295–299.

Thiambutosine

Antileprotic
$C_{19}H_{25}N_3OS$ = 343.5
CAS—500-89-0
IUPAC Name 1-(4-Butoxyphenyl)-3-(4-dimethylaminophenyl)thiourea
Synonym DPT
Proprietary Name *Ciba-1906*



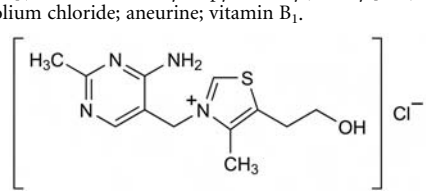
Chemical Properties A white or creamy-white crystalline powder. Mp 123° to 127°. Practically insoluble in water; soluble 1 in 1.5 of chloroform and 1 in 300 of ether; soluble in acetone.
Thin-layer Chromatography System TA—R_f 0.76; system TL—R_f 0.69; system TB—R_f 0.06; system TC—R_f 0.77.
Gas Chromatography System GA—RI 1715.
Ultraviolet Spectrum Ethanol—270 nm (A^1_1=720a).
Infrared Spectrum Principal peaks at wavenumbers 1234, 720, 1307, 1499, 813, 1093 cm^{-1}.
Mass Spectrum Principal ions at *m/z* 151, 109, 136, 178, 177, 29, 207, 135.



Disposition in the Body Thiambutosine is poorly absorbed after oral administration; aqueous suspensions are slowly and possibly incompletely absorbed after IM injection. About 10% of a dose is metabolised to water-soluble compounds and rapidly excreted in the urine; about 75% of a dose is eliminated unchanged in the faeces.
Dose 1 to 3 g daily.

Thiamine

Vitamin
$C_{12}H_{17}ClN_4OS$ = 300.8
CAS—59-43-8
IUPAC Name 2-[3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium-5-yl]ethanol chloride
Synonyms 3-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride; aneurine; vitamin B_1.



Chemical Properties pK_a 4.8 (20°). Log *P* (octanol/water), −3.9.

Thiamine Hydrochloride

$C_{12}H_{17}ClN_4OS,HCl$ = 337.3
CAS—67-03-8
Synonym Aneurine chloride hydrochloride
Proprietary Names *Benerva*; *Beneuran*; *Betabion*; *Betamin(e)*; *Beta-Sol*; *Betaxin*; *Bevitine*; *Bevitol*; *Bewon*; *Neuramin*; *Vita-B1*. It is an ingredient of *Labiton* and *Quiet Life*.

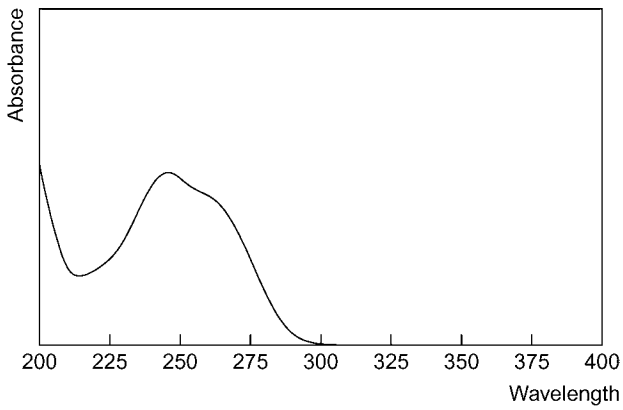
T

Chemical Properties Colourless crystals or white crystalline powder. Mp 248°, with decomposition. Soluble 1 in about 1 of water, 1 in 100 of 95% ethanol and 1 in 18 of glycerol; practically insoluble in dehydrated alcohol, benzene, hexane, chloroform and ether; soluble in methanol.

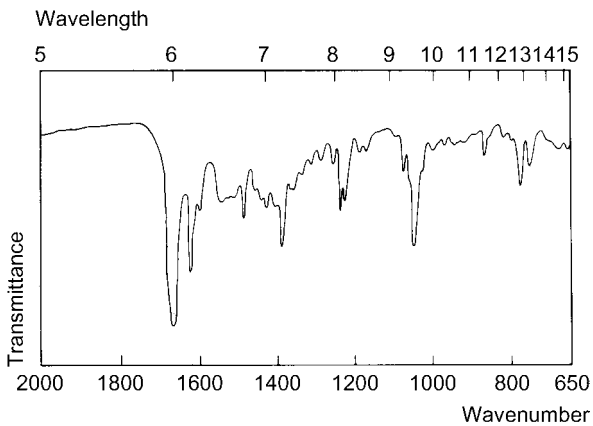
Thiamine Mononitrate

$C_{12}H_{17}N_5O_4S = 327.4$
CAS—532-43-4
Synonyms Aneurine mononitrate; thiamine nitrate; vitamin B_1 mononitrate.
Proprietary Name *B_1-Vicotrat*
Chemical Properties White crystals or crystalline powder. Mp 196° to 200°, with decomposition. Soluble 1 in 44 of water; slightly soluble in ethanol and chloroform.

Colour Test Dissolve about 5 mg of thiamine hydrochloride in a mixture of 1 mL of lead acetate solution and 1 mL of 10% sodium hydroxide solution—yellow; after heating on a steam-bath—brown; on standing—a black precipitate of lead sulfide.
Thin-layer Chromatography Thiamine hydrochloride: system TA—R_f 0.01; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.01; system TL—R_f 0.00; system TAE—R_f 0.02; system TAF—R_f 0.18 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—not eluted.
High Performance Liquid Chromatography System HA—*k* 2.0; system HX—RI 09 (hydrochloride); system HY—RI 21 (hydrochloride).
Ultraviolet Spectrum Aqueous acid—246 nm (A_1^1=450a); aqueous alkali—232 nm (A_1^1=566b), 336 nm.



Infrared Spectrum Principal peaks at wavenumbers 1660, 1618, 1048, 1237, 1595, 1228 cm^{-1} (thiamine hydrochloride, KBr disk).



Quantification
Plasma **HPLC** Fluorescence detection. Limit of detection, 150 ng/L [Weber, Kewitz 1985].
Disposition in the Body Well absorbed from the gastrointestinal tract and widely distributed throughout the body. It is converted in the body to thiamine pyrophosphate. Thiamine is not stored to any appreciable extent in the body and amounts in excess of the body's requirements are excreted in the urine unchanged or as metabolites. About 1 mg of thiamine is metabolised in the body daily.
Blood Concentration Endogenous blood concentrations are about 0.010 to 0.015 mg/L and concentrations of thiamine pyrophosphate in erythrocytes are usually 0.03 to 0.11 mg/L.

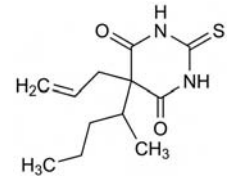
Endogenous plasma concentrations in 91 subjects ranged from 0.002 to 0.013 mg/L (median, 0.004) [Weber, Kewitz 1985].

Note For a general review of thiamine, see Inui and Nakano [1999].
Dose 10 to 100 mg daily.

Inui H, Nakano Y (1999). Vitamin B1. *Nippon Rinsho* 57: 2187–2192.
Weber W, Kewitz H (1985). Determination of thiamine in human plasma and its pharmacokinetics. *Eur J Clin Pharmacol* 28: 213–219.

Thiamylal

Barbiturate
$C_{12}H_{18}N_2O_2S = 254.3$
CAS—77-27-0
IUPAC Name 5-Pentan-2-yl-5-prop-2-enyl-2-sulfanylidene-1,3-diazinane-4,6-dione
Synonym Dihydro-5-(1-methylbutyl)-5-(2-propenyl)-2-thioxo-4,6(1*H*,5*H*)-pyrimidinedione



Chemical Properties Crystals. Mp 132° to 133°. pK_a 7.5. Log *P* (octanol/water), 3.23.

Sodium Thiamylal

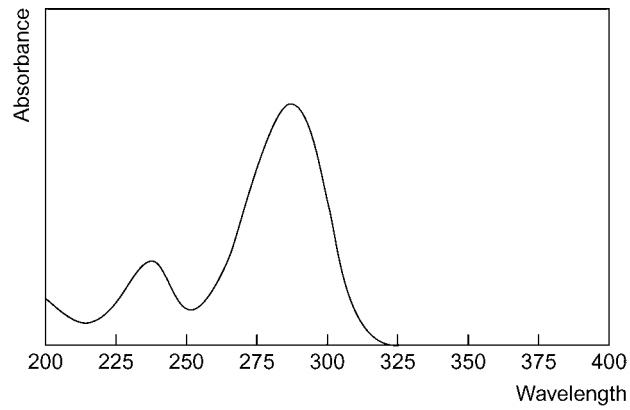
$C_{12}H_{17}N_2NaO_2S = 276.3$
CAS—337-47-3
Proprietary Name *Surital*
Chemical Properties Thiamylal sodium for injection is a mixture of sodium thiamylal with anhydrous sodium carbonate. It is a pale yellow hygroscopic powder. Soluble in water.

Thin-layer Chromatography System TE—R_f 0.55; system TF—R_f 0.75.
Gas Chromatography System GA—RI 1899.

Column: glass coil packed with 3% poly-A-103 on Gas Chrom Q (80–100 mesh) (0.5 m × 2.6 mm i.d.). Column and injector temperatures: 255° and 270°, respectively. Carrier gas: helium, flow rate 40 mL/min. IS: thiopental. MS detection (EI, SIM at *m/z* 184 for thiamylal and 172 for IS). Retention time: thiamylal, 1.2 min; IS, 1.1 min [Kudo *et al.* 1988].

High Performance Liquid Chromatography System HX—RI 516; system HY—RI 476.

Column: Chiral-AGP (100 × 4.0 mm i.d.); guard, Chiral-AGP (10 × 3.0 mm). Mobile phase: 20 mmol/L potassium dihydrogen phosphate containing 3% 2-propanol, pH 4.7, flow rate 0.9 mL/min. UV detection (λ = 288 nm). Retention time: *R*-(+)-thiamylal, 10.0 min; *S*-(−)-thiamylal, 11.7 min [Sueyasu *et al.* 1995].
Ultraviolet Spectrum Aqueous acid—238, 287 nm; aqueous alkali—305 nm.



Infrared Spectrum Principal peaks at wavenumbers 1668, 1541, 1731, 1170, 1318, 1291 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 43, 41, 184, 168, 167, 97, 55, 53.
Quantification
Serum **HPLC** UV detection (λ = 288 nm). Limit of quantification, 0.05 mg/L [Sueyasu *et al.* 1995].
Biological Samples **GC-MS** Limit of detection, 0.01 µg/g [Kudo *et al.* 1988].
Disposition in the Body
Therapeutic Concentration

Six patients with liver dysfunction, aged 0.3 to 72 years, were IV administered with a total dose of 15.1 to 307.4 mg/kg (concentration of 25 or 50 g/L; infusion rate 0.45 to 4.0 mg/kg/h, over a period of 4.7 to 108.3 h). The unbound *R*-(+)-thiamylal serum concentration was 0.57 to 3.11 mg/L and the total concentration was 2.94 to 18.51 mg/L. The unbound *S*-(−)-thiamylal concentration was 0.78 to 3.74 mg/L and total 5.28 to 33.35 mg/L. All concentrations were observed after 10 min following administration [Sueyasu *et al.* 1997].

Toxicity

A fatal intoxication of IV thiamylal administration is reported. After toxicological analysis, thiamylal was detected in blood at a concentration of 129 mg/L; in liver 366 µg/g and in brain 116 µg/g. It was not detected in urine, gastric contents or bile [Stockham *et al.* 1991].

A 28-year-old student was found dead at his hotel room, hanging by his neck, and was IV connected to a bottle of thiamylal sodium. Thiamylal was detected at a concentration of 29 mg/L in blood, 1.4 mg/L in urine, 16 mg/L in bile and was present in liver at 135 µg/g, in kidney 25 µg/g and was present in his stomach contents at 0.4 mg. Alcohol and other drugs were not detected in the blood [Costantino *et al.* 1990].

Dose Initially 3 to 6 mL of a 2.5% solution of sodium thiamylal IV.

Costantino AG *et al.* (1990). Thiamylal: review of the literature and report of a suicide. *J Forensic Sci* 35(1): 89–96.
Kudo K *et al.* (1988). Toxicological analysis of thiamylal in biological materials by gas chromatography/mass spectrometry. *Forensic Sci Int* 37(3): 193–200.
Stockham TL *et al.* (1991). Report of a fatal thiamylal intoxication. *J Anal Toxicol* 15(3): 155–156.
Sueyasu M *et al.* (1995). Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 665: 133–137.
Sueyasu M *et al.* (1997). Pharmacokinetics of thiamylal enantiomers in humans. *Int J Clin Pharmacol Ther* 35(3): 128–132.

Thiazinamium Metilsulfate

Antihistamine

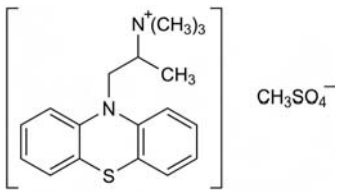
$C_{19}H_{26}N_2O_4S_2 = 410.6$

CAS—2338-21-8 (thiazinamium); 58-34-4 (metilsulfate)

IUPAC Name Methyl sulfate; trimethyl(1-phenothiazin-10-ylpropan-2-yl)azanium

Synonyms Methylpromethazinium methylsulfuricum; RP-3554; *N*,*N*,*N*-α-tetramethyl-10*H*-phenothiazine-10-ethanaminium methyl sulfate; thiazinamium methylsulfate.

Proprietary Name *Multergan*



Chemical Properties A white crystalline powder which discolours on exposure to light. Mp 206° to 210°, with decomposition. Soluble 1 in 10 of water; very soluble in ethanol; practically insoluble in ether. Log *P* (octanol/water), 2.7.

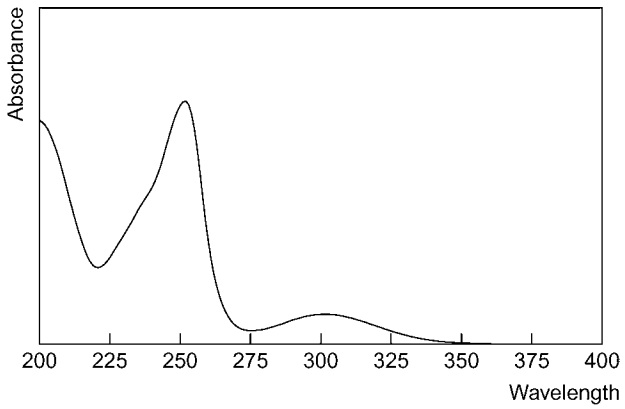
Colour Tests Formaldehyde-Sulfuric acid—brown-violet; Forrest reagent—red; FPN reagent—red; Liebermann's reagent—red.

Thin-layer Chromatography System TA—R_f 0.02; system TAE—thiazinamium metilsulfate R_f 0.01, M (thiazinamium sulfoxide) R_f 0.00; system TAF—R_f 0.25; system TL—R_f 0.00; system TB—thiazinamium metilsulfate R_f 0.00, M (thiazinamium sulfoxide) R_f 0.00; system TC—R_f 0.00; system TE—thiazinamium metilsulfate R_f 0.00, M (thiazinamium sulfoxide) R_f 0.00; system TF—M (thiazinamium sulfoxide) R_f 0.00 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2339 Art (promethazine); system GB—RI 2383 Art (promethazine).

High Performance Liquid Chromatography System HX—RI 326 (M thiazinamium sulfoxide); system HZ—retention time 6.4 min.

Ultraviolet Spectrum Aqueous acid—251 (A^{1}_{1}=450b), 299 nm.



Infrared Spectrum Principal peaks at wavenumbers 1224, 1010, 1059, 764, 743, 1036 cm^{-1}.

Quantification

Plasma **GC** AFID. Limit of detection, 20 µg/L [Jonkman *et al.* 1975].

Urine **GC** See Plasma [Jonkman *et al.* 1975].

Disposition in the Body Thiazinamium metilsulfate is incompletely absorbed after oral administration and undergoes considerable first-pass metabolism to thiazinamium sulfoxide; it is well absorbed after IM administration. About 40% of a parenteral dose is excreted in the urine unchanged in 8 h, together with 9% of the dose as the sulfoxide.

Therapeutic Concentration

Following single oral doses equivalent to 300 mg and 900 mg of thiazinamium to 10 subjects, peak plasma concentrations of 0.03 to 0.13 (mean, 0.07) mg/L and 0.06 to 0.36 (mean, 0.17) mg/L were attained in about 2.4 h and 5 h, respectively [Jonkman *et al.* 1977].

Dose 0.6 to 1.2 g daily.

Jonkman JG *et al.* (1975). Determination of low cencentrations of the quaternary ammonium compound thiazinamium methylsulphate in plasma and urine. *J Pharm Pharmacol* 27: 849–854.
Jonkman JH *et al.* (1977). Variations in the bioavailability of thiazinamium methylsulfate. *Clin Pharmacol Ther* 21: 457–463.

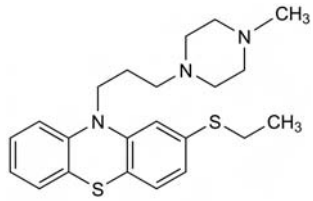
Thiethylperazine

Antiemetic

$C_{22}H_{29}N_3S_2 = 399.6$

CAS—1420-55-9

IUPAC Name 2-Ethylsulfanyl-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine



Chemical Properties Crystals. Mp 62° to 64°. Practically insoluble in water. Log *P* (octanol/water), 5.4.

Thiethylperazine Malate

$C_{22}H_{29}N_3S_2.2C_4H_6O_5 = 667.8$

CAS—52239-63-1

Proprietary Name *Torecan (injection)*

Chemical Properties A white to faintly yellow crystalline powder. Mp 139°. Soluble 1 in 40 of water, 1 in 90 of ethanol, 1 in 525 of chloroform and 1 in 3400 of ether.

Thiethylperazine Maleate

$C_{22}H_{29}N_3S_2.2C_4H_4O_4 = 631.8$

CAS—1179-69-7

Proprietary Name *Torecan (suppositories and tablets)*

Chemical Properties A yellowish granular powder. Mp about 188° to 190°, with decomposition. Soluble 1 in 1700 of water and 1 in 530 of ethanol; practically insoluble in chloroform and ether.

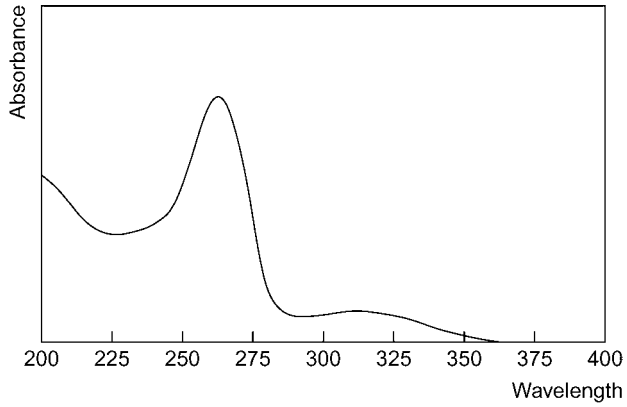
Colour Tests Formaldehyde-Sulfuric acid—green; Forrest reagent—blue; FPN reagent—blue; Mandelin's test—violet; Marquis test—red→green.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.30; system TC—R_f 0.41; system TE—thiethylperazine R_f 0.52, M R_f 0.30; system TL—R_f 0.08; system TAE—thiethylperazine R_f 0.27, M R_f 0.13; system TAJ—R_f 0.22; system TAK—R_f 0.11; system TAL—R_f 0.83 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 3247.

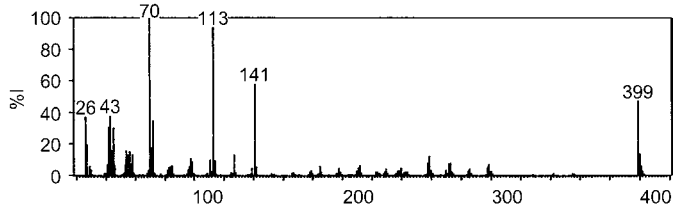
High Performance Liquid Chromatography System HA—*k* 3.8; system HX—RI 487; system HZ—retention time 16.3 min.

Ultraviolet Spectrum Aqueous acid—255 (A^{1}_{1}=857b), 309 nm (A^{1}_{1}=98b).



Infrared Spectrum Principal peaks at wavenumbers 1563, 864, 1613, 1212, 1105, 1067 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 70, 113, 141, 399, 43, 26, 72, 42.



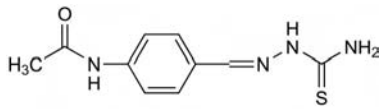
Quantification
Blood **GC** SID. Thiethylperazine and other phenothiazines. Limit of detection, 250 to 500 ng/L [Hattori *et al.* 1992].
HPLC Limit of detection, 0.5 µg/L [Seno *et al.* 1999].
Urine **GC** See Blood [Hattori *et al.* 1992].
Dose Usually 20 to 30 mg of thiethylperazine maleate daily.

Hattori H *et al.* (1992). Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 579(2): 247–252.
Seno H *et al.* (1999). High performance liquid chromatography/electrospray tandem mass spectrometry for phenothiazines with heavy side chains in whole blood. *Rapid Commun Mass Spectrom* 13: 2394–2398.

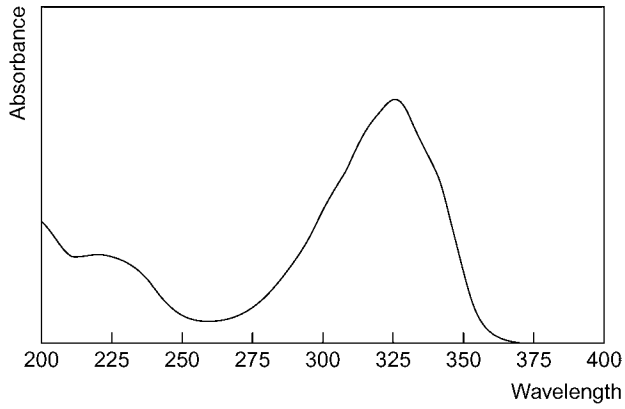
Thioacetazone

Antibacterial, Tuberculostatic
$C_{10}H_{12}N_4OS = 236.3$
CAS—104-06-3

IUPAC Name *N*-[4-[(Carbamothioylhydrazinylidene)methyl]phenyl]acetamide
Synonyms *N*-[4-[[(Aminothioxomethyl)hydrazono]methyl]phenyl]acetamide; amithiozone; TbI-698; tebezonum; thiacetazone.
Proprietary Names *Conteben*; *Livazore*; *Neustab*; *Panrone*; *Seroden*.



Chemical Properties Pale yellow crystals or crystalline powder. Mp 225° to 230°, with decomposition. Insoluble in water, acetone, benzene and chloroform; soluble 1 in 500 of ethanol and 1 in 100 of propylene glycol; practically insoluble in ether. Log *P* (octanol/water), 1.1.
Colour Tests Nessler's reagent (100°)—black; palladium chloride—orange.
Thin-layer Chromatography System TA—R_f 0.78 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2038.
Ultraviolet Spectrum Ethanol—328 nm (A^1_1=1870a).



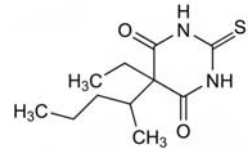
Infrared Spectrum Principal peaks at wavenumbers 1515, 1603, 1580, 1661, 1250, 1305 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** UV detection. Limit of detection, 3 µg/L [Jenner 1983; Jenner *et al.* 1984].
Urine **HPLC** See Plasma [Jenner 1983; Jenner *et al.* 1984].
Disposition in the Body Thioacetazone is slowly absorbed after oral administration. Most of a dose is metabolised and excreted in the urine; about 20% of a dose is excreted in the urine unchanged.
Therapeutic Concentration
Following a single oral dose of 4 mg/kg to 10 subjects, peak serum concentrations of 1.3 to 4.0 (mean, 3.1) mg/L were attained in about 5 h [Sen *et al.* 1973].
Half-life About 8 to 12 h.
Dose Up to 150 mg daily.

Jenner PJ (1983). High-performance liquid chromatographic determination of thiacetazone in body fluids. *J Chromatogr* 276: 463–470.
Jenner PJ *et al.* (1984). A study of thiacetazone blood levels and urinary excretion in man, using high performance liquid chromatography. *Lepr Rev* 55: 121–128.
Sen PK *et al.* (1973). Thiacetazone and INH in tuberculosis. Synchronised peak concentration drug administration for greater action. *J Indian Med Assoc* 61: 306–308.

Thiopental

Barbiturate
$C_{11}H_{18}N_2O_2S = 242.3$
CAS—76-75-5

IUPAC Name 5-Ethyl-5-pentan-2-yl-2-sulfanylidene-1,3-diazinane-4,6-dione
Synonyms 5-Ethyldihydro-5-(1-methylbutyl)-2-thio-4,6(1*H*,3*H*,5*H*)-pyrimidinedione; penthiobarbital; thiomebumal; thiopentone.

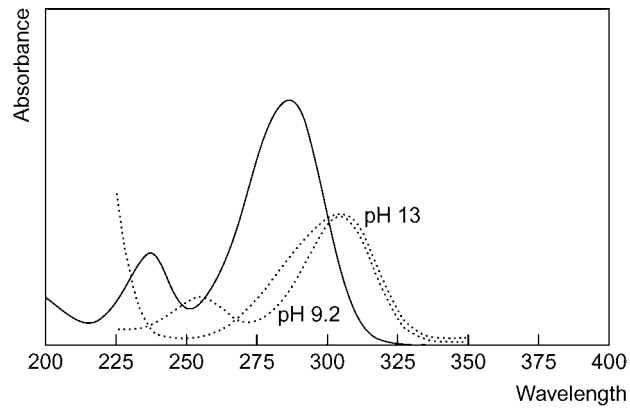


Chemical Properties pK_a 7.6 (20°). Log *P* (octanol/water), 2.85. Extraction yield, 0.9 [Demme *et al.* 2005].

Thiopental Sodium

$C_{11}H_{17}N_2NaO_2S = 264.3$
CAS—71-73-8
Synonyms Penthiobarbital sodique; soluble thiopentone; thiomebumalnatrium cum natrii carbonate; thiopental sodium and sodium carbonate; thiopentalum natricum; thiopentobarbitalum solubile; thiopentone sodium.
Proprietary Names *Farmotal*; *Intraval Sodium*; *Nesdonal*; *Pentothal*; *Trapanal*.
Chemical Properties A white to yellowish-white to pale green, hygroscopic powder. It usually contains anhydrous sodium carbonate in the proportion of 6 parts to each 100 parts of thiopental sodium. Soluble 1 in 1.5 of water; partly soluble in ethanol; insoluble in ether, benzene and petroleum ether.

Colour Tests Koppanyi–Zwikker test—violet; palladium chloride—orange; vanillin reagent—brown-red/violet.
Thin-layer Chromatography System TD—R_f 0.77; system TE—R_f 0.49; system TF—R_f 0.74; system TH—R_f 0.80; system TAD—R_f 0.68. Thiopental sodium: system TAJ—R_f 0.73; system TAK—R_f 0.71; system TAL—R_f 0.92 (mercuric chloride–diphenylcarbazone reagent, positive; mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, green).
Gas Chromatography System GA—thiopental RI 1857, thiopental-Me_2 RI 1825, pentobarbital RI 1735; system GB—thiopental RI 1923, M (OH-) RI 2134; system GF—RI 2600; system GAJ—RRT 0.948 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HX—RI 485; system HY—RI 433; system HZ—retention time 6.9 min; system HAA—retention time 19.2 min.
Column: ODS C_{18} (150 × 4.6 mm i.d., 4 µm). Mobile phase: water : methanol : acetonitrile (50 : 40 : 10), adjusted to pH 2.70 with phosphoric acid, flow rate 1.5 mL/min. UV detection (λ = 290 nm). IS: tolylbarb. Retention time: thiopental, 9.5 min; thiopental isomer, 8.4 min; IS, 5.4 min [Coppa *et al.* 2001].
Column: C_8 Whatman PatriSphere (110 × 4.6 mm i.d.). Mobile phase: 50% acetonitrile : phosphate buffer (120 mmol/L, pH 6.2). UV detection (270 nm). Retention time 4.3 min [Schmid, Wolf 1989].
Column: Chiral AGP (100 × 4.0 mm i.d.); guard, (10 × 3.0 mm i.d.). Mobile phase: 20 mmol/L phosphate buffer : 2-propanol : methanol (93.5 : 5.0 : 1.5), pH 5.0, flow rate 0.9 mL/min. UV detection. Retention time: (*R*)-(+)-thiopental, 2.7 (4.8 min); (*S*)-(−)-thiopental, 3.7 (6.2 min) [Jones *et al.* 1996].
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—255, 304 nm (A^1_1=1138b); 1 mol/L sodium hydroxide (pH 13)—303 nm (A^1_1=1170b).



Infrared Spectrum Principal peaks at wavenumbers 1670, 1540, 1300, 1170, 1735, 1220 cm^{-1} (KBr disk).

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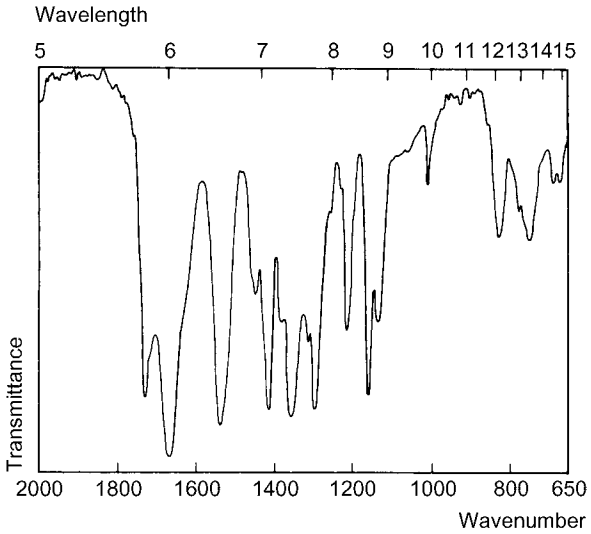
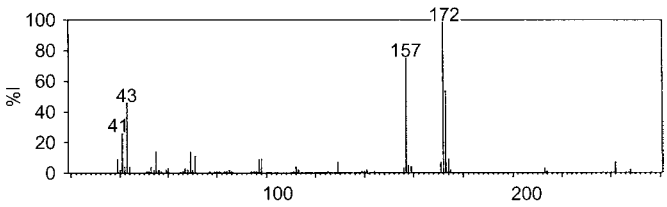


Mass Spectrum Principal ions at *m/z* 172, 157, 173, 43, 41, 55, 69, 71.



Quantification

Blood **HPLC** UV detection (λ = 270 nm). Limit of detection, 0.2 mg/L [Schmid, Wolf 1989]. UV detection. Limit of detection, 1 mg/L [Levine *et al.* 1983].

Plasma **GC** AFID. Limit of detection, 0.025 mg/L [Jung *et al.* 1981].

HPLC UV detection (λ = 280 nm). Limit of quantification, 0.01 mg/L [Jones *et al.* 1996]. UV detection. Limit of detection, 0.2 mg/L [Salvadori *et al.* 1981].

Serum **HPLC** UV detection (λ = 280 nm). Limit of detection, 0.1 mg/L [Altmayer *et al.* 1987].

Tissues **HPLC** See Blood [Levine *et al.* 1983].

See also under Amobarbital.

Disposition in the Body About 10% of a dose is concentrated in the brain within 1 min of an IV injection and it is then rapidly distributed throughout the body, eventually accumulating in body fat; about 50% of a dose accumulates in this way after 30 to 90 min. The very short action of thiopental is due to the brief α-phase half-life and the redistribution of the drug from the brain to other fatty tissues. Metabolic reactions include ω-hydroxylation, further oxidation and, to a lesser extent, desulfuration to pentobarbital. Metabolism occurs almost exclusively in the liver and mostly inactive metabolites are produced. Less than 1% of a dose is excreted in the urine as unchanged drug. 5-(3-Carboxy-1-methylpropyl)-5-ethyl-2-thiobarbituric acid has been reported to be a urinary metabolite. Repeated or continuous administration of thiopental may result in accumulation of the drug in fatty tissues which may result in extended anaesthesia and possibly respiratory and/or cardiovascular depression. Thiopental readily diffuses across the placenta and has been detected in breast milk.

Therapeutic Concentration

Plasma concentrations of 4.2 to 134 mg/L (mean, 28) were reported in 22 patients after IV injections [Becker 1976].

After multiple IV doses of 250 (5 subjects) or 500 mg (2 subjects) every 2 h for up to 7 days to neurosurgical patients, the steady-state trough plasma concentrations were 4.8 to 30 mg/L (mean, 16.0) and the peak concentrations were 8.35 to 45 mg/L (mean, 25.4) [Russo *et al.* 1995].

Toxicity The estimated minimum lethal dose is 1 g. Numerous fatalities have occurred owing to accidental or intentional overdosage, and blood concentrations from 6 to 392 mg/L have been reported in fatalities.

The following postmortem tissue distribution was reported in one suicide case: blood 6 mg/L, brain 24 μg/g, heart 22 μg/g, kidney 31 μg/g, and liver 63 μg/g [Bruce *et al.* 1977].

In a fatality caused by IV self-administration of thiopental, the following postmortem tissue concentrations were reported: blood 285 mg/L, brain 414 μg/g, kidney 195 μg/g, liver 440 μg/g [Reed, Monforte 1973].

The blood concentration of an individual who died following IV administration of a clinical dose was 0.6 mg/L. The brain concentration was 11.9 mg/kg and thiopental was also detected in the thymus at a concentration of 7.66 μg/g [Yasuda *et al.* 1993].

Half-life Plasma half-life, 4 to 20 h (mean, 9), increased after high doses (25 mg/min), up to 60 h.

Volume of Distribution 0.5 to 4 L/kg.

Clearance Plasma clearance, 1 to 4 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.0.

Protein Binding About 75 to 90%.

Dose Premedicated adults, 100 to 150 mg of thiopental sodium IV over 10 to 15 s (25 g/L solution), repeated if necessary, or up to 4 mg/kg. Children, 2 to 7 mg/kg.

Altmayer P *et al.* (1987). Sensitive HPLC assay for thiopental in human serum after simple preparation of the samples. Its application for clinical research. *Methods Find Exp Clin Pharmacol* 9 (12): 817–822.

Becker KE (1976). Gas chromatographic assay for free and total plasma levels of thiopental. *Anesthesiology* 45: 656–660.

Bruce AM *et al.* (1977). A suicide by thiopentone injection. *Forensic Sci* 9: 205–207.

Coppa G *et al.* (2001). Fast, simple and cost-effective determination of thiopental in human plasma by a new HPLC technique. *Clin Chim Acta* 305: 41–45.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Jones DJ *et al.* (1996). Determination of (R)-(+)- and (S)-(-)-isomers of thiopentone in plasma by chiral high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 67: 174–179.

Jung D *et al.* (1981). Gas-chromatographic assay for thiopental in plasma, with use of a nitrogen-specific detector. *Clin Chem* 27: 113–115.

Levine B *et al.* (1983). Liquid chromatographic analysis of thiopental in blood and tissues. *J Anal Toxicol* 7: 207–208.

Reed E, Monforte JR (1973). *Bull Int Assoc Forensic Toxicol* 9 (3&4)12.

Russo H *et al.* (1995). Pharmacokinetics of thiopental after single and multiple intravenous doses in critical care patients. *Eur J Clin Pharmacol* 49: 127–137.

Salvadori C *et al.* (1981). Liquid chromatography determination of thiopentone in human plasma. *Ther Drug Monit* 3: 171–176.

Schmid RW, Wolf C (1989). Simultaneous determination of thiopental and its metabolite, pentobarbital, in blood by high-performance liquid chromatography and post-column photochemical reaction. *J Pharm Biomed Anal* 7(12): 1749–1755.

Yasuda T *et al.* (1993). Postmortem concentrations of thiopental in tissues: a sudden death case. *Int J Legal Med* 105(4): 239–241.

Thiopropazate

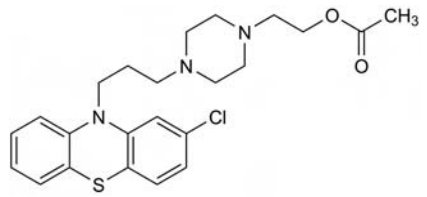
Tranquilliser

$C_{23}H_{28}ClN_3O_2S$ = 446.0

CAS—84-06-0

IUPAC Name 2-[4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl] ethyl acetate

Synonym 4-[3-(2-Chlorophenothiazin-10-yl)propyl]-1-piperazineethanol acetate



Chemical Properties Practically insoluble in water; soluble in chloroform and ether. pK_a 7.3 (24°). Log *P* (octanol/water), 4.8.

Thiopropazate Hydrochloride

$C_{23}H_{28}ClN_3O_2S,2HCl$ = 518.9

CAS—146-28-1

Proprietary Names *Dartal*; *Dartalan*.

Chemical Properties A white or pale yellow crystalline powder. Mp 223° to 229°, with decomposition. Soluble 1 in 4 of water, 1 in 130 of ethanol and 1 in 65 of chloroform; practically insoluble in ether.

Caution Thiopropazate hydrochloride may cause severe dermatitis in sensitised persons.

Colour Tests Formaldehyde-sulfuric acid—red-violet; Forrest reagent—red; FPN reagent—red; Liebermann's reagent—green-brown; Mandelin's test—violet; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.61; system TB—R_f 35; system TC—R_f 0.53; system TE—thiopropazate R_f 0.74, M R_f 0.46; system TL—R_f 0.42; system TAE—thiopropazate R_f 0.62, M R_f 0.48; system TAF—R_f 0.59; system TAJ—R_f 0.52; system TAK—R_f 0.36; system TAL—R_f 0.91. (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, red; Ninhydrin spray, positive).

Gas Chromatography System GA—RI 3467 (metabolised to perphenazine, see monograph for data).

High Performance Liquid Chromatography System HA—*k* 1.0; system HX—RI 483.

Ultraviolet Spectrum Aqueous acid—255 (A^1_1=733a), 305 nm; aqueous alkali—257 nm.

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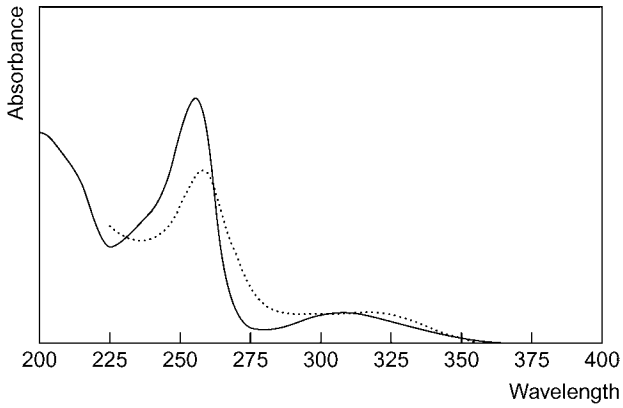
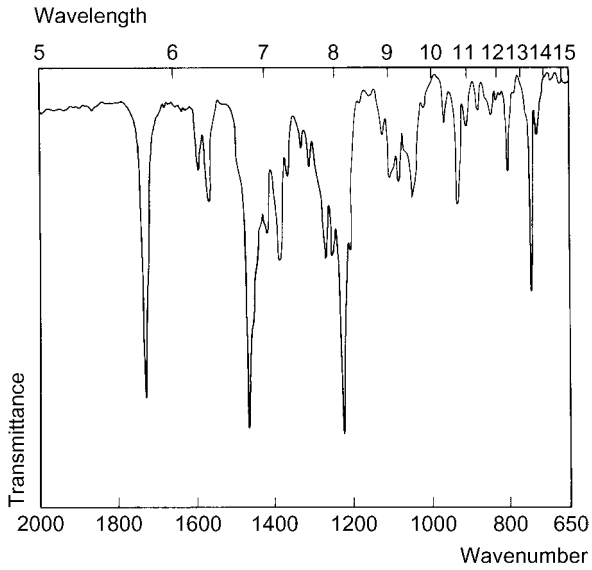
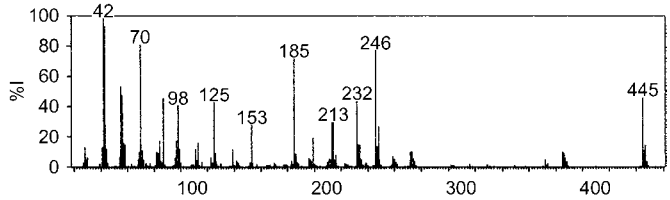


Infrared Spectrum Principal peaks at wavenumbers 1222, 1728, 746, 1265, 1250, 1200 cm^{-1} (thiopropazate hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 42, 43, 70, 246, 185, 55, 56, 445.



Dose Initially 15 to 30 mg of thiopropazate hydrochloride daily; maximum of 100 mg daily.

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Thioproperazine

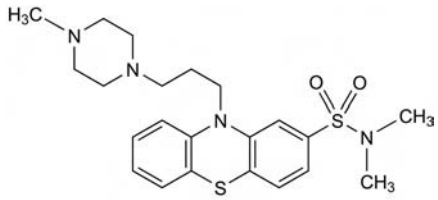
Tranquilliser

$C_{22}H_{30}N_4O_2S_2$ = 446.6

CAS—316-81-4

IUPAC Name *N*,*N*-Dimethyl-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine-2-sulfonamide

Synonyms RP-7843; SKF-5883.



Chemical Properties Crystals. Mp 140°. Practically insoluble in water. Log *P* (octanol/water), 2.9.

Thioproperazine Mesilate

$C_{22}H_{30}N_4O_2S_2,2CH_4O_3S$ = 638.8

CAS—2347-80-0

Synonyms Thioproperazine dimethanesulfonate; thioproperazine mesylate; thioproperazine methanesulfonate.

Proprietary Name *Majeptil*; *Vontil*

Chemical Properties A fine, white or pale cream powder, which becomes coloured on exposure to light. Mp about 227°. Soluble in water; slightly soluble in ethanol.

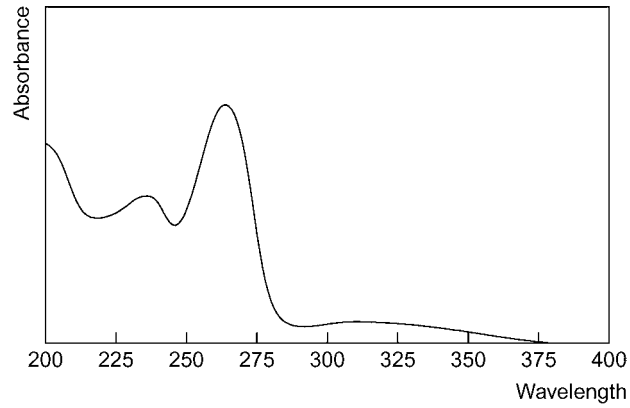
Colour Tests Formaldehyde-sulfuric acid—pink; Forrest reagent—red; FPN reagent—orange; Mandelin's test—brown→green→violet; Marquis test—red.

Thin-layer Chromatography System TA—R_f 0.46; system TB—R_f 0.07; system TC—R_f 0.34; system TE—thioproperazine R_f 0.43, M R_f 0.26; system TL—R_f 0.06; system TAE—thioproperazine R_f 0.22, M R_f 0.13; system TAF—R_f 0.22 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—thioproperazine RI 3552, M (ring) RI 3200.

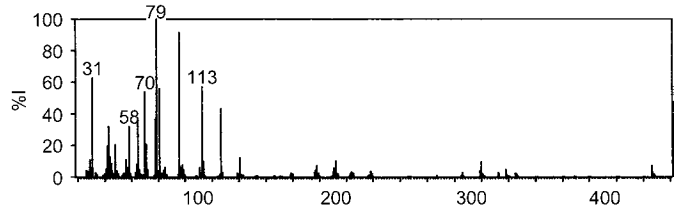
High Performance Liquid Chromatography System HA—*k* 4.1; system HX—RI 427; system HY—RI 305; system HZ—retention time 15.4 min; system HAA—retention time 15.2 min.

Ultraviolet Spectrum Aqueous acid—234, 263 (A_1^1=668a), 312 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1156, 1042, 1212, 720, 960, 762 cm^{-1} (thioproperazine mesilate, Nujol mull).

Mass Spectrum Principal ions at *m/z* 79, 96, 31, 113, 81, 70, 127, 78.



Quantification

Blood **GC** SID. Limit of detection, 250–500 ng/L [Hattori *et al.* 1992].

Urine **GC** See Blood [Hattori *et al.* 1992].

Dose Usually 15 to 30 mg of thioproperazine mesilate daily; up to 90 mg daily has been given.

Hattori H *et al.* (1992). Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J Chromatogr* 579(2): 247–252.

Thioridazine

Tranquilliser

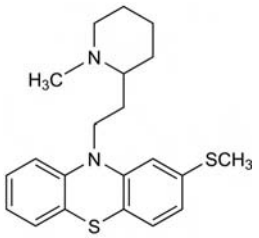
$C_{21}H_{26}N_2S_2$ = 370.6

CAS—50-52-2

IUPAC Name 10-[2-(1-Methylpiperidin-2-yl)ethyl]-2-methylsulfanylphenothiazine

Synonym 10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylthio)-10*H*-phenothiazine

Proprietary Names *Mellaril-S*; *Melleretten*; *Melleril*; *Rideril*.



Chemical Properties A white or slightly yellow crystalline powder that darkens on exposure to light. Mp 72° to 74°. Practically insoluble in water; soluble 1 in 6 of ethanol, 1 in 0.8 of chloroform and 1 in 3 of ether; freely soluble in dehydrated alcohol. pK_a 9.5 (24°). Log *P* (octanol/water), 5.90. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Serum samples were stable for up to 8 h at room temperature and up to 2 weeks when stored at 4° and -20° [Tanaka *et al.* 2007]. Stock standard solutions were stable for up to 2 months at 4° [Svendsen, Bird 1986].

Thioridazine Hydrochloride

$C_{21}H_{26}N_2S_2$,HCl = 407.0
CAS—130-61-0

Proprietary Names *Aldazine*; *Mallorol*; *Mellaril*; *Melleretten* (tablets); *Melleril* (tablets); *Melzine*; *Novoridazine*; *Orsanil*; *Thioril*; *Thiozine*.

Chemical Properties A white or slightly yellow crystalline powder. Mp 158° to 160° (crystals from acetone). Soluble 1 in 9 of water, 1 in 10 of ethanol and 1 in 1.5 of chloroform; freely soluble in methanol; practically insoluble in ether.

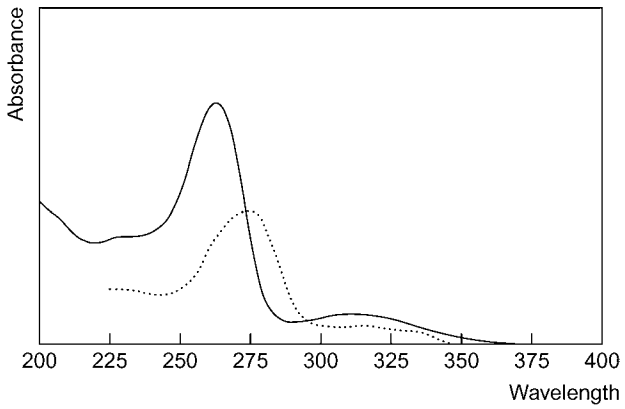
Colour Tests Formaldehyde–sulfuric acid—blue; Forrest reagent—blue; FPN reagent—blue; Mandelin's test—blue→violet; Marquis test—violet-red→blue-green.

Thin-layer Chromatography System TA—R_f 0.48; system TB—R_f 0.42; system TC—R_f 0.30; system TE—R_f 0.67; system TL—R_f 0.13; system TAE—R_f 0.20; system TAF—R_f 0.55; system TAJ—R_f 0.09; system TAK—R_f 0.02; system TAL—R_f 0.51 (Dragendorff spray—positive; Marquis reagent—grey-violet).

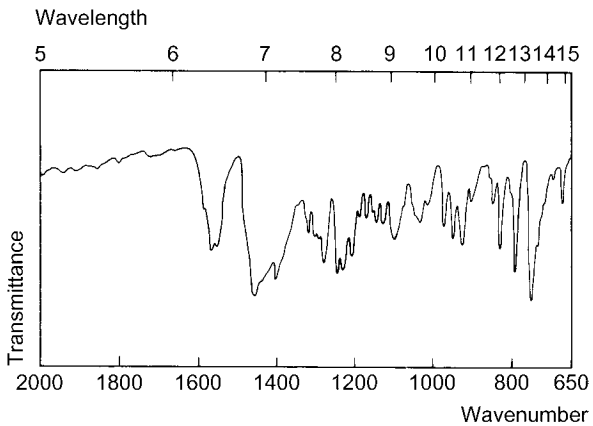
Gas Chromatography System GA—thioridazine RI 3115, mesoridazine RI 3380, M (oxo-) RI 3500, M (ring sulfone) RI 3420, M (side chain sulfone) RI 3800; system GB—thioridazine RI 3292, M (nor-) RI 3275, mesoridazine RI 3629, M (ring) RI 2639, M (ring sulfone) RI 3626; system GW—RT 31.1 min.

High Performance Liquid Chromatography System HA—thioridazine *k* 5.2, mesoridazine RI 5.0; system HX—RI 490; system HY—RI 427; system HZ—RT 13.5 min; system HAA—RT 17.2 min; system HAM—not detected; system HAX—not eluted; system HAY—RT 9.8 min; system HAZ—*k* 3.88.

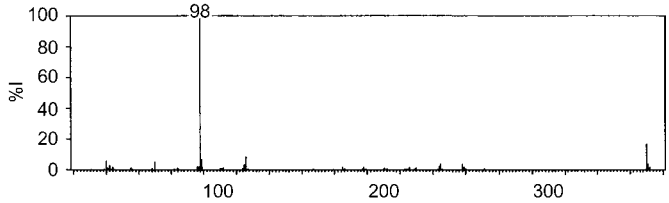
Ultraviolet Spectrum Aqueous acid—262 nm (A_1^1 = 987a), 310 nm; (0.1 mol/L hydrochloric acid)—230, 263 nm; aqueous alkali—275 nm; (0.1 mol/L sodium hydroxide)—313 nm.



Infrared Spectrum Principal peaks at wavenumbers 754, 1248, 796, 1234, 1281, 1211 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 98, 370, 126, 99, 40, 70, 371, 258 (thioridazine); 98, 70, 99, 42, 386, 126, 55, 41 (mesoridazine).



Quantification

Blood **GC** Column: cross-linked fused silica (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Retention time: 12.8 min. Limit of quantification, 122–218 µg/L, limit of detection, 37– 66 µg/L [Sánchez de la Torre *et al.* 2005].

HPLC UV detection (λ= 263 nm). Limit of quantification, 0.05 mg/L [Jortani *et al.* 1994]. Column: Bondapak CN (300 × 4 mm i.d., 10 µm). Mobile phase: methanol : 0.01% *n*-octylamine (58 : 42), flow rate 2.0 mL/min. UV detection (λ= 265 nm). Retention time: 12.5 min. Limit of detection, 4 mg/L [Allender 1985]. Column: Spherosil XOC-005 (250 × 2.8 mm i.d., 9 µm). Mobile phase: 2,2,4-trimethylpentane : 2-aminopropane : acetonitrile : ethanol. Fluorescence detection (λ_{ex}= 365 nm, λ_{em}= 440 nm). Limit of detection, 3 ng [Muusze, Huber 1974].

Plasma **GC** Column: CBP1-bonded methyl silicone (12 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. NPD. Limit of detection, 200 µg/L [Tokunaga *et al.* 1996]. Column: 3% OV-17 Gaschrom Q on 80/120 mesh (1 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 264°. FID. Retention time: 3.1 min. Limit of detection, 2 ng [Ng, Crammer 1977]. Column: 3% OV-17 on Chromosorb Q 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: He, 100 mL/min. Temperature: 275°. FID. Retention time: 3.6 min. Limit of detection, 0.05 mg/L [Dinovo *et al.* 1976]. Column: 3% XE-60 on GasChrom Q 80/100 mesh (1.83 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 240° for 15 min to 270° at 10°/min for 30 min. FID. Retention time: 2.47 min. Limit of detection, 100 µg/L [Vanderheeren, Theunis 1976]. Column: 3% OV-17 on GasChromQ 100/120 mesh. Carrier gas: N_2, 60 mL/min. Temperature: 260°. FID. Limit of detection, 0.05 mg/L [Curry, Mould 1969].

HPLC Column: Cyclobond I 2000 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 1% TEA (pH 3.0, 16 : 84), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex}= 262 nm, λ_{em}= 458 nm). Limit of quantification, 15 µg/L for thioridazine, 5 µg/L for the metabolites [Eap *et al.* 1995]. Column: IBM cyano. Mobile phase: acetonitrile : 50 mmol/L ammonium acetate (pH 6, 70 : 30), flow rate 1.5 mL/min. UV detection (λ= 254 nm). Limit of detection, 1 µg/L [Cohen *et al.* 1989]. Column: Cyano-bonded (300 × 3.9 mm i.d.). Mobile phase: 20 mmol/L disodium EDTA : THF (70 : 30), flow rate 1.5 mL/min. Electrochemical detection. Limit of detection, 0.1 ng/injection [Stoll *et al.* 1984]. Column: Spherisorb ODS C_{18}-bonded (250 × 4.6 mm i.d., 5 µm). Mobile phase: 2,2,4-trimethylpentane : methylene chloride : methanol (8 : 1 : 1) containing 0.036% methylamine, flow rate 2.25 mL/min. UV detection (λ= 254 nm). Limit of detection, 10 µg/L [Kilts *et al.* 1982].

Serum **GC** Column: SE-30 (11.5 m × 0.39 mm i.d., 0.4 µm). Carrier gas: N_2, 50 cm/s. Temperature: 260°. NSD. Retention time: 4 min 36 s. Limit of detection, 6 ng [Debruyne *et al.* 1980]. Column: 3% OV 17 on GasChrom Q (1 m × 3 mm i.d.). Carrier gas: N_2, 50 mL/min. Temperature: 260°. FID. Limit of detection, 50 mg/L [Mårtensson, Roos 1973].

HPLC Column: Inersil ODS-SP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 300 : 200 : 500), flow rate 0.9 mL/min. UV detection (λ= 250 nm). Retention time: 31.7 min. Limit of quantification, 4.0 µg/L [Tanaka *et al.* 2007]. Column: Spherisorb Chiral 1 (250 × 4.6 mm i.d.). Mobile phase: hexane : methylene chloride : methanol : 1 mol/L ammonium acetate in methanol (450 : 450 : 100 : 0.075), flow rate 1.0 mL/min. UV detection (λ= 263 nm). Limit of quantification, 50 µg/L, limit of detection, 12.5 µg/L [Jortani, Poklis 1993]. Column: Spherisorb ODS-2 Superpac (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 85% orthophosphoric acid suprrapur-TEA (1 : 2 : 1), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Retention time: 10.95 min. Limit of detection, 0.5 µmol/L [Svensson *et al.* 1990]. Column: (15 cm, 5 µm). Mobile phase: *n*-butyl chloride-propan-2-ol-water-diethylamine (92 : 7.9 : 0.08 : 0.02) : *n*-butyl chloride-propan-2-ol-water-diethylamine (91.8 : 7.9 : 0.08 : 2.0) (50 : 50), flow rate 1.7 mL/min to 2.0 mL/min over 5 min. UV detection, (λ= 279 nm). Retention time: 2.1 min. Limit of detection not reported [Hale, Poklis 1985].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 2.7 µg/L [Kirchherr, Kühn-Velten 2006]. Column: Uptisphere (125 × 2 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0)-acetonitrile : acetonitrile (90 : 10), flow rate 300 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 54 nmol/L [Gutteck, Rentsch 2003].

Urine **GC** Column: 3% OV-17 on GasChrom Q 80/120 mesh (1 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 264° or 280°. FID. Retention time:

T

4.8 min. Limit of detection not reported [Papadopoulos, Crammer 1986]. See Plasma [Ng, Crammer 1977].

HPLC Column: LiChrosorb C_8 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : TEA (40 : 40 : 20 : 2), flow rate 0.5 mL/min. UV detection (λ = 250 nm). Limit of quantification, 71.3 µg/L, limit of detection, 21.4 µg/L [Cruz-Vera *et al.* 2009]. Column: Symmetry C_8 (150 × 3.9 mm i.d., 5.0 µm). Mobile phase: 0.03 mol/L ammonium acetate (pH 5.5) : acetonitrile (60 : 40), flow rate 1.0 mL/min. UV detection (λ = 238 nm). Limit of quantification, 25 µg/L, limit of detection, 8 µg/L [Xiong *et al.* 2009]. Column: Spherisorb cyano (150 × 4.6 mm i.d., 3 µm). Mobile phase: 2,2,4-trimethylpentane : dichloromethane : methanol : diethylamine (82 : 10 : 8 : 0.1), flow rate 1.1 mL/min. UV detection (λ = 254 nm). Retention time: 2.9 min. Limit of detection, 50 µg/L [Lin *et al.* 1993]. See Blood [Allender 1985].

Bile **HPLC** See Blood [Allender 1985; Jortani *et al.* 1994].

CSF **GC** Column: 0.85% OV-225 on GasChrom Q 80/100 mesh (1 m × 2 mm i.d.). Temperature: 260°. NSD. Limit of detection not reported [Nyberg *et al.* 1981].

Gastric Contents **HPLC** See Blood [Allender 1985].

Brain **HPLC** See Blood [Jortani *et al.* 1994]. Column: µBondapak (300 × 3.9 mm i.d.). Mobile phase: 100 mmol/L ammonium acetate, 20 mmol/L citric acid and 100 µL butylamine in acetonitrile : water (38 : 62, pH 3.4), flow rate 1.5 mL/min. Electrochemical detection. Retention time: 26.7 min. Limit of detection, 50 pg/mg [Svendsen, Bird 1986].

Hair **GC-MS** Column: BP-5 (12 m × 0.53 mm i.d., 1.0 µm). Carrier gas: He, 3.0 mL/min. Temperature programme: 100° for 2 min to 310° at 10°/min for 10 min. NPD. Limit of detection, 0.1–0.25 µg/g [Couper *et al.* 1995].

LC-MS Column: RP-C8-select B (125 × 2 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium formate-0.1% formic acid (pH 3) : acetonitrile-0.1% formic acid (90 : 10 to 70 : 30 at 6.6 min to 30 : 70 at 26.6 min to 10 : 90 at 33.3 min. MRM acquisition mode. Limit of quantification, 0.042 ng/mg, limit of detection, 0.014 ng/mg [Weinmann *et al.* 2002].

Liver **HPLC** See Blood [Allender 1985; Jortani *et al.* 1994].

Disposition in the Body Readily absorbed after oral administration. It is metabolised mainly by sulfoxidation to give the side-chain sulfoxide (mesoridazine) and the side-chain sulfone (sulforidazine), both of which are active, and the ring sulfoxide and sulfone; *N*-demethylation also occurs. Other metabolites are produced by combinations of these metabolic reactions [Lin *et al.* 1993]. It is metabolised in the liver, secreted in the bile and excreted mainly in the faeces. Less than ~10% of a dose is excreted in the urine, with <1% as unchanged drug; mesoridazine and sulforidazine are also excreted in the urine in amounts slightly greater than unchanged drug.

Therapeutic Concentration Plasma concentrations of drug and metabolites show considerable intersubject variation [Mårtensson, Roos 1973]. Concentrations appear to be independent of dose and tend to decrease during chronic treatment. The therapeutic serum concentration is 0.2–1.0 mg/L (thioridazine); 0.3 mg/L (mesoridazine).

After a single oral dose of 100 mg administered in 5 subjects, the following peak serum concentrations were found: thioridazine 0.05–0.50 mg/L (mean 0.24), attained in 1–4 h; mesoridazine 0.10–0.51 mg/L (mean 0.32), attained in 2–6 h; sulforidazine 0.02–0.11 mg/L (mean 0.07), attained in 5–8 h; ring sulfoxide 0.07–0.38 mg/L (mean 0.18), attained in 4–8 h [Axelsson, Martensson 1977].

Following daily oral doses of 100–800 mg (mean 382) to 17 subjects, serum concentrations, determined 12 h after a dose, were thioridazine 0.4–2.0 mg/L (mean 0.9), mesoridazine 0.2–1.6 mg/L (mean 0.8), sulforidazine 0–0.6 mg/L (mean 0.2) and ring sulfoxide 0.06–4.0 mg/L (mean 1.5) [Axelsson *et al.* 1982].

Eight patients between 20 and 40 years of age and 8 patients over 65 years were administered a single oral dose of 25 mg thioridazine. At 4 h post-dosing, the young and elderly patient groups had concentrations of 47.6 and 106 µg/L, respectively, and at 8 h the concentrations were 33.2 and 66.6 µg/L, respectively [Cohen, Sommer 1988].

The following pharmacokinetic parameters of thioridazine, mesoridazine and sulforidazine were measured after the administration of 25, 50 or 100 mg thioridazine to 11 healthy volunteers:

Dose (mg)	C_{max} *(µg/L)*	t_{max} *(h)*
Thioridazine		
25	110.8	1.77
50	197.1	1.36
100	371.8	1.48
Mesoridazine		
25	168.5	2.63
50	334.0	3.32
100	514.1	3.17
Sulforidazine		
25	31.7	4.65
50	53.3	4.91
100	89.9	6.43

[Chakraborty *et al.* 1989]

Toxicity The estimated minimum lethal dose is 1 g. Blood concentrations greater than ~2 mg/L may produce toxic effects and can be lethal; and with sulforidazine, a total concentration of 3 mg/L drug and metabolite is toxic.

A 68-year-old man with known severe depressive illness self-administered a high dose of thioridazine; ~ 80 tablets (each 200 mg slow release). He was admitted to hospital 4 h after ingestion and gastric lavage was able to remove 25 tablets. On arrival at hospital, he was drowsy and progressively lost consciousness over the next 6 h, resulting in coma and failing respiration. Over a period of 9 days, the central nervous, cardiovascular and gastrointestinal systems showed thioridazine toxicity. At high toxic levels of the drug, 6.06–6.48 mg/L, life-threatening malignant ventricular arrhythmias occurred as well as bradycardia. The thioridazine concentration was 3.34 mg/L when the patient slipped into a coma. The patient recovered following the appropriate treatment; 10 days after ingestion, he was transferred from the intensive care unit [Schmidt, Lang 1997].

In 8 deaths involving the ingestion of thioridazine, the following thioridazine and metabolite concentrations (range in parentheses) were found as shown in table below.

In 2 deaths attributed to thioridazine overdose, the following postmortem concentrations were reported:

	Blood (mg/L)	*Liver (µg/g)**
Thioridazine	8.85, 2.43	138, 50
Mesoridazine	26.8, 0.29	–
Sulforidazine	0.87, 0	–

*Total thioridazine plus metabolites determined by a non-specific fluorimetric assay. The determination of 2 stereoisomeric ring sulfoxides is also discussed [Poklis *et al.* 1982].

Note For a study of a possible relationship between thioridazine cardiotoxicity and CYP2D6 hydroxylation capacity, see LLerena *et al.* [2002]. For a study of the postmortem distribution of thioridazine, see Dinovo *et al.* [1978]; for uptake into the human retina, see Kimbrough, Campbell [1981].

Half-life Plasma half-life, 10–36 h.

Distribution in Blood Plasma : whole blood ratio, 0.79.

Protein Binding Thioridazine >99.5%; mesoridazine and sulforidazine, ~99%.

Dose Daily dosage 50 to 300 mg thioridazine hydrochloride; maximum 600 mg daily.

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Cohen BM *et al.* (1989). A fixed dose study of the plasma concentration and clinical effects of thioridazine and its major metabolites. *Psychopharmacology (Berl)* 97: 481–488.

Drug/metabolite	*Blood (mg/L)*	*Bile (mg/L)*	*Urine (mg/L)*	*Liver (µg/g)*	*Stomach plus contents (mg)*
Thioridazine	2.5 (0.3–8.5)	34.4 (1.9–128)	1.4 (0.7–2)	39.3 (3.6–154)	390 (0.3–2500)
Thioridazine disulfoxide	2.3 (0.66–7)	12.5 (7–20.8)	3.1 (0.9–5.2)	4.1 (0.3–15)	3.6 (2.4–4.8)
Thioridazine sulfoxide	2.76 (0.2–11.4)	25.8 (1.6–57)	2.1 (0–2.1)	9.2 (0.96–29.3)	8.6 (0.03–37)
Mesoridazine	2.15 (0.07–7.2)	83.5 (4.1–157)	11.6 (7.3–15.9)	7.7 (0.15–28)	3.8 (1.9–6.8)
Sulforidazine	0.13 (0–0.5)	4.4 (0–10)	1.3 (0–7.8)	1.0 (0–2)	1.8 (0–2.1)
Desmethyl thioridazine	0.2 (0–1)	1.24 (0–2.4)	ND	0.7 (0–1.5)	ND

ND, none detected
[Allender 1985]

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Dinovo EC *et al.* (1978). Distribution of thioridazine and its metabolites in human tissues and fluids obtained postmortem. *Clin Chem* 24: 1828–1830.
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Thiotepa

Antineoplastic

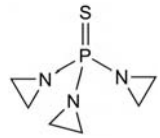
$C_6H_{12}N_3PS = 189.2$

CAS—52-24-4

IUPAC Name Tris(aziridin-1-yl)-sulfanylidene-λ^5-phosphane

Synonyms 1,1′,1″-Phosphinothioylidynetrisaziridine; TESPA; thiophosphamide; triethylenethiophosphoramide; TSPA.

Proprietary Names *Ledertepa*; *Tespamin*; *Thioplex*; *Tifosyl*.



Chemical Properties Fine white crystalline flakes. Mp 51.5°. Soluble 1 in 13 of water, 1 in about 8 of ethanol, 1 in 2 of chloroform and 1 in about 4 of ether. Log *P* (octanol/water), 0.5.

Caution Thiotepa is irritant; avoid contact with skin and mucous membranes.

Thin-layer Chromatography System TAE—R_f 0.75.

Gas Chromatography System GA—RI 1504.

Ultraviolet Spectrum No significant absorption, 230 to 360 nm.

Infrared Spectrum Principal peaks at wavenumbers 932, 1255, 729, 718, 669, 815 cm^{-1} (solution in carbon disulfide).

Quantification

Plasma **GC** Selective NPD. Thiotepa and metabolites [van Maanen *et al.* 2000].

HPLC [Tinsley *et al.* 1989].

Urine **GC** See Plasma [van Maanen *et al.* 2000].

Dose Up to 60 mg in single or divided doses by injection or by instillation.

Tinsley PW *et al.* (1989). High-performance liquid chromatographic analysis of N,N′,N″-triethylenethiophosphoramide in human plasma. *J Chromatogr* 495: 318–323.
van Maanen MJ *et al.* (2000). Stability of thioTEPA and its metabolites, TEPA, monochloroTEPA and thioTEPA-mercapturate, in plasma and urine. *Int J Pharm* 200: 187–194.

Thonzylamine

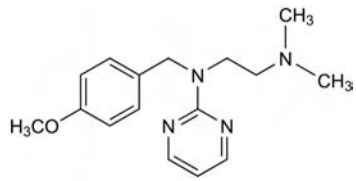
Antihistamine

$C_{16}H_{22}N_4O = 286.4$

CAS—91-85-0

IUPAC Name *N*′-[(4-Methoxyphenyl)methyl]-*N*,*N*-dimethyl-*N*′-pyrimidin-2-ylethane-1,2-diamine

Synonym Histylamine



Chemical Properties An oily liquid. Practically insoluble in water; soluble in chloroform. pK_a 2.1, 8.8 (25°).

Thonzylamine Hydrochloride

$C_{16}H_{22}N_4O,HCl = 322.8$

CAS—63-56-9

Proprietary Name *Tonamil*

Chemical Properties A white crystalline powder. Mp 173° to 176°. Soluble 1 in 1 of water, 1 in 6 of ethanol and 1 in 4 of chloroform; practically insoluble in ether.

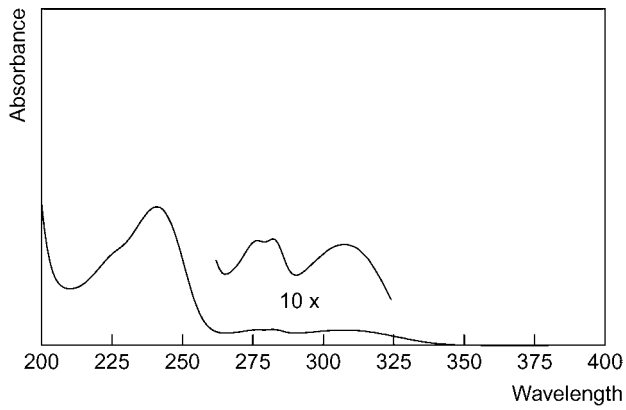
Colour Test Mandelin's test—red-violet.

Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.38; system TC—R_f 0.28; system TE—R_f 0.65; system TL—R_f 0.14; system TAE—R_f 0.22; system TAF—R_f 0.31 (Dragendorff spray, positive; Acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2203; system GC—RI 2576.

High Performance Liquid Chromatography System HA—*k* 3.2.

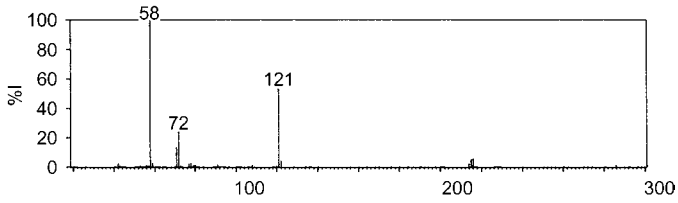
Ultraviolet Spectrum Aqueous acid—236 (A^1_1=857a), 274, 316 nm.



Infrared Spectrum Principal peaks at wavenumbers 1515, 1593, 1550, 1250, 1613, 1175 cm^{-1} (KBr disk).

T

Mass Spectrum Principal ions at *m/z* 58, 121, 72, 71, 216, 215, 122, 78.



Dose Usually 50 to 100 mg of thonzylamine hydrochloride daily.

Thozalinone

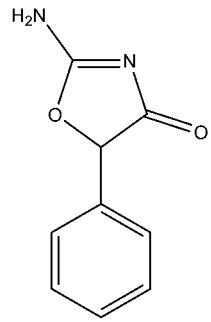
Antidepressant

$C_{11}H_{12}N_2O_2 = 204.2$

IUPAC Name 2-(Dimethylamino)-5-phenyl-1,3-oxazol-4-one

Synonyms CL-39808; 2-dimethylamino-4-oxo-5-phenyl-2-oxazoline; tozalinon(e).

Proprietary Name *Stimsen*



Chemical Properties White crystalline powder. Mp 133° to 136°. Soluble in dilute acetic acid. Thozalinone is extracted by chloroform from aqueous alkaline solutions.

Colour Test Ammonium molybdate test—pale blue, developing slowly (limit of detection, 1.0 μg).

Thin-layer Chromatography System T1—R_f 0.59 (location reagent potassium permanganate spray, positive reaction).

Ultraviolet Spectrum 0.1 N sulfuric acid—226 (E1%, 1 cm 1240), 262 (E1%, 1 cm 20) and 268 nm (E1%, 1 cm 13).

Infrared Spectrum Principal peaks at wavenumbers 1623, 1391 or 1412, 1274 cm^{-1} (KBr disk).

Disposition in the Body

Toxicity LD_{50} in mice 2 g/kg (oral).

Thurfyl Nicotinate

Peripheral Vasodilator, Rubefacient

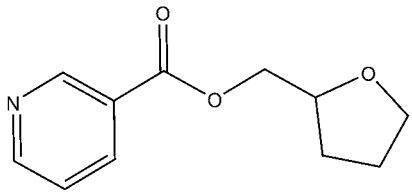
$C_{11}H_{13}NO_3 = 207.2$

CAS—70-19-9

IUPAC Name Oxolan-2-ylmethyl pyridine-3-carboxylate

Synonyms Nicotafuryl; nicotinic acid tetrahydrofurfuryl ester; tetrahydrofurfuryl nicotinate; tetrahydrofurfuryl pyridine-3-carboxylate.

Proprietary Name *Trafuril*



Chemical Properties Colourless liquid. Bp 114° to 116°. Soluble in water, oil and chloroform. Log *P* (octanol/water) 1.16 [Meylan, Howard 1995], 1.1 [National Institutes of Health 2008].

Thin-layer Chromatography System T1—R_f 0.62 (location reagent acidified iodoplatinate spray, positive reaction).

Quantification

Note For a photoelectric reflectometry and plethysmography investigation of thurfyl nicotinate in atopic dermatitis, see Thyne and Rajka [1974].

Disposition in the Body

Therapeutic Concentration

Thurfyl nicotinate has been used as a rubefacient in people with inflammatory joint diseases and in the management of mild disorders of the blood circulation of the hands and feet. Atopic individuals do not react with the erythema or urticaria of 'nonatopics'. The FDA has not approved its topical use [Fisher 1993; Fisher 1995].

Dose It is used as a topical application in creams and ointments.

Fisher AA (1993). Four flushers: topical agents producing facial flushing simulating the systemic variety. *Cutis* 51: 225–227.

Fisher AA (1995). Contactants that produce nonimmunologic erythema or urticaria: part I. *Cutis* 55: 132.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

National Institutes of Health (2008). *Thurfyl Nicotinate.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6260&loc=ec_rcs. (accessed 20 June 2008).

Thyne P, Rajka G (1974). Investigations of thurfyl nicotinate and methacholine reactions in atopic dermatitis by photoelectric reflectometry and plethysmography. *Arch Dermatol Forsch* 250: 285–294.

Thymol

Antiseptic

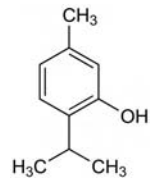
$C_{10}H_{14}O = 150.2$

CAS—89-83-8

IUPAC Name 5-Methyl-2-propan-2-ylphenol

Synonyms Acido timico; isopropylmetacresol; 5-methyl-2-(1-methylethyl)phenol; timol.

Proprietary Names *Flavinol*; *Intrasol*; *Medophyll.*



Chemical Properties Colourless crystals or white crystalline powder. Mp 51.5°. Soluble 1 in 1000 of water, 1 in 1 of ethanol, 1 in 0.6 of chloroform, 1 in 1.5 of ether and 1 in 1.7 of olive oil. pK_a 10.6 (20°). Log *P* (octanol/water), 3.3.

Colour Test Liebermann's reagent—black.

Thin-layer Chromatography System TD—R_f 0.73; system TE—R_f 0.81; system TF—R_f 0.68; system TAD—R_f 0.65; system TAF—R_f 0.92.

Gas Chromatography System GA—RI 1260.

High Performance Liquid Chromatography System HY—RI 544.

Ultraviolet Spectrum Aqueous acid—272 nm (A^1_1=137b); aqueous alkali—292 nm (A^1_1=257b).

Infrared Spectrum Principal peaks at wavenumbers 817, 1248, 1290, 1095, 1160, 952 cm^{-1}.

Mass Spectrum Principal ions at *m/z* 135, 150, 91, 107, 117.

Quantification

Plasma **GC** FID. Thymol metabolites [Kohlert *et al.* 2002].

Urine **GC** See Plasma [Kohlert *et al.* 2002].

Kohlert C *et al.* (2002). Systemic availability and pharmacokinetics of thymol in humans. *J Clin Pharmacol* 42: 731–737.

Tiabendazole

Anthelmintic

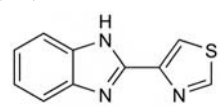
$C_{10}H_7N_3S = 201.3$

CAS—148-79-8

IUPAC Name 4-(1*H*-Benzimidazol-2-yl)-1,3-thiazole

Synonym TBZ; thiabendazole; 2-(4-thiazolyl)-1*H*-benzimidazole.

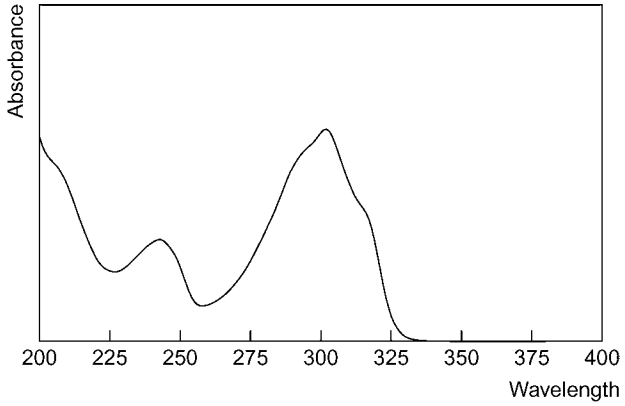
Proprietary Names *Eprofil*; *Equizole* (vet.); *Foldan*; *Folderm*; *Mintezol*; *Storite*; *Tecto*; *Thiaben*; *Thiprazole* (vet.); *Tiabiose*; *Triasox*; *Tutiverm*.



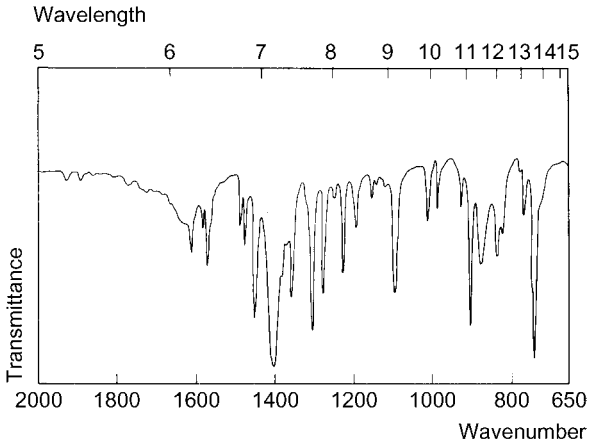
Chemical Properties A white or cream-coloured powder. Mp 300° to 305°. Practically insoluble in water; soluble 1 in 150 of ethanol, 1 in 300 of chloroform and 1 in 2000 of ether; soluble in dilute mineral acids. pK_a 4.6 (25°). Log *P* (octanol/water), 2.5.

Thin-layer Chromatography System TA—R_f 0.67; system TB—R_f 0.07; system TC—R_f 0.54; system TL—R_f 0.53; system TAE—R_f 0.75; system TAJ—R_f 0.46; system TAK—R_f 0.02; system TAL—R_f 0.59 (acidified iodoplatinate solution, positive).

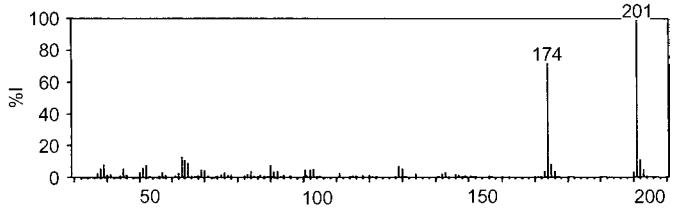
Gas Chromatography System GA—RI 2040; system GK—RRT 1.18 relative to caffeine.
High Performance Liquid Chromatography System HY—RI 234.
Ultraviolet Spectrum Aqueous acid—243, 302 nm (A^{1}_{1}=1230a).



Infrared Spectrum Principal peaks at wavenumbers 740, 1306, 902, 1095, 1279, 1231 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 201, 174, 63, 202, 64, 65, 175, 90.

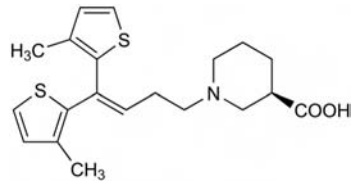


Quantification
Serum **HPLC** Fluorescence detection. Tiabendazole and 5-hydroxythiabendazole. Limit of detection, 100 µg/L and 400 µg/L, respectively [Watts *et al.* 1982].
Disposition in the Body Tiabendazole is readily absorbed after oral administration; peak plasma concentrations are attained in 1 to 2 h. It is metabolised to the 5-hydroxy derivative and excreted in the urine as conjugates. About 90% of a dose is excreted in the urine in 48 h and 5% is eliminated in the faeces. <1% of a dose is excreted in the urine unchanged.
Half-life Plasma half-life, about 1 h.
Dose Usually 25 mg/kg twice daily, to a maximum daily dose of 3 g.

Watts MT *et al.* (1982). Determination of thiabendazole and 5-hydroxythiabendazole in human serum by fluorescence-detected high-performance liquid chromatography. *J Chromatogr* 230: 79–86.

Tiagabine

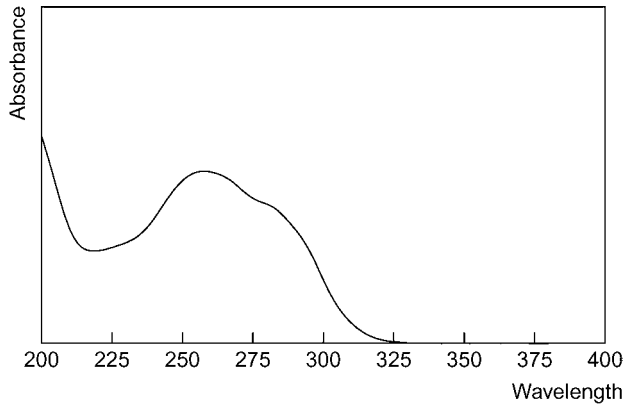
Anticonvulsant, Antidiabetic
$C_{20}H_{25}NO_2S_2$ = 375.6
CAS—115103-54-3
IUPAC Name (3*R*)-1-[4,4-Bis(3-methylthiophen-2-yl)but-3-enyl]piperidine-3-carboxylic acid
Synonyms Abbot-70569; ABT-569; NNC-05-0328; NO-05-0328; TGB.
Proprietary Name *Gabitril*



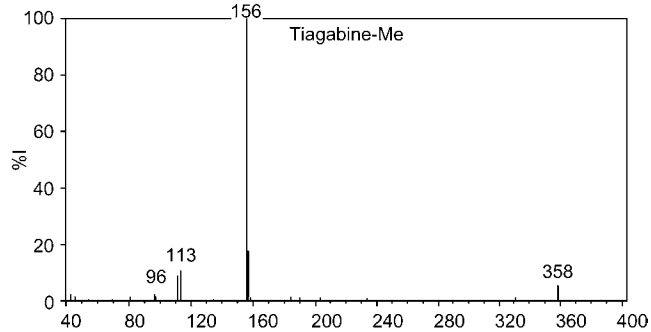
Chemical Properties pK_a 3.3. Extraction yield (chlorobutane), 0.5 [Demme *et al.* 2005].

Tiagabine Hydrochloride
$C_{20}H_{25}NO_2S_2$.HCl = 412.0
CAS—145821-59-6
Proprietary Name *Gabitril*
Chemical Properties A white to off-white odourless crystalline powder. Mp 192°. It is sparingly soluble in water; practically insoluble in hexane and heptane; soluble in aqueous base. pK_a 9.4.

Gas Chromatography-Mass Spectrometry Column: cross-linked 5% phenyl methyl siloxane HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Column temperature: 100° increased at 20°/min to 300°. Carrier gas: He. EI ionisation, SIM acquisition mode, *m/z*: 156 IS: monomethyl analogue of tiagabin. Retention time: tiagabin, 11.9 min; IS, 11.5 min [Chollet *et al.* 1999].
High Performance Liquid Chromatography Column: OD Chiracel (250 × 4.6 mm i.d.). Mobile phase: ethanol : hexane : isopropanol : trifluoroacetic acid (6 : 80 : 14 : 0.5). UV detection (λ=290 nm). Retention time: (*S*-(+)-tiagabine), 10.1 min; (*R*-(−)-tiagabine) 14.2 min [Rustum, Estrad 1998].
Column: l-phenylglycine (250 × 4.6 mm i.d., 5 µm). Mobile phase: ammonium acetate (0.1 mol/L, pH 3.7) : acetonitrile (69 : 31), flow rate 1.5 mL/min. UV detection (λ=230 nm). Retention time: *S*-(+)-tiagabine, 13.0 min; *R*-(−)-tiagabine, 15.7 min [Rustum *et al.* 1998].
Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 156, 157, 113, 111, 358, 96, 97, 81 (Tiagabine -Me).



Quantification
Serum **GC-MS** Limit of quantification, 5 µg/L, limit of detection 0.4 µg/L [Chollet *et al.* 1999].
Plasma **HPLC** Limit of quantification, 2 µg/L [Gustavson, Chu 1992].
Disposition in the Body Tiagabine is readily and virtually completely absorbed after oral administration, within 1 h of administration. Food prolongs absorption, however this reduces the rate but not the extent. Absorption and elimination pharmacokinetics are linear within the therapeutic dosage range. The drug is widely distributed throughout the body. It is extensively metabolised in the liver and excreted as the metabolite in faeces (64%) or to a lesser extent in urine (25%). Only 2% is eliminated as the unchanged drug. Hepatic metabolism occurs via cytochrome P450 enzymes CYP3A subfamily and no active metabolites have been identified. Two metabolic pathways have been identified: thiophene ring

oxidation leading to the formation of 5-oxo-tiagabine and glucuronidation. There is no drug accumulation after multiple doses and no change in pharmacokinetics in patients with renal impairment.

Therapeutic Concentration

Twenty-five patients, aged 19–64 years (mean, 44 years) with varying degrees of renal function–group (1): healthy volunteers; (2): mild renal impairment (creatinine clearance, 40–80 mL/min); (3): moderate (20–39 mL/min); (4): severe (5–19 mL/min); (5): patients requiring haemodialysis, were involved in the study. Groups 1 to 4 were orally administered with 4 mg doses of tiagabine hydrochloride as a single dose on day 1, multiple doses every 12 h for days 2 to 4 and a single dose on day 5. Group 5 was administered with a single 4 mg dose 2 h after haemodialysis. The mean total tiagabine plasma concentrations, after single doses, were 68.64, 108.09, 82.18, 80.50 and 51.65 µg/L for the 5 groups observed at 0.60, 0.50, 0.60, 0.63 and 1.20 h, respectively. For the multiple doses, the peak concentrations were 89.64, 125.25, 104.51 and 80.50 µg/L for groups 1 to 4 at 0.80, 0.44, 0.55 and 0.75 h, respectively [Cato *et al.* 1998].

Four patients with mild hepatic impairment, 2 with moderate hepatic impairment and 6 normal, healthy subjects were administered twice daily with oral tiagabine hydrochloride for 5.5 days. The healthy individuals produced peak plasma concentrations of 117 µg/L, the patients with mild hepatic impairment, 172 µg/L and moderate hepatic impairment, 172 µg/L. Minimum concentrations were 13, 27 and 28 µg/L for the 3 groups, respectively [Lau *et al.* 1997].

Toxicity

Reports of overdose are few and all episodes have recovered within 24 h. There is a broad therapeutic index. Median lethal doses are at least 50-fold higher than the maximum recommended doses (56 mg/day) [Graves *et al.* 1998].

Bioavailability 90–95%.

Half-life 7–9 h. May be reduced to 2–3 h by the concomitant administration of liver enzyme-inducing drugs.

Volume of Distribution 1–1.3 L/kg.

Clearance Mean systemic plasma clearance is 109 mL/min.

Protein Binding About 96% mainly to serum albumin and α_1 acid glycoprotein over the concentration range 10–10 000 µg/L.

Note For a review of the pharmacokinetics of tiagabine, see Adkins and Noble [1998].

Dose The usual dose is 30 to 45 mg daily.

Adkins JC, Noble S (1998). Tiagabine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the management of epilepsy. *Drugs* 55: 437–460.

Cato A *et al.* (1998). Effect of renal impairment on the pharmacokinetics and tolerability of tiagabine. *Epilepsia* 39(1): 43–47.

Chollet D *et al.* (1999). Gas chromatography-mass spectrometry assay method for the therapeutic drug monitoring of the antiepileptic drug tiagabine. *J Pharm Biomed Anal* 21: 641–646.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Graves NM *et al.* (1998). The new generation of antiepileptic drugs: unresolved questions. *Ann Pharmacother* 32(11): 1239–1243.

Gustavson L, Chu S (1992). High-performance liquid chromatographic procedure for the determination of tiagabine concentrations in human plasma using electrochemical detection. *J Chromatogr B Biomed Appl* 574;: 313–318.

Lau AH *et al.* (1997). Pharmacokinetics and safety of tiagabine in subjects with various degrees of hepatic function. *Epilepsia* 38(4): 445–451.

Rustum A, Estrad V (1998). Separation and quantitation of the S-(+)-enantiomer in the bulk drug tiagabine x HCl by chiral high-performance-liquid chromatography using a Chiralcel-OD column. *J Chromatogr B Biomed Sci Appl* 705: 111–117.

Rustum AM *et al.* (1998). Separation of the S(+) and R(-)-enantiomers of tiagabine.HCl and its two chiral precursors by chiral chromatography: application to chiral inversion studies. *J Pharm Biomed Anal* 17: 1439–1447.

Tianeptine

Antidepressant

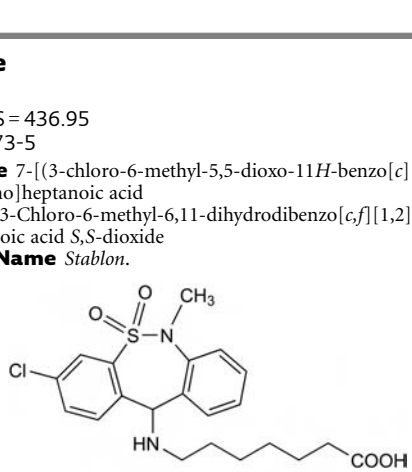
$C_{21}H_{25}ClN_2O_4S$ = 436.95

CAS—66981-73-5

IUPAC Name 7-[(3-chloro-6-methyl-5,5-dioxo-11*H*-benzo[*c*][2,1]benzothiazepin-11-yl)amino]heptanoic acid

Synonym 7-(3-Chloro-6-methyl-6,11-dihydrodibenzo[*c,f*][1,2]thiazepin-11-ylamino)heptanoic acid *S,S*-dioxide

Proprietary Name *Stablon*.



Tianeptine Sodium

$C_{21}H_{25}ClN_2NaO_4S$ = 459.9

Chemical Properties Mp 180°.

High Performance Liquid Chromatography System HAA—RT 14.9 min.

Column: microbore Hypersil ODS (100 × 2.1 mm i.d.). Mobile phase: 20 mmol/L phosphate buffer containing TEA at 500 µL/L : acetronitrile (85 : 15 to 60 : 40 at 10 min to 25 : 75 in 3.5 min to 20 : 80 in 2.5 min), flow rate 0.4 mL/min. IS: prazepam. UV detection (λ= 230 nm). Retention time: tianeptine, 9.0 min; IS, 13.7 min [Turcant *et al.* 1991].

Quantification

Blood **HPLC** Column: Nova-Pack, C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : THF: 10 mmol/L potassium dihydrogen phosphate (0.68 g/L, pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. UV detection (λ= 210 nm). Retention time: 5.40 min. Limit of detection, <120 µg/L [Tracqui *et al.* 1995]. Column: Hypersil ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (45 : 55), flow rate 1.3 mL/min. UV detection (λ= 220 nm). Limit of detection, 10 µg/L [Nicot *et al.* 1986].

Plasma **HPLC** Column: Nucleosil, C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1.5 g/L sodium heptane sulfonate buffer (pH 3, 40 : 60), flow rate 1.3 mL/min. IS: pentanoic acid. UV detection (λ= 220 nm). Retention time: tianeptine 9.5 min, IS 6.2 min. Limit of quantification, 5 µg/L for tianeptine and its metabolite pentanoic acid (MC_5) [Gaulier *et al.* 2000].

Urine **HPLC** See Blood [Nicot *et al.* 1986].

Disposition in the Body Tianeptine drug is completely absorbed, although food delays absorption. It is completely metabolised by the liver and is not subject to first-pass metabolism. Metabolism occurs mainly by extrarenal routes. β-Oxidation is the major metabolic pathway, and the principal metabolites in plasma and urine are propanoic acid (MC_3, inactive metabolite) and pentanoic acid (MC_5, active metabolite). Pharmacokinetics are linear to the dosage. Less than 3% of the dose is excreted unchanged in urine. MC_5 half-life is 7.2 h. It is eliminated partly unchanged by the kidney, and undergoes biotransformation. Plasma concentrations reach steady state in 1 month and are maintained at month 3.

Therapeutic Concentration

Twelve elderly patients, aged between 72 and 81 years, were administered a single oral dose of 12.5 mg tianeptine twice daily for 18 days. The maximum plasma concentration of tianeptine was 353 µg/L after 1.81 h and for its metabolite MC_5, 81 µg/L after 2.96 h from the single dose [Demotes-Mainard *et al.* 1991].

Twelve healthy volunteers were administered a single oral dose of 12.5 mg tianeptine following either an overnight fast or a standard breakfast. Peak plasma concentrations reached 322 µg/L in the fasted individuals after 1.29 h and were lower, 241.5 µg/L at ~1.79 h, in those who had eaten a meal before taking their dose [Dresse *et al.* 1988].

Half-life 2.5 h; increased in patients with renal failure; prolonged in patients suffering from depression (6.3 h).

Bioavailability ≈99%; 85% in the elderly,.

Volume of Distribution 0.5–0.8 L/kg; 0.41 L/kg in the elderly.

Clearance Total plasma clearance, 14.5 L/h; 40% lower in the elderly.

Protein Binding 95%.

Dose Oral administration: 12.5 mg three times daily, which may be reduced to a maximum of 25 mg daily in the elderly and patients with renal impairment.

Demotes-Mainard F *et al.* (1991). Pharmacokinetics of the antidepressant tianeptine at steady state in the elderly. *J Clin Pharmacol* 31: 174–178.

Dresse A *et al.* (1988). Influence of food on tianeptine and its main metabolite kinetics. *J Clin Pharmacol* 28: 1115–1119.

Gaulier JM *et al.* (2000). High-performance liquid chromatographic determination of tianeptine in plasma applied to pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 748: 407–414.

Nicot G *et al.* (1986). Ion-pair extraction and high-performance liquid chromatographic determination of tianeptine and its metabolites in human plasma, urine and tissues. *J Chromatogr* 381: 115–126.

Tracqui A *et al.* (1995). Systematic toxicological analysis using HPLC/DAD. *J Forensic Sci* 40: 254–262.

Turcant A *et al.* (1991). Toxicological screening of drugs by microbore high-performance liquid chromatography with photodiode-array detection and ultraviolet spectral library searches. *Clin Chem* 37: 1210–1215.

Tiaprofenic Acid

Analgesic

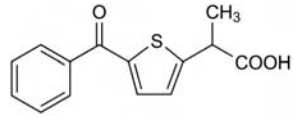
$C_{14}H_{12}O_3S$ = 260.3

CAS—33005-95-7

IUPAC Name 2-(5-Benzoylthiophen-2-yl)propanoic acid

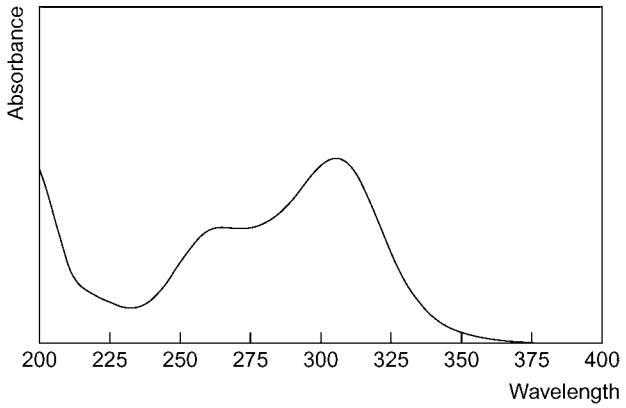
Synonyms 5-Benzoyl-α-methyl-2-thiopheneacetic acid; FC-3001; RU-15060.

Proprietary Names *Anafen*; *Artiflam*; *Artroreuma*; *Fengam*; *Flamirex*; *Gasam*; *Lindotab*; *Suralgan*; *Surdolin*; *Surgam*; *Surgamic*; *Surgamyl*; *Tiaprofen*; *Tiaprorex*.

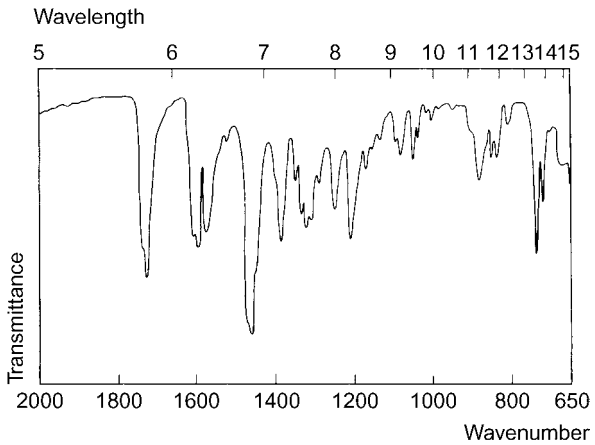


Chemical Properties A white microcrystalline powder. Mp 96°. Slightly soluble in water; soluble in ethanol, chloroform and ether. pK_a 3.0. Log *P* (octanol/water), 2.8.

Thin-layer Chromatography System TE—R_f 0.04; system TF—R_f 0.05; system TAE—R_f 0.86; system TAJ—R_f 0.30; system TAK—R_f 0.80; system TAL—R_f 0.98.
Gas Chromatography System GA—tiaprofenic acid RI 1976, tiaprofenic acid-Me_2 RI 2180.
High Performance Liquid Chromatography System HAA—retention time 17.6 min; system HX—RI 484; system HY—RI 452; system HZ—retention time 5.8 min.
Ultraviolet Spectrum Ethanolic acid—260 (A^1_1=370a), 305 nm (A^1_1=575a).



Infrared Spectrum Principal peaks at wavenumbers 1727, 718, 1593, 1198, 1605, 1572 cm^{-1} (Nujol mull).



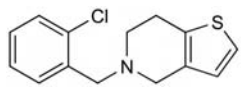
Quantification

***Plasma* HPLC** UV detection. Tiaprofenic acid enantiomers. Limit of detection, 25 µg/L [Geisslinger *et al.* 1996; Vakily, Jamali 1996]. UV detection. Limit of detection, 500 µg/L [Ward *et al.* 1982].
***Urine* GC-MS** Tiaprofenic acid and other NSAIDs. Limits of detection, 10 to 50 µg/L [Maurer *et al.* 2001].
Disposition in the Body Tiaprofenic acid is rapidly and almost completely absorbed after oral administration. It is metabolised by reduction to 2-(5-α-hydroxybenzyl-2-thienyl)propionic acid, oxidation to 2-(5-*p*-hydroxybenzoyl-2-thienyl) propionic acid and conjugation with glucuronic acid. About 60% of an oral dose is excreted in the urine in 24 h, about 55% as tiaprofenic acid (excreted mainly as an acylglucuronide conjugate), and about 5% as the two metabolites, excreted partly as acylglucuronides.
Therapeutic Concentration
Following daily oral administration of 300 mg twice a day to 6 subjects, maximum steady-state serum concentrations of 18.6 to 73.3 (mean, 48) mg/L were reported 0.5 to 1.5 h after a dose [Daymond, Herbert 1983].
Half-life Plasma half-life, 1 to 2 h.
Clearance Plasma clearance, about 1.4 mL/min/kg.
Protein Binding About 98%.
Note For a review of tiaprofenic acid, see Sorkin and Brogden [1985] and Davies [1996].
Dose 600 mg daily.

Davies NM (1996). Clinical pharmacokinetics of tiaprofenic acid and its enantiomers. *Clin Pharmacokinet* 31: 331–347.
Daymond TJ, Herbert R (1983). *Br J Clin Pharmacol* 15157P–158P.
Geisslinger G *et al.* (1996). Stereospecific determination of tiaprofenic acid in plasma: problems with drug degradation. *J Chromatogr B, Biomed Appl* 675: 77–81.
Maurer HH *et al.* (2001). Screening procedure for detection of non-steroidal anti-inflammatory drugs and their metabolites in urine as part of a systematic toxicological analysis procedure for acidic drugs and poisons by gas chromatography-mass spectrometry after extractive methylation. *J Anal Toxicol* 25: 237–244.
Sorkin E, Brogden RN (1985). Tiaprofenic acid. A review of its pharmacological properties and therapeutic efficacy in rheumatic diseases and pain states. *Drugs* 29: 208–235.
Vakily M, Jamali F (1996). Pharmacokinetics of tiaprofenic acid in humans: lack of stereoselectivity in plasma using both direct and precolumn derivatization methods. *J Pharm Sci* 85: 638–642.
Ward GT *et al.* (1982). A rapid and specific method for the determination of tiaprofenic acid in human plasma by high-performance liquid chromatography. *J Liq Chromatogr* 5(1): 165–174.

Ticlopidine

Antithrombotic
$C_{14}H_{14}ClNS$ = 263.8
CAS—55142-85-3
IUPAC Name 5-[(2-Chlorophenyl)methyl]-6,7-dihydro-4*H*-thieno[3,2-*c*]pyridine
Synonym 5-[(2-Chlorophenyl)-methyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine

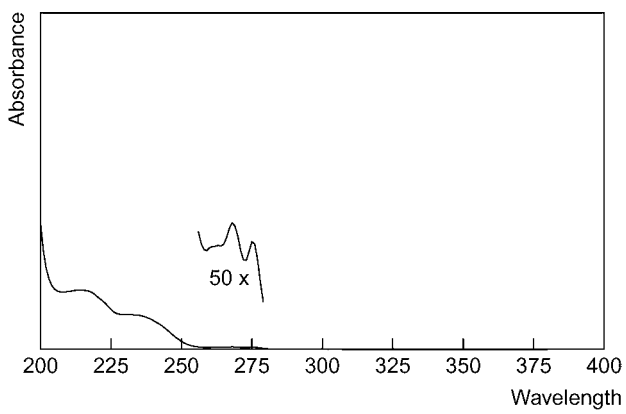


Chemical Properties pK_a 7.6. Extraction yield, 1 [Demme *et al.* 2005].

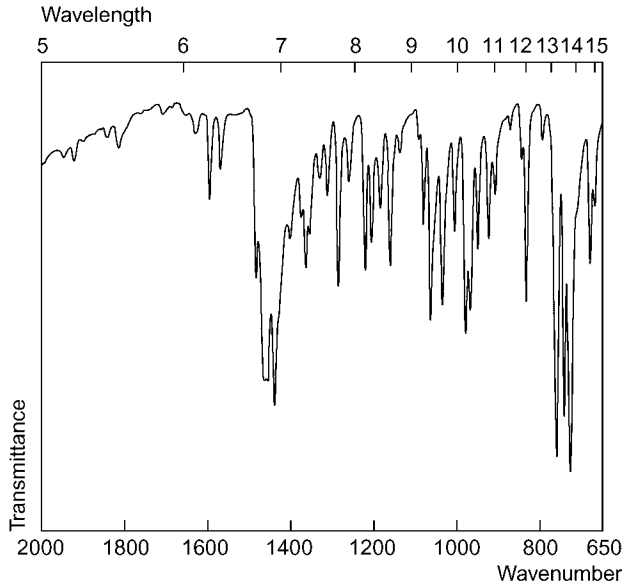
Ticlopidine Hydrochloride

$C_{14}H_{14}ClNS,HCl$ = 300.2
CAS—53885-35-1
Proprietary Names *Anagregal*; *Antigreg*; *Aplaket*; *Clox*; *Fluilast*; *Klodin*; *Opteron*; *Parsilid*; *Thrombodine*; *Thrombopat*; *Ticlid*; *Ticlodone*; *Ticloproge*; *Ticlosan*; *Tiklid*; *Tiklyd*.
Chemical Properties A white or almost white crystalline powder. Sparingly soluble in water and in dehydrated alcohol; very slightly soluble in ethyl acetate; practically insoluble in ether.

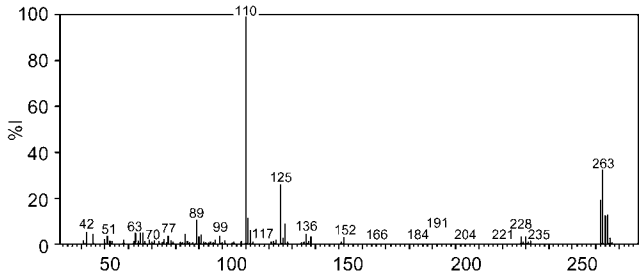
Gas Chromatography Column: methyl silicone HP1 (0.2 mm i.d., 0.33 µm). Column temperature: 250°. Carrier gas: helium, flow rate 0.9 mL/min. MS detection. RI: 2167 [Mills, Roberson 1996].
Column: 3% OV1 on Gas Chrom Q, 80 to 100 mesh glass (1.8 m × 3 mm i.d.). Column temperature: 252°. Carrier gas: helium, flow rate 30 mL/min. IS: *S*-(2,4-dichlorophenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine. MS detection (SIM at *m/z* 110). Retention time: ticlodipine, 70 s; IS, 100 s [Knudsen *et al.* 1992].
High Performance Liquid Chromatography System HAA—retention time 13.8 min.
Column: LC-8-DB Supelcosil (150 × 4.6 mm i.d., 5 µm); (guard) LC-8-DB Supelguard (20 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : potassium dihydrogen phosphate (0.05 mol/L, pH 3, containing 0.2% triethylamine) (20 : 25 : 55), flow rate 1 mL/min. UV detection (λ=235 nm). Retention time: 7.6 min [Dal Bo *et al.* 1995].
Column: ODS Hypersil (100 × 4.5 mm i.d., 5 µm). Mobile phase: methanol : water : concentrated ammonia solution (80 : 20 : 1), flow rate 1 mL/min. IS: *S*-(2,4-dichlorophenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine. UV detection (λ=210 nm). Retention time: ticlodipine, 5.5 min; IS, 10 min [Knudsen *et al.* 1992].
Column: ODS Hypersil (100 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : dihydrogen potassium phosphate buffer (0.01 mol/L, pH 3.5) (90 : 10), flow rate 1 mL/min. UV detection. Retention time: 2.5 min [Mills, Roberson 1996].
Ultraviolet Spectrum Aqueous acid—214, 231, 268, 275 nm.



Infrared Spectrum Principal peaks at wavenumber 1457, 971, 717 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 110, 263, 125, 262, 265, 264, 111, 89.



Quantification

Plasma **GC** NPD. Limit of detection, 0.01 mg/L [Shah *et al.* 1991]. NPD. Limit of detection, 0.002 mg/L [Desager *et al.* 1990].

GC-MS Limit of detection, 5 g/L [Knudsen *et al.* 1992].

HPLC UV detection (λ=215 nm). Limit of quantification, 0.01 mg/L, limit of detection, 0.005 mg/L [Róna *et al.* 1997]. UV detection (λ=235 nm). Limit of detection, 0.005 mg/L [Dal Bo *et al.* 1995].

Disposition in the Body Ticlopidine is rapidly and almost completely absorbed (80 to 90%) after oral administration. The bioavailability is improved when the drug is administered with food, especially a high-fat meal, and the rate and extent of absorption is slightly improved. Peak plasma concentrations occur after about 2 h. Pharmacokinetics are nonlinear, with clearance decreasing markedly on repeated dosing. The drug is rapidly and extensively metabolised in the liver by *N*-dealkylation and oxidation with a number of highly polar urinary and biliary metabolites, which have not been identified. Further metabolism and conjugation may also occur. Approximately 60% of an administered dose is excreted in urine as metabolites, and 25% is eliminated in faeces. <1% of the drug appears in urine unchanged. Accumulation of the drug may occur with repeated dosing.

Therapeutic Concentration

Administration of a single oral dose of 250 mg to 12 young subjects (mean, 28.6 years) and 13 elderly subjects (mean, 69.5 years) after a light breakfast, resulted in mean peak plasma concentrations of 0.41 and 0.70 mg/L, respectively at 2 h. Administration of 250 mg twice a day for 21 days resulted in mean peak plasma concentrations of 0.89 mg/L in the young group and 1.42 mg/L in the elderly group after 1 and 2 h, respectively [Shah *et al.* 1991].

Twelve healthy male volunteers with a mean age of 32 years were fasted from midnight the evening before and administered with a single dose of 250 mg in a fasted state, 30 min after a high-fat breakfast, or immediately after ingestion of an antacid. The peak concentrations were 0.573 mg/L for the fasted individuals, 0.695 mg/L for the fed individuals and 0.375 mg/L for those who also took antacid. These concentrations were observed 1.917, 1.708 and 2.000 h after administration [Shah *et al.* 1990].

Half-life Plasma half-life, 7.9 h (in young volunteers) and 12.7 h (elderly) after a single dose; 91 h (young) and 98 h (elderly) after repeated dosing.

Protein Binding 98%.

Note For reviews of ticlopidine, see Saltiel and Ward [1987] and McTavish *et al.* [1990].

For a review of the pharmacokinetics of ticlopidine, see Desager [1994].

Dose 250 mg of the hydrochloride twice daily.

Dal Bo L *et al.* (1995). Determination of ticlopidine in human plasma by high-performance liquid chromatography and ultraviolet absorbance detection. *J Chromatogr B Biomed Appl* 665: 404–409.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Desager JP (1994). Clinical pharmacokinetics of ticlopidine. *Clin Pharmacokinet* 26: 347–355.

Desager JP *et al.* (1990). Pharmacokinetic profile and bioavailability of a new galenic formulation of ticlopidine. *Int J Clin Pharmacol Res* 10(4): 247–250.

Knudsen JB *et al.* (1992). Pharmacokinetics of ticlopidine during chronic oral administration to healthy volunteers and its effects on antipyrine pharmacokinetics. *Xenobiotica* 22(5): 579–589.

McTavish D *et al.* (1990). Ticlopidine. An updated review of its pharmacology and therapeutic use in platelet-dependent disorders. *Drugs* 40: 238–259.

Mills T, Roberson JC (1996). *Instrumental Data for Drug Analysis*, 2nd edn. Boca Raton, FL: CRC Press, Vol. 5, 392–393.

Róna K *et al.* (1997). Liquid chromatographic method for the determination of ticlopidine in human plasma. *J Chromatogr B Biomed Sci Appl* 693: 393–398.

Saltiel E, Ward A (1987). Ticlopidine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in platelet-dependent disease states. *Drugs* 34: 222–262.

Shah J *et al.* (1990). Effect of food and antacid on absorption of orally administered ticlopidine hydrochloride. *J Clin Pharmacol* 30: 733–736.

Shah J *et al.* (1991). Single and multiple dose pharmacokinetics of ticlopidine in young and elderly subjects. *Br J Clin Pharmacol* 32: 761–764.

Tienilic Acid

Diuretic

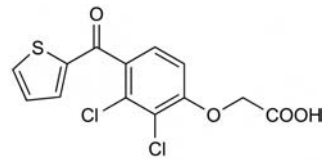
$C_{13}H_8Cl_2O_4S = 331.2$

CAS—40180-04-9

IUPAC Name 2-[2,3-Dichloro-4-(thiophene-2-carbonyl)phenoxy]acetic acid

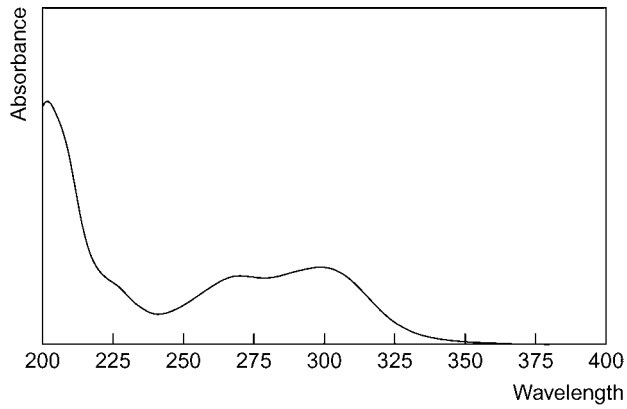
Synonyms Thienylic acid; ticrynafen; ANP-3624; CE-3624; SKF-62698.

Proprietary Names *Diflurex*; *Selacryn*.

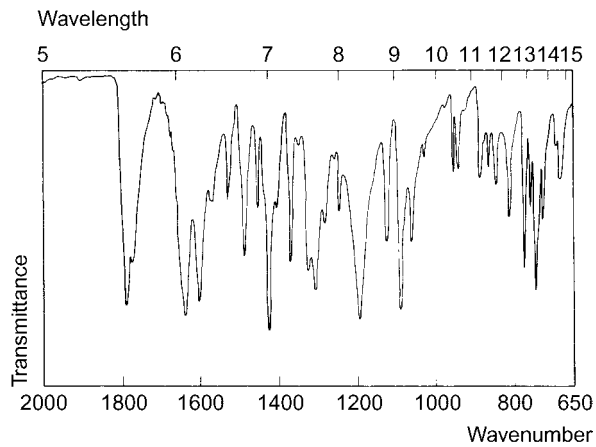


Chemical Properties A white crystalline powder. Mp 148° to 149°; also reported as 157°. Very slightly soluble in water; soluble 1 in 14 of ethanol, 1 in about 5 of acetone, 1 in 77 of chloroform and 1 in about 6 of methanol. pK_a 2.7 (25°). Log *P* (octanol/water), 3.6.

Ultraviolet Spectrum Ethanol—267 (A_1^1=343a), 296 nm (A_1^1=384a).



Infrared Spectrum Principal peaks at wavenumbers 1185, 1620, 1075, 1762, 1580, 1290 cm^{-1} (KBr disk).



T

Quantification
Plasma **GC** ECD. Tienilic acid and metabolites. Limit of detection, 10 µg/L [Hwang *et al.* 1978].

HPLC UV detection. Tienilic acid and monohydroxy metabolite. Limit of detection, 200 µg/L [Kerremans *et al.* 1982].
Serum **GC** See Plasma [Hwang *et al.* 1978].
Urine **GC** See Plasma [Hwang *et al.* 1978].

HPLC Tienilic acid and 5-hydroxy-tienilic acid [Mansuy *et al.* 1984]. See Plasma [Kerremans *et al.* 1982].
Disposition in the Body Tienilic acid is absorbed after oral administration. It is metabolised by hydroxylation and oxidation. About 40% of a dose is excreted in the urine in 24 h; about 20% of the dose is excreted in the urine as unchanged drug, up to 10% as [2,3-dichloro-4-(α-hydroxy-2-thenyl)phenoxy]acetic acid, about 15% as a dihydroxylated metabolite, and 1 to 2% as (2,3-dichloro-4-carboxyphenoxy) acetic acid.
Therapeutic Concentration

After a single oral dose of 250 mg to 8 subjects, mean peak plasma concentrations of 11 mg/L of tienilic acid, 0.7 mg/L of the monohydroxylated metabolite, and 0.07 mg/L of the *p*-carboxy metabolite were attained in 3 to 4 h [Hwang *et al.* 1978].

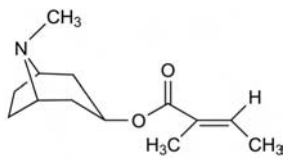
Following a single oral dose of 250 mg to 8 subjects, peak plasma concentrations of 7.7 to 27.4 (mean, 16) mg/L of tienilic acid were attained in 1 to 2 h [Kerremans *et al.* 1982].

Half-life Plasma half-life, 1.5 to 4 h.
Distribution in Blood Plasma : whole blood ratio, about 1.6.
Protein Binding About 99%.
Dose Tienilic acid has been given in doses of 0.25 to 1 g daily.

Hwang B *et al.* (1978). GLC determination of tricrynafen and its metabolites in urine, serum, and plasma of humans and animals. *J Pharm Sci* 67: 1095–1098.
Kerremans AL *et al.* (1982). Pharmacokinetic and pharmacodynamic studies of tienilic acid in healthy volunteers. *Eur J Clin Pharmacol* 22: 515–521.
Mansuy D *et al.* (1984). Metabolic hydroxylation of the thiophene ring: isolation of 5-hydroxy-tienilic acid as the major urinary metabolite of tienilic acid in man and rat. *Biochem Pharmacol* 33: 1429–1435.

Tigloidine

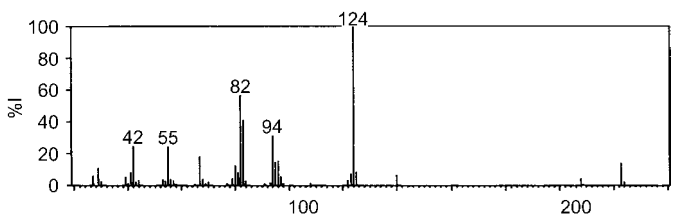
Anticholinergic
$C_{13}H_{21}NO_2$ = 223.3
CAS—495-83-0
IUPAC Name (8-Methyl-8-azabicyclo[3.2.1]octan-3-yl) (*E*)-2-methylbut-2-enoate
Synonyms 2-Methyl-2-butenoic acid [1α,3α(*E*),5α]-8-methyl-8-azabicyclo [3.2.1]oct-3-yl ester; tiglylpseudotropeine.



Chemical Properties Tigloidine was first isolated from *Duboisia myoporoides* (*Solanaceae*) but is now synthesised. A thin, syrupy liquid.

Tigloidine Hydrobromide
$C_{13}H_{21}NO_2$,HBr = 304.2
CAS—22846-83-9
Proprietary Name *Tiglyssin*
Chemical Properties A white crystalline solid. Mp 234° to 235°. Soluble in water and chloroform.

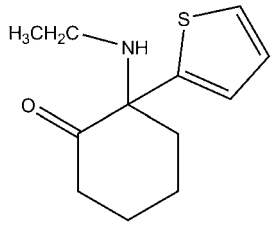
Thin-layer Chromatography System TA—R_f 0.42; system TL—R_f 0.07; system TB—R_f 0.39; system TC—R_f 0.21 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1687.
High Performance Liquid Chromatography System HA—*k* 3.6 (tailing peak).
Infrared Spectrum Principal peaks at wavenumbers 1700, 1266, 1136, 1036, 1156, 1073 cm^{-1} (thin film).
Mass Spectrum Principal ions at *m/z* 124, 82, 83, 94, 55, 42, 67, 96.



Dose 1 to 2 g of tigloidine hydrobromide daily.

Tiletamine

Anaesthetic, Anticonvulsant
$C_{12}H_{17}NOS$ = 223.3
IUPAC Name 2-(Ethylamino)-2-thiophen-2-ylcyclohexan-1-one
Synonym 2-Ethylamino-2-thien-2-ylcyclohexanone



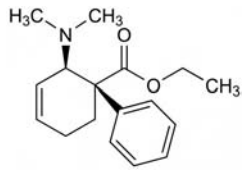
Chemical Properties Tiletamine is extracted by chloroform from aqueous alkaline solutions.

Tiletamine Hydrochloride
Synonyms CI-634; CL-399; CN-54521-2.
Chemical Properties White crystalline powder. Mp 193° to 194°. Soluble in water and chloroform.

Colour Test Vitali's test— –/–/blue-green (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.68 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 2.67 relative to diphenhydramine, retention time 0.60 relative to codeine; System G4/225—retention time 2.70 relative to diphenhydramine retention time 0.30 relative to codeine.
Ultraviolet Spectrum 0.1 N sulfuric acid—234 nm (E1%, 1 cm 265) (tiletamine hydrochloride).
Infrared Spectrum Principal peaks at wavenumbers 1698, 697, 1116 or 1437 cm^{-1} (tiletamine base) (KBr disk)
Disposition in the Body
Toxicity LD_{50} in mice 95.5 mg/kg (IP).

Tilidate

Narcotic Analgesic
$C_{17}H_{23}NO_2$ = 273.4
CAS—20380-58-9
IUPAC Name Ethyl (1*S*,2*R*)-2-(dimethylamino)-1-phenylcyclohex-3-ene-1-carboxylate
Synonyms (1*R*,2*S*)-*rel*-2-(Dimethylamino)-1-phenyl-3-cyclohexene-1-carboxylic acid ethyl ester; tilidine.



Chemical Properties Log *P* (octanol/water), 3.7. Extraction yield (chlorobutane), 1 (tilidine) [Demme *et al.* 2005].

Tilidate Hydrochloride
$C_{17}H_{23}NO_2$,HCl,$\frac{1}{3}H_2O$ = 318.8
CAS—27107-79-5 (anhydrous)
Proprietary Names *Findol N*; *Tili*; *Tilicomp*; *Tilidalor*; *Tilidin*; *Tiligetic*; *Tilimerck*; *Tilitrate*; *tilnalox*; *Valoron*; *Valtran*.
Chemical Properties A white crystalline powder. Mp 125°. Soluble in water.

Thin-layer Chromatography System TAE—R_f 0.61; system TE—R_f 0.84.
Gas Chromatography System GA—tilidate RI 1838, bisnortilidate RI 1827, nortilidate RI 1830.
High Performance Liquid Chromatography System HY—RI 290.
Ultraviolet Spectrum Aqueous acid—251 nm (A^1_1 = 6.2b), 257 nm (A^1_1 = 7.6b), 262 nm (A^1_1 = 6.1b).
Infrared Spectrum Principal peaks at wavenumbers 1714, 1238, 1182, 1263, 1167, 703 cm^{-1} (tilidate hydrochloride; see below).

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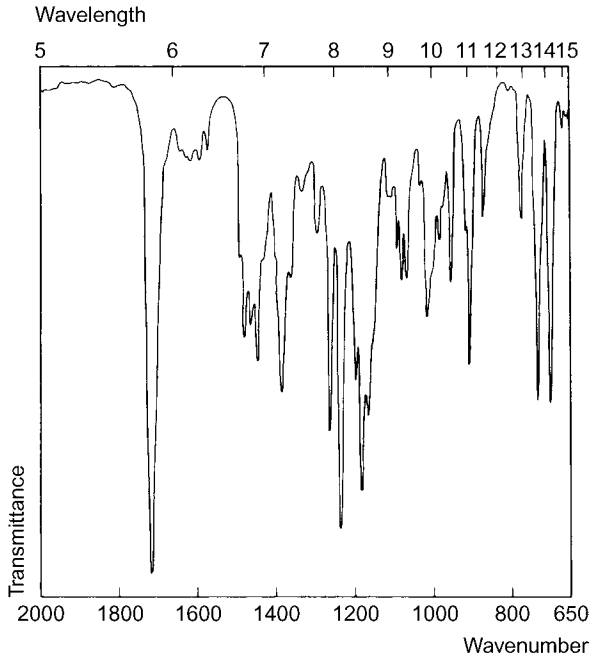


Mass Spectrum Principal ions at *m/z* 103, 77, 82, 29, 97, 42, 51, 104 (tilidate); 69, 68, 70, 77, 103, 56, 54, 51 (bisnortilidate); 83, 68, 82, 72, 84, 77, 103, 115 (nortilidate).

Quantification

Plasma **GC** Column: Chrompack Sil-19 (25 m × 0.22 mm i.d., 0.2 µm). Carrier gas: He, 0.8 bar. Temperature programme: 160° for 2 min to 225° at 5°/min for 10 min. NPD. Limit of detection, 6 µg/L [Cordonnier *et al.* 1987a]. Column: 3% OV25 on GasChrom Q 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 220°. Retention time: 8.2 and 9.2 min for (+)- and (–)-nortilidine, repectively. Limit of detection, 25 ng for (+)- or (–)-nortilidine [Hengy *et al.* 1978a]. Column: 1% CRS 101 and 1.5% LAC-4-*R*-886 on 80/100 mesh GasChrom Q (1.8 m × 2 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 165°. NSD. Retention time: 3.7 min. Limit of detection, 1 µg/L [Hengy *et al.* 1978b].

GC-MS See GC. EI ionistaion at 70 eV, SIM acquisition mode. Limit of detection, 10 µg/L [Cordonnier *et al.* 1987a].

LC-MS Column: Phenomenex C_{12} MAX-RP (150 × 2 mm i.d., 4 µm). Mobile phase: water-acetonitrile (90 : 10) with 5 mmol/L ammonium formate (pH 3.5) : water-acetonitrile (10 : 90) with 5 mmol/L ammonium formate (pH 3.5, 100 : 0 for 5 min to 0 : 100 at 19 min for 7 min to 100 : 0 in 3 min for 6 min), flow rate 200 µL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.8 µg/L, limit of detection, 0.2 µg/L [Musshoff *et al.* 2006].

Urine **GC** See Plasma [Hengy *et al.* 1978a; Hengy *et al.* 1978b].

Hair **LC-MS** Phenomenex C_{12} Synergi Max-RP (150 × 2 mm i.d., 4 µm). Mobile phase: water-acetonitrile (90 : 10) with 5 mmol/L ammonium formate (pH 3.5) : water-acetonitrile (10 : 90) with 5 mmol/L ammonium formate (pH 3.5, 100 : 0 for 5 min to 0 : 100 at 19 min for 7 min to 100 : 0 in 3 min for 6 min), flow rate 200 µL/min. TIS, ESI, positive ion mode. Limit of quantification, 8.7 pg/mg, limit of detection, 2.4 pg/mg [Musshoff *et al.* 2007].

Disposition in the Body Tilidate is rapidly and almost completely absorbed after oral administration. It is rapidly metabolised by *N*-demethylation to form nortilidate and bisnortilidate; these metabolites may then be conjugated with glucuronic acid. CYP3A4 and CYP2C19 have been identified as the main isoforms responsible for the elimination of tilidate [Weiss *et al.* 2008]. Approximately 80% of an oral dose is excreted in the urine in 24 h, with less than 0.1% of a dose as unchanged drug, approx. 1.5% as nortilidate, 2.5% as bisnortilidate and the rest as unknown metabolites. Up to 10% may be eliminated in the faeces.

Therapeutic Concentration

Eight patients with severe hepatic impairment were administered 100 mg tilidate. The maximum tilidate concentration varied from 66 to 290 µg/L, with a mean of 139 µg/L. The elimination half-lives ranged between 2.1 and 19.8 h with a mean of 8.5 h [Brennscheidt *et al.* 2007].

Following a single oral dose of 50 mg in one subject, peak plasma concentrations of 0.127 mg/mL (+)-nortilidate and 0.121 mg/mL (−)-nortilidate were attained in ~2 h [Hengy *et al.* 1978b].

Toxicity

In a fatal intoxication involving the ingestion of an unknown quantity of tilidate by a 29-year-old man, the postmortem tissue concentrations of tilidate, nortilidate and bisnortilidate, respectively, were as follows: blood 1.74, 4.41 and 0.15 mg/L; lung 5.75, 21.70 and 2.12 µg/g; liver 9.40, 13.70 and 3.51 µg/g; bile 2.88, 6.54 and 0.90 mg/L; kidney 2.78, 12.30 and 2.01 µg/g; urine 0.94, 6.75 and 4.10 mg/L [Cordonnier *et al.* 1987b].

A 28-year-old woman had a serum tilidate concentration of 38.1 mg/L 3 h after ingesting 100 mL of a preparation containing 6.94 g tilidate (and approx. 0.56 g naloxone) in a suicide attempt; the concentration of nortilidate was 18.8 mg/L [Regenthal *et al.* 1998].

Half-life After oral dosing, 3.3 h; after IV administration, 4.9 h; after multiple dosing, 3.6 h [Vollmer *et al.* 1989].

Dose The equivalent of up to 400 mg of the anhydrous hydrochloride daily.

Brennscheidt U *et al.* (2007). Pharmacokinetics of tilidine and naloxone in patients with severe hepatic impairment. *Arzneimittelforschung* 57: 106–111.
Cordonnier J *et al.* (1987). Comparison of a GLC-NPD method with a GLC-MS-SIM procedure for the determination of tilidine and its metabolites in plasma. *J Anal Toxicol* 11: 144–148.
Cordonnier J *et al.* (1987). Disposition of tilidine in a fatal poisoning in man. *J Anal Toxicol* 11: 105–109.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Hengy H *et al.* (1978a). Gas-chromatographic determination of nanogram amounts of enantiomers of nortilidine, a main metabolite of tilidine, in biological specimens. *Clin Chem* 24: 692–697.
Hengy H *et al.* (1978b). GLC determination of tilidine, nortilidine, and bisnortilidine in biological fluids with a nitrogen-sensitive detector. *J Pharm Sci* 67: 1765–1768.
Musshoff F *et al.* (2007). Determination of opioid analgesics in hair samples using liquid chromatography/tandem mass spectrometry and application to patients under palliative care. *Ther Drug Monit* 29: 655–661.
Musshoff F *et al.* (2006). An automated and fully validated LC-MS/MS procedure for the simultaneous determination of 11 opioids used in palliative care, with 5 of their metabolites. *J Mass Spectrom* 41: 633–640.
Regenthal R *et al.* (1998). Poisoning with tilidine and naloxone: toxicokinetic and clinical observations. *Hum Exp Toxicol* 17: 593–597.
Vollmer KO *et al.* (1989). Pharmacokinetics of tilidine and metabolites in man. *Arzneimittelforschung* 39: 1283–1288.
Weiss J *et al.* (2008). In vitro metabolism of the opioid tilidine and interaction of tilidine and nortilidine with CYP3A4, CYP2C19, and CYP2D6. *Naunyn Schmiedebergs Arch Pharmacol* 378: 275–282.

Tilmicosin

Antibacterial (Veterinary)

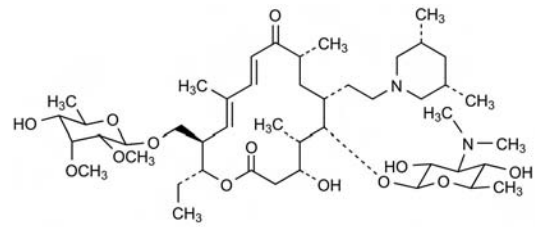
$C_{46}H_{80}N_2O_{13}$ = 869.2

CAS—108050-54-0

IUPAC Name (5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-6-[(2*R*,3*R*,4*S*,5*S*,6*R*)-4-(Dimethylamino)-3,5-dihydroxy-6-methyloxan-2-yl]oxy-7-[2-(3,5-dimethylpiperidin-1-yl)ethyl]-16-ethyl-4-hydroxy-15-[[(2*R*,3*R*,4*R*,5*R*,6*R*)-5-hydroxy-3,4-dimethoxy-6-methyloxan-2-yl]oxymethyl]-5,9,13-trimethyl-1-oxacyclohexadeca-11,13-diene-2,10-dione

Synonyms 4^A-*O*-De(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-20-deoxo-20-(3,5-dimethyl-1-piperidinyl)tylosin; EL-870; LY-177370.

Proprietary Names *Micotil*; *Pulk motil.*

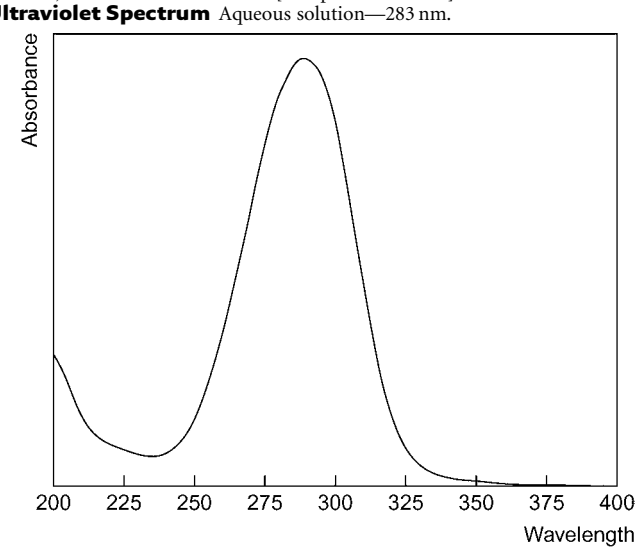


cis-form

Chemical Properties Tilmicosin contains not less than 85% $C_{46}H_{80}N_2O_{13}$, calculated on an anhydrous basis. The content of tilmicosin *cis*-isomers is between 82% and 88%, and the content of tilmicosin *trans*-isomers is between 12% and 18%. pK_a (25°) 8.18. Log *P* (octanol/water), 3.80.

High Performance Liquid Chromatography Column: RP18 Lichrospher (125 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : trifluroacetic acid (0.1%) in water (20 : 20 : 60 to 25 : 55 : 20 in 10 min), flow rate 0.5 mL/min. Detection: MS–MS (NCI: isotopic M^-, $m/z \rightarrow 869$; M^-, $m/z \rightarrow 868$; isotopic, $m/z \rightarrow 678$). Retention time: 5.7 min [Delepine *et al.* 1996].

Ultraviolet Spectrum Aqueous solution—283 nm.



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Infrared Spectrum Principal peaks at wavenumber 1592, 1167, 1141, 1081, 1058 cm^{-1} (acetate).

Quantification

Serum **HPLC** Limit of detection, 0.05 mg/L [Moran *et al.* 1997].

Disposition in the Body

Toxicity May be fatal in humans.

There are 36 cases of accidental exposure where less than 1 mL was given. All medical outcomes were benign [McGuigan 1994].

Delepine B *et al.* (1996). Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry. *J AOAC Int* 79: 397–404.

McGuigan M (1994). Human exposures to tilmicosin (MICOTIL). *Vet Hum Toxicol* 36: 306–308.

Moran JW *et al.* (1997). Determination of tilmicosin in bovine and porcine sera by liquid chromatography. *J AOAC Int* 80(6): 1183–1189.

Tiludronic Acid

Bone Resorption Inhibitor

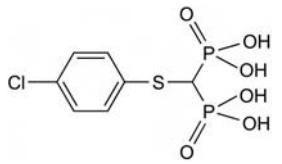
$C_7H_9ClO_6P_2S$ = 318.6

CAS—89987-06-4

IUPAC Name [(4-Chlorophenyl)sulfanyl-phosphonomethyl]phosphonic acid

Synonyms ACPMD; Cl-TMBP; [[(4-chlorophenyl)thio]methylene]bisphosphonic acid; ME-3737; SR-41319; tiludronate.

Proprietary Name *Skelid*



Disodium Tiludronate

$C_7H_7ClNa_2O_6P_2S$ = 362.6

CAS—149845-07-8 (anhydrous); 155453-10-4 (hemihydrate)

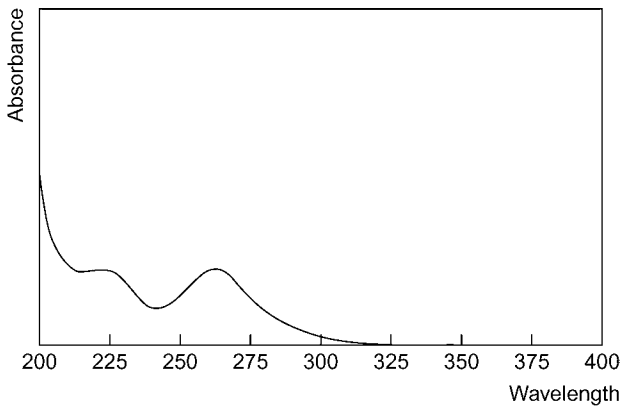
Synonyms Disodium dihydrogen; SR-41319B; Tiludronate disodium.

Proprietary Name *Skelid*

Chemical Properties Mp 253°.

High Performance Liquid Chromatography Column: PRP-1 Hamilton (5 µm, 150 × 4.1 mm i.d.). Mobile phase: tetrabutyl ammonium phosphate (0.005 mol/L)-sodium hydrogen phosphate (0.05 mol/L) : acetonitrile, (pH 11.8) (87 : 13), flow rate 1.0 mL/min. UV detection (λ=280 nm). Retention time: 2.5 min [Fels *et al.* 1988].

Ultraviolet Spectrum



Quantification

Plasma **HPLC** Limit of quantification, 0.05 mg/L [Fels *et al.* 1988].

Urine **HPLC** Limit of quantification, 0.2 mg/L, see Plasma [Fels *et al.* 1988].

Disposition in the Body The drug is rapidly absorbed following oral administration and is absorbed as the free acid. Absorption is decreased by food, especially by products containing calcium or other polyvalent cations. Approximately half of the absorbed portion is bound to bone, and is only very slowly excreted. The remainder is excreted unchanged in urine. After an IV dose, ~50% of a dose is excreted in urine over 13 days. Peak plasma concentrations are reached within 1 to 2 h but are below the limit of detection within 24 h. Steady state is achieved within 9 to 12 days. Concentration of the drug in the bone continues to increase for around 30 days.

Therapeutic Concentration

Twelve healthy individuals were administered with 400 mg tiludronate 1 h after an antacid containing aluminium and magnesium hydroxide was administered. Peak plasma concentrations were 3.35, 1.59 and 2.90 mg/L for the three dosing regimens observed at 1.7, 1.3 and 1.9 h, respectively [Sansom *et al.* 1995].

Bioavailability Approximately 6% in the fasting state. Reduced by 90% when administered within 2 h of food.

Half-life 40 to 150 h.

Volume of Distribution 30 to 60 L.

Clearance Plasma, 1.3 L/h.

Distribution in Blood Binding to red blood cells is <5% of the total concentration.

Protein Binding 90%, mainly to albumin.

Dose 400 mg daily for 3 months.

Fels JP *et al.* (1988). Determination of (4-chlorophenyl)thiomethylene bisphosphonic acid, a new bisphosphonate, in biological fluids by high-performance liquid chromatography. *J Chromatogr* 430: 73–79.

Sansom LN *et al.* (1995). Human pharmacokinetics of tiludronate. *Bone* 17(5): 479S–483S.

Timolol

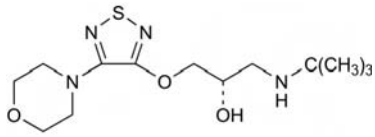
β-*Blocker*

$C_{13}H_{24}N_4O_3S$ = 316.4

CAS—26839-75-8

IUPAC Name (2*S*)-1-(Tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol

Synonym (2*S*)-1-[(1,1-Dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thia-diazol-3-yl]oxy]-2-propanol



Chemical Properties Log *P* (octanol/water), 1.8. Extraction yield, 0.6 [Demme *et al.* 2005].

Timolol Maleate

$C_{13}H_{24}N_4O_3S,C_4H_4O_4$ = 432.5

CAS—26921-17-5

Synonym MK-950

Proprietary Names *Aquanil*; *Betim(ol)*; *Blocadren*; *Cusimolol*; *Dispatim*; *Glaucopt*; *Glaucol*; *Nyogel*; *Nyolol*; *Oftamolol*; *Oftan*; *Optimol*; *Timabak*; *Timacor*; *Timoftal*; *Timoptic*; *Timoptol*; *Timosil*. It is an ingredient of *Cosopt*, *Moducren* and *Prestim*.

Chemical Properties A white crystalline powder. Mp 201.5° to 202.5°. Soluble in water, ethanol and methanol; sparingly soluble in chloroform; practically insoluble in ether.

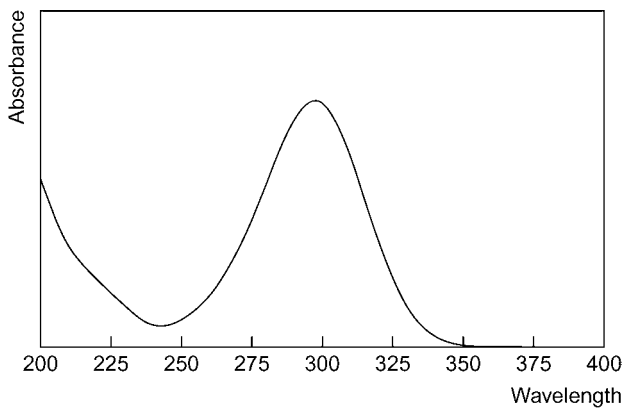
Colour Test Liebermann's reagent (100°)—violet.

Thin-layer Chromatography System TA—R_f 0.52; system TB—R_f 0.06; system TC—R_f 0.11; system TE—R_f 0.50; system TL—R_f 0.09; system TAE—R_f 0.20; system TAF—R_f 0.75.

Gas Chromatography System GA—timolol RI 2266, timolol-Art RI 2275; system GB—timolol RI 2373, timolol-Art RI 2380.

High Performance Liquid Chromatography System HA—*k* 1.2; system HAA—retention time 10.3 min; system HX—RI 317; system HY—RI 250; system HZ—retention time 2.1 min.

Ultraviolet Spectrum Aqueous acid—295 nm (A^1_1=279a). No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1497, 1527, 1120, 1230, 1590, 1620 cm^{-1} (timolol maleate, KBr disk).

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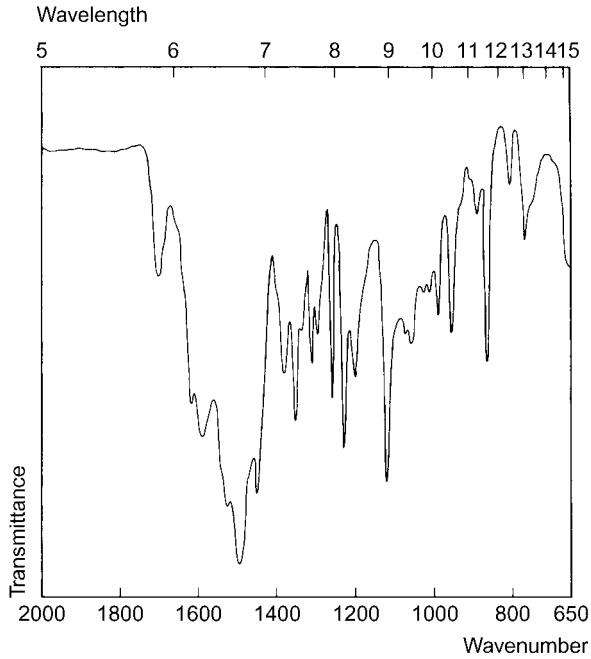
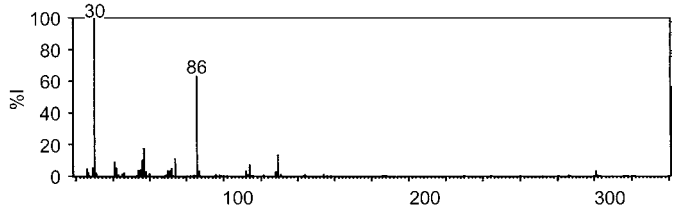


Mass Spectrum Principal ions at *m/z* 30, 86, 57, 129, 74, 56, 41, 114.



Quantification

Plasma **GC** ECD. Limit of detection, 2 µg/L [Tocco *et al.* 1975].

GC-MS Limit of detection, 5 µg/L [Fourtillan *et al.* 1981a].

HPLC Timolol and other beta-blockers. Limit of detection, 4 to 27 µg/L [Tracqui *et al.* 1988; 1989]. ECD [Gregg, Jack 1984]. UV detection. Limit of detection, 40 µg/L [Lefebvre *et al.* 1981].

HPLC-MS Limit of detection, 0.5 µg/L [Olah *et al.* 1993].

Serum **HPLC-GC** Timolol and other beta-blockers. Limit of detection, 18 to 44 µg/L [Hyotylainen *et al.* 1997].

Urine **GC** Limit of detection, 20 µg/L, see Plasma [Tocco *et al.* 1975].

GC-MS See Plasma [Fourtillan *et al.* 1981a].

HPLC Amperometric detection. Timolol and other beta-blockers. Limit of detection, 15 ppb to 500 ppb [Maguregui *et al.* 1995]. See Plasma [Lefebvre *et al.* 1981; Tracqui *et al.* 1988; Tracqui *et al.* 1989].

HPLC-GC See Serum [Hyotylainen *et al.* 1997].

Electrophoresis UV detection. Limit of detection, 190 µg/L [Maguregui *et al.* 2002].

Breast Milk **HPLC** See Plasma [Gregg, Jack 1984].

Hair **GC** Timolol and other beta-blockers. Limit of detection, 2 to 10 ng/g [Kintz *et al.* 2000].

Disposition in the Body Timolol is almost completely absorbed after oral administration. It is metabolised mainly by oxidation and hydrolytic cleavage of the morpholine ring. About 70% of a dose is excreted in the urine in 24 h, with about 20% of the dose as unchanged drug.

Therapeutic Concentration

After a single oral dose of 20 mg to 5 subjects, peak plasma concentrations of 0.05 to 0.11 (mean, 0.08) mg/L were attained in 0.5 to 3 h [Fourtillan *et al.* 1981b].

Following daily oral administration of 15 mg three times a day to 8 subjects, maximum steady-state plasma concentrations of 0.04 to 0.23 (mean, 0.11) mg/L were reported [Singh *et al.* 1980].

One, two or three patches, each releasing 97.5 mg timolol, were applied at weekly intervals to subjects' inner arm for 48 h and produced mean plasma timolol concentrations of 5, 11 and 14 µg/L, respectively. A study of suppression of exercise heart rate and plasma timolol concentrations showed the 50% inhibitory concentration of timolol to be 2 to 4 µg/L [McCrea *et al.* 1990].

Bioavailability 50 to 75%.

Half-life Plasma half-life, about 2 to 6 h.

Volume of Distribution 1 to 3 L/kg.

Clearance Plasma clearance, about 5 to 10 mL/min/kg.

Dose 10 to 60 mg of timolol maleate daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Fourtillan JB *et al.* (1981a). Mass fragmentographic determination of timolol in human plasma and urine. *J Pharm Sci* 70: 573–575.

Fourtillan JB *et al.* (1981b). Pharmacokinetics of oral timolol studied by mass fragmentography. *Eur J Clin Pharmacol* 19: 193–196.

Gregg MR, Jack DB (1984). Determination of timolol in plasma and breast milk using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 305: 244–249.

Hyotylainen T *et al.* (1997). Liquid chromatographic sample cleanup coupled on-line with gas chromatography in the analysis of beta-blockers in human serum and urine. *J Chromatogr Sci* 35: 280–286.

Kintz P *et al.* (2000). Doping control for beta-adrenergic compounds through hair analysis. *J Forensic Sci* 45: 170–174.

Lefebvre MA *et al.* (1981). β-Blocking agents: determination of biological levels using high performance liquid chromatography. *J Liq Chromatogr* 4(3): 483–500.

Maguregui MI *et al.* (1995). High-performance liquid chromatography with amperometric detection applied to the screening of beta-blockers in human urine. *J Chromatogr B Biomed Appl* 674: 85–91.

Maguregui MI *et al.* (2002). Quantitative determination of oxprenolol and timolol in urine by capillary zone electrophoresis. *J Chromatogr A* 949(1-2): 91–97.

McCrea JB *et al.* (1990). Transdermal timolol: beta blockade and plasma concentrations after application for 48 hours and 7 days. *Pharmacotherapy* 10: 289–293.

Olah TV *et al.* (1993). Determination of the beta-adrenergic blocker timolol in plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J Pharm Biomed Anal* 11: 157–163.

Singh BN *et al.* (1980). Plasma timolol levels and systolic time intervals. *Clin Pharmacol Ther* 28: 159–166.

Tocco DJ *et al.* (1975). Electron-capture GLC determination of timolol in human plasma and urine. *J Pharm Sci* 64: 1879–1881.

Tracqui A *et al.* (1988). A specific HPLC method for determination of beta-blockers topically used in ophthalmological diseases. *Forens Sci Int* 38: 37–41.

Tracqui A *et al.* (1989). Adverse events of anti-glaucoma beta-blockers: presentation of an original HPLC determination procedure. *Acta Med Leg Soc (Liege)* 39: 397–400.

Tinidazole

Antiprotozoal

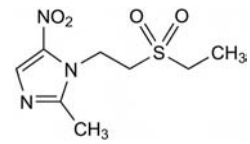
$C_8H_{13}N_3O_4S$ = 247.3

CAS—19387-91-8

IUPAC Name 1-(2-Ethylsulfonylethyl)-2-methyl-5-nitroimidazole

Synonym CP-12574

Proprietary Names *Amebysol*; *Ametricid*; *Amplium*; *Asiazole-TN*; *Dyzole*; *Estovyn-T*; *Fa-Cyl*; *Fasigin*; *Fasigyn(e)*; *Funida*; *Ginosutin*; *Idazole*; *Pletil*; *Protocide*; *Simplotan*; *Sorquetan*; *Sporinex*; *Tini*; *Tonid*; *Trichonas*; *Tricolam*; *Trimonase*; *Trinizol*; *Triseptil*.

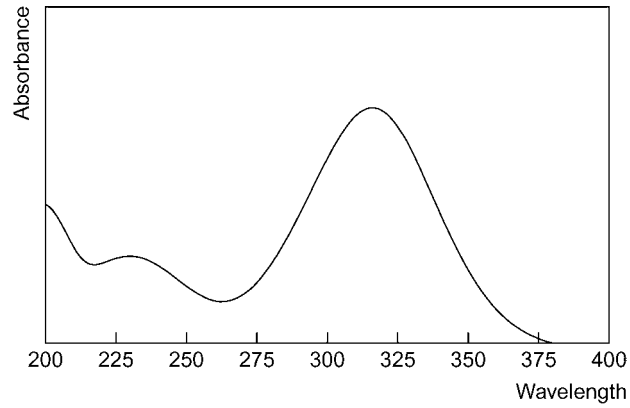


Chemical Properties Colourless crystals. Mp 127° to 128°. Practically insoluble in water; soluble in acetone; sparingly soluble in methanol. Log *P* (octanol/water), −0.4.

Gas Chromatography System GA—RI 2024.

High Performance Liquid Chromatography System HAA—retention time 10.6 min.

Ultraviolet Spectrum Aqueous acid—277 nm (A^1_1=245b); methanol—230 (A^1_1=148b), 311 nm (A^1_1=354b).



Quantification

Plasma **GC** AFID. Limit of detection, 100 µg/L [Laufen *et al.* 1979].

Serum **HPLC** UV/vis detection. Limit of detection, 50 µg/L [Rajnarayana *et al.* 2002]. Limit of detection, 300 µg/L [Nilsson-Ehle *et al.* 1981].

Urine **HPLC** Limit of detection, 600 µg/L, see Serum [Nilsson-Ehle *et al.* 1981].

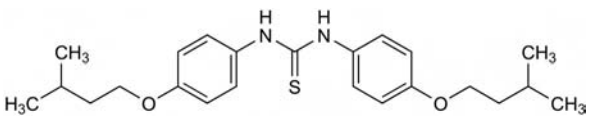
Tissues **GC** Limit of detection, 50 ng/g, see Plasma [Laufen *et al.* 1979].
Disposition in the Body Tinidazole is well absorbed after oral administration. It is excreted in the bile. About 25% of a dose is excreted in the urine unchanged in 72 h together with small amounts of 2-hydroxymethyltinidazole and its glucuronide.
Therapeutic Concentration
Following a single oral dose of 2 g to 3 subjects, peak serum concentrations of 35.7, 35.7 and 46.3 mg/L were attained in 2 to 3 h; serum concentrations of 0.19, 0.24 and 0.46 mg/L of the hydroxymethyl metabolite were reported, 6 h after the dose [Robson *et al.* 1984].
A single oral dose of 2 g in 10 subjects produced serum tinidazole concentrations of 3.2 to 46.5 mg/L (concentrations in gingival crevicular fluid were similar); 24 h after the dose, the mean serum tinidazole concentration was 13 ± 3 mg/L [Liew 1991].
Bioavailability >90%.
Half-life Plasma half-life, about 12 to 17 h.
Volume of Distribution About 0.7 L/kg.
Clearance Plasma clearance, about 0.6 mL/min/kg.
Protein Binding About 12%.
Note For a review of tinidazole, see Carmine *et al.* [1982].
Dose Usually 2 g initially, orally, followed by 1 g daily.

Carmine AA *et al.* (1982). Tinidazole in anaerobic infections: a review of its antibacterial activity, pharmacological properties and therapeutic efficacy. *Drugs* 24: 85–117.
Laufen H *et al.* (1979). Sensitive gas chromatographic assay of tinidazole in tissue and plasma. *J Chromatogr* 163: 217–220.
Liew V (1991). Single-dose concentrations of tinidazole in gingival crevicular fluid, serum, and gingival tissue in adults with periodontitis. *J Dent Res* 70: 910–912.
Nilsson-Ehle I *et al.* (1981). Liquid chromatographic assay for metronidazole and tinidazole: pharmacokinetic and metabolic studies in human subjects. *Antimicrob Agents Chemother* 19: 754–760.
Rajnarayana K *et al.* (2002). Validated HPLC method for the determination of tinidazole in human serum and its application in a clinical pharmacokinetic study. *Pharmazie* 57: 535–537.
Robson RA *et al.* (1984). Tinidazole pharmacokinetics in severe renal failure. *Clin Pharmacokinet* 9: 88–94.

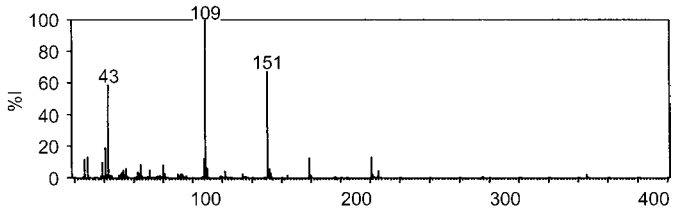
Tiocarlide

Tuberculostatic
$C_{23}H_{32}N_2O_2S = 400.6$
CAS—910-86-1

IUPAC Name 1,3-Bis[4-(3-methylbutoxy)phenyl]thiourea
Synonyms Thiocarlide; DATC
Proprietary Names *Disocarban*; *Datamil*; *Isoxyl*



Chemical Properties A white crystalline powder. Mp 134° to 145°. Practically insoluble in water; soluble in ethanol and chloroform. Log *P* (octanol/water), 7.2.
Colour Tests Liebermann's reagent—green; Mandelin's test—green→yellow.
Thin-layer Chromatography System TA—R_f 0.80; system TB—R_f 0.07; system TC—R_f 0.78; system TL—R_f 0.72 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2005.
Infrared Spectrum Principal peaks at wavenumbers 1228, 1499, 1534, 823, 1163, 1285 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 109, 151, 43, 41, 221, 179, 29, 108.

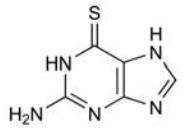


Dose 6 g daily.

Tioguanine

Antineoplastic
$C_5H_5N_5S = 167.2$
CAS—154-42-7 (anhydrous); 5580-03-0 (hemihydrate)

IUPAC Name 2-Amino-3,7-dihydropurine-6-thione
Synonyms 2-Amino-1,7-dihydro-6*H*-purine-6-thione; 6-TG; thioguanine.
Proprietary Name *Lanvis*



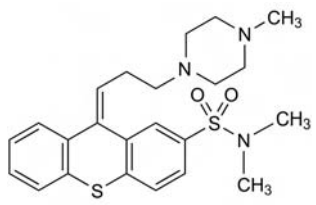
Chemical Properties A pale yellow crystalline powder. Mp >360°. Practically insoluble in water, ethanol and chloroform; freely soluble in dilute solutions of alkali hydroxides. Log *P* (octanol/water), −0.1.
Ultraviolet Spectrum Aqueous acid—258 (A_1^1=490b), 348 nm (A_1^1=1240a); aqueous alkali—320 nm (A_1^1=1015b).
Infrared Spectrum Principal peaks at wavenumbers 1625, 1668, 1257, 1539, 1231, 971 cm^{-1} (KBr disk).
Quantification
Plasma **Spectrofluorimetry** Limit of detection, 5 µg/L [Dooley, Maddocks 1980].
HPLC [Rudy *et al.* 1988]. UV detection. Tioguanine and metabolites. Limit of detection, about 130 µg/L [Andrews *et al.* 1982]. UV detection. Limit of detection, 200 µg/L [Breithaupt, Goebel 1981].
Urine **HPLC** See Plasma [Rudy *et al.* 1988]; [Breithaupt, Goebel 1981].
Cerebrospinal Fluid **HPLC** See Plasma [Rudy *et al.* 1988].
Disposition in the Body Tioguanine is incompletely and variably absorbed after oral administration. It is rapidly activated by intracellular conversion to thioguanylic acid. It is inactivated by methylation to aminomethylthiopurine and by deamination to thioxanthine. About 40% of an oral dose is excreted in the urine as metabolites in 24 h; only traces of tioguanine have been detected.
Dose Initially 2 to 2.5 mg/kg daily.

Andrews PA *et al.* (1982). Reversed-phase high-performance liquid chromatography analysis of 6-thioguanine applicable to pharmacologic studies in humans. *J Chromatogr* 227: 83–91.
Breithaupt H, Goebel G (1981). Quantitative high pressure liquid chromatography of 6-thioguanine in biological fluids. *J Chromatogr Sci* 19: 496–499.
Dooley T, Maddocks JL (1980). Assay of 6-thioguanine in human plasma. *Br J Clin Pharmacol* 9: 77–82.
Rudy JL *et al.* (1988). HPLC analysis of 6-mercaptopurine and metabolites in extracellular body fluids. *Ann Clin Biochem* 25: 504–509.

Tiotixene

Tranquilliser
$C_{23}H_{29}N_3O_2S_2 = 443.6$
CAS—5591-45-7; 3313-26-6 (*Z*-)

IUPAC Name (9*Z*)-*N*,*N*-Dimethyl-9-[3-(4-methylpiperazin-1-yl)propylidene]thioxanthene-2-sulfonamide
Synonyms *N*,*N*-Dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene]thioxanthene-2-sulfonamide; thiothixene.
Proprietary Names *Navane* (capsules and tablets); *Orbinamon*.



Chemical Properties A white to tan-coloured crystalline powder. Mp 147° to 149°. Practically insoluble in water; soluble 1 in 110 of dehydrated alcohol, 1 in 2 of chloroform and 1 in 120 of ether; slightly soluble in acetone and in methanol. Log *P* (octanol/water), 3.8.

Tiotixene Hydrochloride

$C_{23}H_{29}N_3O_2S_2,2HCl,2H_2O = 552.6$
CAS—58513-59-0 (anhydrous); 49746-04-5 (anhydrous, *Z*); 22189-31-7 (dihydrate); 49746-09-0 (dihydrate, *Z*)
Proprietary Names *Navane* (oral concentrate); *Thixit*.
Chemical Properties A white crystalline powder. Soluble 1 in 8 of water, 1 in 270 of dehydrated alcohol and 1 in 280 of chloroform; practically insoluble in acetone, in ether and in benzene.

Colour Tests Formaldehyde-sulfuric acid—red; Liebermann's reagent—red; Mandelin's test—red; Marquis test—red; sulfuric acid—orange.
Thin-layer Chromatography System TA—R_f 0.49; system TAE—tiotixene R_f 0.26, M R_f 0.12; system TAF—R_f 0.24; system TL—R_f 0.07; system TB—R_f 0.10; system TC—R_f 0.40; system TE—tiotixene R_f 0.44, M R_f 0.36; system TAJ—R_f 0.19; system TAK—R_f 0.05; system TAL—R_f 0.71 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3060.
High Performance Liquid Chromatography System HA—*k* 3.8; system HX—RI 442; system HY—RI 374; system HZ—retention time 6.8 min.
Ultraviolet Spectrum Aqueous acid—308 nm (A_1^1=191a).

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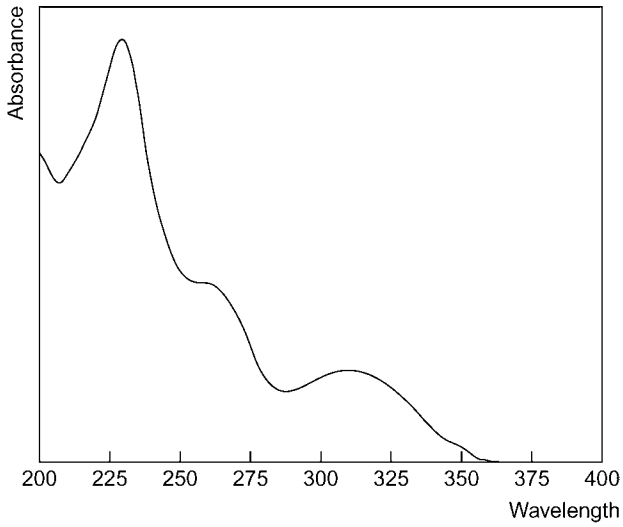
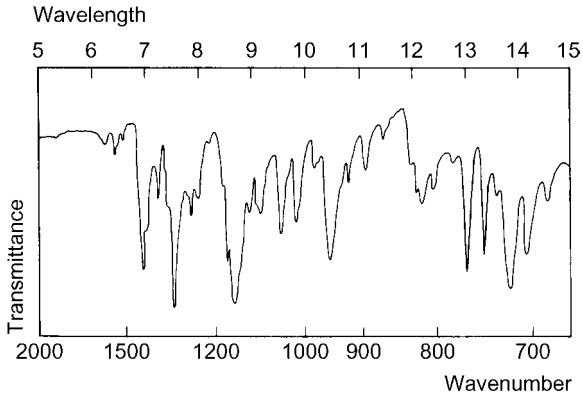
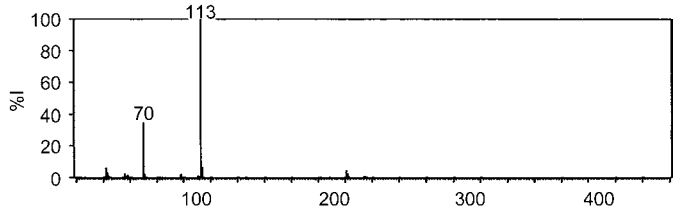


Infrared Spectrum Principal peaks at wavenumbers 1156, 719, 765, 1176, 957, 747 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 113, 70, 114, 42, 221, 222, 56, 43.



Quantification

Plasma **GC-MS** Limit of detection, <1 µg/L [Hobbs *et al.* 1974].

HPLC Coulometric detection. *cis*-tiotixene. Limit of detection, 200 ng/L [Hariharan *et al.* 1991]. UV detection. *cis*- and *trans*-tiotixene [Bogema *et al.* 1982].

Serum **HPLC** UV detection. Limit of detection, 0.05 µg/L [Dilger *et al.* 1988].

Disposition in the Body Tiotixene is rapidly absorbed after oral administration. The *N*-desmethyl derivative accounts for about 10% of the plasma concentration; other unidentified metabolites may accumulate on chronic administration.

Therapeutic Concentration

Following chronic oral administration of 15 to 60 mg daily in divided doses to 15 subjects, plasma concentrations, determined 2 to $2^1/_2$ h after the last daily dose, were in the range 0.010 to 0.023 (mean, 0.016) mg/L [Hobbs *et al.* 1974].

A study in 42 schizophrenic patients showed that a median plasma concentration of 12 to 15 µg/L might be expected with a daily dose of 60 mg of tiotixene [Hollister *et al.* 1987].

Toxicity

In a fatality involving the ingestion of 250 mg of tiotixene and an unknown quantity of doxepin, a postmortem blood concentration of 0.13 mg/L of tiotixene was reported [Baselt 2000].

A blood concentration of 0.53 mg/L was detected in acute overdosage with tiotixene before it declined to 0.047 mg/L in 12 h [Kemal, Imami 1985].

Half-life Plasma half-life, about 34 h.

Dose Usually 10 to 30 mg daily; up to 60 mg daily may be given.

Baselt RC (2000). *Disposition of Toxic Drugs and Chemicals in Man*, 5th edn. Davis, California: Biomedical Publications, 830–831.

Bogema SC *et al.* (1982). Separation and quantitation of cis- and trans-thiothixene in human plasma by high-performance liquid chromatography. *J Chromatogr* 233: 257–267.

Dilger C *et al.* (1988). Improved high-performance liquid chromatographic method for the determination of tiotixene in human serum. *Arzneimittelforschung* 38: 1522–1525.

Hariharan M *et al.* (1991). A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection. *Ther Drug Monit* 13: 79–85.

Hobbs DC *et al.* (1974). Pharmacokinetics of thiothixene in man. *Clin Pharmacol Ther* 16: 473–478.

Hollister LE *et al.* (1987). Plasma concentrations of thiothixene and clinical response in treatment-resistant schizophrenics. *Int Clin Psychopharmacol* 2: 77–82.

Kemal M, Imami RH (1985). Acute thiothixene overdose. *J Anal Toxicol* 9: 94–95.

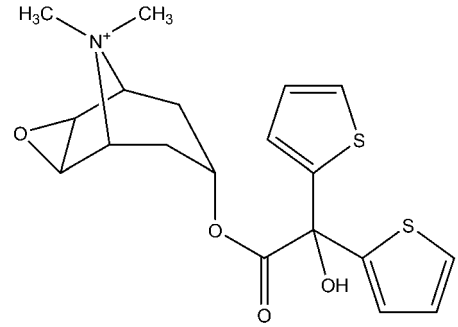
Tiotropium

Anticholinergic, Antimuscarinic, Quaternary Ammonium

$C_{19}H_{22}NO_4S_2 = 391.5$

CAS—186691-13-4

Synonym 6β,7β-Epoxy-3β-hydroxy-8-methyl-1α*H*,5α*H*-tropanium di-2-thienylglycolate



Tiotropium Bromide

$C_{19}H_{22}BrNO_4S_2$=472.4

CAS—139404-48-1 (anhydrous or hydrate); 136310-93-5 (anhydrous); 411207-31-3 (monohydrate)

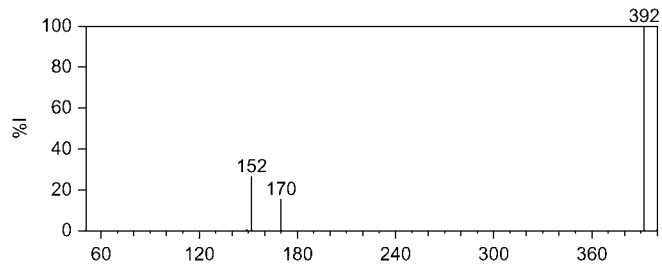
IUPAC Name 6β,7β-Epoxy-3β-hydroxy-8-methyl-1αH,5αH-tropanium bromide di-2-thienylglycolate

Synonyms Ba-679; Ba-679BR.

Proprietary Names *Spiriva*. It is also an ingredient in *Duova*.

Chemical Properties Sparingly soluble in water; Mp 218° to 220°. Log *P* (octanol/water), −0.86 [Wishart 2006].

Mass Spectrum Principal ions at *m/z* 392, 152, 170.



Quantification

Plasma **HPLC** Column: C_8. Mobile phase: acetonitrile : 8 mmol/L 1-heptane sulfonic acid (pH 3.2; 330 : 700). UV detection (λ = 240 nm). Limit of quantification not reported [Brand *et al.* 2007].

LC-MS Column: C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate buffer-1% formic acid (40 : 60), flow rate 0.8 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 2.4 min. Limit of quantification, 0.5 ng/L; limit of detection, 0.1 ng/L [Wang *et al.* 2007]. Column: C_{18} (125 × 1.6 mm i.d., 5 µm). Mobile phase: 1.36 mL triethanolamine in 800 mL water, pH 2.0 with phosphoric acid and 1.92 g pentanesulfonic acid sodium salt made up to 1 L water and diluted 1 : 100 (A) : 800 mL acetonitrile and 200 mL A (pH 3.0 with 9.6 g pentanesulfonic acid sodium salt; 60 : 40 to 20 : 80). API, SRM acquisition mode. Limit of quantification, 2.43 ng/L [Türck *et al.* 2004].

Urine **LC-MS** See Plasma [Türck *et al.* 2004].

Disposition in the Body On inhalation of the dry powder, some tiotropium bromide is absorbed from the lung. It is non-enzymatically cleaved to the inactive *N*-methylscopine and dithienylglycolic acid compounds. Human *in vitro* studies suggest that 25% of a dose may be metabolised by CYP2D6 and CYP3A4, with subsequent glutathione conjugation into a variety of phase II metabolites. It is excreted largely unchanged in the urine.

Therapeutic Concentration

Twenty subjects (5 healthy volunteers and 15 patients with varying degree of chronic obstructive pulmonary disease [COPD]) were administered a daily dose of 18 µg tiotropium as a dry powder inhalation formulation for 14 days.

Mean peak plasma tiotropium concentrations on day 14 were reported as follows:

Subjects	C_{max} *(ng/L)*
Healthy	7.50
Mild COPD	16.2
Moderate COPD	12.4
Severe COPD	13.5

[Brand *et al.* 2007].

Twenty-four subjects (6 healthy volunteers, 18 patients with varying degree of renal impairment) were administered a single IV dose of 4.8 µg tiotropium. Mean peak plasma concentrations of tiotropium and other pharmacokinetic parameters were reported as follows:

Parameter	*Renal function*			
	Normal	*Mild impairment*	*Moderate impairment*	*Severe impairment*
C_{max} (ng/L)	147	200	223	223
Half-life (days)	4	5	4	6
Total clearance (mL/min)	831	465	345	249
Volume of distribution (L)	2703	2209	1427	1659

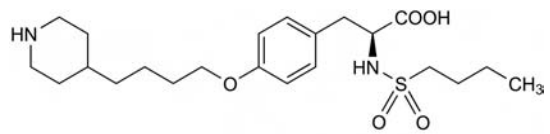
[Türck *et al.* 2004].

Toxicity Since such a small part of a dose is eliminated through enzymatic metabolism, clinically relevant drug interactions are not expected.
Bioavailability Approximately 20% with inhlation powder and 2.5% with oral solution.
Half-life Between 5 and 6 days.
Volume of Distribution Approximately 32 L/kg, varies with renal impairment, see Türck *et al.* [2004].
Protein Binding Approximately 72%.
Note For a review of tiotropium bromide, see Hvizdos and Goa [2002].
Dose Given as inhalation powder in capsules containing 22.5 µg tiotropium bromide monohydrate, equivalent to 18 µg of tiotropium, and supplying 10 µg of tiotropium from the mouthpiece of the inhaler device. The contents of one capsule are inhaled daily, at the same time each day.

Brand P *et al.* (2007). Lung deposition of radiolabeled tiotropium in healthy subjects and patients with chronic obstructive pulmonary disease. *J Clin Pharmacol* 47: 1335–1341.
Hvizdos KM, Goa KL (2002). Tiotropium bromide. *Drugs* 62: 1195–1203.
Türck D *et al.* (2004). Pharmacokinetics of intravenous, single-dose tiotropium in subjects with different degrees of renal impairment. *J Clin Pharmacol* 44: 163–172.
Wang J *et al.* (2007). Highly sensitive assay for tiotropium, a quaternary ammonium, in human plasma by high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 21: 1755–1758.
Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Tirofiban

Antithrombotic
$C_{22}H_{36}N_2O_5S = 440.6$
CAS—144494-65-5
IUPAC Name (2*S*)-2-(Butylsulfonylamino)-3-[4-(4-piperidin-4-ylbutoxy)phenyl]propanoic acid
Synonym *N*-(Butylsulfonyl)-*O*-[4-(4-piperidinyl)butyl]-L-tyrosine

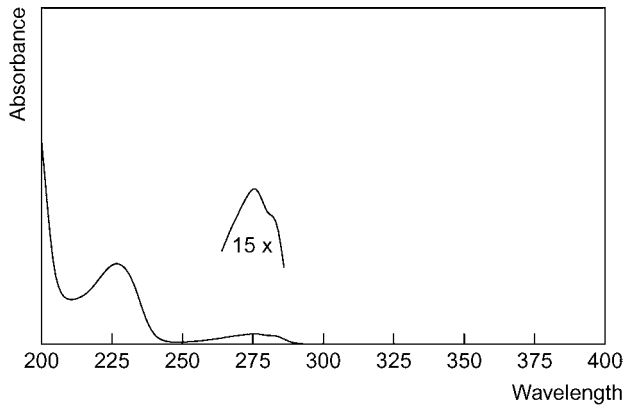


Chemical Properties A white solid. Mp 223° to 225°.

Tirofiban Hydrochloride Monohydrate

$C_{22}H_{36}N_2O_5S,HCl,H_2O = 495.1$
CAS—142373-60-2 (anhydrous); 150915-40-5 (monohydrate)
Synonyms L-700462; MK-0383; MK-383.
Proprietary Name *Aggrastat*
Chemical Properties A white to off-white, non-hygroscopic, free-flowing powder. Mp 131° to 132°. Very slightly soluble in water.

Ultraviolet Spectrum

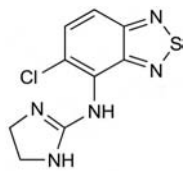


Quantification
Plasma **HPLC–MS** Column: RX-C_{18} Zorbax (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 1 mmol/L ammonium acetate (pH 6.0, 41.5 : 58.5), flow rate 1 mL/min. IS: L-702, 128. Detection: MS–MS (Aggrastat, *m/z* 535 → 415; IS, *m/z* 555 → 141). Retention time: Tirofiban, 5.2 min; IS, 5.1 min. Limit of quantification, 0.4 µg/L [Ellis *et al.* 1997].
Disposition in the Body Tirofiban is mainly cleared from plasma by renal excretion. Approximately 65% of a dose is recovered in urine and 25% in faeces mainly as the unchanged drug. Metabolism is limited.
Therapeutic Concentration
Eighteen healthy males were administered 0.05, 0.10, 0.15, 0.25 and 0.40 µg/kg/min doses of tirofiban hydrochloride as a 1 h infusion. A separate group of 20 healthy males were administered 0.10, 0.15 and 0.20 µg/kg/min doses as a 4 h infusion. The peak plasma concentrations after the 1 h infusion were 5.4, 10.9, 12.0, 30.1 and 47.8 µg/L for the 5 doses and after the 4 h infusion, 20.7, 42.2 and 42.6 µg/L for the 0.10, 0.15 and 0.20 µg/kg/min doses, respectively. As part of the study, the volunteers were administered 0.15 µg/kg/min tirofiban hydrochloride as a 4 h infusion with 325 mg aspirin administered the day before treatment and approximately 1 h before the tirofiban dose. The peak concentration of tirofiban was 29.3 µg/L [Barrett *et al.* 1994].
Toxicity Overdosing may result in bleeding, primarily minor mucocutaneous bleeding and minor bleeding at the sites of cardiac catheterisation. Tirofiban has also been associated with a higher incidence of thrombocytopenia.
Half-life 1.2 to 2.0 h.
Volume of Distribution Steady state, 22 to 42 L.
Clearance Plasma, 213 to 329 mL/min; 152 to 267 mL/min in patients with coronary artery disease. This value is decreased further in elderly patients, and is reduced in patients with severe renal insufficiency (creatinine clearance, <30 mL/min) by ~50%.
Protein Binding Not highly bound.
Dose IV infusion, 0.4 µg/kg/min initially for 30 min followed by 0.1 µg/kg/min for at least 48 h (continued during and for 12 to 24 h after percutaneous coronary invention). Maximum duration is 108 h.

Barrett JS *et al.* (1994). Pharmacokinetics and pharmacodynamics of MK-383, a selective non-peptide platelet glycoprotein-IIb/IIIa receptor antagonist, in healthy men. *Clin Pharmacol Ther* 56: 377–388.
Ellis JD *et al.* (1997). Use of LC-MS/MS to cross-validate a radioimmunoassay for the fibrinogen receptor antagonist, Aggrastat (tirofiban hydrochloride) in human plasma. *J Pharm Biomed Anal* 15(5): 561–569.

Tizanidine

α_2*-Adrenoceptor Agonist, Muscle Relaxant*
$C_9H_8ClN_5S = 253.7$
CAS—51322-75-9
IUPAC Name 6-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazol-7-amine
Synonyms AN-021; 5-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine; DS-103-282; DS-103-282-ch.
Proprietary Names *Sirdalud*; *Temelin*; *Zanaflex*.



Chemical Properties A white to off white fine crystalline odourless powder with Mp 221° to 223°. It is slightly soluble in water and methanol. Stable in plasma

at room temperature and in the autosampler for 24 h, after 3 freeze-thaw cycles and for 24 days when stored below -50°. Stock solutions were stable at room temperature for 4 h, 21 h and after refrigeration at -4° for 2 months [Nirogi *et al.* 2006].

Tizanidine Hydrochloride

$C_9H_8ClN_5S,HCl = 290.2$
CAS—64461-82-1

Chemical Properties A white to off white fine crystalline odourless powder. It is slightly soluble in water and methanol. Its solubility in water decreases as the pH increases.

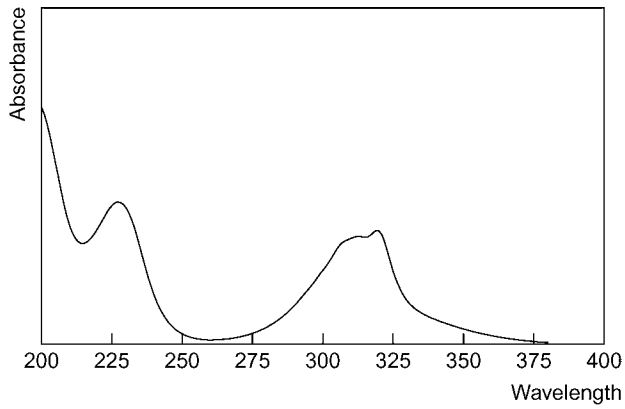
Thin-layer Chromatography System TAE—R_f 0.51; system TB—R_f 0.04; system TE—R_f 0.56.

High Performance Liquid Chromatography System HX—RI 247; system HZ—RT 2.3 min.

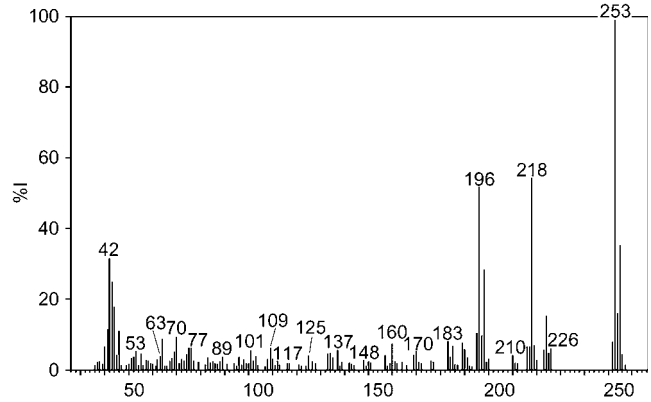
Column: Spherisorb ODS (150 × 4.6 mm i.d., 0.5 µm). Mobile phase: 1% TEA (pH 2.5) : acetonitrile (55 : 45), flow rate 0.8 mL/min. UV detection (λ= 303 nm). Retention time: 1.6 min. Limit of quantification, 80 µg/L, limit of detection, 10 µg/L [Gandhimathi *et al.* 2005].

Column: Kromasil C_{18} (250 × 4.6 mm i.d., 5.0 µm) or Finepak SIL-5 C_{18} (250 ×4.6 mm i.d., 5.0 µm). Mobile phase: phosphate buffer (pH 5.5) : methanol (45 : 55), flow rate 1.0 mL/min. UV detection (λ= 235 nm). Limit of quantification, 0.05 mg/L, limit of detection, 0.01 mg/L [Kaul *et al.* 2005].

Ultraviolet Spectrum Aqueous acid (pH 2.38)—196, 226, 316 nm.



Mass Spectrum Principal ions at *m/z* 253, 218, 196, 255, 42, 198, 43, 224.



Quantification

Blood **GC-MS** Column: RTx-200 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 70° for 1 min to 290° at 20°/min for 1 min. MSD, SIM acquisition mode. Limit of quantification, 0.01 mg/L [Sklerov *et al.* 2006].

Plasma **GC-MS** Column: Ultra-1 SE-30 (17 m × 0.20 mm i.d., 0.33 µm). Carrier gas: He, 100 kPa. Temperature programme: 210° for 1 min to 230° at 3°/min to 300° at 15°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.5 µg/L [Lee *et al.* 2002].

LC-MS Column: Waters Symmetry C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 2 mmol/L ammonium formate (pH 2.8) : acetonitrile (10 : 90), flow rate 1.0 mL/min. API, TIS, positive ion mode. Retention time: 1.49 min. Limit of quantification, 50 ng/L [Nirogi *et al.* 2006]. Column: XTerra RP C_{18} (100 × 3.9 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate (pH 9.5) : acetonitrile. APCI, positive ion mode. Limit of quantification, 0.02 µg/L [Granfors *et al.* 2004]

Urine **GC-MS** See Blood [Sklerov *et al.* 2006].

Bile **GC-MS** See Blood [Sklerov *et al.* 2006].

Gastric Contents **GC-MS** See Blood [Sklerov *et al.* 2006].

Liver **GC-MS** See Blood [Sklerov *et al.* 2006].

Disposition in the Body Tizanidine is rapidly absorbed from the gastrointestinal tract, with 53 to 66% of the dose being absorbed. Peak plasma concentrations are reached within 1 to 2 h after oral administration. The drug is widely distributed throughout the body and undergoes rapid and extensive first-pass metabolism in the liver (~95% of a dose). Metabolism is via oxidation of the imidazoline moiety, the aromatic system and the sulfur atom. Metabolism is mainly via CYP1A2 [Granfors *et al.* 2004]. Approximately 70% of the dose is excreted in urine as the inactive metabolite, and 20% is excreted in faeces. Renal excretion of the parent compound is approximately 53% after a single 5 mg dose. Tizanidine has a short duration of action. Food has no influence on its pharmacokinetics. The drug displays linear pharmacokinetics within the dosage of 1 to 20 mg.

Therapeutic Concentration

Fifteen healthy males, aged between 19 and 27 years (mean 23.5 years), were administered 4 mg tizanidine twice daily for seven consecutive doses. The volunteers fasted for 10 h before the first dose and for 2 h after each subsequent dose. The mean peak plasma concentration was 2.5 µg/L observed at 0.9 h [Shellenberger *et al.* 1999].

Toxicity Low order of acute toxicity.

A 57-year-old woman was found unresponsive. Postmortem tizanidine concentrations were as follows:

Specimen	*Concentration (mg/L, mg/kg or mg)*
Heart blood	2.340
Urine	0.055
Bile	3.370
Gastric contents	10.00
Liver	9.190

[Sklerov *et al.* 2006]

A 46-year-old man with multiple sclerosis fell into coma very shortly after ingesting 100 tablets of 4 mg tizanidine. Respiratory depression occurred but the patient recovered [*Physicians Desk Reference* 1999].

Half-life 2.1–4.2 h.

Bioavailability 34–40%.

Volume of Distribution Steady state, 2.4 L/kg.

Clearance Reduced by 50% in the elderly, renal impairment and women taking oral contraceptives.

Protein Binding 30%.

Dose The usual initial dose is 2 mg, which may be increased in steps of 2 mg daily over 3 to 4 days, depending on response. The maximum is 36 mg daily and the usual is 24 mg.

Gandhimathi M *et al.* (2005). Simultaneous LC determination of tizanidine and rofecoxib in tablets. *J Pharm Biomed Anal* 37: 183–185.

Granfors MT *et al.* (2004). Ciprofloxacin greatly increases concentrations and hypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2-mediated presystemic metabolism. *Clin Pharmacol Ther* 76: 598–606.

Kaul N *et al.* (2005). Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form. *J Pharm Biomed Anal* 37: 27–38.

Lee J *et al.* (2002). Determination of tizanidine in human plasma by gas chromatography–mass spectrometry. *Analyst* 127: 917–920.

Nirogi RV *et al.* (2006). Quantification of tizanidine in human plasma by liquid chromatography coupled to tandem mass spectrometry. *Rapid Commun Mass Spectrom* 20: 2286–2292.

Physicians Desk Reference (1999). Medical Economics Company, Montvale, New Jersey.

Shellenberger MK *et al.* (1999). A controlled pharmacokinetic evaluation of tizanidine and baclofen at steady state. *Drug Metab Dispos* 27: 201–204.

Sklerov JH *et al.* (2006). Tizanidine distribution in a postmortem case. *J Anal Toxicol* 30: 331–334.

Tobramycin

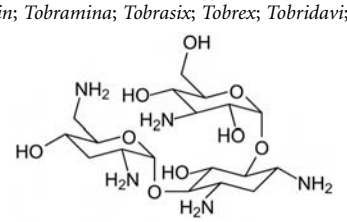
Antibiotic

$C_{18}H_{37}N_5O_9 = 467.5$
CAS—32986-56-4; 49842-07-1 (sulfate: xH_2SO_4); 79645-27-5 (sulfate: $5H_2SO_4$)

IUPAC Name (2*S*,3*R*,4*S*,5*S*,6*R*)-4-Amino-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,5*S*, 6*R*)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-6-(hydroxymethyl)oxane-3,5-diol

Synonyms *O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine; nebramycin factor 6.

Proprietary Names *AkTob*; *Brulamycin*; *Defy*; *Distobram*; *Gernebcin*; *Mytobrin*; *Nebcin(a)*; *Nebcine*; *Obracin*; *Tobi*; *Tobra(l)*; *Tobra-cell*; *Tobracil*; *Tobradistin*; *Tobralex*; *Tobramaxin*; *Tobramina*; *Tobrasix*; *Tobrex*; *Tobridavi*; *Tomycin(e)*; *Trazil.*



Chemical Properties A white hygroscopic powder. A solution in water is dextro-rotatory. Soluble 1 in 1.5 of water and 1 in 2000 of ethanol; practically insoluble in chloroform and ether. pK_a 6.7, 8.3, 9.9. Log *P* (octanol/water), −5.8.

T

Thin-layer Chromatography System TAE—R_f 0.00.
High Performance Liquid Chromatography System HAA—retention time 13.4 min.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1040, 1590, 845, 770, 660, 1260 cm^{-1} (KBr disk).
Quantification See also under Amikacin.
Serum **HPLC** For a method for the quantification of tobramycin and other aminoglycosides, see Essers [1984]. UV detection. Limit of detection, 500 µg/L [Barends *et al.* 1981]. Fluorescence detection. Limit of detection, 200 µg/L [Haughey *et al.* 1980].
Urine **HPLC** See Serum [Haughey *et al.* 1980].
Disposition in the Body Tobramycin is poorly absorbed after oral administration, but rapidly absorbed after IM injection. Up to 90% of a dose is excreted unchanged in the urine in 24 h, most being excreted within the first 6 h. It accumulates in the tissues and may be detected in serum and urine for several days after cessation of treatment.
Therapeutic Concentration During treatment, the serum concentration should be in the range 4 to 10 mg/L and should be monitored regularly, especially in patients who have renal insufficiency. During multiple dosing the trough concentration immediately preceding a dose should not exceed 2 mg/L.

After an IV infusion of 100 mg administered over 1 h to 4 subjects, peak serum concentrations of 4.1 to 5.1 (mean, 4.6) mg/L were achieved at the end of the infusion. After a single IM injection of 100 mg administered to 3 subjects, a mean peak serum concentration of 5.16 mg/L was achieved after 0.3 to 0.75 h [Regamey *et al.* 1973].

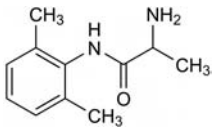
Cystic fibrosis patients inhaling 300 mg tobramycin twice daily as an aerosol, in 28-day on/off treatment cycles, had a mean serum concentration of 0.95 mg/L 1 hour after the dose. The mean peak sputum concentration of tobramycin was 1.237 mg/g [Geller *et al.* 2002].

Toxicity Toxic effects may be produced at serum concentrations of 12 mg/L or more or, during chronic treatment, if the trough serum concentration exceeds 2 mg/L.
Half-life Plasma half-life, about 2 to 3 h, increased in renal failure; a long terminal elimination phase of about 6 days has also been reported.
Volume of Distribution About 0.3 L/kg.
Clearance Plasma clearance, about 1.5 mL/min/kg.
Protein Binding Up to 10%.
Dose Usually the equivalent of 3 to 5 mg/kg of tobramycin daily, given parenterally.

Barends DM *et al.* (1981). Micro-determination of tobramycin in serum by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 225: 417–426.
Essers L (1984). An automated high-performance liquid chromatographic method for the determination of aminoglycosides in serum using pre-column sample clean-up and derivatization. *J Chromatogr* 305: 345–352.
Geller DE *et al.* (2002). Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest* 122: 219–226.
Haughey DB *et al.* (1980). High-pressure liquid chromatography analysis and single-dose disposition of tobramycin in human volunteers. *Antimicrob Agents Chemother* 17: 649–653.
Regamey C *et al.* (1973). Comparative pharmacokinetics of tobramycin and gentamicin. *Clin Pharmacol Ther* 14: 396–403.

Tocainide

Antiarrhythmic
$C_{11}H_{16}N_2O$ = 192.3
CAS—41708-72-9
IUPAC Name 2-Amino-*N*-(2,6-dimethylphenyl)propanamide

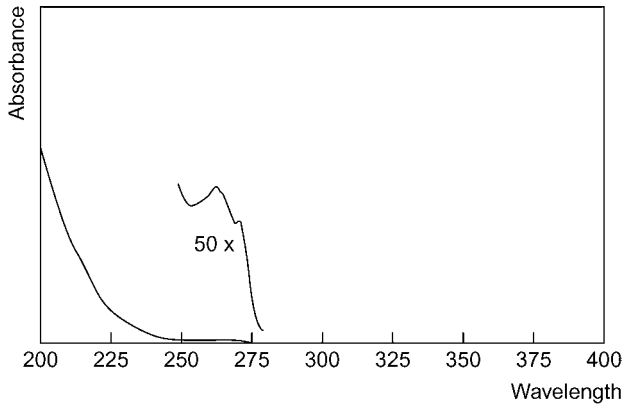


Chemical Properties A white waxy solid. Mp 53° to 55°. Sparingly soluble in water; freely soluble in organic solvents. pK_a 7.8. Log *P* (octanol/water), 0.8. Extraction yield (chlorobutane), 0.3 [Demme *et al.* 2005].

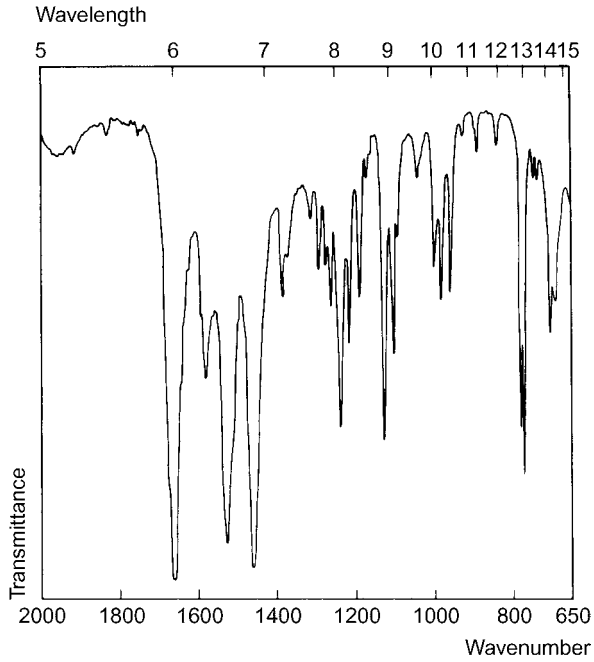
Tocainide Hydrochloride

$C_{11}H_{16}N_2O$,HCl = 228.7
CAS—71395-14-7 ((±)-form); 53984-74-0 (R-(−)-form); 53984-76-2 (S-(−)-form)
Proprietary Names *Tonocard*; *Xylotocan*.
Chemical Properties A white crystalline powder. Mp about 245°, with decomposition. Freely soluble in water and ethanol; practically insoluble in chloroform and ether.

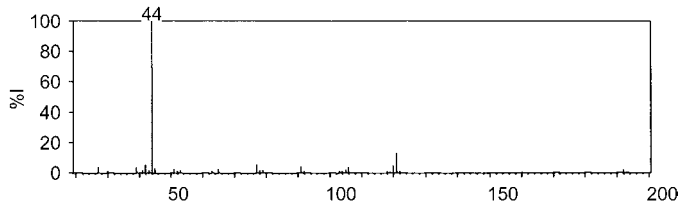
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.02; system TC—R_f 0.23; system TE—R_f 0.44; system TAE—R_f 0.42; system TAF—R_f 0.74.
Gas Chromatography System GA—RI 1714; system GB—RI 1769.
High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 247; system HY—RI 208; system HZ—retention time 2.1 min.
Ultraviolet Spectrum Aqueous acid—262 (A^1_1=22a), 270 nm (A^1_1=18b).



Infrared Spectrum Principal peaks at wavenumbers 1675, 1540, 765, 1128, 772, 1240 cm^{-1} (tocainide hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 44, 121, 77, 120, 42, 106, 91, 39.



Quantification

Plasma **GC** ECD. Limit of detection, 2 pg [Pillai *et al.* 1982]. FID. Limit of detection, 200 µg/L [Gettings *et al.* 1981].

HPLC Fluorescence detection. Limit of detection, 250 µg/L [Carr *et al.* 1991]. Fluorescence or UV detection. For a method of quantification for tocainide and other anti-arrhythmics, see Verbesselt *et al.* [1991]. For a method of quantification for tocainide and other anti-arrhythmics, see Annesley *et al.* [1986]; vasBinder, Annesley [1991]. Fluorescence detection. Limit of detection, 100 µg/L [Sedman, Gal 1982].

Serum **GC** FID. Limit of detection, 200 µg/L [Gettings *et al.* 1981].

HPLC See Plasma [Annesley *et al.* 1986]; [vasBinder, Annesley 1991]. Limit of detection, 100 to 200 µg/L for tocainide and other anti-arrhythmics and metabolites [Proelss, Townsend 1986].

Urine **GC** See Plasma [Pillai *et al.* 1982].
Disposition in the Body Tocainide is almost completely absorbed after oral administration. About 20 to 50% of a dose is excreted in the urine as unchanged drug in 24 h, and about 20 to 30% as a glucuronide conjugate, which is thought to be

tocainide carbamoyl *O*-β-D-glucuronide (TOCG); an additional metabolite, lactoxylidide has also been detected in urine.

Therapeutic Concentration In plasma, usually in the range 4 to 12 mg/L. Following a single oral dose of 400 mg to 3 subjects, peak plasma concentrations of 1.6 to 1.8 mg/L were attained in 0.5 to 2 h. After oral administration of 400 mg three times a day to 4 subjects, steady-state plasma concentrations of 4.9 to 7.1 (mean, 5.9) mg/L were reported. [Graffner *et al.* 1980].

Toxicity
A 26-year-old woman who committed suicide by ingesting a large amount of tocainide was found to have a serum concentration of 68 mg/L. [Sperry *et al.* 1987].

Half-life Plasma half-life, 8 to 25 (mean, 14) h.

Volume of Distribution About 1 to 3 L/kg.

Clearance Plasma clearance, about 2 to 3 mL/min/kg.

Protein Binding Variously reported as 10 to 50%.

Note For a review of tocainide, see Holmes *et al.* [1983].

Dose 1.2 to 2.4 g of tocainide hydrochloride daily, orally.

Annesley T *et al.* (1986). A high performance liquid chromatographic assay for tocainide with alternate application for the determination of lidocaine, procainamide, and N-acetylprocainamide. *Res Commun Chem Pathol Pharmacol* 51: 173–181.

Carr RA *et al.* (1991). Stereospecific high-performance liquid chromatographic determination of tocainide. *J Chromatogr* 566: 155–162.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Gettings SD *et al.* (1981). Simple method for the measurement of tocainide and lignocaine in blood plasma or serum using gas–liquid chromatography with flame ionisation detection. *J Chromatogr* 225: 469–475.

Graffner C *et al.* (1980). Tocainide kinetics after intravenous and oral administration in healthy subjects and in patients with acute myocardial infarction. *Clin Pharmacol Ther* 27: 64–71.

Holmes B *et al.* (1983). Tocainide. A review of its pharmacological properties and therapeutic efficacy. *Drugs* 26: 93–123.

Pillai GK *et al.* (1982). Electron-capture gas-liquid chromatographic determination of tocainide in biological fluids using fused silica capillary columns. *J Chromatogr* 229: 103–109.

Proelss HF, Townsend TB (1986). Simultaneous liquid-chromatographic determination of five antiarrhythmic drugs and their major active metabolites in serum. *Clin Chem* 32: 1311–1317.

Sedman AJ, Gal J (1982). Pre-column derivatization with fluorescamine and high-performance liquid chromatographic analysis of drugs. Application to tocainide. *J Chromatogr* 232: 315–326.

Sperry K *et al.* (1987). Fatal intoxication by tocainide. *J Forensic Sci* 32: 1440–1446.

vasBinder E, Annesley T (1991). Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide. *Biomed Chromatogr* 5: 19–22.

Verbesselt R *et al.* (1991). High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Ther Drug Monit* 13: 157–165.

Tofenacin

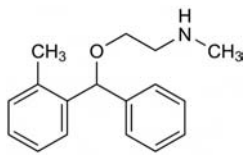
Antidepressant

$C_{17}H_{21}NO$ = 255.4

CAS—15301-93-6

IUPAC Name *N*-Methyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine

Synonyms *N*-Demethylorphenadrine; desmethylorphenadrine.



Chemical Properties A liquid. Log *P* (octanol/water), 3.4.

Tofenacin Hydrochloride

$C_{17}H_{21}NO,HCl$ = 291.8

CAS—10488-36-5

Synonym BS-7331

Proprietary Names *Elamol*; *Tofacine*.

Chemical Properties A white to off-white crystalline powder. Mp 147° to 148°. Soluble 1 in about 3 of water, 1 in 8 of ethanol and 1 in 3 of chloroform; slightly soluble in acetone; practically insoluble in ether.

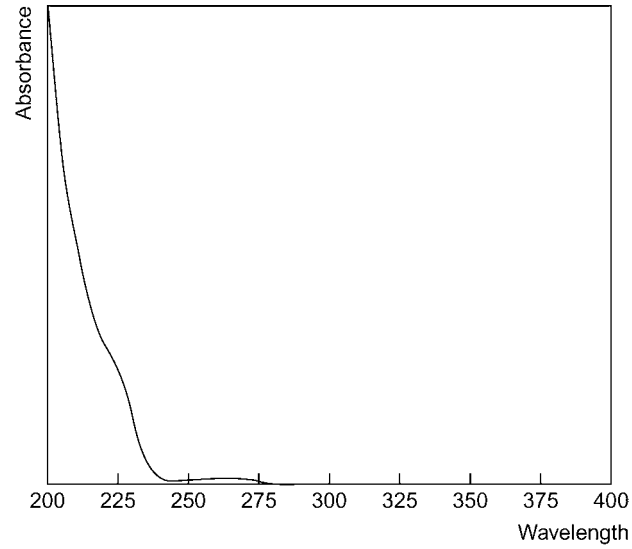
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—yellow; Marquis test—yellow; sulfuric acid—orange.

Thin-layer Chromatography System TA—R_f 0.45; system TB—R_f 0.26; system TC—R_f 0.21; system TE—R_f 0.48; system TL—R_f 0.07; system TAE—R_f 0.14 (acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 1920; system GB—RI 2013; system GM—RRT 0.420(relative to iprindole).

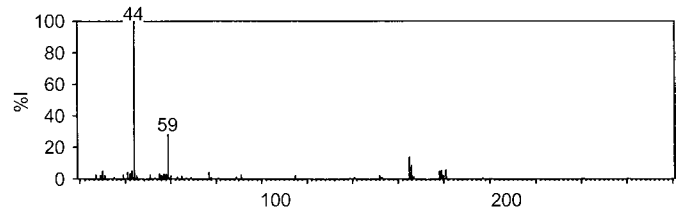
High Performance Liquid Chromatography System HA—*k* 1.7; system HZ—retention time 5.3 min.

Ultraviolet Spectrum Ethanol—259, 265, 272 nm.



Infrared Spectrum Principal peaks at wavenumbers 1102, 704, 757, 1117, 1042, 917 cm^{-1} (tofenacin hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 44, 59, 165, 166, 181, 179, 178, 43.



Quantification See under Orphenadrine.

Disposition in the Body Tofenacin is a metabolite of orphenadrine.

Dose Up to 240 mg of tofenacin hydrochloride daily.

Tolazamide

Antidiabetic

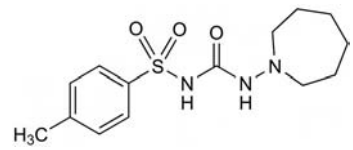
$C_{14}H_{21}N_3O_3S$ = 311.4

CAS—1156-19-0

IUPAC Name 1-(Azepan-1-yl)-3-(4-methylphenyl)sulfonylurea

Synonym *N*-[[(Hexahydro-1*H*-azepin-1-yl)amino]carbonyl]-4-methylbenzenesulfonamide

Proprietary Names *Diabewas*; *Norglycin*; *Tolanase*; *Tolinase*.



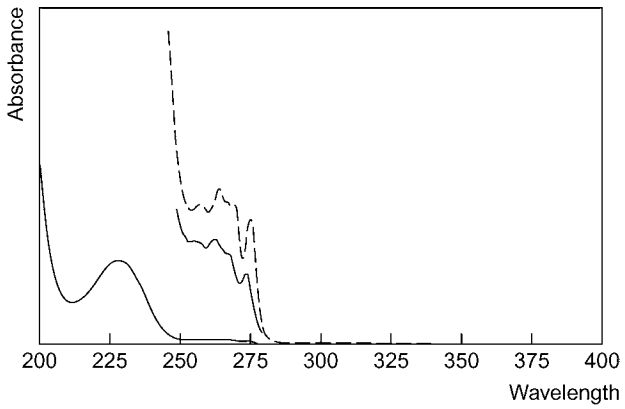
Chemical Properties A white crystalline powder. Mp 170° to 173°. Very slightly soluble in water; slightly soluble in ethanol; soluble in acetone; freely soluble in chloroform. pK_a 3.5 (25°), 5.7 (37.5°). Log *P* (octanol/water), 2.7.

Thin-layer Chromatography System TAD—R_f 0.66; system TAE—R_f 0.86; system TB—R_f 0.00; system TD—R_f 0.52; system TE—R_f 0.07; system TF—R_f 0.50; system TAJ—R_f 0.65; system TAK—R_f 0.71; system TAL—R_f 0.95 (acidified potassium permanganate solution, positive).

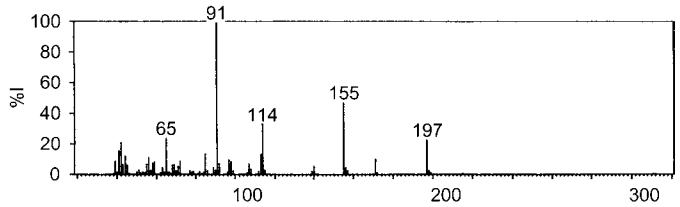
Gas Chromatography System GA—RI 1651.

High Performance Liquid Chromatography System HX—RI 452; system HY—RI 445; system HZ—retention time 6.8 min.

Ultraviolet Spectrum Methanol—257, 263 (A^1_1=21a), 268, 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1694, 1176, 884, 819, 675, 1086 cm^{-1} (KBr disk). Polymorphism may occur.
Mass Spectrum Principal ions at *m/z* 91, 155, 114, 65, 197, 42, 41, 85.



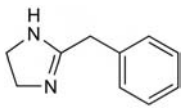
Quantification

Serum **HPLC** UV detection. Limit of detection, 1 mg/L [Welling *et al.* 1982].
Urine **Micellar electrokinetic chromatography** Limit of detection, 50 µg/L for tolazamide and other hypoglycaemic drugs [Núñez *et al.* 1995].
Disposition in the Body Tolazamide is slowly absorbed after oral administration. About 85% of a dose is excreted in the urine in 5 days, mostly in the first 24 h. Of the urinary material, about 17% is the *p*-carboxy derivative, 25% is 4-hydroxytolazamide, 10% is *p*-hydroxymethyltolazamide, 26% is *p*-toluenesulfonamide and 7% is unchanged drug.
Therapeutic Concentration
Following a single oral dose of 500 mg to 20 subjects, a mean peak serum concentration of 27.8 mg/L was attained in 3 h [Welling *et al.* 1982].
Half-life Plasma half-life, about 5 h.
Protein Binding About 94%.
Note For a review of the pharmacokinetics of sulfonylurea hypoglycaemic drugs, see Jackson and Bressler [1981].
Dose 0.1 to 1 g daily.

Jackson JE, Bressler R (1981). Clinical pharmacology of sulphonylurea hypoglycaemic agents: part 1. *Drugs* 22: 211–245.
Núñez M *et al.* (1995). Detection of hypoglycemic drugs in human urine using micellar electrokinetic chromatography. *Anal Chem* 67: 3668–3675.
Welling PG *et al.* (1982). Bioavailability of tolazamide from tablets: comparison of in vitro and in vivo results. *J Pharm Sci* 71: 1259–1263.

Tolazoline

Vasodilator
$C_{10}H_{12}N_2$ = 160.2
CAS—59-98-3
IUPAC Name 2-Benzyl-4,5-dihydro-1*H*-imidazole
Synonyms Benzazoline; 4,5-dihydro-2-(phenylmethyl)-1*H*-imidazole.



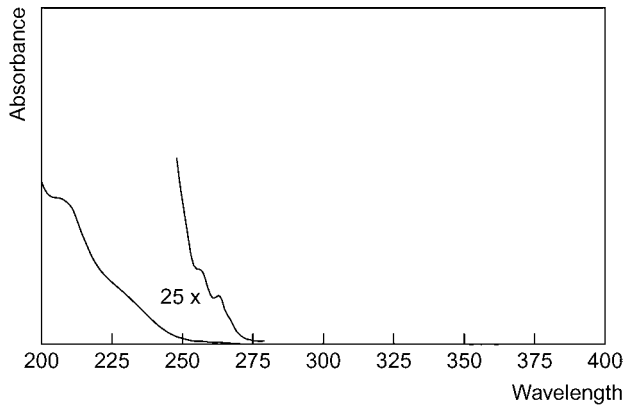
Chemical Properties Soluble in water and chloroform. pK_a 10.6 (20°). Log *P* (octanol/water), 2.7.

Tolazoline Hydrochloride

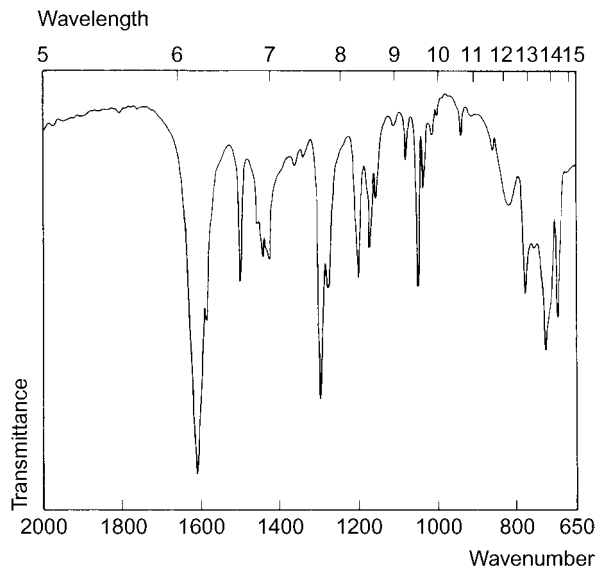
$C_{10}H_{12}N_2$,HCl = 196.7
CAS—59-97-2
Proprietary Names *Priscol*; *Priscoline*; *Vaso-Dilatan*.
Chemical Properties A white or creamy-white crystalline powder. Mp 174°. Soluble 1 in 0.5 of water, 1 in 2 of ethanol and 1 in 3 of chloroform; practically insoluble in ether.

Colour Tests Aromaticity (method 2)—colourless/orange; Liebermann's reagent—orange.

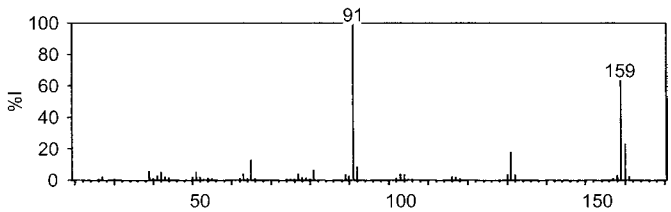
Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 0.02; system TC—R_f 0.02; system TE—R_f 0.25; system TL—R_f 0.02; system TAE—R_f 0.03; system TAF—R_f 0.55 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 1598.
High Performance Liquid Chromatography System HA—*k* 2.1; system HX—RI 225; system HY—RI 179.
Ultraviolet Spectrum Aqueous acid—257 (A^{1}_{1}=14.7a), 263 nm.



Infrared Spectrum Principal peaks at wavenumbers 1608, 1296, 721, 1580, 690, 775 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 91, 159, 160, 131, 65, 92, 81, 39.



Quantification

Plasma **HPLC** UV detection. Limit of detection, 200 µg/L [Rovei *et al.* 1982].
Serum **HPLC** UV detection. Limit of detection, 50 to 250 µg/L [Todesco *et al.* 1987]. UV detection. Limit of detection, 100 µg/L [Cwik *et al.* 1985].
Capillary isotachophoresis Limit of detection, 40 mg/L [Hercegova *et al.* 1998].
Urine **HPLC** See Serum [Cwik *et al.* 1985].
Capillary isotachophoresis Limit of detection, 3 mg/L [Hercegova *et al.* 1998].
Disposition in the Body Tolazoline is well absorbed after oral or parenteral administration. It is excreted in the urine, about 90% of an oral dose being eliminated unchanged in 12 h.
Therapeutic Concentration
After a bolus intravenous injection of 2 mg/kg followed by continuous IV infusion to provide a maintenance dose of 2 mg/kg to 1 neonate, plasma

concentrations attained a steady-state of 7 to 8 mg/L and declined with a half-life of 5 h at the end of the infusion [Rovei *et al.* 1982].

Toxicity

A 54-year-old woman found comatose after ingesting 2.5 g of tolazoline hydrochloride died about 3 h later. Postmortem concentrations were: femoral blood 130 mg/L, liver 102 µg/g [Turner LK, personal communication, 1964].

Dose 12.5 to 200 mg of tolazoline hydrochloride daily.

Cwik MJ *et al.* (1985). Quantitative determination of tolazoline in serum and urine. *J Chromatogr* 338: 123–130.

Hercegova A *et al.* (1998). Isotachophoretic determination of bisoprolol, clonidine, disopyramide and tolazoline in human fluids. *Acta Pol Pharm* 55: 167–171.

Rovei V *et al.* (1982). Determination of tolazoline in plasma by high-performance liquid chromatography. *J Chromatogr* 231: 210–215.

Todesco LM *et al.* (1987). Quantitative determination of tolazoline in human serum by high performance liquid chromatography. *Ther Drug Monit* 9: 78–84.

Tolbutamide

Antidiabetic

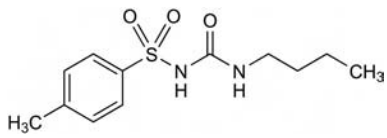
$C_{12}H_{18}N_2O_3S = 270.3$

CAS—64-77-7

IUPAC Name 1-Butyl-3-(4-methylphenyl)sulfonylurea

Synonyms Butamidum; *N*-[(butylamino)carbonyl]-4-methylbenzenesulfonamide; tolglybutamide.

Proprietary Names *Arcosal*; *Artosin*; *Diabetil*; *Diatelan*; *Diatol*; *Diaval*; *Dolipol*; *Flusan*; *Glyconon*; *Ifumelus*; *Ipoglusan*; *Mobenol*; *Novobutamide*; *Orabet*; *Orinase*; *Orsinon*; *Rastinon* (tablets); *Tol-Tab*.



Chemical Properties A white crystalline powder. Mp about 129°. Practically insoluble in water; soluble 1 in 10 of ethanol and 1 in 3 of acetone; soluble in chloroform; slightly soluble in ether. pK_a 5.3 (20°). Log *P* (octanol/water), 2.3.

Tolbutamide Sodium

$C_{12}H_{17}N_2NaO_3S = 292.3$

CAS—473-41-6

Proprietary Names *Orinase Diagnostic*; *Rastinon (injection)*.

Chemical Properties A white crystalline powder. Mp 130° to 133°. Freely soluble in water; soluble in ethanol and chloroform; very slightly soluble in ether.

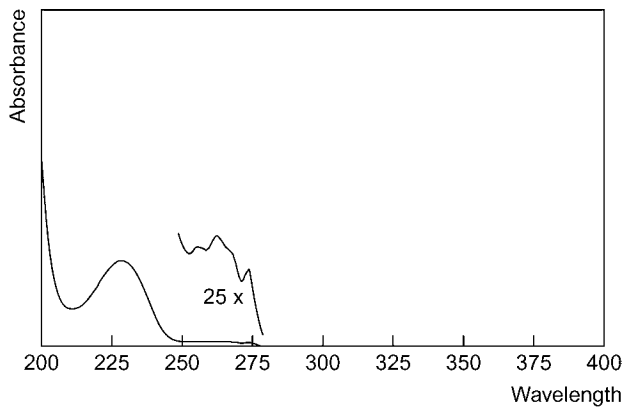
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black.

Thin-layer Chromatography System TA—R_f 0.76; system TD—R_f 0.51; system TE—R_f 0.12; system TF—R_f 0.55; system TT—R_f 0.98; system TU—R_f 0.35; system TV—R_f 0.04; system TAD—R_f 0.62; system TAE—R_f 0.88; system TAF—R_f 0.88; system TAJ—R_f 0.69; system TAK—R_f 0.74; system TAL—R_f 0.93.

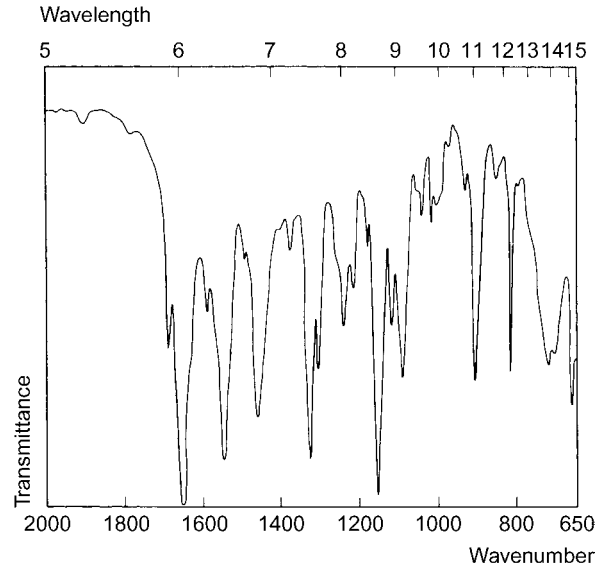
Gas Chromatography System GA—RI 1683.

High Performance Liquid Chromatography System HX—RI 477; system HY—RI 424; system HZ—retention time 5.9 min.

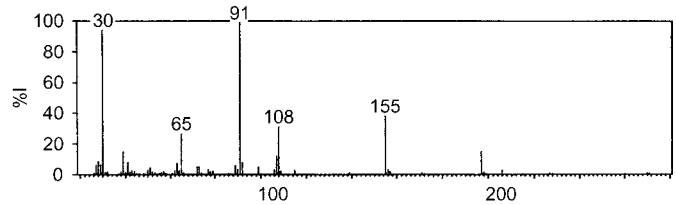
Ultraviolet Spectrum Methanol—257, 263 (A^1_1=22a), 268, 275 nm.



Infrared Spectrum Principal peaks at wavenumbers 1658, 1157, 1552, 668, 1090, 905 cm^{-1} (KBr disk). Polymorphism may occur.



Mass Spectrum Principal ions at *m/z* 91, 30, 155, 108, 65, 197, 39, 107.



Quantification See also under Chlorpropamide.

Plasma **GC** ECD. Limit of detection, 1 mg/L for tolbutamide and two metabolites [Matin, Rowland 1973].

HPLC UV detection. Limit of detection, 0.1 to 1.5 µM for tolbutamide and metabolites [Hansen, Brosen 1999]. Photodiode-array detection. For a method for the quantification of tolbutamide and metabolites, see Csillag *et al.* [1989]. UV detection. Limit of detection, 2 mg/L for tolbutamide, 100 µg/L for the carboxy metabolite [Raghow, Meyer 1981]. UV detection. Limit of detection, 200 µg/L [Hill, Chamberlain 1978].

Serum **MS** Limit of detection, 2 µg/L, limit of quantification, 10 µg/L for tolbutamide and other sulfonylureas [Magni *et al.* 2000].

Urine **GC** See Plasma [Matin, Rowland 1973].

HPLC See Plasma. Limit of detection, 0.5 to 2 µmol/L for tolbutamide and metabolites [Hansen, Brosen 1999]. See Plasma [Csillag *et al.* 1989].

Disposition in the Body Tolbutamide is readily absorbed after oral administration. About 85% of an oral dose is excreted in the urine in 48 h, of which about two-thirds is the 4-carboxy metabolite and about one-third is the 4-hydroxymethyl metabolite; <5% is excreted as unchanged drug. About 9% of a dose is eliminated in the faeces in 48 h.

Therapeutic Concentration

Following a single oral dose of 500 mg to 1 subject, a peak plasma-tolbutamide concentration of 49 mg/L was attained in about 4 h and a peak plasma concentration of the 4-carboxy metabolite of about 2.2 mg/L was attained in 6 h [Raghow, Meyer 1981].

In 20 patients treated for non-insulin-dependent diabetes mellitus with tolbutamide (average daily dose, 2 g), the plasma concentration was 19.8 (range, 4.3 to 58.2) mg/L before their morning dose and a maximum of 92.2 (39.8 to 154.7) mg/L 85 (35 to 370) min after the dose [Ferner *et al.* 1991].

Toxicity Several fatalities from hypoglycaemia have been reported.

The following postmortem tissue distribution was reported in one suicide after the ingestion of 50 g: blood 640 mg/L, kidney 1073 µg/g, liver 930 µg/g, urine 3510 mg/L [Pribilla 1968].

Half-life Plasma half-life, usually in the range 4 to 12 (mean, 7) h but may vary up to 70 h during concomitant administration of other drugs such as sulfonamides, phenylbutazone, or dicoumarol.

Volume of Distribution 0.1 to 0.2 L/kg.

Clearance Plasma clearance, about 0.3 mL/min/kg.

Protein Binding About 95% at concentrations up to 100 µg/mL, decreasing to about 90% at plasma concentrations of 200 µg/mL.

Note For a review of sulfonylurea hypoglycaemic drugs, see Jackson and Bressler [1981].

Dose 0.25 to 3 g daily.

Csillag K *et al.* (1989). Simple high-performance liquid chromatographic method for the determination of tolbutamide and its metabolites in human plasma and urine using photodiode-array detection. *J Chromatogr* 490: 355–363.

Ferner RE *et al.* (1991). The relationships between dose and concentration of tolbutamide and insulin and glucose responses in patients with non-insulin-dependent diabetes. *Eur J Clin Pharmacol* 40: 163–168.
Hansen LL, Brosen K (1999). Quantitative determination of tolbutamide and its metabolites in human plasma and urine by high-performance liquid chromatography and UV detection. *Ther Drug Monit* 21: 664–671.
Hill HM, Chamberlain J (1978). Determination of oral anti-diabetic agents in human body fluids using high-performance liquid chromatography. *J Chromatogr* 149: 349–358.
Jackson JE, Bressler R (1981). Clinical pharmacology of sulphonylurea hypoglycaemic agents: part 1. *Drugs* 22: 211–245.
Magni F *et al.* (2000). Identification of sulfonylureas in serum by electrospray mass spectrometry. *Anal Biochem* 282: 136–141.
Matin SB, Rowland M (1973). *Anal Lett (Part B)* 6: 865–876.
Pribilla O (1968). On fatal poisoning of a non-diabetic person by tolbutamide (Rastinon). *Arch Toxikol* 23: 153–159.
Raghow G, Meyer MC (1981). High-performance liquid chromatographic assay of tolbutamide and carboxytolbutamide in human plasma. *J Pharm Sci* 70: 1166–1168.

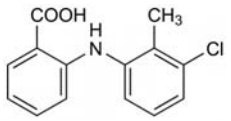
Tolfenamic Acid

Analgesic

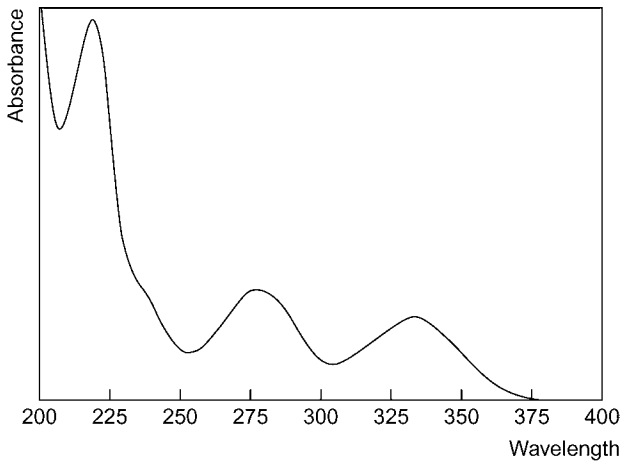
$C_{14}H_{12}ClNO_2$ = 261.7

CAS—13710-19-5

IUPAC Name 2-(3-Chloro-2-methylanilino)benzoic acid
Synonym 2-[(3-Chloro-2-methylphenyl)amino]benzoic acid
Proprietary Names *Bifenac*; *Clotam*; *Clotan*; *Flocur*; *Migea*; *Rociclyn*.



Chemical Properties A white crystalline powder. Mp about 207°. Practically insoluble in water; soluble 1 in 70 of ethanol, 1 in 140 of chloroform, 1 in 15 of dimethylformamide and 1 in 45 of ether. Log *P* (octanol/water) 5.2.
Thin-layer Chromatography System TE—R_f 0.14; system TF—R_f 0.31.
Gas Chromatography System GL—Tolfenamic acid-ME RI 2255.
High Performance Liquid Chromatography System HX—RI 690; system HZ—retention time 37.9 min.
Ultraviolet Spectrum Dehydrated alcohol—294, 330 nm.



Infrared Spectrum Principal peaks at wavenumbers 1670, 1270, 1590, 755, 1500, 1575 cm^{-1} (KBr disk).
Quantification
Plasma **HPLC** UV detection. Limit of detection, 20 µg/L [Pentikäinen *et al.* 1981].
Urine **HPLC** UV detection. Limit of detection, about 2 ng for tolfenamic acid and other anthranilic acid derivatives [Mikami *et al.* 2000]. See Plasma [Pentikäinen *et al.* 1981].
Disposition in the Body Readily absorbed after oral administration. It is metabolised by hydroxylation to *N*-[(2-hydroxymethyl-3-chlorophenyl)amino] benzoic acid and *N*-[(2-methyl-3-chloro-4-hydroxyphenyl)-amino]benzoic acid, which are the major metabolites. About 50% of an oral dose is excreted in the urine in 48 h mainly as glucuronide conjugates of the two hydroxylated metabolites. Less than 10% of a dose is excreted in the urine as unchanged drug or its glucuronide conjugate. Tolfenamic acid and its metabolites are excreted in the bile.
Therapeutic Concentration
Following single oral doses of 100 and 200 mg to 6 subjects, mean peak plasma concentrations of 2.0 and 3.0 mg/L were attained in 1.3 and 1.5 h respectively [Pentikäinen *et al.* 1981].
Bioavailability About 60%.
Half-life Plasma half-life, about 2.5 h.
Volume of Distribution About 0.5 L/kg.
Clearance Plasma clearance about 2.5 mL/min/kg.
Protein Binding >99.5%.
Dose Tolfenamic acid has been given in doses of 300 to 600 mg daily.

Mikami E *et al.* (2000). Simultaneous analysis of anthranilic acid derivatives in pharmaceuticals and human urine by high-performance liquid chromatography with isocratic elution. *J Chromatogr B Biomed Sci Appl* 744: 81–89.
Pentikäinen PJ *et al.* (1981). Human pharmacokinetics of tolfenamic acid, a new anti-inflammatory agent. *Eur J Clin Pharmacol* 19: 359–365.

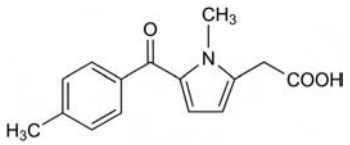
Tolmetin

Analgesic

$C_{15}H_{15}NO_3$ = 257.3

CAS—26171-23-3

IUPAC Name 2-[1-Methyl-5-(4-methylbenzoyl)pyrrol-2-yl]acetic acid
Synonym 1-Methyl-5-(4-methylbenzoyl)-1*H*-pyrrole-2-acetic acid



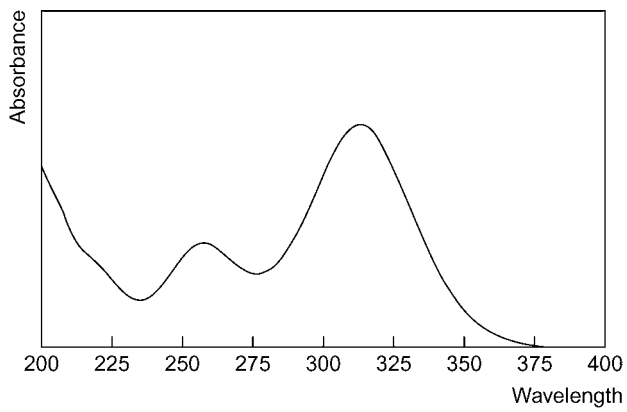
Chemical Properties Crystals. Mp 155° to 157°, with decomposition. pK_a 3.5. Log *P* (octanol/water) 2.8.

Tolmetin Sodium

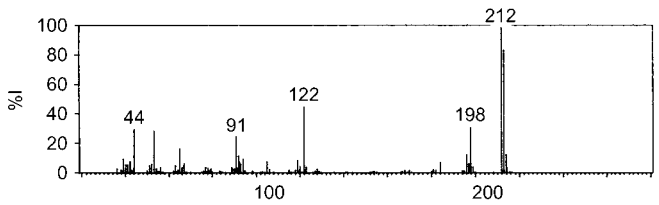
$C_{15}H_{14}NNaO_3,2H_2O$ = 315.3

CAS—35711-34-3 (anhydrous); 64490-92-2 (dihydrate)
Proprietary Names *Artrocaptin*; *Tolectin*.

Colour Tests Formaldehyde-Sulfuric acid—brown-red; Liebermann's reagent (100°)—red.
Thin-layer Chromatography System TD—R_f 0.13; system TE—R_f 0.05; system TF—R_f 0.20; system TG—R_f 0.10; system TAD—R_f 0.30; system TAE—R_f 0.85; system TAJ—R_f 0.20; system TAK—R_f 0.59; system TAL—R_f 0.83 (Ludy Tenger reagent, black).
Gas Chromatography System GA—RI 1890; tolmetin-ME RI 2247; System GD—methyl derivative RRT 1.77, relative to n-$C_{16}H_{34}$; system GL—tolmetin-ME RI 2235; M (COOH-)-ME_2 RI 2600.
High Performance Liquid Chromatography System HD—*k* 2.05; system HV—RRT 0.60 andRRT 0.99 relative to meclofenamic acid; system HX—RI 470; system HY—RI 434; system HZ—retention time 5.4 min.
Ultraviolet Spectrum Aqueous acid—262 nm (A^1_1=345b), 315 nm (A^1_1=735b); aqueous alkali—260 nm (A^1_1=333b), 323 nm (A^1_1=762b).



Mass Spectrum Principal ions at *m/z* 212, 213, 122, 198, 44, 53, 91, 65.



T

Quantification
Plasma **GC** ECD. Limit of detection, 100 µg/L [Ng 1978].

HPLC UV detection. Range of detection, 0.05 to 50 mg/L for tolmetin, tolmetin glucuronide and isomeric conjugates [Hyneck *et al.* 1987]. UV detection. Limit of detection, 40 µg/L for tolmetin and the dicarboxylated metabolite [Desiraju *et al.* 1982].

Urine **HPLC** See Plasma. Range of detection, 0.025 to 50 mg/L for tolmetin, tolmetin glucuronide and isomeric conjugates [Hyneck *et al.* 1987]. See Plasma. For a method of quantification for tolmetin and the dicarboxylated metabolite, see Desiraju *et al.* [1982].

Disposition in the Body Rapidly and almost completely absorbed after oral administration. Metabolised mainly by oxidation to inactive metabolites. >90% of a dose is excreted in the urine in 24 h, mostly in the first 8 h. The urinary material consists mostly of free and conjugated (glucuronide) 5-(4-carboxybenzoyl)-1-methylpyrrol-2-ylacetic acid and conjugated (glucuronide) tolmetin; up to 15% is excreted in the urine as unchanged drug.

Therapeutic Concentration

After a single oral dose of 300 mg to 5 subjects, a mean peak plasma concentration of 29.3 mg/L of tolmetin was attained in 0.25 to 1 h; plasma concentrations of the dicarboxylated metabolite reached a peak of about 5 mg/L in 1.5 to 3 h [Selley *et al.* 1975].

Following oral administration of 400 mg four times a day to 5 subjects for 7 days, maximum steady-state plasma concentrations of 8.3 to 78.9 mg/L (mean, 45) of tolmetin and 4.1 to 13.6 mg/L (mean, 10) of the dicarboxylated metabolite were reported about 0.75 and 1.75 h after a dose, respectively [Dromgoole *et al.* 1982].

Toxicity Toxic effects are associated with plasma concentrations greater than about 60 µg/mL.

Half-life Plasma half-life, about 6 h.

Clearance Plasma clearance, about 1 to 2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.8.

Protein Binding Tolmetin >99% but displaced by salicylic acid; dicarboxylated metabolite about 70%.

Note For a review of the pharmacokinetics of tolmetin, see Brogden *et al.* [1978].

Dose The equivalent of 0.6 to 1.8 g of tolmetin daily.

Brogden RN *et al.* (1978). Tolmetin: a review of its pharmacological properties and therapeutic efficacy in rheumatic diseases. *Drugs* 15: 429–450.

Desiraju RK *et al.* (1982). Simultaneous determination of tolmetin and its metabolite in biological fluids by high-performance liquid chromatography. *J Chromatogr* 232: 119–128.

Dromgoole SH *et al.* (1982). Tolmetin kinetics and synovial fluid prostaglandin E levels in rheumatoid arthritis. *Clin Pharmacol Ther* 32: 371–377.

Hyneck ML *et al.* (1987). High-performance liquid chromatographic determination of tolmetin, tolmetin glucuronide and its isomeric conjugates in plasma and urine. *J Chromatogr* 420: 349–56.

Ng KT (1978). Micro-determination of tolmetin in plasma by electron-capture gas chromatography. *J Chromatogr* 166: 527–535.

Selley ML *et al.* (1975). Pharmacokinetic studies of tolmetin in man. *Clin Pharmacol Ther* 17: 599–605.

Tolnaftate

Antifungal

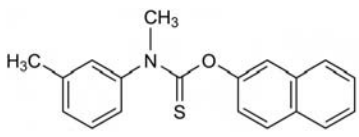
$C_{19}H_{17}NOS = 307.4$

CAS—2398-96-1

IUPAC Name *O*-Naphthalen-2-yl *N*-methyl-*N*-(3-methylphenyl)carbamothioate

Synonym Methyl(3-methylphenyl)-carbamothioic acid *O*-2-naphthalenyl ester

Proprietary Names *Absorbine Antifungal*; *Aftate*; *Blis-To-Sol*; *Curatin*; *Devorfungi*; *Excelsior*; *Micoisdin*; *Mycil*; *Pitrex*; *Podactin*; *Quinsana Plus*; *Sorgoa*; *Sorgoran*; *Sporiline*; *Tinactin*; *Tinatox*; *Tineafax*; *Tinaderm(e)*; *Tinasol*; *Tolnaderm*; *Tonoftal*; *Trinaderm*.



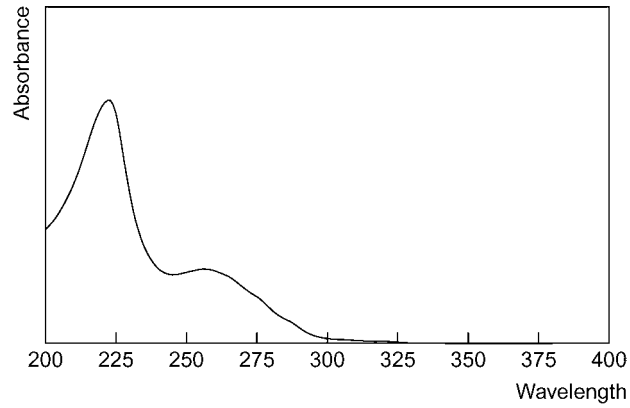
Chemical Properties A white to creamy-white powder. Mp about 111°. Practically insoluble in water; sparingly soluble in ethanol and methanol; soluble 1 in 8 of acetone, 1 in 2.5 of chloroform and 1 in 55 of ether. Log *P* (octanol/water) 5.8.

Colour Tests Mandelin's test—brown; Marquis test—blue-green.

Thin-layer Chromatography System TAJ—R_f 0.96; system TAK—R_f 0.83; system TAL—R_f 0.97.

High Performance Liquid Chromatography System HY—RI 867.

Ultraviolet Spectrum Methanol—257 nm (A^1_1=708a).



Infrared Spectrum Principal peaks at wavenumbers 1211, 1170, 1156, 754, 1234, 810 cm^{-1} (KBr disk).

Use Topically as a 1% solution, powder or cream.

Tolonium Chloride

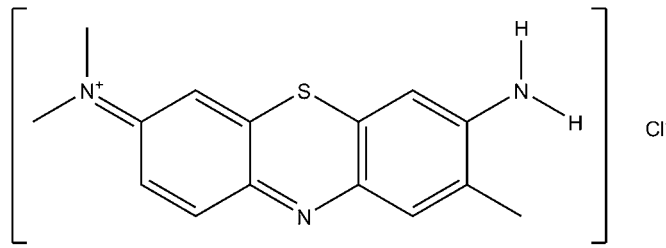
Quaternary Ammonium, Phenothiazine, Dye, Antiheparin Compound, Tumour Marker

$C_{15}H_{16}N_3S,Cl = 305.8$

IUPAC Name (7-Amino-8-methylphenothiazin-3-ylidene)-dimethylazanium chloride

Synonyms 3-Amino-7-dimethylamino-2-methyl-phenazothionium chloride; Basic Blue 17; colour index number 52040; toluidine blue O.

Proprietary Name *Blutene Chloride*



Chemical Properties Green, crystalline powder with a bronze lustre. Soluble 1 in 20 of water; slightly soluble in ethanol; almost insoluble in ether; very slightly soluble in chloroform. Tolonium chloride has been used as a diagnostic aid for oral [Epstein *et al.* 2007 Gupta *et al.* 2007] and gastric [Hallissey, Fielding 1988] neoplasms and in the identification of the parathyroid gland in thyroid surgery [Archer *et al.* 1972; National Institutes of Health 2008].

Colour Tests Ammonium molybdate test—(blue) green (limit of detection; 0.1 µg); ammonium vanadate test—(blue) purple→green (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—(blue) green (limit of detection, 0.1 µg); Vitali's test—(blue) blue/blue/dull red (limit of detection, 0.5 µg).

Thin-layer Chromatography System T1—R_f 0.01 (location under ultraviolet light, red fluorescence; location reagent acidified iodoplatinate spray, positive reaction).

Ultraviolet Spectrum 0.1 N sulfuric acid—245 and 285 nm.

Note McKamey and Spitznagle [1975] have analysed the impurities of commercial tolonium chloride and Marshall and Lewis [1974] have looked at batch variations.

Disposition in the Body Tolonium chloride inhibits the anticoagulant effect of heparinoid substances in the blood.

Note For a study of the pharmacokinetics of tolonium chloride in sheep, see Cudd *et al.* [1996].

Therapeutic Concentration A muco-adhesive patch containing 50 or 100 mg tolonium chloride/cm^2 has been shown to be effective at treating mucocutaneous oropharyngeal candidiasis caused by *Candida albicans* [Donnelly *et al.* 2007].

Toxicity

Note For case studies involving tolonium chloride as an antidote, see Lindenmann *et al.* [2006], Iwersen-Bergmann and Schmoldt [2000] and Ewert *et al.* [1998].

Dose Up to 300 mg daily.

Note During treatment with tolonium chloride the urine of patients assumes a blue-green colour.

Archer *et al.* (1972). Tissue distribution of 125 I-toluidine blue in the rat. *J Nucl Med* 13: 85–91.

Cudd LA *et al.* (1996). Pharmacokinetics and toxicity of tolonium chloride in sheep. *Vet Hum Toxicol* 38: 329–332.

Donnelly RF *et al.* (2007). Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. *J Photochem Photobiol B* 86: 59–69.

Epstein JB *et al.* (2007). Utility of toluidine blue in oral premalignant lesions and squamous cell carcinoma: continuing research and implications for clinical practice. *Head Neck* 29: 948–958.
Ewert R *et al.* (1998). Intravenous injection of India ink with suicidal intent. *Int J Legal Med* 111: 91–92.
Gupta A *et al.* (2007). Utility of toluidine blue staining and brush biopsy in precancerous and cancerous oral lesions. *Acta Cytol* 51: 788–794.
Hallissey MT, Fielding JW (1988). In-vivo stain for gastric cancer. *Lancet* 1: 115.
Iwersen-Bergmann S, Schmoldt A (2000). Acute intoxication with aniline: detection of acetaminophen as aniline metabolite. *Int J Legal Med* 113: 171–174.
Lindenmann J *et al.* (2006). Hyperbaric Oxygenation in the treatment of life-threatening isobutyl nitrite-induced methemoglobinemia−−a case report. *Inhal Toxicol* 18: 1047–1049.
Marshall PN, Lewis SM (1974). Batch variations in commercial dyes employed for Romanowsky-type staining: a thin-layer chromatographic study. *Stain Technol* 49: 351–358.
McKamey MR, Spitznagle LA (1975). Chromatographic, mass spectral, and visible light absorption characteristics of toluidine blue O and related dyes. *J Pharm Sci* 64: 1456–1462.
National Institutes of Health (2008). *Toluidine Blue.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=7083&loc=ec_rcs. (accessed 23 June 2008).

Toloxatone

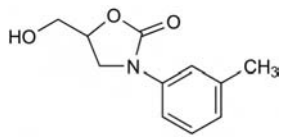
Antidepressant

$C_{11}H_{13}NO_3 = 207.2$

CAS—29218-27-2

IUPAC Name 5-(Hydroxymethyl)-3-*m*-tolyl-2-oxazolidinone

Proprietary Name *Humoryl*



Chemical Properties White to off-white crystalline powder with Mp 76°. It is slightly soluble in water (4.5 g/L), 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide solution. Log *P* (octanol/water), 1.88.

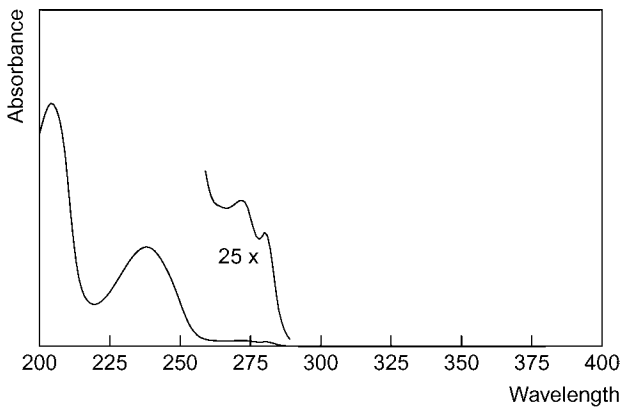
Thin-layer Chromatography

Plate: silica gel coated plates (Kieselgel 60). Mobile phase: chloroform : diethyl ether : methanol : 25% ammonia (85 : 15 : 20 : 0.5). Detection: colour reaction with nitrous gas. R_f 0.68. Limit of detection, 0.005 mg/L. [Benedetti *et al.* 1982].

High Performance Liquid Chromatography System HAA—RT 14.1 min.

Column: Symmetry C_8 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium phosphate buffer (pH 3.8) : acetonitrile (85 : 15 for 6.5 min to 65 : 35 for 18.5 min to 20 : 80 for 3 min), flow rate 1 mL/min for 6.5 min, to 1.5 mL/min over 18.5 min for 3 min. UV detection (λ = 204 nm). RT: 14.11 min [Gaillard, Pepin 1997].

Ultraviolet Spectrum Aqueous acid—330 nm.



Quantification

Blood **HPLC** Column: Nova-Pack C_{18} (300 × 3.9 mm, 4 µm). Mobile phase: methanol : tetrahydrofuran : 10 mmol/L potassium dihydrogen phosphate (pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. UV detection (λ = 238 nm). Retention time: 3.35 min. Limit of detection, <0.12 mg/L [Tracqui *et al.* 1995].

LC-MS Column: XTerra RP18 (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2, 5 : 95 for 2 min to 20 : 80 in 1 min to 30 : 70 over 12 min for 2 min to 5 : 95 in 30 s for 2.5 min), flow rate 0.15 mL/min. ESI, MRM acquisition mode, positive ion mode. Retention time: min. Limit of quantification, 2 µg/L [Titier *et al.* 2007].

Plasma **GC** Column: 3% OV-101 Supelcoport 80/100 mesh (2000 × 2 mm i.d.), silanised glass. Temperature: 200°. Carrier gas: He, 35 mL/min. NPD. Retention time: 2.5 min (TMS derivative) [Vajta *et al.* 1983].

HPLC Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8, 25 : 75 to 40 : 60 in 10 min to 44 : 56 at 8 min), flow rate 1.0 mL/min. UV detection (λ = 240 nm). Retention time: 9.3 min. Limit of quantification, 25 µg/L, limit of detection, 10 µg/L [Duverneuil *et al.* 2003].

Disposition in the Body Toloxatone is rapidly absorbed from the gastrointestinal tract after oral administration. It is rapidly and extensively metabolised and no unchanged drug is found in urine. The major metabolite is 3-(3-carboxyphenyl)-5-hydroxymethyl-2-oxozalidinone produced by methyl-oxidation of the drug. The drug is eliminated extensively in urine (80%), mostly as its metabolites.

Therapeutic Concentration

Five healthy adult volunteers, aged between 23 and 42 years, were administered with a single oral dose of 1-mg/kg 1 h after a standard breakfast. Three weeks later, the volunteers received a 1-mg/kg IV infusion of toloxatone. The mean peak toloxatone concentration for the oral dose was 0.474 (range, 0.384–0.640) mg/L at 0.83 h (0.53–1.00 h). The drug concentration after the IV dose was 0.623–0.840 mg/L observed at the end of the 30 min infusion [Benedetti *et al.* 1982].

Toxicity The minimum toxic dose reported in cases of toloxatone poisoning is 2 g. The first symptoms, including drowsiness and mild adrenergic effects, are observed after 1 h. In cases of massive overdose, coma and jerks have occurred. In cases where toloxatone was co-administered with tricyclic antidepressants, 2 patients died and symptoms included muscular rigidity, hyperthermia and cardiovascular collapse [Azoyan *et al.* 1990].

Half-life 0.88–2.46 h.

Bioavailability 50–62%.

Volume of Distribution 1.09–1.64 L/kg.

Clearance Plasma, 0.462–0.860 L/h/kg.

Protein Binding 49–53% (mainly to albumin).

Dose Orally, 200 mg three times a day.

Azoyan P *et al.* (1990). [Acute toloxatone poisoning. Apropos of 122 cases]. *Therapie* 45: 139–144.
Benedetti *et al.* (1982). Pharmacokinetics of toloxatone in man following intravenous and oral administrations. *Arzneimittelforschung* 32: 276–280.
Duverneuil C *et al.* (2003). A high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. *Ther Drug Monit* 25: 565–573.
Gaillard Y, Pepin G (1997). Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology. *J Chromatogr A* 763: 149–163.
Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography-tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.
Tracqui A *et al.* (1995). Systematic toxicological analysis using HPLC/DAD. *J Forensic Sci* 40: 254–262.
Vajta S *et al.* (1983). Gas–liquid chromatographic determination of toloxatone in human plasma. Routine analysis of a wide range of drug concentrations using a nitrogen-selective detector. *J Chromatogr* 274: 139–148.

Tolpropamine

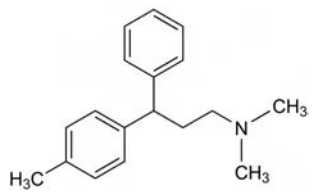
Antihistamine

$C_{18}H_{23}N = 253.4$

CAS—5632-44-0

IUPAC Name *N*,*N*-Dimethyl-3-(4-methylphenyl)-3-phenylpropan-1-amine

Synonym *N*,*N*,4-Trimethyl-γ-phenylbenzenepropanamine



Chemical Properties Practically insoluble in water; soluble in chloroform. Log *P* (octanol/water) 4.4.

Tolpropamine Hydrochloride

$C_{18}H_{23}N,HCl = 289.8$

CAS—3339-11-5

Proprietary Name *Pragman*

Chemical Properties A white crystalline powder. Mp 182° to 184°. Soluble in water and ethanol; practically insoluble in chloroform and ether.

Colour Tests Mandelin's test—brown; Marquis test—red.

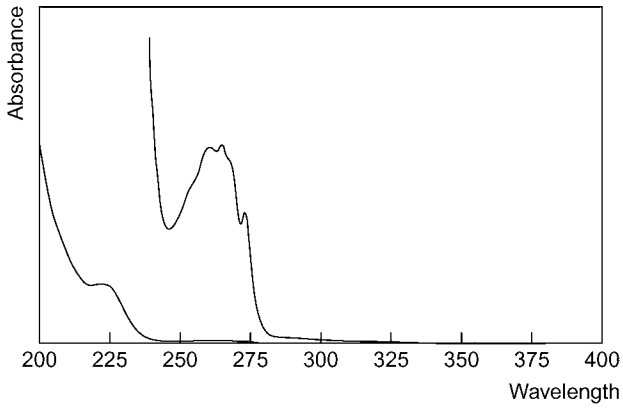
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.52; system TC—R_f 0.32; system TE—R_f 0.68; system TL—R_f 0.15; system TAE—R_f 0.26 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—tolpropamine RI 1900; M (OH-) RI 2150; M (nor-) RI 2100; M (nor-OH-) RI 2200; M (*N*-oxide-) RI 1750.

High Performance Liquid Chromatography System HA—*k* 2.9.

Ultraviolet Spectrum Aqueous acid—260 nm (A^{1}_{1}=25a), 265 nm (A^{1}_{1}=25a), 273 nm.

T

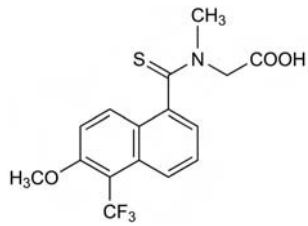


Infrared Spectrum Principal peaks at wavenumbers 727, 776, 700, 802, 1515, 970 cm^{-1} (tolpropamine hydrochloride, KBr disk).
Use Topically in a concentration of 1% of the hydrochloride.

Tolrestat

Antidiabetic
$C_{16}H_{14}F_3NO_3S = 357.3$
CAS—82964-04-3

IUPAC Name 2-[[6-Methoxy-5-(trifluoromethyl)naphthalene-1-carbothioyl]-methylamino]acetic acid
Synonyms AY-27773; *N*-[[6-methoxy-5-(trifluoromethyl)-1-naphthalenyl]thioxomethyl]-*N*-methylglycine.
Proprietary Names *Alredase*; *Lorestat*.



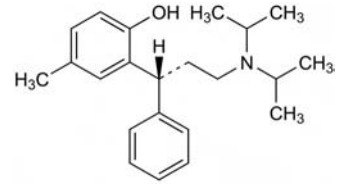
Chemical Properties Mp 164° to 165°.
Mass Spectrum Principal ions at *m/z* 357, 269, 226, 356, 207, 312, 279, 324.
Quantification
***Serum* HPLC** Column: ODS Hypersil (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 100 mmol/L phosphate buffer (pH 6.0, 30 : 70), flow rate 1.1 mL/min. UV detection (λ=240 nm). Retention time: 6.4 min. Limit of detection, 25 µg/L (2 mL serum), 200 µg/L (1 mL serum) [Hicks, Kraml 1984].
***Tissue* HPLC** See Serum. Limit of detection, 50 ng/g [Hicks, Kraml 1984].
Disposition in the Body Tolrestat is well absorbed within 2 h and rapidly distributed throughout the body. Elimination is renal (63 to 68%) and faecal (25 to 27%) mainly as the unchanged drug, along with conjugated tolrestat and sulfotolrestat as minor metabolites. No accumulation was observed after multiple dosing. The majority of the dose (~95%) is excreted within 7 days of administration.
Therapeutic Concentration
Twenty healthy, young females (mean age, 25 years) and males (mean age, 31 years), were administered with a single 200 mg dose of tolrestat after an overnight fast, and were fasted for an additional 4 h after dosing. Multiple dosing of 200 mg every 12 h for 5 days was administered. 25 healthy, elderly males (mean age, 74 years), and females (mean age, 73 years), were also included in the study. Mean peak plasma concentrations were 27 (19 to 34), 21 (12 to 30) mg/L, observed at mean times 1.2 (0.6 to 1.8), 1.3 (0.5 to 2.1) h, for the females and males, respectively [Fruncillo *et al.* 1996].
Bioavailability Linearly proportional to dose over 25 to 800 mg.
Half-life 10 to 13 h.
Volume of Distribution 0.27 to 0.41 L/kg.
Clearance Oral clearance, 18 to 30 mL/h/kg.
Protein Binding 99%.
Dose 200 mg.

Fruncillo R *et al.* (1996). Pharmacokinetics of the aldose reductase inhibitor tolrestat: studies in healthy young and elderly male and female subjects and in subjects with diabetes. *Clin Pharmacol Ther* 59(6): 603–612.
Hicks D, Kraml M (1984). Determination of tolrestat, a novel aldose reductase inhibitor, in serum and tissues. *Ther Drug Monit* 6: 328–333.

Tolterodine

Antimuscarinic
$C_{22}H_{31}NO = 325.5$
CAS—124937-51-5

IUPAC Name 2-[(1*R*)-3-[Di(propan-2-yl)amino]-1-phenylpropyl]-4- methylphenol
Synonym 2-[(1*R*)-3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol
Proprietary Names *Detrol*; *Difluorasone Topical.*

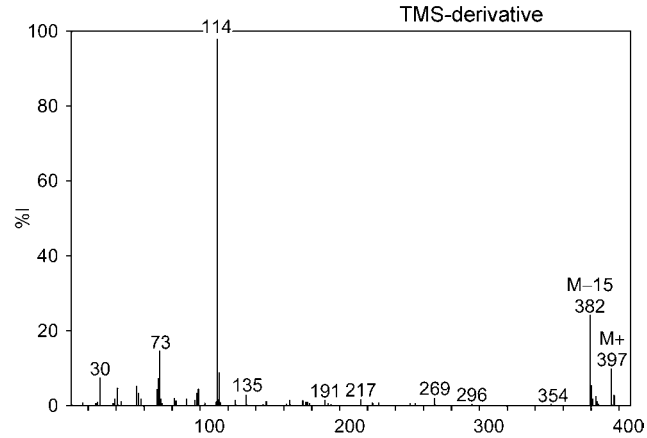


Chemical Properties pK_a 9.9.

Tolterodine Tartrate

$C_{22}H_{31}NO,C_4H_6O_6 = 475.6$
Proprietary Names *Detrol*; *Detrusitol.*
Chemical Properties A white crystalline powder. Soluble in water (12 g/L at room temperature); soluble in methanol; slightly soluble in ethanol; practically insoluble in toluene.

High Performance Liquid Chromatography Column: PKB 100 Supelco (150 × 4.5 mm i.d.). Mobile phase: methanol : 20 mmol/L ammonium acetate (pH 4.5, 10 : 90 to 20 : 80 in 5 min, to 45 : 55 in 30 min, to 100 : 0 in 5 min, for 10 min), flow rate 1 mL/min. UV detection (λ=280 nm). RT: 29 min [Andersson *et al.* 1998].
Mass Spectrum Principal ions at *m/z* 114, 382, 73, 397, 116, 30, 72, 135 (TMS derivative).



Quantification
***Plasma* GC–MS** Column: fused silica capillary with 5% phenyl methyl silicon (25 m × 0.2 mm i.d., 0.33 µm). Temperature: 120° for 0.5 min, to 300° at 35°/min for 5–10 min. Carrier gas: He. IS: [$^2H_5$] tolterodine. EI ionisation, SIM acquisition mode at *m/z* 382 for tolterodine and 387 for IS. Retention time: tolterodine, (plasma) 7.45 min, (urine) 8.25 min; IS, (plasma) 7.41 min, (urine) 8.22 min. Limit of quantification, 0.5 µg/L [Palmer *et al.* 1997].
***Serum* GC–MS** See Plasma [Palmer *et al.* 1997].
***Urine* GC–MS** See Plasma [Palmer *et al.* 1997].
Disposition in the Body Tolterodine is rapidly absorbed, and levels increase when co-administered with food. Peak plasma concentrations are reached within 1–3 h following an oral dose. Metabolism is mainly by the cytochrome P450 isoenzyme, CYP2D6 to the active 5-hydroxymethyl derivative. In a minority of people with no functional gene of CYP2D6, it is metabolised by CYP3A4 to the inactive *N*-dealkylated derivative. Tolterodine is excreted primarily in urine (77%) with ~17% appearing in faeces. <1% of a dose is excreted as the unchanged drug, and 4% as its metabolites. The half-life is prolonged in poor metabolisers and results in a 7-fold increase in the concentration of tolterodine. Steady state concentrations are achieved within 2 days. Pharmacokinetics are effected by hepatic impairment.
Therapeutic Concentration
Nineteen healthy male and female volunteers with a mean age of 33 years (range, 18–55 years), were classified as being either a poor metaboliser of tolterodine (i.e. no functional gene of CYP2D6) or extensive metabolisers. They were then administered with 4 mg once daily of extended release formulation of tolterodine or 2 mg instant release twice daily, for 6 days. A washout period of 7 days was allowed between the doses. The peak plasma tolterodine concentrations for the extended release dose were 6.1 (1.1–59.0) nmol/L at 4 (2–6) h for the extensive metabolisers and 35

(28–130) nmol/L at 4 (3–6) h for the poor metabolisers. After the instant release formulation, concentrations observed for the extensive metabolisers were 12.0 (1.2–57.0) nmol/L after the morning dose and 9.2 (1.5–50.0) nmol/L after the evening dose. For the poor metabolisers, 51 (40–143) nmol/L in the morning and 45 (34–99) nmol/L in the evening. After the morning dose, peak concentrations were observed at ≈1 h, and in the evening, between 1.5 and 1.8 h (and up to 3 h). These steady state concentrations were observed on day 6 [Olsson, Szamosi 2001].

Toxicity The highest dose of tolterodine L-tartrate given to human volunteers is 12.8 mg as a single dose. The most severe adverse effects were accommodation disturbances and micturition difficulties. Overdose can potentially result in severe central anticholinergic effects.

Bioavailability 17% in extensive metabolisers; 65% in poor metabolisers.

Half-life 2–3 h in extensive metabolisers; 10 h in poor metabolisers.

Volume of Distribution Mean, 113 L.

Clearance In extensive metabolisers: systemic clearance is 30 L/h, apparent oral clearance is ≈8900 following a single 4 mg dose of the drug, and 6919 mL/min following multiple oral doses of the drug, respectively. Poor metabolisers (deficient of CYP2D6) show reduced clearance of 283 mL/min, and apparent oral clearance of 183 mL/min. Clearance of oral tolterodine, ≈18 mL/min/kg in individuals with liver cirrhosis or 95 mL/min/kg in healthy individuals.

Distribution in Blood Neither tolterodine nor the metabolite distributes significantly to erythrocytes. Blood : serum ratio 0.6 (tolterodine); 0.8 (5-hydroxy metabolite).

Protein Binding 96.3% (tolterodine) 64% (5-hydroxymethyl metabolite), primarily to orosomucoids and α_1-acid glycoprotein.

Dose 2 mg twice daily.

Andersson S *et al.* (1998). Biotransformation of tolterodine, a new muscarinic receptor antagonist, in mice, rats, and dogs. *Drug Metab Dispos* 26: 528–535.

Olsson B, Szamosi J (2001). Multiple dose pharmacokinetics of a new once daily extended release tolterodine formulation versus immediate release tolterodine. *Clin Pharmacokinet* 40(3): 227–235.

Palmer L *et al.* (1997). Determination of tolterodine and the 5-hydroxymethyl metabolite in plasma, serum and urine using gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 16: 155–165.

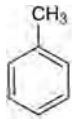
Toluene

Solvent

$C_6H_5{\cdot}CH_3 = 92.1$

CAS—108-88-3

Synonyms Methylbenzene; phenylmethane; toluol(e).

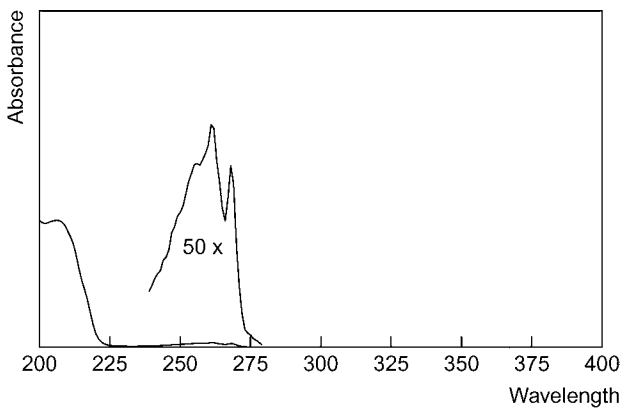


Chemical Properties A colourless, mobile, highly flammable liquid which burns with a smoky flame. Wt per mL about 0.87 g. Bp about 111°. Practically insoluble in water; miscible with ethanol, chloroform, ether, acetone and glacial acetic acid. Log *P* (octanol/water) 2.7.

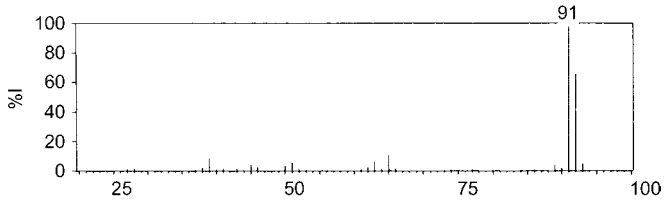
Gas Chromatography System GA—RI 756; system GI—retention time 24.8 min.

High Performance Liquid Chromatography System HAA—retention time 21.9 min.

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 91, 92, 65, 39, 63, 93, 51, 89.



Quantification

Blood **GC** Headspace analysis. Limit of detection, 0.5 to 5 µg/0.5 L for toluene and other volatile compounds [Lee *et al.* 1995; 1998]; [Watanabe-Suzuki *et al.* 2002]. FID. Limit of detection, 1 mg/L [Garriott *et al.* 1981].

GC and GC–MS H-SPME (solid phase micro-extraction). For a method of quantification for toluene, see Kim and Park [2000]]. Headspace analysis. For a method for the quantification of toluene and other volatile compounds, see Bellanca *et al.* [1982].

Urine **GC** See Blood [Lee *et al.* 1995; 1998]; [Watanabe-Suzuki *et al.* 2002].

GC and GC–MS See Blood [Kim and Park 2000].

HPLC Fluorescence and UV detection. Limit of detection, 50 mg/L for hippuric acid, 5 µg/L for *o*-cresol [Hansen, Døssing 1982].

Breath **GC** See Blood [Garriott *et al.* 1981].

Tissues **GC and GC–MS** See Blood [Bellanca *et al.* 1982].

Disposition in the Body Toluene is absorbed from the gastrointestinal tract, through the skin and mucous membranes, and from the lungs. It has an affinity for lipid-rich tissues from which it is slowly released. About 80% of an inhaled dose is oxidised to benzoic acid which is conjugated with glycine to give hippuric acid and excreted in the urine. Conjugation with glucuronic acid also occurs when large amounts of toluene are absorbed. About 20% of the dose is excreted unchanged in expired air with <0.1% in the urine.

Blood Concentration

Exposure to a concentration of 100 ppm of toluene produced a mean venous-blood concentration of about 0.39 mg/L in 6 healthy subjects at rest and about 1.16 mg/L in 6 subjects during light exercise, after 20 to 30 min. Exposure to 200 ppm for the same time produced a mean blood concentration of about 0.55 mg/L in 4 subjects at rest [Astrand *et al.* 1972].

Toxicity Toluene has about the same acute toxicity as benzene but is a less serious industrial hazard. The maximum permissible atmospheric concentration of toluene is 100 ppm. Exposure to air concentrations of 10 000 to 30 000 ppm may cause unconsciousness within a few minutes. A blood concentration of >10 µg/mL may be lethal, although higher concentrations have been found in habitual abusers.

Seven children aged from 9 to 13 years, with an acute encephalopathy caused by toluene intoxication from glue-sniffing were found to have blood-toluene concentrations of 0.8 to 7.7 µg/g (mean, 2.3) [King *et al.* 1981].

A boy of 13 years found dead after sniffing glue had the following postmortem concentrations of toluene in body tissues: blood 11 mg/L, brain 44 µg/g, kidney 39 µg/g, liver 47 µg/g [Collom, Winek 1970].

The following postmortem tissue concentrations of toluene were reported in a 16-year-old boy found dead with a plastic bag over his head: blood 20.6 mg/L, brain 297 µg/g, liver 89 µg/g; acetone was also detected in blood at a concentration of 3 mg/L [Paterson, Sarvesvaran 1983].

Half-life Blood half-life about 7.5 h.

Astrand I *et al.* (1972). *Scand J Work Environ Health* 9: 119–130.

Bellanca JA *et al.* (1982). Detection and quantitation of multiple volatile compounds in tissues by GC and GC/MS. *J Anal Toxicol* 6: 238–240.

Collom WD, Winek CL (1970). Detection of glue constitutents in fatalities due to "glue sniffing". *Clin Toxicol* 3: 125–130.

Garriott JC *et al.* (1981). Measurement of toluene in blood and breath in cases of solvent abuse. *Clin Toxicol* 18: 471–479.

Hansen SH, Døssing M (1982). Determination of urinary hippuric acid and o-cresol, as indices of toluene exposure, by liquid chromatography on dynamically modified silica. *J Chromatogr* 229: 141–148.

Kim NY, Park SW (2000). The comparison of toluene determination between headspace-solid phase microextraction and headspace methods in glue-sniffer's blood and urine samples. *J Forensic Sci* 45: 702–770.

King MD *et al.* (1981). Solvent encephalopathy. *Brit Med J* 283: 663–665.

Lee XP *et al.* (1995). A simple analysis of 5 thinner components in human body fluids by headspace solid-phase microextraction (SPME). *Int J Legal Med* 107: 310–313.

Lee XP *et al.* (1998). Determination of solvent thinner components in human body fluids by capillary gas chromatography with trapping at low oven temperature for headspace samples. *Analyst* 123: 147–150.

Paterson SC, Sarvesvaran R (1983). Plastic bag death–a toluene fatality. *Med Sci Law* 23: 64–66.

Watanabe-Suzuki K *et al.* (2002). Cryogenic oven-trapping gas chromatography for analysis of volatile organic compounds in body fluids. *Anal Bioanal Chem* 373: 75–80.

Tolycaine

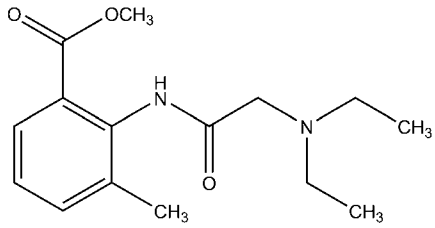
Anaesthetic (Local)

$C_{15}H_{22}N_2O_3 = 278.4$

CAS—3686-58-6

IUPAC Name Methyl 2-[(2-diethylaminoacetyl)amino]-3-methylbenzoate

Synonyms 2-(2-Diethylaminoacetoacetamido)-*m*-toluic acid methyl ester; 2-methyl-6-carbomethoxy-*N*-diethylaminoacetanilide; methyl 2-diethylaminoacetamido-*m*-toluate; 3-methyl-2-diethylaminoacetylaminobenzoic acid methyl ester.



Chemical Properties Oil. Bp 190° to 192° [O'Neil *et al.* 2006]. Insoluble in water, soluble in chloroform. Log *P* (octanol/water) 2.2 [National Institutes of Health 2008]; 2.3 [Meylan, Howard 1995].

Tolycaine Hydrochloride

$C_{15}H_{22}N_2O_3$,HCl = 314.8
CAS—7210-92-6
IUPAC Name Methyl 2-[(2-diethylaminoacetyl)amino]-3-methylbenzoate hydrochloride
Proprietary Name *Baycain*
Chemical Properties Crystals. Mp 139° to 140.5° [O'Neil *et al.* 2006]. Soluble in water.

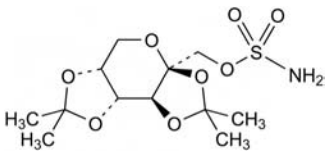
Colour Test Vitali's test— –/–/yellow (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.70 (location under ultraviolet light, green fluorescence; location reagent acidified iodoplatinate spray, positive reaction).
Ultraviolet Spectrum Water—283 nm.
Note For a spectrophotometric assay of tolycaine, see de Freitas [1977].
Dose Used as a 3% solution with adrenaline and noradrenaline.

deFreitas JF (1977). A rapid spectrophotometric assay of amide type dental anaesthetic agents. *Aust Dent J* 22: 182–189.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health (2008). *Tolycaine.* http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=72137&loc=ec_rcs. (accessed 16 June 2008).
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.

Topiramate

Antiepileptic
$C_{12}H_{21}NO_8S$ = 339.4
CAS—97240-79-4

Synonyms 2,3:4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate; McN-4853; RWJ-17021-000.
Proprietary Names *Topamax*; *Topimax*.



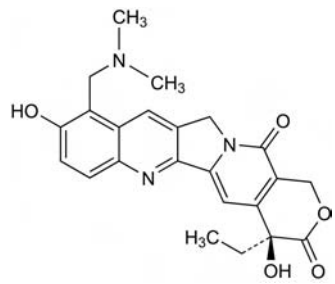
Chemical Properties A white crystalline powder. Extraction yield (chlorobutane), 0.2 [Demme *et al.* 2005]. Freely soluble in acetone, in alcohol, in chloroform and in dimethyl sulfoxide.
Gas Chromatography System GB—RI 2253.
Column: DB-1 methylsilicone (30 m × 0.25 mm, 0.25 µm). Temperature: 230°. Carrier gas: H_2, flow rate 31 mL/min. Detection: flame ionisation. Retention time: 3.2 min [Gidal, Lensmeyer 1999].
Quantification
***Plasma* GC** Column: DB-5 fused silica capillary (25 m × 0.32 mm i.d., 0.25 µm). Temperature: 165° to 205° at 9°/min. Carrier gas: He, flow rate 3 mL/min. Detection: flame ionisation. Retention time: 5 min. Limit of detection, 0.1 mg/L [Holland *et al.* 1988].
Disposition in the Body Topiramate is rapidly and well absorbed after oral administration. The rate of absorption is delayed with the presence of food but the extent is not affected. Peak plasma concentrations are attained after about 2 h. It displays linear pharmacokinetics. Topiramate undergoes rapid distribution and there is significant binding to red blood cells. It is not extensively metabolised (about 20%); up to 50% of a dose may undergo metabolism in patients taking other enzyme-inducing anti-epileptics, for example, phenytoin. The presence of these antiepileptics may also reduce the plasma concentration of topiramate, increase the clearance of the drug and decrease its half-life. Metabolic pathways include, hydroxylation, hydrolysis and glucuronidation and in total six metabolites have been identified. The drug is eliminated mainly in urine as the unchanged drug (a total of 55 to 66% of the dose) and its metabolites (35%). There is some renal tubular reabsorption. It is removed by haemodialysis.
Therapeutic Concentration
Healthy volunteers were administered with 100, 200, 400, 800 and 1200 mg topiramate and peak concentrations of 1.7, 3.7, 7.7, 18.4 and 28.7 mg/L were observed, respectively. These concentrations were seen 1.8 to 4.3 h after administration of the drug [Easterling *et al.* 1988].
Group 1: Seven renally impaired patients with creatinine clearance <30 mL/min. Group 2: Seven renally impaired patients with creatinine clearance between 30 and 69 mL/min. Groups 3 and 4: Seven individuals with normal renal function, creatinine clearance ≥70 mL/min. Each group was administered with a single, oral dose of 100 mg topiramate and peak concentrations were 2.1, 2.2, 1.9 and 1.6 mg/L, respectively, for the 4 groups [Gisclon *et al.* 1993].
Bioavailability 80 to 95%.
Half-life Plasma, 20 to 30 h.
Volume of Distribution 0.6 to 0.8 L/kg. In women it is approximately half of that in men although this difference is not considered clinically significant.
Clearance Plasma, 1.2 to 2.4 L/h; decreased in patients with renal or hepatic impairment.
Distribution in Blood Topiramate significantly binds red blood cells and the major proportion of the circulating drug is bound to erythrocytes. Saturation of the binding sites occurs rapidly with higher drug concentrations and hence, the blood:plasma ratio decreases with increasing drug concentration.
Protein Binding 13 to 17%.
Note For reviews of topiramate, see Langtry *et al.* [1997] and Sachdeo [1998]; for reviews of the pharmacokinetics of topiramate, see Bialer [1993] and Perucca and Bialer [1996].
Dose Usually 200 to 400 mg daily.

Bialer M (1993). Comparative pharmacokinetics of the newer antiepileptic drugs. *Clin Pharmacokinet* 24: 441–452.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Easterling DE *et al.* (1988). *Epilepsia* 29662.
Gidal BE, Lensmeyer GL (1999). Therapeutic monitoring of topiramate: evaluation of the saturable distribution between erythrocytes and plasma of whole blood using an optimized high-pressure liquid chromatography method. *Ther Drug Monit* 21: 567–576.
Gisclon LG *et al.* (1993). *Pharm Res* 10 (Suppl. 10)S397.
Holland ML *et al.* (1988). Automated capillary gas chromatographic assay using flame ionization detection for the determination of topiramate in plasma. *J Chromatogr* 433: 276–281.
Langtry HD *et al.* (1997). Topiramate. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in the management of epilepsy. *Drugs* 54: 752–773.
Perucca E, Bialer M (1996). The clinical pharmacokinetics of the newer antiepileptic drugs. Focus on topiramate, zonisamide and tiagabine. *Clin Pharmacokinet* 31: 29–46.
Sachdeo RC (1998). Topiramate. Clinical profile in epilepsy. *Clin Pharmacokinet* 34: 335–346.

Topotecan

Antineoplastic
$C_{23}H_{23}N_3O_5$ = 421.5
CAS—123948-87-8

Synonyms (4*S*)-10-[(Dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1*H*-pyrano-[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione; hycamptamine; SKF-104864A; SKFS-104864-A.
Proprietary Name *Hycamtin*

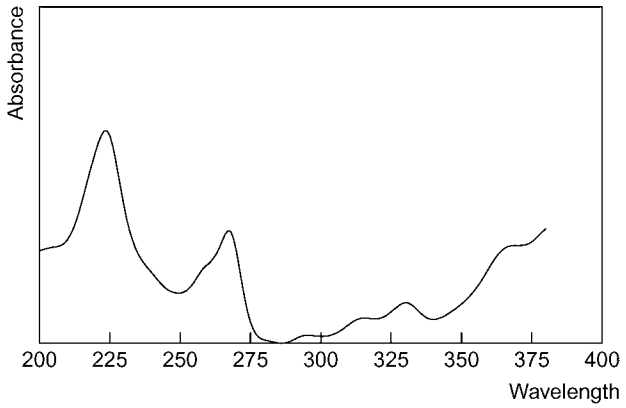


Chemical Properties Soluble in water, methanol and dimethylformamide; insoluble in ethanol. pK_a 10.5. Log *P* (octanol/water), −0.88.

Topotecan Hydrochloride

$C_{23}H_{23}N_3O_5$,HCl = 457.9
CAS—119413-54-6
Synonym NSC-609669
Proprietary Name *Hycamtin*
Chemical Properties A light yellow to greenish powder. Mp 213° to 218°. Soluble in water up to 1 g/L.

Ultraviolet Spectrum Aqueous acid—223, 267, 295, 316 nm.



Mass Spectrum Principal ions at *m/z* 44, 45, 42, 58, 43, 59, 41, 57.

Quantification

Plasma **HPLC** Column: BDS C_{18} Hypersil (100 × 3 mm i.d., 3 m). Mobile phase: 10 mmol/L potassium hydrogen phosphate (pH 6.0) containing methanol (25%) and ethylamine (0.2%), flow rate 0.70 mL/min. Fluorescence detection (λ_{ex}=381 nm, λ_{em}=525 nm). Retention time: topotecan (closed ring form), 6.5 min; (open ring form), 2.5 min. Limit of quantification, 0.10 µg/L [Loos *et al.* 1996]. Fluorescence detection (λ_{ex}=361 nm, λ_{em}=527 nm). Limit of quantification, 0.05 µg/L [Rosing *et al.* 1995]. Column: RP-18 Lichrosorb (125 × 4 mm i.d., 5 µm). Mobile phase: methanol : water : 0.25 mol/L sodium dioctylsulfosuccinate (DOSS) : phosphate buffer : 1.0 mol/L triethylamine (pH 6.0, 567 : 375 : 35 : 20 : 3), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=381 nm; λ_{em}=527 nm). Retention time: 5.52 min. Limit of quantification, 1.0 µg/L, limit of detection, 0.2 µg/L [Beijnen *et al.* 1990].

Disposition in the Body Topotecan is rapidly absorbed following oral administration. It is widely and rapidly distributed throughout the body following IV administration. Topotecan undergoes reversible hydrolysis of the lactone ring to the inactive hydroxy acid form, and only small amounts are demethylated in the liver. A significant proportion (30%) of a dose is excreted in urine via the kidneys. Peak maximum concentrations are reached within 45 min. The concentration of the drug in cerebrospinal fluid is 29% of the plasma concentration. Topotecan exhibits substantial inter-individual pharmacokinetics.

Therapeutic Concentration

The lactone mean peak plasma concentrations following a 30 min infusion ranged from 9 to 35 µg/L after dosages. Following 21 days of continuous infusion of dose 0.4 to 0.6 mg/m^2 per day, steady state plasma lactone and total concentrations were 2.3 to 5.5 µg/L and 6.7 to 8.9 µg/L, respectively. Following oral administration peak plasma lactone concentrations were 5.9 and 48 µg/L after 1.5 and 14 mg/m^2 [Herben *et al.* 1996].

Toxicity One patient on a single dose regimen of 17.5 mg/m^2 was given on day one of a 21-day cycle a single dose of 35 mg/m^2. They experienced severe neutropenia 14 days later but recovered without incident.

Bioavailability Oral, 20 to 55%.

Half-life 2 to 3 h.

Volume of Distribution Initial, 17 to 22 L/m^2. At steady state, 75 to 91 L/m^2.

Clearance Approximately 24%. Clearance is higher in males than females. It decreases to about 67% of the normal value in patients with mild renal impairment; to about 34% of the normal value in those with moderate renal impairment; and about 67% of the norm in patients with hepatic impairment.

Protein Binding Approximately 35%.

Dose 1.5 mg/m^2.

Beijnen JH *et al.* (1990). High-performance liquid chromatographic analysis of the new antitumour drug SK&F 104864-A (NSC 609699) in plasma. *J Pharm Biomed Anal* 8: 789–794.

Herben *et al.* (1996). Clinical pharmacokinetics of topotecan. *Clin Pharmacokinet* 31(2): 85–102.

Loos WJ *et al.* (1996). Sensitive high-performance liquid chromatographic fluorescence assay for the quantitation of topotecan (SKF 104864-A) and its lactone ring-opened product (hydroxy acid) in human plasma and urine. *J Chromatogr B Biomed Appl* 678: 309–315.

Rosing H *et al.* (1995). High-performance liquid chromatographic determination of the novel antitumour drug topotecan and topotecan as the total of the lactone plus carboxylate forms, in human plasma. *J Chromatogr B Biomed Appl* 668: 107–115.

Torasemide

Diuretic

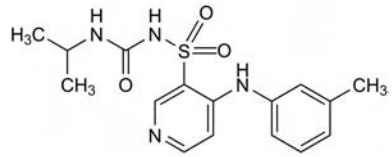
$C_{16}H_{20}N_4O_3S$ = 348.4

CAS—56211-40-6

IUPAC Name 1-[4-(3-Methylanilino)pyridin-3-yl]sulfonyl-3-propan-2-ylurea

Synonyms AC-4464; BM-02015; JDL-464; *N*-[[(1-methylethyl)amino]carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfonamide; torsemide.

Proprietary Names *Demadex*; *Dilutol*; *Isodiur*; *Sutril*; *Torem*, *Torrem*; *Unat*.



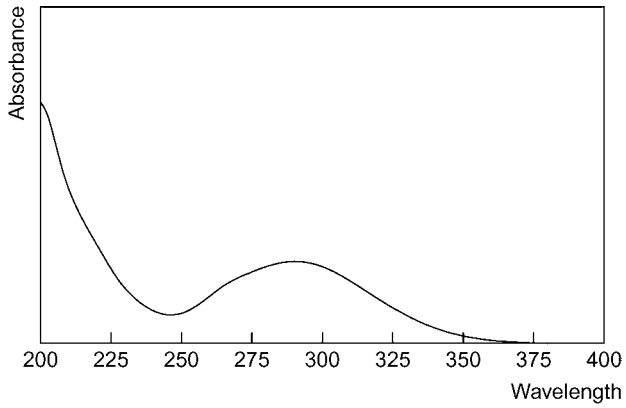
Chemical Properties Mp 163° to 164°. pK_a 6.44, 7.1.

Torasemide Sodium

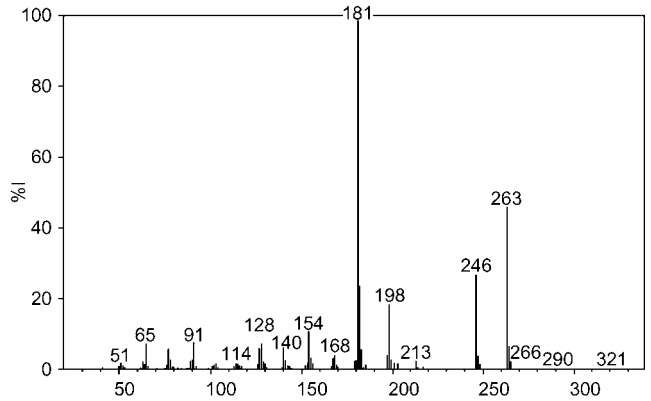
CAS—72810-59-4

High Performance Liquid Chromatography Column: ODS Ultrasphere (75 × 4.6 mm i.d., 3 µm). Mobile phase: acetonitrile : 100 mmol/L ammonium acetate (pH 3; 10 : 90 to 15 : 85 in 2 min, to 55 : 45 in 3 min, to 60 : 40 in 3 min, for 1 min, to 10 : 90 in 1 min, for 2 min, flow rate 1 mL/min. UV detection (λ= 270, 290 nm). Retention time: 5.2 min [Ventura *et al.* 1993].

Ultraviolet Spectrum Principal peak at 289 nm.



Mass Spectrum Principal ions at *m/z* 181, 263, 246, 182, 198, 154, 91, 65.



Quantification

Plasma **HPLC** Column: Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 100 mmol/L potassium dihydrogen phosphate (pH 4.5; 14 : 86 for 8 min, to 30 : 70 for 3.5 min, to 21 : 79 for 8.5 min, to 40 : 60 for 2 min, to 21 : 79 for 10 min, to 14 : 86 for 6 min), flow rate 1.3 mL/min. UV detection (λ= 290 nm). Retention time: 18.2 min. Limit of quantification, 0.01 mg/L [March *et al.* 1990]. Column: C_{18} Nucleosil 10 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.01 mol/L sodium dihydrogen phosphate (pH 3.0; 78 : 22 to 60 : 40 in 5 min, to 40 : 60 in 5 min, to 25 : 75) in 5 min, to 78 : 22 in 20 min. Retention time: 10 min. Limit of detection, 0.04 mg/L [Besenfelder 1987]. UV detection (λ= 290 nm). Limit of detection, 0.02 mg/L [Broekhuysen *et al.* 1986].

Urine **GC-MS** Column: DB-1701 capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature: 40°, held 1 min, then 20°/min to 300°. Carrier gas: He. EI, MID at *m/z* 197 and 305). Retention time: 18.24 min. Limit of quantification, 0.5 ng (1 µL injection) [Barroso *et al.* 1997].

HPLC See Plasma [Broekhuysen *et al.* 1986]. See Plasma. Limit of quantification, 0.02 mg/L [March *et al.* 1990].

Disposition in the Body Torasemide is almost completely absorbed rapidly from the gastrointestinal tract with little first-pass metabolism. Food does not alter the absorption and is unaffected by renal or hepatic dysfunction. Peak serum concentrations are achieved within 1 h of oral administration. It is cleared from the circulation by both hepatic metabolism and excretion into urine (≈20% of total clearance). Very little drug enters urine via glomerular filtration. Most renal clearance occurs via active secretion of the drug by the proximal tubules into tubular urine. Three metabolites are formed (M1, M3 and M5) by stepwise oxidation,

hydroxylation or ring hydroxylation. The total amount of torasemide and metabolites recovered in urine is 83%; 25% torasemide, 11% M1, 3% M3 (both active) and 44% M5 (inactive). Kinetics are linear with dosage.

Therapeutic Concentration

Fifteen patients with moderate chronic renal insufficiency (creatinine clearance, 30–60 mL/min), mean age of 63.3 years, and 16 patients with severe insufficiency (creatinine clearance, <30 mL/min), mean age 52.8 years, were administered with 20, 50 and 100 mg oral doses of torasemide, and 50, 100 and 200 mg doses, respectively. The mean peak drug concentrations were 2.95, 6.68 and 11.1 mg/L for the 20, 50 and 100 mg doses received by the patients with moderate impairment. These concentrations were observed at 0.94, 1.03 and 1.36 h, respectively. The mean drug concentration for the 50, 100 and 200 mg doses administered to the patients with severe impairment were 6.11, 11.3 and 18.5 mg/L at 1.18, 1.05 and 1.56 h, respectively [Gehr *et al.* 1994].

Twelve patients with ascites caused by cirrhosis, aged between 38 and 66 years, with a creatinine clearance 63 to 127 mL/min, were administered with an oral dose of 10 mg or IV over a 30-min period. The patients fasted overnight and for 4 h after dosing. The peak plasma concentrations were 1.7 mg/L for the IV dose observed at 0.52 h and 1.45 mg/L at 0.71 h for the oral dose. For healthy individuals receiving the same oral dose, the peak concentration was 1.27 mg/L at 0.86 h [Schwartz *et al.* 1993].

Sixteen patients with congestive heart failure (New York Heart Association class II or III), aged between 39 and 81 years, were administered with 10 mg orally or IV (over a 30-min infusion). The peak concentration with the IV dose was 2.9 mg/L after 0.6 h and 1.5 mg/L at 1.1 h for the orally administered dose [Vargo *et al.* 1995].

Bioavailability 80–90%; 76% (elderly).

Half-life 2.2–3.5 h.

Volume of Distribution 11–16.5 L or 0.09–0.31 L/kg; this value may increase by approximately double in individuals with hepatic cirrhosis.

Clearance Following a 10 mg dose, total clearance is 0.72 ±0.23 mL/s; renal clearance 0.11 ±0.04 mL/s. After a 20 mg oral dose, total clearance is 3.4 L/h; renal clearance 0.43 L/h. After an IV dose, total clearance is 2.2–2.6 L/h and renal clearance 0.38 to 0.53 L/h. Systemic clearance is 41 mL/min. Renal clearance is reduced in individuals with renal impairment.

Protein Binding >99%.

Dose Oedema: usual dose is 5 mg, increased to 20 mg once daily if necessary, maximum 40 mg. Hypertension: 2.5 mg daily, increased to 5 mg if necessary.

Barroso MB *et al.* (1997). Gas chromatographic-mass spectrometric analysis of the loop diuretic torasemide in human urine. *J Chromatogr B Biomed Sci Appl* 690(1–2): 105–113.

Besenfelder E (1987). The determination of torasemide and metabolites in plasma by high-performance liquid chromatography. *J Pharm Biomed Anal* 5(3): 259–266.

Broekhuysen J *et al.* (1986). Torasemide, a new potent diuretic. Double-blind comparison with furosemide. *Eur J Clin Pharmacol* 31: 29–34.

Gehr TW *et al.* (1994). The pharmacokinetics of intravenous and oral torsemide in patients with chronic renal insufficiency. *Clin Pharmacol Ther* 56: 31–38.

March C *et al.* (1990). Solid-phase extraction and liquid chromatography of torsemide and metabolites from plasma and urine. *J Pharm Sci* 79: 453–457.

Schwartz S *et al.* (1993). Bioavailability, pharmacokinetics, and pharmacodynamics of torsemide in patients with cirrhosis. *Clin Pharmacol Ther* 54(1): 90–97.

Vargo DL *et al.* (1995). Bioavailability, pharmacokinetics, and pharmacodynamics of torsemide and furosemide in patients with congestive heart failure. *Clin Pharmacol Ther* 57(6): 601–609.

Ventura R *et al.* (1993). *J Chromatogr A* 655: 233–242.

Toremifene

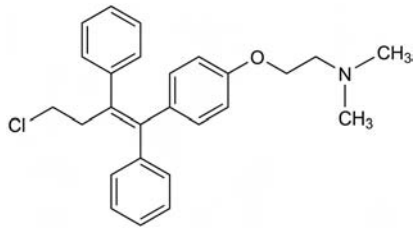
Antioestrogen, Antineoplastic

$C_{26}H_{28}ClNO$ = 406.0

CAS—89778-26-7

IUPAC Name 2-[4-[(*Z*)-4-Chloro-1,2-diphenylbut-1-enyl]phenoxy]-*N*,*N*-dimethylethanamine

Proprietary Name *Fareston*



Chemical Properties Mp 108° to 110°. pK_a 8.0.

Toremifene Citrate

$C_{26}H_{28}ClNO,C_6H_8O_7$ = 598.1

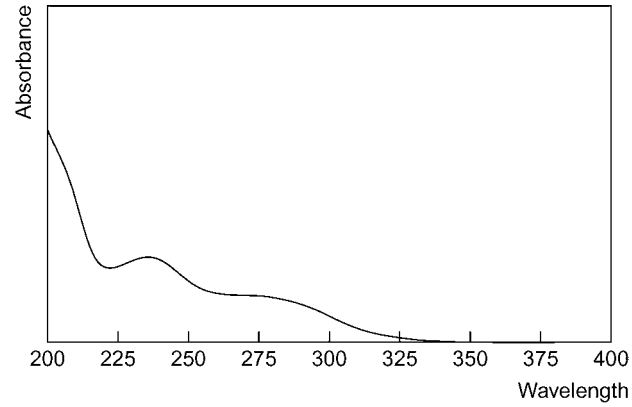
CAS—89778-27-8

Proprietary Name *Fareston*

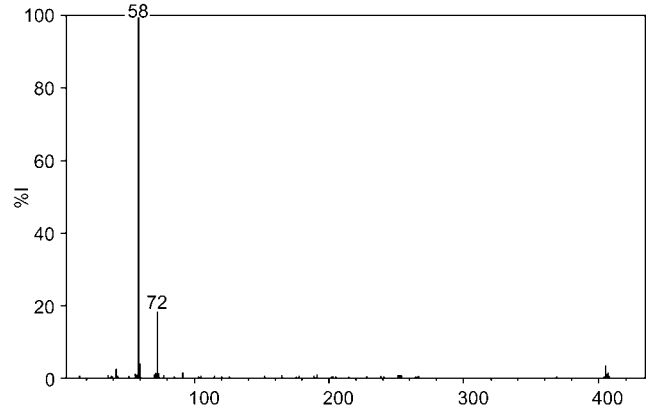
Chemical Properties Mp 160° to 162°.

High Performance Liquid Chromatography Column: C_{18} NovaPak (250 × 4 mm i.d., 5 µm). Mobile phase: acetonitrile : 100 mmol/L mmonium acetate : triethylamine (pH 6, 46 : 54 : 0.05), flow rate 1 mL/min. UV detection (λ=220 nm). Retention time: 25 min [Berthou, Dréano 1993].

Ultraviolet Spectrum Aqueous acid—236 nm.



Mass Spectrum Principal ions at *m/z* 58, 72, 59, 405.



Quantification

Plasma **HPLC** UV detection (λ=277 nm). Limit of detection, about 0.2 mg/L for toremifene and metabolites [Webster *et al.* 1991].

Urine **HPLC** See Plasma. For a method of quantification for toremifene and metabolites, see Webster *et al.* [1991].

HPLC–MS Column: CN Zorbax (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.1 mol/L ammonium acetate (pH 8) in 70% methanol, flow rate 1.2 mL/min. MS detection (API, SIM at toremifene, *m/z* 392; *N*-desmethyltoremifene, *m/z* 406; 4-hydroxytoremifene, m/z 408). Retention time: toremifene, 19.5 min; *N*-desmethyltoremifene, 16.0 min; 4-hydroxytoremifene, 13.7 min. For a method of quantification for metabolites, see Watanabe *et al.* [1989].

Disposition in the Body Toremifene is well absorbed after oral administration and absorption is not influenced by food. Peak plasma concentrations are attained within 3 h. This drug shows linear pharmacokinetics. Toremifene is extensively metabolised primarily by CYP3A4. It is metabolised by *N*-demethylation, *p*-hydroxylation and side chain oxidation to *N*-desmethyltoremifene, which also has antioestrogenic properties but only weak *in vitro* antitumour activity. Other metabolites include deamino-hydroxy-toremifene, 4-hydroxy-toremifene and di-desmethyltoremifene. It also undergoes entero-hepatic circulation. The drug is eliminated as metabolites mainly in faeces (70%). About 10% is excreted in urine during a 1-week period as metabolites. Toremifene accumulates during repeated dosing.

Therapeutic Concentration

Four groups of subjects, aged between 32 and 75 years, were administered with a single oral dose of 120 mg after an overnight fast and fasting was continued for 3 h after dosing. Group 1 comprised 10 normal, healthy subjects; group 2 comprised 10 patients with impaired liver function (alcohol related); group 3 comprised 10 patients with drug-activated liver dysfunction; and group 4 comprised 10 subjects with impaired renal function (stable, chronic renal impairment). Mean peak plasma drug concentrations were 0.414, 0.523, 0.384 and 0.498 mg/L for the four groups, respectively. These concentrations were observed after 2 h (range, 0.5 to 8 h). Peak concentrations for the metabolite, *N*-desmethyltoremifene, were 0.130, 0.142, 0.196 and 0.136 µg/L for groups 1, 2, 3 and 4 observed at approx. 72, 7, 16 and 24 h, respectively [Anttila *et al.* 1995].

Half-life Plasma half-life, toremifene, 5 days; *N*-desmethyltoremifene, 6 days.

Protein Binding >99.5%, mainly to albumin.

Note For a review of toremifene, see Wiseman and Goa [1997].

Dose The equivalent of 60 mg of toremifene daily.

Anttila M *et al.* (1995). Pharmacokinetics of the novel antiestrogenic agent toremifene in subjects with altered liver and kidney function. *Clin Pharmacol Ther* 57: 628–635.
Berthou F, Dréano Y (1993). High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites. *J Chromatogr* 616: 117–127.
Watanabe N *et al.* (1989). Liquid chromatographic-atmospheric pressure ionization mass spectrometric analysis of toremifene metabolites in human urine. *J Chromatogr* 497: 169–180.
Webster LK *et al.* (1991). High-performance liquid chromatographic method for the determination of toremifene and its major human metabolites. *J Chromatogr* 565: 482–487.
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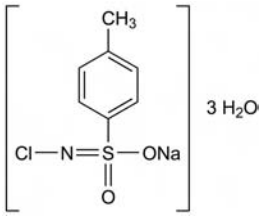
Tosylchloramide Sodium

Disinfectant
$C_7H_7ClNNaO_2S,3H_2O = 281.7$
CAS—127-65-1 (anhydrous)

IUPAC Name Sodium *N*-chloro-4-methylbenzenesulfonamide trihydrate
Synonyms Chloramidum; chloramine; chloramine T; cloramina; mianin; natrium sulfaminochloratum.
Note The name chloramin is applied to a preparation of chlorphenamine maleate.
Proprietary Names *Hydroclonazone*; *Klortee*.

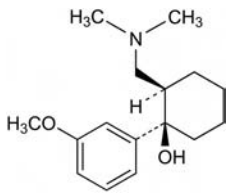


Chemical Properties A white or slightly yellow crystalline powder. It effloresces in air, losing chlorine, becoming yellow in colour and less soluble in water. Soluble 1 in 7 of water and 1 in 2 of boiling water; soluble 1 in 12 of ethanol, with slow decomposition; practically insoluble in benzene, chloroform and ether.
Thin-layer Chromatography System TAJ—R_f 0.40; system TAK—R_f 0.18; system TAL—R_f 0.68.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Infrared Spectrum Principal peaks at wavenumbers 1165, 1316, 1105, 825, 710, 920 cm^{-1} (KCl disk).
Use As a 2% solution for wound irrigation.

Tramadol

Narcotic Analgesic
$C_{16}H_{25}NO_2 = 263.4$
CAS—27203-92-5

IUPAC Name (1*R*,2*R*)-2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol-1-ol



Chemical Properties pK_{a1} 8.3; pK_{a2} 9.41. Log *P* (water pH 7/*n*-octanol), 1.35; (octanol/water), 3.01. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Tramadol Hydrochloride

$C_{16}H_{25}NO_2,HCl = 299.8$
CAS—22204-88-2
Synonyms CG-315; CG-315E; U-26225A.
Proprietary Names *Dromadol*; *Tramake*; *Tramal*; *Ultram*; *Zamadol*; *Zydol*.
Chemical Properties A white, bitter, odourless crystalline powder. Mp 180° to 181°. Readily soluble in water and ethanol.

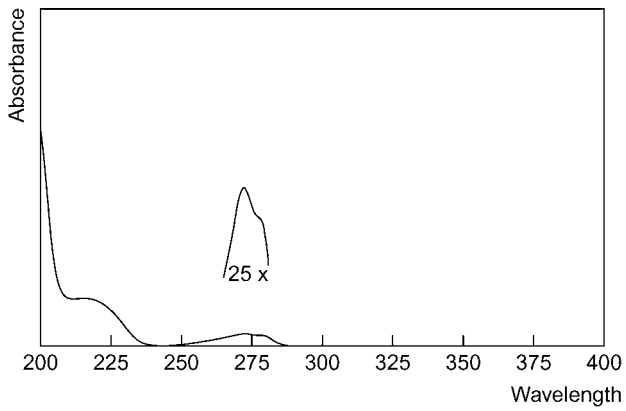
Thin-layer Chromatography System TE—R_f 0.78; system TAE—R_f 0.30.
Gas Chromatography System GA—tramadol RI 1943, M (nor-)-AC RI 2295, M (*O*-desmethyl-) RI 1995, M (*O*-desmethyl-)-AC RI 1998, M (*N*,*O*-didesmethyl-)-AC_2RI 2464, M (OH-) RI 2200; system GB—tramadol RI 2021, M (nor-) RI 2049, M (*O*-desmethyl-) RI 2093, M (*N*,*O*-didesmethyl-) RI 2122, M (OH-) RI 2252.
High Performance Liquid Chromatography System HX—RI 328; system HY—RI 267; system HZ—RT 2.9 min.

Column: LiChrosorb RP-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.01 mol/L phosphate buffer (pH 5.9) : acetonitrile with 0.1% triethylamine (70 : 30), flow rate 0.75 mL/min. UV detection (λ = 218 nm). Retention time 11.4 min. *k* value 2.09 [Gan, Ismail 2001].

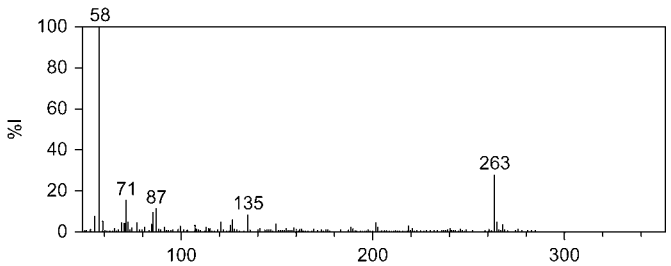
Column: Asahipack ODP-50 (125 × 4.0 mm i.d., 5 µm). Mobile phase: 0.01 mol/L borate buffer (pH 9) : methanol (40 : 60), flow rate 0.7 mL/min. Electochemical detection. Retention time: 10.5 min [Valle *et al.* 1999].

Column (1): Separon SGX C_{18} compact glass cartridge (150 × 3.3 mm i.d., 5 µm). Mobile phase: 4 g hexanesulfonic acid sodium salt monohydrate dissolved in 400 mL 0.02 mol/L phosphoric acid (1.28 mL 85% phosphoric acid in 1000 mL water), and mixed with 600 mL acetonitrile, flow rate 0.9 mL/min. UV detection (λ = 275 nm) and fluorescence detection (λ_{ex} = 202 nm, λ_{em} = 296 nm). Retention time: 4.34 min.

Column (2): LiChrospher 100 RP-18 (250 × 4 mm i.d., 5 µm). Mobile phase: 4 g hexanesulfonic acid sodium salt monohydrate in 600 mL phosphoric acid and 400 mL acetonitrile. Retention time: 4.4 min [Nobilis *et al.* 1996].
Ultraviolet Spectrum Aqueous acid—272 (A^1_1 = 70a), shoulder at 279 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1284, 1601, 1042, 1238, 1575, 702 cm^{-1} (tramadol hydrochloride, KBr disk).
Mass Spectrum



Quantification

Blood **HPLC** Column: Varian Pursuit 3 C_{18} (100 × 3.0 mm i.d., 3 µm). Mobile phase: methanol : ammonium formate. Limit of quantification, 0.5–10 µg/kg [Bjork *et al.* 2010].

LC-MS Column: Gemini C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile containing 0.1% formic acid : ammonium acetate (pH 3.2, 15 : 85 for 9 min to 30 : 70 in 13 min to 80 : 20 in 10 min to 95 : 5 in 1 min), flow rate 150 µL/min. TIS, MRM acquisition mode. Retention time: 6.3 min. Limit of detection, 0.002 mg/L [Gergov *et al.* 2009].

Plasma **GC** Column: RTX 10024 capillary (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 100° to 250° at 10°/min for 8 min. Carrier gas: N_2, 1 mL/min. FID. Retention time: 11.53 min. *k* value 13.41. Limit of detection, 8 µg/L [Ho *et al.* 1999].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 280° at 20°/min for 15 min. EI ionisation at 70 eV. Limit of detection, 0.2 µg/L [Sha *et al.* 2005]. Column: SGE BPX5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 100 for 1 min to 300° at 40°/min for 2 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 5 µg/L [Leis *et al.* 2004]. Column: HP-1 cross-linked methyl silicone (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 70° to 210° at 40°/min to 230° at 8°/min to 300° at 10°/min. SIM acquisition mode. Limit of quantification, 40 µg/L, limit of detection, 10 µg/L [Gambaro *et al.* 2003]. Column: HP-5-MS capillary (30 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 140° to 260° at 24°/min for 4 min. Carrier gas: He, 1 mL/min. SIM acquisition mode (*m*/*z* 263, 179). Retention time: 6.03 min. Limit of detection, 1 µg/L [Merslavic, Zupancic-Kralj 1997].

HPLC Column: RP-18. Mobile phase: *o*-phosphoric acid : triethylamine : acetonitrile : methanol. Fluorescence detection (λ_{ex} = 200 nm, λ_{em} = 300 nm; λ_{ex} = 200 nm, λ_{em} = 295 nm; λ_{ex} = 212 nm, λ_{em} = 305 nm). Limit of quantification, 4.078 µg/L [Curticapean *et al.* 2008]. Column: AGP, α_1-acid glycoprotein chiral

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selector. Mobile phase: 30 µmol/L diammonium hydrogen phosphate buffer: acetonitrile : triethylamine (pH 7, 98.9 : 1 : 0.1), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}= 200 nm, λ_{em}= 301 nm). Limit of quantification, 2 µg/L [Ardakani *et al.* 2008]. Column: RP-18e (100 × 4.6 mm i.d.). Mobile phase: methanol : water (pH 2.5, 19 : 81), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex}= 200 nm, λ_{em}= 301 nm). Limit of detection, 0.12 µg/L [Ebrahimzadeh *et al.* 2008]. Column: Chiralpak AD. Mobile phase: hexane : ethanol : diethylamine (94 : 6 : 0.2), flow rate 1 mL/min. Fluorescence detection (λ_{ex}= 275 nm, λ_{em}= 300 nm). Limit of quantification, 2.5 µg/L [Mehvar *et al.* 2007]. Column: Chromolith Performance RP-18e (50 × 4.6 mm i.d.). Mobile phase: methanol : water (pH 2.5, 13 : 87), flow rate 2 mL/min. Fluorescence detection (λ_{ex}= 200 nm, λ_{em}= 301 nm). Limit of quantification, 2.5 µg/L [Rouini *et al.* 2006]. See also Campanero *et al.* [2004], Gan, Ismail [2001], Gu, Fawcett [2005], Nobilis *et al.* [1996], Nobilis *et al.* [2002], Qu *et al.* [2003], Valle *et al.* [1999] and Yeh *et al.* [1999].

LC-MS Column: Diamonsil C_{18}. Mobile phase: 5 mmol/L ammonium acetate : methanol (50 : 50), flow rate 0.8 mL/min. Limit of quantification, 1 µg/L [Liu *et al.* 2009]. Column: Zorbax SB-C_{18} (100 × 3.0 mm i.d., 3.5 µm). Mobile phase: acetonitrile : 0.2% trifluoroacetic acid in water (10 : 90), flow rate 1 mL/min. ESI, SIM acquisition mode. Retention time, 3.5 min. Limit of quantification, 2.1 µg/L [Vlase *et al.* 2008].

Serum **GC** Column: FS-SE30-CB-0,25 (25 m × 0.32 mm i.d.). Carrier gas: He, 60 cm/s. Temperature programme: 100° to 240° at 32°/min. NSD. Limit of detection, ≈3 µg/L [Becker, Lintz 1986].

GC-MS SIM acquisition mode. Limit of detection, 21 µg/L [Zhu *et al.* 2006]. Column: ARNC-SE-30 (25 m). Carrier gas: He, 2 mL/min. Temperature programme: 80° to 240° at 25.5°/min. SIM acquisition mode. Limit of detection, ≈4 µg/L [Lintz, Uragg 1985].

Urine **GC-MS** Column: Rt-βDEXcst, (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 70 kPa. Temperature programme: 120° to 230° at 2.5°/min for 10 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 0.1 mg/L, limit of detection, 0.01 mg/L [Chytil *et al.* 2009]. Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 130° to 170° at 10°/min to 200° at 5°/min to 250° at 10°/min. EI ionisation, SIM acquisition mode. Limit of quantification, <30 µg/L [Cheng *et al.* 2008].

HPLC See Plasma [Ebrahimzadeh *et al.* 2008].

LC-MS See Blood. Limit of detection, 0.007 mg/L [Gergov *et al.* 2009]. See Plasma [Liu *et al.* 2009].

Hair **GC-MS** Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 5 min to 280° at 15°/min for 5 min. TIC, SIM acquisition mode. Limit of detection, 0.2 ng/mg [Hadidi *et al.* 2003].

Liver **HPLC** Column: reversed phase C_{18}. Mobile phase: methanol : ammonium hydrogen carbonate solution : ammonium hydroxide solution. Fluorescence detection (λ_{ex}= 280 nm, λ_{em}= 310 nm). Limit of detection, 500 µg/L [Paar *et al.* 1996].

Disposition in the Body

Tramadol is rapidly and almost completely absorbed after oral or parenteral administration; bioavailability is ~75%. The presence of food does not significantly affect the rate or extent of absorption. As the drug is administered as the racemate, both (−)- and (+)-tramadol and metabolites are detected in circulation. Peak serum concentrations are achieved in ~2 h. Tramadol is extensively metabolised. The main metabolic reactions are *N*- and *O*-demethylation and conjugation with glucuronic acid and sulfate. The major metabolites formed are *O*-monodesmethyltramadol, *N*,*O*-didesmethyltramadol and their conjugates, and *N*-monodesmethyltramadol. *O*-Monodesmethyltramadol is an active metabolite and has a greater analgesic activity than the parent drug. The extent of metabolism is reduced in patients with hepatic impairment. Approximately 90% of an oral dose is excreted in the urine in 3 days, ~30% of the dose as unchanged drug and the rest as metabolites. The remainder of the dose is eliminated in the faeces. The rate and extent of excretion is reduced in patients with renal impairment. The drug is widely distributed throughout the body and crosses the placenta; it has also been detected in breast milk. Steady-state concentrations of both the drug and main metabolite are achieved within 2 days of multiple dosing.

Therapeutic Concentration The therapeutic tramadol blood concentration is 100–800 µg/L.

Twelve male and female healthy subjects (mean age 30.1 years; range, 21–41) were fasted overnight for 12 h and administered a single oral 150-mg dose tramadol hydrochloride (as drops without ethanol) in the morning and as an IV 30-min infusion, with a 1-week washout period between doses. The mean peak serum concentration was 136 µg/L observed at a median of 1.1 h (minimum 0.74 h and maximum 1.5 h). After the IV dose, a maximum concentration of 286.8 µg/L at 0.52 h was observed [Lintz *et al.* 2000].

Twelve healthy males received a single 50-mg dose tramadol hydrochloride either as an IM injection or as a 30-min IV infusion, after an overnight fast (with a 1-week washout period between doses). After the IM injection, a mean peak serum tramadol concentration of 0.166 mg/L was reached after 0.75 h; after the IV infusion, a peak of 0.293 mg/L was reached at 0.5 h [Lintz *et al.* 1999].

Ninety-two patients who were recovering from major gynaecological surgery were given a maximum IV dose of 200 mg (+)-tramadol, (−)-tramadol or racemic tramadol; a 20-mg dose of each was also administered, as requested, for 24 h. Drug and metabolite serum concentrations were measured before each additional dose. The mean tramadol concentration was 470 µg/L for (+)-tramadol, 590 µg/L for the racemate and 771 µg/L for (−)-tramadol. The mean *O*-desmethyltramadol concentration was 57, 84 and 96 µg/L for the 3 drug types, respectively [Grond *et al.* 1999].

Ten healthy, female subjects (aged 38–57 years; mean, 48.7) were administered with 100 mg tramadol hydrochloride as a rectal suppository and as an IV injection with a washout period of 1 week between doses. Doses were given after a 12-h overnight fast. The mean maximum serum concentration was 294 µg/L observed at 3.3 h (range, 2–6) for the suppository dose. After the IV dose, a concentration of 603.1 µg/L was observed at 0.25 h [Lintz *et al.* 1998].

Toxicity The toxic tramadol blood concentration is 1.0 mg/L; lethal, 2.0 mg/L.

A 26-year-old male nurse returned home from work after a night shift and went to bed to sleep. Later that evening, he was found dead face down on his bed. A bottle of tramadol was found in his pocket. The peripheral blood tramadol concentration was 9.6 mg/L, which is ≈30 times the observed therapeutic concentration range (0.1–0.3 mg/L). The heart blood concentration was 13.1 mg/L, urine 46.0 mg/L, bile 46.1 mg/L, liver 6.2 µg/g and kidney 3.1 µg/g. There was also a trace of the drug detected in the gastric contents. No other drugs or alcohol were detected [Musshoff, Madea 2001].

A 30-year-old woman was found dead at her home after ingestion of unknown amounts of alprazolam, tramadol and alcohol (empty packages of each found at the scene). She had a history of depression. Toxicological analysis showed concentrations of tramadol in her peripheral blood at 38.3 mg/L (~100 times the therapeutic concentration). Alprazolam was found at a concentration of 0.21 mg/L and alcohol at 1.29 mg/g. Tramadol was also detected at concentrations of 44.0 mg/L in bile, 27.6 µg/g in liver and 130 mg total in gastric contents; alprazolam was found in these samples at 0.27 mg/L, 0.23 µg/g and 2.73 mg, respectively [Michaud *et al.* 1999].

In a fatal overdose case involving tramadol, the tramadol concentration in various tissues and fluids was blood 20 mg/L, liver 68.9 µg/g, kidney 37.5 µg/g and urine 110.2 mg/L. In all body tissues and blood, the drug concentration was greater than the metabolites *N*-desmethyl and *O*-desmethyltramadol; *O*-desmethyltramadol was always greater in concentration than the *N*-desmethyl metabolite [Moore *et al.* 1999].

Analysis of 12 blood samples from tramadol-related death and 4 from non-fatal tramadol intoxications revealed concentrations ranging from 0.03–22.59 mg/L for tramadol, 0.02–1.84 mg/L for *O*-desmethyltramadol and 0.01 to 2.08 mg/L for *N*-desmethyltramadol. Three of the deaths were attributable to morphine and 1 to doxapin; 6 were multiple drug overdoses [Goeringer *et al.* 1997].

Half-life Plasma half-life: tramadol ~6 h (increases to 7 h with multiple dosing); *O*-monodesmethyltramadol, ~9 h.

Volume of Distribution ≈3 L/kg; slightly higher in females than in males (2.9 L/kg versus 2.6 L/kg).

Clearance Plasma clearance, ~6 mL/min/kg; slightly higher in males than in females (6.4 mL/min/kg versus 5.7 mL/min/kg).

Protein Binding 20%.

Dose

Tramadol hydrochloride is given in doses of 50 to 100 mg orally, not more often than every 4 h (no more than 400 mg daily required); IM injection or IV injection/infusion, 50 to 100 mg every 4 to 6 h (for postoperative pain, 100 mg initially followed by 50 mg every 10 to 20 min; total maximum, 250 mg in first hour, then as before).

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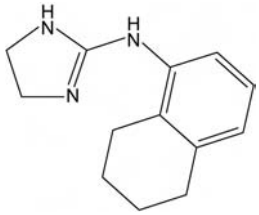
Tramazoline

Sympathomimetic
$C_{13}H_{17}N_3$ = 215.3
CAS—1082-57-1

IUPAC Name *N*-(5,6,7,8-Tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine
Synonym 4,5-Dihydro-*N*-(5,6,7,8-tetrahydro-1-naphthalenyl)-1*H*-imidazol-2-amine

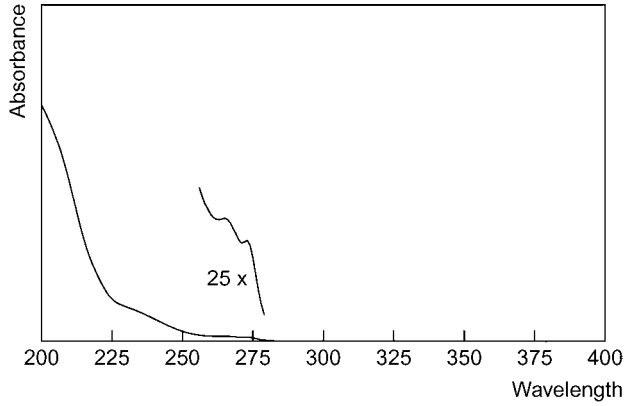


Chemical Properties Crystals. Mp 142° to 143°. Log *P* (chloroform/pH 7.4) 0.8; (octanol/water) 2.5.

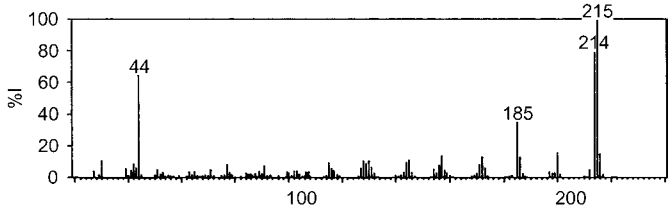
Tramazoline Hydrochloride

$C_{13}H_{17}N_3$,HCl = 251.8
CAS—3715-90-0
Proprietary Names *Biciron*; *Bisolnasal*; *Ellatun*; *Rhinospray*; *Rinogutt Spray-Fher*; *Spray-Tish*. It is an ingredient of *Dexa-Rhinaspray*.
Chemical Properties A white crystalline solid. Mp 171°. Soluble 1 in 6 of water.

Colour Tests Mandelin's test—brown; Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.06; system TAE—R_f 0.04; system TL—R_f 0.02; system TB—R_f 0.04; system TC—R_f 0.02; system TE—R_f 0.30 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2440.
High Performance Liquid Chromatography System HA—*k* 1.8; system HX—RI 341.
Ultraviolet Spectrum Aqueous acid—267, 275 nm.



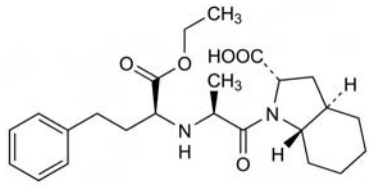
Infrared Spectrum Principal peaks at wavenumbers 1667, 1626, 1590, 1302, 797, 1289 cm^{-1} (tramazoline hydrochloride, KBr disk).
Mass Spectrum Principal ions at *m/z* 215, 214, 44, 185, 200, 216, 157, 186.



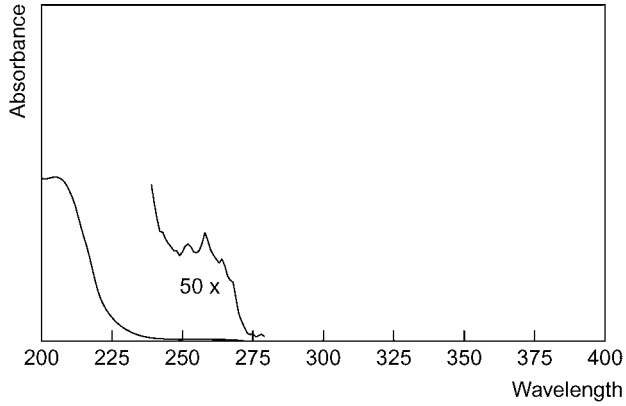
Trandolapril

Antihypertensive
$C_{24}H_{34}N_2O_5$ = 430.5
CAS—87679-37-6

IUPAC Name (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid
Synonyms (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid; RU-44570; RU-44403.
Proprietary Names *Gopten*; *Mavik*; *Odrik*; *Zeddan*.

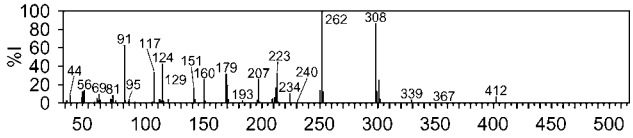


Gas Chromatography System GP—trandolapril-ME RI 2970; M perindoprilate-ME3 RI 3005; M perindoprilate-H_2O-ME3 RI 3070.
High Performance Liquid Chromatography System HAA—RT 17.0 min; system HZ—trandolapril retention time 6.1 min; trandolaprilat retention time 2.1 min.
Ultraviolet Spectrum



T

Mass Spectrum Principal ions at *m/z* 262, 308, 91, 124, 117, 223, 179, 160.



Quantification

Urine **GC–MS** Limit of detection, 10 µg/L [Maurer *et al.* 1998].

Disposition in the Body Trandolapril is very rapidly absorbed after oral administration and is not affected by food. It is a pro-drug of its metabolite, diacid trandolaprilat, which is a specific angiotensin-converting enzyme (ACE) inhibitor. The effective half-life for accumulation of trandolaprilat is 16 to 24 h following multiple dosing. Trandolapril is metabolised in the liver to trandolaprilat and some inactive metabolites. Peak plasma concentrations of trandolapril following an oral dose are achieved within 30 to 60 min. Peak plasma concentrations of trandolaprilat range from 4 to 6 h. Approximately 33% of an oral dose is excreted in urine, mainly as trandolaprilat, and the remainder is excreted in faeces. Excretion is virtually complete (99.2%) after 7 days, except in patients with impaired renal function where excretion is decreased. Steady state concentrations are reached in about 4 days. Trandolaprilat is eliminated in urine in the unchanged form and accounts for 10 to 15% of the dose of trandolapril administered. Modest accumulation of trandolapril occurs during multiple dosing.

Therapeutic Concentration

Ten patients with a mean age of 44.1 years, and 14 patients with a mean age 69.3 years, all with mild to moderate hypertension, were administered 2 mg trandolapril for 10 consecutive days. Steady state plasma concentrations were reached after 4 days and absorption is rapid, in less than 1 h. The steady state concentration was 7.49 µg/L for the young individuals and 8.35 µg/L in the elderly [Arner *et al.* 1994].

Bioavailability Trandolapril ~5%; trandolaprilat, 40 to 60%.

Half-life Trandopril, 1.3 h (healthy individuals and patients with mild to moderate hypertension); trandolaprilat, steady state, 16 to 24 h.

Protein Binding Trandolapril, >80%, not saturable over 0.1 to 1.0 µg/L; trandolaprilat, 65% at 1000 µg/L to 94% at 0.1 µg/L; will bind to saturation with a strong affinity in serum.

Dose The usual dose is 1 to 2 mg, maximum 4 mg.

Arner P *et al.* (1994). Pharmacokinetics and pharmacodynamics of trandolapril after repeated administration of 2 mg to young and elderly patients with mild-to-moderate hypertension. *J Cardiovasc Pharmacol* 23 (Suppl.4): S44–S49.

Maurer HH *et al.* (1998). Screening for the detection of angiotensin-converting enzyme inhibitors, their metabolites, and AT II receptor antagonists. *Ther Drug Monit* 20: 706–713.

Tranylcypromine

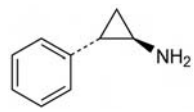
Antidepressant, Monoamine Oxidase Inhibitor

$C_9H_{11}N = 133.2$

CAS—155-09-9

IUPAC Name (1*R*,2*S*)-*rel*-2-Phenylcyclopropanamine

Synonyms Tranilcipromina; transamine.



Chemical Properties Liquid. Log *P* (octanol/water), 1.58 [Hansch *et al.* 1995]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable in plasma and urine for up to 24 months at –20° or –80° [Spahn-Langguth *et al.* 1992].

Tranylcypromine Sulfate

$(C_9H_{11}N)_2,H_2SO_4 = 364.5$

CAS—13492-01-8

Proprietary Names *Jatrosom N*; *Parnate*; *Tylciprine*. It is an ingredient of *Parmodalin*; *Parstelin* and *Stelapar*.

Chemical Properties White crystalline powder. Soluble 1 in 20 to 1 in 25 of water and 1 in 2000 of ether; very slightly soluble in ethanol; practically insoluble in chloroform. pK_a 8.2. Log *P* (octanol/water), 1.57 [Meylan, Howard 1995], (octanol/chloroform), 0.6.

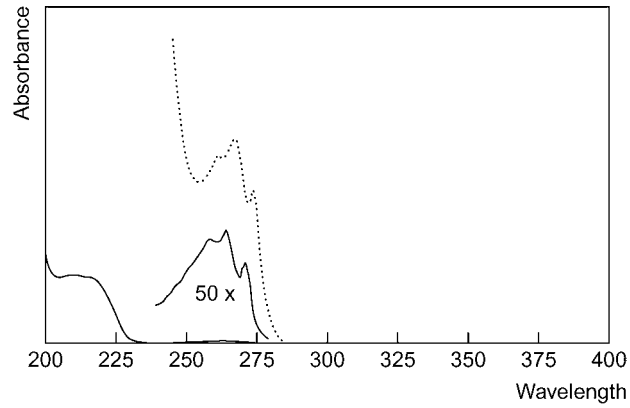
Colour Tests Liebermann's reagent—red-orange; Mandelin's test—green→violet; Marquis test—red→brown.

Thin-layer Chromatography System TA—R_f 0.54; system TAE—R_f 0.41; system TAF—R_f 0.67; system TAG—R_f 0.48; system TB—R_f 0.39; system TC—R_f 0.33; system TE—R_f 0.58 (Dragendorff spray, positive; FPN reagent, yellow; acidified iodoplatinate solution, positive; Marquis reagent, orange-brown; ninhydrin spray, positive).

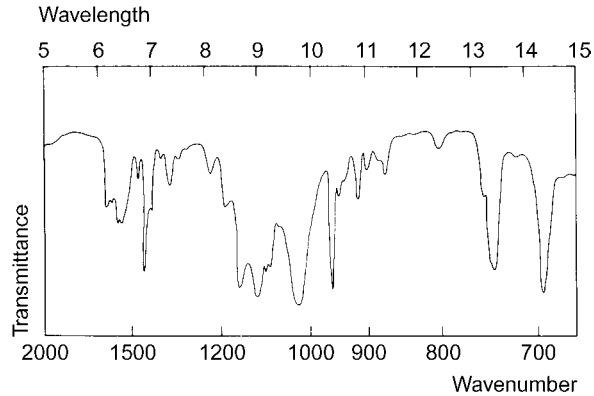
Gas Chromatography System GA—RI 1220; system GB—RI 1252; system GC—RI 1759; system GF—RI 1455.

High Performance Liquid Chromatography System HA—*k* 1.0; system HC—*k* 0.26; system HX—RI 230; system HY—RI 196; system HZ—RT 2.0 min.

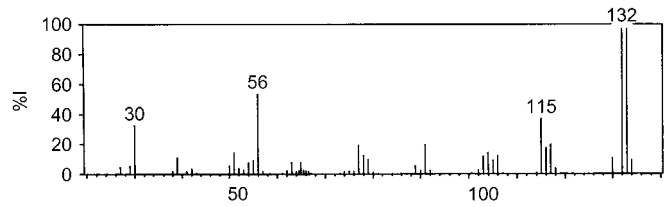
Ultraviolet Spectrum Aqueous acid—258 nm, 264 nm (A_1^1 = 22a), 271 nm; aqueous alkali—260, 266, 273 nm.



Infrared Spectrum Principal peaks at wavenumbers 1023, 1112, 695, 963, 1153, 743 cm^{-1} (tranylcypromine sulfate (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 133, 132, 56, 115, 30, 117, 91, 77.



Quantification

Blood **GC** Column: HP-1 (12.5 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 80° to 100° at 20°/min to 200° at 10°/min to 300° at 20°/min for 5 min. NPD. Limit of quantification, 0.2 mg/L [Boniface 1991].

GC-MS Column: DB-5 (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 100° for 1 min to 210° at 15°/min for 1 min to 270° at 30°/min for 1 min. SIM acquisition mode. Limit of detection not reported [Crifasi, Long 1997].

Plasma **GC** Column: OV-225 (20 m × 0.3 mm i.d.). Carrier gas: He, 2 mL/min. Temperature: 180°. NSD. Limit of detection 2.5 µg/L [Bailey, Barron 1980].

GC-MS Column: 3% OV-1 on 80/100 mesh Supelcoport (1.8 m × 2 mm i.d.). Carrier gas: CH_4, 0.8 Torr. Temperature: 130°. CI. Retention time: 2.95 min. Limit of detection, 1 µg/L [Edwards *et al.* 1985].

HPLC Column: Novapak C_{18} (150 × 3.9 mm i.d.). Mobile phase: acetonitrile : 0.1 mol/L sodium dihydrogen phosphate containing 0.3 g/L sodium chloride and 0.76 g/L sodium 1-octanesulfoate (pH 3, 20 : 80), flow rate 0.5 mL/min. Electrochemical detection. *k'*: 4.18. Limit of detection, 5 µg/L [Krugers Dagneaux *et al.* 1992]. Column: Zorbax ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L sodium phosphate solution (pH 6.5) : methanol : tetrahydrofuran (50 : 60 : 1), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 344 nm, λ_{em} = 442 nm). Limit of detection, 0.5 µg/L [Spahn-Langguth *et al.* 1992].

Serum **GC** Column: CP-Sil-5CB fused silica capillary. Carrier gas: N_2. Temperature programme: 80° to 140° at 10°/min for 1 min to 200° at 30°/min for 2 min. NPD. Limit of detection not reported [Pennings *et al.* 1997]. Column: 2% OV-1 on 100/120 mesh Chromosorb G-HP (6′ × 2 mm i.d.). Carrier gas: CH_3 : Ar (5 : 95), 33 mL/min. Temperature: 185°. ECD. Retention time: 3.1 min for the trichloroacetyl derivatives. Limit of detection, 1 µg/L [Baselt *et al.* 1977b].

Urine **GC** See Blood [Boniface 1991]. See Plasma [Bailey, Barron 1980]. See Serum [Baselt *et al.* 1977b].

GC-MS See Blood [Crifasi, Long 1997]. Column: 5% phenyl-methylpolysiloxane HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 130° for 3 min to 146° at 4°/min for 4 min to 260° at 30°/min for 10 min. MSD. Limit of quantification, 0.06 mg/L [Iwersen, Schmoldt 1996].

HPLC Column: Crownpak CR (15 cm × 4 mm i.d., 5 µm). Mobile phase: 0.1 N perchloric acid : methanol (88 : 12), flow rate 0.6 mL/min. UV detection (λ= 256 nm). Retention time: 12.9 and 16.4 min for *R*-(+)- and *S*-(–)-tranylcypromine, respectively [Aboul-Enein, Serignese 1995]. See Plasma. Limit of detection, 2 µg/L [Spahn-Langguth *et al.* 1992].

Gastric Contents **GC** See Blood [Boniface 1991].

Brain **GC-MS** See Blood [Crifasi, Long 1997].

Liver **GC** See Blood [Boniface 1991].

GC-MS See Blood [Crifasi, Long 1997].

Disposition in the Body Readily absorbed after oral administration and extensively metabolised. <2% of a dose is excreted as unchanged drug in the urine in 24 h; this increases to ~8% if the urine is maintained at an acid pH.

Therapeutic Concentration

After a single dose of 10 mg tranylcypromine to healthy volunteers the mean maximum plasma concentration ranged from 10.6–28.0 µg/L at 50–150 min [Krugers Dagneaux *et al.* 1992].

Three healthy volunteers were administered 20 mg oral racemic tranylcypromine. Mean maximum plasma concentrations were 57.5 and 6.3 µg/L for the *S*-(–)- and *R*-(+)-isomers, respectively [Spahn-Langguth *et al.* 1992].

Following a single oral dose of 20 mg tranylcypromine to 9 depressed patients, peak plasma concentrations of 64.5–190 µg/L were attained at 0.67–3.5 h. Volume of distribution and clearance were 1.1–5.7 L/kg and 6.4–40.6 mL/min/kg, respectively in these patients [Mallinger *et al.* 1986].

A single oral dose of 30 mg administered to 2 subjects produced a peak serum concentration of about 0.04 mg/L in 1 h. During chronic treatment of 42 patients with 10–30 mg daily, serum concentrations of 0.005–0.01 mg/L were found when measured 12 h after the last dose [Baselt *et al.* 1977b].

Toxicity Several cases of addiction to tranylcypromine have been reported.

The following postmortem tissue concentrations were reported following the ingestion of 300 mg of tranylcypromine: blood 3.7 mg/L, brain 1.0 µg/g, liver 7.3 µg/g, urine 25 mg/L; several other drugs were detected [Baselt *et al.* 1977a].

A 58-year-old was found dead. The following concentrations of tranylcypromine were found: heart blood, 5.0 mg/L, iliac blood, 9.1 mg/L, liver, 21.0 mg/kg, and stomach contents, 227 mg. A 50-year-old suffered a seizure and died despite resuscitation attempts. Tranylcypromine concentrations in the urine and blood were 50.8 and 1.7 mg/L, respectively [Boniface 1991].

A man who ingested 400 mg tranylcypromine with suicidal intent had a blood tranylcypromine concentration of 0.51 mg/L ~3 h later; another man, who was found dead 48 h after taking an unknown quantity of tranylcypromine, had the following tranylcypromine tissue concentrations: blood 0.7 mg/L, liver 1.9 mg/g, urine 238.0 mg/L [Iwersen, Schmoldt 1996].

A 41-year-old male survived after ingesting 4 g of tranylcypromine in a suicide attempt. Approximately 8 h after ingestion of the overdose, the tranylcypromine concentration in the blood was 19.9 mg/L and decreased to 1.14 mg/L on day 2, 0.028 mg/L on day 3, 0.006 mg/L on day 4 and 0.001 mg/L on day 8 [Pennings *et al.* 1997].

Half-life Plasma half-life, ~2 h.

Dose The equivalent of 10–30 mg of tranylcypromine daily.

Aboul-Enein HY, Serignese V (1995). Direct separation of tranylcypromine enantiomers and their profile in an atypical depressive patient. *Biomed Chromatogr* 9: 98–101.

Bailey E, Barron EJ (1980). Determination of tranylcypromine in human plasma and urine using high-resolution gas-liquid chromatography with nitrogen-sensitive detection. *J Chromatogr* 183: 25–31.

Baselt RC *et al.* (1977a). Tranylcypromine concentrations and monoamine oxidase activity in tissues from a fatal poisoning. *J Anal Toxicol* 1: 168–170.

Baselt RC *et al.* (1977b). Determination of serum and urine concentrations of tranylcypromine by electron-capture gas-liquid chromatography. *J Anal Toxicol* 1: 215–217.

Boniface PJ (1991). Two cases of fatal intoxication due to tranylcypromine overdose. *J Anal Toxicol* 15: 38–40.

Crifasi J, Long C (1997). The GCMS analysis of tranylcypromine (Parnate) in a suspected overdose. *Forensic Sci Int* 86: 103–108.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Edwards DJ *et al.* (1985). Determination of tranylcypromine in plasma using gas chromatography-chemical-ionization mass spectrometry. *J Chromatogr* 344: 356–361.

Hansch C *et al.* (1995). *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Washington DC: American Chemical Society.

Iwersen S, Schmoldt A (1996). One fatal and one nonfatal intoxication with tranylcypromine. Absence of amphetamines as metabolites. *J Anal Toxicol* 20: 301–304.

Krugers Dagneaux PG *et al.* (1992). Liquid chromatographic estimation of tranylcypromine in human plasma. *Pharm Weekbl Sci* 14: 46–49.

Mallinger AG *et al.* (1986). Pharmacokinetics of tranylcypromine in patients who are depressed: relationship to cardiovascular effects. *Clin Pharmacol Ther* 40: 444–450.

Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.

Pennings EJ *et al.* (1997). Tranylcypromine intoxication with malignant hyperthermia, delirium, and thrombocytopenia. *J Clin Psychopharmacol* 17: 430–432.

Spahn-Langguth H *et al.* (1992). Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase inhibitor tranylcypromine and its applicability in pharmacokinetic studies. *J Chromatogr* 584: 229–237.

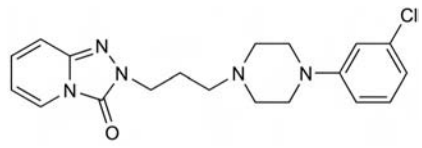
Trazodone

Antidepressant

$C_{19}H_{22}ClN_5O$ = 371.9

CAS—19794-93-5

IUPAC Name 2-[3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl]-[1,2,4]triazolo[4,3-*a*]pyridin-3-one



Chemical Properties Crystals. Mp 86° to 88°. Also reported as 96°. Soluble 1 in 50 of ethanol and methanol; freely soluble in acetone and benzene; slightly soluble in ether. Log *P* (octanol/water), 3.2. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stable after freeze-thaw and long-term storage at −20°. Stock solutions were stable both at room temperature for 6 h and at -20° for 3 months [Mercolini *et al.* 2008].

Trazodone Hydrochloride

$C_{19}H_{22}ClN_5O,HCl$ = 408.3

CAS—25332-39-2

Proprietary Names *Azona*; *Deprax*; *Depyrel*; *Desirel*; *Desyrel*; *Molipaxin*; *Sideril*; *Thombran*; *Trazodil*; *Trazolan*; *Trazone*; *Trazorel*; *Triticum*; *Trittico*.

Chemical Properties White crystals. Mp 22°. Sparingly soluble in water, ethanol and methanol; soluble in chloroform; practically insoluble in common organic solvents.

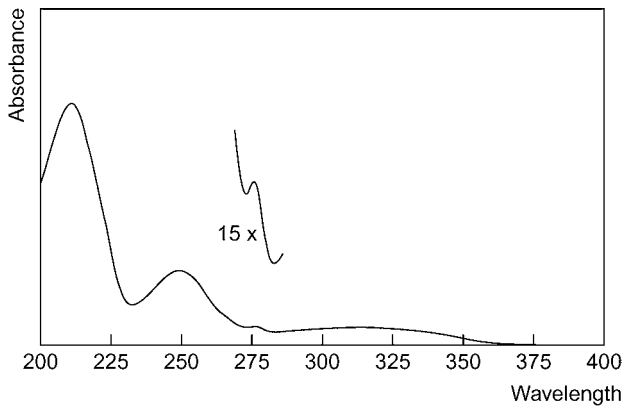
Colour Tests Liebermann's reagent—violet (transient); Mandelin's test—grey→violet.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.10; system TC—R_f 0.58; system TE—R_f 0.66; system TAE—R_f 0.64; system TAF—R_f 0.61; system TAG—R_f 0.37 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; location under ultraviolet light—violet fluorescence.)

Gas Chromatography System GA—trazodone RI 3330, M (OH-acetyl-) RI 3380; system GB—trazodone RI 3562, M (mCPP) RI 1806, M (OH-acetyl-) RI 3640, M (desalkylacetyl-) RI 2261; system GM—trazodone not eluted.

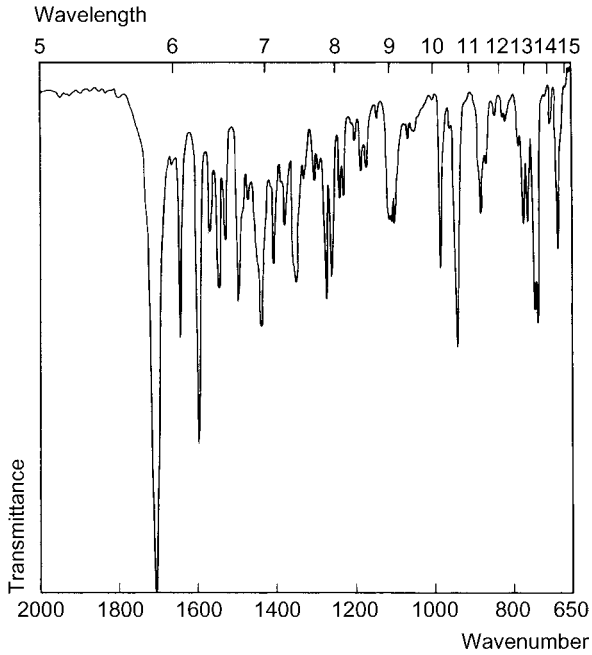
High Performance Liquid Chromatography System HA—*k* 0.6; system HX—RI 378; system HY—RI 305; system HZ—RT 3.3 min; system HAA—RT 12.7 min.

Ultraviolet Spectrum Aqueous acid—246 (A^1_1= 314a), 274, 310 nm. No alkaline shift.

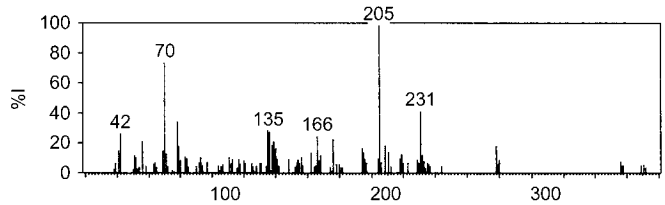


Infrared Spectrum Principal peaks at wavenumbers 1705, 1595, 943, 1640, 742, 1272 cm^{-1} (trazodone hydrochloride, KBr disk, see below).

T



Mass Spectrum Principal ions at *m/z* 205, 70, 231, 78, 135, 136, 42, 166.



Quantification

Plasma **GC-MS** Column: 3% OV-1 80/100 mesh Gas-Chrom Q (1 m × 3 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 270°. EI ionisation at 70 eV. Limit of detection, 50 µg/L [Caccia *et al.* 1981].

HPLC Column: reversed phase C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: aqueous phosphate buffer containing 0.3% triethylamine (pH 3.5) : acetonitrile (70 : 30), flow rate 1.2 mL/min. UV detection (λ = 255 nm). Retention time, 3.6 min. Limit of quantification, 10 µg/L, limit of detection, 4 µg/L [Mercolini *et al.* 2008]. Column: Spherisorb S5 ODS (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L sulfuric acid (18 : 1), flow rate 2 mL/min. UV detection (λ = 254 nm). Limit of detection, 20 µg/L [Ankier *et al.* 1981].

LC-MS Column: Inertsil C-8. Mobile phase: methanol : 10 µmol/L ammonium acetate (pH 5.0) : acetonitrile (70 : 20 : 10), flow rate 0.10 mL/min. Limit of detection, 30 and 630 µg/L [Shinozuka *et al.* 2006].

Serum **HPLC** Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 6.7 µg/L [Kirchherr, Kühn-Velten 2006]. Column: RP-Select B C_{18} (250 cm × 4 mm i.d., 4 µm). Mobile phase: acetonitrile : buffer (30 : 70), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 300 nm). Limit of quantification, 76 µg/L, limit of detection, 5.3 mg/L [Waschgler *et al.* 2002].

Urine **HPLC** Column: C_{18}. Mobile phase: 0.2 mol/L SDS : 8% 1-butanol (pH 3). Fluorimetric detection. Limit of quantification, 9.5 µg/L [Carda-Broch *et al.* 2007].

Brain **GC-MS** See Plasma [Caccia *et al.* 1981].

Disposition in the Body Trazodone is rapidly and almost completely absorbed after oral administration. It is extensively metabolised by hydroxylation and oxidation. Approximately 75% of a dose is excreted in the urine as metabolites and 15% is eliminated in the faeces in 72 h. <1% of a dose is excreted in the urine as unchanged drug. The major urinary metabolites are a propionic acid derivative (3-oxo-1,2,4-triazolo[4,3-*a*]pyridine-2-propionic acid), *p*-hydroxytrazodone and a dihydrodiol derivative and its glucuronide and sulfate conjugates, which account for ~35%, 20% and 15% of the urinary material respectively; an *N*-oxide has also been identified. In plasma, the major metabolite is 1-(3-chlorophenyl)piperazine (mCPP, active), but this accounts for <1% of a dose in the urine.

Therapeutic Concentration

After an oral dose of 50 mg to 13 subjects, peak plasma concentrations of 0.49–2.3 mg/L (mean, 1.1) were attained in 0.5–2.5 h [Ankier *et al.* 1981].

Following a single oral dose of 150 mg to 4 subjects, mean peak plasma concentrations of 2.05 mg/L trazodone and 0.01 mg/L 1-(3-chlorophenyl) piperazine were reported at ~2 h and 2–4 h, respectively [Caccia *et al.* 1982].

Following oral administration of 25 mg three times a day to 10 subjects, steady-state serum concentrations averaged 0.7 mg/L on the 12th day [Catanese *et al.* 1978].

Toxicity

In a fatality in which a woman was found drowned in a bath following the ingestion of ~2–4 g trazodone, the following postmortem concentrations were reported: blood 15 mg/L/mL, bile 45 mg/L, liver 57 µg/g and urine 2.5 mg/L [Demorest 1983].

Postmortem tissue concentrations of trazodone in 2 women, aged 53 and 48 years, were, respectively, as follows: blood 14.4 and 15.5 mg/L, brain 48.6 and 20.9 µg/g, liver 73.7 and 82.4 µg/g, lung 12.9 to 13.3 and 35.3 to 40.1 µg/g, fat 18.5 and 16.5 µg/g, kidneys 34.7 and 39.6 µg/g and heart 30.9 and 28.9 µg/g [Martin, Pounder 1992].

Trazodone was the primary cause of death in 3 subjects (2 suicides and 1 probable suicide), all of whom had blood concentrations >9 mg/L (9.06, 24.32 and 32.91 mg/L) [Goeringer *et al.* 2000].

Half-life Plasma half-life, ~4–7 h.

Protein Binding ≈90%.

Dose Trazodone hydrochloride 100 to 300 mg daily; maximum 600 mg daily.

Ankier SI *et al.* (1981). Trazodone: a new assay procedure and some pharmacokinetic parameters. *Br J Clin Pharmacol* 11: 505–509.

Caccia S *et al.* (1981). Determination of plasma and brain concentrations of trazodone and its metabolite, 1-*m*-chlorophenylpiperazine, by gas–liquid chromatography. *J Chromatogr* 210: 311–318.

Caccia S *et al.* (1982). Plasma concentrations of trazodone and 1-(3-chlorophenyl)piperazine in man after a single oral dose of trazodone. *J Pharm Pharmacol* 34: 605–606.

Carda-Broch S *et al.* (2007). Determination of trazodone in urine and pharmaceuticals using micellar liquid chromatography with fluorescence detection. *J Chromatogr A* 1156: 254–258.

Catanese B *et al.* (1978). A comparative study of trazodone serum concentrations in patients with normal or impaired renal function. *Boll Chim Farm* 117: 424–427.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Demorest D (1983). Death involving trazodone. *J Anal Toxicol* 763.

Goeringer KE *et al.* (2000). Postmortem forensic toxicology of trazodone. *J Forensic Sci* 45: 850–856.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Martin A, Pounder DJ (1992). Post-mortem toxico-kinetics of trazodone. *Forensic Sci Int* 56: 201–207.

Mercolini L *et al.* (2008). HPLC analysis of the antidepressant trazodone and its main metabolite m-CPP in human plasma. *J Pharm Biomed Anal* 47: 882–887.

Shinozuka T *et al.* (2006). Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Sci Int* 162: 108–112.

Waschgler R *et al.* (2002). Simultaneous quantification of citalopram, clozapine, fluoxetine, norfluoxetine, maprotiline, desmethylmaprotiline and trazodone in human serum by HPLC analysis. *Int J Clin Pharmacol Ther* 40: 554–559.

Trenbolone

Anabolic Steroid

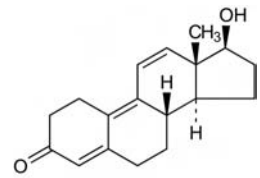
$C_{18}H_{22}O_2$ = 270.4

CAS—10161-33-8

IUPAC Name (8*S*,13*S*,14*S*,17*S*)-17-Hydroxy-13-methyl-2,6,7,8,14,15,16,17-octahydro-1*H*-cyclopenta[*a*]phenanthren-3-one

Synonyms 4,9,11-Estratrien-17β-ol-3-one; (17β)-17-hydroxy-estra-4,9,11-trien-3-one; 17β-hydroxyestra-4,9,11-trien-3-one; RU-1697; β-TBOH; trienbolone; trienolone.

Proprietary Names *Parabolan*; *Regumate*.



Chemical Properties Crystals. Mp 183° to 186°.

Trenbolone Acetate

$C_{20}H_{24}O_3$ = 312.4

CAS—10161-34-9

Proprietary Names *Finajet*; *Finaplix*.

Chemical Properties Crystals. Mp 96° to 97°.

Trenbolone Hexahydrobenzylcarbonate

$C_8H_{13}O_2$ = 411

Proprietary Names *Hexabolan*; *Parabolan*.

Gas Chromatography System GAI—urinary metabolite: 17α-trenbolone RRT 0.943 (relative to 17α-methyl-5α-androstan-3β,17β-diol).

Gas Chromatography-Mass Spectrometry Column: HP1 methyl silicone (0.2 mm i.d., 0.33 µm). Temperature: 280°. Carrier gas: He, 0.9 mL/min. Retention index: trenbolone acetate, 2811 [Mills, Roberson 1993].

Column: HP1 methyl silicone (0.2 mm i.d., 0.33 µm). Temperature: 250°. Carrier gas: He, 0.9 mL/min. Retention index: 2775 [Mills, Roberson 1993].

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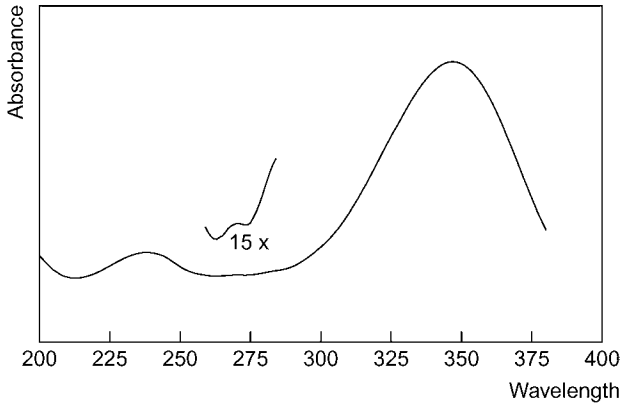
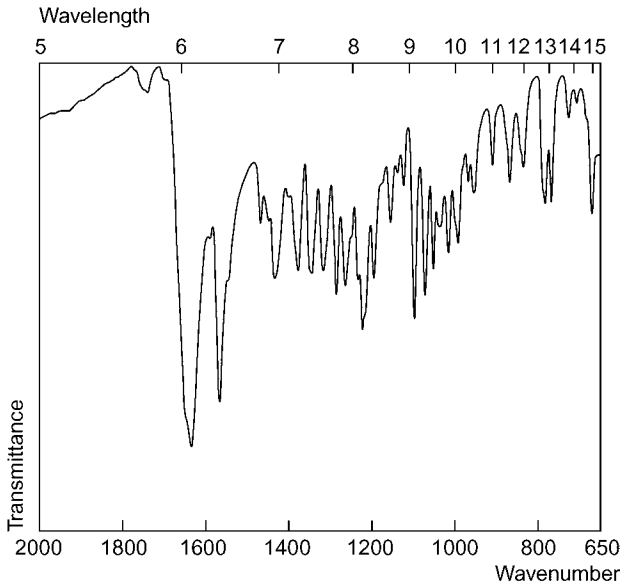
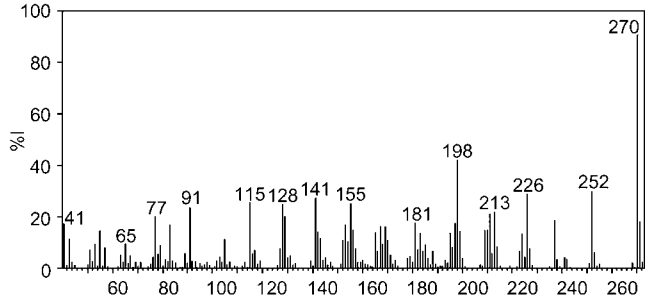
High Performance Liquid Chromatography System HATa—trenbolone hexahydrobenzylcarbonate RRT 1.65 (relative to testosterone); system HATb—trenbolone acetate RRT 1.71 (relative to testosterone).
Ultraviolet Spectrum Alcohol—237, 340 nm (A^{1}_{1}= between 92.0 and 97.6); ethanol—338 nm.



Infrared Spectrum Principal peaks at wavenumber 1636, 1568, 1225, 1100 cm^{-1}; principal peaks at wavenumber 1652, 1739, 1244, 1023 cm^{-1} (trenbolone acetate).



Mass Spectrum Principal ions at *m/z* 270, 198, 252, 226, 141, 115, 155, 128; for acetate *m/z*252, 312, 253, 237, 141, 198, 213, 270.



Quantification

Urine **GC-MS** Column: HP-1 capillary (17 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 140° for 1 min to 180° at 40°/min to 240° at 3°/min to 300° at 40°/min for 3 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 6 µg/L, limit of detection, 3 µg/L [Marques *et al.* 2007].

HPLC Column: Zorbax ODS (250 × 4 mm i.d., 5 µm). Mobile phase: methanol-water (10 : 90) : acetonitrile (85 : 15 to 63 : 37 over 10 min for 10 min, to 0 : 100 over 5 min), flow rate 1 mL/min. UV detection (λ= 340 nm). RT: 17β-trenbolone, 33 min; 17α-trenbolone, 35 min. Limit of detection not reported [Spranger, Metzler 1991].

LC-MS Column: Purospher Star RP-18e (15 × 4 mm i.d., 3 µm). Mobile phase: 5 mmol/L ammonium acetate containing 0.1% acetic acid : acetonitrile (85 : 15 to 0 : 100 within 7 min), flow rate 500 µL/min. ESI, positive ion mode. Limit of detection, 1 µg/L [Thevis *et al.* 2009] Column: C_{18} (250 × 4.6 mm i.d., 4 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 6.7) : acetonitrile-methanol (50 : 50, 80 : 20 to 50 : 50 at 7 min to 20 : 80 at 8 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 1 µg/L, limit of detection, 0.03 µg/L [Kosanam *et al.* 2007].

Bile **LC-MS** Column: LiChrosorb RP-18 (125 × 4 mm i.d.). Mobile phase: acetonitrile : 100 mmol/L ammonium acetate (45 : 55), flow rate 1 mL/min. Retention time: 4 min for 17α-trenbolone. Limit of detection 0.5 ng/g [Hewitt *et al.* 1993].

Faeces **HPLC** See Bile. Limit of detection, 0.5 µg/L [Hewitt *et al.* 1993].

Hair **GC-MS** Column: CP-Sil 5 CB (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min for 5 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 10.3 min. Limit of quantification, 0.05 ng/mg, limit of detection, 0.005 ng/mg [Deng *et al.* 1999].

Other **LC-MS** Human Liver Microsomes. Column: Agilent Eclipse XDB C_{18} (50 × 2.1 mm i.d., 5 µm). Mobile phase: 2.5 mmol/L ammonium acetate in 0.1% acetic acid : methanol (75 : 25 to 5 : 95 in 7 min for 3 min to 75 : 25 for 3 min), flow rate, 200 µL/min. ESI, positive ion mode. Limit of detection not reported [Kuuranne *et al.* 2008].

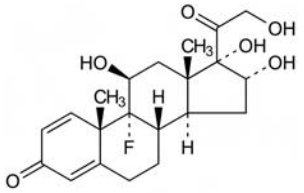
Disposition in the Body After administration, trenbolone undergoes metabolism to epitrenbolone and hydroxytrenbolone (possibly the main metabolites). These metabolites, mainly as the glucuronide form, have been identified in urine, along with a little trenbolone and estra-4,9,11-trien-3,17-dione and other polar metabolites in the unconjugated and sulfate forms.

Deng XS *et al.* (1999). Detection of anabolic steroids in head hair. *J Forensic Sci* 44: 343–346.
Hewitt SA *et al.* (1993). Liquid chromatography-thermospray mass spectrometric assay for trenbolone in bovine bile and faeces. *J Chromatogr* 639: 185–191.
Kosanam H *et al.* (2007). Rapid screening of doping agents in human urine by vacuum MALDI-linear ion trap mass spectrometry. *Anal Chem* 79: 6020–6026.
Kuuranne T *et al.* (2008). Screening of *in vitro* synthesised metabolites of 4,9,11-trien-3-one steroids by liquid chromatography mass spectrometry. *Eur J Mass Spectrom (Chichester)* 14: 181–189.
Marques MA *et al.* (2007). Analysis of synthetic 19-norsteroids trenbolone, tetrahydrogestrinone and gestrinone by gas chromatography-mass spectrometry. *J Chromatogr A* 1150: 215–225.
Mills TI, Roberson JC (1993). *Instrumental Data for Drug Analysis, Vol. 4-5*, 2nd edn. Boca Raton, FL: CRC Press.
Spranger B, Metzler M (1991). Disposition of 17 beta-trenbolone in humans. *J Chromatogr* 564: 485–492.
Thevis M *et al.* (2009). Doping control analysis of trenbolone and related compounds using liquid chromatography-tandem mass spectrometry. *Steroids* 74: 315–321.

Triamcinolone

Corticosteroid
$C_{21}H_{27}FO_6$ = 394.4
CAS—124-94-7

IUPAC Name (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*S*)-9-Fluoro-11,16,17-trihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one
Synonyms (11β,16α)-9-Fluoro-11,16,17,21-tetrahydroxypregna-1,4-diene-3,20-dione; 9α-fluoro-16α-hydroxyprednisolone; fluoxiprednisolonum.
Proprietary Names *Aristocort*; *Atolone*; *Berlicort*; *Delphicort*; *Ipercortis*; *Kenacort*; *Ledercort*; *Sterocort*; *Triam-Oral*; *Triamsicort*; *Volon*.



Chemical Properties A white, slightly hygroscopic, crystalline powder. Mp about 269° to 271°. Soluble 1 in 500 of water and 1 in 240 of ethanol; slightly soluble in methanol; very slightly soluble in chloroform and ether. Log *P* (octanol/water), 1.2.

Triamcinolone Acetonide

$C_{24}H_{31}FO_6$ = 434.5
CAS—76-25-5

Proprietary Names *Adcortyl*; *Aftab*; *Aftach*; *Albicort*; *Aristocort*; *Arutrin*; *Azmacort*; *Berlicort*; *Delphi(cort)*; *Denkacort*; *Dermacort*; *Extracort*; *Facort*; *Generlog*; *Kanolone*; *Kela*; *Kemzid*; *Kenacort*; *Kenalog*; *Kenalone*; *Ledercort*; *Nasacor (t)*; *Omcilon*; *Oracort*; *Orcilone*; *Sta-Cort A*; *Steronase*; *Triaderm*; *Triam*; *Triamonide*; *Tri-Anemul*; *Triderm*; *Trigon*; *Trilog*; *Trimacort*; *Volon A*; *Volonimat*; *Zamacort*.
Chemical Properties A white or cream-coloured crystalline powder. Mp about 292° to 294°. Very slightly soluble in water; soluble 1 in 150 of ethanol, 1 in 11 of acetone and 1 in 40 of chloroform; sparingly soluble in dehydrated alcohol and methanol.

Triamcinolone Diacetate

$C_{25}H_{31}FO_8$ = 478.5
CAS—67-78-7
Proprietary Names *Aristocort*; *Articulose LA*; *Delphicort*; *Delphimix*; *Kenacort*; *Ledercort*; *Proctosteroid*; *Tracilon*; *Triamolone*; *Trilone*; *Tristoject*.

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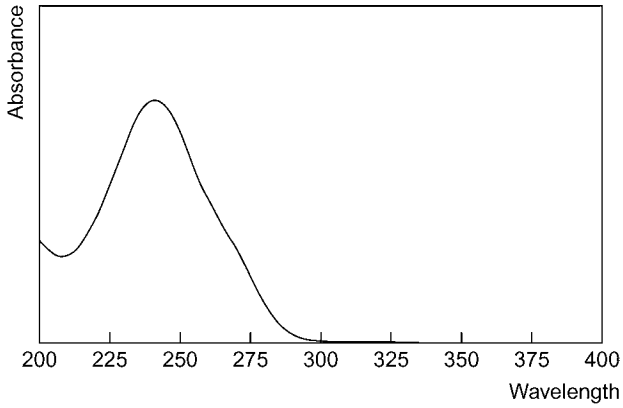
Chemical Properties A fine, white, crystalline powder. Practically insoluble in water; soluble 1 in 13 of ethanol, 1 in 80 of chloroform and 1 in 40 of methanol; slightly soluble in ether.

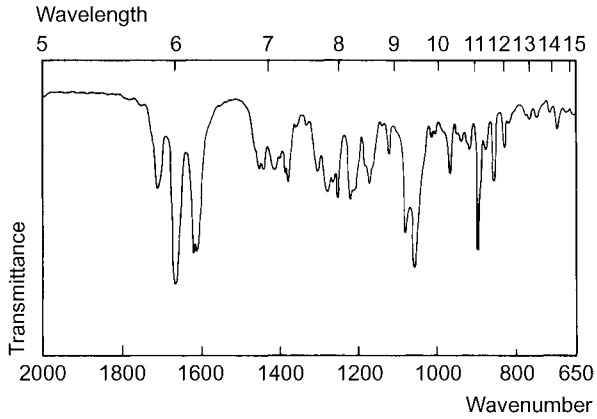
Triamcinolone Hexacetonide

$C_{30}H_{41}FO_7$ = 532.6
CAS—5611-51-8
Synonym Triamcinolone acetonide 21-(3,3-dimethylbutyrate)
Proprietary Names *Aristospan*; *Hexatrione*; *Lederlon*; *Lederspan*.
Chemical Properties A white to cream-coloured powder. Mp 295° to 296°, with decomposition. Practically insoluble in water; soluble in chloroform; slightly soluble in methanol.

Colour Tests Naphthol-Sulfuric acid—green, yellow dichroism/yellow; sulfuric acid—orange.
Thin-layer Chromatography Triamcinolone: System TA—R_f 0.79; system TE—R_f 0.27; system TP—R_f 0.09; system TQ—R_f 0.00; system TR—R_f 0.00; system TS—R_f 0.00; system TAJ—R_f 0.14; system TAK—R_f 0.06; system TAL—R_f 0.65; system TAM—R_f 0.33. (DPST solution.) Triamcinolone acetonide: System TP—R_f 0.32; system TQ—R_f 0.00; system TR—R_f 0.20; system TS—R_f 0.06 (DPST solution).
Gas Chromatography System GA—RI 2970 and RI 3107.
High Performance Liquid Chromatography Triamcinolone: System HX—RI 438; system HY—RI 312. Triamcinolone acetonide: System HT—*k* 2.5.
Ultraviolet Spectrum Methanol—238 nm (A^1_1=390a).



Infrared Spectrum Principal peaks at wavenumbers 1663, 1057, 1618, 1609, 902, 1080 cm^{-1} (triamcinolone acetonide, KBr disk).



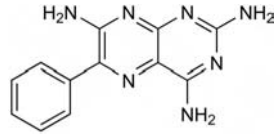
Quantification

Blood **HPLC** UV detection. Limit of detection, 200 pg [Saito *et al.* 1979].
Plasma **HPLC** UV detection. Limit of detection, 0.6 µg/L for triamcinolone acetonide [Doppenschmitt *et al.* 1996].
Radioimmunoassay Limit of detection, <12.5 µg/L [Loo, Jordan 1979].
Urine **HPLC** See Blood Saito *et al.* 1979].
Bronchoalveolar Lavage **GC–MS** Limit of detection, 6.0 ng/L for triamcinolone acetonide [Hubbard *et al.* 2001].
Hair **HPLC–MS** Limit of detection, 0.03 to 0.17 µg/g [Cirimele *et al.* 2000].
Note For a review of the pharmacokinetics of intranasal corticosteroids, see Szefler [2001].
Dose 4 to 48 mg daily.

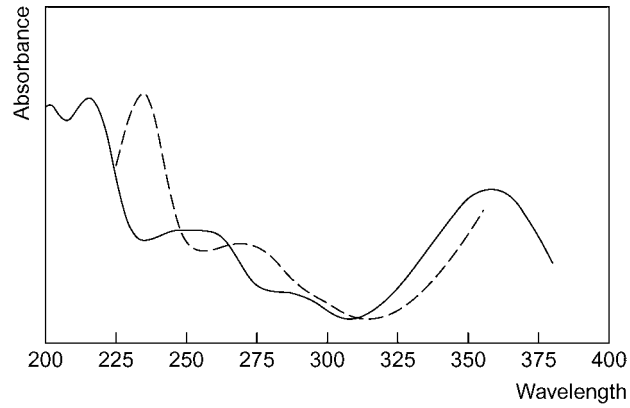
Cirimele V *et al.* (2000). Identification of ten corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry. *Forensic Sci Int* 107: 381–388.
Doppenschmitt SA *et al.* (1996). Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 682: 79–88.
Hubbard WC *et al.* (2001). Measurement of low picomolar levels of triamcinolone acetonide in human bronchoalveolar lavage fluid by gas chromatography-electron-capture negative-ion mass spectrometry. *Anal Biochem* 290: 18–25.
Loo JC, Jordan N (1979). A radio-immunological assay for triamcinolone in plasma. *Res Commun Chem Pathol Pharmacol* 23: 493–504.
Saito Z *et al.* (1979). The high pressure liquid chromatography of corticoids. II. Analysis of synthetic corticoids in blood and urine (author's transl). *Nippon Naibunpi Gakkai Zasshi* 55: 1296–1306.
Szefler SJ (2001). Pharmacokinetics of intranasal corticosteroids. *J Allergy Clin Immunol* 108: S26–S31.

Triamterene

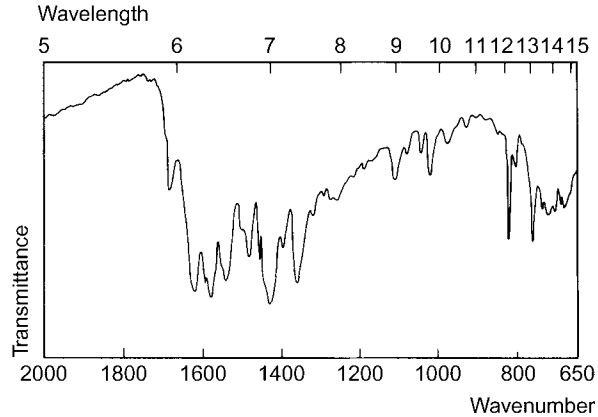
Diuretic
$C_{12}H_{11}N_7$ = 253.3
CAS—396-01-0
IUPAC Name 6-Phenylpteridine-2,4,7-triamine
Synonyms NSC-77625; SKF-8542; triamterenum; triantereno.
Proprietary Names *Dyrenium*; *Dytac*; *Jatropur*. It is an ingredient of *Dyazide*, *Dytenzide*, *Dytide*, *Frusene*, *Kalspare*, *Maxzide*, *Triamaxco* and *Triamco*.



Chemical Properties A yellow crystalline powder. Acidified solutions give a blue fluorescence. Mp 316°. Soluble 1 in 1000 of water, 1 in 3000 of ethanol, 1 in 4000 of chloroform and 1 in 30 of formic acid; soluble in dilute ammonia, dilute aqueous ammonium hydroxide and dimethylformamide; very slightly soluble in acetic acid and dilute mineral acids; practically insoluble in ether and dilute solutions of alkali hydroxides. pK_a 6.2. Log *P* (octanol/water), 0.98.
Colour Tests Liebermann's reagent—red-orange; Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.01; system TC—R_f 0.08; system TE—R_f 0.30; system TL-R_f 0.04; system TAD—R_f 0.13; system TAE—R_f 0.50; system TAF—R_f 0.65; system TAJ—R_f 0.04; system TAK—R_f 0.00; system TAL—R_f 0.40 (location under ultraviolet light, blue fluorescence).
Gas Chromatography System GA—triamterene RI 2010, triamterene-Me_6 RI 2875; system GX—triamterene-Me_6 retention time 9.2 min.
High Performance Liquid Chromatography System HX—RI 298; system HY—RI 263; system HZ—retention time 2.3 min; system HAA—retention time 8.7 min; system HAX—retention time 6.7 min; system HAY—retention time 3.9 min.
Ultraviolet Spectrum Aqueous acid—250 (A^1_1=634b), 360 nm (A^1_1=840a); (0.1 mol/L hydrochloric acid)—228 (A^1_1=1618), 360 nm (A^1_1=840); aqueous alkali (0.1 mol/L sodium hydroxide)—232 (A^1_1=1633), 270 (A^1_1=555a), 370 nm (A^1_1=741); methanol—233 (A^1_1=1570a), 267 nm (A^1_1=580a).



Infrared Spectrum Principal peaks at wavenumbers 1574, 1610, 1584, 1536, 761, 822 cm^{-1} (KBr disk).



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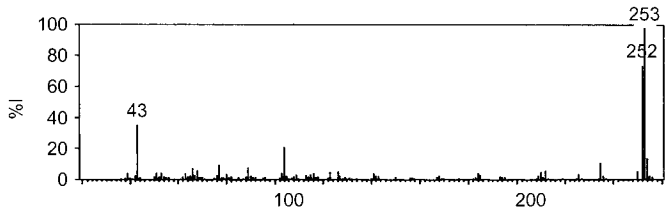
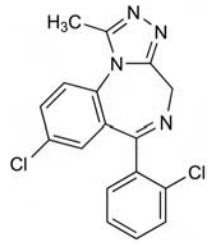
Mass Spectrum Principal ions at *m/z* 253, 252, 43, 104, 254, 235, 77, 89.





Chemical Properties A white or pale yellow crystalline powder. Mp 233° to 235°. Practically insoluble in water and ether; soluble 1 in 1000 of alcohol; 1 in 25 chloroform; 1 in 600 0.1 mol/L hydrochloric acid. Log *P* (octanol/water), 2.42. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Stock standard solutions in acetic acid were stable for at least 3 months when kept at 4°. Plasma samples were stable for 4 weeks when kept at 4° [Lee *et al.* 2006].

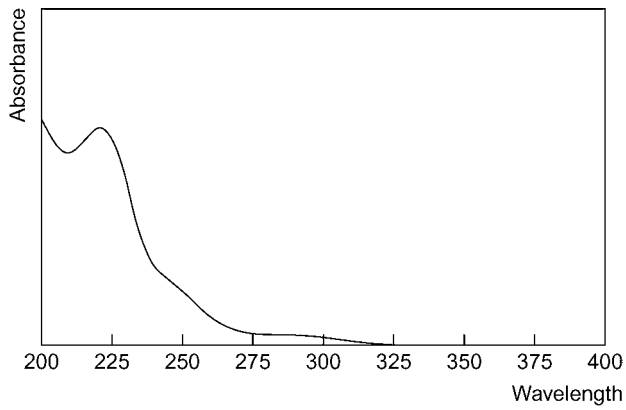
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.01; system TC—R_f 0.40; system TD—R_f 0.05; system TE—R_f 0.44; system TF—R_f 0.02; system TL—R_f 0.16; system TAD—R_f 0.41; system TAE—R_f 0.68; system TAF—R_f 0.65.

Gas Chromatography System GA—triazolam RI 3080, M (α-OH-) RI 3000; system GB—triazolam RI 3219, M(α-OH-) not eluted, M (α-OH-)-TMS RI 3308.

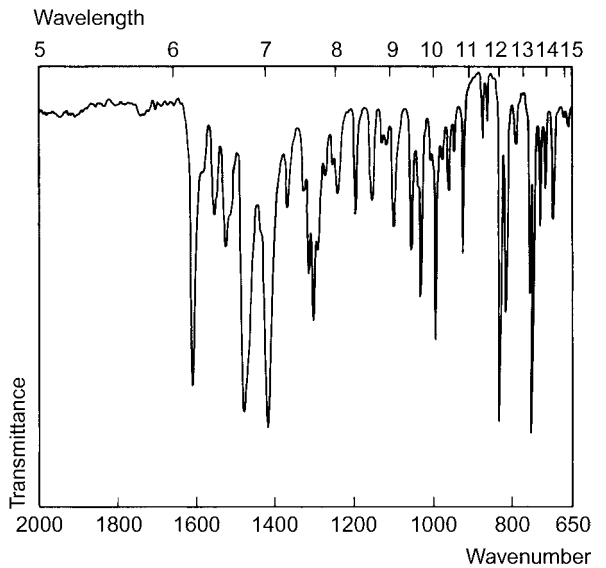
Gas Chromatography-Mass Spectrometry Column: SGE BP1 (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 65° for 1 min to 250° at 15°/min for 8 min to 300° at 10°/min for 2 min. EI ionisation, scan or SIM acquisition mode. Retention time: 23.2 min. Limit of quantification, 2.5–5 µg/L and 0.1–0.5 µg/L for scan and SIM modes, respectively [Borrey *et al.* 2001a].

High Performance Liquid Chromatography System HI—*k* 4.38; system HK—*k* 1.83; system HX—RI 476; system HY—RI 390; system HZ—RT 4.2 min; system HAA—RT 17.4 min; system HAF—RT 13.7 min; system HAL—RT 6.6 min; system HAM—not detected; system HAX—RT 6.4 min; system HAY—RT 6.7 min; system HAZ—*k* 1.07; system HBH—*k* 5.18; system HBI—*k* 1.13.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 761, 842, 1618, 1003, 1310, 827 cm^{-1} (KBr disk).



Quantification

Blood **HPLC** Fluorescence detection. Limit of detection, 1 µg/L for triamterene, 2 µg/L for *p*-hydroxytriamterene sulfate [Sörgel *et al.* 1984].

Plasma **HPLC** For method of quantification for triamterene and *p*-hydroxytriamterene sulfate, see Sörgel *et al.* [1984]. UV detection. Limit of detection, 20 µg/L for triamterene and *p*-hydroxytriamterene [Yakatan, Cruz 1981].

Urine **HPLC** See Blood. Limit of detection, 40 µg/L for triamterene, 100 µg/L for *p*-hydroxytriamterene sulfate [Sörgel *et al.* 1984].

Disposition in the Body Rapidly but incompletely absorbed after oral administration and extensively metabolised. After oral administration, about 30 to 70% of a dose is excreted in the urine, mainly as the sulfate conjugate of *p*-hydroxytriamterene, with about 5 to 10% of a dose as unchanged drug; variable amounts are excreted in the bile. *p*-Hydroxytriamterene sulfate is an active metabolite. Triamterene crosses the placenta and it may be detected in breast milk.

Therapeutic Concentration

After single oral doses of 150 to 300 mg to 7 subjects, peak plasma-triamterene concentrations of 0.03 to 0.15 mg/L (mean, about 0.1) were attained in 1 to 2 h; peak plasma concentrations of *p*-hydroxytriamterene sulfate averaged about 1 mg/L at 2 to 4 h; both unchanged drug and the metabolite exhibited a second peak concentration several hours later, possibly due to enterohepatic circulation [Gundert-Remy *et al.* 1979].

Following a single oral dose of 100 mg to 6 subjects, peak plasma concentrations of 0.06 to 0.18 mg/L (mean, 0.13) of triamterene and 1.3 to 3.2 mg/L (mean, 1.8) of *p*-hydroxytriamterene sulfate were attained in about 1 to 2 h [Hasegawa *et al.* 1982].

Triamterene, 200 mg daily for 10 days, administered to 7 healthy subjects, produced an average plasma concentration during the dosage interval of 0.045 mg/L triamterene and 0.967 mg/L of hydroxytriamterene sulfate in healthy subjects; in 6 patients with cirrhosis and ascites, the respective levels were 0.586 and 0.747 mg/L (a 13-fold increase compared with healthy subjects) [Dao, Villeneuve 1988].

Toxicity The toxic low oral dose for a woman is 42 mg/kg body weight at 3 weekly intervals. It is moderately toxic by SC administration. May be poisonous by ingestion and IP routes.

Bioavailability 30 to 70%.

Half-life Plasma half-life, triamterene about 2 to 4 h, *p*-hydroxytriamterene sulfate, about 3 h; half-life derived from urinary excretion data, 6 to 12 h.

Distribution in Blood Plasma : whole blood ratio, triamterene 0.97, *p*-hydroxytriamterene sulfate, 1.7.

Protein Binding Triamterene about 45 to 70%, *p*-hydroxytriamterene sulfate about 90%.

Dose 150 to 250 mg daily; reduced to alternate days after 1 week.

Dao MT, Villeneuve JP (1988). Kinetics and dynamics of triamterene at steady-state in patients with cirrhosis. *Clin Invest Med* 11: 6–9.

Gundert-Remy U *et al.* (1979). Plasma and urinary levels of triamterene and certain metabolites after oral administration to man. *Eur J Clin Pharmacol* 16: 39–44.

Hasegawa J *et al.* (1982). Pharmacokinetics of triamterene and its metabolite in man. *J Pharmacokinet Biopharm* 10: 507–523.

Sörgel F *et al.* (1984). Liquid chromatographic analysis of triamterene and its major metabolite, hydroxytriamterene sulfate, in blood, plasma, and urine. *J Pharm Sci* 73: 831–833.

Yakatan GJ, Cruz JE (1981). High-performance liquid chromatographic analysis of triamterene and p-hydroxytriamterene in plasma. *J Pharm Sci* 70: 949–951.

Triazolam

Benzodiazepine, Hypnotic

$C_{17}H_{12}Cl_2N_4 = 343.2$

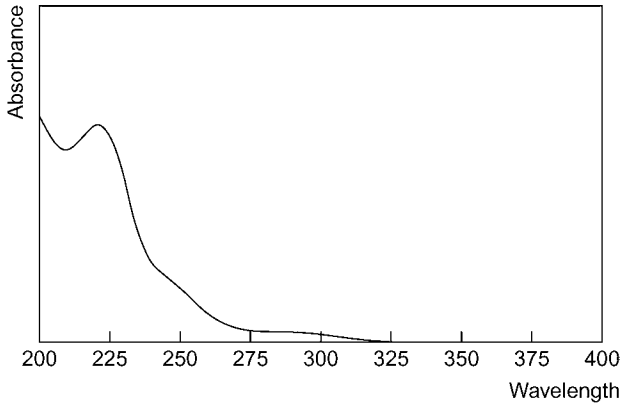
CAS—28911-01-5

IUPAC Name 8-Chloro-6-(2-chlorophenyl)-1-methyl-4*H*-1,2,4-triazolo[4,3-*a*]-1,4-benzodiazepine

Synonyms Clorazolam; U-33030.

Proprietary Names *Apo-Triazo*; *Halcion*; *Hypam*; *Rilamir*; *Somese*; *Songar*; *Trilam*; *Trycam*.

Mass Spectrum Principal ions at *m/z* 313, 238, 342, 315, 75, 344, 239, 137.



Quantification

Blood **GC** Column: TC-1 dimethyl silicone (15 m × 0.53 mm i.d., 1.5 µm) or TC-5 5% phenylmethyl silicone (15 m × 0.53 mm i.d., 1.5 µm) or TC-17 50% phenylmethyl silicone (15 m × 0.53 mm i.d., 1 µm). Carrier gas: N_2 or He, 15 kPa. Temperature programme: 150° for 2 min to 260° at 10°/min for TC-1 and TC-17 to 300° at 20°/min for the TC-5, for 27 and 10.5 min, respectively. FID. Limit of detection, 2 µg/L [Moriya, Hashimoto 2003]. Column: HP-1 (13 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 280° at 20°/min for 3 min. NPD. Limit of detection, 100 ng/g [Kudo *et al.* 1997a]. Column: HP5 cross-linked 5% phenylmethyl silicone (25 m × 0.32 mm i.d., 0.52 µm). Carrier gas: Ar : CH_4 (95 : 5), 3 mL/min. Temperature programme: 90° to 300° at 30°/min. Limit of detection, 0.002 mg/L [Steentoft, Worm 1993]. Column: Shimadzu CBP1 methyl silicone (10 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 20 mL/min. Temperature programme: 220° to 260° at 5°/min. NPD. Limit of detection, 0.5 ng/g [Kudo *et al.* 1991].

GC-MS Column: HP-1 capillary (12 m × 0.2 mm i.d., 0.3 µm) Carrier gas: He, 10 psi. Temperature programme: 70° to 280° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Rossi *et al.* 2009]. Column: DB-1 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 10.0 psi. Temperature programme: 140° to 225° at 25°/min to 245° at 12°/min to 300° at 30°/min. EI ionisation. Limit of quantification, 0.02 mg/L [Levine *et al.* 2002]. Column: CBJ1-S30-100 (30 m × 0.32 mm i.d., 1 µm). Temperature programme: 50° to 220° at 30°/min for 1 min to 280° at 5°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Uemura, Komura 1995]. Column: Ultra-2 (25 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 40 cm/s. Temperature programme: 150° for 1 min to 280° at 30°/min for 12 min. NICI. Limit of quantification, 0.5 µg/L [Cairns *et al.* 1994]. Column: DB1 (15 m). Temperature programme: 185° to 300° at 11°/min. ECD. Retention time: 9.97 min. Limit of detection not reported [Joynt 1993]. See also Gjerde *et al.* [1992], Koves, Wells [1986].

HPLC Column: µBondapak C_{18} (30 cm). Mobile phase: 0.05% acetic acid : acetonitrile (61 : 39), flow rate 1.5 mL/min. UV detection (λ = 221 nm). Limit of detection not reported [Wong 1984].

Plasma **GC** Column: Ultra 2 5% phenylmethyl silicone (25 m × 0.32 mm i.d.). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. NPD and ECD. Limit of quantification, 1 µg/L [Gaillard *et al.* 1993].

GC-MS Column: fused silica (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He with auxilliary gas of 5% Ar in CH_4, 0.6 and 30 mL/min, respectively. Temperature programme: 200° for 1 min, to 235° at 35°/min to 265° at 5°/min for 1 min. IS: flunitrazepam. ECD. Retention time: triazolam, 8.66 min; IS, 4.65 min. Limit of detection, 0.02 µg/L [Edeki *et al.* 1992]. Column: DB-17 (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 30 psi. Temperature programme: 40° for 1 min to 250° at 30°/min to 280° at 10°/min to 300° at 5°/min for 8 min. EI ionisation at 70 eV. Retention time: 21.2 min. Limit of quantification, 0.5 µg/L [Karl *et al.* 1997]. Column: OV-1701 (15 m × 0.32 mm i.d., 0.32 µm). Carrier gas: H_2, 4 mL/min. Temperature programme: 80° to 260° at 30°/min. ECD. Retention time: 15 min. Limit of detection, 0.5 µg/L [Baktir *et al.* 1985]. Column: WSCOT (25 m × 0.32 mm i.d.). Carrier gas: He, 0.8 bar. Temperature programme: 150° to 300° at 2/min. ECD. Limit of detection, 0.1–0.2 µg/L [Coassolo *et al.* 1983]. Column: 1% OV-17 on 80/100 Chromosorb W HP (1.83 m × 2 mm i.d.). Carrier gas: He, 50 mL/min. Temperature: 290°. ECD. Retention time: 4.1 min. Limit of detection, 0.25 µg/L [Greenblatt *et al.* 1981]. See also Jochemsen, Breimer [1981].

HPLC Column: Develosil C18-5 and C8-5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.5% monobasic potassium phosphate (pH 4.5) : acetonitrile (65 : 35), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 19 min. Limit of detection, 0.5 µg/L [Yasui *et al.* 1997]. Column: µBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: methanol : 10 mmol/L phosphate buffer (pH 6.0, 50 : 50), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of detection not reported [Kinirons *et al.* 1996]. Column: Nova Pak C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 6 mmol/L phosphate buffer (23 : 13 : 64), flow rate 1.3 mL/min. UV detection (λ = 242 nm). Limit of detection, 3.0 µg/L [Boukhabza *et al.* 1991].

LC-MS Column: C_{18} (50 × 2.1 mm i.d., 3 µm). Mobile phase: acetonitrile : water : formic acid (35 : 65 : 0.2), flow rate 0.25 mL/min. ESI, CID, MRM acquisition mode. Limit of quantification, 0.05 µg/L [Pan *et al.* 2008]. Column: Shodex MSpak GF-310 4B (50 × 4.6 mm i.d., 6 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 3.56) with 0.1% formic acid : acetonitrile (100 : 0 for 5 min to 40 : 60 for 30 min to 30 : 70 for 5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Limit of quantification, 0.25 µg/L, limit of detection, 0.1 µg/L [Kratzsch *et al.* 2004; Lee *et al.* 2006]. Column: ODS-HG-5 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate : methanol or acetonitrile (50 : 50 to 0 : 100 at 15 min. Positive ion mode. Retention time, 7.9 min. Limit of quantification, 20 ng/L [Senda *et al.* 1995].

Serum **GC-MS** Column: OV-17 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 40 mL/min. Temperature programme: 110° for 1 min to 300° at 10°/min for 5 min. ECD. Limit of detection, 0.5 µg/L [Nishioka 1996].

HPLC [Tanaka *et al.* 1996]. UV detection. Limit of detection, 0.001 µg [Adams *et al.* 1980].

LC-MS [Nakamura *et al.* 2009].

Urine **GC** See Blood [Moriya, Hashimoto 2003]. See Plasma [Coassolo *et al.* 1983].

GC-MS See Blood [Black *et al.* 1994; Borrey *et al.* 2001b; Levine *et al.* 2002; Needleman, Porvaznik 1995; Uemura, Komura 1995]. See also Dickson *et al.* [1992], Edinboro, Poklis [1994], Joern [1992], Jones *et al.* [1989]

HPLC See Plasma. Limit of detection, 10 µg/L for α-hydroxytriazolam [Kinirons *et al.* 1996]. Column: Radial-Pak C_{18} (100 × 8 mm i.d., 10 µm). Mobile phase: methanol : 10 mmol/L phosphate buffer (pH 8.0, 65 : 35), flow rate 1.0 mL/min. UV detection (λ = 220 nm). Limit of detection, 5 µg/L [Inoue, Suzuki 1987]. Column: Partisil PXS-10/25 ODS (25 cm). Mobile phase: acetonitrile : water (35 : 65), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection not reported [Eberts *et al.* 1981].

LC-MS Column: Mightysil RP-18 (150 × 2.0 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0) : methanol (57 : 43), flow rate 0.2 mL/min. ESI, positive ion mode. Limit of detection, 0.5 µg/L [Tsujikawa *et al.* 2005].

Bile **GC** See Blood [Moriya, Hashimoto 2003].

CSF **GC** See Blood [Moriya, Hashimoto 2003].

GC-MS See Blood [Levine *et al.* 2002].

Oral Fluid **LC-MS** [Kintz *et al.* 2005; Moore *et al.* 2007].

Gastric Contents **GC** See Blood [Moriya, Hashimoto 2003].

GC-MS See Blood [Rossi *et al.* 2009]. See Blood [Uemura, Komura 1995].

HPLC See Blood [Wong 1984].

Pericardial Fluid **GC** See Blood [Moriya, Hashimoto 2003].

Vitreous Humour **GC** See Blood [Moriya, Hashimoto 2003].

Brain **GC** See Blood. Limit of detection, 8 ng/g [Moriya, Hashimoto 2003]. See Blood [Kudo *et al.* 1997a].

GC-MS See Blood [Uemura, Komura 1995].

Hair **GC-MS** Column: HP5-MS 5% phenyl-95% methylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 60° for 1 min to 295° at 30°/min for 6 min. NCI, SIM acquisition mode. Retention time: 13.5 min. Limit of detection, 1 pg/mg [Cirimele *et al.* 1997].

LC-MS [Irving, Dickson 2007; Villain *et al.* 2005]. Column: Mightysil RP-18 (100 × 2.0 mm i.d., 3 µm). Mobile phase: water : acetonitrile : ethanol (75 : 25 : 1 to 74 : 26 : 1 in 7 min for 10 min to 60 : 40 : 1 in 8 min to 10 : 90 : 1 in 10 min for 10 min), flow rate 0.15 mL/min. ESI. Limit of detection, 0.025 ng [Toyo'oka *et al.* 2001].

Kidney **GC** See Brain [Moriya, Hashimoto 2003]. See Blood [Kudo *et al.* 1997a; Kudo *et al.* 1991].

GC-MS See Blood [Levine *et al.* 2002].

Liver **GC** See Brain [Moriya, Hashimoto 2003]. See Blood [Kudo *et al.* 1997a; Kudo *et al.* 1991].

GC-MS See Blood [Levine *et al.* 2002; Uemura, Komura 1995; Cairns *et al.* 1994].

HPLC See Blood [Wong 1984].

Lung **GC** See Brain [Moriya, Hashimoto 2003]. See Blood [Kudo *et al.* 1997a].

Muscle **GC** See Blood [Kudo *et al.* 1997a; Kudo *et al.* 1991].

GC-MS Column: DB-5 (15 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 50 mL/min. Temperature programme: 150° for 1 min to 300° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 0.5 ng/g [Inoue *et al.* 1997].

Myocardium **GC** See Brain [Moriya, Hashimoto 2003].

Nail **LC-MS** See Hair [Irving, Dickson 2007].

Skeletal Remains **GC-MS** Column: HP-1 (13 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 2 min to 300° at 20°/min for 2 min. NICI. Limit of detection, 0.2 ng/g [Kudo *et al.* 1997b].

Spleen **GC** See Blood [Kudo *et al.* 1997a].

Disposition in the Body Triazolam is rapidly and almost completely absorbed after oral administration. It is metabolised mainly by hydroxylation. The major metabolite, 1-hydroxymethyltriazolam, has a hypnotic potency approximately equivalent to that of triazolam. Other metabolites include 4-hydroxytriazolam, 1-hydroxymethyl-4-hydroxytriazolam and three dichlorotriazolylbenzophenone derivatives. CYP3A4, and not CYP2C19, is involved in the metabolism of triazolam [Yasui *et al.* 1997]. Approximately 80% of a dose is excreted in the urine in 48 h mainly as the unconjugated metabolite (only a small amount as the unchanged drug) and ~7% is eliminated in the faeces in 72 h.

Therapeutic Concentration

Following oral administration of 0.88 mg [^{14}C]-labelled triazolam to six subjects, peak plasma concentrations of 6 to 17 µg/L (mean 9 µg/L) triazolam were attained in 0.75–3 h; peak plasma concentrations of the

glucuronide conjugates of the 1-hydroxymethyl and 4-hydroxy metabolites of 3–10 μg/L (mean 6) and 2–9 μg/L (mean 6 μg/L) were attained in 0.75–2.5 h and 1.5–5 h, respectively [Eberts *et al.* 1981].

Six male volunteers were administered 0.125, 0.250 or 0.375 mg triazolam. The mean peak plasma concentrations observed were 2.2, 4.3 and 5.0 μg/L, respectively, for the 3 doses and the times to reach these concentrations were 0.7, 0.6 and 0.8 h, respectively [Edeki *et al.* 1992].

Nine healthy children, aged 6 to 9 years were given oral does of 0.025 mg/kg triazolam before dental treatment; the total dose administered to each child ranged from 0.5 to 0.9 mg (median dose 0.75 mg). The mean peak plasma concentration was 8.5 μg/L (range, 4.7–13.7 μg/L), which was reached in 74 min (range, 45–128 min) [Karl *et al.* 1997].

A mean peak plasma triazolam concentration of 4.9 μg/L (SD, 2.0) was attained in 75 min in 9 healthy children given 0.25 or 0.375 mg of sublingual triazolam before dental treatment [Tweedy *et al.* 2001].

Three healthy Chinese volunteers were administered 0.25 mg triazolam. The mean maximum plasma concentration was 1.4 μg/L, reached at 1.2 h [Pan *et al.* 2008].

Toxicity Manifestations of overdosage, including somnolence, confusion, impaired coordination, slurred speech and even coma, may occur with doses of 2 mg (four times the recommended therapeutic dose). Seizures, respiratory depression and even death have also been reported. The lowest triazolam blood concentration that has been associated with death is 7 μg/L in whole blood. Ethanol at a concentration of 2300 mg/L was also detected in the blood of this individual [Uemura, Komura 1995].

A 76-year-old woman was found dead in her kitchen. The concentration of triazolam in her blood and gastric contents was 1100 and 1300 μg/L, respectively [Rossi *et al.* 2009].

A 77-year-old woman who was found dead in her bathtub (her head was above the water line and an empty bottle of triazolam was discovered in the bin) was revealed to have a heart blood triazolam concentration of 120 μg/L; the cause of death was ruled to be triazolam intoxication and the manner of death was suicide [Levine *et al.* 2002].

A 57-year-old man was found dead. Blood triazolam concentrations decreased in the order inferior vena cava, right cardiac chambers, pulmonary arteries, pulmonary veins, left cardiac chambers/aorta, right femoral vein [Moriya, Hashimoto 2003].

Of 6 fatal poisonings by triazolam alone or in combination with ethanol, in 1 case where only triazolam was detected, the blood concentration was 110 μg/L. Because of putrefaction, blood was not available for analysis in the other case involving only triazolam. In the 4 cases also involving ethanol (blood concentration 1100–2020 mg/L), the triazolam concentration was 40–220 μg/L [Steentoft, Worm 1993].

A 39-year-old man was found dead in bed next to empty packages each providing 40 Halcion tablets (25 mg each). The triazolam concentration was 104.4, 153.1 and 398.8 ng/g in skeletal muscle, kidney and liver, respectively [Kudo *et al.* 1991].

Three individuals, aged 76, 59 and 75 years, were all found dead in bed. Blood triazolam concentrations were 16, 20 and 26 μg/L, respectively [Bal *et al.* 1989].

A 38-year-old drug addict who was being treated with methadone was found dead at home. At the scene were syringes containing a liquid later identified to be blood with traces of triazolam and a number of bottles containing Halcion (triazolam). Toxicological analysis of blood, bile, urine and stomach contents showed concentrations of triazolam (blood, 15 μg/L; urine, traces); morphine (blood, 0.12 mg/L; bile, total of 0.0037 mg; urine, traces) and codeine (blood, 030 μg/L; urine, traces). The blood drug concentrations were also found to be at therapeutic concentrations only. The cause of death was determined as the combined adverse effects of triazolam and morphine. This is the first case reported where triazolam was injected rather that taken orally [Kammie Hon *et al.* 1988].

Half-life Plasma half-life, triazolam 1.5–3 h, 1-hydroxymethyltriazolam ~4 h.

Volume of Distribution ~1–2 L/kg, also quoted as 65 L (mean).

Clearance Plasma clearance, ~10 mL/min/kg, also quoted as 300 mL/min (mean).

Protein Binding ~89%.

Note For a review of the pharmacokinetics of triazolam see Garzone, Kroboth [1989], Gupta *et al.* [1990], Klett [1992] and Pakes *et al.* [1981].

Dose As a hypnotic, 125 to 250 μg.

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Kudo K *et al.* (1997b). Detection of triazolam in skeletal remains buried for 4 years. *Int J Legal Med* 110: 281–283.

Lee XP *et al.* (2006). Simple method for determination of triazolam in human plasma by high-performance liquid chromatography/tandem mass spectrometry. *J Pharm Biomed Anal* 41: 64–69.

Levine B *et al.* (2002). Distribution of triazolam and alpha-hydroxytriazolam in a fatal intoxication case. *J Anal Toxicol* 26: 52–54.

Moore C *et al.* (2007). Determination of benzodiazepines in oral fluid using LC-MS-MS. *J Anal Toxicol* 31: 596–600.

Moriya F, Hashimoto Y (2003). A case of fatal triazolam overdose. *Leg Med (Tokyo)* 5(Suppl1): S91–S95.

Nakamura M *et al.* (2009). Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography–tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds. *Biomed Chromatogr* 23: 357–364.

Needleman SB, Porvaznik M (1995). Identification of parent benzodiazepines by gas chromotography/mass spectroscopy (GC/MS) from urinary extracts treated with B-glucuronidase. *Forensic Sci Int* 73: 49–60.

Nishioka R (1996). Determination of triazolam in serum by deactivated metal capillary gas chromatography with electron-capture detection. *J Chromatogr B Biomed Appl* 681: 401–404.

Pakes GE *et al.* (1981). Triazolam: a review of its pharmacological properties and therapeutic efficacy in patients with insomnia. *Drugs* 22: 81–110.

Pan RN *et al.* (2008). Simultaneous determination of triazolam and its metabolites in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 872: 58–62.

Rossi R *et al.* (2009). Acute intoxication by triazolam and promazine: a case report. *Med Sci Law* 49: 65–68.

Senda N *et al.* (1995). A highly sensitive method to quantify triazolam and its metabolites with liquid chromatography–mass spectrometry. *Biomed Chromatogr* 9: 48–51.

Steentoft A, Worm K (1993). Cases of fatal triazolam poisoning. *J Forensic Sci Soc* 33: 45–48.

Tanaka E *et al.* (1996). Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-microns porous microspherical silica gel. *J Chromatogr B Biomed Appl* 682: 173–178.

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Tsujikawa K *et al.* (2005). Urinary excretion profiles of two major triazolam metabolites, alpha-hydroxytriazolam and 4-hydroxytriazolam. *J Anal Toxicol* 29: 240–243.
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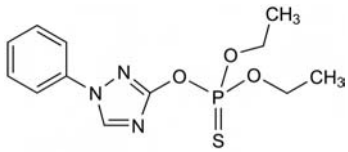
Villain M *et al.* (2005). Screening method for benzodiazepines and hypnotics in hair at pg/mg level by liquid chromatography–mass spectrometry/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 825: 72–78.
Wong RJ (1984). The determination of the triazolobenzodiazepine triazolam in post mortem samples. *J Anal Toxicol* 8: 10–13.
Yasui N *et al.* (1997). Single-dose pharmacokinetics and pharmacodynamics of oral triazolam in relation to cytochrome P4502C19 (CYP2C19) activity. *Ther Drug Monit* 19: 371–374.

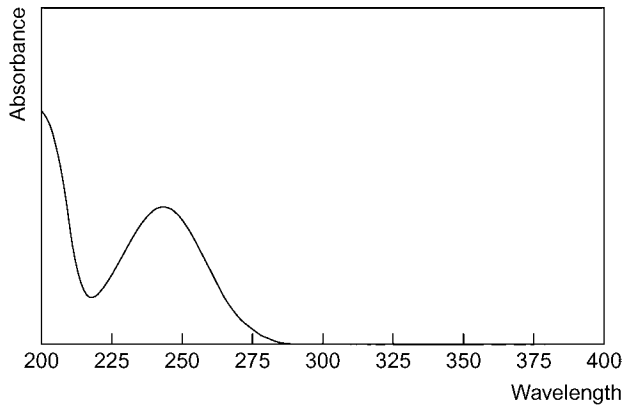
Triazophos

Acaricide, Insecticide
$C_{12}H_{16}N_3O_3PS = 313.3$
CAS—24017-47-8

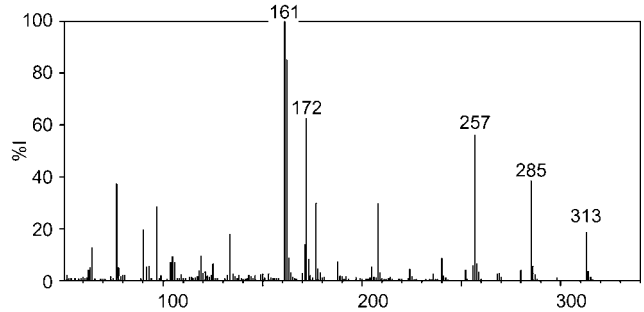
IUPAC Name Dethoxy-[(1-phenyl-1,2,4-triazol-3-yl)oxy]-sulfanylidene-λ^5-phosphane
Synonyms HOE 2960; HOE 2960 OJ; posphorothioic acid *O*,*O*-diethyl-*O*-(1-phenyl-1*H*-1,2,4-triazol-3-yl)-ester; triazofos.
Proprietary Name *Hostathion*



Chemical Properties A yellowish to light brown oil. Mp 2° to 5° (technical); 0.5°. Soluble in water (39 mg/L at 20°), *n*-hexane (0.7 g/100 mL at 25°), toluene (30 g/100 mL at 25°), ethyl acetate (30 g/100 mL at 25°), acetone (30 g/100 mL at 25°) and ethanol (30 g/100 mL at 25°). Log *P* (octanol/water), 3.34.
Thin-layer Chromatography System TX—R_f 0.21; system TY—R_f 0.38.
Gas Chromatography System GA—RI 2258.
Ultraviolet Spectrum Principal peak at 242 nm.



Mass Spectrum Principal ions at *m/z* 161, 162, 172, 257, 285, 77, 208, 177.

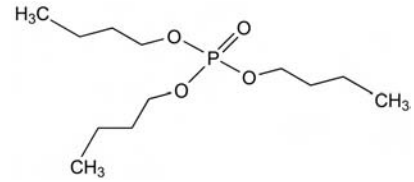


Disposition in the Body Triazophos is absorbed through the skin, respiratory tract and gastrointestinal tract. It acts on the central nervous system.
Toxicity The allowed daily intake is 0.001 mg/kg bodyweight and the highest dose to show no toxic effect is 0.0125 mg/kg.

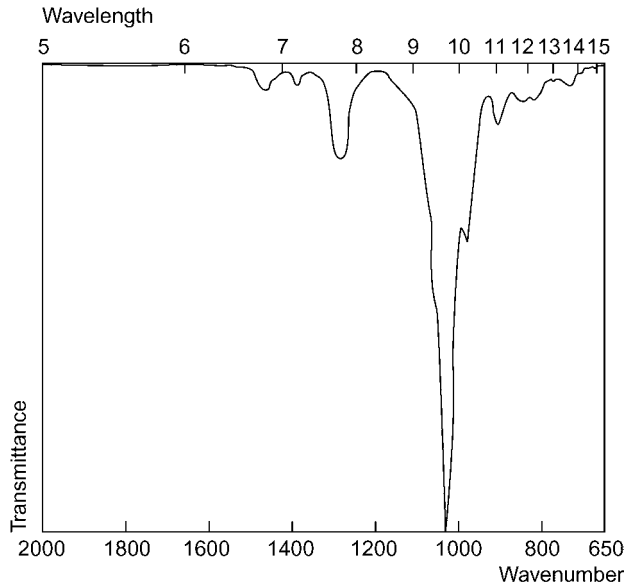
Tributyl Phosphate

Plasticiser
$C_{12}H_{27}O_4P = 266.3$
CAS—126-73-8

IUPAC Name Tributyl phosphate
Synonyms BP; tributhylphosphate; tributoxyphosphine oxide.
Proprietary Names *Celluphos 4*; *Disflammol TB.*



Chemical Properties A colourless to pale yellow liquid. Mp −79° to 80°. Bp 289°. Slightly soluble in water (<1 mg/L at 20.5°); soluble in DMSO, 95% ethanol and acetone (≥100 g/L at 20.5°), ether and benzene. Log *P* (octanol/water), 4.00.
Gas Chromatography System GA—RI 1690.
Infrared Spectrum Principal peaks at wavenumbers 1031, 1293, 907 cm^{-1}.

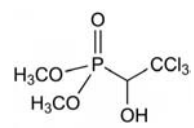


Mass Spectrum Principal ions at *m/z* 99, 156, 41, 29, 57, 211, 125, 137.
Disposition in the Body Tributyl phosphate is absorbed through the skin and after ingestion. During metabolism, oxidation and dealkylation occur to produce dibutylhydrogen phosphate, butyldihydrogen phosphate and butyl-bis(3-hydroxybutyl)phosphate. Excretion occurs mainly within the first 48 h in urine mostly but also in faeces and via exhalation.
Toxicity Tributyl phosphate is highly toxic via IV and IP routes and moderately toxic via oral administration.

Trichlorfon

Insecticide, Anthelmintic
$C_4H_8Cl_3O_4P = 257.4$
CAS—52-68-6

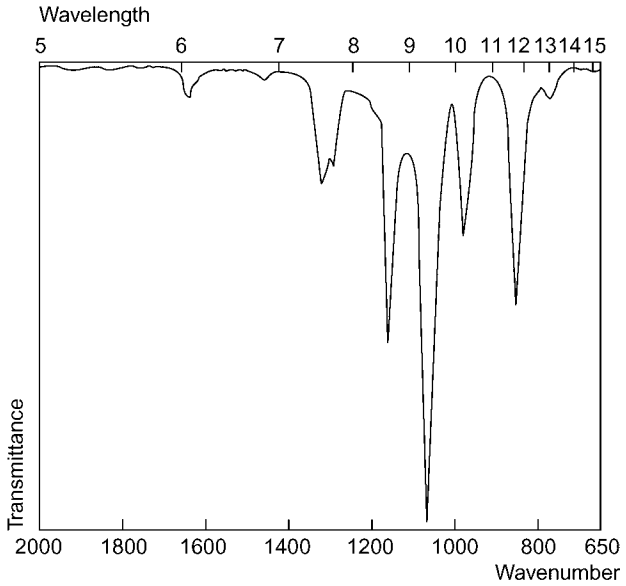
IUPAC Name 2,2,2-Trichloro-1-dimethoxyphosphorylethanol
Synonyms BayerL13/59; chlorofos; DEP; DETF; *O*,*O*-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate; dipherex; foschlor; metrifonate; metrifonatum; metriphonate; TCF; trichlorofon; trichlorphon.
Proprietary Names *Anthon*; *Bilarcil*; *Bovinox*; *Briten*; *Chlorofos*; *Chlorophos*; *Ciclosom*; *Combot*; *Dipterex*; *Ditrifon*; *Dylox*; *Dyrex*; *Leivasom*; *Neguvon*; *Proxol*; *Trinex*; *Vermicide*; *Masoten*; *Promem.*



Chemical Properties A white crystalline powder. Mp 75°. Very soluble in water (15.4 g/100 mL at 20°); soluble in dichloromethane, 2-propanol, diethyl ether (17 g/100 mL at 20°), alcohol, acetone, chloroform (75 g/100 mL at 20°), benzene (15.2 g/100 mL at 20°) and toluene; very slightly soluble in *n*-hexane (0.08 g/100 mL at 20°) and pentane. Log *P* (octanol/water), 0.52.

T

Thin-layer Chromatography System TE—R_f 0.61; system TX—R_f 0.07; system TY—R_f 0.02; system TZ—R_f 0.15; system TAA—R_f 0.05; system TAB—R_f 0.02; system TAC—R_f 0.01; system TAE—R_f 0.90.
Gas Chromatography System GA—trichlorfon RI 1438; trichlorfon-Me RI 1395; system GK—RRT 0.27(relative to caffeine).
High Performance Liquid Chromatography System HY—RI 738.
Infrared Spectrum Principal peaks at wavenumbers 1109, 1154, 854, 978, 1323 cm^{-1}.



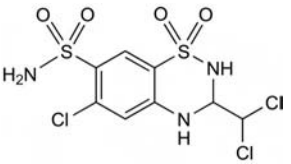
Mass Spectrum Principal ions at *m/z* 109, 79, 110, 145, 139, 47, 15, 112.
Quantification
Blood **GC** ECD. Limit of quantification, 0.01 mg/L [Heinig *et al.* 2000]. In serum: limit of detection 2.5 µg/L, FTD [Ameno *et al.* 1989].
GC–MS Limit of quantification, 0.001 mg/L for the metabolite dichlorvos (DDVP) [Heinig *et al.* 2000].
Plasma **HPLC** Limit of quantification, 1 mg/L for trichlorfos, 0.04 mg/L for the metabolite DDVP [Unni *et al.* 1992].
Serum **GC** See Blood [Ameno *et al.* 1989].
GC–MS Limit of detection, 0.06 mg/L for the metabolite, dimethylphosphate (DMP) [Tarbah *et al.* 1998].
Urine **GC** See Blood [Heinig *et al.* 2000].
GC–MS See Blood [Heinig *et al.* 2000].
Disposition in the Body Trichlorfon is rapidly absorbed after oral administration and some of the dose is converted to dichlorvos (DDVP), which is the active moiety. Another metabolite observed is dimethylphosphate (DMP) as well as dimethylphosphoric acid, phosphoric acid, desmethyldichlorvos and monomethylphosphoric acid. It is excreted via the kidneys mainly as glucuronides. Approximately 70% of a dose is recovered in urine and also a small amount in faeces. Trichlorfon is readily absorbed through the skin and absorption, distribution and excretion are quick. It is distributed to the main organs with the highest concentrations observed in the liver, kidney and lungs.
Blood Concentration
Six healthy male volunteers were administered with a single oral dose of 7.5 mg/kg trichlorfon. Trichlorfon was quickly absorbed and peak plasma concentrations were observed 0.7 to 1 h after administration at levels of 50.5 µmol/L. DDVP concentrations were ~1% of that for trichlorfon [Abdi, Villen 1991].
Toxicity Moderately toxic by ingestion and dermal absorption. The allowed daily intake is 0.01 mg/kg.
A 76-year-old man committed suicide by ingesting 50 mL of Dipterex and died after 8 h. An autopsy revealed that his trichlorfon blood concentration was 215 µg/g and his gastric lavage liquid sample contained 15 mg/g of the pesticide. These samples were taken 1 h after admission [Yashiki *et al.* 1988].
A 70-year-old woman died from acute trichlorfon poisoning after ingesting 50% emulsifiable concentrate. Postmortem toxicology showed a blood concentration of 310 µg/g, liver 487 µg/g, brain 465 µg/g, kidney 416 µg/g, urine 2240 µg/g and a total of 7.2 g in her stomach contents [Yashiki *et al.* 1982].
Half-life The mean half-life is 2.07 h for healthy volunteers.
Clearance The mean clearance, for healthy males, is 0.34 L/h/kg.
Dose (As an anthelmintic agent), 7.5 to 10 mg/kg bodyweight every two weeks for three doses.

Abdi YA, Villen T (1991). Pharmacokinetics of metrifonate and its rearrangement product dichlorvos in whole blood. *Pharmacol Toxicol* 68(2): 137–139.
Ameno K *et al.* (1989). A rapid and sensitive quantitation of Dipterex in serum by solid-phase extraction and gas chromatography with flame thermionic detection. *J Anal Toxicol* 13: 150–151.
Heinig R *et al.* (2000). Development, validation and application of assays to quantify metrifonate and 2,2-dichlorovinyl dimethylphosphate in human body fluids. *J Chromatogr Biomed Sci Appl* 741(2): 257–269.
Tarbah FA *et al.* (1998). *TIAFT Poster Session 4.*
Unni LK *et al.* (1992). High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma. *J Chromatogr* 573(1): 99–103.
Yashiki M *et al.* (1988). [Gas chromatographic and gas chromatographic-mass spectrometric determination of trichlorfon in biological fluids collected in a fatal Dipterex poisoning]. *Nippon Hoigaku Zasshi* 42: 94–98.
Yashiki M *et al.* (1982). *Nippon Hoigaku Zasshi* 36: 426–430.

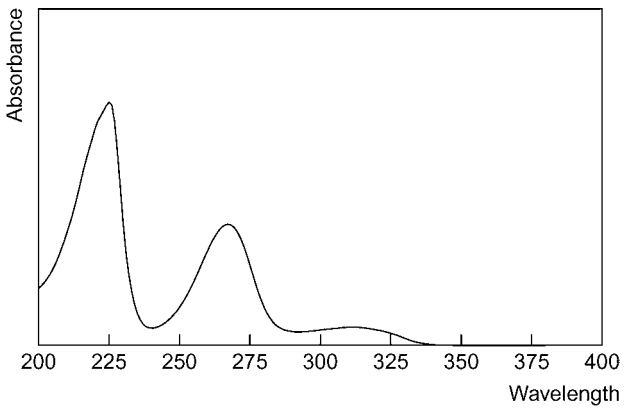
Trichlormethiazide

Diuretic
$C_8H_8Cl_3N_3O_4S_2$ = 380.7
CAS—133-67-5

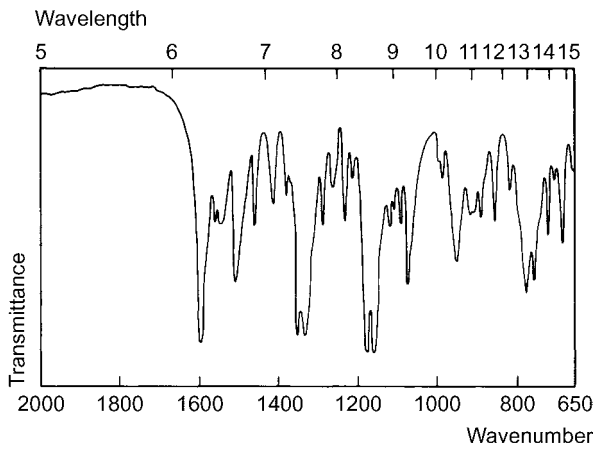
IUPAC Name 6-Chloro-3-(dichloromethyl)-1,1-dioxo-3,4-dihydro-2*H*-benzo[e][1,2,4]thiadiazine-7-sulfonamide
Synonyms 6-Chloro-3-(dichloromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; hydrotrichlorothiazide.
Proprietary Names *Diurese*; *Metahydrin*; *Naqua*. It is an ingredient of *Esmalorid*, *Metatensin* and *Rulun*.



Chemical Properties A white crystalline powder. Mp about 270°, with decomposition. Very slightly soluble in water, chloroform and ether; soluble 1 in 48 of ethanol, 1 in 17 of methanol, 1 in about 9 of dioxan and 1 in about 4 of dimethylformamide; freely soluble in acetone. pK_a 8.6. Log *P* (octanol/water) 0.6.
Colour Test Sulfuric acid—yellow.
Thin-layer Chromatography System TA—R_f 0.80; system TD—R_f 0.15; system TE—R_f 0.14; system TF—R_f 0.60; system TAD—R_f 0.23; system TAE—R_f 0.88; system TAJ—R_f 0.24; system TAK—R_f 0.05; system TAL—R_f 0.61 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—RI 2810; system GX—retention time 10.7 min (both for trichlormethiazide-ME_4).
High Performance Liquid Chromatography System HN—*k* 3.10; system HY—RI 341; system HAA—retention time 14.9 min.
Ultraviolet Spectrum Principal peaks at 225, 267, 314 nm.



Infrared Spectrum Principal peaks at wavenumbers 1158, 1177, 1597, 772, 1073, 1509 cm^{-1} (KBr disk).



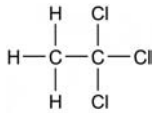
T

Mass Spectrum Principal ions at *m/z* 296, 298, 279, 205, 36, 64, 117, 62.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 10 µg/L [Meyer, Hwang 1981].
Urine **HPLC** See Plasma [Meyer and Hwang 1981].
Disposition in the Body Readily absorbed after oral administration. About 70% of a dose is excreted in the urine unchanged in 48 h, mostly in the first 8 h.
Therapeutic Concentration
Following a single oral dose of 4 mg to 7 subjects, peak plasma concentrations of 0.027 to 0.067 mg/L (mean, 0.04) were attained in 1 to 3.5 h (mean, 2) [Sketris *et al.* 1981].
Half-life Plasma half-life, about 1 to 4 h (mean, 2), increased in renal impairment.
Dose Usually 2 to 4 mg daily; initial doses of 4 to 8 mg daily may be given.

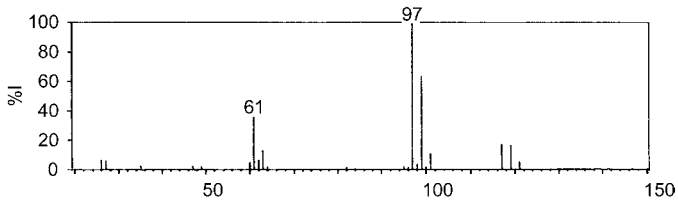
Meyer MC, Hwang PTR (1981). Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography. *J Chromatogr* 223: 466–472.
Sketris IS *et al.* (1981). The pharmacokinetics of trichlormethiazide in hypertensive patients with normal and compromised renal function. *Eur J Clin Pharmacol* 20: 453–457.

Trichloroethane

Solvent
CCl_3CH_3 = 133.4
CAS—71-55-6
IUPAC Name 1,1,1-Trichloroethane
Synonyms Chlorothene; methylchloroform; α-trichloroethane.
Proprietary Name *Zoff*



Chemical Properties A colourless non-flammable liquid. Mp −32.5°. Bp 74°. Practically insoluble in water; miscible with ethanol, methanol, acetone, benzene, ether and chloroform. Log *P* (octanol/water) 2.5.
Colour Test Fujiwara test—red.
Gas Chromatography System GA—RI 634; system GI—retention time 8.2 min.
Mass Spectrum Principal ions at *m/z* 97, 99, 61, 117, 119, 63, 101, 62.

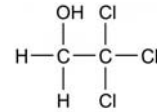


Quantification
Blood **GC** Headspace analysis and FID. For method, see Caplan *et al.* [1976].
Urine **GC** FID. For method, see Ghittori *et al.* [1987].
Bile **GC** See Blood [Caplan *et al.* 1976].
Tissues **GC** See Blood [Caplan *et al.* 1976].
Breath **GC–MS** Sensitivity, in ppb range [Thomas *et al.* 1991].
Disposition in the Body Rapidly absorbed from the gastrointestinal tract and the lungs; poorly absorbed through the skin. It is metabolised by oxidation and conjugation; only a small proportion of an absorbed dose is excreted in the urine as trichloroethanol glucuronide (urochloralic acid) and trichloroacetic acid. About 60 to 80% of a dose is excreted unchanged in expired air in one week.
Toxicity The estimated minimum lethal dose by ingestion or inhalation is 5 mL and the maximum permissible atmospheric concentration is 350 ppm. Exposure to air concentrations of more than 5000 ppm may cause unconsciousness within a short time. Fatalities have been associated with blood concentrations >15 mg/L.
The following postmortem tissue concentrations were reported in a fatality involving the accidental inhalation of trichloroethane: blood 18 mg/L, brain 80 µg/g, liver 80 µg/g, lungs 31 µg/g, urine 0.9 mg/L [Franc 1983].
A postmortem blood tetrachloroethane concentration of 1.7 mg/L was found following fatal cerebral oedema in a 15-year-old boy who had been sniffing typewriter correction fluid [D'Costa, Gunasekera 1990].
There was a broad correlation between blood tetrachloroethane concentration and severity of poisoning in 48 patients referred to a poisons unit (plus data from 18 other deaths); concentrations ranged from 0.1 to 60 mg/L [Meredith *et al.* 1989].
Half-life Blood half-life, about 10 to 12 h.

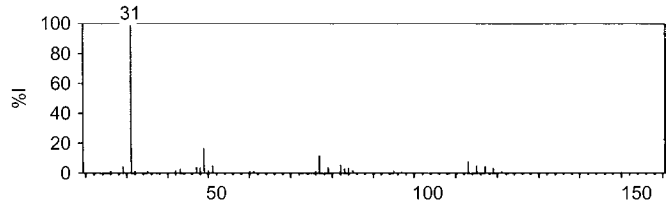
Caplan YH *et al.* (1976). 1,1,1-trichloroethane: report of a fatal intoxication. *Clin Toxicol* 9: 69–74.
D'Costa DF, Gunasekera NPR (1990). Fatal cerebral oedema following trichloroethane abuse. *J R Soc Med* 83: 533–534.
Franc A (1983). *Bull Int Assoc Forensic Toxicol* 17(2): 22–25.
Ghittori S *et al.* (1987). [Use of gas chromatography with flame ionization (GC-FID) in the measurement of solvents in the urine]. *G Ital Med Lav* 9: 21–24.
Meredith TJ *et al.* (1989). Diagnosis and treatment of acute poisoning with volatile substances. *Hum Toxicol* 8: 277–286.
Thomas KW *et al.* (1991). A canister-based method for collection and GC/MS analysis of volatile organic compounds in human breath. *J Anal Toxicol* 15: 54–59.

Trichloroethanol

Hypnotic
$C_2H_3Cl_3O$ = 149.4
CAS—115-20-8
IUPAC Name 2,2,2-Trichloroethanol
Synonym 2,2,2-Trichloroethyl alcohol



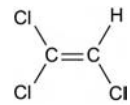
Chemical Properties A hygroscopic liquid or crystals. Mp 18°. Bp 151° to 153°. Soluble 1 in about 12 of water; miscible with ethanol and ether. pK_a 12.2 (25°). Log *P* (octanol/water) 1.4.
Colour Test Fujiwara test—yellow.
Gas Chromatography System GA—RI 857; system GI—retention time 25.5 min.
Mass Spectrum Principal ions at *m/z* 31, 49, 77, 113, 115, 82, 51, 117.



Quantification See under Chloral Hydrate.
Disposition in the Body Trichloroethanol is a metabolite of cloral hydrate, trichloroethane, trichloroethylene and triclofos; it has hypnotic activity. It is further metabolised by conjugation with glucuronic acid to form urochloralic acid and by oxidation to trichloroacetic acid.
Toxicity See under Chloral Hydrate.
Half-life Plasma half-life, about 8 h.
Volume of Distribution About 0.6 L/kg.
Distribution in Blood Plasma : whole blood ratio, about 0.9.
Protein Binding About 35%.

Trichloroethylene

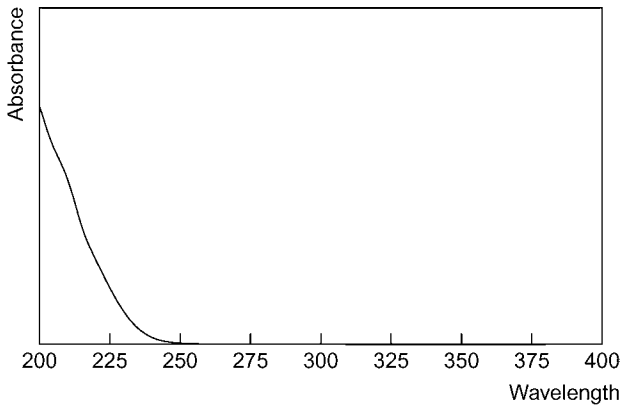
Anaesthetic (General), Solvent
C_2HCl_3 = 131.4
CAS—79-01-6
IUPAC Name 1,1,2-Trichloroethene
Synonyms Trichlorethylene; trichloroethene.
Proprietary Name *Trilene.*



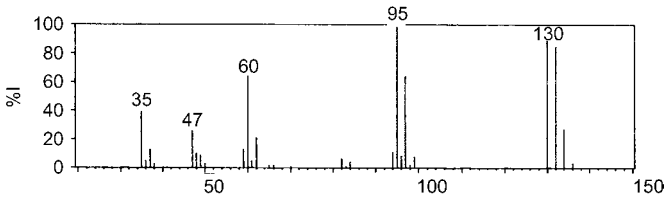
Chemical Properties A clear, colourless or pale blue, mobile, non-flammable liquid. It decomposes in light in the presence of moisture with the formation of hydrochloric acid. Relative density 1.464 to 1.470. Bp 87°. Trichloroethylene (*B.P.*) contains thymol 0.01% w/w as a preservative and may contain not more than 0.001% w/w of a suitable blue colouring matter to distinguish it from chloroform. Practically insoluble in water; miscible with dehydrated alcohol, chloroform and ether. Log *P* (octanol/water) 2.4.
Colour Test Fujiwara test—red.
Gas Chromatography System GA—RI 710; system GI—retention time 14.8 min.

T

Ultraviolet Spectrum



Mass Spectrum Principal ions at *m/z* 95, 130, 132, 60, 97, 35, 134, 47; trichloroacetic acid 44, 83, 85, 36, 28.



Quantification

Blood **GC** ECD using headspace analysis. Trichloroethanol and trichloroacetic acid. Limit of detection, 500 µg/L for trichloroethanol [Breimer *et al.* 1974]. FID using headspace analysis. For method, see Prior [1972].

Urine **GC** Headspace analysis. Trichloroethanol and trichloroacetic acid. Limit of detection, 5 µg/L for trichloroethanol [Ohara *et al.* 1991]. See Blood [Breimer *et al.* 1974].

GC–MS Headspace analysis. For a method for the quantification of trichloroethylene, see Imbriani *et al.* [2001].

HPLC Limit of detection, 0.5 µg for trichloroacetic acid [Ogata, Yamazaki 1979].

Disposition in the Body Rapidly absorbed from the lungs; about 50 to 65% of an inhaled dose is retained. Absorbed from the gastrointestinal tract and through the skin. It is highly soluble in lipid-rich tissues, from which it is slowly released. Cloral hydrate is a transient metabolite which is further metabolised by reduction to trichloroethanol and oxidation to trichloroacetic acid. Trichloroethanol is mainly excreted as its glucuronide conjugate (urochloralic acid). Monochloroacetic acid is a minor metabolite. About 45% of an absorbed dose is excreted as urochloralic acid and about 30% as trichloroacetic acid in the urine in 3 weeks; small amounts are eliminated in the faeces. About 16% is excreted unchanged in expired air.

Therapeutic Concentration In blood, for anaesthesia, usually in the range 26 to 82 mg/L.

During anaesthesia, exposure to an induction level of 1% of trichloroethylene for 30 min to 20 patients, produced blood concentrations of 34 to 90 mg/L (mean, 65) [Prior 1972].

Toxicity The estimated minimum lethal dose by ingestion or inhalation is 5 mL and the maximum permissible atmospheric concentration is 100 ppm.

A man aged 56 years was found dead after exposure to trichloroethylene fumes. The following postmortem concentrations of trichloroethylene were reported: blood about 90 mg/L, brain about 270 µg/g, lung about 40 µg/g [Alha *et al.* 1974].

In 19 cases of fatal trichloroethylene poisoning, the following postmortem concentrations were reported: blood 1 to 41 mg/mL (mean, 14, 15 cases), brain 4 to 74 µg/g (mean, 31, 5 cases), kidney 4 to 140 µg/g (mean, 30, 8 cases), liver 1 to 250 µg/g (mean, 64, 9 cases), lung 1 to 15 µg/g (mean, 7, 6 cases), urine a trace to 91 mg/L (mean, 35, 5 cases) [Bonnichsen, Maehly 1966].

A 17-year-old male ingested about 70 mL of trichloroethylene in a suicide attempt. The highest blood concentration of trichloroethylene (4.05 mg/L) was reached at 13 h after ingestion [Brüning *et al.* 1998].

Half-life About 30 to 38 h.

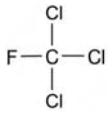
Dose To produce light anaesthesia, 0.5 to 2% of the vapour by inhalation.

Alha A *et al.* (1974). *Bull Int Assoc Forensic Toxicol* 10(1): 14–15.
Bonnichsen R, Maehly AC (1966). Poisoning by volatile compounds. II. Chlorinated aliphatic hydrocarbons. *J Forensic Sci* 11: 414–427.
Breimer DD *et al.* (1974). *J Chromatogr* 88: 55–63.
Brüning T *et al.* (1998). Acute intoxication with trichloroethene: clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. *Toxicol Sci* 41: 157–165.
Imbriani M *et al.* (2001). Trichloroethylene in urine as biological exposure index. *Ind Health* 39: 225–230.
Ogata M, Yamazaki Y (1979). Quantitative determination of urinary trichloroacetic acid as an index of trichloroethylene exposure by high performance liquid chromatography. *Acta Med Okayama* 33: 479–81.
Ohara A *et al.* (1991). Determination of trichloroacetic acid and trichloroethanol by head-space gas chromatography (HS.GC). *Sangyo Igaku* 33: 94–103.
Prior FN (1972). Blood levels of trichlorethylene during major surgery. A gas chromatographic study. *Anaesthesia* 27: 379–389.

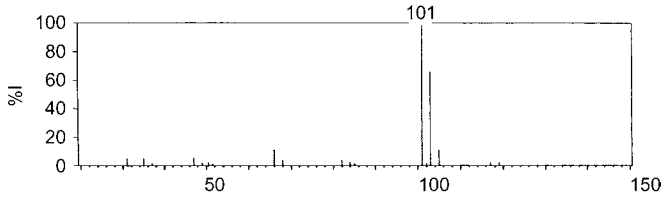
Trichlorofluoromethane

Aerosol Propellent, Refrigerant
$CCl_3F = 137.4$
CAS—75-69-4

IUPAC Name Trichloro(fluoro)methane
Synonyms Fluorocarbon 11; fluorotrichloromethane; propellent 11; refrigerant 11; trichloromonofluoromethane.
Proprietary Name It was formerly an ingredient of *PR Spray*.



Chemical Properties A clear, colourless, non-flammable volatile liquid. Bp about 24°. In the liquid state it is practically immiscible with water but miscible with dehydrated alcohol, ether and other organic solvents. Log *P* (octanol/water) 2.5.
Gas Chromatography System GA—RI 484; system GI—retention time 3.0 min.
Mass Spectrum Principal ions at *m/z* 101, 103, 105, 66, 47, 35, 31, 82.



Disposition in the Body

Toxicity

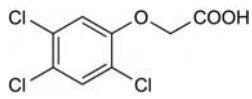
A 33-year-old man, working in an expanded polyurethane factory where trichlorofluoromethane was used as a foaming agent, was found dead on the floor in a 4 m^2 room. Postmortem examination revealed the following concentrations: blood, 62.8 mg/L; brain 108.9 mg/kg; lung, 149.1 mg/kg; liver 74.1 mg/kg and <0.1 mg/L in urine [Groppi *et al.* 1994].

Groppi A *et al.* (1994). A fatal case of trichlorofluoromethane (Freon 11) poisoning. Tissue distribution study by gas chromatography-mass spectrometry. *J Forensic Sci* 39: 871–876.

Trichlorophenoxyacetic Acid

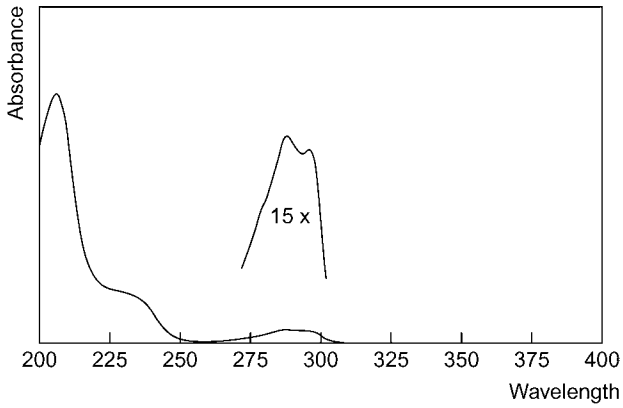
Herbicide
$C_8H_5Cl_3O_3 = 255.5$
CAS—93-76-5;

IUPAC Name (2,4,5-Trichlorophenoxy)acetic acid
Synonym 2,4,5-T
Proprietary Names *Boots Nettle Killer*; *Marks Brushwood Killer*; *Phortox*; *Silvapron T*; *Trioxone 50*. It is an ingredient of *Econal*, *Nettle Ban*, *Spontox* and *Stancide BWK 75*.



Chemical Properties A white crystalline solid. Mp 153°. Very slightly soluble in water; soluble in ethanol, acetone and ether. pK_a 2.8 (25°). Log *P* (octanol/water), 3.3.
Colour Test Liebermann's reagent—pink→brown.
Thin-layer Chromatography System TAB—R_f 0.04; system TAC—R_f 0.02.
Gas Chromatography System GA—2,4,5-T RI 1850, 2,4,5-T-methyl ester RI 1750, 2,4,5-T-isobutyl ester RI 2280, 2,4,5-T-isopropyl ester RI 1825, 2,4,5-T-isooctil ester RI 2320, M (trichlorophenol) RI 1440; system GK—2,4,5-T-methyl ester RRT 0.80; 2,4,5-T-isooctil ester RRT 1.24 and RRT 1.31 (both relative to caffeine).
Ultraviolet Spectrum Principal peaks at 206, 236, 289 nm.

T

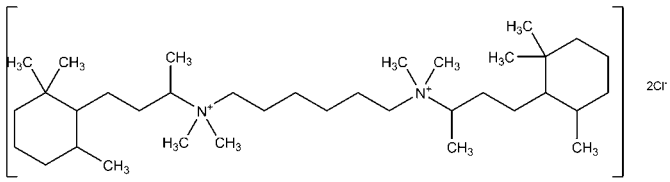


Mass Spectrum Principal ions at *m/z* 233, 45, 42, 59, 179, 146, 109, 235 (methyl ester); 43, 57, 41, 55, 71, 69, 56, 70 (isooctil ester).

Triclobisonium Chloride

Antimicrobial, Antiseptic
$C_{36}H_{74}Cl_2N_2 = 605.9$
CAS—79-90-3

IUPAC Name 6-[Dimethyl-[4-(2,2,6-trimethylcyclohexyl)butan-2-yl]azaniumyl]hexyl-dimethyl-[4-(2,2,6-trimethylcyclohexyl)butan-2-yl]azanium dichloride
Synonyms *N,N′*-Bis[1-methyl-3-(2,2,6-trimethylcyclohexyl)propyl]-*N,N′*-dimethyl-1,6-hexanediamine bis(methochloride); hexamethylenebis[dimethyl[1-methyl-3(2,2,6-trimethylcyclohexyl)propyl]ammonium chloride]; Ro-5-0810/1; *N,N,N′,N′*-tetramethyl-*N,N′*-bis[1-methyl-3-(2,2,6-trimethylcyclohexyl)propyl]-1,6-hexanediaminium dichloride.



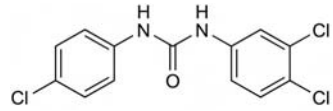
Chemical Properties White crystalline powder. Mp 243° to 253° with decomposition. Soluble in water, chloroform, and alcohol [O'Neil *et al.* 2006]. Insoluble in ether. Log *P* (octanol/water) 3.74 [Meylan, Howard 1995].
Colour Test Ammonium molybdate test—blue (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.01 (location reagent acidified iodoplatinate spray, positive reaction).
Disposition in the Body
Therapeutic Concentration A 0.1% ointment has been shown to be effective topically versus pyogenic dermatoses [Robinson, Harmon 1958], primary and secondary bacterial infections of the skin [Edelson *et al.* 1959], vaginitis [Demetry, Hansen 1960], and cevicitis [Fromhagen 1961], and after plastic surgery following third-degree burns [Georgiade *et al.* 1959]. Triclobisonium chloride has a marked activity against most skin pathogens, including *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus pyogenes* and *Bacillus proteus* [Borsanyi, Blanchard 1961; Gorosin, Blanchard 1961; Hunt, James 1960; Svenson 1960].
Dose Used topically as a 0.1% ointment.

Borsanyi SJ, Blanchard CL (1961). Triclobisonium chloride (Triburon) in ear infections. *Laryngoscope* 71: 1419–1425.
Demetry JP, Hansen RR (1960). Treatment of vaginitis with triclobisonium chloride. *Obstet Gynecol* 16: 189–193.
Edelson E *et al.* (1959). Clinical studies of the effectiveness of a new topical antimicrobial, triclobisonium chloride. *Ann NY Acad Sci* 82: 124–130.
Fromhagen C (1961). Triclobisonium chloride (Triburon) in the treatment of vaginitis and cervicitis. *J New Drugs* 1: 43–47.
Georgiade N *et al.* (1959). The use of triclobisonium chloride (Triburon) in plastic surgery. *Ann NY Acad Sci* 82: 161–168.
Gorosin R, Blanchard C (1961). Clinical trial with triclobisonium chloride in the treatment of *B. proteus* ear infections. A preliminary report. *Laryngoscope* 71: 78–81.
Hunt JA, James BM (1960). Triclobisonium chloride and triclobisonium-hydrocortisone ointments in dermatologic therapy. *Antibiotic Med Clin Ther* 7: 477–480.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.
Robinson RC, Harmon LE (1958). Local application of triclobisonium chloride in the treatment of pyogenic dermatoses. *Antibiot Annu* 6: 113–116.
Svenson SE (1960). Triclobisonium chloride (triburon), a topical microbicide. *Curr Ther Res Clin Exp* 2: 161–170.

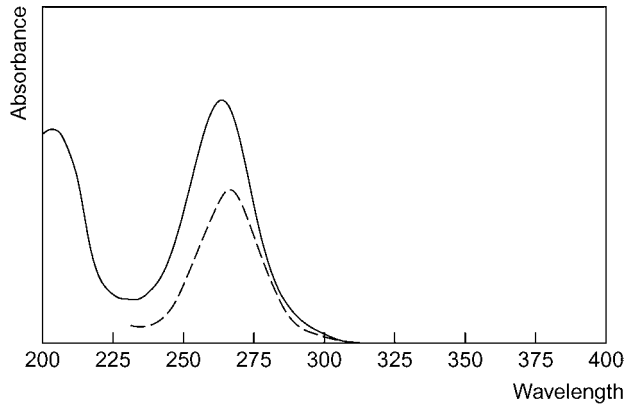
Triclocarban

Disinfectant, Antiseptic
$C_{13}H_9Cl_3N_2O = 315.6$
CAS—101-20-2

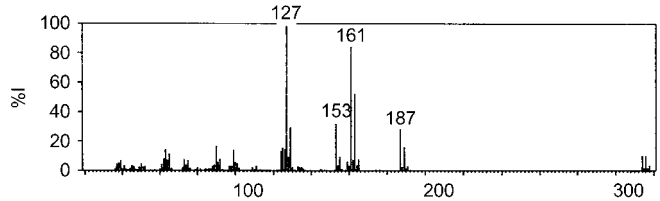
IUPAC Name 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)urea
Synonyms TCC; 3,4,4′-trichlorocarbanilide.
Proprietary Names *Citrosil Sapone*; *Cuticura*; *Cutisan*; *Derso TCC*; *Nobacter*; *Solubacter*; *Valderma*.



Chemical Properties Fine white plates. Mp 255° to 256°. Practically insoluble in water; soluble 1 in 25 of acetone and 1 in 100 of propylene glycol. Log *P* (octanol/water), 4.9.
Thin-layer Chromatography System TD—R_f 0.60; system TE—R_f 0.75; system TF—R_f 0.59; system TAD—R_f 0.63.
Ultraviolet Spectrum Ethanol—265 nm (A_1^1=1391 b).



Infrared Spectrum Principal peaks at wavenumbers 1634, 1587, 1550, 820, 1089, 1232 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 127, 161, 163, 153, 129, 187, 90, 189.



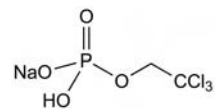
Quantification
Blood **GC** ECD. Limit of detection, 12.5 ng/mL [Hoar, Bowen 1977].
Plasma **GC–MS** Limit of detection, 1.5 ng/mL for 2′-hydroxy sulfate metabolite [Gruenke *et al.* 1997].
Urine **GC–MS** Limit of detection, 3 ng/mL for *N*-glucuronide in urine [Gruenke *et al.* 1997].
Use In concentrations of 1 to 2%.

Gruenke LD *et al.* (1997). *J Anal Toxicol* 11: 75–80.
Hoar DR, Bowen MH (1977). GLC determination of free triclocarban in blood. *J Pharm Sci* 66: 725–726.

Triclofos Sodium

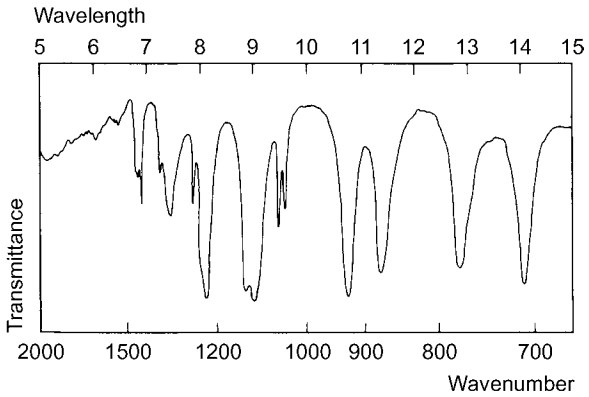
Sedative, Hypnotic
$C_2H_3Cl_3NaO_4P = 251.4$
CAS—306-52-5 (triclofos); 7246-20-0 (sodium salt)

IUPAC Name Sodium 2,2,2-trichloroethanol hydrogen phosphate
Proprietary Names *Triclonam*; *Tricloryl*; *Triclos*.

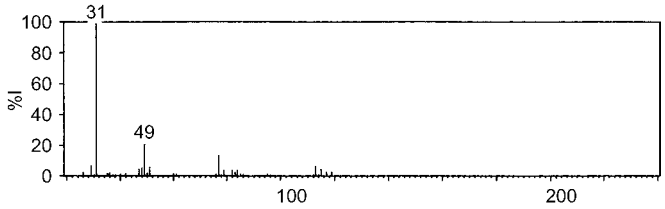


Chemical Properties A white hygroscopic powder. Soluble 1 in 2 of water and 1 in 250 of ethanol; practically insoluble in ether. Log *P* (octanol/water), 1.1.

Colour Test Phosphorus test—yellow precipitate.
Gas Chromatography System GB—RI 1952.
Infrared Spectrum Principal peaks at wavenumbers 1107, 1227, 926, 1124, 709, 876 cm^{-1} (triclofos sodium, (Nujol mull)).



Mass Spectrum Principal ions at *m/z* 31, 49, 77, 29, 113, 51, 48, 115, no peaks above 120 (triclofos); trichloroacetic acid 44, 83, 85, 36, 28; trichloroethanol 31, 49, 77, 113, 115, 82, 51, 117.



Quantification Determination of trichloroethanol – see under Chloral Hydrate.
Disposition in the Body Rapidly hydrolysed in the stomach to trichloroethanol which is readily absorbed. Trichloroethanol, the active metabolite, is conjugated with glucuronic acid to give urochloralic acid, and oxidised to trichloroacetic acid. About 12% of a dose is excreted in the urine in 24 h as metabolites.
Therapeutic Concentration In plasma, trichloroethanol, usually in the range 1.5 to 15 mg/L.

After an oral dose of 22 mg/kg given to 7 subjects, peak plasma concentrations of trichloroethanol and urochloralic acid of about 8 mg/L and 2 mg/L, respectively, were reported; no unchanged triclofos was detectable in plasma [Sellers *et al.* 1973].

Toxicity Plasma concentrations >40 mg/L of trichloroethanol are likely to produce toxic effects, and concentrations >100 mg/L may be fatal.

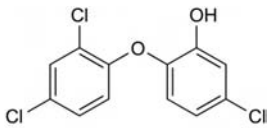
The following postmortem tissue concentrations of trichloroethanol were reported in a fatality due to triclofos ingestion: blood 335 µg/mL, bile 696 mg/L, brain 326 µg/g, kidney 928 µg/g, liver 690 µg/g, urine 195 mg/L; other drugs were also detected [Doedens, Benz 1976].

Half-life Plasma half-life, trichloroethanol about 8 h, urochloralic acid about 7 h, trichloroacetic acid about 4 days.
Volume of Distribution Trichloroethanol, about 0.6 L/kg.
Distribution in Blood Plasma : whole blood ratio, trichloroethanol about 0.9.
Protein Binding Trichloroethanol about 35% and trichloroacetic acid about 94%.
Dose 1 to 2 g, as a hypnotic.

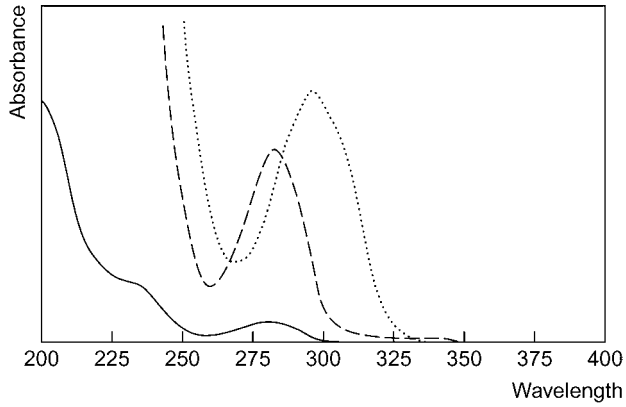
Doedens DJ, Benz JA (1976). *Bull Int Assn Forens Toxicol* 12(2): 10–11.
Sellers EM *et al.* (1973). *Clin Pharmacol Ther* 14147.

Triclosan

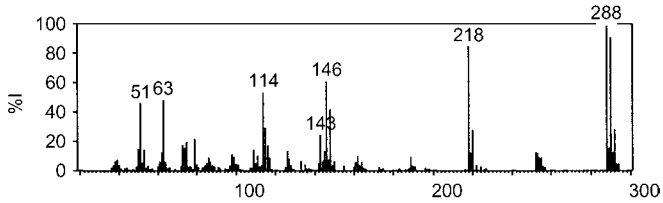
Disinfectant
$C_{12}H_7Cl_3O_2$ = 289.5
CAS—3380-34-5
IUPAC Name 5-Chloro-2-(2,4-dichlorophenoxy)phenol
Synonym Cloxifenol
Proprietary Names *Acneclear*; *Adasept*; *Aquasept*; *Bioband*; *Dermax*; *Dermo-Steril*; *Fisohex*; *Gamophen*; *Geroderm*; *Irgasan DP300*; *Novaderm*; *Procoutol*; *Proderm*; *Sapoderm*; *Septisol*; *Solyptol*; *Ster-Zac*; *Stri-Dex*; *Tersaseptic*; *Zalclense*.



Chemical Properties A white crystalline powder or soft agglomerates. Mp 54° to 57°. Practically insoluble in water; very soluble in most organic solvents. pK_a 7.9. Log *P* (octanol/water), 4.8.
Ultraviolet Spectrum Ethanol—283 nm (A^{1}_{1}=179 b); alkaline ethanol—295 nm.



Mass Spectrum Principal ions at *m/z* 288, 290, 218, 146, 114, 63, 51, 148.



Quantification
Plasma **GC** ECD. Limit of detection, 2 µg/L [Sioufi *et al.* 1977].
Urine **GC** See Plasma [Sioufi *et al.* 1977].
Disposition in the Body
Therapeutic Concentration

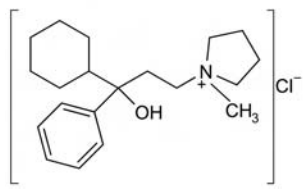
In a single dose study, subjects brushed one time with 1.25 g dentifrice containing 0.3% triclosan (3.75 mg) and ingested all of the dentifrice. Blood samples were collected at multiple time points from pre-dose to 72 h post-dose. In a multiple dose study, the same subjects brushed 3 times daily as in the single dose study. After 12 days of 3 times daily toothbrushing and ingestion of the dental slurry, the mean triclosan plasma concentration was 352 µg/L in the steady state period. There was no significant difference between results obtained in the single dose study, suggesting a complete elimination of daily triclosan dose and no increase in the triclosan level during repeated brushing/ingestion [Bagley, Lin 2000].

Use In concentrations of 0.05 to 2%.

Bagley DM, Lin YJ (2000). Clinical evidence for the lack of triclosan accumulation from daily use in dentifrices. *Am J Dent* 13: 148–152.
Sioufi A *et al.* (1977). GLC determination of free and conjugated triclosan in human plasma and urine. *J Pharm Sci* 66: 1166–1168.

Tricyclamol Chloride

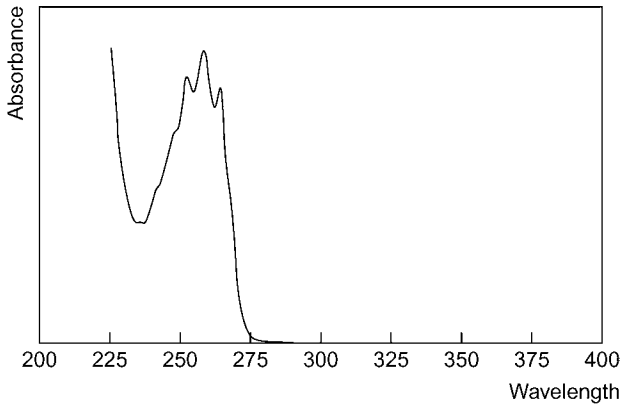
Anticholinergic
$C_{20}H_{32}ClNO$ = 337.9
CAS—3818-88-0
IUPAC Name 1-Cyclohexyl-3-(1-methylpyrrolidin-1-ium-1-yl)-1-phenylpropan-1-ol chloride
Synonym 1-(3-Cyclohexyl-3-hydroxy-3-phenylpropyl)-1-methylpyrrolidinium chloride



Chemical Properties A white crystalline powder. Mp 165° to 168°. Soluble 1 in 3 of water, 1 in 3 of ethanol and 1 in 25 of chloroform; practically insoluble in ether.
Colour Tests Mandelin's test—grey-violet; Marquis test—violet.
Thin-layer Chromatography System TA—R_f 0.06. (acidified iodoplatinate solution, positive).

T

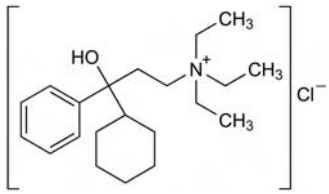
Ultraviolet Spectrum Aqueous acid—251, 257 (A_1^1=5.7 a), 263 nm. No alkaline shift.



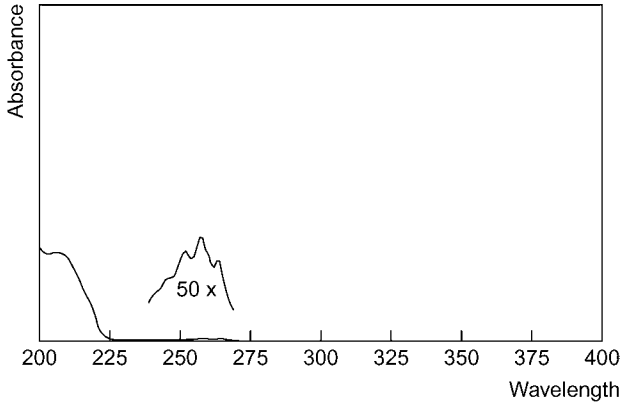
Infrared Spectrum Principal peaks at wavenumbers 705, 920, 1115, 772, 1045, 1061 cm^{-1}(KBr disk).
Dose Tricyclamol chloride has been given in doses of 50 to 100 mg.

Tridihexethyl Chloride

Anticholinergic
$C_{21}H_{36}ClNO$ = 354.0
CAS—60-49-1 (tridihexethyl); 4310-35-4 (chloride)
IUPAC Name γ-Cyclohexyl-*N*,*N*,*N*-triethyl-γ-hydroxybenzenepropanaminium chloride
Proprietary Names *Pathilon*. It is an ingredient of *Milpath*.



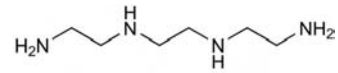
Chemical Properties A white crystalline powder. Mp 196° to 202°. Soluble 1 in 3 of water, 1 in 3 of ethanol and 1 in 2 of chloroform; practically insoluble in ether.
Colour Tests Mandelin's test—violet; Marquis test—brown-violet.
Thin-layer Chromatography System TA—R_f 0.07. (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—251, 257, 263 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 717, 1492, 710, 769, 1162, 729 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 86, 58, 88, 105, 206, 87, 55, 77.
Dose 75 to 300 mg daily.

Trientine

Copper Chelator
$C_6H_{18}N_4$ = 146.2
CAS—112-24-3
IUPAC Name *N*,*N*′-Bis(2-aminoethyl)ethane-1,2-diamine
Synonyms TECZA; TETA; trien; triethylenetetramine.



Chemical Properties An oily liquid. Mp 12°. Bp 266° to 267°. Soluble in water and alcohol, and is corrosive. pK_a 9.92 (20°). Log *P* (octanol/water), −2.65.

Trientine Dihydrochloride

$C_6H_{18}N_4$,2HCl = 219.2
CAS—38260-01-4
Synonyms MK-0681; TECZA; TETA; trien hydrochloride; trientine hydrochloride; triethylenetetramine dihydrochloride.
Proprietary Name *Syprine*
Chemical Properties A white to pale yellow crystalline powder. Mp 115° to 118°. Freely soluble in water; soluble in methyl alcohol; slightly soluble in alcohol; practically insoluble in chloroform and ether.

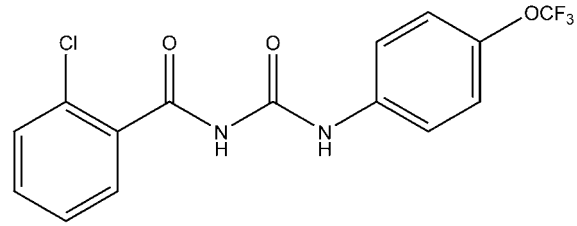
Quantification

Blood **HPLC** Column: Nucleosil 5-CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 140 mmol/L ammonium chloride (containing 48 mmol/L sodium benzene sulfonate, and 9.2 mmol/L acetic acid (pH 6, 27 : 73)), flow rate 0.5 mL/min. Fluorescence detection (λ_{ex}=380 nm, λ_{em}=485 nm). RT: 9.5 min. Limit of detection, 100 µg/L [Miyazaki *et al.* 1990].
Disposition in the Body Trientine is rapidly absorbed after oral administration. High concentrations of the metabolites are detected in the blood at the early stages following administration. It is widely distributed throughout the body. Urine and faeces are the major routes of elimination, with a large amount of metabolites being detected in urine. 1-*N*-acetyltriethylene tetramine (acetyltrien) has been identified in urine. This metabolite has chelating activity which is significantly lower than trientine. Both drug and metabolite are capable of combining with copper, iron and zinc. After oral administration, approx. 1.6% of an administered dose is detected in urine unchanged, and 8% as acetyltrien. The majority of the drug is excreted within the first 6 h, and the metabolite excreted over 26 h.
Bioavailability 90 to 95%.
Dose Adults: (US guidelines) the usual initial dose is 750 to 1250 mg daily which may be increased to a maximum of 2 g daily; (UK guidelines) 1.2 to 2.4 g daily is recommended. Children: the usual initial dose is 500 to 750 mg which may be increased to a maximum of 1.5 g daily if necessary.

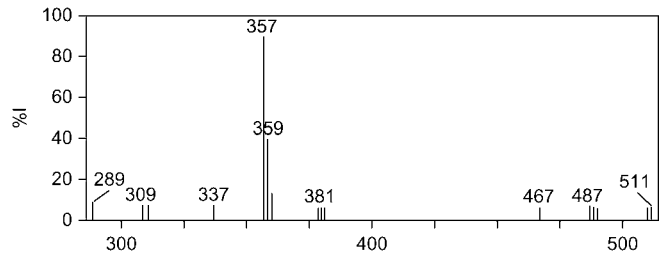
Miyazaki K *et al.* (1990). Determination of triethylenetetramine in plasma of patients by high-performance liquid chromatography. *Chem Pharm Bull* 38: 1035–1038.

Triflumuron

Benzoylurea, Insecticide
$C_{15}H_{10}ClF_3N_2O_3$ = 358.7
CAS—64628-44-0
IUPAC Name 2-Chloro-*N*-[[4-(trifluoromethoxy)phenyl]carbamoyl]benzamide
Synonyms BAY SIR-8514; *N*-(2-chlorobenzoyl)-*N*′-[4-(trifluoromethoxy)phenyl]urea, 2-chloro-*N*-[[[4-trifluoromethoxy)phenyl]amino]carbonyl]benzamide; SIR-8514; trifluron.
Proprietary Names *Alsystin*; *Baycidal*; *Starycide*.



Chemical Properties Crystals. Mp 198°. Slightly soluble in water (0.025 mg/L). Log *P* (octanol/water), 4.91 [Ticha *et al.* 2007].
Mass Spectrum Principal peaks at *m/z* 357, 359, 289, 309, 337, 487, 467, 381.



Quantification No data have been reported for triflumuron concentrations in human fluids or tissues.

***Other* HPLC** Tomatoes. Column: C_{18} (250 × 4.6 mm i.d., 5.0 µm). Mobile phase: methanol : water (80 : 20), flow rate 0.6 mL/min. UV detection (λ = 260 nm). Retention time: 11 min. Limit of quantification, 40 µg/L; limit of detection, 10 µg/L [Markoglou *et al.* 2007]. Column: Spherisorb C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : water (80 : 20), flow rate 0.4 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 410 nm). Retention time: ≈8 min. Limit of quantification, 50 µg/L; limit of detection, 13 µg/L [Martinez-Galera *et al.* 2001]. Grapes and Wine. Column: Hypersil C_{18} (250 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water (10 : 90 for 5 min, to 64 : 36 over 15 min, to 100 : 0 over 20 min), flow rate 0.3 mL/min. UV detection (λ = 260 nm). Retention time: ≈31 min. Limit of quantification, 4 to 5 µg/L [Miliadis *et al.* 1999]. Water. Column: Zorbax SB-C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water with orthophosphoric acid (pH 2.5; 90 : 10 for 5 min, to 10 : 90 over 30 min), flow rate 2 mL/min. Retention time: 27 min. Limit of detection, 6 µg/L [Dommarco *et al.* 1998].

LC-MS Apples. Column: C_{18} (150 × 3.0 mm i.d., 5 µm). Mobile phase: water : methanol (50 : 50 to 0 : 100 over 6 min for 11 min). ESI, positive ion mode, MRM acquisition mode. Limit of detection, 4 µg/kg [Ticha *et al.* 2007]. Tomatoes. Column: C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water (80 : 20), flow rate 0.6 mL/min. APCI, negative ion mode, SIM acquisition mode. Retention time: 11 min. Limit of quantification, 30 µg/L, limit of detection, 8 µg/L [Markoglou *et al.* 2007].

Dose Used in agriculture and as a topical ectoparasiticide in veterinary practice.

Dommarco R *et al.* (1998). Simultaneous quantitative determination of thirteen urea pesticides at sub-ppb levels on a Zorbax SB-C18 column. *J Chromatogr A* 825: 200–204.

Markoglou AN *et al.* (2007). Determination of benzoylurea insecticide residues in tomatoes by high-performance liquid chromatography with ultraviolet-diode array and atmospheric pressure chemical ionization-mass spectrometry detection. *J AOAC Int* 90: 1395–1401.

Martinez-Galera M *et al.* (2001). Determination of benzoylureas in tomato by high-performance liquid chromatography using continuous on-line post-elution photoirradiation with fluorescence detection. *J Chromatogr A* 918: 79–85.

Miliadis GE *et al.* (1999). High-performance liquid chromatographic determination of benzoylurea insecticides residues in grapes and wine using liquid and solid-phase extraction. *J Chromatogr A* 835: 113–120.

Ticha J *et al.* (2007). Safe apples for baby-food production: survey of pesticide treatment regimes leaving minimum residues. *Food Addit Contam* 24: 605–620.

Trifluomeprazine

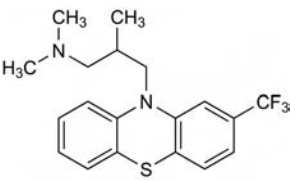
Tranquilliser (Veterinary)

$C_{19}H_{21}F_3N_2S$ = 366.4

CAS—2622-37-9

IUPAC Name *N*,*N*,2-Trimethyl-3-[2-(trifluoromethyl)phenothiazin-10-yl]propan-1-amine

Synonyms Triflutrimeprazine; *N*,*N*,β-trimethyl-2-(trifluoromethyl)-10*H*-phenothiazine-10-propanamine.



Trifluomeprazine Maleate

$C_{19}H_{21}F_3N_2S,C_4H_4O_4$ = 482.5

Proprietary Name *Nortran*

Chemical Properties A white crystalline powder. Mp about 178°. Very slightly soluble in water; soluble 1 in 25 of ethanol and 1 in 25 of chloroform; practically insoluble in ether.

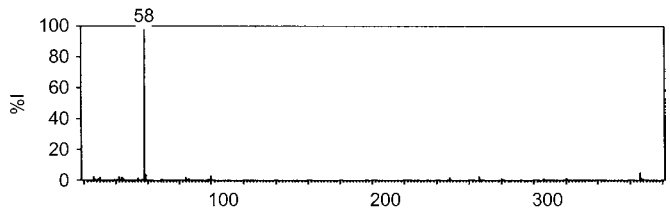
Colour Tests Mandelin's test—green→red-violet; Marquis test—red-violet; sulfuric acid—red-violet.

Thin-layer Chromatography System TA—R_f 0.65; system TB—R_f 0.60; system TC—R_f 0.58; system TL—R_f 0.52 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2250.

Ultraviolet Spectrum Aqueous acid—255 (A^1_1=803 a), 305 nm, less well-defined.

Mass Spectrum Principal ions at *m/z* 58, 366, 59, 100, 266, 248, 84, 44.



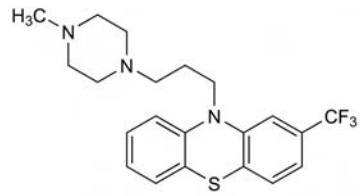
Trifluoperazine

Neuroleptic, Phenothiazine, Tranquilliser

$C_{21}H_{24}F_3N_3S$ = 407.5

CAS—117-89-5

IUPAC Name 10-[3-(4-Methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-10*H*-phenothiazine



Chemical Properties Serum samples were stable for up to 8 h at room temperature and up to 2 weeks when stored at 4° and –20° [Tanaka *et al.* 2007]. Standard solutions were stable for up to 3 weeks when kept at –16° in the dark [Javaid *et al* 1982]. Plasma samples were stable for 3 weeks at -4° [Midha *et al.* 1982]. Plasma samples were stable for several days when stored at 9° [Gillespie, Sipes 1981].

Trifluoperazine Hydrochloride

$C_{21}H_{24}F_3N_3S,2HCl$ = 480.4

CAS—440-17-5

Synonym Triphthazinum

Proprietary Names *Eskazine*; *Flupazine*; *Jatroneural*; *Modalina*; *Novoflurazine*; *Stelazine*; *Terfluzine*; *Triplex*. It is an ingredient of *Parstelin* and *Stelabid*.

Chemical Properties White to pale-yellow, hygroscopic, crystalline powder. Mp 242° to 243°. Soluble 1 in 2 of water, 1 in 11 of ethanol, and 1 in 100 of chloroform; practically insoluble in ether. pK_a 8.1 (24°). Log *P* (octanol/water), 5.0.

Colour Tests Formaldehyde-sulfuric acid—pink; Forrest reagent—orange; FPN reagent—orange; Liebermann's reagent—red; Mandelin's test—red-brown; Marquis test—red-violet.

Thin-layer Chromatography System TA—R_f 0.53; system TAE—R_f 0.30; system TAF—R_f 0.29; system TAG—R_f 0.08; system TB—R_f 0.33; system TC—R_f 0.30; system TE—R_f 0.55 (Dragendorff spray, positive; FPN reagent, pink; acidified iodoplatinate solution, positive; Marquis reagent, pink).

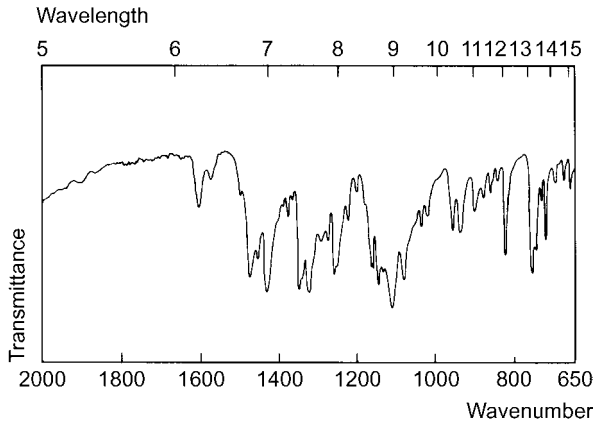
Gas Chromatography System GA—trifluoperazine RI 2683; M (ring) RI 2190; M (sulfoxide) RI 2990; system GB—trifluoperazine RI 2798 ; M (sulfoxide) RI 3145; M (norsulfoxide) RI 3191; system GF—RI 3050.

High Performance Liquid Chromatography System HA—*k* 3.0; system HAA—RT 17.7 min; system HX—RI 480; system HY—RI 344; system HZ—RT 13.2 min.

Column: Discovery C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 575 mg ammonium formate with 1.3 mL formic acid in 500 mL water : acetonitrile (60 : 40 for 3 min to 20 : 80 at 11 min to 0 : 100 at 12 min to 60 : 40 at 13 min for 2 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 378 nm, λ_{em}= 502 nm at 0.01 min to λ_{ex}= 344 nm; λ_{em}= 380 nm at 4.75 min to λ_{ex}= 351 nm, λ_{em}= 436 nm at 8.35 min to λ_{ex}= 344 nm, λ_{em}= 389 nm at 10 min; λ_{ex}= 344 nm, λ_{em}= 379 nm). Limit of detection, 4 nmol/L [Diehl, Karst 2000].

Ultraviolet Spectrum Aqueous acid—256 (A^1_1 = 743a), 305 nm; aqueous alkali—258, 308 nm.

Infrared Spectrum Principal peaks at wavenumbers 1114, 1316, 1145, 1081, 755, 1255 cm^{-1} (trifluoperazine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 113, 70, 407, 43, 141, 42, 127, 71.

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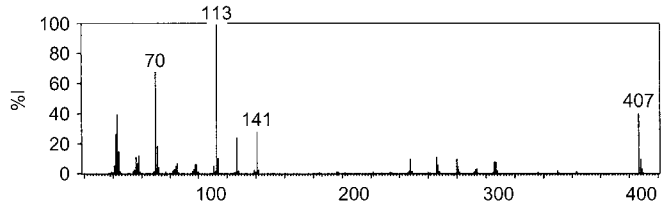


Quantification

Plasma **GC** Column: 3% OV-101 on 80/100 mesh (0.9 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 250°. NPD. Limit of detection, 0.2 µg/L [Javaid *et al.* 1982]. Column: 3% phenylmethylsilicone 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 310°. NPD. Retention time: 0.89 min. Limit of detection, 0.5 µg/L [Roscoe *et al.* 1982]. Column: 3% OV-17 on Gas-Chrom Q, 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 260°. NPD. Limit of detection, 0.1 µg/L [Gillespie, Sipes 1981].

GC-MS Column: 3% OV-1 dimethylchlorosilane Gas Chrom Q on 100/120 mesh (1.22 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 280°. EI ionisation, SIR acquisition mode. Retention time: 1 min 17 s. Limit of quantification, 78 ng/L [Midha *et al.* 1982]. Column: 3% OV-225 on 80/100 Chromosorb W HP (1 m × 4 mm i.d.). Carrier gas: He, 20 mL/min. Temperature: 245°. SIM acquisition mode. Limit of detection, 0.2 µg/L [Whelpton *et al.* 1982].

HPLC Column: SymmetryShield RP8 (150 × 2.1 mm i.d., 3.5 µm). Mobile phase: 10 mmol/L ammonium formate and 0.1% formic acid : acetonitrile (70 : 30 to 0 : 100 at 15 min), flow rate 0.2 mL/min. UV detection (λ= 250 nm). Limit of detection, 0.021 mg/L [Marumo *et al.* 2005].

Serum **HPLC** Column: Inersil ODS-SP C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol: 30 mmol/L sodium dihydrogen phosphate (pH 5.6, 300 : 200 : 500), flow rate 0.9 mL/min. UV detection (λ= 250 nm). Retention time: 62.0 min. Limit of quantification, 5.2 µg/L [Tanaka *et al.* 2007].

Urine **GC** Column: 5% OV-1 on Diatoport S, 80/100 mesh or 2% FFAP on Diatoport S, 80/100 mesh. Carrier gas: N_2, 60–80 mL/min. Temperature: 230°. FID. Retention time: 6.9 and 13.6 min for each column, respectively. Limit of detection, 5–10 µg/L [De Leenheer 1974].

HPLC Column: LiChrosorb C_8 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : water : acetic acid : triethylamine (40 : 40 : 20 : 2), flow rate 0.5 mL/min. UV detection (λ= 250 nm). Limit of quantification, 112.2 µg/L, limit of detection, 33.6 µg/L [Cruz-Vera *et al.* 2009]. See Plasma. Limit of detection, 0.3 mg/L [Marumo *et al.* 2005].

Hair **GC** Column: AC-5 capillary (15 m × 0.25 mm i.d.). Temperature programme: 180° to 270° at 10°/min for 10 min. NPD. Limit of detection not reported [Shen *et al.* 2002].

LC-MS Column: Luna C_{18} (15 cm × 4.6 mm i.d.). Mobile phase: 0.02 mol/L ammonium acetate and 0.1% acetic acid in water : acetonitrile (35 : 65), flow rate 0.5 mL/min. ESI. Limit of detection, 60 nmol/L [McClean *et al.* 2000].

Note For a radioimmunoassay method for the detection of trifluoperazine see Midha *et al.* [1981].

Disposition in the Body Absorbed after oral administration. About 1% of a daily dose is excreted in the 24 h urine as unchanged drug and up to 6% of a dose as the sulfoxide [West *et al.* 1974]. Metabolism to 7-hydroxytrifluoperazine and desmethyltrifluoperazine has been reported.

Therapeutic Concentration

After a single oral dose of 5 mg to 5 healthy subjects, peak plasma concentrations of 0.5–3.0 µg/L (mean, 1.4 µg/L) were reached between 1–4 h. [Midha *et al.* 1983].

After a single oral dose of 20 mg to 4 subjects, peak plasma concentrations of 0.001–0.004 µg/mL (mean 0.002) were attained in 3–6 h [Gillespie, Sipes 1981].

After daily oral doses of 80 mg to 1 subject, a peak plasma concentration of 0.028 µg/mL was reported 8 h after a dose [Curry *et al.* 1981].

Toxicity

Postmortem blood and liver concentrations of 0.06 µg/mL and 0.31 µg/g respectively were reported in a woman who died of suspected trifluoperazine overdose whilst receiving daily oral doses of 40 mg [Baselt 2005].

The following postmortem concentrations were reported in a fatality attributed to trifluoperazine ingestion: blood 0.4 µg/mL, kidney 83 µg/g, liver 198 µg/g; 7-hydroxytrifluoperazine, kidney 61 µg/g, liver 161 µg/g; desmethyltrifluoperazine, kidney 89 µg/g, liver 205 µg/g [Quai *et al.* 1985].

Half-life Plasma half-life, 7–18 h (mean 12).

Dose For psychoses, the equivalent of 10 to 20 mg of trifluoperazine daily; more than 40 mg daily has been given.

Baselt RC (2005). *Disposition of Toxic Drugs and Chemicals in Man*, 7th edn. Foster City, CA: Chemical Toxicology Institute.

Cruz-Vera M *et al.* (2009). Determination of phenothiazine derivatives in human urine by using ionic liquid-based dynamic liquid-phase microextraction coupled with liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 37–42.

Curry SH *et al.* (1981). Plasma-trifluoperazine concentrations during high dose therapy. *Lancet* 1: 395–396.

DeLeenheer AP (1974). Identification and quantitative determination of phenothiazine drugs in urine samples of psychiatric patients. *J Pharm Sci* 63: 389–394.

Diehl G, Karst U (2000). Post-column oxidative derivatization for the liquid chromatographic determination of phenothiazines. *J Chromatogr A* 890: 281–287.

Gillespie TJ, Sipes IG (1981). Sensitive gas chromatographic determination of trifluoperazine in human plasma. *J Chromatogr* 223: 95–102.

Javaid JI *et al.* (1982). GLC analysis of trifluoperazine in human plasma. *J Pharm Sci* 71: 63–66.

Marumo A *et al.* (2005). Analysis of phenothiazines in human body fluids using disk solid-phase extraction and liquid chromatography. *J AOAC Int* 88: 1655–1660.

McClean S *et al.* (2000). Electrospray ionisation-mass spectrometric characterisation of selected anti-psychotic drugs and their detection and determination in human hair samples by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 740: 141–157.

Midha KK *et al.* (1981). Radioimmunoassay for trifluoperazine in human plasma. *Br J Clin Pharmacol* 12: 189–193.

Midha KK *et al.* (1982). A gas chromatographic mass spectrometric assay for plasma trifluoperazine concentrations following single doses. *Biomed Mass Spectrom* 9: 186–190.

Midha KK *et al.* (1983). Plasma concentrations of trifluoperazine following single low doses. *Can Med Assoc J* 129: 324.

Quai I *et al.* (1982). A GLC-nitrogen phosphorous detector assay for trifluoperazine in plasma. *J Pharm Sci* 71: 625–627.

Roscoe RM *et al.* (1982). A GLC-nitrogen phosphorous detector assay for trifluoperazine in plasma. *J Pharm Sci* 71: 625–627.

Shen M *et al.* (2002). Detection of antidepressant and antipsychotic drugs in human hair. *Forensic Sci Int* 126: 153–161.

Tanaka E *et al.* (2007). Simple and simultaneous determination for 12 phenothiazines in human serum by reversed-phase high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 854: 116–120.

West NR *et al.* (1974). Assay procedures for thioridazine, trifluoperazine, and their sulfoxides and determination of urinary excretion of these compounds in mental patients. *J Pharm Sci* 63: 417–420.

Whelpton R *et al.* (1982). Analysis of plasma trifluoperazine by gas chromatography and selected ion monitoring. *J Chromatogr* 228: 321–326.

Trifluoromethylphenylpiperazine

5-HT_1 Receptor Agonist, Stimulant

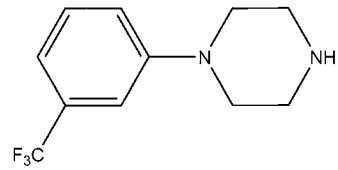
$C_{11}H_{13}F_3N_2$=230.2

CAS—15532-75-9

IUPAC Name 1-[3-Trifluoromethylphenyl]piperazine

Synonyms TFMPP; *m*-trifluoromethylphenylpiperazine.

Street Names TFMPP; Legal E; Legal X.



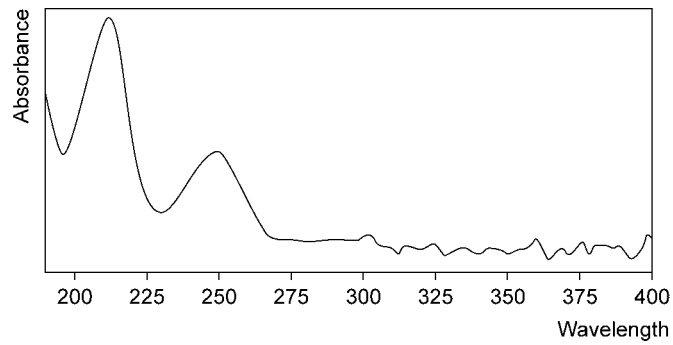
Chemical Properties Mp 89° to 92°. Bp 65° to 71°. pK_a 8.66 [Bishop *et al.* 2005].

Trifluoromethylphenylpiperazine Hydrochloride

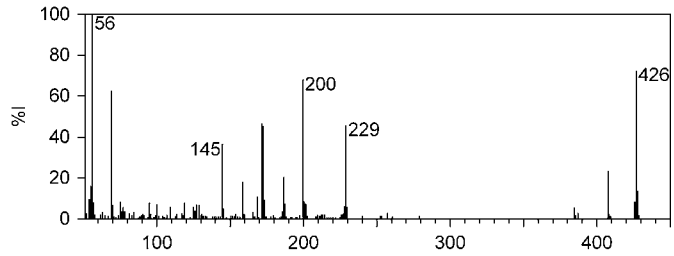
$C_{11}H_{13}F_3N_2$,HCl = 266.7

Chemical Property Solid

UV Spectrum Peaks at 220, 250 nm.



Mass Spectrum Principal ions at *m/z* 272, 229, 200, 188, 145, 172, 56 (AC derivative); 326, 229, 200, 187, 175, 56 (Trifluroacetic acid derivative); 426, 229, 200, 172, 145, 56 (HFBA derivative).



Quantification

Plasma **GC-MS** Column: HP-5MS capillary 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 250 nm). Carrier gas: He, 0.6 mL/min. Temperature programme: 100° to 250° at 10°/min to 310° at 30°/min for 1 min. EI ionisation, SIM acquisition mode. Limit of quantification, 5 µg/L, limit of detection, ~1 µg/L [Peters *et al.* 2003]. Column: HP-5MS (19 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 1 min to 300° at 15°/min for 5 min. EI ionisation at 70 eV. Limit of quantification not reported [de Boer *et al.* 2001].

Urine **GC-MS** Column: Fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 80° for 1 min to 250° at 10°/min. EI ionisation at 70 eV. Limit of detection, 100 µg/L for TFMPP and 500 µg/L for 4-hydroxy-TFMPP [Tsutsumi *et al.* 2005].

LC-MS Column: SCX (150 × 0.2 mm i.d.). Mobile phase: acetonitrile : 40 mmol/L ammonium acetate (pH 4; 75 : 25), flow rate 0.15 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 0.2 µg/L for TFMPP and 1 µg/L for 4-hydroxy-TFMPP [Tsutsumi *et al.* 2005].

Disposition in the Body TFMPP is metabolised by isoenzymes CYP2D6 (predominant catalyser), CYP1A2, and CYP3A4, forming *N*-(3-trifluoromethylphenyl)ethylenediamine, *N*-(hydroxyl-3-trifluoromethylphenyl)ethylenediamine, 3-trifluoromethylaniline and hydroxyl-3-trifluoromethylalanine. Subsequent glucuronidation, sulfatation and acetylation also occur.

Note TFMPP is a metabolite of antrafenine.

Toxicity At the time of writing (2007), there have been no published cases of fatal TFMPP toxicity. It has CNS activity, acting on the serotonergic system and may produce effects similar to MDMA. For further reading, see Maurer *et al.* [2004] and Murphy *et al.* [1991].

Bishop SC *et al.* (2005). Simultaneous separation of different types of amphetamine and piperazine designer drugs by capillary electrophoresis with a chiral selector. *J Forensic Sci* 50: 326–335.

de Boer D *et al.* (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Sci Int* 121: 47–56.

Maurer HH *et al.* (2004). Chemistry, pharmacology, toxicology, and hepatic metabolism of designer drugs of the amphetamine (ecstasy), piperazine, and pyrrolidinophenone types: a synopsis. *Ther Drug Monit* 26: 127–131.

Murphy DL *et al.* (1991). Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans. *Pharmacol Rev* 43: 527–552.

Peters FT *et al.* (2003). Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom* 38: 659–676.

Tsutsumi H *et al.* (2005). Development of simultaneous gas chromatography–mass spectrometric and liquid chromatography–electrospray ionization mass spectrometric determination method for the new designer drugs, *N*-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and their main metabolites in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 819: 315–322.

Trifluperidol

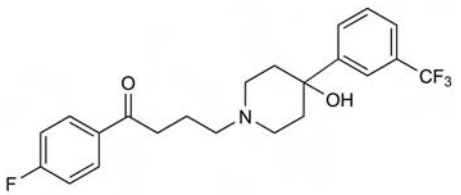
Butyrophenone, Tranquilliser

$C_{22}H_{23}F_4NO_2$ = 409.4

CAS—749-13-3

IUPAC Name 1-(4-Fluorophenyl)-4-[4-hydroxy-4-[3-(trifluoromethyl)phenyl]-1-piperidinyl]-1-butanone

Synonym Flumoperone



Chemical Properties White crystalline powder. Mp 93° to 95°. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Trifluperidol Hydrochloride

$C_{22}H_{23}F_4NO_2$,HCl = 445.9

CAS—2062-77-3

Proprietary Names *Psicoperidol*; *Triperidol*.

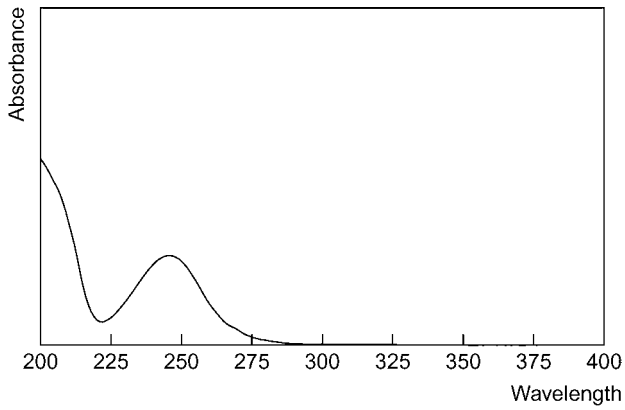
Chemical Properties White, amorphous or crystalline, flocculent powder which is stable in air but darkens slowly on exposure to light. Mp about 201°. Slightly soluble in water and chloroform; soluble in ethanol; practically insoluble in ether; freely soluble in methanol. Log *P* (octanol/water), 4.5.

Thin-layer Chromatography System TA—R_f 0.73; system TAD—R_f 0.26; system TAE—R_f 0.55; system TAF—R_f 0.76; system TB—R_f 0.13; system TD—R_f 0.02; system TE—R_f 0.77; system TF—R_f 0.05 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2675; M (N-desalkyl-oxo-2H_2O) RI 1570; M (N-desalkyl-) RI 1970.

High Performance Liquid Chromatography System HA—*k* 1.2; system HAA—RT 21.6 min; system HX—RI 459; system HY—RI 306.

Ultraviolet Spectrum Aqueous acid—248 nm (A^1_1 = 308a).



Infrared Spectrum Principal peaks at wavenumbers 1317, 1150, 1665, 1170, 1110, 1595 cm^{-1} (trifluperidol hydrochloride , KBr disk).

Mass Spectrum Principal ions at *m/z* 42, 271, 258, 123, 56, 83, 240, 95.

Quantification

Plasma **GC**Column: 3% OV-17 on Gas Chrom Q 100/120 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 270°. ECD. Limit of detection, 1 ng [Marcucci *et al.* 1971].

HPLC [Przyborowski, Misztal 1991].

Tissues **GC** See Plasma [Marcucci *et al.* 1971].

Dose Initially the equivalent of 500 µg of trifluperidol daily, increased to a maximum dose of 8 mg daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Marcucci F *et al.* (1971). A method for the gas chromatographic determination of butyrophenones. *J Chromatogr* 59: 174–177.

Przyborowski L, Misztal G (1991). [HPLC-determination of trifluperidol in blood plasma]. *Pharmazie* 46: 813–814.

Triflupromazine

Antiemetic, Antipsychotic, Phenothiazine, Tranquilliser

$C_{18}H_{19}F_3N_2S$ = 352.4

CAS—146-54-3

IUPAC Name *N,N*-Dimethyl-3-[2-(trifluoromethyl)phenothiazin-10-yl]propan-1-amine

Synonyms *N,N*-Dimethyl-2-(trifluoromethyl)-10*H*-phenothiazine-10-propanamine; fluopromazine.

Chemical Properties A pale-amber viscous oily liquid which forms large irregular crystals during prolonged storage. Practically insoluble in water. pK_a 9.2 (24°). Log *P* (octanol/water), 5.5. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Triflupromazine Hydrochloride

$C_{18}H_{19}F_3N_2S$,HCl = 388.9

CAS—1098-60-8

Proprietary Names *Psyquil*; *Siquil*; *Vesprin*.

Chemical Properties White to pale-tan crystalline powder. Mp 170° to 178°. Soluble 1 in less than 1 of water and of ethanol, and 1 in 1.7 of chloroform; soluble in acetone; practically insoluble in ether.

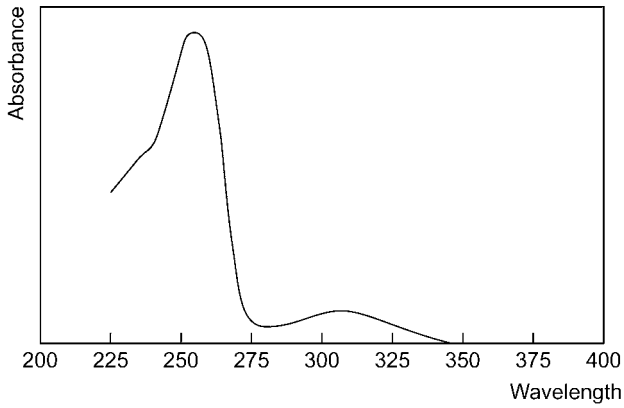
Colour Tests Formaldehyde–sulfuric acid—red; Forrest reagent—orange; FPN reagent—orange; Mandelin's test—red-brown; Marquis test—red-violet.

Thin-layer Chromatography System TA—R_f 0.54; system TB—R_f 0.47; system TC—R_f 0.35; system TE—R_f 0.75; system TL—R_f 0.22; system TAE—R_f 0.32; system TAF—R_f 0.49 (acidified iodoplatinate solution, positive).

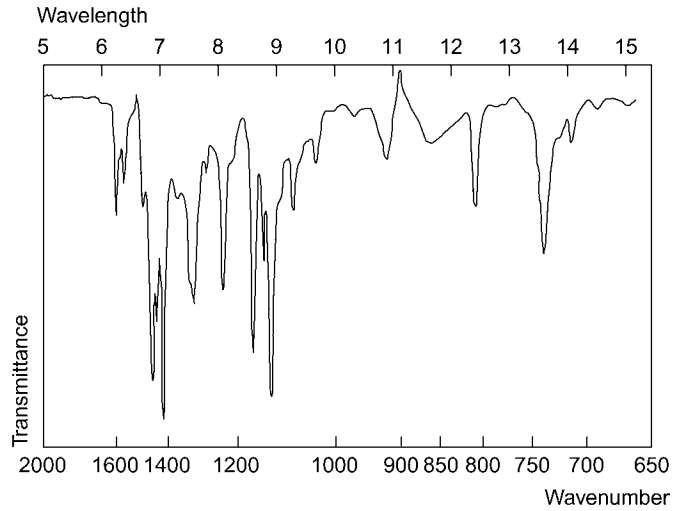
Gas Chromatography System GA—triflupromazine RI 2230, M-ring RI 2190; system GB—triflupromazine RI 2318; system GF—triflupromazine RI 2550; system GW—triflupromazine RT 19.3 min.

High Performance Liquid Chromatography System HA—*k* 2.7; system HX—RI 484; system HY—RI 454; system HZ—RT 12.3 min; system HAX—RT 17.3 min; system HAY—RT 8.9 min.

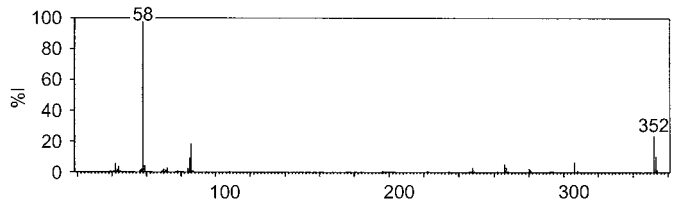
Ultraviolet Spectrum Aqueous acid—256 (A^1_1 = 874a), 305 nm.



Infrared Spectrum Principal peaks at wavenumbers 1117, 1316, 1159, 1237, 1075, 1030 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 352, 86, 353, 85, 306, 42, 266.



Quantification

Blood **GC** Column: Supelco Equity 5 fused silica (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 3 mL/min. Temperature programme: 140° for 1 min to 300° at 15°/min. NPD. Limit of detection, 2.4 µg/L [Shinmen *et al.* 2008].

Plasma **GC** See Blood. Limit of detection, 0.6 µg/L [Shinmen *et al.* 2008].

Urine **GC-MS** Column: HP (12 m × 0.12 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 5 min. EI ionisation at 70 eV, scan mode. Limit of detection not reported [Maurer, Pfleger 1988].

Disposition in the Body

Toxicity A concentration of 10 µmol/L trifluoropromazine was found to be phytotoxic in human erythrocytes [Eberlein-König *et al.* 1997]

Dose 20 to 150 mg of triflupromazine hydrochloride daily.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Eberlein-König B *et al.* (1997). Phototoxic properties of neuroleptic drugs. *Dermatology* 194: 131–135.

Maurer H, Pfleger K (1988). Identification of phenothiazine antihistamines and their metabolites in urine. *Arch Toxicol* 62: 185–191.

Shinmen N *et al.* (2008). Simultaneous determination of some phenothiazine derivatives in human blood by headspace solid-phase microextraction and gas chromatography with nitrogen-phosphorus detection. *J AOAC Int* 91: 1354–1362.

Trihexyphenidyl

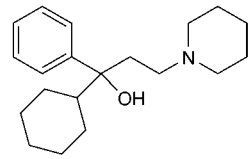
Anticholinergic

$C_{20}H_{31}NO = 301.5$

CAS—144-11-6

IUPAC Name 1-Cyclohexyl-1-phenyl-3-piperidin-1-ylpropan-1-ol

Synonyms Benzhexol; α-cyclohexyl-α-phenyl-1-piperidinepropanol.



Chemical Properties Mp 114.3° to 115.0°. Blood and urine samples containing trihexylphenidyl proved stable when stored for 6 months at –20° or 4° and for 3 months at 25° [Battah, Hadidi 1998]. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Trihexyphenidyl Hydrochloride

$C_{20}H_{31}NO,HCl = 337.9$

CAS—52-49-3

Synonyms Cyclodolum; trihexyphenidylium chloride.

Proprietary Names *Aparkane*; *Apo-Trihex*; *Artandyl*; *Artane*; *Broflex*; *Cyclodol*; *Hipokinon*; *Kexidil*; *Novohexidyl*; *Pacitane*; *Paralest*; *Pargitan*; *Parkinane*; *Parkopan*; *Partane*; *Peragit*; *Pipanol*; *Sedrena*; *Tremin, Trihexy*; *Triphedinon*; *Triphedinyl*; *Tsiklodol*; *Trixyl*. It is an ingredient of *Largatrex* and *Spasman*.

Chemical Properties White or creamy-white crystalline powder. Mp ≈258.5°, with decomposition. Soluble 1 in 100 of water, 1 in 6 of alcohol, 1 in 5 chloroform. More soluble in methanol, very slightly soluble in ether and benzene. Log *P* (octanol/water), 5.27.

Colour Tests Mandelin's test—grey-green; Marquis test—grey-violet.

Thin-layer Chromatography System TA—R_f 0.68; system TB—R_f 0.66; system TC—R_f 0.61; system TE—R_f 0.83; system TL—R_f 0.59; system TAE—R_f 0.43; system TAF—R_f 0.75; system TAJ—R_f 0.38; system TAK—R_f 0.22; system TAL—R_f 0.80 (acidified iodoplatinate solution, positive).

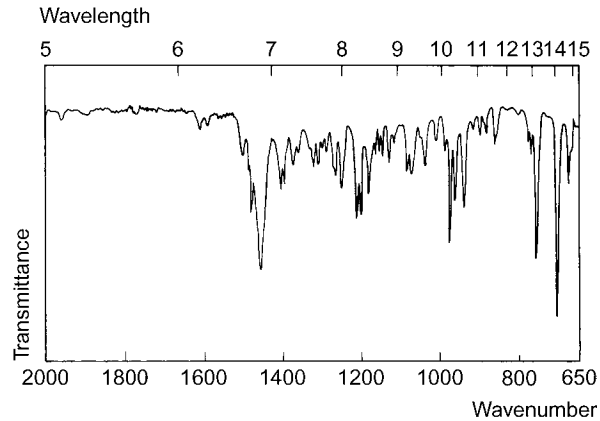
Gas Chromatography System GA—trihexyphenidyl RI 2245, M (OH-) RI 2500; system GB—trihexyphenidyl RI 2354, M (OH-) RI 2618.

Gas Chromatography-Mass Spectrometry Column: BP1 methyl silicone (12.5 m × 0.22 mm i.d., 0.12 µm). Carrier gas: He, 1 mL/min. Temperature programme: 90° for 45 s to 270° at 25°/min for 3 min. Retention time: 8.05 min [Desage *et al.* 1991].

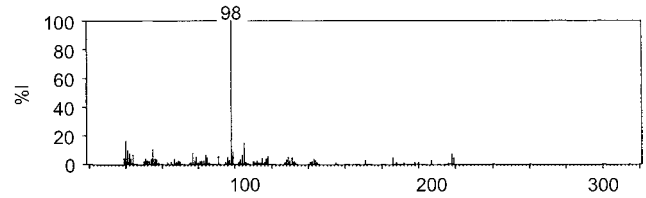
High Performance Liquid Chromatography System HA—*k* 1.8; system HX—RI 429; system HY—RI 381; system HZ—RT 7.6 min; system HAA—RT 15.3 min.

Ultraviolet Spectrum Aqueous acid—252 ($A_1^1 = 6c$), 258 ($A_1^1 = 7c$), 264 nm ($A_1^1 = 6c$).

Infrared Spectrum Principal peaks at wavenumbers 702, 756, 973, 1206, 1196, 935 cm^{-1} (trihexyphenidyl hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 98, 105, 55, 41, 99, 77, 218, 84.



Quantification

Blood **GC** Column: HP1 (5 m × 0.53 mm i.d., 2.65 µm). Carrier gas: N_2, 10 mL/min. Temperature programme: 180° for 2 min to 280° at 8°/min for 3 min. NPD. Limit of detection not reported [Battah, Hadidi 1998].

HPLC Spherisorb-ODS (10 × 0.5 cm). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 3.0; 55 : 45), flow rate 1.5 mL/min. UV detection (λ = 214 nm). Limit of detection not reported [Gall *et al.* 1995].

Plasma GC Column: 3% OV-17 on 100/120 mesh (6′ × 2 mm i.d.). Carrier gas: N_2, 35 psi. Temperature: 248°. NPD. Limit of detection 2 and 1 μg/L for trihexyphenidyl and its hydroxylated metabolite, respectively, [Kintz *et al.* 1989]. Column: OV-17 fused silica capillary (25 m × 0.25 mm i.d., 0.1 μm). Carrier gas: He, 2.3 kg/cm^2. Temperature programme: 150° for 0.6 min to 230° at 30°/min to 245° at 5°/min to 280° at 30°/min. FTD. Limit of detection, 50 ng/L [Owen *et al.* 1989].

GC-MS Column: Teknokroma TRACSIL Meta.X5 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 250° at 25°/min for 1 min to 280° at 8°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 8.92 min. Limit of detection, 0.5 μg/L [Hadidi 2004]. Column: BP1 methyl silicone capillary (12.5 m × 0.22 mm i.d., 0.12 μm). Carrier gas: He, 0.4 kg/cm^2. Temperature programme: 90° for 90 s to 270° at 25°/min for 3 min. EI ionisation. Limit of quantification, 5 ng/mL, limit of detection, 1 ng/mL [Desage *et al.* 1991].

Serum **GC** See Plasma [Owen *et al.* 1989].

LC-MS Column: Cyclobond I 2000 native β-cyclodextrin (250 × 2.0 mm i.d., 0.5 μm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (90 : 10), flow rate 1.0 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 1 μg/L [Capka, Xu 2001]. Column: Cyclobond I 2000 native β-cyclodextin (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : methanol : acetic acid : triethylamine (95 : 1 : 0.5 : 0.3). ESI, SIM acquisition mode. Limit of detection, 0.1 μg/L [Capka *et al.* 1999].

CE Capillary: untreated fused silica (57/49.5 cm total/effective length or 66.5/59.8 cm total/effective length, 75 μm i.d.). Buffer: 50 mmol/L sodium phosphate and 50 mmol/L phosphoric acid, with or without β-cyclodextrin. UV detection (λ = 190 nm). Limit of detection, 220 mg/L [Vargas *et al.* 1998].

Urine **GC** See Blood [Battah, Hadidi 1998]. See Plasma [Kintz *et al.* 1989]. See Plasma [Owen *et al.* 1989].

GC-MS See Plasma [Hadidi 2004].

Gastric Contents **HPLC** See Blood [Gall *et al.* 1995].

Hair **GC** Column: AC-5 capillary (15 m × 0.25 mm i.d.). Temperature programme: 180° to 270° at 10°/min for 10 min. NPD. Limit of detection, 0.1–0.5 μg/g [Shen *et al.* 2002].

GC-MS Column: DB-5 (30 m × 0.25 mm i.d.). Carrier gas: CH_4. Temperature programme: 100° for 2 min to 150° at 10°/min to 280° at 25°/min. CI, full scan mode. Retention time: 10.0 min [Shen *et al.* 2002].

Liver **HPLC** See Blood [Gall *et al.* 1995].

Disposition in the Body Absorbed after oral administration; disappears rapidly from the tissues.

Note For a radioimmunoassay for the detection of trihexyphenidyl, see He *et al.* [1995].

Toxicity In some patients, doses >12 mg daily may produce severe mental disturbance and excitement.

The following postmortem concentrations were observed in a 48-year-old schizophrenic male: femoral artery blood 0.12 mg/L, liver 0.5 μg/g, gastric contents 0.4 mg trihexyphenidyl (benzhexol). Other drugs were not detected. It is suggested that for fatalities to occur following trihexyphenidyl intoxication, secondary contributory factors, which probably further alter the conscious state, are necessary [Gall *et al.* 1995].

The following postmortem concentrations were observed in a 16-year-old youth who experienced hallucinations and was found dead in a nearby lake 2 days after the ingestion of 20 mg of trihexyphenidyl: blood 0.03 mg/L, urine 0.38 mg/L [Kopjak, Jennison 1976].

Dose 1 mg of trihexyphenidyl hydrochloride daily, increased gradually; usual maintenance dose 5 to 15 mg in 3 to 4 divided doses.

Battah AH, Hadidi KA (1998). Stability of trihexyphenidyl in stored blood and urine specimens. *Int J Legal Med* 111: 111–114.

Capka V, Xu Y (2001). Simultaneous determination of enantiomers of structurally related anticholinergic analogs in human serum by liquid chromatography-electrospray ionization mass spectrometry with on-line sample cleanup. *J Chromatogr B Biomed Sci Appl* 762: 181–192.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Desage M *et al.* (1991). Quantitation of trihexyphenidyl from plasma using a mass-selective detector and electron-impact ionization. *J Chromatogr* 571: 250–256.

Gall JA *et al.* (1995). Death due to benzhexol toxicity. *Forensic Sci Int* 71: 9–14.

Hadidi KA (2004). Development of a screening method for the most commonly abused anticholinergic drugs in Jordan; trihexyphenidyl, procyclidine and biperiden. *Leg Med (Tokyo)* 6: 233–241.

He H *et al.* (1995). Development and application of a specific and sensitive radioimmunoassay for trihexyphenidyl to a pharmacokinetic study in humans. *J Pharm Sci* 84: 561–567.

Kintz P *et al.* (1989). Identification and quantification of trihexyphenidyl and its hydroxylated metabolite by gas chromatography with nitrogen-phosphorus detection. *J Anal Toxicol* 13: 47–49.

Kopjak L, Jennison TA (1976). *TIAFT Bulletin* 128.

Owen JA *et al.* (1989). Capillary gas chromatography of trihexyphenidyl, procyclidine and cycrimine in biological fluids. *J Chromatogr* 494: 135–142.

Shen M *et al.* (2002). Detection of antidepressant and antipsychotic drugs in human hair. *Forensic Sci Int* 126: 153–161.

Vargas G *et al.* (1998). Determination of drugs used as anti-Parkinson's disease drugs in urine and serum by capillary electrophoresis. *J Capillary Electrophor* 5: 153–158.

Trilostane

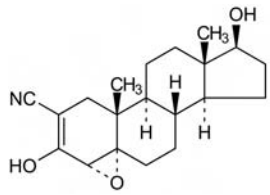
Adrenocortical Suppressant

$C_{20}H_{27}NO_3$ = 329.4

CAS—13647-35-3

Synonym (4α,5α,17β)-4,5-Epoxy-3,17-dihydroxyandrost-2-ene-2-carbonitrile

Proprietary Name *Modrenal*

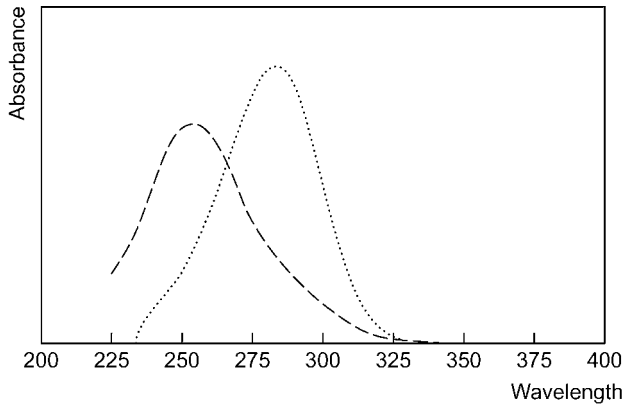


Chemical Properties Tan crystals. Mp 258° to 270°, with some decomposition. Practically insoluble in water, chloroform and ether; slightly soluble in a mixture of equal parts of chloroform and methanol. Log *P* (octanol/water) 2.0.

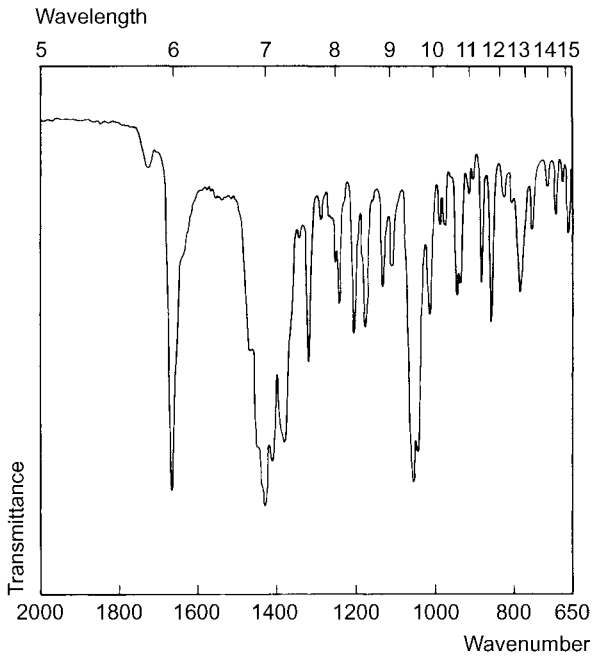
Thin-layer Chromatography System TA—R_f 0.88; system TB—R_f 0.00; system TC—R_f 0.18; system TD—R_f 0.30; system TE—R_f 0.07; system TF—R_f 0.45 (Marquis reagent, red; mercuric chloride–diphenylcarbazone, violet, fades to pink; acidified potassium permanganate solution, positive).

Gas Chromatography System GA—RI 3075, decomposition occurs.

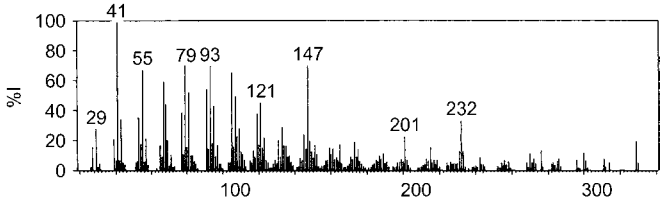
Ultraviolet Spectrum Aqueous alkali—281 nm; ethanol—253 nm (A^1_1=234b).



Infrared Spectrum Principal peaks at wavenumbers 1665, 1060, 1050, 1320, 1205, 1180 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 41, 147, 79, 93, 55, 105, 67, 91.



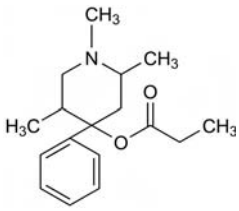
Quantification
***Plasma* HPLC** For a method for the quantification of trilostane and ketotrilostane, see Powles *et al.* [1984]; Brown *et al.* [1995].
Dose 120 to 480 mg daily.

Brown RR *et al.* (1995). *J Chromatogr* 339: 440–444.
Powles P *et al.* (1984). Determination of trilostane and ketotrilostane in human plasma by high-performance liquid chromatography. *J Chromatogr* 311: 434–442.

Trimeperidine

Narcotic Analgesic
$C_{17}H_{25}NO_2$ = 275.4
CAS—64-39-1

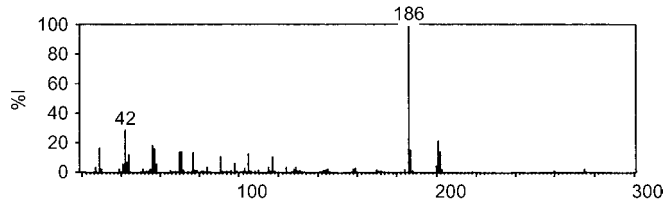
IUPAC Name 1,2,5-Trimethyl-4-phenyl-4-piperidinol propanoate
Synonym Promedol.



Chemical Properties Soluble 1 in about 27 of water.

Trimeperidine Hydrochloride
$C_{17}H_{25}NO_2$,HCl = 311.9
CAS—125-80-4
Chemical Properties White crystalline powder. Freely soluble in water and chloroform; soluble in ethanol; practically insoluble in ether. Log *P* (octanol/water), 3.1.

Colour Test Marquis test—red-violet.
Thin-layer Chromatography System TA—R_f 0.58; system TAG—R_f 0.17; system TB—R_f 0.41; system TC—R_f 0.41 (Dragendorff spray, positive; acidified iodoplatinate solution, positive; Marquis reagent, red-violet).
Gas Chromatography System GA—RI 1808; system GB—RI 1895.
High Performance Liquid Chromatography System HA—*k* 2.1.
Ultraviolet Spectrum Aqueous acid—251, 257 nm (A_1^1 = 7.6b), 263 nm. No alkaline shift.
Infrared Spectrum Principal peaks at wavenumbers 1738, 1181, 1141, 1162, 1195, 1077 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 186, 42, 201, 56, 57, 187, 202, 71.

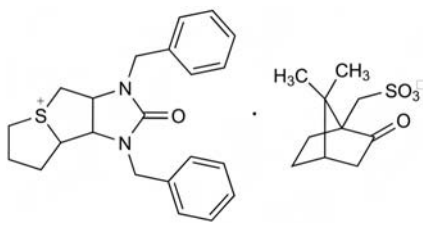


Dose Trimeperidine hydrochloride has been given in doses of up to 200 mg daily.

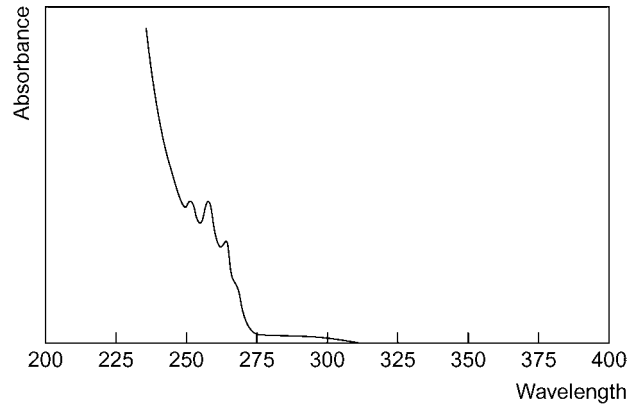
Trimetaphan Camsilate

Antihypertensive
$C_{22}H_{25}N_2OS$,$C_{10}H_{15}O_4S$ = 596.8
CAS—7187-66-8 (trimetaphan); 68-91-7(camsilate)

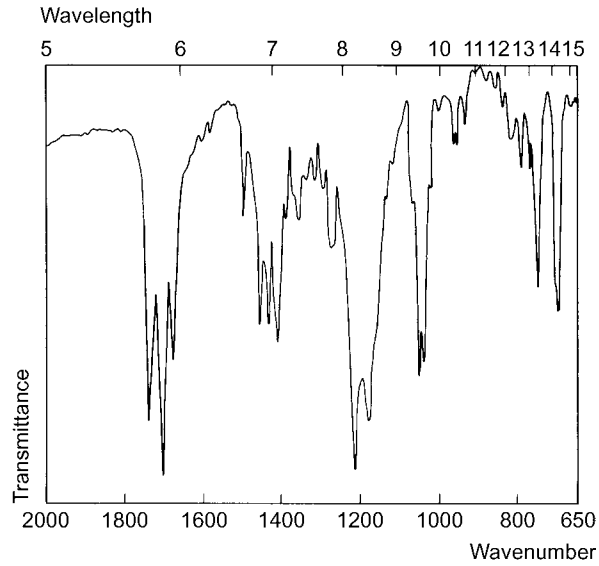
Synonyms Decahydro-2-oxo-1,3-bis(phenylmethyl)thieno[1′,2′:1,2]thieno-[3,4-*d*]imidazol-5-ium salt with (1*S*)-7,7-dimethyl-2-oxobicyclo[2,2,1]heptane-1-methanesulfonic acid (1 : 1); méthioplégium; trimetaphan camphorsulfonate; trimethaphan camsylate.
Proprietary Name *Arfonad*



Chemical Properties Colourless crystals or white crystalline powder. Mp about 245°, with decomposition. Soluble 1 in <5 of water and 1 in 2 of ethanol; freely soluble in chloroform; slightly soluble in acetone and ether. Log *P* (octanol/water) 6.9.
Colour Tests Aromaticity (method 2)—colourless/yellow; Liebermann's reagent—orange.
Thin-layer Chromatography System TA—R_f 0.02; system TB—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.00; system TAJ—R_f 0.00; system TAK—R_f 0.01; system TAL—R_f 0.20 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HX—RI 386.
Ultraviolet Spectrum Aqueous acid—252 nm (A_1^1=8.2a), 258 nm (A_1^1=8.3a), 264 nm. No alkaline shift.



Infrared Spectrum Principal peaks at wavenumbers 1701, 1220, 1184, 1735, 1052, 1672 cm^{-1} (KBr disk).

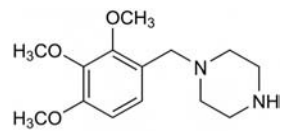


Mass Spectrum Principal ions at *m/z* 91, 65, 187, 92, 277, 259, 90, 273.
Dose Initially 3 to 4 mg/min by IV infusion.

Trimetazidine

Antianginal Vasodilator
$C_{14}H_{22}N_2O_3$ = 266.3
CAS—5011-34-7

IUPAC Name 1-[(2,3,4-Trimethoxyphenyl)methyl]piperazine
Synonym Trimetazine

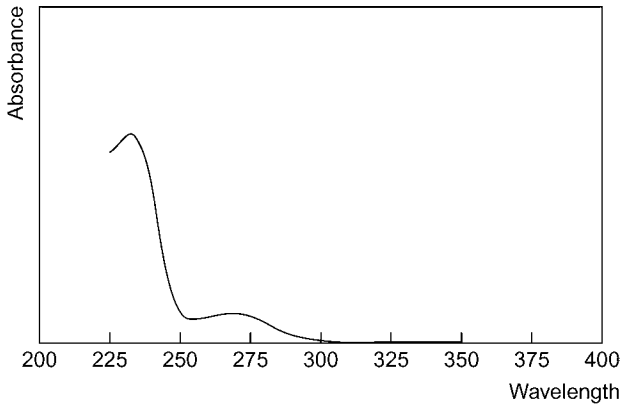


Chemical Properties Log *P* (octanol/water), 0.6.

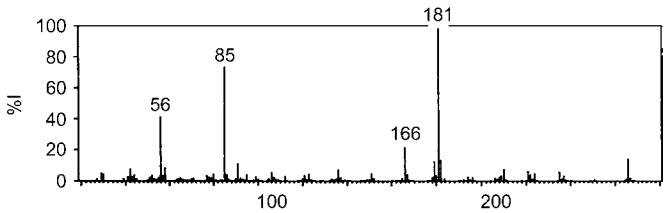
Trimetazidine Hydrochloride
$C_{14}H_{22}N_2O_3$,2HCl = 339.3
CAS—13171-25-0

Proprietary Names *Cardiazidine*; *Centrophene*; *Idaptan*; *Oxygirex*; *Vastarel*; *Vastinol*.
Chemical Properties A white crystalline powder. Mp 225° to 228°. Freely soluble in water; sparingly soluble in ethanol.

Colour Tests Mandelin's test—violet; Marquis test—yellow (fades).
Thin-layer Chromatography System TA—R_f 0.22; system TB—R_f 0.05; system TC—R_f 0.04; system TL—R_f 0.01 (acidified iodoplatinate solution, positive).
High Performance Liquid Chromatography System HA—*k* 3.0 (tailing peak); system HAA—RT 6.1 min.
Ultraviolet Spectrum Aqueous acid—231 ($A_1^1 = 424$ a), 269 nm.



Infrared Spectrum Principal peaks at wavenumbers 1093, 1494, 1280, 1011, 1042, 1600 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 181, 85, 56, 166, 266, 182, 179, 91.



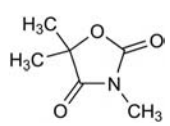
Quantification
Plasma **HPLC** Fluorescence detection. For method, see Courte and Bromet [1981].
Biological Fluids **GC–MS** For method, see Fay *et al.* [1989].
Dose Trimetazidine hydrochloride has been given in doses of 40 to 60 mg daily.

Courte S, Bromet N (1981). Trace determination of trimetazidine in plasma by high-performance liquid chromatography using fluorescence detection. *J Chromatogr* 224: 162–167.
Fay L *et al.* (1989). Determination of trimetazidine in biological fluids by gas chromatography-mass spectrometry. *J Chromatogr* 490: 198–205.

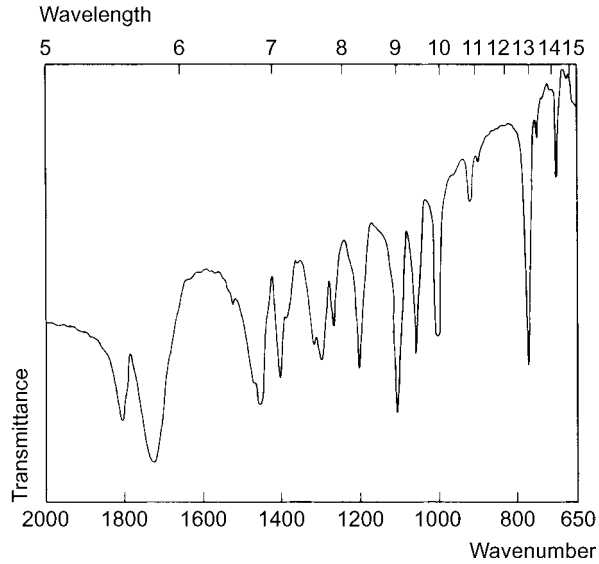
Trimethadione

Anticonvulsant
$C_6H_9NO_3 = 143.1$
CAS—127-48-0

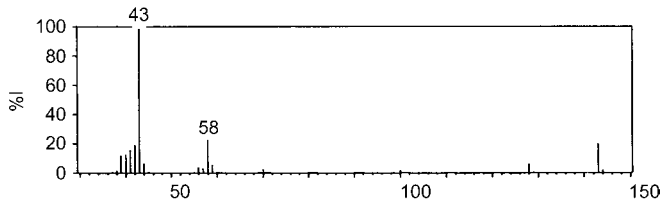
IUPAC Name 3,5,5-Trimethyl-1,3-oxazolidine-2,4-dione
Synonyms Trimetadiona; trimethadionum; trimethinum; troxidone.
Proprietary Names *Absentol*; *Epidione*; *Petidon*; *Ptimal*; *Tridione*.



Chemical Properties Colourless or white granular crystals. Mp 46°. Soluble 1 in 20 of water; freely soluble in ethanol, chloroform, benzene and ether. Log *P* (octanol/water), 0.6.
Thin-layer Chromatography System TE—R_f 0.00; system TF—R_f 0.00; system TAE—R_f 0.00.
Gas Chromatography System GA—trimethadione RI 1090, M (nor-) RI 1060; system GE—RRT 0.04 (relative to phenytoin).
High Performance Liquid Chromatography System HX—RI 286.
Infrared Spectrum Principal peaks at wavenumbers 1730, 1802, 1098, 1200, 770, 1295 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 58, 143, 42, 41, 40, 39, 128.



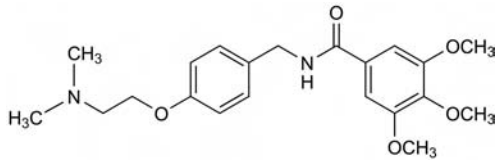
Quantification
Plasma **GC** FID. For a method of quantification for trimethadione and dimethadione, see Bius *et al.* [1979].
Serum **GC** Limit of detection, 10 µg/L for trimethadione, 50 µg/L for dimethadione [Tanaka, Misawa 1992].
Disposition in the Body Readily absorbed after oral administration and extensively metabolised in the liver to the active metabolite, dimethadione, which is thought to be primarily responsible for the pharmacological activity. It is very slowly excreted in the urine over a period of several days, almost entirely in the form of dimethadione; the excretion of dimethadione is increased in alkaline urine.
Therapeutic Concentration Dimethadione attains plasma concentrations about 20 times greater than those of trimethadione and accumulates on chronic administration; there is considerable intersubject variation in plasma concentrations.
Steady-state plasma concentrations of 19 to 41 mg/L (mean, 25) of trimethadione and 350 to 1033 mg/L (mean, 550) of dimethadione were reported in 5 subjects during chronic treatment with trimethadione [Bius *et al.* 1979.]
Toxicity The estimated minimum lethal dose is 5 g. Toxic effects may be associated with plasma-dimethadione concentrations >1000 mg/L.
Half-life Plasma half-life, trimethadione about 16 h; dimethadione, half-life derived from urinary excretion data, 6 to 13 days.
Protein Binding Not significantly bound.
Dose Initially 900 mg daily, increasing to 1.8 g daily.

Bius DL *et al.* (1979). *Ther Drug Monit* 1: 495–505.
Tanaka E, Misawa S (1992). Improved method for the determination of trimethadione and its demethylated metabolite, dimethadione, in human serum by gas chromatography. *J Chromatogr* 584: 267–269.

Trimethobenzamide

Antihistamine
$C_{21}H_{28}N_2O_5 = 388.5$
CAS—138-56-7

IUPAC Name *N*-[[4-(2-Dimethylaminoethyloxy)phenyl]methyl]-3,4,5-trimethoxybenzamide

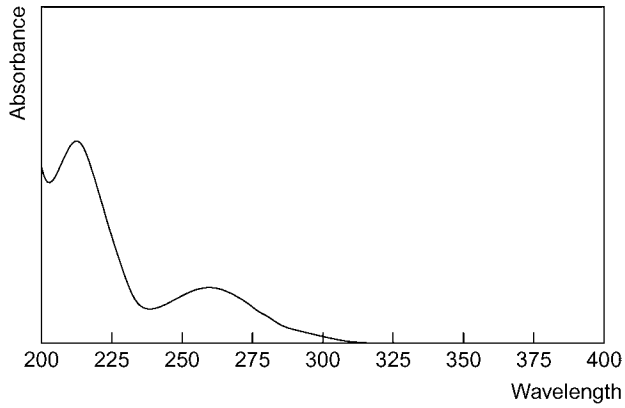


Chemical Properties pK_a 8.8. Log *P* (octanol/water), 2.3.

Trimethobenzamide Hydrochloride

$C_{21}H_{28}N_2O_5$,HCl = 424.9
CAS—554-92-7
Proprietary Names *Tebamide*; *T-Gen*; *Ticon*; *Tigan*; *Trimazide*.
Chemical Properties A white crystalline powder. Mp 188° to 190°. Soluble 1 in 2 of water, 1 in about 60 of ethanol, 1 in 67 of chloroform and 1 in 720 of ether.

Colour Tests Liebermann's reagent—black; Mandelin's test—violet-brown.
Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.02; system TE—R_f 0.47; system TAE—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 3281.
High Performance Liquid Chromatography System HA—*k* 4.7; system HX—RI 347.
Ultraviolet Spectrum Aqueous acid—258 nm (A_1^1 = 312 b). No alkaline shift.



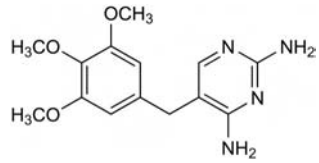
Infrared Spectrum Principal peaks at wavenumbers 1123, 1230, 1575, 1505, 1616, 990 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 58, 195, 59, 72, 388, 89, 315, 42.
Quantification
Serum **HPLC** UV detection. For method, see Robert *et al.* [1982].
Saliva **GC–MS** Limit of detection, <100 µg/L [Robert *et al.* 1981].
Disposition in the Body
Therapeutic Concentration
Following a single oral dose of 250 mg to 4 subjects, peak blood concentrations of 1.3 to 2.4 µg/mL (mean, 1.7) were attained in 1.5 h [Baselt, Cravey 1977].
Toxicity
In a fatality involving the ingestion of trimethobenzamide, postmortem blood and bile concentrations of 184 mg/L and 1150 mg/L, respectively, were reported; a postmortem blood-alcohol concentration of 1100 mg/L was also reported [Harrill 1976].
Dose 0.75 to 1 g daily.

Baselt RC, Cravey RH (1977). *J Anal Toxicol* 1: 81–103.
Harrill JC (1976). *Bull Int Assn Forens Toxicol* 12(2): 29–30.
Robert TA *et al.* (1981). Analysis of trimethobenzamide in human saliva by gas chromatography–mass spectrometry. *J Chromatogr* 224: 116–121.
Robert TA *et al.* (1982). The relationship between serum and saliva trimethobenzamide concentrations in man. *J Clin Pharmacol* 22: 53–58.

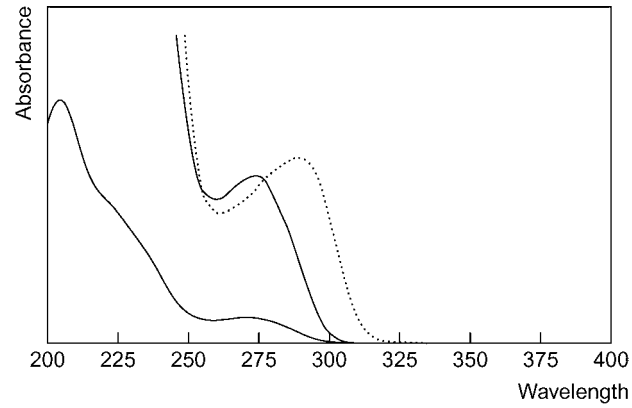
Trimethoprim

Antimicrobial
$C_{14}H_{18}N_4O_3$ = 290.3
CAS—738-70-5

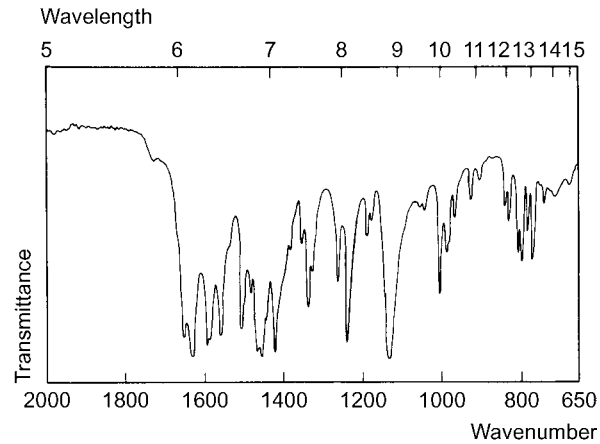
IUPAC Name 5-[(3,4,5-Trimethoxyphenyl)methyl]pyrimidine-2,4-diamine
Synonyms BW-56-72; NSC-106568; trimethoprimum; trimethoxyprim.
Proprietary Names *Ipral*; *Monotrim*; *Primsol*; *Proloprim*; *Tiempe*; *Trimogal*; *Trimopan*; *Trimpex*; *Uretrim*; *Wellcoprim*. It is an ingredient of *Bactrim*, *Borgal* (vet.), *Chemotrim*, *Cotrim*, *Fectrim*, *Laratrim*, *Polytrim*, *Septra*, *Septrin*, *SMZ-TMP*, *Sulfatrimand Trivetrin* (vet.).



Chemical Properties White or yellowish-white crystals or crystalline powder. Mp 199° to 203°. Soluble 1 in 2500 of water, 1 in 300 of ethanol, 1 in 55 of chloroform and 1 in 80 of methanol; practically insoluble in ether. pK_a 7.2; 6.6. Log *P* (octanol/water), 0.91.
Colour Tests Aromaticity (method 2)—yellow/red; Mandelin's test—yellow-brown; Marquis test—orange; nitric acid, cold—red, which fades to yellow on heating.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.00; system TC—R_f 0.22; system TE—R_f 0.45; system TL—R_f 0.12; system TAD—R_f 0.20; system TAE—R_f 0.45; system TAF—R_f 0.59; system TAJ—R_f 0.14; system TAK—R_f 0.08; system TAL—R_f 0.66 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—trimethoprim RI 2558, trimethoprim-AC_2 RI 3000.
High Performance Liquid Chromatography System HA—*k* 1.2; system HX—RI 299; system HY—RI 254; system HZ—retention time 2.1 min; system HAA—retention time 8.3 min; system HAX—retention time 6.7 min; system HAY—retention time 3.7 min.
Ultraviolet Spectrum Aqueous acid—271 nm (A_1^1=218a); aqueous alkali—287 nm (A_1^1=250a).



Infrared Spectrum Principal peaks at wavenumbers 1126, 1630, 1596, 1235, 1650, 1565 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 290, 259, 275, 291, 243, 123, 200, 43.
Quantification
Plasma **GC** AFID. Limit of detection, 0.1 mg/L [Land *et al.* 1978].
Serum **HPLC** Electrochemical detection. Limit of detection, 0.01 mg/L [Nordholm, Dalgaard 1982]. UV detection. Limit of detection, 0.1 mg/L [Gochin *et al.* 1981].
Urine **GC** See Plasma [Land *et al.* 1978].
HPLC See Serum [Nordholm, Dalgaard 1982]. See Serum [Gochin *et al.* 1981].
Tissues **HPLC–MS** SIM: *m/z* 291. Limit of detection, 4 µg/kg [Cannavan *et al.* 1997].
Disposition in the Body Readily and rapidly absorbed after oral administration. Trimethoprim is widely distributed around the body to tissues and fluids including kidneys, liver, lung and bronchial secretions, saliva, aqueous humour and vaginal secretions. Metabolic reactions include oxidation of the methylene

group to a hydroxymethyl group, *N*-oxidation, *O*-demethylation, hydroxylation and conjugation with glucuronic acid or sulfate. 10 to 20% of a dose is metabolised. Metabolites are excreted in the urine as conjugates but the greater part of the dose is excreted as unchanged drug. Urinary excretion is pH-dependent and is increased in acid urine. About 40 to 75% of a dose is excreted in 24 h, up to 60% being in the form of unchanged drug, with about 4% each as the 3′-hydroxymethyl and 4′-hydroxymethyl metabolites, and 2% as the N^1-oxide. <4% is eliminated in the faeces. It readily crosses the placenta and has been detected in breast milk.

Therapeutic Concentration The serum therapeutic concentration ranges from 1.5 to 2.5 mg/L.

Following oral administration of 300 mg once daily to 6 subjects, maximum steady-state plasma concentrations of 3.1 to 9.5 mg/L (mean, 6.0) were attained in 1 to 4 h (mean, 2) [Odlind *et al.* 1984].

For further references to trimethoprim when administered with sulfamethoxazole, see under Sulfamethoxazole.

Toxicity Toxic effects have been associated with plasma concentrations >20 mg/L. Bone marrow depression may result owing to the administration of high doses of trimethoprim or use for extended periods of time.

A 28-year-old man ingested 8 g of trimethoprim and 14 h later the plasma concentration was 19.6 mg/L. It was estimated that the actual amount of trimethoprim absorbed was about 3 g. The patient subsequently recovered [Hoppu *et al.* 1980].

Half-life Plasma half-life, 8 to 17 h (mean, 11).

Volume of Distribution About 1.4 L/kg.

Clearance Plasma clearance, about 2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, about 0.78.

Saliva Plasma : saliva ratio, about 1.3.

Protein Binding 40 to 70%.

Note For a review of the clinical pharmacokinetics of sulfamethoxazole and trimethoprim, see Patel and Welling [1980].

Dose Doses of 1.5 g daily for 3 days have been given for malaria. For acute infection (oral): adults, 100 to 200 mg twice daily or a 100 to 300 mg single dose; children, 6 to 8 mg/kg daily; (6 to 12 years old) 100 mg twice daily; (6 month to 5 years old) 50 mg twice daily; (6 weeks to 5 months) 25 mg twice daily. (IV): 200 mg every 12 h (adults); 6 to 9 mg/kg daily (children).

Cannavan A *et al.* (1997). Determination of trimethoprim in tissues using liquid chromatography-thermospray mass spectrometry. *Analyst* 122(11): 1379–1381.

Gochin R *et al.* (1981). Simultaneous determination of trimethoprim, sulphamethoxazole and N4-acetylsulphamethoxazole in serum and urine by high-performance liquid chromatography. *J Chromatogr* 223: 139–145.

Hoppu K *et al.* (1980). Trimethoprim poisoning. *Lancet* 1: 778.

Land G *et al.* (1978). The gas–liquid chromatographic analysis of trimethoprim in plasma and urine. *J Chromatogr* 146: 143–147.

Nordholm L, Dalgaard L (1982). Assay of trimethoprim in plasma and urine by high-performance liquid chromatography using electrochemical detection. *J Chromatogr* 233: 427–431.

Odlind B *et al.* (1984). Steady state pharmacokinetics of trimethoprim 300 mg once daily in healthy volunteers assessed by two independent methods. *Eur J Clin Pharmacol* 26: 393–397.

Patel RB, Welling PG (1980). Clinical pharmacokinetics of co-trimoxazole (trimethoprim-sulphamethoxazole). *Clin Pharmacokinet* 5: 405–423.

Trimethoxyamfetamine

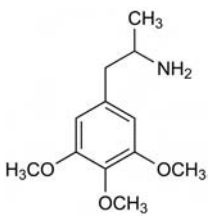
Hallucinogen, Phenethylamine

$C_{12}H_{19}NO_3$ = 225.3

CAS—1082-88-8

IUPAC Name 1-(3,4,5-Trimethoxyphenyl)propan-2-amine

Synonym α-Methyl-3,4,5–trimetoxyphenethylamine; trimethoxyamphetamine.



Chemical Properties Trimethoxyamfetamine is the α-methyl homologue of mescaline. An oil. Practically insoluble in water; soluble in chloroform. The hydrochloride is soluble in water. Mp 219° to 220°. Log *P* (octanol/water), 1.2. Extraction yield (chlorobutane), 0.5 (2,4,6-TMA), 0.2 (2,4,5-TMA) [Demme *et al.* 2005].

Colour Test Marquis test—orange.

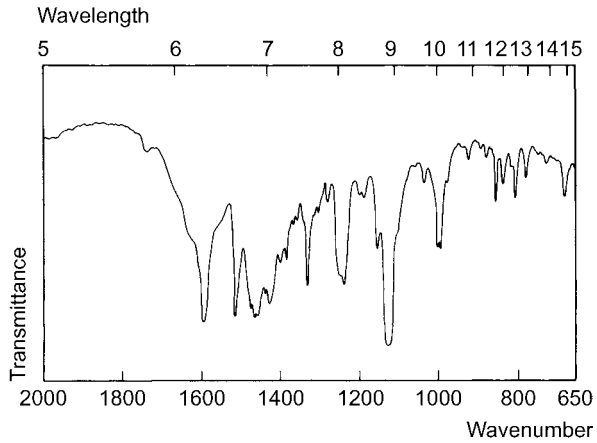
Thin-layer Chromatography System TA—(2,4,6-TMA) R_f 0.33; system TB—(2,4,6-TMA) R_f 0.06; system TC—(2,4,6-TMA) R_f 0.11; system TE—(2,4,6-TMA) R_f 0.29; system TAE—(2,4,6-TMA) R_f 0.07; system TAF—(2,4,6-TMA) R_f 0.70; system TAG—(2,4,6-TMA) R_f 0.12 (acidified iodoplatinate solution—positive).

Gas Chromatography System GA—RI 1748.

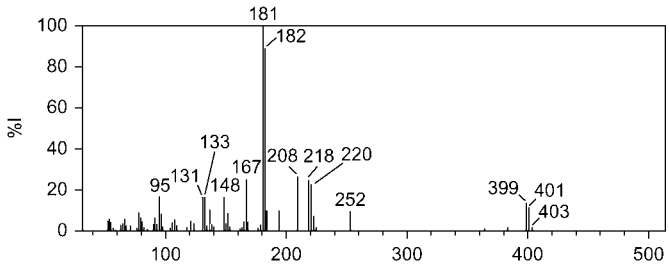
High Performance Liquid Chromatography System HC—*k* 1.48; system HX— (2,4,6-TMA) RI 290; system HY—(3,4,5-TMA) RI 232 , (2,4,6-TMA) RI 298.

Ultraviolet Spectrum Aqueous acid—269 nm (A_1^1 = 26 b). No alkaline shift.

Infrared Spectrum Principal peaks at wavenumbers 1130, 1595, 1510, 1240, 1000, 1151 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 43, 182, 167, 225, 181, 183, 151, 142.



Demme U *et al.* (2005). Systemic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. In *Proceedings of the 12th Annual Meeting of the International Association of Forensic Toxicologists*, Seoul: 481–486.

Trimetozine

Tranquilliser

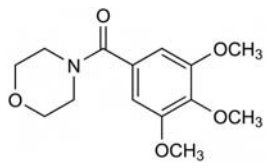
$C_{14}H_{19}NO_5$ = 281.3

CAS—635-41-6

IUPAC Name 4-(3,4,5-Trimethoxybenzoyl)morpholine

Synonyms Trimethoxazine; trimolide.

Proprietary Names *Opalene*; *Trioxazine*.



Chemical Properties White crystalline powder. Mp ≈120° to 122°. Slightly soluble in water and ethanol; freely soluble in chloroform and methanol. Log *P* (octanol/water) −0.2.

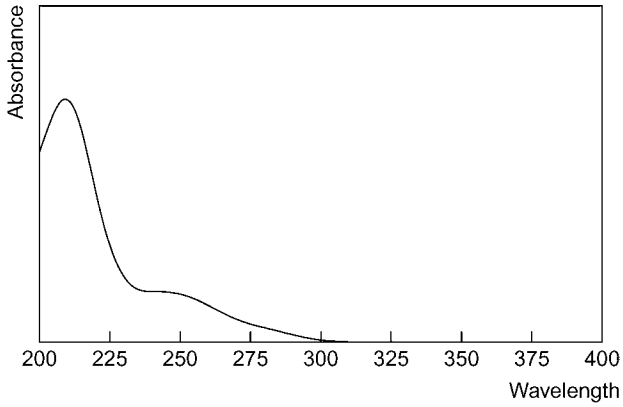
Colour Tests Liebermann's reagent—black; Marquis test—red-brown.

Thin-layer Chromatography System TA—R_f 0.61; system TAE—R_f 0.80; system TAG—R_f 0.52; system TB—R_f 0.11; system TC—R_f 0.72; system TE—R_f 0.68 (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2253.

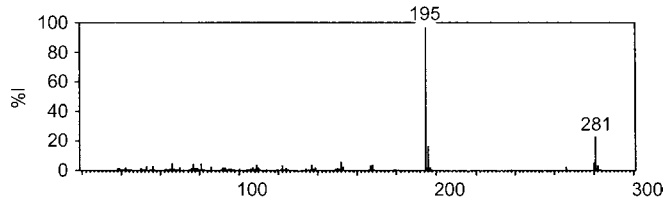
Ultraviolet Spectrum Methanol—245 nm (A_1^1 = 259b).

T



Infrared Spectrum Principal peaks at wavenumbers 1131, 1642, 1229, 1110, 1592, 1001 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 195, 281, 196, 152, 280, 81, 77, 66.



Dose Usually 0.6 to 1.8 g daily; maximum of 3 g daily.

Trimetrexate

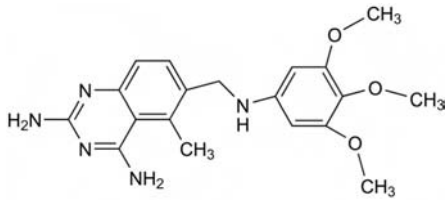
Antineoplastic, Dihydrofolate Reductase Inhibitor

$C_{19}H_{23}N_5O_3 = 369.4$

CAS—52128-35-5

IUPAC Name 5-Methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline-2,4-diamine

Synonyms CI-898; JB-11; NSC-249008.



Chemical Properties Mp 215° to 217°. It is practically insoluble in water (>0.1 g/L).

Trimetrexate D-Glucuronate

$C_{25}H_{33}N_5O_{10} = 563.6$

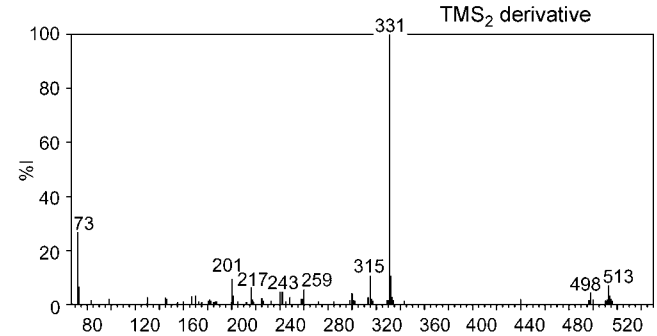
CAS—82952-64-5

Synonyms 2,4-diamino-5-methyl-6-[(3,4,5-triethoxyanilino)methyl] quinazoline mono-*d*-glucuronate; NSC-352122; trimetrexate *d*-glucuronate; *d*-glucuronic acid compound with 5-methyl-6-{[(3,4,5-trimethoxyphenyl)amino]methyl.

Proprietary Names *NeuTrexin*; *Oncotrex*.

Chemical Properties A pale greenish to yellow powder or cake. It is soluble in water (>50 g/L). pK_a (50% methanol/water), 8.0. Log *P* (octanol/water), 1.63, 2.55.

Mass Spectrum



Quantification

Blood **HPLC** Column: Ultrasphere ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L sodium monobasic monohydrate containing 17% acetonitrile and 0.8% acetic acid (pH 5.5), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Limit of quantification, 0.02 µg/L [Lin *et al.* 1987]. Column: Spherisorb-ODS (100 × 4.6 mm i.d., 5 µm). Mobile phase: 0.04% phosphoric acid–0.08% triethylamine : acetonitrile (85 : 15 to 60 : 40 over 10 min), flow rate 1.5 mL/min. DAD (λ= 241 nm). Limit of quantification, 100 µg/L [Hudes *et al.* 1989]. Column: Zorbax TMS (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile: 50 mmol/L ammonium phosphate containing 0.8% triethylamine and 0.2% phosphoric acid (pH 4.5), flow rate 1.2 mL/min. UV detection (λ= 241 nm). Limit of detection, 2.4 µg/L [Bullen *et al.* 1990].

Plasma **GC-MS** Column: fused silica (5000 × 0.32 mm i.d., 0.17 mm). Carrier gas: He, 1.0 mL/min. Temperature programme: 200° for 0.1 min to 300° at 15°/min. EI ionisation, SIM acquisition mode [Stetson, Ensminger 1986].

HPLC Column: HP RPC_{18} ODS (100 × 4.6 mm i.d., 5 µm). Mobile phase: water containing 0.16% trimethylamine and 0.08% phosphoric acid : acetonitrile (91 : 9 to 65 : 35 over 15 min). DAD (λ= 241 nm). Limit of detection, 0.1 mg/L [Hudes *et al.* 1991].

Urine **HPLC** See Plasma [Hudes *et al.* 1991]. Column: Spherisorb ODS (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.08% triethylamine (15 : 85 to 40 : 60 over 10 min), flow rate 1.5 mL/min. UV detection (λ= 241 nm). Limit of detection, 100 µg/L [Tinsley, LaCreta 1990]. See Blood [Hudes *et al.* 1989]. See Blood. Limit of quantification, 0.05 µg/L [Lin *et al.* 1987].

Faeces **HPLC** See Blood. Limit of quantification, 0.05 µg/L [Lin *et al.* 1987].

Disposition in the Body Metabolism has not been characterised in humans for trimetrexate. However, pre-clinical data suggest that the major metabolic pathway is oxidative *O*-demethylation, followed by conjugation to either its glucuronide or the sulfate. *N*-Demethylation and oxidation are related minor pathways. Preliminary findings in humans indicate the presence of a glucuronide conjugate with dihydrofolate reductase inhibition and a demethylated metabolite in urine. Trimetrexate is extensively metabolised by the liver and eliminated by hepatic metabolism via the cytochrome P450 system. At least 2 metabolites are active and 2 are conjugated with glucuronic acid. Hepatic degradation may account for the low and variable oral absorption. Approximately 50% of dose is excreted in urine, with 10–30% as the unchanged drug, and 0.1–8% in faeces.

Therapeutic Concentration

Forty-nine adult patients with AIDS and *Pneumocystis jiroveci* (was *P. carinii*) pneumonia (in combination with calcium folinate) were administered with 30 mg/m^2 trimetrexate for 21 days. Peak plasma concentrations of 4.4 mg/L were observed by the end of infusion [Allegra *et al.* 1987].

Six adult patients with AIDS and *P. jiroveci* pneumonia were administered with 30 mg/m^2 trimetrexate daily (in combination with calcium folinate) for 21 days. Peak plasma concentrations of 3.1 mg/L trimetrexate were detected. A dose of 60 mg/m^2 daily for 3 days produced peak concentrations of 1.2 mg/L [Rogers *et al.* 1988].

A child with AIDS and *P. jiroveci* pneumonia was administered with a 30 mg/m^2 dose of trimetrexate daily for 21 days. A peak plasma concentration of 8 mg/L was reached [Smit *et al.* 1990].

Toxicity Administration without concurrent leucovorin (folinic acid) can cause lethal complications.

Half-life 11–16.4 h.

Bioavailability Mean oral bioavailability, 44% (range, 19–67%).

Volume of Distribution Plasma, 0.17 L/kg; steady state, 0.62 L/kg (20 L/m^2); steady state in AIDS patients, 11–20 L/m^2; in cancer patients, 12–42 L/m^2.

Clearance Plasma clearance, 53 mL/min.

Protein Binding >97%.

Dose Trimetrexate daily 45 mg/m^2 (with folinic acid).

Allegra CJ *et al.* (1987). Trimetrexate for the treatment of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 317: 978–985.

Bullen WW *et al.* (1990). High-performance liquid chromatographic assay for trimetrexate in human plasma. *J Chromatogr* 526: 266–272.

Hudes GR *et al.* (1989). Phase I clinical and pharmacologic trial of trimetrexate in combination with 5-fluorouracil. *Cancer Chemother Pharmacol* 24: 117–122.

Hudes GR *et al.* (1991). Pharmacokinetic study of trimetrexate in combination with cisplatin. *Cancer Res* 51: 3080–3087.

Lin JT *et al.* (1987). Phase I studies with trimetrexate: clinical pharmacology, analytical methodology, and pharmacokinetics. *Cancer Res* 47: 609–616.

Rogers P *et al.* (1988). Bioavailability of oral trimetrexate in patients with acquired immunodeficiency syndrome. *Antimicrob Agents Chemother* 32: 324–326.

Smit MJ *et al.* (1990). Trimetrexate efficacy and pharmacokinetics during treatment of refractory *Pneumocystis carinii* pneumonia in an infant with severe combined immunodeficiency disease. *Pediatr Infect Dis J* 9: 212–214.

Stetson PL, Ensminger WD (1986). Determination of plasma trimetrexate levels using gas chromatography–mass spectrometry with selected-ion monitoring. *J Chromatogr* 383: 69–76.

Tinsley PW, LaCreta FP (1990). Improved chromatographic method for the determination of trimetrexate in urine. *J Chromatogr* 529: 468–472.

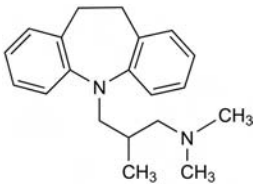
Trimipramine

Antidepressant

$C_{20}H_{26}N_2 = 294.4$

CAS—739-71-9

IUPAC Name 10,11-Dihydro-*N*,*N*,β-trimethyl-5*H*-dibenz[*b*,*f*]azepine-5-propanamine
Synonyms IL-6001; 7162-R; trimeprimine; trimeproprimine.
Proprietary Name *Tydamine*



Chemical Properties Pale yellowish-white waxy solid. Mp 45°. Practically insoluble in water; readily soluble in ethanol. pKa 7.7 [Baselt 2008]. Log *P* (octanol/water), 5.43. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005]. Plasma samples were stable for several months at –20° [Bougerolle *et al.* 1988; Gulaid *et al.* 1991].

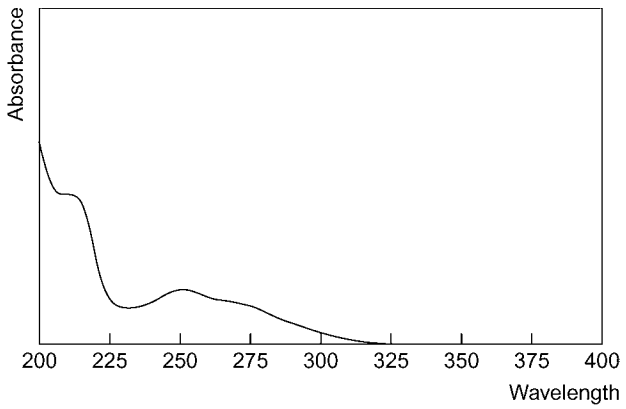
Trimipramine Maleate

$C_{20}H_{26}N_2,C_4H_4O_4 = 410.5$
CAS—521-78-8
Synonym Trimipramine hydrogen maleate
Proprietary Names *Apo-Trimip*; *Herphonal*; *Novo-Tripramine*; *Rhotrimine*; *Stangyl*; *Surmontil*; *Tripress*.
Chemical Properties White to almost-white crystalline powder. Mp 142°. Slightly soluble in water and ethanol; soluble in chloroform; practically insoluble in ether.

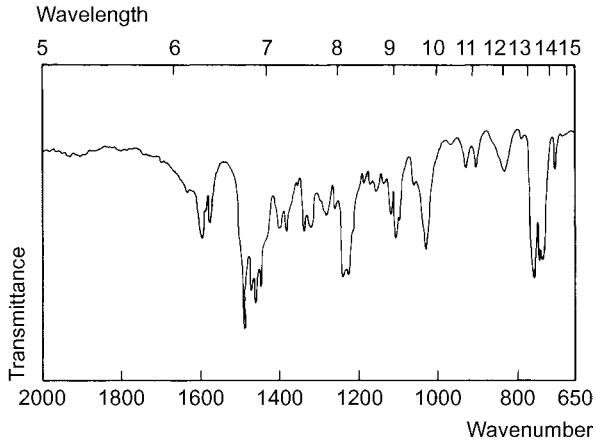
Colour Tests Forrest reagent—blue; FPN reagent—blue; Mandelin's test (add water)—blue.
Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.62; system TC—R_f 0.54; system TE—R_f 0.80; system TL—R_f 0.37; system TAE—R_f 0.36; system TAF—R_f 0.56 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—trimipramine RI 2215; M (OH-) RI 2575; M (OH-methoxy-) RI 2590; M nor- (ring) RI 1930; system GB—trimipramine RI 2302; M (nor-) RI 2335; M (OH-) RI 2631; M (nor-OH-) RI 2662; M (OH-methoxy-) RI 2715; M nor-(ring) RI 2107; system GF—RI 2505; system GS—RT 16.3 min.

Column: OV-1 fused silica (25 m × 0.32 mm i.d., 0.2 μm). Temperatures: 195°. Carrier gas: He, 2.5 mL/min. NPD. ISs: clomipramine and maprotiline. RT: trimipramine, 5.9 min; desmethyltrimipramine, 6.9 min; 2-hydroxytrimipramine, 16.0 min; 2-hydroxydesmethyltrimipramine, 18.0 min; clomipramine, 12.0 min; maprotiline, 8.5 min [Jourdil *et al.* 1993].
High Performance Liquid Chromatography System HA—trimipramine *k* 2.7; M (nor-) *k* 1.8; system HF—*k* 6.17; system HX—RI 454; system HY—RI 345; system HZ—RT 8.3 min; system HAA—RT 15.9 min; system HAX—RT 15.5 min; system HAY—RT 7.7 min.

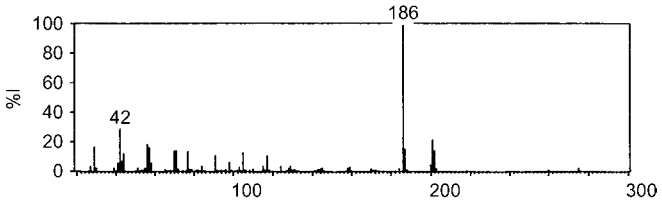
Column: Chiralcel OD-R (250 × 4.6 mm i.d., 10 μm). Mobile phase: 0.3 mol/L aqueous sodium perchlorate : acetonitrile (58 : 42), flow rate 0.5 mL/min. UV detection (λ = 210 nm). *k* values (RTs): *R*-trimipramine, 2.41 (19.6 min); *S*-trimipramine, 2.87 (22.3 min) [Liu, Stewart 1997].
Ultraviolet Spectrum Aqueous acid—250 nm ($A_1^1 = 300a$)



Infrared Spectrum Principal peaks at wavenumbers 1490, 762, 1240, 1230, 741, 749 (KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 249, 208, 99, 193, 234, 84, 248.



Quantification

Blood **GC** Column: DB-1 (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 4 mL/min. Temperature programme: 100° for 1 min to 300° at 30°/min. FID. Limit of detection, 32 μg/L [Lee *et al.* 1997]. Column: 3% SP2100 mesh supelcoport 100/120 mesh (1.8 m × 2 mm i.d.). Carrier gas: N_2, 25 mL/min. Temperature programme: 170° to 275° at 8°/min for 5 min. FID. Limit of detection not reported [Meatherall *et al.* 1983]. Column: 3% OV-101 on Chromosorb WHP 80/100 mesh (1.2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 190° for 2 min to 240° at 20°/min for 5 min. FID. Limit of detection, 0.5 mg/L [Wu Chen *et al.* 1983].

HPLC Column: RP-8 (250 × 4.6 mm i.d., 5 μm). Mobile phase: 0.01 mol/L potassium dihydrogen phosphate : acetonitrile : *n*-nonylamine (pH 3.2, 550 : 450 : 0.6), flow rate 1.6 mL/min. UV detection (λ = 205 nm). Retention time: ≈7 min. Limit of detection not reported [Fraser *et al.* 1987].

Plasma **GC** Column: DB-17 (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: N_2, 0.7 mL/min. Temperature programme: 140° to 220° at 20°/min to 270° at 2°/min. NPD, EI ionisation, SIM acquisition mode. Retention time: 16.3 min. Limit of quantification, 125 μg/L [Ulrich, Martens 1997]. NPD. Limit of detection, 3 μg/L for trimipramine and 4 μg/L for desmethyltrimipramine and hydroxytrimipramine [Jourdil *et al.* 1993]. Column: 3% SP-2250 on 80/100 Supelcoport (6′ × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature: 230°. NPD. Retention time: 4.14 min. Limit of detection, 0.5 μg/L [Abernethy *et al.* 1984].

GC-MS Column: DB-5MS fused silica capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He, 2.0 mL/min. Temperature programme: 100° for 1 min to 300° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 5 μg/L, limit of detection, 2.0 μg/L [Lee *et al.* 2008]. Column: SE-54 (12 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 60 mL/min. Temperature programme: 160° to 260° at 30°/min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 3.11 min. Limit of quantification, 4 μg/L for trimipramine and 2 μg/L for desmethyltrimipramine, limit of detection, 1 μg/L [Eap *et al.* 1994]. Column: SE 30 (25 m × 0.25 mm i.d.). Carrier gas: He. Temperature programme: 80° for 1 min to 270° at 30°/min. EI ionisation. Retention time: 9.62 min. Limit of detection, 1 μg/L [Bougerolle *et al.* 1988].

HPLC Column: Spherisorb (25 cm × 4.6 mm i.d., 5 μm). Mobile phase: dipotassium hydrogen orthophosphate (pH 6.5) : methanol : acetonitrile (1 : 1 : 1), flow rate, 1.0 mL/min. Electrochemical detection. Limit of quantification, 1 μg/L [Gulaid *et al.* 1991]. Column: trimethyl silyl (25 cm × 4.6 mm i.d., 5 μm). Mobile phase: 0.05 mol/L monobasic potassium phosphate : acetonitrile (65 : 35) plus 1.2 mL/L *n*-butylamine and 1.0 mL/L phosphoric acid, flow rate 1.5 mL/min. Electrochemical detection. Limit of quantification, ≈3 μg/L [Suckow, Cooper 1984]. Column: μBondapak CN (30 cm × 3.9 mm i.d., 10 μm). Mobile phase: acetonitrile : methanol : phosphate buffer (pH 7.0, 625 : 155 : 220), flow rate 1.5 mL/min. UV detection (λ = 254 nm). Retention time: 3.59 min. Limit of detection, 2 μg/L [Visser *et al.* 1984].

Serum **GC** Column: 1.4% Carbowax 20M and 1.4% KOH on Gas-Chrom Q 60/80 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 35 mL/min. Temperature: 200°. Relative retention time: 0.44. Limit of detection not reported [Nyberg, Martensson 1977].

T

HPLC Column: LiChrospher 100 CN. Mobile phase: 5 mmol/L sodium phosphate buffer (pH 6.0) : methanol : acetonitrile (22 : 32 : 46), flow rate 1.5 mL/min. UV detection (λ = 250 nm). Limit of quantification, 1 µg/L [Kirchheiner *et al.* 2003]. UV detection (λ = 210 nm). Limit of quantification, 15 µg/L, limit of detection, 10 µg/L [Liu, Stewart 1997]. Column: µBondapak C_{18} (30 cm × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.025 mol/L potassium dihydrogen phosphate : water (45 : 50 : 5), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Retention time: 10.1 min. Limit of detection, 4–5 µg/L [Pok Phak *et al.* 1986].

Red Blood Cells **GC** See Plasma [Jourdil *et al.* 1993].

Urine **GC-MS** Column: HP capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 10°/min for 5 min. EI ionisation, scan mode. Limit of detection not reported [Maurer 1989]. Column: SE-54 (25 m × 0.32 mm i.d.). Temperature programme: 75° to 300° at 25°. EI ionisation at 70 eV, CI. Limit of detection not reported [Köppel, Tenczer 1988].

HPLC See Serum. Mobile phase: 5 mmol/L sodium phosphate buffer (pH 6.0) : methanol : acetonitrile (25 : 32 : 43), flow rate 0.8 mL/min [Kirchheiner *et al.* 2003]. See Blood [Fraser *et al.* 1987].

Hair **LC-MS** Column: Luna C_{18} (15 cm × 4.6 mm i.d.). Mobile phase: 0.02 mol/L ammonium acetate and 0.1% acetic acid in water : acetonitrile (35 : 65), flow rate 0.5 mL/min. ESI. Limit of detection, 20 nmol/L [McClean *et al.* 2000].

Liver **HPLC** See Blood [Fraser *et al.* 1987].

Disposition in the Body Readily absorbed after oral administration but undergoes considerable first-pass metabolism to the active metabolite, *N*-monodesmethyltrimipramine; bioavailability ~40% with wide inter-subject variation. Didesmethyltrimipramine, 2-hydroxytrimipramine and 2-hydroxydesmethyltrimipramine have also been detected as metabolites. Demethylation is catalysed not only by CYP2C9 and CYP2C19 but also CYP1A2 and CYP3A4 [Kirchheiner *et al.* 2003]. Excreted in the urine mainly as metabolites.

Therapeutic Concentration

A single oral dose of 50 mg administered to 9 subjects, resulted in peak plasma concentrations of 15–51 µg/L (mean 28) in 1–6 h [Abernethy *et al.* 1984].

After daily oral doses of 75 or 150 mg to 29 subjects, the following steady-state plasma concentrations were reported: trimipramine 11–241 µg/L (mean 86), *N*-monodesmethyltrimipramine 3–382 µg/L (mean 65), 2-hydroxytrimipramine 3–40 µg/L (mean 16), and 2-hydroxy-*N*-monodesmethyltrimipramine 3–49 µg/L (mean 17) [Suckow, Cooper 1984].

Ten patients receiving 75 mg of trimipramine daily had a mean steady-state plasma concentration of 53.8 µg/L trimipramine and 26.3 µg/L desmethyltrimipramine, whereas 10 others receiving 150 mg daily had a mean concentration of 122.5 µg/L trimipramine and 133.8 µg/L desmethyltrimipramine, representing a markedly disproportionate increase [Musa 1989].

After a single oral dose of 50 mg to a healthy volunteer the maximum plasma concentration was 16 µg/L at 3–4 h [Gulaid *et al.* 1991].

Toxicity Toxic effects have been associated with plasma concentrations >1 mg/L, serum concentrations >0.5 mg/L. A lethal serum concentration of 8.7 mg/L has been noted.

In a fatality involving the ingestion of up to 6 g of trimipramine, the following postmortem tissue concentrations were reported: blood 9.5 mg/L, brain 27 µg/g, kidney 42 µg/g, liver 224 µg/g, spleen 31 µg/g [Rousseau, Rousseau 1970].

In a fatality involving the ingestion of trimipramine and clomipramine, postmortem concentrations were: blood, trimipramine 12 (11.6) mg/L, clomipramine 2.1 mg/L; liver, trimipramine 544 µg/g, clomipramine 56 µg/g [Hucker 1983]

A 25-year-old was found dead in his kitchen. The trimipramine and its metabolites were found at postmortem as shown below.

	Blood (mg/L)	*Urine (mg/L)*	*Liver (mg/kg)*
Trimipramine	4.8	0.58	51
Desmethyltrimipramine	2.1	0.1	16
2-OH trimipramine	–	0.1	1.5
2-OH desmethyltrimipramine	–	0.09	1.1
Ethyl alcohol	200	1500	–
Gastric contents	Trimipramine positive		
Vitreous humour	Ethylalcohol 450 mg/L		

[Fraser *et al.* 1987]

Investigation into the death of a 53-year-old female concluded that she had taken an acute (suicidal) overdose of trimipramine and citalopram. Postmortem revealed the tissue concentrations (in mg/L or µg/kg; TM = trimipramine; DTM = desmethyltrimipramine; CP = citalopram; DCP = desmethylcitalopram) shown in the table above.

	TM	*DTM*	*CP*	*DCP*
Heart blood	11.81	4.07	4.82	2.30
Femoral blood	2.33	1.13	4.81	2.45
Liver	38.35	12.06	11.89	5.89
Kidney	30.57	–	13.34	–
Urine	8.46	–	13.00	–
Brain	10.39	–	1.98	–
Bile	–	–	3.31	–

Zolpidem was also detected (0.07 mg/L in heart blood, 1.12 mg/L in femoral blood, 0.19 µg/g in the kidney) [Musshoff *et al.* 1999].

Half-life Plasma half-life, 16–40 h (mean 24).

Volume of Distribution ≈20–50 L/kg (mean 30).

Clearance Plasma clearance, 10–25 mL/min/kg (mean 16).

Distribution in Blood Plasma : whole blood ratio, 1.2.

Protein Binding ≈94%.

Dose Initially, 50 to 75 mg trimipramine daily; increased as necessary to 150 to 300 mg. In the elderly, 10 to 25 mg is administered initially and increased as necessary. (In the USA, initially 50 mg is administered and a maximum of 100 mg to the elderly).

Abernethy DR *et al.* (1984). Trimipramine kinetics and absolute bioavailability: use of gas-liquid chromatography with nitrogen-phosphorus detection. *Clin Pharmacol Ther* 35: 348–353.

Baselt RC (2008). *Disposition of Toxic Drugs and Chemicals in Man*, 8th edn, Foster City, CA: Biomedical Publications.

Bougerolle AM *et al.* (1988). Simultaneous determination of trimipramine and its demethylated metabolites in plasma by gas chromatography-mass spectrometry. *J Chromatogr* 434: 232–238.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Eap CB *et al.* (1994). Determination of trimipramine and its demethylated and hydroxylated metabolites in plasma by gas chromatography-mass spectrometry. *J Chromatogr* 652: 97–103.

Fraser AD *et al.* (1987). Distribution of trimipramine and its major metabolites in a fatal overdose case. *J Anal Toxicol* 11: 168–170.

Gulaid AA *et al.* (1991). Simultaneous determination of trimipramine and its major metabolites by high-performance liquid chromatography. *J Chromatogr* 566: 228–233.

Hucker RS (1983). A fatal clomipramine and trimipramine poisoning. *TIAFT Bull* 17: 20–22.

Jourdil N *et al.* (1993). Simultaneous determination of trimipramine and desmethyl- and hydroxytrimipramine in plasma and red blood cells by capillary gas chromatography with nitrogen-selective detection. *J Chromatogr* 613: 59–65.

Kirchheiner J *et al.* (2003). Trimipramine pharmacokinetics after intravenous and oral administration in carriers of CYP2D6 genotypes predicting poor, extensive and ultrahigh activity. *Pharmacogenetics* 13: 721–728.

Köppel C, Tenczer J (1988). Gas chromatographic-mass spectrometric study of the urinary metabolism of trimipramine. *J Chromatogr* 431: 197–202.

Lee XP *et al.* (1997). Detection of tricyclic antidepressants in whole blood by headspace solid-phase microextraction and capillary gas chromatography. *J Chromatogr Sci* 35: 302–308.

Lee XP *et al.* (2008). Determination of tricyclic antidepressants in human plasma using pipette tip solid-phase extraction and gas chromatography-mass spectrometry. *J Sep Sci* 31: 2265–2271.

Liu J, Stewart JT (1997). Quantitation of trimipramine enantiomers in human serum by enantioselective high-performance liquid chromatography and mixed-mode disc solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 700: 175–182.

Maurer H (1989). Metabolism of trimipramine in man. *Arzneimittelforschung* 39: 101–103.

McClean S *et al.* (2000). Electrospray ionisation-mass spectrometric characterisation of selected anti-psychotic drugs and their detection and determination in human hair samples by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 740: 141–157.

Meatherall RC *et al.* (1983). Toxicological findings in a death resulting from the ingestion of trimipramine. *J Forensic Sci* 28: 1023–1029.

Musa MN (1989). Nonlinear kinetics of trimipramine in depressed patients. *J Clin Pharmacol* 29: 746–747.

Musshoff F *et al.* (1999). Fatality caused by a combined trimipramine-citalopram intoxication. *Forensic Sci Int* 106: 125–131.

Nyberg G, Martensson E (1977). Quantitative analysis of tricyclic antidepressants in serum from psychiatric patients. *J Chromatogr* 143: 491–497.

Pok Phak R *et al.* (1986). Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography. *J Chromatogr* 375: 339–347.

Rousseau JJ, Rousseau M (1970). Fatal trimipramine poisoning. *TIAFT Bull* 7: 5–6.

Suckow RF, Cooper TB (1984). Determination of trimipramine and metabolites in plasma by liquid chromatography with electrochemical detection. *J Pharm Sci* 73: 1745–1748.

Ulrich S, Martens J (1997). Solid-phase microextraction with capillary gas-liquid chromatography and nitrogen-phosphorus selective detection for the assay of antidepressant drugs in human plasma. *J Chromatogr B Biomed Sci Appl* 696: 217–234.

Visser T *et al.* (1984). Reliable routine method for the determination of antidepressant drugs in plasma by high-performance liquid chromatography. *J Chromatogr* 309: 81–93.

Wu Chen NB *et al.* (1983). Analysis of blood and tissue for amoxapine and trimipramine. *J Forensic Sci* 28: 116–121.

Trioxysalen

Pigmenting Agent

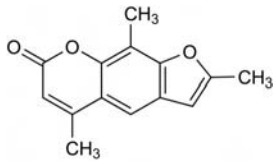
$C_{14}H_{12}O_3$ = 228.2

CAS—3902-71-4

IUPAC Name 2,5,9-Trimethylfuro[3,2-*g*]chromen-7-one

Synonyms 2,5,9-Trimethyl-7*H*-furo[3,2-*g*][1]benzopyran-7-one; 4,5′,8-trimethylpsoralen; trioxsalen.

Proprietary Names *Puvadin*; *Tripsor*; *Trisoralen*.

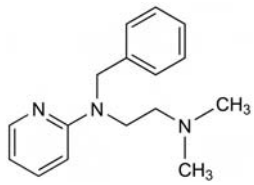


Chemical Properties A white or greyish crystalline solid. Mp about 230°. Practically insoluble in water; soluble 1 in 1150 of ethanol, 1 in 84 of chloroform and 1 in 43 of methylene chloride. Log *P* (octanol/water), 3.7.
Gas Chromatography System GA—RI 2155.
Ultraviolet Spectrum Methanol—248 (A^{1}_{1}=981b), 296 (A^{1}_{1}=428b), 338 nm (A^{1}_{1}=276b).
Infrared Spectrum Principal peaks at wavenumbers 1710, 1600, 1110, 880, 930, 1620 cm^{-1} (Nujol mull).
Mass Spectrum Principal ions at *m/z* 228, 200, 199, 128, 229, 185, 115, 201.
Quantification
Blood **GC–MS** For method, see Ros *et al.* [1988].
HPLC UV detection. Limit of detection, 2 µg/L [Chakrabarti *et al.* 1982].
Ophthalmic Fluid **HPLC** See Blood [Chakrabarti *et al.* 1982].
Skin **GC–MS** See Blood [Ros *et al.* 1988].
HPLC See Blood [Chakrabarti *et al.* 1982].
Dose 5 to 10 mg daily.

Chakrabarti SG *et al.* (1982). Determination of trimethylpsoralen in blood, ophthalmic fluids, and skin. *J Invest Dermatol* 79: 374–377.
Ros AM *et al.* (1988). Concentration of trimethylpsoralen in blood and skin after oral administration. *Photodermatology* 5: 121–125.

Tripelennamine

Antihistamine
$C_{16}H_{21}N_3$ = 255.4
CAS—91-81-6
IUPAC Name *N*-Benzyl-*N*′,*N*′-dimethyl-*N*-pyridin-2-ylethane-1,2-diamine
Synonym *N*,*N*-Dimethyl-*N*′-(phenylmethyl)-*N*′-2-pyridinyl-1,2-ethanediamine



Chemical Properties An oily liquid. Miscible with water. pK_a 4.2, 8.71 [Martínez-Algaba *et al.* 2006], 3.9, 9.0(25°). Log *P* (octanol/water), 2.85 [Martínez-Algaba *et al.* 2006], 2.7.

Tripelennamine Citrate

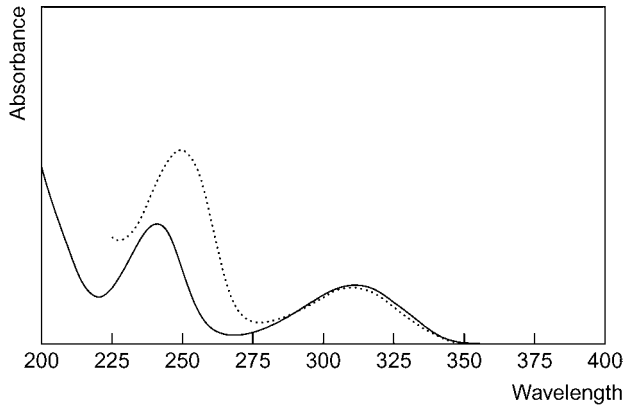
$C_{16}H_{21}N_3,C_6H_8O_7$ = 447.5
CAS—6138-56-3
Proprietary Name *PBZ* (elixir)
Chemical Properties A white crystalline powder. Mp 106° to 110°. Freely soluble in water and ethanol; practically insoluble in benzene and chloroform; very slightly soluble in ether.

Tripelennamine Hydrochloride

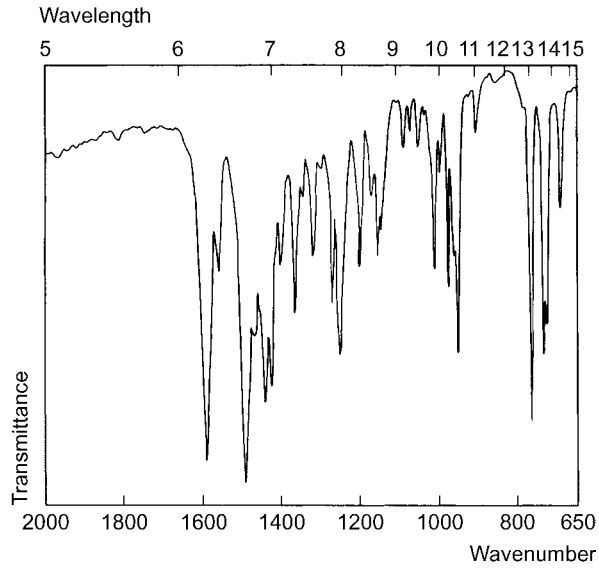
$C_{16}H_{21}N_3$,HCl = 291.8
CAS—154-69-8
Proprietary Names *Azaron*; *Etono*; *Fenistil*; *PBZ* (tablets); *Pyribenzamine*; *Vaginex*.
Chemical Properties A white crystalline powder which slowly darkens on exposure to light. Mp 192° to 193°. Soluble 1 in approx. 0.8 of water, 1 in 6 of ethanol, 1 in 6 of chloroform and 1 in ~350 of acetone; practically insoluble in ether and benzene.

Colour Tests Cyanogen bromide—yellow; Liebermann's reagent—orange→brown; Mandelin's test—yellow-brown; Marquis test—red-brown.
Thin-layer Chromatography System TA—R_f 0.55; system TB—R_f 0.44; system TC—R_f 0.27; system TE—R_f 0.68; system TL—R_f 0.15; system TAE—R_f 0.22; system TAF—R_f 0.34 (Dragendorff spray—positive; acidified iodoplatinate solution—positive; Marquis test—brown).
Gas Chromatography System GA—tripelennamine RI 1976, M (OH-) RI 2400 , M (OH-)(-AC) RI 2390, M (benzylpyridylamine) RI 1650 , M (nor-) RI 2420, M (nor-)(-AC) RI 2420 , M (nor-OH-)(-AC) RI 2860.
High Performance Liquid Chromatography System HA—*k* 3.6; system HX—RI 336; system HY—RI 265.

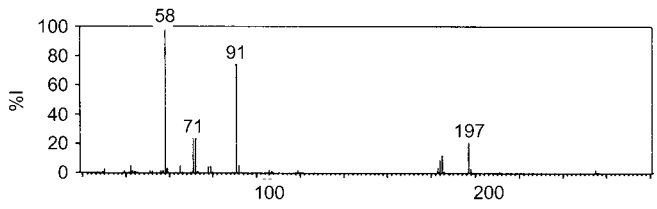
Column: Spherisorb ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.04 mol/L CTAB : butan-1-ol (pH 3.0, 90 : 10), flow rate 1.0 mL/min. UV detection (λ= 240 nm). Limit of detection, 0.3 mg/L [Martínez-Algaba *et al.* 2006]. Column: ODS-2 (120 × 4.6 mm i.d., 5 m). Mobile phase: methanol : water (60 : 40). UV detection (λ= 285 nm). Retention time: 2.1 min. Limit of detection, 40 µg/L [Gil-Agusti *et al.* 2001].
Ultraviolet Spectrum Aqueous acid—239 nm (A^{1}_{1} = 562a), 314 nm; aqueous alkali—249, 312 nm.



Infrared Spectrum Principal peaks at wavenumbers 1496, 1592, 770, 1250, 955, 735 cm^{-1} (tripelennamine hydrochloride, KBr disk).



Mass Spectrum Principal ions at *m/z* 58, 91, 72, 71, 197, 185, 184, 92.



Quantification
Blood **GC** FID [Monforte *et al.* 1983]. Column: 3% OV-17 on Chromosorb W HP 80/100 mesh (1.8 m × 4 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 220°. AFID. Retention time: 6.9 min. Limit of detection, 0.1 mg/L [Mackell, Poklis 1982]. Column: 3% OV-17 Gas-Chrom W HP (6 ft). Carrier gas: He, 50 psi. Temperature programme: 115° for 3 min to 260° at 16°/min for 12 min. FID. Retention time: 0.72 min. Limit of detection not reported [Bayley *et al.* 1975].
Plasma **GC** Column: 3% OV-17 on 120/140 mesh GasChrom Q (1.8 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature programme: 190° to 260° at 10°/min. TID. Limit of detection, 5 µg/L [Yeh *et al.* 1986].
Urine **GC** Column: 3% OV-17 on 120/140 mesh GasChrom Q (180 cm × 2 mm i.d.). Temperature programme: 190° to 250° at 10°/min. NPD. Retention time: 3.1 min. Limit of detection, 0.5 mg/L [Yeh 1991]. Column: 3% OV-17 on Gas-Chrom Q 80/100 mesh (1.8 m × 2 mm i.d.). Carrier gas: CH_4, 30 mL/min. Temperature programme: 150° to 250° at 10°/min. FID. Limit of detection, not reported [Reid, Gerbeck 1981]. See Blood [Bayley *et al.* 1975].

HPLC Column: (250 × 4.6 mm i.d.). Mobile phase: 0.005 mol/L tetraethylammonium in dichloromethane: propan-2-ol (99 : 1), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 310 nm, λ_{em} = 360 nm). Retention time: 7.4 min. Limit of detection, 10 ng/g [Thompson *et al.* 1983]

Gastric Contents **GC** See Blood [Bayley *et al.* 1975].

Brain **GC** See Blood [Bayley *et al.* 1975].

Kidney **GC** See Blood [Bayley *et al.* 1975; Monforte *et al.* 1983].

Liver **GC** See Blood [Bayley *et al.* 1975; Monforte *et al.* 1983].

Other **GC** Bovine Milk. Column: borosilicate 100/120 GasChrom S (2.4 m × 4 mm i.d.). Carrier gas: N_2, 60 mL/min. Temperature: 218°. Limit of detection, 10 ppb [Luders *et al.* 1970].

HPLC Bovine Plasma and Milk. Column: 3CN (100 × 4.6 mm i.d.). Mobile phase: water: acetonitrile-0.05 mol/L acetate buffer (pH 7.2, 70 : 30), flow rate 0.8 mL/min. UV detection (λ = 246 nm). Limit of detection, 2 µg/L [Dadgar, Power 1987].

Disposition in the Body Tripelennamine is rapidly absorbed after oral administration. It is metabolised by ring hydroxylation and demethylation, followed by conjugation with glucuronic acid. It is excreted in the urine, mainly as metabolites, and in the bile, and it appears to undergo enterohepatic circulation. The major urinary metabolites are tripelennamine-*N*-glucuronide, conjugated hydroxytripelennamine and the conjugated hydroxydesmethyl metabolite; the *N*-oxide is a minor metabolite.

Therapeutic Concentration After a single oral dose of 100 mg, a peak plasma concentration of 0.06 mg/L was reported to have been attained in 2–3 h [Bayley *et al.* 1975].

Toxicity

Fatal intracranial haemorrhage occurred in a 30-year-old man following IV abuse of pentazocine and tripelennamine (~125 mg of each was injected over the 6 h before admission) together with oral phenylpropanolamine (a 75 mg extended-release preparation was taken 20 h before onset of neurological symptoms); gastric aspirate at admission contained pentazocine 8.2 mg/L and tripelennamine 2.0 mg/L [Jackson *et al.* 1985].

In a fatality arising from the ingestion of ~1 g, in which death occurred ~7 h after ingestion, the following postmortem tissue distribution was reported: blood 10 mg/L, brain 43 µg/g, kidney 35 µg/g, liver 83 µg/g and urine 287 mg/L [Bayley *et al.* 1975].

In 17 fatalities involving the IV abuse of tripelennamine and pentazocine, tripelennamine blood concentrations of 0 to 3.0 mg/L (mean 0.5) were reported; kidney concentrations of 0.4 to 2.0 µg/g (mean 1.1) were reported in 4 subjects and liver concentrations of 1.0–6.5 µg/g (mean 3.1 µg/g) in 5 subjects; pentazocine blood concentrations ranged from 0 to 11 mg/L (mean 3 mg/L) [Monforte *et al.* 1983].

Dose Tripelennamine hydrochloride 25 to 50 mg every 4 to 6 h; up to 600 mg daily has been given.

Bayley M *et al.* (1975). Report of a fatal, acute tripelennamine intoxication. *J Forensic Sci* 20: 539–543.

Dadgar D, Power A (1987). Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk. *J Chromatogr* 421: 216–222.

Gil-Agusti M *et al.* (2001). Quantitation of antihistamines in pharmaceutical preparations by liquid chromatography with a micellar mobile phase of SDS and pentanol. *J AOAC Int* 84: 1687–1694.

Jackson C *et al.* (1985). Fatal intracranial hemorrhage associated with phenylpropanolamine, pentazocine, and tripelennamine overdose. *J Emerg Med* 3: 127–132.

Luders RC *et al.* (1970). Determination of pyribenzamine (tripelennamine) residues in bovine milk. *J Agric Food Chem* 18: 1153–1155.

Mackell MA, Poklis A (1982). Determination of pentazocine and tripelennamine in blood of T's and Blue addicts by gas–liquid chromatography with a nitrogen detector. *J Chromatogr* 235: 445–452.

Martínez-Algaba C *et al.* (2006). Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography. *J Pharm Biomed Anal* 40: 312–321.

Monforte JR *et al.* (1983). Toxicological and pathological findings in fatalities involving pentazocine and tripelennamine. *J Forensic Sci* 28: 90–101.

Reid RW, Gerbeck CM (1981). Detection of pentazocine and tripelennamine in urine. *Clin Chem* 27: 10–13.

Thompson HCJr *et al.* (1983). Trace determination of the antihistamines tripelennamine hydrochloride, thenyldiamine hydrochloride, and chlorothen citrate in admixture in animal feed, human urine, and wastewater by high-pressure liquid chromatography and use of a fluorescence detector. *Talanta* 30: 251–260.

Yeh SY (1991). Metabolic profile of tripelennamine in humans. *J Pharm Sci* 80: 815–819.

Yeh SY *et al.* (1986). The pharmacokinetics of pentazocine and tripelennamine. *Clin Pharmacol Ther* 39: 669–676.

Triphenyltetrazolium Chloride

Diagnostic Agent

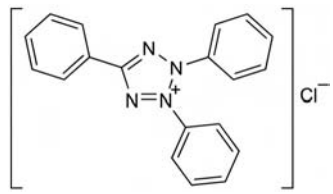
$C_{19}H_{15}ClN_4$ = 334.8

CAS—298-96-4

IUPAC Name 2,3,5-Triphenyltetrazol-2-ium chloride

Synonyms Red tetrazolium; RT; TPTZ; 2,3,5-triphenyl-2*H*-tetrazolium chloride; TTC.

Proprietary Name *VitaStain*



Chemical Properties A white crystalline powder which turns yellow on exposure to light. Mp 243°, with decomposition. Soluble in water, ethanol and acetone; insoluble in ether. Log *P* (octanol/water), −2.4.

Colour Tests Liebermann's reagent—red-orange; Mandelin's test—blue (slow).

Thin-layer Chromatography System TA—R_f 0.61 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—246 nm (A^1_1=680b).

Infrared Spectrum Principal peaks at wavenumbers 691, 769, 1529, 720, 1164, 998 cm^{-1} (KBr disk).

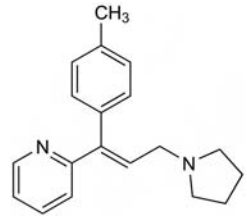
Triprolidine

Antihistamine

$C_{19}H_{22}N_2$ = 278.4

CAS—486-12-4

IUPAC Name 2-[(*E*)-1-(4-Methylphenyl)-3-pyrrolidin-1-ylprop-1-enyl]pyridine



Chemical Properties Crystals. Mp 59° to 61°. pK_a 6.5. Log *P* (octanol/water), 3.9.

Triprolidine Hydrochloride

$C_{19}H_{22}N_2$,HCl, H_2O = 332.9

CAS—550-70-9 (anhydrous); 6138-79-0 (monohydrate)

Proprietary Names *Actidil*; *Actidilon*; *Myidyl*; *Pro-Actidil*. It is an ingredient of *Actifed*.

Chemical Properties A white crystalline powder. Mp 116° to 118°. Soluble 1 in about 2 of water, 1 in about 1.5 of ethanol, 1 in <1 of chloroform and 1 in 2000 of ether.

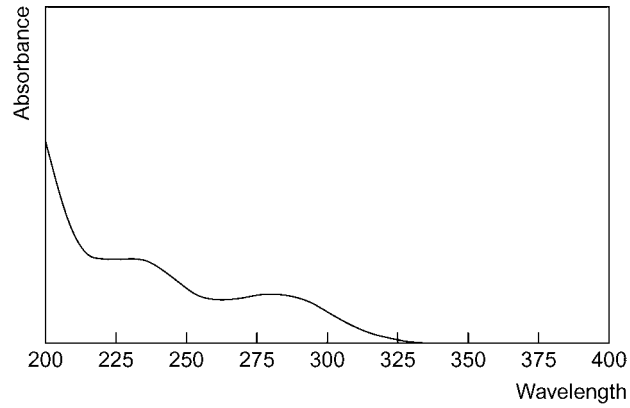
Colour Tests Cyanogen bromide—orange-pink (after 1 to 2 min); Liebermann's reagent—orange.

Thin-layer Chromatography System TA—R_f 0.51; system TB—R_f 0.11; system TC—R_f 0.20; system TE—R_f 0.55; system TL—RF 06; system TAE—R_f 0.19; system TAF—R_f 0.30; system TAJ—R_f 0.95; system TAK—R_f 0.03; system TAL—R_f 0.69 (Dragendorff spray, positive; acidified iodoplatinate solution, positive).

Gas Chromatography System GA—RI 2253; system GB—RI 2340; system GC—RI 2954; system GF—RI 2600.

High Performance Liquid Chromatography System HA—*k* 3.2; system HX—RI 388; system HY—RI 270; system HAA—retention time 13.1 min.

Ultraviolet Spectrum Aqueous acid—290 nm (A^1_1=347a).



Infrared Spectrum Principal peaks at wavenumbers 775, 1580, 825, 990, 1509, 1146 cm^{-1} (triprolidine hydrochloride, KBr disk).

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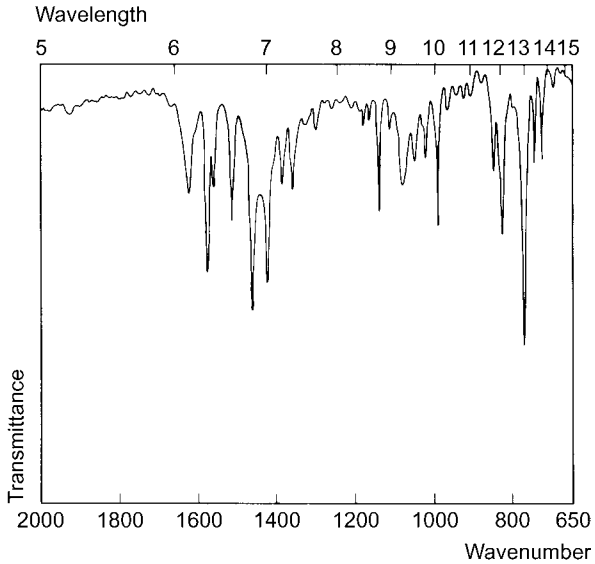
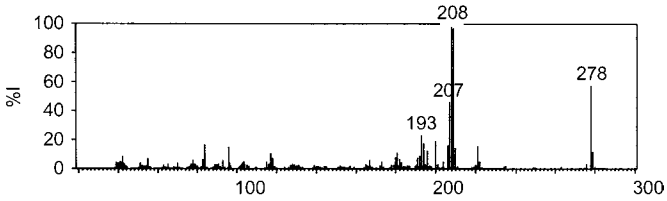


Mass Spectrum Principal ions at *m/z* 208, 209, 278, 207, 193, 200, 194, 84.



Quantification
Plasma **TLC–densitometry** Limit of detection, 800 ng/L [DeAngelis *et al.* 1977].
Biological Samples **HPLC–MS** Limit of detection, 10 µg for triprolidine and its metabolite hydroxymethyltriprolidine [Hansen *et al.* 1989].
Disposition in the Body Irregularly absorbed after oral administration.
Therapeutic Concentration

Following oral administration of 15 mL of a syrup containing 3.75 mg of triprolidine hydrochloride to 16 subjects, peak plasma concentrations of 0.004 to 0.017 mg/L (mean, 0.009) were attained in about 2 h [DeAngelis *et al.* 1977].

Following oral administration of 2.5 mg of triprolidine hydrochloride and 60 mg of pseudoephedrine hydrochloride four times a day to 17 subjects for 5 days, peak plasma-triprolidine concentrations of 0.004 to 0.044 mg/L (mean, 0.013) were reported about 1.6 h after a dose [Perkins *et al.* 1980].

In 6 subjects receiving single oral doses of triprolidine hydrochloride (2.5 mg), peak plasma concentrations of 0.0027 to 0.0085 mg/L (mean, 0.0056) were achieved at 0.8 to 3.2 h (mean, 2.0). After wearing transdermal patches releasing triprolidine (base) 5 or 10 mg for 24 h, the same subjects had peak plasma concentrations of 0.001 to 0.003 mg/L (mean, 0.002) at 6.1 to 17.9 h (mean, 12.0) and 0.0022 to 0.0062 mg/L (mean, 0.0042) at 4.4 to 24.2 h (mean, 14.3), respectively [Miles *et al.* 1990].

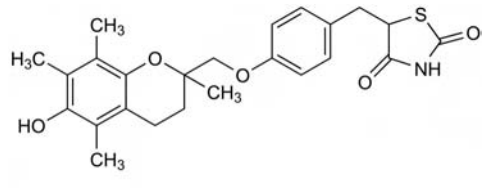
Half-life Plasma half-life, 1.5 to 20 h (mean, 5).
Dose Usually 7.5 to 15 mg of triprolidine hydrochloride daily.

DeAngelis RL *et al.* (1977). Determination of triprolidine in human plasma by quantitative TLC. *J Pharm Sci* 66: 841–843.
Hansen EB *et al.* (1989). Application of HPLC-thermospray ionization mass spectrometry for the analysis of triprolidine and its metabolite hydroxymethyltriprolidine in biological samples. *J Anal Toxicol* 13: 185–187.
Miles MV *et al.* (1990). Pharmacokinetics of oral and transdermal triprolidine. *J Clin Pharmacol* 30: 572–575.
Perkins JG *et al.* (1980). *Curr Ther Res* 28: 650–668.

Troglitazone

Antidiabetic
$C_{24}H_{27}NO_5S = 441.5$
CAS—97322-87-7

IUPAC Name 5-[[4-[(6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydrochromen-2-yl)methoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione
Synonyms CL-991; CS-045; 5-[[4-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)-methoxy]phenyl]methyl]-2,4-thiazolidineodione; GR-92132X; romglizone.
Proprietary Names *Noscal*; *Rezulin*; *Romozin*.



Chemical Properties A white to yellowish crystalline compound with a characteristic odour. Mp 184° to 186°. Practically insoluble in water; soluble in *N,N*-dimethylformamide and acetone; sparingly soluble in ethyl acetate; slightly soluble in acetonitrile, anhydrous ethanol and ether.
Ultraviolet Spectrum Acetonitrile—225 nm.
Quantification
Blood **HPLC** Column: ODS (300 × 6.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water : phosphoric acid (60 : 40 : 0.08), flow rate 1.2 mL/min. UV detection (λ=230 nm). Retention time: 23.2 min. Limit of quantification, 0.1 mg/L [Loi *et al.* 1997].
Plasma **GC** Limit of quantification, 40 nmol/L [Bjork, Pettersson 1990].
Other **HPLC** Tablets. UV detection (λ=225 nm). Limit of detection, 0.05 mg/L [Lambropoulos, Bergholdt 2000].
Disposition in the Body Troglitazone is rapidly absorbed and widely distributed into body tissues following oral administration. Absorption is increased in the presence of food, and peak plasma concentrations are reached generally 1–3 h after administration. It is extensively metabolised in the liver primarily by conjugation, and is excreted largely in the faeces as its metabolites. A few metabolites are also excreted in urine (3%). Steady state concentrations are achieved between 2 and 6 weeks. Three metabolites have been identified, a sulfate conjugate, a quinone conjugate and a glucuronide conjugate. The sulfate conjugate is the most abundant (7–10 times that of the parent drug). Eighty-five percent of the drug is recovered in faeces and 3.1% in urine. Pharmacokinetics are linear over clinical dosage range of 200–600 mg. No accumulation of the drug or its metabolites has been noted.
Therapeutic Concentration

Twelve healthy volunteers and 12 patients with type 2 diabetes were administered 400 mg troglitazone once a day for 15 days. Steady state plasma concentrations were reached by day 5 and peaked at 1.42 mg/L for the healthy individuals and 1.54 mg/L for those with diabetes. These concentrations were observed at 2.63 and 3.25 h, respectively [Loi *et al.* 1997].

Toxicity

A 44-year-old woman with a history of type 1 diabetes began treatment with 200 mg troglitazone daily for 4 weeks and then the dose was increased to 400 mg daily. On hospital admission she was icteric, her liver was tender and enlarged with subacute hepatic necrosis, fibrosis and cholestasis. Troglitazone treatment was stopped and after 3 months the patient gradually improved.

A 65-year-old woman with a history of type 2 diabetes was admitted with a 3-week history of nausea, anorexia, pruritus and jaundice. She was currently being treated with 400 mg daily troglitazone (had been for 6 weeks). Histologic tests showed chronic hepatitis (severe activity), hepatocyte necrosis and fibrosis. 3 months after treatment was discontinued, the patient showed improvement [Gitlin *et al.* 1998].

Bioavailability 53%.
Half-life 10–39 h.
Volume of Distribution Steady state 2.5 L/kg.
Clearance Systemic plasma clearance, 172 mL/min.
Distribution in Blood Distribution into red blood cells is low.
Protein Binding ≥99% at concentrations of 0.5–3 mg/L. Primarily to albumin.
Dose 200 mg.

Bjork M, Pettersson K (1990). Capillary gas chromatographic method for the simultaneous determination of local anaesthetics in plasma samples. *J Chromatogr* 533: 229–234.
Gitlin N *et al.* (1998). Two cases of severe clinical and histologic hepatotoxicity associated with troglitazone. *Ann Intern Med* 129(1): 36–38.
Lambropoulos J, Bergholdt AB (2000). Method development and validation for the HPLC potency assay of troglitazone tablets. *J Pharm Biomed Anal* 24(2): 251–258.
Loi CM *et al.* (1997). Lack of effect of type II diabetes on the pharmacokinetics of troglitazone in a multiple-dose study. *J Clin Pharmacol* 37(12): 1114–1120.

Troleandomycin

Antibiotic
$C_{41}H_{67}NO_{15} = 814.0$
CAS—2751-09-9

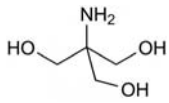
IUPAC Name [6-[3-Acetyloxy-4-(dimethylamino)-6-methyloxan-2-yl]oxy-8-(5-acetyloxy-4-methoxy-6-methyloxan-2-yl)oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-14-yl] acetate
Synonym Triacetyloleandomycin
Proprietary Names *TAO*; *Triocetin*.
Chemical Properties The triacetyl ester of oleandomycin. A white crystalline powder. Mp 176° with decomposition. Slightly soluble in water and ether; soluble 1 in 10 of ethanol and 1 in 1 of chloroform. pK_a 6.6. Log *P* (octanol/water), 2.5.

Colour Tests Mandelin's test—violet (slow); Marquis test—yellow-brown.
Thin-layer Chromatography System TA—R_f 0.65 (acidified iodoplatinate solution, positive).
Infrared Spectrum Principal peaks at wavenumbers 1744, 1239, 1735, 1053, 1004, 1123 cm^{-1} (KBr disk).
Dose The equivalent of 1 to 2 g of oleandomycin daily.

Trometamol

Treatment of Acidosis
$C_4H_{11}NO_3$ = 121.1
CAS—77-86-1

IUPAC Name 2-Amino-2-(hydroxymethyl)propane-1,3-diol
Synonyms THAM; trihydroxymethylaminomethane; TRIS; tris(hydroxymethyl)aminomethane; tromethamine.
Proprietary Names *Addex-THAM*; *Pleomix-Alpha*; *Tham: Thamacet*; *Thamesol*; *Thioctacid*; *Tris*; *TromLipon*.



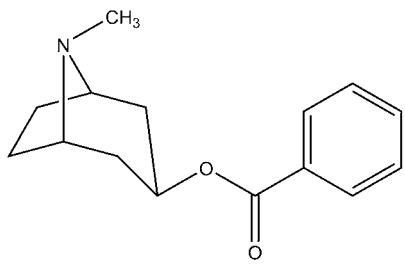
Chemical Properties A white crystalline powder. Mp 171° to 172°. Soluble 1 in 1.8 of water, 1 in 45 of ethanol and 1 in 38 of methanol; practically insoluble in chloroform; slightly soluble in acetone and ether. pK_a 8.3 (20°). Log *P* (octanol/water), −1.6.
Thin-layer Chromatography System TA—R_f 0.57 (Ninhydrin spray, positive).
Gas Chromatography System GA—RI 1645.
Infrared Spectrum Principal peaks at wavenumbers 1031, 1018, 1076, 975, 1582, 1279 cm^{-1} (KBr disk).
Quantification
Plasma **GC** FID. Limit of detection, 5 µg/L [Hulshoff, Kostenbauder 1978].
HPLC Fluorescence detection. For method, see Morris and Hieh [1993]. UV detection. Limit of detection, about 0.28 mg/L [Gumbhir, Mason 1992].
Serum **TLC** Limit of detection, 20 µg [Andermann, Andermann 1980].
Urine **HPLC** See Plasma [Morris and Hieh 1993].
Disposition in the Body About 75% of a dose is excreted in the urine as unchanged drug in 8 h and the remainder is excreted over 3 days. Trometamol may accumulate in the body if large doses are given frequently.
Therapeutic Concentration
Following an IV injection of 109 mg to an infant, a plasma concentration of 430 mg/L was reported at 0.5 h, declining to 86 mg/L at 4.5 h [Hulshoff, Kostenbauder 1978.]
Dose Up to 500 mg/kg by IV infusion.

Andermann G, Andermann C (1980). *J High Resolut Chromatogr Chromatogr Commun* 3: 36–37.
Gumbhir K, Mason WD (1992). High-performance liquid chromatographic method for the determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma. *J Chromatogr* 583: 99–104.
Hulshoff A, Kostenbauder HB (1978). Gas chromatographic method for the quantitative determination of tris(hydroxymethyl)aminomethane in plasma. *J Chromatogr* 145: 155–159.
Morris MJ, Hieh JY (1993). Determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma and urine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 622: 87–92.

Tropacocaine

Anaesthetic (Local)
$C_{15}H_{19}NO_2$ = 245.3
CAS—537-26-8

IUPAC Name (8-Methyl-8-azabicyclo[3.2.1]octan-3-yl) benzoate
Synonyms Benzoylpseudotropeine; benzoylpseudotropine; benzoyl-Ψ-tropeine; benzoyltropein; benzoyltropine; *o*-benzoyltropine; exo-8-methyl-8-azabicyclo[3.2.1]octan-3-ol benzoate (ester); pseudotropine benzoate; 1α*H*,5α*H*-tropan-3β-ol benzoate; Ψ-tropine benzoate; tropacaine; tropacocain.



Chemical Properties White crystalline powder. Mp 49°. Freely soluble in alcohol, ether, chloroform, benzene, petroleum ether, and dilute acids. Slightly soluble in water [O'Neil *et al.* 2006]. Log *P* (octanol/water) 2.9 [National Institutes of Health 2008]; 2.7 [Meylan, Howard 1995]. pK_a 9.9 [Polasek *et al.* 1992].

Tropacocaine Hydrochloride

$C_{15}H_{19}NO_2$,HCl = 280.8
IUPAC Name 2 (8-Methyl-8-azabicyclo[3.2.1]octan-3-yl) benzoate chloride
Synonyms Benzoic acid tropine ester hydrochloride; *o*-benzoyl-pseudotropine hydrochloride; benzoyltropine hydrochloride; NSC76018; pseudotropine benzoate hydrochloride; tropacaoine hydrochloride; 1α-*H*,5α-*H*-tropan-3α-ol, benzoate (ester), hydrochloride.
Chemical Properties White crystalline powder. Mp 283°. Soluble in water, slightly soluble in absolute ethanol, practically insoluble in ether.

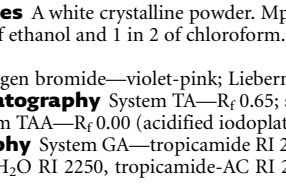
Thin-layer Chromatography System T1—R_f 0.35 (location reagent acidified iodoplatinate solution, positive reaction).
UV Spectrum Ethanol : water (1 : 1)—230, 273 µm.
Quantification
Plasma **HPLC** Nucleosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L citric acid : 0.1 mol/L dibasic sodium phosphate buffer (pH 3.0; 4 : 1) with 18% acetonitrile and 0.3% triethylamine, flow rate 1.5 mL/min. UV detection (λ = 235 nm). Limit of detection not reported [Virag *et al.* 1996].
Other **GC** Illicit Cocaine Samples. Column: 3% OV-1 Chromosorb WHP or 3% OV-17 Chromosorb WHP (both 1.52 m × 2 mm). Carrier gas: N_2, 30 mL/min. Temperature programme: 100° for 2 min to 320° at 12°/min for 5 min. Retention time, ≈11 min on OV-1 and ≈12.5 min on OV-17. Limit of detection not reported [Ensing *et al.* 1992].
GC-MS Greenhouse-cultivated Coca Leaves. Column: DB-1 fused silica capillary (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: H_2, 30–40 cm/s. Temperature programme: 150° for 1.0 min to 275° at 6.0°/min for 8.0 min. ^{63}Ni ECD or FID. Limit of detection not reported [Moore *et al.* 1994].
Note For a comparison of illicit cocaine samples using its ratio with 4 alkaloids, including tropacocaine, see Janzen *et al.* [1992].
Disposition in the Body
Toxicity Anaesthesia is produced more quickly than with cocaine but is more transitory. LD_{50} in rats (IV) is 20 mg/kg.
Dose Up to 300 mg daily.

Ensing JG *et al.* (1992). A rapid gas chromatographic method for the fingerprinting of illicit cocaine samples. *J Forensic Sci* 37: 446–459.
Janzen KE *et al.* (1992). Comparison analysis of illicit cocaine samples. *J Forensic Sci* 37: 436–445.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Moore JM *et al.* (1994). Determination and in-depth chromatographic analyses of alkaloids in South American and greenhouse-cultivated coca leaves. *J Chromatogr A* 659: 163–175.
National Institutes of Health (2008). *Benzoyltropein*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=10834&loc=ec_rcs. (accessed 11 June 2008).
O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14 edn. Whitehouse Station, NJ: Merck & Co., Inc.
Polasek M *et al.* (1992). Determination of limiting ionic mobilities and dissociation constants of some local anaesthetics. *J Chromatogr* 596: 265–270.
Virag L *et al.* (1996). Determination of cocaine, norcocaine, benzoylecgonine and ecgonine methyl ester in rat plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B BiomedAppl* 681: 263–269.

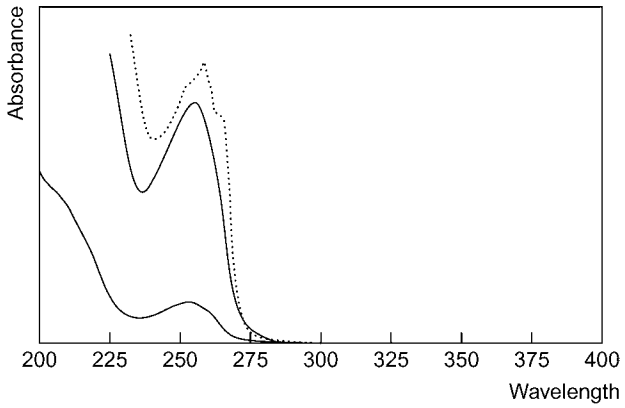
Tropicamide

Anticholinergic
$C_{17}H_{20}N_2O_2$ = 284.4
CAS—1508-75-4

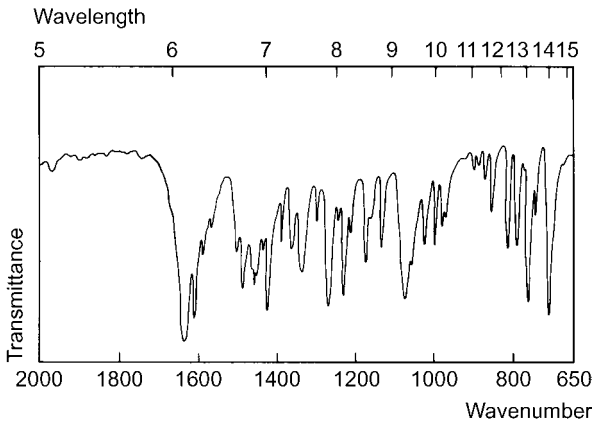
IUPAC Name *N*-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide
Synonyms Bistropamide; *N*-ethyl-α-(hydroxymethyl)-*N*-(4-pyridinylmethyl) benzeneacetamide.
Proprietary Names *Diotrope*; *Mydral*; *Mydramide*; *Mydriacil*; *Mydriacyl*; *Mydrian*; *Mydriaticum*; *Mydrum*; *Myriacyl*; *Ocu-Tropic*; *Opticyl*; *Tropicacyl*; *Tropicil Top*; *Tropicol*; *Tropimil*; *Visumidriatic*.



Chemical Properties A white crystalline powder. Mp 96° to 97°. Soluble 1 in 160 of water, 1 in 3.5 of ethanol and 1 in 2 of chloroform. pK_a 5.2. Log *P* (octanol/water), 1.2.
Colour Tests Cyanogen bromide—violet-pink; Liebermann's reagent—yellow.
Thin-layer Chromatography System TA—R_f 0.65; system TE—R_f 0.51; system TF—R_f 0.03; system TAA—R_f 0.00 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—tropicamide RI 2335, tropicamide-CH_2O RI 2230, tropicamide-H_2O RI 2250, tropicamide-AC RI 2410; system GB—tropicamide RI 2442.
High Performance Liquid Chromatography System HX—RI 311.
Ultraviolet Spectrum Aqueous acid—254 nm (A_i^1=180a); aqueous alkali—256 nm.



Infrared Spectrum Principal peaks at wavenumbers 1627, 1601, 709, 1266, 761, 1070 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 92, 91, 65, 103, 93, 39, 163, 77.

Quantification

Aqueous Humour **HPLC** UV detection. For a method of quantification for tropicamide and phenylephrine, see Galmier *et al.* [2000].

Use As a 0.5 to 1% ophthalmic solution.

Galmier MJ *et al.* (2000). High-performance liquid chromatographic determination of phenylephrine and tropicamide in human aqueous humor. *Biomed Chromatogr* 14: 202–204.

Tropine

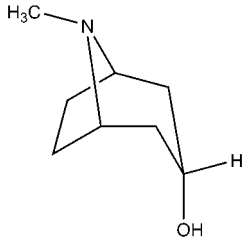
Anticholinergic

$C_8H_{15}NO = 141.2$

CAS—120-29-6

IUPAC Name 8-Methyl-8-azabicyclo[3.2.1]octan-3-ol

Synonyms 2,3-Dihydro-3α-hydroxytropidine; (3-endo)-8-methyl-8-azabicyclo [3.2.1]octan-3-ol; 5α*H*-tropan-3α-ol; tropan-3-ol.

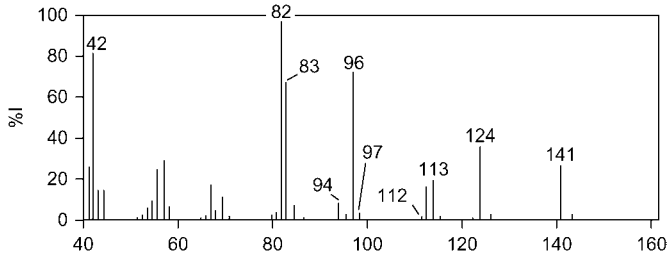


Chemical Properties White powder that may be obtained by the hydrolysis of atropine. Mp 63°. Bp 233°. Soluble in water, ethanol, ether, and chloroform. pK_{a1} 10.4, pK_{a2} 9.85 [Kugel *et al.* 1994]. Log *P* (chloroform/buffer pH 9.0) 0.0124 [Eckert, Hinderling 1981]. Esters of tropine are known as tropeines

Thin-layer Chromatography System T1—R_f 0.40 (location reagent acidified iodoplatinate spray, positive reaction).

Plates: silica gel 60 F_{254}. Solvent system: A) ethyl acetate: isopropanol: concentrated ammonia (45 : 35 : 15); B) 70% ethanol : concentrated ammonia (95 : 5). UV detection (λ = 254 nm) or Dragendorff's reagent and 5% copper chloride solution. R_f: 0.27 in system A, 0.07 in system B. Limit of detection not reported [van der Meer *et al.* 1986].

Mass Spectrum Principal ions at *m/z* 82, 83, 42, 96, 141, 124, 55, 67, 113, Witte *et al.* [1987], Blossey *et al.* [1964].



Capillary Electrophoresis Capillary: fused silica (85 cm in length, 5 μm i.d.). Running buffer: 60 mmol/L ammonium acetate (pH 8.5) containing 5% isopropanol. ESI-TOF, positive ion mode. Migration time: 10.1 min. Limit of detection not reported [Arraez-Roman *et al.* 2008].

Quantification

Blood **GC-MS** Column: Ultra-1 cross-linked methyl silicone (25 m × 0.20 mm i.d., 0.33 μm). Temperature programme: 100° to 240° at 20°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 μg/L for atropine derivatised with PFPA [Saady, Poklis 1989].

Plasma **GC-MS** Column: SE-54 (20 m × 0.31 mm i.d.) or glass packed with 15% GE SF-96 (3 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 90° to 140° at 10°/min for the capillary column, isothermic at 200°. EI ionisation at 70 eV. Retention time: 1.4 and 2.2 min for the capillary and glass column, respectively. Limit of detection, 2 μg/L [Eckert, Hinderling 1981].

Urine **HPLC** Column: Radial Pak C_{18} ODS silica (10 cm × 8 mm i.d., 10 μm). Mobile phase: acetonitrile : 20 mmol/L aqueous tetrabutyl ammonium hydrogen sulphate-50 mmol/L sodium acetate (pH 5; 25 : 75), flow rate 0.75 mL/min. UV detection (λ = 230 nm) or scintillation counting. Limit of detection not reported [van der Meer *et al.* 1986].

GC-MS Column: SE-54 (20 m × 0.31 mm i.d.) or glass packed with 15% GE SF-96 (3 m × 2 mm i.d.). Carrier gas: He, 30 mL/min. Temperature programme: 90° to 140° at 10°/min for the capillary column, isothermic at 200°. EI ionisation at 70 eV. Retention time: 1.4 and 2.2 min for the capillary and glass column, respectively. Limit of detection, 20 μg/L [Eckert, Hinderling 1981].

Other **GC-MS** Seeds from *Erythroxylum Coca* var. *Coca*. Column: DB-1 fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm). Temperature programme: 100° to 300° at 6°/min for 5.67 min. EI ionisation at 70 eV. Limit of detection not reported [Casale *et al.* 2005]. Rat Urine. Column: DB-1 capillary (30 m × 0.32 nm i.d., 0.25 μm). Carrier gas: He, 2 mL/min. Temperature programme: 200° for 1 min to 280° at 10°/min for 10 min. EI ionisation at 70 eV, positive ion mode. Limit of detection not reported [He *et al.* 1995]. *Convolvulus arvensis*. Column: HP1 100% dimethylpolysiloxane (12 m × 0.2 mm i.d.). Temperature programme: 80° for 2 min to 140° at 10°/min to275° at 50°/min for 3 min. EI ionisation at 70 eV. Retention time: 7.6 min [Todd *et al.* 1995]. *Daturia innoxia* Root Extracts. Column: fused silica methylsilicone DB1 (15 m × 0.25 mm i.d.). FID and NPD. RI 1167. Limit of detection not reported [Witte *et al.* 1987].

LC-MS Rat Urine. Column: Zorbax C_{18} (100 × 3.0 mm i.d., 3.5 μm). Mobile phase: methanol : 2 mmol/L ammonium acetate (pH 3.5; 70 : 30), flow rate, 0.2 mL/min. Positive ion mode. Retention time: 2.63 min. Limit of detection, <5 μg/L [Chen *et al.* 2006]. Seeds from *Erythroxylum Coca* var. *Coca*. Column: Phenomenex C_{18} (150 × 4.6 mm i.d.). Mobile phase: 0.1% trifluoroacetic acid : acetonitrile (95 : 5 to 75 : 25 in 15 min for 5 min to 5 : 95 in 5 min to 95 : 5 in 1 min), flow rate 1.0 mL/min. APCI, positive ion mode. Limit of detection not reported [Casale *et al.* 2005].

Disposition in the Body Tropine is one of the metabolites of atropine.

Arraez-Roman D *et al.* (2008). Characterization of *Atropa belladonna* L. compounds by capillary electrophoresis-electrospray ionization-time of flight-mass spectrometry and capillary electrophoresis-electrospray ionization-ion trap-mass spectrometry. *Electrophoresis* 29: 2112–2116.

Blossey EC *et al.* (1964). Mass spectrometry in structural and stereochemical problems–XXXIX: Tropane Alkaloids. *Tetrahedron* 20: 585–595.

Casale JF *et al.* (2005). Alkaloid content of the seeds from *Erythroxylum Coca* var. Coca. *J Forensic Sci* 50: 1402–1406.

Chen H *et al.* (2006). Sensitive and specific liquid chromatographic-tandem mass spectrometric assay for atropine and its eleven metabolites in rat urine. *J Pharm Biomed Anal* 40: 142–150.

Eckert M, Hinderling PH (1981). Atropine: a sensitive gas chromatography-mass spectrometry assay and prepharmacokinetic studies. *Agents Actions* 11: 520–531.

He H, McKay *et al.* (1995). Phase I and II metabolites of benztropine in rat urine and bile. *Xenobiotica* 25: 857–872.

Kugel C *et al.* (1994). Determination of distribution coefficients for some 5-HT3 receptor antagonists by reversed-phase high-performance liquid chromatography. *J Chromatogr A* 667: 29–35.

Saady JJ, Poklis A (1989). Determination of atropine in blood by gas chromatography/mass spectrometry. *J Anal Toxicol* 13: 296–299.

Todd FG *et al.* (1995). Tropane alkaloids and toxicity of *Convolvulus arvensis*. *Phytochemistry* 39: 301–303.

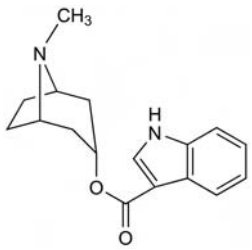
van derMeer *et al.* (1986). The metabolism of atropine in man. *J Pharm Pharmacol* 38: 781–784.

Witte L *et al.* (1987). Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass spectrometry. *Planta Med* 53: 192–197.

Tropisetron

Antiemetic, 5-HT$_3$ Receptor Antagonist
$C_{17}H_{20}N_2O_2$ = 284.4
CAS—89565-68-4

IUPAC Name [(1*R*,5*S*)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl] 1*H*-indole-3-carboxylate
Synonyms ICS-205-930; 1*H*-indole-3-carboxylic acid (3-*endo*)-8-methyl-azabicyclo[3.2.1]oct-3-yl ester.

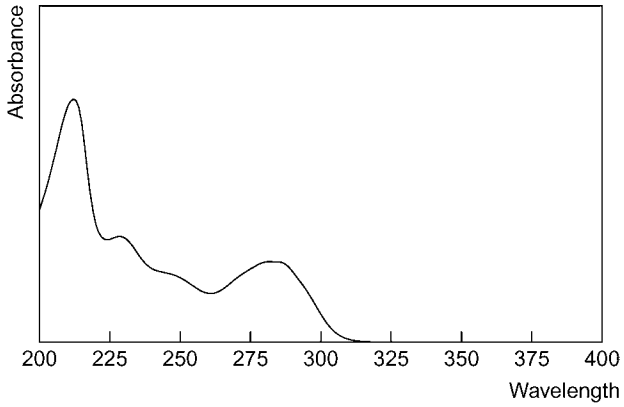


Chemical Properties Mp 201° to 202°.

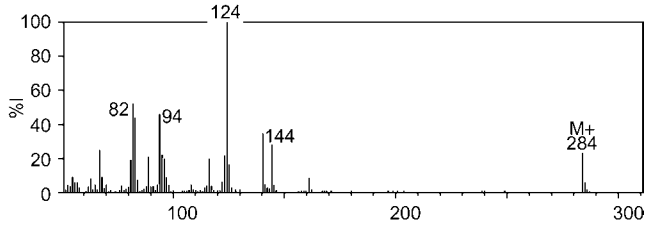
Tropisetron Hydrochloride

$C_{17}H_{21}ClN_2O_2$ = 320.8
CAS—105826-92-4
Proprietary Names *Navaban*; *Navoban*.
Chemical Properties Mp 283° to 285°.

Ultraviolet Spectrum Principal peaks at 213, 231, 249, 283 nm.



Mass Spectrum Principal ions at *m/z* 124, 82, 94, 83, 140, 144, 67, 284.



Quantification

Plasma **HPLC** Column: Lichrosorb RP18 (250 × 7 mm i.d., 7 µm). Mobile phase: acetonitrile : water and 0.3% triethylamine (5 : 95 to 15 : 85 over 30 min, to 20 : 80 over 10 min, to 90 : 10 over 20 min). UV detection (λ=283 nm). Retention time: 60 min. Limit of quantification, 0.3 µg/L [Fischer *et al.* 1992].

Disposition in the Body Tropisetron is rapidly and extensively absorbed from the gastrointestinal tract in ~20 min. Peak plasma concentrations are reached in about 3 h. Metabolism is saturable, which results in non-linear pharmacokinetics. It occurs by hydroxylation at the 5, 6 or 7 position of the indole ring, followed by a conjugation reaction to form the glucuronide or sulfate. These metabolites are excreted in urine or bile with a urine : bile ratio of 5 : 1. Metabolism is linked to a genetically determined sparteine/debrisoquin polymorphism. Eight percent of the Caucasian population are thought to be poor metabolisers, but there is no change in metabolism in cases of renal impairment or in the elderly. Tropisetron is not accumulative.

Therapeutic Concentration In serum, the trough therapeutic concentration is 20 to 30 mg/L and the peak serum concentrations are 50 to 100 mg/L.

Following an oral dose of 20 or 100 mg, the mean and peak plasma concentrations are 24.7 and 173 µg/L, respectively, which occurred after 1.3 and 1.1 h, respectively [Lee *et al.* 1993].

Twelve healthy males were administered 20 or 100 mg single, oral doses and the maximum concentrations observed were 0.087 and 0.608 µg/L, respectively [Fischer *et al.* 1992].

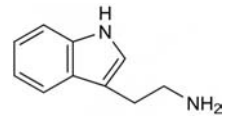
Toxicity At very high doses of 100 mg for 5 days, visual hallucinations may occur.
Bioavailability ≥95%. Absolute bioavailability depends on the dose.
Half-life 7.3 h (IV), 8.6 h (oral) in extensive metabolisers; 30.3 h (IV) and 41.9 h (oral) in poor metabolisers.
Volume of Distribution 400–600 L.
Clearance In extensive metabolisers, the total clearance is 1 L/min, renal clearance is 10%. In poor metabolisers, total clearance is 0.1–0.2 L/min and the renal clearance is unchanged.
Protein Binding 59–71%, mainly to α_1 glycoprotein.
Dose 2 to 5 mg IV; 5 mg orally.

Fischer V *et al.* (1992). Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans. *Drug Metab Dispos* 20(4): 603–607.
Lee C *et al.* (1993). Tropisetron. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential as an antiemetic. *Drugs* 46: 925–943.

Tryptamine

Putrefactive Base
$C_{10}H_{12}N_2$ = 160.2
CAS—61-54-1

IUPAC Name 2-(1*H*-Indol-3-yl)ethanamine

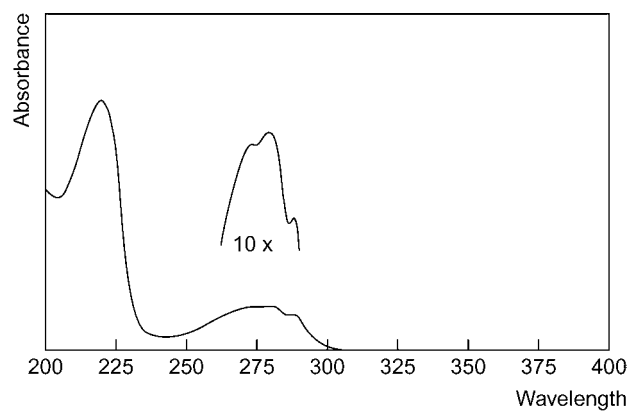


Chemical Properties A white crystalline powder. Mp 118°. Practically insoluble in water, chloroform, benzene and ether; soluble in ethanol and acetone. Log *P* (octanol/water), 1.6.

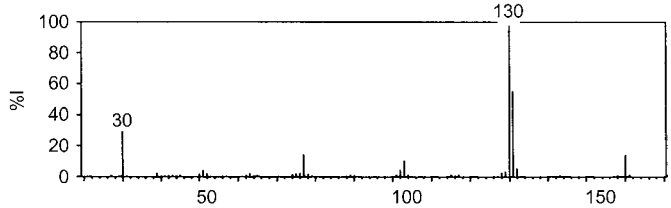
Tryptamine Hydrochloride

$C_{10}H_{12}N_2$,HCl = 196.7
CAS—343-94-2
Chemical Properties A white crystalline powder. Mp 248°. Soluble in water.

Colour Tests *p*-Dimethylaminobenzaldehyde—red/violet; formaldehyde-sulfuric acid—brown; Marquis test—orange.
Thin-layer Chromatography System TA—R_f 0.23; system TB—R_f 0.01; system TC—R_f 0.02; system TE—R_f 0.26; system TL—R_f 0.11; system TAE—R_f 0.06; system TAJ—R_f 0.00; system TAK—R_f 0.05; system TAL—R_f 0.36 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—Tryptamine RI 1725, tryptamine-AC RI 2390, tryptamine-AC$_2$ RI 2440.
High Performance Liquid Chromatography System HA—*k* 1.2; system HY—RI 190.
Ultraviolet Spectrum Aqueous acid—278 (A^1_1=353a), 287 nm.



Infrared Spectrum Principal peaks at wavenumbers 742, 1585, 1233, 1035, 909, 1494 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 130, 131, 30, 160, 77, 103, 132, 102.

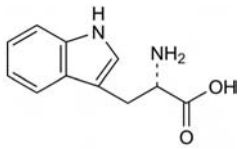


T

Quantification See under Tryptophan.

Tryptophan

Amino Acid
$C_{11}H_{12}N_2O_2$ = 204.2
CAS—73-22-3
IUPAC Name (2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid
Synonyms (*S*)-α-Amino-1*H*-indole-3-propanoic acid; L-Trp; L-tryptophan; tryptophanum.
Proprietary Names *Ardeytropin*; *Kalma*; *Optimax*; *Pacitron*; *Trofan*; *Tryptan*.

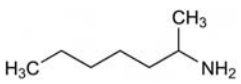


Chemical Properties White to slightly yellowish-white crystals or crystalline powder. Soluble 1 in ≈100 of water; very slightly soluble in ethanol; soluble in hot ethanol; insoluble in chloroform; practically insoluble in ether; soluble in solutions of dilute mineral acids and alkali hydroxides. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].
Gas Chromatography System GA—tryptophan-Me-AC RI 2150, trytophan-Me_2-AC RI 2170; M (OH-skatole) RI 1370, M (indole acetic acid)-Me RI 1900, M (indole formic acid)-Me RI 1940; M (tryptamine) RI 1725.
Ultraviolet Spectrum Aqueous acid—278 (A^1_1=290b), 286 nm (A^1_1=234b); aqueous alkali—280, 288 nm.
Quantification
Blood **LC-MS** For a method of quantification for tryptophan and other amino acids in dried blood, see Casetta *et al.* [2000].
Plasma **LC-MS** See Blood [Casetta *et al.* 2000].
Serum **HPLC** Limit of quantification, 5 µmol/L for tryptophan and other amino acids [Tcherkas *et al.* 2001]. UV detection. Limit of detection, ≈0.6 µmol/L [Tang *et al.* 1998].
Urine **HPLC** Fluorescence detection. For a method of quantification for tryptamine, see Tsuchiya *et al.* [1989].
Note For a review of the quantification of tryptophan and its metabolites, see Molnar-Perl [1999].
Dose 3 to 6 g daily.

Casetta B *et al.* (2000). Development of a method for rapid quantitation of amino acids by liquid chromatography-tandem mass spectrometry (LC-MSMS) in plasma. *Clin Chem Lab Med* 38: 391–401.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Molnar-Perl I (1999). Advances in the analysis of tryptophan and its related compounds by chromatography. *Adv Exp Med Biol* 467: 801–816.
Tang A *et al.* (1998). [Determination of tryptophan in human serum by high-performance liquid chromatography]. *Hunan Yi Ke Da Xue Xue Bao* 23: 605–607, 610.
Tcherkas YV *et al.* (2001). Analysis of amino acids in human serum by isocratic reversed-phase high-performance liquid chromatography with electrochemical detection. *J Chromatogr A* 913: 303–308.
Tsuchiya H *et al.* (1989). High-performance liquid-chromatographic analysis for serotonin and tryptamine excreted in urine after oral loading with L-tryptophan. *Clin Chem* 35: 43–47.

Tuaminoheptane

Sympathomimetic
$C_7H_{17}N$ = 115.2
CAS—123-82-0
IUPAC Name Heptan-2-amine

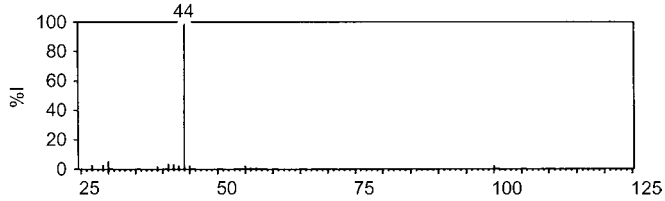


Chemical Properties A colourless or pale yellow volatile liquid. Bp 142° to 144°. It absorbs carbon dioxide on exposure to air, forming a white precipitate of tuaminoheptane carbonate. Sp. gr. 0.760 to 0.763. Slightly soluble in water; freely soluble in ethanol, ether, chloroform and benzene. pK_a 10.6. Log *P* (octanol/water), 2.4.

Tuaminoheptane Sulfate

$(C_7H_{17}N)_2,H_2SO_4$ = 328.5
CAS—6411-75-2
Proprietary Names *Tuamine Sulfate*. It is an ingredient of *R(h)inofluimucil*.
Chemical Properties A white powder. Freely soluble in water; soluble in ethanol; sparingly soluble in ether.

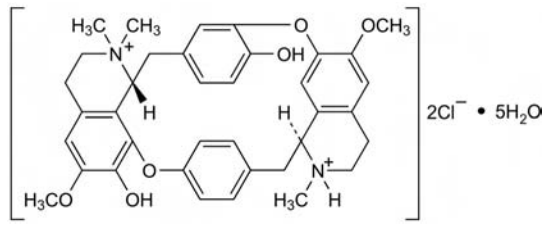
Thin-layer Chromatography System TA—R_f 0.33; system TB—R_f 0.01; system TC—R_f 0.07; system TL—R_f 0.24 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 888; system GC—RI 1287.
Ultraviolet Spectrum No significant absorption, 230 to 360 nm.
Mass Spectrum Principal ions at *m/z* 44, 30, 42, 41, 29, 27, 100, 55.



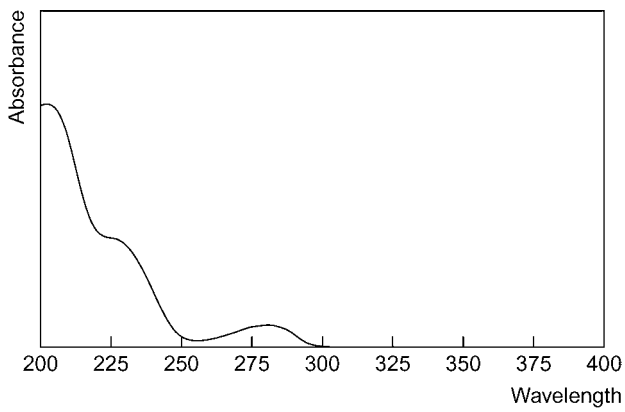
Use As a 1% solution of the sulfate for nasal drops or spray.

Tubocurarine Chloride

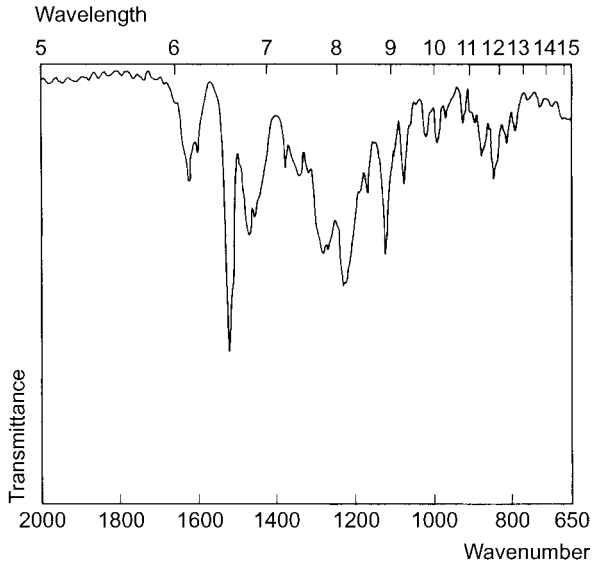
Muscle Relaxant
$C_{37}H_{42}Cl_2N_2O_6,5H_2O$ = 771.7
CAS—57-95-4 (tubocurarine); 57-94-3 (chloride, anhydrous); 6989-98-6 (chloride, pentahydrate)
Synonyms 7′,12′-Dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethyltubocuraranium dichloride pentahydrate; dextrotubocurarine chloride; D-tubocurarine chloride.
Proprietary Names *Curarina Miro*; *Curarine*; *Jexin*; *Tubarine*.



Chemical Properties The chloride of an alkaloid, (+)-tubocurarine. It may be obtained from extracts of the stems of *Chondodendron tomentosum* (Menispermaceae). A white or slightly yellowish-white or greyish-white crystalline powder. Mp about 270°, with decomposition. Soluble 1 in 20 of water; soluble in ethanol and methanol; insoluble in chloroform, benzene, acetone and ether. pK_a 8.0, 9.2 (22°).
Colour Tests Aromaticity (method 2)—yellow/brown; Folin–Ciocalteu reagent—blue; Liebermann's reagent—black; Mandelin's test—brown; Millon's reagent (hot)—red; nitric acid, cold—gives a black colour.
Thin-layer Chromatography System TA—R_f 0.00; system TB—R_f 0.00; system TC—R_f 0.00; system TE—R_f 0.00; system TL—R_f 0.00; system TN—R_f 0.85; system TO—R_f 0.40; system TAE—R_f 0.00; system TAF—R_f 0.11 (acidified iodoplatinate solution, positive).
Gas Chromatography System GA—RI 2495.
High Performance Liquid Chromatography System HX—RI 321; system HY—RI 257.
Ultraviolet Spectrum Water—280 nm (A^1_1=119a).



Infrared Spectrum Principal peaks at wavenumbers 1516, 1228, 1120, 1280, 1265, 1165 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 298, 594, 58, 593, 299, 609, 595, 564.

Quantification

Plasma **HPLC** UV detection. Limit of detection, 25 µg/L [Meulemans *et al.* 1981].

Serum **Radioimmunoassay** Limit of detection, 5 µg/L [Horowitz, Spector 1973].

Urine **Radioimmunoassay** See Serum [Horowitz, Spector 1973].

Postmortem Tissues **UV spectrophotometry** For method, see Stevens and Fox [1971].

Disposition in the Body After IV administration it is widely distributed throughout the tissues and is concentrated at the neuromuscular junctions; slowly and irregularly absorbed following IM injection. About 45% of a dose is excreted in the urine unchanged in 24 h.

Therapeutic Concentration In plasma, usually in the range 0.7 to 1 mg/L. After an IV bolus of 540 µg/kg followed by constant IV infusion of 2.9 µg/kg/min to 9 subjects, a mean steady-state plasma concentration of 1.1 mg/L was attained in about 1 h [Ramzan *et al.* 1980].

Toxicity
In a fatality caused by tubocurarine, a postmortem liver concentration of about 0.8 µg/g was reported [Stevens, Fox 1971].

Half-life Plasma half-life, about 2 to 4 h.

Volume of Distribution About 0.3 to 0.6 L/kg.

Clearance Plasma clearance, about 2 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, 1.9.

Protein Binding 33 to 50%.

Note For a review of the pharmacokinetics of neuromuscular blocking agents, see Ramzan *et al.* [1981].

Dose Initially 10 to 15 mg IV, with additional doses of 5 mg.

Horowitz PE, Spector S (1973). Determination of serum d-tubocurarine concentration by radioimmunoassay. *J Pharmacol Exp Ther* 185: 94–100.

Meulemans A *et al.* (1981). Quantitation of D-tubocurarine in human plasma using high-performance liquid chromatography. *J Chromatogr* 226: 255–258.

Ramzan MI *et al.* (1980). Pharmacokinetics of tubocurarine administered by combined i.v. bolus and infusion. *Br J Anaesth* 52: 893–899.

Ramzan MI *et al.* (1981). Clinical pharmacokinetics of the non-depolarising muscle relaxants. *Clin Pharmacokinet* 6: 25–60.

Stevens HM, Fox RH (1971). A method for detecting tubocurarine in tissue. *J Forensic Sci Soc* 11: 177–182.

T

Tybamate

Tranquilliser

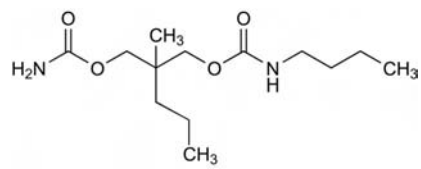
$C_{13}H_{26}N_2O_4 = 274.4$

CAS—4268-36-4

IUPAC Name [2-(Carbamoyloxymethyl)-2-methylpentyl] *N*-butylcarbamate

Synonym Butylcarbamic acid 2-[[(aminocarbonyl)oxy]methyl]-2-methylpentyl ester.

Proprietary Names *Nospan*; *Solacen*; *Tybatran*.



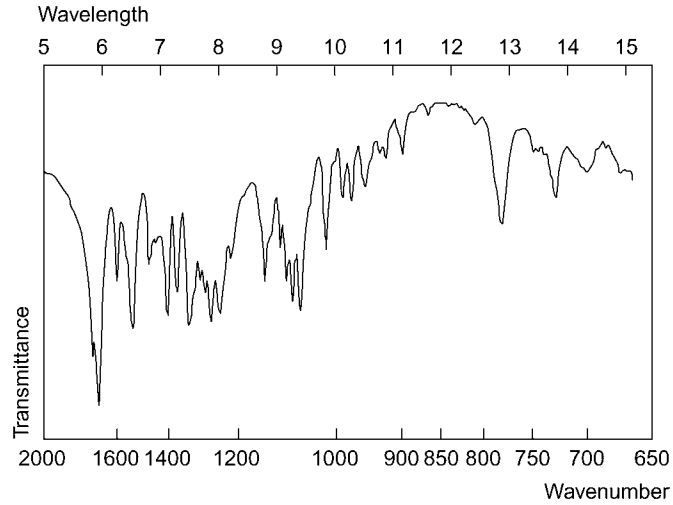
Chemical Properties White crystalline powder or clear viscous liquid which may congeal to a solid form on standing. Mp of the powder 49° to 51°. Very slightly soluble in water; very soluble in ethanol and acetone; freely soluble in ether. Log *P* (octanol/water), 2.9.

Thin-layer Chromatography System TA—R_f 0.77; system TD—R_f 0.35; system TE—R_f 0.65; system TF—R_f 0.68 (furfuraldehyde reagent, positive).

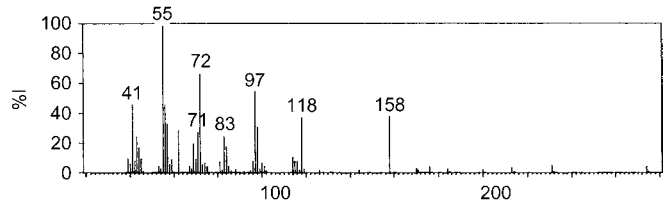
Gas Chromatography System GA—RI 1725; system GF—RI 2130.

Ultraviolet Spectrum No significant absorption, 230–360 nm.

Infrared Spectrum Principal peaks at wavenumbers 1695, 1250, 1065, 1538, 1600, 1140 cm^{-1} (KBr disk) [Douglas *et al.* 1966]].



Mass Spectrum Principal ions at *m/z* 55, 72, 97, 41, 56, 158, 118, 57.



Quantification

Plasma **GC** Column: 3.8% UC-W98 methyl silicone 80/100 mesh Diataport S (121.9 cm). Carrier gas: He, 65 mL/min. Temperature: 180°. FID. Retention time: 5.0 min. Limit of detection, 1 mg/L [Douglas *et al.* 1969].

Urine See Plasma [Douglas *et al.* 1969].

Disposition in the Body Readily absorbed after oral administration. Metabolised in the liver and excreted in the urine, mainly as hydroxylated compounds. Meprobamate can appear as a minor metabolite [Douglas *et al.* 1966].

Half-life Plasma half-life, about 3 h.

Dose Usually 0.75 to 2 g daily; maximum of 3 g daily.

Douglas JF *et al.* (1966). The metabolic fate of tybamate in the rat and dog. *Biochem Pharmacol* 15: 2087–2095.

Douglas JF *et al.* (1969). Gas chromatographic determination of mebutamate, carisoprodol, and tybamate in plasma and urine. *J Pharm Sci* 58: 145–146.

Tylosin

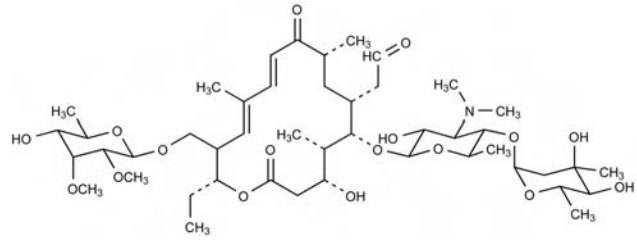
Antibiotic (Veterinary)

$C_{46}H_{77}NO_{17} = 916.1$

CAS—1401-69-0

IUPAC Name 2-[(4*R*,5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-6-[(2*S*,4*S*,5*R*,6*S*)-5-[(2*R*,4*S*,5*R*,6*R*)-4,5-Dihydroxy-4,6-dimethyloxan-2-yl]oxy-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-16-ethyl-4-hydroxy-15-[[(1R,2S,3R,4R,5S)-4-hydroxy-2,3-dimethoxy-5-methylcyclohexyl]oxymethyl]-5,9,13-trimethyl-2,10-dioxo-1-oxacyclohexadeca-11,13-dien-7-yl]acetaldehyde

Proprietary Names *Bilosin*; *Norotyl*; *Tylacre*; *Tylamix*; *Tylan*; *Tyluvet* (includes proprietary names of Tylosin salts). Tylosin Tartrate is an ingredient of *Tylan Soluble*, *Tylo* and *Tylomix*. Tylosin phosphate is an ingredient of *Tylan G* and *Tylasul-G*.



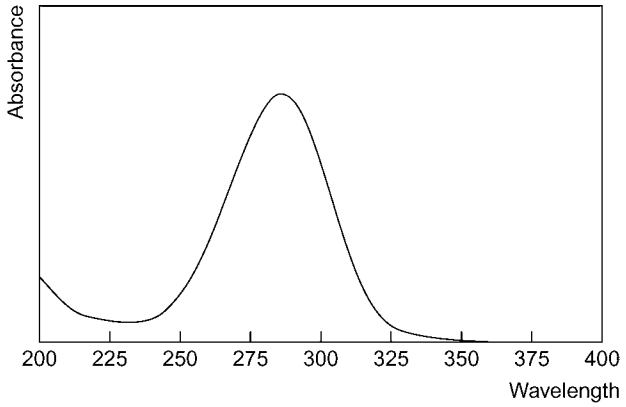
Chemical Properties An antimicrobial substance with a macrolide structure, produced by a strain of *Streptomyces fradiae*. An almost white to buff-coloured powder. Mp 128° to 132°. Slightly soluble in water; soluble in lower alcohols, esters, ketones, chlorinated hydrocarbons, benzene and ether. pK_a 7.73 (25°). Log *P* (octanol/water), 1.6.

Tylosin Tartrate

CAS—1405-54-5

Chemical Properties A white to buff-coloured hygroscopic powder. Mp 140° to 146°. Soluble 1 in 10 of water; slightly soluble in ethanol; very slightly soluble in chloroform; practically insoluble in ether.

Colour Tests Mandelin's test—yellow→yellow—brown; Marquis test—yellow-brown; sulfuric acid—yellow-brown (slow).
Thin-layer Chromatography System TA—R_f 0.72 (acidified iodoplatinate solution, positive).
Ultraviolet Spectrum Aqueous acid—290 nm (A^1_1=225b).

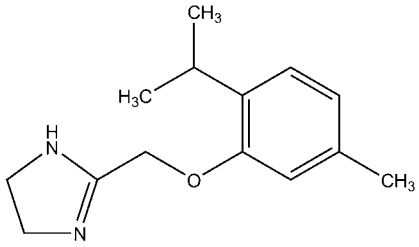


Infrared Spectrum Principal peaks at wavenumbers 1075, 1052, 1162, 1111, 1010, 1587 cm^{-1}.

Tymazoline

Nasal Decongestant, Sympathomimetic
$C_{14}H_{20}N_2O$ = 232.3
CAS—24243-97-8

IUPAC Name 2-[(5-Methyl-2-propan-2-ylphenoxy)methyl]-4,5-dihydro-1*H*-imidazole
Synonyms 4,5-Dihydro-2-[[5-methyl-2-(1-methylethyl)phenoxy]methyl]-1*H*-imidazole; 2-[(*p*-mentha-1,3,5-trien-2-yloxy)methyl]-2-imidazoline; 2-(thymyloxymethyl)glyoxalidine; 2-[(thymyloxy)methyl]-2-imidazoline.



Tymazoline Hydrochloride

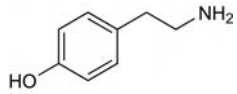
$C_{14}H_{20}N_2O$,HCl = 268.8
CAS—28120-03-8
IUPAC Name 2-[(5-Methyl-2-propan-2-ylphenoxy)methyl]-4,5-dihydro-1*H*-imidazole hydrochloride
Proprietary Names *Pernazene*; *Thymazen*.
Chemical Properties Crystals. Mp 215° to 217°, also reported as 223.5° to 225° [O'Neil *et al.* 2006].

O'Neil MJ *et al.* (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck & Co., Inc.

Tyramine

Diagnostic Agent, Sympathomimetic
$C_8H_{11}NO$ = 137.2
CAS—51-67-2

IUPAC Name 4-(2-Aminoethyl)phenol
Synonyms *p*-Tyramine; tyrosamine.

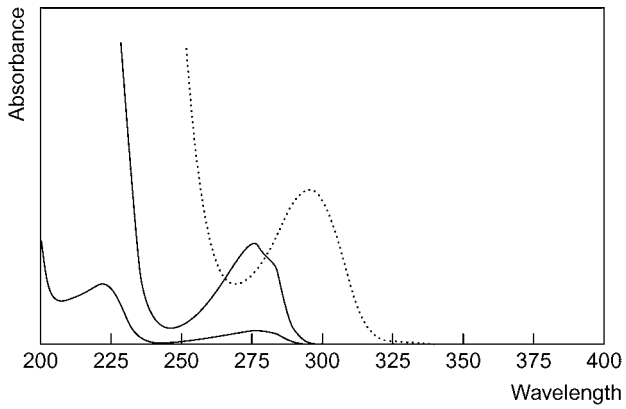


Chemical Properties Colourless crystals. Mp 164° to 165°. Soluble 1 in 95 of water and 1 in 10 of boiling ethanol; sparingly soluble in benzene and xylene. pK_a 9.5 (phenol), 10.8 (-NH_2). Log *P* (octanol/water), 0.9.

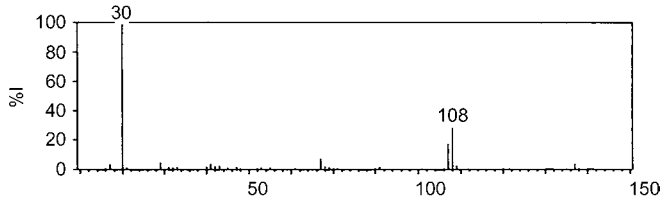
Tyramine Hydrochloride

$C_8H_{11}NO$,HCl = 173.6
CAS—60-19-5
Proprietary Name *Mydrial*
Chemical Properties Crystals. Mp 269°. Soluble in water.

Colour Tests *p*-Dimethylaminobenzaldehyde—orange/violet; Marquis test—brown→green.
Thin-layer Chromatography System TA—R_f 0.31 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—tyramine RI 1745; tyramine-AC_2 RI 1950.
High Performance Liquid Chromatography System HA—*k* 1.2; system HB—*k* 0.81; system HC—*k* 1.47; system HY—RI 77.
Ultraviolet Spectrum Aqueous acid—274 nm (A^1_1=110a); aqueous alkali—294 nm (A^1_1=182b).



Infrared Spectrum Principal peaks at wavenumbers 1266, 1518, 1595, 822, 1495, 1612 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 30, 108, 107, 77, 39, 51, 137, 27.



Quantification

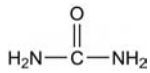
Urine **GC** ECD. Limit of detection, 25 ng [Coutts *et al.* 1980].
Dose Tyramine hydrochloride has been given orally in doses of up to 250 mg daily in the investigation of migraine.

Coutts RT *et al.* (1980). A rapid, sensitive method of measuring meta- and para-tyramine levels in urine using electron-capture gas chromatography. *Res Commun Chem Pathol Pharmacol* 28: 177–184.

Urea

Diuretic
CH_4N_2O = 60.1
CAS—57-13-6

IUPAC Name Urea
Synonyms Carbamide; carbonyldiamide; ureia; ureum.
Proprietary Names *Aquacare*; *Aquadrate*; *Balisa*; *Basodexan*; *Calmurid*; *Calmuril*; *Carmol*; *Dermaflex*; *Elacutan*; *Fenuril*; *Gormel*; *Karbasal*; *Lanaphilic*; *Monilen*; *Nubral*; *Nutraplus*; *Ti-U-Lac*; *Ultra-Mide*; *Ureacin*; *Ureaphil*; *Urecare*; *Urecrem*; *Urederm*; *Uree*; *Uremol*; *Urisec*.



Chemical Properties Colourless, slightly hygroscopic, prismatic crystals or pellets, or white crystalline powder. Mp 133°; decomposes on further heating. Soluble 1 in about 1 of water, 1 in 6 of methanol, 1 in 10 of ethanol, 1 in 1 of boiling ethanol and 1 in 2 of glycerol; practically insoluble in chloroform and ether. pK_a 0.1 (21°). Log *P* (octanol/water), −2.1.
Colour Test Nessler's reagent—brown-orange.
Thin-layer Chromatography System TA—R_f 0.55; system TE—R_f 0.15; system TAJ—R_f 0.05; system TAK—R_f 0.15; system TAL—R_f 0.30.
Gas Chromatography System GA—not eluted.
Mass Spectrum Principal ions at *m/z* 60, 44, 28.
Disposition in the Body Rapidly absorbed after oral administration. Excreted in the urine as unchanged drug and also metabolised to ammonium salts in the colon. Endogenous urinary excretion is in the range 10 to 35 g in 24 h; urea is not usually found in the faeces.
Therapeutic Concentration Endogenous concentrations in blood are about 300 mg/L in adults and lower in infants.

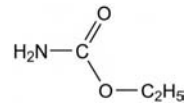
After IV infusion of 0.6 to 1.2 g/kg of a solution containing 30% urea and 10% invert sugar to 7 subjects over a period of 0.5 to 2 h, plasma concentrations at the end of the infusion were reported to range from 1160 to 3400 mg/L (mean, 2430) [Yarnell *et al.* 1972].
Half-life Biological half-life, about 1 h.
Dose 40 to 80 g by IV infusion, not exceeding 1.5 g/kg daily.

Yarnell PR *et al.* (1972). Plasma urea kinetics in urea infusion. *Clin Pharmacol Ther* 13: 558–562.

Urethane

Antineoplastic
$C_3H_7NO_2$ = 89.1
CAS—51-79-6

IUPAC Name Ethyl carbamate
Synonyms Carbamic acid ethyl ester; ethyl carbamate; ethylurethane; urethan.

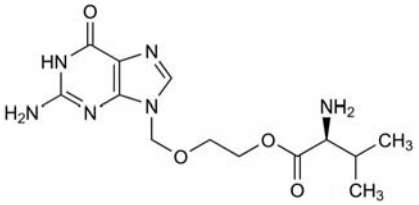


Chemical Properties Colourless crystals or white granular powder. Mp 48° to 50°. Bp 182° to 184°. Soluble 1 in 0.5 of water, 1 in 0.8 of ethanol, 1 in 0.9 of chloroform, 1 in 1.5 of ether, 1 in 2.5 of glycerol and 1 in 32 of olive oil. Log *P* (octanol/water), −0.2.
Gas Chromatography System GA—RI 838.
Infrared Spectrum Principal peaks at wavenumbers 1713, 1075, 1610, 805, 1010 cm^{-1} (KBr disk).
Dose Formerly given in doses of 3 g daily; maximum of 6 g daily.

Valaciclovir

Antiviral
$C_{13}H_{20}N_6O_4 = 324.3$
CAS—124832-26-4

IUPAC Name 2-[(2-Amino-6-oxo-3*H*-purin-9-yl)methoxy]ethyl (2*S*)-2-amino-3-methylbutanoate
Synonyms 256U87; valacyclovir; L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl ester.

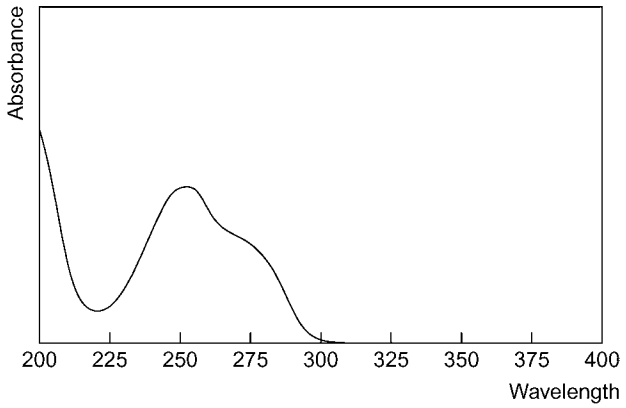


Chemical Properties pK_a 1.90, 7.47, 9.43.

Valaciclovir Hydrochloride

$C_{13}H_{21}ClN_6O_4 = 360.8$
CAS—124832-27-5
Proprietary Names *Valtrex*; *Zelitrex*.
Chemical Properties A white to off-white crystalline powder. Soluble in water (174 g/L at 25°).

Ultraviolet Spectrum Aqueous solution—252.8 nm.



Quantification
Plasma **HPLC** Limit of detection, 0.625 mg/L [Poirier *et al.* 1999].
Serum **HPLC** Column: RP-8 SymmetryShield (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.025 mol/L monoammonium phosphate buffer (pH 4.0), flow rate 1.0 mL/min. UV detection (λ=254 nm). Retention time: 9.7 min. Limit of detection, 0.07 mg/L, lower limit of quantification, 0.25 mg/L [Pham-Huy *et al.* 1999].
Tissue **HPLC** Limit of quantification, 0.001 mg/L [Sinko *et al.* 1998].
Disposition in the Body Valaciclovir is absorbed easily and rapidly from the gastrointestinal tract after oral administration. Absorption is unaffected by administration with food. Valaciclovir is rapidly converted to aciclovir (99% of a dose) and valine by first-pass intestinal and/or hepatic metabolism, mediated by valaciclovir hydrolase. Plasma concentrations of valaciclovir are undetectable after 3 h and plasma aciclovir concentrations can be detected as early as 15 min. Elimination is renal as aciclovir, and the metabolites 9-carboxymethoxymethylguanine (CMMG) and 8-hydroxy-aciclovir (8-OHACV). <1% of the administered dose of valaciclovir is recovered unchanged in urine over a 24 h period. Peak plasma valaciclovir concentrations are generally less than 0.5 mg/L at all doses. Hepatic impairment does not affect pharmacokinetics. No accumulation of aciclovir occurs after administration of valaciclovir at the recommended dosage regimens in healthy individuals.
Therapeutic Concentration

Thirty-nine elderly normotensive or hypertensive volunteers with a mean age of 72 years were administered the following dose regimen: 500 or 1000 mg valaciclovir twice on day 1; 500 or 1000 mg three times a day for days 2 to 7; and either doses once on day 8 (a total of 21 doses). Peak alaciclovir concentrations for the normotensive patients receiving 500 mg valaciclovir were 4.14 mg/L on day 1 and 4.30 mg/L on day 8 (steady state) observed at 1.41 and 1.22 h, respectively. For the 1000 mg dose, however, peak concentrations were 5.66 mg/L at 1.67 h on day 1 and 5.98 mg/L at 2.06 h at steady state. For the hypertensive patients, also receiving thiazide diuretics, peak concentrations with the 500 mg dose were 3.56 mg/L on day 1 observed after 1.22 h and 3.89 mg/L in the steady state at 1.47 h [Wang *et al.* 1996].

Twenty pregnant women (at 36 weeks' gestation) with a history of reoccurring genital herpes simplex virus infections (with positive herpes simplex virus 2 serological results) were administered 500 mg valaciclovir twice daily for 2 weeks. Peak aciclovir plasma concentrations were 3.14 mg/L after the initial dose and 3.03 mg/L at steady state (after 2 weeks dosing) [Kimberlin *et al.* 1998].

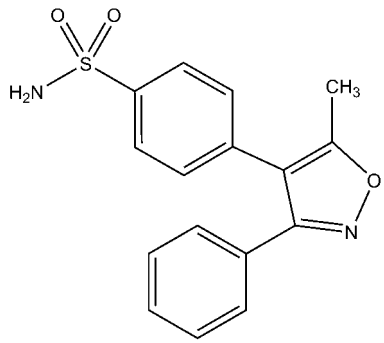
Bioavailability Approximately 54%.
Half-life Three hours as aciclovir which may be increased to 14 h in patients with end stage renal disease.
Volume of Distribution Has not been calculated, as the drug has not been administered IV and the elimination rate constant has not been accurately measured. Aciclovir is 50 L in a typical adult.
Clearance Aciclovir, 679 mL/min/1.73 m^2 in healthy individuals and 86 mL/min/1.73 m^2 in dialysis patients. The mean renal clearance is 15.3 L/h after a single 1000 mg dose of valaciclovir.
Protein Binding 13.5 to 17.9%.
Dose 1 g oral dose.

Kimberlin DF *et al.* (1998). Pharmacokinetics of oral valacyclovir and acyclovir in late pregnancy. *Am J Obstet Gynecol* 179(4): 846–851.
Pham-Huy C *et al.* (1999). Rapid determination of valaciclovir and acyclovir in human biological fluids by high-performance liquid chromatography using isocratic elution. *J Chromatogr B Biomed Sci Appl* 732: 47–53.
Poirier JM *et al.* (1999). Determination of acyclovir in plasma by solid-phase extraction and column liquid chromatography. *Ther Drug Monit* 21: 129–133.
Sinko PJ *et al.* (1998). Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats. *Biopharm Drug Dispos* 19: 209–217.
Wang LH *et al.* (1996). Pharmacokinetics and safety of multiple-dose valaciclovir in geriatric volunteers with and without concomitant diuretic therapy. *Antimicrob Agents Chemother* 40(1): 80–85.

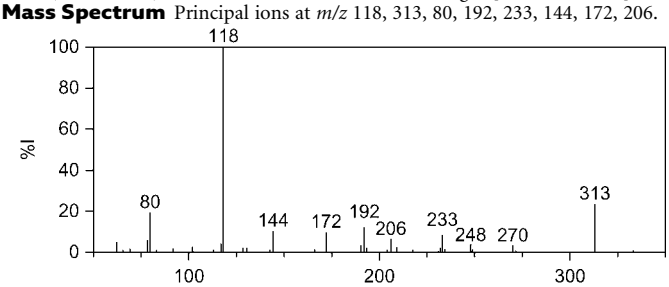
Valdecoxib

Analgesic, Antiinflammatory, COX-2 Inhibitor, Sulfonamide
$C_{16}H_{14}N_2O_3S = 314.4$
CAS—181695-72-7

IUPAC Name 4-(5-Methyl-3-phenyl-1,2-oxazol-4-yl)benzenesulfonamide
Synonyms 4-(5-Methyl-3-phenyl-4-isoxazolyl)benzenesulfonamide; SC-65872.
Proprietary Names *Bextra*; *Valus Insta*; *Valus-XT*; *Vectra-P*; *Vorth Insta*; *Vorth-XT*.



Chemical Properties A white crystalline powder. Mp 155° to 157°. Freely soluble in organic solvents and alkaline aqueous solutions; soluble in methanol and ethanol; insoluble in water (10 mg/L at 25°). Stability in plasma was established for 21 days at –20°, in frozen plasma at –20° for at least 3 freeze-thaw cycles, over a period of 24 h at 5°, and on the benchtop for 8 h [Pavan Kumar *et al.* 2006]. Stability of valdecoxib in plasma stored at -80°C including 2 freeze-thaw cycles was >90%. Standard solutions were found to be stable at –80° for at least 2 months and a minimum of 2 weeks when stored protected from light at room temperature [Werner *et al.* 2005]. Stability in plasma was excellent with no evidence of degradation with 30 days freezer storage. [Ramakrishna *et al.* 2004]. Samples were stable in urine for 133 days at –20°. [Zhang *et al.* 2003a].
High Performance Liquid Chromatography Column: Reversed-phase Intersil C_{18}. Mobile phase: methanol : 0.05% aqueous glacial acetic acid (68 : 32). DAD (λ = 230 nm). Limit of detection, 0.127 to 1.04 mg/L [Rao *et al.* 2005].
Mass Spectrum Principal ions at *m/z* 118, 313, 80, 192, 233, 144, 172, 206.



Quantification

Plasma **HPLC** Column: Kromasil KR 100-5 C_{18} (250 × 4.6 mm i.d., 5 µm) Mobile phase: 0.05 mol/L formic acid (pH 3.0) : water-acetonitrile (5 : 95) : methanol-water (90 : 10; 100 : 0 : 0 at 0 min to 90 : 0 : 10 at 2 min to 50 : 20 : 30 at 9 min to 30 : 50 : 20 at 25 min to 10 : 85 : 5 at 35 min to 100 : 0 : 0 at 36 min until 45 min), flow rate 1.0 mL/min. UV detection (λ = 235 nm). Limit of quantification, 0.1 mg/L [Pavan Kumar *et al.* 2006]. Column: C_{18}. Mobile phase: acetonitrile : water (pH 3.2; 60 : 40), flow rate 1 mL/min. UV detection (λ = 240 nm). Limit of detection, 10 µg/L [Keshetty *et al.* 2006]. Column: Reversed phase ODS-AQ. Mobile phase: methanol : water (53 : 47). UV detection (λ = 210 nm). Limit of quantification, 10 µg/L, [Ramakrishna *et al.* 2004].

LC-MS Limit of quantification, valdecoxib 0.5 µg/L, valdecoxib hydroxy metabolite 0.5 µg/L [Zhang *et al.* 2003b]. Column: Nucleosil C_8 guard column (120-5, 8 × 3 mm). Mobile phase: methanol : water (50 : 50), flow rate 300 µL/min. APCI, SRM acquisition mode. Limit of quantification, 5 µg/L [Werner *et al.* 2005].

Urine **LC-MS** Column: Reversed phase. Mobile phase: acetonitrile : water (50 : 50) containing 10 mmol/L 4-methylmorpholine (pH 6.0). Limit of quantification, valdecoxib 1 µg/L, valdecoxib hydroxy metabolite 1 µg/L, valdecoxib carboxylic acid metabolite 2 µg/L [Zhang *et al.* 2003a].

Disposition in the Body Extensively metabolised in the liver after absorption primarily via CYP2C9 and CYP3A4 to valdecoxib *N*-glucuronide, a hydroxylated metabolite, and a carboxylic acid metabolite. Approximately 70% is excreted in urine as the glucuronide conjugates of valdecoxib and its hydroxylated metabolite [Zhang *et al.* 2003a], and 20% in faeces as the metabolites, with less than 5% of the parent drug excreted unchanged.

Therapeutic Concentration

Eight healthy men (aged 20 to 42 years) were administered 10 mg daily for 14 days. Peak plasma concentrations reached 161 µg/L after 2.25 h and the minimum concentration was 21.9 µg/L. Steady state concentrations were reached by day 4 [Pfizer 2004].

Toxicity On April 7 2005 valdecoxib was withdrawn from the US market because of an increased risk of cardiovascular events as well as the risk of serious, sometimes fatal, skin reactions, including toxic epidermal necrolysis, Stevens–Johnson syndrome, and arythema multiforme. To date (2008) the FDA has received 7 reported deaths from serious skin reactions following valdecoxib therapy.

Bioavailability Absolute, 83%.

Half-life Elimination, 8 to 11 h which increases with age.

Clearance Oral, 6 L/h.

Distribution in Blood Blood : plasma ratio, 2.5 : 1.

Protein Binding Approximately 98%.

Dose 10 mg daily.

Keshetty S *et al.* (2006). Determination of valdecoxib in serum using a HPLC-diode array detector and its application in a pharmacokinetic study. *Pharmazie* 61: 245–246.

Pavan Kumar VV *et al.* (2006). Simultaneous quantitation of etoricoxib, salicylic acid, valdecoxib, ketoprofen, nimesulide and celecoxib in plasma by high-performance liquid chromatography with UV detection. *Biomed Chromatogr* 20: 125–132.

Pfizer (2004). Valdecoxib Prescribing Information. New York: Pfizer; (see for health warning http://www.fda.gov/medwatch/SAFETY/2004/Bextra_PI.pdf. 2004; accessed 16 September 2008).

Ramakrishna NV *et al.* (2004). Quantitation of valdecoxib in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection using liquid–liquid extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 802: 271–275.

Rao RN *et al.* (2005). Development and validation of a reversed-phase liquid chromatographic method for separation and simultaneous determination of COX-2 inhibitors in pharmaceuticals and its application to biological fluids. *Biomed Chromatogr* 19: 362–368.

Werner U *et al.* (2005). A liquid chromatography–mass spectrometry method for the quantification of both etoricoxib and valdecoxib in human plasma. *Biomed Chromatogr* 19: 113–118.

Zhang JY *et al.* (2003). Determination of valdecoxib and its metabolites in human urine by automated solid-phase extraction-liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 785: 123–134.

Zhang JY *et al.* (2003). Development and validation of an automated SPE-LC-MS/MS assay for valdecoxib and its hydroxylated metabolite in human plasma. *J Pharm Biomed Anal* 33: 61–72.

Valethamate Bromide

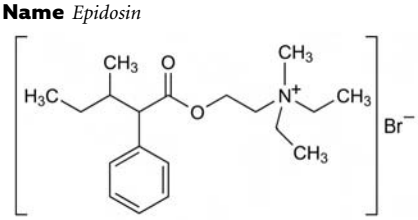
Anticholinergic

$C_{19}H_{32}BrNO_2$ = 386.4

CAS—16376-74-2 (valethamate); 90-22-2 (bromide)

IUPAC Name *N*,*N*-Diethyl-*N*-methyl-2-[(3-methyl-1-oxo-2-phenylpentyl)oxy]-ethanaminium bromide

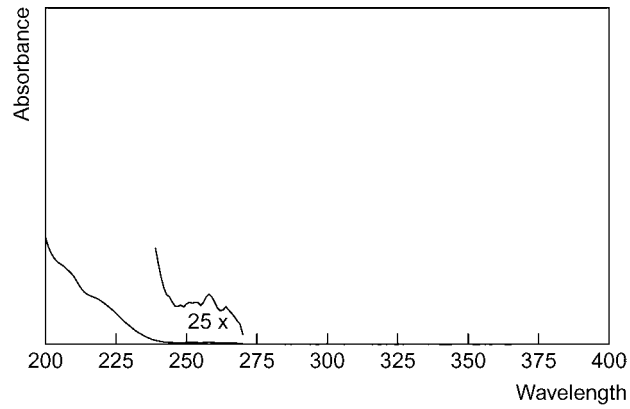
Proprietary Name *Epidosin*



Chemical Properties A white crystalline powder. Mp 100° to 101°. Freely soluble in water; very soluble in ethanol; practically insoluble in ether. Log *P* (octanol/water), 1.1.

Thin-layer Chromatography System TA—R_f 0.02 (acidified iodoplatinate solution, positive).

Ultraviolet Spectrum Aqueous acid—253, 259 (A_1^1=5.4b), 265 nm.



Infrared Spectrum Principal peaks at wavenumbers 1730, 1230, 695, 726, 1163, 1265 cm^{-1} (KBr disk).

Dose 30 to 80 mg daily.

Valproic Acid

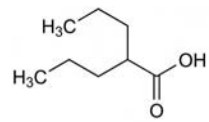
Anticonvulsant, Antiepileptic

$C_8H_{16}O_2$ = 144.2

CAS—99-66-1

IUPAC Name 2-Propylpentanoic acid

Proprietary Names *Convulex (capsules)*; *Cryoval*; *Depakene (capsules)*; *Deproic*; *Ergenyl*; *Mylproin*; *Proteval*; *Provetal*; *Valcaps*; *Valken*; *Valporal*; *Valprosid*.



Chemical Properties A colourless liquid. Very slightly soluble in water. pK_a 4.6. Log *P* (octanol/water), 2.8. Extraction yield (chlorobutane), 0.07 [Demme *et al.* 2005].

Sodium Valproate

$C_8H_{15}NaO_2$ = 166.2

CAS—1069-66-5

Proprietary Names *Absenor*; *Convulex (solution and tablets)*; *Delepsine*; *Depacon*; *Depakene (syrup)*; *Depakin(e)*; *Deplexil*; *Deproic*; *Encorate*; *Epilim*; *Ergenyl*; *Leptilan*; *Leptilanil*; *Orfiril*; *Orlept*; *Selenica*; *Valpakine*; *Valparin*; *Valporal*; *Valpro*; *Valprosid*.

Chemical Properties A white, crystalline, deliquescent powder. Soluble 1 in 0.4 of water, 1 in 5 of ethanol; practically insoluble in common organic solvents.

Colour Test Ferric chloride—orange.

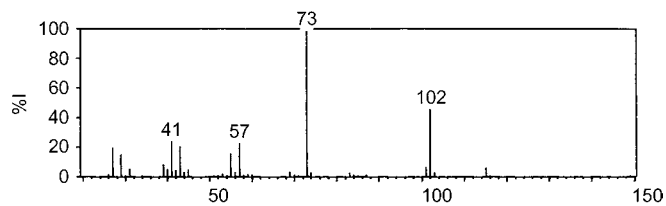
Thin-layer Chromatography System TD—R_f 0.00; system TE—R_f 0.00; system TF—R_f 0.52; system TAD—R_f 0.00.

Gas Chromatography System GA—RI 1064; system GB—RI 1098; system GE—RRT 0.09 (relative to phenytoin); system GAJ—RRT 0.350 (relative to methylphenobarbital).

High Performance Liquid Chromatography System HZ—RT 8.0 min.

Infrared Spectrum Principal peaks at wavenumbers 1708, 1213, 1250, 1278, 946, 1107 cm^{-1} (thin film).

Mass Spectrum Principal ions at *m*/*z* 73, 102, 41, 57, 43, 27, 55, 29.



Quantification

Plasma **GC** Column: Stabilwax (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: H_2, 45 cm/s. Temperature programme: 40° for 0.5 min to 120° at 50°/min to 150° at 2°/min to 250° at 50°/min for 5 min. ECD. Limit of quantification, 0.1 mg/L [Dills, Shen 1997]. Column: Nukol (30 m × 0.2 mm i.d., 0.25 µm). Temperature programme: 60° for 2 min to 150° at 30°/min to 190° at 10°/min for 4 min. FID. Limit of detection, 1 µg/L [Krogh *et al.* 1995]. Column: Chrom Q 100 mesh (1.2 m × 4 mm i.d.). Carrier gas: N_2, 0.55 kg/cm^2. Temperature: 160°. FID. Limit of detection, 2 mg/L [Pokrajac *et al.* 1992]. Column: DB-1 (60 m × 0.25 mm i.d., 0.25 µm).

Carrier gas: H_2, 40 cm/s. Temperature programme: 40° for 0.5 min to 80° at 40°/min for 2 min to 250° at 10°/min for 3 min. FID. Retention time: 9.39 min. Limit of detection, 200 µg/L [Semmes, Shen 1988]. Column: 10% Carbowax 6000 on Chromosorb WAW 80/100 (1.8 m [6 ft] × 2 mm i.d.). Temperature: 170°. FID. Limit of detection, 1 mg/L [Lin, Kelly 1985]. FID. Limit of detection, 1–2 mg/L [Krogh *et al.* 1995; Riva *et al.* 1982].

GC-MS Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° to 300° at 10°/min. EI ionisation at 70 eV. Limit of quantification, 300 µg/L [Deng *et al.* 2006]

HPLC Column: CN (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 40 µmol/L aqueous sodium dihydrogen phosphate (pH 3.5, 30 : 70), flow rate 1 mL/min. UV detection (λ= 210 nm). Limit of quantification, 1.25 mg/L, limit of detection, 100 µg/L [Amini *et al.* 2006]. Column: Zorbax Eclipse XDB-C_8 (150 × 4.6 mm i.d.). Mobile phase: 30 mmol/L sodium acetate buffer (pH 4.8) : acetonitrile (61.5 : 38.5 for 4 min to 15 : 85 at 20 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 325 nm, λ_{em}= 398 nm). Limit of quantification, 5.0 mg/L [Zhong *et al.* 2006]. See also Kushida, Ishizaki [1985], Liu *et al.* [1992] and van der Horst *et al.* [1988].

LC-MS Column: Non-porous silica. Mobile phase: ammonium acetate : methanol. MRM acquisition mode. Limit of quantification, 500 µg/L. Limit of detection, 170 µg/L [Matsuura *et al.* 2008]. Column: Thermo Beta Basic C_8 (500 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile-water (20 : 80) containing 0.1% formic acid : acetonitrile-water (70 : 30) containing 0.1% formic acid (100 : 0 for 11 min to 0 : 100 in 1 min for 4 min to 100 : 0 in 1 min for 3 min), flow rate 0.2 mL/min. ESI, MRM acquisition mode. Limit of quantification, 200 µg/L [Cheng *et al.* 2007].

Serum **GC** Column: DB WAX capillary (30 × 0.25 mm i.d., 0.25 µm). Carrier gas: N_2, 1.2 and 45 mL/min. Temperature programme: 150° and 250°. FID. Limit of detection, 1.7 mg/L [Farajzadeh *et al.* 2009]. Column: cyanopropylphenyldimethylsiloxane fused silica (25 m × 0.22 mm i.d., 2.5 µm). Carrier gas: He, 1.8 mL/min. Temperature programme: 80° for 2 min to 140° at 15°/min for 1 min to 280° at 40°/min for 8 min. FID. Limit of detection, 800 µg/L [Shahdousti *et al.* 2007]. See Plasma [Lin, Kelly 1985].

GC-MS Column: OV-1701 bonded phase (25 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 15 psi. Temperature programme: 50° to 140° at 30°/min to 250° at 5°/min. Limit of detection, 2 µg/L [Kassahun *et al.* 1989]. Column: 3% OV-17 on 120/140 mesh Gas-Chrom Q (2 m × 2.5 mm i.d.). Temperature programme: 80° for 1 min to 140° at 12.5/min to 250° at 30°/min for 1 min. EI ionisation. Limit of detection, 2.8 to 18 µg/L [Nau *et al.* 1981].

HPLC Column: reversed phase C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L phosphate buffer, (pH 3.0, 45 : 55 [v/v]), flow rate 1.2 mL/min. Limit of quantification, 1 mg/L [Kishore *et al.* 2003]. Column: YMC Pack ODS-A reversed phase. Fluorescence detection (λ_{ex}= 365 nm, λ_{em}= 440 nm). Limit of detection, 100 µg/L [Hara *et al.* 1999]. Column: HP Hypersil-ODS (100 × 2.1 mm i.d., 5 µm). Mobile phase: methanol : water (80 : 20), flow rate 0.3 mL/min. DAD and fluorescence detection (λ_{ex}= 322 nm, λ_{em}= 695 nm). Limit of detecton, 2.5 mg/L [Liu *et al.* 1992]. Column: Hypersil ODS (5 µm). Mobile phase: acetonitrile : water (65 : 35), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 310 nm, λ_{em}= 405 nm). Limit of detection, 5 mg/L [Bousquet *et al.* 1991]. Column: Hypersil ODS (100 × 5.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.003 mol/L potassium dihydrogen phosphate (30 : 70), flow rate 2 mL/min. UV detection (λ= 254 nm). Limit of detection, 9 mg/L [Moody, Allan 1983].

LC-MS Column: Inertsil ODS C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L ammonium acetate buffer : methanol (15 : 85), flow rate 1.0 mL/min. T-ESI, negative ion mode, SIM acquisition mode. Limit of quantification, 500 µg/L [Ramakrishna *et al.* 2005]. Column: Shim-pack CLC-ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: 90% methanol : 0.1 mol/L ammonium acetate buffer (pH 4.0, 96 : 4), flow rate 1 mL/min. SIM acquisition mode. Limit of detection, 500 µg/L [Mino *et al.* 2001].

Urine **GC-MS** See Serum [Nau *et al.* 1981].

Breast Milk **GC-MS** See Serum [Nau *et al.* 1981].

Oral Fluid **GC-MS** See Serum [Kassahun *et al.* 1989].

Brain **GC** See Plasma [Dills, Shen 1997]. See Plasma. Limit of detection, 0.1 µg/g [Semmes, Shen 1988].

Disposition in the Body Valproic acid is well absorbed after oral administration; bioavailability is almost 100%. It is metabolised mainly by oxidation to a number of metabolites, including 3-oxovalproic acid, 2-propylglutaric acid, 3-hydroxyvalproic acid, 4-hydroxyvalproic acid, 5-hydroxyvalproic acid and valpro-1,4-lactone. Approximately 20% of a dose is excreted in the urine as the glucuronide of valproic acid in 72 h; most of the remainder is excreted as glucuronides of metabolites, <5% of a dose being excreted as unchanged drug.

Therapeutic Concentration In plasma, usually in the range 40–100 mg/L, but there is no clear correlation with therapeutic effect.

Following a single oral dose of 500 mg to 6 subjects, peak plasma concentrations of 36–73 mg/L (mean, 58) were attained in 1–5 h (mean, 3) [May, Garnett 1983].

Daily oral doses in the range 1–3.75 g were administered to 20 patients; at the end of 10 weeks, minimum serum concentrations were 30–135 mg/L (mean, 82) and maximum concentrations were 70–190 mg/L (mean, 109). Approximately 75% of the patients had serum concentrations within 55–100 mg/L [Bruni *et al.* 1978].

Rapid IV infusion of valproic acid in 21 subjects (dose range 21–28 mg/kg; mean, 24.2) at a rate of 3 or 6 mg/kg/min produced peak serum concentrations of 105–204.1 mg/L (mean, 132.6) 20 min after the end of infusion; in 2 subjects the serum valproic acid levels were 64 and 74 mg/L at 30–40 min after the infusion [Venkataraman, Wheless 1999].

Toxicity Plasma concentrations >200 mg/L are associated with toxic effects but valproic acid is relatively non-toxic after overdose and recovery after the ingestion of up to 75 g has been reported. Hepatotoxicity has been reported during therapeutic administration.

A 43-year-old female recovered following haemodialysis after becoming comatose owing to ingesting a large amount of valproic acid; her serum valproic acid concentration on admission to hospital was 1380 mg/L [Johnson *et al.* 1999].

A 25-year-old female became comatose after ingesting an unknown quantity of valproic acid (concentrations increased to >1200 mg/L); she was successfully treated with haemodialysis [Kane *et al.* 2000].

A multicentre case series study of valproic acid intoxication revealed that, of 335 reports, 186 had serum levels of >100 mg/L. Peak serum valproic acid concentrations were 110–1840 mg/L (mean, 378.3) at 1–18 h (mean, 7.4). A peak concentration of >850 mg/L was more likely to be associated with coma, respiratory depression, aspiration or metabolic acidosis [Spiller *et al.* 2000].

Half-life Plasma half-life, ~6–20 h, reduced when administered in combination with other anticonvulsants.

Volume of Distribution ≈0.1–0.2 L/kg.

Clearance Plasma clearance, ~0.1–0.3 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.8.

Protein Binding Approximately 90% (concentration dependent; decreased at plasma concentration >100 µg/mL).

Note For a review of the clinical pharmacokinetics of valproic acid, see Gugler, von Unruh [1980]; for a review of its toxicity, see Sztajnkrycer [2002]; for a review of its pharmacological and therapeutic properties, see Perucca [2002].

Dose Initially 600 mg of sodium valproate daily, increasing to 2.6 g daily.

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Kane SL *et al.* (2000). High-flux hemodialysis without hemoperfusion is effective in acute valproic acid overdose. *Ann Pharmacother* 34: 1146–1151.

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Kishore P *et al.* (2003). HPLC determination of valproic acid in human serum. *Pharmazie* 58: 378–380.

Krogh M *et al.* (1995). Solid-phase microextraction for the determination of the free concentration of valproic acid in human plasma by capillary gas chromatography. *J Chromatogr B Biomed Appl* 673: 299–305.

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Lin W, Kelly AR (1985). Determination of valproic acid in plasma or serum by solid-phase column extraction and gas–liquid chromatography. *Ther Drug Monit* 7: 336–343.

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Matsuura K *et al.* (2008). A simple and rapid determination of valproic acid in human plasma using a non-porous silica column and liquid chromatography with tandem mass spectrometric detection. *Biomed Chromatogr* 22: 387–393.

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Moody JP, Allan SM (1983). Measurement of valproic acid in serum as the 4-bromophenacyl ester by high performance liquid chromatography. *Clin Chim Acta* 127: 263–269.

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Valsartan

Angiotensin II Receptor Antagonist

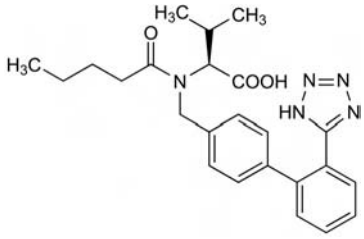
$C_{24}H_{29}N_5O_3 = 435.5$

CAS—137862-53-4

IUPAC Name (2*S*)-3-Methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid

Synonyms CGP-48933; *N*-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-*N*-valeryl-L-valine.

Proprietary Names *Diovan*; *Tareg*.

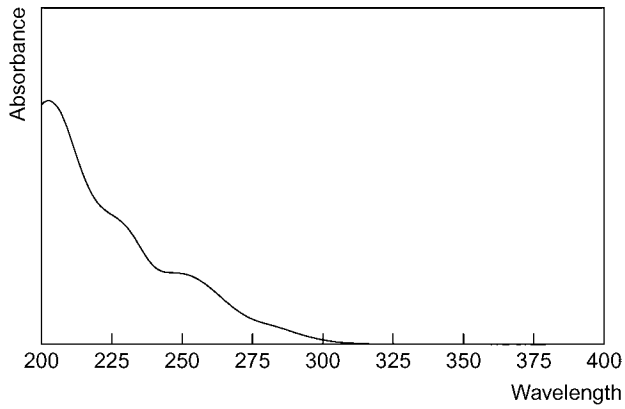


Chemical Properties A white to practically white fine powder. Mp 116° to 117°. Soluble in ethanol and methanol; slightly soluble in water.

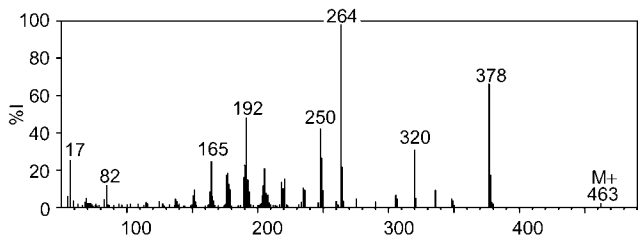
Gas Chromatography System GP—RI 3420valsartan-ME_2.

High Performance Liquid Chromatography Column: Chiral AGP (ChromTech) silica bonded α_1-acid glycoprotein (100 × 4 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 7.0, containing 2% 2-propanol), flow rate 0.8 mL/min. UV detection (λ=227 nm). Retention time: 10 min; CGP 49309, 6 min [Francotte *et al.* 1996].

Ultraviolet Spectrum Aqueous acid—203, 248 nm.



Mass Spectrum Principal ions at *m/z* 264, 378, 192, 250, 320, 58, 165, 251 (valsartan-ME_2).



Quantification

Plasma **HPLC** Fluorometric detection. Limit of quantification, 5 µg/L [Brookman *et al.* 1997]. Column: ODS Hypersil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 2.8) : acetonitrile (50 : 50), flow rate 1.3 mL/min. Fluorescence detection (λ_{ex}=265 nm; λ_{em}=378 nm). Retention time: 5 min. Limit of quantification, 5 µg/L [Sioufi *et al.* 1994].

Urine **GC–MS** Limit of detection, 10 µg/L [Maurer *et al.* 1998].

HPLC See Plasma. Limit of quantification, 10 µg/L [Brookman *et al.* 1997]. See Plasma. Limit of quantification, 10 µg/L [Sioufi *et al.* 1994].

Bile **HPLC** See Plasma. Limit of quantification, 10 µg/L [Brookman *et al.* 1997]. See Plasma. Limit of quantification, 10 µg/L [Sioufi *et al.* 1994].

Disposition in the Body Valsartan is rapidly absorbed following oral administration. It is quickly eliminated from the body and no significant metabolism takes place. A pharmacologically inactive metabolite, valeryl-4-hydroxyvalsartan, is formed by oxidative biotransformation, accounting for 9% of the dose, and can be detected in plasma after 2 h. Following an oral dose, 86% is excreted in faeces, mainly as the unchanged drug and 13% in urine, 10% as the unchanged drug. After IV administration, 30% of a dose is detected unchanged in urine. The bulk of the drug is excreted within 4 days; and 99% of a dose is excreted in 7 days. The drug displays linear pharmacokinetics. Little accumulation occurs following multiple dosing.

Therapeutic Concentration

A peak plasma concentration of 1.64 mg/L occurs in 2 h following a single 80 mg dose and 3.46 mg/L following 200 mg [Markham, Goa 1997].

Twelve patients, 6 with mild impairment of liver function and 6 with moderate impairment, and 12 healthy individuals were orally administered a single dose of 160 mg valsartan. The patients with mild impairment produced mean peak concentrations of 5.91 (range, 4.06 to 7.16) mg/L at 3.5 h; those with moderate impairment, 3.91 (2.44 to 6.5) mg/L at 4.0 h and the healthy individuals produced peak concentrations of 3.30 (1.27 to 5.12) mg/L at 2.5 h [Brookman *et al.* 1997].

Bioavailability 23%.

Half-life Mean 7.5 h (range, 5 to 9 h).

Volume of Distribution Steady state, 16.9 L.

Clearance Plasma, 2.2 L/h.

Protein Binding 94 to 97% mainly to albumin.

Dose Usually 80 to 160 mg and a maximum of 320 mg.

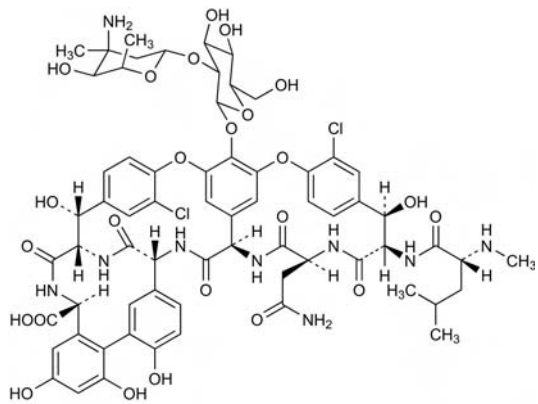
Brookman LJ *et al.* (1997). Pharmacokinetics of valsartan in patients with liver disease. *Clin Pharmacol Ther* 62(3): 272–278.
Francotte E *et al.* (1996). Development and validation of chiral high-performance liquid chromatographic methods for the quantitation of valsartan and of the tosylate of valinebenzyl ester. *J Chromatogr B* 686: 77–83.
Markham A, Goa KL (1997). Valsartan. A review of its pharmacology and therapeutic use in essential hypertension. *Drugs* 54: 299–311.
Maurer HH *et al.* (1998). Screening for the detection of angiotensin-converting enzyme inhibitors, their metabolites, and AT II receptor antagonists. *Ther Drug Monit* 20: 706–713.
Sioufi A *et al.* (1994). *J Liq Chromatogr* 17(10): 2179–2186.

Vancomycin

Antibiotic

$C_{66}H_{75}Cl_2N_9O_{24} = 1449.3$

CAS—1404-90-6



Chemical Properties An amphoteric glycopeptide antimicrobial substance produced by the growth of certain strains of *Streptomyces orientalis*.

Vancomycin Hydrochloride

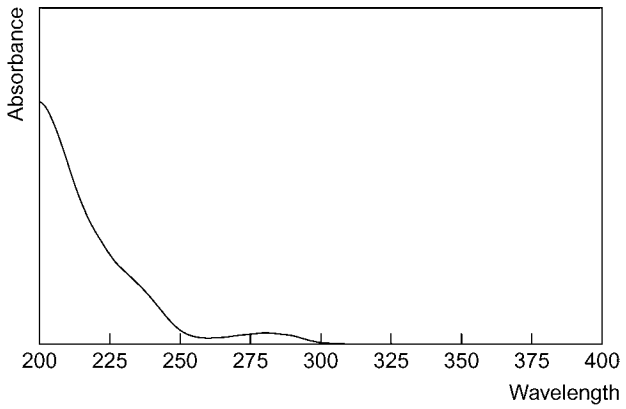
$C_{66}H_{75}Cl_2N_9O_{24}.HCl = 1485.7$

CAS—1404-93-9

Proprietary Names *Balcoran*; *Diatracin*; *Edicin*; *Glipep*; *Lyphocin*; *Orivan*; *Vancocin(e)*; *Vancocina*; *Vancoled*; *Vancoscand*; *Vancox*

Chemical Properties A light brown powder. Soluble 1 in 10 of water; moderately soluble in dilute methanol; insoluble in higher alcohols, acetone and ether.

Colour Test Marquis test—yellow.
Thin-layer Chromatography System TA—R_f 0.22 (acidified potassium permanganate solution, positive).
Ultraviolet Spectrum Vancomycin hydrochloride: aqueous acid—281 nm (A^1_1=34b).



Quantification

Plasma **HPLC** Electrochemical detection. For comparison with EMIT, see Favetta *et al.* [2001]. UV detection. For comparison with FPIA, see Farin *et al.* [1998]. UV detection. For method, see Li *et al.* [1995]. For method, see Luksa and Marusic [1995]. UV detection. Limit of detection, 3 mg/L [Greene *et al.* 1987].
Serum **HPLC** UV detection (comparison with FPIA). Limit of detection, 0.5 mg/L [Furuta *et al.* 2000]. UV detection. For a method for the quantification of vancomycin and CDP-1, see Backes *et al.* [1998]. See Plasma [Li *et al.* 1995]. For comparison with EMIT, see Demotes-Mainard *et al.* [1994]. For the quantification of vancomycin and its crystalline degradation product CDP-1 (comparison with EMIT and fluorescence polarisation immunoassay (FPIA)), see Hu *et al.* [1990]. Limit of detection, 100 µg/L [Jehl *et al.* 1985]. UV detection. Limit of detection, 2 mg/L [Hoagland *et al.* 1984].

Fluorescence Polarisation Immunoassay (FPIA) Limit of detection, <2 mg/L [Adamczyk *et al.* 1998]. For comparison with HPLC and EMIT, see Smith *et al.* [1998].

Radioimmunoassay Limit of detection, 40 ng/L [Fong *et al.* 1981].
Urine **Radioimmunoassay** See Serum [Fong *et al.* 1981].
Cerebrospinal Fluid **HPLC** See Serum [Jehl *et al.* 1985].
Pericardial Fluid **HPLC** See Plasma [Greene *et al.* 1987].
Peritoneal Fluid **HPLC** See Serum [Jehl *et al.* 1985].
Bone Atrial Appendage Tissue **HPLC** See Plasma. Limit of detection, 0.75 µg/g [Greene *et al.* 1987].
Tissue **HPLC** See Plasma [Farin *et al.* 1998].
Disposition in the Body Not absorbed from the gastrointestinal tract. About 90% of an IV dose is excreted in the urine with about 50% of the dose being excreted in 4 h.
Therapeutic Concentration In plasma, usually in the range 10 to 40 mg/L. In 101 infants who received vancomycin 15 mg/kg by IV infusion over 60 min, every 12 or 18 h, trough serum concentrations of 0.6 to 23.4 mg/L (median, 7.1) and peak serum concentrations of 9.0 to 47.2 mg/L (median, 25.5) were attained. Target trough concentrations of 1 to 10 mg/L were achieved by 46.5% and 1 to 12 mg/L by 55.4%; peak concentrations of 20 to 40 mg/L were found in 83.2% and 15 to 60 mg/L in 99.0% [Tan *et al.* 2002].
Half-life Plasma half-life, about 4 to 10 h.
Volume of Distribution About 0.4 to 1 L/kg.
Clearance Plasma clearance, about 1 mL/min/kg.
Protein Binding About 55%.
Note For a review of vancomycin, see Cunha and Ristuccia [1983].
Dose The equivalent of up to 2 g of vancomycin daily, orally or by IV infusion.

Adamczyk M *et al.* (1998). Development of a quantitative vancomycin immunoassay for the Abbott AxSYM analyzer. *Ther Drug Monit* 20: 191–201.
Backes DW *et al.* (1998). Quantitation of vancomycin and its crystalline degradation product (CDP-1) in human serum by high performance liquid chromatography. *J Pharm Biomed Anal* 16: 1281–1287.
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Demotes-Mainard F *et al.* (1994). Column-switching high-performance liquid chromatographic determination of vancomycin in serum. *Ther Drug Monit* 16: 293–297.
Farin D *et al.* (1998). A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX). *J Pharm Biomed Anal* 18: 367–372.
Favetta P *et al.* (2001). New sensitive assay of vancomycin in human plasma using high-performance liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Sci Appl* 751: 377–382.
Fong KL *et al.* (1981). Sensitive radioimmunoassay for vancomycin. *Antimicrob Agents Chemother* 19: 139–143.
Furuta I *et al.* (2000). Rapid serum vancomycin assay by high-performance liquid chromatography using a semipermeable surface packing material column. *Clin Chim Acta* 301: 31–39.
Greene SV *et al.* (1987). High-performance liquid chromatographic analysis of vancomycin in plasma, bone, atrial appendage tissue and pericardial fluid. *J Chromatogr* 417: 121–128.
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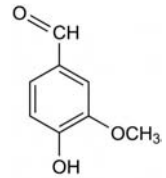
Jehl F *et al.* (1985). Determination of vancomycin in human serum by high-pressure liquid chromatography. *Antimicrob Agents Chemother* 27: 503–507.
Li L *et al.* (1995). An improved micromethod for vancomycin determination by high-performance liquid chromatography. *Ther Drug Monit* 17: 366–370.
Luksa J, Marusic A (1995). Rapid high-performance liquid chromatographic determination of vancomycin in human plasma. *J Chromatogr B Biomed Appl* 667: 277–281.
Smith PF *et al.* (1998). New modified fluorescence polarization immunoassay does not falsely elevate vancomycin concentrations in patients with end-stage renal disease. *Ther Drug Monit* 20: 231–235.
Tan WH *et al.* (2002). Dose regimen for vancomycin not needing serum peak levels? *Arch Dis Child Fetal Neonatal Ed* 87: F214–F216.

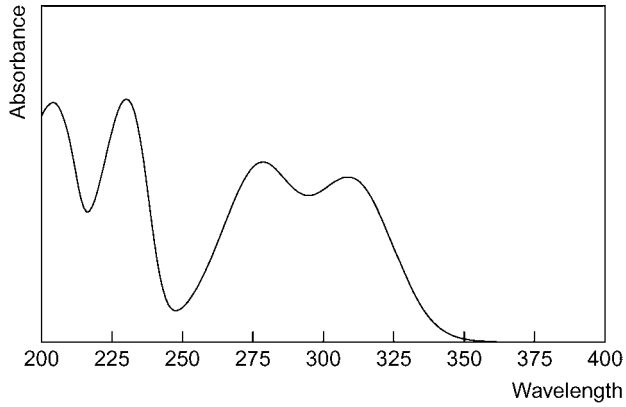
Vanillin

Flavouring Agent
$C_8H_8O_3$ = 152.1
CAS—121-33-5

IUPAC Name 4-Hydroxy-3-methoxybenzaldehyde
Synonyms Vainillina; vanillic aldehyde; vanillinum.



Chemical Properties White or slightly yellow crystalline needles or powder. Mp 81° to 83°. Soluble 1 in 100 of water; freely soluble in ethanol, chloroform and ether; soluble in glycerol and hot water. pK_a 7.4 (20°). Log *P* (octanol/water), 1.2.
Gas Chromatography System GA—RI 1630; system GB—RI 1632; system GN—retention time 86.5 min.
High Performance Liquid Chromatography System HAA—retention time 11.7 min.
Ultraviolet Spectrum Aqueous acid—230, 278 (A^1_1=685b), 309 nm; aqueous alkali—248, 348 nm (A^1_1=1640b).



Infrared Spectrum Principal peaks at wavenumbers 1660, 1153, 1267, 735, 1590, 1300 cm^{-1} (KCl disk).
Mass Spectrum Principal ions at *m/z* 152, 151, 81, 109, 51.
Quantification
Plasma **HPLC** UV detection. Limit of detection, 1 mg/L for vanillin and vanillic acid [Farthing *et al.* 1999].
Urine **HPLC** See Plasma [Farthing *et al.* 1999].
Red Blood Cells **HPLC** See Plasma [Farthing *et al.* 1999].

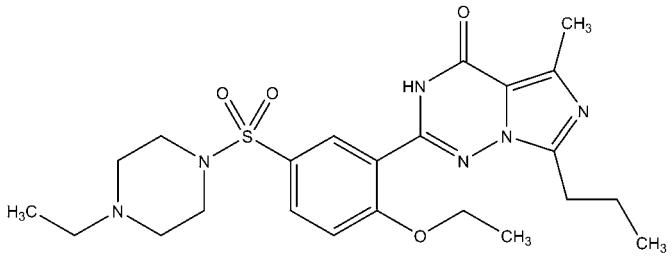
Farthing D *et al.* (1999). High-performance liquid chromatographic method for determination of vanillin and vanillic acid in human plasma, red blood cells and urine. *J Chromatogr B Biomed Sci Appl* 726: 303–307.

Vardenafil

Phosphodiesterase 5 Inhibitor, Treatment of ED
$C_{23}H_{32}N_6O_4S$ = 488.6
CAS—224785-90-4

IUPAC Name 2-[2-Ethoxy-5-(4-ethylpiperazin-1-yl)sulfonylphenyl]-5-methyl-7-propyl-1*H*-imidazo[5,1-*f*][1,2,4]triazin-4-one

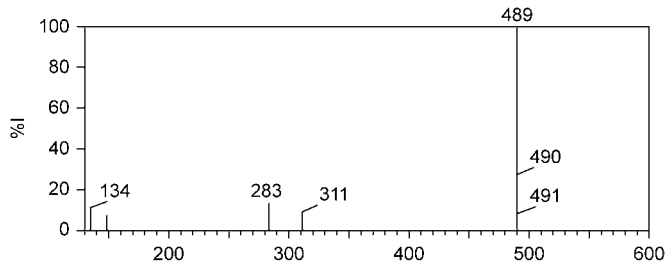
Synonyms 1-[[3-(1,4-Dihydro-5-methyl-4-oxo-7-propylimidazo-[5,1-f][1,2,4] triazin-2-yl)-4-ethoxy-phenyl]sulfonyl]-4-ethyl-piperazine; 4-[2-ethoxy-5-(4-ethyl-piperazin-1-yl)sulfonyl-phenyl]-9-methyl-7-propyl-; 3,5,6,8-tetrazabicyclo[4.3.0]nona -3,7,9-trien-2-one.



Vardenafil Dihydrochloride
$C_{23}H_{32}N_6O_4S,2HCl = 561.5$
CAS—224789-15-5

Vardenafil Hydrochloride Trihydrate
$C_{23}H_{32}N_6O_4S,HCl,3H_2O = 579.1$
CAS—330808-88-3; 224785-91-5 (anhydrous)
Synonym Bay-38-9456
Proprietary Names *Levitra*; *Nuviva*.
Chemical Properties Almost colourless solid. Mp 218°. Slightly soluble in water. pK_{a1} 3.4, pK_{a2} 6.7, pK_{a3} 8.8 [Rajagopalan *et al.* 2003]. Pure samples are stable when refrigerated or frozen. Plasma samples are stable at 20° for up to 4 h, at 4° for up to 24 h and at –20° for up to 28 days. Samples are stable following freeze–thaw and in processed samples left at room temperature for up to 16 h [Cheng *et al.* 2007].

Mass Spectrum Principal ions at *m/z* 489, 490, 283, 134, 311, 491.



Quantification
Blood **LC-MS** Mobile phase: acetonitrile : buffer (70 : 30), flow rate 1.0 mL/min. APCI. Limit of quantification, 0.39 µg/L; limit of detection, 0.19 µg/L [Johnson *et al.* 2007].
Bile **LC-MS** See Blood [Johnson *et al.* 2007].
Kidney **LC-MS** See Blood [Johnson *et al.* 2007].
Liver **LC-MS** Mobile phase: acetonitrile : buffer (70 : 30), flow rate 1.0 mL/min. APCI. Limit of quantification, 0.39 µg/L; limit of detection, 0.19 µg/L [Johnson *et al.* 2007].
Skeletal Muscle **LC-MS** See Blood [Johnson *et al.* 2007].
Heart Muscle **LC-MS** See Blood [Johnson *et al.* 2007].
Other **HPLC** Rat Plasma and Bile. Column: Hypersil-100 C_{18} (25 cm × 4.6 mm i. d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L ammonium acetate (pH 6.8; 40 : 60), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 470 nm). Limit of detection, 0.2 mg/L [Cheng *et al.* 2007]. Dietary Supplements. Column: C_{18} silica (5 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate and 0.2% formic acid (35 : 65 for 10 min to 80 : 20 at 15 min for 5 min to 100 : 0 for 5 min to 35 : 65 for 10 min), flow rate 1 mL/min. UV detection (λ = 292 nm). Limit of detection, 1.1 µg/L [Zhu *et al.* 2005].

LC-MS Dietary Supplements. Column: Zorbax stable bond C_{18} (150 × 2.1 mm, 5 µm). Mobile phase: acetonitrile : 0.1% formic acid in water (15 : 85 for 5 min to 90 : 10 at 15 min for 5 min), flow rate 400 µL/min. ESI, positive ion mode. Limit of detection, 1.4 mg/L [Gratz *et al.* 2004].

CE Pharmaceutical Formulations. Capillary: fused silica (60 cm × 75 µm i.d.). Electrolyte buffer: 10 mmol/L phosphate buffer (pH 12) with 25 mmol/L SDS. UV detection (λ = 222 nm). Limit of quantification, 1 mg/L [Rodriguez Flores *et al.* 2004].

Disposition in the Body Vardenafil is rapidly absorbed after administration, with peak plasma concentrations reached by 30 min to 2 h. Drug concentrations can decrease when the drug is administered with food. It is metabolised by a number of hepatic enzymes but predominantly by CYP3A4. The enzymes CYP3A5 and CYP2C also contribute to the metabolism of the drug. Desethylation of vardenafil results in M1 (major circulating metabolite), which is pharmacologically active with an estimated efficacy contribution of around 7%. Vardenafil can be extensively distributed between tissues and is excreted as metabolites in faeces (91 to 95% of dose) and also in urine (2 to 6%).

Therapeutic Concentration

Twenty-five healthy males were administered a single 20 mg dose after an overnight fast, after a high fat breakfast, in the evening on an empty stomach, or after an evening meal with a moderately high fat content. Mean peak plasma drug concentrations reached 17.1, 14.0, 14.2 and 13.0 µg/L, respectively, after 0.5 to 4 h. Metabolite concentrations reached 17.4, 9.3, 15.3 and 14.0 µg/L, respectively, for the four groups, [Rajagopalan *et al.* 2003].

Toxicity

Postmortem analysis of fluid and tissue samples from a victim of an aviation accident yielded the following levels:

Specimen	*Concentration (µg/L or µg/kg)*
Heart blood	291
Bile	1665
Liver	86
Kidney	15
Skeletal muscle	8
Heart muscle	26
Lung	234

Postmortem redistribution could explain the abnormally high blood concentration [Johnson *et al.* [2007].

Administration of 5 mg vardenafil in addition to ritonavir 600 mg twice daily resulted in a 13-fold increase in vardenafil peak plasma concentrations. Similar effects have been observed with concomitant administration of vardenafil with ketoconazole or erythromycin [Keating, Scott 2003].

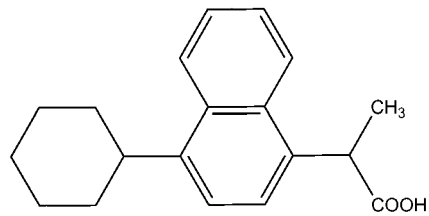
Bioavailability Absolute, 20%.
Half-life Elimination, 4 to 5 h.
Volume of Distribution 208 L at steady state.
Clearance Total body, 56 L/h.
Protein Binding Approximately 95%.
Dose Vardenafil hydrochloride trihydrate, 10 to 20 mg daily.

Cheng CL *et al.* (2007). Development and validation of a high-performance liquid chromatographic method using fluorescence detection for the determination of vardenafil in small volumes of rat plasma and bile. *J Chromatogr A* 1154: 222–229.
Gratz SR *et al.* (2004). Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal* 36: 525–533.
Johnson RD *et al.* (2007). The postmortem distribution of vardenafil (Levitra) in an aviation accident victim with an unusually high blood concentration. *J Anal Toxicol* 31: 328–333.
Keating G *et al.* (2003). Vardenafil: a review of its use in erectile dysfunction. *Drugs* 63: 2673–2703.
Rajagopalan P *et al.* (2003). Effect of high-fat breakfast and moderate-fat evening meal on the pharmacokinetics of vardenafil, an oral phosphodiesterase-5 inhibitor for the treatment of erectile dysfunction. *J Clin Pharmacol* 43: 260–267.
Rodriguez Flores J *et al.* (2004). Development of a micellar electrokinetic capillary chromatography method for the determination of three drugs employed in the erectile dysfunction therapy. *J Chromatogr B Analyt Technol Biomed Life Sci* 811: 231–236.
Zhu X *et al.* (2005). Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr A* 1066: 89–95.

Vedaprofen

Propionic Acid, NSAID
$C_{19}H_{22}O_2 = 282.4$
CAS—71109-09-6

IUPAC Name 2-(4-Cyclohexylnaphthalen-1-yl)propanoic acid
Synonyms CERM-10202; (±)-4-cyclohexyl-α-methyl-1-napthalene acetic acid; 2-(4-cyclohexyl-1-naphthyl)-propionic acid; PM-150.
Proprietary Name *Quadrisol*



Chemical Properties Crystals. Mp 150°.
Quantification No data have been reported for vedaprofen concentrations in human fluids or tissues.
Other **GC-MS** Horse Faeces. Column: DB5-MS (30 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 0.8 mL/min. Temperature programme: 100° for 0.5 min to 220° at 15°/min, to 305° at 8°/min for 8 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, 10 µg/g [Popot *et al.* 2006].

HPLC Horse Plasma. Column: Lichrosorb C_{18} (100 mm). Mobile phase: acetonitrile : water (65 : 35) in 1% acetic acid, flow rate 0.6 mL/min. UV detection (λ = 288 nm). Limits of detection, 3 µg/L for the (*R*)-enantiomer and 6 µg/L for the (*S*)-enantiomer [Lees *et al.* 1999].
Dose Used in veterinary medicine for the treatment of inflammation and pain. It is available as a 100 mg/mL gel.

Lees P *et al.* (1999). A pharmacodynamic and pharmacokinetic study with vedaprofen in an equine model of acute nonimmune inflammation. *J Vet Pharmacol Ther* 22: 96–106.
Popot MA *et al.* (2006). Use of accelerating solvent extraction for detecting non-steroidal anti-inflammatory drugs in horse feces. *J Anal Toxicol* 30: 323–330.

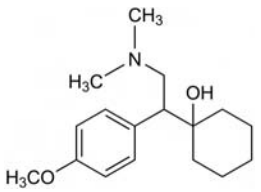
Venlafaxine

Antidepressant, Phenethylamine, Serotonin/Noradrenaline Reuptake Inhibitor (SNRI)

$C_{17}H_{27}NO_2 = 277.4$

CAS—93413-69-5

IUPAC Name 1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol
Synonym Venlafexine



Chemical Properties Both (−)- and (+)-forms crystalyse from ethyl acetate. Mp 102° to 104°. Log *P* (octanol/water), 3.28 [Meylan, Howard 1995], 2.68 [Matoga *et al.* 2001], 2.9 [Rodda, Drummer 2006], 0.43. Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005]. Stock solutions stable at room temperature for 7 h and at 4° for 25 days. Stable in methanol : water (50 : 50) for 24 days. Stable in plasma at room temperature for up to 24 h and after 6 freeze-thaw cycles [Patel *et al.* 2008]. Stable in methanol at 4° and room temperature for at least 48 h. Stable in plasma when stored at -20° in polypropylene tubes for at least 74 days [Theron *et al.* 2007]. Stock solution in methanol is stable at 4° for a week. Plasma samples were stable at -20° for 14 days and after 5 freeze-thaw cycles [Wei *et al.* 2007]. Serum samples were stable for a minimum of 4 months at -20° and a minimum of 4 days at room temperature [Reis *et al.* 2002]. Venlaflaxine is stable at -20° for 15 days, at 4° for 48 h and 20° for 24 h [Matoga *et al.* 2001]. Venlafaxine is stable at -20° for at least 370 days in human plasma, 182 days in rat and dog plasma, and 91 days in human urine [Hicks *et al.* 1994].

Venlafaxine Hydrochloride

$C_{17}H_{27}NO_2$,HCl = 313.9

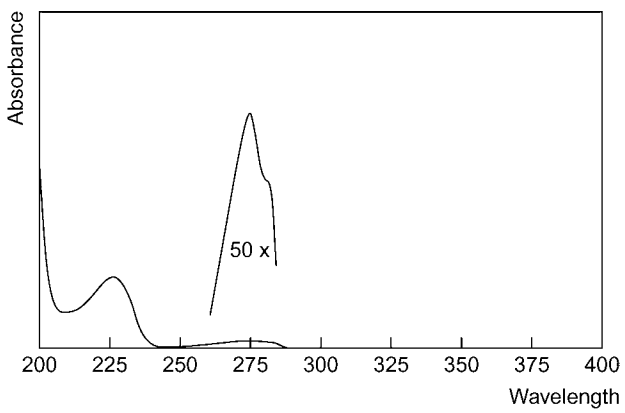
CAS—99300-78-4

Synonym Wy-45030

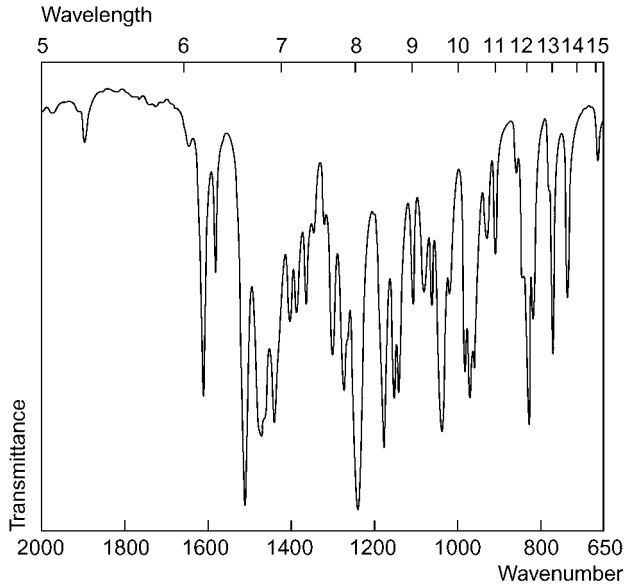
Proprietary Names *Dobupal*; *Efexor*; *Effexor*; *Trevilor*; *Trewilor*; *Vandral*.

Chemical Properties A white to off-white crystalline solid. Mp 215° to 217°. It is soluble in water at 572 g/L. The (−)- and (+)-forms crystalyse from methanol/ether. Mp 240°.

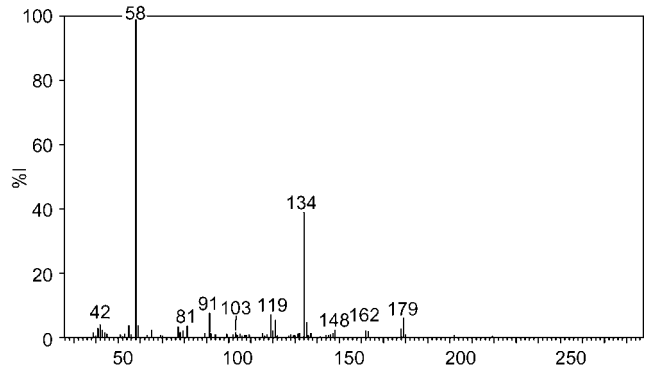
Gas Chromatography System GB—venlafaxine RI 2163, M (*N*-desmethyl-) RI 2196, M (*O*-desmethyl-) RI 2230, M (*NO*-didesmethyl) RI 2264, M (nor-OH-) RI 2450, M (*O*-desmethyl OH-) isomer 1 RI 2373, M (*O*-desmethyl OH-) isomer 2 RI 2408; system GM—venlafaxine RRT 0.544, M (*N*-desmethyl-) RRT 0.570, M (*O*-desmethyl- RRT 0.625), M (*N*,*O*-didesmethyl) RRT 0.687 (all relative to iprindole).
High Performance Liquid Chromatography System HZ—venlafaxine RT 3.1, M (*O*-desmethyl-) RT 2.2 min.
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid)—225, 273, 280 nm; (0.025 mol/L sulfuric acid)—226.5, 274.5 nm; basic—274, 280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1513, 1243, 1179 cm^{-1} (venlafaxine hydrochloride; KBr pellet).



Mass Spectrum Principal ions at *m/z* 58, 134, 91, 121, 179, 42, 77, 277.



Quantification

Blood **GC** Column: DB-17 (30 m × 0.32 mm i.d., 0.25 µm) or HP-5 (30 m × 0.32 mm i.d., 0.25 µm). Temperature programme: 120° for 1 min to 280° at 8°/min [Long *et al.* 1997].

GC-MS Column: DB-5 cross-linked 5% phenylmethylsiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Tempertaure programme: 50° for 2 min to 180° at 30°/min to 280° at 5°/min for 19 min. Full scan mode. Retention time: 15.7 min. Limit of quantification, 0.05 mg/L [Paterson *et al.* 2004].

HPLC Column: XTerra RP18 (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2, 15 : 85 for 1 min to 35 : 65 over 12 min for 1 min), flow rate 1 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 5 µg/L [Castaing *et al.* 2007]. Column: Novapak phenyl (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L potassium phosphate buffer (pH 3.0, 55 : 45), flow rate 1.5 mL/min. UV detection (λ = 214 nm). Limit of detection, 1 mg/L [Jaffe *et al.* 1999]. Column: ODS (150 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 4.0) : methanol (71 : 29), flow rate 1 mL/min. DAD (λ = 220 nm) [Long *et al.* 1997].

LC-MS Column: Zorbax Extend C_{18} (150 × 2.1 mm i.d., 5.0 µm). Mobile phase: 0.05 mol/L ammonia : methanol: tetrahydrofuran (pH 10.0, 32.5 : 67.0 : 0.5), flow rate 0.25 mL/min. MSD, SIM acquisition mode, positive ion mode. Limit of quantification, 50 ng/g [Goeringer *et al.* 2001].

Plasma **GC** Column: HP-5 cross-linked 5% phenylmethyl silicone (25 m × 0.32 mm i.d., 0.17 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 250° at 5°/min. NPD. Limit of detection, 0.1 mg/L [Levine *et al.* 1996].

GC-MS Column: J, W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min for 2.5 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 9 µg/L [Wille *et al.* 2007]. Column: Varian factor FOUR VF-5 ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 0.5 min to 180° at 50°/min for 10 min to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection, not reported [Wille *et al.* 2005].

HPLC Column: Diamonsil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.0) : TEA (33.5 : 66.5 : 0.4), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 276 nm, λ_{em} = 596 nm). Limit of quantification,

10 µg/L, limit of detection, 2.0 µg/L [Qin *et al.* 2008]. Column: Zorbax C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile (pH 6.8) : 40 mmol/L phosphate buffer containing 0.25% TEA (25 : 75), flow rate 1.0 mL/min for 3.5 min to 2.0 mL/min at 4 min for 11 min to 1.0 mL/min at 15.5 min. Fluorescence detection (λ_{ex} = 238 nm, λ_{em} = 300 nm). Limit of quantification, 1.0 µg/L, limit of detection, 0.3 µg/L [Mandrioli *et al.* 2007]. Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8, 25 : 75 to 40 : 60 in 10 min to 44 : 66 in 8 min), flow rate 1.0 mL/min. DAD (λ = 290 nm). Retention time: 8.2 min. Limit of quantification, 25 µg/L, limit of detection, 2 µg/L [Duverneuil *et al.* 2003]. Column: Symmetry C_8 (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 10 mmol/L phosphate buffer (pH 3.8, 25 : 75 to 36 : 64 over 7 min for 7 min to 25 : 75 for 3 min), flow rate 1 mL/min. UV detection (λ = 230 to 290 nm for 1.5 min to 230 nm). Retention time: 7.0 min. Limit of quantification, 100 µg/L [Titier *et al.* 2003] Column: Spherisorb S5 C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 62.4 mmol/L phosphate buffer with diethylamine and Pic B5 (pH 5.5, 30 : 70) , flow rate 1.4 mL/min. UV detection (λ = 229 nm). Limit of quantification, 100 µg/L [Matoga *et al.* 2001].

See also Alfaro *et al.* [2000], Clement *et al.* [1998], Hicks *et al.* [1994], Ilett *et al.* [1998]; Ilett *et al.* [2002], Vu *et al.* [1997] and Wang *et al.* [1992].

LC-MS Column: Xbridge C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 8.1) : acetonitrile (84 : 16 to 66.5 : 33.5 at 1.31 min to 40 : 60 from 7.51 to 10.9 min to 15 : 85 from 11 to 13 min to 84 : 16 for 5 min), flow rate 300 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 2 µg/L [Choong *et al.* 2009]. Column: Zorbax SB C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L ammonium acetate (80 : 20), flow rate 1.0 mL/min. ESI, TIS, positive ion mode. Retention time: 1.6 min. Limit of quantification, 4.005 µg/L [Rajasekhar *et al.* 2009]. Column: Sunfire C_{18} (20 × 2.1 mm i.d., 3.5 µm). Mobile phase: 2 mmol/L ammonium formate (pH 3.0) : acetonitrile (85 : 15 for 0.5 min to 50 : 50 at 4 min to 30 : 70 at 5 min for 3 min), flow rate 0.4 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 0.77 min. Limit of quantification, 2 µg/L [De Castro *et al.* 2008]. Column: Hypurity cyano (50 × 4.6 mm i.d., 5.0 µm). Mobile phase: methanol : water: 1.0 mol/L ammonium trifluoroacetate (350 : 650 : 1.5), flow rate 700 µL/min. API, TIS, positive ion mode, MRM acquisition mode. Limit of detection, 2 µg/L [Patel *et al.* 2008]. Column: BDS Hypersil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 30 mmol/L ammonium acetate-2.6 mmol/L formic acid-0.13 mmol/L trifluoroacetic acid : acetonitrile (60 : 40 to 46 : 54 at 7 min to 60 : 40 at 7.1 min to 40 : 60 at 8.5 min), flow rate 1.0 mL/min for 7 min to 1.5 mL/min at 7.1 min for 1.4 min. ESI, positive ion mode, SIR acquisition mode. Limit of quantification, 3.5 µg/L [Liu *et al.* 2007a]. Column: CHIROBIC V (250 × 4.6 mm i.d., 5 µm). Mobile phase: 30 mmol/L ammonium acetate : methanol (15 : 85, pH 6.0), flow rate 1.0 mL/min. ESI, positive ion mode, SIR acquisition mode. Limit of quantification, 5.0 and 5.2 µg/L for (*S*)-(+)- and (*R*)-(–)-venlaflaxine, respectively, limit of detection, 1.0 µg/L [Liu *et al.* 2007b]. See also Bhatt *et al.* [2005], Juan *et al.* [2005], Smyth *et al.* [2006], Theron *et al.* [2007] and Wei *et al.* [2007].

Serum **HPLC** Column: C_{18}. Mobile phase: acetonitrile : buffer (30 : 70), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex} = 227 nm, λ_{em} = 300 nm). Limit of quantification, 14.8 µg/L, limit of detection, 4.93 µg/L [Waschgler *et al.* 2004]. Column: Nucleosil 100-5-Protect 1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 25 mmol/L potassium dihydrogen phosphate (pH 7.0) : acetonitrile (60 : 40), flow rate 1.0 mL/min. UV detection (λ = 230 nm). Retention time: 7.3 min. Limit of detection not reported [Frahnert *et al.* 2003]. Column: Lichrosphere RP-select B (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : 20 mmol/L potassium dihydrogen phosphate (pH 4.4, 10 : 20 : 70), flow rate 1.0 mL/min. Limit of quantification, 20 nmol/L [Reis *et al.* 2002]. Column: Beckman ODS C_{18} (250 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L sodium phosphate buffer (pH 3.8, 50 : 50), flow rate 1 mL/min. UV detection (λ = 200.4 nm). Retention time: 4.46 min. Limit of detection, 25 ng [Tournel *et al.* 2001].

LC-MS Column: Chromolith Speed ROD C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9) in ammonia (20 : 80 to 70 : 30 at 4 min for 1 min to 20 : 80), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 12.7 µg/L [Kirchherr, Kühn-Velten 2006].

Urine **GC** See Plasma [Levine *et al.* 1996].

GC-MS Column: CP-SIL 8 CB (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.2 mL/min. Temperature programme: 60° for 2 min to 200° at 20°/min to 280° at 5°/min. EI ionisaton at 70 eV, positive ion mode. Limit of detection, 0.21 µg/L [Salgado-Petinal *et al.* 2005]. Column: HP-1 cross-linked methyl silicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

HPLC See Blood [Jaffe *et al.* 1999]. See Plasma. Limit of detection, 100 µg/L [Hicks *et al.* 1994]. Column: Supelco LC-18-DB (250 × 4.6 mm i.d., 5 µm). Mobile phase: 100 mmol/L sodium dihydrogen phosphate : acetonitrile (90 : 10 to 60 : 40), flow rate 1.0 mL/min. UV detection (λ = 229 nm). Limit of detection not reported [Howell *et al.* 1993].

LC-MS See Blood [Goeringer *et al.* 2001].

Bile **GC** See Plasma [Levine *et al.* 1996].

HPLC See Blood [Jaffe *et al.* 1999].

LC-MS See Blood [Goeringer *et al.* 2001]

Gastric Contents **HPLC** See Blood [Jaffe *et al.* 1999].

Milk **HPLC** See Plasma [Ilett *et al.* 1998]

Oral Fluid **LC-MS** See Plasma [De Castro *et al.* 2008].

Vitreous Humour **HPLC** See Blood [Jaffe *et al.* 1999].

LC-MS See Blood [Goeringer *et al.* 2001].

Kidney **GC** See Plasma [Levine *et al.* 1996].

Liver **GC** See Plasma [Levine *et al.* 1996].

HPLC See Blood [Jaffe *et al.* 1999].

LC-MS See Blood [Goeringer *et al.* 2001].

Disposition in the Body Venlafaxine is well absorbed after oral administration and undergoes extensive first-pass metabolism in the liver, mainly to the active metabolite *O*-desmethylvenlafaxine by P450 CYP2D6 [Fukuda *et al.* 2000]. Two lesser active minor metabolites are also produced: *N*-desmethylvenlafaxine and *N,O*-didesmethylvenlafaxine. Venlafaxine is excreted predominantly in urine, mainly in the form of its metabolites (either free or conjugated), 1–10% as the unchanged drug, 30% *O*-desmethylvenlafaxine, 6–19% *N,O*-didesmethylvenlafaxine and 1% *N*-desmethylvenlafaxine. Approximately 2% is excreted in faeces. It is not removed by dialysis. The serum therapeutic range is 250–750 µg/L for the total drug and metabolites.

Therapeutic Concentration

Eighteen healthy male volunteers (mean age of 27 years) were administered single oral doses of 25, 75 or 150 mg on day 1, and the same dose every 8 h on days 2 to 4. A single dose was then administered on day 5. The mean peak concentrations from day 1 were 37, 102 and 163 µg/L, respectively, for the 3 doses, attained at at 2.4, 2.1 and 2.1 h, respectively. The mean peak *O*-desmethylvenlafaxine concentrations were 61, 168 and 325 µg/L, respectively, for the three doses, attained att 4.0 h (range, 1.5–7.8), 4.4 h (range, 1.2–6.7) and 4.6 h (range, 3.1–5.9) respectively [Klamerus *et al.* 1992].

Toxicity The toxic range is 1.0–1.5 mg/L for the drug and metabolites together.

A 21-year-old woman ingested ~14.7 g venlaflaxine. Postmortem venlaflaxine and *O*-desmethylvenlafaxine concentrations were 95 and 8 mg/L, respectively, in femoral blood [Hojer *et al.* 2008].

A 39-year-old woman ingested a large undefinable amount of venlaflaxine. Postmortem venlaflaxine and *O*-desmethylvenlafaxine concentrations were 79 and 17.7 mg/L, respectively, in femoral blood [Hojer *et al.* 2008].

A 13-year-old girl ingested 48 tablets of 150 mg venlaflaxine. A serum sample obtained after 3 h revealed a venlaflaxine concentration of 24 460 µg/L and an *O*-desmethylvenlaflaxine concentration of 3930 µg/L [Bond *et al.* 2003].A 39-year-old woman ingested 30 g of extended-release venlaflaxine capsules. Concentrations of venlaflaxine and desmethylvenlaflaxine were as follows:

Day after ingestion	*Venlaflaxine (mg/L)*	*O-Desmethylvenlaflaxine (mg/L)*
1	21.82	3.33
2	10.36	1.44
3	13.33	1.94
4	12.11	2.07
7	2.39	1.04
8	2.43	0.84
10	0.42	0.18
11	0.19	0.11
12	0.10	0.07
13	0.04	0.02
16	<0.03	<0.03

[Mazur *et al.* 2003]

Postmortem drug concentrations were reported for 2 women who died after taking venflaxine [Jaffe *et al.* 1999]. The first woman was 41 years old and took 84 tablets of 75 mg venlaflaxine. The second was 36 years of age and took 56 tablets of 75 mg venlaflaxine. The following concentrations were found at postmortem:

	Case 1	*Case 2*
Blood (mg/L)	53	78
Liver (mg/kg)	81	110
Bile (mg/L)	90	200
Vitreous humour (mg/L)	22	58
Urine (mg/L)	9	NA
Gastric contents (mg)	NA	400

NA, not analysed

Two venlafaxine-related deaths were reported [Long *et al.* 1997]. Blood concentrations in the first were 7.27 mg/L venlafaxine, and 6.03 mg/L *O*-desmethylvenlafaxine. Blood concentrations in the second were 84.30 mg/L venlafaxine and 3.44 mg/L *O*-desmethylvenlafaxine.

In 3 postmortem cases, heart blood concentrations were 6.6, 84, and 44 mg/L [Levine *et al.* 1996].

Half-life Plasma half-life: venlafaxine 4 h, *O*-desmethylvenlafaxine 10 h.

Volume of Distribution 8 L/kg [Rodda, Drummer 2006]; venlafaxine 6.8 L/kg; *O*-desmethylvenlafaxine 5.7 L/kg.

Clearance Plasma clearance: venlafaxine 1.2–1.7 L/h/kg, *O*-desmethylvenlafaxine 0.4 L/h/kg; decreased by ~55% in patients on dialysis [Troy *et al.* 1994].

Milk : plasma ratio 4.14 [Ilett *et al.* 1998].

V

Protein Binding 27% [Rodda, Drummer 2006]; venlafaxine and *O*-desmethylvenlafaxine, 30%.

Note For a review of venlafaxine see Holliday, Benfield [1995].

Dose The usual dose is 75 mg venlafaxine daily with a maximum of 375 mg. The dose is reduced by 50% in those with moderate hepatic impairment and by 25% in mild to moderate renal impairment.

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Verapamil

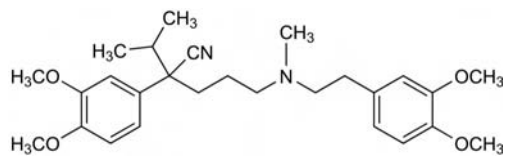
Antiarrhythmic

$C_{27}H_{38}N_2O_4$ = 454.6

CAS—52-53-9

IUPAC Name 2-(3,4-Dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile

Synonyms CP-16533-1; D-365; iproveratril.



Chemical Properties A pale yellow viscous oil. Practically insoluble in water; freely soluble in the lower alcohols, acetone, ethyl acetate and chloroform; soluble in benzene and ether. pK_a 8.9. Log *P* (octanol/water), 3.8. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Verapamil Hydrochloride

$C_{27}H_{38}N_2O_4$,HCl = 491.1

CAS—152-11-4

Proprietary Names *Angimon*; *Berkatens*; *Calan*; *Cordilox*; *Covera*; *Half Securon*; *Isoptin(e)*; *Securon*; *Univer*; *Verapress*; *Verelan*; *Vertab*; *Zolvera*.

Chemical Properties A white crystalline powder. Mp 138.5° to 140.5°, with decomposition. Soluble 1 in 20 of water, 1 in 25 of ethanol and 1 in 1.5 of chloroform; practically insoluble in ether.

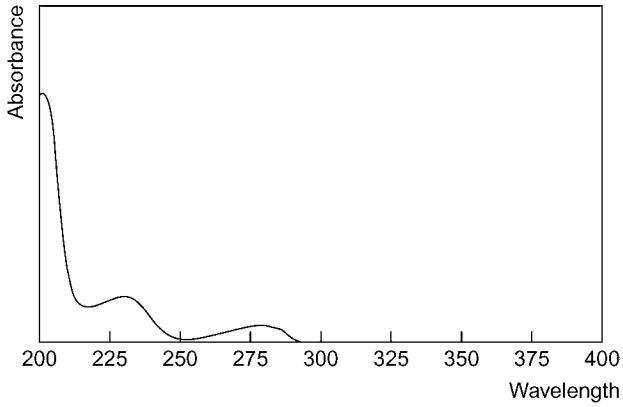
Colour Tests Liebermann's reagent—black; Marquis test—yellow-green→grey.

Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.23; system TC—R_f 0.70; system TE—R_f 0.73; system TL—R_f 0.42; system TAE—R_f 0.43; system TAF—R_f 0.61 (acidified iodoplatinate solution—positive).

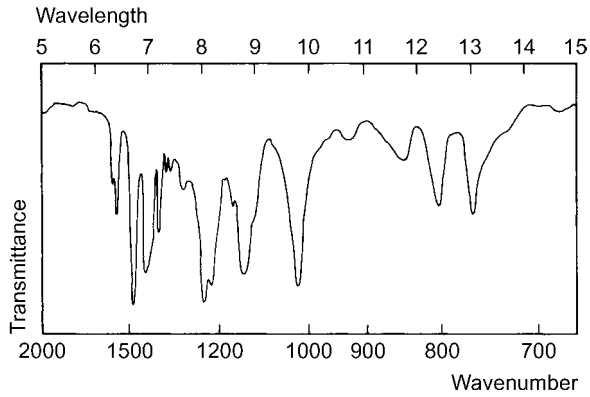
Gas Chromatography System GA—verapamil RI 3150, M (nor-) RI 3180, M (*N*-desalkyl-) RI 2100, M (*N*-desalkyl-acetyl-) RI 2460, M (*N*-didesalkyl-acetyl-) RI 2545; system GB—verapamil RI 3305, M (nor-) RI 3371, M (*N*-desalkyl-) RI 2193, M (*O*-desmethyl-didesalkyl-) RI 2169, M (*O*-desmethyl-desalkyl-) RI 2246, M (didesalkyl-) RI 2300, M (*N*-desalkyl-acetyl-) RI 2546, M (*N*-didesalkyl-acetyl-) RI 2579.

High Performance Liquid Chromatography System HA—verapamil *k* 2.6, M (nor-) *k* 1.7; system HX—RI 447; system HY—RI 386; system HZ—verapamil RT 7.0 min, M (nor-) RT 6.6 min; system HAA—RT 15.4 min; system HAV—*k* 8.2; system HAX—RT 13.3 min; system HAY—RT 7.0 min [Stagni, Gillespie 1995].

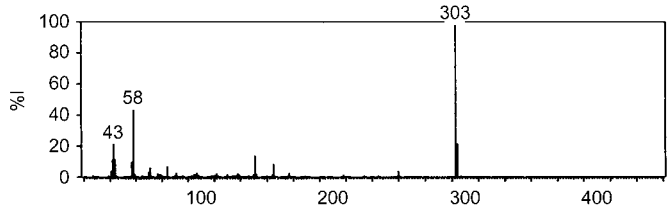
Ultraviolet Spectrum Aqueous acid—278 nm (A_1^1 = 127a).



Infrared Spectrum Principal peaks at wavenumbers 1510, 1253, 1026, 1232, 1149, 1587 cm^{-1} (thin film).



Mass Spectrum Principal ions at *m/z* 303, 58, 43, 304, 151, 44, 42, 57.



Quantification

Blood **HPLC** Column: Nova-Pak C_{18} RP (150 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : 0.04 mol/L ammonium acetate : acetonitrile : triethylamine (pH 7.1, 2 : 2 : 1 : 0.04), flow rate 1.2 mL/min. UV detection (λ = 210 nm). Retention time: verapamil 4.9 min, norverapamil 3.6 min. Limit of detection, 5 µg/L [Garcia *et al.* 1997].

Plasma **GC** Column: SE-54 fused silica capillary cross-linked with 5% phenyl methyl silicone (25 m × 0.2 mm i.d., 0.3 µm). Temperature programme: 100° to 310° at 20°/min for 10 min. Carrier gas: He, 0.9 mL/min. Reference compound: zipeprol. NPD. Retention time: 17.53 min. Limit of detection, 2 µg/L [Shin *et al.* 1996].

HPLC Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 315 nm). Limit of detection, 10 µg/L [Ho *et al.* 2000]. Column: Macherey-Nagel cyanopropyl silica (11 × 4 mm i.d., 30 µm) or ODS (10 × 4 mm i.d., 10 µm). Mobile phase: acetonitrile : 2-aminoheptane : 0.01 mol/L sodium acetate buffer (pH 3.0, 25 : 0.5 : 75), flow rate 0.9 mL/min. Fluorometric detection (λ_{ex} = 275 nm, λ_{em} = 310 nm). Limit of quantification, 4.3 µg/L, limit of detection, 1.3 µg/L [Ceccato *et al.* 1996]. Column: Chiral-AGP (150 × 4 mm). Mobile phase: 0.01 mol/L phosphate buffer (pH 6.65) : acetonitrile (91 : 9), flow rate 0.9 mL/min. Fluorescence detection (λ_{ex} = 227 nm; λ_{em} = 308 nm). Resolution: 3.3. Limit of quantification, 3 µg/L [Stagni, Gillespie 1995].

Serum **HPLC** Column: α_1-acid glycoprotein (100 × 4.0 mm i.d.). Mobile phase: 0.01 mol/L disodium hydrogen phosphate (pH 7.0) : acetonitrile (90 : 10), flow rate 0.9 mL/min. Fluorescence detection (λ_{ex} = 276 nm, λ_{em} = 310 nm). Limit of quantification, 3 µg/L [Brandsteterova, Wainer 1999].

Urine **HPLC** See Plasma [Ho *et al.* 2000]. Column: Chiralcel OD-R CSP (250 × 4.6 mm i.d., 10 µm). Mobile phase: acetonitrile : 0.2 mol/L sodium perchlorate (40 : 60), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 312 nm). Limit of quantification, 3 µg/L [Asafu-Adjaye, Shiu 1998].

Disposition in the Body Verapamil is almost completely absorbed after oral administration but undergoes extensive first-pass metabolism. The main metabolic reactions are *N*-dealkylation (*N*-demethylation gives norverapamil, which is thought to be active) and *O*-demethylation of the resulting compounds. Approximately 70% of a dose is excreted in the urine in 5 days, with ~50% in the first 24 h; <5% is excreted as unchanged drug. The urinary metabolites consist mainly of *N*-dealkylated compounds together with ~10% of the dose as norverapamil or its *O*-demethylated derivative; conjugated *O*-demethylated products account for ~17% of the urinary material. Approximately 16% of a dose is eliminated in the faeces.

Therapeutic Concentration

Following single oral doses of 80 mg to 20 subjects, mean peak plasma concentrations of 38 µg/L verapamil and 26 µg/L norverapamil were attained in ~3 h; after oral doses of 120 mg to the same subjects, mean peak plasma concentrations of 68 µg/L verapamil and 59 µg/L norverapamil were reported at 2 h [McAllister, Kirsten 1982].

Twelve healthy subjects, aged 20–38 years, were administered with a single dose of verapamil 20 mg either as a buccal formulation or orally as a tablet, with a 7-day gap between doses. A mean peak verapamil plasma concentration of 51.28 µg/L was attained at 1.5 h after buccal administration and 31.67 µg/L at 1 h after oral administration. The equivalent concentrations of norverapamil were 5.03 µg/L at 6 h after buccal administration and 27.35 µg/L at 1 h after oral administration [Sawicki, Janicki 2002].

Healthy subjects, 30 young and 30 elderly (~half of each age group were males and half were females), were administered with verapamil 180 mg via a controlled-release gastrointestinal therapeutic system. Mean steady-state plasma concentrations were reported as follows: (*R*)-verapamil, 155.0 µg/L in elderly and 108.6 µg/L in young subjects (in 10.0 h); (*S*)-verapamil, 40.9 µg/L in elderly and 24.2 µg/L in young subjects (in 10.2 and 10.0 h, respectively); (*R*)-norverapamil, 150.3 µg/L in elderly and 121.3 µg/L in young patients (in 11.2 and 10.9 h, respectively); (*S*)-norverapamil, 56.9 µg/L in elderly and 43.2 µg/L in young patients (in 11.0 h) [Gupta *et al.* 1995].

Twelve healthy men (aged 19–37 years) received a single 240-mg oral dose of racemic verapamil as either an immediate-release formulation (in the fasting state) or as a sustained-release formulation. A peak plasma verapamil concentration of 327 µg/L was attained at 1.71 h with the immediate-release preparation and 73 µg/L at 10.8 h after the sustained-release preparation. Measurement of the total (*R*)-verapamil and (*S*)-verapamil levels in the plasma revealed the *R*/*S* ratio (at C_{max} and at several other times) to be lower with the immediate-release preparation for both verapamil (4.52 versus 5.83%) and norverapamil (2.48 versus 3.04%) [Karim, Piergies 1995].

Nine healthy, male volunteers (mean age 26 years; range, 19–40) were administered 120 mg verapamil twice daily for 3 days before the actual study day. Grapefruit juice or orange juice (control), 200 mL, was also taken twice daily for 5 days beforehand. On the study day, a single oral dose of 120 mg verapamil was administered with either orange or grapefruit juice, after an overnight fast. The steady-state maximum plasma concentration of (*S*)-verapamil was 41 µg/L for the dose taken with grapefruit juice compared with 26 µg/L for the dose taken with orange juice. For (*R*)-verapamil, the maximum concentration was 132 µg/L with grapefruit juice and 94 µg/L with orange juice. These concentrations were observed at 4.6 and 3.9 h, respectively, for (*S*)-verapamil, and 4.3 and 4.0 h, respectively, for (*R*)-verapamil. The peak (*S*)-norverapamil concentrations were 33 and 29 µg/L at 5.8 and 5.1 h for grapefruit and orange, respectively. For (*R*)-norverapamil, these concentrations were 84 and 75 µg/L at 5.2 and 4.0 h, respectively [Ho *et al.* 2000].

Toxicity

In a fatality caused by verapamil ingestion, the following postmortem tissue concentrations were reported: blood 8.8 mg/L, kidney 28 µg/g and liver 165 µg/g [Thomson, Pannell 1981].

Serum concentrations of 3 mg/L verapamil and 2.5 mg/L norverapamil were reported in a 17-year-old girl 12 h after ingestion of an unknown overdose of verapamil; death occurred after 19 h [Orr *et al.* 1982].

A 30-year-old woman being treated with verapamil for supraventricular tachycardia, ingested the equivalent of 2.4 g verapamil with an unknown quantity of alcohol. On admission to hospital, she was unconscious; her blood pressure could not be recorded and she was hypotensive and bradycardic. Toxicological analysis showed that her blood verapamil concentration was 5.18 mg/L. She deteriorated rapidly but was stabilised with plasmapheresis (this reduced the verapamil blood concentration by ~40%). After 4 h of treatment, she developed acute lung, liver and renal failure. She died 38 h after admission to hospital owing to multi-organ failure [Kuhlmann *et al.* 2000]

A 45-year-old woman overdosed on 4 g ordinary-release verapamil tablets. At 1–2 h after ingestion, she was admitted to hospital unconscious, hypotensive and with a low heart beat. The maximum plasma verapamil concentration observed was 2.7 mg/L 2–3 h after the overdose. This concentration was 10- to 20-fold higher than the expected therapeutic range [Kivistö *et al.* 1999].

A 22-year-old male ingested 3.2 mg verapamil with an unknown quantity of alcohol. He was admitted to hospital 2 h after the overdose with complete heart block and a low blood pressure. The maximum plasma concentration detected was 2.2 mg/L 2–3 h after ingestion. As in the case above, this concentration was 10- to 20-fold higher than the expected therapeutic range [Kivistö *et al.* 1999].

A man was found dead in his home with an empty container of verapamil and a partly emptied bottle of vodka in the room. Verapamil was found in his blood at a concentration of 402 mg/L, in his stomach at 49.3 µg/g and in his liver at 42.1 µg/g. His blood alcohol concentration was 1100 mg/L [Janowska, Chudzikiewicz 1996].

Bioavailability ≈20%.

Half-life Plasma half-life verapamil ~2–7 h, it is increased during long-term oral dosing and in subjects with liver disease; norverapamil ~5–13 h.

Volume of Distribution ≈2–6 L/kg.

Clearance Plasma clearance, ~10–20 mL/min/kg.

Distribution in Blood Plasma : whole blood ratio, ~1.2.

Protein Binding ≈90%.

Note For a general review of verapamil, see McTavish, Sorkin [1989].

Dose 120 to 480 mg of verapamil hydrochloride daily.

Asafu-Adjaye EB, Shiu GK (1998). Solid-phase extraction-high-performance liquid chromatography determination of verapamil and norverapamil enantiomers in urine. *J Chromatogr B Biomed Sci Appl* 707: 161–167.

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Veratrine

Counterirritant (Topical)

$C_{32}H_{49}NO_9$ = 591.7

CAS—8051-02-3 (mixture)

Chemical Properties A mixture of alkaloids from sabadilla, the dried ripe seeds of *Schoenocaulon officinale* (Liliaceae). A white or greyish-white powder. Mp 145° to 155°. Soluble 1 in 1800 of water; soluble 1 in 2.8 of ethanol, 1 in 0.7 of chloroform, 1 in 4.2 of ether and 1 in 80 of olive oil; freely soluble in benzene and amyl alcohol; slightly soluble in glycerol. Log *P* (octanol/water), 4.0.

Note Veratrine should be distinguished from protoveratrines obtained from veratrum.

Caution Veratrine has a violent irritant action on mucous membranes, even in minute doses, and must be handled with great care.

Colour Tests Liebermann's reagent—brown; Mandelin's test—yellow→orange→violet-brown; Marquis test—orange; sulfuric acid—yellow→violet.

Thin-layer Chromatography System TA—R_f 0.59; system TB—R_f 0.04; system TC—R_f 0.35; system TE—R_f 0.71; system TL—R_f 0.17; system TAE—R_f 0.17.

High Performance Liquid Chromatography System HAA—retention time 13.6 min.

Ultraviolet Spectrum Aqueous acid—262 (A^1_1=67b), 292 nm.

Infrared Spectrum Principal peaks at wavenumbers 1270, 1085, 1111, 1136, 1234, 1035 cm^{-1} (KBr disk).

Vetrabutine

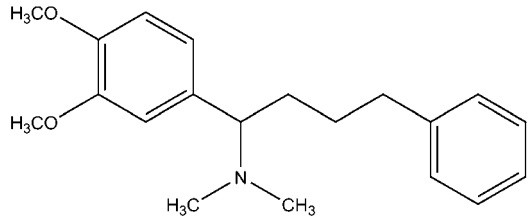
Uterine Relaxant (Veterinary)

$C_{20}H_{27}NO_2$ = 313.4

CAS—3735-45-3

IUPAC Name 1-(3,4-Dimethoxyphenyl)-*N*,*N*-dimethyl-4-phenylbutan-1-amine

Synonyms 3,4-Dimethoxy-*N*,*N*-dimethyl-α-(3-phenylpropyl)-benzylamine; 1-(3,4-dimethoxyphenyl)-1-dimethylamino-4-phenylbutane; α-(3,4-dimethoxyphenyl)-*N*,*N*-dimethylbenzenebutanamine; *N*,*N*-dimethyl-α-(3-phenylpropyl)veratrylamine; dimophebumine.



Chemical Properties A white crystalline powder. Mp 137° to 148°. Bp 166° to 168° at 0.1 mmHg. Soluble 1 in 3 water, of ethanol, and of methanol; slightly soluble in ether, benzene and acetone. Vetrabutine is extracted by chloroform from aqueous alkaline solutions.

Vetrabutine Hydrochloride

$C_{20}H_{27}NO_2$,HCl = 349.9

CAS—5974-09-4

IUPAC Name 1-(3,4-Dimethoxyphenyl)-*N*,*N*-dimethyl-4-phenylbutan-1-amine hydrochloride

Synonym Sp 281

Proprietary Names *Monzal*; *Monzaldon*.

Chemical Properties Solid. Mp 146° to 148°.

Colour Tests Ammonium molybdate test—grey-blue (limit of detection, 1.0 µg); ammonium vanadate test—green (limit of detection, 0.1 µg); sulfuric acid-formaldehyde test—dull red→red-brown (limit of detection, 0.1 µg).

Thin-layer Chromatography System T1—R_f 0.47 (location reagent acidified iodoplatinate spray, strong reation).

Gas Chromatography System G2/225—retention time 0.79 (relative to codeine); system G4/225—retention time 5.30 (relative to diphenhydramine), retention time 0.70 (relative to codeine).

Ultraviolet Spectrum 0.001 N hydrochloric acid—278 nm (E1%, 1 cm 83).

Infrared Spectrum Principal peaks at wavenumbers 462, 1515 cm^{-1} (KBr disk, hydrochloride).

Disposition in the Body

Metabolism After the IM administration of 50 mg/kg of ^{14}C-labelled vetrabutine to rats, 20 to 32% was excreted in the urine within 24 h. Approximately half of the radioactivity was excreted in the faeces in the same period.

Toxicity LD_{50} in rats 500 mg/kg (oral).

Dose Cattle up to 1.5 g, pigs up to 400 mg, sheep up to 250 mg and dogs up to 100 mg, IM.

Vigabatrin

Antiepileptic

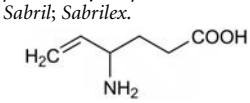
$C_6H_{11}NO_2$ = 129.2

CAS—60643-86-9

IUPAC Name 4-Amino-5-hexenoic acid

Synonym Gamma-vinyl-GABA; γ-vinyl-GABA; GVG; MDL-71754; RMI-71754.

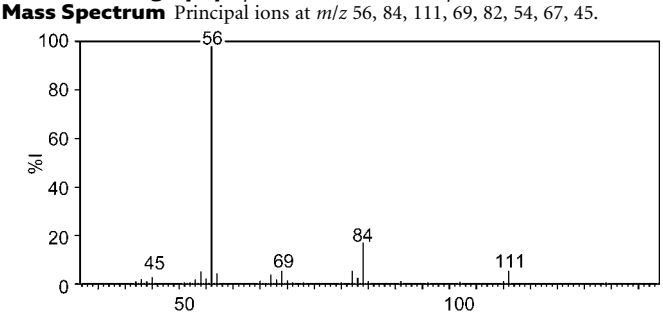
Proprietary Names *Sabril*; *Sabrilex*.



Chemical Properties A white to almost white powder. Mp 209°. It is very soluble in water. pK_{a1} 4.02, pK_{a2}9.72. Extraction yield (chlorobutane), 0 [Demme *et al.* 2005].

Gas Chromatography System GB—not eluted; system GAJ—not eluted.

Mass Spectrum Principal ions at *m*/*z* 56, 84, 111, 69, 82, 54, 67, 45.



Quantification

Plasma GC Column: Chirasil-Val non-packed (20 m × 0.53 mm i.d., 0.125 µm). Carrier gas: He, 2.0 mL/min. Temperature programme: 80° for 1 min to 129° at 20°/min for 2 min to 135° at 1°/min for 20 min to 200° at 20°/min for 10 min. TSD. Limit of detection, 1 mg/L for (*R*)-(−)-vigabatrin and 0.5 mg/L (*S*)-(+)-vigabatrin [Schramm *et al.* 1993]. Column: Chirasil-LVal (25 m × 0.2 mm i.d.). SIM acquisition mode. Limit of detection, 5 mg/L [Rey *et al.* 1990].

GC-MS Column: Chirasil-Val (25 m × 0.92 mm o.d.). Temperature programme: 90° for 1 min to 130° at 4°/min. EI ionisation at 70 eV. Limit of detection, 2.5 nmol [Haegele *et al.* 1983].

HPLC Column: Synergy Hydro-RP (150 × 4.6 mm i.d., 4 µm). Mobile phase: 50 mmol/L phosphate buffer : acetonitrile (55 : 45). Fluorescence detection (λ_{ex} = 300 nm, λ_{em} = 500 nm). Limit of detection, 0.1 mg/L [Mercolini *et al.* 2010]. Column: Chiralcel-ODR (250 × 4.6 mm i.d., 10 µm). Mobile phase: 0.05 mmol/L potassium hexafluorophosphate (pH 4.5) : acetonitrile : ethanol (50 : 40 : 10), flow rate 0.9 mL/min. UV detection (λ = 340 nm). Limit of detection, 0.5 mg/L for both enantiomers [Franco *et al.* 2007]. Column: Shim-Pack CLS-ODS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L orthophosphoric acid (pH 2.5, 20 : 80 for 2 min, to 25 : 75 for 9 min, to 98 : 2 for 9 min), flow rate 1 mL/min. UV detection (λ = 448 nm). Limit of detection, 0.5 mg/L [Cetin, Atmaca 2004]. Column: Microsorb C_{18}.(150 × 4.6 mm i.d., 5 µm). Mobile phase: 10 mmol/L orthophosphoric acid : acetonitrile : methanol (6 : 3 : 1), flow rate 2.0 mL/min. Fluorescence detection (λ_{ex} = 370 nm, λ_{em} = 418 nm). Retention time: 5.6 min. Limit of quantification, 0.08 mg/L, limit of detection, 0.54 mg/L [Tsanaclis *et al.* 1991]. Column: Zorbax C_8 (250 × 4.6 mm i.d., 6 µm). Mobile phase: acetonitrile : dioxane: 0.5 mol/L orthophosphoric acid (35 : 15 : 50), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 345 nm, λ_{em} = 418 nm). Limit of detection, 0.5 mg/L [Smithers *et al.* 1985].

Serum **GC-MS** VF-5 MS (30 m × 0.25 mm i.d., 0.25 um). Carrier gas: He, 1.0 mL/min. Temperature programme: 40° to 300° at 30°/min. EI ionisation, SIM acquisition mode. Limit of quantification, 2 mg/L [Borrey *et al.* 2005].

HPLC Column: XDB-C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: 50 mmol/L acetate buffer : methanol (60 : 40), flow rate 1.2 mL/min. Fluorescence detection (λ_{ex} = 230 nm, λ_{em} = 350 nm). Limit of detection, 2.5 nmol/L [Hsieh *et al.* 2008]. Column: RP Spherisorb 3ODS2 (150 × 4.6 mm i.d.). Mobile phase: acetonitrile : 55 mmol/L sodium acetate (pH 7.6, 5 : 95 to 17.5 : 82.5 in 20 min, to 35 : 65 in 1 min for 3 min to 5 : 95 in 1 min for 2 min), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 450 nm). Limit of detection, 0.20 mg/L (*R*)-(−)-vigabatrin and 0.14 mg/L (*S*)-(+)-vigabatrin [Vermeij, Edelbroek 1998]. Column: Alltima $3C_{18}$ (150 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : 20 mmol/L phosphate buffer (pH 7.0), flow rate 0.8 mL/min. Fluorescence detection (λ_{ex} = 330 nm, λ_{em} = 450 nm). Limit of quantification, 0.06 mg/L [Vermeij, Edelbroek 1994].

Urine **GC-MS** See Plasma [Rey *et al.* 1990].

HPLC See Plasma. Limit of detection, 10 mg/L [Smithers *et al.* 1985]. See Plasma [Cetin, Atmaca 2004].

Disposition in the Body Vigabatrin is rapidly and completely absorbed after oral administration and food does not alter the extent of absorption. The inactive (*R*)-(−)-enantiomer is reported to be present at much higher plasma concentrations than the active (*S*)-(+)-enantiomer. It is widely distributed and readily crosses the blood–brain barrier. Concentrations in CSF are ~10–15% of those in plasma. Most of a dose is excreted in urine as the unchanged drug as little or no metabolism occurs: 50% is excreted as the (*S*)-(+)-enantiomer and 45% as (*R*)-(−)-enatiomer. There does not appear to be any correlation between plasma concentrations and efficacy or toxicity. Haemodialysis reduces plasma concentrations by ~40–60%.

Therapeutic Concentration Plasma concentrations show considerable interpatient variation.

Eight healthy, male subjects aged between 18 and 40 years, were fasted overnight and administered 1 g vigabatrin orally on an empty stomach or 1 h after breakfast. The individuals were fasted for a further 4 h after dosing. Mean peak plasma concentrations were 30.5 mg/L and 25.3 mg/L for the fasted and fed individuals, respectively, attained at 1.1 and 1.6 h, respectively [Frisk-Holmberg *et al.* 1989].

Twelve individuals (one group of six with a mean age of 12.1 months (infants) and the other group of six with a mean age of 8.7 years (children)) suffering from refractory epilepsy were administered a single oral dose of 50 mg/kg (not exceeding 1.5 g in the older children). At 24 h after the single treatment, multiple dosing at 50 mg/kg twice daily was continued. The mean peak concentration after the single dose was 13.8 mg/L (*S*)-(+)-vigabatrin and 20.6 mg/L (*R*)-(−)-vigabatrin, attained at 2.85 and 2.35 h, respectively, for those in the infant group. The peak concentrations for the children group were 19.4 and 34.7 mg/L for the (*S*)-(+)- and (*R*)-(−)-enantiomers, respectively, observed at 1.36 and 1.28 h, respectively. The mean concentrations for the multiple dosing were 13.6 mg/L (range, 1.2 to 29.2) and 20.7 mg/L (range, 0.6 to 37.5) for the (*S*)-(+)- and (*R*)-(−)-enantiomers, respectively, 1 h after dosing for the infant group. In the group of children, these concentrations were 11.8 mg/L (range, 5.0–24.0) and 21.0 mg/L (range, 3.5–55.1) for the 2 enantiomers, respectively [Rey *et al.* 1990].

Half-life Plasma half-life, 5–8 h

Bioavailability 60–80%.

Volume of Distribution 0.8 L/kg.

Clearance Plasma clearance, 1.7 mL/min/kg.

Protein Binding Not bound.

Dose Vigabatrin 3 g daily; increased in increments of 0.5 to 1 g daily up to a maximum of 4 mg daily or 50 mg/kg.

Note For a review of vigabatrin, see Grant, Heel [1991]; for reviews of the pharmacokinetics of vigabatrin, see Bialer [1993], Elwes, Binnie [1996] and Rey *et al.* [1990].

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Schramm TM *et al.* (1993). Gas chromatographic assay of vigabatrin enantiomers in plasma. *J Chromatogr* 616: 39–44.

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Tsanaclis LM *et al.* (1991). Determination of vigabatrin in plasma by reversed-phase high-performance liquid chromatography. *Ther Drug Monit* 13: 251–253.

Vermeij TA, Edelbroek PM (1994). High-performance liquid chromatographic and megabore gas–liquid chromatographic determination of levetiracetam (ucb L059) in human serum after solid-phase extraction. *J Chromatogr B Biomed Appl* 662: 134–139.

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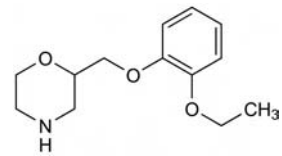
Viloxazine

Antidepressant

$C_{13}H_{19}NO_3$ = 237.3

CAS—46817-91-8

IUPAC Name 2-[(2-Ethoxyphenoxy)methyl]morpholine



Chemical Properties pK_a 8.1. Log *P* (octanol/water), 1.8. Extraction yield (chlorobutane), 0.85 [Demme *et al.* 2005]. Stable in plasma for at least a month at -20° and overnight when stored at -4° [Norman *et al.* 1979].

Viloxazine Hydrochloride

$C_{13}H_{19}NO_3$,HCl = 273.8

CAS—35604-67-2

Proprietary Names *Vicilan*; *Vivalan*; *Vivarint*.

Chemical Properties Mp 185° to 186°.

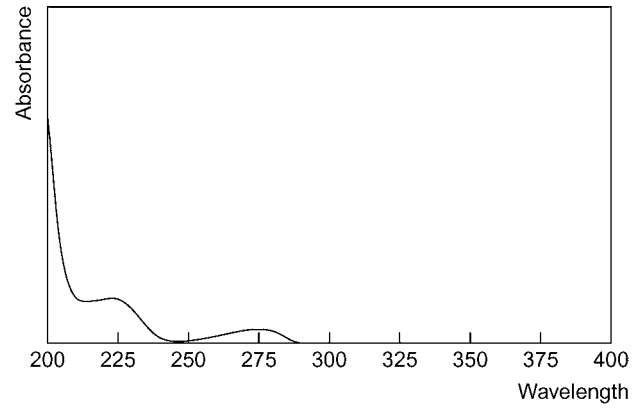
Colour Tests Liebermann's reagent—black; Mandelin's test—blue-green; Marquis test—violet.

Thin-layer Chromatography System TA—R_f 0.42; system TB—R_f 0.06; system TC—R_f 0.23; system TE—R_f 0.36; system TL—R_f 0.06; system TAE—R_f 0.25.

Gas Chromatography System GA—viloxazine RI 1855, M (-AC) RI 2220, M (OH-)(-AC_2) RI 2590, M (*O*-desethyl-) RI 2360, M (di-oxo-) RI 2325; system GB—viloxazine RI 1923.

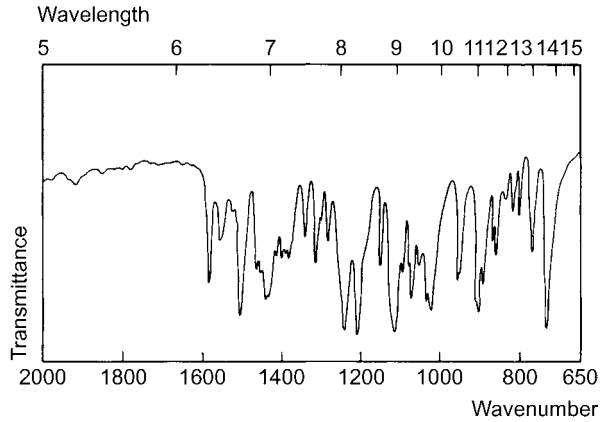
High Performance Liquid Chromatography System HF—*k* 2.70; system HX—RI 325; system HY—RI 273; system HAA—RT 11.0 min

Ultraviolet Spectrum Aqueous acid—273 nm (A^{1}_{1} = 92b) No alkaline shift

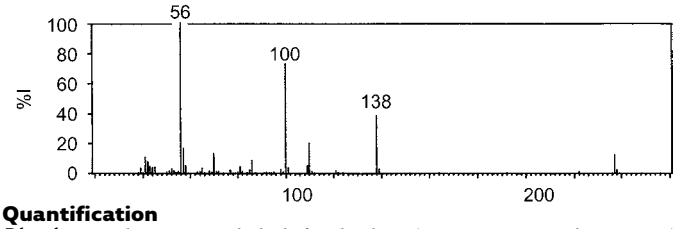


V

Infrared Spectrum Principal peaks at wavenumbers 1217, 1121, 1252, 739, 1511, 1031 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 56, 100, 138, 110, 57, 237, 70, 41.



Quantification

Blood **GC** Column: cross-linked fused silica (25 m × 0.2 mm i.d., 0.11 µm). Carrier gas: He, 195 kPa. Temperature programme: 180° for 1 min to 300° at 10°/min for 3 min. NPD. Limit of quantification, 76 µg/L, limit of detection, 23 µg/L [Martinez *et al.* 2002].

LC-MS Column: Xterra RP_{18} (100 × 2.1 mm i.d.). Mobile phase: acetonitrile : 4 mmol/L ammonium formate buffer (pH 3.2, 5 : 95 for 2 min to 20 80 over 2 min to 30 70 over 12 min for 2 min), flow rate 0.3 mL/min. ESI, positive ion mode, MRM acquisition mode. Retention time: 5.3 min. Limit of quantification, 2 µg/L [Titier *et al.* 2007]

Plasma **GC** Column: GP 3% SP2250 on 80/100 Supelcoport (1.8 m × 0.4 cm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 225°. NPD. Retention time: 2.6 min. Limit of detection, 10 µg/L [Altamura *et al.* 1983] Column: GP 3% SP2250 on 80/100 Supelcoport (1.8 m × 0.4 cm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 225°. NPD. Retention time: 2.6 min. Limit of detection, 10 µg/L [Altamura *et al.* 1983]. Column: 3% OV-17 80/100 mesh GasChrom Q (1.8 m × 4 mm i.d.). Carrier gas: N_2, 20 mL/min. Temperature: 260°. FID. Limit of detection, 200 µg/L [Norman *et al.* 1979].

GC-MS Column: J, W-5 MS (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 1 min to 180° at 50°/min for 10 min to 300° at 10°/min for 2.5 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of quantification, 4 µg/L [Wille *et al.* 2007]. Column: Varian factor FOUR VF-5ms (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.3 mL/min. Temperature programme: 90° for 0.5 min to 180° at 50°/min for 10 min to 300° at 10°/min for 10 min. EI ionisation at 70 eV, MSD, SIM acquisition mode. Limit of detection not reported [Wille *et al.* 2005].

HPLC Column: Hypurity C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8, 25 : 75 to 40 : 60 over 10 min to 44 : 56 in 8 min), flow rate 1.0 mL/min. DAD (λ = 240 nm). Retention time: 6.0 min. Limit of quantification, 25 µg/L, limit of detection, 3 µg/L [Duverneuil *et al.* 2003]. Column: Chrompack C_{18} (15 cm, 5 µm). Mobile phase: acetonitrile : sodium hydrogen phosphate (25 : 75, pH 2.5), flow rate 0.9 mL/min. UV detection (λ = 220 nm). Limit of detection not reported [Kergueris *et al.* 1989]. Mobile phase: acetonitrile : 6 mmol/L TEA : 0.2 mol/L phosphate buffer (pH 3.2), flow rate 1.3 mL/min. Fluorescence detection. Retention time: 8.2 min. Limit of detection, 25 µg/L [Gillilan, Mason 1981].

Serum **GC** Column: GP-3% SP-2250 on 80/100 mesh Supelcoport (2.0 m × 3 mm i.d.). Carrier gas: N_2, 40 mL/min. Temperature: 245°. NPD. Retention time: ~6 min. Limit of detection, 100 µg/L [Fazio *et al.* 1984].

GC-MS Column: PTE5 capillary (30 m × 0.32 mm i.d., 0.25 µm). EI ionisation at 70 eV, full scan mode. Limit of detection, 500 µg/L [Lacassie *et al.* 1999].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.31 µg/L [Kirchherr, Kühn-Velten 2006]

Urine **HPLC** See Plasma. Limit of detection, 1 µg/L [Gillilan, Mason 1981].

Disposition in the Body Viloxazine is rapidly and completely absorbed after oral administration and crosses the blood–brain barrier. It is extensively metabolised by hydroxylation. Approximately 90% of an oral dose is excreted in the urine in 24 h, of which 12–15% is unchanged drug, 3% is the free 4- and 5-hydroxy metabolites, and more than 40% is the glucuronide conjugate of 5-hydroxyviloxazine; ~16% is excreted as the glucuronide of a hydroxylated 5-oxo metabolite [Case, Reeves 1975].

Therapeutic Concentration

Following single oral doses of 100 mg to 10 subjects, peak plasma concentrations of 1.11–2.93 mg/L (mean 1.7 mg/L) were attained in ~1.5 h [Vandel *et al.* 1982a].

Following oral doses of 400 mg daily in divided doses to 13 subjects, a mean steady-state blood concentration of ~1.3 mg/L was reported [Bayliss, Case 1975].

In 11 subjects receiving 300 mg viloxazine, peak blood concentrations of 3.599 mg/L were achieved in 86 min; when the same dose was given as a sustained-release preparation, peak concentrations of 1.917 mg/L were achieved in 215 min. At 12 h after administration, plasma levels were 0.54–1.6 mg/L and 0.66–2.12 mg/L with the conventional and sustained-release dosage forms, respectively [Kergueris *et al.* 1989].

Toxicity A number of attempted suicides have been reported but recovery has occurred after the ingestion of up to 6.5 g viloxazine together with other drugs.

The following postmortem tissue concentrations were reported in a fatality attributed to viloxazine: blood 45 mg/L, liver 185 µg/g and urine 640 mg/L; death occurred ~8–12 h after ingestion [Bailey 1976].

Note For cases of overdose with viloxazine, see Brosnan *et al.* [1976].

Half-life Plasma half-life, 2 to 5 h.

Volume of Distribution ~0.5–1.5 L/kg [Vandel *et al.* 1982b].

Protein Binding ~85–90%.

Note For a review of viloxazine, see Pinder *et al.* [1977].

Dose The equivalent of 100 to 400 mg viloxazine daily.

Altamura AC *et al.* (1983). Age-related differences in kinetics and side-effects of viloxazine in man and their clinical implications. *Psychopharmacology (Berl)* 81: 281–285.

Bailey M (1976). Four assorted deaths. *TIAFT Bull* 12(3): 22.

Bayliss PE, Case DE (1975). Blood level studies with viloxazine hydrochloride in man. *Br J Clin Pharmacol* 2: 209–214.

Brosnan RD *et al.* (1976). Cases of overdosage with viloxazine hydrochloride (Vivalan). *J Int Med Res* 4: 83–85.

Case DE, Reeves PR (1975). The disposition and metabolism of I.C.I. 58,834 (viloxazine) in humans. *Xenobiotica* 5: 113–129.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Duverneuil C *et al.* (2003). A high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. *Ther Drug Monit* 25: 565–573.

Fazio A *et al.* (1984). A sensitive gas chromatographic assay for the determination of serum viloxazine concentration using a nitrogen–phosphorus-selective detector. *Ther Drug Monit* 6: 484–488.

Gillilan R, Mason WD (1981). High-pressure liquid chromatographic determination of viloxazine in human plasma and urine. *J Pharm Sci* 70: 220–221.

Kergueris MF *et al.* (1989). Comparative pharmacokinetic study of conventional and sustained-release viloxazine in normal volunteers. *Neuropsychobiology* 20: 136–140.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.

Lacassie E *et al.* (1999). [A specific dosage method for the analysis of 24 antidepressants using gas chromatography–mass spectrometry (GC/SM)]. *Acta Clin Belg Suppl* 1: 20–24.

Martinez MA *et al.* (2002). Simultaneous determination of viloxazine, venlafaxine, imipramine, desipramine, sertraline, and amoxapine in whole blood: comparison of two extraction/cleanup procedures for capillary gas chromatography with nitrogen–phosphorus detection. *J Anal Toxicol* 26: 296–302.

Norman TR *et al.* (1979). Determination of viloxazine in plasma by GLC. *Br J Clin Pharmacol* 8: 169–171.

Pinder RM *et al.* (1977). Viloxazine: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs* 13: 401–421.

Titier K *et al.* (2007). Quantification of tricyclic antidepressants and monoamine oxidase inhibitors by high-performance liquid chromatography–tandem mass spectrometry in whole blood. *J Anal Toxicol* 31: 200–207.

Vandel B *et al.* (1982a). Pharmacokinetics of viloxazine hydrochloride in man. *Eur J Drug Metab Pharmacokinet* 7: 65–68.

Vandel B *et al.* (1982b). Pharmacokinetics of viloxazine hydrochloride in man. *Eur J Drug Metab Pharmacokinet* 7: 65–68.

Wille SM *et al.* (2005). Development of a solid phase extraction for 13 'new' generation antidepressants and their active metabolites for gas chromatographic-mass spectrometric analysis. *J Chromatogr A* 1098: 19–29.

Wille SM *et al.* (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic-mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *J Chromatogr A* 1176: 236–245.

Vinbarbital

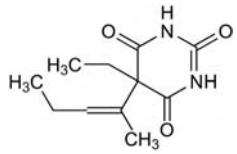
Sedative, Barbiturate

$C_{11}H_{16}N_2O_3 = 224.3$

CAS—125-42-8

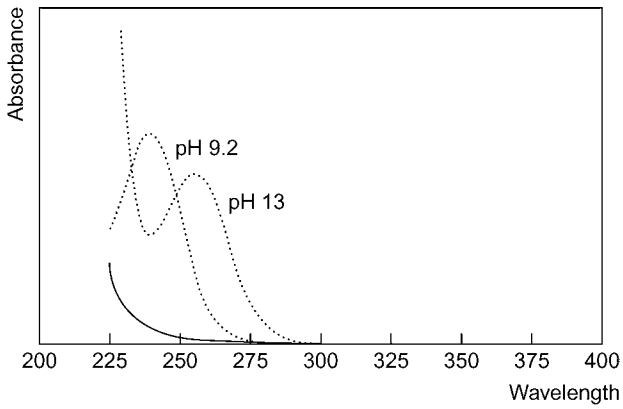
IUPAC Name 5-Ethyl-5-[(*E*)-pent-2-en-2-yl]-1,3-diazinane-2,4,6-trione

Synonyms Butenemal; 5-ethyl-5-(1-methyl-1-butenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione; vinbarbitone.

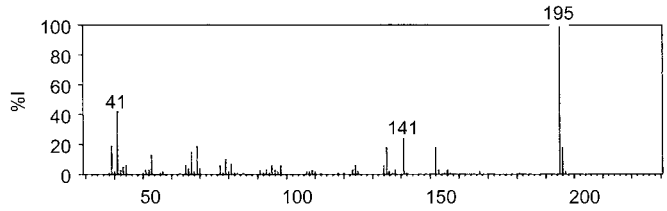


V

Chemical Properties A white powder. Mp 160° to 163°. Very slightly soluble in water; soluble in ethanol; sparingly soluble in ether. pK_a 7.5 (20°).
Colour Tests Koppanyi–Zwikker test—violet; mercurous nitrate—black; vanillin reagent—violet-brown/colourless.
Thin-layer Chromatography System TD—R_f 0.50; system TE—R_f 0.34; system TF—R_f 0.65; system TH—R_f 0.56; system TAD—R_f 0.57; system TAE—R_f 0.89; system TAJ—R_f 0.56; system TAK—R_f 0.62; system TAL—R_f 0.91 (mercurous nitrate spray, black; acidified potassium permanganate solution, yellow-brown; Zwikker's reagent, pink).
Gas Chromatography System GA—vinbarbital RI 1753, vinbarbital-Me_2 RI 1670, M (5-(1-ethylpropyl)-5-hydroxybarbituric acid) RI 1790, M (5-(1-ethylpropyl)barbituric acid) RI 1660, M (5-(3-hydroxy-1-methylbutyl)-5-vinylbarbituric acid) RI 1930, M (OH-) RI 2070, M (OH-)-H_2O RI 2020; system GF—RI 2495.
High Performance Liquid Chromatography System HG—*k* 4.83; system HH—*k* 2.32; system HX—RI 379; system HY—RI 363.
Ultraviolet Spectrum Borax buffer 0.05 mol/L (pH 9.2)—238 nm (A^1_1=452a); M sodium hydroxide (pH 13)—254 nm (A^1_1=374b).



Infrared Spectrum Principal peaks at wavenumbers 1675, 1760, 1725, 1234, 1282, 1298 cm^{-1} (KBr disk).
Mass Spectrum Principal ions at *m/z* 195, 41, 141, 69, 39, 152, 135, 196.

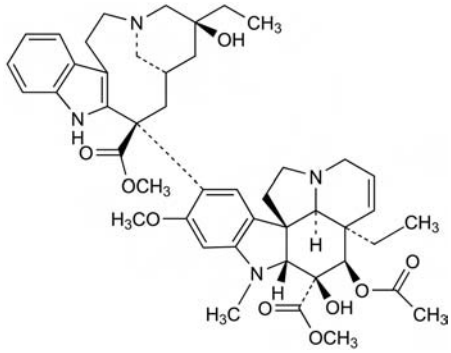


Quantification See under Amobarbital.
Dose 100 to 200 mg daily.

Vinblastine

Antineoplastic
$C_{46}H_{58}N_4O_9$ = 811.0
CAS—865-21-4

Synonyms Vinblastini; vincaleucoblastine; vincaleukoblastine; VLB.



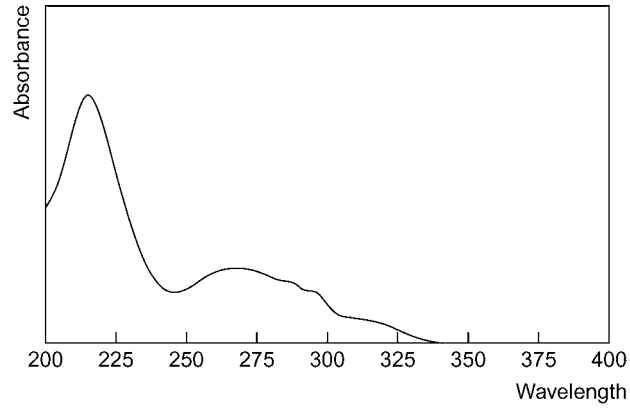
Chemical Properties An alkaloid extracted from periwinkle, *Vinca rosea* (*Catharanthus roseus*) (Apocynaceae). Crystals. Mp 211° to 216°. Practically insoluble in water; soluble in ethanol, acetone and chloroform. pK_a 5.4, 7.4. Log *P* (octanol/water), 3.7.

Vinblastine Sulfate

$C_{46}H_{58}N_4O_9,H_2SO_4$ = 909.1
CAS—143-67-9

Proprietary Names *Blastovin*; *Cellblastin*; *Lemblastin*; *Solblastin*; *Velban*; *Velbe*.
Chemical Properties A white to slightly yellow, very hygroscopic, amorphous or crystalline powder. Mp 284° to 285°. Soluble 1 in 10 of water and 1 in 50 of chloroform; very slightly soluble in ethanol; practically insoluble in ether.

Colour Test Marquis test—red.
Thin-layer Chromatography System TA—R_f 0.60; system TB—R_f 0.01; system TC—R_f 0.60; system TE—R_f 0.57; system TL—RF 29; system TAE—R_f 0.46.
High Performance Liquid Chromatography System HY—RI 355; system HAA—retention time 8.4 min.
Ultraviolet Spectrum Aqueous acid—268 nm (A^1_1=176b).



Infrared Spectrum Principal peaks at wavenumbers 1227, 1136, 1111, 1724, 1176, 1613 cm^{-1} (vinblastine sulfate, KBr disk).
Quantification
***Plasma* HPLC** Fluorescence detection. Vinblastine and metabolites. Limit of detection, 1 µg/L for vinblastine [van Tellingen *et al.* 1991]. Electrochemical detection. Limit of detection, 100 pg for vinblastine, desacetylvinblastine, vincristine and vindesine [Vendrig *et al.* 1988]. For a method for the quantification of vinblastine, vincristine and vindesine, see de Smet *et al.* [1985].

Radioimmunoassay Limit of detection, 2.1 µg/L for vinblastine, 3.8 µg/L for vincristine [Teale *et al.* 1977].

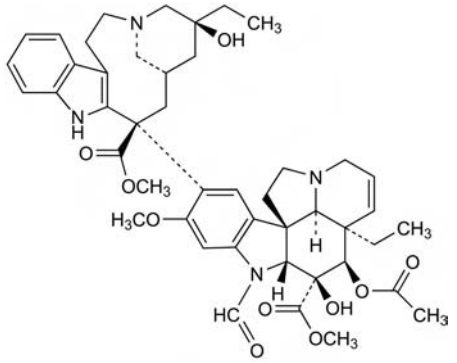
***Urine* HPLC** See Plasma [van Tellingen *et al.* 1991]. See Plasma [Vendrig *et al.* 1988]. For a method of quantification for vinblastine, vincristine and vindesine, see de Smet *et al.* [1985].
Disposition in the Body Poorly absorbed after oral administration. After IV administration, it is metabolised to desacetylvinblastine which is active. About 14% of a radioactively labelled dose is excreted in the urine in 72 h and 10% is eliminated in the faeces in the same period.
Therapeutic Concentration
After a bolus IV dose of 3 mg/m^2 given before or after cisplatin (100 mg/m^2) to 16 subjects, plasma vinblastine concentrations at 10 h were 3.2 to 6.3 µg/L (mean, 4.8) and 2.7 to 7.1 µg/L (mean, 4.9), respectively. Neutropenia was less severe in patients with plasma vinblastine levels <2.75 µg/L at 10 h [Links *et al.* 1999].
Toxicity
A vinblastine overdose in an 18-month-old child, who received 1.5 mg/kg instead of 0.15 mg/kg, was succesfully treated with steroids and citrovorum factor [Conter *et al.* 1991].
Half-life Plasma half-life (total radioactivity), about 20 h.
Protein Binding About 99%.
Dose Initially 100 µg/kg of vinblastine sulfate weekly, by IV injection.

Conter V *et al.* (1991). Overdose of vinblastine in a child with Langerhans' cell histiocytosis: toxicity and salvage therapy. *Pediatr Hematol Oncol* 8: 165–169.
de Smet M *et al.* (1985). High-performance liquid chromatographic determination of vinca-alkaloids in plasma and urine. *J Chromatogr* 345: 309–321.
Links M *et al.* (1999). Vinblastine pharmacokinetics in patients with non-small cell lung cancer given cisplatin. *Cancer Invest* 17: 479–485.
Teale JD *et al.* (1977). Radioimmunoassay of vinblastine and vincristine. *Br J Clin Pharmacol* 4: 169–172.
van Tellingen O *et al.* (1991). High-performance liquid chromatographic determination of vinblastine, 4-O-deacetylvinblastine and the potential metabolite 4-O-deacetylvinblastine-3-oic acid in biological fluids. *J Chromatogr* 553: 47–53.
Vendrig DE *et al.* (1988). Analysis of vinca alkaloids in plasma and urine using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 424: 83–94.

Vincristine

Antineoplastic
$C_{46}H_{56}N_4O_{10}$ = 825.0
CAS—57-22-7

Synonyms Leurocristine; 22-oxovincaleukoblastine.



Chemical Properties An alkaloid obtained from *Vinca rosea* (*Catharanthus roseus*) (Apocynaceae). Crystals. Mp 218° to 220°. pK_a 5.0, 7.4. Log *P* (octanol/water), 2.8.

Vincristine Sulfate

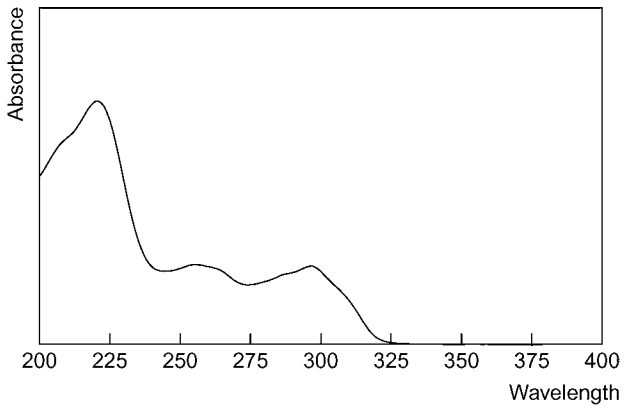
$C_{46}H_{56}N_4O_{10},H_2SO_4 = 923.0$
CAS—2068-78-2

Proprietary Names *Cellcristin*; *Citomid RU*; *Farmistin*; *Faulcris*; *Filcrin*; *Oncovin*; *Pericristine*; *Vincasar*; *Vincrin*; *Vincrisul*; *Vintec*.

Chemical Properties A white to slightly yellow, very hygroscopic, amorphous or crystalline powder. Mp about 277°. Soluble 1 in 2 of water and 1 in 30 of chloroform; slightly soluble in ethanol; practically insoluble in ether; soluble in methanol.

High Performance Liquid Chromatography System HAA—retention time 13.8 min.

Ultraviolet Spectrum Aqueous acid—256 (A_1^1=181b), 297 nm (A_1^1=179b).



Infrared Spectrum Principal peaks at wavenumbers 1230, 1745, 1170, 1685, 1030, 1130 cm^{-1} (vincristine sulfate, KBr disk).

Quantification See also under Vinblastine.

Plasma **HPLC** Electrochemical detection. For method, see Koopmans *et al.* [2001]. UV detection. Limit of detection, <28 µg/L [Embree *et al.* 1997].

Serum **HPLC** Electrochemical detection. Limit of detection, 0.3 µg/L [Bloemhof *et al.* 1991].

Disposition in the Body Poorly absorbed after oral administration. After IV injection it disappears rapidly from the blood and is excreted mainly in the bile. About 12% of a dose is excreted in the urine in 72 h and 70% is eliminated in the faeces in the same period.

Toxicity

A vincristine overdose (7.5 mg/m^2) was accidentally administered to 3 children, 2 of whom survived and 1 of whom died. The children were treated with double-volume exchange transfusions. In the 2 children who survived, the vincristine plasma levels were, respectively: pre-exchange 39.5 and 14.4 µg/L, post-exchange 16.7 and 4.1 µg/L, 8th day 2.6 and 2.9 µg/L; in the child who died the levels were: pre-exchange 26.0 µg/L, post-exchange 24.0 µg/L, 8th day 5.6 µg/L [Kosmidis *et al.* 1991.]

Half-life Plasma half-life, about 3 h; a terminal elimination half-life of about 23 h has also been reported.

Dose Initially 25 to 75 µg/kg of vincristine sulfate weekly, by IV injection.

Bloemhof H *et al.* (1991). Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction. *J Chromatogr* 572: 171–179.

Embree L *et al.* (1997). Validation of a high-performance liquid chromatographic assay method for quantification of total vincristine sulfate in human plasma following administration of vincristine sulfate liposome injection. *J Pharm Biomed Anal* 16: 675–687.

Koopmans P *et al.* (2001). An automated method for the bioanalysis of vincristine suitable for therapeutic drug monitoring and pharmacokinetic studies in young children. *Ther Drug Monit* 23: 406–409.

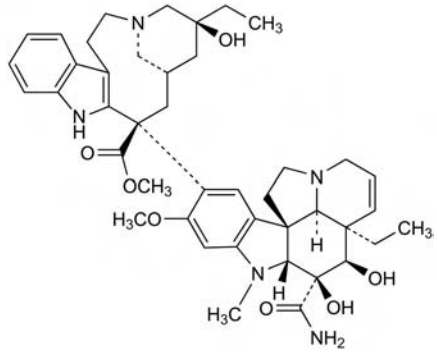
Kosmidis HV *et al.* (1991). Vincristine overdose: experience with 3 patients. *Pediatr Hematol Oncol* 8: 171–178.

Vindesine

Antineoplastic

$C_{43}H_{55}N_5O_7 = 753.9$
CAS—53643-48-4

Synonyms 3-(Aminocarbonyl)-*O*^{4}-deacetyl-3-de(methoxycarbonyl)-vincaleukoblastine; desacetyl vinblastine amide; VDS.



Chemical Properties Crystals. Mp 230° to 232°. pK_a (DMF 66%) 5.4, 7.4; (water) 6.0, 7.7. Log *P* (octanol/water), 4.4.

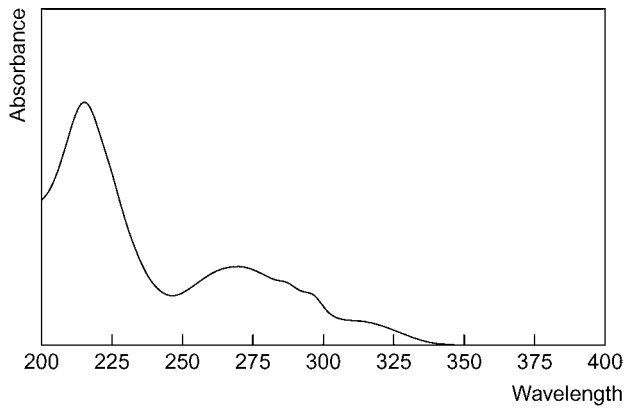
Vindesine Sulfate

$C_{43}H_{55}N_5O_7,H_2SO_4 = 852.0$
CAS—59917-39-4

Proprietary Names *Eldisin(e)*; *Enison*; *Gesidine*.

Chemical Properties An amorphous solid. Freely soluble in water.

Ultraviolet Spectrum Methanol—270 nm (A_1^1=235b).



Infrared Spectrum Principal peaks at wavenumbers 1120, 1230, 1680, 1175, 1610, 1038 cm^{-1} (vindesine sulfate, KBr disk).

Quantification See also under Vinblastine.

Plasma **Radioimmunoassay** Limit of detection, 50 ng/L [Rahmani *et al.* 1983].

Disposition in the Body Poorly absorbed after oral administration. After IV injection, about 13% of a dose is excreted in the urine in 24 h.

Therapeutic Concentration

Fifteen subjects received therapy with vindesine either by IV bolus injection at doses ranging from 0.7 to 1.2 mg/m^2 or by 5 day infusions (total dose 5 mg/m^2) in combination with cisplatin (20 mg/m^2 daily) and bleomycin (6 mg/m^2 daily) for 5 days. Steady state vindesine concentrations of 4 to 15 µg/L were reached after about 30 h of infusion administration [Rahmani *et al.* 1985].

Half-life Plasma half-life, about 24 h.

Dose Initially 3 mg/m^2 of vindesine sulfate weekly, by IV injection.

Rahmani R *et al.* (1983). A 125I-radiolabelled probe for vinblastine and vindesine radioimmunoassays: applications to measurements of vindesine plasma levels in man after intravenous injections and long-term infusions. *Clin Chim Acta* 129: 57–69.

Rahmani R *et al.* (1985). Clinical pharmacokinetics of vindesine infusion. *Cancer Treat Rep* 69: 839–844.

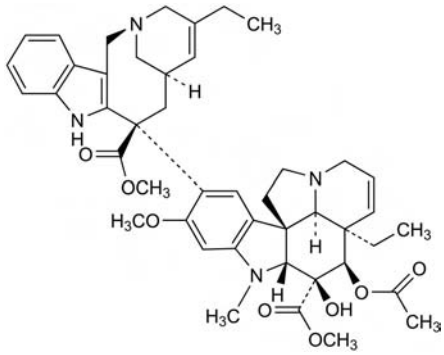
Vinorelbine

Antineoplastic

$C_{45}H_{54}N_4O_8 = 779.0$
CAS—71486-22-1

Synonyms nor-5′-Anhydrovinblastine; 3′,4′-didehydro-4′-deoxy-*C*′-norvincaleukoblastine; NVBKW-2307.

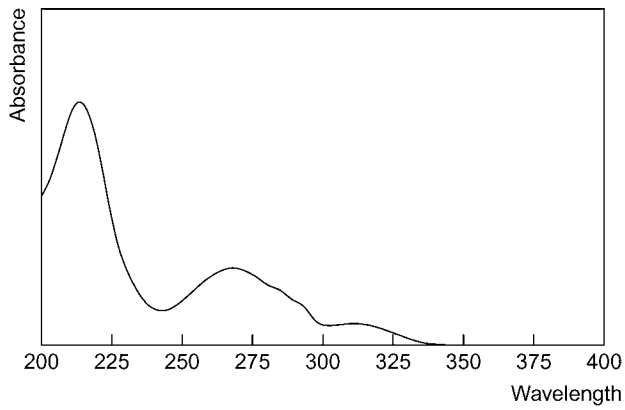
Proprietary Name *Navelbine*



Chemical Properties pK_a 5.4. Log *P* (octanol/buffer, pH 7.2), 16.

Vinorelbine Tartrate
$C_{45}H_{54}N_4O_8,2C_4H_6O_6 = 1079.1$
CAS—125317-39-7
Synonym Vinorelbine ditartrate
Proprietary Names *Biovelbin*; *Eunades*; *Navelbine*.
Chemical Properties A yellow to white amorphous powder. Soluble in water and in alcohol.

High Performance Liquid Chromatography Column: cyano Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 80 mmol/L ammonium acetate (pH 2.5, 50 : 50), flow rate 1 mL/min. IS: vinoblastine sulfate. Fluorescence detection (λ_{ex}=280 nm; λ_{em}=360 nm). Retention time: vinorelbine, 5.4 min; IS, 4.5 min [Gauvin *et al.* 2000].
Ultraviolet Spectrum Ethanol—215, 268, 282, 293, 310 nm.



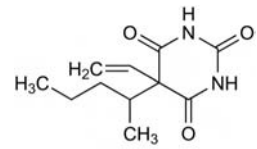
Quantification
Plasma **HPLC** Column: C_{18} Novapak (300 × 3.9 mm i.d.). Mobile phase: acetonitrile : phosphate buffer (pH 2.7, 60 : 40), flow rate 0.9 mL/min. Fluorescence detection (λ_{ex}=280 nm; λ_{em}=360 nm). Retention time: 13 min. Limit of detection, 2 µg/L [Robieux *et al.* 1996]. Fluorescence detection (λ_{ex}=270 nm; λ_{em}=320 nm). Limit of detection, 1.5 µg/L [van Tellingen *et al.* 1992]. Electrochemical detection. Limit of detection, about 1 µg/L [Nicot *et al.* 1990].
Serum **HPLC** UV detection (λ=268 nm). Limit of detection, 2.5 µg/L for vinorelbine and desacetylvinorelbine [Jehl *et al.* 1990].
Urine **HPLC** See Plasma. Limit of detection, 15 µg/L [van Tellingen *et al.* 1992]. UV detection (λ=268 nm). Limit of detection, 5.0 µg/L for vinorelbine and desacetylvinorelbine [Jehl *et al.* 1990].
Disposition in the Body Vinorelbine is rapidly absorbed and peak plasma concentrations increase with dose between 0.9 and 2.0 h after administration. The drug shows triphasic pharmacokinetics after IV administration. It is widely distributed and highly bound to platelets (78%) and lymphocytes. Vinorelbine is metabolised in the liver to desacetylvinorelbine which has antineoplastic activity. It is excreted primarily in faeces via bile, and to a lesser extent it is also excreted in urine as the unchanged drug and as metabolites. Recovery of the drug is incomplete which suggests that it may be retained in tissue.
Therapeutic Concentration
Eight male patients with lung cancer (stage III or IV), aged between 51 and 74 years, were IV administered a weekly dose of 30 mg/m^2 over 15 min. A mean peak plasma concentration of 780 µg/L (range, 260 to 1230 µg/L) was observed [Marquet *et al.* 1992].

Five patients, with a mean age of 56.7 years, with advanced breast cancer were treated with 30 mg/m^2 vinorelbine IV over a 20 min infusion. Mean peak plasma concentrations of 179 µg/L were observed at 0.5 h and plasma concentrations remained above 2 µg/L for 48 h or longer after administration [Robieux *et al.* 1996].
Toxicity
In a study carried out in 8 male patients with lung cancer, 2 patients died resulting from haematological toxicity, namely anaemia. Other toxicological effects observed were leucopenia and neurological toxicity [Marquet *et al.* 1992].
Half-life Plasma half-life, 28 to 44 h.
Volume of Distribution 25 to 100 L/kg; steady-state mean 47.6 L/kg (range, 13.8 to 156).
Clearance Plasma clearance, 16 to 21 mL/min/kg (mean, 1.28 L/h/kg; range, 0.58 to 2.61).
Distribution in Blood Vinorelbine, 84% bound to red blood cells, mainly platelets.
Protein Binding 14%.
Note For a review of the pharmacokinetics of vinorelbine, see Levêque and Jehl [1996].
Dose Initially the equivalent of 25 to 30 mg per m^2 of vinorelbine IV weekly.

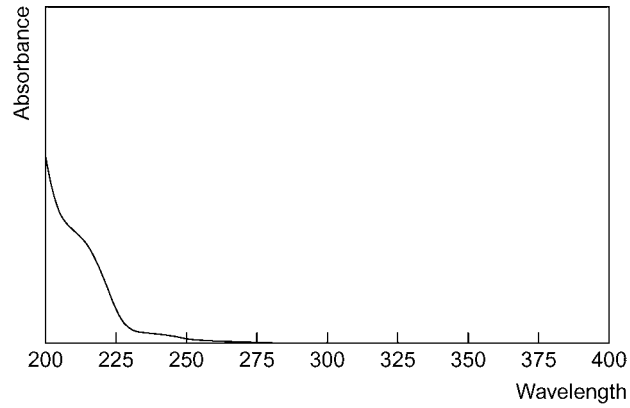
Gauvin A *et al.* (2000). High-performance liquid chromatographic determination of vinorelbine in human plasma and blood: application to a pharmacokinetic study. *J Chromatogr B Biomed Sci Appl* 748 (2): 389–399.
Jehl F *et al.* (1990). Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography. *J Chromatogr* 525: 225–233.
Levêque D, Jehl F (1996). Clinical pharmacokinetics of vinorelbine. *Clin Pharmacokinet* 31: 184–197.
Marquet P *et al.* (1992). Pharmacokinetics of vinorelbine in man. *Eur J Clin Pharmacol* 42: 545–547.
Nicot G *et al.* (1990). High-performance liquid chromatographic determination of navelbine in human plasma and urine. *J Chromatogr* 528: 258–266.
Robieux I *et al.* (1996). Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations. *J Chromatogr B Biomed Appl* 675: 183–187.
van Tellingen O *et al.* (1992). Bio-analysis of vinorelbine by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 573: 328–332.

Vinylbital

Hypnotic, Barbiturate
$C_{11}H_{16}N_2O_3 = 224.3$
CAS—2430-49-1
IUPAC Name 5-Ethenyl-5-pentan-2-yl-1,3-diazinane-2,4,6-trione
Synonyms Butyvinal; 5-ethenyl-5-(1-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione; vinylbitone; vinymalum.
Proprietary Names *Optanox*; *Speda*.



Chemical Properties Crystals. Mp 90° to 92°. Log *P* (octanol/water), 1.9.
Thin-layer Chromatography System TD—R_f 0.38; system TE—R_f 0.39; system TF—R_f 0.64; system TAD—R_f 0.66; system TAE—R_f 0.89.
Gas Chromatography System GA—vinylbital RI 1729, vinylbital-Me_2 RI 1655, M (OH-)-H_2O RI 1995, M (desvinyl-) RI 1665; system GAJ—vinylbital RRT 0.798 (relative to methylphenobarbital).
High Performance Liquid Chromatography System HX—RI 424; system HZ—retention time 4.1 min; system HAA—retention time 16.6 min.
Ultraviolet Spectrum Aqueous alkali—247 nm (A^1_1=298b).

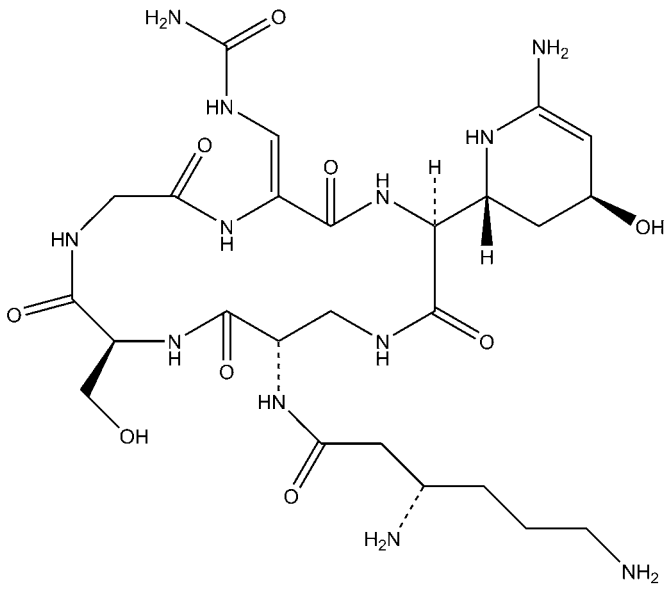


Infrared Spectrum Principal peaks at wavenumbers 1692, 1730, 1750, 1318, 1220, 1630 cm^{-1}.
Quantification See also under Amobarbital.
Plasma **GC** AFID. Limit of detection, <125 µg/L [Breimer, de Boer 1976].
Disposition in the Body Readily absorbed after oral administration. Metabolites which have been identified in urine are 3′-hydroxyvinylbital and the corresponding desvinyl derivative, 5-(3-hydroxy-1-methylbutyl)barbituric acid. <5% of a dose is excreted in the urine unchanged in 72 h.
Therapeutic Concentration
Following a single oral dose of 150 mg to 6 subjects, peak plasma concentrations of 2.2 to 3.9 mg/L were attained in 1 to 2 h [Breimer, de Boer 1976].
Half-life Plasma half-life, 18 to 34 h (mean, 24).
Volume of Distribution About 0.7 L/kg.
Dose 100 to 200 mg, as a hypnotic.

Breimer DD, de Boer AG (1976). Pharmacokinetics and relative bioavailability of vinylbital in man after oral and rectal administration. *Arzneimittelforschung* 26: 448–454.

Viomycin

Antibiotic, Tuberculostatic
$C_{25}H_{43}N_{13}O_{10}$ = 685.7
CAS—32988-50-4
IUPAC Name (3*S*)-3,6-Diamino-*N*-[(3*S*,6*Z*,9*S*,12*S*,15*S*)-3-[(4*R*,6*S*)-2-amino-6-hydroxy-3,4,5,6-tetrahydropyrimidin-4-yl]-6-[(carbamoylamino)methylidene]-9,12-bis(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-15-yl]hexanamide
Synonyms Celiomycin; florimycin.

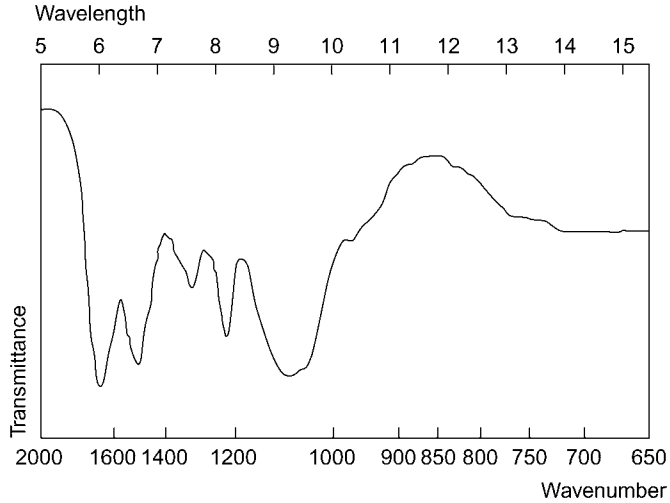


Chemical Properties An antimicrobial polypeptide base produced by certain strains of *Streptomyces griseus* var. *purpureus*. Log *P* (octanol/water) −11.4 [Meylan, Howard 1995].

Viomycin Sulfate

$C_{25}H_{43}N_{13}O_{10},H_2SO_4$
CAS—37883-00-4
IUPAC Name (3*S*)-3,6-Diamino-*N*-[(3*S*,6*Z*,9*S*,12*S*,15*S*)-3-[(4*R*,6*S*)-2-amino-6-hydroxy-3,4,5,6-tetrahydropyrimidin-4-yl]-6-[(carbamoylamino)methylidene]-9,12-bis(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-15-yl]hexanamide sulfuric acid
Synonym Tuberactinomycin B
Proprietary Names *Viocin*; *Vionactane*.
Chemical Properties White hygroscopic powder. Mp 252° (anhydrous) or 280° with decomposition. Very soluble in water, slightly soluble in ethanol, almost insoluble in ether.

Colour Test Ammonium molybdate test—faint blue (limit of detection, 1.0 µg).
Thin-layer Chromatography System T1—R_f 0.00 (location reagent potassium permanganate spray., positive reaction).
Ultraviolet Spectrum Aqueous acid (0.1 N H_2SO_4)—269 nm, neutral—269 nm.
Infrared Spectrum Principal peaks at wavenumber 1700, 1075, 1500, 3250, 1250 cm^{-1} (KBr disk) [Phillips, Ragheb 1965].



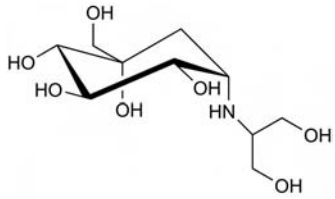
Mass Spectrum Principal ions at *m/z* 686, 668, 670, 728, 700 [Pittenauer *et al.* 2006].
Quantification
Note For an ELISA assay for the analysis of viomycin, see Kitagawa *et al.* [1976]. For a review of the chromatographic analysis of antituberculosis drugs, see Holdiness [1985].
Disposition in the Body Viomycin is only partially absorbed if given by mouth, and therapeutic blood levels are difficult to attain by this route of administration. It does not readily pass the normal blood–brain barrier or into peritoneal or pleural effusions. It is mainly excreted by the kidneys with ~85% of a dose being recovered unchanged in the urine within 24 h [Holdiness 1984].
Therapeutic Concentration
Serum concentrations of viomycin have been reported to range from 12 to 40 mg/L 1 h after a dose of 500 mg IM, falling to ~5 mg/L after 8 h.
A dose of 1000 mg given IM resulted in a mean maximum plasma concentration of 25 to 50 µg/mL at 2 h [Pyle 1970].
Practically all strains of *M. tuberculosis* are inhibited by viomycin concentrations in the 1 to 10 µg/mL range [Holdiness 1984].
Toxicity
In large doses (30 to 75 mg/kg) viomycin suppresses serum potassium and calcium concentrations as early as 6 weeks after the initiation of therapy [Adcock *et al.* 1954; Schaffeld *et al.* 1954; Werner *et al.* 1951].
One 58-year-old male who received acceptably low doses (in combination with pyrazinamide) developed profound electrolyte imbalances that eventually resulted in mental confusion and grand mal seizures [Vanasin *et al.* 1972].
The coadministration of viomycin and pyrazinamide resulted in electrolyte imbalances, including hypocalcaemia, hypomagnesaemia, hypokalaemia and hypophosphataemia in a 42-year-old woman [Clarke, McCarthy 1961].
Damage to the eighth cranial nerve with deafness and loss of vestibular function has occurred in humans. The potential for toxicity could be avoided by limiting the daily dose to 1 to 2 g IM in 1 or 2 doses and restricting daily administration to a maximum of 3 weeks [Holdiness 1987].
A study of 75 patients found vestibular function disturbances in 28%, vertigo in 5.3%, dizziness alone in 24% and mild and severe hearing loss in 2.7% of individuals receiving 1 g daily [Garfield *et al.* 1966].
The LD_{50} in mice (IV) is 165 to 240 mg/kg.
Dose Up to 1 mega unit daily, IM.

Adcock JD *et al.* (1954). The use of viomycin in patients with pulmonary tuberculosis. *Am Rev Tuberc* 69: 543–553.
Clarke M, McCarthy CF (1961). Electrolyte changes due to viomycin. *Tubercle* 42: 358–361.
Garfield JW *et al.* (1966). The auditory, vestibular and renal effects of capreomycin in humans. *Ann NY Acad Sci* 135: 1039–1046.
Holdiness MR (1984). Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet* 9: 511–544.
Holdiness MR (1985). Chromatographic analysis of antituberculosis drugs in biological samples. *J Chromatogr* 340: 321–359.
Holdiness MR (1987). Neurological manifestations and toxicities of the antituberculosis drugs. A review. *Med Toxicol* 2: 33–51.
Kitagawa T *et al.* (1976). Enzyme-coupled immunoassay of viomycin. *J Antibiot (Tokyo)* 29: 1343–1345.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
Phillips WF, Ragheb HS (1965). Chromatographic fractionation of antibiotic 136 and characterization of its components. *J Chromatogr* 19: 147–159.
Pittenauer E *et al.* (2006). Comparison of CID spectra of singly charged polypeptide antibiotic precursor ions obtained by positive-ion vacuum MALDI IT/RTOF and TOF/RTOF. AP-MALDI-IT and ESI-IT mass spectrometry. *J Mass Spectrom* 41: 421–447.
Pyle MM (1970). Ethambutol and viomycin. *Med Clin North Am* 54: 1317–1327.
Schaffeld HG *et al.* (1954). Viomycin therapy in human tuberculosis. *Am Rev Tuberc* 69: 520–542.
Vanasin B *et al.* (1972). Hypocalcemia, hypomagnesemia and hypokalemia during chemotherapy of pulmonary tuberculosis. *Chest* 61: 496–499.
Werner CA *et al.* (1951). The toxicity of viomycin in humans. *Am Rev Tuberc* 63: 49–61.

Voglibose

Antidiabetic, α-Glucosidase Inhibitor
$C_{10}H_{21}NO_7 = 267.3$
CAS—83480-29-9

IUPAC Name 5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane- 1,2,3,4-tetrol
Synonyms AO-128; 3,4-dideoxy-4-[[2-hydroxy-1-(hydroxymethyl)ethyl] amino]-2-*C*-(hydroxymethyl)-D-epi-inositol.
Proprietary Name *Basen*

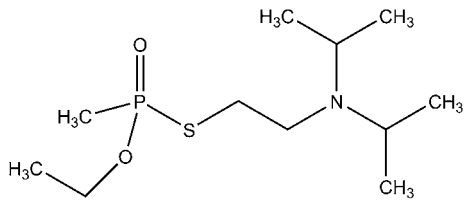


Chemical Properties Colourless crystals, Mp 162° to 163°
Disposition in the Body Voglibose is slowly and poorly absorbed, and is rapidly excreted. Little metabolism occurs and no metabolites have as yet been identified. Within 48 h of oral administration, it is almost totally excreted in the faeces (93%) as the unchanged drug with a small amount in urine (4%). Maximum concentrations are reached in tissues up to 1 h after oral administration. Penetration of the blood–brain barrier and erythrocyte membrane is low.
Toxicity Voglibose competitively and reversibly inhibits the alpha-glucosidase enzymes; glucoamylase, sucrase, maltase and isomaltase, found in the small intestine, and this delays hydrolysis of complex carbohydrates. It is unlikely to produce hypoglycaemia in overdose, but abdominal discomfort and diarrhoea may occur.
Bioavailability <6%.
Volume of Distribution Low tissue affinity.
Protein Binding <15%.
Dose 200 to 300 μg orally 3 times a day.

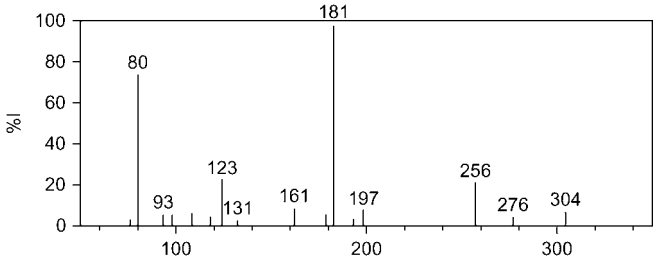
VX

Anticholinesterase, Organophosphate Nerve Agent
$C_{11}H_{26}NO_2PS = 267.4$
CAS—50782-69-9

IUPAC Name Methylphosphonothioic acid *S*-[2-[bis(1-methylethyl)amino] ethyl] *O*-ethyl ester
Synonyms *O*-Ethyl *S*-[2-(diisopropylamino)ethyl]methylphosphonothioate; Tx 60.



Chemical Properties Odourless liquid. Mp −39°. Bp 300°. Soluble in water. pK_a 8.6 [Epstein *et al.* 1974], 9.4 [van der Schans *et al.* 2003]. Log *P* (octanol/water), 2.09 [Munro *et al.* 1999]. Samples were stable after 8 and 48 h at room temperature and after 3 freeze–thaw cycles [McGuire *et al.* 2008a]. At a pH of ≈6.0, stock solutions were stable at 4° for 2 weeks [Wils, Hulst 1990].
Gas Chromatography-Mass Spectrometry Column: DB5-MS (25 m × 0.22 mm i.d., 0.33 μm). Carrier gas: He, 0.9 mL/min. Temperature programme: 40° for 2 min to 160° at 20°/min to 310° at 30°/min for 5 min. ATD, full scan mode. Limit of detection, 50 ng [Carrick *et al.* 2001].
Liquid Chromatography-Mass Spectrometry Column: Waters Atlantis HILIC (50 × 2.1 mm i.d., 3 μm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate (86 : 14). ESI, negative ion mode, SRM acquisition mode. Limit of detection, ethylmethylphosphonic acid (EMPA) 160 ng/L [Mawhinney *et al.* 2007a]. The effect of post-column addition of organic solvents on this method is reported in [Mawhinney *et al.* 2007b].
Mass Spectrum Principal ions at *m/z* 181, 80, 123, 256, 161, 197, 304, 93 (EMPA-PFB derivative).



Quantification
Blood **GC-MS** Column: Restek Rtx-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 35° for 2 min to 125° at 15°/min to 325° at 30°/min. CI, MRM acquisition mode. Limit of detection, 4 μg/L [McGuire *et al.* 2008b].
Plasma **GC-MS** Column: Phenomenex ZB-5 (60 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 68° for 2.5 min to 175° at 6.7° for 16 min to 310° at 50°/min for 2.5 min. EI ionisation mode. Limit of detection, 5.5 ng/L for G-VX [Solano *et al.* 2008].
LC-MS Column: Chiralcel OD-H (25 cm × 4.6 mm i.d.). Mobile phase: hexane : isopropyl alcohol (92 : 08), flow rate: 0.8 mL/min. DAD (λ = 210 nm) followed by positive ion mode, full scan mode. Limit of detection not reported [Smith 2004].
Serum **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, EMPA 10 μg/L [Miki *et al.* 1999]. Column: Hewlett Packard Ultra 2 (25 m × 0.32 mm i.d., 0.52 μm). Carrier gas: He, 45 cm/s. Temperature programme: 70° for 2 min to 300° at 10°/min. EI ionisation at 70 eV. Limit of detection, EMPA 0.1 mg/L [Tsuchihashi *et al.* 1998].
HPLC Column: Shim-pack IC-A3 (150 × 4.6 mm i.d., 5 μm). Mobile phase: 0.5 mmol/L phthalic acid : 0.1 mmol/L Tris : 5% acetonitrile, flow rate 1 mL/min. UV detection (λ = 266 nm). Limit of detection, EMPA 40 μg/L [Katagi *et al.* 1997].
LC-MS Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45, containing 0.1% glycerol), flow rate 100 μL/min. FAB ionisation. Limit of detection, EMPA 3 μg/L [Katagi *et al.* 1999].
Urine **GC-MS** Column: Restek Rtx-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 40 cm/s. Temperature programme: 60° for 1 min to 180° at 30°/min to 280° at 10°/min for 2 min. CI, negative ion mode, SRM acquisition mode. Limit of detection, EMPA 0.5 μg/L [Riches *et al.* 2005]. Column: DB-5MS capillary (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 70° for 1.5 min to 250° in 5 min at 40°/min for 3 min. MRM acquisition mode. Limit of detection, EMPA 0.9 μg/L [Barr *et al.* 2004]. Column: DB-5 capillary (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 50° for 1.5 min to 250° at 40°/min in 5 min for 5 min. SRM acquisition mode. Limit of detection, 4 μg/L [Driskell *et al.* 2002]. Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, EMPA 5 μg/L [Miki *et al.* 1999].
Oral Fluid **GC-MS** Column: DB-1 megabore (30 m × 0.53 mm i.d., 1.5 μm). Carrier gas: He, 6.5 mL/min. Temperature programme: 50° to 270° at 10°/min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection, EMPA 20 μg/L [Miki *et al.* 1999].
Other **GC** Water. Column: CP-Sil 19 (50 m × 0.32 mm i.d., 0.4 μm). Temperature programme: 60° for 3 min to 70° at 5°/min for 7 min to 180° at 10°/min to 240° at 15°/min for 12 min. Limit of detection, ng/L range [Degenhardt-Langelaan, Kientz 1996].
GC-MS Minipig Blood. Column: Restek Rtx-5MS 5% diphenyl 95% dimethyl polysiloxane (30 m × 0.25 mm i.d.). Carrier gas: He, 1 mL/min. Temperature programme: 35° for 2 min to 100° at 15°/min for 0.1 min to 280° at 35°/min for 1 min. CI, MRM acquisition mode. Limit of quantification, 5 μg/L [Byers *et al.* 2008]. Minipig Plasma. Column: Restek Rtx-5MS (30 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He, 1 mL/min. Temperature programme: 70° for 2 min to 300° at 25°/min for 2 min. Retention times: VX and deuterated VX 9.2 to 9.3 min. Limit of quantification, 10 μg/L [McGuire *et al.* 2008a].
LC-MS River Water. Column: CAPCELL PAK UG C_{18} (150 × 1.5 mm i.d.). Mobile phase: 5 mmol/L ammonium acetate : acetonitrile (55 : 45) containing 0.1% glycerol, flow rate 100 μL/min. FAB ionisation. Limit of detection, EMPA 1 μg/L [Katagi *et al.* 1999]. Spiked Soil Samples. Column: LiChrosorb C_{18} (250 × 5 mm i.d., 7 μm). Mobile phase: methanol : 0.1 mol/L ammonium acetate (80 : 20) or acetonitrile: methanol : 0.25 mol/L ammonium acetate (70 : 20 : 10), flow rate 1.2 mL/min. ESI, positive ion mode, SIM acquisition mode. Limit of detection, 5 μg/L [Wils, Hulst 1990].
Note For a review article on the chromatographic analysis of chemical warfare agents, see Witkiewicz *et al.* [1990].
Disposition in the Body At pH <6 and >10, the formation of EMPA and diisopropylethyl mercaptoamine (DESH) predominates. The latter can be oxidised to bis(2-diisopropylaminoethyl) disulfide (EA 4196) or react with the diisopropylethyleneimmonium ion $(CH_2)2N^+(C_3H_7)_2$ to form bis(2-diisopropylaminoethyl) sulfide. At neutral and alkaline pH values (7 to 10), this pathway competes with dealkylation of the ethoxy group to yield the environmentally stable EA 2192 and ethanol. Although EMPA can theoretically be slowly hydrolysed to methylphosphonic acid (MPA), it has not been demonstrated in aqueous solutions [Munro *et al.* 1999].
Toxicity

On June 1, 2006 a laboratory worker was accidentally exposed to VX. Red blood cell samples were obtained from the worker at 1, 6, 8, 20 and 27 days following exposure. Levels were determined as follows:

Days post exposure	*G-VX concentration (ng/L)*
1	219.9 ± 97.10
6	144.8 ± 57.80
8	279.1 ± 170.5
20	128.9 ± 17.50
27	96.90 ± 60.40

[McGuire *et al.* 2008b].

A 28-year-old was attacked by 2 members of a cult. They sprinkled VX on his neck and he collapsed. He was carried to hospital and treated but he died 10 days later. Serum that was collected ≈1 h after his exposure to VX and stored at –20° was analysed for its VX content. The concentration of 2-(diisopropylaminoethyl)methyl sulfide in the serum sample was estimated to be 143 μg/L [Tsuchihashi *et al.* 1998].

For a review of the toxicity of tabun, sarin and VX, see Munro [1994].

Barr JR *et al.* (2004). Quantitation of metabolites of the nerve agents sarin, soman, cyclohexylsarin, VX, and Russian VX in human urine using isotope-dilution gas chromatography–tandem mass spectrometry. *J Anal Toxicol* 28: 372–378.

Byers CE *et al.* (2008). Gas chromatography–tandem mass spectrometry analysis of red blood cells from Göttingen minipig following whole-body vapor exposure to VX. *J Anal Toxicol* 32: 57–62.

Carrick WA *et al.* (2001). Retrospective identification of chemical warfare agents by high-temperature automatic thermal desorption-gas chromatography–mass spectrometry. *J Chromatogr A* 925: 241–249.

Degenhardt-Langelaan CE, Kientz CE (1996). Capillary gas chromatographic analysis of nerve agents using large volume injections. *J Chromatogr A* 723: 210–214.

Driskell WJ *et al.* (2002). Quantitation of organophosphorus nerve agent metabolites in human urine using isotope dilution gas chromatography–tandem mass spectrometry. *J Anal Toxicol* 26: 6–10.

Epstein J *et al.* (1974). The kinetics and mechanisms of hydrolysis of phosphonothiolates in dilute agueous solution. *Phosphorus* 4: 157–163.

Katagi M *et al.* (1997). Determination of the main hydrolysis products of organophosphorus nerve agents, methylphosphonic acids, in human serum by indirect photometric detection ion chromatography. *J Chromatogr B Biomed Sci Appl* 698: 81–88.

Katagi M *et al.* (1999). On-line solid-phase extraction liquid chromatography–continuous flow FRIT fast atom bombardment mass spectrometric and tandem mass spectrometric determination of hydrolysis products of nerve agents alkyl methylphosphonic acids by *p*-bromophenacyl derivatization. *J Chromatogr A* 833: 169–179.

Mawhinney DB *et al.* (2007a). The determination of organophosphonate nerve agent metabolites in human urine by hydrophilic interaction liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 852: 235–243.

Mawhinney DB *et al.* (2007b). Enhancing the response of alkyl methylphosphonic acids in negative electrospray ionization liquid chromatography tandem mass spectrometry by post-column addition of organic solvents. *J Am Soc Mass Spectrom* 18: 1821–1826.

McGuire JM *et al.* (2008a). A Rapid and sensitive technique for assessing exposure to VX via GC–MS–MS analysis. *J Anal Toxicol* 32: 63–67.

McGuire JM *et al.* (2008b). Determination of VX-G analogue in red blood cells via gas chromatography–tandem mass spectrometry following an accidental exposure to VX. *J Anal Toxicol* 32: 73–77.

Miki A *et al.* (1999). Determination of alkylmethylphosphonic acids, the main metabolites of organophosphorus nerve agents, in biofluids by gas chromatography–mass spectrometry and liquid–liquid–solid-phase-transfer-catalyzed pentafluorobenzylation. *J Anal Toxicol* 23: 86–93.

Munro N (1994). Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: implications for public protection. *Environ Health Perspect* 102: 18–37.

Munro NB *et al.* (1999). The sources, fate, and toxicity of chemical warfare agent degradation products. *Environ Health Perspect* 107: 933–974.

Riches J *et al.* (2005). The trace analysis of alkyl alkylphosphonic acids in urine using gas chromatography–ion trap negative ion tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 816: 251–258.

Smith JR (2004). Analysis of the enantiomers of VX using normal-phase chiral liquid chromatography with atmospheric pressure chemical ionization-mass spectrometry. *J Anal Toxicol* 28: 390–392.

Solano MI *et al.* (2008). Quantification of nerve agent VX–butyrylcholinesterase adduct biomarker from an accidental exposure. *J Anal Toxicol* 32: 68–72.

Tsuchihashi H *et al.* (1998). Identification of metabolites of nerve agent VX in serum collected from a victim. *J Anal Toxicol* 22: 383–388.

van der Schans MJ *et al.* (2003). Toxicokinetics of the nerve agent (+/−)-VX in anesthetized and atropinized hairless guinea pigs and marmosets after intravenous and percutaneous administration. *Toxicol Appl Pharmacol* 191: 48–62.

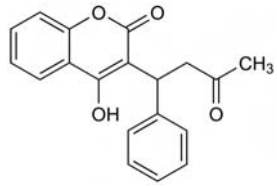
Wils ER, Hulst AG (1990). Determination of O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate (VX) by thermospray liquid chromatography–mass spectrometry. *J Chromatogr* 523: 151–161.

Witkiewicz Z *et al.* (1990). Chromatographic analysis of chemical warfare agents. *J Chromatogr* 503: 293–357.

Warfarin

Anticoagulant, Rodenticide
$C_{19}H_{16}O_4$ = 308.3
CAS—81-81-2

IUPAC Name 2-Hydroxy-3-(3-oxo-1-phenylbutyl)chromen-4-one
Synonym 4-Hydroxy-3-(3-oxo-1-phenylbutyl)coumarin
Proprietary Names *Biotrol*; *Dethmor*; *Sorexa* (all rodenticides).



Chemical Properties Colourless crystals. Mp of the purified compound 159° to 160°; of the technical grade 157°. Freely soluble in alkaline aqueous solutions; soluble in acetone and dioxane; moderately soluble in alcohols; practically insoluble in water, benzene and cyclohexane. pK_a 4.5 [Hou *et al.* 2007], 5.0 (20°). Log *P* (octanol/pH 8.0), 0.0; (octanol/water), 3.47 [Hou *et al.* 2007], 2.60. Quality control samples were stable for 281 days at −20°, extracted samples were stable in polypropylene plates for at least 7 days when stored at 5°, and stock solutions were stable at 5° for 112 days in polypropylene containers [Coe *et al.* 2006].

Warfarin Potassium

$C_{19}H_{15}KO_4$ = 346.4
CAS—2610-86-8
Proprietary Name *Athrombin-K*
Chemical Properties A white crystalline powder that discolours on exposure to light. Soluble 1 in 1.5 of water and 1 in 1.9 of ethanol; very slightly soluble in chloroform and ether.

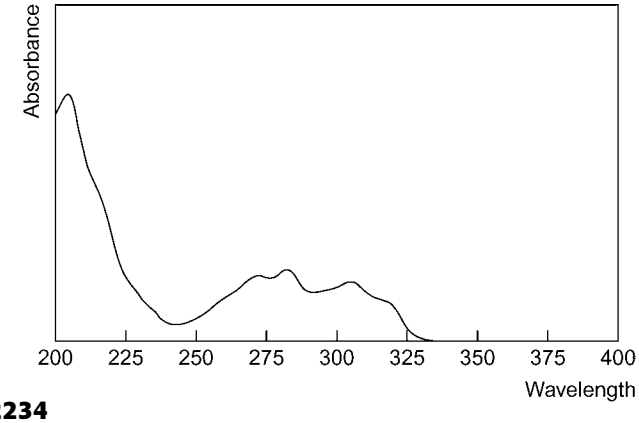
Warfarin Sodium

$C_{19}H_{15}NaO_4$ = 330.3
CAS—129-06-6
Proprietary Names *Aldocumar*; *Coumadin(e)*; *Jantoven*; *Marevan*; *Panwarfin*; *Sofarin*; *Varfine*; *Waran*; *Warfilone*.
Chemical Properties A white amorphous or crystalline powder which discolours on exposure to light. Soluble 1 in <1 of water and ethanol; very slightly soluble in chloroform and ether.

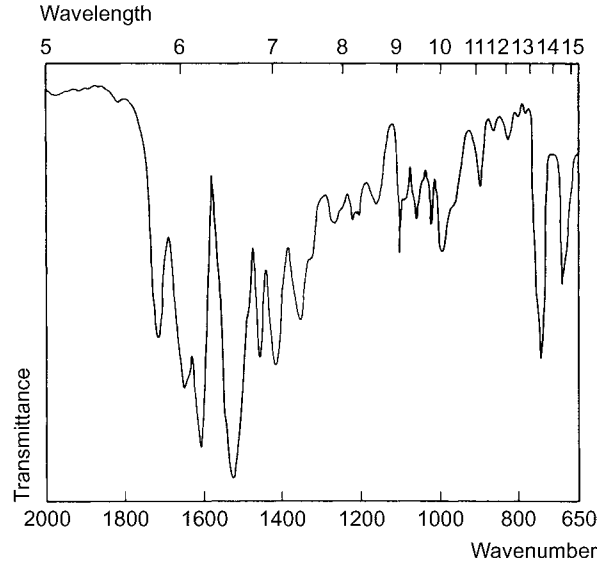
Colour Test Liebermann's reagent—red-orange.
Thin-layer Chromatography System TD—R_f 0.64; system TE—R_f 0.18; system TF—R_f 0.62; system TX—R_f 0.12; system TY—R_f 0.11; system TAD—R_f 0.64 (acidified potassium permanganate solution—positive).
Gas Chromatography System GA—warfarin RI 1432, warfarin-Me RI 2580, M (OH-) isomer 1 Me_2 RI 2810, M (OH-) isomer 2 Me_2 RI 2830, M (OH-) isomer 3 Me_2 RI 2870.
High Performance Liquid Chromatography System HX—RI 546; system HY—RI 514; system HZ—RT 9.4 min; system HAX—RT 8.0 min; system HAY—RT 8.3 min.

Column: Astec β-cyclodextrin (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : glacial acetic acid : triethylamine (1000 : 3 : 2.5), flow rate 1.0 mL/min. UV detection (λ = 320 nm). Retention time: (*S*)-warfarin 6.9 min, (*R*)-warfarin 7.7 min [Ring, Bostick 2000].

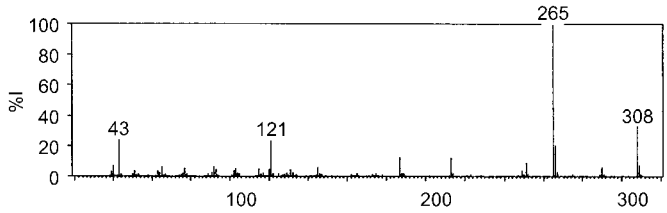
Column: Chiralcel OD (250 × 4.6 mm i.d., 10 µm). Mobile phase: isopropanol : acetic acid : hexane (18 : 0.5 : 81.5), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 320 nm, λ_{em} = 415 nm). Resolution factor: warfarin, 5.6, 7-hydroxywarfarin 4.5. Retention time: (*R*)-warfarin 13.1 min, for (*S*)-warfarin 25.3 min. Limit of detection, 20 µg/L for (*R*)-warfarin, 40 µg/L for (*S*)-warfarin [Takahashi *et al.* 1997].
Ultraviolet Spectrum Aqueous acid—270, 280, 303 nm; aqueous alkali—293, 308 nm (A_1^1 = 462a).



Infrared Spectrum Principal peaks at wavenumbers 1517, 1599, 1640, 750, 1700, 692 cm^{-1} (warfarin sodium, KBr disk).



Mass Spectrum Principal ions at *m/z* 265, 308, 121, 43, 266, 187, 213, 251.



Quantification

Blood **HPLC** Column: C_8 Symmetry (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : phosphate buffer (pH 3.8, 13 : 87 for 1 min to 35 : 65 at 9 min to 80 : 20 at 28 min for 2 min to 13 : 87 until 35 min), flow rate 0 mL/min. UV detection (λ = 200–400 nm). Limit of quantification, 400 µg/L [Denooz *et al.* 2009]. Column: XDB C (150 × 2.1 mm i.d., 5 µm). Mobile phase: 2% methanol and 2% aqueous acetic acid (88 : 12), flow rate 0.5 mL/min. Fluorescence detection. Limit of quantification, 0.05 mg/L for warfarin and 0.01 mg/L for other 4-hydroxycoumarin rodenticides [Jin *et al.* 2007].

Plasma **GC** FID. Limit of detection, 0.3 mg/L [Hanna *et al.* 1978].

HPLC UV detection [Sadrai *et al.* 2008]. Column: Chiracel OD-RH (150 × 4.6 mm i.d.). Mobile phase: phosphate buffer : acetonitrile (pH 2.0, 45 : 55). UV detection (λ = 312 nm). Limit of quantification, 3.0 µg/L for warfarin and 7-hydroxywarfarin enantiomers [Uno *et al.* 2007]. Column: Phenomenex C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L ammonium acetate buffer (pH 3.74, 67 : 33), flow rate 1.2 mL/min. UV detection (λ = 308 nm). Limit of quantification, 0.12 mg/L [Sun *et al.* 2006]. Column: Pirkle (R,R) Whelk-01 (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : acetonitrile : water (50 : 10 : 40) with 0.1% glacial acetic acid, flow rate 1 mL/min. UV detection (λ = 305 nm). Limit of detection, 16 µg/L for (*S*)-warfarin and 18 µg/L for (*R*)-warfarin [Osman *et al.* 2005]. Column: chiral AGP (100 × 3.0 mm i.d., 5 µm) or ODS Hypersil C_{18} (100 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 50 mmol/L phosphate buffer (pH 7.0, 15 : 85), flow rate 0.9 mL/min. UV detection (λ = 310 nm). Limit of detection, 25 µg/L for warfarin enantiomers and 4′-, 10-, 6-, and 7-hydroxywarfarin; 35 µg/L for 8-hydroxywarfarin; and 50 µg/L for racemic warfarin [Locatelli *et al.* 2005]. Column: reversed phase ovomucoid silica (150 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate (pH 4.7) : acetonitrile (90 : 10), flow rate 1.0 mL/min. Fluorometric detection (λ_{ex} = 313 nm, λ_{em} = 400 nm). Limit of quantification, 25 µg/L for warfarin enantiomers [Boppana *et al.* 2002]. See also Cai *et al.* [1994], Huang *et al.* [2001], Lombardi *et al.* [2003], Naidong, Lee [1993], Ring, Bostick [2000] and Takahashi *et al.* [1997].

LC-MS Column: Acquity UPLC BEH C_{18} (50 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.1% formic acid : acetonitrile (50 : 50). MRM acquisition mode. Limit of quantification, 0.25 µg/L free warfarin [Huang *et al.* 2008]. Column: reverse phase Luna C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 2% acetonitrile-25 mmol/L formic acid : 70% acetonitrile-25 mmol/L formic acid (45 : 55 for 5 min to 0 : 100 for 2 min to 45 : 55 for 4 min), flow rate 0.3 mL/min. ESI, SIM acquisition mode. Limit of quantification, ≤40 nmol/L for warfarin and phenprocoumon and

≤25 nmol/L for metabolites, limit of detection, ≤1 nmol/L [Ufer *et al.* 2004]. Column: Symmetry C_{18} (150 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 1 g/L formic acid (75 : 25), flow rate 0.5 mL/min. ESI, positive ion mode. Retention time: 2.53 min. Limit of detection, 1 µg/L [Kollroser, Schober 2002]. Column: β-cyclodextrin. Mobile phase: acetonitrile : acetic acid : triethylamine (1000 : 3 : 2.5). ESI. Limit of quantification, 1 µg/L [Naidong *et al.* 2001].

SFC-MS Column: Chiralpak AD (250 × 4.6 mm i.d.). Mobile phase: ethanol : CO_2 (30 : 70), flow rate 4.9 mL/min. APCI, MRM acquisition mode. Limit of quantification, 13.6 µg/L for (*R*)- and (*S*)-warfarin [Coe *et al.* 2006].

CE-MS Capillary: fused silica (1.2 m × 50 µm i.d.). Mobile phase: methanol : water (80 : 20) containing 5 mmol/L ammonium acetate (pH 6.8), flow rate 5 µL/min. ESI, SIM acquisition mode. Limit of detection, 0.1 mg/L for warfarin enantiomers [Hou *et al.* 2007].

Immunoassay See Fitzpatrick, O'Kennedy [2004].

Urine **GC-MS** Column: HP-1 capillary (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV, full scan mode. Limit of detection not reported [Maurer, Arlt 1998].

HPLC Column: cellulose derivative. UV and fluorescence detection. Limit of detection, 2.5 µg/L for for 7-hydroxywarfarin enantiomers and 4.5 µg/L for (*S*)-hydroxywarfarin [Takahashi *et al.* 1997].

LC-MS See Plasma. Limit of detection, ≤2.5 nmol/L [Ufer *et al.* 2004].

Disposition in the Body Warfarin is readily and almost completely absorbed after oral administration, with peak concentrations observed within the first 4 h. The (*R*)-enantiomer of warfarin is metabolised mainly by reduction to (*R,S*)-3′-hydroxywarfarin although 6-hydroxylation also occurs. The (*S*)-enantiomer is metabolised mainly by 6- and 7-hydroxylation with smaller amounts of the (*S*)-3′-hydroxy metabolite; the reduced metabolites are less active than warfarin; the 6- and 7-hydroxy metabolites are inactive. Other metabolites include warfarin sodium alcohols, including dehydrowarfarin sodium and 2 diastereoisomer alcohols, which have minimum activity. <1% of a dose is excreted in the urine as unchanged drug. Approximately 16–43% of a single dose is excreted in the urine as free or conjugated metabolites in 6 days. Excretion also occurs, to a lesser extent, through bile.

Therapeutic Concentration In plasma, usually in the range 1–3 mg/L but there are considerable inter-subject variations in sensitivity to warfarin and measurement of the plasma concentration is generally of little value since the pharmacological effect can be easily measured.

In 6 subjects receiving daily oral doses of 5–11 mg warfarin, steady-state plasma concentrations of 1.2–2.6 mg/L were attained [Orme *et al.* 1977].

Following a single oral dose of 50 mg warfarin to 8 subjects, peak plasma concentrations of 5.2–10.9 mg/L (mean, 7.2) were attained in 0.3–3 h (mean, 1.5) [Welle-Watne, Jahren. 1980].

Six men with ischemic stroke, aged between 61 and 69 years, participating in a warfarin–aspirin recurrent stroke study, were administered 4–6 mg warfarin daily for at least 1 week. The mean plasma concentration observed was 0.47 mg/L (range, 0.16–0.66) for (*S*)-warfarin and 0.69 mg/L (0.42–0.97) for (*R*)-warfarin [Cai *et al.* 1994].

Toxicity Toxic effects are associated with plasma concentrations >10 mg/L. The maximum permissible atmospheric concentration is 0.1 mg/m^3.

In a patient who took an overdose of warfarin (2000 mg by mouth plus 25×10^4 U administered SC), a plasma concentration of 111 mg/L was reported on the day of admission (the day after the overdose). Concentrations fell steadily in an approximate first-order fashion to 0.2 mg/L 10 days after the overdose [Hackett *et al.* 1985].

In a study involving 226 patients (mean age 62.7 years), the mean starting dose of warfarin was 3.4 mg daily (range, 1.3–10.0) and the mean maintenance dose was 3.1 mg daily (range, 1.2–7.7). Of these 226 patients, 55 survived a haemorrhage, with the most commonplace being the gastrointestinal tract. Three of the patients died as a result of their haemorrhage, 23 required medical treatment and 29 were uneventful. One female, who had breast cancer with multiple metastases, was being treated with warfarin for deep-vein thrombosis. She suffered a gastrointestinal tract haemorrhage 1 month after warfarin treatment had started and died. The second fatality was a male patient who was treated with warfarin for a period of 1.8 months for cerebrovascular disease. He died of an intracranial haemorrhage. The final fatality was also treated for cerebrovascular disease and was treated for 5 months before haemorrhaging from multiple sites [Chenhsu *et al.* 2000].

A 15-year-old boy who took an overdose of warfarin (50 tablets of 5 mg each and 100 tablets of 1 mg each) as well as allopurinol (14 tablets of 300 mg each) had an international normalised ratio (INR) on admission of 1.1. He was given 10 mg vitamin K injections on day 1 and 5. On day 3, his INR peaked at 5.0, at which point he was given the first of two infusions of fresh frozen plasma. His INR became normal after 5 days [Ramanan *et al.* 2002].

For a report of warfarin poisoning from ingestion of a rodenticide, see Matsuda *et al.* [2008].

Half-life Plasma half-life, 15–85 h (mean, 42), decreased in subjects with renal disease. The half-life of the (*R*)-enantiomer (mean, ~45 h; range, 37–89) appears to be longer than that of the (*S*)-enantiomer (mean,~30 h; range, 21–43).

Volume of Distribution 0.05–0.25 L/kg (mean, 0.15).

Clearance Plasma clearance, 0.02–0.08 mL/min/kg (mean, 0.04).

Distribution in Blood Plasma : whole blood ratio, ~1.8.

Protein Binding 97–99%, reduced in renal impairment.

Note For a review of warfarin metabolism and mode of action, see Park [1988].

Dose Maintenance, usually 3 to 9 mg warfarin sodium daily.

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Fitzpatrick B, O'Kennedy R (2004). The development and application of a surface plasmon resonance-based inhibition immunoassay for the determination of warfarin in plasma ultrafiltrate. *J Immunol Meth* 291: 11–25.

Hackett LP *et al.* (1985). Plasma warfarin concentrations after a massive overdose. *Med J Aust* 142: 642–643.

Hanna S *et al.* (1978). GLC determination of warfarin in human plasma. *J Pharm Sci* 67: 84–86.

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Huang C *et al.* (2008). Measurement of free concentrations of highly protein-bound warfarin in plasma by ultra performance liquid chromatography–tandem mass spectrometry and its correlation with the international normalized ratio. *Clin Chim Acta* 393: 85–89.

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Kollroser M, Schober C (2002). Determination of coumarin-type anticoagulants in human plasma by HPLC-electrospray ionization tandem mass spectrometry with an ion trap detector. *Clin Chem* 48: 84–91.

Locatelli I *et al.* (2005). Determination of warfarin enantiomers and hydroxylated metabolites in human blood plasma by liquid chromatography with achiral and chiral separation. *J Chromatogr B Analyt Technol Biomed Life Sci* 818: 191–198.

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Naidong W, Lee JW (1993). Development and validation of a high-performance liquid chromatographic method for the quantitation of warfarin enantiomers in human plasma. *J Pharm Biomed Anal* 11: 785–792.

Naidong W *et al.* (). Development and validation of a sensitive and robust LC-tandem MS method for the analysis of warfarin enantiomers in human plasma. *J Pharm Biomed Anal* 25: 219–226.

Orme ML *et al.* (1977). May mothers given warfarin breast-feed their infants? *Br Med J* 1: 1564–1565.

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Uno T *et al.* (2007). Simultaneous determination of warfarin enantiomers and its metabolite in human plasma by column-switching high-performance liquid chromatography with chiral separation. *Ther Drug Monit* 29: 333–339.

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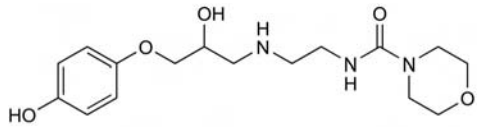
Xamoterol

Cardiotonic, β-Adrenoceptor Partial Agonist
$C_{16}H_{25}N_3O_5 = 339.4$
CAS—81801-12-9

IUPAC Name *N*-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl] morpholine-4-carboxamide



Chemical Properties Crystals. Mp 168° to 169°. Log *P* (octanol/water), −1.11.

Xamoterol Hemifumarate

$(C_{16}H_{25}N_3O_5)_2,C_4H_4O_4 = 794.8$
CAS—90730-93-1
Synonym ICI-118587
Proprietary Names *Corwil*; *Corwin*; *Xamtol*.

Thin-layer Chromatography System TB—R_f 0.00; system TE—R_f 0.15; system TF—R_f 0.00; system TAE—R_f 0.18.

Plate: silica gel Merck 60 F_{254}. Mobile phase: toluene : ethanol : ethyl acetate : ammonium hydroxide (6 : 4 : 2 : 1). R_f value: 0.10 (sulfate conjugate) [Marten *et al.* 1984].
High Performance Liquid Chromatography System HX—RI 239.
Ultraviolet Spectrum Aqueous acid—280 nm (xamoterol); 270 nm (sulfate metabolite); 275 nm (glucuronide metabolite); aqueous alkali—300 nm (xamoterol).
Quantification
Blood **HPLC** Column: Hypersil-ODS (150 × 4.1 mm i.d., 5 µm). Mobile phase: methanol : tetrahydrofuran (THF) : 30 mmol/L perchloric acid (8.0 : 0.6 : 91.4), flow rate 1.5 mL/min. Retention time: 8 min. Electrochemical detection. Limit of detection, 0.002 mg/L [Davis 1987]. Column: Lichrospher 60 RP Select B (125 × 4 mm i. d., 5 µm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate (55 : 45), flow rate 2 mL/min. IS: prenalterol. Fluorescence detection (λ_{ex}=190 nm; λ_{em}=320 to 400 nm). Retention time: xamoterol, 6.9 min; IS, 5.8 min. Limit of detection, 0.001 mg/L [Oddie *et al.* 1984].
Urine **HPLC** See Blood. Limit of detection, 0.01 mg/L [Oddie *et al.* 1984].
Disposition in the Body The drug is poorly absorbed from the gastrointestinal tract. A small amount is metabolised to inactive sulfate conjugates. Glucuronide metabolites have also been identified. Xamoterol is excreted mainly in urine as the unchanged drug and its metabolite(s). Most of the absorbed drug is eliminated within 24 h. Maximum plasma concentrations are reached within 1 to 2 h, although in the elderly the plasma concentrations are increased.
Therapeutic Concentration

The mean maximal plasma concentration after a 200 mg oral dose is 0.189 mg/L in young healthy individuals, and 0.134 mg/L in the elderly. In individuals with ischaemic heart disease, the mean maximum plasma concentrations were 0.128, 0.264 and 0.403 mg/L after 200, 400 and 600 mg doses, respectively [Furlong, Brogden 1988].

Six patients with liver disease (mostly alcohol induced), aged between 29 and 69 years (mean, 56.5 years) and 6 healthy individuals (34 to 68 years; mean, 56 years) were administered 0.2 mg/kg xamoterol IV over 5 min followed by a single oral dose of 200 mg. The peak plasma concentrations after the IV dose were 0.715 mg/L for the patients and 0.691 mg/L for the healthy individuals. For the oral dose, peak concentrations were 0.149 and 0.138 mg/L, respectively [Nicholls *et al.* 1989].

Bioavailability 5%.
Half-life 16 h.
Volume of Distribution 1.12 L/kg.
Clearance Total plasma clearance, 0.18 to 0.3 L/h/kg.
Protein Binding 3%.
Dose 200 to 400 mg daily.

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Furlong R, Brogden R (1988). Xamoterol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* 36(4): 455–474.
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Nicholls DP *et al.* (1989). The pharmacokinetics of xamoterol in liver disease. *Br J Clin Pharmacol* 28: 718–721.
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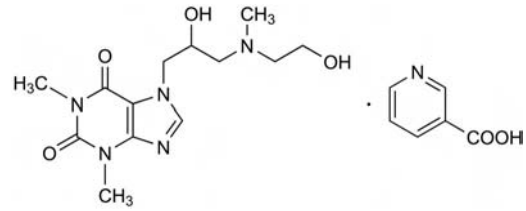
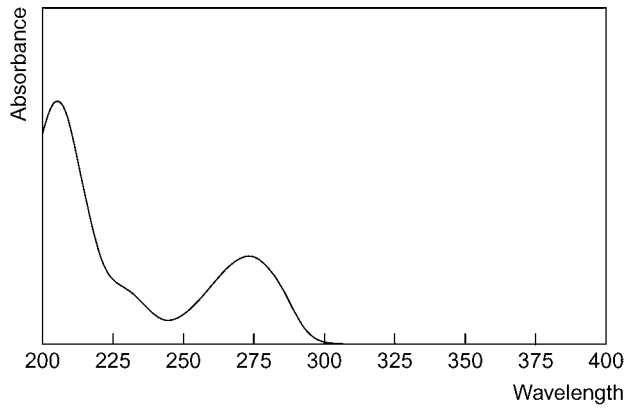
Xantinol Nicotinate

Vasodilator
$C_{13}H_{21}N_5O_4,C_6H_5NO_2 = 434.5$
CAS—2530-97-4 (xantinol); 437-74-1 (nicotinate)

IUPAC Name 7-[2-Hydroxy-3-[2-hydroxyethyl(methyl)amino]propyl]-1,3-dimethylpurine-2,6-dione; pyridine-3-carboxylic acid
Synonyms 3-Pyridinecarboxylic acid compound with 3,7-dihydro-7-[2-hydroxy-3-[(2-hydroxyethyl)methylamino]propyl]-1,3-dimethyl-1*H*-purine-2,6-dione (1 : 1); xanthinol nicotinate; xantinol niacinate
Proprietary Names *Complamin*; *Frigol*; *Vedrin*.



Chemical Properties Colourless crystals. Mp 180°. Freely soluble in water. Log *P* (octanol/water), −1.8.
Colour Tests Amalic acid test—orange/violet; cyanogen bromide—orange.
Thin-layer Chromatography System TA—R_f 0.41; system TE—R_f 0.21; system TF—R_f 0.00; system TAE—R_f 0.26 (acidified potassium permanganate solution, positive).
Gas Chromatography System GA—xantinol-AC_2 RI 2870.
High Performance Liquid Chromatography System HX—RI 215.
Ultraviolet Spectrum Aqueous acid—267 nm (A^1_1=300a). Xantinol: aqueous acid—273 nm (A^1_1=270b).

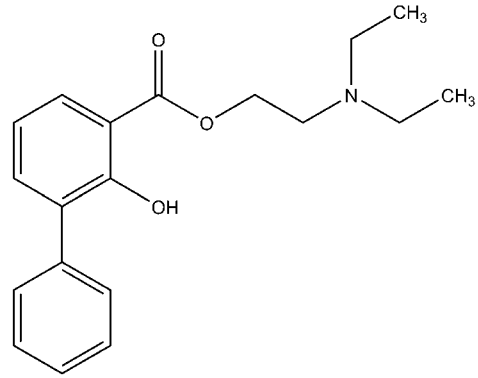


Infrared Spectrum Principal peaks at wavenumbers 1658, 1695, 1548, 763, 750, 1029 cm^{-1} (xantinol, KBr disk).
Dose 450 to 900 mg daily.

Xenysalate

Anaesthetic (Local), Antibacterial, Antifungal
$C_{19}H_{23}NO_3 = 313.4$
CAS—3572-52-9

IUPAC Name 2-Diethylaminoethyl 2-hydroxy-3-phenylbenzoate
Synonyms Biphenamine; 2-diethylaminoethyl 2-hydroxy-3-biphenylcarboxylate; 2-diethylaminoethyl 3-phenylsalicylat; 2-diethylaminoethyl 3-phenylsalicylate; 3-phenylsalicylic acid 2-diethylaminoethyl ester.



Chemical Properties Oily liquid. Soluble in water. Log *P* (octanol/water) 5.05 [Meylan, Howard 1995]; 5.2 [National Institutes of Health 2008].

Xenysalate Hydrochloride

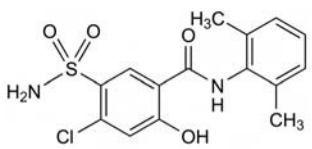
$C_{19}H_{23}NO_3$,HCl = 349.9
CAS—5560-62-3
IUPAC Name 2-Diethylaminoethyl 2-hydroxy-3-phenylbenzoate hydrochloride
Proprietary Names *Sebaclen*; *Sebaklen*.

Colour Tests Ammonium molybdate test—blue (limit of detection, 0.1 µg); ammonium vandate test—green (limit of detection, 0.1 µg); sulfuric acid–formaldehyde test—olive-green (limit of detection, 0.25 µg); Vitali's test— –/faint yellow/bright yellow (limit of detection, 0.1 µg).
Thin-layer Chromatography System T1—R_f 0.62 (location reagent acidified iodoplatinate spray, positive reaction).
Gas Chromatography System G2/225—retention time 1.20 relative to codeine.
Ultraviolet Spectrum 0.1 N sulfuric acid—231, 319 nm.
Dose Used in shampoos for the control of seborrhoeic dermatitis and dandruff [Diamond 1962; Lubowe 1963].

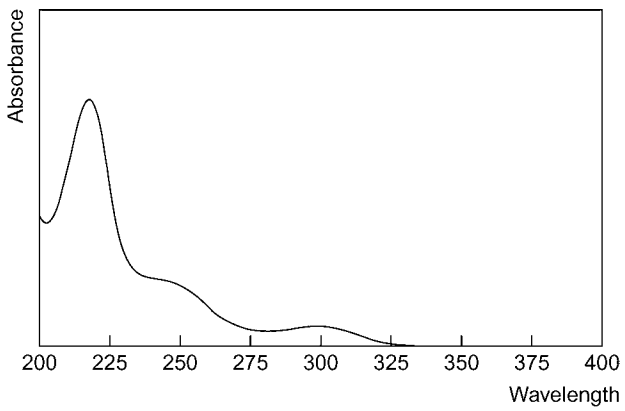
Diamond S (1962). Biphenamine HC1 in seborrheic scalp disorders. *West Med Med J West* 3: 215–216.
Lubowe II (1963). Treatment of seborrhea capitis with biphenamine hydrochloride. *Clin Med (Northfield.Il)* 70: 71–75.
Meylan WM, Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J Pharm Sci* 84: 83–92.
National Institutes of Health (2008). *Biphenamine*. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=21720&loc=ec_rcs. (accessed 19 June 2008).

Xipamide

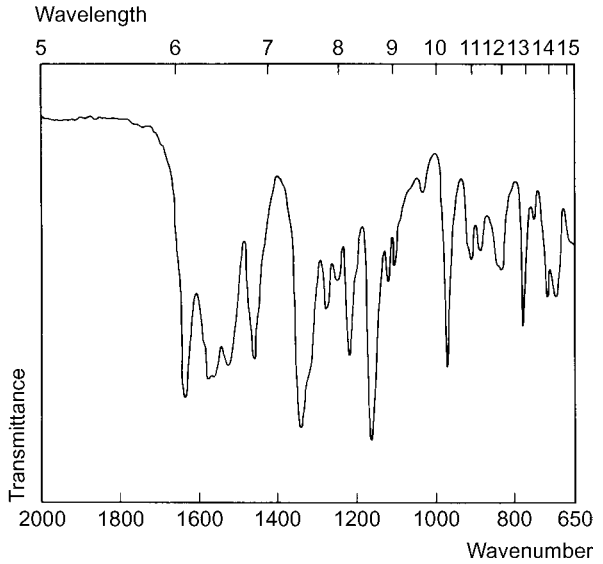
Diuretic
$C_{15}H_{15}ClN_2O_4S$ = 354.8
CAS—14293-44-8
IUPAC Name 4-Chloro-*N*-(2,6-dimethylphenyl)-2-hydroxy-5-sulfamoylbenzamide
Synonym 4-Chloro-5-sulfamoyl-2′,6′-salicyloxylidide
Proprietary Names *Aquaphor*; *Aquaphoril*; *Chronexan*; *Demiax*; *Diurexan*; *Lumitens*.



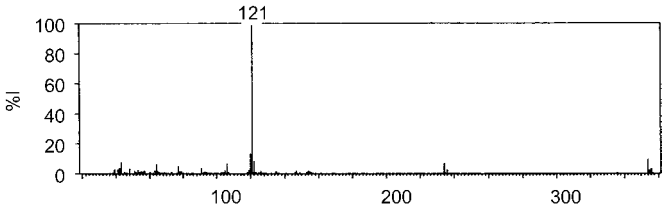
Chemical Properties A white crystalline substance. Mp about 260°, with decomposition. Practically insoluble in water; soluble in ethanol; very soluble in acetone; slightly soluble in chloroform and ether. pK_a 4.8 (phenol), 10.0 (sulfonamide). Log *P* (octanol/water), 2.2.
Colour Tests Koppanyi–Zwikker test—violet; Liebermann's reagent—orange; Mandelin's test—blue; mercurous nitrate—black
Thin-layer Chromatography System TB—R_f 0.00; system TD—R_f 0.38; system TE—R_f 0.13; system TF—R_f 0.64; system TAD—R_f 0.36; system TAE—R_f 0.93.
Gas Chromatography System GA—xipamide-Me_2 RI 3350, xipamide-Me_4 RI 2780, M (OH-)-Me_4 RI 3000, xipamide isomer-1Me_3 RI 2800, xipamide isomer-2Me_3 RI 3320, art (-SO_2NH) RI 2385, art (-SO_2NH)-Me RI 2480, art -(SO_2NH)-Me_2 RI 2115.
High Performance Liquid Chromatography System HX—RI 488; system HAA—retention time 18.8 min.
Ultraviolet Spectrum Aqueous acid—296 nm (A^1_1=92b); aqueous alkali—330 nm (A^1_1=160b).



Infrared Spectrum Principal peaks at wavenumbers 1163, 1635, 1578, 1560, 975, 1525 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 121, 120, 354, 122, 43, 234, 106, 64.

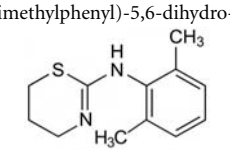


Quantification
Plasma **TLC–spectrofluorimetry** Limit of detection, <10 µg/L [Sobel, Mutschler 1980].
Urine **HPLC** UV detection. For method, see Rosado-Maria *et al.* [2000]. UV detection. For method, see Diembeck *et al.* [1982].
TLC–spectrofluorimetry For method, see Sobel and Mutschler [1980].
Disposition in the Body Completely and fairly rapidly absorbed after oral administration. About 88% of a dose is excreted in the urine in 48 h with about 50% as unchanged drug and 30% as xipamide-*O*-glucuronide; free amine metabolites have also been detected. In normal subjects, only traces are excreted in the bile, but biliary excretion may be extensive in renal failure.
Half-life Plasma half-life, about 5 to 8 h.
Protein Binding About 99%.
Dose Usually 20 to 40 mg daily; doses of 80 mg daily have been given.

Diembeck W *et al.* (1982). [Pharmacokinetics of xipamide and triamterene in healthy probands]. *Arzneimittelforschung* 32: 1482–1485.
Rosado-Maria A *et al.* (2000). High-performance liquid chromatographic separation of a complex mixture of diuretics using a micellar mobile phase of sodium dodecyl sulphate. Application to human urine samples. *J Chromatogr B Biomed Sci Appl* 748: 415–424.
Sobel M, Mutschler E (1980). Fluorimetric determination of xipamide in biological materials using a new fluorescence reagent. *J Chromatogr* 183: 124–130.

Xylazine

α_2-Adrenoceptor Agonist, Analgesic (Veterinary), Sedative (Veterinary)
$C_{12}H_{16}N_2S$ = 220.3
CAS—7361-61-7
IUPAC Name *N*-(2,6-Dimethylphenyl)-5,6-dihydro-4*H*-1,3-thiazin-2-amine



Chemical Properties Colourless crystals. Mp 140° to 142°; also reported as 136° to 139°. Practically insoluble in water and alkalis; soluble in dilute acids, benzene, acetone and chloroform; sparingly soluble in petroleum ether. Log *P* (octanol/water), 4.52.

Xylazine Hydrochloride

$C_{12}H_{16}N_2S$,HCl = 256.8
CAS—23076-35-9
Synonym Bay-Va-1470
Proprietary Names *Narcoxyl*; *Rompun*; *Solvazine*; *Xylapan*; *Xylasol*.

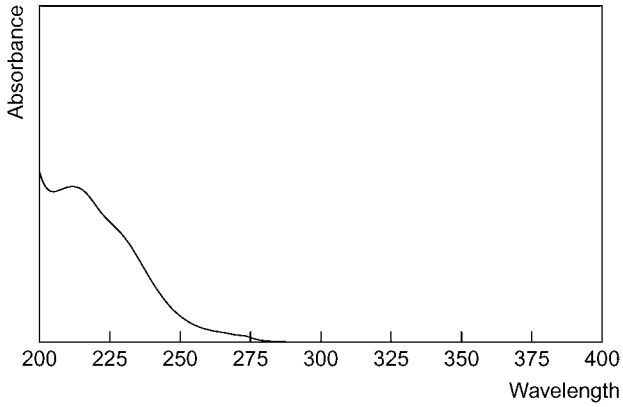
Chemical Properties A white powder. Mp 164° to 167°. Readily soluble in water, ethanol and methanol; very slightly soluble in chloroform and ether.

Colour Tests Liebermann's reagent—red; Mandelin's test—blue.

Thin-layer Chromatography System TA—R_f 0.60; system TE—R_f 0.78; system TAJ—R_f 0.08; system TAK—R_f 0.06; system TAL—R_f 0.70 (acidified iodoplatinate solution—positive).

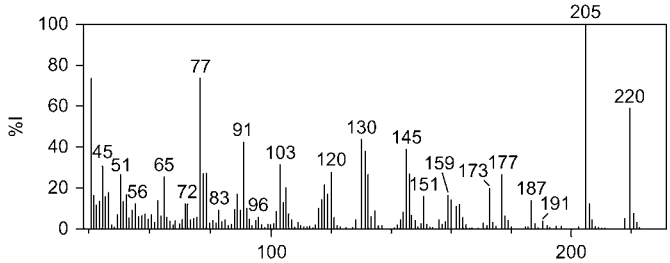
Gas Chromatography System GV—RT 11.6 min.

Ultraviolet Spectrum



Infrared Spectrum Principal peaks at wavenumbers 1613, 1585, 1164, 1314, 1085, 1099 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m/z* 205, 77, 41, 39, 220, 130, 145, 131.



Quantification

Blood **GC-MS** Column: 5% phenylmethylsiloxane (12 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1 mL/min. Temperature programme: 120° for 1 min to 270° at 20°/min for 7 min. EI ionisation at 70 eV, SIM acquisition mode. Retention time: 5.91 min. Limit of quantification, 10 µg/L, limit of detection, 2 µg/L [Barroso *et al.* 2007]. Column: 3% OV-17 on 100/120 mesh Gas Chrom Q (1.8 m × 2 mm i.d.). Carrier gas: He, 20 mL/min. Temperature programme: 180° for 2 min to 230° at 20°/min. EI ionisation at 70 eV. Limit of detection, 100 µg/L [Gallanosa *et al.* 1981].

Plasma **HPLC** UV detection. Limit of quantification, 25 µg/L, limit of detection, 5 µg/L [Hoffmann *et al.* 2001].

Urine **GC-MS** Limit of detection, 20 µg/L [Olmos-Carmona, Hernandez-Carrasquilla 1999]. See Blood [Gallanosa *et al.* 1981].

Disposition in Body Xylazine is metabolised in the liver to ~20 metabolites. A major metabolite is 2,6-dimethylaniline. Both the unchanged drug and metabolites have been detected in urine.

Toxicity

A fatal case of multiple drug abuse in a 36-year-old veterinarian is reported involving injection of xylazine and ingestion of alcohol and clorazepate. Xylazine levels were 0.2 mg/L in blood, 0.4 mg/kg in brain, 0.6 mg/kg in kidney, 0.9 mg/kg in liver, 1.1 mg/kg in lung, 0.05 mg/kg in omentum adipose and 7.0 mg/L in urine [Poklis *et al.* 1985].

A 59-year-old woman who was a known former alcoholic was found dead at her home. Various empty containers of a number of drugs were found in her bedroom. After toxicological analysis, xylazine was found in her urine at a concentration of 30 mg/L, in her blood at 16 mg/L and there was a large amount in her stomach contents. Traces of lorazepam could also be detected and ethanol was found in her blood at 0.78 mg/g [Briellmann *et al.* (1994).

A 27-year-old farmer attempted to commit suicide by injecting himself IM with 1.5 g xylazine (~75 mL of a 2% xylazine solution). As a result, he became comatose with no light/pain response; he was hypotensive, bradycardic and mildly glycemic and suffered from respiratory depression. He was admitted to hospital 2 h after ingestion and was treated in the intensive care unit with artificial respiration and IV fluid therapy. The highest concentration of xylazine in plasma was 4.6 mg/L; xylazine was 446 mg/L in gastric fluid and 194 mg/L in urine. The level of drug decreased over 12 h and he regained consciousness after 20 h. He was discharged from hospital 4 days after admission [Hoffmann *et al.* 2001].

The 16-year-old son of a horse breeder developed signs consistent with α-adrenergic agonist toxicity 2 h after an overdose of xylazine (the xylazine solution had been dried on a CD case, scraped up into a bag and then snorted). The patient recovered after appropriate supportive measures. A sample of blood taken ~2 h after inhalation contained 0.54 mg/L xylazine [Capraro *et al.* 2001].

In a suicide by hanging, xylazine was the sole drug detected in tissues; the concentrations were heart blood 2.3 mg/L, peripheral (subclavian) blood 2.9 mg/L, bile 6.3 mg/L, urine 0.01 mg/L, liver 6.1 µg/g and kidney 7.8 µg/g [Moore *et al.* 2003].

Xylazine levels in blood of 0.57 mg/L were reported in a case of suspected impaired driving involving self-administration of xylazine (*Xyla-Ject*) and paroxetine [Stillwell 2003]. Fatal toxicity in two homicide victims is reported with xylazine up to 1800 mg being used to cause death [Mittleman *et al.* 1998].

Seven cases of drug-related deaths in which illicit drugs (such as heroin and cocaine) were adulterated with xylazine have been described. Xylazine was detected in urine in all 7 cases and blood in 3 cases (levels ranged from trace–130 µg/L) [Wong *et al.* 2008].

For a further report of toxicity from inhaled xylazine, see Elejalde *et al.* [2003]; for a literature review of xylazine toxicity, see [Mittleman *et al.* 1998]; for a further report of xylazine overdose, see Gallanosa *et al.* [1981].

Barroso M *et al.* (2007). Solid-phase extraction and gas chromatographic- mass spectrometric determination of the veterinary drug xylazine in human blood. *J Anal Toxicol* 31: 165–169.

Briellmann, TA *et al.* (1994) A fatal intoxication with xylazine *TIAFT Bull Case Notes* 24(1) (http://www.tiaft.org/tmembers/cnrarchive/24_1_1.php (last accessed 07 December 2010).

Capraro AJ *et al.* (2001). Severe intoxication from xylazine inhalation. *Pediatr Emerg Care* 17: 447–448.

Elejalde JI *et al.* (2003). Drug abuse with inhalated xylazine. *Eur J Emerg Med* 10: 252–253.

Gallanosa AG *et al.* (1981). Human xylazine overdose: a comparative review with clonidine, phenothiazines, and tricyclic antidepressants. *Clin Toxicol* 18: 663–678.

Hoffmann U *et al.* (2001). Severe intoxication with the veterinary tranquilizer xylazine in humans. *J Anal Toxicol* 25: 245–249.

Mittleman RE *et al.* (1998). Xylazine toxicity: literature review and report of two cases. *J Forensic Sci* 43: 400–402.

Moore KA *et al.* (2003). Tissue distribution of xylazine in a suicide by hanging. *J Anal Toxicol* 27: 110–112.

Olmos-Carmona ML, Hernandez-Carrasquilla M (1999). Gas chromatographic-mass spectrometric analysis of veterinary tranquillizers in urine: evaluation of method performance. *J Chromatogr B Biomed Sci Appl* 734: 113–120.

Poklis A *et al.* (1985). Xylazine in human tissue and fluids in a case of fatal drug abuse. *J Anal Toxicol* 9: 234–236.

Stillwell ME (2003). A reported case involving impaired driving following self-administration of xylazine. *Forensic Sci Int* 134: 25–28.

Wong SC *et al.* (2008). Concurrent detection of heroin, fentanyl, and xylazine in seven drug-related deaths reported from the Philadelphia Medical Examiner's Office. *J Forensic Sci* 53: 495–498.

Xylene

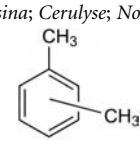
Solvent

C_8H_{10} = 106.2

CAS—1330-20-7; 108-38-3 (*m*-Xylene); 95-47-6 (*o*-Xylene); 106-42-3 (*p*-Xylene)

Synonym Xylol(e)

Proprietary Names *Cerulisina*; *Cerulyse*; *Novo-Cerusol*.

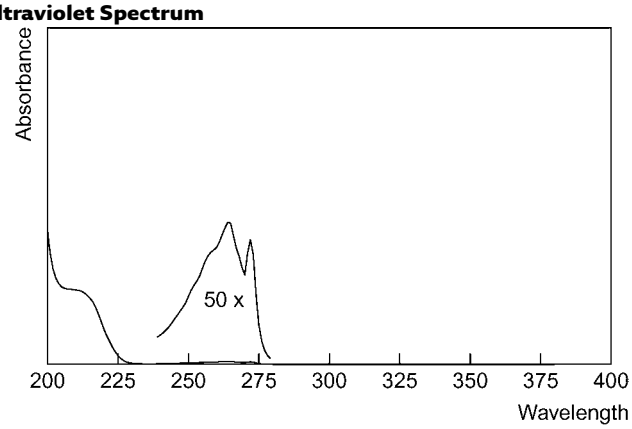


Chemical Properties A mixture of *o*-, *m*- and *p*-dimethylbenzene in which the *m*-isomer predominates. A colourless, flammable liquid. Mass per mL about 0.86 g. Bp 137° to 140°. Practically insoluble in water; miscible with absolute ethanol, ether and other organic solvents. Log *P* (octanol/water), 3.2.

Gas Chromatography System GA—*o*-xylene RI 884, *m*-xylene RI 863, *p*-xylene RI 860; system GI—*o*-xylene retention time 34.5 min, *m*-xylene retention time 33.2, *p*-xylene retention time 34.2 min.

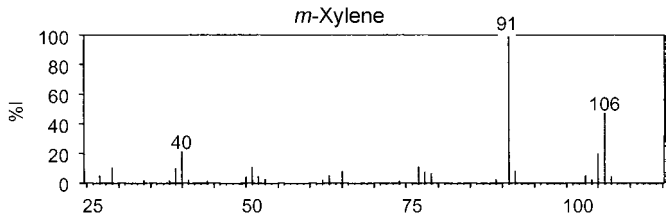
High Performance Liquid Chromatography System HAA—retention time 24.0 min.

Ultraviolet Spectrum



X

Mass Spectrum Principal ions at *m/z* 91, 106, 105, 77, 51, 39, 29, 92 (*m*-xylene).



Quantification

Blood **GC** Limit of detection, about 20 µg/L for *o*-, *m*- and *p*-xylene [Hattori *et al.* 1998]. ECD. For a method for the quantification of musk xylene (a synthetic musk), see Angerer and Kafferlein [1997]. Ion-trap detection. For a method for the quantification of xylene isomers and other volatile organic compounds, see Schuberth [1996].

GC and GC–MS For a method for the quantification of xylenes and other volatile compounds, using headspace analysis, see Bellanca *et al.* [1982].

Urine **GC** PID, ECD, FID. Limit of detection, 26 to 67 ng/L for xylene and other volatile compounds [Schroers *et al.* 1998]. Limits of detection, 0.1 to 0.2 mg/L for xylenol isomers and other phenolics [Bieniek 1996]. AFID. Limit of detection, 1.0 mg/L for *m*- and *p*-methylhippuric acids [Morin *et al.* 1981].

GC and GC–MS Limit of detection, 12 to 34 ng/L for xylenes, benzene, toluene and ethylbenzene, using headspace analysis [Fustinoni *et al.* 1999].

HPLC For a method of quantification for *o*-, *m*- and *p*-methylhippuric acids and other aromatic hydrocarbon metabolites, see Burrini [1998]. For a method of quantification for xylene isomers and metabolites and other organic solvents, see Moon *et al.* [1997]. For a method for the quantification of *o*-, *m*- and *p*-xylene metabolites, see Ogata and Taguchi [1987]; Ogata and Taguchi [1988]; Astier [1992].

Body Fluids **GC** For a method for the quantification of xylene isomers and other volatile organic compounds, see Watanabe-Suzuki *et al.* [2002].

Postmortem Tissue **GC** See Blood [Schuberth 1996].

Tissues **GC and GC–MS** See Blood [Bellanca *et al.* 1982].

Disposition in the Body The three isomers of xylene are absorbed from the gastrointestinal tract, through the skin and from the lungs. The main metabolic reactions are oxidation of a methyl group to give the corresponding *o*-, *m*- or *p*-toluic acid and conjugation with glycine to *o*-, *m*- or *p*-methylhippuric acid; about 70% of the absorbed material is excreted as these conjugates in the urine in 18 h. Hydroxylation of the xylenes to the corresponding xylenols also occurs and these may be excreted in the urine as conjugates. About 5% of a dose is excreted unchanged in expired air and <0.01% in the urine.

Blood Concentration

Exposure to a concentration of 100 ppm of *m*-xylene, for 6 h, produced a mean peak blood-xylene concentration of about 1.0 mg/L in 4 subjects at rest; when the concentration was increased to 200 ppm for 3 h, the mean peak blood concentration was about 2.1 mg/L [Savolainen *et al.* 1979].

In a paint factory, average ambient-air concentrations of xylene were 5 to 58 ppm (average, 29) during production and 3 to 21 ppm (average, 8) during spraying; the blood concentrations of xylenes in 10 production workers were 63 to 715 µg/L (average, 380) and 49 to 308 µg/L (average, 130) in 10 spray workers [Kramer *et al.* 1999].

Toxicity Xylene has about the same acute toxicity as benzene or toluene. The maximum permissible atmospheric concentration is 100 ppm. Exposure to air concentrations of 10 000 ppm has been reported to cause unconsciousness or death.

Two men, aged 51 and 55 years, died in two separate incidents after ingestion of petrol which contained xylenes. The following postmortem concentrations of xylene in body tissues were reported: blood 20 and 3.0 mg/L, and liver 1.0 µg/g for both subjects. A woman of 55, found dead, had the following postmortem concentrations of xylene in body tissues: blood 40 mg/L, liver 1.0 µg/g [Bonnichsen *et al.* 1966].

A 27-year-old man committed suicide by ingesting a large quantity of xylene. A high level was detected in his blood (0.11 g/L), gastric contents (8.8 g/L), and duodenal contents (33 g/L) [Abu Al Ragheb *et al.* 1986].

A 20-year-old man who attempted suicide by IV injecting 8 mL of xylene (0.1 mL/kg) developed acute pulmonary failure after 10 min and eventually recovered following haemoperfusion and haemodialysis [Sevcik *et al.* 1992].

Half-life Blood half-life, about 20 to 30 h.

Abu Al Ragheb S *et al.* (1986). Suicide by xylene ingestion. A case report and review of literature. *Am J Forensic Med Pathol* 7: 327–329.

Angerer J, Kafferlein HU (1997). Gas chromatographic method using electron-capture detection for the determination of musk xylene in human blood samples. Biological monitoring of the general population. *J Chromatogr B Biomed Sci Appl* 693: 71–78.

Astier A (1992). Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene. *J Chromatogr* 573: 318–322.

Bellanca JA *et al.* (1982). Detection and quantitation of multiple volatile compounds in tissues by GC and GC/MS. *J Anal Toxicol* 6: 238–240.

Bieniek G (1996). Simultaneous determination of phenol, cresol, xylenol isomers and naphthols in urine by capillary gas chromatography. *J Chromatogr B Biomed Appl* 682: 167–172.

Bonnichsen R *et al.* (1966). Poisoning by volatile compounds. I. Aromatic hydrocarbons. *J Forensic Sci* 11: 186–204.

Burrini C (1998). [The simultaneous determination of hippuric acid, o-, m-, p-methylhippuric acids, mandelic acid and phenylglyoxylic acid in urine by HPLC]. *Med Lav* 89: 404–411.

Fustinoni S *et al.* (1999). Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. *J Chromatogr B Biomed Sci Appl* 723: 105–115.

Hattori H *et al.* (1998). Sensitive determination of xylenes in whole blood by capillary gas chromatography with cryogenic trapping. *J Chromatogr B Biomed Sci Appl* 718: 285–289.

Kramer A *et al.* (1999). Occupational chronic exposure to organic solvents XVII. Ambient and biological monitoring of workers exposed to xylenes. *Int Arch Occup Environ Health* 72: 52–55.

Moon DH *et al.* (1997). Analysis of some metabolites of organic solvents in urine by high-performance liquid chromatography with beta-cyclodextrin. *J Chromatogr B Biomed Sci Appl* 694: 367–374.

Morin M *et al.* (1981). Measurement of exposure to xylenes by separate determination of m- and p-methylhippuric acids in urine. *J Chromatogr* 210: 346–349.

Ogata M, Taguchi T (1987). Quantitation of urinary metabolites of toluene, xylene, styrene, ethylbenzene, benzene and phenol by automated high performance liquid chromatography. *Int Arch Occup Environ Health* 59: 263–272.

Ogata M, Taguchi T (1988). Simultaneous determination of urinary creatinine and metabolites of toluene, xylene, styrene, ethylbenzene and phenol by automated high performance liquid chromatography. *Int Arch Occup Environ Health* 61: 131–140.

Savolainen K *et al.* (1979). Effects of short-term xylene exposure on psychophysiological functions in man. *Int Arch Occup Environ Health* 44: 201–211.

Schroers HJ *et al.* (1998). Determination of physiological levels of volatile organic compounds in blood using static headspace capillary gas chromatography with serial triple detection. *Analyst* 123: 715–720.

Schuberth J (1996). A full evaporation headspace technique with capillary GC and ITD: a means for quantitating volatile organic compounds in biological samples. *J Chromatogr Sci* 34: 314–319.

Sevcik P *et al.* (1992). Intravenous xylene poisoning. *Intensive Care Med* 18: 377–378.

Watanabe-Suzuki K *et al.* (2002). Cryogenic oven-trapping gas chromatography for analysis of volatile organic compounds in body fluids. *Anal Bioanal Chem* 373: 75–80.

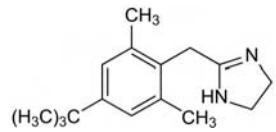
Xylometazoline

Sympathomimetic

$C_{16}H_{24}N_2 = 244.4$

CAS—526-36-3

IUPAC Name 2-[(4-Tert-butyl-2,6-dimethylphenyl)methyl]-4,5-dihydro-1*H*-imidazole



Chemical Properties Mp 131° to 133°. Log *P* (chloroform/water), 5.4.

Xylometazoline Hydrochloride

$C_{16}H_{24}N_2,HCl = 280.8$

CAS—1218-35-5

Proprietary Names *Amidrin*; *Balkis*; *Dorenasin*; *Gelonasal*; *Nasolin*; *Olynth*; *Otalgicin*; *Otradrops*; *Otraspray*; *Otrivin(e)*; *Otrivina*; *Otrix*; *Passagen*; *Rhinidine*; *Rinosedin*; *Xylo*; *Xylolin*; *Xyloma*; *Xylovit*; *Zymelin*.

Chemical Properties A white crystalline powder. Mp 300°, with decomposition. Soluble 1 in 33 of water; soluble in ethanol and methanol; practically insoluble in ether or benzene.

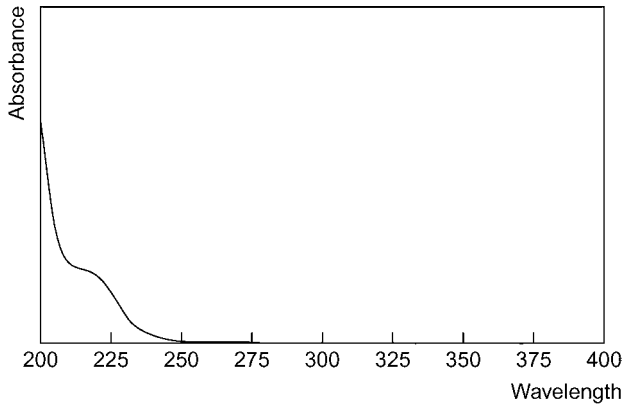
Colour Tests Mandelin's test—red; Marquis test—orange.

Thin-layer Chromatography System TA—R_f 0.13; system TB—R_f 0.07; system TC—R_f 0.05; system TE—R_f 0.30; system TL—R_f 0.03; system TAE—R_f 0.05; system TAF—R_f 0.64. (acidified iodoplatinate solution, positive).

Gas Chromatography System GA—xylometazoline RI 2020, xylometazoline-AC RI 2260.

High Performance Liquid Chromatography System HA—*k* 1.6; system HX—RI 413.

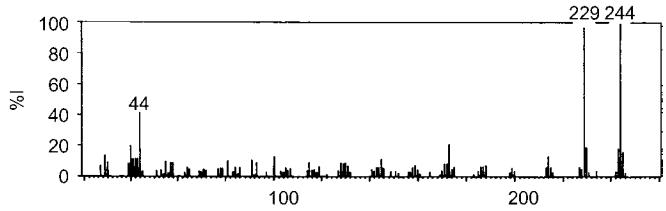
Ultraviolet Spectrum Aqueous acid—265 nm (A_1^1=11.5a).



Infrared Spectrum Principal peaks at wavenumbers 1602, 1253, 1298, 871, 1237, 800 cm^{-1} (xylometazoline hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 244, 229, 44, 173, 40, 230, 243, 245.

X



Quantification

Plasma **GC** ECD. For method, see Sioufi *et al.* [1989].

Urine **GC** See Plasma [Sioufi *et al.* 1989].

Disposition in the Body

Toxicity

The following postmortem concentrations were reported in a 23-year-old man who died after the IV self-administration of xylometazoline: blood 0.14 mg/L, liver blood 0.37 mg/L [Vanezis, Toseland 1980].

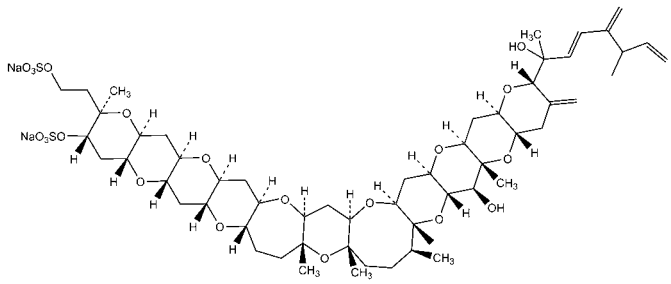
Use In concentrations of 0.05 to 0.1% of the hydrochloride, as nasal drops or spray.

Sioufi A *et al.* (1989). Determination of xylometazoline in plasma and urine by gas chromatography using a fused-silica capillary column and an electron-capture detector. *J Chromatogr* 487: 81–89.

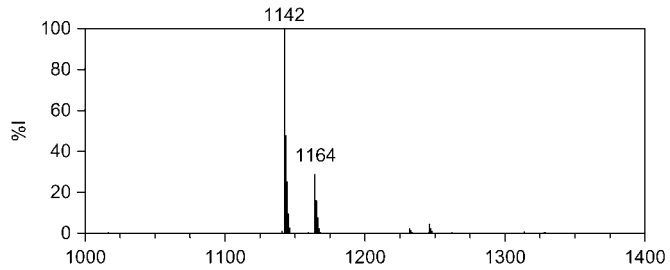
Vanezis P, Toseland PA (1980). Xylometazoline poisoning–report of a case. *Med., Sci Law* 20: 35–36.

Yessotoxin

Cardiotoxin, Sulfated Polyether
$C_{55}H_{78}O_{21}S_2Na_2 = 1185.3$
CAS—112514-54-2
Synonym YTX



Chemical Properties Brevetoxin-type toxin produced by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum*. It was first isolated in 1986 from the scallop *Patinopecten yessoensis* [Draisci *et al.* 1996; Van Egmond *et al.* 1993]. Almost 40 yessotoxin (YTX) analogues have since been detected and characterised in different shellfish and dinoflagellate samples. The major analogues are homo-YTX, 45-hydroxyhomo-YTX, and their carboxy derivatives [Paz *et al.* 2004].
Mass Spectrum Principal ions at *m/z* 1142, 1164.



Quantification

Other **HPLC** Dinoflagellate Cultures (*P. reticulatum*). Column: Lichrospher C_{18} (125 × 4.6 mm i.d., 5 µm). Mobile phase: 100 mmol/L ammonium acetate (pH 5.8) : methanol (3 : 7), flow rate 0.75 mL/min. Fluorescence detection (λ_{ex} = 370 nm, λ_{em} = 440 nm). Retention time: 2 peaks ≈17 min (4-(2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalimylethyl)-1,2,4-triazoline-3,5-dione), DMEQ-TAD derivative). Limit of quantification not reported [Paz *et al.* 2004, 2006; Satake *et al.* 1999; Yasumoto, Takizawa 1997]. Mussel Samples. Column: Cosmosil C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: 40 mmol/L phosphate buffer (pH 5.8) : methanol (3 : 7), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 370 nm, λ_{em} = 440 nm). Retention time: YTX two peaks ≈15 min, 1-desulfo-YTX two peaks ≈30 min (both DMEQ-TAD derivatives). Limit of quantification not reported [Daiguji *et al.* 1998].

LC-MS Mussel Hepatopancreas Samples (*Mytilus galloprovincialis*). Column: Hypersil C_8 (50 × 2.0 mm i.d., 3 µm). Mobile phase: water : acetonitrile-water (95 : 5) both containing 5 mmol/L ammonium acetate (pH 7; 80 : 20 to 0 : 100 over 10 min for 15 min), flow rate 0.2 mL/min. Turbospray, negative ion mode, SIM acquisition mode. Retention times: YTX 9 min, 45-hydroxy-YTX 8 min, carboxy-YTX 7 min. Limit of quantification not reported [Morton *et al.* 2007]. Dinoflagellate Cultures (*P. reticulatum*). Column: Hypersil C_8 (50 × 2.1 mm i.d., 3 µm). Mobile phase: water : acetonitrile-water (95 : 5)-2 mmol/L ammonium formate-50 mmol/L formic acid (80 : 20 to 0 : 100 over 10 min for 15 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention times: YTX 10.1 min, noroxo-YTX-enone 8.2 min, 45,46,47-trinor-YTX 9.5 min, 45,46,47-trino-1-homo-YTX 9.5 min, 1-homo-YTX 10.1 min. Limit of quantification not reported [Suzuki *et al.* 2007]. Column: Lichrospher C_{18} (125 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 5.8) : methanol (3 : 7), flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Retention time: 9.8 min. Limit of quantification not reported [Paz *et al.* 2004, 2006]. Column: C_{18} (150 mm × 75 µm, 3 µm). Mobile phase: acetonitrile : water (90 : 10)-1 mmol/L ammonium acetate, flow rate 400 nL/min. NanoESI, negative ion mode, MRM acquisition mode. Retention time: 14 min. Limit of quantification, 0.75 µg/L [Ruppen Canas *et al.* 2004]. Mussel Tissue Samples. Column: Hypersil C_8 (50 × 2.0 mm i.d., 3 µm). Mobile phase: acetonitrile : water-3.5 mmol/L ammonium formate-50 mmol/L formic acid (9.5 : 90.5 to 95 : 5 over 10 min for 15 min), flow rate 0.2 mL/min. ESI, negative ion mode, MRM acquisition mode. Retention times: YTX 8.98 min, homo-YTX 9.01 min, 45-hydroxy-YTX 8.73 min, 45-hydroxy-homo-YTX 8.76 min, carboxy-YTX 8.18 min, carboxyhomo-YTX 8.21 min, 42,43,44,45,46,47,55-heptanor-41-oxohomo-YTX 8.04 min. Limit of detection, 7 µg/kg [Ciminiello *et al.* 2002]. Column: amide C_{16} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water (60 : 40) containing 0.5 mmol/L ammonium acetate, flow rate 0.5 mL/min. ESI, negative ion mode, MRM acquisition mode. Limit of detection, 3 µg/kg [Fernandez Amandi *et al.* 2002]. Column: C_{18} (300 × 1.0 mm i.d., 5 µm). Mobile phase: acetonitrile : water (80 : 20) containing 2 mmol/L ammonium acetate, flow rate 0.04 mL/min. ESI, negative ion mode, SIM acquisition mode. Retention time: 4.3 min (other diarrhetic shellfish poisoning [DSP] also detected but resolved under different conditions). Limit of quantification not reported [Draisci *et al.* 1999].

CE Algae and Shellfish Samples. Column: uncoated bare fused silica capillary (total/effective length: 48.5/40.0 cm, 50 µm i.d.). Running buffer: CZE, 50 mmol/L disodium hydrogen phosphate, 40% methanol (pH 8.5); MEKC, 10 mmol/L disodium hydrogen phosphate, 35 mmol/L SDS, 20% methanol (pH 8.5). UV detection (λ = 230 nm). Retention time: CZE, 11 min; MEKC, 13.5 min. Limit of quantification, CZE 0.9 mg/L, MEKC 0.5 mg/L; limit of detection, CZE 0.3 mg/L, MEKC 0.2 mg/L [de la Iglesia *et al.* 2007].

CE-MS Algae and Shellfish Samples. Column: uncoated bare fused-silica capillary (total/effective length: 80.0/21.6 cm, 50 µm i.d.). Running buffer: 10 mmol/L ammonium acetate (pH 8.5). ESI, negative ion mode, SIM. Limit of quantification, 0.08 mg/L; limit of detection, 0.02 mg/L [de la Iglesia *et al.* 2007].

Note For the complete NMR characterisation of YTX analogues in *P. reticulatum* cultures, see Miles *et al.* [2004a, b, 2006]. For the characterisation of further analogues in mussel samples, see Ciminiello *et al.* [1997, 1999, 2000, 2001, 2003, 2007], MacKenzie *et al.* [2002], Miles *et al.* [2005], Satake *et al.* [1997], Souto *et al.* [2005]. For a large-scale purification method of YTX from *P. reticulatum* cultures and the characterisation of a novel furano-YTX, see Loader *et al.* [2007]. For extraction and cleaning methods to detect YTXs in mussels, see Alfonso *et al.* [2007]. For ELISA methods for the detection of YTX and its analogues, see Briggs *et al.* [2004]; Garthwaite *et al.* [2001] and Samdal *et al.* [2005].

Distribution in the Body

Toxicity The YTXs were formerly included in the causative toxins associated with DSP because they were found in shellfish relating to DSP together with okadaic acid and dinophysistoxins. However, YTX and its analogues do not actually induce diarrhoea, but cardiotoxic effects have been demonstrated in mice as well as neurotoxicity on cultured cerebellar neurons. The YTXs are no longer included in the DSP group [de la Iglesia *et al.* 2007; Perez-Gomez *et al.* 2006; Ruppen Canas *et al.* 2004]. At the time of writing, there are no reports of human intoxication caused by YTXs [Satake *et al.* 1999; Toyofuku 2006]. For toxicity studies of YTX and its analogues in mice, see Aune *et al.* [2002], Tubaro *et al.* [2003, 2004]. LD_{50} in mice (IP): 80 to 100 µg/kg [Ogino *et al.* 1997].

Alfonso C *et al.* (2007). Extraction and cleaning methods to detect yessotoxins in contaminated mussels. *Anal Biochem* 363: 228–238.
Aune T *et al.* (2002). Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. *Toxicon* 40: 77–82.
Briggs LR *et al.* (2004). Enzyme-linked immunosorbent assay for the detection of yessotoxin and its analogues. *J Agric Food Chem* 52: 5836–5842.
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Ciminiello P *et al.* (2003). Complex yessotoxins profile in *Protoceratium reticulatum* from north-western Adriatic sea revealed by LC-MS analysis. *Toxicon* 42: 7–14.
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de lalglesia P *et al.* (2007). Advanced studies for the application of high-performance capillary electrophoresis for the analysis of yessotoxin and 45-hydroxyyessotoxin. *J Chromatogr A* 1156: 160–166.
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Fernandez Amandi M *et al.* (2002). Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of yessotoxins in shellfish. *J Chromatogr A* 976: 329–334.
Garthwaite I *et al.* (2001). Integrated enzyme-linked immunosorbent assay screening system for amnesic, neurotoxic, diarrhetic, and paralytic shellfish poisoning toxins found in New Zealand. *J AOAC Int* 84: 1643–1648.
Loader JI *et al.* (2007). Convenient large-scale purification of yessotoxin from *Protoceratium reticulatum* culture and isolation of a novel furanoyessotoxin. *J Agric Food Chem* 55: 11093–11100.
MacKenzie L *et al.* (2002). Complex toxin profiles in phytoplankton and Greenshell mussels (*Perna canaliculus*), revealed by LC-MS/MS analysis. *Toxicon* 40: 1321–1330.
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Miles CO *et al.* (2004b). Isolation of 41a-homoyessotoxin and the identification of 9-methyl-41a-homoyessotoxin and nor-ring A-yessotoxin from *Protoceratium reticulatum*. *Chem Res Toxicol* 17: 1414–1422.
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Miles CO *et al.* (2006). Isolation of yessotoxin 32-O-[beta-L-arabinofuranosyl-(5′→1″)-beta-L-arabinofuranoside] from *Protoceratium reticulatum*. *Toxicon* 47: 510–516.
Morton SL *et al.* (2007). Identification of yessotoxin in mussels from the Caucasian Black Sea Coast of the Russian Federation. *Toxicon* 50: 581–584.
Ogino H *et al.* (1997). Toxicologic evaluation of yessotoxin. *Nat Toxins* 5: 255–259.
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Paz B *et al.* (2006). Detection and identification of glycoyessotoxin A in a culture of the dinoflagellate *Protoceratium reticulatum*. *Toxicon* 48: 611–619.
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Ruppen Canas I *et al.* (2004). Nano liquid chromatography with hybrid quadrupole time-of-flight mass spectrometry for the determination of yessotoxin in marine phytoplankton. *J Chromatogr A* 1056: 253–256.
Samdal IA *et al.* (2005). Comparison of ELISA and LC-MS analyses for yessotoxins in blue mussels (*Mytilus edulis*). *Toxicon* 46: 7–15.
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Souto ML *et al.* (2005). Glycoyessotoxin a, a new yessotoxin derivative from cultures of *Protoceratium reticulatum*. *J Nat Prod* 68: 420–422.
Suzuki T *et al.* (2007). Yessotoxin analogues in several strains of *Protoceratium reticulatum* in Japan determined by liquid chromatography–hybrid triple quadrupole/linear ion trap mass spectrometry. *J Chromatogr A* 1142: 172–177.
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Van Egmond HP *et al.* (1993). Paralytic and diarrhoeic shellfish poisons: occurrence in Europe, toxicity, analysis and regulation. *J Nat Toxins* 2: 41–83.
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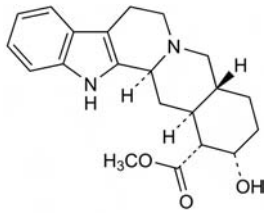
Yohimbine

α_2-Adrenoceptor Antagonist, Alkaloid
$C_{21}H_{26}N_2O_3 = 354.4$
CAS—146-48-5

Synonyms Aphrodine; corynine; (16α,17α)-17-hydroxy-yohimban-16-carboxylic acid methyl ester; québrachine.



Chemical Properties The principal alkaloid of the bark of the yohimbe tree, *Pausinystalia yohimbe* (*Corynanthe yohimbi*) (Rubiaceae). Crystals. Mp ~234°. Sparingly soluble in water; soluble in ethanol and chloroform; moderately soluble in ether. Log *P* (octanol/water), 2.7.

Yohimbine Hydrochloride

$C_{21}H_{26}N_2O_3$,HCl = 390.9
CAS—65-19-0

Proprietary Names *Antagonil*; *Aphrodine*; *Dayto Himbin*; *Erex*; *Pluriviron mono*; *Prowess Plain*; *Urobine*; *Yobine*; *Yocon*; *Yocoral*; *Yohimex*; *Yohydrol*; *Yomax*; *Yovital*; *Zumba*.

Chemical Properties A white crystalline powder. Mp ~302°, with decomposition. Soluble 1 in 100 of water, 1 in 400 of ethanol; more soluble in hot water and ethanol.

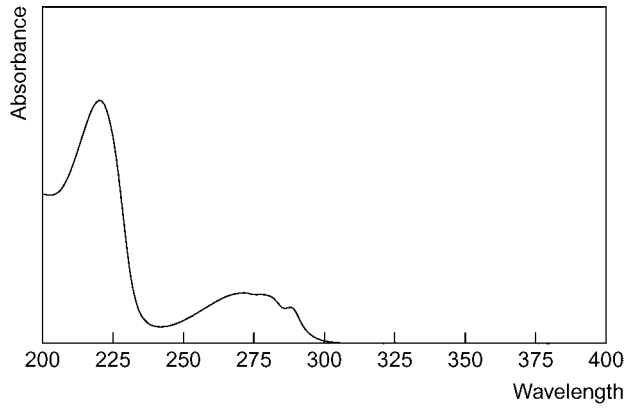
Colour Tests Liebermann's reagent—blue; Mandelin's test—blue→green.

Thin-layer Chromatography System TA—R_f 0.63; system TB—R_f 0.06; system TC—R_f 0.38; system TE—R_f 0.64; system TAE—R_f 0.66; system TAF—R_f 0.70; system TAG—R_f 0.52 (acidified iodoplatinate solution—positive; Dragendorff spray—positive; Marquis test—grey).

Gas Chromatography System GA—yohimbine RI 3155, yohimbine-AC RI 3190.

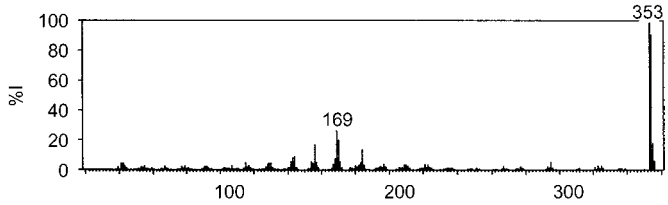
High Performance Liquid Chromatography System HX—RI 348; system HY—RI 279; system HZ—RT 2.6 min; system HAA—RT 11.5 min.

Ultraviolet Spectrum Aqueous acid—271 (A^1_1 = 211a), 277 (A^1_1 = 207a), 287 nm; aqueous alkali—280 nm.



Infrared Spectrum Principal peaks at wavenumbers 1705, 741, 1197, 1160, 1290, 1135 cm^{-1} (yohimbine hydrochloride, KBr disk).

Mass Spectrum Principal ions at *m/z* 353, 354, 169, 170, 355, 156, 184, 144.



Quantification

Blood **HPLC** Column: Partisil 5 (250 × 2.1 mm i.d.). Mobile phase: methanol : water (95 : 5). Fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 360 nm). Limit of detection, 50 ng/L [Owen *et al.* 1985].

Plasma **HPLC** Electrochemical detection. Limit of detection, 10 µg/L [Goldberg *et al.* 1984].

LC-MS Column: LiChrosorb Si 60. Mobile phase: 0.02 mol/L sodium acetate (pH 5) : methanol (5 : 95), flow rate 1 mL/min. Fluorometric detection (λ_{ex} = 280 nm, λ_{em} = 320 nm). Limit of detection, 1 µg/L (yohimbine and 11-hydroxyyohimbine) and 0.5 µg/L (10-hydroxyyohimbine) [Le Verge *et al.* 1992].

Serum **HPLC** Column: TSK-gel ODS-120T. Mobile phase: methanol : potassium phosphate buffer. Limit of detection, 0.80 µg/L [Chiba, Isii 1990].

Urine **LC-MS** Column: Hypersil GOLD (100 × 2.1 mm i.d., 5 µm). Mobile phase: 1% acetonitrile in 10 mmol/L formic acid : 60% acetonitrile in 10 mmol/L formic acid (100 : 0 to 0 : 100 in 10 min to 100 : 0 for 4 min), flow rate 200 µL/min. ESI, positive ion mode SRM acquisition mode. Limit of detection, 2 µg/L [Björnstad *et al.* 2009]

Other **HPLC** Rat plasma. Column: Bondapak C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: water : methanol (52 : 48) containing 0.01 mol/L ammonium phosphate, flow rate 1.0 mL/min. Electrochemical detection. Retention time: 7.2 min. Limit of quantification, 1 µg/L [Diquet *et al.* 1984].

Disposition in the Body

Therapeutic Concentration

In 13 healthy subjects receiving an IV bolus dose of 0.25 or 0.5 mg/kg yohimbine, peak plasma concentrations of 150 to 1140 µg/L were achieved [Hedner *et al.* 1992].

Toxicity

A 38-year-old man experienced atrial fibrillation and memory loss after ingesting 350 mg yohimbine [Varkey 1992].

A 62-year-old male who ingested 200 mg yohimbine experienced only mild adverse effects (tachycardia, hypertension, anxiety) [Friesen *et al.* 1993].

Dose Yohimbine hydrochloride has been given in doses of up to 30 mg daily.

Björnstad K *et al.* (2009). A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1162–1168.
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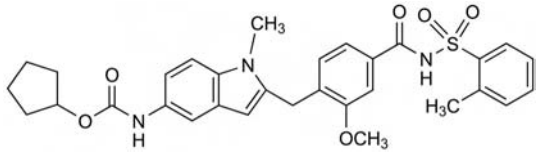
LeVerge R *et al.* (1992). Determination of yohimbine and its two hydroxylated metabolites in humans by high-performance liquid chromatography and mass spectral analysis. *J Chromatogr* 574: 283–292.
Owen JA *et al.* (1985). Sub-nanogram analysis of yohimbine and related compounds by high-performance liquid chromatography. *J Chromatogr* 342: 333–340.
Varkey S (1992). Overdose of yohimbine. *Br Med J* 304: 548.

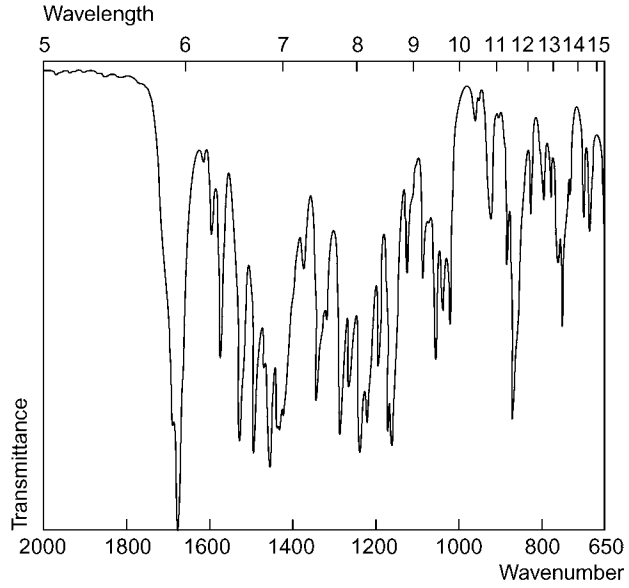
Zafirlukast

Leukotriene D_4 Antagonist, Antiasthmatic
$C_{31}H_{33}N_3O_6S = 575.7$
CAS—107753-78-6

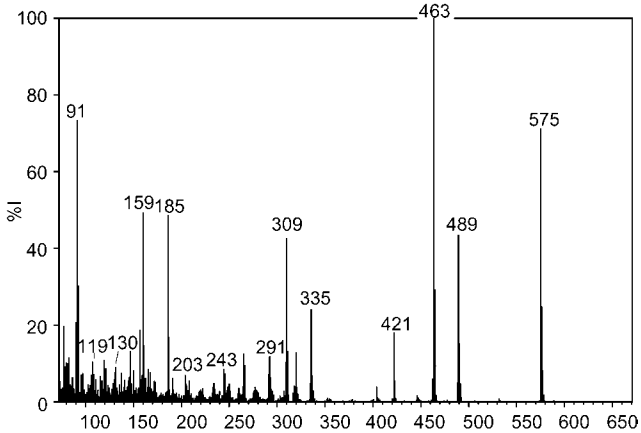
IUPAC Name Cyclopentyl *N*-[3-[[2-methoxy-4-[(2-methylphenyl)sulfonylcarbamoyl]phenyl]methyl]- 1-methylindol-5-yl]carbamate
Synonyms ICI-204219; [3-[[2-methoxy-4-[[[(2-methylphenyl)sulfonyl]amino]carbonyl]phenyl]-methyl]-1-methyl-1*H*-indol-5-yl]carbamic acid cyclopentyl ester; MK-571.
Proprietary Name *Accolate*



Chemical Properties A white to pale yellow powder. Mp 138° to 140°. Practically insoluble in water; slightly soluble in methanol; freely soluble in tetrahydrofuran (THF), dimethylsulfoxide and acetone.
High Performance Liquid Chromatography Column: C_{18} Vydac reversed-phase (250 × 4.6 mm, i.d., 10 µm). Temperature: 24°. Mobile phase: acetonitrile : water (80 : 20), flow rate 1 mL/min. IS: flavone. UV detection (λ=245 nm). Retention time: zafirlukast 3.22 min; IS, 3.78 min [Ficarra *et al.* 2000].
Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 463, 91, 575, 159, 185, 489, 309, 92.



Quantification
***Blood* HPLC** Column: Zorbax CN (150 × 4.6 mm i.d., 5 µm). Mobile phase: THF : hexane : glacial acetic acid (90%) (30 : 70 : 0.1). Fluorescence detection (λ_{ex}=250 nm; λ_{em}=452 nm). Retention time: 9 to 10 min. Limit of quantification, 0.75 µg/L [Bui *et al.* 1997].

Disposition in the Body Zafirlukast is absorbed from the gastrointestinal tract, 1 to 2 h after oral administration. Food decreases the rate and the extent of absorption. It is distributed to tissue, although there is minimal distribution across the blood–brain barrier. The drug is extensively metabolised in the liver by hepatic cytochrome P450; CYO2C9 and CYP3A4. It is excreted primarily in faeces as the unchanged drug and its metabolite. Ten percent of the dose is excreted in urine following oral administration. The metabolites are at least 90 times less potent than zafirlukast. The onset of action is 1 to 2 h and peak plasma concentrations are reached within 3 h of dosing. Peak serum levels after 20 mg and 40 mg oral dose are about 150 µg/L and 250 µg/L, respectively. The steady-state plasma concentration is proportional to the dose and is achieved within 3 days. Pharmacokinetics are not changed in individuals with renal impairment but are with increased age and hepatic impairment.

Therapeutic Concentration

Five healthy male volunteers (mean age, 25.5 years) were administered 40 mg zafirlukast orally in solution, directly into the colon via an oro-enteric tube. Peak concentrations of 697 µg/L and 194 µg/L were reached for the oral and direct administration, respectively. Median times to reach these concentrations were 2.0 (1.1 to 3.0) and 1.35 (0.5 to 10.0) h, respectively [Fischer *et al.* 2000].

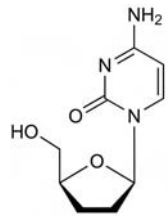
Half-life 10 h.
Clearance Apparent oral clearance, 11.4 L/h; decreased by 50 to 60% in the elderly and those with cirrhosis.
Protein Binding 99%; predominately to albumin.
Dose 20 mg twice daily.

Bui K *et al.* (1997). Determination of zafirlukast, a selective leukotriene antagonist, human plasma by normal-phase high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl* 696: 131–136.
Ficarra R *et al.* (2000). Validation of a LC method for the analysis of zafirlukast in a pharmaceutical formulation. *J Pharm Biomed Anal* 23: 169–174.
Fischer JD *et al.* (2000). Comparison of zafirlukast (Accolate) absorption after oral and colonic administration in humans. *Pharm Res* 17(2): 154–159.

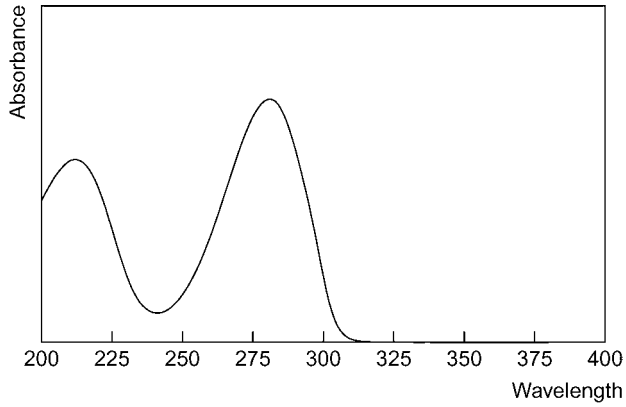
Zalcitabine

Antiviral
$C_9H_{13}N_3O_3 = 211.2$
CAS—7481-89-2

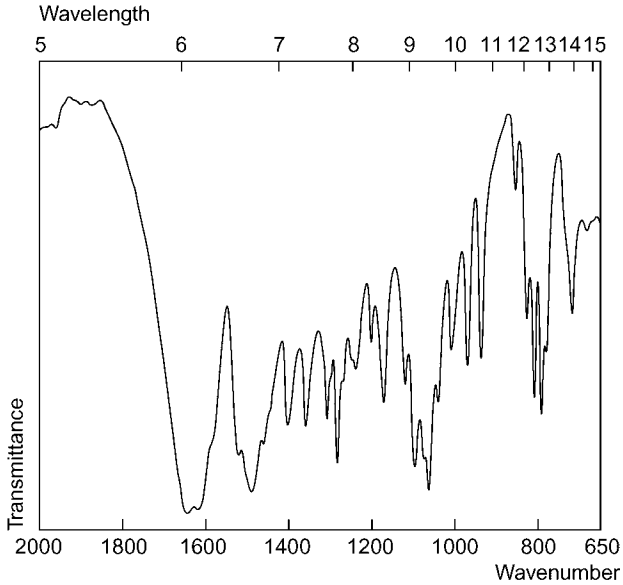
IUPAC Name 4-Amino-1-[5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2- one
Synonyms DDC; ddC; ddCyd; dideoxycytidine; 2′,3′-dideoxycytidine; NSC-606170; Ro-24-2027; Ro-24-2027/000.
Proprietary Name *Hivid*



Chemical Properties A white to off-white crystalline powder. Mp 215° to 217°. Soluble in water and methyl alcohol; sparingly soluble in alcohol in acetonitrile, chloroform and dichloromethane; slightly soluble in cyclohexane. Log *P* (octanol/water), −1.30.
High Performance Liquid Chromatography Column: RSP cyclobond I (250 × 4.6 mm, i.d., 5 µm). Mobile phase: aqueous triethylamine (0.25%), flow rate 0.25 mL/min. IS: 2*S*-cis, 2*R*-trans and 2*S*-trans stereoisomers. Retention time: 27.1 min. UV detection (λ=270 nm). Retention time: zalcitabine, 27.1 min; 2*S*-cis, 24.9 min; 2*S*-trans, 29.4 min; 2*R*-trans, 32.2 min [Scypinski, Ross 1994].
Ultraviolet Spectrum Aqueous acid (0.1 mol/L hydrochloric acid)—212, 280 nm; aqueous alkali (0.1 mol/L sodium hydroxide)—270 nm.



Infrared Spectrum Principal peaks at wavenumbers 1646, 1490, 1064 cm^{-1}.



Quantification

Blood **HPLC** Column: Ultrasphere ODS (250 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 50 mmol/L ammonium acetate (10 : 90), flow rate 1 mL/min. MS detection (SIM at *m/z* 212). Retention time: zalcitabine, 8.9 min. Limit of quantification, 0.25 µg/L [Jajoo *et al.* 1992]. Column: phenyl Hypersil NC-04 (250 × 4 mm, i.d., 5 µm). Mobile phase: 1.4 g/L methanol : sodium acetate (pH 6.55), flow rate 1 mL/min. Retention time: 10 min. Limit of detection, 50 µg/L [Frijus-Plessen *et al.* 1990].

Plasma **GC–MS** Limit of quantification, 2 µg/L [Rubio *et al.* 1988].

HPLC MS detection. Limit of quantification, 0.2 µg/L [Bazunga *et al.* 1998].

Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 440 µg/L [Simon *et al.* 2001].

Urine **HPLC** See Blood. Limit of quantification, 5 µg/L [Bazunga *et al.* 1998].

Cell Suspensions **HPLC** See Blood [Frijus-Plessen *et al.* 1990].

Perfusate **HPLC** See Blood [Frijus-Plessen *et al.* 1990].

Disposition in the Body Zalcitabine is well absorbed from the gastrointestinal tract. Absorption shows interindividual variation and the rate of absorption is decreased by administration with food. Peak plasma concentrations in the fasting state are achieved in about 1 to 2 h. The cerebrospinal fluid concentration of the drug is 9 to 37% of those in plasma. The drug is phosphorylated intracellularly to an active antiviral triphosphate for HIV-reverse transcriptase. The metabolites have been identified primarily as dideoxyuridine (ddU). The drug does not appear to undergo any substantial hepatic metabolism. Renal excretion is the primary route of elimination (75% excreted in urine after an IV dose, 60 to 70% after an oral dose). Approximately 10% of an orally administered dose appears in faeces, comprised primarily of unchanged drug and ddU. Accumulation is negligible.

Therapeutic Concentration

Five individuals were fasted overnight and administered 0.75 mg zalcitabine orally, followed by a further 2 h fasting. Peak plasma concentrations reached 17.27, 16.95 and 12.50 µg/L at 1.3, 1.58 and 1.22 h, respectively [Bazunga *et al.* 1998].

One hundred HIV-infected patients with $CD4^+$ cell counts between 50 and 301 cells/mm^3, mean age 31.4 years, were administered 0.75 mg zalcitabine three times a day plus 200 mg zidvudine three times a day for 24 weeks. The mean peak zalcitabine concentration was 5.31 µg/L after 1.75 h [Vanhove *et al.* 1997].

Toxicity Use of zalcitabine has been associated with significant clinical adverse reactions, some of which are potentially fatal. However, there have been no reports of acute overdose associated with high zalcitabine doses (over 1.5 mg/kg).

Bioavailability Adults: >80%. Children: 29 to 100%. Reduced by 14% with food.

Half-life Adults: 1 to 3 h; children: 0.8 h. Prolonged in patients with renal impairment, up to 8.5 h (with creatinine clearance <55 mL/min).

Volume of Distribution Adults: 0.54 L/kg; children: 9.3 L/m^3.

Clearance Adults: total mean clearance, 285 mL/min (range, 165 to 447); mean plasma clearance, 227 mL/min/m^2. Children: mean plasma clearance, 149 mL/min/m^2.

Protein Binding <4%.

Dose 0.75 mg. Dose may be reduced in patients with renal and hepatic impairment.

Bazunga M *et al.* (1998). The effects of renal impairment on the pharmacokinetics of zalcitabine. *J Clin Pharmacol* 38: 28–33.

Frijus-Plessen N *et al.* (1990). Determination of 3′-azido-3′-deoxythymidine, 2′,3′-dideoxycytidine, 3′-fluoro-3′-deoxythymidine and 2′,3′-dideoxyinosine in biological samples by high-performance liquid chromatography. *J Chromatogr* 534: 101–107.

Jajoo H *et al.* (1992). Thermospray liquid chromatographic-mass spectrometric analysis of anti-AIDS nucleosides: quantification of 2′,3′-dideoxycytidine in plasma samples. *J Chromatogr* 577: 299–304.

Rubio FR *et al.* (1988). Quantification of dideoxycytidine in human plasma by gas chromatography/mass spectrometry. *Biomed Environ Mass Spectrom* 17: 399–404.

Scypinski S, Ross A (1994). Liquid chromatographic separation of zalcitabine and its stereoisomers. *J Pharm Biomed Anal* 12(10): 1271–1276.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

Vanhove GF *et al.* (1997). Pharmacokinetics of saquinavir, zidovudine, and zalcitabine in combination therapy. *Antimicrob Agents Chemother* 41(11): 2428–2432.

Zaleplon

Hypnotic, Pyrazolopyrimidine, Sedative

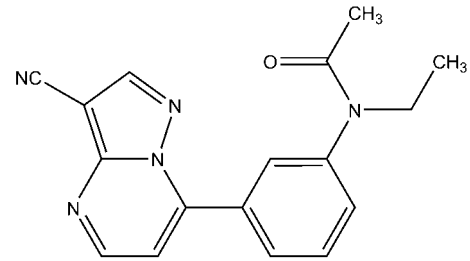
$C_{17}H_{15}N_5O$ = 305.3

CAS—151319-34-5

IUPAC Name *N*-[3-(3-Cyanopyrazolo[5,1-*b*]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide

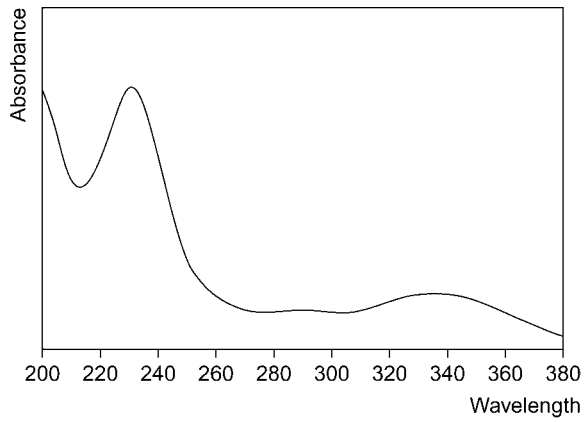
Synonyms CL-284846; *N*-[3-(3-Cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide; *N*-[3-(9-cyano-2,6,7-triazabicyclo[4.3.0]nona-2,4,7,9-tetraen-5-yl)phenyl]-*N*-ethyl-ethanamide.

Proprietary Name *Sonata*

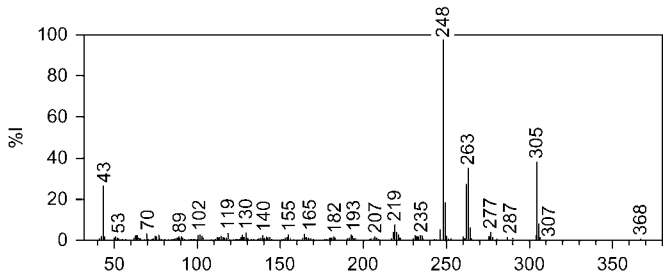


Chemical Properties White to off-white powder. Mp 186° to 187°. Sparingly soluble in ethanol and propylene glycol; practically insoluble in water. Log *P* (octanol/water), 2.47 [Wishart 2006], 1.23 [O'Neil *et al.* 2006]. Extraction yield (chlorobutane), + 1 [Demme *et al.* 2005]. There were no stability related problems during routine analysis for pharmacokinetic, bioavailability or bioequivalence studies in human plasma [Zhang *et al.* 2006].

Ultraviolet Spectrum Aqueous acid—230, 289, 335 nm.



Mass Spectrum Principal ions at *m/z* 248, 305, 263, 262, 43, 249, 306, 219.



Quantification

Blood **GC** Column: J & W DB-5 5% phenylmethylsilicone fused capillary (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 1 min to 200° at 30°/min to 300° at 15°/min for 6 min. NPD. Limit of quantification, 0.5 mg/L [Moore *et al.* 2003].

GC-MS Column: Cross-linked DB-35 fused silica capillary (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode, full scan mode. Limit of quantification, 10 µg/L; limit of detection, <5 µg/L [Gunnar *et al.* 2006].

LC-MS Column: Chrompack Inertsil ODS-3 (150 × 2.0 mm i.d., 3 μm). Mobile phase: acetonitrile : 1 mmol/L ammonium formate (pH 4.0; 10 : 90 for 2 min to 60 : 40 at 15 min for 3 min to 10 : 90 in 1 min), flow rate 200 μL/min. API, SIM acquisition mode. Limit of quantification, 1 μg/L [Giroud *et al.* 2003].

Plasma **HPLC** Column: Beckman C_8 reversed phase (150 × 4.6 mm i.d., 5 μm). Mobile phase: acetonitrile : water (25 : 75), flow rate 1.0 mL/min. Fluorescence detection ($\lambda_{ex} = 345$ nm, $\lambda_{em} = 460$ nm). Limit of quantification, 0.5 μg/L [Beer *et al.* 1994].

LC-MS Column: Shim-pack VP-ODS C_{18} (250 × 2.0 mm i.d., 5 μm). Mobile phase: methanol : water (70 : 30), flow rate 0.2 mL/min. APCI , positive ion mode, SIM acquisition mode. Limit of quantification, 0.2 μg/L; limit of detection, 0.1 μg/L [Zhang *et al.* 2006]. Column: Phenomenex Luna $C_8(2)$ (250 × 4.6 mm i.d.). Mobile phase: methanol : water (75 : 25). Limit of quantification, 0.1 μg/L [Feng *et al.* 2003].

Urine **LC-MS** Positive ion mode, MRM acquisition mode. Limit of detection, 15 μg/L [Nordgren *et al.* 2005]. Column: LiChrospher RP 8 (125 × 4 mm i.d., 5 μm). Mobile phase: acetonitrile: 50 mmol/L ammonium acetate (10 : 90 for 10 min to 20 : 80 at 13 min for 10 min to 45 : 55 at 25 min), flow rate 1 mL/min. ESI, positive ion mode. UV detection ($\lambda = 245$ nm). Limit of detection not reported [Horstkotter *et al.* 2003].

CE Column: Fused silica capillary (total/effective length 27/20 cm, 50 μm i.d.). Fluorescence detection ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 425$ nm). Limit of quantification, 10 μg/L for zaleplon and *N*-desethylzaleplon, 100 μg/L for 5-oxozaleplon and 5-oxo-*N*-desethylzaleplon [Horstkotter *et al.* 2003].

Oral Fluid **LC-MS** Column: Xterra MS C_{18} (100 × 2.1 mm i.d., 3.5 μm). Mobile phase: acetonitrile : 0.1% formic acid (5 : 95 to 80 : 20 at 10 min), flow rate 200 μL/min. API, positive ion mode. Limit of quantification, 0.1 μg/L [Kintz *et al.* 2005].

Hair **LC-MS** Column: XTerra MS C_{18} (100 × 2.1 mm i.d, 3.5 μm). Mobile phase: acetonitrile : 1% formic acid (5 : 95 to 80 : 20 at 10 min). API, positive ion mode. Limit of quantification, 1 pg/mg [Villain *et al.* 2005].

Disposition in the Body Zaleplon is rapidly absorbed from the gastrointestinal tract but undergoes significant first-pass hepatic metabolism. Plasma concentrations are usually reached in approximately 1 h and increase linearly with dose. A heavy meal or one with a high fat content delays absorption and reduces peak concentrations. It is metabolised primarily by aldehyde oxidase to form 5-oxozaleplon and, to a lesser extent, by CYP3A4 to *N*-desethylzaleplon, which is further metabolised by aldehyde oxidase to 5-oxo-*N*-desethylzaleplon. About 70% of a dose is excreted in urine as these inactive metabolites or their glucuronides; less than 1% is excreted unchanged. About 17% of a dose is eliminated in the faeces, mainly as 5-oxozaleplon. Zaleplon is distributed into breast milk.

Therapeutic Concentration

Seventeen healthy subjects (mean age 25 years) were administered a single 10 mg oral dose of zaleplon. Mean peak plasma concentration was 37.1 μg/L after 45 min [Sanchez Garcia *et al.* 2000].

Five healthy non-pregnant lactating mothers aged 18 to 35 years old were administered 10 mg zaleplon following an overnight fast of at least 8 h. Venous blood and milk samples were obtained just before and at 1, 2, 3, 4, 6 and 8 h after dose administration. Mean peak plasma concentration for the 5 subjects was 28.7 μg/L and peak breast milk concentration reached 14.0 μg/L, both after 1.2 h. The mean peak plasma concentration for 5-oxozaleplon was 71.7 μg/L after 1.4 h [Darwish *et al.* 1999].

Ten healthy subjects (5 men and 5 women; aged 23 to 31 years) with no history of sleeping disorder were administered 10 and 20 mg oral doses of zaleplon after an overnight fast, with a wash-out period between doses. Peak plasma concentration (C_{max}) and time to peak concentration (t_{max}) were reported:

	Blood zaleplon	
	Arterial	*Venous*
10 mg dose		
C_{max} (μg/L)	31.8	27.1
t_{max} (min)	78.4	84.5
20 mg dose		
C_{max} (μg/L)	81.7	61.0
t_{max} (min)	46.6	55.5

[Drover *et al.* 2000].

Healthy male volunteers were assigned to receive single oral doses of 1, 5, 15, 30 or 60 mg zaleplon or placebo in a randomised, double-blind study. The following pharmacokinetic values were measured:

Dose (mg)	*C_{max} (ng/mL [SD])*	*t_{max} (h [SD])*	*AUC (ng•L/mL [SD])*	*$t_{1/2}$ (h [SD])*
1	3.1 (1.0)	0.9 (0.2)	5.9 (2.1)	1.1 (0.2)
5*	10.0 (3.5)	1.0 (0.0)	20.3 (4.7)	0.9 (0.2)
15	27.3 (13.3)	1.2 (0.8)	59.4 (25.6)	1.0 (0.2)
30	71.2 (26.2)	0.9 (0.2)	171.8 (53.1)	1.0 (0.1)
60	109.0 (40.4)	1.5 (1.0)	259.3 (76.4)	1.1 (0.2)

n = 5 unless otherwise specified; *4 subjects. [Beer *et al.* 1994].

Toxicity

A 41-year-old Caucasian woman was found lying on her floor dead surrounded by multiple empty pill vials. No medical history was available except that she had been prescribed alprazolam, zaleplon, and butalbital at various different times. Zaleplon was found at the following concentrations: blood, 2.2 mg/L; bile 8.6 mg/L, and urine 1.4 mg/L. The blood zaleplon concentration was around 40 times greater than normal therapeutic levels. Promethazine and butalbital were also found in blood at 0.8 and 9.9 mg/L, respectively [Moore *et al.* 2003].

A 20-year-old man involved in a two-car collision was observed to be very unsteady on his feet. He also had slow movements and reactions, poor co-ordination, lack of balance, and poor attention. He admitted inhaling 3 crushed 10 mg zaleplon tablets and ingesting another three. The time of inhalation and ingestion were not determined. Blood was drawn approx. 1 h after the accident. The following blood concentrations were reported: zaleplon, 0.13 mg/L, chlorphenamine, 0.03 mg/L, dextromethorphan, 0.08 mg/L. No alcohol was detected [Stillwell 2003].

Bioavailability Approximately 30%.

Clearance Approximately 3.5 L/min.

Half-life Approximately 1 h.

Volume of Distribution Approximately 1.4 L/kg.

Protein Binding Approximately 60%.

Dose Usual dose is 10 mg before bed although occasionally patients may require 20 mg. Elderly or debilitated patients and those with mild to moderate hepatic impairment should be given 5 mg. When taking cimetidine concomitantly, it is recommended that the dose of zaleplon be reduced to 5 mg.

Beer B *et al.* (1994). A placebo-controlled evaluation of single, escalating doses of CL 284 846 a non-benzodiazepine hypnotic. *J Clin Pharmacol* 34: 335–344.

Darwish M *et al.* (1999). Rapid disappearance of zaleplon from breast milk after oral administration to lactating women. *J Clin Pharmacol* 39: 670–674.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Drover D *et al.* (2000). Pharmacokinetics, pharmacodynamics, and relative pharmacokinetic/pharmacodynamic profiles of zaleplon and zolpidem. *Clin Ther* 22: 1443–1461.

Feng F *et al.* (2003). Development and validation of a high-performance liquid chromatography–electrospray ionization–mass spectrometry assay for the determination of zaleplon in human plasma. *J Chromatogr Sci* 41: 17–21.

Giroud C *et al.* (2003). Determination of zaleplon and zolpidem by liquid chromatography–turboionspray mass spectrometry: application to forensic cases. *J Chromatogr B Analyt Technol Biomed Life Sci* 789: 131–138.

Gunnar T *et al.* (2006). Fast gas chromatography–negative-ion chemical ionization mass spectrometry with microscale volume sample preparation for the determination of benzodiazepines and alpha-hydroxy metabolites, zaleplon and zopiclone in whole blood. *J Mass Spectrom* 41: 741–754.

Horstkotter C *et al.* (2003). Separation and identification of zaleplon metabolites in human urine using capillary electrophoresis with laser-induced fluorescence detection and liquid chromatography-mass spectrometry. *J Chromatogr A* 1014: 71–81.

Kintz P *et al.* (2005). Screening and confirmatory method for benzodiazepines and hypnotics in oral fluid by LC-MS/MS. *Forensic Sci Int* 150: 213–220.

Moore KA *et al.* (2003). Mixed drug intoxication involving zaleplon ("Sonata'). *Forensic Sci Int* 134: 120–122.

Nordgren HK *et al.* (2005). Application of direct urine LC-MS-MS analysis for screening of novel substances in drug abusers. *J Anal Toxicol* 29: 234–239.

O'Neil MJ *et al.* (2006). *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 14th edn. Whitehouse Station, NJ: Merck Research Laboratories.

Sanchez Garcia P *et al.* (2000). Absence of an interaction between ibuprofen and zaleplon. *Am J Health Syst Pharm* 57: 1137–1141.

Stillwell ME (2003). Zaleplon and driving impairment. *J Forensic Sci* 48: 677–679.

Villain M *et al.* (2005). Screening method for benzodiazepines and hypnotics in hair at pg/mg level by liquid chromatography-mass spectrometry/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 825: 72–78.

Wishart DS (2006). Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34: D668–D672.

Zhang B *et al.* (2006). High-performance liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry determination of zaleplon in human plasma. *J Pharm Biomed Anal* 40: 707–714.

Zanamivir

Antiviral

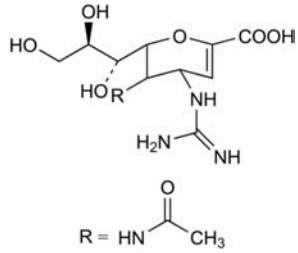
$C_{12}H_{20}N_4O_7 = 332.31$

CAS—139110-80-8

IUPAC Name (2*R*,3*R*,4*S*)-3-Acetamido-4-(diaminomethylideneamino)-2-[(1*S*,2*S*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid

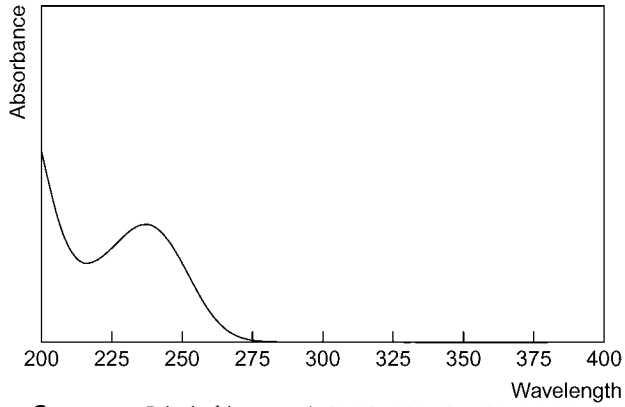
Synonyms 5-(Acetylamino)-4-[(aminoiminomethyl)amino]-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid; GG-167; GR-121167X.

Proprietary Name *Relenza*

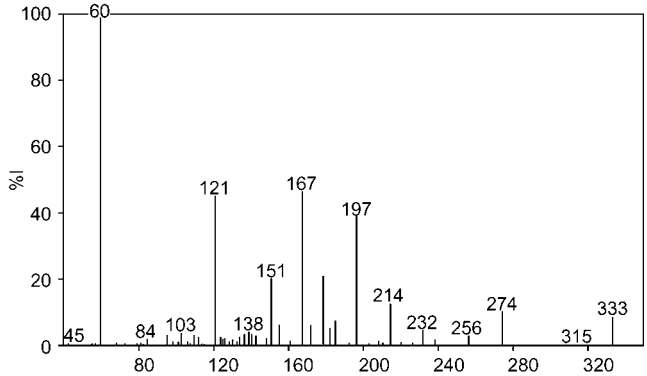


Chemical Properties A white to off-white powder which is soluble in water (18 g/L at 20°).

Ultraviolet Spectrum Principal peak at 238 nm.



Mass Spectrum Principal ions at *m/z* 60, 167, 121, 197, 180, 151, 214, 274.



Quantification

Serum **HPLC** MS–MS detection (*m/z* 333 to 60). Limit of detection, 10 µg/L [Allen *et al.* 1999]. Column: ODS Hypersil (100 × 4.6 mm i.d.). Mobile phase: acetonitrile : 0.05 mol/L Tris-HCl buffer (pH 8.5, 20 : 80), flow rate 1 mL/min. Fluorescent detection (λ_{ex}=325 nm; λ_{em}=442 nm). Retention time: not specified. Limit of quantification, 10 µg/L [Stubbs, Harker 1995].

Urine **HPLC** Column: Nucleosil-Diol (250 × 4.6 mm, 7 µm). Mobile phase: 20 mmol/L phosphate (pH 2.5) : acetonitrile (18 : 82), flow rate 2.0 mL/min. UV detection (λ=238 nm). Retention time 17 min. Limit of quantification, 300 µg/L [Morris *et al.* 1995].

Disposition in the Body Zanamivir is not well absorbed. Systemic absorption is between 10 and 20% after an inhaled dose. It does not undergo extensive metabolism or biotransformation, and no metabolites have been detected in humans. The unchanged drug is excreted in urine with 90% of a dose being detected within 24 h. Unabsorbed drug has been detected in faeces.

Therapeutic Concentration

Ninety-two healthy male volunteers and 12 females, aged 18 to 45 years, were administered with the following: 16 mg zanamivir, single doses, IV over a period of 20 to 30 min; 600 mg IV as a single dose and repeated doses; 500 mg, single oral dose; and 16 mg administered daily for five days as separate studies. The single 16 mg IV dose generated mean maximum plasma concentrations of 1.28 (range, 1.14 to 1.47) mg/L at 0.32 h. For the single 600 mg dose concentrations reached 32.76 mg/L at 0.5 h. Steady-state concentrations were observed with the repeated dosing, and the maximum concentration was 39.71 mg/L at 0.5 h on day 5. After the single intranasal dose, the maximum serum concentration was 0.042 (0.020 to 0.054) mg/L observed within 1.8 (0.75 to 3.0) h. For the repeated dosing, concentration was 0.086 (0.039 to 0.107) mg/L at 0.5 to 3.0 h. The steady-state concentration was 0.071 mg/L. For the oral dose, the maximum concentration was 0.057 to 0.500 mg/L observed at 4 (0.75 to 8.0) h [Cass *et al.* 1999].

Bioavailability 1 to 5% after oral administration.

Half-life Serum half-life, 2.5 to 5.1 h.

Volume of Distribution 16 L; decreases in patients with renal impairment (13.4 L in those with severe impairment; 15.1 L for mild–moderate impairment).

Clearance Total serum clearance, 6.07 to 7.22 (mean, 6.67) L/h; decreases in patients with renal impairment.

Protein Binding <10%.

Dose 10 mg daily.

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Zearalenone

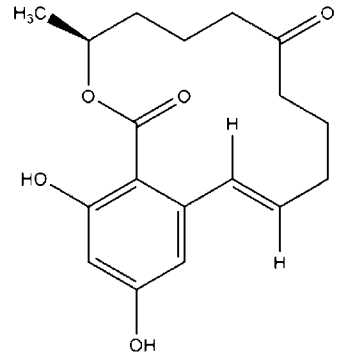
Oestrogenic Mycotoxin

$C_{18}H_{22}O_5$ = 318.4

CAS—17924-92-4

IUPAC Name (2*E*,11*S*)-15,17-Dihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca1(14),2,15,17-tetraene-7,13-dione

Synonyms Compound F-2; FES; 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl-β-resorcylic acid lactone; *trans*-zearalenone.



Chemical Properties An oestrogenic mycotoxin produced by *Fusarium graminearum* and other *Fusarium* spp and also by some *Aspergillus* spp. Crystals. Mp 164° to 165°. Soluble in aqueous alkali, ether, benzene and alcohols. Practically insoluble in water [O'Neil *et al.* 2006].

Thin-layer Chromatography Plates: RP-18 and KC-18. Solvent systems: (A) methanol : water; (B) acetonitrile : water; (C) tetrahydrofuran : water. Location reagents: 4-(*p*-nitrobenzyl)pyridine for trichothecenes; UV (λ= 365 nm) for aflatoxins, ochratoxin A, citrinin; aluminium trichloride for sterigmatocystin; Azoene Fast Violet B for zearalenone; 3-methyl-2-benzthiazolinone hydrazone hydrochloride for patulin and penicillic acid. Solvent composition and R_f values reported as follows:

	Mobile phase solvent ratio, R_f value								
	A	***RP-18***	***KC-18***	***B***	***RP-18***	***KC-18***	***C***	***RP-18***	***KC-18***
Aflatoxins B_1	70 : 30	0.38	0.53	70 : 30	0.55	0.60	60 : 40	0.44	N/A
Aflatoxins B_2	70 : 30	0.46	0.53	70 : 30	0.63	0.60	60 : 40	0.46	N/A
Aflatoxins G_1	70 : 30	0.60	0.61	70 : 30	0.69	0.73	60 : 40	0.60	N/A
Aflatoxins G_2	70 : 30	0.68	0.61	70 : 30	0.74	0.73	60 : 40	0.65	N/A
Citrinin	60 : 40	0.24	0.71	60 : 40	N/A	0.95	N/A	N/A	N/A
Diacetoxyscirpenol	65 : 35	0.31	0.42	65 : 35	0.63	0.59	N/A	N/A	N/A
Deoxynivalenol	65 : 35	0.63	0.74	65 : 35	0.76	0.81	N/A	N/A	N/A
3-Acetyldeoxynivalenol	65 : 35	0.48	0.59	65 : 35	0.71	0.78	N/A	N/A	N/A
Fusarenone-X	65 : 35	0.63	0.73	65 : 35	0.76	0.75	N/A	N/A	N/A
HT-2 toxin	65 : 35	0.23	0.32	65 : 35	0.65	0.58	N/A	N/A	N/A
Neosolaniol	65 : 35	0.61	0.71	65 : 35	0.76	0.75	N/A	N/A	N/A
Nivalenol	65 : 35	0.78	0.87	65 : 35	0.86	0.85	N/A	N/A	N/A
Ochratoxin A	65 : 35	0.13	0.71	60 : 40	N/A	0.78	60 : 40	0.14	N/A
Patulin	60 : 40	0.70	0.94	60 : 40	0.66	0.85	60 : 40	0.49	0.60
Penicillic acid	70 : 30	0.70	0.85	60 : 40	0.62	0.90	60 : 40	0.45	0.98
Sterigmatocystin	90 : 10	0.37	0.62	90 : 10	0.59	0.85	70 : 30	N/A	N/A
T-2 toxin	65 : 35	0.17	0.22	65 : 35	0.51	0.43	N/A	N/A	N/A
Zearalenone	**90 : 10**	**0.53**	**0.70**	**80 : 20**	**0.59**	**0.85**	**65 : 35**	**0.32**	**N/A**

[Abramson *et al.* 1989]

Ultraviolet Spectrum See Rajakylä *et al.* [1987] and Sydenham *et al.* [1996].
Infrared Spectrum See Sydenham *et al.* [1996].
Mass Spectrum See Sydenham *et al.* [1996].
Quantification
Plasma **GC-MS** Zearalenone and its congeners (α-zearalenol and β-zearalenol) [Pillay *et al.* 2002].

HPLC Zearalenone and its congeners (α-zearalenol and β-zearalenol) [Pillay *et al.* 2002].
Urine **HPLC** ECD using a carbon nanotube modified glassy carbon electrode (CNT). Limit of detection, 1.3–1.4 μg/L for zearalenone and its metabolites [de Andres *et al.* 2008].
Other **TLC** Cereals and Feed. TLC with ELISA. Zearalenone and other mycotoxins [Klaric *et al.* 2009]. Grain. Comparison with HLPC [Schaafsma *et al.* 1998].

GC Bananas. Fluorescence detection. Zearalenone, zearalenols and other mycotoxins [Jimenez, Mateo 1997]. Cereal Samples. Column: SE-52 (12 m × 0.25 mm i. d.). Carrier gas: H_2, 40 kPa. Temperature programme: 180° to 260° at 4°/min. FID. Limit of detection, 100 ppb for zearalenone and other fusariotoxins [Bata *et al.* 1983].

GC-MS Veal Liver. Zearalenone, resorcylic acid lactones and stilbene anabolic steroids. Limit of detection, 0.08–0.19 μg/kg [Dickson *et al.* 2009]. Barley, Wheat, Potato, Sugar Beet. Zearalenone and other mycotoxins [Burlakoti *et al.* 2008]. Meat and Urine. NICI. Zearalenone and other resorcylic acid lactones [Blokland *et al.* 2006]. Bovine Muscle. Limit of quantification, 1.0 μg/kg, limit of detection, 0.5 μg/kg for zeranol and related compounds [Zhang *et al.* 2006]. Cereals. Zearalenone and trichothecene mycotoxins [Tanaka *et al.* 2000]. Barley. Limit of detection, 0.1–0.5 mg/kg for zearalenone and other *Fusarium* mycotoxins [Onji *et al.* 1998]

HPLC Edible Oils. Fluorescence detection. Limit of quantification, 30 μg/kg, limit of detection, 10 μg/kg [Siegel *et al.* 2010]. Wheat and Wheat Products (e.g. cornflakes, milk wheat mash, rusk). Mobile phase: acetonitrile : water (50 : 50). Fluorescence detection (for zearalenone), UV detection (for deoxynivalenol) [Brenn-Struckhofova *et al.* 2009]. Leghorn Broiler Meat. Column: Hypersil Gold C_{18}. Mobile phase: 50 mmol/L aqueous ammonium acetate : acetonitrile : methanol (45 : 8 : 47), flow rate 1 mL/min. Zearalenone and its metabolites [Duca *et al.* 2009]. Surface Water, Groundwater and Wastewater (with different natural organic matter content). Column: Zearala Test immunoaffinity [Gromadzka *et al.* 2009]. Maize Grain. Zearalenone and other mycotoxins [Ofitserova *et al.* 2009]. Bovine and Swine Urine. ECD using CNT. Limit of detection, 1.3–1.4 μg/L for zearalenone and its metabolites [de Andres *et al.* 2008]. Air Samples (for airborne mycotoxins in poultry house). Zearalenone and aflatoxins and ochratoxin A [Wang *et al.* 2008]. Maize Flour. Zearalenone and other macrocyclic lactone mycotoxins. Limit of quantification, 30 μg/kg [Zougagh, Rios 2008]. Baby Food and Animal Feed. Column: reversed phase. Fluorimetric detection (λ_{ex} = 274 nm, λ_{em} = 446 nm) [Arranz *et al.* 2007]. Cereal Grains, Swine Feed, Dairy Feed. Column: reversed phase. Fluorescence detection [Campbell, Armstrong 2007]. Maize-based Foods. Fluorescence detection [Cerveró *et al.* 2007]. Soya Food. Fluorescence and UV detection. Limit of detection, 1–19 μg/kg for zearalenone and α- and β-zearalenol and other *Fusarium* toxins [Schollenberger 2007]. Cereal grains. Column: Nova-Pak (150 × 4.6 mm i.d., 4 μm). Mobile phase: methanol : acetonitrile : 1% phosphoric acid. Zearalenone and other mycotoxins. Limit of detection, 4 μg/kg for zearalenone [Li *et al.* 2006]. Wheat Grain and Wholemeal Wheat Flours (conventional and organic). Fluorescence or double-mode UV fluorescence detection. Limit of detection, 1.5 μg/kg for zearalenone, 50 μg/kg for deoxynivalenol and 0.05 μg/kg for ochratoxin A [Pussemier *et al.* 2006]. Barley, Maize and Wheat Flours, Polenta, Maize-based Baby Food. Column: reversed phase. Mobile phase: acetonitrile. Fluorescence detection [MacDonald *et al.* 2005]. Maize Flour and Cheese Snacks. Fluorescence detection. Limit of detection, 0.01 mg/L [Reza Oveisi *et al.* 2005]. Grains. Fluorescence detection. Zearalenone and other mycotoxins. Limit of quantification, 5 μg/kg, limit of detection, 1–3 μg/kg for zearalenone [Göbel, Lusky 2004]. Soil Samples. Column: phenyl hexyl. Fluorescence detection. Limit of detection, 1 μg/kg for zearalenone and 0.1 μg/kg for ochratoxin A [Mortensen *et al.* 2003]. Maize, Rice, and Wheat Grains. Mobile phase: methanol : water (80 : 20). DAD and fluorescence detection [Llorens *et al.* 2002]. Cereal Cultures. Secondary toxic metabolites of *Fusarium* spp. [Mateo *et al.* 2002]. Maize, Wheat, Barley, Swine Feed, Poultry Feed. Column: reversed phase C_{18}. Mobile phase: acetonitrile : water (50 : 50). Fluorescence detection. Limit of detection, 10 ng/g [Fazekas, Tar 2001]. Maize. Fluorescence detection, 0.10–0.0025 μg/g. Comparison with fluorometry. Limit of detection, 0.1 μg/g [Kruger *et al.* 1999]. Maize. Column: reversed phase. Mobile phase: acetonitrile : water : methanol (46 : 46 : 8). Fluorimetric detection (λ_{ex} = 274 nm, λ_{em} = 400 nm). Limit of detection, 3 ng/g [Visconti, Pascale 1998]. Cereal Grains (Wheat and Barley). Limit of detection, 0.02–0.15 mg/kg for zearalenone and other mycotoxins [Stratton *et al.* 1993].

LC-MS Cereals. QuEChers-like method and accelerated solvent extraction. ESI. 17 mycotoxins. Limit of quantification, 5–100 μg/kg [Desmarchelier *et al.* 2010] Maize Silage. Limit of detection, 1–739 μg/kg for zearalenone and other mycotoxins [Rasmussen *et al.* 2010]. Bovine and Swine Urine. ESI, negative ion mode. Limit of detection, 0.56–0.68 μg/L for zearalenone and other resorcylic acid lactones [Dusi *et al.* 2009]. Bovine Urine. APCI. Limit of detection, 0.11–0.49 μg/L for zearalenone and other anabolic steroids [Kaklamanos *et al.* 2009a]. Muscle Tissue. MRM acquisition mode. Limit of detection, 0.05–0.24 μg/kg for zearalenone and other anabolic steroids [Kaklamanos *et al.* 2009b]. Oilseed Cakes. Zearalenone and other mycotoxins [Lanier *et al.* 2009]. Sweet Pepper. Mobile phase: variable mixtures of water and methanol, 1% acetic acid and 5 mmol/L ammonium acetate. ESI, positive ion mode. Limit of detection, 0.32–42.48 μg/kg for zearalenone and other mycotoxins [Monbaliu *et al.* 2009]. Beers. Ultra-HPLC Limit of quantification, <0.5 μg/L for zearalenone and other mycotoxins [Romero-Gonzalez *et al.* 2009]. Rat Serum. MRM acquisition mode [Shin *et al.* 2009a]. Cereal-based Food (maize, flour, porridge, beer, pasta). Zearalenone, deoxynivalenol and their major masked metabolites [Vendl *et al.* 2009]. Bovine Milk. Ultra-HPLC. Column: Waters Acquity BEH C_{18}. ESI, MRM acquisition mode, negative ion mode. Limit of quantification, 0.05–0.2 μg/L, limit of detection, 0.01–0.05 μg/L for zearalenone and other resorcylic acid lactones [Xia *et al.* 2009]. Wheat Field Drainage Water and River Water. Zearalenone and deoxynivalenol [Bucheli *et al.* 2008]. Beer. ESI. Zearalenone and oestrogenic compounds. Limit of detection, 1.3 μg/L for zearalenone [Maragou *et al.* 2008]. Bovine Urine. Zearalenone and other resorcylic acid lactones and stilbenes [Schmidt *et al.* 2008]. Food (peanuts, pistachios, wheat, maize, cornflakes, raisins, figs). Column: reversed phase. Mobile phase: acetonitrile : water. ESI, MRM acquisition mode, positive ion mode. Limit of quantification, 10–200 μg/kg for zearalenone and 32 other mycotoxins [Spanjer *et al.* 2008]. Aqueous Environmental Samples (drainage water, river water, wastewater treatment plant effluent). Limit of detection, 0.4–12.4 ng/L for zearalenone and other oestrogenic mycotoxins [Hartmann *et al.* 2007]. Maize. Column: reversed phase. Mobile phase: methanol : water containing 0.5% acetic acid and 1 mmol/L ammonium acetate. ESI. Limit of detection, 0.3–4.2 μg/kg for zearalenone and other mycotoxins [Lattanzio *et al.* 2007]. Plant Extract of *Arabidopsis thaliana*. Zearalenone metabolites [Berthiller *et al.* 2006]. Cellulose Filters and Fungal Cultures. Column: C_{18} reversed phase SunFire. Mobile phase: variable mixtures of 10 mmol/L ammonium acetate and 20 μmol/L sodium acetate in water and in methanol. ESI, MRM acquisition mode. Limit of detection, 0.009–50 μg/L for filter samples and 0.04–75 μg/L for fungal culture samples [Delmulle *et al.* 2006]. Horse Plasma, Urine and Faeces. APCI. Limit of quantification, 0.5–1.0 μg/L or 0.5–1.0 μg/kg, limit of detection, 0.1–0.5 μg/L or 0.1–0.5 μg/kg for zearalenone and its metabolites [Songsermsakul *et al.* 2006]. Maize, Wheat, Cornflakes and Biscuits. Column: Zorbax Eclipse XDB-C_{18} (150 × 2.1 mm i.d., 3.5 μm). Mobile phase: 10 mmol/L ammonium acetate : methanol (90 : 10 to 0 : 100 in 40 min), flow rate 200 μL/min. APCI. Limit of detection, 0.1–6.1 ng/g for zearalenone and other mycotoxins [Tanaka *et al.* 2006]. Cereals and Cereal-based Foods. ESI. Limit of detection, 0.3–5 μg/kg for zearalenone and trichothecenes [Klötzel *et al.* 2006]. Maize. APCI. Limit of detection, 0.3–3.8 μg/kg for zearalenone and trichothecenes [Berthiller *et al.* 2006]. Cereals and Cereal-based Food. Column: SB-RP18-Zorbax (150 × 3.0 mm i.d., 3.5 μm). Mobile phase: methanol : water (30 : 70 to 100 : 0 in 8 min), flow rate 250 μL/min. ESI, negative and positive ion mode, MRM acquisition mode. Retention time: 13.6 min. Limit of quantification, 0.02–10 ppb [Biselli, Hummert 2005]. Maize Meal. Column: C_{18}. Mobile phase: methanol : water-10 mmol/L formate buffer (pH 3.8). Limit of detection, 2 to 14 μg/kg for zearalenone and most other *Fusarium* mycotoxins [Cavaliere *et al.* 2005]. Bovine Milk. Column: Hypersil ENV or Luna C_{18}. Positive ion mode. Limit of detection, 0.020–0.15 μg/L for zearalenone and 17 other mycotoxins [Sorensen, Elbaek 2005]. Fungal Liquid Cultures, Maize Grain, Insect Larvae, Pig Serum. Limit of detection, 8 ppb for zearalenone, deoxynivalenol and 15-acetyldeoxynivalenol [Bily *et al.* 2004]. Bovine Urine. ESI. Zearalenone and related mycotoxins and zeranol and taleranol. Limit of detection, 15 μg/L for zearalenone and 2 related mycotoxins [Launay *et al.* 2004]. Fish Tissue. Mobile phase: acetonitrile : methanol : water. ESI or APCI. Limit of detection, 0.1–1.0 ng/g for zearalenone, α-zearalenol and β-zearalenol [Lagana *et al.* 2003]. Sewage Treatment Plant Samples. Column: reversed-phase. APCI, negative ion mode. Zearalenone and anabolic macrocyclic lactones. Limit of detection, ng/L range for zearalenone [Lagana *et al.* 2001]. Beer. APCI, negative ion mode. Limit of quantification, 0.07–0.15 μg/L, limit of detection, 0.03–0.06 μg/L for zearalenone and its metabolites [Zöllner *et al.* 2000]. Grains. APCI, negative ion mode. Limit of quantification, 1 μg/kg, limit of detection, 0.5 μg/kg [Zöllner *et al.* 1999]. Food and Feed. Column: C_{18}. Mobile phase: acetonitrile : water (40 : 60), flow rate 1 mL/min. APCI. Comparison with HPLC fluorescence detection [Rosenberg *et al.* 1998].

CE Grains. Zearalenone and its metabolites. Limit of detection, 0.0084 mg/L for zearalenone [Zeng *et al.* 2003].

Note For a review of analytical methods for the analysis of oestrogenic mycotoxins in cereals, see Krska, Josephs [2001]; for a review of the analysis of mycotoxins and its quality assurance, see Krska *et al.* [2005]. For an LC-MS method for the determination of 90 major mycotoxins and other secondary fungal metabolites in cereals, see Berthiller *et al.* [2007]; for a review of LC-MS methods of mycotoxin analysis in biological and food matrices, see Zöllner, Mayer-Helm [2006]; for the determination of 39 mycotoxins in wheat and maize by LC-MS, see Sulyok *et al.* [2006]; for the development of an extraction method using less organic solvents for the LC-MS determination of zearalenone in maize, see Pallaroni, von Holst [2004] and for the influence of extraction parameters such as temperature and solvent extraction mixture, see Pallaroni, von Holst [2003]; for the development of an accelerated extraction method for the LC-MS determination of zearalenone in wheat, see Royer *et al.* [2004]. For an HPLC screening method for mycotoxins, see Kuronen [1989]. For the development of a one-step immunochromatographic strip test for the detection of zearalenone and ochratoxin A in maize, see Shim *et al.* [2009a] and for the development of a gold nanoparticle immunochromatographic assay for the detection of zearalenone in maize, see Shim *et al.* [2009b]. For a fluorescence polarization immunoassay for the detection of zearalenone in maize, see Chun *et al.* [2009]; for an ELISA assay for the detection of zearalenone in cereals, see Thongrussamee *et al.* [2008].

Disposition in the Body Zearalenone is metabolised *in vitro* and *in vivo*to its main metabolites α-zearalenol and β-zearalenol [Kolf-Clauw *et al.* 2008].

In goats, zearalenone given as a single IV injection was excreted in urine and faeces as unchanged compound, and its metabolites, α- and β-zearalenol, were excreted mainly as glucuronide and/or sulfate conjugates, with the β-metabolite being the predominant form [Dong *et al.* 2010].

In pigs fed zearalenone-contaminated oats, urine samples showed that 60% of zearalenone was transformed to α- and β-zearalenol (in ratio of 3 : 1). Trace amounts of zeanol and taleranol were also found in urine. Liver samples contained mainly α-zearalenol with small amounts of β-zearalenol and zearalenone; the ratio of α-/β-zearalenol was 2.5 : 1. In both urine and liver, α- and β-zearalenol were mainly in the form of glucuronide conjugates [Zöllner *et al.* 2002].

After IV administration of zearalenone to rats, minimal quantities were excreted unchanged in urine (0.5%) and bile (0.9%) [Shin *et al.* 2009b].

In rats, steady-state blood levels of zearalenone after oral doses of 0.1 mg/kg daily were predicted to be 0.014 μg/L. A daily oral dose in humans to achieve this blood levels was determined to be 0.0312 mg/kg daily [Shin *et al.* 2009c]. In rats, zearalenone is rapidly absorbed after oral administration, but absolute oral bioavailability is low.

Toxicity The α-reduced metabolites of zearalenone are activated forms, but β-reduced metabolites are less oestrogenic than zearalenone [Kolf-Clauw *et al.* 2008].

The LD_{50} (oral) is 500 mg/kg in mice [Ouanes *et al.* 2003] and >10 g/kg in rats [Baldwin *et al.* 1983].

Note For a review of the toxicology of mycotoxins including zearalenone, see He, Xu [2005] and Paterson, Lima [2010]; for other reviews of mycotoxin toxicology and potential effects on humans, see Pestka, Smolinski [2005], Rotter *et al.* [1996] and Sudakin [2003].

Half-life Distribution half-life 3.15 h and elimination half-life 28.58 h (in goats, both after IV administration) [Dong *et al.* 2010].

Bioavailability Absolute oral bioavailability in rats 2.7% [Shin *et al.* 2009b].

Volume of Distribution In rats after IV administration, 2–4.7 L/kg [Shin *et al.* 2009b].

Clearance In rats after IV administration, 5.0–6.6 L/h/kg [Shin *et al.* 2009b].

Dose For a report of the mycotoxin content of UK organic and conventional oats, see Edwards [2009]. It has been reported that *Fusarium* toxins were used as an agent of biological/chemical warfare ("yellow rain') [Mirocha *et al.* 1983; Rosen, Rosen 1982; Stark 2005].

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Zeranol

Anabolic Steroid (Veterinary), Oestrogen

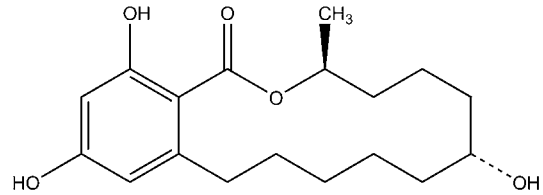
$C_{18}H_{26}O_5 = 322.4$

CAS—26538-44-3

IUPAC Name (7*R*,11*S*)-7,15,17-Trihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(14),15,17-trien-13-one

Synonyms (3*S*,7*R*)-3,4,5,6,7,8,9,10,11,12-Decahydro-7,14,16-trihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1-one; 6-(6-10-dihydroxyundecyl)-β-resorcyclic acid μ-lactone; MK-188; P-1496; THFES (HM); α-zearalanol.

Proprietary Names *Ralabol*; *Ralgro*; *Ralone*.



Chemical Properties Crystals. Mp 182° to 183°. Log *P* (octanol/water), 3.86 [Meylan, Howard 1995]. Zeranol was stable for 14 days at 4°, in LC mobile phase for 4 days at 4°, and in milk brought to boiling [Scott, Lawrence 1988].

Ultraviolet Spectrum Methanol—218, 263, 303 nm.

Mass Spectrum Principal ions at *m/z* 73, 433, 307, 335, 434, 312, 74, 295 (TMS derivative).

Quantification

Plasma **HPLC** Column: LiChrosorb RP-8 reversed-phase (250 × 3.0 mm i.d., 10.0 μm). Mobile phase: methanol : acetonitrile : water (125 : 205 : 250), flow rate 1.75 mL/min. Fluorescence detection ($\lambda_{ex} = 236$ nm, $\lambda_{em} = 418$ nm). Limit of detection, 0.6 μg/L [Trenholm *et al.* 1981].

Urine **GC-MS** Column: J & W DB-1 methylsilicone capillary (15 m × 0.25 mm i.d., 0.25 μm). Carrier gas: He. Temperature programme: 100° for 1 min to 220° at 16°/min to 301° at 3.8°/min for 5.5 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of detection not reported [Ayotte *et al.* 1996].

Milk **LC**. Column: Waters Guard-PAK precolumn module with Resolve C_{18} precolumn insert (255 × 4.6 mm i.d., 5.0 μm). Mobile phase: methanol : water : acetonitrile (61 : 35 : 4), flow rate 1.2 mL/min. Fluorescence detection ($\lambda_{ex} = 236$ nm, $\lambda_{em} = 470$ nm). Limit of detection, 2 μg/mL [Scott, Lawrence 1988].

Other **GC-MS** Bovine Muscle. Column: HP-5MS capillary (30 m × 250 μm i.d., 0.25 μm). Carrier gas: He, 1.0 mL/min. Temperature programme: 120° to 280° at 30°/min for 9 min. EI ionisation, SIM acquisition mode. Limit of quantification, 1.0 μg/kg; limit of detection, 0.5 μg/kg [Zhang *et al.* 2006].

HPLC Hand Gauze Patches. Column: octadecylsiloxane C_{18}. Mobile phase: methanol : water (60 : 40), flow rate 2 mL/min. UV detection ($\lambda = 236$ nm). Limit of quantification, 20 μg/L; limit of detection, 7 μg/L. Fluorescence detection ($\lambda_{ex} = 236$ nm, $\lambda_{em} = 418$ nm). Limit of quantification, 150 μg/L; limit of detection, 50 μg/L [Neumeister 1987].

LC-MS Chicken and Rabbit Liver. Column: Waters Xtettra C_{18} (50 × 2.1 mm i.d., 5.0 μm). Mobile phase: acetonitrile : 20 mmol/L ammonium acetate. ESI, negative ion mode, MRM acquisition mode. Limit of quantification, 1 μg/kg [Fang *et al.* 2002]. Bovine Muscle and Liver. Column: Zorbax Eclipse XDB-C_{18} (150 × 2.1 mm i.d., 5.0 μm). Mobile phase: 0.005% acetic acid : acetonitrile (60 : 40), flow rate 0.2 mL/min. ESI, negative ion mode, SIM acquisition mode. Limit of detection, 0.5 μg/kg [Hori, Nakazawa 2000].

Disposition in the Body Zeranol is rapidly metabolised to zearalanone, the main active metabolite. Approximately 55% of a dose is excreted in urine, with less than 1% as free zeranol and free zearalanone compared with 19% and 13% of their respective conjugates.

Therapeutic Concentration

Six healthy volunteers were each administered a single tablet containing 75 mg [^{3}H]zeranol (corresponding to an average dosage of 1.23 mg/kg). A maximum blood concentration of 0.32 mg/L was reached after 1 h [Migdalof *et al.* 1983].

Half-life Approximately 22 h (for [^{3}H]zeranol).

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Zidovudine

Antiviral

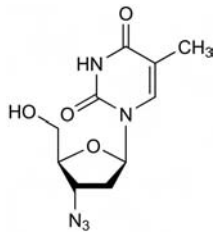
$C_{10}H_{13}N_5O_4$ = 267.2

CAS—30516-87-1

IUPAC Name 1-[4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione

Synonyms Azidodeoxythymidine; 3′-azido-3′-deoxythymidine; azidothymidine; AZT; BW-A509U; BW-509U; compound-S; zidovudinum.

Proprietary Names *Combivir*; *Novo-AZT*; *Retrovir*.



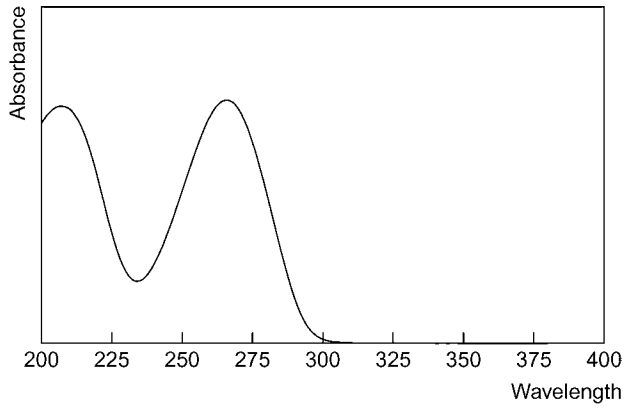
Chemical Properties A white to yellowish or brownish odourless crystalline powder. Mp 124°. Sparingly soluble in water, freely soluble in alcohol and soluble in dehydrated alcohol. pK_a 9.68; 3′-azido-3′-deoxy-5′-β′D-glucopyranuronosyl (GZDV), 3.5. Log *P* (octanol/water), 0.05.

Gas Chromatography Column: 3% OV-1 Chromosorb WHP 80/100 mesh (4 inch × 1/4 inch). Temperature: 280°. Carrier gas: N_2, flow rate 32 mL/min. Detection: flame ionisation. Retention index: 2307 [Mills, Roberson 1993].

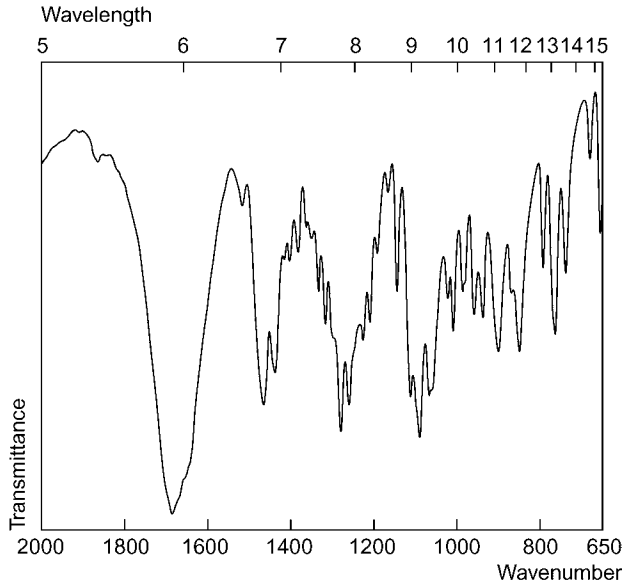
High Performance Liquid Chromatography System HAD—*k* 6.60.

Column: Novapak phenyl (150 × 3.9 mm i.d., 4 µm). Mobile phase: isopropanol : 20 mmol/L, pH 5 sodium citrate (2.5 : 97.5), flow rate 1 mL/min. UV detection (λ=250 nm). Retention time: 22.4 min [Rosell-Rovira *et al.* 1996].

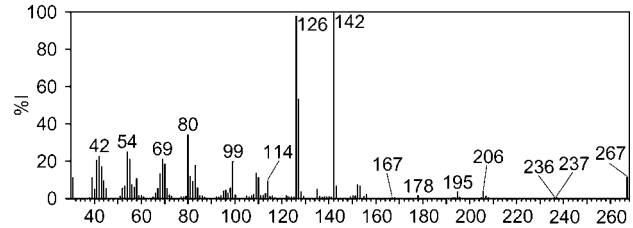
Ultraviolet Spectrum Aqueous acid—207, 266 nm: basic—266 nm.



Infrared Spectrum Principal peaks at wavenumbers 2077, 1675, 1084 cm^{-1} (KBr disk).



Mass Spectrum Principal ions at *m/z* 142, 126, 127, 80, 54, 42, 55, 69.



Quantification

Blood **HPLC** Column: Ultrasphere ODS (75 × 4.6 mm, 3 µm). Mobile phase: phosphoric acid (pH 6.55) : MeCN (95 : 5 to 70 : 30 over 7 min, to 20 : 80 over 1.5 min, return to initial ratio over 1 min), flow rate 1 mL/min. IS: G-AZT. UV detection (λ=266). Retention time: zidovudine, 3.6 min; IS, 5.0 min. Limit of detection, 7 µg/L, limit of quantification, 22 µg/L [Nadal *et al.* 1996].

Plasma **HPLC** Limit of detection, 10 µg/L [Aymard *et al.* 2000]. Column: RPC_{18} Novapak (300 × 3 mm, i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : 0.025 mol/L potassium phosphate (pH 3.1, 3.7 : 2.8 : 93.5), flow rate 1.0 mL/min. UV detection (λ=270 nm). Retention time: zidovudine, 16.8 min; metabolite GZDV, 11.7 min. Limit of quantification, 20 µg/L for zidovudine, 10 µg/L for the 5′-glucuronyl form, GZDV [Schrive, Plasse 1994].

Serum **HPLC** UV detection (λ=250 nm). Limit of detection, 30 µg/L [Simon *et al.* 2001]. MS–MS detection. Limit of detection, 2.5 µg/L [Kenney *et al.* 2000]. Limit of detection, 0.05 mg/L [Nebinger, Koel 1994].

Urine **HPLC** See Plasma [Schrive, Plasse 1994].

Disposition in the Body Zidovudine is rapidly absorbed from the gastrointestinal tract (66 to 70%), although absorption is delayed if the drug is administered with food. It undergoes extensive first-pass metabolism in the liver where ~40% of the oral dose is lost. Metabolism of zidovudine by the kidney is minimal but rapid metabolism occurs intracellularly in the liver to GZDV. Two other hepatic metabolites have been identified, 3′-amino-3′-deoxythymidine (AMT), and its 5′-*O*-glucuronide derivative GAMT. 5′-glucuronide is the major metabolite in plasma and urine accounting for 50 to 80% of an administered dose. Metabolism of zidovudine to AMT occurs in the liver and gastrointestinal microsomes. Zidovudine is highly lipophilic and is widely distributed. It concentrates in the semen where it may exhibit delayed clearance compared to serum. It is excreted in urine as the unchanged drug and its metabolite (63 to 95%), via both glomerular filtration and tubular secretion. After an oral dose, 72 to 74% is excreted as the metabolite and 14 to 18% as unchanged drug. After IV administration, 45 to 60% is excreted as metabolite and 18 to 29% as the unchanged drug. There is no evidence that zidovudine accumulates in plasma or tissues. There is significant penetration into CNS, with a CNS: plasma ratio of about 0.5.

Therapeutic Concentration The trough serum therapeutic concentration range is 0.1 to 0.3 mg/L and peak, 1.0 to 1.5 mg/L.

Thirteen HIV-positive asymptomatic adults with an average age of 33.1 years were administered 300 mg zidovudine after an 8 h fast. These individuals did not have CD4 cell counts ≤200 cells/mm^3. The mean peak zidovudine concentration was 1.64 mg/L at 0.52 h and the peak GZDV concentration 9.41 mg/L at 0.71 h [Wang *et al.* 1999].

Eleven HIV-positive, asymptomatic men (average age, 37.4 years, and with CD4 cell counts ≥300 cells/mm^3) were administered 200 mg zidovudine every 8 h for 4 days. Mean peak zidovudine concentrations were 0.714, 0.594 and 0.394 mg/L for three separate dosing regimens (with washout periods of ~9 days), observed at 0.9, 0.9 and 1.8 h, respectively. Peak GZDV concentrations were 4.655, 3.346 and 2.508 mg/L at 1.0, 1.2 and 1.7 h, respectively for the three dose intervals [Cato *et al.* 1998].

One-hundred HIV-infected patients with CD4 cell counts between 50 and 301 cells/mm^3, mean age 31.4 years, were administered 0.75 mg zalcitabine three times a day, plus 200 mg zidvudine three times a day for 24 weeks. The mean weekly peak zidovudine concentration was 347 µg/L after 1.29 h [Vanhove *et al.* 1997].

Toxicity The serum toxic concentration range is 0.5 to 3 mg/L.

A 34-year-old man with AIDS took a hundred 200 mg tablets of zidovudine with temazepam, after 6 h he experienced symptoms of drowsiness. The blood levels of zidovudine were 185 µmol/L and 5′-gluconuride, 114 µmol/L [Hargreaves *et al.* 1988].

A 20-year-old man took an entire month's supply of zidovudine 2 h before presentation. His blood pressure was 90/40 mmHg, and he had tachycardia of 140 bpm. Serum levels 2 h after the overdose were 339.43 µmol/L (zidovudine) and 565.51 µmol/L (G-AZT); after 12 h, 0.33 µmol/L (zidovudine) and 0.88 µmol/L (G-AZT); after 24 h, <0.05 µmol/L (zidovudine) and <0.13 µmol/L (G-AZT) [Valentine *et al.* 1993].

A 26-year-old male with AIDS ingested 110 to 220 mg/kg zidovudine and unknown amounts of phenobarbital and triazolam. 8 h after ingestion, the man experienced headache and nausea, although the vital signs were normal. 7.75 h after administration, serum levels were 23.82 mg/L (zidovudine) and 89.39 mg/L (G-AZT); after 8.75 h, serum levels were 9.18 mg/L (zidovudine)

and 64.29 mg/L (G-AZT); after 9.75 h, serum levels were 9.18 mg/L (zidovudine) and 26.60 mg/L (G-AZT); after 16 h, serum levels were 0.12 mg/L (zidovudine) and 0.47 mg/L (G-AZT) [Spear *et al.* 1988].

Bioavailability 60 to 70%. This value is erratic in HIV-positive individuals.

Half-life Zidovudine, 0.9 to 1 h; AMT, 2.7 h; GZDV, 1.6 h.

Volume of Distribution Adults (at steady state): 1.4 to 1.6 L/kg, apparent 3.0 L/kg. Children (at steady state): 22 to 64 L/m^2.

Clearance Adults: total body clearance, 22 to 27 mL/min/kg; plasma clearance, 1.3 L/h/kg. Children: mean total body clearance, 30.9 mL/min/kg. Reduced clearance in hepatic and renal impairment.

Protein Binding 34 to 38% (predominately to albumin); 20% plasma.

Dose Adults: oral dose, 500 to 1200 mg daily; IV dose, 1 to 2 mg/kg/dose. Children: oral dose, maximum 200 mg; IV dose, 100 mg/m^2/dose.

Aymard G *et al.* (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 744: 227–240.

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Schrive I, Plasse JC (1994a). Quantification of zidovudine and one of its metabolites in plasma and urine by solid-phase extraction and high-performance liquid chromatography. *J Chromatogr Biomed Appl* 657: 233–237.

Simon VA *et al.* (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. *J Chromatogr A* 913(1-2): 447–453.

Spear JB *et al.* (1988). Zidovudine overdosage. *Ann Intern Med* 109: 76–77.

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Zimeldine

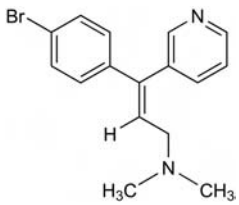
Antidepressant, Selective Serotonin Reuptake Inhibitor (SSRI)

$C_{16}H_{17}BrN_2$ = 317.2

CAS—56775-88-3

IUPAC Name 3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-pyridin-3-ylprop-2-en-1-amine

Note Zimeldine was previously known as zimelidine.



Chemical Properties A white crystalline solid. pK_a 3.8, 8.6. Log *P* (octanol/water), 3.18.

Zimeldine Hydrochloride

$C_{16}H_{17}BrN_2,2HCl,H_2O$ = 408.2

CAS—60525-15-7 (anhydrous) ; 61129-30-4 (monohydrate).

Proprietary Names *Normud*; *Zelmid*.

Chemical Properties A white to yellowish-white crystalline substance. Mp 193°. Soluble 1 in less than 1 of water and 1 in 1000 of ethanol; practically insoluble in chloroform and ether.

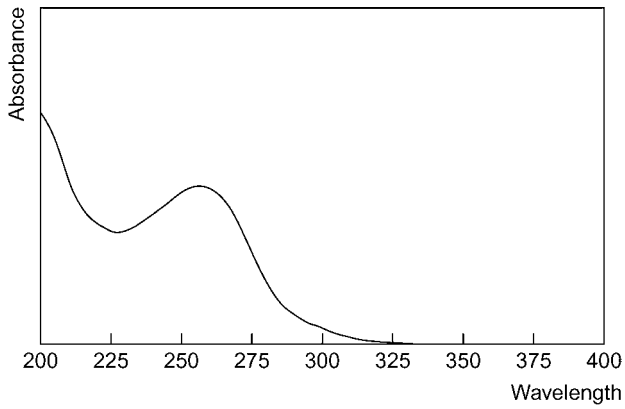
Colour Tests Cyanogen bromide (30 s)—red; Liebermann's reagent—brown.

Thin-layer Chromatography System TA—R_f 0.47; system TB—R_f 0.27; system TC—R_f 0.25; system TE—R_f 0.48; system TAE—R_f 0.20. (Dragendorff spray—positive; location under ultraviolet light—violet fluorescence).

Gas Chromatography System GA—zimeldine RI 2270, M (nor-) RI 2223; system GM—zimeldine RRT 0.820, M (nor-) RRT 0.941 (both relative to iprindole).

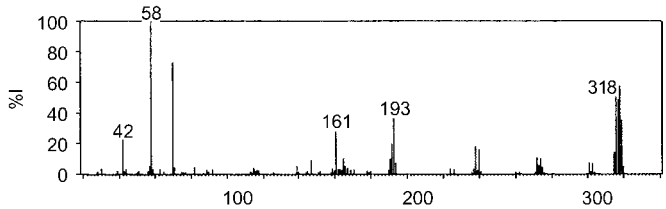
High Performance Liquid Chromatography System HA—zimeldine *k* 3.2 (tailing peak), M (nor-) *k* 2.9 (tailing peak); system HF—*k* 0.67; system HY—RI 270.

Ultraviolet Spectrum Aqueous acid—250 nm (A_1^1 = 630a); aqueous alkali—258 nm.



Infrared Spectrum Principal peaks at wavenumbers 815, 1492, 699, 953, 1075, 840 cm^{-1} (KBr disk).

Mass Spectrum Principal ions at *m*/*z* 58, 70, 318, 316, 317, 193, 319, 161.



Quantification

Plasma **GC** Column: 3% OV-17 Supelcoport 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: N_2, 30 mL/min. Temperature: 255°. Retention time: 3.4 min. Limit of detection, 10 µg/L [Högberg *et al.* 1979].

GC-MS Column: 3% OV-17 on GasChrom Q 80/100 mesh (2 m × 2 mm i.d.). Carrier gas: Ar : CH_4, 60 mL/min. ECD. Limit of detection, 5 µg/L [Caillé *et al.* 1983].

HPLC Column: Nucleosil C_{18} (100 × 3 or 4 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 2.0) : acetonitrile (91 : 9), flow rate 1.0 mL/min. UV detection (λ = 254 nm). Limit of detection, 1.5 for zimeldine and 0.7 µg/L for norzimeldine [Westerlund, Erixson 1979]. Column: µBondapak-CN (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : methanol : phosphate buffer (pH 7.0, 625 : 155 : 220), flow rate 1.5 mL/min. Limit of detection, 5 µg/L [Visser *et al.* 1984].

Disposition in the Body Zimeldine is readily absorbed after oral administration and rapidly demethylated by first-pass metabolism to norzimeldine, which is active. Other metabolites include zimeldine *N*′-oxide and 3-(4-bromophenyl)-3-(3-pyridyl)acrylic acid; very little unchanged drug is excreted in the urine.

Therapeutic Concentration

Following a single oral dose of 100 mg to 10 subjects, peak plasma zimeldine concentrations of 0.048–0.164 mg/L (mean 0.10 mg/L) were attained in 2–3 h and peak plasma norzimeldine concentrations of 0.012–0.092 mg/L (mean 0.04 mg/L) were attained in 1.5–8 h [Caillé *et al.* 1983].

Following oral doses averaging 225 mg daily, given twice a day to 8 subjects, steady-state plasma concentrations of 0.029–0.102 mg/L (mean 0.07 mg/L) zimeldine and 0.145–0.554 mg/L (mean 0.25 mg/L) norzimeldine were reported. The corresponding steady-state concentrations in CSF were 0.003–0.007 mg/L (mean 0.006 mg/L) and 0.027–0.072 mg/L (mean 0.043 mg/L) for zimeldine and norzimeldine, respectively [Calil *et al.* 1982].

Toxicity Zimeldine has been withdrawn from use because of potentially severe neurological side-effects [Montgomery *et al.* 1989]. Recovery within 48 h of ingestion of 3 g zimeldine together with half a bottle of vodka has been reported.

The following postmortem tissue distribution was reported in a female subject who had been receiving 300 mg zimeldine daily and had been found drowned: zimeldine 0.3 mg/L and norzimeldine 0.9 mg/L in blood, zimeldine 5.0 mg/L and norzimeldine 15 mg/L in bile, and zimeldine 4.0 µg/g and norzimeldine 16 µg/g in liver [Geyer 1981].

A 30-year-old woman who was undergoing psychiatric treatment died from a self-inflicted gunshot wound. However, zimeldine concentrations exceeded the normal therapeutic levels (ranging from 0.02–0.25 mg/L). It was known that she was being treated with 100 mg twice daily and other drugs. The postmortem blood concentrations were 0.71 mg/L for zimeldine and 2.2 mg/L for norzimeldine. Zimeldine was also detected at a concentration of 24 µg/g in liver, 1.5 mg/L in urine, 0.77 µg/g in brain tissue and 0.77 µg/g in bile, a total of 3.8 mg/L. Norzimeldine was detected at concentrations of 8.2 mg/kg, 11 mg/L, 3.7 mg/kg and 16 mg/L, respectively [Semple 1984].

A 35-year-old woman attempted suicide by ingesting 5 g zimeldine at 9 a.m. Her plasma zimeldine concentrations were 747, 1985 and 433 µg/L, respectively, at 2, 6 and 12 h later [Ansseau *et al.* 1985].

Bioavailability 20–50%

Half-life Plasma half-life, zimeldine ~4–9 h; norzimeldine ~15–25 h.

Volume of Distribution Zimeldine, ~4 L/kg; norzimeldine, ~9 L/kg.

Clearance Plasma clearance, zimeldine ~7–11 mL/min/kg; norzimeldine ~8 mL/min/kg.
Protein Binding ~90% for zimeldine; ~80% for norzimeldine.
Note For a review of the pharmacokinetics of zimeldine see Heel *et al.* [1982].
Dose Zimeldine hydrochloride has been given in doses of 100 to 300 mg daily.

Ansseau M *et al.* (1985). Extrapyramidal signs following zimelidine overdose. *J Clin Psychopharmacol* 5: 347–349.
Caillé G *et al.* (1983). Pharmacokinetic study of zimelidine using a new GLC method. *Clin Pharmacokinet* 8: 530–540.
Calil HM *et al.* (1982). Zimelidine and norzimelidine protein binding measured by equilibrium dialysis and cerebrospinal fluid. *Clin Pharmacol Ther* 31: 522–527.
Geyer R (1981). *TIAFT Bull* 16: 30–31.
Heel RC *et al.* (1982). Zimelidine: a review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drugs* 24: 169–206.
Högberg K *et al.* (1979). Simultaneous GLC determination of zimelidine and norzimelidine. Synthesis of secondary amines as closely related internal standards. *Acta Pharm Suec* 16: 299–308.
Montgomery SA *et al.* (1989). The specificity of the zimelidine reaction. *Int Clin Psychopharmacol* 4: 19–23.
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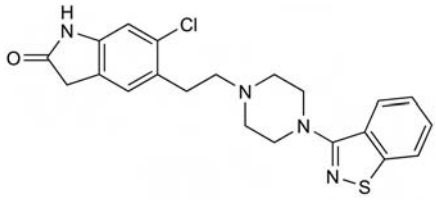
Visser T *et al.* (1984). Reliable routine method for the determination of antidepressant drugs in plasma by high-performance liquid chromatography. *J Chromatogr* 309: 81–93.
Westerlund D, Erixson E (1979). Reversed-phase chromatography of zimelidine and similar dibasic amines. I. Analysis in biological material. *J Chromatogr* 185: 593–603.

Ziprasidone

Atypical Antipsychotic
$C_{21}H_{21}ClN_4OS$ = 412.9
CAS—146939-27-7

IUPAC Name 5-[2-[4-(1,2-Benzothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydroindol-2-one
Synonym CP-88059
Proprietary Names *Geoden*; *Zeldox.*



Chemical Properties White to off white powder. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Ziprasidone Hydrochloride Monohydrate

$C_{21}H_{24}Cl_2N_4O_2S$ = 467.4
CAS—138982-67-9

Synonym

CP-88059-1

Quantification

Blood **LC-MS** Column: Zorbax Stable Bond Cyano (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 2 : 8 : 90) : methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 24 : 36 : 40, 80 : 20 to 20 : 80 in 4.5 min for 2.5 min to 80 : 20 for 2.5 min). TIS, MRM acquisition mode. Retention time: 5.0 min. Limit of detection, 0.8 µg/L [Roman *et al.* 2008].

Plasma **HPLC** Limit of quantification, 10 µg/L [Schlotterbeck *et al.* 2009]. Column: Lichrospher 60 RP-Select B. Mobile phase: acetonitrile : water (40 : 60) containing 0.01 mol/L SDS and 0.5% glacial acetic acid. UV detection (λ=245 nm). Limit of quantification, 10 µg/L [Mauri *et al.* 2007]. Column: Supelcosil LC-1 (250 × 4.6 mm i.d., 5 µm). Mobile phase: 0.05 mol/L monobasic potassium phosphate : acetonitrile : *o*-phosphoric acid : *n*-butylamine (720 : 280 : 1.0 : 1.2), flow rate 1.5 mL/min. Fluorescence detection (λ_{ex}=320 nm, λ_{em}=410 nm). Retention time: 6.6 min. Limit of quantification, 0.5 µg/L [Suckow *et al.* 2004].

LC-MS Column: Luna C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: 0.65 mmol/L aqueous ammonium acetate : methanol : acetonitrile (10 : 45 : 45), flow rate 0.6 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.25 µg/L [Aravagiri *et al.* 2007].

Serum **HPLC** Column: Hypersil ODS C_{18} (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : water : tetramethylethylendiamine (50 : 49.6 : 0.4, pH 6.5), flow rate 1.0 mL/min. UV detection (λ=254 nm). Retention time: 12 min. Limit of quantification, 10 µg/L [Sachse *et al.* 2005]. Column: Supelco, LC-18 DB (250 × 2.1 mm i.d.). Mobile phase: water : acetonitrile : trifluoroacetic acid : TEA (600 : 300 : 0.45 : 0.72), flow rate 0.27 mL/min. UV detection (λ= 215 nm). Retention time: 9.22–9.74 min. Limit of quantification, 0.5–50 µg/L [Janiszewski *et al.* 1995].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.87 µg/L [Kirchherr, Kühn-Velten 2006]. Limit of quantification, 0.5 µg/L [Prakash *et al.* 1997a].

Milk **HPLC** See Plasma [Schlotterbeck *et al.* 2009].

Other **HPLC** Human Liver Microsomes. Column: YMC basic (150 × 4.6 mm i.d., 3 µm). Mobile phase: 10 mmol/L ammonium acetate (pH 5.0) : methanol, flow rate 1.0 mL/min. ESI. Retention time: ~40 min. Limit of detection not reported [Miao *et al.* 2005]. Rat Urine. Column: YMC basic (250 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 5.00) : methanol (90 : 10 for 10 min to 20 : 80 over 50 min for 7 min to 90 : 10 over 8 min. Limit of detection not reported [Prakash *et al.* 1997b].

Disposition in the Body Ziprasidone is rapidly absorbed and extensively metabolised by *N*-dealkylation, oxidation, reductive cleavage, hydration and *N*-dearylation. Twelve metabolites have been identified in urine and serum; the major ones in urine are oxindole acetic acid and its glucuronide conjugate, benzisothiazole-3-yl-piperazine (BITP), BITP sulfoxide, BITP sulfone and its lactam, ziprasidone sulfoxide, and ziprasidone sulfone (the major metabolites in serum). Other metabolites include *S*-methyldihydroziprasidone, *S*-methyldihydroziprasidone sulfoxide and 6-chloro-5-(2-piperazin-1-yl-ethyl)-1,3-dihydro-indol-2-one. After 10 days of treatment with ziprasidone, approx. 20% of an administered dose is excreted in urine, with only a small proportion as the unchanged drug. In patients with mild to moderate impairment of liver function, the serum concentrations were 30% higher than in normal patients. No significant differences in the pharmacokinetics of ziprasidone have been observed in patients with impaired renal function. Less than a third of the clearance of ziprasidone is mediated by P450 CYP iosforms but CYP3A4 appears to be the main isoform involved, leading to interactions with the classic inhibitor ketoconozole [Trenton *et al.* 2003].

Therapeutic Concentration

Four healthy male volunteers were fasted overnight and administered a 20 mg suspension of ziprasidone after a standard breakfast for 11 days. The mean peak plasma concentration was 45.4 µg/L (range 28.8–62.0 µg/L) for the 4 subjects which was observed at ~3.5 h (range 2–6 h) [Prakash *et al.* 1997a].

Thirty-nine healthy males, 18 to 45 years of age, were administered fixed doses of 10, 40, 40 escalated to 80, and 40 escalated to 120 mg daily. For all doses, a dose of 20 mg was administered on day 1 and a 48 h wash-out period allowed. On subsequent days, the dose was administered twice daily and escalated to 80 mg or 120 mg on days 7 and 10, respectively. The peak plasma concentrations on day 1 were 12.2, 26.6, 60.0 and 34.3 µg/L for the 4 dosing regimens, respectively, observed at 5.0, 4.8, 3.8 and 4.0 h, respectively. On day 18, the peak plasma concentrations were 14.8, 44.6, 118.6 and 139.4 µg/L for the 10, 40, 80 and 120 mg doses, respectively, observed at 5.2, 3.8, 3.7 and 4.7 h [Miceli *et al.* 2000].

Sixty-seven patients treated with 40 to 280 mg ziprasidone for at least 7 days reached a median steady-state serum concentration of 76 µg/L. A concentration of 40–130 µg/L may be considered the recommended target plasma concentration [Sachse *et al.* 2005].

Thirteen schizophrenic patients were administered doses of 80–160 mg/day ziprasidone for 8 weeks. Plasma concentrations ranged from 16–160 µg/L with a mean of 75.8 ± 51 µg/L. No correlation was found between the oral dose and plasma concentrations [Mauri *et al.* 2007]

Toxicity Common clinical effects following ziprasidone overdose are drowsiness and tachycardia [Klein-Schwartz *et al.* 2007]. Ziprasidone is widely reported to cause QTc prolongation, which in one patient has been associated with torsades de pointes [Manini *et al.* 2007]. Ziprasidone has been associated with instances of pathological laughing when used for drug-induced psychosis in Parkinson's disease [Schindehütte, Trenkwalder 2007], neuroleptic malignant syndrome [Ozen *et al.* 2007] and tardive dyskinesia [[Sinha *et al.* 2007]. For a review of exposures, see Forrester [2008] or LoVecchio *et al.* [2005]. For overdose, see Burton *et al.* [2000], Fasano *et al.* [2009], Gómez-Criado *et al.* [2005], House [2002], Insa Gómez, Gutierrez [2005], Prieto *et al.* [2005] and MacVane, Baumann [2009].

Half-life Normally 4 h but is increased by ~2 h in patients with mild to moderately impaired liver function.

Bioavailability Oral, 60% [MacVane, Baumann 2009].

Milk : plasma ratio 0.06 [Schlotterbeck *et al.* 2009].

Protein Binding 99% [Trenton *et al.* 2003].

Aravagiri M *et al.* (2007). Determination of ziprasidone in human plasma by liquid chromatography–electrospray tandem mass spectrometry and its application to plasma level determination in schizophrenia patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 847: 237–244.
Burton S *et al.* (2000). Ziprasidone overdose. *Am J Psychiatry* 157: 835.
Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.
Fasano CJ *et al.* (2009). Pediatric ziprasidone overdose. *Pediatr Emerg Care* 25: 258–259.
Forrester MB (2008). Pattern of ziprasidone exposures reported to Texas poison centers, 2001–2005. *Hum Exp Toxicol* 27: 355–361.
Gómez-Criado MS *et al.* (2005). Ziprasidone overdose: cases recorded in the database of Pfizer-Spain and literature review. *Pharmacotherapy* 25: 1660–1665.
House M (2002). Overdose of ziprasidone. *Am J Psychiatry* 159: 1061–1062.
Insa Gómez FJ, Gutierrez CJr (2005). Ziprasidone overdose: cardiac safety. *Actas Esp Psiquiatr* 33: 398–400.
Janiszewski JS *et al.* (1995). Development and validation of a high-sensitivity assay for an antipsychotic agent, CP-88,059, with solid-phase extraction and narrow-bore high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 668: 133–139.

Kirchherr H, Kühn-Velten WN (2006). Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B Analyt Technol Biomed Life Sci* 843: 100–113.
Klein-Schwartz W *et al.* (2007). Prospective observational multi-poison center study of ziprasidone exposures. *Clin Toxicol (Phila)* 45: 782–786.
LoVecchio F *et al.* (2005). Three-year experience with ziprasidone exposures. *Am J Emerg Med* 23: 586–587.
MacVane CZ, Baumann MR (2009). Unintentional ingestion of ziprasidone in a 22-month-old. *Pediatr Emerg Care* 25: 460–462.
Manini AF *et al.* (2007). QT prolongation and torsades de pointes following overdose of ziprasidone and amantadine. *J Med Toxicol* 3: 178–181.
Mauri MC *et al.* (2007). Ziprasidone outcome and tolerability: a practical clinical trial with plasma drug levels. *Pharmacopsychiatry* 40: 89–92.
Miao Z *et al.* (2005). Characterization of a novel metabolite intermediate of ziprasidone in hepatic cytosolic fractions of rat, dog, and human by ESI-MS/MS, hydrogen/deuterium exchange, and chemical derivatization. *Drug Metab Dispos* 33: 879–883.
Miceli JJ *et al.* (2000). Single- and multiple-dose pharmacokinetics of ziprasidone under non-fasting conditions in healthy male volunteers. *Br J Clin Pharmacol* 49(Suppl1): 5S–13S.
Ozen ME *et al.* (2007). Neuroleptic malignant syndrome induced by ziprasidone on the second day of treatment. *World J Biol Psychiatry* 8: 42–44.
Prakash C *et al.* (1997a). Metabolism and excretion of a new antipsychotic drug, ziprasidone, in humans. *Drug Metab Dispos* 25: 863–872.
Prakash C *et al.* (1997b). Characterization of the novel benzisothiazole ring-cleaved products of the antipsychotic drug ziprasidone. *Drug Metab Dispos* 25: 897–901.
Prieto T *et al.* (2005). The highest intentional ziprasidone overdose was not fatal. *Acta Psychiatr Scand* 112: 79–80.
Roman M *et al.* (2008). Quantitation of seven low-dosage antipsychotic drugs in human postmortem blood using LC-MS-MS. *J Anal Toxicol* 32: 147–155.
Sachse J *et al.* (2005). Automated determination of ziprasidone by HPLC with column switching and spectrophotometric detection. *Ther Drug Monit* 27: 158–162.
Schindehütte J, Trenkwalder C (2007). Treatment of drug-induced psychosis in Parkinson's disease with ziprasidone can induce severe dose-dependent off-periods and pathological laughing. *Clin Neurol Neurosurg* 109: 188–191.
Schlotterbeck P *et al.* (2009). Low concentration of ziprasidone in human milk: a case report. *Int J Neuropsychopharmacol* 12: 437–438.
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Suckow RF *et al.* (2004). Determination of plasma ziprasidone using liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 799: 201–208.
Trenton A *et al.* (2003). Fatalities associated with therapeutic use and overdose of atypical antipsychotics. *CNS Drugs* 17: 307–324.

Zolmitriptan

5-HT_1 Receptor Agonist, Antimigraine

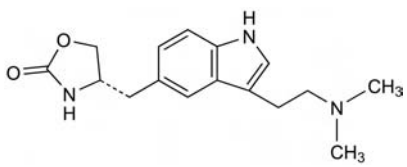
$C_{16}H_{21}N_3O_2$ = 287.4

CAS—139264-17-8

IUPAC Name (4*S*)-4-[[3-(2-Dimethylaminoethyl)-1*H*-indol-5-yl]methyl]- 1,3-oxazolidin-2-one

Synonym 311C90

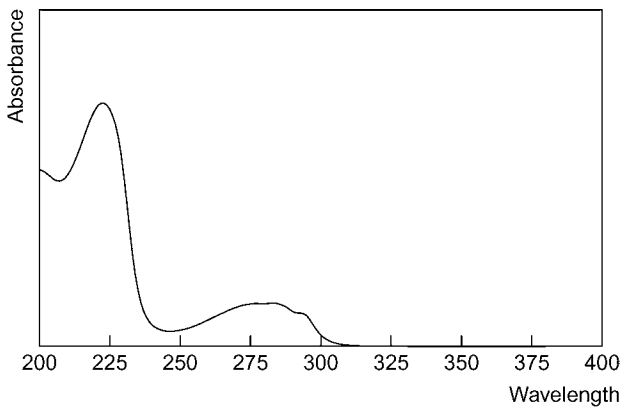
Proprietary Name *Zomig*



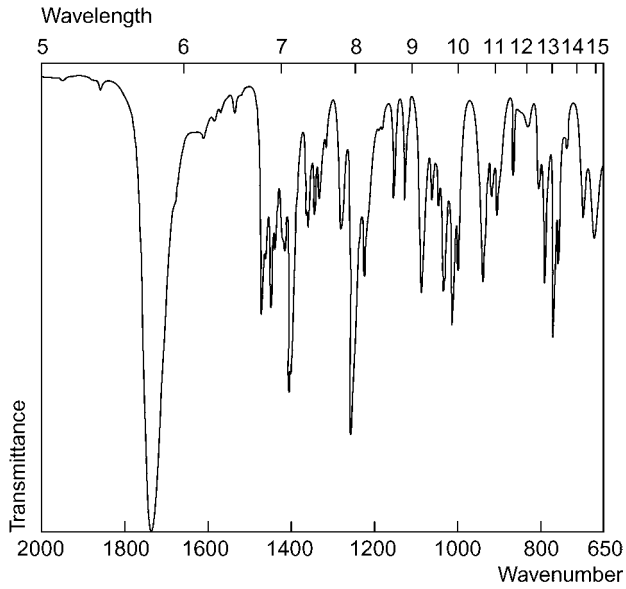
Chemical Properties A white to almost white crystalline powder. Mp 139° to 141°. Readily soluble in water. pK_a 9.6.

High Performance Liquid Chromatography System HZ—retention time 1.8 min.

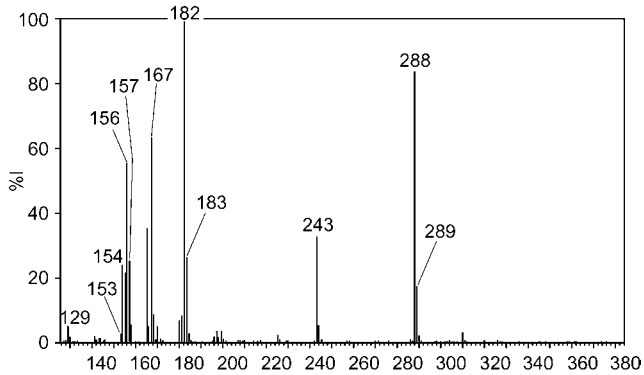
Ultraviolet Spectrum Principal peaks at 225, 301 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 182, 288, 167, 156, 165, 243, 183, 157.



Quantification

Plasma **HPLC** MS–MS detection. Limit of detection, 0.1 µg/L [Seaber *et al.* 1998]. UV detection (λ=230 nm). Limit of detection, 2 µg/L [Seaber *et al.* 1997].

Serum **HPLC** Column: silica Alltech (150 × 2.1 mm i.d., 5 µm). Mobile phase: 20 mmol/L ammonium acetate (pH 4); formic acid : methanol : acetonitrile (80 : 10 : 10) (pH 2.7). Detection: MS–MS. Retention time: 2.95 min. Limit of detection, 0.1 µg/L [Vishwanathan *et al.* 2000].

Urine **HPLC** See Plasma. Limit of detection, 100 µg/L [Seaber *et al.* 1997].

Disposition in the Body Zolmitriptan is absorbed after oral administration, which is unaffected by food. There is extensive hepatic metabolism, principally to the indole acetic acid, and also the *N*-oxide and *N*-desmethyl analogues. The *N*-desmethyl metabolite is more active than the parent compound in animal studies. Metabolism is primarily mediated by CYP1A2, and monoamine oxidase is responsible for further metabolism of the *N*-desmethyl metabolite. At least 60% of the dose is excreted in urine, mainly as the indole acetic acid. Approximately 30% is excreted in faeces, mainly as the unchanged drug. There is no evidence of accumulation on multiple dosing.

Therapeutic Concentration

Twenty-four normal individuals, 12 aged between 18 and 39 years, and 12 aged from 65 to 76 years old, were fasted overnight and administered zolmitriptan (5, 10 and 15 mg doses). The peak plasma drug concentrations for the young individuals were 9.1, 18.9 and 23.3 µg/L for the three respective doses. The group aged from 65 to 76 years showed peak zolmitriptan concentrations of 9.9, 18.6 and 22.7 µg/L for the respective doses [Peck *et al.* 1998].

Twenty healthy individuals (average age of 23 years) were administered a single oral dose of 2.5 mg and the equivalent IV dosage. Peak drug concentrations were observed within the hour and were sustained for 6 h at levels of 3.3 µg/L (for men), and 3.8 µg/L (for women). For the IV doses, the females and males produced peak concentrations of 5.8 and 10.6 µg/L, respectively, when administered 2.5 mg [Seaber *et al.* 1998].

Toxicity Doses of 50 mg result in sedation. Other side effects include transient increase in blood pressure, myalgia, and muscle weakness.

Bioavailability 40 to 50%.

Half-life 2.5 to 3 h, prolonged in patients with hepatic impairment.

Volume of Distribution About 7.0 L/kg.

Clearance Total plasma clearance, about 31.5 mL/min/kg.

Protein Binding 25%.

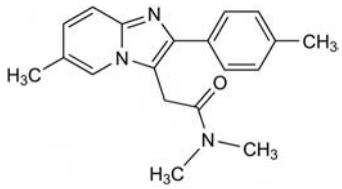
Dose The usual dose is 2.5 mg with a maximum dose of 15 mg in 24 h.

Peck RW *et al.* (1998). The pharmacodynamics and pharmacokinetics of the 5HT1B/1D-agonist zolmitriptan in healthy young and elderly men and women. *Clin Pharmacol Ther* 63(3): 342–353.
Seaber EJ *et al.* (1998). The absolute bioavailability and effect of food on the pharmacokinetics of zolmitriptan in healthy volunteers. *Br J Clin Pharmacol* 46(5): 433–439.
Seaber E *et al.* (1997). The absolute bioavailability and metabolic disposition of the novel antimigraine compound zolmitriptan (311C90). *Br J Clin Pharmacol* 43(6): 579–587.
Vishwanathan K *et al.* (2000). Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14(3): 168–172.

Zolpidem

Hypnotic
$C_{19}H_{21}N_3O = 307.4$
CAS—82626-48-0

IUPAC Name *N*,*N*-Dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide
Synonyms SL-80.0750; *N*,*N*-6-trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide.

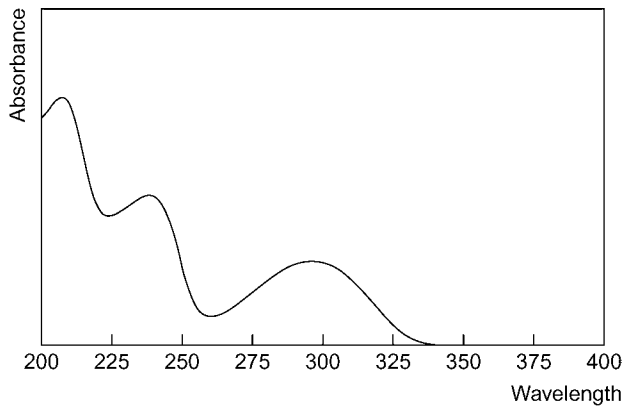


Chemical Properties Mp 196°. Insoluble in water. pK_a 6.2. Log *P* (octanol/water) 3.85. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

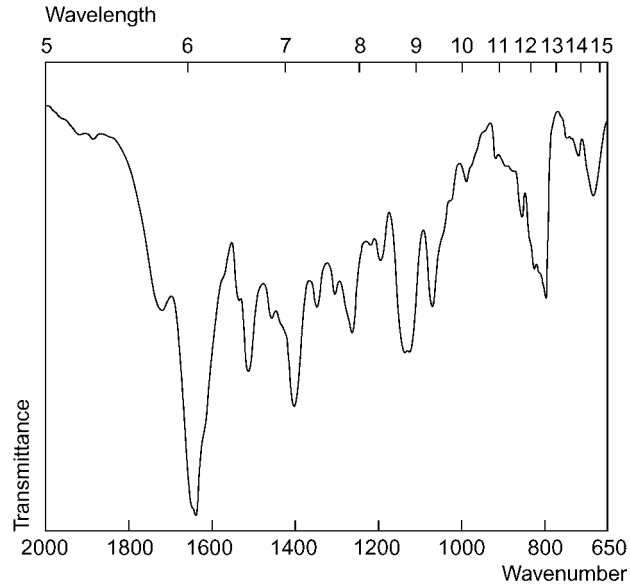
Zolpidem Tartrate

$(C_{19}H_{21}N_3O)_2,C_4H_6O_6 = 764.9$
CAS—99294-93-6
IUPAC Name (2*R*,3*R*)-2,3-Dihydroxybutanedioic acid; *N*,*N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide
Synonyms SL-80.0750-23N; zolpidem hemitartrate.
Proprietary Names *Ambien*; *Bikalm*; *Cedrol*; *Edluar*; *Ivadal*; *Niotal*; *Stilnoct*; *Stilnox*; *Tovalt*.
Chemical Properties A white or almost white hygroscopic crystalline powder. Mp 193° to 197°. It is slightly soluble in water (23 g/L at 20°); practically insoluble in dichloromethane; sparingly soluble in methanol.

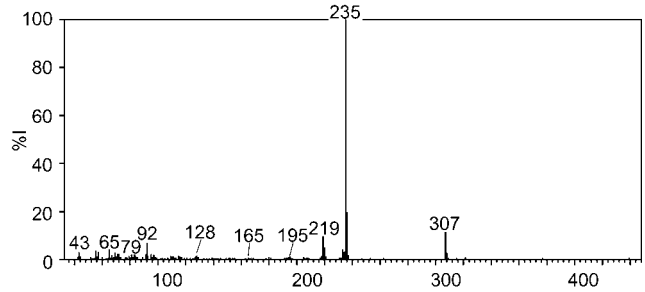
Gas Chromatography System GB—RI 2941; system GT—RI 2715.
Column: Ultra 2 5% phenyl methyl silicone fused silica capillary (25 m × 0.32 mm i.d., 0.52 µm). Carrier gas: He, 2.1 mL/min. Temperature programme: 210° to 285° at 2.5°/min. IS: prazepam. ECD/NPD. Retention time: 1.2 min for zolpidem, 1 min for IS [Gaillard *et al.* 1993].
High Performance Liquid Chromatography System HY—RI 291; system HZ—RT 3.2 min; system HAA—RT 11.9 min.
Column: µBondapak C_{18} (300 × 3.9 mm i.d.). Mobile phase: acetonitrile : 50 nmol/L potassium dihydrogen phosphate (50 : 50), flow rate 1.5 mL/min. IS: propylzolpidem. Fluorescence detection (λ_{ex} = 254 nm, λ_{ex} = 390 nm). Retention time: 3.10 min for zolpidem, 4.75 min for IS [Durol, Greenblatt 1997].
Ultraviolet Spectrum Aqueous acid (pH 2.6)—207 nm; (methanol)—243, 314 nm; (0.1 mol/L hydrochloric acid)—236, 294 nm; (0.2 mol/L sulfuric acid)—237, 295 nm; (0.025 mol/L sulfuric acid)—238, 294.5 nm; (0.1 mol/L ammonium formate, pH 3)—238, 296 nm; aqueous alkali (0.1 mol/L sodium hydroxide)—242 nm.



Infrared Spectrum Principal peaks at wavenumbers 1637, 1402, 1511, 1136 cm^{-1} (zolpidem tartate; KBr pellet).



Mass Spectrum Principal ions at *m*/*z* 235, 307, 219, 92, 65, 42, 115, 102.



Quantification
Blood **GC-MS** SIM acquisition mode [Rohrig *et al.* 2010]. EI ionisation, full scan mode. Comparison with ELISA [Reidy *et al.* 2008]. Column: DB 35MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 330° at 15°/min for 2.8 min. EI ionisation at 70 eV. Retention time: 15.12 min. Limit of quantification, 30 µg/L, limit of detection, 1.5 µg/L [Gunnar *et al.* 2005]. Column: DB-35 MS (30 m × 0.32 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 25 µg/L, limit of detection, <10 µg/L [Gunnar *et al.* 2004]. Column: HP-5 MS 95% dimethyl polysiloxane, 5% diphenylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 280° for 6.5 min to 320° at 20°/min for 1.5 min. MSD, SIM acquisition mode. Retention time: 5.28 min. Limit of detection not reported [Keller *et al.* 1999].

HPLC Fluorescence detection (λ = 254 nm). Limit of detection, 10 µg/L [Guinebault *et al.* 1986].

LC-MS Column: Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: methanol: ammonium formate. MRM acquisition mode, positive ion mode. Limit of quantification, 0.0005–0.01 mg/kg for zolpidem and other drugs of abuse and metabolites [Bjork *et al.* 2010]. Zolpidem, zopiclone and benzodiazepines [Deveaux *et al.* 2008]. Column: XTerra MS C_{18}. Mobile phase: methanol : formate buffer. MRM acquisition mode. Limit of quantification, 1–2 µg/L for zolpidem, zopiclone, benzodiazepines and metabolites [Laloup *et al.* 2005]. Column: Inertsil ODS-3 (150 × 2.0 mm i.d., 3 µm). Mobile phase: 1 mmol/L ammonium formate buffer : acetonitrile (90 : 10 for 2 min to 40 : 60 in 15 min for 3 min to 90 : 10 in 1 min. APCI, positive ion mode, SIM acquisition mode. Limit of detection, 0.1 µg/L [Giroud *et al.* 2003].

Plasma **GC** Column: OV-1 fused silica (25 m × 0.32 mm i.d., 0.2 µm). Temperature: 235°. Carrier gas: He, 1.3 mL/min. IS: clonazepam. NPD. Retention time: 10.02 min for zolpidem, 10.72 min for IS, limit of detection, 1 µg/L for zolpidem and 2 µg/L for zopiclone [Stanke *et al.* 1996]. Column: Ultra 2 (5% phenylmethylsiloxane). NPD and ECD. Zolpidem and benzodiazepines. Limit of detection, 10 µg/L for zolpidem [Gaillard *et al.* 1993].

HPLC Column:YMC-Pack C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: 20 mmol/L phosphate buffer (pH 3) : acetonitrile (73 : 27), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}=320 nm, λ_{em}= 388 nm). Limit of quantification, 1.8 µg/L [Nirogi *et al.* 2006]. Column: Shiseido Capcell PAK UG C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L potassium phosphate (pH 6.0, 4 : 6). IS: trazodone. Fluorescence detection (λ_{ex} = 254 nm, λ_{em} = 400 nm). Retention time: 4.6 min for zolpidem, 5.3 min for IS. Limit of quantification, 1 µg/L [Ring, Bostick 2000]. Column: Nucleosil 100-3 C_{18} (150 × 4.6 mm i.d.).

Temperature programme: 35°. Mobile phase: methanol : 30 mmol/L potassium dihydrogen phosphate (pH 6.8) : TEA (30 : 69 : 1), flow rate 0.7 mL/min. Fluorescence detection (λ_{ex} = 244 nm, λ_{em} = 388 nm). Retention time: 4.7 min. Limit of quantification, 1.5 µg/L [Ptácek *et al.* 1997]. Column: Nova-Pak C_{18} (300 × 3.9 mm i.d., 4 µm). Temperature: 30°. Mobile phase: methanol : tetrahydrofuran : phosphate buffer (pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. DAD (λ = 210 nm). Retention time: 4.66 min. Limit of detection, ~24 µg/L [Tracqui *et al.* 1993a]. Column: Supelcosil LC_{18}-DB (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.05 mol/L phosphate buffer (pH 6.0, 60 : 40) containing 0.75% methanol, flow rate 1.0 mL/min. Fluorescence detection (λ_{ex} = 254 nm, λ_{em} = 390 nm). Limit of detection, 0.2 µg/L [Ascalone *et al.* 1992]. See Blood [Guinebault *et al.* 1986].

LC-MS Column: Acquity UPLC BEH C_{18} (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.05% formic acid in water : 0.05% formic acid in acetonitrile (75 : 25 for 0.5 min to 70 : 30 at 6 min to 59.5 : 40.5 at 9.5 min to 10 : 90 at 13 min to 75 : 25 at 17 min), flow rate 0.4 mL/min. SIR acquisition mode. Retention time: 1.56 min. Limit of detection, 0.2–8 µg/L for zopiclone, zolpidem, benzodiazepines and metabolites [Ishida *et al.* 2009]. Column: C_8 reversed phase (50 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 10 mmol/L ammonium acetate (pH 4.6, 80 : 20), flow rate 0.7 mL/min. ESI, SRM acquisition mode. Limit of quantification, 2.5 µg/L [Bhatt *et al.* 2006]. Column: Merck LiChroCART with Superspher 60 RP Select B stationary phase. Mobile phase: ammonium formate : acetonitrile. APCI. Zolpidem and benzodiazepines and related drugs [Kratzsch *et al.* 2004].

Serum **GC** Column: DB 5 (5 m × 0.32 mm i.d., 0.17 µm). Carrier gas: N_2, 50 cm/s. Temperature: 230°. NSD. Limit of quantification, 5 µg/L for zopiclone and zolpidem [Debruyne *et al.* 1991].

Urine **GC-MS** Column: HP-1 (12 m × 0.2 mm i.d., 0.33 µm). Carrier gas: He, 1.0 mL/min. Temperature programme: 150° for 1 min to 310° at 30°/min for 3 min. SIM acquisition mode. Limit of detection, 2 µg/L for zolpidem carboxylic acid [Lewis, Vine 2007]. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001]. See Blood [Keller *et al.* 1999; Reidy *et al.* 2008].

HPLC See Plasma. Limit of detection, 1 µg/L [Ascalone *et al.* 1992].

LC-MS Limit of detection, <0.5 µg/L for zolpidem, zopiclone and benzodiazepines [Deveaux *et al.* 2008]. Column: Waters XTerra MS C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile-2 mmol/L ammonium formate (pH 3, 90 : 10) : 2 mmol/L ammonium formate (pH 3, 30 : 70 to 36 : 64 in 4 min for 4.5 min to 90 : 10 in 3.5 min to 30 : 70 in 1.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 2.8 min. Limit of detection, 0.01 µg/L [Quintela *et al.* 2006]. Column: XTerra MS C_{18}. Mobile phase: methanol : formate buffer. MRM acquisition mode. Limit of quantification, 10–25 µg/L for zolpidem, zopiclone, benzodiazepines and metabolites [Laloup *et al.* 2005]. Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : formate buffer (5 : 95 at time 0 to 60 : 40 at 3 min to 80 : 20 at 7 min until 10 min to 5 : 95 at 10.5 min for 9.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.1 ng/mg, limit of detection, 0.01 µg/L [Villain *et al.* 2004].

Immunoassay EMIT. Limit of detection, 5 µg/L. Comparison with LC-MS [Huynh *et al.* 2009].

Stomach Contents **GC-MS** See Blood [Keller *et al.* 1999].

Hair **LC-MS** Column Zorbax SB-Phenyl (100 × 2.1 mm i.d., 3.5 µm). Positive mode ESI. Zolpidem and benzodiazepines and other drugs [Hegstad *et al.* 2008]. Column: XTerra MS C_{18}. Mobile phase: methanol : formate buffer. MRM acquisition mode. Limit of quantification, 0.5–10 ng/g for zolpidem, zopiclone, benzodiazepines and metabolites [Laloup *et al.* 2005]. See Urine [Villain *et al.* 2004].

Disposition in the Body Zolpidem is absorbed after oral administration; bioavailability is ~70%. Peak plasma concentrations are attained within 0.5 and 3 h. It displays linear pharmacokinetics within the therapeutic dose range and undergoes first-pass metabolism by the liver. Metabolic pathways include oxidation and hydroxylation. The inactive metabolites are excreted in urine (56%) and faeces (37%), with <1% excreted as the unchanged drug. It is distributed into breast milk and crosses the placenta. It is not removed by dialysis.

Therapeutic Concentration The therapeutic concentration range in serum is 80–150 µg/L.

Twelve men aged between 23 and 36 years (mean, 28) were administered 10 mg oral zolpidem. The peak concentration observed at 2.6 h was 76 µg/L [Colle *et al.* 1991].

Eighteen male volunteers (average age 26 years) were administered single oral and IV doses of zolpidem. The 12 men who received 20 mg orally showed a mean peak concentration of 55 µg/L at 1.0 h. The remaining men were administered 5 mg IV, and the peak concentration was 244 µg/L at 1.1 h [Patat *et al.* 1994].

In 16 healthy woman (including smokers and some taking oral contraceptives) given 5 mg zolpidem tartrate, maximum serum levels were 60 µg/L. Clearance was higher (445 vs. 345 mL/min) and half-life shorter (1.8 vs. 2.7 h) in smokers than in non-smokers. Those taking oral contraceptives also had higher clearance and a shorter zolpidem half-life [Olubodun *et al.* 2002].

After 5 mg zolpidem, elderly subjects had higher maximum serum levels than younger subjects (93 vs. 40 µg/L) and half-life was also increased in elderly subjects (2.7 vs. 1.5 h) [Olubodun *et al.* 2003].

Toxicity The serum toxic concentration is 500 µg/L.

In a fatality, zolpidem blood concentration was 3.29 mg/L; acepromazine was also detected [Tracqui *et al.* 1993b].

In an analysis of 344 cases of acute overdose with zolpidem, ingested doses ranged from 10–1400 mg. Half the patients had also taken other drugs (psychotropic drugs or alcohol). Signs of intoxication were attributed to zolpidem in 105 cases. Drowsiness occurred at doses of 140–440 mg [Garnier *et al.* 1994].

A 39-year-old man who died after an overodse of zolpidem had levels of 2.91, 1.40 and 2.13 mg/L in heart blood, peripheral blood and urine, respectively. The zolpidem concentration in the liver was 4.74 µg/g and the gastric contents contained a total of 172 mg [Meeker *et al.* 1995].

A 68-year old woman died after ingesting at least 300 mg zolpidem. Postmortem zolpidem blood concentration was 4.1 mg/L; mebrobamate and carisoprodol were also detected [Winek *et al.* 1996].

In a review of cases of zolpidem poisoning, only mild symptoms were found with ingestion of up to 0.6 g, although 1 anorexic patient became comatose after ingestion of 0.6 g [Wyss *et al.* 1996].

An elderly woman who was found dead in the bath and who had taken up to a possible 60 tablets of 10 mg zolpidem each had blood and urine levels of 7.9 and 4.1 mg/L, respectively. A total of 7 mg of unabsorbed zolpidem was found in the gastric contents. However, death was attributed to drowning and death solely from the zolpidem overdosage was considered an unlikely occurrence [Lichtenwalner, Tully 1997].

Caffeine, risperidone and zolpidem were found in the urine of 36-year-old woman who was found dead. The following postmortem levels of zolpidem were found: 4.6 mg/L in subclavian blood, 7.7 mg/L in iliac blood, 1.6 mg/L in vitreous humour, 8.9 mg/L in bile, 1.2 mg/L in urine, 22.6 mg/L in liver and 42 mg in gastric contents. Serum also contained 5.6 µg/L 9-hydroxyrisperidone. Death was attributed to acute zolpidem overdose [Gock *et al.* 1999].

In a 58-year-old woman who was found dead, zolpidem and carbamazepine were found in the urine. Postmortem zolpidem levels were 1.6 mg/L in iliac blood, 0.52 mg/L in vitreous humour, 2.6 mg/L in bile, 12 mg/L in liver and 0.9 g in gastric contents. Death was attributed to acute zolpidem overdose [Gock *et al.* 1999].

In 8 fatalities where zolpidem was identified at postmortem, zolpidem was found at therapeutic levels in 3. Five deaths were attributed to drug intoxication and in 3 of these, zolpidem was considered an incidental finding. In 2 deaths, zolpidem levels were elevated and considered to contribute to the drug intoxication [Levine *et al.* 1999].

A 23-year-old woman who was a victim of drug-facilitated sexual assault had blood and urine zolpidem levels of 16 and 32 ng/L, respectively, 6 days later. Zolpidem level in hair was 0.75 ng/g [Kintz *et al.* 2005].

In another report of drug-facilitated sexual assault, the blood level of zolpidem 11 h after the last supposed intake was 47 µg/L [Maravelias *et al.* 2009].

For another report of zolpidem intoxication, see [Sadeg *et al.* 1999].

Bioavailability ≈70%.

Half-life Plasma half-life, 1.7–2.5 h in normal subjects; increased to ~10 h in patients with cirrhosis.

Volume of Distribution 0.54–0.68 L/kg.

Clearance Plasma clearance, 0.26 L/h/kg.

Protein Binding 92%.

Dose The usual dose is 10 mg, occasionally 15 or 20 mg, but this is associated with increased risk of side-effects. In the elderly, the dose is reduced to 5 mg.

Ascalone V *et al.* (1992). Determination of zolpidem, a new sleep-inducing agent, and its metabolites in biological fluids: pharmacokinetics, drug metabolism and overdosing investigations in humans. *J Chromatogr* 581: 237–250.

Bhatt J *et al.* (2006). Quantification of zolpidem in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry. *Biomed Chromatogr* 20: 736–742.

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Bjork MK *et al.* (2010). Determination of 19 drugs of abuse and metabolites in whole blood by high-performance liquid chromatography–tandem mass spectrometry. *Anal Bioanal Chem* 396: 2393–2401.

Colle M *et al.* (1991). Nocturnal profile of growth hormone secretion during sleep induced by zolpidem: a double-blind study in young adults and children. *Horm Res* 35: 30–34.

Debruyne D *et al.* (1991). Determination of zolpidem and zopiclone in serum by capillary column gas chromatography. *J Pharm Sci* 80: 71–74.

Demme U *et al.* (2005). Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs. *Proceedings of the 12th TIAFT*, Seoul: 481–486.

Deveaux M *et al.* (2008). The role of liquid chromatography–tandem mass spectrometry (LC-MS/MS) to test blood and urine samples for the toxicological investigation of drug-facilitated crimes. *Ther Drug Monit* 30: 225–228.

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Garnier R *et al.* (1994). Acute zolpidem poisoning: analysis of 344 cases. *J Toxicol Clin Toxicol* 32: 391–404.

Giroud C *et al.* (2003). Determination of zaleplon and zolpidem by liquid chromatography–turboionspray mass spectrometry: application to forensic cases. *J Chromatogr B Analyt Technol Biomed Life Sci* 789: 131–138.

Gock SB *et al.* (1999). Acute zolpidem overdose: report of two cases. *J Anal Toxicol* 23: 559–562.

Guinebault P *et al.* (1986). High-performance liquid chromatographic determination of zolpidem, a new sleep inducer, in biological fluids with fluorimetric detection. *J Chromatogr* 383: 206–211.

Gunnar T *et al.* (2004). Validated semiquantitative/quantitative screening of 51 drugs in whole blood as silylated derivatives by gas chromatography–selected ion monitoring mass spectrometry and gas chromatography electron capture detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 806: 205–219.
Gunnar T *et al.* (2005). Determination of 14 benzodiazepines and hydroxy metabolites, zaleplon and zolpidem as *tert*-butyldimethylsilyl derivatives compared with other common silylating reagents in whole blood by gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 818: 175–189.
Hegstad S *et al.* (2008). Drug screening of hair by liquid chromatography–tandem mass spectrometry. *J Anal Toxicol* 32: 364–372.
Huynh K *et al.* (2009). Development of a homogeneous immunoassay for the detection of zolpidem in urine. *J Anal Toxicol* 33: 486–490.
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Keller T *et al.* (1999). GC/MS determination of zolpidem in postmortem specimens in a voluntary intoxication. *Forensic Sci Int* 106: 103–108.
Kintz P *et al.* (2005). Drug-facilitated sexual assault and analytical toxicology: the role of LC-MS/MS. *A case involving zolpidem. J Clin Forensic Med* 12: 36–41.
Kratzsch C *et al.* (2004). Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. *J Mass Spectrom* 39: 856–872.
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Zomepirac

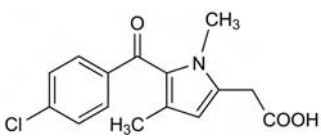
Analgesic

$C_{15}H_{14}ClNO_3 = 291.7$

CAS—33369-31-2

IUPAC Name 2-[5-(4-Chlorobenzoyl)-1,4-dimethylpyrrol-2-yl]acetic acid

Synonym 5-(4-Chlorobenzoyl)-1,4-dimethyl-1*H*-pyrrole-2-acetic acid



Chemical Properties White crystals. Mp 178° to 179°, with decomposition. Log *P* (octanol/water), 3.21.

Zomepirac Sodium

$C_{15}H_{13}ClNNaO_3,2H_2O = 349.7$

CAS—64092-48-4 (anhydrous); 64092-49-5 (dihydrate)

Proprietary Names *Zomax*; *Zomaxin*; *Zopirac*.

Chemical Properties A pale yellow crystalline powder. Mp 295° to 296°.

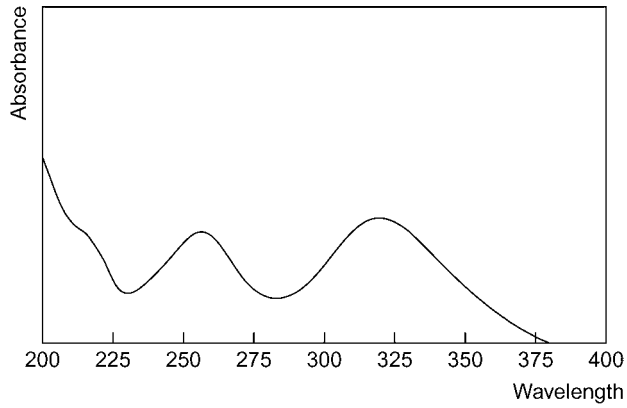
Colour Tests Formaldehyde-sulfuric acid—red; Liebermann's reagent (100°)—orange; Marquis test—yellow→orange (at 100°); sulfuric acid—yellow.

Thin-layer Chromatography System TD—R_f 0.12; system TE—R_f 0.04; system TF—R_f 0.12; system TAE—R_f 0.88; system TAJ—R_f 0.19; system TAK—R_f 0.65; system TAL—R_f 0.88 (Marquis reagent, yellow → green, slow; mercuric chloride–diphenylcarbazone reagent, blue, pink after heating; acidified potassium permanganate solution, positive; Van Urk reagent, positive, red after heating).

Gas Chromatography System GA—zomepirac-ME RI 2343; M ($-CO_2$) RI 2025.

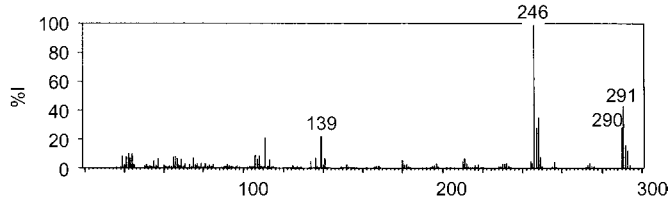
High Performance Liquid Chromatography System HD—*k* 3.7; system HY—RI 495.

Ultraviolet Spectrum Aqueous acid 262 (A_1^1=418b), 324 nm (A_1^1 = 492b); aqueous alkali 261, 330 nm (A_1^1=525a).



Infrared Spectrum Principal peaks at wavenumbers 1587, 1560, 760, 1275, 958, 1493 cm^{-1}(zomepirac sodium, KBr disk).

Mass Spectrum Principal ions at *m/z* 246, 291, 248, 290, 247, 139, 111, 292.



Quantification

Plasma **GC** ECD. Limit of detection, 5 µg/L [Ng, Kalbron 1983].

HPLCUV detection. Limit of detection, 50 µg/L [Welch *et al.* 1982].

Serum **HPLC** See Plasma [Welch *et al.* 1982].

Urine **HPLC** UV detection. Limit of detection, about 100 µg/L for zomepirac and zomepirac glucuronide [Pietta, Calatroni 1983].

Disposition in the Body Rapidly and completely absorbed after oral administration. About 90% of a dose is excreted in the urine in 48 h with about 5 to 20% as unchanged drug and about 60 to 80% as a glucuronide conjugate; about 5% of the dose is excreted as a hydroxylated metabolite; 4-chlorobenzoic acid has been reported to be a minor metabolite. <2% of a dose is eliminated in the faeces.

Therapeutic Concentration

After a single oral dose of 100 mg to 21 subjects, peak plasma concentrations averaged 4.7 mg/L at about 1 h; following oral administration of 100 mg four times a day to 10 subjects, steady-state plasma concentrations of about 3 to 4 mg/L were reported [Nayak *et al.* 1980].

Following a single oral dose of 200 mg to 5 subjects, peak plasma concentrations of 4.5 to 11.3 mg/L (mean, 9) were attained in about 1 h; mean peak plasma concentrations of zomepirac glucuronide and hydroxyzomepirac of 3.9 mg/L and 0.2 mg/L, respectively, were attained in 0.5 to 3 h [O'Neill *et al.* 1982].

Toxicity Zomepirac has been withdrawn from use because of reports of allergic anaphylactoid reactions. In a fatality caused by the ingestion of 9 g of zomepirac sodium, a postmortem blood concentration of 152 mg/L and an ante-mortem blood concentration, obtained 9 h prior to death, of 286 mg/L were reported [Backer *et al.* 1983].

Half-life Plasma half-life, about 4 to 10 h.

Distribution in Blood Plasma : whole blood ratio, 1.9.

Protein Binding 98 to 99%.

Note For a review of zomepirac, see Morley *et al.* [1982]; for a review on the acute toxicity of zomepirac, see Joubert [1982].

Dose Zomepirac sodium has been given in doses equivalent to 400 to 600 mg of zomepirac daily.

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Nayak RK *et al.* (1980). Zomepirac kinetics in healthy males. *Clin Pharmacol Ther* 27: 395–401.
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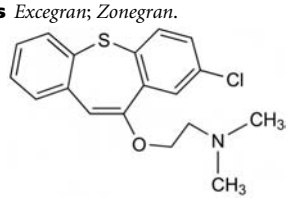
O'Neill PJ *et al.* (1982). Disposition of zomepirac sodium in man. *J Clin Pharmacol* 22: 470–476.
Pietta P, Calatroni A (1983). High-performance liquid chromatographic assay for zomepirac and its main metabolite in urine. *J Chromatogr* 275: 217–222.
Welch CL *et al.* (1982). Liquid-chromatographic determination of zomepirac in serum and plasma. *Clin Chem* 28: 481–484.

Zonisamide

Anticonvulsant
$C_8H_8N_2O_3S = 212.2$
CAS—68291-97-4

IUPAC Name 1,2-Benzoxazol-3-ylmethanesulfonamide
Synonyms AD-810; CI-912; PD-110843
Proprietary Names *Excegran*; *Zonegran*.



Chemical Properties White needles. Mp 160° to 163°; also reported as 162° to 166°. Sparingly soluble in water, chloroform, hexane; soluble in methanol, ethanol ethyl acetate and acetic acid. pK_a 9.66 [Kawada *et al.* 2002], 10.2. For the stability of zonisamide in oral suspensions, see Abobo *et al.* [2009].

Quantification

Blood **HPLC** Column: Develosil ODS-7 (250 × 4.6 mm i.d.). Mobile phase: 1% acetic acid : acetonitrile : isopropylalcohol (70 : 11 : 10), flow rate 1.0 mL/min. UV detection (λ = 285 nm). Limit of detection, 0.1 mg/L [Nishiguchi *et al.* 1992]

Plasma **HPLC** Column: Supelcosil LC-18 (250 × 4.6 mm i.d., 5 µm). Mobile phase: aqueous 30 mmol/L potassium phosphate buffer (pH 3.7) : acetonitrile (65 : 35), flow rate 1.2 mL/min. UV detection (λ = 270 nm). Retention time: 4.5 min. Limit of quantification, 0.5 mg/L, limit of detection, 0.75 mg/L [Greiner-Sosanko *et al.* 2007a]. Column: µBondapack C_{18}. Mobile phase: aqueous 30 mmol/L potassium phosphate buffer (pH 3.7) : acetonitrile (65 : 35), flow rate 1.2 mL/min. UV detection (λ = 270 nm). Limit of detection, 0.5 mg/L [Greiner-Sosanko *et al.* 2007b]. Column: Luna phenylhexyl (50 × 2.0 mm i.d., 5 µm). Mobile phase: water : dibutylammonium phosphate : methanol (1000: 1 vial: 100), flow rate 0.5 mL/min. UV detection (λ = 220 nm). Limit of quantification, 5 mg/L [Juenke *et al.* 2006]. Column: Shim-pack CLC-DDS (150 × 6.0 mm i.d.). Mobile phase: water : methanol (70 : 30), flow rate 1.5 mL/min. UV detection (λ = 238 nm). Limit of detection not reported [Kawada *et al.* 2002]. Column: Presto FT-C_{18} (30 × 4.6 mm i.d., 2 µm). Mobile phase: 10 mmol/L sodium phosphate buffer (pH 7.5) : 10 mmol/L sodium phosphate buffer (pH 7.5)-acetonitrile (60 : 40, 100 : 0 for 3 min to 95 : 5 in 3 min to 0 : 100 in 1 min for 4 min to 100 : 0 in 0.5 min), flow rate 0.5 mL/min. UV detection (λ = 235 nm). Limit of detection, 0.1 mg/L [Nakamura *et al.* 2001]. See also Berry [1990], Shimoyama *et al.* [1999].

LC-MS Column: Shimadzu Shimpack XR-ODS (50 × 4.6 mm i.d., 2.2 µm). Mobile phase: 20 mmol/L acetate buffer (pH 5.6) : acetonitrile-methanol-tetrahydrofuran (61.3 : 32.2 : 6.5; 86 : 14 for 2 min to 77 : 23 at 5 min for 4 min to 64 : 36 at 12 min to 59 : 41 at 16.5 min to 86 : 14 at 17.5 min), flow rate 0.8 mL/min. APCI, SIM acquisition mode. Limit of detection, 0.78 µg/L [Subramanian *et al.* 2008].

Serum **HPLC** Column: Alltima $3C_{18}$ (150 × 4.6 mm i.d.). Mobile phase: methanol : acetonitrile : 25 mmol/L phosphate buffer containing 12.5 mmol/L sodium chloride (14.5 : 19.5 : 66), flow rate 0.9 mL/min. Fluorescence detection (λ_{ex} = 215 nm, λ_{em} = 275 nm). Retention time: 4.45 min. Limit of quantification, 0.027 mg/L, limit of detection, 0.016 mg/L. [Vermeij, Edelbroek 2007]. See Plasma [Juenke *et al.* 2006]. Column: Cadenza CD-C_{18} (150 × 4.6 mm i.d., 3 µm). Mobile phase: 25 mmol/L potassium phosphate buffer (pH 4.6) : methanol : acetonitrile (65 : 20 : 15), flow rate 1.0 mL/min. UV detection (λ = 235 nm). Limit of quantification, 2.5 mg/L [Yoshida *et al.* 2006]. Column: ODS µBondapk C_{18} (300 × 3.9 mm i.d., 10 µm). Mobile phase: acetonitrile : methanol : water (17 : 20 : 63). UV detection (λ = 246 nm). Retention time: 3.9 min. Limit of detection, 0.1 mg/L [Furuno *et al.* 1994]. Column: Chemcosorb 5-ODS-UH (150 × 4.6 mm i.d.). Mobile phase: 1% acetic acid : propan-2-ol : acetonitrile (80 : 11 : 10). UV detection (λ = 235 nm). Limit of detection not reported [Hashimoto *et al.* 1994]. Column: Hypersil R ODS (125 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer-acetonitrile (90 : 10) : phosphate buffer-acetonitrile (40 : 60, 85 : 15 for 0.5 min to 55 : 45 at 9.5 min for 3.5 min to 85 : 15 at 15 min), flow rate, 1.2 mL/min. UV detection (λ = 205 nm). Limit of detection, 6 mg/L [Juergens 1987].

CE Running buffer: 50 mmol/L SDS with 10 or 25 mmol/L phosphate (pH 8.0). UV detection (λ = 210 nm). Limit of detection not reported [Pucci, Raggi 2005].

Brain **HPLC** See Blood. Limit of detection, 0.05 µg/g [Nishiguchi *et al.* 1992].

Breast Milk **HPLC** See Plasma [Kawada *et al.* 2002]. See Plasma. Mobile phase: potassium dihydrogen phosphate buffer (pH 3.5) : acetonitrile [Shimoyama *et al.* 1999].

Disposition in the Body Zonisamide is rapidly and almost completely absorbed from the gastrointestinal tract following oral administration [Neels *et al.* 2004]. Peak plasma concentrations are achieved within 2–6 h of oral administration; however, steady-state concentrations are not achieved for up to 2 weeks owing to the drug's long half-life. It is metabolised via acetylation to *N*-acetylzonisamide and reduction mediated by CYP3A4 to 2-sulfamoylacetylphenol (SMAP); further glucuronide conjugation also occurs. Excretion is mainly in the urine, 35% as unchanged drug, 15% as *N*-acetylzonisamide and 50% as the glucuronide of SMAP.

Therapeutic Concentration

After single dose oral administration of 200 mg and 800 mg zonisamide to healthy volunteers, peak plasma concentrations were 2.3 and 12.5 mg/L, respectively, reached within 2.4–3.6 h [Peters, Sorkin 1993].

In a study investigating the steady-state pharmacokinetics of zonisamide, 24 healthy volunteers were administered the following drug regimen: 100 mg/day for a week, followed by 200 mg/day for another week and finishing on 400 mg/day until day 35. The volunteers were split into 2 groups: the 11 in group A received their dose divided every 12 h after the first week and the 8 in group B received their entire dose in the morning. Peak plasma concentrations were 30.3 and 23.8 mg/L for group A and group B, reached after 2.1 and 1.8 h, respectively. Steady-state concentrations were 28.3 mg/L for group A and 23.8 mg/L for group B and were reached in an average of 15 days from the last dose adjustment [Kochak *et al.* 1998].

Note For a study of zonisamide pharmacokinetics in epileptic patients, see Hashimoto *et al.* [1994] and patients also taking phenytoin or carbamazepine, see Ojemann *et al.* [1986]

Toxicity Adverse events, most notably drowsiness, loss of appetite, gastrointestinal problems and CNS toxicity have been noted with plasma zonisamide concentrations exceeding 30 mg/L.

Note For a case of zonisamide-induced mania, see Charles *et al.* [1990].

Bioavailability Oral, 100% [Neels *et al.* 2004].

Half-life In plasma, ~63 h.

Volume of Distribution Apparent, 1.45 L/kg.

Clearance Plasma, 0.30–0.35 mL/min/kg.

Distribution Plasma : red blood cells 1 : 8.

Protein Binding 40–60% [Neels *et al.* 2004].

Note For a review of zonisamide and its pharmacodynamic and pharmacokinetic properties and therapeutic potential in epilepsy, see Peters, Sorkin [1993].

Dose Initially, 100 mg once daily orally, increased to 200 mg daily in one or several doses. Doses of up to 600 mg daily have been administered.

Abobo CV *et al.* (2009). Stability of zonisamide in extemporaneously compounded oral suspensions. *Am J Health SystPharm* 66: 1105–1109.
Berry DJ (1990). Determination of zonisamide (3-sulphamoylmethyl-1,2-benzisoxazole) in plasma at therapeutic concentrations by high-performance liquid chromatography. *J Chromatogr* 534: 173–181.
Charles CL *et al.* (1990). Zonisamide-induced mania. *Psychosomatics* 31: 214–217.
Furuno K *et al.* (1994). Simple and sensitive assay of zonisamide in human serum by high-performance liquid chromatography using a solid-phase extraction technique. *J Chromatogr B Biomed Appl* 656: 456–459.
Greiner-Sosanko E *et al.* (2007a). Drug monitoring: simultaneous analysis of lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide by HPLC-UV and a rapid GC method using a nitrogen-phosphorus detector for levetiracetam. *J Chromatogr Sci* 45: 616–622.
Greiner-Sosanko E *et al.* (2007b). Simultaneous determination of lamotrigine, zonisamide, and carbamazepine in human plasma by high-performance liquid chromatography. *Biomed Chromatogr* 21: 225–228.
Hashimoto Y *et al.* (1994). Population analysis of the dose-dependent pharmacokinetics of zonisamide in epileptic patients. *Biol Pharm Bull* 17: 323–326.
Juenke J *et al.* (2006). Drug monitoring and toxicology: a procedure for the monitoring of levetiracetam and zonisamide by HPLC-UV. *J Anal Toxicol* 30: 27–30.
Juergens U (1987). Simultaneous determination of zonisamide and nine other anti-epileptic drugs and metabolites in serum. A comparison of microbore and conventional high-performance liquid chromatography. *J Chromatogr* 385: 233–240.
Kawada K *et al.* (2002). Pharmacokinetics of zonisamide in the perinatal period. *Brain Dev* 24: 95–97.
Kochak GM *et al.* (1998). Steady-state pharmacokinetics of zonisamide, an antiepileptic agent for treatment of refractory complex partial seizures. *J Clin Pharmacol* 38: 166–171.
Nakamura M *et al.* (2001). High-performance liquid chromatographic assay of zonisamide in human plasma using a non-porous silica column. *J Chromatogr B Biomed Sci Appl* 755: 337–341.
Neels HM *et al.* (2004). Therapeutic drug monitoring of old and newer anti-epileptic drugs. *Clin Chem Lab Med* 42: 1228–1255.
Nishiguchi K *et al.* (1992). Pharmacokinetics of zonisamide; saturable distribution into human and rat erythrocytes and into rat brain. *J Pharmacobiodyn* 15: 409–415.
Ojemann LM *et al.* (1986). Comparative pharmacokinetics of zonisamide (CI-912) in epileptic patients on carbamazepine or phenytoin monotherapy. *Ther Drug Monit* 8: 293–296.
Peters DH, Sorkin EM (1993). Zonisamide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in epilepsy. *Drugs* 45: 760–787.
Pucci V, Raggi MA (2005). Analysis of antiepileptic drugs in biological fluids by means of electrokinetic chromatography. *Electrophoresis* 26: 767–782.
Shimoyama R *et al.* (1999). Monitoring of zonisamide in human breast milk and maternal plasma by solid-phase extraction HPLC method. *Biomed Chromatogr* 13: 370–372.
Subramanian M *et al.* (2008). High-speed simultaneous determination of nine antiepileptic drugs using liquid chromatography–mass spectrometry. *Ther Drug Monit* 30: 347–356.
Vermeij TA, Edelbroek PM (2007). Robust isocratic high performance liquid chromatographic method for simultaneous determination of seven antiepileptic drugs including lamotrigine, oxcarbazepine and zonisamide in serum after solid-phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 40–46.
Yoshida T *et al.* (2006). Simultaneous determination of zonisamide, carbamazepine and carbamazepine-10,11-epoxide in infant serum by high-performance liquid chromatography. *J Pharm Biomed Anal* 41: 1386–1390.

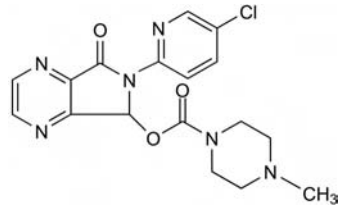
Zopiclone

Hypnotic
$C_{17}H_{17}ClN_6O_3 = 388.8$
CAS—43200-80-2

IUPAC Name [6-(5-Chloropyridin-2-yl)-5-oxo-7*H*-pyrrolo[3,4-*b*]pyrazin-7-yl] 4-methylpiperazine-1-carboxylate

Synonyms 4-Methyl-1-piperazine-carboxylic acid 6-(5-chloro-2-pyridinyl)-6,7-dihydro-7-oxo-5*H*-pyrrolo [3,4-b] pyrazin-5-yl ester; RP-27267.

Proprietary Names *Amoban*; *Datolan*; *Imovane*; *Limovan*; *Rhovane*; *Siaten*; *Sopivan*; *Ximovan*; *Zileze*; *Zimovane*.



Chemical Properties A white or slightly yellow powder. Mp 178°. It is practically insoluble in water and in alcohol; sparingly soluble in acetone; freely soluble in dichloromethane; dissolves in dilute mineral acids. Extraction yield (chlorobutane), 0.9 [Demme *et al.* 2005].

Thin-layer Chromatography System TAE—R_f 0.04; system TB—R_f 0.04; system TE—R_f 0.47; system TF—R_f 0.00.

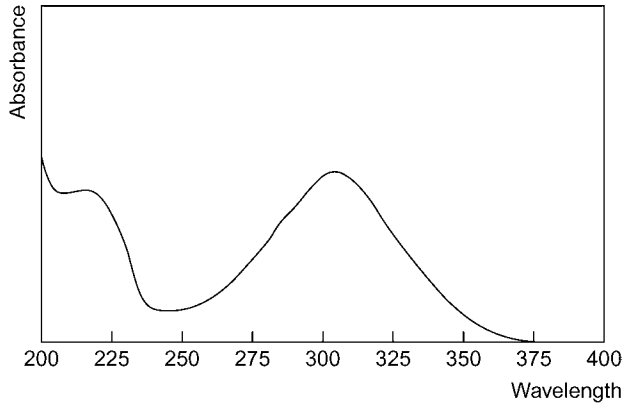
Gas Chromatography System GB—RI 3263, art (aminochloropyridine) RI 1261; system GT—RI 2950.

High Performance Liquid Chromatography System HX—RI 331; system HY—RI 269; system HZ—RT 2.3 min; system HAX—RT 7.5 min; system HAY—RT 3.8 min.

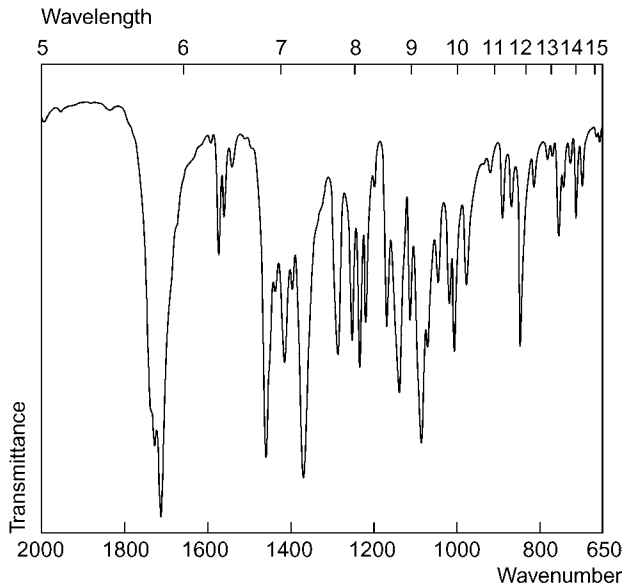
Column: Symmetry C_8 (150 × 3.9 mm i.d., 5 µm). Mobile phase: 0.05 mol/L ammonium acetate : acetonitrile (70 : 30), flow rate 1.7 mL/min. IS: zopiclone chloride. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 470 nm). Retention time not specified [Bramness *et al.* 2001].

Column: Chiralcel OD-H chiral (250 × 4.6 mm i.d., 5 µm). Mobile phase: ethanol : hexane (60 : 40), flow rate 0.6 mL/min. IS: chlordiazepoxide. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 470 nm). Retention time: 19 min for (−)-zopiclone, 28 min for (+)-zopiclone and 9.5 min for IS [Foster *et al.* 1994].

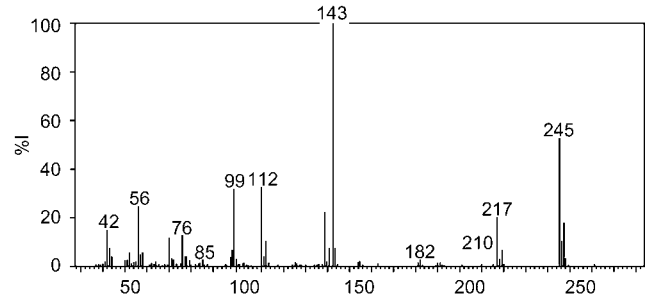
Ultraviolet Spectrum Aqueous acid (0.2 mol/L sulfuric acid/methanol)—304 nm; (0.1 mol/L hydrochloric acid)—303 nm; (0.1 mol/L ammonium formate, pH 3)—306, 214 nm; aqueous alkali (0.1 mol/L sodium hydroxide)—237, 277 nm; basic—304 nm.



Infrared Spectrum Principal peaks at wavenumbers 1715, 1372, 1463, 1087 cm^{-1}.



Mass Spectrum Principal ions at *m/z* 143, 245, 218, 247, 98, 112, 159, 56.



Quantification

Blood **GC** Column: DB 17 (15 m × 0.2 mm i.d., 0.25 µm). Carrier gas: H_2. Temperature programme: 220° for 5 min to 280° at 10°/min for 11 min. ECD. Limit of quantification, 4 µg/L [Boniface *et al.* 1992].

GC-MS Column: RTX-5MS (15 m × 0.25 mm i.d., 0.25 µm). Temperature programme: 100° for 1.0 min to 300° at 30°/min for 1.33 min. Limit of quantification, 10 µg/L [Rohrig *et al.* 2010]. Column: DB-5HT (30 m × 0.32 mm i.d., 0.10 µm). Carrier gas: H_2, 2.5 mL/min. Temperature programme: 180° for 1 min to 350° at 50°/min. NI and PICI, SIM acquisition mode. Retention time: 3.88 min. Limit of quantification, 10 µg/L [Gunnar *et al.* 2006]. Column: DB-35MS (30 m × 0.2 mm i.d., 0.25 µm). Carrier gas: He, 1.5 mL/min. Temperature programme: 120° for 1 min to 320° at 15°/min for 4 min. EI ionisation at 70 eV, SIM acquisition mode. Limit of quantification, 10 µg/L, limit of detection, 10 µg/L [Gunnar *et al.* 2004].

LC-MS Column: Varian Pursuit 3 C_{18} (100 × 3 mm i.d., 3 µm). Mobile phase: methanol : ammonium formate. MRM acquuisition mode, positive ion mode. Limit of quantification, 0.010 mg/kg, limit of detection, 0.0003 mg/kg [Bjork *et al.* 2010]. Zopiclone, zolpidem and benzodiazepines [Deveaux *et al.* 2008]. Column: XTerra MS C_{18}.(150 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol : 0.1% formate buffer (10 : 90 to 50 : 50 over 5 min to 70 : 30 in 20 min to 10 : 90 in 0.1 min for 14.9 min), flow rate 0.2 mL/min. MRM acquisition mode. Limit of quantification, 2 µg/L [Laloup *et al.* 2005].

Plasma **GC** Column: OV-1 fused silica (25 m × 0.32 mm i.d., 0.2 µm). Temperature: 235°. Carrier gas: He, 1.3 mL/min. NPD. Retention time: 19.59 min. Limit of detection, 2 µg/L for zopiclone and 1 µg/L for zolpidem [Stanke *et al.* 1996]. Column: 5% phenyl methyl silicone (25 m × 0.32 mm i.d., 0.52 µm). Carrier gas: He, 1.8 mL/min. Temperature programme: 210° to 300° at 2.5°/min for 9 min. ECD. Retention time: 11.8 min. Limit of quantification, 2 µg/L [Gaillard *et al.* 1993]. AFID. Limit of detection, 5 µg/L [Kennel *et al.* 1990].

HPLC Column: reversed phase C_{18}. Fluorescence detection (λ_{ex}= 307 nm, λ_{em}= 483ynm). Limit of quantification, 3 µg/L for zopiclone and 6 µg/L for desmethylzopiclone [Nirogi *et al.* 2006]. Column: Daicel OD-H (250 × 4.6 mm i.d.). Mobile phase: heptane : ethanol (2 : 3), flow rate 0.6 mL/min. UV detection (λ= 306 nm). Limit of quantification, 2.5 µg/L for zopiclone enantiomers [Gebauer, Alderman 2002]. Column: Nova-Pak C_{18} (300 × 3.9 mm i.d., 4 µm). Mobile phase: methanol : tetrahydrofuran : phosphate buffer (pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. DAD (λ= 210 nm). *k* value 0.56. Retention time: 4.05 min. Limit of detection, ~25 µg/L [Tracqui *et al.* 1993]. Column: Ultraspere ODS C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : tetrahydrofuran : 10 mmol/L TMA (pH 2.5, 15 : 5 : 2 : 78), flow rate 1.0 mL/min. UV detection (λ= 305 nm). Limit of detection, 5 µg/L [Royer-Morrot *et al.* 1992]. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 470 nm). Limit of detection, 5 µg/L for zopiclone enantiomers [Fernandez *et al.* 1993]. See Urine [Le Liboux *et al.* 1987]. See also Miller *et al.* [1986] and Stanley *et al.* [1985].

LC-MS Column: Acquity UPLC BEH C_{18} (100 × 2.1 mm i.d., 1.7 µm). Mobile phase: 0.05% formic acid in water : 0.05% formic acid in acetonitrile (75 : 25 for 0.5 min to 70 : 30 at 6 min to 59.5 : 40.5 at 9.5 min to 10 : 90 at 13 min to 75 : 25 at 17 min), flow rate 0.4 mL/min. SIR acquisition mode. Retention time: 1.04 min. Limit of detection, 0.2–8 µg/L for zopiclone, zolpidem, benzodiazepines and metabolites [Ishida *et al.* 2009]. Column: Symmetry Shield RP8 (150 × 4.6 mm i.d., 3.5 µm). Mobile phase: 0.05% formic acid in water : acetonitrile : methanol (25 : 65 : 10), flow rate 0.5 mL/min. TIS. MRM acquisition mode, positive ion mode. Limit of quantification, 0.5 µg/L for zopiclone and *N*-desmethylzopiclone and 1 µg/L for zopiclone *N*-oxide [Mistri *et al.* 2008]. Column: Merck LiChroCART with Superspher 60 RP Select B stationary phase. Mobile phase: ammonium formate : acetonitrile. APCI. Zopiclone and benzodiazepines and related drugs [Kratzsch *et al.* 2004].

Serum **HPTLC** Plate: silica gel 60 F_{254} (100 × 100 mm). Mobile phase 1: cyclohexane : toluene : diethylamine (75 : 15 : 10); mobile phase 2: chloroform : methanol (90 : 10); mobile phase 3: chlorofrom : acetone (80 : 20). Dragendorff's Reagent. UV detection (λ= 366 nm). R_f 0.21 (mobile phase1); R_f 0.71 (mobile phase 2); R_f 0.60 (mobile phase 3). Limit of detection, 100 µg/L [Otsubo *et al.* 1995].

GC Column: DB 5 (5 m × 0.32 mm i.d., 0.17 µm). Carrier gas: N_2, 50 cm/s. Temperature: 230°. NSD. Limit of quantification, 5 µg/L for zopiclone and zolpidem [Debruyne *et al.* 1991].

HPLC Column: LiChroCART 15-25-4 packed with LiChrospher 60 RP select B (5 µm). Mobile phase: acetonitrile : monopotassium phosphate (20 : 80). Limit of detection, 15 µg/L [Yang, Rochholz 2002].

Urine **GC-MS** See Blood [Rohrig *et al.* 2010].

HPLC Column: Nucleosil C_{18} (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1 mol/L sodium dihydrogen phosphate (pH 5.1, 35 : 65), flow rate 1.0 mL/min. UV detection (λ= 300 nm). Limit of detection, 100 µg/L [Nordgren *et al.* 2002]. Column: Spherisorb ODS-2 (200 × 4.6 mm i.d., 5 µm). Mobile phase: 0.025 mol/L monobasic sodium phosphate: methanol (45 : 55), flow rate 1.0 mL/min. Fluorescence detection (λ_{ex}= 300 nm, λ_{em}= 470 nm). Limit of detection, 5 µg/L for zopiclone and 10 µg/L for metabolites [Le Liboux *et al.* 1987].

LC-MS Limit of detection, <0.5 µg/L for zopiclone, zolpidem and benzodiazepines [Deveaux *et al.* 2008]. Column: Waters XTerra MS C_{18} (150 × 2.1 mm i.d., 5 µm). Mobile phase: acetonitrile-2 mmol/L ammonium formate (pH 3, 90 : 10) : 2 mmol/L ammonium formate (pH 3, 30 : 70 to 36 : 64 in 4 min for 4.5 min to 90 : 10 in 3.5 min to 30 : 70 in 1.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, SRM acquisition mode. Retention time: 2.3 min. Limit of detection, 0.01 µg/L [Quintela *et al.* 2006] Column: XTerra MS C_{18}. Mobile phase: methanol : formate buffer. MRM acquisition mode. Limit of quantification, 10–25 µg/L for zopiclone, zolpidem, benzodiazepines and metabolites [Laloup *et al.* 2005]. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].

CE Capillary: fused silica (400/470 mm effective/total length, 50 µm). Mobile phase: 100 mmol/L phosphate buffer (pH 2.75): 16.3 mmol/L β-cyclodextrin. UV laser-induced fluorescence detection (λ_{ex}=325 nm, λ_{em}=450 nm). Limit of detection, 6 µg/L [Hempel, Blaschke 1996].

Oral Fluid **CE** See Urine [Hempel, Blaschke 1996].

Hair **LC-MS** Column Zorbax SB-Phenyl (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: 25 mmol/L formic acid : acetonitrile (98 : 2 to 80 : 20 for 3 min to 10 : 90 in 4 min for 2 min), flow rate 0.25 mL/min. ESI, positive ion mode. Limit of quantification, 0.005 ng/mg [Hegstad *et al.* 2008]. Column: XTerra MS C_{18}. Mobile phase: methanol : formate buffer. MRM acquisition mode. Limit of quantification, 0.5–10 ng/g for zopiclone, zolpidem, benzodiazepines and metabolites [Laloup *et al.* 2005]. Column: XTerra MS C_{18} (100 × 2.1 mm i.d., 3.5 µm). Mobile phase: acetonitrile : formate buffer (5 : 95 at time 0 to 60 : 40 at 3 min to 80 : 20 at 7 min until 10 min to 5 : 95 at 10.5 min for 9.5 min), flow rate 0.2 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of detection, 0.3 pg/mg [Villain *et al.* 2004].

Disposition in the Body Zopiclone is rapidly absorbed after oral administration and is also widely distributed into breast milk. It is extensively metabolised in the liver by demethylation, oxidation and decarboxylation. The 2 major metabolites excreted in urine are zopiclone *N*-oxide and *N*-demethylzopiclone (inactive). Approximately 50% of a dose is converted by decarboxylation to inactive metabolites, some of which are partly eliminated via the lungs as carbon dioxide. Only 5% of a dose is excreted unchanged in urine and ~16% appears in faeces, the remaining dose being excreted as metabolites.

Therapeutic Concentration The serum therapeutic concentration range is 0.01–0.05 mg/L, with a peak of 0.04–0.07 mg/L.

Twelve male Caucasians, 19–36 years old, were administered a single oral dose of 15 mg zopiclone after an overnight fast. After 93.8 min, the total drug concentration was 0.131 mg/L (0.087 mg/L as the (+)-enantiomer and 0.044 mg/L as the (−)-enantiomer, observed at 98.8 and 88.8 min, respectively) [Fernandez *et al.* 1993].

Twelve healthy, lactating women between 2 and 6 days after childbirth and aged 20–45 years were administered 7.5 mg oral zopiclone. The mean peak plasma concentration was 0.08 mg/L at 1.6 h, and the mean milk concentration was 0.034 mg/L at 2.4 h [Matheson *et al.* 1990].

Toxicity The serum toxic concentration is 0.15 mg/L.

A 25-year-old man had ingested 300 mg zopiclone in a suicide attempt The maximum plasma concentration was measured as 1.6 mg/L at 4.5 h post-ingestion [Royer-Morrot *et al.* 1992].

The postmortem blood concentrations of 2 individuals, 1 who had taken 30 tablets of 7.5 mg zopiclone and 1 who had taken up to 100 tablets of 7.5 mg zopiclone, were 0.62 mg/L and 1.70 mg/L, respectively [Boniface *et al.* 1992].

Blood concentrations of zopiclone in a 29-year-old woman who had committed suicide, were between 0.9 and 2.0 mg/L using 10 distinct blood samples. The following postmortem tissue concentrations were reported: portal venous blood 3.0 mg/L, spleen 5.8 mg/L, brainstem 2.8 mg/L, myocardium 1.6 mg/L, kidney 1.7 mg/L and liver 4.9 µg/g [Pounder, Davies 1994].

A 72-year-old man who took 90 mg zopiclone, died 4 to 10 h after ingestion. Postmortem zopliclone levels were 254 µg/L in femoral blood, 408 µg/L in cardiac blood, 94 µg/L in vitreous humour, 7.330 mg/L in urine and 114.700 mg/L in bile [Meatherall 1997].

A 72-year-old woman who had taken an overdose of zopiclone (probably 200–350 mg) had postmortem femoral blood levels of 1.9 mg/L [Bramness *et al.* 2001].

A case report describes excessive use of zopiclone in a 67-year-old man who increased his dose of zopiclone from 7.5 mg daily up to 337.5 mg daily [Kuntze *et al.* 2002].

Half-life Plasma half-lives are zopiclone 3.5–6.5 h, zopiclone *N*-oxide 3.5–6.0 h and *N*-desmethylzopiclone 7–11 h.

Volume of Distribution ≈100 L/kg.

Clearance Plasma clearance, 13.9 L/h, also reported as 17.3 L/h (7.5 mg dose).

Protein Binding 45–80%.

Note For reviews of zopiclone, see Goa, Heel [1986] and Noble *et al.* [1998]; for a review of the pharmacokinetics of zopiclone, see Fernandez *et al.* [1995].

Dose The usual dose is 7.5 mg at night, increased to 15 mg if necessary. Initially 3.75 mg may be given to the elderly and those with hepatic/renal impairment and increased if necessary.

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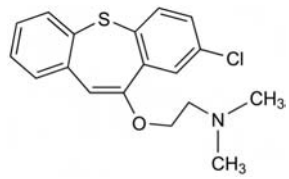
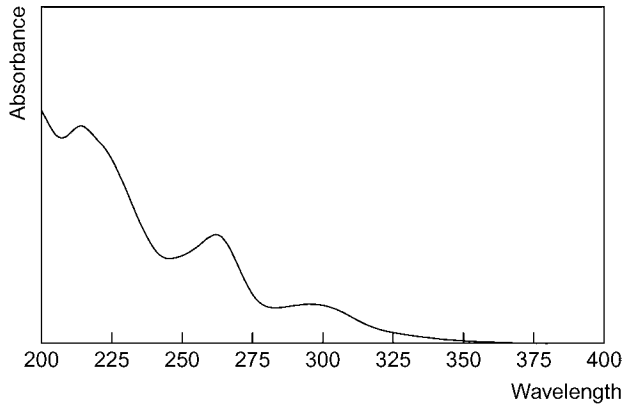
Zotepine

Antipsychotic
$C_{18}H_{18}ClNOS$ = 331.9
CAS—26615-21-4

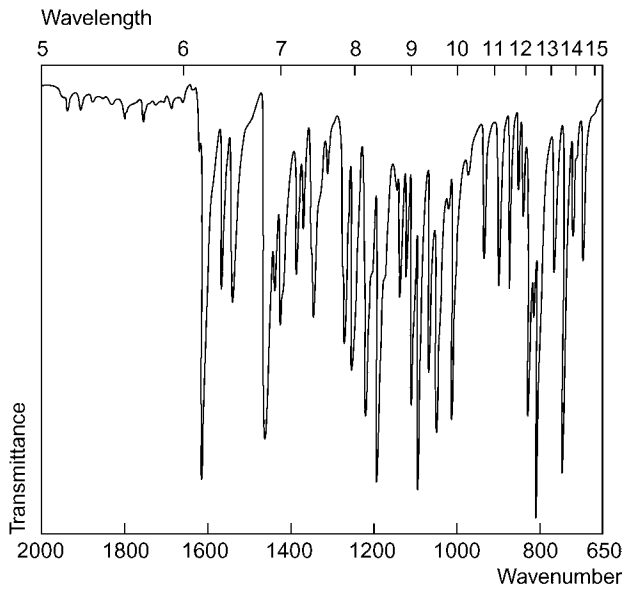
IUPAC Name 2-(8-Chlorobenzo[*b*][1]benzothiepin-6-yl)oxy-*N*,*N*-dimethylethanamine
Synonym 2-[(8-Chlorodibenzo[*b*,*f*]-thiepin-10-yl)oxy]-*N*,*N*-dimethylethylamine
Proprietary Names *Lodopin*; *Nipolept*; *Zoleptil*.



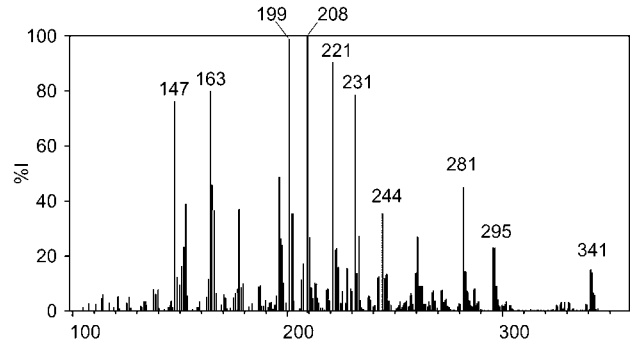
Chemical Properties Extraction yield (chlorobutane), 0.95 [Demme *et al.* 2005].
Gas Chromatography System GA—RI 2660; system GT—RI 2440 [Tanaka *et al.* 1996].
High Performance Liquid Chromatography Column: Nova-Pak, C_{18} (150 × 3.9 mm i.d., 4 µm). Mobile phase: acetonitrile : 0.067 mol/L phosphate (pH 7.95, 45 : 55), flow rate 1.0 mL/min. IS: diazepam. UV detection (λ = 304 nm). Retention time: 2.6 min zotepine, 4.7 min IS [Paw, Misztal 2000].
Ultraviolet Spectrum Aqueous acid (methanol)—215, 264, 297 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m*/*z* 208, 199, 221, 163, 231, 147, 281, 244.



Quantification
Blood **GC** Column: Shimadzu CBP-1-bonded methyl silicone (12 m × 0.53 mm i.d., 1.0 µm). Carrier gas: He, 10 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min for 2 min. NPD. Limit of detection, not reported [Jitsufuchi *et al.* 1995].
Plasma **GC** Column: CBP1-bonded methyl silicone (12 m × 0.53 mm i.d., 1 µm). Carrier gas: He, 20 mL/min. Temperature programme: 200° for 2 min to 300° at 10°/min. NPD. Limit of detection, 10 µg/L [Tokunaga *et al.* 1996]. Column: HP-5 capillary (25 m × 0.2 mm i.d., 0.33 µm). Temperature programme: 260° to 280° at 2/min. NPD. Retention time: 7.36 min. Limit of detection, 1.0 µg/L [Ulrich *et al.* 1996].
GC-MS Column: HP-5 cross-linked 5% methyl silicone (30 m). Temperature programme: 50° for 1 min, to 300° at 40°/min. IS: clothiapine. EI ionisation, SIM acquisition mode. Retention time: zotepine 8.9 min; IS, 8.4 min. Limit of detection, 1 µg/L [Tanaka *et al.* 1996].
Serum **GC** See Plasma [Ulrich *et al.* 1996].
LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 1.03 µg/L [Kirchherr, Kühn-Velten 2006].
Urine **GC-MS** Column: HP-1 cross-linked methyl silicone (12 m × 0.2 mm i.d., 330 nm). Carrier gas: He, 1.0 mL/min. Temperature programme: 100° for 3 min to 310° at 30°/min for 8 min. EI ionisation at 70 eV. Limit of detection, <100 µg/L [Bickeboeller-Friedrich, Maurer 2001].
Brain **GC** See Blood [Jitsufuchi *et al.* 1995].
Kidney **GC** See Blood [Jitsufuchi *et al.* 1995].
Liver **GC** See Blood [Jitsufuchi *et al.* 1995].
Muscle **GC** See Blood [Jitsufuchi *et al.* 1995].
Disposition in the Body Zotepine is absorbed after oral administration. Peak plasma concentrations are attained within 2–3 h. The drug undergoes extensive first-pass metabolism to the equipotent metabolite norzotepine, and inactive metabolites (hydroxylated, demethylated and *S*-oxide derivatives). CYP1A2 and CYP3A4 are the major isozymes involved [Shiraga *et al.* 1999]. It is excreted mainly in urine and faeces as metabolites (ratio 4 : 6) and is distributed into breast milk.
Therapeutic Concentration
Two healthy individuals were administered a single oral dose of 50 mg zotepine. The maximum plasma concentrations observed at 6 and 3 h were 31.7 and 31.0 µg/L, respectively. A second peak was also observed 10–12 h after administration [Tanaka *et al.* 1996].
Toxicity
A 46-year-old schizophrenic man died in hospital. The concentrations of zotepine, amongst other antipsychotic drugs, in his blood, brain, kidney, liver and muscle were 612, 832, 3801, 8605 and 1251 ng/g respectively. Cause of death was asphyxia [Jitsufuchi *et al.* 1995].
Note For a case of neuroleptic malignant syndrome induced by zotepine therapy see Tsai *et al.* [2005].
Half-life ≈15–16 h in plasma.
Volume of Distribution 1851–2856 L.
Clearance Oral clearance, 81–1323 L/h.
Protein Binding ≈97%.
Dose Up to 300 mg daily.

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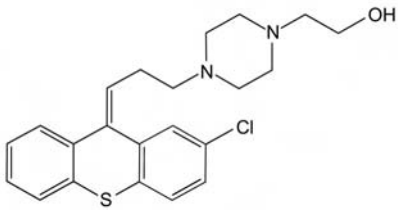
Zuclopenthixol

Antipsychotic, Neuroleptic
$C_{22}H_{25}ClN_2OS = 401.0$
CAS—53772-83-1

IUPAC Name 2-[4-[(3*Z*)-3-(2-chlorothioxanthen-9-ylidene)propyl]piperazin-1-yl] ethanol
Synonyms (*Z*)-4[3-(2-Chloro-9*H*-thioxan-9-ylidene)propyl]-1-piperazine ethanol; *Z*-clopenthixol; *cis*-clopenthixol; α-clopenthixol; Lu 0-108.
Proprietary Names *Ciatyl*; *Cisordinol*.



Chemical Properties An off-white, granular powder (base). It is very slightly soluble in water; sparingly soluble in diethyl ether; soluble in chloroform; readily soluble in methanol and ethanol (96%). pK_a 7.6, 3.3. Log *P* (octanol/phosphate buffer pH 7.4), 3.9; Log *P* (octanol/water [pH 5.0]), 2.22. Extraction yield (chlorobutane), 1 [Demme *et al.* 2005].

Zuclopenthixol Acetate

$C_{24}H_{27}ClN_2O_2S = 443.0$
CAS—85721-05-7
Proprietary Name *Viscoleo*
Chemical Properties A yellowish, viscous oil. It is very slightly soluble in water; very soluble in alcohol, dichloromethane and ether.

Zuclopenthixol Decanoate

$C_{32}H_{43}ClN_2O_2S = 555.2$
CAS—64053-00-5
Chemical Properties A yellowish, viscous oil. It is very slightly soluble in water; very soluble in alcohol, dichloromethane and ether.

Zuclopenthixol Hydrochloride

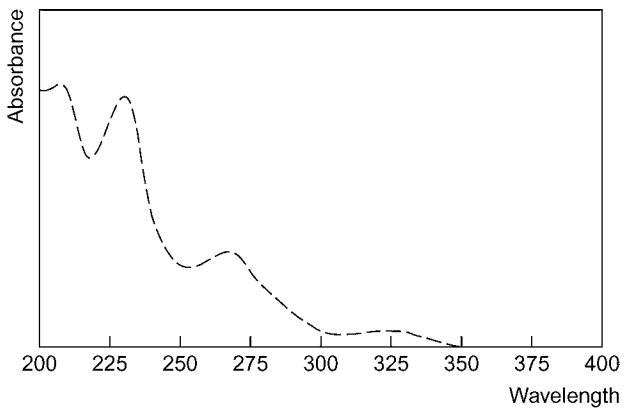
$C_{22}H_{25}ClN_2OS,2HCl = 473.9$
CAS—633-59-0
Synonym Zuclopenthixol dihydrochloride
Proprietary Name *Clopixol*
Chemical Properties An off white granular powder. It is very soluble in water; sparingly soluble in alcohol; slightly soluble in chloroform; very slightly soluble in ether. A 1% solution has a pH of 2.0–3.0.

Thin-layer Chromatography System TA—R_f 0.56; system TB—R_f 0.07; system TC—R_f 0.32; system TE—R_f 0.44; system TAE—R_f 0.45; system TAG—R_f 0.11.
High Performance Liquid Chromatography System HAA—RT 16.3 min; system HZ—RT 8.0 min.

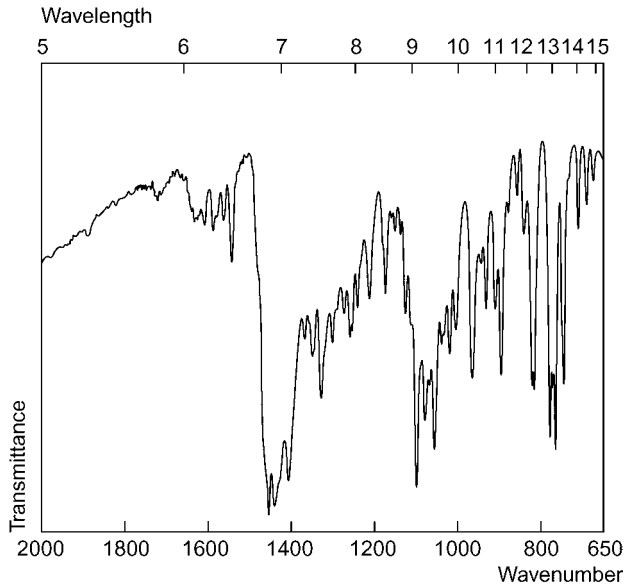
Column: LiChrosorb RP-18 ODS silica (120 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : water: 0.2 mol/L potassium phosphate buffer (pH 4.0, 60 : 35 : 5), flow rate 1.0 mL/min. UV detection (λ= 254 nm). Retention time: ~24 and ~26 min for *cis*- and *trans*-isomers, respectively [Helboe 1990]. Column: Spherisorb (250 × 4.6 mm i.d., 5 µm). Mobile phase ethyl acetate : methanol : 3% ammonia (85 : 15 : 1), flow rate 1.0 mL/min. UV detection (λ= 260 nm). Retention time: 8.02 and 8.89 min for the *cis* and *trans*-isomers, respectively. Limit of detection, 1.0 mg/L [Li Wan Po, Irwin 1979].

Liquid Chromatography-Mass Spectrometry Column: NovaPak, RP C_{18} (150 × 2 mm, 4 µm). Mobile phase: methanol : 2 mmol/L ammonium acetate (pH 3, 90 : 10), flow rate 0.2 mL/min. ESI. Retention time: 14.95 min [Tracqui *et al.* 1997].

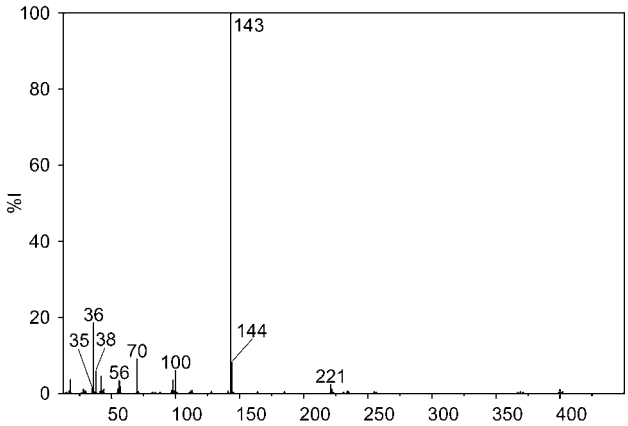
Ultraviolet Spectrum Aqueous solution (water)—(dihydrochloride) 208, 230, 268, 325 nm; aqueous acid (ethanol)—(acetate) 208, 231, 268, 326 nm; aqueous solution (water)—(decanoate) 208, 230, 272, 325 nm.



Infrared Spectrum



Mass Spectrum Principal ions at *m/z* 143, 70, 100, 221, 98, 42, 222, 234 (zuclopenthixol); 143, 36, 70, 144, 38, 100, 35, 56 (dihydrochloride); 185, 36, 186, 98, 87, 43, 70, 221 (acetate); 297, 298, 36, 98, 125, 143, 70, 43 (decanoate).



Quantification

Blood **GC-MS** Column: BPX5 (15 m × 0.22 mm i.d, 0.25 µm). Carrier gas: He, 30 mL/min. Temperature programme: 70° for 1 min to 220° at 15°/min to 260° at 5°/min to 330° at 25°/min. MSD, EI ionisation. Retention time: 22.3 and 22.4 min for (*E*)- and (*Z*)-isomers, respectively [Rop 2001].

HPLC Column: Symmetry (250 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : methanol : 0.1 mol/L ammonium acetate (30 : 30 : 40), flow rate 1.0 mL/min. UV detection (λ= 230 nm). Limit of quantification, 40 and 45 µg/L for (*Z*)- and (*E*)-isomers, respectively [Rop 2001]. Column: Spherisorb, S5 CN (120 × 4.6 mm). Mobile phase: acetonitrile : 200 mmol/L potassium phosphate (pH 6.5, 36 : 5 : 59), flow rate 1 mL/min. Fluorescence detection (λ_{ex}= 260 nm, λ_{em}= 435 nm). Retention time: 9 min. Limit of detection, 0.05 µg/L [Hansen, Hansen 1994]. Column: NovaPak, C_{18} (300 × 3.9 mm, 4 µm). Mobile phase: methanol : tetrahydrofuran : 10 mmol/L potassium dihydrogen phosphate (pH 2.6, 65 : 5 : 30), flow rate 0.8 mL/min. UV detection (λ= 228 nm). Retention time: 8.98 min. Limit of detection, 7 µg/L [Tracqui *et al.* 1997]. Column: Spherisorb S5W (250 × 4.6 mm i.d., 5 µm). Mobile phase: *n*-heptane : propan-2-ol : concentrated ammonia : water (85 : 15 : 0.4 : 0.2), flow rate 0.1 mL/min. Limit of detection, 0.5 µg/L [Aaes-Jorgensen 1980].

LC-MS Column: Zorbax Stable Bond Cyano (50 × 2.1 mm i.d., 3.5 µm). Mobile phase: methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 2 : 8 : 90) : methanol-acetonitrile-20 mmol/L ammonium formate buffer (pH 4, 24 : 36 : 40, 80 : 20 to 20 : 80 in 4.5 min for 2.5 min to 80 : 20 for 2.5 min). TIS, MRM acquisition mode. Retention time: 6.0 min. Limit of detection, 0.8 µg/L [Roman *et al.* 2008]. Column: Symmetry C_{18} (150 × 3.0 mm i.d., 5 µm). Mobile phase: acetonitrile : 0.1% formic acid (10 : 90 to 90 : 10 in 10 min to 10 : 90 in 1 min). ESI. Limit of detection, 1 µg/L [Kollroser *et al.* 2001].

Plasma **HPLC** Column: XTerra MS C_{18} (150 × 3.9 mm i.d., 5 µm). Mobile phase: acetonitrile : 62.4 mmol/L phosphate buffer (pH 4.2, 38 : 62), flow rate 1.0 mL/min. UV detection (λ= 220 nm). Relative retention time: 4.29 min. Limit of quantification, 20 µg/L, limit of detection, 9 µg/L [Garay Garcia *et al.* 2003]. Column:

Microsorb MV C_8 (150 × 4.6 mm i.d., 5 µm). Mobile phase: acetonitrile : 25 mmol/L phosphate buffer (pH 3.0, 35 : 65,) with 0.25% TEA. UV detection (λ= 230 nm). Retention time: 7.4 and 7.9 min for the (*Z*)- and (*E*)-isomers, respectively. Limit of quantification, 1 µg/L, limit of detection, 0.3 µg/L [Pucci *et al.* 2003]. Column: Phenomenex Luna C_{18} (100 × 2.0 mm i.d., 3 µm). Mobile phase: 40 mmol/L phosphate buffer (pH 7.5) : acetonitrile (52 : 48), flow rate 0.3 mL/min. UV detection (λ= 258 nm). Retention time: 7.12 min. Limit of detection, 1.0 nmol/L [Jaanson *et al.* 2002].

Serum **HPLC** Column: S5 CN Spherisorb (150 × 4.6 mm i.d.). Mobile phase: 10 mmol/L ammonium acetate buffer : methanol (1 : 9), flow rate 0.6–1.7 mL/min. UV detection (λ= 256 and 245 nm). Limit of quantification, 5 nmol/L [Angelo, Petersen 2001]. Limit of detection, 0.5 µg/L [Aaes-Jorgensen *et al.* 1986].

LC-MS Column: C_{18} (50 × 4.6 mm i.d., 5 µm). Mobile phase: methanol : 5 mmol/L acetic acid (pH 3.9, 20 : 80 to 70 : 30 over 4 min for 1 min), flow rate 1.0 mL/min. ESI, positive ion mode, MRM acquisition mode. Limit of quantification, 0.47 µg/L [Kirchherr, Kühn-Velten 2006]. Column: Uptisphere (125 × 2 mm i.d., 5 µm). Mobile phase: 50 mmol/L ammonium acetate (pH 4.0)-acetonitrile : acetonitrile (92 : 8), flow rate 200 µL/min. ESI, positive ion mode, SIM acquisition mode. Limit of quantification, 5 nmol/L [Gutteck, Rentsch 2003].

Urine **HPLC** See Blood [Hansen, Hansen 1994; Rop 2001; Tracqui *et al.* 1997] UV detection (λ= 228 nm).

Breast Milk **HPLC** See Serum [Aaes-Jorgensen *et al.* 1986].

Gastric Contents **HPLC** See Blood [Rop 2001]. See Blood [Tracqui *et al.* 1997].

Vitreous Humour **HPLC** See Blood [Tracqui *et al.* 1997].

Hair **LC-MS** Column: RP-C8-select B (125 × 2 mm i.d., 5 µm). Mobile phase: 1 mmol/L ammonium formate with 0.1% formic acid (pH 3) : acetonitrile with 0.1% formic acid (90 : 10 to 70 : 30 at 6.6 min to 30 : 70 at 26.6 min to 10 : 90 at 33.3 min. MRM acquisition mode. Limit of quantification, 0.024 ng/mg, limit of detection, 0.008 ng/mg [Weinmann *et al.* 2002].

Disposition in the Body Following IM injections, the acetate and decanoate esters of zuclopenthixol are hydrolysed to release zuclopenthixol. The acetate has a quick onset after injection, with a duration of action of 2–3 h, which is shorter than for the decanoate. The drug is rapidly absorbed from the gastrointestinal tract. First-pass metabolism occurs in the gut wall, and the drug is also extensively metabolised in the liver. Metabolism occurs by sulfoxidation, side-chain *N*-dealkylation and glucuronic acid conjugation [Khan 1969]. One known metabolite of zuclopenthixol is *trans*-(*E*)-clopenthixol. The drug is widely distributed in the body, with higher concentrations in the liver, lung, intestine and kidney. It is excreted mainly in faeces, but also in urine, in the form of numerous metabolites. There is evidence of enterohepatic recycling and wide intersubject variation in plasma concentration. The drug also displays linear kinetics. CYP2D6 plays a significant role in the systemic elimination of zuclopenthixol [Jaanson *et al.* 2002].

Therapeutic Concentration

Nineteen acutely disturbed Asian patients aged between 18 and 67 years, the majority of whom were suffering from schizophrenia, acute psychosis or mania, were administered 50 mg zuclopenthixol acetate (Viscoleo) by IM injection. The mean serum drug concentrations were taken at 24, 48 and 72 h after administration, and were 19.9 µg/L (range 8–41.5), 31.5 µg/L (range 12.8–52) and 17.8 µg/L (range 8–28.8), respectively. The *trans*-(*E*)-clopenthixol concentrations ranged between negligible and 39.5 µg/L [Tan *et al.* 1993].

Toxicity

Postmortem samples were obtained from a 16-year-old Caucasian female who died 40 h after ingesting an undetermined number of clopixol 10 tablets (10 mg each). The following postmortem tissue concentrations were reported: blood 391 µg/L, urine 957 µg/L, gastric contents 105.4 µg/g, liver 0.75 µg/g, kidney 1.413 µg/g, lung 5.21 µg/g, brain 0.12 µg/g [Tracqui *et al.* 1997].

A 26-year-old man was found dead and the postmortem zuclopenthixol concentrations (µg/L) were as follows:

	Peripheral blood (µg/L)	*Cardiac blood (µg/L)*	*Gastric contents (µg/L)*	*Urine (µg/L)*
(*Z*)-*cis*-Clopenthixol	278	455	29880	505
(*E*)-*trans*-Clopenthixol	177	245	1680	230
Total	455	700	31560	735

[Rop 2001]

A 39-year-old woman was found dead. Heart and peripheral blood concentrations zuclopenthixol were 0.68 and 0.60 mg/L, respectively [Kollroser *et al.* 2001].

Note For a case of an 8-year-old child with X-linked cerebral adrenoleukodystrophy developing neuroleptic malignant syndrome when his zuclopenthixol was increased, see Rubio-Gozalbo *et al.* [2001]; for a 14-year-old girl with neuroleptic malignant syndrome, see Erermis *et al.* [2007]; for three patients with neuroleptic malignant syndrome, see Kemperman, van den Hoofdakker [1990].

Half-life Zuclopenthixol, mean 20 h (range 12–29 h); zuclopenthixol hydrochloride, 1 day.

Volume of Distribution 15–20 L/kg.

Clearance Systemic clearance, 0.9 L/min.

Protein Binding 98%.

Note For a review of the clinical physiognomy of zuclopenthixol see Rimestad [1974].

Dose Zuclopenthixol acetate 50 to 150 mg (1 to 3 mL); zuclopenthixol decanoate 200 to 500 mg every 1 to 4 weeks, with a maximum of 600 mg weekly; zuclopenthixol hydrochloride doses between 4 and 150 mg daily have been administered but the usual dose is 20 to 50 mg.

Aaes-Jorgensen T (1980). Specific high-performance liquid chromatographic method for estimation of the *cis*-(Z)- and *trans*-(E)-isomers of clopenthixol and a *N*-dealkyl metabolite. *J Chromatogr* 183: 239–245.

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PART THREE

Indexes of Analytical Data

1 CAS Numbers

CAS no.	Compound
50-00-0	Formaldehyde Solution
50-02-2	Dexamethasone
50-03-3	Hydrocortisone Acetate
50-04-4	Cortisone Acetate
50-06-6	Phenobarbital
50-09-9	Hexobarbital Sodium
50-10-2	Oxyphenonium Bromide
50-11-3	Metharbital
50-12-4	Mephenytoin
50-13-5	Pethidine Hydrochloride
50-14-6	Ergocalciferol
50-18-0	Cyclophosphamide (anhydrous)
50-19-1	Hydroxyphenamate
50-23-7	Hydrocortisone
50-24-8	Prednisolone (anhydrous)
50-27-1	Estriol
50-28-2	Estradiol
50-29-3	Clofenotane
50-33-9	Phenylbutazone
50-34-0	Propantheline Bromide
50-35-1	Thalidomide
50-36-2	Cocaine
50-37-3	Lysergide
50-41-9	Clomifene Citrate
50-42-0	Adiphenine Hydrochloride
50-44-2	Mercaptopurine (anhydrous)
50-47-5	Desipramine
50-48-6	Amitriptyline
50-49-7	Imipramine
50-50-0	Estradiol Benzoate
50-52-2	Thioridazine
50-53-3	Chlorpromazine
50-54-4	Quinidine Sulfate (anhydrous)
50-55-5	Reserpine
50-58-8	Phendimetrazine Tartrate
50-59-9	Cefaloridine
50-60-2	Phentolamine
50-62-4	Hexobendine Hydrochloride
50-63-5	Chloroquine Phosphate
50-65-7	Niclosamide
50-67-9	Serotonin
50-76-0	Dactinomycin
50-78-2	Aspirin
50-81-7	Ascorbic Acid
50-98-6	Ephedrine Hydrochloride
51-03-6	Piperonyl Butoxide
51-05-8	Procaine Hydrochloride
51-06-9	Procainamide
51-12-7	Nialamide
51-15-0	Pralidoxime Chloride
51-21-8	Fluorouracil
51-30-9	Isoprenaline Hydrochloride
51-34-3	Hyoscine
51-40-1	Noradrenaline Acid Tartrate (anhydrous)
51-41-2	Noradrenaline
51-42-3	Adrenaline Acid Tartrate
51-43-4	Adrenaline
51-45-6	Histamine
51-48-9	Levothyroxine
51-52-5	Propylthiouracil
51-55-8	Atropine
51-56-9	Homatropine Hydrobromide
51-57-0	Metamfetamine Hydrochloride
51-60-5	Neostigmine Methylsulfate
51-61-6	Dopamine
51-63-8	Dexamfetamine Sulfate
51-64-9	Dexamfetamine
51-67-2	Tyramine
51-68-3	Meclofenoxate
51-71-8	Phenelzine
51-74-1	Histamine Acid Phosphate
51-79-6	Urethane
51-83-2	Carbachol
51-98-9	Norethisterone Acetate
52-01-7	Spironolactone
52-21-1	Prednisolone Acetate
52-24-4	Thiotepa
52-26-6	Morphine Hydrochloride (anhydrous)
52-28-8	Codeine Phosphate (anhydrous)
52-31-3	Cyclobarbital
52-39-1	Aldosterone
52-43-7	Allobarbital
52-49-3	Trihexyphenidyl Hydrochloride
52-53-9	Verapamil
52-62-0	Pentolonium Tartrate
52-67-5	Penicillamine
52-68-6	Trichlorfon
52-76-6	Lynestrenol
52-78-8	Norethandrolone
52-85-7	Famphur
52-86-8	Haloperidol
52-88-0	Atropine Methonitrate
53-03-2	Prednisone
53-06-5	Cortisone
53-16-7	Estrone
53-21-4	Cocaine Hydrochloride
53-33-8	Paramethasone
53-36-1	Methylprednisolone Acetate
53-39-4	Oxandrolone
53-41-8	Androsterone
53-43-0	Dehydroepiandrosterone
53-46-3	Methanthelinium Bromide
53-60-1	Promazine Hydrochloride
53-86-1	Indometacin
54-03-5	Hexobendine
54-04-6	Mescaline
54-05-7	Chloroquine
54-11-5	Nicotine
54-21-7	Sodium Salicylate
54-31-9	Furosemide
54-32-0	Moxisylyte
54-35-3	Procaine Benzylpenicillin (anhydrous)
54-36-4	Metyrapone
54-42-2	Idoxuridine
54-49-9	Metaraminol
54-71-7	Pilocarpine Hydrochloride
54-85-3	Isoniazid
54-87-5	Nitrofurantoin Sodium
54-91-1	Pipobroman
54-92-2	Iproniazid
54-95-5	Pentetrazol
55-312	Adrenaline Hydrochloride
55-03-8	Levothyroxine Sodium (anhydrous)
55-06-1	Liothyronine Sodium
55-38-9	Fenthion
55-48-1	Atropine Sulfate (anhydrous)
55-56-1	Chlorhexidine
55-63-0	Glyceryl Trinitrate
55-65-2	Guanethidine
55-73-2	Bethanidine
55-92-5	Methacholine
55-94-7	Suxamethonium Bromide
55-97-0	Hexamethonium Bromide
55-98-1	Busulfan
56-04-2	Methylthiouracil
56-23-5	Carbon Tetrachloride
56-25-7	Cantharidin
56-29-1	Hexobarbital
56-38-2	Parathion
56-47-3	Desoxycortone Acetate
56-53-1	Diethylstilbestrol
56-54-2	Quinidine (anhydrous)
56-65-5	Adenosine Triphosphate
56-72-4	Coumaphos
56-75-7	Chloramphenicol
56-92-8	Histamine Hydrochloride
56-94-0	Demecarium Bromide
56-95-1	Chlorhexidine Diacetate
57-12-5	Cyanide
57-13-6	Urea
57-15-8	Chlorobutanol (anhydrous)
57-22-7	Vincristine
57-24-9	Strychnine
57-27-2	Morphine (anhydrous)
57-29-4	Nalorphine Hydrochloride
57-30-7	Phenobarbital Sodium
57-33-0	Pentobarbital Sodium
57-37-4	Benactyzine Hydrochloride
57-41-0	Phenytoin
57-42-1	Pethidine
57-43-2	Amobarbital
57-44-3	Barbital
57-47-6	Physostigmine
57-53-4	Meprobamate
57-62-5	Chlortetracycline
57-63-6	Ethinylestradiol
57-64-7	Physostigmine Salicylate
57-66-9	Probenecid
57-67-0	Sulfaguanidine
57-68-1	Sulfadimidine
57-74-9	Chlordane
57-83-0	Progesterone
57-85-2	Testosterone Propionate
57-88-5	Cholesterol
57-92-1	Streptomycin
57-94-3	Tubocurarine Chloride (anhydrous)
57-95-4	Tubocurarine
57-96-5	Sulfinpyrazone
58-00-4	Apomorphine
58-08-2	Caffeine
58-14-0	Pyrimethamine
58-15-1	Aminophenazone
58-18-4	Methyltestosterone
58-19-5	Drostanolone
58-20-8	Testosterone Cipionate
58-22-0	Testosterone
58-25-3	Chlordiazepoxide
58-27-5	Menadione
58-28-6	Desipramine Hydrochloride
58-32-2	Dipyridamole
58-33-3	Promethazine Hydrochloride
58-34-4	Thiazinamium Metilsulfate
58-37-7	Aminopromazine
58-38-8	Prochlorperazine
58-39-9	Perphenazine
58-40-2	Promazine
58-46-8	Tetrabenazine
58-54-8	Etacrynic Acid
58-55-9	Theophylline
58-56-0	Pyridoxine Hydrochloride
58-61-7	Adenosine
58-71-9	Cefalothin Sodium
58-73-1	Diphenhydramine
58-74-2	Papaverine
58-89-9	Benzene Hexachloride (γ-isomer)
58-89-9	Lindane
58-93-5	Hydrochlorothiazide
58-94-6	Chlorothiazide
59-01-8	Kanamycin
59-05-2	Methotrexate
59-26-7	Nikethamide
59-30-3	Folic Acid
59-32-5	Chloropyramine
59-33-6	Mepyramine Maleate
59-39-2	Piperoxan
59-40-5	Sulfaquinoxaline
59-41-6	Bretylium
59-42-7	Phenylephrine
59-43-8	Thiamine

CAS no.	*Compound*
59-46-1	Procaine
59-47-2	Mephenesin
59-50-7	Chlorocresol
59-52-9	Dimercaprol
59-63-2	Isocarboxazid
59-66-5	Acetazolamide
59-67-6	Nicotinic Acid
59-87-0	Nitrofurazone
59-92-7	Levodopa
59-96-1	Phenoxybenzamine
59-97-2	Tolazoline Hydrochloride
59-98-3	Tolazoline
59-99-4	Neostigmine
60-13-9	Amfetamine Sulfate
60-19-5	Tyramine Hydrochloride
60-26-4	Hexamethonium
60-29-7	Ether
60-30-0	Azamethonium
60-31-1	Acetylcholine Chloride
60-32-2	Aminocaproic Acid
60-40-2	Mecamylamine
60-41-3	Strychnine Sulfate (anhydrous)
60-44-6	Penthienate Methobromide
60-45-7	Fenimide
60-46-8	Dimevamide
60-49-1	Tridihexethyl
60-51-5	Dimethoate
60-54-8	Tetracycline (anhydrous)
60-56-0	Thiamazole
60-57-1	HEOD
60-79-7	Ergometrine
60-80-0	Phenazone
60-87-7	Promethazine
60-89-9	Pecazine
60-91-3	Diethazine
60-93-5	Quinine Dihydrochloride
60-99-1	Levomepromazine
61-00-7	Acepromazine
61-01-8	Methoxypromazine
61-12-1	Cinchocaine Hydrochloride
61-16-5	Methoxamine Hydrochloride
61-25-6	Papaverine Hydrochloride
61-32-5	Meticillin
61-33-6	Benzylpenicillin
61-49-4	*N*-Methyltryptamine
61-50-7	Dimethyltryptamine
61-51-8	Diethyltryptamine
61-54-1	Tryptamine
61-56-3	Sultiame
61-57-4	Niridazole
61-68-7	Mefenamic Acid
61-72-3	Cloxacillin
61-75-6	Bretylium Tosilate
61-76-7	Phenylephrine Hydrochloride
61-78-9	Aminohippuric Acid
61-82-5	Aminotriazole
62-13-5	Adrenalone Hydrochloride
62-31-7	Dopamine Hydrochloride
62-38-4	Phenylmercuric Acetate
62-44-2	Phenacetin
62-49-7	Choline (cation)
62-51-1	Methacholine Chloride
62-53-3	Aniline
62-67-9	Nalorphine
62-73-7	Dichlorvos
62-74-8	Sodium Fluoroacetate
62-76-0	Sodium Oxalate
62-90-8	Nandrolone Phenylpropionate
62-97-5	Diphemanil Metilsulfate
63-05-8	Androstenedione
63-12-7	Benzquinamide
63-25-2	Carbaryl
63-45-6	Primaquine Phosphate
63-56-9	Thonzylamine Hydrochloride
63-74-1	Sulfanilamide
63-75-2	Arecoline
63-89-8	Colfosceril Palmitate
63-92-3	Phenoxybenzamine Hydrochloride
63-98-9	Phenacemide
64-04-0	Phenethylamine
64-17-5	Ethanol
64-18-6	Formic Acid
64-19-7	Acetic Acid Glacial
64-31-3	Morphine Sulfate (anhydrous)
64-39-1	Trimeperidine
64-43-7	Amobarbital Sodium
64-47-1	Physostigmine Sulfate
64-55-1	Mebutamate
64-65-3	Bemegride
64-72-2	Chlortetracycline Hydrochloride
64-73-3	Demeclocycline Hydrochloride
64-75-5	Tetracycline Hydrochloride
64-77-7	Tolbutamide
64-85-7	Desoxycortone
64-86-8	Colchicine
64-95-9	Adiphenine
65-19-0	Yohimbine Hydrochloride
65-23-6	Pyridoxine
65-28-1	Phentolamine Mesilate
65-29-2	Gallamine Triethiodide
65-45-2	Salicylamide
65-49-6	Aminosalicylic Acid
65-64-5	Mebanazine
65-85-0	Benzoic Acid
66-23-9	Acetylcholine Bromide
66-32-0	Strychnine Nitrate
66-76-2	Dicoumarol
66-86-4	Neomycin C
66-97-7	Furanocoumarins
67-03-8	Thiamine Hydrochloride
67-04-9	Oxedrine Tartrate (±)
67-20-9	Nitrofurantoin (anhydrous)
67-45-8	Furazolidone
67-48-1	Choline Chloride
67-52-7	Barbituric Acid
67-56-1	Methanol
67-63-0	Isopropyl Alcohol
67-64-1	Acetone
67-66-3	Chloroform
67-68-5	Dimethyl Sulfoxide
67-73-2	Fluocinolone Acetonide
67-78-7	Triamcinolone Diacetate
67-92-5	Dicycloverine Hydrochloride
67-96-9	Dihydrotachysterol
67-97-0	Colecalciferol
68-19-9	Cyanocobalamin
68-22-4	Norethisterone
68-23-5	Noretynodrel
68-35-9	Sulfadiazine
68-41-7	Cycloserine
68-88-2	Hydroxyzine
68-89-3	Dipyrone (anhydrous)
68-90-6	Benziodarone
68-91-7	Trimetaphan Camsilate
68-96-2	Hydroxyprogesterone
69-05-6	Mepacrine Hydrochloride (anhydrous)
69-09-0	Chlorpromazine Hydrochloride
69-22-7	Caffeine Citrate
69-23-8	Fluphenazine
69-43-2	Prenylamine Lactate
69-44-3	Amodiaquine Hydrochloride (anhydrous)
69-52-3	Ampicillin Sodium
69-53-4	Ampicillin
69-57-8	Benzylpenicillin Sodium
69-65-8	Mannitol
69-72-7	Salicylic Acid
69-74-9	Cytarabine Hydrochloride
69-81-8	Carbazochrome
70-19-9	Thurfyl Nicotinate
70-30-4	Hexachlorophene
70-51-9	Desferrioxamine
71-23-8	Propanol
71-27-2	Suxamethonium Chloride (anhydrous)
71-43-2	Benzene
71-55-6	Trichloroethane
71-58-9	Medroxyprogesterone Acetate
71-63-6	Digitoxin
71-68-1	Hydromorphone Hydrochloride
71-73-8	Thiopental Sodium
71-78-3	Pipradrol Hydrochloride
71-81-8	Isopropamide Iodide
71-82-9	Levallorphan Tartrate
72-14-0	Sulfathiazole
72-20-8	Endrin
72-33-3	Mestranol
72-43-5	Methoxychlor
72-44-6	Methaqualone
72-63-9	Methandienone
72-69-5	Nortriptyline
72-80-0	Chlorquinaldol
73-05-2	Phentolamine Hydrochloride
73-22-3	Tryptophan
73-48-3	Bendroflumethiazide
73-49-4	Quinethazone
73-78-9	Lidocaine Hydrochloride (anhydrous)
74-55-5	Ethambutol
74-83-9	Methyl Bromide
74-90-8	Hydrogen Cyanide
75-07-0	Acetaldehyde
75-09-2	Methylene Chloride
75-44-5	Phosgene
75-60-5	Dimethylarsinic Acid
75-69-4	Trichlorofluoromethane
75-71-8	Dichlorodifluoromethane
76-06-2	Chloropicrin
76-14-2	Cryofluorane
76-22-2	Camphor
76-25-5	Triamcinolone Acetonide
76-38-0	Methoxyflurane
76-40-4	Butylchloral Hydrate
76-41-5	Oxymorphone
76-42-6	Oxycodone
76-43-7	Fluoxymesterone
76-44-8	Heptachlor
76-57-3	Codeine (anhydrous)
76-58-4	Ethylmorphine
76-68-6	Cyclopentobarbital
76-73-3	Secobarbital
76-74-4	Pentobarbital
76-75-5	Thiopental
76-90-4	Mepenzolate Bromide
76-94-8	Phenylmethylbarbituric Acid
76-99-3	Methadone
77-01-0	Fenpipramide
77-02-1	Aprobarbital
77-07-6	Levorphanol
77-09-8	Phenolphthalein
77-10-1	Phencyclidine
77-14-5	Proheptazine
77-15-6	Ethoheptazine
77-19-0	Dicycloverine
77-20-3	Alphaprodine
77-21-4	Glutethimide
77-22-5	Caramiphen
77-23-6	Carbetapentane
77-26-9	Butalbital
77-27-0	Thiamylal
77-28-1	Butobarbital
77-30-5	Hexethal
77-36-1	Chlortalidone
77-37-2	Procyclidine
77-38-3	Chlorphenoxamine
77-39-4	Cycrimine
77-41-8	Mesuximide
77-51-0	Isoaminile
77-65-6	Carbromal
77-66-7	Acecarbromal
77-67-8	Ethosuximide
77-75-8	Methylpentynol
77-81-6	Tabun
77-86-1	Trometamol
77-91-8	Choline Dihydrogen Citrate
78-11-5	Pentaerithrityl Tetranitrate
78-28-4	Emylcamate
78-34-2	Dioxathion
78-44-4	Carisoprodol
78-93-3	Methyl Ethyl Ketone
79-01-6	Trichloroethylene
79-34-5	Tetrachloroethane
79-55-0	Pempidine
79-57-2	Oxytetracycline Dihydrate (anhydrous)
79-64-1	Dimethisterone (anhydrous)
79-90-3	Triclobisonium Chloride
79-93-6	Phenaglycodol
80-08-0	Dapsone
80-13-7	Halazone
80-32-0	Sulfachlorpyridazine
80-35-3	Sulfamethoxypyridazine
80-49-9	Homatropine Methylbromide
80-50-2	Octatropine Methylbromide
80-74-0	Acetyl Sulfafurazole
80-77-3	Chlormezanone

CAS no.	Compound
80-78-4	Solanidine
81-07-2	Saccharin
81-13-0	Dexpanthenol
81-81-2	Warfarin
82-54-2	Cotarnine
82-58-6	Lysergic Acid
82-66-6	Diphenadione
82-88-2	Phenindamine
82-92-8	Cyclizine
82-93-9	Chlorcyclizine
82-95-1	Buclizine
82-98-4	Piperidolate
83-12-5	Phenindione
83-43-2	Methylprednisolone
83-67-0	Theobromine
83-73-8	Diiodohydroxyquinoline
83-74-9	Ibogaine
83-75-0	Quinine Ethyl Carbonate
83-79-4	Rotenone
83-88-5	Riboflavin
83-89-6	Mepacrine
83-98-7	Orphenadrine
84-02-6	Prochlorperazine Maleate
84-04-8	Pipamazine
84-06-0	Thiopropazate
84-12-8	Phanquinone
84-16-2	Hexestrol (meso)
84-17-3	Dienestrol
84-22-0	Tetryzoline
84-36-6	Syrosingopine
84-66-2	Diethyl Phthalate
84-74-2	Dibutyl Phthalate
84-80-0	Phytomenadione
84-96-8	Alimemazine
84-97-9	Perazine
85-00-7	Diquat Dibromide
85-73-4	Phthalylsulfathiazole
85-79-0	Cinchocaine
86-12-4	Thenalidine
86-13-5	Benzatropine
86-14-6	Diethylthiambutene
86-21-5	Pheniramine
86-22-6	Brompheniramine
86-34-0	Phensuximide
86-35-1	Ethotoin
86-42-0	Amodiaquine
86-43-1	Propoxycaine
86-50-0	Azinphos-(Me)
86-54-4	Hydralazine
86-78-2	Pentaquin
86-80-6	Quinisocaine
87-00-3	Homatropine
87-08-1	Phenoxymethylpenicillin
87-33-2	Isosorbide Dinitrate
87-67-2	Choline Bitartrate
87-86-5	Pentachlorophenol
88-04-0	Chloroxylenol
89-78-1	Menthol
89-83-8	Thymol
90-22-2	Valethamate Bromide (bromide)
90-34-6	Primaquine
90-39-1	Sparteine
90-45-9	Aminoacridine
90-49-3	Pheneturide
90-64-2	Mandelic Acid
90-69-7	Lobeline
90-81-3	Racephedrine
90-82-4	Pseudoephedrine
90-84-6	Diethylpropion
90-89-1	Diethylcarbamazine
91-20-3	Naphthalene
91-33-8	Benzthiazide
91-75-8	Antazoline
91-79-2	Thenyldiamine
91-80-5	Methapyrilene
91-81-6	Tripelennamine
91-82-7	Pyrrobutamine
91-84-9	Mepyramine
91-85-0	Thonzylamine
92-12-6	Phenyltoloxamine
92-13-7	Pilocarpine
92-52-4	Diphenyl
92-62-6	Proflavine
92-84-2	Phenothiazine
93-14-1	Guaifenesin
93-30-1	Methoxyphenamine
93-60-7	Methyl Nicotinate
93-76-5	Trichlorophenoxyacetic Acid
93-88-9	Phenylpropylmethylamine
94-07-5	Oxedrine
94-09-7	Benzocaine
94-10-0	Etoxazene
94-13-3	Propyl Hydroxybenzoate
94-15-5	Dimethocaine
94-16-6	Sodium Aminohippurate
94-19-9	Sulfaethidole
94-20-2	Chlorpropamide
94-24-6	Tetracaine
94-25-7	Butyl Aminobenzoate
94-35-9	Styramate
94-44-0	Benzyl Nicotinate
94-63-3	Pralidoxime Iodide
94-74-6	Methylchlorophenoxyacetic Acid
94-75-7	Dichlorophenoxyacetic Acid
94-78-0	Phenazopyridine
95-05-6	Sulfiram
95-25-0	Chlorzoxazone
95-27-2	Diamthazole
95-47-6	*o*-Xylene
95-48-7	*o*-Cresol
95-51-2	2-Chloroaniline
95-81-8	Chloromethylaniline
96-48-0	γ-Butyrolactone
96-64-0	Soman
96-88-8	Mepivacaine
97-23-4	Dichlorophen
97-24-5	Fenticlor
97-31-4	Normetadrenaline
97-44-9	Acetarsone
97-53-0	Eugenol
97-59-6	Allantoin
97-77-8	Disulfiram
98-50-0	Arsanilic Acid
98-92-0	Nicotinamide
98-95-3	Nitrobenzene
98-96-4	Pyrazinamide
99-43-4	Oxybuprocaine
99-45-6	Adrenalone
99-66-1	Valproic Acid
99-76-3	Methyl Hydroxybenzoate
100-33-4	Pentamidine
100-51-6	Benzyl Alcohol
100-52-7	Benzaldehyde
100-55-0	Nicotinyl Alcohol
100-88-9	Cyclamic Acid
100-91-4	Eucatropine
100-92-5	Mephentermine
100-97-0	Methenamine
101-08-6	Diperodon (anhydrous)
101-20-2	Triclocarban
101-26-8	Pyridostigmine Bromide
101-31-5	Hyoscyamine
101-40-6	Propylhexedrine
101-62-2	Phenamidine
101-93-9	Phenacaine
102-29-4	Resorcinol Monoacetate
102-45-4	Cyclopentamine
103-84-4	Acetanilide
103-86-6	Hydroxyamfetamine
103-90-2	Paracetamol
104-06-3	Thioacetazone
104-29-0	Chlorphenesin
104-31-4	Benzonatate
104-32-5	Propamidine
105-20-4	Ametazole
105-41-9	Methylhexaneamine
106-42-3	*p*-Xylene
106-44-5	*p*-Cresol
106-46-7	Paradichlorobenzene
106-47-8	4-Chloroaniline
106-48-9	Parachlorophenol
106-50-3	Paraphenylenediamine
107-02-8	Acrolein
107-15-3	Ethylenediamine Hydrate (anhydrous)
107-21-1	Ethylene Glycol
107-43-7	Betaine
107-44-8	Sarin
108-38-3	*m*-Xylene
108-39-4	*m*-Cresol
108-42-9	3-Chloroaniline
108-46-3	Resorcinol
108-88-3	Toluene
108-95-2	Phenol
110-44-1	Sorbic Acid
110-46-3	Amyl Nitrite
110-60-1	Putrescine
110-85-0	Piperazine
111-00-2	Suxethonium Bromide
111-48-8	Thiodiglycol (metabolite)
112-24-3	Trientine
113-07-5	Doxapram Hydrochloride (anhydrous)
113-15-5	Ergotamine
113-18-8	Ethchlorvynol
113-42-8	Methylergometrine
113-45-1	Methylphenidate
113-52-0	Imipramine Hydrochloride
113-53-1	Dosulepin
113-59-7	Chlorprothixene
113-69-9	Benzquinamide Hydrochloride
113-92-8	Chlorphenamine Maleate
113-98-4	Benzylpenicillin Potassium
114-07-8	Erythromycin A
114-49-8	Hyoscine Hydrobromide (trihydrate)
114-80-7	Neostigmine Bromide
114-85-2	Bethanidine Sulfate
114-86-3	Phenformin
114-90-9	Obidoxime Chloride
114-91-0	Metazocine
114-91-0	Methyridine
115-09-3	Methylmercuric Chloride
115-20-8	Trichloroethanol
115-29-7	Endosulfan
115-33-3	Oxyphenisatine Acetate
115-37-7	Thebaine
115-38-8	Methylphenobarbital
115-44-6	Talbutal
115-46-8	Azacyclonol
115-51-5	Ambutonium Bromide
115-63-9	Hexocyclium Metilsulfate
115-67-3	Paramethadione
115-77-5	Pentaerithrityl
115-79-7	Ambenonium Chloride (anhydrous)
116-06-3	Aldicarb
116-38-1	Edrophonium Chloride
116-43-8	Succinylsulfathiazole (anhydrous)
117-10-2	Dantron
117-37-3	Anisindione
117-74-8	Berberine
117-89-5	Trifluoperazine
118-08-1	Hydrastine
118-10-5	Cinchonine
118-23-0	Bromazine
118-42-3	Hydroxychloroquine
118-55-8	Salol
119-04-0	Neomycin B
119-36-8	Methyl Salicylate
120-29-6	Tropine
120-47-8	Ethyl Hydroxybenzoate
120-51-4	Benzyl Benzoate
120-97-8	Diclofenamide
121-19-7	Roxarsone
121-21-1	Pyrethrin I
121-25-5	Amprolium
121-29-9	Pyrethrin II
121-33-5	Vanillin
121-54-0	Benzethonium Chloride
121-59-5	Carbarsone
121-75-5	Malathion
122-06-5	Stilbamidine
122-09-8	Phentermine
122-11-2	Sulfadimethoxine
122-14-5	Fenitrothion
122-18-9	Cetalkonium Chloride
122-34-9	Simazine
123-03-5	Cetylpyridinium Chloride (anhydrous)
123-30-8	4-Aminophenol
123-31-9	Hydroquinone
123-51-3	3-Methyl-1-butanol
123-56-8	Succinimide
123-63-7	Paraldehyde
123-82-0	Tuaminoheptane
123-92-2	Amyl Acetate (iso)

CAS no.	*Compound*
124-58-3	Methanearsonic Acid
124-65-2	Sodium Cacodylate
124-87-8	Picrotoxin
124-90-3	Oxycodone Hydrochloride (anhydrous)
124-92-5	Metopon Hydrochloride
124-94-7	Triamcinolone
124-97-0	Protaveratrine B
125-02-0	Prednisolone Sodium Phosphate
125-04-2	Hydrocortisone Sodium Succinate
125-10-0	Prednisone Acetate
125-13-3	Oxyphenisatine
125-24-6	Pseudomorphine (anhydrous)
125-28-0	Dihydrocodeine
125-29-1	Hydrocodone
125-30-4	Ethylmorphine Hydrochloride (anhydrous)
125-33-7	Primidone
125-40-6	Secbutabarbital
125-42-8	Vinbarbital
125-51-9	Pipenzolate Bromide
125-52-0	Oxyphencyclimine Hydrochloride
125-53-1	Oxyphencyclimine
125-56-4	Methadone Hydrochloride (±)
125-64-4	Methyprylon
125-68-8	Racemethorphan Hydrobromide (*l*-Form)
125-69-9	Dextromethorphan Hydrobromide (anhydrous)
125-69-9	Racemethorphan Hydrobromide (*d*-Form)
125-71-3	Dextromethorphan
125-72-4	Levorphanol Tartrate (anhydrous)
125-73-5	Dextrorphan
125-80-4	Trimeperidine Hydrochloride
125-84-8	Aminoglutethimide
125-85-9	Caramiphen Hydrochloride
125-86-0	Caramiphen Edisilate
125-88-2	Aprobarbital Sodium
126-02-3	Cycrimine Hydrochloride
126-07-8	Griseofulvin
126-12-5	Anileridine Hydrochloride
126-27-2	Oxetacaine
126-52-3	Ethinamate
126-73-8	Tributyl Phosphate
126-75-0	Demeton-*S*
127-07-1	Hydroxycarbamide
127-18-4	Tetrachloroethylene
127-31-1	Fludrocortisone
127-33-3	Demeclocycline
127-35-5	Phenazocine
127-48-0	Trimethadione
127-56-0	Sulfacetamide Sodium (anhydrous)
127-57-1	Sulfapyridine Sodium Monohydrate (anhydrous)
127-58-2	Sulfamerazine Sodium
127-65-1	Tosylchloramide Sodium (anhydrous)
127-69-5	Sulfafurazole
127-79-7	Sulfamerazine
127-85-5	Sodium Arsanilate
128-37-0	Butylated Hydroxytoluene
128-44-9	Saccharin Sodium (anhydrous)
128-46-1	Dihydrostreptomycin
128-49-4	Docusate Calcium
128-62-1	Noscapine
129-03-3	Cyproheptadine
129-06-6	Warfarin Sodium
129-20-4	Oxyphenbutazone (anhydrous)
129-49-7	Methysergide Maleate
129-50-0	Ergometrine Tartrate
129-51-1	Ergometrine Maleate
129-74-8	Buclizine Hydrochloride
129-77-1	Piperidolate Hydrochloride
130-26-7	Clioquinol
130-37-0	Menadione Sodium Bisulfite (anhydrous)
130-61-0	Thioridazine Hydrochloride
130-80-3	Diethylstilbestrol Dipropionate
130-83-6	Azapetine Phosphate
130-89-2	Quinine Hydrochloride (anhydrous)
130-95-0	Quinine (anhydrous)
131-01-1	Deserpidine
131-11-3	Dimethyl Phthalate
131-28-2	Narceine (anhydrous)
131-69-1	Phthalylsulfacetamide
132-17-2	Benzatropine Mesilate
132-18-3	Diphenylpyraline Hydrochloride
132-18-3	Diphenylpyraline Hydrobromide
132-19-4	Diethylthiambutene Hydrochloride
132-20-7	Pheniramine Maleate
132-21-8	Dexbrompheniramine
132-22-9	Chlorphenamine
132-60-5	Cinchophen
132-69-4	Benzydamine Hydrochloride
132-73-0	Chloroquine Sulfate (anhydrous)
132-92-3	Meticillin Sodium (anhydrous)
132-93-4	Pheneticillin Potassium
132-98-9	Phenoxymethylpenicillin Potassium
133-09-5	Potassium Aminosalicylate
133-10-8	Sodium Aminosalicylate (anhydrous)
133-11-9	Phenyl Aminosalicylate
133-15-3	Calcium Aminosalicylate (anhydrous)
133-16-4	Chloroprocaine
133-67-5	Trichlormethiazide
134-03-2	Sodium Ascorbate
134-31-6	Hydroxyquinoline Sulfate
134-36-1	Erythromycin Propionate
134-49-6	Phenmetrazine
134-50-9	Aminoacridine Hydrochloride (anhydrous)
134-53-2	Amprotropine Phosphate
134-62-3	Diethyltoluamide
134-63-4	Lobeline Hydrochloride
134-64-5	Lobeline Sulfate
134-71-4	Racephedrine Hydrochloride
134-72-5	Ephedrine Sulfate
134-80-5	Diethylpropion Hydrochloride
134-95-2	Calcium Mandelate
135-07-9	Methyclothiazide
135-09-1	Hydroflumethiazide
135-14-8	Quinuronium Sulfate
135-19-3	Betanaphthol
135-23-9	Methapyrilene Hydrochloride
135-31-9	Pyrrobutamine Phosphate
135-44-4	Leucinocaine Mesylate
136-40-3	Phenazopyridine Hydrochloride
136-47-0	Tetracaine Hydrochloride
136-69-6	Protokylol Hydrochloride
136-70-9	Protokylol
136-77-6	Hexylresorcinol
136-82-3	Piperocaine
136-96-9	Diamthazole Hydrochloride
137-32-6	2-Methyl-1-butanol
137-58-6	Lidocaine
137-88-2	Amprolium Hydrochloride
138-14-7	Desferrioxamine Mesilate
138-37-4	Mafenide Hydrochloride
138-39-6	Mafenide
138-56-7	Trimethobenzamide
138-84-1	Potassium Aminobenzoate
138-92-1	Ametazole Hydrochloride
139-05-9	Sodium Cyclamate
139-06-0	Anhydrous Calcium Cyclamate
139-10-6	Amfetamine Phosphate
139-40-2	Propazine
139-62-8	Cyclomethycaine
139-91-3	Furaltadone
140-36-3	Hydroxyamfetamine Hydrobromide (±)
140-40-9	Aminitrozole
140-59-0	Stilbamidine Isethionate
140-63-6	Propamidine Isetionate
140-64-7	Pentamidine Isetionate
140-65-8	Pramocaine
141-43-5	Monoethanolamine
141-78-6	Ethyl Acetate
141-94-6	Hexetidine
142-63-2	Piperazine Hydrate
142-88-1	Piperazine Adipate
143-52-2	Metopon
143-57-7	Protoveratrine A and B
143-67-9	Vinblastine Sulfate
143-71-5	Hydrocodone Tartrate (anhydrous)
143-76-0	Cyclobarbital Calcium
143-81-7	Secbutabarbital Sodium
144-00-3	Hexethal Sodium
144-02-5	Barbital Sodium
144-11-6	Trihexyphenidyl
144-14-9	Anileridine
144-21-8	Disodium Methanearsonate
144-29-6	Piperazine Citrate (anhydrous)
144-44-5	Pentolonium
144-49-0	Fluoroacetic Acid
144-49-0	Fluoroacetic Acid
144-62-7	Oxalic Acid
144-74-1	Sulfathiazole Sodium (anhydrous)
144-80-9	Sulfacetamide
144-82-1	Sulfamethizole
144-83-2	Sulfapyridine
145-12-0	Oxymesterone
146-22-5	Nitrazepam
146-28-1	Thiopropazate Hydrochloride
146-36-1	Azapetine
146-48-5	Yohimbine
146-54-3	Triflupromazine
146-56-5	Fluphenazine Hydrochloride
147-20-6	Diphenylpyraline
147-24-0	Diphenhydramine Hydrochloride
147-27-3	Dimoxyline
147-48-8	Phenoxymethylpenicillin Calcium (anhydrous)
147-55-7	Pheneticillin
147-94-4	Cytarabine
148-01-6	Dinitolmide
148-18-5	Ditiocarb Sodium
148-24-3	Hydroxyquinoline
148-32-3	Amprotropine
148-64-1	Chloropyrilene Citrate
148-65-2	Chloropyrilene
148-72-1	Pilocarpine Nitrate
148-79-8	Tiabendazole
148-82-3	Melphalan
149-15-5	Butacaine Sulfate
149-16-6	Butacaine
149-29-1	Patulin
149-64-4	Hyoscine Butylbromide
150-13-0	Aminobenzoic Acid
150-59-4	Alverine
151-06-4	Chlorphentermine Hydrochloride
151-67-7	Halothane
151-73-5	Betamethasone Sodium Phosphate
151-83-7	Methohexital
152-02-3	Levallorphan
152-11-4	Verapamil Hydrochloride
152-47-6	Sulfametopyrazine
152-62-5	Dydrogesterone
152-72-7	Acenocoumarol
152-97-6	Fluocortolone
153-00-4	Metenolone
153-61-7	Cefalothin
153-76-4	Gallamine
153-87-7	Oxypertine
154-21-2	Lincomycin
154-41-6	Phenylpropanolamine Hydrochloride
154-42-7	Tioguanine (anhydrous)
154-68-7	Antazoline Phosphate
154-69-8	Tripelennamine Hydrochloride
154-93-8	Carmustine
154-97-2	Pralidoxime Mesilate
155-09-9	Tranylcypromine
155-41-9	Hyoscine Methobromide
155-97-5	Pyridostigmine
156-08-1	Benzfetamine
156-34-3	Levamfetamine
156-51-4	Phenelzine Sulfate
156-74-1	Decamethonium
244-63-3	Norharman
257-07-8	CR Gas
297-76-7	Etynodiol Diacetate
297-88-1	Methadone (±)
297-90-5	Racemorphan
298-04-4	Disulfoton
298-46-4	Carbamazepine
298-50-0	Propantheline
298-57-7	Cinnarizine
298-59-9	Methylphenidate Hydrochloride
298-81-7	Methoxsalen
298-96-4	Triphenyltetrazolium Chloride
299-26-3	α-Methyltryptamine
299-39-8	Sparteine Sulfate (anhydrous)
299-42-3	Anhydrous Ephedrine
299-95-6	Isoprenaline Sulfate (anhydrous)
300-08-3	Arecoline Hydrobromide
300-62-9	Amfetamine
300-76-5	Naled
301-04-2	Lead Acetate
302-17-0	Chloral Hydrate

CAS no.	*Compound*
302-22-7	Chlormadinone Acetate
302-27-2	Aconitine
302-27-2	Lithium Carbonate
302-31-8	Morphine Tartrate (anhydrous)
302-40-9	Benactyzine
302-41-0	Piritramide
302-66-9	Methylpentynol Carbamate
303-25-3	Cyclizine Hydrochloride
303-40-2	Fluocortolone Hexanoate
303-42-4	Metenolone Enantate
303-47-9	Ochratoxin A
303-49-1	Clomipramine
303-53-7	Cyclobenzaprine
303-69-5	Prothipendyl
304-20-1	Hydralazine Hydrochloride
304-21-2	Harmaline
304-84-7	Etamivan
305-03-3	Chlorambucil
305-33-9	Iproniazid Phosphate
306-03-6	Hyoscyamine Hydrobromide
306-07-0	Pargyline Hydrochloride
306-21-8	Hydroxyamfetamine Hydrobromide
306-40-1	Suxamethonium
306-52-5	Triclofos
306-53-6	Azamethonium Bromide
309-00-2	Aldrin
309-29-5	Doxapram
309-36-4	Methohexital Sodium
309-43-3	Secobarbital Sodium
312-48-1	Edrophonium
313-06-4	Estradiol Cipionate
314-19-2	Apomorphine Hydrochloride (anhydrous)
314-35-2	Etamiphylline
315-22-0	Monocrotaline
315-30-0	Allopurinol
315-37-7	Testosterone Enantate
315-72-0	Opipramol
315-80-0	Dibenzepin Hydrochloride
316-05-2	Mepacrine Mesilate (anhydrous)
316-42-7	Emetine Hydrochloride (anhydrous)
316-81-4	Thioproperazine
317-34-0	Aminophylline (anhydrous)
318-23-0	Imolamine
318-98-93	Propranolol Hydrochloride
319-84-6	Benzene Hexachloride (α-isomer)
319-85-7	Benzene Hexachloride (β-isomer)
319-86-8	Benzene Hexachloride (δ-isomer)
321-55-1	Haloxon
321-64-2	Tacrine
322-35-0	Benserazide
326-43-2	Fenyramidol Hydrochloride
329-56-6	Noradrenaline Hydrochloride
330-54-1	Diuron
330-55-2	Linuron
333-31-3	Methacholine Bromide
333-41-5	Dimpylate
337-03-1	Flugestone
337-47-3	Sodium Thiamylal
339-43-5	Carbutamide
340-56-7	Methaqualone Hydrochloride
340-57-8	Mecloqualone
341-69-5	Orphenadrine Hydrochloride
341-70-8	Diethazine Hydrochloride
343-94-2	Tryptamine Hydrochloride
345-78-8	Pseudoephedrine Hydrochloride
346-18-9	Polythiazide
356-12-7	Fluocinonide
357-07-3	Oxymorphone Hydrochloride
357-08-4	Naloxone Hydrochloride (dihydrate)
357-56-2	Dextromoramide
357-57-3	Brucine (anhydrous)
357-70-0	Galantamine
359-83-1	Pentazocine
360-70-3	Nandrolone Decanoate
361-37-5	Methysergide
362-29-8	Propiomazine
364-62-5	Metoclopramide
364-98-7	Diazoxide
365-26-4	Hydroxyephedrine
366-70-1	Procarbazine Hydrochloride
370-14-9	Pholedrine
372-66-7	Heptaminol
378-44-9	Betamethasone
379-79-3	Ergotamine Tartrate
389-08-2	Nalidixic Acid
390-28-3	Methoxamine
390-64-7	Prenylamine
395-28-8	Isoxsuprine
396-01-0	Triamterene
404-82-0	Fenfluramine Hydrochloride
426-13-1	Fluorometholone
427-00-9	Desomorphine
428-37-5	Profadol
432-60-0	Allylestrenol
434-03-7	Ethisterone
434-05-9	Metenolone Acetate
434-07-1	Oxymetholone
434-22-0	Nandrolone
435-97-2	Phenprocoumon
437-38-7	Fentanyl
437-74-1	Xantinol Nicotinate
438-41-5	Chlordiazepoxide Hydrochloride
438-60-8	Protriptyline
439-14-5	Diazepam
440-17-5	Trifluoperazine Hydrochloride
441-61-2	Ethylmethylthiambutene
442-16-0	Ethacridine
442-51-3	Harmine
442-52-4	Clemizole
443-48-1	Metronidazole
446-86-6	Azathioprine
447-41-6	Buphenine
452-35-7	Ethoxzolamide
456-59-7	Cyclandelate
458-24-2	Fenfluramine
458-88-8	Coniine
461-78-9	Chlorphentermine
462-94-2	Cadaverine
464-49-3	Camphor (+)
465-65-6	Naloxone
466-40-01	Isomethadone
466-90-0	Thebacon
466-97-7	Normorphine
466-99-9	Hydromorphone
467-14-1	Neopine
467-15-2	Norcodeine
467-18-5	Myrophine
467-36-7	Thialbarbital
467-60-7	Pipradrol
467-83-4	Dipipanone
467-84-5	Phenadoxone
467-85-6	Normethadone
467-86-7	Dioxaphetyl Butyrate
468-07-5	Phenomorphan
468-51-9	Betameprodine
468-59-7	Betaprodine
468-61-1	Oxeladin
469-21-6	Doxylamine
469-62-5	Dextropropoxyphene
469-79-4	Ketobemidone
469-81-8	Morpheridine
469-82-9	Etoxeridine
470-82-6	Cineole
471-53-4	Enoxolone
473-41-6	Tolbutamide Sodium
475-81-0	*d*-Glaucine
476-32-4	Chelidonine
477-30-5	Demecolcine
477-93-0	Dimethoxanate
478-43-3	Rhein
478-94-4	Lysergamide
479-18-5	Diprophylline
479-92-5	Propyphenazone
480-30-8	Dichloralphenazone
481-06-1	Santonin
481-37-8	Ecgonine
482-15-5	Isothipendyl
483-17-0	Cephaëline
483-18-1	Emetine
483-63-6	Crotamiton
484-23-1	Dihydralazine
485-35-8	Cytisine
485-71-2	Cinchonidine
486-12-4	Triprolidine
486-16-8	Carbinoxamine
486-17-9	Captodiame
486-67-9	Mersalyl Acid
486-84-0	Harman
487-93-4	Bufotenine
490-55-1	Amiphenazole
492-18-2	Mersalyl Sodium
492-39-7	Cathine
493-75-4	Bialamicol
493-78-7	Methaphenilene
493-80-1	Histapyrrodine
493-92-5	Prolintane
495-70-5	Meprylcaine
495-83-0	Tigloidine
495-84-1	Salinazid
495-99-8	Hydroxystilbamidine
496-00-4	Dibrompropamidine
496-67-3	Bromisoval
499-67-2	Proxymetacaine
500-34-5	Benzamine
500-55-0	Apoatropine
500-89-0	Thiambutosine
500-92-5	Proguanil
501-68-8	Beclamide
502-59-0	Octamylamine
502-85-2	Sodium-4-hydroxybutyrate
503-01-5	Isometheptene
505-60-2	Sulfur Mustard
506-09-0	Propranolol Hydrochloride (±)
506-68-3	Cyanogen Bromide
506-77-4	Cyanogen Chloride
508-99-6	Hydrocortisone Cipionate
509-15-9	Gelsemine
509-60-4	Dihydromorphine
509-67-1	Pholcodine (anhydrous)
509-78-4	Dimenoxadole
509-86-4	Heptabarb
510-53-2	Racemethorphan
511-13-7	Clofedanol Hydrochloride
512-15-2	Cyclopentolate
513-10-0	Ecothiopate Iodide
513-77-9	Barium Carbonate
513-78-0	Cadmium Carbonate
514-36-3	Fludrocortisone Acetate
514-65-8	Biperiden
515-64-0	Sulfisomidine
516-21-2	Cycloguanil
517-18-0	Methallenestril
518-63-8	Dimethoxanate Hydrochloride
518-75-2	Citrinin
519-09-5	Benzoylecgonine
519-37-9	Etofylline
519-88-0	Ambucetamide
520-52-5	Psilocybine
520-53-6	Psilocin
520-85-4	Medroxyprogesterone
521-10-8	Methandriol
521-11-9	Mestanolone
521-12-0	Propionate
521-18-6	Androstanolone
521-74-4	Broxyquinoline
521-78-8	Trimipramine Maleate
522-00-9	Profenamine
522-40-7	Diethylstilbestrol Diphosphate
522-48-5	Tetryzoline Hydrochloride
522-51-0	Dequalinium Chloride
522-66-7	Hydroquinine
523-87-5	Dimenhydrinate
524-61-8	Cinchonidine Sulfate (anhydrous)
524-81-2	Mebhydrolin
524-84-5	Dimethylthiambutene
525-66-6	Propranolol
526-08-9	Sulfaphenazole
526-36-3	Xylometazoline
528-92-7	Apronal
530-08-5	Isoetarine
530-31-4	Ammonium Mandelate
530-43-8	Chloramphenicol Palmitate
530-78-9	Flufenamic Acid
532-03-6	Methocarbamol
532-27-4	CN Gas
532-32-1	Sodium Benzoate
532-43-4	Thiamine Mononitrate
532-59-2	Amylocaine Hydrochloride
532-76-3	Hexylcaine Hydrochloride
532-77-4	Hexylcaine
533-06-2	Mephenesin Carbamate
533-22-2	Hydroxystilbamidine Isetionate

CAS no.	*Compound*
533-28-8	Piperocaine Hydrochloride
533-45-9	Clomethiazole
534-52-1	Dinitro-orthocresol
534-87-2	Etilefrine Hydrochloride
536-24-3	Ethylnoradrenaline
536-25-4	Orthocaine
536-33-4	Ethionamide
536-43-6	Dyclonine Hydrochloride
536-71-0	Diminazene
536-93-6	Eucatropine Hydrochloride
537-12-2	Diperodon Hydrochloride
537-21-3	Chlorproguanil
537-26-8	Tropacocaine
537-46-2	Metamfetamine
537-61-1	Cyclomethycaine (hydrochloride)
538-71-6	Domiphen Bromide
539-15-1	Hordenine
539-21-9	Ambazone (anhydrous)
541-19-5	Suxamethonium Iodide
541-22-0	Decamethonium Bromide
541-25-3	Lewisite
541-73-8	Dithiazanine Iodide
542-42-5	1,2-Naphthoquinone
542-62-1	Barium Cyanide
543-15-7	Heptaminol Hydrochloride
543-80-6	Barium Acetate
545-80-2	Poldine Metilsulfate
545-91-5	Phenadoxone Hydrochloride
545-93-7	Ibomal
546-06-5	Conessine
546-48-5	Pempidine Tartrate
547-32-0	Sulfadiazine Sodium
547-44-4	Sulfacarbamide (anhydrous)
548-00-5	Ethyl Biscoumacetate
548-73-2	Droperidol
548-84-5	Pyrvinium Chloride
549-18-8	Amitriptyline Hydrochloride
549-49-5	Quinine Hydrobromide (anhydrous)
549-56-4	Quinine Bisulfate (anhydrous)
549-68-8	Octaverine
550-01-6	Metabutethamine Hydrochloride
550-01-6	Metabutoxycaine Hydrochloride
550-28-7	Amisometradine
550-70-9	Triprolidine Hydrochloride (anhydrous)
550-81-2	Amopyroquine
550-83-4	Propoxycaine Hydrochloride
550-99-2	Naphazoline Hydrochloride
551-01-9	Plasmocide
551-27-9	Propicillin
551-48-4	Guanoclor Sulfate
551-74-6	Mannomustine
551-92-8	Dimetridazole
552-25-0	Diampromide
552-79-4	Methylephedrine
552-94-3	Salsalate
553-30-0	Proflavine Hemisulfate (sulfate, anhydrous)
553-69-5	Fenyramidol
554-57-4	Methazolamide
554-92-7	Trimethobenzamide Hydrochloride
555-30-6	Methyldopa (anhydrous)
555-57-7	Pargyline
555-65-7	Brocresine
561-10-4	Isomethadone (*l*-Form)
561-27-3	Diamorphine
561-48-8	Norpipanone
561-76-2	Properidine
561-78-4	Alphaprodine Hydrochloride (±)
561-83-1	Nealbarbital
561-86-4	Brallobarbital
562-09-4	Chlorphenoxamine Hydrochloride
562-10-7	Doxylamine Succinate
562-26-5	Phenoperidine
563-72-4	Calcium Oxalate
564-25-0	Doxycycline (anhydrous)
566-48-3	Formestane
568-69-4	Amotriphene Hydrochloride
569-57-3	Chlorotrianisene
569-59-5	Phenindamine Tartrate
569-65-3	Meclozine
573-20-6	Acetomenaphthone
573-41-1	Theophylline Monoethanolamine
575-74-6	Buclosamide
577-11-7	Docusate Sodium
577-48-0	Butyl Aminobenzoate Picrate
578-94-9	Adamsite
579-38-4	Diloxanide
579-56-6	Isoxsuprine Hydrochloride
581-88-4	Debrisoquine Sulfate
583-52-8	Potassium Oxalate
586-06-1	Orciprenaline
586-60-7	Dyclonine
587-23-5	Methenamine Mandelate
587-61-1	Propyliodone
590-00-1	Potassium Sorbate
590-46-5	Betaine Hydrochloride
590-63-6	Bethanechol Chloride
591-81-1	γ-Hydroxybutyrate
593-74-8	Dimethylmercury
595-33-5	Megestrol Acetate
596-15-6	Morphine Acetate (anhydrous)
596-50-9	Poldine
596-51-0	Glycopyrronium Bromide
598-31-2	Bromoacetone
599-79-1	Sulfasalazine
603-00-9	Proxyphylline
603-50-9	Bisacodyl
604-51-3	Deptropine
604-75-1	Oxazepam
607-91-0	Myristicin
609-78-9	Cycloguanil Embonate
611-72-3	Mandelic Acid (±)
611-75-6	Bromhexine Hydrochloride
614-18-6	Ethyl Nicotinate
614-39-1	Procainamide Hydrochloride
614-87-9	Dibrompropamidine Isetionate
616-91-1	Acetylcysteine
620-61-1	Hyoscyamine Sulfate (anhydrous)
620-90-6	Phenamidine Isethionate
620-99-5	Phenacaine Hydrochloride Monohydrate (anhydrous)
622-64-0	Hordenine Sulfate (anhydrous)
628-63-7	Amyl Acetate (*n*)
630-56-8	Hydroxyprogesterone Caproate
630-60-4	Ouabain (anhydrous)
630-86-4	Benzylmorphine Hydrochloride
630-93-3	Phenytoin Sodium
632-00-8	Sulfasomizole
633-47-6	Cropropamide
633-59-0	Clopenthixol Hydrochloride
633-59-0	Zuclopenthixol Hydrochloride
633-65-8	Berberine Hydrochloride (anhydrous)
633-66-9	Berberine Sulfate
634-03-7	Phendimetrazine
635-41-6	Trimetozine
636-54-4	Clopamide
637-07-0	Clofibrate
637-32-1	Proguanil Hydrochloride
637-49-0	Coniine Hydrobromide
637-58-1	Pramocaine Hydrochloride
638-94-8	Desonide
639-48-5	Nicomorphine
640-19-7	Fluoroacetamide
642-44-4	Aminometradine
642-72-8	Benzydamine
642-78-4	Cloxacillin Sodium (anhydrous)
643-22-1	Erythromycin Stearate
644-26-8	Amylocaine
644-62-2	Meclofenamic Acid
645-05-6	Altretamine
645-43-2	Guanethidine Monosulfate
651-06-9	Sulfametoxydiazine
651-48-9	Dehydroepiandrosterone Sulfate
653-03-2	Butaperazine
655-35-6	Carbocromen Hydrochloride
657-24-9	Metformin
665-66-7	Amantadine Hydrochloride
671-16-9	Procarbazine
671-88-5	Disulfamide
673-31-4	Phenprobamate
674-38-4	Bethanechol
692-13-7	Buformin
695-53-4	Dimethadione
709-55-7	Etilefrine
709-98-8	Propanil
721-50-6	Prilocaine
723-46-6	Sulfamethoxazole
729-99-7	Sulfamoxole
734-32-7	Norandrostenedione
738-70-5	Trimethoprim
739-71-9	Trimipramine
742-20-1	Cyclopenthiazide
745-65-3	Alprostadil
747-36-4	Hydroxychloroquine Sulfate
747-45-5	Quinidine Bisulfate (anhydrous)
749-13-3	Trifluperidol
750-90-3	Quinine Salicylate (anhydrous)
751-94-0	Sodium Fusidate
751-97-3	Rolitetracycline
768-94-5	Amantadine
791-35-5	Clofedanol
804-10-4	Carbocromen
804-63-7	Quinine Sulfate (anhydrous)
808-48-0	Desoxycortone Pivalate
826-39-1	Mecamylamine Hydrochloride
831-61-8	Ethyl Gallate
834-12-8	Ametryne
834-28-6	Phenformin Hydrochloride
835-31-4	Naphazoline
841-06-5	Methoprotryne
846-48-0	Boldenone
846-49-1	Lorazepam
846-50-4	Temazepam
847-84-7	Normethadone Hydrochloride
848-53-3	Homochlorcyclizine
848-75-9	Lormetazepam
849-55-8	Buphenine Hydrochloride
855-19-6	Clostebol Acetate
856-87-1	Dipipanone Hydrochloride
859-18-7	Lincomycin Hydrochloride (anhydrous)
865-04-3	Methoserpidine
865-21-4	Vinblastine
870-62-2	Hexamethonium Iodide
886-50-0	Terbutryne
886-74-8	Chlorphenesin Carbamate
894-71-3	Nortriptyline Hydrochloride
897-06-3	Boldione
897-15-4	Dosulepin Hydrochloride
904-04-1	Captodiame Hydrochloride
908-54-3	Diminazene Aceturate (anhydrous)
909-39-7	Opipramol Hydrochloride
910-86-1	Tiocarlide
911-45-5	Clomifene
911-65-9	Etonitazene
912-60-7	Noscapine Hydrochloride (anhydrous)
914-00-1	Methacycline
915-30-0	Diphenoxylate
919-16-4	Lithium Citrate (anhydrous)
919-86-8	Demeton-*S*-(Me)
926-93-2	Metallibure
938-73-8	Etenzamide
942-31-4	Amiphenazole Hydrochloride
942-51-8	Hydroxyephedrine Hydrochloride
952-54-5	Morinamide
956-03-6	Meprylcaine Hydrochloride
956-90-1	Phencyclidine Hydrochloride
958-93-0	Thenyldiamine Hydrochloride
959-24-0	Sotalol Hydrochloride
959-98-8	Endosulfan (α-)
963-39-3	Demoxepam
964-52-3	Moxisylyte Hydrochloride
965-90-2	Ethylestrenol
968-81-0	Acetohexamide
969-33-5	Cyproheptadine Hydrochloride (anhydrous)
972-02-1	Difenidol
979-32-8	Estradiol Valerate
980-71-2	Brompheniramine Maleate
982-24-1	Clopenthixol
982-57-0	Chloramphenicol Sodium Succinate
987-24-6	Betamethasone Acetate
990-73-8	Fentanyl Citrate
991-42-4	Norbormide
992-21-2	Lymecycline
1007-33-6	Propylhexedrine Hydrochloride
1010-95-3	5-Methyltryptamine Hydrochloride
1014-69-3	Desmetryne
1019-45-0	5-Methoxy-*N*,*N*-dimethyltryptamine
1028-33-7	Pentifylline
1041-90-3	Nalorphine Hydrobromide
1050-48-2	Benzilonium Bromide
1055-55-6	Bunamidine Hydrochloride

CAS no.	*Compound*
1063-55-4	Butaperazine Maleate
1069-66-5	Sodium Valproate
1070-11-7	Ethambutol Hydrochloride
1071-83-6	Glyphosate
1082-57-1	Tramazoline
1082-88-8	Trimethoxyamfetamine
1088-11-5	Nordazepam
1093-58-9	Clostebol
1094-08-2	Profenamine Hydrochloride
1095-90-5	Methadone Hydrochloride
1098-60-8	Triflupromazine Hydrochloride
1099-87-2	Dehydroepiandrosterone Sodium Sulfate
1104-22-9	Meclozine Hydrochloride (anhydrous)
1107-99-9	Prednisolone Pivalate
1111-39-3	Acetyldigitoxin
1113-02-6	Omethoate
1115-70-4	Metformin Hydrochloride
1131-64-2	Debrisoquine
1134-47-0	Baclofen
1142-70-7	Butallylonal
1143-38-0	Dithranol
1156-05-4	Phenglutarimide
1156-19-0	Tolazamide
1162-65-8	Aflatoxin B1
1163-36-6	Clemizole Hydrochloride
1164-38-1	Lachesine Chloride
1165-39-5	Aflatoxin G1
1165-48-6	Dimefline
1166-52-5	Dodecyl Gallate
1172-18-5	Flurazepam Hydrochloride
1176-08-5	Phenyltoloxamine Citrate
1177-87-3	Dexamethasone Acetate (anhydrous)
1179-69-7	Thiethylperazine Maleate
1181-54-0	Clomocycline
1190-53-0	Buformin Hydrochloride
1197-21-3	Phentermine Hydrochloride
1200-55-1	Pralidoxime Metilsulfate
1209-98-9	Fencamfamin
1211-28-5	Prolintane Hydrochloride
1212-72-2	Mephentermine Sulfate (anhydrous)
1213-06-5	Etebenecid
1218-35-5	Xylometazoline Hydrochloride
1225-55-4	Protriptyline Hydrochloride
1225-60-1	Isothipendyl Hydrochloride
1225-65-6	Prothipendyl Hydrochloride (anhydrous)
1229-29-4	Doxepin Hydrochloride
1229-35-2	Methdilazine Hydrochloride
1229-69-2	Bamipine Hydrochloride
1231-93-2	Etynodiol
1235-15-0	Norbolethone
1235-82-1	Biperiden Hydrochloride
1239-04-9	Phenazocine Hydrobromide (anhydrous)
1239-45-8	Homidium Bromide
1240-15-9	Propiomazine Hydrochloride
1242-56-4	Stenbolone Acetate
1245-44-9	Propicillin Potassium
1249-84-9	Azacosterol
1253-28-7	Gestonorone Caproate
1254-35-9	Oxabolone Cipionate
1255-49-8	Testosterone Phenylpropionate
1257-78-9	Prochlorperazine Edisilate
1264-62-6	Erythromycin Ethylsuccinate
1300-94-3	Amylmetacresol
1303-00-0	Gallium Arsenide
1303-28-2	Arsenic Pentoxide
1304-28-5	Barium Oxide
1304-56-9	Beryllium Oxide
1306-19-0	Cadmium Oxide
1306-23-6	Cadmium Sulfide
1309-64-4	Antimony Trioxide
1314-60-9	Antimony Pentoxide
1314-87-0	Lead Sulfide
1315-04-4	Antimony Pentasulfide
1317-36-8	Lead Oxide
1319-77-3	Cresol
1320-42-9	Aminonitrothiazole
1327-41-9	Aluminium Chlorhydrate
1327-53-3	Arsenic Trioxide
1327-53-3	Arsenic Trioxide
1330-20-7	Xylene
1330-43-4	Sodium Borate Decahydrate
1344-28-1	Aluminium Oxide
1344-48-5	Mercuric (II) Sulfide
1345-04-6	Antimony Trisulfide
1397-89-3	Amphotericin B
1400-61-9	Nystatin
1401-69-0	Tylosin
1403-66-3	Gentamicin
1404-04-2	Neomycin
1404-90-6	Vancomycin
1404-93-9	Vancomycin Hydrochloride
1405-10-3	Neomycin Sulfate
1405-37-4	Capreomycin Sulfate
1405-41-0	Gentamicin Sulfate
1405-54-5	Tylosin Tartrate
1405-89-6	Bacitracin Zinc
1406-04-8	Neomycin Undecylenate
1415-73-2	Barbaloin
1420-53-7	Codeine Sulfate (anhydrous)
1420-55-9	Thiethylperazine
1421-14-3	Propanidid
1421-28-9	Dihydromorphine Hydrochloride
1421-68-7	Amidefrine Mesilate
1421-86-9	Strychnine Hydrochloride (anhydrous)
1422-07-7	Codeine Hydrochloride (anhydrous)
1424-00-6	Mesterolone
1424-27-7	Acetazolamide Sodium
1435-55-8	Hydroquinidine
1473-73-0	Morinamide Hydrochloride
1476-98-8	Hydroquinidine Hydrochloride
1477-39-0	Noracymethadol
1477-40-3	Levomethadyl Acetate
1480-19-9	Fluanisone
1490-04-6	Menthol
1491-59-4	Oxymetazoline
1501-84-4	Rimantadine Hydrochloride
1502-95-0	Diamorphine Hydrochloride (anhydrous)
1508-65-2	Oxybutynin Hydrochloride
1508-75-4	Tropicamide
1508-76-5	Procyclidine Hydrochloride
1518-86-1	Hydroxyamfetamine (±)
1524-88-5	Fludroxycortide
1531-12-0	Norlevorphanol
1538-09-6	Anhydrous Benzathine Benzylpenicillin
1553-34-0	Metixene Hydrochloride (anhydrous)
1597-82-6	Paramethasone Acetate
1600-27-7	Mercuric Acetate
1620-21-9	Chlorcyclizine Hydrochloride
1622-61-3	Clonazepam
1622-62-4	Flunitrazepam
1639-60-7	Dextropropoxyphene Hydrochloride
1641-17-4	Mexenone
1641-74-3	Nicametate Citrate
1642-54-2	Diethylcarbamazine Citrate
1649-18-9	Azaperone
1665-48-1	Metaxalone
1668-19-5	Doxepin
1674-96-0	Phenglutarimide Hydrochloride
1684-40-8	Tacrine Hydrochloride
1689-89-0	Nitroxinil
1707-14-8	Phenmetrazine Hydrochloride
1715-33-9	Prednisolone Succinate (sodium salt)
1722-62-9	Mepivacaine Hydrochloride
1744-22-5	Riluzole
1746-81-2	Monolinuron
1764-85-8	Epithiazide
1786-81-8	Prilocaine Hydrochloride
1798-50-1	Azacyclonol Hydrochloride
1808-12-4	Bromazine Hydrochloride
1812-30-2	Bromazepam
1821-47-2	5-Methyltryptamine
1824-58-4	Ethiazide
1837-57-6	Ethacridine Lactate
1841-19-6	Fluspirilene
1847-24-1	Flucloxacillin Sodium (anhydrous)
1861-21-8	Enallylpropymal
1867-58-9	Clomethiazole Edisilate
1867-66-9	Ketamine Hydrochloride
1892-80-4	Fenetylline Hydrochloride
1893-33-0	Pipamperone
1910-42-5	Paraquat Dichloride
1910-68-5	Metisazone
1912-24-9	Atrazine
1918-00-9	Dicamba
1944-12-3	Fenoterol Hydrobromide
1951-25-3	Amiodarone
1953-04-4	Galantamine Hydrobromide
1961-77-9	Chlormadinone
1972-08-3	Δ^9-Tetrahydrocannabinol
1972-08-3	Δ^9-Tetrahydrocannabinol
1977-10-2	Loxapine
1981-58-4	Sulfadimidine Sodium
1982-37-2	Methdilazine
2002-29-1	Flumetasone Pivalate
2016-36-6	Choline Salicylate
2016-63-9	Bamifylline
2016-88-8	Amiloride Hydrochloride (anhydrous)
2022-85-7	Flucytosine
2030-63-9	Clofazimine
2058-46-0	Oxytetracycline Hydrochloride
2058-52-8	Clotiapine
2061-86-1	Methandriol Diacetate
2062-77-3	Trifluperidol Hydrochloride
2062-78-4	Pimozide
2062-84-2	Benperidol
2066-89-9	Isoniazid Aminosalicylate
2068-78-2	Vincristine Sulfate
2078-54-8	Propofol
2081-65-4	Butanilicaine Phosphate
2086-83-1	Berberine
2090-89-3	Butethamine
2127-01-7	Clorexolone
2135-17-3	Flumetasone
2139-47-1	Nifenazone
2152-34-3	Pemoline
2152-44-5	Betamethasone Valerate
2153-98-2	Cathine Hydrochloride
2163-80-6	Sodium Methanearsonate
2165-19-7	Guanoxan
2167-85-3	Pipazetate
2169-44-0	Lauroscholtzine
2169-75-7	Deptropine Citrate
2180-92-9	Bupivacaine
2188-67-2	Naepaine
2192-20-3	Hydroxyzine Hydrochloride
2203-97-6	Hydrocortisone Hydrogen Succinate
2216-51-5	Menthol (−)
2218-68-0	Chloral Betaine
2219-30-9	Penicillamine Hydrochloride
2228-39-9	Dehydroemetine Hydrochloride
2240-14-4	Fencamfamin Hydrochloride
2259-96-3	Cyclothiazide
2265-64-7	Dexamethasone Isonicotinate
2272-11-9	Monoethanolamine Oleate
2276-52-0	Pentazocine Hydrochloride
2277-92-1	Oxyclozanide
2298-55-7	Dibenzylpiperazine
2313-87-3	Etoxazene Hydrochloride
2315-02-8	Oxymetazoline Hydrochloride
2324-94-9	Profadol Hydrochloride
2338-21-8	Thiazinamium
2338-37-6	Levopropoxyphene
2347-80-0	Thioproperazine Mesilate
2375-03-3	Methylprednisolone Sodium Succinate
2385-81-1	Furethidine
2391-03-9	Dexbrompheniramine Maleate
2392-39-4	Dexamethasone Sodium Phosphate
2398-96-1	Tolnaftate
2413-38-9	Flupentixol Hydrochloride
2426-63-3	5-Methoxy-*N*,*N*-diisopropyltryptamine Hydrochloride
2430-49-1	Vinylbital
2438-32-6	Dexchlorpheniramine Maleate
2438-32-6	Dexchlorphenamine Maleate
2438-72-4	Bufexamac
2446-23-3	Dehydrochloromethyltestosterone
2447-57-6	Sulfadoxine
2448-68-2	Pipamperone Hydrochloride
2454-11-7	Formebolone
2470-73-7	Dixyrazine
2507-91-7	Gloxazone
2508-72-7	Antazoline Hydrochloride
2508-79-4	Methyldopate Hydrochloride
2529-45-5	Flugestone Acetate
2530-97-4	Xantinol
2544-09-4	Methyldopate
2545-39-3	Clamoxyquin
2552-55-8	Ibotenic Acid
2576-92-3	Isoetarine Hydrochloride
2577-32-4	Sulfamethoxypyridazine Sodium
2589-47-1	Prajmalium Bitartrate
2609-46-3	Amiloride
2610-86-8	Warfarin Potassium
2622-26-6	Pericyazine

CAS no.	*Compound*
2622-30-2	Carfenazine
2622-37-9	Trifluomeprazine
2624-43-3	Cyclofenil
2642-71-9	Azinphos-(Et)
2698-41-1	CS Gas
2709-56-0	Flupentixol
2740-04-7	Dimefline Hydrochloride
2746-81-8	Fluphenazine Enantate
2751-09-9	Troleandomycin
2751-68-0	Acetophenazine
2753-45-9	Mebeverine Hydrochloride
2759-28-6	*N*-Benzylpiperazine
2763-96-4	Muscimol
2764-72-9	Diquat
2773-92-4	Quinisocaine Hydrochloride
2784-73-8	6-Monoacetylmorphine
2870-71-5	Atropine Methobromide
2898-12-6	Medazepam
2920-86-7	Prednisolone Succinate
2921-57-5	Methylprednisolone Hemisuccinate
2921-88-2	Chlorpyrifos
2922-20-5	Butoxamine
2922-44-3	Dextromoramide Tartrate
2955-38-6	Prazepam
2971-90-6	Clopidol
2975-34-0	Carfenazine Maleate
2975-36-2	Pecazine Hydrochloride
3056-17-5	Stavudine
3088-37-7	Lewisite
3093-35-4	Halcinonide
3099-52-3	Nicametate
3131-32-6	Antazoline Mesilate
3146-66-5	Idobutal
3198-07-0	Ethylnoradrenaline Hydrochloride
3200-06-4	Naftidrofuryl Oxalate
3215-70-1	Hexoprenaline
3254-89-5	Difenidol Hydrochloride
3269-83-8	Pheniramine Aminosalicylate
3313-26-6	Tiotixene (Z-)
3321-80-0	Methylpiperidyl Benzilate
3329-14-4	Fenpiprane Hydrochloride
3339-11-5	Tolpropamine Hydrochloride
3354-67-4	Amidefrine
3362-45-6	Noxiptiline
3380-34-5	Triclosan
3416-26-0	Lidoflazine
3459-06-1	Cyclopentamine Hydrochloride
3459-20-9	Glymidine Sodium
3459-96-9	Amicarbalide
3485-62-9	Clidinium Bromide
3505-38-2	Carbinoxamine Maleate
3521-62-8	Erythromycin Estolate
3540-95-2	Fenpiprane
3545-67-3	Chloroquine Hydrochloride
3546-29-0	Thialbarbital Sodium
3546-41-6	Pyrvinium Embonate
3562-63-8	Megestrol
3562-84-3	Benzbromarone
3565-72-8	Embramine
3568-23-8	Propiomazine Maleate
3568-43-2	Acetyl Sulfamethoxypyridazine
3570-46-5	Ethomoxane
3571-53-7	Estradiol Undecylate
3572-43-8	Bromhexine
3572-52-9	Xenysalate
3572-80-3	Cyclazocine
3576-64-5	Clefamide
3593-85-9	Methandriol Dipropionate
3595-11-7	Propylhexedrine (±)
3598-37-6	Acepromazine Maleate
3614-30-0	Emepronium Bromide
3614-69-5	Dimetindene Maleate
3615-24-5	Isopropylaminophenazone
3616-05-5	Pyritidium
3624-87-1	Metabutoxycaine
3624-96-2	Bialamicol Hydrochloride
3625-06-7	Mebeverine
3627-49-4	Phenoperidine Hydrochloride
3639-12-1	Butetamate Citrate
3671-72-5	Amicarbalide Isetionate
3684-46-6	Broxaldine
3685-84-5	Meclofenoxate Hydrochloride
3686-58-6	Tolycaine
3688-62-8	Aminopromazine Fumarate
3688-66-2	Nicocodine
3691-78-9	Benzethidine
3693-39-8	Fluclorolone Acetonide
3697-42-5	Chlorhexidine Hydrochloride
3703-79-5	Bamethan
3704-09-4	Mibolerone
3706-26-1	Methoxyamfetamine Hydrochloride
3715-90-0	Tramazoline Hydrochloride
3717-88-2	Flavoxate Hydrochloride
3734-33-6	Denatonium Benzoate
3735-45-3	Vetrabutine
3736-08-1	Fenetylline
3736-81-0	Diloxanide Furoate
3737-09-5	Disopyramide
3748-77-4	Bunamidine
3751-730-9	Acebutolol
3784-99-4	Stilbazium Iodide
3785-21-5	Butanilicaine
3810-74-0	Streptomycin Sulfate
3810-80-8	Diphenoxylate Hydrochloride
3811-25-4	Clorprenaline
3818-37-9	Phenoxypropazine
3818-37-9	Phenoxypropazine Maleate
3818-50-6	Hydroxynaphthoate
3818-88-0	Tricyclamol Chloride
3819-00-9	Piperacetazine
3820-67-5	Glafenine
3847-29-8	Erythromycin Lactobionate
3858-89-7	Chloroprocaine Hydrochloride
3861-72-1	Acetyldihydrocodeine
3861-76-5	Clonitazene
3902-71-4	Trioxysalen
3922-90-5	Oleandomycin
3930-20-9	Sotalol
3947-65-7	Neomycin A
3963-95-9	Methacycline Hydrochloride
3964-81-6	Azatadine
3978-86-7	Azatadine Maleate
4008-48-4	Nitroxoline
4021-34-5	5-Methoxy-*N*,*N*-diisopropyltryptamine
4028-98-2	Dequalinium Acetate
4044-65-9	Bitoscanate
4075-96-1	Dimethylamfetamine
4185-80-2	Levomepromazine Hydrochloride
4205-90-7	Clonidine
4205-91-8	Clonidine Hydrochloride
4255-23-6	Aletamine
4255-24-7	Aletamine Hydrochloride
4267-05-4	Teclothiazide
4268-36-4	Tybamate
4268-37-5	Anileridine Phosphate
4291-63-8	Cladribine
4299-60-9	Sulfafurazole Diethanolamine
4304-40-9	Closilate
4310-35-4	Tridihexethyl Chloride
4323-43-7	Hexoprenaline Hydrochloride
4330-99-8	Alimemazine Tartrate
4360-12-7	Ajmaline
4368-28-9	Tetrodotoxin
4378-36-3	Fenbutrazate
4394-00-7	Niflumic Acid
4406-22-8	Cyprenorphine
4419-39-0	Beclometasone
4438-22-6	Atropine Oxide
4439-25-2	Metabutethamine
4498-32-2	Dibenzepin
4499-40-5	Choline Theophyllinate
4544-15-4	Pipethanate Hydrochloride
4546-39-8	Pipethanate
4546-39-8	Piperoxan Hydrochloride (*dl*-Form)
4574-60-1	Atropine Oxide Hydrochloride
4682-36-4	Orphenadrine Citrate
4685-14-7	Paraquat
4697-36-3	Carbenicillin
4721-69-1	Oxabolone
4724-59-8	Clamoxyquin Hydrochloride
4759-48-2	Isotretinoin
4764-17-4	Methylenedioxyamfetamine
4800-94-6	Carbenicillin Sodium
4825-53-0	Hexestrol Dipropionate
4825-86-9	Ochratoxin B
4845-99-2	Brucine Sulfate (anhydrous)
4865-85-4	Ochratoxin C
4884-68-8	Hydrastinine Hydrochloride
4901-03-5	Narceine Hydrochloride
4914-30-1	Dehydroemetine
4936-47-4	Nifuratel
4945-47-5	Bamipine
4969-02-2	Metixene
4985-15-3	Noxiptiline Hydrochloride
5001-32-1	Guanoclor
5001-33-2	Metadrenaline
5002-47-1	Fluphenazine Decanoate
5003-48-5	Benorilate
5011-34-7	Trimetazidine
5026-62-0	Sodium Methyl Hydroxybenzoate
5036-02-2	Tetramisole
5060-55-9	Prednisolone Steaglate
5086-74-8	Tetramisole Hydrochloride
5103-74-2	Chlordane (*trans*-)
5104-49-4	Flurbiprofen
5111-12-6	Dihydroergotamine
5144-52-5	Naphazoline Nitrate
5152-30-7	Metocurine Iodide (metocurine)
5189-11-7	Pizotifen Malate
5197-58-0	Stenbolone
5250-39-5	Flucloxacillin
5321-63-1	*N*-Benzylpiperazine Dihydrochloride
5355-16-8	Diaveridine
5355-48-6	β-Acetyldigoxin
5370-01-4	Mexiletine Hydrochloride
5377-20-8	Metomidate
5411-22-3	Benzfetamine Hydrochloride
5428-64-8	Pentaquin Phosphate
5490-27-7	Dihydrostreptomycin Sulfate
5511-98-8	α-Acetyldigoxin
5534-09-8	Beclometasone Dipropionate
5560-59-8	Alverine Citrate
5560-62-3	Xenysalate Hydrochloride
5560-72-5	Iprindole
5579-84-0	Betahistine Hydrochloride
5579-93-1	Iopydone
5580-03-0	Tioguanine (hemihydrate)
5585-64-8	Amotriphene
5585-73-9	Butriptyline Hydrochloride
5588-10-3	Methoxyphenamine Hydrochloride
5588-20-5	Clodantoin
5588-22-7	Clorprenaline Hydrochloride (monohydrate)
5588-33-0	Mesoridazine
5591-29-7	Etafedrine Hydrochloride
5591-45-7	Tiotixene
5593-20-4	Betamethasone Dipropionate
5598-13-0	Chlorpyrifos-(Me)
5611-51-8	Triamcinolone Hexacetonide
5630-11-5	*dl*-Glaucine
5632-44-0	Tolpropamine
5633-20-5	Oxybutynin
5633-25-0	Noracymethadol Hydrochloride
5634-34-4	Bufylline
5635-50-7	Hexestrol
5636-83-9	Dimetindene
5638-76-6	Betahistine
5650-44-2	Methcathinone
5667-46-9	Dimoxyline Phosphate
5697-56-3	Carbenoxolone
5714-00-1	Acetophenazine Dimaleate
5714-04-5	Guanoxan Sulfate
5714-73-8	Methenamine Hippurate
5714-90-9	Levopropoxyphene Napsilate (anhydrous)
5716-20-1	Bamethan Sulfate
5743-12-4	Caffeine Hydrate
5786-21-0	Clozapine
5796-31-6	Ecgonine Hydrochloride
5818-17-7	Methanthelinium
5836-29-3	Coumatetralyl
5843-53-8	Clobenzorex Hydrochloride
5853-29-2	Cephaëline Hydrochloride
5870-29-1	Cyclopentolate Hydrochloride
5874-97-5	Orciprenaline Sulfate
5875-06-9	Proxymetacaine Hydrochloride
5892-31-9	Chlortetracycline Calcium
5892-48-8	Acetarsone Sodium (anhydrous)
5897-16-5	Calcium Cyclamate Dihydrate
5897-19-8	Cyclizine Lactate
5907-38-0	Dipyrone (monohydrate)
5908-99-6	Atropine Sulfate (monohydrate)

CAS no.	*Compound*
5913-76-8	Codeine Phosphate (sesquihydrate)
5913-82-6	Conessine Hydrobromide
5915-41-3	Terbutylazine
5928-83-6	Benzathine Benzylpenicillin Monohydrate
5934-55-4	Ibogaine Hydrochloride
5936-28-7	Hydrastine Hydrochloride
5949-11-1	Cinchonine Hydrochloride (anhydrous)
5949-44-0	Testosterone Undecylate
5956-60-5	Berberine Hydrochloride (dihydrate)
5964-56-7	Octamylamine Hydrochloride
5965-13-9	Dihydrocodeine Tartrate
5965-49-1	Ketobemidone Hydrochloride
5967-84-0	Theophylline Hydrate
5974-09-4	Vetrabutine Hydrochloride
5974-11-8	Morphine Acetate (trihydrate)
5985-38-6	Levorphanol Tartrate (dihydrate)
5987-82-6	Oxybuprocaine Hydrochloride
5989-77-5	Dihydroergotamine Tartrate
5991-71-9	Clorazepate Monopotassium
5995-96-0	Physostigmine Aminoxide Salicylate
5996-06-5	Glaucine Hydrobromide (*d*-form)
6000-74-4	Hydrocortisone Sodium Phosphate
6001-64-5	Chlorobutanol (hemihydrate)
6004-24-6	Cetylpyridinium Chloride (monohydrate)
6004-98-4	Hexocyclium
6009-81-0	Morphine (monohydrate)
6011-12-7	Ambazone (monohydrate)
6018-19-5	Sodium Aminosalicylate (dihydrate)
6031-86-3	Racemethorphan Hydrobromide
6032-59-3	Morphine Tartrate (trihydrate)
6033-41-6	Norpipanone Hydrochloride
6033-42-7	Norpipanone Hydrobromide
6038-78-4	Ethomoxane Hydrochloride
6054-98-4	Olsalazine Sodium
6055-06-7	Morphine Hydrochloride (trihydrate)
6055-19-2	Cyclophosphamide (monohydrate)
6056-11-7	Pipazetate Hydrochloride
6059-47-8	Codeine (monohydrate)
6080-58-6	Lithium Citrate (tetrahydrate)
6101-04-8	Strychnine Hydrochloride (dihydrate)
6101-15-1	Suxamethonium Chloride (dihydrate)
6101-35-5	Sulfacarbamide (monohydrate)
6101-41-3	Sulfapyridine Sodium Monohydrate (monohydrate)
6106-46-3	Hyoscine Methonitrate
6108-05-0	Lidocaine Hydrochloride (monohydrate)
6108-10-7	Benzene Hexachloride (-isomer)
6112-76-1	Mercaptopurine (monohydrate)
6113-17-3	Histapyrrodine Hydrochloride
6114-26-7	Pholedrine Sulfate
6119-47-7	Quinine Hydrochloride (dihydrate)
6119-70-6	Quinine Sulfate (dihydrate)
6130-64-9	Procaine Benzylpenicillin (monohydrate)
6138-47-2	Liothyronine Hydrochloride
6138-56-3	Tripelennamine Citrate
6138-79-0	Triprolidine Hydrochloride (monohydrate)
6147-37-1	Menadione Sodium Bisulfite (trihydrate)
6151-30-0	Mepacrine Hydrochloride (dihydrate)
6151-39-9	Quinidine Bisulfate (tetrahydrate)
6153-19-1	Phenacaine Hydrochloride Monohydrate
6153-33-9	Mebhydrolin Napadisilate
6153-64-6	Oxytetracycline Dihydrate
6155-57-3	Saccharin Sodium (dihydrate)
6160-12-9	Sparteine Sulfate (pentahydrate)
6164-87-0	Nicotinyl Tartrate
6168-76-9	Crotetamide
6168-86-1	Isometheptene Hydrochloride
6170-42-9	Chloropyramine Hydrochloride
6183-68-2	Quinine Bisulfate (heptahydrate)
6190-39-2	Dihydroergotamine Mesilate
6190-55-2	Sulfaguanidine Monohydrate
6190-60-9	Mephentermine Sulfate (dihydrate)
6192-95-6	Propylhexedrine Hydrochloride (±)
6202-17-1	Hordenine Sulfate (dihydrate)
6202-23-9	Cyclobenzaprine Hydrochloride
6209-17-2	Sulfacetamide Sodium (monohydrate)
6211-15-0	Morphine Sulfate (pentahydrate)
6236-05-1	Nifuroxime
6292-91-7	Methylenedioxyamfetamine Hydrochloride
6381-91-5	Saccharin Calcium (hydrate)
6385-02-0	Meclofenamate Sodium
6398-98-7	Amodiaquine Hydrochloride (dihydrate)
6411-75-2	Tuaminoheptane Sulfate
6416-04-2	Tetracycline (trihydrate)
6452-71-7	Oxprenolol
6452-73-9	Oxprenolol Hydrochloride
6469-93-8	Chlorprothixene Hydrochloride
6472-73-7	Pseudomorphine (trihydrate)
6474-85-7	Fenbutrazate Hydrochloride
6485-34-3	Saccharin Calcium (anhydrous)
6493-05-6	Pentoxifylline
6500-81-8	Etacrynate Sodium
6506-37-2	Nimorazole
6509-18-8	Aconitine Nitrate
6536-18-1	Morazone
6556-11-2	Inositol Nicotinate
6591-63-5	Quinidine Sulfate (dihydrate)
6592-85-4	Hydrastinine
6621-47-2	Perhexiline
6640-24-0	m-Chlorophenylpiperazine
6673-35-4	Practolol
6700-34-1	Dextromethorphan Hydrobromide (monohydrate)
6700-39-6	Isoprenaline Sulfate (dihydrate)
6700-56-7	Ethoheptazine Citrate
6703-27-1	Acetylcodeine
6707-58-0	Dequalinium
6724-53-4	Perhexiline Maleate
6735-59-7	Pralidoxime
6736-03-4	Ecothiopate
6740-88-1	Ketamine
6746-59-4	Ethylmorphine Hydrochloride (dihydrate)
6775-26-4	Octaverine Hydrochloride
6780-13-8	Ethylenediamine Hydrate (monohydrate)
6791-71-5	Sulfathiazole Sodium (pentahydrate)
6795-23-9	Aflatoxin M_1
6823-79-6	Pentamidine Mesilate
6835-16-1	Hyoscyamine Sulfate (dihydrate)
6854-40-6	Codeine Sulfate (trihydrate)
6885-57-0	Aflatoxin M_2
6888-11-5	Diphenhydramine Acefyllinate
6893-02-3	Liothyronine
6923-22-4	Monocrotophos
6933-90-0	Clorprenaline Hydrochloride (anhydrous)
6989-98-6	Tubocurarine Chloride (pentahydrate)
6990-06-3	Fusidic Acid (anhydrous)
6998-60-3	Rifamycin SV
7009-54-3	Pentapiperide
7020-55-5	Clidinium
7054-25-3	Quinidine Gluconate
7060-74-4	Oleandomycin Phosphate
7081-38-1	Oxyphenbutazone (monohydrate)
7081-40-5	Metixene Hydrochloride (monohydrate)
7081-44-9	Cloxacillin Sodium (monohydrate)
7081-52-9	Piminodine Esilate
7081-53-0	Doxapram Hydrochloride (monohydrate)
7082-21-5	Terodiline Hydrochloride
7083-71-8	Emetine Hydrochloride (hydrate)
7084-07-3	Methaphenilene Hydrochloride
7085-44-1	Chlorothiazide Sodium
7085-45-2	Biperiden Lactate
7104-38-3	Levomepromazine Maleate
7177-48-2	Ampicillin Trihydrate
7179-49-9	Lincomycin Hydrochloride (monohydrate)
7181-73-9	Bephenium
7187-66-8	Trimetaphan
7195-27-9	Mefruside
7210-92-6	Tolycaine Hydrochloride
7220-81-7	Aflatoxin B2
7232-21-5	Metoclopramide Hydrochloride (monohydrate)
7241-98-7	Aflatoxin G2
7246-14-2	Meticillin Sodium (monohydrate)
7246-20-0	Triclofos Sodium
7247-57-6	Heteronium Bromide
7261-97-4	Dantrolene
7279-75-6	Isoetarine Mesilate
7287-19-6	Prometryne
7327-87-9	Dihydralazine Sulfate
7331-52-4	(S)-3-Hydroxy-γ Butyrolactone
7361-61-7	Xylazine
7389-45-9	Butaperazine Phosphate
7416-34-4	Molindone
7421-40-1	Carbenoxolone Sodium
7429-90-5	Aluminium
7439-92-1	Lead
7439-97-6	Mercury
7440-36-0	Antimony
7440-38-2	Arsenic
7440-39-3	Barium
7440-41-7	Beryllium
7440-43-9	Cadmium
7446-14-2	Lead Sulfate
7446-70-0	Aluminium Chloride
7455-39-2	Dimetotiazine Mesilate
7456-24-8	Dimetotiazine
7460-12-0	Pseudoephedrine Sulfate
7481-89-2	Zalcitabine
7487-81-2	Isomethadone Hydrochloride (*l*-Form)
7487-94-7	Mercuric (II) Chloride
7491-09-0	Docusate Potassium
7492-31-1	Isometheptene Mucate
7492-32-2	Isopropamide
7527-91-5	Aminoacridine Hexylresorcinate
7528-00-9	Dexamfetamine Phosphate
7542-37-2	Paromomycin
7554-65-6	Fomepizole
7563-42-0	Pentobarbital Calcium
7599-79-3	Clomifene Citrate (*E*-)
7601-55-0	Metocurine Iodide
7619-53-6	Clomifene Citrate (*Z*-)
7647-15-6	Sodium Bromide
7648-98-8	Ambenonium
7664-39-3	Hydrofluoric Acid
7681-14-3	Prednisolone Tebutate (anhydrous)
7681-49-4	Sodium Fluoride
7681-78-9	Mebezonium Iodide
7681-79-0	Etafedrine
7681-80-3	Pentapiperide Metilsulfate
7681-93-8	Natamycin
7683-36-5	Obidoxime
7683-59-2	Isoprenaline
7716-59-8	Etisazole Hydrochloride
7716-60-1	Etisazole
7758-02-3	Potassium Bromide
7758-04-5	Potassium Cyclamate
7758-95-4	Lead Chloride
7758-97-6	Lead Chromate
7758-98-7	Copper
7759-35-5	Elcometrine
7773-52-6	Cetylpyridinium
7778-39-4	Arsenic Acid
7778-43-0	Sodium Arsenate
7778-44-1	Calcium Arsenate
7783-47-3	Stannous Fluoride
7784-30-7	Aluminium Phosphate
7784-46-5	Sodium Arsenite
7786-34-7	Mevinphos
7787-47-5	Beryllium Chloride
7787-49-7	Beryllium Fluoride
7789-41-5	Calcium Bromide
7803-52-3	Stibine
8000-10-0	Theophylline Sodium Glycinate
8000-33-47	Pyrethrum
8001-54-5	Benzalkonium Bromide
8001-54-5	Benzalkonium Chloride
8006-25-5	Ergotoxine
8008-45-5	Nutmeg Oil
8015-51-8	Prethcamide
8015-51-8	Prethcamide
8015-61-0	Aloin
8017-89-8	Amyl Nitrite
8025-81-8	Spiramycin
8044-71-1	Cetrimide
8047-28-7	Ergotoxine Esilate
8051-02-3	Veratrine (mixture)
8053-18-7	Protoveratrines A and B
8063-14-7	Cannabis
8063-24-9	Acriflavinium Chloride
8065-48-3	Demeton
8067-24-1	Codergocrine Mesilate
8067-69-4	Halquinol
9002-61-3	Chorionic Gonadotropin
9002-91-9	Metaldehyde
9004-10-8	Insulin
9009-86-3	Ricin
9014-67-9	Aloxiprin

CAS no.	*Compound*
9015-68-3	Asparaginase
9041-08-1	Bemiparin
9041-08-1	Enoxaparin Sodium
9041-93-4	Bleomycin Sulfate
10018-19-6	Cotarnine Chloride (anhydrous)
10025-91-9	Antimony Trichloride
10041-19-7	[1,4-bis(2-Ethylhexyl) Sulfosuccinate]
10043-01-3	Aluminium Sulfate
10043-35-3	Boric Acid
10048-13-2	Sterigmatocystin
10061-32-2	Levophenacylmorphan
10075-24-8	Imipramine Embonate
10085-81-1	Benzoctamine Hydrochloride
10099-74-8	Lead Nitrate
10101-63-0	Lead Iodide
10108-64-2	Cadmium Chloride
10112-91-1	Mercurous (I) Chloride
10118-90-8	Minocycline
10124-36-4	Cadmium Sulfate
10161-33-8	Trenbolone
10161-34-9	Trenbolone Acetate
10238-21-8	Glibenclamide
10246-75-0	Hydroxyzine Embonate
10262-69-8	Maprotiline
10265-92-6	Methamidophos
10347-81-6	Maprotiline Hydrochloride
10350-81-9	Amopyroquine Hydrochloride
10361-37-2	Barium Chloride
10379-14-3	Tetrazepam
10418-03-8	Stanozolol
10488-36-5	Tofenacin Hydrochloride
10592-13-9	Doxycycline Hydrochloride
10596-23-3	Clodronic Acid
11003-38-6	Capreomycin
11005-63-3	Strophanthin-K
11018-89-6	Ouabain (octahydrate)
11030-71-0	Amatoxins
11032-41-0	Codergocrine
11050-21-8	Ciguatoxin-1
11056-06-7	Bleomycin
11061-68-0	Insulin (human)
11097-68-0	Aluminium Chlorhydrate
12001-72-8	Mafenide Propionate
12002-30-1	Piperazine Calcium Edetate
12124-97-9	Ammonium Bromide
12629-01-5	Somatropin
12650-69-0	Mupirocin
12789-03-6	Chlordane (technical grade)
13009-99-9	Mafenide Acetate
13013-17-7	Propranolol (±)
13029-44-2	Dienestrol (*E,E*-)
13051-01-9	Carbazochrome Salicylate
13055-82-8	Reproterol Hydrochloride
13103-34-9	Boldenone Undecylenate
13115-40-7	Dimetotiazine Mesilate
13171-25-0	Trimetazidine Hydrochloride
13292-46-1	Rifampicin
13327-32-7	Beryllium Hydroxide
13364-32-4	Clobenzorex
13392-18-2	Fenoterol
13392-28-4	Rimantadine
13422-51-0	Hydroxocobalamin
13424-46-9	Lead Azide
13457-18-6	Pyrazophos
13473-38-6	Pipenzolate
13473-90-0	Aluminium Nitrate
13492-01-8	Tranylcypromine Sulfate
13494-90-1	Gallium Nitrate (anhydrous)
13495-09-5	Piminodine
13501-04-7	Bunamidine Hydroxynaphthoate
13510-48-0	Beryllium Nitrate
13523-86-9	Pindolol
13539-59-8	Azapropazone (anhydrous)
13597-99-4	Beryllium Nitrate
13609-67-1	Hydrocortisone Butyrate
13614-98-7	Minocycline Hydrochloride
13647-35-3	Trilostane
13655-52-2	Alprenolol
13669-70-0	Nefopam
13678-97-2	Alprenolol Hydrochloride (±)
13707-88-5	Alprenolol Hydrochloride
13710-19-5	Tolfenamic Acid
13739-02-1	Diacerein
13764-49-3	Etorphine Hydrochloride
13838-16-9	Enflurane
13900-14-6	Domiphen
13912-80-6	Butoxyethyl Nicotinate
13930-31-9	Oxetacaine Hydrochloride
13931-75-4	Phenmetrazine Teoclate
13956-29-1	Cannabidiol
13977-28-1	Embramine Hydrochloride
13997-19-8	Methyl Benzoquate
14007-49-9	Ambutonium
14007-53-5	Fenpipramide Hydrochloride (anhydrous)
14007-64-8	Butetamate
14008-44-7	Metopimazine
14028-44-5	Amoxapine
14046-64-1	Benserazide Hydrochloride
14107-37-0	Alfadolone
14116-06-4	Methylthioamfetamine
14214-84-7	Oxyphenonium
14222-46-9	Pyritidium Bromide
14222-60-7	Protionamide
14252-80-3	Bupivacaine Hydrochloride (monohydrate)
14255-87-9	Parbendazole
14277-97-5	Domoic Acid
14293-44-8	Xipamide
14297-87-1	Benzylmorphine
14357-78-9	Diprenorphine
14362-31-3	Chlorcyclizine Hydrochloride
14399-14-5	Chloramphenicol Cinnamate
14437-41-3	Clioxanide
14484-47-0	Deflazacort
14521-96-1	Etorphine
14538-56-8	Piperazine Phosphate (anhydrous)
14611-51-9	Selegiline
14611-52-0	Selegiline Hydrochloride
14648-14-7	Norcodeine Hydrochloride (anhydrous)
14663-23-1	Dantrolene Sodium (anhydrous)
14759-06-9	Sulforidazine
14769-73-4	Levamisole
14777-25-4	Perazine Dimalonate
14779-78-3	Padimate
14838-15-4	Phenylpropanolamine
14897-39-3	Rifamycin Sodium
14919-77-8	Benserazide Hydrochloride
14976-57-9	Clemastine Fumarate
15096-52-3	Cryolite
15105-92-7	Rifamycin Sodium
15180-03-7	Alcuronium Chloride
15251-48-6	Oxytetracycline Calcium
15262-86-9	Testosterone Isocaproate
15301-48-1	Bezitramide
15301-50-5	Cloponone
15301-69-6	Flavoxate
15301-93-6	Tofenacin
15307-79-6	Diclofenac Sodium
15307-86-5	Diclofenac
15351-13-0	Nicofuranose
15356-70-4	Menthol (±)
15500-66-0	Pancuronium Bromide
15532-75-9	Trifluoromethylphenylpiperazine
15537-76-5	Chlorproguanil Hydrochloride
15574-96-6	Pizotifen
15585-70-3	Bibenzonium Bromide
15588-95-1	DOM
15589-00-1	DOM Hydrochloride
15599-39-0	Noxytiolin
15622-65-8	Molindone Hydrochloride
15662-33-6	Ryanodine
15663-27-1	Cisplatin
15676-16-1	Sulpiride
15676-23-0	(+)-Norlevorphanol
15686-51-8	Clemastine
15686-61-0	Fenproporex
15686-71-2	Cefalexin (anhydrous)
15686-83-6	Pyrantel
15687-14-6	Embutramide
15687-27-1	Ibuprofen
15687-33-9	Metindizate
15687-40-8	Octafonium Chloride (anhydrous)
15690-55-8	Clomifene (*Z*-)
15690-57-0	Clomifene (*E*-)
15722-48-2	Olsalazine
15793-40-5	Terodiline
15823-89-9	Imolamine Hydrochloride
15826-37-6	Sodium Cromoglicate (disodium salt)
15867-21-7	Alphaprodine (±)
15876-67-2	Distigmine Bromide
15879-93-3	Alphachloralose
15972-60-8	Alachlor
16008-36-9	Methyldesorphine
16051-77-7	Isosorbide Mononitrate
16110-51-3	Cromoglycic Acid
16203-97-7	Dithranol Triacetate
16210-52-9	Cotarnine Chloride (dihydrate)
16320-04-0	Gestrinone
16376-74-2	Valethamate
16509-35-6	Thenalidine Tartrate
16589-24-5	Oxedrine Tartrate
16590-41-3	Naltrexone
16595-80-5	Levamisole Hydrochloride
16676-29-2	Naltrexone Hydrochloride
16776-64-0	Thenium
16808-86-9	Diprenorphine Hydrochloride
16893-85-9	Sodium Fluorosilicate
16915-70-1	Nifursol
17021-26-0	Calusterone
17086-03-2	Amitriptyline Embonate
17086-28-1	Doxycycline (monohydrate)
17086-29-2	Levomepromazine Maleate
17140-78-2	Dextropropoxyphene Napsilate (anhydrous)
17140-81-7	Nitrofurantoin (monohydrate)
17146-95-1	Pentazocine Lactate
17194-00-2	Barium Hydroxide
17230-88-5	Danazol
17243-39-9	Benzoctamine
17321-77-6	Clomipramine Hydrochloride
17466-45-4	Phallotoxin
17560-51-9	Metolazone
17575-22-3	Lanatoside C
17598-65-1	Deslanoside
17617-23-1	Flurazepam
17692-31-8	Dropropizine
17693-51-5	Promethazine Teoclate
17737-65-4	Clonixin
17762-90-2	Butylone
17780-72-2	Clorgiline
17780-75-5	Clorgiline Hydrochloride
17924-92-4	Zearalenone
18010-40-7	Bupivacaine Hydrochloride (anhydrous)
18174-58-8	Pipoxolan Hydrochloride
18305-29-8	Fenproporex Hydrochloride
18323-44-9	Clindamycin
18472-51-0	Chlorhexidine Gluconate
18507-89-6	Decoquinate
18534-18-4	Piperazine Phosphate (monohydrate)
18559-94-9	Salbutamol
18652-93-2	Methohexital
18833-13-1	Acefylline Piperazine
19216-56-9	Prazosin
19237-84-4	Prazosin Hydrochloride
19326-29-5	Etamiphylline Camsilate
19387-91-8	Tinidazole
19774-82-4	Amiodarone Hydrochloride
19794-93-5	Trazodone
19982-08-2	Memantine
20236-82-2	Thebacon Hydrochloride
20380-58-9	Tilidate
20432-64-8	Iprindole Hydrochloride
20537-88-6	Amifostine
20562-02-1	Solanine
20574-50-9	Morantel
20594-83-6	Nalbuphine
20684-06-4	Bamifylline Hydrochloride
20685-78-3	Rolitetracycline Nitrate (anhydrous)
20788-07-2	Resorantel
20830-75-5	Digoxin
20830-81-3	Daunorubicin
20859-73-8	Aluminium Phosphide
21150-22-1	β-Amanitin
21187-98-4	Gliclazide
21245-01-2	Padimate
21245-02-3	Padimate O
21259-20-1	T-2 Toxin
21462-39-5	Clindamycin Hydrochloride (anhydrous)
21466-07-9	Bromofenofos
21498-08-8	Lofexidine Hydrochloride
21535-47-7	Mianserin Hydrochloride
21645-51-2	Aluminium Hydroxide

CAS no.	*Compound*
21679-14-1	Fludarabine
21829-25-4	Nifedipine
21898-19-1	Clenbuterol Hydrochloride
21962-82-3	Dibutoline
22059-60-5	Disopyramide Phosphate
22064-27-3	Penthienate
22071-15-4	Ketoprofen
22131-35-7	Butalamine
22131-79-9	Alclofenac
22161-81-5	Dexketoprofen
22189-31-7	Tiotixene Hydrochloride (dihydrate)
22204-24-6	Pyrantel Embonate
22204-29-1	Cetoxime Hydrochloride
22204-53-1	Naproxen
22204-88-2	Tramadol Hydrochloride
22212-52-8	Chlordane (*cis*-)
22224-92-6	Fenamiphos
22232-54-8	Carbimazole
22232-71-9	Mazindol
22254-24-6	Ipratropium Bromide
22260-51-1	Bromocriptine Mesilate
22298-29-9	Betamethasone Benzoate
22316-47-8	Clobazam
22348-32-9	Diphenylprolinol
22487-42-9	Benaprizine
22494-42-4	Diflunisal
22500-92-1	Sorbic Acid
22560-50-5	Disodium Clodronate
22662-39-1	Rafoxanide
22664-55-7	Metipranolol
22832-87-7	Miconazole Nitrate
22846-83-9	Tigloidine Hydrobromide
22881-35-2	Famprofazone
22916-47-8	Miconazole
22972-97-0	Oxprenolol Hydrochloride (±)
22972-98-1	Oxprenolol (±)
23031-25-6	Terbutaline
23031-32-5	Terbutaline Sulfate
23047-25-8	Lofepramine
23067-13-2	Erythromycin Gluceptate
23076-35-9	Xylazine Hydrochloride
23093-74-5	Bunitrolol Hydrochloride
23109-05-9	α-Amanitin
23142-01-0	Carbetapentane Citrate
23182-46-9	Pipethanate Ethobromide
23214-92-8	Doxorubicin
23214-96-2	Alcuronium
23239-32-9	Methoxyamfetamine
23239-51-2	Ritodrine Hydrochloride
23277-43-2	Nalbuphine Hydrochloride
23282-55-5	Sulfachlorpyridazine Sodium
23313-50-0	Practolol (±)
23325-78-2	Cefalexin (monohydrate)
23327-57-3	Nefopam Hydrochloride
23541-50-6	Daunorubicin Hydrochloride
23593-75-1	Clotrimazole
23597-82-2	Hexyl Nicotinate
23651-95-8	Droxidopa
23744-24-3	Pipoxolan
23779-99-9	Floctafenine
23846-70-0	Alprenolol (±)
23887-31-2	Clorazepic Acid
23930-19-0	Alfaxalone
23930-37-2	Alfadolone Acetate
23978-85-0	Δ^9-Tetrahydrocannabinolic Acid
23983-43-9	Dehydroepiandrosterone Enanthate
24017-47-8	Triazophos
24169-02-6	Econazole Nitrate
24204-13-5	Dihydrocodeine Phosphate
24219-97-4	Mianserin
24243-97-8	Tymazoline
24280-93-1	Mycophenolic Acid
24359-81-7	Antazoline Sulfate (anhydrous)
24390-14-5	Doxycycline Hydrochloride
24526-64-5	Nomifensine
24584-09-6	Dexrazoxane
24634-61-5	Potassium Sorbate
24668-75-5	Dexamethasone Tebutate
24729-96-2	Clindamycin Phosphate
24815-24-5	Rescinnamine
24868-20-0	Dantrolene Sodium (hemiheptahydrate)
25013-16-5	Butylated Hydroxyanisole
25122-41-2	Clobetasol
25122-46-7	Clobetasol Propionate
25122-57-0	Clobetasone Butyrate
25126-76-5	Androstanediol
25316-40-9	Doxorubicin Hydrochloride
25332-20-1	Dimetridazole Hydrochloride
25332-39-2	Trazodone Hydrochloride
25333-77-1	Acetorphine
25333-78-2	Acetorphine Hydrochloride
25334-00-3	Bemegride Sodium
25384-17-2	Allylprodine
25389-94-0	Kanamycin Sulfate
25416-65-3	Levothyroxine Sodium (hydrate)
25451-15-4	Felbamate
25507-04-4	Clindamycin Palmitate Hydrochloride (palmitate hydrochloride)
25523-97-1	Dexchlorphenamine
25523-97-1	Dexchlorpheniramine
25567-68-4	Chloronitrotoluene
25573-43-7	Physostigmine Aminoxide
25614-03-3	Bromocriptine
25967-29-7	Flutoprazepam
25968-91-6	Hydrocodone Hydrochloride (anhydrous)
25990-43-6	Mepenzolate
26097-80-3	Cambendazole
26155-31-7	Morantel Tartrate
26159-34-2	Naproxen Sodium
26171-23-3	Tolmetin
26309-95-5	Pivampicillin Hydrochloride
26372-86-1	Pentapiperium
26538-44-3	Zeranol
26570-10-5	Dextropropoxyphene Napsilate (monohydrate)
26594-41-2	Isomethadone (*d*-Form)
26615-21-4	Zotepine
26628-22-8	Sodium Azide
26652-09-5	Ritodrine
26657-13-6	Rolitetracycline Nitrate (sesquihydrate)
26786-32-3	Lofepramine Hydrochloride
26787-78-0	Amoxicillin
26807-65-8	Indapamide
26839-75-8	Timolol
26844-12-2	Indoramin
26864-56-2	Penfluridol
26921-17-5	Timolol Maleate
26934-87-2	HT-2 Toxin
26944-48-9	Glibornuride
27060-91-9	Flutazolam
27107-79-5	Tilidate Hydrochloride (anhydrous)
27134-26-5	Chloroaniline
27203-92-5	Tramadol
27220-47-9	Econazole
27223-35-4	Ketazolam
27262-47-1	Levobupivacaine
27262-48-2	Levobupivacaine Hydrochloride
27555-34-6	Quinidine Polygalacturonate (anhydrous)
27833-64-3	Loxapine Succinate
27848-84-6	Nicergoline
27885-92-3	Imidocarb
27892-33-7	Emepronium
27912-14-7	Levobunolol Hydrochloride
27917-82-4	Nitroxinil Eglumine Salt
28120-03-8	Tymazoline Hydrochloride
28159-98-0	Irgarol
28300-74-5	Antimony Potassium Tartrate
28319-77-9	Choline Alfoscerate
28395-03-1	Bumetanide
28416-66-2	Isoaminile Citrate
28523-86-6	Sevoflurane
28721-07-5	Oxcarbazepine
28797-61-7	Pirenzepine
28860-95-9	Carbidopa (anhydrous)
28911-01-5	Triazolam
28981-97-7	Alprazolam
29094-61-9	Glipizide
29122-68-7	Atenolol
29205-06-9	Fluocortolone Pivalate
29216-28-2	Mequitazine
29218-27-2	Toloxatone
29560-58-5	Moracizine Hydrochloride
29868-97-1	Pirenzepine Hydrochloride
29883-15-6	Amygdalin
30272-08-3	Amineptine Hydrochloride
30516-87-1	Zidovudine
30544-47-9	Etofenamate
30652-11-0	Deferiprone
30685-43-9	Metildigoxin
30685-43-9	Metildigoxin
30748-29-9	Feprazone
30909-51-4	Flupentixol Decanoate
31036-80-3	Lofexidine
31329-57-4	Naftidrofuryl
31377-23-8	Amantadine Sulfate
31431-39-7	Mebendazole
31677-93-7	Bupropion Hydrochloride
31828-50-9	Cefradine (dihydrate)
31828-71-4	Mexiletine
31842-01-0	Indoprofen
31842-61-2	Rimiterol Hydrobromide
31853-38-0	1,2-Naphthoquinone 2-Semicarbazone
31868-18-5	Mexazolam
31879-05-7	Fenoprofen
31883-05-3	Moracizine
31884-77-2	Meclozine Hydrochloride (monohydrate)
32266-10-7	Hexoprenaline Sulfate
32672-69-8	Mesoridazine Benzenesulfonate
32702-55-9	Benaprizine Hydrochloride
32780-64-6	Labetalol Hydrochloride
32795-44-1	Acecainide
32795-47-4	Nomifensine Maleate
32808-09-6	Papaverine Sulfate (anhydrous)
32828-81-2	Picotamide
32865-01-3	Brompheniramine Maleate (±)
32953-89-2	Rimiterol
32986-56-4	Tobramycin
32988-50-4	Viomycin
33005-95-7	Tiaprofenic Acid
33032-12-1	Methapyrilene Fumarate
33069-62-4	Paclitaxel
33124-53-7	Indoramin Hydrochloride
33125-97-2	Etomidate
33159-27-2	Ecabet
33213-65-9	Endosulfan (−)
33237-74-0	Aprindine Hydrochloride
33286-22-5	Diltiazem Hydrochloride
33342-05-1	Gliquidone
33369-31-2	Zomepirac
33386-08-2	Buspirone Hydrochloride
33401-94-4	Pyrantel Tartrate
33402-03-8	Metaraminol Tartrate
33419-42-0	Etoposide
33580-30-2	Tertatolol Hydrochloride
33774-52-6	Detajmium Bitartrate
33817-20-8	Pivampicillin
34118-92-8	Acecainide Hydrochloride
34123-59-6	Isoproturon
34154-59-1	Meptazinol Hydrochloride (±)
34156-56-4	Foscarnet Sodium (hexahydrate)
34195-34-1	Hydrocodone Tartrate (hemipentahydrate)
34214-51-2	Flucloxacillin Sodium (monohydrate)
34262-84-5	Mesocarb
34366-67-1	Hydrocodone Phosphate
34368-04-2	Dobutamine
34381-68-5	Acebutolol Hydrochloride
34552-83-5	Loperamide Hydrochloride
34580-13-7	Ketotifen
34580-14-8	Ketotifen Fumarate
34597-40-5	Fenoprofen Calcium (anhydrous)
34642-77-8	Amoxicillin Sodium
34645-84-6	Fenclofenac
34784-64-0	Tertatolol
34911-55-2	Bupropion
34915-68-9	Bunitrolol
35055-78-8	Nicomorphine Hydrochloride
35080-11-6	Prajmalium
35144-63-9	Dimevamide Sulfate
35285-68-8	Sodium Ethyl Hydroxybenzoate
35285-69-9	Sodium Propyl Hydroxybenzoate
35554-08-6	Saxitoxin
35604-67-2	Viloxazine Hydrochloride
35711-34-3	Tolmetin Sodium (anhydrous)
35941-65-2	Butriptyline
35944-74-2	Metomidate Hydrochloride
36104-80-0	Camazepam
36105-20-1	Flurazepam Monohydrochloride
36141-82-9	Diamfenetide

CAS no.	*Compound*
36167-63-2	Halofantrine Hydrochloride
36322-90-4	Piroxicam
36330-85-5	Fenbufen
36393-56-3	Cathine
36418-22-1	6-Monoacetylmorphine Hydrochloride
36505-84-7	Buspirone
36637-18-0	Etidocaine
36637-19-1	Etidocaine Hydrochloride
36653-54-0	Fazadinium
36688-78-5	Clindamycin Palmitate Hydrochloride (palmitate)
36735-22-5	Quazepam
36894-69-6	Labetalol
37091-65-9	Azlocillin Sodium
37091-66-0	Azlocillin
37148-27-9	Clenbuterol
37350-58-6	Metoprolol
37517-26-3	Pipotiazine Palmitate
37517-28-5	Amikacin
37612-13-8	Encainide
37640-71-4	Aprindine
37661-08-8	Bacampicillin Hydrochloride
37883-00-4	Viomycin Sulfate
38194-50-2	Sulindac
38260-01-4	Trientine Dihydrochloride
38304-91-5	Minoxidil
38455-90-2	Methylephedrine Hydrochloride
38641-94-0	Glyphosate Isopropylammonium
38677-85-9	Flunixin
38821-49-7	Carbidopa (monohydrate)
38821-53-3	Cefradine (anhydrous)
39562-70-4	Nitrendipine
39718-89-3	Alminoprofen
39809-25-1	Penciclovir
39831-55-5	Amikacin Sulfate
39860-99-6	Pipotiazine
40054-69-1	Etizolam
40077-57-4	Aviptadil
40180-04-9	Tienilic Acid
40542-65-2	Gestrinone
40828-46-4	Suprofen
41100-52-1	Memantine Hydrochloride
41294-56-8	Alfacalcidol
41340-25-4	Etodolac
41342-53-4	Erythromycin Ethylsuccinate
41354-29-4	Cyproheptadine Hydrochloride (sesquihydrate)
41354-30-7	Dimethisterone (monohydrate)
41372-02-5	Benzathine Benzylpenicillin Tetrahydrate
41372-08-1	Methyldopa (sesquihydrate)
41372-10-5	Piperazine Citrate (hydrate)
41372-20-7	Apomorphine Hydrochloride (hemihydrate)
41444-62-6	Codeine Phosphate (hemihydrate)
41510-23-0	Biriperone
41587-36-4	Chloronitroaniline
41708-72-9	Tocainide
41859-67-0	Bezafibrate
42200-33-9	Nadolol
42399-41-7	Diltiazem
42408-82-2	Butorphanol
42461-84-7	Flunixin Meglumine
42542-10-9	Methylenedioxymetamfetamine
42864-78-8	Bevantolol Hydrochloride
42882-96-2	Chlorphenamine (±)
42924-53-8	Nabumetone
43033-72-3	Levomethadyl Acetate Hydrochloride
43200-80-2	Zopiclone
43210-67-9	Fenbendazole
43229-80-7	Formoterol Fumarate (dihydrate)
46817-91-8	Viloxazine
47141-42-4	Levobunolol
47719-70-0	Detajmium
48141-64-6	Etafedrine
49562-28-9	Fenofibrate
49564-56-9	Fazadinium Bromide
49656-78-2	Methcathinone Hydrochloride
49745-95-1	Dobutamine Hydrochloride
49746-04-5	Tiotixene Hydrochloride (anhydrous, Z)
49746-09-0	Tiotixene Hydrochloride (dihydrate, Z)
49842-07-1	Tobramycin (sulfate)
50321-35-2	Morazone Hydrochloride
50782-69-9	VX
50906-05-3	Ephedrine
50972-17-3	Bacampicillin
50978-10-4	Cyclomethycaine Sulfate
51022-70-9	Salbutamol Sulfate
51022-71-0	Nabilone
51037-30-0	Acipimox
51218-45-2	Metolachlor
51264-14-3	Amsacrine
51322-75-9	Tizanidine
51460-26-5	Carbazochrome Sodium Sulfonate
51481-10-8	Deoxynivalenol
51481-61-9	Cimetidine
51552-99-9	Diperodon (monohydrate)
51781-06-7	Carteolol
51781-21-6	Carteolol Hydrochloride
51888-09-6	Prochlorperazine Mesilate
52022-31-8	Ambenonium Chloride (tetrahydrate)
52128-35-5	Trimetrexate
52214-84-3	Ciprofibrate
52225-20-4	Alpha Tocoferil Acetate
52239-63-1	Thiethylperazine Malate
52432-72-1	Oxeladin Citrate
52438-85-4	Prednisolone (sesquihydrate)
52479-85-3	Exifone
52485-79-7	Buprenorphine
52712-76-2	Bunazosin Hydrochloride
52918-63-5	Deltamethrin
53016-31-2	Norelgestromin
53043-14-4	Amylmetacresol
53123-88-9	Sirolimus
53152-21-9	Buprenorphine Hydrochloride
53164-05-9	Acemetacin
53179-11-6	Loperamide
53267-01-9	Cibenzoline
53496-15-4	Amyl Acetate (sec)
53597-27-6	Fendosal
53643-48-4	Vindesine
53648-55-8	Dezocine
53714-56-0	Leuprorelin
53746-45-5	Fenoprofen Calcium (dihydrate)
53772-83-1	Zuclopenthixol
53862-81-0	Detajmium Bitartrate
53885-35-1	Ticlopidine Hydrochloride
53910-25-1	Pentostatin
53984-74-0	Tocainide Hydrochloride (R-(−))
53984-76-2	Tocainide Hydrochloride (S-(−))
53994-73-3	Cefaclor (anhydrous)
54063-32-0	Clobetasone
54063-35-3	Dofamium Chloride
54063-54-6	Reproterol
54143-55-4	Flecainide
54143-56-5	Flecainide Acetate
54187-04-1	Rilmenidene
54340-58-8	Meptazinol
54350-48-0	Etretinate
54573-75-0	Doxercalciferol
54739-18-3	Fluvoxamine
54910-89-3	Fluoxetine
54965-21-8	Albendazole
55079-83-9	Acitretin
55096-26-9	Nalmefene
55142-85-3	Ticlopidine
55242-55-2	Propentofylline
55294-15-0	Muzolimine
55557-30-7	Levopropoxyphene Napsilate (monohydrate)
55812-90-3	Dexamethasone Acetate (monohydrate)
55837-18-8	Butibufen
56030-54-7	Sufentanil
56180-94-0	Acarbose
56211-40-6	Torasemide
56281-37-9	2C-B Hydrochloride
56341-08-3	Mabuterol
56390-09-1	Epirubicin Hydrochloride
56392-17-7	Metoprolol Tartrate
56420-45-2	Epirubicin
56775-88-3	Zimeldine
56974-46-0	Butalamine Hydrochloride
56980-93-9	Celiprolol
56995-20-1	Flupirtine
57041-67-5	Desflurane
57109-90-7	Clorazepate Dipotassium
57149-07-2	Naftopidil
57149-08-3	Naftopidil Dihydrochloride
57262-94-9	Setiptiline
57432-61-8	Methylergometrine Maleate
57470-78-7	Celiprolol Hydrochloride
57524-89-7	Hydrocortisone Valerate
57526-81-5	Prenalterol
57574-09-1	Amineptine
57576-44-0	Aclarubicin
57801-81-7	Brotizolam
57852-57-0	Idarubicin Hydrochloride
57982-77-1	Buserelin
57982-78-2	Budipine
58186-27-9	Idebenone
58207-19-5	Clindamycin Hydrochloride (monohydrate)
58513-59-0	Tiotixene Hydrochloride (anhydrous)
58579-51-4	Anagrelide Hydrochloride
58786-99-5	Butorphanol Tartrate
58895-64-0	Nalmefene Hydrochloride
58934-46-6	Lorcainide Hydrochloride
58957-92-9	Idarubicin
59122-46-2	Misoprostol
59170-23-9	Bevantolol
59263-76-2	Meptazinol Hydrochloride
59277-89-3	Aciclovir
59302-14-6	Furaltadone (±)
59333-67-4	Fluoxetine Hydrochloride
59392-53-9	Maitotoxin-1
59467-70-8	Midazolam
59729-31-6	Lorcainide
59729-32-7	Citalopram Hydrobromide
59729-33-8	Citalopram
59804-37-4	Tenoxicam
59865-13-3	Ciclosporin
59866-76-1	Bibenzonium
59917-39-4	Vindesine Sulfate
60142-96-3	Gabapentin
60205-81-4	Ipratropium
60491-10-3	Strychnine Sulfate (pentahydrate)
60525-15-7	Zimeldine Hydrochloride (anhydrous)
60560-33-0	Pinacidil
60561-17-3	Sufentanil Citrate
60583-39-3	Brucine Sulfate (heptahydrate)
60643-86-9	Vigabatrin
60682-24-8	Butibufen Sodium
60929-23-9	Indeloxazine
60991-48-2	Ajmaline Monoethanolate
61036-62-2	Teichoplanin
61036-64-4	Teichoplanin A_2
61129-30-4	Zimeldine Hydrochloride (monohydrate)
61197-73-7	Loprazolam
61260-05-7	Prenalterol Hydrochloride
61336-70-7	Amoxicillin Trihydrate
61337-67-5	Mirtazapine
61413-54-5	Rolipram
61718-82-9	Fluvoxamine Maleate
61802-93-5	Metaclazepam Hydrochloride
61825-94-3	Oxaliplatin
61869-08-7	Paroxetine
62013-04-1	Dirithromycin
62571-86-2	Captopril
62613-82-5	Oxiracetam
62658-63-3	Bopindolol
62666-20-0	Progabide
63074-08-8	Terazosin Hydrochloride
63547-13-7	Adrafinil
63585-09-1	Foscarnet Sodium
63590-64-7	Terazosin
63659-18-7	Betaxolol
63659-19-8	Betaxolol Hydrochloride
63661-61-0	Budipine Hydrochloride
63675-72-9	Nisoldipine
63717-04-4	Quinidine (dihydrate)
63732-85-4	Norlevorphanol Hydrobromide
63814-06-2	Isomethadone Hydrochloride (*d*-Form)
63968-64-9	Artemisinin
64024-15-3	Pentazocine Hydrochloride
64053-00-5	Zuclopenthixol Decanoate
64057-70-1	Methylenedioxymetamfetamine Hydrochloride
64092-48-4	Zomepirac Sodium (anhydrous)
64092-49-5	Zomepirac Sodium (dihydrate)
64228-79-1	Atracurium
64228-81-5	Atracurium Besilate

CAS no.	*Compound*
64241-34-5	Cadralazine
64436-13-1	Arsenobetaine
64461-82-1	Tizanidine Hydrochloride
64490-92-2	Tolmetin Sodium (dihydrate)
64628-44-0	Triflumuron
65043-22-3	Indeloxazine Hydrochloride
65141-46-0	Nicorandil
65271-80-9	Mitoxantrone
65277-42-1	Ketoconazole
65369-76-8	*m*CPP Hydrochloride
65484-56-2	Quinidine Polygalacturonate (hydrate)
66051-63-6	Halofantrine (racemic)
66085-59-4	Nimodipine
66104-22-1	Pergolide
66104-23-2	Pergolide Mesilate
66142-81-2	2C-B
66357-35-5	Ranitidine
66357-59-3	Ranitidine Hydrochloride
66376-36-1	Alendronic Acid
66711-21-5	Apraclonidine
66722-44-9	Bisoprolol
66722-45-0	Bisoprolol Fumarate (hemifumarate)
66778-36-7	Encainide
66794-74-9	Encainide Hydrochloride
66981-73-5	Tianeptine
66985-17-9	Ipratropium Bromide
67037-37-0	Eflornithine
67227-56-9	Fenoldopam
67227-57-0	Fenoldopam Methanesulfonate
67392-87-4	Drospirenone
67434-14-4	Benoxaprofen
68206-94-0	Cloricromen
68278-23-9	Eflornithine Hydrochloride
68291-97-4	Zonisamide
68359-37-5	Cyfluthrin
68475-42-3	Anagrelide
68497-62-1	Pramiracetam
68630-75-1	Buserelin Acetate
68693-11-8	Modafinil
68767-14-6	Loxoprofen
68797-31-9	Econazole Nitrate
68844-77-9	Astemizole
69049-06-5	Alfentanil Hydrochloride (anhydrous)
69049-73-6	Nedocromil
69049-74-7	Nedocromil Sodium
69525-81-1	Morantel Citrate
69655-05-6	Didanosine
69657-51-8	Aciclovir Sodium
69756-53-2	Halofantrine
69815-49-2	Noradrenaline Acid Tartrate (monohydrate)
69975-86-6	Doxofylline
70024-40-7	Terazosin Hydrochloride Dihydrate
70111-54-5	Loprazolam Mesilate (anhydrous)
70356-03-5	Cefaclor (monohydrate)
70374-39-9	Lornoxicam
70393-85-0	Glyphosate Sesquisodium Salt
70434-82-1	CP 47,497
70476-82-3	Mitoxantrone Hydrochloride
70879-28-6	Alfentanil Hydrochloride (monohydrate)
70918-01-3	Doxazosin Hydrochloride
70958-86-0	Amosulalol Monohydrochloride
71109-09-6	Vedaprofen
71125-38-7	Meloxicam
71195-58-9	Alfentanil
71320-77-9	Moclobemide
71395-14-7	Tocainide Hydrochloride (±)
71486-22-1	Vinorelbine
71620-89-8	Reboxetine
71675-85-9	Amisulpride
71771-90-9	Denopamine
71939-50-9	Dihydroartemisinin
71963-77-4	Artemether
72150-17-5	Eptazocine Hydrobromide
72432-03-2	Miglitol
72432-10-1	Aniracetam
72509-76-3	Felodipine
72522-13-5	Eptazocine
72558-82-8	Ceftazidime
72559-06-9	Rifabutin
72810-59-4	Torasemide Sodium
72869-16-0	Pramiracetam Sulfate
72878-35-4	*N*-Benzylpiperazine Hydrochloride
72956-09-3	Carvedilol
73121-56-9	Enprostil
73217-88-6	Apraclonidine Dihydrochloride
73218-79-8	Apraclonidine Hydrochloride
73220-03-8	Remoxipride Hydrochloride (anhydrous)
73239-87-9	Glaucine Phosphate (*dl*-form)
73368-74-8	Phenoxymethylpenicillin Calcium (dihydrate)
73384-59-5	Ceftriaxone
73573-87-2	Formoterol
73590-58-6	Omeprazole
73963-72-1	Cilostazol
74011-58-8	Enoxacin
74011-58-8	Enoxaparin
74050-98-9	Ketanserin
74090-97-8	Haloperidol Decanoate
74103-06-3	Ketorolac
74103-07-4	Ketorolac Tromethamine
74197-85-8	Doxazosin
74258-86-9	Alacepril
74381-53-6	Leuprorelin Acetate
74397-12-9	Limaprost
74536-44-0	Metadoxine
74578-69-1	Ceftriaxone Sodium (anhydrous)
74639-40-0	Docarpamine
74697-28-2	Cloricromen Hydrochloride
74863-84-6	Argatroban (anhydrous)
75272-39-8	Nemonapride
75330-75-5	Lovastatin
75438-57-2	Moxonidine
75438-58-3	Moxonidine Hydrochloride
75507-68-5	Flupirtine Maleate
75530-68-6	Nilvadipine
75607-67-9	Fludarabine Phosphate
75695-93-1	Isradipine
75696-02-5	Cinolazepam
75706-12-6	Leflunomide
75847-73-3	Enalapril
75887-54-6	Arteether
75975-70-1	Cefradine (monohydrate)
76095-16-4	Enalapril Maleate
76420-72-9	Enalaprilat (anhydrous)
76547-98-3	Lisinopril
76568-02-0	Flosequinan
76824-35-6	Famotidine
76932-56-4	Nafarelin
76963-41-2	Nizatidine
77337-73-6	Acamprosate Calcium
77337-76-9	Acamprosate
77472-70-9	Carphedon
77671-31-9	Enoximone
77883-43-3	Doxazosin Mesilate
78110-38-0	Aztreonam
78111-17-8	Okadaic Acid
78415-72-2	Milrinone
78439-06-2	Ceftazidime Pentahydrate
78628-80-5	Terbinafine Hydrochloride
78755-81-4	Flumazenil
79350-37-1	Cefixime
79449-98-2	Cabastine (racemate of levocabastine)
79516-68-0	Levocabastine
79517-01-4	Octreotide Acetate
79547-78-7	Levocabastine Hydrochloride
79559-97-0	Sertraline Hydrochloride
79617-96-2	Sertraline
79645-27-5	Tobramycin (sulfate)
79794-75-5	Loratadine
79902-63-9	Simvastatin
79983-71-4	Hexaconazole
80012-43-7	Epinastine
80125-14-0	Remoxipride
80214-83-1	Roxithromycin
80382-23-6	Sodium Loxoprofen
80474-14-2	Fluticasone Propionate
80573-04-2	Balsalazide
80755-51-7	Bunazosin
81093-37-0	Pravastatin
81098-60-4	Cisapride
81103-11-9	Clarithromycin
81131-70-6	Pravastatin Sodium
81161-17-3	Esmolol Hydrochloride
81403-68-1	Alfuzosin Hydrochloride
81403-80-7	Alfuzosin
81409-90-7	Cabergoline
81591-81-3	Glyphosate Trimesium
81732-46-9	Bambuterol Hydrochloride
81732-65-2	Bambuterol
81801-12-9	Xamoterol
82101-18-6	Balsalazide Disodium Dihydrate (anhydrous)
82159-09-9	Epalrestat
82248-59-7	Atomoxetine Hydrochloride
82410-32-0	Ganciclovir
82419-36-1	Ofloxacin
82586-52-5	Moexipril Hydrochloride
82586-55-8	Quinapril Hydrochloride
82586-57-0	Moexipril Diacid Hydrochloride
82626-01-5	Alpidem
82626-48-0	Zolpidem
82640-04-8	Raloxifene Hydrochloride
82747-56-6	Cicletanine Hydrochloride
82752-99-6	Nefazodone Hydrochloride
82768-85-2	Quinaprilat (diacid of quinapril)
82834-16-0	Perindopril
82857-38-3	Bopindolol Malonate
82952-64-5	Trimetrexate D-Glucuronate
82964-04-3	Tolrestat
83015-26-3	Atomoxetine
83150-76-9	Octreotide
83348-52-1	Doxacurium Chloride (total racemate)
83366-66-9	Nefazodone
83380-47-6	Ofloxacin
83435-66-9	Delapril
83435-67-0	Delapril Hydrochloride
83480-29-9	Voglibose
83602-05-5	Spiraprilat
83647-97-6	Spirapril
83799-24-0	Fexofenadine
83846-83-7	Ketanserin Tartrate
83881-51-0	Cetirizine
83881-52-1	Cetirizine Dihydrochloride
83905-01-5	Azithromycin (anhydrous)
83915-83-7	Lisinopril Dihydrate
83919-23-7	Mometasone Furoate
84031-17-4	Metaclazepam
84057-84-1	Lamotrigine
84057-94-3	Esmolol
84057-95-4	Ropivacaine
84294-96-2	Enoxacin Sesquihydrate
84371-65-3	Mifepristone
84449-90-1	Raloxifene
84625-61-6	Itraconazole
84680-54-6	Enalaprilat (dihydrate)
84861-98-3	Aluminium Chlorhydrate
85320-68-9	Amosulalol
85329-89-1	Cabergoline Diphosphate
85371-64-8	Pinacidil Monohydrate
85409-38-7	Rilmenidene Phosphate
85441-61-8	Quinapril
85622-93-1	Temozolomide
85721-05-7	Zuclopenthixol Acetate
85721-33-1	Ciprofloxacin
86189-69-7	Felodipine
86197-47-9	Dopexamine
86220-42-0	Nafarelin Acetate Hydrate
86386-73-4	Fluconazole
86393-32-0	Ciprofloxacin Hydrochloride (monohydrate)
86408-72-2	Ecabet Sodium
86483-48-9	Ciprofloxacin Hydrochloride (anhydrous)
86484-91-5	Dopexamine Dihydrochloride
86541-74-4	Benazepril Hydrochloride
86541-75-5	Benazepril
87056-78-8	Quinagolide
87233-61-2	Emedastine
87233-62-3	Emedastine Difumarate
87269-97-4	Ramiprilat
87333-19-5	Ramipril
87679-37-6	Trandolapril
87760-53-0	Tandospirone
87848-99-5	Acrivastine
87913-26-6	Bromantane
88150-42-9	Amlodipine
88150-47-4	Amlodipine Maleate
88495-63-0	Artesunate
88852-12-4	Limaprost Alfadex

CAS no.	*Compound*
88889-14-9	Fosinopril Sodium
89365-50-4	Salmeterol
89371-37-9	Imidapril
89371-44-8	Imidaprilat
89396-94-1	Imidapril Monohydrochloride
89419-40-9	Clospipramine
89565-68-4	Tropisetron
89778-26-7	Toremifene
89778-27-8	Toremifene Citrate
89796-99-6	Aceclofenac
89943-82-8	Cicletanine
89987-06-4	Tiludronic Acid
89998-15-2	Citronella Oil
90098-04-7	Rebamipide
90101-16-9	Droxicam
90357-06-5	Bicalutamide
90729-43-4	Ebastine
90730-93-1	Xamoterol Hemifumarate
91161-71-6	Terbinafine
91374-20-8	Ropinirole Hydrochloride
91374-21-9	Ropinirole
91714-93-1	Bromfenac Sodium
91714-94-2	Bromfenac
91832-40-5	Cefdinir
92077-78-6	Cilazapril
92134-98-0	Fosphenytoin Sodium
92528-87-5	Bacitracin
92623-85-3	Milnacipran
93384-43-1	Botulinum Toxin A
93384-44-2	Botulinum Toxin B
93390-81-9	Fosphenytoin
93413-69-5	Venlafaxine
93479-97-1	Glimepiride
93633-92-2	Amosulalol Monohydrochloride
93664-94-9	Nemonapride
93957-54-1	Fluvastatin
93957-55-2	Fluvastatin Sodium
94188-84-8	Clopidogrel
94424-50-7	Quinagolide Hydrochloride
94749-08-3	Salmeterol Xinafoate
94841-17-5	Spirapril Hydrochloride
95058-81-4	Gemcitabine
95153-31-4	Perindoprilat
95233-18-4	Atovaquone
95382-33-5	Omeprazole Magnesium
95510-70-6	Omeprazole Sodium
95656-48-7	*dl*-Mabuterol Hydrochloride
95656-54-5	*d*-Mabuterol Hydrochloride
95656-55-6	*l*-Mabuterol Hydrochloride
95737-68-1	Pyriproxyfen
96020-91-6	Eflornithine Hydrochloride Monohydrate
96829-58-2	Orlistat
96946-42-8	Cisatracurium Besilate
97240-79-4	Topiramate
97322-87-7	Troglitazone
97682-44-5	Irinotecan
97845-62-0	Penciclovir Sodium
97867-33-9	Ciprofloxacin Lactate
98043-60-8	Clospipramine Dihydrochloride
98048-97-6	Fosinopril
98319-26-7	Finasteride
98418-47-4	Metoprolol Succinate
98717-15-8	Ropivacaine Hydrochloride
98769-81-4	Reboxetine
98769-82-5	Reboxetine Methanesulfonate (Mesylate)
99200-09-6	Nebivolol
99283-10-0	Molgramostim
99291-24-4	Levodropropizine
99294-93-6	Zolpidem Tartrate
99300-78-4	Venlafaxine Hydrochloride
99495-87-1	Flecainide (±)
99614-01-4	Ondansetron Hydrochloride Dihydrate
99614-02-5	Ondansetron
100286-90-6	Irinotecan Hydrochloride
100286-97-3	Milrinone Lactate
100643-71-8	Desloratadine
100678-32-8	Cibenzoline Succinate
100986-85-4	Levofloxacin
100986-85-4	Levofloxacin (ofloxacin *S*-(–)-form)
101152-94-7	Milnacipran Hydrochloride
101626-68-0	Nedocromil Calcium
102625-70-7	Pantoprazole
102767-28-2	Levetiracetam
103177-37-3	Pranlukast
103300-74-9	Taltirelin
103577-45-3	Lansoprazole
103628-46-2	Sumatriptan
103628-46-2	Sumatriptan Succinate
103628-47-3	Sumatriptan Hemisuccinate
103745-39-7	Fasudil
103775-10-6	Moexipril
103890-78-4	Lacidipine
103909-75-7	Maxacalcitol
104227-87-4	Famciclovir
104344-23-2	Bisoprolol Fumarate (hemifumarate)
104376-79-6	Ceftriaxone Sodium (hemiheptahydrate)
104632-26-0	Pramipexole
104987-11-3	Tacrolimus
105462-24-6	Risedronic Acid
105628-07-7	Fasudil Hydrochloride
105816-04-4	Nateglinide
105826-92-4	Tropisetron Hydrochloride
105827-78-9	Imidacloprid
105857-23-6	Alteplase
106133-20-4	Tamsulosin
106266-06-2	Risperidone
106463-17-6	Tamsulosin Hydrochloride
106516-24-9	Sertindole
106819-53-8	Doxacurium Chloride (*meso* isomer)
106861-44-3	Mivacurium Chloride
107007-99-8	Granisetron Hydrochloride
107133-36-8	Perindopril Erbumine (perindopril *tert*-butylamine)
107452-89-1	Ziconitide
107753-78-6	Zafirlukast
107868-30-4	Exemestane
107910-75-8	Ganciclovir Sodium
108050-54-0	Tilmicosin
108612-45-9	Mizolastine
108736-35-2	Lanreotide
108929-04-0	Epinastine Hydrochloride
109581-93-3	Tacrolimus Monohydrate
109889-09-0	Granisetron
110429-35-1	Paroxetine Hydrochloride Hemihydrate
110942-02-4	Aldesleukin
111025-46-8	Pioglitazone
111470-99-6	Amlodipine Besilate
111911-87-6	Rebamipide
111974-69-7	Quetiapine
111974-72-2	Quetiapine Fumarate
112457-95-1	Tandospirone Citrate
112514-54-2	Yessotoxin
112529-15-4	Pioglitazone Hydrochloride
112809-51-5	Letrozole
112856-44-7	Losigamone
112887-68-0	Raltitrexed
113665-84-2	Clopidogrel
113775-47-6	Dexmedetomidine
113852-37-2	Cidofovir
114084-78-5	Ibandronic Acid
114798-26-4	Losartan
114870-03-0	Fondaparinux Sodium
114977-28-5	Docetaxel
115007-34-6	Mycophenolate Mofetil
115103-54-3	Tiagabine
115256-11-6	Dofetilide
115436-72-1	Risedronate Sodium
115956-12-2	Dolasetron
115956-13-3	Dolasetron Methanesulfonate
116355-83-0	Fumonisin B_1
116355-84-1	Fumonisin B_2
116539-59-4	Duloxetine
116644-53-2	Mibefradil
116666-63-8	Mibefradil Dihydrochloride
116836-09-0	Isomethadone (*dl*-Form)
117591-79-4	Remoxipride Hydrochloride (monohydrate)
117772-70-0	Azithromycin (dihydrate)
117976-89-3	Rabeprazole
117976-90-6	Rabeprazole Sodium
119141-88-7	Esomeprazole
119302-91-9	Rocuronium Bromide
119413-54-6	Topotecan Hydrochloride
120011-70-3	Donepezil Hydrochloride
120014-06-4	Donepezil
120202-66-6	Clopidogrel Bisulfate
120279-96-1	Dorzolamide
120511-73-1	Anastrozole
120608-46-0	Duteplase
120638-55-3	Bromfenac Sodium
121124-29-6	Emamectin
121268-17-5	Alendronate Sodium
121424-52-0	Emamectin
121679-13-8	Naratriptan
121679-19-4	Naratriptan Hydrochloride
122111-03-9	Gemcitabine Hydrochloride
122320-73-4	Rosiglitazone
122647-31-8	Ibutilide
122647-32-9	Ibutilide Fumarate
123441-03-2	Rivastigmine
123948-87-8	Topotecan
124750-99-8	Losartan Potassium
124832-26-4	Valaciclovir
124832-27-5	Valaciclovir Hydrochloride
124937-51-5	Tolterodine
125317-39-7	Vinorelbine Tartrate
127779-20-8	Saquinavir
127984-74-1	Lanreotide Acetate
128196-01-0	Escitalopram
128345-62-0	Ranitidine Bismuth Citrate
128794-94-5	Mycophenolate Mofetil
129101-54-8	Rivastigmine Hydrogen Tartrate
129273-38-7	Perospirone Hydrochloride
129618-40-2	Nevirapine
129722-12-9	Aripiprazole
130636-43-0	Nifekalant
130656-51-8	Nifekalant Hydrochloride
130693-82-2	Dorzolamide Hydrochloride
130929-57-6	Entacapone
132036-88-5	Ramosetron
132112-35-7	Ropivacaine Hydrochloride
132539-06-1	Olanzapine
132875-61-7	Remifentanil
132907-88-5	Ramosetron Hydrochloride
133242-30-5	Landiolol
133652-38-7	Reteplase
134523-00-5	Atorvastatin
134523-03-8	Atorvastatin Calcium
134678-17-4	Lamivudine
135062-02-1	Repaglinide
135886-70-3	Gallium Nitrate (nonahydrate)
136310-93-5	Tiotropium Bromide
136426-54-5	Fluquinconazole
136434-34-9	Duloxetine Hydrochloride
136470-78-5	Abacavir
136572-09-3	Irinotecan Hydrochloride Trihydrate
136817-59-9	Delavirdine
137335-79-6	Emamectin
137512-74-4	Emamectin Benzoate
137862-53-4	Valsartan
138068-37-8	Lepirudin
138199-71-0	Levofloxacin Hemihydrate
138261-41-3	Imidacloprid
138402-11-6	Irbesartan
138452-21-8	Fexofenadine Hydrochloride
138729-47-2	Eszopiclone
138926-19-9	Ibandronate Sodium
138982-67-9	Ziprasidone Hydrochloride Monohydrate
139110-80-8	Zanamivir
139264-17-8	Zolmitriptan
139341-09-6	Ciguatoxin-3
139404-48-1	Tiotropium Bromide
139481-59-7	Candesartan
139755-83-2	Sildenafil
141396-28-3	Argatroban Monohydrate
141505-33-1	Levosimendan
142185-85-1	Ciguatoxin-2
142373-60-2	Tirofiban Hydrochloride Monohydrate (anhydrous)
142435-64-1	Erythrityl Tetranitrate
143201-11-0	Cerivastatin Sodium
143322-58-1	Eletriptan
143388-64-1	Naratriptan Hydrochloride
143558-00-3	Rocuronium
144034-80-0	Rizatriptan
144481-98-1	Landiolol Hydrochloride
144494-65-5	Tirofiban

CAS no.	*Compound*
144689-24-7	Olmesartan
144689-63-4	Olmesartan Medoxomil
144701-48-4	Telmisartan
145040-37-5	Candesartan Cilexetil
145158-71-0	Tegaserod
145202-66-0	Rizatriptan Benzoate
145599-86-6	Cerivastatin
145821-59-6	Tiagabine Hydrochloride
146939-27-7	Ziprasidone
147098-20-2	Rosuvastatin Calcium
147221-93-0	Delavirdine Monomethanesulfonate
148408-66-6	Docetaxel Trihydrate
148553-50-8	Pregabalin
149824-15-7	Ilodecakin
149845-06-7	Saquinavir Mesilate
149845-07-8	Disodium Tiludronate (anhydrous)
150378-17-9	Indinavir
150399-21-6	Balsalazide Disodium Dihydrate
150915-40-5	Tirofiban Hydrochloride Monohydrate
150915-41-6	Perospirone
151096-09-2	Moxifloxacin
151319-34-5	Zaleplon
151767-02-1	Montelukast Sodium
154323-57-6	Almotriptan
154361-50-9	Capecitabine
154598-52-4	Efavirenz
155141-29-0	Rosiglitazone Maleate
155213-67-5	Ritonavir
155453-10-4	Disodium Tiludronate (hemihydrate)
156137-99-4	Rapacuronium Bromide
156604-79-4	Dexketoprofen Trometamol
157810-81-6	Indinavir Sulfate
158966-92-8	Montelukast
159445-63-3	Nateplase
159989-64-7	Nelfinavir
159989-65-8	Nelfinavir Mesilate
161814-49-9	Amprenavir
162011-90-7	Rofecoxib
163222-33-1	Ezetimibe
164579-32-2	Pantoprazole Sodium Sesquihydrate
164656-23-9	Dutasteride
168146-84-7	Abacavir Succinate
169590-42-5	Celecoxib
171596-29-5	Tadalafil
171599-83-0	Sildenafil Citrate
174722-31-7	Rituximab
177834-92-3	Eletriptan Hydrobromide
181183-52-8	Almotriptan Maleate
181695-72-7	Valdecoxib
182815-44-7	Colesevelam Hydrochloride
183325-78-2	Calfactant
185243-69-0	Etanercept
186028-79-5	Methylone
186691-13-4	Tiotropium
186826-86-8	Moxifloxacin Hydrochloride
188062-50-2	Abacavir Sulfate
189188-57-6	Tegaserod Maleate
191217-81-9	Pramipexole Dihydrochloride Monohydrate
192725-17-0	Lopinavir
196597-26-9	Ramelteon
196618-13-0	Oseltamivir
201677-75-0	Taltirelin Tetrahydrate
202409-33-4	Etoricoxib
204255-11-8	Oseltamivir Phosphate
207572-68-7	Rilmenidene Fumarate
207740-24-7	2C-T-2
207740-26-9	2C-T-7
217087-09-7	Esomeprazole Magnesium
219861-08-2	Escitalopram Oxalate
221373-18-8	Olanzapine Embonate
222535-22-0	Alefacept
224785-90-4	Vardenafil
224785-91-5	Vardenafil Hydrochloride Trihydrate (anhydrous)
224789-15-5	Vardenafil Dihydrochloride
242138-07-4	Omalizumab
287714-41-4	Rosuvastatin
330808-88-3	Vardenafil Hydrochloride Trihydrate
411207-31-3	Tiotropium Bromide
618903-56-3	Tetrahydrogestrinone

2 Molecular Formulae

Al
Aluminium
$AlCl_3$
Aluminium Chloride
AlF_6Na_3
Cryolite
$AlN_3O_9,9H_2$
Aluminium Nitrate
$Al(OH)_3$
Aluminium Hydroxide
AlP
Aluminium Phosphide
$AlPO_4$
Aluminium Phosphate
$[Al_2(OH)_5Cl]_x$
Aluminium Chlorhydrate
Al_2O_3
Aluminium Oxide
$Al_2(SO_4)_3$
Aluminium Sulfate
$Al_3O_2[C_6H_4(OOCCH_3)COO]_5$
Aloxiprin
As
Arsenic
AsH_3O_4
Arsenic Acid
As_2O_3
Arsenic Trioxide
As_2O_5
Arsenic Pentoxide
Be
Beryllium
$BeCl_2$
Beryllium Chloride
BeF_2
Beryllium Fluoride
$Be(NO_3)_2$
Beryllium Nitrate
BeO
Beryllium Oxide
$Be(OH)_2$
Beryllium Hydroxide
Bi
Bismuth
BrH_4N
Ammonium Bromide
BrK
Potassium Bromide
BrNa
Sodium Bromide
Br_2Ca
Calcium Bromide
$BaCl_2$
Barium Chloride
$Ba(CH_3CO_2)_2$
Barium Acetate
$Ba(CN)_2$
Barium Cyanide
$BaCO_3$
Barium Carbonate
BaO
Barium Oxide
$Ba(OH)_2$
Barium Hydroxide
$BaSO_4$
Barium Sulfate
Ba^{2+}
Barium
Cd
Cadmium
$CdCl_2$
Cadmium Chloride
$CdCO_3$
Cadmium Carbonate
CdO
Cadmium Oxide
CdS
Cadmium Sulfide
$CdSO_4$
Cadmium Sulfate
Cu
Copper
$CuSO_4$
Copper Sulfate
$CClF_2{\cdot}CClF_2$
Cryofluorane
CCl_2F_2
Dichlorodifluoromethane
CCl_3CH_3
Trichloroethane
CCl_3F
Trichlorofluoromethane
$CC_{l3}NO_2$
Chloropicrin
CCl_4
Carbon Tetrachloride
$CF_3CHBrCl$
Halothane
$CHCl_3$
Chloroform
CH_2Cl_2
Methylene Chloride
$CH_2Cl_2Na_2O_6P_2$
Disodium Clodronate
CH_2O
Formaldehyde
$(CH_3)_2As(O)OH$
Dimethylarsinic Acid
$(CH_3)_3As^+CH_2CO_2$
Arsenobetaine
CH_3Br
Methyl Bromide
$CH_3Br,20H_2O$
Crystal Hydrate
CH_3CH_2OH
Ethanol
$CH_3{\cdot}COOH$
Acetic Acid Glacial
$CH_3C_6H_4OH$
Cresol
$CH_3H_2AsO_3$
Methanearsonic Acid
$CH_3NaHAsO_3$
Sodium Methanearsonate
$CH_3Na_2AsO_3$
Disodium Methanearsonate
CH_3OH
Methanol
$CH_4Cl_2O_6P_2$
Clodronic Acid
CH_4N_2O
Urea
$CH_4N_2O_2$
Hydroxycarbamide
CN
Cyanide
CNBr
Cyanogen Bromide
CNCl
Cyanogen Chloride
CNa_3O_5P
Foscarnet Sodium
$COCl_2$
Phosgene
$Ca(C_5H_{10}NO_4S)_2$
Acamprosate Calcium
$Ca_3(AsO_4)_2$
Calcium Arsenate
C_2Cl_4
Tetrachloroethylene
C_2CaO_4
Calcium Oxalate
C_2HCl_3
Trichloroethylene
$C_2H_2AsCl_3$
Lewisite
$C_2H_2Cl_4$
Tetrachloroethane
$C_2H_2FNaO_2$
Sodium Fluoroacetate
$C_2H_2O_4$
Oxalic Acid
$C_2H_3Cl_3NaO_4P$
Triclofos Sodium
$C_2H_3Cl_3O$
Trichloroethanol
$C_2H_3Cl_3O_2$
Chloral Hydrate
$C_2H_3FO_2$
Fluoroacetic Acid
$C_2H_4N_4$
Aminotriazole
C_2H_4O
Acetaldehyde
$(C_2H_4O)_x$
Metaldehyde
$(C_2H_4O)_3$
Paraldehyde
$(C_2H_5)_2O$
Ether
$C_2H_6AsNaO_2$
Sodium Cacodylate
C_2H_6Hg
Dimethylmercury
C_2H_6OS
Dimethyl Sulfoxide
$C_2H_6O_2$
Ethylene Glycol
C_2H_7NO
Monoethanolamine
$C_2H_7NO,C_{18}H_{34}O_2$
Monoethanolamine Oleate
$C_2H_8NO_2PS$
Methamidophos
$C_2H_8N_2,H_2O$
Ethylenediamine Hydrate
$C_2K_2O_4$
Potassium Oxalate
$C_2Na_2O_4$
Sodium Oxalate
$C_3H_2ClF_5O$
Enflurane
$C_3H_2F_6O$
Desflurane
$C_3H_3N_3O_2S$
Aminonitrothiazole
$C_3H_4Cl_2F_2O$
Methoxyflurane
C_3H_4O
Acrolein
C_3H_5BrO
Bromoacetone
$C_3H_5N_3O_9$
Glyceryl Trinitrate
$C_3H_6N_2O_2$
Cycloserine
C_3H_6O
Acetone
$C_3H_7NO_2$
Urethane
C_3H_7OH
Propanol

$C_3H_8NO_5P$
Glyphosate
$C_3H_8N_2OS$
Noxytiolin
C_3H_8O
Isopropyl Alcohol
$C_3H_8OS_2$
Dimercaprol
$C_4H_3FN_2O_2$
Fluorouracil
$C_4H_3F_7O$
Sevoflurane
$C_4H_4FN_3O$
Flucytosine
$C_4H_4N_2O_3$
Barbituric Acid
$C_4H_5NO_2$
Succinimide
$C_4H_5N_4NaO_3S_2$
Acetazolamide Sodium
$C_4H_6(NO_3)_4$
Erythrityl Tetranitrate
$C_4H_6N_2$
Fomepizole
$C_4H_6N_2O_2$
Muscimol
$C_4H_6N_2S$
Thiamazole
$C_4H_6N_4O_3$
Allantoin
$C_4H_6N_4O_3S_2$
Acetazolamide
$C_4H_6O_2$
γ-Butyrolactone
$C_4H_6O_3$
(S)-3-Hydroxy-γ Butyrolactone
$C_4H_7Br_2Cl_2O_4P$
Naled
$C_4H_7Cl_2O_4P$
Dichlorvos
$C_4H_7Cl_3O$
Chlorobutanol
$C_4H_7Cl_3O_2$
Butylchloral Hydrate
$C_4H_8Cl_2S$
Sulfur Mustard
$C_4H_8Cl_3O_4P$
Trichlorfon
$C_4H_8NaO_3$
Sodium-4-hydroxybutyrate
C_4H_8O
Methyl Ethyl Ketone
$C_4H_8O_2$
Ethyl Acetate
$C_4H_8O_3$
γ-Hydroxybutyrate
$C_4H_{10}FO_2P$
Sarin
$C_4H_{10}N_2$
Piperazine
$(C_4H_{10}N_2)_3,2C_6H_8O_7,xH_2O$
Piperazine Citrate
$C_4H_{10}N_2,C_6H_{10}O_4$
Piperazine Adipate
$C_4H_{10}N_2,C_{10}H_{14}CaN_2O_8$
Piperazine Calcium Edetate
$C_4H_{10}N_2,H_3PO_4,H_2O$
Piperazine Phosphate
$C_4H_{10}N_2,6H_2O$
Piperazine Hydrate
$C_4H_{10}O_2S$
Thiodiglycol (metabolite)
$C_4H_{11}NO_3$
Trometamol
$C_4H_{11}N_5$
Metformin
$C_4H_{11}N_5,HCl$
Metformin Hydrochloride
$C_4H_{12}NNaO_7P_2,3H_2O$
Alendronate Sodium
$C_4H_{13}NO_7P_2$
Alendronic Acid
$C_5H_3I_2NO$
Iopydone
$C_5H_4N_2O_4$
Nifuroxime
$C_5H_4N_4O$
Allopurinol
$C_5H_4N_4S,H_2O$
Mercaptopurine
$C_5H_5N_3O$
Pyrazinamide
$C_5H_5N_3O_3S$
Aminitrozole
$C_5H_5N_5S$
Tioguanine
$C_5H_6N_2OS$
Methylthiouracil
$C_5H_6N_2O_4$
Ibotenic Acid
$C_5H_7NO_3$
Dimethadione
$C_5H_7N_3O_2$
Dimetridazole
$C_5H_7N_3O_2,HCl$
Dimetridazole Hydrochloride
$C_5H_8N_4O_3S_2$
Methazolamide
$C_5H_8N_4O_{12}$
Pentaerithrityl Tetranitrate
$C_5H_9Cl_2N_3O_2$
Carmustine
$C_5H_9NO_3S$
Acetylcysteine
$C_5H_9N_3$
Ametazole
$C_5H_9N_3$
Histamine
$C_5H_9N_3,2HCl$
Ametazole Hydrochloride
$C_5H_9N_3,2HCl$
Histamine Hydrochloride
$C_5H_9N_3,2H_3PO_4$
Histamine Acid Phosphate
$C_5H_{10}NNaS_2$
Ditiocarb Sodium
$C_5H_{10}NNaS_2,3H_2O$
Ditiocarb Sodium Trihydrate
$C_5H_{10}NS_2$
Ditiocarb
$C_5H_{11}Cl_2N$
Mustine
$C_5H_{11}NO_2$
Amyl Nitrite
$C_5H_{11}NO_2$
Betaine
$C_5H_{11}NO_2,HCl$
Betaine Hydrochloride
$C_5H_{11}NO_2S$
Penicillamine
$C_5H_{11}NO_2S,HCl$
Penicillamine Hydrochloride
$C_5H_{11}N_2O_2P$
Tabun
$C_5H_{12}NO_3PS_2$
Dimethoate
$C_5H_{12}NO_4PS$
Omethoate
$C_5H_{12}O$
Amyl Alcohol
$C_5H_{14}ClNO$
Choline Chloride
$[C_5H_{14}NO]^+$
Choline
$C_5H_{14}N_2$
Cadaverine
$C_5H_{15}NO_2$
Choline Hydroxide
$C_5H_{15}N_2O_3PS$
Amifostine
C_6HCl_5O
Pentachlorophenol
$C_6H_4Cl_2$
Paradichlorobenzene
$(C_6H_4NH_2)H_2AsO_3$
Arsanilic Acid
$C_6H_5ClN_2O_2$
Chloronitroaniline
C_6H_5ClO
Parachlorophenol
C_6H_5CHO
Benzaldehyde
$C_6H_5{\cdot}CH_3$
Toluene
$C_6H_5Li_3O_7,4H_2O$
Lithium Citrate
$C_6H_5NH_2$
Aniline
$C_6H_5NO_2$
Nicotinic Acid
$C_6H_5NO_2$
Nitrobenzene
C_6H_5O
Phenol
C_6H_6
Benzene
$C_6H_6AsNO_6$
Roxarsone
C_6H_6ClN
Chloroaniline
C_6H_6ClN
2-Chloroaniline
C_6H_6ClN
3-Chloroaniline
C_6H_6ClN
4-Chloroaniline
$C_6H_6Cl_2N_2O_4S_2$
Diclofenamide
$C_6H_6Cl_6$
Benzene Hexachloride
$C_6H_6Cl_6$
Lindane
$C_6H_6N_2O$
Nicotinamide
$C_6H_6N_2O_3$
Acipimox
$C_6H_6N_4O_3S$
Niridazole
$C_6H_6N_4O_4$
Nitrofurazone
$C_6H_6N_6O_2$
Temozolomide
$C_6H_6O_2$
Hydroquinone
$C_6H_6O_2$
Resorcinol
$C_6H_7AsNNaO_3$
Sodium Arsanilate
$C_6H_7KO_2$
Potassium Sorbate
C_6H_7NO
4-Aminophenol
C_6H_7NO
Nicotinyl Alcohol
$C_6H_7NO,C_4H_6O_6$
Nicotinyl Tartrate
$C_6H_7NaO_6$
Sodium Ascorbate
$C_6H_7N_3O$
Isoniazid
$C_6H_7N_3O,C_7H_7NO_3$
Isoniazid Aminosalicylate
C_6H_8ClNS
Clomethiazole
$C_6H_8ClN_7O$
Amiloride
$C_6H_8ClN_7O,HCl,2H_2O$
Amiloride Hydrochloride
$C_6H_8N_2$
Paraphenylenediamine
$C_6H_8N_2O_2S$
Sulfanilamide
$C_6H_8N_2O_8$
Isosorbide Dinitrate
$C_6H_8O_2$
Sorbic Acid
$C_6H_8O_6$
Ascorbic Acid
$C_6H_9NO_3$
Trimethadione
$C_6H_9NO_6$
Isosorbide Mononitrate
$C_6H_9N_3O_3$
Metronidazole
$C_6H_{10}N_2O_3$
Oxiracetam
$C_6H_{10}N_4$
Pentetrazol

$C_6H_{10}O$
Methylpentynol
$C_6H_{11}BrN_2O_2$
Bromisoval
$C_6H_{11}NO_2$
Vigabatrin
$C_6H_{12}F_2N_2O_2$
Eflornithine
$C_6H_{12}NNaO_3S$
Sodium Cyclamate
$C_6H_{12}NO_3SK$
Potassium Cyclamate
$C_6H_{12}N_3PS$
Thiotepa
$C_6H_{12}N_4$
Methenamine
$C_6H_{12}N_4,C_8H_8O_3$
Methenamine Mandelate
$C_6H_{12}N_4,C_9H_9NO_3$
Methenamine Hippurate
$C_6H_{13}ClF_2N_2O_2$
Eflornithine Hydrochloride
$C_6H_{13}NO_2$
Aminocaproic Acid
$C_6H_{13}NO_3S$
Cyclamic Acid
$C_6H_{13}N_2Na_3O_{10}P_2$
Glyphosate Sesquisodium Salt
$C_6H_{14}O_2S_3$
1,1′-Sulfonylbis-[2-*S*-(methylthio)ethane] (metabolite)
$C_6H_{14}O_6$
Mannitol
$C_6H_{14}O_6S_2$
Busulfan
$C_6H_{15}ClF_2N_2O_3$
Eflornithine Hydrochloride Monohydrate
$C_6H_{15}ClN_2O_2$
Carbachol
$C_6H_{15}N_5$
Buformin
$C_6H_{15}N_5,HCl$
Buformin Hydrochloride
$C_6H_{15}O_3PS_2$
Demeton-*S*-(Me)
$C_6H_{17}N_2O_5P$
Glyphosate Isopropylammonium
$C_6H_{18}N_4$
Trientine
$C_6H_{18}N_4,2HCl$
Trientine Dihydrochloride
$C_7H_3IN_2O_3$
Nitroxinil
$C_7H_3IN_2O_3,C_8H_{19}NO_5$
Nitroxinil Eglumine Salt
$C_7H_4ClNO_2$
Chlorzoxazone
$C_7H_4NNaO_3S,2H_2O$
Saccharin Sodium
$C_7H_5ClN_3NaO_4S_2$
Chlorothiazide Sodium
$C_7H_5Cl_2NO_4S$
Halazone
$C_7H_5NO_3S$
Saccharin
$C_7H_5NaO_3$
Sodium Salicylate
$C_7H_5O_2Na$
Sodium Benzoate
$C_7H_6ClNO_2$
Chloronitrotoluene
$C_7H_6ClN_3O_4S_2$
Chlorothiazide
$C_7H_6KNO_2$
Potassium Aminobenzoate
$C_7H_6KNO_3$
Potassium Aminosalicylate
$C_7H_6NNaO_3,2H_2O$
Sodium Aminosalicylate
$(C_7H_6NO_3)_2Ca,3H_2O$
Calcium Aminosalicylate
$C_7H_6N_2O_5$
Dinitro-orthocresol
$C_7H_6O_2$
Benzoic Acid
$C_7H_6O_3$
Salicylic Acid
$C_7H_6O_4$
Patulin
$C_7H_7ClNNaO_2S,3H_2O$
Tosylchloramide Sodium
$C_7H_7ClNa_2O_6P_2S$
Disodium Tiludronate
C_7H_7ClO
Chlorocresol
$C_7H_7Cl_2NO$
Clopidol
$C_7H_7Cl_3NO_3PS$
Chlorpyrifos-(Me)
$C_7H_7NO_2$
Aminobenzoic Acid
$C_7H_7NO_2$
Methyl Nicotinate
$C_7H_7NO_2$
Salicylamide
$C_7H_7NO_3$
Aminosalicylic Acid
$C_7H_7N_4NaO_2:C_2H_5NO_2$
Theophylline Sodium Glycinate
$C_7H_8BrNO_2$
Brocresine
C_7H_8ClN
Chloromethylaniline
$C_7H_8ClN_3O_4S_2$
Hydrochlorothiazide
$C_7H_8N_4O_2$
Theobromine
$C_7H_8N_4O_2$
Theophylline
$(C_7H_8N_4O_2)_2,C_2H_4(NH_2)_2,2H_2O$
Aminophylline
$C_7H_8N_4O_2,C_2H_7NO$
Theophylline Monoethanolamine
$C_7H_8N_4O_2,H_2O$
Theophylline Hydrate
C_7H_8O
Benzyl Alcohol
$C_7H_9AsN_2O_4$
Carbarsone
$C_7H_9ClN_2O$
Pralidoxime Chloride
$C_7H_9ClN_2O_4S_2$
Disulfamide
C_7H_9ClO
Ethchlorvynol
$C_7H_9ClO_6P_2S$
Tiludronic Acid
$C_7H_9IN_2O$
Pralidoxime Iodide
$C_7H_9NO_2$
Deferiprone
$C_7H_9N_3O_3S,H_2O$
Sulfacarbamide
$C_7H_{10}NNaO_7P_2$
Risedronate Sodium
$C_7H_{10}N_2OS$
Propylthiouracil
$C_7H_{10}N_2O_2S$
Carbimazole
$C_7H_{10}N_2O_2S$
Mafenide
$C_7H_{10}N_2O_2S,C_2H_4O_2$
Mafenide Acetate
$C_7H_{10}N_2O_2S,C_3H_6O_2$
Mafenide Propionate
$C_7H_{10}N_2O_2S,HCl$
Mafenide Hydrochloride
$C_7H_{10}N_4O_2S$
Sulfaguanidine
$C_7H_{10}N_4O_2S,H_2O$
Sulfaguanidine Monohydrate
$C_7H_{11}NO_2$
Ethosuximide
$C_7H_{11}NO_2$
Methylpentynol Carbamate
$C_7H_{11}NO_3$
Paramethadione
$C_7H_{11}NO_7P_2$
Risedronic Acid
$C_7H_{12}ClN_5$
Simazine
$C_7H_{12}Cl_3NO_3,H_2O$
Chloral Betaine
$C_7H_{13}BrN_2O_2$
Carbromal
$C_7H_{13}O_6P$
Mevinphos
$C_7H_{14}NO_5P$
Monocrotophos
$C_7H_{14}N_2O_2S$
Aldicarb
$C_7H_{14}N_4S_2$
Metallibure
$C_7H_{14}O_2$
Amyl Acetate
$C_7H_{15}Cl_2N_2O_2P,H_2O$
Cyclophosphamide
$C_7H_{15}NO_2$
Emylcamate
$C_7H_{16}BrNO_2$
Acetylcholine Bromide
$C_7H_{16}ClNO_2$
Acetylcholine Chloride
$C_7H_{16}FO_2P$
Soman
$C_7H_{16}N_4O_4S_2$
Taurolin
$C_7H_{16}O_4S_2$
Sulfonal
$C_7H_{17}ClN_2O_2$
Bethanechol Chloride
$C_7H_{17}N$
Methylhexaneamine
$C_7H_{17}N$
Tuaminoheptane
$(C_7H_{17}N)_2,H_2SO_4$
Tuaminoheptane Sulfate
$C_7H_{27}NO_4$
Nadolol
$C_8H_4N_2S_2$
Bitoscanate
$C_8H_5Cl_3O_3$
Trichlorophenoxyacetic Acid
$C_8H_5F_3N_2OS$
Riluzole
$C_8H_5N_4NaO_5$
Nitrofurantoin Sodium
$C_8H_6Cl_2O_3$
Dicamba
$C_8H_6Cl_2O_3$
Dichlorophenoxyacetic Acid
$C_8H_6N_4O_5$
Nitrofurantoin
$C_8H_7ClN_2O_2S$
Diazoxide
C_8H_7ClO
CN Gas
$C_8H_7C_{l4}N_3O_4S_2$
Teclothiazide
$C_8H_7NaO_3$
Sodium Methyl Hydroxybenzoate
$C_8H_7N_3O_5$
Dinitolmide
$C_8H_7N_3O_5$
Furazolidone
$(C_8H_7O_3)_2Ca$
Calcium Mandelate
$C_8H_7O_3NH_4$
Ammonium Mandelate
$C_8H_8Cl_3N_3O_4S_2$
Trichlormethiazide
$C_8H_8F_3N_3O_4S_2$
Hydroflumethiazide
$C_8H_8N_2O_3S$
Zonisamide
$C_8H_8N_4$
Hydralazine
$C_8H_8N_4,HCl$
Hydralazine Hydrochloride
$C_8H_8O_3$
Mandelic Acid
$C_8H_8O_3$
Methyl Hydroxybenzoate
$C_8H_8O_3$
Methyl Salicylate
$C_8H_8O_3$
Resorcinol Monoacetate

$C_8H_8O_3$
Vanillin
$C_8H_9AsNNaO_5,5H_2O$
Acetarsone Sodium
C_8H_9ClO
Chloroxylenol
$C_8H_9I_2NO_3$
Iopydol
C_8H_9NO
Acetanilide
$C_8H_9NO_2$
Ethyl Nicotinate
$C_8H_9NO_2$
Paracetamol
$C_8H_9NO_3$
Orthocaine
$C_8H_9N_2NaO_3S,H_2O$
Sulfacetamide Sodium
$C_8H_9N_3O_4$
Nicorandil
C_8H_{10}
Xylene
$C_8H_{10}AsNO_5$
Acetarsone
$C_8H_{10}N_2O_3S$
Sulfacetamide
$C_8H_{10}N_2S$
Ethionamide
$C_8H_{10}N_4O_2$
Caffeine
$C_8H_{10}N_4O_2,C_6H_8O_7$
Caffeine Citrate
$C_8H_{10}N_4O_2,H_2O$
Caffeine Hydrate
$C_8H_{10}N_5NaO_3$
Aciclovir Sodium
$C_8H_{10}N_6$
Dihydralazine
$C_8H_{10}N_6,H_2SO_4$
Dihydralazine Sulfate
$C_8H_{11}Cl_3O_6$
Alphachloralose
$C_8H_{11}N$
Phenethylamine
$C_8H_{11}NO$
Methyridine
$C_8H_{11}NO$
Tyramine
$C_8H_{11}NO,HCl$
Tyramine Hydrochloride
$C_8H_{11}NO_2$
Dopamine
$C_8H_{11}NO_2,HCl$
Dopamine Hydrochloride
$C_8H_{11}NO_3$
Noradrenaline
$C_8H_{11}NO_3$
Pyridoxine
$C_8H_{11}NO_3,C_4H_6O_6,H_2O$
Noradrenaline Acid Tartrate
$C_8H_{11}NO_3,HCl$
Noradrenaline Hydrochloride
$C_8H_{11}NO_3,HCl$
Pyridoxine Hydrochloride
$C_8H_{11}N_2NaO_3$
Barbital Sodium
$C_8H_{11}N_3O_3S$
Lamivudine
$C_8H_{11}N_5O_3$
Aciclovir
$C_8H_{11}N_7S,H_2O$
Ambazone
$C_8H_{12}NNaO_2$
Bemegride Sodium
$C_8H_{12}N_2$
Betahistine
$C_8H_{12}N_2$
Mebanazine
$C_8H_{12}N_2$
Phenelzine
$C_8H_{12}N_2,H_2SO_4$
Phenelzine Sulfate
$C_8H_{12}N_2O_3$
Barbital
$C_8H_{12}N_2O_4S$
Pralidoxime Mesilate
$C_8H_{12}N_2O_5S$
Pralidoxime Metilsulfate
$C_8H_{12}N_2,2HCl$
Betahistine Hydrochloride
$C_8H_{13}NO_2$
Arecoline
$C_8H_{13}NO_2$
Bemegride
$C_8H_{13}NO_2,HBr$
Arecoline Hydrobromide
$C_8H_{13}N_3O_4S$
Tinidazole
$C_8H_{13}O_2$
Trenbolone Hexahydrobenzylcarbonate
$C_8H_{14}ClN_5$
Atrazine
$C_8H_{14}N_2O_2$
Levetiracetam
$C_8H_{14}N_2O_4Pt$
Oxaliplatin
$C_8H_{14}N_3O_6P$
Cidofovir
$C_8H_{15}NO$
Tropine
$C_8H_{15}NaO_2$
Sodium Valproate
$C_8H_{15}N_5S$
Desmetryne
$C_8H_{15}N_7O_2S_3$
Famotidine
$C_8H_{16}N_6OS_2$
Gloxazone
$C_8H_{16}O_2$
Valproic Acid
$C_8H_{17}N$
Coniine
$C_8H_{17}N,HBr$
Coniine Hydrobromide
$C_8H_{17}NO_2$
Pregabalin
$C_8H_{17}NO_5$
Miglitol
$C_8H_{18}BrNO_2$
Methacholine Bromide
$C_8H_{18}ClNO_2$
Methacholine Chloride
$C_8H_{19}N$
Methylaminoheptane
$C_8H_{19}N,HCl$
Methylaminoheptane Hydrochloride
$C_8H_{19}NO$
Heptaminol
$C_8H_{19}NO,HCl$
Heptaminol Hydrochloride
$C_8H_{19}O_2PS_3$
Disulfoton
$C_8H_{19}O_3PS_2$
Demeton-*O*
$C_8H_{19}O_3PS_2$
Demeton-*S*
$C_8H_{20}BrN$
Tetraethylammonium Bromide
$C_8H_{20}NO_6P$
Choline Alfoscerate
$C_9H_5Br_2NO$
Broxyquinoline
C_9H_5ClINO
Clioquinol
$C_9H_5I_2NO$
Diiodohydroxyquinoline
$C_9H_6Cl_6O_3S$
Endosulfan
$C_9H_6N_2O_3$
Nitroxoline
$C_9H_7Cl_2N_5$
Lamotrigine
C_9H_7NO
Hydroxyquinoline
$(C_9H_7NO)_2,H_2SO_4$
Hydroxyquinoline Sulfate
$C_9H_7N_7O_2S$
Azathioprine
$C_9H_8ClN_5S$
Tizanidine
$C_9H_8ClN_5S,HCl$
Tizanidine Hydrochloride
$C_9H_8N_2O_2$
Pemoline
$C_9H_8N_3NaO_2S_2,5H_2O$
Sulfathiazole Sodium
$C_9H_8O_4$
Aspirin
$C_9H_9ClO_3$
Methylchlorophenoxyacetic Acid
$C_9H_9Cl_2NO$
Propanil
$C_9H_9Cl_2NO_2$
Diloxanide
$C_9H_9Cl_2N_3$
Clonidine
$C_9H_9Cl_2N_3,HCl$
Clonidine Hydrochloride
$C_9H_9NaO_3$
Sodium Ethyl Hydroxybenzoate
$C_9H_9N_2NaO_3$
Sodium Aminohippurate
$C_9H_9N_3O_2S_2$
Sulfathiazole
$C_9H_9N_3S$
Amiphenazole
$C_9H_9N_3S,HCl$
Amiphenazole Hydrochloride
$C_9H_{10}ClN_5O_2$
Imidacloprid
$C_9H_{10}Cl_2N_2O$
Diuron
$C_9H_{10}Cl_2N_2O_2$
Linuron
$C_9H_{10}Cl_2N_4$
Apraclonidine
$C_9H_{10}Cl_2N_4,HCl$
Apraclonidine Hydrochloride
$C_9H_{10}Cl_2N_4,2HCl$
Apraclonidine Dihydrochloride
$C_9H_{10}N_2O_3$
Aminohippuric Acid
$C_9H_{10}N_2O_3S_2$
Ethoxzolamide
$C_9H_{10}N_2S$
Etisazole
$C_9H_{10}N_2S,HCl$
Etisazole Hydrochloride
$C_9H_{10}N_4O_2S_2$
Sulfamethizole
$(C_9H_{10}N_4O_4)_2,C_4H_{10}N_2$
Acefylline Piperazine
$C_9H_{10}O_3$
Ethyl Hydroxybenzoate
$C_9H_{10}O_5$
Ethyl Gallate
$C_9H_{11}ClN_2O_2$
Monolinuron
$C_9H_{11}ClO_3$
Chlorphenesin
$C_9H_{11}Cl_2N_3O_4S_2$
Methyclothiazide
$C_9H_{11}Cl_3NO_3PS$
Chlorpyrifos
$C_9H_{11}F_2N_3O_4$
Gemcitabine
$C_9H_{11}F_2N_3O_4,HCl$
Gemcitabine Hydrochloride
$C_9H_{11}IN_2O_5$
Idoxuridine
$C_9H_{11}N$
Tranylcypromine
$(C_9H_{11}N)_2,H_2SO_4$
Tranylcypromine Sulfate
$C_9H_{11}NO_2$
Benzocaine
$C_9H_{11}NO_2$
Etenzamide
$C_9H_{11}NO_3$
Adrenalone
$C_9H_{11}NO_3$
Styramate

$C_9H_{11}NO_3$,HCl
Adrenalone Hydrochloride
$C_9H_{11}NO_4$
Levodopa
$C_9H_{11}NO_5$
Droxidopa
$C_9H_{12}ClN_3O_4S_2$
Ethiazide
$C_9H_{12}ClN_5O$
Moxonidine
$C_9H_{12}ClN_5O.xHCl$
Moxonidine Hydrochloride
$C_9H_{12}Cl_2N_4O$
Guanoclor
$(C_9H_{12}Cl_2N_4O)_2,H_2SO_4$
Guanoclor Sulfate
$C_9H_{12}NO_5PS$
Fenitrothion
$C_9H_{12}N_2S$
Protionamide
$C_9H_{12}N_4O_3$
Etofylline
$C_9H_{12}N_5NaO_4$
Ganciclovir Sodium
$C_9H_{13}BrN_2O_2$
Pyridostigmine Bromide
$C_9H_{13}N$
Amfetamine
$C_9H_{13}N$
Dexamfetamine
$C_9H_{13}N$
Levamfetamine
$(C_9H_{13}N)_2,H_2SO_4$
Amfetamine Sulfate
$(C_9H_{13}N)_2,H_2SO_4$
Dexamfetamine Sulfate
$C_9H_{13}N$,HCl
Amfetamine Hydrochloride
$C_9H_{13}N,H_3PO_4$
Amfetamine Phosphate
$C_9H_{13}N,H_3PO_4$
Dexamfetamine Phosphate
$C_9H_{13}NO$
Cathine
$C_9H_{13}NO$
Hydroxyamfetamine
$C_9H_{13}NO$
Phenylpropanolamine
$C_9H_{13}NO$,HBr
Hydroxyamfetamine Hydrobromide
$C_9H_{13}NO$,HCl
Cathine Hydrochloride
$C_9H_{13}NO$,HCl
Phenylpropanolamine Hydrochloride
$C_9H_{13}NO_2$
Ethinamate
$C_9H_{13}NO_2$
Metaraminol
$C_9H_{13}NO_2$
Oxedrine
$C_9H_{13}NO_2$
Phenylephrine
$(C_9H_{13}NO_2)_2,C_4H_6O_6$
Oxedrine Tartrate
$C_9H_{13}NO_2,C_4H_6O_6$
Metaraminol Tartrate
$C_9H_{13}NO_2$,HCl
Phenylephrine Hydrochloride
$C_9H_{13}NO_3$
Adrenaline
$C_9H_{13}NO_3$
Nordefrin
$C_9H_{13}NO_3$
Levonordefrin (()-isomer)
$C_9H_{13}NO_3$
Normetadrenaline
$C_9H_{13}NO_3,C_4H_6O_6$
Adrenaline Acid Tartrate
$C_9H_{13}NO_3$,HCl
Adrenaline Hydrochloride
$C_9H_{13}NO_3$,HCl
Nordefrin Hydrochloride
$C_9H_{13}N_3O$
Iproniazid
$C_9H_{13}N_3O,H_3PO_4$
Iproniazid Phosphate
$C_9H_{13}N_3O_2$
Aminometradine
$C_9H_{13}N_3O_2$
Amisometradine
$C_9H_{13}N_3O_3$
Zalcitabine
$C_9H_{13}N_3O_5$
Cytarabine
$C_9H_{13}N_3O_5$,HCl
Cytarabine Hydrochloride
$C_9H_{13}N_5O_4$
Ganciclovir
$C_9H_{14}N_2O$
Phenoxypropazine
$C_9H_{14}N_2O,C_4H_4O_4$
Phenoxypropazine Maleate
$C_9H_{14}N_2O_3$
Metharbital
$C_9H_{14}N_2O_3$
Probarbital
$C_9H_{14}N_4O_3$
Nimorazole
$C_9H_{15}BrN_2O_3$
Acecarbromal
$C_9H_{15}NO_3$
Ecgonine
$C_9H_{15}NO_3$,HCl
Ecgonine Hydrochloride
$C_9H_{15}NO_3S$
Captopril
$C_9H_{15}N_5O$
Minoxidil
$C_9H_{16}ClN_5$
Propazine
$C_9H_{16}ClN_5$
Terbutylazine
$C_9H_{16}N_2O_2$
Apronal
$C_9H_{17}NO_2$
Gabapentin
$C_9H_{17}N_5S$
Ametryne
$C_9H_{18}N_2O_4$
Meprobamate
$C_9H_{18}N_6$
Altretamine
$C_9H_{19}N$
Cyclopentamine
$C_9H_{19}N$
Isometheptene
$(C_9H_{19}N)_2,C_6H_{10}O_8$
Isometheptene Mucate
$C_9H_{19}N$,HCl
Cyclopentamine Hydrochloride
$C_9H_{19}N$,HCl
Isometheptene Hydrochloride
$C_9H_{19}NO_4$
Dexpanthenol
$C_9H_{19}NO_7$
Choline Bitartrate
$C_9H_{22}NNaO_7P_2,H_2O$
Ibandronate Sodium
$C_9H_{23}INO_3PS$
Ecothiopate Iodide
$C_9H_{23}NO_7P_2$
Ibandronic Acid
$C_{10}H_5ClN_2$
CS Gas
$C_{10}H_5Cl_7$
Heptachlor
$C_{10}H_6Cl_8$
Chlordane
$C_{10}H_6O_2$
1,2-Naphthoquinone
$C_{10}H_6O_2,CH_3N_3$
1,2-Naphthoquinone 2-Semicarbazone
$C_{10}H_7Cl_2NO$
Chlorquinaldol
$C_{10}H_7Cl_2N_3O$
Anagrelide
$C_{10}H_7N_3S$
Tiabendazole
$C_{10}H_8$
Naphthalene
$C_{10}H_8ClN_4O_2S$,Na
Sulfachlorpyridazine Sodium
$C_{10}H_8Cl_3N_3O$
Anagrelide Hydrochloride
$C_{10}H_8O$
Betanaphthol
$C_{10}H_9ClN_4O_2S$
Sulfachlorpyridazine
$C_{10}H_9N_4NaO_2S$
Sulfadiazine Sodium
$C_{10}H_{10}N_3NaO_2S_2,H_2O$
Sulfasomizole Sodium Monohydrate
$C_{10}H_{10}N_4OS$
Metisazone
$C_{10}H_{10}N_4O_2S$
Sulfadiazine
$C_{10}H_{10}O_4$
Dimethyl Phthalate
$C_{10}H_{11}BrN_2O_3$
Brallobarbital
$C_{10}H_{11}ClF_3N_3O_4S_3$
Epithiazide
$C_{10}H_{11}I_2NO_3$
Propyliodone
$C_{10}H_{11}NaO_3$
Sodium Propyl Hydroxybenzoate
$C_{10}H_{11}N_3O_2S_2$
Sulfasomizole
$C_{10}H_{11}N_3O_3S$
Sulfamethoxazole
$C_{10}H_{11}N_3O_5S$
Nifuratel
$C_{10}H_{11}N_4NaO_5S$
Carbazochrome Sodium Sulfonate
$C_{10}H_{12}ClNO$
Beclamide
$C_{10}H_{12}ClNO_2$
Baclofen
$C_{10}H_{12}ClNO_4$
Chlorphenesin Carbamate
$C_{10}H_{12}ClN_3O_3S$
Quinethazone
$C_{10}H_{12}ClN_5O_3$
Cladribine
$C_{10}H_{12}FN_5O_4$
Fludarabine
$C_{10}H_{12}N_2$
Tolazoline
$C_{10}H_{12}N_2$
Tryptamine
$C_{10}H_{12}N_2$,HCl
Tolazoline Hydrochloride
$C_{10}H_{12}N_2$,HCl
Tryptamine Hydrochloride
$C_{10}H_{12}N_2O$
Serotonin
$C_{10}H_{12}N_2O_3$
Allobarbital
$C_{10}H_{12}N_2O_4$
Stavudine
$C_{10}H_{12}N_3O_3PS_2$
Azinphos-(Me)
$C_{10}H_{12}N_4OS$
Thioacetazone
$C_{10}H_{12}N_4O_2S_2$
Sulfaethidole
$C_{10}H_{12}N_4O_3$
Carbazochrome
$C_{10}H_{12}N_4O_3$
Didanosine
$C_{10}H_{12}O_2$
Eugenol
$C_{10}H_{12}O_3$
Propyl Hydroxybenzoate
$C_{10}H_{12}O_4$
Cantharidin
$C_{10}H_{13}BrN_2O_3$
Ibomal
$C_{10}H_{13}ClN_2$
m-Chlorophenylpiperazine
$C_{10}H_{13}ClN_2$,HCl
mCPP Hydrochloride

$C_{10}H_{13}ClN_2O_3S$
Chlorpropamide
$C_{10}H_{13}FN_5O_7P$
Fludarabine Phosphate
$C_{10}H_{13}N$
Debrisoquine
$C_{10}H_{13}NO$
Methcathinone
$C_{10}H_{13}NO,HCl$
Methcathinone Hydrochloride
$C_{10}H_{13}NO_2$
Methylenedioxyamfetamine
$C_{10}H_{13}NO_2$
Phenacetin
$C_{10}H_{13}NO_2$
Phenprobamate
$C_{10}H_{13}NO_2,HCl$
Methylenedioxyamfetamine Hydrochloride
$C_{10}H_{13}NO_4,1½H_2O$
Methyldopa
$C_{10}H_{13}N_2NaO_3$
Aprobarbital Sodium
$(C_{10}H_{13}N_3)_2,H_2SO_4$
Debrisoquine Sulfate
$C_{10}H_{13}N_3O_2$
Guanoxan
$(C_{10}H_{13}N_3O_2)_2,H_2SO_4$
Guanoxan Sulfate
$C_{10}H_{13}N_5O_4$
Adenosine
$C_{10}H_{13}N_5O_4$
Zidovudine
$C_{10}H_{14}BrNO_2$
2C-B
$C_{10}H_{14}BrNO_2,HCl$
2C-B Hydrochloride
$C_{10}H_{14}ClN$
Chlorphentermine
$C_{10}H_{14}ClN,HCl$
Chlorphentermine Hydrochloride
$C_{10}H_{14}NO_5PS$
Parathion
$C_{10}H_{14}N_2$
Nicotine
$C_{10}H_{14}N_2O$
Nikethamide
$C_{10}H_{14}N_2O_3$
Aprobarbital
$C_{10}H_{14}N_2O_4,H_2O$
Carbidopa
$C_{10}H_{14}N_2O_4S_2$
Sultiame
$C_{10}H_{14}N_4O_2$
Morinamide
$C_{10}H_{14}N_4O_2,HCl$
Morinamide Hydrochloride
$C_{10}H_{14}N_4O_3$
Proxyphylline
$C_{10}H_{14}N_4O_4$
Diprophylline
$C_{10}H_{14}O$
Thymol
$C_{10}H_{14}O_3$
Mephenesin
$C_{10}H_{14}O_4$
Guaifenesin
$C_{10}H_{15}N$
Metamfetamine
$C_{10}H_{15}N$
Phentermine
$C_{10}H_{15}N$
Phenylpropylmethylamine
$C_{10}H_{15}N,HCl$
Metamfetamine Hydrochloride
$C_{10}H_{15}N,HCl$
Phentermine Hydrochloride
$C_{10}H_{15}N,HCl$
Phenylpropylmethylamine Hydrochloride
$C_{10}H_{15}NO$
Anhydrous Ephedrine
$C_{10}H_{15}NO$
Hordenine
$C_{10}H_{15}NO$
Methoxyamfetamine
$C_{10}H_{15}NO$
Pholedrine
$C_{10}H_{15}NO$
Pseudoephedrine
$C_{10}H_{15}NO$
Racephedrine
$(C_{10}H_{15}NO)_2,H_2SO_4$
Ephedrine Sulfate
$(C_{10}H_{15}NO)_2,H_2SO_4$
Pholedrine Sulfate
$(C_{10}H_{15}NO)_2,H_2SO_4$
Pseudoephedrine Sulfate
$(C_{10}H_{15}NO)_2,H_2SO_4,2H_2O$
Hordenine Sulfate
$C_{10}H_{15}NO,HCl$
Ephedrine Hydrochloride
$C_{10}H_{15}NO,HCl$
Methoxyamfetamine Hydrochloride
$C_{10}H_{15}NO,HCl$
Pseudoephedrine Hydrochloride
$C_{10}H_{15}NO,HCl$
Racephedrine Hydrochloride
$C_{10}H_{15}NO,½H_2O$
Ephedrine
$C_{10}H_{15}NO_2$
Etilefrine
$C_{10}H_{15}NO_2$
Hydroxyephedrine
$C_{10}H_{15}NO_2,HCl$
Etilefrine Hydrochloride
$C_{10}H_{15}NO_2,HCl$
Hydroxyephedrine Hydrochloride
$C_{10}H_{15}NO_3$
Ethylnoradrenaline
$C_{10}H_{15}NO_3$
Metadrenaline
$C_{10}H_{15}NO_3,HCl$
Ethylnoradrenaline Hydrochloride
$C_{10}H_{15}NS$
Methylthioamfetamine
$C_{10}H_{15}N_2NaO_3$
Secbutabarbital Sodium
$C_{10}H_{15}N_3$
Bethanidine
$(C_{10}H_{15}N_3)_2,H_2SO_4$
Bethanidine Sulfate
$C_{10}H_{15}N_3O_5$
Benserazide
$C_{10}H_{15}N_3O_5,HCl$
Benserazide Hydrochloride
$C_{10}H_{15}N_5$
Phenformin
$C_{10}H_{15}N_5,HCl$
Phenformin Hydrochloride
$C_{10}H_{15}N_5O_3$
Penciclovir
$C_{10}H_{15}N_5O_3Na,H_2O$
Penciclovir Sodium
$C_{10}H_{15}O_3PS_2$
Fenthion
$C_{10}H_{16}Br_2N_2O_2$
Pipobroman
$C_{10}H_{16}ClNO$
Edrophonium Chloride
$C_{10}H_{16}NO_5PS_2$
Famphur
$C_{10}H_{16}N_2O$
Rilmenidene
$C_{10}H_{16}N_2O,½C_4H_4O_4$
Rilmenidene Fumarate
$C_{10}H_{16}N_2O,H_3PO_4$
Rilmenidene Phosphate
$C_{10}H_{16}N_2O_3$
Butobarbital
$C_{10}H_{16}N_2O_3$
Secbutabarbital
$C_{10}H_{16}N_2O_3S$
Amidefrine
$C_{10}H_{16}N_2O_3S,CH_3SO_3H$
Amidefrine Mesilate
$C_{10}H_{16}N_2O_4S_3$
Dorzolamide
$C_{10}H_{16}N_2O_4S_3,HCl$
Dorzolamide Hydrochloride
$C_{10}H_{16}N_5O_{13}P_3$
Adenosine Triphosphate
$C_{10}H_{16}N_6S$
Cimetidine
$C_{10}H_{16}O$
Camphor
$C_{10}H_{17}N$
Amantadine
$C_{10}H_{17}N,HCl$
Amantadine Hydrochloride
$C_{10}H_{17}N,½H_2SO_4$
Amantadine Sulfate
$C_{10}H_{17}NO_2$
Methyprylon
$C_{10}H_{17}N_3S$
Pramipexole
$C_{10}H_{17}N_3S,2HCl,H_2O$
Pramipexole Dihydrochloride Monohydrate
$[C_{10}H_{17}N_7O_4]^{2+}$
Saxitoxin
$C_{10}H_{18}O$
Cineole
$C_{10}H_{19}N_5S$
Prometryne
$C_{10}H_{19}N_5S$
Terbutryne
$C_{10}H_{19}O_6PS_2$
Malathion
$C_{10}H_{20}N_2O_4$
Mebutamate
$C_{10}H_{20}N_2S_3$
Sulfiram
$C_{10}H_{20}N_2S_4$
Disulfiram
$C_{10}H_{20}O$
Menthol
$C_{10}H_{21}N$
Pempidine
$C_{10}H_{21}N$
Propylhexedrine
$C_{10}H_{21}N,C_4H_6O_6$
Pempidine Tartrate
$C_{10}H_{21}N,HCl$
Propylhexedrine Hydrochloride
$C_{10}H_{21}NO_7$
Voglibose
$C_{10}H_{21}N_3O$
Diethylcarbamazine
$C_{10}H_{21}N_3O,C_6H_8O_7$
Diethylcarbamazine Citrate
$C_{10}H_{22}Cl_2N_2O_4,2HCl$
Mannomustine
$C_{10}H_{22}N_4$
Guanethidine
$C_{10}H_{22}N_4,H_2SO_4$
Guanethidine Monosulfate
$C_{10}H_{24}N_2O_2$
Ethambutol
$C_{10}H_{24}N_2O_2,2HCl$
Ethambutol Hydrochloride
$C_{11}H_6O_3$
Furanocoumarins
$C_{11}H_8N_2$
Norharman
$C_{11}H_8O_2$
Menadione
$C_{11}H_8O_2NaHSO_3,3H_2O$
Menadione Sodium Bisulfite
$C_{11}H_9Cl_4NO_2$
Cloponone
$C_{11}H_{10}FNO_2S$
Flosequinan
$C_{11}H_{10}N_2O_3$
Phenylmethylbarbituric Acid
$C_{11}H_{10}N_3NaO_2S,H_2O$
Sulfapyridine Sodium Monohydrate
$C_{11}H_{11}ClO_3$
Alclofenac
$C_{11}H_{11}Cl_2N_3O$
Muzolimine
$C_{11}H_{11}NO_2$
Phensuximide
$C_{11}H_{11}NO_2,HCl$
Hydrastinine Hydrochloride

$C_{11}H_{11}N_3O_2S$
Sulfapyridine
$C_{11}H_{11}N_4NaO_2S$
Sulfamerazine Sodium
$C_{11}H_{11}N_5$
Phenazopyridine
$C_{11}H_{11}N_5$,HCl
Phenazopyridine Hydrochloride
$C_{11}H_{12}ClNO_3S$
Chlormezanone
$C_{11}H_{12}Cl_2N_2O$
Lofexidine
$C_{11}H_{12}Cl_2N_2O$,HCl
Lofexidine Hydrochloride
$C_{11}H_{12}Cl_2N_2O_5$
Chloramphenicol
$C_{11}H_{12}N_2O$
Phenazone
$C_{11}H_{12}N_2O_2$
Ethotoin
$C_{11}H_{12}N_2O_2$
Thozalinone
$C_{11}H_{12}N_2O_2$
Tryptophan
$C_{11}H_{12}N_2S$
Levamisole
$C_{11}H_{12}N_2S$
Tetramisole
$C_{11}H_{12}N_2S$,HCl
Levamisole Hydrochloride
$C_{11}H_{12}N_2S$,HCl
Tetramisole Hydrochloride
$C_{11}H_{12}N_4NaO_3S$
Sulfamethoxypyridazine Sodium
$C_{11}H_{12}N_4O_2$
Panidazole
$C_{11}H_{12}N_4O_2S$
Sulfamerazine
$C_{11}H_{12}N_4O_3S$
Sulfamethoxypyridazine
$C_{11}H_{12}N_4O_3S$
Sulfametopyrazine
$C_{11}H_{12}N_4O_3S$
Sulfametoxydiazine
$C_{11}H_{12}O_3$
Myristicin
$C_{11}H_{13}ClF_3N_3O_4S_3$
Polythiazide
$C_{11}H_{13}F_3N_2$
Trifluoromethylphenylpiperazine
$C_{11}H_{13}F_3N_2$,HCl
Trifluoromethylphenylpiperazine Hydrochloride
$C_{11}H_{13}N$
Pargyline
$C_{11}H_{13}N$,HCl
Pargyline Hydrochloride
$C_{11}H_{13}NO_2$
Fenmetramide
$C_{11}H_{13}NO_3$
Hydrastinine
$C_{11}H_{13}NO_3$
Methylone
$C_{11}H_{13}NO_3$
Thurfyl Nicotinate
$C_{11}H_{13}NO_3$
Toloxatone
$C_{11}H_{13}N_3O_3S$
Sulfafurazole
$C_{11}H_{13}N_3O_3S$
Sulfamoxole
$C_{11}H_{13}N_3O_3S,C_4H_{11}NO_2$
Sulfafurazole Diethanolamine
$C_{11}H_{14}ClNO_2$
Buclosamide
$C_{11}H_{14}ClN_5$
Cycloguanil
$(C_{11}H_{14}ClN_5)_2,(C_{23}H_{16}O_6)$
Cycloguanil Embonate
$C_{11}H_{14}N_2$
5-Methyltryptamine
$C_{11}H_{14}N_2$
α-Methyltryptamine
$C_{11}H_{14}N_2$
N-Methyltryptamine
$C_{11}H_{14}N_2$,HCl
5-Methyltryptamine Hydrochloride
$C_{11}H_{14}N_2O$
Cytisine
$C_{11}H_{14}N_2O_2$
Pheneturide
$C_{11}H_{14}N_2O_4$
Felbamate
$C_{11}H_{14}N_2S$
Iminodimethylphenylthiazolidine
$C_{11}H_{14}N_2S$
Pyrantel
$C_{11}H_{14}N_2S,C_4H_6O_6$
Pyrantel Tartrate
$C_{11}H_{14}N_2S,C_{23}H_{16}O_6$
Pyrantel Embonate
$C_{11}H_{14}N_4O_4$
Doxofylline
$C_{11}H_{15}BrN_2O_3$
Butallylonal
$C_{11}H_{15}BrN_2O_3$
Narcobarbital
$C_{11}H_{15}ClO_2$
Phenaglycodol
$C_{11}H_{15}Cl_2N_5$
Chlorproguanil
$C_{11}H_{15}Cl_2N_5$,HCl
Chlorproguanil Hydrochloride
$C_{11}H_{15}N$
Aletamine
$C_{11}H_{15}N$,HCl
Aletamine Hydrochloride
$C_{11}H_{15}NO$
Phenmetrazine
$C_{11}H_{15}NO,C_7H_7ClN_4O_2$
Phenmetrazine Teoclate
$C_{11}H_{15}NO$,HC1
Phenmetrazine Hydrochloride
$C_{11}H_{15}NO_2$
Butyl Aminobenzoate
$C_{11}H_{15}NO_2$
Isobutyl Aminobenzoate
$C_{11}H_{15}NO_2$
Methylenedioxymetamfetamine
$(C_{11}H_{15}NO_2)_2,C_6H_3N_3O_7$
Butyl Aminobenzoate Picrate
$C_{11}H_{15}NO_2$,HCl
Methylenedioxymetamfetamine Hydrochloride
$C_{11}H_{15}NO_3$
Hydroxyphenamate
$C_{11}H_{15}NO_4$
Mephenesin Carbamate
$C_{11}H_{15}NO_4S$
Etebenecid
$C_{11}H_{15}NO_5$
Methocarbamol
$C_{11}H_{16}ClNO$
Clorprenaline
$C_{11}H_{16}ClNO$,HCl,H_2O
Clorprenaline Hydrochloride
$C_{11}H_{16}ClN_5$
Proguanil
$C_{11}H_{16}ClN_5$,HCl
Proguanil Hydrochloride
$C_{11}H_{16}N_2$
N-Benzylpiperazine
$C_{11}H_{16}N_2$,HCl
N-Benzylpiperazine Hydrochloride
$C_{11}H_{16}N_2O$
Tocainide
$C_{11}H_{16}N_2O$,HCl
Tocainide Hydrochloride
$C_{11}H_{16}N_2O_2$
Pilocarpine
$C_{11}H_{16}N_2O_2$,HCl
Pilocarpine Hydrochloride
$C_{11}H_{16}N_2O_2,HNO_3$
Pilocarpine Nitrate
$C_{11}H_{16}N_2O_3$
Butalbital
$C_{11}H_{16}N_2O_3$
Enallylpropymal
$C_{11}H_{16}N_2O_3$
Idobutal
$C_{11}H_{16}N_2O_3$
Talbutal
$C_{11}H_{16}N_2O_3$
Vinbarbital
$C_{11}H_{16}N_2O_3$
Vinylbital
$C_{11}H_{16}N_4O_4$
Dexrazoxane
$C_{11}H_{16}N_4O_4$
Pentostatin
$C_{11}H_{16}N_2$,2HCl
N-Benzylpiperazine Dihydrochloride
$C_{11}H_{16}O_2$
Butylated Hydroxyanisole
$C_{11}H_{17}BrN,C_7H_7O_3S$
Bretylium Tosilate
$C_{11}H_{17}ClNO_2$
DOC
$C_{11}H_{17}Cl_3N_2O_2S$
Clodantoin
$C_{11}H_{17}N$
Dimethylamfetamine
$C_{11}H_{17}N$
Mephentermine
$(C_{11}H_{17}N)_2,H_2SO_4,2H_2O$
Mephentermine Sulfate
$C_{11}H_{17}NO$
Methoxymetamfetamine
$C_{11}H_{17}NO$
Methoxyphenamine
$C_{11}H_{17}NO$
Methylephedrine
$C_{11}H_{17}NO$
Mexiletine
$C_{11}H_{17}NO$,HCl
Methoxyphenamine Hydrochloride
$C_{11}H_{17}NO$,HCl
Methylephedrine Hydrochloride
$C_{11}H_{17}NO$,HCl
Mexiletine Hydrochloride
$C_{11}H_{17}NO_3$
Isoprenaline
$C_{11}H_{17}NO_3$
Mescaline
$C_{11}H_{17}NO_3$
Methoxamine
$C_{11}H_{17}NO_3$
Orciprenaline
$(C_{11}H_{17}NO_3)_2,H_2SO_4$
Orciprenaline Sulfate
$(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$
Isoprenaline Sulfate
$(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$
Mescaline Sulfate Dihydrate
$C_{11}H_{17}NO_3$,HCl
Isoprenaline Hydrochloride
$C_{11}H_{17}NO_3$,HCl
Mescaline Hydrochloride
$C_{11}H_{17}NO_3$,HCl
Methoxamine Hydrochloride
$C_{11}H_{17}NO_3,H_2SO_4$
Mescaline Acid Sulfate
$C_{11}H_{17}N_2NaO_2S$
Thiopental Sodium
$C_{11}H_{17}N_2NaO_3$
Amobarbital Sodium
$C_{11}H_{17}N_2NaO_3$
Pentobarbital Sodium
$(C_{11}H_{17}N_2O_3)_2Ca$
Pentobarbital Calcium
$C_{11}H_{17}N_3O_3S$
Carbutamide
$C_{11}H_{17}N_3O_8$
Tetrodotoxin
$C_{11}H_{18}N_2O_2S$
Thiopental
$C_{11}H_{18}N_2O_3$
Amobarbital
$C_{11}H_{18}N_2O_3$
Pentobarbital
$C_{11}H_{19}N_5O_3$
Bufylline
$C_{11}H_{19}N_5S$
Irgarol

$C_{11}H_{21}N$
Mecamylamine
$C_{11}H_{21}N,HCl$
Mecamylamine Hydrochloride
$C_{11}H_{21}NO_8$
Choline Dihydrogen Citrate
$C_{11}H_{21}N_5OS$
Methoprotryne
$C_{11}H_{26}NO_2PS$
VX
$C_{12}H_6N_2O_2$
Phanquinone
$C_{12}H_7Br_4O_5P$
Bromofenofos
$C_{12}H_7Cl_3O_2$
Triclosan
$C_{12}H_7N_5O_9$
Nifursol
$C_{12}H_8Cl_2O_2S$
Fenticlor
$C_{12}H_8Cl_6$
Aldrin
$C_{12}H_8Cl_6O$
Dieldrin
$C_{12}H_8Cl_6O$
Endrin
$C_{12}H_8O_4$
Methoxsalen
$C_{12}H_9AsClN$
Adamsite
$C_{12}H_9F_3N_2O_2$
Leflunomide
$C_{12}H_9NS$
Phenothiazine
$C_{12}H_9N_3O$
Milrinone
$C_{12}H_9N_3O,C_3H_6O_3$
Milrinone Lactate
$C_{12}H_{10}$
Diphenyl
$C_{12}H_{10}N_2$
Harman
$C_{12}H_{11}ClN_2O_5S$
Furosemide
$C_{12}H_{11}ClO_4$
Losigamone
$C_{12}H_{11}NO_2$
Carbaryl
$C_{12}H_{11}N_2NaO_3$
Phenobarbital Sodium
$C_{12}H_{11}N_7$
Triamterene
$C_{12}H_{12}Br_2N_2$
Diquat Dibromide
$C_{12}H_{12}N_2$
Diquat
$C_{12}H_{12}N_2O_2S$
Dapsone
$C_{12}H_{12}N_2O_2S$
Enoximone
$C_{12}H_{12}N_2O_3$
Nalidixic Acid
$C_{12}H_{12}N_2O_3$
Phenobarbital
$C_{12}H_{13}ClN_4$
Pyrimethamine
$C_{12}H_{13}NO_2$
Mesuximide
$C_{12}H_{13}NO_3$
Aniracetam
$C_{12}H_{13}N_3O_2$
Isocarboxazid
$C_{12}H_{13}N_4NaO_2S$
Sulfadimidine Sodium
$C_{12}H_{14}ClNO_3,2H_2O$
Cotarnine Chloride
$C_{12}H_{14}Cl_2N_2$
Paraquat Dichloride
$C_{12}H_{14}N_2$
Paraquat
$C_{12}H_{14}N_2O_2$
Carphedon
$C_{12}H_{14}N_2O_2$
Mephenytoin
$C_{12}H_{14}N_2O_2$
Primidone
$C_{12}H_{14}N_2O_3$
Cyclopentobarbital
$C_{12}H_{14}N_4O_2S$
Sulfadimidine
$C_{12}H_{14}N_4O_2S$
Sulfisomidine
$C_{12}H_{14}N_4O_4S$
Sulfadimethoxine
$C_{12}H_{14}N_4O_4S$
Sulfadoxine
$C_{12}H_{14}O_4$
Diethyl Phthalate
$C_{12}H_{15}ClO_3$
Clofibrate
$C_{12}H_{15}NO_3$
Butylone
$C_{12}H_{15}NO_3$
Ethylone
$C_{12}H_{15}NO_3$
Metaxalone
$C_{12}H_{15}NO_4$
Cotarnine
$C_{12}H_{15}N_2NaO_3$
Hexobarbital Sodium
$(C_{12}H_{15}N_2O_3)_2Ca$
Cyclobarbital Calcium
$C_{12}H_{15}N_3O_2S$
Albendazole
$C_{12}H_{16}ClNO_3$
Meclofenoxate
$C_{12}H_{16}ClNO_3,HCl$
Meclofenoxate Hydrochloride
$C_{12}H_{16}F_3N$
Fenfluramine
$C_{12}H_{16}F_3N,HCl$
Fenfluramine Hydrochloride
$C_{12}H_{16}N_2$
Dimethyltryptamine
$C_{12}H_{16}N_2$
Fenproporex
$C_{12}H_{16}N_2O$
Bufotenine
$C_{12}H_{16}N_2O$
Psilocin
$C_{12}H_{16}N_2O_3$
Cyclobarbital
$C_{12}H_{16}N_2O_3$
Hexobarbital
$C_{12}H_{16}N_2S$
Morantel
$C_{12}H_{16}N_2S$
Xylazine
$C_{12}H_{16}N_2S,C_4H_6O_6$
Morantel Tartrate
$C_{12}H_{16}N_2S,C_6H_8O_7,H_2O$
Morantel Citrate
$C_{12}H_{16}N_2S,HCl$
Xylazine Hydrochloride
$C_{12}H_{16}N_3O_3PS$
Triazophos
$C_{12}H_{16}N_3O_3PS_2$
Azinphos-(Et)
$C_{12}H_{17}ClN_4OS$
Thiamine
$C_{12}H_{17}ClN_4OS,HCl$
Thiamine Hydrochloride
$C_{12}H_{17}NO$
Diethyltoluamide
$C_{12}H_{17}NO$
Phendimetrazine
$C_{12}H_{17}NO,C_4H_6O_6$
Phendimetrazine Tartrate
$C_{12}H_{17}NOS$
Tiletamine
$C_{12}H_{17}NO_2$
Hexyl Nicotinate
$C_{12}H_{17}NO_3$
Bufexamac
$C_{12}H_{17}NO_3$
Butoxyethyl Nicotinate
$C_{12}H_{17}NO_3$
Etamivan
$C_{12}H_{17}NO_3$
Norbudrine
$C_{12}H_{17}NO_3$
Phenisonone
$C_{12}H_{17}NO_3$
Rimiterol
$C_{12}H_{17}NO_3,HBr$
Phenisonone Hydrobromide
$C_{12}H_{17}NO_3,HBr$
Rimiterol Hydrobromide
$C_{12}H_{17}NO_3,HCl$
Norbudrine Hydrochloride
$C_{12}H_{17}NO_4$
Methyldopate
$C_{12}H_{17}NO_4,HCl$
Methyldopate Hydrochloride
$C_{12}H_{17}N_2NaO_2S$
Sodium Thiamylal
$C_{12}H_{17}N_2NaO_3$
Secobarbital Sodium
$C_{12}H_{17}N_2NaO_3S$
Tolbutamide Sodium
$C_{12}H_{17}N_2O_4P$
Psilocybine
$C_{12}H_{17}N_5O_4S$
Thiamine Mononitrate
$C_{12}H_{18}C_{12}N_2O$
Clenbuterol
$C_{12}H_{18}N_2O$
Isoproturon
$C_{12}H_{18}N_2O_2$
Nicametate
$C_{12}H_{18}N_2O_2,C_6H_8O_7,H_2O$
Nicametate Citrate
$C_{12}H_{18}N_2O_2S$
Thiamylal
$C_{12}H_{18}N_2O_3$
Nealbarbital
$C_{12}H_{18}N_2O_3$
Secobarbital
$C_{12}H_{18}N_2O_3S$
Tolbutamide
$C_{12}H_{18}O$
Amylmetacresol
$C_{12}H_{18}O$
Propofol
$C_{12}H_{18}O_2$
Hexylresorcinol
$C_{12}H_{19}BrN_2O_2$
Neostigmine Bromide
$C_{12}H_{19}NO$
Etafedrine
$C_{12}H_{19}NO,HCl$
Etafedrine Hydrochloride
$C_{12}H_{19}NO_2$
Bamethan
$C_{12}H_{19}NO_2$
DOM
$(C_{12}H_{19}NO_2)_2,H_2SO_4$
Bamethan Sulfate
$C_{12}H_{19}NO_2,HCl$
DOM Hydrochloride
$C_{12}H_{19}NO_2S$
2C-T-2
$C_{12}H_{19}NO_2S,HCl$
2C-T-2 Hydrochloride
$C_{12}H_{19}NO_3$
N-Methylmescaline
$C_{12}H_{19}NO_3$
Prenalterol
$C_{12}H_{19}NO_3$
Terbutaline
$C_{12}H_{19}NO_3$
Trimethoxyamfetamine
$(C_{12}H_{19}NO_3)_2,H_2SO_4$
Terbutaline Sulfate
$C_{12}H_{19}NO_3,HCl$
Prenalterol Hydrochloride
$C_{12}H_{19}NO_4$
Choline Salicylate
$C_{12}H_{19}N_2NaO_3$
Hexethal Sodium
$C_{12}H_{19}N_3O$
Procarbazine

$C_{12}H_{19}N_3O$,HCl
Procarbazine Hydrochloride
$C_{12}H_{20}N_2O_3$
Hexethal
$C_{12}H_{20}N_2O_3S$
Sotalol
$C_{12}H_{20}N_2O_3S$,HCl
Sotalol Hydrochloride
$C_{12}H_{20}N_4O_7$
Zanamivir
$C_{12}H_{21}N$
Memantine
$C_{12}H_{21}N$
Rimantadine
$C_{12}H_{21}N$,HCl
Memantine Hydrochloride
$C_{12}H_{21}N$,HCl
Rimantadine Hydrochloride
$C_{12}H_{21}NO_8S$
Topiramate
$C_{12}H_{21}N_2O_3PS$
Dimpylate
$C_{12}H_{21}N_5O_2S_2$
Nizatidine
$C_{12}H_{21}N_5O_3$
Cadralazine
$C_{12}H_{21}N_5O_3$
Choline Theophyllinate
$C_{12}H_{22}N_2O_2$
Crotetamide
$C_{12}H_{24}CaN_2O_6S_2,2H_2O$
Calcium Cyclamate
$C_{12}H_{24}N_2O_4$
Carisoprodol
$C_{12}H_{26}O_6P_2S_4$
Dioxathion
$C_{12}H_{27}O_4P$
Tributyl Phosphate
$C_{12}H_{30}Br_2N_2$
Hexamethonium Bromide
$C_{12}H_{30}I_2N_2$
Hexamethonium Iodide
$C_{12}H_{32}NO_5PS_3$
Glyphosate Trimesium
$C_{13}H_6Cl_5NO_3$
Oxyclozanide
$C_{13}H_6Cl_6O_2$
Hexachlorophene
$C_{13}H_8Cl_2N_2O_4$
Niclosamide
$C_{13}H_8Cl_2O_4S$
Tienilic Acid
$C_{13}H_8F_2O_3$
Diflunisal
$C_{13}H_9Cl_3N_2O$
Triclocarban
$C_{13}H_9F_3N_2O_2$
Niflumic Acid
$C_{13}H_9ON$
CR Gas
$C_{13}H_{10}BrNO_3$
Resorantel
$C_{13}H_{10}ClN_3O_4S_2$
Lornoxicam
$C_{13}H_{10}Cl_2O_2$
Dichlorophen
$C_{13}H_{10}N_2$
Aminoacridine
$C_{13}H_{10}N_2,C_{12}H_{18}O_2$
Aminoacridine Hexylresorcinate
$C_{13}H_{10}N_2$,HCl,H_2O
Aminoacridine Hydrochloride
$C_{13}H_{10}N_2O_4$
Thalidomide
$C_{13}H_{10}O_3$
Salol
$C_{13}H_{10}O_7$
Exifone
$C_{13}H_{11}ClN_2O_2$
Clonixin
$C_{13}H_{11}Cl_2NaO_4$
Etacrynate Sodium
$C_{13}H_{11}NO_2$
Benzyl Nicotinate
$C_{13}H_{11}NO_3$
Phenyl Aminosalicylate
$(C_{13}H_{11}N_3)_2,H_2SO_4,2H_2O$
Proflavine Hemisulfate
$C_{13}H_{11}N_3O_2$
Salinazid
$C_{13}H_{11}N_3O_4S_2$
Tenoxicam
$C_{13}H_{12}Cl_2O_4$
Etacrynic Acid
$C_{13}H_{12}F_2N_6O$
Fluconazole
$C_{13}H_{12}N_2O$
Harmine
$C_{13}H_{13}N_3O_5S_2,H_2O$
Succinylsulfathiazole
$C_{13}H_{14}Cl_2O_3$
Ciprofibrate
$C_{13}H_{14}N_2$
Tacrine
$C_{13}H_{14}N_2$,HCl
Tacrine Hydrochloride
$C_{13}H_{14}N_2O$
Fenyramidol
$C_{13}H_{14}N_2O$
Harmaline
$C_{13}H_{14}N_2O$,HCl
Fenyramidol Hydrochloride
$C_{13}H_{14}N_2O_2$
Metomidate
$C_{13}H_{14}N_2O_2$,HCl
Metomidate Hydrochloride
$C_{13}H_{14}N_2O_3$
Methylphenobarbital
$C_{13}H_{14}N_3NaO_4S$
Glymidine Sodium
$C_{13}H_{14}N_4O_4S$
Acetyl Sulfamethoxypyridazine
$C_{13}H_{14}O_5$
Citrinin
$C_{13}H_{15}Cl_2NO$
Clorgiline
$C_{13}H_{15}Cl_2NO$,HCl
Clorgiline Hydrochloride
$C_{13}H_{15}NO_2$
Fenimide
$C_{13}H_{15}NO_2$
Glutethimide
$C_{13}H_{15}N_2NaO_2S$
Thialbarbital Sodium
$C_{13}H_{15}N_3O_4S$
Acetyl Sulfafurazole
$C_{13}H_{15}N_5Na_2O_8S_2$
Aztreonam Disodium
$C_{13}H_{16}ClNO$
Ketamine
$C_{13}H_{16}ClNO$,HCl
Ketamine Hydrochloride
$C_{13}H_{16}HgNNaO_6$
Mersalyl Sodium
$C_{13}H_{16}N_2$
Dexmedetomidine
$C_{13}H_{16}N_2$
Pethidine Intermediate A
$C_{13}H_{16}N_2$
Tetryzoline
$C_{13}H_{16}N_2$,HCl
Tetryzoline Hydrochloride
$C_{13}H_{16}N_2O_2$
Aminoglutethimide
$C_{13}H_{16}N_2O_2S$
Thialbarbital
$C_{13}H_{16}N_3NaO_4S,H_2O$
Dipyrone
$C_{13}H_{16}N_4O_2$
Diaveridine
$C_{13}H_{16}N_4O_6$
Furaltadone
$C_{13}H_{17}ClN_2O_2$
Moclobemide
$C_{13}H_{17}ClN_2O_2$,HCl
Moclobemide Hydrochloride
$C_{13}H_{17}HgNO_6$
Mersalyl Acid
$C_{13}H_{17}N$
Selegiline
$C_{13}H_{17}N$,HCl
Selegiline Hydrochloride
$C_{13}H_{17}NO$
Crotamiton
$C_{13}H_{17}NO_2$
Alminoprofen
$C_{13}H_{17}N_3$
Tramazoline
$C_{13}H_{17}N_3$,HCl
Tramazoline Hydrochloride
$C_{13}H_{17}N_3O$
Aminophenazone
$C_{13}H_{17}N_3O_2$
Parbendazole
$C_{13}H_{17}N_3O_3$
Dioxyamidopyrine
$C_{13}H_{17}N_5O_8S_2$
Aztreonam
$C_{13}H_{18}ClF_3N_2O$
Mabuterol
$C_{13}H_{18}ClF_3N_2O$,HCl
dl-Mabuterol Hydrochloride
$C_{13}H_{18}ClNO$
Bupropion
$C_{13}H_{18}ClN_3O_4S_2$
Cyclopenthiazide
$C_{13}H_{18}Cl_2N_2O_2$
Melphalan
$C_{13}H_{18}N_2O$
5-Methoxy-*N*,*N*-dimethyltryptamine
$C_{13}H_{18}N_2O_3$
Heptabarb
$C_{13}H_{18}N_2O_6$
Metadoxine
$C_{13}H_{18}N_4O_3$
Pentoxifylline
$C_{13}H_{18}O_2$
Ibuprofen
$C_{13}H_{19}ClN_2O$
Butanilicaine
$C_{13}H_{19}ClN_2O,H_3PO_4$
Butanilicaine Phosphate
$C_{13}H_{19}ClN_2O_2$
Chloroprocaine
$C_{13}H_{19}ClN_2O_2$,HCl
Chloroprocaine Hydrochloride
$C_{13}H_{19}ClN_2O_5S_2$
Mefruside
$C_{13}H_{19}Cl_2NO$
Bupropion Hydrochloride
$C_{13}H_{19}NO$
Diethylpropion
$C_{13}H_{19}NO$,HCl
Diethylpropion Hydrochloride
$C_{13}H_{19}NO_3$
Viloxazine
$C_{13}H_{19}NO_3$,HCl
Viloxazine Hydrochloride
$C_{13}H_{19}NO_4$
N-Acetylmescaline
$C_{13}H_{19}NO_4S$
Probenecid
$C_{13}H_{19}N_5$
Pinacidil
$C_{13}H_{19}N_5,H_2O$
Pinacidil Monohydrate
$C_{13}H_{20}N_2O$
Prilocaine
$C_{13}H_{20}N_2O$,HCl
Prilocaine Hydrochloride
$C_{13}H_{20}N_2O_2$
Butethamine
$C_{13}H_{20}N_2O_2$
Dropropizine
$C_{13}H_{20}N_2O_2$
Levodropropizine
$C_{13}H_{20}N_2O_2$
Metabutethamine
$C_{13}H_{20}N_2O_2$
Procaine
$C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$
Procaine Benzylpenicillin

$C_{13}H_{20}N_2O_2$,HCl
Metabutethamine Hydrochloride
$C_{13}H_{20}N_2O_2$,HCl
Procaine Hydrochloride
$C_{13}H_{20}N_4O_2$
Pentifylline
$C_{13}H_{20}N_6O_4$
Valaciclovir
$C_{13}H_{21}ClN_6O_4$
Valaciclovir Hydrochloride
$C_{13}H_{21}NO_2$
Tigloidine
$C_{13}H_{21}NO_2$,HBr
Tigloidine Hydrobromide
$C_{13}H_{21}NO_2S$
2C-T-7
$C_{13}H_{21}NO_2S$,HCl
2C-T-7 Hydrochloride
$C_{13}H_{21}NO_3$
Isoetarine
$C_{13}H_{21}NO_3$
Salbutamol
$C_{13}H_{21}NO_3$,CH_4O_3S
Isoetarine Mesilate
$C_{13}H_{21}NO_3$,HCl
Isoetarine Hydrochloride
$C_{13}H_{21}NO_3$,$^1/_2H_2SO_4$
Salbutamol Sulfate
$C_{13}H_{21}N_3O$
Procainamide
$C_{13}H_{21}N_3O$,HCl
Procainamide Hydrochloride
$C_{13}H_{21}N_5O_2$
Etamiphylline
$C_{13}H_{21}N_5O_4$,$C_6H_5NO_2$
Xantinol Nicotinate
$C_{13}H_{22}NO_3PS$
Fenamiphos
$C_{13}H_{22}N_2O_6S$
Neostigmine Methylsulfate
$C_{13}H_{22}N_4O_3S$
Ranitidine
$C_{13}H_{22}N_4O_3S.C_6H_5BiO_7$
Ranitidine Bismuth Citrate
$C_{13}H_{22}N_4O_3S$,HCl
Ranitidine Hydrochloride
$C_{13}H_{24}N_2O_2$
Cropropamide
$C_{13}H_{24}N_4O_3S$
Timolol
$C_{13}H_{24}N_4O_3S$,$C_4H_4O_4$
Timolol Maleate
$C_{13}H_{26}N_2O_4$
Tybamate
$C_{13}H_{29}N$
Octamylamine
$C_{13}H_{29}N$,HCl
Octamylamine Hydrochloride
$C_{13}H_{33}Br_2N_3$
Azamethonium Bromide
$C_{14}H_8CaN_2O_6S_2$,$3^1/_2H_2O$
Saccharin Calcium
$C_{14}H_8N_2Na_2O_6$
Olsalazine Sodium
$C_{14}H_8O_4$
Dantron
$C_{14}H_9ClF_3NO_2$
Efavirenz
$C_{14}H_9Cl_5$
Clofenotane
$C_{14}H_9N_4NaO_5$,$3^1/_2H_2O$
Dantrolene Sodium
$C_{14}H_{10}BrN_3O$
Bromazepam
$C_{14}H_{10}Cl_2NNaO_2$
Diclofenac Sodium
$C_{14}H_{10}Cl_2NNaO_2$
Meclofenamate Sodium
$C_{14}H_{10}Cl_2O_3$
Fenclofenac
$C_{14}H_{10}F_3NO_2$
Flufenamic Acid
$C_{14}H_{10}N_2O_6$
Olsalazine
$C_{14}H_{10}N_4O_5$
Dantrolene
$C_{14}H_{10}O_3$
Dithranol
$C_{14}H_{10}O_5$
Salsalate
$C_{14}H_{11}ClN_2O_4S$
Chlortalidone
$C_{14}H_{11}Cl_2NO_2$
Diclofenac
$C_{14}H_{11}Cl_2NO_2$
Meclofenamic Acid
$C_{14}H_{11}Cl_2NO_4$
Diloxanide Furoate
$C_{14}H_{11}F_3N_2O_2$
Flunixin
$C_{14}H_{11}F_3N_2O_2$,$C_7H_{17}NO_5$
Flunixin Meglumine
$C_{14}H_{11}N_4O_2S$,Na
Sulfaquinoxaline Sodium
$C_{14}H_{12}ClNO_2$
Cicletanine
$C_{14}H_{12}ClNO_2$
Tolfenamic Acid
$C_{14}H_{12}ClNO_2$,HCl
Cicletanine Hydrochloride
$C_{14}H_{12}N_4O_2S$
Sulfaquinoxaline
$C_{14}H_{12}N_6O$
Levosimendan
$C_{14}H_{12}O_2$
Benzyl Benzoate
$C_{14}H_{12}O_3$
Trioxysalen
$C_{14}H_{12}O_3S$
Suprofen
$C_{14}H_{12}O_3S$
Tiaprofenic Acid
$C_{14}H_{13}NaO_3$
Naproxen Sodium
$C_{14}H_{13}N_3O_3$
Phthivazid
$C_{14}H_{13}N_3O_4S_2$
Meloxicam
$C_{14}H_{13}N_5O_5S_2$
Cefdinir
$C_{14}H_{14}ClNS$
Ticlopidine
$C_{14}H_{14}ClNS$,HCl
Ticlopidine Hydrochloride
$C_{14}H_{14}Cl_3O_6P$
Haloxon
$C_{14}H_{14}N_2$
Naphazoline
$C_{14}H_{14}N_2$,HCl
Naphazoline Hydrochloride
$C_{14}H_{14}N_2$,HNO_3
Naphazoline Nitrate
$C_{14}H_{14}N_2O$
Metyrapone
$C_{14}H_{14}N_4O$
Phenamidine
$C_{14}H_{14}N_4O$,$2C_2H_6O_4S$
Phenamidine Isethionate
$C_{14}H_{14}N_4O_2S$
Cambendazole
$C_{14}H_{14}O_3$
Naproxen
$C_{14}H_{15}N_3O_5$
Entacapone
$C_{14}H_{15}N_7$
Diminazene
$C_{14}H_{16}ClN_3O_4S_2$
Cyclothiazide
$C_{14}H_{16}ClO_5PS$
Coumaphos
$C_{14}H_{16}Cl_2N_4O_3$
Obidoxime Chloride
$C_{14}H_{16}N_2O_2$
Etomidate
$C_{14}H_{16}N_2O_2$
Rolicyprine
$C_{14}H_{16}N_4O$
Etoxazene
$C_{14}H_{16}N_4O$,HCl
Etoxazene Hydrochloride
$C_{14}H_{17}ClN_2O_3S$
Clorexolone
$C_{14}H_{17}Cl_2N_3O$
Hexaconazole
$C_{14}H_{17}NO_2$
Indeloxazine
$C_{14}H_{17}NO_2$,HCl
Indeloxazine Hydrochloride
$C_{14}H_{17}NS_2$
Dimethylthiambutene
$C_{14}H_{17}NS_2$,HCl
Dimethylthiambutene Hydrochloride
$C_{14}H_{17}N_2NaO_3$
Methohexital Sodium
$C_{14}H_{17}N_3O_2S$
Fasudil
$C_{14}H_{17}N_3O_2S$,HCl
Fasudil Hydrochloride
$C_{14}H_{18}ClN_3S$
Chloropyrilene
$C_{14}H_{18}ClN_3S$,$C_6H_8O_7$
Chloropyrilene Citrate
$C_{14}H_{18}N_2O$
Propyphenazone
$C_{14}H_{18}N_2O_3$
Methohexital
$C_{14}H_{18}N_4O_3$
Trimethoprim
$C_{14}H_{18}N_6O$
Abacavir
$(C_{14}H_{18}N_6O)_2$,H_2SO_4
Abacavir Sulfate
$C_{14}H_{19}ClN_4$,HCl
Amprolium Hydrochloride
$C_{14}H_{19}Cl_2NO_2$
Chlorambucil
$C_{14}H_{19}H_5O_4$
Famciclovir
$C_{14}H_{19}NO_2$
Methylphenidate
$C_{14}H_{19}NO_2$
Piperoxan
$C_{14}H_{19}NO_2$,HCl
Methylphenidate Hydrochloride
$C_{14}H_{19}NO_2$,HCl
Piperoxan Hydrochloride (*dl*-Form)
$C_{14}H_{19}NO_5$
Trimetozine
$C_{14}H_{19}NaO_2$
Butibufen Sodium
$C_{14}H_{19}N_3O$
Isopropylaminophenazone
$C_{14}H_{19}N_3S$
Methapyrilene
$C_{14}H_{19}N_3S$
Thenyldiamine
$(C_{14}H_{19}N_3S)_2$,$3C_4H_4O_4$
Methapyrilene Fumarate
$C_{14}H_{19}N_3S$,HCl
Methapyrilene Hydrochloride
$C_{14}H_{19}N_3S$,HCl
Thenyldiamine Hydrochloride
$C_{14}H_{20}Br_2N_2$
Bromhexine
$C_{14}H_{20}Br_2N_2$,HCl
Bromhexine Hydrochloride
$C_{14}H_{20}ClNO_2$
Alachlor
$C_{14}H_{20}ClN_3O_3S$
Clopamide
$C_{14}H_{20}C_{l6}N_2$
Chlorisondamine Chloride
$C_{14}H_{20}N_2$
Diethyltryptamine
$C_{14}H_{20}N_{2O}$
Pyrrocaine
$C_{14}H_20N_2O$
Tymazoline
$C_{14}H_{20}N_2O$,HCl
Tymazoline Hydrochloride
$C_{14}H_{20}N_2O_2$
Bunitrolol

$C_{14}H_{20}N_2O_2$
Pindolol
$C_{14}H_{20}N_2O_2$,HCl
Bunitrolol Hydrochloride
$C_{14}H_{20}N_3O_5PS$
Pyrazophos
$C_{14}H_{20}N_4O$
Imolamine
$C_{14}H_{20}N_4O$,HCl
Imolamine Hydrochloride
$C_{14}H_{20}O_2$
Butibufen
$C_{14}H_{21}NO$
Profadol
$C_{14}H_{21}NO$,HCl
Profadol Hydrochloride
$C_{14}H_{21}NO_2$
Amylocaine
$C_{14}H_{21}NO_2$
Meprylcaine
$C_{14}H_{21}NO_2$
Padimate
$C_{14}H_{21}NO_2$,HCl
Amylocaine Hydrochloride
$C_{14}H_{21}NO_2$,HCl
Meprylcaine Hydrochloride
$C_{14}H_{21}N_3O_2S$
Sumatriptan
$C_{14}H_{21}N_3O_2S$,$C_4H_6O_4$
Sumatriptan Succinate
$C_{14}H_{21}N_3O_3S$
Tolazamide
$C_{14}H_{22}ClN_3O_2$
Metoclopramide
$C_{14}H_{22}ClN_3O_2$,HCl,H_2O
Metoclopramide Hydrochloride
$C_{14}H_{22}Cl_2N_2O_6S_4$
Clomethiazole Edisilate
$C_{14}H_{22}N_2O$
Lidocaine
$C_{14}H_{22}N_2O$
Octacaine
$C_{14}H_{22}N_2O$,HCl,H_2O
Lidocaine Hydrochloride
$C_{14}H_{22}N_2O_2$
Naepaine
$C_{14}H_{22}N_2O_2$
Rivastigmine
$C_{14}H_{22}N_2O_2$, $C_4H_6O_6$
Rivastigmine Hydrogen Tartrate
$C_{14}H_{22}N_2O_2$,HCl
Naepaine Hydrochloride
$C_{14}H_{22}N_2O_3$
Atenolol
$C_{14}H_{22}N_2O_3$
Practolol
$C_{14}H_{22}N_2O_3$
Trimetazidine
$C_{14}H_{22}N_2O_3$,2HCl
Trimetazidine Hydrochloride
$C_{14}H_{27}N_3O_2$
Pramiracetam
$C_{14}H_{27}N_3O_2$,H_2SO_4
Pramiracetam Sulfate
$C_{14}H_{30}Br_2N_2O_4$,$2H_2O$
Suxamethonium Bromide
$C_{14}H_{30}Cl_2N_2O_4$,$2H_2O$
Suxamethonium Chloride
$C_{14}H_{30}I_2N_2O_4$
Suxamethonium Iodide
$C_{15}H_8O_6$
Rhein
$C_{15}H_{10}BrClN_4S$
Brotizolam
$C_{15}H_{10}ClF_3N_2O_3$
Triflumuron
$C_{15}H_{10}ClI_2NO_3$
Clioxanide
$C_{15}H_{10}ClN_3O_3$
Clonazepam
$C_{15}H_{10}Cl_2N_2O_2$
Lorazepam
$C_{15}H_{10}I_4NNaO_4$,xH_2O
Levothyroxine Sodium
$C_{15}H_{10}O_2$
Phenindione
$C_{15}H_{11}BrNNaO_3$,$1\frac{1}{2}H_2O$
Bromfenac Sodium
$C_{15}H_{11}ClN_2O$
Mecloqualone
$C_{15}H_{11}ClN_2O$
Nordazepam
$C_{15}H_{11}ClN_2O_2$
Demoxepam
$C_{15}H_{11}ClN_2O_2$
Oxazepam
$C_{15}H_{11}I_3NNaO_4$
Liothyronine Sodium
$C_{15}H_{11}I_4NO_4$
Levothyroxine
$C_{15}H_{11}N_2NaO_2$
Phenytoin Sodium
$C_{15}H_{11}N_3O_3$
Nitrazepam
$C_{15}H_{12}BrNO_3$
Bromfenac
$C_{15}H_{12}I_3NO_4$
Liothyronine
$C_{15}H_{12}I_3NO_4$,HCl
Liothyronine Hydrochloride
$C_{15}H_{12}N_2O$
Carbamazepine
$C_{15}H_{12}N_2O_2$
Oxcarbazepine
$C_{15}H_{12}N_2O_2$
Phenytoin
$C_{15}H_{13}ClNNaO_3$,$2H_2O$
Zomepirac Sodium
$C_{15}H_{13}FO_2$
Flurbiprofen
$C_{15}H_{13}NO_3$
Ketorolac
$C_{15}H_{13}NO_3S_2$
Epalrestat
$C_{15}H_{13}N_3O_2S$
Fenbendazole
$C_{15}H_{13}N_3O_4S$
Piroxicam
$(C_{15}H_{13}O_3)_2Ca$,$2H_2O$
Fenoprofen Calcium
$C_{15}H_{14}ClNO_3$
Zomepirac
$C_{15}H_{14}ClN_3O_4S$,H_2O
Cefaclor
$C_{15}H_{14}ClN_3O_4S_3$
Benzthiazide
$C_{15}H_{14}FN_3O_3$
Flumazenil
$C_{15}H_{14}F_3N_3O_4S_2$
Bendroflumethiazide
$C_{15}H_{14}NNaO_3$,$2H_2O$
Tolmetin Sodium
$C_{15}H_{14}N_4O$
Nevirapine
$C_{15}H_{14}N_4O_2S$
Sulfaphenazole
$C_{15}H_{14}O_3$
Fenoprofen
$C_{15}H_{14}O_3$
Mexenone
$C_{15}H_{14}O_4$
Acetomenaphthone
$C_{15}H_{15}ClN_2O_4S$
Xipamide
$C_{15}H_{15}Cl_2N_2NaO_8$
Chloramphenicol Sodium Succinate
$C_{15}H_{15}NO_2$
Mefenamic Acid
$C_{15}H_{15}NO_2S$
Modafinil
$C_{15}H_{15}NO_3$
Tolmetin
$C_{15}H_{15}NO_3S$
Adrafinil
$C_{15}H_{15}N_3O$
Ethacridine
$C_{15}H_{15}N_3O$,$C_3H_6O_3$
Ethacridine Lactate
$C_{15}H_{16}N_2O$,$2H_3PO_4$
Phenatine
$C_{15}H_{16}N_3S$,Cl
Tolonium Chloride
$C_{15}H_{16}N_6O$
Amicarbalide
$C_{15}H_{16}O_2$
Nabumetone
$C_{15}H_{17}FN_4O_2$
Flupirtine
$C_{15}H_{17}FN_4O_2$,$C_4H_4O_4$
Flupirtine Maleate
$C_{15}H_{17}FN_4O_2$,HCl
Flupirtine Hydrochloride
$C_{15}H_{17}FN_4O_3$
Enoxacin
$C_{15}H_{17}FN_4O_3$,$^3/_2H_2O$
Enoxacin Sesquihydrate
$C_{15}H_{17}N$
Benethamine
$C_{15}H_{17}NaO_3$,$2H_2O$
Sodium Loxoprofen
$C_{15}H_{17}N_3O$
Cetoxime
$C_{15}H_{18}Cl_6N_2O_5$
Dichloralphenazone
$C_{15}H_{18}O_3$
Loxoprofen
$C_{15}H_{18}O_3$
Santonin
$C_{15}H_{19}NO_2$
Tropacocaine
$C_{15}H_{19}NO_2$,HCl
Tropacocaine Hydrochloride
$C_{15}H_{19}NS_2$
Ethylmethylthiambutene
$C_{15}H_{19}NS_2$,HCl
Ethylmethylthiambutene Hydrochloride
$C_{15}H_{19}N_5$
Rizatriptan
$C_{15}H_{19}N_5$,C_6H_5COOH
Rizatriptan Benzoate
$C_{15}H_{20}N_2O_4S$
Acetohexamide
$C_{15}H_{20}N_2S$
Methaphenilene
$C_{15}H_{20}N_2S$,HCl
Methaphenilene Hydrochloride
$C_{15}H_{20}O_6$
Deoxynivalenol
$C_{15}H_{21}F_3N_2O_2$
Fluvoxamine
$C_{15}H_{21}F_3N_2O_2$,$C_4H_4O_4$
Fluvoxamine Maleate
$C_{15}H_{21}N$
Fencamfamin
$C_{15}H_{21}N$,HCl
Fencamfamin Hydrochloride
$C_{15}H_{21}NO$
Eptazocine
$C_{15}H_{21}NO$
Metazocine
$C_{15}H_{21}NO_2$
Benzamine
$C_{15}H_{21}NO_2$
Ketobemidone
$C_{15}H_{21}NO_2$
Pethidine
$C_{15}H_{21}NO_2$,HCl
Ketobemidone Hydrochloride
$C_{15}H_{21}NO_2$,HCl
Pethidine Hydrochloride
$C_{15}H_{21}NO_3$
Hydroxypethidine
$C_{15}H_{21}NO_6$
Domoic Acid
$C_{15}H_{21}N_3O$
Primaquine
$C_{15}H_{21}N_3O_2$
Physostigmine
$(C_{15}H_{21}N_3O_2)_2$,H_2SO_4
Physostigmine Sulfate
$C_{15}H_{21}N_3O_2$,$C_7H_6O_3$
Physostigmine Salicylate

$C_{15}H_{21}N_3O,2H_3PO_4$
Primaquine Phosphate
$C_{15}H_{21}N_3O_3$
Physostigmine Aminoxide
$C_{15}H_{21}N_3O_3,C_7H_6O_3$
Physostigmine Aminoxide Salicylate
$C_{15}H_{21}N_3O_3S$
Gliclazide
$C_{15}H_{22}BrNO$
Eptazocine Hydrobromide
$C_{15}H_{22}ClNO_2$
Metolachlor
$C_{15}H_{22}FN_3O_6$
Capecitabine
$C_{15}H_{22}N_2O$
Mepivacaine
$C_{15}H_{22}N_2O$
Milnacipran
$C_{15}H_{22}N_2O,HCl$
Mepivacaine Hydrochloride
$C_{15}H_{22}N_2O,HCl$
Milnacipran Hydrochloride
$C_{15}H_{22}N_2O_3$
Tolycaine
$C_{15}H_{22}N_2O_3,HCl$
Tolycaine Hydrochloride
$C_{15}H_{22}N_4O_3$
Propentofylline
$C_{15}H_{22}O_5$
Artemisinin
$C_{15}H_{23}N$
Prolintane
$C_{15}H_{23}N,HCl$
Prolintane Hydrochloride
$C_{15}H_{23}NO$
Meptazinol
$C_{15}H_{23}NO,HCl$
Meptazinol Hydrochloride
$C_{15}H_{23}NO_2$
Alprenolol
$C_{15}H_{23}NO_2,HCl$
Alprenolol Hydrochloride
$C_{15}H_{23}NO_3$
Ethomoxane
$C_{15}H_{23}NO_3$
Oxprenolol
$C_{15}H_{23}NO_3,HCl$
Ethomoxane Hydrochloride
$C_{15}H_{23}NO_3,HCl$
Oxprenolol Hydrochloride
$C_{15}H_{23}N_3OS$
Diamthazole
$C_{15}H_{23}N_3OS,2HCl$
Diamthazole Hydrochloride
$C_{15}H_{23}N_3O_2$
Acecainide
$C_{15}H_{23}N_3O_2,HCl$
Acecainide Hydrochloride
$C_{15}H_{23}N_3O_4S$
Sulpiride
$C_{15}H_{24}N_2O_2$
Tetracaine
$C_{15}H_{24}N_2O_2,HCl$
Tetracaine Hydrochloride
$C_{15}H_{24}O$
Butylated Hydroxytoluene
$C_{15}H_{24}O_5$
Dihydroartemisinin
$C_{15}H_{25}NO_3$
Butoxamine
$C_{15}H_{25}NO_3$
Metoprolol
$(C_{15}H_{25}NO_3)_2,C_4H_6O_4$
Metoprolol Succinate
$(C_{15}H_{25}NO_3)_2,C_4H_6O_6$
Metoprolol Tartrate
$C_{15}H_{26}N_2$
Sparteine
$C_{15}H_{26}N_2,H_2SO_4,5H_2O$
Sparteine Sulfate
$(C_{15}H_{33}N_2O_2)_2$
Dibutoline
$(C_{15}H_3{}_3N_2O_2)_2,SO_4$
Dibutoline Sulfate

$C_{16}H_8Cl_2FN_5O$
Fluquinconazole
$C_{16}H_{10}ClKN_2O_3$
Clorazepate Monopotassium
$C_{16}H_{10}ClKN_2O_3,KOH$
Clorazepate Dipotassium
$C_{16}H_{11}ClN_2O_3$
Clorazepic Acid
$C_{16}H_{11}NO_2$
Cinchophen
$C_{16}H_{11}N_3O_5S$
Droxicam
$C_{16}H_{12}ClNO_3$
Benoxaprofen
$C_{16}H_{12}Cl_2N_2O_2$
Lormetazepam
$C_{16}H_{12}FN_3O_3$
Flunitrazepam
$C_{16}H_{12}O_3$
Anisindione
$C_{16}H_{13}ClN_2O$
Diazepam
$C_{16}H_{13}ClN_2O$
Mazindol
$C_{16}H_{13}ClN_2O_2$
Clobazam
$C_{16}H_{13}ClN_2O_2$
Temazepam
$C_{16}H_{13}Cl_2NO_4$
Aceclofenac
$C_{16}H_{13}N_2Na_2O_6P$
Fosphenytoin Sodium
$C_{16}H_{13}N_3O_3$
Mebendazole
$C_{16}H_{13}N_5Na_2O_7S$
Cefixime Disodium Salt
$C_{16}H_{14}ClN_3O$
Chlordiazepoxide
$C_{16}H_{14}ClN_3O,HCl$
Chlordiazepoxide Hydrochloride
$C_{16}H_{14}F_2N_3NaO_4S,{}^3/_2H_2O$
Pantoprazole Sodium Sesquihydrate
$C_{16}H_{14}F_3NO_3S$
Tolrestat
$C_{16}H_{14}F_3N_3O_2S$
Lansoprazole
$C_{16}H_{14}N_2O$
Methaqualone
$C_{16}H_{14}N_2O,HCl$
Methaqualone Hydrochloride
$C_{16}H_{14}N_2O_3S$
Valdecoxib
$C_{16}H_{14}N_2O_6S$
Phthalylsulfacetamide
$C_{16}H_{14}O_3$
Dexketoprofen
$C_{16}H_{14}O_3$
Fenbufen
$C_{16}H_{14}O_3$
Ketoprofen
$C_{16}H_{14}O_3.C_4H_{11}NO_3$
Dexketoprofen Trometamol
$C_{16}H_{15}ClN_2$
Medazepam
$C_{16}H_{15}Cl_3O_2$
Methoxychlor
$C_{16}H_{15}F_2N_3O_4S$
Pantoprazole
$C_{16}H_{15}N_2NaO_6S_2$
Cefalothin Sodium
$C_{16}H_{15}N_2O_6P$
Fosphenytoin
$C_{16}H_{15}N_3$
Epinastine
$C_{16}H_{15}N_3,HBr$
Epinastine Hydrobromide
$C_{16}H_{15}N_5O_7S_2$
Cefixime
$C_{16}H_{16}ClNO_2S$
Clopidogrel
$C_{16}H_{16}ClNO_2S,H_2SO_4$
Clopidogrel Bisulfate
$C_{16}H_{16}ClNO_3$
Fenoldopam

$C_{16}H_{16}ClN_3$
Epinastine Hydrochloride
$C_{16}H_{16}ClN_3O_3S$
Indapamide
$C_{16}H_{16}ClN_3O_3S$
Metolazone
$C_{16}H_{16}N_2O_2$
Lysergic Acid
$C_{16}H_{16}N_2O_6S_2$
Cefalothin
$C_{16}H_{16}N_4$
Stilbamidine
$C_{16}H_{16}N_4O$
Hydroxystilbamidine
$C_{16}H_{16}N_4O,2C_2H_6O_4S$
Hydroxystilbamidine Isetionate
$C_{16}H_{16}N_4,2HCl$
Stilbamidine Dihydrochloride
$C_{16}H_{17}BrClNO_3$
Fenoldopam Hydrobromide
$C_{16}H_{17}BrN_2$
Zimeldine
$C_{16}H_{17}BrN_2,2HCl,H_2O$
Zimeldine Hydrochloride
$C_{16}H_{17}ClN_2O$
Tetrazepam
$C_{16}H_{17}KN_2O_4S$
Benzylpenicillin Potassium
$C_{16}H_{17}KN_2O_5S$
Phenoxymethylpenicillin Potassium
$C_{16}H_{17}NO_3$
Normorphine
$C_{16}H_{17}N_2NaO_4S$
Benzylpenicillin Sodium
$(C_{16}H_{17}N_2O_5S)_2Ca,2H_2O$
Phenoxymethylpenicillin Calcium
$C_{16}H_{17}N_3O$
Lysergamide
$C_{16}H_{17}N_3O_4S,H_2O$
Cefalexin
$C_{16}H_{18}ClN$
Clobenzorex
$C_{16}H_{18}ClN,HCl$
Clobenzorex Hydrochloride
$C_{16}H_{18}N_2$
Nomifensine
$C_{16}H_{18}N_2,C_4H_4O_4$
Nomifensine Maleate
$C_{16}H_{18}N_2O_4S$
Benzylpenicillin
$C_{16}H_{18}N_2O_5S$
Phenoxymethylpenicillin
$C_{16}H_{18}N_3NaO_4S$
Ampicillin Sodium
$C_{16}H_{18}N_3NaO_5S$
Amoxicillin Sodium
$C_{16}H_{18}N_4O_2$
Nialamide
$C_{16}H_{19}BrN_2$
Brompheniramine
$C_{16}H_{19}BrN_2$
Dexbrompheniramine
$C_{16}H_{19}BrN_2,C_4H_4O_4$
Brompheniramine Maleate
$C_{16}H_{19}BrN_2,C_4H_4O_4$
Dexbrompheniramine Maleate
$C_{16}H_{19}ClN_2$
Chlorphenamine
$C_{16}H_{19}ClN_2$
Dexchlorphenamine
$C_{16}H_{19}ClN_2,C_4H_4O_4$
Chlorphenamine Maleate
$C_{16}H_{19}ClN_2,C_4H_4O_4$
Dexchlorphenamine Maleate
$C_{16}H_{19}ClN_2O$
Carbinoxamine
$C_{16}H_{19}ClN_2O,C_4H_4O_4$
Carbinoxamine Maleate
$C_{16}H_{19}NO_4$
Benzoylecgonine
$C_{16}H_{19}N_3O_4S$
Ampicillin
$C_{16}H_{19}N_3O_4S$
Cefradine

$C_{16}H_{19}N_3O_4S,3H_2O$
Ampicillin Trihydrate
$C_{16}H_{19}N_3O_5S$
Amoxicillin
$C_{16}H_{19}N_3O_5S,3H_2O$
Amoxicillin Trihydrate
$C_{16}H_{19}N_3S$
Isothipendyl
$C_{16}H_{19}N_3S$
Prothipendyl
$C_{16}H_{19}N_3S,HCl$
Isothipendyl Hydrochloride
$C_{16}H_{19}N_3S,HCl,H_2O$
Prothipendyl Hydrochloride
$C_{16}H_{20}BrN$
Bromantane
$C_{16}H_{20}ClN_3$
Chloropyramine
$C_{16}H_{20}ClN_3,HCl$
Chloropyramine Hydrochloride
$C_{16}H_{20}N_2$
Pheniramine
$C_{16}H_{20}N_2,C_4H_4O_4$
Pheniramine Maleate
$C_{16}H_{20}N_2,C_7H_7NO_3$
Pheniramine Aminosalicylate
$C_{16}H_{20}N_2(C_{16}H_{18}N_2O_4S)_2$
Benzathine Benzylpenicillin
$C_{16}H_{20}N_4O_3S$
Torasemide
$C_{16}H_{20}N_4O_2,2H_2O$
Azapropazone
$C_{16}H_{21}NO$
Norlevorphanol
$C_{16}H_{21}NO_2$
Propranolol
$C_{16}H_{21}NO_2$
Ramelteon
$C_{16}H_{21}NO_2,HCl$
Propranolol Hydrochloride
$C_{16}H_{21}NO_3$
Homatropine
$C_{16}H_{21}NO_3$
Rolipram
$C_{16}H_{21}NO_3,CH_3Br$
Homatropine Methylbromide
$C_{16}H_{21}NO_3,HBr$
Homatropine Hydrobromide
$C_{16}H_{21}NS_2$
Diethylthiambutene
$C_{16}H_{21}NS_2,HCl$
Diethylthiambutene Hydrochloride
$C_{16}H_{21}N_3$
Tripelennamine
$C_{16}H_{21}N_3,C_6H_8O_7$
Tripelennamine Citrate
$C_{16}H_{21}N_3,HCl$
Tripelennamine Hydrochloride
$C_{16}H_{21}N_3O_2$
Zolmitriptan
$C_{16}H_{21}N_5O_{10}S_2$
Cefixime Trihydrate
$C_{16}H_{22}N_4O$
Thonzylamine
$C_{16}H_{22}N_4O,HCl$
Thonzylamine Hydrochloride
$C_{16}H_{22}O_4$
Dibutyl Phthalate
$C_{16}H_{23}BrN_2O_3$
Remoxipride
$C_{16}H_{23}BrN_2O_3,HCl,H_2O$
Remoxipride Hydrochloride
$C_{16}H_{23}NO$
Dezocine
$C_{16}H_{23}NO,HBr$
Dezocine Hydrobromide
$C_{16}H_{23}NO_2$
Alphaprodine
$C_{16}H_{23}NO_2$
Betaprodine
$C_{16}H_{23}NO_2$
Ethoheptazine
$C_{16}H_{23}NO_2$
Hexylcaine
$C_{16}H_{23}NO_2$
Piperocaine
$C_{16}H_{23}NO_2$
Properidine
$C_{16}H_{23}NO_2,C_6H_8O_7$
Ethoheptazine Citrate
$C_{16}H_{23}NO_2,HCl$
Alphaprodine Hydrochloride
$C_{16}H_{23}NO_2,HCl$
Hexylcaine Hydrochloride
$C_{16}H_{23}NO_2,HCl$
Piperocaine Hydrochloride
$C_{16}H_{23}NO_2,HCl$
Properidine Hydrochloride
$C_{16}H_{23}NO_6$
Monocrotaline
$C_{16}H_{23}N_5O$
Tegaserod
$C_{16}H_{23}N_5O,C_4H_4O_4$
Tegaserod Maleate
$C_{16}H_{24}N_2$
Isoaminile
$C_{16}H_{24}N_2$
Xylometazoline
$C_{16}H_{24}N_2,C_6H_8O_7$
Isoaminile Citrate
$C_{16}H_{24}N_2,HCl$
Xylometazoline Hydrochloride
$C_{16}H_{24}N_2O$
Oxymetazoline
$C_{16}H_{24}N_2O$
Ropinirole
$C_{16}H_{24}N_2O,HCl$
Oxymetazoline Hydrochloride
$C_{16}H_{24}N_2O_2$
Molindone
$C_{16}H_{24}N_2O_2,HCl$
Molindone Hydrochloride
$C_{16}H_{24}N_2O_3$
Carteolol
$C_{16}H_{24}N_2O_3,HCl$
Carteolol Hydrochloride
$C_{16}H_{25}ClN_2O$
Ropinirole Hydrochloride
$C_{16}H_{25}NO_2$
Butetamate
$C_{16}H_{25}NO_2$
Tramadol
$C_{16}H_{25}NO_2,C_6H_8O_7$
Butetamate Citrate
$C_{16}H_{25}NO_2,HCl$
Tramadol Hydrochloride
$C_{16}H_{25}NO_2S$
Tertatolol
$C_{16}H_{25}NO_2S,HCl$
Tertatolol Hydrochloride
$C_{16}H_{25}NO_3$
Moxisylyte
$C_{16}H_{25}NO_3,HCl$
Moxisylyte Hydrochloride
$C_{16}H_{25}NO_4$
Esmolol
$C_{16}H_{25}NO_4,HCl$
Esmolol Hydrochloride
$C_{16}H_{25}N_3O_5$
Xamoterol
$(C_{16}H_{25}N_3O_5)_2,C_4H_4O_4$
Xamoterol Hemifumarate
$C_{16}H_{26}N_2O_2$
Amydricaine
$C_{16}H_{26}N_2O_2$
Dimethocaine
$C_{16}H_{26}N_2O_2,HCl$
Amydricaine Hydrochloride
$C_{16}H_{26}N_2O_3$
Propoxycaine
$C_{16}H_{26}N_2O_3$
Proxymetacaine
$C_{16}H_{26}N_2O_3,HCl$
Propoxycaine Hydrochloride
$C_{16}H_{26}N_2O_3,HCl$
Proxymetacaine Hydrochloride
$C_{16}H_{26}O_5$
Artemether
$C_{16}H_{28}N_2O_4$
Oseltamivir
$C_{16}H_{28}N_2O_4,H_3PO_4$
Oseltamivir Phosphate
$C_{16}H_{34}Br_2N_2O_4$
Suxethonium Bromide
$C_{16}H_{38}Br_2N_2$
Decamethonium Bromide
$C_{17}H_2ON_4S$
Olanzapine
$C_{17}H_{11}Br_2NO_2$
Broxaldine
$C_{17}H_{11}ClF_4N_2S$
Quazepam
$C_{17}H_{11}N_5$
Letrozole
$C_{17}H_{12}Br_2O_3$
Benzbromarone
$C_{17}H_{12}C_{l2}N_4$
Triazolam
$C_{17}H_{12}I_2O_3$
Benziodarone
$C_{17}H_{12}O_6$
Aflatoxin B1
$C_{17}H_{12}O_7$
Aflatoxin G1
$C_{17}H_{12}O_7$
Aflatoxin M_1
$C_{17}H_{13}ClN_4$
Alprazolam
$C_{17}H_{13}N_3O_5S_2$
Phthalylsulfathiazole
$C_{17}H_{14}F_3N_3O_2S$
Celecoxib
$C_{17}H_{14}O_4S$
Rofecoxib
$C_{17}H_{14}O_6$
Aflatoxin B2
$C_{17}H_{14}O_7$
Aflatoxin G2
$C_{17}H_{14}O_7$
Aflatoxin M_2
$C_{17}H_{15}ClN_4S$
Etizolam
$C_{17}H_{15}NO_3$
Indoprofen
$C_{17}H_{15}NO_5$
Benorilate
$C_{17}H_{15}N_3O_6$
Balsalazide
$C_{17}H_{15}N_5O$
Zaleplon
$C_{17}H_{16}ClFN_2O_2$
Progabide
$C_{17}H_{16}ClN_3O$
Amoxapine
$C_{17}H_{16}Cl_2N_2O_5$
Clefamide
$C_{17}H_{16}N_2Na_2O_6S$
Carbenicillin Sodium
$C_{17}H_{16}N_4O_2$
Nifenazone
$C_{17}H_{17}ClN_6O_3$
Eszopiclone
$C_{17}H_{17}ClN_6O_3$
Zopiclone
$C_{17}H_{17}ClO_6$
Griseofulvin
$C_{17}H_{17}Cl_2N$
Sertraline
$C_{17}H_{17}Cl_2N,HCl$
Sertraline Hydrochloride
$C_{17}H_{17}N$
Azapetine
$C_{17}H_{17}N,H_3PO_4$
Azapetine Phosphate
$C_{17}H_{17}NO_2$
Apomorphine
$C_{17}H_{17}NO_2,HCl,{}^1/_2H_2O$
Apomorphine Hydrochloride
$C_{17}H_{17}N_3Na_2O_8,2H_2O$
Balsalazide Disodium Dihydrate
$C_{17}H_{17}N_3O$
Ramosetron

$C_{17}H_{17}N_3O,HCl$
Ramosetron Hydrochloride
$C_{17}H_{18}Br_2N_4O_2$
Dibrompropamidine
$C_{17}H_{18}Br_2N_4O_2,2C_2H_6O_4S$
Dibrompropamidine Isetionate
$C_{17}H_{18}FN_3O_3$
Ciprofloxacin
$C_{17}H_{18}FN_3O_3,C_3H_6O_3$
Ciprofloxacin Lactate
$C_{17}H_{18}FN_3O_3,HCl,H_2O$
Ciprofloxacin Hydrochloride
$C_{17}H_{18}F_3NO$
Fluoxetine
$C_{17}H_{18}F_3NO,C_2H_4$
Fluoxetine Oxalate
$C_{17}H_{18}F_3NO,HCl$
Fluoxetine Hydrochloride
$C_{17}H_{18}N_2O$
Diphenazoline
$C_{17}H_{18}N_2O_6$
Nifedipine
$C_{17}H_{18}N_2O_6S$
Carbenicillin
$C_{17}H_{18}N_3O_3S,Na$
Omeprazole Sodium
$C_{17}H_{19}ClN_2S$
Chlorpromazine
$(C_{17}H_{19}ClN_2S)_2,C_{23}H_{16}O_6$
Chlorpromazine Embonate
$C_{17}H_{19}ClN_2S,HCl$
Chlorpromazine Hydrochloride
$C_{17}H_{19}KN_2O_5S$
Pheneticillin Potassium
$C_{17}H_{19}NO$
Nefopam
$C_{17}H_{19}NO,HCl$
Nefopam Hydrochloride
$C_{17}H_{19}NO_2$
Diphenylprolinol
$C_{17}H_{19}NO_3$
Hydromorphone
$C_{17}H_{19}NO_3$
Norcodeine
$(C_{17}H_{19}NO_3)_2,C_4H_6O_6,3H_2O$
Morphine Tartrate
$(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$
Morphine Sulfate
$C_{17}H_{19}NO_3,C_2H_4O_2,3H_2O$
Morphine Acetate
$C_{17}H_{19}NO_3,HCl$
Hydromorphone Hydrochloride
$C_{17}H_{19}NO_3,HCl,3H_2O$
Morphine Hydrochloride
$C_{17}H_{19}NO_3,HCl_3,H_2O$
Norcodeine Hydrochloride
$C_{17}H_{19}NO_3,H_2O$
Morphine
$C_{17}H_{19}NO_4$
Morphine N-oxide
$C_{17}H_{19}NO_4$
Oxymorphone
$C_{17}H_{19}NO_4,HCl$
Oxymorphone Hydrochloride
$C_{17}H_{19}N_2NaO_6S,H_2O$
Meticillin Sodium
$C_{17}H_{19}N_3$
Antazoline
$C_{17}H_{19}N_3$
Mirtazapine
$(C_{17}H_{19}N_3)_2,H_2SO_4,2H_2O$
Antazoline Sulfate
$C_{17}H_{19}N_3,CH_3SO_3H$
Antazoline Mesilate
$C_{17}H_{19}N_3,HCl$
Antazoline Hydrochloride
$C_{17}H_{19}N_3,H_3PO_4$
Antazoline Phosphate
$C_{17}H_{19}N_3O$
Phentolamine
$C_{17}H_{19}N_3O,CH_3SO_3H$
Phentolamine Mesilate
$C_{17}H_{19}N_3O,HCl$
Phentolamine Hydrochloride
$C_{17}H_{19}N_3O_3S$
Omeprazole
$C_{17}H_{19}N_5$
Anastrozole
$C_{17}H_{20}BrNO$
Bromazine
$C_{17}H_{20}BrNO,HCl$
Bromazine Hydrochloride
$C_{17}H_{20}ClNO$
Clofedanol
$C_{17}H_{20}ClNO,HCl$
Clofedanol Hydrochloride
$C_{17}H_{20}ClNO_6S$
Fenoldopam Methanesulfonate
$C_{17}H_{20}F_6N_2O_3$
Flecainide
$C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$
Flecainide Acetate
$C_{17}H_{20}N_2O_2$
Tropicamide
$C_{17}H_{20}N_2O_2$
Tropisetron
$C_{17}H_{20}N_2O_5S$
Bumetanide
$C_{17}H_{20}N_2O_5S$
Pheneticillin
$C_{17}H_{20}N_2O_6S$
Meticillin
$C_{17}H_{20}N_2S$
Promazine
$C_{17}H_{20}N_2S$
Promethazine
$(C_{17}H_{20}N_2S)_2,C_{23}H_{16}O_6$
Promazine Embonate
$C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$
Promethazine Teoclate
$C_{17}H_{20}N_2S,HCl$
Promazine Hydrochloride
$C_{17}H_{20}N_2S,HCl$
Promethazine Hydrochloride
$C_{17}H_{20}N_4O_2$
Propamidine
$C_{17}H_{20}N_4O_6$
Riboflavin
$C_{17}H_{20}N_4O_2,2C_2H_6O_4S$
Propamidine Isetionate
$C_{17}H_{20}O_6$
Mycophenolic Acid
$C_{17}H_{21}ClN_2O_2$
Tropisetron Hydrochloride
$C_{17}H_{21}N$
Benzfetamine
$C_{17}H_{21}N,HCl$
Benzfetamine Hydrochloride
$C_{17}H_{21}NO$
Atomoxetine
$C_{17}H_{21}NO$
Diphenhydramine
$C_{17}H_{21}NO$
Phenyltoloxamine
$C_{17}H_{21}NO$
Tofenacin
$C_{17}H_{21}NO,C_6H_8O_7$
Phenyltoloxamine Citrate
$C_{17}H_{21}NO,C_7H_7ClN_4O_2$
Dimenhydrinate
$C_{17}H_{21}NO,HCl$
Atomoxetine Hydrochloride
$C_{17}H_{21}NO,HCl$
Diphenhydramine Hydrochloride
$C_{17}H_{21}NO,HCl$
Tofenacin Hydrochloride
$C_{17}H_{21}NO_2$
Apoatropine
$C_{17}H_{21}NO_2$
Desomorphine
$C_{17}H_{21}NO,2C_9H_{10}N_4O_4$
Diphenhydramine Acefyllinate
$C_{17}H_{21}NO_3$
Dihydromorphine
$C_{17}H_{21}NO_3$
Etodolac
$C_{17}H_{21}NO_3$
Galantamine
$C_{17}H_{21}NO_3$
N-Benzoylmescaline
$C_{17}H_{21}NO_3$
Ritodrine
$C_{17}H_{21}NO_3,HBr$
Galantamine Hydrobromide
$C_{17}H_{21}NO_3,HCl$
Dihydromorphine Hydrochloride
$C_{17}H_{21}NO_3,HCl$
Galantamine Hydrochloride
$C_{17}H_{21}NO_3,HCl$
Ritodrine Hydrochloride
$C_{17}H_{21}NO_4$
Cocaine
$C_{17}H_{21}NO_4$
Fenoterol
$C_{17}H_{21}NO_4$
Hydromorphinol
$C_{17}H_{21}NO_4$
Hyoscine
$C_{17}H_{21}NO_4,HBr$
Fenoterol Hydrobromide
$C_{17}H_{21}NO_4,HBr,3H_2O$
Hyoscine Hydrobromide
$C_{17}H_{21}NO_4,HCl$
Cocaine Hydrochloride
$C_{17}H_{22}C_{12},N_{62}H_2O$
Quinapyramine Dichloride
$C_{17}H_{22}N_2O$
Doxylamine
$C_{17}H_{22}N_2O,C_4H_6O_4$
Doxylamine Succinate
$C_{17}H_{22}N_2S$
Thenalidine
$C_{17}H_{22}N_2S,C_4H_6O_6$
Thenalidine Tartrate
$C_{17}H_{22}O_8$
Fusarenone-X
$C_{17}H_{23}NO$
Dextrorphan
$C_{17}H_{23}NO$
Levorphanol
$C_{17}H_{23}NO$
Racemorphan
$C_{17}H_{23}NO,C_4H_6O_6,2H_2O$
Levorphanol Tartrate
$C_{17}H_{23}NO_2$
Tilidate
$C_{17}H_{23}NO_2,HCl,{}^1/_2H_2O$
Tilidate Hydrochloride
$C_{17}H_{23}NO_3$
Atropine
$C_{17}H_{23}NO_3$
Hyoscyamine
$(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$
Atropine Sulfate
$(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$
Hyoscyamine Sulfate
$C_{17}H_{23}NO_3,HBr$
Hyoscyamine Hydrobromide
$C_{17}H_{23}NO_4$
Atropine Oxide
$C_{17}H_{23}NO_4,HCl$
Atropine Oxide Hydrochloride
$C_{17}H_{23}N_3O$
Mepyramine
$C_{17}H_{23}N_3O,C_4H_4O_4$
Mepyramine Maleate
$C_{17}H_{23}N_7O_5$
Taltirelin
$C_{17}H_{23}N_7O_5,4H_2O$
Taltirelin Tetrahydrate
$C_{17}H_{24}ClN_3O$
Clamoxyquin
$C_{17}H_{24}ClN_3O,HCl$
Clamoxyquin Hydrochloride
$C_{17}H_{24}N_2O$
Quinisocaine
$C_{17}H_{24}N_2O,HCl$
Quinisocaine Hydrochloride
$C_{17}H_{24}N_2O_2$
Phenglutarimide
$C_{17}H_{24}N_2O_2,HCl$
Phenglutarimide Hydrochloride

$C_{17}H_{24}O_3$
Cyclandelate
$C_{17}H_{25}N$
Phencyclidine
$C_{17}H_{25}N$,HCl
Phencyclidine Hydrochloride
$C_{17}H_{25}NO_2$
Alphameprodine
$C_{17}H_{25}NO_2$
Betameprodine
$C_{17}H_{25}NO_2$
Proheptazine
$C_{17}H_{25}NO_2$
Trimeperidine
$C_{17}H_{25}NO_2$,HBr
Proheptazine Hydrobromide
$C_{17}H_{25}NO_2$,HCl
Proheptazine Hydrochloride
$C_{17}H_{25}NO_2$,HCl
Trimeperidine Hydrochloride
$C_{17}H_{25}NO_3$
Cyclopentolate
$C_{17}H_{25}NO_3$
Eucatropine
$C_{17}H_{25}NO_3$
Levobunolol
$C_{17}H_{25}NO_3$,HCl
Cyclopentolate Hydrochloride
$C_{17}H_{25}NO_3$,HCl
Eucatropine Hydrochloride
$C_{17}H_{25}NO_3$,HCl
Levobunolol Hydrochloride
$C_{17}H_{25}N_3O$
Plasmocide
$C_{17}H_{25}N_3O$,2HCl
Plasmocide Dihydrochloride
$C_{17}H_{25}N_3O_2S$
Almotriptan
$C_{17}H_{25}N_3O_2S$
Naratriptan
$C_{17}H_{25}N_3O_2S$,HCl
Naratriptan Hydrochloride
$C_{17}H_{26}NO_2$
Ropivacaine
$C_{17}H_{26}N_2O$
5-Methoxy-*N*,*N*-diisopropyltryptamine
$C_{17}H_{26}N_2O$
Phenampromide
$C_{17}H_{26}N_2O$,HCl
5-Methoxy-*N*,*N*-diisopropyltryptamine Hydrochloride
$C_{17}H_{26}N_2O$,HCl,H_2O
Ropivacaine Hydrochloride
$C_{17}H_{26}N_4O$
Emedastine
$C_{17}H_{26}N_4O$,2$C_4H_4O_4$
Emedastine Difumarate
$C_{17}H_{27}NO_2$
Padimate O
$C_{17}H_{27}NO_2$
Venlafaxine
$C_{17}H_{27}NO_2$,HCl
Venlafaxine Hydrochloride
$C_{17}H_{27}NO_3$
Embutramide
$C_{17}H_{27}NO_3$
Pramocaine
$C_{17}H_{27}NO_3$,HCl
Pramocaine Hydrochloride
$C_{17}H_{27}NO_4$
Metipranolol
$C_{17}H_{27}N_3O_4S$
Amisulpride
$C_{17}H_{28}N_2O$
Etidocaine
$C_{17}H_{28}N_2O$,HCl
Etidocaine Hydrochloride
$C_{17}H_{28}N_2O_2$
Ambucetamide
$C_{17}H_{28}N_2O_2$
Leucinocaine
$C_{17}H_{28}N_2O_2$,CH_3SO_3H
Leucinocaine Mesylate
$C_{17}H_{28}N_2O_3$
Metabutoxycaine
$C_{17}H_{28}N_2O_3$
Oxybuprocaine
$C_{17}H_{28}N_2O_3$,HCl
Metabutoxycaine Hydrochloride
$C_{17}H_{28}N_2O_3$,HCl
Oxybuprocaine Hydrochloride
$C_{17}H_{28}N_2O_5$
Perindoprilat
$C_{17}H_{28}O_5$
Arteether
$C_{17}H_{32}BrNO_2$
Octatropine Methylbromide
$C_{17}H_{38}BrN$
Cetrimide
$C_{18}H_{12}O_6$
Sterigmatocystin
$C_{18}H_{13}ClFN_3$
Midazolam
$C_{18}H_{13}ClFN_3O_2$
Cinolazepam
$C_{18}H_{14}Cl_4N_2O$
Miconazole
$C_{18}H_{14}Cl_4N_2O$,HNO_3
Miconazole Nitrate
$C_{18}H_{14}F_4N_2O_4S$
Bicalutamide
$C_{18}H_{14}N_4O_5S$
Sulfasalazine
$C_{18}H_{15}ClN_2O_2S$
Etoricoxib
$C_{18}H_{15}Cl_3N_2O$
Econazole
$C_{18}H_{15}Cl_3N_2O$,HNO_3
Econazole Nitrate
$C_{18}H_{16}Cl_2N_2O_2$
Mexazolam
$C_{18}H_{16}N_8Na_2O_7S_3$,$3\frac{1}{2}H_2O$
Ceftriaxone Sodium
$C_{18}H_{16}O_3$
Phenprocoumon
$C_{18}H_{18}BrClN_2O$
Metaclazepam
$C_{18}H_{18}BrClN_2O$,HCl
Metaclazepam Hydrochloride
$C_{18}H_{18}ClNOS$
Zotepine
$C_{18}H_{18}ClNS$
Chlorprothixene
$C_{18}H_{18}ClNS$,CH_3SO_3H,H_2O
Chlorprothixene Mesilate
$C_{18}H_{18}ClNS$,HCl
Chlorprothixene Hydrochloride
$C_{18}H_{18}ClN_3O$
Loxapine
$C_{18}H_{18}ClN_3O$,$C_4H_6O_4$
Loxapine Succinate
$C_{18}H_{18}ClN_3O$,HCl
Loxapine Hydrochloride
$C_{18}H_{18}ClN_3S$
Clotiapine
$C_{18}H_{18}F_3NO_4$
Etofenamate
$C_{18}H_{18}N_2$
Cibenzoline
$C_{18}H_{18}N_2$,$C_4H_6O_4$
Cibenzoline Succinate
$C_{18}H_{18}N_4O_2$
Mesocarb
$C_{18}H_{18}N_8O_7S_3$
Ceftriaxone
$C_{18}H_{18}O_2$
Dienestrol
$C_{18}H_{19}ClN_4$
Clozapine
$C_{18}H_{19}Cl_2NO_4$
Felodipine
$C_{18}H_{19}F_3N_2S$
Triflupromazine
$C_{18}H_{19}F_3N_2S$,HCl
Triflupromazine Hydrochloride
$C_{18}H_{19}N$
Benzoctamine
$C_{18}H_{19}N$,HCl
Benzoctamine Hydrochloride
$C_{18}H_{19}NOS$
Duloxetine
$C_{18}H_{19}NOS$,HCl
Duloxetine Hydrochloride
$C_{18}H_{19}N_3O$
Ondansetron
$C_{18}H_{19}N_3O$,HCl,$2H_2O$
Ondansetron Hydrochloride Dihydrate
$C_{18}H_{19}N_3O_3S$
Rosiglitazone
$C_{18}H_{19}N_3O_3S$,$C_4H_4O_4$
Rosiglitazone Maleate
$C_{18}H_{20}FN_3O_4$
Levofloxacin
$C_{18}H_{20}FN_3O_4$
Ofloxacin
$C_{18}H_{20}FN_3O_4$
Levofloxacin (Ofloxacin *S*-(–)-form)
$C_{18}H_{20}FN_3O_4$,$\frac{1}{2}H_2O$
Levofloxacin Hemihydrate
$C_{18}H_{20}NO_2$
Methyldesorphine
$C_{18}H_{20}N_2$
Mianserin
$C_{18}H_{20}N_2$,HCl
Mianserin Hydrochloride
$C_{18}H_{20}N_2O_6$
Nitrendipine
$C_{18}H_{20}N_2S$
Methdilazine
$C_{18}H_{20}N_2S$,HCl
Methdilazine Hydrochloride
$C_{18}H_{20}N_3NaO_3S$
Rabeprazole Sodium
$C_{18}H_{20}O_2$
Diethylstilbestrol
$C_{18}H_{21}ClN_2$
Chlorcyclizine
$C_{18}H_{21}ClN_2$,HCl
Chlorcyclizine Hydrochloride
$C_{18}H_{21}KN_2O_5S$
Propicillin Potassium
$C_{18}H_{21}NO$
Azacyclonol
$C_{18}H_{21}NO$
Pipradrol
$C_{18}H_{21}NO$,HCl
Azacyclonol Hydrochloride
$C_{18}H_{21}NO$,HCl
Pipradrol Hydrochloride
$C_{18}H_{21}NO_3$
Hydrocodone
$C_{18}H_{21}NO_3$
Metopon
$C_{18}H_{21}NO_3$
Neopine
$(C_{18}H_{21}NO_3)_2$,H_2SO_4,$3H_2O$
Codeine Sulfate
$C_{18}H_{21}NO_3$,$C_4H_6O_6$,$2\frac{1}{2}H_2O$
Hydrocodone Tartrate
$C_{18}H_{21}NO_3$,HBr
Neopine Hydrobromide
$C_{18}H_{21}NO_3$,HCl
Metopon Hydrochloride
$C_{18}H_{21}NO_3$,HCl,$2H_2O$
Codeine Hydrochloride
$C_{18}H_{21}NO_3$,HCl,$2\frac{1}{2}H_2O$
Hydrocodone Hydrochloride
$C_{18}H_{21}NO_3$,H_2O
Codeine
$C_{18}H_{21}NO_3$,H_3PO_4,$\frac{1}{2}H_2O$
Codeine Phosphate
$C_{18}H_{21}NO_4$
Codeine *N*-oxide
$C_{18}H_{21}NO_4$
Oxycodone
$C_{18}H_{21}NO_4$,HCl,$3H_2O$
Oxycodone Hydrochloride
$C_{18}H_{21}NO_5$
Protokylol
$C_{18}H_{21}NO_5$,HCl
Protokylol Hydrochloride
$C_{18}H_{21}NO_3$,$1\frac{1}{2}H_3PO_4$
Hydrocodone Phosphate

$C_{18}H_{21}N_3O$
Dibenzepin
$C_{18}H_{21}N_3O,HCl$
Dibenzepin Hydrochloride
$C_{18}H_{21}N_3O_3S$
Rabeprazole
$C_{18}H_{22}BrNO$
Embramine
$C_{18}H_{22}BrNO,HCl$
Embramine Hydrochloride
$C_{18}H_{22}BrNO_3S$
Heteronium Bromide
$C_{18}H_{22}ClNO$
Chlorphenoxamine
$C_{18}H_{22}ClNO$
Phenoxybenzamine
$C_{18}H_{22}ClNO,HCl$
Chlorphenoxamine Hydrochloride
$C_{18}H_{22}ClNO,HCl$
Phenoxybenzamine Hydrochloride
$C_{18}H_{22}N_2$
Cyclizine
$C_{18}H_{22}N_2$
Desipramine
$C_{18}H_{22}N_2$
Dibenzylpiperazine
$C_{18}H_{22}N_2,C_3H_6O_3$
Cyclizine Lactate
$C_{18}H_{22}N_2,C_4H_6O_6$
Cyclizine Tartrate
$C_{18}H_{22}N_2,HCl$
Cyclizine Hydrochloride
$C_{18}H_{22}N_2,HCl$
Desipramine Hydrochloride
$C_{18}H_{22}N_2OS$
Methoxypromazine
$C_{18}H_{22}N_2O_2$
Phenacaine
$C_{18}H_{22}N_2O_2,HCl,H_2O$
Phenacaine Hydrochloride Monohydrate
$C_{18}H_{22}N_2O_5S$
Propicillin
$C_{18}H_{22}N_2S$
Alimemazine
$C_{18}H_{22}N_2S$
Diethazine
$(C_{18}H_{22}N_2S)_2,C_4H_6O_6$
Alimemazine Tartrate
$C_{18}H_{22}N_2S,HCl$
Diethazine Hydrochloride
$C_{18}H_{22}O_2$
Estrone
$C_{18}H_{22}O_2$
Hexestrol
$C_{18}H_{22}O_2$
Trenbolone
$C_{18}H_{22}O_3$
Methallenestril
$C_{18}H_{22}O_5$
Zearalenone
$C_{18}H_{22}O_8P_2$
Diethylstilbestrol Diphosphate
$C_{18}H_{23}N$
Tolpropamine
$C_{18}H_{23}N,HCl$
Tolpropamine Hydrochloride
$C_{18}H_{23}NO$
Orphenadrine
$C_{18}H_{23}NO,C_6H_8O_7$
Orphenadrine Citrate
$C_{18}H_{23}NO,HCl$
Orphenadrine Hydrochloride
$C_{18}H_{23}NO_3$
Dihydrocodeine
$C_{18}H_{23}NO_3$
Dobutamine
$C_{18}H_{23}NO_3$
Isoxsuprine
$C_{18}H_{23}NO_3$
Methyldihydromorphine
$C_{18}H_{23}NO_3,C_4H_6O_6$
Dihydrocodeine Tartrate
$C_{18}H_{23}NO_3,HCl$
Dobutamine Hydrochloride
$C_{18}H_{23}NO_3,HCl$
Isoxsuprine Hydrochloride
$C_{18}H_{23}NO_3,H_3PO_4$
Dihydrocodeine Phosphate
$C_{18}H_{23}NO_4$
Denopamine
$C_{18}H_{23}NO_4,HCl$
l-Denopamine Hydrochloride
$C_{18}H_{23}N_3O_6$
Imidaprilat
$C_{18}H_{23}N_5O_2$
Fenetylline
$C_{18}H_{23}N_5O_2,HCl$
Fenetylline Hydrochloride
$C_{18}H_{23}N_5O_5$
Reproterol
$C_{18}H_{23}N_5O_5,HCl$
Reproterol Hydrochloride
$C_{18}H_{24}BrNO_4$
Hyoscine Methobromide
$C_{18}H_{24}N_2O_5,2H_2O$
Enalaprilat
$C_{18}H_{24}N_2O_5S$
Amosulalol
$C_{18}H_{24}N_2O_7$
Hyoscine Methonitrate
$C_{18}H_{24}N_4O$
Granisetron
$C_{18}H_{24}N_4O,HCl$
Granisetron Hydrochloride
$C_{18}H_{24}N_6O_5$
Abacavir Succinate
$C_{18}H_{24}O_2$
Estradiol
$C_{18}H_{24}O_2$
Norandrostenedione
$C_{18}H_{24}O_3$
Estriol
$C_{18}H_{25}ClN_2O_5S$
Amosulalol Monohydrochloride
$C_{18}H_{25}NO$
Cyclazocine
$C_{18}H_{25}NO$
Dextromethorphan
$C_{18}H_{25}NO$
Levomethorphan
$C_{18}H_{25}NO$
Racemethorphan
$C_{18}H_{25}NO,HBr$
Racemethorphan Hydrobromide
$C_{18}H_{25}NO,HBr,H_2O$
Dextromethorphan Hydrobromide
$C_{18}H_{25}NO_2$
Allylprodine
$C_{18}H_{25}NO_2,HCl$
Allylprodine Hydrochloride
$C_{18}H_{26}BrNO_3$
Atropine Methobromide
$C_{18}H_{26}ClN_3$
Chloroquine
$C_{18}H_{26}ClN_3,H_2SO_4,H_2O$
Chloroquine Sulfate
$C_{18}H_{26}ClN_3O$
Hydroxychloroquine
$C_{18}H_{26}ClN_3O,H_2SO_4$
Hydroxychloroquine Sulfate
$C_{18}H_{26}ClN_3,2HCl$
Chloroquine Hydrochloride
$C_{18}H_{26}ClN_3,2H_3PO_4$
Chloroquine Phosphate
$C_{18}H_{26}N_2O_4S$
Glibornuride
$C_{18}H_{26}N_2O_6$
Atropine Methonitrate
$C_{18}H_{26}O_2$
Nandrolone
$C_{18}H_{26}O_3$
Oxabolone
$C_{18}H_{26}O_5$
Zeranol
$C_{18}H_{27}NO_2$
Caramiphen
$C_{18}H_{27}NO_2$
Dyclonine
$C_{18}H_{27}NO_2,{}^1/_2C_2H_6O_6S_2$
Caramiphen Edisilate
$C_{18}H_{27}NO_2,HCl$
Caramiphen Hydrochloride
$C_{18}H_{27}NO_2,HCl$
Dyclonine Hydrochloride
$C_{18}H_{27}NO_4$
Etoxeridine
$C_{18}H_{27}NO_5$
Propanidid
$C_{18}H_{27}N_3O$
Pentaquin
$C_{18}H_{27}N_3O,H_2PO_4$
Pentaquin Phosphate
$C_{18}H_{28}N_2O$
Bupivacaine
$C_{18}H_{28}N_2O$
Levobupivacaine
$C_{18}H_{28}N_2O,HCl$
Levobupivacaine Hydrochloride
$C_{18}H_{28}N_2O,HCl,H_2O$
Bupivacaine Hydrochloride
$C_{18}H_{28}N_2O_4$
Acebutolol
$C_{18}H_{28}N_2O_4,HCl$
Acebutolol Hydrochloride
$C_{18}H_{28}N_4O$
Butalamine
$C_{18}H_{28}N_4O,HCl$
Butalamine Hydrochloride
$C_{18}H_{29}NO_3$
Amprotropine
$C_{18}H_{29}NO_3$
Betaxolol
$C_{18}H_{29}NO_3,HCl$
Betaxolol Hydrochloride
$C_{18}H_{29}N_3O_5$
Bambuterol
$C_{18}H_{29}N_3O_5$
Bambuterol Hydrochloride
$C_{18}H_{30}BrNO_3S$
Penthienate Methobromide
$C_{18}H_{30}N_2O_2$
Butacaine
$(C_{18}H_{30}N_2O_2)_2,H_2SO_4$
Butacaine Sulfate
$C_{18}H_{31}NO_4$
Bisoprolol
$(C_{18}H_{31}NO_4)_2,C_4H_4O_4$
Bisoprolol Fumarate (hemifumarate)
$C_{18}H_{33}ClN_2O_5S$
Clindamycin
$C_{18}H_{33}ClN_2O_5S,HCl,H_2O$
Clindamycin Hydrochloride
$C_{18}H_{34}ClN_2O_8PS$
Clindamycin Phosphate
$C_{18}H_{34}N_2O_6S$
Lincomycin
$C_{18}H_{34}N_2O_6S,HCl,H_2O$
Lincomycin Hydrochloride
$C_{18}H_{36}N_4O_{11}$
Kanamycin
$C_{18}H_{36}N_4O_{11},H_2SO_4$
Kanamycin Sulfate
$C_{18}H_{36}N_4O_{11},1.7H_2SO_4$
Kanamycin Acid Sulfate
$C_{18}H_{37}N_5O_9$
Tobramycin
$C_{19}H_{11}Cl_2I_2NO_3$
Rafoxanide
$C_{19}H_{12}O_6$
Dicoumarol
$C_{19}H_{12}O_8$
Diacerein
$C_{19}H_{15}ClN_2O_4$
Rebamipide
$C_{19}H_{15}ClN_4$
Triphenyltetrazolium Chloride
$C_{19}H_{15}CaNO_7$
Nedocromil Calcium
$C_{19}H_{15}KO_4$
Warfarin Potassium
$C_{19}H_{15}NO_6$
Acenocoumarol

$C_{19}H_{15}NO_7$
Nedocromil
$C_{19}H_{15}NO_7,Na_2$
Nedocromil Sodium
$C_{19}H_{15}NaO_4$
Warfarin Sodium
$C_{19}H_{16}ClFN_2O$
Flutoprazepam
$C_{19}H_{16}ClFN_3NaO_5S,H_2O$
Flucloxacillin Sodium
$C_{19}H_{16}ClNO_4$
Indometacin
$C_{19}H_{16}O_3$
Coumatetralyl
$C_{19}H_{16}O_4$
Warfarin
$C_{19}H_{17}ClFN_3O_5S$
Flucloxacillin
$C_{19}H_{17}ClN_2O$
Prazepam
$C_{19}H_{17}ClN_2O_4$
Glafenine
$C_{19}H_{17}ClN_3NaO_5S,H_2O$
Cloxacillin Sodium
$C_{19}H_{17}NOS$
Tolnaftate
$C_{19}H_{17}N_3O_4S_2$
Cefaloridine
$C_{19}H_{18}ClFN_2O_3$
Flutazolam
$C_{19}H_{18}ClN_3O_3$
Camazepam
$C_{19}H_{18}ClN_3O_5S$
Cloxacillin
$C_{19}H_{18}NO_4$
Californine
$C_{19}H_{19}ClN_2$
Desloratadine
$C_{19}H_{19}N$
Phenindamine
$C_{19}H_{19}N$
Setiptiline
$C_{19}H_{19}N,C_4H_6O_6$
Phenindamine Tartrate
$C_{19}H_{19}NOS$
Ketotifen
$C_{19}H_{19}NOS,C_4H_4O_4$
Ketotifen Fumarate
$C_{19}H_{19}N_3O_6$
Nilvadipine
$C_{19}H_{19}N_7O_6$
Folic Acid
$C_{19}H_{20}ClNO_4$
Bezafibrate
$C_{19}H_{20}ClN_3$
Clemizole
$C_{19}H_{20}ClN_3,HCl$
Clemizole Hydrochloride
$C_{19}H_{20}FNO_3$
Paroxetine
$C_{19}H_{20}FNO_3,HCl,{}^1/_2H_2O$
Paroxetine Hydrochloride Hemihydrate
$C_{19}H_{20}N_2$
Mebhydrolin
$(C_{19}H_{20}N_2)_2,C_{10}H_8O_6S_2$
Mebhydrolin Napadisilate
$C_{19}H_{20}N_2O_2$
Phenylbutazone
$C_{19}H_{20}N_2O_3$
Dolasetron
$C_{19}H_{20}N_2O_3.CH_3SO_3H$
Dolasetron Methanesulfonate
$C_{19}H_{20}N_2O_3,H_2O$
Oxyphenbutazone
$C_{19}H_{20}N_2O_3S$
Pioglitazone
$C_{19}H_{20}N_2O_3S,HCl$
Pioglitazone Hydrochloride
$C_{19}H_{20}N_{60}$
Imidocarb
$C_{19}H_{21}F_3N_2S$
Trifluomeprazine
$C_{19}H_{21}F_3N_2S,C_4H_4O_4$
Trifluomeprazine Maleate
$C_{19}H_{21}N$
Nortriptyline
$C_{19}H_{21}N$
Protriptyline
$C_{19}H_{21}N,HCl$
Nortriptyline Hydrochloride
$C_{19}H_{21}N,HCl$
Protriptyline Hydrochloride
$C_{19}H_{21}NO$
Doxepin
$C_{19}H_{21}NO,HCl$
Doxepin Hydrochloride
$C_{19}H_{21}NO_3$
Nalorphine
$C_{19}H_{21}NO_3$
Thebaine
$C_{19}H_{21}NO_3,HBr$
Nalorphine Hydrobromide
$C_{19}H_{21}NO_3,HCl$
Nalorphine Hydrochloride
$C_{19}H_{21}NO_4$
6-Monoacetylmorphine
$C_{19}H_{21}NO_4$
Naloxone
$C_{19}H_{21}NO_4,HCl$
6-Monoacetylmorphine Hydrochloride
$C_{19}H_{21}NO_4,HCl$
Naloxone Hydrochloride
$C_{19}H_{21}NS$
Dosulepin
$C_{19}H_{21}NS$
Pizotifen
$C_{19}H_{21}NS,C_4H_6O_5$
Pizotifen Malate
$C_{19}H_{21}NS,HCl$
Dosulepin Hydrochloride
$C_{19}H_{21}N_3O$
Zolpidem
$(C_{19}H_{21}N_3O)_2,C_4H_6O_6$
Zolpidem Tartrate
$C_{19}H_{21}N_3O_5$
Isradipine
$C_{19}H_{21}N_5O_2$
Pirenzepine
$C_{19}H_{21}N_5O_4$
Prazosin
$C_{19}H_{21}N_5O_4,HCl$
Prazosin Hydrochloride
$C_{19}H_{21}N_5O_2,2HCl$
Pirenzepine Hydrochloride
$C_{19}H_{22}ClN_5O$
Trazodone
$C_{19}H_{22}ClN_5O,HCl$
Trazodone Hydrochloride
$C_{19}H_{22}FN_3O$
Azaperone
$C_{19}H_{22}NO_4S_2$
Tiotropium
$C_{19}H_{22}N_2$
Methadone Intermediate
$C_{19}H_{22}N_2$
Triprolidine
$C_{19}H_{22}N_2,HCl,H_2O$
Triprolidine Hydrochloride
$C_{19}H_{22}N_2O$
Cinchonidine
$C_{19}H_{22}N_2O$
Cinchonine
$C_{19}H_{22}N_2O$
Noxiptiline
$(C_{19}H_{22}N_2O)_2,H_2SO_4,7H_2O$
Cinchonidine Sulfate
$C_{19}H_{22}N_2O,HCl$
Noxiptiline Hydrochloride
$C_{19}H_{22}N_2O,HCl,2H_2O$
Cinchonine Hydrochloride
$C_{19}H_{22}N_2OS$
Acepromazine
$C_{19}H_{22}N_2OS,C_4H_4O_4$
Acepromazine Maleate
$C_{19}H_{22}N_2O_3S$
Dimethoxanate
$C_{19}H_{22}N_2O_3S,HCl$
Dimethoxanate Hydrochloride
$C_{19}H_{22}N_2S$
Pecazine
$C_{19}H_{22}N_2S,HCl,H_2O$
Pecazine Hydrochloride
$C_{19}H_{22}O_2$
Vedaprofen
$C_{19}H_{23}ClN_2$
Clomipramine
$C_{19}H_{23}ClN_2$
Homochlorcyclizine
$C_{19}H_{23}ClN_2,HCl$
Clomipramine Hydrochloride
$C_{19}H_{23}NO$
Diphenylpyraline
$C_{19}H_{23}NO,HBr$
Diphenylpyraline Hydrobromide
$C_{19}H_{23}NO,HCl$
Diphenylpyraline Hydrochloride
$C_{19}H_{23}NO_3$
Ethylmorphine
$C_{19}H_{23}NO_3$
Reboxetine
$C_{19}H_{23}NO_3$
Xenysalate
$C_{19}H_{23}NO_3,CH_3SO_3H$
Reboxetine Methanesulfonate (Mesylate)
$C_{19}H_{23}NO_3,HCl$
Xenysalate Hydrochloride
$C_{19}H_{23}NO_3,HCl,2H_2O$
Ethylmorphine Hydrochloride
$C_{19}H_{23}N_3O$
Benzydamine
$C_{19}H_{23}N_3O,HCl$
Benzydamine Hydrochloride
$C_{19}H_{23}N_3O_2$
Ergometrine
$(C_{19}H_{23}N_3O_2)_2,C_4H_6O_6$
Ergometrine Tartrate
$C_{19}H_{23}N_3O_2,C_4H_4O_4$
Ergometrine Maleate
$C_{19}H_{23}N_5O_3$
Trimetrexate
$C_{19}H_{24}N_2$
Bamipine
$C_{19}H_{24}N_2$
Histapyrrodine
$C_{19}H_{24}N_2$
Imipramine
$(C_{19}H_{24}N_2)2,C_{23}H_{16}O_6$
Imipramine Embonate
$C_{19}H_{24}N_2,HCl$
Bamipine Hydrochloride
$C_{19}H_{24}N_2,HCl$
Histapyrrodine Hydrochloride
$C_{19}H_{24}N_2,HCl$
Imipramine Hydrochloride
$C_{19}H_{24}N_2O$
Dimevamide
$C_{19}H_{24}N_2O,H_2SO_4$
Dimevamide Sulfate
$C_{19}H_{24}N_2OS$
Levomepromazine
$C_{19}H_{24}N_2OS,C_4H_4O_4$
Levomepromazine Maleate
$C_{19}H_{24}N_2OS,HCl$
Levomepromazine Hydrochloride
$C_{19}H_{24}N_2O_3$
Labetalol
$C_{19}H_{24}N_2O_3,HCl$
Labetalol Hydrochloride
$C_{19}H_{24}N_2O_4$
Formoterol
$(C_{19}H_{24}N_2O_4)_2,C_4H_4O_4.2H_2O$
Formoterol Fumarate Dihydrate
$C_{19}H_{24}N_2O_6$
Ketorolac Tromethamine
$C_{19}H_{24}N_2S$
Profenamine
$C_{19}H_{24}N_2S,C_{14}H_{10}O_4$
Profenamine Hibenzate
$C_{19}H_{24}N_2S,HCl$
Profenamine Hydrochloride
$C_{19}H_{24}N_4O_2$
Pentamidine

$C_{19}H_{24}N_4O_2,2CH_4O_3S$
Pentamidine Mesilate
$C_{19}H_{24}N_4O_2,2C_2H_6O_4S$
Pentamidine Isetionate
$C_{19}H_{24}O_2$
Boldione
$C_{19}H_{25}NO$
Levallorphan
$C_{19}H_{25}NO,C_4H_6O_6$
Levallorphan Tartrate
$C_{19}H_{25}NO_2$
Buphenine
$C_{19}H_{25}NO_2,HCl$
Buphenine Hydrochloride
$C_{19}H_{25}N_3OS$
Thiambutosine
$C_{19}H_{25}N_3O_2S,C_4H_6O_5$
Almotriptan Maleate
$C_{19}H_{25}N_3O_2S_2$
Dimetotiazine
$C_{19}H_{25}N_3O_2S_2,CH_3SO_3H$
Dimetotiazine Mesilate
$C_{19}H_{25}N_3S$
Aminopromazine
$(C_{19}H_{25}N_3S)_2,C_4H_4O_4$
Aminopromazine Fumarate
$C_{19}H_{25}N_5O_4$
Terazosin
$C_{19}H_{25}N_5O_4,HCl$
Terazosin Hydrochloride
$C_{19}H_{25}N_5O_4,HCl,2H_2O$
Terazosin Hydrochloride Dihydrate
$C_{19}H_{26}BrNO$
Bibenzonium Bromide
$C_{19}H_{26}N_2O_4S_2$
Thiazinamium Metilsulfate
$C_{19}H_{26}N_2S$
Pergolide
$C_{19}H_{26}N_2S,CH_3SO_3H$
Pergolide Mesilate
$C_{19}H_{26}O_2$
Androstenedione
$C_{19}H_{26}O_2$
Boldenone
$C_{19}H_{26}O_3$
Formestane
$C_{19}H_{26}O_7$
Diacetoxyscirpenol
$C_{19}H_{27}ClO_2$
Clostebol
$C_{19}H_{27}NO$
Pentazocine
$C_{19}H_{27}NO,C_3H_6O_3$
Pentazocine Lactate
$C_{19}H_{27}NO,HCl$
Pentazocine Hydrochloride
$C_{19}H_{27}NO_3$
Nateglinide
$C_{19}H_{27}NO_3$
Tetrabenazine
$C_{19}H_{27}NaO_5S$
Dehydroepiandrosterone Sodium Sulfate
$C_{19}H_{27}NaO_5S,2H_2O$
Dehydroepiandrosterone Sodium Sulfate Dihydrate
$C_{19}H_{27}NaO_8$
Artesunate Sodium
$C_{19}H_{27}N_3O_5S$
Dofetilide
$C_{19}H_{27}N_5O_3$
Bunazosin
$C_{19}H_{27}N_5O_4$
Alfuzosin
$C_{19}H_{27}N_5O_4,HCl$
Alfuzosin Hydrochloride
$C_{19}H_{27}N_5O_5$
Nifekalant
$C_{19}H_{27}N_5O_5,HCl$
Nifekalant Hydrochloride
$C_{19}H_{28}BrNO_3$
Glycopyrronium Bromide
$C_{19}H_{28}ClN_5O_3$
Bunazosin Hydrochloride
$C_{19}H_{28}N_2$
Iprindole
$C_{19}H_{28}N_2,HCl$
Iprindole Hydrochloride
$C_{19}H_{28}N_6O_9S_2$
Amicarbalide Isetionate
$C_{19}H_{28}O_2$
Dehydroepiandrosterone
$C_{19}H_{28}O_2$
Testosterone
$C_{19}H_{28}O_5S$
Dehydroepiandrosterone Sulfate
$C_{19}H_{28}O_8$
Artesunate
$C_{19}H_{29}NO$
Cycrimine
$C_{19}H_{29}NO$
Procyclidine
$C_{19}H_{29}NO,HCl$
Cycrimine Hydrochloride
$C_{19}H_{29}NO,HCl$
Procyclidine Hydrochloride
$C_{19}H_{29}N_3O,C_{23}H_{16}O_6$
Pamaquin
$C_{19}H_{30}NO_2,CH_3SO_4$
Pentapiperide Metilsulfate
$C_{19}H_{30}O_2$
Androstanolone
$C_{19}H_{30}O_2$
Androsterone
$C_{19}H_{30}O_3$
Oxandrolone
$C_{19}H_{30}O_5$
Dodecyl Gallate
$C_{19}H_{30}O_5$
Idebenone
$C_{19}H_{30}O_5$
Piperonyl Butoxide
$C_{19}H_{32}BrNO_2$
Valethamate Bromide
$C_{19}H_{32}N_2O_5$
Perindopril
$C_{19}H_{32}N_2O_5,C_4H_{11}N$
Perindopril Erbumine (Perindopril Tert-Butylamine)
$C_{19}H_{32}O_2$
Androstanediol
$C_{19}H_{35}N$
Perhexiline
$C_{19}H_{35}N,C_4H_4O_4$
Perhexiline Maleate
$C_{19}H_{35}NO_2$
Dicycloverine
$C_{19}H_{35}NO_2,HCl$
Dicycloverine Hydrochloride
$C_{19}H_{40}I_2N_2$
Mebezonium Iodide
$C_{20}H_{14}O_4$
Phenolphthalein
$C_{20}H_{15}NO_3$
Oxyphenisatine
$C_{20}H_{16}O_6$
Dithranol Triacetate
$C_{20}H_{17}ClN_2O_3$
Ketazolam
$C_{20}H_{17}FO_3S$
Sulindac
$C_{20}H_{17}F_3N_2O_4$
Floctafenine
$C_{20}H_{18}ClNO_6$
Ochratoxin A
$C_{20}H_{18}ClNO_4,2H_2O$
Berberine Hydrochloride
$C_{20}H_{18}Cl_2N_2O_6$
Chloramphenicol Cinnamate
$[C_{20}H_{18}NO_4]^+$
Berberine
$C_{20}H_{18}NO_4,HSO_4$
Berberine Sulfate
$C_{20}H_{19}NO_3$
Pyriproxyfen
$C_{20}H_{19}NO_5$
Chelidonine
$C_{20}H_{19}NO_6$
Ochratoxin B
$C_{20}H_{20}ClN_3O$
Amopyroquine
$C_{20}H_{20}ClN_3O,2HCl$
Amopyroquine Hydrochloride
$C_{20}H_{20}N_2O_2$
Feprazone
$C_{20}H_{21}ClO_4$
Fenofibrate
$C_{20}H_{21}FN_2O$
Citalopram
$C_{20}H_{21}FN_2O$
Escitalopram
$C_{20}H_{21}FN_2O,C_2H_2O_4$
Escitalopram Oxalate
$C_{20}H_{21}FN_2O,HBr$
Citalopram Hydrobromide
$C_{20}H_{21}N$
Cyclobenzaprine
$C_{20}H_{21}N,HCl$
Cyclobenzaprine Hydrochloride
$C_{20}H_{21}NO_3$
Dimefline
$C_{20}H_{21}NO_3,HCl$
Dimefline Hydrochloride
$C_{20}H_{21}NO_4$
Papaverine
$(C_{20}H_{21}NO_4)_2,H_2SO_4,5H_2O$
Papaverine Sulfate
$C_{20}H_{21}NO_4,HCl$
Papaverine Hydrochloride
$C_{20}H_{22}ClN$
Pyrrobutamine
$C_{20}H_{22}ClN,2H_3PO_4$
Pyrrobutamine Phosphate
$C_{20}H_{22}ClN_3O$
Amodiaquine
$C_{20}H_{22}ClN_3O,2HCl,2H_2O$
Amodiaquine Hydrochloride
$C_{20}H_{22}N_2$
Azatadine
$C_{20}H_{22}N_2O_2$
Gelsemine
$C_{20}H_{22}N_2S$
Mequitazine
$C_{20}H_{22}N_5NaO_6S$
Azlocillin Sodium
$C_{20}H_{22}N_8O_5$
Methotrexate
$C_{20}H_{22}N_2,2C_4H_4O_4$
Azatadine Maleate
$C_{20}H_{23}ClN_4O_2$
Clonitazene
$C_{20}H_{23}N$
Amitriptyline
$C_{20}H_{23}N$
Maprotiline
$(C_{20}H_{23}N)_2,C_{23}H_{16}O_6$
Amitriptyline Embonate
$C_{20}H_{23}N,HCl$
Amitriptyline Hydrochloride
$C_{20}H_{23}N,HCl$
Maprotiline Hydrochloride
$C_{20}H_{23}NO_2$
Amolanone
$C_{20}H_{23}NO_2,HCl$
Amolanone Hydrochloride
$C_{20}H_{23}NO_3$
Methylpiperidyl Benzilate
$C_{20}H_{23}NO_4$
Acetylcodeine
$C_{20}H_{23}NO_4$
Lauroscholtzine
$C_{20}H_{23}NO_4$
Naltrexone
$C_{20}H_{23}NO_4$
Thebacon
$C_{20}H_{23}NO_4,HCl$
Naltrexone Hydrochloride
$C_{20}H_{23}NO_4,HCl$
Thebacon Hydrochloride
$C_{20}H_{23}NS$
Metixene
$C_{20}H_{23}NS,HCl$
Metixene Hydrochloride
$C_{20}H_{23}N_5O_6S$
Azlocillin

$C_{20}H_{24}ClNO_2$
Metofoline
$C_{20}H_{24}ClN_3S$
Prochlorperazine
$C_{20}H_{24}ClN_3S,C_2H_6O_6S_2$
Prochlorperazine Edisilate
$C_{20}H_{24}ClN_3S,2CH_3SO_3H$
Prochlorperazine Mesilate
$C_{20}H_{24}ClN_3S,2C_4H_4O_4$
Prochlorperazine Maleate
$C_{20}H_{24}Cl_2N_{10}$
Picloxydine
$C_{20}H_{24}N,CH_3SO_4$
Diphemanil Metilsulfate
$C_{20}H_{24}N_2$
Dimetindene
$C_{20}H_{24}N_2,C_4H_4O_4$
Dimetindene Maleate
$C_{20}H_{24}N_2OS$
Lucanthone
$C_{20}H_{24}N_2OS$
Propiomazine
$C_{20}H_{24}N_2OS,C_4H_4O_4$
Propiomazine Maleate
$C_{20}H_{24}N_2OS,HCl$
Propiomazine Hydrochloride
$(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$
Quinidine Sulfate
$(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$
Quinine Sulfate
$C_{20}H_{24}N_2O_2,(C_6H_{10}O_7)_x,xH_2O$
Quinidine Polygalacturonate
$C_{20}H_{24}N_2O_2,C_6H_{12}O_7$
Quinidine Gluconate
$C_{20}H_{24}N_2O_2,C_7H_6O_3,H_2O$
Quinine Salicylate
$C_{20}H_{24}N_2O_2,HBr,2H_2O$
Quinine Hydrobromide
$C_{20}H_{24}N_2O_2,HCl,2H_2O$
Quinine Hydrochloride
$C_{20}H_{24}N_2O_2,H_2SO_4,4H_2O$
Quinidine Bisulfate
$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$
Quinine Bisulfate
$C_{20}H_{24}N_2O_5$
Diamfenetide
$C_{20}H_{24}N_2O_6$
Nisoldipine
$C_{20}H_{24}N_2O_2,2HBr,3H_2O$
Quinine Dihydrobromide
$C_{20}H_{24}N_2O_2,2HCl$
Quinine Dihydrochloride
$C_{20}H_{24}N_2O_2,2H_2O$
Quinidine
$C_{20}H_{24}N_2O_2,3H_2O$
Quinine
$C_{20}H_{24}O_2$
Ethinylestradiol
$C_{20}H_{24}O_2$
Exemestane
$C_{20}H_{24}O_3$
Trenbolone Acetate
$C_{20}H_{25}ClNO_5,HCl$
Cloricromen Hydrochloride
$C_{20}H_{25}ClN_2O_5$
Amlodipine
$C_{20}H_{25}ClN_2O_5,C_4H_4O_4$
Amlodipine Maleate
$C_{20}H_{25}ClN_2O_5,C_6H_5SO_3H$
Amlodipine Besilate
$C_{20}H_{25}N$
Fenpiprane
$C_{20}H_{25}N,HCl$
Fenpiprane Hydrochloride
$C_{20}H_{25}NO$
Normethadone
$C_{20}H_{25}NO,HCl$
Normethadone Hydrochloride
$C_{20}H_{25}NO_2$
Adiphenine
$C_{20}H_{25}NO_2,HCl$
Adiphenine Hydrochloride
$C_{20}H_{25}NO_2S_2$
Tiagabine
$C_{20}H_{25}NO_2S_2,HCl$
Tiagabine Hydrochloride
$C_{20}H_{25}NO_3$
Benactyzine
$C_{20}H_{25}NO_3$
Dimenoxadole
$C_{20}H_{25}NO_3,HCl$
Benactyzine Hydrochloride
$C_{20}H_{25}NO_4$
Acetyldihydrocodeine
$C_{20}H_{25}NO_4,HCl$
Acetyldihydrocodeine Hydrochloride
$C_{20}H_{25}N_3O$
Lysergide
$C_{20}H_{25}N_3O_2$
Methylergometrine
$C_{20}H_{25}N_3O_2,C_4H_4O_4$
Methylergometrine Maleate
$C_{20}H_{25}N_3S$
Perazine
$C_{20}H_{25}N_3S,2C_3H_4O_4$
Perazine Dimalonate
$C_{20}H_{26}ClNO_3$
Lachesine Chloride
$C_{20}H_{26}ClNO_5$
Cloricromen
$C_{20}H_{26}N_2$
Trimipramine
$C_{20}H_{26}N_2,C_4H_4O_4$
Trimipramine Maleate
$C_{20}H_{26}N_2O$
Ibogaine
$C_{20}H_{26}N_2O,HCl$
Ibogaine Hydrochloride
$C_{20}H_{26}N_2O_2$
Ajmaline
$C_{20}H_{26}N_2O_2$
Hydroquinidine
$C_{20}H_{26}N_2O_2$
Hydroquinine
$C_{20}H_{26}N_2O_2,C_2H_6O$
Ajmaline Monoethanolate
$C_{20}H_{26}N_2O_2,HCl$
Hydroquinidine Hydrochloride
$C_{20}H_{26}N_2O_5S$
Alacepril
$C_{20}H_{26}N_2O_5S_2$
Spiraprilat
$C_{20}H_{26}N_2O_2,2HCl,2H_2O$
Ajmaline Hydrochloride
$C_{20}H_{26}N_2S$
Ethylisobutrazine
$C_{20}H_{26}O_2$
Norethisterone
$C_{20}H_{26}O_2$
Noretynodrel
$C_{20}H_{27}BrN_2O$
Ambutonium Bromide
$C_{20}H_{27}N$
Alverine
$C_{20}H_{27}N$
Terodiline
$C_{20}H_{27}N,C_6H_8O_7$
Alverine Citrate
$C_{20}H_{27}N,HCl$
Terodiline Hydrochloride
$C_{20}H_{27}NO_2$
Vetrabutine
$C_{20}H_{27}NO_2,HCl$
Vetrabutine Hydrochloride
$C_{20}H_{27}NO_3$
Trilostane
$C_{20}H_{27}NO_4$
Bevantolol
$C_{20}H_{27}NO_5$
Carbocromen
$C_{20}H_{27}NO_5,HCl$
Carbocromen Hydrochloride
$C_{20}H_{27}NO_{11}$
Amygdalin
$C_{20}H_{27}NaO_5S,5H_2O$
Ecabet Sodium
$C_{20}H_{27}N_3O_6$
Imidapril
$C_{20}H_{27}N_3O_6,HCl$
Imidapril Monohydrochloride
$C_{20}H_{27}N_5O_3$
Bamifylline
$C_{20}H_{27}N_5O_3,HCl$
Bamifylline Hydrochloride
$C_{20}H_{27}N_5O_5$
Cilostazol
$C_{20}H_{27}O_2Cl$
Dehydrochloromethyltestosterone
$C_{20}H_{28}BrN$
Emepronium Bromide
$C_{20}H_{28}ClNO_4$
Bevantolol Hydrochloride
$C_{20}H_{28}N_2O_3$
Oxyphencyclimine
$C_{20}H_{28}N_2O_3,HCl$
Oxyphencyclimine Hydrochloride
$C_{20}H_{28}N_2O_5$
Enalapril
$C_{20}H_{28}N_2O_5$
Remifentanil
$C_{20}H_{28}N_2O_5,C_4H_4O_4$
Enalapril Maleate
$C_{20}H_{28}N_2O_5,HCl$
Remifentanil Hydrochloride
$C_{20}H_{28}N_2O_5S$
Tamsulosin
$C_{20}H_{28}N_2O_5S,HCl$
Tamsulosin Hydrochloride
$C_{20}H_{28}N_4O_8S_2$
Stilbamidine Isethionate
$C_{20}H_{28}O$
Lynestrenol
$C_{20}H_{28}O_2$
Isotretinoin
$C_{20}H_{28}O_2$
Methandienone
$C_{20}H_{28}O_5S$
Ecabet
$C_{20}H_{29}FO_3$
Fluoxymesterone
$C_{20}H_{29}N_3O_2$
Cinchocaine
$C_{20}H_{29}N_3O_2,HCl$
Cinchocaine Hydrochloride
$C_{20}H_{30}BrNO_3,H_2O$
Ipratropium Bromide
$C_{20}H_{30}NO_3$
Ipratropium
$C_{20}H_{30}N_2O_3$
Morpheridine
$C_{20}H_{30}N_2O_3,2HCl$
Morpheridine Dihydrochloride
$C_{20}H_{30}O_2$
Metenolone
$C_{20}H_{30}O_2$
Methyltestosterone
$C_{20}H_{30}O_2$
Mibolerone
$C_{20}H_{30}O_2$
Norethandrolone
$C_{20}H_{30}O_2$
Stenbolone
$C_{20}H_{30}O_3$
Oxymesterone
$C_{20}H_{31}NO$
Trihexyphenidyl
$C_{20}H_{31}NO,HCl$
Trihexyphenidyl Hydrochloride
$C_{20}H_{31}NO_3$
Carbetapentane
$C_{20}H_{31}NO_3,C_6H_8O_7$
Carbetapentane Citrate
$C_{20}H_{32}ClNO$
Tricyclamol Chloride
$C_{20}H_{32}O$
Ethylestrenol
$C_{20}H_{32}O_2$
Mestanolone
$C_{20}H_{32}O_2$
Mesterolone
$C_{20}H_{32}O_2$
Methandriol

$C_{20}H_{33}NO_3$
Oxeladin
$C_{20}H_{33}NO_3,C_6H_8O_7$
Oxeladin Citrate
$C_{20}H_{33}N_2O,CH_3SO_4$
Hexocyclium Metilsulfate
$C_{20}H_{33}N_3O_3S$
Quinagolide
$C_{20}H_{33}N_3O_3S,HCl$
Quinagolide Hydrochloride
$C_{20}H_{33}N_3O_4$
Celiprolol
$C_{20}H_{33}N_3O_4,HCl$
Celiprolol Hydrochloride
$C_{20}H_{34}O_5$
Alprostadil
$C_{20}H_{36}N_2O_3S$
Ibutilide
$(C_{20}H_{36}N_2O_3S)_2,C_4H_4O_4$
Ibutilide Fumarate
$C_{20}H_{37}KO_7S$
Docusate Potassium
$C_{20}H_{37}NaO_7S$
Docusate Sodium
$C_{21}H_{18}ClNO_6$
Acemetacin
$C_{21}H_{19}N_3O_3S$
Amsacrine
$C_{21}H_{20}BrN_3$
Homidium Bromide
$C_{21}H_{20}N_4O_3$
Picotamide
$C_{21}H_{20}N_4O_3,H_2O$
Picotamide Monohydrate
$C_{21}H_{21}ClN_2O_8$
Demeclocycline
$C_{21}H_{21}ClN_2O_8,HCl$
Demeclocycline Hydrochloride
$C_{21}H_{21}ClN_4OS$
Ziprasidone
$C_{21}H_{21}N$
Cyproheptadine
$C_{21}H_{21}N,HCl,1½H_2O$
Cyproheptadine Hydrochloride
$C_{21}H_{21}NO_6$
Hydrastine
$C_{21}H_{21}NO_6,HCl$
Hydrastine Hydrochloride
$C_{21}H_{22}N_2O_2$
Strychnine
$(C_{21}H_{22}N_2O_2)_2,H_2SO_4,5H_2O$
Strychnine Sulfate
$C_{21}H_{22}N_2O_2,HCl,2H_2O$
Strychnine Hydrochloride
$C_{21}H_{22}N_2O_2,HNO_3$
Strychnine Nitrate
$C_{21}H_{22}N_4O_6S$
Raltitrexed
$C_{21}H_{22}O_9$
Aloin
$C_{21}H_{23}ClFNO_2$
Haloperidol
$C_{21}H_{23}ClFN_3O$
Flurazepam
$C_{21}H_{23}ClFN_3O,HCl$
Flurazepam Monohydrochloride
$C_{21}H_{23}ClFN_3O,2HCl$
Flurazepam Hydrochloride
$C_{21}H_{23}Cl_2N_3O$
Alpidem
$C_{21}H_{23}NO_5$
Diamorphine
$C_{21}H_{23}NO_5,HCl,H_2O$
Diamorphine Hydrochloride
$C_{21}H_{23}N_3OS$
Pericyazine
$C_{21}H_{24}ClNO_4S_2$
Thenium Closilate
$C_{21}H_{24}ClN_3OS$
Pipamazine
$C_{21}H_{24}ClN_3OS,HCl$
Pipamazine Hydrochloride
$C_{21}H_{24}Cl_2N_4O_2S$
Ziprasidone Hydrochloride Monohydrate
$C_{21}H_{24}FN_3O_4$
Moxifloxacin
$C_{21}H_{24}FN_3O_4,HCl$
Moxifloxacin Hydrochloride
$C_{21}H_{24}F_3N_3S$
Trifluoperazine
$C_{21}H_{24}F_3N_3S,2HCl$
Trifluoperazine Hydrochloride
$C_{21}H_{24}O_2$
Gestrinone
$C_{21}H_{25}ClN_2NaO_4S$
Tianeptine Sodium
$C_{21}H_{25}ClN_2O_3$
Cetirizine
$C_{21}H_{25}ClN_2O_4S$
Tianeptine
$C_{21}H_{25}FN_2O_2$
Fluanisone
$C_{21}H_{25}N$
Terbinafine
$C_{21}H_{25}N,HCl$
Terbinafine Hydrochloride
$C_{21}H_{25}NO$
Benzatropine
$C_{21}H_{25}NO,CH_4O_3S$
Benzatropine Mesilate
$C_{21}H_{25}NO_2$
Piperidolate
$C_{21}H_{25}NO_2,HCl$
Piperidolate Hydrochloride
$C_{21}H_{25}NO_3$
Ethylpiperidyl Benzilate
$C_{21}H_{25}NO_3$
Moramide Intermediate
$C_{21}H_{25}NO_3$
Nalmefene
$C_{21}H_{25}NO_3$
Pipethanate
$C_{21}H_{25}NO_3,HCl$
Nalmefene Hydrochloride
$C_{21}H_{25}NO_3,HCl$
Pipethanate Hydrochloride
$C_{21}H_{25}NO_4$
Glaucine
$(C_{21}H_{25}NO_4)_2,3H_3PO_4$
Glaucine Phosphate
$C_{21}H_{25}NO_4,HBr$
Glaucine Hydrobromide
$C_{21}H_{25}NO_4,HCl,3H_2O$
Glaucine Hydrochloride Trihydrate
$C_{21}H_{25}NO_5$
Demecolcine
$C_{21}H_{25}N_3O_2S$
Quetiapine
$(C_{21}H_{25}N_3O_2S)_2C_4H_4O_4$
Quetiapine Fumarate
$C_{21}H_{25}N_3O_3S$
Pipazetate
$C_{21}H_{25}N_3O_3S,HCl$
Pipazetate Hydrochloride
$C_{21}H_{26}BrNO_3$
Mepenzolate Bromide
$C_{21}H_{26}BrNO_3$
Methanthelinium Bromide
$C_{21}H_{26}ClNO$
Clemastine
$C_{21}H_{26}ClNO,C_4H_4O_4$
Clemastine Fumarate
$C_{21}H_{26}ClN_3OS$
Perphenazine
$C_{21}H_{26}ClN_3O_2$
Nemonapride
$C_{21}H_{26}NO_3,CH_3SO_4$
Poldine Metilsulfate
$C_{21}H_{26}N_2O$
Fenpipramide
$C_{21}H_{26}N_2O,HCl,H_2O$
Fenpipramide Hydrochloride
$C_{21}H_{26}N_2OS_2$
Mesoridazine
$C_{21}H_{26}N_2OS_2,C_6H_6O_3S$
Mesoridazine Benzenesulfonate
$C_{21}H_{26}N_2O_2S_2$
Sulforidazine
$C_{21}H_{26}N_2O_3$
Yohimbine
$C_{21}H_{26}N_2O_3,HCl$
Yohimbine Hydrochloride
$C_{21}H_{26}N_2O_7$
Nimodipine
$C_{21}H_{26}N_2S_2$
Thioridazine
$C_{21}H_{26}N_2S_2,HCl$
Thioridazine Hydrochloride
$C_{21}H_{26}O_2$
Mestranol
$C_{21}H_{26}O_3$
Acitretin
$C_{21}H_{26}O_5$
Prednisone
$C_{21}H_{27}ClN_2O_2$
Hydroxyzine
$C_{21}H_{27}ClN_2O_2,C_{23}H_{16}O_6$
Hydroxyzine Embonate
$C_{21}H_{27}ClN_2O_2,2HCl$
Hydroxyzine Hydrochloride
$C_{21}H_{27}C_{l3}N_2O_3$
Cetirizine Dihydrochloride
$C_{21}H_{27}FO_6$
Triamcinolone
$C_{21}H_{27}N$
Budipine
$C_{21}H_{27}N$
Butriptyline
$C_{21}H_{27}N,HCl$
Butriptyline Hydrochloride
$C_{21}H_{27}NO$
Difenidol
$C_{21}H_{27}NO$
Isomethadone
$C_{21}H_{27}NO$
Methadone
$C_{21}H_{27}NO,HBr$
Isomethadone Hydrobromide (*dl*-Form)
$C_{21}H_{27}NO,HCl$
Difenidol Hydrochloride
$C_{21}H_{27}NO,HCl$
Isomethadone Hydrochloride (*d*-Form)
$C_{21}H_{27}NO,HCl$
Methadone Hydrochloride
$C_{21}H_{27}NO,HCl,H_2O$
Isomethadone Hydrochloride Monohydrate (*d*-Form)
$C_{21}H_{27}NO_2$
Diethylaminoethyl Diphenylpropionate
$C_{21}H_{27}NO_3$
Benaprizine
$C_{21}H_{27}NO_3,HCl$
Benaprizine Hydrochloride
$C_{21}H_{27}NO_4$
Nalbuphine
$C_{21}H_{27}NO_4,HCl$
Nalbuphine Hydrochloride
$C_{21}H_{27}Na_2O_8P$
Prednisolone Sodium Phosphate
$C_{21}H_{27}N_3O_2$
Methysergide
$C_{21}H_{27}N_3O_2,C_4H_4O_4$
Methysergide Maleate
$C_{21}H_{27}N_3O_7S$
Bacampicillin
$C_{21}H_{27}N_3O_7S,HCl$
Bacampicillin Hydrochloride
$C_{21}H_{27}N_5O_4S$
Glipizide
$C_{21}H_{28}ClN$
Budipine Hydrochloride
$C_{21}H_{28}N_2O$
Diampromide
$C_{21}H_{28}N_2O_5$
Ramiprilat
$C_{21}H_{28}N_2O_5$
Trimethobenzamide
$C_{21}H_{28}N_2O_5,HCl$
Trimethobenzamide Hydrochloride
$C_{21}H_{28}O_2$
Dydrogesterone
$C_{21}H_{28}O_2$
Ethisterone

$(C_{22}H_{27}NO_2)_2,H_2SO_4$
Lobeline Sulfate
$C_{22}H_{27}NO_2,HCl$
Lobeline Hydrochloride
$C_{22}H_{27}NO_3$
Dioxaphetyl Butyrate
$C_{22}H_{27}N_3O_3S_2$
Metopimazine
$C_{22}H_{27}N_3O_4,HCl$
Diperodon Hydrochloride
$C_{22}H_{27}N_3O_4,H_2O$
Diperodon
$C_{22}H_{28}BrNO_3$
Benzilonium Bromide
$C_{22}H_{28}BrNO_3$
Pipenzolate Bromide
$C_{22}H_{28}ClNO_2$
Amineptine Hydrochloride
$C_{22}H_{28}FNa_2O_8P$
Betamethasone Sodium Phosphate
$C_{22}H_{28}FNa_2O_8P$
Dexamethasone Sodium Phosphate
$C_{22}H_{28}F_2O_5$
Flumetasone
$C_{22}H_{28}N_2O$
Fentanyl
$C_{22}H_{28}N_2O,C_6H_8O_7$
Fentanyl Citrate
$C_{22}H_{28}N_2O_2$
Anileridine
$C_{22}H_{28}N_2O_2$
Encainide
$C_{22}H_{28}N_2O_2,HCl$
Encainide Hydrochloride
$C_{22}H_{28}N_2O_2,H_3PO_4$
Anileridine Phosphate
$C_{22}H_{28}N_2O_2,2HCl$
Anileridine Hydrochloride
$C_{22}H_{28}N_4O_3$
Etonitazene
$C_{22}H_{28}N_4O_3,HCl$
Etonitazene Hydrochloride
$C_{22}H_{28}N_4O_6$
Mitoxantrone
$C_{22}H_{28}N_4O_6,2HCl$
Mitoxantrone Hydrochloride
$C_{22}H_{28}N_6O_3S$
Delavirdine
$C_{22}H_{28}N_6O_3S,CH_4O_3S$
Delavirdine Monomethanesulfonate
$C_{22}H_{28}O_3$
Norethisterone Acetate
$C_{22}H_{28}O_5$
Pyrethrin II
$C_{22}H_{29}ClO_5$
Beclometasone
$C_{22}H_{29}FO_4$
Fluocortolone
$C_{22}H_{29}FO_4$
Fluorometholone
$C_{22}H_{29}FO_5$
Betamethasone
$C_{22}H_{29}FO_5$
Dexamethasone
$C_{22}H_{29}FO_5$
Paramethasone
$C_{22}H_{29}NO_2$
Dextropropoxyphene
$C_{22}H_{29}NO_2$
Levopropoxyphene
$C_{22}H_{29}NO_2$
Noracymethadol
$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O$
Dextropropoxyphene Napsilate
$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O$
Levopropoxyphene Napsilate
$C_{22}H_{29}NO_2,HCl$
Dextropropoxyphene Hydrochloride
$C_{22}H_{29}N_3O_6S$
Pivampicillin
$C_{22}H_{29}N_3O_6S,HCl$
Pivampicillin Hydrochloride
$C_{22}H_{29}N_3S_2$
Thiethylperazine
$C_{22}H_{29}N_3S_2,2C_4H_4O_4$
Thiethylperazine Maleate
$C_{22}H_{29}N_3S_2,2C_4H_6O_5$
Thiethylperazine Malate
$C_{22}H_{29}N_9O_6,4H_2O$
Diminazene Aceturate
$C_{22}H_{30}Cl_2N_{10}$
Chlorhexidine
$C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$
Chlorhexidine Diacetate
$C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$
Chlorhexidine Digluconate
$C_{22}H_{30}Cl_2N_{10},2HCl$
Chlorhexidine Hydrochloride
$C_{22}H_{30}N_2$
Aprindine
$C_{22}H_{30}N_2,HCl$
Aprindine Hydrochloride
$C_{22}H_{30}N_2O_2S$
Sufentanil
$C_{22}H_{30}N_2O_2S,C_6H_8O_7$
Sufentanil Citrate
$C_{22}H_{30}N_2O_5S_2$
Spirapril
$C_{22}H_{30}N_2O_5S_2,HCl$
Spirapril Hydrochloride
$C_{22}H_{30}N_2O_8S_2$
Epalrestat *N*-Methyl-D-Glucamine
$C_{22}H_{30}N_4O_2S_2$
Thioproperazine
$C_{22}H_{30}N_4O_2S_2,2CH_4O_3S$
Thioproperazine Mesilate
$C_{22}H_{30}N_6O_4S$
Sildenafil
$C_{22}H_{30}N_6O_4S,C_6H_8O_7$
Sildenafil Citrate
$C_{22}H_{30}O_4$
Δ^9-Tetrahydrocannabinolic Acid
$C_{22}H_{30}O_5$
Methylprednisolone
$C_{22}H_{31}NO$
Tolterodine
$C_{22}H_{31}NO,C_4H_6O_6$
Tolterodine Tartrate
$C_{22}H_{31}NO_3$
Oxybutynin
$C_{22}H_{31}NO_3,HCl$
Oxybutynin Hydrochloride
$C_{22}H_{31}N_3O_5,H_2O$
Cilazapril
$C_{22}H_{32}Br_2N_4O_4$
Distigmine Bromide
$C_{22}H_{32}N_2O_2$
Dopexamine
$C_{22}H_{32}N_2O_5$
Benzquinamide
$C_{22}H_{32}N_2O_5,HCl$
Benzquinamide Hydrochloride
$C_{22}H_{32}N_2O_6$
Hexoprenaline
$C_{22}H_{32}N_2O_6,H_2SO_4$
Hexoprenaline Sulfate
$C_{22}H_{32}N_2O_2,2HCl$
Dopexamine Dihydrochloride
$C_{22}H_{32}N_2O_6,2HCl$
Hexoprenaline Hydrochloride
$C_{22}H_{32}O_3$
Metenolone Acetate
$C_{22}H_{32}O_3$
Stenbolone Acetate
$C_{22}H_{32}O_3$
Testosterone Propionate
$C_{22}H_{32}O_8$
HT-2 Toxin
$C_{22}H_{33}NO_3$
Cyclomethycaine
$C_{22}H_{33}NO_3,H_2SO_4$
Cyclomethycaine Sulfate
$C_{22}H_{34}INO_4$
Furethidine Methiodide
$C_{22}H_{36}N_2O_5S$
Tirofiban
$C_{22}H_{36}N_2O_5S,HCl,H_2O$
Tirofiban Hydrochloride Monohydrate
$C_{22}H_{36}O_5$
Limaprost
$C_{22}H_{38}O_5$
Misoprostol
$C_{22}H_{40}BrNO$
Domiphen Bromide
$C_{22}H_{43}N_5O_{13}$
Amikacin
$C_{22}H_{43}N_5O_{13},2H_2SO_4$
Amikacin Sulfate
$C_{23}H_{14}Na_2O_{11}$
Sodium Cromoglicate
$C_{23}H_{16}O_3$
Diphenadione
$C_{23}H_{16}O_6,C_{17}H_{20}N_4S,H_2O$
Olanzapine Embonate
$C_{23}H_{20}N_2O_3S$
Sulfinpyrazone
$C_{23}H_{21}ClN_6O_3$
Loprazolam
$C_{23}H_{21}ClN_6O_3,CH_4SO_3,H_2O$
Loprazolam Mesilate
$C_{23}H_{21}ClO_3$
Chlorotrianisene
$C_{23}H_{22}O_6$
Rotenone
$C_{23}H_{23}N_2S_2,I$
Dithiazanine Iodide
$C_{23}H_{23}N_3O_5$
Topotecan
$C_{23}H_{23}N_3O_5,HCl$
Topotecan Hydrochloride
$C_{23}H_{24}ClN_2NaO_9$
Clomocycline Sodium
$C_{23}H_{24}O_4$
Cyclofenil
$C_{23}H_{25}ClN_2O_9$
Clomocycline
$C_{23}H_{25}F_3N_2OS$
Flupentixol
$C_{23}H_{25}F_3N_2OS,2HCl$
Flupentixol Hydrochloride
$C_{23}H_{25}N_5O_5$
Doxazosin
$C_{23}H_{25}N_5O_5,CH_3SO_3H$
Doxazosin Mesilate
$C_{23}H_{25}N_5O_5,HCl$
Doxazosin Hydrochloride
$C_{23}H_{26}N_2O_4$
Brucine
$(C_{23}H_{26}N_2O_4)_2,H_2SO_4,7H_2O$
Brucine Sulfate
$C_{23}H_{26}N_2O_5$
Quinaprilat (Diacid of Quinapril)
$C_{23}H_{26}N_2O_4,4H_2O$
Brucine tetrahydrate
$C_{23}H_{26}N_4O_9S_2$
Quinuronium Sulfate
$C_{23}H_{27}Cl_2N_3O_2$
Aripiprazole
$C_{23}H_{27}FN_4O_2$
Risperidone
$C_{23}H_{27}NO$
Deptropine
$C_{23}H_{27}NO,C_6H_8O_7$
Deptropine Citrate
$C_{23}H_{27}NO_5$
Octaverine
$C_{23}H_{27}NO_5,HBr$
Noracymethadol Hydrochloride
$C_{23}H_{27}NO_5,HBr$
Norlevorphanol Hydrobromide
$C_{23}H_{27}NO_5,HCl$
Octaverine Hydrochloride
$C_{23}H_{27}NO_8$
Narceine
$C_{23}H_{27}NO_8,HCl$
Narceine Hydrochloride
$C_{23}H_{27}N_3O_2$
Morazone
$C_{23}H_{27}N_3O_2,HCl$
Morazone Hydrochloride
$C_{23}H_{27}N_3O_7$
Minocycline

$C_{23}H_{27}N_3O_7$,HCl
Minocycline Hydrochloride
$C_{23}H_{28}ClN_3O_2S$
Thiopropazate
$C_{23}H_{28}ClN_3O_2S$,2HCl
Thiopropazate Hydrochloride
$C_{23}H_{28}ClN_3O_5S$
Glibenclamide
$C_{23}H_{28}N_2O_3$
Bopindolol
$C_{23}H_{28}N_2O_4$
Quinine Ethyl Carbonate
$C_{23}H_{28}O_6$
Enprostil
$C_{23}H_{28}O_6$
Prednisone Acetate
$C_{23}H_{29}ClFN_3O_4$
Cisapride
$C_{23}H_{29}ClFN_3O_4,H_2O$
Cisapride Monohydrate
$C_{23}H_{29}ClO_4$
Chlormadinone Acetate
$C_{23}H_{29}NO$
Norpipanone
$C_{23}H_{29}NO$,HBr
Norpipanone Hydrobromide
$C_{23}H_{29}NO$,HCl
Norpipanone Hydrochloride
$C_{23}H_{29}NO_2$
Phenadoxone
$C_{23}H_{29}NO_2$,HCl
Phenadoxone Hydrochloride
$C_{23}H_{29}NO_3$
Benzethidine
$C_{23}H_{29}NO_3$
Fenbutrazate
$C_{23}H_{29}NO_3$
Phenoperidine
$C_{23}H_{29}NO_3$,HCl
Fenbutrazate Hydrochloride
$C_{23}H_{29}NO_3$,HCl
Phenoperidine Hydrochloride
$C_{23}H_{29}N_3O$
Opipramol
$C_{23}H_{29}N_3O_2$
Oxypertine
$C_{23}H_{29}N_3O$,2HCl
Opipramol Hydrochloride
$C_{23}H_{29}N_3O_2S$
Acetophenazine
$C_{23}H_{29}N_3O_2S_2$
Tiotixene
$C_{23}H_{29}N_3O_2S$,2$C_4H_4O_4$
Acetophenazine Dimaleate
$C_{23}H_{29}N_3O_2S_2$,2HCl,2H_2O
Tiotixene Hydrochloride
$C_{23}H_{30}BrNO_3$
Pipethanate Ethobromide
$C_{23}H_{30}BrNO_3$
Propantheline Bromide
$C_{23}H_{30}ClN_3O$
Mepacrine
$C_{23}H_{30}ClN_3O$,2CH_3SO_3H,H_2O
Mepacrine Mesilate
$C_{23}H_{30}ClN_3O$,2HCl,2H_2O
Mepacrine Hydrochloride
$C_{23}H_{30}N_2O_2$
Piminodine
$C_{23}H_{30}N_2O_2$,$C_2H_6O_3S$
Piminodine Esilate
$C_{23}H_{30}N_2O_4$,H_2O
Pholcodine
$C_{23}H_{30}N_2O_4$,2$C_4H_6O_6$,3H_2O
Pholcodine Tartrate
$C_{23}H_{30}N_4O_2S$
Perospirone
$C_{23}H_{30}N_4O_2S$,HCl
Perospirone Hydrochloride
$C_{23}H_{30}O_3$
Etretinate
$C_{23}H_{30}O_4$
Elcometrine
$C_{23}H_{30}O_6$
Cortisone Acetate
$C_{23}H_{30}O_6$
Prednisolone Acetate
$C_{23}H_{31}FO_5$
Flugestone Acetate
$C_{23}H_{31}FO_6$
Fludrocortisone Acetate
$C_{23}H_{31}NO_2$
Levomethadyl Acetate
$C_{23}H_{31}NO_2$
Proadifen
$C_{23}H_{31}NO_2$,HCl
Levomethadyl Acetate Hydrochloride
$C_{23}H_{31}NO_2$,HCl
Proadifen Hydrochloride
$C_{23}H_{31}NO_7$
Mycophenolate Mofetil
$C_{23}H_{32}N_2O_2S$
Tiocarlide
$C_{23}H_{32}N_2O_5$
Ramipril
$C_{23}H_{32}N_6O_4S$
Vardenafil
$C_{23}H_{32}N_6O_4S$,HCl,3H_2O
Vardenafil Hydrochloride Trihydrate
$C_{23}H_{32}N_6O_4S$,2HCl
Vardenafil Dihydrochloride
$C_{23}H_{32}O_2$,H_2O
Dimethisterone
$C_{23}H_{32}O_3$
Estradiol Valerate
$C_{23}H_{32}O_4$
Desoxycortone Acetate
$C_{23}H_{32}O_6$
Hydrocortisone Acetate
$C_{23}H_{33}IN_2O$
Isopropamide Iodide
$C_{23}H_{33}N_2O_2$,$C_4H_5O_6$
Prajmalium Bitartrate
$C_{23}H_{34}NO_5P$
Fosinopril Diacid
$C_{23}H_{34}O_5$
Alfadolone Acetate
$C_{23}H_{34}O_6$
Pravastatin Lactone
$C_{23}H_{35}O_7Na$
Pravastatin Sodium
$C_{23}H_{36}N_2O_2$
Finasteride
$C_{23}H_{36}N_6O_5S$
Argatroban
$C_{23}H_{36}N_6O_5S$,H_2O
Argatroban Monohydrate
$C_{23}H_{36}O_3$
Drostanolone Propionate
$C_{23}H_{36}O_7$
Pravastatin
$C_{23}H_{37}N_5O_6S$
Etamiphylline Camsilate
$C_{23}H_{42}N_2O_{12}$
Pentolonium Tartrate
$C_{23}H_{45}N_5O_{14}$
Paromomycin
$C_{23}H_{46}N_6O_{13}$
Neomycin
$C_{24}H_{19}NO_5$
Oxyphenisatine Acetate
$C_{24}H_{20}N_6O_3$
Candesartan
$C_{24}H_{21}F_2NO_3$
Ezetimibe
$C_{24}H_{23}NO$
JWH-018
$C_{24}H_{24}N_2O_4$
Nicocodine
$C_{24}H_{25}FNNaO_4$
Fluvastatin Sodium
$C_{24}H_{25}FN_6O$
Mizolastine
$C_{24}H_{25}NO_3$
Benzylmorphine
$C_{24}H_{25}NO_3$,HCl
Benzylmorphine Hydrochloride
$C_{24}H_{25}NO_4$
Flavoxate
$C_{24}H_{25}NO_4$,HCl
Flavoxate Hydrochloride
$C_{24}H_{26}BrN_3O_3$
Nicergoline
$C_{24}H_{26}ClFN_4O$
Sertindole
$C_{24}H_{26}FNO_4$
Fluvastatin
$C_{24}H_{26}FN_3O$
Biriperone
$C_{24}H_{26}N_2O_4$
Carvedilol
$C_{24}H_{26}N_6O_3$
Olmesartan
$C_{24}H_{27}ClN_2O_2S$
Zuclopenthixol Acetate
$C_{24}H_{27}N$
Prenylamine
$C_{24}H_{27}N$,$C_3H_6O_3$
Prenylamine Lactate
$C_{24}H_{27}NO_2$
Levophenacylmorphan
$C_{24}H_{27}NO_5S$
Troglitazone
$C_{24}H_{28}N_2O_3$
Naftopidil
$C_{24}H_{28}N_2O_5$
Benazepril
$C_{24}H_{28}N_2O_5$,HCl
Benazepril Hydrochloride
$C_{24}H_{28}N_2O_3$,2HCl
Naftopidil Dihydrochloride
$C_{24}H_{28}O_4$
Diethylstilbestrol Dipropionate
$C_{24}H_{29}Cl_2FO_5$
Fluclorolone Acetonide
$C_{24}H_{29}NO$
Phenomorphan
$C_{24}H_{29}NO$,HBr
Phenomorphan Hydrobromide
$C_{24}H_{29}NO_3$
Donepezil
$C_{24}H_{29}NO_3$,HCl
Donepezil Hydrochloride
$C_{24}H_{29}N_5O_3$
Valsartan
$C_{24}H_{30}F_2O_6$
Fluocinolone Acetonide
$C_{24}H_{30}N_2O_2$
Doxapram
$C_{24}H_{30}N_2O_2$,HCl,H_2O
Doxapram Hydrochloride
$C_{24}H_{30}N_2O_2S$
Piperacetazine
$C_{24}H_{30}O_3$
Drospirenone
$C_{24}H_{30}O_4$
Hexestrol Dipropionate
$C_{24}H_{31}FO_6$
Betamethasone Acetate
$C_{24}H_{31}FO_6$
Dexamethasone Acetate
$C_{24}H_{31}FO_6$
Paramethasone Acetate
$C_{24}H_{31}FO_6$
Triamcinolone Acetonide
$C_{24}H_{31}NO$
Dipipanone
$C_{24}H_{31}NO$,HCl,H_2O
Dipipanone Hydrochloride
$C_{24}H_{31}N_3O$
Famprofazone
$C_{24}H_{31}N_3OS$
Butaperazine
$C_{24}H_{31}N_3OS$,2$C_4H_4O_4$
Butaperazine Maleate
$C_{24}H_{31}N_3OS$,2H_3PO_4
Butaperazine Phosphate
$C_{24}H_{31}N_3O_2S$
Carfenazine
$C_{24}H_{31}N_3O_2S$,2$C_4H_4O_4$
Carfenazine Maleate
$C_{24}H_{32}ClFO_5$
Halcinonide

$C_{24}H_{32}O_4$
Etynodiol Diacetate
$C_{24}H_{32}O_4$
Megestrol Acetate
$C_{24}H_{32}O_4S$
Spironolactone
$C_{24}H_{32}O_6$
Desonide
$C_{24}H_{32}O_6$
Methylprednisolone Acetate
$C_{24}H_{33}FO_6$
Fludroxycortide
$C_{24}H_{33}NO_3,C_2H_2O_4$
Naftidrofuryl Oxalate
$C_{24}H_{33}N_3O_2S$
Dixyrazine
$C_{24}H_{33}N_3O_3S_2$
Pipotiazine
$C_{24}H_{34}N_2O_5$
Trandolapril
$C_{24}H_{34}N_4O_5S$
Glimepiride
$C_{24}H_{34}O_4$
Medroxyprogesterone Acetate
$C_{24}H_{34}O_9$
T-2 Toxin
$C_{24}H_{35}NO_5$
Decoquinate
$C_{24}H_{36}O_3$
Nabilone
$C_{24}H_{36}O_4$
Methandriol Diacetate
$C_{24}H_{36}O_5$
Lovastatin
$C_{24}H_{40}N_2$
Conessine
$C_{24}H_{40}N_8O_4$
Dipyridamole
$C_{24}H_{40}N_2,2HBr$
Conessine Hydrobromide
$C_{25}H_{19}NO_3$
Fendosal
$C_{25}H_{27}ClN_2$
Meclozine
$C_{25}H_{27}ClN_2,2HCl,H_2O$
Meclozine Hydrochloride
$C_{25}H_{28}N_6O$
Irbesartan
$C_{25}H_{28}O_3$
Estradiol Benzoate
$C_{25}H_{29}I_2NO_3$
Amiodarone
$C_{25}H_{29}I_2NO_3,HCl$
Amiodarone Hydrochloride
$C_{25}H_{30}N_2O_5$
Quinapril
$C_{25}H_{30}N_2O_5,HCl$
Quinapril Hydrochloride
$C_{25}H_{31}FO_8$
Triamcinolone Diacetate
$C_{25}H_{31}F_3O_5S$
Fluticasone Propionate
$C_{25}H_{31}NO_3$
Metindizate
$C_{25}H_{31}NO_3,HCl$
Metindizate Hydrochloride
$C_{25}H_{31}NO_6$
Deflazacort
$C_{25}H_{32}ClFO_5$
Clobetasol Propionate
$C_{25}H_{32}ClN_5O_2$
Nefazodone
$C_{25}H_{32}ClN_5O_2,HCl$
Nefazodone Hydrochloride
$C_{25}H_{32}N_2O_2$
Levomoramide
$C_{25}H_{32}N_2O_2$
Racemoramide
$C_{25}H_{32}N_2O_2,C_4H_6O_6$
Dextromoramide Tartrate
$C_{25}H_{32}O_8$
Prednisolone Succinate
$C_{25}H_{33}NO_4$
Etorphine
$C_{25}H_{33}NO_4,HCl$
Etorphine Hydrochloride
$C_{25}H_{33}N_5O_{10}$
Trimetrexate D-Glucuronate
$C_{25}H_{34}O_8$
Hydrocortisone Hydrogen Succinate
$C_{25}H_{35}NO_5$
Mebeverine
$C_{25}H_{35}NO_5,HCl$
Mebeverine Hydrochloride
$C_{25}H_{35}NaO_8$
Hydrocortisone Sodium Succinate
$C_{25}H_{35}N_3O_6S$
Amprenavir
$C_{25}H_{35}O_9$
Ryanodine
$C_{25}H_{36}O_6$
Hydrocortisone Butyrate
$C_{25}H_{37}NO_4$
Salmeterol
$C_{25}H_{37}NO_4,C_{11}H_8O_3$
Salmeterol Xinafoate
$C_{25}H_{38}N_2O$
Bunamidine
$C_{25}H_{38}N_2O,HCl$
Bunamidine Hydrochloride
$C_{25}H_{38}O_3$
Testosterone Isocaproate
$C_{25}H_{38}O_5$
Simvastatin
$C_{25}H_{39}N_3O_8$
Landiolol
$C_{25}H_{39}N_3O_8,HCl$
Landiolol Hydrochloride
$C_{25}H_{43}NO_{18}$
Acarbose
$C_{25}H_{43}N_{13}O_{10}$
Viomycin
$C_{25}H_{43}N_{13}O_{10},H_2SO_4$
Viomycin Sulfate
$C_{25}H_{44}ClN_3O_2$
Dofamium Chloride
$C_{25}H_{44}N_2O$
Azacosterol
$C_{25}H_{44}N_{14}O_8$
Capreomycin
$C_{25}H_{46}ClN$
Cetalkonium Chloride
$C_{25}H_{48}N_6O_8$
Desferrioxamine
$C_{25}H_{48}N_6O_8,CH_3SO_3H$
Desferrioxamine Mesilate
$C_{26}H_{27}Br_2N_7$
Pyritidium Bromide
$C_{26}H_{27}ClN_2O$
Lofepramine
$C_{26}H_{27}ClN_2O,HCl$
Lofepramine Hydrochloride
$C_{26}H_{27}NO_9$
Idarubicin
$C_{26}H_{27}NO_9,HCl$
Idarubicin Hydrochloride
$C_{26}H_{27}N_3,HCl$
Pyrvinium Chloride
$C_{26}H_{28}ClNO$
Clomifene
$C_{26}H_{28}ClNO$
Toremifene
$C_{26}H_{28}ClNO,C_6H_8O_7$
Clomifene Citrate
$C_{26}H_{28}ClNO,C_6H_8O_7$
Toremifene Citrate
$C_{26}H_{28}Cl_2N_4O_4$
Ketoconazole
$C_{26}H_{28}N_2$
Cinnarizine
$C_{26}H_{29}FN_2O_2$
Levocabastine
$C_{26}H_{29}FN_2O_2,HCl$
Levocabastine Hydrochloride
$C_{26}H_{29}NO_3$
Amotriphene
$C_{26}H_{29}NO_3,HCl$
Amotriphene Hydrochloride
$C_{26}H_{30}Cl_2F_3NO$
Halofantrine
$C_{26}H_{30}Cl_2F_3NO,HCl$
Halofantrine Hydrochloride
$C_{26}H_{30}O_5$
Boldenone Benzoate
$C_{26}H_{32}ClFO_5$
Clobetasone Butyrate
$C_{26}H_{32}F_2O_7$
Fluocinonide
$C_{26}H_{32}N_2O_5$
Delapril
$C_{26}H_{32}N_2O_5,HCl$
Delapril Hydrochloride
$C_{26}H_{32}N_2O_7$
Bopindolol Malonate
$C_{26}H_{33}FNNaO_5$
Cerivastatin Sodium
$C_{26}H_{33}NO_4$
Cyprenorphine
$C_{26}H_{33}NO_6$
Lacidipine
$C_{26}H_{33}NaO_8$
Methylprednisolone Sodium Succinate
$C_{26}H_{34}FNO_5$
Cerivastatin
$C_{26}H_{34}O_8$
Methylprednisolone Hemisuccinate
$C_{26}H_{35}NO_4$
Diprenorphine
$C_{26}H_{35}NO_4,HCl$
Diprenorphine Hydrochloride
$C_{26}H_{36}O_3$
Estradiol Cipionate
$C_{26}H_{36}O_6$
Prednisolone Pivalate
$C_{26}H_{37}N_5O_2$
Cabergoline
$C_{26}H_{37}N_5O_2,2H_3PO_4$
Cabergoline Diphosphate
$C_{26}H_{38}O_4$
Desoxycortone Pivalate
$C_{26}H_{38}O_4$
Gestonorone Caproate
$C_{26}H_{38}O_4$
Oxabolone Cipionate
$C_{26}H_{38}O_6$
Hydrocortisone Valerate
$C_{26}H_{40}O_3$
Dehydroepiandrosterone Enanthate
$C_{26}H_{40}O_3$
Testosterone Enantate
$C_{26}H_{40}O_4$
Methandriol Dipropionate
$C_{26}H_{42}O_4$
Maxacalcitol
$C_{26}H_{44}O_9$
Mupirocin
$C_{27}H_{22}Cl_2N_4$
Clofazimine
$C_{27}H_{23}N_5O_4$
Pranlukast
$C_{27}H_{25}Cl_2N_6$
Acriflavinium Chloride
$C_{27}H_{29}NO_{10}$
Daunorubicin
$C_{27}H_{29}NO_{10},C_6H_8O_7$
Daunorubicin Lactate
$C_{27}H_{29}NO_{10},HCl$
Daunorubicin Hydrochloride
$C_{27}H_{29}NO_{11}$
Doxorubicin
$C_{27}H_{29}NO_{11}$
Epirubicin
$C_{27}H_{29}NO_{11},HCl$
Doxorubicin Hydrochloride
$C_{27}H_{29}NO_{11},HCl$
Epirubicin Hydrochloride
$C_{27}H_{30}Cl_2O_6$
Mometasone Furoate
$C_{27}H_{30}F_6N_2O_2$
Dutasteride
$C_{27}H_{30}N_2O_7,HCl$
Moexipril Diacid Hydrochloride

$C_{27}H_{32}N_2O_7$
Bopindolol Maleate
$C_{27}H_{33}N_3O_6S$
Gliquidone
$C_{27}H_{33}N_3O_8$
Rolitetracycline
$C_{27}H_{33}N_3O_8,HNO_3,1½H_2O$
Rolitetracycline Nitrate
$C_{27}H_{34}N_2O_7$
Moexipril
$C_{27}H_{34}N_2O_7,HCl$
Moexipril Hydrochloride
$C_{27}H_{34}N_4O$
Piritramide
$C_{27}H_{34}O_3$
Nandrolone Phenylpropionate
$C_{27}H_{35}NO_5$
Acetorphine
$C_{27}H_{35}NO_5,HCl$
Acetorphine Hydrochloride
$C_{27}H_{36}F_2O_6$
Flumetasone Pivalate
$C_{27}H_{36}N_2O_4$
Repaglinide
$C_{27}H_{37}FO_5$
Fluocortolone Pivalate
$C_{27}H_{37}FO_6$
Betamethasone Valerate
$C_{27}H_{38}N_2O_4$
Verapamil
$C_{27}H_{38}N_2O_4,HCl$
Verapamil Hydrochloride
$C_{27}H_{38}O_6,H_2O$
Prednisolone Tebutate
$C_{27}H_{40}O_3$
Testosterone Cipionate
$C_{27}H_{40}O_4$
Hydroxyprogesterone Caproate
$C_{27}H_{42}ClNO,H_2O$
Octafonium Chloride
$C_{27}H_{42}ClNO_2$
Benzethonium Chloride
$C_{27}H_{42}Cl_2N_2O_6$
Chloramphenicol Palmitate
$C_{27}H_{42}N_3O_3$
Detajmium
$C_{27}H_{42}O_3$
Metenolone Enantate
$C_{27}H_{43}NO$
Solanidine
$C_{27}H_{44}O$
Colecalciferol
$C_{27}H_{44}O_2$
Alfacalcidol
$C_{27}H_{46}O$
Cholesterol
$C_{28}H_{24}Br_2N_6$
Fazadinium Bromide
$C_{28}H_{26}ClN_7$
Isometamidium
$C_{28}H_{27}ClF_5NO$
Penfluridol
$C_{28}H_{27}NO_4S$
Raloxifene
$C_{28}H_{27}NO_4S,HCl$
Raloxifene Hydrochloride
$C_{28}H_{29}F_2N_3O$
Pimozide
$C_{28}H_{29}NO_4$
Bephenium Hydroxynaphthoate
$C_{28}H_{31}FN_4O$
Astemizole
$C_{28}H_{32}FNO_6$
Dexamethasone Isonicotinate
$C_{28}H_{33}ClN_2$
Buclizine
$C_{28}H_{33}ClN_2,2HCl$
Buclizine Hydrochloride
$C_{28}H_{33}N_5O_5$
Ergotoxine
$C_{28}H_{34}N_2O_2$
Etymide
$C_{28}H_{34}N_2O_3$
Denatonium Benzoate
$C_{28}H_{35}ClN_4O$
Clospipramine
$C_{28}H_{35}ClN_4O,2HCl$
Clospipramine Dihydrochloride
$C_{28}H_{36}O_3$
Testosterone Phenylpropionate
$C_{28}H_{37}ClO_7$
Beclometasone Dipropionate
$C_{28}H_{37}FO_7$
Betamethasone Dipropionate
$C_{28}H_{38}N_2O_4$
Cephaëline
$C_{28}H_{38}N_2O_4,2HCl$
Cephaëline Hydrochloride
$C_{28}H_{39}FO_5$
Fluocortolone Hexanoate
$C_{28}H_{39}FO_6$
Dexamethasone Tebutate
$C_{28}H_{40}N_2O_2$
Bialamicol
$C_{28}H_{40}N_2O_2,2HCl$
Bialamicol Hydrochloride
$C_{28}H_{41}N_3O_3$
Oxetacaine
$C_{28}H_{42}Cl_4N_4O_2$
Ambenonium Chloride
$C_{28}H_{44}O$
Ergocalciferol
$C_{28}H_{44}O_2$
Doxercalciferol
$C_{28}H_{44}O_3$
Nandrolone Decanoate
$C_{28}H_{46}O$
Dihydrotachysterol
$C_{29}H_{25}N_3O_5$
Nicomorphine
$C_{29}H_{25}N_3O_5,HCl$
Nicomorphine Hydrochloride
$C_{29}H_{30}N_6O_6$
Olmesartan Medoxomil
$C_{29}H_{31}F_2N_3O$
Fluspirilene
$C_{29}H_{32}O_{13}$
Etoposide
$C_{29}H_{33}ClN_2O_2$
Loperamide
$C_{29}H_{33}ClN_2O_2,HCl$
Loperamide Hydrochloride
$C_{29}H_{33}FO_6$
Betamethasone Benzoate
$C_{29}H_{33}O_{16}P$
Etoposide Phosphate
$C_{29}H_{35}NO_2$
Mifepristone
$C_{29}H_{38}FN_3O_3$
Mibefradil
$C_{29}H_{38}FN_3O_3,2HCl$
Mibefradil Dihydrochloride
$C_{29}H_{38}F_3N_3O_2S$
Fluphenazine Enantate
$C_{29}H_{38}N_2O_4$
Dehydroemetine
$C_{29}H_{38}N_2O_4,2HCl$
Dehydroemetine Hydrochloride
$C_{29}H_{38}N_4O_{10}$
Lymecycline
$C_{29}H_{39}Cl_2F_3NO_7P$
Halofantrine β-Glycerophosphate
$C_{29}H_{40}N_2O_4$
Emetine
$C_{29}H_{40}N_2O_4,2HCl,7H_2O$
Emetine Hydrochloride
$C_{29}H_{41}NO_4$
Buprenorphine
$C_{29}H_{41}NO_4,HCl$
Buprenorphine Hydrochloride
$C_{29}H_{42}O_6$
Hydrocortisone Cipionate
$C_{29}H_{44}O_3$
Estradiol Undecylate
$C_{29}H_{44}O_{12},8H_2O$
Ouabain
$C_{29}H_{48}O_3$
Testosterone Undecylate
$C_{29}H_{53}NO_5$
Orlistat
$C_{30}H_{24}N_4O_{10}$
Nicofuranose
$C_{30}H_{32}N_2O_2$
Diphenoxylate
$C_{30}H_{32}N_2O_2,HCl$
Diphenoxylate Hydrochloride
$C_{30}H_{34}O_{13}$
Picrotoxin
$C_{30}H_{35}F_2N_3O$
Lidoflazine
$C_{30}H_{40}Cl_2N_4$
Dequalinium Chloride
$C_{30}H_{41}FO_7$
Triamcinolone Hexacetonide
$C_{30}H_{44}N_2O_{10}$
Hexobendine
$C_{30}H_{44}N_2O_{10},2HCl$
Hexobendine Hydrochloride
$C_{30}H_{44}O_3$
Boldenone Undecylenate
$C_{30}H_{45}NNaO_7P$
Fosinopril Sodium
$C_{30}H_{46}NO_7P$
Fosinopril
$C_{30}H_{46}O_4$
Enoxolone
$C_{30}H_{53}NO_{17}$
Benzonatate
$C_{30}H_{60}I_3N_3O_3$
Gallamine Triethiodide
$C_{31}H_{32}N_4O_2$
Bezitramide
$C_{31}H_{33}N_3O_6S$
Zafirlukast
$C_{31}H_{36}IN_3$
Stilbazium Iodide
$C_{31}H_{39}N_5O_5,C_2H_5SO_{3H}$
Ergotoxine Esilate
$C_{31}H_{41}ClFNO_3$
Haloperidol Decanoate
$C_{31}H_{43}N_3Na_{10}O_{49}S_8$
Fondaparinux Sodium
$C_{31}H_{46}O_2$
Phytomenadione
$C_{31}H_{47}NaO_6$
Sodium Fusidate
$C_{31}H_{47}N_3O_9,H_2O$
Detajmium Bitartrate
$C_{31}H_{48}O_6,½H_2O$
Fusidic Acid
$C_{31}H_{52}O_3$
Alpha Tocoferil Acetate
$C_{31}H_{66}Cl_2N_4O,HCl$
Colesevelam Hydrochloride
$C_{32}H_{38}N_2O_8$
Deserpidine
$C_{32}H_{39}NO_2$
Ebastine
$C_{32}H_{39}NO_4$
Fexofenadine
$C_{32}H_{39}NO_4,HCl$
Fexofenadine Hydrochloride
$C_{32}H_{39}NO_6,C_4H_4O_4$
Ebastine Fumarate
$C_{32}H_{40}BrN_5O_5$
Bromocriptine
$C_{32}H_{40}BrN_5O_5,CH_4O_3S$
Bromocriptine Mesilate
$C_{32}H_{43}ClN_2O_2S$
Clopenthixol Decanoate
$C_{32}H_{43}ClN_2O_2S$
Zuclopenthixol Decanoate
$C_{32}H_{44}F_3N_3O_2S$
Fluphenazine Decanoate
$C_{32}H_{45}N_3O_4S$
Nelfinavir
$C_{32}H_{45}N_3O_4S,CH_3SO_3H$
Nelfinavir Mesilate
$C_{32}H_{49}NO_9$
Veratrine
$C_{32}H_{52}Br_2N_4O_4$
Demecarium Bromide

$C_{32}H_{53}BrN_2O_4$
Rocuronium Bromide
$[C_{32}H_{53}N_2O_4]^+$
Rocuronium
$C_{33}H_{25}N_3O_3$
Norbormide
$C_{33}H_{30}N_4O_2$
Telmisartan
$C_{33}H_{33}FN_2O_4$
Atorvastatin Lactone
$C_{33}H_{34}FN_2NaO_5$
Atorvastatin Sodium
$C_{33}H_{34}N_6O_6$
Candesartan Cilexetil
$C_{33}H_{35}FN_2O_5$
Atorvastatin
$C_{33}H_{35}N_5O_5$
Ergotamine
$(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$
Ergotamine Tartrate
$C_{33}H_{37}N_5O_5$
Dihydroergotamine
$(C_{33}H_{37}N_5O_5)_2,C_4H_6O_6$
Dihydroergotamine Tartrate
$C_{33}H_{37}N_5O_5,CH_3SO_3H$
Dihydroergotamine Mesilate
$C_{33}H_{38}N_4O_6$
Irinotecan
$C_{33}H_{38}N_4P_6,HCl,3H_2O$
Irinotecan Hydrochloride Trihydrate
$C_{33}H_{39}ClN_4O_6$
Irinotecan Hydrochloride
$C_{33}H_{40}N_2O_9$
Methoserpidine
$C_{33}H_{40}N_2O_9$
Reserpine
$C_{33}H_{43}F_3N_2O_2S$
Flupentixol Decanoate
$C_{33}H_{47}NO_{13}$
Natamycin
$C_{34}H_{36}MgN_6O_6S_2,3H_2O$
Esomeprazole Magnesium
$C_{34}H_{36}N_2O_6,3H_2O$
Pseudomorphine
$C_{34}H_{36}N_6O_6S_2$
Esomeprazole
$C_{34}H_{36}N_6O_6S_2,Mg$
Omeprazole Magnesium
$C_{34}H_{46}N_4O_4$
Dequalinium Acetate
$C_{34}H_{47}NO_{11}$
Aconitine
$C_{34}H_{47}NO_{11},HNO_3$
Aconitine Nitrate
$C_{34}H_{48}Na_2O_7$
Carbenoxolone Sodium
$C_{34}H_{50}O_7$
Carbenoxolone
$C_{34}H_{59}NO_{14}$
Fumonisin B_2
$C_{34}H_{59}NO_{15}$
Fumonisin B_1
$C_{34}H_{63}ClN_2O_6S,HCl$
Clindamycin Palmitate Hydrochloride
$C_{35}H_{35}ClNNaO_3S$
Montelukast Sodium
$C_{35}H_{36}ClNO_3S$
Montelukast
$C_{35}H_{38}Cl_2N_8O_4$
Itraconazole
$C_{35}H_{41}N_5O_5,CH_4O_3S$
Codergocrine Mesilate
$C_{35}H_{42}N_2O_9$
Rescinnamine
$C_{35}H_{42}N_2O_{11}$
Syrosingopine
$C_{35}H_{48}N_8O_{11}S$
Phallotoxin
$C_{35}H_{51}N_3O_4S_2$
Pipotiazine Undecylenate
$C_{35}H_{60}Br_2N_2O_4$
Pancuronium Bromide
$C_{35}H_{61}NO_{12}$
Oleandomycin
$C_{35}H_{61}NO_{12},H_3PO_4$
Oleandomycin Phosphate
$C_{36}H_{46}N_2O_4$
Bunamidine Hydroxynaphthoate
$C_{36}H_{47}N_5O_4$
Indinavir
$C_{36}H_{47}N_5O_4,H_2SO_4$
Indinavir Sulfate
$C_{36}H_{74}C_{l2}N_2$
Triclobisonium Chloride
$C_{37}H_{42}Cl_2N_2O_6,5H_2O$
Tubocurarine Chloride
$C_{37}H_{46}NNaO_{12}$
Rifamycin Sodium
$C_{37}H_{47}NO_{12}$
Rifamycin SV
$C_{37}H_{48}N_4O_5$
Lopinavir
$C_{37}H_{48}N_6O_5S_2$
Ritonavir
$C_{37}H_{56}O_{13}$
Strophanthin-K
$C_{37}H_{61}N_2O_4,Br$
Rapacuronium Bromide
$C_{37}H_{67}NO_{13}$
Erythromycin
$C_{37}H_{67}NO_{13},C_7H_{14}O_8$
Erythromycin Gluceptate
$C_{37}H_{67}NO_{13},C_{12}H_{22}O_{12}$
Erythromycin Lactobionate
$C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$
Erythromycin Stearate
$C_{38}H_{50}N_6O_5$
Saquinavir
$C_{38}H_{50}N_6O_5,CH_3SO_3H$
Saquinavir Mesilate
$C_{38}H_{51}NO_4$
Myrophine
$C_{38}H_{51}NO_4,HCl$
Myrophine Hydrochloride
$C_{38}H_{69}NO_{13}$
Clarithromycin
$C_{38}H_{72}N_2O_{12}$
Azithromycin
$C_{39}H_{53}N_9O_{15}S$
β-Amanitin
$C_{39}H_{54}N_{10}O_{14}S$
α-Amanitin
$C_{40}H_{48}I_2N_2O_6$
Metocurine Iodide
$C_{40}H_{63}N_3O_4S_2$
Pipotiazine Palmitate
$C_{40}H_{71}NO_{14}$
Erythromycin Propionate
$C_{40}H_{71}NO_{14},C_{12}H_{26}O_4S$
Erythromycin Estolate
$C_{40}H_{74}CaO_{14}S_2$
Docusate Calcium
$C_{40}H_{80}NO_8P$
Colfosceril Palmitate
$C_{41}H_{63}NO_{14}$
Protoveratrine A and B
$C_{41}H_{63}NO_{15}$
Protaveratrine B
$C_{41}H_{64}O_8$
Prednisolone Steaglate
$C_{41}H_{64}O_{13}$
Digitoxin
$C_{41}H_{64}O_{14}$
Digoxin
$C_{41}H_{67}NO_{15}$
Troleandomycin
$C_{41}H_{76}N_2O_{15}$
Roxithromycin
$C_{42}H_{30}N_6O_{12}$
Inositol Nicotinate
$C_{42}H_{53}NO_{15}$
Aclarubicin
$C_{42}H_{53}NO_{15},HCl$
Aclarubicin Hydrochloride
$C_{42}H_{66}O_{14}$
Metildigoxin
$C_{42}H_{78}N_2O_{14}$
Dirithromycin
$C_{43}H_{53}NO_{14}$
Docetaxel
$C_{43}H_{55}N_5O_7$
Vindesine
$C_{43}H_{55}N_5O_7,H_2SO_4$
Vindesine Sulfate
$C_{43}H_{58}N_2O_{13}$
Rifamide
$C_{43}H_{58}N_4O_{12}$
Rifampicin
$C_{43}H_{59}NO_{17}$
Docetaxel Trihydrate
$C_{43}H_{66}O_{14}$
Acetyldigitoxin
$C_{43}H_{66}O_{15}$
α-Acetyldigoxin
$C_{43}H_{75}NO_{16}$
Erythromycin Ethylsuccinate
$C_{44}H_{46}CaN_4O_{18}$
Oxytetracycline Calcium
$C_{44}H_{50}Cl_2N_4O_2,5H_2O$
Alcuronium Chloride
$C_{44}H_{68}O_{13}$
Okadaic Acid
$C_{44}H_{69}NO_{12}$
Tacrolimus
$C_{44}H_{69}NO_{12},H_2O$
Tacrolimus Monohydrate
$C_{45}H_{54}N_4O_8$
Vinorelbine
$C_{45}H_{54}N_4O_8,2C_4H_6O_6$
Vinorelbine Tartrate
$C_{45}H_{73}NO_{15}$
Solanine
$C_{46}H_{56}N_4O_{10}$
Vincristine
$C_{46}H_{56}N_4O_{10},H_2SO_4$
Vincristine Sulfate
$C_{46}H_{58}N_4O_9$
Vinblastine
$C_{46}H_{58}N_4O_9,H_2SO_4$
Vinblastine Sulfate
$C_{46}H_{62}N_4O_{11}$
Rifabutin
$C_{46}H_{77}NO_{17}$
Tylosin
$C_{46}H_{78}N_2O_{15}$
Spiramycin
$C_{46}H_{80}N_2O_{13}$
Tilmicosin
$C_{47}H_{51}NO_{14}$
Paclitaxel
$C_{47}H_{73}NO_{17}$
Amphotericin B
$C_{47}H_{74}O_{19}$
Deslanoside
$C_{47}H_{75}NO_{17}$
Nystatin
$C_{49}H_{66}N_{10}O_{10}S_2$
Octreotide
$C_{49}H_{66}N_{10}O_{10}S_2,xC_2H_4O_2$
Octreotide Acetate
$C_{49}H_{69}ClO_{14}$
Brevetoxin C
$C_{49}H_{70}O_{13}$
Brevetoxin A
$C_{49}H_{75}NO_{13}:C_{48}H_{73}NO_{13}$
Emamectin
$C_{49}H_{76}O_{20}$
Lanatoside C
$C_{50}H_{70}O_{14}$
Brevetoxin B
$C_{51}H_{79}NO_{13}$
Sirolimus
$C_{52}H_{56}N_6,C_{23}H_{14}O_6$
Pyrvinium Embonate
$C_{52}H_{74}N_2O_8,C_2H_6O_8S_2$
Laudexium Methyl Sulfate
$C_{53}H_{72}N_2O_{12}$
Atracurium
$C_{53}H_{72}N_2O_{12}$
Cisatracurium
$C_{53}H_{72}N_2O_{12},2C_6H_5O_3S$
Atracurium Besilate

$C_{54}H_{69}N_{11}O_{10}S_2$
Lanreotide
$C_{54}H_{69}N_{11}O_{10}S_2,xC_2H_4O_2$
Lanreotide Acetate
$C_{55}H_{78}O_{21}S_2Na_2$
Yessotoxin
$C_{55}H_{84}N_{17}O_{21}S_3$
Bleomycin
$C_{56}H_{78}Cl_2N_2O_{16}$
Doxacurium Chloride
$C_{58}H_{80}Cl_2N_2O_{14}$
Mivacurium Chloride
$C_{59}H_{84}N_{16}O_{12}$
Leuprorelin
$C_{59}H_{84}N_{16}O_{12},C_2H_4O_2$
Leuprorelin Acetate
$C_{60}H_{86}N_{16}O_{13}$
Buserelin
$C_{60}H_{86}N_{16}O_{13},C_2H_4O_2$
Buserelin Acetate
$C_{60}H_{86}O_{18}$
Ciguatoxin-2
$C_{60}H_{86}O_{18}$
Ciguatoxin-3
$C_{60}H_{86}O_{19}$
Ciguatoxin-1
$C_{62}H_{86}N_{12}O_{16}$
Dactinomycin
$C_{62}H_{90}ClCoN_{13}O_{15}P$
Hydroxocobalamin
$C_{62}H_{111}N_{11}O_{12}$
Ciclosporin
$C_{63}H_{88}CoN_{14}O_{14}P$
Cyanocobalamin
$C_{65}H_{82}N_2O_{18}S_2$
Cisatracurium Besilate
$C_{66}H_{68}CaF_2N_4O_{10},3H_2O$
Atorvastatin Calcium
$C_{66}H_{75}Cl_2N_9O_{24}$
Vancomycin
$C_{66}H_{75}Cl_2N_9O_{24},HCl$
Vancomycin Hydrochloride
$C_{66}H_{83}N_{17}O_{13}$
Nafarelin
$C_{66}H_{83}N_{17}O_{13},xC_2H_4O_2,yH_2O$
Nafarelin Acetate Hydrate
$C_{66}H_{83}N_{17}O_{13},C_2H_4O_2$
Nafarelin Monoacetate
$C_{102}H_{172}N_{36}O_{32}S_7$
Ziconitide
$C_{136}H_{210}N_{40}O_{31}S$
Tetracosactrin
$C_{147}H_{238}N_{44}O_{42}S$
Aviptadil
$C_{164}H_{256}O_{68}S_2Na_2$
Maitotoxin-1
$C_{287}H_{440}N_{80}O_{111}S_6$
Lepirudin
$C_{639}H_{1007}N_{171}O_{196}S_{18}$
Molgramostim
$C_{690}H_{1115}N_{177}O_{203}S_6$
Aldesleukin
$C_{990}H_{1529}N_{263}O_{299}S_7$
Somatropin
$C_{1736}H_{2653}N_{499}O_{522}S_{22}$
Reteplase
$C_{(20–22)}H_{(28,30)}O_{(3,5)}$
Pyrethrum
$C_{2569}H_{3894}N_{746}O_{781}S_{40}$
Alteplase
$C_{2736}H_{4174}N_{914}O_{824}S_{46}$
Duteplase
FCH_2CONH_2
Fluoroacetamide
FNa
Sodium Fluoride
F_2Sn
Stannous Fluoride
GaAs
Gallium Arsenide
GaN_3O_9
Gallium Nitrate
Hg
Mercury
$HgCl_2$
Mercuric (II) Chloride
$HgCH_3Cl$
Methylmercuric Chloride
$HgC_4H_6O_4$
Mercuric Acetate
$HgC_8H_8O_2$
Phenylmercuric Acetate
HgS
Mercuric (II) Sulfide
Hg_2Cl_2
Mercurous (I) Chloride
HCN
Hydrogen Cyanide
HCOOH
Formic Acid
HF
Hydrofluoric Acid
HN_3
Azide
H_3BO_3
Boric Acid
Li_2CO_3
Lithium Carbonate
$NH_2[CH_2]_4NH_2$
Putrescine
$(NH_3)_2,PtCl_2$
Cisplatin
$NaAsO_2$
Sodium Arsenite
$Na_2B_4O_7,10H_2O$
Sodium Borate Decahydrate
Na_2HAsO_4
Sodium Arsenate
Na_2SiF_6
Sodium Fluorosilicate
N_3Na
Sodium Azide
Pb
Lead
$PbCl_2$
Lead Chloride
$PbCrO_4$
Lead Chromate
$PbC_4H_6O_4$
Lead Acetate
PbI_2
Lead Iodide
PbN_2O_6
Lead Nitrate
PbN_6
Lead Azide
PbO
Lead Oxide
PbS
Lead Sulfide
$PbSO_4$
Lead Sulfate
Sb
Antimony
$SbCl_3$
Antimony Trichloride
SbH_3
Stibine
$Sb_2C_8H_4K_2O_{12},3H_2O$
Antimony Potassium Tartrate
Sb_2O_3
Antimony Trioxide
Sb_2O_5
Antimony Pentoxide
Sb_2S_3
Antimony Trisulfide
Sb_2S_5
Antimony Pentasulfide

3 Functional Classes: Therapeutic

Adrenocortical Suppressant

Trilostane

Aid to Smoking Cessation

Nicotine

Anabolic Steroid (Veterinary)

Zeranol

Anaesthetic (General)

Chloroform

Anaesthetic (Local)

Amolanone
Amydricaine
Benzamine
Bupivacaine
Butanilicaine
Butethamine
Butyl Aminobenzoate
Chloroprocaine
Cocaine
Dimethocaine
Etidocaine
Isobutyl Aminobenzoate
Leucinocaine
Lidocaine
Mepivacaine
Metabutethamine
Metabutoxycaine
Naepaine
Octacaine
Orthocaine
Oxetacaine
Prilocaine
Procaine
Pyrrocaine
Ropivacaine
Tetracaine
Tolycaine
Tropacocaine
Xenysalate

Analgesic

Acetanilide
Alclofenac
Aletamine
Aloxiprin
Aminophenazone
Aspirin
Azapropazone
Benorilate
Benoxaprofen
Benzydamine
Bufexamac
CP 47,497
Cinchophen
Clonixin
Dexmedetomidine
Diclofenac
Diflunisal
Dioxyamidopyrine
Dipyrone
Droxicam
Eptazocine
Etenzamide
Etofenamate
Etoricoxib
Etoxazene
Etymide
Famprofazone
Fenbufen
Fenclofenac
Fendosal
Fenoprofen
Fenyramidol
Feprazone
Floctafenine
Flufenamic Acid
Flunixin
Flupirtine
Flurbiprofen
Glafenine
Ibuprofen
Indometacin
Indoprofen
JWH-018
Ketoprofen
Ketorolac
Lornoxicam
Loxoprofen
Meclofenamic Acid
Mefenamic Acid
Methyl Salicylate
Metofoline
Morazone
Naproxen
Nefopam
Nifenazone
Niflumic Acid
Oxyphenbutazone
Paracetamol
Phenacetin
Phenazone
Phenazopyridine
Phenylbutazone
Piroxicam
Propyphenazone
Remifentanil
Salicylamide
Salicylic Acid
Salol
Salsalate
Sulindac
Suprofen
Tenoxicam
Tiaprofenic Acid
Tolfenamic Acid
Tolmetin
Valdecoxib
Zomepirac

Analgesic (Veterinary)

Isopropylaminophenazone
Xylazine

Anaplasmodastat (Veterinary)

Gloxazone

Anorectic

Benzfetamine
Cathine
Chlorphentermine
Clobenzorex
Dexamfetamine
Diethylpropion
Fenbutrazate
Fenfluramine
Fenproporex
Levamfetamine
Mazindol
Phendimetrazine
Phenmetrazine
Phentermine
Propylhexedrine

Anthelmintic

Bephenium Hydroxynaphthoate
Bitoscanate
Bromofenofos
Dichlorophen
Fenbendazole
Hexylresorcinol
Levamisole
Mebendazole
Niclosamide
Niridazole
Piperazine
Pyrantel
Pyrvinium Embonate
Santonin
Stilbazium Iodide
Tetrachloroethylene
Tiabendazole
Trichlorfon

Anthelmintic (Veterinary)

Albendazole
Bunamidine
Clioxanide
Diamfenetide
Haloxon
Methyridine
Morantel
Nitroxinil
Oxyclozanide
Parbendazole
Phenothiazine
Rafoxanide
Resorantel
Tetramisole
Thenium Closilate

Antiallergic

Ketotifen
Omalizumab
Sodium Cromoglicate

Antiamoebic

Bialamicol
Carbarsone
Clamoxyquin
Clefamide
Clioquinol
Conessine
Diiodohydroxyquinoline
Diloxanide
Emetine
Panidazole
Phanquinone

Antianginal

Diltiazem
Lidoflazine
Limaprost
Metoprolol
Nicorandil
Perhexiline
Prenylamine
Sotalol

Antianginal Vasodilator

Amyl Nitrite
Carbocromen
Dipyridamole
Glyceryl Trinitrate
Hexobendine
Imolamine
Isosorbide Dinitrate
Isosorbide Mononitrate
Nifedipine
Pentaerithrityl Tetranitrate
Trimetazidine

Antiarrhythmic

Acecainide
Adenosine
Ajmaline
Amiodarone
Aprindine
Bretylium Tosilate
Cibenzoline
Detajmium
Diltiazem
Disopyramide
Dofetilide
Encainide
Flecainide
Hydroquinidine
Ibutilide
Ipratropium
Landiolol
Lidocaine
Lorcainide
Metoprolol
Mexiletine
Moracizine
Nifekalant
Prajmalium Bitartrate
Procainamide
Quinidine
Sotalol
Tocainide
Verapamil

Antiarrhythmic (Coronary)

Amotriphene

Antiarthritic

Diacerein

Antiasthmatic

Formoterol
Montelukast
Nedocromil
Pranlukast
Zafirlukast

Antibacterial

Amikacin
Amoxicillin
Azithromycin
Azlocillin
Aztreonam
Bacampicillin
Capreomycin
Cefaclor
Cefdinir
Cefixime
Ceftazidime
Ceftriaxone
Chlortetracycline
Ciprofloxacin
Clarithromycin
Clomocycline
Cloponone
Demeclocycline
Dequalinium Chloride
Dirithromycin
Levofloxacin
Lymecycline
Metronidazole
Minocycline
Moxifloxacin
Ofloxacin
Oxytetracycline Dihydrate
Phanquinone
Phthalylsulfacetamide
Phthalylsulfathiazole
Procaine Benzylpenicillin
Propicillin
Rifamycin SV
Rolitetracycline
Roxithromycin
Streptomycin
Succinylsulfathiazole
Sulfachlorpyridazine
Sulfadiazine
Sulfadimethoxine
Sulfadimidine
Sulfadoxine
Sulfaethidole
Sulfafurazole
Sulfaguanidine
Sulfamerazine
Sulfamethizole
Sulfamethoxypyridazine
Sulfametopyrazine
Sulfametoxydiazine
Sulfamoxole
Sulfanilamide
Sulfaphenazole
Sulfapyridine
Sulfasalazine
Sulfasomizole
Sulfathiazole
Sulfisomidine
Taurolin
Teicoplanin
Thioacetazone
Xenysalate

Antibacterial (Topical)

Mupirocin

Antibacterial (Urinary)

Mandelic Acid
Methenamine
Nalidixic Acid
Nitrofurantoin
Nitroxoline

Antibacterial (Veterinary)

Furaltadone
Tilmicosin

Antibiotic

Ampicillin
Benzathine Benzylpenicillin
Benzylpenicillin
Carbenicillin
Cefalexin
Cefaloridine
Cefalothin
Cefradine
Chloramphenicol
Clindamycin
Cloxacillin
Cycloserine
Dihydrostreptomycin
Doxycycline
Enoxacin
Erythromycin
Flucloxacillin
Fusidic Acid
Gentamicin
Kanamycin
Lincomycin
Methacycline
Meticillin
Neomycin
Oleandomycin
Paromomycin
Pheneticillin
Phenoxymethylpenicillin
Pivampicillin
Rifamide
Spiramycin
Tetracycline
Tobramycin
Troleandomycin
Vancomycin
Viomycin

Antibiotic (Veterinary)

Tylosin

Anticholesteraemic

Azacosterol

Anticoagulant

Acenocoumarol
Anisindione
Bemiparin
Dicoumarol
Diphenadione
Enoxaparin
Ethyl Biscoumacetate
Lepirudin
Phenindione
Phenprocoumon
Picotamide
Warfarin

Anticonvulsant

Beclamide
Carbamazepine
Chlordiazepoxide
Cinolazepam
Clobazam
Clonazepam
Dimethadione
Ethosuximide
Ethotoin
Felbamate
Fosphenytoin
Gabapentin
Lamotrigine
Mephenytoin
Mesuximide
Metharbital
Oxcarbazepine
Paraldehyde
Paramethadione
Phenacemide
Pheneturide
Phensuximide
Phenylmethylbarbituric Acid
Phenytoin
Primidone
Progabide
Sultiame
Tiagabine
Tiletamine
Trimethadione
Valproic Acid
Zonisamide

Antidepressant

Bupropion
Citalopram
Clomipramine
Clorgiline
Desipramine
Dibenzepin
Dosulepin
Duloxetine
Escitalopram
Fenmetramide
Fluoxetine
Fluvoxamine
Imipramine
Iprindole
Iproniazid
Isocarboxazid
Mebanazine
Mianserin
Milnacipran
Mirtazapine
Moclobemide
Nefazodone
Nialamide
Nomifensine
Nortriptyline
Noxiptiline
Opipramol
Paroxetine
Phenelzine
Phenoxypropazine
Reboxetine
Rolicyprine
Rolipram
Sertraline
Setiptiline
Tandospirone
Thozalinone
Tianeptine
Tofenacin
Toloxatone
Tranylcypromine
Trazodone
Trimipramine
Venlafaxine
Viloxazine
Zimeldine

Antidiabetic

Acarbose
Acetohexamide
Buformin
Butoxamine
Carbutamide
Chlorpropamide
Epalrestat
Glibenclamide
Glibornuride
Gliclazide
Glimepiride
Glipizide
Gliquidone
Glymidine Sodium
Insulin
Metformin
Miglitol
Nateglinide
Phenformin
Pioglitazone
Repaglinide
Rosiglitazone
Tiagabine
Tolazamide
Tolbutamide
Tolrestat
Troglitazone
Voglibose

Antidiarrhoeal

Loperamide

Antidote (Barbiturate Poisoning)

Bemegride

Antidote (Nerve Gas and Organophosphate Insecticide Poisoning)

Pralidoxime Chloride

Antidote (Organophosphate Insecticide Poisoning)

Obidoxime Chloride

Antidote (Parametamol)

Acetylcysteine

Antiemetic

Benzquinamide
Cyclizine
Difenidol
Dolasetron
Embramine
Granisetron
Metoclopramide
Metopimazine
Nabilone
Ondansetron
Pipamazine
Ramosetron
Thiethylperazine
Trifluprom azine
Tropisetron
Δ^{9-} Tetrahydrocannabinol

Antiepileptic

Lamotrigine
Levetiracetam
Losigamone
Pregabalin
Topiramate
Valproic Acid
Vigabatrin

Antifilarial

Diethylcarbamazine

Antifungal

Amphotericin B
Citronella Oil
Dequalinium Chloride
Fluconazole
Flucytosine
Griseofulvin
Hydroxystilbamidine
Ketoconazole
Miconazole
Nifuroxime
Terbinafine
Xenysalate

Antifungal (Veterinary)

Etisazole

Antiglaucoma

Acetazolamide
Apraclonidine
Dorzolamide

Antigonadotropic (Veterinary)

Mibolerone

Antihistamine

Acrivastine
Alimemazine
Antazoline
Astemizole
Azatadine
Bamipine
Bromazine
Brompheniramine
Buclizine
Carbinoxamine
Cetoxime
Chlorcyclizine
Chloropyramine
Chloropyrilene
Chlorphenamine
Cinnarizine
Clemastine
Clemizole
Cyclizine
Cyproheptadine
Deptropine
Desloratadine
Dexbrompheniramine
Dexchlorphenamine
Dimenhydrinate
Dimetindene
Dimetotiazine
Diphenazoline
Diphenhydramine
Diphenylpyraline
Doxylamine
Ebastine
Embramine
Emedastine
Epinastine
Fexofenadine
Histapyrrodine
Homochlorcyclizine
Hydroxyzine
Isothipendyl
Mebhydrolin
Meclozine
Mepyramine
Mequitazine
Methaphenilene
Methapyrilene
Methdilazine
Mizolastine
Phenindamine
Pheniramine
Phenyltoloxamine
Promethazine
Propiomazine
Pyrrobutamine
Thenalidine
Thenyldiamine
Thiazinamium Metilsulfate
Thonzylamine
Tolpropamine
Trimethobenzamide
Tripelennamine
Triprolidine

Antihistamine (Non-sedating)

Loratadine

Antihistamine (Sedating)

Pizotifen

Antihistamine (Topical)

Levocabastine

Antihypercalcaemic

Gallium Nitrate

Antihyperlipidaemic

Colesevelam Hydrochloride
Ezetimibe
Fenofibrate
Rosuvastatin
Simvastatin

Antihyperlipoproteinaemic

Fluvastatin
Lovastatin
Pravastatin

Antihyperparathyroid

Doxercalciferol
Maxacalcitol

Antihypertensive

Alacepril
Amosulalol
Azamethonium Bromide
Benazepril
Benzthiazide
Bethanidine
Bopindolol
Bretylium Tosilate
Bunazosin
Cadralazine
Captopril
Chlorisondamine Chloride
Cicletanine
Clonidine
Clopamide
Debrisoquine
Delapril
Deserpidine
Diazoxide
Dihydralazine
Diltiazem
Doxazosin
Enalapril
Felodipine
Fenoldopam
Flosequinan
Fosinopril
Guanethidine
Guanoclor
Guanoxan
Hexamethonium Bromide
Hydralazine
Imidapril
Indoramin
Ketanserin
Labetalol
Lisinopril
Lofexidine
Mecamylamine
Methoserpidine
Methyldopa
Methyldopate
Metoprolol
Minoxidil
Moexipril
Moxonidine
Naftopidil
Nilvadipine
Olmesartan
Pargyline
Pempidine
Pentolonium Tartrate
Perindopril
Phenoxybenzamine
Phentolamine
Pinacidil
Piperoxan
Prazosin
Protoveratrine A and B
Quinapril
Ramipril
Rescinnamine
Reserpine
Rilmenidene
Sotalol
Spirapril
Syrosingopine
Tertatolol
Tetraethylammonium Bromide
Trandolapril
Trimetaphan Camsilate

Antihyperthyroid

Propylthiouracil

Antiinflammatory

Aceclofenac
Alminoprofen
Balsalazide
Deflazacort
Diacerein
Diclofenac
Dipyrone
Droxicam
Etanercept
Etodolac
Fluticasone Propionate
Ilodecakin
Mometasone Furoate
Olsalazine
Rofecoxib
Salol
Sulfasalazine
Valdecoxib

Antiinflammatory Corticosteroid

Desonide

Antileprotic

Clofazimine
Dapsone
Thiambutosine

Antimalarial

Amodiaquine
Amopyroquine
Arteether
Artemether
Artemisinin

Artesunate
Chloroquine
Chlorproguanil
Cinchonidine
Cinchonine
Cycloguanil
Halofantrine
Hydroxychloroquine
Mepacrine
Pamaquin
Pentaquin
Plasmocide
Primaquine
Proguanil
Pyrimethamine
Quinine

Antimicrobial

Furazolidone
Halquinol
Nifuratel
Triclobisonium Chloride
Trimethoprim

Antimigraine

Almotriptan
Eletriptan
Methysergide
Naratriptan
Pizotifen
Rizatriptan
Sumatriptan
Zolmitriptan

Antimycobacterial

Rifabutin

Antineoplastic

Aclarubicin
Aldesleukin
Altretamine
Aminoglutethimide
Amsacrine
Anastrozole
Arsenic Trioxide
Asparaginase
Bicalutamide
Bleomycin
Buserelin
Busulfan
Calusterone
Capecitabine
Carmustine
Chlorambucil
Cisplatin
Cladribine
Cyclophosphamide
Cytarabine
Dactinomycin
Daunorubicin
Docetaxel
Doxorubicin
Eflornithine
Epirubicin
Etoposide
Exemestane
Fludarabine
Fluorouracil
Gemcitabine
Hydroxycarbamide
Idarubicin
Irinotecan
Lanreotide
Letrozole
Mannomustine
Melphalan
Mercaptopurine
Methotrexate
Mitoxantrone
Molgramostim
Mustine
Oxaliplatin
Paclitaxel
Pentostatin
Pipobroman
Procarbazine
Raltitrexed
Sunitinib
Temozolomide
Thiotepa
Tioguanine
Topotecan
Toremifene
Trimetrexate
Urethane
Vinblastine
Vincristine
Vindesine
Vinorelbine

Antineoplastic (Hormonal)

Diethylstilbestrol
Leuprorelin

Antineoplastic Aromatase Inhibitor

Formestane

Antineutropenic

Molgramostim

Antiobesity Agent

Orlistat

Antiosteoporotic

Raloxifene

Antiparkinsonian

Amantadine
Apomorphine
Benserazide
Entacapone
Levodopa
Pergolide
Phenglutarimide
Pramipexole
Ropinirole
Selegiline

Antiplatelet

Aspirin
Clopidogrel

Antiprotozoal

Acetarsone
Aminitrozole
Antimony
Atovaquone
Broxaldine
Broxyquinoline
Dehydroemetine
Eflornithine
Hydroxystilbamidine
Mepacrine
Metronidazole
Nifuratel
Nimorazole
Pentamidine
Stilbamidine
Tinidazole

Antiprotozoal (Veterinary)

Amicarbalide
Aminonitrothiazole
Dimetridazole
Imidocarb
Nifursol
Phenamidine
Quinuronium Sulfate

Antipruritic

Crotamiton
Menthol

Antipsoriatic

Alefacept
Maxacalcitol

Antipsychotic

Acetophenazine
Amisulpride
Aripiprazole
Benperidol
Biriperone
Carfenazine
Chlorprothixene
Clospipramine
Clozapine
Fenimide
Haloperidol
Loxapine
Nemonapride
Olanzapine
Perospirone
Quetiapine
Remoxipride
Triflupromazine
Zotepine
Zuclopenthixol

Antipyretic

Aspirin
Diclofenac
Dipyrone
Droxicam
Salol

Antiretroviral

Abacavir
Lopinavir
Nelfinavir
Nevirapine

Antirheumatic

Alminoprofen
Leflunomide

Antiseptic

Acriflavinium Chloride
Aminoacridine
Amylmetacresol
Benzalkonium Bromide
Benzalkonium Chloride
Benzethonium Chloride
Naphthalene
Thymol
Triclobisonium Chloride
Triclocarban

Antiseptic (Topical)

Proflavine Hemisulfate

Antispasmodic

Alverine
Ambucetamide
Benactyzine
Butetamate
Dibutoline
Dimoxyline
Flavoxate
Hexocyclium Metilsulfate
Mebeverine
Octamylamine
Octaverine
Papaverine
Pipethanate
Pipoxolan

Antispasmodic (Veterinary)

Aminopromazine
Fenpipramide
Fenpiprane
Metindizate

Antithrombotic

Alteplase
Anagrelide
Argatroban
Cilostazol
Cloricromen
Dipyridamole
Fondaparinux Sodium
Picotamide
Sulfinpyrazone
Ticlopidine
Tirofiban

Antithyroid Agent

Carbimazole
Methylthiouracil
Thiamazole

Antitrichomonal

Nifuroxime

Antituberculosis

Aminosalicylic Acid
Ethambutol
Ethionamide

Antitussive

Dextromethorphan
Hydrocodone
Levomethorphan
Nicocodine
Racemethorphan

Antiulcerative

Ecabet
Enprostil
Esomeprazole
Famotidine
Omeprazole
Pirenzepine
Rebamipide

Antiurolithic

Succinimide

Antiviral

Aciclovir
Amantadine
Amprenavir
Cidofovir
Delavirdine
Didanosine
Efavirenz
Famciclovir
Foscarnet Sodium
Ganciclovir
Idoxuridine
Indinavir
Lamivudine
Metisazone
Oseltamivir
Penciclovir
Rimantadine
Ritonavir
Saquinavir
Stavudine
Valaciclovir
Zalcitabine
Zanamivir
Zidovudine

Anxiolytic

Alpidem
Alprazolam
Bromazepam
Buspirone
Californine
Clobazam
Dexmedetomidine
Diazepam
Etizolam
Flutazolam
Flutoprazepam
Lauroscholtzine
Metaclazepam
Mexazolam
Tandospirone

Atypical Antipsychotic

Risperidone
Sertindole
Ziprasidone

Benzodiazepine

Alprazolam
Bromazepam
Clobazam
Clonazepam
Diazepam
Flunitrazepam
Flurazepam
Ketazolam
Lormetazepam
Medazepam
Midazolam
Nordazepam
Nitrazepam
Oxazepine
Quazepam
Temazepam

Biological Response Modifier

Aldesleukin

Bone Modulator

Alendronic Acid
Raloxifene

Bone Resorption Inhibitor

Ibandronic Acid
Risedronic Acid
Tiludronic Acid

Bronchodilator

Bambuterol
Bamifylline
Butetamate
Clenbuterol
Doxofylline
Ipratropium
Mabuterol
Norbudrine
Phenisonone
Protokylol
Reproterol
Rimiterol
Salbutamol
Salmeterol

CNS Depressant

Ethanol
Gelsemine
Muscimol

CNS Stimulant

Amineptine
Bemegride
Brucine
Fencamfamin
Levamfetamine
Meclofenoxate
Mesocarb
Methylphenidate

Modafinil
Pemoline
Pipradrol
Prolintane

Calcium Regulator

Clodronic Acid

Cardiac Stimulant

Heptaminol

Cardioprotective

Dexrazoxane

Cardiotonic

Denopamine
Docarpamine
Levosimendan
Milrinone
Ouabain
Xamoterol

Coccidiostat (Veterinary)

Amprolium Hydrochloride
Clopidol
Decoquinate
Diaveridine
Dinitolmide
Methyl Benzoquate
Sulfaquinoxaline

Contraceptive

Drospirenone
Elcometrine
Norelgestromin

Contraceptive (Veterinary)

Azacosterol

Cough Suppressant

Acetyldihydrocodeine
Benzonatate
Bibenzonium Bromide
Carbetapentane
Clofedanol
Dextromethorphan
Dextrorphan
Dimethoxanate
Dropropizine
Glaucine
Isoaminile
Levodropropizine
Levomethorphan
Levopropoxyphene
Noscapine
Oxeladin
Pholcodine
Pipazetate

Counterirritant (Topical)

Veratrine

Cytoprotective

Amifostine

Decongestant

Menthol
Phenylpropylmethylamine

Dermatological Agent

Acitretin
Cadmium
Dithranol
Enoxolone
Etretinate
Isotretinoin
Resorcinol
Salicylic Acid

Diagnostic Aid (Pheochromocytoma)

Piperoxan

Diuretic

Acetazolamide
Amiloride
Aminometradine
Amisometradine
Bendroflumethiazide
Benzthiazide
Bumetanide
Chlorothiazide
Chlortalidone
Cicletanine
Clopamide
Clorexolone
Cyclopenthiazide
Cyclothiazide
Disulfamide
Epithiazide
Etacrynic Acid
Ethiazide
Ethoxzolamide
Furosemide
Hydrochlorothiazide
Hydroflumethiazide
Indapamide
Mannitol
Mefruside
Mersalyl Acid
Methyclothiazide
Metolazone
Muzolimine
Polythiazide
Quinethazone
Spironolactone
Tienilic Acid
Torasemide
Triamterene
Trichlormethiazide
Urea
Xipamide

Emetic

Apomorphine
Cephaëline

Expectorant

Cephaëline
Guaifenesin

Filaricide

Lucanthone

Gout Suppressant

Colchicine

Growth Stimulant

Somatropin

Haemopoietic Vitamin

Cyanocobalamin
Hydroxocobalamin

Haemostatic

1,2-Naphthoquinone
Adrenalone
Aminocaproic Acid
Carbazochrome
Cotarnine

Hydroxyanthraquinone

Rhein

Hyperglycaemic

Diazoxide

Hypnotic

Acecarbromal
Apronal
Bromisoval
Brotizolam
Butobarbital
Butylchloral Hydrate
Carbromal
Chloral Betaine
Chloral Hydrate
Cinolazepam
Clomethiazole
Cyclobarbital
Dichloralphenazone
Eszopiclone
Ethchlorvynol
Ethinamate
Flunitrazepam
Flurazepam
Glutethimide
Ibomal
Idobutal
Loprazolam
Lormetazepam
Mecloqualone
Methaqualone
Methylpentynol
Methyprylon
Midazolam
Nitrazepam
Paraldehyde
Pentobarbital
Phenobarbital
Quazepam
Sulfonal
Temazepam
Thalidomide
Triazolam

Trichloroethanol
Triclofos Sodium
Vinylbital
Zaleplon
Zolpidem
Zopiclone

Hypothalamus and Pituitary Suppressant

Metallibure

Immunomodulator

Aldesleukin
Ditiocarb

Immunosuppressant

Alefacept
Azathioprine
Ciclosporin
Mycophenolate Mofetil
Rituximab
Sirolimus
Tacrolimus
Thalidomide

Induction of Ovulation

Clomifene
Cyclofenil

Inotrope

Enoximone

Laxative

Rhein

Lipid-Regulating Agent

Bezafibrate
Cerivastatin
Ciprofibrate
Clofibrate

Lung Surfactant

Colfosceril Palmitate

Mucolytic

Acetylcysteine

Mucolytic Expectorant

Bromhexine

Muscle Relaxant

Alcuronium Chloride
Aviptadil
Baclofen
Chlorphenesin Carbamate
Chlorzoxazone
Dantrolene
Decamethonium Bromide
Doxacurium Chloride
Emylcamate
Fazadinium Bromide
Fenyramidol
Gallamine Triethiodide
Laudexium Methyl Sulfate
Mebezonium Iodide
Mephenesin
Methocarbamol
Metocurine Iodide
Mivacurium Chloride
Pancuronium Bromide
Rocuronium
Styramate
Suxamethonium Chloride
Suxethonium Bromide
Tetrazepam
Tizanidine
Tubocurarine Chloride

NSAID

Acemetacin
Alminoprofen
Aspirin
Bromfenac
Butibufen
Celecoxib
Dexketoprofen
Droxicam
Ibuprofen
Ketorolac
Lornoxicam
Loxoprofen
Meloxicam
Nabumetone
Vedaprofen

Narcotic Analgesic

Alfentanil
Allylprodine
Alphameprodine
Alphamethadol
Alphaprodine
Anileridine
Benzethidine
Benzylmorphine
Bezitramide
Buprenorphine
Butorphanol
Codeine
Desomorphine
Dextromoramide
Dextropropoxyphene
Dezocine
Diamorphine
Dihydrocodeine
Dihydromorphine
Dimenoxadole
Dioxaphetyl Butyrate
Dipipanone
Embutramide
Ethoheptazine
Ethylmorphine
Etonitazene
Fentanyl
Hydrocodone
Hydromorphinol
Hydromorphone
Ketobemidone
Levomethadyl Acetate
Levomethorphan
Levomoramide
Levorphanol
Meptazinol
Methadone
Metopon
Morphine
Nalbuphine
Nicomorphine
Noracymethadol
Norcodeine
Norlevorphanol
Normethadone
Normorphine
Norpipanone
Opium
Oxycodone
Oxymorphone
Pentazocine
Pethidine
Phenadoxone
Phenampromide
Phenazocine
Phenoperidine
Piminodine
Piritramide
Profadol
Properidine
Racemoramide
Racemorphan
Sufentanil
Thebacon
Tilidate
Tramadol
Trimeperidine

Narcotic Analgesic (Veterinary)

Acetorphine
Diethylthiambutene
Etorphine

Narcotic Antagonist (Veterinary)

Diprenorphine

Narcotic Antidiarrhoeal

Diphenoxylate

Neuroleptic

Droperidol
Trifluoperazine
Zuclopenthixol

Neuroprotective

Riluzole

Nitrovasodilator

Nicorandil

Nootropic

Donepezil

Peristaltic Stimulant

Cisapride

Progestational Steroid

Chlormadinone Acetate
Dimethisterone
Dydrogesterone
Elcometrine
Ethisterone
Etynodiol Diacetate
Gestonorone Caproate
Hydroxyprogesterone
Lynestrenol
Medroxyprogesterone Acetate
Megestrol Acetate
Norelgestromin
Norethisterone
Noretynodrel
Progesterone

Progestational Steroid (Veterinary)

Flugestone

Psychomimetic

Cannabis

Psychostimulant

Bromantane
Carphedon

Pulmonary Surfactant

Calfactant
Pumactant

Purgative

Aloin
Arecoline
Bisacodyl
Dantron
Oxyphenisatine
Phenolphthalein

Quinolone Antibiotic

Ofloxacin

Respiratory Stimulant

Amiphenazole
Cropropamide
Crotetamide
Dimefline
Doxapram
Etamivan
Lobeline
Nikethamide
Pentetrazol
Picrotoxin

Rifamycin Antibiotic

Rifabutin

Rubefacient

Camphor
Teclothiazide
Thurfyl Nicotinate

Rubefacient (Topical)

Benzyl Nicotinate

Sclerosing Agent

Monoethanolamine

Sedative

Acecarbromal
Alimemazine
Allobarbital
Aprobarbital
Apronal
Barbital
Brallobarbital
Bromisoval
Brotizolam
Butalbital
Chloral Betaine
Chloral Hydrate
Cinolazepam
Clomethiazole
Cyclopentobarbital
Dexmedetomidine
Dichloralphenazone
Eszopiclone
Heptabarb
Hexobarbital
Loprazolam
Methaqualone
Methylpentynol
Methylphenobarbital
Nealbarbital
Paraldehyde
Phenobarbital
Propiomazine
Quazepam
Ramelteon
Secbutabarbital
Talbutal
Triclofos Sodium
Vinbarbital
Zaleplon

Sedative (Veterinary)

Metomidate
Xylazine

Skeletal Muscle Relaxant

Atracurium
Botulinum Toxin
Carisoprodol
Cinolazepam
Cisatracurium
Cyclobenzaprine
Phenprobamate

Tetracyclic Antidepressant

Maprotiline

Thrombolytic

Duteplase
Lepirudin
Nateplase
Reteplase

Thyroid Agent

Levothyroxine
Liothyronine
Taltirelin

Tranquilliser

Acepromazine
Acetophenazine
Azacyclonol
Benzoctamine
Bromazepam
Butaperazine
Camazepam
Captodiame
Carfenazine
Chlordiazepoxide
Chlormezanone
Chlorpromazine
Chlorprothixene
Clobazam
Clopenthixol
Clorazepic Acid
Clotiapine
Demoxepam
Diazepam
Dixyrazine
Droperidol
Emylcamate
Ethomoxane
Ethylisobutrazine
Fluanisone
Flupentixol
Fluphenazine
Fluspirilene
Hydroxyphenamate
Hydroxyzine
Ketazolam
Levomepromazine
Lithium Carbonate
Lorazepam
Loxapine
Mebutamate
Medazepam
Meprobamate
Mesoridazine
Methoxypromazine
Molindone
Nordazepam
Oxazepam
Oxypertine
Pecazine
Penfluridol
Perazine
Pericyazine
Perphenazine
Phenaglycodol
Phenprobamate
Pimozide
Pipamperone
Piperacetazine
Pipethanate
Pipotiazine
Prazepam
Prochlorperazine
Promazine
Prothipendyl
Sulforidazine
Sulpiride
Tetrabenazine

Tetrazepam
Thiopropazate
Thioproperazine
Thioridazine
Tiotixene
Trifluoperazine
Trifluperidol
Triflupromazine
Trimetozine
Tybamate

Tranquilliser (Veterinary)

Azaperone
Trifluomeprazine

Treatment of ADHD

Atomoxetine
Methylphenidate

Treatment of Acidosis

Trometamol

Treatment of Acute Myocardial Infarction

Reteplase

Treatment of Alcohol Poisoning

Metadoxine

Treatment of Alzheimers Disease

Galantamine
Memantine

Treatment of Amnesia

Carphedon

Treatment of BPH

Dutasteride
Finasteride
Naftopidil
Tamsulosin

Treatment of Cardiac Insufficiency

Sparteine

Treatment of ED

Sildenafil
Tadalafil
Vardenafil

Treatment of Endometriosis

Nafarelin

Treatment of Gastric Ulcers

Pirenzepine

Treatment of Hypochlorhydria

Betaine

Treatment of Irritable Bowel Syndrome

Tegaserod

Treatment of Migraine

Dihydroergotamine

Treatment of Peptic Ulcer

Carbenoxolone

Treatment of Urinary Incontinence

Duloxetine
Oxybutynin

Triazole Antifungal

Itraconazole

Tricyclic Antidepressant

Amineptine
Amitriptyline
Amoxapine
Butriptyline
Lofepramine
Protriptyline

Trypanocide (Veterinary)

Homidium Bromide
Isometamidium
Pyritidium Bromide
Quinapyramine

Tuberculostatic

Cycloserine
Isoniazid
Morinamide
Phthivazid
Protionamide
Pyrazinamide
Rifampicin
Salinazid
Thioacetazone
Tiocarlide
Viomycin

Ulcer-healing Drug

Nizatidine

Uricosuric

Benzbromarone
Benziodarone
Etebenecid
Probenecid
Sulfinpyrazone

Uterine Stimulant

Methylergometrine

Vasoconstrictor

Nordefrin

Vasodilator

Alprostadil
Amotriphene
Azapetine
Bamethan
Benziodarone
Betahistine
Buphenine
Butalamine
Codergocrine Mesilate
Cyclandelate
Enoximone
Fasudil
Heptaminol
Inositol Nicotinate
Isoxsuprine
Moxisylyte
Naftidrofuryl Oxalate
Nicametate
Nicergoline
Nicofuranose
Nicotinic Acid
Nicotinyl Alcohol
Pentifylline
Pentoxifylline
Sildenafil
Tolazoline
Xantinol Nicotinate

Vasodilator (Coronary)

Cloricromen
Erythrityl Tetranitrate

Vasodilator (Topical)

Butoxyethyl Nicotinate
Ethyl Nicotinate
Hexyl Nicotinate
Methyl Nicotinate

Xanthine Bronchodilator

Acefylline Piperazine
Bufylline
Caffeine
Diprophylline
Etamiphylline
Etofylline
Proxyphylline
Theophylline

4 Functional Classes: Pesticides

Acaricide

Aldicarb
Azinphos-(Et)
Azinphos-(Me)
Benzyl Benzoate
Chlorpyrifos
Crotamiton
Dioxathion
Fenthion
Methamidophos
Monocrotophos
Naled
Omethoate
Triazophos

Aid to Smoking Cessation

Nicotine

Anaplasmodastat (Veterinary)

Gloxazone

Anthelmintic

Cambendazole
Dithiazanine Iodide
Naphthalene
Tetrachloroethylene

Anthelmintic (Veterinary)

Albendazole
Bunamidine
Carbon Tetrachloride
Clioxanide
Diamfenetide
Haloxon
Methyridine
Morantel
Nitroxinil
Oxyclozanide
Parbendazole
Phenothiazine
Rafoxanide
Resorantel
Tetramisole
Thenium Closilate

Antibacterial

Bacitracin
Cefixime
Chlorquinaldol
Dibrompropamidine
Diminazene
Hydroxyquinoline
Mafenide
Metronidazole
Nitrofurazone
Noxytiolin
Propamidine
Sulfacetamide

Antibacterial (Topical)

Mupirocin

Antibacterial (Urinary)

Mandelic Acid
Methenamine
Nalidixic Acid
Nitrofurantoin
Nitroxoline

Antibacterial (Veterinary)

Furaltadone
Tilmicosin

Antifungal

Buclosamide
Chlorphenesin
Chlorquinaldol
Clodantoin
Clotrimazole
Diamthazole
Dibrompropamidine
Econazole
Fenticlor
Hydroxyquinoline
Natamycin
Noxytiolin
Nystatin
Propamidine
Tolnaftate

Antifungal (Veterinary)

Etisazole

Antimicrobial

Hexetidine

Antiprotozoal

Broxyquinoline
Diminazene
Metronidazole

Antiprotozoal (Veterinary)

Amicarbalide
Aminonitrothiazole
Dimetridazole
Imidocarb
Nifursol
Phenamidine
Quinuronium Sulfate

Bacteriostat

Ambazone

Coccidiostat (Veterinary)

Amprolium Hydrochloride

Ectoparasiticide

Coumaphos
Deltamethrin
Rotenone

Filaricide

Lucanthone

Fungicide

Hexaconazole
Pyrazophos

Fungistatic

Diphenyl

Herbicide

Acrolein
Alachlor
Ametryne
Aminotriazole
Atrazine
Desmetryne
Dicamba
Dichlorophenoxyacetic Acid
Dinitro-orthocresol
Diquat
Diuron
Glyphosate
Isoproturon
Linuron
Methoprotryne
Methylchlorophenoxyacetic Acid
Metolachlor
Monolinuron
Paraquat
Prometryne
Propanil
Propazine
Simazine
Terbutryne
Terbutylazine
Trichlorophenoxyacetic Acid

Insect Fumigant

Hydrogen Cyanide
Methyl Bromide

Insecticide

Aldicarb
Aldrin
Azinphos-(Et)
Azinphos-(Me)
Benzene Hexachloride
Carbaryl
Chlordane
Chloroaniline
Chloromethylaniline
Chloronitroaniline
Chlorpyrifos
Clofenotane
Coumaphos
Deltamethrin
Demeton-*O*
Demeton-*S*
Dichlorvos
Dieldrin
Dimethoate
Dimpylate
Dinitro-orthocresol
Dioxathion
Disulfoton
Emamectin
Endosulfan
Endrin
Famphur
Fenitrothion
Fenthion
Fluquinconazole
Heptachlor
Imidacloprid
Irgarol
Lindane
Malathion
Methamidophos
Methoxychlor
Mevinphos
Monocrotophos
Naled
Nicotine
Omethoate
Paradichlorobenzene
Parathion
Pyrethrum
Pyriproxyfen
Ryanodine
Triazophos
Trichlorfon
Triflumuron

Insecticide Synergist

Piperonyl Butoxide

Molluscicide

Metaldehyde

Nematocide

Aldicarb
Cadmium
Chloropicrin
Coumaphos
Fenamiphos
Propanil

Organophosphate

Malathion

Parasiticide

Betanaphthol
  Carbaryl
  Sulfiram

Pesticide

Chloronitrotoluene

Pyrethroid Insecticide

Cyfluthrin

Rodenticide

Alphachloralose
Coumatetralyl
Diphenadione
Fluoroacetamide
Fluoroacetic Acid
Hydrogen Cyanide
Norbormide
Warfarin

Scabicide

Pyrethrum

Trypanocide (Veterinary)

Homidium Bromide
Isometamidium
Pyritidium Bromide
Quinapyramine

5 Functional Classes: Other Substances

1,4-Benzodiazepine

Alprazolam
Chlorazepic Acid
Chlordiazepoxide
Flumazenil
Ketazolam
Medazepam
Metaclazepam
Quazepam
Triazolam

2-Azetidinone

Ezetimibe

5-HT Receptor Antagonist

Cyproheptadine

5-HT Receptor Releasing Agent

Methoxymetamfetamine
Methylthioamfetamine

5-HT_1 Receptor Agonist

Aripiprazole
Eletriptan
Naftopidil
Rizatriptan
Sumatriptan
Trifluoromethylphenylpiperazine
Zolmitriptan

5-HT_2 Receptor Agonist

2C-T-7

5-HT_2 Receptor Antagonist

Nefazodone

5-HT_{2A} Receptor Agonist

2C-T-2

5-HT_{2A} Receptor Antagonist

2C-B
Aripiprazole
Ketanserin

5-HT_{2C} Receptor Agonist

m-Chlorophenylpiperazine

5-HT_3 Receptor Antagonist

Dolasetron
Ondansetron
Ramosetron
Tropisetron

5α-HT_4 Partial Agonist

Tegaserod

5α-Reductase Inhibitor

Dutasteride
Finasteride

12,13-Epoxytrichothecene

Diacetoxyscirpenol
Fusarenone-X

ACE Inhibitor

Alacepril
Benazepril
Cilazapril
Delapril
Fosinopril

Acetamide

Dimevamide
Etymide
Oxiracetam
Pramiracetam

Acid

Acetic Acid Glacial
Formic Acid
Hydrochloric
Sulfuric
Nitric Acid

Adenosine α_1 Antagonist

Bamifylline
Propentofylline

Aerosol Propellent

Cryofluorane
Dichlorodifluoromethane
Trichlorofluoromethane

Alcohol Dehydrogenase Inhibitor

Fomepizole

Alcohol Denaturant

Denatonium Benzoate

Alcohol Deterrent

Acamprosate Calcium
Disulfiram

Aldehyde

Acetaldehyde
Acrolein

Aldosterone Antagonist

Drospirenone

Alkaloid

Aconitine
Apoatropine
Benzoylecgonine
Berberine
Chelidonine
Coniine
Cytisine
Ecgonine
Harman
Hydrastine
Hydrastinine
Hydroquinine
Ibogaine
Monocrotaline
Narceine
Neopine
Ryanodine
Solanidine
Solanine
Strychnine
Thebaine
Yohimbine

Alkylamine

Chlorphenamine

α-Adrenergic Receptor Agonist

Adrafinil
Methylhexaneamine
Phenylpropylmethylamine

α-Adrenergic Receptor Antagonist

Ergotamine
Labetalol

α-Glucosidase Inhibitor

Miglitol
Voglibose

α_1-Adrenergic Receptor Agonist

Modafinil

α_1-Adrenergic Receptor Antagonist

Alfuzosin
Naftopidil
Tamsulosin
Terazosin

α_2-Adrenergic Receptor Agonist

Apraclonidine
Clonidine
Tizanidine
Xylazine

α_2-Adrenergic Receptor Antagonist

Piperoxan
Yohimbine

Amide

Pranlukast

Amine

Cathine
Duloxetine
Memantine

Amino Acid

Tryptophan

Aminobiphosphonate

Ibandronic Acid
Risedronic Acid

Aminoketone

Bupropion

Aminoquinoline

Amopyroquine

Aminosalicylate

Olsalazine

Anabolic Steroid

Androstanediol
Androstanolone
Androstenedione
Boldenone
Boldione
Calusterone
Clostebol
Dehydrochloromethyltestosterone
Drostanolone Propionate
Ethylestrenol
Formebolone
Gestrinone
Mestanolone
Metenolone
Methandienone
Methandriol
Mibolerone
Nandrolone
Norandrostenediol
Norandrostenedione
Norbolethone
Norethandrolone
Oxabolone
Oxandrolone
Oxymesterone
Oxymetholone
Stanozolol
Stenbolone
Tetrahydrogestrinone
Trenbolone

Anaesthetic

Levobupivacaine
Methohexital
Propofol
Rapacuronium Bromide
Sevoflurane
Thialbarbital
Tiletamine
γ-Hydroxybutyrate

Anaesthetic (General)

Alfadolone
Alfaxalone
Desflurane
Enflurane
Ether
Etomidate
Halothane
Ketamine
Methoxyflurane
Propanidid
Trichloroethylene

Anaesthetic (Local)

Amylocaine
Benzocaine
Benzyl Alcohol
Butacaine
Cinchocaine
Cyclomethycaine
Diperodon
Dyclonine
Hexylcaine
Meprylcaine
Oxybuprocaine
Phenacaine
Piperocaine
Pramocaine
Propoxycaine
Proxymetacaine
Quinisocaine

Anaesthetic (Topical)

Phenol

Androgen

Androsterone
Danazol
Fluoxymesterone
Mesterolone
Methyltestosterone
Oxandrolone
Oxymesterone
Testosterone

Angiotensin II Receptor Antagonist

Candesartan
Irbesartan
Losartan
Olmesartan
Telmisartan
Valsartan

Anion

Azide
Borates
Bromides
Fluorides
Oxalates

Anionic Surfactant

Docusate Sodium

Anti-factor Xa Activity

Bemiparin

Antiandrogen

Bicalutamide
Dutasteride

Anticholinergic

Adiphenine
Ambutonium Bromide
Atropine
Atropine Methobromide
Atropine Methonitrate
Atropine Oxide
Benaprizine
Benzatropine
Benzilonium Bromide
Biperiden
Caramiphen
Chlorphenoxamine
Clidinium Bromide
Cyclopentolate
Cycrimine
Dicycloverine
Diethazine
Diphemanil Metilsulfate
Emepronium Bromide
Eucatropine
Glycopyrronium Bromide
Heteronium Bromide
Hexocyclium Metilsulfate
Homatropine
Homatropine Methylbromide
Hyoscine
Hyoscine Butylbromide
Hyoscine Methobromide
Hyoscine Methonitrate
Hyoscyamine
Isopropamide Iodide
Lachesine Chloride
Mepenzolate Bromide

Methanthelinium Bromide
Metixene
Octatropine Methylbromide
Orphenadrine
Oxyphencyclimine
Oxyphenonium Bromide
Pentapiperide Metilsulfate
Penthienate Methobromide
Phenglutarimide
Pipenzolate Bromide
Piperidolate
Poldine Metilsulfate
Procyclidine
Profenamine
Propantheline Bromide
Tigloidine
Tiotropium
Tricyclamol Chloride
Tridihexethyl Chloride
Trihexyphenidyl
Tropicamide
Tropine
Valethamate Bromide

Anticholinesterase

Ambenonium Chloride
Carbaryl
Demecarium Bromide
Demeton-*O*
Distigmine Bromide
Ecothiopate Iodide
Edrophonium Chloride
Galantamine
Neostigmine Bromide
Physostigmine
Physostigmine Aminoxide
Pyridostigmine Bromide
Rivastigmine
Sarin
Soman
Tabun
Tacrine
VX

Antifreeze

Ethylene Glycol

Antiheparin Compound

Tolonium Chloride

Antimitotic

Demecolcine

Antimuscarinic

Dimevamide
Ipratropium
Oxybutynin
Pipethanate
Terodiline
Tiotropium
Tolterodine

Antimycotic

Cloponone

Antioestrogen

Toremifene

Antioxidant

Butylated Hydroxyanisole
Butylated Hydroxytoluene
Dodecyl Gallate
Ethyl Gallate
Hydroquinone

Antiprogestogenic Steroid

Mifepristone

Appetite Stimulant

Δ^9-Tetrahydrocannabinol

Aromatase Inhibitor

Exemestane

Arylpiperazine

Dibenzylpiperazine
m-Chlorophenylpiperazine

Atropine Derivative

Amprotropine

Azasteroid

Finasteride

B Vitamin

Choline

Barbiturate

Allobarbital
Amobarbital
Aprobarbital
Barbital
Barbituric Acid
Brallobarbital
Butalbital
Butallylonal
Butobarbital
Cyclobarbital
Cyclopentobarbital
Enallylpropymal
Heptabarb
Hexethal
Hexobarbital
Ibomal
Idobutal
Metharbital
Methohexital
Methylphenobarbital
Narcobarbital
Nealbarbital
Pentobarbital
Phenobarbital
Phenylmethylbarbituric Acid
Probarbital
Secbutabarbital
Secobarbital
Talbutal
Thialbarbital
Thiamylal
Thiopental
Vinbarbital
Vinylbital

Benzamide

Remoxipride

Benzimidazole

Esomeprazole

Benzodiazepine

Alprazolam
Bromazepam
Camazepam
Clobazam
Clonazepam
Clozapine
Diazepam
Flunitrazepam
Flurazepam
Loprazolam
Lormetazepam
Medazepam
Metaclazepam
Nitrazepam
Nordazepam
Olanzapine
Oxazepam
Prazepam
Temazepam
Tetrazepam
Triazolam

Benzodiazepine Antagonist

Flumazenil

Benzodioxane

Piperoxan

Benzoic Acid Ester

Amydricaine

Benzothiophene

Raloxifene

Benzoxazocine

Nefopam

Benzoylurea

Triflumuron

β-Adrenoceptor Agonist

Dopexamine

β-Adrenoceptor Antagonist

Acebutolol
Alprenolol

Atenolol
Labetalol
Metipranolol
Metoprolol
Propranolol

β-Adrenoceptor Partial Agonist

Xamoterol

β-Blocker

Betaxolol
Bevantolol
Bisoprolol
Bunitrolol
Carteolol
Carvedilol
Celiprolol
Esmolol
Levobunolol
Nadolol
Nebivolol
Oxprenolol
Pindolol
Practolol
Tertatolol
Timolol

β-Carboline

Harman

β-Methoxybutenolide

Losigamone

β_1-Adrenergic Receptor Agonist

Denopamine

β_1-Adrenergic Receptor Antagonist

Landiolol

β_2-Adrenergic Receptor Agonist

Bambuterol
Clenbuterol
Gestrinone
Mabuterol
Salmeterol

β_2-Adrenergic Receptor Antagonist

Butoxamine
Salbutamol

Bile Acid Sequestrant

Colesevelam Hydrochloride

Butyric Acid

Butibufen

Butyrolactone

Amolanone

Butyrophenone

Benperidol
Haloperidol
Trifluperidol

Cannabinoid CB_1 and CB_2 Receptor Agonist

JWH-018

COX Inhibitor

Ibuprofen
Ketorolac
Lornoxicam
Rofecoxib

COX-2 Inhibitor

Alminoprofen
Celecoxib
Etoricoxib
Meloxicam
Valdecoxib

Calcium Antagonist

Isradipine
Lacidipine
Mibefradil
Nimodipine
Nisoldipine
Nitrendipine
Terodiline

Calcium Channel Blocker

Amlodipine
Diltiazem
Nilvadipine
Nimodipine
Nisoldipine
Nitrendipine

Cannabinoid Receptor Agonist

CP 47,497

Capsaicinoid

Oleoresin of Capsicum

Carbamate

Aldicarb
Carbaryl

Carbonic Anhydrase Inhibitor

Acetazolamide
Diclofenamide
Dorzolamide
Ethoxzolamide
Methazolamide

Carcinogen

Sterigmatocystin

Cardiac Glycoside

Acetyldigitoxin
Deslanoside
Digitoxin
Digoxin
Lanatoside C
Metildigoxin
Ouabain
Strophanthin-K

Cardiotoxin

Yessotoxin

Catecholamine Metabolite

Metadrenaline
Normetadrenaline

Catecholeamine

Noradrenaline

Cathinone Derivative

Butylone
Ethylone
Methylone

Cationic Disinfectant

Benzalkonium Chloride
Benzethonium Chloride
Cetalkonium Chloride
Cetrimide
Cetylpyridinium Chloride
Chlorhexidine
Dofamium Chloride
Domiphen Bromide
Octafonium Chloride

Central Stimulant

Dexamfetamine
Dimethylamfetamine
Metamfetamine
Phenatine
Tacrine

Cefalosporin

Cefdinir

Chelating Agent

Desferrioxamine
Dimercaprol
Ditiocarb
Penicillamine

Chemical Warfare Agent

Adamsite

Cholesterol Absorption Inhibitor

Ezetimibe

Cholinesterase Inhibitor

Famphur
Fenitrothion
Fenthion

Cholinesterase Reactivator

Pralidoxime Chloride

Cognition Enhancer

Aniracetam

Copper Chelator

Trientine

Corticosteroid

Aldosterone
Beclometasone
Betamethasone
Clobetasol Propionate
Clobetasone Butyrate
Cortisone
Desoxycortone
Dexamethasone
Fluclorolone Acetonide
Fludrocortisone
Fludroxycortide
Flumetasone
Fluocinolone Acetonide
Fluocinonide
Fluocortolone
Fluorometholone
Halcinonide
Hydrocortisone
Methylprednisolone
Mometasone Furoate
Paramethasone
Prednisolone
Prednisone
Triamcinolone

Corticotrophic Peptide

Tetracosactrin

Coumarin

Demecolcine

CNS Depressant

Alcohol
Muscimol

Cyanogenic Glycoside

Amygdalin

Cyclic Sulfamic Acid

Cyclamic Acid

Cyclopyrrolone

Eszopiclone

Dehydroquinoline

Aripiprazole

Depigmenting Agent

Hydroquinone

Depressant

γ-Butyrolactone

Dermatoxin

Sterigmatocystin

Desoxynojirimycin Derivative

Miglitol

Diagnostic Agent

Triphenyltetrazolium Chloride
Tyramine

Diagnostic Agent (Gastric Secretion)

Ametazole
Histamine

Diagnostic Agent (Pituitary Function)

Metyrapone

Diagnostic Agent (Renal Function)

Aminohippuric Acid

Diagnostic Aid (Radiopaque Medium)

Barium
Propyliodone

Dibenzoxazepine

Amoxapine

Difuranocoumarin

Aflatoxins B
Aflatoxins G
Aflatoxins M

Dihydrofolate Reductase Inhibitor

Trimetrexate

Dihydropyridine

Nilvadipine

Diphenylacetic Acid Derivative

Dimenoxadole

Diphenylheptane Derivative

Levomethadyl Acetate

Disinfectant

Benzyl Alcohol
Chlorocresol
Chloroxylenol
Cresol
Ethacridine
Formaldehyde
Halazone
Hexachlorophene
Parachlorophenol
Phenol
Picloxydine
Proflavine Hemisulfate
Tosylchloramide Sodium
Triclocarban
Triclosan

Diterpine

Aconitine

Dopa-decarboxylase Inhibitor

Benserazide
Carbidopa

Dopamine Antagonist

Clopenthixol

Dopamine D_1 Receptor Agonist

Fenoldopam

Dopamine D_2 Receptor Agonist

Aripiprazole
Cabergoline
Pramipexole
Ropinirole

Dopamine D_2 Receptor Antagonist

Remoxipride

Dopamine Prodrug

Docarpamine

Dopamine Reuptake Inhibitor

Amineptine

Dopamine Uptake Inhibitor

Benzatropine

Dopamine and Noradrenaline Reuptake Inhibitor

Diphenylprolinol

Dopaminergic Agent

Bromocriptine
Budipine

Docarpamine
Droxidopa
Entacapone
Pergolide
Quinagolide

Drug of Abuse

Dibenzylpiperazine
Glaucine
m-Chlorophenylpiperazine

Dye

Aniline
Paraphenylenediamine
Tolonium Chloride

Electron Transport Chain Inhibitor

Cyanide

Endogenous Steroid

Dehydroepiandrosterone

Entactogen

2C-B

Epoxysesquiterpenoid

Deoxynivalenol

Ergot Alkaloid

Ergometrine
Ergotamine

Ergot Derivative

Ergotoxine

Essential Oil

Cineole
Citronella oil
Eugenol
Pulegium Oil

Ester

Oseltamivir

Fibric Acid Derivative

Fenofibrate

Flavouring Agent

Benzaldehyde
Ethyl Acetate
Vanillin

Fluoroquinolone

Enoxacin
Moxifloxacin

Food Additive

Oleoresin of Capsicum

Fumigant

Chloropicrin

Furopyridine

Cicletanine

GABA Analogue

Progabide

GABA Receptor Agonist

Eszopiclone
Progabide

$GABA_A$ Receptor Agonist

Muscimol

Ganglion Blocker

Azamethonium Bromide

Gastric and Pancreatic Lipase Inhibitor

Orlistat

Glutamatergic Agonist

Domoic Acid

Glutamatergic Antagonist

Riluzole

Glycine Receptor Antagonist

Strychnine

Gonad Stimulating Hormone

Chorionic Gonadotropin

Gonadorelin Analogue

Buserelin
Leuprorelin
Nafarelin

Guanidine

Pinacidil

HMG-CoA Reductase Inhibitor

Atorvastatin
Lovastatin
Pravastatin
Rosuvastatin calcium
Simvastatin

Hallucinogen

2C-B
2C-T-2
2C-T-7
5-Methoxy-*N*,*N*-diisopropyltryptamine
5-Methoxy-*N*,*N*-dimethyltryptamine
5-Methyltryptamine
Bufotenine
DOM
Diethyltryptamine
Dimethyltryptamine
Ethylpiperidyl Benzilate
Harmaline
Harmine
Lysergamide
Lysergic Acid
Lysergide
Mescaline
Methoxyamfetamine
Methylenedioxyamfetamine
Methylenedioxymetamfetamine
Methylpiperidyl Benzilate
Myristicin
N-Methyltryptamine
Phencyclidine
Psilocin
Psilocybine
Trimethoxyamfetamine
Δ^9-Tetrahydrocannabinol
-Methyltryptamine

Halocarbon

Phosgene

Halochondrine

Okadaic Acid

Heparin

Bemiparin

Histamine H_1- and H_2 Receptor Antagonist

Doxepin

Histamine H_1 Receptor Antagonist

Cetirizine
Desloratadine

Histamine H_2 Receptor Antagonist

Cimetidine
Famotidine
Nizatidine
Ranitidine

Histidine Decarboxylase Inhibitor

Brocresine

Hormone

Aviptadil

Hydroxyanthraquinone

Rhein

Hyperlipidaemic

Acipimox

Imidazopyridine

Alpidem

Incapacitant

Oleoresin of Capsicum

Inhibitor of Activated Factor Xa

Fondaparinux Sodium

Insect Repellent

Citronella oil
Dibutyl Phthalate
Diethyltoluamide
Dimethyl Phthalate

Interleukin I Inhibitor

Diacerein

Iron Chelator

Deferiprone

Isocoumarin Derivative

Ochratoxins

Isoquinoline Alkaloid

Lauroscholtzine

Isoxazole

Ibotenic Acid

Kainoid

Domoic Acid

LH-RH Receptor Agonist

Leuprorelin

Lachrymator

Acrolein
Bromoacetone

Leukotriene D_4 Antagonist

Pranlukast
Zafirlukast

Macrocyclic lactone

Emamectin

Macrolide

Dirithromycin

Marine Neurotoxin

Maitotoxin

Meglitinide

Nateglinide

Melatonin Antagonist

Ramelteon

Mesalamine

Balsalazide

Metal

Aluminium
Antimony
Arsenic
Barium
Beryllium
Bismuth
Cadmium
Copper
Lead
Mercury

Military Poison Gas

Cyanogen Chloride

Monoamine Oxidase Inhibitor

Phenelzine
Phenoxypropazine
Tranylcypromine

Monoterpene

Citronella oil

Morphine Impurity

Acetylcodeine
Pseudomorphine

μ Opioid Receptor Agonist

Levomethadyl Acetate

Mycotoxin

Aflatoxins B
Aflatoxins G
Aflatoxins M
Citrinin
Deoxynivalenol
Diacetoxyscirpenol
Fumonisins
Fusarenone-X
Neosolaniol
Ochratoxins
Okadaic Acid
Phallotoxin
Saxitoxin
Sterigmatocystin
Yessotoxin

NMDA Antagonist

Levomethorphan
Memantine

Narcotic

Betameprodine
Betaprodine
Clonitazene
Codeine *N*-oxide
Diampromide
Dimethylthiambutene
Ethylmethylthiambutene
Etoxeridine
Furethidine
Hydroxypethidine
Isomethadone
Levophenacylmorphan
Metazocine
Methyldesorphine
Methyldihydromorphine
Morpheridine
Morphine *N*-oxide
Myrophine
Nicocodine
Phenomorphan
Proheptazine
Racemethorphan

Narcotic Antagonist

Amiphenazole
Cyclazocine
Cyprenorphine
Levallorphan
Nalmefene
Nalorphine
Naloxone
Naltrexone

Narcotic Intermediate

Methadone Intermediate
Moramide Intermediate
Pethidine Intermediate A

Nasal Decongestant

Tymazoline

Natural Penicillin

Benzathine Benzylpenicillin

Natural Toxin

Tetrodotoxin

Neuraminidase Inhibitor

Oseltamivir

Neuromuscular Blocker

Rocuronium

Neurotoxin

Botulinum Toxin
Brevetoxin
Ciguatoxins
Conotoxins
Domoic Acid
Ibotenic Acid
Saxitoxin

Neurotransmitter

Acetylcholine Chloride
Dopamine
GABA
Glutamate
5–HT /Serotonin
Nitric Oxide
Noradrenaline

Nicotinic Acid Analogue

Acipimox

Non-Nucleoside Reverse Transcriptase Inhibitor

Nevirapine

Non-Specific Synergist

Proadifen

Nootropic

Aniracetam
Choline Alfoscerate
Exifone
Idebenone
Indeloxazine
Oxiracetam
Pramiracetam
Propentofylline
Rivastigmine
Taltirelin

Nucleoside Reverse Transcriptase Inhibitor

Abacavir
Lamivudine
Stavudine

Oestrogen

Chlorotrianisene
Dienestrol
Estradiol
Estriol
Estrone
Ethinylestradiol
Hexestrol
Mestranol
Methallenestril
Zeranol

Oestrogenic Mycotoxin

Zearalenone

Opiate

Etonitazene
Proheptazine

Opioid Derivative

6-Monoacetylmorphine
Acetyldihydrocodeine
Cyprenorphine

Opioid Receptor Antagonist

Dezocine

Organic Arsenical

Adamsite
Lewisite

Organocyanide

Dithiazanine Iodide

Organonitrile

CS Gas

Organophosphate

Bromofenofos
Chlorpyrifos
Demeton-*O*
Dioxathion
Famphur
Fenitrothion
Naled

Organophosphate Nerve Agent

Sarin
Soman
Tabun
VX

Oxazolidine

Metaxalone

Parasympatholytic

Amprotropine
Dibutoline
Diethylaminoethyl Diphenylpropionate

Parasympathomimetic

Acetylcholine Chloride
Bethanechol Chloride
Carbachol
Methacholine Chloride
Pilocarpine

Pavine Alkaloid

Californine

Pentasaccharide

Fondaparinux Sodium

Peripheral Vasodilator

Teclothiazide
Thurfyl Nicotinate

Pharmaceutical Adjuvant

Benethamine
Ethylenediamine Hydrate

Phenethylamine

2C-B
2C-T-2
2C-T-7
Amfetamine
Butylone
Dimethylamfetamine
Ethylone
Metamfetamine
Methoxyamfetamine
Methylenedioxymetamfetamine
Methylone
Trimethoxyamfetamine
Venlafaxine

Phenol

4-Aminophenol

Phenothiazine

Acepromazine
Alimemazine
Levomepromazine
Tolonium Chloride
Trifluoperazine
Triflupromazine

Phenylacetic Acid Derivative

Bromfenac

Phenylketone

CN Gas

Phenylpiperidine

Etoxeridine

Phenylpropionic Acid

Alminoprofen

Phosphodiesterase 4 Inhibitor

Rolipram

Phosphodiesterase 5 Inhibitor

Sildenafil
Tadalafil
Vardenafil

Phosphodiesterase Inhibitor

Dipyridamole
Milrinone

Photosensitiser

Furanocoumarins

Phthalane Derivative

Escitalopram

Phycotoxin

Okadaic Acid

Phytoalexin

Furanocoumarins

Pigmenting Agent

Methoxsalen
Trioxysalen

Piperazinoazepine

Mirtazapine

Piperidine

Benzamine
Betaprodine

Piperidine Derivative

Desloratadine

Plasticiser

Diethyl Phthalate
Tributyl Phosphate

Polyether Macrolactone

Pectenotoxins

Polyhydroxyl Alkylamine

Fumonisins

Potassium Channel Activator

Nicorandil
Pinacidil

Potassium Channel Antagonist

Barium
Nifekalant

Preservative

Benzoic Acid
Chlorobutanol
Ethyl Hydroxybenzoate
Methyl Hydroxybenzoate
Pentachlorophenol
Propyl Hydroxybenzoate
Sorbic Acid

Progestational Steroid

Norgestrel

Progestogen

Allylestrenol
Drospirenone
Elcometrine
Norelgestromin

Prolactin Inhibitor

Quinagolide

Propanamide

Ramelteon

Propionic Acid

Rebamipide
Vedaprofen

Prostaglandin

Alprostadil
Enprostil
Limaprost

Prostaglandin Analogue

Misoprostol

Prostaglandin Inhibitor

Droxicam

Protease Inhibitor

Indinavir
Lopinavir
Nelfinavir
Ritonavir

Protein

Alefacept
Etanercept

Protein Phosphatase 1 and 2A Inhibitor

Cantharidin

Protein Phosphatase Inhibitor

Okadaic Acid

Protein Synthesis Inhibitor

Amatoxins

Protein Toxin

Ricin

Proton Pump Inhibitor

Esomeprazole
Lansoprazole
Misoprostol
Omeprazole
Pantoprazole
Rabeprazole

Psoralens

Furanocoumarins

Psychedelic

DOC

Putrefactive Base

Cadaverine
Norharman
Phenethylamine
Putrescine
Tryptamine

Pyrazolopyrimidine

Zaleplon

Pyrimidine

Amisometradine

Pyrimidinedione

Aminometradine

Pyrimidinyl alcohol

Tetrodotoxin

Quaternary Ammonium

Azamethonium Bromide
Benzalkonium Bromide
Chlorisondamine Chloride
Dibutoline
Ipratropium
Laudexium Methyl Sulfate
Tiotropium
Tolonium Chloride

Quinoline

Glaucine

Quinolinol

Clamoxyquin

Radio-opaque Substance

Iopydol
Iopydone

Reagent for Synthesis of Cyanamides

Cyanogen Bromide
Cyanogen Chloride

Refrigerant

Cryofluorane
Dichlorodifluoromethane
Trichlorofluoromethane

Retinoid

Acitretin

Riot Control Agent

Adamsite
CN Gas
CR Gas
CS Gas

Serotonin Norepinephrine Reuptake Inhibitor

Atomoxetine
Duloxetine

Selective Nicotinic Receptor Antagonist

Chlorisondamine Chloride

Selective Oestrogen Receptor Modulator

Raloxifene

Selective Serotonin Reuptake Inhibitor (SSRI)

Citalopram
Escitalopram
Fluvoxamine
Paroxetine
Zimeldine

Serotonin Noradrenaline Reuptake Inhibitor (SNRI)

Venlafaxine

Skeletal Muscle Relaxant

Metaxalone

Sodium Channel Activator

Brevetoxin
Ciguatoxins

Sodium Channel Blocker

Cibenzoline
Tetrodotoxin

Sodium Transport Inhibitor

Saxitoxin

Solvent

Acetone
Amyl Acetate
Amyl Alcohol
Benzene
Carbon Tetrachloride
Diethyl Phthalate
Dimethyl Sulfoxide
Ethyl Acetate
Ethylene Glycol
Isopropyl Alcohol
Methanol
Methyl Ethyl Ketone
Methylene Chloride
Nitrobenzene
Propanol
Tetrachloroethane
Tetrachloroethylene
Toluene
Trichloroethane
Trichloroethylene
Xylene

Somatostatin Analogue (octopeptide)

Octreotide

Statin

Rosuvastatin

Steroid hormone

Drospirenone

Sterol

Cholesterol

Stimulant

Butylone
Ethylone
Methcathinone
Methylenedioxymetamfetamine
Methylone
N-Benzylpiperazine
Rhein
Trifluoromethylphenylpiperazine

Stimulant (Central)

Amfetamine

Succinimide

Fenimide

Sulfated Polyether

Yessotoxin

Sulfonamide

Dorzolamide
Mafenide
Phthalylsulfacetamide
Phthalylsulfathiazole
Succinylsulfathiazole
Sulfacarbamide
Sulfacetamide
Sulfachlorpyridazine
Sulfadiazine
Sulfadimethoxine
Sulfadimidine
Sulfadoxine
Sulfaethidole
Sulfafurazole
Sulfaguanidine
Sulfamerazine
Sulfamethizole
Sulfamethoxazole
Sulfamethoxypyridazine
Sulfametopyrazine
Sulfametoxydiazine
Sulfamoxole
Sulfanilamide
Sulfaphenazole
Sulfapyridine
Sulfasalazine
Sulfasomizole
Sulfathiazole
Sulfisomidine
Sumatriptan
Valdecoxib

Sunscreen Agent

Aminobenzoic Acid
Mexenone
Padimate

Sweetener

Cyclamic Acid
Saccharin

Sympathomimetic

Adrenaline
Adrenalone
Amfetamine
Amidefrine
Cathine
Clorprenaline
Cyclopentamine
Dobutamine
Dopamine
Ephedrine
Etafedrine
Ethylnoradrenaline
Etilefrine
Fenoterol
Hexoprenaline
Hordenine
Hydroxyamfetamine
Hydroxyephedrine
Iminodimethylphenylthiazolidine
Isoetarine
Isometheptene
Isoprenaline
Mabuterol
Mephentermine
Metaraminol
Methoxamine
Methoxyphenamine
Methylaminoheptane
Methylephedrine
Naphazoline
Noradrenaline
Orciprenaline
Oxedrine
Oxymetazoline
Phenylephrine
Phenylpropanolamine
Pholedrine
Prenalterol
Propylhexedrine
Pseudoephedrine
Racephedrine
Ritodrine
Salbutamol
Terbutaline
Tetryzoline
Tramazoline
Tuaminoheptane
Tymazoline
Tyramine
Xylometazoline

T-cell Activation Inhibitor

Alefacept

TNFα-fusion Protein

Etanercept

Taxane

Paclitaxel

Tertiary Amine

Oxybutynin

Tertiary Tricyclic Antidepressant

Doxepin

Tetrahydropurine

Saxitoxin

Theophylline Derivative

Doxofylline

Thiazolidine

Epalrestat

Thiazolidinedione

Pioglitazone
Rosiglitazone

Thienotriazolodiazepine

Brotizolam

Thienyl Opioid Derivative

Dimethylthiambutene

Thioxanthene

Clopenthixol

Thyrotropin Releasing Hormone Analogue

Taltirelin

Toxin

Amatoxins
Botulinum Toxin
Brevetoxin
Ciguatoxins
Conotoxins
Deoxynivalenol
Diacetoxyscirpenol
Maitotoxins
Pectenotoxins
Phallotoxin
Ricin
Saxitoxin
Tetrodotoxin
Yessotoxin
Zearalenone

Trichothecene Mycotoxin

HT-2 Toxin
T-2 Toxin

Tricyclic Amine

Cyclobenzaprine

Tricyclic Dibenzoxazepine

Loxapine

Tropane

Apoatropine

Tumour Marker

Tolonium Chloride

Type 2 Ribosome Inactivating Protein

Ricin

Uracil

Nifekalant

Urea Derivative

Allantoin

Uterine Relaxant (Veterinary)

Vetrabutine

Vasoconstrictor

Adrenalone

Vesicant

Cantharidin
Lewisite
Phosgene
Sulfur Mustard

Vitamin

Alfacalcidol
Ascorbic Acid
Colecalciferol
Ergocalciferol
Folic Acid
Nicotinamide
Nicotinic Acid
Phytomenadione
Pyridoxine
Riboflavin
Thiamine

Vitamin B Activity

Dexpanthenol

Vitamin D Activity

Dihydrotachysterol
Doxercalciferol
Maxacalcitol

Vitamin E Activity

α-Tocoferil Acetate

Vitamin K Activity

Acetomenaphthone

Vitamin K Analogue

Menadione

Vulnerary

Allantoin

War Gas

CS Gas
  Chloropicrin
Phosgene

Xanthine Derivative

Propentofylline
Theobromine

Xanthine Oxidase Inhibitor

Allopurinol

Xanthine Stimulant

Caffeine
Fenetylline

6 Molecular Weights

Mol. wt	Compound
3.89	Lithium Carbonate
9.01	Beryllium
11.4	Nalorphine
13.4	Noscapine
20.01	Hydrofluoric Acid
22	Azide
22.4	Mesocarb
25.01	Beryllium Oxide
25.4	Methylpiperidyl Benzilate
26.02	Cyanide
26.98	Aluminium
27.0	Hydrogen Cyanide
30.03	Formaldehyde
32.04	Methanol
32.3	Mebutamate
33.3	Methylphenidate
35.2	Lormetazepam
37.5	Lobeline
41.9	Sodium Fluoride
43.03	Beryllium Hydroxide
44.1	Acetaldehyde
44.5	Mitoxantrone
45.9	Loxapine Succinate
46.03	Formic Acid
46.07	Ethanol
47.01	Beryllium Fluoride
51.4	Meloxicam
56.1	Acrolein
57.4	Nalbuphine
57.7	Meclofenoxate
58.0	Aluminium Phosphide
58.08	Acetone
60.1	Acetic Acid Glacial
60.10	Isopropyl Alcohol
60.1	Propanol
60.1	Urea
61.1	Monoethanolamine
61.5	Cyanogen Chloride
61.8	Boric Acid
62.07	Ethylene Glycol
63.55	Copper
64.9	Loprazolam
65.01	Sodium Azide
66	Ricin
70.8	Medazepam
72.1	Methyl Ethyl Ketone
72.5	Nabilone
73.0	Lobeline Sulfate
74.12	Ether
74.9	Arsenic
76.1	Hydroxycarbamide
77.1	Fluoroacetamide
78.0	Aluminium Hydroxide
78.0	Fluoroacetic Acid
78.1	Ethylenediamine Hydrate
78.11	Benzene
78.13	Dimethyl Sulfoxide
79.92	Beryllium Chloride
82.1	Fomepizole
83.2	Methyprylon
84.1	Aminotriazole
84.93	Methylene Chloride
85.3	Norcodeine
86.1	γ-Butyrolactone
86.1	Piperazine
86.6	Mesoridazine
88.10	Ethyl Acetate
88.15	Amyl Alcohol
88.2	Putrescine
89.1	Urethane
90.03	Oxalic Acid
92.1	Toluene
92.2	Myristicin
93.1	Aniline
94.11	Phenol
94.9	Methyl Bromide
97.9	Ammonium Bromide
98.14	Methylpentynol
98.3	Nialamide
98.9	Phosgene
99.1	Succinimide
100.0	Sodium Fluoroacetate
101.9	Aluminium Oxide
102.0	(S)-3-Hydroxy-γ Butyrolactone
102.1	Cycloserine
102.2	Cadaverine
102.9	Sodium Bromide
104.1	γ-Hydroxybutyrate
104.2	Choline
105.9	Cyanogen Bromide
106.1	Benzaldehyde
106.2	Xylene
108.1	Benzyl Alcohol
108.1	Paraphenylenediamine
109.1	4-Aminophenol
109.1	Nicotinyl Alcohol
110.1	Hydroquinone
110.1	Resorcinol
111.1	Ametazole
111.1	Histamine
112.1	Sorbic Acid
112.4	Cadmium
114.1	Muscimol
114.2	Thiamazole
115.2	Methylhexaneamine
115.2	Tuaminoheptane
117.1	Amyl Nitrite
117.2	Betaine
119.0	Potassium Bromide
119.4	Chloroform
120.2	Noxytiolin
120.9	Dichlorodifluoromethane
121.1	Trometamol
121.2	Choline Hydroxide
121.2	Phenethylamine
121.8	Antimony
122.0	Aluminium Phosphate
122.1	Benzoic Acid
122.1	Nicotinamide
122.2	Thiodiglycol (metabolite)
123.1	Nicotinic Acid
123.1	Nitrobenzene
123.1	Pyrazinamide
124.2	Dimercaprol
124.8	Stibine
127.1	Sodium-4-hydroxybutyrate
127.2	Coniine
127.6	Chloroaniline
127.6	2-Chloroaniline
127.6	3-Chloroaniline
127.6	4-Chloroaniline
128.1	Barbituric Acid
128.1	Calcium Oxalate
128.2	Naphthalene
128.4	Cadmium Oxide
128.6	Parachlorophenol
129.1	Dimethadione
129.1	Flucytosine
129.2	Metformin
129.2	Methylaminoheptane
129.2	Vigabatrin
129.9	Sodium Arsenite
130.1	Fluorouracil
130.2	Amyl Acetate
131.2	Aminocaproic Acid
131.4	Trichloroethylene
132.2	Paraldehyde
133.0	Beryllium Nitrate
133.2	Tranylcypromine
133.3	Aluminium Chloride
133.4	Trichloroethane
134.0	Methanearsonic Acid
134.0	Sodium Oxalate
135.2	Acetanilide
135.2	Amfetamine
135.2	Dexamfetamine
135.2	Levamfetamine
136.1	Allopurinol
136.2	Betahistine
136.2	Mebanazine
136.2	Phenelzine
136.9	Bromoacetone
137.1	Aminobenzoic Acid
137.1	Isoniazid
137.1	Methyl Nicotinate
137.1	Salicylamide
137.2	Methyridine
137.2	Tyramine
137.3	Barium
137.4	Trichlorofluoromethane
138.0	Dimethylarsinic Acid
138.1	Salicylic Acid
138.2	Pentetrazol
139.2	Deferiprone
139.6	Choline Chloride
140.1	Sarin
140.2	Methenamine
141.1	Dimetridazole
141.1	Methamidophos
141.2	Ethosuximide
141.2	Methylpentynol Carbamate
141.2	Tropine
141.3	Cyclopentamine
141.3	Isometheptene
141.6	Chloromethylaniline
141.7	Amfetamine Hydrochloride
142.2	Methylthiouracil
142.6	Chlorocresol
143.1	Trimethadione
144.1	Sodium Benzoate
144.2	Betanaphthol
144.2	Valproic Acid
144.5	Cadmium Sulfide
144.6	Gallium Arsenide
144.6	Ethchlorvynol
145.1	Aminonitrothiazole
145.2	Emylcamate
145.2	Heptaminol
145.2	Hydroxyquinoline
146.2	Trientine
147.0	Paradichlorobenzene
147.5	Aluminium Chlorhydrate
148.3	Ditiocarb
149.2	Metamfetamine
149.2	Penicillamine
149.2	Phentermine
149.2	Phenylpropylmethylamine
149.3	Botulinum Toxin A
149.4	Trichloroethanol
150.2	Potassium Sorbate
150.2	Thymol
150.6	Botulinum Toxin B
151	Arsenic Acid
151.2	Cathine
151.2	Ethyl Nicotinate
151.2	Hydroxyamfetamine
151.2	Paracetamol
151.2	Phenylpropanolamine
151.3	Amantadine
152.1	Mandelic Acid
152.1	Methyl Hydroxybenzoate

Mol. wt	*Compound*
152.1	Methyl Salicylate
152.1	Resorcinol Monoacetate
152.1	Vanillin
152.2	Camphor
153.1	Aminosalicylic Acid
153.2	Dopamine
153.3	Barium Oxide
153.6	Betaine Hydrochloride
153.8	Carbon Tetrachloride
154.1	Acipimox
154.1	Patulin
154.2	Diphenyl
154.3	Cineole
154.6	CN Gas
155.2	Arecoline
155.2	Bemegride
155.3	Pempidine
155.3	Propylhexedrine
156.1	Mustine
156.1	Nifuroxime
156.3	Menthol
156.6	Chloroxylenol
156.7	Stannous Fluoride
157.2	Buformin
157.2	Paramethadione
158.1	Allantoin
158.1	Ibotenic Acid
158.2	1,2-Naphthoquinone
158.2	Oxiracetam
159.1	Sulfur Mustard
159.2	Pargyline
159.2	Pregabalin
159.6	Copper Sulfate
160.0	Sodium Cacodylate
160.1	Sodium Salicylate
160.2	Hydralazine
160.2	Tolazoline
160.2	Tryptamine
161.2	Aletamine
161.7	Clomethiazole
162.0	Sodium Methanearsonate
162.1	Tabun
162.2	Nicotine
163.2	Acetylcysteine
163.2	Methcathinone
163.3	Dimethylamfetamine
163.3	Mephentermine
164.2	Eugenol
164.4	Chloropicrin
165.0	Methoxyflurane
165.2	Benzocaine
165.2	Anhydrous Ephedrine
165.2	Etenzamide
165.2	Hordenine
165.2	Methoxyamfetamine
165.2	Pholedrine
165.2	Pseudoephedrine
165.2	Racephedrine
165.4	Chloral Hydrate
165.6	Metformin Hydrochloride
165.7	Methylaminoheptane Hydrochloride
165.8	Tetrachloroethylene
166.2	Ethionamide
166.2	Ethyl Hydroxybenzoate
166.2	Potassium Oxalate
166.2	Phenoxypropazine
166.2	Sodium Valproate
167.2	Ethinamate
167.2	Metaraminol
167.2	Orthocaine
167.2	Oxedrine
167.2	Phenylephrine
167.2	Tioguanine
167.3	Mecamylamine
167.8	Tetrachloroethane
168.0	Desflurane
168.2	Norharman
169.1	Glyphosate
169.2	Ammonium Mandelate
169.2	Noradrenaline
169.2	Pyridoxine
169.6	Chlorzoxazone
170.2	Levetiracetam
170.2	Mercaptopurine
170.2	Propylthiouracil
170.9	Cryofluorane
171.2	Gabapentin
171.2	Metronidazole
171.3	Barium Hydroxide
171.3	Ditiocarb Sodium
171.6	Chloronitrotoluene
172.2	Menadione
172.2	Sulfanilamide
172.4	Cadmium Carbonate
172.6	Chloronitroaniline
172.6	Pralidoxime Chloride
173.6	Tyramine Hydrochloride
174.1	Sodium Methyl Hydroxybenzoate
174.2	Ephedrine
174.2	5-Methyltryptamine
174.2	α-Methyltryptamine
174.2	*N*-Methyltryptamine
175.2	Potassium Aminobenzoate
175.2	Debrisoquine
176.1	Ascorbic Acid
176.2	Pemoline
176.2	Serotonin
176.3	*N*-Benzylpiperazine
177.2	Bemegride Sodium
177.2	Bethanidine
177.2	Phenmetrazine
177.5	Chlorobutanol
177.6	Dimetridazole Hydrochloride
177.7	Cyclopentamine Hydrochloride
177.7	Isometheptene Hydrochloride
178.1	Arsenobetaine
178.2	Nikethamide
178.3	Amylmetacresol
178.3	Etisazole
178.3	Propofol
179.2	Cyclamic Acid
179.2	Iproniazid
179.2	Methylenedioxyamfetamine
179.2	Phenacetin
179.2	Phenprobamate
179.3	Memantine
179.3	Methoxymetamfetamine
179.3	Methoxyphenamine
179.3	Methylephedrine
179.3	Mexiletine
179.3	Rimantadine
180.2	Aspirin
180.2	Butylated Hydroxyanisole
180.2	Propyl Hydroxybenzoate
180.2	Rilmenidene
180.2	Theobromine
180.2	Theophylline
180.3	Protionamide
181.2	Adrenalone
181.2	Etilefrine
181.2	Hydroxyephedrine
181.2	Styramate
181.3	Methylthioamfetamine
181.7	Acetylcholine Chloride
181.7	Heptaminol Hydrochloride
182.2	Eflornithine
182.2	Harman
182.2	Mannitol
182.2	Mephenesin
182.2	Soman
182.6	Carbachol
183.2	Adrenaline
183.2	Nordefrin
183.2	Levonordefrin (()-isomer)
183.2	Normetadrenaline
183.2	Saccharin
183.3	Cadmium Chloride
183.7	Chlorphentermine
183.9	Disodium Methanearsonate
184.1	Ametazole Hydrochloride
184.1	Histamine Hydrochloride
184.2	Apronal
184.2	Barbital
184.2	Diquat
184.5	Enflurane
185.2	Ecgonine
185.7	Metamfetamine Hydrochloride
185.7	Penicillamine Hydrochloride
185.7	Phentermine Hydrochloride
185.7	Phenylpropylmethylamine Hydrochloride
185.9	Sodium Arsenate
186.2	Carbimazole
186.2	Furanocoumarins
186.2	Mafenide
186.3	Paraquat
187.2	Aminitrozole
187.3	Selegiline
187.7	Amantadine Hydrochloride
187.7	Cathine Hydrochloride
187.7	Phenylpropanolamine Hydrochloride
188.1	Sodium Fluorosilicate
188.2	Sodium Ethyl Hydroxybenzoate
188.2	Phenazone
188.3	Dimethyltryptamine
188.3	Fenproporex
188.6	CS Gas
189.2	Phensuximide
189.2	Thiotepa
189.4	Barium Cyanide
189.6	Dopamine Hydrochloride
190.2	Cytisine
190.2	Dihydralazine
190.2	Nitroxoline
190.3	Aldicarb
191.1	Isosorbide Mononitrate
191.2	Potassium Aminosalicylate
191.2	Fenmetramide
191.3	Amiphenazole
191.3	Diethyltoluamide
191.3	Phendimetrazine
191.7	Propylhexedrine Hydrochloride
192.0	Clopidol
192.0	Foscarnet Sodium
192.3	Bitoscanate
192.3	Tocainide
193.2	Butyl Aminobenzoate
193.2	Isobutyl Aminobenzoate
193.2	Methylenedioxymetamfetamine
193.3	Etafedrine
193.5	Butylchloral Hydrate
193.7	Buformin Hydrochloride
194.2	Aminoacridine
194.2	Aminohippuric Acid
194.2	Caffeine
194.2	Dimethyl Phthalate
194.2	Piperazine Hydrate
194.2	Temozolomide
194.3	Hexylresorcinol
195.2	Aminometradine
195.2	Amisometradine
195.2	CR Gas
195.7	Methacholine Chloride
195.7	Pargyline Hydrochloride
196.2	Cantharidin
196.6	Hydralazine Hydrochloride
196.7	Bethanechol Chloride
196.7	*m*-Chlorophenylpiperazine
196.7	Tolazoline Hydrochloride
196.7	Tryptamine Hydrochloride
197.2	Ethylnoradrenaline
197.2	Levodopa
197.2	Metadrenaline
197.3	Barium Carbonate
197.4	Halothane
197.7	Aletamine Hydrochloride
197.7	Beclamide
197.8	Arsenic Trioxide
198.1	Sodium Ascorbate
198.1	Dinitro-orthocresol
198.1	Nitrofurazone
198.2	Ethyl Gallate
198.2	Guaifenesin
198.2	Metharbital
198.2	Probarbital
198.2	Theophylline Hydrate
198.3	Guanethidine
198.3	Tacrine
199.3	Diethylcarbamazine
199.3	Phenothiazine
199.4	Octamylamine

Mol. wt	*Compound*
199.7	Methcathinone Hydrochloride
199.9	Calcium Bromide
200.1	Sevoflurane
200.3	Amantadine Sulfate
200.3	Dexmedetomidine
200.3	Pethidine Intermediate A
200.3	Tetryzoline
200.6	Mercury
200.6	Methylchlorophenoxyacetic Acid
201.2	Carbaryl
201.2	Sodium Cyclamate
201.3	Tiabendazole
201.7	Edrophonium Chloride
201.7	Ephedrine Hydrochloride
201.7	Methoxyamfetamine Hydrochloride
201.7	Pseudoephedrine Hydrochloride
201.7	Racephedrine Hydrochloride
201.7	Simazine
202.1	Piperazine Phosphate
202.1	Theophylline Sodium Glycinate
202.2	Sodium Propyl Hydroxybenzoate
202.6	Chlorphenesin
203.2	Mesuximide
203.3	Crotamiton
203.7	Phenylephrine Hydrochloride
203.8	Mecamylamine Hydrochloride
204.2	Ethotoin
204.2	Thozalinone
204.2	Tryptophan
204.3	Bufotenine
204.3	Ethambutol
204.3	Levamisole
204.3	Psilocin
204.3	Tetramisole
205.3	Dexpanthenol
205.3	Diethylpropion
205.3	Phenformin
205.6	Noradrenaline Hydrochloride
205.6	Pyridoxine Hydrochloride
206.2	Barbital Sodium
206.2	Pheneturide
206.3	Ibuprofen
206.3	Iminodimethylphenylthiazolidine
206.3	Isoproturon
206.3	Pyrantel
207.2	Guanoxan
207.2	Hydrastinine
207.2	Lead
207.2	Methylone
207.2	Miglitol
207.2	Thurfyl Nicotinate
207.2	Toloxatone
207.3	Hexyl Nicotinate
207.3	Lewisite
208.1	Coniine Hydrobromide
208.2	Allobarbital
208.2	Barium Chloride
208.3	Pilocarpine
208.5	Cadmium Sulfate
208.9	Bismuth
209.1	Betahistine Hydrochloride
209.2	Hydroxyphenamate
209.3	Bamethan
209.3	DOM
209.3	Minoxidil
209.9	Cryolite
210.2	Aprobarbital
210.2	Phanquinone
210.2	Tetraethylammonium Bromide
210.3	Altretamine
210.3	Naphazoline
210.7	5-Methyltryptamine Hydrochloride
211.1	Sodium Aminosalicylate
211.2	Milrinone
211.2	Nicorandil
211.2	Zalcitabine
211.3	Benethamine
211.3	Isoprenaline
211.3	Mescaline
211.3	Methoxamine
211.3	Orciprenaline
211.3	Pramipexole
212.2	Benzyl Benzoate
212.2	Caffeine Hydrate
212.2	Secbutabarbital
212.2	Zonisamide
212.3	Butobarbital
212.3	Harmine
212.8	*N*-Benzylpiperazine Hydrochloride
213.2	Benzyl Nicotinate
213.2	Droxidopa
213.2	Omethoate
213.2	Phenazopyridine
213.3	Desmetryne
213.7	Baclofen
213.7	Clorprenaline
213.7	Phenmetrazine Hydrochloride
214.0	Carmustine
214.2	Amifostine
214.2	Niridazole
214.2	Salol
214.2	Sulfacetamide
214.3	Fenyramidol
214.3	Harmaline
214.3	Sulfaguanidine
214.4	1,1′-Sulfonylbis-[2-*S*-(methylthio) ethane] (metabolite)
214.6	Monolinuron
214.7	Etisazole Hydrochloride
214.7	Phenaglycodol
215.2	1,2-Naphthoquinone 2-Semicarbazone
215.3	Fencamfamin
215.3	Tramazoline
215.7	Atrazine
215.7	Methoxyphenamine Hydrochloride
215.7	Methylenedioxyamfetamine Hydrochloride
215.7	Methylephedrine Hydrochloride
215.7	Mexiletine Hydrochloride
215.8	Memantine Hydrochloride
215.8	Rimantadine Hydrochloride
216.2	Sodium Aminohippurate
216.2	Methoxsalen
216.3	Diethyltryptamine
217.1	Arsanilic Acid
217.3	Captopril
217.3	Potassium Cyclamate
217.3	Fenimide
217.3	Glutethimide
217.4	Prolintane
217.7	Adrenalone Hydrochloride
217.7	Etilefrine Hydrochloride
217.7	Hydroxyephedrine Hydrochloride
218.0	Brocresine
218.1	Propanil
218.2	Phenylmethylbarbituric Acid
218.3	Carphedon
218.3	Mephenytoin
218.3	Meprobamate
218.3	Metallibure
218.3	5-Methoxy-*N*,*N*-dimethyltryptamine
218.3	Primidone
218.7	Eflornithine Hydrochloride
219.2	Aniracetam
219.2	Trientine Dihydrochloride
219.3	Alminoprofen
219.3	Profadol
219.7	Adrenaline Hydrochloride
219.7	Nordefrin Hydrochloride
220.1	Chlorphentermine Hydrochloride
220.3	Butibufen
220.3	Morantel
220.3	Prilocaine
220.3	Xylazine
220.4	Butylated Hydroxytoluene
221.0	Dicamba
221.0	Dichlorophenoxyacetic Acid
221.0	Dichlorvos
221.2	Butylone
221.2	Ethylone
221.3	Metaxalone
221.3	Procarbazine
221.7	Ecgonine Hydrochloride
222.2	Diethyl Phthalate
222.2	Morinamide
222.2	Phenindione
222.3	Acetazolamide
222.3	Nicametate
222.7	Mafenide Hydrochloride
223.1	Bromisoval
223.2	Lead Oxide
223.2	Monocrotophos
223.3	Bufexamac
223.3	Butoxyethyl Nicotinate
223.3	Etamivan
223.3	Norbudrine
223.3	Phenisonone
223.3	Rimiterol
223.3	Tigloidine
223.3	Tiletamine
223.7	Selegiline Hydrochloride
224.1	Mevinphos
224.2	Etofylline
224.2	Stavudine
224.3	Butalbital
224.3	Enallylpropymal
224.3	Idobutal
224.3	Talbutal
224.3	Vinbarbital
224.3	Vinylbital
225.2	Aciclovir
225.2	Dinitolmide
225.2	Furazolidone
225.2	Mephenesin Carbamate
225.3	Ditiocarb Sodium Trihydrate
225.3	*N*-Methylmescaline
225.3	Prenalterol
225.3	Terbutaline
225.3	Trimethoxyamfetamine
225.7	Hydrastinine Hydrochloride
226.1	Acetylcholine Bromide
226.2	Dithranol
226.2	Nimorazole
226.3	Amobarbital
226.3	Crotetamide
226.3	Metyrapone
226.3	Pentobarbital
226.7	Alclofenac
227.1	Glyceryl Trinitrate
227.3	Ametryne
227.7	Amiphenazole Hydrochloride
227.7	Buclosamide
228.1	Antimony Trichloride
228.1	Chlorquinaldol
228.2	Glyphosate Isopropylammonium
228.2	Trioxysalen
228.3	Nabumetone
228.3	Sulfonal
228.7	Tocainide Hydrochloride
229.2	Phenyl Aminosalicylate
229.3	Dimethoate
229.3	Lamivudine
229.6	Amiloride
229.7	Propazine
229.7	Terbutylazine
229.75	Methylenedioxymetamfetamine Hydrochloride
229.8	Arsenic Pentoxide
229.8	Etafedrine Hydrochloride
230.1	Clonidine
230.2	Trifluoromethylphenylpiperazine
230.3	Demeton-*S*-(Me)
230.3	Metomidate
230.3	Naproxen
230.3	Propyphenazone
230.7	Diazoxide
230.7	DOC
230.7	Dimethylmercury
231.3	Aminophenazone
231.3	Eptazocine
231.3	Fenfluramine
231.3	Indeloxazine
231.3	Isocarboxazid
231.3	Metazocine
232.1	Hydroxyamfetamine Hydrobromide
232.2	Aprobarbital Sodium
232.2	Nalidixic Acid
232.2	Panidazole
232.2	Phenobarbital
232.3	Aminoglutethimide
232.3	Piperazine Adipate

Mol. wt	*Compound*
232.3	Pralidoxime Mesilate
232.3	Pyrrocaine
232.3	Sulfaguanidine Monohydrate
232.3	Tymazoline
232.7	Mercuric (II) Sulfide
233.1	Diuron
233.2	Amfetamine Phosphate
233.2	*m*CPP Hydrochloride
233.2	Dexamfetamine Phosphate
233.2	Sulfacarbamide
233.3	Piperoxan
233.4	Barium Sulfate
233.4	Meptazinol
233.7	Ethylnoradrenaline Hydrochloride
234.1	Diloxanide
234.2	Riluzole
234.2	Secbutabarbital Sodium
234.3	Cyclopentobarbital
234.3	Lidocaine
234.3	Metisazone
234.3	Octacaine
234.3	Phenelzine Sulfate
234.4	Sparteine
234.7	Tacrine Hydrochloride
235.3	Amylocaine
235.3	Azapetine
235.3	Meprylcaine
235.3	Padimate
235.3	Procainamide
235.8	Octamylamine Hydrochloride
236.1	Arecoline Hydrobromide
236.1	Isosorbide Dinitrate
236.2	Carbazochrome
236.2	Didanosine
236.3	Butethamine
236.3	Carbamazepine
236.3	Cyclobarbital
236.3	Dropropizine
236.3	Hexobarbital
236.3	Levodropropizine
236.3	Metabutethamine
236.3	Methazolamide
236.3	Procaine
236.3	Thioacetazone
236.6	Eflornithine Hydrochloride Monohydrate
236.7	Tetryzoline Hydrochloride
237.1	Carbromal
237.3	Cotarnine
237.3	Viloxazine
237.7	Ketamine
238.2	Felbamate
238.2	Methyldopa
238.2	Nitrofurantoin
238.2	Proxyphylline
238.3	Nealbarbital
238.3	Nomifensine
238.3	Rilmenidene Fumarate
238.3	Secobarbital
239.1	Sodium Arsanilate
239.3	Flosequinan
239.3	Isoetarine
239.3	Lead Sulfide
239.3	Methyldopate
239.3	Salbutamol
239.4	Benzfetamine
239.7	Bupropion
240.1	Methacholine Bromide
240.2	Dantron
240.3	Cropropamide
240.3	Hexethal
240.3	Pheniramine
240.8	Levamisole Hydrochloride
240.8	Tetramisole Hydrochloride
241.2	Methocarbamol
241.2	Saccharin Sodium
241.2	Salinazid
241.2	Theophylline Monoethanolamine
241.3	Mefenamic Acid
241.3	Choline Salicylate
241.4	2C-T-2
241.4	Prometryne
241.4	Terbutryne
241.7	Moxonidine
241.7	Phenformin Hydrochloride
241.8	Diethylpropion Hydrochloride
242.3	Butibufen Sodium
242.3	Fenoprofen
242.3	Mexenone
242.3	Thiopental
242.7	Clofibrate
243.2	Cytarabine
243.4	Norlevorphanol
243.4	Phencyclidine
244.2	Acetazolamide Sodium
244.2	Carbidopa
244.3	Amidefrine
244.3	Etomidate
244.3	Flurbiprofen
244.3	Rolicyprine
244.4	Isoaminile
244.4	Xylometazoline
244.7	Pilocarpine Hydrochloride
244.9	Clodronic Acid
245.1	Apraclonidine
245.2	Glyphosate Trimesium
245.3	Isopropylaminophenazone
245.3	Pinacidil
245.3	Tropacocaine
245.4	Dezocine
245.7	Chlorphenesin Carbamate
245.7	DOM Hydrochloride
246.3	Busulfan
246.3	Loxoprofen
246.3	Mafenide Acetate
246.3	Methylphenobarbital
246.3	Santonin
246.4	Mepivacaine
246.4	Milnacipran
246.7	Naphazoline Hydrochloride
247.2	Aciclovir Sodium
247.3	Benzamine
247.3	Ketobemidone
247.3	Parbendazole
247.3	Pethidine
247.3	Tinidazole
247.7	Isoprenaline Hydrochloride
247.7	Methoxamine Hydrochloride
247.8	Mescaline Hydrochloride
248.3	Amobarbital Sodium
248.3	Bunitrolol
248.3	Dapsone
248.3	Enoximone
248.3	Pentobarbital Sodium
248.3	Pindolol
248.3	Pralidoxime Metilsulfate
248.7	Aminoacridine Hydrochloride
248.7	Pyrimethamine
249.1	Alendronic Acid
249.1	Linuron
249.3	*N*-Benzylpiperazine Dihydrochloride
249.3	Cinchophen
249.3	Epinastine
249.3	Sulfapyridine
249.4	Alprenolol
249.4	Benzoctamine
249.7	Phenazopyridine Hydrochloride
250.2	Diflunisal
250.3	Citrinin
250.3	Heptabarb
250.3	Methaqualone
250.3	Naepaine
250.3	Rivastigmine
250.3	Sulfadiazine
250.7	Fenyramidol Hydrochloride
251.1	Methylmercuric Chloride
251.4	Triclofos Sodium
251.7	Cycloguanil
251.8	Fencamfamin Hydrochloride
251.8	Tramazoline Hydrochloride
252.2	Naproxen Sodium
252.3	Anisindione
252.3	Oxcarbazepine
252.3	Phenytoin
252.4	Cimetidine
253.3	Choline Bitartrate
253.3	Diphenylprolinol
253.3	Ethacridine
253.3	*N*-Acetylmescaline
253.3	Nefopam
253.3	Penciclovir
253.3	Sulfamethoxazole
253.3	Triamterene
253.4	Irgarol
253.4	Tolpropamine
253.7	Proguanil
253.7	Tizanidine
253.8	Prolintane Hydrochloride
254.2	Diprophylline
254.2	Phenobarbital Sodium
254.2	Sulfacetamide Sodium
254.3	Dexketoprofen
254.3	Fenbufen
254.3	Ketoprofen
254.3	Phenamidine
254.3	Thiamylal
254.7	Losigamone
254.8	Butanilicaine
255.2	Ganciclovir
255.3	Ambazone
255.3	Cetoxime
255.3	Ketorolac
255.3	Sulfathiazole
255.4	Atomoxetine
255.4	2C-T-7
255.4	Diphenhydramine
255.4	Phenyltoloxamine
255.4	Tofenacin
255.4	Tripelennamine
255.5	Barium Acetate
255.5	Trichlorophenoxyacetic Acid
255.7	Gallium Nitrate
255.7	Imidacloprid
255.8	Profadol Hydrochloride
256.1	Anagrelide
256.1	Lamotrigine
256.3	Etoxazene
256.8	Prilocaine Hydrochloride
256.8	Xylazine Hydrochloride
257.2	Benserazide
257.2	Choline Alfoscerate
257.2	Paraquat Dichloride
257.3	Etebenecid
257.3	Tolmetin
257.4	Dextrorphan
257.4	Levorphanol
257.4	Racemorphan
257.4	Trichlorfon
257.8	Procarbazine Hydrochloride
258.2	Salsalate
258.2	Thalidomide
258.3	Acetomenaphthone
258.3	Demeton-*O*
258.3	Demeton-*S*
258.3	Ethoxzolamide
258.3	Hexobarbital Sodium
258.7	Morinamide Hydrochloride
259.1	Lofexidine
259.2	Nicotinyl Tartrate
259.3	Propranolol
259.3	Ramelteon
259.4	Primaquine
259.8	Clobenzorex
259.8	Norbudrine Hydrochloride
260.1	Carbarsone
260.1	2C-B
260.1	Nitrofurantoin Sodium
260.3	Carisoprodol
260.3	Diaveridine
260.3	Imolamine
260.3	Mafenide Propionate
260.3	Secobarbital Sodium
260.3	Suprofen
260.3	Tiaprofenic Acid
260.4	Methaphenilene
260.4	Oxymetazoline
260.4	Ropinirole
261.1	Pyridostigmine Bromide
261.4	Alphaprodine
261.4	Betaprodine
261.4	Ethoheptazine
261.4	Hexylcaine
261.4	Methapyrilene

Mol. wt	*Compound*
261.4	Phenindamine
261.4	Piperocaine
261.4	Properidine
261.4	Setiptiline
261.4	Thenyldiamine
261.7	Cicletanine
261.7	Prenalterol Hydrochloride
261.7	Tolfenamic Acid
262.3	Hexethal Sodium
262.3	Methohexital
262.4	Cibenzoline
262.7	Clonixin
263.1	Roxarsone
263.1	Guanoclor
263.2	Gemcitabine
263.3	Dioxyamidopyrine
263.3	Hydroxypethidine
263.3	Pinacidil Monohydrate
263.4	Butetamate
263.4	Dimethylthiambutene
263.4	Nortriptyline
263.4	Protriptyline
263.4	Tramadol
263.8	Ticlopidine
264.1	Pralidoxime Iodide
264.3	Pentifylline
264.3	Stilbamidine
264.3	Sulfamerazine
264.3	Thialbarbital
264.3	Thiopental Sodium
264.4	Mianserin
264.4	Tetracaine
264.5	Sulfiram
265.3	Albendazole
265.4	Antazoline
265.4	Ethomoxane
265.4	Mirtazapine
265.4	Oxprenolol
266.3	Atenolol
266.3	Dienestrol
266.3	Diphenazoline
266.3	Doxofylline
266.3	Nevirapine
266.3	Pentachlorophenol
266.3	Practolol
266.3	Tributyl Phosphate
266.3	Trimetazidine
266.4	Cyclizine
266.4	Desipramine
266.4	Dibenzylpiperazine
266.6	Clonidine Hydrochloride
266.7	Metomidate Hydrochloride
266.7	Trifluoromethylphenylpiperazine Hydrochloride
267.2	Adenosine
267.2	Zidovudine
267.3	Apomorphine
267.3	Lysergamide
267.3	Sulfafurazole
267.3	Sulfamoxole
267.3	Voglibose
267.4	Azacyclonol
267.4	Butoxamine
267.4	Metoprolol
267.4	Pipradrol
267.4	VX
267.7	Fenfluramine Hydrochloride
267.8	Indeloxazine Hydrochloride
268.2	Clorprenaline Hydrochloride
268.3	Dexrazoxane
268.3	Lysergic Acid
268.3	Pentostatin
268.4	Diethylstilbestrol
268.7	Moclobemide
268.8	Tymazoline Hydrochloride
269.1	Dichlorophen
269.3	Bufylline
269.3	Sulfasomizole
269.4	Orphenadrine
269.4	Pramiracetam
269.4	Rizatriptan
269.8	Alachlor
269.8	Meptazinol Hydrochloride
269.8	Methylphenidate Hydrochloride
269.8	Piperoxan Hydrochloride (*dl*-Form)
270.1	Halazone
270.2	Leflunomide
270.3	Sulfamethizole
270.3	Tolbutamide
270.4	Doxylamine
270.4	Estrone
270.4	Hexestrol
270.4	Trenbolone
270.7	Mecloqualone
270.7	Nordazepam
270.8	Chloroprocaine
271.3	Carbutamide
271.3	Normorphine
271.3	Pilocarpine Nitrate
271.4	Apoatropine
271.4	Cyclazocine
271.4	Desomorphine
271.4	Dextromethorphan
271.4	Levomethorphan
271.4	Methoprotryne
271.4	Racemethorphan
271.5	Mercuric (II) Chloride
271.8	Amylocaine Hydrochloride
271.8	Meprylcaine Hydrochloride
271.8	Procainamide Hydrochloride
272.1	Muzolimine
272.2	Clorgiline
272.3	Sulfadiazine Sodium
272.4	Estradiol
272.4	Norandrostenedione
272.4	Quinisocaine
272.4	Sotalol
272.8	Metabutethamine Hydrochloride
272.8	Procaine Hydrochloride
273.3	Naphazoline Nitrate
273.4	Modafinil
273.4	Tilidate
273.7	Chlormezanone
273.8	Viloxazine Hydrochloride
274.2	Ketamine Hydrochloride
274.3	Phenytoin Sodium
274.4	Disulfoton
274.4	5-Methoxy-*N*,*N*-diisopropyltryptamine
274.4	Nandrolone
274.4	Phenampromide
274.4	Ropivacaine
274.4	Tybamate
274.8	Chlorphenamine
274.8	Dexchlorphenamine
275.1	Acetarsone
275.3	Homatropine
275.3	Physostigmine
275.3	Rolipram
275.4	Alphameprodine
275.4	Betameprodine
275.4	Cyclobenzaprine
275.4	Proheptazine
275.4	Trimeperidine
275.7	Methyldopate Hydrochloride
275.8	Benzfetamine Hydrochloride
275.8	Isoetarine Hydrochloride
276.2	Bupropion Hydrochloride
276.3	Sodium Thiamylal
276.4	Cyclandelate
276.4	Etidocaine
276.4	Gloxazone
276.4	Mebhydrolin
276.4	Molindone
276.7	Chlorpropamide
277.2	Clenbuterol
277.2	Ethambutol Hydrochloride
277.2	Fenitrothion
277.2	Ganciclovir Sodium
277.2	Iproniazid Phosphate
277.3	Azathioprine
277.4	Acecainide
277.4	Amitriptyline
277.4	Maprotiline
277.4	Padimate O
277.4	Venlafaxine
277.5	Ethylmethylthiambutene
277.5	Perhexiline
277.6	Adamsite
277.9	2C-T-2 Hydrochloride
278.1	Lead Chloride
278.2	Exifone
278.2	Rilmenidene Phosphate
278.3	Dibutyl Phthalate
278.3	Fenthion
278.3	Pentoxifylline
278.3	Sulfadimidine
278.3	Sulfisomidine
278.4	Amydricaine
278.4	Dimethocaine
278.4	Methadone Intermediate
278.4	Tolycaine
278.4	Triprolidine
279.1	Acecarbromal
279.1	Cyclophosphamide
279.2	Cidofovir
279.3	Etamiphylline
279.3	Ramosetron
279.4	Doxepin
279.4	Fenpiprane
279.4	Moxisylyte
279.7	Cytarabine Hydrochloride
279.9	Phencyclidine Hydrochloride
280.3	Hydroxystilbamidine
280.3	Levosimendan
280.3	Phenprocoumon
280.3	Sulfamethoxypyridazine
280.3	Sulfametopyrazine
280.3	Sulfametoxydiazine
280.4	Bamipine
280.4	Histapyrrodine
280.4	Imipramine
280.8	Tropacocaine Hydrochloride
280.8	Xylometazoline Hydrochloride
281.2	Flufenamic Acid
281.3	Diminazene
281.3	Indoprofen
281.3	Nitrazepam
281.3	Trimetozine
281.4	Alverine
281.4	Diphenylpyraline
281.4	Phentolamine
281.4	Terodiline
281.6	Apraclonidine Hydrochloride
281.7	Tosylchloramide Sodium
282.0	Lithium Citrate
282.2	Niflumic Acid
282.3	Artemisinin
282.3	Phenoxypropazine Maleate
282.4	Methyldesorphine
282.4	Vedaprofen
282.6	Chloral Betaine
282.8	Mepivacaine Hydrochloride
282.8	Milnacipran Hydrochloride
283.1	Risedronic Acid
283.3	Cadralazine
283.3	Choline Theophyllinate
283.4	Levallorphan
283.8	Ketobemidone Hydrochloride
283.8	Metolachlor
283.8	Pethidine Hydrochloride
284.2	Rhein
284.3	Dihydroartemisinin
284.3	Methohexital Sodium
284.3	Psilocybine
284.4	Boldione
284.4	Iprindole
284.4	Lynestrenol
284.4	Promazine
284.4	Promethazine
284.4	Sulfaethidole
284.4	Taurolin
284.4	Tropicamide
284.4	Tropisetron
284.7	Disulfamide
284.7	Mazindol
284.7	Sulfachlorpyridazine
284.8	Bunitrolol Hydrochloride
284.8	Diazepam
285.2	Fludarabine
285.3	Hydromorphone
285.3	Letrozole
285.3	Nifuratel

Mol. wt	*Compound*
285.4	Isothipendyl
285.4	Mepyramine
285.4	Pentazocine
285.4	Probenecid
285.4	Prothipendyl
285.7	Cladribine
285.8	Alprenolol Hydrochloride
285.8	Benzoctamine Hydrochloride
285.8	Epinastine Hydrochloride
286.3	Abacavir
286.3	Sulfamerazine Sodium
286.3	Thialbarbital Sodium
286.4	Androstenedione
286.4	Boldenone
286.4	Methallenestril
286.4	Thenalidine
286.4	Thonzylamine
286.7	Demoxepam
286.7	Oxazepam
286.8	Methaqualone Hydrochloride
286.8	Naepaine Hydrochloride
287.1	Brallobarbital
287.2	Fenticlor
287.4	Allylprodine
287.4	Cycrimine
287.4	Cyproheptadine
287.4	Dihydromorphine
287.4	Etodolac
287.4	Galantamine
287.4	*N*-Benzoylmescaline
287.4	Plasmocide
287.4	Procyclidine
287.4	Ritodrine
287.4	Zolmitriptan
288.2	Chlorproguanil
288.3	Dihydralazine Sulfate
288.4	Bupivacaine
288.4	Dehydroepiandrosterone
288.4	Estriol
288.4	Levobupivacaine
288.4	Phenglutarimide
288.4	Salbutamol Sulfate
288.4	Testosterone
288.5	Ethylestrenol
288.8	Lidocaine Hydrochloride
288.8	Tetrazepam
288.9	Disodium Clodronate
289.1	Ibomal
289.2	Ciprofibrate
289.3	Benzoylecgonine
289.3	Phthivazid
289.3	Sulfapyridine Sodium Monohydrate
289.4	Adrafinil
289.4	Atropine
289.4	Caramiphen
289.4	Dyclonine
289.4	Hyoscyamine
289.5	Triclosan
289.7	Quinethazone
289.8	Chloropyramine
289.8	Clofedanol
289.8	Nefopam Hydrochloride
289.8	Tolpropamine Hydrochloride
290.0	Nitroxinil
290.2	Proguanil Hydrochloride
290.2	Tizanidine Hydrochloride
290.3	Isoniazid Aminosalicylate
290.3	Trimethoprim
290.4	Androstanolone
290.4	Androsterone
290.4	Azatadine
290.4	Oxabolone
290.4	Sultiame
290.8	Benzene Hexachloride
290.8	Carbinoxamine
290.8	Lindane
291.3	Lead Azide
291.3	Parathion
291.3	Physostigmine Aminoxide
291.4	Cyclopentolate
291.4	Eucatropine
291.4	Fasudil
291.4	Levobunolol
291.4	Terbinafine
291.5	Antimony Trioxide
291.5	Diethylthiambutene
291.7	Cotarnine Chloride
291.7	Zomepirac
291.8	Atomoxetine Hydrochloride
291.8	Diphenhydramine Hydrochloride
291.8	Tofenacin Hydrochloride
291.8	Tripelennamine Hydrochloride
291.9	2C-T-7 Hydrochloride
292.3	Coumatetralyl
292.3	Methenamine Mandelate
292.3	Tolbutamide Sodium
292.4	Ambucetamide
292.4	Carteolol
292.4	Dimetindene
292.4	Leucinocaine
292.5	Anagrelide Hydrochloride
292.5	Androstanediol
292.8	Etoxazene Hydrochloride
293.4	Anastrozole
293.4	Budipine
293.4	Diamthazole
293.4	Embutramide
293.4	Ondansetron
293.4	Pramocaine
293.5	Butriptyline
293.7	Benserazide Hydrochloride
294.2	Meclofenoxate Hydrochloride
294.3	Penciclovir Sodium
294.4	Cinchonidine
294.4	Cinchonine
294.4	Noxiptiline
294.4	Propoxycaine
294.4	Proxymetacaine
294.4	Trimipramine
295.3	Choline Dihydrogen Citrate
295.3	Mebendazole
295.4	Dibenzepin
295.4	Dosulepin
295.4	Esmolol
295.4	Normethadone
295.4	Pizotifen
295.4	Sumatriptan
295.4	Tertatolol
295.6	Lofexidine Hydrochloride
295.7	Chlorothiazide
295.8	Chloropyrilene
295.8	Propranolol Hydrochloride
296.2	Clobenzorex Hydrochloride
296.2	Diclofenac
296.2	Flunixin
296.2	Meclofenamic Acid
296.3	Amicarbalide
296.3	Deoxynivalenol
296.4	Dimevamide
296.4	Ethinylestradiol
296.4	Exemestane
296.4	Guanethidine Monosulfate
296.4	Methdilazine
296.5	Disulfiram
296.6	2C-B Hydrochloride
296.8	Imolamine Hydrochloride
296.8	Oxymetazoline Hydrochloride
296.8	Ropinirole Hydrochloride
296.9	Methaphenilene Hydrochloride
297.1	Fenclofenac
297.4	Duloxetine
297.7	Hydrochlorothiazide
297.8	Alphaprodine Hydrochloride
297.8	Hexylcaine Hydrochloride
297.8	Methapyrilene Hydrochloride
297.8	Piperocaine Hydrochloride
297.8	Thenyldiamine Hydrochloride
297.9	Properidine Hydrochloride
298.2	Cicletanine Hydrochloride
298.3	Metadoxine
298.4	Alimemazine
298.4	Artemether
298.4	Diethazine
298.4	Norethisterone
298.4	Noretynodrel
298.4	Phenacaine
299.3	Fenbendazole
299.3	Saxitoxin
299.4	Buphenine
299.4	Hydrocodone
299.4	Metopon
299.4	Neopine
299.7	Gemcitabine Hydrochloride
299.8	Chlordiazepoxide
299.8	Metoclopramide
299.8	Nortriptyline Hydrochloride
299.8	Protriptyline Hydrochloride
299.8	Tramadol Hydrochloride
299.9	Dimethylthiambutene Hydrochloride
300.1	Cisplatin
300.2	Ticlopidine Hydrochloride
300.3	Sulfadimidine Sodium
300.3	Sulfaquinoxaline
300.4	Isotretinoin
300.4	Methandienone
300.5	Allylestrenol
300.7	Clobazam
300.7	Temazepam
300.8	Chlorcyclizine
300.8	Mianserin Hydrochloride
300.8	Tetracaine Hydrochloride
300.8	Thiamine
301.3	Milrinone Lactate
301.3	Morphine N-oxide
301.3	Oxymorphone
301.4	Dihydrocodeine
301.4	Dobutamine
301.4	Isoxsuprine
301.4	Methyldihydromorphine
301.4	Pentaquin
301.4	Tegaserod
301.5	Trihexyphenidyl
301.7	Benoxaprofen
301.8	Antazoline Hydrochloride
301.8	Ethomoxane Hydrochloride
301.8	Oxprenolol Hydrochloride
302.1	Amiloride Hydrochloride
302.1	Erythrityl Tetranitrate
302.2	Olsalazine
302.3	Pramipexole Dihydrochloride Monohydrate
302.4	Cambendazole
302.4	Emedastine
302.4	Formestane
302.5	Metenolone
302.5	Methyltestosterone
302.5	Mibolerone
302.5	Norethandrolone
302.5	Stenbolone
302.8	Cyclizine Hydrochloride
302.8	Desipramine Hydrochloride
303.0	Broxyquinoline
303.1	Etacrynic Acid
303.2	Butallylonal
303.2	Narcobarbital
303.2	Neostigmine Bromide
303.3	Flumazenil
303.3	Lead Sulfate
303.3	Sulfamethoxypyridazine Sodium
303.4	Cocaine
303.4	Fenamiphos
303.4	Fenoterol
303.4	Hydromorphinol
303.4	Hyoscine
303.4	Morphine
303.8	Azacyclonol Hydrochloride
303.8	Chlorphenoxamine
303.8	Phenoxybenzamine
303.8	Pipradrol Hydrochloride
304.2	Chlorambucil
304.2	Phenisonone Hydrobromide
304.2	Rimiterol Hydrobromide
304.2	Tigloidine Hydrobromide
304.3	Flupirtine
304.3	Sodium Loxoprofen
304.4	Dimpylate
304.5	Mestanolone
304.5	Mesterolone
304.5	Methandriol
305.1	Risedronate Sodium
305.2	Diclofenamide
305.2	Melphalan

Mol. wt	*Compound*
305.2	Moclobemide Hydrochloride
305.3	Entacapone
305.3	Zaleplon
305.4	Atropine Oxide
305.4	Pempidine Tartrate
305.5	Clioquinol
305.8	Fenoldopam
305.8	Orphenadrine Hydrochloride
305.8	Tolonium Chloride
306.2	Bromantane
306.2	Sertraline
306.3	Fluconazole
306.4	Butacaine
306.4	Oxandrolone
306.4	Propentofylline
306.7	Sulfachlorpyridazine Sodium
307.1	Histamine Acid Phosphate
307.2	Chloroprocaine Hydrochloride
307.4	Amprotropine
307.4	Benzatropine
307.4	Betaxolol
307.4	Tolnaftate
307.4	Zolpidem
308.1	Resorantel
308.3	Nifenazone
308.3	Warfarin
308.4	Gestrinone
308.4	Metabutoxycaine
308.4	Oxybuprocaine
308.4	Phenylbutazone
308.6	Clorgiline Hydrochloride
308.8	Alprazolam
308.8	Sotalol Hydrochloride
308.9	Quinisocaine Hydrochloride
309.3	Fluoxetine
309.3	Acetyl Sulfafurazole
309.3	Sulfasomizole Sodium Monohydrate
309.4	Amolanone
309.4	Benzydamine
309.4	Difenidol
309.4	Ketotifen
309.4	Mescaline Acid Sulfate
309.4	Metipranolol
309.4	Nadolol
309.5	Alphachloralose
309.5	Dicycloverine
309.5	Isomethadone
309.5	Methadone
309.5	Metixene
310.3	Sulfadimethoxine
310.3	Sulfadoxine
310.4	Ibogaine
310.4	Mestranol
310.5	Pecazine
310.7	Mabuterol
310.8	Desloratadine
310.9	5-Methoxy-*N*,*N*-diisopropyltryptamine Hydrochloride
311.3	Domoic Acid
311.4	Adiphenine
311.4	Thebaine
311.4	Tolazamide
311.5	Alphamethadol
311.5	Biperiden
311.9	Cyclobenzaprine Hydrochloride
311.9	Proheptazine Hydrochloride
311.9	Pyrrobutamine
311.9	Trimeperidine Hydrochloride
312.2	Eptazocine Hydrobromide
312.3	Aflatoxin B1
312.4	Arteether
312.4	Ethisterone
312.4	Granisetron
312.4	Olanzapine
312.4	Oseltamivir
312.4	Propamidine
312.4	Trenbolone Acetate
312.5	Dydrogesterone
312.5	Norgestrel
312.5	Profenamine
312.5	Tetrahydrogestrinone
312.8	Apomorphine Hydrochloride
312.8	Molindone Hydrochloride
312.9	Etidocaine Hydrochloride
313.3	Benorilate
313.3	Flunitrazepam
313.3	Triazophos
313.4	Ethylmorphine
313.4	Reboxetine
313.4	Vetrabutine
313.4	Xenysalate
313.8	Acecainide Hydrochloride
313.8	Amoxapine
313.9	Amitriptyline Hydrochloride
313.9	Maprotiline Hydrochloride
313.9	Venlafaxine Hydrochloride
314	Ethylmethylthiambutene Hydrochloride
314.2	Hexaconazole
314.3	Aflatoxin B2
314.3	Dantrolene
314.4	Ranitidine
314.4	Rofecoxib
314.4	Sulfaphenazole
314.4	Valdecoxib
314.5	Cannabidiol
314.5	Methoxypromazine
314.5	Pergolide
314.5	Progesterone
314.5	Δ^9-Tetrahydrocannabinol
314.7	Clorazepic Acid
314.8	Tolycaine Hydrochloride
314.9	Amydricaine Hydrochloride
314.9	Clomipramine
314.9	Homochlorcyclizine
315.2	Amprolium Hydrochloride
315.3	Tolmetin Sodium
315.4	Codeine *N*-oxide
315.4	Oxycodone
315.6	Triclocarban
315.7	Clonazepam
315.7	Efavirenz
315.8	Doxepin Hydrochloride
315.8	Moxisylyte Hydrochloride
315.8	Ramosetron Hydrochloride
315.9	Chlorprothixene
315.9	Fenpiprane Hydrochloride
316–374	Pyrethrum
316.1	Pentaerithrityl Tetranitrate
316.2	Bromazepam
316.4	Butalamine
316.4	Timolol
316.5	Calusterone
316.5	Norbolethone
316.9	Bamipine Hydrochloride
316.9	Histapyrrodine Hydrochloride
316.9	Imipramine Hydrochloride
317.2	Zimeldine
317.3	Azinphos-(Me)
317.3	Metaraminol Tartrate
317.3	Oxyphenisatine
317.4	Codeine
317.4	Denopamine
317.4	Nateglinide
317.4	Tetrabenazine
317.7	Chlorothiazide Sodium
317.8	Phentolamine Hydrochloride
317.9	Diphenylpyraline Hydrochloride
317.9	Terodiline Hydrochloride
318.1	Apraclonidine Dihydrochloride
318.1	Diclofenac Sodium
318.1	Meclofenamate Sodium
318.3	Fluvoxamine
318.3	Phenolphthalein
318.4	Zearalenone
318.5	CP 47,497
318.5	Oxymesterone
318.6	Tiludronic Acid
318.7	Mercuric Acetate
318.8	Tilidate Hydrochloride
319.2	Brompheniramine
319.2	Dexbrompheniramine
319.2	Ibandronic Acid
319.3	Tetrodotoxin
319.4	Epalrestat
319.4	Methenamine Hippurate
319.9	Chloroquine
319.9	Chlorpromazine
320.3	Enoxacin
320.3	Mycophenolic Acid
320.4	Feprazone
320.8	Tropisetron Hydrochloride
320.9	Iprindole Hydrochloride
320.9	Promazine Hydrochloride
320.9	Promethazine Hydrochloride
321.2	Lorazepam
321.3	Famciclovir
321.4	Etoxeridine
321.4	Pyriproxyfen
321.5	Phenazocine
321.8	Clopidogrel
321.8	Hydromorphone Hydrochloride
321.9	Clamoxyquin
321.9	Isothipendyl Hydrochloride
321.9	Pentazocine Hydrochloride
322.3	Carbazochrome Sodium Sulfonate
322.3	Acetyl Sulfamethoxypyridazine
322.3	Sulfaquinoxaline Sodium
322.4	Fenpipramide
322.4	Gelsemine
322.4	Zeranol
322.5	Aprindine
322.5	Chlorpyrifos-(Me)
322.5	Mequitazine
322.8	Thonzylamine Hydrochloride
322.87	Clostebol
323.1	Chloramphenicol
323.2	Lead Chromate
323.4	Dimefline
323.4	Gliclazide
323.4	Lysergide
323.4	Piperidolate
323.5	Antimony Pentoxide
323.8	Dihydromorphine Hydrochloride
323.8	Galantamine Hydrochloride
323.8	Ritodrine Hydrochloride
323.9	Allylprodine Hydrochloride
323.9	Cycrimine Hydrochloride
323.9	Procyclidine Hydrochloride
324.28	Sterigmatocystin
324.3	Furaltadone
324.3	Norlevorphanol Hydrobromide
324.3	Valaciclovir
324.4	Acetohexamide
324.4	Californine
324.4	Citalopram
324.4	Cresol
324.4	Dolasetron
324.4	Dorzolamide
324.4	Escitalopram
324.5	Diampromide
324.6	Chlorproguanil Hydrochloride
324.8	Phenglutarimide Hydrochloride
324.8	Prazepam
324.9	Levobupivacaine Hydrochloride
325.1	Alendronate Sodium
325.1	Etacrynate Sodium
325.3	Famphur
325.3	Lead Acetate
325.4	Ergometrine
325.4	Monocrotaline
325.5	Bisoprolol
325.5	Diethylaminoethyl Diphenylpropionate
325.5	Tolterodine
325.8	Clemizole
325.8	Ethiazide
325.8	Midazolam
325.9	Caramiphen Hydrochloride
325.9	Dyclonine Hydrochloride
326.3	Chloropyramine Hydrochloride
326.3	Clofedanol Hydrochloride
326.3	Dezocine Hydrobromide
326.4	Acitretin
326.4	Ajmaline
326.4	Hydroquinidine
326.4	Hydroquinine
326.5	Acepromazine
326.5	Ethylisobutrazine
326.8	Clozapine
327.1	Niclosamide
327.4	Azaperone
327.4	Benactyzine
327.4	Dimenoxadole

Mol. wt	*Compound*
327.4	6-Monoacetylmorphine
327.4	Naloxone
327.4	Thiamine Mononitrate
327.5	Aminopromazine
327.5	Butorphanol
327.5	Norelgestromin
327.8	Fasudil Hydrochloride
327.8	Loxapine
327.9	Cyclopentolate Hydrochloride
327.9	Diethylthiambutene Hydrochloride
327.9	Eucatropine Hydrochloride
327.9	Levobunolol Hydrochloride
327.9	Terbinafine Hydrochloride
328.2	Diloxanide Furoate
328.3	Aflatoxin G1
328.3	Aflatoxin M_1
328.4	Labetalol
328.5	Boldenone Acetate
328.5	Levomepromazine
328.5	Pyrethrin I
328.5	Stanozolol
328.5	Tuaminoheptane Sulfate
328.8	Carteolol Hydrochloride
328.8	Clorexolone
328.9	Ropivacaine Hydrochloride
329.0	Cloponone
329.4	Paroxetine
329.4	Trilostane
329.5	Prenylamine
329.9	Budipine Hydrochloride
329.9	Butriptyline Hydrochloride
329.9	Pramocaine Hydrochloride
330.2	Epinastine Hydrobromide
330.3	Aflatoxin G2
330.3	Aflatoxin M_2
330.3	Menadione Sodium Bisulfite
330.3	Warfarin Sodium
330.4	Malathion
330.5	Desoxycortone
330.5	Hydroxyprogesterone
330.7	Furosemide
330.9	Noxiptiline Hydrochloride
330.9	Propoxycaine Hydrochloride
330.9	Proxymetacaine Hydrochloride
331.2	Lead Nitrate
331.2	Tienilic Acid
331.3	Glymidine Sodium
331.3	Hydroflumethiazide
331.3	Piroxicam
331.4	Ciprofloxacin
331.4	Protokylol
331.5	Nizatidine
331.8	Dibenzepin Hydrochloride
331.8	Esmolol Hydrochloride
331.9	Dosulepin Hydrochloride
331.9	Normethadone Hydrochloride
331.9	Tertatolol Hydrochloride
331.9	Zotepine
332.31	Zanamivir
332.5	Alfaxalone
332.5	Ipratropium
332.5	Oxymetholone
332.9	Methdilazine Hydrochloride
332.9	Triprolidine Hydrochloride
333.3	Adrenaline Acid Tartrate
333.3	Azapetine Phosphate
333.5	Carbetapentane
333.5	Deptropine
333.9	Antimony Potassium Tartrate
333.9	Duloxetine Hydrochloride
334.2	Bromfenac
334.3	Bromazine
334.4	Benzylpenicillin
334.4	Neostigmine Methylsulfate
334.4	Strychnine
334.8	Progabide
334.8	Triphenyltetrazolium Chloride
334.9	Dehydrochloromethyltestosterone
334.9	Diethazine Hydrochloride
335.4	Isoetarine Mesilate
335.4	Naratriptan
335.5	Almotriptan
335.5	Norpipanone
335.5	Oxeladin
335.8	Metopon Hydrochloride
335.9	Buphenine Hydrochloride
335.9	Hydroxychloroquine
336.2	Chlordiazepoxide Hydrochloride
336.3	Dicoumarol
336.4	Acebutolol
336.4	Azapropazone
336.4	Berberine
336.4	Cetrimide
336.4	Fluoxymesterone
336.5	Fentanyl
336.8	Phenylmercuric Acetate
337.3	Chlorcyclizine Hydrochloride
337.3	Noradrenaline Acid Tartrate
337.3	Stilbamidine Dihydrochloride
337.3	Thiamine Hydrochloride
337.4	Propanidid
337.4	Tenoxicam
337.5	Amineptine
337.5	Danazol
337.5	Famotidine
337.8	Dobutamine Hydrochloride
337.8	Isoxsuprine Hydrochloride
337.8	Oxymorphone Hydrochloride
337.9	Tricyclamol Chloride
337.9	Trihexyphenidyl Hydrochloride
338.4	Dodecyl Gallate
338.4	Idebenone
338.4	Piperonyl Butoxide
338.8	Chlortalidone
339.3	Trimetazidine Hydrochloride
339.4	Ethylpiperidyl Benzilate
339.4	Methylergometrine
339.4	Moramide Intermediate
339.4	Nalmefene
339.4	Papaverine
339.4	Pipethanate
339.4	Topiramate
339.4	Xamoterol
339.5	Dextropropoxyphene
339.5	Disopyramide
339.5	Levopropoxyphene
339.5	Noracymethadol
339.5	Perazine
339.6	Hexetidine
339.7	Antimony Trisulfide
339.8	Cocaine Hydrochloride
339.9	Prothipendyl Hydrochloride
340.3	Chlorphenoxamine Hydrochloride
340.3	Phenoxybenzamine Hydrochloride
340.4	Amidefrine Mesilate
340.4	Diphenadione
340.4	Pentamidine
340.4	Perindoprilat
340.5	Lucanthone
340.5	Norethisterone Acetate
340.5	Propiomazine
340.8	Flupirtine Hydrochloride
341.4	Acetylcodeine
341.4	Benaprizine
341.4	Fenetylline
341.4	Lauroscholtzine
341.4	Naltrexone
341.4	Phendimetrazine Tartrate
341.4	Sulpiride
341.4	Thebacon
341.5	JWH-018
341.8	Atropine Oxide Hydrochloride
342.1	Aluminium Sulfate
342.4	Calcium Mandelate
342.4	Oxyphenbutazone
342.7	Sertraline Hydrochloride
342.8	Flutoprazepam
342.9	Bupivacaine Hydrochloride
342.9	Etizolam
343.2	Triazolam
343.4	Acetyldihydrocodeine
343.4	Ethacridine Lactate
343.5	Cinchocaine
343.5	Monoethanolamine Oleate
343.5	Thiambutosine
343.9	Betaxolol Hydrochloride
343.9	Clemastine
343.9	Clotiapine
344.0	Diquat Dibromide
344.4	Formebolone
344.4	Formoterol
344.5	Metenolone Acetate
344.5	Oxyphencyclimine
344.5	Stenbolone Acetate
344.5	Testosterone Propionate
344.8	Clotrimazole
344.9	Metabutoxycaine Hydrochloride
344.9	Oxybuprocaine Hydrochloride
345.4	Azinphos-(Et)
345.4	Bevantolol
345.4	Esomeprazole
345.4	Omeprazole
345.7	Methoxychlor
345.8	Fluoxetine Hydrochloride
345.9	Amolanone Hydrochloride
345.9	Benzydamine Hydrochloride
345.9	Clopamide
345.9	Difenidol Hydrochloride
345.9	Isomethadone Hydrochloride (*d*-Form)
345.9	Methadone Hydrochloride
345.9	Metixene Hydrochloride
345.9	Metofoline
346.0	Dicycloverine Hydrochloride
346.3	Nifedipine
346.4	Warfarin Potassium
346.5	Morpheridine
346.9	Ibogaine Hydrochloride
346.9	Iopydone
347.2	*dl*-Mabuterol Hydrochloride
347.3	Enoxacin Sesquihydrate
347.5	Indoramin
347.5	Phenomorphan
347.7	Clodantoin
347.8	Nalorphine Hydrochloride
347.9	Adiphenine Hydrochloride
347.9	Biperiden Hydrochloride
348.3	Embramine
348.4	Acrivastine
348.4	Imidocarb
348.4	Torasemide
348.5	Alfadolone
348.9	Granisetron Hydrochloride
348.9	Profenamine Hydrochloride
349.4	Ampicillin
349.4	Cefradine
349.5	Dipipanone
349.7	Zomepirac Sodium
349.9	Vetrabutine Hydrochloride
349.9	Xenysalate Hydrochloride
350.4	Phenoxymethylpenicillin
350.6	Chlorpyrifos
350.9	Cyproheptadine Hydrochloride
350.9	Ranitidine Hydrochloride
351.3	Clomipramine Hydrochloride
351.4	Dipyrone
351.4	Pipoxolan
351.4	Pirenzepine
351.5	Phenadoxone
352.3	Dithranol Triacetate
352.3	Racemethorphan Hydrobromide
352.4	Chlorprothixene Hydrochloride
352.4	Triflupromazine
352.5	Anileridine
352.5	Encainide
352.8	Butanilicaine Phosphate
352.8	Clorazepate Monopotassium
352.8	Griseofulvin
352.9	Butalamine Hydrochloride
352.9	Phenacaine Hydrochloride Monohydrate
353.3	Acenocoumarol
353.3	Dioxaphetyl Butyrate
353.4	Chelidonine
353.5	Levomethadyl Acetate
353.5	Methysergide
353.5	Proadifen
353.9	Amopyroquine
353.9	*l*-Denopamine Hydrochloride
354.0	Tridihexethyl Chloride
354.1	Idoxuridine
354.2	Aceclofenac
354.3	Metoclopramide Hydrochloride
354.4	Fusarenone-X

Mol. wt	*Compound*
354.4	Nomifensine Maleate
354.4	Yohimbine
354.5	Alprostadil
354.5	Clofenotane
354.5	Etretinate
354.8	Xipamide
355.3	Chlorpromazine Hydrochloride
355.4	Glaucine
355.9	Amodiaquine
356.1	Pipobroman
356.3	Homatropine Hydrobromide
356.3	Proheptazine Hydrobromide
356.4	Benzylpenicillin Sodium
356.4	Fluanisone
356.4	Pheniramine Maleate
356.4	Pioglitazone
356.4	Pyrantel Tartrate
356.4	Sulindac
356.5	Cyclizine Lactate
356.5	Dopexamine
356.5	Estradiol Valerate
356.6	Conessine
357.3	Balsalazide
357.3	Droxicam
357.3	Tolrestat
357.4	Rosiglitazone
357.5	Oxybutynin
357.8	Cinolazepam
357.8	Indometacin
358.0	Cetylpyridinium Chloride
358.4	Clamoxyquin Hydrochloride
358.4	Prednisone
358.5	Δ^9-Tetrahydrocannabinolic Acid
358.5	Dimethisterone
358.5	Dimethoxanate
358.7	Triflumuron
358.8	Etoricoxib
359.0	Aprindine Hydrochloride
359.2	Ibandronate Sodium
359.2	Obidoxime Chloride
359.4	Capecitabine
359.4	Rabeprazole
359.5	Cyclomethycaine
359.6	Captodiame
359.9	Dimefline Hydrochloride
359.9	Piperidolate Hydrochloride
360.2	Methyclothiazide
360.32	Plasmocide Dihydrochloride
360.4	Aldosterone
360.4	Cortisone
360.4	Nitrendipine
360.4	Prednisolone
360.5	Drostanolone Propionate
360.5	Quinidine
360.8	Fenofibrate
360.8	Valaciclovir Hydrochloride
360.9	Dorzolamide Hydrochloride
361.4	Bisacodyl
361.4	Carbocromen
361.4	Levofloxacin
361.4	Ofloxacin
361.4	Levofloxacin (Ofloxacin *S*-(–)-form)
361.5	Antazoline Mesilate
361.5	Furethidine
361.5	Levophenacylmorphan
361.8	Bezafibrate
362.2	Hexamethonium Bromide
362.3	Clemizole Hydrochloride
362.3	Diphenylpyraline Hydrobromide
362.3	Fosphenytoin
362.3	Octatropine Methylbromide
362.4	Emepronium Bromide
362.4	Phthalylsulfacetamide
362.5	Hydrocortisone
362.6	Disodium Tiludronate
362.8	Coumaphos
362.9	Hydroquinidine Hydrochloride
363.2	Mexazolam
363.4	Antazoline Phosphate
363.5	Opipramol
363.8	6-Monoacetylmorphine Hydrochloride
363.8	Naloxone Hydrochloride
363.9	Benactyzine Hydrochloride
363.9	Isomethadone Hydrochloride Monohydrate (*d*-Form)
363.9	Lachesine Chloride
364.2	Olsalazine Sodium
364.3	Bibenzonium Bromide
364.3	Loxapine Hydrochloride
364.4	Bumetanide
364.4	Cyclofenil
364.4	Pheneticillin
364.5	Flugestone
364.5	Tranylcypromine Sulfate
364.9	Aldrin
364.9	Clostebol Acetate
364.9	Labetalol Hydrochloride
364.9	Levomepromazine Hydrochloride
364.9	Pecazine Hydrochloride
365.2	Fludarabine Phosphate
365.2	Nifursol
365.4	Amoxicillin
365.4	Cefalexin
365.4	Methyl Benzoquate
365.5	Pericyazine
365.8	Indapamide
365.8	Metolazone
365.9	Ondansetron Hydrochloride Dihydrate
366.4	Atropine Methonitrate
366.4	Diacetoxyscirpenol
366.4	Diamthazole Hydrochloride
366.4	Trifluomeprazine
366.5	Drospirenone
366.5	Glibornuride
366.5	Piminodine
366.8	Atovaquone
366.9	Cinchonine Hydrochloride
367.4	Dimoxyline
367.4	Omeprazole Sodium
367.4	Sulfathiazole Sodium
367.5	Bambuterol
367.5	Benzethidine
367.5	Fenbutrazate
367.5	Phenoperidine
367.5	Pramiracetam Sulfate
367.8	Protokylol Hydrochloride
368.3	Diacerein
368.3	Galantamine Hydrobromide
368.5	Amfetamine Sulfate
368.5	Cinnarizine
368.5	Dehydroepiandrosterone Sulfate
368.5	Dexamfetamine Sulfate
368.5	Perindopril
368.8	Ketazolam
369.3	Etofenamate
369.4	Diamorphine
369.4	Lansoprazole
369.4	Ochratoxin B
369.4	Trimetrexate
369.5	Amisulpride
369.5	Cilostazol
369.85	Paroxetine Hydrochloride Hemihydrate
370.3	Dextromethorphan Hydrobromide
370.3	Homatropine Methylbromide
370.3	Hyoscyamine Hydrobromide
370.4	Levofloxacin Hemihydrate
370.4	Morantel Tartrate
370.5	Elcometrine
370.6	Thioridazine
370.7	Bromazine Hydrochloride
370.8	Rebamipide
370.9	Lorcainide
371.3	Nedocromil
371.3	Remoxipride
371.4	Ampicillin Sodium
371.4	Demecolcine
371.4	Isradipine
371.8	Camazepam
371.8	Lornoxicam
371.9	Codeine Hydrochloride
371.9	Naratriptan Hydrochloride
371.9	Norpipanone Hydrochloride
371.9	Trazodone
372.4	Diamfenetide
372.4	Sulfafurazole Diethanolamine
372.5	Ajmaline Monoethanolate
372.5	Benzylpenicillin Potassium
372.5	Desoxycortone Acetate
372.5	Pyrethrin II
372.6	Finasteride
372.8	Glafenine
372.9	Acebutolol Hydrochloride
373.3	Heptachlor
373.4	Pyrazophos
373.4	Succinylsulfathiazole
373.5	Bunazosin
373.9	Amineptine Hydrochloride
373.9	Lobeline Hydrochloride
373.9	Prochlorperazine
374.5	Methylprednisolone
374.9	Hydroxyzine
375.1	Aluminium Nitrate
375.3	Crystal Hydrate
375.4	Dexketoprofen Trometamol
375.5	Benzylmorphine
375.5	Pentazocine Lactate
375.5	Pipamperone
375.6	Tiagabine
375.8	Morphine Hydrochloride
375.8	Norcodeine Hydrochloride
375.9	Dextropropoxyphene Hydrochloride
375.9	Haloperidol
375.9	Nalmefene Hydrochloride
375.9	Noracymethadol Hydrochloride
375.9	Papaverine Hydrochloride
375.9	Pipethanate Hydrochloride
376.1	Bromhexine
376.2	Fluquinconazole
376.4	Ketorolac Tromethamine
376.4	Picotamide
376.4	Riboflavin
376.5	Enalapril
376.5	Estradiol Benzoate
376.5	Fluocortolone
376.5	Fluorometholone
376.5	Remifentanil
376.8	Flutazolam
376.9	Fenpipramide Hydrochloride
376.9	Propiomazine Hydrochloride
377.4	Imidaprilat
377.5	Famprofazone
377.5	Morazone
377.5	Phentolamine Mesilate
377.9	Benaprizine Hydrochloride
377.9	Fenetylline Hydrochloride
377.9	Naltrexone Hydrochloride
377.9	Thebacon Hydrochloride
378.1	Mannomustine
378.4	Carbenicillin
378.4	Propicillin
378.5	Doxapram
378.5	Quinine
379.4	Droperidol
379.5	Celiprolol
379.5	Donepezil
379.5	Oxypertine
379.9	Acetyldihydrocodeine Hydrochloride
379.9	Cinchocaine Hydrochloride
379.9	Cyclopenthiazide
380.3	Neopine Hydrobromide
380.4	Cibenzoline Succinate
380.4	Hyoscine Methonitrate
380.4	Meticillin
380.5	Amosulalol
380.5	Bopindolol
380.5	Diethylstilbestrol Dipropionate
380.5	Ecabet
380.5	Fludrocortisone
380.5	Limaprost
380.7	Trichlormethiazide
380.8	Naled
380.9	Chlorotrianisene
380.9	Dieldrin
380.9	Endrin
380.9	Hydrocodone Hydrochloride
380.9	Oxyphencyclimine Hydrochloride
381.2	Sodium Borate Decahydrate
381.4	Benperidol
381.4	Celecoxib
381.4	Fendosal
381.4	Rabeprazole Sodium
381.7	Econazole
381.9	Bevantolol Hydrochloride
382.5	Eletriptan
382.5	Hexestrol Dipropionate

Mol. wt	*Compound*
382.5	Misoprostol
382.6	Bunamidine
382.9	Loratadine
382.9	Mefruside
383.2	Bromfenac Sodium
383.2	Ecothiopate Iodide
383.4	Hydrastine
383.4	Pantoprazole
383.4	Prazosin
383.5	Quetiapine
383.5	Tandospirone
383.9	Indoramin Hydrochloride
384.3	Atropine Methobromide
384.3	Felodipine
384.3	Fenoterol Hydrobromide
384.4	Artesunate
384.4	Enalaprilat
384.5	Caramiphen Edisilate
384.5	Etynodiol Diacetate
384.5	Megestrol Acetate
384.6	Colecalciferol
384.6	Ibutilide
384.7	Embramine Hydrochloride
385.4	Nilvadipine
385.5	Bamifylline
385.5	Buspirone
385.8	Cefaclor
385.8	Ciprofloxacin Hydrochloride
385.9	Ethylmorphine Hydrochloride
386.3	Caffeine Citrate
386.4	Valethamate Bromide
386.5	Medroxyprogesterone Acetate
386.6	Sufentanil
386.6	Testosterone Isocaproate
386.7	Cholesterol
386.7	Fenoldopam Hydrobromide
386.8	Quazepam
386.9	Clonitazene
387.2	Acetarsone Sodium
387.4	Amoxicillin Sodium
387.4	Terazosin
387.9	Flurazepam
387.9	Nemonapride
387.9	Pentaquin Phosphate
387.9	Phenadoxone Hydrochloride
387.9	Pipoxolan Hydrochloride
388.4	Hydroxyquinoline Sulfate
388.4	Nisoldipine
388.5	Aminoacridine Hexylresorcinate
388.5	Doxylamine Succinate
388.5	Leucinocaine Mesylate
388.5	Methandriol Diacetate
388.5	Phenoxymethylpenicillin Potassium
388.5	Ramiprilat
388.5	Trimethobenzamide
388.6	Azacosterol
388.8	Eszopiclone
388.8	Zopiclone
388.9	Cetirizine
388.9	Encainide Hydrochloride
388.9	Triflupromazine Hydrochloride
389.4	Reproterol
389.4	Tadalafil
389.5	Alfuzosin
389.5	Diphemanil Metilsulfate
389.5	Proadifen Hydrochloride
389.9	Cyclothiazide
390	Levomethadyl Acetate Hydrochloride
390.4	Isomethadone Hydrobromide (*dl*-Form)
390.5	Alfadolone Acetate
390.5	Dehydroepiandrosterone Sodium Sulfate
390.9	Chlorphenamine Maleate
390.9	Dexchlorphenamine Maleate
390.9	Yohimbine Hydrochloride
391.0	Meclozine
391.3	Azamethonium Bromide
391.4	Ambutonium Bromide
391.4	Diethylcarbamazine Citrate
391.5	Biriperone
391.5	Flavoxate
391.5	Rizatriptan Benzoate
391.5	Tiotropium
391.6	Dimetotiazine
391.9	Phenmetrazine Teoclate
392.3	Nalorphine Hydrobromide
392.5	Betamethasone
392.5	Dexamethasone
392.5	Dextromoramide
392.5	Levomoramide
392.5	Naftopidil
392.5	Paramethasone
392.5	Racemoramide
392.8	Chloroquine Hydrochloride
392.9	Pioglitazone Hydrochloride
393.5	Amsacrine
393.5	Metindizate
393.5	Pheniramine Aminosalicylate
393.6	Perhexiline Maleate
393.7	Brotizolam
393.7	Metaclazepam
393.9	Nalbuphine Hydrochloride
393.9	Oxybutynin Hydrochloride
394.3	Homidium Bromide
394.4	Picotamide Monohydrate
394.4	Rotenone
394.4	Triamcinolone
394.5	Brucine
394.5	Dimevamide Sulfate
394.9	Dimethoxanate Hydrochloride
395.4	Cefdinir
395.4	Ketanserin
395.6	Quinagolide
395.9	Cloricromen
396.1	Captodiame Hydrochloride
396.1	Cetalkonium Chloride
396.4	Cefalothin
396.5	Etonitazene
396.5	Quinine Ethyl Carbonate
396.6	Estradiol Cipionate
396.7	Ergocalciferol
396.9	Quinine Hydrochloride
397.0	Diiodohydroxyquinoline
397.3	Oxaliplatin
397.3	Quinine Dihydrochloride
397.3	Suxamethonium Chloride
397.4	Fluoxetine Oxalate
397.4	Strychnine Nitrate
397.5	Octaverine
397.6	Solanidine
397.9	Carbocromen Hydrochloride
398.1	Calcium Arsenate
398.3	Glycopyrronium Bromide
398.3	Hyoscine Methobromide
398.4	Calcium Aminosalicylate
398.4	Sulfasalazine
398.7	Dihydrotachysterol
399.2	Clefamide
399.3	Dantrolene Sodium
399.4	Colchicine
399.4	Dihydrocodeine Phosphate
399.4	Morphine Acetate
399.5	Pipazetate
399.6	Thiethylperazine
400.0	Mepacrine
400.4	Rivastigmine Hydrogen Tartrate
400.5	Acamprosate Calcium
400.5	Enprostil
400.5	Prednisone Acetate
400.6	Alfacalcidol
400.6	Dehydroepiandrosterone Enanthate
400.6	Testosterone Enantate
400.6	Tiocarlide
401.0	Clopenthixol
401.0	Zuclopenthixol
401.4	Moxifloxacin
401.4	Oxyphenisatine Acetate
401.5	Biperiden Lactate
401.5	Mepyramine Maleate
401.5	Oxyclozanide
401.9	Fenoldopam Methanesulfonate
402	Pipamazine
402.5	Cortisone Acetate
402.5	Pheneticillin Potassium
402.5	Prednisolone Acetate
402.6	Sulforidazine
403.4	Ampicillin Trihydrate
403.4	Phthalylsulfathiazole
403.5	Amotriphene
403.5	Benzatropine Mesilate
403.8	Antimony Pentasulfide
403.8	Ochratoxin A
403.9	Bambuterol Hydrochloride
403.9	Fenbutrazate Hydrochloride
403.9	Phenoperidine Hydrochloride
404.0	Dipipanone Hydrochloride
404.0	Perphenazine
404.1	Glyphosate Sesquisodium Salt
404.3	Alpidem
404.4	Abacavir Succinate
404.5	Benzquinamide
404.5	Hydrocortisone Acetate
404.5	Lovastatin
404.5	Nicocodine
404.5	Sulfinpyrazone
404.9	Chlormadinone Acetate
405.3	Citalopram Hydrobromide
405.4	Imidapril
405.4	Nebivolol
405.4	Taltirelin
405.5	Lisinopril
405.5	Nifekalant
405.9	Oxycodone Hydrochloride
406.0	Clomifene
406.0	Toremifene
406.2	Fosphenytoin Sodium
406.4	Artesunate Sodium
406.4	Codeine Phosphate
406.4	Floctafenine
406.5	Alacepril
406.5	Carvedilol
406.5	Flugestone Acetate
406.5	Lincomycin
406.5	Pravastatin Lactone
406.6	Nandrolone Phenylpropionate
406.9	Carbinoxamine Maleate
406.9	Endosulfan
406.9	Hexachlorophene
406.9	Strychnine Hydrochloride
407.0	Thioridazine Hydrochloride
407.4	Lorcainide Hydrochloride
407.5	Trifluoperazine
407.8	Berberine Hydrochloride
408.2	Zimeldine Hydrochloride
408.3	Trazodone Hydrochloride
408.4	Ethyl Biscoumacetate
408.5	Dimetindene Maleate
408.5	Tamsulosin
408.9	Amlodipine
408.9	Beclometasone
408.9	Clorazepate Dipotassium
409.4	Ezetimibe
409.4	Trifluperidol
409.5	Reboxetine Methanesulfonate (Mesylate)
409.6	Butaperazine
409.8	Chlordane
409.9	Bunazosin Hydrochloride
410.4	Oseltamivir Phosphate
410.5	Flumetasone
410.5	Quinaprilat (Diacid of Quinapril)
410.5	Risperidone
410.5	Trimipramine Maleate
410.6	Pergolide Mesilate
410.6	Piperacetazine
410.6	Thiazinamium Metilsulfate
411	Trenbolone Hexahydrobenzylcarbonate
411.4	Phenazocine Hydrobromide
411.5	Etorphine
411.5	Fluvastatin
411.5	Nedocromil Calcium
411.5	Phenindamine Tartrate
411.6	Acetophenazine
411.9	Benzylmorphine Hydrochloride
412.0	Tiagabine Hydrochloride
412.3	Heteronium Bromide
412.6	Bromhexine Hydrochloride
412.6	Doxercalciferol
412.6	Testosterone Cipionate
412.9	Remifentanil Hydrochloride
412.9	Ziprasidone
413.5	Physostigmine Salicylate
413.5	Sumatriptan Succinate
413.9	Morazone Hydrochloride

Mol. wt	*Compound*
414.3	Flecainide
414.4	Bretylium Tosilate
414.4	Escitalopram Oxalate
414.5	Diltiazem
414.5	Domiphen Bromide
414.6	Desoxycortone Pivalate
414.6	Gestonorone Caproate
414.6	Metenolone Enantate
414.6	Oxabolone Cipionate
415.1	Teclothiazide
415.3	Nedocromil Sodium
415.4	Norpipanone Hydrobromide
415.5	Cefaloridine
415.5	Diperodon
415.5	Pentapiperide Metilsulfate
415.6	Haloxon
415.6	Salmeterol
415.8	Acemetacin
416.0	Celiprolol Hydrochloride
416.0	Donepezil Hydrochloride
416.1	Miconazole
416.4	Piperazine Calcium Edetate
416.5	Alfentanil
416.5	Cyclizine Tartrate
416.5	Desonide
416.5	Methylprednisolone Acetate
416.5	Pholcodine
416.5	Propicillin Potassium
416.5	Ramipril
416.6	Methandriol Dipropionate
416.6	Spironolactone
416.9	Amosulalol Monohydrochloride
417.3	Quinapyramine Dichloride
417.5	Decoquinate
417.5	Tegaserod Maleate
417.9	Benperidol Hydrochloride
418.0	Pyrvinium Chloride
418.3	Decamethonium Bromide
418.4	Aloin
418.4	Cefalothin Sodium
418.4	Nimodipine
418.6	Maxacalcitol
418.6	Simvastatin
419.0	Bunamidine Hydrochloride
419.0	Lofepramine
419.4	Amoxicillin Trihydrate
419.4	Morpheridine Dihydrochloride
419.6	Prenylamine Lactate
419.9	Clopidogrel Bisulfate
419.9	Hydrastine Hydrochloride
419.9	Prazosin Hydrochloride
420.0	Tandospirone Hydrochloride
420.3	Mepenzolate Bromide
420.3	Methanthelinium Bromide
420.4	Flupirtine Maleate
420.4	Meticillin Sodium
420.4	Penthienate Methobromide
420.5	Dolasetron Methanesulfonate
420.5	Hexoprenaline
420.5	Levocabastine
420.6	Testosterone Phenylpropionate
421.0	Iopydol
421.1	Broxaldine
421.4	Bendroflumethiazide
421.4	Ciprofloxacin Lactate
421.5	Topotecan
421.9	Bamifylline Hydrochloride
422.0	Buspirone Hydrochloride
422.4	Carbenicillin Sodium
422.5	Boldenone Benzoate
422.5	Fludrocortisone Acetate
422.5	Sparteine Sulfate
422.9	Losartan
423.6	Cyprenorphine
423.9	Diamorphine Hydrochloride
423.9	Terazosin Hydrochloride
424.1	Benzbromarone
424.3	Flurazepam Monohydrochloride
424.3	Pirenzepine Hydrochloride
424.4	Neosolaniol
424.5	Benazepril
424.5	HT-2 Toxin
424.9	Trimethobenzamide Hydrochloride
425.0	Clindamycin
425.4	Anileridine Hydrochloride
425.5	Ketotifen Fumarate
425.5	Pravastatin
425.6	Carfenazine
425.6	Diprenorphine
425.8	Epithiazide
425.8	Remoxipride Hydrochloride
425.9	Alfuzosin Hydrochloride
425.9	Reproterol Hydrochloride
426.6	Dehydroepiandrosterone Sodium Sulfate Dihydrate
426.6	Perospirone
426.8	Amopyroquine Hydrochloride
427.5	Moracizine
427.6	Dixyrazine
427.9	Flavoxate Hydrochloride
428.3	Diethylstilbestrol Diphosphate
428.4	Oxyphenonium Bromide
428.4	Phenomorphan Hydrobromide
428.5	Ephedrine Sulfate
428.5	Irbesartan
428.5	Pholedrine Sulfate
428.5	Pseudoephedrine Sulfate
428.6	Hexocyclium Metilsulfate
428.6	Hydroxyprogesterone Caproate
428.7	Nandrolone Decanoate
429.0	Chlorisondamine Chloride
429.4	Dopexamine Dihydrochloride
429.5	Physostigmine Aminoxide Salicylate
429.5	Pizotifen Malate
429.6	Mebeverine
429.6	Mifepristone
430.0	Chlorprothixene Mesilate
430.0	Metindizate Hydrochloride
430.2	Metaclazepam Hydrochloride
430.4	Bicalutamide
430.4	Ipratropium Bromide
430.5	Morantel Citrate
430.5	Trandolapril
430.6	Etymide
430.6	Piritramide
431.9	Benzthiazide
431.9	Ochratoxin C
432.0	Quinagolide Hydrochloride
432.3	Cloricromen Hydrochloride
432.4	Clidinium Bromide
432.4	Nicametate Citrate
432.5	Mizolastine
432.5	Pantoprazole Sodium Sesquihydrate
432.5	Timolol Maleate
432.6	Calcium Cyclamate
432.6	Hydrocortisone Butyrate
432.9	Etonitazene Hydrochloride
433.0	Buclizine
433.0	Doxapram Hydrochloride
433.0	Hydroxychloroquine Sulfate
433.4	Berberine Sulfate
433.5	Fluvastatin Sodium
433.5	Levallorphan Tartrate
433.5	Mycophenolate Mofetil
433.9	Diperodon Hydrochloride
433.9	Octaverine Hydrochloride
434.3	Cyfluthrin
434.4	Benzilonium Bromide
434.4	Fluvoxamine Maleate
434.4	Pipenzolate Bromide
434.5	Betamethasone Acetate
434.5	Dexamethasone Acetate
434.5	Flupentixol
434.5	Paramethasone Acetate
434.5	Triamcinolone Acetonide
434.5	Xantinol Nicotinate
435.3	Brompheniramine Maleate
435.3	Dexbrompheniramine Maleate
435.4	Ajmaline Hydrochloride
435.4	Aztreonam
435.5	Cilazapril
435.5	Fosinopril Diacid
435.5	Valsartan
435.9	Cloxacillin
436.0	Chloroquine Sulfate
436.0	Pipazetate Hydrochloride
436.1	Glaucine Hydrobromide
436.3	Phenatine
436.4	Opipramol Hydrochloride
436.5	Fludroxycortide
436.5	Isoaminile Citrate
436.5	Thenalidine Tartrate
436.6	Bialamicol
436.95	Tianeptine
437.3	Balsalazide Disodium Dihydrate
437.5	Disopyramide Phosphate
437.5	Fluphenazine
437.9	Moxifloxacin Hydrochloride
438.3	Hyoscine Hydrobromide
438.4	Pipamazine Hydrochloride
438.5	Quinapril
438.6	Spiraprilat
439.9	Amotriphene Hydrochloride
439.9	Polythiazide
440.4	Hyoscine Butylbromide
440.5	Candesartan
440.6	Tirofiban
440.7	Estradiol Undecylate
440.9	Sertindole
441.0	Benzquinamide Hydrochloride
441.4	Folic Acid
441.4	Quinine Hydrobromide
441.5	Deflazacort
441.5	Ergometrine Maleate
441.5	Lisinopril Dihydrate
441.5	Troglitazone
441.6	Dofetilide
441.6	Perindopril Erbumine (Perindopril Tert-Butylamine)
441.9	Imidapril Monohydrochloride
441.9	Nebivolol Hydrochloride
441.9	Nifekalant Hydrochloride
442.4	Methacycline
442.5	Acepromazine Maleate
443.0	Zuclopenthixol Acetate
443.5	Bephenium Hydroxynaphthoate
443.5	Levorphanol Tartrate
443.6	Tiotixene
444.4	Tetracycline
444.5	Levomepromazine Maleate
444.6	Docusate Sodium
444.6	Prednisolone Pivalate
444.7	Econazole Nitrate
444.7	Testosterone Undecylate
445.0	Tamsulosin Hydrochloride
445.2	Chloramphenicol Sodium Succinate
445.5	Glipizide
445.5	Narceine
445.6	Metopimazine
445.9	Glaucine Hydrochloride Trihydrate
445.9	Trifluperidol Hydrochloride
446.0	Thiopropazate
446.4	Hydrocodone Phosphate
446.5	Olmesartan
446.5	Pravastatin Sodium
446.6	Denatonium Benzoate
446.6	Hydrocortisone Valerate
446.6	Thioproperazine
447.0	Propyliodone
447.5	Phenyltoloxamine Citrate
447.5	Tripelennamine Citrate
447.8	Hydroxyzine Hydrochloride
448.0	Etorphine Hydrochloride
448.1	Benzethonium Chloride
448.4	Aripiprazole
448.4	Pipamperone Hydrochloride
448.4	Pipethanate Ethobromide
448.4	Propantheline Bromide
448.5	Debrisoquine Sulfate
450.1	Octafonium Chloride
450.5	Anileridine Phosphate
450.7	Phytomenadione
451.0	Diltiazem Hydrochloride
451.5	Dihydrocodeine Tartrate
451.5	Doxazosin
451.5	Poldine Metilsulfate
451.6	Cabergoline
452.5	Delapril
452.5	Fluocinolone Acetonide
452.6	Bethanidine Sulfate
452.6	Diphenoxylate
452.6	Repaglinide

Mol. wt	*Compound*
452.7	Boldenone Undecylenate
453.3	Chloramphenicol Cinnamate
453.5	Cefixime
453.5	Ethoheptazine Citrate
453.6	Acetorphine
453.9	Flucloxacillin
454.0	Thenium Closilate
454.1	Dofamium Chloride
454.4	Methotrexate
454.6	Verapamil
455.0	Halcinonide
455.3	Primaquine Phosphate
455.4	Lofepramine Hydrochloride
455.5	Butetamate Citrate
455.5	Lacidipine
455.5	Methylergometrine Maleate
456.2	Hexamethonium Iodide
456.5	Dioxathion
456.5	Aminophylline
456.6	Delavirdine
456.6	Propiomazine Maleate
456.7	Detajmium
457.0	Levocabastine Hydrochloride
457.4	Amygdalin
457.5	Minocycline
457.6	Cyclomethycaine Sulfate
457.9	Topotecan Hydrochloride
458.5	Raltitrexed
458.6	Astemizole
459.6	Cerivastatin
459.9	Terazosin Hydrochloride Dihydrate
459.9	Tianeptine Sodium
460.0	Clemastine Fumarate
460.5	Prednisolone Succinate
460.6	Fluocortolone Pivalate
460.6	Mephentermine Sulfate
460.7	Docusate Potassium
460.8	Flurazepam Hydrochloride
461.0	Benazepril Hydrochloride
461.0	Lead Iodide
461.0	Lincomycin Hydrochloride
461.0	Losartan Potassium
461.5	Azlocillin
461.5	Orphenadrine Citrate
461.6	Pimozide
461.8	Cetirizine Dihydrochloride
462.0	Diprenorphine Hydrochloride
462.5	Doxycycline
462.5	Hydrocortisone Hydrogen Succinate
463.1	Perospirone Hydrochloride
463.4	Eletriptan Hydrobromide
463.5	Pivampicillin
464.0	Moracizine Hydrochloride
464.6	Hordenine Sulfate
464.8	Amodiaquine Hydrochloride
464.9	Demeclocycline
465.4	Cefixime Disodium Salt
465.4	Dimoxyline Phosphate
465.4	Naftopidil Dihydrochloride
465.5	Bacampicillin
466.0	Cisapride
466.0	Mebeverine Hydrochloride
466.5	Brucine tetrahydrate
466.5	T-2 Toxin
466.6	Cephaëline
466.6	Spirapril
467.0	Clobetasol Propionate
467.4	Ziprasidone Hydrochloride Monohydrate
467.5	Saccharin Calcium
467.5	Tobramycin
467.6	Buprenorphine
467.6	Oxetacaine
467.9	Noscapine Hydrochloride
469.5	Methysergide Maleate
469.6	Almotriptan Maleate
469.7	Ebastine
470.0	Dimenhydrinate
470.0	Nefazodone
470.2	Dibrompropamidine
470.5	Docarpamine
470.7	Enoxolone
471.0	Alfentanil Hydrochloride
472.1	Mercurous (I) Chloride
472.8	Alpha Tocoferil Acetate
473.4	Clofazimine
473.5	Rosiglitazone Maleate
473.6	Alverine Citrate
473.6	Naftidrofuryl Oxalate
473.6	Raloxifene
473.9	Clopenthixol Hydrochloride
473.9	Zuclopenthixol Hydrochloride
474.4	Flecainide Acetate
474.5	Methylprednisolone Hemisuccinate
474.6	Fluocortolone Hexanoate
474.6	Sildenafil
475.0	Quinapril Hydrochloride
475.4	Picloxydine
475.6	Fluspirilene
475.6	Tolterodine Tartrate
475.7	Pipotiazine
475.9	Cloxacillin Sodium
476.6	Betamethasone Valerate
476.6	Piminodine Esilate
476.6	Prednisolone Tebutate
477.0	Loperamide
477.4	Taltirelin Tetrahydrate
477.6	Butorphanol Tartrate
478.3	Suxethonium Bromide
478.5	Triamcinolone Diacetate
478.6	Dehydroemetine
478.9	Chlortetracycline
478.9	Methacycline Hydrochloride
479.0	Clobetasone Butyrate
479.1	Clospipramine
479.1	Miconazole Nitrate
479.4	Aztreonam Disodium
479.5	Clindamycin Hydrochloride
480.4	Isopropamide Iodide
480.4	Trifluoperazine Hydrochloride
480.6	Emetine
480.6	Quinine Salicylate
480.9	Tetracycline Hydrochloride
481.5	Cerivastatin Sodium
481.5	Pranlukast
481.5	Rosuvastatin
481.9	Meclozine Hydrochloride
481.9	Narceine Hydrochloride
482.5	Trifluomeprazine Maleate
483.5	Azlocillin Sodium
483.9	Mersalyl Acid
484.0	Cisapride Monohydrate
484.4	Nicergoline
484.4	Prednisolone Sodium Phosphate
484.5	Bopindolol Malonate
484.5	Hydrocortisone Sodium Succinate
484.5	Kanamycin
484.5	Oxedrine Tartrate
486.2	Suxamethonium Bromide
486.4	Hydrocortisone Sodium Phosphate
486.6	Hydrocortisone Cipionate
487.4	Fluclorolone Acetonide
487.7	Dimetotiazine Mesilate
488.0	Chloropyrilene Citrate
488.0	Doxazosin Hydrochloride
488.6	Vardenafil
489.0	Delapril Hydrochloride
489.1	Diphenoxylate Hydrochloride
490.0	Acetorphine Hydrochloride
490.6	Dexamethasone Tebutate
490.6	Glimepiride
490.6	Pentobarbital Calcium
491.1	Verapamil Hydrochloride
491.4	Morphine Tartrate
491.5	Flunixin Meglumine
491.6	Lidoflazine
492.5	Enalapril Maleate
492.6	Bezitramide
492.6	Ecabet Sodium
492.7	Isometheptene Mucate
493.4	Hexoprenaline Hydrochloride
493.6	Ryanodine
493.9	Flucloxacillin Sodium
493.9	Minocycline Hydrochloride
494.0	Glibenclamide
494.5	Fluocinonide
494.5	Hydrocodone Tartrate
494.6	Flumetasone Pivalate
494.6	Quinidine Bisulfate
495.1	Tirofiban Hydrochloride Monohydrate
495.5	Nicomorphine
495.6	Mibefradil
495.7	Orlistat
496.0	Isometamidium
496.5	Methylprednisolone Sodium Succinate
496.5	Oxytetracycline Dihydrate
496.6	Betamethasone Benzoate
496.6	Bopindolol Maleate
496.9	Oxytetracycline Hydrochloride
497.5	Idarubicin
497.6	Dexamethasone Isonicotinate
498.6	Moexipril
499.0	Promethazine Teoclate
499.3	Nitroxinil Eglumine Salt
500.0	Pivampicillin Hydrochloride
500.4	Halofantrine
500.6	Fluticasone Propionate
500.6	Mupirocin
501.3	Demeclocycline Hydrochloride
501.7	Fexofenadine
502.0	Bacampicillin Hydrochloride
503.1	Spirapril Hydrochloride
503.4	Furethidine Methiodide
504.1	Buprenorphine Hydrochloride
504.6	Betamethasone Dipropionate
504.6	Dipyridamole
505.0	Clindamycin Phosphate
505.2	Deltamethrin
505.4	Acriflavinium Chloride
505.5	Chlorhexidine
505.6	Amprenavir
505.9	Mersalyl Sodium
506.0	Buclizine Hydrochloride
506.5	Nefazodone Hydrochloride
506.6	Phenamidine Isethionate
507.2	Adenosine Triphosphate
507.4	Flupentixol Hydrochloride
507.5	Cefixime Trihydrate
507.8	Pyrrobutamine Phosphate
508.6	Argatroban
508.9	Clomocycline
508.9	Mepacrine Hydrochloride
509.6	Bialamicol Hydrochloride
509.6	Landiolol
510.0	Raloxifene Hydrochloride
510.4	Fluphenazine Hydrochloride
510.6	Cyclobarbital Calcium
511.6	Etamiphylline Camsilate
511.6	Norbormide
512.3	Sodium Cromoglicate
512.5	Guanoxan Sulfate
512.9	Doxycycline Hydrochloride
513.5	Clomethiazole Edisilate
513.5	Loperamide Hydrochloride
514.6	Epalrestat *N*-Methyl-D-Glucamine
514.6	Telmisartan
515.3	Chlortetracycline Hydrochloride
515.9	Chloroquine Phosphate
516.4	Betamethasone Sodium Phosphate
516.4	Dexamethasone Sodium Phosphate
516.6	Stilbamidine Isethionate
516.7	Bamethan Sulfate
517.4	Mitoxantrone Hydrochloride
518.1	Benziodarone
518.4	Conessine Hydrobromide
518.5	Dithiazanine Iodide
518.6	Hexoprenaline Sulfate
518.6	Prajmalium Bitartrate
518.9	Thiopropazate Hydrochloride
519.0	Dichloralphenazone
519.6	Ergotoxine
520.6	Orciprenaline Sulfate
520.6	Quinidine Gluconate
521.0	Beclometasone Dipropionate
521.4	Mometasone Furoate
522.6	Azatadine Maleate
524.0	Penfluridol
525.0	Amlodipine Maleate
525.6	Carbetapentane Citrate
525.6	Deptropine Citrate
525.7	Fusidic Acid
526.7	Argatroban Monohydrate
527.5	Daunorubicin

Mol. wt	*Compound*
527.6	Dequalinium Chloride
527.6	Gliquidone
527.6	Oxeladin Citrate
527.6	Rolitetracycline
528.5	Dutasteride
528.6	Fentanyl Citrate
529.8	Rocuronium
530.1	Haloperidol Decanoate
530.9	Clomocycline Sodium
531.0	Moexipril Diacid Hydrochloride
531.4	Ketoconazole
532.0	Nicomorphine Hydrochloride
532.6	Hydroxystilbamidine Isetionate
532.6	Pentamidine Mesilate
532.6	Triamcinolone Hexacetonide
534.0	Idarubicin Hydrochloride
534.6	Emedastine Difumarate
535.0	Moexipril Hydrochloride
536.9	Halofantrine Hydrochloride
538.1	Fexofenadine Hydrochloride
538.6	Pentolonium Tartrate
538.7	Sodium Fusidate
539.5	Cephaëline Hydrochloride
540.3	Quinine Dihydrobromide
540.6	Atorvastatin Lactone
540.7	Mescaline Sulfate Dihydrate
541.5	Clioxanide
542.6	Dextromoramide Tartrate
543.5	Doxorubicin
543.5	Epirubicin
544.2	Suxamethonium Iodide
544.7	Mesoridazine Benzenesulfonate
545.5	Ketanserin Tartrate
546.1	Landiolol Hydrochloride
546.6	Ceftazidime
546.8	Dibutoline
547.6	Doxazosin Mesilate
547.6	Perazine Dimalonate
548.6	Amicarbalide Isetionate
548.6	Quinine Bisulfate
548.6	Terbutaline Sulfate
549.7	Fluphenazine Enantate
550.3	Mebezonium Iodide
551.6	Dehydroemetine Hydrochloride
552.0	Clospipramine Dihydrochloride
552.6	Proflavine Hemisulfate
552.6	Tiotixene Hydrochloride
552.7	Delavirdine Monomethanesulfonate
554.6	Ceftriaxone
554.7	Profenamine Hibenzate
555.2	Clopenthixol Decanoate
555.2	Zuclopenthixol Decanoate
556.6	Isoprenaline Sulfate
558.6	Fenoprofen Calcium
558.6	Olmesartan Medoxomil
558.7	Atorvastatin
560.7	Desferrioxamine
561.5	Chloramphenicol Palmitate
561.5	Vardenafil Dihydrochloride
562.5	Acefylline Piperazine
563.6	Trimetrexate D-Glucuronate
563.7	Fosinopril
564.0	Daunorubicin Hydrochloride
564.1	Prochlorperazine Edisilate
564.6	Propamidine Isetionate
565.7	Dextropropoxyphene Napsilate
565.7	Levopropoxyphene Napsilate
566.2	Prochlorperazine Mesilate
566.6	Quinuronium Sulfate
567.1	Amlodipine Besilate
567.8	Nelfinavir
568.6	Mibefradil Dihydrochloride
570.8	Bunamidine Hydroxynaphthoate
570.8	Carbenoxolone
574.8	Dequalinium Acetate
575.6	Tandospirone Citrate
575.7	Zafirlukast
576.3	Distigmine Bromide
577.6	Stilbazium Iodide
578.4	Chlorhexidine Hydrochloride
578.7	Deserpidine
578.7	Sufentanil Citrate
579.0	Loprazolam Mesilate
579.1	Vardenafil Hydrochloride Trihydrate
580.0	Doxorubicin Hydrochloride
580.0	Epirubicin Hydrochloride
580.6	Atorvastatin Sodium
581.6	Streptomycin
581.7	Ergotamine
581.8	Bromofenofos
582.6	Kanamycin Sulfate
583.6	Dihydrostreptomycin
583.7	Dihydroergotamine
585.6	Amikacin
585.6	Fosinopril Sodium
585.7	Ebastine Fumarate
585.8	Myrophine
586.2	Montelukast
586.7	Irinotecan
587.6	Diminazene Aceturate
588.6	Etoposide
588.7	Procaine Benzylpenicillin
588.8	Flupentixol Decanoate
591.7	Veratrine
591.8	Fluphenazine Decanoate
592.7	Hexobendine
592.7	Pentamidine Isetionate
594.7	Pyrantel Embonate
596.8	Trimetaphan Camsilate
597.4	Pyritidium Bromide
598.1	Clomifene Citrate
598.1	Toremifene Citrate
600.5	Nicofuranose
602.6	Picrotoxin
603	Lymecycline
603.7	Benzonatate
603.7	Salmeterol Xinafoate
604.3	Fazadinium Bromide
605.6	Butaperazine Phosphate
605.9	Triclobisonium Chloride
606.1	Prochlorperazine Maleate
608.2	Montelukast Sodium
608.5	Ambenonium Chloride
608.7	Methoserpidine
608.7	Reserpine
609.7	Rocuronium Bromide
610.2	Mepacrine Mesilate
610.7	Candesartan Cilexetil
613.8	Indinavir
614.6	Neomycin
614.7	Carbenoxolone Sodium
615.6	Butyl Aminobenzoate Picrate
615.6	Paromomycin
617.6	Rolitetracycline Nitrate
618.3	Colesevelam Hydrochloride
622.3	Myrophine Hydrochloride
622.7	Pseudomorphine
623.2	Irinotecan Hydrochloride
623.7	Detajmium Bitartrate
624.3	Guanoclor Sulfate
625.6	Chlorhexidine Diacetate
626.0	Rafoxanide
628.8	Lopinavir
631.8	Thiethylperazine Maleate
634.7	Rescinnamine
636.7	Ceftazidime Pentahydrate
638.8	Thioproperazine Mesilate
641.7	Butaperazine Maleate
641.9	Pipotiazine Undecylenate
642.7	Piperazine Citrate
642.9	Dibutoline Sulfate
643.7	Acetophenazine Dimaleate
645.3	Amiodarone
645.6	Acarbose
645.7	Aconitine
647.6	Cabergoline Diphosphate
648.8	Physostigmine Sulfate
651.0	Liothyronine
651.2	Kanamycin Acid Sulfate
652.8	Metoprolol Succinate
654.6	Bromocriptine
656.8	Desferrioxamine Mesilate
657.7	Carfenazine Maleate
661.6	Ceftriaxone Sodium
663.9	Nelfinavir Mesilate
664.8	Antazoline Sulfate
665.6	Hexobendine Hydrochloride
665.7	Natamycin
666.7	Sildenafil Citrate
666.7	Syrosingopine
667.8	Thiethylperazine Malate
668.6	Etoposide Phosphate
668.7	Capreomycin
670.8	Abacavir Sulfate
670.8	Saquinavir
671.8	Ergotoxine Esilate
672.5	Halofantrine β-Glycerophosphate
673.0	Liothyronine Sodium
677.2	Irinotecan Hydrochloride Trihydrate
677.8	Rapacuronium Bromide
679.7	Emetine Hydrochloride
679.8	Dihydroergotamine Mesilate
681.8	Amiodarone Hydrochloride
684.8	Metoprolol Tartrate
684.9	Prednisolone Steaglate
685.7	Viomycin
687.4	Liothyronine Hydrochloride
687.9	Oleandomycin
691	Fumonisin B_2
694.8	Atropine Sulfate
697.8	Rifamycin SV
699.9	Clindamycin Palmitate Hydrochloride
703.8	Pamaquin
705.6	Itraconazole
707.8	Codergocrine Mesilate
708.8	Aconitine Nitrate
708.8	Strophanthin-K
711.0	Butacaine Sulfate
711.9	Indinavir Sulfate
712.5	Ranitidine Bismuth Citrate
712.9	Hyoscyamine Sulfate
713.1	Omeprazole Magnesium
714.1	Pipotiazine Palmitate
716.6	Demecarium Bromide
718.8	Olanzapine Embonate
719.6	Daunorubicin Lactate
719.8	Rifamycin Sodium
721.0	Ritonavir
721.8	Fumonisin B_1
722.4	Dibrompropamidine Isetionate
728.8	Ouabain
731.8	Diphenhydramine Acefyllinate
732.7	Pancuronium Bromide
733.9	Erythromycin
734.0	Colfosceril Palmitate
747.0	Alimemazine Tartrate
748.0	Clarithromycin
749.0	Azithromycin
750.7	Bromocriptine Mesilate
750.9	Codeine Sulfate
752.7	Pholcodine Tartrate
753.9	Vindesine
758.8	Morphine Sulfate
763.3	Hydroxyzine Embonate
764.9	Digitoxin
764.9	Zolpidem Tartrate
767.0	Bisoprolol Fumarate (hemifumarate)
767.0	Saquinavir Mesilate
767.2	Esomeprazole Magnesium
771.1	Aminopromazine Fumarate
771.7	Tubocurarine Chloride
774.9	Phenoxymethylpenicillin Calcium
776.9	Levothyroxine
779.0	Vinorelbine
780.9	Digoxin
781.8	Amikacin Sulfate
783.0	Quinidine Sulfate
783.0	Quinine Sulfate
785.9	Oleandomycin Phosphate
788.9	Phallotoxin
790.0	Erythromycin Propionate
793.9	Protoveratrine A and B
794.8	Xamoterol Hemifumarate
795.0	Metildigoxin
798.9	Levothyroxine Sodium
800.9	Ergometrine Tartrate
804.0	Tacrolimus
804.9	Formoterol Fumarate Dihydrate
805.0	Okadaic Acid
807.0	Acetyldigitoxin
807.9	Docetaxel
809.9	Protaveratrine B

Mol. wt	*Compound*
810.7	Inositol Nicotinate
810.9	Rifamide
811.0	Vinblastine
811.8	Aclarubicin
813.0	Cinchonidine Sulfate
814.0	Troleandomycin
822.0	Tacrolimus Monohydrate
822.9	Rifampicin
823.0	α-Acetyldigoxin
825.0	Vincristine
827.9	Alcuronium Chloride
835.1	Dirithromycin
837.0	Roxithromycin
841.1	Mebhydrolin Napadisilate
847.0	Rifabutin
848.3	Aclarubicin Hydrochloride
852.0	Vindesine Sulfate
853.9	Paclitaxel
857.0	Strychnine Sulfate
861.9	Docetaxel Trihydrate
862.1	Erythromycin Ethylsuccinate
866.9	Papaverine Sulfate
867.1	Brevetoxin A
868.1	Solanine
869.2	Tilmicosin
871.0	Methapyrilene Fumarate
883.1	Quetiapine Fumarate
883.2	Docusate Calcium
885.2	Ibutilide Fumarate
886.13	Emamectin
891.5	Gallamine Triethiodide
891.8	Cycloguanil Embonate
895.1	Brevetoxin B
897.8	Chlorhexidine Digluconate
899.1	Spiramycin
906.6	Metocurine Iodide
909.1	Benzathine Benzylpenicillin

Mol. wt	*Compound*
909.1	Vinblastine Sulfate
914.2	Sirolimus
916.1	Tylosin
917.5	Brevetoxin C
919	α-Amanitin
920	β-Amanitin
923.0	Vincristine Sulfate
924.1	Amphotericin B
926.1	Nystatin
929.2	Atracurium
929.2	Cisatracurium
943.1	Deslanoside
943.2	Amitriptyline Embonate
949.2	Imipramine Embonate
957.2	Promazine Embonate
958.9	Oxytetracycline Calcium
960.1	Erythromycin Gluceptate
985.1	Lanatoside C
1001.1	Rosuvastatin Calcium
1004.8	Glaucine Phosphate
1013	Brucine Sulfate
1018.4	Erythromycin Stearate
1019.3	Octreotide
1026	Chlorpromazine Embonate
1056.4	Erythromycin Estolate
1060	Maitotoxin-3
1077.4	Laudexium Methyl Sulfate
1079.1	Vinorelbine Tartrate
1092	Erythromycin Lactobionate
1095.3	Ciguatoxin-2
1095.3	Ciguatoxin-3
1096.3	Lanreotide
1100.2	Mivacurium Chloride
1106.1	Doxacurium Chloride
1111.3	Ciguatoxin-1
1151.4	Pyrvinium Embonate
1185.3	Yessotoxin

Mol. wt	*Compound*
1202.6	Ciclosporin
1209.4	Atorvastatin Calcium
1209.4	Leuprorelin
1239.4	Buserelin
1243.5	Atracurium Besilate
1243.5	Cisatracurium Besilate
1255.5	Dactinomycin
1269.5	Leuprorelin Acetate
1299.5	Buserelin Acetate
1313.4	Ergotamine Tartrate
1317.4	Dihydroergotamine Tartrate
1322.5	Nafarelin
1346.4	Hydroxocobalamin
1355.4	Cyanocobalamin
1382.6	Nafarelin Monoacetate
1415.6	Bleomycin
1449.3	Vancomycin
1457.4	Streptomycin Sulfate
1461.4	Dihydrostreptomycin Sulfate
1485.7	Vancomycin Hydrochloride
1728.1	Fondaparinux Sodium
2639.13	Ziconitide
2933.5	Tetracosactrin
3298	Maitotoxin-2
3325.8	Aviptadil
3422	Maitotoxin-1
4000–6000	Enoxaparin
6979.4	Lepirudin
14798.03	Molgramostim
15330.7	Aldesleukin
22124.1	Somatropin
37 000	Chorionic Gonadotropin
39571.1	Reteplase
59007.6	Alteplase
64529.1	Duteplase
136 000	Asparaginase

7 Melting Points

d = with decomposition

°C	*Compound*
−126	Desflurane
−123.5	Acetaldehyde
−118	Phosgene
−94	Acetone
−93.7	Methyl Bromide
−88	Acrolein
−88	Stibine
−79–80	Tributyl Phosphate
−69	Chloropicrin
−57	Sarin
−50	Tabun
−43	γ-Butyrolactone
−42	Soman
−39	VX
−38.87	Mercury
−36.5	Bromoacetone
−32.5	Trichloroethane
−28	Omethoate
−20	Dioxathion
−20	Myristicin
−18	Lewisite
−16	Thiodiglycol (metabolite)
−13.4	Hydrogen Cyanide
−10.3	3-Chloroaniline
−6	Cyanogen Chloride
−1.9	2-Chloroaniline
0	Cineole
0.5	Triazophos
2–5	Triazophos
3.4	Fenitrothion
6	Parathion
7.5	Fenthion
8–9	Ethyl Nicotinate
10	Ethylenediamine Hydrate
10–13	Paraldehyde
12	Trientine
14.4	Sulfur Mustard
15	Acetic Acid Glacial
15.5–18.5	Fomepizole
17–20	*N*-Benzylpiperazine
18	Trichloroethanol
18.3	Dimethyl Sulfoxide
19	Propofol
22	Trazodone Hydrochloride
23–25	Nikethamide
23–24	Putrescine
24	Amylmetacresol
<25	Dichlorvos
25	Foscarnet Sodium
25–30	Monocrotophos
<25	Piperonyl Butoxide
25	Sevoflurane
26.5–27.5	Naled
27–28	Menthol
≈28	Furethidine
28.5–32	Sulfiram
29.5	Chloromethylaniline
30–135	Chlorphenamine Maleate
30–32	Fluphenazine Decanoate
30–32	Menthol
31d	Carmustine
32–35	Nandrolone Decanoate
33	Fluoroacetic Acid
33	Fluoroacetic Acid
33	Picloxydine Digluconate
34	Pilocarpine
35	Mescaline

°C	*Compound*
35.5	Arsenic Acid
36	Mescaline
36–38	Testosterone Enantate
36.5–37.5	Prenylamine
37–38	Prilocaine
38–40	Phenoxybenzamine
38–40	Pyrazophos
38.1	Anhydrous Ephedrine
39	Methyl Nicotinate
40–41	Alachlor
40	Ephedrine
40–43	Orlistat
40–41	Phenol
41	Barium Acetate
41–42	Chlorpyrifos
41–45	Cyclophosphamide
41–42.5	Fenpiprane
41–43	Menthol
41	Myrophine
41–43	Salol
41–46	Tetracaine
43–44	Amolanone
43	Parachlorophenol
43	Piperazine Hydrate
44	Tetrachloroethane
44.6–46.8	Dimethyltryptamine
45–46	Butanilicaine
45	Trimipramine
45.5–46.5	Chlorpyrifos-(Me)
46–52	Ethosuximide
46–74	Heptachlor
46–50	Idebenone
46–47	Octacaine
46–46.5	Phencyclidine
46	Pyriproxyfen
46	Trimethadione
47–49	Diethylcarbamazine
47–48	Pramiracetam
48–55	Butylated Hydroxyanisole
48–50	Esmolol
48–49	Pyrrobutamine
48–50	Urethane
49–60	Aldrin
49	Fenamiphos
49	Tropacocaine
49–51	Tybamate
49.5–50.0	Choline Salicylate
50	Chloral Hydrate
50–56	Mesuximide
50–53	Metyrapone
51	Benactyzine
51–53	Butibufen
51–53	Perazine
51.5	Thiotepa
51.5	Thymol
52	Cyanogen Bromide
52–52.5	Dimethoate
52–53	Idebenone
53	Azinphos-(Et)
53	Famphur
53–56	Methylpentynol Carbamate
53–54	Paradichlorobenzene
53–55	Profenamine
53–55	Tocainide
54	CN Gas
54	Methamidophos
54–55	Monocrotophos

°C	*Compound*
54–57	Triclosan
55.0–56.5	Cyclandelate
55–57	Dosulepin
55–56.5	Hydroxyphenamate
55.5	Chlorocresol
56–60	Chlorpromazine
56	Emylcamate
56–57	Enallylpropymal
56.5	CN Gas
57	Alprenolol
57–59	Butyl Aminobenzoate
57–60	Pentetrazol
57	Quinine
58–60	Cetalkonium Chloride
58–59	CN Gas
59.	Alprenolol
59	Hyoscine
59–61	Triprolidine
60–62	Apoatropine
60	Cyfluthrin
60–65	Halofantrine β-Glycerophosphate
60–61.5	Levamisole
60	Promethazine
60.5	Beryllium Nitrate
60.5–61.0	DOM
≈61	Erythrityl Tetranitrate
61	Procaine
62–68	Hexylresorcinol
62–64	Thiethylperazine
63–64	Isoaminile Citrate
63	Tropine
64	Cinchocaine
64–67	Dichloralphenazone
64–66	Naepaine
65	Isobutyl Aminobenzoate
66	Cannabidiol
66	Chlorambucil
66	Lucanthone
67	Cannabidiol
67	Etomidate
67.5–68.5	Fluanisone
68	Alimemazine
68–69	Isometheptene Hydrochloride
68–69	Lidocaine
68–70	Methoprotryne
68.5–70	Dioxaphetyl Butyrate
69–71	Diphenyl
69–70	5-Methoxy-*N*,*N*-dimethyltryptamine
69–70	Propentofylline
69–70	Ranitidine
70–72	Betaxolol
70	Butylated Hydroxytoluene
70	Disulfiram
70	Embutramide
70–100	Endosulfan
70	Isosorbide Dinitrate
70–71	Mephenesin
71	Chloronitrotoluene
71–73	Phensuximide
72	CR Gas
72–75	Taltirelin
72–74	Thioridazine
72.5	4-Chloroaniline
73	Aluminium Nitrate
73–75	Azaperone
73–74	Azinphos-(Me)
73–75	Budipine

°C	Compound
73.4	Antimony Trichloride
74	Emetine
74–75	Methylphenidate
74–77	Methyprylon
75–115	Aminometradine
75d	Antimony Pentasulfide
75	Sodium Borate Decahydrate
75–76	Dextropropoxyphene
75	Etamiphylline
75–77	Hexobendine
75	Ibuprofen
75–76	Levopropoxyphene
75–80	Oxprenolol
75–76	Phenadoxone
75	Racephedrine
75	Trichlorfon
75.5	Clonitazene
75.5–76.5	Salmeterol
76–77	Dimethadione
76–78	Ethylestrenol
76	Hydroxyquinoline
76	Toloxatone
77–78	Cathine
77–83	Cetylpyridinium Chloride
77–79	Chlorphenesin
77	Flurazepam
77	Ibuprofen
77–78	Lidocaine Hydrochloride
77–79	Mebutamate
77–78	Mupirocin
77–78	Phenaglycodol
≈78	Butylchloral Hydrate
78	Chlorobutanol
78	Etisazole
78–82	Guaifenesin
78	Methadone
79–82	Fenofibrate
80	Allylestrenol
80	Anileridine
80–82	Arteether
80–80.5	Bamifylline
80–81	Fenofibrate
80	Isopropylaminophenazone
80–85	Lysergide
80–83	Monolinuron
80	Nabumetone
80	Testosterone Isocaproate
80.2	Naphthalene
81–82	Anastrozole
81–83	Vanillin
82–85	Fenyramidol
82	Flurazepam
82–83	Pentifylline
83	Fentanyl
83–84	Histamine
83–84	Nefazodone
84–86	Ametryne
84	Colecalciferol
84–86	Desmetryne
84	Fentanyl
84	Pyrrocaine
85–90	Atracurium Besilate
85–90	Docarpamine
85–86	Esmolol Hydrochloride
85–89	Glutethimide
85.5–86.5	Procyclidine
86–88	Artemether
86–92	Benzoylecgonine
86	Dinitro-orthocresol
86–87	Physostigmine
86–88	Trazodone
86.8	Econazole
87–95	Chloramphenicol Palmitate
87–92	Chloroquine
87–89	Diethyltryptamine Hydrochloride
87–91	Etidocaine
87–88	Methdilazine
87–89	Tetramisole
87.5–88.8	Ethambutol
88–92	Benzocaine
88–93	Haloxon
88–90	Metaclazepam
88–90	Noxytiolin
88–90	Ritodrine
89	Anileridine
89–91	Hydroxychloroquine
89–91	Isosorbide Mononitrate
89	Methoxychlor
89–92	Trifluoromethylphenylpiperazine
90–100	Aldicarb
90	Chlorphenesin Carbamate
90–98d	Cinchocaine Hydrochloride
90–100	Clobetasone Butyrate
90–96	Cycrimine
90	Ethotoin
90	Ochratoxin A
90–91	Oxeladin Citrate
90–91	Phenindamine
90	Physostigmine Aminoxide Salicylate
90–92	Vinylbital
91	Alclofenac
91–94	Beclamide
91	Buclosamide
91	Pholcodine
91–93	Propanil
91–95	Quinine Ethyl Carbonate
92–93	Carisoprodol
92–97	Dexketoprofen
92	Ketamine
92–94	Maprotiline
92–96	Methohexital
92–93	Nicorandil
93	Carbetapentane Citrate
93–96	Halofantrine Hydrochloride
93	Ketamine
93–96	Ketoprofen
93–94	Linuron
93	Mephenesin Carbamate
93–94	Mycophenolate Mofetil
93–95	Trifluperidol
94–102	Ditiocarb Sodium Trihydrate
94–99	Etamivan
94	*N*-Acetylmescaline
94–90	Methocarbamol
94–110	Perphenazine
95–97	Cilazapril
95–92	Coumaphos
95–96	CS Gas
≈95	Disopyramide
95	Ditiocarb Sodium
95–140	Erythromycin Gluceptate
95–96	Heptachlor
95	Mebhydrolin
95–97	Medazepam
95–96	Nandrolone Phenylpropionate
95–97	Thenalidine
96–97.5	Dodecyl Gallate
96–98	Ethinamate
96–98	Metadoxine
96	Oxyphenbutazone
96	Propranolol
96–97	Propyl Hydroxybenzoate
96	Tiaprofenic Acid
96	Trazodone
96–97	Trenbolone Acetate
96–97	Tropicamide
96.6	Sufentanil
97–99	Bretylium Tosilate
97	Chlorobutanol
97–98	Chlorprothixene
97–100	Limaprost
98	Cocaine
98–101	Deltamethrin
98–99	α-Methyltryptamine
99–100	Homatropine
99–102	Mexenone
99–100	Phenatine
99–100	Rabeprazole
100–103	Alverine Citrate
100	Antimony Potassium Tartrate
100	Bisoprolol Fumarate (hemifumarate)
100–103	Butacaine Sulfate
100–104	Doxylamine Succinate
100–101	Mepyramine Maleate
100–115	Methylchlorophenoxyacetic Acid
100–101	Opipramol
100	Orciprenaline
100–110	Piperacetazine
100	Secobarbital
100–101	Valethamate Bromide
101	Biperiden
101–104	Phenprobamate
101	Phenylpropanolamine
101–102	Testosterone Cipionate
102–103	Clorgiline Hydrochloride
102–107	Dimenhydrinate
102	Dimethisterone
102–103	Diphenazoline
102–104	Famciclovir
102–108	Gentamicin
102–104d	Noradrenaline Acid Tartrate
102–105	Pentoxifylline
102–104	Venlafaxine
102.5–103.5	Isoxsuprine
103–104	Cibenzoline
103–113	Dexbrompheniramine Maleate
103–107	Dexketoprofen Trometamol
103–104	Difenidol
103	Propyphenazone
104	Acetylcysteine
104	Aldrin
104	Diethylstilbestrol Dipropionate
104–105	Etretinate
104–105	Levodropropizine
104	Meprobamate
104	Oxetacaine
104	Terbutryne
105.0–107.5	Choline Dihydrogen Citrate
105–106	Dioxyamidopyrine
105–108	Docarpamine
105–110	Fenoprofen Calcium
105–107	Flecainide
105–108	Isocarboxazid
105–107	Menadione
105–110	Methenamine Hippurate
105–109	Metipranolol
105–108	Patulin
105–107	Penfluridol
105	Phenylbutazone
105–106	Physostigmine
105–107	Prednisolone Steaglate
105.5–107.5	Cyclizine
106	Captopril
106–109	Hyoscyamine
106	Lofepramine
106	Meprobamate
106	Piperazine
106–107	Pipobroman
106–110d	Procaine Benzylpenicillin
106–107	Rilmenidene
106–110	Tripelennamine Citrate
107	Alminoprofen
107–109	Aminophenazone
107–110	Butetamate Citrate
107–109	Oxprenolol Hydrochloride
107	Pheniramine Maleate
107–110	Phenoxypropazine Maleate
107.5	Bupivacaine
108	Alprenolol Hydrochloride
108	Bupivacaine
108	Disulfoton
108–109	Dropropizine
108–110	Endosulfan
108	Mustine Hydrochloride
108	Naftidrofuryl Oxalate
108–112	Prothipendyl Hydrochloride
108	Talbutal
108–110	Toremifene
108.5–111	Loxoprofen
109	Acecarbromal
109–110	Butyl Aminobenzoate Picrate
109	Clofenotane
109–113	Dextromethorphan
109–110	Loxapine
109	Ramipril
109–111	Resorcinol
109.8	Cisapride Monohydrate
110	Acetylcysteine
110	Alprostadil
110–112	Celiprolol
110–115	Cetirizine
110	Flurbiprofen

°C	*Compound*
110	Gallium Nitrate
110	Nimorazole
110	Patulin
110–111	Styramate
111	Alprenolol Hydrochloride
111–112	Buphenine
111–115	Clomifene
111–112	Diethylaminoethyl Diphenylpropionate Hydrochloride
111	Hexaconazole
111–113	Phenazone
111	Pralidoxime Metilsulfate
111	Tolnaftate
112–115	Acetomenaphthone
112–115	Adiphenine Hydrochloride
112–116	Chloropyrilene Citrate
112–113	Dihydrocodeine
112–113	Domiphen Bromide
112–113	Indeloxazine
112	Nandrolone
112	Norelgestromin
112–113.5	Tandospirone
112.5	Lindane
113–115	Acetanilide
113–115	Azithromycin
113–117	Betaxolol Hydrochloride
113	Chlorquinaldol
113–115	Cyclopentamine Hydrochloride
≈113	Cyproheptadine
113–115	Dexchlorphenamine Maleate
113	Dihydrotachysterol
113–119	Ergocalciferol
113	Iproniazid
113–115	Levorphanol Tartrate
113–115	Ramelteon
114	Atropine
114–118	Busulfan
114–115	Carvedilol
114–116	Chloroxylenol
114–116	Ciprofibrate
114–116	Dicamba
114–116	Diloxanide Furoate
114	Miglitol
114–116	Mirtazapine
114–116	Perazine Dimalonate
114–117	Testosterone Phenylpropionate
114.3–115.0	Trihexyphenidyl
115–119	Sodium Methanearsonate
115	Bamipine
115–116	Caramiphen Edisilate
115–116	Cephaëline
115–116	Flupirtine
115–118	Trientine Dihydrochloride
116–119	Carbromal
116–122	Clotiapine
116–117	Dibenzepin
116	Ethyl Hydroxybenzoate
116	Hydrastine Hydrochloride
116–117	Pericyazine
116–118	Triprolidine Hydrochloride
116–117	Valsartan
116.2–118.2	Chlormezanone
116.5–118	Clomifene Citrate
117–119	Carbinoxamine Maleate
117–120	Etoxazene
117–118	Hordenine
117	Hydrastinine
117–119	Ibutilide Fumarate
117	Levetiracetam
117–120	Metabutoxycaine Hydrochloride
117.5–118.5	Nifekalant
118	Atropine
118	Chlorotrianisene
118–122	Flutoprazepam
118–128	Hydrocodone Tartrate
118–119	Methylchlorophenoxyacetic Acid
118–120	Prometryne
118.0–118.7	Pseudoephedrine
118–122	Testosterone Propionate
118	Tryptamine
118.5–119.5	Morinamide
119–123	Acebutolol
119	Chloramphenicol Cinnamate
119–121	Mandelic Acid
119–121.5	Quinapril Hydrochloride
119	Riluzole
119–124	Sulfafurazole Diethanolamine
119–122	Terbutaline
120–122	Antazoline
120	Clofedanol
120–121.5	Fluvoxamine Maleate
120	Glaucine
120–124	Hydroxyprogesterone Caproate
120–116	Methaqualone
120	Metoprolol Tartrate
120	Naphazoline
120–123	Panidazole
120–128d	Phenoxymethylpenicillin
120–130	Quinapril Hydrochloride
120–121	Rizatriptan
≈120–122	Trimetozine
121–122	Aniracetam
121–123	Betanaphthol
121–122	Etacrynic Acid
121	Etilefrine Hydrochloride
121	*N*-Benzoylmescaline
121–125	Metaxalone
121	Octamylamine Hydrochloride
121	Progesterone
121–123	Sulforidazine
122–125	Carbimazole
122–124d	Dextromethorphan Hydrobromide
122	Maxacalcitol
122–123	Proadifen Hydrochloride
122–124	Racemethorphan Hydrobromide (*d*-Form)
122–123	Rosiglitazone Maleate
122–124	Succinimide
122.4	Benzoic Acid
122.5–124	Quinagolide
123–129	Dihydrotachysterol
123–126	Dipipanone Hydrochloride
≈123d	Ethylmorphine Hydrochloride
123–124	Gestonorone Caproate
123–125	Rivastigmine Hydrogen Tartrate
123–127	Thiambutosine
123.5–125	Bamethan
124–127	Butobarbital
124d	Chloral Betaine
124–125	Dimoxyline
124–125	Flufenamic Acid
124–127	Lopinavir
124–136	Nadolol
124	Nandrolone
124	Picotamide
124–126	Racemethorphan Hydrobromide (*l*-Form)
124	Suprofen
124	Zidovudine
124.5–126.0	Pipamperone Hydrochloride
125–130	Amygdalin
125–140d	Chloropyrilene Citrate
125	Diazepam
125–126	Hydroxyamfetamine
125–129	Naftopidil
125	Nimodipine
125	Penthienate Methobromide
125–127	Phenglutarimide
125–127	Sulfacarbamide
125–127	Sulfonal
125–128	Tetrabenazine
125	Tilidate Hydrochloride
125.4	Landiolol Hydrochloride
126	Amisulpride
126–128	Bemegride
126–127	Butanilicaine Phosphate
126–130	Chlorpropamide
126–129	Clomethiazole Edisilate
126	Diazepam
126–127	Dipipanone Hydrochloride
126–131	Etynodiol Diacetate
126–127	Galantamine
126	Hexethal Sodium
≈126	Levomepromazine
126–128	Lofexidine
126–128	Mecloqualone
126–127	Protokylol Hydrochloride
126–128	Repaglinide
127	Amisulpride
127–130	Aprindine Hydrochloride
127–128	Atropine Oxide
127–129	Calusterone
127–129	Conessine
127–133	Drostanolone Propionate
127–128	Etoricoxib
127–128	Hexestrol Dipropionate
127–133	Meptazinol
127	Neopine
127d	Pentobarbital Sodium
127–131	Progesterone
127–128d	Propylhexedrine Hydrochloride
127–129	Tacrolimus Monohydrate
127–128	Tinidazole
128	Chlorphenoxamine Hydrochloride
128	Idobutal
128–133	Irgarol
128d	Isoprenaline Sulfate
128–130	Methenamine Mandelate
128	Mibefradil Dihydrochloride
128–131	Nicotinamide
128–135	Piminodine Esilate
128	Prenalterol Hydrochloride
128–132	Tylosin
129–133d	Benzathine Benzylpenicillin
129–131	Methoxyphenamine Hydrochloride
129–130	Nateglinide
129–130	Oxybutynin Hydrochloride
129–131	Paroxetine Hydrochloride Hemihydrate
129–130	Pentobarbital
129	Physostigmine Aminoxide
129	Proguanil
129–130	Pyrrobutamine Phosphate
129	Tolbutamide
130–131.5	Benzquinamide
130–132	Bitoscanate
130–133	Butallylonal
130	Clonidine
130–133	Histamine Acid Phosphate
130–131	Lobeline
130–132	Nizatidine
130–132	Thialbarbital Sodium
130–133	Tolbutamide Sodium
131	Benzfetamine Hydrochloride
131–132	Captodiame Hydrochloride
131–136	Mebeverine Hydrochloride
131	Methyl Hydroxybenzoate
131–132	Tirofiban Hydrochloride Monohydrate
131–133	Xylometazoline
131.5–132.5	Encainide Hydrochloride
132	Benzylmorphine
132–134	Brompheniramine Maleate
132–137d	Cotarnine
132–134	Etenzamide
132–133	Famprofazone
132	Hydrastine
132–143	Nicofuranose
132–134	Octacaine Hydrochloride
132	Rolipram
132–135d	Thebacon Hydrochloride
132–133	Thiamylal
133d	Hydroxycarbamide
133–135	Progabide
133–134	Prolintane Hydrochloride
133–134	Ranitidine Hydrochloride
133–136	Thozalinone
133	Urea
134–135	Acetylcodeine
134–136	Alfacalcidol
134	Ambucetamide
134–142	Benperidol Hydrochloride
134	Chlorhexidine
134–137	Clefamide
134–136	Flufenamic Acid
134–136	Loratadine
134–137	Muzolimine
134–138	Orphenadrine Citrate
134–135	Phenacetin
134–136	Practolol
134	Sorbic Acid
134–135	Spironolactone

°C	*Compound*
134–147	Tetracaine Hydrochloride
134–145	Tiocarlide
135–136	Acepromazine Maleate
135–140	Alfentanil Hydrochloride
135	Aspirin
135d	Atropine Oxide
135–137d	Ceftazidime Pentahydrate
135–136	Chelidonine
135–138	Cyclopentolate Hydrochloride
135–140	Erythromycin
135–140d	Erythromycin Estolate
135–139	Flunixin Meglumine
135–137	Levobupivacaine
135–136	Methapyrilene Fumarate
135	Norethandrolone
135d	Omethoate
135–136	(*R*)-Oxiracetam
135–136	(*S*)-Oxiracetam
135–136	Proxyphylline
135–138	Simvastatin
136–139	Butethamine Formate
136	Fenclofenac
136–144	Imidacloprid
136	Meclofenoxate Hydrochloride
136–137	Mephenytoin
136–138	Nicergoline
136–139	Nitroxinil
136d	Sparteine Sulfate
136–137	Sulfinpyrazone
136–139	Xylazine
136.5	Sufentanil Citrate
137–138	Bevantolol Hydrochloride
137–140	Cyclofenil
137–138	Ethylmethylthiambutene Hydrochloride
137	Moclobemide
137–141	Phenoxybenzamine Hydrochloride
137–138	Salmeterol Xinafoate
137–148	Vetrabutine
137.5–139	Quazepam
138	Bisacodyl
138–140	Bufotenine
138–139	Butalbital
138–139.5	*l*-Denopamine Hydrochloride
138–141	Dimetridazole
138–140	Doxercalciferol
138–124.5	Ecothiopate Iodide
138–140	Fluconazole
138	Fluoxetine Hydrochloride
138–140	Formoterol Fumarate Dihydrate
138	Metenolone Acetate
138	Methallenestril
138–140	Phenyltoloxamine Citrate
138–142	Pravastatin Lactone
138–140	Zafirlukast
138.5–140.5d	Verapamil Hydrochloride
139–139.5	Aripiprazole
139–140	Cyclopentobarbital
139–140	Imidapril
139–140	Pantoprazole
139	Phenazopyridine
139	Thiethylperazine Malate
139–140.5	Tolycaine Hydrochloride
139–141	Zolmitriptan
140	Ajmaline Hydrochloride
140–141	Alpidem
140	Amitriptyline Embonate
140–141.5	Aprobarbital
140–150	2C-T-7
140–141	Dehydroepiandrosterone
≈140	Dichlorophenoxyacetic Acid
≈140	Ethoheptazine Citrate
140–142	Fenyramidol Hydrochloride
140–144	Hyoscine Butylbromide
140–142	Isoniazid Aminosalicylate
140	Pentaerithrityl Tetranitrate
140–148	Pentapiperide Metilsulfate
140–145	Phenylephrine Hydrochloride
140	Physostigmine Sulfate
≈140	Pipamazine
140–142	Prenylamine Lactate
140–141	Rabeprazole Sodium
140d	Rifamycin SV
140	Salicylamide
140	Thioproperazine
140–146	Tylosin Tartrate
140–142	Xylazine
140.7	Nebivolol
141–143.	Acebutolol Hydrochloride
141–145	Benzatropine Mesilate
141–143	Cimetidine
141–143	Clindamycin Hydrochloride
141–142	Desoxycortone
141–143	Diethylcarbamazine Citrate
141–146	Emamectin Benzoate
141–146	Ethinylestradiol
141–161	Moexipril Hydrochloride
141	Mycophenolic Acid
141–143	Phenisonone Hydrobromide
141–142	Selegiline Hydrochloride
142–145	Amprotropine Phosphate
142–144	Bibenzonium Bromide
142	Carbaryl
142–150	Colchicine
142–143	Fexofenadine
142–147	Flutazolam
142–142.5	Indeloxazine
142d	Pheniramine Aminosalicylate
142	Protionamide
142–143	Tramazoline
142	Trimipramine Maleate
142.5	Choline Alfoscerate
143	Acetylcholine Bromide
143–144	Aminometradine
143–144.5	Enalapril Maleate
143	Mequitazine
143–148	Methoxsalen
143	Orthocaine
143–147	Raloxifene
144–145	Carbutamide
144–150	Diphenadione
144–145.5	Doxofylline
144–145	Estradiol Valerate
144	Iprindole Hydrochloride
144–149	Neostigmine Methylsulfate
144–145	Norbolethone
144–148	Phenylpropylmethylamine Hydrochloride
144–148	Quinisocaine Hydrochloride
144–149	Rolicyprine
144–146	Ropivacaine
144	Tetrazepam
144–147	Thiamazole
145–154	Azatadine Maleate
145	Berberine
145–149	Bezitramide
145	Butalamine Hydrochloride
145–146	Caramiphen Hydrochloride
145–146.5	Droperidol
145–150	Erythromycin Lactobionate
145–148	Etodolac
145	Felodipine
145–147	Flecainide Acetate
145–147	Hexobarbital
145–146	Methandriol Diacetate
145–170	Moexipril Diacid Hydrochloride
145–147	Paraphenylenediamine
145–146	Prazepam
145–146	Profadol Hydrochloride
145–146	Reboxetine Methanesulfonate (Mesylate)
145–155	Veratrine
145.2–146.4	Noradrenaline Hydrochloride
145.4–147.2	Pentazocine
146–148	Atenolol
146–148	Benserazide Hydrochloride
146	Cycloguanil
146	Ketoconazole
146–154	Mestranol
146–147	Oxetacaine Hydrochloride
146–149	Stenbolone Acetate
146–149	T-2 Toxin
146–148	Vetrabutine Hydrochloride
146.5–148.0	Metoclopramide
147–152d	Adrenaline Acid Tartrate
147–149	Bromisoval
147–150	Cholesterol
147–148	Cloricromen
147–149	Clotrimazole
147–148	Etilefrine
147–148	Etizolam
147–148	Methylenedioxymetamfetamine Hydrochloride
147–148	Nicotinyl Tartrate
147	Papaverine
147–149	Salsalate
147–149	Tiotixene
147–148	Tofenacin Hydrochloride
148–80	Aloin
148–152	Bromazine Hydrochloride
148–151	Emedastine Difumarate
148–151	Enalaprilat
148.0–149.4	Haloperidol
148–149	Mefruside
148	Methacholine Bromide
148–150	Nilvadipine
148–150	Propoxycaine Hydrochloride
148–150	Pyrantel Tartrate
148	Tetracaine Hydrochloride
148–150	Thialbarbital
148–149	Tienilic Acid
148.5	Benazepril
149–150	Aceclofenac
149–153	Chloramphenicol
149d	Distigmine Bromide
149–153	Estradiol Cipionate
149	Fentanyl Citrate
149–153	Fosinopril Diacid
149–150	Isomethadone Hydrobromide (*dl*-Form)
149	Phenylmercuric Acetate
149–150	Morazone
149–151	Phenindione
149–150	Piritramide
149–152	Prajmalium Bitartrate
149.1	Astemizole
149.5–152	Metenolone
150–153	Acemetacin
150	Acetylcholine Chloride
150–151	Aminosalicylic Acid
150–151	Desloratadine
150–152	Embramine Hydrochloride
150	Heptaminol Hydrochloride
150–153	Indinavir Sulfate
150–151	Mepivacaine
150–153	Meprylcaine Hydrochloride
150	Mifepristone
150	Nemonapride
150	Vedaprofen
151	Aminoglutethimide
151	Benzbromarone
151–153	Boldenone Acetate
151–153	Deoxynivalenol
151–154	Ethyl Gallate
151–152	Felbamate
151	Fentanyl Citrate
151–152	HT-2 Toxin
151–152	Ibotenic Acid
151–153	Ketotifen
151–154	Mafenide
151–152	Mafenide Acetate
151–155	Metharbital
151–153	Nialamide
151–152	Nisoldipine
151	Salbutamol
151–152	T-2 Toxin
152–153	Amolanone Hydrochloride
152–158	Anisindione
152	Benzamine Lactate
152	Betahistine Hydrochloride
152–153	Dehydroepiandrosterone
152–153	Diethylthiambutene Hydrochloride
152–154	Hydroxyephedrine
152	Hyoscyamine Hydrobromide
152–153	Ibogaine
152	Isometheptene Mucate
152–154	Lofepramine Hydrochloride
152	Methylenedioxymetamfetamine Hydrochloride
152–154	Naproxen
152–153	Nemonapride
152–154	Pyridostigmine Bromide

°C	Compound
152–157	Testosterone
153	Phenyl Aminosalicylate
153–155d	Bufexamac
153–155	Cabergoline Diphosphate
153–156	Entacapone
153–154	Indinavir
153–155	Ketorolac Tromethamine
153	Methylenedioxymetamfetamine Hydrochloride
153	Naepaine Hydrochloride
153–156	Procaine Hydrochloride
153–155	Rosiglitazone
153	Trichlorophenoxyacetic Acid
154–160	Adrafinil
154–155	Chlorhexidine Diacetate
154–156	Codeine
154–157	Cytisine
154	Dehydroepiandrosterone Sodium Sulfate
154–157	Ethyl Biscoumacetate
154	Fenbutrazate Hydrochloride
154	Gestrinone
154–155	Imolamine Hydrochloride
154–156	Ketorolac Tromethamine
154–155	Pargyline Hydrochloride
154–155	Poldine Metilsulfate
154	Thebacon
155–156	Alacepril
>155d	Ceftriaxone Sodium
155–156d	Cycloserine
155–161	Desoxycortone Acetate
155	Esomeprazole
155–156	Indeloxazine
155–162	Indometacin
155	Isoprenaline
155–157	Nealbarbital
155–160	Oxybuprocaine Hydrochloride
155	Pralidoxime Mesilate
155	Rosuvastatin Calcium
155	Stanozolol
155–158	Stenbolone
155	Tegaserod
155–157d	Tolmetin
155–157	Valdecoxib
156–158	Amobarbital
156	Amobarbital Sodium
156–157	Artemisinin
156–158	Diclofenac
156–157	Ketobemidone
156–157	Moracizine
156	Norandrostenedione
156	Omeprazole
156–157	Orphenadrine Hydrochloride
156–163	Suxamethonium Chloride
156–159	Temazepam
157–159	Celecoxib
157d	Dihydromorphine
157	Feprazone
157–159	Leucinocaine Mesylate
157–158	Salbutamol
157	Tienilic Acid
157	Warfarin
158	Amodiaquine Hydrochloride
158–160	Amosulalol Monohydrochloride
158	Androstenedione
158–165	Dextropropoxyphene Napsilate
158	Diprophylline
158–159	Diuron
158–165	Ethionamide
158–161	Furanocoumarins
158	Isoproturon
158–165	Levopropoxyphene Napsilate
158–160	Lynestrenol
158	Mafenide Propionate
158	Mescaline Acid Sulfate
158–160	Metronidazole
158–160	Midazolam
158	Nitrendipine
158	Suxethonium Bromide
158–160	Thioridazine Hydrochloride
159–163	Alimemazine Tartrate
159–161	Amidefrine
159	Aminotriazole
159	Benzene Hexachloride
159–160	Carbocromen Hydrochloride
159–161	Dimetindene Maleate
159–161	Lidoflazine
159–160	Metindizate Hydrochloride
159	Phenazocine
159–160	Propantheline Bromide
159	Salicylic Acid
159–160	Thenium Closilate
159–160	Warfarin
159.2–160.7	Atorvastatin Lactone
160	Amantadine
160	Amifostine
160–161	Azacyclonol
160–165	Benzethonium Chloride
160	Benzydamine Hydrochloride
160–162	Cadralazine
160	Cefalothin
160	Cetoxime Hydrochloride
160	Cilostazol
160	Clamoxyquin Hydrochloride
160–163	Didanosine
160–163	Ethylisobutrazine Hydrochloride
160–161	Ketorolac
160–162	Lamivudine
160–161	Metenolone
160–162	Mitoxantrone
160	Pempidine Tartrate
160d	Phenindamine Tartrate
160–161	Pipazetate Hydrochloride
160–161	Propiomazine Maleate
160–180	Sertraline Hydrochloride
160–162	Toremifene Citrate
160–163	Vinbarbital
160–163	Zonisamide
161	Amiodarone Hydrochloride
161	Methanearsonic Acid
161–162	Butaperazine Phosphate
161–163d	Dimethoxanate Hydrochloride
161	Dofetilide
161–166	Etofylline
161–167	Hexachlorophene
161–169	Rocuronium Bromide
162–164	Atomoxetine Hydrochloride
162–166	Cyclomethycaine Sulfate
162–167d	Demecarium Bromide
162	Ergometrine
162–164	Etonitazene Hydrochloride
162–166	Gabapentin
162–165	Hexoprenaline
162–168	Isoetarine Mesilate
162	Methapyrilene Hydrochloride
162–168	Methyltestosterone
162–166	Phenatine
162–163	Pholedrine
162–165d	Rolitetracycline
162–163	Voglibose
162–166	Zonisamide
163–164d	Amiphenazole
163	Atropine Methonitrate
163	Candesartan Cilexetil
163–164	Clorprenaline Hydrochloride
163–169	Dextropropoxyphene Hydrochloride
163–165	Epalrestat *N*-Methyl-D-Glucamine
163–164	Famotidine
163–164	Furanocoumarins
163–167	Methandienone
163	Norethisterone Acetate
163–164	Propranolol Hydrochloride
163–165	Spiraprilat
163–164	Torasemide
164	Aldosterone
164–166	Boldenone
164	Bunitrolol Hydrochloride
164–167	*dl*-Denopamine Hydrochloride
164–166	Dicycloverine Hydrochloride
164–167	Dipyridamole
164d	Econazole Nitrate
164–166	Ezetimibe
164–165	Guanoxan
164–166	Modafinil
164–166	Okadaic Acid
164–168	Phenelzine Sulfate
164–165	Pinacidil Monohydrate
164–165	Tolrestat
164–165	Tyramine
164–167	Xylazine Hydrochloride
164–165	Zearalenone
165	Abacavir
165d	Acetophenazine Dimaleate
165–171	Alfaxalone
165–168	Antazoline Mesilate
≈165	Betamethasone Acetate
165–168	Dimethyltryptamine Hydrochloride
165–170d	Edrophonium Chloride
165–167	Gabapentin
165–170d	Isoprenaline Hydrochloride
165–167	Leflunomide
165	Norandrostenedione
165–168	Oxiracetam
165–169	Procainamide Hydrochloride
165–166	Rotenone
165–168	Secbutabarbital
165–166	Stavudine
165	Sulfanilamide
165–168	Tricyclamol Chloride
166–170d	Aminopromazine Fumarate
166–168	Atomoxetine Hydrochloride
166	Benaprizine Hydrochloride
166–168	Clobazam
166–170	Delapril Hydrochloride
166–170	Denatonium Benzoate
166	Diphenhydramine Hydrochloride
166	Lansoprazole
166–168	Lorazepam
166–168	Mannitol
166–170	Phenazocine Hydrobromide
166–168	Quinaprilat (Diacid of Quinapril)
166	Sertindole
166.2	Itraconazole
167–170	Alfadolone
167	Benziodarone
167	Clemizole
167–171	Dydrogesterone
167d	Ergometrine Maleate
167–170	Morantel Tartrate
167–174	Naltrexone
167–169	Piperocaine Hydrochloride
167–168	Prilocaine Hydrochloride
167	Serotonin Hydrochloride
167	Sulfamethoxazole
167.5–168.5	Dioxaphetyl Butyrate Hydrochloride
167.5–168	Indinavir
168–169	Brallobarbital
168–169	Chlortetracycline
168d	Diethylpropion
168–169	Dimethylthiambutene Hydrochloride
168–172	Fenfluramine Hydrochloride
168–170	Isradipine
168	Naphazoline Nitrate
168–172	Phenglutarimide Hydrochloride
168–170	Rafoxanide
168–169	Xamoterol
≈169	Amydricaine Hydrochloride
169–171	Arecoline Hydrobromide
169–170	Bunamidine Hydroxynaphthoate
169–172	Diethylstilbestrol
169–179	Furanocoumarins
169–170	Glafenine
169.0–171.2	Hydrocortisone Sodium Succinate
169	Hydroquinidine
169–170	Indeloxazine
169–170	Ketorolac Tromethamine
169	Ochratoxin A
169–171	Oxymesterone
169.0–170.5	Paracetamol
169–171	Plasmocide Diphosphate
≈169–171	Protriptyline Hydrochloride
169–171	Sumatriptan
169.5–170	Tandospirone Citrate
170d	Amphotericin B
170–171.8	Benperidol
170–171	Bephenium Hydroxynaphthoate
170–179d	Betamethasone Dipropionate
170d	Cefdinir
170d	Cloxacillin Sodium
170–172	Dimenoxadole
170	Diphenhydramine Hydrochloride

°C	*Compound*
170	Flunitrazepam
170–174	Hexobendine Hydrochloride
170–173	Hydrocortisone Hydrogen Succinate
170–171	Hydroquinone
170–174	Isoniazid
170	Methylmercuric Chloride
170–175	Metamfetamine Hydrochloride
170–173	Methacholine Chloride
170–171	Metopimazine
170–171	Naratriptan
170d	Norbudrine Hydrochloride
170–171	Pipethanate Hydrochloride
170–171	Reboxetine
170d	Rifamide
170	Risperidone
170–172	Thenalidine Tartrate
170	Thenyldiamine Hydrochloride
170–173	Tolazamide
170–178	Triflupromazine Hydrochloride
171–174	Atrazine
171	Bacampicillin Hydrochloride
171	Boric Acid
171–174	Cyclobarbital
171–177	Methanthelinium Bromide
171d	Methoserpidine
171–172d	Morazone Hydrochloride
171–176d	Neostigmine Bromide
171–175	Okadaic Acid
171–173	Pindolol
171	Tramazoline Hydrochloride
171–172	Trometamol
172	Altretamine
172	Chloropyramine Hydrochloride
172–176	Coumatetralyl
172	Dipyrone
172–174	Glibenclamide
172–173	Hydralazine
172	Hydroquinine
172–184	Potassium Hydroxyquinoline Sulfate
172–174	Idarubicin Hydrochloride
172–174	Laudexium Methyl Sulfate
172d	Methylergometrine
172	Metomidate Hydrochloride
172–175	Mexazolam
172–174	Nifedipine
172–174	Nifekalant Hydrochloride
172–175.5	*L*-Norbolethone
172–180	Oxymetholone
172–173	Quetiapine Fumarate
172–174	Taurolin
173	Allobarbital
173–174	Camazepam
173	Diamorphine
173–179	Estradiol
173–174	Isomethadone Hydrochloride Monohydrate (*l*-Form)
173–174d	Pilocarpine Nitrate
173–176	Psilocin
173	Remoxipride Hydrochloride
173	Santonin
173–176	Thonzylamine Hydrochloride
174–177	Buformin Hydrochloride
174	Carbarsone
174–175.5	Clenbuterol Hydrochloride
174	Etamiphylline Camsilate
174	Furethidine
174	Heptabarb
174–175	Imipramine Hydrochloride
174–175	Isotretinoin
174	Ketorolac Tromethamine
174–175	Lacidipine
174–177	Levallorphan Tartrate
174–184	Noretynodrel
174–175	Normethadone Hydrochloride
174–178	Phenobarbital
174–175	Phentolamine
174	Phenylephrine
174–179	Pseudoephedrine Sulfate
174–175	Quinidine
174	Stavudine
174	Tolazoline Hydrochloride
174.5	Lovastatin
175–177	Alfadolone Acetate
175	Amisometradine
175–176	Amoxapine
175–178	Astemizole
175d	Citrinin
175	Cyclamic Acid
175–181	Dapsone
175	Dichlorophen
175	Diloxanide
175	Dimetotiazine Mesilate
175–176	Dyclonine Hydrochloride
175–179	Floctafenine
175–178	Hydroxyquinoline Sulfate
175–184	Iproniazid Phosphate
175	Muscimol
175–177	Naepaine Hydrochloride
175–177	Nefazodone
175–176	*D*-Norbolethone
175–180	Nordefrin Hydrochloride
175d	Penicillamine Hydrochloride
175–178	Phenformin Hydrochloride
175	Phenobarbital Sodium
175.0–176.5	Quinidine Gluconate
175–179	Syrosingopine
175.5–176	Flupirtine Maleate
176–180	Argatroban Monohydrate
176–185d	Carfenazine Maleate
176–178	Chloroprocaine Hydrochloride
176–177	Dieldrin
176–181	Dithranol
176	Fenticlor
176–177	Isomethadone Hydrochloride Monohydrate (*d*-Form)
176–177	Metaraminol Tartrate
176–177	Methcathinone Hydrochloride
176	Methylphenobarbital
176	Narceine
176d	Noscapine
176–177d	Phenglutarimide Hydrochloride
176	Sulfadimidine
176	Sulfametopyrazine
176d	Troleandomycin
177–180	Acipimox
177–181	Benactyzine Hydrochloride
177–178	Clemastine Fumarate
177–181	Dinitolmide
177–182	Ethyl Biscoumacetate
177–179	Ibomal
177–180d	Melphalan
177–178	Methoxymetamfetamine
177	Naloxone
177–181	Phentolamine Mesilate
177–179	Terbutylazine
178–179	Amlodipine Maleate
178–181	Benorilate
178	Brucine
178–179d	Clioquinol
178–181	Dimevamide Sulfate
178–179	Elcometrine
178	Gelsemine
178	Gliquidone
178–180	Mercuric Acetate
178d	Mesoridazine Benzenesulfonate
178–178.5	Nicomorphine
178–179	Pyrantel
178–180	Rizatriptan Benzoate
178–179	Sulfadimidine
178–180	Sulpiride
178	Trifluomeprazine Maleate
178–179d	Zomepirac
178	Zopiclone
178.5–179.5	Ondansetron Hydrochloride Dihydrate
179	Camphor
179–180d	Chlorpromazine Hydrochloride
179–182	Fluoxetine Oxalate
179–182	Nitroxoline
179–181	Nomifensine
179–180	Phenprocoumon
179–180	Pipenzolate Bromide
179–183	Sulfaphenazole
180–181	Cathine Hydrochloride
180	2C-T-2 Hydrochloride
180–184	Dextromoramide
180d	Dihydralazine
180d	Ergotamine Tartrate
180	Fenbufen
180–181	Irbesartan
≈180	Labetalol Hydrochloride
180–182	Levallorphan
180	Lobeline Hydrochloride
180–181	5-Methoxy-*N*, *N*-diisopropyltryptamine Hydrochloride
180	Milnacipran Hydrochloride
180–181	Molindone
180–185	Nalmefene Hydrochloride
180	Naloxone
180–182	Olmesartan Medoxomil
180d	Oxytetracycline Hydrochloride
180–181	Pecazine Hydrochloride
180	Pentamidine Isetionate
180d	Quinidine Polygalacturonate
180–184	Raltitrexed Monohydrate
180–182	Sultiame
180–183	Tertatolol
180–183	Tertatolol Hydrochloride
180	Tianeptine Sodium
180–181	Tramadol Hydrochloride
180	Xantinol Nicotinate
181	Androstanolone
181	Gliclazide
181–183	Letrozole
181	Mescaline Hydrochloride
181–184	Metabutethamine Hydrochloride
181–182	Norpipanone Hydrochloride
181–183	Oxymetazoline
181	Phenazocine
181–183	Pramocaine Hydrochloride
181d	Promazine Hydrochloride
182–187	Acetohexamide
182–184	Alphachloralose
182–183	Clobenzorex Hydrochloride
182–184	Ethinylestradiol
182–186	Etisazole Hydrochloride
182–184	Heteronium Bromide
182–184	Hexylcaine Hydrochloride
182–184	Methcathinone
182–189	Methotrexate
182	Miconazole Nitrate
182–187d	1,2-Naphthoquinone 2-Semicarbazone
182	Phenmetrazine Hydrochloride
182–183	Proxymetacaine Hydrochloride
182–184	Sulfacetamide
182–183	Sulfamethoxypyridazine
182–185	Sulindac
182–184	Tolpropamine Hydrochloride
182–183	Zeranol
182.5–184.0	Metoclopramide Hydrochloride
182.5–183.5	Pseudoephedrine Hydrochloride
≈183–184d	Betamethasone Valerate
183–186	Bezafibrate
183–185	Candesartan
183–184	Clozapine
183–184	Dimevamide
183	Eflornithine Hydrochloride
183	Eflornithine Hydrochloride Monohydrate
183–184	Etafedrine Hydrochloride
183–186	Eucatropine Hydrochloride
183–185	Idarubicin Hydrochloride
183–190	Iopydol
183	Ketazolam
183–187	Meptazinol Hydrochloride
183	Mescaline Sulfate Dihydrate
183–184	Pioglitazone
183–188d	Rifampicin
183–185	Sirolimus
183–184	Tacrine
183–186	Trenbolone
183.5–184.5	Losartan
184–186	Biriperone
184	Clopidogrel Bisulfate
184–186	Diethazine Hydrochloride
184–186	Dobutamine Hydrochloride
184–186	Doxepin Hydrochloride
184–185	Letrozole
184–189	Methaphenilene Hydrochloride
184–190	Methdilazine Hydrochloride

°C	*Compound*
184–185	Oxedrine
184–186	Succinylsulfathiazole
184–186	Troglitazone
185	Androsterone
185–186	Bamifylline Hydrochloride
185d	Epirubicin Hydrochloride
185–188	Hexestrol
185–195d	Methylergometrine Maleate
185	Norcodeine
185	Phenothiazine
185–187	Physostigmine Salicylate
185–186d	Pizotifen Malate
185–195d	Psilocybine
185–186	Rotenone
185–192	Choline Theophyllinate
185–186	Viloxazine Hydrochloride
186–187	Allylprodine Hydrochloride
186–189	Aminobenzoic Acid
186	4-Aminophenol
≈186	Butriptyline Hydrochloride
186	Citalopram Hydrobromide
186	Demecolcine
186–190	Dihydrocodeine Tartrate
186–189	Dirithromycin
186–192	Ethylpiperidyl Benzilate Hydrochloride
186	Mescaline Sulfate Dihydrate
186–188	Nifuratel
186–189	Pethidine Hydrochloride
186–188	Pregabalin
186–187	Propyliodone
186	Sulfaethidole
186–187d	Acetyl Sulfamethoxypyridazine
186–187	Zaleplon
187	Fluocortolone Pivalate
187–189	Sildenafil
187.5–190	Fluspirilene
188–191	Argatroban
188–192	Barbital
188–190	Clostebol
188–190d	Daunorubicin Hydrochloride
188–189	Doxepin Hydrochloride
188	Fenpipramide
188–190.5	Fluocortolone
188–190	Nafarelin Monoacetate
188–190	Nalmefene
188–190d	Oxedrine Tartrate
188–191	Perhexiline Maleate
188d	Profenamine Hibenzate
188–190d	Thiethylperazine Maleate
188–190	Trimethobenzamide Hydrochloride
189–190	Benoxaprofen
189–193	Carbamazepine
189	Desomorphine
189–192d	Dextromoramide Tartrate
189–189.5	DOM Hydrochloride
189–195	Ethoxzolamide
189–192	Hydroxyamfetamine Hydrobromide
189	Moracizine Hydrochloride
189	Moxonidine Hydrochloride
189–191	Noxiptiline Hydrochloride
189	Oxabolone
189–191	Pyrazinamide
189	Racephedrine Hydrochloride
189	Sulfasomizole
190–193	Acecainide Hydrochloride
190	Aluminium Chloride
190	Amantadine
190	4-Aminophenol
190–192d	Ascorbic Acid
≈190d	Atropine Sulfate
190	Barbital Sodium
190–192	Betaprodine Hydrochloride
190–194	Chlorzoxazone
190–193	Cinolazepam
190–191	Clofedanol Hydrochloride
190–192	Diprenorphine
190d	Ergotoxine
190–198	Estradiol Benzoate
190	Flurazepam Hydrochloride
190	Homatropine Methylbromide
≈190d	Levomepromazine Maleate
190	Levomoramide
190–198	Norbormide
190d	Ouabain
190	Oxyphenonium Bromide
190–191	Pentachlorophenol
190–192	Phenacaine Hydrochloride Monohydrate
190–194	Phenylpropanolamine Hydrochloride
190	Piperazine Citrate
190–194	Sulfadoxine
190–193	Sulfaguanidine Monohydrate
190–192	Sulfapyridine
190	Suxamethonium Chloride
191–193	Bicalutamide
191–197	Dexrazoxane
191–194	Sulfafurazole
191.5–193	Fluquinconazole
192–194d	Ambazone
192–193	Atropine Oxide Hydrochloride
192–196d	Bromocriptine Mesilate
192–196	Butethamine Hydrochloride
192	Clomipramine Hydrochloride
192–193	Dihydrocodeine Tartrate
192–194	Etebenecid
192	Fencamfamin Hydrochloride
192–195	Glibornuride
192d	Ketotifen Fumarate
192–193	Mestanolone
192–193	Narceine Hydrochloride
192–193	Norpipanone Hydrobromide
192–193	Perospirone Hydrochloride
192–194d	Spirapril Hydrochloride
192–195	Succinylsulfathiazole
192	Tiagabine Hydrochloride
192–193	Tripelennamine Hydrochloride
193	Acetorphine
193–195	Chloroquine Phosphate
193–197	Dantron
193	Fusidic Acid
193–198	Glycopyrronium Bromide
193	Hydroxyzine Hydrochloride
193–196	Metaclazepam Hydrochloride
193–194	Pioglitazone Hydrochloride
193–195d	Ritodrine Hydrochloride
193–194	Acetyl Sulfafurazole
193	Sulfamoxole
193	Thebaine
193–194	Tiletamine Hydrochloride
193	Zimeldine Hydrochloride
193–197	Zolpidem Tartrate
194–198	Antazoline Phosphate
194	Apronal
194–196	Chlorpromazine Hydrochloride
194–195	Diphemanil Metilsulfate
194	Fluvastatin Sodium
194–199	Hyoscine Methonitrate
>194	*d*-Mabuterol Hydrochloride
>194	*l*-Mabuterol Hydrochloride
194–196	Methysergide
194–196	Probenecid
195	Adamsite
195d	Ajmaline
195d	Aminohippuric Acid
195d	Apomorphine
195–196	Dimethylarsinic Acid
195d	Benzoylecgonine
195–197	Bethanidine
195	Butaperazine Maleate
195d	Cocaine Hydrochloride
195	Dimetridazole Hydrochloride
195d	Halazone
195	Hyoscine Hydrobromide
195–198	Lucanthone Hydrochloride
≈195	Nomifensine Maleate
195	Olanzapine
195–196	Piperidolate Hydrochloride
195–197d	Propicillin Potassium
195–198	Terbinafine Hydrochloride
195.5–197	Clobetasol Propionate
196–199	Acenocoumarol
196–197	Amitriptyline Hydrochloride
196	Broxyquinoline
196–197.5d	Carbazochrome Salicylate
196–206d	Codergocrine Mesilate
196–197	Dimethocaine Hydrochloride
196–197	Ethomoxane Hydrochloride
196	Histapyrrodine Hydrochloride
196–208d	Isoetarine Hydrochloride
196–197d	Meticillin Sodium
196	Morinamide Hydrochloride
196	Phthalylsulfacetamide
≈196–197	Pipamazine Hydrochloride
196–200d	Thiamine Mononitrate
196–202	Tridihexethyl Chloride
196	Zolpidem
197–199	Brevetoxin A
197–200d	Celiprolol Hydrochloride
197	Cotarnine Chloride
197–198	Ebastine Fumarate
197–198	Hexoprenaline Hydrochloride
197	Hordenine Sulfate
197–198d	Monocrotaline
197–198	Primaquine Phosphate
198–206	Desoxycortone Pivalate
198–199	Dextrorphan
198d	Dimoxyline Phosphate
198	Diperodon Hydrochloride
198d	Ecgonine
198	Hydrocodone
198	Hydroxychloroquine Sulfate
198–201d	Isopropamide Iodide
198–199	Levorphanol
198–199	Mazindol
198–200d	Metallibure
198–204	Mexiletine Hydrochloride
198–199	Myrophine Hydrochloride
198	Phentermine Hydrochloride
198–200	Piroxicam
198–199	Sulfadimidine
198	Triflumuron
198.5–200.3	Ethambutol Hydrochloride
199–201	Ethylmorphine
199–200d	Ethylnoradrenaline Hydrochloride
199–202	Formestane
199	Norharman
199–200	Octaverine Hydrochloride
199–203	Trimethoprim
200d	Aconitine Nitrate
200	Ambenonium Chloride
200–204	Amicarbalide Isetionate
200d	Aminonitrothiazole
200	Ampicillin
200d	Betamethasone Acetate
200–204d	Carbachol
200–215d	Diiodohydroxyquinoline
200d	Doxycycline Hydrochloride
200–202d	Sodium Fluoroacetate
200–210	Hexocyclium Metilsulfate
200d	Isoxsuprine Hydrochloride
200d	Lymecycline
200–205	Naloxone Hydrochloride
≈200	Noscapine Hydrochloride
200–202	Phenoperidine Hydrochloride
200–205	Pyrrocaine Hydrochloride
200d	Strychnine Sulfate
201–204d	Amikacin
201–204	Cyclazocine
201–202	Diphenylpyraline Hydrobromide
201–203	Flumazenil
201–202	Ketobemidone Hydrochloride
201–208	Norethisterone
201–204	Sulfadimethoxine
201	Trifluperidol Hydrochloride
201–202	Tropisetron
201.3	Drospirenone
201.5–202.5	Buspirone Hydrochloride
201.5–202.5	Timolol Maleate
201.8–202.6	Ethambutol Hydrochloride
202–205d	Etidocaine Hydrochloride
202–203d	Liothyronine Hydrochloride
202–206	Penicillamine
202	Sulfathiazole
203–204	Benzilonium Bromide
203–205d	Carbidopa
203d	Dihydroergotamine Tartrate
203–204	Halofantrine Hydrochloride
203d	Hyoscyamine Sulfate
203–205	Mitoxantrone Hydrochloride
203d	Pentolonium Tartrate
203	Picrotoxin
203–204	Rimiterol
203.5–206	Formestane

°C	Compound
204	Acetorphine Hydrochloride
204d	Aconitine
204d	Aminocaproic Acid
204–210	Arsenobetaine
204–205	Cefalothin Sodium
204–209	Diphenylpyraline Hydrochloride
204–205d	Doxorubicin Hydrochloride
204	Emepronium Bromide
204	Medroxyprogesterone Acetate
204	Niflumic Acid
204–209.5	Pentostatin
204–205	Pilocarpine Hydrochloride
205d	Ampicillin Sodium
205–8d	Betamethasone Acetate
205–210	Chloroquine Sulfate
205–207	Diphenazoline Hydrochloride
≈205d	Disopyramide Phosphate
205	Ecgonine
205–208	Epinastine
205–208	Fludrocortisone Acetate
205	Furaltadone
205	Glipizide
205–206	*dl*-Mabuterol Hydrochloride
205d	Methacycline Hydrochloride
205–208	Methylprednisolone Acetate
205	Orciprenaline Sulfate
205–206	Oxazepam
205d	Prednisolone Succinate
205–212d	Pyridoxine Hydrochloride
205–207	Sulfadimidine
205–213	Tenoxicam
205.5–206.5	Methandriol
206–207	Epithiazide
206d	Furosemide
206	Guanoxan Sulfate
206–208	Levorphanol Tartrate
≈206d	Nalorphine Hydrobromide
206–208.5	Norgestrel
206–209	Pergolide
206–207d	Sotalol Hydrochloride
206–210d	Thiazinamium Metilsulfate
207–209	Amidefrine Mesilate
207	Baclofen
207	Cinchonidine Sulfate
207–210	Eptazocine Hydrobromide
207	Glimepiride
207–208	Methylephedrine Hydrochloride
207–209	Pipoxolan Hydrochloride
207d	Quinidine Sulfate
207	Sertraline
207	Sotalol
207	Tolfenamic Acid
207.5–212.0	Diltiazem Hydrochloride
208–210	Albendazole
208	Amodiaquine
208–212	Chlormadinone Acetate
208–209	Daunorubicin
208–210	Endosulfan
208	Guanoclor Sulfate
208	Moclobemide Hydrochloride
208–210	Moxisylyte Hydrochloride
208	Nalorphine
208–209	Ochratoxin B
208	Sulfamethizole
209–210	Bialamicol Hydrochloride
209	Buprenorphine
209–212	Formebolone
209–211	Hydroxyephedrine Hydrochloride
209–211	Levobunolol Hydrochloride
209–211d	Lormetazepam
209–211	Oxyclozanide
209	Vigabatrin
210	Bunamidine Hydrochloride
210d	Chlortetracycline Hydrochloride
210d	Cinchonidine
210–212	Clofazimine
210–217	Epalrestat
210–214	Hydrocortisone Hydrogen Succinate
210–214	Levosimendan
210	Mesterolone
210	Levonordefrin ((−)-isomer)
≈210d	Opipramol Hydrochloride
210–215	Pyrvinium Embonate
211–215d	Azapetine Phosphate
211	Bethanechol Chloride
≈211	Coniine Hydrobromide
211–216	Vinblastine
≈212d	Adrenaline
212–215	Azamethonium Bromide
212d	Beclometasone Dipropionate
212–214	Brotizolam
212–213	Cytarabine
212	Diflunisal
212–214d	Ergotamine
212d	Hydrastinine Hydrochloride
212	Indoprofen
212d	Isothipendyl Hydrochloride
212–214	Lachesine Chloride
212–216	Methoxamine Hydrochloride
212–218d	Methylpiperidyl Benzilate Hydrochloride
212–213	Naftopidil Dihydrochloride
212–215d	Nordefrin
212–216	Phenacemide
212	Temozolomide
213d	Chlordiazepoxide Hydrochloride
213–216	Cinchophen
213d	Dimefline Hydrochloride
213	Stannous Fluoride
213	Methazolamide
213	Nortriptyline Hydrochloride
213	Propazine
213–218	Topotecan Hydrochloride
214–217d	Benzylpenicillin Potassium
214–216d	Cyproheptadine Hydrochloride
214–221d	Difenidol Hydrochloride
214–217	Etorphine
214–215	Flupirtine Hydrochloride
214d	Hexoprenaline Sulfate
214–217d	Homatropine Hydrobromide
214d	Hydrocortisone
214–216	Imidapril Monohydrochloride
214–215	Loprazolam
214–225d	Nifursol
214–218	Phencyclidine Hydrobromide
214–218	Pimozide
214d	Polythiazide
214–216	Sulfametoxydiazine
214d	Tetracycline Hydrochloride
215–218d	Bromocriptine
215–217	Butorphanol
215	Chloroquine Phosphate
215d	Cinchonine Hydrochloride
215–216	Clioxanide
215–219	Cyclobenzaprine Hydrochloride
215–216	Desipramine Hydrochloride
215	Fazadinium Bromide
215	Levomethadyl Acetate Hydrochloride
215–217d	Mephentermine Sulfate
215	Nortriptyline Hydrochloride
215–216	Oxcarbazepine
215	Pancuronium Bromide
215–216	Phenamidine
215–230	Ramosetron Hydrochloride
215–217	Trimetrexate
215–217	Tymazoline Hydrochloride
215–217	Venlafaxine Hydrochloride
215–217	Zalcitabine
216–219	Atovaquone
216–217	Fenpiprane Hydrochloride
216–218	Lamotrigine
216	Methyclothiazide
216	Metixene Hydrochloride
216–217	Noracymethadol Hydrochloride
216	Nordazepam
216–217d	Paclitaxel
216.5–218d	Noradrenaline
217–221	Acetyldigitoxin
217–219d	Butorphanol Tartrate
217–220	Clarithromycin
217–224d	Cortisone
217–225	Cyclothiazide
217–218	Diacerein
217d	Diminazene Aceturate
217	Domoic Acid Dihydrate
217	Ephedrine Hydrochloride
217–224	Griseofulvin
217–218	Isomethadone Hydrobromide (*l*-Form)
217–224d	Meclozine Hydrochloride
217	Megestrol Acetate
217	Mizolastine
217–219	Moxonidine
217	Nordazepam
218–220	Alphaprodine Hydrochloride
218d	Sodium Ascorbate
218	Cantharidin
218–225	Cefixime Trihydrate
218	Chloroquine Phosphate
218–221	Dosulepin Hydrochloride
218–237	Gentamicin Sulfate
218–220	Mometasone Furoate
218–220	Oxycodone
218	Pentazocine Hydrochloride
218–220	Plasmocide Dihydrochloride
218	Sertraline Hydrochloride
218–219d	Solanidine
218–220	Tiotropium Bromide
218	Vardenafil Hydrochloride Trihydrate
218–220	Vincristine
219–228	Cicletanine Hydrochloride
219–220	Cloricromen Hydrochloride
219–221	Propylthiouracil
219–220	Trimethoxyamfetamine
219.3	Fasudil Hydrochloride
220	Alcuronium Chloride
220	Amygdalin
220d	Bendroflumethiazide
220d	Chlortalidone
220	Cladribine
220	Deslanoside
220	Doxapram Hydrochloride
220–224	Enoxacin
220	Ephedrine Hydrochloride
220	Flurazepam Hydrochloride
220d	Hydrocortisone Acetate
220–225	Papaverine Hydrochloride
220–225	Pentostatin
220d	Rimiterol Hydrobromide
220.5–222	Diphenoxylate Hydrochloride
221	Chlorprothixene Hydrochloride
221–226	Glymidine Sodium
221–223	Lofexidine Hydrochloride
221	Ochratoxin B
221–223	Tizanidine
222	Acrivastine
222–223	Atropine Methobromide
222d	Carbazochrome
222–225	Clarithromycin
222–223	Hydroxyprogesterone
222–223	Irinotecan
222–224	Norlevorphanol Hydrobromide
223–226	Buphenine Hydrochloride
223–226d	Procarbazine Hydrochloride
223–225d	Profenamine Hydrochloride
223–229d	Thiopropazate Hydrochloride
223–225	Tirofiban
223.5–225	Tymazoline Hydrochloride
224	Donepezil Hydrochloride
224–226	Methylphenidate Hydrochloride
224	Nitrazepam
224–225	Phenadoxone Hydrochloride
224.4–226.8d	Danazol
225	Alfuzosin Hydrochloride
≈225–228d	Betamethasone Benzoate
225	Cetirizine Dihydrochloride
225d	Dexamethasone Acetate
225d	α-Acetyldigoxin
225d	Hyoscine Methobromide
225–227d	Levofloxacin Hemihydrate
225d	Loperamide Hydrochloride
225–230	Lornoxicam
225	Metformin Hydrochloride
225d	Methylone
225d	Minoxidil
225–230	Niclosamide
225d	Obidoxime Chloride
225–227	Levofloxacin (Ofloxacin *S*-(−)-form)
225–227d	Parbendazole
225	Pergolide Mesilate
225d	Phenamidine Isethionate
225–226d	Pralidoxime Iodide
225	Suxamethonium Bromide

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°C	*Compound*
225–230d	Thioacetazone
225–228	Trimetazidine Hydrochloride
226–230	Amineptine Hydrochloride
226–227	Chlorcyclizine Hydrochloride
226–228	Delavirdine
226	Dibrompropamidine Isetionate
226	Ethacridine
226–228	Flosequinan
226–228	Fludrocortisone Acetate
226–228	Flunixin
226–228	Methylone
226	Nitrazepam
226	Phenylmethylbarbituric Acid
226–232d	Prednisone Acetate
226–227d	Procyclidine Hydrochloride
226–227	Simazine
227–228	Androstanediol
227d	Aztreonam
227–228d	Carbazochrome Sodium Sulfonate
227–234	Dienestrol
227	Metildigoxin
227–229	Fenetylline Hydrochloride
227–235	Ketanserin
227–229	Levamisole Hydrochloride
227–231	Metildigoxin
227	Metolazone
227–229	Tandospirone Hydrochloride
227	Thioproperazine Mesilate
228–230	Acitretin
228–228.5	Alprazolam
228–229d	Ambutonium Bromide
228	Azapropazone
228–220	Brevetoxin A
228–230	Clostebol Acetate
228–229d	Mepenzolate Bromide
228–237	Methylprednisolone
228–241d	Paramethasone Acetate
228	Prochlorperazine Maleate
228–230	Saccharin
228–230	Tamsulosin Hydrochloride
229–231	Doxorubicin
229–230	Nalidixic Acid
229–230	Resorantel
>230	Apraclonidine
230d	Betaine Hydrochloride
230–240	Buclizine Hydrochloride
230	Bumetanide
230–234d	Deserpidine
230d	Fenoterol Hydrobromide
230–236	Flavoxate Hydrochloride
230–240	Flupentixol Hydrochloride
230	Fluphenazine Hydrochloride
230	Glyphosate
230–232	Indoramin Hydrochloride
230–232	Ipratropium Bromide
230–232	Lofexidine Hydrochloride
230–232	Maprotiline Hydrochloride
230–231	Mefenamic Acid
230–232d	Pheneticillin Potassium
230–232d	Promethazine Hydrochloride
230	Trioxysalen
230–232	Vindesine
231–232	Benzthiazide
231–234	Cycloguanil Embonate
231	Metildigoxin
231–232	Isomethadone Hydrochloride (*d*-Form)
231–233	Isomethadone Hydrochloride (*l*-Form)
≈231	Nalbuphine
231–232	Ondansetron
231–232d	Oxyphencyclimine Hydrochloride
232	Arsanilic Acid
232–247	Cetrimide
232	Docetaxel
232–235d	Droxidopa
232	Glaucine Hydrochloride Trihydrate
232–235	Metazocine
232–237	Piperoxan Hydrochloride (*dl*-Form)
232–233	Salinazid
233–235d	Alendronic Acid
233–234	Bupropion Hydrochloride
233–235	Dexamethasone Sodium Phosphate
233	Diaveridine
233d	Dihydralazine Sulfate
233d	Fenbendazole
233–234	Fludrocortisone Acetate
233–235	Phencyclidine Hydrochloride
233–236	Prednisolone Pivalate
233–235d	Prednisone
233	Pyrimethamine
233–235	Triazolam
234	Adenosine
234–239	Caffeine Hydrate
234	Chlorphentermine Hydrochloride
234	Clonixin
234–235	Colfosceril Palmitate
234	Cyprenorphine
234–237	Nicotinic Acid
234–236	Quinagolide Hydrochloride
234–238d	Sulfamerazine
234–235	Tigloidine Hydrobromide
234	Yohimbine
235	Alfuzosin Hydrochloride
235	Aminoacridine Hydrochloride
235	Bromhexine Hydrochloride
235–238d	Cortisone Acetate
235d	Cyclopenthiazide
235–255d	Emetine Hydrochloride
235d	Gallamine Triethiodide
235	Glaucine Hydrobromide
235	Hydroxystilbamidine
235–236d	Levothyroxine
235	Methadone Hydrochloride
235–238	Oxandrolone
235d	Phenazopyridine Hydrochloride
235–238d	Pralidoxime Chloride
235	Propamidine Isetionate
≈235	Stanozolol
235.5	Adrenalone
236	Amiphenazole Hydrochloride
236–236.5	Chlordiazepoxide
236–251	Etoposide
236–237d	Liothyronine
236–240d	Nitrofurazone
236.5–238.5	Clonazepam
237–240	Aflatoxin G2
237–241	Antazoline Hydrochloride
237–238.5d	Bromazepam
237–239	Fenetylline Hydrochloride
237–238	Lauroscholtzine
237–239	Naratriptan Hydrochloride
237–239d	Prednisolone Acetate
237d	Quinuronium Sulfate
238	Allantoin
238d	Azathioprine
238	Biperiden Hydrochloride
238	Caffeine
238–240d	Cambendazole
238	Dibenzepin
238	Harman
238–242	Moxifloxacin
≈238–242	Nefopam Hydrochloride
238–239	Rescinnamine
239–237	2C-B Hydrochloride
239	Dihydroergotamine
239–241	Fendosal
239–241	Imidaprilat
239–241	Morantel
239–240	Phentolamine Hydrochloride
240d	Acetarsone
240	Ametazole Hydrochloride
240–242	Amiloride
≈240d	Betamethasone
240–243	Diamthazole Hydrochloride
240	Diclofenamide
240d	Digoxin
240	Hydroxychloroquine Sulfate
240d	Lanatoside C
240d	Lysergic Acid
240	Olsalazine Sodium
240–241d	Prednisolone
240–242	Pyrimethamine
240–245d	Sulfasalazine
240	Venlafaxine Hydrochloride
241	Aminoacridine
241d	Dopamine Hydrochloride
241d	Mannomustine
241–242d	Sodium Cromoglicate
241.5–243d	Dactinomycin
242	Clidinium Bromide
242d	Cycrimine Hydrochloride
242	Decoquinate
242–250	Dutasteride
242–245	Fluocortolone Hexanoate
242–245d	Loprazolam Mesilate
242	Oxyphenisatine Acetate
242	Prochlorperazine Mesilate
242–243	Trifluoperazine
243	Adrenalone Hydrochloride
243–244	Diamorphine Hydrochloride
243–245	Metopon
243–244	Proguanil Hydrochloride
243–250	Ropinirole Hydrochloride
243	Sertraline Hydrochloride
243	Sulfisomidine
243–245	Suxamethonium Iodide
243–253d	Triclobisonium Chloride
243d	Triphenyltetrazolium Chloride
244–246	Aflatoxin G1
244–245	Isometamidium Hydrochloride
244–246	Naproxen Sodium
244–245	Pranlukast
245d	Endrin
245d	Ephedrine Sulfate
245d	Ethacridine Lactate
245d	Fluclorolone Acetonide
245d	Histamine Hydrochloride
245d	Homidium Bromide
245d	Mecamylamine Hydrochloride
245	Sertraline Hydrochloride
245d	Tocainide Hydrochloride
245d	Trimetaphan Camsilate
246–248	Capreomycin
246–247	Chlorproguanil Hydrochloride
246	Clemizole Hydrochloride
246	Clopamide
246	Ecgonine Hydrochloride
246–247	Galantamine Hydrobromide
246–248	Terbutaline Sulfate
247d	Amprolium Hydrochloride
247–248	Azapropazone
247–255	Fludroxycortide
247–249	Nevirapine
247–251d	Picloxydine
247–248d	Sulfaquinoxaline
248d	Barbituric Acid
248d	Dithiazanine Iodide
248–250	Meclofenamic Acid
248–250d	Mepacrine Hydrochloride
248d	Metisazone
248–249d	Oxymorphone
248–253d	Pirenzepine Hydrochloride
248d	Thiamine Hydrochloride
248	Tryptamine Hydrochloride
249–251	Clodronic Acid
249–250d	Harmaline
249–251d	Pyrvinium Chloride
249–250	Reproterol Hydrochloride
>250	Cefixime Disodium Salt
250–252	Dexamethasone Isonicotinate
250d	Ganciclovir
250d	Guanethidine Monosulfate
250d	Morphine Sulfate
250–257	Ofloxacin
250–252	Quinethazone
251–253	Racemorphan
251	Salinazid
252–254	Finasteride
252	Viomycin Sulfate
253–255	Capreomycin
253	Disodium Tiludronate
254–255	α-Amanitin
254–255	Balsalazide
254–256	Bufylline
254–255	Cyprenorphine Hydrochloride
254	Meloxicam
254–256d	Morphine
254	Pentazocine Hydrochloride
254–256	Tamsulosin
254.3–254.9	Inositol Nicotinate
255d	Aciclovir

°C	Compound
255–257d	Ciprofloxacin
255–256.5	Deflazacort
255–265d	Dihydrostreptomycin Sulfate
255–258d	Enoximone
255–257	Levobupivacaine Hydrochloride
255–265	Methaqualone Hydrochloride
255–260	Naphazoline Hydrochloride
255d	Sulfadiazine
255–256	Triclocarban
256	Digitoxin
256–257	Galantamine Hydrochloride
256–257d	Pemoline
256–257	Piperazine Adipate
256–262d	Protoveratrines A and B
256–257d	Tetryzoline Hydrochloride
256.5	Irinotecan Hydrochloride Trihydrate
≈257d	Clopenthixol Hydrochloride
257	Finasteride
257–259	Meclofenamic Acid
257–267d	Metocurine Iodide
258–265d	Chlorisondamine Chloride
258	Memantine Hydrochloride
258–259	(+)-Norlevorphanol
258–260d	Pergolide Mesilate
258–262	Phenolphthalein
258	Raloxifene Hydrochloride
258–270d	Trilostane
258.5	Bupivacaine Hydrochloride
≈258.5d	Trihexyphenidyl Hydrochloride
259d	Furazolidone
260	Acefylline Piperazine
260d	Acetazolamide
260–262d	Chlorhexidine Hydrochloride
260d	Cidofovir
260	Disulfamide
260d	Estrone
260	Fludarabine
260	Fludrocortisone
260	Mafenide Hydrochloride
260d	Mebezonium Iodide
260–263	Nalorphine Hydrochloride
260–264	Niridazole
260–262	Ropivacaine Hydrochloride
260	Teicoplanin
260d	Xipamide
261d	Harmine
261–263d	Pizotifen
261–263	Telmisartan
262–263	Ketamine
262–264	Mepivacaine Hydrochloride
263	Lorcainide Hydrochloride
263	Methenamine
264–265d	Aminitrozole
264	Cinchonine
264–265d	Halcinonide
264–266d	Morpheridine Dihydrochloride
264d	Prazosin Hydrochloride
264–265d	Reserpine
264–265	Tetramisole Hydrochloride
265d	Decamethonium Bromide
266–268	Clorexolone
266–267	Etorphine Hydrochloride
266–269	Flugestone Acetate
266–267	Hydromorphone
266d	Quinapyramine Dimethosulfate
267–269d	Protoveratrine A and B
268–269	Aflatoxin B1
268–269	Aflatoxin B2
268–271d	Dexamethasone
269–270	Dezocine Hydrobromide
269–270	Ethiazide
269–271	Thalidomide
269–271	Triamcinolone
269	Tyramine Hydrochloride
269.5–270.6	Ropivacaine Hydrochloride
270	Acamprosate Calcium
270d	Anileridine Hydrochloride
270–281	Azacyclonol Hydrochloride
270d	Brevetoxin B
270d	Cisplatin
270	Exifone
270d	Fluocinolone Acetonide
270d	Fluoxymesterone
270–272	Norlevorphanol
270–272d	Oxycodone Hydrochloride
270d	Potassium Sorbate
270–274	Theophylline
270d	Trichlormethiazide
270d	Tubocurarine Chloride
271	Clospipramine Dihydrochloride
271–273	Deferiprone
271	Nitrofurantoin
271–274	Terazosin Hydrochloride Dihydrate
272–276	Ethisterone
272–273	Fluticasone Propionate
272–273	Hydroflumethiazide
272–275	Penciclovir
272–277d	Phthalylsulfathiazole
272–274	Terazosin
272	Theophylline Hydrate
273	Bunazosin Hydrochloride
273–275	Epinastine Hydrochloride
273d	Hydralazine Hydrochloride
273–275	Hydrochlorothiazide
273–274	Hydroquinidine Hydrochloride
273–277	Normorphine
274d	Benzamine Hydrochloride
274d	Debrisoquine Sulfate
274	Fenoldopam Methanesulfonate
274–276	Hexamethonium Bromide
274–276	Naltrexone Hydrochloride
274–278d	Picloxydine Dihydrochloride
275	Arsenic Trioxide
275d	Levodopa
275	Morphine N-oxide
275–277	Penciclovir
275–285	Strychnine
276	Hydroxyprogesterone
277	Fenoldopam Hydrobromide
277	Mercuric (II) Chloride
277	Vincristine Sulfate
278	Carteolol Hydrochloride
278	Dolasetron Methanesulfonate
278–280	Prazosin
278–282d	Riboflavin
278–279	Terazosin Hydrochloride
279–280	Dantrolene
>280	Anagrelide Hydrochloride
280	Bethanidine Sulfate
280d	Codeine Hydrochloride
280d	Dequalinium Acetate
280	Estriol
280d	Fluorometholone
280	Lead Acetate
280d	Mebhydrolin Napadisilate
280–282	Phallotoxin
280	Viomycin Sulfate
281–282	Primidone
282d	Fluorouracil
282–284	Mianserin Hydrochloride
282	Stilbazium Iodide
283–285	Diclofenac Sodium
283–285	Dorzolamide Hydrochloride
283d	Neopine Hydrobromide
283–284	Tacrine Hydrochloride
283	Tropacocaine Hydrochloride
283–285	Tropisetron Hydrochloride
284–286d	Bromfenac Sodium
284–286	Epinastine Hydrobromide
284–285	Vinblastine Sulfate
285–288d	Amiloride Hydrochloride
285d	Cyclizine Hydrochloride
285d	Solanine
286	Hydroxystilbamidine Isetionate
287–292	*N*-Benzylpiperazine Dihydrochloride
287–293	Dicoumarol
287–292	Gemcitabine Hydrochloride
287–288	Methyl Benzoquate
288–290	Rebamipide
289–290	Doxazosin Hydrochloride
289–291	Meclofenamate Sodium
289	5-Methyltryptamine Hydrochloride
289–292	Phenomorphan Hydrobromide
290–292	Granisetron Hydrochloride
290	Mebendazole
291–294	Carbenoxolone
292	5-Methyltryptamine Hydrochloride
292–294	Triamcinolone Acetonide
293d	Aflatoxins M
295–297d	Flucytosine
295d	Phanquinone
295–298	Phenytoin
295–296d	Triamcinolone Hexacetonide
295–296	Zomepirac Sodium
296	Enoxolone
296–301	Pramipexole Dihydrochloride Monohydrate
298–300d	Nedocromil
299d	Aflatoxin M_1
299–300d	Ibogaine Hydrochloride
>300d	Adenosine
300	Aluminium Hydroxide
300	β-Amanitin
300d	Amfetamine Sulfate
>300	Dexamfetamine Sulfate
>300	Ecabet Sodium
300d	Fluocinonide
<300	Milrinone
300–303d	Oxymetazoline Hydrochloride
300	Rifamycin SV
300–305	Tiabendazole
300d	Xylometazoline Hydrochloride
302–303	Tadalafil
302d	Yohimbine Hydrochloride
305	Clonidine Hydrochloride
305–315d	Hydromorphone Hydrochloride
305–306	(–)-Rebamipide
305–306	(+)-Rebamipide
308d	Mercaptopurine
308–309	Pipradrol Hydrochloride
310d	Betaine
310	Methyldopa
310d	Quinapyramine Dichloride
312–313d	Quinapyramine Diiodide
312.3	Arsenic Trioxide
313	Arsenic Trioxide
315d	Arsenic Pentoxide
315d	Dequalinium Chloride
315–318d	Metopon Hydrochloride
316	Triamterene
318–320	Ciprofloxacin Hydrochloride
318–326d	Iopydone
320–322	Benzoctamine Hydrochloride
320	Clopidol
320–323d	Pholedrine Sulfate
321	Cadmium
321–322	Rhein
324–325d	Moxifloxacin Hydrochloride
326–331d	Methylthiouracil
327d	Pseudomorphine
327.4	Lead
329	Octatropine Methylbromide
330–331	Diazoxide
335	Diquat Dibromide
340d	Chlorothiazide
340d	Conessine Hydrobromide
>350	Balsalazide Disodium Dihydrate
>355	Disodium Methanearsonate
360d	Amantadine Hydrochloride
>360	Tioguanine
370d	Imidocarb
373–375	Rimantadine Hydrochloride
380	Antimony Pentoxide
405	Beryllium Chloride
440	Sodium Salicylate
<500d	Cadmium Carbonate
501	Lead Chloride
550	Antimony Trisulfide
555	Beryllium Fluoride
568	Cadmium Chloride
630.5	Antimony
656	Antimony Trioxide
660	Aluminium
710	Barium
720	Lithium Carbonate
730	Calcium Bromide
730	Potassium Bromide
755	Sodium Bromide
817	Arsenic
844	Lead Chromate
886	Lead Oxide
963	Barium Chloride

°C	*Compound*
993	Sodium Fluoride
1000	Cadmium Sulfate
1000	Cryolite
1083	Copper
1114	Lead Sulfide
1170	Lead Sulfate
1200	Barium Oxide
1238	Gallium Arsenide
1287–1292	Beryllium
>1460	Aluminium Phosphate
1600d	Barium Sulfate
1750	Cadmium Sulfide
2000	Aluminium Oxide
2508–2547	Beryllium Oxide

8 Colour Tests

The following table was extracted from a collection of data kindly provided by Professor George Maylin, Cornell University, USA, and gives compound-specific colour reactions from a series of spray reagents applied to thin-layer chromatography systems TA, TE, TAJ, TAK, TAL and TAM.

Name	*D*	*N*	*C-H*	*M*	*FES*	*NIN*	*Gibb's*	*H+:EtOH*	*ME*	*F*	*I_2*
Acepromazine	orange	brown	brown	pink	pink	no rxn	–	–	–	–	–
Acetylaminobenzoic acid, 4-	no rxn	no rxn	no rxn	yellow fl	–	no rxn	–	–	red	–	wk brown
Adenylic acid, 5-	–	–	–	–	no rxn	no rxn	–	–	no rxn	no rxn	no rxn
Alphaprodine	orange	brown	brown	–	–	–	–	–	–	–	brown
Alphaprodine *N*-oxide	orange	brown	brown	–	–	–	–	–	–	–	brown
Ambroxol	wk orange	brown	brown	yellow	no rxn	pink	–	–	red	–	brown
Amcinonide	–	–	–	–	–	–	–	wk yellow	–	–	–
Amfenac	–	–	–	yellow	–	–	–	–	–	–	–
Amfetamine	wk orange	brown	brown	no rxn	no rxn	pink	–	–	–	–	–
Aminocaproic acid	–	–	–	–	–	pink	–	–	–	–	–
Aminopropiophenone, 2-	wk orange	brown	brown	–	–	pink	–	–	–	–	–
Amitriptyline	–	brown	–	–	–	–	–	–	–	–	–
Ampyrone	orange	brown	brown	–	–	wk pink	–	–	red	–	brown
Amrinone	no rxn	brown	brown	no rxn	–	no rxn	–	–	red	blue	–
Anileridine	orange	brown	brown	–	–	–	–	–	–	–	brown
Apomorphine	wk orange	brown	brown	–	–	–	–	–	–	–	green
Aspirin	–	–	–	green	–	–	–	–	–	–	–
Atropine	orange	brown	brown	–	–	–	–	–	–	–	–
Azaperol	–	brown	brown	–	–	–	–	–	–	–	–
Azaperone	–	brown	brown	–	–	–	–	–	–	–	–
Benoxaprofen	no rxn	brown	brown	fluoresce	fluoresce	no rxn	–	fluoresces	no rxn	no rxn	–
Benzoic acid	–	–	–	white	–	–	–	–	–	–	–
Benzoylecgonine	orange	brown	brown	–	–	–	–	–	–	–	brown
Benzthiazide	no rxn	wk brown	wk brown	yellow fl	brown	no rxn	no rxn	brown	no rxn	orange	–
Betamethasone	–	–	–	–	–	–	–	grey	–	–	–
Bolasterone	–	–	–	–	–	–	–	yellow	–	–	–
Boldenone	–	–	–	–	–	–	–	pink	–	–	–
Bromhexine	–	brown	brown	–	–	–	–	–	red	–	–
Bromhexine metabolite A	–	brown	brown	yellow	–	–	–	–	red	–	–
Bromhexine metabolite B	–	brown	brown	yellow	–	–	–	–	red	–	–
Bromhexine metabolite C	–	brown	brown	yellow	–	–	–	–	red	–	–
Bumetanide	–	–	–	–	–	–	–	–	orange	–	–
Bupivacaine	wk orange	brown	brown	no rxn	no rxn	–	–	–	–	–	brown
Bupivacaine, *p*-hydroxy	wk orange	brown	brown	no rxn	M yellow	–	–	–	–	–	brown
Buprenorphine	–	brown	brown	–	–	pink	–	–	–	–	–
Buspirone	orange	brown	brown	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	brown
Butorphanol	wk orange	brown	brown	–	no rxn	–	–	–	–	–	brown
Butylated hydroxytoluene	–	–	–	no rxn	–	–	–	–	–	–	–
Caffeine	no rxn	wk brown	dark brown	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	wk brown
Chlorcyclizine	orange	brown	brown	–	–	–	–	–	–	–	–
(Chlorobenzoyl)benzoic acid, 2-(4-	–	brown	–	–	–	–	–	–	–	–	–
(Chlorophenyl)piperazine, 1-(M-	orange	brown	brown	pink	no rxn	wk pink	–	–	–	–	brown
Chloroprocaine, 2-	wk orange	brown	brown	–	–	pink	–	–	red	–	brown
Chlorothiazide	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	–	–	no rxn	orange	–
Chlorphenamine	orange	brown	brown	–	–	–	–	–	–	–	brown
Chlorphenesin	no rxn	brown	brown	purple/grey	–	–	–	–	red	–	brown
Chlorphenesin carbamate	–	–	–	grey/blue fl	–	–	–	–	–	–	–
Chlorpromazine	–	brown	–	–	pink	–	–	–	–	–	brown

table continues

Name	*D*	*N*	*C-H*	*M*	*FES*	*NIN*	*Gibb's*	*H+:EtOH*	*ME*	*F*	*I_2*
Chlorpromazine metabolite	–	–	–	–	purple	–	–	–	–	–	–
Cholesterol	–	–	–	red/purple	–	–	–	grey	–	–	–
Cimetidine	wk orange	brown	brown	grey	yellow	–	–	–	grey/purple	–	–
Clenbuterol	–	–	–	–	–	–	–	–	red	–	–
Cocaine	orange	brown	brown	no rxn	no rxn	no rxn	–	–	–	–	no rxn
Codeine	wk orange	brown	brown	–	M blue	–	–	–	–	–	brown
Corticosterone	–	–	–	–	–	–	–	grey	–	–	–
Cortisone	–	–	–	–	–	–	–	no rxn	–	–	–
Cyproheptadine	orange	brown	brown	red/yellow fl	M red	no rxn	–	–	–	–	–
Cyproheptadine epoxide	orange	brown	brown	red/yellow fl	M red	no rxn	–	–	–	–	–
Cyproheptadine *N*-oxide	orange	brown	brown	brown/ylw fl	M brown	no rxn	–	–	–	–	–
Deanol	no rxn	wk brown	brown	–	–	wk pink	–	–	no rxn	–	brown
Dehydronorketamine, 5,6-	wk orange	brown	brown	–	pink	–	–	–	orange	–	brown
Deoxycorticosterone acetate	–	–	–	–	–	–	–	brown/grey	–	–	–
Desipramine	–	–	–	–	blue	–	–	–	–	–	–
Desoximetasone	–	–	–	–	–	–	–	grey	–	–	–
Despropionyl fentanyl	orange	brown	brown	–	–	–	–	–	–	–	–
Detomidine	wk orange	brown	brown	no rxn	no rxn	–	–	–	–	no rxn	no rxn
Dexamethasone	–	–	–	–	–	–	–	grey	–	–	–
Diazepam	wk orange	brown	brown	–	–	–	–	–	–	–	wk brown
Diclofenac	wk orange	wk brown	wk brown	red/brown	–	–	–	–	no rxn	no rxn	–
Diethylpropion	wk orange	brown	brown	–	–	pink	–	–	–	–	–
Diflunisal	no rxn	no rxn	wk brown	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	no rxn
Dihydrocodeine	wk orange	brown	brown	–	M blue	–	–	–	–	–	brown
Dihydrocodeine metabolite	wk orange	brown	brown	–	M blue	–	–	–	–	–	brown
Dimethoxycinnamic acid, 3,4-	orange	no rxn	no rxn	grey	grey	no rxn	–	–	no rxn	no rxn	–
Dipyrone	orange	brown	brown	–	–	wk pink	–	–	–	–	brown
Doxepin	wk orange	brown	brown	–	yellow	–	–	–	–	–	brown
Doxylamine	orange	brown	brown	–	–	–	–	–	–	–	–
Doxylamine base urine metabolite 1	orange	brown	brown	–	–	–	–	–	–	–	–
Doxylamine base urine metabolite 2	orange	brown	brown	–	–	–	–	–	–	–	–
Doxylamine enzyme hydrolysis metabolite	orange	brown	brown	–	–	–	–	–	–	–	–
Dyphylline	orange	no rxn	wk brown	–	–	–	–	–	–	–	wk brown
Ecgonine methyl ester	wk orange	brown	brown	no rxn	no rxn	no rxn	–	–	–	–	brown
Embutramide	orange	brown	brown	white	–	–	–	–	–	–	–
Ephedrine	wk orange	brown	brown	–	no rxn	pink	–	–	–	–	–
Estradiol	–	–	–	–	–	–	–	pink	–	–	–
Estrone	–	–	–	–	–	–	–	pink	–	–	–
Etamiphylline	–	brown	–	–	–	–	–	–	–	–	brown
Ethacrynic acid	–	–	–	–	–	–	–	–	–	–	–
Ethinyl estradiol	–	–	–	–	–	–	–	pink	–	–	–
Ethyl aminobenzoate	wk orange	brown	brown	no rxn	no rxn	–	–	–	red	–	brown
Ethylaminopropiophenone, 2-	wk orange	brown	brown	–	–	pink	–	–	–	–	–
Ethylnicotinamide, *N*-	wk orange	brown	brown	–	–	–	–	–	–	–	–
Etidocaine	orange	brown	brown	–	–	–	–	–	–	–	–
Etodolac	–	–	–	blue	–	–	–	–	–	–	–
Fenfluramine	–	brown	brown	–	wk pink	–	–	–	–	–	–
Fenoprofen	–	–	–	green	–	–	–	–	–	–	–
Fludrocortisone	–	–	–	–	–	–	–	grey	–	–	–
Fludroxycortide	–	–	–	–	–	–	–	grey	–	–	–
Flumethasone	–	–	–	–	–	–	–	grey	–	–	–
Flunixin	wk orange	brown	brown	yellow fl	no rxn	–	–	–	no rxn	–	–
Fluocinolone acetonide	–	–	–	–	–	–	–	grey	–	–	–
Fluorometholone	–	–	–	–	–	–	–	grey	–	–	–
Fluoxymesterone	–	–	–	–	–	–	–	grey	–	–	–
Fluprednisolone	–	–	–	–	–	–	–	grey	–	–	–
Fructose	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn	no rxn
Furosemide	no rxn	no rxn	no rxn	black	–	–	–	–	red	no rxn	–
Glycine xylidide	orange	brown	brown	–	–	–	–	–	–	–	no rxn
Glycopyrrolate	orange	brown	brown	–	–	–	–	–	–	–	–
Gramine	orange	brown	brown	blue	blue	no rxn	–	–	dk blue	–	–
Green 242	wk orange	brown	brown	green	M red	M black	–	–	no rxn	no rxn	–
Griseofulvin	orange	no rxn	no rxn	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	–
Guaifenesin	wk orange	wk brown	–	blue	blue	–	–	–	–	–	–

Name	*D*	*N*	*C-H*	*M*	*FES*	*NIN*	*Gibb's*	*H+:EtOH*	*ME*	*F*	*I_2*
Harmine	orange	–	–	–	–	–	–	–	–	–	–
Heptaminol	–	–	–	–	–	pink	–	–	–	–	–
Homatropine	–	brown	brown	–	–	–	–	–	–	–	–
Hordenine	orange	brown	brown	blue	M blue	–	–	–	–	–	brown
Hydrochlorothiazide	–	–	–	–	–	–	–	–	pink	red	–
Hydrocortisone	orange	wk brown	–	–	–	–	–	yellow/grey	–	–	–
Hydromorphone	wk orange	brown	–	–	–	–	–	–	–	–	brown
Hydroxy imipramine, 2-	–	–	–	–	yellow	–	–	–	–	–	brown
Hydroxyazaperol	–	brown	brown	–	–	–	–	–	–	–	–
Hydroxyazaperone	–	brown	brown	–	–	–	–	–	–	–	–
Hydroxybuspirone, 5-	orange	brown	brown	white	no rxn	no rxn	–	–	no rxn	no rxn	brown
Hydroxybutorphanol	wk orange	brown	brown	–	–	–	–	–	–	–	brown
Hydroxycyproheptadine	–	–	–	red	–	–	–	–	–	–	–
Hydroxydesipramine, 2-	–	–	–	–	yellow	–	–	–	–	–	brown
Hydroxydetomidine, 3-	wk orange	brown	brown	no rxn	no rxn	–	–	–	–	no rxn	no rxn
Hydroxydoxepin 1	wk orange	brown	brown	–	yellow	–	–	–	–	–	brown
Hydroxydoxepin 2	wk orange	brown	brown	–	yellow	–	–	–	–	–	brown
(Hydroxyethyl)promazine sulfoxide, 2-(1-	orange	brown	brown	purple	pink	no rxn	–	–	–	–	–
Hydroxylated mepivacaine	wk orange	brown	brown	–	–	–	–	–	–	–	brown
Hydroxylidocaine, 3-	wk orange	brown	brown	–	–	–	–	–	–	–	wk brown
Hydroxymeperidine, *p*-	–	brown	brown	–	grey	–	–	–	–	–	–
Hydroxymethadone, *p*-	wk orange	brown	brown	–	M red	–	–	–	–	–	–
Hydroxynefopam, *p*-	wk orange	brown	brown	–	–	M blue	–	–	–	–	brown
Hydroxyprednisolone, 20-β-	orange	brown	brown	–	–	–	–	grey	–	–	–
Hydroxyprednisone, 20-β-	orange	brown	brown	–	–	–	–	grey	–	–	–
Hydroxypromazine, 3-	no rxn	no rxn	no rxn	red	purple	–	–	–	–	–	–
Hydroxypropranolol, 4-	wk orange	brown	brown	–	–	–	–	–	–	–	green
Hydroxytenoxicam, 5′-	orange	wk brown	wk brown	no rxn	no rxn	no rxn	–	–	no rxn	white	wk brown
Hydroxytrazodone	orange	brown	brown	blue	no rxn	no rxn	–	–	–	–	brown
Hydroxyzine	orange	brown	brown	–	–	–	–	–	–	–	–
Ibuprofen	–	–	–	grey/tan fl	–	–	–	–	–	–	–
Imipramine	–	–	–	–	blue	–	–	–	–	–	brown
Imipramine *N*-oxide	–	–	–	–	blue	–	–	–	–	–	–
Indapamide	orange	brown	brown	red	pink	pink	–	–	pink	pink	brown
Indometacin	–	–	–	grey	–	–	–	–	–	–	–
Ipratropium bromide	orange	brown	–	no rxn	no rxn	–	–	–	–	–	–
Isoetarine	orange	brown	brown	grey	M grey	pink	–	–	–	dk pink	brown
Isoetarine EH metabolite	orange	brown	brown	grey	M grey	pink	–	–	–	no rxn	brown
Isoxsuprine	orange	brown	brown	blue	M grey	–	–	–	–	–	brown
Ketamine	orange	brown	brown	–	–	–	–	–	–	–	brown
Ketoprofen	–	–	–	yellow/yellow fl	–	–	–	–	–	–	–
Ketorolac	–	brown	brown	–	–	–	–	–	red/grey	–	brown
Lenperone	orange	brown	–	–	–	–	–	–	–	–	–
Levallorphan	orange	brown	brown	no rxn	no rxn	–	–	–	–	–	brown
Levorphanol	wk orange	brown	brown	wk blue	no rxn	–	–	–	–	–	brown
Lidocaine	orange	brown	brown	–	–	–	–	–	–	–	wk brown
Maprotiline	orange	brown	brown	–	–	no rxn	–	–	–	–	–
Mazindol	–	brown	–	–	–	–	–	–	–	–	–
Meclofenamic acid	orange	no rxn	no rxn	black	–	pink	–	–	–	–	–
Medroxyprogesterone	–	–	–	–	–	–	–	green/grey	–	–	–
Medrysone	–	–	–	–	–	–	–	grey	–	–	–
Mefenamic acid	–	–	–	blue/green	–	–	–	–	–	–	–
Mephentermine	orange	brown	brown	–	–	wk pink	–	–	–	–	brown
Mepivacaine	orange	brown	brown	–	–	–	–	–	–	–	brown
Meprednisone	–	–	–	–	–	–	–	grey	–	–	–
Metamfetamine	orange	brown	brown	–	–	pink	–	–	–	–	brown
Metaproterenol	–	–	–	–	–	–	blue	–	–	–	–
Methadone	wk orange	brown	brown	–	M yellow	–	–	–	–	–	brown
Methandrostenolone	–	–	–	–	–	–	–	grey	–	–	–

table continues

Name	*D*	*N*	*C-H*	*M*	*FES*	*NIN*	*Gibb's*	*H+:EtOH*	*ME*	*F*	*I_2*
Methapyrilene	orange	brown	brown	red	–	no rxn	–	–	–	–	brown
Methapyrilene BU metabolite	orange	brown	brown	red	–	pink	–	–	–	–	brown
Methapyrilene EH metabolite I	orange	brown	brown	no rxn	–	no rxn	–	–	–	–	brown
Methapyrilene EH metabolite III	orange	brown	brown	no rxn	–	no rxn	–	–	–	–	brown
Methocarbamol	orange	brown	brown	blue	blue	–	–	–	–	–	wk brown
Methoxamine	orange	brown	brown	–	M yellow	pink	–	–	–	–	–
Methyl-6-oxopipecolo-2′,6′ xylidide, 1-	wk orange	brown	brown	–	–	–	–	–	–	–	brown
Methylamino antipyrine, 4-	orange	brown	brown	–	–	wk pink	–	–	–	–	brown
Methylphenidate	orange	brown	brown	no rxn	no rxn	–	–	–	no rxn	–	brown
Methylprednisolone	–	–	–	–	–	–	–	red/grey	–	–	–
Methyltestosterone, 17-	–	–	–	–	–	–	–	grey	–	–	–
Metolazone	orange	wk brown	–	–	–	–	–	–	–	–	–
Monoethylglycine xylidide	no rxn	brown	brown	–	–	–	–	–	–	–	no rxn
Morphine	wk orange	brown	brown	–	M blue	–	–	–	–	–	brown
Nabumetone	–	–	–	blue	–	–	–	–	–	–	–
Nabumetone metabolite M-I	–	–	–	blue	–	–	–	–	–	–	–
Nabumetone metabolite M-II	–	–	–	blue	–	–	–	–	–	–	–
Nabumetone metabolite M-III	–	–	–	blue	–	–	–	–	–	–	–
Nabumetone metabolite M-IV	–	–	–	blue	–	–	–	–	–	–	–
Nabumetone metabolite M-V	–	–	–	blue	–	–	–	–	–	–	–
Nalbuphine	wk orange	brown	brown	–	–	–	–	–	–	–	–
Naloxone	–	–	–	–	–	–	–	–	–	–	brown
Naproxen	–	–	–	green	–	–	–	orange	–	–	–
Nefopam	wk orange	brown	brown	–	M blue	–	–	–	–	–	brown
Niacinamide	wk orange	wk brown	wk brown	–	–	–	–	–	–	yellow	–
Nicotine	orange	brown	brown	–	–	–	–	–	–	–	–
Nikethamide	wk orange	brown	brown	–	–	–	–	–	–	–	–
Nikethamide *N*-oxide	wk orange	brown	brown	–	–	–	–	–	–	–	–
Nivazol	–	–	–	–	–	–	–	grey	–	–	–
Nordazepam	–	brown	brown	–	–	–	–	–	–	–	–
Nordoxepin	wk orange	brown	brown	–	yellow	–	–	–	–	–	brown
Norketamine	wk orange	brown	brown	–	–	–	–	–	–	–	brown
Norpseudoephedrine	wk orange	brown	brown	–	–	pink	–	–	–	–	–
Oxycodone	orange	brown	–	–	–	–	–	–	–	–	–
Oxymorphone	orange	brown	brown	–	–	–	–	–	–	–	brown
Oxyphenbutazone	wk orange	wk brown	wk brown	yellow	–	–	–	–	–	yellow	–
Paracetamol	no rxn	no rxn	wk brown	white/grey	no rxn	no rxn	–	–	–	blue	brown
Paramethasone	–	–	–	–	–	–	–	grey	–	–	–
Paroxetine	–	–	brown	–	blue/green	–	–	–	blue/green	–	brown
Pemoline	–	–	–	–	–	–	–	–	–	purple/pink	–
Pentachlorophenol	orange	no rxn	no rxn	no rxn	–	–	–	–	–	–	–
Pentazocine	orange	brown	brown	–	–	–	–	–	–	–	–
Pentoxifylline	–	brown	brown	–	–	–	–	–	–	–	–
Pentoxifylline, demethyl-	–	brown	brown	–	–	–	–	–	–	–	–
Perphenazine	orange	brown	brown	–	purple	–	–	–	–	–	brown
Pethidine	orange	brown	brown	no rxn	grey	–	–	–	–	–	brown
Pethidine *N*-oxide	orange	brown	brown	–	grey	–	–	–	–	–	–
Phenmetrazine	wk orange	wk brown	wk brown	–	–	–	–	–	–	–	wk brown
Phentermine	orange	fades	–	–	–	–	–	–	–	–	–
Phenylbutazone	wk orange	brown	brown	red	no rxn	no rxn	–	–	–	–	–
Phenylbutazone alchohol metabolite	–	–	–	wk red	–	–	–	–	–	–	–
Phenylpropanolamine	orange	brown	brown	–	–	pink	–	–	–	–	–
Piretanide	no rxn	no rxn	no rxn	no rxn	chars	wk pink	no rxn	wk rxn	wk red	no rxn	wk brown
Piroxicam	–	brown	–	–	–	–	–	–	–	–	–
Piroxicam metabolite	–	brown	–	–	–	–	–	–	–	–	–
Prednisolone	orange	brown	brown	–	–	–	–	grey	–	–	–
Prednisone	orange	brown	brown	–	–	–	–	yellow/grey	–	–	–
Pregnenolone	–	–	–	–	–	–	–	grey	–	–	–
Prilocaine	no rxn	brown	brown	no rxn	no rxn	no rxn	–	–	–	–	brown
Procaine	orange	brown	brown	no rxn	no rxn	no rxn	–	–	red	–	brown
Progesterone	–	–	–	–	–	–	–	wk grey	–	–	–
Promazine	–	–	–	–	pink	–	–	–	–	–	brown
Propiomazine	–	–	–	–	pink	–	–	–	–	–	–
Propiomazine metabolite	–	–	–	–	pink	–	–	–	–	–	–
Propionylpromazine	–	–	–	–	pink/purple	–	–	–	–	–	–
Propoxyphene	orange	–	–	–	–	–	–	–	–	–	–

Name	*D*	*N*	*C-H*	*M*	*FES*	*NIN*	*Gibb's*	*H+:EtOH*	*ME*	*F*	*I_2*
Propoxyphene metabolite I	orange	–	–	–	–	–	–	–	–	–	–
Propoxyphene metabolite II	orange	–	–	–	–	–	–	–	–	–	–
Propoxyphene metabolite III	orange	–	–	–	–	–	–	–	–	–	–
Propranolol	wk orange	brown	brown	green	grey	red	–	–	red	–	brown
Pseudoephedrine	wk orange	brown	brown	–	–	pink	–	–	–	–	–
Pyrilamine	orange	brown	brown	red	pink	no rxn	–	–	–	–	brown
Pyrilamine, acid hydrolysis metabolite	–	brown	brown	–	no rxn	–	–	–	–	–	brown
Pyrilamine, *O*-demethyl	orange	brown	brown	red	pink	no rxn	–	–	–	–	brown
Pyrilamine, *O*-demethyl *N*-demethyl	orange	brown	brown	red	pink	no rxn	–	–	–	–	brown
Ranitidine	orange	brown	brown	grey	yellow	wk pink	–	–	–	–	–
Retinal	wk orange	wk brown	wk brown	purple	yellow/green	–	–	–	–	–	–
Romifidine	orange	brown	brown	no rxn	no rxn	no rxn	–	–	no rxn	wk blue/grey	brown
Salbutamol	no rxn	brown	brown	red	no rxn	no rxn	lt blue	–	no rxn	no rxn	wk brown
Salicylic acid	–	–	–	green	–	–	–	–	–	–	–
Stanozolol	–	–	–	–	–	–	–	grey	–	–	–
Strychnine	orange	brown	brown	blue orange	no rxn	–	–	–	–	–	–
Sulfadimethoxine	orange	brown	brown	no rxn	no rxn	no rxn	–	–	red	–	brown
Tenoxicam	orange	wk brown	wk brown	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	wk brown
Testolactone	–	–	–	–	–	–	–	grey	–	–	–
Testosterone	–	–	–	–	green	no rxn	–	green	–	–	–
Tetramisole (levamisole/dexamisole)	orange	brown	brown	wk wite	no rxn	no rxn	–	–	no rxn	–	brown
Tetramisole, hydroxylated metabolite	orange	brown	brown	–	–	–	–	–	–	–	brown
Theobromine	no rxn	no rxn	brown	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	–
Theophylline	no rxn	brown	brown	no rxn	no rxn	no rxn	–	–	no rxn	no rxn	–
Tiaprofenic acid	no rxn	wk brown	wk brown	no rxn	yellow	no rxn	–	lt yellow	no rxn	no rxn	wk brown
Tralonide	–	–	–	–	–	–	–	grey	–	–	–
Trazodone	orange	brown	brown	pink	no rxn	wk pink	–	–	–	–	brown
Trazodone metabolite, MW 405	orange	brown	brown	pink	no rxn	wk pink	–	–	–	–	brown
Triamcinolone	–	–	–	–	–	–	–	grey	–	–	–
Trichlormethiazide	–	–	–	–	–	–	–	–	–	red	–
Trimethoprim	orange	brown	brown	–	–	–	–	–	–	–	–
Xylazine	orange	brown	brown	–	–	–	–	–	–	–	brown
Xylazine hydroxylated metabolites	orange	brown	brown	–	–	–	–	–	–	–	brown
Zomepirac	–	–	–	–	–	–	–	–	blue	–	–

C-H, cupric chloride; D, Dragendorff spray; F, Fearon's reagent; FES, ferric chloride, ethanol, sulfuric acid; Gibb's, Gibb's reagent; H+:EtOH, conc. hydrochloric + ethanol; I_2; iodine; M, Mandelin's reagent; ME, modified Ehrlich's reagent; N, sodium nitrite; NIN, ninhydrin; no rxn, no reaction

9 Thin-layer Chromatographic Data

The following retention indices are the hR_f values taken from: DFG/TIAFT (1992). *Thin-Layer Chromatographic Rf Values of Toxicologically Relevant Substances on Standardized Systems*, 2nd edn. Germany: VCH Verlagsgesellschaft.

System T

39 Alprazaolam
22 Carbetapentane
55 Etenzamide

System T1

00 Azamethonium Bromide
00 Paromomycin
00 Tetracosactrin
00 Viomycin
01 Chlorisondamine Chloride
01 Laudexium Methyl Sulfate
01 Tolonium Chloride
01 Triclobisonium Chloride
02 Clamoxyquin
02 Dibutoline
02 Quinapyramine
03 Imidocarb
03 Tetraethylammonium Bromide
04 Stilbamidine
05 Isometamidium
10 Picloxydine
12 Norlevorphanol
15 Levophenacylmorphan
18 Apoatropine
20 Pentaquin
21 Metazocine
22 Pamaquin
23 Codeine *N*-oxide
24 Levomethorphan
25 Racemethorphan
27 Diphenazoline
28 Methyldihydromorphine
29 Desomorphine
30 Dimevamide
30 Phenisonone
30 Phenylpropylmethylamine
33 Nordefrin
33 Picloxydine
35 Plasmocide
35 Tropacocaine
39 Methoxypromazine
40 Proheptazine
40 Tropine
41 Amydricaine
42 Diampromide
45 Amopyroquine
47 Hydroxypethidine
47 Vetrabutine
48 Hydromorphinol
48 Iminodimethylphenylthiazolidine
52 Myrophine
52 Pethidine Intermediate A
52 Phenoxypropazine
52 Taurolin
53 Lucanthone
53 Methaphenilene
53 Properidine
55 Dimethylthiambutene
55 Methadone Intermediate
56 Demecolcine
56 Morpheridine
56 Octacaine
56 Panidazole
57 Alphamethadol
57 Benzamine
57 Metofoline
57 Norbudrine
58 Ethylmethylthiambutene
59 Thozalinone
60 Butethamine
60 Butoxamine
60 Dimenoxadole
60 Ethylisobutrazine
60 Metabutethamine
60 Phthivazid
61 Betaprodine
61 Isomethadone
62 Leucinocaine
62 Pipamazine
62 Teclothiazide
62 Thurfyl Nicotinate
62 Xenysalate
63 Naepaine
63 1,2-Naphthoquinone
64 Betameprodine
64 Diethylaminoethyl Diphenylpropionate
64 Etoxeridine
65 Methadone Intermediate
65 Methyridine
65 Piperoxan
66 Amylocaine
66 Furethidine
67 Rolicyprine
68 Amisometradine
68 Fenmetramide
68 Moramide Intermediate
68 Pipethanate
68 Tiletamine
69 Dimethocaine
69 Dioxyamidopyrine
69 Phenatine
70 Clonitazene
70 Etonitazene
70 Levomoramide
70 Proadifen
70 Racemoramide
70 Tolycaine
71 Amolanone
71 Amprotropine
71 Orthocaine
71 Pyrrocaine
72 Dioxaphetyl Butyrate
72 Etymide
72 Phenomorphan
73 Ethylpiperidyl Benzilate
74 Benzethidine
75 Brocresine
75 Cetoxime
75 Cyprenorphine
75 Fenimide
76 Cloponone
77 Isobutyl Aminobenzoate
79 Nifuroxime
85 Resorantel
86 Rifamide
87 Iopydone
93 Sulfasomizole

System T3

74 Methoxypromazine

System T4

58 Methoxypromazine

System T6

67 Methoxypromazine
83 Pipamazine

System T10

04 Phenamidine
23 Morphine *N*-oxide
24 Probarbital
25 Metopon
28 Butallylonal
36 Nicocodine
51 Noracymethadol
73 Phenampromide
75 Octaverine
77 Phenadoxone

System T16

60 Demeton-*O*

System T26a

68 Norgestrel

System T26c

100 Norgestrel

System TA

00 Adrenaline
00 Carbachol
00 Cetrimide
00 Decamethonium
00 Decamethonium Bromide
00 Demecarium Bromide
00 Diminazene
00 Diquat
00 Distigmine Bromide
00 Ethyl Nicotinate
00 Gallamine Triethiodide
00 Hexamethonium Bromide
00 Homatropine Methylbromide
00 Hydrastinine
00 Mebezonium Iodide
00 Neomycin
00 Noradrenaline
00 Paraquat
00 Pentolonium Tartrate
00 Quinuronium Sulfate
00 Streptomycin
00 Tubocurarine Chloride
01 Alcuronium Chloride
01 Benserazide
01 Bethanidine
01 Bretylium Tosilate
01 Debrisoquine
01 Dibrompropamidine
01 Dihydrostreptomycin
01 Guanethidine
01 Guanoxan
01 Hexylcaine
01 Homatropine
01 Hydroxystilbamidine
01 Kanamycin
01 Mepenzolate Bromide
01 Metformin
01 Obidoxime Chloride
01 Pancuronium Bromide
01 Pentamidine
01 Pentapiperide Metilsulfate
01 Propamidine
01 Pyritidium Bromide
01 Suxamethonium Chloride
01 Suxethonium Bromide
01 Thiamine
02 Acetylcholine Chloride
02 Ambenonium Chloride
02 Amprolium Hydrochloride
02 Atropine Methonitrate
02 Bethanechol Chloride
02 Bibenzonium Bromide
02 Buformin
02 Cadaverine
02 Choline
02 Clidinium Bromide
02 Cotarnine
02 Diphemanil Metilsulfate
02 Ecothiopate Iodide
02 Hexocyclium Metilsulfate
02 Hyoscine Methonitrate
02 Lachesine Chloride
02 Methacholine Chloride
02 Methanthelinium Bromide
02 Neostigmine Bromide
02 Octatropine Methylbromide
02 Oxyphencyclimine
02 Penthienate Methobromide
02 Poldine Metilsulfate
02 Thenium Closilate
02 Thiazinamium Metilsulfate
02 Trimetaphan Camsilate
02 Valethamate Bromide
03 Ambutonium Bromide
03 Benzethonium Chloride
03 Benzilonium Bromide
03 Chlorproguanil
03 Dequalinium Chloride
03 Dofamium Chloride
03 Glycopyrronium Bromide
03 Guanoclor
03 Heteronium Bromide
03 Hexoprenaline
03 Oxyphenonium Bromide
03 Phenformin
03 Proguanil
04 Acefylline Piperazine
04 Pipenzolate Bromide
04 Propantheline Bromide
04 Pyridostigmine Bromide
05 Amicarbalide
05 Chlortetracycline
05 Clomocycline
05 Cytarabine
05 Demeclocycline
05 Domiphen Bromide
05 Emepronium Bromide
05 Isopropamide Iodide
05 Lymecycline
05 Methacycline
05 Oxytetracycline Dihydrate
05 Piperazine
05 Pralidoxime Chloride
05 Psilocybine
05 Rolitetracycline
05 Sparteine
05 Tetracycline
06 Carbamazepine
06 Pyrantel
06 Tramazoline
06 Tricyclamol Chloride
07 Acriflavinium Chloride
07 Berberine
07 Edrophonium Chloride
07 Tridihexethyl Chloride
08 Desferrioxamine
08 Hyoscine Butylbromide
09 Oxymetazoline
09 Putrescine
10 Azacyclonol
10 Betahistine
10 Denatonium Benzoate
10 Mequitazine
10 Stilbazium Iodide
11 Δ^9-THC
12 Cetalkonium Chloride
12 Doxorubicin
12 Doxycycline
13 Benzatropine
13 Deptropine
13 Histamine
13 Nitroxoline
13 Norcodeine
13 Tetryzoline
13 Tolazoline
13 Xylometazoline
14 Naphazoline
15 Amidefrine
15 Maprotiline
15 Octafonium Chloride
16 Brucine
16 Mecamylamine
16 Proflavine Hemisulfate
17 Ecgonine
17 Normorphine
18 Atropine
18 Dopamine
18 Hyoscyamine
18 *N*-Methyltryptamine
19 Halquinol
19 Primaquine
19 Protriptyline
20 Cetylpyridinium Chloride
20 Cyclopentamine
20 Mescaline
21 Atropine Oxide
21 Benzoylecgonine
21 Metadrenaline
22 Octamylamine
22 Trimetazidine
22 Vancomycin
23 Amantadine

55 Dihydralazine
55 Dimenhydrinate
55 Diphenhydramine
55 Erythromycin
55 Fenetylline
55 Homidium Bromide
55 Hyoscine
55 Methoxamine
55 Orphenadrine
55 Pentifylline
55 Perphenazine
55 Physostigmine
55 Piperocaine
55 Propiomazine
55 Thonzylamine
55 Trimethoprim
55 Tripelennamine
55 Urea
56 5-Methyltryptamine
56 Chlorprothixene
56 Clioquinol
56 Clopenthixol
56 Dimetotiazine
56 Imolamine
56 Metopimazine
56 Nicametate
56 Nicotinyl Alcohol
56 Nomifensine
56 Normethadone
56 Oxazepam
56 Pipamperone
56 Piperacetazine
56 Zuclopenthixol
57 Chlorcyclizine
57 Clorprenaline
57 Clozapine
57 Cyclizine
57 Cyclopentolate
57 Ergometrine
57 Inositol Nicotinate
57 Levomepromazine
57 Mebhydrolin
57 Methylphenidate
57 Nifenazone
57 Nimorazole
57 Phendimetrazine
57 Tetracaine
57 Trometamol
58 Alimemazine
58 Aminobenzoic Acid
58 Cyclomethycaine
58 Diaveridine
58 Diethazine
58 Lysergic Acid
58 Methyprylon
58 Metronidazole
58 Metyrapone
58 Mianserin
58 Morazone
58 Nicotinic Acid
58 Pericyazine
58 Propoxycaine
58 Proxyphylline
58 Trimeperidine
58 α-Methyltryptamine
59 Aletamine
59 Benzoctamine
59 Butriptyline
59 Chloroprocaine
59 Clotiapine
59 Dimefline
59 Isoetarine
59 Nalorphine
59 Nikethamide
59 Phenazopyridine
59 Phencyclidine
59 Prajmalium Bitartrate
59 Pyridoxine
59 Trimipramine
59 Verapamil
59 Veratrine
60 Clomifene
60 Dihydroergotamine
60 Dyclonine
60 Ethacridine
60 Ethomoxane
60 Histapyrrodine
60 Lysergamide
60 Lysergide
60 Methylpiperidyl Benzilate
60 Morantel
60 Pemoline
60 Prazosin
60 Sulfametoxydiazine
60 Tocainide
60 Triazolam
60 Vinblastine
60 Xylazine
61 Amiphenazole
61 Benzonatate
61 Bromazepam
61 Difenidol
61 Fenpiprane
61 Hydrastine
61 Lobeline
61 Methyl Nicotinate
61 Nicofuranose
61 Papaverine
61 Paraphenylenediamine
61 Pentazocine
61 Pyrimethamine
61 Quinisocaine
61 Thiopropazate
61 Trimetozine
61 Triphenyltetrazolium Chloride
62 Ajmaline
62 Amodiaquine
62 Chlordiazepoxide
62 Clobazam
62 Clonidine
62 Flavoxate
62 Flupentixol
62 Flurazepam
62 Levamisole
62 Methylergometrine
62 Nordazepam
62 Oxybuprocaine
62 Proxymetacaine
62 Styramate
62 Sulfadimidine
62 Thiamazole
63 Benzyl Nicotinate
63 Butoxyethyl Nicotinate
63 Cinchocaine
63 Demoxepam
63 Dimetridazole
63 Ergotamine
63 Flunitrazepam
63 Fluphenazine
63 Harmine
63 Ketamine
63 Mazindol
63 Mebeverine
63 Phenindamine
63 Pyrazinamide
63 Trazodone
63 Yohimbine
64 Adiphenine
64 Benethamine
64 Biperiden
64 Bunamidine
64 Clomethiazole
64 Doxapram
64 Etenzamide
64 Mephenesin
64 Naftidrofuryl Oxalate
64 Nicergoline
64 Norharman
64 Noscapine
64 Sulfadiazine
64 Sulfisomidine
65 Bamifylline
65 Beclamide
65 Benzquinamide
65 Bitoscanate
65 Cocaine
65 Dropropizine
65 Ethionamide
65 Etoxazene
65 Ibogaine
65 Mebendazole
65 Mepivacaine
65 Methyldopate
65 Methysergide
65 Metisazone
65 Naloxone
65 Phenazone
65 Prazepam
65 Protokylol
65 Sulfadimethoxine
65 Sulfafurazole
65 Sulfaguanidine
65 Sulfamerazine
65 Sulfamethizole
65 Sulfamethoxazole
65 Sulfamethoxypyridazine
65 Trifluomeprazine
65 Troleandomycin
65 Tropicamide
66 Alverine
66 Aminophenazone
66 Azaperone
66 Benactyzine
66 Captodiame
66 Caramiphen
66 Chlormezanone
66 Codergocrine Mesilate
66 Cycrimine
66 Diloxanide
66 Dipipanone
66 Ergotoxine
66 Ketazolam
66 Pipobroman
66 Pipotiazine
66 Propanidid
66 Protionamide
66 Sulfasalazine
66 Sulfathiazole
67 Alprazolam
67 Benorilate
67 Benzocaine
67 Clorgiline
67 Droperidol
67 Etomidate
67 Glafenine
67 Haloperidol
67 Hydroxy-*N*-dealkyl-2-oxoquazepam
67 Levallorphan
67 Lincomycin
67 Medazepam
67 Piminodine
67 Profenamine
67 Pyrvinium Embonate
67 Sulfadoxine
67 Sulfaethidole
67 Sulfametopyrazine
67 Sulfamoxole
67 Sulfanilamide
67 Sulfapyridine
67 Tiabendazole
68 6-Hydroxybrotizolam
68 Aconitine
68 Aminotriazole
68 Azapropazone
68 Butalamine
68 Crotetamide
68 Dextropropoxyphene
68 Dicycloverine
68 Dimoxyline
68 Dipyridamole
68 Hydroxyzine
68 Isoaminile
68 Nifedipine
68 Nitrazepam
68 Norpipanone
68 Oxypertine
68 Phenazocine
68 Prenylamine
68 Solanidine
68 Trihexyphenidyl
69 Bupivacaine
69 Butetamate
69 Chloramphenicol
69 Clefamide
69 Fenyramidol

9

69 Fluspirilene
69 Iproniazid
69 Piperidolate
69 Reserpine
69 Sulfaphenazole
69 Tetrabenazine
69 Tetramisole
70 Aminosalicylic Acid
70 Azapetine
70 Clofazimine
70 Cropropamide
70 Dexpanthenol
70 Diethylthiambutene
70 Diperodon
70 Diprenorphine
70 Ethosuximide
70 Fentanyl
70 Harman
70 Hexetidine
70 Hexyl Nicotinate
70 Lidocaine
70 Lidoflazine
70 Loperamide
70 Mebanazine
70 Methaqualone
70 Methocarbamol
70 Nialamide
70 Norbormide
70 Parbendazole
70 Pargyline
70 Piritramide
70 Pramocaine
70 Protoveratrine A and B
70 Sulfacetamide
70 Sulfachlorpyridazine
71 Bezitramide
71 Butacaine
71 Chlorquinaldol
71 Disulfiram
71 Flumazenil
71 Isocarboxazid
71 Metomidate
71 Phenoperidine
71 Pimozide
71 Propyphenazone
71 Sulfaquinoxaline
71 Hydroxy-2-oxo-quazepam
72 Acetorphine
72 Amiodarone
72 Aniline
72 Bromocriptine
72 Brotizolam
72 Carbimazole
72 Chelidonine
72 Chlorpropamide
72 Clindamycin
72 Clonazepam
72 Deserpidine
72 Embutramide
72 Famprofazone
72 Fenbutrazate
72 Methazolamide
72 Methoserpidine
72 Midazolam
72 Pentetrazol
72 Protoveratrine A and B
72 Tylosin
72 α-Hydroxybrotizolam
73 Ambucetamide
73 Amylocaine
73 Anileridine
73 Benzfetamine
73 Cefaloridine
73 Desmetryne
73 Dextromoramide
73 Diethyltoluamide
73 Etorphine
73 Fluanisone
73 Methoxyamfetamine
73 Miconazole
73 Nifuratel
73 Phenoxybenzamine
73 Rescinnamine
73 Ritodrine
73 Simazine
73 Trifluperidol
74 Bialamicol
74 Bisacodyl
74 Broxaldine
74 Buphenine
74 Diphenoxylate
74 Etisazole
74 Hydroxyphenamate
74 Methoprotryne
74 Noxytiolin
74 Quazepam
74 Selegiline
75 Aminonitrothiazole
75 Bromhexine
75 Buclizine
75 Butyl Aminobenzoate
75 Cinchophen
75 Diazepam
75 Dinitolmide
75 Glutethimide
75 Meprobamate
75 *N*-dealkyl-2-oxoquazepam
75 Padimate
75 Phenprobamate
75 Phensuximide
75 Quinethazone
75 Sulfiram
75 Theophylline
76 Ametryne
76 Buprenorphine
76 Butanilicaine
76 Camazepam
76 Cinnarizine
76 Clorexolone
76 Diethylpropion
76 Ethinamate
76 Ethoxzolamide
76 Fenoterol
76 Glymidine Sodium
76 Meclozine
76 Mesuximide
76 Normetaclazepam
76 Penfluridol
76 Pheneturide
76 Prometryne
76 Sulfacarbamide
76 Thiambutosine
76 Tolbutamide
77 Atrazine
77 Bephenium Hydroxynaphthoate
77 Cyclothiazide
77 Gloxazone
77 Meclofenoxate
77 Metaclazepam
77 Nortetrazepam
77 Oxyphenbutazone
77 Phenelzine
77 Pipoxolan
77 Prilocaine
77 Terbutryne
77 Tybamate
78 Carbutamide
78 Clemizole
78 Isoxsuprine
78 Oxo-quazepam
78 Stanozolol
78 Syrosingopine
78 Tetrazepam
78 Thioacetazone
79 Clioxanide
79 Clopamide
79 Metallibure
79 Phenothiazine
79 Phenylbutazone
79 Quazepam -CFTB
79 Rifampicin
79 Triamcinolone
80 Aminitrozole
80 Econazole
80 Glibenclamide
80 Tiocarlide
80 Trichlormethiazide
81 Diamfenetide
81 Propyliodone
82 Chlorphenesin
82 Chlorphenesin Carbamate
82 Diazoxide
82 Diclofenamide
82 Methoxsalen
82 Oxyphenisatine
83 Apomorphine
83 Crotamiton
83 Nitroxinil
84 Azinphos-(Me)
84 Clorazepic Acid
84 Dipyrone
84 Indoramin
84 Rifamycin SV
84 Salinazid
85 Acetazolamide
85 Chlorzoxazone
86 Hydroflumethiazide
86 Methandienone
86 Nifursol
86 Paramethadione
87 Amygdalin
87 Glipizide
87 Methyclothiazide
87 Methylprednisolone
87 Oxyclozanide
88 Dimenhydrinate
88 Ethotoin
88 Primidone
88 Trilostane
89 Methyltestosterone
89 Rafoxanide
90 Aspirin
90 Buclosamide
90 Cortisone
90 Diclofenac
90 Ethinylestradiol
90 Fludrocortisone
91 Etidocaine
91 Niclosamide
91 Paramethasone
92 Fenbufen
94 CBD
94 CBN
94 Etamivan
94 Indometacin
95 Eugenol
95 Fendosal
95 Methyl Hydroxybenzoate
95 Oxymetholone
95 Paracetamol
96 Etacrynic Acid
96 Fenoprofen
96 Flufenamic Acid
96 Flunixin
96 Hydrocortisone
96 Mefenamic Acid
96 Methyl Salicylate
98 Anisindione
98 Boldenone

System TB

00 5-Hydroxypiroxicam
00 Acebutolol
00 Acenocoumarol
00 Adrenaline
00 Alcuronium
00 Ambazone
00 Amidefrine
00 Amiloride
00 Aminoacridine

00 Apomorphine
00 Atenolol
00 Atropine Methonitrate
00 Azacyclonol
00 Bambuterol Monocarbamate
00 Bamifylline
00 Benorilate
00 Benserazide
00 Benzoylecgonine
00 Bethanidine
00 Bromocriptine
00 Broxyquinoline
00 Brucine
00 Buformin
00 Bufotenine
00 Carbachol
00 Ceftriaxone
00 Cetrimide
00 Chloramphenicol
00 Chlorhexidine
00 Chlorphenesin Carbamate
00 Chlorproguanil
00 Chlorpropamide
00 Chlorzoxazone
00 Cimetidine
00 Cinchophen
00 Cisapride
00 Clefamide
00 Clenbuterol-(M_2)
00 Clenbuterol-(M_3)
00 Clidinium Bromide
00 Clindamycin
00 Clioquinol
00 Clonazepam
00 Colchicine
00 Cytarabine
00 Debrisoquine
00 Decamethonium
00 Demoxepam
00 Denatonium Benzoate
00 Dicoumarol
00 Dimenhydrinate
00 Diminazene
00 Dinormetaclazepam
00 Diprophylline
00 Dipyridamole
00 Dipyrone
00 Dobutamine
00 Dopamine
00 Dopexamine
00 Doxazosin
00 Emepronium Bromide
00 Enalapril
00 Epithiazide
00 Ergometrine
00 Ethchlorvynol
00 Ethionamide
00 Etofylline
00 Etoxazene
00 FLA-838
00 Famotidine
00 Fenoterol
00 Fluconazole
00 Flucytosine
00 Fluorometholone
00 Furaltadone
00 Furazolidone
00 Furosemide
00 Glibenclamide
00 Gliclazide
00 Glipizide
00 Glymidine Sodium
00 Guanethidine
00 Guanoclor
00 Guanoxan
00 Harmine
00 Hexachlorophene
00 Hexylresorcinol
00 Histamine
00 Homatropine Methylbromide
00 Hydrocortisone
00 Hydroxy-*N*-dealkyl-2-oxo-quazepam
00 Hydroxystilbamidine
00 Isoetarine
00 Isoprenaline
00 Isopropamide Iodide
00 Ketoconazole
00 Labetalol
00 Levocabastine
00 Lisinopril
00 Lysergamide
00 Lysergic Acid
00 Mebendazole
00 Mebutamate
00 Meprobamate
00 Mercaptopurine
00 Metformin
00 Methanthelinium Bromide
00 Methazolamide
00 Methocarbamol
00 Methylergometrine
00 Methylprednisolone
00 Metopimazine
00 Minoxidil
00 Morphine
00 *N*-Desmethylpirenzepine
00 NCM-001
00 NCM-009
00 Nalbuphine
00 Narceine
00 Neostigmine Bromide
00 Niclosamide
00 Nicotinamide
00 Nifenazone
00 Nifuratel
00 Nitrazepam
00 Nitrofurantoin
00 Nitrofurazone
00 Nizatidine
00 Noradrenaline
00 Norastemizole
00 Norbormide
00 Norcodeine
00 Norharman
00 Obidoxime Chloride
00 Oxazepam
00 Oxcarbazepine
00 Oxyphenbutazone
00 Oxyphenonium Bromide
00 Oxytetracycline Dihydrate
00 Pancuronium Bromide
00 Paracetamol
00 Paraquat
00 Pemoline
00 Pentapiperide Metilsulfate
00 Pentolonium Tartrate
00 Phenformin
00 Pilocarpine
00 Pirenzepine
00 Practolol
00 Pralidoxime Mesilate
00 Prednisolone
00 Prednisone
00 Proguanil
00 Propantheline Bromide
00 Pseudomorphine
00 Psilocybine
00 Pyridostigmine Bromide
00 Pyridoxine
00 Resorcinol
00 Rimiterol
00 Salicylamide
00 Strophanthin-K
00 Sulfacarbamide
00 Sulpiride
00 Suxamethonium Chloride
00 Tetracycline
00 Thiamine
00 Thiazinamium Metilsulfate
00 Thiazinamium Sulfoxide
00 Tolazamide
00 Trilostane
00 Trimetaphan Camsilate
00 Trimethoprim
00 Tubocurarine Chloride
00 Xamoterol
00 Xipamide
01 6-Hydroxybrotizolam
01 Acefylline Piperazine
01 Alprazolam
01 Astemizole
01 Butalbital
01 Carbimazole
01 Cephaëline
01 Chlormezanone
01 Codergocrine Mesilate
01 Cycloserine
01 Cytisine
01 Diazoxide
01 Dihydroergotamine
01 Dropropizine
01 Ergotamine
01 Ergotoxine
01 Ethylnoradrenaline
01 Glafenine
01 Heptaminol
01 Hexoprenaline
01 Inositol Nicotinate
01 Iproniazid
01 Isoniazid
01 Itraconazole
01 Loprazolam
01 Lorazepam
01 Hydroxyclenbuterol
01 Metaraminol
01 Methyldopa
01 Methysergide
01 Metoclopramide
01 Moclobemide
01 Nadolol
01 Nalorphine
01 Nifedipine
01 Nimodipine
01 Niridazole
01 Orciprenaline
01 Oxymetazoline
01 Oxyphencyclimine
01 Penicillamine
01 Pentamidine
01 Phenazopyridine
01 Phentolamine
01 Phenylephrine
01 Pipamperone
01 Piperazine
01 Piritramide
01 Prazosin
01 Prenalterol
01 Procainamide
01 Propamidine
01 Protionamide
01 Protokylol
01 Rescinnamine
01 Salbutamol
01 Salinazid
01 Sotalol
01 Styramate
01 Terbutaline
01 Theobromine
01 Theophylline
01 Thiamazole
01 Triamterene
01 Triazolam
01 Tryptamine
01 Tuaminoheptane
01 Vinblastine
02 Amiphenazole
02 Amlodipine
02 Bambuterol
02 Buclosamide
02 Carbamazepine
02 Chlordiazepoxide
02 Dihydromorphine
02 Droperidol
02 Etilefrine
02 Felodipine
02 Guaifenesin
02 Harman
02 Hexestrol
02 Hydroquinine
02 Hydroxyamfetamine
02 Hydroxychloroquine
02 Ketobemidone
02 Mephenesin
02 Metronidazole
02 *N*-dealkyl-2-oxo-quazepam

24 Ketotifen
24 Methoxamine
24 Methylenedioxymetamfetamine
24 Thebaine
25 Cathine
25 Cinchocaine
25 Mecloqualone
25 Oxycodone
25 Perazine
26 Acepromazine
26 Benzatropine
26 Carbinoxamine
26 Deptropine
26 Etomidate
26 Meclofenoxate
26 Methoxyphenamine
26 Phenoperidine
26 Phentermine
26 Proxymetacaine
26 Tofenacin
27 Diazepam
27 Mebhydrolin
27 Nortriptyline
27 Quazepam
27 Zimeldine
28 Butaperazine
28 Crotetamide
28 Encainide
28 Ibogaine
28 Metamfetamine
28 Octamylamine
28 Phenethylamine
29 Cropropamide
29 Prilocaine
30 Alphaprodine
30 Azaperone
30 Buspirone
30 Diamthazole
30 Flurazepam
30 Methylpiperidyl Benzilate
30 Thiethylperazine
31 Gestonorone Caproate
31 Glutethimide
31 Mepivacaine
31 Moxisylyte
32 Cyclopentamine
32 Cyclopentolate
32 Methdilazine
32 Propyphenazone
32 Tetrazepam
33 Brompheniramine
33 Clemizole
33 Nefopam
33 Trifluoperazine
34 Dihydralazine
34 Ethomoxane
34 Mephentermine
34 Prochlorperazine
34 Propiomazine
34 Propylhexedrine
35 Chlorphenamine
35 Etafedrine
35 Lidocaine
35 Methylphenidate
35 Pheniramine
35 Thiopropazate
36 Benzydamine
36 Dimetindene
36 Flavoxate
36 Loxapine
36 Methaqualone
36 Phendimetrazine
36 Piminodine
36 Prazepam
36 Progesterone
36 Promethazine
37 Aletamine
37 Cyclandelate
37 Famprofazone
37 Ketamine
37 Methyl Nicotinate
37 Pethidine
37 Phenelzine
38 Cotarnine
38 Hydroxyprogesterone
38 Phenyltoloxamine
38 Promazine
38 Thenalidine
38 Thonzylamine
39 Arecoline
39 Coniine
39 Fluanisone
39 Mepyramine
39 Mianserin
39 Nicotine
39 Tigloidine
39 Tranylcypromine
40 Azinphos-(Me)
40 Bamipine
40 Benactyzine
40 Mebeverine
40 Metaclazepam
40 Normethadone
41 Bezitramide
41 Chloropyramine
41 Clofedanol
41 Clotiapine
41 Doxylamine
41 Hydralazine
41 Isothipendyl
41 Medazepam
41 Methapyrilene
41 Nicametate
41 Tetrabenazine
41 Trimeperidine
42 Aminopromazine
42 Benzyl Nicotinate
42 Bupivacaine
42 Chlorcyclizine
42 Clorgiline
42 Dextromethorphan
42 Dextromoramide
42 Diphenoxylate
42 Diphenylpyraline
42 Fenfluramine
42 Nicofuranose
42 Thenyldiamine
42 Thioridazine
43 Fentanyl
43 Noxiptiline
43 Pramocaine
43 Prothipendyl
44 Bromazine
44 Clomethiazole
44 Diphenhydramine
44 Quazepam-CFTB
44 Tripelennamine
45 Butoxyethyl Nicotinate
45 Chlorpromazine
45 Cocaine
45 Cyproheptadine
45 Dimenhydrinate
45 Ethoheptazine
45 Metixene
45 Nisoldipine
45 Phenindamine
45 Pizotifen
46 Hydrastinine
46 Sertraline
47 Chlorphenoxamine
47 Fenbutrazate
47 Levomepromazine
47 Pecazine
47 Triflupromazine
48 Benethamine
48 Carbetapentane
48 Cyclizine
48 Doxepin
48 Hexetidine
48 Imipramine
48 Lorcainide
48 Mebanazine
48 Orphenadrine
49 Captodiame
49 Clemastine
49 Conessine
49 Dosulepin
49 Dyclonine
49 Iprindole
50 Amitriptyline
50 Embramine
50 Halofantrine
50 Rimantadine
51 Chlorprothixene
51 Mecamylamine
51 Oxeladin
52 Broxaldine
52 Naftidrofuryl Oxalate
52 Tolpropamine
53 Azapropazone
53 Clomipramine
53 Piperocaine
53 Pipoxolan
54 Alimemazine
54 Cinnarizine
54 Pyrrobutamine
55 Cyclomethycaine
55 Piperidolate
55 Prenylamine
55 Quinisocaine
56 Adiphenine
56 Clomifene
56 Difenidol
57 Azapetine
57 Benzoctamine
57 Clofazimine
57 Diethazine
57 Perhexiline
57 Selegiline
58 Isoaminile
58 Norpipanone
59 Butetamate
59 Dextropropoxyphene
59 Methadone
59 Pipradrol
60 Amylocaine
60 Dieldrin
60 Diethylthiambutene
60 Trifluomeprazine
61 Buclizine
61 Butriptyline
61 Meclozine
62 Amiodarone
62 Bialamicol
62 Diethylpropion
62 Fencamfamin
62 Procyclidine
62 Terodiline
62 Trimipramine
63 Aprindine
63 Phenoxybenzamine
63 Sulfadoxine
64 Profenamine
65 Alverine
66 Trihexyphenidyl
67 Benzfetamine
67 Bromhexine
67 Cycrimine
67 Dicycloverine
67 Dipipanone
67 Prolintane
67 Sparteine
68 Biperiden
68 Pempidine
72 Chlordane
73 Phencyclidine
74 Heptachlor

System TC

00 Ametazole
00 Bethanidine
00 Buformin
00 Carbachol
00 Debrisoquine
00 Decamethonium

26 Methapyrilene
27 Carfenazine
27 Haloperidol
27 Phenmetrazine
27 Tripelennamine
28 Chloropyramine
28 Diazoxide
28 Dihydroergotamine
28 Diphenylpyraline
28 Phenethylamine
28 Thonzylamine
29 Nomifensine
29 Perphenazine
30 Butacaine
30 Diamthazole
30 Hydroxy-*N*-dealkyl-2-oxo-quazepam
30 Isothipendyl
30 Norharman
30 Promazine
30 Theophylline
30 Thioridazine
30 Trifluoperazine
31 Chloramphenicol
31 Clonidine
31 Procaine
31 Procyclidine
31 Pyrimethamine
31 Theobromine
32 Amitriptyline
32 Clopenthixol
32 Embramine
32 Isoxsuprine
32 Loperamide
32 Meprobamate
32 Nefopam
32 Pilocarpine
32 Pipotiazine
32 Prolintane
32 Tetracaine
32 Tolpropamine
32 Zuclopenthixol
33 Amiphenazole
33 Dimenhydrinate
33 Diphenhydramine
33 Dipipanone
33 Flupentixol
33 Mebutamate
33 Orphenadrine
33 Propoxycaine
33 Proxyphylline
33 Tranylcypromine
34 Cinchocaine
34 Clomipramine
34 Emetine
34 Ergotamine
34 Fencamfamin
34 Iprindole
34 Normethadone
34 Pethidine
34 Thebacon
34 Thioproperazine
35 6-Hydroxybrotizolam
35 Alphaprodine
35 Chlorpromazine
35 Demoxepam
35 Dibenzepin
35 Lobeline
35 Nicametate
35 Nicotine
35 Noxiptiline
35 Phencyclidine
35 Promethazine
35 Triflupromazine
35 Veratrine
36 Chlorphenoxamine
36 Cyclomethycaine
36 Ethionamide
36 Lorazepam
36 Metronidazole
36 Nitrazepam
36 Physostigmine
37 Butaperazine
37 Clofedanol
37 Colchicine
37 Dipyridamole
37 Doxepin
37 Hyoscine
37 Oxymorphone
37 Perazine
37 Piperocaine
37 Prochlorperazine
37 Pyrrobutamine
37 Thebaine
38 Clozapine
38 Diamorphine
38 Glafenine
38 Levomepromazine
38 Pipradrol
38 Protionamide
38 Yohimbine
39 Aconitine
39 Alimemazine
39 Alverine
39 Cyclopentolate
39 Etamiphylline
39 Lysergide
39 Phenazocine
40 Aletamine
40 Amodiaquine
40 Dyclonine
40 Furaltadone
40 Harman
40 Hexetidine
40 Oxazepam
40 Tiotixene
40 Triazolam
41 Bromazepam
41 Cyclizine
41 Glipizide
41 Methylphenidate
41 Naftidrofuryl Oxalate
41 Oxybuprocaine
41 Proxymetacaine
41 Thiethylperazine
41 Trimeperidine
42 Dosulepin
42 Meclofenoxate
42 Propiomazine
42 Pyrazinamide
43 Acetylcodeine
43 Bamipine
43 Bromazine
43 Diethylthiambutene
43 Inositol Nicotinate
43 Mephenesin
44 Cyproheptadine
44 Hexobendine
44 Moxisylyte
44 Nimorazole
44 Niridazole
44 Pecazine
44 Thenalidine
45 Difenidol
45 Fenetylline
45 Mebhydrolin
45 Phanquinone
45 Piritramide
46 Chlorcyclizine
46 Morazone
46 Quinisocaine
46 α-Hydroxybrotizolam
47 Cocaine
47 Ethomoxane
47 Furazolidone
47 Prazosin
47 Profenamine
48 Butriptyline
48 Codergocrine Mesilate
48 Dimefline
48 Dimetotiazine
48 Droperidol
48 Flurazepam
48 Levamisole
48 Loprazolam
48 Phenyltoloxamine
49 Morinamide
50 Chlordiazepoxide
50 Ibogaine
50 Norpipanone
50 Phenazopyridine
51 Benorilate
51 Chlorprothixene
51 Chlorzoxazone
51 Diethazine
51 Oxycodone
51 Phendimetrazine
52 Benzoctamine
52 Brotizolam
52 Clomifene
52 Fenyramidol
52 Thiamazole
53 Benactyzine
53 Clonazepam
53 Mebeverine
53 Thiopropazate
54 Bamifylline
54 Butanilicaine
54 Hydroxyzine
54 Isoaminile
54 Tetramisole
54 Tiabendazole
54 Trimipramine
55 Dextropropoxyphene
55 Nordazepam
55 Pramocaine
55 Hydroxy-2-oxo-quazepam
56 Anileridine
56 Carbamazepine
56 Clefamide
56 Clorazepic Acid
56 Etoxazene
56 *N*-dealkyl-2-oxo-quazepam
56 Nikethamide
57 Alprazolam
57 Benzocaine
57 Butetamate
57 Phenindamine
58 Caffeine
58 Diperodon
58 Methylpiperidyl Benzilate
58 Metyrapone
58 Mianserin
58 Nortetrazepam
58 Pipobroman
58 Trazodone
58 Trifluomeprazine
59 Benethamine
59 Clofazimine
59 Clotiapine
59 Etenzamide
59 Fluspirilene
59 Mebendazole
59 Temazepam
60 Adiphenine
60 Midazolam
60 Penfluridol
60 Pimozide
60 Vinblastine
61 Cycrimine
61 Econazole
61 Etorphine
61 Lormetazepam
61 Trihexyphenidyl
62 Ergotoxine
62 Mepivacaine
62 Norbormide
63 Butyl Aminobenzoate
63 Chlormezanone
63 Diethylpropion
63 Flumazenil
63 Ketamine
63 Lidoflazine
64 Biperiden
64 Cholesterol
64 Dicycloverine
64 Hydrastine
64 Ketazolam
64 Pentetrazol
64 Phenoperidine
64 Piminodine
64 Prilocaine
65 Beclamide
65 Glymidine Sodium
65 Metomidate
65 Nifedipine

9

65 Oxypertine
65 Papaverine
66 Methyl Nicotinate
66 Naloxone
66 Pentifylline
67 Amylocaine
67 Azapetine
67 Buclosamide
67 Crotetamide
67 Flavoxate
67 Miconazole
68 Ambucetamide
68 Amiodarone
68 Buprenorphine
68 Fluanisone
68 Metisazone
68 Normetaclazepam
68 Pipoxolan
68 Prenylamine
69 Benzquinamide
69 Bromocriptine
69 Butoxyethyl Nicotinate
69 Clemizole
69 Clomethiazole
69 Cropropamide
69 Mebanazine
69 Selegiline
69 Tetrazepam
70 Benzfetamine
70 Clobazam
70 Clorgiline
70 Doxapram
70 Nicofuranose
70 Propanidid
70 Verapamil
71 Benzyl Nicotinate
71 Dextromoramide
71 Etomidate
71 Lidocaine
72 Flunitrazepam
72 Trimetozine
73 Bupivacaine
73 Camazepam
73 Diazepam
73 Metaclazepam
74 Diloxanide
74 Famprofazone
74 Fentanyl
74 Isocarboxazid
74 Medazepam
74 Noscapine
74 Prazepam
74 Reserpine
75 Dimoxyline
75 Quazepam
75 Rescinnamine
76 Bisacodyl
76 Phenoxybenzamine
77 Deserpidine
77 Methoserpidine
77 Methoxyamfetamine
77 Quazepam -CFTB
77 Thiambutosine
78 Cinnarizine
78 Disulfiram
78 Fenbutrazate
78 Tetrabenazine
78 Tiocarlide
79 Bezitramide
79 Bromhexine
79 Broxaldine
79 Meclozine
79 Phenothiazine
80 Bialamicol
80 Methaqualone
81 Piperidolate
83 Buclizine
84 Diphenoxylate
89 Oxo-quazepam
99 Azinphos-(Me)

9

System TD

00 Barbituric Acid
00 Carbazochrome
00 Carbidopa
00 Cinchophen
00 Dipyrone
00 Enalapril
00 Levodopa
00 Lysergic Acid
00 Paramethadione
00 Phthalylsulfacetamide
00 Phthalylsulfathiazole
00 Salicyluric Acid
00 Salicyluric Acid
00 Sodium Cromoglicate
00 Succinylsulfathiazole
00 Sulfasalazine
00 Valproic Acid
01 Baclofen
01 Bumetanide
01 Captopril
01 Digoxin
01 Furosemide
01 Mafenide
01 Nicotinic Acid
01 Saccharin
01 Sulfaguanidine
02 Chlorothiazide
02 Nitrofurantoin
02 Prenalterol
02 Trifluperidol
03 Digitoxin
03 Etacrynic Acid
03 Flurazepam
03 Loprazolam
03 Niflumic Acid
04 Acetazolamide
04 Aloxiprin
04 Chlortalidone
04 Clozapine
04 Dichlorophenoxyacetic Acid
04 Ethyl Biscoumacetate
04 Hydrochlorothiazide
04 Quinethazone
04 Sulfinpyrazone
05 Aminosalicylic Acid
05 6-Hydroxybrotizolam
05 Sulfisomidine
05 Triazolam
07 Alprazolam
07 Carbenoxolone
07 Hydroflumethiazide
07 α-Hydroxybrotizolam
07 Methocarbamol
07 Salicylic Acid
07 Salicylic Acid
08 5-Hydroxypiroxicam
08 Diflunisal
08 Primidone
09 Meprobamate
09 Methylchlorophenoxyacetic Acid
09 Sulfathiazole
10 Chlordiazepoxide
10 Mebutamate
11 Bufexamac
11 Butalamine
11 Chloramphenicol
11 Chlorphenesin Carbamate
11 Diphenadione
11 Ethiazide
11 Guaifenesin
12 Sulfamethizole
12 Zomepirac
13 Bromazepam
13 Epithiazide
13 Midazolam
13 Probenecid
13 Styramate
13 Sulfanilamide
13 Tolmetin
14 Benzthiazide
14 Diclofenamide
14 Mephenesin Carbamate
14 Sulfaethidole
14 Sulindac
15 Brotizolam
15 Caffeine
15 Demoxepam
15 Hydroxy-*N*-dealkyl-2-oxo-quazepam
15 Paracetamol
15 Trichlormethiazide
16 Indometacin
16 Sulfapyridine
17 Diiodohydroxyquinoline
17 Sulfacetamide
18 Alclofenac
18 Aspirin
18 Cyclothiazide
18 Dicoumarol
18 Fenbufen
18 Phenazone
19 Aminobenzoic Acid
19 Clopamide
19 Dantrolene
19 Methyclothiazide
19 Sulfamethoxypyridazine
21 4-Aminophenol
21 Cyclopenthiazide
21 Enoxolone
22 Oxazepam
22 Phenacemide
22 Polythiazide
22 Sulfadiazine
23 Lorazepam
23 Metolazone
23 Sulfadimidine
23 Sulfamerazine
23 Sultiame
24 Sulfametoxydiazine
25 Aminophenazone
25 Bendroflumethiazide
25 Diclofenac
25 Sulfafurazole
26 Benzoic Acid
26 Sulfamethoxazole
27 Ketoprofen
29 Phenylmethylbarbituric Acid
29 Sulfaphenazole
30 Flumazenil
30 Flurbiprofen
30 Glibenclamide
30 Lysergide
30 Trilostane
31 Clorexolone
31 Methyprylon
31 Sulfadimethoxine
32 Aminoglutethimide
32 Hexachlorophene
33 Apronal
33 Chlorambucil
33 Naproxen
33 Normetaclazepam
33 Phenytoin
34 Clorazepic Acid
34 *N*-dealkyl-2-oxo-quazepam
34 Nordazepam
35 Alfadolone
35 Bromisoval
35 Clonazepam
35 Nitrazepam
35 Nortetrazepam
35 Tybamate
36 Carisoprodol
37 Sulfadoxine
38 Chlorpropamide
38 Etamivan
38 Indapamide
38 Phenacetin
38 Pheneturide
38 Phenolphthalein

38 Salicylamide
38 Vinylbital
38 Xipamide
39 Acetohexamide
39 Nalidixic Acid
40 Glibornuride
40 Sulfametopyrazine
41 Barbital
41 Mefenamic Acid
42 Fenoprofen
42 Hydroxy-2-oxo-quazepam
43 Ethoxzolamide
45 Acetanilide
45 Ketazolam
45 Mefruside
46 Ibuprofen
46 Lormetazepam
47 Metaclazepam
47 Phenobarbital
47 Phenprobamate
48 Aprobarbital
49 Acecarbromal
49 Ethinamate
49 Methylpentynol
50 Allobarbital
50 Butobarbital
50 Cyclobarbital
50 Cyclopentobarbital
50 Ethosuximide
50 Heptabarb
50 Ibomal
50 Secbutabarbital
50 Vinbarbital
51 Piroxicam
51 Temazepam
51 Tolbutamide
52 Acenocoumarol
52 Amobarbital
52 Bemegride
52 Brallobarbital
52 Griseofulvin
52 Oxyphenbutazone
52 Tolazamide
53 Carbromal
53 Clobazam
53 Ethotoin
53 Hexethal
53 Talbutal
54 Butalbital
54 Chlorzoxazone
54 Flunitrazepam
54 Medazepam
55 Camazepam
55 Idobutal
55 Pentobarbital
55 Secobarbital
56 Benzocaine
57 Tetrazepam
58 Diazepam
58 Nealbarbital
59 Dichlorophen
59 Oxoquazepam
60 Triclocarban
61 Cholesterol
61 Estradiol
61 Propyphenazone
62 Benziodarone
62 Mephenytoin
62 Methyl Hydroxybenzoate
62 Phenprocoumon
63 Carbimazole
63 Glutethimide
63 Methaqualone
64 Ethyl Hydroxybenzoate
64 Prazepam
64 Santonin
64 Warfarin
65 Hexobarbital
65 Phenindione
66 Metharbital
66 Spironolactone
70 Methylphenobarbital
71 Enallylpropymal
71 Glyceryl Trinitrate
71 Phensuximide
73 Coumatetralyl
73 Methohexital
73 Thymol
74 Cyclandelate
74 Quazepam-CFTB
75 Clofibrate
77 Thialbarbital
77 Thiopental
78 Phenylbutazone
78 Quazepam
80 Dantron
81 Ethchlorvynol
82 Clofenotane
91 Azinphos-(Me)

System TE

00 Acetylcysteine
00 Alcuronium Chloride
00 Amidefrine
00 Aniline
00 Atropine Methonitrate
00 Baclofen
00 Barbituric Acid
00 Captopril
00 Carbachol
00 Carbenoxolone
00 Carbidopa
00 Cefaclor
00 Ceftriaxone
00 Cetrimide
00 Ciprofloxacin
00 Decamethonium
00 Doxorubicin
00 Enalapril
00 Enalaprilat
00 Epirubicin
00 Guanoxan
00 Homatropine Methylbromide
00 Levodopa
00 Lisinopril
00 Lysergic Acid
00 Mandelic Acid
00 Metformin
00 Methotrexate
00 Narceine
00 Nedocromil
00 Neostigmine Bromide
00 Nicotinic Acid
00 Obidoxime Chloride
00 Ofloxacin
00 Oxytetracycline Dihydrate
00 Pancuronium Bromide
00 Paraquat
00 Pentolonium Tartrate
00 Phthalylsulfacetamide
00 Phthalylsulfathiazole
00 Pralidoxime Mesilate
00 Psilocybine
00 Pyridostigmine Bromide
00 Salicyluric Acid
00 Salicyluric Acid
00 Sodium Cromoglicate
00 Strophanthin-K
00 Succinylsulfathiazole
00 Sulfasalazine
00 Suxamethonium Chloride
00 Thiazinamium Metilsulfate
00 Thiazinamium Sulfoxide
00 Trimetaphan Camsilate
00 Trimethadione
00 Tubocurarine Chloride
00 Valproic Acid
01 Aminobenzoic Acid
01 Clidinium Bromide
01 Glycopyrronium Bromide
01 Guanethidine
01 Hexocyclium Metilsulfate
01 Mupirocin
01 Oxyphenonium Bromide
01 Thiamine
02 Chlorothiazide
02 Clenbuterol-(M_2)
02 Dimenhydrinate
02 Dipyrone
02 Emepronium Bromide
02 Mercaptopurine
02 Methyldopa
02 Nalidixic Acid
03 5-Hydroxy-piroxicam
03 Acetazolamide
03 Cilazapril
03 Denatonium Benzoate
03 Distigmine Bromide
03 Isopropamide Iodide
03 *N*-Desmethylpirenzepine
03 Noradrenaline
03 Penicillamine
03 Penthienate Methobromide
03 Perindoprilat
04 Alclofenac
04 Bumetanide
04 Dichlorophenoxyacetic Acid
04 Fenbufen
04 Fluorouracil
04 Propantheline Bromide
04 Sulfacetamide
04 Sulfadiazine
04 Sulfamethizole
04 Sulindac
04 Tiaprofenic Acid
04 Zomepirac
05 Etacrynic Acid
05 Glibornuride
05 Glymidine Sodium
05 Indometacin
05 Methylchlorophenoxyacetic Acid
05 Probenecid
05 Sulfamethoxazole
05 Sulfisomidine
05 Tolmetin
05 Xanthanoic Acid
06 Azathioprine
06 Chlorambucil
06 Clenbuterol-(M_3)
06 Fenoprofen
06 Flurbiprofen
06 Furosemide
06 Ibuprofen
06 Idarubicin
06 Ketoprofen
06 Lanatoside C
06 Naproxen
06 Nitrofurantoin
06 Oxyphencyclimine
06 Perindopril
06 Rimiterol
06 Sulfafurazole
07 Aminosalicylic Acid
07 Benzoic Acid
07 Enoxolone
07 Glipizide
07 Histamine
07 Paramethadione
07 Saccharin
07 Tolazamide
07 Trilostane
08 Azapropazone
08 Cinchophen
08 Sulfaethidole
08 Sulfamerazine
08 Sulfametopyrazine

44 Triazolam
44 Zuclopenthixol
45 Fluphenazine
45 Hydrocortisone
45 Methysergide
45 Nicotinyl Alcohol
45 Oxazepam
45 Oxprenolol
45 Paracetamol
45 Pentobarbital
45 Phenazone
45 Prednisone
45 Quinine
45 Secobarbital
45 Thebaine
45 Trimethoprim
45 α-Hydroxybrotizolam
46 Chloroquine
46 Chlorphenamine
46 Dinormetaclazepam
46 Diphenadione
46 Erythromycin
46 Ethyl Hydroxybenzoate
46 Flupentixol
46 Fluvoxamine
46 Furazolidone
46 Glafenine
46 Metronidazole
46 Pheniramine
46 Phenmetrazine
46 Talbutal
46 Thiopropazate
47 Alprazolam
47 Antazoline
47 Dextromethorphan
47 Dimetindene
47 Fluoxetine
47 Methocarbamol
47 Metipranolol
47 Naloxone
47 Norfluoxetine
47 Perazine
47 Psilocin
47 Trimethobenzamide
47 Zopiclone
48 Bisoprolol
48 Chlorphentermine
48 Fenpipramide
48 Hyoscine
48 Phentermine
48 Pipazetate
48 Secbutabarbital
48 Tofenacin
48 Zimeldine
49 Alprenolol
49 Diamorphine
49 Dimethoxanate
49 Dixyrazine
49 Dobutamine
49 Flecainide
49 Hydroxy-*N*-dealkyl-2-oxo-quazepam
49 Propranolol
49 Proxyphylline
49 Quinidine
49 Thebacon
49 Thiopental
50 Carbinoxamine
50 Dimethyltryptamine
50 Ethiazide
50 Fluocortolone
50 Ketoconazole
50 Resorcinol
50 Salicylamide
50 Timolol
51 Betaxolol
51 Granisetron
51 Metoclopramide
51 Norastemizole
51 Pericyazine
51 Sulfanilamide
51 Tropicamide
52 Bendroflumethiazide
52 Brotizolam
52 Butaperazine
52 Caffeine
52 Chlordiazepoxide
52 Chlorphenesin Carbamate
52 Etizolam
52 Gelsemine
52 Harmine
52 Hordenine
52 Ketotifen
52 Moclobemide
52 Thenalidine
52 Thiethylperazine
53 Emetine
53 Hexobarbital
53 Lofexidine
53 Mazindol
53 Methyclothiazide
53 Phenolphthalein
53 Pipotiazine
53 Propyl Hydroxybenzoate
53 Styramate
54 Acetylcodeine
54 Encainide
54 Fenetylline
54 Hydroxyzine
54 Loxapine
54 Metharbital
54 Oxcarbazepine
54 Phanquinone
54 Phenethylamine
54 Pyrazinamide
54 Remoxipride
54 Sulforidazine
55 Clopamide
55 Clozapine
55 Dibenzepin
55 Ethoheptazine
55 Mephenesin Carbamate
55 Mexiletine
55 Pentoxifylline
55 Physostigmine
55 Prochlorperazine
55 Thiamylal
55 Trifluoperazine
55 Triprolidine
56 Ajmaline
56 Amygdalin
56 Carbamazepine
56 Diltiazem
56 Etafedrine
56 Lysergide
56 Meprobamate
56 Tizanidine
57 Acecarbromal
57 Aminoacridine
57 Harman
57 Metolazone
57 Sultiame
57 Vinblastine
58 Apomorphine
58 Clemastine
58 Clenbuterol
58 Droperidol
58 Enallylpropymal
58 Mepyramine
58 Methohexital
58 Morazone
58 Nimorazole
58 Pyrimethamine
58 Tranylcypromine
59 4-Aminophenol
59 Arecoline
59 Cyclothiazide
59 Lormetazepam
59 Nefopam
59 Nikethamide
59 Perhexiline
59 Prazosin
59 Prothipendyl
60 Aminopromazine
60 Citalopram
60 Clorexolone
60 Disopyramide
60 Doxylamine
60 Eucatropine
60 Hexylresorcinol
60 Mebendazole
60 Mebutamate
60 Midazolam
60 Pargyline
60 Pentetrazol
60 Pethidine
61 Desbutylhalofantrine
61 Fenfluramine
61 Flumazenil
61 Metixene
61 Nicotine
61 Piritramide
61 Trichlorfon
62 Alphaprodine
62 Aminophenazone
62 Amiphenazole
62 Buphenine
62 Carbocromen
62 Chlorphenesin
62 Cisapride
62 Isoxsuprine
62 Oxycodone
62 Phendimetrazine
62 Promazine
62 Temazepam
63 Acepromazine
63 Bromazepam
63 Chloropyramine
63 Diethyltryptamine
63 Doxepin
63 Isopropylaminophenazone
63 Methdilazine
63 Methyprylon
63 Polythiazide
64 Astemizole
64 Cyclopentolate
64 Cyproheptadine
64 Isothipendyl
64 Metyrapone
64 Nitrazepam
64 Nomifensine
64 Pizotifen
64 Tetracaine
64 Yohimbine
65 Aminoglutethimide
65 Cyclazocine
65 Diphenhydramine
65 Dosulepin
65 Levamisole
65 Mebhydrolin
65 Pecazine
65 Phenacemide
65 Phenylbutazone
65 Promethazine
65 Psilocin -ethyl
65 Thenyldiamine
65 Thonzylamine
65 Tybamate
66 Cyclopentamine
66 Cyclopenthiazide
66 Ethosuximide
66 Indapamide
66 Mepivacaine
66 Methapyrilene
66 Methylphenidate
66 Noxiptiline
66 Pentifylline
66 Tetramisole
66 Trazodone
67 Apronal
67 Chlorcyclizine
67 Cinchocaine
67 Clonazepam
67 Imipramine
67 Meclofenoxate
67 Mefruside
67 Nifuratel
67 Nordazepam
67 Oxeladin
67 Phenyltoloxamine
67 Thioridazine
68 Bemegride
68 Bromisoval
68 Chlormezanone
68 Clorazepic Acid
68 Cortisone

68 Cyclizine
68 Dimenhydrinate
68 Diphenylpyraline
68 Fluorometholone
68 Flutazolam
68 Mianserin
68 Nicametate
68 Orphenadrine
68 Phenacetin
68 Phenindamine
68 Propiomazine
68 Simvastatin
68 Tolpropamine
68 Trimetozine
68 Tripelennamine
69 Amitriptyline
69 Crotetamide
69 Cyclobenzaprine
69 Griseofulvin
69 Papaverine
69 Hydroxy-2-oxo-quazepam
70 Acetanilide
70 Chlorphenoxamine
70 Chlorpromazine
70 Clonidine
70 Desamide
70 Lidoflazine
70 Pentazocine
70 Phenazopyridine
70 Testosterone
71 Alfadolone
71 Buspirone
71 Ethinylestradiol
71 Ethotoin
71 Etynodiol Diacetate
71 Flurazepam
71 Fluspirilene
71 Hexoprenaline
71 Hydrastine
71 Nifedipine
71 Phenaglycodol
71 Pheneturide
71 Pimozide
71 Procaine
71 Pyrrobutamine
71 Veratrine
72 Alfentanil
72 Androsterone
72 Azaperone
72 Clomipramine
72 Doxapram
72 Etorphine
72 Methyl Nicotinate
72 *N*-dealkyl-2-oxo-quazepam
72 Pentaerithrityl Tetranitrate
72 Propanidid
72 Sertraline
73 Diethylstilbestrol
73 Doxazosin
73 Etomidate
73 Hexestrol
73 Methylpiperidyl Benzilate
73 Methyltestosterone
73 Nicergoline
73 Normetaclazepam
73 Nortetrazepam
73 Phenprobamate
73 Pramocaine
73 Verapamil
74 Amodiaquine
74 Chlorprothixene
74 Cropropamide
74 Etamiphylline
74 Flunitrazepam
74 Indoramin
74 Isradipine
74 Ketazolam
74 Levallorphan
74 Loperamide
74 Mephenytoin
74 Methylpentynol
74 Metomidate
74 Phenazocine
74 Procyclidine
74 Propyphenazone
74 Thiopropazate
75 Aconitine
75 Butanilicaine
75 Camazepam
75 Carbromal
75 Carisoprodol
75 Clobazam
75 Econazole
75 Etidocaine
75 Histapyrrodine
75 Isocarboxazid
75 Lobeline
75 Mevinphos
75 Prilocaine
75 Santonin
75 Triclocarban
75 Trifluprom azine
76 Ambucetamide
76 Aprindine
76 Cholesterol
76 Clomethiazole
76 Clotrimazole
76 Diazepam
76 Estrone
76 Etenzamide
76 Ethambutol
76 Ethinamate
76 Fenyramidol
76 Haloperidol
76 Levomepromazine
76 Mecloqualone
76 Methanthelinium Bromide
76 Methoxsalen
76 Norethisterone
76 Phenoperidine
76 Piperocaine
77 Alimemazine
77 Benzocaine
77 Captodiame
77 Cocaine
77 Diethazine
77 Felodipine
77 Fencamfamin
77 Fenproporex
77 Flavoxate
77 Methadone
77 Phensuximide
77 Reserpine
77 Trifluperidol
78 Azapetine
78 Clemizole
78 Disulfiram
78 Estradiol
78 Etofenamate
78 Fentanyl
78 Loratadine
78 Medazepam
78 Methaqualone
78 Mexazolam
78 Naftidrofuryl Oxalate
78 Nimodipine
78 Noscapine
78 Oxypertine
78 Spironolactone
78 Tramadol
78 Xylazine
79 Anileridine
79 Dextromoramide
79 Hexetidine
79 Itraconazole
79 Ketamine
79 Metaclazepam
79 Progesterone
79 Prolintane
79 Sulfadoxine
79 Tetrabenazine
79 Tetrazepam
80 Bupivacaine
80 Buprenorphine
80 Cyclandelate
80 Glutethimide
80 Hexylcaine
80 Hydralazine
80 Lidocaine
80 Lorcainide
80 Metformin
80 Methandienone
80 Miconazole
80 Nitrendipine
80 Norpipanone
80 Oxo-quazepam
80 Procarbazine
80 Terodiline
80 Trimipramine
81 Butetamate
81 Demeton-*S* -methyl
81 Deserpidine
81 Diethylthiambutene
81 Isoaminile
81 Lacidipine
81 Pipradrol
81 Prazepam
81 Rescinnamine
81 Thymol
82 Amiodarone
82 Clomifene
82 Fluanisone
82 Phenothiazine
82 Piperidolate
83 Adiphenine
83 Biperiden
83 Butacaine
83 Crotamiton
83 Dichlorvos
83 Gestonorone Caproate
83 Oxybuprocaine
83 Phenelzine
83 Profenamine
83 Quazepam
83 Trihexyphenidyl
84 Clofibrate
84 Dicycloverine
84 Methyl Salicylate
84 Penfluridol
84 Phencyclidine
84 Prenylamine
84 Tilidate
85 Diethylpropion
85 Floctafenine
85 Hydroxyprogesterone
85 Nisoldipine
85 Quazepam -CFTB
86 Butalamine
86 Cinnarizine
86 Clofazimine
86 Fenbutrazate
86 Fludrocortisone
86 Glyceryl Trinitrate
86 Ibogaine
86 Lindane
86 Mebeverine
86 Mesuximide
87 Benzfetamine
87 Chlordane
87 Clofenotane
87 Dimoxyline
87 Diphenoxylate
87 Dipipanone
87 Eugenol
87 Famprofazone
87 Meclozine
87 Metenolone
87 Methoxychlor
87 Phenoxybenzamine
88 Halofantrine
88 Paramethasone
88 Piminodine
89 Dieldrin
89 Heptachlor
90 Lofepramine
91 Difenidol
95 CBD
95 CBN
98 Boldenone
99 Azinphos-(Me)
99 Chlorpyrifos
99 Dimethoate
99 Malathion

System TF

00 Acetylcysteine
00 Baclofen
00 Barbituric Acid
00 Betaxolol
00 Bezafibrate
00 Bisoprolol
00 Carbazochrome
00 Cefaclor
00 Ceftriaxone
00 Cilazapril
00 Cinchophen
00 Ciprofloxacin
00 Clenbuterol-(M_2)
00 Dipyrone
00 Dosulepin-*S*-oxide
00 Doxorubicin
00 Enalapril
00 Enalaprilat
00 Epirubicin
00 Fenoterol
00 Idarubicin
00 Levodopa
00 Lisinopril
00 Lysergic Acid
00 Mandelic Acid
00 Mercaptopurine
00 Methotrexate
00 Metipranolol
00 Mupirocin
00 Nedocromil
00 Neostigmine Bromide
00 Nicotinic Acid
00 Ofloxacin
00 Perindopril
00 Perindoprilat
00 Phthalylsulfacetamide
00 Phthalylsulfathiazole
00 Pralidoxime Mesilate
00 Pyridostigmine Bromide
00 Salicyluric Acid
00 Salicyluric Acid
00 Sodium Cromoglicate
00 Succinylsulfathiazole
00 Sulfasalazine
00 Sulforidazine
00 Thiazinamium Sulfoxide
00 Trimetaphan Camsilate
00 Trimethadione
00 Xamoterol
00 Xantinol Nicotinate
00 Zopiclone
01 Alphaprodine
01 Captopril
01 Loprazolam
01 Mafenide
01 Pecazine
01 Saccharin
01 Salicylic Acid
01 Salicylic Acid
01 Tertatolol
02 Alprazolam
02 Carbidopa
02 Etacrynic Acid
02 Levocabastine
02 Prenalterol
02 Triazolam
03 Benperidol
03 Flurazepam
03 Glafenine
03 Niflumic Acid
03 Tropicamide
04 6-Hydroxybrotizolam
04 Sulfinpyrazone
04 Theobromine
05 Brotizolam
05 Buspirone
05 Clozapine
05 Diflunisal
05 Digoxin
05 Midazolam
05 Tiaprofenic Acid
05 Trifluperidol
06 Dichlorophenoxyacetic Acid
06 Etofylline
06 Sulfaguanidine
06 Tenoxicam
06 α-Hydroxybrotizolam
07 Furosemide
07 Miconazole
08 Alfentanil
08 Xanthanoic Acid
09 Dinormetaclazepam
09 Theophylline
10 Aloxiprin
10 Aminophenazone
10 Bumetanide
10 Caffeine
10 Chlordiazepoxide
10 Labetalol
10 Sulindac
11 Itraconazole
11 Methylchlorophenoxyacetic Acid
11 Nitrofurazone
11 Pemoline
12 Zomepirac
13 Indometacin
14 Flumazenil
14 Phenazone
16 Chlorothiazide
16 Sulfisomidine
17 Carbenoxolone
17 Guaifenesin
18 5-Hydroxy-piroxicam
18 Bromazepam
18 Furazolidone
19 Bufexamac
19 Clenbuterol-(M_3)
19 Diiodohydroxyquinoline
20 Fluorouracil
20 Methylenedioxymetamfetamine
20 Oxcarbazepine
20 Sulfathiazole
20 Tolmetin
21 Quinethazone
23 Fluanisone
23 Methocarbamol
23 Primidone
23 Probenecid
23 Sulfamethizole
24 Aminosalicylic Acid
24 Demoxepam
24 Prednisolone
25 Denatonium Benzoate
25 Dinitro-orthocresol
25 Ketoprofen
25 Methyprylon
25 Normetaclazepam
27 Diclofenac
27 Methylprednisolone
28 Alclofenac
28 Benzoic Acid
28 Fluocortolone
28 Hydrocortisone
28 Hydroxy-*N*-dealkyl-2-oxo-quazepam
28 Prednisone
29 Butalamine
29 Loratadine
30 Aspirin
30 Fenbufen
30 Flurbiprofen
30 Glibenclamide
30 Nitrofurantoin
31 Acetazolamide
31 Chloramphenicol
31 Nalidixic Acid
31 Tolfenamic Acid
32 Camazepam
32 Dicoumarol
32 Ethyl Biscoumacetate
32 Paracetamol
33 Diphenadione
34 Chlorphenesin Carbamate
34 Hexachlorophene
34 Hydrochlorothiazide
35 Etamivan
35 Mebutamate
35 Metaclazepam
35 Oxazepam
35 Sulfaethidole
36 Dantrolene
36 Mephenesin Carbamate
36 Meprobamate
37 Griseofulvin
37 Phenacetin
38 Clopamide
38 Fenoprofen
38 Naproxen
38 Piroxicam
39 Styramate
39 Sulfadiazine
39 Sulfamethoxypyridazine
40 4-Aminophenol
40 Alfadolone
40 Chlorambucil
40 Chlortalidone
40 Medazepam
40 Phenacemide
41 Lorazepam
41 Sulfamerazine
42 Sulfacetamide
42 Sulfapyridine
43 Acetohexamide
43 Acitretin
43 Aminobenzoic Acid
43 Chlorpropamide
43 Meclofenamic Acid
43 Nifuratel
43 Sulfametoxydiazine
43 Sultiame
44 Simvastatin
45 Acetanilide
45 Clonazepam
45 Isotretinoin
45 Ketazolam
45 Lormetazepam
45 *N*-dealkyl-2-oxoquazepam
45 Nordazepam
45 Nortetrazepam
45 Sulfadimidine
45 Testosterone
45 Trilostane
46 Clorazepic Acid
46 Enoxolone
46 Nitrazepam
46 Sulfanilamide
47 Aminoglutethimide
47 Carbimazole
47 Clobazam
47 Flunitrazepam
47 Hydroflumethiazide
47 Methyltestosterone
47 Temazepam
48 Acecarbromal
48 Acenocoumarol
48 Mefenamic Acid
49 Benzbromarone
49 Diazepam
49 Propyphenazone
49 Tetrazepam
50 Ethiazide
50 Methyclothiazide
50 Santonin
50 Sulfametopyrazine
50 Tolazamide
51 Benzthiazide
51 Bromisoval
51 Clorexolone
51 Metolazone
51 Spironolactone
51 Sulfadimethoxine
51 Sulfadoxine
51 Sulfaphenazole
52 Androsterone
52 Apronal
52 Fluorometholone
52 Sulfafurazole
52 Valproic Acid
53 Bemegride
53 Carisoprodol

53 Ethosuximide
53 Pheneturide
54 Ethotoin
54 Sulfamethoxazole
55 Carbromal
55 Mexazolam
55 Phenprobamate
55 Phenytoin
55 Prazepam
55 Salicylamide
55 Tolbutamide
56 Phenindione
56 Progesterone
57 Etynodiol Diacetate
57 Ibuprofen
57 Norethisterone
57 Hydroxy-2-oxoquazepam
58 Benziodarone
58 Estradiol
58 Mefruside
58 Mephenytoin
58 Methoxsalen
58 Phenprocoumon
59 Ethinamate
59 Gestonorone Caproate
59 Isradipine
59 Phenolphthalein
59 Phensuximide
59 Triclocarban
60 Cholesterol
60 Cyclothiazide
60 Felodipine
60 Glibornuride
60 Paramethadione
60 Polythiazide
60 Trichlormethiazide
61 Barbital
61 Chlorzoxazone
61 Indapamide
61 Phenylmethylbarbituric Acid
62 Benzocaine
62 Cyclopenthiazide
62 Epithiazide
62 Glutethimide
62 Metenolone
62 Methylpentynol
62 Oxyphenbutazone
62 Warfarin
63 Hydroxyprogesterone
64 Cyclobarbital
64 Diclofenamide
64 Dimpylate
64 Heptabarb
64 Secbutabarbital
64 Vinylbital
64 Xipamide
65 Aprobarbital
65 Butobarbital
65 Cyclopentobarbital
65 Ethoxzolamide
65 Hexobarbital
65 Hexylresorcinol
65 Lacidipine
65 Metharbital
65 Methyl Hydroxybenzoate
65 Nisoldipine
65 Phenobarbital
65 Vinbarbital
66 Allobarbital
66 Amobarbital
66 Clofibrate
66 Ethyl Hydroxybenzoate
66 Ibomal
66 Pentobarbital
67 Butalbital
67 Dichlorophen
67 Hexethal
67 Methylphenobarbital
67 Propyl Hydroxybenzoate
67 Talbutal
68 Brallobarbital
68 Diethylstilbestrol
68 Estrone
68 Methyl Salicylate
68 Nealbarbital
68 Phenylbutazone
68 Secobarbital
68 Thymol
68 Tybamate
69 Dantron
69 Idobutal
70 Clidinium Bromide
70 Hexestrol
71 Bendroflumethiazide
71 Enallylpropymal
71 Quazepam
72 Chlordane
72 Glyceryl Trinitrate
72 Methohexital
72 Methoxychlor
72 Thialbarbital
73 Benzyl Benzoate
73 Quazepam-CFTB
74 Clofenotane
74 Coumatetralyl
74 Dieldrin
74 Ethchlorvynol
74 Thiopental
75 Heptachlor
75 Lindane
75 Thiamylal
77 Cyclandelate
83 Oxoquazepam
93 Azinphos-(Me)

System Tf

00 Salbutamol
10 Digitoxin

System TG

08 Indoprofen
09 Fenbufen
10 Tolmetin
12 Alclofenac
13 Sulindac
14 Benoxaprofen
14 Ketoprofen
14 Naproxen
16 Fenoprofen
16 Flurbiprofen
18 Ibuprofen
19 Furosemide
20 Fenclofenac
20 Indometacin
23 Phenylbutazone
23 Salsalate
25 Oxyphenbutazone
28 Niflumic Acid
29 Diclofenac
30 Clonixin
32 Mefenamic Acid
33 Flunixin
33 Theophylline
36 Bufexamac
37 Diflunisal
37 Flufenamic Acid
38 Meclofenamic Acid
45 Feprazone
47 Theobromine

System TH

03 Barbituric Acid
27 Phenylmethylbarbituric Acid
38 Phenobarbital
47 Brallobarbital
51 Barbital
53 Allobarbital
56 Vinbarbital
59 Cyclobarbital
61 Ibomal
62 Cyclopentobarbital
62 Heptabarb
66 Aprobarbital
67 Butalbital
68 Butobarbital
69 Secbutabarbital
71 Idobutal
71 Talbutal
72 Methylphenobarbital
74 Amobarbital
74 Hexethal
75 Thialbarbital
76 Pentobarbital
78 Nealbarbital
78 Secobarbital
80 Thiopental
85 Hexobarbital
86 Metharbital
87 Enallylpropymal
93 Methohexital

System TI

05 CBD
30 Δ^9-THC
30 Δ^9-Tetrahydrocannabinol
52 CBN

System TJ

20 CBN
29 Δ^9-THC
29 Δ^9-Tetrahydrocannabinol
36 CBD

System TK

72 Digitoxin

System TL

00 Bamethan
00 Benzalkonium Chloride
00 Berberine
00 Buformin
00 Carbachol
00 Cetrimide
00 Cetylpyridinium Chloride
00 Cinchophen
00 Cotarnine
00 Debrisoquine
00 Decamethonium
00 Diminazene (aceturate)
00 Diquat
00 Dopamine
00 Guanethidine
00 Guanoclor
00 Guanoxan
00 Histamine
00 Homatropine Methylbromide
00 Hydrastinine
00 Hydroxystilbamidine
00 Isoetarine
00 Isopropamide Iodide
00 Lysergic Acid
00 Mequitazine
00 Metformin
00 Minoxidil
00 Narceine
00 Noradrenaline
00 Obidoxime Chloride
00 Oxyphencyclimine
00 Oxyphenonium Bromide
00 Pancuronium Bromide
00 Paraquat
00 Pentamidine
00 Pentapiperide Metilsulfate
00 Pentolonium Tartrate
00 Phenformin
00 Phenylephrine
00 Piperazine
00 Propantheline Bromide
00 Pseudomorphine
00 Psilocybine
00 Suxamethonium Chloride
00 Thiamine
00 Thiazinamium Metilsulfate
00 Tubocurarine Chloride
01 Acefylline Piperazine
01 Atropine
01 Azacyclonol
01 Brucine
01 Bufotenine
01 Chlorproguanil
01 Cytarabine
01 Deptropine
01 Dihydralazine
01 Dihydromorphine
01 Dimethoate
01 Ephedrine
01 Hexoprenaline
01 Homatropine
01 Methyldopa
01 Morphine
01 Nadolol
01 Oxymetazoline
01 Proguanil
01 Propamidine
01 Trimetazidine
02 Amidefrine
02 Amiloride
02 Atenolol
02 Chloroquine
02 Chlorphenamine
02 Cyclopentamine
02 Cycloserine
02 Cytisine
02 Dehydroemetine
02 Dihydrocodeine
02 Dipyrone
02 Ethambutol
02 Hydromorphone
02 Maprotiline
02 Mephentermine
02 Methoxyphenamine
02 Phentolamine
02 Pholcodine
02 Strychnine
02 Tetryzoline
02 Tolazoline
02 Tramazoline
03 Acetophenazine
03 Antazoline
03 Benserazide
03 Broxyquinoline
03 Codeine
03 Conessine
03 Desipramine
03 Dextrorphan
03 Dobutamine
03 Etilefrine
03 Hydroquinine
03 Hydroxychloroquine
03 Isoprenaline
03 Methenamine
03 Penicillamine
03 Perazine
03 Pheniramine
03 Pholedrine
03 Xylometazoline
04 Amantadine
04 Azathioprine
04 Carbinoxamine
04 Diamorphine
04 Ethoheptazine
04 Fenoterol
04 Hydrocodone
04 Mecamylamine
04 Neopine
04 Practolol
04 Procarbazine
04 Quinine
04 Salbutamol
04 Triamterene
05 Butaperazine
05 Cinchonine
05 Coniine
05 Diethylcarbamazine
05 Glipizide
05 Heptaminol
05 Hordenine
05 Hydroquinidine
05 Metamfetamine
05 Pyridoxine
05 Sparteine
05 Terbutaline
05 Thebaine
06 Brompheniramine
06 Cinchonidine
06 Dextromethorphan
06 Dimethoxanate
06 Dimethyltryptamine
06 Dimetindene
06 Ethylmorphine
06 Hexobendine
06 Ketobemidone
06 Lysergamide
06 Methdilazine
06 Orciprenaline
06 Perhexiline
06 Pipazetate
06 Protokylol
06 Quinidine
06 Thioproperazine
06 Triprolidine
06 Viloxazine
07 Carfenazine
07 Propranolol
07 Tigloidine
07 Tiotixene
07 Tofenacin
08 Ambazone
08 Benzylmorphine
08 Cephaëline
08 Chlorphentermine
08 Diphenylpyraline
08 Ergometrine
08 Ergometrine
08 Phenglutarimide
08 Pindolol
08 Pipamperone
08 Profadol
08 Sulfacarbamide
08 Thiethylperazine
09 Azaperone
09 Benzydamine
09 Clemastine
09 Clioquinol
09 Doxylamine
09 Mepacrine
09 Metoprolol
09 Perphenazine
09 Psilocin
09 Timolol
10 Dimenhydrinate
10 Fluphenazine
10 Pempidine
10 Tacrine
11 Alphaprodine
11 Clopenthixol
11 Diethyltryptamine
11 Fenfluramine
11 Hydroxyamfetamine
11 Pethidine
11 Promazine
11 Thebacon
11 Theophylline
11 Tryptamine
12 Acetylcodeine
12 Carbocromen
12 Cimetidine
12 Colchicine
12 Diprophylline
12 Emetine
12 Eucatropine
12 Mescaline
12 Methylergometrine
12 Metixene

61 Clonazepam
61 Isocarboxazid
61 Nicofuranose
62 Benorilate
62 Butoxyethyl Nicotinate
62 Clobazam
62 Ibogaine
62 Medazepam
63 Amylocaine
63 Etorphine
63 Flunitrazepam
63 Lidocaine
63 Mebanazine
63 Pentetrazol
63 Prazepam
63 Pseudoephedrine
64 Beclamide
64 Biperiden
64 Butacaine
64 Diethylpropion
64 Hydralazine
64 Ketamine
64 Methoserpidine
64 Norbormide
64 Noscapine
64 Rescinnamine
65 Bupivacaine
65 Cinnarizine
66 Benzocaine
66 Bisacodyl
66 Diperodon
66 Ketazolam
66 Phencyclidine
66 Profenamine
67 Azapropazone
67 Diloxanide
67 Etoxazene
67 Famprofazone
67 Fenbutrazate
67 Tetrabenazine
68 Clefamide
68 Clofazimine
68 Nifedipine
68 Phenoxybenzamine
69 Buprenorphine
69 Cholesterol
69 Disulfiram
69 Methoxyamfetamine
69 Metisazone
69 Thiambutosine
70 Benzfetamine
70 Bezitramide
70 Buclosamide
70 Butyl Aminobenzoate
70 Diphenoxylate
70 Dipipanone
71 Bromhexine
71 Broxaldine
71 Phenothiazine
72 Buclizine
72 Tiocarlide
73 Bialamicol
99 Azinphos-(Me)

System TM

00 Lysergic Acid
26 Ergometrine
27 Lysergamide
31 Methylergometrine
33 Methysergide
40 Dihydroergotamine
48 Ergotamine
64 Codergocrine Mesilate
67 Ergotoxine
70 Lysergide

9

System TN

22 Paraquat
34 Gallamine Triethiodide
35 Suxamethonium Chloride
36 Hexamethonium Bromide
40 Suxethonium Bromide
56 Decamethonium Bromide
56 Guanethidine
60 Choline
70 Acetylcholine Chloride
80 Pancuronium Bromide
85 Tubocurarine Chloride
94 Bretylium Tosilate
95 Atropine Methonitrate
100 Cetrimide

System TO

05 Gallamine Triethiodide
10 Hexamethonium Bromide
10 Paraquat
10 Suxamethonium Chloride
16 Decamethonium Bromide
23 Suxethonium Bromide
35 Atropine Methonitrate
40 Bretylium Tosilate
40 Tubocurarine Chloride
50 Cetrimide
50 Guanethidine
60 Acetylcholine Chloride
60 Choline
70 Meclozine

System TP

00 Hydrocortisone
00 Prednisolone Sodium Phosphate
08 Hydrocortisone
09 Triamcinolone
20 Prednisolone
23 Methylprednisolone
27 Hydrocortisone
30 Betamethasone
32 Dexamethasone
32 Triamcinolone
41 Prednisone
42 Fluocinolone Acetonide
51 Fluoxymesterone
51 Hydrocortisone
58 Betamethasone Valerate
58 Fludrocortisone
59 Alfadolone
60 Alfaxalone
60 Testosterone
65 Diethylstilbestrol
65 Methandienone
69 Oxymetholone
69 Prednisolone Pivalate
70 Methyltestosterone
71 Norethandrolone
71 Norethisterone
72 Cortisone
72 Dienestrol
72 Ethinylestradiol
75 Beclometasone
77 Lynestrenol
78 Androstanolone
78 Ethisterone
78 Fluocortolone
78 Testosterone
79 Estradiol
79 Ethylestrenol
79 Fluocortolone
79 Methallenestril
79 Noretynodrel
80 Dimethisterone
80 Medroxyprogesterone Acetate
80 Megestrol Acetate
81 Hydroxyprogesterone
81 Progesterone
83 Etynodiol Diacetate
86 Desoxycortone
86 Dydrogesterone
86 Mestranol
86 Testosterone
87 Nandrolone Phenylpropionate
87 Norethisterone
88 Chlorotrianisene
88 Nandrolone Decanoate

System TQ

00 Betamethasone
00 Hydrocortisone
00 Hydrocortisone
00 Prednisolone
00 Prednisolone Sodium Phosphate
00 Prednisone
00 Triamcinolone
00 Triamcinolone
02 Hydrocortisone
04 Prednisolone Pivalate
07 Testosterone
08 Dexamethasone
08 Fluocinolone Acetonide
09 Fluoxymesterone
10 Diethylstilbestrol

10 Methandienone
11 Androstanolone
11 Hydrocortisone
12 Fludrocortisone
12 Testosterone
16 Methyltestosterone
18 Methallenestril
20 Norethandrolone
20 Progesterone
22 Alfadolone
22 Alfaxalone
22 Norethisterone
23 Oxymetholone
25 Dienestrol
27 Betamethasone Valerate
28 Cortisone
28 Testosterone
30 Ethinylestradiol
32 Estradiol
32 Noretynodrel
35 Fluocortolone
38 Beclometasone
39 Ethisterone
39 Fluocortolone
39 Norethisterone
42 Dimethisterone
48 Nandrolone Phenylpropionate
49 Nandrolone Decanoate
50 Ethylestrenol
50 Medroxyprogesterone Acetate
50 Megestrol Acetate
52 Desoxycortone
52 Mestranol
53 Dydrogesterone
55 Hydroxyprogesterone
55 Lynestrenol
61 Etynodiol Diacetate
77 Chlorotrianisene
80 Methylprednisolone

System TR

00 Betamethasone
00 Dexamethasone
00 Hydrocortisone
00 Hydrocortisone
00 Prednisolone Sodium Phosphate
00 Triamcinolone
02 Prednisolone
03 Methylprednisolone
08 Hydrocortisone
10 Fluocinolone Acetonide
10 Prednisone
18 Diethylstilbestrol
20 Betamethasone Valerate
20 Triamcinolone
30 Fludrocortisone
34 Dienestrol
38 Fluoxymesterone
38 Hydrocortisone
40 Ethinylestradiol
44 Prednisolone Pivalate
55 Cortisone
70 Methallenestril
80 Alfadolone
80 Ethisterone
85 Oxymetholone
87 Methandienone
87 Norethisterone
88 Fluocortolone
89 Beclometasone
89 Fluocortolone
90 Alfaxalone
90 Androstanolone
90 Mestranol
90 Testosterone
91 Dimethisterone
91 Methyltestosterone
91 Noretynodrel
94 Ethylestrenol
95 Etynodiol Diacetate
95 Norethandrolone
96 Dydrogesterone
96 Estradiol
97 Nandrolone Decanoate
97 Nandrolone Phenylpropionate
98 Chlorotrianisene
98 Desoxycortone
98 Medroxyprogesterone Acetate
98 Megestrol Acetate
98 Norethisterone
99 Hydroxyprogesterone
99 Lynestrenol
99 Progesterone
99 Testosterone
99 Testosterone

System TS

00 Betamethasone
00 Cortisone
00 Dexamethasone
00 Ethisterone
00 Fludrocortisone
00 Fluocortolone
00 Hydrocortisone
00 Hydrocortisone
00 Hydrocortisone
00 Hydrocortisone
00 Methylprednisolone
00 Prednisolone
00 Prednisolone Pivalate
00 Prednisolone Sodium Phosphate
00 Prednisone
00 Triamcinolone
01 Fluocinolone Acetonide
02 Betamethasone Valerate
03 Diethylstilbestrol
05 Dienestrol
06 Triamcinolone
16 Fluoxymesterone
40 Ethinylestradiol
42 Beclometasone
45 Alfadolone
54 Methallenestril
58 Fluocortolone
61 Methandienone
63 Norethisterone
63 Testosterone
71 Methyltestosterone
71 Noretynodrel
72 Alfaxalone
72 Androstanolone
78 Norethandrolone
79 Estradiol
82 Oxymetholone
85 Medroxyprogesterone Acetate
85 Megestrol Acetate
90 Hydroxyprogesterone
90 Mestranol
90 Norethisterone
92 Chlorotrianisene
95 Desoxycortone
95 Dimethisterone
95 Nandrolone Decanoate
95 Nandrolone Phenylpropionate
95 Progesterone
97 Lynestrenol
98 Dydrogesterone
98 Testosterone
98 Testosterone
99 Ethylestrenol
99 Etynodiol Diacetate

System TT

02 Phthalylsulfathiazole
02 Succinylsulfathiazole
11 Sulfisomidine
21 Sulfaguanidine
24 Sulfadiazine
33 Sulfamerazine
46 Sulfamethizole
47 Sulfapyridine
50 Sulfadimidine
53 Sulfacetamide
53 Sulfamethoxypyridazine
53 Sulfathiazole
55 Sulfametoxydiazine
61 Sulfanilamide
74 Sulfafurazole
84 Chlorpropamide
85 Sulfadimethoxine
88 Sulfamethoxazole
89 Sulfaphenazole
90 Carbutamide
98 Tolbutamide

System TU

01 Succinylsulfathiazole
04 Phthalylsulfathiazole
17 Sulfametoxydiazine
18 Sulfamerazine
22 Sulfadiazine
26 Sulfamethoxypyridazine
27 Carbutamide
27 Sulfadimidine
33 Sulfamethoxazole
35 Tolbutamide
36 Sulfamethizole
37 Sulfacetamide
40 Sulfathiazole
43 Chlorpropamide
43 Sulfapyridine
48 Sulfafurazole
49 Sulfisomidine
52 Sulfadimethoxine
70 Sulfaphenazole
90 Sulfaguanidine
96 Sulfanilamide

System TV

01 Succinylsulfathiazole
02 Sulfamethizole
02 Sulfamethoxazole
03 Chlorpropamide
03 Sulfadiazine
04 Phthalylsulfathiazole
04 Sulfacetamide
04 Sulfafurazole
04 Tolbutamide
05 Sulfathiazole
07 Carbutamide
07 Sulfamerazine
13 Sulfaphenazole
15 Sulfametoxydiazine
20 Sulfisomidine
34 Sulfadimethoxine
48 Sulfaguanidine
50 Sulfamethoxypyridazine
62 Sulfadimidine
66 Sulfanilamide
73 Sulfapyridine

System TW

19 Dimethoate
23 Mevinphos
68 Azinphos-(Me)
74 Malathion
77 Parathion Methyl
81 Parathion
82 Dimpylate

System TX

00 Dichlorophenoxyacetic Acid
00 Methylchlorophenoxyacetic Acid
00 Omethoate
01 Methamidophos
01 Monocrotophos
04 Dimethoate
04 Nicotine
06 Dinitro-orthocresol
07 Trichlorfon
12 Dichlorophen
12 Mevinphos
12 Warfarin
14 Coumatetralyl
18 Aldicarb
18 Carbaryl
18 Demeton-*S*-(Me)
19 Methoprotryne
20 Azinphos-(Me)
20 Dichlorvos
21 Desmetryne
21 Triazophos
22 Linuron
22 Simazine
23 Monolinuron
24 Azinphos-(Et)
26 Ametryne
27 Coumaphos
30 Parathion Methyl
31 Malathion
32 Fenitrothion
32 Prometryne
32 Pyrazophos
32 Terbutryne
37 Cyfluthrin
40 Alachlor
40 Deltamethrin
40 Endosulfan
41 Dicamba
41 Fenthion
41 Parathion Ethyl
43 Methoxychlor
47 Dimpylate
51 Lindane
56 Chlorpyrifos-methyl
58 Disulfoton
64 Chlorpyrifos
65 Dieldrin
65 Dieldrin
71 Endrin
84 Heptachlor
89 Aldrin

System TY

00 Methamidophos
00 Methylchlorophenoxyacetic Acid
00 Omethoate
01 Monocrotophos
01 Nicotine
02 Dichlorophenoxyacetic Acid
02 Trichlorfon
04 Dimethoate
10 Mevinphos
11 Methoprotryne
11 Warfarin
12 Aldicarb
13 Demeton-*S* -methyl
16 Desmetryne
16 Dichlorophen
18 Simazine
20 Dichlorvos
23 Ametryne
25 Carbaryl
30 Coumatetralyl
30 Monolinuron
31 Linuron
31 Prometryne
32 Terbutryne
38 Dinitro-orthocresol
38 Triazophos
42 Azinphos-(Me)
45 Alachlor
47 Pyrazophos
48 Azinphos-(Et)
50 Dimpylate
53 Malathion
61 Coumaphos
70 Dicamba
73 Parathion Methyl
76 Fenitrothion
77 Endosulfan
81 Fenthion
84 Methoxychlor
84 Parathion Ethyl
87 Dieldrin
87 Dieldrin
89 Chlorpyrifos-methyl
89 Cyfluthrin
89 Disulfoton
90 Deltamethrin
90 Endrin
92 Lindane
95 Chlorpyrifos
97 Heptachlor
98 Aldrin

System TZ

15 Trichlorfon
37 Dimethoate
65 Aldicarb
68 Carbaryl
75 Dichlorvos
76 Dimpylate
77 Endosulfan
82 Fenitrothion
82 Malathion
90 Fenthion
95 Deltamethrin

System TAA

00 Tropicamide
05 Trichlorfon
08 Dimethoate
12 Aldicarb
17 Carbaryl
21 Dimpylate
33 Malathion
36 Dichlorvos
65 Fenitrothion
65 Methoxychlor
68 Fenthion
72 Deltamethrin
95 Endosulfan

System TAB

00 Dichlorvos
00 Diquat
00 Glyphosate Acid
00 Paraquat
02 Dichlorophenoxyacetic Acid
02 Trichlorfon
03 Dimethoate
03 Mevinphos
04 Aldicarb
04 Atrazine
04 Dicamba
04 Methylchlorophenoxyacetic Acid
04 Trichlorophenoxyacetic Acid
07 Terbutryne
12 Carbaryl
24 Malathion
30 Dimpylate
50 Fenitrothion
51 Dieldrin
51 Dieldrin
52 Disulfoton
55 Endrin
60 Chlorpyrifos-methyl
64 Chlordane
67 Aldrin
69 Heptachlor

System TAC

00 Dichlorvos
00 Diquat
00 Glyphosate Acid
00 Paraquat
01 Trichlorfon
02 Methylchlorophenoxyacetic Acid
02 Mevinphos
02 Trichlorophenoxyacetic Acid
03 Aldicarb
03 Dichlorophenoxyacetic Acid
04 Carbaryl
05 Dicamba
08 Atrazine
12 Terbutryne
14 Malathion
17 Fenitrothion
20 Dimpylate
27 Dieldrin
27 Dieldrin
35 Chlorpyrifos-methyl
35 Disulfoton
35 Endrin
44 Chlordane
51 Heptachlor
52 Aldrin

System TAD

00 Ambazone
00 Amiloride
00 Baclofen
00 Barbituric Acid
00 Chloropyramine
00 Chloroquine
00 Cytarabine
00 Enalapril
00 Ethambutol
00 Levodopa
00 Lysergic Acid
00 Metformin
00 Oxytetracycline Dihydrate
00 Pentamidine
00 Phthalylsulfacetamide
00 Phthalylsulfathiazole
00 Sodium Cromoglicate
00 Valproic Acid
02 Dipyrone
02 Mafenide
02 Succinylsulfathiazole
02 Sulfasalazine
04 Carbidopa
04 Nicotinic Acid
04 Saccharin
05 Etacrynic Acid
06 Bumetanide
06 Captopril
07 Cinchophen
07 Furosemide
07 Sulfaguanidine
09 Benzyl Benzoate
10 Carbazochrome
11 Chlorothiazide
11 Hydrochlorothiazide
13 Hydroflumethiazide
13 Triamterene
14 Sulfacarbamide
15 Aminosalicylic Acid
15 Methylchlorophenoxyacetic Acid
15 Niflumic Acid
15 Quinethazone
18 Acetazolamide
18 Diflunisal
18 Isoniazid
20 Trimethoprim
21 Ethyl Biscoumacetate
22 Aloxiprin
22 Sulfanilamide
23 Chlortalidone
23 Diclofenamide
23 Trichlormethiazide
24 Probenecid
24 Salicylic Acid
24 Salicylic Acid
25 Epithiazide
26 Cyclothiazide
26 Paracetamol
26 Trifluperidol
27 Cyclopenthiazide
27 Methyclothiazide
27 Sulfamethizole
27 Sulfathiazole
27 Sulfisomidine
28 Carbenoxolone
28 Digoxin
28 Primidone
28 Sulfacetamide
29 Clozapine
30 4-Aminophenol
30 Bendroflumethiazide
30 Benzthiazide
30 Styramate
30 Sulfinpyrazone
30 Tolmetin
31 Aminobenzoic Acid
31 Aspirin
31 Bufexamac
32 Chlorphenesin Carbamate
32 Meprobamate
32 Metronidazole
32 Polythiazide
33 Alclofenac
33 Dicoumarol
33 Metolazone
33 Nitrofurantoin
33 Sulfafurazole
34 Chloramphenicol
34 Sulfaethidole
34 Sulfapyridine
34 Sulindac
35 Benzoic Acid
35 Hydroxy-*N*-dealkyl-2-oxo-quazepam
35 Mebutamate
35 Mephenesin Carbamate
36 Loprazolam
36 Xipamide
37 6-Hydroxybrotizolam
38 Indometacin
38 Methocarbamol
38 Sulfadiazine
39 Clopamide
39 Fenbufen
39 Phenolphthalein
40 Alprazolam
40 Guaifenesin
41 Flurazepam
41 Ketoprofen
41 Phenindamine
41 Sulfamethoxazole
41 Triazolam
41 α-Hydroxybrotizolam
42 Demoxepam
42 Digitoxin
42 Lorazepam
42 Oxazepam
42 Sulfamerazine
42 Sultiame
43 Salicylamide
43 Sulfamethoxypyridazine
43 Sulfaphenazole
44 Hexachlorophene
44 Naproxen
44 Pyrazinamide
44 Sulfadimidine
45 Flurbiprofen
46 Butalamine
46 Indapamide
47 Bromazepam
47 Clorexolone
47 Diclofenac
47 Enoxolone
47 Sulfametoxydiazine
48 Sulfadimethoxine
49 Chlorpropamide
50 Chlorambucil
50 Dantrolene
50 Fenoprofen
50 Phenacemide
50 Phenazone
51 Ethoxzolamide
52 Acetanilide
52 Bromisoval
52 Phenacetin
53 Acetohexamide
53 Aminoglutethimide
53 Brotizolam
53 Chlordiazepoxide
53 Diphenadione
53 Midazolam
53 Nitrazepam
53 Phenobarbital
53 Phenytoin
54 Glibornuride
54 Ibuprofen
54 Mefenamic Acid
54 Sulfametopyrazine
55 Caffeine
55 Mefruside
55 Methyl Hydroxybenzoate
55 Methyprylon
55 Sulfadoxine

56 Allobarbital
56 Chlorzoxazone
56 Clonazepam
56 Ibomal
56 Miconazole
56 Nortetrazepam
56 Paramethadione
56 Propyl Hydroxybenzoate
57 Aprobarbital
57 Barbital
57 Brallobarbital
57 Butalbital
57 Clorazepic Acid
57 Dichlorophen
57 Ethyl Hydroxybenzoate
57 Glibenclamide
57 Methylpentynol
57 Nordazepam
57 Oxyphenbutazone
57 Secbutabarbital
57 Vinbarbital
58 Aminophenazone
58 Amobarbital
58 Butobarbital
58 Cyclobarbital
58 Ethinamate
58 Hydroxy-2-oxo-quazepam
59 Carisoprodol
59 Cyclopentobarbital
59 Etamivan
59 Ethosuximide
59 Heptabarb
59 Idobutal
59 Pentobarbital
59 Pheneturide
60 Acecarbromal
60 Acenocoumarol
60 Ethotoin
60 Hexethal
60 Lormetazepam
60 *N*-dealkyl-2-oxo-quazepam
60 Nealbarbital
60 Phenprobamate
60 Talbutal
61 Flumazenil
61 Phenprocoumon
62 Alfadolone
62 Ketazolam
62 Nalidixic Acid
62 Secobarbital
62 Tolbutamide
63 Benzocaine
63 Triclocarban
64 Bemegride
64 Carbromal
64 Cholesterol
64 Estradiol
64 Warfarin
65 Benziodarone
65 Normetaclazepam
65 Propyphenazone
65 Temazepam
65 Thymol
66 Mephenytoin
66 Tolazamide
66 Vinylbital
67 Tetrazepam
68 Carbimazole
68 Clotrimazole
68 Griseofulvin
68 Procarbazine
68 Thiopental
69 Camazepam
69 Hexobarbital
69 Metharbital
69 Santonin
69 Thialbarbital
70 Clioquinol
70 Clobazam
70 Enallylpropymal
70 Glutethimide
70 Methylphenobarbital
70 Oxo-quazepam
70 Phenindione
71 Azathioprine
71 Clofibrate
71 Metaclazepam
71 Methohexital
71 Piroxicam
72 Diazepam
72 Diiodohydroxyquinoline
72 Flunitrazepam
72 Phensuximide
72 Prazepam
73 Cyclandelate
73 Medazepam
75 Spironolactone
76 Phenylbutazone
78 Dantron
78 Quazepam
80 Clofenotane
80 Quazepam -CFTB
82 Ethchlorvynol
94 Azinphos-(Me)

9

System TAE

00 Alcuronium Chloride
00 Amikacin
00 Atropine Methonitrate
00 Busulfan
00 Cetrimide
00 Cetylpyridinium Chloride
00 Decamethonium
00 Dihydralazine
00 Dihydrostreptomycin
00 Diquat
00 Dopexamine
00 Histamine
00 Homatropine Methylbromide
00 Kanamycin
00 Neomycin
00 Neostigmine Bromide
00 Pancuronium Bromide
00 Paraquat
00 Pentamidine
00 Pentolonium Tartrate
00 Pralidoxime Mesilate
00 Pyridostigmine Bromide
00 Rolitetracycline
00 Streptomycin
00 Suxamethonium Chloride
00 Thiazinamium Sulfoxide
00 Tobramycin
00 Trimetaphan Camsilate
00 Trimethadione
00 Tubocurarine Chloride
01 Amidefrine
01 Chlorhexidine
01 Cotarnine
01 Denatonium Benzoate
01 Deptropine
01 Hydrastinine
01 Piperazine
01 Thiazinamium Metilsulfate
02 Distigmine Bromide
02 Oxyphencyclimine
02 Oxyphenonium Bromide
02 Thiamine
03 Azacyclonol
03 Clidinium Bromide
03 Emepronium Bromide
03 Glycopyrronium Bromide
03 Guanethidine
03 Guanoxan
03 Hexocyclium Metilsulfate
03 Isopropamide Iodide
03 Mequitazine
03 Metformin
03 Methanthelinium Bromide
03 Naphazoline
03 Propantheline Bromide
03 Tolazoline
04 Benzalkonium Chloride
04 Berberine
04 Carbachol
04 Chloroquine
04 *N*-Desmethylpirenzepine
04 Sparteine
04 Tramazoline
05 Antazoline
05 Atropine
05 Brucine
05 Coniine
05 Tetryzoline
05 Xylometazoline
06 Amiloride
06 Benzatropine
06 Cyclopentamine
06 Maprotiline
06 Mephentermine
06 Mescaline
06 Phentolamine
06 Protriptyline
06 Tryptamine
07 2,4,6-TMA
07 Amantadine
07 Benserazide
07 Desipramine
07 Ethacridine
07 Homatropine
07 Hydroxychloroquine
07 Methoxyphenamine
07 Proguanil
07 Rimiterol
08 FLA-838
08 Methylenedioxymetamfetamine
08 Oxytetracycline Dihydrate
08 Paroxetine
08 Perhexiline
08 Phenylephrine
08 Prajmalium Bitartrate
08 Strychnine
09 Aminoacridine
09 DOM
09 Disopyramide
09 Metamfetamine
09 Methoxyamfetamine
09 Penthienate Methobromide
09 Pholedrine
09 Pseudoephedrine
10 Bufotenine
10 Dextromethorphan
10 Dextrorphan
10 Dimetindene
10 Ephedrine
10 Methylenedioxyamfetamine
11 Amlodipine
11 Dihydrocodeine
11 Fluoxetine
11 Hydrocodone
11 Levodopa
11 Mesoridazine
11 Norfluoxetine
11 Phentermine
12 Amfetamine
12 Aminopromazine
12 Brompheniramine
12 Chlorphenamine
12 Dihydromorphine
12 Doxylamine
12 Ethambutol
12 Ethoheptazine
12 Hexobendine
12 Hexoprenaline
12 Hydromorphone
12 Methenamine
12 Methoxamine
12 Methylephedrine
12 Pipazetate
12 Tiotixene
13 Acebutolol

13 Carbinoxamine
13 Metaraminol
13 Thiethylperazine
13 Thioproperazine
14 Atenolol
14 Azapetine
14 Carteolol
14 Chlorphentermine
14 Diethyltryptamine
14 Dimethyltryptamine
14 Dopamine
14 Etafedrine
14 Granisetron
14 Heptaminol
14 Isoprenaline
14 Nadolol
14 Pheniramine
14 Propylhexedrine
14 Psilocin
14 Tofenacin
15 Celiprolol
15 Etilefrine
15 Pholcodine
15 Prothipendyl
15 Psilocin -ethyl
16 Benzydamine
16 Betaine
16 Bunitrolol
16 Encainide
16 Methadone
16 Salbutamol
17 Lofexidine
17 Metoclopramide
17 Procainamide
17 Sulpiride
17 Tertatolol
17 Veratrine
18 Bambuterol
18 Carbocromen
18 Fluvoxamine
18 Morphine
18 Pindolol
18 Pirenzepine
18 Promazine
18 Terbutaline
18 Terodiline
18 Xamoterol
19 Bambuterol Monocarbamate
19 Cinchonine
19 Citalopram
19 Desbutylhalofantrine
19 Emetine
19 Oxeladin
19 Pipradrol
19 Sotalol
19 Triprolidine
20 Aprindine
20 Benzylmorphine
20 Fenfluramine
20 Hydroquinidine
20 Metoprolol
20 Oxprenolol
20 Procyclidine
20 Thenalidine
20 Thioridazine
20 Timolol
20 Zimeldine
21 Codeine
21 Dimethoxanate
21 Ethylmorphine
21 Fencamfamin
21 Hydroquinine
21 Imipramine
21 Methapyrilene
21 Metipranolol
21 Metixene
21 Orciprenaline
21 Perazine
21 Propranolol
21 Thenyldiamine
22 Ajmaline
22 Alprenolol
22 Betaxolol
22 Bisoprolol
22 Chloropyramine
22 Clenbuterol
22 Isothipendyl
22 Mafenide
22 Mepyramine
22 Prolintane
22 Thioproperazine
22 Thonzylamine
22 Tripelennamine
23 Bamethan
23 Diphenylpyraline
23 Lobeline
23 Phencyclidine
23 Thebaine
24 Bamipine
24 Cinchonidine
24 Cyclazocine
24 Doxepin
24 Erythromycin
24 Ketotifen
24 Piperocaine
24 Thebacon
24 Trimethobenzamide
25 Chlorpromazine
25 Cropropamide
25 Fenpipramide
25 Gelsemine
25 Mexiletine
25 Orphenadrine
25 Pyrrobutamine
25 Sertraline
25 Viloxazine
26 Acetylcodeine
26 Butaperazine
26 Clomipramine
26 Diamorphine
26 Ketobemidone
26 Loprazolam
26 Prochlorperazine
26 Quinine
26 Remoxipride
26 Tiotixene
26 Tolpropamine
26 Xantinol Nicotinate
27 Amitriptyline
27 Bromazine
27 Diphenhydramine
27 Dipipanone
27 Dosulepin
27 Lisinopril
27 Oxymorphone
27 Pecazine
27 Thiethylperazine
28 Acepromazine
28 Alphaprodine
28 Dimenhydrinate
28 Flecainide
28 Pizotifen
29 Chlorphenoxamine
29 Noxiptiline
29 Phenelzine
30 Adrenaline
30 Clomifene
30 Cyproheptadine
30 Hexetidine
30 Hydroxyclenbuterol
30 Nefopam
30 Oxycodone
30 Promethazine
30 Propiomazine
30 Quinidine
30 Tramadol
30 Trifluoperazine
31 Profenamine
32 Alimemazine
32 Clofedanol
32 Histapyrrodine
32 Labetalol
32 Levomepromazine
32 Mebeverine
32 Phenyltoloxamine
32 Triflupromazine
33 Buphenine
33 Clofazimine
33 Diethazine
33 Pipamperone
34 Acetophenazine
34 Chlorprothixene
34 Narceine
34 Pentazocine
34 Pethidine
34 Phenmetrazine
35 Chlorcyclizine
35 Cocaine
35 Nicametate
35 Opipramol
36 Mebhydrolin
36 Procaine
36 Trimipramine
38 Amodiaquine
38 Benzoctamine
38 Dibenzepin
38 Fenoterol
39 Carfenazine
39 Cyclizine
39 Ibogaine
39 Nicotine
40 Clioquinol
40 Diethylthiambutene
40 Methylphenidate
40 Perphenazine
40 Physostigmine
40 Pipotiazine
41 Arecoline
41 Lorcainide
41 Tranylcypromine
42 Aconitine
42 Cinchocaine
42 Clozapine
42 Levallorphan
42 Tocainide
42 Zopiclone
43 Dimetotiazine
43 Naftidrofuryl Oxalate
43 Nicergoline
43 Noradrenaline
43 Norpipanone
43 Prenylamine
43 Tetracaine
43 Trihexyphenidyl
43 Verapamil
44 Butacaine
44 Clonidine
44 Fenetylline
44 Minoxidil
44 Penicillamine
44 Phenethylamine
45 Biperiden
45 Clopenthixol
45 Fluphenazine
45 Isoaminile
45 Nizatidine
45 Trimethoprim
45 Zuclopenthixol
46 Cyclopentolate
46 Mazindol
46 Meclofenoxate
46 Pericyazine
46 Spiramycin
46 Vinblastine
47 Captodiame
47 Diltiazem
47 Dixyrazine
48 Butetamate
48 Flavoxate
48 Mianserin
48 Thiopropazate
49 Adiphenine
49 Hyoscine
49 Loxapine
49 Phendimetrazine
50 Flupentixol
50 Phenazocine
50 Triamterene
51 Haloperidol
51 Tetramisole
51 Tizanidine
52 Benactyzine
52 Flurazepam
52 Loperamide
52 Pilocarpine

53 Cimetidine
53 Famotidine
53 Levamisole
53 Nomifensine
53 Obidoxime Chloride
54 Amiodarone
54 Apomorphine
54 Oxybuprocaine
54 Piperidolate
55 Dicycloverine
55 Diethylpropion
55 Isoniazid
55 Norastemizole
55 Trifluperidol
56 Astemizole
56 Furazolidone
56 Halofantrine
57 Flucytosine
57 Hydroxyzine
57 Lysergamide
57 Nalorphine
58 Dihydroergotamine
58 Methylpiperidyl Benzilate
58 Nalbuphine
58 Nifenazone
59 Caffeine
59 Dropropizine
59 Hydrastine
59 Theobromine
60 Anileridine
60 Benzfetamine
60 Lysergide
60 Methyldopa
60 Nimorazole
61 Morazone
61 Oxetacaine
61 Tilidate
62 Benperidol
62 Ergometrine
62 Isoxsuprine
62 Pramocaine
62 Prilocaine
62 Thiopropazate
63 Fluspirilene
63 Mepivacaine
63 Meprobamate
63 Nalidixic Acid
63 Piminodine
64 Buspirone
64 Pentoxifylline
64 Trazodone
65 Azaperone
65 Butanilicaine
65 Harmine
65 Moclobemide
66 Cisapride
66 Etofylline
66 Metyrapone
66 Phenazone
66 Pyrimethamine
66 Yohimbine
67 Alprazolam
67 Fluanisone
67 Fluconazole
68 Ergotamine
68 Ketamine
68 Ketoconazole
68 Nialamide
68 Nicotinamide
68 Prazosin
68 Triazolam
69 Bupivacaine
69 Colchicine
69 Cytarabine
69 Doxapram
69 Methylergometrine
69 Midazolam
70 Ambazone
70 Aminophenazone
70 Diprophylline
70 Fentanyl
70 Iproniazid
70 Lidoflazine
70 Lysergic Acid
70 Phenoperidine
71 Bamifylline
71 Doxazosin
71 Droperidol
71 Etacrynic Acid
71 Nikethamide
71 Proxyphylline
71 Pyrazinamide
72 Brotizolam
72 Dextromoramide
72 Etizolam
72 Harman
72 Lidocaine
72 Nicotinic Acid
72 Noscapine
72 Penfluridol
72 Pentetrazol
72 Pentifylline
73 Bromazepam
73 Deserpidine
73 Hydralazine
73 Isoetarine
73 Pimozide
73 Piritramide
74 Cyclophosphamide
74 Methyl Nicotinate
74 Naloxone
74 Nicotinyl Alcohol
74 Oxypertine
74 Papaverine
74 Theophylline
75 4-Aminophenol
75 Allopurinol
75 Carbimazole
75 Ibuprofen
75 Lincomycin
75 Metronidazole
75 Pyridoxine
75 Sulfaguanidine
75 Thiotepa
75 Tiabendazole
76 6-Hydroxybrotizolam
76 Ambucetamide
76 Chlordiazepoxide
76 Clemizole
76 Flumazenil
76 Levocabastine
76 Primidone
76 Reserpine
77 *dl*-Isovalthine
77 Mercaptopurine
77 Metomidate
77 Miconazole
77 Nifuratel
77 Paracetamol
77 Pargyline
77 Protionamide
77 Quazepam -CFTB
77 Rescinnamine
78 Alfentanil
78 Aspirin
78 Broxaldine
78 Disulfiram
78 Econazole
78 Etomidate
78 Griseofulvin
78 Hydrochlorothiazide
78 Isopropylaminophenazone
78 Methyprylon
78 Nitrofurazone
78 Oxcarbazepine
78 Sulfadoxine
78 α-Hydroxybrotizolam
79 Carbamazepine
79 Cinnarizine
79 Clorexolone
79 Medazepam
79 Methaqualone
79 Nifedipine
79 OH-Carbamazepine
79 Sulfadimidine
79 Sulfamethoxazole
79 Sulfathiazole
80 Acetanilide
80 Azathioprine
80 Buprenorphine
80 Clomethiazole
80 Clotrimazole
80 Dimethoate
80 Etidocaine
80 Fenyramidol
80 Flunitrazepam
80 Mebendazole
80 Mecloqualone
80 Meclozine
80 Oxymetazoline
80 Phenazopyridine
80 Propanidid
80 Psilocybine
80 Sulfamerazine
80 Sulfisomidine
80 Tetrabenazine
80 Thiamazole
80 Trimetozine
81 Azinphos-(Et)
81 Azinphos-(Me)
81 Clindamycin
81 Clofibrate
81 Demoxepam
81 Desamide
81 Dimpylate
81 Dinormetaclazepam
81 Glafenine
81 Guaifenesin
81 Methyl Hydroxybenzoate
81 Normetaclazepam
81 Pemoline
81 Propyphenazone
81 Strophanthin-K
81 Sulfacarbamide
81 Sulfadiazine
81 Sulfafurazole
81 Sultiame
82 Bisacodyl
82 Camazepam
82 Cinchophen
82 Diazepam
82 Dipyridamole
82 Lofepramine
82 Lorazepam
82 Lormetazepam
82 Mebutamate
82 Metaclazepam
82 Methoxsalen
82 NCM-001
82 NCM-009
82 Naproxen
82 Nordazepam
82 Oxazepam
82 Phenothiazine
82 Phenprobamate
82 Sulfametoxydiazine
82 Temazepam
83 Butyl Aminobenzoate
83 Clorazepic Acid
83 Crotetamide
83 Indometacin
83 Ketazolam
83 Methocarbamol
83 Phenacetin
83 Progesterone
83 Rifampicin
83 Salicylamide
83 Sulfamethizole
83 Sulfamethoxypyridazine
83 Sulfanilamide
84 Acecarbromal
84 Acetazolamide
84 Barbital
84 Barbituric Acid
84 Benzocaine
84 Bromhexine
84 Bromisoval
84 Bromocriptine
84 Chlorambucil
84 Chlormezanone
84 Cholesterol
84 Clobazam
84 Crotamiton
84 Diazoxide
84 Ethosuximide

84 Etorphine
84 Flufenamic Acid
84 Flutazolam
84 Gliclazide
84 Isocarboxazid
84 Malathion
84 Methazolamide
84 Nitrofurantoin
84 Phenaglycodol
84 Phenoxybenzamine
84 Prazepam
84 Prednisone
84 Spironolactone
84 Tetrazepam
85 Carbromal
85 Carisoprodol
85 Chlorpyrifos
85 Clonazepam
85 Digoxin
85 Dipyrone
85 Enalapril
85 Etamivan
85 Floctafenine
85 Hexobarbital
85 Ketoprofen
85 Methohexital
85 Nortetrazepam
85 Phenobarbital
85 Sulfasalazine
85 Testosterone
85 Tolmetin
86 Aprobarbital
86 Benorilate
86 Benzyl Alcohol
86 Butobarbital
86 Chloramphenicol
86 Demeton-*S* -methyl
86 Ethinamate
86 Ethinylestradiol
86 Furosemide
86 Glipizide
86 Glutethimide
86 Hydrocortisone
86 Hydroxyprogesterone
86 Loratadine
86 Methylphenobarbital
86 Methyltestosterone
86 Mevinphos
86 Mexazolam
86 Nisoldipine
86 Norethisterone
86 Phanquinone
86 Phenolphthalein
86 Phenytoin
86 Prednisolone
86 Resorcinol
86 Salicylic Acid
086 Salicylic Acid
86 Sulfadimethoxine
86 Tiaprofenic Acid
86 Tolazamide
87 Allobarbital
87 Brallobarbital
87 Bumetanide
87 Butalbital
87 Carbutamide
87 Chlorphenesin Carbamate
87 Chlorpropamide
87 Clenbuterol-(M_2)
87 Clenbuterol-(M_3)
87 Cortisone
87 Cyclandelate
87 Dimenhydrinate
87 Dobutamine
87 Estrone
87 Etenzamide
87 Ethchlorvynol
87 Felodipine
87 Hydroflumethiazide
87 Itraconazole
87 Mefenamic Acid
87 Mephenesin
87 Metharbital
87 Methylprednisolone
87 Nimodipine
87 Nitrendipine
87 Oxo-quazepam
87 Phenylbutazone
87 Probenecid
87 Quazepam
87 Saccharin
87 Sulfacetamide
87 Sulindac
87 Tenoxicam
87 Xanthanoic Acid
87 α-(N-Acetylcysteine-S-yl)-isovalerylurea
88 5-Hydroxy-piroxicam
88 Amobarbital
88 Azapropazone
88 Ceftriaxone
88 Chlortalidone
88 Chlorzoxazone
88 Clemastine
88 Cyclobarbital
88 Dicoumarol
88 Digitoxin
88 Doxycycline
88 Epithiazide
88 Ethyl Hydroxybenzoate
88 Fenbutrazate
88 Heptabarb
88 Hexestrol
88 Hexylresorcinol
88 Hydroxy-*N*-dealkyl-2-oxo-quazepam
88 Isradipine
88 Minocycline
88 *N*-dealkyl-2-oxo-quazepam
88 Niflumic Acid
88 Piroxicam
88 Procarbazine
88 Secbutabarbital
88 Secobarbital
88 Tetracycline
88 Tolbutamide
88 Trichlormethiazide
88 Zomepirac
88 Hydroxy-2-oxo-quazepam
89 Bezafibrate
89 Dieldrin
89 Diflunisal
89 Etofenamate
89 Etynodiol Diacetate
89 Indapamide
89 Lanatoside C
89 Sulfaphenazole
89 Vinbarbital
89 Vinylbital
90 Androsterone
90 Beclamide
90 Buclosamide
90 Cyclopentobarbital
90 Diclofenac
90 Diphenoxylate
90 Famprofazone
90 Glibenclamide
90 Mesuximide
90 Nitrazepam
90 Oxyphenbutazone
90 Pentobarbital
90 Propyl Hydroxybenzoate
90 Trichlorfon
91 Chlordane
91 Dichlorophen
91 Fenticlor
91 Fluorometholone
91 Ibomal
92 Acenocoumarol
92 Bezitramide
92 Diethylstilbestrol
92 Feprazone
92 Glibornuride
92 Metenolone
92 Nealbarbital
92 Pentaerithrityl Tetranitrate
92 Talbutal
93 Gliquidone
93 Metformin
93 Niclosamide
93 Phenprocoumon
93 Xipamide
94 Benzbromarone
94 Hexachlorophene

9

System TAF

00 Diquat
00 Paraquat
00 Suxamethonium Chloride
01 Pentolonium Tartrate
01 Psilocybine
02 Decamethonium
03 Histamine
04 Piperazine
07 Brucine
07 Disopyramide
08 Obidoxime Chloride
10 Sparteine
11 Betaine
11 Strychnine
11 Tubocurarine Chloride
12 Homatropine Methylbromide
12 Methenamine
13 Hydrocodone
14 Chloroquine
14 Hydromorphone
14 Narceine
15 Hydrastinine
15 Loprazolam
15 Pancuronium Bromide
16 Carbinoxamine
16 Lysergic Acid
18 Oxyphencyclimine
18 Thiamine
19 Dihydrocodeine
21 Chlorphenamine
22 Codeine
22 Cotarnine
22 Dibenzepin
22 Nicotine
22 Thioproperazine
23 Benzylmorphine
23 Carbachol
23 Morphine
23 Perazine
24 Methapyrilene
24 Tiotixene
25 Acetylcodeine
25 Thebacon
25 Thiazinamium Metilsulfate
26 Ethylmorphine
26 Pheniramine
26 Prochlorperazine
27 Homatropine
28 Atropine
29 Cetrimide
29 Cetylpyridinium Chloride
29 Prothipendyl
29 Trifluoperazine
30 Cocaine
30 Guanethidine
30 Triprolidine
31 Propantheline Bromide
31 Thonzylamine
32 Acetophenazine
32 Thebaine
33 Diamorphine
33 Mepyramine
33 Oxycodone
33 Procainamide
34 Bufotenine
34 Tripelennamine

35 Isothipendyl
35 Promazine
36 Oxymorphone
36 Oxyphenonium Bromide
36 Thenyldiamine
38 Dimethoxanate
38 Physostigmine
39 Dimethyltryptamine
39 Opipramol
39 Tetracaine
40 Perphenazine
40 Pethidine
41 Dosulepin
41 Ethoheptazine
41 Isopropamide Iodide
41 Phendimetrazine
42 Dextromethorphan
42 Procaine
44 Promethazine
45 Chlorpromazine
45 Doxepin
45 Doxepin
45 Flurazepam
45 Phenmetrazine
45 Pilocarpine
46 Alimemazine
46 Dimenhydrinate
46 Mebhydrolin
47 Hyoscine
47 Imipramine
48 Bromazine
48 Dimenhydrinate
48 Diphenhydramine
48 Metformin
48 Psilocin
49 Clemastine
49 Dextrorphan
49 Diphenylpyraline
49 Fluphenazine
49 Isoniazid
49 Levomepromazine
49 Orphenadrine
49 Phenindamine
49 Triflupromazine
50 Cyproheptadine
50 Methylpiperidyl Benzilate
50 Mianserin
51 Amitriptyline
51 Chlorprothixene
51 Lysergamide
52 Chlorcyclizine
52 Cyclizine
52 Levamisole
52 Naphazoline
52 Nomifensine
52 Piperidolate
52 Propiomazine
53 Tetramisole
54 Clomipramine
54 Diethazine
54 Theobromine
55 Caffeine
55 Cimetidine
55 Cinchonidine
55 Profenamine
55 Thioridazine
55 Tolazoline
56 Berberine
56 Butetamate
56 Diethylpropion
56 Diethyltryptamine
56 Piperocaine
56 Trimipramine
57 Benactyzine
57 Hydrastine
59 Diprophylline
59 Dipyrone
59 Dopamine
59 Lysergide
59 Nalorphine
59 Pipotiazine
59 Thiopropazate
59 Trimethoprim
60 Ergometrine
60 Mepivacaine
60 Methadone
60 Pramocaine
60 Tetryzoline
61 Cinchonine
61 Pericyazine
61 Pipamperone
61 Trazodone
61 Verapamil
63 Colchicine
63 Indometacin
63 Mescaline
63 Metamfetamine
63 Quinidine
64 Amiodarone
64 Ephedrine
64 Ergotamine
64 Nialamide
64 Xylometazoline
65 Hydroxyzine
65 Mazindol
65 Quinine
65 Triamterene
65 Triazolam
66 Alprazolam
66 Anileridine
66 Antazoline
66 Benzalkonium Chloride
66 Nicotinamide
66 Phenazone
66 Pyrrobutamine
66 Theophylline
67 Nikethamide
67 Phenylephrine
67 Psilocin -ethyl
67 Pyridoxine
67 Tranylcypromine
68 Aconitine
68 Aminophenazone
68 Cyclopentamine
68 Harmine
68 Procyclidine
69 Benperidol
69 Bromazepam
69 Hydroquinine
69 Iproniazid
69 Isoprenaline
69 Lidocaine
69 Nicotinyl Alcohol
69 Phencyclidine
69 Protriptyline
70 2,4,6-TMA
70 Coniine
70 Hydroquinidine
70 Methylphenidate
70 Metronidazole
70 Midazolam
70 Pyrazinamide
70 Yohimbine
71 Acebutolol
71 Brotizolam
71 Desipramine
71 Maprotiline
72 Cinchophen
72 Dipipanone
72 Flumazenil
72 Ibogaine
72 Ketamine
72 Pentazocine
73 Clemizole
73 Droperidol
73 Levallorphan
73 Methoxamine
74 Amiloride
74 Cyclazocine
74 Etilefrine
74 Methoxyamfetamine
74 Metoprolol
74 Papaverine
74 Pentetrazol
74 Piritramide
74 Prazosin
74 Salbutamol
74 Tocainide
75 Amfetamine
75 Carbamazepine
75 Carbimazole
75 Fluanisone
75 Haloperidol
75 Methyldopa
75 Noscapine
75 Timolol
75 Trihexyphenidyl
76 6-Hydroxybrotizolam
76 Butacaine
76 Clonidine
76 DOM
76 Guanoxan
76 Harman
76 Metaraminol
76 Methylenedioxyamfetamine
76 Trifluperidol
77 Amantadine
77 Chlordiazepoxide
77 Chlorphentermine
77 Fentanyl
77 Lidoflazine
77 Methyl Nicotinate
77 Orciprenaline
77 Piminodine
77 Terbutaline
78 Dextromoramide
78 Fluspirilene
78 Glafenine
78 Mexiletine
78 Oxprenolol
78 Phentermine
78 Pindolol
78 α-Hydroxybrotizolam
79 Bupivacaine
79 Carisoprodol
79 Crotetamide
79 Pipradrol
79 Prilocaine
79 Proguanil
79 Propranolol
79 Rescinnamine
79 Santonin
80 Chlormezanone
80 Econazole
80 Ketazolam
80 Miconazole
80 Reserpine
81 Disulfiram
81 Fenoterol
81 Isoxsuprine
81 Loperamide
81 Metomidate
81 Pemoline
81 Phenazocine
82 Flunitrazepam
82 Lorazepam
82 Lormetazepam
82 Phenelzine
82 Phenoperidine
82 Pimozide
82 Temazepam
83 Buphenine
83 Camazepam
83 Chlortalidone
83 Cropropamide
83 Demoxepam
83 Medazepam
83 Nordazepam
84 Acecarbromal
84 Acetanilide
84 Mebendazole
84 Metaclazepam
84 Methaqualone
84 Normetaclazepam
84 Salicylamide
85 Clobazam
85 Clomethiazole
85 Diazepam
85 Etomidate
85 Mebutamate
85 Prenylamine
86 Clofazimine
86 Fenyramidol
86 Isocarboxazid
86 Methyltestosterone

9

86 Nitrazepam
87 Azinphos-(Me)
87 Benzocaine
87 Bisacodyl
87 Carbromal
87 Cinnarizine
87 Clonazepam
87 Clorazepic Acid
87 Dipyridamole
87 Ethinamate
87 Meprobamate
88 Ambucetamide
88 Bemegride
88 Bromocriptine
88 Chlorpropamide
88 Clioquinol
88 Meclozine
88 *N*-dealkyl-2-oxo-quazepam
88 Testosterone
88 Tolbutamide
89 Glutethimide
89 Hydroxy-*N*-dealkyl-2-oxo-quazepam
89 Mephenesin
89 Penfluridol
89 Phenothiazine
89 Prazepam
89 Progesterone
89 Tetrazepam
90 2-Oxo-quazepam
90 Butyl Aminobenzoate
90 Chloramphenicol
90 Chlorzoxazone
90 Methyl Salicylate
90 Hydroxy-2-oxo-quazepam
91 Hexetidine
91 Oxazepam
91 Resorcinol
92 Acenocoumarol
92 Diethylstilbestrol
92 Diphenoxylate
92 Nortetrazepam
92 Thymol
93 Camphor
93 Quazepam -CFTB
95 Cyclandelate
96 Bezitramide
96 Cholesterol
96 Quazepam
97 Phenoxybenzamine
98 Hexachlorophene

System TAG

00 Adrenaline
01 Mesoridazine
02 Benzatropine
02 Protriptyline
02 Racemorphan
04 Nialamide
05 Loprazolam
05 Sotalol
06 Acebutolol
07 Lysergamide
07 Opipramol
07 Prochlorperazine
08 Aminopromazine
08 Trifluoperazine
09 Mexiletine
09 Procainamide
09 Prothipendyl
11 Alprenolol
11 Zuclopenthixol
12 2,4,6-TMA
12 Acepromazine
13 6-Hydroxybrotizolam
17 Nefopam
17 Trimeperidine
18 Clomipramine
18 Noxiptiline
22 Meclofenoxate
25 Cyclazocine
27 Brotizolam
29 Lobeline
29 Nalorphine
31 Nomifensine
31 α-Hydroxybrotizolam
32 Benperidol
34 Bamifylline
37 Trazodone
38 Butriptyline
38 Hydroxy-*N*-dealkyl-2-oxo-quazepam
42 Piritramide
48 Tranylcypromine
52 Trimetozine
52 Yohimbine
56 Mebutamate
58 *N*-dealkyl-2-oxo-quazepam
58 Normetaclazepam
58 Oxypertine
58 Phenoperidine
59 Hydroxy-2-oxo-quazepam
60 Nortetrazepam
62 Metaclazepam
63 Naloxone
63 Phenelzine
63 Reserpine
65 Bupivacaine
65 Camazepam
66 Deserpidine
66 Tetrazepam
71 Oxo-quazepam
73 Quazepam -CFTB
76 Quazepam

System TAH

45 CBN
50 Δ^9-THC
50 Δ^9-Tetrahydrocannabinol
60 CBD

System TAJ

00 Amiloride
00 Antazoline
00 Benzilonium Bromide
00 Berberine
00 Cefalothin
00 Chloroquine
00 Chlorphenamine
00 Cimetidine
00 Cinchonidine
00 Debrisoquine
00 Demeclocycline
00 Dopamine
00 Ephedrine
00 Ethambutol
00 Ethylnoradrenaline
00 Guanethidine
00 Homatropine
00 Hyoscyamine
00 Isoetarine
00 Isometheptene
00 Isopropamide Iodide
00 Levodopa
00 Mafenide
00 Mecamylamine
00 Mephentermine
00 Metamfetamine
00 Metaraminol
00 Methoxamine
00 Methyldopa
00 Morphine
00 Normorphine
00 Oxymetazoline
00 Oxyphencyclimine
00 Perphenazine
00 Phenformin
00 Phentolamine
00 Phenylephrine
00 Phthalylsulfathiazole
00 Pindolol
00 Procyclidine
00 Propantheline Bromide
00 Propranolol
00 Propylhexedrine
00 Protokylol
00 Pseudoephedrine
00 Pseudomorphine
00 Quinidine
00 Saccharin
00 Sparteine
00 Sulfasalazine
00 Terbutaline
00 Trimetaphan Camsilate
00 Tryptamine
00 Δ^9-THC
00 Δ^9-Tetrahydrocannabinol
01 Aminocaproic Acid
01 Brompheniramine
01 Hydralazine
01 Nortriptyline
01 Phenylpropanolamine
02 Diethyltryptamine
02 Mescaline
02 Metoprolol
02 Minocycline
02 Narceine
02 Pentazocine
02 Phentermine
02 Ranitidine
03 Eucatropine
03 Hordenine
03 Ketobemidone
03 Methylenedioxymetamfetamine
03 Phenethylamine
04 Brucine
04 Carbinoxamine
04 Dimetindene
04 Erythromycin
04 Etafedrine
04 Mazindol
04 Nicotinic Acid
04 Piperacetazine
04 Strychnine
04 Triamterene
05 Acetophenazine
05 Etacrynic Acid
05 Fendosal
05 Methoxyphenamine
05 Urea
06 Aconitine
06 Apomorphine

06 Diflunisal
06 Dihydrocodeine
06 Fluphenazine
06 Procaine
06 Promazine
07 Desipramine
07 Fenfluramine
07 Imipramine
08 Carfenazine
08 Methadone
08 Xylazine
09 Chloroprocaine
09 Clonidine
09 Emetine
09 Hydrochlorothiazide
09 Hydroflumethiazide
09 Isoniazid
09 Nicotine
09 Tetramisole
09 Thioridazine
10 Butaperazine
10 Codeine
10 Dimethoxanate
10 Furosemide
10 Levallorphan
11 Azapropazone
11 Chlorothiazide
11 Chlorphenoxamine
11 Chlorpromazine
11 Methylphenidate
11 Phanquinone
11 Propoxycaine
11 Quinethazone
12 Methdilazine
12 Salicylic Acid
12 Tetracaine
13 6-Monoacetylmorphine
13 Amitriptyline
13 Cocaine
13 Indoramin
13 Isoxsuprine
13 Oxymorphone
14 Orphenadrine
14 Pethidine
14 Triamcinolone
14 Trimethoprim
15 Amodiaquine
16 Metixene
17 Alclofenac
17 Chlortalidone
18 Acetazolamide
18 Bumetanide
18 Physostigmine
19 Normethadone
19 Prednisolone
19 Tiotixene
19 Zomepirac
20 Anileridine
20 Chlorprothixene
20 Methapyrilene
20 Phenmetrazine
20 Tolmetin
21 Chlorcyclizine
22 Epithiazide
22 Pilocarpine
22 Profenamine
22 Sulfanilamide
22 Thebaine
22 Thiethylperazine
23 Alphaprodine
23 Cyclizine
23 Cyclopentolate
23 Doxepin
23 Methysergide
24 Difenidol
24 Molindone
24 Phencyclidine
24 Pyrrobutamine
24 Trichlormethiazide
25 Diamorphine
25 Quinisocaine
26 Hydroxyzine
26 Phenazocine
27 Butacaine
27 Levomepromazine
27 Niflumic Acid
27 Oxycodone
27 Phenyltoloxamine
28 Arecoline
28 Etamiphylline
28 Mepivacaine
28 Probenecid
29 Hexylcaine
29 Primidone
30 Methyclothiazide
30 Paracetamol
30 Tiaprofenic Acid
31 Benzoctamine
31 Benzthiazide
31 Chlorphenesin Carbamate
31 Methylprednisolone
32 Acetylcodeine
32 Chloramphenicol
32 Metronidazole
32 Theobromine
33 Dihydroergotamine
33 Lysergide
33 Prednisone
34 Benactyzine
34 Bromazepam
34 Metolazone
34 Nitrofurazone
34 Procarbazine
34 Propiomazine
34 Resorcinol
34 Sulfamethizole
35 Furaltadone
35 Mebutamate
35 Meprobamate
35 Polythiazide
36 Hydrocortisone
37 Biperiden
37 Diazoxide
37 Flunixin
37 Phenindamine
37 Suprofen
38 Bendroflumethiazide
38 Chlorphenesin
38 Dexamethasone
38 Trihexyphenidyl
39 Colchicine
39 Prazosin
39 Sulfafurazole
39 Sulindac
40 Aspirin
40 Clonixin
40 Diclofenac
40 Sulfadiazine
40 Theophylline
40 Tosylchloramide Sodium
41 Fluoxymesterone
41 Methocarbamol
41 Sulfachlorpyridazine
42 Dicycloverine
42 Mepenzolate Bromide
43 Fenbufen
43 Sulfadimidine
44 Aminobenzoic Acid
44 Carbamazepine
44 Diethylpropion
44 Methandienone
44 Methazolamide
45 Mephenesin
45 Sulfamethoxazole
46 Dantrolene
46 Fluorometholone
46 Indometacin
46 Lorazepam
46 Nitrofurantoin
46 Phenazopyridine
46 Sulfamethoxypyridazine
46 Tiabendazole
47 Flurbiprofen
47 Ketamine
47 Oxazepam
47 Prenylamine
48 Benaprizine
48 Chlordiazepoxide
48 Lysergic Acid
48 Phenytoin
49 Piperidolate
50 Clonazepam
50 Prilocaine
50 Sulfapyridine
51 Chlorzoxazone
51 Cortisone
51 Diphenadione
51 Ethoxzolamide
51 Phenazone
51 Piminodine
52 Etorphine
52 Flavoxate
52 Thiopropazate
53 Aminophenazone
53 Benzquinamide
53 Nitrazepam
53 Nordazepam
53 Sulfadimethoxine
53 Sulfadoxine
54 Amygdalin
54 Azaperone
54 Caffeine
54 Ketoprofen
54 Paramethasone
55 Benzoic Acid
55 Chlormezanone
55 Estradiol
55 Ethinylestradiol
55 Fludrocortisone
55 Flufenamic Acid
55 Lidocaine
56 Benoxaprofen
56 Benzonatate
56 Hexachlorophene
56 Indapamide
56 Oxyphenbutazone
56 Vinbarbital
57 Etoxazene
58 Ethinamate
58 Fenoprofen
58 Mebendazole
58 Phenacetin
59 Azapetine
59 Ibuprofen
59 Meclofenamic Acid
59 Nikethamide
59 Pramocaine
59 Testosterone
60 Acetohexamide
60 Dicoumarol
60 Doxapram
60 Etamivan
60 Methyltestosterone
60 Naproxen
61 Acecarbromal
61 Diphenylpyraline
61 Ethotoin
61 Hyoscine
61 Methyl Hydroxybenzoate
62 Buprenorphine
62 Halcinonide
63 Estrone
64 Aniline
64 Dimoxyline
64 Mephenytoin
65 Chlorpropamide
65 Cholesterol
65 Meclozine
65 Noscapine
65 Phenoxybenzamine
65 Temazepam
65 Tolazamide
66 Cyclopentobarbital
66 Etidocaine
66 Papaverine
66 *p*-Cresol
67 Diazepam
67 Isocarboxazid
68 Acenocoumarol
68 Griseofulvin
68 Mefenamic Acid
69 Piroxicam
69 Tolbutamide
69 *m*-Cresol
70 Medazepam

70 Oxymetholone
70 Pentetrazol
71 Pargyline
73 Spironolactone
73 Thiopental
73 *o*-Cresol
75 Prazepam
76 Progesterone
77 Mecloqualone
78 Desoxycortone
81 Cyclandelate
81 Phensuximide
82 Anisindione
82 Methoxsalen
83 Tetrabenazine
85 Eugenol
85 Mesuximide
87 Paramethadione
88 CBD
90 Boldenone
90 CBN
90 Clofibrate
90 Phenothiazine
90 Phenylbutazone
95 Clofenotane
95 Methyl Salicylate
95 Triprolidine
96 Tolnaftate
98 Bromhexine
98 Camphor

System TAK

00 Acetophenazine
00 Aconitine
00 Amiloride
00 Aminophenazone
00 Amodiaquine
00 Anileridine
00 Aniline
00 Antazoline
00 Apomorphine
00 Arecoline
00 Azaperone
00 Benzilonium Bromide
00 Berberine
00 Brompheniramine
00 Brucine
00 Butaperazine
00 Carbinoxamine
00 Carfenazine
00 Chloroprocaine
00 Chloroquine
00 Chlorothiazide
00 Chlorphenamine
00 Cimetidine
00 Cinchonidine
00 Cocaine
00 Codeine
00 Demeclocycline
00 Dihydrocodeine
00 Dimetindene
00 Dopamine
00 Ethambutol
00 Ethylnoradrenaline
00 Etorphine
00 Fluphenazine
00 Furaltadone
00 Guanethidine
00 Hordenine
00 Hydrochlorothiazide
00 Hydroflumethiazide
00 Hydroxyzine
00 Isoetarine
00 Isopropamide Iodide
00 Levodopa
00 Lidocaine
00 Metaraminol
00 Methadone
00 Methapyrilene
00 Methyldopa
00 Minocycline
00 Molindone
00 Morphine
00 Nicotine
00 Normorphine
00 Oxymorphone
00 Phanquinone
00 Phenylephrine
00 Pilocarpine
00 Piperacetazine
00 Prazosin
00 Procaine
00 Procyclidine
00 Propantheline Bromide
00 Propoxycaine
00 Pseudomorphine
00 Ranitidine
00 Sparteine
00 Terbutaline
00 Tetracaine
00 Tetramisole
00 Triamterene
01 Acetazolamide
01 Ephedrine
01 Hydralazine
01 Hyoscyamine
01 Ketobemidone
01 Narceine
01 Oxycodone
01 Oxymetazoline
01 Oxyphencyclimine
01 Phentolamine
01 Pseudoephedrine
01 Trimetaphan Camsilate
01 Δ^9-THC
01 Δ^9-Tetrahydrocannabinol
02 6-Monoacetylmorphine
02 Chlordiazepoxide
02 Chlorpromazine
02 Clonidine
02 Cyclopentolate
02 Diethylpropion
02 Emetine
02 Erythromycin
02 Etamiphylline
02 Etoxazene
02 Eucatropine
02 Homatropine
02 Imipramine
02 Lysergide
02 Mepivacaine
02 Orphenadrine
02 Phenindamine
02 Phenylpropanolamine
02 Promazine
02 Protokylol
02 Quinidine
02 Strychnine
02 Thioridazine
02 Tiabendazole
03 Benactyzine
03 Debrisoquine
03 Diethyltryptamine
03 Dihydroergotamine
03 Doxapram
03 Isoniazid
03 Mephentermine
03 Metamfetamine
03 Pentazocine
03 Perphenazine
03 Phenformin
03 Physostigmine
03 Prednisolone
03 Procarbazine
03 Triprolidine
04 Bromazepam
04 Buprenorphine
04 Isometheptene
04 Ketamine
04 Mepenzolate Bromide
04 Methylphenidate
04 Methysergide
04 Phencyclidine
04 Phenoxybenzamine
04 Phthalylsulfathiazole
04 Prednisone
05 Acetylcodeine
05 Alphaprodine
05 Amitriptyline
05 Benaprizine
05 Butacaine
05 Chlorphenoxamine
05 Diamorphine
05 Epithiazide
05 Hydrocortisone
05 Isoxsuprine
05 Paracetamol
05 Phentermine
05 Propranolol
05 Sulfanilamide
05 Tiotixene
05 Trichlormethiazide
05 Tryptamine
06 Azapropazone
06 Benzquinamide
06 Benzthiazide
06 Cefalothin
06 Etafedrine
06 Levallorphan
06 Pethidine
06 Pindolol
06 Quinethazone
06 Triamcinolone
06 Xylazine
07 Dexamethasone
07 Lysergic Acid
08 Chloramphenicol
08 Chlorprothixene
08 Colchicine
08 Methyclothiazide
08 Metolazone
08 Papaverine
08 Phenmetrazine
08 Polythiazide
08 Theobromine
08 Trimethoprim
09 Cortisone
09 Mescaline
09 Nitrofurantoin
09 Nortriptyline
10 Chlorcyclizine
10 Chlortalidone
10 Dimoxyline
10 Indoramin
10 Nicotinic Acid
10 Profenamine
11 Bendroflumethiazide
11 Cyclizine
11 Piperidolate
11 Sulfadiazine
11 Sulfafurazole
11 Thebaine
11 Thiethylperazine
12 Biperiden
12 Medazepam
12 Methocarbamol
12 Methoxamine
12 Metoprolol
13 Diazoxide
13 Difenidol
13 Methylprednisolone
13 Quinisocaine
14 Benzoctamine
14 Clonixin
14 Doxepin
14 Metronidazole
14 Sulfamethizole
15 Hexylcaine
15 Methazolamide
15 Phenyltoloxamine
15 Urea
16 Mecamylamine

16 Phenethylamine
16 Propiomazine
17 Methylenedioxymetamfetamine
17 Sulfadimidine
18 Caffeine
18 Chlorphenesin Carbamate
18 Noscapine
18 Phenazone
18 Tosylchloramide Sodium
19 Etidocaine
19 Levomepromazine
19 Methdilazine
19 Nikethamide
19 Saccharin
19 Sulfapyridine
20 Flunixin
20 Methoxyphenamine
20 Normethadone
20 Pargyline
20 Phenazocine
20 Pramocaine
20 Resorcinol
20 Sulfamethoxypyridazine
21 Theophylline
22 Indapamide
22 Metixene
22 Prilocaine
22 Trihexyphenidyl
23 Benzonatate
23 Desipramine
23 Sulfachlorpyridazine
24 Dantrolene
24 Meclozine
24 Piminodine
24 Propylhexedrine
25 Dicycloverine
25 Fenfluramine
25 Furosemide
25 Pyrrobutamine
26 Azapetine
26 Fluorometholone
26 Sulfamethoxazole
28 Bromhexine
28 Sulfadimethoxine
28 Sulfadoxine
29 Mephenesin
29 Meprobamate
30 Sulfasalazine
32 Chlorphenesin
33 Tetrabenazine
35 Fludrocortisone
35 Fluoxymesterone
36 Nitrofurazone
36 Thiopropazate
39 Paramethasone
40 Sulindac
41 Oxyphenbutazone
41 Pentetrazol
41 Phenacetin
42 Bumetanide
42 Etacrynic Acid
42 Lorazepam
44 Aminobenzoic Acid
45 Chlormezanone
45 Piroxicam
47 Mebutamate
47 Oxazepam
48 Diazepam
48 Etamivan
48 Ethoxzolamide
48 Griseofulvin
49 Hyoscine
50 Diphenylpyraline
51 Acenocoumarol
52 Methyl Hydroxybenzoate
52 Nitrazepam
53 Clonazepam
54 Temazepam
56 Niflumic Acid
56 Phenazopyridine
57 Mebendazole
58 Acecarbromal
58 Estradiol
58 Ethinylestradiol
58 Halcinonide
59 Tolmetin
60 Nordazepam
60 Primidone
61 Chlorzoxazone
61 Methandienone
62 Amygdalin
62 Suprofen
62 Vinbarbital
63 Cyclopentobarbital
63 Prenylamine
63 Testosterone
64 Carbamazepine
64 Diclofenac
64 Spironolactone
65 Aspirin
65 Methyltestosterone
65 Zomepirac
66 Acetohexamide
66 Estrone
66 Ethotoin
67 Isocarboxazid
68 Fenbufen
68 Fendosal
68 Mecloqualone
68 Methoxsalen
68 Progesterone
69 Diflunisal
69 Ethinamate
69 Flurbiprofen
69 Prazepam
70 Alclofenac
70 Anisindione
70 Mephenytoin
70 Mesuximide
70 Paramethadione
71 Cyclandelate
71 Desoxycortone
71 Flavoxate
71 Hexachlorophene
71 Phensuximide
71 Probenecid
71 Salicylic Acid
71 Thiopental
71 Tolazamide
72 Mafenide
72 Nitrofurantoin
74 Diphenadione
74 Oxymetholone
74 Tolbutamide
75 Naproxen
76 CBD
76 Ibuprofen
76 Phenylbutazone
77 CBN
77 Meclofenamic Acid
77 Phenothiazine
78 Chlorpropamide
78 Cholesterol
78 Eugenol
78 Fenoprofen
78 Flufenamic Acid
78 *m*-Cresol
78 *p*-Cresol
80 Benoxaprofen
80 Clofibrate
80 Dicoumarol
80 Tiaprofenic Acid
81 Benzoic Acid
82 Ketoprofen
82 *o*-Cresol
83 Tolnaftate
84 Dimethoxanate
84 Phenytoin
86 Mefenamic Acid
86 Methyl Salicylate
88 Boldenone
90 Indometacin
93 Clofenotane
95 Camphor

System TAL

00 Levodopa
00 Phanquinone
02 Cocaine
02 Pseudomorphine
04 Chloroquine
04 Demeclocycline
05 Benzilonium Bromide
05 Ecgonine
06 Chlorpropamide
06 Methyldopa
06 Tetramisole
07 Dopamine
08 Cimetidine
08 Minocycline
09 Amodiaquine
10 Ranitidine
11 Nicotine
13 Oxymorphone
15 Antazoline
15 Ethylnoradrenaline
15 Morphine
16 Guanethidine
19 Isopropamide Iodide
20 Berberine
20 Butacaine
20 Ethambutol
20 Hordenine
20 Metaraminol
20 Propantheline Bromide
20 Trimetaphan Camsilate
22 Apomorphine
22 Phenylephrine
22 Procaine
24 Aconitine
24 Mazindol
24 Oxyphencyclimine
25 Chlorphenamine
25 Hydralazine
25 Oxymetazoline
25 Saccharin
25 Tetracaine
26 Codeine
26 Isoetarine
27 Carbinoxamine
27 Sparteine
28 Arecoline
28 Brompheniramine
28 Lidocaine
28 Normorphine
29 Amiloride
29 Ephedrine
29 Phenformin
29 Phenylpropanolamine
29 Terbutaline
30 Piperacetazine
30 Pseudoephedrine
30 Urea
31 Δ^9-THC
31 Δ^9-Tetrahydrocannabinol
32 Chloroprocaine
33 Acetophenazine
33 Eucatropine
34 Homatropine
34 Hydroxyzine
34 Hyoscyamine
34 Phentolamine
35 Diethylpropion
36 Debrisoquine
36 Mephentermine
36 Narceine
36 Oxycodone
36 Phentermine
36 Procyclidine
36 Tryptamine
37 Molindone

38 Dihydrocodeine
39 Sulfasalazine
40 Azacyclonol
40 Dimetindene
40 Hydrochlorothiazide
40 Ketobemidone
40 Mepivacaine
40 Triamterene
41 Cefalothin
41 Chlorothiazide
41 Diethyltryptamine
41 Fluphenazine
41 Promazine
42 Butaperazine
42 Cyclopentolate
42 Erythromycin
42 Phthalylsulfathiazole
43 Hydroflumethiazide
43 Isometheptene
43 Ketamine
44 Etamiphylline
44 Pilocarpine
44 Strychnine
45 Aminocaproic Acid
45 Metamfetamine
45 Methadone
45 Propoxycaine
47 Chlorpromazine
47 Isoniazid
47 Orphenadrine
48 Benactyzine
48 Methapyrilene
48 Phencyclidine
48 Protokylol
49 Dimethoxanate
49 Etafedrine
49 Propranolol
50 Maprotiline
50 Methoxamine
50 Phenethylamine
50 Physostigmine
50 Sulfanilamide
51 6-Monoacetylmorphine
51 Carfenazine
51 Clonidine
51 Mescaline
51 Phenoxybenzamine
51 Thioridazine
52 Imipramine
52 Mepenzolate Bromide
54 Chlorphenoxamine
54 Prazosin
56 Amitriptyline
56 Nicotinic Acid
56 Perphenazine
56 Pindolol
56 Quinethazone
57 Etacrynic Acid
57 Methylenedioxymetamfetamine
57 Pentazocine
57 Profenamine
58 Benaprizine
58 Emetine
58 Mecamylamine
59 Lysergide
59 Tiabendazole
60 Acetazolamide
60 Alphaprodine
60 Etorphine
60 Isoxsuprine
60 Phenmetrazine
61 Acetylcodeine
61 Azapropazone
61 Trichlormethiazide
62 Furaltadone
63 Bromazepam
63 Chlortalidone
63 Epithiazide
64 Brucine
64 Diamorphine
64 Methoxyphenamine
64 Piperidolate
65 Benzoctamine
65 Chlorprothixene
65 Prednisolone
65 Propylhexedrine
65 Theobromine
65 Triamcinolone
66 Levallorphan
66 Methysergide
66 Quinisocaine
66 Trimethoprim
67 Azaperone
67 Difenidol
68 Anileridine
68 Fenfluramine
68 Nortriptyline
68 Quinidine
68 Resorcinol
68 Tosylchloramide Sodium
69 Diflunisal
69 Methyclothiazide
69 Phenyltoloxamine
69 Prilocaine
69 Triprolidine
70 Chlorcyclizine
70 Cinchonidine
70 Etoxazene
70 Furosemide
70 Hexylcaine
70 Methylphenidate
70 Metoprolol
70 Polythiazide
70 Salicylic Acid
70 Sulfadiazine
70 Sulfafurazole
70 Xylazine
71 Benzthiazide
71 Chloramphenicol
71 Doxepin
71 Tiotixene
72 Bendroflumethiazide
72 Cyclizine
72 Desipramine
72 Methdilazine
72 Methocarbamol
72 Pethidine
73 Biperiden
73 Colchicine
73 Doxapram
73 Metixene
73 Paracetamol
74 Hydrocortisone
74 Prednisone
75 Dexamethasone
75 Diazoxide
75 Etidocaine
75 Metolazone
75 Metronidazole
75 Sulfamethizole
76 Aminophenazone
76 Fenoprofen
76 Thebaine
77 Buprenorphine
77 Indoramin
78 Bromhexine
78 Mephenesin
78 Meprobamate
78 Methazolamide
78 Methylprednisolone
78 Normethadone
78 Theophylline
79 Chlordiazepoxide
79 Lysergic Acid
80 Bumetanide
80 Chlorphenesin Carbamate
80 Clonixin
80 Sulfachlorpyridazine
80 Trihexyphenidyl
81 Caffeine
81 Levomepromazine
81 Propiomazine
81 Sulfamethoxazole
82 Phenindamine
83 Aminobenzoic Acid
83 Cortisone
83 Fendosal
83 Flunixin
83 Nitrofurazone
83 Phenazone
83 Thiethylperazine
83 Tolmetin
84 Diclofenac
84 Dicycloverine
84 Dihydroergotamine
84 Sulfamethoxypyridazine
85 Sulfapyridine
86 Chlorphenesin
86 Lorazepam
86 Primidone
86 Pyrrobutamine
87 Mebutamate
87 Suprofen
88 Zomepirac
89 Nikethamide
89 Oxazepam
89 Phenacetin
90 Acecarbromal
90 Alclofenac
90 Aspirin
90 Azapetine
90 Benzonatate
90 Cyclopentobarbital
90 Dantrolene
90 Fluorometholone
90 Indometacin
90 Niflumic Acid
90 Phenazocine
90 Piminodine
90 Pramocaine
90 Prenylamine
90 Probenecid
90 Procarbazine
90 Sulfadoxine
91 Acetohexamide
91 Benzquinamide
91 Clonazepam
91 Diphenadione
91 Estradiol
91 Estrone
91 Ethinamate
91 Ethinylestradiol
91 Ethotoin
91 Ethoxzolamide
91 Fenbufen
91 Fludrocortisone
91 Fluoxymesterone
91 Flurbiprofen
91 Halcinonide
91 Hexachlorophene
91 Mephenytoin
91 Methyl Hydroxybenzoate
91 Paramethasone
91 Phenazopyridine
91 Thiopropazate
91 Vinbarbital
92 Acenocoumarol
92 Amygdalin
92 Benzoic Acid
92 Chlorzoxazone
92 Diphenylpyraline
92 Flavoxate
92 Indapamide
92 Isocarboxazid
92 Meclofenamic Acid
92 Methandienone
92 Methyltestosterone
92 Nitrazepam
92 Nordazepam
92 Oxyphenbutazone
92 Sulindac
92 Temazepam
92 Testosterone
92 Thiopental
93 Dimoxyline
93 Hyoscine
93 Ibuprofen
93 Mafenide
93 Mebendazole
93 Naproxen
93 Noscapine
93 Papaverine
93 Pargyline
93 Tolbutamide
94 Carbamazepine

94 Chlormezanone
94 Oxymetholone
94 Paramethadione
94 Piroxicam
94 Prazepam
95 Anisindione
95 Cyclandelate
95 Flufenamic Acid
95 Meclozine
95 Medazepam
95 Mefenamic Acid
95 Pentetrazol
95 Progesterone
95 Sulfadimidine
95 Tolazamide
96 Aniline
96 Cholesterol
96 Desoxycortone
96 Diazepam
96 Dicoumarol
96 Etamivan
96 Eugenol
96 Griseofulvin
96 Mecloqualone
96 Methoxsalen
96 Phenothiazine
96 Phensuximide
96 Phenytoin
96 Spironolactone
96 *m*-,*o*-,*p*-Cresol
97 CBD
97 CBN
97 Clofenotane
97 Clofibrate
97 Phenylbutazone
97 Tetrabenazine
97 Tolnaftate
98 Boldenone
98 Camphor
98 Ketoprofen
98 Mesuximide
98 Sulfadimethoxine
98 Tiaprofenic Acid
99 Benoxaprofen

System TAM

09 Estradiol
13 Chlorothiazide
33 Triamcinolone
54 Prednisolone
56 Methylprednisolone
58 Hydrocortisone
60 Prednisone
64 Flumetasone
66 Dexamethasone
68 Fluocinolone Acetonide
74 Fluoxymesterone
75 Metolazone
86 Fluorometholone
86 Oxymetholone
88 Methandienone
90 Fludrocortisone
91 Cortisone
91 Desoxycortone
91 Paramethasone
92 Methyltestosterone
92 Testosterone
94 Ethinylestradiol
95 Estrone
96 Cholesterol
97 Progesterone
99 Boldenone

10 Gas Chromatographic Data

ND = not detected/eluted
t = tailing peak

System G1/180

0.44	Probarbital

System G2/120

1.5	Phenoxypropazine

System G2/225

0.25	Octacaine	0.85	Etoxeridine	1.70	Pentaquin
0.31	Pyrrocaine	0.92	Betameprodine	1.75	Apoatropine
0.32	1,2-Naphthoquinone	0.98	Piperoxan	1.75	Hydroxypethidine
0.35	Cloponone	0.99	Nifuroxime	1.80	Butethamine
0.35	Sulfonal	1.00	Alphameprodine	1.82	Panidazole
0.36	Dioxyamidopyrine	1.00	Methyldihydromorphine	2.00	Dimethocaine
0.41	Panidazole	1.0	Metopon	2.00	Phenatine
0.43	Phenatine	1.10	Dimethylthiambutene	2.53	Benzethidine
0.48	Dimethocaine	1.13	Octacaine	2.55	Alphamethadol
0.48	Proheptazine	1.20	Xenysalate	2.67	Tiletamine
0.50	Racemethorphan	1.27	Acetyldihydrocodeine	3.30	Diphenazoline
0.60	Tiletamine	1.30	Ethylmethylthiambutene	4.70	Racemoramide
0.66	Amolanone	1.30	Methaphenilene	ND	Brocresine
0.70	Levomethorphan	1.30	Pipethanate	ND	Imidocarb
0.75	Diphenazoline	1.32	Pyrrocaine	ND	Isometamidium
0.75	Properidine	1.54	Morpheridine	ND	Phthivazid
0.78	Desomorphine	1.55	Cloponone	ND	Picloxydine
0.79	Vetrabutine	1.60	Dioxyamidopyrine	ND	Resorantel
0.81	Methyldesorphine	1.60	Phenadoxone	ND	Rolicyprine
0.84	Betaprodine	1.70	Amprotropine	ND	Taurolin

System G4

0.40	Betameprodine	0.65	Properidine	1.05	Etoxeridine
0.41	Betaprodine	0.66	Desomorphine	1.15	Sulfonal
0.43	Amolanone	0.74	Dimethylthiambutene	1.70	Benzethidine
0.43	Apoatropine	0.80	Ethylmethylthiambutene	2.40	Amprotropine
0.46	Proheptazine	0.95	Acetyldihydrocodeine	2.44	Alphamethadol
0.48	Alphameprodine	0.96	Phenadoxone		
0.56	Hydroxypethidine	0.96	Piperoxan		

System G4/225

0.13	Dimethocaine	2.70	Tiletamine	ND	Phthivazid
0.30	Tiletamine	5.00	Dioxyamidopyrine	ND	Picloxydine
0.51	Dioxyamidopyrine	5.30	Vetrabutine	ND	Resorantel
0.85	Diphenazoline	ND	Brocresine	ND	Taurolin
0.89	Phenatine	ND	Imidocarb		
1.29	Dimethocaine	ND	Isometamidium		

System G5

0.69	Alphamethadol	1.09	Methyridine

System G7

1.1	Probarbital

System GA

0	Acemetacin
305	Dichlorodifluoromethane
361	Cryofluorane
372	Acetaldehyde
421	Ethanol
462	Enflurane
469	Acetone
484	Trichlorofluoromethane
491	Methanol
515	Ether
515	Methylene Chloride
530	Isopropyl Alcohol
533	Halothane
571	Propanol
579	Methyl Ethyl Ketone
596	Ethyl Acetate
605	Chloroform
634	Trichloroethane
695t	Chloral Hydrate
701	Methoxyflurane
710	Trichloroethylene
715	Methylpentynol
756	Toluene
780	Monoethanolamine
786	Paraldehyde
789	Tetrachloroethylene
798	Ethylene Glycol
813	Piperazine
838	Urethane
857	Trichloroethanol
860	*p*-Xylene
863	*m*-Xylene
882	Allopurinol
884	*o*-Xylene
888	Tuaminoheptane
910	Tetrachloroethane
930	Putrescine
947	Benzaldehyde
949	Chlorobutanol
981	Phenol
1000	Acefylline Piperazine
>1000	Amyl Nitrite
1000	Benzene
1000	Carbon Tetrachloride
1010	Aminotriazole-AC
1015	Ethchlorvynol
1017	Fluspirilene
1020	Metaldehyde
1035	Cadaverine
1040	Benzyl Alcohol
1040	*o*-Cresol
1050	Aminotriazole-Me_2
1052	Isometheptene
1060	Dimethadione
1060	Nortrimethadione
1060	*p*-Cresol
1064	Valproic Acid
1065	*m*-Cresol
1090	Trimethadione
1095	Amfetamine-trifluroacetic Acid
1100	4-Aminophenol-Me
1100	Amfetamine (formyl)
1100	Methyl Nicotinate
1100	Phentermine-TFA
1105	Emylcamate
1110	*p*-Cresol-AC
1111	Phenethylamine
1115	Paramethadione
1120	Heptaminol
1125	Amfetamine
1133	Norfenfluramine
1140	OH-Isovalerianic Acid
1143	Camphor
1155	Phentermine
1158	Aniline
1160	OH-Desacyl-prilocaine
1160	Deschloro-2-OH-clomethiazole
1175	Metamfetamine
1175	Propylhexedrine
1180	2,6-Dimethylaniline
1180	Benzoic Acid
1185	Deschloro-clomethiazole
1186	Naphthalene
1190	Amfetamine-TMS
1190	Br-Isovalerianic Acid
1190	Methamidophos
1195	Arecoline
1195	Methyl Salicylate
1195	Phentermine-TMS
1195	Salicylic Acid (-Me_2)
1195	Salicylic Acid-Me
1200	2,5-Dichloromethoxybenzene
1200	Salicylic Acid (-Me)
1205	Ethosuximide
1206	Menthol
1210	4-Chloroaniline
1210	Desacyl-hyoscine (art)
1210	Methenamine
1212	Sulfafurazole
1214	Pargyline
1215	Carbromide
1215	Nicotinyl Alcohol
1220	(*p*-Aminophenol)-Me_2
1220	Tranylcypromine
1227	Clomethiazole
1230	Cyclopentamine
1230	Fenfluramine
1235	Betahistine
1235	Chlormezanone (art)
1235	Dimethylamfetamine
1235	Deschloro-COOH-clomethiazole
1240	Amantadine
1240	Hydroquinone
1240	Mebanazine
1240	Mephentermine
1250	Pyrazinamide
1253	4-Aminophenol
1253	*p*-Aminophenol
1253	*p*-Aminophenol-aprindine
1258	Resorcinol
1260	Thymol
1265	4-Aminophenol
1270	Oxo-ethosuximide
1275	Dichlorvos
1275	P-phenetidine
1280	Anhydroecgonine Methylester
1293	Aletamine
1303	Disulfoton (sulphoxide)
1303	Octamylamine
1305	Phentermine-PFP
1307	Salicylic Acid
1308	Salicylic Acid
1309	Aminosalicylic Acid
1309	Aspirin
1312	Aminotriazole
1313	Phenylpropanolamine
1320	2,4-Dichlorophenol
1320	Aldicarb
1320	Labetalol
1322	3-OH-Ethosuximide
1323	3,4-Dichloroaniline
1330	Amfetamine-PFP
1331	Apronal
1334	Phendimetrazine
1335	Mephentermine (-TFA)
1335	Nicotinic Acid
1335	Phenelzine
1340	OH-Carbromide
1341	Nicotinamide
1344	Chlorphentermine
1345	Ephedrine (-TFA_2)
1350	Desacyl-prilocaine-(AC)
1350	Dichlorophenylisocyanate
1350	Nicotine
1350	Norselegiline
1350	Desamino-oxo-mexiletine
1353	Dimetridazole
1355	Norephedrine-(TFA_2)
1360	Cathine
1360	Norephedrine
1360	Phenylpropanolamine
1361	Methoxyphenamine
1365	2-OH-Clomethiazole
1365	Ephedrine
1365	Ethinamate
1365	Pseudoephedrine
1368	Acetanilide
1368	Eugenol
1370	Ephedrine (-PFP_2)
1370	OH-Ethyl-ethosuximide
1370	Tryptophan (oH-skatole)
1373	Bemegride
1380	Desbromo-carbromal
1380	Desbromocarbromal
1380	Norephedrine-(PFP_2)
1380	Deschloro-2-OH-ethyl-clomethiazole
1385	Propylhexedrine-PFP
1385	Propylhexedrine-TFA
1388	Rimantadine
1389	Diphenyl
1390	Ametazole
1390	Glyphosate-(Me_4)
1390	Nicotinic Acid-(Me)
1390	Parachlorophenol
1394	Aspirin-(Me)
1395	Hydroquinone (-AC_2)
1395	Trichlorfon (-Me)
1400	4-Chlorobenzoic Acid
1400	COOH-Dimethoate-(Me)
1400	Chlorocresol
1400	Mexiletene
1405	3,4-Dichloroaniline
1405	Methylephedrine
1406	Dimethyl Phthalate
1410	Glyphosate-(Me_3)
1412	Methoxyamfetamine
1413	Piroxicam
1414	(Desethyl)/salicylamide
1414	Salicylamide
1416	Fenoterol
1420	Barbital-Me_2
1420	Chloroxylenol
1430	OH-Dimethoate
1432	Hordenine
1432	Methyl Hydroxybenzoate
1432	Phenmetrazine
1432	Warfarin
1436	Nicotinamide
1438	Trichlorfon
1440	Trichlorophenol
1450	Carbromal (art)
1450	Hydroquinine
1450	Mevinphos
1450	Selegiline
1455	Fenfluramine-PFP
1455	Fenfluramine-TFA
1460	2-OH-Hydroquinone
1460	Dimethylhydroxyaniline
1462	Butylated Hydroxyanisole
1465	Methoxyamfetamine
1465	OH-Methoxy-amfetamine
1465	Pheneturide
1470	Barbital-Me (metharbital)
1470	Metharbital
1472	Methylecgonine
1473	Phenacemide
1475	OH-Propylhexedrine
1480	4-OH-Amfetamine
1480	Hydroxyamfetamine
1480	MDA
1480	Methoxy-methyl hydroxybenzoate
1480	Methylenedioxyamfetamine (MDA)
1485	Mandelic Acid
1486	Diethylpropion
1487	Mandelic Acid
1487	Protokylol
1489	Barbital
1490	1-Naphtholcarbaryl
1490	Butylated Hydroxytoluene
1490	Pholedrine
1492	Cantharidin
1497	Diethylcarbamazine
1497	Histamine
1500	Dichlorophenylmethylcarbamate
1500	Nialamide
1500	Phenyltoloxamine ((*N*-oxide)-ME_2 NOH)
1501	Amfetamine-AC
1501	Mephentermine-(AC)
1504	Thiotepa

1505 1-Naphthol-propranolol
1510 Ibuprofen (-Me)
1510 Norfenfluramine-AC
1510 Phentermine-AC
1510 Desamino-oxo-OH-methoxy-amfetamine
1512 Paracetamol-Me
1513 Carbromal
1515 Pyridostigmine Bromide
1517 Mercaptopurine
1519 Etafedrine
1520 3-OH-Amfetamine-(PFP_2)
1520 Enallylpropymal-(Me_2)
1520 Methylenedioxyamfetamine (art (formyl))
1520 Nordiphenhydramine
1520 Phenprobamate
1520 Desamino-oxo-OH-amfetamine-(AC)
1525 (*p*-Nitrophenol)parathion
1525 Dicamba-Me
1525 Nikethamide
1527 Methyprylon
1528 Hydralazine
1530 Heptaminol-(AC_2)
1530 Phenmetrazine-TFA
1540 Aprobarbital-Me_2
1540 Bromisoval
1540 COOH-Deltamethrin-(Me)
1540 Monocrotophos-(TFA)
1540 OH-Methyprylon (-H_2O)
1542 Clofibate
1545 Aspirin
1545 Gliclazide (art 1-Me)
1547 4-Aminobenzoic Acid
1547 Acetylcysteine
1547 Aminobenzoic Acid
1550 Pentetrazol
1550 Thiamazole
1550 Nor-OH-selegiline
1555 1-Naphtholcarbaryl-(AC)
1555 Benzocaine
1555 *N*-Methyl-4-chlorobenzamide
1555 Norephedrine-(TMS_2)
1560 1-OH-Ethyl-clomethiazole
1560 Doxylamine ((carbinol)-H_2O)
1560 Enallylpropymal
1560 Etenzamide
1560 Gabapentin-(H_2O-Me)
1564 Diethyl Phthalate
1565 Butobarbital-Me_2
1565 DOM (art, formyl)
1565 Secbutabarbital-Me_2
1567 Propyl Hydroxybenzoate
1568 Mephenesin
1570 *N*-Desalkyl-oxo-trifluperidol-($2H_2O$)
1570 OH-Methyl hydroxybenzoate-(AC)
1570 Propylhexedrine-AC
1576 Demeton-*O*
1580 Ethyl Hydroxybenzoate
1580 Fenfluramine-AC
1580 *N*-Desmethoxybenzyl-mepyramine
1580 OH-Selegiline
1583 Diethyltoluamide
1585 Fenproporex
1585 Methylenedioxymetamfetamine
1590 Hydrastinine
1590 Pemoline-Me_2
1592 Metronidazole
1593 2,4-D-Me
1593 Amobarbital-Me_2
1593 Iproniazid
1595 Omethoate
1598 Tolazoline
1600 4-Chloromethylbiphenyl
1600 Allobarbital
1600 Crotamiton
1600 Talbutal (-Me_2)
1600 Desamino-oxo-OH-methoxy-amfetamine (-AC)
1604 Clorprenaline
1605 MDA-PFP
1605 *N*-Ethylnicotinamide
1608 Nicametate
1610 Benzophenone
1610 Idobutal (-Me_2)
1610 Methyprylon Enol-AC
1610 Metipranolol ((phenol)-AC)
1610 Trimethoxyphenylacetonitrile
1615 Ibuprofen
1615 MDA-TFA
1616 DOM
1618 Aprobarbital
1620 Ephedrine (-TMS_2)
1620 Glibornuride (amide)
1620 Gliclazide (amide)
1620 Guaifenesin
1620 Nealbarbital (-Me_2)
1620 Phenmetrazine-TMS
1621 Chlorproguanil
1628 Demeton-*S*-methyl
1630 3-OH-Ibuprofen-(Me)
1630 Pentobarbital-Me_2
1630 Vanillin
1632 Glymidine Sodium
1634 Phensuximide
1634 Prolintane
1636 Allobarbital-Me_2
1640 Amantadine-(AC)
1640 Butoxyethyl Nicotinate
1640 Clofibric Acid
1640 Dipyridamole
1645 Barbituric Acid-Me_3
1645 Diphenylmethanol
1645 Chlorobenzoylpyridine
1645 Trometamol
1650 Benzylpyridylamine
1650 Guaifenesin
1650 *N*-Desalkyl-oxohaloperidol ($2H_2O$)
1651 Tolazamide
1655 Butalbital-Me_2
1655 Secbutabarbital
1655 Vinylbital-(Me_2)
1660 5-(1-Ethylpropyl)barbituric Acid
1660 Butobarbital
1660 Carbidopa-Me_2
1660 Desethyletenzamide-(AC)
1660 Dinitro-orthocresol
1660 Salicylamide-AC
1661 Oxyphencyclimine
1665 (Desvinyl-)vinylbital
1665 Butalbital
1665 Desallyl-secobarbital
1665 Monocrotophos
1665 Paracetamol
1667 Styramate
1668 Etisazole
1670 (Desethyl-deschloro-methoxy-)-atrazine
1670 Gliclazide (art 3)
1670 Isoniazid
1670 Carbinol
1670 Vinbarbital-(Me_2)
1675 Fencamfamin
1675 Monolinuron (-Me)
1675 Phenacetin
1676 Dofamium Chloride
1678 Carbimazole
1678 Dyclonine
1680 (Desethyl-)atrazine
1680 Beclamide-Art
1680 Chlorphenesin
1680 Cyclopentamine-AC
1680 Mescaline
1680 *O*-Desalkyl-phenyltoloxamine
1682 Hydroxyephedrine
1683 Tolbutamide
1684 Demeton-*S*
1685 Etilefrine
1685 Fenproporex-PFP
1685 Isoproturon-(Me)
1685 Salol
1685 Deschloro-di-OH-clomethiazole
1687 Paracetamol
1687 Tigloidine
1688 Crotetamide
1690 Benzene Hexachloride (α-isomer)
1690 Monolinuron ((HOOC-)-Me)
1690 Phenolbisoprolol
1690 Secobarbital-Me_2
1690 Simazine
1690 Tributyl Phosphate
1699 Cetrimide
1700 2,4-D-Isopropyl Ester
1700 Ascorbic Acid ((methylated))
1700 Desacyl-Δ-methrin-(HCN)
1700 Idobutal
1700 Methylbenzophenone
1700 *O*-Desmethylguaifenesin
1700 Desamino-OH-desalkyl-oxprenolol
1703 Talbutal
1705 Fenproporex-TFA
1705 Mesuximide
1710 Amobarbital
1710 Benzene Hexachloride (β-isomer)
1710 MDMA-TMS
1714 Atrazine
1714 Tocainide
1715 Cotinine
1715 Thiambutosine
1720 2-Cl-Benzophenone
1720 Acecarbromal
1720 Beclamide
1720 Chlorothiazide
1720 Desbromo-oxo-ibomal (-Me_2)
1720 MDMA-TFA
1720 Nealbarbital
1724 Hydroxyphenamate
1725 Brallobarbital-Me_2
1725 Dimethoate
1725 Methylphenidate
1725 Tryptamine
1725 Tybamate
1726 Methoxamine
1728 Chlorzoxazone
1729 Vinylbital
1730 Benzimidazolone-AC_2
1730 DOM (-PFP)
1730 Isoprenaline
1730 Methylcaptopril
1730 Methylphenidate-trifluroacetic Acid
1730 Methylsulfonamide
1730 Desbromo-OH-ibomal (-Me_2)
1735 Methohexital (-Me)
1735 Norselegiline-(AC)
1735 Pentobarbital
1735 Desamino-oxo-di-OH-amfetamine (-AC_2)
1738 Benzyl Benzoate
1738 Cropropamide
1738 Propazine
1740 (Methylsulfonamide)-Me
1740 Trimethoxybenzoic Acid-Me
1740 Nor-OH-ketamine (NH_3)
1740 α-Methyltryptamine
1742 Butyl Aminobenzoate
1743 Halquinol
1745 Benzene Hexachloride (γ-isomer)
1745 Hexethal-(Me_2)
1745 Ibomal-(Me_2)
1745 Lindane
1745 Tyramine
1748 Profadol
1748 Trimethoxyamfetamine
1750 Buclizine (carbinol)
1750 Carbinol
1750 Chlorzoxazone-Me
1750 Gabapentin (-H_2O)
1750 (3-OH-)-Me_2-Amobarbital
1750 *N*-Oxide-tolpropamine
1750 Normesuximide
1750 OH-Ibuprofen (-Me)
1750 Trichlorophenoxyacetic Acid-(methyl ester)
1753 Dimethyltryptamine
1753 Vinbarbital
1754 Butetamate
1754 Nitroxinil
1754 Pethidine
1755 Benzene Hexachloride (δ-isomer)
1755 Fencamfamin (-PFP)
1756 Ethionamide
1760 Dimpylate
1760 Pentachlorophenol
1760 Desamino-OH-diphenhydramine
1765 4-Aminophenol-AC_2
1765 COOH-Ibuprofen-(Me_2)
1770 Meclofenoxate
1770 Methohexital
1770 *N*-Methyltryptamine
1770 Neostigmine Bromide

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1770 Desbromo-OH-ibomal
1775 Cyclopentobarbital-Me_2
1780 Fencamfamin (-TMS)
1780 *O*-Desmethyldom-(PFP_2)
1780 Trimethoxybenzoic Acid
1785 Carbaryl-TFA
1785 Linuron-(Me)
1785 Mephenytoin
1785 Meprobamate
1785 Meprobamate
1786 Secobarbital
1790 5-(1-Ethylpropyl)-5-hydroxybarbituric Acid
1790 Desethyl-lidocaine
1790 Phenylmethylbarbituric Acid-Me_2
1791 Chlorpropamide
1792 Alphaprodine
1795 5-Methyltryptamine
1795 Dicamba
1795 Ephedrine (-AC_2)
1795 Desbromo-OH-brallobarbital
1798 Benethamine
1800 2,4-D
1800 Acridine
1800 Caffeine
1800 Desmetryne
1800 Ethotoin
1800 Hexobarbital (-Me)
1800 Monoethylglycinexylidide
1800 *N*-Desalkyl-haloperidol
1800 *N*-Desbenzyl-histapyrrodine
1800 OH-Naproxen (-Me_2)
1801 Sparteine
1802 Chlorquinaldol
1803 Nimorazole
1804 Physostigmine
1805 2,4-D-Isobutyl Ester
1805 Norephedrine-(AC_2)
1805 Pheniramine
1805 Terbutylazine
1807 Dexpanthenol
1807 Theobromine
1808 Cotarnine
1808 Trimeperidine
1810 Norketamine
1810 OH-Methoxymetamfetamine
1810 Phenmetrazine-AC
1810 Salicylic Acid (glycine conjugate-Me)
1811 Pipobroman
1815 (OH-)Aprobarbital
1815 Pentachlorophenol-Me
1816 Protionamide
1819 Saccharin
1820 (*p*-Hydroxyhippuric Acid)-Me
1820 3′-OH-Pentobarbital-(Me_2)
1820 Alprenolol
1820 Lobeline
1823 Prilocaine
1825 Salicylic Acid (glycine conjugate)
1825 Thiopental (-Me_2)
1825 Trichlorophenoxyacetic Acid (-isopropyl ester)
1827 Bisnortilidate
1830 Carisoprodol
1830 Glutethimide
1830 Hexylresorcinol
1830 Isoaminile
1830 MDMA-PFP
1830 Nortilidate
1830 OH-Phenmetrazine (isomer 1)
1830 OH-Phenmetrazine Isomer 1
1830 Desamino-OH-phenyltoloxamine
1832 Moxisylyte
1833 Tetryzoline
1835 2,4-Dichlorophenoxybutyric Acid-Me
1835 Phenazone
1838 Tilidate
1840 2,4-D-Butyl Ester
1840 Benorilate
1840 COOH-Etomidate-(Me)
1840 Ketamine
1844 Dimenhydrinate
1845 Cyclobarbital-Me_2
1845 Salicylic Acid (glycine conjugate-Me_2)
1847 Chlorpyrifos-Me
1850 Trichlorophenoxyacetic Acid
1850 3-OH-Amfetamine-(TMS_2)
1850 4-Chlorobenzophenone
1850 Chlorobenzophenone (ClBP)-buclizine
1850 Dibenzocycloheptanone
1850 Diuron
1850 Hexethal
1850 Isovalerianic Acid Carbamide
1850 Salbutamol (-H_2O)
1851 Norfluoxetine
1852 Lachesine Chloride
1853 Brallobarbital
1855 (Desamino-OH-)-aminophenazone
1855 4-OH-Phenazone
1855 Benzfetamine
1855 Dichloralphenazone
1855 Hexobarbital
1855 Methylphenobarbital (-Me)
1855 Parathion (methyl)
1855 Phenobarbital-Me_2
1855 Viloxazine
1857 Ethoheptazine
1857 Thiopental
1859 Fluoxetine
1860 Demeton-*S*-methylsulfoxide
1860 MDA-AC
1860 Metyrapone
1860 OH-Salicylamide-(AC_2)
1860 OH-Selegiline-(AC)
1865 3′-OH-Secobarbital
1865 Carbaryl
1865 Cyclopentobarbital
1865 Guaifenesin-(AC_2)
1865 OH-Ethyl-glutethimide
1865 OH-Phenmetrazine (isomer 2)
1865 OH-Phenmetrazine Isomer 2
1866 Demeton-*S*-methylsulfone
1870 Diphenhydramine
1870 Etomidate
1870 Lidocaine
1870 Mafenide-(Me_4)
1870 Oxo-methyprylon
1870 Oxprenolol
1875 OH-Ephedrine
1875 OH-Phenylglutethimide
1876 Alachlor
1879 Ametryne
1880 (3′-Oxo-)-butobarbital
1880 (OH-)Methohexital
1880 (Desindane)-AC-aprindine
1880 Diuron-ME
1880 Heptachlor
1880 Ibomal
1880 Phenylmethylbarbituric Acid
1883 Clorgiline
1884 PEMA
1885 4-OH-Metamfetamine (pholedrine)
1885 Flurbiprofen (-Me)
1885 Fluvoxamine
1885 Norpethidine
1889 Mebutamate
1890 Hydroxyamfetamine-(AC)
1890 Malaoxon
1890 Methylphenobarbital
1890 OH-Diphenhydramine
1890 Tolmetin
1892 (Amino-)parathion
1895 Aminophenazone
1895 Clenbuterol (-H_2O)
1895 Oxo-prolintane
1895 Paraoxon
1895 Di-OH-diphenhydramine
1898 Dofamium Chloride
1899 Thiamylal
1900 (Indole Acetic Acid)-(Me))
1900 4-OH-Amfetamine-(AC_2)
1900 Clofedanol (aldehyde)
1900 Etamivan
1900 Flurbiprofen
1900 Hydroxyamfetamine-(AC_2)
1900 Mafenide (-Me_3)
1900 N-Desalkyl-chloropyramine
1900 Nororphenadrine
1900 OH-MeO-Phenmetrazine
1900 OH-Methoxyphenmetrazine
1900 Phencyclidine
1900 Tolpropamine
1902 Digitoxin
1903 Cyclandelate
1905 Carbamazepine (-Me)
1905 Fenitrothion
1906 Fenoprofen (-Me)
1906 Morinamide
1910 Diethyltryptamine
1910 Doxylamine
1910 Monolinuron
1913 Dibutyl Phthalate
1915 Fenproporex-AC
1915 (3-OH-)-Amobarbital
1915 OH-Propylhexedrine-(AC_2)
1919 Terodiline
1920 (3′-OH-)-Butobarbital
1920 1-OH-Harman
1920 Bamethan
1920 Mafenide (-Me_2)
1920 Meptazinol
1920 *N*-Desalkyl-aprindine
1920 *N*-Desalkyl-oxo-penfluridol (-2H_2O)
1920 Propyphenazone
1920 Tofenacin
1920 Nor-di-OH-ketamine (2H_2O)
1923 Bibenzonium Bromide
1925 Bethanidine
1925 Malathion
1926 2′-Hydroxysecbutabarbital
1927 Linuron
1928 Levamisole
1930 3-OH-Amfetamine-(AC_2)
1930 5-(3-Hydroxy-1-methylbutyl)-5-vinylbarbituric Acid
1930 Desipramine (ring)
1930 Imipramine (ring)
1930 Nortrimipramine (ring)
1930 Prometryne
1933 Prenalterol
1935 Orphenadrine
1935 Primidone (diamide)
1938 Fenthion
1939 Azapetine
1940 (3′-OH-)-Butobarbital-(AC)
1940 (Indole Formic Acid)-Me
1940 OH-Butalbital
1940 Phenyltoloxamine
1940 Terbutryne
1940 Desamino-OH-prenylamine-(H_2O)
1943 Aldrin
1943 Tramadol
1945 Nystatin
1948 Bunitrolol
1949 Isocarboxazid
1950 Benzimidazolone
1950 Benzimidazolone-AC_2
1950 Flufenamic Acid
1950 Normephenytoin
1950 Parathion (ethyl)
1950 Tetracycline
1950 Tyramine-(AC_2)
1952 Harman
1953 Phenobarbital
1955 (Bis-nor-)-aminophenazone
1955 3′-OH-Pentobarbital
1955 COOH-Glibornuride ((sulfonamide)-Me_3)
1955 Chlorpyrifos
1955 Cyclobarbital
1955 Desacetylnifenazone
1955 (HOOC-)Gliclazide ((sulfonamide)-Me_3)
1955 Niflumic Acid (-Me)
1955 Bis-desalkyl-dipyrone
1960 Fenyramidol
1960 (COOH-)-Amobarbital
1960 Desamino-OH-doxylamine (-AC)
1960 Nor-OH-ketamine (H_2O)
1964 Padimate
1965 Hexylcaine
1969 Pemoline
1970 Doxepin (*cis*-*N*-oxide)
1970 Etamivan (-AC)
1970 Fencamfamin (-TFA)
1970 *N*-Desalkyl-trifluperidol
1970 OH-Hexobarbital-(H_2O)
1971 Caramiphen

1973 Emepronium Bromide
1974 Dofamium Chloride
1975 Amitriptyline (*n*-oxide)
1975 Clioquinol
1975 Cyclophosphamide-HCl
1975 Ethomoxane
1976 Tiaprofenic Acid
1976 Tripelennamine
1980 Atropine (-CH_2O)
1980 Bunitrolol-Art
1980 Methoxsalen
1980 Naproxen (-Me)
1980 Noraminophenazone
1980 Norhexobarbital
1980 *O*-Desmethyl-naproxen-(Me_2)
1980 Piperocaine
1981 Methapyrilene
1985 Opipramol (ring)
1985 Oxprenolol (art)
1985 Psilocin
1990 Diflunisal-Me_2
1990 Procarbazine
1995 (OH-)Vinylbital-(H_2O)
1995 Normeptazinol
1995 *O*-Desmethyl-tramadol
1996 Chlorphenamine
1998 Iminostilbene
1998 *O*-Desmethyl-tramadol-(AC)
1999 Thenyldiamine
2000 Cyclopentolate-H_2O
2000 Etilefrine (-Me_2-AC)
2005 Desethylfencamfamin-(AC)
2005 Theophylline
2005 Tiocarlide
2008 Diethylthiambutene
2010 Baclofen
2010 Methoxy-diphenhydramine
2010 Procaine
2010 Triamterene
2013 Octafonium Chloride
2014 Norchlorphenamine
2014 Pilocarpine
2015 1-OH-Harman
2015 Dextrorphan-TFA
2016 Fenoprofen
2020 Alimemazine (ring, phenothiazine)
2020 Chlordane
2020 DOM (-AC)
2020 Fenamiphos
2020 Furosemide ((-SO_2NH)-Me)
2020 OH-Vinbarbital (-H_2O)
2020 Oxohexobarbital-(Me)
2020 Phenothiazine
2020 Xylometazoline
2021 2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP)
2022 Cyclopentolate
2022 Isopropamide Iodide
2024 Gliquidone
2024 Tinidazole
2025 Butanilicaine
2025 Cyclizine
2025 Formylacridine
2025 Zomepirac (-CO_2)
2026 Eucatropine
2029 Dimethoxanate
2030 Chlorphenesin-AC
2030 Disopyramide-CHNO
2030 (OH-)Gliclazide ((sulfonamide)-Me_2)
2030 OH-Glibornuride ((sulfonamide)-Me_2)
2030 Quinisocaine
2030 Nor-OH-selegiline (-AC_2)
2033 Isopropylaminophenazone
2035 Metoprolol
2035 Nefopam
2035 Physostigmine
2037 Pipazetate
2038 Thioacetazone
2040 2-OH-Brallobarbital
2040 2-Ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine (EDDP)
2040 Etidocaine
2040 Furosemide (-SO_2NH)
2040 Ketobemidone
2040 Tiabendazole
2045 Naproxen
2045 OH-Pethidine
2045 Prothipendyl (ring)
2045 Desamino-oxo-OH-dom-(PFP_2)
2046 Psilocybine
2050 1,5-Dimethyl-5-(3-oxo-1-cyclohexen-1-yl) barbituric acid
2050 Diflunisal-Me
2050 Furosemide ((-SO_2NH)-Me_2)
2050 Methocarbamol
2050 Norbormide
2050 OH-BPH Isomer 1
2050 OH-Methoxybenzophenone
2050 OH-Methoxybenzophenonediphenylpyraline -(isomer-1)
2050 Oxocyclobarbital-(Me_2)
2055 3′-Oxohexobarbital
2055 Heptabarb
2055 Phenindione
2057 Bufotenine
2060 Dextrorphan-PFP
2060 OH-Ethyl-glutethimide-(AC)
2060 Prilocaine-AC
2065 Ascorbic Acid-(AC)
2065 Cyclophosphamide
2065 Mepivacaine
2065 Metaraminol (-AC_3)
2065 OH-BPH Isomer 1
2065 OH-Benzophenone
2065 OH-Benzophenone-diphenylpyraline-(isomer-1)
2065 OH-Methoxy-amfetamine (-AC_2)
2065 Desamino-OH-propranolol
2069 Mefenamic Acid (-Me)
2070 Berberine
2070 Chlorphenesin-AC_2
2070 (*O*-Methyl-)-dopamine-(AC_2)
2070 *N*-Desalkyl-*O*-methyl-dobutamine-(AC_2)
2070 OH-Methoxy-BPH
2070 OH-Methoxybenzophenone
2070 OH-Methoxybenzophenonediphenylpyraline -(isomer-2)
2070 OH-Vinbarbital
2072 Homatropine
2075 Pentazocine-TFA
2077 Disulfoton (sulphone)
2078 Benzoctamine
2080 Carbinoxamine
2080 Chlorphenoxamine
2080 Norpheniramine
2080 OH-BPH
2080 OH-Benzophenone
2080 OH-Benzophenone-diphenylpyraline -(isomer-2)
2085 4-Aminophenol-AC_3
2085 Atropine (-H_2O)
2085 Clofedanol-(H_2O)
2085 Fencamfamin (-AC)
2085 Methylphenidate-AC
2085 Niflumic Acid
2085 Trimethoxyhippuric Acid
2087 Ranitidine
2090 Clofedanol
2090 Clonidine
2090 DOM (-AC_2)
2090 Endosulfan
2090 Fluorouracil
2090 Ketoprofen (-Me)
2090 Norclofedanol-(H_2O)
2090 *op*-DDE
2092 Brompheniramine
2093 Hexetidine
2095 Desethyl-metoclopramide
2095 Diflunisal
2095 Normethadone
2095 Sulfanilamide-(Me_4)
2098 Methoprotryne
2100 Chlorpromazine (*n*-oxide)
2100 Chlorpromazine (ring)
2100 Clenbuterol
2100 Desacyl-lorcainide
2100 Diphenylpyraline
2100 Dosulepin (*n*-oxide)
2100 *N*-Desalkyl-verapamil
2100 *N*-Desphenyl-oxo-histapyrrodine-(H_2O)
2100 Naphazoline
2100 Nortolpropamine
2100 Perphenazine (ring)
2100 Prochlorperazine (*n*-oxide)
2103 Proxyphylline
2110 Dieldrin
2110 OH-Phenyl-prolintane-(AC)
2111 Dicycloverine
2114 Cycrimine
2115 Acetyl-primidone
2115 Norcocaethylene
2115 OH-Methoxyphenylprolintane-(AC)
2115 Xipamide((-SO_2NH)-Me_2)
2116 Thialbarbital
2120 Ascorbic Acid
2120 Desalkyl-norcyclizine
2120 Glycopyrronium Bromide
2120 *N*-Oxideflupentixol
2120 *N*-Desalkyl-mepyramine
2120 *N*-Desbenzyl-oxo-histapyrrodine
2120 Norcyclizine
2120 Pentazocine-PFP
2120 Phenothiazine
2120 Stanozolol-(AC)
2120 Desamino-OH-fluspirilene
2120 Desamino-OH-penfluridol
2120 Desamino-OH-pimozide
2125 Desaminocarboxy-lidoflazine-(Me)
2125 Etofylline
2125 Etophylline
2130 Dosulepin(OH-*N*-oxide)
2130 Nomifensine
2130 OH-Clofedanol-(H_2O)
2130 Desamino-OH-chlorphenamine-(AC)
2130 Desamino-Carboxy-pimozide
2132 Sulfacetamide
2133 Chloropyrilene
2135 Methaqualone
2135 OH-Phenyl-prolintane
2135 Sulfanilamide (-Me)
2138 Dextromethorphan
2138 Pyrimethamine
2138 Racemetorphan
2140 Dichlorophen
2140 HOOC-Atenolol-(Me)
2140 MDMA-AC
2140 Norphenyltoloxamine
2140 *pp*-DDE
2141 Disulfiram
2142 Alverine
2145 Chlortalidone
2145 Methadone
2145 Methocarbamol-(AC)
2145 OH-Ephedrine-(AC_3)
2145 Pipradrol
2146 Clomipramine (*n*-oxide)
2147 Propranolol
2150 3,4-Di-OH-amfetamine-(AC_3)
2150 Atenolol-(H_2O)
2150 Etilefrine-(AC_3)
2150 Norcarbinoxamine
2150 OH-phenmetrazine (isomer 1-AC_2)
2150 OH-Tolpropamine
2150 Tryptophan-(Me-AC)
2155 Bromazine
2155 OH-Prilocaine
2155 Trioxysalen
2156 Bromocriptine
2156 Procyclidine
2160 Clenbuterol-(H_2O)
2160 Mescaline (-AC)
2160 N-Desalkyl-chloropyramine-(AC)
2162 Norcocaine
2165 Chlorpropamide-Me
2165 Phenindamine
2165 Tacrine
2170 Oxymetazoline
2170 Tryptophan (-Me_2-AC)
2173 Oxyphenonium Bromide (art)
2174 Santonin
2175 Atenolol
2175 Dopamine
2175 Endosulfan II
2177 Imolamine
2180 OH-Flurbiprofen-(Me_2)

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2180 Rescinnamine
2180 Tiaprofenic Acid-(Me_2)
2181 Butriptyline
2183 Endrin
2184 Ergocryptine
2185 Embramine
2185 Sulfanilamide
2186 Adiphenine
2187 Cocaine
2187 Methazolamide
2188 Dextropropoxyphene
2190 Atropine
2190 Chloropyramine
2190 Flupentixol (ring)
2190 Fluphenazine (ring)
2190 Hyoscyamine
2190 Noracetyl-fluoxetine
2190 Oxeladin
2190 Oxocyclobarbital
2190 Physostigmine
2190 Trifluoperazine (ring)
2190 Triflupromazine (ring)
2193 Norracemorphan
2194 Amitriptyline
2195 Clopamide-Art-(SO_2NH)
2195 Cyclobenzaprine
2195 Diclofenac-(Me)
2195 Etacrynic Acid (-Me)
2199 Chlormezanone
2200 4-OH-Phenobarbital-(Me_3)
2200 Desacyl-lorcainide-(AC)
2200 Etofylline (-AC)
2200 OH-Phenmetrazine (isomer 2-AC_2)
2200 OH-Tramadol
2200 Nor-OH-tolpropamine
2200 Oxo-OH-alkyl-prolintane
2201 Mefenamic Acid
2202 Mianserin (*n*-oxide)
2203 Thonzylamine
2205 Dropropizine
2205 OH-Pethidine-(AC)
2210 Etamiphylline
2210 Mianserin
2210 *N*-Desalkyl-penfluridol
2210 Norchloropyramine
2210 Norphenindamine
2214 Norpropoxyphene
2215 Nortriptyline
2215 Trimipramine
2218 *op*-DDT
2220 Carbamazepine (epoxide)
2220 Chlorcyclizine
2220 Diclofenac (-Me_2)
2220 Doxepin (*cis*and *trans*)
2220 Mepyramine
2220 Norisothipendyl
2220 *O*-Desalkyl-OH-phenyltoloxamine
2220 OH-Mesuximide
2220 OH-Methoxycarbinolchlorphenoxamine-(H_2O)
2220 OH-Methoxycarbinolclemastine-(H_2O)
2220 Tetracaine
2220 Viloxazine (-AC)
2223 Norzimeldine
2225 Dichlorophen-Et_2
2225 Isothipendyl
2227 Aminoglutethimide
2230 Clomipramine (ring)
2230 Desaminocarboxy-lidoflazine
2230 Dextrorphan
2230 Dextrorphan-TMS
2230 Hydromorphone-enol-TFA_2
2230 Hyoscine (hydrate)
2230 Imipramine
2230 Levorphanol
2230 Phenindamine (*n*-oxide)
2230 Salbutamol-(AC_2)
2230 Triflupromazine
2230 Tropicamide-(CH_2O)
2230 Bis-nor-isothipendyl
2230 Desamino-carboxy-fluspirilene
2230 Desamino-carboxy-penfluridol
2232 Carbetapentane
2235 Cyclobenzaprine
2235 Desipramine
2235 Medazepam
2235 Normianserin
2235 Phenglutarimide
2235 Phenoxybenzamine
2240 2-Formyl-methaqualone
2240 Aminoacridine
2240 Desipramine (ring)
2240 Dextrorphan
2240 Histapyrrodine
2240 Norpethidine-(AC)
2240 OH-Carbamazepine-(ring)
2240 OH-Imipramine (ring)
2240 OH-Propyphenazonefamprofazone-(AC)
2240 Pentifylline
2240 Desamino-OH-metipranolol (-AC_2)
2240 Di-nor-acetyl-diphenhydramine
2240 Oxo-OH-methoxy-phenyl-prolintane
2241 Chloroprocaine
2241 Metipranolol
2241 Nordextrorphan
2243 Azacyclonol
2245 Dichlorophen-Me_2
2245 Ketoprofen
2245 Perhexiline
2245 Phenazopyridine
2245 Phenytoin (-Me)
2245 Pindolol
2245 Trihexyphenidyl
2245 Bis-desalkyl-disopyramide-(NH_3)
2245 *cis*-Nordoxepin
2245 *trans*-Nor-doxepin
2247 Tolmetin (-ME)
2250 Acetyl-fluoxetine
2250 Bamipine
2250 Chlorpropamide-Me_2
2250 Cocaethylene
2250 Coumatetralyl-HY
2250 Dehydro-nifedipine
2250 Dichlorophen-AC_2
2250 Flecainide
2250 Hydromorphone-PFP
2250 Mirtazapine
2250 Morphine-TFA_2
2250 Norpromethazine
2250 OH-Phenylglutethimide-(AC)
2250 Oxyphencyclimine
2250 Primidone
2250 Salbutamol-(AC_3)
2250 Trifluomeprazine
2250 Tropicamide (-H_2O)
2250 Di-OH-pentifylline-(H_2O isomer-1)
2253 Protriptyline
2253 Sulfinpyrazone
2253 Trimetozine
2253 Triprolidine
2255 Benactyzine
2255 Mecloqualone
2255 Oxo-quazepam
2255 Procainamide
2255 Desalkyl-OH-flurazepam
2255 Oxo-OH-alkyl-prolintane-(AC)
2258 Triazophos
2259 Promethazine
2260 Cl-Thioxanthenone
2260 Cl-Thioxanthenonechlorprothixene
2260 Endosulfan (sulfate)
2260 Metipranolol-(AC)
2260 OH-DOM (-AC_2)
2260 Xylometazoline-(AC)
2265 Dihydrocodeine-TFA
2265 (OH-)Gliclazide ((sulfonamide)-Me)
2265 Noracetyldiphenhydramine
2265 OH-Glibornuride-((sulfonamide)-Me)
2266 Timolol
2269 Bupivacaine
2270 Noxiptyline
2270 Psilocin-(AC)
2270 Zimeldine
2271 Diclofenac
2273 Anisindione
2275 3′-Hydroxyheptabarbital
2275 Atropine (-AC)
2275 Dimetindene
2275 Timolol (-Art)
2275 Dehydro-desethyl-nitrendipine (–CO_2)
2275 Oxo-OH-phenyl-prolintane (-AC)
2276 Biperiden
2280 Codeine-trifluroacetic Acid
2280 Dextrorphan-AC
2280 Diprophylline
2280 Normedazepam
2280 OH-Phenyltoloxamine (isomer 1)
2280 Pentazocine
2280 Trichlorophenoxyacetic Acid (-isobutyl ester)
2280 Bisnordoxylamine-(AC)
2281 Pramocaine
2285 Carbamazepine
2285 Carbamazepine
2285 Galantamine
2285 Di-OH-pentifylline-(H_2O isomer-2)
2290 1-OH-Harman
2290 OH-Methoxyguaifenesin-(AC_2)
2290 Oxycodone (TFA)
2290 Phenylbutazone-Me
2290 Dehydro-COOH-nifedipine
2291 Harmaline-2H (harmine)
2291 Harmine
2293 Normaprotiline
2295 4-OH-Phenobarbital
2295 Nortramadol-(AC)
2295 OH-Pentifylline (isomer-1)
2295 Sulpiride (art-(-SO_2NH))
2295 Di-OH-phenyl-prolintane (-AC_2)
2295 Nor-OH-methylphenobarbital
2298 Diethylstilbestrol
2300 (Desphenyl-)-AC-aprindine
2300 Acetyl-mafenide-(Me)
2300 Coumatetralyl-Me-HY
2300 Hyoscine
2300 Isoxsuprine
2300 OH-ClBP-Buclizine
2300 OH-Chlorobenzophenone
2300 OH-Doxylamine-(AC)
2300 OH-Phenindamine
2300 OH-Phenyltoloxamine (isomer 2)
2300 Nor-OH-mesuximide
2301 Bufylline
2302 Benzatropine
2305 Alimemazine
2305 Desethyl-OH-fencamfamin-(AC_2)
2310 Chloramphenicol
2310 Domiphen Bromide
2310 OH-Methoxymethylphenobarbital
2310 OH-Methoxyflurbiprofen-(Me_2)
2310 Tertatolol
2314 Buphenine
2314 Primaquine
2314 *pp*-DDT
2315 Dihydroergotamine
2315 Fenbufen (-Me)
2315 Promazine
2318 Antazoline
2318 Piperidolate
2318 Thenalidine
2320 3′-Oxoheptabarbitone
2320 Ethylmorphine-TFA
2320 Hydromorphone-enol-PFP_2
2320 OH-Methoxydoxylamine-(AC)
2320 OH-Methoxyphenmetrazine (-AC_2)
2320 OH-Methoxyphenyltoloxamine
2320 Pentazocine-TMS
2320 Phenytoin
2320 Trichlorophenoxyacetic Acid (isooctil ester)
2320 Nor-OH-phenyltoloxamine (isomer 1)
2323 Proxymetacaine
2325 Mazindol
2325 Oxazepam
2325 Di-oxo-viloxazine
2327 Mepenzolate Bromide
2330 Ambucetamide
2330 Dihydromorphine (-PFP_2)
2330 *N*-Desalkyl-CHNO-disopyramide-(AC)
2330 *N*-Desalkyl-*O*-methyl-dobutamine-(AC)
2330 Pentazocine-AC
2330 Dehydro-desmethyl-nitrendipine (–CO_2)
2335 Iprindole
2335 Noralimemazine
2335 Propoxycaine
2335 Tropicamide

2336 Probenecid
2337 Bromhexine
2339 Thiazinamium Metilsulfate (promethazine)
2340 Chlorambucil-Me
2340 Mafenide
2340 Nordoxylamine-(AC)
2340 OH-Carbamazepine-(methoxy-ring)
2340 Profenamine
2340 Psilocin (-AC_2)
2340 Nor-OH-phenyltoloxamine (isomer 2)
2343 Zomepirac (-ME)
2345 Prothipendyl
2348 *cis*-10-OH-Amitriptyline
2348 *trans*-10-OH-Amitriptyline
2350 OH-Lidocaine
2350 Procaine-AC
2350 Trimethoxyhippuric Acid-Me
2355 Cyproheptadine
2355 Levallorphan
2355 Oxypertine
2360 2-OH-Methyl-methaqualone
2360 Dihydrocodeine-PFP
2360 Morphine-PFP_2
2360 *O*-Desethyl-viloxazine
2360 Oxo-OH-methoxy-phenyl-prolintane (-AC)
2365 Nordextropropoxyphene-(AC)
2366 Ergotamine
2367 Phenylbutazone
2370 Betaxolol
2370 Desethyl-phenglutarimide
2370 Diperodon
2370 Noxytiolin
2370 OH-Methoxyimipramine (ring)-(AC)
2370 OH-Methylphenobarbital
2375 Codeine
2375 Dihydro-harmaline
2375 Phenprocoumon (isomer 1-Me)
2375 Pizotifen
2375 *cis*- 10-OH-Nortriptyline
2375 *trans*- 10-OH-Nortriptyline
2376 Piperonyl Butoxide
2377 Azatadine
2377 Diethazine
2378 Bisoprolol
2380 Benzydamine
2380 Desalkyl-pentazocine-(AC_2)
2380 Dosulepin
2380 Feprazone
2380 (OH-Methoxy-)-mephenytoin
2380 OH-Amitriptyline
2380 Polythiazide
2384 Difenidol
2385 Atenolol
2385 Xipamide (-SO_2NH)
2388 2-Amino-5-nitrobenzophenone
2388 Norcodeine
2388 Norcodeine
2390 Acemetacin (art-ME_2)
2390 CBD
2390 Desipramine (ring)
2390 Dihydrocodeine
2390 Dropropizine-AC
2390 Levallorphan (-AC)
2390 Maprotiline
2390 OH-Methoxyimipramine (ring)
2390 OH-Tripelennamine (-AC)
2390 Tryptamine (-AC)
2390 Nor-OH-amitriptyline
2392 Dosulepin (sulfoxide)
2395 Desalkyldipyrone-(AC)
2395 Nandrolone
2395 Neopine
2395 Phenprocoumon-(isomer 2-Me)
2400 2-Carboxy-methaqualone
2400 Betaxolol (art-H_2O)
2400 Bisoprolol (art(-H_2O))
2400 Dihydromorphine
2400 (4-OH-)-Mephenytoin
2400 Nor-*N*-proponyl-dextropropoxyphene
2400 Norcyproheptadine
2400 OH-Tripelennamine
2400 Oxo-mepivacaine
2402 Hexestrol
2405 *N*-Desalkyl-oxo-fluspirilene
2405 Normorphine-(PFP_3)
2405 Norpromazine
2405 OH-Chlorphenamine-(AC)
2406 Di-nordibenzepin
2406 Pentoxifylline
2410 (OH-Alkyl-)-H-$_2$O-azatadine
2410 2′-OH-Methyl-methaqualone
2410 Betaxolol (art)
2410 Clopenthixol
2410 Clopenthixol ((*N*-Oxide)-$C_6H_{14}N_2O_2$)
2410 Lorazepam
2410 *N*-Oxidechlorprothixene (-$(CH_3)_2NOH$)
2410 OH-Mepivacaine
2410 OH-Propyphenazone
2410 Oxo-meptazinol
2410 Tropicamide-(AC)
2413 Chlorpromazine
2413 Sotalol
2415 (*N*-Desalkyl-)-benperidol
2415 Clomipramine
2415 *N*-Desalkyl-pimozide
2417 *op*-Methoxychlor
2419 Pyrrobutamine
2420 Chlorambucil
2420 Diloxanide
2420 Ethylmorphine
2420 Meclofenamic Acid
2420 Nortripelennamine
2420 Nortripelennamine-(AC)
2420 Bisnorchloropyramine-(AC)
2421 Dosulepin (norsulfoxide)
2421 Nordosulepin
2425 Acetyl-mafenide
2425 Clemastine
2428 Diazepam
2430 Codeine-PFP
2430 Dropropizine-AC_2
2430 Ethylmorphine-PFP
2430 Harmaline
2430 Oxo-tetrazepam
2430 Tetrazepam
2432 Norclomipramine
2433 Floctafenine-Me
2433 Propanidid
2434 Aminopromazine
2435 Dantron-Me
2440 Hydrocodone
2440 Normorphine-(PFP_2)
2440 OH-Chloropyramine-(AC)
2440 Quazepam
2440 Tramazoline
2440 Tryptamine-(AC_2)
2444 Ketazolam
2445 Dihydrocodeine-AC
2445 Etynodiol Diacetate
2445 Hydromorphone
2445 Methoxychlor
2445 Morphine
2449 Nordibenzepin
2450 Dibenzepin
2450 Dihydrotestosterone Enol-TMS_2
2450 Mebhydrolin
2450 *N*-Desalkyl-furosemide-(Me_2)
2450 OH-Isothipendyl
2450 OH-Nomifensine
2450 Phenol-acebutolol
2450 Bis-desethyl-profenamine-(AC)
2450 Nor-OH-cyproheptadine
2450 Oxo-di-OH-phenyl-prolintane-(AC_2)
2451 Dihydromorphine
2452 Norchlordiazepoxide
2455 Diprophylline-AC_2
2455 N_5-Desmethyl-dibenzepin
2457 Butacaine
2457 Clorazepic Acid
2459 Morphine
2459 Normorphine
2460 Dantron-AC
2460 Flutazolam
2460 *N*-Desalkyl-acetyl-verapamil
2460 OH-Diclofenac-(Me_2)
2460 Sulfamethoxazole-(Me_2)
2460 *m*-OH-Cocaine
2461 Azapropazone
2462 Aprindine
2464 *N,O*-Didesmethyl-tramadol-(AC_2)
2465 Dantron-TMS
2465 Molindone
2465 Protoveratrine A and B
2467 Methdilazine
2470 3-OH-Bromazepam
2470 Desalkylflurazepam
2470 *N*-Desalkylastemizole
2470 Norchloropyramine-(AC)
2470 OH-Chlorphenoxamine
2471 Oxybuprocaine
2473 Azinphos-(Me)
2473 Δ^9-THC
2473 Δ^9-Tetrahydrocannabinol
2474 Dipipanone
2475 Androsterone
2475 Dantron-Me_2
2475 Oxo-di-OH-phenyl-prolintane
2480 Didesmethyl-chlorpromazine
2480 Dihydrocodeine-TMS
2480 Metixene
2480 N-Desalkylfenetylline-(AC)
2480 Norchlorpromazine
2480 OH-Imipramine-(Me)
2480 Xipamide ((-SO_2NH)-Me)
2485 Benoxaprofen-Me
2485 Dihydrotestosterone-TMS
2485 OH-Perhexiline
2485 Oxo-di-OH-prolintane-(AC_2)
2488 Norpipanone
2490 3′-OH-Methaqualone
2490 Acetylsulfaethidole
2490 Butalamine
2490 Dihydro-chlorprothixene
2490 *N*-Desalkyl-desacyl-lorcainide-(AC_2)
2490 Nordazepam
2490 Nordiazepam
2490 Oxo-procyclidine-(H_2O)
2490 Tetrabenazine
2492 Chlorprothixene
2495 3-Monoacetylmorphine
2495 8-OH-Mianserin
2495 Ergocristine
2495 Tubocurarine Chloride
2500 Androsterone Enol-TMS_2
2500 Cannabigerol
2500 Formylflecainide
2500 *O*-Desmethyl-OH-tetrabenazine
2500 OH-Dosulepin
2500 OH-Histapyrrodine
2500 OH-Trihexyphenidyl
2500 Sulfamethoxazole-(Me)
2502 Sulfadiazine
2503 Acetylcodeine
2503 Codeine-AC
2505 Disopyramide
2505 Emetine
2505 Nomifensine-(isomer 1)
2505 OH-Pentifylline-(isomer-2)
2510 4′-OH-Methaqualone
2510 DiOH-perhexiline-(H_2O)
2510 Dihydrotestosterone
2514 Levomepromazine
2515 Desethyl-profenamine-(AC)
2517 Thebaine
2520 (*N*-Desalkyl-)-meclozine
2520 (*OH*-Alkyl-)-AC-azatadine
2520 (Desalkyl-) (norchlorcyclizine)-buclizine
2520 (Desalkyl-)hydroxyzine
2520 Codeine-TMS
2520 Dihydromorphine-(TMS_2)
2520 Norchlorcyclizine
2524 Oxycodone
2525 6-OH-Methaqualone
2525 6-Monoacetylmorphine
2525 Oxetacaine
2526 Noramidedextropropoxyphene
2529 Demoxepam
2530 Apomorphine
2530 DHEA
2530 Dantron-TMS_2
2530 Ethylmorphine-AC
2530 Norchlorphenamine
2530 Nortetrazepam
2530 OH-Dihydrodoxepin
2530 OH-Methoxymianserin

2533 Thebacon
2535 CBN
2535 OH-Imipramine (ring)-(AC)
2535 Bisnorchlorphenamine-(AC)
2535 *cis*-OH-Doxepin
2538 Oxymorphone
2540 (*OH*-Aryl-)-AC-azatadine
2540 Ethylmorphine-TMS
2540 Nadolol
2540 Pecazine
2540 *cis*-Nor-OH-doxepin
2545 Dihydromorphine-(AC_2)
2545 Harmine-(AC)
2545 *N*-Didesalkyl-acetyl-verapamil
2545 Nifedipine
2545 OH-Pentazocine
2545 Oxyphenbutazone-(Me_2)
2550 (*O*-Desmethyl-)-2*H*-harmaline
2550 Acecainide
2550 Benoxaprofen
2550 Indometacin
2550 *N*-Acetyl-procainamide
2550 *O*-Desmethylharmine
2551 Noretynodrel
2553 2-OH-Desipramine
2555 Desethyl-chloroquine
2555 Dimefline
2555 Drostanolone
2555 Loxapine
2555 OH-Dextrorphan-(AC_2)
2555 Oxycodone-(AC)
2555 Pericyazine (ring)
2555 Prenylamine
2558 Clobazam
2558 Trimethoprim
2560 Azinphos-(Et)
2560 Desethyletamiphylline-(AC)
2560 Morphine-TMS_2
2560 *N*-oxide sulfoxide-chlorprothixene (-$(CH_3)_2$NOH I)
2560 Nadolol (art)
2560 OH-Methoxymethaqualone
2560 OH-Pentifylline-(AC)
2560 Oxycodone-(enol-AC_2)
2560 Desamino-oxo-OH-dom-(AC_2)
2560 Oxo-di-OH-methoxy-prolintane-(AC_2)
2560 *trans*-OH-Doxepin
2565 OH-Imipramine
2566 Sulfamerazine
2569 2-OH-Clomipramine
2570 Benzoylecgonine
2570 Dihydro-oxycodone-(AC_2)
2570 OH-Tetrazepam (isomer 1)
2570 Oxohistapyrrodine
2570 Desamino-di-OH-maprotiline
2573 Coumaphos
2574 10-OH-Clomipramine
2575 (OH-)Trimipramine
2575 Drostanolone-TMS
2575 Midazolam
2578 Ethoxzolamide
2580 (OH-)-Bamipine
2580 Androsterone-AC
2580 DHEA Enol-TMS_2
2580 OH-Tetrazepam-(isomer 2)
2580 Warfarin-(Me)
2580 Nor-OH-promethazine
2588 Carteolol
2590 6-Monoacetylmorphine-TMS
2590 Cinchonidine
2590 Cinchonine
2590 Desacyl-Δ-methrin-(Me)
2590 Methyltestosterone (-TMS)
2590 Nifuratel
2590 Nomifensine (isomer 2)
2590 OH-Chlorpromazine (-Me)
2590 OH-Methoxytrimipramine
2590 OH-Promethazine
2590 OH-Viloxazine-(AC_2)
2590 Pyrazophos
2590 Nor-OH-phenindamine
2595 Bisoprolol (art)
2595 DHEA-H_2O
2595 Dantron-AC_2
2595 Hydromorphone-AC
2595 Temazepam
2600 Acetylsulfanilamide-(Me)
2600 Androstenedione
2600 Clopamide-ME_3
2600 Desipramine (ring)
2600 Flunitrazepam
2600 Mexazolam
2600 *O*-Desmethylharmine-(AC)
2600 OH-Alimemazine-(AC)
2600 OH-Dibenzepin-(isomer-1-AC)
2600 Sulfapyridine
2600 Temazepam-(Me)
2600 Di-OH-imipramine (ring)
2600 Nor-OH-pethidine-(AC_2)
2603 Flupirtine
2605 Chloroquine
2605 Normorphine-(TMS_3)
2607 Ketotifen
2610 Celiprolol
2610 OH-Imipramine-(AC)
2612 Estrone
2612 Mestranol
2613 Sulfadimidine
2615 Deptropine
2615 Diamorphine
2615 Morphine-AC_2(diamorphine)
2617 Phenacaine
2620 Clemizole
2620 Enalapril
2620 Metoclopramide
2620 Nalorphine
2620 *O*-Desmethyl-metoprolol-(AC_3)
2620 Sulfaethidole
2620 Testosterone
2625 Drostanolone-enol-TMS_2
2625 Gallamine Triethiodide
2625 Hydromorphone-enol-AC_2
2625 Norethisterone
2625 Sulfadiazine (-Me)
2625 Sulfamerazine-(Me)
2630 Chlortalidone-Me_4
2630 Dihydrotestosterone-AC
2630 Tri-OH-prolintane-(AC_3)
2635 Coumatetralyl
2635 Nitrendipine
2638 Amoxapine
2640 Clostebol-HCl Enol-TMS_2
2640 Metaclazepam
2640 *N*-Desalkyl-disopyramide-(AC)
2640 Bis-nor-imipramine-(AC)
2641 Oxymorphone (-TMS_3)
2645 (OH-)Biperiden
2645 Methyltestosterone
2648 Prazepam
2650 6-Monoacetylmorphine-PFP
2650 Enalapril-(ME)
2650 OH-Alimemazine
2650 Dehydrodesethyl-OH-nitrendipine-(H_2O)
2653 Hydroflumethiazide (-Me_4)
2655 Bisdesacetylbisacodyl
2655 Coumatetralyl (isomer-1-Me)
2655 OH-phenprocoumon (isomer-1 Me_2)
2659 Estradiol
2660 Acetyl-nortriptyline
2660 DiOH-perhexiline
2660 Lormetazepam
2660 Norlorcainide
2660 Sulfamethizole (-Me)
2660 Zotepine
2662 Noroxymorphone (enol, -TMS_3)
2665 Alimemazine (sulfoxide)
2665 Bromazepam
2665 Methyltestosterone Enol-TMS_2
2670 Acetyldesipramine
2670 Fenbutrazate
2670 Isopropamide Iodide
2670 OH-Methoxycocaine
2670 OH-Temazepam
2672 Methandienone
2675 2-OH-Ethyl-flurazepam
2675 Clostebol-HCl-TMS
2675 Phenprocoumon-(isomer-2 Me_2)
2675 Trifluperidol
2680 Benzthiazide
2680 Methoxybisdesacetylbisacodyl
2680 Noroxycodone-(enol-AC_3)
2680 ter-Nor-dibenzepin
2683 Trifluoperazine
2685 Indometacin
2685 OH-Promazine
2686 Broxaldine
2686 Phenazocine
2690 Acetylsulfanilamide
2690 Carteolol-(art)
2690 Coumatetralyl-(isomer-2-Me)
2690 Dihydro-oxymorphone
2690 Fenpipramide
2690 Normetaclazepam
2690 Tenoxicam-(Me_2)
2690 Testosterone (enol-TMS_2)
2690 Dehydro-desethyl-OH-nitrendipine-(H_2O)
2694 Didesethylflurazepam
2700 Clostebol-HCl-AC
2700 Drostanolone-AC
2700 Griseofulvin
2700 Di-OH-pentifylline
2700 Nor-dihydro-hydrocodone (-AC)
2705 Azaperone
2705 Mazindol-AC
2705 Phenprocoumon (isomer 3 Me_2)
2705 Promazine (sulfoxide)
2708 Indoprofen (-Me)
2710 Nordextrorphan-(AC_2)
2710 Promethazine (sulfoxide)
2712 Clotiapine
2715 Naloxone
2715 *O*-Desmethyl-fluanisone
2715 Oxymorphone (-TMS)
2719 Ethinylestradiol
2720 (Nor-)-AC-azatadine
2720 4-OH-Phenytoin-(Me_2)
2720 Chlorprothixene (sulfoxide)
2720 Dihydro-acepromazine-(H_2O)
2720 Fentanyl
2720 Norflunitrazepam
2720 OH-Prothipendyl
2720 Oxyphenbutazone (-Me_2)
2723 7-Aminoflunitrazepam
2723 Oxymorphone
2727 8-OH-Clomipramine
2727 Lormetazepam (decomposition product)
2728 Oxymorphone-(TMS_2)
2730 *O*-Desmethylmetaclazepam
2730 OH-Metoprolol-(AC_3)
2730 Temazepam-(AC)
2735 Acepromazine
2735 Metoclopramide-(AC)
2738 Propiomazine
2740 4-OH-methoxyphenytoin-(Me_2)
2740 Hydrocortisone
2740 Nitrazepam
2747 Norclobazam
2748 Naftidrofuryl Oxalate
2748 Oxymorphone-(oxime-TMS_3)
2749 Cyfluthrin
2749 Disulfoton
2750 Desacetylbisacodyl
2750 *N*-Desalkyl-furosemide-(Me)
2750 Nordihydrocodeine-(AC_2)
2750 OH-Dihydro-chlorprothixene-(isomer 1)
2750 Prothipendyl (sulfoxide)
2750 Testosterone-(AC)
2760 Nandrolone (tMS)
2760 Norhydrocodone-(AC)
2760 Quinine-AC
2761 Butorphanol
2762 8-OH-Norclomipramine
2765 Amino-dixyrazine-(AC)
2765 Amino-fluphenazine-(AC)
2765 Bis-noralimemazine (-AC)
2770 4-OH-Methoxy-phenytoin
2770 Glafenine (-Me)
2770 Methyltestosterone (-AC)
2770 OH-Dibenzepin-(isomer-2-AC)
2770 OH-Methoxyphenprocoumon (Me_2)
2770 Pseudomorphine
2773 Noroxymorphone (enol-TMS_4)
2774 Captodiame
2775 11-Hydroxy-Δ^9-THC
2779 Etynodiol Diacetate

2780 Flurazepam
2780 Mequitazine
2780 Xipamide (ME_4)
2785 7-Aminonitrazepam
2785 Fluanisone
2788 Noroxymorphone (enol, -TMS_2)
2790 Nordihydromorphine-(AC_3)
2790 OH-Dihydro-chlorprothixene-(isomer 2)
2790 OH-Temazepam (-AC)
2790 Quinidine
2793 Progesterone
2795 Chlordiazepoxide
2795 Hexachlorophene
2795 OH-Phenytoin
2798 Perazine
2800 Clotrimazole
2800 Furosemide-(Me_3)
2800 O-Desalkylamiodarone (art)
2800 OH-Prothipendyl (ring)
2800 Phocoldine-TFA
2800 Quinine
2800 Xipamide (ME_3)
2805 Clopamide-Me_2
2809 Chlorpromazine (sulfoxide)
2810 Hydroquinidine
2810 Lorcainide
2810 Methoxydesacetylbisacodyl
2810 OH-Methoxydihydrochlorprothixene
2810 OH-warfarin (isomer 1 Me_2)
2810 Trichlormethiazide
2811 Acebutolol
2815 Sultiame (-ME_2)
2815 Testosterone Propionate
2818 Bisacodyl
2820 Bismethoxybisdesacetylbisacodyl
2823 Clonazepam
2825 Papaverine
2825 Noraminoflunitrazepam
2830 Apomorphine-AC_2
2830 Clostebol Enol-TMS_2
2830 Fenetylline
2830 OH-warfarin (isomer 2 Me_2)
2830 α-OH-Midazolam
2835 Carbocromen
2835 Fluoxymesterone
2840 Sulfaethidole-(Me_2)
2850 (OH-)-AC-Aprindine
2850 Acebutolol-(H_2O)
2850 Anileridine
2850 Furosemide-(Me_2)
2850 Gelsemine
2860 3-OH-Prazepam
2860 Mefruside-(Me_2)
2860 Nor-OH-tripelennamine-(AC)
2870 Clostebol Acetate-TMS
2870 OH-warfarin (isomer 3 Me_2)
2870 Oxymetholone Enol-TMS_3
2870 Xantinol Nicotinate-(AC_2)
2872 Hydroxychloroquine
2872 Ibogaine
2872 Phenoperidine
2875 Triamterene-(Me_6)
2880 Ajmaline
2880 Dapsone
2880 Hydroxyzine
2880 Naltrexone
2880 OH-Lorcainide-(AC)
2880 Sultiame-(Me)
2880 Desethyl-OH-profenamine-(AC_2)
2884 Piminodine
2890 7-Aminoclonazepam
2890 Bismethoxydesacetylbisacodyl
2890 Cinchocaine
2890 Furosemide-(Me)
2890 Sulindac
2895 Clozapine
2895 Dimoxyline
2895 Methoxy-prajmalium bitartrate
2896 Sulindac-(sulfide)
2900 Chlorpromazine (norsulfoxide)
2900 Deltamethrin
2900 Desethyl-metoclopramide-(AC_2)
2900 Bis-desethyl-OH-profenamine-(AC_2)
2900 Nor-dihydro-oxycodone-(AC_2)
2906 Doxapram
2910 OH-Coumatetralyl-(isomer-1-Me)
2917 Chlorotrianisene
2920 Oxo-pyrrobutamine
2925 OH-Coumatetralyl-(isomer-2-Me_2)
2925 Prajmalium
2925 Sulfametoxydiazine (-Me_3)
2929 Nimodipine
2930 Clomifene
2930 Haloperidol
2930 Norethylmorphine-(AC_2)
2930 Nor-OH-alimemazine-(AC_2)
2931 8-OH-Loxapine
2932 Pipotiazine
2934 Diphenadione
2935 OH-Coumatetralyl-(isomer-3-Me_2)
2935 Amino-OH-clonazepam
2935 Nor-dihydro-oxycodone-(AC_3)
2940 Dextromoramide
2940 OH-Methoxylorcainide-(AC)
2945 Camazepam
2945 Norcodeine-(AC_2)
2945 Quinine ((*N*-oxide)-AC)
2949 Diltiazem
2950 Quinidine (*N*-oxide)
2951 7-OH-Amoxapine
2954 Prochlorperazine
2955 Estriol
2955 Normorphine-(AC_3)
2959 8-OH-Amoxapine
2960 Nalbuphine
2960 Oxo-cyproheptadine
2965 Clostebol Acetate
2965 Famprofazone
2965 Haloperidol-H_2O
2965 Miconazole
2965 Oxoclemizole
2966 Hydrochlorothiazide (-Me_4)
2970 Alfentanil
2970 Bumetanide-Me_3
2970 Dexamethasone
2970 Triamcinolone
2980 Etizolam
2980 OH-Carbinol-doxylamine-(AC)
2980 Phocoldine-PFP
2985 (Amino-)acenocoumarol-(Me_3)
2985 Drostanolone Propionate
2985 Polythiazide-Me3
2988 Hydrastine
2990 Desacetyldiltiazem
2990 OH-Coumatetralyl-(isomer-4-Me_2)
2990 Trifluoperazine (sulfoxide)
2990 Bis-norchlorpromazine (-AC)
2995 (OH-Methoxy-)-AC-aprindine
2995 Sulpiride-(Me_2)
3000 OH-Clobazam
3000 OH-Clotiapine-(AC)
3000 OH-Xipamide (-Me_4)
3000 Sultiame
3000 Trimethoprim-(AC_2)
3000 α-OH-Triazolam
3005 Dihydro-flupentixol-(AC)
3005 Oxymetholone
3005 Di-OH-coumatetralyl-(isomer-1-Me_2)
3010 Desethyl-chloroquine-(AC)
3010 OH-Dimethoxy-lorcainide-(AC)
3010 Pentamidine
3015 Benzylmorphine
3016 Encainide
3025 Stanozolol (-TMS_2)
3030 Cholesterol-H_2O
3030 Norclotiapine-(AC)
3030 Oxoclotiapine
3033 Etorphine
3035 Acenocoumarol-Me
3035 Indapamide-(Me_3)
3035 Meclozine
3040 Pipamperone
3045 Flupentixol-(AC)
3045 Mebeverine
3045 α-OH-Alprazolam
3050 Cinnarizine
3050 Fluphenazine
3050 *O*-Desmethyl-diltiazem
3050 OH-Brotizolam-(CH_2O)
3050 Opipramol
3055 Desalkyldihydro-flupentixol-(AC)
3058 *cis*-Flupentixol
3060 Dimetotiazine
3060 OH-Cyproheptadine
3060 Sulfadimethoxine-(TMS)
3060 Sulfaethidole-(Me)
3060 Tiotixene
3070 Brotizolam
3070 Norchlorpromazine-(AC)
3070 OH-Methoxycoumatetralyl-(Me_2)
3070 Pholcodine
3075 Trilostane
3078 Fenbufen
3080 Nifenazone
3080 Triazolam
3085 Stanozolol
3085 Di-OH-coumatetralyl-(isomer-2-Me_2)
3088 Cholesterol
3089 Methysergide
3095 OH-Dextromoramide
3100 Alprazolam
3100 Bezafibrate
3100 Canrenoic Acid
3100 *O*-Methyldobutamine-(AC_2)
3102 Sulpiride
3105 Norclozapine
3105 Di-OH-coumatetralyl-(isomer-3-Me_3)
3107 Triamcinolone
3110 Fenetylline-AC
3115 Thioridazine
3116 Strychnine
3120 Sulfoxide
3125 Etofylline (clofibrate)
3125 Sulpiride-(Me)
3130 Canrenoic Acid-Me
3130 Colecalciferol-H_2O
3130 Morazone
3130 OH-Prajmalium bitartrate
3132 Floctafenine
3140 Phocoldine-TMS
3145 Desalkylfluphenazine-(AC)
3145 Noscapine
3150 Acemetacin-Me
3150 Astemizole
3150 Colecalciferol
3150 Nordimetotiazine
3150 Verapamil
3152 Haloperidol (reduced)
3155 Yohimbine
3170 Fluphenazine-(AC)
3175 OH-Perazine
3175 Tri-OH-coumatetralyl-(H_2O-Me_2)
3180 Norverapamil
3180 Sulforidazine (ring)
3190 Butaperazine
3190 Yohimbine-(AC)
3194 Clorexolone
3195 OH-Quinine-(AC_2)
3195 Rotenone
3200 Colchicine
3200 *O*-Methyldobutamine
3200 OH-Dimetotiazine-(AC)
3200 OH-Methoxyprajmalium bitartrate
3200 Thioproperazine (ring)
3205 7-Acetamidonitrazepam
3210 OH-Dextromoramide-(AC)
3220 Acemetacin-(Et)
3220 Dixyrazine
3220 Sulindac-(ME)
3225 Fluocortolone
3240 Buprenorphine-(H_2O)
3245 OH-Alprazolam
3247 Thiethylperazine
3250 Canrenone
3250 OH-Pipamperone
3250 Sulfone
3255 N^4-Acetylsulfamethoxazole-(Me)
3255 OH-MeO-Clobazam
3260 Pericyazine
3260 Phocoldine-AC
3265 (Acetamido-)acenocoumarol-(Me_2)
3269 Methoxy-dextromoramide
3280 Brucine
3280 Spironolactone
3281 Trimethobenzamide

10

3287	Phytomenadione
3320	Buprenorphine ((-H_2O)-AC)
3320	Xipamide (me_3)
3330	Trazodone
3335	Amiodarone
3340	Chlormadinone-H_2O
3350	(OH-)Acenocoumarol (isomer 1-Me_2)
3350	*O*-Desalkyl-dixyrazine-(AC)
3350	*O*-Methyldobutamine-(AC_3)
3350	Testosterone Dipropionate
3350	Xipamide-(Me_2)
3355	*N*-Desalkyldixyrazine-(AC)
3360	Buclizine
3360	Buprenorphine
3360	Chlormadinone Acetate
3360	Nordimetotiazine-(AC)
3360	Penfluridol
3360	Di-OH-dihydroquinine-AC_3)
3370	Droperidol-Me-AC_2
3380	Mesoridazine
3380	OH-Acetyl-trazodone
3380	Perphenazine
3380	Bisnordimetotiazine-(AC)
3400	Clopenthixol *cis*-isomer (zuclopenthixol)
3400	Fluocortolone-(AC_2)
3400	Nor-OH-clotiapine-(AC_2)
3410	Acetylsulfaethidole-(Me_2)
3410	Buprenorphine-(AC)
3415	Sulforidazine
3420	Fluocortolone-(AC)
3420	Glipizide-(Me)
3420	Thioridazine (ring sulfone)
3430	2-Oxo-3-OH-lysergide-(TMS_2)
3430	Droperidol-AC_2
3433	Benperidol
3440	Fenoterol-H_2O-AC_4
3440	Gestonorone Caproate
3445	LSD
3450	Desalkyl-dihydro-clopenthixol-(AC)
3455	Glipizide (-Me_2)
3460	Clopenthixol
3460	Clopenthixol-AC
3467	Thiopropazate
3490	Desalkylclopenthixol-(AC)
3490	Nor-acetyl-clozapine
3495	Dobutamine-AC_4
3500	(OH-)Acenocoumarol (isomer 2)-Me_2
3500	Oxo-thioridazine
3514	Diphenoxylate
3515	Iso-LSD-TMS
3515	Norlysergide-(TMS_2)
3530	Dixyrazine-AC
3550	Econazole
3552	Thioproperazine
3560	Piritramide
3590	Carfenazine
3595	LSD-TMS
3620	Acetylsulfametoxydiazine-(Me)
3705	Norlysergide-(TMS)
3710	Acetylsulfadiazine-(Me_2)
3800	Glibenclamide-(Me)
3800	Thioridazine (side chain sulfone)
3840	Glibenclamide-(Me_2)
3850	Gliquidone-(Me)
3870	Lidoflazine
3870	Pimozide
3910	Metolazone-(Me_3)
ND	4-OH-Alprazolam
ND	Acetophenazine
ND	Aconitine
ND	Amidefrine
ND	Aminonitrothiazole
ND	Amodiaquine
ND	Benzonatate
ND	Betaine
ND	Bezitramide
ND	Bunamidine
ND	Captopril
ND	Carbidopa
ND	Cetylpyridinium Chloride
ND	Cimetidine
ND	Cinchophen
ND	Clioxanide
ND	Clofazimine
ND	Clopamide
ND	Coniine
ND	Debrisoquine
ND	Decoquinate
ND	Diazoxide
ND	Dicoumarol
ND	Diquat (dibromide)
ND	Ecgonine
ND	Epithiazide
ND	Etacrynic Acid
ND	Etoxazene
ND	Flavoxate
ND	Furosemide
ND	Glafenine
ND	Guanethidine
ND	Hexamethonium Bromide
ND	Hexobendine
ND	Homatropine Methylbromide
ND	Hydrochlorothiazide
ND	Hydroflumethiazide
ND	Lofepramine
ND	Loprazolam
ND	Metallibure
ND	Metformin
ND	Methyclothiazide
ND	Methyldopa
ND	Methylergometrine
ND	Metolazone
ND	Morantel
ND	Nicergoline
ND	Nifursol
ND	Noroxymorphone
ND	Orciprenaline
ND	Oxyclozanide
ND	Oxyphenbutazone
ND	Pancuronium Bromide
ND	Parbendazole
ND	Pentapiperide Metilsulfate
ND	Phentolamine
ND	Piperacetazine
ND	Practolol
ND	Prazosin
ND	Propantheline Bromide
ND	Propyliodone
ND	Pyrantel
ND	Quinethazone
ND	Rafoxanide
ND	Reserpine
ND	Ritodrine
ND	Sulfaguanidine
ND	Sulfamoxole
ND	Sulfathiazole
ND	Suxamethonium Chloride
ND	Thiamine
ND	Urea

System GB

994	Isometheptene
1060	Ethchlorvynol
1063	Cineole
1098	Valproic Acid
1142	Amfetamine (formyl)
1150	Amfetamine
1150	Amiphenazole
1157	Norfenfluramine
1191	Phentermine
1192	Propylhexedrine
1194	Menthol
1200	Metamfetamine
1228	Methyl Salicylate
1228	Salicylic Acid-(Me)
1228	Salicylic Acid-Me
1245	Chlormezanone (art)
1250	Mephentermine
1252	Fenfluramine
1252	Tranylcypromine
1257	Pargyline
1258	Ethosuximide
1261	Zopiclone (aminochloropyridine)
1269	Clomethiazole
1270	Labetalol
1278	Phenelzine
1280	4-Aminophenol
1280	*p*-Aminophenol
1338	*N*-Didesethyldiethylpropion
1340	Salicylic Acid
1352	Cathine
1352	Phenylpropanolamine
1353	Phenylpropanolamine
1356	Norephedrine
1380	Eugenol
1380	Nicotine
1393	Chlorphentermine
1395	3-OH-Ethosuximide
1395	Desamino-oxomexiletine
1400	Acetanilide
1410	Ephedrine
1410	Methoxyamfetamine
1410	Pseudoephedrine
1416	Methoxyphenamine
1418	Nicotinamide
1423	*N*-Desethyldiethylpropion
1430	Anhydroecgonine Methylester
1431	Mexiletene
1436	OH-Ethyl-ethosuximide
1451	Methylephedrine
1453	Selegiline
1457	Ethylnorephedrine
1465	Diphenylmethane
1472	Norecgonine
1475	Felbamate
1483	Phenmetrazine
1489	Salicylamide
1504	Phendimetrazine
1505	Allobarbital-Me_2
1510	Etafedrine
1511	Codeine
1512	MDA
1530	Methylecgonine
1532	Diethylpropion
1534	1-Naphthol-propranolol
1539	Beclamide-Art
1551	Cyclopentolate-H_2O
1569	Nikethamide
1572	Methylenedioxymetamfetamine
1579	Pentetrazol
1580	Phenyltoloxamine ((*N*-oxide)-ME_2 NOH)
1581	Methyprylon
1596	*N*-Methyl-4-chlorobenzamide
1599	Diethylnorephedrine
1601	OH-Methyprylon-(H_2O)
1602	Ethylecgonine
1609	Iproniazid
1614	Clorprenaline
1629	Levetiracetam
1632	Vanillin
1633	Gabapentin-(H_2O)
1637	Ibuprofen
1645	Bupropion
1645	Cotinine
1645	Diphenylmethanol
1648	Fenproporex
1652	DOM
1660	Methylsulfonamide
1660	Prolintane
1670	Doxylamine ((carbinol)-H_2O)
1673	Benzophenone
1688	4-Chloromethylbiphenylbuclizine
1688	4-Chloromethylbiphenyl
1689	Methylenedioxyamfetamine (art (formyl))
1695	Glibornuride (amide)
1698	Butalbital
1700	Methylbenzophenone
1707	*N*-Desalkyl-oxohaloperidol-($2H_2O$)
1722	Paracetamol
1724	*O*-Desalkyl-phenyltoloxamine

2438 Oxazepam
2440 Maprotiline
2440 Practolol
2442 Tropicamide
2450 Nor-OH-venlafaxine
2452 Norpromazine
2454 *cis*- 10-OH-Amitriptyline
2460 Cyproheptadine
2460 Levallorphan
2466 *trans*- 10-OH-Amitriptyline
2468 Norsertraline
2468 Oxazepam-(TMS_2)
2469 Atenolol
2472 Phenylbutazone
2480 Bisoprolol (art-(H_2O))
2480 CBD
2480 *cis*- 10-OH-Nortriptyline
2481 Sertraline
2486 Dosulepin
2487 Norpropoxyphene
2487 Procyclidine (OH-isomer-1)
2494 OH-Imipramine
2494 *trans*- 10-OH-Nortriptyline
2496 Dihydrocodeine-TMS
2496 Sertraline (ketone)
2499 Azatadine
2499 Citalopram
2500 2′-OH-Methylmethaqualone
2504 Mazindol
2507 Nordosulepin
2508 Betaxolol-(art)
2511 Clomipramine
2511 Codeine
2511 Dihydrocodeine
2514 Nor-*N*-proponyl-dextropropoxyphene
2517 Procyclidine (OH-isomer-2)
2518 Dihydromorphine-(TMS_2)
2519 Betaxolol-(art-H_2O)
2520 Sotalol
2521 10-OH-Desipramine
2526 Norcitalopram
2527 Dihydromorphine
2528 Lorazepam
2528 *cis*-OH-Doxepin
2533 Dosulepin (sulfoxide)
2535 Norcodeine
2535 Norcodeine
2540 Norclomipramine
2544 *trans*-OH-Doxepin
2546 4-OH-Propranolol
2546 *N*-Desalkyl-acetyl-verapamil
2552 Ketazolam
2556 Diazepam
2559 Desalkylflurazepam
2559 Nordihydrocodeine-(TMS_2)
2559 Thebacon
2562 Lamotrigine
2563 Norchlorphenamine
2564 Morphine
2566 Dibenzepin
2566 Lorazepam-TMS_2
2569 Acebutolol-(H_2O)
2574 Dantron-TMS
2575 Mebhydrolin
2576 Quazepam
2578 Δ^9-THC
2579 *N*-Didesalkyl-acetylverapamil
2579 Tetrabenazine
2580 10-OH-Oxcarbazepine
2580 Hydrocodone
2586 Dipipanone
2588 Remoxipride
2592 Codeine-TMS
2592 Metixene
2595 Hydromorphone-enol-TMS_2
2598 Hydromorphone
2599 Norhydrocodone
2599 Nordihydrocodeine
2600 Oxo-meptazinol
2602 Morphine-TMS_2
2602 Oxycodone (-enol-TMS_2)
2603 Desamino-di-OH-maprotiline
2608 Chlorprothixene
2608 Disopyramide
2608 *m*-OH-Cocaine
2608 Nor-OH-cyproheptadine-(H_2O)
2611 Dantron-TMS_2
2618 Chlorpromazine
2618 Clorazepic Acid
2618 OH-Trihexyphenidyl
2621 Hydromorphone-TMS
2621 Noroxycodone-(enol-TMS_2)
2622 Norflunitrazepam-(TMS)
2625 3-Monoacetylmorphine
2625 Nordazepam
2628 8-OH-Mianserin
2631 Norcodeine-(TMS_2)
2631 OH-Trimipramine
2634 7-Amino-nitrazepam-(TMS_3)
2636 OH-Imipramine
2638 *O*-Desmethyl-OH-tetrabenazine
2639 Thioridazine (ring)
2641 Levomepromazine
2642 Nitrazepam-(TMS)
2644 CBN
2644 *cis*-Nor-OH-doxepin
2645 Acetylcodeine
2645 Codeine-AC
2646 6-Monoacetylmorphine
2646 Didesmethyl-chlorpromazine
2649 OH-Pentazocine
2649 Nor-di-OH-nefopam
2650 3-OH-Bromazepam-(TMS_2)
2650 *p*-OH-Cocaine
2656 Norchlorpromazine
2658 Nadolol
2662 Nor-OH-trimipramine
2663 Benzoylecgonine
2663 Methoxycocaethylene
2665 Oxo-mirtazapine
2666 Dihydro-oxycodone
2669 2-OH-Desipramine
2669 Oxo-procyclidine-(H_2O)
2669 Pecazine
2670 Nadolol-(art)
2671 Oxycodone
2671 *trans*-Nor-OH-doxepin
2673 Noramidedextropropoxyphene
2674 Hydrocodone-(TMS)
2678 Hydromorphone Oxime-(TMS_2)
2679 Norchlordiazepoxide
2680 Bisoprolol (art)
2683 Clobazam
2687 Paroxetine
2688 6-Monoacetylmorphine-(TMS)
2691 Paroxetine
2698 10-OH-Clomipramine
2698 OH-Methoxymethaqualone
2702 Bromazepam-(TMS)
2703 Noroxycodone
2703 Oxycodone-(TMS)
2708 Nifedipine
2709 OH-Cocaethylene
2713 Temazepam-(TMS)
2715 OH-Methoxyimipramine
2715 OH-Methoxytrimipramine
2717 Loxapine
2717 Nor-OH-promethazine
2722 Midazolam
2723 Oxymorphone
2724 Acecainide
2724 *N*-Acetylprocainamide
2727 Temazepam
2729 OH-Methoxycocaine
2732 Promethazine (norsulfoxide)
2734 Desmethylenyl-3-methyl-paroxetine
2735 2-OH-Clomipramine
2738 Di-OH-carbamazepine
2739 Bisdesethylflurazepam
2740 Oxycodone-(oxime-TMS_2)
2742 7-Aminoclonazepam-(TMS_2)
2744 Flunitrazepam
2746 Amoxapine
2746 Noroxycodone-(enol-TMS_3)
2749 OH-Methoxydesipramine
2750 Glymidine Sodium
2759 Norclobazam
2760 Bromazepam
2763 Noroxycodone-(TMS_2)
2769 Diamorphine
2770 Lormetazepam
2774 Acetyl-nortriptyline
2775 OH-Midazolam (TMS)
2778 2-OH-Ethylflurazepam-(TMS)
2779 OH-Methoxycocaethylene
2781 Clonazepam-TMS
2781 OH-Promazine
2783 Prazepam
2789 Norlorcainide
2793 Bisdesacetylbisacodyl
2793 Felodipine
2797 Promethazine (sulfoxide)
2797 Nor-OH-promazine
2798 Trifluoperazine
2799 Lormetazepam-TMS_2
2804 7-Amino-flunitrazepam
2805 2-OH-Ethyl-flurazepam
2805 Alimemazine (sulfoxide)
2806 Demoxepam
2811 Acetyldesipramine
2816 Norflunitrazepam
2817 Alimemazine (norsulfoxide)
2824 Dihydro-acepromazine-(H_2O)
2829 OH-Alimemazine
2832 (Desalkyl-)-*cis*-flupentixol
2833 Clotiapine
2833 Phenazocine
2836 7-Amino-flunitrazepam-(TMS)
2839 Dosulepin (norsulfoxide)
2840 Promazine (sulfoxide)
2843 OH-Clomipramine
2844 Acepromazine
2845 Nor-OH-alimemazine
2855 (Desalkyl-)-*trans*-flupentixol
2861 Olanzapine
2866 α-OH-Midazolam-(TMS)
2875 Promazine (norsulfoxide)
2876 Desacetylbisacodyl
2878 7-Amino-nitrazepam
2880 8-OH-Norclomipramine
2882 Norclotiapine
2896 Flurazepam
2899 Fentanyl
2900 Fenetylline
2901 α-OH-Midazolam
2902 Butorphanol
2911 Norolanzapine
2915 Nitrazepam
2923 Lorcainide
2926 Acebutolol
2939 7-OH-Chlorpromazine
2939 Mequitazine
2941 Zolpidem
2949 Norcyclobenzaprine
2956 Bisacodyl
2962 Di-OH-imipramine
2973 Papaverine
2975 11-Hydroxy-Δ^9-THC
2979 Quinidine
2981 Chlordiazepoxide
2982 Amlodipine
2983 Phenoperidine
2995 Di-OH-desipramine
2996 7-Aminoclonazepam
3000 Clonazepam
3000 Hydroxyzine
3003 Chlorpromazine (sulfoxide)
3018 Ketodoxapram
3024 Clozapine
3046 Chlorpromazine (norsulfoxide)
3046 Doxapram
3050 Metformin (nitrobenzoyltriazine)
3054 Doxazosin
3068 7-OH-Loxapine
3076 Diltiazem
3077 8-OH-Loxapine
3086 Quinidine (*n*-oxide)
3088 Norsulfoxide
3092 Desacetyldiltiazem
3092 Norclozapine
3094 Dextromoramide
3094 Haloperidol
3096 Dimetotiazine
3096 Nimodipine
3108 Alfentanil

3108	Alprazolam
3114	Levomepromazine (sulfoxide)
3114	*N*-Desmethyldiltiazem
3119	Rofecoxib
3129	Prochlorperazine
3145	Trifluoperazine (sulfoxide)
3147	*O*-Desmethyl-diltiazem
3152	Haloperidol (reduced)
3160	7-OH-Acepromazine
3162	Camazepam
3191	Trifluoperazine (norsulfoxide)
3193	Meclozine
3194	Fluphenazine
3199	*cis*-Flupentixol
3211	Etorphine
3217	*trans*-Flupentixol
3219	Opipramol
3219	Triazolam
3233	Cinnarizine
3236	Loratadine
3263	Zopiclone
3275	Northioridazine
3292	Thioridazine
3305	Verapamil
3308	α-OH-Triazolam-(TMS)
3310	OH-Dextromoramide
3332	Lysergide
3348	Pholcodine
3358	Noscapine
3371	Norverapamil
3385	Diprenorphine
3400	Quetiapine
3410	Pholcodine-TMS
3461	Buclizine
3486	Pericyazine
3525	7-OH-Amoxapine
3546	8-OH-Amoxapine
3562	Trazodone
3571	Prochlorperazine (norsulfoxide)
3594	Perphenazine
3609	Nor-acetylclozapine
3610	Buprenorphine
3626	Thioridazine (ring sulfone)
3629	Mesoridazine
3640	OH-Acetyl-trazodone
3667	Benperidol
3670	Diphenoxylate
3690	Sulforidazine
3758	Prochlorperazine (sulfoxide)
ND	Benoxaprofen
ND	Buformin
ND	Captopril
ND	Debrisoquine
ND	Diazoxide
ND	Droperidol
ND	Ecgonine
ND	Glibornuride
ND	Lidoflazine
ND	Loprazolam
ND	Metformin
ND	Nalbuphine
ND	Nalorphine
ND	Naloxone
ND	Naltrexone
ND	OH-Alprazolam
ND	Pimozide
ND	Pipotiazine
ND	Sulpiride
ND	Vigabatrin
ND	α-OH-Triazolam

System GC

1253	Bemegride
1287	Tuaminoheptane
1383	Cathine
1383	Norephedrine
1383	Phenylpropanolamine
1429	Dimethylamfetamine
1440	Pargyline
1450	Phentermine
1460	Phenelzine
1467	Ephedrine
1470	Norfenfluramine
1480	Methylephedrine
1500	Propylhexedrine
1536	Amfetamine
1543	Pseudoephedrine
1573	Nicotine
1621	Fenfluramine
1630	Ethoheptazine
1668	Mephentermine
1670	Methoxyphenamine
1715	Diethylpropion
1722	Metamfetamine
1725	Chlorphentermine
1735	Phendimetrazine
1737	Etafedrine
1759	Tranylcypromine
1849	Prolintane
1852	Nikethamide
1873	Phenmetrazine
1880	Clorprenaline
1934	Phenylephrine
2021	Pentetrazol
2025	Pethidine
2111	Cotinine
2172	Benzfetamine
2173	Dextropropoxyphene
2180	Fencamfamin
2200	Meclofenoxate
2200	Methylphenidate
2225	Pentazocine
2230	Doxapram
2230	Levorphanol
2300	Thenyldiamine
2307	Cyproheptadine
2348	Cyclizine
2370	Aminophenazone
2376	Caffeine
2378	Diphenhydramine
2409	Etamivan
2430	Carbinoxamine
2447	Diphenylpyraline
2457	Brompheniramine
2457	Naphazoline
2470	Methadone
2478	Pipradrol
2504	Dihydromorphine
2542	Morphine
2546	Promethazine
2563	Amiphenazole
2576	Thonzylamine
2586	Chlorphenamine
2646	Alimemazine
2669	Dimetindene
2681	Codeine
2702	Dihydrocodeine
2739	Mebhydrolin
2749	Antazoline
2803	Oxazepam
2894	Dipipanone
2926	Phenindamine
2954	Triprolidine
3028	Hydrocodone
3145	Prazepam
3469	Anileridine
3625	Dextromoramide

System GC1

1776 Disulfoton

System GC2

1906 Disulfoton

System GC3

2080 Disulfoton

System GC19

5.53 Oxycodone

System GD

0.40	Salsalate
0.49	Indometacin (methyl)
0.49	Sulindac (methyl derivative)
0.60	Salsalate
0.89	Ibuprofen (-Me)
1.12	Bufexamac

1.13 Alclofenac (methyl derivative)
1.18 Naproxen
1.20 Diflunisal
1.26 Fenclofenac
1.26 Flufenamic Acid
1.30 Flurbiprofen
1.31 Fenoprofen
1.37 Naproxen (-Me)
1.38 Niflumic Acid (methyl)
1.39 Flunixin
1.42 Diclofenac (-Me)
1.45 Ketoprofen (methyl derivative)
1.45 Mefenamic Acid (-Me)
1.55 Fenclofenac
1.55 Indometacin (methyl)
1.61 Clonixin
1.62 Meclofenamic Acid (methyl derivative)
1.77 Tolmetin (methyl derivative)
1.79 Methylfenbufen
1.81 Feprazone
1.98 Methylbenoxaprofen
2.05 Phenylbutazone
2.07 Indoprofen (methyl derivative)
2.11 Methyloxyphenbutazone
2.11 Oxyphenbutazone
2.27 Indoprofen (methyl derivative)
2.64 Furosemide

System GE

0.04 Trimethadione
0.06 Paramethadione
0.09 Valproic Acid
0.18 Ethosuximide
0.22 *N*-Desmethylparamethadione
0.35 Mesuximide
0.40 Phensuximide
0.55 Mephenytoin
0.57 Ethotoin
0.65 PEMA
0.73 5-Ethyl-5-phenylhydantoin
0.74 Phenobarbital
0.83 Carbamazepine
0.89 Primidone
1.35 Cholesterol

System GF

1315 Amfetamine
1335 Metamfetamine
1455 Tranylcypromine
1525 Nicotine
1655 Diethylpropion
1895 Nikethamide
1935 Methylphenidate
1995 Pethidine
2050 Benzfetamine
2090 Methyprylon
2100 Benzocaine
2100 Pheniramine
2105 Diphenhydramine
2110 Ethoheptazine
2130 Tybamate
2150 Phencyclidine
2170 Doxylamine
2185 Orphenadrine
2190 EMDP
2195 Cotinine
2230 Barbital
2240 Lidocaine
2265 Aminophenazone
2265 Dicycloverine
2270 Oxprenolol
2280 Methadone (eDDP)
2305 Methapyrilene
2310 Propyphenazone
2315 Glutethimide
2320 Cyclizine
2325 Phenacetin
2335 Chlorphenamine
2340 Allobarbital
2340 Caffeine
2340 Thenyldiamine
2345 Mepivacaine
2365 Chlorphenoxamine
2370 Dextropropoxyphene
2370 Methadone
2380 Hexobarbital
2380 Nefopam
2390 Butobarbital
2395 Butalbital
2405 Diphenylpyraline
2430 Amobarbital
2445 Benzoctamine
2445 Phenazone
2455 Carbetapentane
2460 Meprobamate
2460 Nealbarbital
2465 Butriptyline
2465 Pentobarbital
2470 Brompheniramine
2480 Bromazine
2485 Procyclidine
2495 Vinbarbital
2505 Trimipramine
2510 Amitriptyline
2510 Secobarbital
2515 Phenindamine
2540 Imipramine
2550 Cocaine
2550 Trifluprom azine
2560 Carbamazepine (epoxide)
2560 Chlorcyclizine
2560 Mepyramine
2570 Doxepin
2580 Methaqualone
2580 Procaine
2590 Protriptyline
2595 Mianserin
2600 Phenoxybenzamine
2600 Pramocaine
2600 Thiopental
2600 Triprolidine
2610 Carbamazepine
2620 Iminostilbene
2640 Medazepam
2660 Atropine
2660 Piperidolate
2670 Nomifensine
2675 Promethazine
2710 Clemastine
2710 Cyproheptadine
2710 Dipipanone
2715 Alimemazine
2715 Tetracaine
2745 Promazine
2745 Theophylline
2765 Brallobarbital
2770 Dosulepin
2775 Profenamine
2795 Clomipramine
2800 Feprazone
2800 Prothipendyl
2803 Oxazepam
2815 Pyrrobutamine
2820 Dosulepin Sulfoxide
2825 Cyclobarbital
2830 10-OH-Amitriptyline
2840 Dihydrocodeine
2845 Phenothiazine
2860 Codeine
2860 Phenylbutazone
2885 Dibenzepin
2895 Metixene
2900 Oxyphencyclimine
2910 Chlorprothixene
2910 Disopyramide
2920 Mebhydrolin
2920 Methdilazine
2930 Hydrocodone
2940 Chlorpromazine
2940 Heptabarb
2960 Phenobarbital
2965 Levomepromazine
2965 Procainamide
3010 Azapropazone
3025 Norpropoxyphene
3030 Pentazocine
3041 Nordazepam
3045 Diazepam
3050 Trifluoperazine
3210 Flurazepam
3225 Propiomazine
3230 Acepromazine
3245 Chloroquine

System GG

2620 Medazepam
2803 Oxazepam
2910 Lorazepam
2940 Diazepam
3041 Nordazepam
3041 Nordiazepam
3043 Demoxepam
3065 Chlordiazepoxide
3125 Clorazepic Acid
3125 Temazepam
3147 Clobazam
3190 Flunitrazepam
3220 Flurazepam
3280 Bromazepam
3450 Nitrazepam
3455 Clozapine
3600 Clonazepam

System GH

2110 Propyl-CBD
2170 Propyl-Δ^9-THC
2270 CBD
2280 Cannabicyclol
2350 Δ^9-THC
2430 CBN

2440 Cannabigerol
2580 8α-OH-Δ^9-THC
2620 11-Hydroxy-Δ^9-THC
2710 8α,11-Dihydroxy-Δ^9-THC
2756 11-Nor-Δ^9-THC-^{9}-carboxylic Acid

System GI

0.70 Acetaldehyde
0.70 Acetaldehyde
0.70 Methanol
0.90 Dichlorodifluoromethane
1.90 Methylene Chloride
2.00 Cryofluorane
2.50 Acetone
3.00 Trichlorofluoromethane
4.00 Isopropyl Alcohol
5.50 Propanol
5.90 Ether
6.20 Chloroform
7.30 Methyl Ethyl Ketone
8.20 Trichloroethane
8.30 Enflurane
8.50 Halothane
8.60 Carbon Tetrachloride
9.40 Ethyl Acetate
12.5t Chloral Hydrate
14.8 Benzene
14.8 Trichloroethylene
17.0t Ethylene Glycol
17.6 Methoxyflurane
20.1 Methylpentynol
20.3 Amyl Nitrite
23.2 Paraldehyde
24.3 Tetrachloroethylene
24.8 Toluene
24.9 Tetrachloroethane
25.5 Trichloroethanol
29.8 Chlorobutanol
33.2 *m*-Xylene
34.2 Benzaldehyde
34.2 *p*-Xylene
34.5 *o*-Xylene
35.2 Ethchlorvynol
38.2 Camphor

System GJ

0.16 Sulfacetamide
0.40 Sulfamethoxazole-(Me)
0.40 Sulfamoxole
0.42 Sulfafurazole
0.47 Sulfapyridine (methyl derivative)
0.49 Sulfathiazole
0.50 Sulfisomidine (methyl derivative)
0.53 Glymidine Sodium
0.66 Sulfadiazine-(Me)
0.69 Sulfamerazine-(Me)
0.69 Sulfametopyrazine-(Me)
0.71 Sulfadimidine (Me)
0.91 N^4-Acetylsulfamethoxazole-(Me)
0.93 Sulfamethoxypyridazine (Me)
0.98 Sulfamethizole (Me)
1.38 Sulfametoxydiazine (Me)
1.69 N^4-Acetylsulfadiazine-(Me)
1.71 Sulfaphenazole (Me)

10

System GK

0.13 3,4-Dichlorophenyl Isocyanate
0.13 3,4-Dichlorophenyl-isocyanate
0.23 Dichlorvos
0.27 Trichlorfon
0.36 3,4-Dichloroaniline
0.36 Monoene Reduction Product
0.40 Diquat (monoene reduction product)
0.47 1-Naphtholcarbaryl
0.49 Dicamba
0.49 Diquat (diene reduction product)
0.50 Mevinphos
0.54 Methylchlorophenoxyacetic Acid
0.58 Diene Reduction Product
0.65 Dichlorophenoxyacetic Acid
0.70 Demeton-*S*
0.73 Omethoate
0.74 Dinitro-orthocresol Methyl Ether
0.76 Lindane
0.78 Monolinuron
0.79 Atrazine
0.79 Dimpylate
0.80 Simazine
0.80 Trichlorophenoxyacetic Acid (-methyl ester)
0.85 Heptachlor
0.86 Dimethoate
0.86 Disulfoton
0.88 Aldrin
0.90 Monocrotophos
0.94 Propanil
0.95 Desmetryne
0.96 Parathion (methyl)
0.97 Linuron
0.99 Terbutryne
1.01 Fenitrothion
1.03 Malathion
1.04 Parathion (ethyl)
1.06 *trans*-Chlordane
1.08 *cis*-Chlordane
1.10 Endosulfan
1.12 Dichlorophenoxyacetic Acid Iso-ocitol Ester
1.13 Dieldrin
1.13 Dieldrin
1.18 Dichlorophenoxyacetic Acid Iso-ocitol Ester
1.18 Tiabendazole
1.19 Endrin
1.24 Trichlorophenoxyacetic Acid (-isooctil ester)
1.24 *op*-DDT
1.27 Pyrethrin I
1.31 Trichlorophenoxyacetic Acid
1.32 *pp*-DDT
1.40 *op*-Methoxychlor
1.46 *pp*-Methoxychlor
1.59 Pyrethrin II
1.60 Azinphos-(Me)
ND Aminotriazole

System GKA

866 Aldicarb
1866 Chlorpyrifos-Me
1944 Fenitrothion
2008 Aldrin
2170 Dieldrin

System GKB

966 Aldicarb
2060 Chlorpyrifos-Me
2096 Aldrin
2112 Fenitrothion
2304 Dieldrin

System GKC

1088 Aldicarb
2226 Aldrin
2234 Chlorpyrifos-Me
2278 Fenitrothion
2528 Dieldrin

System GKD

5.8 Deltamethrin

System GL

1210 Methyl Salicylate
1210 Salicylic Acid-(Me_2)
1505 Ibuprofen-(Me)
1530 5-OH-Salicylic Acid-(Me_3)
1630 Paracetamol-Me
1680 3-OH-Ibuprofen-(Me)
1800 OH-Naproxen-(Me_2)
1875 Flufenamic Acid-(Me)
1880 Flurbiprofen-(Me)
1960 Niflumic Acid-(Me)
1970 Fenoprofen-(Me)
1975 Fenbufen-(Me)
2090 Ketoprofen-(Me)
2115 OH-Flufenamic acid-(Me_2)
2120 Naproxen-(Me)
2120 *O*-Desmethyl-naproxen-(Me_2)
2130 OH-Fenoprofen-(Me_2)
2140 OH-Niflumic acid (Me_2)
2180 OH-Flurbiprofen-(Me_2)
2190 Acetic acid OH-fenbufen-(Me_2)
2200 Diclofenac-(2Me)
2225 Etodolac-(ME)
2235 Tolmetin-(ME)
2240 Meclofenamic Acid-(Me)
2250 OH-Ketoprofen-(Me_2)
2255 Tolfenamic Acid-(ME)
2290 Phenylbutazone-Me
2460 OH-Diclofenac-(Me_2)
2500 OH-Alkylphenylbutazone-(Me)
2600 COOH-Tolmetin-(Me_2)
2770 Indometacin-Me
2880 OH-Indometacin-(Me_2)

System GM

0.284 Norfluoxetine
0.295 Fluvoxamine
0.304 Fluoxetine
0.319 Pethidine
0.357 Norpethidine
0.420 Tofenacin
0.423 Norketamine
0.427 Ketamine
0.428 Normeptazinol
0.429 Meptazinol
0.520 EDDP
0.544 Venlafaxine
0.570 *N*-Desmethylvenlafaxine
0.606 Methadone
0.625 *O*-Desmethylvenlafaxine
0.683 Butriptyline
0.687 *N*,*O*-Didesmethylvenlafaxine
0.723 Amitriptyline
0.761 Norbutriptyline
0.784 Imipramine
0.788 Doxepin (*cis* -Isomer)
0.816 Nortriptyline
0.820 Zimeldine
0.823 Doxepin (*trans* -Isomer)
0.830 *cis*-Nordoxepin
0.850 Cyclobenzaprine
0.850 Nomifensine
0.870 Pentazocine
0.878 Protriptyline
0.879 Mianserin
0.880 Norcyclobenzaprine
0.896 Desipramine
0.933 *trans*-Nordoxepin
0.941 Norzimeldine
0.967 Moclobemide
1.000 Brallobarbital
1.000 Iprindole
1.0861 Maprotiline
1.105 Normianserin
1.107 Normaprotiline
1.12 Citalopram
1.149 *cis*- 10-OH-Amitriptyline
1.166 Sertraline
1.168 *trans*- 10-OH-Amitriptyline
1.172 Clomipramine
1.218 Norsertraline
1.220 Dextropropoxyphene
1.23 Norcitalopram
1.248 Norpropoxyphene
1.259 Dosulepin
1.261 *cis*- 10-OH-Nortriptyline
1.300 Nor-*N*-proponyldextropropoxyphene
1.309 Dipipanone
1.323 *trans*- 10-OH-Nortriptyline
1.374 Norclomipramine
1.449 Acetylcodeine
1.449 Codeine-AC
1.450 Nordosulepin
1.493 Dihydrocodeine
1.519 Codeine
1.735 Dibenzepin
1.969 Noramidedextropropoxyphene
2.047 Paroxetine
2.831 Amoxapine
ND Alfentanil
ND Buprenorphine
ND Diphenoxylate
ND Diprenorphine
ND Etorphine
ND Morphine
ND Trazodone

System GN

86.5 Vanillin

System GO

11.2 Cineole

System GP

2235 Dehydrodesethylfelodipine-(Me)
2255 Dehydronifedipine
2255 Dehydrodesisobutyl-nisoldipine-(Me)
2270 Dehydrodeisopropylisradipine-(Me)
2280 Dehydrofelodipine
2300 Dehydrodesethyl-nitrendipine-(Me)
2300 Dehydrodesisopropyl-desmethoxyethyl-nimodipine-(Me_2)
2360 Dehydroisradipine
2370 Dehydronitrendipine
2390 Dehydrodesmethoxyethylnimodipine-(Me)
2430 (Dehydro2-HOOC-$^-$)-Me-amlodipine
2450 Dehydronisoldipine
2450 Perindopril-Me
2470 (Perindoprilat)-Me_3
2560 (Perindoprilat-H_2O)-Me_3
2615 Dehydro-OH$^-$nisoldipine
2635 (Dehydro-desamino-HOOC-$^-$)-Me-amlodipine
2645 Dehydro-desisopropyl-O-desmethyl-HOOC-nimodipine-(Me)
2655 Dehydro-nimodipine
2665 Dehydro-desisopropyl-O-desmethyl-nimodipine-(Me)
2675 Enalapril (-ME)
2680 Enalapril (-ME3)
2695 Dehydro-2-COOH-nifedipine (-Me)
2695 Dehydro-desisobutyl-2-HOOC-$^-$nisoldipine (-Me_2)
2735 (Enalaprilat-H_2O)-ME
2740 Dehydro-O-desmethyl-HOOC-nimodipine (-Me)
2865 (Ramiprilat)-Me_3
2880 Ramipril-Me
2925 (Ramiprilate-H_2O)-Me_3
2960 Cilazapril-Me_3
2970 Trandolapril (-Me)
2985 Benazepril (-Me_3)
3005 Trandolapril (perindoprilate-ME3)
3010 Cilazapril-Me
3030 Benazepril-(ME)
3070 Trandolapril (perindoprilate-H_2O-Me_3)
3080 (Quinaprilate)-ME_3
3110 Quinapril-Me
3310 (Quinaprilate-H_2O)-ME_3
3420 Valsartan-(ME_2)
3555 Losartan-2ME
3575 Moexipril-(ME)
3580 (Moexiprilate)-ME3
3775 (Moexiprilate-H_2O)-ME3

System GQ

5.50 Monoethylglycinexylidide
5.70 Prilocaine
6.00 Lidocaine
6.80 Procaine
7.20 Mepivacaine
7.85 Cocaine
8.00 Tetracaine
10.40 Cinchocaine

System GR

2.8 Sevoflurane
4.2 Halothane

System GS

16.1 Amitriptyline
16.3 Trimipramine
17.1 Imipramine
17.2 Doxepin (*cis* -Isomer)
17.5 Nortriptyline
17.6 Doxepin (*trans* -Isomer)
18.1 Mianserin
18.7 Desipramine
21.0 Maprotiline
21.2 Chloramitriptyline (IS)

System GT

2210 Moclobemide
2440 Zotepine
2715 Zolpidem
2780 Olanzapine
2950 Zopiclone
4510 Nefazodone

System GU

14.1 Setiptiline

System GV

10.4 Ketamine
11.6 Xylazine
16.6 Chlorpromazine
19.0 Azaperone
23.3 Haloperidol

System GW

19.3 Triflupromazine
20.3 Alimemazine
23.0 Chlorpromazine
23.6 Levomepromazine
31.1 Thioridazine

System GX

4.0 Etacrynic Acid-(Me)
4.4 Hydroflumethiazide-(Me_4)
6.3 Hydroflumethiazide-(Me_4)
6.6 Chlorothiazide-Me_3
7.0 Clopamide-Me_2
7.0 Furosemide-(Me_3)
7.4 Mefruside-(Me_2)
7.7 Bumetanide-Me_3
7.7 Chlortalidone-Me_4
9.0 Hydrochlorothiazide-(Me_4)
9.0 Indapamide-(Me_3)
9.2 Triamterene-(Me_6)
9.8 5-Oxo-mefruside-(Me_2)
9.9 Methyclothiazide-(Me_3)
10.7 Bendrofluazide-(Me_4)
10.7 Trichlormethiazide
11.0 Polythiazide-Me_3

System GY

3.3 Etacrynic Acid-(Me)
4.3 Chlorothiazide-Me_3
4.7 Furosemide-(Me_3)
4.7 Mefruside-(Me_2)
4.8 Chlortalidone-Me_4
4.9 Bumetanide-Me_3
5.0 Hydrochlorothiazide-(Me_4)
5.1 5-Oxomefruside-(Me_2)
5.6 Bendrofluazide-(Me_4)
6.3 Cyclopenthiazide-Me_4

System GZ

6.8 Quazepam

System GAA

499 Ether
543 Halothane
603 Chloroform
661 Carbon Tetrachloride
784 Dimethyl Sulfoxide
811 Tetrachloroethylene

System GAD

7.50 Amfetamine
8.50 Metamfetamine
10.9 Methylenedioxymetamfetamine

System GAG

0.89 Methandriol
1.05 Boldenone
1.05 Methyltestosterone
1.05 Methyltestosterone
1.17 Oxandrolone
1.20 Methandienone

1.21 Testosterone Acetate
1.28 Oxymetholone
1.43 Testosterone Propionate
1.50 Fluoxymesterone
1.54 Testosterone Isobutyrate
1.70 Methandriol Dipropionate
1.77 Testosterone Isocaproate
1.92 Testosterone Enantate
2.19 Testosterone Cipionate
2.36 Testosterone Decanoate
2.56 Testosterone Undecylate
2.62 Boldenone Undecylenate

System GAI

0.57 Clenbuterol
0.905 1-Methylen-5α-androstan-3α-ol-17-one
0.921 17α-Methyl-5β-androst-1-en-3α,17β-diol
0.925 17α-Methyl-5α-androstan-3α,17β-diol
0.925 17α-methyl-5β, (α)-androstan-3α,17β-diol
0.925 Methandienone
0.925 Methandriol
0.943 17α-Trenbolone
0.950 5α-Dihydrotestosterone
0.960 5β-Androst-1-en-17β-ol-3-one
0.961 Boldenone
0.970 Testosterone
0.974 Drostanolone Propionate
0.993 Metenolone
1.078 Norethandrolone
1.106 2-Hydroxymethyl-17α-methyl-5-androstan-3,17-diol
1.111 Oxandrolone
1.117 17α-Methyl-1,4-androstadien-6β,17β-diol-3-one
1.155 Fluoxymesterone
1.180 2-Hydroxymethyl-17α-methyl-5-androstan-3, 6, 17-triol
1.380 3′-OH-Stanozolol
1.393 4β-OH-Stanozolol
310 Stanozolol

System GAJ

0.350 Valproic Acid
0.453 Ethosuximide
0.612 Barbital
0.689 Mesuximide
0.732 Butobarbital
0.775 (COOH-)-Amobarbital
0.778 Butalbital
0.789 Nealbarbital
0.794 Amobarbital
0.798 Methohexital
0.798 Vinylbital
0.803 Pentobarbital
0.865 Secobarbital
0.918 Mephenytoin
0.940 Ethotoin
0.940 Hexobarbital
0.948 Thiopental
1.000 Methylphenobarbital
1.053 (3′-OH-)-Butobarbital
1.065 Iminostilbene
1.074 PEMA
1.087 Phenylmethylbarbituric Acid
1.138 (3-OH-)-Amobarbital
1.142 Cyclobarbital
1.150 Phenobarbital
1.188 Carbamazepine (epoxide)
1.206 3′-OH-Secobarbital
1.282 Heptabarb
1.674 Primidone
1.716 Carbamazepine
1.941 Lamotrigine
ND Vigabatrin

System GAK

4.9 Amfetamine
8.7 Methylenedioxymetamfetamine
12.7 Cocaine
13.3 CBD
13.9 CBN
14.2 Diamorphine

System GAL

7.3 Lysergide
1362 Cathine

System GAM

6.9 Psilocybine

System GAO

4.0 γ-Butyrolactone
5.6 γ-Hydroxybutyrate-(TMS_2)
5.6 γ-Hydroxybutyrate-(TMS_2)

System GAR

11.8 DHEA
11.9 Androsterone
12.3 Methandriol
12.5 Nandrolone
12.8 Boldenone
12.9 Testosterone
13.1 Methyltestosterone
13.1 Methyltestosterone
13.2 Methandienone
13.5 Testosterone Acetate
13.6 Boldenone Acetate
13.7 Oxandrolone
13.7 Oxymetholone
14.2 Testosterone Propionate
14.6 Fluoxymesterone
15.2 Methandriol Dipropionate
15.4 Stanozolol
15.9 Testosterone Isocaproate
16.7 Testosterone Enantate
18.0 Testosterone Benzoate
18.7 Boldenone Benzoate
18.7 Testosterone Cipionate
19.8 Testosterone Decanoate
20.2 Testosterone Phenylpropionate
22.4 Boldenone Undecylenate

System GAS

1882 Ethoheptazine
1938 Dextropropoxyphene

11 High Performance Liquid Chromatographic Data

t = tailing peak

System H

1.45 Ethylmorphine

System HA

0.1 Theobromine
0.1 Acetanilide
0.1 Acetazolamide
0.1 Benzocaine
0.1 Clomethiazole
0.1 Diazepam
0.1 Diazoxide
0.1 Desalkylflurazepam
0.1 Indapamide
0.1 Lorazepam
0.1 Methocarbamol
0.1 Nitrazepam
0.1 Phenothiazine
0.1 Phenoxybenzamine
0.1 Proxyphylline
0.1 Theophylline
0.2 Bezitramide
0.2 Caffeine
0.2 Colchicine
0.2 Nordazepam
0.2 Diphenoxylate
0.2 Dipyridamole
0.2 Mebanazine
0.2 Medazepam
0.2 Mephenesin
0.2 EMDP
0.2 Methaqualone
0.2 Cotinine
0.2 Nifedipine
0.2 Pargyline
0.2 Pemoline
0.2 Perhexiline
0.2 Phenazone
0.3 Aminophenazone
0.3 Benzquinamide
0.3 Ergocristine
0.3 Fenbutrazate
0.3 Noscapine
0.3 Papaverine
0.4 Acetorphine
0.4 Bromhexine
0.4 Buprenorphine
0.4 Cimetidine
0.4 Deserpidine
0.4 Doxapram
0.4 Ergometrine
0.4 Ergotamine
0.4 Ergocornine
0.4 Ergocryptine
0.4 Methylergometrine
0.4 Methysergide
0.5 Lysergamide
0.5 Methoserpidine
0.5 Practolol
0.6 Dihydroergotamine
0.6t Diphenoxylate
0.6 Diprenorphine
0.6 Droperidol
0.6 Etorphine
0.6 Lidocaine
0.6 Lidoflazine
0.6 Iofepramine
0.6 Phentermine
0.6 Piritramide
0.6 Pramocaine
0.6 Rescinnamine
0.6 Trazodone
0.7 Buclizine
0.7 Dextromoramide
0.7 Fenoterol
0.7 Lysergide
0.7 Meclozine
0.7 Morazone
0.7 Narceine
0.7 Oxypertine
0.7 Pimozide
0.8 Cinnarizine
0.8 Fentanyl
0.8 Harmine
0.8 Isoxsuprine
0.8t Lysergic Acid
0.8 Phenoperidine
0.8 Prazosin
0.9 Amfetamine
0.9 Bamethan
0.9 Benzoylecgonine
0.9 Buphenine
0.9 Bupivacaine
0.9 Chlorphentermine
0.9 Norephedrine
0.9 Mepivacaine
0.9 Metaraminol
0.9 Methoxamine
0.9 Nomifensine
0.9 Phendimetrazine
0.9 Phenylpropanolamine
0.9 Terbutaline
1.0 Cathine
1.0 Ephedrine
1.0 Norfenfluramine
1.0 Ephedrine
1.0 Nalorphine
1.0 Phenelzine
1.0 Piminodine
1.0 Prenylamine
1.0 Prilocaine
1.0 Norpseudoephedrine
1.0 Pyrimethamine
1.0 Salbutamol
1.0 Thiopropazate
1.0 Tranylcypromine
1.1 Anileridine
1.1 Benperidol
1.1 Clorprenaline
1.1 Ecgonine
1.1 Dicycloverine
1.1 Hyoscine
1.1 Loxapine
1.1 Desmethylmaprotiline
1.1 Nicotine
1.1t Orphenadrine-*N*-oxide
1.1 4-Hydroxy-propranolol
1.2 Alprenolol
1.2 Azacyclonol
1.2 Benzfetamine
1.2 Butacaine
1.2 Clonidine
1.2 Debrisoquine
1.2 2-OH-desipramine
1.2 Etamiphylline
1.2 Fluphenazine
1.2 Haloperidol
1.2 Hydroxydesipramine
1.2 Monoethylglycinexylidide
1.2 2-Hydroxydesipramine
1.2 Mexiletine
1.2 Nadolol
1.2 Nialamide
1.2 Phenethylamine
1.2 Pindolol
1.2 Pipradrol
1.2 Pseudoephedrine
1.2 Sotalol
1.2 Timolol
1.2 Tocainide
1.2 Trifluperidol
1.2 Trimethoprim
1.2 Tryptamine
1.2 Tyramine
1.3 Atenolol
1.3 Didesmethylimipramine
1.3 Norpropoxyphene
1.3 Fencamfamin
1.3 Fenfluramine
1.3 Flupentixol
1.3 Flurazepam
1.3 Mescaline
1.3 Metoprolol
1.3 Oxprenolol
1.3 Pericyazine
1.3 Phenazocine
1.3 Phenylephrine
1.3 Procainamide
1.3 Propranolol
1.4 Acebutolol
1.4 Diethylcarbamazine
1.4 Hydroxyzine
1.4 Metopimazine
1.4 Naloxone
1.4 Primaquine
1.5 Mephentermine
1.6t Cyclopentolate
1.6 Tacrine
1.6 Xylometazoline
1.7 Benactyzine
1.7 Benzoctamine
1.7 Butetamate

1.7 Norbutriptyline
1.7 Carfenazine
1.7 Cyclopentamine
1.7 Diethylpropion
1.7t Labetalol
1.7 Mecamylamine
1.7 Meclofenoxate
1.7 Methoxyphenamine
1.7 Methylphenidate
1.7 *N*-Monodesmethylorphenadrine
1.7 Oxymetazoline
1.7t Norpethidine
1.7 Phenmetrazine
1.7 Phentolamine
1.7 Piperidolate
1.7 Tofenacin
1.7 Norverapamil
1.8 Adiphenine
1.8 Alverine
1.8 Monodesethylamiodarone
1.8 Nor-10-OH-amitriptyline
1.8 Antazoline
1.8 *N*-Monodesisopropyldisopyramide
1.8 Lorcainide
1.8 Mazindol
1.8 Mianserin
1.8 10-Hydroxynortriptyline
1.8 Pentazocine
1.8 Tramazoline
1.8 Trihexyphenidyl
1.8 Nor-trimipramine
1.9 Acetophenazine
1.9 Cinchocaine
1.9 Dextropropoxyphene
1.9 Etafedrine
1.9t Levallorphan
1.9 Mebeverine
1.9 Perphenazine
1.9 Piperacetazine
1.9 Procaine
2.0 Nortriptyline
2.0 Metamfetamine
2.0 Monodesmethylclomipramine
2.0 Diethylthiambutene
2.0 Procyclidine
2.0 Prolintane
2.0 Tetracaine
2.0 Thiamine
2.1 Cyclazocine
2.1 Desipramine
2.1 Dimetotiazine
2.1 Ibogaine
2.1 Propiomazine
2.1 Protriptyline
2.1 Proxymetacaine
2.1 Quinidine
2.1 Tolazoline
2.1 Trimeperidine
2.2 Nor-chlorpromazine
2.2 Norcyclizine
2.2 Dipipanone
2.2 Monodesmethyldosulepin
2.2 Monodesmethyldoxepin
2.2 Flavoxate
2.2 Maprotiline
2.2 Methadone
2.2 Opipramol
2.2t Prajmalium Bitartrate
2.2 Quinisocaine
2.3 Chlorcyclizine
2.3 Methylephedrine
2.3 Desacetylmoxisylyte
2.3 Ranitidine
2.4 Amiodarone
2.4 Disopyramide
2.4t Isopropamide Iodide
2.4 *N*-Desmethyl-mianserin
2.4 Naphazoline
2.4t Phencyclidine
2.4 Profenamine
2.4 Quinine
2.5 Phenindamine
2.6t Oxyphenonium Bromide
2.6 Physostigmine
2.6 Verapamil
2.7 Bromazine
2.7 Butriptyline
2.7t Dopamine
2.7 Pirenzepine
2.7 Triflupromazine
2.7 Trimipramine
2.8t Ajmaline
2.8t Alphaprodine
2.8 Cocaine
2.8 Dibenzepin
2.8t Ketobemidone
2.8 EDDP
2.8 Oxyphencyclimine
2.8t Pethidine
2.8t Pethidinic Acid
2.8 Pyrrobutamine
2.9 10-OH-Amitriptyline
2.9 Chlorphenoxamine
2.9 Cyclizine
2.9t Normorphine
2.9 Moxisylyte
2.9 Phenglutarimide
2.9 Tolpropamine
2.9t Norzimeldine
3.0 Chlorprothixene
3.0t Diamorphine
3.0 Histapyrrodine
3.0t Mebhydrolin
3.0 Nefopam
3.0 Orphenadrine
3.0 Oxeladin
3.0 *N*-Acetylprocainamide
3.0 Trifluoperazine
3.0t Trimetazidine
3.1 Alimemazine
3.1 Betahistine
3.1 Bufotenine
3.1 Cinchonidine
3.1t Norcodeine
3.1 Hydroxyimipramine
3.1 Meptazinol
3.1t Norcodeine
3.1 Phenyltoloxamine
3.1t Protokylol
3.1t Psilocin
3.2 Cyproheptadine
3.2 Dosulepin
3.2t Glycopyrronium Bromide
3.2 Levomepromazine
3.2t Morphine *N*-oxide
3.2 Penthienate Methobromide
3.2 Thonzylamine
3.2 Triprolidine
3.2t Zimeldine
3.3 Amitriptyline
3.3 Diphenhydramine
3.3t Diphenylpyraline
3.3 Ethoheptazine
3.3t Poldine Metilsulfate
3.4 Butaperazine
3.4 Clomipramine
3.4 Diethazine
3.4 Pizotifen
3.5 Thenalidine
3.6t 6-Monoacetylmorphine
3.6 Metixene
3.6t 6-Monoacetylmorphine
3.6t Tigloidine
3.6 Tripelennamine
3.7t Apomorphine
3.7t Benzatropine
3.7 Clemastine
3.7 Doxepin
3.7 Ethylmorphine
3.7t Hyoscyamine
3.7 Thebacon
3.8t Morphine
3.8t Morphine
3.8 Isothipendyl
3.8t Morphine
3.8 Thiethylperazine
3.8 Tiotixene
3.9t Atropine
3.9 Chlorphenamine
3.9 Mepyramine
3.9 Pecazine
3.9 Prochlorperazine
4.0 Chloropyrilene
4.0 Thenyldiamine
4.1 Acepromazine
4.1 Brompheniramine
4.1 Chlorpromazine
4.1 Iprindole
4.1t Mepenzolate Bromide
4.1 Methapyrilene
4.1 Pheniramine
4.1 Thioproperazine
4.2 Chloropyramine
4.2t Homatropine
4.2 Imipramine
4.3t Bretylium Tosilate
4.4t Benzylmorphine
4.4 Doxylamine
4.4t Levorphanol
4.4 Propantheline Bromide
4.4 Prothipendyl
4.6t Dosulepin Sulfoxide
4.6t Thebaine
4.7t Carbinoxamine
4.7t Dextrorphan
4.7 Neostigmine Bromide
4.7 Trimethobenzamide
4.8t Clemizole
4.8t Codeine
5.0t Deptropine
5.0 Mesoridazine
5.0 Metoclopramide
5.0 Promethazine
5.0 Mesoridazine
5.1 Dimetindene
5.2 Emepronium Bromide
5.2 Thioridazine
5.4 Pipazetate
5.6t Dextromethorphan
5.7t Dihydromorphine
5.8t Dimethoxanate
5.9 Promazine
6.0 Methdilazine
6.0t Pholcodine
6.3t Pyridostigmine Bromide
6.7t Oxymorphone
6.9t Oxycodone
7.1t Emetine
7.1t Hydrocodone
7.2t Dihydrocodeine
7.7t Cephaëline
7.9t Hydromorphone
8.2t Cotarnine
8.3t Mequitazine
11.1t Brucine
13.0t Strychnine
15.2 Chloroquine

System HB

0.10 Noradrenaline
0.27 Oxedrine
0.73 Hydroxyephedrine
0.81 Tyramine
2.00 Hordenine
2.24 Hydroxyamfetamine
3.60 Phenethylamine
3.87 Norephedrine
3.90 Phenylpropanolamine
4.39 Cathine
5.68 Ephedrine
5.90 Pseudoephedrine

5.91 Phenelzine
8.48 Amfetamine
10.52 Metamfetamine
11.08 Dimethylamfetamine
14.95 Methoxyamfetamine
16.82 Mescaline
19.46 Phentermine
29.99 Prazepam
32.17 Methoxyphenamine

System HBA

15.7 Clarithromycin
17.1 Roxithromycin
20.7 Azithromycin

System HBB

6.8 Clarithromycin
9.6 Azithromycin
16.3 Roxithromycin

System HBC

2.1 Amfetamine
2.42 Methylenedioxymetamfetamine
2.9 Diamorphine
5.2 Cocaine

System HBD

3.7 Amfetamine
5.31 Methylenedioxymetamfetamine

System HBE

5.3 Lysergide

System HBF

3.20 Psilocybine

System HBG

3.5 GHB
3.5 γ-Hydroxybutyrate-TMS_2
4.0 GBL

System HBH

1.63 Bromazepam
2.92 Clonazepam
3.22 Nitrazepam
3.34 Flunitrazepam
3.35 Alprazolam
4.14 Clobazam
5.16 Lorazepam
5.18 Triazolam
5.42 Oxazepam
6.68 Chlordiazepoxide
6.80 Temazepam
7.19 Lormetazepam
8.22 Clorazepic Acid
8.97 Nordazepam
10.4 Diazepam
10.78 Ketazolam
12.98 Flurazepam
41.46 Medazepam

System HBI

0.79 Clonazepam
0.80 Bromazepam
0.86 Flunitrazepam
0.97 Nitrazepam
1.09 Clobazam
1.13 Triazolam
1.14 Lorazepam
1.25 Oxazepam
1.28 Alprazolam
1.46 Lormetazepam
1.49 Temazepam
1.65 Chlordiazepoxide
1.84 Clorazepic Acid
1.89 Nordazepam
2.22 Ketazolam
2.29 Diazepam
3.12 Flurazepam
4.28 Prazepam
6.31 Medazepam

System HC

0.05 Buprenorphine
0.09 Dextromoramide
0.1 Pemoline
0.10 Phenoperidine
0.1 Piritramide
0.15 Benzfetamine
0.15 Noscapine
0.16 Diethylpropion
0.16 Papaverine
0.17 Naloxone
0.19 Dextropropoxyphene
0.20 Mazindol
0.26 Caffeine
0.26 Tranylcypromine
0.27 Fenetylline
0.29 Nalorphine
0.3 Phenazocine
0.3 Phendimetrazine
0.35 Norpipanone
0.36 Methylphenidate
0.37 Phenelzine
0.53 Normethadone
0.55 Pethidine
0.63 Adrenaline
0.66 Diamorphine
0.67 Pentazocine
0.69 Pipradrol
0.70 Norephedrine
0.7 Phenylpropanolamine
0.72 Fencamfamin

0.78	Acetylcodeine
0.80	6-Monoacetylmorphine
0.80	6-Monoacetylmorphine
0.82	Chlorphentermine
0.83	Cathine
0.85	Oxycodone
0.85	Thebacon
0.86	Phentermine
0.88	Fenfluramine
0.9	Thebaine
0.98	Amfetamine
0.98	Methylenedioxyamfetamine
1.03	Benzylmorphine
1.03	Methadone
1.06	Ethylmorphine
1.08	Normetadrenaline
1.11	Etorphine
1.11	Fentanyl
1.11	Hydroxyamfetamine
1.13	DOM
1.21	Codeine
1.30	Morphine
1.30	Morphine
1.3	Phenethylamine
1.3	Prolintane
1.46	Levallorphan
1.47	Tyramine
1.48	Trimethoxyamfetamine
1.55	Ethoheptazine
1.56	Morphine 3-glucuronide
1.6	Phenylephrine
1.61	Dipipanone
1.63	Pholcodine
1.77	Pseudoephedrine
1.79	Ephedrine
1.79	Ephedrine
1.83	Methylephedrine
1.89	Dimethylamfetamine
2.04	Norpethidine
2.07	Metamfetamine
2.17	Hydrocodone
2.17	Mescaline
2.48	Mephentermine
2.50	Dihydrocodeine
2.75	Dihydromorphine
3.20	Levorphanol
3.51	Nor-codeine
3.51	Norcodeine
3.92	Normorphine

System HD

0.1	Paracetamol
0.1	Dipyrone
0.1	Nifenazone
0.1	Phenazone
0.2	Aminophenazone
0.4	Morazone
0.4	Salicylamide
0.5	Acetanilide
0.5	Aspirin
0.5	Etenzamide
0.6	Phenacetin
0.6	Piroxicam
0.7	Salicylic Acid
0.7	Benorilate
0.7	Choline Salicylate
1.2	Indoprofen
1.25	Sulindac
1.3	Propyphenazone
1.95	Bufexamac
1.95	Oxyphenbutazone
2.05	Tolmetin
2.4	Ketoprofen
2.5	Famprofazone
2.6	Alclofenac
3.3	Naproxen
3.6	Salsalate
3.7	Zomepirac
3.9	Methyl Salicylate
4.0	Fenbufen
4.1	Diflunisal
6.5	Phenylbutazone
6.95	Indometacin
7.9	Fenoprofen
11.3	Benoxaprofen
11.5	Diclofenac
15.1	Ibuprofen
15.6	Salol
19.7	Flufenamic Acid
21.1	Mefenamic Acid

System HE

0.91	Ethosuximide
1.35	Primidone
1.57	Sultiame
2.76	Phenobarbital
2.76	Primidone
2.81	Ethotoin
6.02	Mesuximide
6.8	Pheneturide
7.97	Glutethimide
8.30	Carbamazepine
9.71	Phenytoin

System HF

0.42	Nomifensine
0.50	Dibenzepin
0.67	Naproxen
0.67	Zimeldine
1.33	Oxypertine
1.63	Noxiptiline
1.63	Opipramol
2.27	Doxepin
2.70	Viloxazine
3.60	Desipramine
3.60	Dosulepin
3.60	Protriptyline
4.17	Imipramine
4.58	Nortriptyline
4.92	Maprotiline
5.42	Amitriptyline
6.17	Trimipramine
7.33	Butriptyline
9.92	Clomipramine
10.83	Iprindole

System HG

1.11	Barbital
1.50	Phenylmethylbarbituric Acid
2.46	Allobarbital
2.69	Metharbital
3.09	Brallobarbital
3.09	Phenobarbital
3.42	Aprobarbital
4.01	Ibomal
4.83	Vinbarbital
4.90	Secbutabarbital
5.25	Cyclobarbital
5.43	Butobarbital
6.00	Cyclopentobarbital
6.17	Butalbital
7.20	Talbutal
7.27	Methylphenobarbital
7.37	Hexobarbital
8.12	Idobutal
8.65	Enallylpropymal
9.90	Heptabarb
10.22	Nealbarbital
10.91	Amobarbital
10.96	Pentobarbital
16.28	Secobarbital
27.61	Methohexital
34.28	Hexethal

System HH

0.63	Barbital
0.9	Phenylmethylbarbituric Acid
1.23	Phenobarbital
1.33	Allobarbital
1.72	Brallobarbital
1.99	Metharbital
2.22	Aprobarbital
2.32	Vinbarbital
2.58	Ibomal
2.61	Cyclobarbital
3.30	Secbutabarbital
3.42	Butobarbital
3.48	Butalbital
3.84	Cyclopentobarbital
3.84	Methylphenobarbital
4.70	Talbutal
4.77	Idobutal
4.93	Heptabarb

5.67 Hexobarbital
6.19 Nealbarbital
6.96 Enallylpropymal
7.05 Amobarbital
8.07 Pentobarbital
11.47 Secobarbital
20.39 Hexethal
20.48 Methohexital

System HI

0.46 7-Aminonitrazepam
0.68 7-Acetamidonitrazepam
1.17 Clorazepic Acid
2.32 Bromazepam
2.42 Demoxepam
2.85 Clonazepam
2.96 Nitrazepam
3.06 Norclobazam
3.15 Flunitrazepam
3.91 Clobazam
4.27 OH-ethylflurazepam
4.38 Triazolam
4.47 Norchlordiazepoxide
4.60 Lorazepam
4.62 Oxazepam
4.70 Alprazolam
5.19 Desalkylflurazepam
5.68 Temazepam
6.32 Lormetazepam
6.41 Chlordiazepoxide
8.00 Nordiazepam
8.00 Nordazepam
9.47 Diazepam
9.75 Midazolam
12.81 Ketazolam

System HJ

2.10 Midazolam
2.29 Diazepam
2.45 Ketazolam
3.19 Flurazepam
4.60 Prazepam
7.05 Medazepam

System HK

0.00 7-Aminonitrazepam
0.01 Norclobazam
0.03 Demoxepam
0.03 Clobazam
0.04 Ketazolam
0.08 Lormetazepam
0.14 Lorazepam
0.35 Clonazepam
0.47 Flunitrazepam
0.60 Temazepam
0.73 Oxazepam
1.43 2-OH-ethylflurazepam
1.49 Nitrazepam
1.52 Desalkylflurazepam
1.83 Triazolam
1.93 7-Acetamidonitrazepam
1.99 Nordiazepam
1.99 Nordazepam
2.00 Clorazepic Acid
2.19 Prazepam
2.39 Norchlordiazepoxide
2.49 Diazepam
2.79 Alprazolam
2.87 Chlordiazepoxide
2.99 Bromazepam
4.44 Medazepam
5.90 Midazolam
6.50 Flurazepamflurazepam

System HL

7.47 CBD
7.47 Cannabivarin
8.18 Cannabigerol
8.18 Tetrahydrocannabivarin
8.76 Cannabidiolic Acid
11.77 CBN
13.35 Δ^9-THC
14.07 Δ^8-THC
14.64 Tetrahydrocannabivaric Acid
14.78 Cannabicyclol
19.09 Cannabichromene
25.83 Tetrahydrocannabinolic Acid

System HM

5.40 Digitoxin
11.3 Digoxin
39.5 Lanatoside C

System HN

0.54 Chlorothiazide
0.67 Quinethazone
0.70 Hydrochlorothiazide
1.28 Chlortalidone
1.30 Hydroflumethiazide
3.10 Trichlormethiazide
3.82 Methyclothiazide
4.01 Clopamide
4.89 Metolazone
7.26 Clorexolone
8.67 Mefruside
9.32 Benzthiazide
10.78 Cyclothiazide
11.91 Cyclothiazide
12.81 Cyclothiazide
15.09 Polythiazide
15.35 Bendroflumethiazide
16.45 Cyclopenthiazide

System HP

0.00 Lysergic Acid
0.33 Lysergamide
0.50 Ergometrine
0.83 Methylergometrine
0.92 2-Oxylysergide
1.83 LSD
2.33 Methysergide
9.58 Ergotamine
10.2 Ergocornine
11.4 Dihydroergotamine
15.2 Ergocryptine
15.9 Codergocrine Mesilate
17.3 Ergocristine
18.3 Codergocrine Mesilate
44.3 Bromocriptine

System HPLC 27

2.37 7-Acetamidoclonazepam
3.43 Clonazepam

System HQ

0.00	Procaine	1.38	Proxymetacaine	8.97	Butacaine
0.24	Chloroprocaine	2.68	Cocaine	16.25	Oxybuprocaine
0.79	Lidocaine	4.42	Butanilicaine	16.25	Tetracaine
1.09	Mepivacaine	4.59	Piperocaine	20.06	Benzocaine
1.10	Propoxycaine	5.68	Benzoylecgonine		
1.38	Prilocaine	7.19	Bupivacaine		

System HR

0.86	Bupivacaine	2.48	Diperodon	5.51	Cinchocaine
0.86	Oxybuprocaine	2.48	Pramocaine	10.31	Cyclomethycaine
1.33	Tetracaine	2.78	Dyclonine	11.24	Quinisocaine
1.61	Benzocaine	4.14	Oxetacaine		

System HS

0.01	Noscapine	0.50	Acetylcodeine	2.02	Quinine
0.04	Papaverine	0.80	Thebaine	2.43	Strychnine
0.21	Caffeine	1.00	6-Monoacetylmorphine	5.16	Morphine
0.35	Diamorphine	1.90	Codeine	5.16	Morphine

System HT

2.4	Cortisone	4.2	Beclometasone	7.5	Methylprednisolone
2.5	Triamcinolone	4.8	Dexamethasone	8.4	Prednisolone
3.4	Prednisone	5.8	Hydrocortisone		

System HU

3.3	Sulfachlorpyridazine	7.1	Sulfadimidine	9.6	*N*-Acetylsulfanilamide
3.8	Sulfapyridine	7.5	Sulfamethoxypyridazine	12.6	Sulfamoxole
4.4	Sulfadoxine	7.7	Sulfacetamide	13.4	Sulfathiazole
4.8	Sulfamethoxazole	8.1	Sulfamerazine	14.0	Phthalylsulfathiazole
4.8	Sulfaquinoxaline	8.2	Sulfametoxydiazine	16.8	Succinylsulfathiazole
4.9	*N*-Acetylsulfamethoxazole	8.7	Sulfadiazine		
6.0	Sulfafurazole	8.9	Sulfanilamide		

System HV

0.45	Furosemide	0.77	Diflunisal	0.92	Feprazone
0.52	Indoprofen	0.78	Sulindac	0.93	Niflumic Acid
0.60	Tolmetin	0.81	Fenbufen	0.95	Mefenamic Acid
0.61	Alclofenac	0.85	Diclofenac	0.95	Phenylbutazone
0.66	Ketoprofen	0.87	Clonixin	0.98	Benoxaprofen
0.69	Oxyphenbutazone	0.87	Indometacin	0.99	Flunixin
0.69	Salsalate	0.89	Flurbiprofen	0.99	Tolmetin
0.70	Oxyphenbutazone	0.91	Fenclofenac	1.00	Flufenamic Acid

System HW

0.30	Paracetamol	2.05	Morazone	4.60	Etenzamide
0.32	Paracetamol	2.30	Acetanilide	4.80	Choline Salicylate
0.32	Aminophenazone	2.50	Salicylamide	7.70	Piroxicam
0.45	Dipyrone	2.70	Aspirin	11.0	Propyphenazone
0.45	Nifenazone	4.40	Phenacetin	22.4	Benorilate
0.95	Phenazone	4.60	Salicylic Acid		

System HX

00	Diquat	56	Decamethonium	72	Flucytosine
00	Paraquat	60	Metformin	79	Arecoline
09	Thiamine Hydrochloride	65	Levodopa	79	Isoprenaline
30	Adrenaline	65	Pholcodine	80	Phenylephrine
35	Benserazide	65	Pyridostigmine Bromide	84	Metaraminol
39	Dihydralazine	65	Pyridoxine	118	Etilefrine
48	Pentolonium Tartrate	69	Methyldopa	149	Acetylcysteine
55	Nicotinyl Alcohol	69	Nicotine	150	Rimiterol
56	Cetrimide	70	Fluorouracil	151	Orciprenaline

377 Clopamide
377 Dextromethorphan
377 Methylpiperidyl Benzilate
377 Opipramol
377 Phenacetin
377 Piritramide
377 Propranolol
377 Secbutabarbital
378 Alfentanil
378 Trazodone
379 Clidinium Bromide
379 Epirubicin
379 Ibomal
379 Isopropamide Iodide
379 Metipranolol
379 Phenobarbital
379 Proguanil
379 Vinbarbital
380 Benzoctamine
381 Tertatolol
382 Benactyzine
382 Colchicine
383 Antazoline
383 Astemizole
384 Butobarbital
384 Cyclobarbital
385 Droperidol
385 Pipazetate
386 Betaxolol
386 Trimetaphan Camsilate
387 Chlorphenesin Carbamate
387 Flumazenil
388 Loprazolam
388 Prothipendyl
388 Triprolidine
389 Tetracaine
390 Butetamate
390 Chloramphenicol
390 Isothipendyl
391 Cyclopentobarbital
391 Mianserin
392 Butacaine
392 Isocarboxazid
393 Benperidol
393 Diphenhydramine
393 Dipyridamole
393 Dinormetaclazepam
394 Butalbital
395 Clomethiazole
396 Apronal
396 Oxcarbazepine
396 Phenoxybenzamine
397 Bromazepam
397 Buprenorphine
397 Chlorzoxazone
397 Flurazepam
397 Phenindamine
398 Amoxapine
398 Chlorhexidine
399 Midazolam
401 Diphenylpyraline
401 Prednisolone
402 Oxypertine
403 Citalopram
403 Doxazosin
403 Hydrocortisone
403 Perazine
403 Talbutal
404 Benzocaine
404 Doxepin
405 Cyclizine
405 Medazepam
405 Oxybuprocaine
406 Procyclidine
407 Loxapine
407 Promazine
409 Phenazocine
409 Promethazine
410 Carbromal
410 Idarubicin
410 Pericyazine
411 Dihydroergotamine
411 Mebhydrolin
413 Xylometazoline
415 Phenyltoloxamine
415 Pramocaine
416 Demoxepam
416 Ergotamine
416 Heptabarb
417 Bromhexine
417 Nealbarbital
418 Carbamazepine
418 Methanthelinium Bromide
418 Orphenadrine
418 Protriptyline
419 Carfenazine
419 (M3)-hydroxyclenbuterol
419 Flecainide
419 Hexobarbital
420 Alimemazine
420 Cilazapril
420 Clemizole
420 Emepronium Bromide
421 Haloperidol
421 Sulforidazine
422 Adiphenine
422 Denatonium Benzoate
423 Fluanisone
424 Amobarbital
424 Desipramine
424 Metomidate
424 Oxyphencyclimine
424 Oxyphenonium Bromide
424 Pentobarbital
424 Vinylbital
425 Lorcainide
425 Normetaclazepam
426 Methylprednisolone
426 Paroxetine
427 Aminopromazine
427 Thioproperazine
428 Amlodipine
428 Dosulepin
428 Perphenazine
429 Acecarbromal
429 Piperidolate
429 Trihexyphenidyl
430 Fluvoxamine
431 Cisapride
431 Levocabastine
431 Phenytoin
431 Pipotiazine
431 Piroxicam
432 Aconitine
432 Nifuratel
433 Aprindine
433 Sulfasalazine
434 Phenoperidine
435 Furosemide
435 Levomepromazine
435 Metharbital
435 Methylphenobarbital
435 Pizotifen
436 Glutethimide
436 Phenolphthalein
437 Hydroxyzine
437 Imipramine
437 Secobarbital
438 Maprotiline
438 Mebendazole
438 Triamcinolone
439 Ketoconazole
440 Amitriptyline
440 Dextromoramide
440 Methadone
440 Propiomazine
441 Oxazepam
441 Propyphenazone
442 Tiotixene
443 Pecazine
444 Beclometasone
444 Bromazine
444 Lorazepam
444 Profenamine
446 5-Hydroxypiroxicam
447 Verapamil
448 Clopenthixol
448 Mebeverine
448 Mepyramine
448 Nitrazepam
450 Chlorpropamide
450 Prochlorperazine
451 Metaclazepam
451 Metixene
452 Tolazamide
454 Propantheline Bromide
454 Trimipramine
456 Bromocriptine
456 Chlorpromazine
457 Epithiazide
457 Methoxsalen
459 Chlorprothixene
459 Methaqualone
459 Mupirocin
459 Trifluperidol
460 Sertraline
462 Clomipramine
462 Fluphenazine
464 Butaperazine
465 Clonazepam
466 Norpipanone
467 Reserpine
468 Bamipine
470 Alprazolam
470 Fluorometholone
470 Nordazepam
470 Tolmetin
471 Deptropine
472 Temazepam
473 Propyl Hydroxybenzoate
475 Clorazepic Acid
475 Etomidate
475 Flupentixol
476 Triazolam
477 Pyrrobutamine
477 Tolbutamide
478 Glipizide
480 Methyl Salicylate
480 Thialbarbital
480 Trifluoperazine
482 Mecloqualone
483 Flunitrazepam
483 Thiopropazate
484 Brotizolam
484 Tiaprofenic Acid
484 Triflupromazine
485 Thiopental
487 Lormetazepam
487 Thiethylperazine
488 Clobazam
488 Sulindac
488 Xipamide
490 Thioridazine
495 Ketoprofen
496 Rescinnamine
499 Xanthanoic Acid
500 Dipipanone
501 Clemastine
501 Naproxen
501 Oxyphenbutazone
503 Methohexital
504 Pimozide
508 Bendroflumethiazide
508 Diflunisal
510 Loperamide
511 Tetrazepam
513 Bezafibrate
516 Thiamylal
520 Fenbufen
521 Etacrynic Acid
523 Loratadine
526 Econazole
526 Probenecid
527 Nifedipine
528 Diazepam
530 Lidoflazine
531 Bisacodyl
534 Testosterone
536 Gliclazide
536 Norethisterone
538 Fluspirilene
539 Ethinylestradiol
544 Estrone
546 Warfarin
549 Etynodiol Diacetate

560 Cinnarizine
561 Captodiame
563 Acenocoumarol
564 Bezitramide
574 Fenoprofen
577 Ketazolam
579 Hexylresorcinol
585 Flurbiprofen
587 Meclozine
592 Diethylstilbestrol
592 Spironolactone
595 Niflumic Acid
599 Dicoumarol
607 Indometacin
616 Diclofenac
616 Ibuprofen
616 Phenprocoumon
618 Dimpylate
618 Desbutylhalofantrine
618 Hexestrol
625 Nitrendipine
632 Isradipine
637 Glibenclamide
648 Prazepam
653 Meclofenamic Acid
659 Penfluridol
661 Mefenamic Acid
663 Pentaerithrityl Tetranitrate
665 Phenothiazine
668 Nimodipine
671 Flufenamic Acid
672 Phenylbutazone
672 Progesterone
683 Amiodarone
690 Felodipine
690 Nisoldipine
690 Tolfenamic Acid
693 Stanozolol
723 Niclosamide
725 Itraconazole
741 Disulfiram
800 Halofantrine
809 Metenolone
814 Simvastatin
860 Benzbromarone
896 Estradiol
900 Lacidipine
900 Methoxychlor
936 Hexachlorophene
939 Isotretinoin
990 CBD
1017 Chlordane
1054 Hydroxyprogesterone
1072 Heptachlor
1080 CBD

System HY

21 Noradrenaline
21 Thiamine hydrochloride
52 Ascorbic Acid
66 *m*-Aminobenzoic Acid
77 Tyramine
79 Phenylephrine
86 Sulfanilamide
92 Pholcodine
92 Sulfaguanidine
95 Suprofen
96 Fluorouracil
128 Allopurinol
131 Orciprenaline
132 Hydralazine
133 Normorphine
142 Etilefrine
156 Dihydromorphine
158 Pilocarpine
160 Procainamide
166 Hydroxyamfetamine
175 Ranitidine
176 Acetylcysteine
178 Nizatidine
179 Tolazoline
181 Bufotenine
182 Dimenhydrinate
182 Morphine
184 Oxymorphone
185 Psilocybine
187 Hydromorphone
190 Amiloride
190 Carbidopa
190 Tryptamine
191 Famotidine
192 Diethylcarbamazine
194 Atenolol
194 Clonidine
194 Dipyrone
196 Tranylcypromine
197 Methapyrilene
201 Phenylpropanolamine
201 Theobromine
203 Pargyline
204 Aminophenazone
206 Pheniramine
208 Dihydrocodeine
208 Tocainide
209 Cinchonine
211 Resorcinol
212 *p*-Aminobenzoic Acid
214 Cinchonidine
216 Metamfetamine
218 Phendimetrazine
222 Tetramisole
223 Homatropine
225 Procaine
226 Acetazolamide
226 Amoxicillin
226 Cimetidine
226 Fenproporex
226 Metronidazole
226 Psilocin
227 Diprophylline
227 Ephedrine
228 Dimethyltryptamine
228 Methoxamine
228 Methoxyamfetamine
230 Diethylpropion
230 Pseudoephedrine
231 Hydrocodone
232 3,4,5-TMA
234 Saccharin
234 Sulfadiazine
234 Tiabendazole
235 *o*-Aminobenzoic Acid
235 Norcodeine
235 Sulpiride
236 Benzoylecgonine
236 Lysergic Acid
237 Codeine
237 Nalorphine
238 Levamisole
238 Naloxone
238 Salbutamol
239 Chlorothiazide
239 Nikethamide
240 Minocycline
240 Physostigmine
241 Paracetamol
241 Phenmetrazine
241 Pipamperone
241 Sulfacetamide
242 Hexobarbital
242 Sulfapyridine
243 Mescaline
244 Ethylmorphine
245 Debrisoquine
245 Ketobemidone
245 Phentermine
245 Quinidine
246 Chloroquine
246 Fenoterol
246 Isoniazid
246 Mephentermine
246 Oxycodone
246 Quinine
247 Sulfamerazine
248 Methylenedioxyamfetamine
249 Nadolol
249 Theophylline
250 Ampicillin
250 Chloroprocaine
250 Lisinopril
250 Timolol
251 Atropine
252 Methylenedioxymetamfetamine
253 Hyoscine
253 Pindolol
254 Trimethoprim
255 Hydrochlorothiazide
256 Apomorphine
257 Mepyramine
257 Strychnine
257 Sulfadimidine
257 Tubocurarine Chloride
258 Barbital
258 Lidocaine
259 Caffeine
259 Doxylamine
260 Ciprofloxacin
260 Mepivacaine
260 Ofloxacin
260 Oxytetracycline Dihydrate
262 Guaifenesin
262 Ketamine
263 Metoclopramide
263 Naphazoline
263 Triamterene
264 Chlorphenamine
265 Levorphanol
265 Methazolamide
265 Tetracycline
265 Tripelennamine
266 Phenacemide
267 Brompheniramine
267 Brucine
267 Tramadol
269 Meptazinol
269 Proxymetacaine
269 Zopiclone
270 Triprolidine
270 Zimeldine
271 Harmine
271 Pemoline
272 Lamotrigine
272 Metoprolol
273 Demeclocycline
273 Viloxazine
274 Pentoxifylline
275 Bretylium Tosilate
275 Sultiame
276 Diethyltryptamine
276 Ergocalciferol
276 Ethosuximide
276 Glafenine
276 Primaquine
276 Thebaine
277 Ajmaline
277 Fenetylline
277 Methylphenidate
278 Mexiletine
279 Yohimbine
280 Butanilicaine
280 Chlortetracycline
280 Hydrastine
281 Acetanilide
281 Disopyramide
281 Pethidine
282 Chlortetracycline
282 Clenbuterol
282 Diamorphine
283 Captopril
284 Clozapine

449 Methyl Salicylate
451 Hexethal
452 Tiaprofenic Acid
453 Cyclopenthiazide
454 Buclizine
454 Triflupromazine
455 Chlorocresol
455 Clobazam
456 Estradiol
458 Propyl Hydroxybenzoate
459 Oxyphenbutazone
461 Fenbufen
462 Sulindac
463 Lormetazepam
464 Ketoconazole
464 Nifedipine
468 Digitoxin
468 Naproxen
469 Flucloxacillin
471 Fluphenazine
474 Sulfinpyrazone
476 Amiodarone
476 Thiamylal
483 Butyl Aminobenzoate
483 Gliclazide
483 Tetrazepam
484 Methohexital
488 Griseofulvin
491 Fluocinolone Acetonide
495 Zomepirac
497 Etacrynic Acid
499 Glyceryl Trinitrate
500 Diloxanide
506 Camazepam
506 Domiphen
506 Linuron
507 Probenecid
508 Testosterone
511 Chloroxylenol
513 Azinphos-(Me)
514 Warfarin
521 Estrone
522 Ketazolam
524 Fenoprofen
528 Hexylresorcinol
530 Niflumic Acid
535 Dienestrol
539 Spironolactone
544 Thymol
554 Nitrendipine
558 Naphthalene
559 Diethylstilbestrol
562 Metolachlor
570 Prazepam
571 Glibenclamide
575 Dicycloverine
583 Diflunisal
584 Betamethasone Valerate
584 Nimodipine
587 Methyltestosterone
590 Indometacin
591 Dichlorophen
592 Diclofenac
598 Coumatetralyl
598 Ibuprofen
603 Dantron
628 Dimpylate
636 Clobetasol Propionate
637 Phenothiazine
643 Phenylbutazone
656 Penfluridol
667 Flufenamic Acid
667 Gliquidone
676 Norethisterone
680 9-Carboxy-11-nor-9-tetrahydrocannabinol
686 Mefenamic Acid
690 Meclofenamic Acid
691 Disulfoton
698 Progesterone
703 Dithranol
711 Beclometasone dipropionate
711 Danazol
734 Disulfiram
738 Trichlorfon
766 Quazepam
824 Carbenoxolone
867 Tolnaftate
894 Testosterone Acetate
902 CBD
1003 Testosterone Propionate
1028 CBN

11 System HZ

1.4 Dipyrone
1.4 Methyldopa
1.5 Aminosalicylic Acid
1.5 Ascorbic Acid
1.5 Enalapril
1.5 Flucytosine
1.5 Folic Acid
1.5 Lisinopril
1.55 Allopurinol
1.57 Acetylcysteine
1.6 Isoniazid
1.6 Perindoprilat
1.6 Theobromine
1.7 Atenolol
1.7 Benzoylecgonine
1.7 Cilazapril
1.7 Dopamine
1.7 Metformin
1.7 Theophylline
1.71 Cotinine
1.73 Nicotine
1.8 Cimetidine
1.8 Ciprofloxacin
1.8 Famotidine
1.8 Morphine
1.8 Olanzapine
1.8 *N*-Acetylprocainamide
1.8 Pyridostigmine Bromide
1.8 Ranitidine
1.8 Zolmitriptan
1.9 Acetazolamide
1.9 Caffeine
1.9 Carbamazepine epoxide
1.9 Codeine
1.9 Hydralazine
1.9 Hydroxychloroquine
1.9 Paracetamol
1.9 Procainamide
1.9 Sulfapyridine
1.9 Sulfasalazine
2.0 Azapropazone
2.0 Baclofen
2.0 Dihydrocodeine
2.0 Naloxone
2.0 Ofloxacin
2.0 Sotalol
2.0 Sulpiride
2.0 Tranylcypromine
2.1 Aminophenazone
2.1 Captopril
2.1 Chloroquine
2.1 Chlorothiazide
2.1 Colchicine
2.1 Ephedrine
2.1 Methylenedioxyamfetamine
2.1 Methylergometrine
2.1 Naratriptan
2.1 Pentoxifylline
2.1 Phenazone
2.1 Primidone
2.1 Timolol
2.1 Tocainide
2.1 Trandolaprilat
2.1 Trimethoprim
2.2 Atropine
2.2 Barbital
2.2 *O*-Desmethylvenlafaxine
2.2 Hydrochlorothiazide
2.2 Methylenedioxymetamfetamine
2.2 Nicorandil
2.2 10-OH-Oxcarbazepine
2.2 Physostigmine
2.2 Strychnine
2.2 Sulfapyridine
2.2 Terazosin
2.25 Acebutolol
2.3 Chlortalidone
2.3 Ethosuximide
2.3 Glafenine
2.3 Lamotrigine
2.3 Desmethylmirtazapine
2.3 Rivastigmine
2.3 Sulfamethizole
2.3 Tizanidine
2.3 Triamterene
2.3 Zopiclone
2.4 Alfuzosin
2.4 Ketamine
2.4 Meprobamate
2.4 Metamfetamine
2.4 Methysergide
2.4 Minoxidil
2.4 Moclobemide
2.4 Nitrofurantoin
2.4 Phentermine
2.4 Pindolol
2.4 Succinimide
2.5 Aminoglutethimide
2.5 Celiprolol
2.5 Clonidine
2.5 Metoprolol
2.5 Prazosin
2.5 Prednisolone
2.6 Di-OH-Carbamazepine
2.6 Crotetamide
2.6 Dapsone
2.6 Flumazenil
2.6 Lidocaine
2.6 Mepivacaine
2.6 Metoclopramide
2.6 Prednisone
2.6 Quinidine
2.6 Quinine
2.6 Yohimbine
2.7 Allobarbital
2.7 Aspirin
2.7 Pipamperone
2.7 Prilocaine
2.8 Aprobarbital
2.8 Diethylpropion
2.8 Dimethoate
2.8 Hydroquinine
2.8 Mirtazapine
2.8 Omeprazole
2.8 9-Hydroxy-risperidone
2.9 Bromisoval
2.9 Ondansetron
2.9 Oxcarbazepine
2.9 Sulfamethoxazole
2.9 Tramadol
3.0 Benzoic Acid
3.0 Brallobarbital
3.0 Bromazepam
3.0 Dipyridamole
3.0 Disopyramide
3.0 Labetalol
3.0 Oxprenolol
3.0 Phenacetin
3.0 Phenobarbital
3.0 Phentolamine
3.0 Remoxipride
3.1 Bisoprolol
3.1 Demoxepam
3.1 Risperidone
3.1 Venlafaxine
3.2 Butobarbital
3.2 Chlordiazepoxide

3.2 Cyclobarbital
3.2 Cyclopentolate
3.2 Doxapram
3.2 Methyl Hydroxybenzoate
3.2 Pethidine
3.2 Zolpidem
3.3 Cocaine
3.3 Cropropamide
3.3 Trazodone
3.4 Butalbital
3.4 Dexamethasone
3.4 Dihydroergotamine
3.4 Ergotamine
3.4 Mesoridazine
3.5 Buspirone
3.5 Carbamazepine
3.5 Chlorphenamine
3.5 Droperidol
3.5 Furosemide
3.5 Metipranolol
3.6 Aldicarb
3.6 Benperidol
3.6 Cetirizine
3.6 Mazindol
3.7 Desmethylcitalopram
3.7 Mephenytoin
3.7 Paraoxon
3.7 Phenytoin
3.7 Propranolol
3.8 Betaxolol
3.8 Oxazepam
3.8 Pentazocine
3.8 Proguanil
3.87 Alprenolol
3.9 Astemizole
3.9 Carbromal
3.9 Clozapine
3.9 Fenfluramine
3.9 Heptabarb
3.9 Opipramol
3.9 Sulindac
4.0 Alprazolam
4.0 Amobarbital
4.0 Dantrolene
4.1 Bupivacaine
4.1 Ketorolac
4.1 Lorazepam
4.1 Pentobarbital
4.1 Vinylbital
4.2 Bevantolol
4.2 Flurazepam
4.2 Midazolam
4.2 Nitrazepam
4.2 Phenolphthalein
4.2 Ramipril
4.2 Triazolam
4.3 Benzocaine
4.3 Hexobarbital
4.4 Carvedilol
4.4 Floctafenine
4.4 Pericyazine
4.4 Tetrabenazine
4.4 Tetracaine
4.5 Cilazapril
4.5 Citalopram
4.5 Diltiazem
4.5 Glipizide
4.6 Brotizolam
4.6 Clonazepam
4.6 Desmethyldoxepin
4.6 Methylphenobarbital
4.6 Mianserin
4.7 Propyphenazone
4.7 Secobarbital
4.7 Sulfinpyrazone
4.8 Cyclizine
4.8 Glutethimide
4.8 Mesuximide
4.8 Sulforidazine
4.9 Amlodipine
4.9 Piroxicam
5.0 Buprenorphine
5.0 Chlorpropamide
5.0 Doxepin
5.2 Carvedilol
5.2 Cisapride
5.2 Flecainide
5.2 Ketoconazole
5.2 Methoxsalen
5.3 Mebhydrolin
5.3 Tofenacin
5.4 Diflunisal
5.4 Malathion
5.4 Medazepam
5.4 Methaqualone
5.4 Pergolide
5.4 Quinapril
5.4 Tolmetin
5.5 Temazepam
5.6 Clorazepic Acid
5.6 Flunitrazepam
5.6 Fluvoxamine
5.6 Paroxetine
5.7 Bromocriptine
5.7 Dosulepin
5.7 Hydroxyzine
5.7 Promethazine
5.8 Diuron
5.8 Haloperidol
5.8 Tiaprofenic Acid
5.9 Desipramine
5.9 Promazine
5.9 Tolbutamide
6.0 Orphenadrine
6.1 Trandolapril
6.2 Clobazam
6.2 Lormetazepam
6.2 Procyclidine
6.3 Perazine
6.4 Biperiden
6.4 Ketoprofen
6.4 Thiazinamium Metilsulfate
6.5 Cyproheptadine
6.5 Paraoxon
6.5 Pramocaine
6.6 Lorcainide
6.6 Maprotiline
6.6 Nortriptyline
6.6 Pizotifen
6.6 Norverapamil
6.7 Desmethyl-fluoxetine
6.7 Imipramine
6.7 Oxyphenbutazone
6.7 Oxyphenbutazone
6.8 Meloxicam
6.8 Naproxen
6.8 Tiotixene
6.8 Tolazamide
6.9 Thiopental
7.0 Desmethylsertraline
7.0 Verapamil
7.1 Mebeverine
7.2 Nifedipine
7.2 Perphenazine
7.2 Sulindac
7.5 Amitriptyline
7.5 Bromhexine
7.5 Levomepromazine
7.6 Dextropropoxyphene
7.6 Fluoxetine
7.6 Trihexyphenidyl
7.7 Nefazodone
7.8 Probenecid
8.0 Valproic Acid
8.0 Zuclopenthixol
8.2 Sertraline
8.3 Trimipramine
8.4 Diazepam
8.5 Methadone
8.8 Gliclazide
9.1 Acenocoumarol
9.1 Chlorpromazine
9.4 Warfarin
9.5 Spironolactone
9.6 Bisacodyl
9.6 Camazepam
9.8 Sertindole
10.1 Chlorprothixene
10.1 Fluphenazine
10.2 Clomipramine
10.3 Deptropine
10.3 Ketazolam
10.4 Prochlorperazine
10.7 Aceclofenac
10.7 Flupentixol
10.9 Fenoprofen
11.8 Flurbiprofen
11.9 Pimozide
12.3 Triflupromazine
12.6 Phenprocoumon
13.2 Trifluoperazine
13.5 Thioridazine
13.6 Nabumetone
14.0 Clemastine
14.0 Loperamide
14.2 Betamethasone
14.4 Glibenclamide
14.4 Indometacin
14.6 Loratadine
14.8 Diclofenac
14.9 Isradipine
15.4 Thioproperazine
16.3 Thiethylperazine
16.5 Ibuprofen
17.6 Prazepam
17.7 Nimodipine
19.5 Phenylbutazone
22.0 Cinnarizine
22.5 Bezitramide
25.8 Felodipine
27.4 Diphenoxylate
33.2 Dimpylate
35.0 Propofol
35.5 Parathion
37.5 Quazepam
37.9 Tolfenamic Acid
43.4 Penfluridol
90.4 Amiodarone

System HAA

2.7 Adenosine
2.7 Pholcodine
2.8 Metformin
2.8 Noradrenaline
2.9 Pralidoxime Chloride
2.9 Pyridoxine
3.0 Acamprosate Calcium
3.0 Methyldopa
3.0 Pancuronium Bromide
3.1 Aciclovir
3.1 Amoxicillin
3.1 Arecoline
3.1 Flucytosine
3.2 Betahistine
3.2 Nicotinic Acid
3.2 Pyridostigmine Bromide
3.3 Morphine
3.3 Nizatidine
3.4 Enalapril
3.4 Fluorouracil
3.5 Famotidine
3.6 Amiloride
3.6 Atenolol
3.6 Cimetidine
3.6 Diprophylline
3.6 Folic Acid
3.6 Levodopa
3.7 Amfetamine
3.7 Ranitidine
3.7 Terbutaline

17.8 Altretamine
18.0 Carbaryl
18.1 Phenindione
18.2 Atrazine
18.2 Estradiol
18.2 Parbendazole
18.3 Bezafibrate
18.3 Clofibrate
18.3 Propyl Hydroxybenzoate
18.4 Clorazepate
18.4 Griseofulvin
18.5 Diuron
18.6 Bendroflumethiazide
18.6 Flunitrazepam
18.6 Temazepam
18.7 Digitoxin
18.8 Xipamide
18.9 Methylprednisolone
19.1 Bumetanide
19.2 Clobazam
19.2 Thiopental
19.3 Cinnarizine
19.3 Fenbufen
19.3 Fenproporex
19.5 Benzene
19.5 Nifedipine
19.6 Ketoprofen
20.0 Meclozine
20.1 Acenocoumarol
20.1 Econazole
20.2 Captodiame
20.2 Penfluridol
20.3 Diazepam
20.5 Gliclazide
20.5 Desbutylhalofantrine
20.7 Spironolactone
20.9 Diethylstilbestrol
20.9 Rifamycin SV
21.2 Ciprofibrate
21.2 Droperidol
21.2 Fenoprofen
21.3 Flurbiprofen
21.3 Linuron
21.5 Etodolac
21.6 Trifluperidol
21.7 Indometacin
21.9 Prometryne
21.9 Toluene
22.0 Glibenclamide
22.0 Niflumic Acid
22.1 Diclofenac
22.1 Nitrendipine
22.4 Chlorambucil
22.4 Isradipine
22.4 Tetrazepam
22.6 Minocycline
22.9 Loratadine
23.0 Halofantrine
23.1 Pentaerithrityl
23.3 Naphthalene
23.4 Prazepam
23.8 Ibuprofen
23.8 Progesterone
23.9 Niclosamide
24.0 Norethisterone
24.0 Xylene
24.1 Chlormadinone Acetate
24.1 Phenylbutazone
24.4 Felodipine
24.9 Fusidic Acid
25.1 Disulfiram
25.8 Dimpylate
26.1 Benzbromarone
26.4 Cyclandelate
27.2 Lacidipine
27.4 Methoxychlor
27.6 Piperonyl Butoxide

System HAB

1.0 Abacavir
4.0 Amprenavir
4.2 Indinavir

System HAC

2.0 Indinavir
2.5 Amprenavir
7.1 Ritonavir
8.5 Efavirenz
9.5 Saquinavir
17.3 Nelfinavir

System HAD

2.70 Lamivudine
3.20 Didanosine
3.80 Stavudine
6.60 Zidovudine
8.10 Abacavir
11.1 Nevirapine

System HAE

6.30 Delavirdine
8.00 Nelfinavir
9.40 Amprenavir
22.2 Ritonavir
22.2 Saquinavir
28.6 Efavirenz

System HAF

5.30 Clonazepam
6.6t Bromazepam
9.10 Nitrazepam
13.7 Triazolam
15.0 Lorazepam
18.4 Etizolam
21.0 Chlordiazepoxide
29.8 Diazepam
32.2 Flutazolam

System HAG

25.5t Bromazepam

System HAI

1.47 Alprazolam
12.03 Flurazepam

System HAK

4.7 Carbamazepine
6.2 Clonazepam
7.6 Nordazepam
9.3 Clobazam
ND Phenobarbital
ND Phenytoin

System HAL

1.4	Barbital	4.4	Oxazepam	7.8	Clobazam
1.45	7-acetamidoclonazepam	4.6	Nitrazepam	7.9	Nordazepam
1.55	7-aminoclonazepam	5.1	Lorazepam	8.1	Bromazepam
2.0	Aprobarbital	6.2	Flunitrazepam	8.2	Medazepam
2.4	Hexobarbital	6.3	Alprazolam	13.2	Diazepam
3.7	Norflunitrazepam	6.6	Triazolam		
4.3	Clonazepam	7.7	Chlordiazepoxide		

System HAM

1.8	Theophylline	4.3	Clonazepam	ND	Ephedrine
1.98	Caffeine	4.4	Oxazepam	ND	Levomepromazine
2.0	Paracetamol	4.5	Nitrazepam	ND	Lidocaine
2.2	Primidone	9.0	Imipramine	ND	Medazepam
2.7	Sulfamethoxazole	9.1	Desipramine	ND	Nortriptyline
2.8	Phenobarbital	10.3	Diazepam	ND	Propranolol
3.1	Chlordiazepoxide	ND	Alprazolam	ND	Thioridazine
3.4	Oxazepam	ND	Bromazepam	ND	Triazolam
3.5	Phenytoin	ND	Clobazam		
4.2	Lorazepam	ND	Codeine		

System HAO

0.00	Aldrin	0.22	Fenitrothion	7.63	Aldicarb
0.00	Chlordane	1.37	Mevinphos	10.75	Atrazine
0.00	Heptachlor	5.22	Dichlorvos	12.09	Terbutryne
0.10	Disulfoton	6.18	Chlorpyrifos-(Me)	99.9	Diquat Dibromide

System HAP

0.00	Diquat Dibromide	1.24	Atrazine	20.8	Heptachlor
0.45	Mevinphos	1.68	Terbutryne	33.86	Aldrin
0.69	Aldicarb	2.86	Fenitrothion	99.9	Chlordane
0.86	Dichlorvos	6.48	Disulfoton		

System HAR

0.74	Boldenone	1.25	Methandriol Dipropionate	2.60	Testosterone Enantate
0.78	Fluoxymesterone	1.76	Testosterone Acetate	2.63	Testosterone Cipionate
0.86	Methandienone	1.90	Clostebol Acetate	3.18	Testosterone Undecanoate
1.17	Methyltestosterone	2.01	Testosterone Propionate		
1.25	Methandriol	2.17	Testosterone Isobutyrate		

System HATa

1.26	Metenolone Acetate	1.65	Trenbolone Hexahydrobenzylcarbonate	2.05	Testosterone Cipionate
1.31	Testosterone Propionate	1.80	Testosterone Enantate	2.53	Testosterone Undecenoate
1.48	Testosterone Phenylpropionate	1.87	Metenolone Enantate	2.78	Testosterone Decanoate
1.62	Testosterone Isocaproate	1.94	Boldenone Undecylenate	3.27	Testosterone Undecylate

System HATb

0.70	Fluoxymesterone	1.27	Methyltestosterone	2.64	Oxymetholone
0.75	Oxandrolone	1.29	Methandriol	2.75	Methandriol Dipropionate
0.76	Boldenone	1.52	Mesterolone	3.54	Metenolone Acetate
0.84	Ethisterone	1.55	Norethandrolone	4.06	Testosterone Propionate
0.87	Methandienone	1.71	Trenbolone Acetate		
1.13	Dehydroepiandrosterone	2.59	Testosterone Acetate		

System HAV

2.2	Celiprolol	5.1	Desmethyldiltiazem	8.2	Verapamil
2.3	Propranolol	6.1	Diltiazem		
3.6	Deacetyldiltiazem	6.4	Imipramine		

System HAW

5.0	Clenbuterol
6.1	Clenbuterol

System HAX

4.1	Methyldopa
4.5	Theophylline
4.7	Methotrexate
4.8	Caffeine
4.8	Paracetamol
5.0	Methocarbamol
5.0	Pentoxifylline
5.1	Colchicine
5.1	Hydrochlorothiazide
5.2	Salicylic Acid
5.4	Phenazone
5.6	Morphine
5.6	Nizatidine
5.6	Phenobarbital
5.7	Fenoterol
5.8	Bromazepam
5.8	Furosemide
5.8	Hydromorphone
5.9	Diflunisal
5.9	Pentobarbital
5.9	Ranitidine
6.0	Chlormezanone
6.0	Dantrolene
6.0	Oxazepam
6.0	Mephenytoin
6.1	Codeine
6.1	Lorazepam
6.1	Phenytoin
6.2	Carbamazepine
6.2	Secobarbital
6.3	Ketorolac
6.3	Nitrazepam
6.4	Alprazolam
6.4	Methoxamine
6.4	Triazolam
6.5	Clonazepam
6.5	Hydralazine
6.5	Oxycodone
6.6	Dobutamine
6.6	Floctafenine
6.6	Glutethimide
6.7	Nordazepam
6.7	Triamterene
6.7	Trimethoprim
6.8	Homatropine
6.8	Methaqualone
6.9	Chlordiazepoxide
6.9	Hydrocodone
6.9	Methylenedioxyamfetamine
6.9	Moclobemide
7.0	Hyoscine
7.0	Naproxen
7.1	Metoprolol
7.2	Clobazam
7.3	Procaine
7.4	Brotizolam
7.4	Nifedipine
7.5	Strychnine
7.5	Zopiclone
7.7	Diazepam
7.7	Ketazolam
7.7	Mepivacaine
7.8	Clonidine
7.9	Diamorphine
7.9	Metoclopramide
8.0	Fenoprofen
8.0	Flurbiprofen
8.0	Lidocaine
8.0	Warfarin
8.1	Ibuprofen
8.3	Quinine
8.4	Chlorpromazine sulfoxide
8.5	Glibenclamide
8.5	Indometacin
8.6	Methylphenidate
8.7	Azacyclonol
8.7	Diclofenac
8.7	Doxapram
8.7	Quinidine
8.8	Remoxipride
8.89	Cetirizine
8.9	Temazepam
9.1	Buspirone
9.1	Naphazoline
9.1	Risperidone
9.2	Pethidine
9.2	Propranolol
9.3	Desacetyldiltiazem
9.4	Encainide
9.5	Pheniramine
9.9	Pentazocine
10.0	Cocaine
10.0	Fluvoxamine
10.1	Mesoridazine
10.1	Propofol
10.2	Midazolam
10.2	Pericyazine
10.5	Flurazepam
10.5	Prazepam
10.7	Azatadine
10.8	Chlorphenamine
10.8	Tetracaine
10.9	Cisapride
10.9	Clozapine
10.9	Loratadine
11.1	Diltiazem
11.1	Haloperidol
11.1	Paroxetine
11.3	Flavoxate
11.4	Fentanyl
11.4	Hydroxyzine
11.7	Anileridine
11.9	Quazepam
12.1	Difenidol
12.2	Diphenhydramine
12.2	Fluoxetine
12.4	Cyclizine
12.7	Chloroquine
12.9	Doxepin
13.0	Desipramine
13.1	Mefenamic Acid
13.1	Perphenazine
13.2	Promethazine
13.3	Verapamil
13.6	Fluphenazine
13.7	Flupentixol
13.7	Nortriptyline
13.8	Lovastatin
14.1	Propiomazine
14.5	Sertraline
14.7	Imipramine
14.9	Alimemazine
15.1	Diethazine
15.2	Levomepromazine
15.2	Methdilazine
15.3	Pecazine
15.5	Trimipramine
15.8	Amitriptyline
16.5	Methadone
16.6	Profenamine
17.0	Chlorpromazine
17.3	Triflupromazine
17.6	Chlorprothixene
18.3	Fluspirilene
ND	Procyclidine
ND	Thioridazine

System HAY

2.8	Methyldopa
2.9	Methotrexate
3.1	Nizatidine
3.2	Morphine
3.3	Ranitidine
3.4	Codeine
3.4	Fenoterol
3.4	Hydromorphone
3.5	Hydralazine
3.5	Theophylline
3.6	Chloroquine
3.6	Homatropine
3.6	Methoxamine
3.7	Hydrocodone
3.7	Hyoscine
3.7	Paracetamol
3.7	Trimethoprim
3.8	Caffeine
3.8	Dobutamine
3.8	Methylenedioxyamfetamine
3.8	Zopiclone
3.9	Methocarbamol
3.9	Moclobemide
3.9	Strychnine
3.9	Triamterene
4.0	Hydrochlorothiazide
4.0	Procaine
4.1	Colchicine
4.1	Diamorphine
4.1	Metoprolol
4.2	Mepivacaine
4.2	Pentoxifylline
4.3	Chlorpromazine sulfoxide
4.3	Clonidine
4.3	Metoclopramide
4.4	Salicylic Acid
4.5	Azacyclonol
4.5	Oxazepam
4.5	Lidocaine
4.5	Phenazone
4.5	Pheniramine
4.5	Quinine
4.6	Azatadine
4.6	Desacetyldiltiazem
4.6	Doxapram
4.6	Quinidine
4.6	Remoxipride
4.6	Risperidone
4.7	Methylphenidate
4.7	Phenobarbital
4.7	Procyclidine
4.8	Naphazoline
4.8	Pethidine
4.9	Encainide
4.9	Propranolol
5.0	Buspirone
5.0	Cocaine
5.0	Furosemide
5.0	Mesoridazine
5.1	Bromazepam
5.1	Pericyazine
5.2	Clozapine
5.2	Dantrolene
5.29	Cetirizine
5.3	Chlordiazepoxide
5.3	Chlormezanone

5.3	Chlorphenamine	6.2	Glutethimide	7.7	Sertraline
5.3	Phenytoin	6.2	Haloperidol	7.7	Trimipramine
5.4	Diltiazem	6.3	Desipramine	7.8	Chlorpromazine
5.4	Mephenytoin	6.3	Hydroxyzine	7.9	Brotizolam
5.4	Tetracaine	6.3	Midazolam	7.9	Nifedipine
5.5	Carbamazepine	6.3	Perphenazine	8.3	Chlorprothixene
5.5	Flurazepam	6.4	Alprazolam	8.3	Profenamine
5.5	Pentazocine	6.4	Promethazine	8.3	Warfarin
5.6	Ketorolac	6.7	Temazepam	8.4	Methadone
5.6	Pentobarbital	6.7	Methdilazine	8.8	Diazepam
5.8	Cisapride	6.7	Triazolam	8.9	Fenoprofen
5.8	Cyclizine	6.8	Nordazepam	8.9	Flurbiprofen
5.8	Floctafenine	6.8	Imipramine	8.9	Triflupromazine
5.8	Lorazepam	6.8	Nortriptyline	9.2	Indometacin
5.8	Oxycodone	7.0	Pecazine	9.2	Ketazolam
5.8	Paroxetine	7.0	Verapamil	9.8	Fluspirilene
5.9	Anileridine	7.07	Fluoxetine	9.8	Glibenclamide
5.9	Fluvoxamine	7.1	Alimemazine	9.8	Mefenamic Acid
5.9	Secobarbital	7.1	Clobazam	9.8	Thioridazine
6.0	Clonazepam	7.1	Propiomazine	10.0	Diclofenac
6.0	Diflunisal	7.2	Fluphenazine	10.5	Ibuprofen
6.0	Diphenhydramine	7.2	Levomepromazine	12.8	Prazepam
6.0	Fentanyl	7.2	Naproxen	13.3	Loratadine
6.0	Flavoxate	7.3	Amitriptyline	14.1	Diphenoxylate
6.0	Nitrazepam	7.4	Diethazine	14.8	Lovastatin
6.1	Difenidol	7.4	Methaqualone	15.2	Propofol
6.1	Doxepin	7.5	Flupentixol	17.7	Quazepam

System HAZ

0.02	Sulpiride	0.72	Haloperidol	1.62	Imipramine
0.14	4-OH-Phenobarbital	0.73	Flurazepam	1.68	Chlordiazepoxide
0.18	Caffeine	0.79	Clonazepam	1.71	Nortriptyline
0.19	OH-Mianserin	0.82	Carbamazepine	1.76	Amitriptyline
0.22	7-aminoflunitrazepam	0.86	Flunitrazepam	1.82	Levomepromazine
0.38	Carbamazepine epoxide	0.88	Normianserin	1.85	Imipramine *N*-oxide
0.39	2-OH-desipramine	0.88	Nitrazepam	1.88	Nordazepam
0.39	OH-Imipramine	1.07	Triazolam	2.25	Diazepam
0.39	Phenobarbital	1.18	Mianserin	2.64	Chlorpromazine
0.53	Mianserin *N*-oxide	1.23	Oxazepam	3.28	Perphenazine
0.60	Phenytoin	1.43	Temazepam	3.88	Thioridazine
0.62	Chlorpromazine sulfoxide	1.44	Maprotiline		
0.65	Norflunitrazepam	1.52	Desipramine		

12 Ultraviolet Absorption Data

In the following tables no values below 190 nm have been included although some have been recorded in the monographs. Details of A (1%, 1 cm) values are given in the monographs.

Table A gives the wavelengths of maximum absorption, together with the wavelengths of any subsidiary peaks in the range 230–360 nm, for the compounds in **acid** solution. Table B gives the wavelengths of maximum absorption in **alkaline** solution when values in acid solution are not recorded in the monographs, or when the values in alkaline solution differ significantly from the values in acid solution. Table C gives the values in **neutral** solution for compounds for which values in acid or alkaline solution are not recorded in the monographs, or when the values in neutral solution differ significantly from these in acid or alkaline solution.

For an unknown compound, the wavelength of maximum absorption should be compared with the tables using a tolerance of ±2 nm to give a list of possible compounds; reference may then be made to the monographs.

A. In acid solution—wavelengths of maximum absorption from 192 nm

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
192	Cetirizine	258
192	Esmolol	220, 276
194	Risperidone	236, 270
196	Moclobemide	238
196	Tizanidine	226, 316
198	Methylenedioxymetamfetamine	234.5, 282.5
202	Carbocromen	216, 321
203	Benserazide	269
203	Cisplatin	285, 301, 362
203	Dibenzepin	223
203	5-Methoxy-*N*,*N*-dimethyltryptamine	217, 275
203	Valsartan	248
204	Gemcitabine	274
204	Salsalate	235, 310
204	Saquinavir	238, 292
205	Citalopram	238
205	Methylthioamfetamine	255
205	Nevirapine	216, 280
205	Ropinirole	248
206	Diprophylline	273
207	Bendroflumethiazide	273, 325
207	Zidovudine	266, 266
207	Zolpidem	
208	Dimenhydrinate	276
208	Lamotrigine	264.5
208	Mizolastine	229, 281, 288
208	Rifabutin	237, 277, 326
208	Zuclopenthixol	231, 268, 326
209	Adenosine	259
209	Astemizole	277
210	Indinavir	259
210	Ondansetron	248, 266, 310
212	Formoterol	283
212	Lamivudine	279
212	Zalcitabine	280
213	Clotiapine	297
214	Ticlopidine	231, 268, 275
215	Biriperone	250, 286
215	Zotepine	264, 297
216	Pramipexole	264
217	Bicalutamide	272
218	Aflatoxins G	345
218	Dicycloverine	252, 280
219	Astemizole	249, 286
219	Carteolol	252, 288
220	Chlorphentermine	259, 267, 274
220	Nordefrin	280, 249
221	Irinotecan	254, 359, 372
222	Ethylisobutrazine	275
222	Pentaquin	265, 277, 331, 400
222	Pergolide	279
223	Levobunolol	209, 256, 313
223	Lucanthone	257, 331, 280
223	Topotecan	267, 295, 316
224	Amopyroquine	238, 342
224	Famciclovir	246, 310
224	Irbesartan	246
224	Naratriptan	278, 284, 295
225	Bisoprolol	273
225	Diampromide	257, 263
225	Gallamine Triethiodide	266
225	Ofloxacin	226, 256, 326
225	Rizatriptan	282
225	Tamsulosin	280
225	Venlafaxine	273, 280
226	Embramine	258
226	Fluoxetine	263, 275
226	Fomepizole	
226	Thozalinone	262, 268
226.5	Fluoxetine	
226.5	Venlafaxine	274.5
227	Chlorocresol	279
227	Decamethonium Bromide	
227	Fluoxetine	264, 268, 275
227	Paclitaxel	273
227	Sertindole	258, 302
227	Sumatriptan	277, 283, 292
228	Butalamine	
228	Butethamine	280, 275, 290
228	Butylated Hydroxyanisole	292
228	Clorgiline	282
228	Ibutilide	267
228	Metabutethamine	275, 280
228	Phthivazid	273, 307, 246, 297
228	Triamterene	360
229	Aniline	255, 261
229	Clomocycline	268
229	Dolasetron	282
229	Enalapril	
229	Tetracaine	281, 312, 227, 303
230	Amiphenazole	261
230	Amisulpride	285
230	Amoxicillin	272
230	Aspirin	278
230	Benzoic Acid	273
230	Bromazine	
230	Chloroprocaine	288
230	Chlorprothixene	268, 325
230	Chlorpyrifos	289
230	Clobazam	289
230	Dehydroemetine	283
230	Dichloralphenazone	
230	Diprenorphine	286
230	Dipyridamole	285
230	Docetaxel	275, 283
230	Donepezil	271, 316
230	Dosulepin	303
230	Emetine	282
230	Ethionamide	275
230	Etonitazene	282
230	Flupentixol	
230	Gliclazide	
230	Methysergide	322
230	Minoxidil	281
230	Nabumetone	261, 270, 333
230	Phenazone	
230	Stanozolol	
230	Thioridazine	263
230	Vanillin	278, 309
230	Zaleplon	289, 335

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
231	Aloxiprin	
231	Amicarbalide	256
231	Benperidol	249, 279
231	Celiprolol	324
231	Clopenthixol	269, 325
231	Laudexium Methyl Sulfate	280
231	Lormetazepam	311
231	Lovastatin	238, 247
231	Nifuroxime	340
231	Piperocaine	274
231	Pseudomorphine	
231	Rifampicin	263, 336
231	Trimetazidine	269
231	Xenysalate	319
232	Bunitrolol	292
232	Chlorcyclizine	
232	Chlorpropamide	
232	Clonitazene	285
232	Cloponone	
232	Cytisine	302
232	Diperodon	
232	Etisazole	323
232	Hexylcaine	275
232	Hydroxyzine	258, 263, 270
232	Meprylcaine	
232	Pericyazine	268
232	Procarbazine	
233	Benzamine	274
233	Cocaine	275
233	Doxorubicin	253, 290
233	Floctafenine	348
233	Homochlorcyclizine	258, 263, 270
233	Methylenedioxyamfetamine	285
233	Paroxetine	264, 270, 293
233	Phenisonone	284, 313
233	Picloxydine	253
234	Acebutolol	320
234	Aconitine	275
234	Aminosalicylic Acid	300
234	Benzoylecgonine	274
234	Etenzamide	293
234	Heteronium Bromide	
234	Mebendazole	288
234	Methaqualone	269
234	Methylenedioxymetamfetamine	285
234	Oxazepam	280
234	Thenalidine	
234	Tiletamine	
235	Ambucetamide	
235	Cambendazole	319
235	Cinchonine	315
235	Clomifene	292
235	Dimetotiazine	262
235	Disulfamide	276, 285
235	Fluvastatin	305
235	Furosemide	274, 342
235	Hydroxychloroquine	256, 329, 343
235	Nisoldipine	331
235	Oxybuprocaine	298
235	Propoxycaine	298
235	Rotenone	292
235	Salicylamide	298
236	Cinchonidine	316
236	Diltiazem	
236	Flurazepam	284
236	Foscarnet Sodium	
236	Nitrendipine	203, 350
236	Salicylic Acid	303
236	Thonzylamine	274, 316
236	Toremifene	
236	Zolpidem	294
237	Aminitrozole	345
237	Amodiaquine	343
237	Amydricaine	277
237	Benazepril	
237	Benzbromarone	274, 281
237	Chloropyrilene	312
237	Clorazepic Acid	287
237	Demoxepam	305
237	Felodipine	359
237	Fenyramidol	309
237	Halazone	
237	Methapyrilene	314
237	Metolazone	271, 344
237	Oxyphenbutazone	
237	Phenylbutazone	
237	Sulfinpyrazone	
237	Temazepam	284, 358
237	Zolpidem	295
237.2	Benazepril	
238	Dropropizine	
238	Halcinonide	
238	Mesoridazine	262, 310
238	Nifedipine	338
238	Norbormide	300
238	Nordazepam	283, 361
238	Penthienate Methobromide	
238	Pravastatin	
238	Ritonavir	
238	Thiamylal	287
238	Zolpidem	294.5
238	Zolpidem	296
238.5	Risperidone	274.5
239	Acetanilide	815
239	Amitriptyline	
239	Amlodipine	
239	Bromazepam	345
239	Chloropyramine	315
239	Chlorpromazine	274, 300, 341
239	Lacidipine	209, 282, 366
239	Mepyramine	316
239	Niridazole	
239	Nortriptyline	
239	Tetrazepam	284, 345
239	Thenyldiamine	315
239	Tripelennamine	314
240	Altretamine	
240	Dihydralazine	274, 306
240	Letrozole	273, 281
240	Muzolimine	
240	Octacaine	
240	Prazepam	285, 360
240	Propyphenazone	
240	Sulfapyridine	
240	Tacrine	323, 336
240	Thalidomide	300
241	Amiodarone	
241	Antazoline	291
241	Carvedilol	285, 320, 332
241	Cefaloridine	254
241	Dequalinium Chloride	330
241	Flavoxate	293, 320
241	Mexazolam	290, 373
241	Mitoxantrone	273, 608, 658
241	Physostigmine Aminoxide	300
241	Propiomazine	273, 360
242	Azaperone	312
242	Butaperazine	277
242	Cetoxime	290
242	Clenbuterol	295
242	Clopamide	
242	Diazepam	284, 366
242	Homidium Bromide	283
242	Oleandomycin	
242	Phenethylamine	247, 252, 257, 263
242	Pralidoxime Chloride	292
242	Prothipendyl	
242	Spironolactone	
242	Sulfadiazine	
242	Sulfamerazine	304
243	Acepromazine	279
243	Acetophenazine	278
243	Ascorbic Acid	
243	Carfenazine	277
243	Cinchophen	268, 344
243	Etizolam	
243	Famprofazone	279
243	Fluanisone	
243	Isometamidium	252, 280, 231, 248, 270
243	Isotretinoin	359
243	Metallibure	
243	Methacycline	340
243	Methotrexate	307
243	Nitroxinil	350

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
243	Piperacetazine	278
243	Practolol	
243	Propantheline Bromide	281
243	Sulfadimidine	301
243	Tiabendazole	302
244	Diamfenetide	
244	Dofamium Chloride	
244	Ethosuximide	
244	Menadione	249, 261, 340
244	Mitoxantrone	279, 525, 620, 660
245	Ajmaline	289
245	Aloin	253, 300
245	Bromhexine	310
245	Buclosamide	297
245	Chlorhexidine	
245	Clozapine	297
245	Haloperidol	
245	Harman	300
245	Isothipendyl	
245	Nefazodone	275
245	Paracetamol	
245	Sulfasomizole	301
245	Tolonium Chloride	285
246	Amprolium Hydrochloride	262
246	Candesartan	281
246	Chlordiazepoxide	308
246	Doxazosin	331
246	Lisinopril	253, 258, 264, 267
246	Physostigmine	302
246	Prajmalium Bitartrate	290
246	Terazosin	212, 331
246	Thiamine	
246	Trazodone	274, 310
246	Triphenyltetrazolium Chloride	
247	Acetohexamide	
247	Alphameprodine	252, 263
247	Cinchocaine	319
247	Cyclofenil	
247	Flurbiprofen	
247	Gentamicin	
247	Harmine	319
247	Methoxsalen	304
247	Norharman	300
247	Phenelzine	252, 257, 263
247	Piminodine	253, 257, 263
247	Prazosin	331
248	Aminopromazine	297
248	Azapetine	
248	Betaprodine	253, 258, 264, 268
248	Boldenone	
248	Carbenoxolone	
248	Didanosine	
248	Droperidol	276
248	Lobeline	
248	Oxetacaine	252, 258, 264
248	Pipamperone	
248	Probenecid	
248	Quinapyramine	297, 325
248	Sulfaquinoxaline	263, 348
248	Trifluperidol	
249	Bupropion	296
249	Hydrastinine	306, 363
249	Kanamycin	306
249	Nitroxoline	293
249	Promethazine	298
249	Sulfamoxole	
250	Allopurinol	
250	Azapropazone	
250	Chlorproguanil	
250	Desipramine	
250	Diethazine	298
250	Etizolam	294, 362
250	Histapyrrodine	295
250	Hydroquinidine	316, 345
250	Hydroquinine	316, 345
250	Levomepromazine	302
250	Methoxypromazine	278, 303
250	Noxiptiline	
250	Papaverine	
250	Phenampromide	257, 260, 262, 266
250	Phenmetrazine	256, 261, 263, 267
250	Profenamine	299
250	Quinidine	317, 345
250	Quinine	317, 346
250	Triamterene	360
250	Trimipramine	
250	Zimeldine	
251	Aletamine	257, 263
251	Alimemazine	300
251	Alphaprodine	257, 263
251	Amfetamine	
251	Aminoglutethimide	257
251	Bamipine	298
251	Benaprizine	257, 261, 264
251	Bezitramide	274
251	Clomipramine	
251	Cycrimine	256, 263
251	Diflunisal	315
251	Ephedrine	257, 263
251	Etafedrine	257, 263
251	Ethoheptazine	257, 263
251	Ethylisobutrazine	300
251	Exemestane	
251	Felbamate	256, 262
251	Fentanyl	257, 263
251	Hydroxyphenamate	257, 263
251	Hydroxyquinoline	308, 318, 356
251	Hyoscine	257, 263
251	Hyoscine Methonitrate	257, 263
251	Imipramine	
251	Levophenacylmorphan	275, 286
251	Loxapine	292
251	Mandelic Acid	257, 262
251	Mebanazine	257, 261, 267
251	Mephentermine	257, 263
251	Methcathinone	
251	Methenamine	257, 263
251	Methylephedrine	257, 263
251	Methylphenidate	257, 264
251	Methylpiperidyl Benzilate	257
251	Metindizate	257
251	Pargyline	256, 261, 268
251	Perazine	300
251	Pethidine	257, 263
251	Phendimetrazine	257, 261, 267
251	Phenformin	258, 264, 267
251	Phenglutarimide	257, 263
251	Pipazetate	
251	Procyclidine	257, 263
251	Promazine	302
251	Properidine	257, 263
251	Styramate	257, 263
251	Thiazinamium Metilsulfate	299
251	Tilidate	257, 262
251	Tricyclamol Chloride	257, 263
251	Tridihexethyl Chloride	257, 263
251	Trimeperidine	257, 263
252	Allylprodine	258, 264
252	Amoxapine	293
252	Anileridine	258, 264
252	Atropine	258, 264
252	Atropine Methonitrate	258, 264
252	Bacitracin	
252	Beclamide	258, 264
252	Benactyzine	258, 262, 264
252	Benzethidine	258, 264, 267
252	Benzfetamine	258, 262, 268
252	Benzilonium Bromide	258, 264
252	Bethanidine	258, 264
252	Biperiden	259, 264
252	Caramiphen	259, 265
252	Carbetapentane	258, 264
252	Cathine	258, 263
252	Cyclopentolate	258, 264
252	Dextropropoxyphene	257, 263
252	Diethylaminoethyl Diphenylpropionate	264.5
252	Difenidol	258
252	Diphenhydramine	257
252	Eucatropine	258, 264
252	Fenbutrazate	257, 261, 267
252	Fenproporex	257, 263
252	Fenthion	
252	Flunixin	327
252	Gelsemine	

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
252	Glycopyrronium Bromide	258, 264
252	Hexocyclium Metilsulfate	257, 264
252	Homatropine	258, 264
252	Homatropine Methylbromide	258, 264
252	Hyoscine Butylbromide	258, 264
252	Hyoscyamine	258, 264
252	Isoaminile	258, 264
252	Lachesine Chloride	258, 264
252	Mepenzolate Bromide	258, 261, 263
252	Metamfetamine	257, 263
252	Nelfinavir	
252	Oxeladin	258, 264
252	Oxyphenonium Bromide	258, 264
252	Phencyclidine	258, 263, 270
252	Phenylpropylmethylamine	257, 263
252	Pipenzolate Bromide	258, 262
252	Piperidolate	258, 264
252	Pipethanate	258, 262
252	Pipradrol	258
252	Poldine Metilsulfate	258
252	Proadifen	259, 264, 270, 327, 337
252	Prolintane	258, 264
252	Ramipril	258
252	Selegiline	258, 264
252	Thiamazole	
252	Trihexyphenidyl	258, 264
252	Trimetaphan Camsilate	258, 264
253	Adiphenine	258, 264
253	Alverine	259, 264, 268
253	Ambutonium Bromide	259, 265
253	Azacyclonol	259
253	Benzatropine	259
253	Bibenzonium Bromide	258, 264, 268
253	Butetamate	258, 264
253	Clidinium Bromide	259
253	Cotarnine	332
253	Diethylpropion	
253	Dimevamide	259, 265
253	Diphenylpyraline	258
253	Doxapram	259, 265
253	Emepronium Bromide	258, 268
253	Ethoxzolamide	304
253	Fencamfamin	259, 265, 269
253	Fenpipramide	259, 265
253	Hyoscine Methobromide	258, 264
253	Medazepam	
253	Methadone	259, 264, 292
253	Methdilazine	302
253	Noracymethadol	258, 269
253	Normethadone	259, 265, 292
253	Opipramol	
253	Orthocaine	
253	Pecazine	300
253	Pentapiperide Metilsulfate	258, 265
253	Phenadoxone	259, 266, 292
253	Valethamate Bromide	259, 265
253.5	Alphamethadol	259
253.5	Racemoramide	264
254	Celecoxib	
254	Cinnarizine	
254	Clostebol	
254	Dextromoramide	259, 264
254	Dimethoxanate	
254	Enoxolone	
254	Ethyl Hydroxybenzoate	
254	Fluvoxamine	
254	Levomoramide	259, 265
254	Loperamide	260, 266
254	Norbudrine	285
254	Perphenazine	307
254	Prochlorperazine	305
254	Strychnine	
254	Tropicamide	
255	Aciclovir	
255	Alcuronium Chloride	291
255	Furaltadone	362
255	Ganciclovir	254
255	Methazolamide	290
255	Methylthioamfetamine	
255	Molindone	299
255	Niflumic Acid	329
255	Pentaerithrityl Tetranitrate	
255	Pipamazine	
255	Propyl Hydroxybenzoate	
255	Thiethylperazine	309
255	Thiopropazate	305
255	Trifluomeprazine	305
256	Fluphenazine	306
256	Harmaline	
256	Mequitazine	307
256	Oxcarbazepine	306
256	Panidazole	278, 261, 236, 270
256	Pemoline	262, 268
256	Pivampicillin	261, 268
256	Pyritidium Bromide	313
256	Sulfachlorpyridazine	
256	Trifluoperazine	305
256	Triflupromazine	305
256	Vincristine	297
257	Amfetamine	
257	Aminophenazone	
257	Ampicillin	262, 268
257	Carbarsone	
257	Cefradine	
257	Chloroquine	329, 343
257	Clemastine	
257	Cyclizine	262, 268
257	Diminazene	
257	Enalapril	268
257	Nalidixic Acid	315
257	Paraquat	
257	Salinazid	327
257	Tolazoline	263
258	Cefalexin	
258	Cetalkonium Chloride	264, 270
258	Chlormezanone	265, 272
258	Clomethiazole	
258	Desmetryne	
258	Dipyrone	
258	Etoxeridine	252, 264
258	Etymide	263, 270
258	Halofantrine	215, 230, 298
258	Halquinol	
258	Levocabastine	263, 269
258	Morazone	
258	Orphenadrine	264
258	Succinylsulfathiazole	281
258	Tioguanine	348
258	Tranylcypromine	264, 271
258	Trimethobenzamide	
259	Baclofen	266, 274
259	Carbenicillin	265, 327
259	Ceftazidime	
259	Chlorphenoxamine	
259	Clofedanol	265
259	Demecarium Bromide	265
259	Dipipanone	265, 293
259	Fenpiprane	268
259	Nicotine	
259	Nifuratel	367
259	Phenindamine	
260	Alprazolam	
260	Aminoacridine	313, 326
260	Aprindine	266, 272
260	Ceftazidime	
260	Dimetindene	
260	Hexyl Nicotinate	
260	Isopropylaminophenazone	
260	Ketoprofen	
260	Lopinavir	
260	Metyrapone	233
260	Mexiletine	
260	Neostigmine Bromide	266
260	Nicotinyl Alcohol	
260	Norpipanone	294
260	Tiaprofenic Acid	305
260	Tolpropamine	265, 273
261	Betahistine	
261	Ceftriaxone	
261	Cloponone	
261	Cyclomethycaine	
261	Dibrompropamidine	
261	Doxylamine	
261	Ethyl Nicotinate	

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
261	Etoxazene	310
261	Fluconazole	266
261	Inositol Nicotinate	
261	Methyl Benzoquate	
261	Nicametate	
261	Nicocodine	267, 256
261	Nicotinamide	
261	Nitrofurazone	
261	Pheniramine	266
261	Quinisocaine	269, 331
262	Acriflavinium Chloride	
262	Clorprenaline	266
262	Debrisoquine	270
262	Diloxanide	
262	Etidocaine	271
262	Mebeverine	
262	Naproxen	272, 315, 328
262	Nicofuranose	
262	Nifenazone	
262	Pentamidine	
262	Phenatine	242
262	Phenoxybenzamine	268, 274
262	Sulfanilamide	269
262	Sulfisomidine	
262	Thioridazine	310
262	Tocainide	270
262	Tolmetin	315
262	Veratrine	292
262	Zomepirac	324
263	Amfetamine	
263	Benzethonium Chloride	269, 274
263	Bretylium Tosilate	272, 278
263	Broxyquinoline	
263	Bupivacaine	271
263	Carbinoxamine	
263	Chlorquinaldol	330
263	Denatonium Benzoate	269
263	Disopyramide	269
263	Itraconazole	
263	Lidocaine	272
263	Mepivacaine	271
263	Phenoxypropazine	268, 319
263	Pyrrocaine	
263	Tenoxicam	377
264	Benzoctamine	271
264	Berberine	345
264	Bisacodyl	
264	Fenfluramine	271
264	Metopimazine	310
264	Nikethamide	
264	Pindolol	287
264	Sorbic Acid	
264	Stilbazium Iodide	355
264	Sulfadoxine	
264	Sulfaguanidine	271
265	Acetazolamide	
265	Acetone	
265	Alachlor	273
265	Amolanone	269, 276.5
265	Brompheniramine	
265	Brucine	300
265	Butriptyline	
265	Cefaclor	
265	Chlorphenamine	
265	Cladribine	
265	Decoquinate	
265	Econazole	271, 280
265	Famotidine	
265	Hexobendine	
265	Maprotiline	272
265	Minocycline	354
265	Phanquinone	272, 292
265	Phenamidine	
265	Primaquine	282, 334
265	Sertraline	273, 281
265	Sulfafurazole	
265	Sulfamethoxazole	
265	Sulforidazine	311
265	Xylometazoline	
266	Amineptine	
266	Bialamicol	
266	Captodiame	
266	Carbutamide	
266	Chlortetracycline	359
266	Coniine	
266	Fluorouracil	
266	Iproniazid	
266	Isoniazid	
266	Lidoflazine	272
266	Milrinone	322
266	Nialamide	
266	Psilocin	283, 292
266	Stavudine	
266	Sulfacarbamide	272
267	Butanilicaine	275
267	Ethomoxane	
267	Lamotrigine	
267	Mafenide	274
267	Moracizine	
267	Nefopam	275
267	Penfluridol	273
267	Racemoramide	270
267	Riboflavin	
267	Sirolimus	277, 288
267	Tramazoline	275
267	Xantinol Nicotinate	273
268	Aztreonam	281
268	Capreomycin	
268	Demeclocycline	
268	Diethylthiambutene	288
268	Enoxacin	219, 338
268	Ethacridine	
268	Ethylmethylthiambutene	285, 226
268	Fenitrothion	
268	Flucloxacillin	274, 344
268	Mescaline	
268	Metixene	
268	Oxytetracycline Dihydrate	352
268	Phthalylsulfacetamide	
268	Sulfamethizole	
268	Thenium Closilate	275
268	Vinblastine	
268.5	Amisometradine	
269	Chlorpromazine	
269	Deserpidine	
269	Doxycycline	346
269	Isoxsuprine	274
269	Ketamine	276
269	Ketoconazole	
269	Lymecycline	356
269	Morinamide	314
269	Nadolol	276
269	Octafonium Chloride	274, 281
269	Pyrazinamide	312
269	Sotalol	
269	Trimethoxyamfetamine	
269	Viomycin	
270	Alprenolol	276
270	Aminobenzoic Acid	
270	Clopidogrel	278, 308
270	Diamthazole	293
270	Distigmine Bromide	
270	Etacrynic Acid	
270	Etofylline	
270	Mephenesin	276
270	Methyclothiazide	314
270	Phenol	
270	Phenyltoloxamine	276
270	Pyridostigmine Bromide	
270	Tetracycline	356
270	Theophylline	
270	Warfarin	280, 303
271	Ambenonium Chloride	277
271	4-Aminophenol	
271	Bufylline	
271	Clonidine	278
271	Dinitro-orthocresol	
271	Embutramide	276
271	Mazindol	
271	Methoxyphenamine	
271	Naphazoline	281, 288, 291
271	Propofol	
271	Sulfacetamide	
271	Trimethoprim	

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
271	Yohimbine	277, 287
272	Amidefrine	
272	Amosulalol	
272	Apomorphine	
272	Benzocaine	278
272	Butacaine	278
272	Cyclothiazide	314
272	Epithiazide	315
272	Ethiazide	
272	Etilefrine	
272	Etodolac	
272	Fenoprofen	
272	Guanoclor	278
272	Hydrochlorothiazide	318
272	Metaraminol	
272	Profadol	
272	Pyrimethamine	
272	Rolitetracycline	357
272	Theobromine	
272	Thymol	
272	Tramadol	279
273	Anisindione	
273	Buphenine	
273	Caffeine	
273	Cisapride	307
273	Clonazepam	
273	Coumatetralyl	283, 309
273	Diclofenac	
273	Edrophonium Chloride	
273	Glymidine Sodium	299
273	Guaifenesin	
273	Hydroflumethiazide	325
273	Hydroxyephedrine	
273	Metoclopramide	309
273	Naepaine	279, 290
273	Naftidrofuryl Oxalate	283
273	Oxedrine	
273	Oxprenolol	
273	Phenylephrine	
273	Pimozide	280
273	Proxyphylline	
273	Resorcinol	
273	Viloxazine	
274	Acefylline Piperazine	
274	Atenolol	280
274	Chlorisondamine Chloride	282, 290
274	Cyclopenthiazide	319
274	Hordenine	
274	Hydroxocobalamin	351
274	Methoxyamfetamine	
274	Methoxymetamfetamine	
274	Metoprolol	280
274	Pentoxifylline	
274	Piperoxan	
274	Quinuronium Sulfate	323, 359
274	Ritodrine	
274	Tyramine	
275	Bamethan	
275	Clemizole	
275	Fenetylline	
275	Fenoterol	
275	Guanoxan	
275	Hydroxyamfetamine	
275	Moxisylyte	
275	Pentifylline	
275	Pholedrine	
275	Propylthiouracil	
275	Sulfadimethoxine	
275	Tetracosactrin	
276	Diaveridine	
276	Glipizide	
276	Insulin	
276	Methylthiouracil	
276	Orciprenaline	
276	Salbutamol	
276	Serotonin	
276	Terbutaline	
276.5	Reboxetine	
277	Alclofenac	
277	Ciprofloxacin	315
277	Cyclazocine	
277	Desomorphine	225, 282
277	Meclofenoxate	
277	Metadrenaline	
277	Metronidazole	
277	Narceine	
277	Omeprazole	303
277	Oxymetholone	
277	Racemethorphan	283
277	Risperidone	
277	Tinidazole	
278	Amylmetacresol	286
278	Bamifylline	
278	Bufotenine	297
278	Butorphanol	
278	Chlorothiazide	
278	Dextromethorphan	
278	Diethyltryptamine	287
278	Ethylnoradrenaline	
278	Ibogaine	
278	Isoetarine	
278	Levomethorphan	
278	Metipranolol	
278	Normetadrenaline	
278	Pentazocine	
278	Phenazocine	
278	Phentolamine	
278	Procaine Benzylpenicillin	
278	Teicoplanin	
278	Tryptamine	287
278	Tryptophan	286
278	Verapamil	
278	Vetrabutine	
279	Butyl Aminobenzoate	
279	Chloroxylenol	
279	Deferiprone	
279	Dextrorphan	
279	Diamorphine	
279	Dimethyltryptamine	288
279	Dobutamine	
279	Isoprenaline	
279	Leucinocaine	273, 290
279	Levallorphan	
279	Levorphanol	
279	Mefenamic Acid	350
279	Mepacrine	343
279	Methyldopa	
279	α-Methyltryptamine	288
279	Mianserin	
279	Noradrenaline	
279	Phenomorphan	284
279	Racemorphan	
280	Adrenaline	
280	Azathioprine	
280	Brocresine	225, 250
280	Cetylpyridinium Chloride	
280	Chlorzoxazone	
280	Cytarabine	
280	Dihydroergotamine	
280	Dopamine	
280	Etamivan	
280	Hexoprenaline	
280	Hydrocodone	
280	Hydromorphone	290
280	Ketobemidone	
280	Levodopa	
280	Loratadine	
280	Methyldopate	
280	Metocurine Iodide	
280	Metopon	225
280	Mivacurium Chloride	
280	Nitrazepam	
280	Octreotide	288
280	Oxycodone	
280	Oxymetazoline	
280	Pirenzepine	
280	Rafoxanide	335
280	Rimiterol	
280	Sulfathiazole	
280	Xamoterol	270, 275
281	Acetarsone	
281	Naloxone	
281	Naltrexone	
281	Oxymorphone	
281	Parbendazole	288
281	Protokylol	
281	Thebacon	
281	Vancomycin	

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
282	Benzquinamide	
282	Carbidopa	
282	Dichlorophen	
282	Dyclonine	
282	Methanthelinium Bromide	
282	Phthalylsulfathiazole	
283	Acetyldihydrocodeine	
283	Azatadine	
283	Benzthiazide	
283	Cephaëline	
283	Clarithromycin	
283	Clofazimine	
283	Dihydrocodeine	
283	Dihydromorphine	
283	Fazadinium Bromide	
283	Methyldesorphine	278
283	Myrophine	261
283	Quazepam	
284	Acetorphine	
284	Acetylcodeine	
284	Benzylmorphine	
284	Ethylmorphine	
284	Etofenamate	349
284	Hydromorphinol	278
284	Lansoprazole	
284	Nalbuphine	
284	Nalmefene	
284	Norcodeine	
284	Sulindac	327
284	Thebaine	
284.5	Morphine N-oxide	279
285	Amiloride	361
285	Codeine	
285	Dimoxyline	309, 335
285	Methylenedioxymetamfetamine	
285	Morphine	
285	Nalorphine	
285	Nemonapride	314
285	Neopine	
285	Normorphine	
285	Obidoxime Chloride	
286	Buprenorphine	
286	Cyproheptadine	
286	Diclofenamide	296
286	Dimethylthiambutene	268, 227
286	Flucytosine	
286	Mebhydrolin	320
286	Mersalyl Acid	
286	Nicergoline	
286	Remoxipride	
287	Cyprenorphine	
287	Iprindole	293
287	Prenalterol	
287	Remoxipride	
288	Dapsone	
288	Idoxuridine	
288	Propranolol	305, 319
289	DOM	
289	Etorphine	
289	Fenbendazole	302
290	Bleomycin	290
290	Cyclobenzaprine	
290	Haloxon	312
290	Methoxamine	
290	Natamycin	303, 318
290	Noscapine	312
290	Pantoprazole	
290	Pyridoxine	
290	Triprolidine	
290	Tylosin	
291	Carbimazole	
292	Albendazole	
292	Doxepin	
292	Protriptyline	
292	Sulpiride	
292.5	Paroxetine	234.5
294	Hydrastine	
294	Procaine	222
295	Paroxetine	
295	Timolol	
296	Xipamide	
297	Ketotifen	
297	Nimorazole	
298	Bunamidine	
299	Rescinnamine	
301	Oxypertine	
302	Labetalol	
302	Mifepristone	
303	Zopiclone	
304	Zopiclone	
305	Lysergic Acid	
306	Bromocriptine	
306	Zopiclone	214
307	Benzydamine	
308	Tiotixene	
309	Dimefline	
310	Diquat	
310	Ergometrine	
312	Buspirone	
313	Ergometrine	
313	Lysergamide	
313	Metabutoxycaine	
313	Methylergometrine	
314.5	Mirtazapine	252.5
315	Lysergide	
315	Pyrantel	
316	Ergotamine	
316	Sulfamethoxypyridazine	
317	Dimetridazole	226
318	Indometacin	
319	Nizatidine	254
325	Mercaptopurine	
326	Aflatoxins G	216
327	Enoximone	
329	Gloxazone	
330	Toloxatone	
336	Diphenadione	
340	Bumetanide	
344	Hydroxystilbamidine	
345	Glafenine	
358	Olsalazine	221, 249
413	Aflatoxins G	225, 255, 346
464	Metaclazepam	255

B. In alkali solution—wavelengths of maximum absorption

In this table wavelengths of maximum absorption in alkaline solution are given only for those compounds for which maxima in acid solution are not recorded in the monographs, or for which the maxima in alkaline solution differ significantly from the maxima in acid solution.

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
215	m-Chlorophenylpiperazine	250
217	Abacavir	260, 285
224	Allantoin	
228	Sumatriptan	278, 284, 294
229	Halazone	
229	Simvastatin	237, 246
230	Acecarbromal	
230	Buformin	
230	Indometacin	279
231	Aspirin	298
231	Celiprolol	324
231	Flurazepam	312
231	Mercaptopurine	310
231	Oxybuprocaine	310
231	Temazepam	313
232	Chlorhexidine	253

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
232	Harman	284
232	Methylenedioxymetamfetamine	285
232	Thiamine	336
232	Triamterene	270, 370
233	Bromisoval	
233	Niridazole	282
233	Oxazepam	344
233	Paroxetine	264, 270, 292
235	Moxisylyte	301
235	Prenalterol	305
235	Saccharin	268
236	Clamoxyquin	288, 364, 300
236	Dobutamine	292
236	Papaverine	277, 326
237	Bromazepam	348
237	Cambendazole	314
237	Cyclazocine	298
237	Diltiazem	
237	Etilefrine	290
237	Ketotifen	302
237	Profadol	290
237	Tacrine	317
237	Zopiclone	277
238	Amlodipine	
238	Captopril	
238	Cinchocaine	325
238	Clozapine	261, 297
238	Fenbendazole	312
238	Hexethal	
238	Hydroxyamfetamine	294
238	Metaraminol	292
238	Pamaquin	288, 298, 362
238	Phenazocine	298
238	Phenylephrine	291
238	Phenylmethylbarbituric Acid	
238	Pholedrine	295
238	Pravastatin	
238	Vinbarbital	
239	Barbital	
239	Bromocriptine	300
239	Butobarbital	
239	Cresol	290
239	Cyclobarbital	
239	Heptabarb	
239	Idobutal	
239	Lymecycline	267
239	Pentobarbital	
239	Phenobarbital	
239	Secbutabarbital	
239	Secobarbital	
239	Talbutal	
240	Acetazolamide	291
240	Amobarbital	
240	Benzbromarone	281, 355
240	Brallobarbital	
240	Butalbital	
240	Clenbuterol	295
240	Clioxanide	282, 362
240	Dextrorphan	299
240	Edrophonium Chloride	294
240	Ibomal	
240	Levallorphan	299
240	Levorphanol	299
240	Nealbarbital	
240	Nordazepam	340
240	Pentazocine	300
240	Propofol	291
240	Racemorphan	299
240	Sulfadiazine	254
241	Allobarbital	
241	Altretamine	
241	Aprobarbital	
241	Benazepril	
241	Cyclopentobarbital	
241	Demeclocycline	276, 388
241	Diacerein	277, 502
241	Methapyrilene	312
241	Oxedrine	291
241	Salicylamide	328
242	Adrenaline	297
242	Bamethan	290
242	Buphenine	291
242	Ceftriaxone	269
242	Chloroxylenol	296
242	Dichloralphenazone	256
242	Hexestrol	297
242	Hydroxyephedrine	290
242	Iproniazid	307
242	Phenazone	256
242	Phenomorphan	300
242	Sulfadimidine	258
242	Sulfamerazine	255
242	Zolpidem	
243	Chloropyrilene	308
243	Chlorzoxazone	287
243	Demoxepam	255, 310
243	Etizolam	
243	Glymidine Sodium	317
243	Harmine	275, 300
243	Hexobarbital	
243	Hexobarbital	
243	Isoxsuprine	270, 277, 292
243	Methylphenobarbital	
243	Methysergide	320
243	Minocycline	
243	Propiomazine	283
244	Benoxaprofen	309
244	Carbimazole	
244	Chlorocresol	299
244	Enallylpropymal	
244	Flumazenil	
244	Metharbital	
244	Metharbital	
244	Methylphenobarbital	
244	Mexazolam	
244	Morazone	263
245	Acepromazine	283
245	Dichlorophen	304
245	Enallylpropymal	
245	Metadrenaline	291
245	Propyphenazone	265
245	Salbutamol	295
245	Sulfametoxydiazine	
246	Diethylpropion	
246	Diprenorphine	299
246	Droperidol	288
246	Eugenol	296
246	Felbamate	251, 256, 262
246	Labetalol	333
246	Lisinopril	254, 258, 261, 267
246	Methohexital	
246	Methohexital	
246	Oxytetracycline Dihydrate	269
246	Thenyldiamine	310
247	Amoxicillin	291
247	Antazoline	295
247	Buclosamide	325
247	Diminazene	
247	Methcathinone	
247	Spironolactone	
247	Sulfapyridine	
247	Vinylbital	
248	Benperidol	288
248	Bisacodyl	
248	Carbarsone	
248	Chloropyramine	313
248	Loxapine	298
248	Mepyramine	312
248	Rolitetracycline	268
248	Vanillin	348
249	Acetohexamide	
249	Cetoxime	300
249	Floctafenine	359
249	Hexachlorophene	320
249	Pentamidine	
249	Propantheline Bromide	282
249	Sotalol	
249	Sulfaphenazole	
249	Tripelennamine	312
250	Acetarsone	299
250	Amoxapine	299
250	Boldenone	
250	Clomethiazole	
250	Isothipendyl	
250	Prothipendyl	320
250	Sulfamethoxypyridazine	

λ *max* *nm*	*Compound*	*Subsidiary Peaks* *nm*
250	Sulfamoxole	
250	Sulfanilamide	
250	Terazosin	273
251	Amiodarone	
251	Candesartan	
251	Clomocycline	283
251	Gentamicin	
251	Nalorphine	298
252	Atovaquone	276
252	Fosinopril	258, 268
252	Imipramine	
252	Loratadine	
252	Pargyline	257, 263
252	Prazosin	345
252	Ramipril	258, 267
252	Sulfaquinoxaline	357
253	Apomorphine	
253	Chlortetracycline	284, 346
253	Doxorubicin	
253	Hexethal	
253	Hydroxychloroquine	330
253	Phenylmethylbarbituric Acid	
253	Profenamine	302
253	Sulfafurazole	
254	Barbital	
254	Butobarbital	
254	Chloroquine	330
254	Clemizole	269, 275, 283
254	Cyclopentobarbital	
254	Didanosine	
254	Idobutal	
254	Oxyphenbutazone	
254	Parbendazole	297, 303
254	Phenobarbital	
254	Promazine	306
254	Promethazine	305
254	Secbutabarbital	
254	Secobarbital	
254	Sorbic Acid	
254	Vinbarbital	
255	Amobarbital	
255	Azapropazone	325
255	Butalbital	
255	Carbutamide	
255	Heptabarb	
255	Methdilazine	308
255	Nealbarbital	
255	Pentobarbital	
255	Strychnine	278
255	Sulfacarbamide	
255	Talbutal	
255	Thiopental	304
256	Alimemazine	310
256	Allobarbital	
256	Brallobarbital	
256	Cyclobarbital	
256	Dapsone	292
256	Folic Acid	283
256	Methylthiouracil	
256	Mifepristone	305
256	Nikethamide	261
256	Sulfacetamide	
256	Sulfamethoxazole	
256	Sulfathiazole	
256	Thialbarbital	307
256	Tropicamide	
257	Allopurinol	
257	Aprobarbital	
257	Barbituric Acid	
257	Carbenoxolone	
257	Ceftazidime	
257	Ibomal	
257	Paracetamol	
257	Perphenazine	310
257	Pivampicillin	311
257	Succinylsulfathiazole	
257	Sulfachlorpyridazine	
257	Sulfasomizole	280
257	Thiopropazate	
258	Methotrexate	303
258	Nalidixic Acid	334
258	Olmesartan	
258	Pheniramine	263, 268
258	Trifluoperazine	308
258	Zimeldine	
259	Barbituric Acid	
259	Chlormezanone	
259	Cinchophen	330
259	Halquinol	343
259	Levomepromazine	323
259	Moracizine	
259	Selegiline	269
259	Sulfaguanidine	
260	Cyclizine	
260	Enoxolone	
260	Phthalylsulfathiazole	
260	Propylthiouracil	
260	Sulfamethizole	
260	Sulfinpyrazone	
260	Tolmetin	323
260	Tranylcypromine	266, 273
261	Aciclovir	
261	Carbinoxamine	
261	Cefaloridine	
261	Disopyramide	
261	Fluconazole	267
261	Naproxen	271, 316, 330
261	Nicotine	
261	Zomepirac	330
262	Bromhexine	317
262	Brompheniramine	269
262	Chlordiazepoxide	
262	Chlorphenamine	
262	Coniine	268
262	Cyclofenil	
262	Ketoprofen	
262	Minoxidil	288
262	Sulfisomidine	
263	Chlorquinaldol	345
263	Gliclazide	
263	Nicofuranose	
263	Phenindamine	
263	Tenoxicam	289
264	Aminophenazone	
264	Fluoxetine	275
264	Ganciclovir	
264	Methyclothiazide	300
264	Phenylbutazone	
264	Salinazid	
265	Aminobenzoic Acid	
265	Aminosalicylic Acid	300
265	Amprenavir	
265	Cladribine	
265	Diethazine	
265	Dinitro-orthocresol	
265	Ibuprofen	273
265	Methaqualone	306
265	Pecazine	
265	Phthalylsulfacetamide	
266	4-Aminophenol	
266	Brucine	304
266	Feprazone	
266	Isradipine	278, 291, 326
266	Sulforidazine	
268	Aloin	
268	Dimetotiazine	
268	Flucloxacillin	274, 318
268	Gelsemine	
269	Mazindol	275
269	Mebeverine	290
269	Sulfadimethoxine	
270	Mebendazole	355
270	Mepacrine	345
270	Psilocin	293
270	Riboflavin	356
270	Zalcitabine	
271	Furosemide	333
271	Isopropylaminophenazone	
271	Nitroxinil	
272	Amosulalol	
272	Ciprofloxacin	322, 334
272	Pericyazine	
272	Sulfadoxine	
273	Amodiaquine	287

λ *max* *nm*	*Compound*	*Subsidiary Peaks* *nm*
273	Bendroflumethiazide	330
273	Captodiame	
274	Cytarabine	
274	Etodolac	
274	Hydrochlorothiazide	324
274	Hydroflumethiazide	333
274	Isocarboxazid	
274	Serotonin	323
274	Theobromine	
274	Venlafaxine	280
275	Bufexamac	
275	Cisapride	308
275	Deserpidine	
275	Diclofenac	
275	Meclofenamic Acid	319
275	Procainamide	
275	Theophylline	
275	Thioridazine	
276	Dithranol	
276	Gliquidone	
277	Diflunisal	
277	Ethionamide	
278	Dimenhydrinate	
278	Phenindione	330
279	Ambucetamide	286
279	Idoxuridine	
279	Omeprazole	
279	Pergolide	
280	Dantron	
280	Diazoxide	
280	Mivacurium Chloride	
280	Nicergoline	
280	Procaine Benzylpenicillin	
280	Quinidine	330
280	Quinine	330
280	Sulindac	327
280	Tryptophan	288
280	Yohimbine	
281	Atracurium	
281	Ethylmorphine	
281	Felodipine	
281	Flunixin	
281	Noscapine	315
281	Quazepam	
281	Trilostane	
282	Butacaine	
282	Clarithromycin	
282	Milrinone	331
283	Acetyldihydrocodeine	
284	Cyproheptadine	
284	Nitrofurazone	
284	Nomifensine	
285	Azathioprine	
285	Benzocaine	
285	Butyl Aminobenzoate	
285	Fenbufen	
285	Mefenamic Acid	
286	Clobazam	
286	Cotarnine	330
286	Famotidine	
286	Pyrimethamine	
287	Ketoconazole	
287	Mupirocin	
287	Phenol	
287	Trimethoprim	
288	Cinchonine	302, 315
288	Methazolamide	
288	Niflumic Acid	
288	Ofloxacin	332
289	Aztreonam	
289	Capreomycin	
290	Mersalyl Acid	
290	Resorcinol	
291	Carbidopa	
291	Nystatin	305, 319
291	Phentolamine	
292	Chlorothiazide	
292	Fendosal	
292	Hydrastine	
292	Oxymorphone	
292	Rabeprazole	
292	Thymol	
293	Flucytosine	
293	Metallibure	
293	Naltrexone	
293	Warfarin	308
294	Pentaerithrityl Tetranitrate	
294	Tyramine	
295	Diethylthiambutene	
295	Dipyridamole	
295	Ethyl Hydroxybenzoate	
295	Fenoterol	
295	Triclosan	
296	Noradrenaline	
296	Propyl Hydroxybenzoate	
297	Benzthiazide	
297	Dihydromorphine	
297	Isoprenaline	
297	Nalbuphine	
297	Orciprenaline	
297	Pirenzepine	
297	Teicoplanin	
297	Terbutaline	
298	Aloxiprin	
298	Isoniazid	
298	Morphine	
298	Nalmefene	
298	Parachlorophenol	
298	Salicylic Acid	
299	Butorphanol	
299	Diamorphine	
300	Buprenorphine	
300	Ketobemidone	
300	Xamoterol	
301	Cyprenorphine	
302	Etorphine	
302	Iprindole	
302	Methyldopa	
303	Gloxazone	
303	Oxymetazoline	
303	Thiopental	
304	Famciclovir	
304	Zopiclone	
305	Nimorazole	
305	Tetrazepam	
305	Thiamylal	
306	Rescinnamine	
306	Thialbarbital	
307	Buspirone	
307	Nialamide	
308	Lysergic Acid	
309	Albendazole	
309	Lysergamide	
310	Bacampicillin	
310	Ergotamine	
310	Hydroxystilbamidine	
310	Lysergide	
310	Methylergometrine	
310	Phenprocoumon	
311	Coumatetralyl	
311	Ethyl Biscoumacetate	
313	Nizatidine	
313	Thioridazine	
314	Dantrolene	
314	Dicoumarol	
315	Oxymetholone	
317	Bumetanide	
319	Liothyronine	
319	Metronidazole	
319	Pentachlorophenol	
320	Tioguanine	
322	Dimetridazole	
324	Nisoldipine	
324	Oxyclozanide	
325	Levothyroxine	
330	Harmaline	
330	Xipamide	
332	Pralidoxime Chloride	280
334	Niclosamide	377
341	Salsalate	
353	Obidoxime Chloride	
371	Lornoxicam	
377	Metaclazepam	274, 236

C. In neutral solution—wavelengths of maximum absorption

The compounds listed below are those whose wavelengths of maximum absorption in acid or alkaline solution are not given in the monographs, or for which the maxima in neutral solution differ significantly from those in acid or alkaline solution.

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
215	m-Chlorophenylpiperazine	250
217	Abacavir	260, 285
224	Allantoin	
228	Sumatriptan	278, 284, 294
229	Halazone	
229	Simvastatin	237, 246
230	Acecarbromal	
230	Buformin	
230	Indometacin	279
231	Aspirin	298
231	Celiprolol	324
231	Flurazepam	312
231	Mercaptopurine	310
231	Oxybuprocaine	310
231	Temazepam	313
232	Chlorhexidine	253
232	Harman	284
232	Methylenedioxymetamfetamine	285
232	Thiamine	336
232	Triamterene	270, 370
233	Bromisoval	
233	Niridazole	282
233	Oxazepam	344
233	Paroxetine	264, 270, 292
235	Moxisylyte	301
235	Prenalterol	305
235	Saccharin	268
236	Clamoxyquin	288, 364, 300
236	Dobutamine	292
236	Papaverine	277, 326
237	Bromazepam	348
237	Cambendazole	314
237	Cyclazocine	298
237	Diltiazem	
237	Etilefrine	290
237	Ketotifen	302
237	Profadol	290
237	Tacrine	317
237	Zopiclone	277
238	Amlodipine	
238	Captopril	
238	Cinchocaine	325
238	Clozapine	261, 297
238	Fenbendazole	312
238	Hexethal	
238	Hydroxyamfetamine	294
238	Metaraminol	292
238	Pamaquin	288, 298, 362
238	Phenazocine	298
238	Phenylephrine	291
238	Phenylmethylbarbituric Acid	
238	Pholedrine	295
238	Pravastatin	
238	Vinbarbital	
239	Barbital	
239	Bromocriptine	300
239	Butobarbital	
239	Cresol	290
239	Cyclobarbital	
239	Heptabarb	
239	Idobutal	
239	Lymecycline	267
239	Pentobarbital	
239	Phenobarbital	
239	Secbutabarbital	
239	Secobarbital	
239	Talbutal	
240	Acetazolamide	291
240	Amobarbital	
240	Benzbromarone	281, 355
240	Brallobarbital	
240	Butalbital	
240	Clenbuterol	295

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
240	Clioxanide	282, 362
240	Dextrorphan	299
240	Edrophonium Chloride	294
240	Ibomal	
240	Levallorphan	299
240	Levorphanol	299
240	Nealbarbital	
240	Nordazepam	340
240	Pentazocine	300
240	Propofol	291
240	Racemorphan	299
240	Sulfadiazine	254
241	Allobarbital	
241	Altretamine	
241	Aprobarbital	
241	Benazepril	
241	Cyclopentobarbital	
241	Demeclocycline	276, 388
241	Diacerein	277, 502
241	Methapyrilene	312
241	Oxedrine	291
241	Salicylamide	328
242	Adrenaline	297
242	Bamethan	290
242	Buphenine	291
242	Ceftriaxone	269
242	Chloroxylenol	296
242	Dichloralphenazone	256
242	Hexestrol	297
242	Hydroxyephedrine	290
242	Iproniazid	307
242	Phenazone	256
242	Phenomorphan	300
242	Sulfadimidine	258
242	Sulfamerazine	255
242	Zolpidem	
243	Chloropyrilene	308
243	Chlorzoxazone	287
243	Demoxepam	255, 310
243	Etizolam	
243	Glymidine Sodium	317
243	Harmine	275, 300
243	Hexobarbital	
243	Hexobarbital	
243	Isoxsuprine	270, 277, 292
243	Methylphenobarbital	
243	Methysergide	320
243	Minocycline	
243	Propiomazine	283
244	Benoxaprofen	309
244	Carbimazole	
244	Chlorocresol	299
244	Enallylpropymal	
244	Flumazenil	
244	Metharbital	
244	Metharbital	
244	Methylphenobarbital	
244	Mexazolam	
244	Morazone	263
245	Acepromazine	283
245	Dichlorophen	304
245	Enallylpropymal	
245	Metadrenaline	291
245	Propyphenazone	265
245	Salbutamol	295
245	Sulfametoxydiazine	
246	Diethylpropion	
246	Diprenorphine	299
246	Droperidol	288
246	Eugenol	296
246	Felbamate	251, 256, 262
246	Labetalol	333
246	Lisinopril	254, 258, 261, 267

λ *max*		*Subsidiary Peaks*
nm	*Compound*	*nm*
246	Methohexital	
246	Methohexital	
246	Oxytetracycline Dihydrate	269
246	Thenyldiamine	310
247	Amoxicillin	291
247	Antazoline	295
247	Buclosamide	325
247	Diminazene	
247	Methcathinone	
247	Spironolactone	
247	Sulfapyridine	
247	Vinylbital	
248	Benperidol	288
248	Bisacodyl	
248	Carbarsone	
248	Chloropyramine	313
248	Loxapine	298
248	Mepyramine	312
248	Rolitetracycline	268
248	Vanillin	348
249	Acetohexamide	
249	Cetoxime	300
249	Floctafenine	359
249	Hexachlorophene	320
249	Pentamidine	
249	Propantheline Bromide	282
249	Sotalol	
249	Sulfaphenazole	
249	Tripelennamine	312
250	Acetarsone	299
250	Amoxapine	299
250	Boldenone	
250	Clomethiazole	
250	Isothipendyl	
250	Prothipendyl	320
250	Sulfamethoxypyridazine	
250	Sulfamoxole	
250	Sulfanilamide	
250	Terazosin	273
251	Amiodarone	
251	Candesartan	
251	Clomocycline	283
251	Gentamicin	
251	Nalorphine	298
252	Atovaquone	276
252	Fosinopril	258, 268
252	Imipramine	
252	Loratadine	
252	Pargyline	257, 263
252	Prazosin	345
252	Ramipril	258, 267
252	Sulfaquinoxaline	357
253	Apomorphine	
253	Chlortetracycline	284, 346
253	Doxorubicin	
253	Hexethal	
253	Hydroxychloroquine	330
253	Phenylmethylbarbituric Acid	
253	Profenamine	302
253	Sulfafurazole	
254	Barbital	
254	Butobarbital	
254	Chloroquine	330
254	Clemizole	269, 275, 283
254	Cyclopentobarbital	
254	Didanosine	
254	Idobutal	
254	Oxyphenbutazone	
254	Parbendazole	297, 303
254	Phenobarbital	
254	Promazine	306
254	Promethazine	305
254	Secbutabarbital	
254	Secobarbital	
254	Sorbic Acid	
254	Vinbarbital	
255	Amobarbital	
255	Azapropazone	325
255	Butalbital	
255	Carbutamide	
255	Heptabarb	
255	Methdilazine	308
255	Nealbarbital	
255	Pentobarbital	
255	Strychnine	278
255	Sulfacarbamide	
255	Talbutal	
255	Thiopental	304
256	Alimemazine	310
256	Allobarbital	
256	Brallobarbital	
256	Cyclobarbital	
256	Dapsone	292
256	Folic Acid	283
256	Methylthiouracil	
256	Mifepristone	305
256	Niketham ide	261
256	Sulfacetamide	
256	Sulfamethoxazole	
256	Sulfathiazole	
256	Thialbarbital	307
256	Tropicamide	
257	Allopurinol	
257	Aprobarbital	
257	Barbituric Acid	
257	Carbenoxolone	
257	Ceftazidime	
257	Ibomal	
257	Paracetamol	
257	Perphenazine	310
257	Pivampicillin	311
257	Succinylsulfathiazole	
257	Sulfachlorpyridazine	
257	Sulfasomizole	280
257	Thiopropazate	
258	Methotrexate	303
258	Nalidixic Acid	334
258	Olmesartan	
258	Pheniramine	263, 268
258	Trifluoperazine	308
258	Zimeldine	
259	Barbituric Acid	
259	Chlormezanone	
259	Cinchophen	330
259	Halquinol	343
259	Levomepromazine	323
259	Moracizine	
259	Selegiline	269
259	Sulfaguanidine	
260	Cyclizine	
260	Enoxolone	
260	Phthalylsulfathiazole	
260	Propylthiouracil	
260	Sulfamethizole	
260	Sulfinpyrazone	
260	Tolmetin	323
260	Tranylcypromine	266, 273
261	Aciclovir	
261	Carbinoxamine	
261	Cefaloridine	
261	Disopyramide	
261	Fluconazole	267
261	Naproxen	271, 316, 330
261	Nicotine	
261	Zomepirac	330
262	Bromhexine	317
262	Brompheniramine	269
262	Chlordiazepoxide	
262	Chlorphenamine	
262	Coniine	268
262	Cyclofenil	
262	Ketoprofen	
262	Minoxidil	288
262	Sulfisomidine	
263	Chlorquinaldol	345
263	Gliclazide	
263	Nicofuranose	
263	Phenindamine	
263	Tenoxicam	289
264	Aminophenazone	
264	Fluoxetine	275
264	Ganciclovir	

λ *max* nm	Compound	*Subsidiary Peaks* nm
264	Methyclothiazide	300
264	Phenylbutazone	
264	Salinazid	
265	Aminobenzoic Acid	
265	Aminosalicylic Acid	300
265	Amprenavir	
265	Cladribine	
265	Diethazine	
265	Dinitro-orthocresol	
265	Ibuprofen	273
265	Methaqualone	306
265	Pecazine	
265	Phthalylsulfacetamide	
266	4-Aminophenol	
266	Brucine	304
266	Feprazone	
266	Isradipine	278, 291, 326
266	Sulforidazine	
268	Aloin	
268	Dimetotiazine	
268	Flucloxacillin	274, 318
268	Gelsemine	
269	Mazindol	275
269	Mebeverine	290
269	Sulfadimethoxine	
270	Mebendazole	355
270	Mepacrine	345
270	Psilocin	293
270	Riboflavin	356
270	Zalcitabine	
271	Furosemide	333
271	Isopropylaminophenazone	
271	Nitroxinil	
272	Amosulalol	
272	Ciprofloxacin	322, 334
272	Pericyazine	
272	Sulfadoxine	
273	Amodiaquine	287
273	Bendroflumethiazide	330
273	Captodiame	
274	Cytarabine	
274	Etodolac	
274	Hydrochlorothiazide	324
274	Hydroflumethiazide	333
274	Isocarboxazid	
274	Serotonin	323
274	Theobromine	
274	Venlafaxine	280
275	Bufexamac	
275	Cisapride	308
275	Deserpidine	
275	Diclofenac	
275	Meclofenamic Acid	319
275	Procainamide	
275	Theophylline	
275	Thioridazine	
276	Dithranol	
276	Gliquidone	
277	Diflunisal	
277	Ethionamide	
278	Dimenhydrinate	
278	Phenindione	330
279	Ambucetamide	286
279	Idoxuridine	
279	Omeprazole	
279	Pergolide	
280	Dantron	
280	Diazoxide	
280	Mivacurium Chloride	
280	Nicergoline	
280	Procaine Benzylpenicillin	
280	Quinidine	330
280	Quinine	330
280	Sulindac	327
280	Tryptophan	288
280	Yohimbine	
281	Atracurium	
281	Ethylmorphine	
281	Felodipine	
281	Flunixin	
281	Noscapine	315
281	Quazepam	
281	Trilostane	
282	Butacaine	
282	Clarithromycin	
282	Milrinone	331
283	Acetyldihydrocodeine	
284	Cyproheptadine	
284	Nitrofurazone	
284	Nomifensine	
285	Azathioprine	
285	Benzocaine	
285	Butyl Aminobenzoate	
285	Fenbufen	
285	Mefenamic Acid	
286	Clobazam	
286	Cotarnine	330
286	Famotidine	
286	Pyrimethamine	
287	Ketoconazole	
287	Mupirocin	
287	Phenol	
287	Trimethoprim	
288	Cinchonine	302, 315
288	Methazolamide	
288	Niflumic Acid	
288	Ofloxacin	332
289	Aztreonam	
289	Capreomycin	
290	Mersalyl Acid	
290	Resorcinol	
291	Carbidopa	
291	Nystatin	305, 319
291	Phentolamine	
292	Chlorothiazide	
292	Fendosal	
292	Hydrastine	
292	Oxymorphone	
292	Rabeprazole	
292	Thymol	
293	Flucytosine	
293	Metallibure	
293	Naltrexone	
293	Warfarin	308
294	Pentaerithrityl Tetranitrate	
294	Tyramine	
295	Diethylthiambutene	
295	Dipyridamole	
295	Ethyl Hydroxybenzoate	
295	Fenoterol	
295	Triclosan	
296	Noradrenaline	
296	Propyl Hydroxybenzoate	
297	Benzthiazide	
297	Dihydromorphine	
297	Isoprenaline	
297	Nalbuphine	
297	Orciprenaline	
297	Pirenzepine	
297	Teicoplanin	
297	Terbutaline	
298	Aloxiprin	
298	Isoniazid	
298	Morphine	
298	Nalmefene	
298	Parachlorophenol	
298	Salicylic Acid	
299	Butorphanol	
299	Diamorphine	
300	Buprenorphine	
300	Ketobemidone	
300	Xamoterol	
301	Cyprenorphine	
302	Etorphine	
302	Iprindole	
302	Methyldopa	
303	Gloxazone	
303	Oxymetazoline	
303	Thiopental	
304	Famciclovir	

λ *max* *nm*	*Compound*	*Subsidiary Peaks nm*
304	Zopiclone	
305	Nimorazole	
305	Tetrazepam	
305	Thiamylal	
306	Rescinnamine	
306	Thialbarbital	
307	Buspirone	
307	Nialamide	
308	Lysergic Acid	
309	Albendazole	
309	Lysergamide	
310	Bacampicillin	
310	Ergotamine	
310	Hydroxystilbamidine	
310	Lysergide	
310	Methylergometrine	
310	Phenprocoumon	
311	Coumatetralyl	
311	Ethyl Biscoumacetate	
313	Nizatidine	
313	Thioridazine	
314	Dantrolene	
314	Dicoumarol	
315	Oxymetholone	
317	Bumetanide	
319	Liothyronine	
319	Metronidazole	
319	Pentachlorophenol	
320	Tioguanine	
322	Dimetridazole	
324	Nisoldipine	
324	Oxyclozanide	
325	Levothyroxine	
330	Harmaline	
330	Xipamide	
332	Pralidoxime Chloride	280
334	Niclosamide	377
341	Salsalate	
353	Obidoxime Chloride	
371	Lornoxicam	
377	Metaclazepam	274, 236

D. Values not covered in Tables A–C

λ *max* *nm*	*Compound*	*Subsidiary Peaks nm*
192	Memantine	
200	Tadalafil	285
201	Aldicarb	
205	Adrenalone	232, 280, 312
205	Detajmium	245
205	Isoproturon	240
206	Emedastine	227, 280, 286
206	Leflunomide	261
206	Trichlorophenoxyacetic Acid	236, 289
207	Granisetron	303
208	Cilazapril	
210	Duloxetine	
211	Bunazosin	244
213	Galantamine	233, 289
213	Tropisetron	231, 249, 283
214	Alfacalcidol	267
214	Mycophenolate Mofetil	251, 303
215	Apraclonidine	252, 300
215	Mevinphos	
215	Monocrotophos	
217	Nebivolol	283
219	Disulfiram	
220	Fusarenone-X	
220	Imidacloprid	270
220	Trifluoromethylphenylpiperazine	250
221	Bopindolol	267
221	Deslanoside	
221	Oxyphencyclimine	
222	Moxonidine	258
224	Aniracetam	288
225	Methoprotryne	
225	Trichlormethiazide	267, 314
225	Zolmitriptan	301
230	Cantharidin	
230	Dienestrol	
230	Imolamine	
231	Padimate	314
233	Epirubicin	253, 292
233	Spiramycin	
234	Amylocaine	275
234	Thioproperazine	263, 312
238	Zanamivir	
240	Oxyphenisatine	
241	Norbolethone	
242	Domoic Acid	
242	Triazophos	
243	Metenolone	
244	Monolinuron	
244	Phenacetin	
247	Efavirenz	293
247	Flupirtine	346
247	Phentermine	251, 257, 263
249	Aldicarb	
249	Alpidem	
250	Citrinin	331
250	Dehydrochloromethyltestosterone	
250	Fenamiphos	
250	Furanocoumarins	
250	Rosuvastatin	
251	Phenylpropanolamine	257, 262
251	Pseudoephedrine	257, 263
252	Azide	
253	Idarubicin	289
253	Penciclovir	278
258	Fludarabine	
264	Pindolol	287
265	Tetryzoline	272
266	Acecainide	
270	Nadolol	278
271	Pheneticillin	275
275	Oxabolone	
280	Ricin	
282	Tetrabenazine	
283	Pholcodine	
286	Pramocaine	
289	Torasemide	
290	Glaucine	310
300	Fenticlor	
302	Amatoxins	

E. No significant absorption

Compound	*Compound*
Acetylcysteine	Dimethadione
Acetyldigitoxin	Dimethyl Sulfoxide
Aldrin	Emylcamate
Allylestrenol	Ethambutol
Amantadine	Ethinamate
Ambazone	Ethylestrenol
Ametazole	Guanethidine
Amikacin	Heptaminol
Aminotriazole	Hexamethonium Bromide
Androsterone	Hexetidine
Apronal	Histamine
Arecoline	Hydroxycarbamide
Bemegride	Isometheptene
Betaine	Isosorbide Dinitrate
Bethanechol Chloride	Isosorbide Mononitrate
Butylchloral Hydrate	Lincomycin
Cadaverine	Lynestrenol
Captopril	Mannomustine
Carbachol	Mebutamate
Carbromal	Mecamylamine
Carisoprodol	Menthol
Chloral Betaine	Meprobamate
Chloral Hydrate	Mesterolone
Chlorobutanol	Methoxyflurane
Choline	Neomycin
Cimetidine	Noretynodrel
Clindamycin	Pancuronium Bromide
Clodantoin	Paramethadione
Cloxacillin	Pentetrazol
Conessine	Pentolonium Tartrate
Cropropamide	Perhexiline
Crotamiton	Piperazine
Crotetamide	Pipobroman
Cyclopentamine	Propylhexedrine
Cyclophosphamide	Sparteine
Cycloserine	Suxamethonium Chloride
Desferrioxamine	Suxethonium Bromide
Dexpanthenol	T-2 Toxin
Dieldrin	Thiotepa
Diethylcarbamazine	Tobramycin
Diethyltoluamide	Tosylchloramide Sodium
Dihydrostreptomycin	Tuaminoheptane
Dimercaprol	Tybamate

13 Infrared Peaks

The table below lists the 6 major absorption bands which have been selected from recorded spectra over the range 3500 to 450 cm^{-1} (5–15 μm). The selected peaks are the 6 most intense peaks, except that peaks in the region where Nujol absorbs (1490–1320 cm^{-1}, 6.7–7.6 μm) have been omitted. The peaks are arranged in descending order of amplitude. Shoulders have been chosen only if they are clearly resolved and the point of maximum absorption can be easily determined. Where there is more than one peak of the same intensity at the sixth intensity level, the choice has been arbitrary. It should be noted that, because of variations in instruments and conditions, other determinations of the spectrum may not give peaks with the same relative intensities. In particular, the order of the 2 most intense peaks may be reversed and this should be taken into consideration when using the index.

To identify an unknown compound, the wavenumbers of the 2 most intense peaks should be compared with the table, initially allowing a tolerance of ±4 cm^{-1}, to produce a list of possible compounds. Comparison of the six most intense peaks with this list may allow a tentative identification; reference should then be made to the spectra given in the monographs.

If a tentative identification cannot be made, it may be necessary to use a wider coverage, or to search on the third most intense peak.

Wavenumbers (cm^{-1})						*Compound*
462	1515	–	–	–	–	Vetrabutine Hydrochloride
675	1262	1305	1556	1215	788	Nicotinyl Alcohol
680	665	695	778	840	950	Lindane
683	1040	1014	1053	813	1266	Lynestrenol
685	1629	776	1174	715	1205	Cetylpyridinium Chloride
688	855	704	781	917	957	Benzene Hexachloride (Nujol Mull)
690	755	1235	1595	1497	811	Phenol (Nujol Mull)
691	769	1529	720	1164	998	Triphenyltetrazolium Chloride
694	751	1086	1041	1010	714	Captodiame
694	1190	740	1075	1020	1694	Alverine
695	745	755	942	1146	1608	Phenethylamine
695	750	1370	–	–	–	Dimevamide
695	1723	1231	1054	1162	751	Benactyzine
696	745	1111	1022	1592	1067	Benethamine
696	1036	835	723	1255	893	Aldrin
696	1117	755	751	1030	1298	Cycrimine
697	701	749	773	1123	1219	Difenidol
697	746	714	724	1052	1515	Pargyline
697	1051	1020	1000	740	1115	Etafedrine
699	1175	760	1199	1292	1070	Mebanazine (Nujol Mull)
700	720	760	940	805	1491	Meclozine Hydrochloride
700	740	1495	1090	1605	825	Amfetamine (Thin Film)
700	746	1030	1500	1055	1590	Phenylpropanolamine Hydrochloride
700	747	1136	1027	1592	1075	Prenylamine
700	756	746	1128	1075	1175	Procyclidine
700	760	1298	1020	892	869	Phencyclidine Hydrochloride
700	1040	762	1025	1605	1200	*N*-Methyltryptamine
700	1590	1123	1086	1041	751	Doxylamine
700	1664	764	1590	1315	979	Ambutonium Bromide
701	740	690	1585	1300	1065	Pipradrol
701	1230	1682	1287	973	1594	Diethylpropion Hydrochloride
702	691	1138	964	740	1000	Cinnarizine
702	726	780	875	1618	1211	Benzalkonium Chloride
702	735	1121	997	760	1153	Biperiden (Nujol Mull)
702	745	1316	1063	971	1155	Azacyclonol
702	753	858	1115	1215	1256	Moexipril
702	756	973	1206	1196	935	Trihexyphenidyl Hydrochloride
704	765	740	1492	1092	981	Selegiline Hydrochloride
704	1036	764	1005	1210	1595	Pseudoephedrine Hydrochloride
704	1265	1020	1075	1612	826	Chlordane
705	920	1115	772	1045	1061	Tricyclamol Chloride
705	1095	749	759	1020	952	Bibenzonium Bromide
708	699	752	1102	742	1134	Emepronium Bromide
708	756	1042	797	738	1492	Fencamfamin Hydrochloride (Nujol Mull)
708	1250	1230	1010	765	699	Diphemanil Metilsulfate
709	1689	1296	667	935	685	Benzoic Acid
710	1709	769	1107	943	1133	Methadone Hydrochloride (Nujol Mull)
712	1022	810	1575	1310	1040	Nicotine (Thin Film)
713	754	1103	1017	1180	991	Diphenhydramine Hydrochloride
714	787	1205	1220	928	1515	Chloroform
716	743	851	833	1244	1618	Diethylthiambutene Hydrochloride
716	756	701	984	1125	1496	Cyclizine Hydrochloride
717	1492	710	769	1162	729	Tridihexethyl Chloride
720	760	775	1112	1130	1038	Nefopam Hydrochloride
722	698	1612	1204	875	790	Cetalkonium Chloride
727	776	700	802	1515	970	Tolpropamine Hydrochloride
728	1695	833	1086	1050	1010	Benoxaprofen
730	1285	705	1495	1610	1510	Phentermine Hydrochloride
731	809	826	931	1035	1064	5-Methoxy-*N*,*N*-diisopropyltryptamine
735	750	1310	710	1300	1590	Phenothiazine
736	809	1036	1384	1489	1204	2C-T-2 Hydrochloride
736	1066	811	746	1245	1260	Pericyazine
737	1134	1316	695	1122	1212	Mebhydrolin
738	1650	1612	1145	1173	1040	Iprindole
740	710	1573	1175	1018	918	Diphenyl
740	747	765	1230	1110	756	Imipramine Hydrochloride
740	1111	1063	1086	1234	724	Diethyltryptamine
740	1306	902	1095	1279	1231	Tiabendazole
742	1039	702	1052	990	1162	Methylephedrine
742	1585	1233	1035	909	1494	Tryptamine
743	1113	1235	1050	812	1010	Dimethyltryptamine
745	1510	1602	1187	1258	1035	Aprindine Hydrochloride
745	1583	697	1019	707	1139	Fenproporex Hydrochloride
745	1593	725	1495	690	1275	Bamipine
746	741	763	1230	1590	1104	Desipramine Hydrochloride
747	698	1060	1491	1590	1085	Metamfetamine Hydrochloride
747	1240	1561	1125	1095	1220	Chlorpromazine
748	765	740	833	1111	1010	Clemizole
748	918	1063	990	1111	1639	Azapetine
748	1245	1587	1282	1562	1219	Diethazine
748	1248	1260	1305	1590	1275	Alimemazine Tartrate
748	1248	1590	1282	1568	1125	Profenamine
748	1279	1244	1163	1143	1570	Perazine
750	729	1052	1162	1086	1280	Metixene
750	768	1219	1250	1162	1282	Pecazine
751	1250	742	1220	729	1042	Mequitazine
751	1250	1234	1287	770	1162	Promazine Hydrochloride
751	1317	1235	1504	1245	1567	Harman
752	1225	805	842	1212	1237	Clomipramine Hydrochloride
752	1271	1124	803	984	795	Azatadine
752	1280	1569	1165	1242	1145	Prochlorperazine
753	1242	1319	1222	1277	1031	Methdilazine Hydrochloride
754	899	1090	800	877	1587	Cinchonidine
754	1248	796	1234	1281	1211	Thioridazine
754	1653	1221	1186	1575	1316	Propiomazine Hydrochloride
754	1656	700	1492	1590	1117	Morazone
755	1155	790	1237	1115	1126	Opipramol
756	700	1035	770	1190	735	Clofedanol
756	741	1103	1092	1046	1280	Ajmaline
756	742	768	720	775	1590	Nortriptyline Hydrochloride
756	770	746	969	1014	1258	Amitriptyline Hydrochloride
757	743	732	1134	1060	1020	Benzoctamine
757	1033	1265	740	1098	1149	Butriptyline
757	1294	917	1145	1247	1155	Perphenazine
757	1295	780	1592	1162	1110	Prothipendyl
758	1229	733	1129	1259	1287	Promethazine Hydrochloride
758	1657	1288	1210	1250	1150	Salicylic Acid
759	1140	1592	1032	1308	935	Maprotiline Hydrochloride
759	1250	1698	699	731	1199	Cinchophen
759	1271	747	1575	1242	932	Levallorphan (Nujol Mull)
760	1110	1505	1590	990	909	Cinchonine
760	1556	1645	1655	750	1612	Aminoacridine
760	1695	1169	946	–	–	Sildenafil
761	842	1618	1003	1310	827	Triazolam
763	727	747	1252	963	717	Dosulepin Hydrochloride

Wavenumbers (cm^{-1})						*Compound*
763	1590	724	747	1570	1050	Dimetindene
763	1656	1063	1175	674	1093	Mazindol
764	778	800	968	988	880	Cyclobenzaprine
764	1410	1615	1380	1040	1524	Etizolam
764	1674	1708	1242	1268	829	Fenbufen
765	752	708	1075	741	1205	Clotrimazole
767	698	1446	1496	1383	1242	Budipine
768	1096	1250	1288	1053	890	Pindolol
775	790	1500	1100	1022	850	Clofenotane
775	1219	1450	1492	1515	1667	Pyrrocaine Hydrochloride
775	1580	825	990	1509	1146	Triprolidine Hydrochloride
776	714	752	1501	1141	1020	Prolintane Hydrochloride
776	1104	832	746	1030	1170	Chlorprothixene
777	756	815	786	1640	960	Cyproheptadine Hydrochloride
787	772	1138	1254	1314	742	Mianserin Hydrochloride
790	833	1145	1186	917	980	Chlorobutanol (Nujol Mull)
792	750	768	1490	730	1595	Protriptyline Hydrochloride
793	1080	1492	1508	1385	843	Methylthioamfetamine Hydrochloride
800	1515	1065	1130	1290	1205	Norcodeine
800	1515	1612	1587	1234	1315	5-Methyltryptamine Hydrochloride
805	1243	1118	945	1086	833	Morphine
815	1492	699	953	1075	840	Zimeldine
817	1248	1290	1095	1160	952	Thymol
818	1493	1092	1020	848	754	Chlorphentermine Hydrochloride
828	1590	1284	902	1148	710	Protionamide
830	1090	1051	1020	690	1300	Butylchloral Hydrate
832	1151	1673	1217	1136	1598	Haloperidol
832	1228	1066	1264	1494	1108	Bamethan
834	1507	1034	1226	1160	1601	Citalopram
835	1083	1300	970	1620	–	Chloral Hydrate
835	1510	1054	1271	1098	1611	Oxedrine
836	1261	1236	1042	1061	733	Psilocin
840	1622	1522	1084	802	1577	Histamine Hydrochloride
840	1630	1085	1130	730	1500	Chloral Betaine
848	811	1038	700	1005	912	Dieldrin
851	750	724	1049	889	1010	Endrin
887	1538	784	823	1297	1584	Acetarsone
896	–	–	–	–	–	Nitrendipine
905	1008	999	1020	1101	985	Allylestrenol (Nujol Mull)
913	970	1630	945	1063	1000	Hexamethonium Bromide
913	1143	1312	–	–	–	Taurolin
917	1126	1156	995	1030	1050	Methylpentynol
932	1255	729	718	669	815	Thiotepa
935	950	830	1010	1063	980	Ethchlorvynol
949	1089	869	1231	1000	1740	Acetylcholine Chloride
952	1123	1162	819	1111	1298	Isopropyl Alcohol
953	1198	808	784	1266	728	Clioquinol
961	888	831	1621	985	1020	Mebezonium Iodide
961	909	952	719	970	724	Cetrimide
967	1049	1226	1278	1158	1473	Fenamiphos
970	980	1185	1616	1150	1205	Hexylresorcinol
970	1080	1033	1060	1115	1495	Nalbuphine
973	1059	1079	1712	893	1000	Ergocalciferol
975	994	964	1299	1142	1165	Ethylestrenol (Nujol Mull)
975	1090	1672	1110	1065	1025	Iproniazid
980	1075	1219	847	1162	1052	Cineole
980	1238	835	1157	1129	792	Hydroxyephedrine
988	725	760	706	1087	870	Chlorcyclizine Hydrochloride (Nujol Mull)
994	699	754	1049	1242	670	Ephedrine Hydrochloride
995	1018	1087	772	1492	820	Pyrrobutamine Phosphate
996	1090	1637	1600	1282	847	Obidoxime Chloride
1000	1560	1570	1650	–	–	Picloxydine Dihydrochloride
1000	1666	833	781	1041	775	Azinphos-(Me)
1002	1131	754	800	1075	694	Buclizine
1009	1095	699	–	–	–	Embramine
1010	1065	1038	1103	1183	1126	Amphotericin B
1010	1666	819	666	1538	1176	Dimethoate
1012	1091	817	1121	951	1000	Paradichlorobenzene
1015	1041	667	1548	1602	1190	Diamthazole
1016	1108	1050	1080	940	699	Phenelzine Sulfate
1017	1030	1070	987	1090	1080	Isothipendyl
1018	1748	1174	1209	824	1250	Malathion (Thin Film)
1019	1083	1421	1280	–	–	Mannitol
1020	925	1515	1219	1587	970	Parathion
1020	1230	1290	1210	1530	1605	Rimiterol Hydrobromide
1020	1250	775	793	826	1190	Demeton-S-methyl
1020	1587	971	1562	823	1159	Dimpylate
1022	1050	990	1270	1235	1170	Dimercaprol
1023	1112	695	963	1153	743	Tranylcypromine Sulfate (Nujol Mull)
1023	1250	1200	970	1718	1585	Nitrofurazone (Nujol Mull)
1026	1111	1312	1136	1653	990	Ascorbic Acid (Nujol Mull)
1030	1143	1127	1070	1050	1630	Guanethidine Sulfate

Wavenumbers (cm^{-1})						*Compound*
1031	952	666	798	2983	2941	Disulfoton
1031	1018	1076	975	1582	1279	Trometamol
1031	1293	907	–	–	–	Tributyl Phosphate
1033	930	805	1489	1442	1189	Methylenedioxymetamfetamine Hydrochloride
1033	1007	1575	1300	1078	1139	Coniine Hydrobromide (Nujol Mull)
1033	1068	1143	1123	980	1516	Kanamycin Sulfate
1033	1153	1458	–	–	–	Acarbose
1035	1257	1222	1497	748	1070	Chelidonine
1040	1590	845	770	660	1260	Tobramycin
1045	695	754	704	1091	976	Cathine
1046	1026	995	1102	1078	979	Menthol (Nujol Mull)
1046	1714	1278	775	2964	599	Omethoate
1047	1248	1124	1493	756	748	Mephenesin (Nujol Mull)
1050	752	1239	1282	1562	1091	Mesoridazine
1050	1168	1074	1010	1108	1734	Erythromycin
1050	1630	1120	1145	1545	940	Amikacin
1052	894	901	967	862	1075	Colecalciferol
1052	1163	1122	994	1016	1730	Spiramycin
1052	1170	1108	1734	–	–	Clarithromycin
1052	1268	1500	1111	793	934	Codeine
1054	700	742	1191	1204	1075	Benzatropine
1055	830	971	1243	1359	1539	Fenitrothion
1056	967	1018	1009	870	980	Dihydrotachysterol
1058	1015	885	913	1153	966	Propylhexedrine Hydrochloride
1059	1022	800	959	952	839	Cholesterol (Nujol Mull)
1059	1040	751	760	738	1114	Deptropine
1061	1158	850	978	1320	2971	Dichlorvos
1062	1002	1563	1709	1101	1258	Natamycin
1062	1618	862	1266	1653	1089	Isosorbide (Nujol Mull)
1064	1045	1129	1499	1185	1264	Ethylmorphine Hydrochloride (Nujol Mull)
1065	1013	1298	1284	1102	1581	Mannomustine Hydrochloride
1065	1025	695	758	1272	890	Amygdalin
1065	1455	1345	–	–	–	Azacosterol
1066	1690	1729	1140	1008	1242	Noretynodrel
1067	1000	1570	1175	1316	847	Nystatin (Nujol Mull)
1067	1094	1007	696	1041	746	Bromazine
1070	709	1050	696	765	1101	Diphenylpyraline Hydrochloride
1070	1620	1650	–	–	–	Norgestrel
1071	1035	1733	1140	1165	1640	Strophanthin-K
1072	1058	1010	1740	1168	990	Digitoxin
1075	775	1587	1041	1030	1123	Clorprenaline
1075	1027	1145	1109	1178	1002	Cytarabine
1075	1038	1263	1228	1213	822	Salbutamol
1075	1052	1162	1111	1010	1587	Tylosin
1075	1709	1055	1020	1160	1110	Digoxin
1078	1123	772	1634	1175	1672	Sulfasalazine
1082	1130	1005	1149	1063	800	Hydroxyzine
1083	757	695	965	990	1030	Phenmetrazine Hydrochloride
1084	1110	1584	1041	763	1010	Carbinoxamine
1085	1014	1730	1163	1130	864	Metildigoxin
1085	1319	827	1302	1038	812	Miconazole Nitrate
1086	1111	1318	1327	–	–	Hydromorphinol
1086	1173	1605	1010	1305	995	Orciprenaline Sulfate
1086	1298	1538	1149	689	917	Sulfaethidole
1088	1490	1010	760	700	990	Chlorphenoxamine Hydrochloride
1090	1011	700	763	1121	1210	Clemastine
1090	1061	1142	987	1050	1009	Ethambutol
1090	1230	795	1264	1247	1724	Dichlorophenoxyacetic Acid (Nujol Mull)
1090	1310	805	825	1010	1040	Econazole Nitrate
1092	1070	1248	1262	1582	1217	Nadolol
1092	1273	1713	1235	710	1020	Aconitine
1093	854	1300	910	1176	1234	Hexetidine
1093	1494	1280	1011	1042	1600	Trimetazidine
1094	1113	1163	1074	975	1205	Metaldehyde
1095	1085	1015	1180	807	830	Phenaglycodol
1095	1170	946	860	846	763	Paraldehyde
1095	1605	1266	1172	1698	1527	Benzonatate
1096	950	1068	982	1175	1125	Stanozolol
1100	1120	1260	1000	780	1600	Gallamine Triethiodide
1101	1153	1270	799	1207	1316	Amidefrine
1102	704	757	1117	1042	917	Tofenacin Hydrochloride
1102	1242	1190	1605	1517	1280	Hexoprenaline Sulfate
1103	1270	772	1580	795	1240	Propranolol Hydrochloride
1106	1702	1365	1458	–	–	Alachlor
1107	1227	926	1124	709	876	Triclofos Sodium (Nujol Mull)
1109	1154	854	978	1323	–	Trichlorfon
1111	711	1162	696	763	1587	Mephentermine
1111	1052	1075	1010	1162	1190	Oleandomycin Phosphate
1111	1068	1078	1592	1098	890	Mecamylamine Hydrochloride (Nujol Mull)
1111	1666	1052	1724	1250	1515	Dihydrostreptomycin

Wavenumbers (cm^1)						Compound
1113	1195	1205	1155	1088	1005	Etorphine
1113	1630	1495	1226	1591	1034	Ethacridine
1114	1316	1145	1081	755	1255	Trifluoperazine Hydrochloride
1115	1176	1653	1284	1265	1600	Flufenamic Acid
1115	1495	1264	1665	1592	748	Guanoxan
1116	767	1245	1144	1084	836	Fluphenazine Hydrochloride
1117	1316	1159	1237	1075	1030	Triflupromazine
1118	874	1288	923	1026	1036	Putrescine
1118	969	1250	1043	1500	1098	Pholcodine
1118	1070	1045	980	962	1015	Sparteine
1118	1098	1069	1044	1195	1210	Pseudomorphine
1119	1320	1160	1081	1253	1287	Flupentixol
1120	1060	1625	1525	1290	880	Gentamicin
1120	1105	697	1083	752	1630	Phendimetrazine
1120	1220	1710	1088	1180	1740	Methoserpidine
1120	1225	1272	805	1584	938	Amylmetacresol
1120	1230	1680	1175	1610	1038	Vindesine Sulfate
1120	1279	1594	1493	1097	1252	Ethomoxane
1121	1222	1508	1252	1205	1022	Dehydroemetine Hydrochloride
1122	1315	1572	1237	1142	1165	Flunixin
1123	1219	1703	1585	1497	1171	Hexobendine
1123	1230	1575	1505	1616	990	Trimethobenzamide
1123	1520	1732	1337	–	–	Mivacurium Chloride
1124	1220	1087	1208	963	1618	Sulfachlorpyridazine
1125	1260	1138	1595	1650	1070	Sulfisomidine
1126	1630	1596	1235	1650	1565	Trimethoprim
1127	1242	1592	996	1513	834	Mescaline Hydrochloride
1130	1092	1267	1247	691	1220	Glymidine Sodium
1130	1515	1587	1612	1639	1315	Ambazone
1130	1527	929	1274	1082	1592	Sulfathiazole
1130	1595	1510	1240	1000	1151	Trimethoxyamfetamine
1131	1642	1229	1110	1592	1001	Trimetozine
1135	1010	1600	1618	1630	720	Homochlorcyclizine
1138	1158	1172	1293	889	1192	Sultiame
1139	1294	1166	1495	1112	812	Diazoxide
1140	1600	1725	3100	–	–	Furanocoumarins
1140	1238	1735	1175	1282	1090	Clofibrate
1141	1405	1253	–	–	–	Quazepam
1143	1585	1091	840	1529	1667	Succinylsulfathiazole
1143	1668	1565	1240	1590	1260	Furosemide
1145	1160	1599	1621	685	1306	Sulfamethoxazole
1145	1264	1552	1090	825	1600	Sulfacetamide
1145	1315	1162	1601	1642	1085	Sulfametopyrazine
1145	1595	1304	1635	682	1090	Sulfadimidine
1148	1507	1313	1592	675	1630	Sulfaphenazole
1148	1733	704	1199	735	990	Cyclopentolate Hydrochloride
1149	1590	1560	1316	1088	1618	Sulfamerazine
1149	1603	774	962	1164	1289	Resorcinol
1149	1603	1316	1637	1099	1294	Sulfanilamide
1150	1090	757	740	1052	1123	Clopenthixol
1150	1276	1592	1107	685	1633	Dapsone
1150	1310	750	1665	1102	1248	Metopimazine
1151	1180	1125	1052	1300	1525	Hydroflumethiazide
1151	1316	1299	897	1089	1592	Mafenide
1153	1597	1140	1284	1090	925	Sulfametoxydiazine
1155	1248	1724	761	1200	755	Methanthelinium Bromide
1156	719	765	1176	957	747	Tiotixene
1156	1042	1212	720	960	762	Thioproperazine Mesilate
1157	1305	1595	1090	1123	1620	Chlorothiazide
1158	1177	1597	772	1073	1509	Trichlormethiazide
1158	1317	1590	1020	858	848	Chloroxylenol
1158	1595	715	1063	1515	1300	Methyclothiazide
1159	1045	1718	1176	700	1212	Atropine Methonitrate (Nujol Mull)
1159	1599	1130	1305	1630	1088	Sulfamethoxypyridazine
1160	947	1311	1092	1078	934	Diprenorphine
1160	992	785	1512	1230	1585	Sotalol Hydrochloride
1162	1120	1597	1071	1240	1500	Polythiazide
1162	1176	819	1041	746	909	Mefruside
1162	1176	882	702	907	1123	Diclofenamide
1163	1172	1603	1312	781	1510	Ethiazide (Nujol Mull)
1163	1635	1578	1560	975	1525	Xipamide
1164	1599	1042	1508	750	700	Antazoline Mesilate
1164	1730	1118	1026	1056	1195	Atropine Oxide
1165	817	1629	1200	1282	1239	Harmine
1165	1031	1681	1531	905	1264	Acetohexamide (Nujol Mull)
1165	1116	1070	698	793	1202	Fenfluramine Hydrochloride
1165	1316	1105	825	710	920	Tosylchloramide Sodium
1165	1735	710	745	1250	955	Pipotiazine
1166	1633	1598	1092	1647	690	Sulfafurazole
1167	1183	1742	1080	833	1768	Meclofenoxate
1167	1548	1316	1665	671	1115	Acetazolamide
1168	1138	1605	1309	1511	1060	Cyclopenthiazide
1169	1221	1291	1637	1083	1549	Flecainide
1170	770	1290	1620	1700	2850	Biriperone

Wavenumbers (cm^1)						Compound
1170	904	814	1322	1619	1638	Nalmefene
1170	1155	1306	1518	1621	750	Bendroflumethiazide
1170	1490	1263	824	1224	1036	Ethoxzolamide
1170	1633	1217	1660	1715	1045	Bromocriptine Mesilate
1170	1681	1527	837	1302	1592	Phthalylsulfacetamide
1170	1682	1520	1710	1050	1100	Glibornuride
1174	1638	1064	1229	1284	850	Paraquat
1175	1523	1220	840	857	1615	Hexestrol
1175	1538	1582	1510	1307	1274	Stilbazium Iodide
1175	1721	1052	874	1072	709	Hyoscine Butylbromide (Nujol Mull)
1176	1314	1167	1126	1603	1269	Epithiazide (Nujol Mull)
1178	934	861	980	962	773	Busulfan
1178	1605	1229	1672	1575	965	Dyclonine Hydrochloride
1180	1160	1310	1502	1620	1590	Benzthiazide
1181	1258	1515	825	746	1119	Dimetridazole
1182	1050	1661	1730	1248	1194	Benorilate
1183	1688	1305	1755	925	1219	Aspirin
1184	1212	1761	1261	1504	931	Moxisylyte Hydrochloride
1185	1512	1490	–	–	–	Paroxetine Hydrochloride
1185	1620	1075	1762	1580	1290	Tienilic Acid
1186	1359	1458	1256	1422	1520	Panidazole
1187	1535	1070	1265	745	1160	Metronidazole
1188	1012	1032	1120	680	815	Bretylium Tosilate
1188	1604	1260	1028	1676	1003	Pentamidine Isetionate
1188	1727	1495	1232	1162	1011	Oxyphenisatine
1190	1227	1041	1031	779	823	Clomethiazole Edisilate
1190	1647	1520	1309	1764	816	Clioxanide (Nujol Mull)
1190	1654	1266	1047	1600	1306	Dibrompropamidine Isetionate
1190	1658	1730	1608	890	1053	Beclometasone Dipropionate
1190	1710	1025	–	–	–	Apoatropine
1192	1715	1163	1101	700	1149	Piperidolate
1195	1225	1582	1627	720	1105	Serotonin Oxalate
1195	1508	1610	1580	1592	1247	Phentolamine
1195	1590	1672	1250	1562	1052	Amicarbalide
1196	1028	1667	1597	742	1053	Hydroxystilbamidine Isetionate
1200	1040	770	760	1595	1090	Mexiletine Hydrochloride
1200	1218	1168	1760	1150	1122	Acetorphine Hydrochloride
1200	1219	1613	1048	1280	1495	Propamidine
1200	1287	1075	1114	1518	1160	Isoetarine
1200	1623	1176	1504	1515	1252	Aminonitrothiazole
1202	1220	1050	805	1185	1294	Butylated Hydroxyanisole
1203	1527	1166	1587	1155	1219	Oxyclozanide
1204	781	806	920	1265	869	Diiodohydroxyquinoline
1204	1219	1754	917	1162	1492	Cyclofenil
1205	1176	833	1250	1512	1610	Diethylstilbestrol
1205	1190	1057	1160	1220	717	Dimetotiazine Mesilate
1206	827	1173	1510	1248	775	Dienestrol
1208	1041	1541	1515	1634	1094	DOM
1208	1138	1744	1500	905	1194	Thebacon
1208	1182	1094	–	–	–	Amlodipine
1208	1499	1392	1040	850	811	2C-T-7
1209	816	1515	741	1176	1105	*p*-Cresol (Nujol Mull)
1209	1130	1247	1180	1770	1727	Syrosingopine
1210	1174	1042	1135	760	1010	Thenium Closilate
1210	1192	1520	760	1240	813	Hydroquinone
1210	1231	1155	1069	1610	1042	Terbutaline Sulfate
1210	1248	1276	1572	1025	1525	Quinuronium Sulfate
1210	1705	1530	1600	1305	1050	Diperodon
1210	1716	692	–	–	–	Ethylpiperidyl Benzilate
1210	1730	1252	755	1008	1162	Pentapiperide Metilsulfate
1210	1755	1075	1105	1160	1250	Alpha Tocoferil Acetate
1210	1760	1163	1070	775	1030	Acetomenaphthone
1211	1170	1156	754	1234	810	Tolnaftate
1212	1198	1754	1162	1500	909	Bisacodyl
1212	1708	712	1112	1132	664	Etomidate
1213	1708	1305	1104	1590	1520	Proxymetacaine
1215	1170	1050	750	1520	790	α-Methyltryptamine Esilate
1215	1562	1592	1257	1087	1275	Rafoxanide
1216	1266	1132	1510	1715	1174	Mebeverine Hydrochloride
1217	1121	1252	739	1511	1031	Viloxazine
1218	1653	695	1560	1137	1275	Pemoline
1219	1180	1112	1135	1304	696	Desflurane
1220	1120	1700	1240	1720	1265	Reserpine
1220	1611	1210	1583	1135	800	Griseofulvin
1220	1620	1192	1570	1590	1260	Ranitidine Hydrochloride
1220	1727	1163	763	1062	1093	Mepenzolate Bromide
1222	701	1061	1008	765	1751	Poldine Metilsulfate
1222	1728	746	1265	1250	1200	Thiopropazate Hydrochloride
1224	1005	748	1052	700	770	Hexocyclium Metilsulfate
1224	1010	1059	764	743	1036	Thiazinamium Metilsulfate
1224	1497	1241	705	1613	758	Phenazocine Hydrobromide (Nujol Mull)
1225	1044	975	1088	945	1128	Cyclophosphamide
1225	1127	1715	1100	1184	1590	Deserpidine

Wavenumbers (cm^{-1})						Compound
1225	1719	729	704	1030	1111	Oxyphenonium Bromide
1225	1745	1031	1056	1013	907	Pancuronium Bromide
1226	817	1111	1162	1245	1170	Dichlorophen
1227	1136	1111	1724	1176	1613	Vinblastine Sulfate
1227	1436	1586	–	–	–	Nizatidine
1227	1739	1015	1250	1101	738	Furazolidone (Nujol Mull)
1228	752	1500	1600	689	1080	Domiphen Bromide
1228	1067	917	1147	1250	1605	Estriol (Nujol Mull)
1228	1499	1534	823	1163	1285	Tiocarlide
1228	1758	1710	1278	1010	1043	Alfadolone Acetate
1230	1745	1170	1685	1030	1130	Vincristine Sulfate
1231	1725	1664	1466	–	–	Moracizine
1232	812	671	1010	1044	724	Methenamine (Nujol Mull)
1232	824	1493	1615	792	1052	Bufotenine
1232	1043	818	1109	1490	710	Chlorphenesin
1233	768	1653	1590	858	726	Bephenium Hydroxynaphthoate
1233	1153	1205	1592	762	1003	Oxypertine
1233	1731	1272	1042	1055	1501	Acetylcodeine
1234	720	1307	1499	813	1093	Thiambutosine
1234	1605	1144	1270	1030	910	Thebaine
1235	1032	1499	925	1075	934	Hydrastinine
1235	1215	1510	1619	1030	1105	Quinine
1236	749	1047	1125	1026	1175	Methoxyphenamine (Nujol Mull)
1237	1306	1580	832	1500	1531	Azathioprine
1238	1264	1609	1214	854	1054	Pentazocine
1238	1495	753	1278	1578	1608	Levorphanol
1238	1724	1646	1219	881	1597	Chlormadinone Acetate
1238	1730	1213	1050	1030	1131	6-Monoacetylmorphine
1240	750	698	1494	1598	1586	Phenoxybenzamine Hydrochloride
1240	840	760	1515	708	1185	Octafonium Chloride (Nujol Mull)
1240	1225	1507	1623	1026	1132	Hydroquinine
1240	1495	1280	1580	756	1610	Dextrorphan
1240	1505	1120	826	1063	769	Benzethonium Chloride
1241	1562	1582	1090	–	–	Demecolcine
1242	746	1031	1492	1079	916	Alprenolol
1242	962	900	1786	1852	1002	Cantharidin (Nujol Mull)
1242	1258	1707	1215	1100	772	Fenclofenac
1242	1280	1580	1495	1505	760	Racemorphan
1242	1667	1744	1718	1203	1610	Desoxycortone Acetate
1244	1655	1513	1555	1265	836	Phenacetin
1244	1666	1510	1595	1078	880	Clefamide
1244	1728	940	1230	922	1050	Naloxone
1244	1733	1633	1204	1515	1123	Benzquinamide
1245	760	940	747	1085	1145	Dihydromorphine
1245	1054	1227	1493	1276	821	Estradiol
1245	1491	752	1030	697	1111	Phenyltoloxamine
1245	1624	1635	1600	1185	1501	Etoxazene
1245	1627	1587	1500	1560	1522	Mepacrine Hydrochloride
1245	1764	1178	1215	911	1736	Diamorphine Hydrochloride (Nujol Mull)
1246	1293	1535	1230	1040	1607	Isoprenaline Hydrochloride
1247	731	747	1630	813	1280	Norharman
1247	1198	1748	1499	1143	806	Methylchlorophenoxyacetic Acid (Nujol Mull)
1247	1437	1614	1192	–	–	Norbutrine Base
1250	752	758	1670	1626	1587	Salicylamide
1250	1500	825	740	750	–	2C-B
1250	1510	1606	1174	813	1184	Chlorotrianisene
1250	1550	1588	1610	1090	1655	Colchicine
1250	1567	976	1098	1064	1650	Rifampicin
1251	1281	1606	1176	1674	1566	Nifursol
1252	1226	1746	1737	1012	1028	Etynodiol Diacetate
1252	1505	1298	1285	1020	1060	Ethinylestradiol
1252	1512	820	1612	1270	869	Hordenine
1253	1274	1500	945	1224	871	Adrenaline
1253	1582	1060	1090	1000	1047	Narceine
1255	761	1208	1733	745	1158	Propantheline Bromide
1255	1060	1035	1612	1291	1241	Mestranol
1255	1510	740	1230	1125	1020	Guaifenesin
1255	1647	1572	1504	757	1163	Mefenamic Acid
1258	750	1500	1120	1092	1037	Oxprenolol Hydrochloride
1258	1497	1527	775	1053	–	Nordefrin
1258	1508	1637	1142	1724	1220	Propanidid
1258	1513	1274	1590	1610	810	Pholedrine
1258	1514	1619	1040	860	820	Quinidine
1258	1689	1235	933	1016	1500	Alclofenac
1259	1517	1599	1102	813	1111	Hydroxyamfetamine
1260	1154	1646	750	678	1120	Saccharin
1260	1598	1637	1211	1613	920	Mexenone
1263	1249	1733	1662	1712	1630	Megestrol Acetate
1263	1304	1216	1591	1136	1063	Metaraminol
1263	1706	1106	713	698	1067	Benzyl Benzoate
1264	740	1620	1597	1152	1197	Dantron

Wavenumbers (cm^{-1})						Compound
1264	1515	1116	1229	1213	1616	Cephaëline Hydrochloride
1265	1293	1216	1200	1066	1137	Noradrenaline Acid Tartrate
1265	1298	1204	752	985	790	Apomorphine
1266	1147	1124	1165	1233	1715	Rescinnamine
1266	1518	1595	822	1495	1612	Tyramine
1266	1600	1779	1692	1176	1120	Procaine Benzylpenicillin
1267	1193	1492	1147	966	913	Disulfiram (Nujol Mull)
1267	1547	1706	1238	1020	966	Sodium Fusidate
1269	825	1209	818	889	1104	Fenticlor
1269	1130	1233	1575	1249	1303	Butorphanol Tartrate
1270	1085	1111	1136	1234	1035	Veratrine
1270	1490	1198	1142	968	915	Sulfiram
1270	1718	1105	710	1068	1022	Hexylcaine
1272	1600	1702	–	–	–	Butethamine
1272	1734	1102	1593	1025	718	Inositol Nicotinate
1273	1595	1681	1630	1111	1163	Butyl Aminobenzoate
1274	1040	1502	1017	763	1056	Benzylmorphine
1275	1165	1598	1694	1639	1111	Butacaine
1275	1500	1118	1050	1250	782	Neopine
1275	1710	710	1110	–	–	Amylocaine
1275	1720	1618	717	1116	1316	Benzoylecgonine
1276	1165	1220	1317	1665	1600	Propyl Hydroxybenzoate
1276	1182	737	1139	960	1213	Hexachlorophene
1277	1212	1015	1540	870	1086	Pyridoxine Hydrochloride
1278	1172	1109	–	–	–	Isobutyl Aminobenzoate
1278	1703	711	1115	1310	1040	Piperocaine
1278	1724	1108	740	699	1587	Benzyl Nicotinate
1279	1508	1263	1026	1292	1238	Papaverine Hydrochloride
1280	1220	1160	1250	760	780	Fazadinium Bromide (Nujol Mull)
1280	1250	1055	1099	801	1124	Clorgiline
1280	1650	1635	852	1090	968	Isosorbide Mononitrate
1280	1680	1598	1170	1315	1634	Benzocaine
1281	751	1718	1306	1075	1103	Nicergoline
1282	820	1709	1244	921	1493	Estrone
1282	990	1010	1587	1612	1204	Doxorubicin Hydrochloride
1282	1668	1506	–	–	–	Phthivazid
1282	1715	710	–	–	–	Amydricaine
1282	1718	1111	741	1022	703	Methyl Nicotinate
1282	1724	1111	740	1020	1587	Butoxyethyl Nicotinate
1284	1601	1042	1238	1575	702	Tramadol Hydrochloride
1285	1650	714	1587	690	1610	Phytomenadione
1286	1724	1110	740	1591	1025	Ethyl Nicotinate
1290	1673	1170	1240	1610	1590	Ethyl Hydroxybenzoate
1290	1680	1565	1270	687	772	Salinazid
1299	1256	1064	1208	1129	676	Pentolonium Tartrate
1300	744	1710	1042	694	1180	Nicotinic Acid
1301	1638	1595	1510	805	1180	Aminosalicylic Acid
1307	774	1222	711	1196	1553	Pentachlorophenol
1308	1261	1210	1136	1063	675	Pempidine
1312	790	1258	1066	1275	1030	Normorphine
1314	1176	1709	1215	818	740	Aminitrozole
1315	1063	1136	833	1204	1234	Methoxyflurane
1316	1190	930	806	1258	782	Broxyquinoline
1317	1150	1665	1170	1110	1595	Trifluperidol Hydrochloride
1318	1180	1150	1168	1602	1060	Hydrochlorothiazide
1319	1295	622	594	–	–	Methyl Bromide
1320	1077	1503	1155	1120	947	Buprenorphine Hydrochloride
1320	1172	1552	965	675	1550	Disulfamide
1330	1260	1165	755	–	–	Amosulalol
1335	1240	1110	–	–	–	Norfluoxetine
1346	811	820	1305	1531	–	Nifuroxime
1353	1541	809	719	1263	1609	Chloronitrotoluene
1387	1347	1444	–	–	–	Clamoxyquin
1392	964	1480	1177	–	–	Chlorisondamine Chloride
1406	1038	850	967	1166	1271	Chlorpyrifos
1412	1121	1028	1285	–	–	Brocresine
1414	774	640	–	–	–	Clenbuterol
1449	1372	1054	942	–	–	Methandriol
1456	1157	1038	775	–	–	Pergolide Mesilate
1457	971	717	–	–	–	Ticlopidine
1458	1393	1355	–	–	–	Pamaquin
1459	1621	1713	1086	–	–	Ofloxacin
1461	1202	748	–	–	–	Propofol
1465	1500	1582	760	1353	–	Phenoxypropazine
1470	1135	825	800	790	760	Sertraline
1470	1356	748	1152	1582	–	Methoxypromazine Maleate
1477	750	1439	768	1006	1198	Doxepin Hydrochloride
1477	1014	968	1620	–	–	Azamethonium Bromide
1477	1202	984	914	–	–	Ditiocarb Sodium
1485	1206	1263	–	–	–	Butoxamine
1490	762	1240	1230	741	749	Trimipramine
1490	1210	1140	810	1020	835	Ibogaine Hydrochloride
1490	1257	1038	1504	799	1188	Methylenedioxyamfetamine

Wavenumbers (cm^{-1})						Compound
1490	1610	697	1316	1540	827	Alprazolam
1492	1250	1271	1227	1036	1128	Protokylol
1492	1512	1598	1247	1175	1035	Mepyramine
1492	1631	1092	698	1010	1600	Fenpiprane
1494	1598	769	1562	1010	1098	Chloropyramine
1495	1271	1040	1250	1055	1149	Dihydrocodeine (Nujol Mull)
1495	1592	1283	1650	1030	757	Alcuronium Chloride
1495	1637	688	750	1665	1050	Amiphenazole
1495	1694	770	1136	1562	1587	Butanilicaine
1496	1219	1022	1276	1179	1050	Methoxamine Hydrochloride
1496	1242	1040	1300	1280	1070	Dextromethorphan
1496	1592	770	1250	955	735	Tripelennamine Hydrochloride
1497	740	698	1028	1125	1220	Benzfetamine
1497	1527	1120	1230	1590	1620	Timolol Maleate
1499	1203	823	–	–	–	Cisapride
1500	1138	1225	1315	1157	825	Penfluridol
1500	1340	1255	1165	–	–	Tamsulosin
1500	1529	1280	1265	1052	1600	Ethylnoradrenaline
1500	1660	1280	1195	1120	1212	Brucine
1500	1665	1593	1195	1570	1265	Benserazide
1503	1287	1190	1174	813	1115	Dopamine
1503	1497	1316	1603	1089	1517	Amantadine Hydrochloride
1504	1247	1208	1145	1225	865	Dimoxyline
1504	1277	1307	1189	1149	1565	Nitroxoline
1505	748	1600	693	1279	755	Thenalidine
1505	1121	1155	1304	805	945	Nalorphine Hydrobromide
1505	1271	1234	1030	1587	1098	Berberine
1505	1645	1220	1155	835	775	Lidoflazine
1506	1217	738	–	–	–	Astemizole
1506	1657	1565	1263	1227	1612	Paracetamol
1507	1279	1144	680	1620	966	Fluconazole
1507	1640	1240	1258	1200	1221	Ketoconazole
1508	1560	1295	1587	747	1083	Clofazimine
1508	1580	1101	903	1681	1248	Carbarsone
1509	779	709	1282	1219	1190	Hydroxyquinoline
1509	1261	1620	1234	854	1030	Hydroquinidine
1510	1209	1256	1610	833	1170	Ritodrine
1510	1220	1265	1280	1125	1240	Metocurine Iodide
1510	1230	1118	1210	824	1635	Pramocaine
1510	1245	1108	1028	1175	810	Metoprolol
1510	1253	1026	1232	1149	1587	Verapamil (Thin Film)
1510	1592	1171	1299	809	1217	Prometryne
1512	1220	750	695	1182	837	Buphenine
1512	1259	1033	808	1190	1623	Methoxyamfetamine Hydrochloride
1512	1282	812	1200	950	785	Halquinol
1513	1243	1179	–	–	–	Venlafaxine Hydrochloride
1513	1604	750	698	1500	732	Histapyrrodine
1513	1664	1080	1050	1302	1250	Clindamycin
1514	1240	745	1220	1043	1500	Isoxsuprine
1514	1256	1228	1110	1200	1150	Emetine
1515	1249	1091	1053	3250	830	Betaxolol
1515	1593	1550	1250	1613	1175	Thonzylamine
1515	1595	1294	1166	806	1140	Ametryne
1515	1603	1580	1661	1250	1305	Thioacetazone
1516	1228	1120	1280	1265	1165	Tubocurarine Chloride
1517	1599	1640	750	1700	692	Warfarin Sodium
1518	1242	1213	1099	840	772	Prenalterol Hydrochloride
1520	1280	1266	1190	1200	1220	Dobutamine Hydrochloride
1520	1562	1620	1600	1128	1290	Chlorproguanil Hydrochloride
1520	1587	1215	806	1266	1133	Terbutryne
1522	1498	1140	1166	1097	1632	Diquat
1524	1160	1623	1718	1276	823	Glibenclamide
1524	1180	1298	1147	1573	770	Piroxicam
1524	1577	1209	1025	1147	1258	Metallibure
1526	1214	1010	1076	1041	1251	Dipyridamole
1527	835	1574	1495	1624	1095	Baclofen
1527	1387	1051	–	–	–	Altretamine
1527	1628	1575	1235	820	1080	Chlorhexidine
1527	1672	1612	738	1595	1289	Dinitolmide
1527	1726	1516	1290	1240	1215	Methyldopate
1528	1690	1650	1159	1032	900	Glipizide
1529	740	1491	1613	1182	1141	Benzydamine
1529	1162	1250	1037	1290	1123	Normetanephrine Hydrochloride
1529	1245	1123	730	1309	1600	Nitroxinil
1529	1537	896	1622	1153	1614	Heptaminol
1530	1300	1230	1275	1580	1715	Acetylcysteine (Nujol Mull)
1530	1634	1666	1562	1587	1149	Buformin
1531	1587	1258	806	1292	1163	Desmetryne
1532	1142	1084	1710	1585	1560	Phthalylsulfathiazole
1534	1635	1492	1575	1600	1170	Proguanil
1535	1115	1250	1000	900	800	Pentetrazol
1538	811	1304	1121	1272	1175	Methoprotryne
1538	1504	753	1618	762	1095	Clopidol
1538	1587	1433	1656	755	1488	Octacaine

Wavenumbers (cm^{-1})						Compound
1538	1600	1266	1277	1160	1117	Rimantadine
1538	1613	1098	1319	1258	1203	Aminocaproic Acid
1539	1612	1295	804	1161	1121	Atrazine
1543	898	1190	1590	1201	1506	Buclosamide
1544	1575	1641	1715	1235	1070	Riboflavin
1549	1134	699	1084	1152	1600	Sulfamethizole
1550	1005	1616	1602	1647	882	Sorbic Acid
1550	1596	1680	719	704	757	Denatonium Benzoate
1553	1621	1289	797	1103	1129	Simazine
1558	1042	1230	987	1026	1517	Noxytiolin
1559	1648	1690	1298	1270	925	Secobarbital Sodium
1560	1691	2676	2982	1458	739	Bupropion
1562	1225	696	1211	1268	1248	Fenoprofen Calcium
1562	1642	1715	1289	1261	1210	Carbenoxolone
1563	864	1613	1212	1105	1067	Thiethylperazine
1563	1316	1647	775	928	1044	Ametazole
1563	1490	1252	–	–	–	Amopyroquine
1565	815	1535	1255	869	847	Amodiaquine
1565	1508	1600	1120	1173	923	Cadaverine
1566	1650	863	1615	1190	740	Methysergide Maleate
1568	1537	1590	1625	1650	1500	Phenformin Hydrochloride
1570	1255	1304	754	1227	1241	Cyclazocine
1570	1271	740	1250	765	675	Thiamazole
1571	1496	1173	1323	–	–	Propazine
1572	756	1504	775	1286	1308	Diclofenac Sodium
1572	1079	1263	1234	763	1511	Bunamidine
1572	1515	1613	1285	1650	1218	Niclosamide
1573	1538	1612	1155	800	870	Chloroquine
1574	1275	740	1246	1150	767	Carbimazole
1574	1610	1584	1536	761	822	Triamterene
1575	1590	1234	752	702	1267	Profadol
1576	1631	1499	1155	1115	751	Quinisocaine
1577	756	1212	1703	1124	1274	Pizotifen
1577	1550	1526	1620	934	877	Cycloserine
1579	1608	1530	1050	1150	810	Hydroxychloroquine
1580	708	795	1225	1205	1300	Meptazinol Hydrochloride
1580	877	741	1000	708	775	Nomifensine Maleate
1580	1030	1620	1180	1080	1260	Δ^8-THC
1580	1040	1620	1180	1130	1050	Δ^9-THC
1580	1040	1620	1180	1130	1050	Δ^9-Tetrahydrocannabinol
1580	1159	1494	682	940	797	Sulfadiazine
1580	1270	1175	1030	1205	752	Levomepromazine
1580	1490	1650	1620	1065	1103	Methylergometrine
1580	1613	1660	1244	1220	1040	Doxycycline Hydrochloride
1580	1620	1063	1075	935	740	Metformin
1582	873	990	730	1029	1081	Perhexiline Maleate
1583	1161	1596	1315	1091	1305	Sulfadoxine
1584	690	860	870	990	1010	Pheniramine Maleate
1584	745	660	1278	1190	765	Aminopromazine Fumarate
1584	1298	1462	1081	–	–	Rabeprazole
1585	750	1003	1067	1565	1040	Brompheniramine
1585	1020	1050	1100	1082	115	Cyclopentamine
1585	1086	746	1010	830	1562	Chlorphenamine
1585	1264	1127	1078	1639	950	Sulfapyridine
1585	1305	1252	1131	1605	1070	Floctafenine
1585	1557	1666	1204	751	1298	Carfenazine
1585	1560	1290	762	1238	1145	Betahistine
1585	1630	1020	1210	1240	1050	CBD
1585	1656	762	685	1248	1493	Phenazopyridine (Nujol Mull)
1587	1079	1228	1259	1575	1022	Gloxazone
1587	1560	760	1275	958	1493	Zomepirac Sodium
1587	1575	1197	699	1150	1248	Levamisole
1587	1669	775	1236	1222	1152	Azaperone
1587	1724	1176	1515	699	1041	Choline Salicylate
1588	808	865	1275	1140	880	Ethionamide
1588	1620	1208	1082	1160	1503	Cimetidine
1589	1742	1202	1192	1229	1245	Captopril
1590	1111	1245	1505	700	1170	Clomifene
1590	1147	1090	1314	685	1066	Sulfadimethoxine
1590	1220	1722	1050	1308	743	Suprofen
1590	1493	1277	1170	1122	1041	Pantoprazole
1590	1603	1564	1109	1188	1248	Loxapine
1590	1614	1530	1496	1254	1311	Metoclopramide Hydrochloride
1590	1640	1500	1133	1270	1695	Azapropazone
1592	765	1558	1250	1158	1316	Methapyrilene
1592	787	758	1308	691	1015	Lysergic Acid
1592	955	870	1080	1055	1115	Piperazine
1592	1167	1141	1081	1058	–	Tilmicosin
1592	1618	1294	1188	1500	1513	Pyrvinium Embonate
1594	1273	784	696	1304	900	Phenylephrine Hydrochloride
1594	1437	1247	–	–	–	Terazosin
1595	767	990	1562	1149	1234	Chloropyrilene
1595	1199	1285	1250	1070	765	Oxymetazoline Hydrochloride
1595	1238	1500	760	1060	925	Dropropizine

Wavenumbers (cm^{-1})						Compound
1595	1525	1615	1550	1090	1055	Penicillamine
1595	1650	1114	914	1313	1248	Methylprednisolone
1595	1698	1240	1625	1111	1315	Chloroprocaine
1596	1153	1168	1307	1500	1075	Cyclothiazide
1596	1570	1548	1295	757	1234	Clotiapine
1597	766	1242	976	1159	1311	Thenyldiamine
1597	1068	797	1305	1279	1222	Etilefrine Hydrochloride
1597	1168	1290	1665	1305	1625	Aminobenzoic Acid
1597	1198	1562	1220	1745	760	Aloxiprin
1597	1470	1062	843	–	–	Danazol
1597	1548	1300	702	1230	830	Dipotassium Clorazepate
1598	1278	1615	1222	1168	1635	Dithranol
1598	1492	1238	3366	873	–	Alfuzosin Hydrochloride
1598	1620	1765	1495	1659	771	Cloxacillin Sodium
1598	1643	1540	766	1236	1574	Cinchocaine
1598	1648	1628	2953	3144	864	Bunazosin Hydrochloride
1600	700	830	765	964	986	Loperamide Hydrochloride
1600	742	1293	1250	763	1500	Tetryzoline Hydrochloride
1600	1225	1510	850	1713	1108	Dantrolene Sodium
1600	1286	1174	1688	1126	1532	Tetracaine Hydrochloride
1600	1288	1261	1530	1123	1219	Methyldopa
1600	1511	1634	1305	1200	1538	Methotrexate (Nujol Mull)
1600	1512	1639	1297	1545	1570	Procainamide Hydrochloride
1600	1560	758	1136	1101	1117	Clozapine
1600	1575	1410	765	–	–	Quetiapine
1600	1634	1501	1307	1205	1235	Clomocycline Sodium
1600	1645	1525	1225	1310	1050	Minocycline Hydrochloride
1600	1650	1160	960	1510	1040	Quinethazone
1600	1757	1669	1224	1314	750	Propicillin
1600	1759	1409	1064	–	–	Fosinopril
1600	1764	1656	1538	715	1143	Cefaloridine
1602	1031	1074	1548	1122	857	Bromhexine
1602	1253	1298	871	1237	800	Xylometazoline Hydrochloride
1603	1098	1381	1222	–	–	Cerivastatin
1603	1643	1293	1125	1252	1540	Prazosin
1603	1767	1622	1495	1660	794	Flucloxacillin Sodium
1604	1592	752	1181	1246	1107	Amoxapine
1604	1680	–	–	–	–	Amisometradine
1605	1086	1490	1655	1505	1300	Cotarnine Chloride
1605	1232	1090	1218	1546	1156	Dinitro-orthocresol (Nujol Mull)
1605	1277	1170	1685	–	–	Naepaine
1605	1278	1246	1670	1210	1700	Propoxycaine
1605	1493	1097	1673	1040	826	Metisazone
1605	1510	700	1160	1575	1200	Fenoterol Hydrobromide
1605	1589	1500	749	702	–	Donepezil
1605	1625	1125	1160	1218	1263	Proflavine Hemisulfate
1605	1626	1127	1145	1276	1094	Sulfamoxole
1605	1660	765	1560	1540	1307	Dequalinium Chloride
1607	1325	1423	–	–	–	Tenoxicam
1607	1683	1313	–	–	–	Isobutyl Aminobenzoate
1607	1766	1673	1500	1093	1260	Meticillin Sodium
1608	820	767	1310	1210	995	Midazolam
1608	1180	1493	1250	1535	1320	Liothyronine
1608	1270	1585	1240	1300	1543	Etamivan
1608	1296	721	1580	690	775	Tolazoline
1608	1314	1292	772	706	1111	Etisazole
1608	1575	1537	1136	769	1100	Dihydralazine
1608	1642	1508	–	–	–	Aminometradine
1609	690	1217	1495	934	1280	Edrophonium Chloride
1610	1178	1298	700	1255	815	Medazepam
1610	1220	1570	1010	865	780	Mercaptopurine
1610	1445	1240	1095	780	–	Lofexidine
1610	1635	1588	1171	1267	1198	Diminazene
1610	1660	1165	1145	962	1012	Metolazone
1610	1778	1510	1238	1690	752	Pheneticillin Potassium
1611	1595	815	1230	1572	1170	Primaquine
1612	1204	1219	1306	1157	935	Oxymetholone
1612	1234	1515	980	1052	1176	Bufexamac
1612	1580	1660	1226	1248	1530	Tetracycline Hydrochloride
1612	1754	1315	1123	1030	1000	Carbenicillin
1613	1524	700	768	1058	752	Fenyramidol
1613	1585	1164	1314	1085	1099	Xylazine
1613	1704	809	1250	1225	1515	Nalidixic Acid
1614	1090	1650	1583	750	715	Debrisoquine Sulfate
1614	1488	1429	1404	–	–	Lamotrigine
1615	762	740	1685	1250	1570	Coumatetralyl
1615	780	1499	791	1211	800	Naphazoline
1615	1570	1450	1210	825	–	Mitoxantrone
1615	1575	1658	1224	1258	1206	Methacycline
1615	1642	1515	1250	1562	1298	Pyritidium Bromide
1616	1224	1512	1591	–	–	Lucanthone Base
1616	1573	1660	1190	1298	1315	Demeclocycline
1616	1584	1665	1235	1180	1138	Oxytetracycline Dihydrate Hydrochloride

Wavenumbers (cm^{-1})						Compound
1618	756	1577	1135	1293	1176	Benziodarone (Nujol Mull)
1618	1258	1217	1712	1105	1149	Diethylcarbamazine
1618	1684	695	712	702	764	Fenpipramide
1620	1050	1580	1030	1120	1228	CBN
1620	1129	1230	1537	1075	1176	Sulfaguanidine
1620	1630	1540	1560	740	1317	Indoramin Hydrochloride Form II (Nujol Mull)
1620	1777	1500	1310	1700	703	Benzylpenicillin
1621	1513	1186	1160	1250	1205	Melphalan
1621	1689	1250	1089	1211	1550	Methyl Benzoquate
1621	1757	1537	1157	3000	–	Ceftazidime
1622	1580	1666	1311	1041	1227	Chlortetracycline Hydrochloride
1623	703	1107	717	858	1002	Dextromoramide (Nujol Mull)
1623	1391	1412	1274	–	–	Thozalinone
1624	1602	1284	1244	–	–	Nemonapride
1625	760	1260	690	1590	850	Chlordiazepoxide
1625	1121	1260	1525	1290	875	Carbidopa
1625	1520	1547	698	670	1600	Nialamide
1625	1668	1257	1539	1231	971	Tioguanine
1626	1219	1003	1266	923	892	Pipobroman
1626	1307	1136	1066	1212	749	Lysergide
1626	1577	1319	1305	1148	955	Morantel
1626	1600	1274	1093	1100	1193	Parbendazole
1627	910	965	1030	1136	1063	Decamethonium Bromide
1627	1601	709	1266	761	1070	Tropicamide
1628	1195	1562	1265	1149	1041	Desferrioxamine
1628	1205	1015	–	–	–	Omeprazole
1628	1492	1260	1312	836	1077	Homidium Bromide
1628	1575	1640	1075	835	805	Pyrimethamine
1628	1585	1308	1185	1240	1148	Levothyroxine
1628	1610	1045	1182	1192	1518	Loprazolam Mesilate
1629	1035	1064	1520	1282	1250	Dexpanthenol
1629	1284	794	1305	1581	1095	Diethyltoluamide
1630	748	1245	1558	1170	998	Amiodarone
1630	1111	1174	701	1192	1066	Bethanidine Sulfate
1630	1645	1598	1250	1510	1570	Diaveridine
1631	1590	1171	1181	1316	1242	Acriflavinium Chloride
1631	1712	750	1208	1136	1160	Ergotamine
1632	1555	1661	1301	861	1176	Procarbazine
1633	1121	1718	1255	759	1272	Flavoxate
1633	1210	1172	1048	1675	1732	Dihydroergocryptine Mesilate
1633	1245	1510	1184	805	820	Atenolol
1633	1662	1720	1705	1558	768	Dihydroergocornine
1634	754	748	1044	1212	1541	Ergometrine
1634	1233	753	1276	1114	1587	Etenzamide
1634	1587	1550	820	1089	1232	Triclocarban
1634	1602	1504	1686	1238	1538	Amiloride Hydrochloride
1635	1260	1590	1730	1230	705	Mebendazole
1635	1305	1264	805	1069	1047	Sodium Cromoglicate
1635	1590	1291	1103	1316	712	Nikethamide
1635	1728	1510	1215	760	1540	Dihydroergocristine
1636	1568	1225	1100	–	–	Trenbolone
1636	1620	953	1075	1040	860	Choline Chloride
1637	1278	1602	1081	699	1136	Dimefline
1637	1402	1511	1136	–	–	Zolpidem Tartate
1637	1494	1422	1341	1323	1287	Mabuterol
1638	698	752	1610	1500	1070	Imolamine
1638	1522	1593	787	1562	1149	Amprolium
1638	1535	1290	781	–	–	Famotidine
1639	751	1603	694	1492	1170	Butalamine
1639	775	761	1603	1250	1316	Dibenzepin Hydrochloride
1639	1047	1534	1595	1211	971	Aminotriazole
1639	1052	1612	1086	1265	1694	Desonide
1639	1492	–	–	–	–	Amisometradine
1639	1509	1527	1696	1600	1259	Acecainide Hydrochloride
1639	1536	1460	1285	1158	1040	Nelfinavir
1639	1592	1111	1492	812	758	Hydroxycarbamide
1639	1721	758	1534	1212	1294	Ergotoxine
1640	696	736	1558	1275	1290	Beclamide
1640	1041	633	–	–	–	Aztreonam
1640	1203	–	–	–	–	Nitrendipine
1640	1535	1316	745	1575	715	Indoramin Hydrochloride Form I (Nujol Mull)
1640	1605	1305	1208	1132	1065	Pyrantel
1640	1610	1550	1231	1210	758	Minoxidil
1640	1685	1118	755	712	1255	Dimenhydrinate
1640	1702	1610	1232	1042	1115	Hydrocortisone
1642	1497	1234	1515	822	1019	Practolol
1644	1455	747	–	–	–	Pipamazine
1645	756	707	1219	1101	1068	Oxetacaine
1645	1170	1564	1198	850	1240	Methylthiouracil
1646	1482	772	735	2365	819	Metaclazepam
1646	1490	1064	–	–	–	Zalcitabine
1646	1520	1256	1300	1046	815	Phenacaine

Wavenumbers (cm^{-1})						Compound
1647	1279	843	1013	903	754	Glyceryl Trinitrate
1647	1379	1041	–	–	–	Ganciclovir
1647	1485	1210	–	–	–	Nisoldipine
1647	1562	1117	1241	1274	1520	Levodopa
1648	1225	1702	1491	–	–	Isradipine
1648	1670	1588	1511	1302	1495	Nifenazone
1649	716	1094	1298	1153	1016	Lorcainide Hydrochloride
1649	1068	1617	689	1272	660	Norethisterone
1650	986	1020	999	1062	1075	Aldosterone
1650	1150	1130	1315	880	1090	Chlormezanone
1650	1263	1612	1210	894	1185	Norethandrolone
1650	1280	1255	1302	1720	1180	Ketotifen Fumarate
1650	1520	765	1235	1272	1180	Etidocaine
1650	1523	760	1220	1123	1265	Mepivacaine
1650	1527	704	749	–	–	Phenatine
1650	1540	1565	1140	790	1311	Cytisine
1650	1576	705	769	757	1500	Isopropamide Iodide
1650	1590	765	1496	774	1176	Tacrine
1650	1618	1131	1590	1500	750	Propyphenazone
1650	1698	1538	729	1041	1205	Bamifylline
1651	1271	1714	1736	1246	1041	Fludrocortisone Acetate
1652	1248	1037	1595	1562	1063	Embutramide
1652	1700	1548	1220	760	750	Pentifylline
1652	1739	1244	1023	–	–	Trenbolone Acetate
1653	1242	1718	816	1220	1495	Fluorouracil
1653	1508	1529	1238	1136	1597	Diamfenetide
1653	1541	1621	676	992	845	Isoniazid
1653	1548	1118	618	–	–	Bleomycin
1653	1600	768	1560	754	1730	Ethyl Biscoumacetate
1653	1610	778	795	1295	1568	Clonidine Hydrochloride
1654	867	1036	1247	926	1282	Fluoxymesterone
1654	1400	1055	–	–	–	Boldenone
1654	1612	1708	887	1112	1085	Prednisolone
1654	1617	1219	1282	1098	1123	Cropropamide
1655	1104	1075	1564	1040	1262	Lincomycin Hydrochloride
1655	1570	1388	741	732	–	Lisinopril
1655	1600	1290	1210	1125	1075	Metenolone
1655	1617	1234	1098	1282	1123	Crotetamide
1655	1699	1755	1275	1205	815	Metharbital
1656	759	696	1613	1163	1113	Phenprocoumon (Nujol Mull)
1656	767	1587	1562	1315	1298	Dichloralphenazone
1656	1513	1176	1250	1026	820	Ambucetamide
1656	1623	1235	1280	1316	1590	Crotamiton
1656	1690	750	1538	763	1282	Proxyphylline
1656	1693	1284	714	690	1226	Ketoprofen
1656	1706	748	1543	1220	1600	Etamiphylline
1656	1737	1227	705	1201	1211	Oxyphencyclimine Hydrochloride
1656	1739	1605	758	1111	1153	Chlorquinaldol
1657	1517	1592	1378	1348	1038	Mifepristone
1658	1157	1552	668	1090	905	Tolbutamide
1658	1163	1622	1722	1176	1747	Fluocortolone Hexanoate
1658	1227	1600	905	1745	1034	Paramethasone (Nujol Mull)
1658	1276	716	–	–	–	Boldenone Benzoate
1658	1563	1193	1625	1166	1241	Propylthiouracil
1658	1605	1275	876	1281	1241	Dimethisterone
1658	1695	1548	763	750	1029	Xantinol
1658	1698	747	1548	1242	760	Caffeine
1659	1278	1370	–	–	–	Atovaquone
1660	701	1493	1263	1273	1236	Fentanyl
1660	770	1318	1305	1590	1580	Phenazone
1660	871	1615	1057	1236	1066	Testosterone
1660	1059	1630	750	699	1115	Styramate
1660	1060	1235	1612	1127	723	Ethisterone
1660	1060	1730	1080	1250	1175	Halcinonide
1660	1090	716	1618	1170	970	Phenacemide
1660	1153	1267	735	1590	1300	Vanillin
1660	1160	1239	950	1612	1090	Methyltestosterone
1660	1210	1712	1053	1140	768	Dihydroergotamine
1660	1315	1126	750	700	1620	Aminophenazone
1660	1497	1575	1070	1150	1220	Cyanocobalamin
1660	1598	752	1538	1315	1500	Acetanilide
1660	1617	1606	1710	1056	907	Betamethasone
1660	1618	1048	1237	1595	1228	Thiamine Hydrochloride
1660	1620	886	1601	1160	1240	Methandienone
1660	1622	1697	1581	1232	1197	Dydrogesterone
1660	1685	1600	1239	1157	1212	Pipamperone
1660	1693	1085	1310	750	869	Methyprylon
1660	1700	1039	747	1562	1234	Diprophylline
1660	1700	1720	1550	762	752	Pentoxifylline
1661	1147	1599	1089	1635	1310	Carbutamide
1661	1159	1553	757	1086	909	Chlorpropamide
1661	1172	1234	1149	978	1613	Clorexolone
1661	1400	1204	1470	1300	1078	Mexazolam
1662	1159	1725	1619	1605	1285	Fluocortolone Pivalate
1662	1495	762	1204	1290	1086	Lidocaine
1662	1614	1700	872	1209	1232	Progesterone
1662	1705	1496	700	765	1120	Primidone
1663	896	1622	1695	1052	1603	Dexamethasone
1663	1057	1618	1609	902	1080	Triamcinolone Acetonide
1663	1172	1038	1532	1285	928	Clopamide
1664	764	1050	1110	1282	775	Strychnine
1664	1585	697	1163	1562	760	Disopyramide
1664	1623	901	1733	1139	1160	Flumetasone Pivalate
1664	1705	1600	1546	746	1219	Fenetylline
1665	790	1582	1000	810	1175	Hydralazine Hydrochloride
1665	1060	1050	1320	1205	1180	Trilostane
1665	1245	1525	1495	1217	1285	Acebutolol Hydrochloride
1665	1703	1318	1300	1278	1593	Pirenzepine Hydrochloride
1665	1712	1636	1562	740	1180	Bufylline
1666	1086	1052	1063	1724	1639	Fluclorolone Acetonide
1666	1574	590	–	–	–	Milrinone
1666	1612	1724	884	1063	1010	Clobetasol Propionate
1667	1036	1117	1020	810	1239	Fluoroacetamide (Nujol Mull)
1667	1101	1499	1125	1002	1019	Morinamide
1667	1248	1119	1215	1010	1080	Methocarbamol
1667	1259	1096	1605	1289	1070	Idoxuridine
1667	1316	740	694	1602	704	Prazepam
1667	1505	1246	1039	2932	2867	Cilostazol
1667	1522	1279	1222	787	944	Bupivacaine Hydrochloride
1667	1626	1590	1302	797	1289	Tramazoline Hydrochloride
1667	1689	1536	–	–	–	Cloponone
1668	1436	1351	–	–	–	Buspirone
1668	1498	1231	1118	–	–	Capreomycin
1668	1541	1731	1170	1318	1291	Thiamylal
1668	1707	904	1622	1610	1246	Prednisone
1669	701	757	1087	1190	1592	Piritramide
1669	1074	1629	910	1056	1615	Fluocinolone Acetonide
1670	750	705	870	815	1595	Isocarboxazid
1670	760	783	1618	1158	1270	Lysergamide
1670	1220	1265	1587	746	1149	Acetophenazine
1670	1240	1730	1750	1070	1635	Fluocinonide
1670	1270	1590	755	1500	1575	Tolfenamic Acid
1670	1540	1300	1170	1735	1220	Thiopental
1670	1626	760	1538	1592	1298	Dofamium Chloride
1670	1658	1198	1173	–	–	Saquinavir
1670	1717	1567	745	980	1190	Theophylline
1672	1064	1179	1163	1208	1639	Dipyrone
1672	1134	1652	1050	765	1243	Guanoclor
1672	1578	1255	714	699	970	Metyrapone
1672	1613	1316	1211	1171	1100	Flurazepam
1672	1700	1093	694	1176	1615	Pheneturide
1673	1515	1628	1570	760	1224	Cycloguanil
1673	1761	1718	1237	1292	1303	Heptabarb
1674	1636	823	591	–	–	Celiprolol
1675	749	1279	1193	1163	1143	Butaperazine
1675	820	1105	1195	1308	1242	Ketazolam
1675	1220	1265	1587	746	1562	Acepromazine
1675	1222	1269	1592	748	1560	Piperacetazine
1675	1242	1500	1615	1298	765	Fendosal
1675	1505	767	806	1136	1190	Ambenonium Chloride
1675	1540	765	1128	772	1240	Tocainide Hydrochloride
1675	1701	1059	1045	1095	1079	Fludroxycortide
1675	1760	1317	1303	1230	853	Secbutabarbital
1675	1760	1725	1234	1282	1298	Vinbarbital
1676	1638	1548	1230	1516	1211	Flucytosine
1676	1704	1092	1620	1185	750	Apronal
1678	690	1240	1265	755	717	Demoxepam
1678	1197	1093	1290	1727	1167	Diloxanide
1678	1594	800	769	787	1298	Carbamazepine
1678	1602	825	1132	1310	800	Tetrazepam
1679	1733	1205	1178	1255	692	Nandrolone Phenylpropionate
1680	700	1602	820	738	790	Nordazepam
1680	705	1310	1220	760	1255	Methyl Salicylate
1680	1277	1730	860	1160	1145	Bemegride
1680	1510	730	1300	1220	1605	Indoprofen
1680	1643	1502	1270	820	1250	Labetalol
1680	1698	703	1618	1594	1026	Nicotinamide
1680	1709	1316	1193	1253	1040	Methohexital Sodium
1680	1718	1193	1318	1110	703	Camazepam
1680	1720	1767	1320	1245	875	Barbital
1681	727	1205	1250	1316	1110	Thalidomide (Nujol Mull)
1681	847	1072	1515	816	1562	Chloramphenicol (Nujol Mull)
1681	1228	1218	1706	1299	1065	Indometacin
1681	1313	705	840	1125	740	Diazepam
1682	1070	1710	1601	1140	788	Mebutamate
1682	1153	1121	1315	1610	843	Lormetazepam
1682	1599	1565	770	1265	697	Methaqualone
1682	1605	768	782	1282	1583	Mecloqualone

Wavenumbers (cm^{-1})						Compound
1683	753	710	1253	696	764	Doxapram Hydrochloride
1683	1156	1285	1307	1125	1180	Probenecid
1683	1492	1248	1220	1150	978	Diflunisal
1683	1736	1275	1512	765	1600	Oxyphenbutazone
1684	1664	704	1490	764	845	Clobazam
1685	825	750	802	1315	1230	Bromazepam
1685	1020	1600	1584	1050	1165	Pyrazinamide
1685	1033	1160	845	1190	1310	Chlortalidone
1685	1149	1317	1120	1605	826	Lorazepam
1685	1610	748	1255	1578	1532	Clonazepam
1685	1610	1300	1130	1270	1000	Thialbarbital Sodium
1685	1705	1595	1230	752	1156	Droperidol
1685	1719	1744	1315	1218	845	Pentobarbital
1686	1275	1637	1111	695	751	Phensuximide
1686	1602	1636	1191	1567	1225	Folic Acid
1686	1616	1508	1070	762	1570	Acenocoumarol
1686	1710	1200	1270	1281	704	Glutethimide
1687	700	1211	1115	1052	769	Lobeline
1687	1315	925	1219	847	1640	Allobarbital
1687	1670	1112	1603	705	1150	Temazepam
1687	1706	693	830	1136	1123	Oxazepam
1688	1069	1090	1590	1310	1140	Meprobamate
1688	1210	1200	1223	1134	1179	Ecgonine Hydrochloride
1688	1636	1215	1588	1150	1607	Repaglinide
1689	1232	1270	857	1606	1149	Methallenestril
1689	1337	1098	–	–	–	Nimodipine
1689	1718	1550	1640	1285	672	Enallylpropymal
1690	1088	1064	700	1605	1623	Phenprobamate
1690	1205	1170	1310	1620	1270	Salsalate
1690	1225	1527	1120	1496	1310	Nifedipine
1690	1235	1497	1598	1155	750	Fluanisone
1690	1274	1605	1174	1116	772	Procaine
1690	1515	1370	1250	–	–	Formoterol
1690	1610	698	1536	745	784	Nitrazepam
1690	1610	1041	1170	1150	1010	Emylcamate
1690	1640	1602	835	665	880	Muzolimine
1690	1665	1221	1550	1595	680	Theobromine
1690	1720	1740	1310	1290	1200	Butalbital
1691	705	1245	1469	–	–	Methcathinone
1691	1113	1342	1346	1237	1458	Metolachlor
1692	1587	916	1224	1235	956	Allopurinol
1692	1730	1750	1318	1220	1630	Vinylbital
1693	1614	1082	–	–	–	Felbamate
1693	1720	1745	1316	1255	860	Aprobarbital
1693	1725	1745	1300	1210	830	Cyclobarbital
1694	660	1600	1094	1212	834	Carbromal
1694	729	1162	1282	1298	934	Etebenecid
1694	757	1587	1162	1204	1219	Benperidol
1694	1149	1587	1086	1612	684	Sulfacarbamide
1694	1176	884	819	675	1086	Tolazamide
1694	1260	1520	1155	1140	1222	Tetrabenazine
1695	1148	1220	1267	1000	1252	Alfaxalone
1695	1185	1200	1515	1623	1267	Aminoglutethimide
1695	1215	1199	1153	1587	1280	Bumetanide
1695	1220	707	930	773	960	Flurbiprofen
1695	1250	1065	1538	1600	1140	Tybamate
1695	1505	753	1220	1230	825	Pimozide
1695	1520	1229	1610	1175	1270	Chlorambucil
1695	1527	1075	1246	1101	1319	Carisoprodol (Nujol Mull)
1695	1543	766	754	1299	1258	Prilocaine Hydrochloride
1695	1613	1497	1263	1205	1558	Decoquinate
1695	1720	1190	1200	1390	1350	Bambuterol
1695	1752	1265	833	1205	775	Nealbarbital
1696	747	1142	1120	712	1027	Ketamine
1696	1260	1020	–	–	–	Nabumetone
1696	1619	1574	1260	1100	1038	Nabilone
1696	1727	1760	1242	850	1215	Butobarbital
1696	1728	1755	835	1290	1207	Idobutal
1697	1620	1490	1528	1107	783	Flunitrazepam
1698	697	1116	1437	–	–	Tiletamine Base
1698	1046	1277	1027	1064	1079	Androstanolone
1698	1194	1181	1250	700	1150	Phenglutarimide Hydrochloride
1698	1496	1206	1099	–	–	Felodipine
1698	1720	1757	1316	1230	850	Hexethal Sodium
1699	1594	1242	–	–	–	Nefazodone
1700	700	766	1219	1740	1280	Phenindione
1700	1075	1500	3250	1250	–	Viomycin
1700	1120	1179	1512	1219	1620	Anileridine
1700	1210	1601	1175	1318	753	Benzaldehyde (Thin Film)
1700	1235	1495	1590	1100	763	Lofepramine Hydrochloride
1700	1266	1136	1036	1156	1073	Tigloidine
1700	1283	1584	1275	1683	950	Phanquinone
1700	1610	1435	1325	830	1090	Enoximone
1700	1652	1160	1285	1295	1530	Gliquidone

Wavenumbers (cm^{-1})						Compound
1700	1660	1235	1720	1275	1750	Cortisone
1700	1660	1612	1538	1298	1219	Acefylline Piperazine
1700	1722	1210	1300	830	1622	Ibomal
1700	1755	1212	815	1319	848	Cyclopentobarbital
1700	1777	1208	1130	1303	730	Ethosuximide
1701	1220	1184	1735	1052	1672	Trimetaphan Camsilate
1702	1720	1751	1240	802	716	Phenylmethylbarbituric Acid
1703	1140	957	–	–	–	Etretinate
1703	1618	1576	1407	–	–	Daunorubicin
1703	1619	1267	–	–	–	Ciprofloxacin
1703	1728	1752	1315	1211	829	Talbutal
1704	1006	1018	1160	1270	1603	Sulindac
1704	1605	1253	775	1170	1235	Cyclomethycaine (Nujol Mull)
1704	1724	1637	1600	1227	1510	Cefalothin Sodium
1704	1754	733	1052	1302	702	Mephenytoin
1705	700	1115	1492	1600	1157	Norpipanone
1705	741	1197	1160	1290	1135	Yohimbine Hydrochloride
1705	1150	1100	1580	1020	1000	Methoxsalen (Nujol Mull)
1705	1282	1030	699	1775	1086	Mesuximide
1705	1595	943	1640	742	1272	Trazodone Hydrochloride
1705	1597	1248	777	699	1285	Ketobemidone
1706	1068	1055	1252	1238	1032	Mesterolone
1707	1162	920	667	1089	997	Gliclazide
1707	1684	1754	720	1298	1050	Methylphenobarbital
1708	698	1491	1110	1092	1030	Dipipanone
1708	701	751	1590	1190	1754	Norbormide
1708	1213	1250	1278	946	1107	Valproic Acid (Thin Film)
1708	1218	1166	1148	688	730	Pethidine Hydrochloride
1708	1608	1210	1755	1178	1085	Carbocromen
1709	1214	1261	1613	1294	1311	Oxybuprocaine Hydrochloride
1709	1435	1226	997	–	–	Loratadine
1710	698	1230	1136	1175	1205	Phenoperidine Hydrochloride
1710	1060	1245	1185	690	720	Mandelic Acid
1710	1505	1596	1220	743	1230	Fluspirilene
1710	1600	1110	880	930	1620	Trioxysalen (Nujol Mull)
1710	1652	1530	–	–	–	Allantoin
1710	1734	1750	1225	775	1190	Barbituric Acid
1710	1738	1275	1110	712	1037	Cocaine
1711	701	1495	1122	1032	760	Normethadone
1711	1215	1154	1176	948	690	Neostigmine Bromide
1712	1014	1300	1062	1094	946	Bethanechol Chloride
1712	1262	1135	1282	1020	1650	Arecoline
1712	1684	1670	1770	1310	1300	Phenobarbital
1713	1041	1694	1030	1052	1250	Ethinamate
1713	1075	1610	805	1010	–	Urethane
1714	1214	1136	1197	1184	1155	Dicycloverine Hydrochloride
1714	1238	1182	1263	1167	703	Tilidate Hydrochloride
1714	1276	711	1108	–	–	Benzamine
1714	1300	1755	755	1492	1275	Phenylbutazone
1715	1072	700	1603	1110	760	Hydroxyphenamate
1715	1100	1200	690	760	1020	Fenbutrazate
1715	1200	1735	1035	1087	1265	Drostanolone Propionate
1715	1300	767	1745	715	703	Feprazone
1715	1372	1463	1087	–	–	Zopiclone
1715	1540	1610	1140	1500	1220	Bezafibrate
1715	1620	1100	747	759	1237	Gelsemine
1716	700	760	820	1780	1100	Ethotoin
1716	1305	750	688	1750	1595	Sulfinpyrazone
1716	1505	1280	1639	1115	909	Naltrexone Hydrochloride
1717	1202	1231	1258	–	–	Oxandrolone
1717	1225	1315	1300	1095	1050	Pipazetate
1717	1632	1485	1104	–	–	Aciclovir
1718	1146	1052	–	–	–	Mupirocin
1718	1154	694	1173	1220	1060	Caramiphen
1718	1180	1174	1042	923	858	Hyoscine Methobromide
1718	1216	1108	1285	1585	1265	Orphenadrine Citrate
1718	1237	1205	1770	1513	1126	Nitrofurantoin
1718	1317	1217	1250	763	1298	Dimethoxanate
1718	1459	1389	1267	–	–	Simvastatin
1719	1501	1033	1255	934	980	Oxycodone
1720	1035	1153	1163	1063	1204	Atropine
1720	1235	1740	1295	1145	1262	Eucatropine
1720	1250	1204	1268	1495	1289	Physostigmine Aminoxide
1720	1270	1500	959	1055	800	Hydrocodone
1720	1665	1748	1200	1275	1045	Hexobarbital
1720	1700	1581	1210	1170	1300	Bromisoval
1720	1740	1774	748	698	785	Phenytoin
1721	1188	1052	–	–	–	Azithromycin
1721	1232	779	1185	1273	870	Ibuprofen
1721	1271	1107	737	1587	1022	Nicofuranose
1722	1606	1532	1772	1272	3295	Azlocillin
1722	1654	1252	1109	715	967	Alfentanil Hydrochloride
1724	1000	1031	1062	1242	1282	Androsterone (Nujol Mull)
1724	1148	1308	961	952	1612	Suxamethonium Chloride

Wavenumbers (cm^{-1})						Compound
1724	1174	1155	1223	1190	1681	Naproxen
1724	1179	1160	1195	1044	935	Homatropine Methylbromide
1724	1216	1149	1178	1124	943	Demecarium Bromide
1724	1219	1250	1111	781	1265	Carbaryl
1724	1234	1028	1692	1111	824	Chlorphenesin Carbamate
1724	1243	1200	1495	1120	1162	Physostigmine
1724	1250	1052	1020	1612	1086	Docusate Sodium
1724	1276	1111	741	1022	1590	Hexyl Nicotinate
1724	1652	1161	–	–	–	Flumazenil
1725	1120	1158	1176	694	1234	Carbetapentane
1725	1189	1075	–	–	–	Methandriol Dipropionate
1725	1231	1034	–	–	–	Boldenone Acetate
1725	1260	1072	1460	–	–	Lovastatin
1725	1372	1062	–	–	–	Dehydroepiandrosterone
1725	1513	1189	–	–	–	Atracurium
1725	1654	1175	–	–	–	Boldenone Undecylenate
1725	1696	1758	1317	1240	850	Amobarbital
1726	698	1220	1160	763	1055	Pipenzolate Bromide
1726	1249	1279	1077	1586	1661	Etacrynic Acid
1727	718	1593	1198	1605	1572	Tiaprofenic Acid
1727	1193	1094	695	1248	1030	Ethoheptazine
1727	1247	1279	1034	757	1500	Hydromorphone
1727	1579	1187	–	–	–	Pravastatin Sodium Salt
1727	1669	1258	1608	967	1188	Medroxyprogesterone
1727	1683	1251	–	–	–	Clostebol
1728	697	1183	1120	1215	1495	Diphenoxylate
1728	1156	1249	1500	1630	1010	Pyridostigmine Bromide
1728	1220	1190	1162	690	1052	Benzilonium Bromide
1728	1670	1177	1188	1716	1224	Hydroxyprogesterone Caproate
1729	1282	1590	1136	–	–	Propanil
1729	1283	1594	1130	1116	743	Nicametate
1730	853	1166	736	705	1047	Hyoscine Hydrobromide
1730	1047	1025	1277	1095	755	Camphor
1730	1083	1056	930	1102	1200	Carbachol
1730	1158	1194	694	1265	1219	Butetamate
1730	1162	1060	1195	1378	–	Amprotropine
1730	1165	722	1280	1620	1070	Naftidrofuryl Oxalate
1730	1172	1030	735	1063	1125	Homatropine
1730	1175	725	704	776	1026	Dextropropoxyphene (Nujol Mull)
1730	1200	1567	1608	751	1227	Propyliodone
1730	1230	695	726	1163	1265	Valethamate Bromide
1730	1230	725	770	1288	1200	Metomidate Hydrochloride
1730	1240	769	1190	712	1011	Clidinium Bromide
1730	1240	1225	1145	941	953	Oxymorphone
1730	1616	1216	–	–	–	Famciclovir
1730	1786	730	1059	766	1309	Clodantoin
1730	1802	1098	1200	770	1295	Trimethadione
1730	1805	1100	1040	1280	1070	Paramethadione
1731	1128	1221	1100	700	1080	Oxeladin
1731	1206	885	994	902	1250	Betaine
1733	755	1708	700	1286	1054	Bezitramide
1733	1287	1086	1132	744	1044	Diethyl Phthalate
1733	1667	1715	1266	1250	1618	Gestonorone Caproate
1734	1192	1212	1274	1104	1074	Cyclandelate
1734	1195	965	685	1160	720	Methylpiperidyl Benzilate Hydrochloride
1735	1155	1178	1040	1237	1203	Octatropine Methylbromide
1735	1175	1185	1317	860	923	Hyoscine Methonitrate
1735	1175	1210	1150	705	740	Methylphenidate Hydrochloride
1735	1219	1085	1234	1063	700	Heteronium Bromide
1735	1255	1170	1507	1135	1295	Distigmine Bromide
1736	1064	1110	1183	1242	1174	Monocrotaline
1736	1145	700	1190	1500	746	Adiphenine
1738	1010	1070	1036	1277	1605	Haloxon
1738	1160	1025	1145	1225	1050	Hyoscyamine Hydrobromide
1738	1181	1141	1162	1195	1077	Trimeperidine
1738	1225	1164	1178	1115	1192	Glycopyrronium Bromide
1739	1227	1234	704	1064	1130	Penthienate Methobromide
1739	1238	1227	702	1058	1093	Metindizate
1739	1241	699	743	1176	1191	Lachesine Chloride
1739	1248	1066	1632	972	1128	Methacholine Chloride
1739	1598	1527	1393	–	–	Ceftriaxone
1739	1674	1211	–	–	–	Benazepril
1740	1163	1638	1270	962	1012	Suxethonium Bromide
1740	1178	1134	694	1030	1055	Alphaprodine
1740	1178	1230	1265	1278	1510	Phenolphthalein
1740	1760	1815	1188	1280	1007	Dimethadione
1742	1200	1182	1240	1150	1055	Protoveratrine A and B
1743	1652	1187	756	–	–	Ramipril
1743	2069	1210	2563	2480	2250	Oxybutynin
1744	1239	1735	1053	1004	1123	Troleandomycin
1745	1276	1038	1010	1498	1080	Noscapine
1745	1668	1590	1195	–	–	Dactinomycin
1746	1412	1034	748	–	–	Etodolac
1748	1253	1232	1520	1027	1126	Nifuratel
1750	1330	1200	–	–	–	Acetone
1750	1645	1425	1390	1187	–	Enalapril Maleate
1751	1302	1585	752	1072	693	Phenindamine Tartrate
1752	1168	1104	660	1505	1020	Pilocarpine
1753	1215	700	690	1180	1020	Benaprizine Hydrochloride,
1754	1582	1678	1271	1235	1190	Cefradine
1754	1582	1681	1271	695	1186	Cefalexin
1754	1660	1181	1202	1174	1532	Phenoxymethylpenicillin
1755	1226	1250	1110	1020	1315	Furaltadone
1760	1501	1037	1260	1020	1111	Hydrastine
1760	1742	1104	1683	972	1150	Pivampicillin
1762	801	962	1149	840	1300	Chlorzoxazone
1770	1075	1788	1263	1687	1744	Bacampicillin Hydrochloride
1773	1676	1170	1127	1115	1185	Spironolactone
1774	1046	1004	888	1609	1151	Coumaphos
1775	1583	1684	1248	1613	1313	Amoxicillin Trihydrate
1775	1610	1515	1485	1250	–	Etoposide
1775	1693	1526	1308	1497	1583	Ampicillin
1775	1693	1600	1365	1500	1560	Cefaclor
1776	1730	1183	1267	1149	1236	Acecarbromal (Nujol Mull)
1777	1145	648	896	–	–	Succinimide
1778	1169	1019	1094	1402	–	Dicamba
1780	1755	1795	1123	990	1162	Picrotoxin
1786	1176	1205	1111	1149	698	Pipoxolan
1795	1025	750	1455	–	–	Amolanone
1820	1163	1047	2993	2909	874	γ-Butyrolactone
2077	1675	1084	–	–	–	Zidovudine
2209	1630	1610	1537	–	–	Entacapone
2269	1507	1729	1594	1132	1282	Linuron
2393	1679	1743	839	781	3056	Diltiazem
2400	2000	1615	–	–	–	Enoxacin
2986	2772	1614	1329	1257	1122	Fluoxetine
3082	2964	1736	1690	1472	1331	Pioglitazone
3295	1619	1535	1215	1169	1035	Indinavir
3355	3328	2964	1714	1618	1522	Ritonavir

14 Mass Spectral Data of Drugs

In the following table the *m/z* values of the eight most intense ions are listed in descending order of intensity for drugs and other compounds of forensic interest. To identify an unknown compound, the *m/z* values of the two most intense ions should be compared with the table in order to produce a list of possible compounds. The eight most intense ions of the unknown can then be compared with this list. Comparison with standard spectra should then be made. Complete mass spectra for most compounds can be found in the monographs.

Principal peaks at m/z								Compound
27	26	12	28	13	14	–	–	Cyanide
28	99	56	27	26	55	42	100	Succinimide
29	30	28	31	32	–	–	–	Formaldehyde
29	41	39	42	69	116	167	168	Ascorbic Acid
29	44	43	42	26	41	28	27	Acetaldehyde
29	46	45	28	44	30	47	–	Formic Acid
30	27	124	56	123	87	78	41	Hexoprenaline
30	42	43	27	44	29	31	41	Ethylenediamine Hydrate
30	44	41	45	86	55	43	69	Octamylamine
30	44	42	568	31	58	27	81	Pseudomorphine
30	57	82	116	99	53	55	42	Cimetidine
30	72	43	135	121	148	52	56	Guanoxan
30	86	57	294	71	310	70	87	Nadolol
30	86	57	41	77	135	29	206	Salbutamol
30	86	57	41	29	39	192	42	Terbutaline
30	86	57	129	74	56	41	114	Timolol
30	91	65	92	51	39	121	103	Phenethylamine
30	108	107	77	39	51	137	27	Tyramine
30	138	195	140	103	197	77	196	Baclofen
30	167	45	168	165	183	166	152	Didesmethyldiphenhydramine
30	313	246	211	273	274	302	183	Didesethylflurazepam
31	32	29	30	28	33	34	27	Methanol
31	45	29	27	46	43	30	42	Ethanol
31	45	46	29	27	59	74	43	Phenelzine
31	49	77	113	115	82	51	117	Trichloroethanol
31	49	77	29	113	51	48	115	Triclofos
31	49	77	113	115	82	51	117	Trichloroethanol
31	59	29	45	74	27	41	43	Ether
31	59	42	60	27	29	45	41	Propanol
33	131	69	79	181	51	151	101	Sevoflurane
41	43	29	55	39	57	73	81	Lanatoside C
41	43	183	167	140	184	124	109	*N*-Hydroxyaprobarbital
41	45	43	168	39	70	69	167	3′-Hydroxysecobarbital
41	81	53	221	79	39	178	233	Methohexital
41	147	79	93	55	105	67	91	Trilostane
41	167	124	39	80	53	68	141	Allobarbital
41	167	168	39	124	97	141	181	Butalbital
41	173	91	135	77	55	44	69	Santonin
41	226	77	143	181	141	39	145	Alclofenac
41	229	43	329	69	344	29	283	Δ9-THC-11-oic Acid
42	28	41	29	27	86	56	39	γ-Butyrolactone
42	31	61	43	29	27	44	41	Monoethanolamine
42	43	70	246	185	55	56	445	Thiopropazate
42	128	85	43	44	41	70	69	Barbituric Acid
42	140	112	41	85	43	71	141	Methenamine
42	248	247	32	151	45	201	69	Enoximone
42	271	258	123	56	83	240	95	Trifluperidol
42	292	56	294	109	203	201	44	Penfluridol
43	29	27	72	57	42	44	41	Methyl Ethyl Ketone
43	29	28	59	30	42	74	102	Cycloserine
43	29	39	41	45	57	68	58	Digitoxin
43	31	29	61	60	85	73	44	Isosorbide Dinitrate
43	31	57	73	60	29	55	44	Amygdalin
43	41	184	168	167	97	55	53	Thiamylal
43	44	85	86	42	129	68	30	Metformin
43	44	87	31	45	42	131	71	Paraldehyde
43	45	60	120	42	92	138	44	Aloxiprin
43	45	60	58	95	99	398	206	Rifampicin
43	55	69	81	152	210	95	180	Cyclohexane Pyrolysis Product Of DQHS
43	55	299	91	147	79	253	271	Desoxycortone Acetate
43	58	42	143	85	171	157	102	Bethanechol Chloride
43	58	42	44	30	129	36	143	Carbachol
43	58	59	27	42	26	39	29	Acetone
43	58	143	42	41	40	39	128	Trimethadione
43	59	155	121	156	31	157	158	Phenaglycodol

Principal peaks at m/z								Compound
43	61	45	70	29	27	73	42	Ethyl Acetate
43	69	168	41	85	167	86	169	3′-Ketosecobarbital
43	70	29	71	27	154	41	267	Ergotoxine
43	70	55	42	41	73	61	7	Amyl Acetate
43	70	71	54	44	154	267	55	Ergocornine
43	70	71	154	41	209	69	267	Ergocryptine
43	71	55	79	91	109	123	336	Fluoxymesterone
43	91	134	92	65	39	63	135	Phenylacetone
43	91	227	268	312	79	55	77	Dydrogesterone
43	114	85	30	101	86	44	72	Buformin
43	127	195	85	44	36	152	58	Proguanil
43	129	57	56	41	72	39	58	Paramethadione
43	129	69	41	86	97	55	44	Acecarbromal
43	141	183	42	87	41	140	165	Paracetamol (mercapturic acid conjugate)
43	147	158	91	328	55	93	79	Clostebol
43	180	42	45	44	64	100	222	Acetazolamide
43	182	167	225	181	183	151	142	Trimethoxyamfetamine
43	187	42	171	229	86	144	170	Amiloride
43	221	83	236	56	223	222	55	Methazolamide
43	262	136	202	135	321	83	148	Famciclovir
43	327	299	298	292	329	328	256	7-Acetamidoclonazepam
43	340	298	325	91	41	231	280	Norethisterone Acetate
43	398	337	72	309	95	362	217	Daunorubicin
44	30	42	41	29	27	100	55	Tuaminoheptane
44	42	124	165	163	123	93	65	Adrenaline
44	42	147	120	65	43	45	39	Amidefrine
44	42	159	43	45	184	41	109	Norfenfluramine
44	43	59	56	69	55	41	113	Heptaminol
44	43	79	93	41	135	91	81	Rimantadine
44	45	26	218	215	203	202	42	10-OH-nortriptyline
44	45	26	218	215	203	202	42	10-Hydroxynortriptyline
44	45	42	58	43	59	41	57	Topotecan
44	45	58	76	60	43	42	46	Noradrenaline
44	57	43	40	55	41	79	77	Cathine
44	59	165	166	181	179	178	43	N-Monodesmethylorphenadrine
44	59	165	166	181	179	178	43	Tofenacin
44	69	41	208	210	55	71	43	Carbromal
44	70	59	277	71	191	278	203	Maprotiline
44	70	179	178	207	280	250	236	10,11-Dihydro-10, 11-dihydroxyprotriptyline
44	71	268	227	193	42	269	229	Monodesmethylclomipramine
44	76	77	29	45	42	95	65	Phenylephrine
44	77	76	29	95	39	58	42	Metaraminol
44	77	79	51	45	42	107	105	Norephedrine
44	77	79	51	45	42	107	105	Phenylpropanolamine
44	82	80	107	77	108	81	79	Hydroxyamfetamine
44	83	85	36	28	–	–	–	Trichloroacetic Acid
44	83	180	182	137	143	139	41	Bromisoval
44	91	40	42	65	45	39	43	Amfetamine
44	100	41	60	29	27	30	86	Piperazine Adipate
44	100	57	111	139	75	84	224	Bupropion
44	100	167	266	281	–	–	–	Terodiline
44	108	42	77	107	149	95	123	Oxedrine
44	121	77	120	42	106	91	39	Tocainide
44	136	51	135	77	42	78	45	Methylenedioxyamfetamine
44	138	122	137	121	91	78	45	Methylthioamfetamine
44	144	115	154	–	–	–	–	Duloxetine
44	146	147	39	41	190	42	148	Cytisine
44	148	255	–	–	–	–	–	Atomoxetine
44	166	151	57	43	91	135	209	DOM
44	167	168	165	183	105	152	77	Monodesmethyldiphenhydramine
44	180	209	227	223	77	208	179	3′-Carboxymefenamic acid
44	194	109	67	86	238	56	85	Acefylline Piperazine
44	202	45	220	218	215	91	–	Nortriptyline
44	202	45	220	218	215	91	–	Nortriptyline

Principal peaks at m/z								Compound
44	204	203	202	41	221	57	55	Desmethyldosulepin
44	206	162	108	51	207	201	178	Cromoglycic Acid
44	208	117	58	193	130	57	29	Norpropoxyphene
44	209	211	250	210	224	42	251	2-Hydroxydesipramine
44	209	211	250	210	224	42	251	Hydroxydesipramine
44	209	211	250	210	224	42	251	2-Hydroxydesipramine
44	215	411	324	45	164	42	216	Etorphine
44	238	310	138	208	57	–	–	Desmethylcitalopram
44	309	183	104	251	91	77	59	Fluoxetine
44	329	192	70	138	109	176	147	Paroxetine
45	29	43	60	42	28	44	41	Acetic Acid Glacial
45	43	27	41	39	29	59	44	Isopropyl Alcohol
45	130	44	71	42	61	115	55	2-Ethylbutyrylurea
45	156	141	69	41	43	157	55	3′-Hydroxypentobarbital
45	217	70	41	69	110	285	202	Pentazocine
46	76	57	55	56	60	47	97	Pentaerithrityl Tetranitrate
49	84	86	51	47	35	88	41	Methylene Chloride
51	29	101	149	31	69	99	32	Desflurane
53	204	146	117	91	77	138	159	Bufotenine
55	41	42	43	70	57	29	1	Amyl Alcohol
55	44	142	141	41	61	81	82	Apronal
55	57	43	97	41	56	158	44	Carisoprodol
55	69	83	149	95	410	245	111	Hydroxybrotizolam
55	72	97	41	56	158	118	57	Tybamate
55	82	41	39	54	42	56	109	Pentetrazol
55	83	82	41	113	70	29	69	Bemegride
55	91	316	140	125	98	43	331	Betaxolol Methaneboronate
55	339	110	36	298	82	242	96	Nalmefene
55	378	43	29	57	410	379	84	Buprenorphine
56	42	83	57	77	51	97	54	Dipyrone
56	55	41	43	45	85	42	84	Cadaverine
56	62	42	77	69	49	105	64	Carmustine
56	84	111	69	82	54	67	45	Vigabatrin
56	100	138	110	57	237	70	41	Viloxazine
56	231	97	111	112	42	77	71	Aminophenazone
57	41	141	167	39	83	55	182	Nealbarbital
57	42	44	177	58	219	133	107	Profadol
57	58	70	42	44	188	84	43	Ethoheptazine
57	85	42	56	76	191	70	77	Phendimetrazine
57	139	351	134	121	333	313	109	Fluticasone Propionate
57	233	42	56	158	43	160	103	Norpethidine
57	342	253	268	145	147	81	91	Methandriol Dipropionate
58	30	59	77	29	95	65	57	Etilefrine
58	30	59	56	77	–	–	–	Hydroxyephedrine
58	30	107	59	77	56	42	43	Pholedrine
58	30	363	44	72	29	27	364	Bialamicol
58	41	30	126	59	69	56	44	Cyclopentamine
58	41	56	30	124	65	59	93	Ethylnoradrenaline
58	42	41	125	59	30	89	168	Chlorphentermine
58	42	59	30	77	107	57	51	Hordenine
58	42	69	41	30	215	202	59	10-OH-Amitriptyline
58	43	57	149	71	42	41	55	Acetylcholine Chloride
58	44	31	59	57	42	45	40	Dosulepin Sulfoxide
58	44	41	83	77	69	85	43	Mexiletine
58	55	41	44	43	128	59	56	Isometheptene
58	59	42	215	202	57	189	43	Cyclobenzaprine
58	59	72	45	292	218	42	–	Dimetindene
58	59	179	42	178	72	77	30	Chlorphenoxamine
58	59	202	42	203	214	217	–	Amitriptyline
58	59	221	30	42	222	255	43	Chlorprothixene
58	70	318	316	317	193	319	161	Zimeldine
58	71	26	54	167	72	42	44	Carbinoxamine
58	71	72	207	42	91	59	118	Cyclopentolate
58	71	72	167	182	42	180	59	Doxylamine
58	71	91	115	129	273	–	–	Cyclopentolate-(H_2O)
58	71	150	176	72	193	105	59	Tetracaine
58	71	208	72	59	42	89	57	Noxiptiline
58	71	414	284	296	150	121	344	Diltiazem
58	72	29	42	71	57	59	224	Normethadone
58	72	30	77	56	44	42	73	Methylephedrine
58	72	43	78	271	57	44	77	Camazepam
58	72	59	405	–	–	–	–	Toremifene
58	72	279	234	151	192	166	165	Moxisylyte
58	73	44	45	165	42	40	181	Orphenadrine
58	73	45	165	59	42	166	149	Bromazine
58	73	45	43	57	167	44	165	Dimenhydrinate
58	73	45	167	165	166	44	152	Diphenhydramine
58	77	51	56	42	105	50	43	Methcathinone
58	77	59	56	51	42	105	91	Pseudoephedrine
58	77	135	51	105	63	89	178	Methylenedioxymetamfetamine
58	85	86	57	84	70	43	268	8-Hydroxyclomipramine
58	85	269	268	270	271	314	242	Clomipramine
58	86	318	85	320	272	319	273	Chlorpromazine
58	91	42	41	134	65	59	40	Phentermine
58	91	59	134	65	56	42	57	Metamfetamine
58	91	59	56	30	42	121	78	Methoxyphenamine
58	91	59	134	65	56	42	57	Metamfetamine
58	91	72	71	197	185	184	92	Tripelennamine
58	97	72	71	42	191	79	78	Methapyrilene
58	97	72	71	203	191	190	42	Thenyldiamine
58	107	30	178	77	56	57	137	Dobutamine
58	111	71	42	75	59	141	113	Meclofenoxate
58	116	198	199	72	59	42	44	Dimethoxanate
58	117	208	115	193	91	179	130	Dextropropoxyphene
58	121	72	71	216	215	122	78	Thonzylamine
58	121	78	91	106	148	134	164	Methoxymetamfetamine
58	125	71	72	36	127	79	219	Chloropyramine
58	131	72	71	79	42	30	78	Chloropyrilene
58	134	91	121	179	42	77	277	Venlafaxine
58	140	67	72	83	81	155	156	Propylhexedrine
58	143	115	42	156	211	128	269	Rizatriptan
58	143	142	295	156	59	115	42	Sumatriptan (MSTFA derivative)
58	146	56	105	77	42	106	40	Ephedrine
58	160	218	117	154	130	42	89	5-Methoxy-*N*, *N*-dimethyltryptamine
58	165	255	359	166	73	199	45	Captodiame
58	170	284	213	145	212	159	144	Iprindole
58	179	180	225	178	165	42	210	Nefopam
58	181	43	45	60	44	165	73	Orphenadrine N-oxide
58	188	130	59	42	143	129	115	Dimethyltryptamine
58	195	59	72	388	89	315	42	Trimethobenzamide
58	204	59	42	30	146	77	44	Psilocin
58	213	198	180	214	57	212	270	*N*-Monodesmethylpromethazine-(nor)
58	220	219	59	191	189	42	205	Doxepin
58	224	209	71	225	72	210	180	Dibenzepin
58	233	29	304	72	305	159	288	Entacapone
58	234	193	192	42	235	194	59	Lofepramine
58	235	85	234	236	195	193	208	Imipramine
58	236	40	202	235	203	42	44	Dosulepin
58	238	91	45	239	167	165	56	Prenylamine
58	238	208	42	324	190	–	–	Citalopram
58	249	208	99	193	234	84	248	Trimipramine
58	251	250	211	85	42	209	296	Hydroxyimipramine
58	254	45	44	77	42	59	72	Clofedanol
58	254	111	227	214	45	44	71	Nizatidine
58	255	42	71	59	44	181	165	Phenyltoloxamine
58	282	30	284	355	73	283	44	Amodiaquine
58	284	86	238	198	199	85	42	Promazine
58	285	214	200	86	227	85	213	Prothipendyl
58	293	45	59	193	100	178	294	Butriptyline
58	298	212	198	100	299	252	199	Alimemazine
58	328	100	228	185	329	242	229	Levomepromazine
58	352	86	353	85	306	42	266	Triflupromazine
58	366	59	100	266	248	84	44	Trifluomeprazine
58	427	234	59	50	42	428	91	Narceine
59	157	156	141	43	41	71	69	3′-Hydroxyamylobarbital
59	257	150	256	31	76	42	157	Dextrorphan
59	257	150	256	44	31	200	157	Levorphanol
59	271	150	270	31	214	42	171	Dextromethorphan
60	42	88	30	31	43	29	70	Benserazide
60	44	28	–	–	–	–	–	Urea
60	167	121	197	180	151	214	274	Zanamivir
61	63	47	45	55	–	–	–	Cyanogen Chloride
63	78	45	61	46	62	48	47	Dimethyl Sulfoxide
66	120	39	65	269	205	118	77	Cyclothiazide
67	137	91	138	55	79	41	95	Dimethisterone
67	193	66	41	169	39	65	77	Cyclopentobarbital
69	43	41	44	71	167	165	55	2-Bromo-2-ethylbutyramide
69	56	71	91	261	84	119	70	Fenbutrazate
69	68	70	77	103	56	54	51	Bisnortilidate
69	83	43	79	41	81	53	80	Methylpentynol Carbamate
70	41	69	75	114	42	217	68	Captopril
70	43	55	41	57	42	71	–	Amyl Nitrite
70	43	154	71	41	209	86	195	Bromocriptine
70	44	191	192	188	59	165	71	Protriptyline
70	44	207	178	279	249	–	–	10-Hydroxyprotriptyline
70	71	42	44	57	190	29	247	Ketobemidone
70	71	269	154	195	55	59	57	Dihydroergocornine
70	83	42	257	193	56	228	164	Loxapine
70	91	84	113	245	41	28	224	Lisinopril
70	96	97	71	42	43	44	169	Naratriptan
70	112	293	264	44	42	305	41	Bromhexine
70	113	141	399	43	26	72	42	Thiethylperazine
70	120	43	91	39	103	71	65	Aletamine
70	125	71	91	153	267	154	221	Ergocristine
70	125	91	153	43	41	44	244	Dihydroergotamine
70	150	151	153	77	51	118	60	Chloramphenicol

Principal peaks at m/z								Compound
70	154	125	41	43	155	42	225	Codergocrine
70	155	55	113	69	98	–	–	Oxoethosuximide
71	42	56	43	177	77	178	105	Phenmetrazine
71	58	72	43	159	56	42	201	Quinisocaine
71	70	44	57	42	247	43	246	Pethidine
71	70	57	43	219	42	218	44	Pethidinic Acid
71	72	58	100	83	56	70	44	Diethylcarbamazine
71	86	129	139	142	157	–	–	3-*OH*-ethosuximide
71	91	106	177	57	72	65	30	Bethanidine
71	375	70	246	305	290	–	–	Ofloxacin-Me
72	30	43	77	73	51	41	27	Clorprenaline
72	30	43	122	73	41	106	121.	Sotalol
72	30	56	73	249	98	234	102	Alprenolol
72	30	56	98	43	107	41	73	Atenolol
72	30	56	43	27	41	39	110	Prenalterol
72	30	107	56	45	41	44	43	Metoprolol
72	30	116	56	43	160	41	158	4-Hydroxypropranolol
72	30	151	43	109	56	57	108	Practolol
72	39	42	38	94	56	51	81	Pyridostigmine Bromide
72	41	56	43	221	73	57	45	Oxprenolol
72	42	208	108	65	73	66	39	Neostigmine Bromide
72	43	30	56	151	221	41	98.	Acebutolol
72	43	30	56	151	221	41	98	2C-T-2-(HFB derivative)
72	43	73	41	70	65	40	39	Orciprenaline
72	44	43	124	123	30	42	41	Isoprenaline
72	44	159	73	58	42	109	56	Fenfluramine
72	56	30	43	98	115	144	41	Propranolol
72	58	71	42	73	70	56	44	Isoaminile
72	73	91	293	223	165	85	71	Methadone
72	73	214	200	44	285	86	56	Isothipendyl
72	73	284	198	213	199	180	56	Promethazine
72	73	320	71	70	56	210	198	Dimetotiazine
72	73	340	269	197	71	70	56	Propiomazine
72	86	57	70	367	282	352	334	Bambuterol
72	91	73	44	42	56	70	65	Dimethylamfetamine
72	91	73	56	148	65	57	42	Mephentermine
72	91	114	145	75	160	117	92	Oxetacaine
72	107	116	251	43	56	91	102	Esmolol
72	116	107	100	281	73	43	325	Bisoprolol
72	133	30	116	248	134	56	41	Pindolol
72	171	294	105	207	354	46	–	Levomethadyl Acetate
72	176	91	58	177	41	42	30	Alverine
72	181	85	152	42	44	58	43	Methanthelinium Bromide
72	263	292	307	55	107	192	219	Betaxolol
73	43	84	55	69	41	44	85	Emylcamate
73	58	57	43	41	39	29	45	Digoxin
73	61	103	74	43	56	45	31	Mannitol
73	75	692	115	95	147	–	–	Domoic Acid (*n*-trifluoroacetyl-o-tert-butyldimethylsilyl derivative)
73	102	41	57	43	27	55	29	Valproic Acid
73	243	169	102	114	147	128	–	Muscimol (di-TMS derivative)
73	433	307	335	434	312	74	295	Zeranol (TMS derivative)
73	534	147	143	389	444	519	–	17-Epi-Oxymesterone (Tris-TMS derivative)
74	56	44	269	284	239	255	210	Mitoxantrone
75	–	–	–	–	–	–	–	Arsenic
75	41	57	70	43	59	56	47	Penicillamine
76	173	104	111	148	50	169	130	Thalidomide
76	239	104	240	285	241	50	75	Chlortalidone
77	51	123	50	65	93	30	78	Nitrobenzene
77	106	105	51	50	78	52	39	Benzaldehyde
77	183	308	184	105	55	51	41	Phenylbutazone
77	278	105	78	51	279	252	130	Sulfinpyrazone
78	77	52	51	50	39	79	74	Benzene
78	106	51	137	50	136	107	79	Methyl Nicotinate
78	106	135	136	51	50	79	52	*N*-Methylnicotinamide
78	131	103	503	454	80	217	544	Maxacalcitol (tri-TMS, pyro form)
78	350	135	176	77	97	123	110	Tenoxicam-Me_2
79	77	108	101	51	50	39	40	Benzyl Alcohol
79	78	52	105	304	314	316	51	3-Hydroxybromazepam
79	96	31	113	81	70	127	78	Thioproperazine
79	105	104	51	78	52	50	77	Betahistine
81	53	330	96	82	332	64	63	Furosemide
81	83	46	110	82	112	84	117	Chloral Betaine
81	95	71	41	67	55	138	123	Menthol
81	124	54	53	125	171	45	42	Metronidazole
81	153	67	152	55	54	68	96	Gabapentin-(H_2O)
81	223	79	41	80	157	185	77	Thialbarbital
82	30	81	83	55	54	42	27	Ametazole
82	30	81	54	28	55	83	41	Histamine
82	47	84	29	111	83	113	85	Chloral Hydrate
82	68	91	159	42	158	92	65	Pargyline
82	81	54	28	52	55	53	51	Fomepizole
82	83	42	96	141	124	55	67	Tropine
82	97	42	83	96	57	94	55	Ecgonine
82	182	83	105	303	77	94	96	Cocaine
83	68	82	72	84	77	103	115	Nortilidate
83	70	261	202	217	203	82	232	Setiptiline
83	70	273	244	209	42	71	43	Clotiapine
83	84	55	56	43	71	41	62	Meprobamate
83	85	47	87	48	49	35	82	Chloroform
83	140	82	124	96	97	42	125	Benzatropine
83	140	82	124	43	96	97	42	Deptropine
83	141	55	41	42	100	125	86	Phosgene (bis(2-hydroxymethylpiperidine) derivative)
84	56	85	77	105	55	30	42	Pipradrol
84	71	85	82	80	341	70	356	Conessine
84	82	80	56	43	28	30	41	Coniine
84	91	77	55	182	85	65	104	Antazoline
84	91	85	56	55	150	41	118	Methylphenidate
84	91	212	296	213	297	–	–	OH-Histapyrrodine
84	97	301	56	85	96	218	82	Flecainide
84	128	179	42	85	178	214	98	Clemastine
84	133	42	162	161	105	77	119	Nicotine
84	178	161	133	118	–	–	–	Nicotine-1′-n-oxide
84	190	106	111	122	77	–	–	*N*-Desbenzyl-histapyrrodine
84	194	55	85	56	41	30	99	Perhexiline
84	204	205	85	42	55	105	77	Procyclidine
84	209	67	43	110	41	192	164	Minoxidil
85	43	42	86	44	41	75	110	Mefruside
85	56	69	55	27	44	42	109	Pipobroman
85	58	86	91	84	70	42	225	Benzydamine
85	84	183	105	56	77	55	30	Azacyclonol
85	87	101	50	103	31	66	35	Dichlorodifluoromethane
85	135	87	137	31	101	100	50	Cryofluorane
86	30	57	108	44	29	84	41	Bamethan
86	30	58	29	130	87	77	42	Diethyltryptamine
86	30	58	123	78	51	29	42	Nicametate
86	30	72	44	141	42	29	57	Butanilicaine
86	30	167	99	87	58	165	29	Adiphenine
86	30	216	87	58	100	99	56	Phenglutarimide
86	36	87	84	58	56	44	38	Amiodarone
86	36	126	30	58	38	112	99	Mepacrine
86	44	30	265	41	180	29	87	Ethomoxane
86	44	87	107	43	106	56	41	Prilocaine
86	57	41	127	190	243	90	174	Clenbuterol
86	57	277	70	292	87	114	60	Carteolol
86	57	318	259	99	112	200	333	Levobunolol Acetylated Derivative
86	58	87	42	56	77	44	43	Etafedrine
86	58	88	105	206	87	55	77	Tridihexethyl Chloride
86	58	99	56	162	132	149	205	Acecainide
86	58	99	56	162	132	149	205	*N*-Acetylprocainamide
86	58	144	84	100	76	–	–	Aldicarb
86	58	250	44	57	72	291	71	Celiprolol
86	58	319	87	73	247	245	112	Chloroquine
86	71	99	58	55	56	100	87	Dicycloverine
86	87	58	30	29	84	56	42	Carbocromen
86	87	58	149	111	99	57	41	Cinchocaine
86	87	58	44	72	42	120	85	Lidocaine
86	87	99	58	84	387	315	56	Flurazepam
86	87	100	30	58	44	42	29	Clomifene
86	91	87	145	58	144	30	44	Carbetapentane
86	91	105	87	144	58	100	56	Oxeladin
86	99	29	30	100	71	87	192	Oxybuprocaine
86	99	30	87	58	71	136	100	Proxymetacaine
86	99	91	191	87	119	58	248	Butetamate
86	99	91	144	58	56	41	87	Caramiphen
86	99	100	141	87	71	44	58	Naftidrofuryl
86	99	120	30	92	87	58	65	Procainamide
86	99	120	58	87	30	92	71	Procaine
86	99	136	30	58	178	87	71	Propoxycaine
86	99	154	30	87	58	29	156	Chloroprocaine
86	99	184	58	30	87	201	186	Metoclopramide
86	105	77	87	182	99	183	58	Benactyzine
86	109	30	151	87	81	99	58	Etamiphylline
86	114	100	44	238	115	56	72	Isopropamide Iodide
86	166	251	280	57	151	70	165	Tertatolol
86	181	43	44	41	114	42	152	Propantheline
86	298	87	30	58	299	212	180	Diethazine
86	365	369	112	144	196	158	366	Detajmium

Principal peaks at m/z								Compound
87	79	51	225	42	50	86	80	Furazolidone
87	209	237	383	341	181	–	–	(Nor-)ethylmorphine
88	42	123	124	89	77	51	44	Methyldopa
90	177	105	77	106	51	89	50	5-Phenyloxazolidine-2,4-dione
91	30	92	42	29	146	44	104	Phenformin
91	30	155	108	65	197	39	107	Tolbutamide
91	65	187	92	277	259	90	273	Trimetaphan Camsilate
91	70	208	254	117	56	160	54	Enalapril Maleate
91	79	67	201	77	105	93	120	Lynestrenol
91	79	77	55	67	93	270	105	Danazol
91	81	106	78	39	95	68	43	Ethinamate
91	92	65	39	63	93	51	89	Toluene
91	92	118	44	43	135	65	178	Phenacemide
91	106	105	77	51	39	29	92	M-xylene
91	106	197	162	107	148	27	63	Beclamide
91	120	65	121	92	105	77	118	Benethamine
91	121	65	122	309	64	230	123	Benzthiazide
91	127	106	110	43	92	65	120	Isocarboxazid
91	133	176	174	44	107	92	177	Buphenine
91	146	44	119	206	41	78	77	Pheneturide
91	146	134	132	85	56	218	175	*N*-Benzylpiperazine-(AC)
91	148	149	65	92	42	56	39	Benzfetamine
91	155	114	65	197	42	41	85	Tolazamide
91	155	197	65	84	39	95	41	Glibornuride
91	159	160	131	65	92	81	39	Tolazoline
91	159	216	160	215	84	–	–	*N*-Desphenyl-oxo-histapyrrodine
91	177	44	106	45	78	123	51	Nialamide
91	196	198	92	197	65	77	56	Phenoxybenzamine
91	196	209	197	275	294	–	–	Oxohistapyrrodine
91	215	79	105	77	55	41	298	Noretynodrel
91	216	77	259	–	–	–	–	1-(1-Phenylcyclohexyl)-4-hydroxypiperidine
91	233	30	232	31	276	275	65	Mebhydrolin
91	269	324	55	296	295	323	297	Prazepam
91	272	181	56	69	175	146	196	*N*-Benzylpiperazine-(TFAA)
91	281	372	175	295	–	–	–	*N*-Benzylpiperazine-(HFBA)
92	65	108	214	156	39	109	43	Sulfaguanidine
92	91	65	103	93	39	163	77	Tropicamide
92	156	65	191	108	55	45	174	Succinylsulfathiazole
92	156	65	108	191	45	39	55	Sulfathiazole
92	156	191	65	108	76	104	50	Phthalylsulfathiazole
92	270	65	156	108	106	93	59	Sulfamethizole
92	284	156	108	65	106	93	220	Sulfaethidole
93	135	43	66	65	39	94	92	Acetanilide
94	66	39	65	40	55	38	95	Phenol
94	138	42	108	136	41	96	97	Hyoscine
94	151	57	95	40	41	58	108	Amantadine
95	55	41	43	59	27	39	67	Picrotoxin
95	81	41	69	55	83	67	137	Camphor
95	96	42	109	41	208	54	39	Pilocarpine
95	130	132	60	97	35	134	47	Trichloroethylene
95	327	39	329	96	122	244	67	Diloxanide Furoate
96	42	195	210	71	69	44	152	Altretamine
96	44	43	31	45	27	78	264	Hydroxystilbamidine
96	56	42	91	97	39	65	58	Selegiline
96	94	159	110	97	98	136	137	Granisetron
96	97	98	91	136	31	29	55	Pentapiperide Metilsulfate
96	105	77	97	216	42	218	–	Lobeline
96	109	97	294	95	135	121	242	Astemizole
96	293	198	211	143	183	55	115	Ondansetron
97	43	71	70	96	91	98	42	Bamipine
97	55	69	72	71	98	43	62	Mebutamate
97	56	91	68	132	173	–	–	Fenproporex
97	56	139	91	118	65	–	–	Fenproporex-(AC)
97	70	99	43	98	44	42	188	Thenalidine
97	98	296	199	55	212	96	198	Methdilazine
97	99	61	117	119	63	101	62	Trichloroethane
97	105	77	96	42	183	98	82	Mepenzolate Bromide
97	105	77	183	84	36	42	51	Methylpiperidyl Benzilate
98	41	42	55	99	77	69	44	Cycrimine
98	44	242	70	28	111	149	369	Amisulpride
98	70	99	42	386	126	55	41	Mesoridazine
98	70	214	111	134	341	199	326	Sulpiride
98	84	71	56	41	42	99	124	Mecamylamine
98	99	70	42	96	55	41	40	Mepivacaine
98	99	70	228	230	243	245	185	Remoxipride
98	99	105	77	55	41	127	111	Difenidol
98	105	55	41	99	77	218	84	Trihexyphenidyl
98	111	99	147	55	41	42	96	Flavoxate
98	111	99	42	55	41	29	112	Norpipanone
98	111	99	199	41	288	200	55	Pipazetate
98	112	99	55	42	41	211	84	Fenpipramide
98	119	91	99	124	64	41	55	Diperodon
98	135	99	70	77	97	42	112	Encainide
98	137	97	136	41	193	84	110	Sparteine
98	152	154	42	69	174	208	153	Chlormezanone
98	176	42	118	41	119	51	175	Cotinine
98	194	166	157	55	42	165	99	Pipoxolan
98	215	58	84	91	56	71	186	Fencamfamin
98	218	99	55	41	42	77	84	Biperiden
98	370	126	99	40	70	371	258	Thioridazine
99	56	72	165	300	228	229	242	Chlorcyclizine
99	56	167	207	194	266	195	165	Cyclizine
99	114	98	167	70	165	57	43	Diphenylpyraline
99	125	43	81	41	39	42	47	Sarin
99	156	41	29	57	211	125	137	Tributyl Phosphate
99	197	44	58	112	309	41	42	Metixene
99	404	56	70	312	381	42	474	Sildenafil
100	44	72	101	77	56	42	105	Diethylpropion
100	56	42	176	98	120	70	189	Molindone
100	56	42	101	55	54	41	30	Nimorazole
100	56	101	42	185	184	41	128	Furaltadone
100	56	231	132	103	115	57	70	Indeloxazine
100	58	41	101	43	56	65	30	Isoetarine
100	58	105	77	56	101	41	70	Meprylcaine
100	72	240	340	44	197	254	43	Acepromazine Maleate
100	101	44	72	198	180	42	29	Profenamine
100	113	56	101	87	378	194	91	Doxapram
100	128	70	41	42	293	56	101	Pramocaine
100	142	280	456	116	58	458	42	Halofantrine
100	182	225	250	268	113	56	42	Moclobemide
100	265	128	266	44	98	56	101	Dextromoramide
100	286	142	56	42	239	70	185	Moracizine
101	103	105	66	47	35	31	82	Trichlorofluoromethane
102	30	72	44	116	55	173	71	Ethambutol
102	84	58	56	42	30	103	91	Bamifylline
102	245	247	304	305	306	58	126	Hydroxychloroquine
103	77	82	29	97	42	51	104	Tilidate
104	105	204	77	78	133	51	132	Ethotoin
104	132	218	51	103	77	78	52	Phenylmethylbarbituric Acid
104	189	103	117	78	91	51	146	2-Phenylglutarimide
104	189	103	78	51	77	105	52	Phensuximide
104	209	77	116	115	94	–	–	(*N*-Desalkyl-)aprindine
105	77	51	122	50	39	74	76	Benzoic Acid
105	77	51	–	–	–	–	–	CN Gas
105	77	96	183	51	42	182	94	Clidinium Bromide
105	77	106	44	51	128	78	40	Broxaldine
105	79	81	45	55	–	–	–	Cyanogen Bromide
105	104	77	79	244	106	108	27	Etomidate
105	111	77	96	97	183	42	51	Pipenzolate Bromide
105	129	112	77	42	313	41	55	Oxyphencyclimine
105	135	51	134	77	106	50	78	Hippuric Acid
105	177	77	209	254	210	103	181	Ketoprofen
105	210	255	77	51	132	91	65	Ketorolac
105	554	540	43	45	77	31	29	Aconitine
106	30	77	185	105	104	89	141	Mafenide
106	78	51	137	50	79	107	31	Isoniazid
106	78	177	51	178	107	149	40	Nikethamide
106	119	118	98	77	204	–	–	*N*-Desbenzyl-oxo-histapyrrodine
106	123	78	51	119	79	77	107	Salinazid
107	45	149	242	181	58	–	–	Lewisite Oxide (2-chlorovinylarsonous oxide (CVAO))1, 3-propanedithiol (PDT) derivative)
107	69	125	83	79	55	41	77	Cyclandelate
107	79	77	51	152	105	50	78	Mandelic Acid
107	79	77	51	152	105	50	78	Mandelic Acid
107	108	77	79	51	39	53	50	*p*-Cresol
107	108	78	79	80	77	51	52	Fenyramidol
107	120	79	75	77	44	91	45	Styramate
107	145	268	238	121	133	159	224	Diethylstilbestrol
107	163	223	108	29	164	41	57	Bufexamac
107	165	123	95	121	–	–	–	Azaperone
107	176	90	77	70	105	42	79	Pemoline
108	81	213	54	77	136	51	43	Phenazopyridine
108	107	91	182	109	77	79	31	Mephenesin
108	109	179	137	43	81	80	110	Phenacetin
108	122	179	164	–	–	–	–	Memantine
108	248	140	65	92	141	109	80	Dapsone
109	27	165	198	40	55	57	288	Mizolastine
109	76	104	92	239	65	108	43	Phthalylsulfacetamide
109	79	110	145	139	47	15	112	Trichlorfon
109	80	44	81	53	39	52	54	Aminosalicylic Acid
109	80	53	81	108	52	54	110	4-Aminophenol
109	108	80	53	51	39	91	27	Nicotinyl Alcohol
109	111	158	63	160	73	–	–	Sulfur Mustard
109	151	43	80	108	81	53	52	Paracetamol

Principal peaks at m/z								Compound
109	151	43	41	221	179	29	108	Tiocarlide
109	241	132	83	242	111	–	–	N-Desalkylastemizole
109	304	231	124	258	110	83	123	Flupirtine
110	82	39	81	53	69	55	111	Resorcinol
110	263	125	262	265	264	111	89	Ticlopidine
111	96	167	112	165	71	43	42	Piperidolate
111	112	127	55	139	358	–	–	Clopamide-(Me_2)
111	127	55	83	59	41	112	42	Clopamide
111	127	139	83	96	251	–	–	Clopamide-(SO_2NH) (art)
111	175	75	85	30	276	127	113	Chlorpropamide
112	77	105	139	55	41	96	56	Hexylcaine
112	161	85	45	163	113	114	59	Clomethiazole
112	246	105	77	55	41	44	247	Piperocaine
112	264	113	91	179	110	178	115	Dipipanone
112	344	121	41	67	55	54	345	Cyclomethycaine
113	70	42	211	351	43	71	56	Pirenzepine
113	70	55	42	41	39	85	69	Ethosuximide
113	70	114	42	221	222	56	43	Tiotixene
113	70	373	141	43	72	42	127	Prochlorperazine
113	70	407	43	141	42	127	71	Trifluoperazine
113	70	409	43	141	283	42	127	Butaperazine
113	85	98	69	71	142	–	–	OH-Ethyl-ethosuximide
113	86	116	98	117	115	58	84	Aprindine
113	100	71	43	73	81	96	376	Aclarubicin
113	339	44	70	141	43	60	340	Perazine
114	42	72	113	69	81	54	115	Thiamazole
114	44	142	365	42	223	115	205	Pericyazine
114	72	43	100	29	44	337	86	Propanidid
114	72	275	160	56	89	188	215	5-Methoxy-*N*, *N*-diisopropyltryptamine
114	97	71	140	157	–	–	–	Allantoin
114	100	56	42	115	101	70	398	Pholcodine
114	115	160	72	43	86	229	42	Ropinirole
114	159	142	96	141	–	–	–	Oxiracetam
114	382	73	397	116	30	72	135	Tolterodine (TMS derivative)
115	86	79	57	56	107	100	52	Morinamide
115	117	89	53	109	51	91	39	Ethchlorvynol
115	187	139	145	157	170	259	289	Mannitol Hexaacetate
116	88	29	44	60	148	56	27	Disulfiram
117	119	47	35	121	82	84	49	Carbon Tetrachloride
117	119	121	82	164	–	–	–	Chloropicrin
117	198	196	119	129	67	127	98	Halothane
118	108	182	91	225	107	57	75	Mephenesin Carbamate
118	117	91	92	119	65	77	103	Phenprobamate
118	117	203	103	77	78	119	91	Mesuximide
118	313	80	192	233	144	172	206	Valdecoxib
120	43	138	92	121	39	64	63	Aspirin
120	92	105	148	150	121	133	65	Etenzamide
120	92	137	65	121	39	64	53	Salicylamide
120	92	138	64	39	63	121	65	Salicylic Acid
120	92	152	121	65	64	93	63	Methyl Salicylate
120	106	78	92	51	226	41	39	Metyrapone
120	137	193	92	65	121	138	41	Butyl Aminobenzoate
120	165	92	65	137	39	121	93	Benzocaine
120	263	142	178	100	264	41	29	Butacaine
121	43	122	223	147	91	41	135	Betamethasone
121	58	72	71	214	122	215	78	Mepyramine
121	65	36	185	50	38	76	39	Halazone
121	120	69	92	195	39	93	45	Salicyluric Acid
121	120	354	122	43	234	106	64	Xipamide
121	122	91	147	225	43	135	120	Prednisolone
121	122	315	43	147	223	135	41	Dexamethasone
121	135	190	91	293	161	177	–	Embutramide
121	148	122	149	169	240	361	–	Methoxyamfetamine
121	152	40	93	65	–	–	–	Methyl Hydroxybenzoate
121	163	151	109	43	122	108	65	Benorilate
121	164	77	57	56	165	162	122	Ritodrine
121	180	364	278	205	382	305	261	T-2 Toxin
121	318	317	173	120	362	44	31	Ethyl Biscoumacetate
121	429	280	430	389	134	122	281	Mifepristone
122	78	106	51	50	52	44	123	Nicotinamide
122	119	77	78	91	107	65	63	Formoterol Glucuronide
122	121	91	123	147	77	107	93	Boldenone
122	121	91	147	161	43	107	120	Ethisterone
122	221	105	91	–	–	–	–	Metaxalone
123	31	58	106	79	43	78	51	Iproniazid
123	51	78	106	50	52	105	39	Isonicotinic Acid
123	57	42	103	44	85	124	51	Carbidopa
123	105	78	51	106	77	124	50	Nicotinic Acid
123	106	78	51	105	50	77	52	Nicofuranose
123	124	77	44	51	74	53	39	Levodopa
124	43	314	79	91	229	272	105	Progesterone
124	82	83	94	55	42	67	96	Tigloidine
124	82	94	83	42	96	103	67	Atropine
124	82	94	83	140	144	67	284	Tropisetron
124	82	168	77	105	42	94	83	Benzoylecgonine
124	107	82	83	42	77	79	94	Homatropine
124	109	198	81	77	125	52	31	Guaifenesin
124	118	198	109	43	57	125	77	Methocarbamol
124	276	58	140	56	72	125	41	Eucatropine
125	44	70	91	41	40	244	153	Ergotamine
125	69	138	511	68	458	–	–	Progabide
125	70	91	153	41	244	43	71	Dihydroergocristine
125	109	79	277	47	93	63	260	Fenitrothion
125	189	230	127	191	63	232	90	Diazoxide
125	207	55	369	41	126	134	163	Cilostazol
126	44	30	42	41	55	43	58	Guanethidine
126	56	70	211	127	151	152	85	Pramipexole
126	84	56	–	–	–	–	–	Ropivacaine
126	98	41	69	58	55	127	44	Levetiracetam
126	99	69	82	57	–	–	–	Soman
126	127	174	91	70	69	55	42	Prolintane
127	43	229	44	161	231	85	186	Chlorproguanil
127	161	163	153	129	187	90	189	Triclocarban
128	96	70	39	41	27	42	29	Cantharidin
128	130	65	39	64	63	129	99	Parachlorophenol
128	130	169	87	41	129	242	171	Clofibrate
128	130	202	43	129	111	75	204	Chlorphenesin Carbamate
128	141	268	130	270	77	143	233	Dichlorophen
129	172	128	102	101	130	76	77	Saquinavir Mesilate
130	131	30	160	77	103	132	102	Tryptamine
131	167	41	103	43	132	77	289	Ciprofibrate
131	256	125	42	255	89	258	257	Clemizole
132	104	44	175	103	117	43	130	Debrisoquine
133	132	56	115	30	117	91	77	Tranylcypromine
134	93	135	144	116	296	162	324	Dolasetron
134	104	103	91	77	78	105	44	Felbamate
134	104	191	143	162	77	–	–	Norfluoxetine
135	57	91	77	43	119	105	180	Hydroxyphenamate
135	136	164	29	31	108	178	237	Adenosine
135	150	91	107	117	–	–	–	Thymol
135	358	–	–	–	–	–	–	Rosiglitazone
136	43	123	148	161	113	83	414	Metenolone
136	81	137	42	41	55	130	128	Cinchonidine
136	81	322	188	55	42	41	172	Quinidine
136	123	148	83	93	107	161	67	Metenolone Acetate
136	135	52	28	137	109	29	18	Allopurinol
136	137	81	42	41	189	55	117	Quinine
136	294	81	159	55	42	41	143	Cinchonine
136	310	135	225	149	122	155	311	Ibogaine
137	120	92	65	39	138	121	63	Aminobenzoic Acid
137	120	92	65	39	138	121	63	4-Aminobenzoic acid
138	55	189	82	41	110	326	42	Hydroquinine
138	326	55	110	189	82	160	139	Hydroquinidine
138	352	215	310	214	69	321	354	Griseofulvin
139	141	357	111	359	140	113	75	Indometacin
140	84	56	41	72	69	29	39	Pempidine
140	141	84	41	29	96	56	55	Bupivacaine
141	43	183	44	140	80	108	52	Paracetamol (cysteine conjugate)
141	156	41	55	98	39	142	155	Butobarbital
141	156	41	57	39	98	157	47	Secbutabarbital
141	156	43	41	157	55	39	98	Pentobarbital
141	445	169	123	155	96	42	317	Metopimazine
142	44	140	42	198	170	41	96	Pipotiazine
142	126	127	80	54	42	55	69	Zidovudine
142	143	100	155	44	112	57	29	Butalamine
142	168	228	91	119	287	–	–	Desethyl-OH-fencamfamin
142	170	44	410	42	143	43	41	Piperacetazine
143	36	70	144	38	100	35	56	Zuclopenthixol Dihydrochloride
143	70	100	144	42	56	98	221	Clopenthixol
143	70	100	144	42	98	58	56	Flupentixol
143	70	100	221	98	42	222	234	Zuclopenthixol
143	245	218	247	98	112	159	56	Zopiclone
144	115	116	57	58	63	145	89	Carbaryl
146	190	117	118	161	189	103	91	Primidone
146	233	103	133	91	117	115	77	4-Hydroxyglutethimide
147	43	146	42	193	118	164	44	Carbazochrome
147	73	75	233	117	148	77	59	γ-Hydroxybutyrate TMS_2
147	131	130	132	119	148	365	218	Indapamide
147	191	73	131	45	59	87	117	Ethylene Glycol
149	41	29	57	56	104	32	65	Dibutyl Phthalate
149	177	150	65	76	105	176	104	Diethyl Phthalate
149	204	121	160	–	–	–	–	Methylone (pentafluoropropionyl derivative)

Principal peaks at m/z								Compound
150	121	56	93	39	151	66	94	Glipizide
150	134	121	356	–	–	–	–	Pioglitazone
150	152	165	41	167	43	44	130	2-Bromo-2-ethyl-3-hydroxybutyramide
150	326	163	203	251	281	41	311	Acitretin
151	72	223	123	222	152	52	29	Etamivan
151	94	122	106	51	53	149	150	Pyridoxine
151	96	123	69	–	–	–	–	Phosgene (aminothiophenol derivative)
151	109	136	178	177	29	207	135	Thiambutosine
151	136	121	180	297	77	93	194	Omeprazole
151	164	45	43	165	109	110	108	Aciclovir
152	119	165	163	180	60	179	153	Protionamide
152	151	81	109	51	–	–	–	Vanillin
153	93	30	65	152	154	125	110	Normetanephrine
153	93	30	65	152	154	125	110	Normetadrenaline
153	183	126	75	50	161	99	137	CS Gas
154	31	29	139	133	43	27	–	Deferiprone-o-methylated Metabolite
154	70	155	167	223	225	349	153	Dihydroergocryptine
154	153	182	121	69	155	77	110	Phosgene (dimercaptotoluene derivative)
155	96	140	43	42	81	94	53	Arecoline
155	140	83	98	55	41	84	69	Methyprylon
155	170	112	169	55	82	41	39	Metharbital
155	214	283	–	–	–	–	–	JWH-018
156	92	108	65	140	43	157	42	Sulfafurazole
156	92	108	65	140	253	157	43	Sulfamethoxazole
156	141	45	157	41	29	55	27	3′-Hydroxybutobarbitone
156	141	55	155	98	39	82	43	Barbital
156	141	55	41	157	43	98	39	Hexethal
156	141	55	155	98	39	82	43	Barbital
156	141	157	41	55	142	98	39	Amobarbital
156	157	113	111	358	96	97	81	Tiagabine-Me
156	158	92	77	157	314	108	65	Sulfaphenazole
158	214	57	145	124	45	70	96	Niridazole
159	157	43	198	71	172	143	105	Simvastatin
159	157	64	198	172	143	105	73	Lovastatin
159	161	81	335	333	163	337	205	Miconazole
160	103	89	131	115	76	161	104	Hydralazine
160	300	189	145	188	301	161	42	Azapropazone
162	43	63	164	30	44	98	42	Guanoclor
163	77	164	135	194	76	92	50	Dimethyl Phthalate
163	148	91	103	117	120	44	77	Phenylethylmalondiamide
163	161	119	91	206	117	107	164	Ibuprofen
163	178	91	117	41	164	77	121	Propofol
164	149	131	137	103	77	133	165	Eugenol
165	138	331	123	110	194	42	41	Pipamperone
165	234	194	235	193	196	166	179	Azapetine
166	68	95	41	53	123	–	–	3-Methylxanthine
166	68	95	41	53	123	–	–	3-Methylxanthine
166	68	123	53	42	41	95	–	7-Methylxanthine
166	164	129	131	168	94	133	96	Tetrachloroethylene
166	165	167	138	133	105	60	106	Ethionamide
166	209	221	180	308	123	95	45	Propentofylline
167	41	124	168	97	39	169	45	Aprobarbital
167	41	168	124	39	97	141	67	Idobutal
167	168	41	43	97	124	39	55	Secobarbital
167	168	41	97	124	39	57	53	Talbutal
167	209	43	124	39	41	53	140	Ibomal
168	125	210	170	91	127	212	89	Clobenzorex-(AC)
168	137	44	139	43	152	124	167	Methoxamine
168	140	169	114	141	167	113	63	Norharman
168	167	514	548	40	166	51	–	Ceftazidime
168	227	55	212	303	140	93	42	Remifentanil
169	58	168	170	72	167	44	42	Pheniramine
169	78	171	113	115	63	51	170	Chlorzoxazone
169	92	289	65	290	39	333	184	Sulfasalazine
170	142	58	97	91	84	–	–	Fencamfamin-(AC)
170	142	91	171	115	229	–	–	Desethyl-fencamfamin
171	43	143	41	128	55	141	159	5-(2 3-Dihydroxypropyl) secobarbital
171	228	172	185	128	43	115	141	Nabumetone
172	92	156	65	108	39	44	41	Sulfacetamide
172	92	156	65	108	173	39	174	Sulfanilamide
172	98	73	367	173	56	124	55	Perindopril-(TMS)
172	157	173	43	41	55	69	71	Thiopental
172	187	84	57	42	188	44	43	Alphaprodine
173	117	145	78	104	94	76	147	Piroxicam
173	340	168	167	165	341	174	322	Diphenadione
174	73	291	–	–	–	–	–	Alminoprofen (TMS TFA derivative)
174	77	91	105	132	–	–	–	Norphenazone
174	160	104	145	117	77	91	129	Carphedon

Principal peaks at m/z								Compound
175	56	176	91	104	86	174	85	Reboxetine
175	70	176	132	379	204	56	217	Oxypertine
175	73	70	132	160	104	–	–	Levodropropizine
175	189	190	286	173	162	271	163	Abacavir
176	59	132	150	45	193	105	29	Benzonatate
177	118	45	135	90	91	221	119	Procarbazine
177	277	265	122	148	108	56	70	Buspirone
178	44	135	179	77	84	107	41	Isoxsuprine
178	44	163	135	77	196	176	179	Protokylol
178	192	272	466	244	288	191	273	Cephaëline
179	159	191	247	217	229	–	–	Fusarenone-X
180	55	67	109	82	42	137	70	Theobromine
180	77	181	44	179	51	209	167	*trans*-10,11-Dihydro-10, 11-dihydroxycarbamazepine
180	95	68	41	58	53	123	96	Bufylline
180	95	68	41	53	181	96	40	Theophylline
180	104	223	77	209	252	51	165	Phenytoin
180	179	178	152	44	181	223	51	Carbamazepine-10,11-epoxide
180	209	182	152	181	30	211	138	Ketamine
180	209	252	208	152	179	181	210	Oxcarbazepine
180	223	194	254	109	95	193	166	Diprophylline
180	264	193	109	194	181	67	42	Pentifylline
181	41	182	39	124	53	138	97	Enallylpropymal
181	80	123	256	161	197	304	93	VX (EMPA-PFB Derivative)
181	85	56	166	266	182	179	91	Trimetazidine
181	152	153	254	182	151	76	127	Fenbufen
181	152	153	182	268	237	–	–	Fenbufen -me
181	256	80	303	97	276	161	179	Sarin (IMPA-PFB derivative)
181	256	123	277	97	303	41	80	Soman (PFB derivative)
181	263	246	182	198	154	91	65	Torasemide
181	395	198	251	397	396	199	666	Syrosingopine
182	30	181	167	211	183	151	148	Mescaline
182	57	43	55	40	69	41	181	Harman
182	288	167	156	165	243	183	157	Zolmitriptan
183	43	29	60	41	129	91	73	Ouabain
183	76	90	50	91	120	106	92	Saccharin
183	77	252	320	184	41	69	51	Feprazone
184	92	185	65	39	108	66	186	Sulfapyridine
185	36	186	98	87	43	70	221	Zuclopenthixol Acetate
185	187	255	257	123	117	157	150	Lamotrigine
185	230	141	186	184	115	170	153	Naproxen
186	42	201	56	57	187	202	71	Trimeperidine
186	114	29	72	42	113	27	109	Carbimazole
186	158	102	51	130	50	76	75	Furanocoumarins
186	185	92	65	108	39	93	187	Sulfadiazine
186	218	77	295	263	105	51	158	Mebendazole
187	71	45	276	43	172	145	200	Fluvoxamine
188	47	82	96	29	77	84	56	Dichloralphenazone
188	96	77	56	105	189	55	51	Phenazone
188	155	113	43	45	85	69	146	Famotidine
188	189	159	132	53	173	131	145	Nalidixic Acid
189	104	190	77	44	105	132	103	Mephenytoin
189	105	201	285	165	166	190	134	Meclozine
189	132	117	160	91	115	103	77	Glutethimide
191	121	77	104	122	43	51	192	Amiphenazole
191	192	207	208	209	–	–	–	Amineptine
191	261	274	260	316	192	190	176	Tetrabenazine
192	193	287	176	191	286	270	285	Dehydroemetine
192	201	249	165	450	–	–	–	Losartan-2ME
192	206	272	480	288	246	205	191	Emetine
192	343	342	232	190	165	193	67	Irbesartan
193	118	140	91	56	152	–	–	Fenproporex-(TFA)
193	192	236	191	194	165	190	237	Carbamazepine
193	208	194	250	178	192	29	179	*N*-Desmethylmianserin
193	255	228	256	257	165	230	258	Desmethylmedazepam
193	264	43	72	71	220	192	194	Mianserin
194	109	55	67	82	195	42	110	Caffeine
194	165	123	342	338	134	–	–	*O*-Desmethyl-fluanisone
194	180	109	193	238	95	137	81	Proxyphylline
194	195	238	193	72	178	45	196	Nomifensine
195	41	141	69	39	152	135	196	Vinbarbital
195	194	196	209	180	167	–	–	Desmethylmirtazapine
195	208	42	180	167	71	111	265	Mirtazapine
195	212	114	30	194	72	44	43	Disopyramide
195	281	196	152	280	81	77	66	Trimetozine
196	239	208	200	225	397	396	257	5-Hydroxyl-amosulalol
197	241	198	77	242	104	91	103	Fenoprofen
197	256	154	128	152	198	–	–	Acetic Acid OH-fenbufen
197	256	198	257	91	103	–	–	Fenoprofen-(Me)
198	58	115	70	269	199	72	71	Aminopromazine
198	197	170	199	182	169	77	183	Tacrine
199	167	198	166	99	154	69	77	Phenothiazine
199	200	92	65	108	39	66	201	Sulfamerazine
199	244	200	178	179	184	183	245	Flurbiprofen

Principal peaks at m/z								Compound
199	324	93	77	65	55	121	135	Oxyphenbutazone
200	91	84	86	186	259	–	–	4-Phenyl-4-piperidinocyclohexanol
200	91	243	242	84	186	166	201	Phencyclidine
200	185	199	171	44	84	71	131	Tetryzoline
201	81	98	175	259	176	202	242	Primaquine
201	117	167	202	251	165	118	115	Cinnarizine
201	165	203	56	187	242	299	388	Cetirizine
201	174	63	202	64	65	175	90	Tiabendazole
201	176	202	56	258	70	71	42	Morazone
201	203	165	45	299	166	202	56	Hydroxyzine
202	366	115	77	368	125	105	213	Atovaquone
203	58	44	205	54	204	72	202	Chlorphenamine
203	232	132	175	204	233	160	118	Aminoglutethimide
204	117	146	161	77	103	115	118	Phenobarbital
204	148	73	101	203	127	104	205	Levamisole
205	42	135	173	206	123	145	45	Pyrantel
205	70	231	78	135	136	42	166	Trazodone
205	77	41	39	220	130	145	131	Xylazine
205	218	123	356	219	162	95	190	Fluanisone
205	240	91	84	125	242	206	186	Pyrrobutamine
205	244	191	345	206	72	272	246	Benzquinamide
207	41	39	124	91	165	122	44	Brallobarbital
207	141	81	79	67	80	41	77	Cyclobarbital
208	190	146	172	142	112	–	–	Miglitol
208	193	130	115	91	165	179	207	EMDP
208	199	221	163	231	147	281	244	Zotepine
208	209	278	207	193	200	194	84	Triprolidine
209	70	224	293	115	41	156	266	Anastrozole
209	210	141	115	153	208	46	181	Naphazoline
209	257	180	208	210	77	44	194	3′-Hydroxymethylmefenamic acid
210	56	43	184	211	75	99	76	Acetohexamide
210	144	239	45	321	209	95	211	Quetiapine
210	252	371	284	312	43	178	240	Isradipine
211	143	91	283	197	70	344	97	Cilazapril
211	182	184	210	212	183	155	156	Milrinone
211	285	179	137	107	253	–	–	Thioglycolic Acid Methyl Ester Derivative of As[3]
212	42	187	45	70	180	56	98	Dixyrazine
212	86	271	197	185	156	–	–	DOC-(AC)
212	169	197	213	106	211	170	168	Harmine
212	213	122	198	44	53	91	65	Tolmetin
213	160	159	296	133	145	212	157	Ethinylestradiol
213	214	170	198	169	115	63	143	Harmaline
214	92	65	213	108	42	215	39	Sulfisomidine
214	213	92	65	215	108	39	42	Sulfadimidine
214	216	242	295	215	297	179	178	Diclofenac
215	92	216	65	108	53	69	39	Sulfamethoxypyridazine
215	214	44	185	200	216	157	186	Tramazoline
215	230	56	77	216	96	41	39	Propyphenazone
216	215	92	65	108	54	125	39	Sulfametoxydiazine
217	159	95	43	186	143	160	218	Bicalutamide
217	190	285	215	216	284	102	218	Letrozole
218	42	219	91	165	155	193	115	Diphenoxylic Acid
218	44	191	221	219	178	42	180	Benzoctamine
218	117	118	146	103	77	91	115	Methylphenobarbital
218	174	160	161	275	219	175	162	Physostigmine
219	248	148	220	120	218	133	64	4-Hydroxyphenobarbital
220	191	204	178	192	221	233	410	Risperidone
220	221	205	147	42	193	77	118	Noscapine
221	43	78	93	80	41	141	39	Heptabarb
221	72	325	54	196	55	207	181	Ergometrine
221	81	157	80	79	155	41	77	Hexobarbital
221	199	200	186	395	251	77	214	Rescinnamine
221	263	342	298	368	326	–	–	Nilvadipine
221	314	248	261	193	236	222	315	Δ8-THC
222	165	76	223	166	105	104	90	Phenindione
223	241	208	222	194	180	77	224	Mefenamic Acid
224	42	237	226	123	206	239	56	Haloperidol
224	82	127	55	83	141	125	113	Fluconazole
226	225	183	30	255	153	169	227	2C-T-7
227	265	211	240	312	225	197	181	Tetrahydrogestrinone
227	310	174	284	147	160	173	199	Mestranol
228	182	363	88	229	76	276	257	Clefamide
228	200	199	128	229	185	115	201	Trioxysalen
228	258	287	198	57	229	259	214	Etodolac
229	30	231	172	194	174	200	230	Clonidine
229	215	202	242	301	258	–	–	Oxocyproheptadine
229	257	303	42	230	258	201	215	Flumazenil
230	109	82	187	243	363	42	123	Benperidol
230	187	42	217	83	82	461	96	Pimozide
230	231	58	44	42	105	173	159	Phenazocine

Principal peaks at m/z								Compound
230	232	215	217	77	259	261	91	2C-B
230	271	55	256	164	270	71	124	Cyclazocine
231	42	119	232	92	65	67	74	Azathioprine
231	147	285	232	201	132	165	166	Buclizine
231	246	314	232	121	193	74	174	CBD
232	184	233	201	186	433	95	120	Cisapride
232	250	175	204	176	233	102	78	Diflunisal
233	45	245	316	451	259	42	96	Doxazosin
233	71	316	245	43	387	234	205	Terazosin
233	383	259	245	95	56	246	234	Prazosin
234	91	74	107	70	160	55	404	Imidapril (TMS derivative)
234	146	179	206	131	91	117	118	Metisazone
235	73	250	207	161	115	128	177	Propofol (TMS derivative)
235	195	208	44	234	193	194	71	Desipramine
235	250	91	233	236	65	76	132	Methaqualone
235	307	219	92	65	42	115	102	Zolpidem
236	235	264	235	307	278	321	248	Fluvastatin
236	317	315	288	316	286	208	78	Bromazepam
238	42	239	224	240	56	72	226	Loperamide
238	210	239	354	383	338	178	310	Felodipine
238	210	239	287	150	360	178	67	Nitrendipine
239	196	268	120	197	225	77	104	HPPH
239	298	135	191	107	103	–	–	OH-Indometacin
240	212	241	184	138	92	128	63	Dantron
240	478	143	455	373	333	263	–	Dehydrochloromethyltestosterone Di-TMS derivative
241	77	242	105	44	43	195	57	2-Amino-5-nitrobenzophenone
242	43	270	269	241	103	243	76	Clorazepic Acid
242	207	244	270	243	271	269	165	Medazepam
242	229	213	312	198	42	254	99	Olanzapine
242	244	229	148	301	303	77	201	2C-B-(Ac)
242	269	270	241	243	271	244	272	Desmethyldiazepam
242	269	270	241	243	271	244	272	Nordazepam
243	118	190	56	91	202	–	–	Fenproporex-(PFP)
243	157	136	198	200	242	–	–	Nordextrorphan
243	256	70	245	192	227	258	326	Clozapine
244	42	72	475	109	245	85	476	Fluspirilene
244	59	77	29	43	31	45	55	Glymidine Sodium
244	229	44	173	40	230	243	245	Xylometazoline
244	242	229	231	148	455	457	199	2C-B-(HFB derivative)
245	146	42	189	44	105	29	43	Fentanyl
245	257	247	193	56	246	228	259	Amoxapine
245	260	44	217	218	246	261	259	Oxymetazoline
246	42	247	91	103	165	115	56	Diphenoxylate
246	42	367	247	57	56	91	77	Phenoperidine
246	45	42	366	58	57	43	106	Piminodine
246	92	65	245	108	247	39	260	Sulfadimethoxine
246	143	403	70	404	42	248	113	Perphenazine
246	165	42	123	199	247	214	108	Droperidol
246	243	180	315	248	245	182	167	Efavirenz
246	247	42	120	218	172	106	91	Anileridine
246	291	248	290	247	139	111	292	Zomepirac
247	189	249	191	243	55	29	245	Etacrynic Acid
247	248	249	250	219	212	106	221	Pyrimethamine
247	249	58	72	248	167	250	168	Brompheniramine
247	260	233	373	234	221	245	259	Bunazosin
247	285	328	249	41	287	330	55	Clorexolone
248	44	249	121	136	29	164	192	Ambucetamide
248	261	219	233	247	205	318	389	Alfuzosin
248	305	263	262	43	249	306	219	Zaleplon
249	166	221	306	–	–	–	–	3-Methyl-1-(5-hydroxyhexyl)-7-propylxanthine
250	70	207	91	251	119	148	56	Fenetylline
250	95	39	235	66	207	41	193	3′-Oxohexobarbital
251	70	253	28	41	42	139	18	Mexazolam
251	222	223	250	252	195	110	97	7-Aminonitrazepam
251	266	249	77	143	76	252	39	4′-Hydroxymethaqualone
251	280	118	91	121	119	252	189	Phenprocoumon
252	196	326	169	119	382	69	150	Lacidipine
252	237	253	181	238	77	76	209	Anisindione
252	297	–	–	–	–	–	–	Denopamine O-(TMS)
252	312	253	237	141	198	213	270	Trenbolone Acetate
253	91	213	43	105	271	79	145	Methandriol
253	218	196	255	42	198	43	224	Tizanidine
253	252	43	104	254	235	77	89	Triamterene
253	288	287	289	225	259	254	41	Tetrazepam
254	143	42	70	411	113	56	157	Acetophenazine
254	256	118	255	303	305	63	45	Chlorambucil
256	121	185	43	257	65	42	214	Probenecid
256	283	284	285	257	255	258	286	Diazepam
256	284	283	285	84	257	258	255	Ketazolam
256	301	91	258	119	65	257	303	Benoxaprofen

Principal peaks at m/z								Compound
257	55	311	77	259	313	44	312	3-Hydroxyprazepam
257	59	150	256	31	157	42	200	Racemorphan
257	73	147	359	331	218	–	–	Ibotenic Acid (tri-TMS derivative)
257	77	268	239	205	267	233	259	Oxazepam
257	77	268	239	205	267	233	259	Ketazolam
257	77	268	239	205	267	233	259	Oxazepam
258	260	262	–	–	–	–	–	Lofexidine
259	215	213	147	159	230	–	–	Norlevorphanol
260	259	288	287	261	289	262	290	N^1-Desalkylflurazepam
260	261	42	57	184	215	217	262	Phenindamine
260	262	180	287	261	145	286	124	Quinethazone
261	263	243	245	281	316	–	–	Etacrynic Acid-(Me)
262	264	110	138	125	152	89	77	Clopidogrel Bisulfate
262	308	91	124	117	223	179	160	Trandolapril
263	281	166	92	145	167	235	139	Flufenamic Acid
263	295	235	166	264	92	–	–	Flufenamic Acid-(Me)
264	378	192	250	320	58	165	251	Valsartan-ME_2
265	251	267	133	237	135	78	211	Nevirapine
265	308	121	43	266	187	213	251	Warfarin
266	267	224	220	268	44	250	248	Apomorphine
266	268	264	167	165	270	202	132	Pentachlorophenol
266	268	267	255	231	102	88	176	Mazindol
267	221	207	180	223	154	196	268	Lysergamide
267	284	330	221	253	239	–	–	Arteether
267	299	266	31	51	268	29	77	Fenbendazole
268	143	425	70	269	42	394	157	Carfenazine
268	224	154	180	207	223	192	179	Lysergic Acid
268	390	269	391	413	262	270	389	Tadalafil
269	205	221	297	271	62	285	124	Hydrochlorothiazide
269	240	241	268	270	107	121	213	7-Amino-1-desmethylflunitrazepam
270	198	252	226	141	115	155	128	Trenbolone
271	43	41	311	295	312	297	91	8α-Hydroxy-Δ9-THC
271	43	295	41	297	29	330	272	8β-Hydroxy-Δ9-THC
271	81	150	201	148	110	272	82	Normorphine
271	272	270	165	193	241	–	–	Nor-OH-cyproheptadine
271	273	300	272	256	77	255	257	Temazepam
272	229	200	188	145	172	56	–	Trifluoromethylphenylpiperazine (AC derivative)
272	273	41	327	145	76	42	29	Butorphanol
273	215	216	231	229	272	–	–	Norcyproheptadine
274	159	276	103	262	132	129	161	Sertraline
274	225	318	273	275	226	257	121	Phenolphthalein
276	111	219	42	277	97	29	100	Diethylthiambutene
278	70	56	41	42	91	115	178	Budipine
278	125	109	169	93	–	–	–	Fenthion
279	163	323	58	277	308	280	322	Dimefline
280	143	42	70	437	406	113	56	Fluphenazine
280	253	281	206	234	252	254	264	Nitrazepam
280	266	310	281	267	217	216	245	Desloratadine
280	281	501	262	105	85	–	–	Fexofenadine
280	314	315	286	234	288	316	240	Clonazepam
280	359	238	161	–	–	–	–	Etoricoxib
281	43	282	187	107	91	55	105	Megestrol Acetate
281	120	91	122	280	160	68	282	Phentolamine
282	236	237	281	263	145	44	93	Niflumic Acid
282	299	284	283	241	56	301	253	Chlordiazepoxide
283	44	255	282	254	284	264	256	7-Aminoflunitrazepam
283	282	256	176	157	43	41	57	Levallorphan
284	91	375	81	42	36	285	175	Benzylmorphine
285	81	215	148	286	164	110	115	Norcodeine
285	96	229	228	70	214	115	200	Hydromorphone
285	162	42	215	286	124	44	284	Morphine
285	256	257	258	44	287	110	220	7-Aminoclonazepam
285	268	284	77	286	42	287	233	Desmethylchlordiazepoxide
285	286	269	287	241	242	77	270	Demoxepam
285	312	313	286	266	238	294	284	Flunitrazepam
286	124	244	148	109	107	287	147	Androstenedione
286	227	287	123	91	152	–	–	OH-Fenoprofen
286	229	91	81	287	41	77	228	Famprofazone
286	244	77	218	51	217	288	215	Desmethylclobazam
286	300	96	42	244	230	301	82	Bezitramide
287	70	44	164	42	288	59	230	Dihydromorphine
287	96	286	215	70	44	58	42	Cyproheptadine
287	245	289	331	56	29	41	57	Ciprofloxacin
288	69	178	41	287	43	289	55	Nabilone
288	91	107	105	255	203	93	55	Dehydroepiandrosterone
288	273	331	287	304	290	289	275	N1-[2-Hydroxyethyl]flurazepam
288	290	218	146	114	63	51	148	Triclosan
288	379	91	175	172	178	378	243	Donepezil
289	140	42	106	113	111	44	132	Sufentanil
289	245	246	210	259	211	183	291	Flutazolam
290	67	108	107	79	55	41	93	Androsterone
290	184	185	104	77	168	291	198	Sultiame
290	259	275	291	243	123	200	43	Trimethoprim
291	239	274	293	75	302	276	138	Lorazepam
291	248	43	79	91	93	107	176	Oxandrolone
292	56	166	139	195	138	111	294	m-Chlorophenylpiperazine ((n)-trifluoroacetyl derivative)
293	265	264	292	43	222	223	294	7-Acetamidonitrazepam
294	248	91	110	117	209	160	297	Ramipril
295	296	238	310	119	43	251	239	CBN
296	41	44	110	298	285	55	268	Cyclopenthiazide
296	195	58	297	253	196	212	84	Hexobendine
296	215	217	298	216	152	181	251	Fenclofenac
296	254	196	273	59	67	151	178	Nimodipine
296	298	205	221	64	63	41	125	Ethiazide
296	298	279	205	36	64	117	62	Trichlormethiazide
296	409	380	280	352	266	–	–	Ehtylmorphine-tfa
296	459	280	266	402	430	–	–	Ethylmorphine-(PFP)
297	208	44	254	298	347	236	165	Amlodipine
297	298	36	98	125	143	70	43	Zuclopenthixol Decanoate
298	271	299	224	272	270	252	280	Desmethylflunitrazepam
298	594	58	593	299	609	595	564	Tubocurarine Chloride
299	42	162	124	229	59	300	69	Codeine
299	43	41	67	300	69	231	29	11-Hydroxy-Δ9-THC
299	162	229	123	59	42	44	300	Neopine
299	231	314	43	41	295	55	271	Δ9-THC
299	231	314	43	41	295	55	271	Δ9-Tetrahydrocannabinol
299	242	59	243	42	96	70	214	Hydrocodone
300	258	77	259	283	302	231	256	Clobazam
300	302	193	207	264	246	211	370	Cisplatin
301	–	–	–	–	–	–	–	CP 47,497
301	44	42	59	164	70	302	242	Dihydrocodeine
301	73	460	302	461	300	370	147	Norbolethone (di-TMS derivative)
301	138	41	70	96	84	316	153	Carteolol Methaneboronate
301	216	44	42	70	302	203	57	Oxymorphone
302	57	85	231	91	110	79	215	Norethandrolone
302	124	43	91	79	121	105	122	Methyltestosterone
303	58	43	304	151	44	42	57	Verapamil
303	121	285	177	95	245	227	163	Mibolerone
303	173	–	–	–	–	–	–	Tegaserod
303	203	202	217	304	205	–	–	OH-Cyproheptadine
303	262	135	175	41	55	43	95	Enoxolone
303	274	260	69	454	209	318	469	Nefazodone
303	301	305	115	194	196	114	87	Broxyquinoline
303	331	239	255	30	158	64	159	Hydroflumethiazide
304	306	64	74	109	177	176	48	Diclofenamide
305	150	307	115	152	114	306	123	Clioquinol
305	307	306	309	308	334	102	75	Lormetazepam
306	292	280	218	–	–	–	–	Aripiprazole (TMS derivative)
308	165	309	121	55	154	98	56	Mebeverine
308	279	204	273	77	307	310	309	Alprazolam
309	96	42	58	98	70	57	44	Ketotifen
310	58	199	112	311	111	212	41	Pecazine
310	64	36	312	42	43	62	63	Methyclothiazide
310	121	353	311	43	120	92	296	Acenocoumarol
310	206	42	64	312	299	45	48	Polythiazide
310	312	311	163	325	75	39	297	Midazolam
311	255	42	44	296	310	312	174	Thebaine
311	312	41	188	80	82	81	241	Nalorphine
311	326	235	151	75	–	–	–	Rhein (3Me derivative)
312	43	399	297	356	281	371	311	Colchicine
313	162	314	124	284	59	42	243	Ethylmorphine
313	238	342	315	75	344	239	137	Triazolam
313	315	212	169	214	216	–	–	Chlorpyrifos
314	285	154	87	315	155	144	167	Pergolide
314	315	393	270	283	79	271	394	Amsacrine
315	230	316	70	44	42	258	140	Oxycodone
316	270	91	130	117	104	132	231	Quinapril
317	43	271	147	289	390	95	81	Alfadolone Acetate
317	288	359	401	43	318	289	196	Oxyphenisatine Acetate
321	278	320	292	306	191	322	304	Berberine
321	364	304	240	168	91	322	365	Bumetanide
323	221	181	222	207	72	223	324	Lysergide
325	293	278	250	223	202	–	–	OH-Flufenamic Acid
326	229	200	187	175	56	–	–	Trifluoromethylphenylpiperazine (trifluroacetic acid derivative)
327	43	369	268	310	42	215	204	Diamorphine
327	268	42	43	215	44	328	269	6-Monoacetylmorphine
327	268	46	43	215	44	328	269	6-Monoacetylmorphine
327	328	41	242	286	96	229	70	Naloxone
329	284	224	268	330	285	225	270	Nifedipine
330	118	91	319	219	64	92	421	Bendroflumethiazide
331	43	71	332	121	147	131	41	Clobetasone
332	333	304	335	176	303	334	162	Chelidonine
334	335	162	120	107	144	143	130	Strychnine
335	333	349	305	378	–	–	–	Normetaclazepam

Principal peaks at m/z								*Compound*
336	121	120	215	162	92	337	187	Dicoumarol
339	221	196	181	207	223	222	72	Methylergometrine
339	324	338	325	340	308	154	292	Papaverine
341	43	340	374	267	107	55	342	Spironolactone
341	44	343	440	70	18	42	344	Sertindole
341	55	256	300	243	202	342	115	Naltrexone
341	233	356	246	247	248	234	281	Sulindac
341	282	229	42	43	59	342	204	Acetylcodeine
341	298	43	44	55	284	242	–	Thebacon
342	18	28	266	313	224	239	45	Etizolam
342	189	357	107	274	343	–	–	Oxybutynin
343	70	344	109	42	113	491	56	Lidoflazine
350	91	259	352	348	65	351	107	Metolazone
351	420	73	232	233	260	405	200	Tms Derivative
352	367	338	366	29	368	353	339	Dimoxyline
353	210	235	336	72	54	236	195	Methysergide
353	354	169	170	355	156	184	144	Yohimbine
354	355	80	–	–	–	–	–	Glaucine
355	139	113	371	341	415	–	–	Acemetacin
355	296	327	234	268	204	–	–	Ethylmorphine-(AC)
357	231	356	355	298	315	–	–	OH-Dextrorphan
357	269	226	356	207	312	279	324	Tolrestat
357	359	289	309	337	487	467	381	Triflumuron
360	375	390	405	–	–	–	–	Mestanolone (MO-TMS derivative)
361	277	319	276	199	318	362	43	Bisacodyl
363	206	143	42	70	207	218	113	Opipramol
365	204	91	366	392	347	–	–	Benazepril Methyl ester derivative
369	394	315	370	333	395	–	–	1,2-Naphthoquinone Glucuronide
371	210	270	266	284	254	268	41	Nisoldipine
372	58	110	357	272	57	373	72	Finasteride
372	374	387	373	264	245	–	–	Clopamide-(Me_3)
377	43	55	246	278	59	378	41	Halcinonide
379	204	380	91	144	438	–	–	Benzaprilat
380	223	382	238	152	345	215	113	Chlorotrianisene
381	300	281	140	115	362	360	204	Celecoxib
382	266	245	280	292	384	383	294	Loratadine
384	109	306	246	183	201	–	–	Diacetoxyscirpenol
385	192	146	196	234	357	–	–	Ethylmorphine-(TMS)

Principal peaks at m/z								*Compound*
386	138	387	84	110	42	301	263	Piritramide
386	359	323	245	303	388	342	–	Quazepam
386	368	43	55	275	81	57	95	Cholesterol
388	310	215	198	173	91	–	–	Nemonapride
392	73	183	154	129	222	451	309	Carvedilol (TMSTFA derivative)
392	73	407	376	318	–	–	–	Tetrodotoxin (tri-TMS derivative)
392	152	170	–	–	–	–	–	Tiotropium
394	245	316	210	291	176	365	313	Brotizolam
394	395	379	392	120	197	203	393	Brucine
397	115	242	398	88	143	271	62	Diiodohydroxyquinoline
409	172	452	186	245	228	130	396	Repaglinide
409	271	257	228	208	70	–	–	Tamsulosin
426	229	200	172	145	56	–	–	Trifluoromethylphenylpiperazine (HFBA derivative)
428	450	301	–	–	–	–	–	Perospirone
428	471	82	103	233	151	472	412	Nebivolol (tri-acetylated derivative)
445	460	315	143	355	169	225	–	Calusterone-(MSTFA/TMS)
446	208	193	221	–	–	–	–	Stenbolone (TMS-EE derivative)
447	183	611	429	629	155	308	–	Lopinavir
455	457	472	474	459	456	458	473	Clofazimine
461	69	95	264	420	187	236	–	Dutasteride
463	91	575	159	185	489	309	92	Zafirlukast
470	–	–	–	–	–	–	–	Androstanediol
489	490	283	134	311	491	–	–	Vardenafil
504	473	429	505	221	474	84	430	Dipyridamole
518	173	264	519	373	376	520	249	Benziodarone
527	368	526	73	396	528	306	369	C *N*-Methylene-reproterol tris-TMS
565	884	242	885	566	455	–	–	Emamectin
578	195	577	367	351	579	366	365	Deserpidine
596	513	465	364	338	–	–	–	Indinavir
608	195	607	397	609	395	381	396	Methoserpidine
608	606	195	609	395	397	212	396	Reserpine
686	668	670	728	700	–	–	–	Viomycin
1124	1106	1088	1070	1052	1142	–	–	Ciguatoxins
1142	1164	–	–	–	–	–	–	Yessotoxin

15 Mass Spectral Data of Pesticides

In the following table the *m/z* values of the eight most intense ions are listed in descending order of intensity; the table should be used as described under Mass Spectral Data of Drugs.

The values for *m/z* in this table were obtained using a quadrupole spectrometer, after gas chromatography unless otherwise stated, except for those substances marked the data for which were obtained on a magnetic sector instrument.

Principal peaks at m/z								Compound
30	43	72	102	73	42	118	99	Diminazene
41	132	102	160	96	29	27	44	Propamidine
42	140	112	41	85	43	71	141	Methenamine
43	57	41	55	71	69	56	70	Trichloropheno-xyacetic Acid (isooctil ester)
43	58	44	200	68	215	41	42	Atrazine
45	160	188	146	237	132	117	77	Alachlor
54	108	81	190	111	135	83	80	Diquat Diene reduction product
58	214	229	43	172	187	216	69	Propazine
61	46	126	214	99	60	63	153	Monolinuron
61	160	132	124	46	248	73	109	Linuron
66	91	79	263	65	101	261	265	Aldrin
67	81	263	36	79	82	261	265	Endrin
72	44	73	42	232	187	124	45	Diuron
72	220	148	132	205	–	–	–	Isoproturon (methyl derivative)
73	352	454	253	144	42	212	455	*tert*-Butyldimethylsilyl (BDMS) derivative
77	105	91	51	65	212	50	78	Benzyl Benzoate
77	160	132	44	105	104	93	76	Azinphos-(Me)
79	82	81	263	77	108	265	80	Dieldrin
81	124	54	53	125	171	45	42	Metronidazole
83	108	96	54	111	192	84	55	Diquat Monoene reduction product
84	58	43	44	42	53	55	86	Aminotriazole
84	133	42	162	161	105	77	119	Nicotine
84	178	161	133	118	–	–	–	Nicotine-1′-n-oxide
86	58	144	84	100	76	–	–	Aldicarb
86	100	44	30	29	42	58	56	Diamthazole
87	93	125	58	47	63	79	42	Dimethoate
88	60	109	142	79	47	111	61	Demeton-*S*-(Me)
89	29	97	60	61	27	65	125	Disulfoton
91	58	86	106	231	45	77	230	Norbormide
94	95	141	64	79	80	111	110	Methamidophos
94	96	15	79	81	93	95	14	Methyl Bromide
96	42	70	72	150	122	194	43	Monoene Reduction Product
96	42	148	192	94	122	134	44	Diene Reduction Product
97	109	291	139	125	137	155	123	Parathion
97	109	362	226	65	210	125	137	Coumaphos
98	176	42	118	41	119	51	175	Cotinine
100	56	101	42	185	184	41	128	Furaltadone
100	272	274	270	237	102	65	276	Heptachlor
106	30	77	185	105	104	89	141	Mafenide
106	141	77	143	140	142	52	79	Chloromethylaniline
107	79	77	51	152	105	50	78	Mandelic Acid
109	79	110	145	139	47	15	112	Trichlorfon
109	185	15	79	47	145	187	29	Dichlorvos
110	156	109	79	58	15	47	126	Omethoate
117	119	47	35	121	82	84	49	Carbon Tetrachloride
117	119	121	82	164	–	–	–	Chloropicrin
121	227	228	114	152	122	63	165	*op′*-Methoxychlor
125	93	127	173	158	99	55	79	Malathion
125	109	79	277	47	93	63	260	Fenitrothion
127	67	58	97	109	192	79	223	Monocrotophos
127	192	109	67	43	193	39	79	Mevinphos
128	130	202	31	29	65	43	111	Chlorphenesin
132	77	160	105	29	65	76	97	Azinphos-(Et)
136	89	171	125	63	90	78	77	Chloronitrotoluene
137	179	152	93	153	199	97	43	Dimpylate
142	57	42	197	185	339	240	226	Hexetidine
144	115	116	57	58	63	145	89	Carbaryl
145	117	122	89	105	90	63	146	Hydroxyquinoline
146	148	111	75	50	150	113	147	Paradichloro-benzene
155	157	227	154	185	44	30	156	Buclosamide
161	57	163	217	219	63	165	90	Propanil
161	162	172	257	285	77	208	177	Triazophos
162	238	45	146	240	163	91	77	Metolachlor
163	206	226	127	–	–	–	–	Cyfluthrin
166	164	129	131	168	94	133	96	Tetrachloroethylene
172	92	156	65	108	39	44	41	Sulfacetamide
173	175	220	73	222	174	191	62	Dicamba
173	340	168	167	165	341	174	322	Diphenadione
176	177	149	57	119	193	41	147	Piperonyl Butoxide
181	183	109	219	111	217	51	221	Lindane
182	165	89	90	212	51	65	91	Dinitro-orthocresol Methyl Ether
188	189	159	132	53	173	131	145	Nalidixic Acid
195	36	237	41	241	75	239	170	Endosulfan
199	45	175	145	111	109	234	133	Dichlorophenoxyacetic Acid Methyl Ester
199	167	198	166	99	154	69	77	Phenothiazine
201	44	186	68	173	96	158	203	Simazine
213	57	58	198	82	171	43	99	Demeton-*S*-(Me)
214	141	155	125	46	77	89	143	Methylchloro-phenoxyacetic Acid Methyl Ester
214	173	216	229	132	68	138	175	Terbutylazine
221	232	97	237	373	65	265	193	Pyrazophos
227	228	114	152	212	63	169	115	*pp′*-Methoxychlor
233	45	42	59	179	146	109	235	Trichlorophenoxyacetic Acid (methyl ester)
235	237	165	236	75	239	82	199	*pp′*-DDT
260	302	216	215	243	189	242	188	Cambendazole
265	308	121	43	266	187	213	251	Warfarin
277	279	165	278	241	239	242	240	Clotrimazole
278	125	109	169	93	–	–	–	Fenthion
292	121	188	130	115	91	128	129	Coumatetralyl
303	154	217	44	288	80	260	153	Fenamiphos
303	301	305	115	194	196	114	87	Broxyquinoline
313	315	212	169	214	216	–	–	Chlorpyrifos
357	359	289	309	337	487	467	381	Triflumuron
373	375	377	371	44	109	75	272	*cis*-Chlordane
373	375	377	371	272	237	75	65	*trans*-Chlordane
565	884	242	885	566	455	–	–	Emamectin

16 Reagents

Acetate Buffer (pH 5): dissolve 13.6 g of sodium acetate and 6 mL of acetic acid in sufficient water to produce 1000 mL.

Acetic Acid: Glacial Acetic Acid: contains not less than 99% w/w of $CH_3 \cdot CO_2H$.

Acetic Acid, Dilute: contains approximately 6% w/w of $CH_3 \cdot CO_2H$.

Ammonia Buffer (pH 9.5): dissolve 10.7 g of ammonium chloride in 40 mL of 5 mol/L ammonia and dilute with water to 1000 mL.

Ammonia Solution, Dilute: dilute 37.5 mL of strong ammonia solution to 100 mL with water. It contains about 10% w/w of NH_3.

Ammonia Solution, Strong: contains 27 to 30% w/w of NH_3.

Ammonium Polysulfide Solution: dissolve a sufficient quantity of precipitated sulfur in ammonium sulfide solution to produce a deep orange solution.

Ammonium Sulfide Solution: saturate 120 mL of 5 mol/L ammonia with washed hydrogen sulfide and add 80 mL of 5 mol/L ammonia. It should be recently prepared.

***p*-Anisaldehyde Reagent:** dissolve 0.5 mL of *p*-anisaldehyde in 50 mL of acetic acid and 1 mL of hydrochloric acid.

Ascorbic Acid Reagent: a 2% solution of ascorbic acid.

Barium Chloride Solution: a l0% solution of barium chloride.

Benzalkonium Chloride Solution: a solution containing 50% of a mixture of alkylbenzyldimethylammonium chlorides.

Borax Buffer 0.05 mol/L: dissolve 19.07 g of borax in sufficient water to produce 1000 mL.

Bratton-Marshall Spray: (a) 1% sodium nitrite (w/v) in 1% sulfuric acid (v/v). (Add 1.0 g sodium nitrite to 1.0 mL concentrated sulfuric acid in 99 mL water. Store in refrigerator); (b) 5% ammonium sulfamate (w/v) in water. (Add 5.0 g ammonium sulfamate to 100 mL water. Store in refrigerator); (c) 2% *N*-(1-Naphthyl) ethylenediamine dihydrochloride in ethanol (95%). (Add 2.0 g *N*-(1-naphthyl) ethylenediamine dihydrochloride to 100 mL ethanol (95%). Store in refrigerator in brown bottle); (d) Spray in sequence a + b + c; (e) Spray with concentrated HCl; (f) Heat plate in oven at 125° for 10 min

Bromine Solution (Bromine Water): a freshly-prepared, saturated solution of bromine.

Chlorine Solution: a freshly-prepared, saturated solution of chlorine.

Chromic Acid Solution: dissolve 3 g of potassium dichromate in 10 mL of water, then carefully dilute to 100 mL with sulfuric acid with continuous stirring. The solution is stable for one month.

Cobalt Thiocyanate Solution: dissolve 6 g of cobalt nitrate and 18 g of potassium thiocyanate in 100 mL of water.

Copper Sulfate Solution: a 5% solution of copper sulfate.

Cupric Chloride: Dissolve 125 g cupric chloride in 375 mL water and 125 mL methanol.

Dimethyl Yellow Solution: a 0.2% solution of dimethyl yellow in ethanol (90%).

Dithiobisnitrobenzoic Acid Solution: dissolve 10 mg of 5,5′-dithiobis-2-nitrobenzoic acid in 100 mL of phosphate buffer (pH 7.4).

DPST Solution: dissolve 0.5 g of 2,5-diphenyl-3-(4-styrylphenyl)-tetrazolium chloride in 100 mL of ethanol; dilute 5 mL of this solution to 50 mL with 2 mol/L sodium hydroxide immediately before use.

Dragendorff Spray: (a) mix together 2 g of bismuth subnitrate, 25 mL of acetic acid, and 100 mL of water; (b) dissolve 40 g of potassium iodide in 100 mL of water. Mix together 10 mL of (a), 10 mL of (b), 20 mL of acetic acid, and 100 mL of water. Prepare every 2 days.

Duquenois Reagent: dissolve 2 g of vanillin and 0.3 mL of acetaldehyde in 100 mL of ethanol. The reagent should be stored in the dark.

Fast Blue B Solution: a 1% solution of fast blue B salt (diazotised *o*-dianisidine). It should be freshly prepared.

Fearons Reagent: Dissolve 0.5 g pentacyanoamine ferroate (ammonium disodium salt) in 60 mL 5% sodium hydroxide. Add 3 drops of 3% hydrogen peroxide.

Ferric Ammonium Sulfate Solution: a 10% solution of ferric ammonium sulfate.

Ferric Chloride, Ethanol, Sulfuric Acid: Dissolve 500 mg ferric chloride in 150 mL ethanol and 50 mL concentrated sulfuric acid. DANGER: exothermic reaction. Add acid to ethanol with cooling and stirring.

Ferric Chloride Solution: a 5% solution of ferric chloride.

Fluorescamine: Dissolve 50 mg fluorescamine in 100 mL methanol.

Fluorescein Solution: mix 10 mL of a saturated solution of fluorescein in acetic acid with 15 mL of acetic acid and 25 mL of strong hydrogen peroxide solution. This solution must be freshly prepared.

Formaldehyde Solution: Formalin: contains 34–38% w/w of formaldehyde.

Forrest Reagent: mix together equal volumes of a 0.2% solution of potassium dichromate, a 30% v/v solution of sulfuric acid, a 20% w/w solution of perchloric acid, and a 50% v/v solution of nitric acid.

FPN Reagent: mix together 5 mL of ferric chloride solution, 45 mL of a 20% w/w solution of perchloric acid, and 50 mL of a 50% v/v solution of nitric acid.

Furfuraldehyde Reagent: (a) dilute 2 mL of redistilled furfuraldehyde to 100 mL with acetone; (b) dilute 4 mL of sulfuric acid to 100 mL with acetone; prepare immediately before use. Spray with (a) first, followed by (b).

Furfuraldehyde Solution: a 10% solution of furfuraldehyde in ethanol.

Gibbs Reagent: Dissolve 500 mg 2,6-dichloroquinone-4-chloroimide in 100 mL ethanol.

Hydriodic Acid: contains about 55% w/w of HI.

Hydrochloric Acid: Concentrated: contains 35–39% w/w of HCI.

Hydrochloric Acid, Dilute: mix 274 g of hydrochloric acid with 726 g of water. It contains about 10% w/w of HCl.

Hydrogen Peroxide Solution, Strong: 100 volume: contains 29–3l% w/v of H_2O_2.

Hydroxylamine Hydrochloride Solution: dissolve 3.5 g of hydroxylamine hydrochloride in 95 mL of ethanol (60%), add 0.5 mL of a 0.1% solution of bromophenol blue followed by 0.5 mol/L potassium hydroxide in ethanol until a green tint develops in the solution, then add sufficient ethanol (60%) to produce 100 mL.

Iodine Solution: dissolve 2 g of iodine and 3 g of potassium iodide in sufficient water to produce 100 mL.

Iodobismuthous Acid Solution: mix 40 mg of bismuth subcarbonate with 0.5 mL of 0.5 mol/L sulfuric acid and add 5 mL of a 10% solution of potassium iodide, 1 mL of 0.5 mol/L sulfuric acid, and 25 mL of water.

Iodoplatinate Solution: dissolve 0.25 g of platinic chloride and 5 g of potassium iodide in sufficient water to produce 100 mL.

Iodoplatinate Solution, Acidified: add 5 mL of hydrochloric acid to 100 mL of iodoplatinate solution.

Lead Acetate Paper: immerse white filter paper in a mixture of 10 volumes of lead acetate solution and 1 volume of 2 mol/L acetic acid. After drying, cut the paper into strips. Store in a well-closed container.

Lead Acetate Solution: a 10% solution of lead acetate in carbon dioxide-free water.

Ludy Tenger Reagent: dissolve 5 g of bismuth subcarbonate in 15 mL of hydrochloric acid, add 30 g of potassium iodide and then add 85 mL of water very slowly and with constant stirring. For use as a TLC spray, dilute 1 volume with 4 volumes of water.

Mandelins Reagent: dissolve 0.5 g of ammonium vanadate in 1.5 mL of water and dilute to 100 mL with sulfuric acid. Filter the solution through glass wool.

Marquis Reagent: mix 1 mL of formaldehyde solution with 9 mL of sulfuric acid. Prepare daily.

Mercuric Chloride-Diphenylcarbazone Reagent: (a) dissolve 0.l g of diphenylcarbazone in 50 mL of ethanol; (b) dissolve 1 g of mercuric chloride in 50 mL of ethanol; prepare the solutions daily. Mix (a) and (b) just before spraying.

Mercuric Nitrate Solution: dissolve 40 g of mercuric oxide (red or yellow) in a mixture of 32 mL of nitric acid and 15 mL of water. Store in glass containers protected from light.

Mercurous Nitrate Spray: a saturated solution of mercurous nitrate.

Mercuric Sulfate: Dissolve 4 g mercuric nitrate in 50 mL concentrated sulfuric acid: water (1 : 1). Make up to 250 mL with water.

Molybdate–Antimony Reagent: dissolve 10 g of ammonium molybdate in 40 mL of 2 mol/L sulfuric acid; dissolve 0.1 g of sodium antimony tartrate in 50 mL of 2 mol/L sulfuric acid. Mix the two solutions and dilute to 500 mL with 2 mol/L sulfuric acid.

Naphthoquinone Sulfonate Solution: dissolve 0.3 g of sodium 1,2-naphthoquinone-4-sulfonate in 100 mL of ethanol:water (1 : l).

***N*-(1-Naphthyl)ethylenediamine Solution:** a 0.5% solution of *N*-(1-naphthyl) ethylenediamine hydrochloride.

Ninhydrin Spray: add 0.5 g of ninhydrin to 10 mL of hydrochloric acid and dilute to 100 mL with acetone. Prepare daily.

Nitric Acid: Concentrated: contains 69–71% w/w of HNO_3.

Nitric Acid, Dilute: mix 106 mL of nitric acid with sufficient water to produce 1000 mL. It contains about 10% w/w of HNO_3.

Nitric Acid, Fuming: contains not less than 95% w/w of HNO_3.

Nitrogen Dioxide Vapour: produced by the action of nitric acid on copper turnings. For location in thin-layer chromatography the TLC plate is exposed to the nitrous fumes which are evolved.

Nitroso-naphthol Solution: a 0.2% solution of 2-nitroso-1-naphthol in ethanol.

Perchloric Acid: contains about 72% w/w of $HC1O_4$.

Perchloric Acid Solution: add 15 mL of perchloric acid to sufficient water to produce 100 mL.

Phosphate Buffer (pH 5.6): dissolve 62.4 g of sodium dihydrogen phosphate in sufficient water to produce 100 mL.

Phosphate Buffer (pH 6.88): dissolve 3.40 g (0.025 mol/L) of potassium dihydrogen phosphate and 3.53 g (0.025 mol/L) of anhydrous disodium hydrogen phosphate, both previously dried at 110° to 130° for 2 hours, in sufficient water to produce 1000 mL.

Phosphate Buffer (pH 7.4): (a) dissolve 9.465 g of anhydrous disodium hydrogen phosphate in water and dilute to 1000 mL; (b) dissolve 9.073 g of potassium dihydrogen phosphate in water and dilute to 1000 mL. Mix 80 mL of (a) with 20 mL of (b) to obtain a solution of pH 7.4.

Phosphoric Acid: contains 84 to 90% w/w of H_3PO_4.

Potassium Cupri-tartrate Solution: Fehlings Solution: (a) dissolve 34.64 g of copper sulfate in a mixture of 0.5 mL of sulfuric acid and sufficient water to produce 500 mL; (b) dissolve 176 g of sodium potassium tartrate and 77 g of sodium hydroxide in sufficient water to produce 500 mL. Mix equal volumes of solutions (a) and (b) immediately before use.

Potassium Cyanide Solution: a 10% solution of potassium cyanide.

Potassium Permanganate Solution, Acidified: a 1% solution of potassium permanganate in 0.25 M sulfuric acid.

Silver Nitrate Solution: a freshly-prepared 5% solution of silver nitrate.

Sodium Chloride-Potassium Chloride Solution: mix 140 mL of mol/Lsodium chloride with 50 mL of 0.1 mol/L potassium chloride, and dilute with water to 1000 mL.

Sodium Hydroxide Solution: a 20% solution of sodium hydroxide.

Sodium Hydroxide Solution, Dilute: a 5% solution of sodium hydroxide.

Sodium Hypobromite Solution: dissolve 5 mL of bromine in 50 mL of a 40% solution of sodium hydroxide, with agitation and cooling.

Starch-iodide Paper: impregnate unglazed white paper with starch mucilage diluted with an equal volume of a 0.4% solution of potassium iodide.

Starch Mucilage: triturate 0.5 g of starch with 5 mL of water and add this, with constant stirring, to sufficient water to produce about 100 mL. Boil for a few minutes, cool, and filter. It should be freshly prepared.

Sulfuric Acid: Concentrated: contains not less than 95% w/w of H_2SO_4.

Sulfuric Acid, Dilute: mix carefully 104 g of sulfuric acid with 896 g of water and cool. It contains about 10% w/w of H_2SO_4.

Sulfuric Acid, Fuming: Oleum: prepared by the addition of sulfur trioxide to sulfuric acid and is available containing about 30% of free SO_3.

Sulfuric Acid–Ethanol Reagent: add gradually 10 mL of sulfuric acid to 90 mL of ethanol.

Tetrabutylammonium Phosphate, 0.005 mol/L, pH 7.5: PIC Reagent A. Marketed by Waters Associates Inc., Milford, MA 01757, USA; Waters Associates (Instruments) Ltd, Northwich, Cheshire, UK.

***p*-Toluenesulfonic Acid Solution:** dissolve 4 g of *p*-toluenesulfonic acid in 20 mL of ethanol.

Trinders Reagent: dissolve 40 mg of mercuric chloride in 850 mL of water, add 120 mL of mol/L hydrochloric acid and 40 g of hydrated ferric nitrate, and dilute to 1000 mL with water.

Van Urk Reagent: dissolve 1 g of *p*-dimethylaminobenzaldehyde in 100 mL of ethanol and add 10 mL of hydrochloric acid.

Vanillin: Dissolve 2 g vanillin in 100 mL concentrated sulfuric acid.

Zwikkers Reagent: mix 40 mL of a 10% solution of copper sulfate with 10 mL of pyridine and add sufficient water to produce 100 mL.

Proprietary test materials

Amberlite XAD-2: polymeric adsorbent beads for adsorbing water-soluble organic substances. Marketed by Rohm and Haas Company, Philadelphia, PA 19105, USA: Rohm and Haas (UK) Ltd, Croydon, Surrey, UK.

Anabolic Steroid Radioimmunoassay Kits: for nandrolone and for 17α-methyl substituted anabolic steroids. Obtainable from Prof. R. V. Brooks, Dept of Chemical Pathology, St. Thomas Hospital Medical School, London, UK.

Carbon Monoxide in Blood: Spectrophotometric Reference Standards. Marketed by American Hospital Supply Corporation, Evanston, IL 60201, USA: American Hospital Supply (UK) Ltd, Ashford, Middlesex, UK.

Cholinesterase Activity Assay Kit: colorimetric method for plasma or serum. Marketed by Sigma Chemical Co., St. Louis, MO 63178, USA; Sigma London Chemical Co. Ltd, Poole, Dorset, UK.

Co-oximeter, IL 282: for the determination of carbon monoxide in blood. Marketed by Instrumentation Laboratory Inc., Lexington, MA 02173, USA; Instrumentation Lab. UK Ltd. Warrington, Cheshire, UK.

Dextrostix: reagent strips for the approximate estimation of glucose in blood. Marketed by Ames, Division of Miles Laboratories Inc., Elkhart, IN 46515, USA; Ames Division of Miles Laboratories Ltd, Stoke Poges, Buckinghamshire, UK.

Ethanol Assay Kit: enzymatic method for the determination of ethanol in whole blood, plasma, or serum. Marketed by Sigma Chemical Co., St Louis, MO 63178, USA; Sigma London Chemical Co. Ltd, Poole, Dorset, UK.

Extrelut: a column containing granules of diatomaceous earth with a large pore volume. Marketed by E. Merck, D-6100 Darmstadt, W. Germany; BDH Chemicals Ltd, Poole, Dorset, UK.

High Performance Silica Gel TLC Plates: with fluorescent indicator and concentration zone. Marketed by E. Merck, D-6100 Darmstadt, W. Germany; BDH Chemicals Ltd, Poole, Dorset, UK.

Labstix: reagent strips for urine testing to indicate pH, and the presence of protein, glucose, ketones, and blood. Marketed by Ames, Division of Miles Laboratories Inc., Elkhart, IN 46515, USA; Ames Division of Miles Laboratories Ltd, Stoke Poges, Buckinghamshire, UK.

Paracetamol Assay Kit: enzymatic method for the determination of paracetamol in plasma. Marketed by Cambridge Life Sciences, Cambridge, UK.

Sephadex LH-20: a bead-formed dextran gel with hydrophilic and lipophilic properties. Marketed by Pharmacia (Great Britain) Ltd, Milton Keynes, Buckinghamshire, UK; Pharmacia Fine Chemicals, Division of Pharmacia Inc., Piscataway, NJ 08854, USA.

Sep-Pak C-18 Cartridge: a silica cartridge for column chromatography. Marketed by Waters Associates Inc., Milford, MA 01757, USA; Waters Associates (Instruments) Ltd, Northwich, Cheshire, UK.

Serum-Iron Assay Kit: Marketed by Travenol Laboratories Inc., Deerfield, IL 60015, USA; Lorne Laboratories Ltd, Reading, Berkshire, UK.

Subtilisin A: Marketed by Novo Enzyme Products Ltd, Windsor, Berkshire, UK.

Tox Elut: a column containing granules of diatomaceous earth with a large pore volume. Marketed by Analytichem International Inc., Lawndale, CA 90260, USA.

Urastrat: a complete quantitative system for the determination of urea nitrogen in serum. Marketed by General Diagnostics, Warner-Lambert (UK) Ltd, Eastleigh, Hampshire, UK.

17 Pharmacological Terms

Abdominal: relating to the abdomen (the part of the body between the pelvis and the chest).

Abscess: a localised collection of pus.

Absorption rate constant: a pharmacokinetic measurement used in describing the distribution of a drug.

Acetoacetylcoenzyme A thiolase: an enzyme that catalyses the synthesis of acetoacetyl coenzyme A from two molecules of acetyl coenzyme A; it can also act as a thiolase, catalysing the reverse reaction.

Acetylation: addition of an acetyl group to a substance.

Acetylcholinesterase: a hydrolase enzyme found in red blood cells and other tissues that catalyses the cleavage of acetylcholine to choline and acetate.

α_1-Acid glycoprotein: an acute phase protein found in blood plasma that is an indication of tissue necrosis and inflammation.

Acidification of urine: administration of agents, such as ammonium chloride or ascorbic acid, to produce acidic urine.

Acidosis: disturbance of acid–base balance of the body as a result of accumulation of acid and hydrogen ions or depletion of the alkaline reserve.

Acrodynia: *see* Pink disease.

Active metabolite: the pharmacologically active product of metabolism of a drug.

Acute renal failure: kidney failure of sudden onset.

Addiction: state of being physiologically and psychologically dependent on a drug.

Adenosine triphosphate: a nucleotide that occurs in all cells and acts as an energy store and is required for various metabolic reactions, including the synthesis of nucleic acids and proteins, and to produce mechanical movement.

Adipose tissue: fatty tissue.

Adrenal medulla: the inner part of the adrenal gland that synthesises catecholamines.

Adrenergic nervous system: sympathetic nerves that liberate noradrenaline at their synapses.

Adrenocortical insufficiency: reduced secretion of corticosteroids by the adrenal cortex.

Agglutination: the clumping together of red blood cells or micro-organisms that is brought about by specific antibodies called agglutinins.

Agonal: occurring at the time just before death.

Alanine aminotransferase: an enzyme found in the serum and body tissues, especially the liver, that catalyses the reversible transfer of nitrogen (as an amino group) for excretion or for incorporation into other compounds.

Albumin: the main protein in blood plasma.

Alcohol dehydrogenase: an enzyme that catalyses the oxidation of many alcohols to their corresponding aldehyde.

Aleukia: a reduced number of leukocytes (white blood cells) in the blood.

Algal: relating to or caused by algae.

Alkaline phosphatase: an enzyme that in alkaline conditions catalyses the cleavage of orthophosphate from orthophosphoric monoesters.

Alkalinisation of urine: administration of agents, such as potassium citrate or sodium bicarbonate, in order to produce alkaline urine.

Allele: any alternative form of a gene that can occupy a particular chromosomal locus.

Allergen: an agent capable of producing immediate-type hypersensitivity (allergy).

Allergic reaction: an antigen–antibody reaction that causes tissue damage and that is triggered by exposure of a sensitised individual to the particular antigen or allergen.

Alopecia: hair loss.

Alzheimers disease: a form of dementia in which there is diffuse atrophy of the cerebral cortex.

Amanita phalloides: the death cap poisonous mushroom.

Amino acid: any organic compound containing an amino and a carboxyl group.

Aminoglycoside antibiotic: a generic term used for a bactericidal antibiotic that is derived from bacteria of the *Streptomyces* and *Micromonospora* families, such as gentamicin, neomycin, and framycetin.

Aminotransferase: an enzyme that catalyses the transfer of an amino group to another molecule.

Amnesia: partial or total loss of memory.

Amylase: any enzyme that catalyses the conversion of starches into sugars.

Amyotrophic lateral sclerosis: a fatal progressive degenerative disorder that affects the upper and lower motor neurones in the brain and spinal cord.

Anabolic agent: an agent that promotes synthesis of body protein.

Anaemia: a reduction below normal of the blood-haemoglobin concentration due to lack of red blood cells and/or their haemoglobin content.

Anaerobic: occurring in the absence of molecular oxygen.

Anaesthesia: loss of sensation either by nerve or receptor damage or by the administration of an anaesthetic drug.

Anagen: the part of the hair growth cycle during which hair synthesis occurs.

Analgesic: a drug that relieves pain.

Anaphylactic shock: shock due to an allergic reaction.

Anecdotal: based on individual cases rather than on controlled studies.

Angiotensin-converting enzyme inhibitor: an antihypertensive drug that acts by inhibiting angiotensin-converting enzyme and produces vasodilatation and reduces peripheral resistance.

Anhedonia: total loss of interest or enjoyment in former pleasures or activities.

Anorexia: loss or lack of appetite for food.

Anoxia: a total lack of oxygen in the tissues.

Antagonist: an agent that has no effect alone, reverses the effects of agonists.

Antemortem: before death.

Anti-arrhythmic: a drug that is used in the management of cardiac arrhythmias.

Antibiotic: a general term used to encompass all the different antimicrobial drugs.

Antibody: a specific substance produced in the blood as a reaction to an antigen.

Anti-cancer agent: a drug that is used to treat malignant neoplasms (cancers).

Anticoagulant: a drug, such as heparin or warfarin, that inhibits blood clotting and is used in the treatment of thromboembolic disorders.

Anticonvulsant: a drug that is used in the treatment of epilepsy or other convulsive disorders.

Antidepressant: a drug used to treat depression, includes the tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs).

Antidote: a remedy that counteracts the actions of a poison.

Antiepileptic: a drug that reduces the frequency of epileptic seizures.

Antifungal: a drug, such as ketoconazole, that is used to treat fungal infections.

Antigen: a substance that is capable of initiating the production of a specific antibody (immunoglobulin) that reacts with the antigen.

Antihistamine: a drug that antagonises the actions of histamine at H_1 receptors and is used to treat type I hypersensitivity reactions and to alleviate pruritus, nausea and vomiting.

Antihypertensive: a drug that reduces blood pressure.

Anti-inflammatory: a drug that is used to reduce inflammation, includes aspirin, NSAIDs and antirheumatic drugs.

Antiparkinsonian: a drug that stimulates dopamine receptors or enhances the actions of dopamine and is used to treat Parkinsons disease.

Antipsychotic: a drug used in the treatment of psychoses (formerly called a major tranquilliser).

Antiserum: a serum, obtained from an animal that has previously been immunised against an antigen, which contains immunoglobulins against the antigen.

Anti-ulcer: an agent that prevents formation of ulcers or promotes their healing.

Antivenom: a proteinaceous material containing antitoxins that is used as an antidote to poisoning by certain snakes and arthropods.

Antiviral: a drug used in the treatment of a viral infection.

Anxiolytic: a drug that reduces anxiety.

Aorta: the artery that carries blood out of the left ventricle of the heart.

Aphonia: loss of voice.

Aphrodisiac: a drug that arouses sexual excitement.

Apnoea: cessation of breathing.

Arrhythmia: any variation from the normal rhythm of the heartbeat.

Arterial blood: oxygenated blood (bright red in colour).

Arthralgia: pain in a joint.

Aspartate aminotransferase: (aspartate transaminase, AST) an enzyme that catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate.

Asphyxia: cessation of breathing that occurs as a result of hypoxia and hypercapnia induced by breathing air lacking in oxygen.

Aspiration: removal, by suction, of excess fluid or gas from a body cavity (*see also* Gastric aspiration).

Assay: determination of the amount of a particular constituent of a mixture, or determination of the biological or pharmacological potency of a drug.

Asymptomatic: no symptoms.

Ataxia: jerky and irregular movements that are a result of defective muscular control.

Atrial flutter: a cardiac arrhythmia characterised by a rapid atrial rate.

Attention deficit disorder: a childhood mental disorder characterised by inattention and hyperactivity.

AUC: area under the plasma concentration vs time curve.

Autonomic nerves: the parasympathetic and sympathetic nervous systems that control cardiac and smooth muscle and glands.

Autopsy: postmortem examination of a body for determining the cause of death or presence of any pathological changes.

Axon: that part of a neurone that carries impulses away from the cell body.

Barbiturate: a sedative drug derived from barbituric acid.

Benzodiazepine: any of a group of compounds having a common molecular structure and acting similarly as depressants of the central nervous system.

β-blocker: a competitive antagonist at beta-adrenergic receptor sites.

β-glucuronidase: *see* Glucuronidase.

Bezoar: a deposit of foreign hard material in the gastrointestinal or urinary tract.

Bile: a greenish-yellow fluid stored in the gall bladder and secreted by the liver into the small intestines via the bile ducts; its main constituents are bile salts, the bile pigments (bilirubin and biliverdin), and cholesterol.

Biliary excretion: excretion of a drug or metabolite into the bile.

Bilirubin: a yellow bile pigment that is mainly formed from the breakdown of haemoglobin from red blood cells in the spleen. It circulates in plasma as a complex with albumin and undergoes conjugation in the liver to form bilirubin diglucuronide, which is excreted in bile.

Bioavailability: proportion of a drug or foreign compound absorbed.

Bipolar disorder: a mood disorder in which the patient experiences alternating episodes of hypomania or mania and depression.

Blepharospasm: spasm of the muscles in the eyelid that results in the eye being almost completely closed.

Blood: the red viscid fluid that circulates through the heart and blood vessels; it consists of plasma, erythrocytes (red blood cells), leukocytes (white cells) and platelets (thrombocytes).

Blood bank: a special refrigerator used to store blood after withdrawal from donors until required for transfusion.

Blood clot: a semi-solid mass formed mainly of platelets and fibrin.

Blood urea nitrogen: the urea concentration of blood or serum stated in terms of nitrogen content; it is used as an indication of glomerular filtration rate.

Blood–brain barrier: the membranes between the circulating blood and the brain; some drugs can diffuse through these membranes into the cerebrospinal fluid, others cannot.

Bone marrow: the soft material filling the cavities of the bones.

Botulism: neurotoxicity caused by the exotoxin *Clostridium botulinum* usually as a result of eating contaminated food; symptoms include descending paralysis, gastrointestinal disturbances, postural hypotension, dry mouth, and dilated pupils.

Bronchoconstriction: narrowing of the bronchial lumen usually as a result of bronchial smooth muscle contraction.

Buccal: relating to the cheek of the mouth.

Calcineurin phosphatase: a protein phosphatase, specific for serine and threonine, that plays an important role in activation of T lymphocytes.

Cannabinoid: any of the principles that can be extracted from cannabis, including tetrahydrocannabinol, cannabinol, and cannabidiol.

Capillary: one of the minute blood vessels that connect the arterioles and venules.

Carboxyhaemoglobin: haemoglobin in which the oxygen binding sites are occupied by carbon monoxide.

Carcinogen: any cancer-producing substance.

Carcinogenic: a substance able to cause cancer.

Carcinomatosis: a condition in which cancer is disseminated throughout the body.

Cardiac arrest: sudden cessation of the heart beat.

Cardiac arrhythmia: any abnormality in the rate or regularity of the heartbeat.

Cardiac failure: inability of the heart to pump blood at an adequate rate for tissue requirements.

Cardiac glycoside: a drug, such as digoxin, that is used to slow the heart rate in some cardiac arrhythmias and to treat heart failure.

Cardiac infarction (myocardial infarction): interruption of the blood supply to the heart muscle resulting in necrosis.

Cardiorespiratory arrest: stopping of the heart and breathing.

Cardiovascular: relating to the heart and blood vessels.

Catagen: the brief part of the hair growth cycle when anagen (growth) stops and telogen (resting) begins.

Catalepsy: term used in psychiatry to describe maintenance of a fixed body posture.

Catecholamine: any of the amines produced in the body (e.g. noradrenaline and adrenaline) that are involved in nerve transmission in the sympathetic nervous system.

Catecholmethyltransferase: a cytosolic enzyme found in the kidney, liver and central nervous system that catalyses the methylation of a catechol or catecholamine.

Catheterisation: insertion of a catheter (a tubular, flexible surgical instrument) into a body cavity to withdraw or introduce fluid.

Central nervous system (CNS): the part of the nervous system consisting of the brain and spinal cord.

Cerebral haemorrhage: bleeding into the brain.

Cerebrospinal fluid (CSF): the clear fluid filling the ventricles of the brain, the subarachnoid space, and the central canal of the spinal cord.

Cerebrovascular accident: *see* Stroke.

Charcoal haemoperfusion: the extracorporeal removal of toxic substances from the blood using charcoal as an adsorbent.

Chelating agent: an agent, such as desferrioxamine or EDTA, that inhibits a toxin by reacting with it to form a less active or inactive complex.

Chelation therapy: the use of a chelating agent to manage a case of poisoning.

Cholesterol: a sterol found in the brain, nerves, liver, blood, and bile that is the precursor of bile acids and steroid hormones and is also a constituent of cell membranes.

Cholinergic: receptors that are stimulated by acetylcholine.

Cholinesterase: an enzyme found in various tissues, including plasma, that catalyses the hydrolysis of acetylcholine into choline.

Chorea: brief, involuntary muscle contractions and an inability to sustain voluntary contractions.

Chronic interstitial nephritis: chronic inflammation of the interstitial renal tissue that leads to progressive fibrosis and danger of renal failure.

Circulatory collapse: *see* Shock.

Circulatory shock: *see* Shock.

Cirrhosis: damage to cells of an organ resulting in fibrosis (usually applied to the liver).

Clearance: a pharmacokinetic term that usually refers to plasma clearance and is the rate at which plasma is cleared of a substance by processes such as excretion by the kidneys, metabolism, or dialysis.

Clot: a semi-solid mass, as in a clot of blood or lymph.

Clotting factor: one of the substances in the blood (known as factors) that are essential to the clotting process.

CNS depressant: a substance that reduces activity of the central nervous system.

Coagulated blood: blood that has clotted.

Coagulation factor: *see* Clotting factor.

Cognition: the processes in the mind that are involved in perceiving, thinking and remembering.

Colic: acute abdominal pain.

Coma: a state of complete loss of consciousness from which the patient cannot be roused even by powerful stimulation.

Competitive inhibition: reversible binding of a drug that results in inhibition of enzyme activity or antagonism at a receptor site.

Complex partial epilepsy: a seizure with impaired consciousness in which the neuronal discharges remain localised in 1 area of the brain.

Concretion: a deposit of hard material in a natural cavity or in tissue.

Congener: something that is closely related to another thing or shares the same functions.

Conjugation reaction: a type of metabolic reaction that involves the addition of an endogenous group to a drug molecule to produce a more polar substance that is more readily excreted by the body.

Conjunctiva: the delicate transparent membrane that lines the eyelids and covers the exposed part of the eyeball.

Convulsion: involuntary contraction of the voluntary muscles.

Corticosteroid: one of the 21-carbon steroids synthesised by the adrenal cortex which may possess either mainly glucocorticoid actions (effects on carbohydrate, fat and protein metabolism) or mineralocorticoid actions (effects on fluid and electrolyte balance).

Cotinine: the major urinary metabolite of nicotine.

Coumarin anticoagulant: any derivative of coumarin or any compound that acts like coumarin as an indirect anticoagulant, such as warfarin.

Cramp: an involuntary, painful, spasmodic muscle contraction.

Creatine: an amino acid found mainly in skeletal muscle.

Creatinine: a breakdown product of the decomposition of creatine that is excreted in the urine.

Creatinine clearance: renal clearance of endogenous creatinine (commonly used to provide an estimate of glomerular filtration rate).

Cyanosis: a bluish coloration of skin and mucous membranes caused by excessive amounts of deoxyhaemoglobin (reduced haemoglobin) in the blood.

Cyclo-oxygenase-2 inhibitor: a type of non-steroidal anti-inflammatory drug, such as celecoxib, that selectively inhibits the enzyme cyclo-oxygenase-2 and is used in the management of rheumatic disorders.

CYP3A: one of the forms of cytochrome P450.

Cysteine: a sulfur-containing non-essential amino acid produced by the hydrolysis of proteins.

Cytochrome a-a_3 complex: a complex formed in the electron transport chain that transfers electron to oxygen, reducing it to water.

Cytochrome P450: an enzyme found in the cells of most tissues that catalyses a wide range of oxygenation reactions that are responsible for the detoxification of many drugs.

Cytochrome P450 mixed function oxidase: *see* Cytochrome P450.

Cytochrome-P450 IID6: one of the forms of cytochrome P450.

Cytosolic: relating to or contained in the cytosol (liquid medium of the cytoplasm).

Deacetylation: removal of an acetyl group.

Dealkylation: removal of an alkyl group.

Deamination: removal of an amine group.

Deletion: in genetics, the loss of any part of the genetic material on a chromosome.

Delirium: an acute transient mental disturbance that may be characterised by hallucinations or illusions.

Dementia: a deterioration of cognitive processes or intellectual abilities including memory, judgement, language and abstract thinking.

Demethylation: removal of a methyl group.

Dental caries: tooth decay.

Dependency: a state in which there is a compulsive or chronic need, usually for a drug.

Depolarising substance: a substance that reduces the voltage across a biological membrane.

Depressant: a substance that reduces energy and activity by causing muscular relaxation.

Dialysis: a general term used to describe the removal of waste products and excess water from a patient by diffusion through a semi-permeable membrane, examples of specific procedures include peritoneal dialysis and haemodialysis.

Dialysis dementia: a dementia formerly seen in dialysis patients associated with high levels of aluminium in the dialysis fluid or with aluminium-containing antacids given to control phosphorus levels.

Diaphorase: any flavoprotein that catalyses the oxidation of the cofactors NAD or NADP (see below) using a non-physiological compound (such as methylene blue) as an electron acceptor.

Diaphoresis: *see* Sweating.

Diaphragm: the dome-shaped muscular membrane that separates the thorax from the abdomen.

Diarrhoea: increased frequency and liquidity of the stools.

Diffuse intravascular coagulation: *see* Disseminated intravascular coagulopathy.

Diplopia: double vision.

Disseminated intravascular coagulopathy: a bleeding disorder where there is widespread microvascular thrombosis with tissue and organ ischaemia combined with spontaneous bruising and bleeding.

Diuresis: increased production of urine.

Diuretic: an agent that promotes diuresis.

DNA: deoxyribonucleic acid, the primary genetic material of cellular organisms.

Dopamine receptor: one of several types of receptor that are stimulated by the neurotransmitter dopamine.

Drug compliance: the degree to which a patient complies with instructions for taking their prescribed medicines.

Drug conjugate: the product of a drug metabolism reaction in which the drug is combined with another moiety such as a sugar or amino acid.

Drug receptor: *see* Receptor.

Drug tolerance: the requirement for larger doses of a drug to maintain a constant response or a diminution of response to repeated constant doses of a drug.

Duodenal ulcer: a peptic ulcer (a distinct break in the gastro-intestinal mucosa) in the duodenum.

Dysarthria: impaired speech due to loss of muscular control.

Dysautonomia: dysfunction of the autonomic nervous system.

Dysphagia: difficulty in swallowing.

Dysphoria: disquiet, malaise, restlessness.

Dyspnoea: difficulty in or laboured breathing.

Eating disorder: a disorder, such as anorexia nervosa, where an abnormal feeding habit is accompanied by psychological factors.

Eczema: a term used, often synonymously with dermatitis, to refer to a variety of skin conditions characterised by redness, inflammation and itching.

EEG: *see* Electroencephalogram.

Egestion: the casting out of indigestible material.

Electrocardiogram (ECG): a graphic tracing that represents the electrical currents produced by contraction of the heart muscle.

Electroencephalogram (EEG): a recording of the electrical impulses generated by nerve cells in the brain.

Elimination: a pharmacokinetic term used to describe the removal of a drug from the body, for example by metabolism or excretion in urine or faeces.

Elimination rate constant: a pharmacokinetic definition describing the rate of clearance of a drug.

Emesis: *see* Vomiting.

Emetic: an agent that causes vomiting.

Emetic chemoreceptor: the vomiting centre in the brain, stimulation of which produces vomiting.

Emphysema: an abnormal accumulation of air in tissues or organs; often refers to pulmonary emphysema where there is distension of the alveoli of the lungs.

Encephalopathy: degenerative disease of the brain.

Endocytosis: the uptake of a substance by a cell by invagination of its membrane and formation of a vesicle.

Endogenous: arising from causes within the organism.

Endotoxin: a heat-stable toxin associated with the outer membranes of certain gram-negative bacteria that are not secreted by the cells but are only released when the cells are disrupted.

End-stage renal failure: chronic renal failure that is irreversible.

Enterohepatic recirculation: a recycling of a drug/metabolite that can occur if the compound is excreted in the bile and then undergoes metabolism by the gut flora and is reabsorbed and is subject to metabolism in the liver, excretion in the bile, etc.

Enzymatic hydrolysis: the splitting of a compound by the addition of water and facilitated by an enzyme.

Eosinophilia-myalgia syndrome: a syndrome, sometimes fatal, of eosinophilia (increased white blood cells in the blood) combined with severe generalised myalgia that has been reported in patients taking preparations containing tryptophan.

Epidermal: relating to the external layer of the skin.

Epithelium: the tissue covering internal and external surfaces of the body.

Epigastric: referring to the region of the abdomen lying over the stomach.

Epilepsy: a group of syndromes that are characterised by recurrent epileptic seizures; an epileptic seizure is a paroxysmal discharge of cerebral neurones that may manifest as various clinical signs, including impairment or loss of consciousness, abnormal motor phenomena, or sensory or autonomic disturbances.

EPO: *see* Erythropoietin.

Ergotism: poisoning from eating ergotised grain or from misuse of medicinal ergot.

Erythema: reddening of the skin.

Erythrocytes (red blood cells): non-nucleated cells in circulating blood that contain haemoglobin.

Erythropoietin (EPO): a glycoprotein hormone secreted chiefly by the kidney in the adult and by the liver in the fetus, which acts on the bone marrow cells to stimulate red blood cell production; an erythropoietin that is produced by recombinant DNA technology is available for clinical use.

Esterase: an enzyme that catalyses the breakdown of an ester to produce an alcohol and an acid.

Eumelanin: a type of melanin (black pigment) found in darkly coloured skin and hair.

Euphoria: an exaggerated feeling of well-being (physical and mental).

Exogenous: from outside the organism.

Extracellular: outside the cell.

Extrapyramidal: referring to the parts of the central nervous system that are involved in motor activities but which are outside the pyramidal tract.

Extravascular: situated or occurring outside a blood vessel.

F-actin: a polymerised form of actin, a protein that makes up muscle fibres.

Factor V: a clotting factor found in plasma but not in serum that catalyses the cleavage of prothrombin to the active thrombin.

Factor X: a clotting factor that is involved in both the intrinsic and the extrinsic pathways of blood coagulation.

Faeces: the excrement discharged from the intestines consisting of bacteria, cells exfoliated from the intestines, secretions (mainly from the liver) and a small amount of food residue.

Fatty acid: a straight chain monocarboxylic acid that occurs in fats.

Femoral: relating to the femur or thigh.

Ferrochelatase: an enzyme found in mitochondria that catalyses the insertion of ferrous iron into protoporphyrin IX to form haem.

Fetal: relating to the fetus.

Fetus: the postembryonic developing child in utero (i.e. from 9 weeks after fertilisation until birth).

Fever: elevation of the body temperature above normal.

Fibrin: the insoluble protein matrix that forms part of a blood clot and is formed from soluble fibrinogen by the enzymatic action of thrombin.

Fibrinogen: a soluble plasma protein that is converted to insoluble fibrin by the action of thrombin.

First-pass metabolism: metabolism of an orally taken drug that occurs in the gastro-intestinal tract or in the liver before the drug enters the systemic circulation.

Fit: colloquial term for an epileptic seizure.

Flaccid paralysis: a paralysis that is accompanied by loss of muscle tone and absence of tendon reflexes.

Free drug: drug in the bloodstream that is not bound to plasma proteins.

Fresh frozen plasma: a preparation prepared from centrifuged donated whole blood that is used clinically to replace clotting factors or other plasma proteins.

G-actin: a globular form of actin, a protein that makes up muscle fibres.

Gangrene: death of tissue, usually as a result of inadequate blood supply, that is followed by bacterial infection and putrefaction.

Gastric aspiration: inhalation of the stomach contents into the lungs.

Gastric lavage: irrigation of the stomach.

Gastric ulcer: a peptic ulcer (an ulceration of the mucous membrane) occurring in the stomach.

Gastroenteritis: an acute inflammation of the mucous membranes of the stomach and intestines, usually caused by bacterial infection, with symptoms including nausea, diarrhoea, abdominal pain and weakness.

Gene: a segment of a DNA molecule that contains all the information required for synthesis of a product (polypeptide chain or RNA molecule).

Genome: the complete set of hereditary factors as in chromosomes.

Genomics: the study of the structure and function of the genome.

Genotype: the complete genetic constitution of an individual.

Gilberts syndrome: an inborn error of bilirubin metabolism where blood concentration of unconjugated bilirubin is raised but there is no blood abnormality or liver damage.

β-Globulin: one of the globulins present in serum that constitute the majority of plasma protein.

Glomerulopathy: any disease of the glomeruli of the kidneys.

Glomerulus: a functional unit of the kidney that collects the filtrate from the blood capillaries and directs it into the kidney tubule.

Glucocorticosteroid: any of the corticosteroids that regulate carbohydrate, lipid, and protein metabolism.

Gluconeogenesis: synthesis of glucose from protein or fat that occurs when there is insufficient carbohydrate available.

Glucose-6-phosphate dehydrogenase: an enzyme that catalyses the oxidation of glucose-6-phosphate to a lactone, reducing NADP+ to NADPH, which in turn maintains levels of reduced glutathione in cells.

Glucuronidase: an enzyme that catalyses the cleavage of terminal glucuronic acid residues from a variety of β-glucuronides; it is present in the intestine where it can hydrolyse glucuronide conjugates and therefore can contribute to the recycling that is seen with some compounds (*see* Enterohepatic recirculation).

Glucuronidation: addition of glucuronic acid to form a conjugate (*see* Glucuronide conjugate).

Glucuronide conjugate: a water-soluble compound formed during metabolism of a drug that is a result of combination with α-D-glucuronic acid.

Glucuronyl transferase: an enzyme that catalyses the transfer of glucuronic acid to a substrate to form a glucuronide congugate.

Glutathione: a tripeptide, found in many tissues but especially in the liver, which acts as a cofactor for enzymes and also detoxifies harmful compounds.

Glutathione peroxidase: an enzyme found in high levels in the liver that catalyses the detoxifying reduction of peroxides (such as hydrogen peroxide or organic peroxides) via oxidation of glutathione.

Glutathione S-transferase: an enzyme that catalyses the conjugation of a wide variety of substances with glutathione.

Glycosuria: glucose in the urine.

Granuloma: an imprecise term for any small nodule formed by inflammatory cells.

Granulopenia: a reduction in the number of granulocytes (a type of white blood cell) in the blood.

Growth hormone: an anabolic hormone secreted by the pituitary gland that promotes growth of skeletal, muscular and other tissues; a growth hormone produced by recombinant DNA technology is available for clinical use.

Habituation: an older term used in describing drug dependency.

Haem: the iron protophorphyrin group that is the pigment-carrying part of haemoglobin.

Haematocrit: the proportion of the volume of a blood sample that is red blood cells.

Haematuria: blood in the urine.

Haemocyanin: a blue pigment found in the blood plasma of many molluscs and arthropods that can bind oxygen.

Haemodialysis: the removal of waste products from the blood by passing the blood through a dialysis machine.

Haemofiltration: the removal of waste products from the blood by passing the blood through filters outside the body and returning the blood to the patient.

Haemoglobin: the red oxygen-carrying pigment of red blood cells that comprises an iron-containing group called haem combined with globin; it has the ability to reversibly bind oxygen.

Haemolysis: breakdown of red blood cells causing release of haemoglobin.

Haemolytic uraemic syndrome: a syndrome (seen predominantly in children) in which intravascular platelet clumping occurs mainly in the renal vasculature causing renal impairment.

Haemorrhagic colitis: a bloody diarrhoea caused by a strain of *Escherichia coli* that is generally self-limiting but may be complicated by haemolytic uraemic syndrome.

Haemorrhagic diathesis: a predisposition to abnormal clotting and bleeding.

Haemostatic: an agent that stops bleeding.

Hair follicle: the sheath in which a hair grows.

Half life (plasma half life): the time required for the drug plasma concentration to fall to half of a certain measured concentration.

Hallucination: a false perception of an object that occurs in the absence of such an object.

Hallucinogen: an agent capable of producing hallucinations.

HCG (human chorionic gonadotrophin): a hormone produced by the placenta.

Heart block: impairment of conduction of the normal sinus node impulse through the heart.

Heparinised blood: blood that has had heparin added to it to increase the clotting time.

Hepatic clearance: removal of a drug from the blood by metabolism in the liver.

Hepatic dysfunction: impaired functioning of the liver.

Hepatic encephalopathy: a degenerative disease of the brain that usually occurs as a consequence of advanced liver disease; disturbances of consciousness occur that may progress to coma.

Hepatic failure: liver failure.

Hepatic portal system: the blood supply of the liver.

Hepatitis: inflammation of the liver.

Hepatobiliary: affecting the liver and the bile or bile ducts.

Hepatocellular carcinoma: cancer of the liver cells.

Hepatocyte: a liver cell.

Hepatolenticular degeneration: a rare autosomal disease of impaired excretion of excess copper which leads to copper accumulation in the liver, brain and other organs including the kidneys and corneas; also known as Wilsons disease.

Hepatorenal: relating to the liver and the kidneys.

Hepatotoxicity: having a destructive or harmful effect on liver cells.

Histamine: a naturally occurring substance found in all body tissues that has several functions including capillary dilatation, smooth muscle contraction, increased gastric secretion and acceleration of heart rate.

Homeostasis: the tendency to maintain a stable internal environment, such as constant body temperature or blood pressure.

Homogeneous: of the same quality or consistency throughout.

Homogenous: having a similar structure because of descent from a common ancestor.

Hormone: a chemical substance produced by an endocrine gland and circulated in the bloodstream that regulates the activity of an organ or tissues.

Human chorionic gonadotrophin: *see* HCG.

Hyaluronidase: an enzyme that catalyses the breakdown of hyaluronic acid.

Hydrolysis: the splitting of a compound by the addition of water.

Hydrophilic: a substance that has an affinity with water.

Hydroxylation: addition of a hydroxyl group.

Hyperaemia: an increase of blood in an area.

Hyperaesthesia: excessive sensitivity.

Hyperbaric oxygen: oxygen under greater than atmospheric pressure.

Hyperbilirubinaemia: excessive bilirubin in the blood, which may lead to jaundice.

Hyperimmune: having large quantities of specific antibodies in the serum.

Hyperinsulinaemia: excessively high blood insulin levels.

Hyperkalaemia: abnormally high potassium concentration in the blood.

Hyperkeratosis: thickening of the stratum corneum of the skin.

Hypermagnesaemia: abnormally high magnesium concentration in the blood.

Hypernatraemia: abnormally high sodium concentration in the blood.

Hyperpigmentation: excessive pigmentation of the skin.

Hypertension: a higher than normal arterial blood pressure.

Hyperthermia: abnormally raised core body temperature (to above 37.2°).

Hyperventilation (hyperpnoea): increased breathing that results in reduced carbon dioxide tension and alkalosis.

Hypnotic: a drug used to induce sleep.

Hypocalcaemia: low blood calcium concentration.

Hypoglycaemia: decreased concentration of glucose in the blood that may produce symptoms of anxiety, cold sweat, hypothermia and delirium or coma (hypoglycaemic coma).

Hypoglycaemic agent: an agent that controls blood sugar levels and is used in the management of diabetes mellitus.

Hypoglycaemic coma: *see* Hypoglycaemia.

Hypokalaemia: low potassium concentration in the blood.

Hyponatraemia: low sodium concentration in the blood.

Hypophosphataemia: decreased amounts of phosphates in the blood; signs include haemolysis, weakness and convulsions.

Hypotension: abnormally low blood pressure.

Hypothermia: a below normal core body temperature (35° or lower).

Hypotonia: reduced tone of the skeletal muscles.

Hypotonic: displaying hypotonia; solution with a low osmotic pressure.

Hypoventilation: reduced breathing that results in increased carbon dioxide tension.

Hypoxia: low oxygen level in the tissues despite adequate perfusion by blood.

Iatrogenic: any adverse condition that is a result of treatment by a doctor.

Idiosyncrasy: an abnormal individual reaction to a drug or protein or other administered agent.

IgE: one of the immunoglobulins that act as antibodies.

Iliac: relating to the ilium, part of the hip bone.

Immune response: the response of the immune system to an antigenic stimulus.

Immunogen: a substance capable of inducing an immune response.

Immunoglobulin: one of the 5 classes of glycoproteins (IgA, IgD, IgE, IgG and IgM) that function as antibodies.

Immunostimulant: an agent capable of stimulating immune responses.

Immunosuppressant: an agent that can suppress immune responses, such as a corticosteroid or ciclosporin.

Immunotoxic: toxic to or toxicity involving the immune system.

Immunotoxin: a molecule formed by coupling a toxin to an antibody.

Impotence: failure to initiate an erection or to maintain an erection until ejaculation.

***In vitro*:** in the test tube.

***In vivo*:** in living tissue.

Incontinence: inability to control defaecation or urination.

Induce (metabolism): increase/enhance metabolism of a substance.

Inferior vena cava: the main trunk vein that supplies the lower limbs.

Ingestion: the taking in of food/medicines by mouth.

Inhaler: an apparatus for administering a vapour or volatilised medication by inhalation.

Insomnia: inability to sleep; abnormal wakefulness.

Insulin-like growth factor I: one of a group of polypeptide hormones believed to be responsible for many of the anabolic effects of growth hormone.

Interferon-α: one of a large family of glycoproteins that are produced by virus-infected cells and that confer protection on uninfected cells of the same species.

Interleukin-2: a glycoprotein that stimulates the proliferation of T lymphocytes and therefore amplifies the immune response to an antigen.

International Normalised Ratio: a standardised form of the prothrombin–time ratio that allows for accurate monitoring of patients taking anticoagulant therapy.

Interstitial nephritis: inflammation of the interstitial tissue of the kidney.

Intoxication: Poisoning.

Intracellular: within a cell.

Intramuscular: within a muscle.

Intraperitoneal: within the peritoneal cavity (abdominal and pelvic cavities).

Intravascular: within a blood vessel.

Intravenous: within a vein.

Isoform: proteins that have the same function and similar structure but are specific to different tissues.

Jaundice: a condition characterised by hyperbilirubinaemia. Mild forms are only detectable chemically; more severe forms present with yellow discoloration of sclerae, skin and mucous membranes that results from deposition of bile pigment.

Jejunum: the portion of the small intestine between the duodenum and the ileum.

Keratin: a protein that is one of the main constituents of skin, hair and nails.

Keshan disease: a fatal disease of the heart muscle caused by dietary deficiency of essential trace elements.

Ketosis: accumulation of ketone bodies in the bloodstream and body tissues due to incomplete metabolism of fatty acids.

Kidneys: the two organs in the lumbar region that filter the blood and excrete waste products in urine and regulate the concentrations of various ions in the extracellular fluid.

Kinin: any of a group of polypeptides found in plasma, such as bradykinin, that cause vasodilatation of most vessels but vasoconstriction of the pulmonary vessels and also alter vascular permeability.

Lacrimation: tear formation.

Lactic acidosis: a metabolic acidosis that occurs when cellular respiration is impaired (in conditions such as shock and septicaemia) producing a build up of lactic acid in the blood.

Laryngeal: relating to the larynx (voice box).

Laryngitis: inflammation of the larynx (voice box).

Laxative: an agent that promotes bowel evacuation.

Leptomeningeal: relating to the leptomeninges (the membranes that cover the brain and spinal cord).

Leptomeningeal carcinoma: cancer of the meninges (the membranes covering the brain and spinal cord), specifically the pia mater and arachnoid membranes.

Leukocyte: a white blood cell; there are several different types that are classified into two large groups, granular leukocytes (basophils, eosinophils, and neutrophils), and non-granular leukocytes (lymphocytes, and monocytes).

Leukopenia: reduction in the number of leukocytes in the blood below about 5000/mm^3.

Lipid: a fat or fat-like substance that is characterised by being insoluble in water.

Lipid-lowering drug: a drug that reduces cholesterol and/or triglycerides in the blood and is used to treat hyperlipidaemias and to reduce cardiovascular risk.

Lipophilic: having an affinity for fat.

Loading dose: a large initial dose that is sometimes given so that therapeutic steady-state plasma concentrations are reached more quickly.

Local anaesthetic: an anaesthetic that produces reversible loss of function or sensation near to their site of application or injection by diminishing nerve impulse conduction.

Locus: in anatomy, a general term for a site in the body; in genetics, the position of a gene on a chromosome.

Lyme disease: an infectious disease, caused by *Borrelia burgdorferi* and transmitted largely by *Ixodes* ticks, that is a multi-system disease affecting the skin in the initial stages (erythema migrans), followed by variable effects on the nervous system, heart and joints.

Lymphatic: pertaining to lymph or a lymph vessel; by extension, the term is used alone to designate a lymphatic vessel or, in the plural, to designate the lymphatic system.

Lymphocyte: a type of white blood cell; there are two types, B lymphocytes that provide humoral immunity, and T lymphocytes that are responsible for cellular immunity.

Lymphocytosis: an increase in normal lymphocytes in the blood.

Lysozyme: an enzyme found in saliva and tears that acts as an antiseptic.

Macrocyte: an abnormally large red blood cell.

Malaise: a vague feeling of illness and discomfort.

Malate dehydrogenase: an enzyme found in the mitochondria and cytosol that catalyses the oxidation of L-malate to oxaloacetate.

Mania: undue elation with hyperexcitability and hyperactivity that occurs usually as a phase of bipolar disorder (manic depression).

Manic-depressive disorder: *see* Bipolar disorder.

Meconium: a dark greenish-black viscous substance that forms the first bowel movement in newborns.

Mees lines: white lines on the fingernails that can be a sign of poisoning with trace elements and arsenic.

Melanin: a black pigment found in skin, hair and the choroid of the eye.

Meningitis: inflammation of the meninges (the membranes surrounding the spinal cord and brain) usually caused by either bacterial or viral infection.

Mercapturic acid: a cysteine conjugate excreted in the urine that is the end-product of metabolism of a glutathione conjugate.

Metabolic acidosis: disturbance of the acid–base balance due to loss of base or accumulation of (non-carbonic) acid.

Metabolism: the chemical processes that occur in the maintenance of living tissues i.e. synthesis of new tissue (anabolism) and breakdown of old tissue (catabolism); also used to describe the chemical transformation undergone by drugs in the body (biotransformation).

Metabolism inducer: a substance that can enhance the rate of metabolism by increasing the numbers of metabolising enzymes.

Metabolite: any product of metabolism.

Metallothionein: a protein that is involved in the transport of metals, such as zinc, within the body.

Methaemoglobin: a brown pigment that is a form of haemoglobin (but which is unable to transport oxygen) in which the haem portion is oxidised from the ferrous to ferric state.

Methaemoglobinaemia: the presence of methaemoglobin in the blood; symptoms depend on the amount of methaemoglobin present and can range from breathlessness on exertion through to cyanosis, fatigue, nausea and vomiting, and drowsiness which can progress to stupor and coma.

Methylation: the addition of a methyl group to a compound.

Methyltransferase: an enzyme that catalyses the transfer of a methyl group from one compound to another.

Microflora: micro-organisms present in or characteristic of a specific location.

Microsomal enzyme: a general term used for the enzymes found in the smooth endoplasmic reticulum of cells that are responsible for the metabolism of foreign compounds, the major one being the cytochrome P450 system.

Minor tranquilliser: the old term for an anxiolytic sedative, such as diazepam, used for treating anxiety disorders.

Miosis: contraction of the pupils.

Mixed-function oxidase system: cytochrome P450-dependent oxidation reactions.

Monoamine oxidase inhibitor: a drug used to treat depression that inhibits monoamine oxidase (the enzyme responsible for the metabolism of several biogenic amines).

Motility: capable of spontaneous movement.

Motor: a muscle or nerve that produces movement.

Motor paralysis: paralysis of voluntary muscles.

Mucin: a mixture of glycoproteins that are secreted by many cells and are the main constituent of mucus.

Mucosa: a mucous membrane.

Mucous membrane: a membrane or lining that contains glands that secrete mucus.

Munchausens syndrome: a condition in which the patient repeatedly presents with an apparent acute physical illness and gives a false history.

Mutagen: a substance that causes changes in DNA.

Mutation: a permanent transmissible change in genetic material.

Mycotoxin: a fungal toxin.

Mydriasis: dilatation of the pupil of the eye.

Myocardial infarction: necrosis of heart muscle as a result of interruption of the blood supply to the area usually due to thrombosis at the site of a ruptured atheromatous plaque.

Myoglobinuria: the presence of myoglobin (the oxygen-transporting muscle protein) in the urine.

***N*-acetyltransferase:** an enzyme that catalyses the transfer of an acetyl group, often acetyl coenzyme A, to another compound.

NAD: nicotinamide adenine dinucleotide and its reduced and oxidised forms are used as co-factors in many metabolic drug reactions.

NAD+: *see* NAD.

NADH: *see* NAD.

NADP: nicotine adenine dinucleotide phosphate is used as a cofactor in many metabolic drug reactions.

Narcolepsy: repetitive, uncontrollable episodes of sleep occurring in the daytime.

Narcotic: an agent that produces deep sleep, applied especially to the opioids.

Nasal septum: the partition between the nasal cavities.

Nausea: a feeling of sickness.

***N*-dealkylation:** removal of an alkyl group from a compound where the alkyl group is attached to a nitrogen atom.

Necropsy: examination of a body after death.

Necrosis: localised death of tissue.

Neonatal: the first 4 weeks after birth.

Neonate: newborn infant up to 4 weeks old.

Neoplastic disease: generally used to refer to malignant neoplasms (cancers).

Nephrotoxicity: toxicity to kidney cells.

Neuroleptic: a drug that acts on the nervous system and is used in the treatment of psychoses (antipsychotic).

Neurological: relating to the nervous system.

Neuromuscular blocking drug: a drug that affects transmission at the neuromuscular junction and is used to produce muscle relaxation in anaesthesia.

Neuromuscular junction: the junction between the skeletal muscle fibre and the nerve that innervates it.

Neuropathy: damage to the peripheral nervous system.

Neurotoxic: toxic to the nervous system.

Neurotoxin: a toxin that is poisonous or destructive to nervous tissue.

Neurotransmitter: an endogenous substance involved in the transmission of nerve impulses, such as adrenaline.

Non-compliance: failure of the patient to comply with the dosage instructions for his/her medication.

***N*-oxidation:** formation of an *N*-oxide.

NSAID: a non-steroidal anti-inflammatory drug.

Nystagmus: repetitive involuntary and jerky movement of the eyeball.

Organophosphorous insecticide: an insecticidal compound that contains phosphorus bound to an organic compound.

Ocular: relating to the eye.

***O*-dealkylation:** metabolism of an aromatic alkyl ether to give a phenol and corresponding aldehyde.

Oedema: the presence of abnormally large amounts of fluid in the tissues of the body.

Oesophagus: the tube that extends from the pharynx to the stomach.

Oestrogens: a steroidal hormone that controls the development and maintenance of the female sex organs, secondary sex characteristics, and mammary glands as well as certain functions of the uterus.

Olfactory nerve: the nerve supplying the nose.

Opiate: an agent derived from opium.

Opioid: a narcotic with the same properties as an opiate but that is not derived from opium.

Opisthotonus: a spasm with extreme extension of the body with the head and the heels being bent backwards and the body bowed forwards.

Optic neuritis: inflammation of the optic nerve.

Oral adsorbent: a substance, such as activated charcoal, that is given by mouth to bind poisons in the gastrointestinal tract thereby reducing their absorption.

Oral contraceptive: an oestrogen and progestogen or progestogen-only preparation that is taken by mouth for contraception.

Organic aciduria: excessive excretion of one or more organic acids in the urine.

Osteoporosis: loss of bone density.

Ototoxicity: causing damage to the ear.

Oxyhaemoglobin: oxygenated haemoglobin.

P450 enzymes: *see* Cytochrome P450.

Paediatric: relating to paediatrics; the branch of medicine that deals with the child.

Pancytopenia: reduced amounts of platelets, and red and white cells in the blood.

Papillary necrosis (renal): death of the tissue that forms the papillae in the medulla of the kidneys.

Papule: a small circumscribed elevation of the skin.

Paraesthesia: an abnormal touch sensation (often without any external stimulus), such as tingling, burning or prickling.

Paralysis: loss or impairment of motor function that may be due to damage to the nerves or muscular mechanisms.

Paranoia: behaviour characterised by delusions of persecution or of grandeur, or a combination of the two.

Parasympathetic system: a part of the autonomic nervous system.

Parent drug: the administered drug that undergoes metabolism to form an active metabolite.

Parenteral: administration of a drug (or exposure to a substance) by a route other than the gastro-intestinal tract.

Parkinsons disease: a slowly progressive neurodegenerative disease with reduced brain dopamine activity characterised by tremor, impaired voluntary movement, and loss of postural reflexes.

Parotid gland: the salivary gland.

Pathogenesis: the development and origin of disease.

Pathognomonic: a sign or a symptom that is distinctive to a particular disease and therefore allows a diagnosis to be made.

Peptide: a molecule made up of 2 or more amino acids.

Pericardial: relating to the pericardium, the membranous sac that surrounds the heart.

Perimortem: the time immediately before and after death.

Peripheral blood: blood in the systemic circulation.

Peripheral neuritis: inflammation of a peripheral nerve.

Peristalsis: waves of contraction and relaxation that pass along the intestines and that move the contents along.

Peritoneal dialysis: removal of waste products from the blood by irrigation of the peritoneal cavity with a dialysis solution.

Perspiration: *see* Sweating.

P-glycoprotein: a cell surface protein found in many cells, including tumour cells where it aids transport of anti-cancer drugs out of the tumour cell and is therefore responsible for drug resistance.

Pharmacodynamics: the study of the effects of drugs and the mechanisms of their actions and the correlation of these with their chemical structure.

Pharmacogenetics: the study of the relationship between genetic factors and the effects produced by drugs (often refers to side effects produced by drugs).

Pharmacokinetics: the study of the absorption, distribution and clearance (metabolism and excretion) of drugs with respect to time.

Pharmacological: relating to the properties and reactions of drugs.

Pharyngitis: inflammation of the throat.

Phase I metabolism: alteration of the foreign molecule (drug) so that a functional group is added or uncovered that can then undergo conjugation in a phase II metabolic reaction.

Phase II metabolism: a conjugation reaction (addition of another moiety) that generally increases the water solubility of the product enabling its excretion in the bile or urine.

Phenothiazine: any of a group of antipsychotic agents derived from the phenothiazine structure.

Phenotype: the physical, biochemical and physiological make-up of an individual (includes genetically and environmentally determined traits).

Pheomelanin: a sulfur-containing type of melanin found in red hair.

Phosphodiesterase: an enzyme that catalyses the hydrolysis of one of the ester linkages in a phosphodiester compound.

Phospholipase: an enzyme that catalyses the hydrolysis of an ester bond in a phospholipid.

Phospholipase A_2: an esterase that catalyses the hydrolysis of a membrane phospholipid.

Piloerection: erection of the hair.

Pink disease: a disease of early childhood, usually caused by mercury poisoning, and characterised by pink, swollen fingers and toes and other symptoms that include skin rashes, listlessness, irritability, failure to thrive and photophobia.

Pituitary gland: a gland located at the base of the brain attached to the hypothalamus.

Pituitary hormone: one of the hormones secreted by the pituitary gland, such as gonadotrophin or growth hormone.

Placental transfer: transfer of a substance from the mothers blood to the fetus blood by passage through the placenta.

Plasma: the fluid fraction of blood; plasma is to be distinguished from serum (see below).

Plasma esterase: an enzyme that acts on ester bonds to produce an alcohol and an acid.

Pleural: relating to the pleura, the serous membrane that covers the surface of the lungs.

Polymerase: an enzyme that catalyses polymerisation, especially of nucleotides to polynucleotides.

Polymorphic: occurring in several or many forms.

Polypeptide: a chain of amino acids.

Polyuria: excretion of an excessive volume of urine, as in diabetes mellitus.

Portal vein: the vein that supplies blood to the liver.

Positional nystagmus: nystagmus that occurs or is altered depending on the position of the head.

Postmortem: after death.

Precursor: in biological processes, a substance that undergoes transformation/degradation to form a more active compound.

Presynaptic: situated just before the synapse (the join of 2 adjacent neurones).

Pre-systemic: occurring before the drug is in the systemic circulation.

Procollagen: a precursor of collagen, the protein that forms connective tissue.

Prodrug: an inactive drug that, after administration, is metabolised to produce the active pharmacological agent.

Prognosis: a forecast of the probable outcome of a disease.

Prophylaxis: an intervention that is aimed at the prevention of a disease.

Protease: an enzyme that catalyses the splitting of internal peptide bonds in a polypeptide or protein.

Protein: a nitrogenous organic compound that is composed of amino acids.

Protein binding: Non-covalent binding of a drug to a plasma protein (usually albumin).

Protein phosphatase: a hydrolase enzyme that catalyses the cleavage of phosphoryl groups from phosphoproteins.

Proteolysis: the breaking down of proteins into smaller polypeptides.

Proteolytic enzyme: an enzyme that catalyses the breakdown of proteins.

Proteomics: the study of the proteome (the complete set of proteins produced from the information encoded in a genome) under various conditions, as a means of understanding biological processes.

Prothrombin time: the time taken for a clot to form in plasma on the addition of thromboplastin and calcium.

Protozoal: relating to the Protozoa (unicellular organisms).

Provocation test: the administration of a chemical or antigen in order to assess for a response.

Proximal renal tubule: part of the nephron in the kidney.

Pruritus: Itching.

Pseudocholinesterase: a plasma enzyme that is responsible for the metabolism and inactivation of succinylcholine.

Pseudohallucination: a hallucination that the subject knows is unreal and is generated within the mind.

Psychic: relating to the mind.

Psychoactive drug: a drug that has an effect on mental state (also called a psychotropic drug).

Psychological dependence: a substance dependence in which the drug is used to obtain relief from tension or emotional discomfort rather than being associated with tolerance or withdrawal.

Psychomotor: relating to motor effects of cerebral or psychic activity.

Psychomotor skills: the motor effects of cerebral or psychic activity.

Psychosis: a severe psychiatric disorder in which there is disordered thinking and loss of contact with reality due to delusions and/or hallucinations; there may also be mood and behavioural disturbances.

Psychotropic drug: *see* Psychoactive drug.

Ptosis: drooping of the upper eyelid.

Pulmonary: relating to the lungs.

Pulmonary fibrosis: chronic inflammation and progressive fibrosis of the lungs.

Pulmonary oedema: accumulation of extravascular fluid in the lungs.

Pulse: the expansion of the arteries that is transmitted by the contraction of the left ventricle that is usually palpated at the wrist.

Pupillary dilation: dilation of the pupils of the eye.

Purgative: *see* Laxative.

Putrefaction: the process of decomposition or rotting.

Pyrogenic: causing fever.

QT-time: a measure on the electrocardiogram recording between the Q and the T points of the action potential.

Receptor: a molecular structure within a cell or on its surface that binds a specific substance or drug to elicit a specific physiologic effect.

Recombinant EPO: *see* Erythropoietin.

Recombinant growth hormone: *see* Growth hormone.

Rectal: relating to the rectum, the lower part of the large intestine that ends in the anal canal.

Red blood cells: *see* Erythrocytes.

Red cells: *see* Erythrocytes.

Reduced haemoglobin (deoxyhaemoglobin or deoxygenated haemoglobin): haemoglobin that is formed when oxyhaemoglobin releases its oxygen.

Reflux (gastric): a backward flow; usually refers to gastric reflux where the stomach contents reflux back into the oesophagus.

Regime: a regulated programme of an activity, such as exercise, diet or medication.

Regurgitation: *see* Vomiting.

Renal carcinoma: cancer of the kidney.

Renal clearance: clearance of a drug/metabolite from the blood by excretion by the kidney.

Renal dysfunction: *see* Renal impairment.

Renal failure: the progressive loss of renal function so that waste products and metabolites accumulate in the blood; it usually refers to a glomerular filtration rate of less than 10 mL/min.

Renal impairment: a reduced renal function where glomerular filtration rate is between 10 and 60 mL/min.

Renal tubule: part of the nephron in the kidney.

Respiration: the act of breathing.

Respiratory alkalosis: an increase in tissue pH that is a result of excess loss of carbon dioxide from the body, usually caused by hyperventilation.

Respiratory arrest: when breathing stops.

Respiratory failure: a condition resulting from respiratory insufficiency, in which there is persistent abnormally low arterial oxygen tension or abnormally high carbon dioxide tension.

Reticulocyte: an immature red blood cell.

Retroperitoneal: behind the peritoneum (the membrane that lines the abdominal and pelvic cavities).

Rhabdomyolysis: disintegration of muscle, associated with excretion of myoglobin in the urine.

Rhodanese: an enzyme involved in the metabolism of cyanide.

RNA: ribonucleic acid, the genetic material in RNA viruses.

Salicylate: a compound derived from salicylic acid; it is an inhibitor of prostaglandin synthesis and has analgesic, antipyretic and anti-inflammatory effects.

Saliva: the clear, alkaline secretion from the salivary glands.

Salivation: the secretion of saliva.

Sebaceous glands: the glands of the skin that secrete sebum.

Sebum: the oily secretion of the sebaceous glands.

Sedative: an agent that reduces functional activity (may also be called a tranquiliser).

Seizure: a single episode of epilepsy.

Sensory nerve: a nerve that conducts impulses from a sense organ back to the spinal cord or brain.

Serotonin: a monoamine found in the brain, blood platelets and gastro-intestinal tract that has various functions including involvement in central nervous system transmission, vascular spasm and gastro-intestinal motility.

Serous fluid: pertaining to or resembling serum.

Serum: the cell-free and fibrinogen-free fluid that remains once plasma or blood has clotted.

Shock: failure of the circulatory system to maintain adequate perfusion of vital organs.

Skeletal muscle: striated muscle that is attached to bone.

Small intestine: the proximal part of the intestine, comprising the duodenum, jejunum and ileum.

Sodium channel: a protein channel selective for the passage of sodium ions.

Somatic nerve: a nerve that supplies skeletal muscle.

Spastic paresis: a form of paralysis with associated muscle spasms and hypertonia.

Spleen: a large vascular organ in the upper part of the abdominal cavity that produces lymphocytes and plasma cells and also breaks down red blood cells.

SSRI: selective serotonin re-uptake inhibitor, such as fluoxetine, that is used for the management of depression.

Steady-state concentration: the plasma-drug concentration that is achieved with chronic dosing.

Steroid: a term used to denote a naturally occurring lipid, related to cholesterol, that has a particular ring structure, such as the adrenocortical hormones, sex hormones, bile acids and sterols.

Sterol: a steroid, such as cholesterol or ergosterol, that has a ring structure with a long side-chain at position 17 and at least one alcoholic hydroxyl group, usually at position 3.

Stimulant drug: an agent or remedy that produces stimulation.

Striated muscle: a muscle that has the appearance of fine stripes, e.g. cardiac and voluntary muscle.

Stroke: A stroke, previously known medically as a cerebrovascular accident (CVA), is the rapidly developing loss of brain function(s) due to disturbance in the blood supply to the brain.

Stupor: a state of marked impairment of consciousness that only responds to vigorous stimulation.

Subclavian: below the clavicle (collar-bone).

Subcutaneous: underneath the skin.

Sublingual: underneath the tongue.

Sublingual glands: salivary glands underneath the tongue.

Submandibular glands: salivary glands underneath the jaw.

Substrate: a substance upon which an enzyme acts.

Sudoresis: *see* Sweating.

Sulfhaemoglobin: (sulphaemoglobin, sulphmethaemoglobin) a sulfide oxidation product of haemoglobin that cannot transport oxygen or carbon dioxide and is formed in the body by certain drugs.

Sulfotransferase: an enzyme that catalyses the transfer of a sulfate group.

Sulfoxidation: the addition of an oxygen atom to the sulfur atom of an organic compound to form a sulfoxide.

Suppository: a solid dosage form, administered usually rectally or vaginally, in which the drug is dispersed in a base that is solid at room temperature but melts or dissolves at body temperature.

Surrogate: a substitute.

Sweat: the liquid secreted by the sweat glands.

Sweating: the secretion of sweat.

Sympathetic: a part of the autonomic nervous system.

Symptom: any subjective evidence of disease.

Synaesthesia: a sense impairment where stimulus of one part of the body may produce a sensation in a different part of the body or in a different sense, for example a sound produces a sensation of colour.

Synergism: when the effect produced by 2 substances together is greater than the additive effect of the 2 compounds.

Systemic circulation: the general circulation that carries oxygenated blood from the left side of the heart to the tissues of the body, and returns the venous blood to the heart.

Systemic lupus erythematosus: an auto-immune disease, more common in women, that is characterised by tissue damage involving the skin, joints, kidneys, central nervous system and other organs, with the most common symptom being arthralgia or arthritis.

Tachyarrhythmia: any disturbance of the heart rhythm in which the heart rate is abnormally increased.

Tachycardia: excessive rapidity in the action of the heart.

Telogen phase: the resting third phase of the hair growth cycle that follows catagen.

Teratogen: any agent or factor capable of disrupting fetal development and producing malformation.

Tetanus: uncontrolled muscle spasm that is caused by the neurotoxin produced by the bacillus *Clostridium tetani*.

Therapeutic index: the ratio of the median lethal dose to the median effective dose.

Therapeutic range: *see* Therapeutic window.

Therapeutic window: the range between the minimum and maximum doses of a drug.

Thoracic: relating to or affecting the thorax (chest).

Thrombocytopenic purpura: any form of purpura (spontaneous extravasation of blood from capillaries into the skin) in which the platelet count is decreased.

Thyroid: gland that sits on both sides of the windpipe and secretes thyroxine that controls the rate of metabolism.

Tinnitus: a sound in the ears such as buzzing, ringing, roaring or clicking.

Tissue plasminogen activator: an endogenous enzyme that converts plasminogen in blood clots to fibrinolysin that results in breakdown of the clot; several types of tissue plasminogen activator are available for clinical use for the treatment of thromboembolic disorders.

Tolerance: a decreasing response to a stimulus after prolonged exposure (*see also* Drug tolerance).

Torsade de pointes: a potentially lethal ventricular tachycardia with a characteristic ECG pattern.

Toxin: a poison.

Trachea: the windpipe that descends from the larynx to the bronchi.

Tranquilliser: a drug with a calming or soothing effect (usually refers to an anti-anxiety agent, also known as a minor tranquilliser).

Transferrin: a serum glycoprotein that binds and transports iron.

Transfusion: the introduction of blood (whole blood or blood components) directly into a blood vessel.

Transit time: a measure of the time required for faeces to pass through the colon.

Transplant: an organ or tissue that is removed from its site in the body and grafted into another site or into another individual.

Tremor: an involuntary trembling.

Tricyclic antidepressant: an antidepressant drug that possesses a three-ring molecular structure and that inhibits the neuronal re-uptake of noradrenaline in the central nervous system.

Trophic: relating to nutrition.

Trough concentration: the minimum plasma-drug concentration that occurs before administration of the following dose.

Trypsin: an enzyme present in pancreatic secretions that catalyses the breakdown of protein.

L-tryptophan: an amino acid that is an essential constituent of the diet.

Tuberculostatic drug: a drug, such as isoniazid or rifampicin, that is used in the treatment of tuberculosis.

Tubular necrosis (renal): severe damage or necrosis of the tubule cells of the kidney that causes acute renal failure.

Tyrosine hydroxylase: an enzyme found in the brain that activates molecular oxygen to catalyse the hydroxylation of tyrosine to dopa.

Uraemia: retentionin the blood of urea, creatinine and other nitrogenous products of protein metabolism.

Urea: the end-product of protein metabolism that is excreted in the urine.

Urea nitrogen: the urea concentration of blood or serum expressed in terms of nitrogen content.

Uridine diphosphate glucuronosyltransferase: one of the enzymes involved in glucuronide formation.

Urinary retention: accumulation of urine within the bladder due to inability to urinate.

Urine: the fluid excreted by the kidneys.

Urticaria: a usually transient allergic skin reaction consisting of itchy, pinkish, raised wheals.

Vaccine: a suspension or extract of attenuated or killed micro-organisms used for the prevention or treatment of infectious diseases.

Vaginal: relating to the vagina.

Vasculopathy: any disorder of the blood vessels.

Vasodilator: causing dilation of the blood vessels.

Vasospasm: a spasm of blood vessels producing vasoconstriction.

Venepuncture: insertion of a needle into a vein.

Venous blood: blood obtained from a vein.

Vertical nystagmus: an up-and-down movement of the eyes.

Vesicle: a small bladder or sac containing fluid; a skin blister.

Visual acuity: sharpness of vision.

Vitamin: a substance that is found in many foods in small amounts and is necessary in trace amounts for the normal metabolic functioning of the body.

Vitreous humour: the transparent jelly-like substance that fills the cavity inside the eyeball between the lens and the retina.

Volume of distribution: the apparent volume of body fluids into which a drug is distributed.

Vomiting: the forcible expulsion of the stomach contents through the mouth.

Vomitus: matter that has been vomited.

Washout: irrigation or lavage of a body cavity (often the stomach).

Wheal: an itchy skin reaction, typical of urticaria, with smooth blister-type lesions that are redder or paler than the surrounding skin.

Whole blood: blood from which none of the elements have been removed.

Wilsons disease: *see* Hepatolenticular degeneration.

Withdrawal symptoms: a substance-specific mental disorder that follows the cessation of use or reduction in intake of a psychoactive substance that had been regularly used to induce a state of intoxication.

Wolff-Parkinson-White syndrome: a disturbed heart rhythm where there is atrial fibrillation in association with pre-excitation.

Xanthine: a purine derivative, such as theophylline, used for its bronchodilator properties.

Xenobiotic: a chemical foreign to a given biological system.

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